COMPOSITIONS COMPRISING MODIFIED ANELLOVIRUS CAPSID PROTEINS AND USES THEREOF

CROSS REFERENCE TO RELATED APPLICATIONS

This application claims the benefit of U.S. Provisional Application No. 63/344,019, filed May 19, 2022 and U.S. Provisional Application No. 63/387,337, filed December 14, 2022. The contents of the aforementioned applications are hereby incorporated by reference in their entirety.

BACKGROUND

There is an ongoing need to develop suitable vectors to deliver therapeutic agents to patients.

SUMMARY

The present disclosure provides an anellovector, e.g., a synthetic anellovector, which can be used as a delivery vehicle, e.g., for delivering genetic material, for delivering an effector, e.g., a payload, or for delivering a therapeutic agent or a therapeutic effector to a eukaryotic cell (e.g., a human cell or a human tissue). In some embodiments, an anellovector (e.g., particle, e.g., a viral particle, e.g., an *Anellovirus* particle) comprises a genetic element (e.g., a genetic element comprising a therapeutic DNA sequence) encapsulated in a proteinaceous exterior (e.g., a proteinaceous exterior comprising an *Anellovirus* capsid protein, e.g., an Anellovirus ORF1 molecule or a polypeptide encoded by an *Anellovirus* ORF1 nucleic acid, e.g., as described herein), which is capable of introducing the genetic element into a cell (e.g., a mammalian cell, e.g., a human cell). In some embodiments, the anellovector is a particle comprising a proteinaceous exterior comprising a polypeptide encoded by an *Anellovirus* ORF1 nucleic acid (e.g., an ORF1 nucleic acid of *Betatorquevirus*, e.g., as described herein).

In some embodiments, the proteinaceous exterior of an anellovector or anelloVLP comprises a modified Anellovirus ORF1 molecule. In some embodiments, the Anellovirus ORF1 molecule is modified to delete at least a portion of the structural arginine-rich region (e.g., as described herein). In some embodiments, the Anellovirus ORF1 molecule is modified to delete at least a portion of the structural C-terminal domain (e.g., as described herein). In some embodiments, the Anellovirus ORF1 molecule is a chimeric ORF1 molecule comprising a fragment or domain (e.g., a structural arginine-rich region, a P1 domain, a P2 domain, a P1-1 domain, and/or a P1-2 domain, e.g., as described herein) from a different Anellovirus ORF1 protein (e.g., as described herein). In some embodiments, the Anellovirus ORF1 molecule is a chimeric ORF1 molecule comprising a fragment or domain from a protein other than an Anellovirus ORF1 protein (e.g., a protein from another virus, e.g., as described herein).

In some embodiments, the anellovector or anelloVLP comprises on its exterior surface (e.g., attached to a proteinaceous exterior) a surface moiety as described herein. In some embodiments, the proteinaceous exterior comprises an ORF1 molecule attached to the surface moiety. In some embodiments, the proteinaceous exterior comprises an ORF1 molecule comprising a click handle. In some embodiments, the proteinaceous exterior comprises an ORF1 molecule fused to a polypeptide surface moiety. In some embodiments, the proteinaceous exterior comprises a plurality of ORF1 molecules each attached to a surface moiety, e.g., wherein the plurality of ORF1 molecules form a multimer (e.g., a dimer, trimer, or pentamer).

The genetic element of an anellovector of the present disclosure is typically a circular and/or single-stranded DNA molecule (e.g., circular and single stranded), and generally includes a protein binding sequence that binds to the proteinaceous exterior enclosing it, or a polypeptide attached thereto, which may facilitate enclosure of the genetic element within the proteinaceous exterior and/or enrichment of the genetic element, relative to other nucleic acids, within the proteinaceous exterior. In some instances, the genetic element is circular or linear. In some instances, the genetic element comprises or encodes an effector (e.g., a nucleic acid effector, such as a non-coding RNA, or a polypeptide effector, e.g., a protein), e.g., which can be expressed in the cell. In some embodiments, the effector is a therapeutic agent or a therapeutic effector, e.g., as described herein. In some instances, the effector is an endogenous effector or an exogenous effector, e.g., to a wild-type *Anellovirus* or a target cell. In some embodiments, the effector is exogenous to a wild-type *Anellovirus* or a target cell. In some embodiments, the anellovector can deliver an effector into a cell by contacting the cell and introducing a genetic element encoding the effector into the cell, such that the effector is made or expressed by the cell. In certain instances, the effector is an endogenous effector (e.g., endogenous to the target cell but, e.g., provided in increased amounts by the anellovector). In other instances, the effector is an exogenous effector. The effector can, in some instances, modulate a function of the cell or modulate an activity or level of a target molecule in the cell. For example, the effector can decrease levels of a target protein in the cell. In another example, the anellovector can deliver and express an effector, e.g., an exogenous protein, *in vivo*. Anellovectors can be used, for example, to deliver genetic material to a target cell, tissue or subject; to deliver an effector to a target cell, tissue or subject; or for treatment of diseases and disorders, e.g., by delivering an effector that can operate as a therapeutic agent to a desired cell, tissue, or subject. In some instances, the anellovector is made by *in vitro* assembly. *In vitro* assembly of an anellovector generally involves the formation of a proteinaceous exterior enclosing a genetic element, which occurs outside of a host cell (e.g., in a cell-free suspension, lysate, or supernatant). *In vitro* assembly may, in some instances, utilize components generated in a host cell but does not generally require a host cell for particle assembly.

The present disclosure provides an anelloVLP, e.g., a synthetic anelloVLP, which can be used as a delivery vehicle, e.g., for delivering genetic material, for delivering an effector, e.g., a payload, or for delivering a therapeutic agent or a therapeutic effector to a eukaryotic cell (e.g., a human cell or a human tissue). The anelloVLP generally comprises on its exterior surface (e.g., attached to a proteinaceous exterior) a surface moiety as described herein. In some embodiments, the surface moiety comprises the effector. In some embodiments, the surface moiety comprises a targeting agent (e.g., an agent that targets the anelloVLP to a target cell or tissue). In some embodiments, an anelloVLP (e.g., particle, e.g., a viral particle, e.g., an *Anellovirus* particle) comprises a proteinaceous exterior (e.g., a proteinaceous exterior comprising an *Anellovirus* capsid protein, e.g., an Anellovirus ORF1 molecule or a polypeptide encoded by an *Anellovirus* ORF1 nucleic acid, e.g., as described herein). In some embodiments, the anelloVLP is a particle comprising a proteinaceous exterior comprising a polypeptide encoded by an *Anellovirus* ORF1 nucleic acid (e.g., an ORF1 nucleic acid of *Betatorquevirus*, e.g., as described herein). In some embodiments, the proteinaceous exterior encloses an effector. In some embodiments, the effector is a therapeutic agent or a therapeutic effector, e.g., as described herein. In some instances, the effector is an endogenous effector or an exogenous effector, e.g., to a wild-type *Anellovirus* or a target cell. In some embodiments, the effector is exogenous to a wild-type *Anellovirus* or a target cell. In some embodiments, the anelloVLP can deliver an effector into a cell by contacting the cell and introducing the effector into the cell. In certain instances, the effector is an endogenous effector (e.g., endogenous to the target cell but, e.g., provided in increased amounts by the anelloVLP). In other instances, the effector is an exogenous effector. The effector can, in some instances, modulate a function of the cell or modulate an activity or level of a target molecule in the cell. For example, the effector can decrease levels of a target protein in the cell. In another example, the anelloVLP can deliver an effector, e.g., an exogenous protein, *in vivo*. AnelloVLPs can be used, for example, to deliver an effector to a target cell, tissue or subject; or for treatment of diseases and disorders, e.g., by delivering an effector that can operate as a therapeutic agent to a desired cell, tissue, or subject. In some instances, the anelloVLP is made by *in vitro* assembly. *In vitro* assembly of an anelloVLP generally involves the formation of a proteinaceous exterior in connection with an effector (e.g., the proteinaceous exterior enclosing the effector), which occurs outside of a host cell (e.g., in a cell-free suspension, lysate, or supernatant). *In vitro* assembly of an anelloVLP may, in some instances, utilize components generated in a host cell but does not generally require a host cell for particle assembly.

The invention further provides synthetic anellovectors and synthetic anelloVLPs. A synthetic anellovector or synthetic anelloVLP has at least one structural difference compared to a wild-type virus (e.g., a wild-type *Anellovirus*, e.g., a described herein), e.g., a deletion, insertion, substitution, modification (e.g., enzymatic modification), relative to the wild-type virus. Generally, synthetic

anellovectors and synthetic anelloVLPs include a proteinaceous exterior, which can be used for delivering an effector (e.g., an exogenous effector or an endogenous effector) into eukaryotic (e.g., human) cells. In some embodiments, the anellovector or anelloVLP does not cause a detectable and/or an unwanted immune or inflammarory response, e.g., does not cause more than a 1%, 5%, 10%, 15% increase in a molecular marker(s) of inflammation, e.g., TNF-alpha, IL-6, IL-12, IFN, as well as B-cell response e.g. reactive or neutralizing antibodies, e.g., the anellovector or anelloVLP may be substantially non-immunogenic to the target cell, tissue or subject.

In an aspect, the invention features an anellovector comprising: (i) a genetic element comprising a promoter element and a sequence encoding an effector (e.g., an endogenous or exogenous effector), and a protein binding sequence (e.g., an exterior protein binding sequence, e.g., a packaging signal); and (ii) a proteinaceous exterior; wherein the genetic element is enclosed within the proteinaceous exterior (e.g., a capsid); and wherein the anellovector is capable of delivering the genetic element into a eukaryotic (e.g., mammalian, e.g., human) cell. In some embodiments, the anellovector comprises a surface moiety (e.g., a surface moiety having effector and/or targeting function), e.g., displayed on the exterior surface of the anellovector (e.g., as described herein). In some embodiments, the surface moiety comprises the effector.

In some embodiments, the genetic element is a single-stranded and/or circular DNA. Alternatively or in combination, the genetic element has one, two, three, or all of the following properties: is circular, is single-stranded, it integrates into the genome of a cell at a frequency of less than about 0.0001%, 0.001%, 0.005%, 0.01%, 0.05%, 0.1%, 0.5%, 1%, 1.5%, or 2% of the genetic element that enters the cell, and/or it integrates into the genome of a target cell at less than 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, or 30 copies per genome. In some embodiments, integration frequency is determined as described in Wang et al. (2004, *Gene Therapy* 11: 711-721, incorporated herein by reference in its entirety). In some embodiments, the genetic element is enclosed within the proteinaceous exterior. In some embodiments, the anellovector is capable of delivering the genetic element into a eukaryotic cell. In some embodiments, the genetic element comprises a nucleic acid sequence (e.g., a nucleic acid sequence of between 300-4000 nucleotides, e.g., between 300-3500 nucleotides, between 300-3000 nucleotides, between 300-2500 nucleotides, between 300- 2000 nucleotides, between 300-1500 nucleotides) having at least 75% (e.g., at least 75, 76, 77, 78, 79, 80, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, or 100%) sequence identity to a sequence of a wild-type Anellovirus (e.g., a wild-type Torque Teno virus (TTV), Torque Teno mini virus (TTMV), or TTMDV sequence, e.g., a wild-type Anellovirus sequence as listed in any one of Tables A1-A26 or N1-N26). In some embodiments, the genetic element comprises a nucleic acid sequence (e.g., a nucleic acid sequence of at least 300 nucleotides, 500 nucleotides, 1000 nucleotides, 1500 nucleotides, 2000 nucleotides, 2500 nucleotides, 3000 nucleotides or more) having at least 75% (e.g., at least 75, 76, 77, 78, 79, 80, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, or 100%) sequence identity to a

sequence of a wild-type Anellovirus (e.g., a wild-type Anellovirus sequence as described herein, e.g., as listed in any one of Tables A1-A26 or N1-N26). In some embodiments, the nucleic acid sequence is codon-optimized, e.g., for expression in a mammalian (e.g., human) cell. In some embodiments, at least 50%, 60%, 70%, 80%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% of the codons in the nucleic acid sequence are codon-optimized, e.g., for expression in a mammalian (e.g., human) cell.

In an aspect, the invention features an anelloVLP comprising a proteinaceous exterior (e.g., a capsid) and an effector; wherein the anelloVLP is capable of delivering the effector into a eukaryotic (e.g., mammalian, e.g., human) cell. In some embodiments, the effector is comprised in a surface moiety, e.g., displayed on the exterior surface of the anelloVLP (e.g., as described herein).

In an aspect, the invention features an infectious (to a human cell) particle comprising an Anellovirus capsid (e.g., a capsid comprising an Anellovirus ORF, e.g., ORF1, polypeptide). In some embodiments, the infectious particle encapsulates a genetic element comprising a protein binding sequence that binds to the capsid and a heterologous (to the Anellovirus) sequence encoding a therapeutic effector. In some embodiments, the particle is capable of delivering the genetic element into a mammalian, e.g., human, cell. In some embodiments, the genetic element has less than about 6% (e.g., less than 6%, 5.5%, 5%, 4.5%, 4%, 3.5%, 3%, 2.5%, 2%, 1.5%, or less) identity to a wild type Anellovirus. In some embodiments, the genetic element has no more than 1.5%, 2%, 2.5%, 3%, 3.5%, 4%, 4.5%, 5%, 5.5% or 6% identity to a wild type Anellovirus. In some embodiments, the genetic element has at least about 2% to at least about 5.5% (e.g., 2 to 5%, 3% to 5%, 4% to 5%) identity to a wild type Anellovirus. In some embodiments, the genetic element has greater than about 2000, 3000, 4000, 4500, or 5000 nucleotides of non-viral sequence (e.g., non Anellovirus genome sequence). In some embodiments, the genetic element has greater than about 2000 to 5000, 2500 to 4500, 3000 to 4500, 2500 to 4500, 3500, or 4000, 4500 (e.g., between about 3000 to 4500) nucleotides of non-viral sequence (e.g., non Anellovirus genome sequence). In some embodiments, the genetic element is a single-stranded, circular DNA. Alternatively or in combination, the genetic element has one, two or 3 of the following properties: is circular, is single stranded, it integrates into the genome of a cell at a frequency of less than about 0.001%, 0.005%, 0.01%, 0.05%, 0.1%, 0.5%, 1%, 1.5%, or 2% of the genetic element that enters the cell, it integrates into the genome of a target cell at less than 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, or 30 copies per genome or integrates at a frequency of less than about 0.0001%, 0.001%, 0.005%, 0.01%, 0.05%, 0.1%, 0.5%, 1%, 1.5%, or 2% of the genetic element that enters the cell. In some embodiments, integration frequency is determined as described in Wang et al. (2004, *Gene Therapy* 11: 711-721, incorporated herein by reference in its entirety).

Also described herein are viral vectors and viral particles based on Anelloviruses, which can be used to deliver an agent (e.g., an exogenous effector or an endogenous effector, e.g., a therapeutic

effector) to a cell (e.g., a cell in a subject to be treated therapeutically). In some embodiments, Anelloviruses can be used as effective delivery vehicles for introducing an agent, such as an effector described herein, to a target cell, e.g., a target cell in a subject to be treated therapeutically or prophylactically.

In an aspect, the invention features a polypeptide (e.g., a synthetic polypeptide, e.g., an ORF1 molecule) comprising (e.g., in series):

(i) a first region comprising a structural arginine-rich region, e.g., amino acid sequence having at least 70% (e.g., at least about 70, 80, 90, 95, 96, 97, 98, 99, or 100%) sequence identity to a structural arginine-rich region sequence described herein or a sequence of at least about 40 amino acids comprising at least 60%, 70%, or 80% basic residues (e.g., arginine, lysine, or a combination thereof),

(ii) a second region comprising a structural jelly-roll domain, e.g., an amino acid sequence having at least 30% (e.g., at least about 30, 35, 40, 50, 60, 70, 80, 90, 95, 96, 97, 98, 99, or 100%) sequence identity to a structural jelly-roll region sequence described herein or a sequence comprising at least 6 beta strands,

(iii) a third region comprising an amino acid sequence having at least 30% (e.g., at least about 30, 35, 40, 50, 60, 70, 80, 90, 95, 96, 97, 98, 99, or 100%) sequence identity to an structural N22 domain sequence described herein,

(iv) a fourth region comprising an amino acid sequence having at least 70% (e.g., at least about 70, 80, 90, 95, 96, 97, 98, 99, or 100%) sequence identity to an Anellovirus ORF1 structural C-terminal domain (CTD) sequence described herein, and

(v) optionally wherein the polypeptide has an amino acid sequence having less than 100%, 99%, 98%, 95%, 90%, 85%, 80% sequence identity to a wild type Anellovirus ORF1 protein described herein.

In some embodiments, the polypeptide comprises at least about 70, 80, 90, 95, 96, 97, 98, 99, or 100% sequence identity to an Anellovirus ORF1 molecule as described herein (e.g., as listed in any one of Tables A1-A26). In some embodiments, the polypeptide comprises at least about 70, 80, 90, 95, 96, 97, 98, 99, or 100% sequence identity to a subsequence (e.g., a structural arginine (Arg)-rich domain, a structural jelly-roll domain, a hypervariable region (HVR), an structural N22 domain, or a structural C-terminal domain (CTD)) of an Anellovirus ORF1 molecule as described herein. In one embodiment, the amino acid sequences of the (i), (ii), (iii), and (iv) region have at least 90% sequence identity to their respective references and wherein the polypeptide has an amino acid sequence having less than 100%, 99%, 98%, 95%, 90%, 85%, 80% sequence identity to a wild type Anellovirus ORF1 protein described herein.

In an aspect, the invention features a complex comprising a polypeptide as described herein (e.g., an Anellovirus ORF1 molecule as described herein) and a genetic element comprising a promoter element

and a nucleic acid sequence (e.g., a DNA sequence) encoding an effector (e.g., an exogenous effector or an endogenous effector), and a protein binding sequence.

The present disclosure further provides nucleic acid molecules (e.g., a nucleic acid molecule that includes a genetic element as described herein, or a nucleic acid molecule that includes a sequence encoding a proteinaceous exterior protein as described herein). A nucleic acid molecule of the invention may include one or both of (a) a genetic element as described herein, and (b) a nucleic acid sequence encoding a proteinaceous exterior protein as described herein.

In an aspect, the invention features an isolated nucleic acid molecule comprising a genetic element comprising a promoter element operably linked to a sequence encoding an effector, e.g., a payload, and an exterior protein binding sequence. In some embodiments, the exterior protein binding sequence includes a sequence at least 75% (at least 80%, 85%, 90%, 95%, 97%, 100%) identical to a 5'UTR sequence of an Anellovirus, as disclosed herein. In some embodiments, the genetic element is a single-stranded DNA, is circular, integrates at a frequency of less than about 0.001%, 0.005%, 0.01%, 0.05%, 0.1%, 0.5%, 1%, 1.5%, or 2% of the genetic element that enters the cell, and/or integrates into the genome of a target cell at less than 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, or 30 copies per genome or integrates at a frequency of less than about 0.001%, 0.005%, 0.01%, 0.05%, 0.1%, 0.5%, 1%, 1.5%, or 2% of the genetic element that enters the cell. In some embodiments, integration frequency is determined as described in Wang et al. (2004, *Gene Therapy* 11: 711-721, incorporated herein by reference in its entirety). In embodiments, the effector does not originate from TTV and is not an SV40-miR-S1. In embodiments, the nucleic acid molecule does not comprise the polynucleotide sequence of TTMV-LY2. In embodiments, the promoter element is capable of directing expression of the effector in a eukaryotic (e.g., mammalian, e.g., human) cell.

In some embodiments, the nucleic acid molecule is circular. In some embodiments, the nucleic acid molecule is linear. In some embodiments, a nucleic acid molecule described herein comprises one or more modified nucleotides (e.g., a base modification, sugar modification, or backbone modification).

In some embodiments, the nucleic acid molecule comprises a sequence encoding an ORF1 molecule (e.g., an *Anellovirus* ORF1 protein, e.g., as described herein). In some embodiments, the nucleic acid molecule comprises a sequence encoding an ORF2 molecule (e.g., an *Anellovirus* ORF2 protein, e.g., as described herein). In some embodiments, the nucleic acid molecule comprises a sequence encoding an ORF3 molecule (e.g., an *Anellovirus* ORF3 protein, e.g., as described herein). In an aspect, the invention features a genetic element comprising one, two, or three of: (i) a promoter element and a sequence encoding an effector, e.g., an exogenous or endogenous effector; (ii) at least 72 contiguous nucleotides (e.g., at least 72, 73, 74, 75, 76, 77, 78, 79, 80, 90, 100, or 150 nucleotides) having at least 75% (e.g., at least 75, 76, 77, 78, 79, 80, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, or 100%) sequence identity

to a wild-type *Anellovirus* sequence; or at least 100 (e.g., at least 300, 500, 1000, 1500) contiguous nucleotides having at least 72% (e.g., at least 72, 73, 74, 75, 76, 77, 78, 79, 80, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, or 100%) sequence identity to a wild-type *Anellovirus* sequence; and (iii) a protein binding sequence, e.g., an exterior protein binding sequence, and wherein the nucleic acid construct is a single-stranded DNA; and wherein the nucleic acid construct is circular, integrates at a frequency of less than about 0.001%, 0.005%, 0.01%, 0.05%, 0.1%, 0.5%, 1%, 1.5%, or 2% of the genetic element that enters the cell, and/or integrates into the genome of a target cell at less than 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, or 30 copies per genome In some embodiments, a genetic element encoding an effector (e.g., an exogenous or endogenous effector, e.g., as described herein) is codon optimized. In some embodiments, the genetic element is circular. In some embodiments, the genetic element is linear. In some embodiments, the genetic element comprises an anellovector, e.g., as described herein. In some embodiments, a genetic element described herein comprises one or more modified nucleotides (e.g., a base modification, sugar modification, or backbone modification). In some embodiments, the genetic element comprises a sequence encoding an ORF1 molecule (e.g., an *Anellovirus* ORF1 protein, e.g., as described herein). In some embodiments, the genetic element comprises a sequence encoding an ORF2 molecule (e.g., an *Anellovirus* ORF2 protein, e.g., as described herein). In some embodiments, the genetic element comprises a sequence encoding an ORF3 molecule (e.g., an *Anellovirus* ORF3 protein, e.g., as described herein).

In an aspect, the invention features a host cell or helper cell comprising: (a) a nucleic acid comprising a sequence encoding one or more of an ORF1 molecule, an ORF2 molecule, or an ORF3 molecule (e.g, a sequence encoding an Anellovirus ORF1 polypeptide described herein), wherein the nucleic acid is a plasmid, is a viral nucleic acid, or is integrated into a helper cell chromosome; and (b) a genetic element, wherein the genetic element comprises (i) a promoter element operably linked to a nucleic acid sequence (e.g., a DNA sequence) encoding an effector (e.g., an exogenous effector or an endogenous effector) and (ii) a protein binding sequence that binds the polypeptide of (a), wherein optionally the genetic element does not encode an ORF1 polypeptide (e.g., an ORF1 protein). For example, the host cell or helper cell comprises (a) and (b) either in cis (both part of the same nucleic acid molecule) or in trans (each part of a different nucleic acid molecule). In embodiments, the genetic element of (b) is circular, single-stranded DNA. In some embodiments, the host cell is a manufacturing cell line. In some embodiments, the host cell or helper cell is adherent or in suspension, or both. In some embodiments, the host cell or helper cell is grown in a microcarrier. In some mbodiments, the host cell or helper cell is compatible with cGMP manufacturing practices. In some embodiments, the host cell or helper cell is grown in a medium suitable for promoting cell growth. In certain embodiments, once the

host cell or helper cell has grown sufficiently (e.g., to an appropriate cell density), the medium may be exchanged with a medium suitable for production of anellovectors by the host cell or helper cell.

In an aspect, the invention features a pharmaceutical composition comprising an anellovector (e.g., a synthetic anellovector) as described herein. In embodiments, the pharmaceutical composition further comprises a pharmaceutically acceptable carrier or excipient. In embodiments, the pharmaceutical composition comprises a unit dose comprising about 10^5-10^{14} genome equivalents of the anellovector per kilogram of a target subject. In some embodiments, the pharmaceutical composition comprising the preparation will be stable over an acceptable period of time and temperature, and/or be compatible with the desired route of administration and/or any devices this route of administration will require, e.g., needles or syringes. In some embodiments, the pharmaceutical composition is formulated for administration as a single dose or multiple doses. In some embodiments, the pharmaceutical composition is formulated at the site of administration, e.g., by a healthcare professional. In some embodiments, the pharmaceutical composition comprises a desired concentration of anellovector genomes or genomic equivalents (e.g., as defined by number of genomes per volume).

In an aspect, the invention features a method of treating a disease or disorder in a subject, the method comprising administering to the subject an anellovector, e.g., a synthetic anellovector, e.g., as described herein.

In an aspect, the invention features a method of delivering an effector or payload (e.g., an endogenous or exogenous effector) to a cell, tissue or subject, the method comprising administering to the subject an anellovector, e.g., a synthetic anellovector, e.g., as described herein, wherein the anellovector comprises a nucleic acid sequence encoding the effector. In embodiments, the payload is a nucleic acid. In embodiments, the payload is a polypeptide.

In an aspect, the invention features a method of delivering an anellovector to a cell, comprising contacting the anellovector, e.g., a synthetic anellovector, e.g., as described herein, with a cell, e.g., a eukaryotic cell, e.g., a mammalian cell, e.g., in vivo or ex vivo.

In an aspect, the invention features a method of treating a disease or disorder in a subject, the method comprising administering to the subject an anelloVLP, e.g., a synthetic anelloVLP, e.g., as described herein.

In an aspect, the invention features a method of delivering an effector or payload (e.g., an endogenous or exogenous effector) to a cell, tissue or subject, the method comprising administering to the subject an anelloVLP, e.g., a synthetic anelloVLP, e.g., as described herein, wherein the anelloVLP comprises the effector (e.g., wherein the proteinaceous exterior of the anelloVLP encapsulates the effector). In embodiments, the payload is a nucleic acid. In embodiments, the payload is a polypeptide (e.g., a protein).

In an aspect, the invention features a method of delivering an anelloVLP to a cell, comprising contacting the anelloVLP, e.g., a synthetic anelloVLP, e.g., as described herein, with a cell, e.g., a eukaryotic cell, e.g., a mammalian cell, e.g., in vivo or ex vivo.

In an aspect, the invention features a method of making an anellovector, e.g., a synthetic anellovector. The method includes:

a) providing a host cell comprising:

(i) a first nucleic acid molecule comprising the nucleic acid sequence of a genetic element of an anellovector, e.g., a synthetic anellovector, as described herein, and

(ii) the first nucleic acid or a second nucleic acid molecule encoding one or more of an amino acid sequence chosen from ORF1, ORF2, ORF2/2, ORF2/3, ORF1/1, or ORF1/2, e.g., as listed in any one of Tables A1-A26, or an amino acid sequence having at least 70% (e.g., at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100%) sequence identity thereto; and

b) incubating the host cell under conditions suitable to make the anellovector.

In some embodiments, the method further includes, prior to step (a), introducing the first nucleic acid molecule and/or the second nucleic acid molecule into the host cell. In some embodiments, the second nucleic acid molecule is introduced into the host cell prior to, concurrently with, or after the first nucleic acid molecule. In other embodiments, the second nucleic acid molecule is integrated into the genome of the host cell. In some embodiments, the second nucleic acid molecule is a helper (e.g., a helper plasmid or the genome of a helper virus).

In another aspect, the invention features a method of manufacturing an anellovector composition, comprising:

a) providing a host cell comprising, e.g., expressing one or more components (e.g., all of the components) of an anellovector, e.g., a synthetic anellovector, e.g., as described herein. For example, the host cell comprises (a) a nucleic acid comprising a sequence encoding an Anellovirus ORF1 polypeptide described herein, wherein the nucleic acid is a plasmid, is a viral nucleic acid, or is integrated into a helper cell chromosome; and (b) a genetic element, wherein the genetic element comprises (i) a promoter element operably linked to a nucleic acid sequence (e.g., a DNA sequence) encoding an effector (e.g., an exogenous effector or an endogenous effector) and (i) a protein binding sequence (e.g, packaging sequence) that binds the polypeptide of (a), wherein the host cell or helper cell comprises (a) and (b) either in cis or in trans. In embodiments, the genetic element of (b) is circular, single-stranded DNA. In some embodiments, the host cell is a manufacturing cell line;

b) culturing the host cell under conditions suitable for producing a preparation of anellovectors from the host cell, wherein the anellovectors of the preparation comprise a proteinaceous exterior (e.g,,

comprising an ORF1 molecule) encapsulating the genetic element (e.g., as described herein), thereby making a preparation of anellovectors; and

optionally, c) formulating the preparation of anellovectors, e.g., as a pharmaceutical composition suitable for administration to a subject.

In some embodiments, the components of the anellovector are introduced into the host cell at the time of production (e.g., by transient transfection). In some embodiments, the host cell stably expresses the components of the anellovector (e.g., wherein one or more nucleic acids encoding the components of the anellovector are introduced into the host cell, or a progenitor thereof, e.g., by stable transfection).

In some embodiments, the method further comprises one or more purification steps (e.g., purification by sedimentation, chromatography, and/or ultrafiltration). In some embodiments, the purification steps comprise removing one or more of serum, host cell DNA, host cell proteins, particles lacking the genetic element, and/or phenol red from the preparation. In some embodiments, the resultant preparation or a pharmaceutical composition comprising the preparation will be stable over an acceptable period of time and temperature, and/or be compatible with the desired route of administration and/or any devices this route of administration will require, e.g., needles or syringes.

In an aspect, the invention features a method of manufacturing an anellovector composition, comprising: a) providing a plurality of anellovectors described herein, or a preparation of anellovectors described herein; and b) formulating the anellovectors or preparation thereof, e.g., as a pharmaceutical composition suitable for administration to a subject.

In an aspect, the invention features a method of manufacturing an anelloVLP composition, comprising: a) providing a plurality of anelloVLPs described herein, or a preparation of anelloVLPs described herein; and b) formulating the anelloVLPs or preparation thereof, e.g., as a pharmaceutical composition suitable for administration to a subject.

In an aspect, the invention features a method of making a host cell, e.g., a first host cell or a producer cell (e.g., as shown in Figure 20), e.g., a population of first host cells, comprising an anellovector, the method comprising introducing a genetic element, e.g., as described herein, to a host cell and culturing the host cell under conditions suitable for production of the anellovector. In some embodiments, the method further comprises introducing a helper, e.g., a helper virus, to the host cell. In some embodiments, the introducing comprises transfection (e.g., chemical transfection) or electroporation of the host cell with the anellovector.

In an aspect, the invention features a method of making an anellovector, comprising providing a host cell, e.g., a first host cell or producer cell (e.g., as shown in Figure 20), comprising an anellovector, e.g., as described herein, and purifying the anellovector from the host cell. In some embodiments, the method further comprises, prior to the providing step, contacting the host cell with an anellovector, e.g.,

as described herein, and incubating the host cell under conditions suitable for production of the anellovector. In some embodiments, the host cell is the first host cell or producer cell described in the above method of making a host cell. In some embodiments, purifying the anellovector from the host cell comprises lysing the host cell.

In some embodiments, the method further comprises a second step of contacting the anellovector produced by the first host cell or producer cell with a second host cell, e.g., a permissive cell (e.g., as shown in Figure 20), e.g., a population of second host cells. In some embodiments, the method further comprises incubating the second host cell inder conditions suitable for production of the anellovector. In some embodiments, the method further comprises purifying an anellovector from the second host cell, e.g., thereby producing an anellovector seed population. In some embodiments, at least about 2-100-fold more of the anellovector is produced from the population of second host cells than from the population of first host cells. In some embodiments, purifying the anellovector from the second host cell comprises lysing the second host cell. In some embodiments, the method further comprises a second step of contacting the anellovector produced by the second host cell with a third host cell, e.g., permissive cells (e.g., as shown in Figure 20), e.g., a population of third host cells. In some embodiments, the method further comprises incubating the third host cell inder conditions suitable for production of the anellovector. In some embodiments, the method further comprises purifying an anellovector from the third host cell, e.g., thereby producing an anellovector stock population. In some embodiments, purifying the anellovector from the third host cell comprises lysing the third host cell. In some embodiments, at least about 2-100-fold more of the anellovector is produced from the population of third host cells than from the population of second host cells.

In some embodiments, the host cell is grown in a medium suitable for promoting cell growth. In certain embodiments, once the host cell has grown sufficiently (e.g., to an appropriate cell density), the medium may be exchanged with a medium suitable for production of anellovectors by the host cell. In some embodiments, anellovectors produced by a host cell separated from the host cell (e.g., by lysing the host cell) prior to contact with a second host cell. In some embodiments, anellovectors produced by a host cell are contacted with a second host cell without an intervening purification step.

In an aspect, the invention features a method of making a pharmaceutical anellovector preparation. The method comprises (a) making an anellovector preparation as described herein, (b) evaluating the preparation (e.g., a pharmaceutical anellovector preparation, anellovector seed population or the anellovector stock population) for one or more pharmaceutical quality control parameters, e.g., identity, purity, titer, potency (e.g., in genomic equivalents per anellovector particle), and/or the nucleic acid sequence, e.g., from the genetic element comprised by the anellovector, and (c) formulating the preparation for pharmaceutical use of the evaluation meets a predetermined criterion, e.g, meets a

pharmaceutical specification. In some embodiments, evaluating identity comprises evaluating (e.g., confirming) the sequence of the genetic element of the anellovector, e.g., the sequence encoding the effector. In some embodiments, evaluating purity comprises evaluating the amount of an impurity, e.g., mycoplasma, endotoxin, host cell nucleic acids (e.g., host cell DNA and/or host cell RNA), animal-derived process impurities (e.g., serum albumin or trypsin), replication-competent agents (RCA), e.g., replication-competent virus or unwanted anellovectors (e.g., an anellovector other than the desired anellovector, e.g., a synthetic anellovector as described herein), free viral capsid protein, adventitious agents, and aggregates. In some embodiments, evalating titer comprises evaluating the ratio of functional versus non-functional (e.g., infectious vs non-infectious) anellovectors in the preparation (e.g., as evaluated by HPLC). In some embodiments, evaluating potency comprises evaluating the level of anellovector function (e.g., expression and/or function of an effector encoded therein or genomic equivalents) detectable in the preparation. In some embodiments, the impurities comprise residual denaturant (e.g., urea) or cellular substituents (e.g., proteasomes or ferritin).

In some embodiments, the formulated preparation is substantially free of pathogens, host cell contaminants or impurities; has a predetermined level of non-infectious particles or a predetermined ratio of particles:infectious units (e.g., <300:1, < 200:1, <100:1, or <50:1). In some embodiments, multiple anellovectors can be produced in a single batch. In some embodiments, the levels of the anellovectors produced in the batch can be evaluated (e.g., individually or together).

In an aspect, the invention features a method of making a pharmaceutical anelloVLP preparation. The method comprises (a) making an anelloVLP preparation as described herein, (b) evaluating the preparation (e.g., a pharmaceutical anelloVLP preparation, anelloVLP seed population or the anelloVLP stock population) for one or more pharmaceutical quality control parameters, e.g., identity, purity, titer, potency, and (c) formulating the preparation for pharmaceutical use of the evaluation meets a predetermined criterion, e.g, meets a pharmaceutical specification. In some embodiments, evaluating purity comprises evaluating the amount of an impurity, e.g., mycoplasma, endotoxin, host cell nucleic acids (e.g., host cell DNA and/or host cell RNA), animal-derived process impurities (e.g., serum albumin or trypsin), replication-competent agents (RCA), e.g., replication-competent virus or unwanted VLPs (e.g., an anelloVLP other than the desired anelloVLP, e.g., a synthetic anelloVLP as described herein), free viral capsid protein, adventitious agents, and aggregates. In some embodiments, evalating titer comprises evaluating the ratio of functional versus non-functional (e.g., infectious vs non-infectious) anelloVLPs in the preparation (e.g., as evaluated by HPLC). In some embodiments, evaluating potency comprises evaluating the level of anelloVLP function (e.g., expression and/or function of an effector encoded therein or genomic equivalents) detectable in the preparation. In some embodiments, the impurities comprise residual denaturant (e.g., urea) or cellular substituents (e.g., proteasomes or ferritin).

In some embodiments, the formulated preparation is substantially free of pathogens, host cell contaminants or impurities; has a predetermined level of non-infectious particles or a predetermined ratio of particles:infectious units (e.g., <300:1, < 200:1, <100:1, or <50:1). In some embodiments, multiple anelloVLPs can be produced in a single batch. In some embodiments, the levels of the anelloVLPs produced in the batch can be evaluated (e.g., individually or together).

In an aspect, the invention features a host cell comprising:

(i) a first nucleic acid molecule comprising the nucleic acid sequence of a genetic element of an anellovector as described herein, and

(ii) optionally, a second nucleic acid molecule encoding one or more of an amino acid sequence chosen from ORF1, ORF2, ORF2/2, ORF2/3, ORF1/1, or ORF1/2 as listed in any one of Tables A1-A26, or an amino acid sequence having at least about 70% (e.g., at least about 70, 80, 90, 95, 96, 97, 98, 99, or 100%) sequence identity thereto.

In an aspect, the invention features a reaction mixture comprising an anellovector described herein and a helper virus, wherein the helper virus comprises a polynucleotide, e.g., a polynucleotide encoding an exterior protein, (e.g., an exterior protein capable of binding to the exterior protein binding sequence and, optionally, a lipid envelope), a polynucleotide encoding a replication protein (e.g., a polymerase), or any combination thereof.

In some embodiments, an anellovector (e.g., a synthetic anellovector) is isolated, e.g., isolated from a host cell and/or isolated from other constituents in a solution (e.g., a supernatant). In some embodiments, an anellovector (e.g., a synthetic anellovector) is purified, e.g., from a solution (e.g., a supernatant). In some embodiments, an anellovector is enriched in a solution relative to other constituents in the solution.

In some embodiments of any of the aforesaid anellovectors, compositions or methods, providing an anellovector comprises separating (e.g., harvesting) an anellovector from a composition comprising an anellovector-producing cell, e.g., as described herein. In other embodiments, providing an anellovector comprises obtaining an anellovector or a preparation thereof, e.g., from a third party.

In some embodiments of any of the aforesaid anellovectors, anellovectors, compositions or methods, the genetic element comprises an anellovector genome, e.g., as identified according to the method described in Example 9. In embodiments, the anellovector genome is an anellovector genome capable of self-replication and/or self-amplification. In some embodiments, the anellovector genome is not capable of self-replication and/or self-amplification. In some embodiments, the anellovector genome is capable of replicating and/or being amplified in *trans*, e.g., in the presence of a helper, e.g., a helper virus.

Additional features of any of the aforesaid anellovectors, anelloVLPs, compositions or methods include one or more of the following enumerated embodiments.

Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments of the invention described herein. Such equivalents are intended to be encompassed by the following enumerated embodiments.

Enumerated Embodiments

1. A particle comprising:

a proteinaceous exterior comprising about 40-80 (e.g., about 60), 100-140 (e.g., about 120), or 160-200 (e.g., about 180) copies of an Anellovirus ORF1 molecule,

wherein the particle:

(i) does not comprise (e.g., does not enclose) a polynucleotide (e.g., as determined using a nuclease protection assay as described herein),

(ii) does not comprise (e.g., does not enclose) a polynucleotide of greater than 1000, 500, 200, or 100 nucleotides in length, or

(iii) comprises less than about 1, 2, 3 ,4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 40, 50, 60, 70, 80, 90, 100, 150, 200, 250, 300, 400, 500, 600, 700, 800, 900, or 1000 nucleotides.

2. The particle of embodiment 1, wherein the Anellovirus ORF1 molecule comprises an ORF1 domain and an exogenous surface moiety.

3. The particle of embodiment 1, wherein the Anellovirus ORF1 molecule is bound to an exogenous surface moiety via a noncovalent integration or a covalent bond other than a peptide bond.

4. The particle of embodiment 1, wherein the Anellovirus ORF1 molecule does not comprise a structural arginine-rich domain.

5. The particle of embodiment 1, wherein the particle is a virus-like particle (VLP).

6. A particle comprising:

(a) a proteinaceous exterior comprising about 40-80 (e.g., about 60), 100-140 (e.g., about 120), or 160-200 (e.g., about 180) copies of an Anellovirus ORF1 molecule and an exogenous surface moiety, and

(b) a genetic element comprising a heterologous nucleic acid sequence encoding an exogenous effector.

7. A particle comprising:

a proteinaceous exterior comprising an Anellovirus ORF1 molecule, wherein the ORF1 molecule comprises an ORF1 domain and an exogenous surface moiety;

wherein one or more of:

a) the exogenous surface moiety is chosen from a receptor, a ligand, an antibody molecule (e.g., scFv), an antigen (e.g., a viral antigen, a bacterial antigen, a fungal antigen, or a parasite antigen) an adjuvant (e.g., TLR agonist, e.g., bacterial flagellin);

b) wherein the ORF1 molecule comprises a hypervariable region (HVR);

c) wherein the particle comprises a genetic element that encodes a peptide or polypeptide that boosts an immune response (e.g. an adjuvant, a TCR agonist (e.g., a bacterial flagellin));

d) wherein the exogenos surface moiety is between 1-5, 5-10, 10-20, 20-50, 50-100, 100-200, 200-500, or 500-1000 amino acids in length;

e) wherein a polypeptide linker region is situated between the exogenous surface moiety and the ORF1 molecule,

f) wherein the particle comprises 1-2, 2-5, 5-10, 10-20, 20-40, 40-60, 60-80, 80-100, 100-125, 125-150, 150-175, 175-200, 200-225, 225-250, 250-275, or 275-300 copies of the exogenous surface moiety;

g) wherein the proteinaceous exterior comprises (i) a plurality of ORF1 molecules lacking the exogenous surface moiety (e.g., a wild-type ORF1 molecule) and (ii) a plurality of ORF1 molecules that comprise the exogenous surface moiety, wherein optionally the ratio of (i) : (ii) is between 10:1 – 5:1, 5:1 – 2:1, 2:1 – 1:2, 1:2 – 1:5, or 1:5 – 1:10; and/or

h) wherein the particle further comprises a second exogenous surface moiety.

8. The particle of embodiment 7, wherein the exogenous surface moiety is situated at an insertion point between an N-terminal portion of the ORF1 domain and a C-terminal portion of the ORF1 domain.

9. The particle of embodiment 8, wherein the insertion point is in the HVR.

10. The particle of any of embodiments 7-9, further comprising a genetic element comprising a heterologous nucleic acid sequence encoding an exogenous effector.

11. The particle of any of embodiments 7-10, wherein the particle does not comprise (e.g., does not enclose) a polynucleotide, or does not comprise (e.g., does not enclose) a polynucleotide of greater than 1000, 500, 200, or 100 nucleotides in length, or comprises less than about 1, 2, 3 ,4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 40, 50, 60, 70, 80, 90, 100, 150, 200, 250, 300, 400, 500, 600, 700, 800, 900, or 1000 nucleotides.

12. A particle comprising:

a proteinaceous exterior comprising an Anellovirus ORF1 molecule, and an exogenous surface moiety, wherein the exogenous surface moiety is covalently bound to the ORF1 molecule using a bond other than a peptide bond.

12a. The particle of embodiment 12, wherein the exogenous surface moiety is attached to an NHS moiety, and the exogenous surface moiety is bound to the ORF1 molecule via the NHS moiety.

13. The particle of embodiment 12, wherein a non-polypeptide linker is situated between the exogenous surface moiety and the ORF1 molecule.

14. The particle of embodiment 13, wherein the non-polypeptide linker comprises a click linkage.

14a. The particle of embodiment 13, wherein the non-polypeptide linker is produced by a click reaction between a DBCO moiety and an azide moiety.

14b. The particle of embodiment 14a, wherein, prior to the click reaction, the DBCO moiety is attached to the Anellovirus ORF1 molecule via an NHS moiety.

14c. The particle of embodiment 14a or 14b, wherein, prior to the click reaction, the azide moiety is attached to the exogenous surface moiety via an NHS moiety.

14c1. The particle of embodiment 14b or 14c, wherein the NHS moiety of the DBCO moiety is attached to a lysine residue on the surface of the Anellovirus ORF1 molecule.

14c2. The particle of any of embodiments 14b-14c1, wherein the NHS moiety of the azide moiety is attached to a lysine residue on the surface of the exogenous surface moiety.

14d. The particle of embodiment 14a, wherein, prior to the click reaction, the DBCO moiety is attached to the exogenous surface moiety via an NHS moiety.

14e. The particle of embodiment 14a or 14d, wherein, prior to the click reaction, the azide moiety is attached to the Anellovirus ORF1 molecule via an NHS moiety.

14f. The particle of embodiment 14d or 14e, wherein the NHS moiety of the DBCO moiety is attached to a lysine residue on the surface of the exogenous surface moiety.

14g. The particle of any of embodiments 14d-14f, wherein the NHS moiety of the azide moiety is attached to a lysine residue on the surface of the Anellovirus ORF1 molecule.

15. A particle comprising:

a proteinaceous exterior comprising an Anellovirus ORF1 molecule, and an exogenous surface moiety, wherein the exogenous surface moiety is non-covalently bound to the ORF1 molecule.

16. The particle of embodiment 15, wherein the ORF1 molecule comprises an exogenous binding domain (e.g., MS2 coat protein or avidin), and the exogenous surface moiety comprises a cognate binding moiety (e.g., MS2 hairpin or biotin) that binds the exogenous binding domain.

17. The particle of any of embodiments 12-16, wherein the exogenous surface moiety comprises a polypeptide.

18. The particle of any of embodiments 12-17, wherein the exogenous surface moiety comprises a small molecule or nucleic acid molecule (e.g., polynucleotide).

19. The particle of any of the preceding, wherein the ratio of ORF1 molecule to exogenous surface moiety is between about 60:1 - 30:1, 30:1 - 20:1, 20:1 - 10:1, or 10:1 - 1:1.

20. The particle of any of the preceding embodiments, wherein the antibody molecule is a bispecific antibody molecule.

21. The particle of embodiment 20, wherein the bispecific antibody molecule comprises:

a first antigen-binding domain that binds a first antigen on a first type of host cell, and

a second antigen-binding domain that binds a second antigen on a second type of host cell.

22. The particle of any of the preceding embodiments, which is capable of entering a target cell, e.g., by endocytosis.

23. The particle of embodiment 22, wherein the exogenous surface moiety binds to a cognate moiety in the target cell.

24. The particle of embodiment 22, wherein the particle comprises a genetic element encoding an exogenous effector to be delivered to the interior of the target cell.

25. The particle of any of the preceding embodiments, wherein a genetic element is enclosed within the proteinaceous exterior.

26. The particle of any of the preceding embodiments, which particle does not comprise a polynucleotide, or does not comprise a polynucleotide of greater than 1000, 500, 200, or 100 nucleotides in length.

27. The particle of any of the preceding embodiments, wherein the Anellovirus ORF1 molecule comprises:

(b) a first region comprising an Anellovirus ORF1 structural jelly-roll region, e.g., an amino acid sequence having at least 30% (e.g., at least about 30, 35, 40, 50, 60, 70, 80, 90, 95, 96, 97, 98, 99, or 100%) sequence identity to a Anellovirus ORF1 structural jelly-roll region sequence described herein or a sequence comprising at least 6 beta strands;

(c) a second region comprising an Anellovirus ORF1 structural N22 domain, e.g., an amino acid sequence having at least 30% (e.g., at least about 30, 35, 40, 50, 60, 70, 80, 90, 95, 96, 97, 98, 99, or 100%) sequence identity to an Anellovirus ORF1 structural N22 domain sequence described herein; and

(d) a third region comprising an Anellovirus ORF1 structural C-terminal domain (CTD), e.g., an amino acid sequence having at least 30% (e.g., at least about 30, 35, 40, 50, 60, 70, 80, 90, 95, 96, 97, 98, 99, or 100%) sequence identity to an Anellovirus ORF1 CTD sequence described herein; and

wherein the Anellovirus ORF1 molecule does not comprise an Anellovirus ORF1 structural arginine-rich region, e.g., a sequence of at least about 40 amino acids comprising at least 60%, 70%, or 80% basic residues (e.g., arginine, lysine, or a combination thereof).

28. A preparation comprising the particle of any of the preceding embodiments.

29. The preparation of embodiment 28, wherein the preparation comprises less than 10^{10} - 10^{14} (e.g., less than 10^{10} - 10^{11}, 10^{11} - 10^{12}, 10^{12} - 10^{13}, or 10^{13} - 10^{14}) viral genome equivalents of nucleic acid molecules (e.g., genetic elements, e.g., of an anellovector as described herein) per kilogram of a subject to be administered the composition (e.g., as determined by qPCR or by measuring optical density).

30. A polypeptide, e.g., an Anellovirus ORF1 molecule, comprising:

(b) a first region comprising an Anellovirus ORF1 structural jelly-roll region, e.g., an amino acid sequence having at least 30% (e.g., at least about 30, 35, 40, 50, 60, 70, 80, 90, 95, 96, 97, 98, 99, or 100%) sequence identity to a Anellovirus ORF1 structural jelly-roll region sequence described herein or a sequence comprising at least 6 beta strands;

(c) a second region comprising an Anellovirus ORF1 structural N22 domain, e.g., an amino acid sequence having at least 30% (e.g., at least about 30, 35, 40, 50, 60, 70, 80, 90, 95, 96, 97, 98, 99, or 100%) sequence identity to an Anellovirus ORF1 structural N22 domain sequence described herein; and

(d) a third region comprising an Anellovirus ORF1 structural C-terminal domain (CTD), e.g., an amino acid sequence having at least 30% (e.g., at least about 30, 35, 40, 50, 60, 70, 80, 90, 95, 96, 97, 98, 99, or 100%) sequence identity to an Anellovirus ORF1 CTD sequence described herein; and

wherein the polypeptide does not comprise an Anellovirus ORF1 structural arginine-rich region, e.g., a sequence of at least about 40 amino acids comprising at least 60%, 70%, or 80% basic residues (e.g., arginine, lysine, or a combination thereof).

31. A nucleic acid molecule encoding a polypeptide of embodiment 30.

32. A particle comprising a proteinaceous exterior comprising an Anellovirus ORF1 molecule, wherein the ORF1 molecule comprises an ORF1 domain and an exogenous surface domain;

wherein the particle is made by contacting a plurality of Anellovirus ORF1 molecules in a cell-free solution under conditions suitable to form a proteinaceous exterior comprising the plurality of Anellovirus ORF1 molecules.

33. The particle of embodiment 32, wherein the particle does not comprise (e.g., does not enclose) a polynucleotide, or does not comprise (e.g., does not enclose) a polynucleotide of greater than 1000, 500, 200, or 100 nucleotides in length.

34. A method of making a particle, the method comprising:

contacting a plurality of Anellovirus ORF1 molecules in a cell-free solution under conditions suitable to form a proteinaceous exterior comprising the plurality of Anellovirus ORF1 molecules;

thereby making a particle.

35. A method of modulating a biological activity in a cell, the method comprising:

contacting the cell with a particle of any of the proceeding embodiments;

wherein the cell comprises a moiety on its surface that binds to the exogenous surface moiety of the particle.

36. A method of targeting a particle to a cell, the method comprising:

contacting the cell with a particle of any of the proceeding embodiments;

wherein the cell comprises a moiety on its surface that binds to the exogenous surface moiety of the particle.

37. A polypeptide comprising (e.g., in an N to C-terminal direction):

(i) a structural jelly-roll region of an Anellovirus ORF1 molecule;

(ii) a structural N22 domain of an Anellovirus ORF1 molecule; and

(iii) a portion of a structural C-terminal domain (CTD) of an Anellovirus ORF1 molecule, which comprises a deletion of about 20-30, 30-40 (e.g., about 37), 40-50 (e.g., about 55), 50-60 , 60-70, 70-80, 80-90, 90-100, 100-110, 110-120, 120-130 (e.g., about 129), 130-140 (e.g., about 131), 140-150 (e.g., about 148), or 150-160 (e.g., about 155) amino acids at the C-terminal end of the structural CTD, relative to a corresponding wild-type structural CTD of the Anellovirus ORF1 molecule.

38. A polypeptide comprising (e.g., in an N to C-terminal direction):

(i) optionally a first portion of a structural jelly-roll region (e.g., comprising beta strands B-H of the structural jelly-roll region) of an Anellovirus ORF1 molecule;

(ii) a first portion of a P1 domain of an Anellovirus ORF1 molecule (e.g., a P1-1 domain as described herein);

(iii) a P2 domain of an Anellovirus ORF1 molecule;

(iv) a second portion of a P1 domain of an Anellovirus ORF1 molecule (e.g., a P1-2 domain as described herein);

(v) optionally a second portion of a structural jelly-roll region (e.g., comprising beta strand I of the structural jelly-roll region) of an Anellovirus ORF1 molecule; and

(vi) a portion of a structural C-terminal domain (CTD) of an Anellovirus ORF1 molecule, which comprises a deletion of about 20-30, 30-40 (e.g., about 37), 40-50 (e.g., about 55), 50-60 , 60-70, 70-80, 80-90, 90-100, 100-110, 110-120, 120-130 (e.g., about 129), 130-140 (e.g., about 131), 140-150 (e.g., about 148), or 150-160 (e.g., about 155) amino acids at the C-terminal end of the structural CTD, relative to a corresponding wild-type structural CTD of the Anellovirus ORF1 molecule.

39. A polypeptide comprising (e.g., in an N to C-terminal direction):

(i) a structural jelly-roll region of an Anellovirus ORF1 molecule;

(ii) an structural N22 domain of an Anellovirus ORF1 molecule; and

(iii) a portion of a structural CTD of an Anellovirus ORF1 molecule, wherein the portion consists of the N-terminal-most 1-5, 5-10 (e.g. about 7), 10-20, 30-40, 40-50, 50-60 (e.g. about 52), 60-70 (e.g., about 69), 70-80, 80-90 (e.g., about 88), 90-100 (e.g., about 93), or 100-110 amino acids of a corresponding wild-type structural CTD of an Anellovirus ORF1 molecule, or a sequence having at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity thereto.

40. A polypeptide comprising (e.g., in an N to C-terminal direction):

(i) optionally a first portion of a structural jelly-roll region (e.g., comprising beta strands B-H of the structural jelly-roll region) of an Anellovirus ORF1 molecule;

(ii) a first portion of a P1 domain of an Anellovirus ORF1 molecule (e.g., a P1-1 domain as described herein);

(iii) a P2 domain of an Anellovirus ORF1 molecule;

(iv) a second portion of a P1 domain of an Anellovirus ORF1 molecule (e.g., a P1-2 domain as described herein);

(v) optionally a second portion of a structural jelly-roll region (e.g., comprising beta strand I of the structural jelly-roll region) of an Anellovirus ORF1 molecule; and

(vi) a portion of a structural CTD of an Anellovirus ORF1 molecule, wherein the portion consists of the N-terminal most 1-5, 5-10 (e.g. about 7), 10-20, 30-40, 40-50, 50-60 (e.g. about 52), 60-70 (e.g., about 69), 70-80, 80-90 (e.g., about 88), 90-100 (e.g., about 93), or 100-110 amino acids of a corresponding wild-type structural CTD of an Anellovirus ORF1 molecule, or a sequence having at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity thereto.

41. A polypeptide comprising (e.g., in an N to C-terminal direction):

(i) a structural jelly-roll region of an Anellovirus ORF1 molecule comprising the structural jelly-roll sequence of a Ring2, Ring9, Ring10, Ring 18, or Ring19 Anellovirus ORF1 protein (e.g., as described herein), or an amino acid sequence having at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity thereto; and

(ii) a full-length structural N22 domain of an Anellovirus ORF1 molecule comprising the structural N22 sequence of the Ring2, Ring9, Ring10, Ring 18, or Ring19 Anellovirus ORF1 protein, or an amino acid sequence having at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity thereto;

wherein the polypeptide does not comprise the amino acid sequence of the full-length structural C-terminal domain of the Ring2, Ring9, Ring10, Ring 18, or Ring19 Anellovirus ORF1 protein.

42. A polypeptide comprising (e.g., in an N to C-terminal direction):

(i) optionally the amino acid sequence of beta strands B-H of the structural jelly-roll region of a Ring2, Ring9, Ring10, Ring 18, or Ring19 Anellovirus ORF1 protein (e.g., as described herein), or an amino acid sequence having at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity thereto;

(ii) the amino acid sequence of a full-length P1-1 domain of the Ring2, Ring9, Ring10, Ring 18, or Ring19 Anellovirus ORF1 protein, or an amino acid sequence having at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity thereto;

(iii) the amino acid sequence of a full-length P2 domain of the Ring2, Ring9, Ring10, Ring 18, or Ring19 Anellovirus ORF1 protein, or an amino acid sequence having at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity thereto;

(iv) the amino acid sequence of a full-length P1-2 domain of the Ring2, Ring9, Ring10, Ring 18, or Ring19 Anellovirus ORF1 protein, or an amino acid sequence having at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity thereto; and

(v) optionally the amino acid sequence of beta strand I of the structural jelly-roll region of the Ring2, Ring9, Ring10, Ring 18, or Ring19 Anellovirus ORF1 protein (e.g., as described herein), or an amino acid sequence having at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity thereto;

wherein the polypeptide does not comprise the amino acid sequence of the full-length structural C-terminal domain of the Ring2, Ring9, Ring10, Ring 18, or Ring19 Anellovirus ORF1 protein.

43. The polypeptide of embodiment 41 or 42, which comprises a fragment of the structural C-terminal domain.

44. The polypeptide of embodiment 41 or 42, which does not comprise a fragment of the structural C-terminal domain.

45. The polypeptide of any of embodiments 37-44, which further comprises (e.g., at the C-terminal end of the C-terminal P1 subdomain sequence) a structural jelly-roll I region of an Anellovirus ORF1 molecule comprising the structural jelly-roll I sequence as listed in Table A2, or an amino acid sequence having at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity thereto.

46. The polypeptide of any of embodiments 37-45, wherein the polypeptide does not comprise the 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 most N-terminal amino acid residues of the structural jelly-roll B-H region of the Anellovirus ORF1 molecule.

47. The polypeptide of any of the preceding embodiments, further comprising a structural arginine-rich domain of an Anellovirus ORF1 molecule, e.g., wherein the structural arginine-rich domain is N-terminal of the structural jelly-roll region.

48. The polypeptide of any of embodiments 37-47, further comprising an amino acid sequence having at least 70% (e.g., at least about 70, 80, 90, 95, 96, 97, 98, 99, or 100%) sequence identity to a structural arginine-rich region sequence described herein, or a sequence of at least about 40 amino acids comprising at least 60%, 70%, or 80% basic residues.

49. The polypeptide of any of embodiments 37-48, wherein the polypeptide further comprises a deletion of a structural arginine-rich region of an Anellovirus ORF1 molecule.

50. The polypeptide of any of embodiments 37-49, wherein the polypeptide comprises a deletion of 35-40, 40-45, 45-50, 50-55, 55-60, 60-65, or 65-70 amino acids (e.g., 37, 40, 43, 44, 47 49, 66, or 67 amino acids) relative to a corresponding wild-type arginine-rich region of an Anellovirus ORF1 molecule.

51. The polypeptide of any of embodiments 37-50, wherein the polypeptide does not comprise a structural arginine-rich region of an Anellovirus ORF1 molecule.

52. The polypeptide of any of the preceding embodiments, wherein the polypeptide does not comprise a sequence of at least 60 contiguous amino acids consisting of at least 40%, 45%, 50%, 55%, 60%, 65%, 70%, or 75% (e.g., up to 80%) basic residues.

53. The polypeptide of any of the preceding embodiments, wherein the polypeptide does not comprise a sequence of at least 60 contiguous amino acids consisting of at least 40%, 45%, 50%, 55%, 60%, 65%, or 75% (e.g., up to 80%) arginine residues.

54. The polypeptide of any of the preceding embodiments, wherein the polypeptide does not comprise a sequence of at least 60 contiguous amino acids consisting of at least 2%, 3%, 4%, 5%, 10%, or 15% (e.g., up to 20%) lysine residues.

55. The polypeptide of any of the preceding embodiments, wherein the polypeptide does not comprise a sequence of at least 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, or 70 contiguous amino acids consisting of at least 40%, 41%, 42%, 43%, 44%, 45%, 46%, 47%, 48%, 49%, 50%, 51%, 52%, 53%, 54%, 55%, 56%, 57%, 58%, 59%, or 60% arginine residues.

56. The polypeptide of any of the preceding embodiments, further comprising a structural hypervariable region (HVR) of an Anellovirus ORF1 molecule (e.g., situated between the structural jelly-roll region and the structural N22 domain).

57. A polypeptide comprising (e.g., in an N-terminal to C-terminal direction):

(i) a structural jelly-roll region of an Anellovirus ORF1 molecule; and

(ii) an structural N22 domain of an Anellovirus ORF1 molecule; and

wherein the polypeptide lacks a structural C-terminal domain (CTD) of an Anellovirus ORF1 molecule.

58. A polypeptide comprising (e.g., in an N-terminal to C-terminal direction):

(i) optionally a first portion of a structural jelly-roll region (e.g., comprising beta strands B-H of the structural jelly-roll region) of an Anellovirus ORF1 molecule;

(ii) a first portion of a P1 domain of an Anellovirus ORF1 molecule (e.g., a P1-1 domain as described herein);

(iii) a P2 domain of an Anellovirus ORF1 molecule;

(iv) a second portion of a P1 domain of an Anellovirus ORF1 molecule (e.g., a P1-2 domain as described herein);

(v) optionally a second portion of a structural jelly-roll region (e.g., comprising beta strand I of the structural jelly-roll region) of an Anellovirus ORF1 molecule; and

wherein the polypeptide lacks a structural C-terminal domain (CTD) of an Anellovirus ORF1 molecule.

59. A polypeptide comprising (e.g., in an N-terminal to C-terminal direction):

(i) a structural jelly-roll region of an Anellovirus ORF1 molecule, or an amino acid sequence having at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity thereto; and

(ii) an structural N22 domain of an Anellovirus ORF1 molecule, or an amino acid sequence having at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity thereto; and

wherein the polypeptide lacks a structural arginine-rich region of an Anellovirus ORF1 molecule.

60. A polypeptide comprising (e.g., in an N-terminal to C-terminal direction):

(i) optionally a first portion of a structural jelly-roll region (e.g., comprising beta strands B-H of the structural jelly-roll region) of an Anellovirus ORF1 molecule;

(ii) a first portion of a P1 domain of an Anellovirus ORF1 molecule (e.g., a P1-1 domain as described herein);

(iii) a P2 domain of an Anellovirus ORF1 molecule;

(iv) a second portion of a P1 domain of an Anellovirus ORF1 molecule (e.g., a P1-2 domain as described herein);

(v) optionally a second portion of a structural jelly-roll region (e.g., comprising beta strand I of the structural jelly-roll region) of an Anellovirus ORF1 molecule; and

wherein the polypeptide lacks a structural arginine-rich region of an Anellovirus ORF1 molecule.

61. A polypeptide comprising (e.g., in an N-terminal to C-terminal direction):

(i) a portion of a full-length structural jelly-roll region of an Anellovirus ORF1 molecule, which does not comprise the 1-10 (e.g., 7) N-terminal-most amino acids of the full-length structural jelly-roll region; and

(ii) an structural N22 domain of the Anellovirus ORF1 molecule; and

wherein the polypeptide lacks a structural arginine-rich region of the Anellovirus ORF1 molecule.

62. A polypeptide comprising (e.g., in an N-terminal to C-terminal direction):

(i) a portion of the full-length beta strands B-H of the structural jelly-roll region of an Anellovirus ORF1 molecule, which does not comprise the 1-10 (e.g., 7) N-terminal-most amino acids of the full-length beta strands B-H of the structural jelly-roll region;

(ii) a first portion of a P1 domain of an Anellovirus ORF1 molecule (e.g., a P1-1 domain as described herein);

(iii) a P2 domain of an Anellovirus ORF1 molecule;

(iv) a second portion of a P1 domain of an Anellovirus ORF1 molecule (e.g., a P1-2 domain as described herein);

(v) optionally a second portion of a structural jelly-roll region (e.g., comprising beta strand I of the structural jelly-roll region) of an Anellovirus ORF1 molecule; and

wherein the polypeptide lacks a structural arginine-rich region of the Anellovirus ORF1 molecule.

63. The polypeptide of embodiment 61 or 62, wherein the Anellovirus ORF1 molecule is a Ring2 ORF1 molecule.

64. The polypeptide of embodiment 61 or 62, wherein the Anellovirus ORF1 molecule is a Ring9 ORF1 molecule.

65. The polypeptide of embodiment 61 or 62, wherein the Anellovirus ORF1 molecule is a Ring10 ORF1 molecule.

66. The polypeptide of embodiment 61 or 62, wherein the Anellovirus ORF1 molecule is a Ring18 ORF1 molecule.

67. The polypeptide of embodiment 61 or 62, wherein the Anellovirus ORF1 molecule is a Ring19 ORF1 molecule.

68. The polypeptide of any of embodiments 59-67, wherein the polypeptide does not comprise the 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 most N-terminal amino acid residues of the structural jelly-roll region of the Anellovirus ORF1 molecule.

69. A polypeptide comprising:

(i) a structural jelly-roll region of an Anellovirus ORF1 molecule;

(ii) an structural N22 domain of an Anellovirus ORF1 molecule; and

(iii) an amino acid sequence comprising substitutions of at least 50%, 60%, 70%, 80%, or 90% of basic amino acids relative to the structural arginine-rich region of a wild-type Anellovirus ORF1 molecule.

70. A polypeptide comprising:

(i) optionally a first portion of a structural jelly-roll region (e.g., comprising beta strands B-H of the structural jelly-roll region) of an Anellovirus ORF1 molecule;

(ii) a first portion of a P1 domain of an Anellovirus ORF1 molecule (e.g., a P1-1 domain as described herein);

(iii) a P2 domain of an Anellovirus ORF1 molecule;

(iv) a second portion of a P1 domain of an Anellovirus ORF1 molecule (e.g., a P1-2 domain as described herein);

(v) optionally a second portion of a structural jelly-roll region (e.g., comprising beta strand I of the structural jelly-roll region) of an Anellovirus ORF1 molecule; and

(vi) an amino acid sequence comprising substitutions of at least 50%, 60%, 70%, 80%, or 90% of basic amino acids relative to the structural arginine-rich region of a wild-type Anellovirus ORF1 molecule.

71. The polypeptide of embodiment 69 or 70, wherein (iii) comprises one or more (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10) mutations of lysine to histidine relative to the structural arginine-rich region of the wild-type Anellovirus ORF1 molecule.

72. The polypeptide of embodiment 69 or 71, which comprises the amino acid sequencenumbered 1721 in Table B4-2, or an amino acid sequence having at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity thereto.

73. The polypeptide of embodiment 69, wherein the amino acid sequence of (iii) further comprises one or more substitutions at one or more acidic, hydrophobic, and/or nonpolar amino acids of the structural arginine-rich region of the wild-type Anellovirus ORF1 molecule.

74. The polypeptide of any of embodiments 59-73, which further comprises a structural CTD, e.g., C-terminal of the structural N22 domain.

75. The polypeptide of any of embodiments 59-74, which further comprises a structural HVR, e.g., situated between the structural jelly-roll region and the structural N22 domain.

76. The polypeptide of any of embodiments 59-75, which does not substantially bind DNA.

77. The polypeptide of any of embodiments 59-76, further comprising (e.g., at the N-terminus) an N-terminal portion of a capsid protein from a virus other than an *Anellovirus*, or an amino acid sequence having at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity thereto;

optionally wherein the N-terminal region of the capsid protein from the virus other than an *Anellovirus* comprises at least the N-terminal 10, 20, 30, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 55, or 60 amino acids of the capsid protein.

78. The polypeptide of any of embodiments 59-77, further comprising (e.g., at the N-terminus) a structural arginine-rich motif of a capsid protein from a virus other than an *Anellovirus,* or an amino acid sequence having at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity thereto.

79. The polypeptide of embodiment 78, wherein the virus other than an *Anellovirus* is beak and feather disease virus (BFDV).

80. The polypeptide of any of embodiments 59-79, further comprising (e.g., at the N-terminus) the amino acid sequence MWGTSNCACAKFQIRRRYARPYRRRHIRRYRRRRRHFRRRRFTTNR, or an amino acid sequence having at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity thereto.

81. A polypeptide comprising (e.g., in an N-terminal to C-terminal direction):

(i) a structural arginine-rich region of a first Anellovirus ORF1 molecule, or an amino acid sequence having at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity thereto;

(ii) a structural jelly-roll region of a second Anellovirus ORF1 molecule, or an amino acid sequence having at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity thereto; and

(iii) an structural N22 domain of the second Anellovirus ORF1 molecule, or an amino acid sequence having at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity thereto;

wherein the sequence of (i) comprises at least one amino acid sequence difference relative to the structural arginine-rich region of the second Anellovirus ORF1 molecule.

82. A polypeptide comprising (e.g., in an N-terminal to C-terminal direction):

(i) a structural arginine-rich region of a first Anellovirus ORF1 molecule, or an amino acid sequence having at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity thereto;

(ii) optionally a first portion of a structural jelly-roll region (e.g., comprising beta strands B-H of the structural jelly-roll region) of a second Anellovirus ORF1 molecule;

(iii) a first portion of a P1 domain of a secondAnellovirus ORF1 molecule (e.g., a P1-1 domain as described herein);

(iv) a P2 domain of a secondAnellovirus ORF1 molecule;

(v) a second portion of a P1 domain of a secondAnellovirus ORF1 molecule (e.g., a P1-2 domain as described herein);

(vi) optionally a second portion of a structural jelly-roll region (e.g., comprising beta strand I of the structural jelly-roll region) of a secondAnellovirus ORF1 molecule; and

wherein the sequence of (i) comprises at least one amino acid sequence difference relative to the structural arginine-rich region of the second Anellovirus ORF1 molecule.

83. The polypeptide of embodiment 89 or 90, wherein (i) comprises the structural arginine-rich region of the first Anellovirus ORF1 molecule, or an amino acid sequence having at least 90% identity thereto.

84. The polypeptide of embodiment 89 or 90, wherein (i) comprises the structural arginine-rich region of the first Anellovirus ORF1 molecule, or an amino acid sequence having at least 95% identity thereto.

85. The polypeptide of embodiment 89 or 90, wherein (i) comprises the structural arginine-rich region of the first Anellovirus ORF1 molecule, or an amino acid sequence having at least 97% identity thereto.

86. The polypeptide of embodiment 89 or 90, wherein (i) has 100% sequence identity to the structural arginine-rich region of the first Anellovirus ORF1 molecule.

87. The polypeptide of any of embodiments 89-94, wherein the first Anellovirus ORF1 molecule is Ring 9 and the second Anellovirus ORF1 molecule is Ring 2.

88. The polypeptide of any of embodiments 89-95, wherein the first Anellovirus ORF1 molecule is Ring 2 and the second Anellovirus ORF1 molecule is Ring 9.

89. A polypeptide comprising (e.g., in an N-terminal to C-terminal direction):

(i) an N-terminal portion of a first Anellovirus ORF1 molecule, or an amino acid sequence having at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity thereto, wherein the N-terminal portion of the first Anellovirus ORF1 molecule has a length of between 30-40, 40-50, 50-60, 60-70, 70-80, or 80-90 amino acids; and

(ii) a C-terminal portion of a second Anellovirus ORF1 molecule, or an amino acid sequence having at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity thereto, wherein the C-terminal portion of the second Anellovirus ORF1 molecule has a length of between 590-600, 600-610, 610-620, 620-630, 630-640, 640-650, 650-660, 660-670, 670-680, 680-690, 690-700, 700-710, 710-720, or 720-730 amino acids; and

wherein the sequence of (i) comprises at least one amino acid sequence difference relative to the structural arginine-rich region of the second Anellovirus ORF1 molecule.

90. The polypeptide of embodiment 97, wherein the N-terminal portion of the first Anellovirus ORF1 molecule comprises a structural arginine-rich domain.

91. The polypeptide of embodiment 97 or 98, wherein the N-terminal portion of the first Anellovirus ORF1 molecule further comprises an N-terminal portion of a structural jelly-roll domain (e.g., having a length of between 1-10, 10-20, 20-30, 30-40, or 40-50 amino acids).

92. The polypeptide of any of embodiments 97-99, wherein the N-terminal portion of the first Anellovirus ORF1 molecule further comprises one or more beta strands of a structural jelly-roll domain (e.g., beta strands 1 and/or 2 of the structural jelly-roll domain).

93. The polypeptide of any of embodiments 97-100, wherein the C-terminal portion of the second Anellovirus ORF1 molecule comprises a C-terminal portion of a structural jelly-roll domain (e.g., having a length of between 100-110, 110-120, 120-130, 130-140, 140-150, 150-160, 160-170, 170-180, 180-190, or 190-200 amino acids).

94. A polypeptide comprising (e.g., in an N-terminal to C-terminal direction):

(i) a first portion of a structural jelly-roll region of a first Anellovirus ORF1 molecule, or an amino acid sequence having at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity thereto; and

(ii) a second portion of a structural jelly-roll region of a second Anellovirus ORF1 molecule, or an amino acid sequence having at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity thereto; and

wherein the sequence of (i) and (ii) together comprises at least one amino acid sequence difference relative to each of the sequences of the structural jelly-roll region of the first Anellovirus ORF1 molecule and the second Anellovirus ORF1 molecule.

95. A polypeptide comprising (e.g., in an N-terminal to C-terminal direction):

(i) a structural jelly-roll region of an Anellovirus ORF1 molecule; and

(ii) an structural N22 domain of an Anellovirus ORF1 molecule; and

wherein the polypeptide lacks a structural arginine-rich region of an Anellovirus ORF1 molecule; and

wherein the polypeptide lacks a structural C-terminal domain of an Anellovirus ORF1 molecule.

96. A polypeptide comprising (e.g., in an N-terminal to C-terminal direction):

(i) optionally a first portion of a structural jelly-roll region (e.g., comprising beta strands B-H of the structural jelly-roll region) of an Anellovirus ORF1 molecule;

(ii) a first portion of a P1 domain of an Anellovirus ORF1 molecule (e.g., a P1-1 domain as described herein);

(iii) a P2 domain of an Anellovirus ORF1 molecule;

(iv) a second portion of a P1 domain of an Anellovirus ORF1 molecule (e.g., a P1-2 domain as described herein);

(v) optionally a second portion of a structural jelly-roll region (e.g., comprising beta strand I of the structural jelly-roll region) of an Anellovirus ORF1 molecule; and

wherein the polypeptide lacks a structural arginine-rich region of an Anellovirus ORF1 molecule; and

wherein the polypeptide lacks a structural C-terminal domain of an Anellovirus ORF1 molecule.

97. The polypeptide of any of the preceding embodiments, wherein the polypeptide does not comprise amino acids 2-45 of the amino acid sequence of a Ring2 ORF1 protein as described herein, or an amino acid sequence having at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity thereto.

98. The polypeptide of any of the preceding embodiments, wherein the polypeptide does not comprise amino acids 518-666 of the amino acid sequence of a Ring2 ORF1 protein as described herein, or an amino acid sequence having at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity thereto.

99. The polypeptide of any of the preceding embodiments, wherein the polypeptide does not comprise amino acids 538-666 of the amino acid sequence of a Ring2 ORF1 protein as described herein, or an amino acid sequence having at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity thereto.

100. The polypeptide of any of the preceding embodiments, wherein the polypeptide does not comprise amino acids 545-666 of the amino acid sequence of a Ring2 ORF1 protein as described herein, or an amino acid sequence having at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity thereto.

101. The polypeptide of any of the preceding embodiments, wherein the polypeptide does not comprise amino acids 594-666 of the amino acid sequence of a Ring2 ORF1 protein as described herein, or an amino acid sequence having at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity thereto.

102. The polypeptide of any of the preceding embodiments, wherein the polypeptide does not comprise amino acids 611-666 of the amino acid sequence of a Ring2 ORF1 protein as described herein, or an amino acid sequence having at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity thereto.

103. The polypeptide of any of the preceding embodiments, wherein the polypeptide does not comprise amino acids 630-666 of the amino acid sequence of a Ring2 ORF1 protein as described herein, or an amino acid sequence having at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity thereto.

104. The polypeptide of any of the preceding embodiments, wherein the polypeptide does not comprise amino acids 2-45 of the amino acid sequence of a Ring10 ORF1 protein as described herein, or an amino acid sequence having at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity thereto.

105. The polypeptide of any of the preceding embodiments, wherein the polypeptide does not comprise amino acids 2-51 of the amino acid sequence of a Ring10 ORF1 protein as described herein, or an amino acid sequence having at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity thereto.

106. The polypeptide of any of the preceding embodiments, wherein the polypeptide does not comprise amino acids 542-672 of the amino acid sequence of a Ring10 ORF1 protein as described herein, or an amino acid sequence having at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity thereto.

107. The polypeptide of any of the preceding embodiments, wherein the polypeptide does not comprise amino acids 552-672 of the amino acid sequence of a Ring10 ORF1 protein as described herein, or an amino acid sequence having at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity thereto.

108. The polypeptide of any of the preceding embodiments, wherein the polypeptide does not comprise amino acids 557-672 of the amino acid sequence of a Ring10 ORF1 protein as described herein, or an amino acid sequence having at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity thereto.

109. The polypeptide of any of the preceding embodiments, wherein the polypeptide does not comprise amino acids 560-672 of the amino acid sequence of a Ring10 ORF1 protein as described herein, or an amino acid sequence having at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity thereto.

110. The polypeptide of any of the preceding embodiments, wherein the polypeptide does not comprise amino acids 566-672 of the amino acid sequence of a Ring10 ORF1 protein as described herein, or an amino acid sequence having at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity thereto.

111. The polypeptide of any of the preceding embodiments, wherein the polypeptide does not comprise amino acids 569-672 of the amino acid sequence of a Ring10 ORF1 protein as described herein, or an amino acid sequence having at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity thereto.

112. The polypeptide of any of the preceding embodiments, wherein the polypeptide does not comprise amino acids 610-672 of the amino acid sequence of a Ring10 ORF1 protein as described herein, or an amino acid sequence having at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity thereto.

113. The polypeptide of any of the preceding embodiments, wherein the polypeptide does not comprise amino acids 2-69 of the amino acid sequence of a Ring18 ORF1 protein as described herein, or an amino acid sequence having at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity thereto.

114. The polypeptide of any of the preceding embodiments, wherein the polypeptide does not comprise amino acids 582-736 of the amino acid sequence of a Ring18 ORF1 protein as described herein, or an amino acid sequence having at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity thereto.

115. The polypeptide of any of the preceding embodiments, wherein the polypeptide does not comprise amino acids 651-736 of the amino acid sequence of a Ring18 ORF1 protein as described herein, or an amino acid sequence having at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity thereto.

116. The polypeptide of any of the preceding embodiments, wherein the polypeptide does not comprise amino acids 2-49 of the amino acid sequence of a Ring19 ORF1 protein as described herein, or an amino acid sequence having at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity thereto.

117. The polypeptide of any of the preceding embodiments, wherein the polypeptide does not comprise amino acids 527-655 of the amino acid sequence of a Ring19 ORF1 protein as described herein, or an amino acid sequence having at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity thereto.

118. The polypeptide of any of the preceding embodiments, wherein the polypeptide does not comprise amino acids 537-655 of the amino acid sequence of a Ring19 ORF1 protein as described herein, or an amino acid sequence having at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity thereto.

119. The polypeptide of any of the preceding embodiments, wherein the polypeptide does not comprise amino acids 583-655 of the amino acid sequence of a Ring19 ORF1 protein as described herein, or an amino acid sequence having at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity thereto.

120. The polypeptide of any of the preceding embodiments, wherein the polypeptide does not comprise amino acids 600-655 of the amino acid sequence of a Ring19 ORF1 protein as described herein, or an amino acid sequence having at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity thereto.

121. A polypeptide comprising (e.g., in an N-terminal to C-terminal direction):

(i) a mutant ORF1 structural jelly-roll region; and

(ii) an structural N22 domain of an Anellovirus ORF1 molecule;

wherein the mutant ORF1 structural jelly-roll region comprises one or more mutations (e.g., at least 2, 3, 4, 5, 6, 7, 8, 9, or 10 mutations) in a beta strand relative to the amino acid sequence of a wild-type Anellovirus ORF1 structural jelly-roll region.

122. A polypeptide comprising (e.g., in an N-terminal to C-terminal direction):

(i) a mutant ORF1 structural jelly-roll region; and

(ii) a first portion of a P1 domain of an Anellovirus ORF1 molecule (e.g., a P1-1 domain as described herein);

(iii) a P2 domain of an Anellovirus ORF1 molecule;

(iv) a second portion of a P1 domain of an Anellovirus ORF1 molecule (e.g., a P1-2 domain as described herein);

(v) optionally a second portion of a structural jelly-roll region (e.g., comprising beta strand I of the structural jelly-roll region) of an Anellovirus ORF1 molecule; and

wherein the mutant ORF1 structural jelly-roll region comprises one or more mutations (e.g., at least 2, 3, 4, 5, 6, 7, 8, 9, or 10 mutations) in a beta strand relative to the amino acid sequence of a wild-type Anellovirus ORF1 structural jelly-roll region.

123. The polypeptide of embodiment 121 or 122, wherein the one or more mutations in the beta strand comprise one or more mutations (e.g., at least 2, 3, 4, 5, 6, 7, 8, 9, or 10 mutations) of a basic residue in the amino acid sequence of the wild-type Anellovirus ORF1 structural jelly-roll region, e.g., to a residue other than a basic residue (e.g., a nonpolar residue or an acidic residue).

124. The polypeptide of any of embodiments 121-123, wherein the beta strand is selected from beta strand B, D, G, or I of the wild-type Anellovirus ORF1 structural jelly-roll region.

125. The polypeptide of any of embodiments 121-124, wherein the one or more mutations reduce the binding of the polypeptide to a nucleic acid molecule relative to an otherwise similar polypeptide comprising the wild-type Anellovirus ORF1 structural jelly-roll region.

126. A polypeptide comprising (e.g., in an N to C-terminal direction):

(i) optionally a structural arginine-rich region of an Anellovirus ORF1 (e.g., a full-length arginine-rich region or a portion of a structural arginine-rich region),

(ii) a structural jelly-roll region of an Anellovirus ORF1 molecule,

(iii) a P1 domain of an Anellovirus ORF1 molecule,

(iv) a P2 domain of an Anellovirus ORF1 molecule, and

(v) optionally a structural C-terminal domain (CTD) of an Anellovirus ORF1 molecule (e.g., a full-length structural CTD or a portion of a structural CTD);

wherein:

(a) the P1 domain is from a different Anellovirus than one or more (e.g., 1, 2, 3, or 4) of the structural arginine-rich region, structural jelly-roll region, P2 domain, and/or the structural CTD;

(b) the P2 domain is from a different Anellovirus than one or more (e.g., 1, 2, 3, or 4) of the structural arginine-rich region, structural jelly-roll region, P1 domain, and/or the structural CTD; or

(c) the P1 domain and the P2 domain are from a different Anellovirus than one or more (e.g., 1, 2, or 3) of the structural arginine-rich region, structural jelly-roll region, and/or the

structural CTD, optionally wherein the P1 domain and the P2 domain are from the same Anellovirus.

127. A polypeptide comprising (e.g., in an N to C-terminal direction):

(i) optionally a structural arginine-rich region of an Anellovirus ORF1 molecule (e.g., a full-length arginine-rich region or a portion of a structural arginine-rich region),

(ii) a structural jelly-roll region of an Anellovirus ORF1 molecule,

(iii) a P1 domain of an Anellovirus ORF1 molecule,

(iv) a P2 domain of a viral capsid protein, wherein the viral capsid protein is not an Anellovirus ORF1 molecule, and

(v) optionally a structural C-terminal domain (CTD) of an Anellovirus ORF1 molecule (e.g., a full-length structural CTD or a portion of a structural CTD).

128. A polypeptide comprising (e.g., in an N to C-terminal direction):

(i) optionally a structural arginine-rich region of an Anellovirus ORF1 (e.g., a full-length arginine-rich region or a portion of a structural arginine-rich region),

(ii) a structural jelly-roll region of an Anellovirus ORF1 molecule,

(iii) a P1 domain of a viral capsid protein, wherein the viral capsid protein is not an Anellovirus ORF1 molecule,

(iv) a P2 domain of an Anellovirus ORF1 molecule, and

(v) optionally a structural C-terminal domain (CTD) of an Anellovirus ORF1 molecule (e.g., a full-length structural CTD or a portion of a structural CTD).

129. The polypeptide of embodiment 127 or 128, wherein the viral capsid protein is a hepatitis virus capsid protein (e.g., a hepatitis E virus capsid protein).

131. A polypeptide comprising (e.g., in an N to C-terminal direction):

(i) a portion (e.g., an N-terminal portion) of a viral capsid protein, wherein the viral capsid protein is not from an Anellovirus; and

(ii) a P2 domain of an Anellovirus ORF1 molecule (e.g., a wild-type Anellovirus ORF1 protein), or an amino acid sequence having at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% amino acid sequence identity thereto.

132. The polypeptide of embodiment 131, wherein the viral capsid protein is a hepatitis virus capsid protein (e.g., a hepatitis E virus (HEV) capsid protein), or a protein having at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% amino acid sequence identity thereto.

133. The polypeptide of embodiment 131 or 132, wherein the portion of the viral capsid protein comprises the amino acid sequence MAVAPAPDTAPVPDVDSRGAILRRQYNLSTSPLTSTIATGTNLVLYAAPLSSLLPLQDGTNTHIM ATEASNYAQYRVVRATIRYRPLVPSAVGGYAISISFWPQTTTTPTSVDMNSITSTDVRILVQPGIAS ELVIPSERLHYRNQGWRSVETSGVAEEEATSGLVMLCIHGSPVNSYTNTPYTGALGLLDFALELE FRNLTPGNTNTRVSRYSSSARHKLRRGPDGTAELTTTAATRFMKDLHFTGTNDVGEVGRGIALT LFNLADTLLGGLPTELISSAGGQLFYSRPVVSANGEPTVKLYTSVENAQQDKGIAIPHDIDLGESR VVIQDYDNQHEQDRPTPSPAPSRP, or an amino acid sequence having at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% amino acid sequence identity thereto.

134. The polypeptide of any of embodiments 131-133, wherein the P2 domain of the amino acid sequence comprises the amino acid sequence LNTTYIQNRDWGDRNKTYYCQTLGTQRYFLYGTHSTAQNINDIKLQELIPLTNTQDYVQGFDWT EKDKHNITTYKEFLTKGAGNPFHAEWITAQNPVIHTANSPTQIEQIYTASTTTFQNKKLTDLPTPG YIFITPTV, or an amino acid sequence having at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% amino acid sequence identity thereto.

137. The polypeptide of embodiment 135 or 136, wherein the P2 domain of the amino acid sequence comprises the amino acid sequence LNTTYIQNRDWGDRNKTYYCQTLGTQRYFLYGTHSTAQNINDIKLQELIPLTNTQDYVQGFDWT EKDKHNITTYKEFLTKGAGNPFHAEWITAQNPVIHTANSPTQIEQIYTASTTTFQNKKLTDLPTPG YIFITPTV, or an amino acid sequence having at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% amino acid sequence identity thereto.

138. The polypeptide of any of embodiments 131-137, further comprising one or more additional portions of the viral capsid protein (e.g., at the C-terminal end of the polypeptide).

139. The polypeptide of embodiment 138, wherein the additional portion of the viral capsid protein comprises the amino acid sequence HHHHH (e.g., at the C-terminal end of the polypeptide).

140. The polypeptide of any of embodiments 126-139, wherein the amino acid sequence of the P1 domain is non-contiguous (e.g., wherein the amino acid sequence of the P2 domain is situated between a first portion and a second portion of the amino acid sequence of the P1 domain).

141. A polypeptide comprising (e.g., in an N to C-terminal direction) one or more of:

(i) a structural arginine-rich region of an Anellovirus ORF1 molecule,

(ii) a structural jelly-roll region of the Anellovirus ORF1 molecule,

(iii) a structural N22 domain of the Anellovirus ORF1 molecule, and/or

(iv) a structural C-terminal domain of the Anellovirus ORF1 molecule;

wherein the polypeptide further comprises (e.g., between the structural jelly-roll region and the structural N22 domain e.g., in a structural hypervariable region (HVR) as described herein) the amino acid sequence EQI, e.g., the amino acid sequence SPTQIEQIYT; and

wherein the Anellovirus ORF1 molecule of (i)-(iv) comprises at least one difference relative to a Ring 10 ORF1 protein.

142. A polypeptide comprising (e.g., in an N to C-terminal direction) one or more of:

(i) a structural arginine-rich region of an Anellovirus ORF1 molecule,

(ii) a jelly-roll B-H strands subdomain of the Anellovirus ORF1 molecule,

(iii) a P1-1 subdomain of the Anellovirus ORF1 molecule,

(iv) a P1-2 subdomain of the Anellovirus ORF1 molecule,

(v) a jelly-roll I strand subdomain of the Anellovirus ORF1 molecule, and/or

(vi) a structural C-terminal domain of the Anellovirus ORF1 molecule;

wherein the polypeptide further comprises (e.g., between the P1-1 subdomain and the P1-2 subdomain domain) the amino acid sequence EQI, e.g., the amino acid sequence SPTQIEQIYT (e.g., in a P2 domain as described herein); and

wherein the Anellovirus ORF1 molecule of (i)-(vi) comprises at least one difference relative to a Ring 10 ORF1 protein.

143. A polypeptide comprising a structural hypervariable region (HVR) having at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity to the structural HVR sequence of a Ring 2 ORF1 protein;

wherein the polypeptide comprises one or more (e.g., 1, 2, or all 3) amino acid substitutions relative to the sequence of the Ring 2 ORF1 protein, the substitutions selected from K357E, N358Q, and E359I.

144. A polypeptide comprising a P2 domain having at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity to the P2 domain sequence of a Ring 2 ORF1 protein;

wherein the polypeptide comprises one or more (e.g., 1, 2, or all 3) amino acid substitutions relative to the sequence of the Ring 2 ORF1 protein, the substitutions selected from K357E, N358Q, and E359I.

145. The polypeptide of embodiment 143 or 144, wherein the polypeptide comprises all three of the substitutions selected from K357E, N358Q, and E359I.

146. A method of enriching a polypeptide of any of embodiments X1-X3b, the method comprising:

(a) providing the polypeptide (e.g., wherein the polypeptide is comprised in an anelloVLP or a particle);

(b) contacting the polypeptide with a binding moiety (e.g., an antibody molecule) that binds an epitope comprising the amino acid sequence EQI, and

(c) enriching material bound by the binding moiety relative to material not bound by the binding moiety,

thereby enriching the polypeptide.

147. An anellovector comprising:

(i) a proteinaceous exterior comprising a polypeptide of any of the preceding embodiments; and

(ii) a genetic element enclosed by the proteinaceous exterior, wherein the genetic element comprises a sequence encoding an exogenous effector.

148. An anellovector comprising:

(i) a proteinaceous exterior comprising a plurality of Anellovirus ORF1 molecules, wherein the Anellovirus ORF1 molecule lacks part or all of a structural C-terminal domain (CTD); and

(ii) a genetic element enclosed by the proteinaceous exterior, wherein the genetic element comprises a sequence encoding an exogenous effector.

149. An anellovector comprising:

(i) a proteinaceous exterior comprising a plurality of Anellovirus ORF1 molecules, wherein the Anellovirus ORF1 molecule lacks part or all of a structural arginine-rich region; and

(ii) a genetic element enclosed by the proteinaceous exterior, wherein the genetic element comprises a sequence encoding an exogenous effector.

150. A composition comprising a plurality of the anellovectors of any of embodiments S1-X.

151. A particle comprising:

a proteinaceous exterior comprising a plurality of Anellovirus ORF1 molecules, wherein the Anellovirus ORF1 molecule lacks part or all of a structural C-terminal domain (CTD);

wherein the particle:

(i) does not comprise (e.g., does not enclose) a polynucleotide (e.g., as determined using a nuclease protection assay as described herein),

(ii) does not comprise (e.g., does not enclose) a polynucleotide of greater than 1000, 500, 200, or 100 nucleotides in length, or

(iii) comprises less than about 1, 2, 3 ,4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 40, 50, 60, 70, 80, 90, 100, 150, 200, 250, 300, 400, 500, 600, 700, 800, 900, or 1000 nucleotides.

152. A particle comprising:

a proteinaceous exterior comprising a plurality of Anellovirus ORF1 molecules, wherein the Anellovirus ORF1 molecule lacks part or all of a structural arginine-rich region;

wherein the particle:

(i) does not comprise (e.g., does not enclose) a polynucleotide (e.g., as determined using a nuclease protection assay as described herein),

(ii) does not comprise (e.g., does not enclose) a polynucleotide of greater than 1000, 500, 200, or 100 nucleotides in length, or

(iii) comprises less than about 1, 2, 3 ,4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 40, 50, 60, 70, 80, 90, 100, 150, 200, 250, 300, 400, 500, 600, 700, 800, 900, or 1000 nucleotides.

153. A particle comprising:

a proteinaceous exterior comprising a plurality of Anellovirus ORF1 molecules, wherein the Anellovirus ORF1 molecule comprises a polypeptide of any of the preceding embodiments;

wherein the particle:

(i) does not comprise (e.g., does not enclose) a polynucleotide (e.g., as determined using a nuclease protection assay as described herein),

(ii) does not comprise (e.g., does not enclose) a polynucleotide of greater than 1000, 500, 200, or 100 nucleotides in length, or

(iii) comprises less than about 1, 2, 3 ,4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 40, 50, 60, 70, 80, 90, 100, 150, 200, 250, 300, 400, 500, 600, 700, 800, 900, or 1000 nucleotides.

154. The particle of any of embodiments 151-153, wherein the proteinaceous exterior comprises about 40-80 (e.g., about 60), 100-140 (e.g., about 120), or 160-200 (e.g., about 180) copies of the Anellovirus ORF1 molecules.

155. The particle of any of embodiments 151-154, wherein the particle further comprises an exogenous effector or a nucleic acid sequence encoding an exogenous effector.

156. A composition comprising a plurality of the particles of any of embodiments 151-155.

157. A nucleic acid molecule encoding a polypeptide of any of the preceding embodiments.

158. A method of delivering an exogenous effector to a cell, the method comprising contacting a cell with an anellovector or particle of any of the preceding embodiments, or a particle (e.g., an anellovector) comprising a polypeptide of any of the preceding embodiments, thereby delivering the exogenous effector to the cell.

159. A method of delivering an exogenous effector to a subject, the method comprising administering to the subject an anellovector or particle of any of the preceding embodiments, or a particle (e.g., an anellovector) comprising a polypeptide of any of the preceding embodiments, thereby delivering the exogenous effector to the subject.

160. A method of treating or preventing a disease or disorder in a subject, the method comprising administering to a subject in need thereof an anellovector or particle of any of the preceding embodiments, or a particle (e.g., an anellovector) comprising a polypeptide of any of the preceding embodiments, thereby treating or preventing the disease or disorder in the subject.

161. A method of making an anellovector, the method comprising:

(a) providing a host cell comprising:

(i) a plurality of Anellovirus ORF1 molecules, wherein the Anellovirus ORF1 molecule comprises a polypeptide of any of the preceding embodiments, and

(ii) a genetic element, wherein the genetic element comprises a sequence encoding an exogenous effector; and

(b) maintaining the host cell under conditions that allow for production of a proteinaceous exterior that comprises the plurality of Anellovirus ORF1 molecules, wherein the proteinaceous exterior encloses the genetic element,

thereby producing one or more of the anellovectors from the host cell.

162. A method of making an anellovector, the method comprising:

(a) providing a host cell comprising:

(i) a plurality of Anellovirus ORF1 molecules, wherein the Anellovirus ORF1 molecule lacks part or all of a structural C-terminal domain (CTD), and

(ii) a genetic element, wherein the genetic element comprises a sequence encoding an exogenous effector; and

(b) maintaining the host cell under conditions that allow for production of a proteinaceous exterior that comprises the plurality of Anellovirus ORF1 molecules, wherein the proteinaceous exterior encloses the genetic element;

thereby producing one or more of the anellovectors from the host cell.

163. A method of making an anellovector, the method comprising:

(a) providing a host cell comprising:

(i) a plurality of Anellovirus ORF1 molecules, wherein the Anellovirus ORF1 molecule lacks part or all of a structural arginine-rich region, and

(ii) a genetic element, wherein the genetic element comprises a sequence encoding an exogenous effector; and

(b) maintaining the host cell under conditions that allow for production of a proteinaceous exterior that comprises the plurality of Anellovirus ORF1 molecules, wherein the proteinaceous exterior encloses the genetic element;

thereby producing one or more of the anellovectors from the host cell.

164. The method of any of embodiments 161-163, wherein providing the host cell comprising the plurality of Anellovirus ORF1 molecules comprises maintaining the host cell under conditions that allow for expression of the Anellovirus ORF1 molecules.

165. A method of making an anellovector, the method comprising:

(a) providing a mixture comprising a plurality of Anellovirus ORF1 molecules, wherein the Anellovirus ORF1 molecule comprises a polypeptide of any of the preceding embodiments; optionally subjecting the mixture to denaturing conditions (e.g., providing a denaturant as part of the mixture or contacting the mixture with a denaturant), wherein at least 75%, 80%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% of the plurality of ORF1 molecules are not comprised in a particle comprising about 40-80 (e.g., about 60) copies of an ORF1 molecule;

(b) subjecting the mixture to non-denaturing conditions (e.g., reducing the concentration of the denaturant to a level) suitable for *in vitro* assembly of the Anellovirus ORF1 molecules (e.g., by dialysis); and

(c) incubating the Anellovirus ORF1 molecules with a plurality of genetic elements, under conditions suitable for assembly of the Anellovirus ORF1 molecules into one or more anellovectors each enclosing one or more of the genetic elements.

166. A method of making an anellovector, the method comprising:

(a) providing a mixture comprising a plurality of Anellovirus ORF1 molecules, wherein the Anellovirus ORF1 molecule lacks part or all of a structural C-terminal domain (CTD); optionally subjecting the mixture to denaturing conditions (e.g., providing a denaturant as part of the mixture or contacting the mixture with a denaturant), wherein at least 75%, 80%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% of the plurality of ORF1 molecules are not comprised in a particle comprising about 40-80 (e.g., about 60) copies of an ORF1 molecule;

(b) subjecting the mixture to non-denaturing conditions (e.g., reducing the concentration of the denaturant to a level) suitable for *in vitro* assembly of the Anellovirus ORF1 molecules (e.g., by dialysis); and

(c) incubating the Anellovirus ORF1 molecules with a plurality of genetic elements, under conditions suitable for assembly of the Anellovirus ORF1 molecules into one or more anellovectors each enclosing one or more of the genetic elements.

167. A method of making an anellovector, the method comprising:

(a) providing a mixture comprising a plurality of Anellovirus ORF1 molecules, wherein the Anellovirus ORF1 molecule lacks part or all of a structural arginine-rich region; optionally subjecting the mixture to denaturing conditions (e.g., providing a denaturant as part of the mixture or contacting the mixture with a denaturant), wherein at least 75%, 80%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% of the plurality of ORF1 molecules are not comprised in a particle comprising about 40-80 (e.g., about 60) copies of an ORF1 molecule;

(b) subjecting the mixture to non-denaturing conditions (e.g., reducing the concentration of the denaturant to a level) suitable for *in vitro* assembly of the Anellovirus ORF1 molecules (e.g., by dialysis); and

(c) incubating the Anellovirus ORF1 molecules with a plurality of genetic elements, under conditions suitable for assembly of the Anellovirus ORF1 molecules into one or more anellovectors each enclosing one or more of the genetic elements.

168. A method of making an anelloVLP, the method comprising:

(a) providing a mixture comprising a plurality of Anellovirus ORF1 molecules, wherein the Anellovirus ORF1 molecule comprises a polypeptide of any of the preceding embodiments, and wherein at least 75%, 80%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% of the plurality of ORF1 molecules are not comprised in a particle comprising about 40-80 (e.g., about 60) copies of an ORF1 molecule;

(b) subjecting the mixture to conditions suitable for *in vitro* assembly of the Anellovirus ORF1 molecules; and

(c) incubating the Anellovirus ORF1 molecules with a plurality of effectors (e.g., exogenous effectors), under conditions suitable for assembly of the Anellovirus ORF1 molecules into one or more anelloVLPs each enclosing one or more of the effectors.

169. A method of making an anelloVLP, the method comprising:

(a) providing a mixture comprising a plurality of Anellovirus ORF1 molecules, wherein the Anellovirus ORF1 molecule lacks part or all of a structural C-terminal domain (CTD), and wherein at least 75%, 80%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% of the plurality of ORF1 molecules are not comprised in a particle comprising about 40-80 (e.g., about 60) copies of an ORF1 molecule;

(b) subjecting the mixture to conditions suitable for *in vitro* assembly of the Anellovirus ORF1 molecules; and

(c) incubating the Anellovirus ORF1 molecules with a plurality of effectors (e.g., exogenous effectors), under conditions suitable for assembly of the Anellovirus ORF1 molecules into one or more anelloVLPs each enclosing one or more of the effectors.

170. A method of making an anelloVLP, the method comprising:

(a) providing a mixture comprising a plurality of Anellovirus ORF1 molecules, wherein the Anellovirus ORF1 molecule lacks part or all of a structural arginine-rich region, and wherein at least 75%, 80%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% of the plurality of ORF1 molecules are not comprised in a particle comprising about 40-80 (e.g., about 60) copies of an ORF1 molecule;

(b) subjecting the mixture to conditions suitable for *in vitro* assembly of the Anellovirus ORF1 molecules; and

(c) incubating the Anellovirus ORF1 molecules with a plurality of effectors (e.g., exogenous effectors), under conditions suitable for assembly of the Anellovirus ORF1 molecules into one or more anelloVLPs each enclosing one or more of the effectors.

171. The polypeptide, particle, nucleic acid molecule, or method of any of the preceding embodiments, wherein the polypeptide or Anellovirus ORF1 molecule comprises an exogenous surface moiety.

172. The polypeptide, particle, nucleic acid molecule, or method of embodiment 171, wherein the exogenous surface moiety is fused to the N-terminus of the Anellovirus ORF1 molecule.

173. The polypeptide, particle, nucleic acid molecule, or method of embodiment 171, wherein the exogenous surface moiety is fused to the C-terminus of the Anellovirus ORF1 molecule.

174. The polypeptide, particle, nucleic acid molecule, or method of embodiment 171, wherein the exogenous surface moiety is inserted within the amino acid sequence of the Anellovirus ORF1 molecule.

175. The polypeptide, particle, nucleic acid molecule, or method of any of embodiments 172-174, wherein the Anellovirus ORF1 molecule comprises a portion of a structural C-terminal domain (CTD) of an Anellovirus ORF1 molecule, which comprises a deletion of about 20-30, 30-40 (e.g., about 37), 40-50 (e.g., about 55), 50-60 , 60-70, 70-80, 80-90, 90-100, 100-110, 110-120, 120-130 (e.g., about 129), 130-140 (e.g., about 131), 140-150 (e.g., about 148), or 150-160 (e.g., about 155) amino acids at the C-terminal end of the structural CTD, relative to a corresponding wild-type structural CTD of the Anellovirus ORF1 molecule.

176. The polypeptide, particle, nucleic acid molecule, or method of any of embodiments 172-174, wherein the Anellovirus ORF1 molecule comprises a portion of a structural CTD of an Anellovirus ORF1 molecule, wherein the portion consists of the N-terminal most 1-5, 5-10 (e.g. about 7), 10-20, 30-40, 40-50, 50-60 (e.g. about 52), 60-70 (e.g., about 69), 70-80, 80-90 (e.g., about 88), 90-100 (e.g., about 93), or 100-110 amino acids of a corresponding wild-type structural CTD of an Anellovirus ORF1 molecule, or a sequence having at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity thereto.

177. The polypeptide, particle, nucleic acid molecule, or method of any of the preceding embodiments, wherein the polypeptide or Anellovirus ORF1 molecule comprises a detectable marker or effector (e.g., exogenous effector).

178. The polypeptide, particle, nucleic acid molecule, or method of embodiment 177, wherein the detectable marker or effector is fused to the N-terminus of the Anellovirus ORF1 molecule.

179. The polypeptide, particle, nucleic acid molecule, or method of embodiment 177, wherein the detectable marker or effector is fused to the C-terminus of the Anellovirus ORF1 molecule.

180. The polypeptide, particle, nucleic acid molecule, or method of embodiment 177, wherein the detectable marker or effector is inserted within the amino acid sequence of the Anellovirus ORF1 molecule.

181. The polypeptide, particle, nucleic acid molecule, or method of any one of embodiments 177-180, wherein the detectable marker comprises an epitope tag, e.g., a His tag or a FLAG tag.

182. The polypeptide, particle, nucleic acid molecule, or method of any one of embodiments 177-181, wherein the detectable marker comprises a fluorescent protein (e.g., GFP).

183. The polypeptide, particle, nucleic acid molecule, or method of any one of embodiments 177-182, wherein the polypeptide or Anellovirus ORF1 molecule comprises a protease recognition sequence (e.g., a 3C protease recognition sequence) between the detectable marker or effector and the remainder of the polypeptide or Anellovirus ORF1 molecule.

184. The polypeptide, particle, nucleic acid molecule, or method of any one of embodiments 177-183, wherein the effector is a therapeutic effector (e.g., a therapeutic polypeptide or therapeutic nucleic acid molecule).

185. The polypeptide, particle, nucleic acid molecule, or method of any of the preceding embodiments, wherein the polypeptide or Anellovirus ORF1 molecule comprises at its N-terminus a methionine residue corresponding to the N-terminal methionine residue of an Anellovirus structural arginine-rich region.

186. The polypeptide, particle, nucleic acid molecule, or method of any of the preceding embodiments, wherein the Anellovirus ORF1 molecule is a Ring 2 ORF1 molecule (e.g., as described herein), or an ORF1 molecule having at least 75%, 80%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity thereto.

187. The polypeptide, particle, nucleic acid molecule, or method of any of the preceding embodiments, wherein the Anellovirus ORF1 molecule is an ORF1 molecule of SEQ ID NO: 58, or an ORF1 molecule having at least 75%, 80%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity thereto.

188. The polypeptide, particle, nucleic acid molecule, or method of any of the preceding embodiments, wherein the Anellovirus ORF1 molecule is a Ring 9 ORF1 molecule (e.g., as described herein), or an ORF1 molecule having at least 75%, 80%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity thereto.

189. The polypeptide, particle, nucleic acid molecule, or method of any of the preceding embodiments, wherein the Anellovirus ORF1 molecule is an ORF1 molecule of SEQ ID NO: 1005, or an ORF1 molecule having at least 75%, 80%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity thereto.

190. The polypeptide, particle, nucleic acid molecule, or method of any of the preceding embodiments, wherein the Anellovirus ORF1 molecule is a Ring 10 ORF1 molecule (e.g., as described herein), or an ORF1 molecule having at least 75%, 80%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity thereto.

191. The polypeptide, particle, nucleic acid molecule, or method of any of the preceding embodiments, wherein the Anellovirus ORF1 molecule is an ORF1 molecule of SEQ ID NO: 1012, or an ORF1 molecule having at least 75%, 80%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity thereto.

192. The polypeptide, particle, nucleic acid molecule, or method of any of the preceding embodiments, wherein the Anellovirus ORF1 molecule is a Ring 18 ORF1 molecule (e.g., as described herein), or an ORF1 molecule having at least 75%, 80%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity thereto.

193. The polypeptide, particle, nucleic acid molecule, or method of any of the preceding embodiments, wherein the Anellovirus ORF1 molecule is an ORF1 molecule of SEQ ID NO: 1100, or an ORF1 molecule having at least 75%, 80%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity thereto.

194. The polypeptide, particle, nucleic acid molecule, or method of any of the preceding embodiments, wherein the Anellovirus ORF1 molecule is a Ring 19 ORF1 molecule (e.g., as described herein), or an ORF1 molecule having at least 75%, 80%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity thereto.

195. The polypeptide, particle, nucleic acid molecule, or method of any of the preceding embodiments, wherein the Anellovirus ORF1 molecule is an ORF1 molecule of Table B1-11a, or an ORF1 molecule having at least 75%, 80%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity thereto.

196. The polypeptide, particle, nucleic acid molecule, or method of any of the preceding embodiments, wherein the polypeptide or Anellovirus ORF1 molecule comprises an amino acid sequence as shown in any of Tables B1-1 to B4-5, or an amino acid sequence having at least 75%, 80%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity thereto.

197. The polypeptide, particle, nucleic acid molecule, or method of any of the preceding embodiments, wherein the polypeptide or Anellovirus ORF1 molecule comprises an amino acid sequence as shown in any of Tables B1-1 to B4-5, or an amino acid sequence having at least 90% sequence identity thereto.

198. The polypeptide, particle, nucleic acid molecule, or method of any of the preceding embodiments, wherein the polypeptide or Anellovirus ORF1 molecule comprises an amino acid sequence as shown in any of Tables B1-1 to B4-5, or an amino acid sequence having at least 95% sequence identity thereto.

199. The polypeptide, particle, nucleic acid molecule, or method of any of the preceding embodiments, wherein the polypeptide or Anellovirus ORF1 molecule comprises an amino acid sequence as shown in any of Tables B1-1 to B4-5, or an amino acid sequence having at least 99% sequence identity thereto.

200. The polypeptide, particle, nucleic acid molecule, or method of any of the preceding embodiments, wherein the polypeptide or Anellovirus ORF1 molecule comprises the structural arginine-rich region sequence of Ring 2, Ring 9, Ring 10, Ring 18, or Ring 19, or an amino acid sequence having at least 75%, 80%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity thereto.

201. The polypeptide, particle, nucleic acid molecule, or method of any of the preceding embodiments, wherein the polypeptide or Anellovirus ORF1 molecule comprises the structural jelly-roll domain sequence of Ring 2, Ring 9, Ring 10, Ring 18, or Ring 19, or an amino acid sequence having at least 75%, 80%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity thereto.

202. The polypeptide, particle, nucleic acid molecule, or method of any of the preceding embodiments, wherein the polypeptide or Anellovirus ORF1 molecule comprises the structural hypervariable domain sequence of Ring 2, Ring 9, Ring 10, Ring 18, or Ring 19, or an amino acid sequence having at least 75%, 80%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity thereto.

203. The polypeptide, particle, nucleic acid molecule, or method of any of the preceding embodiments, wherein the polypeptide or Anellovirus ORF1 molecule comprises the structural N22 domain sequence of Ring 2, Ring 9, Ring 10, Ring 18, or Ring 19, or an amino acid sequence having at least 75%, 80%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity thereto.

204. The polypeptide, particle, nucleic acid molecule, or method of any of the preceding embodiments, wherein the polypeptide or Anellovirus ORF1 molecule comprises the structural C-terminal domain sequence of Ring 2, Ring 9, Ring 10, Ring 18, or Ring 19, or an amino acid sequence having at least 75%, 80%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity thereto.

205. The polypeptide, particle, nucleic acid molecule, or method of any of the preceding embodiments, wherein the polypeptide or Anellovirus ORF1 molecule does not comprise the structural arginine-rich region sequence of Ring 2, Ring 9, Ring 10, Ring 18, or Ring 19, or an amino acid sequence having at least 75%, 80%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity thereto.

206. The polypeptide, particle, nucleic acid molecule, or method of any of the preceding embodiments, wherein the polypeptide or Anellovirus ORF1 molecule does not comprise the structural jelly-roll domain sequence of Ring 2, Ring 9, Ring 10, Ring 18, or Ring 19, or an amino acid sequence having at least 75%, 80%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity thereto.

207. The polypeptide, particle, nucleic acid molecule, or method of any of the preceding embodiments, wherein the polypeptide or Anellovirus ORF1 molecule does not comprise the structural hypervariable domain sequence of Ring 2, Ring 9, Ring 10, Ring 18, or Ring 19, or an amino acid sequence having at least 75%, 80%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity thereto.

208. The polypeptide, particle, nucleic acid molecule, or method of any of the preceding embodiments, wherein the polypeptide or Anellovirus ORF1 molecule does not comprise the structural N22 domain sequence of Ring 2, Ring 9, Ring 10, Ring 18, or Ring 19, or an amino acid sequence having at least 75%, 80%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity thereto.

209. The polypeptide, particle, nucleic acid molecule, or method of any of the preceding embodiments, wherein the polypeptide or Anellovirus ORF1 molecule does not comprise the structural C-terminal domain sequence of Ring 2, Ring 9, Ring 10, Ring 18, or Ring 19, or an amino acid sequence having at least 75%, 80%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity thereto.

210. The polypeptide, particle, nucleic acid molecule, or method of any of the preceding embodiments, wherein the polypeptide or Anellovirus ORF1 molecule comprises the structural arginine-rich region sequence of Ring 2, Ring 9, Ring 10, Ring 18, or Ring 19, or an amino acid sequence having at least 75%, 80%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity thereto.

211. The polypeptide, particle, nucleic acid molecule, or method of any of the preceding embodiments, wherein the polypeptide or Anellovirus ORF1 molecule comprises beta strands B-H of the structural jelly-roll domain sequence of Ring 2, Ring 9, Ring 10, Ring 18, or Ring 19, or an amino acid sequence having at least 75%, 80%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity thereto.

212. The polypeptide, particle, nucleic acid molecule, or method of any of the preceding embodiments, wherein the polypeptide or Anellovirus ORF1 molecule comprises the P1-1 domain sequence of Ring 2, Ring 9, Ring 10, Ring 18, or Ring 19, or an amino acid sequence having at least 75%, 80%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity thereto.

213. The polypeptide, particle, nucleic acid molecule, or method of any of the preceding embodiments, wherein the polypeptide or Anellovirus ORF1 molecule comprises the P2 domain sequence of Ring 2, Ring 9, Ring 10, Ring 18, or Ring 19, or an amino acid sequence having at least 75%, 80%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity thereto.

214. The polypeptide, particle, nucleic acid molecule, or method of any of the preceding embodiments, wherein the polypeptide or Anellovirus ORF1 molecule comprises the P1-2 domain sequence of Ring 2, Ring 9, Ring 10, Ring 18, or Ring 19, or an amino acid sequence having at least 75%, 80%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity thereto.

215. The polypeptide, particle, nucleic acid molecule, or method of any of the preceding embodiments, wherein the polypeptide or Anellovirus ORF1 molecule comprises beta strand I of the structural jelly-roll domain sequence of Ring 2, Ring 9, Ring 10, Ring 18, or Ring 19, or an amino acid sequence having at least 75%, 80%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity thereto.

216. The polypeptide, particle, nucleic acid molecule, or method of any of the preceding embodiments, wherein the polypeptide or Anellovirus ORF1 molecule comprises the structural C-terminal domain sequence of Ring 2, Ring 9, Ring 10, Ring 18, or Ring 19, or an amino acid sequence having at least 75%, 80%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity thereto.

217. The polypeptide, particle, nucleic acid molecule, or method of any of the preceding embodiments, wherein the polypeptide or Anellovirus ORF1 molecule does not comprise the structural arginine-rich region sequence of Ring 2, Ring 9, Ring 10, Ring 18, or Ring 19, or an amino acid sequence having at least 75%, 80%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity thereto.

218. The polypeptide, particle, nucleic acid molecule, or method of any of the preceding embodiments, wherein the polypeptide or Anellovirus ORF1 molecule does not comprise beta strands B-H of the structural jelly-roll domain sequence of Ring 2, Ring 9, Ring 10, Ring 18, or Ring 19, or an amino acid sequence having at least 75%, 80%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity thereto.

219. The polypeptide, particle, nucleic acid molecule, or method of any of the preceding embodiments, wherein the polypeptide or Anellovirus ORF1 molecule does not comprise the P1-1 domain sequence of

Ring 2, Ring 9, Ring 10, Ring 18, or Ring 19, or an amino acid sequence having at least 75%, 80%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity thereto.

220. The polypeptide, particle, nucleic acid molecule, or method of any of the preceding embodiments, wherein the polypeptide or Anellovirus ORF1 molecule does not comprise the P2 domain sequence of Ring 2, Ring 9, Ring 10, Ring 18, or Ring 19, or an amino acid sequence having at least 75%, 80%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity thereto.

221. The polypeptide, particle, nucleic acid molecule, or method of any of the preceding embodiments, wherein the polypeptide or Anellovirus ORF1 molecule does not comprise the P1-2 domain sequence of Ring 2, Ring 9, Ring 10, Ring 18, or Ring 19, or an amino acid sequence having at least 75%, 80%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity thereto.

222. The polypeptide, particle, nucleic acid molecule, or method of any of the preceding embodiments, wherein the polypeptide or Anellovirus ORF1 molecule does not comprise beta strand I of the structural jelly-roll domain sequence of Ring 2, Ring 9, Ring 10, Ring 18, or Ring 19, or an amino acid sequence having at least 75%, 80%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity thereto.

223. The polypeptide, particle, nucleic acid molecule, or method of any of the preceding embodiments, wherein the polypeptide or Anellovirus ORF1 molecule does not comprise the structural C-terminal domain sequence of Ring 2, Ring 9, Ring 10, Ring 18, or Ring 19, or an amino acid sequence having at least 75%, 80%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity thereto.

224. The polypeptide, particle, nucleic acid molecule, or method of any of the preceding embodiments, wherein the polypeptide or Anellovirus ORF1 molecule comprises one or more (e.g., 1, 2, or all 3) amino acid substitutions relative to the sequence of the Ring 2 ORF1 protein, the substitutions selected from K357E, N358Q, and E359I.

225. The polypeptide, particle, nucleic acid molecule, or method of any of the preceding embodiments, wherein the polypeptide or Anellovirus ORF1 molecule comprises the amino acid sequence SPTQIEQIYT (e.g., in a structural HVR domain as described herein).

226. The polypeptide, particle, nucleic acid molecule, or method of any of the preceding embodiments, wherein the polypeptide or Anellovirus ORF1 molecule comprises the amino acid sequence SPTQIEQIYT (e.g., in a P2 domain as described herein).

227. An anellovector comprising:

(i) a proteinaceous exterior comprising an Anellovirus ORF1 protein as listed in Table A26, or a polypeptide comprising an amino acid sequence having at least about 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity thereto, and

(ii) a genetic element enclosed by the proteinaceous exterior, wherein the genetic element comprises a promoter element operably linked to a nucleic acid sequence (e.g., a DNA sequence) encoding an exogenous effector.

228. An anellovector comprising:

(i) a proteinaceous exterior comprising an Anellovirus ORF1 protein as listed in Table A26, or a polypeptide comprising an amino acid sequence having at least about 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity thereto, and

(ii) a genetic element enclosed by the proteinaceous exterior, wherein the genetic element comprises a promoter element operably linked to a nucleic acid sequence (e.g., a DNA sequence) encoding an effector (e.g., an exogenous effector or an endogenous effector);

wherein the proteinaceous exterior and/or the genetic element comprises at least one difference (e.g., a mutation, chemical modification, or epigenetic alteration) relative to a wild-type Anellovirus ORF1 protein and/or wild-type Anellovirus genome, respectively (e.g., as described herein), e.g., an insertion, substitution, chemical or enzymatic modification, and/or deletion, e.g., a deletion of a domain (e.g., one or more of a structural arginine-rich region, structural jelly-roll domain, structural HVR, structural N22, structural CTD, P1 domain, or P2 domain, e.g., as described herein) or genomic region (e.g., one or more of a TATA box, cap site, transcriptional start site, 5' UTR, open reading frame (ORF), poly(A) signal, or GC-rich region, e.g., as described herein).

229. An anellovector comprising:

(i) a proteinaceous exterior comprising a polypeptide encoded by an Anellovirus ORF1 nucleic acid sequence as listed in Table N24, or a polypeptide encoded by a nucleic acid sequence having at least about 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity to the Anellovirus ORF1 nucleic acid sequence, and

(ii) a genetic element enclosed by the proteinaceous exterior, wherein the genetic element comprises a promoter element operably linked to a nucleic acid sequence (e.g., a DNA sequence) encoding an exogenous effector.

230. An anellovector comprising:

(i) a proteinaceous exterior comprising a polypeptide encoded by an Anellovirus ORF1 nucleic acid sequence as listed in Table N24, or a polypeptide encoded by a nucleic acid sequence having at least about 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity to the Anellovirus ORF1 nucleic acid sequence, and

(ii) a genetic element enclosed by the proteinaceous exterior, wherein the genetic element comprises a promoter element operably linked to a nucleic acid sequence (e.g., a DNA sequence) encoding an effector (e.g., an exogenous effector or an endogenous effector);

wherein the proteinaceous exterior and/or the genetic element comprises at least one difference (e.g., a mutation, chemical modification, or epigenetic alteration) relative to a wild-type Anellovirus ORF1 protein and/or wild-type Anellovirus genome, respectively (e.g., as described herein), e.g., an insertion, substitution, chemical or enzymatic modification, and/or deletion, e.g., a deletion of a domain (e.g., one or more of a structural arginine-rich region, structural jelly-roll domain, structural HVR, structural N22, structural CTD, P1 domain, or P2 domain, e.g., as described herein) or genomic region (e.g., one or more of a TATA box, cap site, transcriptional start site, 5' UTR, open reading frame (ORF), poly(A) signal, or GC-rich region, e.g., as described herein).

231. An anellovector comprising:

(i) a proteinaceous exterior (e.g., comprising an Anellovirus ORF1 molecule, e.g., as described herein, or a polypeptide comprising an amino acid sequence having at least about 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity thereto), and

(ii) a genetic element enclosed by the proteinaceous exterior, wherein the genetic element comprises: (a) a 5' UTR conserved domain as listed in Table N24, or a nucleic acid sequence having at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity thereto, and (b) a promoter element operably linked to a nucleic acid sequence (e.g., a DNA sequence) encoding an exogenous effector.

232. An anellovector comprising:

(i) a proteinaceous exterior (e.g., comprising an Anellovirus ORF1 molecule, e.g., as described herein, or a polypeptide comprising an amino acid sequence having at least about 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity thereto), and

(ii) a genetic element enclosed by the proteinaceous exterior, wherein the genetic element comprises: (a) a 5' UTR conserved domain as listed in Table N24, or a nucleic acid sequence having at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity thereto, and (b) a promoter element operably linked to a nucleic acid sequence (e.g., a DNA sequence) encoding an effector (e.g., an exogenous effector or an endogenous effector);

wherein the proteinaceous exterior and/or the genetic element comprises at least one difference (e.g., a mutation, chemical modification, or epigenetic alteration) relative to a wild-type Anellovirus ORF1 protein and/or wild-type Anellovirus genome, respectively (e.g., as described herein), e.g., an insertion, substitution, chemical or enzymatic modification, and/or deletion, e.g., a deletion of a domain (e.g., one or more of a structural arginine-rich region, structural jelly-roll domain, structural HVR, structural N22, structural CTD, P1 domain, or P2 domain, e.g., as described herein) or genomic region (e.g., one or more of a TATA box, cap site, transcriptional start site, 5' UTR, open reading frame (ORF), poly(A) signal, or GC-rich region, e.g., as described herein).

233. An anellovector comprising:

(i) a proteinaceous exterior (e.g., comprising an Anellovirus ORF1 molecule, e.g., as described herein, or a polypeptide comprising an amino acid sequence having at least about 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity thereto), and

(ii) a genetic element enclosed by the proteinaceous exterior, wherein the genetic element comprises a promoter element operably linked to a nucleic acid sequence (e.g., a DNA sequence) encoding an exogenous effector, and wherein the genetic element has at least about 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to an Anellovirus genome sequence as listed in Table N24.

234. An anellovector comprising:

(i) a proteinaceous exterior (e.g., comprising an Anellovirus ORF1 molecule, e.g., as described herein, or a polypeptide comprising an amino acid sequence having at least about 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity thereto), and

(ii) a genetic element enclosed by the proteinaceous exterior, wherein the genetic element comprises a promoter element operably linked to a nucleic acid sequence (e.g., a DNA sequence) encoding an effector (e.g., an exogenous effector or an endogenous effector), and wherein the genetic

element has at least about 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to an Anellovirus genome sequence as listed in Table N24;

wherein the proteinaceous exterior and/or the genetic element comprises at least one difference (e.g., a mutation, chemical modification, or epigenetic alteration) relative to a wild-type Anellovirus ORF1 protein and/or wild-type Anellovirus genome, respectively (e.g., as described herein), e.g., an insertion, substitution, chemical or enzymatic modification, and/or deletion, e.g., a deletion of a domain (e.g., one or more of a structural arginine-rich region, structural jelly-roll domain, structural HVR, structural N22, or structural CTD, e.g., as described herein) or genomic region (e.g., one or more of a TATA box, cap site, transcriptional start site, 5' UTR, open reading frame (ORF), poly(A) signal, or GC-rich region, e.g., as described herein).

235. An isolated ORF1 molecule comprising the amino acid sequence of an ORF1 as listed in Table A26, or an amino acid sequence having at least about 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity thereto;

wherein the ORF1 molecule comprises at least one difference (e.g., a mutation, chemical modification, or epigenetic alteration) relative to a wild-type ORF1 protein (e.g., as described herein), e.g., an insertion, substitution, chemical or enzymatic modification, and/or deletion, e.g., a deletion of a domain (e.g., one or more of a structural arginine-rich region, structural jelly-roll domain, structural HVR, structural N22, structural CTD, P1 domain, or P2 domain, e.g., as described herein).

236. An isolated ORF1 molecule comprising the amino acid sequence of the structural jelly-roll domain of an ORF1 as listed in Table A26, or an amino acid sequence having at least about 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity thereto;

wherein the ORF1 molecule comprises at least one difference (e.g., a mutation, chemical modification, or epigenetic alteration) relative to a wild-type ORF1 protein (e.g., as described herein), e.g., an insertion, substitution, chemical or enzymatic modification, and/or deletion, e.g., a deletion of a domain (e.g., one or more of a structural arginine-rich region, jelly-roll domain, structural HVR, structural N22, structural CTD, P1 domain, or P2 domain, e.g., as described herein).

237. An isolated ORF2 molecule comprising the amino acid sequence of an ORF2 as listed in Table A26, or an amino acid sequence having at least about 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity thereto;

wherein the ORF2 molecule comprises at least one difference (e.g., a mutation, chemical modification, or epigenetic alteration) relative to a wild-type ORF2 protein (e.g., as described herein),

e.g., an insertion, substitution, chemical or enzymatic modification, and/or deletion, e.g., a deletion of a domain.

238. An isolated nucleic acid molecule (e.g., a genetic element construct or a genetic element) comprising the nucleic acid sequence of a 5' UTR conserved domain as listed in Table N24, or a nucleic acid sequence having at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity thereto.

239. An isolated nucleic acid molecule (e.g., a genetic element construct or a construct for providing an ORF1 molecule in *trans*, e.g., as described herein) comprising the nucleic acid sequence of an ORF1 gene as listed in Table N24, or a nucleic acid sequence having at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity thereto.

240. An isolated nucleic acid molecule (e.g., a genetic element construct or a construct for providing an ORF2 molecule in *trans*, e.g., as described herein) comprising the nucleic acid sequence of an ORF2 gene as listed in Table N24, or a nucleic acid sequence having at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity thereto.

241. An isolated nucleic acid molecule (e.g., a genetic element construct, a genetic element, or a construct for providing an ORF1 or ORF2 molecule in *trans*, e.g., as described herein) comprising an Anellovirus genome sequence as listed in Table N24, or a nucleic acid sequence having at least 50%, 60%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity thereto.

242. A genetic element comprising:

(a) a 5' UTR conserved domain as listed in Table N24, or a nucleic acid sequence having at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity thereto, and

(b) a promoter element operably linked to a nucleic acid sequence (e.g., a DNA sequence) encoding an exogenous effector.

243. A method of manufacturing an anellovector composition, the method comprising:

(a) providing a cell, e.g., a host cell as described herein;

(b) introducing a nucleic acid molecule encoding an ORF1 polypeptide as listed in Table A26 (or an amino acid sequence having at least about 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity thereto) into the cell;

(c) introducing a genetic element construct into the cell (e.g., before, after, or simultaneously with (b)),

(d) incubating the cell under conditions that allow the cell to produce anellovector; and

(e) formulating the anellovectors, e.g., as a pharmaceutical composition suitable for administration to a subject,

thereby making the anellovector composition.

244. A method of manufacturing an anellovector composition, the method comprising:

(a) providing a cell, e.g., a host cell as described herein;

(b) introducing a nucleic acid molecule encoding an ORF1 polypeptide into the cell;

(c) introducing a genetic element construct into the cell as listed in Table N24 (or a nucleic acid sequence having at least about 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity thereto) (e.g., before, after, or simultaneously with (b)),

(d) incubating the cell under conditions that allow the cell to produce anellovector; and

(e) formulating the anellovectors, e.g., as a pharmaceutical composition suitable for administration to a subject,

thereby making the anellovector composition.

245. A method of making an anellovector, e.g., a synthetic anellovector, comprising:

(a) providing a host cell comprising:

(i) a nucleic acid molecule, e.g., a first nucleic acid molecule, comprising the nucleic acid sequence of a Anellovirus genome as listed in Table N24 (or a nucleic acid sequence having at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity thereto), and

(ii) a nucleic acid molecule, e.g., a second nucleic acid molecule, encoding one or more of an amino acid sequence chosen from ORF1, ORF2, ORF2/2, ORF2/3, ORF1/1, or ORF1/2, e.g., as listed in Table A1, or an amino acid sequence having at least 70% 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity thereto; and

(b) culturing the host cell under conditions suitable to make the anellovector.

246. A method of delivering an effector to an eye of a subject, the method comprising administering to the eye of the subject an anellovector of any of embodiments 227-234.

247. A method of modulating a biological function in an eye of a subject, the method comprising administering the anellovector of any of embodiments 227-234 to the subject.

248. A method of treating an eye disease or disorder in a subject in need thereof, the method comprising administering to the subject an anellovector of any of embodiments 227-234.

249. An ORF1 molecule comprising an exogenous surface moiety, wherein the exogenous surface moiety is fused to, replaces, and/or is situated within an insertion point of an ORF1 domain (e.g., within an HVR or P2).

250. An ORF1 molecule comprising an exogenous surface moiety, wherein the exogenous surface moiety is fused to, replaces, and/or is situated at an insertion point between residues corresponding to positions 284-285 of Ring 10 ORF1, e.g., in an ORF1 domain (e.g., within the HVR or P2 domain).

251. An ORF1 molecule comprising an exogenous surface moiety, wherein the exogenous surface moiety is fused to, replaces, and/or is situated at an insertion point between residues corresponding to positions 328-329 of Ring 10 ORF1, e.g., in an ORF1 domain (e.g., within the HVR or P2 domain).

252. An ORF1 molecule comprising an exogenous surface moiety, wherein the exogenous surface moiety is fused to, replaces, and/or is situated at an insertion point between residues corresponding to positions 256-383 of Ring 10 ORF1, e.g., in an ORF1 domain (e.g., within the HVR or P2 domain).

253. An ORF1 molecule comprising an exogenous surface moiety, wherein the exogenous surface moiety is fused to, replaces, and/or is situated at an insertion point between residues corresponding to positions 251-383 of Ring 10 ORF1, e.g., in an ORF1 domain (e.g., within the HVR or P2 domain).

254. An ORF1 molecule comprising an exogenous surface moiety, wherein the exogenous surface moiety is fused to, replaces, and/or is situated at an insertion point between residues corresponding to positions 251-384 of Ring 10 ORF1, e.g., in an ORF1 domain (e.g., within the HVR or P2 domain).

255. An ORF1 molecule comprising an exogenous surface moiety, wherein the exogenous surface moiety is attached to (e.g., conjugated to) the amino acid residue (e.g., a cysteine residue) corresponding to position 254, 263, 264, 265, 272, 273, 274, 276, 283, 284, 285, 287, 288, 290, 291, 308, 311, 312, 313, 314, 316, 317, 318, 319, 321, 324, 328, 329, 341, 343, 354, 358, 361, 362, 363, 364, 365, 368, 369, 371, 374, 376, 378, 380, or 381 of Ring 10 ORF1, e.g., in an ORF1 domain (e.g., within the HVR or P2 domain).

256. The ORF1 molecule of any of embodiments 249-255, wherein the exogenous surface moiety forms a pentamer when the ORF1 molecule is complexed with four other ORF1 molecules (e.g., four other copies of the ORF1 molecule).

257. The ORF1 molecule of any of embodiments 249-256, wherein the exogenous surface moiety forms a trimer when the ORF1 molecule is complexed with four other ORF1 molecules (e.g., four other copies of the ORF1 molecule).

258. The ORF1 molecule of any of embodiments 249-257, wherein the exogenous surface moiety forms a dimer when the ORF1 molecule is complexed with four other ORF1 molecules (e.g., four other copies of the ORF1 molecule).

259. A protein complex comprising five ORF1 molecules, wherein each of the ORF1 molecules comprises:

(i) an ORF1 domain, and

(ii) an exogenous surface moiety;

wherein the exogenous surface moieties of the five ORF1 molecules forms a pentamer.

260. The protein complex of embodiment 259, wherein each of the exogenous surface moieties is fused to, replaces, and/or is situated within an insertion point of an ORF1 domain (e.g., within an HVR or P2) of the corresponding ORF1 molecule.

261. The protein complex of embodiment 259, wherein each of the exogenous surface moieties is fused to, replaces, and/or is situated within an insertion point between residues corresponding to positions 284-285 in an ORF1 domain (e.g., within the HVR or P2 domain) of Ring 10 ORF1.

262. The protein complex of embodiment 259, wherein each of the exogenous surface moieties is fused to, replaces, and/or is situated within an insertion point between residues corresponding to positions 328-329 in an ORF1 domain (e.g., within the HVR or P2 domain) of Ring 10 ORF1.

263. The protein complex of embodiment 259, wherein each of the exogenous surface moieties is fused to, replaces, and/or is situated within an insertion point between residues corresponding to positions 256-383 in an ORF1 domain (e.g., within the HVR or P2 domain) of Ring 10 ORF1.

264. The protein complex of embodiment 259, wherein each of the exogenous surface moieties is fused to, replaces, and/or is situated within an insertion point between residues corresponding to positions 251-383 in an ORF1 domain (e.g., within the HVR or P2 domain) of Ring 10 ORF1.

265. The protein complex of embodiment 259, wherein each of the exogenous surface moieties is fused to, replaces, and/or is situated within an insertion point between residues corresponding to positions 251-384 in an ORF1 domain (e.g., within the HVR or P2 domain) of Ring 10 ORF1.

266. The protein complex of embodiment 259, wherein each of the exogenous surface moieties is attached to (e.g., conjugated to) the amino acid residue (e.g., a cysteine residue) corresponding to position 254, 263, 264, 265, 272, 273, 274, 276, 283, 284, 285, 287, 288, 290, 291, 308, 311, 312, 313, 314, 316, 317, 318, 319, 321, 324, 328, 329, 341, 343, 354, 358, 361, 362, 363, 364, 365, 368, 369, 371, 374, 376, 378, 380, or 381 of Ring 10 ORF1, e.g., in an ORF1 domain (e.g., within the HVR or P2 domain).

267. The protein complex of any of embodiments 259-266, wherein the exogenous surface moieties of the ORF1 molecules have the same amino acid sequences.

268. The protein complex of any of embodiments 259-267, wherein at least two (e.g., at least 2, 3, 4, or 5) of the exogenous surface moieties of the ORF1 molecules have different amino acid sequences.

269. A protein complex comprising three ORF1 molecules, wherein each of the ORF1 molecules comprises:

(i) an ORF1 domain, and

(ii) an exogenous surface moiety;

wherein the exogenous surface moieties of the three ORF1 molecules forms a trimer.

270. The protein complex of embodiment 269, wherein each of the exogenous surface moieties is fused to, replaces, and/or is situated within an insertion point of an ORF1 domain (e.g., within an HVR or P2) of the corresponding ORF1 molecule.

271. The protein complex of embodiment 269, wherein each of the exogenous surface moieties is fused to, replaces, and/or is situated within an insertion point between positions 284-285 in an ORF1 domain (e.g., within the HVR or P2 domain) of the corresponding ORF1 molecule.

272. The protein complex of embodiment 269, wherein each of the exogenous surface moieties is fused to, replaces, and/or is situated within an insertion point between positions 328-329 in an ORF1 domain (e.g., within the HVR or P2 domain) of the corresponding ORF1 molecule.

273. The protein complex of embodiment 269, wherein each of the exogenous surface moieties is fused to, replaces, and/or is situated within an insertion point between positions 256-383 in an ORF1 domain (e.g., within the HVR or P2 domain) of the corresponding ORF1 molecule.

274. The protein complex of embodiment 269, wherein each of the exogenous surface moieties is fused to, replaces, and/or is situated within an insertion point between positions 251-383 in an ORF1 domain (e.g., within the HVR or P2 domain) of the corresponding ORF1 molecule.

275. The protein complex of embodiment 269, wherein each of the exogenous surface moieties is fused to, replaces, and/or is situated within an insertion point between positions 251-384 in an ORF1 domain (e.g., within the HVR or P2 domain) of the corresponding ORF1 molecule.

276. The protein complex of embodiment 269, wherein each of the exogenous surface moieties is attached to (e.g., conjugated to) the amino acid residue (e.g., a cysteine residue) at position 254, 263, 264, 265, 272, 273, 274, 276, 283, 284, 285, 287, 288, 290, 291, 308, 311, 312, 313, 314, 316, 317, 318, 319, 321, 324, 328, 329, 341, 343, 354, 358, 361, 362, 363, 364, 365, 368, 369, 371, 374, 376, 378, 380, or 381 of the corresponding ORF1 molecule, e.g., in an ORF1 domain (e.g., within the HVR or P2 domain).

277. The protein complex of any of embodiments 269-276, wherein the exogenous surface moieties of the ORF1 molecules have the same amino acid sequences.

278. The protein complex of any of embodiments 269-277, wherein at least two (e.g., at least 2 or 3) of the exogenous surface moieties of the ORF1 molecules have different amino acid sequences.

279. A protein complex comprising two ORF1 molecules, wherein each of the ORF1 molecules comprises:

(i) an ORF1 domain, and

(ii) an exogenous surface moiety;

wherein the exogenous surface moieties of the two ORF1 molecules forms a dimer.

280. The protein complex of embodiment 279, wherein each of the exogenous surface moieties is fused to, replaces, and/or is situated within an insertion point of an ORF1 domain (e.g., within an HVR or P2) of the corresponding ORF1 molecule.

281. The protein complex of embodiment 279, wherein each of the exogenous surface moieties is fused to, replaces, and/or is situated within an insertion point between residues corresponding to positions 284-285 in an ORF1 domain (e.g., within the HVR or P2 domain) of Ring 10 ORF1.

282. The protein complex of embodiment 279, wherein each of the exogenous surface moieties is fused to, replaces, and/or is situated within an insertion point between residues corresponding to positions 328-329 in an ORF1 domain (e.g., within the HVR or P2 domain) of Ring 10 ORF1.

283. The protein complex of embodiment 279, wherein each of the exogenous surface moieties is fused to, replaces, and/or is situated within an insertion point between residues corresponding to positions 256-383 in an ORF1 domain (e.g., within the HVR or P2 domain) of Ring 10 ORF1.

284. The protein complex of embodiment 279, wherein each of the exogenous surface moieties is fused to, replaces, and/or is situated within an insertion point between residues corresponding to positions 251-383 in an ORF1 domain (e.g., within the HVR or P2 domain) of Ring 10 ORF1.

285. The protein complex of embodiment 279, wherein each of the exogenous surface moieties is fused to, replaces, and/or is situated within an insertion point between residues corresponding to positions 251-384 in an ORF1 domain (e.g., within the HVR or P2 domain) of Ring 10 ORF1.

286. The protein complex of embodiment 279, wherein each of the exogenous surface moieties is attached to (e.g., conjugated to) the amino acid residue (e.g., a cysteine residue) corresponding to position 254, 263, 264, 265, 272, 273, 274, 276, 283, 284, 285, 287, 288, 290, 291, 308, 311, 312, 313, 314, 316, 317, 318, 319, 321, 324, 328, 329, 341, 343, 354, 358, 361, 362, 363, 364, 365, 368, 369, 371, 374, 376, 378, 380, or 381 of Ring 10 ORF1, e.g., in an ORF1 domain (e.g., within the HVR or P2 domain).

287. The protein complex of any of embodiments 279-286, wherein the exogenous surface moieties of the two ORF1 molecules have the same amino acid sequences.

288. The protein complex of any of embodiments 279-287, wherein the exogenous surface moieties of the two ORF1 molecules have different amino acid sequences.

289. The polypeptide, particle, nucleic acid molecule, method, or protein complex of any of the preceding embodiments, wherein the polypeptide or ORF1 molecule comprises one or more substitutions of a cysteine residues (e.g., one or more cysteine to alanine substitutions or one or more cysteine to serine substitutions).

290. The polypeptide, particle, nucleic acid molecule, method, or protein complex of any of the preceding embodiments, wherein the polypeptide or ORF1 molecule comprises a cysteine to serine mutation at one or more (e.g., 1, 2, 3, 4, 5, 6, 7, or 8) residues corresponding to position 63, 70, 137, 269, 403, 460, 503, and/or 515 of a Ring 10 ORF1 protein (e.g., as described herein).

291. The polypeptide, particle, nucleic acid molecule, method, or protein complex of any of the preceding embodiments, wherein the polypeptide or ORF1 molecule comprises a cysteine to alanine mutation at one or more (e.g., 1, 2, 3, 4, 5, 6, or 7) residues corresponding to position 63, 137, 269, 403, 460, 503, and/or 515 of a Ring 10 ORF1 protein (e.g., as described herein).

292. The polypeptide, particle, nucleic acid molecule, method, or protein complex of any of the preceding embodiments, wherein the polypeptide or ORF1 molecule comprises a cysteine to serine mutation at the residue corresponding to position 70 of a Ring 10 ORF1 protein (e.g., as described herein).

293. The polypeptide, particle, nucleic acid molecule, method, or protein complex of any of the preceding embodiments, wherein the polypeptide or ORF1 molecule comprises a substitution of an amino acid residue (e.g., a threonine, serine, asparagine, alanine, glutamine, or lysine residue) to cysteine.

294. The polypeptide, particle, nucleic acid molecule, method, or protein complex of embodiment 293, wherein the polypeptide or ORF1 molecule comprises a threonine to cysteine substitution, e.g., at the position corresponding to position 365 of a Ring 10 ORF1 protein (e.g., as described herein).

295. The polypeptide, particle, nucleic acid molecule, method, or protein complex of embodiment 293, wherein the polypeptide or ORF1 molecule comprises a serine to cysteine substitution, e.g., at the position corresponding to position 284 of a Ring 10 ORF1 protein (e.g., as described herein).

296. The polypeptide, particle, nucleic acid molecule, method, or protein complex of embodiment 293, wherein the polypeptide or ORF1 molecule comprises an asparagine to cysteine substitution, e.g., at the position corresponding to position 290 of a Ring 10 ORF1 protein (e.g., as described herein).

297. The polypeptide, particle, nucleic acid molecule, method, or protein complex of embodiment 293, wherein the polypeptide or ORF1 molecule comprises a lysine to cysteine substitution, e.g., at the position corresponding to position 317 of a Ring 10 ORF1 protein (e.g., as described herein).

298. The polypeptide, particle, nucleic acid molecule, method, or protein complex of embodiment 293, wherein the polypeptide or ORF1 molecule comprises a lysine to cysteine substitution, e.g., at the position corresponding to position 324 of a Ring 10 ORF1 protein (e.g., as described herein).

299. The polypeptide, particle, nucleic acid molecule, method, or protein complex of embodiment 293, wherein the polypeptide or ORF1 molecule comprises an alanine to cysteine substitution, e.g., at the position corresponding to position 362 of a Ring 10 ORF1 protein (e.g., as described herein).

300. The polypeptide, particle, nucleic acid molecule, method, or protein complex of embodiment 293, wherein the polypeptide or ORF1 molecule comprises a serine to cysteine substitution, e.g., at the position corresponding to position 363 of a Ring 10 ORF1 protein (e.g., as described herein).

301. The polypeptide, particle, nucleic acid molecule, method, or protein complex of embodiment 293, wherein the polypeptide or ORF1 molecule comprises an asparagine to cysteine substitution, e.g., at the position corresponding to position 369 of a Ring 10 ORF1 protein (e.g., as described herein).

302. The polypeptide, particle, nucleic acid molecule, method, or protein complex of embodiment 293, wherein the polypeptide or ORF1 molecule comprises a lysine to cysteine substitution, e.g., at the position corresponding to position 371 of a Ring 10 ORF1 protein (e.g., as described herein).

303. The polypeptide, particle, nucleic acid molecule, method, or protein complex of embodiment 293, wherein the polypeptide or ORF1 molecule comprises a glutamine to cysteine substitution, e.g., at the position corresponding to position 287 of a Ring 10 ORF1 protein (e.g., as described herein).

304. The polypeptide, particle, nucleic acid molecule, method, or protein complex of embodiment 293, wherein the polypeptide or ORF1 molecule comprises one or more substitutions to cysteine at one or more positions corresponding to Y254, R263, N264, K265, L272, G273, T274, R276, H283, T285, N288, D291, Q308, D311, W312, T313, E314, D316, H318, N319, T321, T328, K329, T341, Q343, T354, Q358, T361, T364, Q368, D374, P376, P378, Y380, and/or I381 of a Ring 10 ORF1 protein (e.g., as described herein).

305. A particle comprising:

(a) a proteinaceous exterior comprising an ORF1 molecule; and

(b) a genetic element comprising a heterologous nucleic acid sequence encoding an exogenous effector;

wherein the genetic element is enclosed within the proteinaceous exterior; and

wherein the particle has one or more of the following characteristics:

(i) the genetic element (e.g., a DNA genetic element) does not comprise an Anellovirus 5' UTR or an origin of replication;

(ii) the sequence encoding the exogenous effector takes up at least 90%, 95%, 96%, 97%, 98%, 99% or 100% of the genetic element (e.g., a DNA genetic element);

(iii) the heterologous nucleic acid sequence takes up at least 90%, 95%, 96%, 97%, 98%, 99% or 100% of the genetic element (e.g., a DNA genetic element);

(iv) the particle does not comprise a detectable amount of (e.g., any) polypeptides from a host cell, or comprises less than 5, 10, 15, 20, 25, 30, 40, or 50 copies of a polypeptide from a host cell;

(v) the particle does not comprise a detectable amount of (e.g., any) nucleic acid molecules from a host cell, or comprises less than 2, 3, 4, or 5 copies of a nucleic acid molecule from a host cell;

(vi) the particle comprises a denaturant in a concentration of less than about 0.01M, 0.1M, 0.2M, 0.3M, 0.4M, 0.5M, 0.6M, 0.7M, 0.8M, 0.9M, 1M, 1.1M, 1.2M, 1.3M, 1.5M, 1.5M, 1.6M, 1.7M, 1.8M, 1.9M, or 2M;

(vii) does not substantially replicate when introduced into a cell (e.g., a human cell); and/or

(viii) has a symmetrical morphology.

306. The particle of embodiment 305, wherein the heterologous nucleic acid sequence is about 60-100, 100-200, 200-300, 300-400, 400-500, 500-600, 600-700, 700-800, 800-900, 900-1000, 1000-1500, or 1500-2000, 2000-3000, 3000-4000, or 4000-5000 nucleotides in length.

307. A population of the particles of embodiment 305, wherein at least 75%, 80%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% of the particles in the population comprise at least 50, 55, or 60 copies of an Anellovirus ORF1 molecule.

308. A population of the particles of embodiment 305, wherein at least 90% of the particles in the population have a diameter of at least 30, 31, 32, 33, 34, or 35 nm.

309. A population of the particles of embodiment 305, wherein at least 90%, 95%, 96%, 97%, 98%, 99%, or 100% of the particles have a symmetrical morphology.

310. A population of the particles of embodiment 305, wherein the population does not comprise a detectable amount of polypeptides from a host cell, or comprises less than 1, 2, 3, 4, 5, 10, 15, 20, 25, 30, 40, or 50 copies per particle of a polypeptide from a host cell.

311. A population of the particles of embodiment 305, wherein the population does not comprise a detectable amount of nucleic acid molecules from a host cell, or comprises less than 1, 2, 3, 4, 5, 10, 15, 20, 25, 30, 40, or 50 copies per particle of a nucleic acid molecule from a host cell.

312. A population of the particles of embodiment 305, wherein the population comprises less than 10 ng of nucleic acids.

313. A population of the particles of embodiment 305, wherein the population does not comprise a detectable amount of nucleic acid molecules from a host cell, or comprises less than 1, 2, 3, 4, 5, 10, 15, 20, 25, 30, 40, or 50 copies per particle of a nucleic acid molecule having a length of 200 bp or less from a host cell.

314. A particle comprising:

a proteinaceous exterior comprising about 40-80 (e.g., about 60) copies of an ORF1 molecule; and

wherein the particle:

(i) does not comprise (e.g., does not enclose) a polynucleotide,

(ii) does not comprise (e.g., does not enclose) detectable levels of polynucleotides,

(iii) does not comprise (e.g., does not enclose) a polynucleotide of greater than 1000, 500, 200, or 100 nucleotides in length,

(iv) does not comprise (e.g., does not enclose) a polynucleotide comprising any contiguous nucleic acid sequences of at least 50, 100, 150, 200, 300, 400, 500, 600, 700, 800, 900, or 1000 nucleotides in length having least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to contiguous sequence in a wild-type *Anellovirus* genome (e.g., as described herein), and/or

(v) does not comprise a polynucleotide comprising an Anellovirus 5' UTR or an origin of replication.

315. The particle of embodiment 314, further comprising an exogenous effector.

316. The particle of embodiment 315, wherein the exogenous effector is enclosed within the proteinaceous exterior.

317. The particle of embodiment 314 or 315, wherein the exogenous effector is a polypeptide.

318. The particle of any of embodiments 314-317, wherein the exogenous effector is a small molecule.

319. A composition comprising a plurality of particles, the particles comprising a proteinaceous exterior comprising about 40-80 (e.g., about 60) copies of an ORF1 molecule;

wherein at least 50%, 60%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, 99.5%, or 99.9% of the particles do not comprise (e.g., do not enclose):

(i) a polynucleotide,

(ii) a nucleic acid molecule of greater than 1000, 500, 200, or 100 nucleotides in length,

(iii) a plurality of polynucleotides,

(iv) a circular nucleic acid molecule,

(v) a single-stranded nucleic acid molecule, and/or

(vi) a genetic element (e.g., a genetic element of an anellovector), e.g., as described herein; or

wherein the composition comprises less than 10^{10} - 10^{14} (e.g., less than 10^{10} - 10^{11}, 10^{11} - 10^{12}, 10^{12} - 10^{13}, or 10^{13} - 10^{14}) viral genome equivalents of nucleic acid molecules (e.g., genetic elements, e.g., of an anellovector as described herein) per kilogram of a subject to be administered the composition (e.g., as determined by qPCR or by measuring optical density).

320. The composition of embodiment 319, further comprising a denaturant (e.g., urea), e.g., in concentration of less than about 0.01M, 0.1M, 0.2M, 0.3M, 0.4M, 0.5M, 0.6M, 0.7M, 0.8M, 0.9M, 1M, 1.1M, 1.2M, 1.3M, 1.5M, 1.5M, 1.6M, 1.7M, 1.8M, 1.9M, or 2M; proteasomes; or ferritin.

321. The composition of embodiment 319 or 320, wherein the composition comprises 0.01-100 mg of the particles (e.g., 0.01-1, 1-10, 10-20, 20-30, 30-40, 40-50, 50-60, 60-70, 70-80, 80-90, or 90-100 mg of the particles).

322. A method of disassembling a particle, the method comprising:

(a) providing a mixture comprising a particle and a denaturant, wherein the particle comprises:

(i) a proteinaceous exterior comprising a plurality of Anellovirus ORF1 molecules, and

(ii) a nucleic acid molecule (e.g., a nucleic acid endogenous to a host cell or a nucleic acid exogenous to a host cell, e.g., an anellovirus genome); and

(b) incubating the mixture under conditions suitable for:

disassembly of the proteinaceous exterior, and

dissociation of the nucleic acid molecule from the proteinaceous exterior.

323. The method of embodiment 322, wherein the Anellovirus ORF1 molecules were made in mammalian cells.

324. The method of embodiment 322 or 323, wherein the conditions suitable for disassembly of the proteinaceous exterior comprises one or more of: a predetermined conductivity, a detergent (e.g., SDS (e.g., 0.1% SDS), Tween, or Triton), a chaotropic agent (e.g, urea), a high salt solution (e.g., a solution comprising NaCl, e.g., at a concentration of at least about 1M, e.g., at least about 0.5, 0.6, 0.7, 0.8, 0.9, 1.0, 1.1, 1.2, 1.3, 1.4, 1.5, 2, 3, 4, or 5M), or conditions involving a predetermined temperature.

325. The method of any of embodiments 322-324, wherein the mixture comprises a population of the particles.

326. The method of any of embodiments 322-325, wherein the incubating of (b) results in at least 50%, 60%... 95%, or 100% of the population of particles being disassembled.

327. The method of any of embodiments 322-326, further comprising a step of (c) removing (partially or completely) the nucleic acid molecule from the mixture, e.g., by washing.

328. The method of any of embodiments 322-327, wherein the host cell is a human cell.

329. A method of making an anellovector, the method comprising:

(a) providing a mixture comprising a plurality of Anellovirus ORF1 molecules, wherein at least 75%, 80%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% of the plurality of ORF1 molecules are not comprised in a particle comprising about 40-80 (e.g., about 60) copies of an ORF1 molecule;

(b) subjecting the mixture to conditions suitable for *in vitro* assembly of the Anellovirus ORF1 molecules; and

(c) incubating the Anellovirus ORF1 molecules with a plurality of genetic elements, under conditions suitable for assembly of the Anellovirus ORF1 molecules into one or more anellovectors each enclosing one or more of the genetic elements.

330. The method of embodiment 329, wherein the mixture provided in (a) is under denaturing conditions, e.g., wherein the mixture comprises a denaturant at a level sufficient to disassemble a complex (e.g., a proteinaceous exterior) comprising at least about 20, 30, 40, 50, or 60 copies, or 20-30, 30-40, 40-50, or 50-60 copies, of the Anellovirus ORF1 molecule.

331. The method of embodiment 329 or 330, wherein the conditions suitable for *in vitro* assembly comprise reducing the concentration of a denaturant or removing the mixture from denaturing conditions.

332. A method of making an anellovector, the method comprising:

(a) providing a mixture comprising a plurality of Anellovirus ORF1 molecules and subjecting the mixture to denaturing conditions (e.g., providing a denaturant as part of the mixture, e.g., contacting the mixture with a denaturant), wherein at least 75%, 80%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% of the plurality of ORF1 molecules are not comprised in a particle comprising about 40-80 (e.g., about 60) copies of an ORF1 molecule;

(b) subjecting the mixture to non-denaturing conditions (e.g., reducing the concentration of the denaturant to a level) suitable for *in vitro* assembly of the Anellovirus ORF1 molecules (e.g., by dialysis); and

(c) incubating the Anellovirus ORF1 molecules with a plurality of genetic elements, under conditions suitable for assembly of the Anellovirus ORF1 molecules into one or more anellovectors each enclosing one or more of the genetic elements.

333. The method of embodiment 332, wherein (b) and (c) are performed concurrently.

334. The method of embodiment 332, wherein (b) is performed prior to (c).

335. The method of any of embodiments 332-334, wherein the genetic elements are introduced into a mixture comprising the Anellovirus ORF1 molecules prior to, concurrently with, or after (b).

336. The method of any of embodiments 332-335, wherein at least 75%, 80%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% of the plurality of ORF1 molecules in the mixture of (a) are comprised in capsomers (e.g., decamers or particles of 25-40 nm in diameter, e.g., 25-30, 30-32, 32-35, or 35-40 nm in diameter or about 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, or 40 nm).

337. The method of any of embodiments 332-3336, wherein the ratio of ORF1 molecules in the mixture of (a) comprised in capsomers (e.g., decamers) compared to ORF1 molecules in the mixture of (a) comprised in particles is at least 2:1, 3:1, 4:1, 5:1, 10:1, 50:1, 100:1, 500:1, 1000:1, 5000:1, or 10,000:1.

338. The method of any of embodiments 332-3337, wherein at least 75%, 80%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% of the plurality of ORF1 molecules after the incubating of (c) are comprised in anellovectors (e.g., 60mers or particles of at least 30, 31, 32, 33, 34, or 35 nm in diameter).

339. The method of any of embodiments 332-338, wherein the genetic element encodes an exogenous effector.

340. The method of any of embodiments 332-339, wherein the genetic element is an oligonucleotide.

341. The method of any of embodiments 332-340, wherein the genetic element does not encode a polypeptide or functional nucleic acid.

342. The method of any of embodiments 332-341, wherein the concentration of the denaturant after step (b) is no more than about 0.01M, 0.1M, 0.2M, 0.3M, 0.4M, 0.5M, 0.6M, 0.7M, 0.8M, 0.9M, 1M, 1.1M, 1.2M, 1.3M, 1.5M, 1.5M, 1.6M, 1.7M, 1.8M, 1.9M, or 2M.

343. The method of any of embodiments 332-342, wherein, after the incubating of (c), at least 75%, 80%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% of the particles in the mixture comprise at least 50, 55, or 60 copies of an Anellovirus ORF1 molecule.

344. The method of any of embodiments 332-343, wherein, after the incubating of (c), at least 90% of the particles in the mixture have a diameter of at least 30, 31, 32, 33, 34, or 35 nm.

