

ENHANCED VIRUS-LIKE PARTICLES AND METHODS OF USE THEREOF FOR DELIVERY TO CELLS

CLAIM OF PRIORITY

This application claims the benefit of U.S. Provisional Patent Application Serial No. 63/056,125, filed on July 24, 2020. The entire contents of the foregoing are hereby incorporated by reference.

5 **FEDERALLY SPONSORED RESEARCH OR DEVELOPMENT**

This invention was made with Government support under Grant No. GM118158 awarded by the National Institutes of Health. The Government has certain rights in the invention.

TECHNICAL FIELD

10 Described herein are enhanced virus-like particles (eVLPs), comprising a membrane comprising a phospholipid bilayer with one or more virally-derived glycoproteins on the external side; and a cargo disposed in the core of the eVLP on the inside of the membrane, wherein the eVLP does not comprise a protein from any human endogenous or exogenous viral gag or pol, and methods of use thereof for delivery of the
15 cargo to cells.

BACKGROUND

Delivery of cargo such as proteins, nucleic acids, and/or chemicals into the cytosol of living cells has been a significant hurdle in the development of biological therapeutics.

20 **SUMMARY**

Described herein are enhanced virus-like particles (eVLPs) that are capable of packaging and delivering a wide variety of payloads, e.g., biomolecules including nucleic acids (DNA, RNA) or proteins, chemical compounds including small molecules, and/or other molecules, and any combination thereof, into eukaryotic cells. The non-viral eVLP

systems described herein have the potential to be simpler, more efficient and safer than conventional, artificially-derived lipid/gold nanoparticles and viral particle-based delivery systems, at least because eVLPs have no virus-derived components except for ENV, eVLPs can utilize but do not require chemical-based dimerizers, and eVLPs have the ability to package and deliver specialty single and/or double-stranded DNA molecules (e.g., plasmid, mini circle, closed-ended linear DNA, AAV DNA, episomes, bacteriophage DNA, homology directed repair templates, etc.), single and/or double-stranded RNA molecules (e.g., single guide RNA, prime editing guide RNA, messenger RNA, transfer RNA, long non-coding RNA, circular RNA, RNA replicon, circular or linear splicing RNA, micro RNA, small interfering RNA, short hairpin RNA, piwi-interacting RNA, toehold switch RNA, RNAs that can be bound by RNA binding proteins, bacteriophage RNA, internal ribosomal entry site containing RNA, etc.), proteins, chemical compounds and/or molecules, and combinations of the above listed cargos (e.g. AAV particles) . The eVLPs described herein are different from conventional retroviral particles, virus-like particles (VLPs), exosomes and other previously described extracellular vesicles that can be loaded with cargo because of the membrane configuration, vast diversity of possible cargos that are enabled by novel, innovative loading strategies, the lack of a limiting DNA/RNA length constraint, the lack of proteins derived from any viral gag or pol, and the mechanism of cellular entry.

Described herein are compositions and methods for cargo delivery that can be used with a diverse array of protein and nucleic acid molecules, including genome editing, epigenome modulation, transcriptome editing and proteome modulation reagents, that are applicable to many disease therapies.

Thus, provided herein are eVLPs that include a membrane comprising a phospholipid bilayer with one or more virally-derived glycoproteins (e.g., as shown in Table 1) on the external side; and optionally a cargo disposed in the core of the eVLP on the inside of the membrane, wherein the eVLP does not comprise any gag and/or pol protein.

Also provided herein are methods for delivering a cargo to a target cell, e.g., a cell *in vivo* or *in vitro*. The methods include contacting the cell with an eVLP as described herein comprising the biomolecule and/or chemical as cargo.

Additionally provided herein are methods for producing an eVLP, e.g., comprising a biomolecular cargo. The methods include providing a cell expressing one or more virally-derived glycoproteins (ENV) (e.g., as shown in Table 1), and a cargo biomolecule and/or chemical, wherein the cell does not express an exogenous gag and/or pol protein; and maintaining the cell under conditions such that the cells produce eVLPs.

In some embodiments, the methods include harvesting and optionally purifying and/or concentrating the produced eVLPs.

In some embodiments, the methods include using cells that have or have not been manipulated to express any exogenous proteins except for an ENV (e.g., as shown in Table 1), and, if desired, a plasma membrane recruitment domain (e.g., as shown in Table 6). In this embodiment, the “empty” particles that are produced can be loaded with cargo by utilizing nucleofection, lipid, polymer, or CaCl₂ transfection, sonication, freeze thaw, and/or heat shock of purified particles mixed with cargo. In all embodiments, producer cells do not express any viral gag protein. This type of loading allows for cargo to be unmodified by fusions to plasma membrane recruitment domains and represents a significant advancement from previous VLP technology.

Also provided herein are cells expressing one or more virally-derived glycoproteins (e.g., as shown in Table 1), and a cargo, wherein the cell does not express an exogenous gag protein. In some embodiments, the cells are primary or stable human cell lines, e.g., Human Embryonic Kidney (HEK) 293 cells or HEK293 T cells.

In some embodiments, the outer surface of the particle could contain scFvs, nanobodies, darpins, and/or other targeting peptides to enable cell-specific entry.

In some embodiments, the biomolecule cargo is a therapeutic or diagnostic protein or nucleic acid encoding a therapeutic or diagnostic protein.

In some embodiments, the cargo is a chemical compound or molecule.

In some embodiments, the chemical molecule is a trigger for protein-protein dimerization or multimerization, such as the A/C heterodimerizer or rapamycin.

In some embodiments, the chemical compound is a DNA PK inhibitor, such as M3814, NU7026, or NU7441 which potentially enhance homology directed repair gene editing.

In some embodiments, the cargo is a gene editing reagent.

In some embodiments, the gene editing reagent comprises a zinc finger (ZF), transcription activator-like effector (TALE), and/or CRISPR-based genome editing or modulating protein; a nucleic acid encoding a zinc finger (ZF), transcription activator-like effector (TALE), and/or CRISPR-based genome editing or modulating protein; or a
5 ribonucleoprotein complex (RNP) comprising a CRISPR-based genome editing or modulating protein.

In some embodiments, the gene editing reagent is selected from the proteins listed in Tables 2, 3, 4 & 5.

In some embodiments, the gene editing reagent comprises a CRISPR-based
10 genome editing or modulating protein, and the eVLP further comprises one or more guide RNAs that bind to and direct the CRISPR-based genome editing or modulating protein to a target sequence.

In some embodiments, the cargo comprises a covalent or non-covalent connection to a plasma membrane recruitment domain, preferably as shown in Table 6. Covalent
15 connections, for example, can include direct protein-protein fusions generated from a single reading frame, inteins that can form peptide bonds, other proteins that can form covalent connections at R-groups and/or RNA splicing. Non-covalent connections, for example, can include DNA/DNA, DNA/RNA, and/or RNA/RNA hybrids (nucleic acids base pairing to other nucleic acids via hydrogen-bonding interactions), protein domains
20 that dimerize or multimerize with or without the need for a chemical compound/molecule to induce the protein-protein binding, single chain variable fragments, nanobodies, affibodies, proteins that bind to DNA and/or RNA, proteins with quaternary structural interactions, optogenetic protein domains that can dimerize or multimerize in the presence of certain light wavelengths, and/or naturally reconstituting split proteins.

25 In some embodiments, the cargo comprises a fusion to a dimerization domain or protein-protein binding domain that may or may not require a molecule to trigger dimerization or protein-protein binding.

In some embodiments, the producer cells are FDA-approved cells lines, allogenic cells, and/or autologous cells derived from a donor.

30 In some embodiments, the full or active peptide domains of human CD47 may be incorporated in the eVLP surface to reduce immunogenicity.

Examples of AAV proteins included here are AAV REP 52, REP 78, and VP1-3. The capsid site where proteins can be inserted is T138 starting from the VP1 amino acid counting. Dimerization domains could be inserted at this point in the capsid, for instance.

5 Examples of dimerization domains included here that may or may not need a small molecule inducer are dDZF1, dDZF2, DmrA, DmrB, DmrC, FKBP, FRB, GCN4 scFv, 10x/24x GCN4, GFP nanobody and GFP.

 Examples of split inteins included here are Npu DnaE, Cfa, Vma, and Ssp DnaE.

 Examples of other split proteins included here that make a covalent bond together are Spy Tag and Spy Catcher.

10 Examples of RNA binding proteins included here are MS2, Com, and PP7.

 Examples of synthetic DNA-binding zinc fingers included here are ZF6/10, ZF8/7, ZF9, MK10, Zinc Finger 268, and Zinc Finger 268/NRE.

 Examples of proteins that multimerize as a result of quaternary structure included here are E. coli ferritin, and the other chimeric forms of ferritin.

15 Examples of optogenetic “light-inducible proteins” included here are Cry2, CIBN, and Lov2-Ja.

 Examples of peptides the enhance transduction included here are L17E, Vectofusin, KALA, and the various forms of nisin.

20 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. Methods and materials are described herein for use in the present invention; other, suitable methods and materials known in the art can also be used. The materials, methods, and examples are illustrative only and not intended to be limiting. All publications, patent applications, patents, sequences, database entries, and other
25 references mentioned herein are incorporated by reference in their entirety. In case of conflict, the present specification, including definitions, will control.

 Other features and advantages of the invention will be apparent from the following detailed description and figures, and from the claims.

DESCRIPTION OF DRAWINGS

FIG 1: Depiction of exemplary T2eVLP/T4eVLP production and transduction for RNP/protein delivery. All eVLP expression constructs are stably integrated in the genome of the producer cell. Construct 1-1 corresponds to the phospholipid bilayer recruitment domain. 1-2 corresponds to the cargo. 2 corresponds to an optional guide RNA. 1-1 and 1-2 is translated in the cytosol where it complexes with guide RNA before it is recruited to the phospholipid bilayer. 3 corresponds to a virally-derived glycoprotein (VSVG). The virally-derived glycoprotein is expressed as a transmembrane protein on the plasma membrane and helps to drive budding of cargo-containing eVLPs from the plasma membrane to extracellular space. These particles are purified and are able to fuse with target cells and deliver cargo by interacting with surface receptors at the target cell surface.