345. The method of any of embodiments 332-344, wherein, after the incubating of (c), at least 90%, 95%, 96%, 97%, 98%, 99%, or 100% of the particles in the mixture have a symmetrical morphology.

346. The method of any of embodiments 332-41, wherein the denaturant is selected from a chaotropic agent (e.g., urea), heat (e.g., temperature above about 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, or 95°C), or pH (e.g., acidic pH or basic pH).

347. A method of making an anelloVLP, the method comprising:

(a) providing a mixture comprising a plurality of Anellovirus ORF1 molecules, wherein at least 75%, 80%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% of the plurality of ORF1 molecules are not comprised in a particle comprising about 40-80 (e.g., about 60) copies of an ORF1 molecule;

(b) subjecting the mixture to conditions suitable for *in vitro* assembly of the Anellovirus ORF1 molecules; and

(c) incubating the Anellovirus ORF1 molecules with a plurality of effectors (e.g., exogenous effectors), under conditions suitable for assembly of the Anellovirus ORF1 molecules into one or more anelloVLPs each enclosing one or more of the effectors.

348. The method of embodiment 347, wherein the mixture provided in (a) is under denaturing conditions, e.g., wherein the mixture comprises a denaturant at a level sufficient to disassemble a complex (e.g., a proteinaceous exterior) comprising at least about 20, 30, 40, 50, or 60 copies, or 20-30, 30-40, 40-50, or 50-60 copies, of the Anellovirus ORF1 molecule.

349. The method of embodiment 347 or 348, wherein the conditions suitable for *in vitro* assembly comprise reducing the concentration of a denaturant or removing the mixture from denaturing conditions.

350. A method of making an anelloVLP, the method comprising:

(a) providing a mixture comprising a plurality of Anellovirus ORF1 molecules and a denaturant, wherein at least 75%, 80%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% of the plurality of ORF1 molecules are not comprised in a particle comprising about 40-80 (e.g., about 60) copies of an ORF1 molecule;

(b) reducing the concentration of the denaturant to a level suitable for *in vitro* assembly of the Anellovirus ORF1 molecules; and

(c) incubating the Anellovirus ORF1 molecules with a plurality of effectors (e.g., exogenous effectors), under conditions suitable for *in vitro* assembly of the Anellovirus ORF1 molecules into one or more anelloVLPs each enclosing one or more of the effectors.

351. The method of embodiment 350, wherein the effectors are introduced into a mixture comprising the Anellovirus ORF1 molecules prior to, concurrently with, or after (b).

352. The method of embodiment 350 or 351, wherein at least 75%, 80%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% of the plurality of ORF1 molecules in the mixture of (a) are comprised in capsomers (e.g., decamers or particles of at most 25-40 nm in diameter, e.g., 25-30, 30-32, 32-35, or 35-40 nm in diameter or about 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, or 40 nm).

353. The method of any of embodiments 350-352, wherein the ratio of ORF1 molecules in the mixture of (a) comprised in capsomers (e.g., decamers) compared to ORF1 molecules in the mixture of (a) comprised in particles is at least 2:1, 3:1, 4:1, 5:1, 10:1, 50:1, 100:1, 500:1, 1000:1, 5000:1, or 10,000:1.

354. The method of any of embodiments 350-353, wherein at least 75%, 80%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% of the plurality of ORF1 molecules after the incubating of (c) are comprised in anelloVLPs (e.g., 60mers or particles of at least 30, 31, 32, 33, 34, or 35 nm in diameter).

355. The method of any of embodiments 350-354, wherein the anelloVLP has one or more of the following characteristics:

(i) does not comprise (e.g., does not enclose) a polynucleotide,

(ii) does not comprise (e.g., does not enclose) detectable levels of polynucleotides,

(iii) does not comprise (e.g., does not enclose) a polynucleotide of greater than 1000, 500, 200, or 100 nucleotides in length,

(iv) does not comprise (e.g., does not enclose) a polynucleotide comprising any contiguous nucleic acid sequences of at least 50, 100, 150, 200, 300, 400, 500, 600, 700, 800, 900, or 1000 nucleotides in length having least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to contiguous sequence in a wild-type *Anellovirus* genome (e.g., as described herein), and/or

(v) does not comprise a polynucleotide comprising an Anellovirus 5' UTR or an origin of replication.

356. The method of any of embodiments 350-355, wherein the concentration of the denaturant after step (b) is no more than about 0.01M, 0.1M, 0.2M, 0.3M, 0.4M, 0.5M, 0.6M, 0.7M, 0.8M, 0.9M, 1M, 1.1M, 1.2M, 1.3M, 1.5M, 1.5M, 1.6M, 1.7M, 1.8M, 1.9M, or 2M.

357. The method of any of embodiments 350-356, wherein, after the incubating of (c), at least 75%, 80%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% of the particles in the mixture comprise at least 50, 55, or 60 copies of an Anellovirus ORF1 molecule.

358. The method of any of embodiments 350-357, wherein, after the incubating of (c), at least 90% of the particles in the mixture have a diameter of at least 30, 31, 32, 33, 34, or 35 nm.

359. The method of any of embodiments 350-358, wherein, after the incubating of (c), at least 90%, 95%, 96%, 97%, 98%, 99%, or 100% of the particles in the mixture have a symmetrical morphology.

360. The method of any of embodiments 350-359, wherein the denaturant is selected from a chaotropic agent (e.g., urea), heat (e.g., temperature above about 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, or 95°C), or pH (e.g., acidic pH or basic pH).

361. A method of making an anelloVLP, the method comprising:

(a) providing a mixture comprising a particle and a denaturant, wherein the particle comprises:

(i) a proteinaceous exterior comprising a plurality of Anellovirus ORF1 molecules, and

(ii) a nucleic acid molecule (e.g., a host cell nucleic acid molecule); and

(b) incubating the mixture under conditions suitable for:

disassembly of the proteinaceous exterior, and

dissociation of the nucleic acid molecule from the proteinaceous exterior;

(c) providing a mixture comprising a plurality of Anellovirus ORF1 molecules and a denaturant, wherein at least 75%, 80%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% of the plurality of ORF1 molecules are not comprised in a particle comprising about 40-80 (e.g., about 60) copies of an ORF1 molecule;

(d) reducing the concentration of the denaturant to a level suitable for *in vitro* assembly of the Anellovirus ORF1 molecules; and

(e) incubating the Anellovirus ORF1 molecules with a plurality of effectors (e.g., exogenous effectors), under conditions suitable for assembly of the Anellovirus ORF1 molecules into one or more anelloVLPs each enclosing one or more of the effectors.

362. A polypeptide, e.g., an ORF1 molecule, comprising one or more of:

(a) a first region comprising an amino acid sequence having at least 70% (e.g., at least about 70, 80, 90, 95, 96, 97, 98, 99, or 100%) sequence identity to an arginine-rich region sequence described herein (e.g., MPYYYRRRRYNYRRPRWYGRGWIRRPFRRRFRRKRRVR (SEQ ID NO: 216) or MAWGWWKRRRRWWFRKRWTRGRLRRRWPRSARRRPRRRRVRRRRRWRRGRRKTRTYRRRR

RFRRRGRK (SEQ ID NO: 186), or as listed in any one of Tables A1-A26) or a sequence of at least about 40 amino acids comprising at least 60%, 70%, or 80% basic residues (e.g., arginine, lysine, or a combination thereof),

(b) a second region comprising an amino acid sequence having at least 30% (e.g., at least about 30, 35, 40, 50, 60, 70, 80, 90, 95, 96, 97, 98, 99, or 100%) sequence identity to a jelly-roll region sequence described herein (e.g., PTYTTIPLKQWQPPYKRTCYIKGQDCLIYYSNLRLGMNSTMYEKSIVPVHWPGGGSFSVSMLTLD ALYDIHKLCRNWWTSTNQDLPLVRYKGCKITFYQSTFTDYIVRIHTELPANSNKLTYPNTHPLM MMMSKYKHIIPSRQTRRKKKPYTKIFVKPPPQFENKWYFATDLYKIPLLQIHCTACNLQNPFVKP DKLSNNVTLWSLNT (SEQ ID NO: 217), or as listed in any of any one of Tables A1-A26) or a sequence comprising at least 6 (e.g., at least 6, 7, 8, 9, 10, 11, or 12) beta strands;

(c) a third region comprising an amino acid sequence having at least 30% (e.g., at least about 30, 35, 40, 50, 60, 70, 80, 90, 95, 96, 97, 98, 99, or 100%) sequence identity to an N22 domain sequence described herein (e.g., TMALTPFNEPIFTQIQYNPDRDTGEDTQLYLLSNATGTGWDPPGIPELILEGFPLWLIYWGFADFQ KNLKKVTNIDTNYMLVAKTKFTQKPGTFYLVILNDTFVEGNSPYEKQPLPEDNIKWYPQVQYQL EAQNKLLQTGPFTPNIQGQLSDNISMFYKFYFK (SEQ ID NO: 219), or as listed in any of any one of Tables A1-A26); and

(d) a fourth region comprising an amino acid sequence having at least 30% (e.g., at least about 30, 35, 40, 50, 60, 70, 80, 90, 95, 96, 97, 98, 99, or 100%) sequence identity to an Anellovirus ORF1 C-terminal domain (CTD) sequence described herein (e.g., WGGSPPKAINVENPAHQIQYPIPRNEHETTSLQSPGEAPESILYSFDYRHGNYTTTALSRISQDWA LKDTVSKITEPDRQQLLKQALECLQISEETQEKKEKEVQQLISNLRQQQQLYRERIISLLKDQ (SEQ ID NO: 220), or as listed in any of any one of Tables A1-A26);

wherein the ORF1 molecule comprises at least one difference (e.g., a mutation, chemical modification, or epigenetic alteration) relative to a wild-type ORF1 protein (e.g., as described herein), e.g., an insertion, substitution, chemical or enzymatic modification, and/or deletion, e.g., a deletion of a domain (e.g., one or more of an arginine-rich region, jelly-roll domain, HVR, N22, or CTD, e.g., as described herein).

363. The polypeptide of embodiment 362, wherein the amino acid sequences of the region of (a), (b), (c), and (d) have at least 90% sequence identity to their respective references.

364. The polypeptide of embodiment 362, wherein the polypeptide comprises:

(i) the first region and the second region;

(ii) the first region and the third region;

(iii) the first region and the fourth region;

(iv) the second region and the third region;

(v) the second region and the fourth region;

(vi) the third region and the fourth region;

(vii) the first region, the second region, and the third region;

(viii) the first region, the second region, and the fourth region;

(ix) the first region, the third region, and the fourth region; or

(x) the second region, the third region, and the fourth region.

365. The polypeptide of any of embodiments 362-364, wherein:

the first region comprises an amino acid sequence having at least 70% (e.g., at least about 70, 80, 90, 95, 96, 97, 98, 99, or 100%) sequence identity to an arginine-rich region sequence as listed in any one of Tables A1-A26;

the second region comprises an amino acid sequence having at least 70% (e.g., at least about 70, 80, 90, 95, 96, 97, 98, 99, or 100%) sequence identity to a jelly-roll region sequence as listed in any one of Tables A1-A26;

the third region comprises an amino acid sequence having at least 70% (e.g., at least about 70, 80, 90, 95, 96, 97, 98, 99, or 100%) sequence identity to an N22 domain sequence as listed in any one of Tables A1-A26; and/or

the fourth region comprises an amino acid sequence having at least 70% (e.g., at least about 70, 80, 90, 95, 96, 97, 98, 99, or 100%) sequence identity to a CTD sequence as listed in any one of Tables A1-A26.

366. The polypeptide according to embodiment 365, wherein the amino acid sequences of the first, second, third and fourth region have at least 90% sequence identity to their respective references.

367. The polypeptide of any of the preceding embodiments, wherein the polypeptide comprises, in N-terminal to C-terminal order, the first region, the second region, the third region, and the fourth region.

368. The polypeptide of any of the preceding embodiments, wherein the at least one difference comprises at least one difference in the first region relative to the arginine-rich region of a wild-type ORF1 protein.

369. The polypeptide of any of the preceding embodiments, wherein the first region comprises an arginine-rich region from the ORF1 protein of an Anellovirus other than the wild-type Anellovirus to which the polypeptide, or the portion thereof excluding the first region, has greatest sequence identity.

370. The polypeptide of any of the preceding embodiments, wherein the first region comprises an amino acid sequence having at least 70% sequence identity to the arginine-rich region from an Anellovirus other than the wild-type Anellovirus to which the polypeptide has greatest sequence identity.

371. The polypeptide of any of the preceding embodiments, wherein the second region comprises a jelly-roll region from the ORF1 protein of an Anellovirus other than the wild-type Anellovirus to which the polypeptide, or the portion thereof excluding the second region, has greatest sequence identity.

372. The polypeptide of any of the preceding embodiments, wherein the second region comprises an amino acid sequence having at least 70% sequence identity to the jelly-roll region from an Anellovirus other than the wild-type Anellovirus to which the polypeptide has greatest sequence identity.

373. The polypeptide of any of the preceding embodiments, wherein the third region comprises an N22 domain from the ORF1 protein of an Anellovirus other than the wild-type Anellovirus to which the polypeptide, or the portion thereof excluding the third region, has greatest sequence identity.

374. The polypeptide of any of the preceding embodiments, wherein the third region comprises an amino acid sequence having at least 70% sequence identity to the N22 region from an Anellovirus other than the wild-type Anellovirus to which the polypeptide has greatest sequence identity.

375. The polypeptide of any of the preceding embodiments, wherein the fourth region comprises a CTD domain from the ORF1 protein of an Anellovirus other than the wild-type Anellovirus to which the polypeptide, or the portion thereof excluding the fourth region, has greatest sequence identity.

376. The polypeptide of any of the preceding embodiments, wherein the fourth region comprises an amino acid sequence having at least 70% sequence identity to the CTD region from an Anellovirus other than the wild-type Anellovirus to which the polypeptide has greatest sequence identity.

377. The polypeptide of any of embodiments 362-376, wherein the HVR sequence is positioned between the second region and the third region.

378. The polypeptide of embodiment 377, wherein the HVR sequence comprises an amino acid sequence having at least 70% (e.g., at least about 70, 80, 90, 95, 96, 97, 98, 99, or 100%) sequence identity to the HVR from an Anellovirus other than the wild-type Anellovirus to which the ORF1 protein has greatest sequence identity.

379. The polypeptide of any of embodiment 377or 378, wherein the HVR sequence is heterologous relative to one or more of the first region, second region, third region, and/or fourth region.

380. The polypeptide of any of embodiments 377-379, wherein the HVR sequence comprises an HVR from the ORF1 protein of an Anellovirus other than the wild-type Anellovirus to which the polypeptide, or the portion thereof excluding the HVR sequence, has greatest sequence identity.

381. The polypeptide of any of embodiments 377-380, wherein the HVR sequence comprises an amino acid sequence having at least 70% sequence identity to the HVR from an Anellovirus other than the wild-type Anellovirus to which the polypeptide has greatest sequence identity.

382. The anellovector of any of the preceding embodiments, wherein the proteinaceous exterior comprises a polypeptide of any of embodiments 362-381.

383. The particle of any of the preceding embodiments, wherein the proteinaceous exterior comprises a polypeptide of any of embodiments 362-381.

384. The anelloVLP of any of the preceding embodiments, wherein the proteinaceous exterior comprises a polypeptide of any of embodiments 362-381.

385. A method of making two or more different Anellovirus ORF molecules, the method comprising:

(i) providing an insect cell comprising a nucleic acid construct encoding two or more different Anellovirus ORF molecules (e.g., two or more of an ORF1, ORF2, ORF2/2, ORF2/3, ORF1/1, and/or ORF1/2 molecule);

(ii) incubating the insect cell under conditions suitable for expression of the two or more different Anellovirus ORF molecules.

386. The method of embodiment 385, wherein the nucleic acid construct comprises sequences encoding all of an ORF1, ORF2, ORF2/2, ORF2/3, ORF1/1, and/or ORF1/2 molecule.

387. The method of embodiment 385, further comprising incubating the insect cell under conditions suitable for secretion of the Anellovirus ORF molecule.

388. The method of embodiment 385, further isolating the Anellovirus ORF molecule from the insect cell.

389. The method of embodiment 388, wherein the isolating step comprises lysing the insect cell.

390. The method of any of embodiments 385-389, wherein the Anellovirus ORF comprises an Anellovirus ORF1 molecule.

391. A method of making an Anellovirus ORF1 molecule, the method comprising:

(i) providing an insect cell comprising a nucleic acid construct encoding an Anellovirus ORF1 molecule, wherein:

(a) the Anellovirus ORF1 molecule has a molecular weight of at least 101 kDa,

(b) the Anellovirus ORF1 molecule is a full-length Anellovirus ORF1 protein,

(c) a plurality of the Anellovirus ORF1 molecules, when in the presence of an Anellovirus genetic element, enclose the Anellovirus genetic element,

(d) the Anellovirus ORF1 molecule is not a TTV ORF1 protein,

(e) the Anellovirus ORF1 molecule is a *Betatorquevirus* or *Gammatorquevirus* ORF1 molecule; or

(f) the Anellovirus ORF1 molecule comprises an Anellovirus ORF1 Arginine-rich region and an Anellovirus C-terminal domain;

(ii) incubating the insect cell under conditions suitable for expression of the Anellovirus ORF1 molecule.

392. The method of embodiment 391, further comprising incubating the insect cell under conditions suitable for secretion of the Anellovirus ORF1 molecule.

393. The method of embodiment 391, further isolating the Anellovirus ORF1 molecule from the insect cell.

394. The method of embodiment 393, wherein the isolating step comprises lysing the insect cell.

395. The method of any of the preceding embodiments, wherein the incubation step produces an amount of the Anellovirus ORF1 molecule detectable by Western blot, e.g., as described herein.

396. A method of making an Anellovirus ORF molecule (e.g., an ORF1, ORF2, ORF2/2, ORF2/3, ORF1/1, and/or ORF1/2 molecule), the method comprising:

(i) providing an insect cell (e.g., an Sf9 cell) comprising a nucleic acid construct encoding the Anellovirus ORF molecule;

(ii) incubating the insect cell under conditions suitable for expression of a plurality of the Anellovirus ORF molecules; and

(iii) optionally isolating, purifying, and/or enriching the plurality of Anellovirus ORF molecules from the insect cell or other components or constituents thereof;

thereby making the Anellovirus ORF molecule.

397. The method of embodiment 396, wherein the Anellovirus ORF molecule is fused to a marker (e.g., a His tag), e.g., at its N-terminal end or at its C-terminal end (e.g., as described in Table E1 and/or Example 9).

398. The method of embodiment 396 or 397, wherein the insect cell further comprises a nucleic acid construct encoding one or more additional Anellovirus ORF molecules (e.g., one or more of an ORF1, ORF2, ORF2/2, ORF2/3, ORF1/1, and/or ORF1/2 molecule), and wherein the method further comprises:

incubating the insect cell under conditions suitable for expression of a plurality of the one or more additional Anellovirus ORF molecules, e.g., prior to, concurrently with, or subsequent to step (ii); and

optionally isolating, purifying, and/or enriching the plurality of the one or more additional Anellovirus ORF molecules from the insect cell or other components or constituents thereof, e.g., prior to, concurrently with, or subsequent to step (iii).

399. The method of embodiment 398, wherein the nucleic acid construct encoding the one or more additional Anellovirus ORF molecules is the same as the nucleic acid construct of (i).

400. The method of embodiment 399, wherein the nucleic acid construct of (i) comprises sequences encoding 2, 3, 4, 5, or all 6 of an Anellovirus ORF1, ORF2, ORF2/2, ORF2/3, ORF1/1, and/or ORF1/2 molecule.

401. The method of embodiment 399, wherein the nucleic acid construct of (i) encodes an Anellovirus ORF1, ORF2, ORF2/2, ORF2/3, ORF1/1, and ORF1/2 molecule.

402. The method of embodiment 399, wherein the nucleic acid construct of (i) comprises the full open reading frame region of an Anellovirus genome.

403. The method of embodiment 398, wherein the nucleic acid construct encoding the one or more additional Anellovirus ORF molecules is different from the nucleic acid construct of (i).

404. The method of any of embodiments 398-403, wherein the Anellovirus ORF molecules are from the same Anellovirus genome.

405. The method of any of embodiments 398-403, wherein the Anellovirus ORF molecules are from a plurality of Anellovirus genomes (e.g., wherein the ORF1 molecule is from one Anellovirus genome and the ORF2 molecule is from a different Anellovirus genome).

406. The method of any of embodiments 398-405, wherein one or more of the Anellovirus ORF molecules are from an Alphatorquevirus (e.g., as listed in Table E2).

407. The method of any of embodiments 398-406, wherein one or more of the Anellovirus ORF molecules are from a Betatorquevirus (e.g., as listed in Table E2).

408. The method of any of embodiments 398-407, wherein one or more of the Anellovirus ORF molecules are from a Gammatorquevirus (e.g., as listed in Table E2).

409. The method of any of embodiments 398-408, wherein the nucleic acid construct or constructs each comprises a promoter (e.g., a promoter controlling expression of one or more of the Anellovirus ORF molecules, e.g., a baculovirus polyhedron promoter).

410. The method of any of embodiments 398-409, further comprising incubating the insect cell under conditions suitable for secretion of the Anellovirus ORF molecules.

411. The method of any of embodiments 398-410, wherein the isolating step comprises lysing the insect cell.

412. The method of any of embodiments 398-411, wherein the incubation step produces an amount of the Anellovirus ORF molecule (e.g., ORF1 molecule) detectable by Western blot, e.g., as described herein.

413. The method of any of emcodiments 398-412, wherein the incubation step produces at least 1, 2, 3, 4, 5, or 6 mg of the Anellovirus ORF1 molecule per 1 L of cell culture (e.g., Sf9 culture).

414. The method of any of the preceding embodiments, wherein the Anellovirus ORF molecules are isolated, purified, or enriched by isopycnic centrifugation.

415. The method of any of the preceding embodiments, wherein the Anellovirus ORF molecule is an Anellovirus ORF1 molecule, and wherein the method further comprises:

contacting, *in vitro,* the isolated, purified, or enriched Anellovirus ORF1 molecule with a genetic element under conditions suitable for enclosure of the genetic element by a proteinaceous exterior comprising the Anellovirus ORF1 molecule, e.g., as described herein.

Other features, objects, and advantages of the invention will be apparent from the description and drawings, and from the claims.

Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. All publications,

patent applications, patents, and other references mentioned herein are incorporated by reference in their entirety. In addition, the materials, methods, and examples are illustrative only and not intended to be limiting.

BRIEF DESCRIPTION OF THE DRAWINGS

The following detailed description of the embodiments of the invention will be better understood when read in conjunction with the appended drawings. For the purpose of illustrating the invention, there are shown in the drawings embodiments that are presently exemplified. It should be understood, however, that the invention is not limited to the precise arrangement and instrumentalities of the embodiments shown in the drawings. The patent or application file contains at least one drawing executed in color. Copies of this patent or patent application publication with color drawing(s) will be provided by the Office upon request and payment of the necessary fee.

Figure 1 is a series of diagrams showing production of exemplary malaria peptide conjugation constructs comprising the C-terminal region of a CS protein.

Figure 2 is a series of diagrams showing the structure of an exemplary malaria peptide conjugation construct on the surface of an anellovector capsid.

Figure 3 is a diagram showing separation of *in vitro* circularized DNA into positive and negative sense circularized single-stranded DNA (ssDNA) after denaturation.

Figures 4A and 4B are a series of diagrams showing successful expression of Anellovirus ORF1 molecules in cells. (A) Anellovirus strains for which ORF1 expression was detected from Sf9 cells included Ring2, Ring3, Ring4, Ring5, Ring6, Ring9, and Ring10 (e.g., as described herein). (B) Virus-like particles (VLPs) were observed by electron microscopy after production of Anellovirus ORF1 proteins for Ring2 and Ring10, as well as for chicken anemia virus (CAV) VP1.

Figures 5A-5C are a series of diagrams showing successful expression and purification of Ring2 ORF1 and ORF2 proteins from Sf9 cells. (A) Exemplary workflow for purification of ORF1 and ORF2 proteins from Sf9 cells. (B) Western blot using anti-ORF1 antibody, showing detection of Ring2 ORF1 proteins produced in Sf9 cells as described. (C) Electron micrograph showing formation of virus-like particles (VLPs) from Ring2 ORF1 proteins produced in Sf9 cells as described.

Figures 6A-6C are a series of diagrams showing disassembly of Ring2 VLPs using urea as a chaotropic denaturant. (A) Ring2 ORF1 protein was produced and purified from Sf9 cells as described, and VLPs were detected by electron microscopy. (B) After treatment with urea, VLPs are no longer observed, but instead the solution contains small ORF1 capsomers. (C) Expanded view of the boxed area shown in Figure 6B, showing detail of the capsomers.

Figures 7A-7B are a series of electron micrographs showing exemplary symmetrical (A) and asymmetrical (B) particle morphologies.

Figure 8 depicts expression of Ring2 ORF1 with a C-terminal His tag in insect cells.

Figure 9 depicts expression of Ring1 ORF1 and ORF1/1 with a C-terminal His tag in insect cells.

Figure 10 depicts expression of Ring2 ORF1 with an N-terminal His-tag, with or without PreScission cleavage sequence, in insect cells.

Figure 11 depicts expression of Ring1 ORFs 1/1, 1/2, 2, 2/2, and 2/3 as C-terminal His-tagged recombinant proteins in insect cells.

Figure 12 depicts expression of individual Ring2 ORFs in insect cells. Two exposures of the same blot are shown in the middle and right panels. The left panel shows the structures of Ring2 constructs tested as indicated.

Figure 13 depicts baculovirus-mediated co-expression of Ring2 ORF1 + "FullORF", ORF1 + ORF2, ORF1 + ORF2/2, and ORF1 + ORF2/3 in insect cells.

Figure 14 depicts simultaneous co-expression of multiple Ring2 proteins in insect cells using baculovirus.

Figure 15 depicts expression of ORFs from Anellovirus genome delivered into insect cells by baculovirus and by transfection.

Figure 16 shows that expression of Ring1 ORF2 is independent of the polyhedron promoter (arrow labeled pH) in Sf9 cells.

Figure 17 depicts co-delivery of Ring2 ORF1-His and Ring2 genomic DNA into Sf9 cells, followed by incubation and fractionation on a CsCl linear density gradient. An anti-His tag Western blot of fractions is shown at the top of the figure, as well as a qPCR assay of each fraction. Bottom panels show transmission electron microscopy images of two individual fractions and a pool of fractions, as indicated by boxes on the Western blot. The inset in the middle panel is a zoomed-in view showing proteasome-like structures.

Figure 18 depicts characterization of Sf9 isopycnic fractions by immunogold electron microscopy.

Figure 19 depicts expression of ORF1 from additional Anellovirus strains.

Figure 20 is a schematic showing an exemplary workflow for production of anellovectors (e.g., replication-competent or replication-deficient anellovectors as described herein).

Figure 21 is a graph showing primer specificity for primer sets designed for quantification of TTV and TTMV genomic equivalents. Quantitative PCR based on SYBR green chemistry shows one distinct peak for each of the amplification products using TTMV or TTV specific primer sets, as indicated, on plasmids encoding the respective genomes.

Figure 22 is a graph showing an exemplary amplification plot for linear amplification of TTMV (Target 1) or TTV (Target 2) over a 7 log10 of genome equivalent concentrations. Genome equivalents were quantified over 7 10-fold dilutions with high PCR efficiencies and linearity (R^2 TTMV: 0.996; R^2 TTV: 0.997).

Figures 23A and 23B are a series of diagrams showing that a tandem Anellovirus plasmid can increase anellovirus or anellovector production. (A) Plasmid map for an exemplary tandem Anellovirus plasmid. (B) Transfection of HEK293T cells with a tandem Anellovirus plasmid resulted in production of four times the number of viral genomes compared to single-copy harboring plasmids.

Figure 23C is a gel electrophoresis image showing circularization of TTMV-LY2 plasmids pVL46-063 and pVL46-240.

Figure 23D is a chromatogram showing copy numbers for linear and circular TTMV-LY2 constructs, as determined by size exclusion chromatography (SEC).

Figure 24 is a schematic showing the domains of an Anellovirus ORF1 molecule and the hypervariable region to be replaced with a structural hypervariable domain from a different Anellovirus.

Figure 25 is a schematic showing the domains of ORF1 and the hypervariable region that will be replaced with a protein or peptide of interest (POI) from a non-anellovirus source.

Figure 26 is a series of diagrams showing the design of an exemplary anellovector genetic element based on an Anellovirus genome. The protein-coding region was deleted from the anellovirus genome (left), leaving the anelloviral non-coding region (NCR), including the viral promoter, 5'UTR conserved domain (5CD), and GC-rich region. Payload DNA was inserted into the non-coding region at the protein-coding locus (right). The resulting anellovector harbored the payload DNA (including open reading frames, genes, non-coding RNAs, etc.) and the essential anellovirus cis replication and packaging elements, but lacked the essential protein elements for replication and packaging.

Figure 27 is a diagram showing an alignment of 36-nucleotide GC-rich regions from nine Anellovirus genome sequences, and a consensus sequence based thereon (SEQ ID NOS 818-827, respectively, in order of appearance).

Figure 28 is a series of diagrams showing ORF1 structures from Anellovirus strains LY2 and CBD203. Putative domains are labeled: arginine-rich region (arg-rich), core region comprising a structural jelly-roll domain, hypervariable region (HVR), structural N22 region, and structural C-terminal domain (CTD), as indicated.

Figure 29 is a graph showing the ability of an in vitro circularized (IVC) TTV-tth8 genome (IVC TTV-tth8) compared to a TTV-tth8 genome in a plasmid to yield TTV-tth8 genome copies at the expected density in HEK293T cells.

Figure 30 is a series of graphs showing the ability of an in vitro circularized (IVC) LY2 genome (WT LY2 IVC) and a wild-type LY2 genome in plasmid (WT LY2 Plasmid) to yield LY2 genome copies at the expected density in Jurkat cells.

Figure 31A is a schematic presentation of full-length Ring 10 ORF1 (also referred to herein as Ly1) labeled and colored by domains. The structural arginine-rich motif (ARM) is shown in purple, the structural jelly roll (JR) domain is shown in red, the spike P1 domain is shown in blue, the spike P2 domain is shown in green, and the C-terminal domain is shown in cyan. Residue numbers beginning each domain and the structural C-terminal domain (also referred to as the C-terminus) are indicated above.

Figure 31B is the sequence of full-length Ring 10 ORF1 colored as in Figure 31A with residue numbers indicated above. In bold are residues included in the Ring 10 delARM construct including the initial residue, K46, which is labeled. A dashed line above the sequence indicates residues not observed in the density. Secondary structure elements are indicated above with β-strands as arrows and α-helices as zig-zag lines. The JR β-strands are labeled B-I per convention while additional secondary structures are numbered by their domain. Three peptides used to generate polyclonal antibodies are underlined.

Figure 31C shows western blot analysis of Ring 10 delARM after expression (Expression) and after purification and storage (Purification). A molecular weight marker is labeled to the left of the gels, while arrows on the right indicate the band of Ring 10 delARM before (Ring 10 delARM) and after proteolysis (Ring 10 delARM Fragment). Polyclonal antibodies used to probe the western blots are indicated below and colored by the peptides used to generate them.

Figure 31D is an overlay of the 3D reconstruction of Ring 10 delARM VLP electron density and 60-mer VLP molecular structure colored as in Figure 31A. The spike P1 and P2 domains are labeled.

Figure 31E depicts one ORF1 protomer, shown in its electron density with domains labeled and colored as in Figure 31A.

Figure 31F depicts the electron density of Ring 10 delARM VLP colored by its local resolution. The bar (left) indicates the resolution (unit in angstrom) scale by color. The particle (right) is oriented as in Figure 31D.

Figure 32A depicts 60 Ring 10 structural jelly roll (JR) domains with one uniquely colored in red. Sixty Ring 10 structural jelly roll (JR) domains form the core of anellovirus particles.

Figure 32B depicts two JR domains, shown in red, with the observed C-terminal domain backbone colored in cyan. The JR domains are arbitrarily labeled JR^1 and JR^2 with the first (K48) and last (V562) observed residues for each protomer labeled with the corresponding number for clarity.

Figure 32C depicts a single JR domain oriented to show the β-sheet on the interior of the particle core. Sidechains of basic residues in position to contact with the viral genome are shown and labeled.

Figure 32D depicts the structural arginine-rich region, JR, and structural C-terminal domains of Ring 10 aligned with corresponding ORF1 sequences from different anellovirus genera (indicated in parentheses). Residues of Ring 10 are colored as in Figure 31A-31D. Basic residues of Ring 10 positioned to potentially contact the viral genome are indicated with asterisks.

Figure 33A depicts the anellovirus particle structure as shown as a surface rendering. The particle is shown in gray with 5 spikes forming a crown structure, numbered for clarity and colored as in Figure 31A-31D. The spike domains extend from the core on the 5-fold axis.

Figure 33B depicts the exterior of the crown structure as shown from the side. Five spike domains are colored as in Figure 33A. The hydrophobic and hydrophilic conserved residues are colored in light blue and magenta, respectively.

Figure 33C depicts the same spike domain from Figure 33B rotated to view residues on the interior of the crown structure.

Figure 33D depicts the spike domain of Ring 10 (colored as in Figure 31A-31D) aligned with ORF1 sequences representative of different anellovirus genera (indicated in parentheses). Magenta and black asterisks indicate the surface-exposed residues of the P1 domain and P2 domain, respectively. Below the alignment are >30% consensus residues, or Ø or γ indicating the residues are >70% hydrophobic or >60% hydrophilic, respectively.

Figure 34A is a schematic representation of full-length Ring 10 (top), which shows highly heterogeneous particles by negative-stained electron microscopy (bottom). Scale bar = 100 nm.

Figure 34B is a schematic representation of Ring 10 delARM (arginine-rich motif; top), which demonstrates a structural homology virus-like particle (VLP) as shown by negative-stained electron microscopy (bottom). Scale bar = 100 nm.

Figure 34C is a schematic representation of Ring 10 delARM delCTD (top), wherein further truncation of the structural C-terminal domain (Δ552-672) preserves a structured VLP as shown by negative-stained electron microscopy (bottom). Scale bar = 100 nm.

Figure 35 depicts a data processing procedure of the Ring 10 delARM cryogenic electron microscopy (cryo-EM) reconstruction. In short, crYOLO picked 58,391 particles from 11,083 micrographs. Several rounds of 2D classification resulted in 11,185 particles. After Relion de novo initial model reconstruction, Relion 3D refinement was implemented to obtain the orientation parameters. All particles with parameters were fed in a 3D classification. The class with the most abundant particle population resulted in 3.98 Å resolution.

Figure 36A is a representative negative-stained micrograph of Ring 10 delARM. The micrograph was imaged at NanoImaging Service.

Figure 36B is a representative cryo-EM micrograph of Ring 10 delARM. The micrograph was imaged at NanoImaging Service.

Figure 37A and 37B depict circular dichroism (CD) results of the TTMV-Ring 10 C-terminal peptide (CSEEEEESNLFERLLRQRTKQLQLKRRIIQTLKDLQKLE). Figure 37A is a table showing averages of secondary structure fractions estimated by different packages of CDPro. α-helix dominates the secondary structure assignment from the CD spectrum. Figure 37B shows an experimental spectrum of the C-terminal peptide (shown in red) overlaid with the calculated and averaged reference set spectra (shown in blue) from three different packages (SELCON3, CDSSTR, and CONTINLL).

Figure 38 depicts sequence alignment of 15 known anelloviruses within different genera indicated in parentheses. The conserved amino acids are shown in the first blue row underneath the sequence. The top blue row underneath the sequence alignment indicates the homology sequence if the conservation is larger than 30%. The bottom blue row shows either hydrophobic (ø, within 70% similarity) or 60% positive charged (γ, within 60% similarity) of amino acids, respectively. The alignment was done by Geneious-implemented Clustal Omega.

Figure 39A-39D depict the spikes of Ring 10 or alpha-fold predicted JA20 and MN779270.1. Figure 39A depicts the sphere representation of Ring 10 spike (identical to Figure 36C). Figure 39B and 39C are the spike representations for alpha-fold predicted JA20 and MN779270.1, respectively. P1 and P2 domains are demonstrated in blue and green. The light blue and magenta are the conserved hydrophobic and basic residues. Figure 39D shows the sequence alignment between Ring 10, JA20, and MN779210.1.

Figure 40 is a series of graphs showing that a polyclonal antibody (i.e., AB3725) that recognizes the surface-exposed Ring 10 HVR helix consisting of amino acid residues 352-361 of Ring 10 ORF1 specifically binds to Ring 10 anelloVLPs, but polyclonal antibodies generated against the HVR of different strains (shown as Strain 1 and Strain 2) did not bind to the Ring10 ORF1.

Figures 41A-41B show that AB3725 recognized Ring2 ORF1 mutants into which point mutations K357E, N358Q and E359I from Ring10 ORF1 were introduced.

Figures 42A-42B are a series of diagrams showing a Coomassie stain (Fig. 42A) and a Western blot (Fig. 42B) for Ring2 virus-like particles (VLPs).

Figure 43 is an electron micrograph showing Ring2 VLPs obtained after Capto400 purification.

Figures 44A-44B are a series of diagrams showing a Coomassie stain (Fig. 44A) and a Western blot (Fig. 44B) for Ring19 VLPs.

Figure 45 is an electron micrograph showing Ring19 VLPs obtained after Capto400 purification.

Figure 46 is a diagram showing an exemplary workflow for conjugating NHS ester moieties to surface lysines of anelloVLPs using click chemistry.

Figures 47A-47B are a series of diagrams showing a Coomassie stain (Fig. 47A) and a Western blot (Fig. 47B) for Ring2 VLPs conjugated with NHS Ester 647.

Figures 48A-48B are a series of diagrams showing Western blots for Ring2 anelloVLPs conjugated with NHS Ester biotin, labeled using a streptavidin CV 800 antibody (Fig. 48A) or a Ring19 HVR3 primary antibody and a goat anti-rabbit secondary antibody (Fig. 48B).

Figure 49 is a diagram showing an exemplary two-step process for conjugating a surface effector moiety to the surface of an anelloVLP.

Figure 50 is a diagram showing Coomassie staining, Western blot, and UV labeling for SARS-CoV-2 receptor binding domains (RBD) attached to DBCO, CalFluor 488, Azide, or Alexa488, as shown.

Figure 51 is a diagram showing Coomassie staining and Western blot for pRTx-2652 (a Ring 2 ORF1 variant) produced using SE-FPLC.

Figure 52 is a diagram showing Coomassie staining and Western blot showing conjugation of RBD attached to an azide moiety to pRTx-2652 ORF1 polypeptide attached to DBCO, to produce RBD-pRTx-2652 conjugate anelloVLPs.

Figure 53A is the 60-mer icosahedral structure of Ring10delARM produced from Sf9 cells.

Figure 53B is the 60-mer icosahedral structure of Ring10-ORF1delCterm Helix produced from Expi293 cells.

Figure 53C is a superposition of ORF1 protomers from the Ring10delARM and Ring10-ORF1delCterm Helix structures.

DETAILED DESCRIPTION OF CERTAIN EMBODIMENTS

Definitions

The present invention will be described with respect to particular embodiments and with reference to certain figures, but the invention is not limited thereto but only by the claims. Terms as set forth hereinafter are generally to be understood in their common sense unless indicated otherwise.

Where the term "comprising" is used in the present description and claims, it does not exclude other elements. For the purposes of the present invention, the term "consisting of" is considered to be a preferred embodiment of the term "comprising of". If hereinafter a group is defined to comprise at least a certain number of embodiments, this is to be understood to preferably also disclose a group which consists only of these embodiments.

Where an indefinite or definite article is used when referring to a singular noun, e.g. "a", "an" or "the", this includes a plural of that noun unless something else is specifically stated.

The wording "compound, composition, product, etc. for treating, modulating, etc." is to be understood to refer a compound, composition, product, etc. *per se* which is suitable for the indicated purposes of treating, modulating, etc. The wording "compound, composition, product, etc. for treating, modulating, etc." additionally discloses that, as an embodiment, such compound, composition, product, etc. is for use in treating, modulating, etc.

The wording "compound, composition, product, etc. for use in …", "use of a compound, composition, product, etc in the manufacture of a medicament, pharmaceutical composition, veterinary composition, diagnostic composition, etc. for …", or "compound, composition, product, etc. for use as a medicament…" indicates that such compounds, compositions, products, etc. are to be used in therapeutic methods which may be practiced on the human or animal body. They are considered as an equivalent disclosure of embodiments and claims pertaining to methods of treatment, etc. If an embodiment or a claim thus refers to "a compound for use in treating a human or animal being suspected to suffer from a disease", this is considered to be also a disclosure of a "use of a compound in the manufacture of a medicament for treating a human or animal being suspected to suffer from a disease" or a "method of treatment by administering a compound to a human or animal being suspected to suffer from a disease". The wording "compound, composition, product, etc. for treating, modulating, etc." is to be understood to refer a compound, composition, product, etc. *per se* which is suitable for the indicated purposes of treating, modulating, etc.

If hereinafter examples of a term, value, number, etc. are provided in parentheses, this is to be understood as an indication that the examples mentioned in the parentheses can constitute an embodiment. For example, if it is stated that "in embodiments, the nucleic acid molecule comprises a nucleic acid sequence having at least about 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to the *Anellovirus* ORF1-encoding nucleotide sequence of Table 1 (e.g., nucleotides 571 – 2613 of the nucleic acid sequence of Table 1)", then some embodiments relate to nucleic acid molecules comprising a nucleic acid sequence having at least about 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to nucleotides 571 – 2613 of the nucleic acid sequence of Table 1.

As used herein, the term "anellovector" refers to a vehicle comprising a genetic element, e.g., an episome, e.g., circular DNA, enclosed in a proteinaceous exterior. A "synthetic anellovector," as used herein, generally refers to an anellovector that is not naturally occurring, e.g., has a sequence that is different relative to a wild-type virus (e.g., a wild-type *Anellovirus* as described herein). In some embodiments, the proteinaceous exterior comprises an ORF1 molecule (e.g., an Anellovirus ORF1 protein), e.g., as described herein. In some embodiments, the proteinaceous exterior comprises a plurality of ORF1 molecules (e.g., an Anellovirus ORF1 protein), e.g., at least about 40, 45, 50, 55, 60, 65, or 70

ORF1 molecules. In some embodiments, the synthetic anellovector is engineered or recombinant, e.g., comprises a genetic element that comprises a difference or modification relative to a wild-type viral genome (e.g., a wild-type *Anellovirus* genome as described herein). In some embodiments, enclosed within a proteinaceous exterior encompasses 100% coverage by a proteinaceous exterior, as well as less than 100% coverage, e.g., 95%, 90%, 85%, 80%, 70%, 60%, 50% or less. For example, gaps or discontinuities (e.g., that render the proteinaceous exterior permeable to water, ions, peptides, or small molecules) may be present in the proteinaceous exterior, so long as the genetic element is retained in the proteinaceous exterior, e.g., prior to entry into a host cell. In some embodiments, the anellovector is purified, e.g., it is separated from its original source and/or substantially free (>50%, >60%, >70%, >80%, >90%) of other components.

An anellovector may, in some embodiments, comprise a nucleic acid vector that comprises sufficient nucleic acid sequence derived from or highly similar to (e.g., at least 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to) an *Anellovirus* genome sequence or a contiguous portion thereof to allow packaging into a proteinaceous exterior (e.g., a capsid), and further comprises a heterologous sequence. In some embodiments, the anellovector is a viral vector or a naked nucleic acid. In some embodiments, the anellovector comprises at least about 50, 60, 70, 71, 72, 73, 74, 75, 80, 90, 100, 150, 200, 300, 400, 500, 600, 700, 800, 900, 1000, 1100, 1200, 1300, 1400, 1500, 1600, 1700, 1800, 1900, 2000, 2500, 3000, or 3500 consecutive nucleotides of a native *Anellovirus* sequence or a sequence highly similar (e.g., at least 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical) thereto. In some embodiments, the anellovector further comprises one or more of an *Anellovirus* ORF1, ORF2, or ORF3. In some embodiments, the heterologous sequence comprises a multiple cloning site, comprises a heterologous promoter, comprises a coding region for a therapeutic protein, or encodes a therapeutic nucleic acid. In some embodiments, the capsid is a wild-type *Anellovirus* capsid. In embodiments, an anellovector comprises a genetic element described herein, e.g., comprises a genetic element comprising a promoter, a sequence encoding a therapeutic effector, and a capsid binding sequence.

As used herein, the term "anelloVLP" refers to a vehicle (e.g., a virus-like particle) comprising a proteinaceous exterior and an effector (e.g., an exogenous effector). In some instances, an anelloVLP does not comprise a substantial amount of a nucleic acid. In some embodiments, the proteinaceous exterior comprises an ORF1 molecule (e.g., an Anellovirus ORF1 protein), e.g., as described herein. In some embodiments, the proteinaceous exterior comprises a plurality of ORF1 molecules (e.g., an Anellovirus ORF1 protein), e.g., at least about 40, 45, 50, 55, 60, 65, or 70 ORF1 molecules. In some embodiments, the effector is enclosed in the proteinaceous exterior. In some embodiments, the effector is on the surface of the proteinaceous exterior (e.g., comprised in a surface moiety as described herein). In some embodiments, the anelloVLP does not comprise a polynucleotide of greater than 50, 100, 150, 200,

300, 400, 500, 600, 700, 800, 900, 1000, 1100, 1200, 1300, 1400, 1500, 1600, 1700, 1800, 1900, or 2000 nucleotides in length. In some embodiments, the anelloVLP does not comprise a polynucleotide comprising an Anellovirus 5' UTR or Anellovirus origin of replication. In some embodiments, the anelloVLP does not comprise a polynucleotide comprising any contiguous nucleic acid sequences of at least 50, 100, 150, 200, 300, 400, 500, 600, 700, 800, 900, or 1000 nucleotides in length having least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to contiguous sequence in a wild-type *Anellovirus* genome (e.g., as described herein).

As used herein, the term "antibody molecule" refers to a protein, e.g., an immunoglobulin chain or fragment thereof, comprising at least one immunoglobulin variable domain sequence. The term "antibody molecule" encompasses full-length antibodies and antibody fragments (e.g., scFvs). In some embodiments, an antibody molecule is a multispecific antibody molecule, e.g., the antibody molecule comprises a plurality of immunoglobulin variable domain sequences, wherein a first immunoglobulin variable domain sequence of the plurality has binding specificity for a first epitope and a second immunoglobulin variable domain sequence of the plurality has binding specificity for a second epitope. In embodiments, the multispecific antibody molecule is a bispecific antibody molecule. A bispecific antibody molecule is generally characterized by a first immunoglobulin variable domain sequence which has binding specificity for a first epitope and a second immunoglobulin variable domain sequence that has binding specificity for a second epitope.

The term "deletion," as used herein with respect to an amino acid sequence or a nucleic acid sequence, refers to a portion of a sequence that is absent relative to a reference sequence. In some embodiments, a deletion is actively removed from the sequence (e.g., by cleavage and/or by an enzyme). In some embodiments, the sequence is produced *de novo* without the deletion (e.g., a nucleic acid molecule synthesized *de novo* without the deletion sequence, a nucleic acid molecule produced using a template sequence in which the deletion sequence has already been removed, or a polypeptide translated from a nucleic acid sequence that does not encode the deletion sequence).

The term "disassembly," as used herein with respect to a particle, such as a virus-like particle (VLP), or a proteinaceous exterior, refers to disassociating one or more components of the particle (e.g., a capsid protein, e.g., an ORF1 molecule as described herein) from the remainder of the particle. In some instances, disassembly of a particle (e.g., a VLP) comprises separating enough of the ORF1 molecules from each other that they no longer form a proteinaceous exterior. In some instances, a ORF1 molecules separated from each other via disassembly of a particle form capsomers (e.g., decameric capsomers), e.g., as described herein. In some embodiments, disassembly reduces the particle to individual monomers. In some embodiments, after disassembly, multimers, e.g., decamers, monomers, and/or pentamers remain. In some instances, disassembly comprises denaturation of protein complexes of the particle (e.g.,

breaking noncovalent bounds between ORF1 molecules in the proteinaceous exterior). In some instances, disassembly is driven by a denaturant as described herein

The term "*in vitro* assembly," as used herein with respect to an anellovector or an anelloVLP, refers to the formation of a proteinaceous exterior comprising an ORF1 molecule, wherein the formation does not take place inside of a cell (e.g., takes place in a cell-free system such as a cell-free suspension, a lysate, or a supernatant). In some instances, *in vitro* assembly of an anellovector comprises enclosure, outside of a cell, of a genetic element (e.g., as described herein) within the proteinaceous exterior. In some instances, *in vitro* assembly of an anelloVLP comprises association, outside of a cell, of an effector (e.g., an exogenous effector, e.g., as described herein) with the proteinaceous exterior (e.g., enclosed within the proteinaceous exterior). *In vitro* assembly of a proteinaceous exterior may occur, in some instances, under conditions suitable for multimerization of a plurality of ORF1 molecules (e.g., nondenaturing conditions), e.g., to form a multimer of more than 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 ORF1 molecules. In some instances, *in vitro* assembly results in the formation of a proteinaceous exterior comprising at least about 20, 30, 40, 50, or 60 ORF1 molecules, or about 20-30, 30-40, 40-50, 50-60, or 60-70 ORF1 molecules). In some instances, the proteinaceous exterior is formed from ORF1 molecules that were produced in a cell and then purified therefrom. In some instances, the *in vitro* assembly takes place in a solution free of cells or constituents thereof. In other instances, the *in vitro* assembly takes place in a solution comprising cell debris (e.g., from lysed cells). In some instances, the *in vitro* assembly takes place in a solution substantially free of cellular nucleic acid molecules (e.g., genomic DNA, mitochondrial DNA, mRNA, and/or noncoding RNA from a cell). As used herein, a nucleic acid "encoding" refers to a nucleic acid sequence encoding an amino acid sequence or a functional polynucleotide (e.g., a non-coding RNA, e.g., an siRNA or miRNA).

An "exogenous" agent (e.g., an effector, a nucleic acid (e.g., RNA), a gene, payload, protein) as used herein refers to an agent that is either not comprised by, or not encoded by, a corresponding wild-type virus, e.g., an Anellovirus as described herein. In some embodiments, the exogenous agent does not naturally exist, such as a protein or nucleic acid that has a sequence that is altered (e.g., by insertion, deletion, or substitution) relative to a naturally occurring protein or nucleic acid. In some embodiments, the exogenous agent does not naturally exist in the host cell. In some embodiments, the exogenous agent exists naturally in the host cell but is exogenous to the virus. In some embodiments, the exogenous agent exists naturally in the host cell, but is not present at a desired level or at a desired time.

A "heterologous" agent or element (e.g., an effector, a nucleic acid sequence, an amino acid sequence), as used herein with respect to another agent or element (e.g., an effector, a nucleic acid sequence, an amino acid sequence), refers to agents or elements that are not naturally found together, e.g., in a wild-type virus, e.g., an Anellovirus. In some embodiments, a heterologous nucleic acid sequence

may be present in the same nucleic acid as a naturally occurring nucleic acid sequence (e.g., a sequence that is naturally occurring in the Anellovirus). In some embodiments, a heterologous agent or element is exogenous relative to an Anellovirus from which other (e.g., the remainder of) elements of the anellovector are based.

As used herein, the term "genetic element" refers to a nucleic acid sequence, generally in an anellovector. It is understood that the genetic element can be produced as naked DNA and optionally further assembled into a proteinaceous exterior. It is also understood that an anellovector can insert its genetic element into a cell, resulting in the genetic element being present in the cell and the proteinaceous exterior not necessarily entering the cell.

As used herein, the term "ORF1 molecule" refers to a polypeptide having an activity and/or a structural feature of an Anellovirus ORF1 protein (e.g., an Anellovirus ORF1 protein as described herein, e.g., as listed in any one of Tables A1-A26), or a functional fragment thereof. An ORF1 molecule may, in some instances, comprise one or more of (e.g., 1, 2, 3 or 4 of): a first region comprising at least 60% basic residues (e.g., at least 60% arginine residues), a second region compising at least about six beta strands (e.g., at least 4, 5, 6, 7, 8, 9, 10, 11, or 12 beta strands), a third region comprising a structure or an activity of an Anellovirus structural N22 domain (e.g., as described herein, e.g., an structural N22 domain from an Anellovirus ORF1 protein as described herein), and/or a fourth region comprising a structure or an activity of an Anellovirus structural C-terminal domain (CTD) (e.g., as described herein, e.g., a CTD from an Anellovirus ORF1 protein as described herein). In some instances, the ORF1 molecule comprises, in N-terminal to C-terminal order, the first, second, third, and fourth regions. In some instances, an anellovector comprises an ORF1 molecule comprising, in N-terminal to C-terminal order, the first, second, third, and fourth regions. An ORF1 molecule may, in some instances, comprise a polypeptide encoded by an Anellovirus ORF1 nucleic acid (e.g., as listed in any one of Tables N1-N26). An ORF1 molecule may, in some instances, further comprise a heterologous sequence, e.g., a hypervariable region (HVR), e.g., an HVR from an Anellovirus ORF1 protein, e.g., as described herein. An "Anellovirus ORF1 protein," as used herein, refers to an ORF1 protein encoded by an Anellovirus genome (e.g., a wild-type Anellovirus genome, e.g., as described herein), e.g., an ORF1 protein having the amino acid sequence as listed in any one of Tables A1-A26, or as encoded by the ORF1 gene as listed in any one of Tables N1-N26.

The term "ORF1 domain," as used herein with respect to an ORF1 molecule, refers to the portion of the ORF1 molecule having the structure or function of an Anellovirus ORF1 protein. The ORF1 domain is generally capable of forming a multimer with other copies of the ORF1 domain (e.g., in other ORF1 molecules), or with other ORF1 molecules, e.g., to form a proteinaceous exterior (e.g., of an anellovector or anelloVLP as described herein). In some instances, the ORF1 molecule may comprise

one or more additional domains other than the ORF1 domain (for example, a domain comprising or attached to a surface effector, e.g., as described herein). In some instances, the amino acid sequence of an ORF1 domain comprises an insertion (e.g., an insertion encoding a surface moiety or a domain capable of binding to a surface moiety), e.g., between the N-terminal end and C-terminal end of the ORF1 domain. In certain instances, the insertion does not substantially disrupt the structure and/or function of the ORF1 domain, e.g., such that the ORF1 domain remains capable of forming a multimer with other ORF1 domains or ORF1 molecules. The position within the ORF1 domain sequence into which the insertion is made is referred to herein as the "insertion point." An insertion can be made into an ORF1 domain by any genetic or polypeptide engineering method known in the art. In some embodiments, an ORF1 molecule consists of an ORF1 domain. In other embodiments, an ORF1 molecule comprises an ORF1 domain and a heterologous domain (e.g., a surface moiety as described herein). In some embodiments, an ORF1 domain is connected to a surface moiety by a polypeptide linker region.

As used herein, the term "ORF2 molecule" refers to a polypeptide having an activity and/or a structural feature of an Anellovirus ORF2 protein (e.g., an Anellovirus ORF2 protein as described herein, e.g., as listed in any one of Tables A1-A26), or a functional fragment thereof. An "Anellovirus ORF2 protein," as used herein, refers to an ORF2 protein encoded by an Anellovirus genome (e.g., a wild-type Anellovirus genome, e.g., as described herein), e.g., an ORF2 protein having the amino acid sequence as listed in any one of Tables A1-A26, or as encoded by the ORF2 gene as listed in any one of Tables N1-N26.

As used herein, the term "particle" refers to a vehicle having a diameter of less than 100 nm (e.g., about 20-25, 25-30, 30-35, or 35-40 nm) comprising a proteinaceous exterior. In some instances, the particle comprises a plurality of ORF1 molecules. The proteinaceous exterior of the particle generally forms an enclosure capable of limiting or preventing movement of certain molecules between the inside and outside of the proteinaceous exterior. In some embodiments, gaps or discontinuities (e.g., that render the proteinaceous exterior permeable to water, ions, peptides, or small molecules) may be present in the proteinaceous exterior. In certain embodiments, the gaps or discontinuities are of a sufficiently small size (e.g., diameter) that the proteinaceous exterior limits or prevents one or more large macromolecules (e.g., peptides, polypeptides, polynucleotides, lipids, or polysaccharides) from passing through the proteinaceous exterior.

As used herein, the term "proteinaceous exterior" refers to an exterior component that is predominantly (e.g., >50%, >60%, > 70%, >80%, > 90%) protein.

As used herein, the term "regulatory nucleic acid" refers to a nucleic acid sequence that modifies expression, e.g., transcription and/or translation, of a DNA sequence that encodes an expression product. In embodiments, the expression product comprises RNA or protein.

As used herein, the term "regulatory sequence" refers to a nucleic acid sequence that modifies transcription of a target gene product. In some embodiments, the regulatory sequence is a promoter or an enhancer.

As used herein, the term "replication protein" refers to a protein, e.g., a viral protein, that is utilized during infection, viral genome replication/expression, viral protein synthesis, and/or assembly of the viral components.

When viewed by electron microscopy, anellovector or anelloVLP particles typically adopt one of two conformations: a symmetrical morphology (e.g., as exemplified in Figure 7A) and an asymmetrical, or less symmetrical, morphology (e.g., as exemplified in Figure 7B). Accordingly, the term "symmetrical morphology," as used herein with respect to anellovector or anelloVLP particle morphology, refers to a particle having a shape that is predominantly symmetrical. The particle having symmetrical morphology may, in some instances, be approximately round. The particle having symmetrical morphology may, in some instances, not be perfectly circular or spherical (e.g., may be ovoid). In some instances, the particle having symmetrical morphology may include one or more deviations from a circular or spherical shape (e.g., one or more protrusions or indentations from its surface).

As used herein, the term "structural arginine-rich region" refers to a domain of an Anellovirus ORF1 molecule having a structural arginine-rich region sequence as listed in any of Tables B1-1 to B1-12, or a corresponding sequence in another ORF1 molecule.

As used herein, the term "structural jelly-roll region" refers to a domain of an Anellovirus ORF1 molecule having a structural jelly-roll region sequence as listed in any of Tables B1-1 to B1-12, or a corresponding sequence in another ORF1 molecule.

As used herein, the term "structural N22 domain" refers to a domain of an Anellovirus ORF1 molecule having a structural N22 domain sequence as listed in any of Tables B1-1 to B1-12, or a corresponding sequence in another ORF1 molecule.

As used herein, the term "structural C-terminal domain region" refers to a domain of an Anellovirus ORF1 molecule having a structural C-terminal domain sequence as listed in any of Tables B1-1 to B1-12, or a corresponding sequence in another ORF1 molecule.

As used herein, the term "jelly-roll B-H strands subdomain" refers to a domain of an Anellovirus ORF1 molecule having a jelly-roll B-H strands subdomain sequence as listed in any of Tables B1-1 to B1-12, or a corresponding sequence in another ORF1 molecule.

As used herein, the term "P1 domain" generally refers to a noncontiguous domain comprising a P1-1 subdomain and a P1-2 subdomain, e.g., of an Anellovirus ORF1 molecule.

As used herein, the term "P1-1 subdomain" refers to a domain of an Anellovirus ORF1 molecule having a P1-1 domain sequence as listed in any of Tables B1-1 to B1-12, or a corresponding sequence in another ORF1 molecule.

As used herein, the term "P2 domain " refers to a domain of an Anellovirus ORF1 molecule having a P2 domain sequence as listed in any of Tables B1-1 to B1-12, or a corresponding sequence in another ORF1 molecule.

As used herein, the term "P1-2 subdomain" refers to a domain of an Anellovirus ORF1 molecule having a P1-2 subdomain sequence as listed in any of Tables B1-1 to B1-12, or a corresponding sequence in another ORF1 molecule.

As used herein, the term "jelly-roll I strand subdomain" refers to a domain of an Anellovirus ORF1 molecule having a jelly-roll I strand subdomain sequence as listed in any of Tables B1-1 to B1-12, or a corresponding sequence in another ORF1 molecule.

As used herein, the term "mutant ORF1," as applied to a particular domain or region of an ORF1 molecule, refers to a non-naturally occurring ORF1 domain or region comprising at least one sequence difference (e.g., addition, deletion, or substitution) relative to the closest naturally-occurring ORF1 domain or region sequence. For instance, a "mutant ORF1 structural jelly-roll region" comprises at least one sequence difference (e.g., addition, deletion, or substitution) relative to the closest naturally-occurring Anellovirus ORF1 structural jelly-roll region.

As used herein, a "substantially non-pathogenic" organism, particle, or component, refers to an organism, particle (e.g., a virus or an anellovector, e.g., as described herein), or component thereof that does not cause or induce a detectable disease or pathogenic condition, e.g., in a host organism, e.g., a mammal, e.g., a human. In some embodiments, administration of an anellovector to a subject can result in minor reactions or side effects that are acceptable as part of standard of care.

As used herein, the term "non-pathogenic" refers to an organism or component thereof that does not cause or induce a detectable disease or pathogenic condition, e.g., in a host organism, e.g., a mammal, e.g., a human.

As used herein, a "substantially non-integrating" genetic element refers to a genetic element, e.g., a genetic element in a virus or anellovector, e.g., as described herein, wherein less than about 0.01%, 0.05%, 0.1%, 0.5%, or 1% of the genetic element that enter into a host cell (e.g., a eukaryotic cell) or organism (e.g., a mammal, e.g., a human) integrate into the genome. In some embodiments the genetic element does not detectably integrate into the genome of, e.g., a host cell. In some embodiments, integration of the genetic element into the genome can be detected using techniques as described herein, e.g., nucleic acid sequencing, PCR detection and/or nucleic acid hybridization.

As used herein, a "substantially non-immunogenic" organism, particle, or component, refers to an organism, particle (e.g., a virus or anellovector, e.g., as described herein), or component thereof, that does not cause or induce an undesired or untargeted immune response, e.g., in a host tissue or organism (e.g., a mammal, e.g., a human). In some embodiments, the substantially non-immunogenic organism, particle, or component does not produce a detectable immune response. In some embodiments, the substantially non-immunogenic anellovector does not produce a detectable immune response against a protein comprising an amino acid sequence or encoded by a nucleic acid sequence shown in any one of Tables N1-N26. In some embodiments, an immune response (e.g., an undesired or untargeted immune response) is detected by assaying antibody presence or level (e.g., presence or level of an anti-anellovector antibody, e.g., presence or level of an antibody against an anellovector as described herein) in a subject, e.g., according to the anti-TTV antibody detection method described in Tsuda et al. (1999; *J. Virol. Methods* 77: 199-206; incorporated herein by reference) and/or the method for determining anti-TTV IgG levels described in Kakkola et al. (2008; *Virology* 382: 182-189; incorporated herein by reference). Antibodies against an *Anellovirus* or an anellovector based thereon can also be detected by methods in the art for detecting anti-viral antibodies, e.g., methods of detecting anti-AAV antibodies, e.g., as described in Calcedo et al. (2013; *Front. Immunol.* 4(341): 1-7; incorporated herein by reference).

A "subsequence" as used herein refers to a nucleic acid sequence or an amino acid sequence that is comprised in a larger nucleic acid sequence or amino acid sequence, respectively. In some instances, a subsequence may comprise a domain or functional fragment of the larger sequence. In some instances, the subsequence may comprise a fragment of the larger sequence capable of forming secondary and/or tertiary structures when isolated from the larger sequence similar to the secondary and/or tertiary structures formed by the subsequence when present with the remainder of the larger sequence. In some instances, a subsequence can be replaced by another sequence (e.g., a subseqence comprising an exogenous sequence or a sequence heterologous to the remainder of the larger sequence, e.g., a corresponding subsequence from a different *Anellovirus*).

As used herein, the term "surface moiety" refers to a moiety for which at least a portion is exposed on the exterior surface of a particle (e.g., exposed to the solution surrounding the particle). The surface moiety is generally attached, directly or indirectly, to a component of the proteinaceous exterior of the particle (e.g., an ORF1 molecule). In some instances, the surface moiety is covalently attached to the component of the proteinaceous exterior of the particle (e.g., the ORF1 molecule). In some instances, the surface moiety is noncovalently attached to the component of the proteinaceous exterior of the particle (e.g., the ORF1 molecule). In some instances, the surface moiety is bound to a binding moiety that is in turn attached (e.g., covalently or noncovalently) to the component of the proteinaceous exterior of the particle (e.g., the ORF1 molecule). In some instances, the surface moiety is comprised in an ORF1

molecule (e.g., is a heterologous domain of an ORF1 molecule). In some instances, a surface moiety is exogenous relative to an Anellovirus (e.g., the Anellovirus from which the ORF1 molecule was derived and/or an Anellovirus for which the ORF1 protein has at least 50%, 60%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to the ORF1 molecule). In some instances, a surface moiety is exogenous relative a target cell (e.g., a mammalian cell, e.g., a human cell) to be infected by the particle.

As used herein, “treatment”, "treating" and cognates thereof refer to the medical management of a subject with the intent to improve, ameliorate, stabilize, prevent or cure a disease, pathological condition, or disorder. This term includes active treatment (treatment directed to improve the disease, pathological condition, or disorder), causal treatment (treatment directed to the cause of the associated disease, pathological condition, or disorder), palliative treatment (treatment designed for the relief of symptoms), preventative treatment (treatment directed to preventing, minimizing or partially or completely inhibiting the development of the associated disease, pathological condition, or disorder); and supportive treatment (treatment employed to supplement another therapy).

As used herein, the term “virome” refers to viruses in a particular environment, e.g., a part of a body, e.g., in an organism, e.g. in a cell, e.g. in a tissue.

This invention relates generally to anellovectors, e.g., synthetic anellovectors, and uses thereof. The present disclosure provides anellovectors, compositions comprising anellovectors, and methods of making or using anellovectors. Anellovectors are generally useful as delivery vehicles, e.g., for delivering a therapeutic agent to a eukaryotic cell. Generally, an anellovector will include a genetic element comprising a nucleic acid sequence (e.g., encoding an effector, e.g., an exogenous effector or an endogenous effector) enclosed within a proteinaceous exterior. An anellovector may include one or more deletions of sequences (e.g., regions or domains as described herein) relative to an *Anellovirus* sequence (e.g., as described herein). Anellovectors can be used as a substantially non-immunogenic vehicle for delivering the genetic element, or an effector encoded therein (e.g., a polypeptide or nucleic acid effector, e.g., as described herein), into eukaryotic cells, e.g., to treat a disease or disorder in a subject comprising the cells.

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I. Anellovectors and AnelloVLPs

In some aspects, the invention described herein comprises compositions and methods of using and making an anellovector, anellovector preparations, anelloVLPs, anelloVLP preparations, and therapeutic compositions.

Anellovectors

In some embodiments, the anellovector has a sequence, structure, and/or function that is based on an *Anellovirus* (e.g., an *Anellovirus* as described herein, e.g., an *Anellovirus* comprising a nucleic acid or polypeptide comprising a sequence as shown in any one of Tables A1-A26 or N1-N26), or fragments or portions thereof, or other substantially non-pathogenic virus, e.g., a symbiotic virus, commensal virus, native virus. In some embodiments, an *Anellovirus*-based anellovector comprises at least one element exogenous to that *Anellovirus*, e.g., an exogenous effector or a nucleic acid sequence encoding an exogenous effector disposed within a genetic element of the anellovector. In some embodiments, an *Anellovirus*-based anellovector comprises at least one element heterologous to another element from that *Anellovirus*, e.g., an effector-encoding nucleic acid sequence that is heterologous to another linked nucleic acid sequence, such as a promoter element. In some embodiments, an anellovector comprises a genetic element (e.g., circular DNA, e.g., single stranded DNA), which comprise at least one element that

is heterologous relative to the remainder of the genetic element and/or the proteinaceous exterior (e.g., an exogenous element encoding an effector, e.g., as described herein). An anellovector may be a delivery vehicle (e.g., a substantially non-pathogenic delivery vehicle) for a payload into a host, e.g., a human. In some embodiments, the anellovector is capable of replicating in a eukaryotic cell, e.g., a mammalian cell, e.g., a human cell. In some embodiments, the anellovector is substantially non-pathogenic and/or substantially non-integrating in the mammalian (e.g., human) cell. In some embodiments, the anellovector is substantially non-immunogenic in a mammal, e.g., a human. In some embodiments, the anellovector is replication-deficient. In some embodiments, the anellovector is replication-competent.

In some embodiments the anellovector comprises a curon, or a component thereof (e.g., a genetic element, e.g., comprising a sequence encoding an effector, and/or a proteinaceous exterior), e.g., as described in PCT Application No. PCT/US2018/037379, which is incorporated herein by reference in its entirety.

In an aspect, the invention includes an anellovector comprising (i) a genetic element comprising a promoter element, a sequence encoding an effector, (e.g., an endogenous effector or an exogenous effector, e.g., a payload), and a protein binding sequence (e.g., an exterior protein binding sequence, e.g., a packaging signal), wherein the genetic element is a single-stranded DNA, and has one or both of the following properties: is circular and/or integrates into the genome of a eukaryotic cell at a frequency of less than about 0.001%, 0.005%, 0.01%, 0.05%, 0.1%, 0.5%, 1%, 1.5%, or 2% of the genetic element that enters the cell; and (ii) a proteinaceous exterior; wherein the genetic element is enclosed within the proteinaceous exterior; and wherein the anellovector is capable of delivering the genetic element into a eukaryotic cell.

In some embodiments of the anellovector described herein, the genetic element integrates at a frequency of less than about 0.001%, 0.005%, 0.01%, 0.05%, 0.1%, 0.5%, 1%, 1.5%, or 2% of the genetic element that enters a cell. In some embodiments, less than about 0.01%, 0.05%, 0.1%, 0.5%, 1%, 2%, 3%, 4%, or 5% of the genetic elements from a plurality of the anellovectors administered to a subject will integrate into the genome of one or more host cells in the subject. In some embodiments, the genetic elements of a population of anellovectors, e.g., as described herein, integrate into the genome of a host cell at a frequency less than that of a comparable population of AAV viruses, e.g., at about a 50%, 60%, 70%, 75%, 80%, 85%, 90%, 95%, 100%, or more lower frequency than the comparable population of AAV viruses.

In an aspect, the invention includes an anellovector comprising: (i) a genetic element comprising a promoter element and a sequence encoding an effector (e.g., an endogenous effector or an exogenous effector, e.g., a payload), and a protein binding sequence (e.g., an exterior protein binding sequence), wherein the genetic element has at least 75% (e.g., at least 75, 76, 77, 78, 79, 80, 90, 91, 92, 93, 94, 95,

96, 97, 98, 99, or 100%) sequence identity to a wild-type Anellovirus sequence (e.g., a wild-type Torque Teno virus (TTV), Torque Teno mini virus (TTMV), or TTMDV sequence, e.g., a wild-type Anellovirus sequence as listed in any one of Tables N1-N26); and (ii) a proteinaceous exterior; wherein the genetic element is enclosed within the proteinaceous exterior; and wherein the anellovector is capable of delivering the genetic element into a eukaryotic cell.

In one aspect, the invention includes an anellovector comprising:

a) a genetic element comprising (i) a sequence encoding an exterior protein (e.g., a non-pathogenic exterior protein), (ii) an exterior protein binding sequence that binds the genetic element to the non-pathogenic exterior protein, and (iii) a sequence encoding an effector (e.g., an endogenous or exogenous effector); and

b) a proteinaceous exterior that is associated with, e.g., envelops or encloses, the genetic element.

In some embodiments, the anellovector includes sequences or expression products from (or having >70%, 75%, 80%, 85%, 90%, 95%, 97%, 98%, 99%, 100% homology to) a non-enveloped, circular, single-stranded DNA virus. Animal circular single-stranded DNA viruses generally refer to a subgroup of single strand DNA (ssDNA) viruses, which infect eukaryotic non-plant hosts, and have a circular genome. Thus, animal circular ssDNA viruses are distinguishable from ssDNA viruses that infect prokaryotes (i.e. Microviridae and Inoviridae) and from ssDNA viruses that infect plants (i.e. Geminiviridae and Nanoviridae). They are also distinguishable from linear ssDNA viruses that infect non-plant eukaryotes (i.e. Parvoviridiae).

In some embodiments, the anellovector modulates a host cellular function, e.g., transiently or long term. In certain embodiments, the cellular function is stably altered, such as a modulation that persists for at least about 1 hr to about 30 days, or at least about 2 hrs, 6 hrs, 12 hrs, 18 hrs, 24 hrs, 2 days, 3, days, 4 days, 5 days, 6 days, 7 days, 8 days, 9 days, 10 days, 11 days, 12 days, 13 days, 14 days, 15 days, 16 days, 17 days, 18 days, 19 days, 20 days, 21 days, 22 days, 23 days, 24 days, 25 days, 26 days, 27 days, 28 days, 29 days, 30 days, 60 days, or longer or any time therebetween. In certain embodiments, the cellular function is transiently altered, e.g., such as a modulation that persists for no more than about 30 mins to about 7 days, or no more than about 1 hr, 2 hrs, 3 hrs, 4 hrs, 5 hrs, 6 hrs, 7 hrs, 8 hrs, 9 hrs, 10 hrs, 11 hrs, 12 hrs, 13 hrs, 14 hrs, 15 hrs, 16 hrs, 17 hrs, 18 hrs, 19 hrs, 20 hrs, 21 hrs, 22 hrs, 24 hrs, 36 hrs, 48 hrs, 60 hrs, 72 hrs, 4 days, 5 days, 6 days, 7 days, or any time therebetween.

In some embodiments, the genetic element comprises a promoter element. In some embodiments, the promoter element is selected from an RNA polymerase II-dependent promoter, an RNA polymerase III-dependent promoter, a PGK promoter, a CMV promoter, an EF-1α promoter, an SV40 promoter, a CAGG promoter, or a UBC promoter, TTV viral promoters, Tissue specific, U6 (pollIII), minimal CMV promoter with upstream DNA binding sites for activator proteins (TetR-VP16, Gal4-VP16, dCas9-VP16,

etc). In some embodiments, the promoter element comprises a TATA box. In some embodiments, the promoter element is endogenous to a wild-type *Anellovirus*, e.g., as described herein.

In some embodiments, the genetic element comprises one or more of the following characteristics: single-stranded, circular, negative strand, and/or DNA. In some embodiments, the genetic element comprises an episome. In some embodiments, the portions of the genetic element excluding the effector have a combined size of about 2.5-5 kb (e.g., about 2.8-4kb, about 2.8-3.2kb, about 3.6-3.9kb, or about 2.8-2.9kb), less than about 5kb (e.g., less than about 2.9kb, 3.2 kb, 3.6kb, 3.9kb, or 4kb), or at least 100 nucleotides (e.g., at least 1kb).

The anellovectors, compositions comprising anellovectors, methods using such anellovectors, etc., as described herein are, in some instances, based in part on the examples which illustrate how different effectors, for example miRNAs (e.g. against IFN or miR-625), shRNA, etc and protein binding sequences, for example DNA sequences that bind to capsid protein such as Q99153, are combined with proteinaceious exteriors, for example a capsid disclosed in Arch Virol (2007) 152: 1961-1975, to produce anellovectors which can then be used to deliver an effector to cells (e.g., animal cells, e.g., human cells or non-human animal cells such as pig or mouse cells). In embodiments, the effector can silence expression of a factor such as an interferon. The examples further describe how anellovectors can be made by inserting effectors into sequences derived, e.g., from an *Anellovirus*. It is on the basis of these examples that the description hereinafter contemplates various variations of the specific findings and combinations considered in the examples. For example, the skilled person will understand from the examples that the specific miRNAs are used just as an example of an effector and that other effectors may be, e.g., other regulatory nucleic acids or therapeutic peptides. Similarly, the specific capsids used in the examples may be replaced by substantially non-pathogenic proteins described hereinafter. The specifc *Anellovirus* sequences described in the examples may also be replaced by the *Anellovirus* sequences described hereinafter. These considerations similarly apply to protein binding sequences, regulatory sequences such as promoters, and the like. Independent thereof, the person skilled in the art will in particular consider such embodiments which are closely related to the examples.

In some embodiments, an anellovector, or the genetic element comprised in the anellovector, is introduced into a cell (e.g., a human cell). In some embodiments, the effector (e.g., an RNA, e.g., an miRNA), e.g., encoded by the genetic element of an anellovector, is expressed in a cell (e.g., a human cell), e.g., once the anellovector or the genetic element has been introduced into the cell. In some embodiments, introduction of the anellovector, or genetic element comprised therein, into a cell modulates (e.g., increases or decreases) the level of a target molecule (e.g., a target nucleic acid, e.g., RNA, or a target polypeptide) in the cell, e.g., by altering the expression level of the target molecule by the cell. In some embodiments, introduction of the anellovector, or genetic element comprised therein,

decreases level of interferon produced by the cell. In some embodiments, introduction of the anellovector, or genetic element comprised therein, into a cell modulates (e.g., increases or decreases) a function of the cell. In some embodiments, introduction of the anellovector, or genetic element comprised therein, into a cell modulates (e.g., increases or decreases) the viability of the cell. In some embodiments, introduction of the anellovector, or genetic element comprised therein, into a cell decreases viability of a cell (e.g., a cancer cell).

In some embodiments, an anellovector (e.g., a synthetic anellovector) described herein induces an antibody prevalence of less than 70% (e.g., less than about 60%, 50%, 40%, 30%, 20%, or 10% antibody prevalence). In some embodiments, antibody prevalence is determined according to methods known in the art. In some embodiments, antibody prevalence is determined by detecting antibodies against an *Anellovirus* (e.g., as described herein), or an anellovector based thereon, in a biological sample, e.g., according to the anti-TTV antibody detection method described in Tsuda et al. (1999; *J. Virol. Methods* 77: 199-206; incorporated herein by reference) and/or the method for determining anti-TTV IgG seroprevalence described in Kakkola et al. (2008; *Virology* 382: 182-189; incorporated herein by reference). Antibodies against an *Anellovirus* or an anellovector based thereon can also be detected by methods in the art for detecting anti-viral antibodies, e.g., methods of detecting anti-AAV antibodies, e.g., as described in Calcedo et al. (2013; *Front. Immunol.* 4(341): 1-7; incorporated herein by reference).

In some embodiments, a replication deficient, replication defective, or replication incompetent genetic element does not encode all of the necessary machinery or components required for replication of the genetic element. In some embodiments, a replication defective genetic element does not encode a replication factor. In some embodiments, a replication defective genetic element does not encode one or more ORFs (e.g., ORF1, ORF1/1, ORF1/2, ORF2, ORF2/2, ORF2/3, and/or ORF2t/3, e.g., as described herein). In some embodiments, the machinery or components not encoded by the genetic element may be provided in *trans* (e.g., using a helper, e.g., a helper virus or helper plasmid, or encoded in a nucleic acid comprised by the host cell, e.g., integrated into the genome of the host cell), e.g., such that the genetic element can undergo replication in the presence of the machinery or components provided in *trans*.

In some embodiments, a packaging deficient, packaging defective, or packaging incompetent genetic element cannot be packaged into a proteinaceous exterior (e.g., wherein the proteinaceous exterior comprises a capsid or a portion thereof, e.g., comprising a polypeptide encoded by an ORF1 nucleic acid, e.g., as described herein). In some embodiments, a packaging deficient genetic element is packaged into a proteinaceous exterior at an efficiency less than 10% (e.g., less than 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, 1%, 0.5%, 0.1%, 0.01%, or 0.001%) compared to a wild-type *Anellovirus* (e.g., as described herein). In some embodiments, the packaging defective genetic element cannot be packaged into a proteinaceous exterior even in the presence of factors (e.g., ORF1, ORF1/1, ORF1/2, ORF2, ORF2/2,

ORF2/3, or ORF2t/3) that would permit packaging of the genetic element of a wild-type *Anellovirus* (e.g., as described herein). In some embodiments, a packaging deficient genetic element is packaged into a proteinaceous exterior at an efficiency less than 10% (e.g., less than 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, 1%, 0.5%, 0.1%, 0.01%, or 0.001%) compared to a wild-type *Anellovirus* (e.g., as described herein), even in the presence of factors (e.g., ORF1, ORF1/1, ORF1/2, ORF2, ORF2/2, ORF2/3, or ORF2t/3) that would permit packaging of the genetic element of a wild-type *Anellovirus* (e.g., as described herein).

In some embodiments, a packaging competent genetic element can be packaged into a proteinaceous exterior (e.g., wherein the proteinaceous exterior comprises a capsid or a portion thereof, e.g., comprising a polypeptide encoded by an ORF1 nucleic acid, e.g., as described herein). In some embodiments, a packaging competent genetic element is packaged into a proteinaceous exterior at an efficiency of at least 20% (e.g., at least 20%, 30%, 40%, 50%, 60%, 70%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, 100%, or higher) compared to a wild-type *Anellovirus* (e.g., as described herein). In some embodiments, the packaging competent genetic element can be packaged into a proteinaceous exterior in the presence of factors (e.g., ORF1, ORF1/1, ORF1/2, ORF2, ORF2/2, ORF2/3, or ORF2t/3) that would permit packaging of the genetic element of a wild-type *Anellovirus* (e.g., as described herein). In some embodiments, a packaging competent genetic element is packaged into a proteinaceous exterior at an efficiency of at least 20% (e.g., at least 20%, 30%, 40%, 50%, 60%, 70%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, 100%, or higher) compared to a wild-type *Anellovirus* (e.g., as described herein) in the presence of factors (e.g., ORF1, ORF1/1, ORF1/2, ORF2, ORF2/2, ORF2/3, or ORF2t/3) that would permit packaging of the genetic element of a wild-type *Anellovirus* (e.g., as described herein).

AnelloVLPs

In some embodiments, the anelloVLP has a sequence, structure, and/or function that is based on an *Anellovirus* (e.g., an *Anellovirus* as described herein, e.g., an *Anellovirus* comprising a nucleic acid or polypeptide comprising a sequence as shown in any one of Tables A1-A26), or fragments or portions thereof, or other substantially non-pathogenic virus, e.g., a symbiotic virus, commensal virus, native virus. In some embodiments, an *Anellovirus*-based anelloVLP comprises at least one element exogenous to that *Anellovirus*, e.g., an exogenous effector or a nucleic acid sequence encoding an exogenous effector. In some embodiments, the anelloVLP comprises a surface moiety comprising the exogenous effector. In some embodiments, an *Anellovirus*-based anelloVLP comprises at least one element heterologous to another element from that *Anellovirus*, e.g., an effector-encoding nucleic acid sequence that is heterologous to another linked nucleic acid sequence, such as a promoter element. An anelloVLP may be a delivery vehicle (e.g., a substantially non-pathogenic delivery vehicle) for a payload into a host, e.g., a human. In some embodiments, the anelloVLP is not capable of replicating in a eukaryotic cell,

e.g., a mammalian cell, e.g., a human cell. In some embodiments, the anelloVLP is substantially non-pathogenic and/or substantially non-integrating in the mammalian (e.g., human) cell. In some embodiments, the anelloVLP is substantially non-immunogenic in a mammal, e.g., a human.

In an aspect, the invention includes an anelloVLP comprising a proteinaceous exterior and an effector (e.g., an exogenous effector); wherein the anelloVLP is capable of delivering the exogenous effector into a eukaryotic cell. In some embodiments, the exogenous effector is enclosed within the proteinaceous exterior. In some embodiments, the exogenous effector is comprised in a surface moiety on the surface of the anelloVLP (e.g., as described herein). In some embodiments, the proteinaceous exterior comprises one or more ORF1 molecules (e.g., an Anellovirus ORF1 protein, e.g., as described herein, or a polypeptide having at least 50%, 60%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity thereto).

In some embodiments, the anelloVLP includes sequences or expression products from (or having >70%, 75%, 80%, 85%, 90%, 95%, 97%, 98%, 99%, 100% homology to) a non-enveloped, circular, single-stranded DNA virus. Animal circular single-stranded DNA viruses generally refer to a subgroup of single strand DNA (ssDNA) viruses, which infect eukaryotic non-plant hosts, and have a circular genome. Thus, animal circular ssDNA viruses are distinguishable from ssDNA viruses that infect prokaryotes (i.e. Microviridae and Inoviridae) and from ssDNA viruses that infect plants (i.e. Geminiviridae and Nanoviridae). They are also distinguishable from linear ssDNA viruses that infect non-plant eukaryotes (i.e. Parvoviridiae).

In some embodiments, the anelloVLP modulates a host cellular function, e.g., transiently or long term. In certain embodiments, the cellular function is stably altered, such as a modulation that persists for at least about 1 hr to about 30 days, or at least about 2 hrs, 6 hrs, 12 hrs, 18 hrs, 24 hrs, 2 days, 3, days, 4 days, 5 days, 6 days, 7 days, 8 days, 9 days, 10 days, 11 days, 12 days, 13 days, 14 days, 15 days, 16 days, 17 days, 18 days, 19 days, 20 days, 21 days, 22 days, 23 days, 24 days, 25 days, 26 days, 27 days, 28 days, 29 days, 30 days, 60 days, or longer or any time therebetween. In certain embodiments, the cellular function is transiently altered, e.g., such as a modulation that persists for no more than about 30 mins to about 7 days, or no more than about 1 hr, 2 hrs, 3 hrs, 4 hrs, 5 hrs, 6 hrs, 7 hrs, 8 hrs, 9 hrs, 10 hrs, 11 hrs, 12 hrs, 13 hrs, 14 hrs, 15 hrs, 16 hrs, 17 hrs, 18 hrs, 19 hrs, 20 hrs, 21 hrs, 22 hrs, 24 hrs, 36 hrs, 48 hrs, 60 hrs, 72 hrs, 4 days, 5 days, 6 days, 7 days, or any time therebetween.

The anelloVLPs, compositions comprising anelloVLPs, methods using such anelloVLPs, etc., as described herein are, in some instances, based in part on the examples which illustrate how different effectors, for example miRNAs (e.g. against IFN or miR-625), shRNA, etc and protein binding sequences, for example DNA sequences that bind to capsid protein such as Q99153, are combined with proteinaceious exteriors, for example a capsid disclosed in Arch Virol (2007) 152: 1961-1975, to produce

anelloVLPs which can then be used to deliver an effector to cells (e.g., animal cells, e.g., human cells or non-human animal cells such as pig or mouse cells). In embodiments, the effector can silence expression of a factor such as an interferon. The examples further describe how anelloVLPs can be made by inserting effectors into sequences derived, e.g., from an *Anellovirus*. It is on the basis of these examples that the description hereinafter contemplates various variations of the specific findings and combinations considered in the examples. For example, the skilled person will understand from the examples that the specific miRNAs are used just as an example of an effector and that other effectors may be, e.g., other regulatory nucleic acids or therapeutic peptides. Similarly, the specific capsids used in the examples may be replaced by substantially non-pathogenic proteins described hereinafter. The specifc *Anellovirus* sequences described in the examples may also be replaced by the *Anellovirus* sequences described hereinafter. These considerations similarly apply to protein binding sequences, regulatory sequences such as promoters, and the like. Independent thereof, the person skilled in the art will in particular consider such embodiments which are closely related to the examples.

In some embodiments, an anelloVLP is introduced into a cell (e.g., a human cell). In some embodiments, the exogenous effector is delivered to the cell. In some embodiments, delivery of the exogenous effector to a cell modulates (e.g., increases or decreases) the level of a target molecule (e.g., a target nucleic acid, e.g., RNA, or a target polypeptide) in the cell, e.g., by altering the expression level of the target molecule by the cell. In some embodiments, delivery of the exogenous effector to a cell modulates (e.g., increases or decreases) a function of the cell. In some embodiments, delivery of the exogenous effector to a cell modulates (e.g., increases or decreases) the viability of the cell. In some embodiments, delivery of the exogenous effector to a cell decreases viability of a cell (e.g., a cancer cell).

In some embodiments, an anelloVLP (e.g., a synthetic anelloVLP) described herein induces an antibody prevalence of less than 70% (e.g., less than about 60%, 50%, 40%, 30%, 20%, or 10% antibody prevalence). In some embodiments, antibody prevalence is determined according to methods known in the art. In some embodiments, antibody prevalence is determined by detecting antibodies against an *Anellovirus* (e.g., as described herein), or an anelloVLP based thereon, in a biological sample, e.g., according to the anti-TTV antibody detection method described in Tsuda et al. (1999; *J. Virol. Methods* 77: 199-206; incorporated herein by reference) and/or the method for determining anti-TTV IgG seroprevalence described in Kakkola et al. (2008; *Virology* 382: 182-189; incorporated herein by reference). Antibodies against an *Anellovirus* or an anelloVLP based thereon can also be detected by methods in the art for detecting anti-viral antibodies, e.g., methods of detecting anti-AAV antibodies, e.g., as described in Calcedo et al. (2013; *Front. Immunol.* 4(341): 1-7; incorporated herein by reference).

Anelloviruses

In some embodiments, an anellovector or anelloVLP, e.g., as described herein, comprises sequences or expression products derived from an *Anellovirus*. In some embodiments, an anellovector or anelloVLP includes one or more sequences or expression products that are exogenous relative to the *Anellovirus*. In some embodiments, an anellovector or anelloVLP includes one or more sequences or expression products that are endogenous relative to the *Anellovirus*. In some embodiments, an anellovector or anelloVLP includes one or more sequences or expression products that are heterologous relative to one or more other sequences or expression products in the anellovector. *Anelloviruses* generally have single-stranded circular DNA genomes with negative polarity. *Anelloviruses* have not generally been linked to any human disease. However, attempts to link *Anellovirus* infection with human disease are confounded by the high incidence of asymptomatic *Anellovirus* viremia in control cohort population(s), the remarkable genomic diversity within the anellovirus viral family, the historical inability to propagate the agent in vitro, and the lack of animal model(s) of *Anellovirus* disease (Yzebe et al., Panminerva Med. (2002) 44:167-177; Biagini, P., Vet. Microbiol. (2004) 98:95-101).

Anelloviruses are generally transmitted by oronasal or fecal-oral infection, mother-to-infant and/or in utero transmission (Gerner et al., Ped. Infect. Dis. J. (2000) 19:1074-1077). Infected persons can, in some instances, be characterized by a prolonged (months to years) *Anellovirus* viremia. Humans may be co-infected with more than one genogroup or strain (Saback, et al., Scad. J. Infect. Dis. (2001) 33:121-125). There is a suggestion that these genogroups can recombine within infected humans (Rey et al., Infect. (2003) 31:226-233). The double stranded isoform (replicative) intermediates have been found in several tissues, such as liver, peripheral blood mononuclear cells and bone marrow (Kikuchi et al., J. Med. Virol. (2000) 61:165-170; Okamoto et al., Biochem. Biophys. Res. Commun. (2002) 270:657-662; Rodriguez-Inigo et al., Am. J. Pathol. (2000) 156:1227-1234).

In some embodiments, an anellovector or anelloVLP as described herein comprises one or more polypeptides (e.g., ORF1 molecules) comprising an amino acid sequence having at least about 50%, 60%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to an *Anellovirus* sequence, e.g., as described herein, or a fragment thereof. In embodiments, the polypeptide comprises an amino acid sequence encoded by a nucleic acid sequence selected from a sequence as shown in any one of Tables N1-N26, or a sequence having at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity thereto. In embodiments, the polypeptide comprises a sequence as shown in any one of Tables A1-A26, or a sequence having at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity thereto.

In some embodiments, the genetic element comprises a nucleotide sequence encoding an amino acid sequence or a functional fragment thereof or a sequence having at least about 60%, 70% 80%, 85%,

90% 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to any one of the amino acid sequences described herein, e.g., an *Anellovirus* amino acid sequence.

In some embodiments, an anellovector as described herein comprises one or more nucleic acid molecules (e.g., a genetic element as described herein) comprising a sequence having at least about 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to an *Anellovirus* sequence, e.g., as described herein, or a fragment thereof. In embodiments, the anellovector comprises a nucleic acid sequence selected from a sequence as shown in any one of Tables N1-N26, or a sequence having at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity thereto. In embodiments, the anellovector comprises a polypeptide comprising a sequence as shown in any one of Tables A1-A26, or a sequence having at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity thereto.

In some embodiments, an anellovector as described herein comprises one or more nucleic acid molecules (e.g., a genetic element as described herein) comprising a sequence having at least about 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to one or more of a TATA box, cap site, initiator element, transcriptional start site, 5' UTR conserved domain, ORF1, ORF1/1, ORF1/2, ORF2, ORF2/2, ORF2/3, ORF2t/3, three open-reading frame region, poly(A) signal, GC-rich region, or any combination thereof, of any of the *Anelloviruses* described herein (e.g., an *Anellovirus* sequence as annotated, or as encoded by a sequence listed, in any one of Tables N1-N26. In some embodiments, the nucleic acid molecule comprises a sequence encoding a capsid protein, e.g., an ORF1, ORF1/1, ORF1/2, ORF2, ORF2/2, ORF2/3, ORF2t/3 sequence of any of the *Anelloviruses* described herein (e.g., an *Anellovirus* sequence as annotated, or as encoded by a sequence listed, in any one of Tables N1-N26). In some embodiments, the nucleic acid molecule comprises a sequence encoding a capsid protein comprising an amino acid sequence having at least about 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to an *Anellovirus* ORF1 or ORF2 protein (e.g., an ORF1 or ORF2 amino acid sequence as shown in any one of Tables A1-A26, or an ORF1 or ORF2 amino acid sequence encoded by a nucleic acid sequence as shown in any one of Tables N1-N26). In embodiments, the nucleic acid molecule comprises a sequence encoding a capsid protein comprising an amino acid sequence having at least about 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to an *Anellovirus* ORF1 protein (e.g., an ORF1 amino acid sequence as shown in any one of Tables A1-A26, or an ORF1 amino acid sequence encoded by a nucleic acid sequence as shown in any one of Tables N1-N26).

Nucleic acid sequences

In some embodiments, the nucleic acid molecule comprises a nucleic acid sequence having at least about 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to the *Anellovirus* ORF1 nucleotide sequence of any one of Tables N1-N26. In some embodiments, the nucleic acid molecule comprises a nucleic acid sequence having at least about 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to the *Anellovirus* ORF2 nucleotide sequence of any one of Tables N1-N26. In some embodiments, the nucleic acid molecule comprises a nucleic acid sequence having at least about 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to the *Anellovirus* ORF3 nucleotide sequence of any one of Tables N1-N26. In some embodiments, the nucleic acid molecule comprises a nucleic acid sequence having at least about 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to the *Anellovirus* GC-rich region nucleotide sequence of any one of Tables N1-N26. In some embodiments, the nucleic acid molecule comprises a nucleic acid sequence having at least about 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to the *Anellovirus* 5' UTR conserved domain nucleotide sequence of any one of Tables N1-N26.

Amino acid sequences encoded by nucleic acid sequences

In embodiments, the nucleic acid molecule comprises a nucleic acid sequence encoding an amino acid sequence having at least about 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to the *Anellovirus* ORF1 amino acid sequence of any one of Tables A1-A26. In embodiments, the nucleic acid molecule comprises a nucleic acid sequence encoding an amino acid sequence having at least about 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to the *Anellovirus* ORF2 amino acid sequence of any one of Tables A1-A26. In embodiments, the nucleic acid molecule comprises a nucleic acid sequence encoding an amino acid sequence having at least about 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to the *Anellovirus* ORF3 amino acid sequence of any one of Tables A1-A26.

Proteins comprising amino acid sequences

In embodiments, the anellovector described herein comprises a protein having an amino acid sequence having at least about 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to the *Anellovirus* ORF1 amino acid sequence of any one of Tables A1-A26. In embodiments, the anellovector described herein comprises a protein having an amino acid sequence having at least about 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to the *Anellovirus* ORF2 amino acid sequence of any one of Tables A1-A26. In embodiments, the anellovector described herein comprises a protein having an amino acid sequence having at least about

70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to the *Anellovirus* ORF3 amino acid sequence of any one of Tables A1-A26. In some embodiments, the ORF1 molecule (e.g., comprised in the anellovector) comprises an Anellovirus ORF1 protein of any one of Tables A1-A26 or a splice variant or post-translationally processed (e.g., proteolytically processed) variant thereof. In some embodiments, the ORF2 molecule (e.g., comprised in the anellovector) comprises an Anellovirus ORF2 protein of any one of Tables A1-A26 or a splice variant or post-translationally processed (e.g., proteolytically processed) variant thereof. In some embodiments, the ORF3 molecule (e.g., comprised in the anellovector) comprises an Anellovirus ORF3 protein of any one of Tables A1-A26 or a splice variant or post-translationally processed (e.g., proteolytically processed) variant thereof.

Polypeptides comprising amino acid sequences

In some embodiments, the polypeptide described herein comprises an amino acid sequence having at least about 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to an *Anellovirus* ORF1 amino acid sequence described herein. In embodiments, the polypeptide described herein comprises an amino acid sequence having at least about 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to the *Anellovirus* ORF1 amino acid sequence of any one of Tables A1-A26.

In some embodiments, the polypeptide described herein comprises an amino acid sequence having at least about 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to an ORF1 molecule encoded by an *Anellovirus* ORF1 nucleic acid described herein. In some embodiments, the polypeptide described herein comprises an amino acid sequence having at least about 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to an ORF1 molecule encoded by an *Anellovirus* ORF1 nucleic acid as listed in any one of Tables A1-A26.

In some embodiments, the polypeptide described herein comprises an amino acid sequence having at least about 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to an *Anellovirus* ORF2 amino acid sequence described herein. In embodiments, the polypeptide described herein comprises an amino acid sequence having at least about 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to the *Anellovirus* ORF2 amino acid sequence of any one of Tables A1-A26.

In some embodiments, the polypeptide described herein comprises an amino acid sequence having at least about 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to an ORF2 molecule encoded by an *Anellovirus* ORF2 nucleic acid described herein. In some embodiments, the polypeptide described herein comprises an amino acid sequence having at least about

70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to an ORF2 molecule encoded by an *Anellovirus* ORF2 nucleic acid as listed in any one of Tables A1-A26.

In some embodiments, the polypeptide described herein comprises an amino acid sequence having at least about 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to an *Anellovirus* ORF3 amino acid sequence described herein. In embodiments, the polypeptide described herein comprises an amino acid sequence having at least about 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to the *Anellovirus* ORF3 amino acid sequence of any one of Tables A1-A26.

In some embodiments, the polypeptide described herein comprises an amino acid sequence having at least about 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to an ORF3 molecule encoded by an *Anellovirus* ORF3 nucleic acid described herein. In some embodiments, the polypeptide described herein comprises an amino acid sequence having at least about 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to an ORF3 molecule encoded by an *Anellovirus* ORF3 nucleic acid as listed in any one of Tables A1-A26.

In some embodiments, the polypeptide comprises an amino acid sequence (e.g., an ORF1, ORF1/1, ORF1/2, ORF2, ORF2/2, ORF2/3, or ORF2t/3 sequence) as shown in any one of Tables A1-A26, or a sequence having at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity thereto.

Table N1. Novel *Anellovirus* nucleic acid sequence (*Alphatorquevirus*)

Name	TTV-RTx1
Genus/Clade	*Alphatorquevirus*, Clade 6
Accession Number	SRR2167793

Full Sequence: 3648 bp

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1         10         20         30         40         50
|         |          |          |          |          |
CGTCACTAACCACGTGACTCCCACAGGCCAACCACAGTGTACGTGATTCA
CTTCCTGGGAGTGGTTTACATTATAATATAAGCAACTGCACTTCCGAATG
GCTGAGTTTTCCACGCCCGTCCGCAGCGAGAACACCACGGAGGGGAGTCC
GCGCGTCCCGTGGGCGGGTGCCGAAGGTGAGTTTACACACCGCAGTCAAG
GGGCAATTCGGGCACGGGACTGGCCGGGCTATGGGCAAGGCTCTTAAAAA
GCTATGTTTCTTGGTAGGCCGTACCGAAAGAAAAGGAAACTGCTACTGCT
ACCACTGCATTCTACACCGAAAACTAGCCGGGTTATGAGCTGGTCTAGGC
CTGTACATAATGCCACAGGCATTGAAAGAAACTGGTGGGAGTCCTGTCTT
AGATCCCACGCAAGTTCTTGTGGCTGCGGTAATTTTGTTAATCATATTAA
TGTACTGGCTAATCGGTATGGCTTTGCTGGTTCCACGGAGACGCCGGGTA
ATCCTCGGCCGAGGCCCCCGGTACTGAGCTCCACCACCAGCACTCCTACC
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GATCAATCCAGACCAGCTCTACCATGGCATGGGGATACTGGTGGAGAAGG
CGCTTCTGGAGACCCCGCAGGAGATGGAGAACGTGGCGCCGCAGAAGGAG
ACTACGGCCCAGAAGATCTAGACGCACTTTTCGACGCACTCGACGAAGAG
TAAGGAGGCGACGGTGGGGGAGGCGTGCACGCAGGCGGGGATGGCGACGC
AGGACTTATATTAGAGCCAGGCGACGCAGGAGACGAAAAAGACTTGTACT
GACTCAGTGGCATCCCGCAGTTAGAAGAAAATGTAAAATTACAGGCTACA
TGCCTATAGTATACTGTGGACATGGCAGAGCTAGTTTTAACTATGCCTGG
CACTCTGATGACTGTATAAAACAACCACTACCCTTTGGAGGCTCACTATC
TACAGTGTCCTTCAACCTAAAAGTACTATTTGACGAAAACCAAAGAGGAC
TAAACAAATGGAGCTACCCAAATGACCAACTAGACCTCGCCAGATACAAA
GGCTGTAGACTAACATTTTACAGAAAAAAAAACACAGACTACATAGCTCA
ATATGACATATCAGAACCTTATCAACTAGACAAATATAGCTGTGCAAACT
ATCACCCCTCAAAAATGATGTTTGCAAAAAACAAAATTTTAATTCCTAGC
TATGATACAAAACCTAGAGGCAGACAAAGAGTTAGAGTTAGAATAGGGCC
CCCTAAACTATTTACAGACAAGTGGTACAGTCAATCAGACTTATGCAAGG
TAAACCTTGTGTCACTTGCGGTTTCTGCGGCTTCCTTTCTCCACCCATTC
GGCTCACCACAAACTGCCAACTTTTGTGCAACCTTCCAGGTGCTGCAACC
GTTCTACTACCAGGCTATAGGCATTAGTTCTACAAAACACTCAGAAGTTA
TAGACATTTTATATAAGAAAAATACATACTGGCAAAGCAACATTACCTCT
TGGTTTTTAACTAATGTTAAAAACCCAAAAAATATGTCCACAAAAATGTT
TGAGGACATTAATGTTAAATCAAACAAAGACAGTAATTATGACTGGTTTC
CATTTACCCCATACACTACAGAAAACTATTCAAAAATTCAAAATGCAGCT
CAAGAATACTGGAAATATTTAACTAGTGACCACCCACAAGCTACTAATAG
CAATGAAGGCCTAGTACAACCATGGACTAATGCCACTATAAAACAATATG
AATACCACCTCGGTATGTTTAGTCCTATATTTATAGGACCTACCAGAGCT
AAAACTAAATTTAAAACAGCATACTTTGACTGCACTTATAACCCACTACT
AGACAAAGGAATGGGAAACAGAATATGGTATCAATACGCAACCAAAGCTG
ACACACAAATATCAAAAACAGGGTGCTACTGCATGTTAGAAGACATTCCA
ATATATGCAGCATTTTATGGATACGTAGACTTTATAGAAATGGAAATAGG
TAAAGGACAAGACATTAAAGAGAACGGACTTATTTGCTGCATATGTAGAT
ACACAGACCCCCCAATGTACAATGAACAACATCCAGACATGGGATTTGTA
TTTTATAACACTAACTTTGGAAATGGAAAATGGATAGATGGACGGGGCGA
CATACCTACTTACTGGATGCAAAGATGGAGACCTGTTGTATTATTTCAAA
CTGATGTTATTAGAGACTTAGTAGAAACTGGACCTTTTAGTTACAAAGAT
GACCTAGCAAATACCTCACTGACTATGAAATATGAATTCTATTTTACCTG
GGGCGGAAACCAGGCGTACCACCAGACAATCAAAAACCCTTGTAAAGACG
AAGGTACCGGACCCCATAGACAGCCTAGAGACGTACAAGTTACGGACCCG
ACAACCGTGGGACCTGAATATGTGTTCCACGCGTGGGACTGGAGACGGGG
CTTCCTTAGCGAGCGAGCTCTCAGACGCATGTTCGAAAAACCTCTCAACT
ATGATGAGTATTCTAAAAAACCAAAAAGACCTAGAATATTTCCTCCAACA
GAAACAGAGTCCCGAAACCAAGAGCTCGAAGAAAGCTCGCTTTCAGAGGA
AGAAAAGTCGCTACTCTCCACAGAAGAGATCCAGAAAGAGGAGATACAGC
GACAGTTCAAGCGACAGCTCAAGCGACAGCTGCGCCTCGGGCAGCAGCTC
AAACTCCTCCAACAACAACTCCTCAAGACGCAAGCGGGCCTGCACCTAAA
CCCCCTTTCATATTTCCCGCAATAAATAAAGTGTACCTGTTCCCAGACAG
AGCTCCAAAACCTAAACCCACCTCTGGAGACTGGGAAACAGAGTATGCAG
CTTGCAGTGCCTTTGACAGACCCGCTAGAACCAACCTTAGCTCACCCCCT
TACTACCCAGGAGTACCTACTCCCTGGCAAGTAAAATTCAGCCTTAAATT
TCAATAAAGTGCATTTTTACTACAGCTGGGCCGTGGGAGTTTCACTTGTC
GGTGTCTACCTCTTAAGGTCACTAAGCACTCCGAGCGCAGCGAGGAGTGC
GACCCTTAACCCTGGGTCAACGCCTTCGGAGCCGCGCGCTACGCCTTCGG
CTGCGCGCGGCACCTCAGACCCCCGCTCGTGCTGACGCGCTTGCGCGCGT
CAGACCACTTCGGGCTCGCGGGGGTCGGGAACTTTGCTAACAGACTCCGA
GGTGCCATTGGACACAGAGTGGGCGTTCAGCAACGAAAGTGAGTGGGGCC
AGACTTCGCCATAAGGCCTTTATCTTCTTGCCATTTGTCAGTATAAGGGG
TTGCCATAGGCTTCGGCCTCAATTTTAGGCCTTCCGGACTACCAAAATGG
CCGATTTAGTGACGTCACGGCGGCCATTTTAAGTAAGGCGGAAGTAACTC

CACTATTTACAAAATGGCGGCGGAGCACTTCCGGCTTGCCCAAAATGGCG
GCAAAAAACATCCGGGTCAAAGGTCGTTACCACGTCACAAGTCACGTGGG
AGGGTGGTGCTGTAAACCCGGAAGCAATCCTCTCACGTGGCTAGTCACGT
GACTAACACGTCACACCCGCCATTTTGTTTTACAAAATGGCCGACTTCCT
TCCGCTTTTTTAAAAATAACGGCTCAGCGGCGGCGCGCGCGCTACGCG
(SEQ ID NO: 830)

Annotations:

Putative Domain	*Base range*
TATA Box	77 – 81
Initiator Element	95-110
Transcriptional Start Site	105
5' UTR Conserved Domain	165 - 235
ORF2	335 - 703
ORF2/2	335 – 699 ; 2326 – 2759
ORF2/3	335 – 699 ; 2552 – 2957
ORF2t/3	335-465 ; 2552 - 2957
ORF1	574 – 2775
ORF1/1	574 – 699 ; 2326 – 2775
ORF1/2	574 – 699 ; 2552 – 2759
Three open-reading frame region	2535 – 2746
Poly(A) Signal	2953 - 2958
GC-rich region**	3620 – 3648

Table A1. Novel *Anellovirus* amino acid sequences (*Alphatorquevirus*, Clade 6)

TTV-RTx1 (*Alphatorquevirus* Clade 6)	
ORF2	MSWSRPVHNATGIERNWWESCLRSHASSCGCGNFVNHINVLANRYGFAGS TETPGNPRPRPPVLSSTTSTPTDQSRPALPWHGDTGGEGASGDPAGDGERGA AEGDYGPEDLDALFDALDEE (SEQ ID NO: 831)
ORF2/2	MSWSRPVHNATGIERNWWESCLRSHASSCGCGNFVNHINVLANRYGFAGS TETPGNPRPRPPVLSSTTSTPTDQSRPALPWHGDTGGEGASGDPAGDGERGA AEGDYGPEDLDALFDALDEEQSKTLVKTKVPDPIDSLETYKLRTRQPWDLN MCSTRGTGDGASLASELSDACSKNLSTMMSILKNQKDLEYFLQQKQSPETK

	SSKKARFQRKKSRYSPQKRSRKRRYSDSSSDSSSDSCASGSSSNSSNNNSSRR KRACT (SEQ ID NO: 832)
ORF2/3	MSWSRPVHNATGIERNWWESCLRSHASSCGCGNFVNHINVLANRYGFAGS TETPGNPRPRPPVLSSTTSTPTDQSRPALPWHGDTGGEGASGDPAGDGERGA AEGDYGPEDLDALFDALDEENRVPKPRARRKLAFRGRKVATLHRRDPERG DTATVQATAQATAAPRAAAQTPPTTTPQDASGPAPKPPFIFPAINKVYLFPD RAPKPKPTSGDWETEYAACSAFDRPARTNLSSPPYYPGVPTPWQVKFSLKF Q (SEQ ID NO: 833)
ORF2t/3	MSWSRPVHNATGIERNWWESCLRSHASSCGCGNFVNHINVLANRNRVPKP RARRKLAFRGRKVATLHRRDPERGDTATVQATAQATAAPRAAAQTPPTTTP QDASGPAPKPPFIFPAINKVYLFPDRAPKPKPTSGDWETEYAACSAFDRPART NLSSPPYYPGVPTPWQVKFSLKFQ (SEQ ID NO: 834)
ORF1	MAWGYWWRRRFWRPRRRWRTWRRRRRLRPRRSRRTFRRTRRRVRRRRW GRRARRRGWRRRTYIRARRRRRRKRLVLTQWHPAVRRKCKITGYMPIVYC GHGRASFNYAWHSDDCIKQPLPFGGSLSTVSFNLKVLFDENQRGLNKWSYP NDQLDLARYKGCRLTFYRKKNTDYIAQYDISEPYQLDKYSCANYHPSKMM FAKNKILIPSYDTKPRGRQRVRVRIGPPKLFTDKWYSQSDLCKVNLVSLAVS AASFLHPFGSPQTANFCATFQVLQPFYYQAIGISSTKHSEVIDILYKKNTYWQ SNITSWFLTNVKNPKNMSTKMFEDINVKSNKDSNYDWFPFTPYTTENYSKIQ NAAQEYWKYLTSDHPQATNSNEGLVQPWTNATIKQYEYHLGMFSPIFIGPT RAKTKFKTAYFDCTYNPLLDKGMGNRIWYQYATKADTQISKTGCYCMLED IPIYAAFYGYVDFIEMEIGKGQDIKENGLICCICRYTDPPMYNEQHPDMGFVF YNTNFGNGKWIDGRGDIPTYWMQRWRPVVLFQTDVIRDLVETGPFSYKDD LANTSLTMKYEFYFTWGGNQAYHQTIKNPCKDEGTGPHRQPRDVQVTDPT TVGPEYVFHAWDWRRGFLSERALRRMFEKPLNYDEYSKKPKRPRIFPPTET ESRNQELEESSLSEEEKSLLSTEEIQKEEIQRQFKRQLKRQLRLGQQLKLLQQ QLLKTQAGLHLNPLSYFPQ (SEQ ID NO:835)
ORF1/1	MAWGYWWRRRFWRPRRRWRTWRRRRRLRPRRSRRTFRRTRRRTIKNPCK DEGTGPHRQPRDVQVTDPTTVGPEYVFHAWDWRRGFLSERALRRMFEKPL NYDEYSKKPKRPRIFPPTETESRNQELEESSLSEEEKSLLSTEEIQKEEIQRQFK RQLKRQLRLGQQLKLLQQQLLKTQAGLHLNPLSYFPQ (SEQ ID NO: 836)

ORF1/2	MAWGYWWRRRFWRPRRRWRTWRRRRRLRPRRSRRTFRRTRRRKQSPETK SSKKARFQRKKSRYSPQKRSRKRRYSDSSSDSSSDSCASGSSSNSSNNNSSRR KRACT (SEQ ID NO: 837)

Table N2. Novel *Anellovirus* nucleic acid sequence (*Alphatorquevirus*)

Name	TTV-RTx2
Genus/Clade	*Alphatorquevirus*, Clade 6
Accession Number	SRR3479021

Full Sequence: 3704 bp

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1         10         20         30         40         50
|         |          |          |          |          |
CCCCGAAGTCCGTCACTAACCACGTGACTCCCACAGGCCAATCAGATGCT
ATGTCGTGCACTTCCTGGGCTGTGTCTACGTCCTCATATAAGTAACTGCA
CTTCCGAATGGCTGAGTTTTCCACGCCCGTCCGCAGCGGCAGCACCACGG
AGGGTGATCCCCGCGTCCCGTGGGCGGGTGCCGGAGGTGAGTTTACACAC
CGCAGTCAAGGGGCAATTCGGGCACGGGACTGGCCGGGCTATGGGCAAGG
CTCTTAAAAAGCTATGTTCTTCGGTAGGTGCTGGAGAAAGAAAAGGAAAG
TGCTTCTGCAAGATCTGTCAACTCCACCGAAAAAACCTGCTATGAGTGTG
TGGCTTCCTCCCATAGACAATGTTACCGAGCGTGAGAGGAGCTGGCTCTC
TAGCATTCTTCAGTCTCACAGAGCTTTTTGTGGGTGCCATGATGCTATCT
ATCATCTTAGCAGTCTGGCTGCTCGCTTTAATATGCAACCAGGGCCGTCG
CCGGGTGGTGATTCTAGGCCGCCGCGACCGCCACTAAGACGCCTGCCCGC
GCTCCCGGGTCCCAGAGACCCCCCTAGCGACACCAACAACCGCAGGTCAT
GGCCTACTGGGGATGGTGGAGACGGAGGCGCTGGCCAAGGCGCAGGTGGA
GGCGCTACCGCTACCGAAGAAGACTACCGCGCCGAAGACCTAGACGAGCT
GTACGCCGCCCTCGAAGGAGACGAGTAAGGAGGCGCCGCGGTAGGGGGTG
GTACAGAGGGCGACGCTACTCCCGCAGACGGTACAGACGTAGATATGTGA
GGCGAAAGAGAAAGACTCTAGTTTGGAGACAGTGGCAGCCTCAAAATATC
AGAAAATGCAGGATCAGGGGCATAATTCCCATCCTGATATGCGGACACGG
GAGGGGGGCCAGAAACTATGCGCTCCACAGCGACGACATAACCCCCCAGA
ACACCCCCTTCGGGGGAGGACTGAGCACCACCTCCTGGAGCCTAAAAGTG
CTATATGACCAGCACACCAGGGGACTCAACAGGTGGTCTGCCAGTAACGA
GAGCCTAGACCTTGCCAGATACAATGGCTGTAGTTTCACTTTCTACAGAG
ACAAAAAGACTGACTTTATAGTGACCTATGACACCTCTGCTCCCTACAAA
CTAGACAAATACAGCTCCCCCAGCTACCACCCAGGGTCCATGATGCTCAT
GACAAAACACAAAATCCTGATCCCCAGTTTTGACACAAAACCCAAAGGTC
CTGCCAAAATTAGAGTCAGAATCAAGCCCCCCAAAATGTTCTTAGATAAA
TGGTACACTCAAGACGACCTCTGTTCCGTTAATCTTGTGTCACTTGCGGT
TAGCGCAGCTTCCTTTACACATCCGTTCTGCCCACCACTAACTGACACTC
CTTGTGTAACGCTGCAGGTGTTGAAAGACTTCTACTACACAACCATAGGC
TACTCCTCTAATGCAGACAAAGTAGAGTCTGTATTCACTAACACTCTCTA
CAAACACTGCTGCTACTATCAGTCCTTTCTCACCACTCAATTTATAGCCA
AAATCACTCGCACACCAGATGGACAACCAGTAGCCACATTCTCTCCTCCT
ACCTCTTTCCCTGGCACAACTGTAACAAAAAGTTCCATAGAATCATTTAA
CCAATGGGTAACTTCCACAGGTACAAGTGGCTGGCTAACAAATGCAAACC
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AACACTTTCATTTCTGTAACTATAAACCAGATGCCACAAAGCTAAAATGG
CTCAGACAGTACTACTTTGACTGGGAAACATACAAATTAGCAGATGTAAA
GCCAGACGGCCTTACACCCTCAGTAAACTGGTATGAGTACAGAATAGGCC
TCTTTAGTCCTATTTTCCTGAGCCCCTTCAGATCTAGCAGTCTAGACTTT
CCCAGAGCCTACCAGGATGTGAACTACAACCCCCTGGTAGACAAAGGAGT
GGGCAACATCATATGGTTCCAATACAACACAAAACCAGACACACAGCTGT
CAGTACCCAGCTGCAAGTGTGTCATAGAAGACAAACCCCTATGGGCAGCC
TTCTATGGCTACAGTGACTTTGTACAACAAGAGATAGGAGACTACACAGA
CGCAGAGGCCGTGGGCTTCGTCTGTGTCATCTGTCCATACACCAAACCCC
CTCTAAAAAACCCAGACAACCCCATGCAAGGGTTCATATTCTATGACAGC
CTTTTTGGCAATGGCAAGTGGATAGATGGCACGGGGCACGTCCCCCTTTA
CTGGCAGAGCAGGTGGAGGCCAGAGATGCTCTTCCAAGAAAACACCATGA
GAGACATCACACTATCTGGGCCCTTCAGCTACAAGGACGACTATAAGAAC
TGTGTACTGACTTGCAAATACAAATTTAACTTTCGATTCGGGGGCAATCT
TCTCCACGAACAGACGATCAGAAACCCATGCCCCACGGACGGACATCCCA
GTACCGGTAGACAGCCTAGAGACGTACAAGTGGTTGACCCGATCAAAGTG
GGCCCCCGGTTCGTGTTCCACTCCTGGGACTGGCGCAGAGGCTACCTTAG
CCCAGCAGCTCTCAAAAGAATTGGAGAGCAACCGCTCGATTATGAAGCTT
ATTCGTACCGCCCAAAGAGACCTAGAATCTTTCCTCCCACAGAAGGAGAC
CAGCTCGCCCGAAGTCGAGAAGAAGACTCATTTTCAGAGGAAGAAAGTCC
CCATATCTCGTTCGAAGAGGGGCAGGAACCGAAAGCCCAGGCGGTACAGC
AGCACCTCCTCCGACACCTCAGAAAGCAGCGAGAACTCCGAAAGCGACTC
CGAGCCCTGTTCCAAAGCCTCCAAAAGACGCAGGCGGGTCTCCACGTAAA
TCCATTATTATTCAACCAGCCTGCAATCAGGTTCTGATGTTCCCAGAGAT
GGGGCCTAAGCCAGCTCCCACTGCCCAAGACTGGCAGTGCGAATACGAGA
CATGTAAGCACTGGGATAGACCCCCCAGAAAGTTTCTCACAGACCCCCCT
TTCTATCCCTGGGCCCCTACTACTTACAATGTATCTTTCAAGCTAAACTT
CAAATAAACTAGGCCGTGGGAGTCTCACTTGTCGGTGTCTACCTCTTAAG
GTCACTAAGCACTCCGAGCGTCAGCGAGGAGTGCGACCCTTCCCCCTGGT
GCAACGCCCTCGGCGGCCGCGCGCTACGCCTTCGGCTGCGCCGGCACCT
CGGACCCCCGCTCGTGCTGACGCGCTCGCGCGCGTCAGACCACTTCGGGC
TCGCGGGGGTCGGGAAATTTGCTAAACAGACTCCGAGTTGCCATTGGACA
CAGGAGCTGTGAATCAGTAACGAAAGTGAGTGGGGCCAGACTTCGCCATA
AGGCCTTTATCTTCTTGCCATTTGTCCGTGAGGAGGGGTCGCCAAGACGC
GGACCCCGTTTTCGGACCTTCCGAACTACCAAAATGGCCGATTCAGTGAC
GTCACGGCAGCCATTTTGTGTAAGCACCGCCCAGGACAGACGTCACAGTT
CAAAGGTCATCCTCGAGCGGAACTTACAGAAAATGGCGGTCAATTGCTTC
CGGGTCAAAGGTCACGCCTACGTCATAAGTCACGTGGTGGAGGCTACTGC
GCATACACGGAAGTAGGCCCCGCCACGTGACCGACCACGTGGGTGCTGCG
TCACGGCCGCCATTTTGTATCACAAAATGGCCGACTTCCTTCCTCTTTTT
CAAA (SEQ ID NO: 838)

Annotations:

Putative Domain	*Base range*
TATA Box	87 – 91
Initiator Elemcnt	105-120
Transcriptional Start Site	115
5’ UTR Conserved Domain	175 - 245
ORF2	342 - 728

ORF2/2	342 – 724 ; 2414 – 2849
ORF2/3	342 – 724 ; 2643 – 3057
ORF1	599 – 2887
ORF1/1	599 – 724 ; 2414 – 2887
ORF1/2	599 – 724 ; 2643 – 2849
Three open-reading frame region	2626 – 2846
Poly(A) Signal	3052 - 3058

Table A2. Novel *Anellovirus* amino acid sequences (*Alphatorquevirus*, Clade 6)

TTV-RTx2 (*Alphatorquevirus* Clade 6)	
ORF2	MSVWLPPIDNVTERERSWLSSILQSHRAFCGCHDAIYHLSSLAARFNMQPGP SPGGDSRPPRPPLRRLPALPGPRDPPSDTNNRRSWPTGDGGDGGAGQGAGG GATATEEDYRAEDLDELYAALEGDE (SEQ ID NO: 839)
ORF2/2	MSVWLPPIDNVTERERSWLSSILQSHRAFCGCHDAIYHLSSLAARFNMQPGP SPGGDSRPPRPPLRRLPALPGPRDPPSDTNNRRSWPTGDGGDGGAGQGAGG GATATEEDYRAEDLDELYAALEGDERSETHAPRTDIPVPVDSLETYKWLTR SKWAPGSCSTPGTGAEATLAQQLSKELESNRSIMKLIRTAQRDLESFLPQKE TSSPEVEKKTHFQRKKVPISRSKRGRNRKPRRYSSTSSDTSESSENSESDSEPC SKASKRRRRVST (SEQ ID NO: 840)
ORF2/3	MSVWLPPIDNVTERERSWLSSILQSHRAFCGCHDAIYHLSSLAARFNMQPGP SPGGDSRPPRPPLRRLPALPGPRDPPSDTNNRRSWPTGDGGDGGAGQGAGG GATATEEDYRAEDLDELYAALEGDERRPARPKSRRRLIFRGRKSPYLVRRG AGTESPGGTAAPPPTPQKAARTPKATPSPVPKPPKDAGGSPRKSIIIQPACNQ VLMFPEMGPKPAPTAQDWQCEYETCKHWDRPPRKFLTDPPFYPWAPTTYN VSFKLNFK (SEQ ID NO: 841)
ORF1	MAYWGWWRRRRWPRRRWRRYRYRRRLPRRRPRRAVRRPRRRRVRRRRG RGWYRGRRYSRRRYRRRYVRRKRKTLVWRQWQPQNIRKCRIRGIIPILICGH GRGARNYALHSDDITPQNTPFGGGLSTTSWSLKVLYDQHTRGLNRWSASNE SLDLARYNGCSFTFYRDKKTDFIVTYDTSAPYKLDKYSSPSYHPGSMMLMT KHKILIPSFDTKPKGPAKIRVRIKPPKMFLDKWYTQDDLCSVNLVSLAVSAA SFTHPFCPPLTDTPCVTLQVLKDFYYTTIGYSSNADKVESVFTNTLYKHCCY

	YQSFLTTQFIAKITRTPDGQPVATFSPPTSFPGTTVTKSSIESFNQWVTSTGTS GWLTNANQHFHFCNYKPDATKLKWLRQYYFDWETYKLADVKPDGLTPSV NWYEYRIGLFSPIFLSPFRSSSLDFPRAYQDVNYNPLVDKGVGNIIWFQYNTK PDTQLSVPSCKCVIEDKPLWAAFYGYSDFVQQEIGDYTDAEAVGFVCVICPY TKPPLKNPDNPMQGFIFYDSLFGNGKWIDGTGHVPLYWQSRWRPEMLFQE NTMRDITLSGPFSYKDDYKNCVLTCKYKFNFRFGGNLLHEQTIRNPCPTDGH PSTGRQPRDVQVVDPIKVGPRFVFHSWDWRRGYLSPAALKRIGEQPLDYEA YSYRPKRPRIFPPTEGDQLARSREEDSFSEEESPHISFEEGQEPKAQAVQQHLL RHLRKQRELRKRLRALFQSLQKTQAGLHVNPLLFNQPAIRF (SEQ ID NO: 842)
ORF1/1	MAYWGWWRRRRWPRRRWRRYRYRRRLPRRRPRRAVRRPRRRRTIRNPCP TDGHPSTGRQPRDVQVVDPIKVGPRFVFHSWDWRRGYLSPAALKRIGEQPL DYEAYSYRPKRPRIFPPTEGDQLARSREEDSFSEEESPHISFEEGQEPKAQAV QQHLLRHLRKQRELRKRLRALFQSLQKTQAGLHVNPLLFNQPAIRF (SEQ ID NO: 843)
ORF1/2	MAYWGWWRRRRWPRRRWRRYRYRRRLPRRRPRRAVRRPRRRRKETSSPE VEKKTHFQRKKVPISRSKRGRNRKPRRYSSTSSDTSESSENSESDSEPCSKAS KRRRRVST (SEQ ID NO: 844)

Table N3. Novel *Anellovirus* nucleic acid sequence (*Alphatorquevirus*)

Name	TTV-RTx3
Genus/Clade	*Alphatorquevirus*, Clade 4
Accession Number	SRR3479781

Full Sequence: 3653 bp

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1         10         20         30         40         50
|         |          |          |          |          |
CCAACCAGAGTCTATGTCGTGCACTTCCTGGGCATGGTCTACGTAATAAT
ATAAAGCGGTGCACTTCCGAATGGCTGAGTTTTCCACGCCCGTCCGCAGC
GAGATCGCGACGGAGGAGCGATCGAGCGTCCCGAGGGCGGGTGCCGGAGG
TGAGTTTACACACCGCAGTCAAGGGGCAATTCGGGCTCGGGACTGGCCGG
GCTATGGGCAAGGCTCTTAAAAAGCCATGTTTCTCGGTAAACTTTACAGG
CAGAAAAGGAAACTGCTACTGCAGCCTGTGCGTGCTCCACAGACGCCATC
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TTCCATGAGCTCTACCTGGCGAGTGCCCCGCGGCGATGTCTCCGCCCGCG
AGCTATGTTGGTACCGCTCAGTTCGAGAGAGCCACGATGCTTTTTGTGGC
TGTCGTGATCCTGTTTTTCATCTTTCTCGTCTGGCTGCACGTTCTAACCA
TCAGGGACCTCCGACGCCCCCCACGGACGAGCGCCCGTCGGCGTCTACCC
CAGTGAGGCGCCTGCTGCCGCTGCCCTCCTACCCCGGCGAGGGTCCCCAG
GCTAGATGGCCTGGTGGGGATGGAGAAGGCGCTGGTGGCGCCCGCGGAGG
CGCTGGAGATGGCGGCGCCCGCGCAGGCGAAGAAGAGTACCGGCCCGAAG
ACCTCGACGAGCTGTTCGACGCTATCGAACAAGAACAGTAAGGAGACGGA
GGCGAGGGTGGCGGAGGGGCTACAGGCGCCGTTACAGACTGAGACGCTAC
CGTAGAAGGGGCAGGCGACGCAAAAAAATAGTACTGACTCAGTGGAACCC
CCAGACTGTCAGAAAGTGCTTTATCAGAGGACTGATGCCAGTACTATGGG
CGGGCATGGGCACGGGGGGCCACAACTACGCCGTCCGCTCAGATGACTTT
GTGGTAGACAGAGGCTTCGGGGGCTCCTTCGCCACAGAAACTTTCTCCCT
GAGGGTCCTCTTTGACCAGTACCAGAGAGGATTTAATAGGTGGTCTCACA
CCAACGAAGACCTAGACCTGGCCCGCTACACGGGCTGCAAATGGACATTT
TACAGACACCAAGACACAGACTTTATAGTGTACTTTACAAACAATCCCCC
CATGAAAACCAACCAGCACACAGCCCCTCTCACAACTCCAGGCATGCTCA
TGAGGAGCAAGTATAAAATACTAGTGCCCAGTTTTAAAACAAGACCAAAG
GGCAGAAAAACAGTGTCAGTGAGAGTTAGACCCCCCAAACTGTTTCAGGA
CAAATGGTATACTCAACAGGACCTCTGTCCAGTACCCCTCGTCCAACTGA
ACGTGACCGCAGCGGATTTCACACATCCGTTCGGCTCACCACTAACTGAC
ACGCCTTGCATAAGATTCCAAGTTTTAGGGAACTTATACAACAAGTGCCT
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CACCTTATAACAGAGAAAACAAAGAAGATCTTAAAAAGCTTTATAAAACT
CTATTTGTAGATGAACACGCAGGCAATTATTGGCAGACATTCTTAACCAA
CACAATGGTAAAGTCACACATAGATGCAAACCAAGCAAAGACATACGATC
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CCAAAAGACCAATTCACTACCTGGAACAAGAAACTAGTAGACCCTAGAGA
CAGCAACTTTCTCTTTGCCACATATCACCCAAAAAACATTAAAAAAGCTA
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ATATATGGAGACTACACCGCGGCCTACACCAGAAACACCCACATGCTAGA
CTACTACCTAGGCTTTTATAGCCCCATATTTCTTTCCAGCGGTAGGTCCA
ACACAGAGTTCTGGACCGCCTACAGAGACATAGTATATAATCCCCTCTTA
GACAAAGGCACAGGCAACATGATCTGGTTCCAATATCACACAAAAACAGA
CAATATATACAAAAAACCAGAGTGCCACTGGGAGATACTAGACATGCCCC
TGTGGGCCCTCTGCAACGGGTATGTAGAGTACCTAGAGAGCCAAATAAAG
TACGGGGACATCCTAGTAGAGGGCAAAGTCCTCATCAGATGCCCCTACAC
CAAACCCGCACTGGTAGACCCCAATAACAGCCTAGCTGGTTACGTGGTAT
TCAACACCACCTTCGGCCAGGGAAAATGGATAGATGGCAAAGGCTACATC
CCCCTACACGAGAGGAGCAAGTGGTACGTCATGCTCAGATACCAGACCGA
CGTACTCCATGACATAGTGACTTGTGGACCCTGGCAGTACAGAGACGATA
ACAAAAACTCTCAGCTAATAGCCAAGTACAGATTCAAGTTCTACTGGGGA
GGTAACATGGTACATTCTCAGGTCATCAGAAACCCGTGCAAAGACACCCA
AGTATCCGGACCCCGTCGACAGCCTCGCGAAGTACAAGTCGTTGACCCGC
AACTCATTACGCCGCCGTGGGTCCTCCACTCGTTCGACCAGAGACGAGGA
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AGTTCCAGCGAGAGGGAGAAGATGCAGAAAGCGGCTCAGGTTCACCACCC
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CTCGGAGGGGCCGGCGGAGACGACGAGGGAGCTCCTCGAGCGAAAGCTCA
GAGAGCAGCGAGTCCTCAACCTCCAACTCCAGCATGTCGCAGTACAACTC
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GTCCCCAATCCTGAAATAAAGGTCAGCTTTAAGCTCGGTTTCAAATCTTA
CAAGGCCGTGGGAGTTTCACTGGTCGGTGTCTACCTCTTAAGGTCACTAA

GCACTCCGAGCGTCAGCGAGGAGTGCGACCCTTCCCCCTGGTGCAACGCC
CTCGGCGGCCGCGCGCTACGCCTTCGGCTGCGCGCGGCACCTCGGACCCC
CGCTCGTGCTGACGCGCTCGCGCGCGTCAGACCACTTCGGGCTCGCGGGG
GTCGGGAATTTTGCTAAACAGACTCCGAGTTGCCATTGGACACTGTAGCT
GTGAATCAGTAACGAAAGTGAGTGGGGCCAGACTTCGCCATAAGGCCTTT
ATCTTCTTGCCATTGGTCCGTGTAGGGGGTCGCCATAGGCTTCGGGTTCG
GTTTTAGGCCTTCCGGACTACAAAAATGGCGGATTTAGTGACGTCACGGC
CGCCATTTTAAGTAGGTGCCGTCCAGGACTGCTGTTCCGGGTCACAGGGC
ATCCTCGGCGGAACTTACACAAAATGGCGGTCAAAAACATCCGGGTCAAA
GGTCGCAGCTACGTCATAAGTCACGTGCAGGGGTCCTGCTGCGTCATATG
CGG (SEQ ID NO: 845)

Annotations:

Putative Domain	*Base range*
TATA Box	50 – 55
Initiator Element	68-83
Transcriptional Start Site	78
5' UTR Conserved Domain	138 - 208
ORF2	305 - 691
ORF2/2	305 – 687 ; 2422 – 2878
ORF2/3	305 – 687 ; 2564 – 3317
ORF2t/3	305 – 360 ; 2564 – 3317
ORF1	556 – 2904
ORF1/1	556 – 687 ; 2422 – 2904
ORF1/2	556 – 687 ; 2564 – 2878
Three open-reading frame region	2626 – 2846
Poly(A) Signal	3316 - 3319

Table A3. Novel *Anellovirus* amino acid sequences (*Alphatorquevirus*, Clade 4)

TTV-RTx3 (*Alphatorquevirus* Clade 4)	
ORF2	MSSTWRVPRGDVSARELCWYRSVRESHDAFCGCRDPVFHLSRLAARSNHQ GPPTPPTDERPSASTPVRRLLPLPSYPGEGPQARWPGGDGEGAGGARGGAG DGGARAGEEEYRPEDLDELFDAIEQEQ (SEQ ID NO: 846)
ORF2/2	MSSTWRVPRGDVSARELCWYRSVRESHDAFCGCRDPVFHLSRLAARSNHQ GPPTPPTDERPSASTPVRRLLPLPSYPGEGPQARWPGGDGEGAGGARGGAG DGGARAGEEEYRPEDLDELFDAIEQEQSSETRAKTPKYPDPVDSLAKYKSLT RNSLRRRGSSTRSTRDEECLLQELSNVCSSNQYLASMLLHHSGSRSSFPPQSS

	SEREKMQKAAQVHHPRDRDSGRKRPTRRKRSPRRGRRRRRGSSSSESSESSE SSTSNSSMSQYNSPKPKRTST (SEQ ID NO: 847)
ORF2/3	MSSTWRVPRGDVSARELCWYRSVRESHDAFCGCRDPVFHLSRLAARSNHQ GPPTPPTDERPSASTPVRRLLPLPSYPGEGPQARWPGGDGEGAGGARGGAG DGGARAGEEEYRPEDLDELFDAIEQEQSYQTSAQATNTWRVCSYTTQGPAP LSLLRVPARGRRCRKRLRFTTQETATLAGRGQPDANGVLGGAGGDDEGAP RAKAQRAASPQPPTPACRSTTRQNPSEPPHKPPIILPALNKVYLFPPDKPTPIQ XXXXXXNTEFEACQAFDRPPRKYLSDTPTYPWLPVPNPEIKVSFKLGFKSYK AVGVSLVGVYLLRSLSTPSVSEECDPSPWCNALGGRALRLRLRAAPRTPAR ADALARVRPLRARGGREFC (SEQ ID NO: 848)
ORF2t/3	MSSTWRVPRGDVSARELCWSYQTSAQATNTWRVCSYTTQGPAPLSLLRVP ARGRRCRKRLRFTTQETATLAGRGQPDANGVLGGAGGDDEGAPRAKAQR AASPQPPTPACRSTTRQNPSEPPHKPPIILPALNKVYLFPPDKPTPIQXXXXXX NTEFEACQAFDRPPRKYLSDTPTYPWLPVPNPEIKVSFKLGFKSYKAVGVSL VGVYLLRSLSTPSVSEECDPSPWCNALGGRALRLRLRAAPRTPARADALAR VRPLRARGGREFC (SEQ ID NO: 849)
ORF1	MAWWGWRRRWWRPRRRWRWRRPRRRRRVPARRPRRAVRRYRTRTVRR RRRGWRRGYRRRYRLRRYRRRGRRRKKIVLTQWNPQTVRKCFIRGLMPVL WAGMGTGGHNYAVRSDDFVVDRGFGGSFATETFSLRVLFDQYQRGFNRW SHTNEDLDLARYTGCKWTFYRHQDTDFIVYFTNNPPMKTNQHTAPLTTPG MLMRSKYKILVPSFKTRPKGRKTVSVRVRPPKLFQDKWYTQQDLCPVPLVQ LNVTAADFTHPFGSPLTDTPCIRFQVLGNLYNKCLNIDLPQFDEDGEILTSTP YNRENKEDLKKLYKTLFVDEHAGNYWQTFLTNTMVKSHIDANQAKTYDQ EKTAAEQGKDPFPTNPPKDQFTTWNKKLVDPRDSNFLFATYHPKNIKKAIKT MRDNNFALTTGKNDIYGDYTAAYTRNTHMLDYYLGFYSPIFLSSGRSNTEF WTAYRDIVYNPLLDKGTGNMIWFQYHTKTDNIYKKPECHWEILDMPLWAL CNGYVEYLESQIKYGDILVEGKVLIRCPYTKPALVDPNNSLAGYVVFNTTFG QGKWIDGKGYIPLHERSKWYVMLRYQTDVLHDIVTCGPWQYRDDNKNSQ LIAKYRFKFYWGGNMVHSQVIRNPCKDTQVSGPRRQPREVQVVDPQLITPP WVLHSFDQRRGMFTAGAIKRLLKQPIPGEYAPTPLRVPLLFPSSEFQREGED

	AESGSGSPPKRPRLWQEEANQTQTESSEGPAETTRELLERKLREQRVLNLQL QHVAVQLAKTQANLHINPLLYSQP (SEQ ID NO: 850)
ORF1/1	MAWWGWRRRWWRPRRRWRWRRPRRRRRVPARRPRRAVRRYRTRTVIRN PCKDTQVSGPRRQPREVQVVDPQLITPPWVLHSFDQRRGMFTAGAIKRLLK QPIPGEYAPTPLRVPLLFPSSEFQREGEDAESGSGSPPKRPRLWQEEANQTQT ESSEGPAETTRELLERKLREQRVLNLQLQHVAVQLAKTQANLHINPLLYSQP (SEQ ID NO: 852)
ORF1/2	MAWWGWRRRWWRPRRRWRWRRPRRRRRVPARRPRRAVRRYRTRTELSN VCSSNQYLASMLLHHSGSRSSFPPQSSSEREKMQKAAQVHHPRDRDSGRKR PTRRKRSPRRGRRRRRGSSSSESSESSESSTSNSSMSQYNSPKPKRTST (SEQ ID NO: 853)

Table N4. Novel *Anellovirus* nucleic acid sequence (*Alphatorquevirus*)

Name	TTV-RTx4
Genus/Clade	*Alphatorquevirus*, Clade 4
Accession Number	SRR3481579

Full Sequence: 3742 bp

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1         10         20         30         40         50
|         |          |          |          |          |
AAAGTGCTACGTCACTAACCACGTGACACCCACAGGCCAACCGAATGCTA
TGTCGTGCACTTCCTGGGCCGGGTCTACGTCCTCATATAACTACCTGCAC
TTCCGAATGGCTGAGTTTTCCACGCCCGTCCGCAGCGGTGAAGCCACGGA
GGGAGATCAGCGCGTCCCGAGGGCGGGTGCCGAAGGTGAGTTTACACACC
GAAGTCAAGGGGCAATTCGGGCTCGGGACTGGCCGGGCTATGGGCAAGGC
TCTGAAAAAAGCATGTTTATTGGCAGGCATTACAGAAAGAAAAGGGCGCT
GCCACTGTGTGCTGTGCGATCAACAAAGAAGGCTTGCAAACTACTAATAG
TAATGTGGACCCCACCTCGCAATGACCAACAGTACCTTAACTGGCAATGG
TACTCAAGTATACTTAGCTCCCACGCTGCTATGTGCGGGTGTCCCGACGT
TGTTGCTCATTTTAATCATCTTGCTTCTGTGCTTCGCGCCCCGCAAAATC
CACCCCCACCCGGTCCCCAGCGAAACCTGCCCCTCCGACGGCTGCCGGCT
CTCCCGGCTGCGCCAGAGGCGCCCGGAGATAGAGCACCATGGCCTATGGC
TGGTGGCGCCGGAGGAGAAGACGGTGGCGCAGGTGGAGACGCAGACCATG
GAGGCGCCGCTGGAGGACCAGAAGACGCAGACCTGTTAGACGCCGTGGCC
GCCGCAGAAACGTAAGGAGACGCCGCAGAGGAGGGAGGTGGAGGAGGAGG
TACAGGAGATGGAAAAGAAAGGGCAGACGCAGAAAAAAAGCTAAAATAAT
AATAAGACAATGGCAACCTAACTACAGAAGGAGATGTAACATAGTAGGCT
ATATTCCTGTACTGATATGTGGCGAAAATACTGTCAGCAGAAACTATGCC
ACACACTCAGACGATACTAACTACCCAGGACCCTTTGGGGGGGGTATGAC
TACAGACAAATTTACCTTAAGAATTCTGTATGACGAGTACAAAAGGTTTA
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TACACCCAGGAATGCTAGCCTTAGACAAAAGAGCAAGATGGATACCTAGC
TTAAAATCTAGACCAGGAAAAAAACACTATATTAAAATAAGAGTAGGGGC
GCCTAAAATGTTCACAGATAAATGGTACCCCCAAACAGATCTTTGTGACA
TGGTGCTGCTAACTGTCTATGCAACCGCAGCGGATATGCAATATCCGTTC
GGCTCACCACTAACTGACTCTGTGGTTGTGAACTTCCAGGTTCTGCAATC
CATGTATGATGAAACCATTAGCATATTACCAGATCAAAAGGAGAAAAGAA
TAACGCTGCTCACTAGTATAGCCTTTTATAACACCACACAAACTATAGCC
CAATTAAAGCCATTTATAGATGCAGGCAATATGACTTCAACTACAACAGC
AACAACATGGGGATCATACATAAACACAACCAAATTTAATACAGCAGCCA
CTACAACATACACATACCCAGGCAGTACTACAACTACAGTAACTATGTTA
ACTTGTAATGACTCCTGGTACAGAGGAACAGTATATAACGACCAAATTAA
AAATTTACCAAAGGAAGCAGCTCAATTATACTTAAAAGCAACAAAAACCT
TACTAGGAAACACCTTCACAAATGACGACCACACACTAGAATACCATGGA
GGACTGTACAGCTCAATTTGGCTGTCCCCCGGCAGATCTTACTTTGAAAC
ACCAGGAGCATACACAGACATAAAATACAACCCATTTACAGACAGAGGAG
AAGGAAACATGCTATGGATAGACTGGCTAAGCAAAAAAAATATGAACTAT
GACAAACTACAAAGTAAATGTTTAATATCAGACCTACCTTTATGGGCAGC
AGCATATGGATATTTAGAATTTTGTGCAAAAAGTACAGGAGACCAAAATA
TACACATGAATGCCAGACTACTAATAAGAAGTCCCTTTACAGACCCCCAA
CTACTAGTACACACAAACCCCACAAAAGGCTTTGTTCCCTACTCTTTAAA
CTTTGGAAATGGTAAAATGCCAGGAGGTAGTAGTAATGTTCCTATTAGAA
TGAGAGCTAAATGGTATCCAACATTGTTTCACCAGCAAGAAGTACTAGAG
GCCTTAGCACAGTCAGGCCCCTTTGCATACCACTCAGACATTAAAAAAGT
ATCTCTGGGTATGAAATACCGTTTTAAGTGGATCTGGGGTGGAAACCCCG
TTCGCCAACAGGTTGTTAGAAATCCCTGCAAAGACTCCCACTCCTCGGTC
AATAGAGTCCCTAGAAGCTTACAAATCGTTGACCCGAAATACAACTCACC
GGAACTCACATTCCATACGTGGGACTTCAGACGTGGCCTCTTTGGCCAGA
AAGCTATTGAGAGAATGCAACAACAACCAACAACTACTGACATTTTTTCA
GCAGGCCGCAAGAGACCCAGGAGGGACACCGAGGTGTACCACTCCAGCCA
AGAAGGGGAGCAAAAAGAAAGCTTACTTTTCCCCCCAGTCAAGCTCCTCA
GACGAGTCCCCCCGTGGGAAGACTCGCAGCAGGAGGAAAGCGGGTCGCAA
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GGCCTGTAGAATATGGGACAGACCCCCAAGAGGCAATCTAAGAGACACCC
CCTTTTACCCCTGGGCCCCCAAAGAAAACCAGTACCGTGTAAACTTTAAA
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CTTATAAAAGTAACCAAGCACTCCGAGCGAAGCGAGGAGTGCGACCCTTG
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ACCTCAGACCCCCGCTCGTGCTGACACGCTTGCGCGTGTCAGACCACTTC
GGGCTCGCGGGGGTCGGGAAATTTATTAAACAGACTCCGAGTTGCCATTG
GACACAGTAGTCTATGAACAGCAACGAAAGTGAGTGGGGCCAGACTTCGC
CATAAGGCCTTTATCTTCTTGCCATTTGTCAGTATAGAGGGTCGCCATAG
GCTTCGGTCTCCATTTTAACCTGTAAAAACTACCAAAATGGCCGTTCCAG
TGACGTGACAGCCGCCATTTTAAGTAGCTGACGTCAAGGATTGACGTAAA
GGTTAAAGGTCATCCTCGGCGGAAGCTACACAAAATGGTGGACAACATCT
TCCGGGTCAAAGGTCGTGCACACGTCAAAAGTCACGTGGTGGGGACCCGC
TGTAACCCGGAAGTAGGCCCCGTCACGTGATTTGTCACGTGTGTACACGT
CACAGCCGCCATTTTGTTTTACAAAATGGCTGACTTCCTTCCTCTTTTTT
GAAAAAAGGCGCCAAAAAAGGCTCCGCCCCCCGGCCCCCCCC (SEQ ID NO: 854)

Annotations:

Putative Domain	*Base range*
TATA Box	86 – 90
Initiator Element	104 – 119
Transcriptional Start Site	114
5' UTR Conserved Domain	174 – 244
ORF2	353 – 715
ORF2/2	353 – 711 ; 2362 – 2863
ORF2/3	353 – 711 ; 2555 – 3065
ORF2t/3	353 – 432; 2555 – 3065
ORF1	589 – 2889
ORF1/1	589 – 711 ; 2362 – 2889
ORF1/2	589 – 711 ; 2555 – 2863
Three open-reading frame region	2555 – 2863
Poly(A) Signal	3062 – 3066
GC-rich region, or a portion thereof**	3720 – 3742

Table A4. Novel *Anellovirus* amino acid sequences (*Alphatorquevirus*, Clade 4)

TTV-RTx4 (*Alphatorquevirus* Clade 4)	
ORF2	MWTPPRNDQQYLNWQWYSSILSSHAAMCGCPDVVAHFNHLASVLRAPQN PPPPGPQRNLPLRRLPALPAAPEAPGDRAPWPMAGGAGGEDGGAGGDADH GGAAGGPEDADLLDAVAAAET (SEQ ID NO: 855)
ORF2/2	MWTPPRNDQQYLNWQWYSSILSSHAAMCGCPDVVAHFNHLASVLRAPQN PPPPGPQRNLPLRRLPALPAAPEAPGDRAPWPMAGGAGGEDGGAGGDADH GGAAGGPEDADLLDAVAAAETLLEIPAKTPTPRSIESLEAYKSLTRNTTHRN SHSIRGTSDVASLARKLLRECNNNQQLLTFFQQAARDPGGTPRCTTPAKKGS KKKAYFSPQSSSSDESPRGKTRSRRKAGRKAQRKRRRPSPSSSSSSCSNSESW ESNSYSCSTKSKKSNKIKISTLPCYQGGGI (SEQ ID NO: 856)
ORF2/3	MWTPPRNDQQYLNWQWYSSILSSHAAMCGCPDVVAHFNHLASVLRAPQN PPPPGPQRNLPLRRLPALPAAPEAPGDRAPWPMAGGAGGEDGGAGGDADH GGAAGGPEDADLLDAVAAAETPQETQEGHRGVPLQPRRGAKRKLTFPPSQ

	APQTSPPVGRLAAGGKRVAKLRGRDADRLPAAQAAAAATANPGSQTHTPV QPSPKNPTKSRYQPYLVTKGGGSSILISNSTINMFGDPKPYNPSSNDWKEEYE ACRIWDRPPRGNLRDTPFYPWAPKENQYRVNFKLGFQ (SEQ ID NO: 857)
ORF2t/3	MWTPPRNDQQYLNWQWYSSILSSHAAMPQETQEGHRGVPLQPRRGAKRK LTFPPSQAPQTSPPVGRLAAGGKRVAKLRGRDADRLPAAQAAAAATANPGS QTHTPVQPSPKNPTKSRYQPYLVTKGGGSSILISNSTINMFGDPKPYNPSSND WKEEYEACRIWDRPPRGNLRDTPFYPWAPKENQYRVNFKLGFQ (SEQ ID NO: 858)
ORF1	MAYGWWRRRRRRWRRWRRRPWRRRWRTRRRRPVRRRGRRRNVRRRRR GGRWRRRYRRWKRKGRRRKKAKIIIRQWQPNYRRRCNIVGYIPVLICGENT VSRNYATHSDDTNYPGPFGGGMTTDKFTLRILYDEYKRFMNYWTASNEDL DLCRYLGVNLYFFRHPEVDFIIKINTMPPFLDTELTAPSIHPGMLALDKRARW IPSLKSRPGKKHYIKIRVGAPKMFTDKWYPQTDLCDMVLLTVYATAADMQ YPFGSPLTDSVVVNFQVLQSMYDETISILPDQKEKRITLLTSIAFYNTTQTIAQ LKPFIDAGNMTSTTTATTWGSYINTTKFNTAATTTYTYPGSTTTTVTMLTCN DSWYRGTVYNDQIKNLPKEAAQLYLKATKTLLGNTFTNDDHTLEYHGGLY SSIWLSPGRSYFETPGAYTDIKYNPFTDRGEGNMLWIDWLSKKNMNYDKLQ SKCLISDLPLWAAAYGYLEFCAKSTGDQNIHMNARLLIRSPFTDPQLLVHTN PTKGFVPYSLNFGNGKMPGGSSNVPIRMRAKWYPTLFHQQEVLEALAQSGP FAYHSDIKKVSLGMKYRFKWIWGGNPVRQQVVRNPCKDSHSSVNRVPRSL QIVDPKYNSPELTFHTWDFRRGLFGQKAIERMQQQPTTTDIFSAGRKRPRRD TEVYHSSQEGEQKESLLFPPVKLLRRVPPWEDSQQEESGSQSSEEETQTVSQ QLKQQLQQQRILGVKLILLFNQVQKIQQNQDINPTLLPRGGDLASLFQIAP (SEQ ID NO: 859)
ORF1/1	MAYGWWRRRRRRWRRWRRRPWRRRWRTRRRRPVRRRGRRRNVVRNPC KDSHSSVNRVPRSLQIVDPKYNSPELTFHTWDFRRGLFGQKAIERMQQQPTT TDIFSAGRKRPRRDTEVYHSSQEGEQKESLLFPPVKLLRRVPPWEDSQQEES GSQSSEEETQTVSQQLKQQLQQQRILGVKLILLFNQVQKIQQNQDINPTLLPR GGDLASLFQIAP (SEQ ID NO: 860)
ORF1/2	MAYGWWRRRRRRWRRWRRRPWRRRWRTRRRRPVRRRGRRRNAARDPG GTPRCTTPAKKGSKKKAYFSPQSSSSDESPRGKTRSRRKAGRKAQRKRRRPS

	PSSSSSSCSNSESWESNSYSCSTKSKKSNKIKISTLPCYQGGGI (SEQ ID NO: 861)

Table N5. Novel *Anellovirus* nucleic acid sequence (*Alphatorquevirus*)

Name	TTV-RTx5b
Genus/Clade	*Alphatorquevirus*, Clade 5
Accession Number	SRR3481639

Full Sequence: 3553 bp

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|         |          |          |          |          |
ATACCTCATCATATAAAGCGGCGCACTTCCGAATGGCTGAGTTTTCCACG
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GGGTGCCGGAGGTGAGTTTACACACCGCAGTCAAGGGGCAATTCGGGCTC
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CAATGGGATCGAGAGAAACCTGTGGGAGGCATTCTTTCGCATGCATGCTT
CAGCTTGTGGTTGTGGCGATCTTGTTGGCCATCTTACTGTACTGGCTGGT
CGGTATGGTGCTCCTCCTCGTCCCCCGGCCCCCGGCGCTCCCAGACCACC
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GGGGAGGCGGAGGTGGAGACGGGGGTACAGACGCAGACTGAGACTAAGAC
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AAAAAGTACAGAGACAAAAGATTCGACTGGCTAACAAACATGCCAGTAAC
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GGGAGAAGGCAACATTATCTGGTTCCAGTACCTCACTAAACCCACCACAG
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CTACATGTTTCCCCCCGACAAGCCCATGCCCATACACGGGTACCACGGGT
GGGAGACGGAGTACCAGGCCTGCAAGGCCTTCAACAGGCCCCCCAGAAAC
TACCTTTCAGACAAACCCATCTACCCTTGGCTCCCTCGCCCCGAACCCGA
AATAATAGTGAGCTTTAGGTTCGGTTTCAAATAAACAAGGCCGCAAATAA
ACAAGGCCGTGGGAGTTTCACTGGTCGGTGTCTACCTCTTAAGGTCACTA
AGCACTCCGAGCGTTAGCGAGGAGTGCGACCCTTCCCCCTGGTGCCACGC
CCTCGGCGGCCGCGCGCTACGCCTNNNNNNNNNNNNNNNNNNNNNNNNN
NNNNNNNNNNNNNNNNNNNNNNNNNNTGAATCAGTAACGAAAGTGAGTGG
GGCCAGACTTCGCCATAAGGCCTTTATCTTCTTGCCATTGGTCCGTGTGG
GGAGTCGCCATAGGCTTCGGGCTCGGTTTTAGGCCTTCCGGACTACAAAA
ACCGCCATTTTAGTGACGTCACGGCGGCCATTTTAAGTAAGCATGGCGGG
CGGTGACGTACAAGTTGAAAGGTCACCGCGCTTCCGTGTTTACTCAAAAT
GGTGGCCAACTGCTTCCGGGTCAAAGGTCGGCGGCCACGTCATAAGTCAC
GTGGGAGGGCTGCGTCACAAACACGGAAGTGGCTGTCCCACGTGACTTGT
CACGTGATTGCTACGTCACGGCCGCCATTTTAGTTCACAAAATGGCGGAC
TTC (SEQ ID NO: 862)

Annotations:

Putative Domain	*Base range*
TATA Box	12 – 17
Initiator Element	30 – 45
Transcriptional Start Site	40
5' UTR Conserved Domain	100 – 171
ORF2	272 – 637
ORF2/2	272 – 633 ; 2326 – 2767
ORF2/3	272 – 633 ; 2525 – 2984
ORF2t/3	272 – 633 ; 2525 – 2984
ORF1	511 – 2793

ORF1/1	511 – 711 ; 2326 – 2793
ORF1/2	511 – 711 ; 2525 – 2767
Three open-reading frame region	2525 – 2767
Poly(A) Signal	2981 – 2985
Unknown sequence	3125-3176

*Note: Modifications made to maintain reading frames:

-"C" inserted into ORF2	430
-"N" inserted into ORF1	1842

Table A5. Novel *Anellovirus* amino acid sequences (*Alphatorquevirus*, Clade 5)

TTV-RTx5b (*Alphatorquevirus* Clade 5)	
ORF2	MSWRPPVHNPNGIERNLWEAFFRMHASACGCGDLVGHLTVLAGRYGAPPR PPAPGAPRPPLIRQLALPAPPADPQQANPQWPGGDGGEDGAGGPAAGGAVA DAEYQEDELNALFDAVEQEE (SEQ ID NO: 863)
ORF2/2	MSWRPPVHNPNGIERNLWEAFFRMHASACGCGDLVGHLTVLAGRYGAPPR PPAPGAPRPPLIRQLALPAPPADPQQANPQWPGGDGGEDGAGGPAAGGAVA DAEYQEDELNALFDAVEQEELLKTPAQTAGYDLHPVESVAQYKSLARSQW GQSTYSTGGTGDGGSLIKKLSKECLKNQLMMESIQQAQRSLDGFPHSTTKSK KAPQVQRRQGRSPRKKKPLKKPSKKSKRRRYSSTSSSSTESSDGSESNSNSSC CSSPRRRATCT (SEQ ID NO: 864)
ORF2/3	MSWRPPVHNPNGIERNLWEAFFRMHASACGCGDLVGHLTVLAGRYGAPPR PPAPGAPRPPLIRQLALPAPPADPQQANPQWPGGDGGEDGAGGPAAGGAVA DAEYQEDELNALFDAVEQEEPKGPSMVSPTRQPRARRRLRFRGDKVAVLAR RSRSRSPPRSPRGVATAAPPPAVPRAATDRKATPTRHAAAHQDAEQPAHKP PCSWPCINKVYMFPPDKPMPIHGYHGWETEYQACKAFNRPPRNYLSDKPIY PWLPRPEPEIIVSFRFGFK (SEQ ID NO:865)
ORF2t/3	MSWRPPVHNPNGIERNLWEAFFRMHASACGCGDLVGHLTVLAGRPKGPSM VSPTRQPRARRRLRFRGDKVAVLARRSRSRSPPRSPRGVATAAPPPAVPRAA TDRKATPTRHAAAHQDAEQPAHKPPCSWPCINKVYMFPPDKPMPIHGYHG