FIG 2: Depiction of purified eVLPs entering a target cell and delivering cargo to the cytosol. Importantly, the phospholipid bilayer recruitment domain allows cargo to enter the target cell nucleus as long as cargo possesses a nuclear localization sequence.

FIG 3: Cas9 RNP was delivered in VSVG-pseudotyped T2eVLPs with or without a PH domain from hPLC δ 1 or hAKT1. The PH domains were fused to the N-terminus of Cas9 via a 10 amino acid glycine/serine polypeptide linker. HepG2, U2OS, HEK293T, CFPAC1, BeWo, Jurkat, K562, and primary T cells were treated with purified and 100x concentrated T2eVLPs for 72 hours. Percent targeted gene modification of VEGF site #3 was determined by amplicon sequencing. The x-axis labels correspond to the contents of each T2eVLP preparation. Cas9 (no fusion) lacked a PH domain fusion. Benzonase (Benz) was used to degrade RNA and DNA outside of VLPs, and a Benzonase treated sample was included as a control.

FIG 4: Depiction of T1eVLP/T3eVLP production. Plasmid DNA constructs involved in the transfection encode cargo, an optional guide RNA and a virally-derived glycoprotein (VSVG). Plasmids, or other types of DNA molecules, will be distributed throughout the production cell, so constructs located in the nucleus will express eVLP components and cargo, and constructs located near the plasma membrane or endosomes will be encapsulated within budding eVLPs.

FIGs. 5A-B: Exemplary T1eVLP-delivered spCas9 genome editing in vitro. A) U2OS eGFP and HEK293 eGFP cell lines transduced with VLPs containing Rous sarcoma virus gag fused to spCas9 and sgRNA, T1eVLPs containing PLC pleckstrin homology (PH) fused to spCas9 and sgRNA, or VLPs containing Rous sarcoma virus gag fused to the SV40 nuclear localization sequence (NLS) and sgRNA. The sgRNA targets GFP. Flow cytometry or T7E1 is performed 72 hours after transduction. The Rous sarcoma virus gag VLPs serve as controls. B) T7E1 analysis of a subpopulation of U2OS or 293 cells from the experiment in FIG 5A. eVLPs and VLPs are pseudotyped with VSVG

FIG 6: Exemplary T1eVLP-delivered spCas9 genome editing in vitro. U2OS cells transduced with T1eVLPs containing PLC PH fused to spCas9 targeted to HEK site #3 or VEGF site #2. eVLPs are pseudotyped with VSVG. Gene modification is measured by amplicon sequencing.

FIG 7: Exemplary T1eVLP-delivered spCas9 genome editing in vitro. U2OS cells transduced with T1eVLPs containing PLC PH or hAkt PH fused to spCas9 targeted to VEGF site #3. eVLPs are pseudotyped with VSVG. Gene modification is measured by amplicon sequencing.

FIG 8: Exemplary T1eVLP-delivered spCas9 base editing in vitro. HEK293T cells transduced with VLPs containing Rous sarcoma virus gag fused to spCas9 BE3 or Gam-BE4 with sgRNA targeted to VEGF site #2, or T1eVLPs containing PLC PH fused to spCas9 BE3 or Gam-BE4 with sgRNA targeted to VEGF site #2. eVLPs and VLPs are pseudotyped with VSVG. Gene modification is measured by amplicon sequencing. The Rous sarcoma virus gag VLP serves as a positive control.

FIG 9: Exemplary T1eVLP-delivered spCas9 base editing in vitro. HEK293T cells transduced with T1eVLPs containing PLC PH fused to codon optimized spCas9 BE4 targeted to HEK site #3. eVLPs are pseudotyped with VSVG. Gene modification is measured by amplicon sequencing.

FIG 10: Exemplary T1eVLP-delivered spCas9 base editing in vitro. HEK293T cells transduced with T1eVLPs containing PLC PH fused to codon optimized spCas9 ABE targeted to VEGF site #3. eVLPs are pseudotyped with VSVG. Gene modification is measured by amplicon sequencing.

FIG 11: Exemplary T1eVLP-delivered spCas9 base editing in vitro. HEK293T cells transduced with T1eVLPs containing PLC PH fused to codon optimized spCas9 ABE targeted to HEK site #3. eVLPs are pseudotyped with VSVG. Gene modification is measured by amplicon sequencing.

FIG 12: Exemplary T1eVLP-delivered asCas12a genome editing in vitro. HEK293 cells transduced with VLPs containing Rous sarcoma virus gag or T1eVLPs containing PLC PH fused to asCas12a. VLPs and eVLPs are targeted to FANCF site #1 by crRNA. Gene modification is measured by T7E1. The Rous sarcoma virus gag VLP serves as a positive control.

FIG 13: Exemplary T1eVLP-delivered spCas9 genome editing in vitro. HEK293 cells transduced with T1eVLPs containing PLC PH fused to RNA binding protein MS2. MS2 binds to MS2 stem loops in the sgRNA, which is complexed with Cas9, and MS2 is fused to a PH domain for efficient eVLP loading. eVLPs are targeted to GFP site #1 by sgRNA. Gene modification is measured by T7E1.

FIG 14: Exemplary T1eVLP-delivered spCas9 genome editing in vitro. HEK293 cells transduced with T1eVLPs containing PLC PH fused to dimerization domain (DmrC). In the presence of A/C Heterodimerizer molecule, DmrC binds to DmrA which is directly fused to Cas9. eVLPs are targeted to GFP site #1 by sgRNA. Gene modification is measured by T7E1.

FIG 15: Exemplary T1eVLP-delivered asCas9 genome editing in vitro. HEK293 cells transduced with T1eVLPs containing PLC PH fused to GNC4 protein domain repeats. An scFv binds to the GCN4 repeats, and scFvs are directly fused to Cas9. eVLPs are targeted to GFP site #1 by sgRNA. Gene modification is measured by T7E1.

FIGS. 16-40: Schematic illustrations of various exemplary eVLP configurations and possible cargo.

DETAILED DESCRIPTION

Therapeutic proteins and nucleic acids hold great promise, but for many of these large biomolecules delivery into cells is a hurdle to clinical development. Genome editing reagents such as zinc finger nucleases (ZFNs) or RNA-guided, enzymatically active/inactive DNA binding proteins such as Cas9 have undergone rapid advancements

in terms of specificity and the types of edits that can be executed, but the hurdle of safe in vivo delivery still precludes efficacious gene editing therapies. Described herein are various embodiments of enhanced virus-like particles (eVLPs), as well as characteristics of various embodiments of eVLPs that provide a novel and optimal platform for the delivery of genome editing reagents, and contrasts eVLPs with canonical delivery modalities.

Retroviral particles, such as lentivirus, have been developed to deliver RNA that is reverse transcribed to DNA that may or may not be integrated into genomic DNA. VLPs have been developed that mimic virus particles in their ability to self-assemble, but are not infectious as they lack some of the core viral genes. Both lentiviral and VLP vectors are typically produced by transiently transfecting a producer cell line with plasmids that encode all components necessary to produce lentiviral particles or VLP. One major flaw that we have discovered regarding lentiviral particles and VSVG-based VLPs that are produced by this conventional transient transfection method is that, in addition to their conventional cargo, these particles package and deliver plasmid DNA that was used in the initial transient transfection. This unintended plasmid DNA delivery can be immunogenic and cause undesirable effects, such as plasmid DNA being integrated into genomic DNA. It is important to specify the type of biomolecules/chemicals that are to be delivered within particles, and eVLPs have been designed to possess this germane capability.

The eVLPs described herein can deliver a wide variety of cargo including DNA only, DNA+RNA+protein, or RNA+protein. Importantly, eVLPs are the first VSVG-based VLP delivery modality that can control the form of the cargo (DNA, protein, and/or RNA). Previously described VSVG-based vesicles and viral particles package and deliver unwanted plasmid DNA (or other types of DNA-based gene expression constructs) introduced into particle producer cells via transient transfection in addition to the intended protein and/or RNA cargo(s).

Another aspect of eVLPs is the ENV protein on the surface of the eVLP. Without wishing to be bound by theory, the ENV protein alone is responsible for eVLP particle generation and the ability of eVLPs to efficiently deliver cargo into cells. Lentivirus and VLPs commonly require GAG and ENV proteins to drive particle formation via budding

off of the plasma membrane of producer cells into the cell culture medium. In addition, the majority of retroviral ENV proteins require post-translational modifications in the form of proteolytic cleavage of the intracellular domain (ICD) of the ENV protein in order to activate the fusogenicity of the ENV protein; this is essential for viral infectivity.

5 The envelope proteins described in Table 1 are all derived from viruses. However, these eVLP ENV proteins do not require exogenous GAG for particle formation and they do not require ICD cleavage for fusogenicity.¹⁻³ The ENV is the only virally-derived component of eVLPs, and these ENV glycoproteins on the external surface of the eVLPs are used to facilitate fusion/entry of eVLPs into the target cell because they are known to
10 be naturally fusogenic. In addition, eVLPs are different from previously described viral particles, VLPs, and extracellular vesicles because eVLPs are composed of a mixture of ectosomes and exosomes which can be separated by purification, if desired. Because of the above mentioned design simplifications and optimizations, eVLPs are particularly suited for delivery of cargo including DNA, RNA, protein, or combinations of
15 biomolecules and/or chemicals, such as DNA-encoded or RNP-based genome editing reagents.