	WETEYQACKAFNRPPRNYLSDKPIYPWLPRPEPEIIVSFRFGFK (SEQ ID NO: 866)
ORF1	MAWWGRWRRWRWRPRRWRRRRRRRVPRRRAQRPVRRRRARRVRRRRW GRRRWRRGYRRRLRLRRRRRRKRKIVLTQWNPAKVRRCTIKGVLPMILCG AGRSGFNYGLHSDDYTVQKPLGQNPHGGGMSTVTFSLQVLYDQYQRFMN KWSYSNDQLDLARYFGCTFWFYRHPEVDFVAQFDNVPPMKMDENTAPNT HPSFLLQNKHKVKIPSFKTKPFGKKRVRVTVGPPKLFEDKWYSQHDLCKVP LVSWRLTAADFRFPFCSPQTDNPCYTFQVLHEEYYPVIGTSALENGSNYNSS AITALEKFLYEKCTHYQTFATDTRLNPQRPVSSTNANKTYTPSGSQETIVWG QSDFNLFKKHTDSNYGYCTYCPTNDLATKIKKYRDKRFDWLTNMPVTNTC HINATFARGKIKEWEYHLGWFSNIFIGNLRHNLAFRAAYIDITXTDKGEGNII WFQYLTKPTTEYIEAQAKCSITNIPLYAAFYGYEDYLQRTLGPYQDVETLGII CVKCPYTDPPLVHKSTDKKNWGYVFYDVHFGNGKTPEGLGQVHPYWMQR WRPYVQFQKDTMNKIARTGPFSYRDETPSITLTAGYKFHFNWGGDSIFPQIIK NPCPDSGVRPSSSRERRSVQVVSPLTMGPEYIFHRWDWRRGFFNQKALKRM LEKSINDGEYPTGPKVPRWFPPLDNQEQEGASGSEETRSQSSQEEAAQEALQ EVQEASLQQHLLQQYREQRRIGKQLQLVMLQLTKTQSNLHINPRVLGHA (SEQ ID NO: 867)
ORF1/1	MAWWGRWRRWRWRPRRWRRRRRRRVPRRRAQRPVRRRRARRIIKNPCPD SGVRPSSSRERRSVQVVSPLTMGPEYIFHRWDWRRGFFNQKALKRMLEKSI NDGEYPTGPKVPRWFPPLDNQEQEGASGSEETRSQSSQEEAAQEALQEVQE ASLQQHLLQQYREQRRIGKQLQLVMLQLTKTQSNLHINPRVLGHA (SEQ ID NO: 868)
ORF1/2	MAWWGRWRRWRWRPRRWRRRRRRRVPRRRAQRPVRRRRARRAQRSLDG FPHSTTKSKKAPQVQRRQGRSPRKKKPLKKPSKKSKRRRYSSTSSSSTESSD GSESNSNSSCCSSPRRRATCT (SEQ ID NO: 869)

Table N6. Novel *Anellovirus* nucleic acid sequence (*Alphatorquevirus*)

Name	TTV-RTx6
Genus/Clade	*Alphatorquevirus*, Clade 5

Accession Number SRR3438066

Full Sequence: 3896 bp

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1         10         20         30         40         50
|         |          |          |          |          |
TAAACTTCCTCTTTTAATAGGAAACCACAAAATTTGCATTGCCGACCACA
AACGCATATGCAAATTTACTTCCCCAAAAACTCAACCACAAAATTTGCAT
TGCCGCCCACAAACGTCTACTTTAACCACATCCTCTAACATGTTAGAAAC
TCCACCCAACTACTTCATTAGTATACAGCATCACAAGGGAGGAGCCAAAC
AACTATATAACCAAGTGTACTTCCGAATGGCTGAGTTTATGCCGCCAGAC
GGAGACGGGATCGCGACGGAGGAGCGATCGAGCGTCCCGAGGGCGGGTGC
CGGAGGTGAGTTTACACACCGCAGTCAAGGGGCAATTCGGGCTCGGGACT
GGCCGGGCTATGGGCAAGGCTCTTAAAAAAGCCATGTTTCTCGGTCGACC
TTACAGAAAGAAAAGGGCACTGTCACTGCTACGCGTGCGAGCTCCAGAGG
CGAAACCACCTGCTATGAGCTGGAGGCCCCCGGTGCACAACCCTAATGGG
ATCCAGAGAAACCTGTGGGAGGCATTCTTTCGCATGCATGCTGCAGCTTG
TGGTTGTGGCGATCTTGTTGGCCATATTACTGTACTGGCTGGTCGGTATG
GTGCTCCTCCTCGTCCCCCGGCCCCCGGGGCTCCCAGACCACCGCTGATA
CGCCAGCTGGCCCTTCCGGCGCCCCCCGCCGATCCTCAACAGGCTAACCC
ACAATGGCCTGGTGGGGACGGTGGAGAAGATGGCGCTGGAGGCCCCGCCG
CTGGCGGCGCCGTCGCAGACGCCGAGTACCAAGAAGACGAGCTCAACGCC
CTGTTCGACGCCGTCGAGCAAGAAGAGTAAGGAGGAGGCGATGGGGGAGG
CGGAGGTGGAGACGGGGGTACAGACGCAGACTAAGACTGAGACGCAGACG
CAGACGAAAGAAAATAAGACTGACTCAGTGGAACCCAGCCAAAGTCAGGA
GATGTACTATTAAGGGGGTGCTACCCATGATCTTATGCGGCGCCGGCCGC
TCGGGGTTTAACTATGGACTGCACAGCGACGACTACACGGTGCAGAAACC
CCTGGGGCAGAACCCCCACGGGGGCGGCATGAGCACAGTAACTTTTAGCC
TACAAGTACTATTTGACCAGTACCAGAGGTTTATGAACCGGTGGTCGTAC
TCCAACGACCAGCTAGACCTCGCCAGGTACTTTGGCTGCACCTTCTACTT
TTACAGACACCCTGAAATTGACTTTGTAGCTCAGTATGACAATGTACCCC
CAATGAAAATGGACGAGAACACGGCNCCTAACACTCACCCCTCTTTTCTA
CTACAAAACAAACGCAAAATTAAAATCCCCAGCTTTAAAACCAAGCCATT
TGGCAGAAAAAGAGTAAAAGTAACAGTGGGGCCCCCCAAACTGTTTGAAG
ATAAATGGTACAGCCAGCATGACTTGTGTAAGGTGCCCCTAGTCAGTTGG
CGGTTAACCGCATGTGACTTCAGGTTTCCGTTCTGCTCACCACTAACTGA
CAACCCTTGCTACACCTTCCAGGTATTGCATGAAAACTATTACCCAGTCA
TAGGCACTTCCTCTTTAGAAAACGGTACAAACTACAATAACACTGCTATA
ACTACCCTTGAGACATGGCTATATGGAAAATGCACACACTATCAAACATT
TGCCACAGACACCAGACTTAATCCACAGAGACCTGTATCTTCAAGTAATG
CAAATGAAACTTATACTCCTAGTGGTTCTAAAGAATCAATAATATGGGGA
CAGTCTGACTGGGCAAACTTTAAAAAGAACACAGACAGCAACTATGGCTA
CTGTTCCTACTGCCCCTCAAATGGCACTAACGGAACAGTAGATAAAATTA
AAAAATACAGAGACCAAAGATTTAGATGGCTTACAGAAATGCCAGTACCT
AACACCTGTCACATACATGCCACCTTCGCCCGAGGCACTATTAAATACTG
GGAGTACCACCTAGGCTGGTACTCAAACATATTTATTGGCAACCTCAGAC
ACAACTTAGCCTTCAGACCAGCCTACATAGACATTACCTACAATCCCATC
ACTGACAAAGGAGAGGGCAACATTATCTGGTTCCAGTACCTCACTAAGCC
CACCACAGAATACATAGAAACCCAGGCAAAATGCACCATTACTAACATTC
CCCTTTATGCTGCTTTCTATGGCTACGAAGACTACCTCCAGAGAACACTA
GGCCCCTACCAAGATGTAGAAACCCTAGGCATAATCTGTGTTAAATGTCC
CTACACAGATCCCCCTCTAGTTCACAAAGACAAAAGTAAAACCAACTGGG
GCTACGTATTCTACGACGCCCACTTTGGCAACGGAAAGACCCCAGAGGGA
CTAGGCCAAGTACACCCTTACTGGATGCAGAGATGGAGACCCTATGTACA
GTTTCAAAAAGACACCATGCACAAAATATCCAGAACGGGACCCTTCAGCT
ACAGAGACGACACGCCTTCCATCACCCTCACTGCCGAATACAAGTTTCGT
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TTTAACTGGGGGGGCGACTCTATATTTCCACAGATTATTAAAAACCCCTG
CCCAGACACCGGGGTTCGACCTTCAACCGGTAGAGACCGTCGCTCAGTAC
AAGTCGTTAGCCCGCTCACAATGGGACCCCAGTTTATATTCCACTCATGG
GACTGGAGACGGGGGTTCTTTAATCAAAAAACTCTCAAAAGAATGCTTGA
AAAACCAGTTAATGATGGAGAATATCCAACAGGCCCAAAGGTGCCTCGAT
GGTTTCCCCCACTCGACAACCAAGAGCAAGAAGGCGTCTCAGATACAGAG
ACGACAACCTCGCAGTCCTCGCAAGAAGAAGCCGCTCAAGAAGCCCTCCA
AGAAGTCCAAGAGGCGTCGCTACAGCAGCACCTCCTCCAGCAGTACCGAG
AGCAGCGAAGAATCGGAAAGCAACTCCAACTCGTCATGCTCCAACTCACC
AAGACGCAGAGCAACCTGCACATAAATCCCCGTGTCCTTGGCCATGCATA
AATAAAGTGTACATGTTTCCCCCCGAAAAGCCAATGCCCATACACGGCTA
CCACGGGTGGGAGACAGAGTATCAGGCCTGCAAGGCCTTTGACAGGCCCC
CTAGAAACTACCTATCAGACAAACCCATCTACCCCTGGCTTCCCCGCTCC
CAACCAGAATTTAAAGTGAGTTTTAAGCTTGGCTGTCAATAAACAAGNNN
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NNNNNGTTTACACAAAATGGTGGCCAAGTCCTTCCGGGTGAAAGGTCGGC
GCCTACGTCATAAGTCACGTGGGGAGGGCTGCGTCACAACCAGGAAGCAA
TCCTCACCACGTGATTTGTCACGTGATCGCTACGTCACGGCCGCCATTTT
AGTTTACAAAATGGCGGACTTCCTTCCTCTTTTTCAAAAATAACGGCCCT
GCGGCGGCGCGCGCGCTGCGCGCGCGCCGGGGGCTGCCGCCCCA (SEQ ID NO: 870)

Annotations:

Putative Domain	*Base range*
TATA Box	206 – 210
Initiator Element	224 – 239
Transcriptional Start Site	234
5' UTR Conserved Domain	294 – 364
ORF2	465 – 830
ORF2/2	465 – 826 ; 2534 – 2975
ORF2/3	465 – 826; 2721 – 3192
ORF2t/3	465 – 595 ; 2721 – 3192
ORF1	704 – 3001
ORF1/1	704 – 826; 2534 – 3001
ORF1/2	704 – 826; 2721 – 2975
Three open-reading frame region	2721 – 2975
Poly(A) Signal	3189 – 3193

Unknown sequence	3198 – 3655
GC-rich region, or a portion thereof**	3844 – 3895

Table A6. Novel *Anellovirus* amino acid sequences (*Alphatorquevirus*, Clade 5)

TTV-RTx6 (*Alphatorquevirus* Clade 5)	
ORF2	MSWRPPVHNPNGIQRNLWEAFFRMHAAACGCGDLVGHITVLAGRYGAPPR PPAPGAPRPPLIRQLALPAPPADPQQANPQWPGGDGGEDGAGGPAAGGAVA DAEYQEDELNALFDAVEQEE (SEQ ID NO: 871)
ORF2/2	MSWRPPVHNPNGIQRNLWEAFFRMHAAACGCGDLVGHITVLAGRYGAPPR PPAPGAPRPPLIRQLALPAPPADPQQANPQWPGGDGGEDGAGGPAAGGAVA DAEYQEDELNALFDAVEQEELLKTPAQTPGFDLQPVETVAQYKSLARSQW DPSLYSTHGTGDGGSLIKKLSKECLKNQLMMENIQQAQRCLDGFPHSTTKS KKASQIQRRQPRSPRKKKPLKKPSKKSKRRRYSSTSSSSTESSEESESNSNSSC SNSPRRRATCT (SEQ ID NO: 872)
ORF2/3	MSWRPPVHNPNGIQRNLWEAFFRMHAAACGCGDLVGHITVLAGRYGAPPR PPAPGAPRPPLIRQLALPAPPADPQQANPQWPGGDGGEDGAGGPAAGGAVA DAEYQEDELNALFDAVEQEEISNRPKGASMVSPTRQPRARRRLRYRDDNLA VLARRSRSRSPPRSPRGVATAAPPPAVPRAAKNRKATPTRHAPTHQDAEQP AHKSPCPWPCINKVYMFPPEKPMPIHGYHGWETEYQACKAFDRPPRNYLSD KPIYPWLPRSQPEFKVSFKLGCQ (SEQ ID NO: 873)
ORF2t/3	MSWRPPVHNPNGIQRNLWEAFFRMHAAACGCGDLVGHITVLAGRISNRPK GASMVSPTRQPRARRRLRYRDDNLAVLARRSRSRSPPRSPRGVATAAPPPA VPRAAKNRKATPTRHAPTHQDAEQPAHKSPCPWPCINKVYMFPPEKPMPIH GYHGWETEYQACKAFDRPPRNYLSDKPIYPWLPRSQPEFKVSFKLGCQ (SEQ ID NO: 874)
ORF1	MAWWGRWRRWRWRPRRWRRRRRRRVPRRRAQRPVRRRRARRVRRRRW GRRRWRRGYRRRLRLRRRRRRKKIRLTQWNPAKVRRCTIKGVLPMILCGA GRSGFNYGLHSDDYTVQKPLGQNPHGGGMSTVTFSLQVLFDQYQRFMNR WSYSNDQLDLARYFGCTFYFYRHPEIDFVAQYDNVPPMKMDENTAPNTHP SFLLQNKRKIKIPSFKTKPFGRKRVKVTVGPPKLFEDKWYSQHDLCKVPLVS WRLTACDFRFPFCSPLTDNPCYTFQVLHENYYPVIGTSSLENGTNYNNTAIT

	TLETWLYGKCTHYQTFATDTRLNPQRPVSSSNANETYTPSGSKESIIWGQSD WANFKKNTDSNYGYCSYCPSNGTNGTVDKIKKYRDQRFRWLTEMPVPNTC HIHATFARGTIKYWEYHLGWYSNIFIGNLRHNLAFRPAYIDITYNPITDKGEG NIIWFQYLTKPTTEYIETQAKCTITNIPLYAAFYGYEDYLQRTLGPYQDVETL GIICVKCPYTDPPLVHKDKSKTNWGYVFYDAHFGNGKTPEGLGQVHPYWM QRWRPYVQFQKDTMHKISRTGPFSYRDDTPSITLTAEYKFRFNWGGDSIFPQ IIKNPCPDTGVRPSTGRDRRSVQVVSPLTMGPQFIFHSWDWRRGFFNQKTLK RMLEKPVNDGEYPTGPKVPRWFPPLDNQEQEGVSDTETTTSQSSQEEAAQE ALQEVQEASLQQHLLQQYREQRRIGKQLQLVMLQLTKTQSNLHINPRVLGH A (SEQ ID NO: 875)
ORF1/1	MAWWGRWRRWRWRPRRWRRRRRRRVPRRRAQRPVRRRRARRIIKNPCPD TGVRPSTGRDRRSVQVVSPLTMGPQFIFHSWDWRRGFFNQKTLKRMLEKPV NDGEYPTGPKVPRWFPPLDNQEQEGVSDTETTTSQSSQEEAAQEALQEVQE ASLQQHLLQQYREQRRIGKQLQLVMLQLTKTQSNLHINPRVLGHA (SEQ ID NO: 876)
ORF1/2	MAWWGRWRRWRWRPRRWRRRRRRRVPRRRAQRPVRRRRARRNIQQAQR CLDGFPHSTTKSKKASQIQRRQPRSPRKKKPLKKPSKKSKRRRYSSTSSSSTE SSEESESNSNSSCSNSPRRRATCT (SEQ ID NO: 877)

Table N7. Exemplary *Anellovirus* nucleic acid sequence (*Alphatorquevirus*, Clade 1)

Name	TTV-CT30F
Genus/Clade	*Alphatorquevirus*, Clade 1
Accession Number	AB064597.1

Full Sequence: 3570 bp

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1         10         20         30         40         50
|         |          |          |          |          |
ATTTTGTGCAGCCCGCCAATTCTCGTTCAAACAGGCCAATCAGGAGGCTC
TACGTACACTTCCTGGGGGTGTGTCTTCGAAGAGTATATAAGCAGAGGCGG
TGACGAATGGTAGAGTTTTTCCTGGCCCGTCCGCGGCGAGAGCGCGAGCG
GAGCGAGCGATCGAGCGTCCCGTGGGCGGGTGCCGTAGGTGAGTTTACAC
ACCGCAGTCAAGGGGCAATTCGGGCTCGGGACTGGCCGGGCTATGGGCAA
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GATTCTTAAAAAATTCCCCCGATCCCTCTGTCGCCAGGACATAAAAACAT
GCCGTGGAGACCGCCGGTGCATAGTGTCCAGGGGCGAGAGGATCAGTGGT
TCGCGAGCTTTTTTCACGGCCACGCTTCATTTTGCGGTTGCGGTGACGCT
GTTGGCCATCTTAATAGCATTGCTCCTCGCTTTCCTCGCGCCGGTCCACC
AAGGCCCCCTCCGGGGCTAGAGCAGCCTAACCCCCCGCAGCAGGGCCCGG
CCGGGCCCGGAGGGCCGCCCGCCATCTTGGCGCTGCCGGCTCCGCCCGCG
GAGCCTGACGACCCGCAGCCACGGCGTGGTGGTGGGGACGGTGGCGCCGC
CGCTGGCGCCGCAGGCGACCGTGGAGACCGAGACTACGACGAAGAAGAGC
TAGACGAGCTTTTCCGCGCCGCCGCCGAAGACGATTTGTAAGTAGGAGAT
GGCGCCGGCCTTACAGGCGCAGGAGGAGACGCGGGCGACGCAGACGCAGA
CGCAGACGCAGACATAAGCCCACCCTAGTACTCAGACAGTGGCAACCTGA
CGTTATCAGACACTGTAAGATAACAGGACGGATGCCCCTCATTATCTGTG
GAAAGGGGTCCACCCAGTTCAACTACATCACCCACGCGGACGACATCACC
CCCAGGGGAGCCTCCTACGGGGGCAACTTCACAAACATGACTTTCTCCCT
GGAGGCAATATACGAACAGTTTCTGTACCACAGAAACAGGTGGTCAGCCT
CCAACCACGACCTCGAACTCTGCAGATACAAGGGTACCACCCTAAAACTG
TACAGGCACCCAGATGTAGACTACATAGTCACCTACAGCAGAACGGGACC
CTTTGAGATCAGCCACATGACCTACCTCAGCACTCACCCCCTTCTCATGC
TGCTAAACAAACACCACATAGTGGTGCCCAGCCTAAAGACTAAGCCCAGG
GGCAGAAAGGCCATAAAAGTCAGAATAAGACCCCCCAAACTCATGAACAA
CAAGTGGTACTTCACCAGAGACTTCTGTAACATAGGCCTCTTCCAGCTCT
GGGCCACAGGCTTAGAACTCAGAAACCCCTGGCTCAGAATGAGCACCCTG
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CAGCAACCTACCTCAGCACAGAGAAGACAGACTTAACATTATTAACAACA
CATTACACCCACATGACATAACAGGACCAAACAATAAAAAATGGCAGTAC
ACATATACCAAACTCATGGCCCCCATTTACTATTCAGCAAACAGGGCCAG
CACCTATGACTTACTACGAGAGTATGGCCTCTACAGTCCATACTACCTAA
ACCCCACAAGGATAAACCTTGACTGGATGACCCCCTACACACACGTCAGG
TACAATCCACTAGTAGACAAGGGCTTCGGAAACAGAATATACATACAGTG
GTGCTCAGAGGCAGATGTAAGCTACAACAGGACTAAATCCAAGTGTCTCT
TACAAGACATGCCCCTGTTTTTCATGTGCTATGGCTACATAGACTGGGCA
ATTAAAAACACAGGGGTCTCCTCACTAGCGAGAGACGCCAGAATCTGCAT
CAGGTGTCCCTACACAGAGCCACAGCTGGTGGGCTCCACAGAAGACATAG
GGTTCGTACCCATCACAGAGACCTTCATGAGGGGCGACATGCCGGTACTT
GCACCATACATACCGTTGAGCTGGTTTTGCAAGTGGTATCCCAACATAGC
TCACCAGAAGGAAGTACTTGAGGCAATCATTTCCTGCAGCCCCTTCATGC
CCCGTGACCAGGGCATGAACGGTTGGGATATTACAATAGGTTACAAAATG
GACTTCTTATGGGGCGGTTCCCCTCTCCCCTCACAGCCAATCGACGACCC

CTGCCAGCAGGGAACCCACCCGATTCCCGACCCCGATAAGCACCCTCGCC
TCCTACAAGTGTCGAACCCGAAACTGCTCGGACCGAGGACAGTGTTCCAC
AAGTGGGACATCAGACGTGGGCAGTTTAGCAAAAGAAGTATTAAAAGAGT
GTCAGAATACTCATCGGATGATGAATCTCTTGCGCCAGGTCTCCCATCAA
AGCGAAACAAGCTCGACTCGGCCTTCAGAGGAGAAAACCCAGAGCAAAAA
GAATGCTATTCTCTCCTCAAAGCACTCGAGGAAGAAGAGACCCCAGAAGA
AGAAGAACCAGCACCCCAAGAAAAAGCCCAGAAAGAGGAGCTACTCCACC
AGCTCCAGCTCCAGAGACGCCACCAGCGAGTCCTCAGACGAGGGCTCAAG
CTCGTCTTTACAGACATCCTCCGACTCCGCCAGGGAGTCCACTGGAACCC
CGAGCTCACATAGAGCCCCCACCTTACATACCAGACCTACTTTTTCCCAA
TACTGGTAAAAAAAAAAAATTCTCTCCCTTCGACTGGGAAACGGAGGCCC
AGCTAGCAGGGATATTCAAGCGTCCTATGCGCTTCTATCCCTCAGACACC
CCTCACTACCCGTGGTTACCCCCCAAGCGCGATATCCCGAAAATATGTAA
CATAAACTTCAAAATAAAGCTGCAAGAGTGAGTGATTCGAGGCCCTCCTC
TGTTCACTTAGCGGTGTCTACCTCTTAAAGTCACCAAGCACTCCGAGCGT
CAGCGAGGAGTGCGACCCTCCACCAAGGGGCAACTTCCTCGGGGTCCGGC
GCTACGCGCTTCGCGCTGCGCCGGACGCCTCGGACCCCCCCCCGACCCGA
ATCGCTCGCGCGATTCGGACCTGCGGCCTCGGGGGGGGTCGGGGGCTTTA
CTAAACAGACTCCGAGTTGCCACTGGACTCAGGAGCTGTGAATCAGTAAC
GAAAGTGAGTGGGGCCAGACTTCGCCATAGGGCCTTTAACTTGGGGTCGT
CTGTCGGTGGCTTCCGGGTCCGCCTGGGCGCCGCCATTTTAGCTTTAGAC
GCCATTTTAGGCCCTCGCGGGCACCCGTAGGCGCGTTTTAATGACGTCAC
GGCAGCCATTTTGTCGTGACGTTTGAGACACGTGATGGGGGCGTGCCTAA
ACCCGGAAGCATCCCTGGTCACGTGACTCTGACGTCACGGCGGCCATTTT
GTGCTGTCCGCCATCTTGTGACTTCCTTCCGCTTTTTCAAAAAAAAAGAG
GAAGTATGACAGTAGCGGCGGGGGGGCGGCCGCGTTCGCGCGCCGCCCAC
CAGGGGGTGCTGCGCGCCCCCCCCCGCGCATGCGCGGGGCCCCCCCCCGG
GGGGGCTCCGCCCCCCCGGCCCCCCCCCGTGCTAAACCCACCGCGCATGC
GCGACCACGCCCCCGCCGCC (SEQ ID NO: 1)

Annotations:

Putative Domain	*Base range*
TATA Box	84 – 90
Cap Site	107 – 114
Transcriptional Start Site	114
5' UTR Conserved Domain	177 – 247
ORF2	299 – 691

ORF2/2	299 – 687 ; 2137 – 2659
ORF2/3	299 – 687 ; 2339 – 2831
ORF2t/3	299 – 348 ; 2339 – 2831
ORF1	571 – 2613
ORF1/1	571 – 687 ; 2137 – 2613
ORF1/2	571 – 687 ; 2339 – 2659
Three open-reading frame region	2325 – 2610
Poly(A) Signal	2813 – 2818
GC-rich region	3415 – 3570

Table A7. Exemplary *Anellovirus* amino acid sequences (*Alphatorquevirus*, Clade 1)

TTV-CT30F (*Alphatorquevirus* Clade 1)	
ORF2	MPWRPPVHSVQGREDQWFASFFHGHASFCGCGDAVGHLNSIAPRFPRAGPPRPPPG LEQPNPPQQGPAGPGGPPAILALPAPPAEPDDPQPRRGGGDGGAAAGAAGDRGDRD YDEEELDELFRAAAEDDL (SEQ ID NO: 2)
ORF2/2	MPWRPPVHSVQGREDQWFASFFHGHASFCGCGDAVGHLNSIAPRFPRAGPPRPPPG LEQPNPPQQGPAGPGGPPAILALPAPPAEPDDPQPRRGGGDGGAAAGAAGDRGDRD YDEEELDELFRAAAEDDFQSTTPASREPTRFPTPISTLASYKCRTRNCSDRGQCSTSG TSDVGSLAKEVLKECQNTHRMMNLLRQVSHQSETSSTRPSEEKTQSKKNAILSSKH SRKKRPQKKKNQHPKKKPRKRSYSTSSSSRDATSESSDEGSSSSLQTSSDSARESTGT PSSHRAPTLHTRPTFSQYW (SEQ ID NO: 3)
ORF2/3	MPWRPPVHSVQGREDQWFASFFHGHASFCGCGDAVGHLNSIAPRFPRAGPPRPPPG LEQPNPPQQGPAGPGGPPAILALPAPPAEPDDPQPRRGGGDGGAAAGAAGDRGDRD YDEEELDELFRAAAEDDLSPIKAKQARLGLQRRKPRAKRMLFSPQSTRGRRDPRRR RTSTPRKSPERGATPPAPAPETPPASPQTRAQARLYRHPPTPPGSPLEPRAHIEPPPYIP DLLFPNTGKKKKFSPFDWETEAQLAGIFKRPMRFYPSDTPHYPWLPPKRDIPKICNIN FKIKLQE (SEQ ID NO: 4)
ORF2t/3	MPWRPPVHSVQGREDQWSPIKAKQARLGLQRRKPRAKRMLFSPQSTRGRRDPRRR RTSTPRKSPERGATPPAPAPETPPASPQTRAQARLYRHPPTPPGSPLEPRAHIEPPPYIP DLLFPNTGKKKKFSPFDWETEAQLAGIFKRPMRFYPSDTPHYPWLPPKRDIPKICNIN FKIKLQE (SEQ ID NO: 5)
ORF1	TAWWWGRWRRRWRRRRPWRPRLRRRRARRAFPRRRRRRFVSRRWRRPYRRRRR RGRRRRRRRRRHKPTLVLRQWQPDVIRHCKITGRMPLIICGKGSTQFNYITHADDIT

	PRGASYGGNFTNMTFSLEAIYEQFLYHRNRWSASNHDLELCRYKGTTLKLYRHPD VDYIVTYSRTGPFEISHMTYLSTHPLLMLLNKHHIVVPSLKTKPRGRKAIKVRIRPPK LMNNKWYFTRDFCNIGLFQLWATGLELRNPWLRMSTLSPCIGFNVLKNSIYTNLSN LPQHREDRLNIINNTLHPHDITGPNNKKWQYTYTKLMAPIYYSANRASTYDLLREY GLYSPYYLNPTRINLDWMTPYTHVRYNPLVDKGFGNRIYIQWCSEADVSYNRTKSK CLLQDMPLFFMCYGYIDWAIKNTGVSSLARDARICIRCPYTEPQLVGSTEDIGFVPIT ETFMRGDMPVLAPYIPLSWFCKWYPNIAHQKEVLEAIISCSPFMPRDQGMNGWDITI GYKMDFLWGGSPLPSQPIDDPCQQGTHPIPDPDKHPRLLQVSNPKLLGPRTVFHKW DIRRGQFSKRSIKRVSEYSSDDESLAPGLPSKRNKLDSAFRGENPEQKECYSLLKALE EEETPEEEEPAPQEKAQKEELLHQLQLQRRHQRVLRRGLKLVFTDILRLRQGVHWN PELT (SEQ ID NO: 6)
ORF1/1	TAWWWGRWRRRWRRRRPWRPRLRRRRARRAFPRRRRRRFPIDDPCQQGTHPIPDP DKHPRLLQVSNPKLLGPRTVFHKWDIRRGQFSKRSIKRVSEYSSDDESLAPGLPSKR NKLDSAFRGENPEQKECYSLLKALEEEETPEEEEPAPQEKAQKEELLHQLQLQRRH QRVLRRGLKLVFTDILRLRQGVHWNPELT (SEQ ID NO: 7)
ORF1/2	TAWWWGRWRRRWRRRRPWRPRLRRRRARRAFPRRRRRRFVSHQSETSSTRPSEE KTQSKKNAILSSKHSRKKRPQKKKNQHPKKKPRKRSYSTSSSSRDATSESSDEGSSS SLQTSSDSARESTGTPSSHRAPTLHTRPTFSQYW (SEQ ID NO: 8)

Table N8. Exemplary *Anellovirus* nucleic acid sequence (*Alphatorquevirus*, Clade 2)

Name	TTV-P13-1
Genus/Clade	*Alphatorquevirus*, Clade 2
Accession Number	KT163896.1

Full Sequence: 3451 bp

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1         10         20         30         40         50
|         |          |          |          |          |
AATTTTGCTAAACAGACTCCGAGGTGCTCTTGGACACTGAGTGGGCGTAC
AGCAACGAAAGTGAGTGGGGCCAGACTTCGCCATAAGGCCTTTATCTTCG
GGTCTACATCATAATATAAAGATGTGCACTTCCGAATGGCTGAGTTTTTC
ACGCCATTCCGCAGCGGTGGAGCAGCGCAGCCACGACCCCCGCGTCCCGA
GGGCGGGTGCCGGAGGTGAGTTTACACACCGCAGTCAAGGGGCAATTCGG
GCTCGGGACTGGCCGGGCCCGGGCAAGGCTCTTAAAGCGAAACCATGTTC
CTCGGCAGGCCCTACCGCCACAGAAAGCGGCACCAGGCCGGCAAGAAAGG
GCCACTGCCACTGCCAAATCTGCAACCTGCACAGGAGAAACGGGCTGGTG
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GTCCGTCCTTGATGGCCTCCGGACGCAGGGGATGGATGCCCCCGGACCTG
ACGGTCCAGGAGAGGGAGGATGCCTGGTGGACCAGCTTCTGCGCTAGCCA
CCGCAGCTTTTGTAGCTGCGACGATCCTGTGGGCCATATTAATACTCTCG
CCCGCGATAATAGTCCTCTGGCCCAGACTCCTACTACAACTTCAGGCCAG
GGGCCGCCGCCGCCGCCTACGCCTCCGCGGACGCCGGGGCCGCGCCCTGG
GTCTGCTCCGGACCAGGGGGGAAGGATCAGGGCCTCCTGGACCTACCCCC
TAGCCCCCGGAGGTCCCGGTAGCACGCCATGGCCTACTGGTGGGGCCGGA
GACGCCGGTGGCGCCGCTGGAGGAGGCGCCGGCGTCCTCTCCGCCGCCGC
CGGCGGTGGCGGAGAAGGCGACGCTGGCCCAGAAGGCGCCGGTGGAGGCG
AAGGAGACGACGTGCGAGACCTGCTCGCCGCTATCGAAGGAGACGTGGGC
GCAGACGGGTAAGGAGACGCCGTCGCCCCCAGAAACTAGTACTGACTCAG
TGGAATCCCCAGACTGTGAGAAAGTGTGTTATTAGGGGGTTTCTGCCCCT
GTTCTTCTGCGGACAGGGGGCCTACCACAGAAACTTTACAGACCACTATG
ACGATGTGTTCCCCAAGGGACCCAGCGGAGGTGGGCACGGGAGCATGGTG
TTCAACCTGTCCTTTCTGTACCAAGAGTTTAAGAAGCACCACAATAAGTG
GTCGCGCAGCAACCTGGACTTTGACTTAGTGAGATACAAGGGCACAGTGA
TAAAGCTGTACAGACACCAGGACTTTGACTACATAGTGTGGATAAGCAGG
ACCCCTCCCTTCCAGGAGAGCCTGCTCACAGTAATGACCCACCAGCCCAG
CGTCATGCTGCAGGCAAAAAAGTGCATAATAGTAAAGAGCTACAGGACCC
ACCCGGGGGGCAAACCCTATGTAACTGCAAAAGTTAGGCCCCCCAGACTC
CTAACTGACAAGTGGTACTTCCAGTCAGACTTCTGCAACGTTCCGCTTTT
TAGCCTACAGTTTGCCCTTGCGGAACTGCGGTTTCCGATCTGCTCACCAC
AAACTGACACCAATTGCATTAACTTCCTGGTGTTAGATGACATCTACTAC
AAGTTTCTAGATAATAAGCCTAAACAGAGTTCAGACCCTAATGACGAAAA
CAGAATAAAATTCTGGCACGGCCTATGGTCCACTATGAGATATTTAAACA
CCACCTACATAAACACACTGTTTCCAGGCACAGACAGTCTAGTGGCCGCC
AAAGATACTGACAATAGTGTAAATAAATACCCCAGCACAGCCACTAAACA
GCCCTACAAAGACAGTCAGTACATGCAAAATATATGGAATACATCAAAAA
TACATGCCTTATATACGTGGGTAGCAGAGACAAACTACAAAAGACTGCAG
GCCTACTACACACAGACCTACGGAGGCTACCAGAGACAATTTTTCACAGG
AAAACAGTACTGGGACTACAGAGTAGGCATGTTTAGTCCAGCCTTCCTGA
GTCCCAGCAGACTAAATCCCCAGAACCCAGGGGCATACACAGAGGTCTCC
TACAACCCCTGGACAGACGAGGGCACGGGCAACGTAGTGTGCCTGCAGTA
TCTGACTAAAGAGACCTCAGACTACAAACCAGGTGGTGGGAGCAAGTTCT
GCATAGAAGGTGTGCCTCTATGGGCAGCGCTGGTGGGATACGTAGACATG
TGTAAAAAAGAGGGCAAGGACCCGGGCATCAGACTAAACTGTCTCCTGTT
AGTCAAGTGTCCCTATACAAAGCCTCAGCTGTATGACAAAAAAAACCCCG
AGAAACTGTTTGTACCTTACTCCTATAACTTTGGGCACGGCAAGATGCCG

GGGGGAGACAAATACATACCCATAGAGTTCAAAGACAGGTGGTACCCCTG
CCTGCTCCACCAAGAGGAGTGGATAGAGGACATTGTCAGGTCGGGACCCT
TCGTTCCAAAAGACATGCCCAGCAGCGTCACCTGCATGATGAGGTACAGC
TCTCTTTTTAACTGGGGCGGTAATATAATCCAAGAACAGGCCGTGGAAGA
CCCCTGTAAGAAAGGCACCTTCGTCGTTCCCGGAACCAGTGGCATCGCTC
GCATACTACAAGTCAGCAACCCGGCCAAGCAGACCCCCACGACAACCTGG
CACTCGTGGGACTGGAGACGATCCCTCTTTACAGAGACGGGTCTTAAAAG
AATGCGCGAACAACAACCATATGATGAACTGTCTTATACGGGCCCTAAAA
AGCCAAAACTGTCCCTTCCCGCAGGGCCCGCCGTCCCCGGTGCCGCCGTC
GCCTCCTCCTGGTGGGAAACAAAACAGGTCACCTCGCCAGACGTCAGCGA
GACGGAGACCGAAGCAGAAGCCCACCAAGAGGAAGAGACGGAGCCGGAGG
AGGGAGTCCAGCTCCAGCAGCTGTGGGAGCAGCAACTCCTGCAAAAGCGA
CAGCTGGGAGTCGTGTTCCAGCAACTCCTCCGACTCAGACAGGGGGCGGA
GATCCACCCGGGCCTCGTATAATTCCTGGGCCCCAGAACCCGTACCTGCT
TTTCCCGGAGCAGGCCCCTCCAAAAGTGCCTATTTTTGACCCCTTTGGTC
AGAAAACAGAGCTAGAGCTGTGCGGCTGCTTCGACAGGCCGCCCAGGAAC
AACCCCTACGACCACCCCTTCTACCCCTGGCTGCCCAAAGAGCCTCCCTC
CTACTACCAGGGCTACAAAGTGTCTTTCAAACTAGGGTTCCACCCAGACA
AGCATGTGTGAACCCCGCCAATAAACCACTGCTGCTACACTGATTCTTAG
GCCGTGGGAGTCTCACTGGTCGGTGTCTACCTCTTAAGGTCACTAAGCAC
TCCGAGCGTTAGCGAGGAGTGCGACCCTACCCCCTGGGCCCACTTCTTCG
GAGCCGCGCGCTACGCCTTCGGCTGCGCGCGGCACCTCAGACCCCCGCTC
GTGCTGACACGCTTGCGCGTGTCAGACCACTTCGGGCTCGCGGGGGTCGG
G (SEQ ID NO: 9)

Annotations:

Putative Domain	*Base range*
TATA Box	112 – 119
Initiator Element	128-148
Transcriptional Start Site	148
5' UTR Conserved Domain	204 – 273
ORF2	412-912
ORF2/2	412 – 908 ; 2490 – 3039
ORF2/3	412 – 908 ; 2725 – 3208
ORF1	729 – 2972
ORF1/1	729 – 908 ; 2490 – 2972

ORF1/2	729 – 908 ; 2725 – 3039
Three open-reading frame region	2699 – 2969
Poly(A) Signal	3220 - 3225
GC-rich region	3302 – 3541

Table A8. Exemplary *Anellovirus* amino acid sequences (*Alphatorquevirus*, Clade 2)

TTV-P13-1 (*Alphatorquevirus* Clade 2)	
ORF2	MASGRRGWMPPDLTVQEREDAWWTSFCASHRSFCSCDDPVGHINTLARDN SPLAQTPTTTSGQGPPPPPTPPRTPGPRPGSAPDQGGRIRASWTYPLAPGGPG STPWPTGGAGDAGGAAGGGAGVLSAAAGGGGEGDAGPEGAGGGEGDDV RDLLAAIEGDVGADG (SEQ ID NO: 10)
ORF2/2	MASGRRGWMPPDLTVQEREDAWWTSFCASHRSFCSCDDPVGHINTLARDN SPLAQTPTTTSGQGPPPPPTPPRTPGPRPGSAPDQGGRIRASWTYPLAPGGPG STPWPTGGAGDAGGAAGGGAGVLSAAAGGGGEGDAGPEGAGGGEGDDV RDLLAAIEGDVGADGPWKTPVRKAPSSFPEPVASLAYYKSATRPSRPPRQPG TRGTGDDPSLQRRVLKECANNNHMMNCLIRALKSQNCPFPQGPPSPVPPSPP PGGKQNRSPRQTSARRRPKQKPTKRKRRSRRRESSSSSCGSSNSCKSDSWES CSSNSSDSDRGRRSTRASYNSWAPEPVPAFPGAGPSKSAYF (SEQ ID NO: 11)
ORF2/3	MASGRRGWMPPDLTVQEREDAWWTSFCASHRSFCSCDDPVGHINTLARDN SPLAQTPTTTSGQGPPPPPTPPRTPGPRPGSAPDQGGRIRASWTYPLAPGGPG STPWPTGGAGDAGGAAGGGAGVLSAAAGGGGEGDAGPEGAGGGEGDDV RDLLAAIEGDVGADGARRPRCRRRLLLVGNKTGHLARRQRDGDRSRSPPRG RDGAGGGSPAPAAVGAATPAKATAGSRVPATPPTQTGGGDPPGPRIIPGPQN PYLLFPEQAPPKVPIFDPFGQKTELELCGCFDRPPRNNPYDHPFYPWLPKEPP SYYQGYKVSFKLGFHPDKHV (SEQ ID NO: 12)
ORF1	MAYWWGRRRRWRRWRRRRRPLRRRRRWRRRRRWPRRRRWRRRRRRARP ARRYRRRRGRRRVRRRRRPQKLVLTQWNPQTVRKCVIRGFLPLFFCGQGAY HRNFTDHYDDVFPKGPSGGGHGSMVFNLSFLYQEFKKHHNKWSRSNLDFD LVRYKGTVIKLYRHQDFDYIVWISRTPPFQESLLTVMTHQPSVMLQAKKCII VKSYRTHPGGKPYVTAKVRPPRLLTDKWYFQSDFCNVPLFSLQFALAELRF PICSPQTDTNCINFLVLDDIYYKFLDNKPKQSSDPNDENRIKFWHGLWSTMR

	YLNTTYINTLFPGTDSLVAAKDTDNSVNKYPSTATKQPYKDSQYMQNIWNT SKIHALYTWVAETNYKRLQAYYTQTYGGYQRQFFTGKQYWDYRVGMFSP AFLSPSRLNPQNPGAYTEVSYNPWTDEGTGNVVCLQYLTKETSDYKPGGGS KFCIEGVPLWAALVGYVDMCKKEGKDPGIRLNCLLLVKCPYTKPQLYDKK NPEKLFVPYSYNFGHGKMPGGDKYIPIEFKDRWYPCLLHQEEWIEDIVRSGP FVPKDMPSSVTCMMRYSSLFNWGGNIIQEQAVEDPCKKGTFVVPGTSGIARI LQVSNPAKQTPTTTWHSWDWRRSLFTETGLKRMREQQPYDELSYTGPKKP KLSLPAGPAVPGAAVASSWWETKQVTSPDVSETETEAEAHQEEETEPEEGV QLQQLWEQQLLQKRQLGVVFQQLLRLRQGAEIHPGLV (SEQ ID NO: 13)
ORF1/1	MAYWWGRRRRWRRWRRRRRPLRRRRRWRRRRRWPRRRRWRRRRRRARP ARRYRRRRGRRRAVEDPCKKGTFVVPGTSGIARILQVSNPAKQTPTTTWHS WDWRRSLFTETGLKRMREQQPYDELSYTGPKKPKLSLPAGPAVPGAAVASS WWETKQVTSPDVSETETEAEAHQEEETEPEEGVQLQQLWEQQLLQKRQLG VVFQQLLRLRQGAEIHPGLV (SEQ ID NO: 14)
ORF1/2	MAYWWGRRRRWRRWRRRRRPLRRRRRWRRRRRWPRRRRWRRRRRRARP ARRYRRRRGRRRGPPSPVPPSPPPGGKQNRSPRQTSARRRPKQKPTKRKRRS RRRESSSSSCGSSNSCKSDSWESCSSNSSDSDRGRRSTRASYNSWAPEPVPAF PGAGPSKSAYF (SEQ ID NO: 15)

Table N9. Exemplary *Anellovirus* nucleic acid sequence (*Alphatorquevirus*, Clade 3)

Name	Ring1
Genus/Clade	*Alphatorquevirus*, Clade 3
Accession Number	AJ620231.1

Full Sequence: 3753 bp

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1         10         20         30         40         50
|         |          |          |          |          |
TGCTACGTCACTAACCCACGTGTCCTCTACAGGCCAATCGCAGTCTATGT
CGTGCACTTCCTGGGCATGGTCTACATAATTATATAAATGCTTGCACTTC
CGAATGGCTGAGTTTTTGCTGCCCGTCCGCGGAGAGGAGCCACGGCAGGG
GATCCGAACGTCCTGAGGGCGGGTGCCGGAGGTGAGTTTACACACCGAAG
TCAAGGGGCAATTCGGGCTCAGGACTGGCCGGGCTTTGGGCAAGGCTCTT
AAAAATGCACTTTTCTCGAATAAGCAGAAAGAAAAGGAAAGTGCTACTGC
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TTTGCGTGCCAGCAGCTAAGAAAAAACCAACTGCTATGAGCTTCTGGAAA
CCTCCGGTACACAATGTCACGGGGATCCAACGCATGTGGTATGAGTCCTT
TCACCGTGGCCACGCTTCTTTTTGTGGTTGTGGGAATCCTATACTTCACA
TTACTGCACTTGCTGAAACATATGGCCATCCAACAGGCCCGAGACCTTCT
GGGCCACCGGGAGTAGACCCCAACCCCCACATCCGTAGAGCCAGGCCTGC
CCCGGCCGCTCCGGAGCCCTCACAGGTTGATTCGAGACCAGCCCTGACAT
GGCATGGGGATGGTGGAAGCGACGGAGGCGCTGGTGGTTCCGGAAGCGGT
GGACCCGTGGCAGACTTCGCAGACGATGGCCTCGATCAGCTCGTCGCCGC
CCTAGACGACGAAGAGTAAGGAGGCGCAGACGGTGGAGGAGGGGGAGACG
AAAAACAAGGACTTACAGACGCAGGAGACGCTTTAGACGCAGGGGACGAA
AAGCAAAACTTATAATAAAACTGTGGCAACCTGCAGTAATTAAAAGATGC
AGAATAAAGGGATACATACCACTGATTATAAGTGGGAACGGTACCTTTGC
CACAAACTTTACCAGTCACATAAATGACAGAATAATGAAAGGCCCCTTCG
GGGGAGGACACAGCACTATGAGGTTCAGCCTCTACATTTTGTTTGAGGAG
CACCTCAGACACATGAACTTCTGGACCAGAAGCAACGATAACCTAGAGCT
AACCAGATACTTGGGGGCTTCAGTAAAAATATACAGGCACCCAGACCAAG
ACTTTATAGTAATATACAACAGAAGAACCCCTCTAGGAGGCAACATCTAC
ACAGCACCCTCTCTACACCCAGGCAATGCCATTTTAGCAAAACACAAAAT
ATTAGTACCAAGTTTACAGACAAGACCAAAGGGTAGAAAAGCAATTAGAC
TAAGAATAGCACCCCCCACACTCTTTACAGACAAGTGGTACTTTCAAAAG
GACATAGCCGACCTCACCCTTTTCAACATCATGGCAGTTGAGGCTGACTT
GCGGTTTCCGTTCTGCTCACCACAAACTGACAACACTTGCATCAGCTTCC
AGGTCCTTAGTTCCGTTTACAACAACTACCTCAGTATTAATACCTTTAAT
AATGACAACTCAGACTCAAAGTTAAAAGAATTTTTAAATAAAGCATTTCC
AACAACAGGCACAAAAGGAACAAGTTTAAATGCACTAAATACATTTAGAA
CAGAAGGATGCATAAGTCACCCACAACTAAAAAAACCAAACCCACAAATA
AACAAACCATTAGAGTCACAATACTTTGCACCTTTAGATGCCCTCTGGGG
AGACCCCATATACTATAATGATCTAAATGAAAACAAAAGTTTGAACGATA
TCATTGAGAAAATACTAATAAAAAACATGATTACATACCATGCAAAACTA
AGAGAATTTCCAAATTCATACCAAGGAAACAAGGCCTTTTGCCACCTAAC
AGGCATATACAGCCCACCATACCTAAACCAAGGCAGAATATCTCCAGAAA
TATTTGGACTGTACACAGAAATAATTTACAACCCTTACACAGACAAAGGA
ACTGGAAACAAAGTATGGATGGACCCACTAACTAAAGAGAACAACATATA
TAAAGAAGGACAGAGCAAATGCCTACTGACTGACATGCCCCTATGGACTT
TACTTTTTGGATATACAGACTGGTGTAAAAAGGACACTAATAACTGGGAC
TTACCACTAAACTACAGACTAGTACTAATATGCCCTTATACCTTTCCAAA
ATTGTACAATGAAAAAGTAAAAGACTATGGGTACATCCCGTACTCCTACA
AATTCGGAGCGGGTCAGATGCCAGACGGCAGCAACTACATACCCTTTCAG

TTTAGAGCAAAGTGGTACCCCACAGTACTACACCAGCAACAGGTAATGGA
GGACATAAGCAGGAGCGGGCCCTTTGCACCTAAGGTAGAAAAACCAAGCA
CTCAGCTGGTAATGAAGTACTGTTTTAACTTTAACTGGGGCGGTAACCCT
ATCATTGAACAGATTGTTAAAGACCCCAGCTTCCAGCCCACCTATGAAAT
ACCCGGTACCGGTAACATCCCTAGAAGAATACAAGTCATCGACCCGCGGG
TCCTGGGACCGCACTACTCGTTCCGGTCATGGGACATGCGCAGACACACA
TTTAGCAGAGCAAGTATTAAGAGAGTGTCAGAACAACAAGAAACTTCTGA
CCTTGTATTCTCAGGCCCAAAAAAGCCTCGGGTCGACATCCCAAAACAAG
AAACCCAAGAAGAAAGCTCACATTCACTCCAAAGAGAATCGAGACCGTGG
GAGACCGAGGAAGAAAGCGAGACAGAAGCCCTCTCGCAAGAGAGCCAAGA
GGTCCCCTTCCAACAGCAGTTGCAGCAGCAGTACCAAGAGCAGCTCAAGC
TCAGACAGGGAATCAAAGTCCTCTTCGAGCAGCTCATAAGGACCCAACAA
GGGGTCCATGTAAACCCATGCCTACGGTAGGTCCCAGGCAGTGGCTGTTT
CCAGAGAGAAAGCCAGCCCCAGCTCCTAGCAGTGGAGACTGGGCCATGGA
GTTTCTCGCAGCAAAAATATTTGATAGGCCAGTTAGAAGCAACCTTAAAG
ATACCCCTTACTACCCATATGTTAAAAACCAATACAATGTCTACTTTGAC
CTTAAATTTGAATAAACAGCAGCTTCAAACTTGCAAGGCCGTGGGAGTTT
CACTGGTCGGTGTCTACCTCTAAAGGTCACTAAGCACTCCGAGCGTAAGC
GAGGAGTGCGACCCTCCCCCCTGGAACAACTTCTTCGGAGTCCGGCGCTA
CGCCTTCGGCTGCGCCGGACACCTCAGACCCCCCCTCCACCCGAAACGCT
TGCGCGTTTCGGACCTTCGGCGTCGGGGGGGTCGGGAGCTTTATTAAACG
GACTCCGAAGTGCTCTTGGACACTGAGGGGGTGAACAGCAACGAAAGTGA
GTGGGGCCAGACTTCGCCATAAGGCCTTTATCTTCTTGCCATTTGTCAGT
GTCCGGGGTCGCCATAGGCTTCGGGCTCGTTTTTAGGCCTTCCGGACTAC
AAAAATCGCCATTTTGGTGACGTCACGGCCGCCATCTTAAGTAGTTGAGG
CGGACGGTGGCGTGAGTTCAAAGGTCACCATCAGCCACACCTACTCAAAA
TGGTGGACAATTTCTTCCGGGTCAAAGGTTACAGCCGCCATGTTAAAACA
CGTGACGTATGACGTCACGGCCGCCATTTTGTGACACAAGATGGCCGACT
TCCTTCCTCTTTTTCAAAAAAAAGCGGAAGTGCCGCCGCGGCGGCGGGGG
GCGGCGCGCTGCGCGCGCCGCCCAGTAGGGGGAGCCATGCGCCCCCCCCC
GCGCATGCGCGGGGCCCCCCCCCGCGGGGGGCTCCGCCCCCCGGCCCCCC
CCG (SEQ ID NO: 16)

Annotations:

Putative Domain	*Base range*
TATA Box	83 – 88
Cap Site	104 – 111

Transcriptional Start Site	111
5' UTR Conserved Domain	170 – 240
ORF2	336 – 719
ORF2/2	336 – 715 ; 2363 – 2789
ORF2/3	336 – 715 ; 2565 – 3015
ORF2t/3	336 – 388 ; 2565 – 3015
ORF1	599 – 2830
ORF1/1	599 – 715 ; 2363 – 2830
ORF1/2	599 – 715 ; 2565 – 2789
Three open-reading frame region	2551 – 2786
Poly(A) Signal	3011 – 3016
GC-rich region	3632 – 3753

Table A9. Exemplary *Anellovirus* amino acid sequences (*Alphatorquevirus*, Clade 3)

Ring1 (*Alphatorquevirus* Clade 3)	
ORF2	MSFWKPPVHNVTGIQRMWYESFHRGHASFCGCGNPILHITALAETYGHPTGPRPSG PPGVDPNPHIRRARPAPAAPEPSQVDSRPALTWHGDGGSDGGAGGSGSGGPVADFA DDGLDQLVAALDDEE (SEQ ID NO: 17)
ORF2/2	MSFWKPPVHNVTGIQRMWYESFHRGHASFCGCGNPILHITALAETYGHPTGPRPSG PPGVDPNPHIRRARPAPAAPEPSQVDSRPALTWHGDGGSDGGAGGSGSGGPVADFA DDGLDQLVAALDDEELLKTPASSPPMKYPVPVTSLEEYKSSTRGSWDRTTRSGHGT CADTHLAEQVLRECQNNKKLLTLYSQAQKSLGSTSQNKKPKKKAHIHSKENRDRG RPRKKARQKPSRKRAKRSPSNSSCSSSTKSSSSSDRESKSSSSSS (SEQ ID NO: 18)
ORF2/3	MSFWKPPVHNVTGIQRMWYESFHRGHASFCGCGNPILHITALAETYGHPTGPRPSG PPGVDPNPHIRRARPAPAAPEPSQVDSRPALTWHGDGGSDGGAGGSGSGGPVADFA DDGLDQLVAALDDEEPKKASGRHPKTRNPRRKLTFTPKRIETVGDRGRKRDRSPLA REPRGPLPTAVAAAVPRAAQAQTGNQSPLRAAHKDPTRGPCKPMPTVGPRQWLFP ERKPAPAPSSGDWAMEFLAAKIFDRPVRSNLKDTPYYPYVKNQYNVYFDLKFE (SEQ ID NO: 19)
ORF2t/3	MSFWKPPVHNVTGIQRMWPKKASGRHPKTRNPRRKLTFTPKRIETVGDRGRKRDR SPLAREPRGPLPTAVAAAVPRAAQAQTGNQSPLRAAHKDPTRGPCKPMPTVGPRQ WLFPERKPAPAPSSGDWAMEFLAAKIFDRPVRSNLKDTPYYPYVKNQYNVYFDLK FE (SEQ ID NO: 20)

ORF1	MAWGWWKRRRRWWFRKRWTRGRLRRRWPRSARRRPRRRRVRRRRRWRRGRRK TRTYRRRRRFRRRGRKAKLIIKLWQPAVIKRCRIKGYIPLIISGNGTFATNFTSHINDR IMKGPFGGGHSTMRFSLYILFEEHLRHMNFWTRSNDNLELTRYLGASVKIYRHPDQ DFIVIYNRRTPLGGNIYTAPSLHPGNAILAKHKILVPSLQTRPKGRKAIRLRIAPPTLFT DKWYFQKDIADLTLFNIMAVEADLRFPFCSPQTDNTCISFQVLSSVYNNYLSINTFN NDNSDSKLKEFLNKAFPTTGTKGTSLNALNTFRTEGCISHPQLKKPNPQINKPLESQ YFAPLDALWGDPIYYNDLNENKSLNDIIEKILIKNMITYHAKLREFPNSYQGNKAFC HLTGIYSPPYLNQGRISPEIFGLYTEIIYNPYTDKGTGNKVWMDPLTKENNIYKEGQS KCLLTDMPLWTLLFGYTDWCKKDTNNWDLPLNYRLVLICPYTFPKLYNEKVKDY GYIPYSYKFGAGQMPDGSNYIPFQFRAKWYPTVLHQQQVMEDISRSGPFAPKVEKP STQLVMKYCFNFNWGGNPIIEQIVKDPSFQPTYEIPGTGNIPRRIQVIDPRVLGPHYSF RSWDMRRHTFSRASIKRVSEQQETSDLVFSGPKKPRVDIPKQETQEESSHSLQRESR PWETEEESETEALSQESQEVPFQQQLQQQYQEQLKLRQGIKVLFEQLIRTQQGVHV NPCLR (SEQ ID NO: 21)
ORF1/1	MAWGWWKRRRRWWFRKRWTRGRLRRRWPRSARRRPRRRRIVKDPSFQPTYEIPG TGNIPRRIQVIDPRVLGPHYSFRSWDMRRHTFSRASIKRVSEQQETSDLVFSGPKKPR VDIPKQETQEESSHSLQRESRPWETEEESETEALSQESQEVPFQQQLQQQYQEQLKL RQGIKVLFEQLIRTQQGVHVNPCLR (SEQ ID NO: 22)
ORF1/2	MAWGWWKRRRRWWFRKRWTRGRLRRRWPRSARRRPRRRRAQKSLGSTSQNKK PKKKAHIHSKENRDRGRPRKKARQKPSRKRAKRSPSNSSCSSSTKSSSSSDRESKSSS SSS (SEQ ID NO: 23)

Table N10. Exemplary *Anellovirus* nucleic acid sequence (*Alphatorquevirus*, Clade 4)

Name	TTV-HD20a
Genus/Clade	*Alphatorquevirus*, Clade 4
Accession Number	FR751492.1

Full Sequence: 3878 bp

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1         10         20         30         40         50
|         |          |          |          |          |
AAATACGTCACTAACCACGTGACTCCCACAGGCCAACCACAGTCTATGTC
GTGCACTTCCTGGGCATGGTCTACGTGATAATATAAAGCGGTGCACTTCC
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GAATGGCTGAGTTTTCCACGCCCGTCCGCAGCGAGATCGCGACGTAGGAG
CGATCGAGCGTCCCGAGGGCGGGTGCCGGAGGTGAGTTTACACACCGCAG
TCAAGGGGCAATTCGGGCTCGGGAGGCCGGGCCATGGGCAAGGCTCTTAA
AAAGCTATGTTTCTCGGTAAAATCTACAGGAAGAAAAGGAAACTGCTTCT
GCAGGCTGTGCGTGCTCCGCAGACGCCATCTTCCATGAGCCGCTGCTGGT
GTCCCCCTCGGGGTGATGTCTCCTCCCGCGAGTCTCGATGGTACGAGGCG
GTTCGAGGAAGCCACGATGCTTTTTGTGGCTGTAGTGATCCTATTCTTCA
TCTTTCTCGTCTGGCTGCACGTTTTAACCATCAGGGACCTCCGACGCCCC
CCACGGACGACCGTGCGCCGCAGAATACCCCAGTGAGACGCCTGCTGCCT
CTCCCCAGCTACCCCGGCGAGGGTCCCCAGGCTAGATGGCCTGGTGGGGA
TGGAGGCGCCGCTGGTGGCGACCGAAGAGAAGGTGGAGATGGCGGCGCGC
GCGCCGCCGAAGACGAGTACCAGCCCGAAGACCTAGACGAGCTTTTCGGC
GCTATCGAACAAGAACAGTAAGGAGGAGGCGAAGGGGGAGGCGGAGGGGC
TACCGGCGCCGTTACAGACTGAGACGCTATGCCAGACGCAGGTTCCGACG
CAAAAAGATAGTACTGACTCAGTGGAACCCCCAGACTACCAGAAAATGTA
TAATAAGGGGCATGATGCCAGTACTGTGGGCCGGCATGGGTACGGGGGGC
AGAAACTATGCAGTGAGGTCAGATGACTATGTGGTGAACAAAGGGTTCGG
GGGCTCCTTCGCCACGGAGACCTTCTCCCTGAAGGTTCTCTATGACCAGT
TTCAAAGGGGCTTCAACAGGTGGTCCCACACTAACGAGGACCTAGACCTG
GCCCGCTACAGGGGCTGCAGGTGGACTTTTTACAGACATAAAGACACAGA
CTTTATAGTGTACTTTACAAACAATCCTCCCATGAAGACCAACCAGTTCT
CCGCGCCCCTGACGACCCCCGGCATGCTCATGCGCAGTAAATACAAAGTC
CTCATTCCCAGCTTCCAGACCAGACCCAAGGGTCGCAAAACAGTAACCGT
TAAAATAAGACCCCCCAAACTATTTCAAGACAAGTGGTACACCCAGCAGG
ACCTGTGTTCAGTTCCTCTTGTCCAACTGAACGTGACCGCAGCTGATTTC
ACACATCCGTTCGGCTCACCACTAACTGAAACTCCTTGCGTAGAGTTCCA
GGTGCTGGGTGACTTGTACAATACATGTCTCAATATCGACCTTCCGCAAT
TTAGTGAATTAGGAGAAATAACTAGTGCCTACTCAAAACCAAACTCAAAT
AACCTAAAAGAATTATACAAAGAATTGTTCACAAAAGCCACATCAGGACA
CTACTGGCAGACATTCATAACCAACAGCATGGTCAGAGCACACATAGATG
CAGACAAAGCTAAAGAAGCACAAAGAGCATCCACCACACCCTCATACAAC
AATGACCCCTTCCCCACAATACCTGTTAAATCAGAGTTTGCACAGTGGAA
AAAGAAATTCACAGACACTAGAGACAGCCCCTTTCTTTTTGCCACTTACC
ATCCCGAAGCTATAAAAGACACAATTATGAAAATGAGAGAGAACAACTTT
AAGCTAGAGACAGGACCCAATGACAAGTATGGAGACTACACAGCACAGTA
CCAAGGAAACACACACATGCTAGACTACTACCTTGGCTTTTACAGCCCCA
TATTCCTCTCAGATGGAAGGTCTAACGTAGAATTCTTCACTGCCTACAGA
GACATAGTATACAATCCCTTCTTAGACAAGGCCCAGGGCAACATGGTGTG

GTTTCAGTACCACACAAAGACAGACAACAAGTTTAAAAAACCAGAGTGCC
ACTGGGAAATCAAAGACATGCCCCTGTGGGCCCTCCTAAACGGATATGTA
GACTACTTAGAGACTCAAATACAGTATGGTGACCTCAGTAAAGAAGGGAA
AGTCCTCATCAGGTGTCCCTACACCAAGCCAGCACTAGTAGACCCCAGAG
ACGACACTGCAGGATATGTAGTCTACAACAGAAACTTTGGCAGAGGCAAG
TGGATAGACGGAGGGGGCTACATCCCCCTGCACGAGAGGACAAAATGGTA
CGTGATGCTCAGATACCAGACGGACGTCTTCCATGACATAGTGACCTGTG
GGCCCTGGCAGTACAGAGACGACAACAAAAACAGCCAGCTAGTGGCCAAA
TACCGCTTCAGCTTTATATGGGGAGGTAACACTGTCCACTCTCAGGTCAT
CAGAAACCCGTGCAAAGACAACCAAGTATCCGGTCCCCGTCGACAGCCTA
GGGATATACAAGTCGTTGACCCGCAACGCATCACGCCGCCGTGGGTCCTC
CACAGCTTCGACCAGCGAAGAGGCCTCTTTACTGAAACAGCTCTCAGGCG
CCTGCTCCAGGAACCACTACCTGGCGAGTATGCTGTTAGCACCCTCAGGA
CACCCCTCCTCTTTCTACCCTCAGAATACCAGCGAGAAGACGGCGCTGCA
GAAAGCGCCTCAGGTTCACCGGCCAAAAGACCCCGTATCTGGTCAGAAGA
GAGTCAGACGGAGACGATCTCCTCGGAGGAGAACCCGGCGGAGACGACGA
GGGAGCTCCTCCAGCGAAAGCTCCGAGAGCAGCGAGCACTCCAGTTCCAA
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AAACCCCCTGTTATCTTTCCCGCAATGAATAAGGTCTTTCTGTTTCCCCC
AGAGGGTCCCAAGCCCATCCTGGGCAAAGAGGCCTGGCAGGACGAGTACG
AGACCTGCAGGGTCTGGAACAGACCTGCCAGAACCCACCACACAGACACC
CCCTTCTATCCCTGGGCCCCCCACAAGTTCCATGTAAGCTTCAAACTTGG
CTTCCAATAAAATTACTAGGCCGTGGAACTCTCACTGGTCGGTGTCTACC
TCTTAAGGTCACTAAGCACTCCGAGCGTCAGCGAGGAGTGCGACCCTCTA
CCCTGGTGCAACGCCCTCGGCGGCCGCGCGCTACGCCTTCGGCTGCGCGC
GGCACCTCGGACCCCCGCTCGTGCTGACGCGCTCGCGCGCGTCAGACCAC
TTCGGGCTCGCGGGGGTCGGGAATTTTGCTAAACAGACTCCGAGTTGCCA
TTGGACACTGTAGCTGTGAATCAGTAACGAAAGTGAGTGGGGCCAGACTT
CGCCATAGGGCCTTTATCTTCTTGCCATTGGTCCGTGTAGGGGGTCGCCA
TAGGCTTCGACCTCCCTTTTAGGCCTTCCGGACTACAAAAATGGCGGATT
CAGTGACGTCACGGCCGCCATTTTAAGTAGGTGCCGTCCAGGACTGCAGT
TCCGGGTCAGAGTGCATCCTCGGCGGAACCTGCACAAAATGGCGGTCAAT
ATCTTCCGGGTCAAAGGTCACACCTACGTCATAAGTCACGTGACTGGGTC
CTGCTACGTCATATGCGGAAGTAGGCCCCGCCACGTGACTCGTCACGTGG
GCGCTGCGTCACGGCGGCCATTTTGTATCACAAAATGGCGGACTTCCTTC
CTCTTTTTTAAAAATAACGGCCCAGCGGCGGCGCGCGCGCTTCGCGCGCG
CGCCGGGGGGGCTCCGCCCCCCCCCGCGCATGCGCGGGGCCCCCCCCCGCG
GGGGGCTCCGCCCCCCGGTCCCCCCCCG (SEQ ID NO: 24)

Annotations:

Putative Domain	*Base range*
TATA Box	82 – 87
Initiator Element	95-115
Transcriptional Start Site	115
5' UTR Conserved Domain	170 - 238
ORF2	335 - 721
ORF2/2	335 – 717 ; 2446 – 2902
ORF2/3	335 – 717 ; 2675 – 3109
ORF1	586 – 2928
ORF1/1	586 – 717 ; 2446 – 2928
ORF1/2	586 – 717 ; 2675 – 2902
Three open-reading frame region	2640 – 2899
Poly(A) Signal	3106 - 3114
GC-rich region	3768 – 3878

Table A10. Exemplary *Anellovirus* amino acid sequences (*Alphatorquevirus*, Clade 4)

TTV-HD20a (*Alphatorquevirus* Clade 4)	
ORF2	MSRCWCPPRGDVSSRESRWYEAVRGSHDAFCGCSDPILHLSRLAARFNHQG PPTPPTDDRAPQNTPVRRLLPLPSYPGEGPQARWPGGDGGAAGGDRREGGD GGARAAEDEYQPEDLDELFGAIEQEQ (SEQ ID NO: 25)
ORF2/2	MSRCWCPPRGDVSSRESRWYEAVRGSHDAFCGCSDPILHLSRLAARFNHQG PPTPPTDDRAPQNTPVRRLLPLPSYPGEGPQARWPGGDGGAAGGDRREGGD GGARAAEDEYQPEDLDELFGAIEQEQSSETRAKTTKYPVPVDSLGIYKSLTR NASRRRGSSTASTSEEASLLKQLSGACSRNHYLASMLLAPSGHPSSFYPQNT SEKTALQKAPQVHRPKDPVSGQKRVRRRRSPRRRTRRRRRGSSSSESSESSE HSSSNSSTSRSNSPRPRRIST (SEQ ID NO: 26)
ORF2/3	MSRCWCPPRGDVSSRESRWYEAVRGSHDAFCGCSDPILHLSRLAARFNHQG PPTPPTDDRAPQNTPVRRLLPLPSYPGEGPQARWPGGDGGAAGGDRREGGD

	GGARAAEDEYQPEDLDELFGAIEQEQIPARRRRCRKRLRFTGQKTPYLVRRE SDGDDLLGGEPGGDDEGAPPAKAPRAASTPVPTPALRGPTRQDPGESPRKPP VIFPAMNKVFLFPPEGPKPILGKEAWQDEYETCRVWNRPARTHHTDTPFYP WAPHKFHVSFKLGFQ (SEQ ID NO: 27)
ORF1	MAWWGWRRRWWRPKRRWRWRRARRRRRVPARRPRRAFRRYRTRTVRR RRRGRRRGYRRRYRLRRYARRRFRRKKIVLTQWNPQTTRKCIIRGMMPVL WAGMGTGGRNYAVRSDDYVVNKGFGGSFATETFSLKVLYDQFQRGFNRW SHTNEDLDLARYRGCRWTFYRHKDTDFIVYFTNNPPMKTNQFSAPLTTPGM LMRSKYKVLIPSFQTRPKGRKTVTVKIRPPKLFQDKWYTQQDLCSVPLVQL NVTAADFTHPFGSPLTETPCVEFQVLGDLYNTCLNIDLPQFSELGEITSAYSK PNSNNLKELYKELFTKATSGHYWQTFITNSMVRAHIDADKAKEAQRASTTP SYNNDPFPTIPVKSEFAQWKKKFTDTRDSPFLFATYHPEAIKDTIMKMRENN FKLETGPNDKYGDYTAQYQGNTHMLDYYLGFYSPIFLSDGRSNVEFFTAYR DIVYNPFLDKAQGNMVWFQYHTKTDNKFKKPECHWEIKDMPLWALLNGY VDYLETQIQYGDLSKEGKVLIRCPYTKPALVDPRDDTAGYVVYNRNFGRGK WIDGGGYIPLHERTKWYYVMLRYQTDVFHDIVTCGPWQYRDDNKNSQLVA KYRFSFIWGGNTVHSQVIRNPCKDNQVSGPRRQPRDIQVVDPQRITPPWVLH SFDQRRGLFTETALRRLLQEPLPGEYAVSTLRTPLLFLPSEYQREDGAAESAS GSPAKRPRIWSEESQTETISSEENPAETTRELLQRKLREQRALQFQLQHFAVQ LAKTQANLHVNPLLSFPQ (SEQ ID NO: 28)
ORF1/1	MAWWGWRRRWWRPKRRWRWRRARRRRRVPARRPRRAFRRYRTRTVIRN PCKDNQVSGPRRQPRDIQVVDPQRITPPWVLHSFDQRRGLFTETALRRLLQE PLPGEYAVSTLRTPLLFLPSEYQREDGAAESASGSPAKRPRIWSEESQTETISS EENPAETTRELLQRKLREQRALQFQLQHFAVQLAKTQANLHVNPLLSFPQ (SEQ ID NO: 29)
ORF1/2	MAWWGWRRRWWRPKRRWRWRRARRRRRVPARRPRRAFRRYRTRTNTSE KTALQKAPQVHRPKDPVSGQKRVRRRRSPRRRTRRRRRGSSSSESSESSEHS SSNSSTSRSNSPRPRRIST (SEQ ID NO: 30)

Table N11. Exemplary *Anellovirus* nucleic acid sequence (*Alphatorquevirus*, Clade 5)

Name	TTV-16 (TUS01)
Genus/Clade	*Alphatorquevirus*, Clade 5
Accession Number	AB017613.1

Full Sequence: 3818 bp

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1         10         20         30         40         50
|         |          |          |          |          |
AAGTCCGCCACTAACCACGTGACTCCCGCAGGCCAACCCAGTACTATGTC
GTCCACTTCCTGGGACGAGTCTACGTCCTGATATAAGTAAGTGCACTTCC
GAATGGCTGAGTTTTCCACGCCCGTCCGCAGCGAGAACGCCACGGAGGGG
AGTCCGCGCGTCCCGAGGGCGGGTGCCGGAGGTGAGTTTACACACCGCAG
TCAAGGGGCAATTCGGGCTCGGGACTGGCCGGGCCCCGGGCAAGGCTCTT
AAAAAATGCACTTTCGCAGAGTGCGAGCGAAAAGGAAACTGCTACTGCAA
GCTGTGCGAGCTCCACCGAAGGCACCTGCCATGAGCTTCACCACACCTAC
TATTAATGCCGGGATCCGAGAGCAGCAATGGTTCGAGTCCACCCTTAGAT
CCCACCACTCGTTCTGTGGCTGTGGTGATCCCGTGCTTCATTTTACTAAC
CTTGCTACTCGCTTTAACTATCTGCCTGCTACCTCTTCGCCTCTGGACCC
TCCCGGCCCAGCGCCGCGAGGCCGCCCGGCGCTCCGCCGCCTCCCGGCAC
TCCCTTCAGCCCCCGCGACCCCTTCTAGAGAACTAGCATGGCCTACTGGT
TCAGAAGGTGGGGCTGGAGGCCGAGGCGCCGGTGGAGAAGGTGGCGCCGC
CGTCGAAGGAGACTACCGAGAAGAAGAACTAGACGAGCTGTTCGCGGCCT
TGGAAGAAGACGCAAACCAAGGGTAAGGAGGCGCCGCAGAACTCGCAGAC
GTACCTACAGACGGGGGTGGAGACGCAGGAGGTACATAAGACGGGGGCGA
CGCAAAAAGAAACTCATACTGACTCAGTGGAACCCGGCAATAGTTAAGAG
GTGCAACATTAAGGGCGGACTTCCAATAATTATATGCGGAGAGCCCAGGG
CAGCCTTTAACTATGGCTACCACATGGAGGACTACACTCCTCAACCTTTC
CCCTTCGGAGGGGGAATGAGCACAGTGACTTTCTCTCTGAAAGCCTTGTA
TGACCAGTACCTAAAACACCAAAACAGGTGGACTTTCTCAAACGACCAGC
TAGACCTCGCCAGATACAGGGGCTGTAAACTAAGGTTCTACAGAAGCCCC
GTCTGTGACTTTATAGTACACTACAACCTAATACCTCCACTAAAAATGAA
CCAGTTCACAAGTCCCAACACGCACCCGGGACTACTCATGCTCAGCAAAC
ACAAGATAATAATTCCCAGCTTTCAAACAAGACCTGGGGGCAGACGCTTT
GTTAAAATAAGACTTAATCCCCCCAAACTATTTGAAGACAAGTGGTACAC
TCAGCAAGACCTGTGCAAGGTTCCGCTCGTTAGTATTACAGCAACTGCGG
CTGACTTGCGGTATCCGTTCTGCTCACCACAAACGAACAACCCTTGCACC
ACCTTCCAGGTACTGCGCAAGAACTACAATACAGTTATAGGAACTTCCGT
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AAAAGACCAAGAGTCCACACAAGACTTTGAAAATTGGCTTTATAAAACAG
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GCATTTAATCCTGATGGCACTAAAAACACTAAACAGCAGTCGTGGCAAGA
TAACTGGAGCAAAAAAAATTCACCATGGACAGGTAACTCAGGTACATACC
CACAAACAACCAGTGAAATGTACAAAATTCCATATGACAGTAACTTCGGC
TTTCCCACATACAGAGCCCAAAAAGACTACATTTTAGAAAGAAGACAGTG
CAACTTTAACTATGAAGTTAATAATCCAGTTAGCAAAAAAGTATGGCCAC
AACCTAGTACAACAACACCCACAGTAGACTACTATGAATACCACTGTGGA
TGGTTCAGCAACATATTCATAGGCCCCAACAGATACAACCTACAGTTTCA
AACAGCATATGTAGACACCACATACAACCCACTAATGGACAAGGGCAAAG
GCAACAAAATATGGTTTCAATATCTGTCTAAAAAGGGCACAGACTACAAT
GAAAAACAATGCTACTGCACCCTAGAAGACATGCCCCTATGGGCAATATG
CTTTGGATACACTGACTATGTAGAGACTCAACTAGGACCCAATGTGGACC
ATGAAACAGCAGGCTTAATAATTATGATCTGTCCATACACTCAACCACCT
ATGTATGACAAAAACAGACCTAACTGGGGATACGTAGTCTATGACACAAA
CTTTGGCAATGGAAAAATGCCCTCAGGAAGTGGCCAAGTCCCAGTATACT
GGCAATGCCGATGGAGGCCCATGCTGTGGTTCCAACAACAAGTACTCAAT
GACATCTCAAAGACTGGACCGTACGCCTACAGAGACGAATATAAAAATGT
ACAACTGACTCTCTACTACAACTTTATTTTTAACTGGGGGGGCGACATGT
ATTACCCACAGGTCGTTAAAAACCCCTGTGGAGACTCCGGAATCGTTCCC
GGTTCCGGTAGATTCACTCGAGAAGTACAAGTCGTTAGCCCGCTTTCCAT
GGGACCGGCCTACATCTTCCACTACTTCGACTCCAGACGCGGGTTCTTTA
GTGAAAAAGCTCTTAAAAGAATGCAACAACAACAAGAATTTGATGAATCT
TTTACATTCAAACCTAAGAGACCCAAACTTTCTACAGCAGCCGCAGAAAT
CCTCCAGCTCGAAGAAGACTCGACTTCAGGGGAAGGAAAATCGCCACTAC
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CAGCTCCAGCGAAACATCCAGGAGCAGCTCGCAATCAAGCAGCAGCTCCA
ATTCCTCTTGCTCCAACTCCTCAAAACCCAATCCAATTTGCATTTAAACC
CACAATTTTTAAGCCCTTCATAAAATATGACATGTTTGGGGACCCCCTTC
CTCACCCCCCAACAGCCGAAGAGTGGGAAACAGAGTACCAGTGCTGTAAG
GCCTTTAACAGACCACCTAGAACCAACCTAAAAGACACCCCCTTCTACCC
CTGGGTACCTAAACCTAAACCTCAATTCCGTGTATCTTTTAAACTTGGTT
TTCAATAAACAAGGCCGTGGGAGTTTCACTTGTCGGTGTCAACCTCTTAA
GGTCACTAAGCACTCCGAGCGTAAGCGAGGAGTGCGACCCTCCCCCCTGG
GGCAACTCCCTCGAAGTCCGGCGCTACGCGCTTCGCGCTGCGCCGGACAT
CTCGGACCCCCCCTCCACCCGAAACGCTTGCGCGTTTCGGACCTTCGGCG
TCGGGGGGGTCGGGGGCTTTACTAAACAGACTCCGAGGTGCCATTGGACA
CTGAGGGGATGAACAGCAACGAAAGTGAGTGGGGCCAGACTTCGCCATAA

GGCCTTTATCTTCTTGCCATTTGTCAGTATAGAGGGTCGCCATAGGCTTC
GGCCTCCATTTTAACCTCTAAAAACTACCAAAATGGCCGTTCCAGTGACG
TCACAGCCGCCATTTTAAGTAGCTGACGTCAAGGATTGACGTGAAGGTTA
AAGGTCATCCTCGGCGGAAGCTACACAAAATGGTGGACAACATCTTCCGG
GTCAAAGGTCGTGCACACGTCATAAGTCACGTGGTGGGGACCCGCTGTAA
CCCGGAAGTAGGCCCCGTCACGTGATTTGTCACGTGTGTACACGTCACAA
CCGCCATTTTGTTTTACAAAATGGCTGACTTCCTTCCTCTTTTTTAAAAA
AAACGGCCGTGCGGCGGCGCGCGCTTCGCGCGCGCCGGGGGCTGCC
GCCCCCCCCCGCGCATGCGCGCGGGGCCCCCCCCCGCGGGGGGCTCCGCC
CCCCGGCCCCCCCCCCCG (SEQ ID NO: 31)

Annotations:

Putative Domain	*Base range*
TATA Box	82 – 86
Initiator Element	100-115
Transcriptional Start Site	115
5' UTR Conserved Domain	170 - 240
ORF2	331- 726
ORF2/2	331 – 722 ; 2412 – 2847
ORF2/3	331 – 722 ; 2638 – 3058
ORF2t/3	331 – 380 ; 2638 - 3058
ORF1	588 – 2873
ORF1/1	588 – 722 ; 2412 – 2873
ORF1/2	588 – 722 ; 2638 – 2847
Three open-reading frame region	2699 – 2969
Poly(A) Signal	3220 - 3225
GC-rich region	3302 – 3541

Table A11. Exemplary *Anellovirus* amino acid sequences (*Alphatorquevirus*, Clade 5)

TTV-16-TUS01 (*Alphatorquevirus* Clade 5)	
ORF2	MSFTTPTINAGIREQQWFESTLRSHHSFCGCGDPVLHFTNLATRFNYLPATSS PLDPPGPAPRGRPALRRLPALPSAPATPSRELAWPTGSEGGAGGRGAGGEGG AAVEGDYREEELDELFAALEEDANQG (SEQ ID NO: 32)

ORF2/2	MSFTTPTINAGIREQQWFESTLRSHHSFCGCGDPVLHFTNLATRFNYLPATSS PLDPPGPAPRGRPALRRLPALPSAPATPSRELAWPTGSEGGAGGRGAGGEGG AAVEGDYREEELDELFAALEEDANQGSLKTPVETPESFPVPVDSLEKYKSLA RFPWDRPTSSTTSTPDAGSLVKKLLKECNNNKNLMNLLHSNLRDPNFLQQP QKSSSSKKTRLQGKENRHYSKKRKKSKSSKRRQYSSSSSETSRSSSQSSSSSN SSCSNSSKPNPICI (SEQ ID NO: 33)
ORF2/3	MSFTTPTINAGIREQQWFESTLRSHHSFCGCGDPVLHFTNLATRFNYLPATSS PLDPPGPAPRGRPALRRLPALPSAPATPSRELAWPTGSEGGAGGRGAGGEGG AAVEGDYREEELDELFAALEEDANQGSRRNPPARRRLDFRGRKIATTARRE RSRSPPNADSTAPAPAKHPGAARNQAAAPIPLAPTPQNPIQFAFKPTIFKPFIK YDMFGDPLPHPPTAEEWETEYQCCKAFNRPPRTNLKDTPFYPWVPKPKPQF RVSFKLGFQ (SEQ ID NO: 34)
ORF2t/3	MSFTTPTINAGIREQQCSRRNPPARRRLDFRGRKIATTARRERSRSPPNADST APAPAKHPGAARNQAAAPIPLAPTPQNPIQFAFKPTIFKPFIKYDMFGDPLPH PPTAEEWETEYQCCKAFNRPPRTNLKDTPFYPWVPKPKPQFRVSFKLGFQ (SEQ ID NO: 35)
ORF1	MAYWFRRWGWRPRRRWRRWRRRRRRLPRRRTRRAVRGLGRRRKPRVRR RRRTRRRTYRRGWRRRRYIRRGRRKKKLILTQWNPAIVKRCNIKGGLPIIICG EPRAAFNYGYHMEDYTPQPFPFGGGMSTVTFSLKALYDQYLKHQNRWTFS NDQLDLARYRGCKLRFYRSPVCDFIVHYNLIPPLKMNQFTSPNTHPGLLMLS KHKIIIPSFQTRPGGRRFVKIRLNPPKLFEDKWYTQQDLCKVPLVSITATAAD LRYPFCSPQTNNPCTTFQVLRKNYNTVIGTSVKDQESTQDFENWLYKTDSH YQTFATEAQLGRIPAFNPDGTKNTKQQSWQDNWSKKNSPWTGNSGTYPQT TSEMYKIPYDSNFGFPTYRAQKDYILERRQCNFNYEVNNPVSKKVWPQPST TTPTVDYYEYHCGWFSNIFIGPNRYNLQFQTAYVDTTYNPLMDKGKGNKIW FQYLSKKGTDYNEKQCYCTLEDMPLWAICFGYTDYVETQLGPNVDHETAG LIIMICPYTQPPMYDKNRPNWGYVVYDTNFGNGKMPSGSGQVPVYWQCR WRPMLWFQQQVLNDISKTGPYAYRDEYKNVQLTLYYNFIFNWGGDMYYP QVVKNPCGDSGIVPGSGRFTREVQVVSPLSMGPAYIFHYFDSRRGFFSEKAL KRMQQQQEFDESFTFKPKRPKLSTAAAEILQLEEDSTSGEGKSPLQQEEKEV

	EVLQTPTVQLQLQRNIQEQLAIKQQLQFLLLQLLKTQSNLHLNPQFLSPS (SEQ ID NO: 36)
ORF1/1	MAYWFRRWGWRPRRRWRRWRRRRRRLPRRRTRRAVRGLGRRRKPRVVK NPCGDSGIVPGSGRFTREVQVVSPLSMGPAYIFHYFDSRRGFFSEKALKRMQ QQQEFDESFTFKPKRPKLSTAAAEILQLEEDSTSGEGKSPLQQEEKEVEVLQT PTVQLQLQRNIQEQLAIKQQLQFLLLQLLKTQSNLHLNPQFLSPS (SEQ ID NO: 37)
ORF1/2	MAYWFRRWGWRPRRRWRRWRRRRRRLPRRRTRRAVRGLGRRRKPRQPQ KSSSSKKTRLQGKENRHYSKKRKKSKSSKRRQYSSSSSETSRSSSQSSSSSNS SCSNSSKPNPICI (SEQ ID NO: 38)

Table N12. Exemplary *Anellovirus* nucleic acid sequence (*Alphatorquevirus*, Clade 6)

Name	TTV-TJN02
Genus/Clade	*Alphatorquevirus*, Clade 6
Accession Number	AB028669.1

Full Sequence: 3794 bp

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1         10         20         30         40         50
|         |          |          |          |          |
CCCGAAGTCCGTCACTAACCACGTGACTCCTGTCGCCCAATCAGAGTGTA
TGTCGTGCATTTCCTGGGCATGGTCTACATCCTGATATAACTAAGTGCAC
TTCCGAATGGCTGAGTTTTCCACGCCCGTCCGCAGCGAGGGAGCGACGGA
GGAGCTCCCGAGCGTCCCGAGGGCGGGTGCCGGAGGTGAGTTTACACACC
GCAGTCAAGGGGCAATTCGGGCTCGGGACTGGCCGGGCTATGGGCAAGGC
TCTTAGGGTCTTCATTCTTAATATGTTTCTTGGCAGAGTTTACCGCCACA
AGAAAAGGAAAGTGCTACTGTCCACACTGCGAGCTCCACAGGCGTCTCGC
AGGGCTATGAGTTGGCGACCCCCGGTACACGATGCACCCGGCATCGAGCG
CAATTGGTACGAGGCCTGTTTCAGAGCCCACGCTGGAGCTTGTGGCTGTG
GCAATTTTATTATGCACCTTAATCTTTTGGCTGGGCGTTATGGTTTTACT
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GAGAGCCAGGCCTAGTCCCGCCGCACCAGAGCAGCCCGCTGCCCTACCAT
GGCATGGGGATGGTGGAGATGGCGGCGCCGCTGGCCCGCCAGACGCTGGA
GGAGACGCCGTCGCCGGCGCCCCGTACGGAGAACAAGAGCTCGCCGACCT
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GCTCGACGCTATAGAAGACGACGAACAGTAAGAACCAGGCGAAGGCGGTG
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AGGGGCGACACAGAAAAAAGAAAAAGAGACTGATACTGAGACAGTGGCAA
CCAGCCACAAGACGCAGATGTACCATAACTGGGTACCTGCCCATAGTGTT
CTGCGGCCACACTAGGGGCAATAAAAACTATGCACTACACTCTGACGACT
ACACCCCCCAAGGACAACCATTTGGAGGGGCTCTAAGCACTACCTCATTC
TCTTTAAAAGTACTATTTGACCAGCATCAGAGAGGACTAAACAAGTGGTC
TTTTCCAAACGACCAACTAGACCTCGCCAGATATAGAGGCTGCAAATTTA
TATTTTATAGAACAAAACAAACTGACTGGGTGGGCCAGTATGACATATCA
GAACCCTACAAGCTAGACAAATACAGCTGCCCCAACTATCACCCTGGAAA
CATGATTAAGGCAAAGCACAAATTTTTAATACCAAGCTATGACACTAATC
CTAGAGGCAGACAAAAAATTATAGTTAAAATTCCCCCCCCAGACCTCTTT
GTAGACAAGTGGTACACTCAAGAGGATCTGTGTTCCGTTAATCTTGTGTC
ACTTGCGGTTTCTGCGGCTTCCTTTCTCCACCCATTCGGCTCACCACAAA
CTGACAACCCTTGCTACACCTTCCAGGTGTTGAAAGAGTTCTACTATCAG
GCAATAGGCTTCTCTGCAAGCACACAAGCAATGACATCAGTATTAGACAC
GCTATACACACAAAACAGTTATTGGGAATCTAATCTAACTCAGTTTTATG
TACTTAATGCAAAAAAAGGCAGTGATACAACACAGCCTTTAACTAGCAAT
ATGCCAACTCGTGAAGAGTTTATGGCAAAAAAAAATACCAATTACAACTG
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GACAAAGGGGTGGGAAACCACATTTGGTTTCAGTACAACACAAAGGCAGA
CACTCAGCTAATAGTCACAGGAGGGTCCTGCAAAGCACACATACAAGACA
TACCACTGTGGGCGGCCTTCTATGGATACAGTGACTTTATAGAGTCAGAA
CTAGGCCCCTTTGTAGATGCAGAGACGGTAGGCTTAGTGTGTGTAATATG
CCCTTATACAAAACCCCCCATGTACAACAAGACAAACCCCGCCATGGGCT
ACGTGTTCTATGACAGAAACTTTGGTGACGGAAAATGGACTGACGGACGG
GGCAAAATAGAGCCCTACTGGCAAGTTAGGTGGAGGCCCGAAATGCTTTT
CCAAGAAACTGTAATGGCAGACCTAGTTCAGACTGGGCCCTTTAGCTACA
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GACGGACGGACAACCCACCGACTCCAGTAGACACCCTAGAGGAATACAAG
TGGCGGACCCGGAACAAATGGGACCCCGCTGGGTGTTCCACTCCTTTGAC
TGGCGAAGGGGCTATCTTAGCGAGAAAGCTCTCAAACGCCTGCAAGAAAA
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TTCCTCCAACAGAATCAGCAGAGGGAGAGTTCCGAGAGCCCGAAAAAGGC
TCGTATTCAGAGGAAGAAAGGTCGCAAGCCTCTGCCGAAGAGCAGACGCA
GGAGGCGACAGTACTCCTCCTCAAGCGACGACTCAGAGAGCAACAGCAGC
TCCAGCAGCAGCTCCAATTCCTCACCCGAGAAATGTTCAAAACGCAAGCG
GGTCTCCACCTAAACCCTATGTTATTAAACCAGCGATAAACCAAGTGTAC
CTGTTTCCAGAGAGGGCCCCAAAACCCCCTCCTAGCAGCCAAGACTGGCA
GCAGGAGTACGAGGCCTGCGCAGCCTGGGACAGGCCCCCTAGATACAATC
TGTCCTCTCCTCCTTTCTACCCCAGCTGCCCTTCAAAATTCTGTGTAAAA
TTCAGCCTTGGCTTTAAATAAATGGCAACTTTACTGTGCAAGGCCGTGGG
AGTTTCACTGGTCGGTGTCTACCTCTAAAGGTCACTAAGCACTCCGAGCG
TTAGCGAGGAGTGCGACCCTTCCCCCTGACTCAACTTCTTCGGAGCCGCG
CGCTACGCCTTCGGCTGCGCGCGGCACCTCAGACCCCCGCTCGTGCTGAC
ACGCTCGCGCGTGTCAGACCACTTCGGGCTCGCGGGGGTCGGGAATTTTG
CTAAACAGACTCCGAGTTGCTCTTGGACACTGAGGGGGCATATCAGTAAC
GAAAGTGAGTGGGGCCAGACTTCGCCATAAGGCCTTTATCTTCTTGCCAT
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CGGACTACAAAAATGGCCGTTTTAGTGACGTCACGGCCGCCATTTTAAGT
AAGGCGGAAGCAGCTCGGCGTACACAAAATGGCGGCGGAGCACTTCCGGC
TTGCCCAAAATGGTGGGCAACTTCTTCCGGGTCAAAGGTCACAGCTACGT
CACAAGTCACGTGGGGAGGGTTGGCGTTTAACCCGGAAGCCAATCCTCTT
ACGTGGCCTGTCACGTGACTTGTACGTCACGACCACCATTTTGTTTTACA
AAATGGCCGACTTCCTTCCTCTTTTTTAAAAATAACGGTTCGGCGGCGGC
GCGCGCGCTACGCGCGCGCGCCGGGGGGCTGCCGCCCCCCCCCCGCGCAT
GCGCGGGGCCCCCCCCCGCGGGGGGCTCCGCCCCCGGCCCCCC (SEQ ID NO: 39)

Annotations:

Putative Domain	*Base range*
TATA Box	89 – 90
Cap Site	107 – 114
Transcriptional Start Site	114
5' UTR Conserved Domain	174 – 244
ORF2	357 – 731
ORF2/2	357 – 727 ; 2381 – 2813
ORF2/3	357 – 727 ; 2619 – 3021
ORF2t/3	357 – 406 ; 2619 – 3021
ORF1	599 – 2839

ORF1/1	599 – 727 ; 2381 – 2839
ORF1/2	599 – 727 ; 2619 – 2813
Three open-reading frame region	2596 – 2810
Poly(A) Signal	3017 – 3022
GC-rich region	3691 – 3794

Table A12. Exemplary *Anellovirus* amino acid sequences (*Alphatorquevirus*, Clade 6)

TTV-TJN02 (*Alphatorquevirus* Clade 6)	
ORF2	MSWRPPVHDAPGIERNWYEACFRAHAGACGCGNFIMHLNLLAGRYGFTPGSAPPG GPPPGTPQIRRARPSPAAPEQPAALPWHGDGGDGGAAGPPDAGGDAVAGAPYGEQ ELADLLDAIEDDEQ (SEQ ID NO: 40)
ORF2/2	MSWRPPVHDAPGIERNWYEACFRAHAGACGCGNFIMHLNLLAGRYGFTPGSAPPG GPPPGTPQIRRARPSPAAPEQPAALPWHGDGGDGGAAGPPDAGGDAVAGAPYGEQ ELADLLDAIEDDEQRSKTRARRTDNPPTPVDTLEEYKWRTRNKWDPAGCSTPLTGE GAILARKLSNACKKNLLTMTNILHNQKDLESFLQQNQQRESSESPKKARIQRKKGR KPLPKSRRRRRQYSSSSDDSESNSSSSSSSNSSPEKCSKRKRVST (SEQ ID NO: 41)
ORF2/3	MSWRPPVHDAPGIERNWYEACFRAHAGACGCGNFIMHLNLLAGRYGFTPGSAPPG GPPPGTPQIRRARPSPAAPEQPAALPWHGDGGDGGAAGPPDAGGDAVAGAPYGEQ ELADLLDAIEDDEHRGRVPRARKRLVFRGRKVASLCRRADAGGDSTPPQATTQRAT AAPAAAPIPHPRNVQNASGSPPKPYVIKPAINQVYLFPERAPKPPPSSQDWQQEYEA CAAWDRPPRYNLSSPPFYPSCPSKFCVKFSLGFK (SEQ ID NO: 42)
ORF2t/3	MSWRPPVHDAPGIERNCRGRVPRARKRLVFRGRKVASLCRRADAGGDSTPPQATT QRATAAPAAAPIPHPRNVQNASGSPPKPYVIKPAINQVYLFPERAPKPPPSSQDWQQ EYEACAAWDRPPRYNLSSPPFYPSCPSKFCVKFSLGFK (SEQ ID NO: 43)
ORF1	MAWGWWRWRRRWPARRWRRRRRRRPVRRTRARRPARRYRRRRTVRTRRRRWG RRRYRRGWRRRTYVRKGRHRKKKKRLILRQWQPATRRRCTITGYLPIVFCGHTRG NKNYALHSDDYTPQGQPFGGALSTTSFSLKVLFDQHQRGLNKWSFPNDQLDLARY RGCKFIFYRTKQTDWVGQYDISEPYKLDKYSCPNYHPGNMIKAKHKFLIPSYDTNP RGRQKIIVKIPPPDLFVDKWYTQEDLCSVNLVSLAVSAASFLHPFGSPQTDNPCYTF QVLKEFYYQAIGFSASTQAMTSVLDTLYTQNSYWESNLTQFYVLNAKKGSDTTQPL TSNMPTREEFMAKKNTNYNWYTYKAASVKNKLHQMRQTYFEELTSKGPQTTKSE EGYSQHWTTPSTNAYEYHLGMFSAIFLAPDRPVPRFPCAYQDVTYNPLMDKGVGN HIWFQYNTKADTQLIVTGGSCKAHIQDIPLWAAFYGYSDFIESELGPFVDAETVGLV

	CVICPYTKPPMYNKTNPAMGYVFYDRNFGDGKWTDGRGKIEPYWQVRWRPEMLF QETVMADLVQTGPFSYKDELKNSTLVCKYKFYFTWGGNMMFQQTIKNPCKTDGQ PTDSSRHPRGIQVADPEQMGPRWVFHSFDWRRGYLSEKALKRLQEKPLDYDEYFT QPKRPRIFPPTESAEGEFREPEKGSYSEEERSQASAEEQTQEATVLLLKRRLREQQQL QQQLQFLTREMFKTQAGLHLNPMLLNQR (SEQ ID NO: 44)
ORF1/1	MAWGWWRWRRRWPARRWRRRRRRRPVRRTRARRPARRYRRRRTTIKNPCKTDG QPTDSSRHPRGIQVADPEQMGPRWVFHSFDWRRGYLSEKALKRLQEKPLDYDEYF TQPKRPRIFPPTESAEGEFREPEKGSYSEEERSQASAEEQTQEATVLLLKRRLREQQQ LQQQLQFLTREMFKTQAGLHLNPMLLNQR (SEQ ID NO: 45)
ORF1/2	MAWGWWRWRRRWPARRWRRRRRRRPVRRTRARRPARRYRRRRTQRESSESPKK ARIQRKKGRKPLPKSRRRRRQYSSSSDDSESNSSSSSSSNSSPEKCSKRKRVST (SEQ ID NO: 46)

Table N13. Exemplary *Anellovirus* nucleic acid sequence (*Alphatorquevirus*, Clade 7)

Name	TTV-HD16d
Genus/Clade	*Alphatorquevirus*, Clade 7
Accession Number	FR751479.1

Full Sequence: 3866 bp

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1         10         20         30         40         50
|         |          |          |          |          |
AAGTCCGTCACTAACCACGTGACTCCCGCAGGCCAATCAGAGTCTATGTC
GTGCACTTCCTGGGCATGGTCTACGTTCTCATATAACTAACTGCACTTCC
GAATGGCTGAGTTTTCCACGCCCGTCCGCAGCGGCAGCACCACGGAGGGT
GATCCCCGCGTCCCGAGGGCGGGTGCCGAAGGTGAGTTTACACACCGCAG
TCAAGGGGCAATTCGGGCTCGGGACTGGCCGGGCTATGGGCAAGGCTCTT
AGGGCTTTCATTGTTAAAAATGTTTCTCGGCAGGCCTTACAGGAGAAAGA
AAAGGGCGCTGTCACTGCCTGGCGTGCGAGCTGCACAGGCGAAACAACCT
GGTGATATGAGCTGGAGCCGTCCAGTACATAATGCCGCCGGGATCGAAAG
GCAGTGGTTCGAATCCACCTTTAGATCCCACGCTAGTTGCTGTGGCTGCG
GCAATTTTGTTAATCATATTAATGTACTGGCTGCTCGCTACGGCTTTACT
GGGGGGCCGACGCCGCCAGGTGGTCCTGGGCCGCGTCCACAACTGAGGCC
CGCGCTTCCCGCGCCGGACCCCGACCCCCAGGCGCCCAACCGTGAGCCAT
GGCGTGGAGCTGGTGGTGGCAACGATGGAGAAGGCGCCGCTGGAAACCCA
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GGAGGCGCCGCTGGAGACGTCTACGATGGAGAAGACCTAGACGCGCTGTT
CGCCGCCGTCGTCGAGGACGTAGAGTAAGGAGGCGGAGGTGGGCGCGTAG
ACGGGGGCGACGCAGACGGTACGCCACCAGACGAAAGAGACGTTATAGGG
GTCGCCGCTTTAAAAAGAAACTAGTACTGACTCAGTGGCACCCTAATACC
ATGAGACGCTGCTTAATCAAGGGCATAGTCCCCCTGGTAATATGCGGCCA
CACCAGGTGGAACTACAACTACGCCCTCCATAGCAAGGACTACACAGAGG
AGGGTCGCTACCCTCACGGGGGGGCCCTCAGCACCACTACGTGGTCCCTT
AAGGTGCTGTATGACGAGCACCTCAAACACCACGACTTCTGGGGCTATCC
CAACAACCAGCTAGACCTGGCCAGGTACAAGGGGGCCAAGTTCACCTTCT
ACAGACACAAAAAGACTGACTTTATAATATTCTTTAACAGAAAGCCTCCC
TTTAAGCTAAACAAGTACAGCTGTGCCTCCTATCACCCAGGCATGCTGAT
GCAGCAGAGACACAAGATCCTGCTACCCAGCTACGAAACTAAACCCAAGG
GCAGGCCAAAGATAACAGTTAGAATAAAGCCCCCCACTCTGTTAGAGGAC
AAGTGGTACACCCAGCAGGACCTGTGCGACGTTAACCTGTTGCAACTTGT
GGTCACTGCGGCTGACTTTCGACATCCACTCTGCTCACCACAAACGAACA
CTCCAACCACAACCTTCCAGGTGTTGAAAGACATCTATTATGACACTATG
AGCATATCTGAACCCACAGACTCCTACACTAGTGTTAACAATAAAAGTAC
AACACAAACTTTTACTAACTACTCAAACACCTTAGAAAACATTCTGTACA
CACGAGCCTCCTACTGGAACTCGTTCCACGCCACTGAATACCTAAACCCC
AACATCATATACAAAAACGGTGAAAAACTATTCAAAGAACATGAAGACTT
AATAACCTGGATGACCCAAACTAACAATACCGGGTTTCTAACTAAAAACA
ACACAGCTTTTGGCAACAACAGCTACAGGCCCAATGCAGACAAAATTAAA
AAAGCCAGAAAGACATACTGGAACGCCCTAATAGGCACCAACGACCTGGC
CACTAATATAGGCCAGGCCAGAGCAGAAAGGTTCGAGTACCACCTAGGCT
GGTACTCCCCCATATTTCTCAGCAGACACAGGAGCAACATGAACTTTGCC
AGGGCCTACCAAGACGTCACATACAACCCCAACTGTGACAGGGGAGTTAA
CAACAGGGTGTGGGTTCAGCCTCTAACTAAACCCACCACAGAGTTCGACG
AGAAAAGGTGTAAGTGCGTAGTGCAGCACCTGCCTCTGTGGGCGGCTCTG
TACTGCTACCAAGACTTTGTAGAGGAGGAGCTGGGGTCCTCCTCAGAGAT
ATTAAATTCATGCCTACTGGTATTACAGTGCCCTTACACCTTTCCCCCAA
TGTATGACAAAAAGCTACCAGACAAGGGATTCGTGTTTTATGACTCCCTT
TTTGGAGACGGCAAAATGTCTGACGGACGCGGACAGGTGGACATTTTCTG
GCAACAGCGATGGTACCCTCGCTTAGCCACTCAGATGCAAGTCATGCACG
ACATCACCATGACGGGCCCCTTCTCCTACCGAGACGAGCTAGTTAGCACC
CAACTGACTGCCAAGTACACCTTTGACTTTATGTGGGGCGGAAATATGAT
CTCCACACAGATCATCAAGAACCCCTGCAAAGACAGTGGACTGGAACCCG
CCTACCCCGGTAGACAGCGTCGCGACTTACAAATTGTTGACCCATACTCC
ATGGGCCCCCAATTCTCGTTCCACAACTGGGACTACAGACATGGCCTTTT

TGGCCAAGACGCTATCGACAGAGTGTCTAAACAACCAAAAGATGATGCAG
ACTATCCTAACCCATACAAAAGGCCTAGATATTTTCCACCCACAGACCAA
GCCGCCCAAGAGCAAGAAAAAGACTTCAGTTTCCTCAAAACAGCACCGTC
GAACTCAGAAGAGAGCGATCAAGAAGTCCTCCAAGAAACGCAAGTACTCC
GATTCCAGCCAGAGCAGCACAAGCAACTCCACCTGCAGCTCGCAGAGCGG
CAGCGAATCGGAGAGCAACTCCGATACCTACTCCAACAGATGTTCAAAAC
TCAGGCCAATCTCCACCTAAACCCATATACATTTACCCAGCTGTAAAGCA
GGTGTTTATGTTTGACCCCCCGGGCCCTAAGGCTATCTCGGGCGCCAAGG
CCTGGGAGGACGAGTTCCTCACCGCAAAAGTGTGGAACCGCCCGGTACGC
AAGTACTACTCAGACACCCCCTACTACCCCTGGGCCCCCAAACCCCAGTA
CTCTGTCAGTTTCAAACTCGGCTGGAAATAAAAAAAGCCTGCTCCACTGT
ACTAGGCCGTGGGAGTTTCACTCGTCGGTGTCTACCTCTTAAGGTCACCA
AGCACTCCGAGCGTCAGCGAGGAGTGCGACCCTTGGGGGTGGGTGCAACG
CCCTCGGCGGCCGCGCGCTACGCCTTCGGCTGCGCGCGGCACCTCGGACC
CCCGCTCGTGCTGACGCGCTTGCGCGCGTCAGACCACTTCGGGCTCGCGG
GGGTCGGAAATTTTGCTAAACAGACTCCGAGTTGCCATTGGACACTGGAG
CCGTGAATCAGTAACGAAAGTGAGTGGGGCCAGACTTCGCCATAAGGCCT
TTATCTTTTTGCCATTTGTCCGTGGGGAAGGGTCGCTGCAAGCGCGGACC
CCGTTTTCACCCCTTCCGGACTACAAAAATAGCGCATTAGTGACGTCACG
GCCGCCATTTTAAGTAAGGCGGAAGCAACTCCACTTTCTCACAAAATGGC
GGCGGAGCACTTCCGGCTTGCCCAAAATGGCCGCCAAAAACATCCGGGTC
AAAGTTCGCCGCTACGTCATAAGTCACGTGACTGGGGAGGTACTTAAACA
CGGAAGTATCCTCAACCACGTAACTGGTCACGTGGTGCGCACGTCACGGC
AACCATTTTGTTTTACAAAATGGCGCATTTCCTTCCTCTTTTTTAAAAAT
TAACCGTTGGCGGCGGCGCGCGCGCTACGCGCGCGCGCCGGGGAGCTCTG
CCCCCCCCCGCGCATGCGCGCGGGTCCCCCCCCCCGCGGGGGGCTCCGCCC
CCCGGTCCCCCCCCCG (SEQ ID NO: 47)

Annotations:

Putative Domain	*Base range*
TATA Box	82 – 86
Initiator Element	94-115
Transcriptional Start Site	115
5’ UTR Conserved Domain	170 - 240
ORF2	357 - 728

ORF2/2	357 – 724 ; 2411 – 2870
ORF2/3	357 – 724 ; 2646 – 3081
ORF1	599 – 2896
ORF1/1	599 – 724 ; 2411 – 2896
ORF1/2	599 – 724 ; 2646 – 2870
Three open-reading frame region	2629 – 2867
Poly(A) Signal	3076 - 3086
GC-rich region	3759 – 3866

Table A13. Exemplary *Anellovirus* amino acid sequences (*Alphatorquevirus*, Clade 7)

TTV-HD16d (*Alphatorquevirus* Clade 7)	
ORF2	MSWSRPVHNAAGIERQWFESTFRSHASCCGCGNFVNHINVLAARYGFTGGP TPPGGPGPRPQLRPALPAPDPDPQAPNREPWRGAGGGNDGEGAAGNPGGA AGDVYDGEDLDALFAAVVEDVE (SEQ ID NO: 48)
ORF2/2	MSWSRPVHNAAGIERQWFESTFRSHASCCGCGNFVNHINVLAARYGFTGGP TPPGGPGPRPQLRPALPAPDPDPQAPNREPWRGAGGGNDGEGAAGNPGGA AGDVYDGEDLDALFAAVVEDVESSRTPAKTVDWNPPTPVDSVATYKLLTH TPWAPNSRSTTGTTDMAFLAKTLSTECLNNQKMMQTILTHTKGLDIFHPQT KPPKSKKKTSVSSKQHRRTQKRAIKKSSKKRKYSDSSQSSTSNSTCSSQSGSE SESNSDTYSNRCSKLRPIST (SEQ ID NO: 49)
ORF2/3	MSWSRPVHNAAGIERQWFESTFRSHASCCGCGNFVNHINVLAARYGFTGGP TPPGGPGPRPQLRPALPAPDPDPQAPNREPWRGAGGGNDGEGAAGNPGGA AGDVYDGEDLDALFAAVVEDVEPSRPRARKRLQFPQNSTVELRRERSRSPP RNASTPIPARAAQATPPAARRAAANRRATPIPTPTDVQNSGQSPPKPIYIYPA VKQVFMFDPPGPKAISGAKAWEDEFLTAKVWNRPVRKYYSDTPYYPWAPK PQYSVSFKLGWK (SEQ ID NO: 50)
ORF1	MAWSWWWQRWRRRRWKPRRRRWRRLRWRRPRRAVRRRRRGRRVRRRR WARRRGRRRRYATRRKRRYRGRRFKKKLVLTQWHPNTMRRCLIKGIVPLV ICGHTRWNYNYALHSKDYTEEGRYPHGGALSTTTWSLKVLYDEHLKHHDF WGYPNNQLDLARYKGAKFTFYRHKKTDFIIFFNRKPPFKLNKYSCASYHPG MLMQQRHKILLPSYETKPKGRPKITVRIKPPTLLEDKWYTQQDLCDVNLLQ

	LVVTAADFRHPLCSPQTNTPTTTFQVLKDIYYDTMSISEPTDSYTSVNNKSTT QTFTNYSNTLENILYTRASYWNSFHATEYLNPNIIYKNGEKLFKEHEDLITW MTQTNNTGFLTKNNTAFGNNSYRPNADKIKKARKTYWNALIGTNDLATNI GQARAERFEYHLGWYSPIFLSRHRSNMNFARAYQDVTYNPNCDRGVNNRV WVQPLTKPTTEFDEKRCKCVVQHLPLWAALYCYQDFVEEELGSSSEILNSC LLVLQCPYTFPPMYDKKLPDKGFVFYDSLFGDGKMSDGRGQVDIFWQQRW YPRLATQMQVMHDITMTGPFSYRDELVSTQLTAKYTFDFMWGGNMISTQII KNPCKDSGLEPAYPGRQRRDLQIVDPYSMGPQFSFHNWDYRHGLFGQDAID RVSKQPKDDADYPNPYKRPRYFPPTDQAAQEQEKDFSFLKTAPSNSEESDQE VLQETQVLRFQPEQHKQLHLQLAERQRIGEQLRYLLQQMFKTQANLHLNPY TFTQL (SEQ ID NO: 51)
ORF1/1	MAWSWWWQRWRRRRWKPRRRRWRRLRWRRPRRAVRRRRRGRRIIKNPC KDSGLEPAYPGRQRRDLQIVDPYSMGPQFSFHNWDYRHGLFGQDAIDRVSK QPKDDADYPNPYKRPRYFPPTDQAAQEQEKDFSFLKTAPSNSEESDQEVLQE TQVLRFQPEQHKQLHLQLAERQRIGEQLRYLLQQMFKTQANLHLNPYTFTQ L (SEQ ID NO: 52)
ORF1/2	MAWSWWWQRWRRRRWKPRRRRWRRLRWRRPRRAVRRRRRGRRTKPPK SKKKTSVSSKQHRRTQKRAIKKSSKKRKYSDSSQSSTSNSTCSSQSGSESESN SDTYSNRCSKLRPIST (SEQ ID NO: 53)

Table N14. Exemplary *Anellovirus* nucleic acid sequence (*Betatorquevirus*)