Large biomolecules including proteins and protein complexes such as genome editing reagents, especially CRISPR-CAS, zinc finger, and TAL-nuclease-based reagents, have the potential to become in vivo therapeutics for the treatment of a number of
20 diseases including genetic diseases, but techniques for delivering these reagents into cells are severely limiting or unsafe for patients. Conventional therapeutic monoclonal antibody delivery is successful at utilizing direct injection for proteins. Unfortunately, strategies for direct injection of gene editing proteins, such as Cas9, are hampered by immunogenicity, degradation, ineffective cell specificity, and inability to cross the plasma
25 membrane or escape endosomes/lysosomes.⁴⁻¹⁰ More broad applications of protein therapy and gene editing could be achieved by delivering therapeutic protein cargo to the inside of cells. Cas9, for example, cannot efficiently cross the phospholipid bilayer to enter into cells, and has been shown to have innate and adaptive immunogenic potential.⁴⁻⁸ Therefore, it is not practical or favorable to deliver Cas9 by direct injection
30 or as an external/internal conjugate to lipid, protein or metal-based nanoparticles that

have cytotoxic and immunogenic properties and often yield low levels of desired gene modifications.⁹⁻²⁰

Nanoparticles that encapsulate cargo are another delivery strategy that can be used to deliver DNA, protein, RNA and RNPs into cells.⁹⁻¹⁸ Nanoparticles can be engineered for cell specificity and can trigger endocytosis and subsequent endosome lysis. However, nanoparticles can have varying levels of immunogenicity due to an artificially-derived vehicle shell.⁹⁻²⁰ Many nanoparticles rely on strong opposing charge distributions to maintain particle structural integrity, and the electrostatics can make it toxic and unfit for many in vivo therapeutic scenarios.⁹ Nanoparticles that deliver RNA have had successes in recent clinical trials, but most have only been used to deliver siRNA or shRNA. Toxicity from such nanoparticles is still a major concern.⁹ Nanoparticles that deliver mRNA coding for genome editing RNPs have also been a recent success, but these create a higher number of off-target effects compared to protein delivery and RNA stability is lower than that of protein.¹⁷ Nanoparticles that deliver genome editing RNPs and DNA have been a significant breakthrough because they can leverage both homology directed repair (HDR) and non-homologous end joining (NHEJ), but exhibit prohibitively low gene modification frequencies in vitro and in vivo, and therefore currently have limited applications in vivo as a gene editing therapeutic.¹⁵

Currently, the clinical standard vehicles for delivering genome editing therapeutics are adeno-associated virus (AAV). Although AAV vectors are a promising delivery modality that can successfully deliver DNA into eukaryotic cells, AAV cannot efficiently package and deliver DNA constructs larger than 4.5 kb and this precludes delivery of many CRISPR-based gene editing reagents that require larger DNA expression constructs. CRISPR-based gene editing reagents can be split into multiple different AAV particles, but this strategy drastically reduces delivery and editing efficiency. Depending on the dose required, AAV and adenoviral vectors can have varying levels of immunogenicity. In addition, inverted-terminal repeats (ITRs) in the AAV DNA construct can promote the formation of spontaneous episomes leading to prolonged expression of genome editing reagents and increased off-target effects. ITRs can also promote the undesired integration of AAV DNA into genomic DNA.²¹⁻²⁴

Recently, VLPs have been utilized to deliver mRNA and protein cargo into the cytosol of cells.^{2,3,25-30} VLPs have emerged as a substitute delivery modality for retroviral particles. VLPs can be designed to lack the ability to integrate retroviral DNA, and to package and deliver protein/RNP/DNA. However, most VLPs, including recently conceived VLPs that deliver genome editing reagents known to date, utilize HIV or other virally-derived *gag-pol* protein fusions and viral proteases to generate retroviral-like particles.^{25-27,29,30} Secondly, some VLPs containing RGNs also must package and express guide RNAs from a lentiviral DNA transcript.²⁷ Thirdly, some VLPs require a viral protease in order to form functional particles and release genome editing cargo.^{25-27,29} Since this viral protease recognizes and cleaves at multiple amino acid motifs, it can cause damage to the protein cargo which could be hazardous for therapeutic applications. Fourthly, most published VLP modalities that deliver genome editing proteins to date exhibit low *in vitro* and *in vivo* gene modification efficiencies due to low packaging and transduction efficiency.²⁵⁻²⁷ Fifthly, the complex viral genomes utilized for these VLP components possess multiple reading frames and employ RNA splicing that could result in spurious fusion protein products being delivered.^{25-27,29,30} Sixthly, the presence of reverse transcriptase, integrase, capsid and a virally-derived envelope protein in these VLPs is not ideal for most therapeutic applications because of immunogenicity and off target editing concerns. Lastly, most retroviral particles, such as lentiviral particles, are pseudotyped with VSVG and nearly all described VLPs that deliver genome editing reagents hitherto possess and rely upon VSVG.^{2,3,25-30} We have discovered that VSVG-based particles that are formed by transiently transfecting producer cells package and deliver DNA that was transfected. The current versions of VSVG-based VLPs cannot prevent this inadvertent delivery of DNA and this impedes the use of VLPs in scenarios that necessitate minimal immunogenicity and off target effects.

Extracellular vesicles are another delivery modality that can package and deliver cargo within exosomes and ectosomes.^{31,32} Similar to VLPs, extracellular vesicles are comprised of a phospholipid bilayer from a mammalian cell. Unlike VLPs, extracellular vesicles lack viral components and therefore have limited immunogenicity. Whereas VLPs have a great ability to enter cells due to external fusogenic glycoproteins (VSVG)

extracellular vesicles mainly rely on cellular uptake via micropinocytosis and this limits the delivery efficiency of extracellular vesicles.

eVLPs are a safer and more effective alternative than previously described VLPs, extracellular vesicles, AAVs and nanoparticles-especially for delivery of genome editing reagents-because eVLPs are composed of all human components except for a virally-derived glycoprotein that has been demonstrated to be safe in humans in a clinical trial of a HIV-1 gag vaccine (VSVG),³³ eVLPs lack all other retroviral components besides a safe glycoprotein, eVLPs have the ability to deliver DNA+RNP, or RNP alone while other previously described VLPs cannot prevent transient transfection DNA from being unintentionally packaged and delivered, eVLPs can deliver specialty DNA molecules while previously described VLPs, nanoparticles and AAVs cannot or do not, and eVLPs can be produced with cells that have been derived from patients (autologous eVLPs) and other FDA-approved cell lines (allogenic eVLPs) to further reduce the risks of adverse immune reactions. Here, we describe methods and compositions for producing, purifying, and administering eVLPs for *in vitro* and *in vivo* applications, e.g., of genome editing, epigenome modulation, transcriptome editing and proteome modulation. The desired editing outcome depends on the therapeutic context and will require different gene editing reagents. *Streptococcus pyogenes* Cas9 (spCas9) and *acidaminococcus* sp. Cas12a (functionalize) are two of the most popular RNA-guided enzymes for editing that leverages NHEJ for introducing stop codons or deletions, or HDR for causing insertions.³⁴⁻³⁶ Cas9-deaminase fusions, also known as base editors, are the current standard for precise editing of a single nucleotide without double stranded DNA cleavage.^{37,38} Importantly, these methods address the phenomenon of inadvertent DNA delivery in VLPs and the first to control for the type of biomolecule to be delivered (DNA, RNA, and/or protein) thereby increasing the types of therapeutic *in vivo* genome modifications that are possible and minimizing deleterious off target effects.

Section 1: eVLP-mediated delivery of DNAs, proteins and RNAs

Conventional VLPs that have been engineered to encapsulate and deliver protein-based cargo commonly fuse cargo to the INT or GAG polyprotein.^{25-27,29,30,39,40} After transient transfection of production plasmid DNA constructs, these protein fusions are

translated in the cytosol of conventional VLP production cell lines, the gag matrix is acetylated and recruited to the cell membrane, and the gag fusions are encapsulated (transient transfection DNA is also unintentionally encapsulated) within VLPs as VLPs bud off of the membrane into extracellular space.

5 In contrast, in some embodiments the eVLPs described herein can package protein-based cargo by integrating all production DNA into the genomic DNA of production cell lines. Once cell lines are created, protein delivery eVLPs can be produced in a constitutive or inducible fashion. Proteins are packaged into eVLP by fusing select human-derived phospholipid bilayer recruitment domains to protein-based cargo (e.g., as
10 shown in Table 6). One such human-derived phospholipid bilayer recruitment domain used for this purpose is a human pleckstrin homology (PH) domain. PH domains interact with phosphatidylinositol lipids and proteins within biological membranes, such as PIP2, PIP3, $\beta\gamma$ -subunits of GPCRs, and PKC.^{41,42} Alternatively, the human Arc protein can be fused to protein-based cargo to recruit cargo to the cytosolic side of the phospholipid
15 bilayer.⁴³ These human-derived phospholipid bilayer recruitment domains can be fused to the N-terminus or C-terminus of protein-based cargo via polypeptide linkers of variable length regardless of the location or locations of one or more nuclear localization sequence(s) (NLS) within the cargo. Preferably, the linker between protein-based cargo and the phospholipid bilayer recruitment domain is a polypeptide linker 5-20, e.g., 8-12,
20 e.g., 10, amino acids in length primarily composed of glycines and serines. The human-derived phospholipid bilayer recruitment domain localizes the cargo to the phospholipid bilayer and this protein cargo is packaged within eVLPs that utilize a glycoprotein to trigger budding off of particles from the producer cell into extracellular space (FIG 1). These human-derived domains and proteins can facilitate for localization of cargo to the
25 cytosolic face of the plasma membrane within the eVLP production cells, and they also allow for cargo to localize to the nucleus of eVLP-transduced cells without the utilization of exogenous retroviral gag/pol or chemical and/or light-based dimerization systems (FIG 2). The delivery of Cas9, for example, is significantly more efficient with a fusion to a plasma membrane recruitment domain compared to without a plasma membrane
30 recruitment domain (FIG 3).