Name	Ring2
Genus/Clade	*Betatorquevirus*
Accession Number	JX134045.1

Full Sequence: 2797 bp

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1         10         20         30         40         50
|         |          |          |          |          |
TAATAAATATTCAACAGGAAAACCACCTAATTTAAATTGCCGACCACAAA
CCGTCACTTAGTTCCCCTTTTTGCAACAACTTCTGCTTTTTTCCAACTGC
CGGAAAACCACATAATTTGCATGGCTAACCACAAACTGATATGCTAATTA
ACTTCCACAAAACAACTTCCCCTTTTAAAACCACACCTACAAATTAATTA
```

TTAAACACAGTCACATCCTGGGAGGTACTACCACACTATAATACCAAGTG
CACTTCCGAATGGCTGAGTTTATGCCGCTAGACGGAGAACGCATCAGTTA
CTGACTGCGGACTGAACTTGGGCGGGTGCCGAAGGTGAGTGAAACCACCG
AAGTCAAGGGGCAATTCGGGCTAGTTCAGTCTAGCGGAACGGGCAAGAAA
CTTAAAATTATTTTATTTTTCAGATGAGCGACTGCTTTAAACCAACATGC
TACAACAACAAAACAAAGCAAACTCACTGGATTAATAACCTGCATTTAAC
CCACGACCTGATCTGCTTCTGCCCAACACCAACTAGACACTTATTACTAG
CTTTAGCAGAACAACAAGAAACAATTGAAGTGTCTAAACAAGAAAAAGAA
AAAATAACAAGATGCCTTATTACTACAGAAGAAGACGGTACAACTACAGA
CGTCCTAGATGGTATGGACGAGGTTGGATTAGACGCCCTTTTCGCAGAAG
ATTTCGAAGAAAAAGAAGGGTAAGACCTACTTATACTACTATTCCTCTAA
AGCAATGGCAACCGCCATATAAAAGAACATGCTATATAAAAGGACAAGAC
TGTTTAATATACTATAGCAACTTAAGACTGGGAATGAATAGTACAATGTA
TGAAAAAAGTATTGTACCTGTACATTGGCCGGGAGGGGGTTCTTTTTCTG
TAAGCATGTTAACTTTAGATGCCTTGTATGATATACATAAACTTTGTAGA
AACTGGTGGACATCCACAAACCAAGACTTACCACTAGTAAGATATAAAGG
ATGCAAAATAACATTTTATCAAAGCACATTTACAGACTACATAGTAAGAA
TACATACAGAACTACCAGCTAACAGTAACAAACTAACATACCCAAACACA
CATCCACTAATGATGATGATGTCTAAGTACAAACACATTATACCTAGTAG
ACAAACAAGAAGAAAAAAGAAACCATACACAAAAATATTTGTAAAACCAC
CTCCGCAATTTGAAAACAAATGGTACTTTGCTACAGACCTCTACAAAATT
CCATTACTACAAATACACTGCACAGCATGCAACTTACAAAACCCATTTGT
AAAACCAGACAAATTATCAAACAATGTTACATTATGGTCACTAAACACCA
TAAGCATACAAAATAGAAACATGTCAGTGGATCAAGGACAATCATGGCCA
TTTAAAATACTAGGAACACAAAGCTTTTATTTTTACTTTTACACCGGAGC
AAACCTACCAGGTGACACAACACAAATACCAGTAGCAGACCTATTACCAC
TAACAAACCCAAGAATAAACAGACCAGGACAATCACTAAATGAGGCAAAA
ATTACAGACCATATTACTTTCACAGAATACAAAAACAAATTTACAAATTA
TTGGGGTAACCCATTTAATAAACACATTCAAGAACACCTAGATATGATAC
TATACTCACTAAAAAGTCCAGAAGCAATAAAAAACGAATGGACAACAGAA
AACATGAAATGGAACCAATTAAACAATGCAGGAACAATGGCATTAACACC
ATTTAACGAGCCAATATTCACACAAATACAATATAACCCAGATAGAGACA
CAGGAGAAGACACTCAATTATACCTACTCTCTAACGCTACAGGAACAGGA
TGGGACCCACCAGGAATTCCAGAATTAATACTAGAAGGATTTCCACTATG
GTTAATATATTGGGGATTTGCAGACTTTCAAAAAAACCTAAAAAAAGTAA
CAAACATAGACACAAATTACATGTTAGTAGCAAAAACAAAATTTACACAA
AAACCTGGCACATTCTACTTAGTAATACTAAATGACACCTTTGTAGAAGG
CAATAGCCCATATGAAAAACAACCTTTACCTGAAGACAACATTAAATGGT

ACCCACAAGTACAATACCAATTAGAAGCACAAAACAAACTACTACAAACT
GGGCCATTTACACCAAACATACAAGGACAACTATCAGACAATATATCAAT
GTTTTATAAATTTTACTTTAAATGGGGAGGAAGCCCACCAAAAGCAATTA
ATGTTGAAAATCCTGCCCACCAGATTCAATATCCCATACCCCGTAACGAG
CATGAAACAACTTCGTTACAGAGTCCAGGGGAAGCCCCAGAATCCATCTT
ATACTCCTTCGACTATAGACACGGGAACTACACAACAACAGCTTTGTCAC
GAATTAGCCAAGACTGGGCACTTAAAGACACTGTTTCTAAAATTACAGAG
CCAGATCGACAGCAACTGCTCAAACAAGCCCTCGAATGCCTGCAAATCTC
GGAAGAAACGCAGGAGAAAAAAGAAAAAGAAGTACAGCAGCTCATCAGCA
ACCTCAGACAGCAGCAGCAGCTGTACAGAGAGCGAATAATATCATTATTA
AAGGACCAATAACTTTTAACTGTGTAAAAAAGGTGAAATTGTTTGATGAT
AAACCAAAAAACCGTAGATTTACACCTGAGGAATTTGAAACTGAGTTACA
AATAGCAAAATGGTTAAAGAGACCCCCAAGATCCTTTGTAAATGATCCTC
CCTTTTACCCATGGTTACCACCTGAACCTGTTGTAAACTTTAAGCTTAAT
TTTACTGAATAAAGGCCAGCATTAATTCACTTAAGGAGTCTGTTTATTTA
AGTTAAACCTTAATAAACGGTCACCGCCTCCCTAATACGCAGGCGCAGAA
AGGGGGCTCCGCCCCCTTTAACCCCCAGGGGGCTCCGCCCCCTGAAACCC
CCAAGGGGGCTACGCCCCCTTACACCCCC (SEQ ID NO: 54)

Annotations:

Putative Domain	*Base range*
TATA Box	237– 243
Cap Site	260 – 267
Transcriptional Start Site	267
5’ UTR Conserved Domain	323 – 393
ORF2	424 – 723
ORF2/2	424 – 719 ; 2274 – 2589
ORF2/3	424 – 719 ; 2449 – 2812
ORF1	612 – 2612
ORF1/1	612 – 719 ; 2274 – 2612
ORF1/2	612 – 719 ; 2449 – 2589
Three open-reading frame region	2441 – 2586
Poly(A) Signal	2808 – 2813
GC-rich region	2868 – 2929

Table A14. Exemplary *Anellovirus* amino acid sequences (*Betatorquevirus*)

Ring2 (*Betatorquevirus*)	
ORF2	MSDCFKPTCYNNKTKQTHWINNLHLTHDLICFCPTPTRHLLLALAEQQETIEVSKQE KEKITRCLITTEEDGTTTDVLDGMDEVGLDALFAEDFEEKEG (SEQ ID NO: 55)
ORF2/2	MSDCFKPTCYNNKTKQTHWINNLHLTHDLICFCPTPTRHLLLALAEQQETIEVSKQE KEKITRCLITTEEDGTTTDVLDGMDEVGLDALFAEDFEEKEGFNIPYPVTSMKQLRY RVQGKPQNPSYTPSTIDTGTTQQQLCHELAKTGHLKTLFLKLQSQIDSNCSNKPSNA CKSRKKRRRKKKKKYSSSSATSDSSSSCTESE (SEQ ID NO: 56)
ORF2/3	MSDCFKPTCYNNKTKQTHWINNLHLTHDLICFCPTPTRHLLLALAEQQETIEVSKQE KEKITRCLITTEEDGTTTDVLDGMDEVGLDALFAEDFEEKEGARSTATAQTSPRMP ANLGRNAGEKRKRSTAAHQQPQTAAAAVQRANNIIIKGPITFNCVKKVKLFDDKPK NRRFTPEEFETELQIAKWLKRPPRSFVNDPPFYPWLPPEPVVNFKLNFTE (SEQ ID NO: 57)
ORF1	MPYYYRRRRYNYRRPRWYGRGWIRRPFRRRFRRKRRVRPTYTTIPLKQWQPPYKR TCYIKGQDCLIYYSNLRLGMNSTMYEKSIVPVHWPGGGSFSVSMLTLDALYDIHKL CRNWWTSTNQDLPLVRYKGCKITFYQSTFTDYIVRIHTELPANSNKLTYPNTHPLM MMMSKYKHIIPSRQTRRKKKPYTKIFVKPPPQFENKWYFATDLYKIPLLQIHCTACN LQNPFVKPDKLSNNVTLWSLNTISIQNRNMSVDQGQSWPFKILGTQSFYFYFYTGA NLPGDTTQIPVADLLPLTNPRINRPGQSLNEAKITDHITFTEYKNKFTNYWGNPFNK HIQEHLDMILYSLKSPEAIKNEWTTENMKWNQLNNAGTMALTPFNEPIFTQIQYNP DRDTGEDTQLYLLSNATGTGWDPPGIPELILEGFPLWLIYWGFADFQKNLKKVTNID TNYMLVAKTKFTQKPGTFYLVILNDTFVEGNSPYEKQPLPEDNIKWYPQVQYQLEA QNKLLQTGPFTPNIQGQLSDNISMFYKFYFKWGGSPPKAINVENPAHQIQYPIPRNE HETTSLQSPGEAPESILYSFDYRHGNYTTTALSRISQDWALKDTVSKITEPDRQQLLK QALECLQISEETQEKKEKEVQQLISNLRQQQQLYRERIISLLKDQ (SEQ ID NO: 58)
ORF1/1	MPYYYRRRRYNYRRPRWYGRGWIRRPFRRRFRRKRRIQYPIPRNEHETTSLQSPGE APESILYSFDYRHGNYTTTALSRISQDWALKDTVSKITEPDRQQLLKQALECLQISEE TQEKKEKEVQQLISNLRQQQQLYRERIISLLKDQ (SEQ ID NO: 59)
ORF1/2	MPYYYRRRRYNYRRPRWYGRGWIRRPFRRRFRRKRRSQIDSNCSNKPSNACKSRK KRRRKKKKKYSSSSATSDSSSSCTESE (SEQ ID NO: 60)

Table N15. Exemplary *Anellovirus* nucleic acid sequence (*Gammatorquevirus*)

Name	TTMDV-MD1-073
Genus/Clade	*Gammatorquevirus*
Accession Number	AB290918.1

Full Sequence: 3242 bp

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1         10         20         30         40         50
|         |          |          |          |          |
AGGTGGAGACTCTTAAGCTATATAACCAAGTGGGGTGGCGAATGGCTGAG
TTTACCCCGCTAGACGGTGCAGGGACCGGATCGAGCGCAGCGAGGAGGTC
CCCGGCTGCCCGTGGGCGGGAGCCCGAGGTGAGTGAAACCACCGAGGTCT
AGGGGCAATTCGGGCTAGGGCAGTCTAGCGGAACGGGCAAGAAACTTAAA
AATATTTCTTTTACAGATGCAAAACCTATCAGCCAAAGACTTCTACAAAC
CATGCAGATACAACTGTGAAACTAAAAACCAAATGTGGATGTCTGGCATT
GCTGACTCCCATGACAGTTGGTGTGACTGTGATACTCCTTTTGCTCACCT
CCTGGCTAGTATTTTTCCTCCTGGTCACACAGATCGCACACGAACCATCC
AAGAAATACTTACCAGAGATTTTAGGAAAACATGCCTTTCTGGTGGGGCC
GACGCAACAAATTCTGGTATGGCCGAAACTATAGAAGAAAAAAGAGAAGA
TTTCCAAAAAGAAGAAAAAGAAGATTTTACAGAAGAACAAAATATAGAAG
ACCTGCTCGCCGCCGTCGCAGACGCAGAAGGAAGGTAAGAAGAAAAAAAA
AAACTCTTATAGTAAGACAATGGCAGCCAGACTCTATTGTACTCTGTAAA
ATTAAAGGGTATGACTCTATAATATGGGGAGCTGAAGGCACACAGTTTCA
ATGTTCTACACATGAAATGTATGAATATACAAGACAAAAGTACCCTGGGG
GAGGAGGATTTGGTGTACAACTTTACAGCTTAGAGTATTTGTATGACCAA
TGGAAACTTAGAAATAATATATGGACTAAAACAAATCAACTCAAAGATTT
GTGTAGATACTTAAAATGTGTTATGACCTTTTACAGACACCAACACATAG
ATTTTGTAATTGTATATGAAAGACAACCCCCATTTGAAATAGATAAACTA
ACATACATGAAATATCATCCATATATGTTATTACAAAGAAAGCATAAAAT
AATTTTACCTAGTCAAACAACTAATCCTAGAGGTAAATTAAAAAAAAAGA
AAACTATTAAACCTCCCAAACAAATGCTCAGCAAATGGTTTTTTCAACAA
CAATTTGCTAAATATGATCTACTACTTATTGCTGCAGCAGCATGTAGTTT
AAGATACCCTAGAATAGGCTGCTGCAATGAAAATAGAATGATAACCTTAT
ACTGTTTAAATACTAAATTTTATCAAGATACAGAATGGGGAACTACAAAA
CAGGCCCCCCACTACTTTAAACCATATGCAACAATTAATAAATCCATGAT
ATTTGTCTCTAACTATGGAGGTAAAAAAACAGAATATAACATAGGCCAAT
GGATAGAAACAGATATACCTGGAGAAGGTAATCTAGCAAGATACTACAGA
TCAATAAGTAAAGAAGGAGGTTACTTTTCACCTAAAATACTGCAAGCATA
TCAAACAAAAGTAAAGTCTGTAGACTACAAACCTTTACCAATTGTTTTAG
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GTAGATATAACCCAGCAATAGATGATGGAAAAGGCAACAAAATTTACTTA
CAAACTATAATGAATGGCCATTGGGGCCTACCTCAAAAAACACCAGATTA
TATAATAGAAGAGGTCCCTCTTTGGCTAGGCTTCTGGGGATACTATAACT
ACTTAAAACAAACAAGAACTGAAGCTATATTTCCACTACACATGTTTGTA
GTGCAAAGCAAATACATTCAAACACAACAAACAGAAACACCTAACAATTT
TTGGGCATTTATAGACAACAGCTTTATACAGGGCAAAAACCCATGGGACT
CAGTTATTACTTACTCAGAACAAAAGCTATGGTTTCCTACAGTTGCATGG
CAACTAAAAACCATAAATGCTATTTGTGAAAGTGGACCATATGTACCTAA
ACTAGACAATCAAACATATAGTACCTGGGAACTAGCAACTCATTACTCAT
TTCACTTTAAATGGGGTGGTCCACAGATATCAGACCAACCAGTTGAAGAC
CCAGGAAACAAAAACAAATATGATGTGCCCGATACAATCAAAGAAGCATT
ACAAATTGTTAACCCAGCAAAAAACATTGCTGCCACGATGTTCCATGACT
GGGACTACAGACGGGGTTGCATTACATCAACAGCTATTAAAAGAATGCAA
CAAAACCTCCCAACTGATTCATCTCTCGAATCTGATTCAGACTCAGAACC
AGCACCCAAGAAAAAAAGACTACTACCAGTCCTCCACGACCCACAAAAGA
AAACGGAAAAGATCAACCAATGTCTCCTCTCTCTCTGCGAAGAAAGTACA
TGCCAGGAGCAGGAAACGGAGGAAAACATCCTCAAGCTCATCCAGCAGCA
GCAGCAGCAGCAGCAGAAACTCAAGCACAACCTCTTAGTACTAATCAAGG
ACTTAAAAGTGAAACAAAGATTATTACAACTACAAACGGGGGTACTAGAA
TAACCCTTACCAGATTTAAACCAGGATTTGAGCAAGAAACTGAAAAAGAG
TTAGCACAAGCATTTAACAGACCCCCTAGACTGTTCAAAGAAGATAAACC
CTTTTACCCCTGGCTACCCAGATTTACACCCCTTGTAAACTTTCACCTTA
ATTTTAAAGGCTAGGCCTACACTGCTCACTTAGTGGTGTATGTTTATTAA
AGTTTGCACCCCAGAAAAATTGTAAAATAAAAAAAAAAAAAAAAAATAAA
AAATTGCAAAAATTCGGCGCTCGCGCGCGCTGCGCGCGCGAGCGCCGTCA
CGCGCCGGCGCTCGCGCGCCGCGCGTATGTGCTAACACACCACGCACCTA
GATTGGGGTGCGCGCGTAGCGCGCGCACCCCAATGCGCCCCGCCCTCGTT
CCGACCCGCTTGCGCGGGTCGGACCACTTCGGGCTCGGGGGGGCGCGCCT
GCGGCGCTTATTTACTAAACAGACTCCGAGTCGCCATTGGGCCCCCCCTA
AGCTCCGCCCCCCTCATGAATATTCATAAAGGAAACCACAAAATTAGAAT
TGCCGACCACAAACTGCCATATGCTAATTAGTTCCCCTTTTACACAGTAA
AAAGGGGAAGTGGGGGGGCAGAGCCCCCCCACACCCCCCGCGGGGGGGGC
AGAGCCCCCCCCGCACCCCCCCTACGTCACAGGCCACGCCCCCGCCGCCA
TCTTGGGTGCGGCAGGGCGGGGACTAAAATGGCGGGACCCAATCATTTTA
TACTTTCACTTTCCAATTAAAACCCGCCACGTCACACAAAAG (SEQ ID NO: 61)

Annotations:

Putative Domain	*Base range*
TATA Box	21– 25
Cap Site	42 – 49
Transcriptional Start Site	49
5’ UTR Conserved Domain	117 – 187
ORF2	283 – 588
ORF2/2	283 – 584 ; 1977 – 2388
ORF2/3	283 – 584 ; 2197 – 2614
ORF1	432 – 2453
ORF1/1	432 – 584 ; 1977 – 2453
ORF1/2	432 – 584 ; 2197 – 2388
Three open-reading frame region	2186 – 2385
Poly(A) Signal	2676 – 2681
GC-rich region	3054 – 3172

Table A15. Exemplary *Anellovirus* amino acid sequences (*Gammatorquevirus*)

TTMDV-MD1-073 (*Gammatorquevirus*)	
ORF2	MWMSGIADSHDSWCDCDTPFAHLLASIFPPGHTDRTRTIQEILTRDFRKTCLSGGAD ATNSGMAETIEEKREDFQKEEKEDFTEEQNIEDLLAAVADAEGR (SEQ ID NO: 62)
ORF2/2	MWMSGIADSHDSWCDCDTPFAHLLASIFPPGHTDRTRTIQEILTRDFRKTCLSGGAD ATNSGMAETIEEKREDFQKEEKEDFTEEQNIEDLLAAVADAEGRYQTNQLKTQETK TNMMCPIQSKKHYKLLTQQKTLLPRCSMTGTTDGVALHQQLLKECNKTSQLIHLSN LIQTQNQHPRKKDYYQSSTTHKRKRKRSTNVSSLSAKKVHARSRKRRKTSSSSSSSS SSSSRNSSTTS (SEQ ID NO: 63)
ORF2/3	MWMSGIADSHDSWCDCDTPFAHLLASIFPPGHTDRTRTIQEILTRDFRKTCLSGGAD ATNSGMAETIEEKREDFQKEEKEDFTEEQNIEDLLAAVADAEGRTSTQEKKTTTSPP RPTKENGKDQPMSPLSLRRKYMPGAGNGGKHPQAHPAAAAAAAETQAQPLSTNQ GLKSETKIITTTNGGTRITLTRFKPGFEQETEKELAQAFNRPPRLFKEDKPFYPWLPRF TPLVNFHLNFKG (SEQ ID NO: 64)
ORF1	MPFWWGRRNKFWYGRNYRRKKRRFPKRRKRRFYRRTKYRRPARRRRRRRRKVR RKKKTLIVRQWQPDSIVLCKIKGYDSIIWGAEGTQFQCSTHEMYEYTRQKYPGGGG FGVQLYSLEYLYDQWKLRNNIWTKTNQLKDLCRYLKCVMTFYRHQHIDFVIVYER QPPFEIDKLTYMKYHPYMLLQRKHKIILPSQTTNPRGKLKKKKTIKPPKQMLSKWFF

	QQQFAKYDLLLIAAAACSLRYPRIGCCNENRMITLYCLNTKFYQDTEWGTTKQAPH YFKPYATINKSMIFVSNYGGKKTEYNIGQWIETDIPGEGNLARYYRSISKEGGYFSPK ILQAYQTKVKSVDYKPLPIVLGRYNPAIDDGKGNKIYLQTIMNGHWGLPQKTPDYII EEVPLWLGFWGYYNYLKQTRTEAIFPLHMFVVQSKYIQTQQTETPNNFWAFIDNSFI QGKNPWDSVITYSEQKLWFPTVAWQLKTINAICESGPYVPKLDNQTYSTWELATH YSFHFKWGGPQISDQPVEDPGNKNKYDVPDTIKEALQIVNPAKNIAATMFHDWDY RRGCITSTAIKRMQQNLPTDSSLESDSDSEPAPKKKRLLPVLHDPQKKTEKINQCLLS LCEESTCQEQETEENILKLIQQQQQQQQKLKHNLLVLIKDLKVKQRLLQLQTGVLE (SEQ ID NO: 65)
ORF1/1	MPFWWGRRNKFWYGRNYRRKKRRFPKRRKRRFYRRTKYRRPARRRRRRRRKISD QPVEDPGNKNKYDVPDTIKEALQIVNPAKNIAATMFHDWDYRRGCITSTAIKRMQQ NLPTDSSLESDSDSEPAPKKKRLLPVLHDPQKKTEKINQCLLSLCEESTCQEQETEEN ILKLIQQQQQQQQKLKHNLLVLIKDLKVKQRLLQLQTGVLE (SEQ ID NO: 66)
ORF1/2	MPFWWGRRNKFWYGRNYRRKKRRFPKRRKRRFYRRTKYRRPARRRRRRRRKISD QPVEDPGNKNKYDVPDTIKEALQIVNPAKNIAATMFHDWDYRRGCITSTAIKRMQQ NLPTDSSLESDSDSEPAPKKKRLLPVLHDPQKKTEKINQCLLSLCEESTCQEQETEEN ILKLIQQQQQQQQKLKHNLLVLIKDLKVKQRLLQLQTGVLE (SEQ ID NO: 67)

Table N16. Exemplary *Anellovirus* nucleic acid sequence (*Gammatorquevirus*)

Name Ring3.1

Genus/Clade *Gammatorquevirus*

Accession Number

Full Sequence: 3264 bp

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1         10         20         30         40         50
|         |          |          |          |          |
TAAAATGGCGGCAACCAATCATTTTATACTTTCACTTTCCAATTACAAGC
CGCCACGTCACAGAACAGGGGTGGAGACTTTAAAACTATATAACCAAGTG
ATGTGACGAATGGCTGAGTTTACCCCGCTAGACGGTGCAGGGACCGGATC
GAGCGCAGCGAGGAGGTCCCCGGCTGCCCGTGGGCGGGAGCCCGAGGTGA
GTGAAACCACCGAGGTCTAGGGGCAATTCGGGCTAGGGCAGTCTAGCGGA
ACGGGCAAGAAACTTAAAATATGTTTTGTTTCAGATGCAGACACCTGCTT
CACAGATAAGCTCAGACGACTTCTTTGTACACACTCCATTTAATGCAGTA
ACTAAACAGCAAATATGGATGTCTCAAATTGCTGATGGACATGACAACAT
TTGTCACTGCCACCGTCCTTTTGCTCACCTGCTTGCTAATATTTTTCCTC
CTGGTCATAAAGACAGGGATCTTACCATTAATCAAATACTTGCTAGAGAT
CTTACAGAAACATGCCATTCTGGTGGAGACGAAGGAACAAGCGGTGGTGG
GGTCGCCGCTTCCGCTACCGCCGCTACAACAAATATAAAACCAGAAGGAG
ACGCAGAATACCCAGAAGACGAAATAGAAGATTTACTAAGACACGCAGGA
GAAGAAAAAGAAAGAAGGTAAGAAGAAAACTTAAAAAAATTACTATTAAA
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CAATGGCAGCCAGATTCAGTGAAAAAATGTAAAATTAAAGGATATAGTAC
TTTAGTTATGGGTGCACAAGGAAAACAATACAACTGTTACACAAACCAAG
CAAGTGACTATGTTCAGCCTAAAGCACCACAAGGTGGGGGCTTTGGCTGT
GAAGTATTTAATTTAAAATGGCTATACCAAGAATATACTGCACACAGAAA
TATTTGGACAAAAACAAATGAATATACAGACCTTTGTAGATACACTGGAG
CTCAAATAATTTTATACAGGCACCCAGATGTTGATTTTATAGTCAGCTGG
GACAATCAGCCACCTTTTTTTACTTAACAAATATACATATCCAGAACTGCA
ACCACAAAACCTTTTACTAGCTAGAAGGAAAAGAATTATTCTTAGTCAAA
AATCAAACCCCAAAGGAAAACTAAGAATTAAACTAAGAATACCACCACCA
AAACAAATGATAACAAAATGGTTTTTTCAAAGAGACTTTTGTGATGTGAA
TCTGTTTAAACTATGTGCTTCTGCTGCTTCTTTCCGCTACCCAGGTATCA
GTCATGGAGCTCAAAGTACTATTTTTTCTGCATATGCTTTAAACACTGAC
TTTTATCAATGCAGTGACTGGTGCCAAACTAACACAGAAACTGGCTACCT
AAACATTAAAACACAACAAATGCCACTATGGTTTCATTACAGAGAGGGTG
GCAAAGAGAAATGGTATAAATACACCAACAAAGAACACAGACCATATACA
AATACATATCTTAAAAGTATTAGCTATAATGATGGATTGTTTTCTCCTAA
AGCCATGTTTGCATTTGAAGTAAAAGCGGGGGGTGAAGGAACAACAGAAC
CACCACAAGGCGCCCAATTAATTGCTAACCTTCCACTCATTGCACTAAGA
TATAATCCACATGAAGACACAGGCCATGGCAATGAAATTTACCTTACATC
AACTTTTAAAGGTACATATGACAAACCTAAAGTTACTGATGCTCTATACT
TTAACAATGTACCCCTGTGGATGGGATTTTATGGCTACTGGGACTTTATA
TTACAAGAAACAAAAAACAAAGGTGTCTTTGATCAACATATGTTTGTTGT
TAAATGTCCTGCCTTAAGGCCCATATCACAAGTCACAAAACAAGTATACT
ACCCACTTGTAGACATGGACTTTTGTTCAGGGAGACTGCCATTTGATGAA
TATTTATCCAAAGACATTAAAAGTCATTGGTATCCCACTGCAGAAAGACA
AACAGTTACAATAAATAATTTTGTTACAGCAGGTCCATACATGCCTAAAT
TTGAACCCACAGACAAAGACAGTACATGGCAATTAAACTATCACTATAAA
TTTTTTTTTAAGTGGGGTGGTCCACAAGTCACAGACCCAACTGTTGAAGA
CCCATGCAGCAGAAACAAATATCCTGTCCCCGATACAATGCAACAAACAA
TACAAATTAAAAACCCTGAAAAGCTGCACCCAGCAACCCTCTTCCATGAC
TGGGACCTTAGAAGGGGCTTCATTACACAAGCAGCTATTAAAAGAATGTC
AGAAAACCTCCAAATTGATTCATCTTTCGAATCTGATGGCACAGAATCAC
CCAAAAAAAAGAAAAGATGCACCAAAGAAATCCCAACACAAAACCAAAAG
CAAGAAGAGATCCAAGAATGTCTCCTCTCACTCTGCGAAGAGCCTACATG
CCAAGAAGAAACAGAGGACCTCCAGCTCTTCATCCAGCAGCAGCAGCAGC
AGCAGTACAAGCTCAGAAAAAACCTCTTCAAACTCCTCACTCACCTGAAA
AAAGGACAGAGAATAAGTCAACTACAAACGGGACTTTTAGAGTAATACCA
TTTAAACCAGGTTTTGAACAAGAAACAGAAAAAGAACTTGCCATAGCTTT
CTGCAGACCACCTAGAAAATATAAAAATGATCCCCCTTTTTATCCCTGGT
TACCATGGACACCCCTTGTACACTTTAACCTTAATTACAAAGGCTAGGCC
AACACTGTTCACTTAGTGGTGTATGTTTAATAAAGTTTCACCCCCAAAAA
AAAAAAAAAAAAAAAAAAAAAAAAAAAAAATAAAAAATTGCAAAAATTCG
GCGCTCGCGCGCGCTGCGCGCGCGAGCGCCGTCACGCGCCGGCGCTCG
CGCGCCGCGCGTATGTGCTAACACACCACGCACCTAGATTGGGGTGCGCG
CGCTAGCGCGCGCACCCCAATGCGCCCCGCCCTCGTTCCGACCCGCTTGC
GCGGGTCGGACCACTTCGGGCTCGGGGGGGCGCGCCTGCGGCGCTTTTTT
ACTAAACAGACTCCGAGCCGCCATTTGGCCCCCCCCTAAGCTCCGCCCCCC
TCATGAATATTCATAAAGGAAACCACATAATTAGAATTGCCGACCACAAA
CTGCCATATGCTAATTAGTTCCCCTTTTACACAGTAAAAAGGGGAAGTGG
GGGGGCATAGCCCCCCCACACCCCCCGCGGGGGGGGCAGAGCCCCCCCCC
GCACCCCCCCCCTACGTCACAATCCACGCCCCCGCCGCCATCTTGGGTGC
GGCAGGGCGGGGGC (SEQ ID NO: 878)

Annotations:

Putative Domain *Base range*

TATA Box	87– 93
Cap Site	110 – 117
Transcriptional Start Site	117
5' UTR Conserved Domain	185 – 255
ORF2	285 – 671
ORF2/2	285 – 667 ; 2063 – 2498
ORF2/3	285 – 667 ; 2295 – 2697
TAIP	385 - 585
ORF1	512 – 2545
ORF1/1	512 – 667 ; 2063 – 2545
ORF1/2	512 – 667 ; 2295 – 2498
Three open-reading frame region	2295 – 2495
Poly(A) Signal	2729 – 2734
GC-rich region	3141 – 3264

Table A16. Exemplary *Anellovirus* amino acid sequences (*Gammatorquevirus*)

Ring 3.1 (*Gammatorquevirus*)	
ORF2	MQTPASQISSDDFFVHTPFNAVTKQQIWMSQIADGHDNICHCHRPFAHLLAN IFPPGHKDRDLTINQ ILARDLTETCHSGGDEGTSGGGVAASATAATTNIKPEGDAEYPEDEIEDLLR HAGEEKERR (SEQ ID NO: 879)
ORF2/2	MQTPASQISSDDFFVHTPFNAVTKQQIWMSQIADGHDNICHCHRPFAHLLAN IFPPGHKDRDLTINQ ILARDLTETCHSGGDEGTSGGGVAASATAATTNIKPEGDAEYPEDEIEDLLR HAGEEKERSGVVHKSQTQLLKTHAAETNILSPIQCNKQYKLKTLKSCTQQPS SMTGTLEGASLHKQLLKECQKTSKLIHLSNLMAQNHPKKRKDAPKKSQHK TKSKKRSKNVSSHSAKSLHAKKKQRTSSSSSSSSSSSTSSEKTSSNSSLT (SEQ ID NO: 880)
ORF2/3	MQTPASQISSDDFFVHTPFNAVTKQQIWMSQIADGHDNICHCHRPFAHLLAN IFPPGHKDRDLTINQ ILARDLTETCHSGGDEGTSGGGVAASATAATTNIKPEGDAEYPEDEIEDLLR HAGEEKERRITQKKEKMHQRNPNTKPKARRDPRMSPLTLRRAYMPRRNRG

	PPALHPAAAAAAVQAQKKPLQTPHSPEKRTENKSTTNGTFRVIPFKPGFEQE TEKELAIAFCRPPRKYKNDPPFYPWLPWTPLVHFNLNYKG (SEQ ID NO: 881)
TAIP	MDMTTFVTATVLLLTCLLIFFLLVIKTGILPLIKYLLEILQKHAILVETKEQAV VGSPLPLPPLQQI (SEQ ID NO: 882)
ORF1	MPFWWRRRNKRWWGRRFRYRRYNKYKTRRRRRIPRRRNRRFTKTRRRRK RKKVRRKLKKITIKQWQP DSVKKCKIKGYSTLVMGAQGKQYNCYTNQASDYVQPKAPQGGGFGCEVF NLKWLYQEYTAHRNIWTKTNEYTDLCRYTGAQIILYRHPDVDFIVSWDNQP PFLLNKYTYPELQPQNLLLARRKRIILSQKSNPKGKLRIKLRIPPPKQMITKWF FQRDFCDVNLFKLCASAASFRYPGISHGAQSTIFSAYALNTDFYQCSDWCQT NTETGYLNIKTQQMPLWFHYREGGKEKWYKYTNKEHRPYTNTYLKSISYN DGLFSPKAMFAFEVKAGGEGTTEPPQGAQLIANLPLIALRYNPHEDTGHGNE IYLTSTFKGTYDKPKVTDALYFNNVPLWMGFYGYWDFILQETKNKGVFDQ HMFVVKCPALRPISQVTKQVYYPLVDMDFCSGRLPFDEYLSKDIKSHWYPT AERQTVTINNFVTAGPYMPKFEPTDKDSTWQLNYHYKFFFKWGGPQVTDP TVEDPCSRNKYPVPDTMQQTIQIKNPEKLHPATLFHDWDLRRGFITQAAIKR MSENLQIDSSFESDGTESPKKKKRCTKEIPTQNQKQEEIQECLLSLCEEPTCQ EETEDLQLFIQQQQQQQYKLRKNLFKLLTHLKKGQRISQLQTGLLE (SEQ ID NO: 883)
ORF1/1	MPFWWRRRNKRWWGRRFRYRRYNKYKTRRRRRIPRRRNRRFTKTRRRRK RKKWGGPQVTDPTVEDPCSRNKYPVPDTMQQTIQIKNPEKLHPATLFHDWD LRRGFITQAAIKRMSENLQIDSSFESDGTESPKKKKRCTKEIPTQNQKQEEIQE CLLSLCEEPTCQEETEDLQLFIQQQQQQQYKLRKNLFKLLTHLKKGQRISQL QTGLLE (SEQ ID NO: 884)
ORF1/2	MPFWWRRRNKRWWGRRFRYRRYNKYKTRRRRRIPRRRNRRFTKTRRRRK RKKNHPKKRKDAPKKSQHKTKSKKRSKNVSSHSAKSLHAKKKQRTSSSSSS SSSSSSTSSEKTSSNSSLT (SEQ ID NO: 885)

Table N17. Exemplary *Anellovirus* nucleic acid sequence (*Gammatorquevirus*)

Name	Ring4
Genus/Clade	*Gammatorquevirus*

Accession Number

Full Sequence: 3176 bp

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1         10         20         30         40         50
|         |          |          |          |          |
TAAAATGGCGGGAGCCAATCATTTTATACTTTCACTTTCCAATTAAAAAT
GGCCACGTCACAAACAAGGGGTGGAGCCATTTAAACTATATAACTAAGTG
GGGTGGCGAATGGCTGAGTTTACCCCGCTAGACGGTGCAGGGACCGGATC
GAGCGCAGCGAGGAGGTCCCCGGCTGCCCATGGGCGGGAGCCGAGGTGAG
TGAAACCACCGAGGTCTAGGGGCAATTCGGGCTAGGGCAGTCTAGCGGAA
CGGGCAAGAAACTTAAAACAATATTTGTTTTACAGATGGTTAGTATATCC
TCAAGTGATTTTTTTAAGAAAACGAAATTTAATGAGGAGACGCAGAACCA
AGTATGGATGTCTCAAATTGCTGACTCTCATGATAATATCTGCAGTTGCT
GGCATCCATTTGCTCACCTTCTTGCTTCCATATTTCCTCCTGGCCACAAA
GATCGTGATCTTACTATTAACCAAATTCTTCTAAGAGATTATAAAGAAAA
ATGCCATTCTGGTGGAGAAGAAGGAGAAAATTCTGGACCAACAACAGGTT
TAATTACACCAAAAGAAGAAGATATAGAAAAAGATGGCCCAGAAGGCGCC
GCAGAAGAAGACCATACAGACGCCCTGTTCGCCGCCGCCGTAGAAAACTT
CGAAAGGTAAAGAGAAAAAAAAAATCTTTAATTGTTAGACAATGGCAACC
AGACAGTATAAGAACTTGTAAAATTATAGGACAGTCAGCTATAGTTGTTG
GGGCTGAAGGAAAGCAAATGTACTGTTATACTGTCAATAAGTTAATTAAT
GTGCCCCCAAAAACACCATATGGGGGAGGCTTTGGAGTAGACCAATACAC
ACTGAAATACTTATATGAAGAATACAGATTTGCACAAAACATTTGGACAC
AATCTAATGTACTGAAAGACTTATGCAGATACATAAATGTTAAGCTAATA
TTCTACAGAGACAACAAAACAGACTTTGTCCTTTCCTATGACAGAAACCC
ACCTTTTCAACTAACAAAATTTACATACCCAGGAGCACACCCACAACAAA
TCATGCTTCAAAAACACCACAAATTCATACTATCACAAATGACAAAGCCT
AATGGAAGACTAACAAAAAAACTCAAAATTAAACCTCCTAAACAAATGCT
TTCTAAATGGTTCTTTTCAAAACAATTCTGTAAATACCCTTTACTATCTC
TTAAAGCTTCTGCACTAGACCTTAGGCACTCTTACCTAGGCTGCTGTAAT
GAAAATCCACAGGTATTTTTTTATTATTTAAACCATGGATACTACACAAT
AACAAACTGGGGAGCACAATCCTCAACAGCATACAGACCTAACTCCAAGG
TGACAGACACAACATACTACAGATACAAAAATGACAGAAAAAATATTAAC
ATTAAAAGCCATGAATACGAAAAAAGTATATCATATGAAAACGGTTATTT
TCAATCTAGTTTCTTACAAACACAGTGCATATATACCAGTGAGCGTGGTG
AAGCCTGTATAGCAGAAAAACCACTAGGAATAGCTATTTACAATCCAGTA
AAAGACAATGGAGATGGTAATATGATATACCTTGTAAGCACTCTAGCAAA
CACTTGGGACCAGCCTCCAAAAGACAGTGCTATTTTAATACAAGGAGTAC
CCATATGGCTAGGCTTATTTGGATATTTAGACTACTGTAGACAAATTAAA
GCTGACAAAACATGGCTAGACAGTCATGTACTAGTAATTCAAAGTCCTGC
TATTTTTACTTACCCAAATCCAGGAGCAGGCAAATGGTATTGTCCACTAT
CACAAAGTTTTATAAATGGCAATGGTCCGTTTAATCAACCACCTACACTG
CTACAAAAAGCAAAGTGGTTTCCACAAATACAATACCAACAAGAAATTAT
TAATAGCTTTGTAGAATCAGGACCATTTGTTCCCAAATATGCAAATCAAA
CTGAAAGCAACTGGGAACTAAAATATAAATATGTTTTTACATTTAAGTGG
GGTGGACCACAATTCCATGAACCAGAAATTGCTGACCCTAGCAAACAAGA
GCAGTATGATGTCCCCGATACTTTCTACCAAACAATACAAATTGAAGATC
CAGAAGGACAAGACCCCAGATCTCTCATCCATGATTGGGACTACAGACGA
GGCTTTATTAAAGAAAGATCTCTTAAAAGAATGTCAACTTACTTCTCAAC
TCATACAGATCAGCAAGCAACTTCAGAGGAAGACATTCCCAAAAAGAAAA
AGAGAATTGGACCCCAACTCACAGTCCCACAACAAAAAGAAGAGGAGACA
CTGTCATGTCTCCTCTCTCTCTGCAAAAAAGATACCTTCCAAGAAACAGA
GACACAAGAAGACCTCCAGCAGCTCATCAAGCAGCAGCAGGAGCAGCAGC
TCCTCCTCAAGAGAAACATCCTCCAGCTCATCCACAAACTAAAAGAGAAT
CAACAAATGCTTCAGCTTCACACAGGCATGTTACCTTAACCAGATTTAAA
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CCTGGATTTGAAGAGCAAACAGAGAGAGAATTAGCAATTATATTTCATAG
GCCCCCTAGAACCTACAAAGAGGACCTTCCATTCTATCCCTGGCTACCAC
CTGCACCCCTTGTACAATTTAACCTTAACTTCAAAGGCTAGGCCAACAAT
GTACACTTAGTAAAGCATGTTTATTAAAGCACAACCCCCAAAATAAATGT
AAAAATAAAAAAAAAAAAAAAAAAATAAAAAATTGCAAAAATTCGGCGCT
CGCGCGCATGTGCGCCTCTGGCGCAAATCACGCAACGCTCGCGCGCCCGC
GTATGTCTCTTTACCACGCACCTAGATTGGGGTGCGCGCGCTAGCGCGCG
CACCCCAATGCGCCCCGCCCTCGTTCCGACCCGCTTGCGCGGGTCGGACC
ACTTCGGGCTCGGGGGGGCGCGCCTGCGGCGCTTTTTTACTAAACAGACT
CCGAGCCGCCATTTGGCCCCCTAAGCTCCGCCCCCCTCATGAATATTCAT
AAAGGAAACCACATAATTAGAATTGCCGACCACAAACTGCCATATGCTAA
TTAGTTCCCCTTTTACAAAGTAAAAGGGGAAGTGAACATAGCCCCACACC
CGCAGGGGCAAGGCCCCGCACCCCTACGTCACTAACCACGCCCCCGCCGC
CATCTTGGGTGCGGCAGGGCGGGGGC (SEQ ID NO: 886)

Annotations:

Putative Domain	*Base range*
TATA Box	87– 93
Cap Site	110 – 117
Transcriptional Start Site	117
5’ UTR Conserved Domain	185 – 254
ORF2	286 – 660
ORF2/2	286 – 656 ; 1998 – 2442
ORF2/3	286 – 656 ; 2209 – 2641
TAIP	385 - 484
ORF1	501 – 2489
ORF1/1	501 – 656 ; 1998 – 2489
ORF1/2	501 – 656 ; 2209 – 2442
Three open-reading frame region	2209 – 2439
Poly(A) Signal	2672 – 2678
GC-rich region	3076 – 3176

Table A17. Exemplary *Anellovirus* amino acid sequences (*Gammatorquevirus*)

Ring 4 (*Gammatorquevirus*)	
ORF2	MVSISSSDFFKKTKFNEETQNQVWMSQIADSHDNICSCWHPFAHLLASIFPP GHKDRDLTINQILLR DYKEKCHSGGEEGENSGPTTGLITPKEEDIEKDGPEGAAEEDHTDALFAAAV ENFER (SEQ ID NO: 887)

ORF2/2	MVSISSSDFFKKTKFNEETQNQVWMSQIADSHDNICSCWHPFAHLLASIFPP GHKDRDLTINQILLRDYKEKCHSGGEEGENSGPTTGLITPKEEDIEKDGPEGA AEEDHTDALFAAAVENFESGVDHNSMNQKLLTLANKSSMMSPILSTKQYKL KIQKDKTPDLSSMIGTTDEALLKKDLLKECQLTSQLIQISKQLQRKTFPKRKR ELDPNSQSHNKKKRRHCHVSSLSAKKIPSKKQRHKKTSSSSSSSSRSSSSSSR ETSSSSSTN (SEQ ID NO: 888)
ORF2/3	MVSISSSDFFKKTKFNEETQNQVWMSQIADSHDNICSCWHPFAHLLASIFPP GHKDRDLTINQILLRDYKEKCHSGGEEGENSGPTTGLITPKEEDIEKDGPEGA AEEDHTDALFAAAVENFERSASNFRGRHSQKEKENWTPTHSPTTKRRGDTV MSPLSLQKRYLPRNRDTRRPPAAHQAAAGAAAPPQEKHPPAHPQTKRESTN ASASHRHVTLTRFKPGFEEQTERELAIIFHRPPRTYKEDLPFYPWLPPAPLVQ FNLNFKG (SEQ ID NO: 889)
TAIP	MRRRRTKYGCLKLLTLMIISAVAGIHLLTFLLPYFLLATKIVILLLTKFF (SEQ ID NO: 890)
ORF1	MPFWWRRRRKFWTNNRFNYTKRRRYRKRWPRRRRRRRPYRRPVRRRRRK LRKVKRKKKSLIVRQWQPDSIRTCKIIGQSAIVVGAEGKQMYCYTVNKLINV PPKTPYGGGFGVDQYTLKYLYEEYRFAQNIWTQSNVLKDLCRYINVKLIFY RDNKTDFVLSYDRNPPFQLTKFTYPGAHPQQIMLQKHHKFILSQMTKPNGR LTKKLKIKPPKQMLSKWFFSKQFCKYPLLSLKASALDLRHSYLGCCNENPQ VFFYYLNHGYYTITNWGAQSSTAYRPNSKVTDTTYYRYKNDRKNINIKSHE YEKSISYENGYFQSSFLQTQCIYTSERGEACIAEKPLGIAIYNPVKDNGDGNM IYLVSTLANTWDQPPKDSAILIQGVPIWLGLFGYLDYCRQIKADKTWLDSHV LVIQSPAIFTYPNPGAGKWYCPLSQSFINGNGPFNQPPTLLQKAKWFPQIQYQ QEIINSFVESGPFVPKYANQTESNWELKYKYVFTFKWGGPQFHEPEIADPSK QEQYDVPDTFYQTIQIEDPEGQDPRSLIHDWDYRRGFIKERSLKRMSTYFST HTDQQATSEEDIPKKKKRIGPQLTVPQQKEEETLSCLLSLCKKDTFQETETQE DLQQLIKQQQEQQLLLKRNILQLIHKLKENQQMLQLHTGMLP (SEQ ID NO: 891)
ORF1/1	MPFWWRRRRKFWTNNRFNYTKRRRYRKRWPRRRRRRRPYRRPVRRRRRK LRKWGGPQFHEPEIADPSKQEQYDVPDTFYQTIQIEDPEGQDPRSLIHDWDY RRGFIKERSLKRMSTYFSTHTDQQATSEEDIPKKKKRIGPQLTVPQQKEEETL

	SCLLSLCKKDTFQETETQEDLQQLIKQQQEQQLLLKRNILQLIHKLKENQQM LQLHTGMLP (SEQ ID NO: 892)
ORF1/2	MPFWWRRRRRKFWTNNRFNYTKRRRYRKRWPRRRRRRRRPYRRPVRRRRRK LRKISKQLQRKTFPKRKR ELDPNSQSHNKKKRRHCHVSSLSAKKIPSKKQRHKKTSSSSSSSSRSSSSSSR ETSSSSSTN (SEQ ID NO: 893)

Table N18. Exemplary *Anellovirus* nucleic acid sequence (*Alphatorquevirus*) – Clade 1

Name Ring5.2

Genus/Clade *Alphaatorquevirus* Clade 1

Accession Number

Full Sequence: 3696 bp

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1         10         20         30         40         50
|         |          |          |          |          |
ATTTTGTTCAGCCCGCCAATTTCTCTTTCAAACAGGCCAATCAGCTACTA
CTTCGTGCACTTCCTGGGGCGTGTCCTGCCGCTCTATATAAGCAGAGGCG
GTGACGAATGGTAGAGTTTTTCTTGGCCCGTCCGCGGCGAGAGCGCGAGC
GAAGCGAGCGATCGAGCGTCCCGAGGGCGGGTGCCGGAGGTGAGTTTACA
CACCGCAGTCAAGGGGCAATTCGGGCTCGGGACTGGCCGGGCTATGGGCA
AGATTCTTAAAAAATTCCCCCGATCCCTTTGCCGCCAGGACATAAAAACA
TGCCGTGGAGACCGCCGGTCCATAGTGTCCAGGGGCGAGAGGATCAGTGG
TTCGCAAGCTTTTTTCACGGCCACGATTCGTTTTGCGGCTGCGGTGACCC
TCTTGGCCATATTAATAGCATTGCTCATCGCTTTCCTCGCGCCGGTCCAC
CAAGGCCCCCTCCGGGGCTAGATCAGCCTAACCCCCGGGAGCAGGGCCCG
GCCGGACCCGGAGGGCCGCCCGCCATCTTGGCCCTGCCGGCTCCGCCCGC
GGAGCCTGACGACCCGCAGCCACGGCGTGGTGGTGGGGACGGTGGCGCCG
CCGCTGGCGCCGCAGACGACCATACACAACGAGACTACGACGAAGAAGAG
CTAGACGAGCTTTTCCGCGCCGCCGCCGAAGACGATTTGTAAGTAGGAGA
TGGCGCCGGCCTTACAGGCGCAGGAGGAGACGCGGGCGACGCAGACGCAG
ACGCAGACGCAGACATAAGCCCACCCTAATACTCAGACAGTGGCAACCTG
ACTGTATCAGACACTGTAAAATAACAGGATGGATGCCCCTCATTATCTGT
GGAAAGGGGTCCACCCAGTTCAACTACATCACCCACGCGGACGATATCAC
CCCCAGGGGAGCCTCCTACGGAGGCAATTTCACAAACATGACTTTCTCCC
TGGAGGCCATATATGAACAGTTCCTATACCACAGAAACAGGTGGTCGGCC
TCTAACCACGACCTAGAACTGTGCAGATACAAGGGGACCACCTTAAAACT
CTACAGACACCCAGAAGTAGACTACATAGTTACCTACAGCAGAACAGGAC
CCTTTGAAATCAGCCACATGACCTACCTCAGCACTCACCCCATGCTAATG
CTGCTAAACAAGCACCACATTGTGGTGCCCAGCTTAAAGACTAAGCCCAG
AGGCAGAAAGGCCATAAAAGTCAGGATAAGGCCCCCAAAACTCATGAACA
ACAAGTGGTACTTCACCAGAGACTTCTGTAACATAGGCCTCTTCCAGCTC
TGGGCCACAGGCTTAGAACTCAGAAACCCCTGGCTCAGAATGAGCACCCT
GAGCCCCTGCATAGGCTTTAATGTCCTCAAAAACAGCATTTACACAAACC
TCAGCAACCTGCCACAATACAAAAACGAAAGACTAAACATCATTAACAAC
ATACTTCACCCACAAGAAATTACAGGTACAAACAACAAAAAGTGGCAGTA
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CACATACACCAAACTCATGGCCCCTATTTACTATTCAGCAAACAGGGCCA
GCACCTATGACTGGGAAAATTACAGCAAAGAAACAAACTACAATAATACA
TATGTTAAATTTACCCAGAAAAGACAGGAAAAACTAACTAAAATTAGAAA
AGAGTGGCAGATGCTTTATCCACAACAACCCACAGCACTGCCAGACTCCT
ATGACCTCCTACAAGAGTATGGCCTCTACAGTCCATACTACCTAAACCCC
ACAAGAATAAACCTAGACTGGATGACCCCATACACACACGTCAGATACAA
TCCCCTAGTAGACAAGGGCTTTGGAAACAGAATATACATCCAGTGGTGCT
CAGAAGCAGATGTTAGCTACAACAGGACAAAATCCAAGTGTCTGCTACAA
GACATGCCCCTGTTTTTCATGTGCTATGGCTACATAGACTGGGCAATAAA
AAACACTGGAGTGTCATCTCTAGTGAAGGACGCCAGAATCTGCATCAGGT
GTCCCTACACAGAGCCACAACTAGTTGGCTCCACAGAAGACATAGGCTTT
GTACCCATCTCAGAAACCTTCATGAGGGGCGACATGCCGGTACTTGCACC
ATACATACCGTTAAGCTGGTTTTGCAAGTGGTATCCCAACATAGCTCACC
AAAAGGAAGTCCTTGAGTCAATCATTTCCTGCAGCCCCTTCATGCCCCGT
GACCAAGACATGAACGGTTGGGATATCACAATCGGTTACAAAATGGACTT
CTTATGGGGCGGTTCCCCTCTCCCCTCACAGCCAATCGACGACCCCTGCC
AGCAGGGAACCCACCCGATTCCCGACCCCGATAAACACCCTCGCCTCCTA
CAAGTCTCGAACCCGAAACTACTCGGACCGAGGACAGTGTTCCACAAGTG
GGACATCAGACGTGGGCAGTTTAGCAAAAGAAGTATTAAGAGAGTGTCAG
AATACTCAAGCGATGATGAATCTCTTGCGCCAGGTCTCCCATCAAAGCGA
AACAAGCTCGACTCGGCGTTCCGAGGAGAAAATCGAGAGCAAAAAGAATG
CTATTCTCTCCTCAAAGCGCTCGAGGAAGAAGAGACCCCAGAAGAAGAAG
AACCAGCACCCCAAGAAAAAGCCCAGAAAGAGGAGCTACTCCACCAGCTC
CAGCTCCAGAGACGCCACCAGCGAGTCCTCAGACGAGGGCTCAAGCTCGT
CTTTACAGACATCCTCCGACTCCGCCAGGGAGTCCACTGGAACCCGGAGC
TCACATAGCGCCCCCACCTTACATACCAGACCTGCTTTTTCCCAATACTG
GTAAAAAAAAAAAATTCTCTCCCTTCGATTGGGAGACAGAGGCGCAAATA
GCGGGGTGGATGCGGCGGCCCATGCGCTTCTATCCCTCAGACACCCCTCA
CTACCCGTGGCTACCCCCCGAGCGAGATATCCCGAAAATATGTAACATAA
ACTTCAAAATAAAGCTTCAAGAGTGAGTGATTCGAGGCCCTCCTCTGTTC
ACTTAGCGGTGTCTACCTCTTAAGGTCACTAAGCACTCCGAGCGTAAGCG
AGGAGTGCGACCCTCTACCAAGGGGCAACTTCCTCGGGGTCCGGCGCTAC
GCGCTTCGCGCTGCGCCGGACATCTCGGACCCCTCGACCCGAATCGCTTG
CGCGATTCGGACCTGCGGCCTCGGGGGGGGTCGGGGGCTTTACTAAACAGA
CTCCGAGGTGCCATTGGACACTGTAGGGGGGTGAACAGCAACGAAAGTGAG
TGGGGCCAGACTTCGCCATAAGGCCTTTATCTTCTTGCCATTGGATAGTG
ACTTCCGGGTCCGCCTGGGGGCCGCCATTTTAGCTTCGGCCGCCATTTTA
GGCCCTCGCGGGCCTCCGTAGGCGCGCTTTAGTGACGTCACGGCAGCCAT
TTTGTCGTGACGTTTGAGACACGTGATGGGGGCGTGCCTAAACCCGGAAG
CATCCCTGGTCACGTGACTCTGACGTCACGGCGGCCATCTTGTGCTGTCC
GCCATCTTGTAACTTCCTTCCGCTTTTTCAAAAAAAAAGAGGAAGTGTGA
CGTAGCGGCGGGGGGGCGGCGCGCTTCGCGCGCCGCCCACCAGGGGGCGC
TGCGCGCCCCCCGCGCATGCGCAGGGGCCTCTCGAGGGGCTCCGCCCCCC
CCCCGTGCTAAATTTACCGCGCATGCGCGACCACGCCCCCGCCGCC (SEQ ID NO: 894)

Annotations:

Putative Domain	*Base range*
TATA Box	85– 91
Cap Site	108 – 115
Transcriptional Start Site	115
5' UTR Conserved Domain	178 – 248
ORF2	300 – 692

ORF2/2	300 – 688 ; 2282 – 2804
ORF2/3	300 – 688 ; 2484 – 2976
ORF2t/3	300 – 349 : 2484 - 2976
TAIP	322 - 471
ORF1	572 – 2758
ORF1/1	572 – 688 ; 2282 – 2758
ORF1/2	572 – 688 ; 2484 – 2804
Three open-reading frame region	2484 – 2755
Poly(A) Signal	3018 –3023
GC-rich region	3555 – 3696

Table A18. Exemplary *Anellovirus* amino acid sequences (*Alphatorquevirus*) Clade 1

Ring 5.2 (*Alphaatorquevirus*) Clade 1	
ORF2	MPWRPPVHSVQGREDQWFASFFHGHDSFCGCGDPLGHINSIAHRFPRAGPP RPPPGLDQPNPREQGPAGPGGPPAILALPAPPAEPDDPQPRRGGGDGGAAAG AADDHTQRDYDEEELDELFRAAAEDDL (SEQ ID NO: 895)
ORF2/2	MPWRPPVHSVQGREDQWFASFFHGHDSFCGCGDPLGHINSIAHRFPRAGPP RPPPGLDQPNPREQGPAGPGGPPAILALPAPPAEPDDPQPRRGGGDGGAAAG AADDHTQRDYDEEELDELFRAAAEDDFQSTTPASREPTRFPTPINTLASYKS RTRNYSDRGQCSTSGTSDVGSLAKEVLRECQNTQAMMNLLRQVSHQSETSS TRRSEEKIESKKNAILSSKRSRKKRPQKKKNQHPKKKPRKRSYSTSSSSRDAT SESSDEGSSSSLQTSSDSARESTGTRSSHSAPTLHTRPAFSQYW (SEQ ID NO: 896)
ORF2/3	MPWRPPVHSVQGREDQWFASFFHGHDSFCGCGDPLGHINSIAHRFPRAGPP RPPPGLDQPNPREQGPAGPGGPPAILALPAPPAEPDDPQPRRGGGDGGAAAG AADDHTQRDYDEEELDELFRAAAEDDLSPIKAKQARLGVPRRKSRAKRMLF SPQSARGRRDPRRRRTSTPRKSPERGATPPAPAPETPPASPQTRAQARLYRHP PTPPGSPLEPGAHIAPPPYIPDLLFPNTGKKKKFSPFDWETEAQIAGWMRRPM RFYPSDTPHYPWLPPERDIPKICNINFKIKLQ (SEQ ID NO: 897)
ORF2t/3	MPWRPPVHSVQGREDQWSPIKAKQARLGVPRRKSRAKRMLFSPQSARGRR DPRRRRTSTPRKSPERGATPPAPAPETPPASPQTRAQARLYRHPPTPPGSPLEP

	GAHIAPPPYIPDLLFPNTGKKKKFSPFDWETEAQIAGWMRRPMRFYPSDTPH YPWLPPERDIPKICNINFKIKLQE (SEQ ID NO: 898)
TAIP	IVSRGERISGSQAFFTATIRFAAAVTLLAILIALLIAFLAPVHQGPLRG (SEQ ID NO: 899)
ORF1	TAWWWGRWRRRWRRRRPYTTRLRRRRARRAFPRRRRRRFVSRRWRRPYR RRRRRGRRRRRRRRRHKPTLILRQWQPDCIRHCKITGWMPLIICGKGSTQFN YITHADDITPRGASYGGNFTNMTFSLEAIYEQFLYHRNRWSASNHDLELCRY KGTTLKLYRHPEVDYIVTYSRTGPFEISHMTYLSTHPMLMLLNKHHIVVPSL KTKPRGRKAIKVRIRPPKLMNNKWYFTRDFCNIGLFQLWATGLELRNPWLR MSTLSPCIGFNVLKNSIYTNLSNLPQYKNERLNIINNILHPQEITGTNNKKWQ YTYTKLMAPIYYSANRASTYDWENYSKETNYNNTYVKFTQKRQEKLTKIR KEWQMLYPQQPTALPDSYDLLQEYGLYSPYYLNPTRINLDWMTPYTHVRY NPLVDKGFGNRIYIQWCSEADVSYNRTKSKCLLQDMPLFFMCYGYIDWAIK NTGVSSLVKDARICIRCPYTEPQLVGSTEDIGFVPISETFMRGDMPVLAPYIPL SWFCKWYPNIAHQKEVLESIISCSPFMPRDQDMNGWDITIGYKMDFLWGGS PLPSQPIDDPCQQGTHPIPDPDKHPRLLQVSNPKLLGPRTVFHKWDIRRGQFS KRSIKRVSEYSSDDESLAPGLPSKRNKLDSAFRGENREQKECYSLLKALEEE ETPEEEEPAPQEKAQKEELLHQLQLQRRHQRVLRRGLKLVFTDILRLRQGVH WNPELT (SEQ ID NO: 900)
ORF1/1	TAWWWGRWRRRWRRRRPYTTRLRRRRARRAFPRRRRRRFPIDDPCQQGT HPIPDPDKHPRLLQVSNPKLLGPRTVFHKWDIRRGQFSKRSIKRVSEYSSDDE SLAPGLPSKRNKLDSAFRGENREQKECYSLLKALEEEETPEEEEPAPQEKAQ KEELLHQLQLQRRHQRVLRRGLKLVFTDILRLRQGVHWNPELT (SEQ ID NO: 901)
ORF1/2	TAWWWGRWRRRWRRRRPYTTRLRRRRARRAFPRRRRRRFVSHQSETSSTR RSEEKIESKKNAILSSK RSRKKRPQKKKNQHPKKKPRKRSYSTSSSSRDATSESSDEGSSSSLQTSSDSA RESTGTRSSHSAPTLHTRPAFSQYW (SEQ ID NO: 902)

Table N19. Exemplary *Anellovirus* nucleic acid sequence (*Alphatorquevirus*) – Clade 3

Name	Ring 6.0
Genus/Clade	*Alphatorquevirus* – Clade 3
Accession Number	

Full Sequence: 3828 bp

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1         10         20         30         40         50
|         |          |          |          |          |
GTGCTACGTCACTAACCTACGTGTCCGTCTCCCATAGGCCGGACACCGTA
TACGTCATACACTTCCTGGGCATGGTCTACGTGATAATATAAGTGGCTGC
ACTTCCGAATGGCTGAGTTTTCCACGCCCGTCCGCAGCGAGGACGCCACG
GAGGGGGATCCGCGCGTCCCGAGGGCGGGTGCCGGAGGTGAGTTTACACA
CCGCAGTCAAGGGGCAATTCGGGCTCGGGACTGGCCGGGCTATGGGCAAG
GCTCTTAAAAATGCACTTTTCTAGGTGCAGTAGAAAGAAAAGGACATTGT
CACTGCTACCACTGTACCATTCACAGAAAGCTAGGCCATCTGTGACAGGT
ATGTGGAGACCCCCGACTCGAAATGCGTTCAATATTCAACGTGACTGGTT
CTACAGTTGCTTTCACTCCCACGCTTCTATGTGCGGCTGTGCTGATTTTA
TTGGTCATTTCAATCATATCGCTGCTATGCTCGGCCGTCCGGAAGACCAG
AACCCTCCTCCGCCACCCGGGGCTCTGAGACCCCTACCCGCTCTCCCGGC
CTCTTCCGAGGCACCCGGTGATCGAGCGCCATGGCCTATGGGTGGTGGCG
GAGGCGACGGAGGCGCCCGTGGTGGAGGAGGAGATGGCGCCGCTGGAGAC
GCCGTCGGAGACCCCGCAGACGCCGACCTCGTCGCCGCTATCGACGCCGC
AGAACAGTAAGGAGGCGCGGCAGGGGGAGGTGGACTAGAGCACACAGGAG
ATGGCGCCGCAAGGGAAAACGCAGTCGCAAAAAAAAGATTATTATAAGAC
AATGGCAGCCCAACTACACTCGCAGATGCAACATAGTGGGCTACATGCCT
CTACTAATATGTGGGGAAAATACTGTTGCTACAAACTATGCCACCCACTC
AGACGACAGCTACTACCCCGGACCCTTTGGGGGGGGAATGACTACAGACA
AATTTACTCTAAGAATACTGTATGATGAGTACAAAAGGTTCATGAACTAC
TGGACCTCTTCAAACGAGGACCTAGACCTATGTAGATACCTGGGATGCAC
TCTATATGTGTTTAGACACCCAGAAGTAGACTTTATAATCATTATAAATA
CCTCTCCTCCATTCCTAGACACAGAAATAACAGGGCCTAGCATACACCCA
GGTATGATGGCCCTTAACAAAAGAAGCAGATGGATACCTAGCATAAAAAA
CAGACCAGGCAGAAAGCACTATATAAAGATTAAAGTAGGAGCCCCCCGAA
TGTTCACAGATAAGTGGTACCCCCAAACAGACCTCTGTGACATGACACTC
CTAACGATCTTTGCCAGTGCGGCGGATATGCAATATCCGTTCGGCTCACC
ACTAACTGACACCATAGTTGTGTCATTCCAAGTTCTGCAATCCATGTACA
ACGACTGCCTGAGTGTACTTCCTGATAATTTTGCAGAGACATCAGGCAAA
GGCACCCAACTACATGAGAACATAATACAACATCTGCCCTACTACAACAC
CACACAAACACAAGCACAATTTAAAAGATTTATAGAAAACATGAATGCAA
CAAATGGAGACAATATATGGGCAAGCTACATAAACACAACCAAGTTCTCA
TCCGCAAACACTCCAAAGAATGACACAGGCATAGGAGGCCCTTACACTAC
ATATTCAGACTCATGGTACAAAGGCACAGTATACAATGACAAAATTAAAA
CCATACCAATAAAAGCAAGCAAGTTATACTACGAGCAAACCAAAAACCTC
ATTGGCATTACATTCACTGGATCCACACACAGACTCCATTACTGTGGAGG
CCTATACTCCTCCGTATGGCTATCAGCAGGTAGATCCTACTTTGAAACCA
AAGGCCCATACACAGACATAACTTACAACCCCTTTTCAGACAGAGGAGAG
GGTAACATGCTATGGATAGACTGGCTAACTAAAAATGACTCAGTGTACTC
AAAAACAAGTAGCAAGTGTCTTATAGAAAACCTGCCCCTGTGGGCCTCAG
TATACGGATATAAAGAATACTGCAGCAAGGTAACAGGAGACACAAACATA
GAACACAACTGTAGATGTGTTATCAGAAGCCCCTACACAGTACCACAACT
GTTAGACCACAACAATCCCTTCAGAGGATACGTGCCTTATAGCTTCAACT
TTGGAAATGGTAAAATGCCAGGCGGTAGCAGCCTAGTGCCCATTAGAATG
AGAGCCAAGTGGTACCCCACTCTGTTCCACCAAAAAGAAGTTCTAGAAGC
CATAGCACAGGCGGGCCCCTTCGCATACCACTCAGATATTAAAAAAGTGT
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CCCTGGGCATAAAGTACAGATTTAAGTGGGTGTGGGGTGGCAACCCCGTG
TCCCAACAGGTTGTTAGAAACCCCTGCAAGACCACCCAAGGTTCCTCGGG
CAATAGAGTGCCTCGATCAATACAAGTCGTTGACCCGCGGTACAACACGC
CAGAACTCACCATACACGCGTGGGACTTCAGACATGGGTTCTTTGGCAGA
AAAGCTATTAAGAGAATGCAAGAACAACCAATACCTCATGACACTTTTTC
AGCAGGGTTCAAGCGCAGTCGCCGAGATACAGAAGCACTCCAATGCAGCC
AAGAAGAGCAACAAAAAGAAAACTTACTTTTCCCAGTCCAGCAGCTCAAG
CGAGTCCCCCCGTGGGAGACCTCGCAAGAGAGCCAAAGCGAGGAAGAAAA
CTCGCAAAAACAGGAGACCCTCTCCCAGCAACTCAGAGACCAGCTGCACA
AGCAGCGGCTCATGGGAGAGCAACTCCGATCGCTCCTCTACCAAATGCAG
AGGGTCCAACAAAATCAACACATAAACCCTATGTTATTGCCAAAGGGTCT
GGCATTAACTTCTATTTCTCACAATGTAATATAGATATGTTTGGTGACCC
CAAACCCTACAAGCCCTCCTCCAATGACTGGAAGGAGGAGTACGAGGCCG
CAAAGTACTGGGACAGACCCCCCAGACGCGACCTGAGGAGCACCCCCTTC
TACCCCTGGGCCCCCACCCCCAAACCATACAATGTCAACTTTGCCCTCAA
CTACAAATAAACGGTGGCCGTGGGAGTTTCACTTGTCGGTGTCTACCTCT
TAAGGTCACTAAGCACTCCGAGCGTAAGCGAGGAGTGCGACCCTTCACCA
AGGGCAACTCCCTCGAAGTCCGGCGCTACGCGCTTCGCGCTGCGCCGGAC
ATCTCGGACCCCCCCTCGACCCGAATCGCTTGCGCGATTCGGACCTGCGG
CCTCGGGGGGGTCGGGGGCTTTACTAAACAGACTCCGAGGTGCCATTGGA
CACTGAGGGGGTGAACAGCAACGAAAGTGAGTGGGGCCAGACTTCGCCAT
AAGGCCTTTATCTTCTTGCCATTTGTCCGCGACCGGGGGTCGCTCCTAGG
CGCGGACCCCGTTTCGGGGTCCTTCCGGGTTCATCGGCGCCGTTCCAGTG
ACGTCACGGGCGCCATGTTAAGTGGCTGTCGCCGAGGATTGACGTCACAG
TTCAAAGGTCATCCTCGGCGGTAACCGCAAACATGGCGGTCAATCTCTTC
CGGGTCAAAGGTCGTGCATACGTCATAAGTCACATGACAGGGGTCCACTT
AAACACGGAAGTAGGCCCCGACATGTGACTCGTCACGTGTGTACACGTCA
CGGCCGCCATTTTGTTTTACAAAATGGCCGACTTCCTTCCTGTTTTTTAA
AAAAAGGCGCGAAAAAACCGTCGGCGGGGGCCGCGCGCTGCGCGCGCGGG
AGGCAATGCCTCCCCCCCCCCGCGCGCATGCGCGCGGGTCCCCCCCCCTC
CGGGGGGCTCCGCCCCCCGGCCCCCCCC (SEQ ID NO: 903)

Annotations:

Putative Domain	*Base range*
TATA Box	85– 92
Cap Site	109 – 116
Transcriptional Start Site	116
5' UTR Conserved Domain	176 – 246
ORF2	351 – 710
ORF2/2	351 – 706 ; 2360 – 2825
ORF2/3	351 – 706 ; 2556 – 3060
TAIP	373 – 528
ORF1	581 – 2884
ORF1/1	581 – 706 ; 2360 – 2884
ORF1/2	581 – 706 ; 2556 – 2825

Three open-reading frame region	2556 – 2821
Poly(A) Signal	3055 – 3061
GC-rich region	3720 – 3828

Table A19. Exemplary *Anellovirus* amino acid sequences (*Alphatorquevirus*) – Clade 3

Ring 6.0 (*Alphatorquevirus*)	
ORF2	MWRPPTRNAFNIQRDWFYSCFHSHASMCGCADFIGHFNHIAAMLGRPEDQ NPPPPPGALRPLPALPASSEAPGDRAPWPMGGGGGDGGARGGGGDGAAGD AVGDPADADLVAAIDAAEQ (SEQ ID NO: 904)
ORF2/2	MWRPPTRNAFNIQRDWFYSCFHSHASMCGCADFIGHFNHIAAMLGRPEDQ NPPPPPGALRPLPALPASSEAPGDRAPWPMGGGGGDGGARGGGGDGAAGD AVGDPADADLVAAIDAAEQLLETPARPPKVPRAIECLDQYKSLTRGTTRQNS PYTRGTSDMGSLAEKLLRECKNNQYLMTLFQQGSSAVAEIQKHSNAAKKS NKKKTYFSQSSSSSESPRGRPRKRAKARKKTRKNRRPSPSNSETSCTSSGSW ESNSDRSSTKCRGSNKINT (SEQ ID NO: 905)
ORF2/3	MWRPPTRNAFNIQRDWFYSCFHSHASMCGCADFIGHFNHIAAMLGRPEDQ NPPPPPGALRPLPALPASSEAPGDRAPWPMGGGGGDGGARGGGGDGAAGD AVGDPADADLVAAIDAAEQVQAQSPRYRSTPMQPRRATKRKLTFPSPAAQA SPPVGDLAREPKRGRKLAKTGDPLPATQRPAAQAAAHGRATPIAPLPNAEG PTKSTHKPYVIAKGSGINFYFSQCNIDMFGDPKPYKPSSNDWKEEYEAAKY WDRPPRRDLRSTPFYPWAPTPKPYNVNFALNYK (SEQ ID NO: 906)
TAIP	MRSIFNVTGSTVAFTPTLLCAAVLILLVISIISLLCSAVRKTRTLLRHPGL (SEQ ID NO: 907)
ORF1	MAYGWWRRRRRRPWWRRRWRRWRRRRRPRRRRPRRRYRRRRTVRRRGR GRWTRAHRRWRRKGKRSRKKKIIIRQWQPNYTRRCNIVGYMPLLICGENTV ATNYATHSDDSYYPGPFGGGMTTDKFTLRILYDEYKRFMNYWTSSNEDLD LCRYLGCTLYVFRHPEVDFIIIIINTSPPFLDTEITGPSIHPGMMALNKRSRWIPS IKNRPGRKHYIKIKVGAPRMFTDKWYPQTDLCDMTLLTIFASAADMQYPFG SPLTDTIVVSFQVLQSMYNDCLSVLPDNFAETSGKGTQLHENIIQHLPYYNTT QTQAQFKRFIENMNATNGDNIWASYINTTKFSSANTPKNDTGIGGPYTTYSD SWYKGTVYNDKIKTIPIKASKLYYEQTKNLIGITFTGSTHRLHYCGGLYSSV

	WLSAGRSYFETKGPYTDITYNPFSDRGEGNMLWIDWLTKNDSVYSKTSSKC LIENLPLWASVYGYKEYCSKVTGDTNIEHNCRCVIRSPYTVPQLLDHNNPFR GYVPYSFNFGNGKMPGGSSLVPIRMRAKWYPTLFHQKEVLEAIAQAGPFAY HSDIKKVSLGIKYRFKWVWGGNPVSQQVVRNPCKTTQGSSGNRVPRSIQVV DPRYNTPELTIHAWDFRHGFFGRKAIKRMQEQPIPHDTFSAGFKRSRRDTEA LQCSQEEQQKENLLFPVQQLKRVPPWETSQESQSEEENSQKQETLSQQLRD QLHKQRLMGEQLRSLLYQMQRVQQNQHINPMLLPKGLALTSISHNVI (SEQ ID NO: 908)
ORF1/1	MAYGWWRRRRRRPWWRRRWRRWRRRRRPRRRRPRRRYRRRRTVVRNPC KTTQGSSGNRVPRSIQVVDPRYNTPELTIHAWDFRHGFFGRKAIKRMQEQPI PHDTFSAGFKRSRRDTEALQCSQEEQQKENLLFPVQQLKRVPPWETSQESQS EEENSQKQETLSQQLRDQLHKQRLMGEQLRSLLYQMQRVQQNQHINPMLL PKGLALTSISHNVI (SEQ ID NO: 909)
ORF1/2	MAYGWWRRRRRRPWWRRRWRRWRRRRRPRRRRPRRRYRRRRTGSSAVA EIQKHSNAAKKSNKKKTYFSQSSSSSESPRGRPRKRAKARKKTRKNRRPSPS NSETSCTSSGSWESNSDRSSTKCRGSNKINT (SEQ ID NO: 910)

Table N20. Exemplary *Anellovirus* nucleic acid sequence (*Alphatorquevirus*) – Clade 7

Name	Ring7
Genus/Clade	*Alphatorquevirus* – Clade 7
Accession Number	

Full Sequence: 3815 bp

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1         10         20         30         40         50
|         |          |          |          |          |
AAGATCGTCACTAACCACGTGACTCCTCTCGCCCAATCAGTGTCTACGTC
GTCCATTTCCTGGGCATGGTCTACATCCTGATATAAAGCGATGCACTTCC
GAATGGCTGAGTTTTCCACGCCCGTCCGCGGCGAGATCGCGACGGAGGAG
CGATCGAGCGTCCCGAGGGCGGGTGCCGGAGGTGAGTTTACACACCGCAG
TCAAGGGGCAATTCGGGCTCGGGACTGGCCGGGCTATGGGCAAGGCTCTT
AAAGCGTACGTCCCCCGCTATGTTTCTCGGCAGGGTGTGGAGGAAACAGA
AAAGGAAAGTGCTTCTGCTGGCTGTGCGAGCTACACAGAAAACATCTTCC
ATGAGTATCTGGCGTCCCCCCCTTGGGAATGTCTCCTACAGGGAGAGAAA
TTGGCTTCAGGCCGTCGAAACATCCCACAGTTCTTTTTGTGGCTGTGGTG
ATTTTATTCTTCATCTTACTAATTTGGCTGCACGCTTTGCTCTCCAGGGG
CCCCCGCCAGAGGGTGGTCCACCTCGGCCGAGGCCGCCGCTCCTGAGAGC
GCTGCCGGCCCCCGAGGTCCGCAGGGAGACGCGCACAGAGAACCGGGGCG
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CCTCCGGTGAGCCATGGCCTGGCGATGGTGGTGGCAGAGACGATGGCGCC
GCCGCCGGTGGCCCCGCAGACGGTGGAGACGCCTACGACGCCGGAGACCT
AGACGACCTGTTCGCCGCCGTCGAAGAAGAACAACAGTAAGGAGGCGGAG
GTGGAGGGGCAGACGTGGGCGACGCACATACACCCGACGCGCGGTCAGAC
GCAGACGCAGACCCAGAAAGAGACTTGTACTGACTCAGTGGAGCCCCCAG
ACAGTCAGAAACTGCTCAATAAGGGGCATAGTGCCCATGGTAATATGCGG
ACACACAAAAGCAGGTAGAAACTATGCTATTCATAGCGAGGACTTCACCA
CACAGATACAACCCTTCGGGGGCAGTTTCAGCACGACCACCTGGTCCCTA
AAAGTGCTGTGGGACGAGCACCAGAAATTCCAGAACAGATGGTCCTACCC
AAACACACAACTAGACCTGGCCAGATACAGAGGGGTCACCTTCTGGTTCT
ACAGAGACCAGAAAACAGACTATATAGTACAGTGGAGTAGGAATCCCCCT
TTTAAACTCAATAAATACAGCAGTGCCATGTACCACCCGGGCATGATGAT
GCAGGCCAAAAGGAAACTAGTTGTACCTAGTTTCCAGACCAGACCCAAAG
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ACCCTTGCATCACCTTCCAGGTTTTGAAAGACATATATGATGAATGCATA
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CAACAGACAGCATAATGACAAAAGCTAATGGCTGGTTTTGGCAAAACCTA
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GATCTTTAGAATTCCCAGCAGCATACCAAGACGTTACATACAACCCTAAC
TGTGATAGAGCAGTTGGAAACGTAGTTTGGTTTCAGTACAGCACTAAAAT
GGATACAAATTTTGATGAAACAAAATGTAAATGTGTCCTTAAAAACATTC
CACTGTGGGCGGCCTTCAATGGCTACTCAGACTTTATAATGCAAGAACTC
AGCATAAGTACAGAAATCCACAACTTTGGCATAGTGTGCTTTCAGTGCCC
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CCCCCCGGAAAGCGCGCCGCTCCTACAGCAGCAGCAGCAGCAGCGGCAGC
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GCAAGTACTGGGATCGCCCTCCCAGAACCAACCACCTAGACATCCCCACT
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CTCTATAAGTCACTAAGCACTCCGAGCGCAGCGAGGAGTGCGACCCTCAG
CGGTGGGTGCAACGCCCTCGGCGGCCGCGCGCTACGCCTTCGGCTGCGCG
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CTTGGACACTGTAGCTGTGAATCAGTAACGAAAGTGAGTGGGGCCAGACT
TCGCCATAAGGCCTTTATCTTCTTGCCATTGGTCCGTCTCGGGGGTCGCC
ATAGGCTTCGGGCTCGGTTTTAGGCCTTCCGGACTACCAAAATGGCGGAT

TCCGTGACGTCATGGCCGCCATTTTAAGTAAGGCGGAACAGGCTGTCACC
CCGTGTCAAAGTTCAGGGGTCAGCCTTCCGCTTTACACAAAATGGAGGTC
AATATCTTCCGGGTCAAAGGTCGCTACCGCGTCATAAGTCACGTGGGGAA
GGCTGCTGTGAATCCGGAAGTAGCTGACCCACGTGACTTGTCACGTGACT
AGCACGTCACGGCAGCCATTTTGAATCACAAAATGGCCGACTTCCTTCCT
CTTTTTTAAAAATAACGGCCCGGCGGCGGCGCGCGCGCTTCGCGCCGCTC
CGCCCCCCCCGCGCATGCGCGGGACCCCCCCCCGCGGGGGGCTCCGCCCC
CCGGTCCCCCCCCCG (SEQ ID NO: 911)

Annotations:

Putative Domain	*Base range*
TATA Box	82– 87
Cap Site	103 – 110
Transcriptional Start Site	110
5' UTR Conserved Domain	170 – 240
ORF2	351 – 740
ORF2/2	351 – 737 ; 2378 – 2843
ORF2/3	351 – 737 ; 2526 – 3057
TAIP	379 - 543
ORF1	614 – 2911
ORF1/1	614 – 737 ; 2378 – 2911
ORF1/2	614 – 737 ; 2526 – 2843
Three open-reading frame region	2526 – 2840
Poly(A) Signal	3056 – 3062
GC-rich region	3716 – 3815

Table A20. Exemplary *Anellovirus* amino acid sequences (*Alphatorquevirus*) – Clade 7

Ring7.0 (*Alphatorquevirus*)	
ORF2	MSIWRPPLGNVSYRERNWLQAVETSHSSFCGCGDFILHLTNLAARFALQGPP PEGGPPRPRPPLLRA LPAPEVRRETRTENRGASGEPWPGDGGGRDDGAAAGGPADGGDAYDAGD LDDLFAAVEEEQQ (SEQ ID NO: 912)
ORF2/2	MSIWRPPLGNVSYRERNWLQAVETSHSSFCGCGDFILHLTNLAARFALQGPP PEGGPPRPRPPLLRA

	LPAPEVRRETRTENRGASGEPWPGDGGGRDDGAAAGGPADGGDAYDAGD LDDLFAAVEEEQQLSRTRVTESSPSLPIPIDNVATYKLLTHQPWARSTPSTHG TGDGGFLVQMLSRECHKNREMLSALQTLSRDPDIFPRQTEKTTDKKKTSLY RKKDGAHPQKKPRTRRAPRKARRSYSSSSSSGSSQSTSRSSSDSESNSDTSSK KSSKRKRVST (SEQ ID NO: 913)
ORF2/3	MSIWRPPLGNVSYRERNWLQAVETSHSSFCGCGDFILHLTNLAARFALQGPP PEGGPPRPRPPLLRA LPAPEVRRETRTENRGASGEPWPGDGGGRDDGAAAGGPADGGDAYDAGD LDDLFAAVEEEQQCYPESVTKTGRCSPLYKPFQETQISSPDRQRRLPTRRRLR FTGKKTAHIHRRSPGRGEPPGKRAAPTAAAAAAAALSPPRGAAATRSPTPIH PPRSPQNASGSPPKPPIIRPATNKVYLFEPSKGLLPIVGKEAWEDEYCTCKYW DRPPRTNHLDIPTYPWMPTNFKVSFKLGFKP (SEQ ID NO: 914)
TAIP	MSPTGREIGFRPSKHPTVLFVAVVILFFILLIWLHALLSRGPRQRVVHLGRGR RS (SEQ ID NO: 915)
ORF1	MAWRWWWQRRWRRRRWPRRRWRRLRRRRPRRPVRRRRRRTTVRRRRW RGRRGRRTYTRRAVRRRRRPRKRLVLTQWSPQTVRNCSIRGIVPMVICGHT KAGRNYAIHSEDFTTQIQPFGGSFSTTTWSLKVLWDEHQKFQNRWSYPNTQ LDLARYRGVTFWFYRDQKTDYIVQWSRNPPFKLNKYSSAMYHPGMMMQA KRKLVVPSFQTRPKGKKRYRVTIKPPNMFADKWYTQEDLCPVPLVQIVVSA ASLLHPFCPPQTNNPCITFQVLKDIYDECIGVNETMKDKYKKLQTTLYTTCT YYQTTQVLAQLSPAFQPAMKPTTTQSAATATTLGNYVPELKYNNGSFHTGQ NAVFGMCSYKPTDSIMTKANGWFWQNLMVDNNLHSSYGKATLECMEYHT GIYSSIFLSPQRSLEFPAAYQDVTYNPNCDRAVGNVVWFQYSTKMDTNFDE TKCKCVLKNIPLWAAFNGYSDFIMQELSISTEIHNFGIVCFQCPYTFPPCFNK NKPLKGYVFYDTTFGNGKMPDGSGHVPIYWQQRWWIRLAFQVQVMHDFV LTGPFSYKDDLANTTLTARYKFKFKWGGNIIPEQIIKNPCHREQSLASYPDRQ RRDLQVVDPSTMGPIYTFHTWDWRRGLFGADAIQRVSQKPGDALRFTNPFK RPRYLPPTDREDYRQEEDFALQEKRRRTSTEEAQDEESPPESAPLLQQQQQQ RQLSVHLAEQQRLGVQLRYILQEVLKTQAGLHLNPLLLGPPQTRSISLSPPKA YSP (SEQ ID NO: 916)

ORF1/1	MAWRWWWQRRWRRRRWPRRRWRRLRRRRPRRPVRRRRRRRTTIIKNPCHR EQSLASYPDRQRRDLQVVDPSTMGPIYTFHTWDWRRGLFGADAIQRVSQKP GDALRFTNPFKRPRYLPPTDREDYRQEEDFALQEKRRRTSTEEAQDEESPPE SAPLLQQQQQQRQLSVHLAEQQRLGVQLRYILQEVLKTQAGLHLNPLLLGP PQTRSISLSPPKAYSP (SEQ ID NO: 917)
ORF1/2	MAWRWWWQRRWRRRRWPRRRWRRLRRRRPRRPVRRRRRRRTTMLSRECH KNREMLSALQTLSRDPDIFPRQTEKTTDKKKTSLYRKKDGAHPQKKPRTRR APRKARRSYSSSSSSGSSQSTSRSSSDSESNSDTSSKKSSKRKRVST (SEQ ID NO: 918)

Table N21. Exemplary *Anellovirus* nucleic acid sequence (*Betatorquevirus*)

Name	Ring9
Genus/Clade	*Betatorquevirus*
Accession Number	MH649263.1

Full Sequence: 2845 bp

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1         10         20         30         40         50
|         |          |          |          |          |
TTATTAATATTCAACAGGAAAACCACCTAATTTAAATTGCCGACCACAAA
CCGTCACTAACTTCCTTATTTAACATTACTTCCCTTTTAACCAATGAATA
TTCATACAACACATCACACTTCCTGGGAGGAGACATAAAACTATATAACT
AACTACACAGACGAATGGCTGAGTTTATGCCGCTAGACGGAGGACGCACA
GCTACTGCTGCGACCTGAACTTGGGCGGGTGCCGAAGGTGAGTGTAACCA
CCGTAGTCAAGGGGCAATTCGGGCTAGTTCAGTCTAGCGGAACGGGCAAG
ATTATTAATACAAACTTATTTTTACAGATGAGCAAACAACTAAAACCAAC
TTTATACAAAGACAAATCATTGGAATTACAATGGCTAAACAACATTTTTA
GCTCTCACGACCTGTGCTGCGGCTGCAACGATCCAGTTTTACATTTACTG
ATTTTAATTAACAAAACCGGAGAAGCACCTAAACCAGAAGAAGACATTAA
AAATATAAAATGCCTCCTTACTGGCGCCAAAAATACTACCGAAGAAGATA
TAGACCTTTCTCCTGGAGAACTAGAAGAATTATTCAAAGAAGAAAAAGAT
GGAGATACCGCAAACCAAGAAAAACATACTGGAGAAGAAAACTGCGGGTA
AGAAAACGTTTTTATAAAAGAAAGTTAAAAAAAATTGTACTTAAACAGTT
TCAACCAAAAATTATTAGAAGATGTACAATATTTGGAACAATCTGCCTAT
TTCAAGGCTCTCCAGAAAGAGCCAACAATAATTATATTCAAACAATCTAC
TCCTACGTACCAGATAAAGAACCAGGAGGAGGGGGATGGACTTTAATAAC
TGAAAGCTTAAGTAGTTTATGGGAAGACTGGGAACATTTAAAAAATGTAT
GGACTCAAAGTAACGCTGGTTTACCACTTGTAAGATACGGGGGAGTAACA
TTATACTTTTATCAATCTGCCTATACTGACTATATTGCTCAAGTTTTCAA
CTGTTATCCTATGACAGACACAAAATACACACATGCAGACTCAGCACCAA
ACAGAATGTTATTAAAAAAACATGTAATAAGAGTACCTAGCAGAGAAACA
CGCAAAAAAAGAAAGCCATACAAAAGAGTTAGAGTAGGACCTCCTTCTCA
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GACTGTGCTAGTAACAACTTAACTCTAACATGTTTAAACCCACTATTGTT
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AACCTGGAGTATATCTATACTCAACACAAAGAAGTAACAAGCCAAGTTCT
TCAGACTGTATATACTTAGGAAACACAAAAGACAATCAAGAAGGTAAATC
TGCAAGTAGTCTAATGACTCTAAAAACACAAAAAATAACAGATTGGGGAA
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TTTAAACCCCCATCACAATTAGACAGCAGCGACTTTGAACACATGACAGA
ATTAGCAGAACCAATGTTTATACAAGTTAGATACAACCCAGAAAGAGACA
CAGGACAAGGAAACTTAATATACGTAACAGAAAACTTTAGAGGACAACAC
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TGACATGTGCTGGGGTTTCATAGACTGGATAGAAAAAGTTCATGAAACAG
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GAAAAAAAAACAGTTTTTATACCTGTAGATCATTCATTTTTAACAGGTTT
TAGCCCATATGAAACTCCAGTTAAATCATCAGACCAAGCTCACTGGCACC
CACAAATAAGATTTCAAACAAAATCAATAAATGACATTTGTTTAACAGGC
CCCGGTTGTGCTAGGTCCCCATATGGCAATTACATGCAGGCAAAAATGAG
TTATAAATTTCATGTAAAATGGGGAGGATGTCCAAAAACTTATGAAAAAC
CATATGATCCTTGTTCACAGCCCAATTGGACTATTCCCCATAACCTCAAT
GAAACAATACAAATCCAGAATCCAAACACATGCCCACAAACAGAACTCCA
AGAATGGGACTGGCGACGTGATATTGTTACAAAAAAAGCTATCGAAAGAA
TTAGACAACACACGGAACCTCATGAAACTTTGCAAATCTCTACAGGTTCC
AAACACAACCCACCAGTACACAGACAAACATCACCGTGGACGGACTCAGA
AACGGACTCGGAAGAGGAAAAAGACCAAACACAAGAGATCCAGATCCAGC
TCAACAAGCTCAGAAAGCATCAACAGCATCTCAAGCAGCAGCTCAAGCAG
TACCTGAAACCCCAAAATATAGAATAGTTGCAAGCAACATAAAAGTTGAA
CTTTTTCCTACTAAAAAACCTTTTAAAAACAGACGCTTTACTCCTTCTGA
AAGAGAAACAGAAAGACAATGTGCTAAAGCTTTTTGTAGACCAGAAAGAC
ATTTCTTTTATGATCCTCCTTTTTACCCTTACTGTGTACCTGAACCTATT
GTAAACTTTGCTTTGGGATATAAAATTTAAGGCCAACAAATTTCACTTAG
TGGTGTCTGTTTATTAAAGTTTAACCTTAATAAGCATACTCCGCCTCCCT
ACATTAAGGCGCCAAAAGGGGGCTCCGCCCCCTTAAACCCCAAGGGGGCT
CCGCCCCCTTAAACCCCCAAGGGGGCTCCGCCCCCTTACACCCCC (SEQ ID NO: 1001)

Annotations:

Putative Domain	*Base range*
TATA Box	142 – 148
Initiation Element	162 – 177
Transcriptional Start Site	172
5' UTR Conserved Domain	226 – 296
ORF2	328 – 651
ORF2/2	328 – 647; 2121 – 2457
ORF2/3	328 – 647; 2296 – 2680
ORF1	510 – 2477
ORF1/1	510 – 647; 2121 – 2477

ORF1/2	510 – 647; 2296 – 2457
Three open-reading frame region	2296 – 2454
GC-rich region	2734 – 2845

Table A21. Exemplary *Anellovirus* amino acid sequences (*Betatorquevirus*)

Ring9 (*Betatorquevirus*)	
ORF2	MSKQLKPTLYKDKSLELQWLNNIFSSHDLCCGCNDPVLHLLILINKTGEAPK PEEDIKNIKCLLTGAKNTTEEDIDLSPGELEELFKEEKDGDTANQEKHTGEEN CG (SEQ ID NO: 1002)
ORF2/2	MSKQLKPTLYKDKSLELQWLNNIFSSHDLCCGCNDPVLHLLILINKTGEAPK PEEDIKNIKCLLTGAKNTTEEDIDLSPGELEELFKEEKDGDTANQEKHTGEEN CGPIGLFPITSMKQYKSRIQTHAHKQNSKNGTGDVILLQKKLSKELDNTRNL MKLCKSLQVPNTTHQYTDKHHRGRTQKRTRKRKKTKHKRSRSSSTSSESIN SISSSSSSST (SEQ ID NO: 1003)
ORF2/3	MSKQLKPTLYKDKSLELQWLNNIFSSHDLCCGCNDPVLHLLILINKTGEAPK PEEDIKNIKCLLTGAKNTTEEDIDLSPGELEELFKEEKDGDTANQEKHTGEEN CGFQTQPTSTQTNITVDGLRNGLGRGKRPNTRDPDPAQQAQKASTASQAAA QAVPETPKYRIVASNIKVELFPTKKPFKNRRFTPSERETERQCAKAFCRPERH FFYDPPFYPYCVPEPIVNFALGYKI (SEQ ID NO: 1004)
ORF1	MPPYWRQKYYRRRYRPFSWRTRRIIQRRKRWRYRKPRKTYWRRKLRVRKR FYKRKLKKIVLKQFQPKIIRRCTIFGTICLFQGSPERANNNYIQTIYSYVPDKE PGGGGWTLITESLSSLWEDWEHLKNVWTQSNAGLPLVRYGGVTLYFYQSA YTDYIAQVFNCYPMTDTKYTHADSAPNRMLLKKHVIRVPSRETRKKRKPYK RVRVGPPSQMQNKWYFQRDICEIPLIMIAATAVDFRYPFCASDCASNNLTLT CLNPLLFQNQDFDHPSDTQGYFPKPGVYLYSTQRSNKPSSSDCIYLGNTKDN QEGKSASSLMTLKTQKITDWGNPFWHYYIDGSKKIFSYFKPPSQLDSSDFEH MTELAEPMFIQVRYNPERDTGQGNLIYVTENFRGQHWDPPSSDNLKLDGFP LYDMCWGFIDWIEKVHETENLLTNYCFCIRSSAFNEKKTVFIPVDHSFLTGFS PYETPVKSSDQAHWHPQIRFQTKSINDICLTGPGCARSPYGNYMQAKMSYK FHVKWGGCPKTYEKPYDPCSQPNWTIPHNLNETIQIQNPNTCPQTELQEWD

	WRRDIVTKKAIERIRQHTEPHETLQISTGSKHNPPVHRQTSPWTDSETDSEEE KDQTQEIQIQLNKLRKHQQHLKQQLKQYLKPQNIE (SEQ ID NO: 1005)
ORF1/1	MPPYWRQKYYRRRYRPFSWRTRRIIQRRKRWRYRKPRKTYWRRKLRPNW TIPHNLNETIQIQNPNTCPQTELQEWDWRRDIVTKKAIERIRQHTEPHETLQIS TGSKHNPPVHRQTSPWTDSETDSEEEKDQTQEIQIQLNKLRKHQQHLKQQL KQYLKPQNIE (SEQ ID NO: 1006)
ORF1/2	MPPYWRQKYYRRRYRPFSWRTRRIIQRRKRWRYRKPRKTYWRRKLRVPNT THQYTDKHHRGRTQKRTRKRKKTKHKRSRSSSTSSESINSISSSSSSST (SEQ ID NO: 1007)

Table N22. Exemplary *Anellovirus* nucleic acid sequence (*Betatorquevirus*)

Name	Ring10
Genus/Clade	*Betatorquevirus*
Accession Number	JX134044.1

Full Sequence: 2912 bp

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1         10         20         30         40         50
|         |          |          |          |          |
TAATAAATATTCAACAGGAAAACCACCTAATTTAAATTGCCGACCACAAA
CCGTCACTTAGTTCCTCTTTTTCCACAACTTCCTCTTTTACTAATGAATA
TTCATGTAATTAATTAATAATCACCGTAATTCCGGGGAGGAGCCTTTAAA
CTATAAAACTAACTACACATTCGAATGGCTGAGTTTATGCCGCCAGACGG
AGACGGGATCACTTCAGTGACTCCAGGCTGATCAAGGGCGGGTGCCGAAG
GTGAGTGAAACCACCGTAGTCAAGGGGCAATTCGGGCTAGATCAGTCTGG
CGGAACGGGCAAGAAACTTAAAATGTACTTTATTTTACAGAAATGTTCAA
ATCTCCAACATACTTAACAACTAAAGGCAAAAACAATGCCTTAATCAACT
GCTTCGTTGGAGACCACGATCTTCTGTGCAGCTGTAACAATCCTGCCTAC
CATTGCCTCCAAATACTTGCAACTACCTTAGCACCTCAACTAAAACAAGA
AGAAAAACAACAAATAATACAATGCCTTGGTGGTACAGACGCCGTAGCTA
CAACCCGTGGAGACGAAGAAATTGGTTTAGAAGACCTAGAAAAACTATTT
ACAGAAGATACAGAAGAAGACGCCGCTGGGTAAGAAGAAAACCTTTTTAC
AAACGTAAAATTAAGAGACTAAATATAGTAGAATGGCAACCTAAATCAAT
TAGAAAATGTAGAATAAAAGGAATGCTATGCTTGTTTCAAACGACAGAAG
ACAGACTGTCATATAACTTTGATATGTATGAAGAGTCTATTATACCAGAA
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CTTATACCAAGAACACATACATGCACACAACATATTTACACACACAAACA
CAGACAGACCACTAGCAAGATACACAGGCTGTTCTTTAAAATTCTACCAA
AGCAAAGACATAGACTACGTAGTAACATATTCTACATCACTCCCACTAAG
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CCATATATTAAAAAACATATATCACCACCAACACAAATGAAATCTCAATG
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GGGAGACAGAAATAAAACTTACTACTGCCAAACATTAGGAACACAAAGAT
ACTTCCTATATGGAACACATTCAACTGCACAAAATATTAATGACATAAAG
CTACAAGAACTAATACCTTTAACAAACACACAAGACTATGTACAAGGCTT
TGATTGGACAGAAAAAGACAAACATAACATAACAACCTACAAAGAATTCT
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ACCTACCACAATGGTTACTATTATTTGGCTACCCAGACTACATAAAAAGA
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ATATGTTGTTCCCAATAACTTCAATGAAACAACTTCGTTACAGAATCCAA
CCACCAGACCAGAGCACTTCTTGTACTCCTTTGACGAAAGGAGGGGACAA
CTTACAGAAAAAGCTACAAAACGCTTGCTTAAAGACTGGGAAACTAAAGA
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TTCGAGCGACTCCTCCGACAGCGAACCAAGCAGCTCCAGCTCAAGCGCAG
AATAATACAAACATTGAAAGACCTACAAAAATTAGAATAACTAACAGCAA
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CATGGGAAATACAAGAAGACAAAGAAATAGCCAATTTATTTGGCAGACCA
CATAGATACTTTTTAAAAGACATTCCTTTCTATTGGGATATACCCCCAGA
GCCTAAAGTAAACTTTGATTTAAATTTTCAATAAAGAAATAAAGGGCAAG
GCCCCATTAACTCAAAGTCGGTGTCTACCTCTTTAAGTTTAACTTTACTA
AACGGACTCCGCCTCCCTAAATTTGGGCGCCAAAAGGGGGCTCCGCCCCC
TTAAACCCCAGGGGGCTCCGCCCCCTAAAACCCCCAAGGGGGCTACGCCC
CCTTACACCCCC (SEQ ID NO: 1008)

Annotations:

Putative Domain	*Base range*
TATA Box	152 – 158
Initiation Element	172 – 187
Transcriptional Start Site	182
5' UTR Conserved Domain	239 – 309
ORF2	343 – 633
ORF2/2	343 – 629; 2196 – 2505
ORF2/3	343 – 629; 2371 – 2734
ORF1	522 – 2540
ORF1/1	522 – 629; 2196 – 2540

ORF1/2	522 – 629; 2371 – 2505
Three open-reading frame region	2276 – 2502
GC-rich region	2803 – 2912

Table A22. Exemplary *Anellovirus* amino acid sequences (*Betatorquevirus*)

Ring10 (*Betatorquevirus*)	
ORF2	MFKSPTYLTTKGKNNALINCFVGDHDLLCSCNNPAYHCLQILATTLAPQLK QEEKQQIIQCLGGTDAVATTRGDEEIGLEDLEKLFTEDTEEDAAG (SEQ ID NO: 1009)
ORF2/2	MFKSPTYLTTKGKNNALINCFVGDHDLLCSCNNPAYHCLQILATTLAPQLK QEEKQQIIQCLGGTDAVATTRGDEEIGLEDLEKLFTEDTEEDAAGQHMLFPI TSMKQLRYRIQPPDQSTSCTPLTKGGDNLQKKLQNACLKTGKLKKLLYCLQ NTDSRSQHKHKPHKRTRPRKKKKRATSSSDSSDSEPSSSSSSAE (SEQ ID NO: 1010)
ORF2/3	MFKSPTYLTTKGKNNALINCFVGDHDLLCSCNNPAYHCLQILATTLAPQLK QEEKQQIIQCLGGTDAVATTRGDEEIGLEDLEKLFTEDTEEDAAGIQIRGANT NTSPTRGPVLGRRRREQPLRATPPTANQAAPAQAQNNTNIERPTKIRITNSKN TVYLFPPEQKNRRLTPWEIQEDKEIANLFGRPHRYFLKDIPFYWDIPPEPKVN FDLNFQ (SEQ ID NO: 1011)
ORF1	MPWWYRRRSYNPWRRRNWFRRPRKTIYRRYRRRRRWVRRKPFYKRKIKR LNIVEWQPKSIRKCRIKGMLCLFQTTEDRLSYNFDMYEESIIPEKLPGGGGFSI KNISLYALYQEHIHAHNIFTHTNTDRPLARYTGCSLKFYQSKDIDYVVTYSTS LPLRSSMGMYNSMQPSIHLMQQNKLIVPSKQTQKRRKPYIKKHISPPTQMKS QWYFQHNIANIPLLMIRTTALTLDNYYIGSRQLSTNVTIHTLNTTYIQNRDW GDRNKTYYCQTLGTQRYFLYGTHSTAQNINDIKLQELIPLTNTQDYVQGFD WTEKDKHNITTYKEFLTKGAGNPFHAEWITAQNPVIHTANSPTQIEQIYTAS TTTFQNKKLTDLPTPGYIFITPTVSLRYNPYKDLAERNKCYFVRSKINAHGW DPEQHQELINSDLPQWLLLFGYPDYIKRTQNFALVDTNYILVDHCPYTNPEK TPFIPLSTSFIEGRSPYSPSDTHEPDEEDQNRWYPCYQYQQESINSICLSGPGTP KIPKGITAEAKVKYSFNFKWGGDLPPMSTITNPTDQPTYVVPNNFNETTSLQ NPTTRPEHFLYSFDERRGQLTEKATKRLLKDWETKETSLLSTEYRFAEPTQT

	QAPQEDPSSEEEEESNLFERLLRQRTKQLQLKRRIIQTLKDLQKLE (SEQ ID NO: 1012)
ORF1/1	MPWWYRRRSYNPWRRRNWFRRPRKTIYRRYRRRRRWPTYVVPNNFNETT SLQNPTTRPEHFLYSFDERRGQLTEKATKRLLKDWETKETSLLSTEYRFAEP TQTQAPQEDPSSEEEEESNLFERLLRQRTKQLQLKRRIIQTLKDLQKLE
ORF1/2	MPWWYRRRSYNPWRRRNWFRRPRKTIYRRYRRRRRWNTDSRSQHKHKPH KRTRPRKKKKRATSSSDSSDSEPSSSSSSAE (SEQ ID NO: 1013)

Table N23. Exemplary *Anellovirus* nucleic acid sequence (*Alphatorquevirus*, Clade 4)

Name	Ring20
Genus/Clade	*Alphatorquevirus* Clade 4
Accession Number	AF122914.3

Full Sequence: 3853 bp

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1         10         20         30         40         50
|         |          |          |          |          |
GGCTTAGTGCGTCACCACCCACGTGACCCGCCTCCGCCAATTAACAGGTA
CTTCGTACACTTCCTGGGCGGGCTTATAAGACTAATATAAGTAGCTGCAC
TTCCGAATGGCTGAGTTTTCCACGCCCGTCCGCAGCGGTGAAGCCACGGA
GGGAGCTCAGCGCGTCCCGAGGGCGGGTGCCGGAGGTGAGTTTACACACC
GCAGTCAAGGGGCAATTCGGGCTCGGGACTGGCCGGGCTTTGGGCAAGGC
TCTTAAAAAAAGCTATGTTTATTGGCAGGCACTACCGAAAGAAAAGGGCGC
TGCTACTGCTATCTGTGCATTCTACAAAGACAAAAGGGAAACTTCTAATA
GCTATGTGGACTCCCCCACGCAATGATCAACAATACCTTAACTGGCAATG
GTACACTTCTGTACTTAGCTCCCACTCTGCTATGTGCGGGTGTTCCGACG
CTATCGCTCATCTTAATCATCTTGCTAATCTGCTTCGTGCCCCGCAAAAT
CCGCCCCCGCCTGATAATCCAAGACCCCTACCCGTGCGAGCACTGCCTGC
TCCCCCGGCTGCCCACGAGGCAGCCGGTGATCGAGCACCATGGCCTATGG
GTGGTGGAGGAGACGCCGGAGGCGCTGGCGCAGGTGGAGACGCCGACCAT
GGAGGCGCCGCTGGAGGACCCGCAGACGCAGACCTGCTAGACGCCGTGGC
CGCCGCAGAAACGTAAGGAGACGGCGCAGAGGGAGGTGGAGAAGGAGGTA
CAGGAGGTGGAAAAGAAAGGGCAGACGTAGAAGAAAAGCAAAAATAATAA
TAAGACAGTGGCAGCCAAACTACAGAAGAAGATGTAATATAGTGGGCTAC
CTCCCTATACTTATCTGTGGTGGAAATACTGTTTCTAGAAACTATGCCAC
ACACTCAGACGATACTAACTATCCAGGACCCTTTGGGGGAGGCATGACCA
CAGACAAATTCAGCCTTAGAATACTATATGATGAATACAAAAGATTTATG
AACTACTGGACAGCCTCAAATGAGGACCTAGATCTCTGTAGATATCTAGG
ATGCACTTTTTACTTCTTTAGACACCCTGAAGTAGACTTTATTATAAAAA
TAAACACCATGCCCCCATTCTTAGATACAACCATAACAGCACCTAGCATA
CACCCAGGCCTCATGGCCCTAGACAAAAGAGCCAGATGGATTCCTTCTCT
TAAAAATAGACCAGGTAAAAAACACTATATAAAAATTAGAGTAGGGGCTC
CTAAAATGTTCACAGATAAATGGTACCCTCAAACAGACCTCTGTGACATG
ACACTGCTAACTATCTATGCAACCGCAGCGGATATGCAATATCCGTTCGG
```

CTCACCACTAACTGACACTGTGGTTGTTAACTCCCAAGTTCTGCAATCCA
TGTATGATGAAACAATTAGCATATTACCTGATGAAAAAACTAAAAGAAAT
AGCCTTCTTACTTCTATAAGAAGCTACATACCTTTTTTATAATACTACACA
AACAATAGCTCAATTAAAACCATTTGTAGATGCAGGAGGACACACAACAG
GCTCAACAACAACTACATGGGGACAACTATTAAACACAACTAAATTTACC
ACTACCACAACAACCACATACACATACCCTGGCACCACAAATACAGCAGT
AACATTTATAACAGCCAATGATACCTGGTACAGGGGAACAGCATATAAAG
ATAACATTAAAGATGTACCACAAAAAGCAGCACAATTATACTTTCAAACA
ACACAAAAACTACTAGGAAACACATTCCATGGCTCAGATGAAACACTTGA
ATACCATGCAGGCCTATACAGCTCTATCTGGCTATCACCAGGTAGATCCT
ACTTTGAAACACCAGGTGCATACACAGACATTAAATATAACCCTTTTACA
GACAGAGGAGAAGGCAACATGCTGTGGATAGACTGGCTAAGTAAAAAAAA
CATGAAATATGACAAAGTGCAAAGTAAGTGCCTAGTAGCAGACCTACCAC
TGTGGGCAGCAGCATATGGTTATGTAGAATTCTGCTCTAAAAGCACAGGA
GACACAAACATACACATGAATGCCAGACTACTAATAAGAAGTCCTTTTAC
AGACCCCCAGCTAATAGTACACACAGACCCCACTAAAGGCTTTGTACCCT
ATTCTTTAAACTTTGGAAATGGTAAAATGCCAGGAGGTAGCAGCAATGTT
CCCATAAGAATGAGAGCTAAGTGGTACCCCACTTTATCCCACCAACAAGA
AGTTCTAGAGGCCTTAGCACAGTCAGGACCCTTTGCTTATCACTCAGACA
TTAAAAAAGTATCTCTAGGCATAAAATACCGTTTTAAGTGGATCTGGGGT
GGAAACCCCGTTCGCCAACAGGTTGTTAGAAATCCCTGCAAGGAACCCCA
CTCCTCGGGCAATAGAGTCCCTAGAAGCATACAAATCGTTGACCCGAGAT
ACAACTCACCGGAACTTACCATCCATGCCTGGGACTTCAGACGTGGCTTC
TTTGGCCCGAAAGCTATTCAAAGAATGCAACAACAACCAACTGCTACTGA
ATTTTTTTCAGCAGGCCGCAAGAGACCCAGAAGGGACACAGAAGTGTATC
AGTCCGACCAAGAAAAGGAGCAAAAAGAAAGCTCGCTTTTCCCCCCAGTC
AAGCTCCTCCGAAGAGTCCCCCCGTGGGAGGACTCGGAACAGGAGCAAAG
CGGGTCGCAAAGCTCAGAGGAAGAGACGGCGACCCTCTCCCAGCAGCTCA
AACAGCAGCTGCAGCAGCAGCGAGTCTTGGGAGTCAAACTCAGACTCCTG
TTCAACCAAGTCCAAAAAATCCAACAAAATCAAGATATCAACCCTACCTT
GTTACCAAGGGGGGGGGATCTAGTATCCTTCTTTCAGGCTGTACCATAAA
TATGTTTCCAGACCCTAAACCTTACTGCCCCTCCAGCAATGACTGGAAAG
AAGAGTATGAGGCCTGTAAATATTGGGATAGACCTCCCAGACACAACCTT
AGAGACCCCCCCTTTTACCCCTGGGCCCCTAAAAACAATCCTTGCAATGT
AAGCTTTAAACTTGGCTTCAAATAAACTAGGCCGTGGGAGTTTCACTTGT
CGGTGTCTACCTCTATAAGTCACTAAGCACTCCGAGCGCAGCGAGGAGTG
CGACCCTTCCCCCTGGTGCAACGCCCTCGGCGGCCGCGCGCTACGCCTTC
GGCTGCGCGCGGCACCTCGGACCCCCGCTCGTGCTGACACGCTTGCGCGT
GTCAGACCACTTCGGGCTCGCGGGGGTCGGGAAATTTGCTAAACAGACTC
CGAGTTGCCATTGGACACTGTAGCTATGAATCAGTAACGAAAGTGAGTGG
GGCCAGACTTCGCCATAAGGCCTTTATCTTCTTGCCATTTGTCAGTATTG
GGGGTCGCCATAAACTTTGGGCTCCATTTTAGGCCTTCCGGACTACAAAA
ATCGCCATATTTGTGACGTCAGAGCCGCCATTTTAAGTCAGCTCTGGGGA
GGCGTGACTTCCAGTTCAAAGGTCATCCTCACCATAACTGGCACAAAATG
GCCGCCAACTTCTTCCGGGTCAAAGGTCACTGCTACGTCATAGGTGACGT
GGGGGGGGACCTACTTAAACACGGAAGTAGGCCCCGACACGTCACTGTCA
CGTGACAGTACGTCACAGCCGCCATTTTGTTTTACAAAATAGCCGACTTC
CTTCCTCTTTTTTAAAAAAAGGCGCCAAAAAACCGTCGGCGGGGGGGCCG
CGCGCTGCGCGCGCGGCCCCCGGGGGAGGCACAGCCTCCCCCCCCCGCGC
GCATGCGCGCGGGTCCCCCCCCCTCCGGGGGGCTCCGCCCCCCGGCCCCC
CCC (SEQ ID NO: 1014)

Annotations:

Putative Domain *Base range*

TATA Box	86 – 90
Initiation Element	104 – 119
Transcriptional Start Site	114
5' UTR Conserved Domain	174 – 244
ORF2	354 – 716
ORF2/2	354 – 712; 2372 – 2873
ORF2/3	354 – 712; 2565 – 3075
ORF2t/3	354 – 400; 2565 – 3075
TAIP	373 – 690
ORF1	590 – 2899
ORF1/1	590 – 712; 2372 – 2899
ORF1/2	590 – 712; 2565 – 2873
Three open-reading frame region	2551 – 2870
Poly(A)-Signal	3071 – 3076
GC-rich region	3733 – 3853

Table A23. Exemplary *Anellovirus* amino acid sequences (*Alphatorquevirus*)

Ring20 (*Alphatorquevirus* Clade 4)	
ORF2	MWTPPRNDQQYLNWQWYTSVLSSHSAMCGCSDAIAHLNHLANLLRAPQN PPPPDNPRPLPVRALPAPPAAHEAAGDRAPWPMGGGGDAGGAGAGGDADH GGAAGGPADADLLDAVAAAET (SEQ ID NO: 1015)
ORF2/2	MWTPPRNDQQYLNWQWYTSVLSSHSAMCGCSDAIAHLNHLANLLRAPQN PPPPDNPRPLPVRALPAPPAAHEAAGDRAPWPMGGGGDAGGAGAGGDADH GGAAGGPADADLLDAVAAAETLLEIPARNPTPRAIESLEAYKSLTRDTTHRN LPSMPGTSDVASLARKLFKECNNNQLLLNFFQQAARDPEGTQKCISPTKKRS KKKARFSPQSSSSEESPRGRTRNRSKAGRKAQRKRRRPSPSSSNSSCSSSESW ESNSDSCSTKSKKSNKIKISTLPCYQGGGI (SEQ ID NO: 1016)
ORF2/3	MWTPPRNDQQYLNWQWYTSVLSSHSAMCGCSDAIAHLNHLANLLRAPQN PPPPDNPRPLPVRALPAPPAAHEAAGDRAPWPMGGGGDAGGAGAGGDADH GGAAGGPADADLLDAVAAAETPQETQKGHRSVSVRPRKGAKRKLAFPPSQ APPKSPPVGGLGTGAKRVAKLRGRDGDPLPAAQTAAAAAASLGSQTQTPV

	QPSPKNPTKSRYQPYLVTKGGGSSILLSGCTINMFPDPKPYCPSSNDWKEEY EACKYWDRPPRHNLRDPPFYPWAPKNNPCNVSFKLGFK (SEQ ID NO: 1017)
ORF2t/3	MWTPPRNDQQYLNWQWPQETQKGHRSVSVRPRKGAKRKLAFPPSQAPPKS PPVGGLGTGAKRVAKLRGRDGDPLPAAQTAAAAAASLGSQTQTPVQPSPK NPTKSRYQPYLVTKGGGSSILLSGCTINMFPDPKPYCPSSNDWKEEYEACKY WDRPPRHNLRDPPFYPWAPKNNPCNVSFKLGFK (SEQ ID NO: 1018)
TAIP	MINNTLTGNGTLLYLAPTLLCAGVPTLSLILIILLICFVPRKIRPRLIIQDPYPCE HCLLPRLPTRQPVIEHHGLWVVEETPEALAQVETPTMEAPLEDPQTQTC (SEQ ID NO: 1019)
ORF1	MAYGWWRRRRRRWRRWRRRPWRRRWRTRRRRPARRRGRRRNVRRRRR GRWRRRYRRWKRKGRRRRKAKIIIRQWQPNYRRRCNIVGYLPILICGGNTV SRNYATHSDDTNYPGPFGGGMTTDKFSLRILYDEYKRFMNYWTASNEDLD LCRYLGCTFYFFRHPEVDFIIKINTMPPFLDTTITAPSIHPGLMALDKRARWIP SLKNRPGKKHYIKIRVGAPKMFTDKWYPQTDLCDMTLLTIYATAADMQYP FGSPLTDTVVVNSQVLQSMYDETISILPDEKTKRNSLLTSIRSYIPFYNTTQTI AQLKPFVDAGGHTTGSTTTTWGQLLNTTKFTTTTTTTYTYPGTTNTAVTFIT ANDTWYRGTAYKDNIKDVPQKAAQLYFQTTQKLLGNTFHGSDETLEYHAG LYSSIWLSPGRSYFETPGAYTDIKYNPFTDRGEGNMLWIDWLSKKNMKYDK VQSKCLVADLPLWAAAYGYVEFCSKSTGDTNIHMNARLLIRSPFTDPQLIVH TDPTKGFVPYSLNFGNGKMPGGSSNVPIRMRAKWYPTLSHQQEVLEALAQS GPFAYHSDIKKVSLGIKYRFKWIWGGNPVRQQVVRNPCKEPHSSGNRVPRSI QIVDPRYNSPELTIHAWDFRRGFFGPKAIQRMQQQPTATEFFSAGRKRPRRD TEVYQSDQEKEQKESSLFPPVKLLRRVPPWEDSEQEQSGSQSSEEETATLSQ QLKQQLQQQRVLGVKLRLLFNQVQKIQQNQDINPTLLPRGGDLVSFFQAVP (SEQ ID NO: 1020)
ORF1/1	MAYGWWRRRRRRWRRWRRRPWRRRWRTRRRRPARRRGRRRNVVRNPC KEPHSSGNRVPRSIQIVDPRYNSPELTIHAWDFRRGFFGPKAIQRMQQQPTAT EFFSAGRKRPRRDTEVYQSDQEKEQKESSLFPPVKLLRRVPPWEDSEQEQSG SQSSEEETATLSQQLKQQLQQQRVLGVKLRLLFNQVQKIQQNQDINPTLLPR GGDLVSFFQAVP (SEQ ID NO: 1021)

ORF1/2	MAYGWWRRRRRRWRRWRRRPWRRRWRTRRRRPARRRGRRRNAARDPEG TQKCISPTKKRSKKKARFSPQSSSSEESPRGRTRNRSKAGRKAQRKRRRPSPS SSNSSCSSSESWESNSDSCSTKSKKSNKIKISTLPCYQGGGI (SEQ ID NO: 1022)

Table N24. Exemplary *Anellovirus* nucleic acid sequence (*Alphatorquevirus*)

Name	Ring18
Genus/Clade	*Alphatorquevirus*
Accession Number	

Full Sequence: 3733 bp

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   1 CACGTGACTC CCGCAGGCCA ACCAGAGTCT ACGTCGTGCA CTTCCTGGGC ATGGTCTACA
  61 TCATAATATA AGAAGGCGCA CTTCCGAATG GCTGAGTTTT CCACGCCCGT CCGCAGCGAG
 121 AACGCCACGG AGGGAGATCC TCGCGTCCCG AGGGCGGGTG CCGGAGGTGA GTTTACACAC
 181 CGCAGTCAAG GGGCAATTCG GGCTCGGGAC TGGCCGGGCC CCGGGCAAGG CTCTTAAAAA
 241 ATGCGCTTTC GCAGGGTTGC TGAGAAAAGG AAAGTGCTTC TGCAAACTGT GCGAGCTGCA
 301 GAGAAGACTA GGCGGCTTCT AGGTATGTGG CAGCCCCCCG CGCACAATGT CCCCGGCATC
 361 GAGAGAAACT GGTACGAGAG CTGTTTTCGA TCCCATGCTG CTGTTTGCGG CTGTGGCGAC
 421 TTTGTTGGCC ATCTTAGTTA TCTGGCAACT ACTCTGGGTC GTCCTCCGCG TCCTGGGCCC
 481 CCAGGCGGAC CCCGCACACC GCAGATAAGA AACCTGCCAG CGCTCCCGGC GCCCCAGGGC
 541 GAGCCCGGTG ACAGAGCGCC ATGGCGTGGG GCTTCTGGGG CCGACGCCGC CGGTGGAGAC
 601 GGTGGAGACC ACGGCGCAGA CGGTGGAGAC CCCGCAGACG TAGGAGACGA CGCCCTGCTC
 661 GCCGCTTTCG AGCTCGTCGA AGAGTAAGGA GGCGCGGGGG GCGGTGGCGC AGACGCTACA
 721 GAAAATGGCG ACGGGGCAGA CGCAGACGAA CTCACAGAAA AAAGATAGTC ATAAAACAGT
 781 GGCAACCAAA CTTTATAAGA CGCTGCTACA TCATAGGGTA CCTACCTTTA ATATTCTGTG
 841 GCGAAAACAC AACCGCCCAG AACTATGCCA CTCACTCAGA CGACATGATA AGCAAAGGAC
 901 CATACGGGGG GGGCATGACT ACCACAAAAT TTACTCTGAG AATACTGTAC GACGAGTTTA
 961 CCAGGTTTAT GAACTTTTGG ACTGTTAGTA ACGAAGACCT AGACCTGTGT AGATACGTGG
1021 GCTGCAAACT CATATTTTTT AAACATCCCA CAGTGGACTT TATAGTACAG ATAAACACTC
1081 AGCCTCCTTT CTTAGACACG CACCTTACCG CGGCCAGCAT ACACCCGGGC ATCATGATGC
1141 TCAGCAAGAG ACGCATACTA ATACCCTCTC TAAAAACCCG GCCAAGCAGA AAACACAGGG
1201 TGGTTGTTAG GGTGGGCGCC CCAAGACTTT TTCAGGACAA GTGGTACCCC CAGTCAGACC
1261 TGTGTGACAC AGTTCTGCTT TCCATATTTG CAACCGCCTG TGACTTGCAA TATCCGTTCG
1321 GCTCACCACT AACTGACAAC CCTTGCGTCA ACTTCCAGAT TCTGGGGCCC CAGTACAAAA
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1381 AACACCTTAG TATTAGCTCT ACTATGGATA CAACTAACAA ACAGCACTAT GACAGCAATT
1441 TGTTTAACCA AACTCAGCTA TACAACACCT TTCAAACTAT AGCTCAGCTT AAAGAGACAG
1501 GACAAACTGC AAACATATCT CCTAGTTGGA GTGCAGTGCA AAATAATATG GCCCTTAGTA
1561 ATACCGGTGA AAATGCAACC CAAAGCAAAG ACACTTGGTA CAAAGGAAAC ACATACAACA
1621 ACCACATTAC AACGTTAGCA CAAAAAACCA GAGAAAGATT TAAAGGTGCA ACAAAAGCAG
1681 CACTACAAAA CTACCCCACC ATAATGTCCA CAGACTTATA TGAATACCAC TCAGGCATAT
1741 ACTCCAGCAT ATTTCTATCA GCTGGCAGGA GCTACTTTGA AACCACCGGG GCCTACTCTG
1801 ACATTATATA CAACCCTTTC ACAGACAAAG GCACAGGCAA CATAATCTGG ATAGACTACC
1861 TCACAAAAGA AGACACCATT TTTGTAAAAA ACAAAAGCAA ATGCGAAATA ATGGACATGC
1921 CCCTGTGGGC GGCCTGCACA GGATATACAG AGTTCTGTGC AAAGTATACA GGAGACTCTG
1981 CCATTATTTA CAATGCCAGA GTACTAATAA GATGCCCGTA CACTGAACCC ATGCTAATAG
2041 ACCACTCAGA CCCGAACAAA GGCTTCGTAC CCTACTCATT TAACTTTGGC AACGGAAAAA
2101 TGCCCGGAGG CAGCTCCAAC GTACCCATAA GAATGAGAGC CAAATGGTAC GTGAACATAT
2161 TCCACCAAAA AGAGGTTCTA GAGACTATAG TACAGAGCGG ACCGTTCGGG TACAAGGGCG
2221 ACATAAAATC AGCTGTACTG GCCATGAAAT ACAGATTTCA CTGGAAATGG GGTGGAAACC
2281 CTATATCCAA ACAGGTCGTC AGGAATCCCT GCTCCAACTC CAGCTCCTCC GCGGCCCATA
2341 GAGGACCTCG CAGCGTACAA GCAGTTGACC CGAAATACAA TACCCCAGAG GTCACGTGGC
2401 ACTCGTGGGA CATCAGACGA GGACTCTTTG GCAAAGCAGG TATTAAAAGA ATGCAACAAG
2461 AATCAGATGC TCTTTACATT CCTCCAGGAC CATTCAAGAG ACCTCGCAGA GACACGAACG
2521 CCCAAGACCC AGAAGAGCAA AACGAAAGCT CAGGTTTCAG AGTCCAGCAG CGACTCCCGT
2581 GGGTCCACTC CAGCCAAGAG ACGCAAAGCT CCCAAGAAGA AACGGAGGCG CAGGGGTCGG
2641 TACAAGACCA ACTACTCCTC CAGCTCCGAG AGCAGCGAGT ACTCCGACTC CAGCTCCAGC
2701 AACTCGCAAC CCAAGTCCTC AAAGTCCAAG CAGGGCACGG CATACACCCC CTATTATCTT
2761 CCCAAGCGTA AACAAAGTCT TTATGTTTGA GCCCCACGGT CCTAAACCCA TACAGGGCTA
2821 CAACGATTGG CTAGAGGAGT ACACTGCTTG TAAATTCTGG GACAGACCCC CAAGAAAGCT
2881 ACACACAGAC TTACCCTTTT ACCCCTGGGC ACCAAAACCC CAAGACCAAG TCAGGGTAAG
2941 CTTTAAACTC AACTTTCAAT AAAAATTCTA GGCCGTGGGA GTTTCACTTG TCGGTGTCTG
3001 CTTCTTAAGG TCGCCAAGCA CTCCGAGCGC CAGCGAGGAG TGCGACCCCC CCTCCGGTAG
3061 CAACGCCTTC GGAGCCGCGC GCTACGCCTT CGGCTGCGCG CGGCACCTCA GACCCCCCCT
3121 CCACCCGAAA CGCTTGCGCG TTTCGGACCT TCGGCGTCGG GGGGGTCGGG AGCTTTATTA
3181 AACAGACTCC GAGTTGCCAT TGGACACTGG AGCTGTGAAT CAGTAACGAA AGTGAGTGGG
3241 GCCAGACTTC GCCATAGGGC CTTTATCTTC TCGCCATTGG ATAGTGTCCG GGGTCGCCGT
3301 AGGCTTCGGC CTCGTTTTTA GGCCTTCCGG ACTACAAAAA TGGCGAACTT GGTGACGTCA
3361 CGGCCGCCAT TTTAAGTAAG GCGGAAGCAG CTCCACTTTC TCACAAAATG GCGGCGGAGC
3421 ACTTCCGGCT TGCCCAAAAT AGCGGGCAAG CTCTTCCGGG TCAAAGGTCA GCAGCTACGT
3481 CACAAGTCAC CTGACTGAGG AGGAGCTAAA ACCCGGAAGT CCTCCTCGGT CACGTGGCTA
3541 GTCACGTGAC TACTACGTCA TCGGCGCCAT CTTGTGTGAC AAAATGGCGG ACAACTTCCG
3601 CTTTTTTGAA AAAAGGCGCG AAAAAACGGC GGCGGCGGCG CGCGCGCTGC GCGCGCGCGC

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3661 CGAGGGGGCG CCAGCGCCCC CACTGTGCGG TCCCCCGCGG GGCTCCGGCC CCCCCCCGAA
3721 GTCCGTCACT AAC
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Annotations:

Putative Domain	*Base range*
TATA Box	67 - 71
Cap Site	88 - 95
Transcriptional Start Site	95
5' UTR Conserved Domain	155 - 225
ORF2	325 - 687
TAIP	347 - 508
ORF2/2	325 - 683, 2295 - 2790
ORF2/3	325- 683, 2488 - 2962
ORF1	561 - 2771
ORF1/1	561 - 683, 2295 - 2771
ORF1/2	561 - 683, 2488 – 2790
Three open-reading frame region	2447 - 2771
Poly(A) Signal	2958 - 2963
GC-rich region	3627 - 3718

Table A24. Exemplary *Anellovirus* amino acid sequences for Ring 18 (*Alphatorquevirus*)

ORF1	MAWGFWGRRRRWRRWRPRRRRWRPRRRRRRRPARRFRARRRVRRRGGR WRRRYRKWRRGRRRRTHRKKIVIKQWQPNFIRRCYIIGYLPLIFCGENTTA QNYATHSDDMISKGPYGGGMTTTKFTLRILYDEFTRFMNFWTVSNEDLDL CRYVGCKLIFFKHPTVDFIVQINTQPPFLDTHLTAASIHPGIMMLSKRRILIPS LKTRPSRKHRVVVRVGAPRLFQDKWYPQSDLCDTVLLSIFATACDLQYPF GSPLTDNPCVNFQILGPQYKKHLSISSTMDTTNKQHYDSNLFNQTQLYNTF QTIAQLKETGQTANISPSWSAVQNNMALSNTGENATQSKDTWYKGNTYN NHITTLAQKTRERFKGATKAALQNYPTIMSTDLYEYHSGIYSSIFLSAGRSY FETTGAYSDIIYNPFTDKGTGNIIWIDYLTKEDTIFVKNKSKCEIMDMPLWA ACTGYTEFCAKYTGDSAIIYNARVLIRCPYTEPMLIDHSDPNKGFVPYSFNF GNGKMPGGSSNVPIRMRAKWYVNIFHQKEVLETIVQSGPFGYKGDIKSAV LAMKYRFHWKWGGNPISKQVVRNPCSNSSSSAAHRGPRSVQAVDPKYNT

	PEVTWHSWDIRRGLFGKAGIKRMQQESDALYIPPGPFKRPRRDTNAQDPEE QNESSGFRVQQRLPWVHSSQETQSSQEETEAQGSVQDQLLLQLREQRVLR LQLQQLATQVLKVQAGHGIHPLLSSQA (SEQ ID NO: 1100)
ORF1/1	MAWGFWGRRRRWRRWRSRRRRWRPRRRRRRRPARRFRARRRVVRNPCS NSSSSAAHRGPRSVQAVDPKYNTPEVTWHSWDIRRGLFGKAGIKRMQQES DALYIPPGPFKRPRRDTNAQDPEEQNESSGFRVQQRLPWVHSSQETQSSQE ETEAQGSVQDQLLLQLREQRVLRLQLQQLATQVLKVQAGHGIHPLLSSQA
ORF1/2	MAWGFWGRRRRWRRWRPRRRRWRPRRRRRRRPARRFRARRRDHSRDLA ETRTPKTQKSKTKAQVSESSSDSRGSTPAKRRKAPKKKRRRRGRYKTNYSS SSESSEYSDSSSSNSQPKSSKSKQGTAYTPYYLPKRKQSLYV
ORF2	MWQPPAHNVPGIERNWYESCFRSHAAVCGCGDFVGHLSYLATTLGRPPRP GPPGGPRTPQIRNLPALPAPQGEPGDRAPWRGASGADAAGGDGGDHGAD GGDPADVGDDALLAAFELVEE
ORF2/2	MWQPPAHNVPGIERNWYESCFRSHAAVCGCGDFVGHLSYLATTLGRPPRP GPPGGPRTPQIRNLPALPAPQGEPGDRAPWRGASGADAAGGDGGDHGAD GGDPADVGDDALLAAFELVEESSGIPAPTPAPPRPIEDLAAYKQLTRNTIPQ RSRGTRGTSDEDSLAKQVLKECNKNQMLFTFLQDHSRDLAETRTPKTQKS KTKAQVSESSSDSRGSTPAKRRKAPKKKRRRRGRYKTNYSSSSESSEYSDS SSSNSQPKSSKSKQGTAYTPYYLPKRKQSLYV
ORF2/3	MWQPPAHNVPGIERNWYESCFRSHAAVCGCGDFVGHLSYLATTLGRPPRP GPPGGPRTPQIRNLPALPAPQGEPGDRAPWRGASGADAAGGDGGDHGAD GGDPADVGDDALLAAFELVEETIQETSQRHERPRPRRAKRKLRFQSPAATP VGPLQPRDAKLPRRNGGAGVGTRPTTPPAPRAASTPTPAPATRNPSPQSPSR ARHTPPIIFPSVNKVFMFEPHGPKPIQGYNDWLEEYTACKFWDRPPRKLHT DLPFYPWAPKPQDQVRVSFKLNFQ

Table N25. Novel *Anellovirus* nucleic acid sequence *(Betatorquevirus)*

Name	RING 19
Genus/Clade	*Betatorquevirus*
Accession	N/A

Full Sequence: 2876 bp

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1         10        20        30        40        50
|         |         |         |         |         |
CGGGAGCCGAAGGTGAGTGCAACCACCGTAGTCTAGGGGCAATTCGGGCT
AGTTCAGTATGGCGGAACGGGCAAGAAACTTAAATATTATTATTTTACAG
ATGCAAATACAACCACCTATTAGAACCTTCAAACAAACAATTTCAGATTG
GAAAAACTTAATTGTCCACGTTCACGACAACATTTGCAACTGCAATAAAC
CATTAGAACACACTATTGATACCTGTATCACCAATCCAGATGAATTAAGA
TTAAACAAATCTACTAAACAACAACTACAAAAATGCCTTGGTACCCCAGA
AGAAGATACCCAAGAAGACGTTATCGATGGCTTCGCAGATGGAGAGCTAG
ACGCCCTTTTCGCCCAAGATACAGAAGAAGATACTGGGTAAGAAACTATT
CTCGAAAGAGAAAACTATTTAAAATAACAACCAAAGAATGGCAACCAAAA
GTTATAAGAAAGACTCATGTAAAGGGCACCTATCCTTTGTTTCTTTGTAC
AAAGCACAGAATTAACAATAATATGATACAATATTTAGACTCTATAGCTC
CAGAACACTATTACGGAGGAGGAGGATTTTCAATAATGCAATTTTCCTTA
CAAGCCTTATATGAAGAATTTATAAAAGCAAAAAACTGGTGGACTAATAC
AAACTGCTTTTTACCACTTGTAAGATATATGGGTTGCTCATTCAAATTTT
ATAAAACTGAATTTTATGATTATATTGTACTAATTGAAAGATGTTATCCA
CTTGCTTGTACTGATGAAATGTACTTATCTACTCAACCTAGTATTATGAT
GCTTACAAGAAAATGTATTTTTGTACCATGCAAACAAAACAGCAAAGGTA
AAAAACCTTACAAAAAAGTTAGAGTAAGACCACCTTCACAAATGACTACA
GGATGGCATTTCTCACAAGACTTAGCAAACATGCCACTTGTAGTACTAAA
AACTTCAGTATGCAGCTTTGACAGATATTACACAGACAGTACAGCTAAAT
CAACCACAATAGGCTTTAAAACACTTAACACACAAACATTTAGATATCAT
GACTGGCAGGAACCACCTACAACAGGATACAAACCACAAAACCTACTATG
GTTTTATGGAGCAGAAAACGGATCACCAGTAGACCCCAACAACACAATAG
TATCAAACCTAATATACTTAGGAGGCACAGGACCTTATGAAAAAGGCACA
CCAATAAAAACAAACATAAGCAATTACTTTTCAGAGCCTAAACTGTGGGG
AAATATATTTCACGATGATTATACATCAGGAACATCACCCGTGTTTGTTA
CAAACAAATCACCATCAGAAATTAAAACCGCATGGAACACTATAAAAGAC
TTAACTGTTAAAGCTAGCGGTGTATTTACATTAAGAACAATTCCACTATG
GCTACCTTGCAGATACAACCCATTTGCAGACAAAGCAACCAACAACAAAA
TATGGCTAGTTTCTATACATTCAGACCACACAGAATGGAAACCAATAGAC
AATCCATTACTACAACGAACAGACCTTCCTTTATGGTTACTTGTATGGGG
TTGGCAAGATTGGCAGAAAAAAAACCAACAAACTTCACAACCTGATATTA
ATTATTTAACAGTAATATCTTCACCATATATATCATGCTACCCAAAATTA
GATTACTATGTGTTACTAGATGAAGGATTTTGGGAGGGTCACTCAACATA
CATAGAGTCAATTACAGACTCAGACAAAAAACACTGGTACCCTAAAAATA
GATTTCAAATAGAAACACTTAATCTAATAGCTAACACAGGTCCAGGAACT
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GTAAAACTAAGAGAAAACCAAGCAGCAGAAGGTCACATGGTATATCGCTT
TAATTTTAAGCTTGGAGGATGTCCCGCACCGATGGAAAAAATATGTGACC
CTAGCAAACAATCCAAATATCCTATTCCCAATAACCAGCAACAAACAACT
TCGTTGCAGAGTCCAGAAAACCCAATTCAAACCTATCTCTACGACTTCGA
CGAAAGGAGGGGCCTACTTACAGAAAGAGCTACAAAAAGAATCAAACAAG
ATCACACATCTGAAAAAACTGTTTTGCCATTTACAGGAGCAGCAACAGAC
CTCCCCATACTCCAAACAACATCACAGGAGGAAAGCTCCTCGGAAGAAGA
AGAAGAGCAACAAGCGGAGAAGAAACTACTCCAGCTCCGAAGAAAGCAGC
ACCGACTCCGGGAGCGAATCCTCCAGCTATTAGACATACAAAATACATAA
TAAAACAAAGTACTGTAAAAATTGATATGTTTGGAGATACTCATGTACCT
AACCGTAGAATGACCCCAGAAGAATTTGAACAAGAACTAATTGTCGCTGG
TGTTTTTCGCAGACCTCCTTGTTACTATATAAAAGATAGACCTACTTATC
CTTATGTACCAAAACCTACTGATGAAAAATGTATGGTAAACTTTGACTTA
AACTTTCCTTAATAAACTACGCCTGCAAACTTTCACTCTCGGTGTCCATT
TATATAAGATAAAACTTAAATAAACATCCACCACTCTCCCAAATACGCAG
GCGCACAAGGGGGCTCCGCCCCCTTAAACCCCCAAGGGGGCTCCGCCCCC
TTAAACCCCCAAGGGGGCTCCGCCCCCTTACACCCCCTAATAAATATTCA
ACAGGAAAACCACCTAATTAGAATTGCCGACCACAAACCGTCACTTACTT
CTCCTTTTTGCACTTACTTCCTCTTTTACTTATTATTATTCATTACATTA
ATTAATAATCACTGTAATTCCGGGGAGGAGCTAACAATCTATATAACTAA
CTACACTTCCGAATGGCTGAGTTTATGCCGCCAGACGGAGACGGGATCAC
TTCAGTGACTCCAGGCTGAACTTGGG
(SEQ ID NO: 1023)

Annotations:

Putative Domain	*Base range*
ORF1	283 – 2250
ORF2	59 – 391
ORF3	2277 – 2462
GC-rich region, or a portion thereof	2515 – 2615
5' UTR Conserved Domain, or a portion thereof	1 – 71

Table A25. Novel *Anellovirus* amino acid sequence *(Betatorquevirus)*

RING 19 (Betatorquevirus)	
ORF1	MPWYPRRRYPRRRYRWLRRWRARRPFRPRYRRRYWVRNYSRK RKLFKITTKEWQPKVIRKTHVKGTYPLFLCTKHRINNNMIQYLDSIA PEHYYGGGGFSIMQFSLQALYEEFIKAKNWWTNTNCFLPLVRYM GCSFKFYKTEFYDYIVLIERCYPLACTDEMYLSTQPSIMMLTRKCIF VPCKQNSKGKKPYKKVRVRPPSQMTTGWHFSQDLANMPLVVLK

	TSVCSFDRYYTDSTAKSTTIGFKTLNTQTFRYHDWQEPPTTGYKP QNLLWFYGAENGSPVDPNNTIVSNLIYLGGTGPYEKGTPIKTNISN YFSEPKLWGNIFHDDYTSGTSPVFVTNKSPSEIKTAWNTIKDLTVK ASGVFTLRTIPLWLPCRYNPFADKATNNKIWLVSIHSDHTEWKPID NPLLQRTDLPLWLLVWGWQDWQKKNQQTSQPDINYLTVISSPYIS CYPKLDYYVLLDEGFWEGHSTYIESITDSDKKHWYPKNRFQIETLN LIANTGPGTVKLRENQAAEGHMVYRFNFKLGGCPAPMEKICDPSK QSKYPIPNNQQQTTSLQSPENPIQTYLYDFDERRGLLTERATKRIK QDHTSEKTVLPFTGAATDLPILQTTSQEESSSEEEEEQQAEKKLLQ LRRKQHRLRERILQLLDIQNT (SEQ ID NO: 1024)
ORF2	MAERARNLNIIILQMQIQPPIRTFKQTISDWKNLIVHVHDNICNCNKP LEHTIDTCITNPDELRLNKSTKQQLQKCLGTPEEDTQEDVIDGFAD GELDALFAQDTEEDTG (SEQ ID NO: 1025)
ORF3	MFGDTHVPNRRMTPEEFEQELIVAGVFRRPPCYYIKDRPTYPYVP KPTDEKCMVNFDLNFP (SEQ ID NO: 1026)