In some embodiments, eVLPs can also package and deliver a combination of DNA and RNA if eVLPs are produced via transient transfection of a production cell line. DNA that is transfected into cells will possess size-dependent mobility such that a fraction of the transfected DNA will remain in the cytosol while another fraction of the transfected DNA will localize to the nucleus.⁴⁴⁻⁴⁶ One fraction of the transfected DNA in the nucleus will express components needed to create eVLPs and the other fraction in the cytosol/near the plasma membrane will be encapsulated and delivered in eVLPs (FIG 4).

eVLP “Cargo” refers to any payload that can be delivered, including chemicals, e.g., small molecule compounds, and biomolecules, including DNA, RNA, RNP, proteins, and combinations thereof, including combinations of DNA and RNP, RNP, combinations of DNA and proteins, or proteins, as well as viruses and portions thereof, e.g., for therapeutic or diagnostic use, or for the applications of genome editing, epigenome modulation, and/or transcriptome modulation. In order to simplify these distinctions, a combination of DNA and RNP will be referred to herein as type 1 cargo (T1eVLPs), RNP will be referred to herein as type 2 cargo (T2eVLPs), a combination of DNA and proteins will be referred to herein as type 3 cargo (T3eVLPs), and proteins will be referred to herein as type 4 cargo (T4eVLPs). RNA in this context includes, for example, single guide RNA (sgRNA), Clustered Regularly Interspaced Palindromic Repeat (CRISPR) RNA (crRNA), and/or mRNA coding for cargo.

As used herein, “small molecules” refers to small organic or inorganic molecules of molecular weight below about 3,000 Daltons. In general, small molecules useful for the invention have a molecular weight of less than 3,000 Daltons (Da). The small molecules can be, e.g., from at least about 100 Da to about 3,000 Da (e.g., between about 100 to about 3,000 Da, about 100 to about 2500 Da, about 100 to about 2,000 Da, about 100 to about 1,750 Da, about 100 to about 1,500 Da, about 100 to about 1,250 Da, about 100 to about 1,000 Da, about 100 to about 750 Da, about 100 to about 500 Da, about 200 to about 1500, about 500 to about 1000, about 300 to about 1000 Da, or about 100 to about 250 Da).

The cargo is limited by the diameter of the particles, e.g., which in some embodiments range from 30nm to 500nm.

Cargo developed for applications of genome editing also includes nucleases and base editors. Nucleases include FokI and AcuI ZFNs and Transcription activator-like effector nucleases (TALENs) and CRISPR based nucleases or a functional derivative thereof (e.g., as shown in Table 2) (ZFNs are described, for example, in United States Patent Publications 20030232410; 20050208489; 20050026157; 20050064474; 20060188987; 20060063231; and International Publication WO 07/014275) (TALENs are described, for example, in United States Patent Publication US9393257B2; and International Publication WO2014134412A1) (CRISPR based nucleases are described, for example, in United States Patent Publications US8697359B1; US20180208976A1; and International Publications WO2014093661A2; WO2017184786A8).³⁴⁻³⁶ Base editors that are described by this work include any CRISPR based nuclease orthologs (wt, nickase, or catalytically inactive (CI)), e.g., as shown in Table 2, fused at the N-terminus to a deaminase or a functional derivative thereof (e.g., as shown in Table 3) with or without a fusion at the C-terminus to one or multiple uracil glycosylase inhibitors (UGIs) using polypeptide linkers of variable length (Base editors are described, for example, in United States Patent Publications US20150166982A1; US20180312825A1; US10113163B2; and International Publications WO2015089406A1; WO2018218188A2; WO2017070632A2; WO2018027078A8; WO2018165629A1).^{37,38} In addition, prime editors are also compatible with eVLP delivery modalities (Prime editors are described, for example, in PMID: 31634902).

sgRNAs complex with genome editing reagents during the packaging process, and are co-delivered within eVLPs. To date, this concept has been validated *in vitro* by experiments that demonstrate the T1eVLP or T2eVLP delivery of RGN and CI RGN fused to deaminase and UGI (base editor) as protein for the purposes of site specific editing of exogenous and endogenous sites (FIGs. 3, 5, 6, 7, 8, 9, 10, 11 & 12). For example, T1eVLPs have been used to deliver Cas9 RNP to U2OS and HEK293 cells for the purposes of editing exogenous GFP, and endogenous HEK site #3 and VEGF site #2 & #3 (FIGs. 4, 5, 6 & 7). In addition, T1eVLPs have been used to deliver BE3 and BE4 RNP to HEK293T cells for the purpose of base editing endogenous VEGF site #2 & #3 and HEK site #3 (FIGs. 8, 9, 10 & 11). T1eVLPs have also been used to deliver Cas12a RNP to HEK293 cells for the purposes of editing endogenous FANCF site #1 (FIG 12).

Cargo designed for the purposes of epigenome modulation includes the CI CRISPR based nucleases, zinc fingers (ZFs) and TALEs fused to an epigenome modulator or combination of epigenome modulators or a functional derivative thereof connected together by one or more variable length polypeptide linkers (Tables 2 & 4).

5 T1-T4 cargo designed for the purposes of transcriptome editing includes CRISPR based nucleases or any functional derivatives thereof in Table 5 or CI CRISPR based nucleases or any functional derivatives thereof in Table 5 fused to deaminases in Table 3 by one or more variable length polypeptide linkers.

The cargo can also include any therapeutically or diagnostically useful protein,
10 DNA, RNP, or combination of DNA, protein and/or RNP. See, e.g., WO2014005219; US10137206; US20180339166; US5892020A; EP2134841B1; WO2007020965A1. For example, cargo encoding or composed of nuclease or base editor proteins or RNPs or derivatives thereof can be delivered to retinal cells for the purposes of correcting a splice site defect responsible for Leber Congenital Amaurosis type 10. In the mammalian inner
15 ear, eVLP delivery of base editing reagents or HDR promoting cargo to sensory cells such as cochlear supporting cells and hair cells for the purposes of editing β -catenin (β -catenin Ser 33 edited to Tyr, Pro, or Cys) in order to better stabilize β -catenin could help reverse hearing loss.