Table N26. Novel *Anellovirus* nucleic acid sequence *(Betatorquevirus)*

Name	RING 19 alternate
Genus/Clade	*Betatorquevirus*
Accession	N/A

Full Sequence: 2876 bp

```
1         10        20        30        40        50
|         |         |         |         |         |
CGGGAGCCGAAGGTGAGTGCAACCACCGTAGTCTAGGGGCAATTCGGGCT
AGTTCAGTATGGCGGAACGGGCAAGAAACTTAAATATTATTATTTTACAG
ATGCAAATACAACCACCTATTAGAACCTTCAAACAAACAATTTCAGATTG
GAAAAACTTAATTGTCCACGTTCACGACAACATTTGCAACTGCAATAAAC
CATTAGAACACACTATTGATACCTGTATCACCAATCCAGATGAATTAAGA
TTAAACAAATCTACTAAACAACAACTACAAAAATGCCTTGGTACCCCAGA
AGAAGATACCCAAGAAGACGTTATCGATGGCTTCGCAGATGGAGAGCTAG
ACGCCCTTTTCGCCCAAGATACAGAAGAAGATACTGGGTAAGAAACTATT
CTCGAAAGAGAAAACTATTTAAAATAACAACCAAAGAATGGCAACCAAAA
GTTATAAGAAAGACTCATGTAAAGGGCACCTATCCTTTGTTTCTTTGTAC
AAAGCACAGAATTAACAATAATATGATACAATATTTAGACTCTATAGCTC
CAGAACACTATTACGGAGGAGGAGGATTTTCAATAATGCAATTTTCCTTA
CAAGCCTTATATGAAGAATTTATAAAAGCAAAAAACTGGTGGACTAATAC
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AAACTGCTTTTTACCACTTGTAAGATATATGGGTTGCTCATTCAAATTTT
ATAAAACTGAATTTTATGATTATATTGTACTAATTGAAAGATGTTATCCA
CTTGCTTGTACTGATGAAATGTACTTATCTACTCAACCTAGTATTATGAT
GCTTACAAGAAAATGTATTTTTGTACCATGCAAACAAAACAGCAAAGGTA
AAAAACCTTACAAAAAAGTTAGAGTAAGACCACCTTCACAAATGACTACA
GGATGGCATTTCTCACAAGACTTAGCAAACATGCCACTTGTAGTACTAAA
AACTTCAGTATGCAGCTTTGACAGATATTACACAGACAGTACAGCTAAAT
CAACCACAATAGGCTTTAAAACACTTAACACACAAACATTTAGATATCAT
GACTGGCAGGAACCACCTACAACAGGATACAAACCACAAAACCTACTATG
GTTTTATGGAGCAGAAAACGGATCACCAGTAGACCCCAACAACACAATAG
TATCAAACCTAATATACTTAGGAGGCACAGGACCTTATGAAAAAGGCACA
CCAATAAAAACAAACATAAGCAATTACTTTTCAGAGCCTAAACTGTGGGG
AAATATATTTCACGATGATTATACATCAGGAACATCACCCGTGTTTGTTA
CAAACAAATCACCATCAGAAATTAAAACCGCATGGAACACTATAAAAGAC
TTAACTGTTAAAGCTAGCGGTGTATTTACATTAAGAACAATTCCACTATG
GCTACCTTGCAGATACAACCCATTTGCAGACAAAGCAACCAACAACAAAA
TATGGCTAGTTTCTATACATTCAGACCACACAGAATGGAAACCAATAGAC
AATCCATTACTACAACGAACAGACCTTCCTTTATGGTTACTTGTATGGGG
TTGGCAAGATTGGCAGAAAAAAAACCAACAAACTTCACAACCTGATATTA
ATTATTTAACAGTAATATCTTCACCATATATATCATGCTACCCAAAATTA
GATTACTATGTGTTACTAGATGAAGGATTTTGGGAGGGTCACTCAACATA
CATAGAGTCAATTACAGACTCAGACAAAAAACACTGGTACCCTAAAAATA
GATTTCAAATAGAAACACTTAATCTAATAGCTAACACAGGTCCAGGAACT
GTAAAACTAAGAGAAAACCAAGCAGCAGAAGGTCACATGGTATATCGCTT
TAATTTTAAGCTTGGAGGATGTCCCGCACCGATGGAAAAAATATGTGACC
CTAGCAAACAATCCAAATATCCTATTCCCAATAACCAGCAACAAACAACT
TCGTTGCAGAGTCCAGAAAACCCAATTCAAACCTATCTCTACGACTTCGA
CGAAAGGAGGGGCCTACTTACAGAAAGAGCTACAAAAAGAATCAAACAAG
ATCACACATCTGAAAAAACTGTTTTGCCATTTACAGGAGCAGCAACAGAC
CTCCCCATACTCCAAACAACATCACAGGAGGAAAGCTCCTCGGAAGAAGA
AGAAGAGCAACAAGCGGAGAAGAAACTACTCCAGCTCCGAAGAAAGCAGC
ACCGACTCCGGGAGCGAATCCTCCAGCTATTAGACATACAAAATACATAA
TAAAACAAAGTACTGTAAAAATTGATATGTTTGGAGATACTCATGTACCT
AACCGTAGAATGACCCCAGAAGAATTTGAACAAGAACTAATTGTCGCTGG
TGTTTTTCGCAGACCTCCTTGTTACTATATAAAAGATAGACCTACTTATC
CTTATGTACCAAAACCTACTGATGAAAAATGTATGGTAAACTTTGACTTA
AACTTTCCTTAATAAACTACGCCTGCAAACTTTCACTCTCGGTGTCCATT
TATATAAGATAAAACTTAAATAAACATCCACCACTCTCCCAAATACGCAG

GCGCACAAGGGGGCTCCGCCCCCTTAAACCCCCAAGGGGGCTCCGCCCCC
TTAAACCCCCAAGGGGGCTCCGCCCCCTTACACCCCCTAATAAATATTCA
ACAGGAAAACCACCTAATTAGAATTGCCGACCACAAACCGTCACTTACTT
CTCCTTTTTGCACTTACTTCCTCTTTTACTTATTATTATTCATTACATTA
ATTAATAATCACTGTAATTCCGGGGAGGAGCTAACAATCTATATAACTAA
CTACACTTCCGAATGGCTGAGTTTATGCCGCCAGACGGAGACGGGATCAC
TTCAGTGACTCCAGGCTGAACTTGGG
(SEQ ID NO: 1027)

Annotations:

Putative Domain	*Base range*
ORF1	283 – 2250
ORF2	101 – 391
ORF3	2277 – 2462
GC-rich region, or a portion thereof	2515 – 2615
5' UTR Conserved Domain, or a portion thereof	1 – 71

Table A26. Novel *Anellovirus* amino acid sequence *(Betatorquevirus)*

RING 19 (Betatorquevirus)	
ORF1	MPWYPRRRYPRRRYRWLRRWRARRPFRPRYRRRYWVRNYSRK RKLFKITTKEWQPKVIRKTHVKGTYPLFLCTKHRINNNMIQYLDSIA PEHYYGGGGFSIMQFSLQALYEEFIKAKNWWTNTNCFLPLVRYM GCSFKFYKTEFYDYIVLIERCYPLACTDEMYLSTQPSIMMLTRKCIF VPCKQNSKGKKPYKKVRVRPPSQMTTGWHFSQDLANMPLVVLK TSVCSFDRYYTDSTAKSTTIGFKTLNTQTFRYHDWQEPPTTGYKP QNLLWFYGAENGSPVDPNNTIVSNLIYLGGTGPYEKGTPIKTNISN YFSEPKLWGNIFHDDYTSGTSPVFVTNKSPSEIKTAWNTIKDLTVK ASGVFTLRTIPLWLPCRYNPFADKATNNKIWLVSIHSDHTEWKPID NPLLQRTDLPLWLLVWGWQDWQKKNQQTSQPDINYLTVISSPYIS CYPKLDYYVLLDEGFWEGHSTYIESITDSDKKHWYPKNRFQIETLN LIANTGPGTVKLRENQAAEGHMVYRFNFKLGGCPAPMEKICDPSK QSKYPIPNNQQQTTSLQSPENPIQTYLYDFDERRGLLTERATKRIK QDHTSEKTVLPFTGAATDLPILQTTSQEESSSEEEEEQQAEKKLLQ LRRKQHRLRERILQLLDIQNT (SEQ ID NO: 1028)
ORF2	MQIQPPIRTFKQTISDWKNLIVHVHDNICNCNKPLEHTIDTCITNPD ELRLNKSTKQQLQKCLGTPEEDTQEDVIDGFADGELDALFAQDTE EDTG (SEQ ID NO: 1029)

ORF3	MFGDTHVPNRRMTPEEFEQELIVAGVFRRPPCYYIKDRPTYPYVP KPTDEKCMVNFDLNFP (SEQ ID NO: 1030)

In some embodiments, an anellovector or anelloVLP as described herein is a chimeric anellovector or anelloVLP. In some embodiments, a chimeric anellovector or anelloVLP further comprises one or more elements, polypeptides, or nucleic acids from a virus other than an *Anellovirus*.

In some embodiments, the chimeric anellovector or anelloVLP comprises a plurality of polypeptides (e.g., Anellovirus ORF1, ORF1/1, ORF1/2, ORF2, ORF2/2, ORF2/3, and/or ORF2t/3) comprising sequences from a plurality of different Anelloviruses (e.g., as described herein).

In some embodiments, the anellovector or anelloVLP comprises a chimeric polypeptide (e.g., Anellovirus ORF1, ORF1/1, ORF1/2, ORF2, ORF2/2, ORF2/3, and/or ORF2t/3), e.g., comprising at least one portion from an Anellovirus (e.g., as described herein) and at least one portion from a different virus (e.g., as described herein).

In some embodiments, the anellovector or anelloVLP comprises a chimeric polypeptide (e.g., Anellovirus ORF1, ORF1/1, ORF1/2, ORF2, ORF2/2, ORF2/3, and/or ORF2t/3), e.g., comprising at least one portion from one Anellovirus (e.g., as described herein) and at least one portion from a different Anellovirus (e.g., as described herein). In some embodiments, the anellovector or anelloVLP comprises a chimeric ORF1 molecule comprising at least one portion of an ORF1 molecule from one Anellovirus (e.g., as described herein), or an ORF1 molecule having at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% amino acid sequence identity thereto, and at least one portion of an ORF1 molecule from a different Anellovirus (e.g., as described herein), or an ORF1 molecule having at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% amino acid sequence identity thereto. In some embodiments, the chimeric ORF1 molecule comprises an ORF1 structural jelly-roll domain from one Anellovirus, or a sequence having at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity thereto, and an ORF1 amino acid subsequence (e.g., as described herein) from a different Anellovirus, or a sequence having at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity thereto. In some embodiments, the chimeric ORF1 molecule comprises an ORF1 structural arginine-rich region from one Anellovirus, or a sequence having at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity thereto, and an ORF1 amino acid subsequence (e.g., as described herein) from a different Anellovirus, or a sequence having at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity thereto. In some embodiments, the chimeric ORF1 molecule comprises an ORF1 structural hypervariable domain from one Anellovirus, or a sequence having at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity thereto, and an ORF1 amino acid subsequence

(e.g., as described herein) from a different Anellovirus, or a sequence having at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity thereto. In some embodiments, the chimeric ORF1 molecule comprises an ORF1 structural N22 domain from one Anellovirus, or a sequence having at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity thereto, and an ORF1 amino acid subsequence (e.g., as described herein) from a different Anellovirus, or a sequence having at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity thereto. In some embodiments, the chimeric ORF1 molecule comprises an ORF1 structural C-terminal domain from one Anellovirus, or a sequence having at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity thereto, and an ORF1 amino acid subsequence (e.g., as described herein) from a different Anellovirus, or a sequence having at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity thereto.

In some embodiments, the anellovector or anelloVLP comprises a chimeric ORF1/1 molecule comprising at least one portion of an ORF1/1 molecule from one Anellovirus (e.g., as described herein), or an ORF1/1 molecule having at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% amino acid sequence identity thereto, and at least one portion of an ORF1/1 molecule from a different Anellovirus (e.g., as described herein), or an ORF1/1 molecule having at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% amino acid sequence identity thereto. In some embodiments, the anellovector or anelloVLP comprises a chimeric ORF1/2 molecule comprising at least one portion of an ORF1/2 molecule from one Anellovirus (e.g., as described herein), or an ORF1/2 molecule having at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% amino acid sequence identity thereto, and at least one portion of an ORF1/2 molecule from a different Anellovirus (e.g., as described herein), or an ORF1/2 molecule having at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% amino acid sequence identity thereto. In some embodiments, the anellovector or anelloVLP comprises a chimeric ORF2 molecule comprising at least one portion of an ORF2 molecule from one Anellovirus (e.g., as described herein), or an ORF2 molecule having at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% amino acid sequence identity thereto, and at least one portion of an ORF2 molecule from a different Anellovirus (e.g., as described herein), or an ORF2 molecule having at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% amino acid sequence identity thereto. In some embodiments, the anellovector or anelloVLP comprises a chimeric ORF2/2 molecule comprising at least one portion of an ORF2/2 molecule from one Anellovirus (e.g., as described herein), or an ORF2/2 molecule having at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% amino acid sequence identity thereto, and at least one portion of an ORF2/2 molecule from a different Anellovirus (e.g., as described herein), or an ORF2/2 molecule having at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% amino acid sequence identity thereto. In some embodiments, the anellovector or anelloVLP comprises a chimeric ORF2/3 molecule comprising at

least one portion of an ORF2/3 molecule from one Anellovirus (e.g., as described herein), or an ORF2/3 molecule having at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% amino acid sequence identity thereto, and at least one portion of an ORF2/3 molecule from a different Anellovirus (e.g., as described herein), or an ORF2/3 molecule having at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% amino acid sequence identity thereto. In some embodiments, the anellovector or anelloVLP comprises a chimeric ORF2T/3 molecule comprising at least one portion of an ORF2T/3 molecule from one Anellovirus (e.g., as described herein), or an ORF2T/3 molecule having at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% amino acid sequence identity thereto, and at least one portion of an ORF2T/3 molecule from a different Anellovirus (e.g., as described herein), or an ORF2T/3 molecule having at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% amino acid sequence identity thereto.

In some embodiments, an anellovector comprises a nucleic acid comprising a sequence listed in PCT Application No. PCT/US2018/037379, incorporated herein by reference in its entirety. In some embodiments, an anellovector or anelloVLP comprises a polypeptide comprising a sequence listed in PCT Application No. PCT/US2018/037379, incorporated herein by reference in its entirety.

In some embodiments, an anellovector comprises an *Anellovirus* genome, e.g., as identified according to the method described in Example 9 of PCT Publication No. WO 2020/123816, incorporated by reference herein in its entirety. In some embodiments, an anellovector or anelloVLP comprises an *Anellovirus* sequence, or a portion thereof, as described in Example 29.

In some embodiments, an anellovector comprises a genetic element comprising a consensus *Anellovirus* motif, e.g., as shown in Table 19. In some embodiments, an anellovector comprises a genetic element comprising a consensus *Anellovirus* ORF1 motif, e.g., as shown in Table 19. In some embodiments, an anellovector comprises a genetic element comprising a consensus *Anellovirus* ORF1/1 motif, e.g., as shown in Table 19. In some embodiments, an anellovector comprises a genetic element comprising a consensus *Anellovirus* ORF1/2 motif, e.g., as shown in Table 19. In some embodiments, an anellovector comprises a genetic element comprising a consensus *Anellovirus* ORF2/2 motif, e.g., as shown in Table 19. In some embodiments, an anellovector comprises a genetic element comprising a consensus *Anellovirus* ORF2/3 motif, e.g., as shown in Table 19. In some embodiments, an anellovector comprises a genetic element comprising a consensus *Anellovirus* ORF2t/3 motif, e.g., as shown in Table 19. In some embodiments, X, as shown in Table 19, indicates any amino acid. In some embodiments, Z, as shown in Table 19, indicates glutamic acid or glutamine. In some embodiments, B, as shown in Table 19, indicates aspartic acid or asparagine. In some embodiments, J, as shown in Table 19, indicates leucine or isoleucine.

Table 19. Consensus motifs in open reading frames (ORFs) of *Anelloviruses*

Consensus Threshold	Open Reading Frame	Position	Motif	SEQ ID NO:
50	ORF1	79	LIJRQWQPXXIRRCXIXGYXPLIXC	68
50	ORF1	111	NYXXHXD	69
50	ORF1	135	FSLXXLYDZ	70
50	ORF1	149	NXWTXSNXDLDLCRYXGC	71
50	ORF1	194	TXPSXHPGXMXLXKHK	72
50	ORF1	212	IPSLXTRPXG	73
50	ORF1	228	RIXPPXLFXDKWYFQXDL	74
50	ORF1	250	LLXIXATA	75
50	ORF1	260	LXXPFXSPXTD	76
50	ORF1	448	YNPXXDKGXGNXIW	77
50	ORF1	519	CPYTZPXL	78
50	ORF1	542	XFGXGXMP	79
50	ORF1	569	HQXEVXEX	80
50	ORF1	600	KYXFXFXWGGNP	81
50	ORF1	653	HSWDXRRG	82
50	ORF1	666	AIKRXQQ	83
50	ORF1	750	XQZQXXLR	84
50	ORF1/1	73	PRXJQXXDP	85
50	ORF1/1	91	HSWDXRRG	86
50	ORF1/1	105	AIKRXQQ	87
50	ORF1/1	187	QZQXXLR	88
50	ORF1/2	97	KXKRRRR	89
50	ORF2/2	158	PIXSLXXYKXXTR	90
50	ORF2/2	189	LAXQLLKECXKN	91
50	ORF2/3	39	HLNXLA	92
50	ORF2/3	272	DRPPR	93

50	ORF2/3	281	DXPFYPWXP	94
50	ORF2/3	300	VXFKLXF	95
50	ORF2t/3	4	WXPPVHBVXGIERXW	96
50	ORF2t/3	37	AKRKLX	97
50	ORF2t/3	140	PSSXDWXXEY	98
50	ORF2t/3	156	DRPPR	99
50	ORF2t/3	167	PFYPW	100
50	ORF2t/3	183	NVXFKLXF	101
50	ORF1	84	JXXXXWQPXXXXXCXIXGXXXJWQP	102
50	ORF1	149	NXWXXXNXXXXLXRY	103
50	ORF1	448	YNPXXDXG	104

ORF1 molecules

In some embodiments, the anellovector or anelloVLP comprises an ORF1 molecule and/or a nucleic acid encoding an ORF1 molecule. Generally, an ORF1 molecule comprises a polypeptide having the structural features and/or activity of an Anellovirus ORF1 protein (e.g., an Anellovirus ORF1 protein as described herein, e.g., as listed in any one of Tables A1-A26), or a functional fragment thereof. In some embodiments, the ORF1 molecule comprises a truncation relative to an Anellovirus ORF1 protein (e.g., an Anellovirus ORF1 protein as described herein, e.g., as listed in any one of Tables A1-A26). In some embodiments, the ORF1 molecule is truncated by at least 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 150, 200, 250, 300, 350, 400, 450, 500, 550, 600, 650, or 700 amino acids of the Anellovirus ORF1 protein. In some embodiments, an ORF1 molecule comprises an amino acid sequence having at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to an Anellovirus ORF1 protein sequence as shown in any one of Tables A1-A26. In some embodiments, an ORF1 molecule comprises an amino acid sequence having at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity to an Betatorquevirus ORF1 protein, e.g., as described herein. An ORF1 molecule can generally bind to a nucleic acid molecule, such as DNA (e.g., a genetic element, e.g., as described herein). In some embodiments, an ORF1 molecule localizes to the nucleus of a cell. In certain embodiments, an ORF1 molecule localizes to the nucleolus of a cell.

Without wishing to be bound by theory, an ORF1 molecule may be capable of binding to other ORF1 molecules, e.g., to form a proteinaceous exterior (e.g., as described herein). Such an ORF1 molecule may be described as having the capacity to form a capsid. In some embodiments, the

proteinaceous exterior may encapsidate a nucleic acid molecule (e.g., a genetic element as described herein). In some embodiments, a plurality of ORF1 molecules may form a multimer, e.g., to produce a proteinaceous exterior. In some embodiments, the multimer may be a homomultimer. In other embodiments, the multimer may be a heteromultimer (e.g., comprising a plurality of distinct ORF1 molecules). It is also contemplated that an ORF1 molecule may have replicase activity.

Anellovirus ORF1 molecule domain structures

Previously, Anellovirus ORF1 molecules have been described as having the following domains, from N-terminus to C-terminus: arginine rich region, jelly-roll region, hypervariable region (HVR), N22 domain, C-terminal domain (CTD). This disclosure, for instance in Examples 32-40 herein, describes the structural analysis that leads to a refined domain structure for Anellovirus ORF1. In particular, domains P1 and P2 have been identified, overlapping with the regions previously referred to as the HVR and N22 domain. In addition, this work has refined the boundaries of the previous domain structures. These refined domain structures are referred to herein as the structural arginine-rich region, the structural jelly-roll region, the structural HVR, the structural N22 domain, and the structural CTD.

Structural ORF1 domain-based nomenclature for Anellovirus ORF1 molecules

In an aspect, the present disclosure provides an ORF1 molecule comprising one or more (e.g., 1, 2, 3, 4, or 5) of domains or domain fragments of an Anellovirus ORF1 protein, wherein the boundaries of the domains are defined based on the refined domain structures obtained via the structural analysis described herein, e.g., in Example 32-40. An ORF1 molecule may include, for example, one or more (e.g., 1, 2, 3, 4, or 5) of (e.g., in an N-terminal to C-terminal direction): a structural arginine-rich region, a structural jelly-roll region, a structural hypervariable region (HVR), a structural N22 domain, and a structural C-terminal domain (e.g., as described herein). In some embodiments, the ORF1 molecule comprises a structural arginine-rich region (e.g., as described herein), or an amino acid sequence having at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity thereto. In some embodiments, the ORF1 molecule comprises a structural jelly-roll region (e.g., as described herein), or an amino acid sequence having at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity thereto. In some embodiments, the ORF1 molecule comprises a structural hypervariable region (e.g., as described herein), or an amino acid sequence having at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity thereto. In some embodiments, the ORF1 molecule comprises a structural N22 domain (e.g., as described herein), or an amino acid sequence having at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity thereto. In some embodiments, the ORF1

molecule comprises a structural C-terminal domain (e.g., as described herein), or an amino acid sequence having at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity thereto.

P1/P2 domain-based nomenclature for Anellovirus ORF1 moelcules

In an aspect, the present disclosure provides an ORF1 molecule comprising one or more (e.g., 1, 2, 3, 4, 5, 6, or 7) domains or domain fragments of an Anellovirus ORF1 protein, wherein the boundaries of the domains are defined based on the structural analysis identifying the P1 and P2 domains of Anellovirus ORF1 proteins (e.g., as described herein, e.g., in Examples 32-40). An ORF1 molecule may include, for example, one or more (e.g., 1, 2, 3, 4, 5, 6, or 7) of (e.g., in an N-terminal to C-terminal direction): a structural arginine-rich region, a jelly-roll B-H strands subdomain, a first P1 domain fragment (e.g., a P1-1 subdomain), a P2 domain, a second P1 domain fragment (e.g., a P1-2 subdomain), a jelly-roll I-strand subdomain, and a structural C-terminal domain (e.g., as described herein). In some embodiments, the ORF1 molecule comprises a structural arginine-rich region (e.g., as described herein), or an amino acid sequence having at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity thereto. In some embodiments, the ORF1 molecule comprises a jelly-roll B-H strands subdomain (e.g., as described herein), or an amino acid sequence having at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity thereto. In some embodiments, the ORF1 molecule comprises a P1-1 region (e.g., as described herein), or an amino acid sequence having at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity thereto. In some embodiments, the ORF1 molecule comprises a P2 domain (e.g., as described herein), or an amino acid sequence having at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity thereto. In some embodiments, the ORF1 molecule comprises a P1-2 region (e.g., as described herein), or an amino acid sequence having at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity thereto. In some embodiments, the ORF1 molecule comprises a jelly-roll I strand subdomain (e.g., as described herein), or an amino acid sequence having at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity thereto. In some embodiments, the ORF1 molecule comprises a structural C-terminal domain (e.g., as described herein), or an amino acid sequence having at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity thereto.

Structural arginine-rich region

An ORF1 molecule may, in some embodiments, also include a region comprising the structure or activity of an Anellovirus structural arginine-rich region (e.g., as described herein, e.g., a structural arginine-rich region from an Anellovirus ORF1 protein as described herein). In some embodiments, the

region comprises the amino acid sequence of a structural arginine-rich region as described herein, or a sequence having at least 70%, 75% 80%, 85%, 90% 95%, 96%, 97%, 98% and 99% amino acid sequence identity thereto.

A structural arginine rich region generally has at least 70% (e.g., at least about 70, 80, 90, 95, 96, 97, 98, 99, or 100%) sequence identity to a structural arginine-rich region sequence described herein or a sequence of at least about 40 amino acids comprising at least 60%, 70%, or 80% basic residues (e.g., arginine, lysine, or a combination thereof). In some embodiments, an ORF1 molecule as described herein does not comprise a full-length structural arginine-rich region (e.g., as described herein). In some embodiments, an ORF1 molecule as described herein comprises a portion of a structural arginine-rich region, e.g., a C-terminal portion of a structural arginine-rich region (e.g., as described herein). In some embodiments, an ORF1 molecule as described herein does not any substantial portion (e.g., a portion consisting of at least 5, 10, 15, 20, 25, 30, 35, 40, 45, or 50 contiguous amino acids) of a structural arginine-rich region (e.g., as described herein). In some embodiments, the structural arginine-rich region of an ORF1 molecule is replaced by a heterologous sequence, e.g., a sequence from another virus (e.g., a different Anellovirus or a virus other than an Anellovirus, e.g., as described herein), e.g., as described herein.

In some embodiments, the first region of an ORF1 molecule as described herein comprises a structural arginine-rich region. In other embodiments, an ORF1 molecule as described herein does not comprise a structural arginine-rich region or only comprises a portion of a structural arginine-rich region.

Structural jelly roll region

An ORF1 molecule may, in some embodiments, also include a region comprising the structure or activity of an Anellovirus structural jelly-roll domain or region (e.g., as described herein, e.g., a structural jelly-roll domain or region from an Anellovirus ORF1 protein as described herein). In some embodiments, the region comprises the amino acid sequence of a structural jelly-roll region as described herein, or a sequence having at least 70%, 75% 80%, 85%, 90% 95%, 96%, 97%, 98% and 99% amino acid sequence identity thereto.

In some embodiments, a structural jelly-roll domain or region comprises (e.g., consists of) a polypeptide (e.g., a domain or region comprised in a larger polypeptide) comprising one or more (e.g., 1, 2, or 3) of the following characteristics:

(i) at least 30% (e.g., at least 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 90%, or more) of the amino acids of the structural jelly-roll domain are part of one or more β-sheets;

(ii) the secondary structure of the structural jelly-roll domain comprises at least four (e.g., at least 4, 5, 6, 7, 8, 9, 10, 11, or 12) β-strands; and/or

(iii) the tertiary structure of the structural jelly-roll domain comprises at least two (e.g., at least 2, 3, or 4) β-sheets; and/or

(iv) the structural jelly-roll domain comprises a ratio of β-sheets to α-helices of at least 2:1, 3:1, 4:1, 5:1, 6:1, 7:1, 8:1, 9:1, or 10:1.

In certain embodiments, a structural jelly-roll domain comprises two β-sheets.

In certain embodiments, one or more (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10) of the β-sheets comprises about eight (e.g., 4, 5, 6, 7, 8, 9, 10, 11, or 12) β-strands. In certain embodiments, one or more (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10) of the β-sheets comprises eight β-strands. In certain embodiments, one or more (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10) of the β-sheets comprises seven β-strands. In certain embodiments, one or more (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10) of the β-sheets comprises six β-strands. In certain embodiments, one or more (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10) of the β-sheets comprises five β-strands. In certain embodiments, one or more (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10) of the β-sheets comprises four β-strands.

In some embodiments, the structural jelly-roll domain comprises a first β-sheet in antiparallel orientation to a second β-sheet. In certain embodiments, the first β-sheet comprises about four (e.g., 3, 4, 5, or 6) β-strands. In certain embodiments, the second β-sheet comprises about four (e.g., 3, 4, 5, or 6) β-strands. In embodiments, the first and second β-sheet comprise, in total, about eight (e.g., 6, 7, 8, 9, 10, 11, or 12) β-strands.

In certain embodiments, a structural jelly-roll domain is a component of a capsid protein (e.g., an ORF1 molecule as described herein). In certain embodiments, a structural jelly-roll domain has self-assembly activity. In some embodiments, a polypeptide comprising a structural jelly-roll domain binds to another copy of the polypeptide comprising the structural jelly-roll domain. In some embodiments, a structural jelly-roll domain of a first polypeptide binds to a structural jelly-roll domain of a second copy of the polypeptide.

In some embodiments, an ORF1 molecule as described herein does not comprise a full-length structural jelly-roll region (e.g., as described herein). In some embodiments, an ORF1 molecule as described herein comprises a portion of a structural jelly-roll region, e.g., an N-terminal portion of a structural jelly-roll region (e.g., as described herein). In some embodiments, an ORF1 molecule as described herein does not comprise any substantial portion (e.g., a portion consisting of at least 5, 10, 15, 20, 25, 30, 35, 40, 45, or 50 contiguous amino acids) of a structural jelly-roll region (e.g., as described herein). In some embodiments, the structural jelly-roll region of an ORF1 molecule is replaced by a heterologous sequence, e.g., a sequence from another virus (e.g., a different Anellovirus or a virus other than an Anellovirus, e.g., as described herein), e.g., as described herein.

In some embodiments, the second region of an ORF1 molecule as described herein comprises a structural jelly-roll region.

Jelly-roll B-H strands subdomain

Structural analysis described herein revealed that the beta strands of the jelly-roll region are in noncontiguous portions of the ORF1 molecule. In particular, a first jelly-roll subdomain comprises beta strands B-H and a second jelly-roll subdomain comprises beta strand I. An ORF1 molecule may thus, in some embodiments, also include a region comprising the structure or activity of the region of an Anellovirus ORF1 protein comprising beta strands B-H of the jelly-roll domain or region (e.g., as described herein). In some embodiments, the region comprises the amino acid sequence of a first portion of a jelly-roll region (e.g., a jelly-roll (B-H) sequence as described herein), or a sequence having at least 70%, 75% 80%, 85%, 90% 95%, 96%, 97%, 98% and 99% amino acid sequence identity thereto.

In some embodiments, the first portion of the jelly-roll domain or region may comprise one or more (e.g., 1, 2, or 3) of the following characteristics:

(i) at least 30% (e.g., at least 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 90%, or more) of the amino acids of the jelly-roll domain are part of one or more β-sheets;

(ii) the secondary structure of the jelly-roll domain comprises at least four (e.g., at least 4, 5, 6, 7, 8, 9, 10, 11, or 12) β-strands; and/or

(iii) the tertiary structure of the jelly-roll domain comprises at least two (e.g., at least 2, 3, or 4) β-sheets; and/or

(iv) the jelly-roll domain comprises a ratio of β-sheets to α-helices of at least 2:1, 3:1, 4:1, 5:1, 6:1, 7:1, 8:1, 9:1, or 10:1.

In certain embodiments, a jelly-roll domain comprises two β-sheets.

In certain embodiments, one or more (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10) of the β-sheets comprises about eight (e.g., 4, 5, 6, 7, 8, 9, 10, 11, or 12) β-strands. In certain embodiments, one or more (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10) of the β-sheets comprises eight β-strands. In certain embodiments, one or more (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10) of the β-sheets comprises seven β-strands. In certain embodiments, one or more (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10) of the β-sheets comprises six β-strands. In certain embodiments, one or more (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10) of the β-sheets comprises five β-strands. In certain embodiments, one or more (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10) of the β-sheets comprises four β-strands.

In some embodiments, the first portion of the jelly-roll domain comprises a first β-sheet in antiparallel orientation to a second β-sheet. In certain embodiments, the first β-sheet comprises about four (e.g., 3, 4, 5, or 6) β-strands. In certain embodiments, the second β-sheet comprises about four (e.g.,

3, 4, 5, or 6) β-strands. In embodiments, the first and second β-sheet comprise, in total, about eight (e.g., 6, 7, 8, 9, 10, 11, or 12) β-strands.

In certain embodiments, the jelly-roll B-H strands subdomain is a component of a capsid protein (e.g., an ORF1 molecule as described herein). In certain embodiments, a jelly-roll B-H strands subdomain has self-assembly activity. In some embodiments, a polypeptide comprising a jelly-roll B-H strands subdomain binds to another copy of the polypeptide comprising the jelly-roll B-H strands subdomain. In some embodiments, the jelly-roll B-H strands subdomain of a first polypeptide binds to a jelly-roll B-H strands subdomain of a second copy of the polypeptide.

In some embodiments, an ORF1 molecule as described herein does not comprise a full-length jelly-roll B-H strands subdomain (e.g., as described herein). In some embodiments, an ORF1 molecule as described herein comprises a portion of a jelly-roll B-H strands subdomain, e.g., an N-terminal portion of a jelly-roll B-H strands subdomain (e.g., as described herein). In some embodiments, an ORF1 molecule as described herein does not comprise any substantial portion (e.g., a portion consisting of at least 5, 10, 15, 20, 25, 30, 35, 40, 45, or 50 contiguous amino acids) of a jelly-roll B-H strands subdomain (e.g., as described herein). In some embodiments, the jelly-roll B-H strands subdomain of an ORF1 molecule is replaced by a heterologous sequence, e.g., a sequence from another virus (e.g., a different Anellovirus or a virus other than an Anellovirus, e.g., as described herein), e.g., as described herein.

In some embodiments, the second region of an ORF1 molecule as described herein comprises a jelly-roll B-H strands subdomain.

Structural N22 domain *(Structural N22 domain-based nomenclature)*

An ORF1 molecule may, in some embodiments, also include a region comprising the structure or activity of an Anellovirus structural N22 domain (e.g., as described herein, e.g., an structural N22 domain from an Anellovirus ORF1 protein as described herein). In some embodiments, the region comprises the amino acid sequence of a structural N22 domain as described herein, or a sequence having at least 70%, 75% 80%, 85%, 90% 95%, 96%, 97%, 98% and 99% amino acid sequence identity thereto.

In some embodiments, the third region of an ORF1 molecule as described herein comprises a structural N22 domain.

Structural hypervariable region (HVR)

An ORF1 molecule may, in some embodiments, further comprise a hypervariable region (HVR), e.g., an HVR from an Anellovirus ORF1 protein, e.g., as described herein. In some embodiments, the region comprises the amino acid sequence of a structural HVR as described herein, or a sequence having at least 70%, 75% 80%, 85%, 90% 95%, 96%, 97%, 98% and 99% amino acid sequence identity thereto.

In some embodiments, the HVR is positioned between the second region and the third region. In some embodiments, the HVR comprises comprises at least about 55 (e.g., at least about 45, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, or 65) amino acids (e.g., about 45-160, 50-160, 55-160, 60-160, 45-150, 50-150, 55-150, 60-150, 45-140, 50-140, 55-140, or 60-140 amino acids).

P1-1 subdomain

In some embodiments, an ORF1 molecule comprises a P1 domain. A P1 domain is generally noncontiguous and comprises a P1-1 subdomain and a P1-2 subdomain, which may be separated by a P2 domain. An ORF1 molecule may, in some embodiments, include a region comprising the structure or activity of a first portion of an Anellovirus ORF1 P1 domain, e.g., an Anellovirus ORF1 P1-1 domain (e.g., as described herein, e.g., a P1-1 domain from an Anellovirus ORF1 protein as described herein). In some embodiments, the region comprises the amino acid sequence of a P1-1 domain as described herein, or a sequence having at least 70%, 75% 80%, 85%, 90% 95%, 96%, 97%, 98% and 99% amino acid sequence identity thereto.

In some embodiments, the third region of an ORF1 molecule as described herein comprises a P1-1 domain.

P2 domain

An ORF1 molecule may, in some embodiments, also include a region comprising the structure or activity of an Anellovirus ORF1 P2 domain (e.g., as described herein, e.g., a P2 domain from an Anellovirus ORF1 protein as described herein). In some embodiments, the region comprises the amino acid sequence of a P2 domain as described herein, or a sequence having at least 70%, 75% 80%, 85%, 90% 95%, 96%, 97%, 98% and 99% amino acid sequence identity thereto.

In some embodiments, the fourth region of an ORF1 molecule as described herein comprises a P2 domain.

P1-2 subdomain

An ORF1 molecule may, in some embodiments, also include a region comprising the structure or activity of a second portion of an Anellovirus ORF1 P1 domain, e.g., an Anellovirus ORF1 P1-2 domain (e.g., as described herein, e.g., a P1-2 domain from an Anellovirus ORF1 protein as described herein). In some embodiments, the region comprises the amino acid sequence of a P1-2 domain as described herein, or a sequence having at least 70%, 75% 80%, 85%, 90% 95%, 96%, 97%, 98% and 99% amino acid sequence identity thereto.

In some embodiments, the fifth region of an ORF1 molecule as described herein comprises a P1-2 domain.

Jelly-roll I-strand subdomain

An ORF1 molecule may, in some embodiments, also include a region comprising the structure or activity of a second portion of an Anellovirus ORF1 jelly-roll domain, e.g., an Anellovirus ORF1 jelly-roll strand I subdomain (e.g., as described herein, e.g., a jelly-roll strand I subdomain from an Anellovirus ORF1 protein as described herein). In some embodiments, the region comprises the amino acid sequence of a jelly-roll strand I subdomain as described herein, or a sequence having at least 70%, 75% 80%, 85%, 90% 95%, 96%, 97%, 98% and 99% amino acid sequence identity thereto.

In some embodiments, the sixth region of an ORF1 molecule as described herein comprises a jelly-roll strand I subdomain.

Structural C-terminal domain

An ORF1 molecule may also include a region comprising the structure or activity of an Anellovirus structural C-terminal domain (CTD) (e.g., as described herein, e.g., a structural CTD from an Anellovirus ORF1 protein as described herein). In some embodiments, the region comprises the amino acid sequence of a structural C-terminal domain as described herein, or a sequence having at least 70%, 75% 80%, 85%, 90% 95%, 96%, 97%, 98% and 99% amino acid sequence identity thereto. In some embodiments, an ORF1 molecule as described herein does not comprise a full-length structural C-terminal domain (e.g., as described herein). In some embodiments, an ORF1 molecule as described herein comprises a portion of a structural C-terminal domain, e.g., an N-terminal portion of a structural C-terminal domain (e.g., as described herein). In some embodiments, an ORF1 molecule as described herein does not comprise any substantial portion (e.g., a portion consisting of at least 5, 10, 15, 20, 25, 30, 35, 40, 45, or 50 contiguous amino acids) of a structural C-terminal domain (e.g., as described herein). In some embodiments, the structural C-terminal domain of an ORF1 molecule is replaced by a heterologous sequence, e.g., a sequence from another virus (e.g., a different Anellovirus or a virus other than an Anellovirus, e.g., as described herein), e.g., as described herein.

In some embodiments, the fourth region of an ORF1 molecule as described herein comprises a structural C-terminal domain. In other embodiments, the seventh region of an ORF1 molecule as described herein comprises a structural C-terminal domain.

ORF1 molecule structures

In some embodiments, the ORF1 molecule comprises, in N-terminal to C-terminal order, a first region (e.g., comprising a structural arginine-rich region), second region (e.g., comprising a structural jelly-roll region), third region (e.g., comprising a structural N22 domain), and fourth region (e.g., comprising a structural C-terminal domain). In certain embodiments, the ORF1 molecule comprises a structural HVR (e.g., between the third and fourth regions).

In some embodiments, the first region can bind to a nucleic acid molecule (e.g., DNA). In some embodiments, the basic residues are selected from arginine, histidine, or lysine, or a combination thereof. In some embodiments, the first region comprises at least 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 100% arginine residues (e.g., between 60%-90%, 60%-80%, 70%-90%, or 70-80% arginine residues). In some embodiments, the first region comprises about 30-120 amino acids (e.g., about 40-120, 40-100, 40-90, 40-80, 40-70, 50-100, 50-90, 50-80, 50-70, 60-100, 60-90, or 60-80 amino acids). In some embodiments, the first region comprises the structure or activity of a viral ORF1 structural arginine-rich region (e.g., a structural arginine-rich region from an Anellovirus ORF1 protein, e.g., as described herein). In some embodiments, the first region comprises a nuclear localization sigal.

In some embodiments, the second region comprises a structural jelly-roll domain, e.g., the structure or activity of a viral ORF1 structural jelly-roll domain (e.g., a structural jelly-roll domain from an Anellovirus ORF1 protein, e.g., as described herein). In some embodiments, the second region is capable of binding to the second region of another ORF1 molecule, e.g., to form a proteinaceous exterior (e.g., capsid) or a portion thereof.

In some embodiments, at least a portion of the fourth region is exposed on the surface of a proteinaceous exterior (e.g., a proteinaceous exterior comprising a multimer of ORF1 molecules, e.g., as described herein).

In some embodiments, the first region, second region, third region, fourth region, and/or HVR each comprise fewer than four (e.g., 0, 1, 2, or 3) beta sheets.

In some embodiments, the ORF1 molecule comprises, in N-terminal to C-terminal order, a first region (e.g., comprising a structural arginine-rich region), second region (e.g., comprising a jelly-roll B-H strands subdomain), third region (e.g., comprising a first portion of a P1 domain), fourth region (e.g., comprising a P2 domain), fifth region (e.g., comprising a second portion of a P1 domain), sixth region (e.g., comprising a jelly-roll strand I subdomain), and seventh region (e.g., comprising a structural C-terminal domain).

In some embodiments, the first region can bind to a nucleic acid molecule (e.g., DNA). In some embodiments, the basic residues are selected from arginine, histidine, or lysine, or a combination thereof. In some embodiments, the first region comprises at least 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 100% arginine residues (e.g., between 60%-90%, 60%-80%, 70%-90%, or 70-80% arginine residues). In

some embodiments, the first region comprises about 30-120 amino acids (e.g., about 40-120, 40-100, 40-90, 40-80, 40-70, 50-100, 50-90, 50-80, 50-70, 60-100, 60-90, or 60-80 amino acids). In some embodiments, the first region comprises the structure or activity of a viral ORF1 structural arginine-rich region (e.g., a structural arginine-rich region from an Anellovirus ORF1 protein, e.g., as described herein). In some embodiments, the first region comprises a nuclear localization sigal.

In some embodiments, at least a portion of the seventh region is exposed on the surface of a proteinaceous exterior (e.g., a proteinaceous exterior comprising a multimer of ORF1 molecules, e.g., as described herein).

In some embodiments, one or more of the domains or regions of an ORF1 molecule may be replaced by a heterologous amino acid sequence (e.g., the corresponding region from a heterologous ORF1 molecule). In some embodiments, the heterologous amino acid sequence has a desired functionality, e.g., as described herein.

In some embodiments, the ORF1 molecule comprises a plurality of conserved motifs (e.g., motifs comprising about 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30, 35, 40, 45, 50, 60, 70, 80, 90, 100, or more amino acids) (e.g., as shown in Figure 34 of PCT Publication No. WO2020/123816). In some embodiments, the conserved motifs may show 60, 70, 80, 85, 90, 95, or 100% sequence identity to an ORF1 protein of one or more wild-type Anellovirus clades (e.g., *Betatorquevirus*). In some embodiments, the conserved motifs each have a length between 1-1000 (e.g., between 5-10, 5-15, 5-20, 10-15, 10-20, 15-20, 5-50, 5-100, 10-50, 10-100, 10-1000, 50-100, 50-1000, or 100-1000) amino acids. In certain embodiments, the conserved motifs consist of about 2-4% (e.g., about 1-8%, 1-6%, 1-5%, 1-4%, 2-8%, 2-6%, 2-5%, or 2-4%) of the sequence of the ORF1 molecule, and each show 100% sequence identity to the corresponding motifs in an ORF1 protein of the wild-type Anellovirus clade. In certain embodiments, the conserved motifs consist of about 5-10% (e.g., about 1-20%, 1-10%, 5-20%, or 5-10%) of the sequence of the ORF1 molecule, and each show 80% sequence identity to the corresponding motifs in an ORF1 protein of the wild-type Anellovirus clade. In certain embodiments, the conserved motifs consist of about 10-50% (e.g., about 10-20%, 10-30%, 10-40%, 10-50%, 20-40%, 20-50%, or 30-50%) of the sequence of the ORF1 molecule, and each show 60% sequence identity to the corresponding motifs in an ORF1 protein of the wild-type Anellovirus clade. In some embodiments, the conserved motifs comprise one or more amino acid sequences as listed in Table 19.

In some embodiments, an ORF1 molecule comprises at least one difference (e.g., a mutation, chemical modification, or epigenetic alteration) relative to a wild-type ORF1 protein, e.g., as described herein.

Conserved ORF1 Motif in Structural N22 Domain

In some embodiments, a polypeptide (e.g., an ORF1 molecule) described herein comprises the amino acid sequence YNPX^{2}DXGX^{2}N (SEQ ID NO: 829), wherein X^n is a contiguous sequence of any *n* amino acids. For example, X^2 indicates a contiguous sequence of any two amino acids. In some embodiments, the YNPX^{2}DXGX^{2}N (SEQ ID NO: 829) is comprised within the structural N22 domain of an ORF1 molecule, e.g., as described herein. In some embodiments, a genetic element described herein comprises a nucleic acid sequence (e.g., a nucleic acid sequence encoding an ORF1 molecule, e.g., as described herein) encoding the amino acid sequence YNPX^{2}DXGX^{2}N (SEQ ID NO: 829), wherein X^n is a contiguous sequence of any *n* amino acids.

In some embodiments, a polypeptide (e.g., an ORF1 molecule) comprises a conserved secondary structure, e.g., flanking and/or comprising a portion of the YNPX^{2}DXGX^{2}N (SEQ ID NO: 829) motif, e.g., in an structural N22 domain. In some embodiments, the conserved secondary structure comprises a first beta strand and/or a second beta strand. In some embodiments, the first beta strand is about 5-6 (e.g., 3, 4, 5, 6, 7, or 8) amino acids in length. In some embodiments, the first beta strand comprises the tyrosine (Y) residue at the N-terminal end of the YNPX^{2}DXGX^{2}N (SEQ ID NO: 829) motif. In some embodiments, the YNPX^{2}DXGX^{2}N (SEQ ID NO: 829) motif comprises a random coil (e.g., about 8-9 amino acids of random coil). In some embodiments, the second beta strand is about 7-8 (e.g., 5, 6, 7, 8, 9, or 10) amino acids in length. In some embodiments, the second beta strand comprises the asparagine (N) residue at the C-terminal end of the YNPX^{2}DXGX^{2}N (SEQ ID NO: 829) motif.

Exemplary YNPX^{2}DXGX^{2}N (SEQ ID NO: 829) motif-flanking secondary structures are described in Example 47 and Figure 48 of PCT Publication No. WO 2020/123816, incorporated by reference herein in its entirety. In some embodiments, an ORF1 molecule comprises a region comprising one or more (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or all) of the secondary structural elements (e.g., beta strands) shown in Figure 48 of PCT Publication No. WO 2020/123816. In some embodiments, an ORF1 molecule comprises a region comprising one or more (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or all) of the secondary structural elements (e.g., beta strands) shown in Figure 48 of PCT Publication No. WO 2020/123816, incorporated by reference herein in its entirety, flanking a YNPX^{2}DXGX^{2}N (SEQ ID NO: 829) motif (e.g., as described herein).

Conserved Secondary Structural Motif in ORF1 Structural Jelly-Roll Domain

In some embodiments, a polypeptide (e.g., an ORF1 molecule) described herein comprises one or more secondary structural elements comprised by an Anellovirus ORF1 protein (e.g., as described herein). In some emboiments, an ORF1 molecule comprises one or more secondary structural elements comprised by the structural jelly-roll domain of an Anellovius ORF1 protein (e.g., as described herein). Generally, an ORF1 structural jelly-roll domain comprises a secondary structure comprising, in order in

the N-terminal to C-terminal direction, a first beta strand, a second beta strand, a first alpha helix, a third beta strand, a fourth beta strand, a fifth beta strand, a second alpha helix, a sixth beta strand, a seventh beta strand, an eighth beta strand, and a ninth beta strand. In some embodiments, an ORF1 molecule comprises a secondary structure comprising, in order in the N-terminal to C-terminal direction, a first beta strand, a second beta strand, a first alpha helix, a third beta strand, a fourth beta strand, a fifth beta strand, a second alpha helix, a sixth beta strand, a seventh beta strand, an eighth beta strand, and/or a ninth beta strand.

In some embodiments, a pair of the conserved secondary structural elements (i.e., the beta strands and/or alpha helices) are separated by an interstitial amino acid sequence, e.g., comprising a random coil sequence, a beta strand, or an alpha helix, or a combination thereof. Interstitial amino acid sequences between the conserved secondary structural elements may comprise, for example, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, or more amino acids. In some embodiments, an ORF1 molecule may further comprise one or more additional beta strands and/or alpha helices (e.g., in the structural jelly-roll domain). In some embodiments, consecutive beta strands or consecutive alpha helices may be combined. In some embodiments, the first beta strand and the second beta strand are comprised in a larger beta strand. In some embodiments, the third beta strand and the fourth beta strand are comprised in a larger beta strand. In some embodiments, the fourth beta strand and the fifth beta strand are comprised in a larger beta strand. In some embodiments, the sixth beta strand and the seventh beta strand are comprised in a larger beta strand. In some embodiments, the seventh beta strand and the eighth beta strand are comprised in a larger beta strand. In some embodiments, the eighth beta strand and the ninth beta strand are comprised in a larger beta strand.

In some embodiments, the first beta strand is about 5-7 (e.g., 3, 4, 5, 6, 7, 8, 9, or 10) amino acids in length. In some embodiments, the second beta strand is about 15-16 (e.g., 13, 14, 15, 16, 17, 18, or 19) amino acids in length. In some embodiments, the first alpha helix is about 15-17 (e.g., 13, 14, 15, 16, 17, 18, 19, or 20) amino acids in length. In some embodiments, the third beta strand is about 3-4 (e.g., 1, 2, 3, 4, 5, or 6) amino acids in length. In some embodiments, the fourth beta strand is about 10-11 (e.g., 8, 9, 10, 11, 12, or 13) amino acids in length. In some embodiments, the fifth beta strand is about 6-7 (e.g., 4, 5, 6, 7, 8, 9, or 10) amino acids in length. In some embodiments, the second alpha helix is about 8-14 (e.g., 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, or 17) amino acids in length. In some embodiments, the second alpha helix may be broken up into two smaller alpha helices (e.g., separated by a random coil sequence). In some embodiments, each of the two smaller alpha helices are about 4-6 (e.g., 2, 3, 4, 5, 6, 7, or 8) amino acids in length. In some embodiments, the sixth beta strand is about 4-5 (e.g., 2, 3, 4, 5, 6, or 7) amino acids in length. In some embodiments, the seventh beta strand is about 5-6 (e.g., 3, 4, 5, 6, 7, 8, or 9) amino acids in length. In some embodiments, the eighth beta strand is about 7-9 (e.g., 5, 6, 7, 8,

9, 10, 11, 12, or 13) amino acids in length. In some embodiments, the ninth beta strand is about 5-7 (e.g., 3, 4, 5, 6, 7, 8, 9, or 10) amino acids in length.

Exemplary structural jelly-roll domain secondary structures are described in Example 47 and Figure 47 of PCT Publication No. WO 2020/123816, incorporated by reference herein in its entirety. In some embodiments, an ORF1 molecule comprises a region comprising one or more (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or all) of the secondary structural elements (e.g., beta strands and/or alpha helices) of any of the structural jelly-roll domain secondary structures shown in Figure 47 of PCT Publication No. WO 2020/123816, incorporated by reference herein in its entirety.

Exemplary ORF1 Sequences

In some embodiments, a polypeptide (e.g., an ORF1 molecule) described herein comprises an amino acid sequence having at least about 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to one or more *Anellovirus* ORF1 subsequences, e.g., as described herein). In some embodiments, an anellovector or anelloVLP described herein comprises an ORF1 molecule comprising an amino acid sequence having at least about 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to one or more *Anellovirus* ORF1 subsequences, e.g., as described herein. In some embodiments, an anellovector or anelloVLP described herein comprises a nucleic acid molecule (e.g., a genetic element) encoding an ORF1 molecule comprising an amino acid sequence having at least about 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to one or more *Anellovirus* ORF1 subsequences, e.g., as described herein.

In some embodiments, the one or more *Anellovirus* ORF1 subsequences comprises one or more of a structural arginine (Arg)-rich domain, a structural jelly-roll domain, a hypervariable region (HVR), an structural N22 domain, or a structural C-terminal domain (CTD) (e.g., as listed herein), or sequences having at least about 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity thereto. In some embodiments, the ORF1 molecule comprises a plurality of subsequences from different *Anelloviruses*. In some embodiments, the ORF1 molecule comprises one or more of an Arg-rich domain, a structural jelly-roll domain, an structural N22 domain, and a CTD from one *Anellovirus*, and an HVR from another. In some embodiments, the ORF1 molecule comprises one or more of a structural jelly-roll domain, an HVR, an structural N22 domain, and a CTD from one *Anellovirus*, and an Arg-rich domain from another. In some embodiments, the ORF1 molecule comprises one or more of an Arg-rich domain, an HVR, an structural N22 domain, and a CTD from one *Anellovirus*, and a structural jelly-roll domain from another. In some embodiments, the ORF1 molecule comprises one or more of an Arg-rich domain, a structural jelly-roll domain, an HVR, and a CTD from one *Anellovirus*, and an structural N22 domain from another. In some embodiments, the ORF1 molecule comprises one or more of an Arg-rich domain,

a structural jelly-roll domain, an HVR, and an structural N22 domain from one *Anellovirus*, and a CTD from another.

In some embodiments, the one or more *Anellovirus* ORF1 subsequences comprises one or more of a structural arginine (Arg)-rich domain, a portion of a jelly-roll domain comprising beta strands B-H (e.g., a jelly-roll B-H strands subdomain as described herein), a first portion of a P1 domain (e.g., a P1-1 sequence), a P2 domain, a second portion of a P1 domain (e.g., a P1-2 sequence), a portion of a jelly-roll domain comprising beta strand I (e.g., a jelly-roll I strand subdomain as described herein), or a structural C-terminal domain (CTD) (e.g., as listed herein), or sequences having at least about 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity thereto. In some embodiments, the ORF1 molecule comprises a plurality of subsequences from different *Anelloviruses*. In some embodiments, the ORF1 molecule comprises one or more of a structural arginine-rich domain, a portion of a jelly-roll domain comprising beta strands B-H (e.g., a jelly-roll B-H strands subdomain as described herein), a first portion of a P1 domain (e.g., a P1-1 sequence), a P2 domain, a second portion of a P1 domain (e.g., a P1-2 sequence), a portion of a jelly-roll domain comprising beta strand I (e.g., a jelly-roll I strand subdomain as described herein), and a structural C-terminal domain (CTD) (e.g., as listed herein) from another (e.g., as described herein).

In some embodiments, a polypeptide (e.g., an ORF1 molecule) described herein comprises an amino acid sequence having at least about 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to one or more *Anellovirus* ORF1 domain subsequences, e.g., as described in any of Tables B1-1 to B1-12). In some embodiments, an anellosome described herein comprises an ORF1 molecule comprising an amino acid sequence having at least about 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to one or more *Anellovirus* ORF1 subsequences, e.g., as described in any of Tables B1-1 to B1-12. In some embodiments, an anellosome described herein comprises a nucleic acid molecule (e.g., a genetic element) encoding an ORF1 molecule comprising an amino acid sequence having at least about 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to one or more *Anellovirus* ORF1 subsequences, e.g., as described in any of Tables B1-1 to B1-12.

Table B1-1: Ring 2 ORF1 amino acid subsequences (Betatorquevirus) with structural HVR and structural N22 domain

Structural Arginine-Rich Region (1-38)	MPYYYRRRRYNYRRPRWYGRGWIRRPFRRRFRRKRRVR

Structural jelly-roll region (39-224)	PTYTTIPLKQWQPPYKRTCYIKGQDCLIYYSNLRLGMNSTM YEKSIVPVHWPGGGSFSVSMLTLDALYDIHKLCRNWWTST NQDLPLVRYKGCKITFYQSTFTDYIVRIHTELPANSNKLTYP NTHPLMMMMSKYKHIIPSRQTRRKKKPYTKIFVKPPPQFEN KWYFATDLYKIPLLQIHCTACN
Structural hypervariable region (225-374)	LQNPFVKPDKLSNNVTLWSLNTISIQNRNMSVDQGQSWPF KILGTQSFYFYFYTGANLPGDTTQIPVADLLPLTNPRINRPG QSLNEAKITDHITFTEYKNKFTNYWGNPFNKHIQEHLDMIL YSLKSPEAIKNEWTTENMKWNQLNNAG
Structural N22 domain (375-541)	TMALTPFNEPIFTQIQYNPDRDTGEDTQLYLLSNATGTGWD PPGIPELILEGFPLWLIYWGFADFQKNLKKVTNIDTNYMLV AKTKFTQKPGTFYLVILNDTFVEGNSPYEKQPLPEDNIKWY PQVQYQLEAQNKLLQTGPFTPNIQGQLSDNISMFYKFYFK WGGS
Structural C-terminal domain (542-666)	PPKAINVENPAHQIQYPIPRNEHETTSLQSPGEAPESILYSFD YRH GNYTTTALSRISQDWALKDTVSKITEPDRQQLLKQALECLQ ISEETQEKKEKEVQQLISNLRQQQQLYRERIISLLKDQ

Table B1-2: Ring 2 ORF1 amino acid subsequence (Betatorquevirus) with P1 and P2 domains

Structural Arg-Rich Region (1-38)	MPYYYRRRRYNYRRPRWYGRGWIRRPFRRRFRRKRRVR
Jelly-roll B-H strands subdomain (39-224)	PTYTTIPLKQWQPPYKRTCYIKGQDCLIYYSNLRLGMNSTM YEKSIVPVHWPGGGSFSVSMLTLDALYDIHKLCRNWWTST NQDLPLVRYKGCKITFYQSTFTDYIVRIHTELPANSNKLTYP NTHPLMMMMSKYKHIIPSRQTRRKKKPYTKIFVKPPPQFEN KWYFATDLYKIPLLQIHCTACN
P1-1 (225-244)	LQNPFVKPDKLSNNVTLWSL
P2 (245-385)	NTISIQNRNMSVDQGQSWPFKILGTQSFYFYFYTGANLPGD TTQIPVADLLPLTNPRINRPGQSLNEAKITDHITFTEYKNKFT

	NYWGNPFNKHIQEHLDMILYSLKSPEAIKNEWTTENMKW NQLNNAGTMALTPFNEPI
P1-2 (386-524)	FTQIQYNPDRDTGEDTQLYLLSNATGTGWDPPGIPELILEGF PLWLIYWGFADFQKNLKKVTNIDTNYMLVAKTKFTQKPG TFYLVILNDTFVEGNSPYEKQPLPEDNIKWYPQVQYQLEAQ NKLLQTGPFTPNIQGQL
Jelly-roll I strand subdomain (525-541)	SDNISMFYKFYFKWGGS
Structural C-terminal domain (542-666)	PPKAINVENPAHQIQYPIPRNEHETTSLQSPGEAPESILYSFD YRH GNYTTTALSRISQDWALKDTVSKITEPDRQQLLKQALECLQ ISEETQEKKEKEVQQLISNLRQQQQLYRERIISLLKDQ

Table B1-3: Ring 9 ORF1 amino acid subsequence (Betatorquevirus) with P1 and P2 domains

Structural Arg-Rich Region (1-54)	MPPYWRQKYYRRRYRPFSWRTRRIIQRRKRWRYRK PRKTYWRRKLRVRKRFYKR
Jelly-Roll B-H strands subdomain (55-239)	KLKKIVLKQFQPKIIRRCTIFGTICLFQGSPERANNNYI QTIYSYVPDKEPGGGGWTLITESLSSLWEDWEHLKN VWTQSNAGLPLVRYGGVTLYFYQSAYTDYIAQVFN CYPMTDTKYTHADSAPNRMLLKKHVIRVPSRETRKK RKPYKRVRVGPPSQMQNKWYFQRDICEIPLIMIAATA VD
P1-1 (240-259)	FRYPFCASDCASNNLTLTCL
P2 (260-369)	NPLLFQNQDFDHPSDTQGYFPKPGVYLYSTQRSNKPS SSDCIYLGNTKDNQEGKSASSLMTLKTQKITDWGNP FWHYYIDGSKKIFSYFKPPSQLDSSDFEHMTELAEPM
P1-2 (370-507)	FIQVRYNPERDTGQGNLIYVTENFRGQHWDPPSSDNL KLDGFPLYDMCWGFIDWIEKVHETENLLTNYCFCIRS SAFNEKKTVFIPVDHSFLTGFSPYETPVKSSDQAHWH PQIRFQTKSINDICLTGPGCARSPYGN
Jelly-Roll I strand subdomain (508-522)	YMQAKMSYKFHVKWG
Structural C-terminal domain (523-622)	GCPKTYEKPYDPCSQPNWTIPHNLNETIQIQNPNTCP QTELQEWDWRRDIVTKKAIERIRQHTEPHETLQISTG SKHNPPVHRQTSPWTDSETDSEEEKDQTQEIQIQLNK LRKHQQHLKQQLKQYLKPQNIE

Table B1-4: Ring10 ORF1 amino acid subsequence (Betatorquevirus) with structural HVR and structural N22 domain

Structural Arg-Rich Region (1-45)	MPWWYRRRSYNPWRRRNWFRRPRKTIYRRYRRRRRWVR RKPFYKR
Structural jelly-roll Domain (46-229)	KIKRLNIVEWQPKSIRKCRIKGMLCLFQTTEDRLSYNFDMY EESIIPEKLPGGGGFSIKNISLYALYQEHIHAHNIFTHTNTDR PLARYTGCSLKFYQSKDIDYVVTYSTSLPLRSSMGMYNSM QPSIHLMQQNKLIVPSKQTQKRRKPYIKKHISPPTQMKSQW YFQHNIANIPLLMIRTTAL
Structural hypervariable domain (230-385)	TLDNYYIGSRQLSTNVTIHTLNTTYIQNRDWGDRNKTYYC QTLGTQRYFLYGTHSTAQNINDIKLQELIPLTNTQDYVQGF DWTEKDKHNITTYKEFLTKGAGNPFHAEWITAQNPVIHTA NSPTQIEQIYTASTTTFQNKKLTDLPTPGYIFITP
Structural N22 domain (386-543)	TVSLRYNPYKDLAERNKCYFVRSKINAHGWDPEQHQELIN SDLPQWLLLFGYPDYIKRTQNFALVDTNYILVDHCPYTNPE KTPFIPLSTSFIEGRSPYSPSDTHEPDEEDQNRWYPCYQYQQ ESINSICLSGPGTPKIPKGITAEAKVKYSFNFKWG
Structural C-terminal domain (544-672)	GDLPPMSTITNPTDQPTYVVPNNFNETTSLQNPTTRPEHFLY SFDERRGQLTEKATKRLLKDWETKETSLLSTEYRFAEPTQT QAPQEDPSSEEEEESNLFERLLRQRTKQLQLKRRIIQTLKDL QKLE

Table B1-5: Ring10 ORF1 amino acid subsequence (Betatorquevirus) with P1 and P2 domains

Structural Arg-Rich Region (1-45)	MPWWYRRRSYNPWRRRNWFRRPRKTIYRRYRRRRRWVR RKPFYKR
Jelly-roll B-H strands subdomain (46-229)	KIKRLNIVEWQPKSIRKCRIKGMLCLFQTTEDRLSYNFDMY EESIIPEKLPGGGGFSIKNISLYALYQEHIHAHNIFTHTNTDR PLARYTGCSLKFYQSKDIDYVVTYSTSLPLRSSMGMYNSM

	QPSIHLMQQNKLIVPSKQTQKRRKPYIKKHISPPTQMKSQW YFQHNIANIPLLMIRTTAL
P1-1 (230-250aa)	TLDNYYIGSRQLSTNVTIHTL
P2 (251-385)	NTTYIQNRDWGDRNKTYYCQTLGTQRYFLYGTHSTAQNIN DIKLQELIPLTNTQDYVQGFDWTEKDKHNITTYKEFLTKGA GNPFHAEWITAQNPVIHTANSPTQIEQIYTASTTTFQNKKLT DLPTPGYIFITP
P1-2 (386-528aa)	TVSLRYNPYKDLAERNKCYFVRSKINAHGWDPEQHQELIN SDLPQWLLLFGYPDYIKRTQNFALVDTNYILVDHCPYTNPE KTPFIPLSTSFIEGRSPYSPSDTHEPDEEDQNRWYPCYQYQQ ESINSICLSGPGTPKIPKGI
Jelly-roll I-strand subdomain (529-543)	TAEAKVKYSFNFKWG
Structural C-terminal domain (544-672)	GDLPPMSTITNPTDQPTYVVPNNFNETTSLQNPTTRPEHFLY SFDERRGQLTEKATKRLLKDWETKETSLLSTEYRFAEPTQT QAPQEDPSSEEEEESNLFERLLRQRTKQLQLKRRIIQTLKDL QKLE

Table B1-9: Ring18 ORF1 amino acid subsequence (Alphatorquevirus) with P1 and P2 domains

Structural Arg-Rich Region (1-67)	MAWGFWGRRRRWRRWRPRRRRWRPRRRRRRRPARRFRA RRRVRRRGGRWRRRYRKWRRGRRRRTHRK
Jelly-roll B-H strands subdomain (68-248)	KIVIKQWQPNFIRRCYIIGYLPLIFCGENTTAQNYATHSDDM ISKGPYGGGMTTTKFTLRILYDEFTRFMNFWTVSNEDLDLC RYVGCKLIFFKHPTVDFIVQINTQPPFLDTHLTAASIHPGIM MLSKRRILIPSLKTRPSRKHRVVVRVGAPRLFQDKWYPQSD LCDTVLLSIFATACD
P1-1 (249-279)	LQYPFGSPLTDNPCVNFQILGPQYKKHLSIS
P2 (280-401)	STMDTTNKQHYDSNLFNQTQLYNTFQTIAQLKETGQTANI SPSWSAVQNNMALSNTGENATQSKDTWYKGNTYNNHITT

	LAQKTRERFKGATKAALQNYPTIMSTDLYEYHSGIYSSIFLS A
P1-2 (402-555)	GRSYFETTGAYSDIIYNPFTDKGTGNIIWIDYLTKEDTIFVK NKSKCEIMDMPLWAACTGYTEFCAKYTGDSAIIYNARVLI RCPYTEPMLIDHSDPNKGFVPYSFNFGNGKMPGGSSNVPIR MRAKWYVNIFHQKEVLETIVQSGPFGYKGDI
Jelly-roll I-strand subdomain (556-574)	KSAVLAMKYRFHWKWGGNP
Structural C-terminal domain (575-736)	ISKQVVRNPCSNSSSSAAHRGPRSVQAVDPKYNTPEVTWH SWDIRRGLFGKAGIKRMQQESDALYIPPGPFKRPRRDTNAQ DPEEQNESSGFRVQQRLPWVHSSQETQSSQEETEAQGSVQ DQLLLQLREQRVLRLQLQQLATQVLKVQAGHGIHPLLSSQ A

Table B1-11a: Ring19 ORF1 subsequences with structural HVR and structural N22 domain

Structural Arg-Rich Region (1-41)	MPWYPRRRYPRRRYRWLRRWRARRPFRPRYRRRYWVRN YSR
Structural jelly-roll region (42-228)	KRKLFKITTKEWQPKVIRKTHVKGTYPLFLCTKHRINNNMI QYLDSIAPEHYYGGGGFSIMQFSLQALYEEFIKAKNWWTN TNCFLPLVRYMGCSFKFYKTEFYDYIVLIERCYPLACTDEM YLSTQPSIMMLTRKCIFVPCKQNSKGKKPYKKVRVRPPSQ MTTGWHFSQDLANMPLVVLKTSVCS
Structural hypervariable domain (229-372)	FDRYYTDSTAKSTTIGFKTLNTQTFRYHDWQEPPTTGYKPQ NLLWFYGAENGSPVDPNNTIVSNLIYLGGTGPYEKGTPIKT NISNYFSEPKLWGNIFHDDYTSGTSPVFVTNKSPSEIKTAW NTIKDLTVKASGVFTLRTIPL
Structural N22 domain (373-528)	WLPCRYNPFADKATNNKIWLVSIHSDHTEWKPIDNPLLQR TDLPLWLLVWGWQDWQKKNQQTSQPDINYLTVISSPYISC

	YPKLDYYVLLDEGFWEGHSTYIESITDSDKKHWYPKNRFQI ETLNLIANTGPGTVKLRENQAAEGHMVYRFNFKLG
Structural C-terminal domain (529-655)	GCPAPMEKICDPSKQSKYPIPNNQQQTTSLQS PENPIQTYLYDFDERRGLLTERATKRIKQDHTSEKTVLPFTG AATDLPILQTTSQEESSSEEEEEQQAEKKLLQLRRKQHRLR ERILQLLDIQNT

Table B1-12: Ring19 ORF1 subsequences with P1 and P2 domains

Structural Arg-Rich Region (1-41)	MPWYPRRRYPRRRYRWLRRWRARRPFRPRYRRRYWVRN YSR
Jelly-roll B-H strands subdomain (42-228)	KRKLFKITTKEWQPKVIRKTHVKGTYPLFLCTKHRINNNMI QYLDSIAPEHYYGGGGFSIMQFSLQALYEEFIKAKNWWTN TNCFLPLVRYMGCSFKFYKTEFYDYIVLIERCYPLACTDEM YLSTQPSIMMLTRKCIFVPCKQNSKGKKPYKKVRVRPPSQ MTTGWHFSQDLANMPLVVLKTSVCS
P1-1 (229-247aa)	FDRYYTDSTAKSTTIGFKT
P2 (248-372)	LNTQTFRYHDWQEPPTTGYKPQNLLWFYGAENGSPVDPN NTIVSNLIYLGGTGPYEKGTPIKTNISNYFSEPKLWGNIFHD DYTSGTSPVFVTNKSPSEIKTAWNTIKDLTVKASGVFTLRTI PL
P1-2 (373-514aa)	WLPCRYNPFADKATNNKIWLVSIHSDHTEWKPIDNPLLQR TDLPLWLLVWGWQDWQKKNQQTSQPDINYLTVISSPYISC YPKLDYYVLLDEGFWEGHSTYIESITDSDKKHWYPKNRFQI ETLNLIANTGPGTVKLRENQA
Jelly-roll I-strand subdomain (515-528)	AEGHMVYRFNFKLG
Structural C-terminal domain (529-655)	GCPAPMEKICDPSKQSKYPIPNNQQQTTSLQSPENPIQTYLY DFDERRGLLTERATKRIKQDHTSEKTVLPFTGAATDLPILQ TTSQEESSSEEEEEQQAEKKLLQLRRKQHRLRERILQLLDIQ NT

Exemplary ORF1 variant construct sequence tables

Provided below are exemplary Anellovirus ORF1 mutant and variant sequences. These types of mutants and variants are described in further detail in the sections below.

Table B1-3a: Exemplary Ring2 variant constructs suitable for expression in 293 cells

#ID	Sequence
1. Ring2 delARM (Δ2-45) (7046)	MLKQWQPPYKRTCYIKGQDCLIYYSNLRLGMNSTMYEKSIVPVHWPG GGSFSVSMLTLDALYDIHKLCRNWWTSTNQDLPLVRYKGCKITFYQST FTDYIVRIHTELPANSNKLTYPNTHPLMMMMSKYKHIIPSRQTRRKKKP YTKIFVKPPPQFENKWYFATDLYKIPLLQIHCTACNLQNPFVKPDKLSN NVTLWSLNTISIQNRNMSVDQGQSWPFKILGTQSFYFYFYTGANLPGDT TQIPVADLLPLTNPRINRPGQSLNEAKITDHITFTEYKNKFTNYWGNPFN KHIQEHLDMILYSLKSPEAIKNEWTTENMKWNQLNNAGTMALTPFNEP IFTQIQYNPDRDTGEDTQLYLLSNATGTGWDPPGIPELILEGFPLWLIYW GFADFQKNLKKVTNIDTNYMLVAKTKFTQKPGTFYLVILNDTFVEGNS PYEKQPLPEDNIKWYPQVQYQLEAQNKLLQTGPFTPNIQGQLSDNISMF YKFYFKWGGSPPKAINVENPAHQIQYPIPRNEHETTSLQSPGEAPESILY SFDYRHGNYTTTALSRISQDWALKDTVSKITEPDRQQLLKQALECLQIS EETQEKKEKEVQQLISNLRQQQQLYRERIISLLKDQ
2. Ring2 delCterm (Δ611-666) (7047)	MPYYYRRRRYNYRRPRWYGRGWIRRPFRRRFRRKRRVRPTYTTIPLKQ WQPPYKRTCYIKGQDCLIYYSNLRLGMNSTMYEKSIVPVHWPGGGSFS VSMLTLDALYDIHKLCRNWWTSTNQDLPLVRYKGCKITFYQSTFTDYI VRIHTELPANSNKLTYPNTHPLMMMMSKYKHIIPSRQTRRKKKPYTKIF VKPPPQFENKWYFATDLYKIPLLQIHCTACNLQNPFVKPDKLSNNVTL WSLNTISIQNRNMSVDQGQSWPFKILGTQSFYFYFYTGANLPGDTTQIP VADLLPLTNPRINRPGQSLNEAKITDHITFTEYKNKFTNYWGNPFNKHI QEHLDMILYSLKSPEAIKNEWTTENMKWNQLNNAGTMALTPFNEPIFT QIQYNPDRDTGEDTQLYLLSNATGTGWDPPGIPELILEGFPLWLIYWGF ADFQKNLKKVTNIDTNYMLVAKTKFTQKPGTFYLVILNDTFVEGNSPY

	EKQPLPEDNIKWYPQVQYQLEAQNKLLQTGPFTPNIQGQLSDNISMFYK FYFKWGGSPPKAINVENPAHQIQYPIPRNEHETTSLQSPGEAPESILYSFD YRHGNYTTTALSRISQDWALKDTVSK
3. Ring2 delCterm (Δ538-666) (7048)	MPYYYRRRRYNYRRPRWYGRGWIRRPFRRRFRRKRRVRPTYTTIPLKQ WQPPYKRTCYIKGQDCLIYYSNLRLGMNSTMYEKSIVPVHWPGGGSFS VSMLTLDALYDIHKLCRNWWTSTNQDLPLVRYKGCKITFYQSTFTDYI VRIHTELPANSNKLTYPNTHPLMMMMSKYKHIIPSRQTRRKKKPYTKIF VKPPPQFENKWYFATDLYKIPLLQIHCTACNLQNPFVKPDKLSNNVTL WSLNTISIQNRNMSVDQGQSWPFKILGTQSFYFYFYTGANLPGDTTQIP VADLLPLTNPRINRPGQSLNEAKITDHITFTEYKNKFTNYWGNPFNKHI QEHLDMILYSLKSPEAIKNEWTTENMKWNQLNNAGTMALTPFNEPIFT QIQYNPDRDTGEDTQLYLLSNATGTGWDPPGIPELILEGFPLWLIYWGF ADFQKNLKKVTNIDTNYMLVAKTKFTQKPGTFYLVILNDTFVEGNSPY EKQPLPEDNIKWYPQVQYQLEAQNKLLQTGPFTPNIQGQLSDNISMFYK FYFK
4. Ring2 delARM delCterm (Δ2-45+ Δ 611-666) (7049)	MLKQWQPPYKRTCYIKGQDCLIYYSNLRLGMNSTMYEKSIVPVHWPG GGSFSVSMLTLDALYDIHKLCRNWWTSTNQDLPLVRYKGCKITFYQST FTDYIVRIHTELPANSNKLTYPNTHPLMMMMSKYKHIIPSRQTRRKKKP YTKIFVKPPPQFENKWYFATDLYKIPLLQIHCTACNLQNPFVKPDKLSN NVTLWSLNTISIQNRNMSVDQGQSWPFKILGTQSFYFYFYTGANLPGDT TQIPVADLLPLTNPRINRPGQSLNEAKITDHITFTEYKNKFTNYWGNPFN KHIQEHLDMILYSLKSPEAIKNEWTTENMKWNQLNNAGTMALTPFNEP IFTQIQYNPDRDTGEDTQLYLLSNATGTGWDPPGIPELILEGFPLWLIYW GFADFQKNLKKVTNIDTNYMLVAKTKFTQKPGTFYLVILNDTFVEGNS PYEKQPLPEDNIKWYPQVQYQLEAQNKLLQTGPFTPNIQGQLSDNISMF YKFYFKWGGSPPKAINVENPAHQIQYPIPRNEHETTSLQSPGEAPESILY SFDYRHGNYTTTALSRISQDWALKDTVSK

5. Ring2-ORF1 delARM delCterm (Δ2-45+ Δ 538-666) (7050)	MLKQWQPPYKRTCYIKGQDCLIYYSNLRLGMNSTMYEKSIVPVHWPG GGSFSVSMLTLDALYDIHKLCRNWWTSTNQDLPLVRYKGCKITFYQST FTDYIVRIHTELPANSNKLTYPNTHPLMMMMSKYKHIIPSRQTRRKKKP YTKIFVKPPPQFENKWYFATDLYKIPLLQIHCTACNLQNPFVKPDKLSN NVTLWSLNTISIQNRNMSVDQGQSWPFKILGTQSFYFYFYTGANLPGDT TQIPVADLLPLTNPRINRPGQSLNEAKITDHITFTEYKNKFTNYWGNPFN KHIQEHLDMILYSLKSPEAIKNEWTTENMKWNQLNNAGTMALTPFNEP IFTQIQYNPDRDTGEDTQLYLLSNATGTGWDPPGIPELILEGFPLWLIYW GFADFQKNLKKVTNIDTNYMLVAKTKFTQKPGTFYLVILNDTFVEGNS PYEKQPLPEDNIKWYPQVQYQLEAQNKLLQTGPFTPNIQGQLSDNISMF YKFYFK
#ID	Sequence
6. Ring2 delARM(Δ2 -45) and Orf2 (5130)	MLKQWQPPYKRTCYIKGQDCLIYYSNLRLGMNSTMYEKSIVPVHWPG GGSFSVSMLTLDALYDIHKLCRNWWTSTNQDLPLVRYKGCKITFYQST FTDYIVRIHTELPANSNKLTYPNTHPLMMMMSKYKHIIPSRQTRRKKKP YTKIFVKPPPQFENKWYFATDLYKIPLLQIHCTACNLQNPFVKPDKLSN NVTLWSLNTISIQNRNMSVDQGQSWPFKILGTQSFYFYFYTGANLPGDT TQIPVADLLPLTNPRINRPGQSLNEAKITDHITFTEYKNKFTNYWGNPFN KHIQEHLDMILYSLKSPEAIKNEWTTENMKWNQLNNAGTMALTPFNEP IFTQIQYNPDRDTGEDTQLYLLSNATGTGWDPPGIPELILEGFPLWLIYW GFADFQKNLKKVTNIDTNYMLVAKTKFTQKPGTFYLVILNDTFVEGNS PYEKQPLPEDNIKWYPQVQYQLEAQNKLLQTGPFTPNIQGQLSDNISMF YKFYFKWGGSPPKAINVENPAHQIQYPIPRNEHETTSLQSPGEAPESILY SFDYRHGNYTTTALSRISQDWALKDTVSKITEPDRQQLLKQALECLQIS EETQEKKEKEVQQLISNLRQQQQLYRERIISLLKDQ
7. Ring2 delARM	MLKQWQPPYKRTCYIKGQDCLIYYSNLRLGMNSTMYEKSIVPVHWPG GGSFSVSMLTLDALYDIHKLCRNWWTSTNQDLPLVRYKGCKITFYQST

Table B1-3b: Exemplary Ring2 Baculovirus constructs

delCterm (Δ2-45+Δ545-666) Orf2 (5131)	FTDYIVRIHTELPANSNKLTYPNTHPLMMMMSKYKHIIPSRQTRRKKKP YTKIFVKPPPQFENKWYFATDLYKIPLLQIHCTACNLQNPFVKPDKLSN NVTLWSLNTISIQNRNMSVDQGQSWPFKILGTQSFYFYFYTGANLPGDT TQIPVADLLPLTNPRINRPGQSLNEAKITDHITFTEYKNKFTNYWGNPFN KHIQEHLDMILYSLKSPEAIKNEWTTENMKWNQLNNAGTMALTPFNEP IFTQIQYNPDRDTGEDTQLYLLSNATGTGWDPPGIPELILEGFPLWLIYW GFADFQKNLKKVTNIDTNYMLVAKTKFTQKPGTFYLVILNDTFVEGNS PYEKQPLPEDNIKWYPQVQYQLEAQNKLLQTGPFTPNIQGQLSDNISMF YKFYFKWGGSPPK
8. Ring2 delARM delCterm (Δ2-45+Δ538-666) ORF2 (5132)	MLKQWQPPYKRTCYIKGQDCLIYYSNLRLGMNSTMYEKSIVPVHWPG GGSFSVSMLTLDALYDIHKLCRNWWTSTNQDLPLVRYKGCKITFYQST FTDYIVRIHTELPANSNKLTYPNTHPLMMMMSKYKHIIPSRQTRRKKKP YTKIFVKPPPQFENKWYFATDLYKIPLLQIHCTACNLQNPFVKPDKLSN NVTLWSLNTISIQNRNMSVDQGQSWPFKILGTQSFYFYFYTGANLPGDT TQIPVADLLPLTNPRINRPGQSLNEAKITDHITFTEYKNKFTNYWGNPFN KHIQEHLDMILYSLKSPEAIKNEWTTENMKWNQLNNAGTMALTPFNEP IFTQIQYNPDRDTGEDTQLYLLSNATGTGWDPPGIPELILEGFPLWLIYW GFADFQKNLKKVTNIDTNYMLVAKTKFTQKPGTFYLVILNDTFVEGNS PYEKQPLPEDNIKWYPQVQYQLEAQNKLLQTGPFTPNIQGQLSDNISMF YKFYFK
9. Ring2 delARM delCterm (Δ2-45+Δ518-666) ORF2 (5136)	MPYYYRRRRYNYRRPRWYGRGWIRRPFRRRFRRKRRVRPTYTTIPLKQ WQPPYKRTCYIKGQDCLIYYSNLRLGMNSTMYEKSIVPVHWPGGGSFS VSMLTLDALYDIHKLCRNWWTSTNQDLPLVRYKGCKITFYQSTFTDYI VRIHTELPANSNKLTYPNTHPLMMMMSKYKHIIPSRQTRRKKKPYTKIF VKPPPQFENKWYFATDLYKIPLLQIHCTACNLQNPFVKPDKLSNNVTL WSLNTISIQNRNMSVDQGQSWPFKILGTQSFYFYFYTGANLPGDTTQIP VADLLPLTNPRINRPGQSLNEAKITDHITFTEYKNKFTNYWGNPFNKHI QEHLDMILYSLKSPEAIKNEWTTENMKWNQLNNAGTMALTPFNEPIFT QIQYNPDRDTGEDTQLYLLSNATGTGWDPPGIPELILEGFPLWLIYWGF

	ADFQKNLKKVTNIDTNYMLVAKTKFTQKPGTFYLVILNDTFVEGNSPY EKQPLPEDNIKWYPQVQYQLEAQNKLLQTGPFT
10. Ring 2 ORF1 delCterm (Δ538-666) ORF2 (5137)	MPYYYRRRRYNYRRPRWYGRGWIRRPFRRRFRRKRRVRPTYTTIPLKQ WQPPYKRTCYIKGQDCLIYYSNLRLGMNSTMYEKSIVPVHWPGGGSFS VSMLTLDALYDIHKLCRNWWTSTNQDLPLVRYKGCKITFYQSTFTDYI VRIHTELPANSNKLTYPNTHPLMMMMSKYKHIIPSRQTRRKKKPYTKIF VKPPPQFENKWYFATDLYKIPLLQIHCTACNLQNPFVKPDKLSNNVTL WSLNTISIQNRNMSVDQGQSWPFKILGTQSFYFYFYTGANLPGDTTQIP VADLLPLTNPRINRPGQSLNEAKITDHITFTEYKNKFTNYWGNPFNKHI QEHLDMILYSLKSPEAIKNEWTTENMKWNQLNNAGTMALTPFNEPIFT QIQYNPDRDTGEDTQLYLLSNATGTGWDPPGIPELILEGFPLWLIYWGF ADFQKNLKKVTNIDTNYMLVAKTKFTQKPGTFYLVILNDTFVEGNSPY EKQPLPEDNIKWYPQVQYQLEAQNKLLQTGPFTPNIQGQLSDNISMFYK FYFK
11. Ring 2 ORF1 delCterm (Δ594-666) ORF2 (5138)	MPYYYRRRRYNYRRPRWYGRGWIRRPFRRRFRRKRRVRPTYTTIPLKQ WQPPYKRTCYIKGQDCLIYYSNLRLGMNSTMYEKSIVPVHWPGGGSFS VSMLTLDALYDIHKLCRNWWTSTNQDLPLVRYKGCKITFYQSTFTDYI VRIHTELPANSNKLTYPNTHPLMMMMSKYKHIIPSRQTRRKKKPYTKIF VKPPPQFENKWYFATDLYKIPLLQIHCTACNLQNPFVKPDKLSNNVTL WSLNTISIQNRNMSVDQGQSWPFKILGTQSFYFYFYTGANLPGDTTQIP VADLLPLTNPRINRPGQSLNEAKITDHITFTEYKNKFTNYWGNPFNKHI QEHLDMILYSLKSPEAIKNEWTTENMKWNQLNNAGTMALTPFNEPIFT QIQYNPDRDTGEDTQLYLLSNATGTGWDPPGIPELILEGFPLWLIYWGF ADFQKNLKKVTNIDTNYMLVAKTKFTQKPGTFYLVILNDTFVEGNSPY EKQPLPEDNIKWYPQVQYQLEAQNKLLQTGPFTPNIQGQLSDNISMFYK FYFKWGGSPPKAINVENPAHQIQYPIPRNEHETTSLQSPGEAPESILYSFD YRHGNYTTT

12. Ring 2 ORF1 delCterm (Δ630-666) ORF2 (5139)	MPYYYRRRRYNYRRPRWYGRGWIRRPFRRRFRRKRRVRPTYTTIPLKQ WQPPYKRTCYIKGQDCLIYYSNLRLGMNSTMYEKSIVPVHWPGGGSFS VSMLTLDALYDIHKLCRNWWTSTNQDLPLVRYKGCKITFYQSTFTDYI VRIHTELPANSNKLTYPNTHPLMMMMSKYKHIIPSRQTRRKKKPYTKIF VKPPPQFENKWYFATDLYKIPLLQIHCTACNLQNPFVKPDKLSNNVTL WSLNTISIQNRNMSVDQGQSWPFKILGTQSFYFYFYTGANLPGDTTQIP VADLLPLTNPRINRPGQSLNEAKITDHITFTEYKNKFTNYWGNPFNKHI QEHLDMILYSLKSPEAIKNEWTTENMKWNQLNNAGTMALTPFNEPIFT QIQYNPDRDTGEDTQLYLLSNATGTGWDPPGIPELILEGFPLWLIYWGF ADFQKNLKKVTNIDTNYMLVAKTKFTQKPGTFYLVILNDTFVEGNSPY EKQPLPEDNIKWYPQVQYQLEAQNKLLQTGPFTPNIQGQLSDNISMFYK FYFKWGGSPPKAINVENPAHQIQYPIPRNEHETTSLQSPGEAPESILYSFD YRHGNYTTTALSRISQDWALKDTVSKITEPDRQQLLKQALECLQI

Table B1-6: 293 constructs for Ring10 delARM/C-terminal deletions

#ID	sequence
13. Ring10 delARM (Δ2-51) (7051)	MIVEWQPKSIRKCRIKGMLCLFQTTEDRLSYNFDMYEESIIPEKLPGGGG FSIKNISLYALYQEHIHAHNIFTHTNTDRPLARYTGCSLKFYQSKDIDYV VTYSTSLPLRSSMGMYNSMQPSIHLMQQNKLIVPSKQTQKRRKPYIKK HISPPTQMKSQWYFQHNIANIPLLMIRTTALTLDNYYIGSRQLSTNVTIH TLNTTYIQNRDWGDRNKTYYCQTLGTQRYFLYGTHSTAQNINDIKLQE LIPLTNTQDYVQGFDWTEKDKHNITTYKEFLTKGAGNPFHAEWITAQN PVIHTANSPTQIEQIYTASTTTFQNKKLTDLPTPGYIFITPTVSLRYNPYK DLAERNKCYFVRSKINAHGWDPEQHQELINSDLPQWLLLFGYPDYIKR TQNFALVDTNYILVDHCPYTNPEKTPFIPLSTSFIEGRSPYSPSDTHEPDE EDQNRWYPCYQYQQESINSICLSGPGTPKIPKGITAEAKVKYSFNFKWG GDLPPMSTITNPTDQPTYVVPNNFNETTSLQNPTTRPEHFLYSFDERRGQ LTEKATKRLLKDWETKETSLLSTEYRFAEPTQTQAPQEDPSSEEEEESNL FERLLRQRTKQLQLKRRIIQTLKDLQKLE

14. Ring10-ORF1 delCterm-Helix (Δ610-672) (7052)	MPWWYRRRSYNPWRRRNWFRRPRKTIYRRYRRRRRWVRRKPFYKRK IKRLNIVEWQPKSIRKCRIKGMLCLFQTTEDRLSYNFDMYEESIIPEKLPG GGGFSIKNISLYALYQEHIHAHNIFTHTNTDRPLARYTGCSLKFYQSKDI DYVVTYSTSLPLRSSMGMYNSMQPSIHLMQQNKLIVPSKQTQKRRKPY IKKHISPPTQMKSQWYFQHNIANIPLLMIRTTALTLDNYYIGSRQLSTNV TIHTLNTTYIQNRDWGDRNKTYYCQTLGTQRYFLYGTHSTAQNINDIKL QELIPLTNTQDYVQGFDWTEKDKHNITTYKEFLTKGAGNPFHAEWITA QNPVIHTANSPTQIEQIYTASTTTFQNKKLTDLPTPGYIFITPTVSLRYNP YKDLAERNKCYFVRSKINAHGWDPEQHQELINSDLPQWLLLFGYPDYI KRTQNFALVDTNYILVDHCPYTNPEKTPFIPLSTSFIEGRSPYSPSDTHEP DEEDQNRWYPCYQYQQESINSICLSGPGTPKIPKGITAEAKVKYSFNFK WGGDLPPMSTITNPTDQPTYVVPNNFNETTSLQNPTTRPEHFLYSFDER RGQLTEKATKRLLKDWETK
15. Ring10-ORF1 delCterm-Helix (Δ542-672) (7053)	MPWWYRRRSYNPWRRRNWFRRPRKTIYRRYRRRRRWVRRKPFYKRK IKRLNIVEWQPKSIRKCRIKGMLCLFQTTEDRLSYNFDMYEESIIPEKLPG GGGFSIKNISLYALYQEHIHAHNIFTHTNTDRPLARYTGCSLKFYQSKDI DYVVTYSTSLPLRSSMGMYNSMQPSIHLMQQNKLIVPSKQTQKRRKPY IKKHISPPTQMKSQWYFQHNIANIPLLMIRTTALTLDNYYIGSRQLSTNV TIHTLNTTYIQNRDWGDRNKTYYCQTLGTQRYFLYGTHSTAQNINDIKL QELIPLTNTQDYVQGFDWTEKDKHNITTYKEFLTKGAGNPFHAEWITA QNPVIHTANSPTQIEQIYTASTTTFQNKKLTDLPTPGYIFITPTVSLRYNP YKDLAERNKCYFVRSKINAHGWDPEQHQELINSDLPQWLLLFGYPDYI KRTQNFALVDTNYILVDHCPYTNPEKTPFIPLSTSFIEGRSPYSPSDTHEP DEEDQNRWYPCYQYQQESINSICLSGPGTPKIPKGITAEAKVKYSFNFK
16. Ring10-ORF1 delARM delCterm-Helix (Δ2-	MIVEWQPKSIRKCRIKGMLCLFQTTEDRLSYNFDMYEESIIPEKLPGGGG FSIKNISLYALYQEHIHAHNIFTHTNTDRPLARYTGCSLKFYQSKDIDYV VTYSTSLPLRSSMGMYNSMQPSIHLMQQNKLIVPSKQTQKRRKPYIKK HISPPTQMKSQWYFQHNIANIPLLMIRTTALTLDNYYIGSRQLSTNVTIH TLNTTYIQNRDWGDRNKTYYCQTLGTQRYFLYGTHSTAQNINDIKLQE

51+610-672) (7054)	LIPLTNTQDYVQGFDWTEKDKHNITTYKEFLTKGAGNPFHAEWITAQN PVIHTANSPTQIEQIYTASTTTFQNKKLTDLPTPGYIFITPTVSLRYNPYK DLAERNKCYFVRSKINAHGWDPEQHQELINSDLPQWLLLFGYPDYIKR TQNFALVDTNYILVDHCPYTNPEKTPFIPLSTSFIEGRSPYSPSDTHEPDE EDQNRWYPCYQYQQESINSICLSGPGTPKIPKGITAEAKVKYSFNFKWG GDLPPMSTITNPTDQPTYVVPNNFNETTSLQNPTTRPEHFLYSFDERRGQ LTEKATKRLLKDWETK
17. Ring10-ORF1 delARM delCterm (Δ2-51+542-672) (7055)	MIVEWQPKSIRKCRIKGMLCLFQTTEDRLSYNFDMYEESIIPEKLPGGGG FSIKNISLYALYQEHIHAHNIFTHTNTDRPLARYTGCSLKFYQSKDIDYV VTYSTSLPLRSSMGMYNSMQPSIHLMQQNKLIVPSKQTQKRRKPYIKK HISPPTQMKSQWYFQHNIANIPLLMIRTTALTLDNYYIGSRQLSTNVTIH TLNTTYIQNRDWGDRNKTYYCQTLGTQRYFLYGTHSTAQNINDIKLQE LIPLTNTQDYVQGFDWTEKDKHNITTYKEFLTKGAGNPFHAEWITAQN PVIHTANSPTQIEQIYTASTTTFQNKKLTDLPTPGYIFITPTVSLRYNPYK DLAERNKCYFVRSKINAHGWDPEQHQELINSDLPQWLLLFGYPDYIKR TQNFALVDTNYILVDHCPYTNPEKTPFIPLSTSFIEGRSPYSPSDTHEPDE EDQNRWYPCYQYQQESINSICLSGPGTPKIPKGITAEAKVKYSFNFK
18. Ring10 ORF1 delARM (Δ2-45) (7092)	MKIKRLNIVEWQPKSIRKCRIKGMLCLFQTTEDRLSYNFDMYEESIIPEK LPGGGGFSIKNISLYALYQEHIHAHNIFTHTNTDRPLARYTGCSLKFYQS KDIDYVVTYSTSLPLRSSMGMYNSMQPSIHLMQQNKLIVPSKQTQKRR KPYIKKHISPPTQMKSQWYFQHNIANIPLLMIRTTALTLDNYYIGSRQLS TNVTIHTLNTTYIQNRDWGDRNKTYYCQTLGTQRYFLYGTHSTAQNIN DIKLQELIPLTNTQDYVQGFDWTEKDKHNITTYKEFLTKGAGNPFHAE WITAQNPVIHTANSPTQIEQIYTASTTTFQNKKLTDLPTPGYIFITPTVSLR YNPYKDLAERNKCYFVRSKINAHGWDPEQHQELINSDLPQWLLLFGYP DYIKRTQNFALVDTNYILVDHCPYTNPEKTPFIPLSTSFIEGRSPYSPSDT HEPDEEDQNRWYPCYQYQQESINSICLSGPGTPKIPKGITAEAKVKYSFN FKWGGDLPPMSTITNPTDQPTYVVPNNFNETTSLQNPTTRPEHFLYSFD

	ERRGQLTEKATKRLLKDWETKETSLLSTEYRFAEPTQTQAPQEDPSSEE EEESNLFERLLRQRTKQLQLKRRIIQTLKDLQKLE
19. Ring 10 delARM delCterm-568 (Δ2-45+Δ569-672) (7093)	MKIKRLNIVEWQPKSIRKCRIKGMLCLFQTTEDRLSYNFDMYEESIIPEK LPGGGGFSIKNISLYALYQEHIHAHNIFTHTNTDRPLARYTGCSLKFYQS KDIDYVVTYSTSLPLRSSMGMYNSMQPSIHLMQQNKLIVPSKQTQKRR KPYIKKHISPPTQMKSQWYFQHNIANIPLLMIRTTALTLDNYYIGSRQLS TNVTIHTLNTTYIQNRDWGDRNKTYYCQTLGTQRYFLYGTHSTAQNIN DIKLQELIPLTNTQDYVQGFDWTEKDKHNITTYKEFLTKGAGNPFHAE WITAQNPVIHTANSPTQIEQIYTASTTTFQNKKLTDLPTPGYIFITPTVSLR YNPYKDLAERNKCYFVRSKINAHGWDPEQHQELINSDLPQWLLLFGYP DYIKRTQNFALVDTNYILVDHCPYTNPEKTPFIPLSTSFIEGRSPYSPSDT HEPDEEDQNRWYPCYQYQQESINSICLSGPGTPKIPKGITAEAKVKYSFN FKWGGDLPPMSTITNPTDQPTYVVPNNFN
20. Ring 10 delARM delCterm (Δ2-45+Δ566-672) (7094)	MKIKRLNIVEWQPKSIRKCRIKGMLCLFQTTEDRLSYNFDMYEESIIPEK LPGGGGFSIKNISLYALYQEHIHAHNIFTHTNTDRPLARYTGCSLKFYQS KDIDYVVTYSTSLPLRSSMGMYNSMQPSIHLMQQNKLIVPSKQTQKRR KPYIKKHISPPTQMKSQWYFQHNIANIPLLMIRTTALTLDNYYIGSRQLS TNVTIHTLNTTYIQNRDWGDRNKTYYCQTLGTQRYFLYGTHSTAQNIN DIKLQELIPLTNTQDYVQGFDWTEKDKHNITTYKEFLTKGAGNPFHAE WITAQNPVIHTANSPTQIEQIYTASTTTFQNKKLTDLPTPGYIFITPTVSLR YNPYKDLAERNKCYFVRSKINAHGWDPEQHQELINSDLPQWLLLFGYP DYIKRTQNFALVDTNYILVDHCPYTNPEKTPFIPLSTSFIEGRSPYSPSDT HEPDEEDQNRWYPCYQYQQESINSICLSGPGTPKIPKGITAEAKVKYSFN FKWGGDLPPMSTITNPTDQPTYVVPN
21. Ring 10 delARM delCterm (Δ2-	MKIKRLNIVEWQPKSIRKCRIKGMLCLFQTTEDRLSYNFDMYEESIIPEK LPGGGGFSIKNISLYALYQEHIHAHNIFTHTNTDRPLARYTGCSLKFYQS KDIDYVVTYSTSLPLRSSMGMYNSMQPSIHLMQQNKLIVPSKQTQKRR KPYIKKHISPPTQMKSQWYFQHNIANIPLLMIRTTALTLDNYYIGSRQLS

45+Δ560-672) (7095)	TNVTIHTLNTTYIQNRDWGDRNKTYYCQTLGTQRYFLYGTHSTAQNIN DIKLQELIPLTNTQDYVQGFDWTEKDKHNITTYKEFLTKGAGNPFHAE WITAQNPVIHTANSPTQIEQIYTASTTTFQNKKLTDLPTPGYIFITPTVSLR YNPYKDLAERNKCYFVRSKINAHGWDPEQHQELINSDLPQWLLLFGYP DYIKRTQNFALVDTNYILVDHCPYTNPEKTPFIPLSTSFIEGRSPYSPSDT HEPDEEDQNRWYPCYQYQQESINSICLSGPGTPKIPKGITAEAKVKYSFN FKWGGDLPPMSTITNPTDQP
22. Ring 10 delARM delCterm (Δ2-45+Δ557-672) (7096)	MKIKRLNIVEWQPKSIRKCRIKGMLCLFQTTEDRLSYNFDMYEESIIPEK LPGGGGFSIKNISLYALYQEHIHAHNIFTHTNTDRPLARYTGCSLKFYQS KDIDYVVTYSTSLPLRSSMGMYNSMQPSIHLMQQNKLIVPSKQTQKRR KPYIKKHISPPTQMKSQWYFQHNIANIPLLMIRTTALTLDNYYIGSRQLS TNVTIHTLNTTYIQNRDWGDRNKTYYCQTLGTQRYFLYGTHSTAQNIN DIKLQELIPLTNTQDYVQGFDWTEKDKHNITTYKEFLTKGAGNPFHAE WITAQNPVIHTANSPTQIEQIYTASTTTFQNKKLTDLPTPGYIFITPTVSLR YNPYKDLAERNKCYFVRSKINAHGWDPEQHQELINSDLPQWLLLFGYP DYIKRTQNFALVDTNYILVDHCPYTNPEKTPFIPLSTSFIEGRSPYSPSDT HEPDEEDQNRWYPCYQYQQESINSICLSGPGTPKIPKGITAEAKVKYSFN FKWGGDLPPMSTITNPT

Table B1-7: Ring10 Baculovirus constructs

#ID	Sequence
23. Ring10 delARM (Δ2-45) (5125)	MKIKRLNIVEWQPKSIRKCRIKGMLCLFQTTEDRLSYNFDMYEESIIPEK LPGGGGFSIKNISLYALYQEHIHAHNIFTHTNTDRPLARYTGCSLKFYQS KDIDYVVTYSTSLPLRSSMGMYNSMQPSIHLMQQNKLIVPSKQTQKRR KPYIKKHISPPTQMKSQWYFQHNIANIPLLMIRTTALTLDNYYIGSRQLS TNVTIHTLNTTYIQNRDWGDRNKTYYCQTLGTQRYFLYGTHSTAQNIN DIKLQELIPLTNTQDYVQGFDWTEKDKHNITTYKEFLTKGAGNPFHAE WITAQNPVIHTANSPTQIEQIYTASTTTFQNKKLTDLPTPGYIFITPTVSLR YNPYKDLAERNKCYFVRSKINAHGWDPEQHQELINSDLPQWLLLFGYP

	DYIKRTQNFALVDTNYILVDHCPYTNPEKTPFIPLSTSFIEGRSPYSPSDT HEPDEEDQNRWYPCYQYQQESINSICLSGPGTPKIPKGITAEAKVKYSFN FKWGGDLPPMSTITNPTDQPTYVVPNNFNETTSLQNPTTRPEHFLYSFD ERRGQLTEKATKRLLKDWETKETSLLSTEYRFAEPTQTQAPQEDPSSEE EEESNLFERLLRQRTKQLQLKRRIIQTLKDLQKLE
24. Ring10 delARM (Δ2-51) (5149)	MIVEWQPKSIRKCRIKGMLCLFQTTEDRLSYNFDMYEESIIPEKLPGGGG FSIKNISLYALYQEHIHAHNIFTHTNTDRPLARYTGCSLKFYQSKDIDYV VTYSTSLPLRSSMGMYNSMQPSIHLMQQNKLIVPSKQTQKRRKPYIKK HISPPTQMKSQWYFQHNIANIPLLMIRTTALTLDNYYIGSRQLSTNVTIH TLNTTYIQNRDWGDRNKTYYCQTLGTQRYFLYGTHSTAQNINDIKLQE LIPLTNTQDYVQGFDWTEKDKHNITTYKEFLTKGAGNPFHAEWITAQN PVIHTANSPTQIEQIYTASTTTFQNKKLTDLPTPGYIFITPTVSLRYNPYK DLAERNKCYFVRSKINAHGWDPEQHQELINSDLPQWLLLFGYPDYIKR TQNFALVDTNYILVDHCPYTNPEKTPFIPLSTSFIEGRSPYSPSDTHEPDE EDQNRWYPCYQYQQESINSICLSGPGTPKIPKGITAEAKVKYSFNFKWG GDLPPMSTITNPTDQPTYVVPNNFNETTSLQNPTTRPEHFLYSFDERRGQ LTEKATKRLLKDWETKETSLLSTEYRFAEPTQTQAPQEDPSSEEEEESNL FERLLRQRTKQLQLKRRIIQTLKDLQKLE
25. Ring10 delARM delCterm (Δ2-51+Δ542-672) (5150)	MIVEWQPKSIRKCRIKGMLCLFQTTEDRLSYNFDMYEESIIPEKLPGGGG FSIKNISLYALYQEHIHAHNIFTHTNTDRPLARYTGCSLKFYQSKDIDYV VTYSTSLPLRSSMGMYNSMQPSIHLMQQNKLIVPSKQTQKRRKPYIKK HISPPTQMKSQWYFQHNIANIPLLMIRTTALTLDNYYIGSRQLSTNVTIH TLNTTYIQNRDWGDRNKTYYCQTLGTQRYFLYGTHSTAQNINDIKLQE LIPLTNTQDYVQGFDWTEKDKHNITTYKEFLTKGAGNPFHAEWITAQN PVIHTANSPTQIEQIYTASTTTFQNKKLTDLPTPGYIFITPTVSLRYNPYK DLAERNKCYFVRSKINAHGWDPEQHQELINSDLPQWLLLFGYPDYIKR TQNFALVDTNYILVDHCPYTNPEKTPFIPLSTSFIEGRSPYSPSDTHEPDE EDQNRWYPCYQYQQESINSICLSGPGTPKIPKGITAEAKVKYSFNFK

26. Ring10 delARM delCterm (Δ2-51+Δ552-672) (5151)	MIVEWQPKSIRKCRIKGMLCLFQTTEDRLSYNFDMYEESIIPEKLPGGGG FSIKNISLYALYQEHIHAHNIFTHTNTDRPLARYTGCSLKFYQSKDIDYV VTYSTSLPLRSSMGMYNSMQPSIHLMQQNKLIVPSKQTQKRRKPYIKK HISPPTQMKSQWYFQHNIANIPLLMIRTTALTLDNYYIGSRQLSTNVTIH TLNTTYIQNRDWGDRNKTYYCQTLGTQRYFLYGTHSTAQNINDIKLQE LIPLTNTQDYVQGFDWTEKDKHNITTYKEFLTKGAGNPFHAEWITAQN PVIHTANSPTQIEQIYTASTTTFQNKKLTDLPTPGYIFITPTVSLRYNPYK DLAERNKCYFVRSKINAHGWDPEQHQELINSDLPQWLLLFGYPDYIKR TQNFALVDTNYILVDHCPYTNPEKTPFIPLSTSFIEGRSPYSPSDTHEPDE EDQNRWYPCYQYQQESINSICLSGPGTPKIPKGITAEAKVKYSFNFKWG GDLPPMST

Table B1-10: Ring18 delARM/C-terminal deletion constructs

#ID	Sequence
27. Ring18 delCterm (Δ651-736) (5152)	MAWGFWGRRRRWRRWRPRRRRWRPRRRRRRRPARRFRARRRVRRR GGRWRRRYRKWRRGRRRRTHRKKIVIKQWQPNFIRRCYIIGYLPLIFCG ENTTAQNYATHSDDMISKGPYGGGMTTTKFTLRILYDEFTRFMNFWTV SNEDLDLCRYVGCKLIFFKHPTVDFIVQINTQPPFLDTHLTAASIHPGIM MLSKRRILIPSLKTRPSRKHRVVVRVGAPRLFQDKWYPQSDLCDTVLLS IFATACDLQYPFGSPLTDNPCVNFQILGPQYKKHLSISSTMDTTNKQHY DSNLFNQTQLYNTFQTIAQLKETGQTANISPSWSAVQNNMALSNTGEN ATQSKDTWYKGNTYNNHITTLAQKTRERFKGATKAALQNYPTIMSTDL YEYHSGIYSSIFLSAGRSYFETTGAYSDIIYNPFTDKGTGNIIWIDYLTKE DTIFVKNKSKCEIMDMPLWAACTGYTEFCAKYTGDSAIIYNARVLIRCP YTEPMLIDHSDPNKGFVPYSFNFGNGKMPGGSSNVPIRMRAKWYVNIF HQKEVLETIVQSGPFGYKGDIKSAVLAMKYRFHWKWGGNPISKQVVR NPCSNSSSSAAHRGPRSVQAVDPKYNTPEVTWHSWDIRRGLFGKAGIK RMQQESDALYIPPGPFKRPRR

28. Ring18 delCterm (Δ582-736) (5153)	MAWGFWGRRRRWRRWRPRRRRWRPRRRRRRRPARRFRARRRVRRR GGRWRRRYRKWRRGRRRRTHRKKIVIKQWQPNFIRRCYIIGYLPLIFCG ENTTAQNYATHSDDMISKGPYGGGMTTTKFTLRILYDEFTRFMNFWTV SNEDLDLCRYVGCKLIFFKHPTVDFIVQINTQPPFLDTHLTAASIHPGIM MLSKRRILIPSLKTRPSRKHRVVVRVGAPRLFQDKWYPQSDLCDTVLLS IFATACDLQYPFGSPLTDNPCVNFQILGPQYKKHLSISSTMDTTNKQHY DSNLFNQTQLYNTFQTIAQLKETGQTANISPSWSAVQNNMALSNTGEN ATQSKDTWYKGNTYNNHITTLAQKTRERFKGATKAALQNYPTIMSTDL YEYHSGIYSSIFLSAGRSYFETTGAYSDIIYNPFTDKGTGNIIWIDYLTKE DTIFVKNKSKCEIMDMPLWAACTGYTEFCAKYTGDSAIIYNARVLIRCP YTEPMLIDHSDPNKGFVPYSFNFGNGKMPGGSSNVPIRMRAKWYVNIF HQKEVLETIVQSGPFGYKGDIKSAVLAMKYRFHWKWGGNPISKQVVR
29. Ring18 delARM delCterm (Δ 2-69 +Δ651-736) (5154)	MVIKQWQPNFIRRCYIIGYLPLIFCGENTTAQNYATHSDDMISKGPYGG GMTTTKFTLRILYDEFTRFMNFWTVSNEDLDLCRYVGCKLIFFKHPTVD FIVQINTQPPFLDTHLTAASIHPGIMMLSKRRILIPSLKTRPSRKHRVVVR VGAPRLFQDKWYPQSDLCDTVLLSIFATACDLQYPFGSPLTDNPCVNFQ ILGPQYKKHLSISSTMDTTNKQHYDSNLFNQTQLYNTFQTIAQLKETGQ TANISPSWSAVQNNMALSNTGENATQSKDTWYKGNTYNNHITTLAQK TRERFKGATKAALQNYPTIMSTDLYEYHSGIYSSIFLSAGRSYFETTGAY SDIIYNPFTDKGTGNIIWIDYLTKEDTIFVKNKSKCEIMDMPLWAACTGY TEFCAKYTGDSAIIYNARVLIRCPYTEPMLIDHSDPNKGFVPYSFNFGNG KMPGGSSNVPIRMRAKWYVNIFHQKEVLETIVQSGPFGYKGDIKSAVL AMKYRFHWKWGGNPISKQVVRNPCSNSSSSAAHRGPRSVQAVDPKYN TPEVTWHSWDIRRGLFGKAGIKRMQQESDALYIPPGPFKRPRR
30. Ring18 delARM delCterm	MVIKQWQPNFIRRCYIIGYLPLIFCGENTTAQNYATHSDDMISKGPYGG GMTTTKFTLRILYDEFTRFMNFWTVSNEDLDLCRYVGCKLIFFKHPTVD FIVQINTQPPFLDTHLTAASIHPGIMMLSKRRILIPSLKTRPSRKHRVVVR VGAPRLFQDKWYPQSDLCDTVLLSIFATACDLQYPFGSPLTDNPCVNFQ ILGPQYKKHLSISSTMDTTNKQHYDSNLFNQTQLYNTFQTIAQLKETGQ

(Δ 2-69 +Δ582-736) (5155)	TANISPSWSAVQNNMALSNTGENATQSKDTWYKGNTYNNHITTLAQK TRERFKGATKAALQNYPTIMSTDLYEYHSGIYSSIFLSAGRSYFETTGAY SDIIYNPFTDKGTGNIIWIDYLTKEDTIFVKNKSKCEIMDMPLWAACTGY TEFCAKYTGDSAIIYNARVLIRCPYTEPMLIDHSDPNKGFVPYSFNFGNG KMPGGSSNVPIRMRAKWYVNIFHQKEVLETIVQSGPFGYKGDIKSAVL AMKYRFHWKWGGNPISKQVVR

Table B1-13b: Ring19 constructs suitable for expression in 293 cells

#ID	Sequence
31. Ring19 delCterm (Δ600-655) (7120)	MPWYPRRRYPRRRYRWLRRWRARRPFRPRYRRRYWVRNYSRKRKLF KITTKEWQPKVIRKTHVKGTYPLFLCTKHRINNNMIQYLDSIAPEHYYG GGGFSIMQFSLQALYEEFIKAKNWWTNTNCFLPLVRYMGCSFKFYKTE FYDYIVLIERCYPLACTDEMYLSTQPSIMMLTRKCIFVPCKQNSKGKKP YKKVRVRPPSQMTTGWHFSQDLANMPLVVLKTSVCSFDRYYTDSTAK STTIGFKTLNTQTFRYHDWQEPPTTGYKPQNLLWFYGAENGSPVDPNN TIVSNLIYLGGTGPYEKGTPIKTNISNYFSEPKLWGNIFHDDYTSGTSPVF VTNKSPSEIKTAWNTIKDLTVKASGVFTLRTIPLWLPCRYNPFADKATN NKIWLVSIHSDHTEWKPIDNPLLQRTDLPLWLLVWGWQDWQKKNQQT SQPDINYLTVISSPYISCYPKLDYYVLLDEGFWEGHSTYIESITDSDKKH WYPKNRFQIETLNLIANTGPGTVKLRENQAAEGHMVYRFNFKLGGCPA PMEKICDPSKQSKYPIPNNQQQTTSLQSPENPIQTYLYDFDERRGLLTER ATKRIKQDHTSEKTVLP
32. Ring19 delCterm (Δ583-655) (7121)	MPWYPRRRYPRRRYRWLRRWRARRPFRPRYRRRYWVRNYSRKRKLF KITTKEWQPKVIRKTHVKGTYPLFLCTKHRINNNMIQYLDSIAPEHYYG GGGFSIMQFSLQALYEEFIKAKNWWTNTNCFLPLVRYMGCSFKFYKTE FYDYIVLIERCYPLACTDEMYLSTQPSIMMLTRKCIFVPCKQNSKGKKP YKKVRVRPPSQMTTGWHFSQDLANMPLVVLKTSVCSFDRYYTDSTAK

	STTIGFKTLNTQTFRYHDWQEPPTTGYKPQNLLWFYGAENGSPVDPNN TIVSNLIYLGGTGPYEKGTPIKTNISNYFSEPKLWGNIFHDDYTSGTSPVF VTNKSPSEIKTAWNTIKDLTVKASGVFTLRTIPLWLPCRYNPFADKATN NKIWLVSIHSDHTEWKPIDNPLLQRTDLPLWLLVWGWQDWQKKNQQT SQPDINYLTVISSPYISCYPKLDYYVLLDEGFWEGHSTYIESITDSDKKH WYPKNRFQIETLNLIANTGPGTVKLRENQAAEGHMVYRFNFKLGGCPA PMEKICDPSKQSKYPIPNNQQQTTSLQSPENPIQTYLYDFDERRGLLTER

Table B1-14: Ring19 delARM/C-terminal deletion: Baculovirus constructs

#ID	Sequence
33. Ring19 delARM (Δ2-49) (5146)	MTKEWQPKVIRKTHVKGTYPLFLCTKHRINNNMIQYLDSIAPEHYYGG GGFSIMQFSLQALYEEFIKAKNWWTNTNCFLPLVRYMGCSFKFYKTEF YDYIVLIERCYPLACTDEMYLSTQPSIMMLTRKCIFVPCKQNSKGKKPY KKVRVRPPSQMTTGWHFSQDLANMPLVVLKTSVCSFDRYYTDSTAKS TTIGFKTLNTQTFRYHDWQEPPTTGYKPQNLLWFYGAENGSPVDPNNTI VSNLIYLGGTGPYEKGTPIKTNISNYFSEPKLWGNIFHDDYTSGTSPVFV TNKSPSEIKTAWNTIKDLTVKASGVFTLRTIPLWLPCRYNPFADKATNN KIWLVSIHSDHTEWKPIDNPLLQRTDLPLWLLVWGWQDWQKKNQQTS QPDINYLTVISSPYISCYPKLDYYVLLDEGFWEGHSTYIESITDSDKKHW YPKNRFQIETLNLIANTGPGTVKLRENQAAEGHMVYRFNFKLGGCPAP MEKICDPSKQSKYPIPNNQQQTTSLQSPENPIQTYLYDFDERRGLLTERA TKRIKQDHTSEKTVLPFTGAATDLPILQTTSQEESSSEEEEEQQAEKKLL QLRRKQHRLRERILQLLDIQNT
34. Ring19 delARM dCterm (Δ2-49+ Δ537-655) (5147)	MTKEWQPKVIRKTHVKGTYPLFLCTKHRINNNMIQYLDSIAPEHYYGG GGFSIMQFSLQALYEEFIKAKNWWTNTNCFLPLVRYMGCSFKFYKTEF YDYIVLIERCYPLACTDEMYLSTQPSIMMLTRKCIFVPCKQNSKGKKPY KKVRVRPPSQMTTGWHFSQDLANMPLVVLKTSVCSFDRYYTDSTAKS TTIGFKTLNTQTFRYHDWQEPPTTGYKPQNLLWFYGAENGSPVDPNNTI VSNLIYLGGTGPYEKGTPIKTNISNYFSEPKLWGNIFHDDYTSGTSPVFV

	TNKSPSEIKTAWNTIKDLTVKASGVFTLRTIPLWLPCRYNPFADKATNN KIWLVSIHSDHTEWKPIDNPLLQRTDLPLWLLVWGWQDWQKKNQQTS QPDINYLTVISSPYISCYPKLDYYVLLDEGFWEGHSTYIESITDSDKKHW YPKNRFQIETLNLIANTGPGTVKLRENQAAEGHMVYRFNFKLGGCPAP MEK
35. Ring19 delARM delCterm (Δ2-49+ Δ527-655) (5148)	MTKEWQPKVIRKTHVKGTYPLFLCTKHRINNNMIQYLDSIAPEHYYGG GGFSIMQFSLQALYEEFIKAKNWWTNTNCFLPLVRYMGCSFKFYKTEF YDYIVLIERCYPLACTDEMYLSTQPSIMMLTRKCIFVPCKQNSKGKKPY KKVRVRPPSQMTTGWHFSQDLANMPLVVLKTSVCSFDRYYTDSTAKS TTIGFKTLNTQTFRYHDWQEPPTTGYKPQNLLWFYGAENGSPVDPNNTI VSNLIYLGGTGPYEKGTPIKTNISNYFSEPKLWGNIFHDDYTSGTSPVFV TNKSPSEIKTAWNTIKDLTVKASGVFTLRTIPLWLPCRYNPFADKATNN KIWLVSIHSDHTEWKPIDNPLLQRTDLPLWLLVWGWQDWQKKNQQTS QPDINYLTVISSPYISCYPKLDYYVLLDEGFWEGHSTYIESITDSDKKHW YPKNRFQIETLNLIANTGPGTVKLRENQAAEGHMVYRFNFK

Table B2-1. Ring2 cysteine deletion

1. Ring2-ORF1 delARM (Δ2-45) + All Cysteine to Serine (C57S, C64S, C112S, C131S, C220S, C223S, C626S) (SB-7078/pRTx-2683)	MLKQWQPPYKRT**S**YIKGQD**S**LIYYSNLRLGMNSTMYEKSIVPV HWPGGGSFSVSMLTLDALYDIHKL**S**RNWWTSTNQDLPLVRYK G**S**KITFYQSTFTDYIVRIHTELPANSNKLTYPNTHPLMMMMSK YKHIIPSRQTRRKKKPYTKIFVKPPPQFENKWYFATDLYKIPLL QIH**S**TA**S**NLQNPFVKPDKLSNNVTLWSLNTISIQNRNMSVDQG QSWPFKILGTQSFYFYFYTGANLPGDTTQIPVADLLPLTNPRIN RPGQSLNEAKITDHITFTEYKNKFTNYWGNPFNKHIQEHLDMIL YSLKSPEAIKNEWTTENMKWNQLNNAGTMALTPFNEPIFTQIQ YNPDRDTGEDTQLYLLSNATGTGWDPPGIPELILEGFPLWLIY WGFADFQKNLKKVTNIDTNYMLVAKTKFTQKPGTFYLVILND TFVEGNSPYEKQPLPEDNIKWYPQVQYQLEAQNKLLQTGPFTP

	NIQGQLSDNISMFYKFYFKWGGSPPKAINVENPAHQIQYPIPRN EHETTSLQSPGEAPESILYSFDYRHGNYTTTALSRISQDWALKD TVSKITEPDRQQLLKQALE**S**LQISEETQEKKEKEVQQLISNLRQ QQQLYRERIISLLKDQ

Table B2-2. Ring10 cysteine deletion with surface moiety grafting

#ID	Sequence
2. Ring10-ORF1 delARM (Δ2-51) + All Cysteine to Serine (C63S, C70S, C137S, C269S, C403S, C460S, C503S, C515S) (SB-7079/pRTx-2684)	MIVEWQPKSIRK**S**RIKGML**S**LFQTTEDRLSYNFDMYEESIIPEK LPGGGGFSIKNISLYALYQEHIHAHNIFTHTNTDRPLARYTG**S**S LKFYQSKDIDYVVTYSTSLPLRSSMGMYNSMQPSIHLMQQNK LIVPSKQTQKRRKPYIKKHISPPTQMKSQWYFQHNIANIPLLMI RTTALTLDNYYIGSRQLSTNVTIHTLNTTYIQNRDWGDRNKTY Y**S**QTLGTQRYFLYGTHSTAQNINDIKLQELIPLTNTQDYVQGF DWTEKDKHNITTYKEFLTKGAGNPFHAEWITAQNPVIHTANSP TQIEQIYTASTTTFQNKKLTDLPTPGYIFITPTVSLRYNPYKDLA ERNK**S**YFVRSKINAHGWDPEQHQELINSDLPQWLLLFGYPDYI KRTQNFALVDTNYILVDH**S**PYTNPEKTPFIPLSTSFIEGRSPYSP SDTHEPDEEDQNRWYP**S**YQYQQESINSI**S**LSGPGTPKIPKGITAE AKVKYSFNFKWGGDLPPMSTITNPTDQPTYVVPNNFNETTSLQ NPTTRPEHFLYSFDERRGQLTEKATKRLLKDWETKETSLLSTE YRFAEPTQTQAPQEDPSSEEEEESNLFERLLRQRTKQLQLKRRII QTLKDLQKLE
3. Ring10-ORF1 delARM (Δ2-51) +Buried Cysteines to Alanines (C63A, C137A, C269A,	MIVEWQPKSIRK**A**RIKGML**S**LFQTTEDRLSYNFDMYEESIIPEK LPGGGGFSIKNISLYALYQEHIHAHNIFTHTNTDRPLARYTG**A**S LKFYQSKDIDYVVTYSTSLPLRSSMGMYNSMQPSIHLMQQNK LIVPSKQTQKRRKPYIKKHISPPTQMKSQWYFQHNIANIPLLMI RTTALTLDNYYIGSRQLSTNVTIHTLNTTYIQNRDWGDRNKTY Y**A**QTLGTQRYFLYGTHSTAQNINDIKLQELIPLTNTQDYVQGF

C403A, C460A, C503A, C515A) + Exposed Cysteines to Serine (C70S) (SB-7082/pRTx-2687)	DWTEKDKHNITTYKEFLTKGAGNPFHAEWITAQNPVIHTANSP TQIEQIYTASTTTFQNKKLTDLPTPGYIFITPTVSLRYNPYKDLA ERNK**A**YFVRSKINAHGWDPEQHQELINSDLPQWLLLFGYPDYI KRTQNFALVDTNYILVDH**A**PYTNPEKTPFIPLSTSFIEGRSPYSP SDTHEPDEEDQNRWYP**A**YQYQQESINSI**A**LSGPGTPKIPKGITA EAKVKYSFNFKWGGDLPPMSTITNPTDQPTYVVPNNFNETTSL QNPTTRPEHFLYSFDERRGQLTEKATKRLLKDWETKETSLLST EYRFAEPTQTQAPQEDPSSEEEEESNLFERLLRQRTKQLQLKRR IIQTLKDLQKLE

Table B2-3. Ring10 delARM grafting with free cysteine on P2 domain

#ID	Sequence
4. Ring10-ORF1 delARM (Δ2-51) +Buried Cysteines to Alanines (C63A, C137A, C269A, C403A, C460A, C503A, C515A) + Exposed Cysteines to Serine (C70S) + P2 Cysteine for conjugation (T365C) (SB-7085/pRTx-2690)	MIVEWQPKSIRK**A**RIKGML**S**LFQTTEDRLSYNFDMYEESIIPEK LPGGGGFSIKNISLYALYQEHIHAHNIFTHTNTDRPLARYTG**A**S LKFYQSKDIDYVVTYSTSLPLRSSMGMYNSMQPSIHLMQQNK LIVPSKQTQKRRKPYIKKHISPPTQMKSQWYFQHNIANIPLLMI RTTALTLDNYYIGSRQLSTNVTIHTLNTTYIQNRDWGDRNKTY Y**A**QTLGTQRYFLYGTHSTAQNINDIKLQELIPLTNTQDYVQGF DWTEKDKHNITTYKEFLTKGAGNPFHAEWITAQNPVIHTANSP TQIEQIYTAST**C**TFQNKKLTDLPTPGYIFITPTVSLRYNPYKDLA ERNK**A**YFVRSKINAHGWDPEQHQELINSDLPQWLLLFGYPDYI KRTQNFALVDTNYILVDH**A**PYTNPEKTPFIPLSTSFIEGRSPYSP SDTHEPDEEDQNRWYP**A**YQYQQESINSI**A**LSGPGTPKIPKGITA EAKVKYSFNFKWGGDLPPMSTITNPTDQPTYVVPNNFNETTSL QNPTTRPEHFLYSFDERRGQLTEKATKRLLKDWETKETSLLST EYRFAEPTQTQAPQEDPSSEEEEESNLFERLLRQRTKQLQLKRR IIQTLKDLQKLE

5. Ring10-ORF1 delARM (Δ2-51) + All Cysteine to Serine (C63S, C70S, C137S, C269S, C403S, C460S, C503S, C515S) + P2 Cysteine for conjugation (T365C)	MIVEWQPKSIRK**S**RIKGML**S**LFQTTEDRLSYNFDMYEESIIPEK LPGGGGFSIKNISLYALYQEHIHAHNIFTHTNTDRPLARYTG**S**S LKFYQSKDIDYVVTYSTSLPLRSSMGMYNSMQPSIHLMQQNK LIVPSKQTQKRRKPYIKKHISPPTQMKSQWYFQHNIANIPLLMI RTTALTLDNYYIGSRQLSTNVTIHTLNTTYIQNRDWGDRNKTY Y**S**QTLGTQRYFLYGTHSTAQNINDIKLQELIPLTNTQDYVQGF DWTEKDKHNITTYKEFLTKGAGNPFHAEWITAQNPVIHTANSP TQIEQIYTAST**C**TFQNKKLTDLPTPGYIFITPTVSLRYNPYKDLA ERNK**S**YFVRSKINAHGWDPEQHQELINSDLPQWLLLFGYPDYI KRTQNFALVDTNYILVDH**S**PYTNPEKTPFIPLSTSFIEGRSPYSP SDTHEPDEEDQNRWYP**S**YQYQQESINSI**S**LSGPGTPKIPKGITA EAKVKYSFNFKWGGDLPPMSTITNPTDQPTYVVPNNFNETTSL QNPTTRPEHFLYSFDERRGQLTEKATKRLLKDWETKETSLLST EYRFAEPTQTQAPQEDPSSEEEEESNLFERLLRQRTKQLQLKRR IIQTLKDLQKLE
6. Ring10-ORF1 delARM (Δ2-51) + All Cysteine to Serine (C63S, C70S, C137S, C269S, C403S, C460S, C503S, C515S) + P2 Cysteine for conjugation (S284C)	MIVEWQPKSIRK**S**RIKGML**S**LFQTTEDRLSYNFDMYEESIIPEK LPGGGGFSIKNISLYALYQEHIHAHNIFTHTNTDRPLARYTG**S**S LKFYQSKDIDYVVTYSTSLPLRSSMGMYNSMQPSIHLMQQNK LIVPSKQTQKRRKPYIKKHISPPTQMKSQWYFQHNIANIPLLMI RTTALTLDNYYIGSRQLSTNVTIHTLNTTYIQNRDWGDRNKTY Y**S**QTLGTQRYFLYGTH**C**TAQNINDIKLQELIPLTNTQDYVQGF DWTEKDKHNITTYKEFLTKGAGNPFHAEWITAQNPVIHTANSP TQIEQIYTASTTTFQNKKLTDLPTPGYIFITPTVSLRYNPYKDLA ERNK**S**YFVRSKINAHGWDPEQHQELINSDLPQWLLLFGYPDYI KRTQNFALVDTNYILVDH**S**PYTNPEKTPFIPLSTSFIEGRSPYSP SDTHEPDEEDQNRWYP**S**YQYQQESINSI**S**LSGPGTPKIPKGITA EAKVKYSFNFKWGGDLPPMSTITNPTDQPTYVVPNNFNETTSL QNPTTRPEHFLYSFDERRGQLTEKATKRLLKDWETKETSLLST EYRFAEPTQTQAPQEDPSSEEEEESNLFERLLRQRTKQLQLKRR IIQTLKDLQKLE

6. Ring10-ORF1 delARM (Δ2-51) + All Cysteine to Serine (C63S, C70S, C137S, C269S, C403S, C460S, C503S, C515S) + P2 Cysteine for conjugation (N290C)	MIVEWQPKSIRK**S**RIKGML**S**LFQTTEDRLSYNFDMYEESIIPEK LPGGGGFSIKNISLYALYQEHIHAHNIFTHTNTDRPLARYTG**S**S LKFYQSKDIDYVVTYSTSLPLRSSMGMYNSMQPSIHLMQQNK LIVPSKQTQKRRKPYIKKHISPPTQMKSQWYFQHNIANIPLLMI RTTALTLDNYYIGSRQLSTNVTIHTLNTTYIQNRDWGDRNKTY Y**S**QTLGTQRYFLYGTHSTAQNI**C**DIKLQELIPLTNTQDYVQGF DWTEKDKHNITTYKEFLTKGAGNPFHAEWITAQNPVIHTANSP TQIEQIYTASTTTFQNKKLTDLPTPGYIFITPTVSLRYNPYKDLA ERNK**S**YFVRSKINAHGWDPEQHQELINSDLPQWLLLFGYPDYI KRTQNFALVDTNYILVDH**S**PYTNPEKTPFIPLSTSFIEGRSPYSP SDTHEPDEEDQNRWYP**S**YQYQQESINSI**S**LSGPGTPKIPKGITA EAKVKYSFNFKWGGDLPPMSTITNPTDQPTYVVPNNFNETTSL QNPTTRPEHFLYSFDERRGQLTEKATKRLLKDWETKETSLLST EYRFAEPTQTQAPQEDPSSEEEEESNLFERLLRQRTKQLQLKRR IIQTLKDLQKLE
8. Ring10-ORF1 delARM (Δ2-51) + All Cysteine to Serine (C63S, C70S, C137S, C269S, C403S, C460S, C503S, C515S) + P2 Cysteine for conjugation (K317C)	MIVEWQPKSIRK**S**RIKGML**S**LFQTTEDRLSYNFDMYEESIIPEK LPGGGGFSIKNISLYALYQEHIHAHNIFTHTNTDRPLARYTG**S**S LKFYQSKDIDYVVTYSTSLPLRSSMGMYNSMQPSIHLMQQNK LIVPSKQTQKRRKPYIKKHISPPTQMKSQWYFQHNIANIPLLMI RTTALTLDNYYIGSRQLSTNVTIHTLNTTYIQNRDWGDRNKTY Y**S**QTLGTQRYFLYGTHSTAQNINDIKLQELIPLTNTQDYVQGF DWTEKD**C**HNITTYKEFLTKGAGNPFHAEWITAQNPVIHTANSP TQIEQIYTASTTTFQNKKLTDLPTPGYIFITPTVSLRYNPYKDLA ERNK**S**YFVRSKINAHGWDPEQHQELINSDLPQWLLLFGYPDYI KRTQNFALVDTNYILVDH**S**PYTNPEKTPFIPLSTSFIEGRSPYSP SDTHEPDEEDQNRWYP**S**YQYQQESINSI**S**LSGPGTPKIPKGITA EAKVKYSFNFKWGGDLPPMSTITNPTDQPTYVVPNNFNETTSL QNPTTRPEHFLYSFDERRGQLTEKATKRLLKDWETKETSLLST

	EYRFAEPTQTQAPQEDPSSEEEEESNLFERLLRQRTKQLQLKRR IIQTLKDLQKLE
9. Ring10-ORF1 delARM (Δ2-51) + All Cysteine to Serine (C63S, C70S, C137S, C269S, C403S, C460S, C503S, C515S) + P2 Cysteine for conjugation (K324C)	MIVEWQPKSIRK**S**RIKGML**S**LFQTTEDRLSYNFDMYEESIIPEK LPGGGGFSIKNISLYALYQEHIHAHNIFTHTNTDRPLARYTG**S**S LKFYQSKDIDYVVTYSTSLPLRSSMGMYNSMQPSIHLMQQNK LIVPSKQTQKRRKPYIKKHISPPTQMKSQWYFQHNIANIPLLMI RTTALTLDNYYIGSRQLSTNVTIHTLNTTYIQNRDWGDRNKTY Y**S**QTLGTQRYFLYGTHSTAQNINDIKLQELIPLTNTQDYVQGF DWTEKDKHNITTY**C**EFLTKGAGNPFHAEWITAQNPVIHTANSP TQIEQIYTASTTTFQNKKLTDLPTPGYIFITPTVSLRYNPYKDLA ERNK**S**YFVRSKINAHGWDPEQHQELINSDLPQWLLLFGYPDYI KRTQNFALVDTNYILVDH**S**PYTNPEKTPFIPLSTSFIEGRSPYSP SDTHEPDEEDQNRWYP**S**YQYQQESINSI**S**LSGPGTPKIPKGITA EAKVKYSFNFKWGGDLPPMSTITNPTDQPTYVVPNNFNETTSL QNPTTRPEHFLYSFDERRGQLTEKATKRLLKDWETKETSLLST EYRFAEPTQTQAPQEDPSSEEEEESNLFERLLRQRTKQLQLKRR IIQTLKDLQKLE
10. Ring10-ORF1 delARM (Δ2-51) + All Cysteine to Serine (C63S, C70S, C137S, C269S, C403S, C460S, C503S, C515S) + P2 Cysteine for conjugation (A362C)	MIVEWQPKSIRK**S**RIKGML**S**LFQTTEDRLSYNFDMYEESIIPEK LPGGGGFSIKNISLYALYQEHIHAHNIFTHTNTDRPLARYTG**S**S LKFYQSKDIDYVVTYSTSLPLRSSMGMYNSMQPSIHLMQQNK LIVPSKQTQKRRKPYIKKHISPPTQMKSQWYFQHNIANIPLLMI RTTALTLDNYYIGSRQLSTNVTIHTLNTTYIQNRDWGDRNKTY Y**S**QTLGTQRYFLYGTHSTAQNINDIKLQELIPLTNTQDYVQGF DWTEKDKHNITTYKEFLTKGAGNPFHAEWITAQNPVIHTANSP TQIEQIYT**C**STTTFQNKKLTDLPTPGYIFITPTVSLRYNPYKDLA ERNK**S**YFVRSKINAHGWDPEQHQELINSDLPQWLLLFGYPDYI KRTQNFALVDTNYILVDH**S**PYTNPEKTPFIPLSTSFIEGRSPYSP SDTHEPDEEDQNRWYP**S**YQYQQESINSI**S**LSGPGTPKIPKGITA EAKVKYSFNFKWGGDLPPMSTITNPTDQPTYVVPNNFNETTSL

	QNPTTRPEHFLYSFDERRGQLTEKATKRLLKDWETKETSLLST EYRFAEPTQTQAPQEDPSSEEEEESNLFERLLRQRTKQLQLKRR IIQTLKDLQKLE
11. Ring10-ORF1 delARM (Δ2-51) + All Cysteine to Serine (C63S, C70S, C137S, C269S, C403S, C460S, C503S, C515S) + P2 Cysteine for conjugation (S363C)	MIVEWQPKSIRK**S**RIKGML**S**LFQTTEDRLSYNFDMYEESIIPEK LPGGGGFSIKNISLYALYQEHIHAHNIFTHTNTDRPLARYTG**S**S LKFYQSKDIDYVVTYSTSLPLRSSMGMYNSMQPSIHLMQQNK LIVPSKQTQKRRKPYIKKHISPPTQMKSQWYFQHNIANIPLLMI RTTALTLDNYYIGSRQLSTNVTIHTLNTTYIQNRDWGDRNKTY Y**S**QTLGTQRYFLYGTHSTAQNINDIKLQELIPLTNTQDYVQGF DWTEKDKHNITTYKEFLTKGAGNPFHAEWITAQNPVIHTANSP TQIEQIYTA**C**TTTFQNKKLTDLPTPGYIFITPTVSLRYNPYKDLA ERNK**S**YFVRSKINAHGWDPEQHQELINSDLPQWLLLFGYPDYI KRTQNFALVDTNYILVDH**S**PYTNPEKTPFIPLSTSFIEGRSPYSP SDTHEPDEEDQNRWYP**S**YQYQQESINSI**S**LSGPGTPKIPKGITA EAKVKYSFNFKWGGDLPPMSTITNPTDQPTYVVPNNFNETTSL QNPTTRPEHFLYSFDERRGQLTEKATKRLLKDWETKETSLLST EYRFAEPTQTQAPQEDPSSEEEEESNLFERLLRQRTKQLQLKRR IIQTLKDLQKLE
12. Ring10-ORF1 delARM (Δ2-51) + All Cysteine to Serine (C63S, C70S, C137S, C269S, C403S, C460S, C503S, C515S) + P2 Cysteine for conjugation (N369C)	MIVEWQPKSIRK**S**RIKGML**S**LFQTTEDRLSYNFDMYEESIIPEK LPGGGGFSIKNISLYALYQEHIHAHNIFTHTNTDRPLARYTG**S**S LKFYQSKDIDYVVTYSTSLPLRSSMGMYNSMQPSIHLMQQNK LIVPSKQTQKRRKPYIKKHISPPTQMKSQWYFQHNIANIPLLMI RTTALTLDNYYIGSRQLSTNVTIHTLNTTYIQNRDWGDRNKTY Y**S**QTLGTQRYFLYGTHSTAQNINDIKLQELIPLTNTQDYVQGF DWTEKDKHNITTYKEFLTKGAGNPFHAEWITAQNPVIHTANSP TQIEQIYTASTTTFQ**C**KKLTDLPTPGYIFITPTVSLRYNPYKDLA ERNK**S**YFVRSKINAHGWDPEQHQELINSDLPQWLLLFGYPDYI KRTQNFALVDTNYILVDH**S**PYTNPEKTPFIPLSTSFIEGRSPYSP SDTHEPDEEDQNRWYP**S**YQYQQESINSI**S**LSGPGTPKIPKGITA

	EAKVKYSFNFKWGGDLPPMSTITNPTDQPTYVVPNNFNETTSL QNPTTRPEHFLYSFDERRGQLTEKATKRLLKDWETKETSLLST EYRFAEPTQTQAPQEDPSSEEEEESNLFERLLRQRTKQLQLKRR IIQTLKDLQKLE
13. Ring10-ORF1 delARM (Δ2-51) + All Cysteine to Serine (C63S, C70S, C137S, C269S, C403S, C460S, C503S, C515S) + P2 Cysteine for conjugation (K371C)	MIVEWQPKSIRK**S**RIKGML**S**LFQTTEDRLSYNFDMYEESIIPEK LPGGGGFSIKNISLYALYQEHIHAHNIFTHTNTDRPLARYTG**S**S LKFYQSKDIDYVVTYSTSLPLRSSMGMYNSMQPSIHLMQQNK LIVPSKQTQKRRKPYIKKHISPPTQMKSQWYFQHNIANIPLLMI RTTALTLDNYYIGSRQLSTNVTIHTLNTTYIQNRDWGDRNKTY Y**S**QTLGTQRYFLYGTHSTAQNINDIKLQELIPLTNTQDYVQGF DWTEKDKHNITTYKEFLTKGAGNPFHAEWITAQNPVIHTANSP TQIEQIYTASTTTFQNK**C**LTDLPTPGYIFITPTVSLRYNPYKDLA ERNK**S**YFVRSKINAHGWDPEQHQELINSDLPQWLLLFGYPDYI KRTQNFALVDTNYILVDH**S**PYTNPEKTPFIPLSTSFIEGRSPYSP SDTHEPDEEDQNRWYP**S**YQYQQESINSI**S**LSGPGTPKIPKGITA EAKVKYSFNFKWGGDLPPMSTITNPTDQPTYVVPNNFNETTSL QNPTTRPEHFLYSFDERRGQLTEKATKRLLKDWETKETSLLST EYRFAEPTQTQAPQEDPSSEEEEESNLFERLLRQRTKQLQLKRR IIQTLKDLQKLE
6. Ring10-ORF1 delARM (Δ2-51) + All Cysteine to Serine (C63S, C70S, C137S, C269S, C403S, C460S, C503S, C515S) + P2 Cysteine for conjugation (Q287C)	MIVEWQPKSIRK**S**RIKGML**S**LFQTTEDRLSYNFDMYEESIIPEK LPGGGGFSIKNISLYALYQEHIHAHNIFTHTNTDRPLARYTG**S**S LKFYQSKDIDYVVTYSTSLPLRSSMGMYNSMQPSIHLMQQNK LIVPSKQTQKRRKPYIKKHISPPTQMKSQWYFQHNIANIPLLMI RTTALTLDNYYIGSRQLSTNVTIHTLNTTYIQNRDWGDRNKTY Y**S**QTLGTQRYFLYGTHSTA**C**NINDIKLQELIPLTNTQDYVQGF DWTEKDKHNITTYKEFLTKGAGNPFHAEWITAQNPVIHTANSP TQIEQIYTASTTTFQNKKLTDLPTPGYIFITPTVSLRYNPYKDLA ERNK**S**YFVRSKINAHGWDPEQHQELINSDLPQWLLLFGYPDYI KRTQNFALVDTNYILVDH**S**PYTNPEKTPFIPLSTSFIEGRSPYSP

	SDTHEPDEEDQNRWYP**S**YQYQQESINSI**S**LSGPGTPKIPKGITA EAKVKYSFNFKWGGDLPPMSTITNPTDQPTYVVPNNFNETTSL QNPTTRPEHFLYSFDERRGQLTEKATKRLLKDWETKETSLLST EYRFAEPTQTQAPQEDPSSEEEEESNLFERLLRQRTKQLQLKRR IIQTLKDLQKLE
Other P2 residues that can be replaced by Cysteines (Bold/Underlined and in Red) Y254, R263, N264, K265, L272, G273, T274, R276, H283, T285, N288, D291, Q308, D311, W312, T313, E314, D316, H318, N319, T321, T328, K329, T341, Q343, T354, Q358, T361, T364, Q368, D374, P376, P378, Y380, I381	MPWWYRRRSYNPWRRRNWFRRPRKTIYRRYRRRRRWVRRK PFYKRKIKRLNIVEWQPKSIRKCRIKGMLCLFQTTEDRLSYNFD MYEESIIPEKLPGGGGFSIKNISLYALYQEHIHAHNIFTHTNTDR PLARYTGCSLKFYQSKDIDYVVTYSTSLPLRSSMGMYNSMQPS IHLMQQNKLIVPSKQTQKRRKPYIKKHISPPTQMKSQWYFQHN IANIPLLMIRTTALTLDNYYIGSRQLSTNVTIHTLNTT**Y**IQNRD WGD**RNK**TYYCQT**LGT**Q**R**YFLYGT**H**S**T**AQ**N**IN**D**IKLQELIPLT NTQDYV**Q**GF**DWTE**K**D**K**HN**I**TTYKEFL**TK**GAGNPFHAEWI**T**A **Q**NPVIHTANSP**T**QIE**Q**IY**T**AS**T**TTF**Q**NKKLT**D**L**P**T**P**G**YI**FITPTV SLRYNPYKDLAERNKCYFVRSKINAHGWDPEQHQELINSDLP QWLLLFGYPDYIKRTQNFALVDTNYILVDHCPYTNPEKTPFIPL STSFIEGRSPYSPSDTHEPDEEDQNRWYPCYQYQQESINSICLS GPGTPKIPKGITAEAKVKYSFNFKWGGDLPPMSTITNPTDQPT YVVPNNFNETTSLQNPTTRPEHFLYSFDERRGQLTEKATKRLL KDWETKETSLLSTEYRFAEPTQTQAPQEDPSSEEEEESNLFERL LRQRTKQLQLKRRIIQTLKDLQKLE

Table B2-4. Ring10 ORF1 molecules with tags (e.g., epitope tags)

# ID	Sequence
6xHis-flag tag on Ring10 N-terminus	**MGSSHHHHHHGSDYKDDDDKSGSLEVLFQGPSGAT**MP WWYRRRSYNPWRRRNWFRRPRKTIYRRYRRRRRWVRRKP FYKRKIKRLNIVEWQPKSIRKCRIKGMLCLFQTTEDRLSYNF DMYEESIIPEKLPGGGGFSIKNISLYALYQEHIHAHNIFTHTN

	TDRPLARYTGCSLKFYQSKDIDYVVTYSTSLPLRSSMGMYN SMQPSIHLMQQNKLIVPSKQTQKRRKPYIKKHISPPTQMKSQ WYFQHNIANIPLLMIRTTALTLDNYYIGSRQLSTNVTIHTLN TTYIQNRDWGDRNKTYYCQTLGTQRYFLYGTHSTAQNINDI KLQELIPLTNTQDYVQGFDWTEKDKHNITTYKEFLTKGAGN PFHAEWITAQNPVIHTANSPTQIEQIYTASTTTFQNKKLTDLP TPGYIFITPTVSLRYNPYKDLAERNKCYFVRSKINAHGWDPE QHQELINSDLPQWLLLFGYPDYIKRTQNFALVDTNYILVDH CPYTNPEKTPFIPLSTSFIEGRSPYSPSDTHEPDEEDQNRWYP CYQYQQESINSICLSGPGTPKIPKGITAEAKVKYSFNFKWGG DLPPMSTITNPTDQPTYVVPNNFNETTSLQNPTTRPEHFLYS FDERRGQLTEKATKRLLKDWETKETSLLSTEYRFAEPTQTQ APQEDPSSEEEEESNLFERLLRQRTKQLQLKRRIIQTLKDLQ KLE
6His-flag tag on Ring10 C-terminus	MPWWYRRRSYNPWRRRNWFRRPRKTIYRRYRRRRRWVRR KPFYKRKIKRLNIVEWQPKSIRKCRIKGMLCLFQTTEDRLSY NFDMYEESIIPEKLPGGGGFSIKNISLYALYQEHIHAHNIFTH TNTDRPLARYTGCSLKFYQSKDIDYVVTYSTSLPLRSSMGM YNSMQPSIHLMQQNKLIVPSKQTQKRRKPYIKKHISPPTQM KSQWYFQHNIANIPLLMIRTTALTLDNYYIGSRQLSTNVTIH TLNTTYIQNRDWGDRNKTYYCQTLGTQRYFLYGTHSTAQN INDIKLQELIPLTNTQDYVQGFDWTEKDKHNITTYKEFLTKG AGNPFHAEWITAQNPVIHTANSPTQIEQIYTASTTTFQNKKL TDLPTPGYIFITPTVSLRYNPYKDLAERNKCYFVRSKINAHG WDPEQHQELINSDLPQWLLLFGYPDYIKRTQNFALVDTNYI LVDHCPYTNPEKTPFIPLSTSFIEGRSPYSPSDTHEPDEEDQN RWYPCYQYQQESINSICLSGPGTPKIPKGITAEAKVKYSFNF KWGGDLPPMSTITNPTDQPTYVVPNNFNETTSLQNPTTRPE HFLYSFDERRGQLTEKATKRLLKDWETKETSLLSTEYRFAE PTQTQAPQEDPSSEEEEESNLFERLLRQRTKQLQLKRRIIQTL

	KDLQKLE**GSSHHHHHHGSDYKDDDDKSGSLEVLFQGPS GAT**
6His-flag tag on Ring10 ΔC-terminus (Δ563-672)	MPWWYRRRSYNPWRRRNWFRRPRKTIYRRYRRRRRWVRR KPFYKRKIKRLNIVEWQPKSIRKCRIKGMLCLFQTTEDRLSY NFDMYEESIIPEKLPGGGGFSIKNISLYALYQEHIHAHNIFTH TNTDRPLARYTGCSLKFYQSKDIDYVVTYSTSLPLRSSMGM YNSMQPSIHLMQQNKLIVPSKQTQKRRKPYIKKHISPPTQM KSQWYFQHNIANIPLLMIRTTALTLDNYYIGSRQLSTNVTIH TLNTTYIQNRDWGDRNKTYYCQTLGTQRYFLYGTHSTAQN INDIKLQELIPLTNTQDYVQGFDWTEKDKHNITTYKEFLTKG AGNPFHAEWITAQNPVIHTANSPTQIEQIYTASTTTFQNKKL TDLPTPGYIFITPTVSLRYNPYKDLAERNKCYFVRSKINAHG WDPEQHQELINSDLPQWLLLFGYPDYIKRTQNFALVDTNYI LVDHCPYTNPEKTPFIPLSTSFIEGRSPYSPSDTHEPDEEDQN RWYPCYQYQQESINSICLSGPGTPKIPKGITAEAKVKYSFNF KWGGDLPPMSTITNPTDQPTYV**GSSHHHHHHGSDYKDDD DKSGSLEVLFQGPSGAT**
SNAP tag attachment on Ring10 C-terminus	MPWWYRRRSYNPWRRRNWFRRPRKTIYRRYRRRRRWVRR KPFYKRKIKRLNIVEWQPKSIRKCRIKGMLCLFQTTEDRLSY NFDMYEESIIPEKLPGGGGFSIKNISLYALYQEHIHAHNIFTH TNTDRPLARYTGCSLKFYQSKDIDYVVTYSTSLPLRSSMGM YNSMQPSIHLMQQNKLIVPSKQTQKRRKPYIKKHISPPTQM KSQWYFQHNIANIPLLMIRTTALTLDNYYIGSRQLSTNVTIH TLNTTYIQNRDWGDRNKTYYCQTLGTQRYFLYGTHSTAQN INDIKLQELIPLTNTQDYVQGFDWTEKDKHNITTYKEFLTKG AGNPFHAEWITAQNPVIHTANSPTQIEQIYTASTTTFQNKKL TDLPTPGYIFITPTVSLRYNPYKDLAERNKCYFVRSKINAHG WDPEQHQELINSDLPQWLLLFGYPDYIKRTQNFALVDTNYI LVDHCPYTNPEKTPFIPLSTSFIEGRSPYSPSDTHEPDEEDQN

	RWYPCYQYQQESINSICLSGPGTPKIPKGITAEAKVKYSFNF KWGGDLPPMSTITNPTDQPTYVVPNNFNETTSLQNPTTRPE HFLYSFDERRGQLTEKATKRLLKDWETKETSLLSTEYRFAE PTQTQAPQEDPSSEEEEESNLFERLLRQRTKQLQLKRRIIQTL KDLQKLE**MDKDCEMKRTTLDSPLGKLELSGCEQGLHRII FLGKGTSAADAVEVPAPAAVLGGPEPLMQATAWLNAYF HQPEAIEEFPVPALHHPVFQQESFTRQVLWKLLKVVKF GEVISYSHLAALAGNPAATAAVKTALSGNPVPILIPCHRV VQGDLDVGGYEGGLAVKEWLLAHEGHRLGKPGLG**
SNAP tag attach on Ring 10 (Δ563-672) C-terminus	MPWWYRRRSYNPWRRRNWFRRPRKTIYRRYRRRRRWVRR KPFYKRKIKRLNIVEWQPKSIRKCRIKGMLCLFQTTEDRLSY NFDMYEESIIPEKLPGGGGFSIKNISLYALYQEHIHAHNIFTH TNTDRPLARYTGCSLKFYQSKDIDYVVTYSTSLPLRSSMGM YNSMQPSIHLMQQNKLIVPSKQTQKRRKPYIKKHISPPTQM KSQWYFQHNIANIPLLMIRTTALTLDNYYIGSRQLSTNVTIH TLNTTYIQNRDWGDRNKTYYCQTLGTQRYFLYGTHSTAQN INDIKLQELIPLTNTQDYVQGFDWTEKDKHNITTYKEFLTKG AGNPFHAEWITAQNPVIHTANSPTQIEQIYTASTTTFQNKKL TDLPTPGYIFITPTVSLRYNPYKDLAERNKCYFVRSKINAHG WDPEQHQELINSDLPQWLLLFGYPDYIKRTQNFALVDTNYI LVDHCPYTNPEKTPFIPLSTSFIEGRSPYSPSDTHEPDEEDQN RWYPCYQYQQESINSICLSGPGTPKIPKGITAEAKVKYSFNF KWGGDLPPMSTITNPTDQPTYV**MDKDCEMKRTTLDSPLG KLELSGCEQGLHRIIFLGKGTSAADAVEVPAPAAVLGGP EPLMQATAWLNAYFHQPEAIEEFPVPALHHPVFQQESFT RQVLWKLLKVVKFGEVISYSHLAALAGNPAATAAVKTA LSGNPVPILIPCHRVVQGDLDVGGYEGGLAVKEWLLAH EGHRLGKPGLG**

Halo-His tag attach on Ring 10 (Δ563-672) C-terminus	MPWWYRRRSYNPWRRRNWFRRPRKTIYRRYRRRRRWVRR KPFYKRKIKRLNIVEWQPKSIRKCRIKGMLCLFQTTEDRLSY NFDMYEESIIPEKLPGGGGFSIKNISLYALYQEHIHAHNIFTH TNTDRPLARYTGCSLKFYQSKDIDYVVTYSTSLPLRSSMGM YNSMQPSIHLMQQNKLIVPSKQTQKRRKPYIKKHISPPTQM KSQWYFQHNIANIPLLMIRTTALTLDNYYIGSRQLSTNVTIH TLNTTYIQNRDWGDRNKTYYCQTLGTQRYFLYGTHSTAQN INDIKLQELIPLTNTQDYVQGFDWTEKDKHNITTYKEFLTKG AGNPFHAEWITAQNPVIHTANSPTQIEQIYTASTTTFQNKKL TDLPTPGYIFITPTVSLRYNPYKDLAERNKCYFVRSKINAHG WDPEQHQELINSDLPQWLLLFGYPDYIKRTQNFALVDTNYI LVDHCPYTNPEKTPFIPLSTSFIEGRSPYSPSDTHEPDEEDQN RWYPCYQYQQESINSICLSGPGTPKIPKGITAEAKVKYSFNF KWGGDLPPMSTITNPTDQPTYV**MAEIGTGFPFDPHYVEVL GERMHYVDVGPRDGTPVLFLHGNPTSSYVWRNIIPHVAP THRCIAPDLIGMGKSDKPDLGYFFDDHVRFMDAFIEALG LEEVVLVIHDWGSALGFHWAKRNPERVKGIAFMEFIRPI PTWDEWPEFARETFQAFRTTDVGRKLIIDQNVFIEGTLP MGVVRPLTEVEMDHYREPFLNPVDREPLWRFPNELPIA GEPANIVALVEEYMDWLHQSPVPKLLFWGTPGVLIPPAE AARLAKSLPNCKAVDIGPGLNLLQEDNPDLIGSEIARWL STLEIS**
CLIP tag attach on Ring 10 (Δ563-672) C-terminus	MPWWYRRRSYNPWRRRNWFRRPRKTIYRRYRRRRRWVRR KPFYKRKIKRLNIVEWQPKSIRKCRIKGMLCLFQTTEDRLSY NFDMYEESIIPEKLPGGGGFSIKNISLYALYQEHIHAHNIFTH TNTDRPLARYTGCSLKFYQSKDIDYVVTYSTSLPLRSSMGM YNSMQPSIHLMQQNKLIVPSKQTQKRRKPYIKKHISPPTQM KSQWYFQHNIANIPLLMIRTTALTLDNYYIGSRQLSTNVTIH TLNTTYIQNRDWGDRNKTYYCQTLGTQRYFLYGTHSTAQN INDIKLQELIPLTNTQDYVQGFDWTEKDKHNITTYKEFLTKG

	AGNPFHAEWITAQNPVIHTANSPTQIEQIYTASTTTFQNKKL TDLPTPGYIFITPTVSLRYNPYKDLAERNKCYFVRSKINAHG WDPEQHQELINSDLPQWLLLFGYPDYIKRTQNFALVDTNYI LVDHCPYTNPEKTPFIPLSTSFIEGRSPYSPSDTHEPDEEDQN RWYPCYQYQQESINSICLSGPGTPKIPKGITAEAKVKYSFNF KWGGDLPPMSTITNPTDQPTYV**MDKDCEMKRTTLDSPLG KLELSGCEQGLHRIIFLGKGTSAADAVEVPAPAAVLGGP EPLIQATAWLNAYFHQPEAIEEFPVPALHHPVFQQESFT RQVLWKLLKVVKFGEVISESHLAALVGNPAATAAVNTA LDGNPVPILIPCHRVVQGDSDVGPYLGGLAVKEWLLAH EGHRLGKPGLG**
Ring10 C-terminus (Δ563-672) + AVI-tag insertion between 284-285	MPWWYRRRSYNPWRRRNWFRRPRKTIYRRYRRRRRWVRR KPFYKRKIKRLNIVEWQPKSIRKCRIKGMLCLFQTTEDRLSY NFDMYEESIIPEKLPGGGGFSIKNISLYALYQEHIHAHNIFTH TNTDRPLARYTGCSLKFYQSKDIDYVVTYSTSLPLRSSMGM YNSMQPSIHLMQQNKLIVPSKQTQKRRKPYIKKHISPPTQM KSQWYFQHNIANIPLLMIRTTALTLDNYYIGSRQLSTNVTIH TLNTTYIQNRDWGDRNKTYYCQTLGTQRYFLYGTHS**GLND IFEAQKIEWHE**TAQNINDIKLQELIPLTNTQDYVQGFDWTE KDKHNITTYKEFLTKGAGNPFHAEWITAQNPVIHTANSPTQI EQIYTASTTTFQNKKLTDLPTPGYIFITPTVSLRYNPYKDLAE RNKCYFVRSKINAHGWDPEQHQELINSDLPQWLLLFGYPD YIKRTQNFALVDTNYILVDHCPYTNPEKTPFIPLSTSFIEGRSP YSPSDTHEPDEEDQNRWYPCYQYQQESINSICLSGPGTPKIP KGITAEAKVKYSFNFKWGGDLPPMSTITNPTDQPTYV
Ring10 delARM (Δ2-47) C-terminus (Δ563-672) + AVI-tag insertion between 284-285	MKRLNIVEWQPKSIRKCRIKGMLCLFQTTEDRLSYNFDMYE ESIIPEKLPGGGGFSIKNISLYALYQEHIHAHNIFTHTNTDRPL ARYTGCSLKFYQSKDIDYVVTYSTSLPLRSSMGMYNSMQPS IHLMQQNKLIVPSKQTQKRRKPYIKKHISPPTQMKSQWYFQ

	HNIANIPLLMIRTTALTLDNYYIGSRQLSTNVTIHTLNTTYIQ NRDWGDRNKTYYCQTLGTQRYFLYGTHS**GLNDIFEAQKIE WHE**TAQNINDIKLQELIPLTNTQDYVQGFDWTEKDKHNITT YKEFLTKGAGNPFHAEWITAQNPVIHTANSPTQIEQIYTAST TTFQNKKLTDLPTPGYIFITPTVSLRYNPYKDLAERNKCYFV RSKINAHGWDPEQHQELINSDLPQWLLLFGYPDYIKRTQNF ALVDTNYILVDHCPYTNPEKTPFIPLSTSFIEGRSPYSPSDTHE PDEEDQNRWYPCYQYQQESINSICLSGPGTPKIPKGITAEAK VKYSFNFKWGGDLPPMSTITNPTDQPTYV
Ring10 C-terminus (Δ563-672) + AVI-tag insertion between 328-329	MPWWYRRRSYNPWRRRNWFRRPRKTIYRRYRRRRRWVRR KPFYKRKIKRLNIVEWQPKSIRKCRIKGMLCLFQTTEDRLSY NFDMYEESIIPEKLPGGGGFSIKNISLYALYQEHIHAHNIFTH TNTDRPLARYTGCSLKFYQSKDIDYVVTYSTSLPLRSSMGM YNSMQPSIHLMQQNKLIVPSKQTQKRRKPYIKKHISPPTQM KSQWYFQHNIANIPLLMIRTTALTLDNYYIGSRQLSTNVTIH TLNTTYIQNRDWGDRNKTYYCQTLGTQRYFLYGTHSTAQN INDIKLQELIPLTNTQDYVQGFDWTEKDKHNITTYKEFL**GL NDIFEAQKIEWHE**TKGAGNPFHAEWITAQNPVIHTANSPT QIEQIYTASTTTFQNKKLTDLPTPGYIFITPTVSLRYNPYKDL AERNKCYFVRSKINAHGWDPEQHQELINSDLPQWLLLFGYP DYIKRTQNFALVDTNYILVDHCPYTNPEKTPFIPLSTSFIEGR SPYSPSDTHEPDEEDQNRWYPCYQYQQESINSICLSGPGTPK IPKGITAEAKVKYSFNFKWGGDLPPMSTITNPTDQPTYV
Ring10 delARM (Δ2-47) C-terminus (Δ563-672) + AVI-tag insertion between 328-329	MKRLNIVEWQPKSIRKCRIKGMLCLFQTTEDRLSYNFDMYE ESIIPEKLPGGGGFSIKNISLYALYQEHIHAHNIFTHTNTDRPL ARYTGCSLKFYQSKDIDYVVTYSTSLPLRSSMGMYNSMQPS IHLMQQNKLIVPSKQTQKRRKPYIKKHISPPTQMKSQWYFQ HNIANIPLLMIRTTALTLDNYYIGSRQLSTNVTIHTLNTTYIQ NRDWGDRNKTYYCQTLGTQRYFLYGTHSTAQNINDIKLQE LIPLTNTQDYVQGFDWTEKDKHNITTYKEFL**GLNDIFEAQK IEWHE**TKGAGNPFHAEWITAQNPVIHTANSPTQIEQIYTAST

	TTFQNKKLTDLPTPGYIFITPTVSLRYNPYKDLAERNKCYFV RSKINAHGWDPEQHQELINSDLPQWLLLFGYPDYIKRTQNF ALVDTNYILVDHCPYTNPEKTPFIPLSTSFIEGRSPYSPSDTHE PDEEDQNRWYPCYQYQQESINSICLSGPGTPKIPKGITAEAK VKYSFNFKWGGDLPPMSTITNPTDQPTYV
Ring10 delARM (Δ2-47) + P2 (Δ256-383) replaced by miRFP670nano	MKRLNIVEWQPKSIRKCRIKGMLCLFQTTEDRLSYNFDMYE ESIIPEKLPGGGGFSIKNISLYALYQEHIHAHNIFTHTNTDRPL ARYTGCSLKFYQSKDIDYVVTYSTSLPLRSSMGMYNSMQPS IHLMQQNKLIVPSKQTQKRRKPYIKKHISPPTQMKSQWYFQ HNIANIPLLMIRTTALTLDNYYIGSRQLSTNVTIHTLNTTYI**A** N**L**D**KMLNTTVTEVR**Q**F**L**QVD**R**V**CVFQFEEDYSGVVVVE AVDDRW**I**SILK**TQVRDRYFMETRGEEYSHGRYQAIADIY TANLTEC**YRDL**LT**QFQVRAILAVPILQGKKL**W**GLL**V**A**H QLAA**P**R**Q**WQTWEID**F**LKQQAVVVGIAIQQS**TPTVSLRYN PYKDLAERNKCYFVRSKINAHGWDPEQHQELINSDLPQWL LLFGYPDYIKRTQNFALVDTNYILVDHCPYTNPEKTPFIPLST SFIEGRSPYSPSDTHEPDEEDQNRWYPCYQYQQESINSICLSG PGTPKIPKGITAEAKVKYSFNFKWGGDLPPMSTITNPTDQPT YV
Ring10 delARM (Δ2-47) + ΔC-terminus (Δ563-672) + P2 (Δ256-383) replaced by SpyCatcher03 Protein + GGGS linkers	MKRLNIVEWQPKSIRKCRIKGMLCLFQTTEDRLSYNFDMY EESIIPEKLPGGGGFSIKNISLYALYQEHIHAHNIFTHTNTDR PLARYTGCSLKFYQSKDIDYVVTYSTSLPLRSSMGMYNSM QPSIHLMQQNKLIVPSKQTQKRRKPYIKKHISPPTQMKSQW YFQHNIANIPLLMIRTTALTLDNYYIGSRQLSTNVTIHTLNT TYI**GGGGSVTTLSGLSGEQGPSGDMTTEEDSATHIKFSK RDEDGRELAGATMELRDSSGKTISTWISDGHVKDFYLY PGKYTFVETAAPDGYEVATPIEFTVNEDGQVTVDGEAT EGDAHTGGGGS**TPTVSLRYNPYKDLAERNKCYFVRSKIN AHGWDPEQHQELINSDLPQWLLLFGYPDYIKRTQNFALVD TNYILVDHCPYTNPEKTPFIPLSTSFIEGRSPYSPSDTHEPDE

	EDQNRWYPCYQYQQESINSICLSGPGTPKIPKGITAEAKVK YSFNFKWGGDLPPMSTITNPTDQPTYV
GFP linker with the Ring10 C-terminus (Δ563-672)	MPWWYRRRSYNPWRRRNWFRRPRKTIYRRYRRRRRWVRR KPFYKRKIKRLNIVEWQPKSIRKCRIKGMLCLFQTTEDRLSY NFDMYEESIIPEKLPGGGGFSIKNISLYALYQEHIHAHNIFTH TNTDRPLARYTGCSLKFYQSKDIDYVVTYSTSLPLRSSMGM YNSMQPSIHLMQQNKLIVPSKQTQKRRKPYIKKHISPPTQM KSQWYFQHNIANIPLLMIRTTALTLDNYYIGSRQLSTNVTIH TLNTTYIQNRDWGDRNKTYYCQTLGTQRYFLYGTHSTAQN INDIKLQELIPLTNTQDYVQGFDWTEKDKHNITTYKEFLTKG AGNPFHAEWITAQNPVIHTANSPTQIEQIYTASTTTFQNKKL TDLPTPGYIFITPTVSLRYNPYKDLAERNKCYFVRSKINAHG WDPEQHQELINSDLPQWLLLFGYPDYIKRTQNFALVDTNYI LVDHCPYTNPEKTPFIPLSTSFIEGRSPYSPSDTHEPDEEDQN RWYPCYQYQQESINSICLSGPGTPKIPKGITAEAKVKYSFNF KWGGDLPPMSTITNPTDQPTYV**SKGEELFTGVVPILVELD GDVNGHKFSVSGEGEGDATYGKLTLKFICTTGKLPVPW PTLVTTLTYGVQCFSRYPDHMKQHDFFKSAMPEGYVQE RTIFFKDDGNYKTRAEVKFEGDTLVNRIELKGIDFKEDG NILGHKLEYNYNSHNVYIMADKQKNGIKVNFKIRHNIED GSVQLADHYQQNTPIGDGPVLLPDNHYLSTQSALSKDPN EKRDHMVLLEFVTAAGITLGMDELYK**
Ring10 delARM (Δ2-51) + P2 (Δ251-383) replaced by C-terminus of Malaria CS protein	MIVEWQPKSIRKCRIKGMLCLFQTTEDRLSYNFDMYEESIIP EKLPGGGGFSIKNISLYALYQEHIHAHNIFTHTNTDRPLARY TGCSLKFYQSKDIDYVVTYSTSLPLRSSMGMYNSMQPSIHL MQQNKLIVPSKQTQKRRKPYIKKHISPPTQMKSQWYFQHNI ANIPLLMIRTTALTLDNYYIGSRQLSTNVTIHTL**ANPNANPN ANPNANPNANPNANPNANPNANPNANPNANPNANPNANP NANPNANPNANPNKNNQGNGQGHNMPNDPNRNVDENA NANSAVKNNNNEEPSDKHIKEYLNKIQNSLSTEWSPCSV**

	TCGNGIQVRIKPGSANKPKDELDYANDIEKKICKMEKCS SVFNVVNSSIGLIMVLSFLFLNTPTVSLRYNPYKDLAERNK CYFVRSKINAHGWDPEQHQELINSDLPQWLLLFGYPDYIKR TQNFALVDTNYILVDHCPYTNPEKTPFIPLSTSFIEGRSPYSPS DTHEPDEEDQNRWYPCYQYQQESINSICLSGPGTPKIPKGIT AEAKVKYSFNFKWGGDLPPMSTITNPTDQPTYVVPNNFNET TSLQNPTTRPEHFLYSFDERRGQLTEKATKRLLKDWETKET SLLSTEYRFAEPTQTQAPQEDPSSEEEEESNLFERLLRQRTK QLQLKRRIIQTLKDLQKLE
Ring10 delARM (Δ2-51) + ΔC-terminus (Δ563-672) replaced by C-Terminus of Malaria CS protein	MIVEWQPKSIRKCRIKGMLCLFQTTEDRLSYNFDMYEESIIP EKLPGGGGFSIKNISLYALYQEHIHAHNIFTHTNTDRPLARY TGCSLKFYQSKDIDYVVTYSTSLPLRSSMGMYNSMQPSIHL MQQNKLIVPSKQTQKRRKPYIKKHISPPTQMKSQWYFQHNI ANIPLLMIRTTALTLDNYYIGSRQLSTNVTIHTLNTTYIQNRD WGDRNKTYYCQTLGTQRYFLYGTHSTAQNINDIKLQELIPL TNTQDYVQGFDWTEKDKHNITTYKEFLTKGAGNPFHAEWI TAQNPVIHTANSPTQIEQIYTASTTTFQNKKLTDLPTPGYIFIT PTVSLRYNPYKDLAERNKCYFVRSKINAHGWDPEQHQELIN SDLPQWLLLFGYPDYIKRTQNFALVDTNYILVDHCPYTNPE KTPFIPLSTSFIEGRSPYSPSDTHEPDEEDQNRWYPCYQYQQ ESINSICLSGPGTPKIPKGITAEAKVKYSFNFKWGGDLPPMST ITNPTDQPTYV**NPNANPNANPNANPNANPNANPNANPNAN PNANPNANPNANPNANPNANPNANPNANPNKNNQGNGQ GHNMPNDPNRNVDENANANSAVKNNNNEEPSDKHIKEY LNKIQNSLSTEWSPCSVTCGNGIQVRIKPGSANKPKDELD YANDIEKKICKMEKCSSVFNVVNSSIGLIMVLSFLFLN**
Ring10 delARM (Δ2-45) with Structured	MKIKRLNIVEWQPKSIRKCRIKGMLCLFQTTEDRLSYNFDM YEESIIPEKLPGGGGFSIKNISLYALYQEHIHAHNIFTHTNTDR PLARYTGCSLKFYQSKDIDYVVTYSTSLPLRSSMGMYNSMQ

Malaria Graft replacing HVR (Δ251-384)	PSIHLMQQNKLIVPSKQTQKRRKPYIKKHISPPTQMKSQWYF QHNIANIPLLMIRTTALTLDNYYIGSRQLSTNVTIHTL**EPSDK HIKEYLNKIQNSLSTEWSPCSVTCGNGIQVRIKPGSANKP KDELDYANDIEKKICKMEKCSS**PTVSLRYNPYKDLAERNK CYFVRSKINAHGWDPEQHQELINSDLPQWLLLFGYPDYIKR TQNFALVDTNYILVDHCPYTNPEKTPFIPLSTSFIEGRSPYSPS DTHEPDEEDQNRWYPCYQYQQESINSICLSGPGTPKIPKGIT AEAKVKYSFNFKWGGDLPPMSTITNPTDQPTYVVPNNFNET TSLQNPTTRPEHFLYSFDERRGQLTEKATKRLLKDWETKET SLLSTEYRFAEPTQTQAPQEDPSSEEEEESNLFERLLRQRTK QLQLKRRIIQTLKDLQKLE
Ring10 delARM (Δ2-45) with NANPx6 Malaria Graft replacing HVR (Δ251-384)	MKIKRLNIVEWQPKSIRKCRIKGMLCLFQTTEDRLSYNFDM YEESIIPEKLPGGGGFSIKNISLYALYQEHIHAHNIFTHTNTDR PLARYTGCSLKFYQSKDIDYVVTYSTSLPLRSSMGMYNSMQ PSIHLMQQNKLIVPSKQTQKRRKPYIKKHISPPTQMKSQWYF QHNIANIPLLMIRTTALTLDNYYIGSRQLSTNVTIHTL**NANP NANPNANPNANPNANPNANP**PTVSLRYNPYKDLAERNKC YFVRSKINAHGWDPEQHQELINSDLPQWLLLFGYPDYIKRT QNFALVDTNYILVDHCPYTNPEKTPFIPLSTSFIEGRSPYSPS DTHEPDEEDQNRWYPCYQYQQESINSICLSGPGTPKIPKGIT AEAKVKYSFNFKWGGDLPPMSTITNPTDQPTYVVPNNFNET TSLQNPTTRPEHFLYSFDERRGQLTEKATKRLLKDWETKET SLLSTEYRFAEPTQTQAPQEDPSSEEEEESNLFERLLRQRTK QLQLKRRIIQTLKDLQKLE

Table B2-5. Ring2 ORF1 molecules with different tags on the surface

Ring2 delARM (Δ2-45) + P2 (Δ249-383) replaced by miRFP670nano + delCTerm (611-666)	MLKQWQPPYKRTCYIKGQDCLIYYSNLRLGMNSTMYEKSI VPVHWPGGGSFSVSMLTLDALYDIHKLCRNWWTSTNQDLP LVRYKGCKITFYQSTFTDYIVRIHTELPANSNKLTYPNTHPL MMMMSKYKHIIPSRQTRRKKKPYTKIFVKPPPQFENKWYFA TDLYKIPLLQIHCTACNLQNPFVKPDKLSNNVTLWSLNTISIQ

	NRNMSVDQGQSWPFKILGTQSFYFYFYTGANLPGDTTQIPV ADLLPLTNPRINRPGQSLNEAKITDHITFTEYKNKFTNYWGN PFNKHIQEHLDMILYSLKSPEAIKNEWTTENMKWNQLNNA GTMALTPFNEPIFTQIQYNPDRDTGEDTQLYLLSNATGTGW DPPGIPELILEGFPLWLIYWGFADFQKNLKKVTNIDTNYMLV AKTKFTQKPGTFYLVILNDTFVEGNSPYEKQPLPEDNIKWYP QVQYQLEAQNKLLQTGPFTPNIQGQLSDNISMFYKFYFKWG GSPPKAINVENPAHQIQYPIPRNEHETTSLQSPGEAPESILYSF DYRHGNYTTTALSRISQDWALKDTVSK

Table B2-6. Ring19 ORF1 molecules with different tag attachments

# ID	Sequence
Ring19 delARM (Δ2-46) + P2 (Δ254-370) replaced by miRFP670nano	MKITTKEWQPKVIRKTHVKGTYPLFLCTKHRINNNMIQYLDSI APEHYYGGGGFSIMQFSLQALYEEFIKAKNWWTNTNCFLPLV RYMGCSFKFYKTEFYDYIVLIERCYPLACTDEMYLSTQPSIMM LTRKCIFVPCKQNSKGKKPYKKVRVRPPSQMTTGWHFSQDL ANMPLVVLKTSVCSFDRYYTDSTAKSTTIGFKTLNTQTF**ANL** D**KMLNT**T**VTEVR**Q**FLQVDRVCVFQFEEDYS**G**V**VVVEA**VD **DRWISILK**T**Q**V**RDRYFMETR**G**EE**Y**SH**G**RYQAIAD**I**YTA**N**LT **ECYRDLLTQFQVRAILAVPILQGK**KLWG**LLVAHQLAAPRQ** W**Q**T**WEIDFL**K**QQAVVVGIAIQQS**PLWLPCRYNPFADKATN NKIWLVSIHSDHTEWKPIDNPLLQRTDLPLWLLVWGWQDWQ KKNQQTSQPDINYLTVISSPYISCYPKLDYYVLLDEGFWEGHS TYIESITDSDKKHWYPKNRFQIETLNLIANTGPGTVKLRENQA AEGHMVYRFNFKLGGCPAPMEKICDPSKQSKYPIPNNQQQTT SLQSPENPIQTYLYDFDERRGLLTERATKRIKQDHTSEK
Ring_19_Cterm 599_SL_His	MPWYPRRRYPRRRYRWLRRWRARRPFRPRYRRRYWVRNYS RKRKLFKITTKEWQPKVIRKTHVKGTYPLFLCTKHRINNNMIQ

Δ600-655	YLDSIAPEHYYGGGGFSIMQFSLQALYEEFIKAKNWWTNTNC FLPLVRYMGCSFKFYKTEFYDYIVLIERCYPLACTDEMYLSTQ PSIMMLTRKCIFVPCKQNSKGKKPYKKVRVRPPSQMTTGWHF SQDLANMPLVVLKTSVCSFDRYYTDSTAKSTTIGFKTLNTQTF RYHDWQEPPTTGYKPQNLLWFYGAENGSPVDPNNTIVSNLIY LGGTGPYEKGTPIKTNISNYFSEPKLWGNIFHDDYTSGTSPVF VTNKSPSEIKTAWNTIKDLTVKASGVFTLRTIPLWLPCRYNPF ADKATNNKIWLVSIHSDHTEWKPIDNPLLQRTDLPLWLLVWG WQDWQKKNQQTSQPDINYLTVISSPYISCYPKLDYYVLLDEG FWEGHSTYIESITDSDKKHWYPKNRFQIETLNLIANTGPGTVK LRENQAAEGHMVYRFNFKLGGCPAPMEKICDPSKQSKYPIPN NQQQTTSLQSPENPIQTYLYDFDERRGLLTERATKRIKQDHTS EKTVLP**GSSGSALEVLFEGPGSHHHHHHHHHH**
Ring_19_Cterm 599_LL_His Δ600-655	MPWYPRRRYPRRRYRWLRRWRARRPFRPRYRRRYWVRNYS RKRKLFKITTKEWQPKVIRKTHVKGTYPLFLCTKHRINNNMIQ YLDSIAPEHYYGGGGFSIMQFSLQALYEEFIKAKNWWTNTNC FLPLVRYMGCSFKFYKTEFYDYIVLIERCYPLACTDEMYLSTQ PSIMMLTRKCIFVPCKQNSKGKKPYKKVRVRPPSQMTTGWHF SQDLANMPLVVLKTSVCSFDRYYTDSTAKSTTIGFKTLNTQTF RYHDWQEPPTTGYKPQNLLWFYGAENGSPVDPNNTIVSNLIY LGGTGPYEKGTPIKTNISNYFSEPKLWGNIFHDDYTSGTSPVF VTNKSPSEIKTAWNTIKDLTVKASGVFTLRTIPLWLPCRYNPF ADKATNNKIWLVSIHSDHTEWKPIDNPLLQRTDLPLWLLVWG WQDWQKKNQQTSQPDINYLTVISSPYISCYPKLDYYVLLDEG FWEGHSTYIESITDSDKKHWYPKNRFQIETLNLIANTGPGTVK LRENQAAEGHMVYRFNFKLGGCPAPMEKICDPSKQSKYPIPN NQQQTTSLQSPENPIQTYLYDFDERRGLLTERATKRIKQDHTS EKTVLP**GSSGSAGSSGSAGSSGSALEVLFEGPGSHHHHHHHH HH**
Ring_19_Cterm 599_SL_GFPHis	MPWYPRRRYPRRRYRWLRRWRARRPFRPRYRRRYWVRNYS RKRKLFKITTKEWQPKVIRKTHVKGTYPLFLCTKHRINNNMIQ

Δ600-655	YLDSIAPEHYYGGGGFSIMQFSLQALYEEFIKAKNWWTNTNC FLPLVRYMGCSFKFYKTEFYDYIVLIERCYPLACTDEMYLSTQ PSIMMLTRKCIFVPCKQNSKGKKPYKKVRVRPPSQMTTGWHF SQDLANMPLVVLKTSVCSFDRYYTDSTAKSTTIGFKTLNTQTF RYHDWQEPPTTGYKPQNLLWFYGAENGSPVDPNNTIVSNLIY LGGTGPYEKGTPIKTNISNYFSEPKLWGNIFHDDYTSGTSPVF VTNKSPSEIKTAWNTIKDLTVKASGVFTLRTIPLWLPCRYNPF ADKATNNKIWLVSIHSDHTEWKPIDNPLLQRTDLPLWLLVWG WQDWQKKNQQTSQPDINYLTVISSPYISCYPKLDYYVLLDEG FWEGHSTYIESITDSDKKHWYPKNRFQIETLNLIANTGPGTVK LRENQAAEGHMVYRFNFKLGGCPAPMEKICDPSKQSKYPIPN NQQQTTSLQSPENPIQTYLYDFDERRGLLTERATKRIKQDHTS EKTVLP**GSSGSALEVLFEGPGSMVSKGEELFTGVVPILVEL DGDVNGHKFSVSGEGEGDATYGKLTLKFICTTGKLPVPW PT LVTTLTYGVQCFSRYPDHMKQHDFFKSAMPEGYVQER TIFFKDDGNYKTRAEVKFEGDTLVNRIELKGIDFKEDGNIL GHKLEYNYNSHNVYIMADKQKNGIKVNFKIRHNIEDGSVQ LADHYQQNTPIGDGPVLLPDNHYLSTQSALSKDPNEKRDH MVLLEFVTAAGITLGMDELYKHHHHHHHHHH**
Ring_19_Cterm 599_LL_GFPHis Δ600-655	MPWYPRRRYPRRRYRWLRRWRARRPFRPRYRRRYWVRNYS RKRKLFKITTKEWQPKVIRKTHVKGTYPLFLCTKHRINNNMIQ YLDSIAPEHYYGGGGFSIMQFSLQALYEEFIKAKNWWTNTNC FLPLVRYMGCSFKFYKTEFYDYIVLIERCYPLACTDEMYLSTQ PSIMMLTRKCIFVPCKQNSKGKKPYKKVRVRPPSQMTTGWHF SQDLANMPLVVLKTSVCSFDRYYTDSTAKSTTIGFKTLNTQTF RYHDWQEPPTTGYKPQNLLWFYGAENGSPVDPNNTIVSNLIY LGGTGPYEKGTPIKTNISNYFSEPKLWGNIFHDDYTSGTSPVF VTNKSPSEIKTAWNTIKDLTVKASGVFTLRTIPLWLPCRYNPF ADKATNNKIWLVSIHSDHTEWKPIDNPLLQRTDLPLWLLVWG WQDWQKKNQQTSQPDINYLTVISSPYISCYPKLDYYVLLDEG FWEGHSTYIESITDSDKKHWYPKNRFQIETLNLIANTGPGTVK

	LRENQAAEGHMVYRFNFKLGGCPAPMEKICDPSKQSKYPIPN NQQQTTSLQSPENPIQTYLYDFDERRGLLTERATKRIKQDHTS EKTVLP**GSSGSAGSSGSAGSSGSALEVLFEGPGSMVSKGEE** **LFTGVVPILVELDGDVNGHKFSVSGEGEGDATYGKLTLKF** **ICTTGKLPVPWPTLVTTLTYGVQCFSRYPDHMKQHDFFKS** **AMPEGYVQERTIFFKDDGNYKTRAEVKFEGDTLVNRIELK** **GIDFKEDGNILGHKLEYNYNSHNVYIMADKQKNGIKVNFK** **IRHNIEDGSVQLADHYQQNTPIGDGPVLLPDNHYLSTQSAL** **SKDPNEKRDHMVLLEFVTAAGITLGMDELYKHHHHHHH** **HH**
Ring_19_Cterm 599_SL_HA Δ600-655	MPWYPRRRYPRRRYRWLRRWRARRPFRPRYRRRYWVRNYS RKRKLFKITTKEWQPKVIRKTHVKGTYPLFLCTKHRINNNMIQ YLDSIAPEHYYGGGGFSIMQFSLQALYEEFIKAKNWWTNTNC FLPLVRYMGCSFKFYKTEFYDYIVLIERCYPLACTDEMYLSTQ PSIMMLTRKCIFVPCKQNSKGKKPYKKVRVRPPSQMTTGWHF SQDLANMPLVVLKTSVCSFDRYYTDSTAKSTTIGFKTLNTQTF RYHDWQEPPTTGYKPQNLLWFYGAENGSPVDPNNTIVSNLIY LGGTGPYEKGTPIKTNISNYFSEPKLWGNIFHDDYTSGTSPVF VTNKSPSEIKTAWNTIKDLTVKASGVFTLRTIPLWLPCRYNPF ADKATNNKIWLVSIHSDHTEWKPIDNPLLQRTDLPLWLLVWG WQDWQKKNQQTSQPDINYLTVISSPYISCYPKLDYYVLLDEG FWEGHSTYIESITDSDKKHWYPKNRFQIETLNLIANTGPGTVK LRENQAAEGHMVYRFNFKLGGCPAPMEKICDPSKQSKYPIPN NQQQTTSLQSPENPIQTYLYDFDERRGLLTERATKRIKQDHTS EKTVLP**GSSGSALEVLFEGPGSYPYDVPDYA**
Ring_19_Cterm 599_LL_HA Δ600-655	MPWYPRRRYPRRRYRWLRRWRARRPFRPRYRRRYWVRNYS RKRKLFKITTKEWQPKVIRKTHVKGTYPLFLCTKHRINNNMIQ YLDSIAPEHYYGGGGFSIMQFSLQALYEEFIKAKNWWTNTNC FLPLVRYMGCSFKFYKTEFYDYIVLIERCYPLACTDEMYLSTQ PSIMMLTRKCIFVPCKQNSKGKKPYKKVRVRPPSQMTTGWHF SQDLANMPLVVLKTSVCSFDRYYTDSTAKSTTIGFKTLNTQTF

	RYHDWQEPPTTGYKPQNLLWFYGAENGSPVDPNNTIVSNLIY LGGTGPYEKGTPIKTNISNYFSEPKLWGNIFHDDYTSGTSPVF VTNKSPSEIKTAWNTIKDLTVKASGVFTLRTIPLWLPCRYNPF ADKATNNKIWLVSIHSDHTEWKPIDNPLLQRTDLPLWLLVWG WQDWQKKNQQTSQPDINYLTVISSPYISCYPKLDYYVLLDEG FWEGHSTYIESITDSDKKHWYPKNRFQIETLNLIANTGPGTVK LRENQAAEGHMVYRFNFKLGGCPAPMEKICDPSKQSKYPIPN NQQQTTSLQSPENPIQTYLYDFDERRGLLTERATKRIKQDHTS EKTVLP**GSSGSAGSSGSAGSSGSALEVLFEGPGSYPYDVPDY A**
Ring_19_Cterm 599_SL_FLAG Δ600-655	MPWYPRRRYPRRRYRWLRRWRARRPFRPRYRRRYWVRNYS RKRKLFKITTKEWQPKVIRKTHVKGTYPLFLCTKHRINNNMIQ YLDSIAPEHYYGGGGFSIMQFSLQALYEEFIKAKNWWTNTNC FLPLVRYMGCSFKFYKTEFYDYIVLIERCYPLACTDEMYLSTQ PSIMMLTRKCIFVPCKQNSKGKKPYKKVRVRPPSQMTTGWHF SQDLANMPLVVLKTSVCSFDRYYTDSTAKSTTIGFKTLNTQTF RYHDWQEPPTTGYKPQNLLWFYGAENGSPVDPNNTIVSNLIY LGGTGPYEKGTPIKTNISNYFSEPKLWGNIFHDDYTSGTSPVF VTNKSPSEIKTAWNTIKDLTVKASGVFTLRTIPLWLPCRYNPF ADKATNNKIWLVSIHSDHTEWKPIDNPLLQRTDLPLWLLVWG WQDWQKKNQQTSQPDINYLTVISSPYISCYPKLDYYVLLDEG FWEGHSTYIESITDSDKKHWYPKNRFQIETLNLIANTGPGTVK LRENQAAEGHMVYRFNFKLGGCPAPMEKICDPSKQSKYPIPN NQQQTTSLQSPENPIQTYLYDFDERRGLLTERATKRIKQDHTS EKTVLP**GSSGSALEVLFEGPGSDYKDDDDK**
Ring_19_Cterm 599_LL_FLAG Δ600-655	MPWYPRRRYPRRRYRWLRRWRARRPFRPRYRRRYWVRNYS RKRKLFKITTKEWQPKVIRKTHVKGTYPLFLCTKHRINNNMIQ YLDSIAPEHYYGGGGFSIMQFSLQALYEEFIKAKNWWTNTNC FLPLVRYMGCSFKFYKTEFYDYIVLIERCYPLACTDEMYLSTQ PSIMMLTRKCIFVPCKQNSKGKKPYKKVRVRPPSQMTTGWHF SQDLANMPLVVLKTSVCSFDRYYTDSTAKSTTIGFKTLNTQTF

	RYHDWQEPPTTGYKPQNLLWFYGAENGSPVDPNNTIVSNLIY LGGTGPYEKGTPIKTNISNYFSEPKLWGNIFHDDYTSGTSPVF VTNKSPSEIKTAWNTIKDLTVKASGVFTLRTIPLWLPCRYNPF ADKATNNKIWLVSIHSDHTEWKPIDNPLLQRTDLPLWLLVWG WQDWQKKNQQTSQPDINYLTVISSPYISCYPKLDYYVLLDEG FWEGHSTYIESITDSDKKHWYPKNRFQIETLNLIANTGPGTVK LRENQAAEGHMVYRFNFKLGGCPAPMEKICDPSKQSKYPIPN NQQQTTSLQSPENPIQTYLYDFDERRGLLTERATKRIKQDHTS EKTVLP**GSSGSAGSSGSAGSSGSALEVLFEGPGSDYKDDDD K**

Table B4-1. Replacement of Ring 10 structural arginine-rich region with BFDV ARM

1. Ring10 ARM replaced with BFDV (1-44)	**MWGTSNCACAKFQIRRRYARPYRRRHIRRYRRRRRHFRRRRFTT NR**KIKRLNIVEWQPKSIRKCRIKGMLCLFQTTEDRLSYNFDMYEESIIP EKLPGGGGFSIKNISLYALYQEHIHAHNIFTHTNTDRPLARYTGCSLKF YQSKDIDYVVTYSTSLPLRSSMGMYNSMQPSIHLMQQNKLIVPSKQT QKRRKPYIKKHISPPTQMKSQWYFQHNIANIPLLMIRTTALTLDNYYI GSRQLSTNVTIHTLNTTYIQNRDWGDRNKTYYCQTLGTQRYFLYGTH STAQNINDIKLQELIPLTNTQDYVQGFDWTEKDKHNITTYKEFLTKGA GNPFHAEWITAQNPVIHTANSPTQIEQIYTASTTTFQNKKLTDLPTPGY IFITPTVSLRYNPYKDLAERNKCYFVRSKINAHGWDPEQHQELINSDL PQWLLLFGYPDYIKRTQNFALVDTNYILVDHCPYTNPEKTPFIPLSTSF IEGRSPYSPSDTHEPDEEDQNRWYPCYQYQQESINSICLSGPGTPKIPK GITAEAKVKYSFNFKWGGDLPPMSTITNPTDQPTYVVPNNFNETTSLQ NPTTRPEHFLYSFDERRGQLTEKATKRLLKDWETKETSLLSTEYRFAE PTQTQAPQEDPSSEEEEESNLFERLLRQRTKQLQLKRRIIQTLKDLQKL E

Table B4-2. Interchange between different Anellovirus sequences, e.g., between Ring 2 and 9

# ID	Sequence

(1426) Ring 2 Nterminal HISFLAG-3CProtease-ORF1	**MGSSHHHHHHGSDYKDDDDKSGSLEVLFQGPSGAT**MPYYYRRR RYNYRRPRWYGRGWIRRPFRRRFRRKRRVRPTYTTIPLKQW QPPYKRTCYIKGQDCLIYYSNLRLGMNSTMYEKSIVPVHWP GGGSFSVSMLTLDALYDIHKLCRNWWTSTNQDLPLVRYKGC KITFYQSTFTDYIVRIHTELPANSNKLTYPNTHPLMMMMSK YKHIIPSRQTRRKKKPYTKIFVKPPPQFENKWYFATDLYKI PLLQIHCTACNLQNPFVKPDKLSNNVTLWSLNTISIQNRNM SVDQGQSWPFKILGTQSFYFYFYTGANLPGDTTQIPVADLL PLTNPRINRPGQSLNEAKITDHITFTEYKNKFTNYWGNPFN KHIQEHLDMILYSLKSPEAIKNEWTTENMKWNQLNNAGTMA LTPFNEPIFTQIQYNPDRDTGEDTQLYLLSNATGTGWDPPG IPELILEGFPLWLIYWGFADFQKNLKKVTNIDTNYMLVAKT KFTQKPGTFYLVILNDTFVEGNSPYEKQPLPEDNIKWYPQV QYQLEAQNKLLQTGPFTPNIQGQLSDNISMFYKFYFKWGGS PPKAINVENPAHQIQYPIPRNEHETTSLQSPGEAPESILYS FDYRHGNYTTTALSRISQDWALKDTVSKITEPDRQQLLKQA LECLQISEETQEKKEKEVQQLISNLRQQQQLYRERIISLLK DQ
(1433) Ring 9 Nterminal HISFLAG-3CProtease-ORF1	**MGSSHHHHHHGSDYKDDDDKSGSLEVLFQGPSGAT**MPPYWRQK YYRRRYRPFSWRTRRIIQRRKRWRYRKPRKTYWRRKLRVRK RFYKRKLKKIVLKQFQPKIIRRCTIFGTICLFQGSPERANN NYIQTIYSYVPDKEPGGGGWTLITESLSSLWEDWEHLKNVW TQSNAGLPLVRYGGVTLYFYQSAYTDYIAQVFNCYPMTDTK YTHADSAPNRMLLKKHVIRVPSRETRKKRKPYKRVRVGPPS QMQNKWYFQRDICEIPLIMIAATAVDFRYPFCASDCASNNL TLTCLNPLLFQNQDFDHPSDTQGYFPKPGVYLYSTQRSNKP SSSDCIYLGNTKDNQEGKSASSLMTLKTQKITDWGNPFWHY YIDGSKKIFSYFKPPSQLDSSDFEHMTELAEPMFIQVRYNP ERDTGQGNLIYVTENFRGQHWDPPSSDNLKLDGFPLYDMCW

	GFIDWIEKVHETENLLTNYCFCIRSSAFNEKKTVFIPVDHS FLTGFSPYETPVKSSDQAHWHPQIRFQTKSINDICLTGPGC ARSPYGNYMQAKMSYKFHVKWGGCPKTYEKPYDPCSQPNWT IPHNLNETIQIQNPNTCPQTELQEWDWRRDIVTKKAIERIR QHTEPHETLQISTGSKHNPPVHRQTSPWTDSETDSEEEKDQ TQEIQIQLNKLRKHQQHLKQQLKQYLKPQNIE
(1707) Ring 2 ORF1 with ARG arm of Ring 9 (Ring 291)	**MPPYWRQKYYRRRYRPFSWRTRRIIQRRKRWRYRKPRKTY WRRKLRVRK** RRVRPTYTTIPLKQWQPPYKRTCYIKGQDCLIYYSNLRLGM NSTMYEKSIVPVHWPGGGSFSVSMLTLDALYDIHKLCRNWWTST NQDLPLVRYKGCKITFYQSTFTDYIVRIHTELPANSNK LTYPNTHPLMMMMSKYKHIIPSRQTRRKKKPYTKIFVKPPP QFENKWYFATDLYKIPLLQIHCTACNLQNPFVKPDKLSNNV TLWSLNTISIQNRNMSVDQGQSWPFKILGTQSFYFYFYTGA NLPGDTTQIPVADLLPLTNPRINRPGQSLNEAKITDHITFT EYKNKFTNYWGNPFNKHIQEHLDMILYSLKSPEAIKNEWTT ENMKWNQLNNAGTMALTPFNEPIFTQIQYNPDRDTGEDTQL YLLSNATGTGWDPPGIPELILEGFPLWLIYWGFADFQKNLK KVTNIDTNYMLVAKTKFTQKPGTFYLVILNDTFVEGNSPYE KQPLPEDNIKWYPQVQYQLEAQNKLLQTGPFTPNIQGQLSD NISMFYKFYFKWGGSPPKAINVENPAHQIQYPIPRNEHETT SLQSPGEAPESILYSFDYRHGNYTTTALSRISQDWALKDTV SKITEPDRQQLLKQALECLQISEETQEKKEKEVQQLISNLR QQQQLYRERIISLLKDQ
(1708) Ring 2 ORF1 with ARG arm and Beta strands 1 + 2 722 epitope of Ring 9	**MPPYWRQK YYRRRYRPFSWRTRRIIQRRKRWRYRKPRKTYWRRKLRVRK RFYKRKLKKIVLKQFQPKIIRRCTIFGTICLFQG**SNLRLGM NSTMYEKSIVPVHWPGGGSFSVSMLTLDALYDIHKLCRNWW TSTNQDLPLVRYKGCKITFYQSTFTDYIVRIHTELPANSNK LTYPNTHPLMMMMSKYKHIIPSRQTRRKKKPYTKIFVKPPP QFENKWYFATDLYKIPLLQIHCTACNLQNPFVKPDKLSNNV

	TLWSLNTISIQNRNMSVDQGQSWPFKILGTQSFYFYFYTGA NLPGDTTQIPVADLLPLTNPRINRPGQSLNEAKITDHITFT EYKNKFTNYWGNPFNKHIQEHLDMILYSLKSPEAIKNEWTT ENMKWNQLNNAGTMALTPFNEPIFTQIQYNPDRDTGEDTQL YLLSNATGTGWDPPGIPELILEGFPLWLIYWGFADFQKNLK KVTNIDTNYMLVAKTKFTQKPGTFYLVILNDTFVEGNSPYE KQPLPEDNIKWYPQVQYQLEAQNKLLQTGPFTPNIQGQLSD NISMFYKFYFKWGGSPPKAINVENPAHQIQYPIPRNEHETT SLQSPGEAPESILYSFDYRHGNYTTTALSRISQDWALKDTV SKITEPDRQQLLKQALECLQISEETQEKKEKEVQQLISNLR QQQQLYRERIISLLKDQ
3. (1721) Ring 9 with LYS/HIS mutations in ARG arm and first beta strand	MPPYWRQKYYRRRYRPFSWRTRRIIQRR**H**RWRYRKPR**H**TYWRR **H**LRVR**H**RFY**H**R**H**L**HH**IVLKQFQPKIIRRCTIFGTICLFQGSPERAN NNYIQTIYSYVPDKEPGGGGWTLITESLSSLWEDWEHLKNVWTQ SNAGLPLVRYGGVTLYFYQSAYTDYIAQVFNCYPMTDTKYTHA DSAPNRMLLKKHVIRVPSRETRKKRKPYKRVRVGPPSQMQNKW YFQRDICEIPLIMIAATAVDFRYPFCASDCASNNLTLTCLNPLLFQ NQDFDHPSDTQGYFPKPGVYLYSTQRSNKPSSSDCIYLGNTKDNQ EGKSASSLMTLKTQKITDWGNPFWHYYIDGSKKIFSYFKPPSQLD SSDFEHMTELAEPMFIQVRYNPERDTGQGNLIYVTENFRGQHWD PPSSDNLKLDGFPLYDMCWGFIDWIEKVHETENLLTNYCFCIRSS AFNEKKTVFIPVDHSFLTGFSPYETPVKSSDQAHWHPQIRFQTKSI NDICLTGPGCARSPYGNYMQAKMSYKFHVKWGGCPKTYEKPYD PCSQPNWTIPHNLNETIQIQNPNTCPQTELQEWDWRRDIVTKKAI ERIRQHTEPHETLQISTGSKHNPPVHRQTSPWTDSETDSEEEKDQT QEIQIQLNKLRKHQQHLKQQLKQYLKPQNIE
4. (1722) Ring 9 with ARG arm of BFDV	**MWGTSNCACAKFQIRRRYARPYRRRHIRRYRRRRRHFRRRR FTTNR**RKRFYKRKLKKIVLKQFQPKIIRRCTIFGTICLFQGSPERA NNNYIQTIYSYVPDKEPGGGGWTLITESLSSLWEDWEHLKNVWT QSNAGLPLVRYGGVTLYFYQSAYTDYIAQVFNCYPMTDTKYTH ADSAPNRMLLKKHVIRVPSRETRKKRKPYKRVRVGPPSQMQNK

	WYFQRDICEIPLIMIAATAVDFRYPFCASDCASNNLTLTCLNPLLF QNQDFDHPSDTQGYFPKPGVYLYSTQRSNKPSSSDCIYLGNTKDN QEGKSASSLMTLKTQKITDWGNPFWHYYIDGSKKIFSYFKPPSQL DSSDFEHMTELAEPMFIQVRYNPERDTGQGNLIYVTENFRGQHW DPPSSDNLKLDGFPLYDMCWGFIDWIEKVHETENLLTNYCFCIRS SAFNEKKTVFIPVDHSFLTGFSPYETPVKSSDQAHWHPQIRFQTKS INDICLTGPGCARSPYGNYMQAKMSYKFHVKWGGCPKTYEKPY DPCSQPNWTIPHNLNETIQIQNPNTCPQTELQEWDWRRDIVTKKA IERIRQHTEPHETLQISTGSKHNPPVHRQTSPWTDSETDSEEEKDQ TQEIQIQLNKLRKHQQHLKQQLKQYLKPQNIE
5. (1723) Ring 9 with beta strands F and G of BFDV capsid protein	MPPYWRQKYYRRRYRPFSWRTRRIIQRRKRWRYRKPRKTYWRR KLRVRKRFYKRKLKKIVLKQFQPKIIRRCTIFGTICLFQGSPERAN NNYIQTIYSYVPDKEPGGGGWTLITESLSSLWEDWEHLKNVWTQ SNAGLPLVRYGGVTLYFYQSAYTDYIAQVFNCYPMTDTKYTHA DSAPNRMLLKKH**AKKWF**SRETRKKRKP**GFKRLL**GPPSQMQNK WYFQRDICEIPLIMIAATAVDFRYPFCASDCASNNLTLTCLNPLLF QNQDFDHPSDTQGYFPKPGVYLYSTQRSNKPSSSDCIYLGNTKDN QEGKSASSLMTLKTQKITDWGNPFWHYYIDGSKKIFSYFKPPSQL DSSDFEHMTELAEPMFIQVRYNPERDTGQGNLIYVTENFRGQHW DPPSSDNLKLDGFPLYDMCWGFIDWIEKVHETENLLTNYCFCIRS SAFNEKKTVFIPVDHSFLTGFSPYETPVKSSDQAHWHPQIRFQTKS INDICLTGPGCARSPYGNYMQAKMSYKFHVKWGGCPKTYEKPY DPCSQPNWTIPHNLNETIQIQNPNTCPQTELQEWDWRRDIVTKKA IERIRQHTEPHETLQISTGSKHNPPVHRQTSPWTDSETDSEEEKDQ TQEIQIQLNKLRKHQQHLKQQLKQYLKPQNIE
6. (1724) Ring 2 with beta C of Ring 9	MPYYYRRRRYNYRRPRWYGRGWIRRPFRRRFRRKRRVRPTYTTI PLKQWQPPYKR**RCTIFGTICLFQG**SNLRLGMNSTMYEKSIVPVH WPGGGSFSVSMLTLDALYDIHKLCRNWWTSTNQDLPLVRYKGC KITFYQSTFTDYIVRIHTELPANSNKLTYPNTHPLMMMMSKYKHII PSRQTRRKKKPYTKIFVKPPPQFENKWYFATDLYKIPLLQIHCTAC NLQNPFVKPDKLSNNVTLWSLNTISIQNRNMSVDQGQSWPFKILG

	TQSFYFYFYTGANLPGDTTQIPVADLLPLTNPRINRPGQSLNEAKI TDHITFTEYKNKFTNYWGNPFNKHIQEHLDMILYSLKSPEAIKNE WTTENMKWNQLNNAGTMALTPFNEPIFTQIQYNPDRDTGEDTQL YLLSNATGTGWDPPGIPELILEGFPLWLIYWGFADFQKNLKKVTN IDTNYMLVAKTKFTQKPGTFYLVILNDTFVEGNSPYEKQPLPEDN IKWYPQVQYQLEAQNKLLQTGPFTPNIQGQLSDNISMFYKFYFK WGGSPPKAINVENPAHQIQYPIPRNEHETTSLQSPGEAPESILYSFD YRHGNYTTTALSRISQDWALKDTVSKITEPDRQQLLKQALECLQI SEETQEKKEKEVQQLISNLRQQQQLYRERIISLLKDQ
7. (1725) Ring 2 with linker 1 of Ring 9	MPYYYRRRRYNYRRPRWYGRGWIRRPFRRRFRRKRRVRPTYTTI PLKQWQPPYKRTCYIKGQDCLIYY**SPERANNNYIQTIYSYVPDK** **EPGGGGWTLITESLSSLWEDWEHLKNVWTQSNAGLP**LVRYK GCKITFYQSTFTDYIVRIHTELPANSNKLTYPNTHPLMMMMSKYK HIIPSRQTRRKKKPYTKIFVKPPPQFENKWYFATDLYKIPLLQIHCT ACNLQNPFVKPDKLSNNVTLWSLNTISIQNRNMSVDQGQSWPFK ILGTQSFYFYFYTGANLPGDTTQIPVADLLPLTNPRINRPGQSLNE AKITDHITFTEYKNKFTNYWGNPFNKHIQEHLDMILYSLKSPEAIK NEWTTENMKWNQLNNAGTMALTPFNEPIFTQIQYNPDRDTGEDT QLYLLSNATGTGWDPPGIPELILEGFPLWLIYWGFADFQKNLKKV TNIDTNYMLVAKTKFTQKPGTFYLVILNDTFVEGNSPYEKQPLPE DNIKWYPQVQYQLEAQNKLLQTGPFTPNIQGQLSDNISMFYKFYF KWGGSPPKAINVENPAHQIQYPIPRNEHETTSLQSPGEAPESILYSF DYRHGNYTTTALSRISQDWALKDTVSKITEPDRQQLLKQALECL QISEETQEKKEKEVQQLISNLRQQQQLYRERIISLLKDQ
8. (1726) Ring 2 with beta strand D of Ring 9	MPYYYRRRRYNYRRPRWYGRGWIRRPFRRRFRRKRRVRPTYTTI PLKQWQPPYKRTCYIKGQDCLIYYSNLRLGMNSTMYEKSIVPVH WPGGGSFSVSMLTLDALYDIHKLCRNWWTSTNQD**LPLVRYGG** **VTLYF**YQSTFTDYIVRIHTELPANSNKLTYPNTHPLMMMMSKYK HIIPSRQTRRKKKPYTKIFVKPPPQFENKWYFATDLYKIPLLQIHCT ACNLQNPFVKPDKLSNNVTLWSLNTISIQNRNMSVDQGQSWPFK ILGTQSFYFYFYTGANLPGDTTQIPVADLLPLTNPRINRPGQSLNE

	AKITDHITFTEYKNKFTNYWGNPFNKHIQEHLDMILYSLKSPEAIK NEWTTENMKWNQLNNAGTMALTPFNEPIFTQIQYNPDRDTGEDT QLYLLSNATGTGWDPPGIPELILEGFPLWLIYWGFADFQKNLKKV TNIDTNYMLVAKTKFTQKPGTFYLVILNDTFVEGNSPYEKQPLPE DNIKWYPQVQYQLEAQNKLLQTGPFTPNIQGQLSDNISMFYKFYF KWGGSPPKAINVENPAHQIQYPIPRNEHETTSLQSPGEAPESILYSF DYRHGNYTTTALSRISQDWALKDTVSKITEPDRQQLLKQALECL QISEETQEKKEKEVQQLISNLRQQQQLYRERIISLLKDQ
9. (1727) Ring 2 with linker 2 of Ring 9	MPYYYRRRRYNYRRPRWYGRGWIRRPFRRRFRRKRRVRPTYTTI PLKQWQPPYKRTCYIKGQDCLIYYSNLRLGMNSTMYEKSIVPVH WPGGGSFSVSMLTLDALYDIHKLCRNWWTSTNQDLPLVRYKGC KITFYQSTFTDYIVRIH**NCYPMTDTKYTHADSAPNRMLLKKH**K HIIPSRQTRRKKKPYTKIFVKPPPQFENKWYFATDLYKIPLLQIHCT ACNLQNPFVKPDKLSNNVTLWSLNTISIQNRNMSVDQGQSWPFK ILGTQSFYFYFYTGANLPGDTTQIPVADLLPLTNPRINRPGQSLNE AKITDHITFTEYKNKFTNYWGNPFNKHIQEHLDMILYSLKSPEAIK NEWTTENMKWNQLNNAGTMALTPFNEPIFTQIQYNPDRDTGEDT QLYLLSNATGTGWDPPGIPELILEGFPLWLIYWGFADFQKNLKKV TNIDTNYMLVAKTKFTQKPGTFYLVILNDTFVEGNSPYEKQPLPE DNIKWYPQVQYQLEAQNKLLQTGPFTPNIQGQLSDNISMFYKFYF KWGGSPPKAINVENPAHQIQYPIPRNEHETTSLQSPGEAPESILYSF DYRHGNYTTTALSRISQDWALKDTVSKITEPDRQQLLKQALECL QISEETQEKKEKEVQQLISNLRQQQQLYRERIISLLKDQ
10. (1728) Ring 2 with beta strand G DNA binding of Ring 9	MPYYYRRRRYNYRRPRWYGRGWIRRPFRRRFRRKRRVRPTYTTI PLKQWQPPYKRTCYIKGQDCLIYYSNLRLGMNSTMYEKSIVPVH WPGGGSFSVSMLTLDALYDIHKLCRNWWTSTNQDLPLVRYKGC KITFYQSTFTDYIVRIHTELPANSNKLTYPNTHPLMMMMSKYKHII PSRQTRRKKKP**YKRVRV**KPPPQFENKWYFATDLYKIPLLQIHCT ACNLQNPFVKPDKLSNNVTLWSLNTISIQNRNMSVDQGQSWPFK ILGTQSFYFYFYTGANLPGDTTQIPVADLLPLTNPRINRPGQSLNE AKITDHITFTEYKNKFTNYWGNPFNKHIQEHLDMILYSLKSPEAIK

	NEWTTENMKWNQLNNAGTMALTPFNEPIFTQIQYNPDRDTGEDT QLYLLSNATGTGWDPPGIPELILEGFPLWLIYWGFADFQKNLKKV TNIDTNYMLVAKTKFTQKPGTFYLVILNDTFVEGNSPYEKQPLPE DNIKWYPQVQYQLEAQNKLLQTGPFTPNIQGQLSDNISMFYKFYF KWGGSPPKAINVENPAHQIQYPIPRNEHETTSLQSPGEAPESILYSF DYRHGNYTTTALSRISQDWALKDTVSKITEPDRQQLLKQALECL QISEETQEKKEKEVQQLISNLRQQQQLYRERIISLLKDQ
11. (1729) Ring 2 with beta strand F interprotomer contact of Ring 9	MPYYYRRRRYNYRRPRWYGRGWIRRPFRRRFRRKRRVRPTYTTI PLKQWQPPYKRTCYIKGQDCLIYYSNLRLGMNSTMYEKSIVPVH WPGGGSFSVSMLTLDALYDIHKLCRNWWTSTNQDLPLVRYKGC KITFYQSTFTDYIVRIHTELPANSNKLTYPNTHPLMMMMSK**HVIR VP**SRQTRRKKKPYTKIFVKPPPQFENKWYFATDLYKIPLLQIHCT ACNLQNPFVKPDKLSNNVTLWSLNTISIQNRNMSVDQGQSWPFK ILGTQSFYFYFYTGANLPGDTTQIPVADLLPLTNPRINRPGQSLNE AKITDHITFTEYKNKFTNYWGNPFNKHIQEHLDMILYSLKSPEAIK NEWTTENMKWNQLNNAGTMALTPFNEPIFTQIQYNPDRDTGEDT QLYLLSNATGTGWDPPGIPELILEGFPLWLIYWGFADFQKNLKKV TNIDTNYMLVAKTKFTQKPGTFYLVILNDTFVEGNSPYEKQPLPE DNIKWYPQVQYQLEAQNKLLQTGPFTPNIQGQLSDNISMFYKFYF KWGGSPPKAINVENPAHQIQYPIPRNEHETTSLQSPGEAPESILYSF DYRHGNYTTTALSRISQDWALKDTVSKITEPDRQQLLKQALECL QISEETQEKKEKEVQQLISNLRQQQQLYRERIISLLKDQ
12. (1730) Ring 2 ORF1 with strand H and I with C-terminal fragment of Ring 9	MPYYYRRRRYNYRRPRWYGRGWIRRPFRRRFRRKRRVRPTYTTI PLKQWQPPYKRTCYIKGQDCLIYYSNLRLGMNSTMYEKSIVPVH WPGGGSFSVSMLTLDALYDIHKLCRNWWTSTNQDLPLVRYKGC KITFYQSTFTDYIVRIHTELPANSNKLTYPNTHPLMMMMSKYKHII PSRQTRRKKKPYTKIFVKPPPQFENKWYFATDLY**KIPLIMIAATA VDFRYPFCASDCASNNLTLTCLNPLLFQNQDFDHPSDTQGYFP KPGVYLYSTQRSNKPSSSDCIYLGNTKDNQEGKSASSLMTLKT QKITDWGNPFWHYYIDGSKKIFSYFKPPSQLDSSDFEHMTELA EPMFIQVRYNPERDTGQGNLIYVTENFRGQHWDPPSSDNLKL**

	DGFPLYDMCWGFIDWIEKVHETENLLTNYCFCIRSSAFNEKK TVFIPVDHSFLTGFSPYETPVKSSDQAHWHPQIRFQTKSINDIC LTGPGCARSPYGNYMQAKMSYKFHVKWGGCPKTYEKPYDP CSQPNWTIPHNLNETIQIQNPNTCPQTELQEWDWRRDIVTKK AIERIRQHTEPHETLQISTGSKHNPPVHRQTSPWTDSETDSEE EKDQTQEIQIQLNKLRKHQQHLKQQLKQYLKPQNIE

Table B4-3. Replacement of hepatitis E virus domains with Anellovirus P2 domain. The engineering of virus capsid protein could expand tropism and alleviate severe immune response. Hepatitis E virus (HEV) is an non-enveloped RNA virus which contains three structural domains: S (shell), M (middle) and P (protruding) domains. Here we propose to swap HEV domains with Anellovirus P2 domain to pursue another novel VLP for encapsulation of mRNA payload.

HEV P domain (462-608) replaced with Anellovirus P2 (250-387)	MAVAPAPDTAPVPDVDSRGAILRRQYNLSTSPLTSTIATGTNL VLYAAPLSSLLPLQDGTNTHIMATEASNYAQYRVVRATIRYR PLVPSAVGGYAISISFWPQTTTTPTSVDMNSITSTDVRILVQPGI ASELVIPSERLHYRNQGWRSVETSGVAEEEATSGLVMLCIHGS PVNSYTNTPYTGALGLLDFALELEFRNLTPGNTNTRVSRYSSS ARHKLRRGPDGTAELTTTAATRFMKDLHFTGTNDVGEVGRG IALTLFNLADTLLGGLPTELISSAGGQLFYSRPVVSANGEPTVK LYTSVENAQQDKGIAIPHDIDLGESRVVIQDYDNQHEQDRPTP SPAPSRP**LNTTYIQNRDWGDRNKTYYCQTLGTQRYFLYGT HSTAQNINDIKLQELIPLTNTQDYVQGFDWTEKDKHNITT YKEFLTKGAGNPFHAEWITAQNPVIHTANSPTQIEQIYTAS TTTFQNKKLTDLPTPGYIFITPTV**HHHHH

Identification of ORF1 protein sequences

In some embodiments, an Anellovirus ORF1 protein sequence, or a nucleic acid sequence encoding an ORF1 protein, can be identified from the genome of an *Anellovirus* (e.g., a putative *Anellovirus* genome identified, for example, by nucleic acid sequencing techniques, e.g., deep sequencing

techniques). In some embodiments, an ORF1 protein sequence is identified by one or more (e.g., 1, 2, or all 3) of the following selection criteria:

(i) Length Selection: Protein sequences (e.g., putative Anellovirus ORF1 sequences passing the criteria described in (ii) or (iii) below) may be size-selected for those greater than about 600 amino acid residues to identify putative Anellovirus ORF1 proteins. In some embodiments, an Anellovirus ORF1 protein sequence is at least about 600, 650, 700, 750, 800, 850, 900, 950, or 1000 amino acid residues in length. In some embodiments, an Alphatorquevirus ORF1 protein sequence is at least about 700, 710, 720, 730, 740, 750, 760, 770, 780, 790, 800, 900, or 1000 amino acid residues in length. In some embodiments, a Betatorquevirus ORF1 protein sequence is at least about 650, 660, 670, 680, 690, 700, 750, 800, 900, or 1000 amino acid residues in length. In some embodiments, a Gammatorquevirus ORF1 protein sequence is at least about 650, 660, 670, 680, 690, 700, 750, 800, 900, or 1000 amino acid residues in length. In some embodiments, a nucleic acid sequence encoding an Anellovirus ORF1 protein is at least about 1800, 1900, 2000, 2100, 2200, 2300, 2400, or 2500 nucleotides in length. In some embodiments, a nucleic acid sequence encoding an Alphatorquevirus ORF1 protein sequence is at least about 2100, 2150, 2200, 2250, 2300, 2400, or 2500 nucleotides in length. In some embodiments, a nucleic acid sequence encoding a Betatorquevirus ORF1 protein sequence is at least about 1900, 1950, 2000, 2500, 2100, 2150, 2200, 2250, 2300, 2400, or 2500 or 1000 nucleotides in length. In some embodiments, a nucleic acid sequence encoding a Gammatorquevirus ORF1 protein sequence is at least about 1900, 1950, 2000, 2500, 2100, 2150, 2200, 2250, 2300, 2400, or 2500 or 1000 nucleotides in length.

(ii) Presence of ORF1 motif: Protein sequences (e.g., putative Anellovirus ORF1 sequences passing the criteria described in (i) above or (iii) below) may be filtered to identify those that contain the conserved ORF1 motif in the structural N22 domain described above. In some embodiments, a putative Anellovirus ORF1 sequence comprises the sequence YNPXXDXGXXN. In some embodiments, a putative Anellovirus ORF1 sequence comprises the sequence Y[NCS]P*XX*D*X*[GASKR]*XX*[NTSVAK].

(iii) Presence of arginine-rich region: Protein sequences (e.g., putative Anellovirus ORF1 sequences passing the criteria described in (i) and/or (ii) above) may be filtered for those that include a structural arginine-rich region (e.g., as described herein). In some embodiments, a putative Anellovirus ORF1 sequence comprises a contiguous sequence of at least about 30, 35, 40, 45, 50, 55, 60, 65, or 70 amino acids that comprises at least 30% (e.g., at least about 20%, 25%, 30%, 35%, 40%, 45%, or 50%) arginine residues. In some embodiments, a putative Anellovirus ORF1 sequence comprises a contiguous sequence of about 35-40, 40-45, 45-50, 50-55, 55-60, 60-65, or 65-70 amino acids that comprises at least 30% (e.g., at least about 20%, 25%, 30%, 35%, 40%, 45%, or 50%) arginine residues. In some embodiments, the structural arginine-rich region is positioned at least about 30, 40, 50, 60, 70, or 80

amino acids downstream of the start codon of the putative Anellovirus ORF1 protein. In some embodiments, the structural arginine-rich region is positioned at least about 50 amino acids downstream of the start codon of the putative Anellovirus ORF1 protein.

In some embodiments, an ORF1 protein is identified in an Anellovirus genome sequence as described in Example 36 of PCT Publication No. WO2020/123816 (incorporated herein by reference in its entirety).

Structural arginine-rich region deletions and truncations

In some embodiments, an ORF1 molecule as described herein comprises a deletion or truncation of a structural arginine-rich region. In some embodiments, the entire structural arginine-rich region is deleted. In embodiments, the ORF1 molecule does not comprise an Anellovirus ORF1 structural arginine-rich region, or an amino acid sequence having at least 70%, 75%, 80%, 8%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity thereto. In embodiments, the ORF1 molecule does not comprise the amino acid sequence of the full-length structural arginine-rich region of an Ring2, Ring9, Ring10, Ring 18, or Ring19 Anellovirus ORF1 protein (e.g., as described herein). In some embodiments, the ORF1 molecule as described herein does not comprise a sequence of at least 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, or 70 contiguous amino acids consisting of at least 40%, 41%, 42%, 43%, 44%, 45%, 46%, 47%, 48%, 49%, 50%, 51%, 52%, 53%, 54%, 55%, 56%, 57%, 58%, 59%, or 60% basic residues (e.g., arginine and/or lysine residues).

In some embodiments, a portion of the structural arginine-rich region (e.g., a N-terminal portion of the structural arginine-rich region) is deleted. In some embodiments, the ORF1 molecule comprises a portion of a structural arginine-rich region of an Anellovirus ORF1 molecule, which comprises a deletion of about 1-5, 5-10, 10-15, 15-20, 20-25, 25-30, 30-35, or 35-40 amino acids, e.g., at the N-terminal end, of the structural arginine-rich region, relative to a corresponding wild-type structural arginine-rich region of the Anellovirus ORF1 molecule, or an amino acid sequence having at least 70%, 75%, 80%, 8%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity thereto. In some embodiments, the ORF1 molecule does not comprise the 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 most N-terminal amino acid residues of the structural jelly-roll region of the Anellovirus ORF1 molecule other than the N-terminal methionine residue. It is understood that the ORF1 molecule generally comprises at its N-terminus a methionine residue, e.g., a methionine residues corresponding to the N-terminal methionine residue of an Anellovirus structural arginine-rich region

In embodiments, the ORF1 molecule comprises a portion of a structural arginine-rich region (e.g., an C-terminal portion of the structural arginine-rich region) consisting of between 1-5, 5-10, 10-15, 15-

20, 20-25, 25-30, 30-35, or 35-40 contiguous amino acids of a structural arginine-rich region sequence as described herein, or an amino acid sequence having at least 70%, 75%, 80%, 8%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity thereto. In embodiments, the ORF1 molecule comprises a portion of a structural arginine-rich region of an Anellovirus ORF1 molecule, wherein the portion consists of the N-terminal most 30-40, 40-50, 50-60, 60-70 (e.g., about 69), 70-80, 80-90, 90-100 (e.g., about 93), or 100-110 amino acids of a corresponding wild-type structural arginine-rich region of an Anellovirus ORF1 molecule, or a sequence having at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity thereto.

In some embodiments, an ORF1 molecule having a deletion or truncation of the structural arginine-rich region further comprises a deletion or truncation of at least a portion of a structural jelly-roll domain as described herein. In some embodiments, an ORF1 molecule having a deletion or truncation of the structural arginine-rich region further comprises a deletion or truncation of at least a portion of a jelly-roll B-H strands subdomain as described herein.

In some embodiments, an ORF1 molecule having a deletion or truncation of the structural arginine-rich region further comprises a deletion or truncation of at least a portion of a structural C-terminal domain (e.g., as described herein).

In some embodiments, an ORF1 molecule comprises an amino acid sequence comprising substitutions of at least 50%, 60%, 70%, 80%, or 90% of basic amino acids (e.g., arginines and/or lysines) relative to the structural arginine-rich region of a wild-type Anellovirus ORF1 molecule.

Structural C-terminal domain deletions and truncations

In some embodiments, an ORF1 molecule as described herein comprises a deletion or truncation of a structural C-terminal domain (CTD). In some embodiments, the entire structural CTD is deleted. In embodiments, the ORF1 molecule does not comprise an Anellovirus ORF1 structural CTD, or an amino acid sequence having at least 70%, 75%, 80%, 8%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity thereto. In embodiments, the ORF1 molecule does not comprise the amino acid sequence of the full-length structural C-terminal domain of an Ring2, Ring9, Ring10, Ring 18, or Ring19 Anellovirus ORF1 protein (e.g., as described herein).

In some embodiments, a portion of the structural CTD (e.g., a C-terminal portion of the structural CTD) is deleted. In some embodiments, the ORF1 molecule comprises a portion of a structural C-terminal domain (CTD) of an Anellovirus ORF1 molecule, which comprises a deletion of about 20-30, 30-40 (e.g., about 37), 40-50 (e.g., about 55), 50-60 , 60-70, 70-80, 80-90, 90-100, 100-110, 110-120, 120-130 (e.g., about 129), 130-140 (e.g., about 131), 140-150 (e.g., about 148), or 150-160 (e.g., about 155) amino acids at the C-terminal end of the structural CTD, relative to a corresponding wild-type

structural CTD of the Anellovirus ORF1 molecule, or an amino acid sequence having at least 70%, 75%, 80%, 8%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity thereto.

In embodiments, the ORF1 molecule comprises a portion of a structural CTD (e.g., an N-terminal portion of the structural CTD) consisting of between 1-5, 5-10, 10-15, 15-20, 20-25, 25-30, 30-40, 40-50, 50-60, 60-70, 70-80, 80-90, 90-100, 100-110, 110-120, 120-130, 130-140, or 140-150 contiguous amino acids of a structural CTD sequence as described herein, or an amino acid sequence having at least 70%, 75%, 80%, 8%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity thereto. In embodiments, the ORF1 molecule comprises a portion of a structural CTD of an Anellovirus ORF1 molecule, wherein the portion consists of the N-terminal most 60-70 (e.g., about 69), 70-80, 80-90, 90-100 (e.g., about 93), or 100-110 amino acids of a corresponding wild-type structural CTD of an Anellovirus ORF1 molecule, or a sequence having at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity thereto.

In some embodiments, an ORF1 molecule having a deletion or truncation of the structural CTD further comprises a deletion or truncation of at least a portion of a structural N22 domain as described herein. In some embodiments, an ORF1 molecule having a deletion or truncation of the structural CTD further comprises a deletion or truncation of at least a portion of a jelly-roll I strand subdomain and/or a P1-2 domain as described herein.

In some embodiments, an ORF1 molecule having a deletion or truncation of the structural CTD further comprises a deletion or truncation of at least a portion of a structural arginine-rich region (e.g., as described herein).

Chimeric ORF1 molecules

In some embodiments, an ORF1 molecule described herein comprises a heterologous amino acid sequence (e.g., an amino acid sequence from a protein other than an Anellovirus protein). In some embodiments, the ORF1 molecule comprises one or more deletions or truncations relative to a corresponding Anellovirus ORF1 protein (e.g., a deleteion or truncation of a structural arginine-rich region and/or a deleteion or truncation of a structural CTD, e.g., as described herein). In some embodiments, the heterologous amino acid sequence is inserted at or within 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 35, 40, 45, or 50 amino acid residues of the position of one of the deletions or truncations relative to the corresponding Anellovirus ORF1 protein. For example, the heterologous amino acid sequence can, in some instances, be inserted at the N-terminus of the ORF1 molecule (e.g., an ORF1 molecule comprising a deletion or truncation of the structural arginine-rich region, e.g., as described herein). In another example, the heterologous amino acid sequence can, in some instances, be inserted at the C-terminus of the ORF1 molecule (e.g., an ORF1 molecule comprising a deletion or truncation of the structural CTD, e.g., as described herein).

In some embodiments, the heterologous amino acid sequence is attached to the remainder of the ORF1 molecule by a linker. In certain embodiments, the linker comprises one or more (e.g., at least 1, 2, 3, 4, or 5) copies of the amino acid sequence GGGGS. In certain embodiments, the linker comprises the amino acid sequence TYTTIP.

N-terminal insertions (e.g., structural arginine-rich region swaps)

In some embodiments, an ORF1 molecule as described herein comprises a heterologous amino acid sequence at or within 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 35, 40, or 50 amino acids of its N-terminus. In some embodiments, the structural arginine-rich region of the ORF1 molecule, or a portion thereof (e.g., consisting of at least about 5, 10, 15, 20, 25, 30, 35, or 40 contiguous amino acids, e.g., of the C-terminal portion of the structural arginine-rich region) is replaced by the heterologous amino acid sequence. In some embodiments, the heterologous amino acid sequence is from another virus (e.g., a non-Anellovirus). In certain embodiments, the heterologous amino acid sequence comprises an arginine-rich motif or arginine-rich region from the other virus, or an amino acid sequence having at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity thereto.

In some embodiments, an N-terminal domain or portion thereof of a heterologous protein (e.g., a capsid protein from a virus other than an Anellovirus, e.g., as described herein), or a sequence having at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity thereto, is inserted into the sequence of the ORF1 molecule (e.g., at the N-terminus and/or at the position of a deletion or truncation of the structural arginine-rich region). In some embodiments, an arginine-rich region or arginine-rich motif, or a portion thereof, of a heterologous protein, or a sequence having at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity thereto, is inserted into the sequence of the ORF1 molecule (e.g., at the N-terminus and/or at the position of a deletion or truncation of the structural arginine-rich region). In some embodiments, the heterologous protein is a capsid protein (e.g., an ORF1 protein) from a different Anellovirus. In certain embodiments the ORF1 molecule comprises a structural arginine-rich region, or a portion thereof, from a different Anellovirus (e.g., replacing one or more of the corresponding residues of the structural arginine-rich region of the ORF1 molecule itself).

In other embodiments, the heterologous protein is a capsid protein from a virus other than an Anellovirus, e.g., as described herein. In certain embodiments, the virus is a beak and feather disease virus (BFDV). In certain embodiments, the ORF1 molecule comprises (e.g., at the N-terminus) the amino acid sequence MWGTSNCACAKFQIRRRYARPYRRRHIRRYRRRRRHFRRRRFTTNR, or an amino acid sequence having at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity thereto.

P1/P2 domain swaps

An ORF1 molecule as described herein may, in some instances, comprise a P1 domain, or a functional variant thereof, from the capsid protein of a different virus (e.g., a different Anellovirus or a non-Anellovirus), or an amino acid sequence having at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity thereto. In certain embodiments, the P1 domain is noncontiguous (e.g., comprising a P1-1 domain and a P1-2 domain, e.g., as described herein). In certain embodiments, the P1 domain or functional fragment thereof from the other virus replaces one or more of the corresponding residues of the P1 domain of the ORF1 molecule. In some instances, an ORF1 molecule as described herein may comprise a P2 domain, or a functional fragment thereof, from the capsid protein of a different virus (e.g., a different Anellovirus or a non-Anellovirus), or an amino acid sequence having at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity thereto. In certain embodiments, the P2 domain or functional fragment thereof from the other virus replaces one or more of the corresponding residues of the P2 domain of the ORF1 molecule.

In some embodiments, an ORF1 molecule comprises a P1 domain, or a functional fragment thereof, from a hepatitive E virus (HEV) capsid protein, or an amino acid sequence having at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity thereto. In some embodiments, an ORF1 molecule comprises a P2 domain, or a functional fragment thereof, from an HEV capsid protein, or an amino acid sequence having at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity thereto.

Other domain mutants

An ORF1 molecule as described herein may comprise one more additional mutations relative to a wild-type ORF1 protein sequence.

In some embodiments, a structural jelly-roll region or portion thereof of a heterologous protein (e.g., a capsid protein from a virus other than an Anellovirus, e.g., as described herein), or a sequence having at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity thereto, is inserted into the sequence of the ORF1 molecule (e.g., at the position of a deletion or truncation of the structural jelly-roll region of the ORF1 molecule). In some embodiments, the structural jelly-roll region of an ORF1 molecule comprises one or more mutations (e.g., at least 2, 3, 4, 5, 6, 7, 8, 9, or 10 mutations) in a beta strand relative to the amino acid sequence of a wild-type Anellovirus ORF1 structural jelly-roll region.

In some embodiments, an C-terminal domain or portion thereof of a heterologous protein (e.g., a capsid protein from a virus other than an Anellovirus, e.g., as described herein), or a sequence having at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity thereto, is inserted into the sequence of the ORF1 molecule (e.g., at the C-terminus and/or at the position of a deletion or truncation of the structural CTD).

ORF2 molecules

In some embodiments, the anellovector or anelloVLP comprises an ORF2 molecule and/or a nucleic acid encoding an ORF2 molecule. Generally, an ORF2 molecule comprises a polypeptide having the structural features and/or activity of an Anellovirus ORF2 protein (e.g., an Anellovirus ORF2 protein as described herein, e.g., as listed in any one of Tables A1-A26), or a functional fragment thereof. In some embodiments, an ORF2 molecule comprises an amino acid sequence having at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to an Anellovirus ORF2 protein sequence as shown in any one of Tables A1-A26.

In some embodiments, an ORF2 molecule comprises an amino acid sequence having at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity to an Alphatorquevirus, Betatorquevirus, or Gammatorquevirus ORF2 protein. In some embodiments, an ORF2 molecule (e.g., an ORF2 molecule having at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity to an Alphatorquevirus ORF2 protein) has a length of 250 or fewer amino acids (e.g., about 150-200 amino acids). In some embodiments, an ORF2 molecule (e.g., an ORF2 molecule having at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity to a Betatorquevirus ORF2 protein) has a length of about 50-150 amino acids. In some embodiments, an ORF2 molecule (e.g., an ORF2 molecule having at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity to a Gammatorquevirus ORF2 protein) has a length of about 100-200 amino acids (e.g., about 100-150 amino acids). In some embodiments, the ORF2 molecule comprises a helix-turn-helix motif (e.g., a helix-turn-helix motif comprising two alpha helices flanking a turn region). In some embodiments, the ORF2 molecule does not comprise the amino acid sequence of the ORF2 protein of TTV isolate TA278 or TTV isolate SANBAN. In some embodiments, an ORF2 molecule has protein phosphatase activity. In some embodiments, an ORF2 molecule comprises at least one difference (e.g., a mutation, chemical modification, or epigenetic alteration) relative to a wild-type ORF2 protein, e.g., as described herein (e.g., as shown in any one of Tables A1-A26).

Conserved ORF2 Motif

In some embodiments, a polypeptide (e.g., an ORF2 molecule) described herein comprises the amino acid sequence [W/F]X^7HX^3CX^1CX^5H (SEQ ID NO: 949), wherein X^n is a contiguous sequence of any *n* amino acids. In embodiments, X^7 indicates a contiguous sequence of any seven amino acids. In some embodiments, X^3 indicates a contiguous sequence of any three amino acids. In some embodiments, X^1 indicates any single amino acid. In some embodiments, X^5 indicates a contiguous sequence of any five amino acids. In some embodiments, the [W/F] can be either tryptophan or phenylalanine. In some

embodiments, the [W/F]X^7HX^3CX^1CX^5H (SEQ ID NO: 949) is comprised within the structural N22 domain of an ORF2 molecule, e.g., as described herein. In some embodiments, a genetic element described herein comprises a nucleic acid sequence (e.g., a nucleic acid sequence encoding an ORF2 molecule, e.g., as described herein) encoding the amino acid sequence [W/F]X^7HX^3CX^1CX^5H (SEQ ID NO: 949), wherein X^n is a contiguous sequence of any *n* amino acids.

Genetic Elements

In some embodiments, the anellovector comprises a genetic element. In some embodiments, the genetic element has one or more of the following characteristics: is substantially non-integrating with a host cell's genome, is an episomal nucleic acid, is a single stranded DNA, is circular, is about 1 to 10 kb, exists within the nucleus of the cell, can be bound by endogenous proteins, produces an effector, such as a polypeptide or nucleic acid (e.g., an RNA, iRNA, microRNA) that targets a gene, activity, or function of a host or target cell. In one embodiment, the genetic element is a substantially non-integrating DNA. In some embodiments, the genetic element comprises a packaging signal, e.g., a sequence that binds a capsid protein. In some embodiments, outside of the packaging or capsid-binding sequence, the genetic element has less than 70%, 60%, 50%, 40%, 30%, 20%, 10%, 5% sequence identity to a wild type Anellovirus nucleic acid sequence, e.g., has less than 70%, 60%, 50%, 40%, 30%, 20%, 10%, 5% sequence identity to an *Anellovirus* nucleic acid sequence, e.g., as described herein. In some embodiments, outside of the packaging or capsid-binding sequence, the genetic element has less than 500, 450, 400, 350, 300, 250, 200, 150, or 100 contiguous nucleotides that are at least 70%, 75%, 80%, 8%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to an *Anellovirus* nucleic acid sequence. In certain embodiments, the genetic element is a circular, single stranded DNA that comprises a promoter sequence, a sequence encoding a therapeutic effector, and a capsid binding protein.

In some embodiments, the genetic element has at least about 70%, 75%, 80%, 8%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to an *Anellovirus* nucleic acid sequence, e.g., as described herein (e.g., as described in any one of Tables N1-N26), or a fragment thereof, or encodes an amino acid sequence having at least about 70%, 75%, 80%, 8%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to an *Anellovirus* amino acid sequence (e.g., as described in any one of Tables A1-A26), or a fragment thereof. In some embodiments, the genetic element comprises a sequence encoding an effector (e.g., an endogenous effector or an exogenous effector, e.g., a payload), e.g., a polypeptide effector (e.g., a protein) or nucleic acid effector (e.g., a non-coding RNA, e.g., a miRNA, siRNA, mRNA, lncRNA, RNA, DNA, an antisense RNA, gRNA).

In some embodiments, the genetic element has a length less than 20kb (e.g., less than about 19kb, 18kb, 17kb, 16kb, 15kb, 14kb, 13kb, 12kb, 11kb, 10kb, 9kb, 8kb, 7kb, 6kb, 5kb, 4kb, 3kb, 2kb, 1kb, or

less). In some embodiments, the genetic element has, independently or in addition to, a length greater than 1000b (e.g., at least about 1.1kb, 1.2kb, 1.3kb, 1.4kb, 1.5kb, 1.6kb, 1.7kb, 1.8kb, 1.9kb, 2kb, 2.1kb, 2.2kb, 2.3kb, 2.4kb, 2.5kb, 2.6kb, 2.7kb, 2.8kb, 2.9kb, 3kb, 3.1kb, 3.2kb, 3.3kb, 3.4kb, 3.5kb, 3.6kb, 3.7kb, 3.8kb, 3.9kb, 4kb, 4.1kb, 4.2kb, 4.3kb, 4.4kb, 4.5kb, 4.6kb, 4.7kb, 4.8kb, 4.9kb, 5kb, or greater). In some embodiments, the genetic element has a length of about 2.5-4.6, 2.8-4.0, 3.0-3.8, or 3.2-3.7 kb. In some embodiments, the genetic element has a length of about 1.5-2.0, 1.5-2.5, 1.5-3.0, 1.5-3.5, 1.5-3.8, 1.5-3.9, 1.5-4.0, 1.5-4.5, or 1.5-5.0 kb. In some embodiments, the genetic element has a length of about 2.0-2.5, 2.0-3.0, 2.0-3.5, 2.0-3.8, 2.0-3.9, 2.0-4.0, 2.0-4.5, or 2.0-5.0 kb. In some embodiments, the genetic element has a length of about 2.5-3.0, 2.5-3.5, 2.5-3.8, 2.5-3.9, 2.5-4.0, 2.5-4.5, or 2.5-5.0 kb. In some embodiments, the genetic element has a length of about 3.0-5.0, 3.5-5.0, 4.0-5.0, or 4.5-5.0 kb. In some embodiments, the genetic element has a length of about 1.5-2.0, 2.0-2.5, 2.5-3.0, 3.0-3.5, 3.1-3.6, 3.2-3.7, 3.3-3.8, 3.4-3.9, 3.5-4.0, 4.0-4.5, or 4.5-5.0 kb.

In some embodiments, the genetic element comprises one or more of the features described herein, e.g., a sequence encoding a substantially non-pathogenic protein, a protein binding sequence, one or more sequences encoding a regulatory nucleic acid, one or more regulatory sequences, one or more sequences encoding a replication protein, and other sequences. In some embodiments, the substantially non-pathogenic protein comprises an amino acid sequence or a functional fragment thereof or a sequence having at least about 60%, 65%, 70%, 75%, 80%, 85%, 90% 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to any one of the amino acid sequences described herein, an *Anellovirus* amino acid sequence, e.g., as listed in any one of Tables A1-A26.

In some embodiments, the genetic element was produced from a double-stranded circular DNA (e.g., produced by in vitro circularization). In some embodiments, the genetic element was produced by rolling circle replication from the double-stranded circular DNA. In some embodiments, the rolling circle replication occurs in a cell (e.g., a host cell, e.g., a mammalian cell, e.g., a human cell, e.g., a HEK cell (e.g., HEK293T cell, an Expi293 cell), an A549 cell, or a Jurkat cell). In some embodiments, the genetic element can be amplified exponentially by rolling circle replication in the cell. In some embodiments, the genetic element can be amplified linearly by rolling circle replication in the cell. In some embodiments, the double-stranded circular DNA or genetic element is capable of yielding at least 2, 4, 8, 16, 32, 64, 128, 256, 518, 1024 or more times the original quantity by rolling circle replication in the cell. In some embodiments, the double-stranded circular DNA was introduced into the cell, e.g., as described herein.

In some embodiments, the double-stranded circular DNA and/or the genetic element does not comprise one or more bacterial plasmid elements (e.g., a bacterial origin of replication or a selectable marker, e.g., a bacterial resistance gene). In some embodiments, the double-stranded circular DNA and/or the genetic element does not comprise a bacterial plasmid backbone.

In one embodiment, the invention includes a genetic element comprising a nucleic acid sequence (e.g., a DNA sequence) encoding (i) a substantially non-pathogenic exterior protein, (ii) an exterior protein binding sequence that binds the genetic element to the substantially non-pathogenic exterior protein, and (iii) a regulatory nucleic acid. In such an embodiment, the genetic element may comprise one or more sequences with at least about 60%, 70% 80%, 85%, 90% 95%, 96%, 97%, 98% and 99% nucleotide sequence identity to any one of the nucleotide sequences to a native viral sequence (e.g., a native *Anellovirus* sequence, e.g., as described herein).

In some embodiments, a genetic element as described herein comprises a sequence (e.g., a TATA box, cap site, transcriptional start site, 5' UTR, open reading frame (ORF), poly(A) signal, or GC-rich region sequence) as listed in any of Tables A1, A3, A5, A7, A9, A11, B1-B5, 1, 3, 5, 7, 9, 11, 13, 15, or 17 of PCT Publication No. WO2020/123816 (incorporated herein by reference in its entirety), or a sequence having at least 70% 80%, 85%, 90% 95%, 96%, 97%, 98% and 99% nucleotide sequence identity thereto.

In some embodiments, a genetic element comprises a sequence encoding an effector (e.g., an exogenous effector). In some embodiments, the effector-encoding sequence is inserted into an Anellovirus genome sequence (e.g., as described herein). In some embodiments, the effector-encoding sequence replaces a contiguous sequence (e.g., of at least 5, 10, 15, 20, 25, 30, 40, 50, 60, 70, 80, 90, 100, 200, 300, 400, 500, 600, 700, 800, 900, 1000, or more nucleotides) from the Anellovirus genome sequence. In some embodiments, the effector-encoding sequence replaces a TATA box, cap site, transcriptional start site, 5' UTR, open reading frame (ORF), poly(A) signal, or GC-rich region sequence, or a portion thereof (e.g., a portion consisting of at least 5, 10, 15, 20, 25, 30, 40, 50, 60, 70, 80, 90, 100, 200, 300, 400, 500, 600, 700, 800, 900, 1000, or more nucleotides) e.g., as listed in any of Tables A1, A3, A5, A7, A9, A11, B1-B5, 1, 3, 5, 7, 9, 11, 13, 15, or 17 of PCT Publication No. WO2020/123816 (incorporated herein by reference in its entirety), or a sequence having at least 70% 80%, 85%, 90% 95%, 96%, 97%, 98% and 99% nucleotide sequence identity thereto.

In some embodiments, the sequence of a first nucleic acid element comprised in a genetic element (e.g., a TATA box, cap site, transcriptional start site, 5' UTR, open reading frame (ORF), poly(A) signal, or GC-rich region) overlaps with the sequence of a second nucleic acid element (e.g., a TATA box, cap site, transcriptional start site, 5' UTR, open reading frame (ORF), poly(A) signal, or GC-rich region), e.g., by at least 5, 10, 15, 20, 25, 30, 40, 50, 60, 70, 80, 90, 100, 150, 200, 250, 300, 400, or 500 nucleotides. In some embodiments, the sequence of a first nucleic acid element comprised in a genetic element (e.g., a TATA box, cap site, transcriptional start site, 5' UTR, open reading frame (ORF), poly(A) signal, or GC-rich region) does not overlap with the sequence of a second nucleic acid element (e.g., a TATA box, cap site, transcriptional start site, 5' UTR, open reading frame (ORF), poly(A) signal, or GC-rich region).

Protein Binding Sequence

A strategy employed by many viruses is that the viral capsid protein recognizes a specific protein binding sequence in its genome. For example, in viruses with unsegmented genomes, such as the L-A virus of yeast, there is a secondary structure (stem-loop) and a specific sequence at the 5' end of the genome that are both used to bind the viral capsid protein. However, viruses with segmented genomes, such as *Reoviridae*, *Orthomyxoviridae* (influenza), *Bunyaviruses* and *Arenaviruses,* need to package each of the genomic segments. Some viruses utilize a complementarity region of the segments to aid the virus in including one of each of the genomic molecules. Other viruses have specific binding sites for each of the different segments. See for example, Curr Opin Struct Biol. 2010 Feb; 20(1): 114–120; and Journal of Virology (2003), 77(24), 13036-13041.

In some embodiments, the genetic element encodes a protein binding sequence that binds to the substantially non-pathogenic protein. In some embodiments, the protein binding sequence facilitates packaging the genetic element into the proteinaceous exterior. In some embodiments, the protein binding sequence specifically binds a structural arginine-rich region of the substantially non-pathogenic protein. In some embodiments, the genetic element comprises a protein binding sequence as described in Example 8 of PCT Publication No. WO 2020/123816, incorporated by reference herein in its entirety. In some embodiments, the genetic element comprises a protein binding sequence having at least 70%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to a 5' UTR conserved domain or GC-rich domain of an *Anellovirus* sequence (e.g., as shown in any one of Tables N1-N26).

In embodiments, the protein binding sequence has at least about 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to the *Anellovirus* 5' UTR conserved domain nucleotide sequence of any one of Tables N1-N26.

5' UTR Regions

In some embodiments, the genetic element (e.g., protein-binding sequence of the genetic element) comprises a nucleic acid sequence having at least about 75% (e.g., at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100%) identity to a nucleic acid sequence shown in Table 38 and/or Figure 20 of PCT Publication No. WO 2020/123816. In some embodiments, the genetic element (e.g., protein-binding sequence of the genetic element) comprises a nucleic acid sequence of the Consensus 5' UTR sequence shown in Table 38, wherein X_1, X_2, X_3, X_4, and X_5 are each independently any nucleotide, e.g., wherein X_1 = G or T, X_2 = C or A, X_3 = G or A, X_4 = T or C, and X_5 = A, C, or T). In some embodiments, the genetic element (e.g., protein-binding sequence of the genetic element) comprises a nucleic acid sequence having at least about 75% (e.g., at least 75%, 80%, 85%, 90%, 95%, 96%, 97%,

98%, 99%, or 100%) identity to the Consensus 5' UTR sequence shown in Table 38. In some embodiments, the genetic element (e.g., protein-binding sequence of the genetic element) comprises a nucleic acid sequence having at least about 75% (e.g., at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100%) identity to the exemplary TTV 5' UTR sequence shown in Table 38. In some embodiments, the genetic element (e.g., protein-binding sequence of the genetic element) comprises a nucleic acid sequence having at least about 75% (e.g., at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100%) identity to the TTV-CT30F 5' UTR sequence shown in Table 38. In some embodiments, the genetic element (e.g., protein-binding sequence of the genetic element) comprises a nucleic acid sequence having at least about 75% (e.g., at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100%) identity to the TTV-HD23a 5' UTR sequence shown in Table 38. In some embodiments, the genetic element (e.g., protein-binding sequence of the genetic element) comprises a nucleic acid sequence having at least about 75% (e.g., at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100%) identity to the TTV-JA20 5' UTR sequence shown in Table 38. In some embodiments, the genetic element (e.g., protein-binding sequence of the genetic element) comprises a nucleic acid sequence having at least about 75% (e.g., at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100%) identity to the TTV-TJN02 5' UTR sequence shown in Table 38. In some embodiments, the genetic element (e.g., protein-binding sequence of the genetic element) comprises a nucleic acid sequence having at least about 75% (e.g., at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100%) identity to the TTV-tth8 5' UTR sequence shown in Table 38.

In some embodiments, the genetic element (e.g., protein-binding sequence of the genetic element) comprises a nucleic acid sequence having at least about 75% (e.g., at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100%) identity to the Alphatorquevirus Consensus 5' UTR sequence shown in Table 38. In some embodiments, the genetic element (e.g., protein-binding sequence of the genetic element) comprises a nucleic acid sequence having at least about 75% (e.g., at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100%) identity to the Alphatorquevirus Clade 1 5' UTR sequence shown in Table 38. In some embodiments, the genetic element (e.g., protein-binding sequence of the genetic element) comprises a nucleic acid sequence having at least about 75% (e.g., at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100%) identity to the Alphatorquevirus Clade 2 5' UTR sequence shown in Table 38. In some embodiments, the genetic element (e.g., protein-binding sequence of the genetic element) comprises a nucleic acid sequence having at least about 75% (e.g., at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100%) identity to the Alphatorquevirus Clade 3 5' UTR sequence shown in Table 38. In some embodiments, the genetic element (e.g., protein-binding sequence of the genetic element) comprises a nucleic acid sequence having at least about 75% (e.g., at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100%) identity to the Alphatorquevirus Clade 4 5' UTR

sequence shown in Table 38. In some embodiments, the genetic element (e.g., protein-binding sequence of the genetic element) comprises a nucleic acid sequence having at least about 75% (e.g., at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100%) identity to the Alphatorquevirus Clade 5 5' UTR sequence shown in Table 38. In some embodiments, the genetic element (e.g., protein-binding sequence of the genetic element) comprises a nucleic acid sequence having at least about 75% (e.g., at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100%) identity to the Alphatorquevirus Clade 6 5' UTR sequence shown in Table 38. In some embodiments, the genetic element (e.g., protein-binding sequence of the genetic element) comprises a nucleic acid sequence having at least about 75% (e.g., at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100%) identity to the Alphatorquevirus Clade 7 5' UTR sequence shown in Table 38.

In some embodiments, the genetic element comprises a nucleic acid sequence having at least about 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to the *Anellovirus* 5' UTR conserved domain nucleotide sequence of any one of Tables N1-N26.

In some embodiments, the genetic element comprises a nucleic acid sequence having at least about 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to the *Anellovirus* 5' UTR conserved domain nucleotide sequence of any one of Tables N1-N26.

Table 38. Exemplary 5' UTR sequences from *Anelloviruses*

Source	Sequence	SEQ ID NO:
Consensus	CGGGTGCCGX$_1$AGGTGAGTTTACACACCGX$_2$AGT CAAGGGGCAATTCGGGCTCX$_3$GGACTGGCCGGG CX$_4$X$_5$TGGG X$_1$ = G or T X$_2$ = C or A X$_3$ = G or A X$_4$ = T or C X$_5$ = A, C, or T	105
Exemplary TTV Sequence	CGGGTGCCGGAGGTGAGTTTACACACCGCAGTC AAGGGGCAATTCGGGCTCGGGACTGGCCGGGCT WTGGG	106
TTV-CT30F	CGGGTGCCGTAGGTGAGTTTACACACCGCAGTC AAGGGGCAATTCGGGCTCGGGACTGGCCGGGCT ATGGG	107

TTV-HD23a	CGGGTGCCGGAGGTGAGTTTACACACCGCAGTC AAGGGGCAATTCGGGCTCGGGACTGGCCGGGCC CTGGG	108
TTV-JA20	CGGGTGCCGGAGGTGAGTTTACACACCGCAGTC AAGGGGCAATTCGGGCTCGGGACTGGCCGGGCT TTGGG	109
TTV-TJN02	CGGGTGCCGGAGGTGAGTTTACACACCGCAGTC AAGGGGCAATTCGGGCTCGGGACTGGCCGGGCT ATGGG	110
TTV-tth8	CGGGTGCCGGAGGTGAGTTTACACACCGAAGTC AAGGGGCAATTCGGGCTCAGGACTGGCCGGGCT TTGGG	111
Alphatorquevirus Consensus 5' UTR	CGGGTGCCGGAGGTGAGTTTACACACCGCAGTC AAGGGGCAATTCGGGCTCGGGACTGGCCGGGC X_1X_2TGGG; wherein X_1 comprises T or C, and wherein X_2 comprises A, C, or T.	112
Alphatorquevirus Clade 1 5' UTR (e.g., TTV-CT30F)	CGGGTGCCGTAGGTGAGTTTACACACCGCAGTC AAGGGGCAATTCGGGCTCGGGACTGGCCGGGCT ATGGG	113
Alphatorquevirus Clade 2 5' UTR (e.g., TTV-P13-1)	CGGGTGCCGGAGGTGAGTTTACACACCGCAGTC AAGGGGCAATTCGGGCTCGGGACTGGCCGGGCC CGGG	114
Alphatorquevirus Clade 3 5' UTR (e.g., TTV-tth8)	CGGGTGCCGGAGGTGAGTTTACACACCGAAGTC AAGGGGCAATTCGGGCTCAGGACTGGCCGGGCT TTGGG	115
Alphatorquevirus Clade 4 5' UTR (e.g., TTV-HD20a)	CGGGTGCCGGAGGTGAGTTTACACACCGCAGTC AAGGGGCAATTCGGGCTCGGGAGGCCGGGCCAT GGG	116
Alphatorquevirus Clade 5 5' UTR (e.g., TTV-16)	CGGGTGCCGGAGGTGAGTTTACACACCGCAGTC AAGGGGCAATTCGGGCTCGGGACTGGCCGGGCC CCGGG	117

Alphatorquevirus Clade 6 5' UTR (e.g., TTV-TJN02)	CGGGTGCCGGAGGTGAGTTTACACACCGCAGTC AAGGGGCAATTCGGGCTCGGGACTGGCCGGGCT ATGGG	118
Alphatorquevirus Clade 7 5' UTR (e.g., TTV-HD16d)	CGGGTGCCGAAGGTGAGTTTACACACCGCAGTC AAGGGGCAATTCGGGCTCGGGACTGGCCGGGCT ATGGG	119

Identification of 5' UTR sequences

In some embodiments, an Anellovirus 5' UTR sequence can be identified within the genome of an *Anellovirus* (e.g., a putative *Anellovirus* genome identified, for example, by nucleic acid sequencing techniques, e.g., deep sequencing techniques). In some embodiments, an Anellovirus 5' UTR sequence is identified by one or both of the following steps:

(i) Identification of circularization junction point: In some embodiments, a 5' UTR will be positioned near a circularization junction point of a full-length, circularized *Anellovirus* genome. A circularization junction point can be identified, for example, by identifying overlapping regions of the sequence. In some embodiments, an overlapping region of the sequence can be trimmed from the sequence to produce a full-length *Anellovirus* genome sequence that has been circularized. In some embodiments, a genome sequence is circularized in this manner using software. Without wishing to be bound by theory, computationally circularizing a genome may result in the start position for the sequence being oriented in a non-biological. Landmarks within the sequence can be used to re-orient sequences in the proper direction. For example, landmark sequence may include sequences having substantial homology to one or more elements within an Anellovirus genome as described herein (e.g., one or more of a TATA box, cap site, initiator element, transcriptional start site, 5' UTR conserved domain, ORF1, ORF1/1, ORF1/2, ORF2, ORF2/2, ORF2/3, ORF2t/3, three open-reading frame region, poly(A) signal, or GC-rich region of an *Anellovirus*, e.g., as described herein).

(ii) Identification of 5' UTR sequence: Once a putative *Anellovirus* genome sequence has been obtained, the sequence (or portions thereof, e.g., having a length between about 40-50, 50-60, 60-70, 70-80, 80-90, or 90-100 nucleotides) can be compared to one or more Anellovirus 5' UTR sequences (e.g., as described herein) to identify sequences having substantial homology thereto. In some embodiments, a putative *Anellovirus* 5' UTR region has at least 50%, 60%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to an *Anellovirus* 5' UTR sequence as described herein.

GC-Rich Regions

In some embodiments, the genetic element (e.g., protein-binding sequence of the genetic element) comprises a nucleic acid sequence having at least about 75% (e.g., at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100%) identity to a nucleic acid sequence shown in any of Table 39 and/or Figures 20 and 32 of PCT Publication No. WO 2020/123816. In some embodiments, the genetic element (e.g., protein-binding sequence of the genetic element) comprises a nucleic acid sequence having at least about 75% (e.g., at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100%) identity to a GC-rich sequence shown in Table 39.

In some embodiments, the genetic element (e.g., protein-binding sequence of the genetic element) comprises a nucleic acid sequence having at least about 75% (e.g., at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100%) identity to a 36-nucleotide GC-rich sequence as shown in Table 39 (e.g., 36-nucleotide consensus GC-rich region sequence 1, 36-nucleotide consensus GC-rich region sequence 2, TTV Clade 1 36-nucleotide region, TTV Clade 3 36-nucleotide region, TTV Clade 3 isolate GH1 36-nucleotide region, TTV Clade 3 sle1932 36-nucleotide region, TTV Clade 4 ctdc002 36-nucleotide region, TTV Clade 5 36-nucleotide region, TTV Clade 6 36-nucleotide region, or TTV Clade 7 36-nucleotide region). In some embodiments, the genetic element (e.g., protein-binding sequence of the genetic element) comprises a nucleic acid sequence comprising at least 10, 15, 20, 25, 30, 31, 32, 33, 34, 35, or 36 consecutive nucleotides of a 36-nucleotide GC-rich sequence as shown in Table 39 (e.g., 36-nucleotide consensus GC-rich region sequence 1, 36-nucleotide consensus GC-rich region sequence 2, TTV Clade 1 36-nucleotide region, TTV Clade 3 36-nucleotide region, TTV Clade 3 isolate GH1 36-nucleotide region, TTV Clade 3 sle1932 36-nucleotide region, TTV Clade 4 ctdc002 36-nucleotide region, TTV Clade 5 36-nucleotide region, TTV Clade 6 36-nucleotide region, or TTV Clade 7 36-nucleotide region).

In some embodiments, the genetic element (e.g., protein-binding sequence of the genetic element) comprises a nucleic acid sequence having at least about 75% (e.g., at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100%) identity to an Alphatorquevirus GC-rich region sequence, e.g., selected from TTV-CT30F, TTV-P13-1, TTV-tth8, TTV-HD20a, TTV-16, TTV-TJN02, or TTV-HD16d, e.g., as listed in Table 39. In some embodiments, the genetic element (e.g., protein-binding sequence of the genetic element) comprises a nucleic acid sequence comprising at least 10, 15, 20, 25, 30, 35, 40, 45, 50, 60, 70, 80, 90, 100, 104, 105, 108, 110, 111, 115, 120, 122, 130, 140, 145, 150, 155, or 156 consecutive nucleotides of an Alphatorquevirus GC-rich region sequence, e.g., selected from TTV-CT30F, TTV-P13-1, TTV-tth8, TTV-HD20a, TTV-16, TTV-TJN02, or TTV-HD16d, e.g., as listed in Table 39.

In some embodiments, the 36-nucleotide GC-rich sequence is selected from:

(i) CGCGCTGCGCGCGCCGCCCAGTAGGGGGAGCCATGC (SEQ ID NO: 160),

(ii) GCGCTX$_1$CGCGCGCGCGCCGGGGGGCTGCGCCCCCCC (SEQ ID NO: 164), wherein X_1 is selected from T, G, or A;

(iii) GCGCTTCGCGCGCCGCCCACTAGGGGGCGTTGCGCG (SEQ ID NO: 165);

(iv) GCGCTGCGCGCGCCGCCCAGTAGGGGGCGCAATGCG (SEQ ID NO: 166);

(v) GCGCTGCGCGCGCGGCCCCCGGGGGAGGCATTGCCT (SEQ ID NO: 167);

(vi) GCGCTGCGCGCGCGCCGGGGGGGCGCCAGCGCCC (SEQ ID NO: 168);

(vii) GCGCTTCGCGCGCGCCGGGGGGCTCCGCCCCCCC (SEQ ID NO: 169);

(viii) GCGCTTCGCGCGCGCCGGGGGGCTGCGCCCCCC (SEQ ID NO: 170);

(ix) GCGCTACGCGCGCGCCGGGGGGCTGCGCCCCCCC (SEQ ID NO: 171); or

(x) GCGCTACGCGCGCGCCGGGGGGCTCTGCCCCCCC (SEQ ID NO: 172).

In some embodiments, the genetic element (e.g., protein-binding sequence of the genetic element) comprises the nucleic acid sequence CGCGCTGCGCGCGCCGCCCAGTAGGGGGAGCCATGC (SEQ ID NO: 160).

In some embodiments, the genetic element (e.g., protein-binding sequence of the genetic element) comprises a nucleic acid sequence of the Consensus GC-rich sequence shown in Table 39, wherein X_1, X_4, X_5, X_6, X_7, X_{12}, X_{13}, X_{14}, X_{15}, X_{20}, X_{21}, X_{22}, X_{26}, X_{29}, X_{30}, and X_{33} are each independently any nucleotide and wherein X_2, X_3, X_8, X_9, X_{10}, X_{11}, X_{16}, X_{17}, X_{18}, X_{19}, X_{23}, X_{24}, X_{25}, X_{27}, X_{28}, X_{31}, X_{32}, and X_{34} are each independently absent or any nucleotide. In some embodiments, one or more of (e.g., all of) X_1 through X_{34} are each independently the nucleotide (or absent) specified in Table 39. In some embodiments, the genetic element (e.g., protein-binding sequence of the genetic element) comprises a nucleic acid sequence having at least about 75% (e.g., at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100%) identity to an exemplary TTV GC-rich sequence shown in Table 39 (e.g., the full sequence, Fragment 1, Fragment 2, Fragment 3, or any combination thereof, e.g., Fragments 1-3 in order). In some embodiments, the genetic element (e.g., protein-binding sequence of the genetic element) comprises a nucleic acid sequence having at least about 75% (e.g., at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100%) identity to a TTV-CT30F GC-rich sequence shown in Table 39 (e.g., the full sequence, Fragment 1, Fragment 2, Fragment 3, Fragment 4, Fragment 5, Fragment 6, Fragment 7, Fragment 8, or any combination thereof, e.g., Fragments 1-7 in order). In some embodiments, the genetic element (e.g., protein-binding sequence of the genetic element) comprises a nucleic acid sequence having at least about 75% (e.g., at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100%) identity to a TTV-HD23a GC-rich sequence shown in Table 39 (e.g., the full sequence, Fragment 1, Fragment 2, Fragment 3, Fragment 4, Fragment 5, Fragment 6, or any combination thereof, e.g., Fragments 1-6 in order). In some embodiments, the genetic element (e.g., protein-binding sequence of the genetic element) comprises a nucleic acid sequence having at least about 75% (e.g., at least 75%, 80%, 85%, 90%, 95%,

96%, 97%, 98%, 99%, or 100%) identity to a TTV-JA20 GC-rich sequence shown in Table 39 (e.g., the full sequence, Fragment 1, Fragment 2, or any combination thereof, e.g., Fragments 1 and 2 in order). In some embodiments, the genetic element (e.g., protein-binding sequence of the genetic element) comprises a nucleic acid sequence having at least about 75% (e.g., at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100%) identity to a TTV-TJN02 GC-rich sequence shown in Table 39 (e.g., the full sequence, Fragment 1, Fragment 2, Fragment 3, Fragment 4, Fragment 5, Fragment 6, Fragment 7, Fragment 8, or any combination thereof, e.g., Fragments 1-8 in order). In some embodiments, the genetic element (e.g., protein-binding sequence of the genetic element) comprises a nucleic acid sequence having at least about 75% (e.g., at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100%) identity to a TTV-tth8 GC-rich sequence shown in Table 39 (e.g., the full sequence, Fragment 1, Fragment 2, Fragment 3, Fragment 4, Fragment 5, Fragment 6, Fragment 7, Fragment 8, Fragment 9, or any combination thereof, e.g., Fragments 1-6 in order). In some embodiments, the genetic element (e.g., protein-binding sequence of the genetic element) comprises a nucleic acid sequence having at least about 75% (e.g., at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100%) identity to Fragment 7 shown in Table 39. In some embodiments, the genetic element (e.g., protein-binding sequence of the genetic element) comprises a nucleic acid sequence having at least about 75% (e.g., at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100%) identity to Fragment 8 shown in Table 39. In some embodiments, the genetic element (e.g., protein-binding sequence of the genetic element) comprises a nucleic acid sequence having at least about 75% (e.g., at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100%) identity to Fragment 9 shown in Table 39.

Table 39. Exemplary GC-rich sequences from *Anelloviruses*

Source	Sequence	SEQ ID NO:
Consensus	CGGCGGX$_1$GGX$_2$GX$_3$X$_4$X$_5$CGCGCTX$_6$CGCGC GCX$_7$X$_8$X$_9$X$_{10}$CX$_{11}$X$_{12}$X$_{13}$X$_{14}$GGGGX$_{15}$X$_{16}$X$_{17}$X$_{18}$ X$_{19}$X$_{20}$X$_{21}$GCX$_{22}$X$_{23}$X$_{24}$X$_{25}$CCCCCCCX$_{26}$CGCGC ATX$_{27}$X$_{28}$GCX$_{29}$CGGGX$_{30}$CCCCCCCCCX$_{31}$X$_{32}$X $_{33}$GGGGGGCTCCGX$_{34}$CCCCCCGGCCCCCC X$_1$ = G or C X$_2$ = G, C, or absent X$_3$ = C or absent X$_4$ = G or C	120

		X_5 = G or C X_6 = T, G, or A X_7 = G or C X_8 = G or absent X_9 = C or absent X_{10} = C or absent X_{11} = G, A, or absent X_{12} = G or C X_{13} = C or T X_{14} = G or A X_{15} = G or A X_{16} = A, G, T, or absent X_{17} = G, C, or absent X_{18} = G, C, or absent X_{19} = C, A, or absent X_{20} = C or A X_{21} = T or A X_{22} = G or C X_{23} = G, T, or absent X_{24} = C or absent X_{25} = G, C, or absent X_{26} = G or C X_{27} = G or absent X_{28} = C or absent X_{29} = G or A X_{30} = G or T X_{31} = C, T, or absent X_{32} = G, C, A, or absent X_{33} = G or C X_{34} = C or absent	
Exemplary TTV Sequence	Full sequence	GCCGCCGCGGCGGCGGSGGNGNSGCGCGCT DCGCGCGCSNNNCRCCRGGGGGNNNNCWG CSNCNCCCCCCCCCGCGCATGCGCGGGKCC CCCCCCCNNCGGGGGGCTCCGCCCCCCGGC	121

		CCCCCCCCGTGCTAAACCCACCGCGCATGC GCGACCACGCCCCCGCCGCC	
	Fragment 1	GCCGCCGCGGCGGCGGSGGNGNSGCGCGCT DCGCGCGCSNNNCRCCRGGGGGNNNNCWG CSNCNCCCCCCCCCGCGCAT	122
	Fragment 2	GCGCGGGKCCCCCCCCCNNCGGGGGGCTC CG	123
	Fragment 3	CCCCCCGGCCCCCCCCCGTGCTAAACCCAC CGCGCATGCGCGACCACGCCCCCGCCGCC	124
TTV-CT30F	Full sequence	GCGGCGG-GGGGGCG-GCCGCG-TTCGCGCGCCGCCCACCAGGGGGTG--CTGCG-CGCCCCCCCCCGCGCAT GCGCGGGGCCCCCCCCCC--GGGGGGGCTCCGCCCCCCCGGCCCCCCCCC GTGCTAAACCCACCGCGCATGCGCGACCAC GCCCCCGCCGCC	125
	Fragment 1	GCGGCGG	126
	Fragment 2	GGGGGCG	127
	Fragment 3	GCCGCG	128
	Fragment 4	TTCGCGCGCCGCCCACCAGGGGGTG	129
	Fragment 5	CTGCG	130
	Fragment 6	CGCCCCCCCCCGCGCAT	131
	Fragment 7	GCGCGGGGCCCCCCCCC	132
	Fragment 8	GGGGGGGCTCCGCCCCCCCGGCCCCCCCCC GTGCTAAACCCACCGCGCATGCGCGACCAC GCCCCCGCCGCC	133
TTV-HD23a	Full sequence	CGGCGGCGGCGGCG-CGCGCGCTGCGCGCGCG---CGCCGGGGGGGCGCCAGCG-CCCCCCCCCCCGCGCAT GCACGGGTCCCCCCCCCCACGGGGGGCTCC G CCCCCCGGCCCCCCCCC	134
	Fragment 1	CGGCGGCGGCGGCG	135

	Fragment 2	CGCGCGCTGCGCGCGCG	136
	Fragment 3	CGCCGGGGGGGCGCCAGCG	137
	Fragment 4	CCCCCCCCCCCGCGCAT	138
	Fragment 5	GCACGGGTCCCCCCCCCCACGGGGGGCTCCG	139
	Fragment 6	CCCCCCGGCCCCCCCCC	140
TTV-JA20	Full sequence	CCGTCGGCGGGGGGGCCGCGCGCTGCGCGCGGCCC-CCGGGGGAGGCACAGCCTCCCCCCCCCGCGCGCATGCGCGCGGGTCCCCCCCCCTCCGGGGGGCTCCGCCCCCCGGCCCCCCCC	141
	Fragment 1	CCGTCGGCGGGGGGGCCGCGCGCTGCGCGCGGCCC	142
	Fragment 2	CCGGGGGAGGCACAGCCTCCCCCCCCCGCGCGCATGCGCGCGGGTCCCCCCCCCCTCCGGGGGGCTCCGCCCCCCGGCCCCCCCC	143
TTV-TJN02	Full sequence	CGGCGGCGGCG-CGCGCGCTACGCGCGCG---CGCCGGGGGG----CTGCCGC-CCCCCCCCCGCGCAT GCGCGGGGCCCCCCCCC-GCGGGGGGCTCCG CCCCCCGGCCCCCC	144
	Fragment 1	CGGCGGCGGCG	145
	Fragment 2	CGCGCGCTACGCGCGCG	146
	Fragment 3	CGCCGGGGGG	147
	Fragment 4	CTGCCGC	148
	Fragment 5	CCCCCCCCCGCGCAT	149
	Fragment 6	GCGCGGGGCCCCCCCCC	150
	Fragment 7	GCGGGGGGCTCCG	151
	Fragment 8	CCCCCCGGCCCCCC	152
TTV-tth8	Full sequence	GCCGCCGCGGCGGCGGGGG-GCGGCGCGCTGCGCGCGCCGCCCAGTAGGGGGAGCCATGCG---CCCCCCCCCGCGCATGCGCGGGGCCCCCCCCCC-	153

		GCGGGGGGCTCCG CCCCCCGGCCCCCCCCG	
	Fragment 1	GCCGCCGCGGCGGCGGGGG	154
	Fragment 2	GCGGCGCGCTGCGCGCGCCGCCCAGTAGG GGGAGCCATGCG	155
	Fragment 3	CCCCCCCCCGCGCAT	156
	Fragment 4	GCGCGGGGCCCCCCCCC	157
	Fragment 5	GCGGGGGGCTCCG	158
	Fragment 6	CCCCCCGGCCCCCCCCG	159
	Fragment 7	CGCGCTGCGCGCGCCGCCCAGTAGGGGGA GCCATGC	160
	Fragment 8	CCGCCATCTTAAGTAGTTGAGGCGGACGGT GGCGTGAGTTCAAAGGTCACCATCAGCCAC ACCTACTCAAAATGGTGG	161
	Fragment 9	CTTAAGTAGTTGAGGCGGACGGTGGCGTGA GTTCAAAGGTCACCATCAGCCACACCTACT CAAAATGGTGGACAATTTCTTCCGGGTCAA AGGTTACAGCCGCCATGTTAAAACACGTGA CGTATGACGTCACGGCCGCCATTTTGTGAC ACAAGATGGCCGACTTCCTTCC	162
Additional GC-rich Sequences (as shown in Figure 32 of PCT Publication No. WO 2020/123816)	36-nucleotide consensus GC-rich region sequence 1	CGCGCTGCGCGCGCCGCCCAGTAGGGGGA GCCATGC	163
	36-nucleotide region consensus sequence 2	GCGCTX$_1$CGCGCGCGCCGGGGGGCTGCG CCCCCCC, wherein X$_1$ is selected from T, G, or A	164
	TTV Clade 1 36-nucleotide region	GCGCTTCGCGCGCCGCCCACTAGGGGGCGT TGCGCG	165

	TTV Clade 3 36-nucleotide region	GCGCTGCGCGCGCCGCCCAGTAGGGGGCG CAATGCG	166
	TTV Clade 3 isolate GH1 36-nucleotide region	GCGCTGCGCGCGCGGCCCCCGGGGGAGGC ATTGCCT	167
	TTV Clade 3 sle1932 36-nucleotide region	GCGCTGCGCGCGCGCCGGGGGGGCGCC AGCGCCC	168
	TTV Clade 4 ctdc002 36-nucleotide region	GCGCTTCGCGCGCGCCGGGGGGCTCCGC CCCCCC	169
	TTV Clade 5 36-nucleotide region	GCGCTTCGCGCGCGCCGGGGGGCTGCGC CCCCCC	170
	TTV Clade 6 36-nucleotide region	GCGCTACGCGCGCGCCGGGGGGCTGCG CCCCCCC	171
	TTV Clade 7 36-nucleotide region	GCGCTACGCGCGCGCCGGGGGGCTCTGC CCCCCC	172
Additional Alphatorquevirus GC-rich region sequences	TTV-CT30F	GCGGCGGGGGGGCGGCCGCGTTCGCGCGC CGCCCACCAGGGGGTGCTGCGCGCCCCCCC CCGCGCATGCGCGGGGCCCCCCCCCGGGG GGGCTCCGCCCCCCCGGCCCCCCCCCGTGC TAAACCCACCGCGCATGCGCGACCACGCCC CCGCCGCC	801
	TTV-P13-1	CCGAGCGTTAGCGAGGAGTGCGACCCTACC CCCTGGGCCCACTTCTTCGGAGCCGCGCGC TACGCCTTCGGCTGCGCGCGGCACCTCAGA	802

		CCCCCGCTCGTGCTGACACGCTTGCGCGTG TCAGACCACTTCGGGCTCGCGGGGGTCGGG	
	TTV-tth8	GCCGCCGCGGCGGCGGGGGGCGGCGCGCT GCGCGCGCCGCCCAGTAGGGGGAGCCATG CGCCCCCCCCCGCGCATGCGCGGGGCCCCC CCCCGCGGGGGGCTCCGCCCCCCGGCCCCC CCCG	803
	TTV-HD20a	CGGCCCAGCGGCGGCGCGCGCTTCGCGC GCGCGCCGGGGGGCTCCGCCCCCCCCCGCG CATGCGCGGGGCCCCCCCCCGCGGGGGGCT CCGCCCCCCGGTCCCCCCCCG	804
	TTV-16	CGGCCGTGCGGCGGCGCGCGCTTCGCGC GCGCGCCGGGGGCTGCCGCCCCCCCCCGCG CATGCGCGCGGGGCCCCCCCCCGCGGGGG GCTCCGCCCCCCGGCCCCCCCCCCG	805
	TTV-TJN02	CGGCGGCGGCGCGCGCGCTACGCGCGCGC GCCGGGGGGCTGCCGCCCCCCCCCCGCGCA TGCGCGGGGCCCCCCCCCGCGGGGGGCTCC GCCCCCCGGCCCCCC	806
	TTV-HD16d	GGCGGCGGCGCGCGCGCTACGCGCGCGCG CCGGGGAGCTCTGCCCCCCCCCGCGCATGC GCGCGGGTCCCCCCCCCGCGGGGGGCTCCG CCCCCCGGTCCCCCCCCCG	807

In some embodiments, the genetic element comprises a nucleic acid sequence having at least about 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to the *Anellovirus* GC-rich nucleotide sequence of any one of Tables N1-N26.

Effector

In some embodiments, the genetic element may include one or more sequences that encode a functional effector, e.g., an endogenous effector or an exogenous effector, e.g., a therapeutic polypeptide or nucleic acid, e.g., cytotoxic or cytolytic RNA or protein. In some embodiments, the functional nucleic acid is a non-coding RNA. In some embodiments, the functional nucleic acid is a coding RNA. The effector may modulate a biological activity, for example increasing or decreasing enzymatic activity, gene

expression, cell signaling, and cellular or organ function. Effector activities may also include binding regulatory proteins to modulate activity of the regulator, such as transcription or translation. Effector activities also may include activator or inhibitor functions. For example, the effector may induce enzymatic activity by triggering increased substrate affinity in an enzyme, e.g., fructose 2,6-bisphosphate activates phosphofructokinase 1 and increases the rate of glycolysis in response to the insulin. In another example, the effector may inhibit substrate binding to a receptor and inhibit its activation, e.g., naltrexone and naloxone bind opioid receptors without activating them and block the receptors' ability to bind opioids. Effector activities may also include modulating protein stability/degradation and/or transcript stability/degradation. For example, proteins may be targeted for degradation by the polypeptide co-factor, ubiquitin, onto proteins to mark them for degradation. In another example, the effector inhibits enzymatic activity by blocking the enzyme's active site, e.g., methotrexate is a structural analog of tetrahydrofolate, a coenzyme for the enzyme dihydrofolate reductase that binds to dihydrofolate reductase 1000-fold more tightly than the natural substrate and inhibits nucleotide base synthesis.

In some embodiments, the sequence encoding an effector is part of the genetic element, e.g., it can be inserted at an insert site as described in Example 10, 12, or 22 of PCT Publication No. WO 2020/123816, incorporated by reference herein in its entirety, and Example 28 herein. In some embodiments, the sequence encoding an effector is inserted into the genetic element at a noncoding region, e.g., a noncoding region disposed 3' of the open reading frames and 5' of the GC-rich region of the genetic element, in the 5' noncoding region upstream of the TATA box, in the 5' UTR, in the 3' noncoding region downstream of the poly-A signal, or upstream of the GC-rich region. In some embodiments, the sequence encoding an effector is inserted into the genetic element at about nucleotide 3588 of a TTV-tth8 plasmid, e.g., as described herein or at about nucleotide 2843 of a TTMV-LY2 plasmid, e.g., as described herein. In some embodiments, the sequence encoding an effector is inserted into the genetic element at or within nucleotides 336-3015 of a TTV-tth8 plasmid, e.g., as described herein, or at or within nucleotides 242-2812 of a TTV-LY2 plasmid, e.g., as described herein. In some embodiments, the sequence encoding an effector replaces part or all of an open reading frame (e.g., an ORF as described herein, e.g., an ORF1, ORF1/1, ORF1/2, ORF2, ORF2/2, ORF2/3, and/or ORF2t/3 as shown in any one of Tables A1-A26 or N1-N26).

In some embodiments, the sequence encoding an effector comprises 100-2000, 100-1000, 100-500, 100-200, 200-2000, 200-1000, 200-500, 500-1000, 500-2000, or 1000-2000 nucleotides. In some embodiments, the effector is a nucleic acid or protein payload, e.g., as described in Example 27.

Regulatory Nucleic Acid

In some embodiments, the effector is a regulatory nucleic acid. Regulatory nucleic acids modify expression of an endogenous gene and/or an exogenous gene. In one embodiment, the regulatory nucleic acid targets a host gene. The regulatory nucleic acids may include, but are not limited to, a nucleic acid that hybridizes to an endogenous gene (e.g., miRNA, siRNA, mRNA, lncRNA, RNA, DNA, an antisense RNA, gRNA as described herein elsewhere), nucleic acid that hybridizes to an exogenous nucleic acid such as a viral DNA or RNA, nucleic acid that hybridizes to an RNA, nucleic acid that interferes with gene transcription, nucleic acid that interferes with RNA translation, nucleic acid that stabilizes RNA or destabilizes RNA such as through targeting for degradation, and nucleic acid that modulates a DNA or RNA binding factor. In some embodiments, the regulatory nucleic acid encodes an miRNA.

In some embodiments, the regulatory nucleic acid comprises RNA or RNA-like structures typically containing 5-500 base pairs (depending on the specific RNA structure, e.g., miRNA 5-30 bps, lncRNA 200-500 bps) and may have a nucleobase sequence identical (or complementary) or nearly identical (or substantially complementary) to a coding sequence in an expressed target gene within the cell, or a sequence encoding an expressed target gene within the cell.

In some embodiments, the regulatory nucleic acid comprises a nucleic acid sequence, e.g., a guide RNA (gRNA). In some embodiments, the DNA targeting moiety comprises a guide RNA or nucleic acid encoding the guide RNA. A gRNA short synthetic RNA can be composed of a "scaffold" sequence necessary for binding to the incomplete effector moiety and a user-defined ~20 nucleotide targeting sequence for a genomic target. In practice, guide RNA sequences are generally designed to have a length of between 17 – 24 nucleotides (e.g., 19, 20, or 21 nucleotides) and complementary to the targeted nucleic acid sequence. Custom gRNA generators and algorithms are available commercially for use in the design of effective guide RNAs. Gene editing has also been achieved using a chimeric "single guide RNA" ("sgRNA"), an engineered (synthetic) single RNA molecule that mimics a naturally occurring crRNA-tracrRNA complex and contains both a tracrRNA (for binding the nuclease) and at least one crRNA (to guide the nuclease to the sequence targeted for editing). Chemically modified sgRNAs have also been demonstrated to be effective in genome editing; see, for example, Hendel et al. (2015) Nature Biotechnol., 985 – 991.

The regulatory nucleic acid comprises a gRNA that recognizes specific DNA sequences (e.g., sequences adjacent to or within a promoter, enhancer, silencer, or repressor of a gene).

Certain regulatory nucleic acids can inhibit gene expression through the biological process of RNA interference (RNAi). RNAi molecules comprise RNA or RNA-like structures typically containing 15-50 base pairs (such as about18-25 base pairs) and having a nucleobase sequence identical (complementary) or nearly identical (substantially complementary) to a coding sequence in an expressed

target gene within the cell. RNAi molecules include, but are not limited to: short interfering RNAs (siRNAs), double-strand RNAs (dsRNA), micro RNAs (miRNAs), short hairpin RNAs (shRNA), meroduplexes, and dicer substrates (U.S. Pat. Nos. 8,084,599 8,349,809 and 8,513,207).

Long non-coding RNAs (lncRNA) are defined as non-protein coding transcripts longer than 100 nucleotides. This somewhat arbitrary limit distinguishes lncRNAs from small regulatory RNAs such as microRNAs (miRNAs), short interfering RNAs (siRNAs), and other short RNAs. In general, the majority (~78%) of lncRNAs are characterized as tissue-specific. Divergent lncRNAs that are transcribed in the opposite direction to nearby protein-coding genes (comprise a significant proportion ~20% of total lncRNAs in mammalian genomes) may possibly regulate the transcription of the nearby gene.

The genetic element may encode regulatory nucleic acids with a sequence substantially complementary, or fully complementary, to all or a fragment of an endogenous gene or gene product (e.g., mRNA). The regulatory nucleic acids may complement sequences at the boundary between introns and exons to prevent the maturation of newly-generated nuclear RNA transcripts of specific genes into mRNA for transcription. The regulatory nucleic acids that are complementary to specific genes can hybridize with the mRNA for that gene and prevent its translation. The antisense regulatory nucleic acid can be DNA, RNA, or a derivative or hybrid thereof.

The length of the regulatory nucleic acid that hybridizes to the transcript of interest may be between 5 to 30 nucleotides, between about 10 to 30 nucleotides, or about 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30 or more nucleotides. The degree of identity of the regulatory nucleic acid to the targeted transcript should be at least 75%, at least 80%, at least 85%, at least 90%, or at least 95%.

The genetic element may encode a regulatory nucleic acid, e.g., a micro RNA (miRNA) molecule identical to about 5 to about 25 contiguous nucleotides of a target gene. In some embodiments, the miRNA sequence targets a mRNA and commences with the dinucleotide AA, comprises a GC-content of about 30-70% (about 30-60%, about 40-60%, or about 45%-55%), and does not have a high percentage identity to any nucleotide sequence other than the target in the genome of the mammal in which it is to be introduced, for example as determined by standard BLAST search.

In some embodiments, the regulatory nucleic acid is at least one miRNA, e.g., 2, 3, 4, 5, 6, or more. In some embodiments, the genetic element comprises a sequence that encodes an miRNA at least about 75%, 80%, 85%, 90% 95%, 96%, 97%, 98%, 99% or 100% nucleotide sequence identity to any one of the nucleotide sequences or a sequence that is complementary to a sequence described herein.

siRNAs and shRNAs resemble intermediates in the processing pathway of the endogenous microRNA (miRNA) genes (Bartel, Cell 116:281-297, 2004). In some embodiments, siRNAs can function as miRNAs and vice versa (Zeng et al., Mol Cell 9:1327-1333, 2002; Doench et al., Genes Dev

17:438-442, 2003). MicroRNAs, like siRNAs, use RISC to downregulate target genes, but unlike siRNAs, most animal miRNAs do not cleave the mRNA. Instead, miRNAs reduce protein output through translational suppression or polyA removal and mRNA degradation (Wu et al., Proc Natl Acad Sci USA 103:4034-4039, 2006). Known miRNA binding sites are within mRNA 3' UTRs; miRNAs seem to target sites with near-perfect complementarity to nucleotides 2-8 from the miRNA's 5' end (Rajewsky, Nat Genet 38 Suppl:S8-13, 2006; Lim et al., Nature 433:769-773, 2005). This region is known as the seed region. Because siRNAs and miRNAs are interchangeable, exogenous siRNAs downregulate mRNAs with seed complementarity to the siRNA (Birmingham et al., Nat Methods 3:199-204, 2006. Multiple target sites within a 3' UTR give stronger downregulation (Doench et al., Genes Dev 17:438-442, 2003).

Lists of known miRNA sequences can be found in databases maintained by research organizations, such as Wellcome Trust Sanger Institute, Penn Center for Bioinformatics, Memorial Sloan Kettering Cancer Center, and European Molecule Biology Laboratory, among others. Known effective siRNA sequences and cognate binding sites are also well represented in the relevant literature. RNAi molecules are readily designed and produced by technologies known in the art. In addition, there are computational tools that increase the chance of finding effective and specific sequence motifs (Lagana et al., Methods Mol. Bio., 2015, 1269:393-412).

The regulatory nucleic acid may modulate expression of RNA encoded by a gene. Because multiple genes can share some degree of sequence homology with each other, in some embodiments, the regulatory nucleic acid can be designed to target a class of genes with sufficient sequence homology. In some embodiments, the regulatory nucleic acid can contain a sequence that has complementarity to sequences that are shared amongst different gene targets or are unique for a specific gene target. In some embodiments, the regulatory nucleic acid can be designed to target conserved regions of an RNA sequence having homology between several genes thereby targeting several genes in a gene family (e.g., different gene isoforms, splice variants, mutant genes, etc.). In some embodiments, the regulatory nucleic acid can be designed to target a sequence that is unique to a specific RNA sequence of a single gene.

In some embodiments, the genetic element may include one or more sequences that encode regulatory nucleic acids that modulate expression of one or more genes.

In one embodiment, the gRNA described elsewhere herein are used as part of a CRISPR system for gene editing. For the purposes of gene editing, the anellovector or anelloVLP may be designed to include one or multiple guide RNA sequences corresponding to a desired target DNA sequence; see, for example, Cong et al. (2013) Science, 339:819–823; Ran et al. (2013) Nature Protocols, 8:2281 – 2308. At least about 16 or 17 nucleotides of gRNA sequence generally allow for Cas9-mediated DNA cleavage to occur; for Cpf1 at least about 16 nucleotides of gRNA sequence is needed to achieve detectable DNA cleavage.

Therapeutic effectors (e.g., peptides or polypeptides)

In some embodiments, the genetic element comprises a therapeutic expression sequence, e.g., a sequence that encodes a therapeutic peptide or polypeptide, e.g., an intracellular peptide or intracellular polypeptide, a secreted polypeptide, or a protein replacement therapeutic, e.g., a wild-type protein or a functional fragment or variant thereof. In some embodiments, the genetic element includes a sequence encoding a protein e.g., a therapeutic protein. Some examples of therapeutic proteins may include, but are not limited to, a hormone, a cytokine, an enzyme, an antibody (e.g., one or a plurality of polypeptides encoding at least a heavy chain or a light chain), a transcription factor, a receptor (e.g., a membrane receptor), a ligand, a membrane transporter, a secreted protein, a peptide, a carrier protein, a structural protein, a nuclease, or a component thereof.

In some embodiments, the genetic element includes a sequence encoding a peptide e.g., a therapeutic peptide. The peptides may be linear or branched. The peptide has a length from about 5 to about 500 amino acids, about 15 to about 400 amino acids, about 20 to about 325 amino acids, about 25 to about 250 amino acids, about 50 to about 200 amino acids, or any range there between.

In some embodiments, the polypeptide encoded by the therapeutic expression sequence may be a functional variant or fragment thereof of any of the above, e.g., a protein having at least 80%, 85%, 90%, 95%, 967%, 98%, 99% identity to a protein sequence which disclosed in a table herein by reference to its UniProt ID.

In some embodiments, the therapeutic expression sequence may encode an antibody or antibody fragment that binds any of the above, e.g., an antibody against a protein having at least 80%, 85%, 90%, 95%, 967%, 98%, 99% identity to a protein sequence which disclosed in a table herein by reference to its UniProt ID. The term "antibody" herein is used in the broadest sense and encompasses various antibody structures, including but not limited to monoclonal antibodies, polyclonal antibodies, multispecific antibodies (e.g., bispecific antibodies), and antibody fragments so long as they exhibit the desired antigen-binding activity. An "antibody fragment" refers to a molecule that includes at least one heavy chain or light chain and binds an antigen. Examples of antibody fragments include but are not limited to Fv, Fab, Fab', Fab'-SH, $F(ab')_2$; diabodies; linear antibodies; single-chain antibody molecules (e.g. scFv); and multispecific antibodies formed from antibody fragments.

In some embodiments, an effector as described herein comprises a cytosolic polypeptide or cytosolic peptide, e.g., a wild-type protein or a functional fragment or variant thereof.

In some embodiments, the effector comprises a regulatory intracellular polypeptide, e.g., a wild-type protein or a functional fragment or variant thereof. In some embodiments, the regulatory

intracellular polypeptide binds one or more molecule (e.g., protein or nucleic acid) endogenous to the target cell. In some embodiments, the regulatory intracellular polypeptide increases the level or activity of one or more molecule (e.g., protein or nucleic acid) endogenous to the target cell. In some embodiments, the regulatory intracellular polypeptide decreases the level or activity of one or more molecule (e.g., protein or nucleic acid) endogenous to the target cell.

In some embodiments, an effector as described herein comprises a secreted polypeptide effector, e.g., a wild-type protein or a functional fragment or variant thereof. Exemplary secreted therapeutics include cytokines and cytokine receptors.

Exemplary cytokines and cytokine receptors are described, e.g., in Akdis et al., "Interleukins (from IL-1 to IL-38), interferons, transforming growth factor β, and TNF-α: Receptors, functions, and roles in diseases" October 2016 Volume 138, Issue 4, Pages 984–1010.

Additional exemplary secreted therapeutics include polypeptide hormones and receptors, e.g., a wild-type protein or a functional fragment or variant thereof.

Additional exemplary secreted therapeutics include growth factors, e.g., a wild-type protein or a functional fragment or variant thereof.Exemplary growth factors and growth factor receptors are described, e.g., in Bafico et al., "Classification of Growth Factors and Their Receptors" Holland-Frei Cancer Medicine. 6th edition.

Additional exemplary secreted therapeutics include clotting-associated factors, e.g., a wild-type protein or a functional fragment or variant thereof.

In some embodiments, an effector described herein comprises a protein replacement therapeutic, e.g., a wild-type protein or a functional fragment or variant thereof. Exemplary protein replacement therapeutics are described herein.

In some embodiments, an effector described herein comprises an enzymatic effector, e.g., a wild-type protein or a functional fragment or variant thereof.

In some embodiments, an effector described herein comprises a non-enzymatic effector, e.g., a wild-type protein or a functional fragment or variant thereof.

In some embodiments, an effector described herein comprises a protein that, when mutated, causes a lysosomal storage disorder, e.g., a wild-type protein or a functional fragment or variant thereof. In some embodiments, an effector described herein comprises a transporter protein, e.g., a wild-type protein or a functional fragment or variant thereof.

In some embodiments, a functional variant of a wild-type protein comprises a protein that has one or more activities of the wild-type protein, e.g., the functional variant catalyzes the same reaction as the corresponding wild-type protein, e.g., at a rate no less than 10%, 20%, 30%, 40%, or 50% lower than the wild-type protein. In some embodiments, the functional variant binds to the same binding partner that

is bound by the wild-type protein, e.g., with a Kd of no more than 10%, 20%, 30%, 40%, or 50% higher than the Kd of the corresponding wild-type protein for the same binding partner under the same conditions. In some embodiments, the functional variant has at a polyeptpide sequence at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to that of the wild-type polypeptide. In some embodiments, the functional variant comprises a homolog (e.g., ortholog or paralog) of the corresponding wild-type protein. In some embodiments, the functional variant is a fusion protein. In some embodiments, the fusion comprises a first region with at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity to the corresponding wild-type protein, and a second, heterologous region. In some embodiments, the functional variant comprises or consists of a fragment of the corresponding wild-type protein.

In some embodiments, an effector as described herein comprises a transformation factor, e.g., a wild-type protein or a fragment or variant thereof. In embodiments, the transformation factor is a protein factor that transforms fibroblasts into differentiated cells.In some embodiments, an effector as described herein comprises a protein that stimulates cellular regeneration, e.g., a wild-type protein or a fragment or variant thereof.

In some embodiments, an effector as described herein modulates STING/cGAS signaling, e.g., a wild-type protein or a fragment or variant thereof. In some embodiments, the STING modulator is a polypeptide, e.g., a viral polypeptide or a functional variant thereof. For instance, the effector may comprise a STING modulator (e.g., inhibitor) described in Maringer et al. "Message in a bottle: lessons learned from antagonism of STING signalling during RNA virus infection" Cytokine & Growth Factor Reviews Volume 25, Issue 6, December 2014, Pages 669-679. Additional STING modulators (e.g., activators) are described, e.g., in Wang et al. "STING activator c-di-GMP enhances the anti-tumor effects of peptide vaccines in melanoma-bearing mice." Cancer Immunol Immunother. 2015 Aug;64(8):1057-66. doi: 10.1007/s00262-015-1713-5. Epub 2015 May 19; Bose "cGAS/STING Pathway in Cancer: Jekyll and Hyde Story of Cancer Immune Response" Int J Mol Sci. 2017 Nov; 18(11): 2456; and Fu et al. "STING agonist formulated cancer vaccines can cure established tumors resistant to PD-1 blockade" Sci Transl Med. 2015 Apr 15; 7(283): 283ra52.Some examples of peptides include, but are not limited to, fluorescent tag or marker, antigen, peptide therapeutic, synthetic or analog peptide from naturally-bioactive peptide, agonist or antagonist peptide, anti-microbial peptide, a targeting or cytotoxic peptide, a degradation or self-destruction peptide, and degradation or self-destruction peptides. Peptides useful in the invention described herein also include antigen-binding peptides, e.g., antigen binding antibody or antibody-like fragments, such as single chain antibodies, nanobodies (see, e.g., Steeland et al. 2016. Nanobodies as therapeutics: big opportunities for small antibodies. Drug Discov Today: 21(7):1076-113).

Such antigen binding peptides may bind a cytosolic antigen, a nuclear antigen, or an intra-organellar antigen.

In some embodiments, the genetic element comprises a sequence that encodes small peptides, peptidomimetics (e.g., peptoids), amino acids, and amino acid analogs. Such therapeutics generally have a molecular weight less than about 5,000 grams per mole, a molecular weight less than about 2,000 grams per mole, a molecular weight less than about 1,000 grams per mole, a molecular weight less than about 500 grams per mole, and salts, esters, and other pharmaceutically acceptable forms of such compounds. Such therapeutics may include, but are not limited to, a neurotransmitter, a hormone, a drug, a toxin, a viral or microbial particle, a synthetic molecule, and agonists or antagonists thereof.

In some embodiments, the composition, anellovector, or anelloVLP described herein includes a polypeptide linked to a ligand that is capable of targeting a specific location, tissue, or cell.

Gene Editing Components

The genetic element of the anellovector may include one or more genes that encode a component of a gene editing system. Alternatively, an anellovector or anelloVLP as described herein may comprise a component of a gene editing system. Exemplary gene editing systems include the clustered regulatory interspaced short palindromic repeat (CRISPR) system, zinc finger nucleases (ZFNs), and Transcription Activator-Like Effector-based Nucleases (TALEN). ZFNs, TALENs, and CRISPR-based methods are described, e.g., in Gaj et al. Trends Biotechnol. 31.7(2013):397-405; CRISPR methods of gene editing are described, e.g., in Guan et al., Application of CRISPR-Cas system in gene therapy: Pre-clinical progress in animal model. DNA Repair 2016 Oct;46:1-8. doi: 10.1016/j.dnarep.2016.07.004; Zheng et al., Precise gene deletion and replacement using the CRISPR/Cas9 system in human cells. BioTechniques, Vol. 57, No. 3, September 2014, pp. 115–124.

CRISPR systems are adaptive defense systems originally discovered in bacteria and archaea. CRISPR systems use RNA-guided nucleases termed CRISPR-associated or "Cas" endonucleases (e. g., Cas9 or Cpf1) to cleave foreign DNA. In a typical CRISPR/Cas system, an endonuclease is directed to a target nucleotide sequence (e. g., a site in the genome that is to be sequence-edited) by sequence-specific, non-coding "guide RNAs" that target single- or double-stranded DNA sequences. Three classes (I-III) of CRISPR systems have been identified. The class II CRISPR systems use a single Cas endonuclease (rather than multiple Cas proteins). One class II CRISPR system includes a type II Cas endonuclease such as Cas9, a CRISPR RNA ("crRNA"), and a trans-activating crRNA ("tracrRNA"). The crRNA contains a "guide RNA", typically about 20-nucleotide RNA sequence that corresponds to a target DNA sequence. The crRNA also contains a region that binds to the tracrRNA to form a partially double-stranded structure which is cleaved by RNase III, resulting in a crRNA/tracrRNA hybrid. The

crRNA/tracrRNA hybrid then directs the Cas9 endonuclease to recognize and cleave the target DNA sequence. The target DNA sequence must generally be adjacent to a "protospacer adjacent motif" ("PAM") that is specific for a given Cas endonuclease; however, PAM sequences appear throughout a given genome.

In some embodiments, the anellovector includes a gene for a CRISPR endonuclease. For example, some CRISPR endonucleases identified from various prokaryotic species have unique PAM sequence requirements; examples of PAM sequences include 5'-NGG (Streptococcus pyogenes), 5'-NNAGAA (Streptococcus thermophilus CRISPR1), 5'-NGGNG (Streptococcus thermophilus CRISPR3), and 5'-NNNGATT (Neisseria meningiditis). Some endonucleases, e. g., Cas9 endonucleases, are associated with G-rich PAM sites, e. g., 5'-NGG, and perform blunt-end cleaving of the target DNA at a location 3 nucleotides upstream from (5' from) the PAM site. Another class II CRISPR system includes the type V endonuclease Cpf1, which is smaller than Cas9; examples include AsCpf1 (from Acidaminococcus sp.) and LbCpf1 (from Lachnospiraceae sp.). Cpf1 endonucleases, are associated with T-rich PAM sites, e. g., 5'-TTN. Cpf1 can also recognize a 5'-CTA PAM motif. Cpf1 cleaves the target DNA by introducing an offset or staggered double-strand break with a 4- or 5-nucleotide 5' overhang, for example, cleaving a target DNA with a 5-nucleotide offset or staggered cut located 18 nucleotides downstream from (3' from) from the PAM site on the coding strand and 23 nucleotides downstream from the PAM site on the complimentary strand; the 5-nucleotide overhang that results from such offset cleavage allows more precise genome editing by DNA insertion by homologous recombination than by insertion at blunt-end cleaved DNA. See, e. g., Zetsche et al. (2015) Cell, 163:759 – 771.

A variety of CRISPR associated (Cas) genes may be included in the anellovector. Specific examples of genes are those that encode Cas proteins from class II systems including Cas1, Cas2, Cas3, Cas4, Cas5, Cas6, Cas7, Cas8, Cas9, Cas10, Cpf1, C2C1, or C2C3. In some embodiments, the anellovector includes a gene encoding a Cas protein, e.g., a Cas9 protein, may be from any of a variety of prokaryotic species. In some embodiments, the anellovector includes a gene encoding a particular Cas protein, e.g., a particular Cas9 protein, is selected to recognize a particular protospacer-adjacent motif (PAM) sequence. In some embodiments, the anellovector includes nucleic acids encoding two or more different Cas proteins, or two or more Cas proteins, may be introduced into a cell, zygote, embryo, or animal, e.g., to allow for recognition and modification of sites comprising the same, similar or different PAM motifs. In some embodiments, the anellovector includes a gene encoding a modified Cas protein with a deactivated nuclease, e.g., nuclease-deficient Cas9.

Whereas wild-type Cas9 protein generates double-strand breaks (DSBs) at specific DNA sequences targeted by a gRNA, a number of CRISPR endonucleases having modified functionalities are known, for example: a "nickase" version of Cas endonuclease (e.g., Cas9) generates only a single-strand

break; a catalytically inactive Cas endonuclease, e.g., Cas9 ("dCas9") does not cut the target DNA. A gene encoding a dCas9 can be fused with a gene encoding an effector domain to repress (CRISPRi) or activate (CRISPRa) expression of a target gene. For example, the gene may encode a Cas9 fusion with a transcriptional silencer (e.g., a KRAB domain) or a transcriptional activator (e.g., a dCas9–VP64 fusion). A gene encoding a catalytically inactive Cas9 (dCas9) fused to FokI nuclease ("dCas9-FokI") can be included to generate DSBs at target sequences homologous to two gRNAs. See, e. g., the numerous CRISPR/Cas9 plasmids disclosed in and publicly available from the Addgene repository (Addgene, 75 Sidney St., Suite 550A, Cambridge, MA 02139; addgene.org/crispr/). A "double nickase" Cas9 that introduces two separate double-strand breaks, each directed by a separate guide RNA, is described as achieving more accurate genome editing by Ran et al. (2013) Cell, 154:1380 – 1389.

CRISPR technology for editing the genes of eukaryotes is disclosed in US Patent Application Publications 2016/0138008A1 and US2015/0344912A1, and in US Patents 8,697,359, 8,771,945, 8,945,839, 8,999,641, 8,993,233, 8,895,308, 8,865,406, 8,889,418, 8,871,445, 8,889,356, 8,932,814, 8,795,965, and 8,906,616. Cpf1 endonuclease and corresponding guide RNAs and PAM sites are disclosed in US Patent Application Publication 2016/0208243 A1.

In some embodiments, the anellovector comprises a gene encoding a polypeptide described herein, e.g., a targeted nuclease, e.g., a Cas9, e.g., a wild type Cas9, a nickase Cas9 (e.g., Cas9 D10A), a dead Cas9 (dCas9), eSpCas9, Cpf1, C2C1, or C2C3, and a gRNA. The choice of genes encoding the nuclease and gRNA(s) is determined by whether the targeted mutation is a deletion, substitution, or addition of nucleotides, e.g., a deletion, substitution, or addition of nucleotides to a targeted sequence. Genes that encode a catalytically inactive endonuclease e.g., a dead Cas9 (dCas9, e.g., D10A; H840A) tethered with all or a portion of (e.g., biologically active portion of) an (one or more) effector domain (e.g., VP64) create chimeric proteins that can modulate activity and/or expression of one or more target nucleic acids sequences.

In some embodiments, the anellovector includes a gene encoding a fusion of a dCas9 with all or a portion of one or more effector domains (e.g., a full-length wild-type effector domain, or a fragment or variant thereof, e.g., a biologically active portion thereof) to create a chimeric protein useful in the methods described herein. Accordingly, in some embodiments, the anellovector includes a gene encoding a dCas9-methylase fusion. In other some embodiments, the anellovector includes a gene encoding a dCas9-enzyme fusion with a site-specific gRNA to target an endogenous gene.

In other aspects, the anellovector includes a gene encoding 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, or more effector domains (all or a biologically active portion) fused with dCas9.

Regulatory Sequences

In some embodiments, the genetic element comprises a regulatory sequence, e.g., a promoter or an enhancer, operably linked to the sequence encoding the effector.

In some embodiments, a promoter includes a DNA sequence that is located adjacent to a DNA sequence that encodes an expression product. A promoter may be linked operatively to the adjacent DNA sequence. A promoter typically increases an amount of product expressed from the DNA sequence as compared to an amount of the expressed product when no promoter exists. A promoter from one organism can be utilized to enhance product expression from the DNA sequence that originates from another organism. For example, a vertebrate promoter may be used for the expression of jellyfish GFP in vertebrates. In addition, one promoter element can increase an amount of products expressed for multiple DNA sequences attached in tandem. Hence, one promoter element can enhance the expression of one or more products. Multiple promoter elements are well-known to persons of ordinary skill in the art.

In one embodiment, high-level constitutive expression is desired. Examples of such promoters include, without limitation, the retroviral Rous sarcoma virus (RSV) long terminal repeat (LTR) promoter/enhancer, the cytomegalovirus (CMV) immediate early promoter/enhancer (see, e.g., Boshart et al, Cell, 41:521-530 (1985)), the SV40 promoter, the dihydrofolate reductase promoter, the cytoplasmic .beta.-actin promoter and the phosphoglycerol kinase (PGK) promoter.

In another embodiment, inducible promoters may be desired. Inducible promoters are those which are regulated by exogenously supplied compounds, either in cis or in trans, including without limitation, the zinc-inducible sheep metallothionine (MT) promoter; the dexamethasone (Dex)-inducible mouse mammary tumor virus (MMTV) promoter; the T7 polymerase promoter system (WO 98/10088); the tetracycline-repressible system (Gossen et al, Proc. Natl. Acad. Sci. USA, 89:5547-5551 (1992)); the tetracycline-inducible system (Gossen et al., Science, 268:1766-1769 (1995); see also Harvey et al., Curr. Opin. Chem. Biol., 2:512-518 (1998)); the RU486-inducible system (Wang et al., Nat. Biotech., 15:239-243 (1997) and Wang et al., Gene Ther., 4:432-441 (1997)]; and the rapamycin-inducible system (Magari et al., J. Clin. Invest., 100:2865-2872 (1997); Rivera et al., Nat. Medicine. 2:1028-1032 (1996)). Other types of inducible promoters which may be useful in this context are those which are regulated by a specific physiological state, e.g., temperature, acute phase, or in replicating cells only.

In some embodiments, a native promoter for a gene or nucleic acid sequence of interest is used. The native promoter may be used when it is desired that expression of the gene or the nucleic acid sequence should mimic the native expression. The native promoter may be used when expression of the gene or other nucleic acid sequence must be regulated temporally or developmentally, or in a tissue-specific manner, or in response to specific transcriptional stimuli. In a further embodiment, other native

expression control elements, such as enhancer elements, polyadenylation sites or Kozak consensus sequences may also be used to mimic the native expression.

In some embodiments, the genetic element comprises a gene operably linked to a tissue-specific promoter. For instance, if expression in skeletal muscle is desired, a promoter active in muscle may be used. These include the promoters from genes encoding skeletal α-actin, myosin light chain 2A, dystrophin, muscle creatine kinase, as well as synthetic muscle promoters with activities higher than naturally-occurring promoters. See Li et al., Nat. Biotech., 17:241-245 (1999). Examples of promoters that are tissue-specific are known for liver albumin, Miyatake et al. J. Virol., 71:5124-32 (1997); hepatitis B virus core promoter, Sandig et al., Gene Ther. 3:1002-9 (1996); alpha-fetoprotein (AFP), Arbuthnot et al., Hum. Gene Ther., 7:1503-14 (1996)], bone (osteocalcin, Stein et al., Mol. Biol. Rep., 24:185-96 (1997); bone sialoprotein, Chen et al., J. Bone Miner. Res. 11:654-64 (1996)), lymphocytes (CD2, Hansal et al., J. Immunol., 161:1063-8 (1998); immunoglobulin heavy chain; T cell receptor a chain), neuronal (neuron-specific enolase (NSE) promoter, Andersen et al. Cell. Mol. Neurobiol., 13:503-15 (1993); neurofilament light-chain gene, Piccioli et al., Proc. Natl. Acad. Sci. USA, 88:5611-5 (1991); the neuron-specific vgf gene, Piccioli et al., Neuron, 15:373-84 (1995)]; among others.

The genetic element may include an enhancer, e.g., a DNA sequence that is located adjacent to the DNA sequence that encodes a gene. Enhancer elements are typically located upstream of a promoter element or can be located downstream of or within a coding DNA sequence (e.g., a DNA sequence transcribed or translated into a product or products). Hence, an enhancer element can be located 100 base pairs, 200 base pairs, or 300 or more base pairs upstream or downstream of a DNA sequence that encodes the product. Enhancer elements can increase an amount of recombinant product expressed from a DNA sequence above increased expression afforded by a promoter element. Multiple enhancer elements are readily available to persons of ordinary skill in the art.

In some embodiments, the genetic element comprises one or more inverted terminal repeats (ITR) flanking the sequences encoding the expression products described herein. In some embodiments, the genetic element comprises one or more long terminal repeats (LTR) flanking the sequence encoding the expression products described herein. Examples of promoter sequences that may be used, include, but are not limited to, the simian virus 40 (SV40) early promoter, mouse mammary tumor virus (MMTV), human immunodeficiency virus (HIV) long terminal repeat (LTR) promoter, MoMuLV promoter, an avian leukemia virus promoter, an Epstein-Barr virus immediate early promoter, and a Rous sarcoma virus promoter.

Replication Proteins

In some embodiments, the genetic element of the anellovector, e.g., synthetic anellovector, may include sequences that encode one or more replication proteins. In some embodiments, the anellovector may replicate by a rolling-circle replication method, e.g., synthesis of the leading strand and the lagging strand is uncoupled. In such embodiments, the anellovector comprises three elements additional elements: i) a gene encoding an initiator protein, ii) a double strand origin, and iii) a single strand origin. A rolling circle replication (RCR) protein complex comprising replication proteins binds to the leading strand and destabilizes the replication origin. The RCR complex cleaves the genome to generate a free 3'OH extremity. Cellular DNA polymerase initiates viral DNA replication from the free 3'OH extremity. After the genome has been replicated, the RCR complex closes the loop covalently. This leads to the release of a positive circular single-stranded parental DNA molecule and a circular double-stranded DNA molecule composed of the negative parental strand and the newly synthesized positive strand. The single-stranded DNA molecule can be either encapsidated or involved in a second round of replication. See for example, Virology Journal 2009, 6:60 doi:10.1186/1743-422X-6-60.

The genetic element may comprise a sequence encoding a polymerase, e.g., RNA polymerase or a DNA polymerase.

Other Sequences

In some embodiments, the genetic element further includes a nucleic acid encoding a product (e.g., a ribozyme, a therapeutic mRNA encoding a protein, an exogenous gene).

In some embodiments, the genetic element includes one or more sequences that affect species and/or tissue and/or cell tropism (e.g. capsid protein sequences), infectivity (e.g. capsid protein sequences), immunosuppression/activation (e.g. regulatory nucleic acids), viral genome binding and/or packaging, immune evasion (non-immunogenicity and/or tolerance), pharmacokinetics, endocytosis and/or cell attachment, nuclear entry, intracellular modulation and localization, exocytosis modulation, propagation, and nucleic acid protection of the anellovector in a host or host cell.

In some embodiments, the genetic element may comprise other sequences that include DNA, RNA, or artificial nucleic acids. The other sequences may include, but are not limited to, genomic DNA, cDNA, or sequences that encode tRNA, mRNA, rRNA, miRNA, gRNA, siRNA, or other RNAi molecules. In one embodiment, the genetic element includes a sequence encoding an siRNA to target a different loci of the same gene expression product as the regulatory nucleic acid. In one embodiment, the genetic element includes a sequence encoding an siRNA to target a different gene expression product as the regulatory nucleic acid.

In some embodiments, the genetic element further comprises one or more of the following sequences: a sequence that encodes one or more miRNAs, a sequence that encodes one or more replication proteins, a sequence that encodes an exogenous gene, a sequence that encodes a therapeutic, a regulatory sequence (e.g., a promoter, enhancer), a sequence that encodes one or more regulatory sequences that targets endogenous genes (siRNA, lncRNAs, shRNA), and a sequence that encodes a therapeutic mRNA or protein.

The other sequences may have a length from about 2 to about 5000 nts, about 10 to about 100 nts, about 50 to about 150 nts, about 100 to about 200 nts, about 150 to about 250 nts, about 200 to about 300 nts, about 250 to about 350 nts, about 300 to about 500 nts, about 10 to about 1000 nts, about 50 to about 1000 nts, about 100 to about 1000 nts, about 1000 to about 2000 nts, about 2000 to about 3000 nts, about 3000 to about 4000 nts, about 4000 to about 5000 nts, or any range therebetween.

Encoded Genes

For example, the genetic element may include a gene associated with a signaling biochemical pathway, e.g., a signaling biochemical pathway-associated gene or polynucleotide. Examples include a disease associated gene or polynucleotide. A "disease-associated" gene or polynucleotide refers to any gene or polynucleotide which is yielding transcription or translation products at an abnormal level or in an abnormal form in cells derived from a disease-affected tissues compared with tissues or cells of a non disease control. It may be a gene that becomes expressed at an abnormally high level; it may be a gene that becomes expressed at an abnormally low level, where the altered expression correlates with the occurrence and/or progression of the disease. A disease-associated gene also refers to a gene possessing mutation(s) or genetic variation that is directly responsible or is in linkage disequilibrium with a gene(s) that is responsible for the etiology of a disease.

Examples of disease-associated genes and polynucleotides are available from McKusick-Nathans Institute of Genetic Medicine, Johns Hopkins University (Baltimore, Md.) and National Center for Biotechnology Information, National Library of Medicine (Bethesda, Md.). Examples of disease-associated genes and polynucleotides are listed in Tables A and B of US Patent No.: 8,697,359, which are herein incorporated by reference in their entirety. Disease specific information is available from McKusick-Nathans Institute of Genetic Medicine, Johns Hopkins University (Baltimore, Md.) and National Center for Biotechnology Information, National Library of Medicine (Bethesda, Md.). Examples of signaling biochemical pathway-associated genes and polynucleotides are listed in Tables A-C of US Patent No.: 8,697,359, which are herein incorporated by reference in their entirety.

Moreover, the genetic elements can encode targeting moieties, as described elsewhere herein. This can be achieved, e.g., by inserting a polynucleotide encoding a sugar, a glycolipid, or a protein, such as an antibody. Those skilled in the art know additional methods for generating targeting moieties.

Viral Sequence

In some embodiments, the genetic element comprises at least one viral sequence. In some embodiments, the sequence has homology or identity to one or more sequence from a single stranded DNA virus, e.g., Anellovirus, Bidnavirus, Circovirus, Geminivirus, Genomovirus, Inovirus, Microvirus, Nanovirus, Parvovirus, and Spiravirus. In some embodiments, the sequence has homology or identity to one or more sequence from a double stranded DNA virus, e.g., Adenovirus, Ampullavirus, Ascovirus, Asfarvirus, Baculovirus, Fusellovirus, Globulovirus, Guttavirus, Hytrosavirus, Herpesvirus, Iridovirus, Lipothrixvirus, Nimavirus, and Poxvirus. In some embodiments, the sequence has homology or identity to one or more sequence from an RNA virus, e.g., Alphavirus, Furovirus, Hepatitis virus, Hordeivirus, Tobamovirus, Tobravirus, Tricornavirus, Rubivirus, Birnavirus, Cystovirus, Partitivirus, and Reovirus.

In some embodiments, the genetic element may comprise one or more sequences from a non-pathogenic virus, e.g., a symbiotic virus, e.g., a commensal virus, e.g., a native virus, e.g., an *Anellovirus*. Recent changes in nomenclature have classified the three *Anelloviruses* able to infect human cells into Alphatorquevirus (TT), Betatorquevirus (TTM), and Gammatorquevirus (TTMD) Genera of the *Anelloviridae* family of viruses. To date *Anelloviruses* have not been linked to any human disease. In some embodiments, the genetic element may comprise a sequence with homology or identity to a Torque Teno Virus (TT), a non-enveloped, single-stranded DNA virus with a circular, negative-sense genome. In some embodiments, the genetic element may comprise a sequence with homology or identity to a SEN virus, a Sentinel virus, a TTV-like mini virus, and a TT virus. Different types of TT viruses have been described including TT virus genotype 6, TT virus group, TTV-like virus DXL1, and TTV-like virus DXL2. In some embodiments, the genetic element may comprise a sequence with homology or identity to a smaller virus, Torque Teno-like Mini Virus (TTM), or a third virus with a genomic size in between that of TTV and TTMV, named Torque Teno-like Midi Virus (TTMD). In some embodiments, the genetic element may comprise one or more sequences or a fragment of a sequence from a non-pathogenic virus having at least about 60%, 70% 80%, 85%, 90% 95%, 96%, 97%, 98% and 99% nucleotide sequence identity to any one of the nucleotide sequences described herein.

In some embodiments, the genetic element comprises one or more sequences with homology or identity to one or more sequences from one or more non-*Anelloviruses*, e.g., adenovirus, herpes virus, pox virus, vaccinia virus, SV40, papilloma virus, an RNA virus such as a retrovirus, e.g., lentivirus, a single-

stranded RNA virus, e.g., hepatitis virus, or a double-stranded RNA virus e.g., rotavirus. Since, in some embodiments, recombinant retroviruses are defective, assistance may be provided order to produce infectious particles. Such assistance can be provided, e.g., by using helper cell lines that contain plasmids encoding all of the structural genes of the retrovirus under the control of regulatory sequences within the LTR. Suitable cell lines for replicating the anellovectors described herein include cell lines known in the art, e.g., A549 cells, which can be modified as described herein. Said genetic element can additionally contain a gene encoding a selectable marker so that the desired genetic elements can be identified.

In some embodiments, the genetic element includes non-silent mutations, e.g., base substitutions, deletions, or additions resulting in amino acid differences in the encoded polypeptide, so long as the sequence remains at least about 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to the polypeptide encoded by the first nucleotide sequence or otherwise is useful for practicing the present invention. In this regard, certain conservative amino acid substitutions may be made which are generally recognized not to inactivate overall protein function: such as in regard of positively charged amino acids (and vice versa), lysine, arginine and histidine; in regard of negatively charged amino acids (and vice versa), aspartic acid and glutamic acid; and in regard of certain groups of neutrally charged amino acids (and in all cases, also vice versa), (1) alanine and serine, (2) asparagine, glutamine, and histidine, (3) cysteine and serine, (4) glycine and proline, (5) isoleucine, leucine and valine, (6) methionine, leucine and isoleucine, (7) phenylalanine, methionine, leucine, and tyrosine, (8) serine and threonine, (9) tryptophan and tyrosine, (10) and for example tyrosine, tryptophan and phenylalanine. Amino acids can be classified according to physical properties and contribution to secondary and tertiary protein structure. A conservative substitution is recognized in the art as a substitution of one amino acid for another amino acid that has similar properties.

Identity of two or more nucleic acid or polypeptide sequences having the same or a specified percentage of nucleotides or amino acid residues that are the same (e.g., about 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or higher identity over a specified region, when compared and aligned for maximum correspondence over a comparison window or designated region) may be measured using a BLAST or BLAST 2.0 sequence comparison algorithms with default parameters described below, or by manual alignment and visual inspection (see, e.g., NCBI web site www.ncbi.nlm.nih.gov/BLAST/ or the like). Identity may also refer to, or may be applied to, the compliment of a test sequence. Identity also includes sequences that have deletions and/or additions, as well as those that have substitutions. As described herein, the algorithms account for gaps and the like. Identity may exist over a region that is at least about 10 amino acids or nucleotides in length, about 15 amino acids or nucleotides in length, about 20 amino acids or nucleotides in length, about 25 amino acids or nucleotides in length, about 30 amino acids or nucleotides in length, about 35 amino acids or

nucleotides in length, about 40 amino acids or nucleotides in length, about 45 amino acids or nucleotides in length, about 50 amino acids or nucleotides in length, or more.

In some embodiments, the genetic element comprises a nucleotide sequence with at least about 75% nucleotide sequence identity, at least about 80%, 85%, 90% 95%, 96%, 97%, 98%, 99% or 100% nucleotide sequence identity to any one of the nucleotide sequences described herein, e.g., as listed in any one of Tables N1-N26. Since the genetic code is degenerate, a homologous nucleotide sequence can include any number of silent base changes, i.e., nucleotide substitutions that nonetheless encode the same amino acid.

Gene Editing Component

The genetic element of the anellovector may include one or more genes that encode a component of a gene editing system. Exemplary gene editing systems include the clustered regulatory interspaced short palindromic repeat (CRISPR) system, zinc finger nucleases (ZFNs), and Transcription Activator-Like Effector-based Nucleases (TALEN). ZFNs, TALENs, and CRISPR-based methods are described, e.g., in Gaj et al. Trends Biotechnol. 31.7(2013):397-405; CRISPR methods of gene editing are described, e.g., in Guan et al., Application of CRISPR-Cas system in gene therapy: Pre-clinical progress in animal model. DNA Repair 2016 Oct;46:1-8. doi: 10.1016/j.dnarep.2016.07.004; Zheng et al., Precise gene deletion and replacement using the CRISPR/Cas9 system in human cells. BioTechniques, Vol. 57, No. 3, September 2014, pp. 115–124.

CRISPR systems are adaptive defense systems originally discovered in bacteria and archaea. CRISPR systems use RNA-guided nucleases termed CRISPR-associated or "Cas" endonucleases (e. g., Cas9 or Cpf1) to cleave foreign DNA. In a typical CRISPR/Cas system, an endonuclease is directed to a target nucleotide sequence (e. g., a site in the genome that is to be sequence-edited) by sequence-specific, non-coding "guide RNAs" that target single- or double-stranded DNA sequences. Three classes (I-III) of CRISPR systems have been identified. The class II CRISPR systems use a single Cas endonuclease (rather than multiple Cas proteins). One class II CRISPR system includes a type II Cas endonuclease such as Cas9, a CRISPR RNA ("crRNA"), and a trans-activating crRNA ("tracrRNA"). The crRNA contains a "guide RNA", typically about 20-nucleotide RNA sequence that corresponds to a target DNA sequence. The crRNA also contains a region that binds to the tracrRNA to form a partially double-stranded structure which is cleaved by RNase III, resulting in a crRNA/tracrRNA hybrid. The crRNA/tracrRNA hybrid then directs the Cas9 endonuclease to recognize and cleave the target DNA sequence. The target DNA sequence must generally be adjacent to a "protospacer adjacent motif" ("PAM") that is specific for a given Cas endonuclease; however, PAM sequences appear throughout a given genome.

In some embodiments, the anellovector includes a gene for a CRISPR endonuclease. For example, some CRISPR endonucleases identified from various prokaryotic species have unique PAM sequence requirements; examples of PAM sequences include 5'-NGG (Streptococcus pyogenes), 5'-NNAGAA (Streptococcus thermophilus CRISPR1), 5'-NGGNG (Streptococcus thermophilus CRISPR3), and 5'-NNNGATT (Neisseria meningiditis). Some endonucleases, e. g., Cas9 endonucleases, are associated with G-rich PAM sites, e. g., 5'-NGG, and perform blunt-end cleaving of the target DNA at a location 3 nucleotides upstream from (5' from) the PAM site. Another class II CRISPR system includes the type V endonuclease Cpf1, which is smaller than Cas9; examples include AsCpf1 (from Acidaminococcus sp.) and LbCpf1 (from Lachnospiraceae sp.). Cpf1 endonucleases, are associated with T-rich PAM sites, e. g., 5'-TTN. Cpf1 can also recognize a 5'-CTA PAM motif. Cpf1 cleaves the target DNA by introducing an offset or staggered double-strand break with a 4- or 5-nucleotide 5' overhang, for example, cleaving a target DNA with a 5-nucleotide offset or staggered cut located 18 nucleotides downstream from (3' from) from the PAM site on the coding strand and 23 nucleotides downstream from the PAM site on the complimentary strand; the 5-nucleotide overhang that results from such offset cleavage allows more precise genome editing by DNA insertion by homologous recombination than by insertion at blunt-end cleaved DNA. See, e. g., Zetsche et al. (2015) Cell, 163:759 – 771.

A variety of CRISPR associated (Cas) genes may be included in the anellovector. Specific examples of genes are those that encode Cas proteins from class II systems including Cas1, Cas2, Cas3, Cas4, Cas5, Cas6, Cas7, Cas8, Cas9, Cas10, Cpf1, C2C1, or C2C3. In some embodiments, the anellovector includes a gene encoding a Cas protein, e.g., a Cas9 protein, may be from any of a variety of prokaryotic species. In some embodiments, the anellovector includes a gene encoding a particular Cas protein, e.g., a particular Cas9 protein, is selected to recognize a particular protospacer-adjacent motif (PAM) sequence. In some embodiments, the anellovector includes nucleic acids encoding two or more different Cas proteins, or two or more Cas proteins, may be introduced into a cell, zygote, embryo, or animal, e.g., to allow for recognition and modification of sites comprising the same, similar or different PAM motifs. In some embodiments, the anellovector includes a gene encoding a modified Cas protein with a deactivated nuclease, e.g., nuclease-deficient Cas9.

Whereas wild-type Cas9 protein generates double-strand breaks (DSBs) at specific DNA sequences targeted by a gRNA, a number of CRISPR endonucleases having modified functionalities are known, for example: a "nickase" version of Cas9 generates only a single-strand break; a catalytically inactive Cas9 ("dCas9") does not cut the target DNA. A gene encoding a dCas9 can be fused with a gene encoding an effector domain to repress (CRISPRi) or activate (CRISPRa) expression of a target gene. For example, the gene may encode a Cas9 fusion with a transcriptional silencer (e.g., a KRAB domain) or a transcriptional activator (e.g., a dCas9–VP64 fusion). A gene encoding a catalytically inactive Cas9

(dCas9) fused to FokI nuclease ("dCas9-FokI") can be included to generate DSBs at target sequences homologous to two gRNAs. See, e. g., the numerous CRISPR/Cas9 plasmids disclosed in and publicly available from the Addgene repository (Addgene, 75 Sidney St., Suite 550A, Cambridge, MA 02139; addgene.org/crispr/). A "double nickase" Cas9 that introduces two separate double-strand breaks, each directed by a separate guide RNA, is described as achieving more accurate genome editing by Ran et al. (2013) Cell, 154:1380 – 1389.

CRISPR technology for editing the genes of eukaryotes is disclosed in US Patent Application Publications 2016/0138008A1 and US2015/0344912A1, and in US Patents 8,697,359, 8,771,945, 8,945,839, 8,999,641, 8,993,233, 8,895,308, 8,865,406, 8,889,418, 8,871,445, 8,889,356, 8,932,814, 8,795,965, and 8,906,616. Cpf1 endonuclease and corresponding guide RNAs and PAM sites are disclosed in US Patent Application Publication 2016/0208243 A1.

In some embodiments, the anellovector comprises a gene encoding a polypeptide described herein, e.g., a targeted nuclease, e.g., a Cas9, e.g., a wild type Cas9, a nickase Cas9 (e.g., Cas9 D10A), a dead Cas9 (dCas9), eSpCas9, Cpf1, C2C1, or C2C3, and a gRNA. The choice of genes encoding the nuclease and gRNA(s) is determined by whether the targeted mutation is a deletion, substitution, or addition of nucleotides, e.g., a deletion, substitution, or addition of nucleotides to a targeted sequence. Genes that encode a catalytically inactive endonuclease e.g., a dead Cas9 (dCas9, e.g., D10A; H840A) tethered with all or a portion of (e.g., biologically active portion of) an (one or more) effector domain (e.g., VP64) create chimeric proteins that can modulate activity and/or expression of one or more target nucleic acids sequences.

As used herein, a "biologically active portion of an effector domain" is a portion that maintains the function (e.g. completely, partially, or minimally) of an effector domain (e.g., a "minimal" or "core" domain). In some embodiments, the anellovector includes a gene encoding a fusion of a dCas9 with all or a portion of one or more effector domains to create a chimeric protein useful in the methods described herein. Accordingly, in some embodiments, the anellovector includes a gene encoding a dCas9-methylase fusion. In other some embodiments, the anellovector includes a gene encoding a dCas9-enzyme fusion with a site-specific gRNA to target an endogenous gene.

In other aspects, the anellovector includes a gene encoding 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, or more effector domains (all or a biologically active portion) fused with dCas9.

Proteinaceous Exterior

In some embodiments, the anellovector, e.g., synthetic anellovector, comprises a proteinaceous exterior that encloses the genetic element. In some embodiments, the anelloVLP, e.g., synthetic

anelloVLP, comprises a proteinaceous exterior and an effector (e.g., an exogenous effector). The proteinaceous exterior can comprise a substantially non-pathogenic exterior protein that fails to elicit an unwanted immune response in a mammal. The proteinaceous exterior of the anellovectors or anelloVLPs typically comprises a substantially non-pathogenic protein that may self-assemble into an icosahedral formation that makes up the proteinaceous exterior.

In some embodiments, the proteinaceous exterior protein is encoded by a sequence of the genetic element of the anellovector (e.g., is in cis with the genetic element). In other embodiments, the proteinaceous exterior protein is encoded by a nucleic acid separate from the genetic element of the anellovector (e.g., is in trans with the genetic element).

In some embodiments, the protein, e.g., substantially non-pathogenic protein and/or proteinaceous exterior protein, comprises one or more glycosylated amino acids, e.g., 2, 3, 4, 5, 6, 7, 8, 9, 10, or more.

In some embodiments, the protein, e.g., substantially non-pathogenic protein and/or proteinaceous exterior protein comprises at least one hydrophilic DNA-binding region, a structural arginine-rich region, a threonine-rich region, a glutamine-rich region, a N-terminal polyarginine sequence, a variable region, a C-terminal polyglutamine/glutamate sequence, and one or more disulfide bridges.

In some embodiments, the protein is a capsid protein, e.g., has a sequence having at least about 60%, 65%, 70%, 75%, 80%, 85%, 90% 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to a protein encoded by any one of the nucleotide sequences encoding a capsid protein described herein, e.g., an Anellovirus ORF1 sequence or a capsid protein sequence as listed in any one of Tables A1-A26. In some embodiments, the protein or a functional fragment of a capsid protein is encoded by a nucleotide sequence having at least about 60%, 70% 80%, 85%, 90% 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to any one of the nucleotide sequences described herein, e.g., an Anellovirus capsid sequence or a capsid protein sequence as listed in any one of Tables A1-A26. In some embodiments, the protein comprises a capsid protein or a functional fragment of a capsid protein that is encoded by a capsid nucleotide sequence or a sequence having at least about 60%, 65%, 70%, 75%, 80%, 85%, 90% 95%, 96%, 97%, 98%, 99%, or 100% nucleotide sequence identity to any one of the nucleotide sequences described herein, e.g., an Anellovirus capsid sequence or a capsid protein sequence as listed in any one of Tables N1-N26.

In some embodiments, the anellovector comprises a nucleotide sequence encoding a capsid protein or a functional fragment of a capsid protein or a sequence having at least about 60%, 70% 80%, 85%, 90% 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to any one of the amino acid sequences described herein, e.g., an Anellovirus capsid sequence or a capsid protein sequence in any one

of Tables A1-A26. In some embodiments, the anellovector comprises a nucleotide sequence encoding a capsid protein or a functional fragment of a capsid protein or a sequence having at least about 60%, 65%, 70%, 75%, 80%, 85%, 90% 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to any one of the amino acid sequences described herein, e.g., an Anellovirus capsid sequence or a capsid protein sequence in any one of Tables A1-A26.

In some embodiments, the anellovector comprises a nucleotide sequence encoding an amino acid sequence having about position 1 to about position 150 (e.g., or any subset of amino acids within each range, e.g., about position 20 to about position 35, about position 25 to about position 30, about position 26 to about 30), about position 150 to about position 390 (e.g., or any subset of amino acids within each range, e.g., about position 200 to about position 380, about position 205 to about position 375, about position 205 to about 371), about 390 to about position 525, about position 525 to about position 850 (e.g., or any subset of amino acids within each range, e.g., about position 530 to about position 840, about position 545 to about position 830, about position 550 to about 820), about 850 to about position 950 (e.g., or any subset of amino acids within each range, e.g., about position 860 to about position 940, about position 870 to about position 930, about position 880 to about 923) of the amino acid sequences described herein, an Anellovirus amino acid sequence, e.g., as listed in any one of Tables A1-A26, or a functional fragment thereof. In some embodiments, the protein comprises an amino acid sequence or a functional fragment thereof or a sequence having at least about 60%, 65%, 70%, 75%, 80%, 85%, 90% 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to about position 1 to about position 150 (e.g., or any subset of amino acids within each range as described herein), about position 150 to about position 390, about position 390 to about position 525, about position 525 to about position 850, about position 850 to about position 950 of the amino acid sequences described herein, an Anellovirus amino acid sequence, e.g., as listed in any one of Tables A1-A26.

In some embodiments, the protein comprises an amino acid sequence or a functional fragment thereof or a sequence having at least about 60%, 65%, 70%, 75%, 80%, 85%, 90% 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to any one of the amino acid sequences or ranges of amino acids described herein, an Anellovirus amino acid sequence, e.g., as listed in any one of Tables A1-A26. In some embodiments, the ranges of amino acids with less sequence identity may provide one or more of the properties described herein and differences in cell/tissue/species specificity (e.g. tropism).

In some embodiments, the anellovector or anelloVLP lacks lipids in the proteinaceous exterior. In some embodiments, the anellovector or anelloVLP lacks a lipid bilayer, e.g., a viral envelope. In some embodiments, the interior of the anellovector or anelloVLP is entirely covered (e.g., 100% coverage) by a proteinaceous exterior. In some embodiments, the interior of the anellovector or anelloVLP is less than 100% covered by the proteinaceous exterior, e.g., 95%, 90%, 85%, 80%, 70%, 60%, 50% or less

coverage. In some embodiments, the proteinaceous exterior comprises gaps or discontinuities, e.g., permitting permeability to water, ions, peptides, or small molecules, e.g., so long as the genetic element is retained in the anellovector.

In some embodiments, the proteinaceous exterior comprises one or more proteins or polypeptides that specifically recognize and/or bind a host cell, e.g., a complementary protein or polypeptide, to mediate entry of the genetic element into the host cell.

In some embodiments, the proteinaceous exterior comprises one or more of the following: one or more glycosylated proteins, a hydrophilic DNA-binding region, a structural arginine-rich region, a threonine-rich region, a glutamine-rich region, a N-terminal polyarginine sequence, a variable region, a C-terminal polyglutamine/glutamate sequence, and one or more disulfide bridges. For example, the proteinaceous exterior comprises a protein encoded by an Anellovirus ORF1 described herein.

In some embodiments, the proteinaceous exterior comprises one or more of the following characteristics: an icosahedral symmetry, recognizes and/or binds a molecule that interacts with one or more host cell molecules to mediate entry into the host cell, lacks lipid molecules, lacks carbohydrates, is pH and temperature stable, is detergent resistant, and is substantially non-immunogenic or non-pathogenic in a host.

Surface Moieties

An anellovector or anelloVLP as described herein may, in some instances, include one or more moieties attached to its surface (e.g., a surface moiety that can act as an effector and/or a targeting agent). In some instances, an anellovector or anelloVLP comprises more than one distinct surface moiety (e.g., a first surface moiety having an effector function as described herein and a second surface moiety that targets the anellovector or anelloVLP to a cell or tissue of interest). In some instances, the surface moiety is covalently attached to the surface of the anellovector or anelloVLP. For example, the surface moiety may be covalently attached to the proteinaceous exterior or a component thereof (e.g., covalently attached to an ORF1 molecule of the proteinaceous exterior). In certain embodiments, the surface moiety is fused to an ORF1 molecule. In some instances, the surface moiety is noncovalently attached to the surface of the anellovector or anelloVLP. For example, the surface moiety may be noncovalently bound to the proteinaceous exterior or a component thereof (e.g., noncovalently bound to an ORF1 molecule of the proteinaceous exterior). In certain embodiments, the surface moiety comprises a region that specifically binds to a cognate moiety on or attached to the ORF1 molecule. In an embodiment, the ORF1 molecule comprises a binding moiety (e.g., an antibody molecule) that specifically recognizes an epitope on the region on the surface moiety. In an embodiment, the surface moiety comprises a binding moiety (e.g., an antibody molecule) that specifically recognizes an epitope on the ORF1 molecule. In an embodiment, the

surface moiety comprises a streptavidin moiety that binds to a biotin moiety on the surface of the anellovector or anelloVLP (e.g., a biotin moiety attached to an ORF1 molecule of the proteinaceous exterior of the anellovector or anelloVLP). In an embodiment, the surface moiety comprises a biotin moiety that binds to a streptavidin moiety on the surface of the anellovector or anelloVLP (e.g., a streptavidin moiety attached to an ORF1 molecule of the proteinaceous exterior of the anellovector or anelloVLP).

In embodiments, all copies of an ORF1 molecule in the proteinaceous exterior of an anellovector or anelloVLP are attached to copies of the surface moiety. In embodiments, some copies of the ORF1 molecule in the proteinaceous exterior of an anellovector or anelloVLP are attached to copies of the surface moiety and some copies of the ORF1 molecule in the proteinaceous exterior of an anellovector or anelloVLP are not attached to copies of the surface moiety. In embodiments, some copies of the ORF1 molecule in the proteinaceous exterior of an anellovector or anelloVLP are attached to copies of a first surface moiety and some copies of the ORF1 molecule in the proteinaceous exterior of an anellovector or anelloVLP are attached to copies of a second surface moiety. In embodiments, some copies of the ORF1 molecule in the proteinaceous exterior of an anellovector or anelloVLP are attached to copies of a third surface moiety.

In some embodiments, all copies of an ORF1 molecule in the proteinaceous exterior of an anellovector or anelloVLP are attached at the same position (e.g., a lysine residue) of the ORF1 molecule to a copy of a surface moiety. In some embodiments, a first copy of an ORF1 molecule in the proteinaceous exterior of an anellovector or anelloVLP is attached at a first position (e.g., a first lysine residue) of the ORF1 molecule to a first copy of a surface moiety, and a second copy of an ORF1 molecule in the proteinaceous exterior of an anellovector or anelloVLP is attached at a second position (e.g., a second lysine residue) of the ORF1 molecule to a second of a surface moiety. In some embodiments, the proteinaceous exterior further comprises one or more copies of an ORF1 molecule having a surface moiety attached to a one or more additional positions (e.g., one or more additional lysine residues). In certain embodiments, the first lysine residue, the second lysine residue, and/or the one or more additional lysine residues are positioned on the surface of the proteinaceous exterior. In some embodiments, the surface moiety is attached to the ORF1 molecule via click chemistry or genetic grafting, e.g., as described herein.

In some instances, the surface moiety comprises an effector function (e.g., as described herein). For example, the surface moiety may modulate a biological activity, e.g., of a target cell or organ. In some instances, the surface moiety induces modulation of the biological activity via binding to a cognate moiety on a target cell. For example, the surface moiety may comprise a ligand that binds to a receptor on the surface of the target cell, e.g., wherein binding of the surface moiety to the receptor initiates a

downstream signaling cascade of interest. In some instances, the effector activity comprises increasing or decreasing enzymatic activity, gene expression, cell signaling, and/or cellular or organ function within a target cell or organ. Effector activities may also include binding regulatory proteins to modulate activity of the regulator, such as transcription or translation. Effector activities also may include activator or inhibitor functions.

In some instances, the surface moiety can target the anellovector or anelloVLP to a target cell. For example, the surface moiety may specifically bind to a cognate moiety on the surface of the target cell. The cognate moiety on the surface of the target cell may be, for example, a molecule specifically expressed or preferentially expressed by the target cell. The cognate moiety may be, for example, a polypeptide, lipid, sugar, or small molecule. In certain embodiments, the cognate moiety is a transmembrane protein (e.g., comprising an extracellular domain that binds to the surface moiety of the anellovector or the anelloVLP). In certain embodiments, the cognate moiety is tethered to the surface of the cell (e.g., via a GPI anchor). In some instances, the surface moiety provides a tropism (e.g., to a target tissue or target cell type) for the anellovector or anelloVLP.

In some instances, the surface moiety comprises an effector function and a targeting function, e.g., as described herein. In some instances, the surface moiety comprises a domain having an effector function as described herein. In some instances, the surface moiety comprises a domain having a targeting function as described herein.

In some instances, the surface moiety binds specifically to one cognate moiety. In some embodiments, the surface moiety binds specifically to more than one cognate moiety. In some embodiments, the surface moiety comprises a plurality of binding regions, for example, each of which specifically binds to a different cognate moiety. For example, the surface moiety may be bispecific or trispecific. In some embodiments, the surface moiety comprises a plurality of binding regions, for example, each of which binds to the same cognate moiety or copies thereof (e.g., at different epitopes of the cognate moiety or the same epitope of the cognate moiety). In certain embodiments, the surface moiety having multiple binding regions that specifically bind to the same cognate moiety results in greater avidity for the target moiety.

Click Chemistry

In an aspect, the disclosure provides an ORF1 molecule comprising: (i) the amino acid sequence of an Anellovirus ORF1 protein, or an amino acid sequence having at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity thereto; and (ii) a click handle (e.g., an NHS click handle or a maleimide click handle, e.g., as described herein). In certain embodiments, the click handle is covalently attached to the ORF1 molecule. In certain embodiments, the click handle is noncovalently attached to the

ORF1 molecule. In certain embodiments, the click handle is used to attach the ORF1 molecule to a surface moiety, e.g., via a click reaction, e.g., as described herein.

A "click handle," as that term is used herein, refers to a chemical moiety that is capable of reacting with a second click handle in a click reaction. In some embodiments, a click handle comprises an NHS moiety and/or a maleimide moiety. In certain embodiments, a click handle comprises a DBCO moiety. In certain embodiments, a click handle comprises an azide moiety. In some embodiments, a click handle is attached to a polypeptide (e.g., an ORF1 molecule). In other embodiments, a click handle comprises a reactive group capable of forming a covalent bond with a polypeptide (e.g., an ORF1 molecule). A "click reaction", as that term is used herein, refers to a range of reactions used to covalently link a first and a second moiety, for convenient production of linked products. It typically has one or more of the following characteristics: it is fast, is specific, is high-yield, is efficient, is spontaneous, does not significantly alter biocompatibility of the linked entities, has a high reaction rate, produces a stable product, favors production of a single reaction product, has high atom economy, is chemoselective, is modular, is stereoselective, is insensitive to oxygen, is insensitive to water, is high purity, generates only inoffensive or relatively non-toxic byproducts that can be removed by nonchromatographic methods (e.g., crystallization or distillation), needs no solvent or can be performed in a solvent that is benign or physiologically compatible, e.g., water, stable under physiological conditions. Examples include an alkyne/azide reaction, a diene/dienophile reaction, or a thiol/alkene reaction. Other reactions can be used. In embodiments, a click reaction has a second order forward rate constant of 10-200 M-1s-1, 1-20 M-1s-1, or at least 1, 2, 3, 5, 10, 20, 50, 60, 100, 200, 500, 1E3, 2E3, 5E3, 1E4, 2E4, 5E4, 1E5, 2E5, 5E5, or 1E6 M-1s-1, e.g., at 20°C in PBS. In some embodiments, a click reaction has at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% yield, e.g., for a reaction time of 1 hour at 20°C in PBS.

In some embodiments, a surface moiety is attached to a polypeptide by conjugation of a lysine residue on the surface of the polypeptide to an NHS click handle on the surface moiety (e.g., as described in Example 2 or 3 herein). In some embodiments, a surface moiety is attached to a polypeptide by conjugation of a lysine residue on the surface of the surface moiety to an NHS click handle on the polypeptide (e.g., as described in Example 2 or 3 herein).

In some embodiments, a surface moiety is attached to a polypeptide by conjugation of a cysteine residue on the surface of the polypeptide to a maleimide click handle on the surface moiety (e.g., as described in Example 4 herein). In some embodiments, a surface moiety is attached to a polypeptide by conjugation of a cysteine residue on the surface of the surface moiety to a maleimide click handle on the polypeptide (e.g., as described in Example 4 herein).

In an aspect, the disclosure provides an ORF1 molecule comprising a surface moiety, wherein the surface moiety was attached to the ORF1 molecule via a click reaction.

In an aspect, the disclosure provides a particle (e.g., an anellovector or anelloVLP) comprising: (i) a proteinaceous exterior comprising an ORF1 molecule; and (ii) a click handle (e.g., an NHS click handle and/or a maleimide click handle, e.g., as described herein). In certain embodiments, the click handle is covalently attached to the ORF1 molecule. In some embodiments, the particle is an anellovector comprising a genetic element enclosed in the proteinaceous exterior. In some embodiments, the particle is an anelloVLP comprising an effector (e.g., an exogenous effector), e.g., enclosed in the proteinaceous exterior.

Mutations of surface lysines

In an aspect, the disclosure provides an ORF1 molecule comprising the amino acid sequence of an Anellovirus ORF1 protein (or an amino acid sequence having at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity thereto), wherein at least 30%, 40%, 50%, 60%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% of the lysine residues in the amino acid sequence of the Anellovirus ORF1 protein has been mutated (e.g., substituted with another amino acid, e.g., threonine, alanine, serine, asparagine, or glutamine).

In some embodiments, all but one lysine residue of the Anellovirus ORF1 protein that are exposed from the surface of a proteinaceous exterior comprising the Anellovirus ORF1 protein are mutated (e.g., substituted with another amino acid, e.g., serine or alanine). In some embodiments, all lysine residues of the Anellovirus ORF1 protein that are exposed from the surface of a proteinaceous exterior comprising the Anellovirus ORF1 protein are mutated (e.g., substituted with another amino acid, e.g., serine or alanine). In some embodiments, all but one of the lysine residues of the Anellovirus ORF1 protein are mutated (e.g., substituted with another amino acid, e.g., serine or alanine). In certain embodiments, the one lysine residue not mutated is exposed on the surface of a proteinaceous exterior comprising the Anellovirus ORF1 protein. In some embodiments, all lysine residues of the Anellovirus ORF1 protein are mutated (e.g., substituted with another amino acid, e.g., serine or alanine). In some embodiments, the ORF1 molecule further comprises a lysine residue not found in the amino acid sequence of the Anellovirus ORF1 protein (e.g., a lysine residue inserted or substituted into the Anellovirus ORF1 protein sequence, or a lysine residue attached to the N-terminal or C-terminal end of the Anellovirus ORF1 protein sequence). Such ORF1 molecules may be useful, for example, for controlling covalent attachment of a surface moiety to a lysine residue in the proteinaceous exterior.

Mutations of surface cysteines

In an aspect, the disclosure provides an ORF1 molecule comprising the amino acid sequence of an Anellovirus ORF1 protein (or an amino acid sequence having at least 75%, 80%, 85%, 90%, 95%,

96%, 97%, 98%, or 99% sequence identity thereto), wherein at least 30%, 40%, 50%, 60%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% of the cysteine residues in the amino acid sequence of the Anellovirus ORF1 protein has been mutated (e.g., substituted with another amino acid, e.g., threonine, alanine, serine, asparagine, or glutamine).

In some embodiments, all but one cysteine residue of the Anellovirus ORF1 protein that are exposed from the surface of a proteinaceous exterior comprising the Anellovirus ORF1 protein are mutated (e.g., substituted with another amino acid, e.g., serine or alanine). In some embodiments, all cysteine residues of the Anellovirus ORF1 protein that are exposed from the surface of a proteinaceous exterior comprising the Anellovirus ORF1 protein are mutated (e.g., substituted with another amino acid, e.g., serine or alanine). In some embodiments, all but one of the cysteine residues of the Anellovirus ORF1 protein are mutated (e.g., substituted with another amino acid, e.g., serine or alanine). In certain embodiments, the one cysteine residue not mutated is exposed on the surface of a proteinaceous exterior comprising the Anellovirus ORF1 protein. In some embodiments, all cysteine residues of the Anellovirus ORF1 protein are mutated (e.g., substituted with another amino acid, e.g., serine or alanine). In some embodiments, the ORF1 molecule further comprises a cysteine residue not found in the amino acid sequence of the Anellovirus ORF1 protein (e.g., a cysteine residue inserted or substituted into the Anellovirus ORF1 protein sequence, or a cysteine residue attached to the N-terminal or C-terminal end of the Anellovirus ORF1 protein sequence). Such ORF1 molecules may be useful, for example, for controlling covalent attachment of a surface moiety to a cysteine residue in the proteinaceous exterior.

Polypeptides

The surface moiety can, in some instances, comprise a polypeptide. In some embodiments, the polypeptide is a about 5-10, 10-15, 15-20, 20-25, 25-30, 30-40, 40-50, 50-100, 100-150, 150-200, 200-250, 250-300, 300-400, 400-500, 500-600, 600-700, 700-800, 800-900, 900-1000, 1000-1500, 1500-2000, 2000-2500, 2500-3000, 3000-4000, or 4000-5000 amino acids in length). In some embodiments, the surface moiety is a polypeptide fused to a protein of the anellovector or anelloVLP (e.g., an ORF1 molecule of the anellovector or anelloVLP). In certain embodiments, the peptide is linear or branched.

In some embodiments, the surface moiety comprises an antibody molecule (e.g., an antibody or an antigen-binding fragment thereof). In certain embodiments, the surface moiety comprises an Fv, Fab, Fab', Fab'-SH, $F(ab')_2$, diabody, linear antibody, single-chain antibody molecule (e.g. scFv), or a multispecific antibody formed from antibody fragments. In certain embodiments, the surface moiety is a multispecific antibody molecule (e.g., a bispecific antibody molecule or a trispecific antibody molecule). In some embodiments, the surface moiety is selected from a hormone, cytokine, enzyme, transcription factor, receptor, ligand, transporter, secreted protein, carrier protein, structural protein, or a functional

fragment thereof (e.g., as described herein). In some embodiments, the surface moiety comprises a polypeptide effector (e.g., as described herein). In certain embodiments, the surface moiety comprises a therapeutic effector (e.g., as described herein). In embodiments, the surface moiety comprises a regulatory intracellular polypeptide (e.g., as described herein). In embodiments, the surface moiety comprises a secreted polypeptide effector (e.g., as described herein). In embodiments, the surface moiety comprises a viral polypeptide or peptide. In embodiments, the surface moiety comprises a SARS-CoV-2 polypeptide or peptide (e.g., a receptor binding domain, e.g., of a spike protein, e.g., as described herein), or a polypeptide having at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity thereto. In embodiments, the surface moiety comprises a polypeptide having at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% amino acid sequence identity to amino acids 319-541 from SARS-CoV-2 coronavirus spike protein (e.g., according to ThermoFisher Catalog# RP-87704). In some embodiments, the surface moiety comprises a binding moiety (e.g., a biotin moiety). In some embodiments, the surface moiety comprises a fluorophore (e.g., an Alexa Fluor moiety, e.g., Alexa Fluor 647).

In some embodiments, a polypeptide surface moiety is displayed on the surface of the anellovector or anelloVLP. In some embodiments, the surface moiety is covalently attached to the surface (e.g., proteinaceous exterior) of the anellovector or anelloVLP. In certain embodiments, the surface moiety is a polypeptide fused to an ORF1 molecule. In embodiments, the surface moiety is a heterologous domain of an ORF1 molecule. In embodiments, the surface moiety replaces a region (e.g., a subdomain as described herein, e.g., an HVR) of an ORF1 protein. In embodiments, all copies of the ORF1 molecule in the proteinaceous exterior of an anellovector or anelloVLP are fused to copies of the surface moiety. In embodiments, some copies of the ORF1 molecule in the proteinaceous exterior of an anellovector or anelloVLP are fused to copies of the surface moiety and some copies of the ORF1 molecule in the proteinaceous exterior of an anellovector or anelloVLP are not fused to copies of the surface moiety. In embodiments, some copies of the ORF1 molecule in the proteinaceous exterior of an anellovector or anelloVLP are fused to copies of a first surface moiety and some copies of the ORF1 molecule in the proteinaceous exterior of an anellovector or anelloVLP are fused to copies of a second surface moiety.

In some embodiments, the surface moiety is noncovalently attached to the surface (e.g., proteinaceous exterior) of the anellovector or anelloVLP. For example, the surface moiety may comprise a binding domain that binds to a region on the surface (e.g., proteinaceous exterior) of the anellovector or anelloVLP. In an embodiment, the surface moiety comprises an antibody molecule that specifically binds to an ORF1 molecule of the proteinaceous exterior of the anellovector or anelloVLP.

Genetic grafting of ORF1 to a surface moiety

In some embodiments, an ORF1 molecule comprises the amino acid sequence of a surface moiety, e.g., as described herein. An ORF1 molecule may be fused (e.g., at the N-terminus or C-terminus) to the surface moiety. In some embodiments, a surface moiety is grafted into the sequence of the ORF1 molecule. For example, a surface moiety may be inserted within or between domains of the ORF1 molecule. In some instances, a surface moiety replaces at least a portion (e.g., at least 5-10, 10-20, 20-30, 30-40, 40-50, 50-60, 60-70, 70-80, 80-90, 90-100, 100-150, 150-200, or 200-300 contiguous amino acid residues) of a domain of the ORF1 molecule. In some embodiments, the surface moiety replaces the entirety of at least one domain of the ORF1 molecule.

In some embodiments, an ORF1 molecule grafted to a surface moiety comprises a deletion or truncation of a structural arginine-rich region. In certain embodiments, the surface moiety replaces the structural arginine-rich region, or a portion thereof (e.g., a portion consisting of 5-10, 10-15, 15-20, 20-30, 30-35, or 35-40 amino acids thereof). In certain embodiments, the surface moiety is grafted at or within 1-5, 5-10, 10-15, 15-20, 20-25, 25-30, 30-35, or 35-40 amino acid residues of a structural jelly-roll region or a jelly-roll B-H strands subdomain as described herein.

In some embodiments, an ORF1 molecule grafted to a surface moiety comprises a deletion or truncation of a structural hypervariable region (HVR) (e.g., as described herein). In certain embodiments, the surface moiety replaces the structural HVR, or a portion thereof (e.g., a portion consisting of 5-10, 10-15, 15-20, 20-30, 30-40, 40-50, 50-60, 60-70, 70-80, 80-90, 90-100, 100-110, 110-120, 120-130, 130-140, or 140-150 amino acids thereof). In some embodiments, an ORF1 molecule grafted to a surface moiety comprises a deletion or truncation of one or more of a P1-1 domain, P2 domain, and/or P1-2 domain (e.g., as described herein). In certain embodiments, the surface moiety replaces the P1-1 domain, P2 domain, and/or P1-2 domain, or a portion thereof (e.g., a portion consisting of 5-10, 10-15, 15-20, 20-30, 30-40, 40-50, 50-60, 60-70, 70-80, 80-90, 90-100, 100-110, 110-120, 120-130, 130-140, or 140-150 amino acids thereof). In certain embodiments, the surface moiety is grafted at or within 1-5, 5-10, 10-15, 15-20, 20-25, 25-30, 30-35, 35-40, 40-50, 50-60, 60-70, 70-80, 80-90, 90-100, 100-125, 125-150, 150-175, or 175-200 amino acid residues of the C-terminal end of a structural jelly-roll region or jelly-roll B-H strands subdomain as described herein. In certain embodiments, the surface moiety is grafted at or within 1-5, 5-10, 10-15, 15-20, 20-25, 25-30, 30-35, 35-40, 40-50, 50-60, 60-70, 70-80, 80-90, 90-100, 100-125, 125-150, 150-175, or 175-200 amino acid residues of the N-terminal end of a structural N22 domain, jelly-roll I strand subdomain, or structural CTD as described herein.

In some embodiments, an ORF1 molecule grafted to a surface moiety comprises a deletion or truncation of a structural C-terminal domain (CTD). In certain embodiments, the surface moiety replaces the structural CTD, or a portion thereof (e.g., a portion consisting of 5-10, 10-15, 15-20, 20-30, 30-40, 40-

50, 50-60, 60-70, 70-80, 80-90, 90-100, 100-110, 110-120, or 120-130 amino acids thereof). In certain embodiments, the surface moiety is grafted at or within 1-5, 5-10, 10-15, 15-20, 20-25, 25-30, 30-35, 35-40, 40-50, 50-60, 60-70, 70-80, 80-90, 90-100, 100-125, 125-150, 150-175, or 175-200 amino acid residues of a structural N22 domain or a jelly-roll I strand subdomain as described herein.

Exemplary ORF1 molecules attached to surface moieties

In some embodiments, a surface moiety and ORF1 fusion protein comprises an amino acid sequence as listed in Table E1 below, or an amino acid sequence having at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity thereto. In some embodiments, a surface moiety comprises an amino acid sequence as listed in Table E1 below, or an amino acid sequence having at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity thereto. In some embodiments, a surface moiety comprises a CS protein, or a functional fragment thereof (e.g., a fragment comprising the 184 C-terminal residues of a CS protein). In some embodiments, a surface moiety comprises a hepatitis B virus surface antigen. In some embodiments, a surface moiety comprises one or more (e.g., 1, 2, 3, 4, 5, or 6) NANP peptides (e.g., NANP-2 peptides). In some embodiments, a surface moiety comprises a cysteine residue (e.g., a C-terminal cysteine residue). In some embodiments, a surface moiety comprises an NHS click handle (e.g., as described herein).

In an aspect, the disclosure provides a nucleic acid molecule encoding a fusion protein comprising a surface moiety and an ORF1 molecule (e.g., as described herein).

In an aspect, the disclosure provides a polypeptide comprising a CCN5 protein and a C-terminal cysteine residue. In some embodiments, the polypeptide comprises the amino acid sequence of CCN5 CTerMCys as listed in Table E1 below, or an amino acid sequence having at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity thereto. In an aspect, the disclosure provides a polypeptide comprising an aflibercept protein and a C-terminal cysteine residue. In some embodiments, the polypeptide comprises the amino acid sequence of aflibercept as listed in Table E1 below, or an amino acid sequence having at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity thereto. In an aspect, the disclosure provides a polypeptide comprising ranibizumab and a C-terminal cysteine residue. In some embodiments, the polypeptide comprises the amino acid sequence of ranibizumab HC malE_CTermCys as listed in Table E1 below, or an amino acid sequence having at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity thereto. In some embodiments, the polypeptide comprises the amino acid sequence of scFab RaniHC-link50-LC malE_CTermCys as listed in Table E1 below, or an amino acid sequence having at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity thereto. In an aspect, the disclosure provides a polypeptide comprising bevacizumab and a C-terminal cysteine residue. In some embodiments, the polypeptide

comprises the amino acid sequence of bevacizumab HC malE_CTermCys as listed in Table E1 below, or an amino acid sequence having at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity thereto.

X-fold symmetry

In some embodiments, an ORF1 molecule as described herein comprises a surface moiety situated at a position such that, when the ORF1 molecule is complexed with one or more additional copies of the ORF1 molecules (each attached to another surface moiety, e.g., copies of the same surface moiety or one or more different surface moieties), the surface moieties form a multimer. In certain embodiments, a complex of two such ORF1 molecules attached to surface moieties results in the formation of a dimer of the surface moieties. In certain embodiments, a complex of three such ORF1 molecules attached to surface moieties results in the formation of a trimer of the surface moieties. In certain embodiments, a complex of five such ORF1 molecules attached to surface moieties results in the formation of a pentamer of the surface moieties.

To form a multimer of surface moieties, the surface moieties are generally attached to the ORF1 molecule at a surface-exposed portion of the ORF1 molecule. In some embodiments, the surface-exposed portion of the ORF1 molecule is part of a structural hypervariable region (HVR) of the ORF1 molecule, e.g., as described herein. In some embodiments, the surface-exposed portion of the ORF1 molecule is part of a P1 domain (e.g., a P1-1 subdomain or a P1-2 subdomain) of the ORF1 molecule, e.g., as described herein. In some embodiments, the surface-exposed portion of the ORF1 molecule is part of a P2 domain of the ORF1 molecule, e.g., as described herein. In certain embodiments, the surface moiety is fused to, replaces, or is attached at or between residues of the ORF1 molecule corresponding to positions 284-285 of a Ring10 ORF1 protein. In certain embodiments, the surface moiety is fused to, replaces, or is attached at or between residues of the ORF1 molecule corresponding to positions 328-329 of a Ring10 ORF1 protein. In certain embodiments, the surface moiety is fused to, replaces, or is attached at or between residues of the ORF1 molecule corresponding to positions 256-383 of a Ring10 ORF1 protein. In certain embodiments, the surface moiety is fused to, replaces, or is attached at or between residues of the ORF1 molecule corresponding to positions 251-383 of a Ring10 ORF1 protein. In certain embodiments, the surface moiety is fused to, replaces, or is attached at or between residues of the ORF1 molecule corresponding to positions 251-384 of a Ring10 ORF1 protein. In certain embodiments, the surface moiety is attached at the amino acid residue (e.g., a cysteine residue) corresponding to position 254, 263, 264, 265, 272, 273, 274, 276, 283, 284, 285, 287, 288, 290, 291, 308, 311, 312, 313, 314, 316, 317, 318, 319, 321, 324, 328, 329, 341, 343, 354, 358, 361, 362, 363, 364, 365, 368, 369, 371, 374, 376, 378, 380, or 381 of Ring 10 ORF1, e.g., in an ORF1 domain (e.g., within the HVR or P2 domain).

In an aspect, the disclosure provides an ORF1 molecule comprising the amino acid sequence of an ORF1 protein comprising at least one mutation (e.g., deletion, substitution, addition, insertion, or frameshift) in a surface-exposed region relative to a wild-type ORF1 protein sequence (e.g., as described herein. In some embodiments, the surface-exposed region comprises the region corresponding amino acids 251-386 of the amino acid sequence of Ring 10 ORF1 (e.g., as described herein).

In an aspect, the disclosure provides an ORF1 molecule comprising the amino acid sequence of an ORF1 protein comprising at least one mutation (e.g., deletion, substitution, addition, insertion, or frameshift) in the HVR relative to a wild-type ORF1 protein sequence (e.g., as described herein).

In some embodiments, the glutamine residue corresponding to Q287 of Ring 10 (e.g., as described herein) has been mutated (e.g., substituted). In some embodiments, the glutamine residue corresponding to Q287 of Ring 10 has been mutated to a cysteine residue. In certain embodiments, the ORF1 molecule comprises the amino acid sequence of the Ring 10 ORF1 protein, or an amino acid sequence having at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity thereto, and a Q287C mutation.

In some embodiments, the threonine residue corresponding to T365 of Ring 10 (e.g., as described herein) has been mutated (e.g., substituted). In some embodiments, the threonine residue corresponding to T365 of Ring 10 has been mutated to a cysteine residue. In certain embodiments, the ORF1 molecule comprises the amino acid sequence of the Ring 10 ORF1 protein, or an amino acid sequence having at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity thereto, and a T365C mutation.

In an aspect, the disclosure provides a polypeptide comprising an amino acid sequence as listed in Table E1 below, or an amino acid sequence having at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity thereto. In some embodiments, the polypeptide further comprises the amino acid sequence of an ORF1 protein (e.g., as described herein), or an amino acid sequence having at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity thereto.

In an aspect, the disclosure provides a nucleic acid molecule encoding a polypeptide comprising an amino acid sequence as listed in Table E1 below, or an amino acid sequence having at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity thereto. In some embodiments, the polypeptide encoded by the nucleic acid molecule further comprises the amino acid sequence of an ORF1 protein (e.g., as described herein), or an amino acid sequence having at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity thereto.In some embodiments, an anellovector as described herein comprises a proteinaceous exterior comprising an ORF1 molecule as described above. In some embodiments, an anellovector as described herein comprises a proteinaceous exterior comprising an

ORF1 molecule encoded by a nucleic acid molecule as described above. In some embodiments, an anelloVLP as described herein comprises a proteinaceous exterior comprising an ORF1 molecule as described above. In some embodiments, an anelloVLP as described herein comprises a proteinaceous exterior comprising an ORF1 molecule encoded by a nucleic acid molecule as described above.

Nucleic acid molecules

The surface moiety may, in some instances, comprise a nucleic acid molecule. In some embodiments, the surface moiety comprises DNA (e.g., single-stranded DNA or double-stranded DNA). In some embodiments, the surface moiety comprises RNA (e.g., single-stranded RNA or double-stranded RNA). In some embodiments, the surface moiety comprises DNA and RNA (e.g., a strand of DNA hybridized to a strand of RNA). In some embodiments, the surface moiety comprises an oligonucleotide (e.g., an oligonucleotide having a length of about 5-10, 10-15, 15-20, 20-25, 25-30, 30-35, 35-40, 40-45, 45-50, 50-60, 60-70, 70-80, 80-90, or 90-100 nucleotides). In some embodiments, the surface moiety comprises a functional nucleic acid molecule (e.g., a functional RNA). In certain embodiments, the surface moiety comprises an mRNA, siRNA, miRNA, or tRNA.

Small molecules

The surface moiety may, in some instances, comprise a small molecule. In some embodiments, the small molecule has a molecular weight less than about 5,000 grams per mole, e.g., organic or inorganic compounds having a molecular weight less than about 2,000 grams per mole, e.g., organic or inorganic compounds having a molecular weight less than about 1,000 grams per mole, e.g., organic or inorganic compounds having a molecular weight less than about 500 grams per mole. In some embodiments, the small molecule is a salt, ester, or other pharmaceutically acceptable form of such compounds. Small molecules may include, but are not limited to, a neurotransmitter, a hormone, a drug, a toxin, a viral or microbial particle, a synthetic molecule, and an agonist or an antagonist. In some embodiments, the small molecule is a pharmaceutically active agent. In one embodiment, the small molecule is an inhibitor of a metabolic activity or component. Useful classes of pharmaceutically active agents include, but are not limited to, antibiotics, anti-inflammatory drugs, angiogenic or vasoactive agents, growth factors and chemotherapeutic (anti-neoplastic) agents (e.g., tumor suppressors). One or a combination of molecules from the categories and examples described herein or from Orme-Johnson 2007, Methods Cell Biol. 2007;80:813-26 (incorporated by reference in its entirety) can be used. In one embodiment, the small molecule comprises an antibiotic, anti-inflammatory drug, angiogenic or vasoactive agent, growth factor or chemotherapeutic agent.

Examples of small molecules that can be used as surface moieties as described herein include, without limitation, those described in, "The Pharmacological Basis of Therapeutics," Goodman and Gilman, McGraw-Hill, New York, N.Y., (1996), Ninth edition, under the sections: Drugs Acting at Synaptic and Neuroeffector Junctional Sites; Drugs Acting on the Central Nervous System; Autacoids: Drug Therapy of Inflammation; Water, Salts and Ions; Drugs Affecting Renal Function and Electrolyte Metabolism; Cardiovascular Drugs; Drugs Affecting Gastrointestinal Function; Drugs Affecting Uterine Motility; Chemotherapy of Parasitic Infections; Chemotherapy of Microbial Diseases; Chemotherapy of Neoplastic Diseases; Drugs Used for Immunosuppression; Drugs Acting on Blood-Forming organs; Hormones and Hormone Antagonists; Vitamins, Dermatology; and Toxicology, all incorporated herein by reference.

Vaccines/ Antigens

In some instances, a surface moiety comprises an antigen (e.g., an antigen recognized by the immune system of a subject to be delivered the anellovector or anelloVLP). In some embodiments, a surface moiety comprises a vaccine. In some embodiments, a surface moiety comprises an epitope from a bacterium, virus, fungus, or parasite. In some embodiments, a surface moiety comprises a vaccine for a pathogen (e.g., the surface moiety comprises an antigen of the pathogen). In certain embodiments, the surface moiety comprises one or more vaccine antigens as listed in Table V1 below. In embodiments, the surface moiety comprises one vaccine antigen listed in Table V1. In embodiments, the surface moiety comprises a plurality of distinct vaccine antigens listed in Table V1 (e.g., a plurality of distinct vaccine antigens listed in a single row of Table V1).

Table V1. Exemplary vaccine antigens that can be included in a surface moiety of an anellovector or anelloVLP as described herein.

Ascomycota, e.g., Eurotiomycetes, e.g., Eurotiales, e.g., Trichocomaceae, e.g., Aspergillus species antigen (e.g., Aspergillus versicolor AVS)
Monodnaviria, e.g., Shotokuvirae, e.g., Cossaviricota, e.g., Papovaviricetes, e.g., Sepolyvirales, e.g., Polyomaviridae, e.g., Betapolyomavirus, e.g., BK Virus antigen
Proteobacteria, e.g., Betaproteobacteria, e.g., Burkholderiales, e.g., Alcaligenaceae, e.g., Bordetella, e.g., Bordetella pertussis antigen (e.g., pertussis toxin, filamentous haemagglutinin, pertactin)
Spirochaetes, e.g., Spirochaetales, e.g., Spriochaetaceae, e.g., Borrelia, e.g., Borrelia burgdorferi antigen
Proteobacteria, e.g., Betaproteobacteria, e.g., Burkholderiales, e.g., Burkholderiaceae, e.g., Burkholderia, e.g., Burkholderia species antigen (e.g., hydratase/aldolase PhnE)
Riboviria, e.g., Orthornavirae, e.g., Kitrinoviricota, e.g. Alsuviricetes, e.g., Martellivirales, e.g., Togaviridae, e.g., Alphavirus, e.g., Chikungunya virus (CHIKV) antigens
Chlamydiae, e.g., Chlamydiales, e.g., Chlamydiaceae, e.g., Chlamydia, e.g., Chlamydia trachomatis antigen
Proteobacteria, e.g., Gammaproteobacteria, e.g., Vibrionales, e.g., Vibrionaceae, e.g., Vibrio, e.g., Vibrio cholerae, e.g., Cholera antigen (e.g., cholera toxin B subunit)

"Firmicutes", e.g., Clostridia, e.g., Clostridiales, e.g., Peptostreptococcaceae, e.g., Clostridioides, e.g., Clostridium, e.g., Clostridium difficile antigen
Alveolata, e.g., Apicomplexa, e.g., Conoidasida, e.g., Eucoccidiorida, e.g., Eimeriorina, e.g., Cryoptosporidiidae, e.g., Cryptosporidium antigen
Duplodnaviria, e.g., Heunggongvirae, e.g., Peploviricota, e.g., Herviviricetes, e.g., Herpesvirales, e.g., Herpesviridae, e.g., Betaherpesvirinae, e.g., Cytomegalovirus (CMV) antigen
"Actinobacteria", e.g., Acintobacteria, e.g., Mycobacteriales, e.g., Corynebacteriaceae, e.g., Corynebacterium, Diphtheria antigen (e.g., diphtheria toxoid)
Amoebozoa, e.g., Entamoebidae, e.g., Entamoeba, e.g., Entamoeba histolytica antigen
Riboviria, e.g., Orthornavirae, e.g., Pisuviricota, e.g., Pisoniviricetes, e.g., Picornavirales, e.g., Picornaviridae, e.g., Enterovirus, e.g., Enterovirus 71 antigen (e.g., VP1)
Duplodnaviria, e.g., Heunggongvirae, e.g., Peploviricota, e.g., Herviviricetes, e.g., Herpesviridae, e.g., Lymphocryptovirus, e.g., Epstein-Barr virus antigen
Proteobacteria, e.g., Gammaproteobacteria, e.g., Enterobacterlaes, e.g., Enterobacteriaceae, e.g., Escherichia, e.g., Escherichia coli antigen (e.g., bifunctional penicillin-binding protein)
Riboviria, e.g., Orthornavirae, e.g., Kitrinoviricota, e.g., Flasuviricetes, e.g., Amarillovirales, Flaviviridae genus (Dengue Virus) antigen
Proteobacteria, e.g., Epsilonproteobacteria, e.g., Campylobacterales, e.g., Helicobacteraceae, e.g., Helicobacter, e.g., Helicobacter pylori antigen
Hepatitis antigen (e.g., hepatitis A (HAV), e.g., B (HBV), e.g., C (HCV), e.g., D (HDV) e.g., G (HGV) virus antigen)
Duplodnaviria, e.g., Heunggongvirae, e.g., Peploviricota, e.g., Herviviricetes, e.g., Herpesviridae, e.g., Alphaherpesvirinae, e.g., Simplexvirus, e.g., Herpes simplex antigen
Varidnaviria, e.g., Bamfordvirae, e.g., Preplasmiviricota, e.g., Tectiliviricetes, e.g., Rowavirales, e.g., Adenoviridae, e.g., Aviadenovirus, e.g., Ichtadenovirus, e..g, Testadenovirus, e.g., Atadenovirus, e.g., Mastadenovirus, e.g., Siadenovirus Human adenovirus antigen (e.g., type 34 hexon protein)
Riboviria, e.g., Pararnavirae, e.g., Artverviricota, e.g., Revtraviricetes, e.g., Ortervirales, e.g., Retroviridae, e.g., Orthoretrovirinae, e.g., Lentivirus, e.g., Human immunodeficiency virus (HIV) antigen (e.g., GP-120, GP-160)
Riboviria, e.g., Orthornavirae, e.g., Negarnaviricota, e.g., Monjiviricetes, e.g., Mononegavirales, e.g., Pneumoviridae, e.g., Metapneumovirus, e.g., Human metapneumovirus antigen
Monodnaviria, e.g., Shotokuvirae, e.g., Cossaviricota, e.g., Papovaviricetes, e.g., Zurhausenvirales, e.g., Papillomaviridae, e.g, Alphapapillomavirus, e.g., Betapapillomavirus, e.g., Chipapillomavirus, e.g., Deltapapillovirus, e.g., Dyochipapillomavirus, e.g., Dyodeltapapillomavirus, e.g., Dyoepsilonpapillomavirus, e.g., Dyoetapapillomavirus, e.g., Dyoiotapapillomavirus, e.g., Dyokappapapillomavirus, e.g., Dyolambdapapillomavirus, e.g., Dyomupapillomavirus, e.g., Dyonupapillomavirus, e.g., Dyoomegapapillomavirus, e.g., Dyoomikronpapillomavirus, e.g., Dyophipapillomavirus, e.g., Dyopipapillomavirus, e.g., Dyopsipapillomavirus, e.g., Dyorhopapillomavirus, e.g., Dyosigmapapillomavirus, e.g., Dyotaupapillomavirus, e.g., Dyothetapapillomavirus, e.g., Dyoupsilonpapillomavirus, e.g., Dyoxipapillomavirus, e.g., Dyozetapapillomavirus e.g., Gammapapillomavirus, e.g., Iotapapillomavirus, e.g., Kappapapillomavirus, e.g., Lambdapapillomavirus, e.g., Mupapillomavirus, e.g., Nupapillomavirus, e.g., Omegapapillomavirus, e.., Omikronpapillomavirus, e.g., Phipapillomavirus, e.g., Pipapillomavirus, e.g., Psipapillomavirus, e.g., Rhopapillomavirus, e.g., Sigmapapillomavirus, e.g., Taupapillomavirus, e.g., Thetapapillomavirus, e.g., Treisdeltapapillomavirus, e.g., Treisepsiolonpapillomavirus, e.g., Treisetapapillomavirus, e.g., Treisiotapapillomavirus, e.g., Trieskappapapillomavirus, e.g., Treisthetapapillomavirus, e.g., Treiszetapapillomavirus, e.g., Upsilonpapillomavirus, e.g., Xipapillomavirus, e.g., Zetapapillomavirus, e.g., Alefpapillomavirus, e.g., Human papilloma virus (HPV) antigen (e.g., L1 major capsid protein)
Riboviria, e.g., Orthornavirae, e.g., Negarnaviricota, e.g., Insthoviricetes, e.g., Articulavirales, e.g., Orthomyxoviridae, e.g., Alphainfluenzavirus, e.g., Betainfluenzavirus, e.g., Gammainfluenzavirus, e.g., Deltainfluenzavirus, e.g., Influenza virus antigen (e.g., HA)
Monodnaviria, e.g., Shotokuvirae, e.g., Cossaviricota, e.g., Papovaviricetes, e.g., Sepolyvirales, e.g., Polyomaviridae, e.g., Betapolyomavirus, e.g., JC Virus / JC polyomavirus antigen
Proteobacteria, e.g., gammaproteobacteria, e.g., Enterobacterales, e.g., Klebsiella, e.g., Klebsiella pneumoniae antigen
Riboviria, e.g., Orthornavirae, e.g., Negarnaviricota, e.g., Ellioviricetes, e.g., Bunyavirales, e.g., Arenaviridae, e.g., Mammarenavirus, e.g., Lassa virus antigen
Euglenozoa, e.g., Kinetoplastea, e.g., Trypanosomatida, e.g., Leishmania promastigotes antigen

Spriochaetes, e.g., Spirochaetales, e.g., Leptospiraceae, e.g., Leptospira, e.g., Leptospira interrogans antigen
Proteobacteria, e.g., Betaproteobacteria, e.g., Neisseriales, e.g., Neisseriaceae, e.g., Neisseria, e.g., Meningococcal antigen (e.g., capsular polysaccharides of serogroup A, B, C, Y and W-135)
Actinobacteria, e.g., Corynebacteriales, e.g., Mycobacteriaceae, e.g., Mycobacterium, e.g., Mycobacterium tuberculosis antigen
Proteobacteria, e.g., etaproteobacteria, e.g., Neisseriales, e.g., Neisseriaceae, e.g., Neisseria, e.g., N. meningitidis antigen (e.g., type Y polysaccharide [MenY], type W polysaccharide [MenW])
Proteobacteria, e.g., etaproteobacteria, e.g., Neisseriales, e.g., Neisseriaceae, e.g., Neisseria, e.g., Neisseriea gonorrhea antigen
Apicomplexa, e.g., Aconoidasida, e.g., Haemospororida, e.g., Plasmodiidae, e.g., Plasmodium, e.g., Plasmodium species antigen (e.g., HRPII, pLDH, pAldo)
"Firmicutes", e.g., Bacilli, e.g., Lactobacillales, e.g., Streptococcaceae, e.g., Streptococcus, e.g., Pneumococcal antigen (e.g., Streptococcus pneumoniae type 3 capsular polysaccharide)
Proteobacteria, e.g., Gammaproteobacteria, e.g., Pseudomonadales, e.g., Pseudomonadaceae, e.g., Pseudomonas, e.g., Pseudomonas species antigen (e.g., ferredoxin reductase component)
Riboviria, e.g., Orthornavirae, e.g., Negarnaviricota, e.g., Monjiviricetes, e.g., Mononegavirales, e.g., Rhabdoviridae, e.g., Lyssavirus, e.g., Rabies antigen
Riboviria, e.g., Orthornavirae, e.g., Negarnaviricota, e.g., Monjiviricetes, e.g., Mononegavirales, e.g., Pneumoviridae, e.g., Orthopneumovirus, e.g., Respiratory syncytial virus (RSV) antigen
Riboviria, e.g., Orthornavirae, e.g., Negarnaviricota, e.g., Ellioviricetes, e.g., Bunyavirales, e.g., Phenuviridae, e.g., Phlebovirus, e.g., Rift Valley fever Virus antigen
Riboviria, e.g., Orthornavirae, e.g., Duplornaviricota, e.g., Resentoviricetes, e.g., Reovirales, e.g., Reoviridae, e.g., Sedoreovirinae, e.g., Rotavirus, e.g., Rotavirus antigen (e.g., VP8, H1N1 M2, H7N9 F)
Proteobacteria, e.g., Gammaproteobacteria, e.g., Enterobacterales, e.g., Enterobacteriaceae, e.., Salmonella, e.g., Salmonella typhi antigen (e.g., Vi polysaccharide)
Platyhelminthes, e.g., Diplostomida, e.g., Schistosomatidae, e.g., Schistosomatinae, e.g., Schistosoma, e.g., Schistosoma species antigen
Proteobacteria, e.g., Gammaproteobacteria, e.g., Enterobacterales, e.g., Enterobacteriaceae, e.g., Shigell, e.g., Shigella antigen (e.g., shigella polysaccharides)
"Firmicutes", e.g., Bacilli, e.g., Bacillales, e.g., Staphylococcaceae, e.g., Staphylococcus, e.g., Staphylococcal antigen (e.g., staphylococcal enterotoxin B)
"Firmicutes", e.g., Bacilli, e.g., Lactobacillales, e.g., Streptococcaceae, e.g., Streptococcus, e.g., Streptococcus agalactiae antigen
"Firmicutes", e.g., Bacilli, e.g., Lactobacillales, e.g., Streptococcaceae, e.g., Streptococcus, e.g., Streptococcus pyogenes antigen
"Firmicutes", e.g., Clostridia, e.g., Clostridiales, e.g., Clostridiaceae, e.g., Clostridium, e.g., Tetanus antigen (e.g., tetanus toxoid)
Riboviria, e.g., Orthornavirae, e.g., Kitrinoviricota, e.g., Flasuviricetes, e.g., Amarillovirales, e.g., Flaviviridae, e.g., Flavivirus, e.g., Tick-borne encephalitis (TBE) antigen
Apicomplexa, e.g., Conoidasida, e.g., Eucoccidiorida, e.g., Sarcocystidae, e.g., Toxoplasmatinae, e.g., Toxoplasma, e.g., Toxoplasma gondii antigen
Spirochaetes, e.g., Spirochaetales, e.g., Spirochaetaceae, e.g., Treponema, e.g., Treponema pallidum antigen
"Actinobacteria", e.g., Actinobacteria, e.g., Mycobacteriales, e.g., Mycobacteriaceae, e.g., Mycobacterium, e.g., Tuberculosis antigen
Riboviria, e.g., Orthornavirae, e.g., Negarnaviricota, e.g., Monjiviricetes, e.g., Mononegavirales, e.g., Rhabdoviridae, e.g., Vesiculovirus, e.g., Vesicular stomatitis virus antigen (e.g., vesicular stomatitis virus glycoprotein)
Proteobacteria, e.g., Gammaproteobacteria, e.g., Vibrionales, e..g, Vibrionaceae, e.g., Vibrio, e.g., Vibrio cholerae antigen
Riboviria, e.g., Orthornavirae, e.g., Kitrinoviricota, e.g., Flasuviricetes, e.g., Amarillovirales, e.g., Flaviviridae, e.g., Flavivirus, e.g., West Nile virus (WNV) antigen
Riboviria, e.g., Orthornavirae, e.g., Kitrinoviricota, e.g., Flasuviricetes, e.g., Amarillovirales, e.g., Flaviviridae, e.g., Flavivirus, e.g., Zika virus antigen
Apicomplexa, e.g., Aconoidasida, e.g., Haemospororida, e.g., Plasmodiidae, e.g., Plasmodium, e.g., Plasmodium vivax, Plasmodium falciparum, Plasmodium malariae, Plasmodium ovale, or Plasmodium knowlesi.

Riboviria, e.g., Orthornavirae, e.g., Pisuviricota, e.g., Pisoniviricetes, e.g., Nidovirales, e.g., Coronaviridae, e.g., Betacoronavirus, e.g., Sebacovirus, e.g., severe acute respiratory syndrome-related coronavirus, e.g., SARS-CoV-2, e.g., SARS-CoV-2 alpha variant, SARS-CoV-2 beta variant, SARS-CoV-2 gamma variant, SARS-CoV-2 delta variant, or SARS-CoV-2 omicron variant

In some embodiments, a vaccine comprising an anellovector or anelloVLP as described herein is administered with an adjuvant. In certain embodiments, the adjuvant is an inorganic adjuvant (e.g., potassium alum, aluminium hydroxide, aluminium phosphate, or calcium phosphate hydroxide). In certain embodiments, the adjuvant is an oil-based adjuvant (e.g., paraffin oil). In certain embodiments, the adjuvant is a saponin. In certain embodiments, the adjuvant is a cytokine. In certain embodiments, the adjuvant is a squalene. In certain embodiments, the adjuvant is Freund's complete adjuvant.

In some embodiments, a vaccine as described herein is administered in a dose comprising about 10^{10} to 10^{14} viral genome equivalents of an anellovector as described herein. In some embodiments, a vaccine as described herein is administered as a dose comprising about 10^{10} to 10^{14} particles (e.g., anellovectors or anelloVLPs) as described herein.

Ligands

In some instances, a surface moiety as described herein comprises a ligand (e.g., a ligand that binds specifically to a receptor on a target cell). In some embodiments, the ligand is a growth factor. In certain embodiments, the ligand binds to a growth factor receptor on the surface of the target cell. In some embodiments, the ligand is a cytokine. In some embodiments, the ligand is a hormone.

II. Compositions and Methods for Making Anellovectors and AnelloVLPs

The present disclosure provides, in some aspects, anellovectors, anelloVLPs, and methods thereof for delivering effectors. In some embodiments, the anellovectors, anelloVLPs, or components thereof can be made as described below. In some embodiments, the compositions and methods described herein can be used to produce a genetic element or a genetic element construct. In some embodiments, the compositions and methods described herein can be used to produce one or more Anellovirus ORF molecules (e.g., an ORF1, ORF2, ORF2/2, ORF2/3, ORF1/1, or ORF1/2 molecule, or a functional fragment or splice variant thereof). In some embodiments, the compositions and methods described herein can be used to produce a proteinaceous exterior or a component thereof (e.g., an ORF1 molecule), e.g., in a host cell.

In some embodiments, the anellovectors, anelloVLPs, or components thereof can be made using a tandem construct, e.g., as described in PCT Application No. PCT/US2021/037091, which is incorporated herein by reference in its entirety.

The present disclosure provides, in some aspects, compositions (e.g., bacmids, donor vectors, baculovirus particles, and cells comprising same) and methods that can be used for producing anellovectors or anelloVLPs, e.g., as described herein. In some embodiments, the compositions and methods described herein can be used to produce a genetic element or a genetic element construct. In some embodiments, the compositions and methods described herein can be used to produce one or more Anellovirus ORF molecules (e.g., an ORF1, ORF2, ORF2/2, ORF2/3, ORF1/1, or ORF1/2 molecule, or a functional fragment or splice variant thereof). In some embodiments, the compositions and methods described herein can be used to produce a proteinaceous exterior or a component thereof (e.g., an ORF1 molecule). In some embodiments, the anellovectors, anelloVLPs, or components thereof can be made using a bacmid/insect cell system, e.g., as described as described in PCT Application No. PCT/US2021/037076, which is incorporated herein by reference in its entirety.

Without wishing to be bound by theory, rolling circle amplification may occur via Rep protein binding to a Rep binding site (e.g., comprising a 5' UTR, e.g., comprising a hairpin loop and/or an origin of replication, e.g., as described herein) positioned 5' relative to (or within the 5' region of) the genetic element region. The Rep protein may then proceed through the genetic element region, resulting in the synthesis of the genetic element. The genetic element may then be circularized and then enclosed within a proteinaceous exterior to form an anellovector.

Components and Assembly of Anellovectors and AnelloVLPs

The compositions and methods herein can be used to produce anellovectors and anelloVLPs. As described herein, an anellovector generally comprises a genetic element (e.g., a single-stranded, circular DNA molecule, e.g., comprising a 5' UTR region as described herein) enclosed within a proteinaceous exterior (e.g., comprising a polypeptide encoded by an Anellovirus ORF1 nucleic acid, e.g., as described herein). In some embodiments, the genetic element comprises one or more sequences encoding Anellovirus ORFs (e.g., one or more of an Anellovirus ORF1, ORF2, ORF2/2, ORF2/3, ORF1/1, or ORF1/2). As described herein, an anelloVLP generally comprises a proteinaceous exterior (e.g., comprising a polypeptide encoded by an Anellovirus ORF1 nucleic acid, e.g., as described herein) and an exogenous effector. As used herein, an Anellovirus ORF or ORF molecule (e.g., an Anellovirus ORF1, ORF2, ORF2/2, ORF2/3, ORF1/1, or ORF1/2) includes a polypeptide comprising an amino acid sequence having at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to a corresponding Anellovirus ORF sequence, e.g., as described herein or as described in PCT/US2018/037379 or PCT/US19/65995 (each of which is incorporated by reference herein in their entirety). In embodiments, the genetic element comprises a sequence encoding an Anellovirus ORF1, or a splice variant or functional fragment thereof (e.g., a structural jelly-roll region, e.g., as described

herein). In some embodiments, the proteinaceous exterior comprises a polypeptide encoded by an Anellovirus ORF1 nucleic acid (e.g., an Anellovirus ORF1 molecule or a splice variant or functional fragment thereof).

In some embodiments, an anellovector is assembled by enclosing a genetic element (e.g., as described herein) within a proteinaceous exterior (e.g., as described herein). In some embodiments, an anellovector is assembled by enclosing a genetic element (e.g., as described herein) within a proteinaceous exterior *in vitro* (e.g., as described herein) (e.g., wherein the enclosing occurs external to a host cell, e.g., in the absence of a host cell). In some embodiments, the genetic element is enclosed within the proteinaceous exterior in a host cell (e.g., as described herein). In some embodiments, a surface moiety is attached to the proteinaceous exterior (e.g., as described herein). In some embodiments, the proteinaceous exterior comprises an ORF1 molecule comprising a surface moiety (e.g., as described herein). In certain embodiments, the ORF1 molecule comprises a surface moiety fused to an ORF1 domain.

In some embodiments, an anelloVLP is assembled by enclosing an exogenous effector within a proteinaceous exterior (e.g., as described herein) *in vitro* (e.g., wherein the enclosing occurs external to a host cell, e.g., in the absence of a host cell). In some embodiments, an anelloVLP is assembled by contacting a plurality of ORF1 molecules (e.g., as described herein) with an effector *in vitro* (e.g., wherein the contacting occurs external to a host cell, e.g., in the absence of a host cell). In some embodiments, an anelloVLP is assembled by attaching an exogenous effector to the exterior surface of a proteinaceous exterior (e.g., as described herein) *in vitro*. In some embodiments, a surface moiety is attached to the proteinaceous exterior (e.g., as described herein). In some embodiments, the proteinaceous exterior comprises an ORF1 molecule comprising a surface moiety (e.g., as described herein). In certain embodiments, the ORF1 molecule comprises a surface moiety fused to an ORF1 domain.

In some embodiments, a host cell expresses one or more polypeptides comprised in the proteinaceous exterior (e.g., a polypeptide encoded by an Anellovirus ORF1 nucleic acid, e.g., an ORF1 molecule). For example, in some embodiments, the host cell comprises a nucleic acid sequence encoding an Anellovirus ORF1 molecule, e.g., a splice variant or a functional fragment of an Anellovirus ORF1 polypeptide (e.g., a wild-type Anellovirus ORF1 protein or a polypeptide encoded by a wild-type Anellovirus ORF1 nucleic acid, e.g., as described herein). In embodiments, the nucleic acid sequence encoding the Anellovirus ORF1 molecule is comprised in a nucleic acid construct (e.g., a plasmid, viral vector, virus, minicircle, bacmid, or artificial chromosome) comprised in the host cell. In embodiments, the nucleic acid sequence encoding the Anellovirus ORF1 molecule is integrated into the genome of the host cell.

In some embodiments, a host cell produces a genetic element from a nucleic acid construct comprising the sequence of the genetic element. In some embodiments, the nucleic acid construct is selected from a plasmid, in vitro circularized nucleic acid molecule, viral nucleic acid molecule, minicircle, bacmid, or artificial chromosome. In some embodiments, the genetic element is excised from the nucleic acid construct and, optionally, converted from a double-stranded form to a single-stranded form (e.g., by denaturation). In some embodiments, the genetic element is generated by a polymerase based on a template sequence in the nucleic acid construct. In some embodiments, the polymerase produces a single-stranded copy of the genetic element sequence, which can optionally be circularized to form a genetic element as described herein. In other embodiments, the nucleic acid construct is a double-stranded minicircle produced by circularizing the nucleic acid sequence of the genetic element *in vitro*. In embodiments, the *in vitro*-circularized (IVC) minicircle is introduced into the host cell, where it is converted to a single-stranded genetic element suitable for enclosure in a proteinaceous exterior, as described herein.

In some embodiments, the host cell comprises a genetic element construct (e.g., a bacmid, plasmid, or minicircle). In some embodiments, the host cell comprises a bacmid comprising one or more sequences encoding Anellovirus ORF molecules (e.g., ORF1, ORF2, ORF2/2, ORF2/3, ORF1/1, and/or ORF1/2 ORF molecules), or functional fragments thereof. In some embodiments, proteinaceous exterior proteins are expressed from the bacmid. In embodiments, the proteinaceous exterior proteins expressed from the bacmid enclose a genetic element, thereby forming an anellovector. In some embodiments, the bacmid comprises a backbone suitable for replication of the nucleic acid construct in insect cells (e.g., Sf9 cells), e.g., a baculovirus backbone region. In some embodiments, the bacmid comprises a backbone region suitable for replication of the genetic element construct in bacterial cells (e.g., *E. coli* cells, e.g., DH 10Bac cells). In some embodiments, the genetic element construct comprises a backbone suitable for replication of the nucleic acid construct in insect cells (e.g., Sf9 cells), e.g., a baculovirus backbone region. In some embodiments, the genetic element construct comprises a backbone region suitable for replication of the genetic element construct in bacterial cells (e.g., *E. coli* cells, e.g., DH 10Bac cells). In some embodiments, the bacmid is introduced into the host cell via a baculovirus particle. In embodiments, the bacmid is produced by a producer cell, e.g., an insect cell (e.g., an Sf9 cell) or a bacterial cell (e.g., an *E. coli* cell, e.g., a DH 10Bac cell). In embodiments, the producer cell comprises a bacmid and/or a donor vector, e.g., as described herein. In embodiments, the producer cell further comprises sufficient cellular machinery for replication of the bacmid and/or donor vector.

ORF1 Molecules, e.g., for assembly of Anellovectors

An anellovector or anelloVLP as described herein generally comprises a proteinaceous exterior comprising a polypeptide encoded by an Anellovirus ORF1 nucleic acid (e.g., an Anellovirus ORF1 molecule or a splice variant or functional fragment thereof, e.g., as described herein). An ORF1 molecule may, in some embodiments, comprise one or more of: a first region comprising a structural arginine rich region, e.g., a region having at least 60% basic residues (e.g., at least 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 100% basic residues; e.g., between 60%-90%, 60%-80%, 70%-90%, or 70-80% basic residues), and a second region comprising structural jelly-roll domain, e.g., at least six beta strands (e.g., 4, 5, 6, 7, 8, 9, 10, 11, or 12 beta strands). In embodiments, the proteinaceous exterior comprises one or more (e.g., 1, 2, 3, 4, or all 5) of an Anellovirus ORF1 structural arginine-rich region, structural jelly-roll region, structural N22 domain, hypervariable region, and/or structural C-terminal domain. In some embodiments, the proteinaceous exterior comprises an Anellovirus ORF1 structural jelly-roll region (e.g., as described herein). In some embodiments, the proteinaceous exterior comprises an Anellovirus ORF1 structural arginine-rich region (e.g., as described herein). In some embodiments, the proteinaceous exterior comprises an Anellovirus ORF1 structural N22 domain (e.g., as described herein). In some embodiments, the proteinaceous exterior comprises an Anellovirus hypervariable region (e.g., as described herein). In some embodiments, the proteinaceous exterior comprises an Anellovirus ORF1 structural C-terminal domain (e.g., as described herein).

In some embodiments, the anellovector comprises an ORF1 molecule and/or a nucleic acid encoding an ORF1 molecule. In some embodiments, the anelloVLP comprises an ORF1 molecule. Generally, an ORF1 molecule comprises a polypeptide having the structural features and/or activity of an Anellovirus ORF1 protein (e.g., an Anellovirus ORF1 protein as described herein), or a functional fragment thereof. In some embodiments, the ORF1 molecule comprises a truncation relative to an Anellovirus ORF1 protein (e.g., an Anellovirus ORF1 protein as described herein). In some embodiments, the ORF1 molecule is truncated by at least 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 150, 200, 250, 300, 350, 400, 450, 500, 550, 600, 650, or 700 amino acids of the Anellovirus ORF1 protein. In some embodiments, an ORF1 molecule comprises an amino acid sequence having at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity to a Betatorquevirus ORF1 protein, e.g., as described herein. An ORF1 molecule can generally bind to a nucleic acid molecule, such as DNA (e.g., a genetic element, e.g., as described herein). In some embodiments, an ORF1 molecule localizes to the nucleus of a cell. In certain embodiments, an ORF1 molecule localizes to the nucleolus of a cell.

Without wishing to be bound by theory, an ORF1 molecule may be capable of binding to other ORF1 molecules, e.g., to form a proteinaceous exterior (e.g., as described herein). Such an ORF1 molecule may be described as having the capacity to form a capsid. In some embodiments, the

proteinaceous exterior may enclose a nucleic acid molecule (e.g., a genetic element as described herein, e.g., produced using a composition or construct as described herein) and/or an effector (e.g., an exogenous effector). In some embodiments, a plurality of ORF1 molecules may form a multimer, e.g., to produce a proteinaceous exterior. In some embodiments, the multimer may be a homomultimer. In other embodiments, the multimer may be a heteromultimer.

In some embodiments, a first plurality of anellovectors or anelloVLPs comprising an ORF1 molecule as described herein is administered to a subject. In some embodiments, a second plurality of anellovectors or anelloVLPs comprising an ORF1 molecule described herein, is subsequently administered to the subject following administration of the first plurality. In some embodiments the second plurality of anellovectors or anelloVLPs comprises an ORF1 molecule having the same amino acid sequence as the ORF1 molecule comprised by the anellovectors or anelloVLPs of the first plurality. In some embodiments the second plurality of anellovectors or anelloVLPs comprises an ORF1 molecule having at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% amino acid sequence identity to the ORF1 molecule comprised by the anellovectors or anelloVLPs of the first plurality.

ORF2 Molecules, e.g., for assembly of Anellovectors

Producing an anellovector or anelloVLP using the compositions or methods described herein may involve expression of an Anellovirus ORF2 molecule (e.g., as described herein), or a splice variant or functional fragment thereof. In some embodiments, the anellovector comprises an ORF2 molecule, or a splice variant or functional fragment thereof, and/or a nucleic acid encoding an ORF2 molecule, or a splice variant or functional fragment thereof. In some embodiments, the anellovector or anelloVLP does not comprise an ORF2 molecule, or a splice variant or functional fragment thereof, and/or a nucleic acid encoding an ORF2 molecule, or a splice variant or functional fragment thereof. In some embodiments, producing the anellovector or anelloVLP comprises expression of an ORF2 molecule, or a splice variant or functional fragment thereof, but the ORF2 molecule is not incorporated into the anellovector or anelloVLP.

Production of protein components

Protein components of an anellovector or anelloVLP, e.g., ORF1, can be produced in a variety of ways, e.g., as described herein. In some embodiments, one or more protein components of an anellovector or anelloVLP, including, e.g., the proteinaceous exterior, are produced in a host cell (e.g., the same host cell that packages the genetic elements into the proteinaceous exteriors, thereby producing the anellovectors). In some embodiments, one or more protein components of an anellovector or anelloVLP, including, e.g., the proteinaceous exterior, are produced in a cell that does not comprise a genetic element

and/or a genetic element construct (e.g., as described herein). In some embodiments, one or more protein components of an anellovector or anelloVLP are produced and then secreted from a host cell. In some embodiments, one or more protein components of an anellovector or anelloVLP are produced and then isolated from a host cell (e.g., by lysing the host cell).

Baculovirus expression systems

A viral expression system, *e.g.*, a baculovirus expression system, may be used to express proteins (e.g., for production of anellovectors or anelloVLPs), e.g., as described herein. Baculoviruses are rod-shaped viruses with a circular, supercoiled double-stranded DNA genome. Genera of baculoviruses include: Alphabaculovirus (nucleopolyhedroviruses (NPVs) isolated from *Lepidoptera*), Betabaculoviruses (granuloviruses (GV) isolated from *Lepidoptera*), Gammabaculoviruses (NPVs isolated from *Hymenoptera*) and Deltabaculoviruses (NPVs isolated from *Diptera*). While GVs typically contain only one nucleocapsid per envelope, NPVs typically contain either single (SNPV) or multiple (MNPV) nucleocapsids per envelope. The enveloped virions are further occluded in granulin matrix in GVs and polyhedrin in NPVs. Baculoviruses typically have both lytic and occluded life cycles. In some embodiments, the lytic and occluded life cycles manifest independently throughout the three phases of virus replication: early, late, and very late phase. In some embodiments, during the early phase, viral DNA replication takes place following viral entry into the host cell, early viral gene expression and shut-off of the host gene expression machinery. In some embodiments, in the late phase late genes that code for viral DNA replication are expressed, viral particles are assembled, and extracellular virus (EV) is produced by the host cell. In some embodiments, in the very late phase the polyhedrin and p10 genes are expressed, occluded viruses (OV) are produced by the host cell, and the host cell is lysed. Since baculoviruses infect insect species, they can be used as biological agents to produce exogenous proteins in baculoviruses-permissive insect cells or larvae. Different isolates of baculovirus, such as *Autographa californica* multiple nuclear polyhedrosis virus (AcMNPV) and *Bombyx mori* (silkworm) nuclear polyhedrosis virus (BmNPV) may be used in exogenous protein expression. Various baculoviral expression systems are commercially available, e.g., from ThermoFisher.

In some embodiments, the proteins described herein (e.g., an Anellovirus ORF molecule, e.g., ORF1, ORF2, ORF2/2, ORF2/3, ORF1/1, or ORF1/2, or a functional fragment or splice variant thereof) may be expressed using a baculovirus expression vector (e.g., a bacmid) that comprises one or more components described herein. For example, a baculovirus expression vector may include one or more of (e.g., all of) a selectable marker (e.g., kanR), an origin of replication (e.g., one or both of a bacterial origin of replication and an insect cell origin of replication), a recombinase recognition site (e.g., an att site), and a promoter. In some embodiments, a baculovirus expression vector (e.g., a bacmid as described herein)

can be produced by replacing the naturally occurring wild-type polyhedrin gene, which encodes for baculovirus occlusion bodies, with genes encoding the proteins described herein. In some embodiments, the genes encoding the proteins described herein are cloned into a baculovirus expression vector (e.g., a bacmid as described herein) containing a baculovirus promoter. In some embodiments, the baculovirual vector comprises one or more non-baculoviral promoters, e.g., a mammalian promoter or an Anellovirus promoter. In some embodiments, the genes encoding the proteins described herein are cloned into a donor vector (e.g., as described herein), which is then contacted with an empty baculovirus expression vector (e.g., an empty bacmid) such that the genes encoding the proteins described herein are transferred (e.g., by homologous recombination or transposase activity) from the donor vector into the baculovirus expression vector (e.g., bacmid). In some embodiments, the baculovirus promoter is flanked by baculovirus DNA from the nonessential polyhedrin gene locus. In some embodiments, a protein described herein is under the transcriptional control of the AcNPV polyhedrin promoter in the very late phase of viral replication. In some embodiments, a strong promoter suitable for use in baculoviral expression in insect cells include, but are not limited to, baculovirus p10 promoters, polyhedrin (polh) promoters, p6.9 promoters and capsid protein promoters. Weak promoters suitable for use in baculoviral expression in insect cells include ie1, ie2, ie0, et1, 39K (aka pp31) and gp64 promoters of baculoviruses.

In some embodiments, a recombinant baculovirus is produced by homologous recombination between a baculoviral genome (*e.g.*, a wild-type or mutant baculoviral genome), and a transfer vector. In some embodiments, one or more genes encoding a protein described herein are cloned into the transfer vector. In some embodiments, the transfer vector further contains a baculovirus promoter flanked by DNA from a nonessential gene locus, e.g., polyhedrin gene. In some embodiments, one or more genes encoding a protein described herein are inserted into the baculoviral genome by homologous recombination between the baculoviral genome and the transfer vector. In some embodiments, the baculoviral genome is linearized at one or more unique sites. In some embodiments, the linearized sites are located near the target site for insertion of genes encoding the proteins described herein into the baculoviral genome. In some embodiments, a linearized baculoviral genome missing a fragment of the baculoviral genome downstream from a gene, e.g., polyhedrin gene, can be used for homologous recombination. In some embodiments, the baculoviral genome and transfer vector are co-transfected into insect cells. In some embodiments, the method of producing the recombinant baculovirus comprises the steps of preparing the baculoviral genome for performing homologous recombination with a transfer vector containing the genes encoding one or more protein described herein and co-transfecting the transfer vector and the baculoviral genome DNA into insect cells. In some embodiments, the baculoviral genome comprises a region homologous to a region of the transfer vector. These homologous regions may enhance the probability of recombination between the baculoviral genome and the transfer vector. In

some embodiments, the homology region in the transfer vector is located upstream or downstream of the promoter. In some embodiments, to induce homologous recombination, the baculoviral genome, and transfer vector are mixed at a weight ratio of about 1:1 to 10:1.

In some embodiments, a recombinant baculovirus is generated by a method comprising site-specific transposition with Tn7, e.g., whereby the genes encoding the proteins described herein are inserted into bacmid DNA, e.g., propagated in bacteria, *e.g.*, *E. coli* (e.g., DH 10Bac cells). In some embodiments, the genes encoding the proteins described herein are cloned into a pFASTBAC® vector and transformed into competent cells, *e.g.*, DH10BAC® competent cells, containing the bacmid DNA with a mini-*att*Tn7 target site. In some embodiments, the baculovirus expression vector, *e.g.*, pFASTBAC® vector, may have a promoter, *e.g.*, a dual promoter (*e.g.*, polyhedrin promoter, p10 promoter). Commercially available pFASTBAC® donor plasmids include: pFASTBAC 1, pFASTBAC HT, and pFASTBAC DUAL. In some embodiments, recombinant bacmid DNA containing-colonies are identified and bacmid DNA is isolated to transfect insect cells.

In some embodiments, a baculoviral vector is introduced into an insect cell together with a helper nucleic acid. The introduction may be concurrent or sequential. In some embodiments, the helper nucleic acid provides one or more baculoviral proteins, e.g., to promote packaging of the baculoviral vector. In some embodiments, recombinant baculovirus produced in insect cells (e.g., by homologous recombination) is expanded and used to infect insect cells (e.g., in the mid-logarithmic growth phase) for recombinant protein expression. In some embodiments, recombinant bacmid DNA produced by site-specific transposition in bacteria, *e.g.*, *E. coli*, is used to transfect insect cells with a transfection agent, e.g., Cellfectin® II. Additional information on baculovirus expression systems is discussed in US patent applications Nos. 14/447,341, 14/277,892, and 12/278,916, which are hereby incorporated by reference.

Insect cell systems

The proteins described herein may be expressed in host cells (e.g., insect cells) infected or transfected with recombinant baculovirus or bacmid DNA, e.g., as described above. In some embodiments, the host or host cell is an insect cell (e.g., an Sf9 cell, Sf21 cell, or Hi5 cell). In some embodiments, the insect cell is derived from *Bombyx mori, Mamestra brassicae, Spodoptera frugiperda, Trichoplusia ni*, or *Drosophila melanogaster*. In some embodiments, the insect cell is selected from Sf9 and Sf21 cells derived from *Spodoptera frugiperda* and Tn-368 and High Five™ BTI-TN-5B1-4 cells (also referred to as Hi5 cells) derived from *Trichoplusia ni*. In some embodiments, insect cell lines Sf21 and Sf9, derived from the ovaries of the pupal fall army worm *Spodoptera frugiperda*, can be used for the expression of recombinant proteins using the baculovirus expression system. In some embodiments, Sf21 and Sf9 insect cells may be cultured in commercially available serum-supplemented or serum-free media.

Suitable media for culturing insect cells include: Grace's Supplemented (TNM-FH), IPL-41, TC-100, Schneider's Drosophila, SF-900 II SFM, and EXPRESS-FIVE™ SFM. In some embodiments, some serum-free media formulations utilize a phosphate buffer system to maintain a culture pH in the range of 6.0-6.4 (Licari et al. Insect cell hosts for baculovirus expression vectors contain endogenous exoglycosidase activity. Biotechnology Progress 9: 146-152 (1993) and Drugmand et al. Insect cells as factories for biomanufacturing. Biotechnology Advances 30:1140-1157 (2012)) for both cultivation and recombinant protein production. In some embodiments, a pH of 6.0-6.8 for cultivating various insect cell lines may be used. In some embodiments, insect cells are cultivated in suspension or as a monolayer at a temperature between 25° to 30°C with aeration. Additional information on insect cells is discussed, for example, in US Patent Application Nos. 14/564,512 and 14/775,154, each of which is hereby incorporated by reference.

Mammalian cell systems

In some embodiments, the proteins described herein may be expressed *in vitro* in animal cell lines infected or transfected with a vector encoding the protein, e.g., as described herein. Animal cell lines envisaged in the context of the present disclosure include porcine cell lines, e.g., immortalised porcine cell lines such as, but not limited to the porcine kidney epithelial cell lines PK-15 and SK, the monomyeloid cell line 3D4/31 and the testicular cell line ST. Also, other mammalian cells lines are included, such as CHO cells (Chinese hamster ovaries), MARC-145, MDBK, RK-13, EEL. Additionally or alternatively, particular embodiments of the methods of the invention make use of an animal cell line which is an epithelial cell line, i.e. a cell line of cells of epithelial lineage. Cell lines suitable for expressing the proteins described herein include, but are not limited to cell lines of human or primate origin, such as human or primate kidney carcinoma cell lines.

Genetic Element Constructs, e.g., for assembly of Anellovectors

The genetic element of an anellovector as described herein may be produced from a genetic element construct that comprises a genetic element region and optionally other sequence such as vector backbone. Generally, the genetic element construct comprises an Anellovirus 5' UTR (e.g., as described herein). A genetic element construct may be any nucleic acid construct suitable for delivery of the sequence of the genetic element into a host cell in which the genetic element can be enclosed within a proteinaceous exterior. In some embodiments, the genetic element construct comprises a promoter. In some embodiments, the genetic element construct is a linear nucleic acid molecule. In some embodiments, the genetic element construct is a circular nucleic acid molecule (e.g., a plasmid, bacmid, or a minicircle, e.g., as described herein). The genetic element construct may, in some embodiments, be

double-stranded. In other embodiments, the genetic element is single-stranded. In some embodiments, the genetic element construct comprises DNA. In some embodiments, the genetic element construct comprises RNA. In some embodiments, the genetic element construct comprises one or more modified nucleotides.

In some aspects, the present disclosure provides a method for replication and propagation of the anellovector as described herein (e.g., in a cell culture system), which may comprise one or more of the following steps: (a) introducing (e.g., transfecting) a genetic element (e.g., linearized) into a cell line sensitive to anellovector infection; (b) harvesting the cells and optionally isolating cells showing the presence of the genetic element; (c) culturing the cells obtained in step (b) (e.g., for at least three days, such as at least one week or longer), depending on experimental conditions and gene expression; and (d) harvesting the cells of step (c), e.g., as described herein.

Plasmids

In some embodiments, the genetic element construct is a plasmid. The plasmid will generally comprise the sequence of a genetic element as described herein as well as an origin of replication suitable for replication in a host cell (e.g., a bacterial origin of replication for replication in bacterial cells) and a selectable marker (e.g., an antibiotic resistance gene). In some embodiments, the sequence of the genetic element can be excised from the plasmid. In some embodiments, the plasmid is capable of replication in a bacterial cell. In some embodiments, the plasmid is capable of replication in a mammalian cell (e.g., a human cell). In some embodiments, a plasmid is at least 300, 400, 500, 600, 700, 800, 900, 1000, 2000, 3000, 4000, or 5000 bp in length. In some embodiments, the plasmid is less than 600, 700, 800, 900, 1000, 2000, 3000, 4000, 5000, 6000, 7000, 8000, 9000, or 10,000 bp in length. In some embodiments, the plasmid has a length between 300-400, 400-500, 500-600, 600-700, 700-800, 800-900, 900-1000, 1000-1500, 1500-2000, 2000-2500, 2500-3000, 3000-4000, or 4000-5000 bp. In some embodiments, the genetic element can be excised from a plasmid (e.g., by *in vitro* circularization), for example, to form a minicircle, e.g., as described herein. In embodiments, excision of the genetic element separates the genetic element sequence from the plasmid backbone (e.g., separates the genetic element from a bacterial backbone).

Small circular nucleic acid constructs

In some embodiments, the genetic element construct is a circular nucleic acid construct, e.g., lacking a backbone (e.g., lacking a bacterial origin of replication and/or selectable marker). In embodiments, the genetic element is a double-stranded circular nucleic acid construct. In embodiments, the double-stranded circular nucleic acid construct is produced by *in vitro* circularization (IVC), e.g., as

described herein. In embodiments, the double-stranded circular nucleic acid construct can be introduced into a host cell, in which it can be converted into or used as a template for generating single-stranded circular genetic elements, e.g., as described herein. In some embodiments, the circular nucleic acid construct does not comprise a plasmid backbone or a functional fragment thereof. In some embodiments, the circular nucleic acid construct is at least 2000, 2100, 2200, 2300, 2400, 2500, 2600, 2700, 2800, 2900, 3000, 3100, 3200, 3300, 3400, 3500, 3600, 3700, 3800, 3900, 4000, 4100, 4200, 4300, 4400, or 4500 bp in length. In some embodiments, the circular nucleic acid construct is less than 2900, 3000, 3100, 3200, 3300, 3400, 3500, 3600, 3700, 3800, 3900, 4000, 4100, 4200, 4300, 4400, 4500, 4600, 4700, 4800, 4900, 5000, 5500, or 6000 bp in length. In some embodiments, the circular nucleic acid construct is between 2000-2100, 2100-2200, 2200-2300, 2300-2400, 2400-2500, 2500-2600, 2600-2700, 2700-2800, 2800-2900, 2900-3000, 3000-3100, 3100-3200, 3200-3300, 3300-3400, 3400-3500, 3500-3600, 3600-3700, 3700-3800, 3800-3900, 3900-4000, 4000-4100, 4100-4200, 4200-4300, 4300-4400, or 4400-4500 bp in length. In some embodiments, the circular nucleic acid construct is a minicircle.

In vitro circularization

In some instances, the genetic element to be packaged into a proteinaceous exterior is a single stranded circular DNA. The genetic element may, in some instances, be introduced into a host cell via a genetic element construct having a form other than a single stranded circular DNA. For example, the genetic element construct may be a double-stranded circular DNA. The double-stranded circular DNA may then be converted into a single-stranded circular DNA in the host cell (e.g., a host cell comprising a suitable enzyme for rolling circle replication, e.g., an *Anellovirus* Rep protein, e.g., Rep68/78, Rep60, RepA, RepB, Pre, MobM, TraX, TrwC, Mob02281, Mob02282, NikB, ORF50240, NikK, TecH, OrfJ, or TraI, e.g., as described in Wawrzyniak et al. 2017, *Front. Microbiol.* 8: 2353; incorporated herein by reference with respect to the listed enzymes). In some embodiments, the double-stranded circular DNA is produced by *in vitro* circularization (IVC), e.g., as described in Example 15 or PCT Publication No. WO 2020/123816, incorporated by reference herein in its entirety.

Generally, *in vitro* circularized DNA constructs can be produced by digesting a genetic element construct (e.g., a plasmid comprising the sequence of a genetic element) to be packaged, such that the genetic element sequence is excised as a linear DNA molecule. The resultant linear DNA can then be ligated, e.g., using a DNA ligase, to form a double-stranded circular DNA. In some instances, a double-stranded circular DNA produced by *in vitro* circularization can undergo rolling circle replication, e.g., as described herein. Without wishing to be bound by theory, it is contemplated that *in vitro* circularization results in a double-stranded DNA construct that can undergo rolling circle replication without further modification, thereby being capable of producing single-stranded circular DNA of a suitable size to be

packaged into an anellovector, e.g., as described herein. In some embodiments, the double-stranded DNA construct is smaller than a plasmid (e.g., a bacterial plasmid). In some embodiments, the double-stranded DNA construct is excised from a plasmid (e.g., a bacterial plasmid) and then circularized, e.g., by *in vitro* circularization.

Tandem Constructs

In some embodiments, a genetic element construct comprises a first copy of a genetic element sequence (e.g., the nucleic acid sequence of a genetic element, e.g., as described herein) and at least a portion of a second copy of a genetic element sequence (e.g., the nucleic acid sequence of the same genetic element, or the nucleic acid sequence of a different genetic element), arranged in tandem. Genetic element constructs having such a structure are generally referred to herein as tandem constructs. Such tandem constructs are used for producing an anellovector genetic element. The first copy of the genetic element sequence and the second copy of the genetic element sequence may, in some instances, be immediately adjacent to each other on the genetic acid construct. In other instances, the first copy of the genetic element sequence and the second copy of the genetic element sequence may be separated, e.g., by a spacer sequence. In some embodiments, the second copy of the genetic element sequence, or the portion thereof, comprises an upstream replication-facilitating sequence (uRFS), e.g., as described herein. In some embodiments, the second copy of the genetic element sequence, or the portion thereof, comprises a downstream replication-facilitating sequence (dRFS), e.g., as described herein. In some embodiments, the uRFS and/or dRFS comprises an origin of replication (e.g., a mammalian origin of replication, an insect origin of replication, or a viral origin of replication, e.g., a non-Anellovirus origin of replication, e.g., as described herein) or portion thereof. In some embodiments, the uRFS and/or dRFS does not comprise an origin of replication. In some embodiments, the uRFS and/or dRFS comprises a hairpin loop (e.g., in the 5' UTR). In some embodiments, a tandem construct produces higher levels of a genetic element than an otherwise similar construct lacking the second copy of the genetic element or portion thereof. Without being bound by theory, a tandem construct described herein may, in some embodiments, replicate by rolling circle replication. In some embodiments, a tandem construct is a plasmid. In some embodiments, a tandem construct is circular. In some embodiments, a tandem construct is linear. In some embodiments, a tandem construct is single-stranded. In some embodiments, a tandem construct is double-stranded. In some embodiments, a tandem construct is DNA.

A tandem construct may, in some instances, include a first copy of the sequence of the genetic element and a second copy of the sequence of the genetic element, or a portion thereof. It is understood that the second copy can be an identical copy of the first copy or a portion thereof, or can comprise one or more sequence differences, e.g., substitutions, additions, or deletions. In some instances, the second copy

of the genetic element sequence or portion thereof is positioned 5' relative to the first copy of the genetic element sequence. In some instances, the second copy of the genetic element sequence or portion thereof is positioned 3' relative to the first copy of the genetic element sequence. In some instances, the second copy of the genetic element sequence or portion thereof and the first copy of the genetic element sequence are adjacent to each other in the tandem construct. In some instances, the second copy of the genetic element sequence or portion thereof and the first copy of the genetic element sequence are separated, e.g., by a spacer sequence.

In some embodiments, the tandem constructs described herein can be used to produce the genetic element of a vector (e.g., anellovector), vehicle, or particle (e.g., viral particle) comprising a capsid (e.g., a capsid comprising an Anellovirus ORF, e.g., an ORF1 molecule, e.g., as described herein) encapsulating a genetic element comprising a protein binding sequence that binds to the capsid and a heterologous (e.g., relative to the Anellovirus from which the ORF1 molecule was derived) sequence encoding a therapeutic effector. In embodiments, the vector is capable of delivering the genetic element into a mammalian, e.g., human, cell. In some embodiments, the genetic element has less than about 50% (e.g., less than 50%, 40%, 30%, 25%, 20%, 15%, 10%, 9%, 8%, 7%, 6%, 5.5%, 5%, 4.5%, 4%, 3.5%, 3%, 2.5%, 2%, 1.5%, or less) identity to a wild type Anellovirus genome sequence. In some embodiments, the genetic element has no more than 1.5%, 2%, 2.5%, 3%, 3.5%, 4%, 4.5%, 5%, 5.5%, 6%, 7%, 8%, 9%, 10%, 15%, 20%, 25%, 30%, 40%, 50%, 60%, 70%, 75%, or 80% identity to a wild type Anellovirus genome sequence. In some embodiments, the genetic element has greater than about 2000, 3000, 4000, 4500, or 5000 contiguous nucleotides of non-Anellovirus genome sequence. In some embodiments, the genetic element has greater than about 2000 to 5000, 2500 to 4500, 3000 to 4500, 2500 to 4500, 3500, or 4000, 4500 (e.g., between about 3000 to 4500) nucleotides nucleotides of non-Anellovirus genome sequence.

In some embodiments of the systems and methods herein, a vector (e.g., an anellovector) is made by introducing into a cell a first nucleic acid molecule that is a genetic element or genetic element construct, e.g., a tandem construct, and a second nucleic acid molecule encoding one or more additional proteins (e.g., a Rep molecule and/or a capsid protein), e.g., as described herein. In some embodiments, the first nucleic acid molecule and the second nucleic acid molecule are attached to each other (e.g., in a genetic element construct described herein, e.g., in cis). In some embodiments, the first nucleic acid molecule and the second nucleic acid molecule are separate (e.g, in trans). In some embodiments, the first nucleic acid molecule is a plasmid, cosmid, bacmid, minicircle, or artificial chromosome. In some embodiments, the second nucleic acid molecule is a plasmid, cosmid, bacmid, minicircle, or artificial chromosome. In some embodiments, the second nucleic acid molecule is integrated into the genome of the host cell.

In some embodiments, the method further includes introducing the first nucleic acid molecule and/or the second nucleic acid molecule into the host cell. In some embodiments, the second nucleic acid molecule is introduced into the host cell prior to, concurrently with, or after the first nucleic acid molecule. In other embodiments, the second nucleic acid molecule is integrated into the genome of the host cell. In some embodiments, the second nucleic acid molecule is or comprises or is part of a helper construct, helper virus or other helper vector, e.g., as described herein.

Cis/Trans Constructs

In some embodiments, a genetic element construct as described herein comprises one or more sequences encoding one or more Anellovirus ORFs, e.g., proteinaceous exterior components (e.g., polypeptides encoded by an Anellovirus ORF1 nucleic acid, e.g., as described herein). For example, the genetic element construct may comprise a nucleic acid sequence encoding an Anellovirus ORF1 molecule. Such genetic element constructs can be suitable for introducing the genetic element and the Anellovirus ORF(s) into a host cell in *cis*. In other embodiments, a genetic element construct as described herein does not comprise sequences encoding one or more Anellovirus ORFs, e.g., proteinaceous exterior components (e.g., polypeptides encoded by an Anellovirus ORF1 nucleic acid, e.g., as described herein). For example, the genetic element construct may not comprise a nucleic acid sequence encoding an Anellovirus ORF1 molecule. Such genetic element constructs can be suitable for introducing the genetic element into a host cell, with the one or more Anellovirus ORFs to be provided in *trans* (e.g., via introduction of a second nucleic acid construct encoding one or more of the Anellovirus ORFs, or via an Anellovirus ORF cassette integrated into the genome of the host cell). In some embodiments, an ORF1 molecule is provided in *trans*, e.g., as described herein. In some embodiments, an ORF2 molecule is provided in *trans*, e.g., as described herein. In some embodiments, an ORF1 molecule and an ORF1 molecule are both provided in *trans*, e.g., as described herein.

In some embodiments, the genetic element construct comprises a sequence encoding an Anellovirus ORF1 molecule, or a splice variant or functional fragment thereof (e.g., a structural jelly-roll region, e.g., as described herein). In embodiments, the portion of the genetic element that does not comprise the sequence of the genetic element comprises the sequence encoding the Anellovirus ORF1 molecule, or splice variant or functional fragment thereof (e.g., in a cassette comprising a promoter and the sequence encoding the Anellovirus ORF1 molecule, or splice variant or functional fragment thereof). In further embodiments, the portion of the construct comprising the sequence of the genetic element comprises a sequence encoding an Anellovirus ORF1 molecule, or a splice variant or functional fragment thereof (e.g., a structural jelly-roll region, e.g., as described herein). In embodiments, enclosure of such a genetic element in a proteinaceous exterior (e.g., as described herein) produces a replication-component

anellovector (e.g., an anellovector that upon infecting a cell, enables the cell to produce additional copies of the anellovector without introducing further nucleic acid constructs, e.g., encoding one or more Anellovirus ORFs as described herein, into the cell).

In other embodiments, the genetic element does not comprise a sequence encoding an Anellovirus ORF1 molecule, or a splice variant or functional fragment thereof (e.g., a structural jelly-roll region, e.g., as described herein). In embodiments, enclosure of such a genetic element in a proteinaceous exterior (e.g., as described herein) produces a replication-incompetent anellovector (e.g., an anellovector that, upon infecting a cell, does not enable the infected cell to produce additional anellovectors, e.g., in the absence of one or more additional constructs, e.g., encoding one or more Anellovirus ORFs as described herein).

Expression Cassettes

In some embodiments, a genetic element construct comprises one or more cassettes for expression of a polypeptide or noncoding RNA (e.g., a miRNA or an siRNA). In some embodiments, the genetic element construct comprises a cassette for expression of an effector (e.g., an exogenous or endogenous effector), e.g., a polypeptide or noncoding RNA, as described herein. In some embodiments, the genetic element construct comprises a cassette for expression of an Anellovirus protein (e.g., an Anellovirus ORF1, ORF2, ORF2/2, ORF2/3, ORF1/1, or ORF1/2, or a functional fragment thereof). The expression cassettes may, in some embodiments, be located within the genetic element sequence. In embodiments, an expression cassette for an effector is located within the genetic element sequence. In embodiments, an expression cassette for an Anellovirus protein is located within the genetic element sequence. In other embodiments, the expression cassettes are located at a position within the genetic element construct outside of the sequence of the genetic element (e.g., in the backbone). In embodiments, an expression cassette for an Anellovirus protein is located at a position within the genetic element construct outside of the sequence of the genetic element (e.g., in the backbone).

A polypeptide expression cassette generally comprises a promoter and a coding sequence encoding a polypeptide, e.g., an effector (e.g., an exogenous or endogenous effector as described herein) or an Anellovirus protein (e.g., a sequence encoding an Anellovirus ORF1, ORF2, ORF2/2, ORF2/3, ORF1/1, or ORF1/2, or a functional fragment thereof). Exemplary promoters that can be included in an polypeptide expression cassette (e.g., to drive expression of the polypeptide) include, without limitation, constitutive promoters (e.g., CMV, RSV, PGK, EF1a, or SV40), cell or tissue-specific promoters (e.g., skeletal α-actin promoter, myosin light chain 2A promoter, dystrophin promoter, muscle creatine kinase promoter, liver albumin promoter, hepatitis B virus core promoter, osteocalcin promoter, bone sialoprotein promoter, CD2 promoter, immunoglobulin heavy chain promoter, T cell receptor a chain

promoter, neuron-specific enolase (NSE) promoter, or neurofilament light-chain promoter), and inducible promoters (e.g., zinc-inducible sheep metallothionine (MT) promoter; the dexamethasone (Dex)-inducible mouse mammary tumor virus (MMTV) promoter; the T7 polymerase promoter system, tetracycline-repressible system, tetracycline-inducible system, RU486-inducible system, rapamycin-inducible system), e.g., as described herein. In some embodiments, the expression cassette further comprises an enhancer, e.g., as described herein.

Design and Production of a Genetic Element Construct

Various methods are available for synthesizing a genetic element construct. For instance, the genetic element construct sequence may be divided into smaller overlapping pieces (e.g., in the range of about 100 bp to about 10 kb segments or individual ORFs) that are easier to synthesize. These DNA segments are synthesized from a set of overlapping single-stranded oligonucleotides. The resulting overlapping synthons are then assembled into larger pieces of DNA, e.g., the genetic element construct. The segments or ORFs may be assembled into the genetic element construct, e.g., by in vitro recombination or unique restriction sites at 5' and 3' ends to enable ligation.

The genetic element construct can be synthesized with a design algorithm that parses the construct sequence into oligo-length fragments, creating suitable design conditions for synthesis that take into account the complexity of the sequence space. Oligos are then chemically synthesized on semiconductor-based, high-density chips, where over 200,000 individual oligos are synthesized per chip. The oligos are assembled with an assembly techniques, such as BioFab®, to build longer DNA segments from the smaller oligos. This is done in a parallel fashion, so hundreds to thousands of synthetic DNA segments are built at one time.

Each genetic element construct or segment of the genetic element construct may be sequence verified. In some embodiments, high-throughput sequencing of RNA or DNA can take place using AnyDot.chips (Genovoxx, Germany), which allows for the monitoring of biological processes (e.g., miRNA expression or allele variability (SNP detection). Other high-throughput sequencing systems include those disclosed in Venter, J., et al. Science 16 Feb. 2001; Adams, M. et al, Science 24 Mar. 2000; and M. J, Levene, et al. Science 299:682-686, January 2003; as well as US Publication Application No. 20030044781 and 2006/0078937. Overall such systems involve sequencing a target nucleic acid molecule having a plurality of bases by the temporal addition of bases via a polymerization reaction that is measured on a molecule of nucleic acid, i.e., the activity of a nucleic acid polymerizing enzyme on the template nucleic acid molecule to be sequenced is followed in real time. In some embodiments, shotgun sequencing is performed.

A genetic element construct can be designed such that factors for replicating or packaging may be supplied in *cis* or in *trans*, relative to the genetic element. For example, when supplied in *cis*, the genetic element may comprise one or more genes encoding an *Anellovirus* ORF1, ORF1/1, ORF1/2, ORF2, ORF2/2, ORF2/3, or ORF2t/3, e.g., as described herein. In some embodiments, replication and/or packaging signals can be incorporated into a genetic element, for example, to induce amplification and/or encapsulation. In some embodiments, an effector is inserted into a specific site in the genome. In some embodiments, one or more viral ORFs are replaced with an effector.

In another example, when replication or packaging factors are supplied in *trans*, the genetic element may lack genes encoding one or more of an *Anellovirus* ORF1, ORF1/1, ORF1/2, ORF2, ORF2/2, ORF2/3, or ORF2t/3, e.g., as described herein; this protein or proteins may be supplied, e.g., by another nucleic acid, e.g., a helper nucleic acid. In some embodiments, minimal *cis* signals (e.g., 5' UTR and/or GC-rich region) are present in the genetic element. In some embodiments, the genetic element does not encode replication or packaging factors (e.g., replicase and/or capsid proteins). Such factors may, in some embodiments, be supplied by one or more helper nucleic acids (e.g., a helper viral nucleic acid, a helper plasmid, or a helper nucleic acid integrated into the host cell genome). In some embodiments, the helper nucleic acids express proteins and/or RNAs sufficient to induce amplification and/or packaging, but may lack their own packaging signals. In some embodiments, the genetic element and the helper nucleic acid are introduced into the host cell (e.g., concurrently or separately), resulting in amplification and/or packaging of the genetic element but not of the helper nucleic acid.

In some embodiments, the genetic element construct may be designed using computer-aided design tools.

General methods of making constructs are described in, for example, Khudyakov & Fields, *Artificial DNA: Methods and Applications*, CRC Press (2002); in Zhao, *Synthetic Biology: Tools and Applications*, (First Edition), Academic Press (2013); and Egli & Herdewijn, *Chemistry and Biology of Artificial Nucleic Acids*, (First Edition), Wiley-VCH (2012).

Effectors

The compositions and methods described herein can be used to produce a genetic element of an anellovector comprising a sequence encoding an effector (e.g., an exogenous effector or an endogenous effector), e.g., as described herein. The compositions and methods described herein can also be used to produce an anelloVLP comprising an effector (e.g., an exogenous effector or an endogenous effector), e.g., as described herein. The effector may be, in some instances, an endogenous effector or an exogenous effector. In some embodiments, the effector is a therapeutic effector. In some embodiments, the effector comprises a polypeptide (e.g., a therapeutic polypeptide or peptide, e.g., as described herein).

In some embodiments, the effector comprises a non-coding RNA (e.g., an miRNA, siRNA, shRNA, mRNA, lncRNA, RNA, DNA, antisense RNA, or gRNA). In some embodiments, the effector comprises a regulatory nucleic acid, e.g., as described herein.

In some embodiments, the effector-encoding sequence may be inserted into the genetic element e.g., at a non-coding region, e.g., a noncoding region disposed 3' of the open reading frames and 5' of the GC-rich region of the genetic element, in the 5' noncoding region upstream of the TATA box, in the 5' UTR, in the 3' noncoding region downstream of the poly-A signal, or upstream of the GC-rich region. In some embodiments, the effector-encoding sequence may be inserted into the genetic element, e.g., in a coding sequence (e.g., in a sequence encoding an Anellovirus ORF1, ORF1/1, ORF1/2, ORF2, ORF2/2, ORF2/3, and/or ORF2t/3, e.g., as described herein). In some embodiments, the effector-encoding sequence replaces all or a part of the open reading frame. In some embodiments, the genetic element comprises a regulatory sequence (e.g., a promoter or enhancer, e.g., as described herein) operably linked to the effector-encoding sequence.

Host Cells

The anellovectors described herein can be produced, for example, in a host cell. Generally, a host cell is provided that comprises an anellovector genetic element and the components of an anellovector proteinaceous exterior (e.g., a polypeptide encoded by an Anellovirus ORF1 nucleic acid or an Anellovirus ORF1 molecule). The host cell is then incubated under conditions suitable for enclosure of the genetic element within the proteinaceous exterior (e.g., culture conditions as described herein). In some embodiments, the host cell is further incubated under conditions suitable for release of the anellovector from the host cell, e.g., into the surrounding supernatant. In some embodiments, the host cell is lysed for harvest of anellovectors from the cell lysate. In some embodiments, an anellovector may be introduced to a host cell line grown to a high cell density. In some embodiments, a host cell is an Expi-293 cell.

Introduction of genetic elements into host cells

The genetic element, or a nucleic acid construct comprising the sequence of a genetic element, may be introduced into a host cell. In some embodiments, the genetic element itself is introduced into the host cell. In some embodiments, a genetic element construct comprising the sequence of the genetic element (e.g., as described herein) is introduced into the host cell. A genetic element or genetic element construct can be introduced into a host cell, for example, using methods known in the art. For example, a genetic element or genetic element construct can be introduced into a host cell by transfection (e.g., stable transfection or transient transfection). In embodiments, the genetic element or genetic element construct

is introduced into the host cell by lipofectamine transfection. In embodiments, the genetic element or genetic element construct is introduced into the host cell by calcium phosphate transfection. In some embodiments, the genetic element or genetic element construct is introduced into the host cell by electroporation. In some embodiments, the genetic element or genetic element construct is introduced into the host cell using a gene gun. In some embodiments, the genetic element or genetic element construct is introduced into the host cell by nucleofection. In some embodiments, the genetic element or genetic element construct is introduced into the host cell by PEI transfection. In some embodiments, the genetic element is introduced into the host cell by contacting the host cell with an anellovector comprising the genetic element

In embodiments, the genetic element construct is capable of replication once introduced into the host cell. In embodiments, the genetic element can be produced from the genetic element construct once introduced into the host cell. In some embodiments, the genetic element is produced in the host cell by a polymerase, e.g., using the genetic element construct as a template.

In some embodiments, the genetic elements or vectors comprising the genetic elements are introduced (e.g., transfected) into cell lines that express a viral polymerase protein in order to achieve expression of the anellovector. To this end, cell lines that express an anellovector polymerase protein may be utilized as appropriate host cells. Host cells may be similarly engineered to provide other viral functions or additional functions.

To prepare the anellovector disclosed herein, a genetic element construct may be used to transfect cells that provide anellovector proteins and functions required for replication and production. Alternatively, cells may be transfected with a second construct (e.g., a virus) providing anellovector proteins and functions before, during, or after transfection by the genetic element or vector comprising the genetic element disclosed herein. In some embodiments, the second construct may be useful to complement production of an incomplete viral particle. The second construct (e.g., virus) may have a conditional growth defect, such as host range restriction or temperature sensitivity, e.g., which allows the subsequent selection of transfectant viruses. In some embodiments, the second construct may provide one or more replication proteins utilized by the host cells to achieve expression of the anellovector. In some embodiments, the host cells may be transfected with vectors encoding viral proteins such as the one or more replication proteins. In some embodiments, the second construct comprises an antiviral sensitivity.

The genetic element or vector comprising the genetic element disclosed herein can, in some instances, be replicated and produced into anellovectors using techniques known in the art. For example, various viral culture methods are described, e.g., in U.S. Pat. No. 4,650,764; U.S. Pat. No. 5,166,057; U.S. Pat. No. 5,854,037; European Patent Publication EP 0702085A1; U.S. patent application Ser. No. 09/152,845; International Patent Publications PCT WO97/12032; WO96/34625; European Patent

Publication EP-A780475; WO 99/02657; WO 98/53078; WO 98/02530; WO 99/15672; WO 98/13501; WO 97/06270; and EPO 780 47SA1, each of which is incorporated by reference herein in its entirety.

Methods for providing protein(s) in cis or trans

In some embodiments (e.g., *cis* embodiments described herein), the genetic element construct further comprises one or more expression cassettes comprising a coding sequence for an Anellovirus ORF (e.g., an Anellovirus ORF1, ORF2, ORF2/2, ORF2/3, ORF1/1, or ORF1/2, or a functional fragment thereof). In embodiments, the genetic element construct comprises an expression cassette comprising a coding sequence for an Anellovirus ORF1, or a splice variant or functional fragment thereof. Such genetic element constructs, which comprise expression cassettes for the effector as well as the one or more Anellovirus ORFs, may be introduced into host cells. Host cells comprising such genetic element constructs may, in some instances, be capable of producing the genetic elements and components for proteinaceous exteriors, and for enclosure of the genetic elements within proteinaceous exteriors, without requiring additional nucleic acid constructs or integration of expression cassettes into the host cell genome. In other words, such genetic element constructs may be used for *cis* anellovector production methods in host cells, e.g., as described herein.

In some embodiments (e.g., *trans* embodiments described herein), the genetic element does not comprise an expression cassette comprising a coding sequence for one or more Anellovirus ORFs (e.g., an Anellovirus ORF1, ORF2, ORF2/2, ORF2/3, ORF1/1, or ORF1/2, or a functional fragment thereof). In embodiments, the genetic element construct does not comprise an expression cassette comprising a coding sequence for an Anellovirus ORF1, or a splice variant or functional fragment thereof. Such genetic element constructs, which comprise expression cassettes for the effector but lack expression cassettes for one or more Anellovirus ORFs (e.g., Anellovirus ORF1 or a splice variant or functional fragment thereof), may be introduced into host cells. Host cells comprising such genetic element constructs may, in some instances, require additional nucleic acid constructs or integration of expression cassettes into the host cell genome for production of one or more components of the anellovector (e.g., the proteinaceous exterior proteins). In some embodiments, host cells comprising such genetic element constructs are incapable of enclosure of the genetic elements within proteinaceous exteriors in the absence of an additional nucleic construct encoding an Anellovirus ORF1 molecule. In other words, such genetic element constructs may be used for *trans* anellovector production methods in host cells, e.g., as described herein.

In some embodiments (e.g., *cis* embodiments described herein), the genetic element construct further comprises one or more expression cassettes comprising a coding sequence for one or more non-Anellovirus ORF (e.g., a non-Anellovirus Rep molecule, e.g., an AAV Rep molecule, e.g., an AAV Rep

protein, e.g., an AAV Rep2 protein). Such genetic element constructs, which comprise expression cassettes for the effector as well as the one or more non-Anellovirus ORFs, may be introduced into host cells. Host cells comprising such genetic element constructs may, in some instances, be capable of producing the genetic elements and components for proteinaceous exteriors, and for enclosure of the genetic elements within proteinaceous exteriors, without requiring additional nucleic acid constructs or integration of expression cassettes into the host cell genome. In other words, such genetic element constructs may be used for *cis* anellovector production methods in host cells, e.g., as described herein.

In some embodiments (e.g., *trans* embodiments described herein), the genetic element does not comprise an expression cassette comprising a coding sequence for one or more non-Anellovirus ORFs (e.g., a non-Anellovirus Rep molecule, e.g., an AAV Rep molecule, e.g., an AAV Rep protein, e.g., an AAV Rep2 protein). Such genetic element constructs, which comprise expression cassettes for the effector but lack expression cassettes for one or more non-Anellovirus ORFs (e.g., a non-Anellovirus Rep molecule, e.g., an AAV Rep molecule, e.g., an AAV Rep protein, e.g., an AAV Rep2 protein), may be introduced into host cells. Host cells comprising such genetic element constructs may, in some instances, require additional nucleic acid constructs or integration of expression cassettes into the host cell genome for production of one or more components of the anellovector (e.g., for replication of the genetic element). In some embodiments, host cells comprising such genetic element constructs are incapable of replicating the genetic elements in the absence of an additional nucleic construct, e.g., encoding a non-Anellovirus Rep molecule, e.g., an AAV Rep molecule, e.g., an AAV Rep protein, e.g., an AAV Rep2 protein. In other words, such genetic element constructs may be used for *trans* anellovector production methods in host cells, e.g., as described herein.

Exemplary cell types

Exemplary host cells suitable for production of anellovectors include, without limitation, mammalian cells, e.g., human cells and insect cells. In some embodiments, the host cell is a human cell or cell line. In some embodiments, the cell is an immune cell or cell line, e.g., a T cell or cell line, a cancer cell line, a hepatic cell or cell line, a neuron, a glial cell, a skin cell, an epithelial cell, a mesenchymal cell, a blood cell, an endothelial cell, a gastrointestinal cell, a progenitor cell, a precursor cell, a stem cell, a lung cell, a cardiac cell, or a muscle cell. In some embodiments, the host cell is an animal cell (e.g., a mouse cell, rat cell, rabbit cell, or hamster cell, or insect cell).

In some embodiments, the host cell is a lymphoid cell. In some embodiments, the host cell is a T cell or an immortalized T cell. In embodiments, the host cell is a Jurkat cell. In embodiments, the host cell is a MOLT cell (e.g., a MOLT-4 or a MOLT-3 cell). In embodiments, the host cell is a MOLT-4 cell. In embodiments, the host cell is a MOLT-3 cell. In some embodiments, the host cell is an acute lymphoblastic leukemia (ALL) cell, e.g., a MOLT cell, e.g., a MOLT-4 or MOLT-3 cell. In some

embodiments, the host cell is a B cell or an immortalized B cell. In some embodiments, the host cell comprises a genetic element construct (e.g., as described herein).

In some embodiments, the host cell is a MOLT cell (e.g., a MOLT-4 or a MOLT-3 cell).

In some embodiments, the host cell is an acute lymphoblastic leukemia (ALL) cell, e.g., a MOLT cell, e.g., a MOLT-4 or MOLT-3 cell.

In some embodiments, the host cell is an Expi-293 cell. In some embodiments, the host cell is an Expi-293F cell.

In an aspect, the present disclosure provides a method of manufacturing an anellovector comprising a genetic element enclosed in a proteinaceous exterior, the method comprising providing a MOLT-4 cell comprising an anellovector genetic element, and incubating the MOLT-4 cell under conditions that allow the anellovector genetic element to become enclosed in a proteinaceous exterior in the MOLT-4 cell. In some embodiments, the MOLT-4 cell further comprises one or more Anellovirus proteins (e.g., an Anellovirus ORF1 molecule) that form part or all of the proteinaceous exterior. In some embodiments, the anellovector genetic element is produced in the MOLT-4 cell, e.g., from a genetic element construct (e.g., as described herein). In some embodiments, the method further comprises introducing the anellovector genetic element construct into the MOLT-4 cell.

In an aspect, the present disclosure provides a method of manufacturing an anellovector comprising a genetic element enclosed in a proteinaceous exterior, the method comprising providing a MOLT-3 cell comprising an anellovector genetic element, and incubating the MOLT-3 cell under conditions that allow the anellovector genetic element to become enclosed in a proteinaceous exterior in the MOLT-3 cell. In some embodiments, the MOLT-3 cell further comprises one or more Anellovirus proteins (e.g., an Anellovirus ORF1 molecule) that form part or all of the proteinaceous exterior. In some embodiments, the anellovector genetic element is produced in the MOLT-3 cell, e.g., from a genetic element construct (e.g., as described herein). In some embodiments, the method further comprises introducing the anellovector genetic element construct into the MOLT-3 cell.

In some embodiments, the host cell is a human cell. In embodiments, the host cell is a human embryonic kidney (HEK) cell (e.g., HEK293T cell, HEK293F cell, Expi293 cell), A549 cell, Jurkat cell, Raji cell, Chang cell, HeLa cell Phoenix cell, MRC-5 cell, NCI-H292 cell, or Wi38 cell. In some embodiments, the host cell is a non-human primate cell (e.g., a Vero cell, CV-1 cell, or LLCMK2 cell). In some embodiments, the host cell is a murine cell (e.g., a McCoy cell). In some embodiments, the host cell is a hamster cell (e.g., a CHO cell or BHK 21 cell). In some embodiments, the host cell is a MARC-

145, MDBK, RK-13, or EEL cell. In some embodiments, the host cell is an epithelial cell (e.g., a cell line of epithelial lineage).

In some embodiments, the anellovector is cultivated in continuous animal cell line (e.g., immortalized cell lines that can be serially propagated). According to one embodiment of the invention, the cell lines may include porcine cell lines. The cell lines envisaged in the context of the present invention include immortalised porcine cell lines such as, but not limited to the porcine kidney epithelial cell lines PK-15 and SK, the monomyeloid cell line 3D4/31 and the testicular cell line ST.

Culture Conditions

Host cells comprising a genetic element and components of a proteinaceous exterior can be incubated under conditions suitable for enclosure of the genetic element within the proteinaceous exterior, thereby producing an anellovector. Suitable culture conditions include those described, e.g., in any of Examples 23, 24, 26, or 27. In some embodiments, the host cells are incubated in liquid media (e.g., Grace's Supplemented (TNM-FH), IPL-41, TC-100, Schneider's Drosophila, SF-900 II SFM, or and EXPRESS-FIVE™ SFM). In some embodiments, the host cells are incubated in adherent culture. In some embodiments, the host cells are incubated in suspension culture. In some embodiments, the host cells are incubated in a tube, bottle, microcarrier, or flask. In some embodiments, the host cells are incubated in a dish or well (e.g., a well on a plate). In some embodiments, the host cells are incubated under conditions suitable for proliferation of the host cells. In some embodiments, the host cells are incubated under conditions suitable for the host cells to release anellovectors produced therein into the surrounding supernatant.

The production of anellovector-containing cell cultures according to the present invention can be carried out in different scales (e.g., in flasks, roller bottles or bioreactors). The media used for the cultivation of the cells to be infected generally comprise the standard nutrients required for cell viability, but may also comprise additional nutrients dependent on the cell type. Optionally, the medium can be protein-free and/or serum-free. Depending on the cell type the cells can be cultured in suspension or on a substrate. In some embodiments, different media is used for growth of the host cells and for production of anellovectors.

Harvest

Anellovectors produced by host cells can be harvested, e.g., according to methods known in the art. For example, anellovectors released into the surrounding supernatant by host cells in culture can be harvested from the supernatant (e.g., as described in Example 23). In some embodiments, the supernatant is separated from the host cells to obtain the anellovectors. In some embodiments, the host cells are lysed

before or during harvest. In some embodiments, the host cells are lysed in a detergent (e.g., Triton, e.g., 0.01%-0.1% Triton). In some embodiments, the anellovectors are harvested from the host cell lysates (e.g., as described in Example 10 of PCT Publication No. WO 2020/123816, incorporated by reference herein in its entirety). In some embodiments, the anellovectors are harvested from both the host cell lysates and the supernatant. In some embodiments, the purification and isolation of anellovectors is performed according to known methods in virus production, for example, as described in Rinaldi, et al., DNA Vaccines: Methods and Protocols (Methods in Molecular Biology), 3rd ed. 2014, Humana Press (incorporated herein by reference in its entirety). In some embodiments, the anellovector may be harvested and/or purified by separation of solutes based on biophysical properties, e.g., ion exchange chromatography or tangential flow filtration, prior to formulation with a pharmaceutical excipient.

In vitro assembly methods for anellovectors

An anellovector may be produced, e.g., by *in vitro* assembly, e.g., in the absence of a host cell, in a cell-free suspension, or in a supernatant. In some embodiments, the genetic element is contacted to an ORF1 molecule *in vitro*, e.g., under conditions that allow for assembly.

In an aspect, the present disclosure provides a particle (e.g., an anellovector as described herein) produced via in vitro assembly (e.g., as described herein). The particle may, in some instances, comprise a proteinaceous exterior comprising an ORF1 molecule and a genetic element encoding an exogenous effector, which is enclosed within the proteinaceous exterior. In some embodiments, a particle produced by in vitro assembly does not include a substantial (e.g., detectable) amount of one or more constituents (e.g., small molecules, peptides, polypeptides, nucleic acids, polynucleotides, lipids, sugars, and/or organelles) from a host cell (e.g., a host cell used to produce the ORF1 molecules and/or the genetic element). In some embodiments, the particle may have one or more (e.g., 1, 2, 3, 4, 5, 6, 7, 8, or all 9) of the following characteristics:

(i) the genetic element (e.g., a DNA genetic element) does not comprise an Anellovirus 5' UTR and/or an Anellovirus origin of replication;

(ii) the sequence encoding the exogenous effector takes up at least 90%, 95%, 96%, 97%, 98%, 99% or 100% of the genetic element (e.g., a DNA genetic element);

(iii) the heterologous nucleic acid sequence takes up at least 90%, 95%, 96%, 97%, 98%, 99% or 100% of the genetic element (e.g., a DNA genetic element);

(iv) the particle does not comprise a detectable amount of (e.g., any) polypeptides from a host cell, or comprises less than 5, 10, 15, 20, 25, 30, 40, or 50 copies of a polypeptide from a host cell;

(v) the particle does not comprise a detectable amount of (e.g., any) nucleic acid molecules from a host cell other than the genetic element (or copies thereof), or comprises less than 2, 3, 4, or 5 copies of a nucleic acid molecule from a host cell;

(vi) the particle comprises a denaturant in a concentration of less than about 0.01M, 0.1M, 0.2M, 0.3M, 0.4M, 0.5M, 0.6M, 0.7M, 0.8M, 0.9M, 1M, 1.1M, 1.2M, 1.3M, 1.5M, 1.5M, 1.6M, 1.7M, 1.8M, 1.9M, or 2M;

(vii) does not substantially replicate when introduced into a cell (e.g., a human cell); and/or

(viii) has a symmetrical morphology and/or a diameter of at least 30, 31, 32, 33, 34, or 35 nm.

In another aspect, the disclosure provides a population of the particles (e.g., the anellovectors).

In some embodiments, Anellovirus proteins to be used for *in vitro* assembly of a particle (e.g., an anellovector) as described herein are produced in a cell. In some embodiments, baculovirus constructs are used to produce Anellovirus proteins (e.g., one or more of an Anellovirus ORF1, ORF2, and/or ORF3 molecule, e.g., as described herein), for example, in insect cells (e.g., Sf9 cells). These proteins may then be used, e.g., for *in vitro* assembly to encapsidate a genetic element, e.g., a genetic element comprising RNA. In some embodiments, a polynucleotide encoding one or more Anellovirus proteins is fused to a promoter for expression in a host cell, *e.g.*, an insect cell or an animal cell. In some embodiments, the polynucleotide is cloned into a baculovirus expression system. In some embodiments, a host cell, *e.g.*, an insect cell, is infected with the baculovirus expression system and incubated for a period of time under conditions suitable for expression of the one or more Anellovirus proteins. In some embodiments, an infected cell is incubated for about 1, 2, 3, 4, 5, 10, 15, or 20 days. In some embodiments, an infected cell is lysed to recover the one or more Anellovirus proteins.

In some embodiments, an Anellovirus protein (e.g., an Anellovirus ORF1 molecule) is produced as described in Example 7. In some embodiments, an Anellovirus protein (e.g., an Anellovirus ORF1 molecule) is produced in insect cells as described in Example 8 or 10. In some embodiments, a plurality of Anellovirus ORF1 molecules has a propensity to self-assemble into a proteinaceous exterior, for example, to form a virus-like particle (VLP). In certain embodiments, the VLPs do not encapsulate a genetic element as described herein. In certain embodiments, the VLP comprises at least 40, 45, 50, 55, 60, 65, or 70 ORF1 molecules in its proteinaceous exterior.

In certain embodiments, a VLP comprising an Anellovirus ORF1 molecule can be denatured as described herein (e.g., using a chaotropic agent, such as urea). In embodiments, a VLP is denatured using one or more of: buffers of different pH, conditions of defined conductivity (salt content), a detergent (such as SDS (e.g., 0.1% SDS), Tween, Triton), a chaotropic agent (such as urea, e.g., as described herein), a high salt solution (e.g., a solution comprising NaCl, e.g., at a concentration of at least about 1M, e.g., at least about 0.5, 0.6, 0.7, 0.8, 0.9, 1.0, 1.1, 1.2, 1.3, 1.4, 1.5, 2, 3, 4, or 5M), or conditions involving

defined temperature and time (reannealing temperatures), for example, as described in Example 12. In embodiments, a VLP is denatured using urea as described in Example 11. In embodiments, a VLP is denatured in urea at a concentration of about 1-10 M (e.g., about 1-2 M, 2-3 M, 3-4 M, 4-5 M, 5-6 M, 6-7 M, 7-8 M, 8-9 M, 9-10M, or 1-6 M). In embodiments, a VLP is denatured in urea at a concentration of about 1-10 M (e.g., about 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10M). In an embodiment, a VLP is denatured in urea at a concentration of 2 M. In embodiments, a VLP is denatured under high salt conditions. In embodiments, denaturation of the VLP results in the ORF1 molecules forming capsomers (e.g., capsomeric decamers comprising about 10 copies of the ORF1 molecule).

In some embodiments, removing the capsomers from the presence of the chaotropic agent (e.g., by purifying the capsomers or removing the chaotropic agent, e.g., by dilution or dialysis) allows the ORF1 molecules to reform VLPs (e.g., VLPs comprising at least 40, 45, 50, 55, 60, 65, or 70 ORF1 molecules). In embodiments, VLPs are reformed by dialyzing out a chaotropic agent (e.g., urea) as described in Example 11. In some embodiments, VLPs are reformed in the presence of a cargo of interest, such as a genetic element (e.g., as described herein), under conditions suitable for enclosure of the cargo in the proteinaceous exterior of the VLP (e.g., as described in Example 12).In some embodiments, an isolated Anellovirus protein (e.g., an ORF1 molecule) is purified (e.g., from a cell). In some embodiments, an Anellovirus protein is purified using purification techniques including but not limited to chelating purification, heparin purification, gradient sedimentation purification, and/or SEC purification. In embodiments, an Anellovirus protein is purified as described in Example 7. In some embodiments, an Anellovirus protein (e.g., an Anellovirus ORF1 molecule) is purified from insect cells as described in Example 8 or 10. In some embodiments, a purified Anellovirus protein is mixed with a genetic element to encapsidate the genetic element, e.g., a genetic element comprising RNA. In some embodiments, a genetic element is encapisdated using an ORF1 protein, ORF2 protein, or modified version thereof. In some embodiments two nucleic acids are encapsidated. For instance, the first nucleic acid may be an mRNA e.g., chemically modified mRNA, and the second nucleic acid may be DNA.

In some embodiments, DNA encoding Anellovirus ORF1 (*e.g.*, wildtype ORF1 protein, ORF1 proteins harboring mutations, *e.g.*, to improve assembly efficiency, yield or stability, chimeric ORF1 protein, or fragments thereof) are expressed in insect cell lines (*e.g.*, Sf9 and/or HighFive), animal cell lines (*e.g.*, chicken cell lines (MDCC)), bacterial cells (*e.g.*, E. coli) and/or mammalian cell lines (*e.g.*, 293expi and/or MOLT4). In some embodiments, DNA encoding Anellovirus ORF1 may be untagged. In some embodiments, DNA encoding Anellovirus ORF1 may contain tags fused N-terminally and/or C-terminally. In some embodiments, DNA encoding Anellovirus ORF1 may harbor mutations, insertions or deletions within the ORF1 protein to introduce a tag, *e.g.*, to aid in purification and/or identity determination, *e.g.*, through immunostaining assays (including but not limited to ELISA or Western Blot).

In some embodiments, DNA encoding Anellovirus ORF1 may be expressed alone or in combination with any number of helper proteins. In some embodiments, DNA encoding Anellovirus ORF1 is expressed in combination with Anellovirus ORF2 and/or ORF3 proteins.

In some embodiments, ORF1 proteins harboring mutations to improve assembly efficiency may include, but are not limited to, ORF1 proteins that harbor mutations introduced into the N-terminal Arginine Arm (ARG arm) to alter the pI of the ARG arm permitting pH sensitive nucleic acid binding to trigger particle assembly (SEQ ID 3-5). In some embodiments, ORF1 proteins harboring mutations that improve stability may include mutations to an interprotomer contacting beta strands F and G of the canonical structural jellyroll beta-barrel to alter hydrophobic state of the protomer surface and improve thermodynamic favorability of capsid formation.

In some embodiments, chimeric ORF1 proteins may include, but are not limited to, ORF1 proteins which have a portion or portions of their sequence replaced with comparable portions from another capsid protein, *e.g.*, Beak and Feather Disease Virus (BFDV) capsid protein, or Hepatitis E capsid protein, *e.g.,* ARG arm or F and G beta strands of Ring 9 ORF1 replaced with the comparable components from BFDV capsid protein. In some embodiments, chimeric ORF1 proteins may also include ORF1 proteins which have a portion or portions of their sequence replaced with comparable portions of another Anellovirus ORF1 protein (*e.g.*, structural jellyroll fragments or the C-terminal portion of Ring 2 ORF1 replaced with comparable portions of Ring 9 ORF1.

In some embodiments, a genetic element to be used for *in vitro* assembly of a particle (e.g., an anellovector) as described herein are produced in a cell. In some embodiments, a cell is transfected with a construct (e.g., a plasmid, tandem construct, and/or an *in vitro* circularized nucleic acid molecule, e.g., as described herein) comprising the sequence of a genetic element. In embodiments, the cell is incubated under conditions suitable for replication of the construct. In embodiments, the cell is incubated under conditions suitable for production and/or replication of the genetic element (e.g., from the construct). In embodiments, the cell is lysed to recover the genetic element. In some embodiments, a genetic element to be used for *in vitro* assembly is a DNA, e.g., a single-stranded DNA (ssDNA). In embodiments, the genetic element is a negative sense ssDNA (e.g., generated as described in Example 6). In some embodiments, a genetic element to be used for *in vitro* assembly comprises RNA (e.g., an mRNA), for example, as described in Example 12. In some embodiments, a genetic element to be used for *in vitro* assembly comprises RNA (e.g., an mRNA) and the ORF1 molecule of the proteinaceous exterior comprises one or more contact residues that binds RNA (e.g., a domain from an RNA-binding protein, e.g., an mRNA-binding protein, e.g., MS2 coat protein), for example, as described in Example 12. In some embodiments, a genetic element to be used for *in vitro* assembly comprises RNA (e.g., an mRNA) and DNA (e.g., a DNA portion comprising a sequence that binds to an Anellovirus ORF1 molecule), for

example, as described in Example 12. In certain embodiments, the RNA portion of the genetic element is covalently bound to the DNA portion of the genetic element. In certain embodiments, the RNA portion of the genetic element is hybridized to the DNA portion of the genetic element. In embodiments, the RNA portion of the genetic element comprises a region capable of hybridizing to (e.g., complementary to) at least a subsequence of the DNA portion of the genetic element.

In some embodiments, the present disclosure describes a method of making an anellovector, the method comprising: (a) providing a mixture comprising: (i) a genetic element (e.g., a genetic element comprising DNA and/or RNA), and (ii) an ORF1 molecule; and (b) incubating the mixture under conditions suitable for enclosing the genetic element within a proteinaceous exterior comprising the ORF1 molecule, thereby making an anellovector; wherein the mixture is not comprised in a cell. In some embodiments, the anellovector is assembled *in vitro* as described in Example 7. In some embodiments, the method further comprises, prior to the providing of (a), expressing the ORF1 molecule, e.g., in a host cell (e.g., an insect cell or a mammalian cell). In some embodiments, the expressing comprises incubating a host cell (e.g., an insect cell or a mammalian cell) comprising a nucleic acid molecule (e.g., a baculovirus expression vector) encoding the ORF1 molecule under conditions suitable for producing the ORF1 molecule. In some embodiments, the method further comprises, prior to the providing of (a), purifying the ORF1 molecule expressed by the host cell. In some embodiments, the method is performed in a cell-free system. In some embodiments, the present disclosure describes a method of manufacturing an anellovector composition, comprising: (a) providing a plurality of anellovectors or compositions according to any of the preceding embodiments; (b) optionally evaluating the plurality for one or more of: a contaminant described herein, an optical density measurement (e.g., OD 260), particle number (e.g., by HPLC), infectivity (e.g., particle:infectious unit ratio, e.g., as determined by fluorescence and/or ELISA); and (c) formulating the plurality of anellovectors, e.g., as a pharmaceutical composition suitable for administration to a subject, e.g., if one or more of the parameters of (b) meet a specified threshold.

In vitro assembly methods for anelloVLPs

An anelloVLP as described herein may be produced, e.g., by *in vitro* assembly, e.g., in a cell-free suspension or in a supernatant. In some embodiments, the anelloVLP is produced by contacting a plurality of ORF1 molecules (e.g., in capsomers) to an effector (e.g., an exogenous effector) *in vitro,* e.g., under conditions that allow for assembly. In some embodiments, the plurality of ORF1 molecules enclose the effector in a proteinaceous exterior. In some embodiments, the effector is attached to the exterior surface of a proteinaceous exterior formed from the plurality of ORF1 molecules (e.g., as a surface moiety as described herein). In some embodiments, production of an anelloVLP comprises expressing a plurality of ORF1 molecules in a cell, purifying the plurality of ORF1 molecules, denaturing

virus-like particles formed by the ORF1 molecules (e.g., as described herein), and then allowing the ORF1 molecules to reform virus-like particles in the presence of an effector (e.g., an exogenous effector), thereby forming anelloVLPs comprising the ORF1 molecules enclosing the effector.

In an aspect, the present disclosure provide a particle (e.g., an anelloVLP as described herein) produced via in vitro assembly (e.g., as described herein). The particle may, in some instances, comprise a proteinaceous exterior comprising an ORF1 molecule and an effector (e.g., an exogenous effector), e.g., as described herein. In some embodiments, the effector is enclosed within the proteinaceous exterior. In some embodiments, the effector is comprised in a surface moiety (e.g., as described herein) attached to the exterior surface of the proteinaceous exterior. In some embodiments, a particle produced by in vitro assembly does not include a substantial (e.g., detectable) amount of one or more constituents (e.g., small molecules, peptides, polypeptides, nucleic acids, polynucleotides, lipids, sugars, and/or organelles) from a host cell (e.g., a host cell used to produce the ORF1 molecules and/or the genetic element). In some embodiments, the particle may have one or more (e.g., 1, 2, 3, 4, or all 5) of the following characteristics:

(i) does not comprise (e.g., does not enclose) a polynucleotide,

(ii) does not comprise (e.g., does not enclose) detectable levels of polynucleotides,

(iii) does not comprise (e.g., does not enclose) a polynucleotide of greater than 1000, 500, 200, or 100 nucleotides in length,

(iv) does not comprise (e.g., does not enclose) a polynucleotide comprising any contiguous nucleic acid sequences of at least 50, 100, 150, 200, 300, 400, 500, 600, 700, 800, 900, or 1000 nucleotides in length having least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to contiguous sequence in a wild-type *Anellovirus* genome (e.g., as described herein), and/or

(v) does not comprise a polynucleotide comprising an Anellovirus 5' UTR or an origin of replication.

In another aspect, the disclosure provides a population of the particles (e.g., the anelloVLPs). In some embodiments, the population further comprises one or more anellovectors. In some embodiments, the population does not comprise anellovectors.

In some embodiments, Anellovirus proteins to be used for *in vitro* assembly of a particle (e.g., an anelloVLP) as described herein are produced in a cell. In some embodiments, baculovirus constructs are used to produce Anellovirus proteins (e.g., one or more of an Anellovirus ORF1, ORF2, and/or ORF3 molecule, e.g., as described herein), for example, in insect cells (e.g., Sf9 cells). These proteins may then be used, e.g., for *in vitro* assembly to form an anelloVLP, e.g., as described herein. In some embodiments, a polynucleotide encoding one or more Anellovirus proteins is fused to a promoter for expression in a host cell, *e.g.*, an insect cell or an animal cell. In some embodiments, the polynucleotide is cloned into a baculovirus expression system. In some embodiments, a host cell, *e.g.*, an insect cell, is infected with the

baculovirus expression system and incubated for a period of time under conditions suitable for expression of the one or more Anellovirus proteins. In some embodiments, an infected cell is incubated for about 1, 2, 3, 4, 5, 10, 15, or 20 days. In some embodiments, an infected cell is lysed to recover the one or more Anellovirus proteins.

In some embodiments, an Anellovirus protein (e.g., an Anellovirus ORF1 molecule) is produced as described in Example 7. In some embodiments, an Anellovirus protein (e.g., an Anellovirus ORF1 molecule) is produced in insect cells as described in Example 8 or 10. In some embodiments, a plurality of Anellovirus ORF1 molecules has a propensity to self-assemble into a proteinaceous exterior, for example, to form a virus-like particle (VLP). In certain embodiments, the VLPs do not encapsulate a genetic element as described herein. In certain embodiments, the VLPs do not encapsulate an effector to be delivered to a target cell, e.g., as described herein. In certain embodiments, the VLP comprises at least 40, 45, 50, 55, 60, 65, or 70 ORF1 molecules in its proteinaceous exterior.

In certain embodiments, a VLP comprising an Anellovirus ORF1 molecule can be denatured as described herein (e.g., using a chaotropic agent, such as urea). In embodiments, a VLP is denatured using one or more of: buffers of different pH, conditions of defined conductivity (salt content), a detergent (such as SDS (e.g., 0.1% SDS), Tween, Triton), a chaotropic agent (such as urea, e.g., as described herein), a high salt solution (e.g., a solution comprising NaCl, e.g., at a concentration of at least about 1M, e.g., at least about 0.5, 0.6, 0.7, 0.8, 0.9, 1.0, 1.1, 1.2, 1.3, 1.4, 1.5, 2, 3, 4, or 5M), or conditions involving defined temperature and time (reannealing temperatures), for example, as described in Example 12. In embodiments, a VLP is denatured using urea as described in Example 11. In embodiments, a VLP is denatured in urea at a concentration of about 1-10 M (e.g., about 1-2 M, 2-3 M, 3-4 M, 4-5 M, 5-6 M, 6-7 M, 7-8 M, 8-9 M, 9-10M, or 1-6 M). In embodiments, a VLP is denatured in urea at a concentration of about 1-10 M (e.g., about 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10M). In an embodiment, a VLP is denatured in urea at a concentration of 2 M. In embodiments, a VLP is denatured under high salt conditions. In embodiments, denaturation of the VLP results in the ORF1 molecules forming capsomers (e.g., capsomeric decamers comprising about 10 copies of the ORF1 molecule).

In some embodiments, removing the capsomers from the presence of the chaotropic agent (e.g., by purifying the capsomers or removing the chaotropic agent, e.g., by dilution or dialysis) allows the ORF1 molecules to reform VLPs (e.g., VLPs comprising at least 40, 45, 50, 55, 60, 65, or 70 ORF1 molecules). In embodiments, VLPs are reformed by dialyzing out a chaotropic agent (e.g., urea) as described in Example 11. In some embodiments, VLPs are reformed in the presence of a cargo of interest (e.g., an effector as described herein), for example, under conditions suitable for enclosure of the cargo in the proteinaceous exterior of the VLP (e.g., as described in Example 12). In some embodiments, an isolated Anellovirus protein (e.g., an ORF1 molecule) is purified (e.g., from a cell). In some

embodiments, an Anellovirus protein is purified using purification techniques including but not limited to chelating purification, heparin purification, gradient sedimentation purification, and/or SEC purification. In embodiments, an Anellovirus protein is purified as described in Example 7. In some embodiments, an Anellovirus protein (e.g., an Anellovirus ORF1 molecule) is purified from insect cells as described in Example 8 or 10. In some embodiments, a plurality of purified Anellovirus proteins are mixed with an effector. In embodiments, the purified Anellovirus proteins encapsulated the effector. In some embodiments, the effector is encapsulated using an ORF1 protein, ORF2 protein, or modified version thereof.

In some embodiments, DNA encoding Anellovirus ORF1 (*e.g.*, wildtype ORF1 protein, ORF1 proteins harboring mutations, *e.g.*, to improve assembly efficiency, yield or stability, chimeric ORF1 protein, or fragments thereof) are expressed in insect cell lines (*e.g.*, Sf9 and/or HighFive), animal cell lines (*e.g.*, chicken cell lines (MDCC)), bacterial cells (*e.g.*, E. coli) and/or mammalian cell lines (*e.g.*, 293expi and/or MOLT4). In some embodiments, DNA encoding Anellovirus ORF1 may be untagged. In some embodiments, DNA encoding Anellovirus ORF1 may contain tags fused N-terminally and/or C-terminally. In some embodiments, DNA encoding Anellovirus ORF1 may harbor mutations, insertions or deletions within the ORF1 protein to introduce a tag, *e.g.*, to aid in purification and/or identity determination, *e.g.*, through immunostaining assays (including but not limited to ELISA or Western Blot). In some embodiments, DNA encoding Anellovirus ORF1 may be expressed alone or in combination with any number of helper proteins. In some embodiments, DNA encoding Anellovirus ORF1 is expressed in combination with Anellovirus ORF2 and/or ORF3 proteins.

In some embodiments, ORF1 proteins harboring mutations to improve assembly efficiency may include, but are not limited to, ORF1 proteins that harbor mutations introduced into the N-terminal Arginine Arm (ARG arm) to alter the pI of the ARG arm permitting pH sensitive nucleic acid binding to trigger particle assembly (SEQ ID 3-5). In some embodiments, ORF1 proteins harboring mutations that improve stability may include mutations to an interprotomer contacting beta strands F and G of the canonical structural jellyroll beta-barrel to alter hydrophobic state of the protomer surface and improve thermodynamic favorability of capsid formation.

In some embodiments, chimeric ORF1 proteins may include, but are not limited to, ORF1 proteins which have a portion or portions of their sequence replaced with comparable portions from another capsid protein, *e.g.*, Beak and Feather Disease Virus (BFDV) capsid protein, or Hepatitis E capsid protein, *e.g.,* ARG arm or F and G beta strands of Ring 9 ORF1 replaced with the comparable components from BFDV capsid protein. In some embodiments, chimeric ORF1 proteins may also include ORF1 proteins which have a portion or portions of their sequence replaced with comparable portions of

another Anellovirus ORF1 protein (*e.g.*, structural jellyroll fragments or the C-terminal portion of Ring 2 ORF1 replaced with comparable portions of Ring 9 ORF1.

In some embodiments, the present disclosure describes a method of making an anelloVLP, the method comprising: (a) providing a mixture comprising: (i) an effector (e.g., an exogenous effector), and (ii) an ORF1 molecule; and (b) incubating the mixture under conditions suitable for enclosing the effector within a proteinaceous exterior comprising the ORF1 molecule, thereby making an anelloVLP; wherein the mixture is not comprised in a cell. In some embodiments, the anelloVLP is assembled *in vitro* as described in Example 7. In some embodiments, the method further comprises, prior to the providing of (a), expressing the ORF1 molecule, e.g., in a host cell (e.g., an insect cell or a mammalian cell). In some embodiments, the expressing comprises incubating a host cell (e.g., an insect cell or a mammalian cell) comprising a nucleic acid molecule (e.g., a baculovirus expression vector) encoding the ORF1 molecule under conditions suitable for producing the ORF1 molecule. In some embodiments, the method further comprises, prior to the providing of (a), purifying the ORF1 molecule expressed by the host cell. In some embodiments, the method is performed in a cell-free system. In some embodiments, the present disclosure describes a method of manufacturing an anelloVLP composition, comprising: (a) providing a plurality of anelloVLPs or compositions according to any of the preceding embodiments; (b) optionally evaluating the plurality for one or more of: a contaminant described herein, an optical density measurement (e.g., OD 260), particle number (e.g., by HPLC), infectivity (e.g., particle:infectious unit ratio, e.g., as determined by fluorescence and/or ELISA); and (c) formulating the plurality of anelloVLPs, e.g., as a pharmaceutical composition suitable for administration to a subject, e.g., if one or more of the parameters of (b) meet a specified threshold.

Enrichment and purification

Anellovectors or anelloVLPs produced as described herein can be further purified and/or enriched, e.g., to produce an anellovector preparation or an anelloVLP preparation, respectively. In some embodiments, the harvested anellovectors or anelloVLPs are isolated from other constituents or contaminants present in the harvest solution, e.g., using methods known in the art for purifying viral particles (e.g., purification by sedimentation, chromatography, and/or ultrafiltration). In some embodiments, the purification steps comprise removing one or more of serum, host cell DNA, host cell proteins, particles lacking the genetic element, and/or phenol red from the preparation. In some embodiments, the harvested anellovectors or anelloVLPs are enriched relative to other constituents or contaminants present in the harvest solution, e.g., using methods known in the art for enriching viral particles.

In some embodiments, the resultant preparation or a pharmaceutical composition comprising the preparation will be stable over an acceptable period of time and temperature, and/or be compatible with the desired route of administration and/or any devices this route of administration will require, e.g., needles or syringes.

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III. Vectors

The genetic element described herein may be included in a vector. Suitable vectors as well as methods for their manufacture and their use are well known in the prior art.

In one aspect, the invention includes a vector comprising a genetic element comprising (i) a
10 sequence encoding a non-pathogenic exterior protein, (ii) an exterior protein binding sequence that binds the genetic element to the non-pathogenic exterior protein, and (iii) a sequence encoding a regulatory nucleic acid.

The genetic element or any of the sequences within the genetic element can be obtained using any suitable method. Various recombinant methods are known in the art, such as, for example screening
15 libraries from cells harboring viral sequences, deriving the sequences from a vector known to include the same, or isolating directly from cells and tissues containing the same, using standard techniques. Alternatively or in combination, part or all of the genetic element can be produced synthetically, rather than cloned.

In some embodiments, the vector includes regulatory elements, nucleic acid sequences
20 homologous to target genes, and various reporter constructs for causing the expression of reporter molecules within a viable cell and/or when an intracellular molecule is present within a target cell.

Reporter genes are used for identifying potentially transfected cells and for evaluating the functionality of regulatory sequences. In general, a reporter gene is a gene that is not present in or expressed by the recipient organism or tissue and that encodes a polypeptide whose expression is
25 manifested by some easily detectable property, e.g., enzymatic activity. Expression of the reporter gene is assayed at a suitable time after the DNA has been introduced into the recipient cells. Suitable reporter genes may include genes encoding luciferase, beta-galactosidase, chloramphenicol acetyl transferase, secreted alkaline phosphatase, or the green fluorescent protein gene (e.g., Ui-Tei et al., 2000 FEBS Letters 479: 79-82). Suitable expression systems are well known and may be prepared using known
30 techniques or obtained commercially. In general, the construct with the minimal 5' flanking region showing the highest level of expression of reporter gene is identified as the promoter. Such promoter regions may be linked to a reporter gene and used to evaluate agents for the ability to modulate promoter-driven transcription.

In some embodiments, the vector is substantially non-pathogenic and/or substantially non-integrating in a host cell or is substantially non-immunogenic in a host.

In some embodiments, the vector is in an amount sufficient to modulate one or more of phenotype, virus levels, gene expression, compete with other viruses, disease state, etc. at least about 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, or more.

IV. Compositions

An anellovector or or anelloVLP described herein may also be included in pharmaceutical compositions with a pharmaceutical excipient, e.g., as described herein. In some embodiments, the pharmaceutical composition comprises at least 10^5, 10^6, 10^7, 10^8, 10^9, 10^{10}, 10^{11}, 10^{12}, 10^{13}, 10^{14}, or 10^{15} anellovectors or anelloVLPs. In some embodiments, the pharmaceutical composition comprises about 10^5-10^{15}, 10^5-10^{10}, or 10^{10}-10^{15} anellovectors or anelloVLPs. In some embodiments, the pharmaceutical composition comprises about 10^8 (e.g., about 10^5, 10^6, 10^7, 10^8, 10^9, or 10^{10}) genomic equivalents/mL of the anellovector or anelloVLP. In some embodiments, the pharmaceutical composition comprises 10^5-10^{10}, 10^6-10^{10}, 10^7-10^{10}, 10^8-10^{10}, 10^9-10^{10}, 10^5-10^6, 10^5-10^7, 10^5-10^8, 10^5-10^9, 10^5-10^{11}, 10^5-10^{12}, 10^5-10^{13}, 10^5-10^{14}, 10^5-10^{15}, or 10^{10}-10^{15} genomic equivalents/mL of the anellovector or anelloVLP, e.g., as determined according to the method of Example 31. In some embodiments, the pharmaceutical composition comprises sufficient anellovectors or anelloVLPs to deliver at least 1, 2, 5, or 10, 100, 500, 1000, 2000, 5000, 8,000, 1×10^4, 1×10^5, 1×10^6, 1×10^7 or greater copies of a genetic element comprised in the anellovectors or anelloVLPs per cell to a population of the eukaryotic cells. In some embodiments, the pharmaceutical composition comprises sufficient anellovectors or anelloVLPs to deliver at least about 1×10^4, 1×10^5, 1×10^6, 1 x or 10^7, or about 1×10^4-1×10^5, 1×10^4-1×10^6, 1×10^4-1×10^7, 1×10^5-1×10^6, 1×10^5-1×10^7, or 1×10^6-1×10^7 copies of a genetic element comprised in the anellovectors or anelloVLPs per cell to a population of the eukaryotic cells.

In some embodiments, the pharmaceutical composition has one or more of the following characteristics: the pharmaceutical composition meets a pharmaceutical or good manufacturing practices (GMP) standard; the pharmaceutical composition was made according to good manufacturing practices (GMP); the pharmaceutical composition has a pathogen level below a predetermined reference value, e.g., is substantially free of pathogens; the pharmaceutical composition has a contaminant level below a predetermined reference value, e.g., is substantially free of contaminants; or the pharmaceutical composition has low immunogenicity or is substantially non-immunogenic, e.g., as described herein.

In some embodiments, the pharmaceutical composition comprises below a threshold amount of one or more contaminants. Exemplary contaminants that are desirably excluded or minimized in the pharmaceutical composition include, without limitation, host cell nucleic acids (e.g., host cell DNA

and/or host cell RNA), animal-derived components (e.g., serum albumin or trypsin), replication-competent viruses, non-infectious particles, free viral capsid protein, adventitious agents, and aggregates. In embodiments, the contaminant is host cell DNA. In embodiments, the composition comprises less than about 10 ng of host cell DNA per dose. In embodiments, the level of host cell DNA in the composition is reduced by filtration and/or enzymatic degradation of host cell DNA. In embodiments, the pharmaceutical composition consists of less than 10% (e.g., less than about 10%, 5%, 4%, 3%, 2%, 1%, 0.5%, or 0.1%) contaminant by weight.

In one aspect, the invention described herein includes a pharmaceutical composition comprising:

a) an anellovector comprising a genetic element comprising (i) a sequence encoding a non-pathogenic exterior protein, (ii) an exterior protein binding sequence that binds the genetic element to the non-pathogenic exterior protein, and (iii) a sequence encoding a regulatory nucleic acid; and a proteinaceous exterior that is associated with, e.g., envelops or encloses, the genetic element; and

b) a pharmaceutical excipient.

In one aspect, the invention described herein includes a pharmaceutical composition comprising:

a) an anelloVLP as described herein; and

b) a pharmaceutical excipient.

Vesicles

In some embodiments, the composition further comprises a carrier component, e.g., a microparticle, liposome, vesicle, or exosome. In some embodiments, liposomes comprise spherical vesicle structures composed of a uni- or multilamellar lipid bilayer surrounding internal aqueous compartments and a relatively impermeable outer lipophilic phospholipid bilayer. Liposomes may be anionic, neutral or cationic. Liposomes are generally biocompatible, nontoxic, can deliver both hydrophilic and lipophilic drug molecules, protect their cargo from degradation by plasma enzymes, and transport their load across biological membranes (see, e.g., Spuch and Navarro, Journal of Drug Delivery, vol. 2011, Article ID 469679, 12 pages, 2011. doi:10.1155/2011/469679 for review).

Vesicles can be made from several different types of lipids; however, phospholipids are most commonly used to generate liposomes as drug carriers. Vesicles may comprise without limitation DOTMA, DOTAP, DOTIM, DDAB, alone or together with cholesterol to yield DOTMA and cholesterol, DOTAP and cholesterol, DOTIM and cholesterol, and DDAB and cholesterol. Methods for preparation of multilamellar vesicle lipids are known in the art (see for example U.S. Pat. No. 6,693,086, the teachings of which relating to multilamellar vesicle lipid preparation are incorporated herein by reference). Although vesicle formation can be spontaneous when a lipid film is mixed with an aqueous solution, it can also be expedited by applying force in the form of shaking by using a homogenizer,

sonicator, or an extrusion apparatus (see, e.g., Spuch and Navarro, Journal of Drug Delivery, vol. 2011, Article ID 469679, 12 pages, 2011. doi:10.1155/2011/469679 for review). Extruded lipids can be prepared by extruding through filters of decreasing size, as described in Templeton et al., Nature Biotech, 15:647-652, 1997, the teachings of which relating to extruded lipid preparation are incorporated herein by reference.

As described herein, additives may be added to vesicles to modify their structure and/or properties. For example, either cholesterol or sphingomyelin may be added to the mixture to help stabilize the structure and to prevent the leakage of the inner cargo. Further, vesicles can be prepared from hydrogenated egg phosphatidylcholine or egg phosphatidylcholine, cholesterol, and dicetyl phosphate. (see, e.g., Spuch and Navarro, Journal of Drug Delivery, vol. 2011, Article ID 469679, 12 pages, 2011. doi:10.1155/2011/469679 for review). Also, vesicles may be surface modified during or after synthesis to include reactive groups complementary to the reactive groups on the recipient cells. Such reactive groups include without limitation maleimide groups. As an example, vesicles may be synthesized to include maleimide conjugated phospholipids such as without limitation DSPE-MaL-PEG2000.

A vesicle formulation may be mainly comprised of natural phospholipids and lipids such as 1,2-distearoryl-sn-glycero-3-phosphatidyl choline (DSPC), sphingomyelin, egg phosphatidylcholines and monosialoganglioside. Formulations made up of phospholipids only are less stable in plasma. However, manipulation of the lipid membrane with cholesterol reduces rapid release of the encapsulated cargo or 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine (DOPE) increases stability (see, e.g., Spuch and Navarro, Journal of Drug Delivery, vol. 2011, Article ID 469679, 12 pages, 2011. doi:10.1155/2011/469679 for review).

In embodiments, lipids may be used to form lipid microparticles. Lipids include, but are not limited to, DLin-KC2-DMA4, C12-200 and colipids disteroylphosphatidyl choline, cholesterol, and PEG-DMG may be formulated (see, e.g., Novobrantseva, Molecular Therapy-Nucleic Acids (2012) 1, e4; doi:10.1038/mtna.2011.3) using a spontaneous vesicle formation procedure. The component molar ratio may be about 50/10/38.5/1.5 (DLin-KC2-DMA or C12-200/disteroylphosphatidyl choline/cholesterol/PEG-DMG). Tekmira has a portfolio of approximately 95 patent families, in the U.S. and abroad, that are directed to various aspects of lipid microparticles and lipid microparticles formulations (see, e.g., U.S. Pat. Nos. 7,982,027; 7,799,565; 8,058,069; 8,283,333; 7,901,708; 7,745,651; 7,803,397; 8,101,741; 8,188,263; 7,915,399; 8,236,943 and 7,838,658 and European Pat. Nos. 1766035; 1519714; 1781593 and 1664316), all of which may be used and/or adapted to the present invention.

In some embodiments, microparticles comprise one or more solidified polymer(s) that is arranged in a random manner. The microparticles may be biodegradable. Biodegradable microparticles

may be synthesized, e.g., using methods known in the art including without limitation solvent evaporation, hot melt microencapsulation, solvent removal, and spray drying. Exemplary methods for synthesizing microparticles are described by Bershteyn et al., Soft Matter 4:1787-1787, 2008 and in US 2008/0014144 A1, the specific teachings of which relating to microparticle synthesis are incorporated herein by reference.

Exemplary synthetic polymers which can be used to form biodegradable microparticles include without limitation aliphatic polyesters, poly (lactic acid) (PLA), poly (glycolic acid) (PGA), co-polymers of lactic acid and glycolic acid (PLGA), polycarprolactone (PCL), polyanhydrides, poly(ortho)esters, polyurethanes, poly(butyric acid), poly(valeric acid), and poly(lactide-co-caprolactone), and natural polymers such as albumin, alginate and other polysaccharides including dextran and cellulose, collagen, chemical derivatives thereof, including substitutions, additions of chemical groups such as for example alkyl, alkylene, hydroxylations, oxidations, and other modifications routinely made by those skilled in the art), albumin and other hydrophilic proteins, zein and other prolamines and hydrophobic proteins, copolymers and mixtures thereof. In general, these materials degrade either by enzymatic hydrolysis or exposure to water, by surface or bulk erosion.

The microparticles' diameter ranges from 0.1-1000 micrometers (μm). In some embodiments, their diameter ranges in size from 1-750 μm, or from 50-500 μm, or from 100-250 μm. In some embodiments, their diameter ranges in size from 50-1000 μm, from 50-750 μm, from 50-500 μm, or from 50-250 μm. In some embodiments, their diameter ranges in size from .05-1000 μm, from 10-1000 μm, from 100-1000 μm, or from 500-1000 μm. In some embodiments, their diameter is about 0.5 μm, about 10 μm, about 50 μm, about 100 μm, about 200 μm, about 300 μm, about 350 μm, about 400 μm, about 450 μm, about 500 μm, about 550 μm, about 600 μm, about 650 μm, about 700 μm, about 750 μm, about 800 μm, about 850 μm, about 900 μm, about 950 μm, or about 1000 μm. As used in the context of microparticle diameters, the term "about" means+/-5% of the absolute value stated.

In some embodiments, a ligand is conjugated to the surface of the microparticle via a functional chemical group (carboxylic acids, aldehydes, amines, sulfhydryls and hydroxyls) present on the surface of the particle and present on the ligand to be attached. Functionality may be introduced into the microparticles by, for example, during the emulsion preparation of microparticles, incorporation of stabilizers with functional chemical groups.

Another example of introducing functional groups to the microparticle is during post-particle preparation, by direct crosslinking particles and ligands with homo- or heterobifunctional crosslinkers. This procedure may use a suitable chemistry and a class of crosslinkers (CDI, EDAC, glutaraldehydes, etc. as discussed in more detail below) or any other crosslinker that couples ligands to the particle surface via chemical modification of the particle surface after preparation. This also includes a process whereby

amphiphilic molecules such as fatty acids, lipids or functional stabilizers may be passively adsorbed and adhered to the particle surface, thereby introducing functional end groups for tethering to ligands.

In some embodiments, the microparticles may be synthesized to comprise one or more targeting groups on their exterior surface to target a specific cell or tissue type (e.g., cardiomyocytes). These targeting groups include without limitation receptors, ligands, antibodies, and the like. These targeting groups bind their partner on the cells' surface. In some embodiments, the microparticles will integrate into a lipid bilayer that comprises the cell surface and the mitochondria are delivered to the cell.

The microparticles may also comprise a lipid bilayer on their outermost surface. This bilayer may be comprised of one or more lipids of the same or different type. Examples include without limitation phospholipids such as phosphocholines and phosphoinositols. Specific examples include without limitation DMPC, DOPC, DSPC, and various other lipids such as those described herein for liposomes.

In some embodiments, the carrier comprises nanoparticles, e.g., as described herein.

In some embodiments, the vesicles or microparticles described herein are functionalized with a diagnostic agent. Examples of diagnostic agents include, but are not limited to, commercially available imaging agents used in positron emissions tomography (PET), computer assisted tomography (CAT), single photon emission computerized tomography, x-ray, fluoroscopy, and magnetic resonance imaging (MRI); and contrast agents. Examples of suitable materials for use as contrast agents in MRI include gadolinium chelates, as well as iron, magnesium, manganese, copper, and chromium.

Carriers

A composition (e.g., pharmaceutical composition) described herein may comprise, be formulated with, and/or be delivered in, a carrier. In one aspect, the invention includes a composition, e.g., a pharmaceutical composition, comprising a carrier (e.g., a vesicle, a liposome, a lipid nanoparticle, an exosome, a red blood cell, an exosome (e.g., a mammalian or plant exosome), a fusosome) comprising (e.g., encapsulating) a composition described herein (e.g., an anellovector, anelloVLP, *Anellovirus*, or genetic element described herein).

In some embodiments, the compositions and systems described herein can be formulated in liposomes or other similar vesicles. Generally, liposomes are spherical vesicle structures composed of a uni- or multilamellar lipid bilayer surrounding internal aqueous compartments and a relatively impermeable outer lipophilic phospholipid bilayer. Liposomes may be anionic, neutral or cationic. Liposomes generally have one or more (e.g., all) of the following characteristics: biocompatibility, nontoxicity, can deliver both hydrophilic and lipophilic drug molecules, can protect their cargo from degradation by plasma enzymes, and can transport their load across biological

membranes and the blood brain barrier (BBB) (see, e.g., Spuch and Navarro, Journal of Drug Delivery, vol. 2011, Article ID 469679, 12 pages, 2011. doi:10.1155/2011/469679; and Zylberberg & Matosevic. 2016. Drug Delivery, 23:9, 3319-3329, doi: 10.1080/10717544.2016.1177136).

Vesicles can be made from several different types of lipids; however, phospholipids are most commonly used to generate liposomes as drug carriers. Methods for preparation of multilamellar vesicle lipids are known (see, for example, U.S. Pat. No. 6,693,086, the teachings of which relating to multilamellar vesicle lipid preparation are incorporated herein by reference). Although vesicle formation can be spontaneous when a lipid film is mixed with an aqueeous solution, it can also be expedited by applying force in the form of shaking by using a homogenizer, sonicator, or an extrusion apparatus (see, e.g., Spuch and Navarro, Journal of Drug Delivery, vol. 2011, Article ID 469679, 12 pages, 2011. doi:10.1155/2011/469679 for review). Extruded lipids can be prepared by, e.g., extruding through filters of decreasing size, as described in Templeton et al., Nature Biotech, 15:647-652, 1997.

Lipid nanoparticles (LNPs) are another example of a carrier that provides a biocompatible and biodegradable delivery system for the pharmaceutical compositions described herein. See, e.g., Gordillo-Galeano et al. European Journal of Pharmaceutics and Biopharmaceutics. Volume 133, December 2018, Pages 285-308. Nanostructured lipid carriers (NLCs) are modified solid lipid nanoparticles (SLNs) that retain the characteristics of the SLN, improve drug stability and loading capacity, and prevent drug leakage. Polymer nanoparticles (PNPs) are an important component of drug delivery. These nanoparticles can effectively direct drug delivery to specific targets and improve drug stability and controlled drug release. Lipid–polymer nanoparticles (PLNs), a new type of carrier that combines liposomes and polymers, may also be employed. These nanoparticles possess the complementary advantages of PNPs and liposomes. A PLN is composed of a core–shell structure; the polymer core provides a stable structure, and the phospholipid shell offers good biocompatibility. As such, the two components increase the drug encapsulation efficiency rate, facilitate surface modification, and prevent leakage of water-soluble drugs. For a review, see, e.g., Li et al. 2017, Nanomaterials 7, 122; doi:10.3390/nano7060122.

Exosomes can also be used as drug delivery vehicles for the compositions and systems described herein. For a review, see Ha et al. July 2016. Acta Pharmaceutica Sinica B. Volume 6, Issue 4, Pages 287-296; doi.org/10.1016/j.apsb.2016.02.001.

Ex vivo differentiated red blood cells can also be used as a carrier for a composition described herein. See, e.g., WO2015073587; WO2017123646; WO2017123644; WO2018102740; WO2016183482; WO2015153102; WO2018151829; WO2018009838; Shi et al. 2014. Proc Natl Acad Sci USA. 111(28): 10131–10136; US Patent 9,644,180; Huang et al. 2017. Nature Communications 8: 423; Shi et al. 2014. Proc Natl Acad Sci USA. 111(28): 10131–10136.

Fusosome compositions, e.g., as described in WO2018208728, can also be used as carriers to deliver a composition described herein.

Membrane Penetrating Polypeptides

In some embodiments, the composition further comprises a membrane penetrating polypeptide (MPP) to carry the components into cells or across a membrane, e.g., cell or nuclear membrane. Membrane penetrating polypeptides that are capable of facilitating transport of substances across a membrane include, but are not limited to, cell-penetrating peptides (CPPs)(see, e.g., US Pat. No.: 8,603,966), fusion peptides for plant intracellular delivery (see, e.g., Ng et al., PLoS One, 2016, 11:e0154081), protein transduction domains, Trojan peptides, and membrane translocation signals (MTS) (see, e.g., Tung et al., Advanced Drug Delivery Reviews 55:281-294 (2003)). Some MPP are rich in amino acids, such as arginine, with positively charged side chains.

Membrane penetrating polypeptides have the ability of inducing membrane penetration of a component and allow macromolecular translocation within cells of multiple tissues in vivo upon systemic administration. A membrane penetrating polypeptide may also refer to a peptide which, when brought into contact with a cell under appropriate conditions, passes from the external environment in the intracellular environment, including the cytoplasm, organelles such as mitochondria, or the nucleus of the cell, in amounts significantly greater than would be reached with passive diffusion.

Components transported across a membrane may be reversibly or irreversibly linked to the membrane penetrating polypeptide. A linker may be a chemical bond, e.g., one or more covalent bonds or non-covalent bonds. In some embodiments, the linker is a peptide linker. Such a linker may be between 2-30 amino acids, or longer. The linker includes flexible, rigid or cleavable linkers.

Combinations

In one aspect, the anellovector, anelloVLP, or composition described herein may also include one or more heterologous moieties. In one aspect, the anellovector or composition comprising an anellovector or anelloVLP described herein may also include one or more heterologous moiety in a fusion. In some embodiments, a heterologous moiety may be linked with the genetic element. In some embodiments, a heterologous moiety may be enclosed in the proteinaceous exterior as part of the anellovector or anelloVLP. In some embodiments, a heterologous moiety may be administered with the anellovector or anelloVLP.

In one aspect, the invention includes a cell or tissue comprising any one of the anellovectors or anelloVLP and heterologous moieties described herein.

In another aspect, the invention includes a pharmaceutical composition comprising an anellovector or anelloVLP and the heterologous moiety described herein.

In some embodiments, the heterologous moiety may be a virus (e.g., an effector (e.g., a drug, small molecule), a targeting agent (e.g., a DNA targeting agent, antibody, receptor ligand), a tag (e.g., fluorophore, light sensitive agent such as KillerRed), or an editing or targeting moiety described herein. In some embodiments, a membrane translocating polypeptide described herein is linked to one or more heterologous moieties. In one embodiment, the heterologous moiety is a small molecule (e.g., a peptidomimetic or a small organic molecule with a molecular weight of less than 2000 daltons), a peptide or polypeptide (e.g., an antibody or antigen-binding fragment thereof), a nanoparticle, an aptamer, or pharmacoagent.

Viruses

In some embodiments, the composition may further comprise a virus as a heterologous moiety, e.g., a single stranded DNA virus, e.g., Anellovirus, Bidnavirus, Circovirus, Geminivirus, Genomovirus, Inovirus, Microvirus, Nanovirus, Parvovirus, and Spiravirus. In some embodiments, the composition may further comprise a double stranded DNA virus, e.g., Adenovirus, Ampullavirus, Ascovirus, Asfarvirus, Baculovirus, Fusellovirus, Globulovirus, Guttavirus, Hytrosavirus, Herpesvirus, Iridovirus, Lipothrixvirus, Nimavirus, and Poxvirus. In some embodiments, the composition may further comprise an RNA virus, e.g., Alphavirus, Furovirus, Hepatitis virus, Hordeivirus, Tobamovirus, Tobravirus, Tricornavirus, Rubivirus, Birnavirus, Cystovirus, Partitivirus, and Reovirus. In some embodiments, the anellovector or anelloVLP is administered with a virus as a heterologous moiety.

In some embodiments, the heterologous moiety may comprise a non-pathogenic, e.g., symbiotic, commensal, native, virus. In some embodiments, the non-pathogenic virus is one or more anelloviruses, e.g., Alphatorquevirus (TT), Betatorquevirus (TTM), and Gammatorquevirus (TTMD). In some embodiments, the anellovirus may include a Torque Teno Virus (TT), a SEN virus, a Sentinel virus, a TTV-like mini virus, a TT virus, a TT virus genotype 6, a TT virus group, a TTV-like virus DXL1, a TTV-like virus DXL2, a Torque Teno-like Mini Virus (TTM), or a Torque Teno-like Midi Virus (TTMD). In some embodiments, the non-pathogenic virus comprises one or more sequences having at least at least about 60%, 70% 80%, 85%, 90% 95%, 96%, 97%, 98% and 99% nucleotide sequence identity to any one of the nucleotide sequences described herein, e.g., as listed in any one of Tables N1-N26.

In some embodiments, the heterologous moiety may comprise one or more viruses that are identified as lacking in the subject. For example, a subject identified as having dyvirosis may be

administered a composition comprising an anellovector and one or more viral components or viruses that are imbalanced in the subject or having a ratio that differs from a reference value, e.g., a healthy subject.

In some embodiments, the heterologous moiety may comprise one or more non-anelloviruses, e.g., adenovirus, herpes virus, pox virus, vaccinia virus, SV40, papilloma virus, an RNA virus such as a retrovirus, e.g., lenti virus, a single-stranded RNA virus, e.g., hepatitis virus, or a double-stranded RNA virus e.g., rotavirus. In some embodiments, the anellovector or the virus is defective, or requires assistance in order to produce infectious particles. Such assistance can be provided, e.g., by using helper cell lines that contain a nucleic acid, e.g., plasmids or DNA integrated into the genome, encoding one or more of (e.g., all of) the structural genes of the replication defective anellovector or virus under the control of regulatory sequences within the LTR. Suitable cell lines for replicating the anellovectors described herein include cell lines known in the art, e.g., A549 cells, which can be modified as described herein.

Targeting Moiety

In some embodiments, the composition, anellovector, or anelloVLP described herein may further comprise a targeting moiety, e.g., a targeting moiety that specifically binds to a molecule of interest present on a target cell. The targeting moiety may modulate a specific function of the molecule of interest or cell, modulate a specific molecule (e.g., enzyme, protein or nucleic acid), e.g., a specific molecule downstream of the molecule of interest in a pathway, or specifically bind to a target to localize the anellovector, or anelloVLP, or genetic element. For example, a targeting moiety may include a therapeutic that interacts with a specific molecule of interest to increase, decrease or otherwise modulate its function.

Tagging or Monitoring Moiety

In some embodiments, the composition, anellovector, or anelloVLP described herein may further comprise a tag to label or monitor the anellovector, anelloVLP, or genetic element described herein. The tagging or monitoring moiety may be removable by chemical agents or enzymatic cleavage, such as proteolysis or intein splicing. An affinity tag may be useful to purify the tagged polypeptide using an affinity technique. Some examples include, chitin binding protein (CBP), maltose binding protein (MBP), glutathione-S-transferase (GST), and poly(His) tag. A solubilization tag may be useful to aid recombinant proteins expressed in chaperone-deficient species such as *E. coli* to assist in the proper folding in proteins and keep them from precipitating. Some examples include thioredoxin (TRX) and poly(NANP). The tagging or monitoring moiety may include a light sensitive tag, e.g., fluorescence. Fluorescent tags are useful for visualization. GFP and its variants are some examples commonly used as

fluorescent tags. Protein tags may allow specific enzymatic modifications (such as biotinylation by biotin ligase) or chemical modifications (such as reaction with FlAsH-EDT2 for fluorescence imaging) to occur. Often tagging or monitoring moiety are combined, in order to connect proteins to multiple other components. The tagging or monitoring moiety may also be removed by specific proteolysis or enzymatic cleavage (e.g. by TEV protease, Thrombin, Factor Xa or Enteropeptidase).

Nanoparticles

In some embodiments, the composition, anellovector, or anelloVLP described herein may further comprise a nanoparticle. Nanoparticles include inorganic materials with a size between about 1 and about 1000 nanometers, between about 1 and about 500 nanometers in size, between about 1 and about 100 nm, between about 50 nm and about 300 nm, between about 75 nm and about 200 nm, between about 100 nm and about 200 nm, and any range therebetween. Nanoparticles generally have a composite structure of nanoscale dimensions. In some embodiments, nanoparticles are typically spherical although different morphologies are possible depending on the nanoparticle composition. The portion of the nanoparticle contacting an environment external to the nanoparticle is generally identified as the surface of the nanoparticle. In nanoparticles described herein, the size limitation can be restricted to two dimensions and so that nanoparticles include composite structure having a diameter from about 1 to about 1000 nm, where the specific diameter depends on the nanoparticle composition and on the intended use of the nanoparticle according to the experimental design. For example, nanoparticles used in therapeutic applications typically have a size of about 200 nm or below.

Additional desirable properties of the nanoparticle, such as surface charges and steric stabilization, can also vary in view of the specific application of interest. Exemplary properties that can be desirable in clinical applications such as cancer treatment are described in Davis et al, Nature 2008 vol. 7, pages 771-782; Duncan, Nature 2006 vol. 6, pages 688-701; and Allen, Nature 2002 vol. 2 pages 750-763, each incorporated herein by reference in its entirety. Additional properties are identifiable by a skilled person upon reading of the present disclosure. Nanoparticle dimensions and properties can be detected by techniques known in the art. Exemplary techniques to detect particles dimensions include but are not limited to dynamic light scattering (DLS) and a variety of microscopies such at transmission electron microscopy (TEM) and atomic force microscopy (AFM). Exemplary techniques to detect particle morphology include but are not limited to TEM and AFM. Exemplary techniques to detect surface charges of the nanoparticle include but are not limited to zeta potential method. Additional techniques suitable to detect other chemical properties comprise by ^{1}H, ^{11}B, and ^{13}C and ^{19}F NMR, UV/Vis and infrared/Raman spectroscopies and fluorescence spectroscopy (when nanoparticle is used in combination with fluorescent labels) and additional techniques identifiable by a skilled person.

Small molecules

In some embodiments, the composition, anellovector, or anelloVLP described herein may further comprise a small molecule. Small molecule moieties include, but are not limited to, small peptides, peptidomimetics (e.g., peptoids), amino acids, amino acid analogs, synthetic polynucleotides, polynucleotide analogs, nucleotides, nucleotide analogs, organic and inorganic compounds (including heterorganic and organomettallic compounds) generally having a molecular weight less than about 5,000 grams per mole, e.g., organic or inorganic compounds having a molecular weight less than about 2,000 grams per mole, e.g., organic or inorganic compounds having a molecular weight less than about 1,000 grams per mole, e.g., organic or inorganic compounds having a molecular weight less than about 500 grams per mole, and salts, esters, and other pharmaceutically acceptable forms of such compounds. Small molecules may include, but are not limited to, a neurotransmitter, a hormone, a drug, a toxin, a viral or microbial particle, a synthetic molecule, and agonists or antagonists.

Examples of suitable small molecules include those described in, "The Pharmacological Basis of Therapeutics," Goodman and Gilman, McGraw-Hill, New York, N.Y., (1996), Ninth edition, under the sections: Drugs Acting at Synaptic and Neuroeffector Junctional Sites; Drugs Acting on the Central Nervous System; Autacoids: Drug Therapy of Inflammation; Water, Salts and Ions; Drugs Affecting Renal Function and Electrolyte Metabolism; Cardiovascular Drugs; Drugs Affecting Gastrointestinal Function; Drugs Affecting Uterine Motility; Chemotherapy of Parasitic Infections; Chemotherapy of Microbial Diseases; Chemotherapy of Neoplastic Diseases; Drugs Used for Immunosuppression; Drugs Acting on Blood-Forming organs; Hormones and Hormone Antagonists; Vitamins, Dermatology; and Toxicology, all incorporated herein by reference. Some examples of small molecules include, but are not limited to, prion drugs such as tacrolimus, ubiquitin ligase or HECT ligase inhibitors such as heclin, histone modifying drugs such as sodium butyrate, enzymatic inhibitors such as 5-aza-cytidine, anthracyclines such as doxorubicin, beta-lactams such as penicillin, anti-bacterials, chemotherapy agents, anti-virals, modulators from other organisms such as VP64, and drugs with insufficient bioavailability such as chemotherapeutics with deficient pharmacokinetics.

In some embodiments, the small molecule is an epigenetic modifying agent, for example such as those described in de Groote et al. Nuc. Acids Res. (2012):1-18. Exemplary small molecule epigenetic modifying agents are described, e.g., in Lu et al. J. Biomolecular Screening 17.5(2012):555-71, e.g., at Table 1 or 2, incorporated herein by reference. In some embodiments, an epigenetic modifying agent comprises vorinostat or romidepsin. In some embodiments, an epigenetic modifying agent comprises an inhibitor of class I, II, III, and/or IV histone deacetylase (HDAC). In some embodiments, an epigenetic modifying agent comprises an activator of SirTI. In some embodiments, an epigenetic modifying agent

comprises Garcinol, Lys-CoA, C646, (+)-JQI, I-BET, BICI, MS120, DZNep, UNC0321, EPZ004777, AZ505, AMI-I, pyrazole amide 7b, benzo[d]imidazole 17b, acylated dapsone derivative (e.e.g, PRMTI), methylstat, 4,4'-dicarboxy-2,2'-bipyridine, SID 85736331, hydroxamate analog 8, tanylcypromie, bisguanidine and biguanide polyamine analogs, UNC669, Vidaza, decitabine, sodium phenyl butyrate (SDB), lipoic acid (LA), quercetin, valproic acid, hydralazine, bactrim, green tea extract (e.g., epigallocatechin gallate (EGCG)), curcumin, sulforphane and/or allicin/diallyl disulfide. In some embodiments, an epigenetic modifying agent inhibits DNA methylation, e.g., is an inhibitor of DNA methyltransferase (e.g., is 5-azacitidine and/or decitabine). In some embodiments, an epigenetic modifying agent modifies histone modification, e.g., histone acetylation, histone methylation, histone sumoylation, and/or histone phosphorylation. In some embodiments, the epigenetic modifying agent is an inhibitor of a histone deacetylase (e.g., is vorinostat and/or trichostatin A).

In some embodiments, the small molecule is a pharmaceutically active agent. In one embodiment, the small molecule is an inhibitor of a metabolic activity or component. Useful classes of pharmaceutically active agents include, but are not limited to, antibiotics, anti-inflammatory drugs, angiogenic or vasoactive agents, growth factors and chemotherapeutic (anti-neoplastic) agents (e.g., tumour suppressers). One or a combination of molecules from the categories and examples described herein or from (Orme-Johnson 2007, Methods Cell Biol. 2007;80:813-26) can be used. In one embodiment, the invention includes a composition comprising an antibiotic, anti-inflammatory drug, angiogenic or vasoactive agent, growth factor or chemotherapeutic agent.

Peptides or proteins

In some embodiments, the composition, anellovector, or anelloVLP described herein may further comprise a peptide or protein. The peptide moieties may include, but are not limited to, a peptide ligand or antibody fragment (e.g., antibody fragment that binds a receptor such as an extracellular receptor), neuropeptide, hormone peptide, peptide drug, toxic peptide, viral or microbial peptide, synthetic peptide, and agonist or antagonist peptide.

Peptides moieties may be linear or branched. The peptide has a length from about 5 to about 200 amino acids, about 15 to about 150 amino acids, about 20 to about 125 amino acids, about 25 to about 100 amino acids, or any range therebetween.

Some examples of peptides include, but are not limited to, fluorescent tags or markers, antigens, antibodies, antibody fragments such as single domain antibodies, ligands and receptors such as glucagon-like peptide-1 (GLP-1), GLP-2 receptor 2, cholecystokinin B (CCKB) and somatostatin receptor, peptide therapeutics such as those that bind to specific cell surface receptors such as G protein-coupled receptors (GPCRs) or ion channels, synthetic or analog peptides from naturally-bioactive peptides, anti-microbial

peptides, pore-forming peptides, tumor targeting or cytotoxic peptides, and degradation or self-destruction peptides such as an apoptosis-inducing peptide signal or photosensitizer peptide.

Peptides useful in the invention described herein also include small antigen-binding peptides, e.g., antigen binding antibody or antibody-like fragments, such as single chain antibodies, nanobodies (see, e.g., Steeland et al. 2016. Nanobodies as therapeutics: big opportunities for small antibodies. Drug Discov Today: 21(7):1076-113). Such small antigen binding peptides may bind a cytosolic antigen, a nuclear antigen, an intra-organellar antigen.

In some embodiments, the composition, anellovector, or anelloVLP described herein includes a polypeptide linked to a ligand that is capable of targeting a specific location, tissue, or cell.

Oligonucleotide aptamers

In some embodiments, the composition, anellovector, or anelloVLP described herein may further comprise an oligonucleotide aptamer. Aptamer moieties are oligonucleotide or peptide aptamers. Oligonucleotide aptamers are single-stranded DNA or RNA (ssDNA or ssRNA) molecules that can bind to pre-selected targets including proteins and peptides with high affinity and specificity.

Oligonucleotide aptamers are nucleic acid species that may be engineered through repeated rounds of *in vitro* selection or equivalently, SELEX (systematic evolution of ligands by exponential enrichment) to bind to various molecular targets such as small molecules, proteins, nucleic acids, and even cells, tissues and organisms. Aptamers provide discriminate molecular recognition, and can be produced by chemical synthesis. In addition, aptamers may possess desirable storage properties, and elicit little or no immunogenicity in therapeutic applications.

Both DNA and RNA aptamers can show robust binding affinities for various targets. For example, DNA and RNA aptamers have been selected for t lysozyme, thrombin, human immunodeficiency virus trans-acting responsive element (HIV TAR),(see en.wikipedia.org/wiki/Aptamer - cite_note-10), hemin, interferon γ, prostate specific antigen (PSA), dopamine, and the non-classical oncogene, heat shock factor 1 (HSF1).

Peptide aptamers

In some embodiments, the composition, anellovector, or anelloVLP described herein may further comprise a peptide aptamer. Peptide aptamers have one (or more) short variable peptide domains, including peptides having low molecular weight, 12–14 kDa. Peptide aptamers may be designed to specifically bind to and interfere with protein-protein interactions inside cells.

Peptide aptamers are artificial proteins selected or engineered to bind specific target molecules. These proteins include of one or more peptide loops of variable sequence. They are typically isolated

from combinatorial libraries and often subsequently improved by directed mutation or rounds of variable region mutagenesis and selection. *In vivo*, peptide aptamers can bind cellular protein targets and exert biological effects, including interference with the normal protein interactions of their targeted molecules with other proteins. In particular, a variable peptide aptamer loop attached to a transcription factor binding domain is screened against the target protein attached to a transcription factor activating domain. In vivo binding of the peptide aptamer to its target via this selection strategy is detected as expression of a downstream yeast marker gene. Such experiments identify particular proteins bound by the aptamers, and protein interactions that the aptamers disrupt, to cause the phenotype. In addition, peptide aptamers derivatized with appropriate functional moieties can cause specific post-translational modification of their target proteins, or change the subcellular localization of the targets

Peptide aptamers can also recognize targets *in vitro*. They have found use in lieu of antibodies in biosensors and used to detect active isoforms of proteins from populations containing both inactive and active protein forms. Derivatives known as tadpoles, in which peptide aptamer "heads" are covalently linked to unique sequence double-stranded DNA "tails", allow quantification of scarce target molecules in mixtures by PCR (using, for example, the quantitative real-time polymerase chain reaction) of their DNA tails.

Peptide aptamer selection can be made using different systems, but the most used is currently the yeast two-hybrid system. Peptide aptamers can also be selected from combinatorial peptide libraries constructed by phage display and other surface display technologies such as mRNA display, ribosome display, bacterial display and yeast display. These experimental procedures are also known as biopannings. Among peptides obtained from biopannings, mimotopes can be considered as a kind of peptide aptamers. All the peptides panned from combinatorial peptide libraries have been stored in a special database with the name MimoDB.

V. Host cells

The invention is further directed to a host or host cell comprising an anellovector or anelloVLP as described herein. In some embodiments, the host or host cell is a plant, insect, bacteria, fungus, vertebrate, mammal (e.g., human), or other organism or cell. In certain embodiments, as confirmed herein, provided anellovectors infect a range of different host cells. Target host cells include cells of mesodermal, endodermal, or ectodermal origin. Target host cells include, e.g., epithelial cells, muscle cells, white blood cells (e.g., lymphocytes), kidney tissue cells, lung tissue cells.

In some embodiments, the anellovector or anelloVLP is substantially non-immunogenic in the host. In certain embodiments, the anellovector or genetic element thereof, or the anelloVLP, fails to produce an undesired substantial response by the host's immune system. Some immune responses

include, but are not limited to, humoral immune responses (e.g., production of antigen-specific antibodies) and cell-mediated immune responses (e.g., lymphocyte proliferation).

In some embodiments, a host or a host cell is contacted with (e.g., infected with) an anellovector or anelloVLP. In some embodiments, the host is a mammal, such as a human. The amount of the anellovector or anelloVLP in the host can be measured at any time after administration. In certain embodiments, a time course of anellovector growth in a culture is determined.

In some embodiments, the anellovector, e.g., an anellovector as described herein, is heritable. In some embodiments, the anellovector is transmitted linearly in fluids and/or cells from mother to child. In some embodiments, daughter cells from an original host cell comprise the anellovector. In some embodiments, a mother transmits the anellovector to child with an efficiency of at least 25%, 50%, 60%, 70%, 80%, 85%, 90%, 95%, or 99%, or a transmission efficiency from host cell to daughter cell at least 25%, 50%, 60%, 70%, 80%, 85%, 90%, 95%, or 99%. In some embodiments, the anellovector in a host cell has a transmission efficiency during meiosis of at 25%, 50%, 60%, 70%, 80%, 85%, 90%, 95%, or 99%. In some embodiments, the anellovector in a host cell has a transmission efficiency during mitosis of at least 25%, 50%, 60%, 70%, 80%, 85%, 90%, 95%, or 99%. In some embodiments, the anellovector in a cell has a transmission efficiency between about 10%-20%, 20%-30%, 30%-40%, 40%-50%, 50%-60%, 60%-70%, 70%-75%, 75%-80%, 80%-85%, 85%-90%, 90%-95%, 95%-99%, or any percentage therebetween.

In some embodiments, the anellovector, e.g., anellovector replicates within the host cell. In one embodiment, the anellovector is capable of replicating in a mammalian cell, e.g., human cell. In other embodiments, the anellovector is replication deficient or replication incompetent.

While in some embodiments the anellovector replicates in the host cell, the anellovector does not integrate into the genome of the host, e.g., with the host's chromosomes. In some embodiments, the anellovector has a negligible recombination frequency, e.g., with the host's chromosomes. In some embodiments, the anellovector has a recombination frequency, e.g., less than about 1.0 cM/Mb, 0.9 cM/Mb, 0.8 cM/Mb, 0.7 cM/Mb, 0.6 cM/Mb, 0.5 cM/Mb, 0.4 cM/Mb, 0.3 cM/Mb, 0.2 cM/Mb, 0.1 cM/Mb, or less, e.g., with the host's chromosomes.

VI. Methods of Use

The anellovectors and compositions comprising anellovectors or anelloVLPs described herein may be used in methods of treating a disease, disorder, or condition, e.g., in a subject (e.g., a mammalian subject, e.g., a human subject) in need thereof. Administration of a pharmaceutical composition described herein may be, for example, by way of parenteral (including intravenous, intratumoral,

intraperitoneal, intramuscular, intracavity, and subcutaneous) administration. The anellovectors or anelloVLP may be administered alone or formulated as a pharmaceutical composition.

The anellovectors or anelloVLP may be administered in the form of a unit-dose composition, such as a unit dose parenteral composition. Such compositions are generally prepared by admixture and can be suitably adapted for parenteral administration. Such compositions may be, for example, in the form of injectable and infusable solutions or suspensions or suppositories or aerosols.

In some embodiments, administration of a anellovector or composition comprising same, e.g., as described herein, may result in delivery of a genetic element comprised by the anellovector to a target cell, e.g., in a subject.

An anellovector or anelloVLP, or composition thereof, described herein, e.g., comprising an effector (e.g., an endogenous or exogenous effector), may be used to deliver the effector to a cell, tissue, or subject. In some embodiments, the anellovector, or anelloVLP, or composition thereof is used to deliver the effector to bone marrow, blood, heart, GI or skin. Delivery of an effector by administration of an anellovector composition or anelloVLP composition described herein may modulate (e.g., increase or decrease) expression levels of a noncoding RNA or polypeptide in the cell, tissue, or subject. Modulation of expression level in this fashion may result in alteration of a functional activity in the cell to which the effector is delivered. In some embodiments, the modulated functional activity may be enzymatic, structural, or regulatory in nature.

In some embodiments, the anellovector or anelloVLP, or copies thereof, are detectable in a cell 24 hours (e.g., 1 day, 2 days, 3 days, 4 days, 5 days, 6 days, 1 week, 2 weeks, 3 weeks, 4 weeks, 30 days, or 1 month) after delivery into a cell. In embodiments, an anellovector, anelloVLP, or composition thereof mediates an effect on a target cell, and the effect lasts for at least 1, 2, 3, 4, 5, 6, or 7 days, 2, 3, or 4 weeks, or 1, 2, 3, 6, or 12 months. In some embodiments (e.g., wherein the anellovector or composition thereof comprises a genetic element encoding an exogenous protein), the effect lasts for less than 1, 2, 3, 4, 5, 6, or 7 days, 2, 3, or 4 weeks, or 1, 2, 3, 6, or 12 months.

Examples of diseases, disorders, and conditions that can be treated with an anellovector or anelloVLP described herein, or a composition comprising the anellovector or anelloVLP, include, without limitation: immune disorders, interferonopathies (e.g., Type I interferonopathies), infectious diseases, inflammatory disorders, autoimmune conditions, cancer (e.g., a solid tumor, e.g., lung cancer, non-small cell lung cancer, e.g., a tumor that expresses a gene responsive to mIR-625, e.g., caspase-3), and gastrointestinal disorders. In some embodiments, the anellovector or anelloVLP modulates (e.g., increases or decreases) an activity or function in a cell with which the anellovector is contacted. In some embodiments, the anellovector or anelloVLP modulates (e.g., increases or decreases) the level or activity of a molecule (e.g., a nucleic acid or a protein) in a cell with which the anellovector or anelloVLP is

contacted. In some embodiments, the anellovector or anelloVLP decreases viability of a cell, e.g., a cancer cell, with which the anellovector or anelloVLP is contacted, e.g., by at least about 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, 99%, or more. In some embodiments, the anellovector or anelloVLP comprises an effector, e.g., an miRNA, e.g., miR-625, that decreases viability of a cell, e.g., a cancer cell, with which the anellovector or anelloVLP is contacted, e.g., by at least about 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, 99%, or more. In some embodiments, the anellovector or anelloVLP increases apoptosis of a cell, e.g., a cancer cell, e.g., by increasing caspase-3 activity, with which the anellovector is contacted, e.g., by at least about 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, 99%, or more. In some embodiments, the anellovector or anelloVLP comprises an effector, e.g., an miRNA, e.g., miR-625, that increases apoptosis of a cell, e.g., a cancer cell, e.g., by increasing caspase-3 activity, with which the anellovector or anelloVLP is contacted, e.g., by at least about 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, 99%, or more.

VII. Methods of Production

Producing the Genetic Element

Methods of making the genetic element of the anellovector are described in, for example, Khudyakov & Fields, *Artificial DNA: Methods and Applications*, CRC Press (2002); in Zhao, *Synthetic Biology: Tools and Applications*, (First Edition), Academic Press (2013); and Egli & Herdewijn, *Chemistry and Biology of Artificial Nucleic Acids*, (First Edition), Wiley-VCH (2012).

In some embodiments, the genetic element may be designed using computer-aided design tools. The anellovector may be divided into smaller overlapping pieces (e.g., in the range of about 100 bp to about 10 kb segments or individual ORFs) that are easier to synthesize. These DNA segments are synthesized from a set of overlapping single-stranded oligonucleotides. The resulting overlapping synthons are then assembled into larger pieces of DNA, e.g., the anellovector. The segments or ORFs may be assembled into the anellovector, e.g., in vitro recombination or unique restriction sites at 5' and 3' ends to enable ligation.

The genetic element can alternatively be synthesized with a design algorithm that parses the anellovector into oligo-length fragments, creating optimal design conditions for synthesis that take into account the complexity of the sequence space. Oligos are then chemically synthesized on semiconductor-based, high-density chips, where over 200,000 individual oligos are synthesized per chip. The oligos are assembled with an assembly techniques, such as BioFab®, to build longer DNA segments from the smaller oligos. This is done in a parallel fashion, so hundreds to thousands of synthetic DNA segments are built at one time.

Each genetic element or segment of the genetic element may be sequence verified. In some embodiments, high-throughput sequencing of RNA or DNA can take place using AnyDot.chips (Genovoxx, Germany), which allows for the monitoring of biological processes (e.g., miRNA expression or allele variability (SNP detection). In particular, the AnyDot-chips allow for 10x-50x enhancement of nucleotide fluorescence signal detection. AnyDot.chips and methods for using them are described in part in International Publication Application Nos. WO 02088382, WO 03020968, WO 0303 1947, WO 2005044836, PCTEP 05105657, PCMEP 05105655; and German Patent Application Nos. DE 101 49 786, DE 102 14 395, DE 103 56 837, DE 10 2004 009 704, DE 10 2004 025 696, DE 10 2004 025 746, DE 10 2004 025 694, DE 10 2004 025 695, DE 10 2004 025 744, DE 10 2004 025 745, and DE 10 2005 012 301.

Other high-throughput sequencing systems include those disclosed in Venter, J., et al. Science 16 Feb. 2001; Adams, M. et al, Science 24 Mar. 2000; and M. J, Levene, et al. Science 299:682-686, January 2003; as well as US Publication Application No. 20030044781 and 2006/0078937. Overall such systems involve sequencing a target nucleic acid molecule having a plurality of bases by the temporal addition of bases via a polymerization reaction that is measured on a molecule of nucleic acid, i.e., the activity of a nucleic acid polymerizing enzyme on the template nucleic acid molecule to be sequenced is followed in real time. The sequence can then be deduced by identifying which base is being incorporated into the growing complementary strand of the target nucleic acid by the catalytic activity of the nucleic acid polymerizing enzyme at each step in the sequence of base additions. A polymerase on the target nucleic acid molecule complex is provided in a position suitable to move along the target nucleic acid molecule and extend the oligonucleotide primer at an active site. A plurality of labeled types of nucleotide analogs are provided proximate to the active site, with each distinguishably type of nucleotide analog being complementary to a different nucleotide in the target nucleic acid sequence. The growing nucleic acid strand is extended by using the polymerase to add a nucleotide analog to the nucleic acid strand at the active site, where the nucleotide analog being added is complementary to the nucleotide of the target nucleic acid at the active site. The nucleotide analog added to the oligonucleotide primer as a result of the polymerizing step is identified. The steps of providing labeled nucleotide analogs, polymerizing the growing nucleic acid strand, and identifying the added nucleotide analog are repeated so that the nucleic acid strand is further extended and the sequence of the target nucleic acid is determined.

In some embodiments, shotgun sequencing is performed. In shotgun sequencing, DNA is broken up randomly into numerous small segments, which are sequenced using the chain termination method to obtain reads. Multiple overlapping reads for the target DNA are obtained by performing several rounds of this fragmentation and sequencing. Computer programs then use the overlapping ends of different reads to assemble them into a continuous sequence.

In some embodiments, factors for replicating or packaging may be supplied in *cis* or in *trans*, relative to the genetic element. For example, when supplied in *cis*, the genetic element may comprise one or more genes encoding an *Anellovirus* ORF1, ORF1/1, ORF1/2, ORF2, ORF2/2, ORF2/3, or ORF2t/3, e.g., as described herein. In some embodiments, replication and/or packaging signals can be incorporated into a genetic element, for example, to induce amplification and/or encapsulation. In some embodiments, this is done both in context of larger regions of the anellovector genome (e.g., inserting effectors into a specific site in the genome, or replacing viral ORFs with effectors).

In another example, when supplied in *trans*, the genetic element may lack genes encoding one or more of an *Anellovirus* ORF1, ORF1/1, ORF1/2, ORF2, ORF2/2, ORF2/3, or ORF2t/3, e.g., as described herein; this protein or proteins may be supplied, e.g., by another nucleic acid, e.g., a helper nucleic acid. In some embodiments, minimal *cis* signals (e.g., 5' UTR and/or GC-rich region) are present in the genetic element. In some embodiments, the genetic element does not encode replication or packaging factors (e.g., replicase and/or capsid proteins). Such factors may, in some embodiments, be supplied by one or more helper nucleic acids (e.g., a helper viral nucleic acid, a helper plasmid, or a helper nucleic acid integrated into the host cell genome). In some embodiments, the helper nucleic acids express proteins and/or RNAs sufficient to induce amplification and/or packaging, but may lack their own packaging signals. In some embodiments, the genetic element and the helper nucleic acid are introduced into the host cell (e.g., concurrently or separately), resulting in amplification and/or packaging of the genetic element but not of the helper nucleic acid.

In vitro circularization

In some instances, the genetic element to be packaged into a proteinaceous exterior is a single stranded circular DNA. The genetic element may, in some instances, be introduced into a host cell in a form other than a single stranded circular DNA. For example, the genetic element may be introduced into the host cell as a double-stranded circular DNA. The double-stranded circular DNA may then be converted into a single-stranded circular DNA in the host cell (e.g., a host cell comprising a suitable enzyme for rolling circle replication, e.g., an *Anellovirus* Rep protein, e.g., Rep68/78, Rep60, RepA, RepB, Pre, MobM, TraX, TrwC, Mob02281, Mob02282, NikB, ORF50240, NikK, TecH, OrfJ, or TraI, e.g., as described in Wawrzyniak et al. 2017, *Front. Microbiol.* 8: 2353; incorporated herein by reference with respect to the listed enzymes). In some embodiments, the double-stranded circular DNA is produced by *in vitro* circularization, e.g., as described in Example 35 of PCT Publication No. WO 2020/123816, incorporated by reference herein in its entirety. Generally, *in vitro* circularized DNA constructs can be produced by digesting a plasmid comprising the sequence of a genetic element to be packaged, such that the genetic element sequence is excised as a linear DNA molecule. The resultant linear DNA can then be

ligated, e.g., using a DNA ligase, to form a double-stranded circular DNA. In some instances, a double-stranded circular DNA produced by *in vitro* circularization can undergo rolling circle replication, e.g., as described herein. Without wishing to be bound by theory, it is contemplated that *in vitro* circularization results in a double-stranded DNA construct that can undergo rolling circle replication without further modification, thereby being capable of producing single-stranded circular DNA of a suitable size to be packaged into an anellovector, e.g., as described herein. In some embodiments, the double-stranded DNA construct is smaller than a plasmid (e.g., a bacterial plasmid). In some embodiments, the double-stranded DNA construct is excised from a plasmid (e.g., a bacterial plasmid) and then circularized, e.g., by *in vitro* circularization.

Producing the Anellovector

The genetic elements and vectors comprising the genetic elements prepared as described herein can be used in a variety of ways to express the anellovector in appropriate host cells. In some embodiments, the genetic element and vectors comprising the genetic element are transfected in appropriate host cells and the resulting RNA may direct the expression of the anellovector gene products, e.g., non-pathogenic protein and protein binding sequence, at high levels. Host cell systems which provide for high levels of expression include continuous cell lines that supply viral functions, such as cell lines superinfected with APV or MPV, respectively, cell lines engineered to complement APV or MPV functions, etc.

In some embodiments, the anellovector is produced as described in any of Examples 21, 24, or 25. In some embodiments, the anellovector is produced as described in any of Examples 1, 2, 5, 6, or 15-17 of PCT Publication No. WO 2020/123816, incorporated by reference herein in its entirety.

In some embodiments, the anellovector is cultivated in continuous animal cell lines in vitro. According to one embodiment of the invention, the cell lines may include porcine cell lines. The cell lines envisaged in the context of the present invention include immortalised porcine cell lines such as, but not limited to the porcine kidney epithelial cell lines PK-15 and SK, the monomyeloid cell line 3D4/31 and the testicular cell line ST. Also, other mammalian cells lines are included, such as CHO cells (Chinese hamster ovaries), MARC-145, MDBK, RK-13, EEL. Additionally or alternatively, particular embodiments of the methods of the invention make use of an animal cell line which is an epithelial cell line, i.e. a cell line of cells of epithelial lineage. Cell lines susceptible to infection with anellovectors include, but are not limited to cell lines of human or primate origin, such as human or primate kidney carcinoma cell lines.

In some embodiments, the genetic elements and vectors comprising the genetic elements are transfected into cell lines that express a viral polymerase protein in order to achieve expression of the

anellovector. To this end, transformed cell lines that express an anellovector polymerase protein may be utilized as appropriate host cells. Host cells may be similarly engineered to provide other viral functions or additional functions.

To prepare the anellovector disclosed herein, a genetic element or vector comprising the genetic element disclosed herein may be used to transfect cells which provide anellovector proteins and functions required for replication and production. Alternatively, cells may be transfected with helper virus before, during, or after transfection by the genetic element or vector comprising the genetic element disclosed herein. In some embodiments, a helper virus may be useful to complement production of an incomplete viral particle. The helper virus may have a conditional growth defect, such as host range restriction or temperature sensitivity, which allows the subsequent selection of transfectant viruses. In some embodiments, a helper virus may provide one or more replication proteins utilized by the host cells to achieve expression of the anellovector. In some embodiments, the host cells may be transfected with vectors encoding viral proteins such as the one or more replication proteins. In some embodiments, a helper virus comprises an antiviral sensitivity.

The genetic element or vector comprising the genetic element disclosed herein can be replicated and produced into anellovector particles by any number of techniques known in the art, as described, e.g., in U.S. Pat. No. 4,650,764; U.S. Pat. No. 5,166,057; U.S. Pat. No. 5,854,037; European Patent Publication EP 0702085A1; U.S. patent application Ser. No. 09/152,845; International Patent Publications PCT WO97/12032; WO96/34625; European Patent Publication EP-A780475; WO 99/02657; WO 98/53078; WO 98/02530; WO 99/15672; WO 98/13501; WO 97/06270; and EPO 780 47SA1, each of which is incorporated by reference herein in its entirety.

The production of anellovector-containing cell cultures according to the present invention can be carried out in different scales, such as in flasks, roller bottles or bioreactors. The media used for the cultivation of the cells to be infected are known to the skilled person and can generally comprise the standard nutrients required for cell viability, but may also comprise additional nutrients dependent on the cell type. Optionally, the medium can be protein-free and/or serum-free. Depending on the cell type the cells can be cultured in suspension or on a substrate. In some embodiments, different media is used for growth of the host cells and for production of anellovectors.

The purification and isolation of anellovectors can be performed according to methods known by the skilled person in virus production and is described for example by Rinaldi, et al., DNA Vaccines: Methods and Protocols (Methods in Molecular Biology), 3rd ed. 2014, Humana Press.

In one aspect, the present invention includes a method for the in vitro replication and propagation of the anellovector as described herein, which may comprise the following steps: (a) transfecting a

linearized genetic element into a cell line sensitive to anellovector infection; (b) harvesting the cells and isolating cells showing the presence of the genetic element; (c) culturing the cells obtained in step (b) for at least three days, such as at least one week or longer, depending on experimental conditions and gene expression; and (d) harvesting the cells of step (c).

In some embodiments, an anellovector may be introduced to a host cell line grown to a high cell density. In some embodiments, the anellovector may be harvested and/or purified by separation of solutes based on biophysical properties, e.g., ion exchange chromatography or tangential flow filtration, prior to formulation with a pharmaceutical excipient.

VIII. Administration/Delivery

The composition (e.g., a pharmaceutical composition comprising an anellovector or anelloVLP as described herein) may be formulated to include a pharmaceutically acceptable excipient. Pharmaceutical compositions may optionally comprise one or more additional active substances, e.g. therapeutically and/or prophylactically active substances. Pharmaceutical compositions of the present invention may be sterile and/or pyrogen-free. General considerations in the formulation and/or manufacture of pharmaceutical agents may be found, for example, in Remington: The Science and Practice of Pharmacy 21st ed., Lippincott Williams & Wilkins, 2005 (incorporated herein by reference).

Although the descriptions of pharmaceutical compositions provided herein are principally directed to pharmaceutical compositions which are suitable for administration to humans, it will be understood by the skilled artisan that such compositions are generally suitable for administration to any other animal, e.g., to non-human animals, e.g. non-human mammals. Modification of pharmaceutical compositions suitable for administration to humans in order to render the compositions suitable for administration to various animals is well understood, and the ordinarily skilled veterinary pharmacologist can design and/or perform such modification with merely ordinary, if any, experimentation. Subjects to which administration of the pharmaceutical compositions is contemplated include, but are not limited to, humans and/or other primates; mammals, including commercially relevant mammals such as cattle, pigs, horses, sheep, cats, dogs, mice, and/or rats; and/or birds, including commercially relevant birds such as poultry, chickens, ducks, geese, and/or turkeys.

Formulations of the pharmaceutical compositions described herein may be prepared by any method known or hereafter developed in the art of pharmacology. In general, such preparatory methods include the step of bringing the active ingredient into association with an excipient and/or one or more other accessory ingredients, and then, if necessary and/or desirable, dividing, shaping and/or packaging the product.

In one aspect, the invention features a method of delivering an anellovector or anelloVLP to a subject. The method includes administering a pharmaceutical composition comprising an anellovector or anelloVLP as described herein to the subject. In some embodiments, the administered anellovector or anelloVLP replicates in the subject (e.g., becomes a part of the virome of the subject).

The pharmaceutical composition may include wild-type or native viral elements and/or modified viral elements. The anellovector may include one or more of the sequences (e.g., nucleic acid sequences or nucleic acid sequences encoding amino acid sequences thereof) in any one of Tables N1-N26 or a sequence with at least about 60%, 65%, 70%, 75%, 80%, 85%, 90% 95%, 96%, 97%, 98% and 99% nucleotide sequence identity to any one of the nucleotide sequences or a sequence that is complementary to the sequence in any one of Tables N1-N26. The anellovector may comprise a nucleic acid molecule comprising a nucleic acid sequence with at least about 60%, 65%, 70%, 75%, 80%, 85%, 90% 95%, 96%, 97%, 98% and 99% sequence identity to one or more of the sequences in any one of Tables N1-N26. The anellovector may comprise a nucleic acid molecule encoding an amino acid sequence with at least about 60%, 65%, 70%, 75%, 80%, 85%, 90% 95%, 96%, 97%, 98% and 99% sequence identity to any one of the amino acid sequences in any one of Tables A1-A26. The anellovector may comprise a polypeptide comprising an amino acid sequence with at least about 60%, 65%, 70%, 75%, 80%, 85%, 90% 95%, 96%, 97%, 98% and 99% sequence identity to any one of the amino acid sequences in any one of Tables A1-A26. The anellovector may include one or more of the sequences in any one of Tables A1-A26 or N1-N26, or a sequence with at least about 60%, 65%, 70%, 75%, 80%, 85%, 90% 95%, 96%, 97%, 98% and 99% nucleotide sequence identity to any one of the nucleotide sequences or a sequence that is complementary to the sequence in any one of Tables N1-N26.

In some embodiments, the anellovector or anelloVLP is sufficient to increase (stimulate) endogenous gene and protein expression, e.g., at least about 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, or more as compared to a reference, e.g., a healthy control. In certain embodiments, the anellovector or anelloVLP is sufficient to decrease (inhibit) endogenous gene and protein expression, e.g., at least about 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, or more as compared to a reference, e.g., a healthy control.

In some embodiments, the anellovector or anelloVLP inhibits/enhances one or more viral properties, e.g., tropism, infectivity, immunosuppression/activation, in a host or host cell, e.g., at least about 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, or more as compared to a reference, e.g., a healthy control.

In some embodiments, the subject is administered the pharmaceutical composition further comprising one or more viral strains that are not represented in the viral genetic information.

In some embodiments, the pharmaceutical composition comprising an anellovector or anelloVLP described herein is administered in a dose and time sufficient to modulate a viral infection. Some non-limiting examples of viral infections include adeno-associated virus, Aichi virus, Australian bat lyssavirus, BK polyomavirus, Banna virus, Barmah forest virus, Bunyamwera virus, Bunyavirus La Crosse, Bunyavirus snowshoe hare, Cercopithecine herpesvirus, Chandipura virus, Chikungunya virus, Cosavirus A, Cowpox virus, Coxsackievirus, Crimean-Congo hemorrhagic fever virus, Dengue virus, Dhori virus, Dugbe virus, Duvenhage virus, Eastern equine encephalitis virus, Ebolavirus, Echovirus, Encephalomyocarditis virus, Epstein-Barr virus, European bat lyssavirus, GB virus C/Hepatitis G virus, Hantaan virus, Hendra virus, Hepatitis A virus, Hepatitis B virus, Hepatitis C virus, Hepatitis E virus, Hepatitis delta virus, Horsepox virus, Human adenovirus, Human astrovirus, Human coronavirus, Human cytomegalovirus, Human enterovirus 68, Human enterovirus 70, Human herpesvirus 1, Human herpesvirus 2, Human herpesvirus 6, Human herpesvirus 7, Human herpesvirus 8, Human immunodeficiency virus, Human papillomavirus 1, Human papillomavirus 2, Human papillomavirus 16, Human papillomavirus 18, Human parainfluenza, Human parvovirus B19, Human respiratory syncytial virus, Human rhinovirus, Human SARS coronavirus, Human spumaretrovirus, Human T-lymphotropic virus, Human torovirus, Influenza A virus, Influenza B virus, Influenza C virus, Isfahan virus, JC polyomavirus, Japanese encephalitis virus, Junin arenavirus, KI Polyomavirus, Kunjin virus, Lagos bat virus, Lake Victoria marburgvirus, Langat virus, Lassa virus, Lordsdale virus, Louping ill virus, Lymphocytic choriomeningitis virus, Machupo virus, Mayaro virus, MERS coronavirus, Measles virus, Mengo encephalomyocarditis virus, Merkel cell polyomavirus, Mokola virus, Molluscum contagiosum virus, Monkeypox virus, Mumps virus, Murray valley encephalitis virus, New York virus, Nipah virus, Norwalk virus, O'nyong-nyong virus, Orf virus, Oropouche virus, Pichinde virus, Poliovirus, Punta toro phlebovirus, Puumala virus, Rabies virus, Rift valley fever virus, Rosavirus A, Ross river virus, Rotavirus A, Rotavirus B, Rotavirus C, Rubella virus, Sagiyama virus, Salivirus A, Sandfly fever sicilian virus, Sapporo virus, Semliki forest virus, Seoul virus, Simian foamy virus, Simian virus 5, Sindbis virus, Southampton virus, St. louis encephalitis virus, Tick-borne powassan virus, Torque teno virus, Toscana virus, Uukuniemi virus, Vaccinia virus, Varicella-zoster virus, Variola virus, Venezuelan equine encephalitis virus, Vesicular stomatitis virus, Western equine encephalitis virus, WU polyomavirus, West Nile virus, Yaba monkey tumor virus, Yaba-like disease virus, Yellow fever virus, and Zika Virus. In certain embodiments, the anellovector or anelloVLP is sufficient to outcompete and/or displace a virus already present in the subject, e.g., at least about 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, or more as compared to a reference. In certain embodiments, the anellovector or anelloVLP is sufficient to compete with chronic or acute viral infection. In certain embodiments, the anellovector or anelloVLP may be administered prophylactically to protect from viral infections (e.g. a provirotic). In some

embodiments, the anellovector or anelloVLP is in an amount sufficient to modulate (e.g., phenotype, virus levels, gene expression, compete with other viruses, disease state, etc. at least about 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, or more).

Redosing

The anellovectors or anelloVLPs described herein can, in some instances, be used as a delivery vehicle that can be administered in multiple doses (e.g., doses administered separately). While not wishing to be bound by theory, in some embodiments, an anellovector or anelloVLP (e.g., as described herein) induces a relatively low immune response (as measured, for example, as 50% GMT values, e.g., as observed in Example 28), e.g., allowing for repeated dosing of a subject with one or more anellovectors or anelloVLPs (e.g., multiple doses of the same anellovector or anelloVLP or different anellovectors or anelloVLPs). In an aspect, the invention provides a method of delivering an effector, comprising administering to a subject a first plurality of anellovectors or anelloVLPs and then a second plurality of anellovectors or anelloVLPs. In some embodiments, the second plurality of anellovectors or anelloVLPs comprise the same proteinaceous exterior as the anellovectors or anelloVLPs of the first plurality. In another aspect, the invention provides a method of selecting a subject (e.g., a human subject) to receive an effector, wherein the subject previously received, or was identified as having received, a first plurality of anellovectors or anelloVLPs comprising a genetic element encoding an effector, in which the method involves selecting the subject to receive a second plurality of anellovectors or anelloVLPs comprising a genetic element encoding an effector (e.g., the same effector as that encoded by the genetic element of the first plurality of anellovectors or anelloVLPs, or a different effector as that encoded by the genetic element of the first plurality of anellovectors or anelloVLPs). In another aspect, the invention provides a method of identifying a subject (e.g., a human subject) as suitable to receive a second plurality of anellovectors or anelloVLPs, the method comprising identifying the subject has having previously received a first plurality of anellovectors or anelloVLPs comprising a genetic element encoding an effector, wherein the subject being identified as having received the first plurality of anellovectors or anelloVLPs is indicative that the subject is suitable to receive the second plurality of anellovectors or anelloVLPs.

In some embodiments, the second plurality of anellovectors or anelloVLPs comprises a proteinaceous exterior with at least one surface epitope in common with the anellovectors or anelloVLPs of the first plurality of anellovectors or anelloVLPs. In some embodiments, the anellovectors or anelloVLPs of the first plurality and the anellovectors or anelloVLPs of the second plurality carry genetic elements encoding the same effector. In some embodiments, the anellovectors or anelloVLPs of the first

plurality and the anellovectors or anelloVLPs of the second plurality carry genetic elements encoding different effectors.

In some embodiments, the second plurality comprises about the same quantity and/or concentration of anellovectors or anelloVLPs as the first plurality (e.g., when normalized to the body mass of the subject at the time of administration), e.g., the second plurality comprises 90-110%, e.g., 95-105% of the number of anellovectors or anelloVLPs in the first plurality when normalized to body mass of the subject at the time of administration. In some embodiments, wherein the first plurality comprises a greater dosage of anellovectors or anelloVLPs than the second plurality, e.g., wherein the first plurality comprises a greater quantity and/or concentration of anellovectors or anelloVLPs relative to the second plurality. In some embodiments, wherein the first plurality comprises a lower dosage of anellovectors or anelloVLPs than the second plurality, e.g., wherein the first plurality comprises a lower quantity and/or concentration of anellovectors or anelloVLPs relative to the second plurality.

In some embodiments, the subject is evaluated between the administration of the first and second pluralities of anellovectors or anelloVLPs, e.g., for the presence (e.g., persistence) of anellovectors or anelloVLPs from the first plurality, or progeny thereof. In some embodiments, the subject is administered the second plurality of anellovectors or anelloVLPs if the presence of anellovectors or anelloVLPs from the first plurality, or the progeny thereof, are not detected.

In some embodiments, the second plurality is administered to the subject at least 1, 2, 3, or 4 weeks, or 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, or 12 months, or 1, 2, 3, 4, 5, 10, or 20 years after the administration of the first plurality to the subject. In some embodiments, the second plurality is administered to the subject between 1-2 weeks, 2-3 weeks, 3-4 weeks, 1-2 months, 3-4 months, 4-5 months, 5-6 months, 6-7 months, 7-8 months, 8-9 months, 9-10 months, 10-11 months, 11-12 months, 1-2 years, 2-3 years, 3-4 years, 4-5 years, 5-10 years, or 10-20 years after the administration of the first plurality to the subject. In some embodiments, the method comprises administering a repeated dose of anellovectors or anelloVLPs over the course of at least 1, 2, 3, 4, or 5 years.

In some embodiments, the method further comprises assessing, after administration of the first plurality and before administration of the second plurality, one or more of:

a) the level or activity of the effector in the subject (e.g., by detecting a protein effector, e.g., by ELISA; by detecting a nucleic acid effector, e.g., by RT-PCR, or by detecting a downstream effect of the effector, e.g., level of an endogenous gene affected by the effector);

b) the level or activity of the anellovector or anelloVLP of the first plurality in the subject (e.g., by detecting the level of the VP1 of the anellovector or anelloVLP);

c) the presence, severity, progression, or a sign or symptom of a disease in the subject that the anellovector or anelloVLP was administered to treat; and/or

d) the presence or level of an immune response, e.g., neutralizing antibodies, against an anellovector or anelloVLP.

In some embodiments, the method further comprises administering to the subject a third, fourth, fifth, and/or further plurality of anellovectors or anelloVLPs, e.g., as described herein.

In some embodiments, the first plurality and the second plurality are administered via the same route of administration, e.g., intravenous administration. In some embodiments, the first plurality and the second plurality are administered via different routes of administration. In some embodiments, the first and the second pluralities are administered by the same entity (e.g., the same health care provider). In some embodiments, the first and the second pluralities are administered by different entities (e.g., different health care providers).

All references and publications cited herein are hereby incorporated by reference.

The following examples are provided to further illustrate some embodiments of the present invention, but are not intended to limit the scope of the invention; it will be understood by their exemplary nature that other procedures, methodologies, or techniques known to those skilled in the art may alternatively be used. Additional examples can be found, for example, in PCT Publication No. WO 2020/123816, incorporated by reference herein in its entirety.

EXAMPLES

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Example 1. Expression of ORF1 constructs for conjugation

In nature, Anelloviruses encapsidate circularized negative sense (ns) single stranded (ss) DNA genomes in part through interactions between the genome and the N-terminal arginine rich motif (ARM). UV absorption evidence suggests recombinant ORF1 containing the ARM forms virus like particles (VLPs) that are bound to nucleic acids, presumably non-specific host cell DNA fragments. To remove these potentially undesired host cell impurities, ORF1 constructs are generated in which the ARM is deleted (delARM ORF1).

Expression of Arginine-rich region deletion (delARM) ORF1 constructs

In one example, a delARM mutant was generated for the Ring 10 ORF1 protein, which was then produced in Sf9 insect cells. Without wishing to be bound by theory, this VLP may not contain the same level of host cell nucleic acid impurities as the structural arginine-rich region, also referred to herein as the structural arginine-rich motif (ARM; also referred to herein as the structural arginine-rich region), is removed (referred to herein as "delARM"). Ring 10 delARM ORF1 is expressed with the complete C-terminal region present. During purification, the structural C-terminal domain or a portion thereof is clipped by protease activity resulting in a C-terminal deletion delARM construct.

Anellovirus ORF1 molecules, such as an ORF1 protein in which the ARM has been deleted, harbor a basic residue (lysine) on the particle surface which would be suitable for conjugating surface effectors including peptides, proteins such as antibodies, glycan groups or neuclic acid entities which are covalently bound to N-hydroxysuccinimide (NHS). NHS is a click-chemistry which binds to amino groups such as those located on lysine residues.

Expression of C-terminal deletion ORF1 constructs

Recombinant ORF1s do not need the C-terminal region to form VLPs. While the biological role of the C-terminal region has yet to be elucidated regarding virus formation or viral infection, we have demonstrated that the C-terminal region can be removed and permit efficient VLP formation. One example is the Ring 2 ORF1 C-terminal truncation at residue 611 generated in 293 cells (e.g., as listed in Table B1-3a). After observing that Ring 10 delARM has the C-terminus cleaved by proteases during the purification process, we explored generating VLPs lacking C-terminal region to make a more uniform

product. Without wishing to be bound by theory, it is contemplated that deleting this region for the AnelloVLP surface effector conjugation approach may eliminate immune responses to the C-terminal region (i.e. eliminate anti-drug responses). In addition, generating ORF1 VLPs in mammalian cells (e.g., 293 cells) further suggests these particles could be generated in other eukaryotic cell lines such as stable CHO cell lines to provide alternative production processes.

Example 2. Conjugation of peptides via NHS click chemistry

Based on the structural analysis described herein, basic residues (lysine residues) of Ring 10 ORF1 were identified as surface exposed on the VLP viral surface. Several surface effectors can be conjugated to the amine groups of lysine by adding the click-chemistry entity NHS. In one example, the short antigenic region of the malaria CS protein (for example, the NANP repeat region known to be immunogenic in the R21 malaria vaccine currently being evaluated in the clinic) can be synthesized with an NHS moiety. By mixing the NANP-NHS peptide with ORF1 VLPs, we can conjugate the antigenic region of the R21 vaccine candidate to the ORF1 VLP generating a novel malaria particle as a vaccine candidate. Conjugation of other entities to the surface lysines though NHS conjugation could also be generated such as anti-sense oligomers (ASOs) or glycans from other bacteria known to provide an immune response in vaccines (such as, for example, the glycans of Prevnar13).

Example 3. Conjugation of polypeptides via two-step click chemistry

As discussed in Example 2, surface exposed lysines are available for NHS conjugation. However, not every surface effector may be available for synthetic addition of NHS such as peptides, oligomers or glycans. In one example, larger surface effectors such as proteins (antibody fragments or larger vaccine antigens) could be generated with free cysteine, which can further be conjugated with click-chemistries, for example, by virtue of maleimide-click-chemistry linkers (maleimide being a click chemistry that binds thiols of free cysteine), which in turn can be added to paired chemistries on conjugated to free lysine on the VLP surface. In one example, a free cysteine could be engineered into a polypeptide encoding the C-terminal (immunogenic) portion of the malaria CS protein. This protein could then be conjugated by virtue of its free cysteine to a click chemistry moiety such as azide. The ORF1 VLP could be further conjugated by NHS-DBCO (DBCO being the click-chemistry partner of azide) by virtue of the surface lysines on ORF1. When the VLP-DBCO species is combined with the Azide-Malaria polypeptide species, the DBCO-Azide conjugation would produce a covalently attached Malaria-VLP particle suitable as a malaria vaccine candidate. This two-step conjugation approach could be suitable for other larger surface effectors where direct synthesis with click-chemistry moieties is not available, including antibodies and larger nucleic acid oligomers.

Example 4. Conjugation of polypeptides via maleimide conjugation

In Examples 2 or 3 above, conjugation of surface effectors is done through conjugation of NHS moieties to surface lysines. This approach could have the added advantage of adding several surface effectors to the ORF1, depending on how many lysines are surface exposed in a given strain of ORF1. However, specific conjugation to ORF1 may be desired for some surface effectors to generate a more controlled product. In this example, a Ring 10 ORF1 structure (determined according to the structural analysis described herein) is used to define surface exposed regions of the VLP and introduce a free cysteine suitable for maleimide conjugation (maleimide being a click-chemistry that binds thiols in free cysteine residues), for example, at residues in the P2 domain of Ring10, such as Threonine 365. These engineered VLPs, perhaps in combination with deletion of the ARM as described in Example 1, would provide a cleaner, more controlled conjugation. By replacing the NHS species in Examples 2 and 3 with maleimide moieties, the conjugation of the surface effector, such as a maleimide malaria peptide, could be specifically targeted to a particular residue of interest, e.g., at a controlled stoichiometry.

To ensure only the desired target cysteines are available for modification with antigenic peptides or other surface effector molecules, the native cysteine residues of ORF1 (e.g., located at positions: 57, 64, 112, 131, 220, 223, 626) will also be replaced with either serine or alanine residues. When the residue to be mutated is on the surface of the molecule and the sidechain is exposed to solvent, replacing a cysteine with a serine is a conservative alteration as the two residues are isosteric and therefore should not disrupt the 3-dimensional structure of the protein. When the residue to be mutated is buried inside of the structure, alanine is a more conservative alteration as the polar side chain of serine is likely to destabilize the structure.

Example 5. Genetic grafting of surface effector to the VLP surface

Using a structure determined for Ring 10, the region of the VLP that is surface exposed can be identified, for example, such as an HVR region. In this example, a VLP construct is generated in which a surface effector coding sequence replaces or is inserted into the HVR region of ORF. One example could be the genetic fusion of the malaria CS immunogenic portion into the HVR region of ORF1 VLPs (FIG. 2). In these genetic fusions, the VLP does not require conjugation, but rather a fusion protein comprises an ORF1 portion and a surface effector portion, such that the surface effector portion is displayed on the outer surface of the VLP. The advantage of this approach would be alleviating the need to conjugate the VLP post purification, which may not be an efficient process. Another advantage would be that each copy of the ORF1 molecule would be known to contain one copy of the surface effector. In some

iterations it may be advantageous to express ORF1 with and without surface effectors generating mixed VLPs with fewer surface effectors.

Without wishing to be bound by theory, it is contemplated that the truncation of N- and C-termini will be beneficial for anelloVLP formation. Positive charged N-termini without nucleic acid appeared to improve the packaging of anelloVLP, as observed by electron microscopy in anelloviruses. Removal of the N-terminal peptide has been shown to boost anelloVLP titer and provide a more homogeneous morphology. On the other end, although the C-terminal domain does not have any charge tendency, the potential post-translational modification may hamper the maturation of anelloVLPs, as shown by electron microscopy. The removal of the C-terminal domain avoids such maturation process and thereby is expected to stabilize anelloVLP particle morphology. Based on these studies, we have engineered both N- and C- termini to carry different tags for purification, fluorescent tag labeling for biodistribution assay, or other epitopes as shown in Tables B2-4, B2-5, or B2-6,. For example, we have inserted an AVI tag on the exterior P2 site for biotinylation or other epitope for vaccine development, e.g. malaria CS protein (see Table B2-4). Thus, a surface effector may be grafted onto the N- and/or C-terminus of an ORF 1 molecule.

In an example, a surface effector and ORF1 fusion protein comprises an amino acid sequence as listed in Table E1 below.

Table E1. Exemplary ORF1-surface effector fusion protein amino acid sequences

Construct Name	Amino Acid Sequence
R21 construct [184 C-Terminal residues of CS protein (CS-Cterm) fused to the Hepatitis B Virus Surface Antigen (HBsAg)]	ANPNANPNANPNANPNANPNANPNANPNANPNANPNANPNANPN ANPNANPNANPNKNNQGNGQGHNMPNDPNRNVDENANANSAVKNNNNE EPSDKHIKEYLNKIQNSLSTEWSPCSVTCGNGIQVRIKPGSANKPKDE LDYANDIEKKICKMEKCSSVFNVVNSSIGLIMVLSFLFLNENTTSGFL GPLLVLQAGFFLLTRILTIPQSLDSWWTSLNFQGGAPTCPGQNSQSPT SNHSPTSCPPICPGYRWMCLRRFIIFLFILLLCLIFLLVLLDYQGMLP VCPLLPGTSTTGTGPCRTCTIPAQGTSMFPSCCCTKPSDGNCTCIPIP SSWAFARFLWEWASVRFSWLSLLVPFVQWFAGLSPTVWLSVIWMMWYR GPSLYNTLSPFLPLLPISFCLWVYI
R21 construct + C-Terminal Cysteine [184 C-Terminal residues of CS protein (CS-Cterm-184) fused to the Hepatitis B Virus Surface Antigen (HBsAg) with a Cysteine residue for conjugation]	ANPNANPNANPNANPNANPNANPNANPNANPNANPNANPNANPN ANPNANPNANPNKNNQGNGQGHNMPNDPNRNVDENANANSAVKNNNNE EPSDKHIKEYLNKIQNSLSTEWSPCSVTCGNGIQVRIKPGSANKPKDE LDYANDIEKKICKMEKCSSVFNVVNSSIGLIMVLSFLFLNENTTSGFL GPLLVLQAGFFLLTRILTIPQSLDSWWTSLNFQGGAPTCPGQNSQSPT SNHSPTSCPPICPGYRWMCLRRFIIFLFILLLCLIFLLVLLDYQGMLP VCPLLPGTSTTGTGPCRTCTIPAQGTSMFPSCCCTKPSDGNCTCIPIP SSWAFARFLWEWASVRFSWLSLLVPFVQWFAGLSPTVWLSVIWMMWYR GPSLYNTLSPFLPLLPISFCLWVYIC

SB-7062 R21 CS C-Terminus [184 C-Terminal residues of CS protein for 293 expression]	MKIKTGARILALSALTTMMFSASALAANPNANPNANPNANPNANPNAN PNANPNANPNANPNANPNANPNANPNANPNANPNANPNKNNQGNGQGH NMPNDPNRNVDENANANSAVKNNNNEEPSDKHIKEYLNKIQNSLSTEW SPCSVTCGNGIQVRIKPGSANKPKDELDYANDIEKKICKMEKCSSVFN VVNSSIGLIMVLSFLFLNPHHHHHHA
SB-7062 R21 CS C-Terminus + C-Terminal Cysteine [184 C-Terminal residues of CS protein with additional C-Terminal Cysteine for conjugation]	MKIKTGARILALSALTTMMFSASALAANPNANPNANPNANPNANPNAN PNANPNANPNANPNANPNANPNANPNANPNANPNANPNKNNQGNGQGH NMPNDPNRNVDENANANSAVKNNNNEEPSDKHIKEYLNKIQNSLSTEW SPCSVTCGNGIQVRIKPGSANKPKDELDYANDIEKKICKMEKCSSVFN VVNSSIGLIMVLSFLFLNPHHHHHHAC
R21 Peptide $NANP_{(6x)}$ [Peptide of N-Terminal residues of R21 6x(NANP) with NHS for conjugation]	NPNANPNANPNANPNANPNANPNA(K/Mal)-amide
R21 Peptide NANP-2 [Peptide of second segment of R21 with NHS for conjugation]	ANPNKNNQGNGQGHNMPNDPNRNVDENANANSAVKNNNNE(K/Mal)-amide
R21 Peptide Solved Structure [Structured portion of C-Terminal segment of R21]	EPSDKHIKEYLNKIQNSLSTEWSPSSVTSGNGIQVRIKPGSANKPKDE LDYANDIEKKISKMEKSS(K/Mal)-amide
Ring 10 (Ly1) Wt protein sequence	MPWWYRRRSYNPWRRRNWFRRPRKTIYRRYRRRRRWVRRKPFYK RKIKRLNIVEWQPKSIRKCRIKGMLCLFQTTEDRLSYNFDMYEE SIIPEKLPGGGGFSIKNISLYALYQEHIHAHNIFTHTNTDRPLA RYTGCSLKFYQSKDIDYVVTYSTSLPLRSSMGMYNSMQPSIHLM QQNKLIVPSKQTQKRRKPYIKKHISPPTQMKSQWYFQHNIANIP LLMIRTTALTLDNYYIGSRQLSTNVTIHTLNTTYIQNRDWGDRN KTYYCQTLGTQRYFLYGTHSTAQNINDIKLQELIPLTNTQDYVQ GFDWTEKDKHNITTYKEFLTKGAGNPFHAEWITAQNPVIHTANS PTQIEQIYTASTTTFQNKKLTDLPTPGYIFITPTVSLRYNPYKD LAERNKCYFVRSKINAHGWDPEQHQELINSDLPQWLLLFGYPDY IKRTQNFALVDTNYILVDHCPYTNPEKTPFIPLSTSFIEGRSPY SPSDTHEPDEEDQNRWYPCYQYQQESINSICLSGPGTPKIPKGI TAEAKVKYSFNFKWGGDLPPMSTITNPTDQPTYVVPNNFNETTS LQNPTTRPEHFLYSFDERRGQLTEKATKRLLKDWETKETSLLST EYRFAEPTQTQAPQEDPSSEEEESNLFERLLRQRTKQLQLKRR IIQTLKDLQKLE
CCN5 CTermCys	MRGTPKTHLLAFSLLCLLSKVRTQLCPTPCTCPWPPPRCPLGVPLVLD GCGCCRVCARRLGEPCDQLHVCDASQGLVCQPGAGPGGRGALCLLAED DSSCEVNGRLYREGETFQPHCSIRCRCEDGGFTCVPLCSEDVRLPSWD CPHPRRVEVLGKCCPEWVCGQGGGLGTQPLPAQGPQFSGLVSSLPPGV PCPEWSTAWGPCSTTCGLGMATRVSNQNRFCRLETQRRLCLSRPCPPS RGRSPQNSAFGGGGSGGGGSGGGGSC
Aflibercept malE_CTermCys	MKIKTGARILALSALTTMMFSASALASDTGRPFVEMYSEIPEIIHMTE GRELVIPCRVTSPNITVTLKKFPLDTLIPDGKRIIWDSRKGFIISNAT YKEIGLLTCEATVNGHLYKTNYLTHRQTNTIIDVVLSPSHGIELSVGE KLVLNCTARTELNVGIDFNWEYPSSKHQHKKLVNRDLKTQSGSEMKKF

	LSTLTIDGVTRSDQGLYTCAASSGLMTKKNSTFVRVHEKDKTHTCPPC PAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNW YVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSN KALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFY PSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGN VFSCSVMHEALHNHYTQKSLSLSPGGGGSGGGGSGGGGSGGGGSC
Aflib_D1-D2 malE_CTermCys	MKIKTGARILALSALTTMMFSASALASDTGRPFVEMYSEIPEIIHMTE GRELVIPCRVTSPNITVTLKKFPLDTLIPDGKRIIWDSRKGFIISNAT YKEIGLLTCEATVNGHLYKTNYLTHRQTNTIIDVVLSPSHGIELSVGE KLVLNCTARTELNVGIDFNWEYPSSKHQHKKLVNRDLKTQSGSEMKKF LSTLTIDGVTRSDQGLYTCAASSGLMTKKNSTFVRVHEKGGGGSGGGG SGGGGSC
Ranibizumab HC malE_CTermCys	MKIKTGARILALSALTTMMFSASALAEVQLVESGGGLVQPGGSLRLSC AASGYDFTHYGMNWVRQAPGKGLEWVGWINTYTGEPTYAADFKRRFTF SLDTSKSTAYLQMNSLRAEDTAVYYCAKYPYYYGTSHWYFDVWGQGTL VTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNS GALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSN TKVDKKVEPKSCDKTHLGGGGSGGGGSGGGGSC
Bevacizumab HC malE_CTermCys	MKIKTGARILALSALTTMMFSASALAEVQLVESGGGLVQPGGSLRLSC AASGYTFTNYGMNWVRQAPGKGLEWVGWINTYTGEPTYAADFKRRFTF SLDTSKSTAYLQMNSLRAEDTAVYYCAKYPHYYGSSHWYFDVWGQGTL VTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNS GALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSN TKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRT PEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVS VLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLP PSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDS DGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGKK DELGGGGSGGGGSGGGGSC
scFab RaniHC-link50-LC malE_CTermCys	MKIKTGARILALSALTTMMFSASALAEVQLVESGGGLVQPGGSLRLSC AASGYDFTHYGMNWVRQAPGKGLEWVGWINTYTGEPTYAADFKRRFTF SLDTSKSTAYLQMNSLRAEDTAVYYCAKYPYYYGTSHWYFDVWGQGTL VTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNS GALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSN TKVDKKVEPKSCDKTHLGGSSGSGSGSTGTSSSGTGTSAGTTGTSAST SGSGSGGGGGSGGGGSAGGDIQLTQSPSSLSASVGDRVTITCSASQDI SNYLNWYQQKPGKAPKVLIYFTSSLHSGVPSRFSGSGSGTDFTLTISS LQPEDFATYYCQQYSTVPWTFGQGTKVEIKRTVAAPSVFIFPPSDEQL KSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTY SLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGECGGGGSGG GGSGGGGSC

Example 6. Generation of circular ssDNA transgene for in vitro assembly of Anellovectors

Anelloviruses encapsidate circularized negative sense (ns) single stranded (ss) DNA genomes. Described in this example is a method for generating circularized ns-ssDNA purified *in vitro*. First, the genome of an *Anellovirus* (e.g., the genome of strain Ring 2 as described herein) was generated in a plasmid for amplification. Next, the DNA encoding the viral genome was cut from the plasmid and re-ligated to form double-stranded *in vitro* circularized (IVC) DNA. The IVC DNA was denatured with heat by boiling at 80°C for 5 minutes in the presence of urea and run on a denaturing gel overnight at 4°C. When the gel is stained with fluorescently-labeled primers from either the positive strand (to bind the negative-sense sequence) or the negative strand (to bind the positive-sense sequence) it was observed that the IVC DNA was separated into positive and negative sense circularized ssDNA (**FIG. 3**).

The ssDNA can be extracted from the gel using conventional DNA purification kits and used in anellovector *in vitro* encapsidation screening. Plasmids can also be cloned that encode viral genomes that also harbor reporter genes or therapeutic genes, for example, such that generation and encapsidation of the resulting circularized ssDNA would generate an anellovector that can transduce cells with the reporter or therapeutic gene.

Example 7. Anellovector ORF1 VLP expression in insect or mammalian cells and purification

Described in this example are expression and purification protocols for Anellovirus ORF1 recombinant proteins and variations that can be used, for example, in generating anellovector strains *in vitro*. Several Anellovirus ORF1 strains have been successfully expressed in insect (Sf9) and/or mammalian (293) cell lines (**FIG. 4A**). As described in Examples 8 and 9 below, several ORF1 proteins from different Anellovirus strains (as well as the related CAV Vp1 capsid protein) were shown to have a propensity to self-assemble into VLPs *in vitro* when observed by electron microscopy (**FIG. 4B**).

In one example, anellovectors can be produced *in vitro* by generating expression constructs for certain Anellovirus proteins (e.g., as described herein), such as an Anellovirus ORF1. Such expression constructs can be introduced into cells (e.g., mammalian cells or insect cells, e.g. Sf9 cells) to produce Anellovirus ORF1 proteins capable of forming capsids. Production of the ORF1 proteins can be done in the presence or absence of a suitable Anellovirus ORF2 protein. The expression constructs can also be engineered to attach (e.g., fuse) an affinity tag to the Anellovirus ORF1 protein. The ORF1 proteins can then be purified from the cells.

In some examples, a desired payload nucleic acid molecule (e.g., an engineered Anellovirus genome, a wild-type Anellovirus genome, an oligonucleotide, a single-stranded DNA, a double-stranded DNA, or an RNA, e.g., an mRNA) can be encapsulated by the ORF1 proteins *in vitro*, under conditions suitable for assembly of the ORF1 proteins into a proteinaceous exterior (e.g., a capsid). Such conditions

may include, for example, a solution comprising one or more of: detergents, buffers, and/or reducing agents (e.g., as described herein), as well as incubation at a preselected temperature for a preselected time. This assembly can result in formation of particles (e.g., comprising assembled ORF1 proteins encapsulating the desired payload nucleic acid molecule). Particle formation can be assessed, for example, by co-migration of ssDNA in size exclusion chromatography and/or by detecting DNase-resistant payload nucleic acid molecules (e.g., by PCR), e.g., as described herein. In addition, particles can be assessed for their capacity to transduce target cells with the payload nucleic acid molecule (e.g., by detecting a gene product encoded by the payload nucleic acid molecule, e.g., a reporter).

In addition to exploring the purification and *in vitro* assembly of ORF1 from different strains, Anellovirus ORF1 fragments or chimeric molecules can be used to improve the efficiency of *in vitro* assembly. Such fragment or chimeric molecules include, but are not limited to, Anellovirus ORF1 proteins that harbor mutations introduced into the N-terminal Arginine-rich region (also referred to as the ARG arm) to alter the pI of the ARG arm, permitting pH sensitive nucleic acid binding to trigger particle assembly (SEQ ID NOs: 563-565). Anellovirus ORF1 mutations that improve stability include, for example, mutations to the interprotomer contacting beta strands F and G of the canonical structural jellyroll beta-barrel (F and G beta strands) to alter the hydrophobic state of the protomer surface to make capsid formation more thermodynamically favored.

Exemplary chimeric ORF1 proteins include, but are not limited to, Anellovirus ORF1 proteins which have a portion or portions of their sequence replaced with comparable portions from another capsid protein, such as BFDV, CAV capsid protein or Hepatitis E (such as the ARG arm or F and G beta strands of Ring 9 ORF1 replaced with the comparable components from BFDV capsid protein; SEQ ID NOs: 566-567). Chimeric ORF1 proteins may also include ORF1 proteins which have a portion or portions of their sequence replaced with comparable portions of another Anellovirus ORF1 protein (such as structural jellyroll fragments or the C-terminal portion of Ring 2 ORF1 replaced with comparable portions of Ring 9 ORF1; SEQ ID NOs: 568-575).

Proteins will be purified using purification techniques, including but not limited to chelating purification, heparin purification, gradient sedimentation purification and/or SEC purification.

Example 8. Expression of Ring2 and Ring10 Anellovirus ORF1s in insect cells

In this example, DNA sequences encoding Ring 2 ORF1 or Ring 10 ORF1, each fused to an N-terminal HIS_6-tag (HIS-ORF1), was codon optimized for insect expression and cloned into the baculovirus expression vector pFASTbac system according to the manufacture's method (ThermoFisher Scientific). Insect cells (Sf9 cells) were infected with Ring HIS-ORF1 baculovirus and the cells were harvested 3-days post-infection by centrifugation. The cells were lysed and the protein was purified using

a chelating resin affinity column (HisTrap, GE Healthcare). The resulting material was purified again using a heparin affinity column (Heparin HiTrap, GE Healthcare) and fractions containing ORF1 were analyzed by negative staining electron microscopy. Both Ring 2 ORF1 and Ring 10 ORF1 exhibited an observed propensity to form ~35 nm virus-like particles (VLPs) in this *in vitro* setting **(FIG. 4B)**.

Example 9. Expression of CAV capsid protein VP1 in mammalian cells

In this example, DNA sequences encoding CAV capsid protein (CAV Vp1), fused to an N-terminal HIS_6-Flag-tag (HIS-Flag-Vp1), and helper protein (Vp2) were codon optimized for mammalian expression and cloned into a mammalian expression vector including a CMV promoter. Mammalian cells (293expi cells) were transfected with CAV Vp1 and Vp2 expression vectors. The cells were harvested 3-days post-infection by centrifugation. The cells were lysed and the lysis was purified using chelation and heparin purification as described in Example 8. The elution fraction containing CAV Vp1 were analyzed by negative staining electron microscopy. As shown in **FIG. 4B**, CAV Vp1 virus-like particles were observed in this *in vitro* setting.

Example 10. Expression of Ring2 ORF1 and ORF2 in insect cells

In this example, untagged Ring 2 ORF1 and ORF2 (a putative zinc-finger Ring 2 protein of unknown function) were cloned into a dual-expression baculovirus and co-expressed in Sf9 insect cells using a baculovirus system as described herein. Frozen pellet from a 1 L preparation of the Sf9 cells were resuspended in 60 mL cytosolic buffer (50mM Tris pH 8, 50mM NaCl, 1x protease inhibiter), vortexed, pipetted up-down and distributed into two 30 mL aliquotes in 50 mL conical tubes. The solutions were spun down for 20 min at 14,000 rpm using a fixed rotor centrifuge. The supernatant was collected into separate 50mL conical tubes ("wash sup"). Each pellet was resuspended with 30mL lysis buffer (50mM Tris pH 8, 50mM NaCl buffer, 1x protease inhibitor, 0.01-0.1% Triton), vortexed, and sonicated with four cycles. 1x benzonase was added and the solutions were spun for 20min at 14,000 rpm using a fixed rotor centrifuge. The lysate was incubated for about 30 minutes to allow the benzonase to react to remove host cell DNA. The ORF1 was purified from the lysates using an affinity purification step (heparin column pH=8 with a high salt gradient elution) and VLPs, partially formed VLPs, and ORF1 proteins (putative capsomers) were separated using size-exclusion purification (SEC; GE Healthcare Sephacryl S-500 column). The resulting material was analyzed by electron microscopy **(FIG. 5A-5C)**. Approximately 10^{11} total VLP particles at approximately 10^9 - 10^{10} particles/ml concentration were purified, as determined by western blot analysis and electron microscopy. Such a high-titer VLP preparation can be used to define *in vitro* disassembly and re-assembly/encapsidation conditions for anellovector generation as described below.

Example 11. Disassembly of Ring2 VLPs using urea

In this example, purified Ring2 VLPs were dissociated using a chaotropic agent. Briefly, purified Ring 2 VLPs were treated with urea at different concentrations, between 1 to 6 molar (M), to identify conditions sufficient to disassemble the particles. In one example, Ring 2 VLPs were treated with urea with a final concentration of 2M for approximately 10 minutes. The sample was observed by electron microscopy before **(FIG. 6A)** and after **(FIG. 6B)** urea treatment. Prior to treatment with urea, VLPs were observed with an estimated particle titer of 1×10^9 to 1×10^{10} particles/ml. After treatment with urea, the VLPs were no longer observed. A new species, which appear to be hollow circles, like capsomers, was also observed **(FIG. 6C)**.

In a further example, Ring 2 VLPs are dissociated with 2M urea, and the urea is dialyzed away in the absence or presence of mRNA encoding the mCherry reporter gene. In this example, mRNA is introduced at a molar concentration estimated to be approximately 5-times greater than the estimated number of VLPs prior to dissociation. The dissociated ORF1 and mRNA solution are incubated for approximately 30 minutes to allow complex formation before reassembly by dialysis. After the incubation period, disassembled ORF1 (i.e. VLPs treated with 2M urea) with or without mRNA are then dialyzed against 50 mM Tris pH 8.0 with 150 mM NaCl and 0.01% poloxamer to permit VLP reassembly. The initial VLPs, dissociated ORF1, reassembled ORF1 in the absence of nucleic acids, and reassembled ORF1 in the presence of nucleic acids are then screened by EM to confirm that the dissociation/re-assembly processes are successful as well as to estimate the amount of VLP recovered. It is contemplated that no VLPs will be observed for disassembled VLP or disassembled VLP dialyzed in the absence of mRNA. However, it is contemplated that VLPs prior to disassembly may be present at a particle titer of $1x10^9$ to $1x10^{10}$ particles/ml and the VLP formation observed after dialysis in the presence of mRNA may have a particle titer of $1x10^7$ to $1x10^8$ particles/ml. It is contemplated that VLP reassembly occurs when disassembled ORF1 is dialyzed/reassembled in the presence of mRNA, resulting in mRNA-encapsidated anellovectors.

Example 12. Dissociation of Anellovirus VLPs and reassembly around nucleic acid cargoes

In this example, Anellovirus ORF1 proteins produced and purified as described herein (e.g., wildtype ORF1 protein, chimeric ORF1 protein, or fragments thereof) will be disassembled and then reassembled *in vitro*. VLPs will be incubated under conditions sufficient to dissociate VLPs or viral capsids (e.g., as described herein), and then under conditions suitable enable reassembly, for example, around nucleic acid cargo (**FIGs. 5A-5C**). Exemplary nucleic acid cargo can include, without limitation, as double stranded

DNA, single stranded DNA (ssDNA), or RNA that encodes a gene of interest to be delivered as a therapeutic agent. Exemplary conditions sufficient to dissociate VLPs or viral capsids include, but are not limited to, buffers of different pH, conditions of defined conductivity (salt content), conditions containing detergents (such as SDS, Tween, Triton), conditions containing chaotropic agents (such as Urea, e.g., as described herein) or conditions involving defined temperature and time (reannealing temperatures). Generally, nucleic acid cargo of defined concentration will be combined with Anellovirus ORF1 proteins of defined concentration and treated with conditions sufficient to permit nucleic acid encapsidation. The resulting particles can be subsequently purified using viral purification procedures in the art and/or as described herein.

Encapsidation of ssDNA cargo

In this example, purified Anellovirus ORF1 proteins are treated with high salt in 2 M Urea to disassemble VLPs into dispersed protein or capsomers. The Anellovirus ORF1 proteins are then mixed with ssDNA and dialyzed against Tris pH 8.0 with 150 mM NaCl to permit VLP formation and ssDNA encapsidation. The subsequent complex is purified by SEC using to isolate anellovectors encapsidating ssDNA from non-encapsidated DNA. Anellovector assembly can be further evaluated by biophysical assessment such as DLS or electron microscopy, e.g., as described herein.

Encapsidation of mRNA cargo with wild-type Anellovirus ORF1

In this example, purified Anellovirus ORF1 proteins are treated with 1 M NaCl with 0.1% SDS dissociate oligomers or VLPs into dispersed protein or capsomers. The Anellovirus ORF1 proteins are then mixed with mRNA, such as an mRNA that encodes a gene of interest (e.g., GFP, mCherry, or EPO), and dialyzed against Tris pH 8.0 with 150 mM NaCl to permit VLP formation. The subsequent complex is purified by SEC using Tris pH 8.0 buffer to isolate anellovectors encapsidating mRNA. Anellovector assembly can be further evaluated by *in vitro* or *in vivo* readout, for example, by transducing cells and observing the expression of a reporter gene (in the case of mCherry or GFP) or through expression of a gene of interest (such as using an ELISA to detect the expression of a gene such as EPO).

Encapsidation of mRNA cargo with modified ORF1 having mRNA binding region

In this example, packaging of mRNA cargo by Anellovirus ORF1 proteins is improved by modifying the Anellovirus ORF1 protein to harbor contact residues known to bind mRNA. For example, the ssDNA contact residues and/or the structural jellyroll beta strands known to contact ssDNA and/or the N-terminal arginine ARM can be replaced with components of an mRNA binding viral protein (e.g., MS2 coat protein) or other mRNA-binding protein to permit efficient binding and packaging of mRNA. This

mRNA-binding chimeric ORF1 could be then treated with 1 M NaCl with 0.1% SDS dissociate oligomers or VLPs into dispersed protein or capsomers. The chimeric ORF1 would then be mixed with mRNA, such as an mRNA that translate a gene of interest such as GFP, mCherry or EPO, and dialyzed against Tris pH 8.0 with 150 mM NaCl to permit VLP formation. The subsequent complex is purified by SEC using Tris pH 8.0 buffer to isolate anellovectors encapsidating mRNA. Anellovector assembly can be further evaluated by *in vitro* or *in vivo* readout, for example, by transducing cells and observing the expression of the reporter gene (in the case of mCherry or GFP) or through expression of a gene of interest (such as using an ELISA to detect the expression of a gene such as EPO).

Encapsidation of ssDNA-mRNA hybrid cargo with ORF1

In this example, packaging of mRNA cargo by Anellovirus ORF1 proteins is improved by binding the mRNA molecule to ssDNA or modifying the mRNA transgene in such a way that that a section of the backbone would permit binding to the ssDNA contact residues of wildtype Anellovirus ORF1. For example, modified ssDNA that can bind Anellovirus ORF1 by virtue of its sugar-chain backbone but pair with mRNA non-covalently is mixed with the mRNA to produce a synthetic mRNA complex. Alternatively, a synthetic mRNA transgene can be synthesized with a section or sections of the mRNA molecule harboring a DNA backbone permitting binding and encapsidation with Anellovirus ORF1, while retaining the portion of the mRNA that encodes the gene to be delivered. Anellovirus ORF1 could be then treated with 1 M NaCl with 0.1% SDS dissociate oligomers or VLPs into dispersed protein or capsomers. The Anellovirus ORF1 would then be mixed with the synthetic mRNA (complexes or molecules), such as an mRNA that translate a gene of interest such as GFP, mCherry or EPO, and dialyzed against Tris pH 8.0 with 150 mM NaCl to permit VLP formation. The subsequent particle is purified by SEC using Tris pH 8.0 buffer to isolate anellovectors encapsidating mRNA. Anellovector assembly can be further evaluated by *in vitro* or *in vivo* readout by transducing cells and observing the expression of the reporter gene (in the case of mCherry or GFP) or through expression of a gene of interest (such as using an ELISA to detect the expression of a gene such as EPO).

Example 13: Production of Anellovirus proteins in a baculovirus expression system

In this example, a baculovirus expression system from Thermofisher Scientific (Cat. no. A38841) was adapted for expression of Anellovirus proteins. Briefly, a gene of interest (e.g., a gene encoding an Anellovirus ORF as described herein) was cloned into the pFastBac plasmid, which was then transformed into DH10Bac *E. coli* cells harboring a baculovirus genome. The transformants were grown on indicator plates according to the manufacturer's instructions and white colonies were selected for liquid culture and

extraction of bacmid DNA. Recombination of the Anellovirus ORFs into the bacmids was validated by PCR.

Validated bacmid constructs showing successful recombination of the anellovirus ORF gene were then transfected into ExpiSf9 insect cells. The cells were incubated in a 27°C non-humidified, non-CO_2 atmosphere incubator on an orbital shaker set at 125 rpm. After 72 hours post-transfection, Passage 0 stock (P0) recombinant baculovirus was harvested from the supernatant.

ExpiSf9 cells were infected using 25-100 µL of P0 baculovirus stock to make Passage 1 (P1) baculovirus for protein production. After 96 hours (approx. 4 days) post-infection, the supernatant was collected to obtain P1 baculovirus.

P1 recombinant baculovirus was titered by preparing five 10-fold serial dilutions of the test virus in fresh ExpiSf CD Medium in 1200 µL total volume. 800 µL of Expisf9 cells at 1.25×10^6 viable cells/mL were seeded in a deep well plate and 1000 µL of the different dilutions of the test virus were added to each well. One well was setup as a negative control. Plate was then incubated overnight at 27°C in a non-humidified incubator on a shaking platform at 225 ± 5 rpm. After approx. 14–16 hours of incubation, the plate was removed from the incubator and everything was transferred to microcentrifuge tubes and spun at 300 x g for 5 minutes. Supernatant was aspirated and each cell pellet was resuspended in 100 µL of dilution buffer (PBS + 2% Fetal Bovine Serum) containing Anti-Baculovirus Envelope gp64 APC antibody at a final concentration of 0.15 µg/mL. Tubes were incubated for 30 mins at room temperature. Samples were then washed with 1 mL PBS followed by a 10 min centrifuge spin at 300 x g. Supernatant was aspirated and cell pellet was resuspended in 1 mL Dilution Buffer. Samples were analyzed on a flow cytometer using the following parameters: red laser excitation: 633-647 nm; emission: 660 nm. Samples with different viral dilutions expressing percent positive gp64 were noted and used to calculate the viral titer.

For this and following examples, a series of recombinant bacmids and baculovirus vectors was produced for expression using the method described above. As shown in Table E2 below, various ORFs from LY2, tth8, and other anellovirus strains were cloned into bacmids. The ORFs were either tagged with an N-terminal His-tag with or without a human rhinovirus 3C (HRV 3C) proteolytic cleavage site, a C-terminal His-tag, or were left untagged, as indicated.

Table E2. Recombinant bacmid constructs produced. "FullORF" = Full ORF-containing region, with noncoding regions removed; ORF2/3 tagged.

Construct #/name	Strain	Ring #	ORF	Tag type	Tag position	pFastBac	Bacmid	Baculovirus
tth8 ORF1	tth8	Ring1	ORF1	no-tag	NA	Yes	Yes	No
tth8 ORF1 N-His	tth8	Ring1	ORF1	6xHis	N-ter	Yes	Yes	Yes
tth8 ORF1 C-His	tth8	Ring1	ORF1	6xHis	C-ter	Yes	Yes	Yes

tth8 ORF2	tth8	Ring1	ORF2	no-tag	NA	Yes	Yes	Yes
tth8 ORF2 C-His	tth8	Ring1	ORF2	6xHis	C-ter	Yes	Yes	Yes
tth8 ORF1/1	tth8	Ring1	ORF1/1	no-tag	NA	Yes	Yes	Yes
tth8 ORF1/1 C-His	tth8	Ring1	ORF1/1	6xHis	C-ter	Yes	Yes	Yes
tth8 ORF1/2	tth8	Ring1	ORF1/2	no-tag	NA	Yes	Yes	Yes
tth8 ORF1/2 C-His	tth8	Ring1	ORF1/2	6xHis	C-ter	Yes	Yes	Yes
tth8 ORF2/2	tth8	Ring1	ORF2/2	no-tag	NA	Yes	Yes	Yes
tth8 ORF2/2 C-His	tth8	Ring1	ORF2/2	6xHis	C-ter	Yes	Yes	Yes
tth8 ORF2/3	tth8	Ring1	ORF2/3	no-tag	NA	Yes	Yes	Yes
tth8 ORF2/3 C-His	tth8	Ring1	ORF2/3	6xHis	C-ter	Yes	Yes	Yes
tth8 FullORF	tth8	Ring1	FullORF	no-tag	NA	Yes	Yes	Yes
tth8 FullORF C-His	tth8	Ring1	FullORF	6xHis	C-ter	Yes	Yes	Yes
tth8 ORF2 C-His	tth8	Ring1	ORF2/ORF1	6xHis	C-ter	Yes	Yes	Yes
Ring 3.1 ORF1	6B.CD8. contig3	Ring3.1	ORF1	no-tag	NA	No	No	No
Ring 3.1 ORF1 C-His	6B.CD8. contig3	Ring3.1	ORF1	6xHis	C-ter	Yes	Yes	Yes
Ring 3.1 ORF2	6B.CD8. contig3	Ring3.1	ORF2	no-tag	NA	No	No	No
Ring 3.1 ORF2 C-His	6B.CD8. contig3	Ring3.1	ORF2	6xHis	C-ter	Yes	Yes	Yes
Ring 3.1 ORF2/ORF1 C-His	6B.CD8. contig3	Ring3.1	ORF2/ORF1	6xHis	C-ter	Yes	Yes	Yes
LY2 FullORF	LY2	Ring2	FullORF	no-tag	NA	Yes	Yes	No
LY2 FullORF N-His	LY2	Ring2	FullORF	6xHis	N-ter	Yes	Yes	Yes
LY2 FullORF C-His	LY2	Ring2	FullORF	6xHis	C-ter	Yes	Yes	Yes
LY2 ORF1	LY2	Ring2	ORF1	no-tag	NA	Yes	Yes	No
LY2 ORF1 N-His	LY2	Ring2	ORF1	6xHis	N-ter	Yes	Yes	Yes
LY2 ORF1 C-His	LY2	Ring2	ORF1	6xHis	C-ter	Yes	Yes	Yes
LY2 ORF1(dR)	LY2	Ring2	ORF1 (delta-argininc rich region)	no-tag	NA	Yes	No	No
LY2 ORF1(dR) N-His	LY2	Ring2	ORF1 (delta-arginine rich region)	6xHis	N-ter	Yes	Yes	Yes
LY2 ORF1(dR) C-His	LY2	Ring2	ORF1 (delta-arginine rich region)	6xHis	C-ter	Yes	Yes	Yes
LY2 ORF1/1	LY2	Ring2	ORF1/1	no-tag	NA	Yes	Yes	No
LY2 ORF1/1 N-His	LY2	Ring2	ORF1/1	6xHis	N-ter	Yes	Yes	Yes

LY2 ORF1/1 C-His	LY2	Ring2	ORF1/1	6xHis	C-ter	Yes	Yes	Yes
LY2 ORF1/2	LY2	Ring2	ORF1/2	no-tag	NA	Yes	Yes	No
LY2 ORF1/2 N-IIis	LY2	Ring2	ORF1/2	6xIIis	N-ter	Yes	Yes	Yes
LY2 ORF1/2 C-His	LY2	Ring2	ORF1/2	6xHis	C-ter	Yes	Yes	Yes
LY2 ORF2	LY2	Ring2	ORF2	no-tag	NA	Yes	Yes	No
LY2 ORF2 N-His	LY2	Ring2	ORF2	6xHis	N-ter	Yes	Yes	Yes
LY2 ORF2 C-His	LY2	Ring2	ORF2	6xHis	C-ter	Yes	Yes	Yes
LY2 ORF2/2	LY2	Ring2	ORF2/2	no-tag	NA	Yes	Yes	No
LY2 ORF2/2 N-His	LY2	Ring2	ORF2/2	6xHis	N-ter	Yes	Yes	Yes
LY2 ORF2/2 C-His	LY2	Ring2	ORF2/2	6xHis	C-ter	Yes	Yes	Yes
LY2 ORF2/3	LY2	Ring2	ORF2/3	no-tag	NA	Yes	Yes	No
LY2 ORF2/3 N-His	LY2	Ring2	ORF2/3	6xHis	N-ter	Yes	Yes	Yes
LY2 ORF2/3 C-His	LY2	Ring2	ORF2/3	6xHis	C-ter	Yes	Yes	Yes
LY2 ORF2/ORF1 C-His	LY2	Ring2	ORF2/ORF1	6xHis	C-ter	Yes	Yes	Yes
LY2 ORF1 HisE354	LY2	Ring2	ORF1	6xHis	After E354	Yes	Yes	No
LY2 ORF1 HisN299	LY2	Ring2	ORF1	6xHis	After N299	Yes	Yes	No
LY2 ORF1 HisL267	LY2	Ring2	ORF1	6xHis	After L267	Yes	Yes	No
tth8 ORF1 (JA20 HVR)	tth8	Ring1	ORF1 (with JA20's hypervariable region)	6xHis	C-ter	Yes	No	No
tth8 ORF1 (TJN02 HVR)	tth8	Ring1	ORF1 (with TJN02's hypervariable region)	6xHis	C-ter	Yes	No	No
tth8 ORF1 (TTV16 HVR)	tth8	Ring1	ORF1 (with TTV16's hypervariable region)	6xHis	C-ter	Yes	No	No
Ring2 ORF1 (CodOpt)	LY2	Ring2	ORF1 (codon optimized)	no-tag	NA	Yes	Yes	Yes
Ring2 ORF1 (CodOpt) HRV3C-6His	LY2	Ring2	ORF1 (codon optimized)	6xHis	C-ter	Yes	Yes	Yes
Ring4 ORF1 (CodOpt)	6B.CD8. contig2	Ring4	ORF1 (codon optimized)	no-tag	NA	Yes	Yes	Yes
RIng4 ORF1 (CodOpt) HRV3C-6His	6B.CD8. contig2	Ring4	ORF1 (codon optimized)	6xHis	C-ter	Yes	Yes	Yes
RIng5.2 ORF1 (CodOpt)	CT30F	Ring5.2	ORF1 (codon optimized)	no-tag	NA	Yes	Yes	Yes

Ring5.2 ORF1 (CodOpt) HRV3C-6His	CT30F	RIng5.2	ORF1 (codon optimized)	6xHis	C-ter	Yes	Yes	Yes
Ring6 ORF1 (CodOpt)	190783.3	Ring6	ORF1 (codon optimized)	no-tag	NA	Yes	Yes	Yes
Ring6 ORF1 (CodOpt) HRV3C-6His	190783.3	Ring6	ORF1 (codon optimized)	6xHis	C-ter	Yes	Yes	Yes
Ring1 ORF1 (CosOpt) His	tth8	Ring1	ORF1 (codon optimized)	6xHis	C-ter	Yes	Yes	Yes
Rig3.1 ORF1 (CodOpt) His	6B.CD8. contig3	Ring3.1	ORF1 (codon optimized)	6xHis	C-ter	Yes	Yes	Yes
Ring7 ORF1 (CodOpt) His	190783.4	Ring7	ORF1 (codon optimized)	6xHis	C-ter	Yes	Yes	Yes
Ring2 (CodOpt) N-His	LY2	Ring2	ORF1 (codon optimized)	6xHis	N-ter	Yes	Yes	Yes
Ring2 (CodOpt) N-His (PS)	LY2	Ring2	ORF1 (codon optimized)	6xHis-PreScision Protease recognition sequence)	N-ter	Yes	Yes	Yes
Ring2 tandem	LY2	Ring2	2x whole genome (without Polyhedrin promoter)	no-tag	NA	Yes	Yes	Yes
WTLY2	LY2	Ring2	whole genome	no-tag	NA	Yes	Yes	Yes
WTtth8	tth8	Ring1	whole genome	no-tag	NA	Yes	Yes	Yes
WTtth8 (Reverse)	tth8	Ring1	whole genome (with Reversed 5' Polyhedrin promoter)	no-tag	NA	Yes	Yes	Yes
LoxPWTLY2	LY2	Ring2	LoxP-whole genome-LoxP	no-tag	NA	Yes	Yes	Yes
Cre-R	NA	NA	Cre recombinase	no-tag	NA	Yes	Yes	Yes

On the day before infection, ExpiSf9 cells were seeded at $5x10^6$ cells/ml in 25 ml room temperature ExpiSf9 CD Medium in 125 ml Nalgene Single-Use PETG Erlenmeyer Plain Bottom Flask [Thermofisher Scientific Catalog no: 4115-0125]. The cell viability was monitored to ensure that it was

maintained at or above 95%. 100 µL of ExpiSf Enhancer solution was added to the cells in a dropwise manner. Cells were incubated with shaking overnight in a 27°C non-humidified, air-regulated, non-CO_2 atmosphere incubator using an orbital shaker at 125 ±5 rpm. On Day 1, approximately 18-24 hours after adding ExpiSf Enhancer, cells were infected with the indicated baculovirus at a multiplicity of infection (MOI) of 5 and incubated under the same conditions. Cells were harvested 72 hours post infection and viability was found to range between 60 and 80%. To analyze samples, cells were lysed by adding 1X Bolt LDS sample buffer [Invitrogen Catalog No.: B0007] and 1X Bolt reducing agent [Invitrogen Catalog No.: B0009] and sonicating for 2.5 minutes. As shown in FIG. 8, C-His-tagged LY2 ORF1 was successfully expressed in infected ExpiSf9 cells by day 2 post-infection as determined by western blotting using an anti-poly-histidine antibody. In addition, baculovirus proteins were detected by Coomassie staining, indicating a successful infection.

As shown in FIG. 9, C-His-tagged tth8 ORF1 and ORF1/1 were also successfully expressed in infected ExpiSf9 cells by day 2 post-infection.

N-terminally His-tagged LY2 ORF1 expression was also detected in infected ExpiSf9 cells (FIG. 10). Here, constructs either comprised an N-terminal His-tag which was immediately followed by the wildtype ORF1 sequence (lanes 1, 2, 9, 10, or 14), or an N-terminal His-tag which was followed by a rhinovirus 3C cleavage sequence (lanes 3, 11). Samples in lanes 1 to 7 are lysates loaded directly onto the gel, whereas samples in lanes 9-15 were prepared by first pelleting protein from conditioned medium via ultracentrifugation and resuspending the pellet in a 100-fold smaller volume. Samples shown in lanes 1-3 and 9-11 were grown at a small (5mL) scale. Samples in lanes 6 and 14 were obtained from a 10L culture. Thus, this example shows that production of ORF1 from a plurality of strains with N or C terminal poly-histidine tags can be successfully carried out at a scale ranging from 5mL to 10L, and that ORF1 can be found in Sf9 lysate or culture supernatant (conditioned medium).

Example 14: Expression of Ring1 ORFs in Sf9 cells

In this example, a series of recombinant baculoviruses were produced with alternate arrangements of tth8 ORFs, each tagged with a C-terminal poly-histidine (FIG. 11). The recombinant baculovirus designs included one baculovirus construct for each of the tth8 ORF splice variants (i.e., ORF1, ORF1/1, ORF1/2, ORF2, ORF2/2, and ORF2/3), as well as a "FullORF" construct containing the full ORF region from tth8, driven by the baculovirus polyhedrin promoter. These baculoviruses were produced as described in Example 13.

Protein expression was then detected by western blot using anti-poly-histidine antibody. As shown in FIG. 11, His-tagged tth8 ORFs ORF1/1, ORF1/2, ORF2, ORF2/2 and ORF2/3 were detected.

Example 15: Expression of Ring2 ORFs in Sf9 cells

In one example, a series of recombinant baculoviruses were produced with alternate arrangements of Ring2 ORFs, each tagged with a poly-histidine tag at the C terminus (FIG. 12). The recombinant baculovirus designs included one baculovirus construct for each of the Ring2 ORF splice variants (i.e., ORF1, ORF1/1, ORF1/2, ORF2, ORF2/2, and ORF2/3), a variant in which the N-terminal arginine-rich region (RRR) is deleted (ORF1ΔRRR), as well as a "FullORF" construct containing the full ORF region from Ring2 driven by the baculovirus polyhedrin promoter. For each experimental condition, ExpiSf9 cells were infected with recombinant baculoviruses expressing individual Ring2 variants at an MOI of 5. The experimental conditions for this were as described in Examples 13 and 14.

Protein expression was then detected by western blot using anti-His. As shown in FIG. 12, His-tagged Ring2 ORFs ORF1, ORF1ΔRRR, ORF1/1, ORF1/2, ORF2, ORF2/2, and ORF2/3 were all detected.

In a further experiment as part of this example, recombinant baculoviruses comprising a Ring2 ORF1-encoding sequence and/or a Ring2 ORF2 splice variant-encoding sequence were used to infect Sf9 cells. The expression conditions tested included ORF1 alone, or co-infection of ORF1 + "FullORF", ORF1 + ORF2, ORF1 + ORF2/2, and ORF1 + ORF2/3, as well as a negative control labeled 'Neg'. ExpiSf9 cells were co-infected with baculoviruses at a MOI of 5 for each condition. Experimental conditions were as described in Examples 13 and 14. Protein expression of ORF1, ORF2, ORF2/2, and ORF2/3 was then assessed for each condition by western blot using either anti-His or anti-Ring2 structural N22. The latter is a monoclonal antibody that was obtained by immunizing mice with the structural N22 fragment of Ring2 ORF1 produced in E. coli, and then generating hybridomas.

As shown in FIG. 13, both Westerns detected ORF1 as a band at ~81kD in each of the ORF1-infected conditions. The ORF1 band is highlighted by a dashed box in the anti-structural N22 Western, and is not visible in the negative control (Neg) sample. The lower molecular weight (~10 kD) band detected by both antibodies is thought to be a C-terminal fragment of ORF1. ORF2, ORF2/2, and ORF2/3 were also detected in the corresponding samples (anti-His blot). Thus, this example illustrates that both ORF1 and individual splice variants of ORF2 can be co-expressed in insect cells.

Example 16: Expression of all Ring2 ORFs simultaneously in Sf9 cells

In one example, a series of six recombinant baculoviruses were produced, each designed to express a particular Ring2 ORF (i.e., ORF1, ORF1/1, ORF1/2, ORF2, ORF2/2, and ORF2/3), each tagged with a His tag (FIG. 14), as described in Example 15. Sf9 cells were infected with various combinations of the Ring2 ORF baculoviruses – specifically, each condition involved infecting cells with all but one ORF construct, as indicated in FIG. 14. Protein expression was then detected by western blot of whole

cell suspension using anti-His. As shown in FIG. 14, His-tagged Ring2 ORFs were detected in the expected pattern. Either all ORFs were detected, or all except for the omitted one.

Example 17: Co-delivery and independent expression of anellovirus genomes and recombinant Anellovirus ORFs in Sf9 cells

In this example, anellovirus ORFs and genomes were co-delivered in Sf9 cells by transfecting an *in vitro* circularized (IVC) anellovirus genome and infecting the cells with baculovirus encoding ORF1 tagged at its C-terminus with hexa-histidine (FIG. 15). Protein expression was then detected by western blot using anti-His, anti-ORF2, and anti-ORF1 monoclonal antibody targeting the structural N22 fragment. As shown (FIG. 15, bullet 1), His-tagged ORF1 was detected in this preparation showing successful recombinant ORF1 expression from the baculovirus vector. Consistent with this result, the same ORF1 protein was detected using the anti-ORF1 antibody (FIG. 15, bottom panel, right-most lane).

In the same sample of treated cells, the native anellovirus promoter was shown to be transcriptionally active in Sf9 cells because ORF2 expression was detected (FIG. 15, bullet 3) and could only have been produced by the IVC genome which was transfected into the cells.

In addition, Anellovirus ORFs were co-delivered and expressed in Sf9 cells using an *in vitro* circularized (IVC) construct and a FullORF baculovirus. Protein expression was then detected by western blot using anti-His, anti-Ring2 ORF2, and anti-Ring2 ORF1 structural N22. ORF1 protein was detected in the cells (FIG. 15, bullet 4) and could be the product of either the IVC or the FullORF baculovirus construct. Surprisingly, ORF2 protein was readily detected and its intensity suggests the expression is derived from the FullORF baculovirus construct (FIG. 15, bullet 2).

As a further test of the ability of the anellovirus genome to express its genes in insect cells, the tth8 anellovirus coding region was cloned into the pFastBac vector in both orientations. This yielded 'FullORF' tth8 baculovirus constructs in which the polyhedrin promoter was positioned upstream of either the sense or the anti-sense direction of the coding region. The latter configuration is highly unlikely to initiate transcription of the anellovirus genes. Consistent with our surprising observations in Ring2, expression of tth8 ORF2 was independent of the orientation of the coding region relative to the baculovirus polyhedrin promoter, suggesting that expression is driven by the anellovirus promoter (FIG. 16, bands at ~15 and 20kDa).

This example shows that IVC transfections and baculovirus infections can co-deliver functional anellovirus genes to Sf9 insect cells and that the native anellovirus promoter is active in these cells.

Example 18: Anellovirus ORF1 associates with DNA in Sf9 cells to form complexes isolated by isopycnic centrifugation

In this example, Sf9 cells were transfected with IVC anellovirus genome LY2, infected with a baculovirus encoding LY2 ORF1 with a C-terminal poly-histidine tag, and then fractionated to determine whether ORF1 expressed using the baculoviral expression system forms protein-DNA complexes that can be isolated in vitro.

CsCl gradients were prepared by adding 8 ml of 1.2 g/ml CsCl solution (in TN buffer; 20 mM Tris pH 8.0, 140 mM NaCl) to ultracentrifuge tubes (Ultra-Clear 17 ml– Beckman #344061) for SW32.1 Ti rotor. Tubes were underlayed with 8 ml of 40% CsCl (in TN buffer), then capped with topper and run on Gradient Master program 5-50% for 13 minutes to prepare linear gradient. The caps were removed and the gradients overlayed with 0.5 ml-2 ml of Sf9 lysate to each tube and topped off to near the top with TN buffer containing 0.001% Poloxamer-188. Ultracentrifugation was for 18.5 hours at 22,500 x RPM. Fractions were collected from the gradient by piercing the bottom of the tube and allowing ~600 ul fractions to flow into wells of a deep well block. The refractive index of each sample was measured to determine their density.

Anelloviral DNA content in the fractions was then determined by first extracting DNA from the fractions, and then by carrying out qPCR. Pure Link Viral DNA extraction Kit [Thermofisher Scientific Catalog no. 12280050] was used to purify viral DNA from 50 uL of the fractions. The samples were treated with Proteinase K and lysed using Lysis buffer by incubating at 56◦C for 15min., washed with 99% ethanol, and transferred to a Viral Spin Column. Samples were centrifuged at 6800 x g, washed twice with 500 uL Wash buffer provided with the kit and centrifuged again. 100 uL of RNase-free water was added to the column to elute the DNA.

For qPCR, 2X TaqMan Gene Expression Master Mix, 100uM LY2 primers Forward (AGCAACAGGTAATGGAGGAC), 100uM LY2 Reverse (TGAAGCTGGGGTCTTTAAC) along with 100uM LY2 Probe (TCTACCTAGGTGCAAAGGGCC) were diluted in 5.83 uL Nuclease Free water for each reaction. The following conditions were used for each qPCR cycle: 50 ◦C hold for 2 minutes, 95 ◦C hold for 10 minutes followed by 40 cycles of 95 ◦C for 15 seconds and 60 ◦C for 1 minute on an Applied Biosystems Quant Studio 3 Real-Time PCR Machine. Each sample was run in triplicate and the entire assay was repeated thrice and used to plot the graph.

As shown in FIG. 17, isopycnic fractions were characterized by western blotting, quantitative PCR, and transmission electron microscopy. Anti-his western blotting of gradient fractions showed clear bands of the expected molecular weight for LY2 ORF1 in fractions having densities of 1.32 g/mL and 1.21 g/mL. In addition, fractions ranging from 1.25 to 1.29 g/mL had clear bands of higher and lower

molecular weights than expected. Also, qPCR indicates the presence of LY2 genomic DNA in certain fractions, with peaks at approximately 1.21 g/mL, 1.29 g/mL, and 1.32 g/mL.

Negative stain transmission electron microscopy was carried out on the 1.32 g/mL and 1.21 g/mL fractions, as well as a pool of fractions ranging from 1.25 to 1.29 g/mL. The pool shows an abundance of particles, including several having the appearance of proteasomes. The presence of proteasomes may explain the western blot bands at low and high molecular weights. The former may be due to proteolytic degradation and the latter due to ubiquitylated ORF1, or ORF1 fragments covalently associated with proteasome proteins in the course of degradation. The 1.21 g/mL fraction shows particles of various sizes, including several which appear to be consistent with lipid-based particles. The 1.32 g/mL fraction shows remarkable DNA-like structures that stain differently than naked DNA, suggesting association with macromolecules such as protein.

To determine if LY2 ORF1 is associated with the structures observed in the electron micrographs, immunogold detection using an anti-poly-histidine antibody was carried out. FIG. 18 shows gold label accumulating on the structures observed in the 1.32 and 1.21 g/mL fractions, consistent with the presence of ORF1-His in association with the DNA seen in the 1.32 g/mL fraction, and in the particles seen in the 1.21 g/mL fraction.

Taken together, these results show that ORF1 expressed in Sf9 cells can associate with DNA to form complexes having a density consistent with anellovirus particles.

Example 19: Expression of ORF1 protein from a diverse array of Anelloviruses using baculovirus

In this example, Sf9 cells were infected with baculoviruses engineered to express C-terminal His-tagged ORF1 proteins from anellovirus strains Ring3.1, Ring4, Ring5.2, Ring6, as well as Ring1 and Ring2. As shown in FIG. 19, ORF1 protein originating from each of the *Anellovirus* strains were successfully expressed in Sf9 cells. As shown in **Table E3**, *Anellovirus* ORF1 from the strains representing all three genera (*Alphatorquevirus, Betatorquevirus, and Gammatorquevirus*), were tested and their expression level is seen in FIGS. 3, 4, 5, and 19. In general, we find that the level of expression in this system is highest for ORF1 from *Betatorqueviruses*, intermediate from *Gammatorqueviruses*, and lowest from *Alphatorqueviruses*.

Example 20: *In vitro* assembly of anellovectors using components produced via baculovirus system

In this example, baculovirus constructs suitable for expression of Anellovirus proteins (e.g., ORF1) are generated by *in vitro* assembly.

DNA encoding Anellovirus ORF1 (wildtype protein, chimeric protein or fragments thereof) which may be untagged or contain tags fused N-terminally, C-terminally, or harbor mutations within the

ORF1 protein itself to introduce a tag to aid in purification and/or identity determination through immunostaining assays (such as, but not limited to, ELISA or Western Blot) is expressed in insect cell lines (Sf9 and/or HighFive). Anellovirus ORF1 may be expressed alone or in combination with any number of helper proteins including, but not limited to, Anellovirus ORF2 and/or ORF3 proteins.

Protein is purified using developed purification techniques potentially including but not limited to chelating purification, heparin purification, gradient sedimentation purification and/or size exclusion purification. ORF1 is evaluated for its ability to form capsomers or VLPs and used in subsequent steps for nucleic acid encapsidation.

In one example, DNA encoding Ring2 ORF1 fused to an N-terminal HIS_6-tag (HIS-ORF1) was codon optimized for insect expression and cloned into the baculovirus expression vector pFASTbac system to generate a baculovirus expressing Ring2 ORF-HIS recombinant protein using the Bac-to-BAC expression system according to manufacturer's method (ThermoFisher Scientific). 10 liters of insect cells (Sf9) were infected with Ring2 HIS-ORF1 baculovirus and the cells were harvested 3-days post-infection by centrifugation. The cells were lysed, and the lysate was purified using a chelating resin column using standard art in the field. The elution fraction containing HIS-ORF1 was dialyzed and treated with DNAse to digest host cell DNA. The resulting material was purified again using a chelating resin column and fractions containing ORF1 were retained for nucleic acid encapsidation and viral vector purification.

Nucleic acid encapsidation and viral vector purification: Ring ORF1 (wildtype protein, chimeric protein or fragments thereof) is treated with conditions sufficient to dissociate VLPs or viral capsids to enable reassembly with nucleic acid cargo. Nucleic acid cargo can be defined as double stranded DNA, single stranded DNA, or RNA which encodes a gene of interest that one wants to deliver as a therapeutic agent. Potential conditions sufficient to dissociate VLPs or viral capsids can, but are not limited to, buffers of different pH, conditions of defined conductivity (salt content), conditions containing detergents (such as SDS, Tween, Triton), conditions containing chaotropic agents (such as Urea) or conditions involving defined temperature and time (reannealing temperatures). Nucleic acid cargo of defined concentration is combined with Ring ORF1 of defined concentration and treated with conditions sufficient to permit nucleic acid encapsidation. The resulting particle, defined as viral vector, is subsequently purified, e.g., using developed standard viral purification procedures.

In one example, single stranded circular DNA of a GFP-expression plasmid is added to a solution of Ring 2 HIS-ORF1 and the resulting sample is treated with 0.1% SDS in 50 mM Tris pH 8 buffer at 37C for 30 minutes. The resulting solution is further purified using a heparin column and the viral vector eluted from the column using a gradient of increasing NaCl concentration. The integrity of the viral vector is tested by transducing the cell lines EKVX and HEK293, and observing GFP production in at

least one of the cell lines by fluorescence microscopy, demonstrating encapsidation of the nucleic acid cargo by the ORF1 protein to form the viral vector.

Table E3. Strains for which recombinant ORF1 expression was successful

Name	Genus
Ring1	Alpha
Ring2	Beta
Ring3.1	Gamma
Ring4	Gamma
Ring5.2	Alpha
Ring6	Alpha
HLH	Beta
ctgh3	Beta
LY1	Beta

Example 21: Identification and use of protein binding sequences

This example describes putative protein-binding sites in the *Anellovirus* genome, which can be used for amplifying and packaging effectors, e.g., in an anellovector as described herein. In some instances, the protein-binding sites may be capable of binding to an exterior protein, such as a capsid protein.

Two conserved domains within the *Anellovirus* genome are putative origins of replication: the 5' UTR conserved domain (5CD) and the GC-rich domain (GCR) (de Villiers et al., Journal of Virology 2011; Okamoto et al., Virology 1999). In one example, in order to confirm whether these sequences act as DNA replication sites or as capsid packaging signals, deletions of each region are made in plasmids harboring an Anellovirus sequence. A539 cells are transfected with the deletion constructs. Transfected cells are incubated for four days, and then virus is isolated from supernatant and cell pellets. A549 cells are infected with virus, and after four days, virus is isolated from the supernatant and infected cell pellets. qPCR is performed to quantify viral genomes from the samples. Disruption of an origin of replication prevents viral replicase from amplifying viral DNA and results in reduced viral genomes isolated from transfected cell pellets compared to wild-type virus. A small amount of virus is still packaged and can be found in the transfected supernatant and infected cell pellets. In some embodiments, disruption of a packaging signal will prevent the viral DNA from being encapsulated by capsid proteins. Therefore, in

embodiments, there will still be an amplification of viral genomes in the transfected cells, but no viral genomes are found in the supernatant or infected cell pellets.

In a further example, in order to characterize additional replication or packaging signals in the DNA, a series of deletions across the entire TTMV-LY2 genome is used. Deletions of 100bp are made stepwise across the length of the sequence. Plasmids harboring Anellovirus genome deletions are transfected into A549 and tested as described above. In some embodiments, deletions that disrupt viral amplification or packaging will contain potential *cis*-regulatory domains.

Replication and packaging signals can be incorporated into effector-encoding DNA sequences (e.g., in a genetic element in an anellovector) to induce amplification and encapsulation. This is done both in context of larger regions of the anellovector genome (i.e., inserting effectors into a specific site in the genome, or replacing viral ORFs with effectors, etc.), or by incorporating minimal *cis* signals into the effector DNA. In cases where the anellovector lacks *trans* replication or packaging factors (e.g., replicase and capsid proteins, etc.), the *trans* factors are supplied by helper genes. The helper genes express all of the proteins and RNAs sufficient to induce amplification and packaging, but lack their own packaging signals. The anellovector DNA is co-transfected with helper genes, resulting in amplification and packaging of the effector but not of the helper genes.

Example 22: Replication-deficient anellovectors and helper viruses

For replication and packaging of an anellovector, some elements (e.g., an ORF1, ORF1/1, ORF1/2, ORF2, ORF2/2, ORF2/3, and/or ORF2t/3 molecule, or a nucleic acid sequence encoding same) can be provided in *trans*. These include proteins or non-coding RNAs that direct or support DNA replication or packaging. *Trans* elements can, in some instances, be provided from a source alternative to the anellovector, such as a helper virus, plasmid, or from the cellular genome.

Other elements are typically provided in *cis* (e.g., a TATA box, cap site, initiator element, transcriptional start site, 5' UTR conserved domain, three open-reading frame region, poly(A) signal, or GC-rich region). These elements can be, for example, sequences or structures in the anellovector DNA that act as origins of replication (e.g., to allow amplification of anellovector DNA) or packaging signals (e.g., to bind to proteins to load the genome into the capsid). Generally, a replication deficient virus or anellovector will be missing one or more of these elements, such that the DNA is unable to be packaged into an infectious virion or anellovector even if other elements are provided in *trans*.

Replication deficient viruses can be useful for controlling replication of an anellovector (e.g., a replication-deficient or packaging-deficient anellovector) in the same cell. In some instances, the virus will lack *cis* replication or packaging elements, but express *trans* elements such as proteins and non-coding RNAs. Generally, the therapeutic anellovector would lack some or all of these *trans* elements and

would therefore be unable to replicate on its own, but would retain the *cis* elements. When co-transfected/infected into cells, the replication-deficient virus would drive the amplification and packaging of the anellovector. The packaged particles collected would thus be comprised solely of therapeutic anellovector, without contamination from the replication-deficient virus.

To develop a replication deficient anellovector, conserved elements in the non-coding regions of *Anellovirus* will be removed. In particular, deletions of the conserved 5' UTR domain and the GC-rich domain will be tested, both separately and together. Both elements are contemplated to be important for viral replication or packaging. Additionally, deletion series will be performed across the entire non-coding region to identify previously unknown regions of interest.

Successful deletion of a replication element will result in reduction of anellovector DNA amplification within the cell, e.g., as measured by qPCR, but will support some infectious anellovector production, e.g., as monitored by assays on infected cells that can include any or all of qPCR, western blots, fluorescence assays, or luminescence assays. Successful deletion of a packaging element will not disrupt anellovector DNA amplification, so an increase in anellovector DNA will be observed in transfected cells by qPCR. However, the anellovector genomes will not be encapsulated, so no infectious anellovector production will be observed.

Example 23: Manufacturing process for replication-competent anellovectors

This example describes a method for recovery and scaling up of production of replication-competent anellovectors. Anellovectors are replication competent when they encode in their genome all the required nucleic acid elements and ORFs necessary to replicate in cells. Since these anellovectors are not defective in their replication they do not need a complementing activity provided in trans. They might, however need helper activity, such as enhancers of transcriptions (e.g. sodium butyrate) or viral transcription factors (e.g. adenoviral E1, E2 E4, VA; HSV Vp16 and immediate early proteins).

In this example, double-stranded DNA encoding the full sequence of a synthetic anellovector either in its linear or circular form is introduced into 5E+05 adherent mammalian cells in a T75 flask by chemical transfection or into 5E+05 cells in suspension by electroporation. After an optimal period of time (e.g., 3-7 days post transfection), cells and supernatant are collected by scraping cells into the supernatant medium. A mild detergent, such as a biliary salt, is added to a final concentration of 0.5% and incubated at 37°C for 30 minutes. Calcium and Magnesium Chloride is added to a final concentration of 0.5mM and 2.5mM, respectively. Endonuclease (e.g. DNAse I, Benzonase), is added and incubated at 25-37°C for 0.5-4 hours. Anellovector suspension is centrifuged at 1000 x g for 10 minutes at 4°C. The clarified supernatant is transferred to a new tube and diluted 1:1 with a cryoprotectant buffer (also known as stabilization buffer) and stored at -80°C if desired. This produces passage 0 of the anellovector (P0).

To bring the concentration of detergent below the safe limit to be used on cultured cells, this inoculum is diluted at least 100-fold or more in serum-free media (SFM) depending on the anellovector titer.

A fresh monolayer of mammalian cells in a T225 flask is overlaid with the minimum volume sufficient to cover the culture surface and incubated for 90 minutes at 37°C and 5% carbon dioxide with gentle rocking. The mammalian cells used for this step may or may not be the same type of cells as used for the P0 recovery. After this incubation, the inoculum is replaced with 40ml of serum-free, animal origin-free culture medium. Cells are incubated at 37°C and 5% carbon dioxide for 3-7 days. 4 ml of a 10X solution of the same mild detergent previously utilized is added to achieve a final detergent concentration of 0.5%, and the mixture is then incubated at 37°C for 30 minutes with gentle agitation. Endonuclease is added and incubated at 25-37°C for 0.5-4 hours. The medium is then collected and centrifuged at 1000 x g at 4°C for 10 minutes. The clarified supernatant is mixed with 40 ml of stabilization buffer and stored at-80°C. This generates a seed stock, or passage 1 of anellovector (P1).

Depending on the titer of the stock, it is diluted no less than 100-fold in SFM and added to cells grown on multilayer flasks of the required size. Multiplicity of infection (MOI) and time of incubation is optimized at smaller scale to ensure maximal anellovector production. After harvest, anellovectors may then be purified and concentrated as needed. A schematic showing a workflow, e.g., as described in this example, is provided in FIG 20.

Example 24: Manufacturing process of replication-deficient anellovectors

This example describes a method for recovery and scaling up of production of replication-deficient anellovectors.

Anellovectors can be rendered replication-deficient by deletion of one or more ORFs (e.g., ORF1, ORF1/1, ORF1/2, ORF2, ORF2/2, ORF2/3, and/or ORF2t/3) involved in replication. Replication-deficient anellovectors can be grown in a complementing cell line. Such cell line constitutively expresses components that promote anellovector growth but that are missing or nonfunctional in the genome of the anellovector.

In one example, the sequence(s) of any ORF(s) involved in anellovector propagation are cloned into a lentiviral expression system suitable for the generation of stable cell lines that encode a selection marker, and lentiviral vector is generated as described herein. A mammalian cell line capable of supporting anellovector propagation is infected with this lentiviral vector and subjected to selective pressure by the selection marker (e.g., puromycin or any other antibiotic) to select for cell populations that have stably integrated the cloned ORFs. Once this cell line is characterized and certified to complement the defect in the engineered anellovector, and hence to support growth and propagation of such anellovectors, it is expanded and banked in cryogenic storage. During expansion and maintenance of

these cells, the selection antibiotic is added to the culture medium to maintain the selective pressure. Once anellovectors are introduced into these cells, the selection antibiotic may be withheld.

Once this cell line is established, growth and production of replication-deficient anellovectors is carried out, e.g., as described herein.

Example 25: Production of anellovectors using suspension cells

This example describes the production of anellovectors in cells in suspension.

In this example, an A549 or 293T producer cell line that is adapted to grow in suspension conditions is grown in animal component-free and antibiotic-free suspension medium (Thermo Fisher Scientific) in WAVE bioreactor bags at 37 degrees and 5% carbon dioxide. These cells, seeded at 1 x 10^6 viable cells/ mL, are transfected using lipofectamine 2000 (Thermo Fisher Scientific) under current good manufacturing practices (cGMP), with a plasmid comprising anellovector sequences, along with any complementing plasmids suitable or required to package the anellovector (e.g., in the case of a replication-deficient anellovector, e.g., as described herein). The complementing plasmids can, in some instances, encode for viral proteins that have been deleted from the anellovector genome (e.g., an anellovector genome based on a viral genoe, e.g., an *Anellovirus* genome, e.g., as described herein) but are useful or required for replication and packaging of the anellovectors. Transfected cells are grown in the WAVE bioreactor bags and the supernatant is harvested at the following time points: 48, 72, and 96 hours post transfection. The supernatant is separated from the cell pellets for each sample using centrifugation. The packaged anellovector particles are then purified from the harvested supernatant and the lysed cell pellets using ion exchange chromatography.

The genome equivalents in the purified prep of the anellovectors can be determined, for example, by using a small aliquot of the purified prep to harvest the anellovector genome using a viral genome extraction kit (Qiagen), followed by qPCR using primers and probes targeted towards the anellovector DNA sequence, e.g., as described in Example 31.

The infectivity of the anellovectors in the purified prep can be quantified by making serial dilutions of the purified prep to infect new A549 cells. These cells are harvested 72 hours post transfection, followed by a qPCR assay on the genomic DNA using primers and probes that are specific to the anellovector DNA sequence.

Example 26: Quantification of anellovector genome equivalents by qPCR

This example demonstrates the development of a hydrolysis probe-based quantitative PCR assay to quantify anellovectors. Sets of primers and probes are designed based on an Anellovirus genome sequence. using the software Geneious with a final user optimization. Exemplary primer sequences for

TTV (Accession No. AJ620231.1) and TTMV (Accession No. JX134045.1) are shown in Table E4 below.

Table E4: Sequences of forward and reverse primers and hydrolysis probes used to quantify TTMV and TTV genome equivalents by quantitative PCR.

TTMV		SEQ ID NO:
Forward Primer	5'-GAAGCCCACCAAAAGCAATT-3'	697
Reverse Primer	5'-AGTTCCCGTGTCTATAGTCGA-3'	698
Probe	5'-ACTTCGTTACAGAGTCCAGGGG-3'	699

TTV		
Forward Primer	5'-AGCAACAGGTAATGGAGGAC-3'	700
Reverse Primer	5'-TGGAAGCTGGGGTCTTTAAC-3'	701
Probe	5'-TCTACCTTAGGTGCAAAGGGCC-3'	702

As a first step in the development process, qPCR is run using the Anellovirus primers with SYBR-green chemistry to check for primer specificity. FIG. 21 shows one distinct amplification peak for each primer pair.

Hydrolysis probes are ordered labeled with the fluorophore 6FAM at the 5' end and a minor groove binding, non-fluorescent quencher (MGBNFQ) at the 3' end. The PCR efficiency of the new primers and probes was evaluated using two different commercial master mixes using purified plasmid DNA as component of a standard curve and increasing concentrations of primers. The standard curve is set up by using purified plasmids containing the target sequences for the different sets of primers-probes. Seven tenfold serial dilutions are performed to achieve a linear range over 7 logs and a lower limit of quantification of 15 copies per 20ul reaction. All primers for qPCR are ordered from a commercial vendor such as IDT. Hydrolysis probes conjugated to the fluorophore 6FAM and a minor groove binding, non-fluorescent quencher (MGBNFQ) as well as all the qPCR master mixes are obtained from Thermo Fisher. An exemplary amplification plot is shown in FIG. 22.

Using these primer-probe sets and reagents, the genome equivalent (GEq)/ml in anellovector stocks is quantified. The linear range is then used to calculate the GEq/ml. Samples with higher concentrations than the linear range can be diluted as needed.

Example 27: Tandem copies of the Anellovirus genome

This example describes plasmid-based expression vectors harboring two copies of a single anelloviral genome, arranged in tandem such that the GC-rich region of the upstream genome is near the 5' region of the downstream genome (FIG. 23A).

Anelloviruses replicate via rolling circle, in which a replicase (Rep) protein binds to the genome at an origin of replication and initiates DNA synthesis around the circle. For anellovirus genomes contained in plasmid backbones, this requires either replication of the full plasmid length, which is longer than the native viral genome, or recombination of the plasmid resulting in a smaller circle comprising the genome with minimal backbone. Therefore, viral replication off of a plasmid can be inefficient. To improve viral genome replication efficiency, plasmids are engineered with tandem copies of TTV-tth8 and TTMV-LY2. These plasmids present every possible circular permutation of the anelloviral genome: regardless of where the Rep protein binds, it will be able to drive replication of the viral genome from the upstream origin of replication to the downstream origin. A similar strategy has been used to produce porcine Anelloviruses (Huang et al., 2012, Journal of Virology 86 (11) 6042-6054).

Tandem anellovector can be assembled, for example, by sequentially cloning copies of the genome into a plasmid backbone, leaving 12bp of non-viral DNA between the two sequences. Alternatively, tandem anellovector can be assembled via Golden-gate assembly, simultaneously incorporating two copies of the genome into a backbone and leaving no extra nucleotides between the genomes.

Plasmid harboring tandem copies of an anellovector genetic element sequence is transfected into HEK239T cells. Cells are incubated for five days, then lysed using 0.1% Triton X-100 and treated with nucleases to digest DNA not protected by viral capsids. qPCR is then performed using Taqman probes for the TTV-tth8 genome sequence and the plasmid backbone. TTV-tth8 genome copies are normalized to backbone copies.

Example 28: *In vitro* circularized Anellovirus genomes

This example describes constructs comprising circular, double stranded Anelloviral genome DNA with minimal non-viral DNA. These circular viral genomes more closely match the double-stranded DNA intermediates found during wild-type Anellovirus replication. When introduced into a cell, such circular, double stranded Anelloviral genome DNA with minimal non-viral DNA can undergo rolling circle replication to produce, for example, a genetic element as described herein.

In one example, plasmids harboring an Anellovirus genome sequence are digested with restriction endonucleases recognizing sites flanking the genomic DNA. The resulting linearized genomes are then ligated to form circular DNA. These ligation reactions are done with varying DNA concentrations to

optimize the intramolecular ligations. The ligated circles are either directly transfected into mammalian cells, or further processed to remove non-circular genome DNA by digesting with restriction endonucleases to cleave the plasmid backbone and exonucleases to degrade linear DNA. To demonstrate the improvements in Anellovirus production, circularized Anellovirus genome constructs are transfected into HEK293T cells. After 7 days of incubation, cells are lysed, and qPCR is performed to compare the levels of anellovirus genome between circularized and plasmid-based anelloviral genomes. Increased levels of Anelloviral genomes show that circularization of the viral DNA is a useful strategy for increasing Anellovirus production.

Digested plasmid can be purified on 1% agarose gels prior to electroelution or Qiagen column purification and ligation with T4 DNA Ligase. Circularized DNA is concentrated on a 100 kDa UF/DF membrane before transfection. Circularization is confirmed by gel electrophoresis. T-225 flasks are seeded with HEK293T at 3 x 10^4 cells/cm^2 one day prior to lipofection with Lipofectamine 2000. Nine micrograms of circularized Anellovirus DNA and 50 μg of circularized Anellovirus-nLuc are co-transfected one day post flask seeding. As a comparison, an additional T-225 flask is co-transfected with 50 μg of linearized Anellovirus and 50 μg of linearized Anellovirus-nLuc.

Anellovector production proceeds for eight days prior to cell harvest in Triton X-100 harvest buffer. Generally, anellovectors can be enriched, e.g., by lysis of host cells, clarification of the lysate, filtration, and chromatography. In this example, harvested cells are nuclease treated prior to sodium chloride adjustment and 1.2 μm / 0.45 μm normal flow filtration. Clarified harvest is concentrated and buffer exchanged into PBS on a 750 kDa MWCO mPES hollow fiber membrane. The TFF retentate is filtered with a 0.45 μm filter before loading on a Sephacryl S-500 HR SEC column pre-equilibrated in PBS. Anellovectors are processed across the SEC column at 30 cm/hr. Individual fractions are collected and assayed by qPCR for viral genome copy number and transgene copy number. Viral genomes and transgene copies are observed beginning at the void volume, Fraction 7, of the SEC chromatogram. Agreement between copy number for Anellovirus genomes and Anellovirus-nLuc transgene for Anellovectors produced using circularized input DNA at Fraction 7 – Fraction 10 indicates packaged Anellovectors containing nLuc transgene. SEC fractions are pooled and concentrated using a 100 kDa MWCO PVDF membrane and then 0.2 μm filtered prior to *in vivo* administration.

Example 29: Production of anellovectors containing chimeric ORF1 with structural hypervariable domains from different Torque Teno Virus strains

This example describes domain swapping of hypervariable regions of ORF1 to produce chimeric anellovectors containing the ORF1 structural arginine-rich region, structural jelly-roll domain, structural

N22, and structural C-terminal domain of one TTV strain, and the structural hypervariable domain from an ORF1 protein of a different TTV strain.

The full-length genome of a first Anellovirus is cloned into expression vectors for expression in mammalian cells. This genome is mutated to remove the structural hypervariable domain of the ORF1 coding sequence and replace it with the structural hypervariable domain of the ORF1 coding sequence from a second Anellovirus genome (FIG. 24). The plasmid containing the first Anellovirus genome with the swapped structural hypervariable domain is then linearized and circularized as described herein. HEK293T cells are transfected with the circularized genome and incubated for 5-7 days to allow anellovector production. After the incubation period anellovectors are purified from the supernatant and cell pellet of transfected cells by gradient ultracentrifugation.

To determine if the chimeric anellovectors are still infectious, the isolated viral particles are added to uninfected cells. The cells are incubated for 5-7 days to allow viral replication. After incubation the ability of the chimeric anellovectors to establish infection will be monitored by immunofluorescence, western blot, and qPCR. The structural integrity of the chimeric viruses is assessed by negative stain and cryo-electron microscopy. Chimeric anellovectors can further be tested for ability to infect cells *in vivo*. Establishment of the ability to produce functional chimeric anellovectors through structural hypervariable domain swapping could allow for engineering of viruses to alter tropism and potentially evade immune detection.

Example 30: Design of an anellovector harboring a payload

This example describes the design of an exemplary anellovector genetic element harboring a *trans* gene. The genetic element is composed of the essential *cis* replication and packaging domains from an Anellovirus genome (e.g., as described herein) along with a non-Anellovirus payload, which may include, e.g., protein or non-coding RNA-expressing genes. The anellovector lacks essential *trans* protein elements for replication and packaging, and requires proteins provided by other sources (e.g., helpers, e.g., replicating viruses, expression plasmids, or genome integrations) for rolling circle replication and encapsidation.

In one set of examples, the entire protein-coding DNA sequence is deleted, from the first start codon to the last stop codon (FIG. 26). The resulting DNA retains the viral non-coding region (NCR), including the viral promoter, the 5' UTR conserved domain, the 3' UTR (which encodes miRNAs in some anellovirus strains), and the GC-rich region. The anellovector NCR harbors essential *cis* domains, including the viral origin of replication and capsid binding domains. However, lacking the anellovirus protein-coding open reading frames, the anellovector is unable to express essential protein factors

required for DNA replication and encapsidation, and therefore cannot amplify or package unless these elements are provided in *trans*.

Payload DNA, including but not limited to protein-encoding sequences, full *trans* genes (including non-anelloviral promoter sequences), and non-coding RNA genes are incorporated into the anellovector genetic element by insertion into the site of the deleted anelloviral open reading frames (FIG. 26). Expression from protein-coding sequences can be driven, for example, by either the native viral promoter or a synthetic promoter incorporated as a *trans* gene.

Replication-deficient or incompetent anellovector genetic elements (e.g., as described herein) may lack the protein-coding sequences for viral replication and/or capsid factors. Therefore, packaged anellovectors are produced by co-transfecting cells with the anellovector DNA described in this example and viral-protein-encoding DNA. The viral proteins are expressed off of replication-competent wild-type viral genomes, non-replicating plasmids harboring the viral proteins under control of the viral promoter, or plasmids harboring the viral proteins under control of a strong constitutive promoter.

Example 31: In vitro circularized genome as input material for producing anellovector genetic elements in vitro

This example demonstrates that in vitro circularized (IVC) double stranded anellovirus DNA, as source material for an anellovector genetic element as described herein, is more robust than an anellovirus genome DNA in a plasmid to yield packaged anellovector genomes of the expected density.

1.2E+07 HEK293T cells (human embryonic kidney cell line) in T75 flasks were transfected with 11.25 ug of either, (i) in vitro circularized double stranded TTV-tth8 genome (IVC TTV-tth8), (ii) TTV-tth8 genome in a plasmid backbone, or (iii) plasmid containing just the ORF1 sequence of TTV-tth8 (non-replicating TTV-tth8). Cells were harvested 7 days post transfection, lysed with 0.1% Triton, and treated with 100 units per ml of Benzonase. The lysates were used for cesium chloride density analysis; density was measured and TTV-tth8 copy quantification was performed for each fraction of the cesium chloride linear gradient. As shown in FIG. 29, IVC TTV-tth8 yielded dramatically more viral genome copies at the expected density of 1.33 as compared to TTV-tth8 plasmid.

1E+07 Jurkat cells (human T lymphocyte cell line) were nucleofected with either in-vitro circularized LY2 genome (LY2 IVC) or LY2 genome in plasmid. Cells were harvested 4 days post transfection and lysed using a buffer containing 0.5% triton and 300 mM sodium chloride, followed by two rounds of instant freeze-thaw. The lysates were treated with 100 units/ ml benzonase, followed by cesium chloride density analysis. Density measurement and LY2 genome quantification was performed on each fraction of the cesium chloride linear gradient. As shown in FIG. 30, transfection of in vitro

circularized LY2 genome in Jurkat cells led to a sharp peak at the expected density, as compared to the transfection of plasmid containing the LY2 genome, which showed no detectable peak in FIG. 30.

In some embodiments, IVC anellovector genetic element constructs can be introduced into insect cells (e.g., Sf9 cells) and enclosed within proteinaceous exterior proteins expressed from a bacmid, e.g., as described herein.

Example 32: Antibody generation and western blot analysis

This example describes generation of Ring 10 (also referred to herein as Ly1) antibodies and western blot analysis for determining expression, e.g., of Ring 10 ORF1 proteins. Ring 10 antibodies were generated by immunizing rabbits with a synthetic peptide representing one of three portions of the ORF1 protein (jelly roll residues 46-58: KIKRLNIVEWQPK, spike domain residues 485-502: SPSDTHEPDEEDQNRWYP, C-terminal domain residues 635-672: SEEEEESNLFERLLRQRTKQLQLKRRIIQTLKDLQKLE) conjugated to a carrier protein by an engineered N-terminal cysteine. Rabbits were immunized twice, and polyclonal antibodies were purified from bleeds using protein A purification (Custom Antibody Production, Life Technologies corporation). Western blot analysis was performed using NUPAGE 4-12% gels (ThermoFisher) transferred to nitrocellulose membranes using the Transblot Turbo system (BioRad). Membranes were blocked with blocking buffer (Licor), probed ~16 hours with primary antibody, and detected using anti-rabbit IRDye infrared secondary antibodies and imaging system (Licor).

Example 33: Construct design, cell culture and protein expression/purification

This example describes design of Ring 10 constructs, cell culture, and expression/purification of Ring 10 proteins. The Ring 10 ORF2 and ORF1 sequences were codon optimized for insect cells and with different ORF1 construct length variations (full-length ORF1, delARM with a deletion of residue 2-45, and delARM/delCTD with deletions of residues 2-45 and 552-672). The ORF2 and ORF1 constructs were cloned into the pFastBac Dual plasmid which was used to generate baculoviruses. To express the ORF1 proteins, Sf9 cells (Gibco™ 11496015) were infected by baculovirus with multiplicity of infection = 1 and the cells were cultured for three days at 27°C and harvested by centrifugation. The cells were lysed by treatment with 0.01% Triton X-100 (Sigma-Aldrich 11332481001), subjected to micro-fluidization, and treated with protease inhibitors (Thermo Scientific Halt Protease Inhibitor Cocktail, PI78438) and DNase (Benzonase®; Sigma). Cell lysate was subsequently purified using HiTrap Heparin affinity chromatography (Cytiva) followed by size-exclusion chromatography (HiPrep 16/60 Sephacryl S-500 HR; Cytiva).

Example 34: Negative-stained EM data collection and analysis

This example describes visualization of anellovirus particles using negative stain electron microscopy. Jeol 1200EX transmission electron microscope was used for screening different Ring 10 constructs. 10 µl of sample was blotted on 400 mesh carbon support film (cf400-cu, EMS) for 30 seconds. After washing by ddH_2O for 30 seconds, the grid was stained by 0.75% of uranyl formate (UF) for 10 seconds before loading on the scope. Ring 10 delARM was further imaged at NanoImaging Service. 3 µl of 0.12 mg/ml was blotted to a continuous carbon grid and stained by 1% UF. Negative-stained electron microscopy was performed using a Thermo Fisher Scientific (Hillsboro, Oregon) Glacios Cryo Transmission Electron Microscope (cryo-TEM) operated at 200 kV and equipped with a TFS CETA-D 4x4 CMOS camera and a Falcon 4 direct electron detector.

Example 35: Cryo-EM data collection and data analysis and molecular refinement

This example describes performing cryogenic electron microscopy (Cryo-EM) and data analysis, e.g., for determination of anellovirus particle structure. For the grid preparation, 3 µl of 0.3 mg/ml VLP sample was applied to a 1.2x1.3 graphene oxide grid. A total of 11,083 micrographs were collected from Glacios cryo-TEM (Thermo Fisher Scientific) operated at 200 kV with a Falcon 4 direct electron detector, at a nominal defocus range of -1.0 – -2.5 µm and accumulated dose of 19.59 $e^-/Å$ for a total of 15 frames in 3 minutes. The pixel size was 0.923 Å, with the magnification 150000x. Automated data-collection was carried out by Leginon software.

All micrographs were motion-corrected by Relion-4.0 implemented MotionCor2, and the contrast transfer function (CTF) parameters were estimated by Gctf. With manually picked particles from 20 micrographs to train the network, SPHIRE-crYOLO automatically picked 58,391 particles along with the PhosaurusNet network. All particles were extracted by Relion-4.0 and rescaled to 2-fold (pixel size 1.846 Å), followed by subsequence 2D classifications with 350 Å mask diameter to remove any junk particles. Two iterations of 2D classifications resulted in 11,185 particles, which were merged and reextracted to generate a de novo 3D initial model by Relion-4.0 with I1 symmetry. Notably, several similar initial models without symmetry imposed were obtained by Relion-4.0 and cisTEM. To obtain a better classification result, all particles were first subjected to a Refine3D with initial angular sampling 3.7° and local angular search 0.9° per step. The alignment parameters of each particle were transferred to a 3D classification with angular sampling interval 0.9° and local angular search 5° per step. 3D classification of the entire particle set attributed most of the particles into a single class. After CtfRefine and Bayesian polishing, the post-processing results in 3.98 Å resolution under the gold standard (with FSC=0.143).

The initial anellovirus TTMV-LY2 capsid monomer structure was predicted by TrRosetta, and Ring 10 structure was further predicted by RosettaCM. Structural refinement was performed by Rosetta and Phenix, and fine-adjusted by COOT. JA 20 and SAFIA structures are predicted by Alphafold.

Example 36: Circular dichroism spectroscopy

This example describes performing circular dichroism spectroscopy to determine secondary structure of, e.g., an anellovirus ORF1 protein. To determine the secondary structure of the Ring 10 C-terminal domain, the peptides used to generate C-terminal antibodies (residues 635-672) were analyzed at 25.8 μM in PBS on a Jasco J-815 circular dichroism spectropolarimeter using 2 mm path length cell at ambient temperature. Each data set is the average of three consecutive scans. The secondary structure was determined by using CDPro software package, compared with a reference set containing 56 proteins (IBasis=10). The final secondary structure fractions were averaged over the results from three programs (SELCON3, CDSSTR, CONTINLL) in CDPro.

Example 37: Anellovirus particle structure

This example describes determination of the particle structure of an exemplary Anellovirus.

Initial efforts to study the structure of a virus-like particle (VLP) derived from a TTMV isolate described herein as Ring 10, were done with the full-length ORF1 (residues 1-672) expressed in insect cells (Sf9). Full-length ORF1 was visualized by electron microscopy (as described in Example 34) and was observed to assemble into particles ~32 nm in diameter (FIG. 34A), similar to previously reported estimates of anellovirus size. However, full-length ORF1 particles lacked the homogeneous symmetry expected of viral particles (FIG. 34A). Genome packaging by ARM-containing viruses is believed to occur when the positive arginine residues bind a negatively charged genome, overcoming electrostatic repulsion of the ARM to permit ORF1 assembly. Therefore, Ring 10 delARM, an ORF1 construct wherein residues 2-45 are deleted, was designed as described in Example 33 (FIG. 31A, 31B, and 34B) to facilitate more efficient ORF1 assembly in the absence of viral DNA. Ring 10 delARM expression was confirmed using a rabbit polyclonal antibody raised against a spike domain peptide (residues 485-502; FIG. 31B) as described in Example 32. A band consistent with Ring 10 delARM was observed by western blot analysis (expected mass of 73.3 kDa; FIG. 31C) performed as described in Example 32. Ring 10 delARM yielded an ORF1 band above the 62-kDa marker (FIG. 31C). The difference in apparent molecular weight of Ring 10 delARM ORF1 after initial expression and after purification suggested proteolysis. To identify the site of proteolysis, polyclonal antibodies to peptides from the extreme N- and C-termini of Ring 10 delARM (residues 46-58 and residues 635-672, respectively; FIG. 31B) were

generated as described in Example 32, and the presence of the N-terminal peptide of Ring 10 delARM on the purified fragment and the absence of the C-terminal peptide were confirmed (FIG. 31C).

Electron microscopy (EM) analysis of the Ring 10 delARM fragment showed the VLPs formed were more homogeneous and symmetric in morphology relative to full-length ORF1 (FIG. 34A and 34B). The formation of homogeneous VLPs following genetic removal of the N-terminus and proteolysis of the C-terminus has been observed in another JR-containing virus, hepatitis E (HEV). To determine if the C-terminus of Ring 10 ORF1 is required for particle formation, VLPs from construct Ring 10 ORF1 delARM delCTD (wherein residues 2-45 and 552-672 are deleted) were generated as described in Example 33. Ring 10 ORF1 delARM delCTD produced VLPs of similar symmetry to Ring 10 ORF1 delARM (FIG. 34B and 34C). These results suggest that proteolysis of the ORF1 C-terminus may be a natural part of anellovirus formation. In light of recent evidence that the C-terminal region of ORF1 is the immunodominant region of anelloviruses, its excision from the mature particle would be consistent with the immune evasion properties of anelloviruses.

The structure of the Ring 10 delARM particle was determined using cryo-EM to 3.98 Å resolution as described in Example 35 (FIG. 31D-31F, 35, 36A, and 36B; Table S1). The anellovirus particle was shown to be formed by sixty ORF1 fragments organized in an icosahedral T=1 symmetry (FIG. 31D). Electron density for residues 48-562 was observed (FIG. 31B). The resulting mass of the observed Ring 10 fragment was calculated to be ~59.8 kDa. The N-terminal region (residues 46-228) forms part of the canonical 8-β–strand JR domain (β strands named B to H by convention). Unexpectedly, the eighth and final β–strand in the JR (strand I, residues 531-542) is located just prior to the C-terminal domain (FIG. 31B). The resulting fold of the ORF1 protomer has residues at the N- and C-termini generating the JR domain at the particle core while the intrastrand residues form the exterior of the particle surface. Intrastrand insertions forming the viral particle exterior can be found in other JR-containing viruses such as adeno-associated virus (AAV) and canine parvovirus (CPV). However, while the intrastrand insertions for AAV2 (228 residues) and CPV (227 residues) are between G and H β–strands, the 298-residue intrastrand insertion in Ring 10 is significantly larger and lies between β–strands H and I. The intrastrand region (residues 229-530) extends from the JR domain to form a structure herein referred to as a spike domain. The spike domain is formed by two globular domains: the spike P1 domain (residues 229-250 and 386-530) and the spike P2 domain (residues 251-385; FIG. 31B, 31E).

Table S1. Cryo-EM data collection, refinement and validation statistics of TTMV-Ring 10 delARM.

	Ring 10 delARM

Data collection and processing	
Magnification	150000x
Voltage (kV)	200
Electron exposure (e–/Å^2)	1.31
Defocus range (μm)	-0.5~ -2.5
Pixel size (Å)	0.923
Symmetry imposed	I1
Initial particle images (no.)	58391
Final particle images (no.)	6271
Map resolution (Å) FSC threshold	3.98/0.143
Map resolution range (Å)	3.9-6
Refinement	
Initial model used (PDB code)	
Model resolution (Å) FSC threshold	3.98/0.143
Model resolution range (Å)	3.9-6
Map sharpening *B* factor (Å^2)	-152.851
Model composition Non-hydrogen atoms Protein residues Ligands	253440/30900/0
B factors (Å^2) Protein Ligand	30.00/243.72/133.17
R.m.s. deviations Bond lengths (Å) Bond angles (°)	0.007/1.282
Validation MolProbity score Clashscore Poor rotamers (%)	2.36/25.43/0
Ramachandran plot Favored (%) Allowed (%) Disallowed (%)	92.6/6.62/0.78

CC (mask)	0.81
MolProbility	2.36
Clash Score	25.43

Example 38: Anellovirus structural jelly roll domains

This example describes the structure of anellovirus structural jelly roll (JR) domains. Sixty Ring 10 JR domains form the core of the virus particle (FIG. 32A-32D). The β–strands form β–sheets which are characterized by a C-H-E-F pattern on the core's exterior and B-I-D-G pattern on the core's interior. The N-terminus of strand B is oriented to place the ARM on the interior of the core, where it is positioned to bind the viral genome. The observed C-terminal residues (545-562) extend from the C-terminus of β-strand I on the interior of the particle and thread through JR domains on the 2-fold axis to contact the neighboring JR domain (FIG. 32B).

In several JR-containing viruses, positively charged residues (arginine and lysine) oriented internally on strands B, I, D, and G are expected to bind the negatively charged viral genome (FIG. 32C). In Ring 10, basic residues Arg61, Lys62, Arg64, Lys66 (β–strand B), Lys140 (β–strand D), Arg197 (β–strand G), Lys533, Lys535, and Lys541 (β–strand I) are all oriented toward the particle interior and are likely responsible, together with the ARM motif, for binding the negatively charged viral genome. Notably, density suggesting bound nucleic acid was not observed, possibly because the ARM deletion prevented nucleic acid binding or because any host cell nucleic acid encapsidated by the VLP would be too heterogeneous for detection. Alignment of anellovirus ORF1 sequences revealed that several of these putative DNA-binding residues are conserved across species, which supports their role in DNA-binding (FIG. 32D).

Example 39: Anellovirus spike domains

This example describes the structure of exemplary anellovirus spike domains, which include the P1 and P2 domains (e.g., as described herein). Residues 229 to 530 form the spike domain that extends ~6 nm from the JR core (FIG. 33A-33D). A β strand (residues 245-250) extending from JR β–strand H is the first component of the spike P1 domain and is N-terminal to the spike P2 domain (residues 251-385). The previously described hypervariable region (HVR) of ORF1 comprises the majority of the spike P2 domain. The remaining residues of the spike domain (residues 386-530) form five additional β–strands and eight helices, which, together with the residues 245-250 strand, fold into the spike P1 domain. The local resolution of P1 is only slightly lower than the JR domain (~4-4.5 Å), while the resolution of P2 is

within 5-6 Å. This is likely a consequence of both being further from the radius of gyration and some flexibility of the HVR residues.

Neighboring spike domains pack together around the five-fold symmetry axis to form a ringed structure of 5 spike domains henceforth called the crown (FIG. 33A, 33B). A receptor for anelloviruses has not been identified to date. Given the diverse tropism of different anellovirus strains, it is possible that residues of the spike P2 HVR, which are the most surface-exposed, are involved in viral attachment and infection. However, the hypervariable sequence of the spike P2 domain may serve to aid in immune evasion rather than harboring a receptor-binding motif. If this is the case, a receptor-binding motif on the better conserved spike P1 domain, or even on the surface of the JR core, may exist.

Sequence alignment of the Ring 10 spike domain with other anelloviruses did not readily identify conserved spike surface residues (FIG. 33B-33D, FIG. 38; sequences reproduced below). In fact, the Ring 10 residues that are more conserved between other anelloviruses (e.g., Asp396, Pro429, Trp431, Gly437, Pro461, Phe477, Pro483, Trp500, Tyr501, Pro502, Gly518 and Pro519) are internal spike P1 residues supporting its globular fold. A few basic residues are semi-conserved on the spike P1 surface (e.g., Arg390, Arg481, Arg499, and Arg523) which could contribute to the particle's ability to bind heparin resin. A few semi-conserved hydrophobic or aromatic residues on spike P1 (e.g., Leu231, Ile236, Val245, Tyr404, Gly414, Ile424, Leu428, Leu432, Val450, Ile478, and Tyr484) are at least partially surface-exposed and could represent a conserved receptor binding surface (FIG. 33B-33D). If this is the case, then it is attractive to posit that anelloviruses have evolved their novel elongated H-I intrastrand spike domain to sterically hinder antibody binding to their cellular receptor-binding site present on the P1 surface using domain P2 which is able to tolerate highly diverse amino acid substitutions. This would allow diverse anelloviruses to repeatedly infect human hosts with minimal recognition and neutralization by the immune system.

Sequences included in sequence alignments (FIG. 38)

>Ly1 (Ring10)_orf1
MPWWYRRRSYNPWRRRNWFRRPRKTIYRRYRRRRRWVRRKPFYKRKIKRLNIVEWQPKSIRKCRIKGMLCLFQT
TEDRLSYNFDMYEESIIPEKLPGGGGFSIKNISLYALYQEHIHAHNIFTHTNTDRPLARYTGCSLKFYQSKDID
YVVTYSTSLPLRSSMGMYNSMQPSIHLMQQNKLIVPSKQTQKRRKPYIKKHISPPTQMKSQWYFQHNIANIPLL
MIRTTALTLDNYYIGSRQLSTNVTIHTLNTTYIQNRDWGDRNKTYYCQTLGTQRYFLYGTHSTAQNINDIKLQE
LIPLTNTQDYVQGFDWTEKDKHNITTYKEFLTKGAGNPFHAEWITAQNPVIHTANSPTQIEQIYTASTTTFQNK
KLTDLPTPGYIFITPTVSLRYNPYKDLAERNKCYFVRSKINAHGWDPEQHQELINSDLPQWLLLFGYPDYIKRT
QNFALVDTNYILVDHCPYTNPEKTPFIPLSTSFIEGRSPYSPSDTHEPDEEDQNRWYPCYQYQQESINSICLSG
PGTPKIPKGITAEAKVKYSFNFKWGGDLPPMSTITNPTDQPTYVVPNNFNETTSLQNPTTRPEHFLYSFDERRG
QLTEKATKRLLKDWETKETSLLSTEYRFAEPTQTQAPQEDPSSEEEEESNLFERLLRQRTKQLQLKRRIIQTLK
DLQKLE
>Ly2 (Ring2) orf1
MPYYYRRRRYNYRRPRWYGRGWIRRPFRRRFRRKRRVRPTYTTIPLKQWQPPYKRTCYIKGQDCLIYYSNLRLG
MNSTMYEKSIVPVHWPGGGSFSVSMLTLDALYDIHKLCRNWWTSTNQDLPLVRYKGCKITFYQSTFTDYIVRIH

TELPANSNKLTYPNTHPLMMMMSKYKHIIPSRQTRRKKKPYTKIFVKPPPQFENKWYFATDLYKIPLLQIHCTA
CNLQNPFVKPDKLSNNVTLWSLNTISIQNRNMSVDQGQSWPFKILGTQSFYFYFYTGANLPGDTTQIPVADLLP
LTNPRINRPGQSLNEAKITDHITFTEYKNKFTNYWGNPFNKHIQEHLDMILYSLKSPEAIKNEWTTENMKWNQL
NNAGTMALTPFNEPIFTQIQYNPDRDTGEDTQLYLLSNATGTGWDPPGIPELILEGFPLWLIYWGFADFQKNLK
KVTNIDTNYMLVAKTKFTQKPGTFYLVILNDTFVEGNSPYEKQPLPEDNIKWYPQVQYQLEAQNKLLQTGPFTP
NIQGQLSDNISMFYKFYFKWGGSPPKAINVENPAHQIQYPIPRNEHETTSLQSPGEAPESILYSFDYRHGNYTT
TALSRISQDWALKDTVSKITEPDRQQLLKQALECLQISEETQEKKEKEVQQLISNLRQQQQLYRERIISLLKDQ
>MN773391.1|Beta|245_2239_2_fow
MPPFRRNWWSNYYKPKWRRRRFPARRRRFTTTFRKRRRRTKKVKRHFYSKKYKRKLKKIILRQWQPSTIKRCKI
EGYLCLFQAGKGRFSFNYASYKESFCPEHEPGGGGWGLQQLSLSNLYSQNCDLMNWWTKSNNHLNLCRYMGCEI
TLFRQDTVDYVFTYFDEQPKNVSKYYYATFHPTKLMLYNRKIVVPSFASQPHKRKPYKRKFIRAPKLMKNQWYF
QQQLAPYPLLQFAATAVSLNKMFGSSKALNNNCTVYCLNTDFFRHPHFQYSAQPFTPDGGNQPTYIYTIQRGEP
PITAYKYFNLTFLGQTMTNDPGIPLSNNNNYTKNEWGNVFFYKYLNNDELTYVSKETIDQVKQKKNQQITSGFT
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Example 40: Evasion of immune system

This Example describes the anellovirus C-terminal region and its relation to immune evasion. Absent from the Ring 10 structure is the majority of the C-terminal region (residues 545-672). Observed density for residues 545-562 showed that, were the C-terminal region present on the wild-type virus, the C-terminus would extend from the JR core toward the surface near the 3-fold axis between the spike domain crowns. The C-terminal region is semi-conserved between all anelloviruses, is predicted to be helical in nature, and has a glutamate-rich region (residues 636-640 on Ring 10) which could N-

terminally cap a conserved helical motif. To determine if the C-terminal residues of Ring 10 would form a helical structure, circular dichroism experiments were performed, as described in Example 36, on the C-terminal peptides used to generate the aforementioned antibodies. Indeed, analysis with circular dichroism suggested that the C-terminal region (residues 635-672) is helical in solution, suggesting the C-terminal region would form a coiled-coil domain (FIG. 37A and 37B), strongly suggesting a leucine-zipper like association of the helices perhaps due to the periodicity of hydrophobic residues. Electron microscopy of full-length Ring 10 ORF1 vs ORF1 which either had the C-terminus proteolytically removed or genetically removed suggested that particle formation is improved with the absence of the semi-conserved C-terminus (FIG. 34A-34C). Given that anelloviruses have evolved to evade the immune system, and that the C-terminal region is the immunodominant region of ORF1 and may be antagonistic to particle formation, the semi-conserved C-terminal region may be required for wild-type particle assembly but processed during viral particle maturation.

Despite anelloviruses constituting the majority of the human virome, their capsid structure was unknown. Determination of the Ring 10 structure as described by the examples included herein demonstrates that ORF1 encodes the capsid protein and that anelloviruses evolved a novel spike domain that extends around the 5-fold axis to form a crown structure. These crowns are capped with hypervariable P2 regions, which likely inhibits the development of antibodies against the better conserved spike P1 domain via steric hindrance. Analysis of the P1 surface revealed conserved residue patches that may have a receptor binding function.

The structure of the Ring 10 *Betatorquevirus* can be used to guide future anellovirus research. In particular, the diversity and immunological stealth of anelloviruses indicates that they could be used to deliver therapeutic genes to cell types not currently addressed by existing vectors, and that they may be less susceptible to pre-existing immunity or to the development of neutralizing antibodies following initial treatment. The availability of a capsid structure will help guide the design of anellovirus-based gene therapy vectors.

Example 41. Grafting of HVR helix motif between Anellovirus ORF1 proteins

In this example, a peptide sequence in the structural hypervariable region (HVR) of the Ring 10 ORF1 protein was identified as a surface epitope. The surface epitope can, for example, be recognized by polyclonal antibodies to aid in analytical and purification development. Briefly, polyclonal antibodies (PAbs) were generated to Betatorquevirus strain Ring 10, guided by structural prediction of the viral particle formed by ORF1. The Ring 10 structure, determined as described herein, confirmed that a key helix (residues 352-361, having the amino acid sequence SPTQIEQIYT) is surface exposed in the viral particle.

A PAb that recognizes Ring 10 HVR helix 352-361 PAb (AB3725) was shown to recognize Ring 10 anelloVLPs purified through SEC (Figure 40). In addition, a series of point mutations at positions 357, 358, and 359 in in Ring 2 ORF1 to the corresponding residues of Ring 10 ORF1 (i.e., point mutations K357E, N358Q and E359I) allowed for recognition of the mutant Ring 2 ORF1s by AB3725 by western blot (Figure 41A-41B). Lastly, a full helix swap of amino acids 357-359 from Ring 10 to Ring 2 ORF1 was recognized by AB3725 via western blot.

Without wishing to be bound by theory, it is contemplated that the HVR helix residues of Ring 10 (i.e., residues 352-361) can be grafted as ELISA epitopes into the ORF1 proteins of other Anelloviruses for purification or analytical purposes.

Example 42: Production and purification of AnelloVLPs

Ring2 AnelloVLP:

A plasmid that encodes Ring2 ORF1 with a deletion of the C-terminus amino acids 611-666 (pRTx-2652; see also **Table B1-3a** construct listed as Ring2delCterm (Δ611-666) (7047)) was transfected into Expi293 cells. The cells were collected and resuspended in lysis buffer (50mM Tris HCl, pH 8, 250mM NaCl, 2mM Magnesium Chloride). EDTA (0.5M) and protease inhibitor were added, and the cells were lysed using a microfluidizer. After lysing, protein inhibitor and triton X were added to the lysate and incubated for 30 minutes. The lysate was centrifuged at 12100 rpm and the supernatant was collected.

The lysate supernatant in 50mM Tris HCl pH8, 250mM NaCl was passed through a HiTrap heparin HP column (Cytiva) to separate 60mer VLPs from other smaller proteins from the mixture. The VLP fractions were dialyzed into Capto buffer (50mM Tris HCl pH 8, 100mM NaCl) overnight and incubated for 1.5hrs with Capto400 resin at a 1:10 ratio of resin to protein. The preparation was spun down and supernatant collected.

Samples were loaded onto a gel and Coomassie stained **(FIG. 42A)** or a Western blot was performed using a Ring2 HVR Linker primary antibody raised against amino acids 284-319 of the HVR region of Ring2 ORF1 and a goat-anti-rabbit secondary antibody **(FIG. 42B)**. The lanes were as follows:

Lane 1: Ladder
Lane 2: Blank
Land 3: 2652 unpurified material
Lane 4: Blank
Lane 5: Ring2 AnelloVLP 2652 after Capto400 purification

Both gels show a relatively pure preparation of Ring2 VLPs after Capto400 purification (see lane 5).

Electron microscopy of the preparation obtained after Capto400 purification confirmed Ring2 VLP formation at 65000x magnification **(FIG. 43).**

Ring19 AnelloVLP:

A plasmid that encodes Ring19 ORF1 with a deletion of the C-terminus amino acids 600-655 (pRTx-2814; see Table B1-13b, construct Ring19delCterm (Δ600-655) (7120)) was similarly transfected as above into expi293 cells. The cells were collected and resuspended in lysis buffer (50mM Tris HCl, pH 8, 250mM NaCl, 2mM Magnesium Chloride). EDTA (0.5M) and protease inhibitor were added, and the cells were lysed using a microfluidizer. After lysing, protein inhibitor and triton X were added to the lysate and incubated for 30 minutes. The lysate was centrifuged at 12100 rpm and the supernatant was collected. The supernatant was then purified through the HiTrap Heparin HP column and dialyzed into the Capto buffer as described above. The VLP preparation was then purified through a Capto400 column to further separate the VLPs from smaller proteins. Fractions "C12", "D1", and "D2" are different fractions collected during purification that showed peaks on the chromatogram. Samples from fractions C12, D1, and D2 were loaded onto a gel and Coomassie stained **(FIG. 44A)** or a Western blot was performed using a Ring19 HVR3 primary antibody that was raised against amino acids 342-352 of the Ring19 ORF1 HVR region **(FIG. 44B)**. The lanes were as follows:

Lane 1: Ladder
Lane 2: Ring19 AnelloVLPs post heparin purification
Lane 3: Ring19 AnelloVLPs post dialysis
Lane 4: Ring19 AnelloVLPs capto400 C12 fraction
Lane 5: Ring19 AnelloVLPs capto400 D1 fraction
Lane 6: Ring19 AnelloVLPs capto400 D2 fraction

Both gels show Ring19 VLPs after capto400 purification.

Electron microscopy of the preparation obtained after Capto400 purification confirmed Ring19 VLP formation at 65000x magnification **(FIG. 45)**.

Example 43: Conjugation of AnelloVLPs

In this example, NHS ester moieties were conjugated via click chemistry to the surface lysines of the AnelloVLPs prepared according to the example above. The conjugation workflow is shown in **FIG. 46**.

The preparations of the AnelloVLPs prepared above were buffer exchanged into 50mM sodium borate pH8.5 overnight and NHS ester 647- Alexa Fluor™ ("NHS Ester 647") (Succinimidyl Ester), (ThermoFisher Catalog number: A20006) (for Ring2 AnelloVLPs) or EZ-Link™ NHS-Biotin ("NHS-Biotin") (ThermoFisher Catalog number: 20217) (for Ring19 AnelloVLPs) were added in varying VLP: NHS Ester molar ratios and reacted for 1hr at room temperature or overnight at 4°C. The reactions were stopped by addition of 10mM Tris HCl, pH7.4. The preparations were further desalted in PBS pH7.4 to remove extra free NHS ester moieties. Gels or Western blots were run to confirm conjugation.

FIG. 47A shows a Coomassie stained gel and **FIG. 47B** shows a gel scanned under 520nm of Ring2 AnelloVLP conjugated with NHS Ester 647. The lanes shown are as follows:

Lane 1: ladder
Lane 2: Ring2 AnelloVLP 2652 unlabeled
Lane 3: Ring2 AnelloVLP 2652 labeled at 1:5 VLP:NHS Ester 647 molar ratio, no desalt
Lane 4: Ring2 AnelloVLP 2652 labeled at 1:10 VLP:NHS Ester 647 molar ratio, no desalt
Lane 5: Ring2 AnelloVLP 2652 labeled at 1:20 VLP:NHS Ester 647 molar ratio, no desalt
Lane 6: Blank
Lane 7: Ring2 AnelloVLP 2652 labeled at 1:5 VLP:NHS Ester 647 molar ratio, desalted
Lane 8: Ring2 AnelloVLP 2652 labeled at 1:10 VLP:NHS Ester 647 molar ratio, desalted
Lane 9: Ring2 AnelloVLP 2652 labeled at 1:20 VLP:NHS Ester 647 molar ratio, desalted

The Coomassie-stained gel shows Ring2 AnelloVLPs at around 62kD as expected in all protein-containing lanes. The gel scanned under 520nm shows no visible band in lane 2 (unlabeled Ring2 AnelloVLP) while the other lanes containing labeled AnelloVPs show visible bands at about 62kD, confirming the conjugation of Ring2 AnelloVLPS with NHS Ester 647.

FIG. 48A shows a Western blot with a streptavidin CV 800 antibody [Licor Bioscience- Catalog number P/N: 926-32211] and **FIG. 48B** shows a Western blot with the R19 HVR3 primary antibody described above and a goat-anti rabbit secondary antibody. The lanes shown are as follows:

Lane 1: Ladder
Lane 2: Ring19 AnelloVLP unlabeled

Lane 3: Ring19 AnellovLP labeled at 1:5 VLP:NHS Ester biotin molar ratio [desalted]

Lane 4: Ring19 AnellovLP labeled at 1:10 VLP:NHS Ester biotin molar ratio [desalted]

The Western blot using the Ring19 specific antibody shows visible bands at around 62kD in both the unlabeled and labeled Ring19 AnelloVLP samples. The Western blot with streptavidin shows a band at around 62kD with Ring19 AnelloVLPs labeled with NHS Ester biotin while the unlabeled Ring19 AnelloVLP shows no visible band, confirming the conjugation of Ring19 AnelloVLPs with NHS Ester biotin.

Example 44: VLP conjugation to SARS-CoV-2 RBD peptide

In this Example, a His-Tag recombinant receptor binding domain (RBD) comprising amino acids 319-541 from SARS-CoV-2 coronavirus spike protein (ThermoFisher Catalog# RP-87704) (referred to in this example as RBD) was conjugated to the surface of AnelloVLPs. As shown in **FIG. 49**, the conjugation involved a first step of conjugating NHS-DBCO to the surface ORF1 of anelloVLPs. Azide-labeled RBD effectors were then conjugated to the anelloVLPs via the DBCO linker. This approach can be generalized to attach azide-labeled effectors (e.g., antigenic peptides and proteins) to anelloVLPs with DBCO linkers on their surfaces.

First, RBD was labeled with either DBCO or Azide to confirm that RBD could be labeled for future conjugation to AnelloVLPs. In brief, 100 µg of the His-tagged recombinant RBD (ThermoFisher Catalog # RP-87704) was resuspended in 1 ml of sodium borate buffer (pH=8.5) to final concentration of 0.1 mg/ml. The RBD amino acids from 319-541 contains 11 lysines that could potentially be labeled by NHS. Thus, in one condition, 2 µl of DBCO-PEG4-NHS 1 mg/ml was added to 200 µl of 0.1 mg/ml RBD and incubated overnight at 4°C (2 µg DBCO-PEG4-NHS : 20 µg RBD). In a second condition, 2 µl of Azide-PEG4-NHS at 1 mg/ml was added to 200 µl of 0.1 mg/ml RBD and then incubated overnight at 4°C (2 µg Azide-PEG4-NHS: 20 µg RBD). The next day, a zeba desalting column was prepared by opening the bottom of a column and placing it into a microfuge tube, spinning at 1500g for 1 min at 4°C, adding 400 µl of Borate buffer (50 mM sodium borate) and spinning at 1500g for one minute and then discarding the flow-through. This process is then repeated 3-4 times and the column is then placed into a fresh microfuge tube.

Unreacted DBCO/Azide was desalted out of the samples by running through a desalting column for two minutes at 1500g and at 4°C. Samples were then split as follows:

a. 100 µl RBD-PEG4-DBCO
b. 100 µl RBD-PEG4-DBCO + CaIFlour488
c. 100 µl RBD-PEG4-Azide

d. 100 µl RBD-PEG4-Azide + Alexa488 sDIBO

RBD-PEG4-DBCO + CaIFlour488 and RBD-PEG4-Azide + Alexa488 sDIBO were each made by adding 1 µl of CaIFluor488 Azide/Clicklt Alexa488 sDIBO to the RBDs. The mixture was incubated for 30 minutes, and this process was then repeated three times. RBD-PEG4-DBCO + CaIFlour488 and RBD-PEG4-Azide + Alexa488 sDIBO samples were then desalted as described. A western blot was then run to determine whether RBD labeling occurred. As shown in **FIG. 50**, labeling of RBD was confirmed with both DBCO and azide for future VLP conjugation. This demonstrated that a linker could be conjugated to the RBD.

To conjugate RBD to a Ring2 VLP, a fraction block of Ring2 ORF1 with a C-terminal deletion of amino acids 611-666 (pRTx-2652; corresponding to Ring2delCterm (Δ611-666) (7047) as listed in **Table X1**) in TBS was produced using SE-FPLC. Fractions were pooled and dialyzed into 2L of PBS + 0.01% poloxamer at 4°C. After 3 hours, buffer was refreshed. This was then repeated twice more and left overnight at 4°C. Approximately 30 ml of the post-SE-FPLC solution was concentrated to approximately 1.5 ml final volume. A Western blot was run to confirm that the sample was retained **(FIG. 51)**. The next day, approximately 1.5 ml of SE-FPLC pRTx-2652 was concentrated down to 220 µl.

1 µl NHS-PEG4-DBCO was added to 100 µl SE-FLPC pRTx-2652 samples generated as described above. The samples were then incubated for 1 hour at room temperature. 3 µl 1M Tris pH 8 was added, and the samples were desalted. RBD was labeled with NHS-PEG4-Azide by resuspending RBD in 200 µl borate buffer (0.5mg/mL final) and 1 µl NHS-PEG4-Azide for 1 hour at room temperature. 3 µl 1M Tris pH 8 was then added and the RBD sample was desalted.

7.5 µl of the RBD peptide was then added to 30 µl of DBCO-2652 to produce a final peptide concentration 0.1 mg/ml. The sample was then incubated at 37°C for 2.5 hours and conjugation was assessed by Coomassie staining and Western blot with an anti-Ring2 ORF1 antibody. As shown in **FIG. 52**, a 90kDa band corresponding to the conjugation of RBD (26kDa) to pRTx-2652 anelloVLPs (~62kDa) was observed.

Example 45: VLP production from a C-term deleted ORF1 and structural analysis

A plasmid that encodes Ring10 ORF1 with a deletion of the C-terminus amino acids 610-672 (see **Table B1-6** construct listed as Ring10-ORF1delCterm Helix (Δ610-672) (7052)) was transfected into Expi293 cells. Cells were harvested three days post-transfection and the cell pellet was stored at -80°C.

1L of cell pellet was thawed by incubating the pellets in the 35-37C water bath for 5 minutes. The pellet was resuspended in MF Buffer (50 mM Tris pH 8.0, 100 mM NaCl, 2 mM $MgCl_2$) with 600ul protease

inhibitor and EDTA and the resuspended lysate was centrifuged at 12000rpm for 20mins. The supernatant was discarded and resuspended in 60mL of MF Buffer (50 mM Tris pH 8.0, 100 mM NaCl, 2 mM $MgCl_2$) and 600µL of Protease inhibitor without EDTA. Cells were gently resuspended using a pipette to remove clumps and make a homogeneous cell lysate.

The homogeneous cell lysate was loaded onto a microfluidizer and the cells were lysed at 100 psi. The lysis cycle was repeated 3 times to obtain the maximum lysis of cells. An additional 20 mL of MF buffer was added to wash the lysate from the MF tubing and the lysate was incubated with 80µL Benzonase and 1% (final v/v) Triton X-100 at 37C for 30min. After 30 min of incubation, thelLysate was centrifuged at 12000rpm for 30 min and the supernatant was collected.

The supernatant was filtered with a 0.45um syringe filtration filter. Filtered lysate was spiked with an appropriate amount of 5M NaCl to make the final concentration of salt to 250mM. In this experiment, 5L of starting cell pellets were used. Therefore, the final clarified lysate volume was around 400 mL. Lysate was further diluted to 1L with Heparin buffer A (50 mM Tris pH 8.0, 250 mM NaCl) to load onto a 100 mL Heparin Sepharose column.

Heparin Sepharose column purification:

100 mL of packed Heparin Sepharose column was equilibrated with buffer A (50 mM Tris pH 8.0, 250 mM NaCl). Lysate was loaded onto the pre-equilibrated column at 1 mL/min flow rate using a sample pump on the Cytiva AKTA pure FPLC system. The column was then washed with 20 CV of buffer A at 15 mL/min flow rate.

After the wash step, 250 mL flasks were placed in the fraction collector and a step gradient elution protocol was started with increasing concentrations of buffer B (50 mM Tris pH 8.0, 2 M NaCl).

Typically, the VLPs eluted at 60% of buffer B concentration. Once the run was completed, all fractions were analyzed by SDS-PAGE and Western blotting. Fractions corresponding to VLPs were pooled together and dialyzed into Capto buffer (50 mM Tris pH 8.0, 100 mM NaCl) to purify further using Capto core 400 resin in batch mode.

Capto core 400 Batch purification:

5 mL bed volume of Capto core 400 was pipetted into a 50 mL conical tube and washed with 20 mL capto buffer by centrifugation at 400x g for 5 min. This step was repeated 3 times. 35 ml of dialyzed VLPs was added on to the beads and incubated at 4°C on gentle shaking for 60 min. After 60 min of incubation, beads were centrifuged at 500 X g for 5 min and supernatant was collected. The supernatant was further centrifuged at 4000 x g to remove any debris of beads. The supernatant from this step was transferred into a fresh flask.

Protein concentration:

Capto purified protein was concentrated by using micro pulse to the required final volume to a concentration of 1 mg/mL. Concentrated protein quality was evaluated by SDS-PAGE, Western blot and Negative stain EM. All these quality control methods showed the VLPs were homogeneous and of high purity.

Results:

The structure of the Ring10 virus like particle derived from both Sf9 ("Ring10delARM" as described in Example 37) and Expi293 cells ("Ring10-ORF1delCterm Helix" as described above in this Example) were produced . Although there were differences in the ORF1 proteins (Ring10delARM had the N-terminal ARM domain removed and had the C-terminal region proteolyzed while the ARM domain was present on Ring10-ORF1delCterm Helix and the C-terminus was genetically removed) both formed the same 60-mer icosahedral fold with the same intra-strand P1/P2 Spike Domain extending from the particle core (see **FIGs 53A-53B**). Structural alignment of an ORF1 protomer from each structure shows the ORF1 fold is very similar with an RMSD of 1.6 angstroms (**FIG. 53C**).

CLAIMS

What is claimed is:

1. A polypeptide comprising (e.g., in an N to C-terminal direction):

(i) a structural jelly-roll region of an Anellovirus ORF1 molecule;

(ii) a structural N22 domain of an Anellovirus ORF1 molecule; and

(iii) a portion of a structural C-terminal domain (CTD) of an Anellovirus ORF1 molecule, which comprises a deletion of about 20-30, 30-40 (e.g., about 37), 40-50 (e.g., about 55), 50-60 , 60-70, 70-80, 80-90, 90-100, 100-110, 110-120, 120-130 (e.g., about 129), 130-140 (e.g., about 131), 140-150 (e.g., about 148), or 150-160 (e.g., about 155) amino acids at the C-terminal end of the structural CTD, relative to a corresponding wild-type structural CTD of the Anellovirus ORF1 molecule.

2. A polypeptide comprising (e.g., in an N to C-terminal direction):

(i) optionally a first portion of a structural jelly-roll region (e.g., comprising beta strands B-H of the structural jelly-roll region) of an Anellovirus ORF1 molecule;

(ii) a first portion of a P1 domain of an Anellovirus ORF1 molecule (e.g., a P1-1 domain as described herein);

(iii) a P2 domain of an Anellovirus ORF1 molecule;

(iv) a second portion of a P1 domain of an Anellovirus ORF1 molecule (e.g., a P1-2 domain as described herein);

(v) optionally a second portion of a structural jelly-roll region (e.g., comprising beta strand I of the structural jelly-roll region) of an Anellovirus ORF1 molecule; and

(vi) a portion of a structural C-terminal domain (CTD) of an Anellovirus ORF1 molecule, which comprises a deletion of about 20-30, 30-40 (e.g., about 37), 40-50 (e.g., about 55), 50-60 , 60-70, 70-80, 80-90, 90-100, 100-110, 110-120, 120-130 (e.g., about 129), 130-140 (e.g., about 131), 140-150 (e.g., about 148), or 150-160 (e.g., about 155) amino acids at the C-terminal end of the structural CTD, relative to a corresponding wild-type structural CTD of the Anellovirus ORF1 molecule.

3. A polypeptide comprising (e.g., in an N-terminal to C-terminal direction):

(i) a structural jelly-roll region of an Anellovirus ORF1 molecule, or an amino acid sequence having at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity thereto; and

(ii) an structural N22 domain of an Anellovirus ORF1 molecule, or an amino acid sequence having at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity thereto; and

wherein the polypeptide lacks a structural arginine-rich region of an Anellovirus ORF1 molecule.

4. A polypeptide comprising (e.g., in an N-terminal to C-terminal direction):

(i) optionally a first portion of a structural jelly-roll region (e.g., comprising beta strands B-H of the structural jelly-roll region) of an Anellovirus ORF1 molecule;

(ii) a first portion of a P1 domain of an Anellovirus ORF1 molecule (e.g., a P1-1 domain as described herein);

(iii) a P2 domain of an Anellovirus ORF1 molecule;

(iv) a second portion of a P1 domain of an Anellovirus ORF1 molecule (e.g., a P1-2 domain as described herein);

(v) optionally a second portion of a structural jelly-roll region (e.g., comprising beta strand I of the structural jelly-roll region) of an Anellovirus ORF1 molecule; and

wherein the polypeptide lacks a structural arginine-rich region of an Anellovirus ORF1 molecule.

5. A polypeptide comprising (e.g., in an N-terminal to C-terminal direction):

(i) a structural arginine-rich region of a first Anellovirus ORF1 molecule, or an amino acid sequence having at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity thereto;

(ii) a structural jelly-roll region of a second Anellovirus ORF1 molecule, or an amino acid sequence having at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity thereto; and

(iii) an structural N22 domain of the second Anellovirus ORF1 molecule, or an amino acid sequence having at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity thereto;

wherein the sequence of (i) comprises at least one amino acid sequence difference relative to the structural arginine-rich region of the second Anellovirus ORF1 molecule.

6. A polypeptide comprising (e.g., in an N-terminal to C-terminal direction):

(i) a structural arginine-rich region of a first Anellovirus ORF1 molecule, or an amino acid sequence having at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity thereto;

(ii) optionally a first portion of a structural jelly-roll region (e.g., comprising beta strands B-H of the structural jelly-roll region) of a second Anellovirus ORF1 molecule;

(iii) a first portion of a P1 domain of the second Anellovirus ORF1 molecule (e.g., a P1-1 domain as described herein);

(iv) a P2 domain of the secondAnellovirus ORF1 molecule;

(v) a second portion of a P1 domain of the second Anellovirus ORF1 molecule (e.g., a P1-2 domain as described herein);

(vi) optionally a second portion of a structural jelly-roll region (e.g., comprising beta strand I of the structural jelly-roll region) of the second Anellovirus ORF1 molecule; and

wherein the sequence of (i) comprises at least one amino acid sequence difference relative to the structural arginine-rich region of the second Anellovirus ORF1 molecule.

7. A polypeptide comprising (e.g., in an N-terminal to C-terminal direction):

(i) an N-terminal portion of a first Anellovirus ORF1 molecule, or an amino acid sequence having at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity thereto, wherein the N-terminal portion of the first Anellovirus ORF1 molecule has a length of between 30-40, 40-50, 50-60, 60-70, 70-80, or 80-90 amino acids; and

(ii) a C-terminal portion of a second Anellovirus ORF1 molecule, or an amino acid sequence having at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity thereto, wherein the C-terminal portion of the second Anellovirus ORF1 molecule has a length of between 590-600, 600-610, 610-620, 620-630, 630-640, 640-650, 650-660, 660-670, 670-680, 680-690, 690-700, 700-710, 710-720, or 720-730 amino acids; and

wherein the sequence of (i) comprises at least one amino acid sequence difference relative to the structural arginine-rich region of the second Anellovirus ORF1 molecule.

8. A polypeptide comprising (e.g., in an N-terminal to C-terminal direction):

(i) a structural jelly-roll region of an Anellovirus ORF1 molecule; and

(ii) an structural N22 domain of an Anellovirus ORF1 molecule; and

wherein the polypeptide lacks a structural arginine-rich region of an Anellovirus ORF1 molecule; and

wherein the polypeptide lacks a structural C-terminal domain of an Anellovirus ORF1 molecule.

9. A polypeptide comprising (e.g., in an N to C-terminal direction):

(i) optionally a structural arginine-rich region of an Anellovirus ORF1 (e.g., a full-length arginine-rich region or a portion of a structural arginine-rich region),

(ii) a structural jelly-roll region of an Anellovirus ORF1 molecule,

(iii) a P1 domain of an Anellovirus ORF1 molecule,

(iv) a P2 domain of an Anellovirus ORF1 molecule, and

(v) optionally a structural C-terminal domain (CTD) of an Anellovirus ORF1 molecule (e.g., a full-length structural CTD or a portion of a structural CTD);

wherein:

(a) the P1 domain is from a different Anellovirus than one or more (e.g., 1, 2, 3, or 4) of the structural arginine-rich region, structural jelly-roll region, P2 domain, and/or the structural CTD;

(b) the P2 domain is from a different Anellovirus than one or more (e.g., 1, 2, 3, or 4) of the structural arginine-rich region, structural jelly-roll region, P1 domain, and/or the structural CTD; or

(c) the P1 domain and the P2 domain are from a different Anellovirus than one or more (e.g., 1, 2, or 3) of the structural arginine-rich region, structural jelly-roll region, and/or the structural CTD, optionally wherein the P1 domain and the P2 domain are from the same Anellovirus.

10. An anellovector comprising:

(i) a proteinaceous exterior comprising an Anellovirus ORF1 protein as listed in Table A26, or a polypeptide comprising an amino acid sequence having at least about 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity thereto, and

(ii) a genetic element enclosed by the proteinaceous exterior, wherein the genetic element comprises a promoter element operably linked to a nucleic acid sequence (e.g., a DNA sequence) encoding an effector (e.g., an exogenous effector or an endogenous effector);

wherein the proteinaceous exterior and/or the genetic element comprises at least one difference (e.g., a mutation, chemical modification, or epigenetic alteration) relative to a wild-type Anellovirus ORF1 protein and/or wild-type Anellovirus genome, respectively (e.g., as described herein), e.g., an insertion, substitution, chemical or enzymatic modification, and/or deletion, e.g., a deletion of a domain (e.g., one or more of a structural arginine-rich region, structural jelly-roll domain, structural HVR, structural N22, structural CTD, P1 domain, or P2 domain, e.g., as described herein) or genomic region (e.g., one or more of a TATA box, cap site, transcriptional start site, 5' UTR, open reading frame (ORF), poly(A) signal, or GC-rich region, e.g., as described herein).

11. An anellovector comprising:

(i) a proteinaceous exterior comprising a polypeptide encoded by an Anellovirus ORF1 nucleic acid sequence as listed in Table N24, or a polypeptide encoded by a nucleic acid sequence having at least about 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity to the Anellovirus ORF1 nucleic acid sequence, and

(ii) a genetic element enclosed by the proteinaceous exterior, wherein the genetic element comprises a promoter element operably linked to a nucleic acid sequence (e.g., a DNA sequence) encoding an effector (e.g., an exogenous effector or an endogenous effector);

wherein the proteinaceous exterior and/or the genetic element comprises at least one difference (e.g., a mutation, chemical modification, or epigenetic alteration) relative to a wild-type Anellovirus ORF1 protein and/or wild-type Anellovirus genome, respectively (e.g., as described herein), e.g., an insertion, substitution, chemical or enzymatic modification, and/or deletion, e.g., a deletion of a domain (e.g., one or more of a structural arginine-rich region, structural jelly-roll domain, structural HVR, structural N22, structural CTD, P1 domain, or P2 domain, e.g., as described herein) or genomic region (e.g., one or more of a TATA box, cap site, transcriptional start site, 5' UTR, open reading frame (ORF), poly(A) signal, or GC-rich region, e.g., as described herein).

12. An ORF1 molecule comprising an exogenous surface moiety, wherein the exogenous surface moiety is attached to (e.g., conjugated to) the amino acid residue (e.g., a cysteine residue) corresponding to position 254, 263, 264, 265, 272, 273, 274, 276, 283, 284, 285, 287, 288, 290, 291, 308, 311, 312, 313, 314, 316, 317, 318, 319, 321, 324, 328, 329, 341, 343, 354, 358, 361, 362, 363, 364, 365, 368, 369, 371, 374, 376, 378, 380, or 381 of Ring 10 ORF1, e.g., in an ORF1 domain (e.g., within the HVR or P2 domain).

13. A protein complex comprising five ORF1 molecules, wherein each of the ORF1 molecules comprises:

(i) an ORF1 domain, and

(ii) an exogenous surface moiety;

wherein the exogenous surface moieties of the five ORF1 molecules forms a pentamer.

14. A protein complex comprising three ORF1 molecules, wherein each of the ORF1 molecules comprises:

(i) an ORF1 domain, and

(ii) an exogenous surface moiety;

wherein the exogenous surface moieties of the three ORF1 molecules forms a trimer.

15. A protein complex comprising two ORF1 molecules, wherein each of the ORF1 molecules comprises:

(i) an ORF1 domain, and

(ii) an exogenous surface moiety;

wherein the exogenous surface moieties of the two ORF1 molecules forms a dimer.

16. A particle comprising:

(a) a proteinaceous exterior comprising an ORF1 molecule; and

(b) a genetic element comprising a heterologous nucleic acid sequence encoding an exogenous effector;

wherein the genetic element is enclosed within the proteinaceous exterior; and

wherein the particle has one or more of the following characteristics:

(i) the genetic element (e.g., a DNA genetic element) does not comprise an Anellovirus 5' UTR or an origin of replication;

(ii) the sequence encoding the exogenous effector takes up at least 90%, 95%, 96%, 97%, 98%, 99% or 100% of the genetic element (e.g., a DNA genetic element);

(iii) the heterologous nucleic acid sequence takes up at least 90%, 95%, 96%, 97%, 98%, 99% or 100% of the genetic element (e.g., a DNA genetic element);

(iv) the particle does not comprise a detectable amount of (e.g., any) polypeptides from a host cell, or comprises less than 5, 10, 15, 20, 25, 30, 40, or 50 copies of a polypeptide from a host cell;

(v) the particle does not comprise a detectable amount of (e.g., any) nucleic acid molecules from a host cell, or comprises less than 2, 3, 4, or 5 copies of a nucleic acid molecule from a host cell;

(vi) the particle comprises a denaturant in a concentration of less than about 0.01M, 0.1M, 0.2M, 0.3M, 0.4M, 0.5M, 0.6M, 0.7M, 0.8M, 0.9M, 1M, 1.1M, 1.2M, 1.3M, 1.5M, 1.5M, 1.6M, 1.7M, 1.8M, 1.9M, or 2M;

(vii) does not substantially replicate when introduced into a cell (e.g., a human cell); and/or

(viii) has a symmetrical morphology.

17. A particle comprising:

a proteinaceous exterior comprising about 40-80 (e.g., about 60) copies of an ORF1 molecule; and

wherein the particle:

(i) does not comprise (e.g., does not enclose) a polynucleotide,

(ii) does not comprise (e.g., does not enclose) detectable levels of polynucleotides,

(iii) does not comprise (e.g., does not enclose) a polynucleotide of greater than 1000, 500, 200, or 100 nucleotides in length,

(iv) does not comprise (e.g., does not enclose) a polynucleotide comprising any contiguous nucleic acid sequences of at least 50, 100, 150, 200, 300, 400, 500, 600, 700, 800, 900, or 1000 nucleotides in length having least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to contiguous sequence in a wild-type *Anellovirus* genome (e.g., as described herein), and/or

(v) does not comprise a polynucleotide comprising an Anellovirus 5' UTR or an origin of replication.

18. A composition comprising a plurality of particles, the particles comprising a proteinaceous exterior comprising about 40-80 (e.g., about 60) copies of an ORF1 molecule;

wherein at least 50%, 60%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, 99.5%, or 99.9% of the particles do not comprise (e.g., do not enclose):

(i) a polynucleotide,

(ii) a nucleic acid molecule of greater than 1000, 500, 200, or 100 nucleotides in length,

(iii) a plurality of polynucleotides,

(iv) a circular nucleic acid molecule,

(v) a single-stranded nucleic acid molecule, and/or

(vi) a genetic element (e.g., a genetic element of an anellovector), e.g., as described herein; or

wherein the composition comprises less than 10^{10} - 10^{14} (e.g., less than 10^{10} - 10^{11}, 10^{11} - 10^{12}, 10^{12} - 10^{13}, or 10^{13} - 10^{14}) viral genome equivalents of nucleic acid molecules (e.g., genetic elements, e.g., of an anellovector as described herein) per kilogram of a subject to be administered the composition (e.g., as determined by qPCR or by measuring optical density).

19. A method of disassembling a particle, the method comprising:

(a) providing a mixture comprising a particle and a denaturant, wherein the particle comprises:

(i) a proteinaceous exterior comprising a plurality of Anellovirus ORF1 molecules, and

(ii) a nucleic acid molecule (e.g., a nucleic acid endogenous to a host cell or a nucleic acid exogenous to a host cell, e.g., an anellovirus genome); and

(b) incubating the mixture under conditions suitable for:

disassembly of the proteinaceous exterior, and

dissociation of the nucleic acid molecule from the proteinaceous exterior.

20. A method of making an anellovector, the method comprising:

(a) providing a mixture comprising a plurality of Anellovirus ORF1 molecules, wherein at least 75%, 80%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% of the plurality of ORF1 molecules are not comprised in a particle comprising about 40-80 (e.g., about 60) copies of an ORF1 molecule;

(b) subjecting the mixture to conditions suitable for *in vitro* assembly of the Anellovirus ORF1 molecules; and

(c) incubating the Anellovirus ORF1 molecules with a plurality of genetic elements, under conditions suitable for assembly of the Anellovirus ORF1 molecules into one or more anellovectors each enclosing one or more of the genetic elements.

21. A method of making an anellovector, the method comprising:

(a) providing a mixture comprising a plurality of Anellovirus ORF1 molecules and subjecting the mixture to denaturing conditions (e.g., providing a denaturant as part of the mixture, e.g., contacting the mixture with a denaturant), wherein at least 75%, 80%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% of the plurality of ORF1 molecules are not comprised in a particle comprising about 40-80 (e.g., about 60) copies of an ORF1 molecule;

(b) subjecting the mixture to non-denaturing conditions (e.g., reducing the concentration of the denaturant to a level) suitable for *in vitro* assembly of the Anellovirus ORF1 molecules (e.g., by dialysis); and

(c) incubating the Anellovirus ORF1 molecules with a plurality of genetic elements, under conditions suitable for assembly of the Anellovirus ORF1 molecules into one or more anellovectors each enclosing one or more of the genetic elements.

22. A method of making an anelloVLP, the method comprising:

(a) providing a mixture comprising a plurality of Anellovirus ORF1 molecules, wherein at least 75%, 80%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% of the plurality of ORF1 molecules are not comprised in a particle comprising about 40-80 (e.g., about 60) copies of an ORF1 molecule;

(b) subjecting the mixture to conditions suitable for *in vitro* assembly of the Anellovirus ORF1 molecules; and

(c) incubating the Anellovirus ORF1 molecules with a plurality of effectors (e.g., exogenous effectors), under conditions suitable for assembly of the Anellovirus ORF1 molecules into one or more anelloVLPs each enclosing one or more of the effectors.

23. A method of making an anelloVLP, the method comprising:

(a) providing a mixture comprising a plurality of Anellovirus ORF1 molecules and a denaturant, wherein at least 75%, 80%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% of the plurality of ORF1 molecules are not comprised in a particle comprising about 40-80 (e.g., about 60) copies of an ORF1 molecule;

(b) reducing the concentration of the denaturant to a level suitable for *in vitro* assembly of the Anellovirus ORF1 molecules; and

(c) incubating the Anellovirus ORF1 molecules with a plurality of effectors (e.g., exogenous effectors), under conditions suitable for *in vitro* assembly of the Anellovirus ORF1 molecules into one or more anelloVLPs each enclosing one or more of the effectors.

24. A method of making an anelloVLP, the method comprising:

(a) providing a mixture comprising a particle and a denaturant, wherein the particle comprises:

(i) a proteinaceous exterior comprising a plurality of Anellovirus ORF1 molecules, and

(ii) a nucleic acid molecule (e.g., a host cell nucleic acid molecule); and

(b) incubating the mixture under conditions suitable for:

disassembly of the proteinaceous exterior, and

dissociation of the nucleic acid molecule from the proteinaceous exterior;

(c) providing a mixture comprising a plurality of Anellovirus ORF1 molecules and a denaturant, wherein at least 75%, 80%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% of the plurality of ORF1 molecules are not comprised in a particle comprising about 40-80 (e.g., about 60) copies of an ORF1 molecule.