In another application, eVLP delivery of RNA editing reagents or proteome
20 perturbing reagents could cause a transitory reduction in cellular levels of one or more specific proteins of interest (potentially at a systemic level, in a specific organ or a specific subset of cells, such as a tumor), and this could create a therapeutically actionable window when secondary drug(s) could be administered (this secondary drug is more effective in the absence of the protein of interest or in the presence of lower levels
25 of the protein of interest). For example, eVLP delivery of RNA editing reagents or proteome perturbing reagents could trigger targeted degradation of MAPK and PI3K/AKT proteins and related mRNAs in vemurafenib/dabrafenib-resistant BRAF-driven tumor cells, and this could open a window for the administration of vemurafenib/dabrafenib because BRAF inhibitor resistance is temporarily abolished
30 (resistance mechanisms based in the MAPK/PI3K/AKT pathways are temporarily

downregulated by eVLP cargo). This example is especially pertinent when combined with eVLPs that are antigen inducible and therefore specific for tumor cells.

In some embodiments, eVLPs could be used deliver factors, e.g., including the Yamanaka factors Oct3/4, Sox2, Klf4, and c-Myc, to cells such as human or mouse fibroblasts, in order to generate induced pluripotent stem cells.

In some embodiments, eVLPs could deliver dominant-negative forms of proteins in order to elicit a therapeutic effect.

eVLPs that are antigen-specific (i.e., tumor-antigen specific) could be targeted to cancer cells in order to deliver proapoptotic proteins BIM, BID, PUMA, NOXA, BAD, BIK, BAX, BAK and/or HRK in order to trigger apoptosis of cancer cells. Tumor antigens are known in the art and include

90% of pancreatic cancer patients present with unresectable disease. Around 30% of patients with unresectable pancreatic tumors will die from local disease progression, so it is desirable to treat locally advanced pancreatic tumors with ablative radiation, but the intestinal tract cannot tolerate high doses of radiation needed to cause tumor ablation. Selective radioprotection of the intestinal tract enables ablative radiation therapy of pancreatic tumors while minimizing damage done to the surrounding gastrointestinal tract. To this end, eVLPs could be loaded with dCas9 fused to the transcriptional repressor KRAB and guide RNA targeting *EGLN*. *EGLN* inhibition has been shown to significantly reduce gastrointestinal toxicity from ablative radiation treatments because it causes selective radioprotection of the gastrointestinal tract but not the pancreatic tumor.⁴⁷ Such fusion proteins, eVLPs, and methods of making and using the same are provided herein.

Unbound steroid receptors reside in the cytosol. After binding to ligands, these receptors will translocate to the nucleus and initiate transcription of response genes. eVLPs could deliver single chain variable fragment (scFv) antibodies to the cytosol of cells that bind to and disrupt cytosolic steroid receptors. For example, the scFv could bind to the glucocorticoid receptor and prevent it from binding dexamethasone, and this would prevent transcription of response genes, such as *metallothionein 1E* which has been linked to tumorigenesis.⁴⁸

eVLPs can be indicated for treatments that involve targeted disruption of proteins. For example, eVLPs can be utilized for targeting and disrupting proteins in the cytosol of cells by delivering antibodies/scFvs to the cytosol of cells. Classically, delivery of antibodies through the plasma membrane to the cytosol of cells has been notoriously difficult and inefficient. This mode of protein inhibition is similar to how a targeted small molecule binds to and disrupts proteins in the cytosol and could be useful for the treatment of a diverse array of diseases.⁴⁹⁻⁵¹ Such fusion proteins, eVLPs, and methods of making and using the same are

In addition, the targeting of targeted small molecules is limited to proteins of a certain size that contain binding pockets which are relevant to catalytic function or protein-protein interactions. scFvs are not hampered by these limitations because scFvs can be generated that bind to many different moieties of a protein in order to disrupt catalysis and interactions with other proteins. For example, RAS oncoproteins are implicated across a multitude of cancer subtypes, and *RAS* is one of the most frequently observed oncogenes in cancer. For instance, the International Cancer Genome Consortium found KRAS to be mutated in 95% of their Pancreatic Adenocarcinoma samples. RAS isoforms are known to activate a variety of pathways that are dysregulated in human cancers, like the PI3K and MAPK pathways. Despite the aberrant roles RAS plays in cancer, no efficacious pharmacologic direct or indirect small molecule inhibitors of RAS have been developed and approved for clinical use. One strategy for targeting RAS could be eVLPs that can deliver specifically to cancer cells scFvs that bind to and disrupt the function of multiple RAS isoforms.⁴⁹⁻⁵¹

Section 2: eVLP composition, production, purification and applications

eVLPs can be produced from producer cell lines that are either transiently transfected with at least one plasmid or stably expressing constructs that have been integrated into the producer cell line genomic DNA. In some embodiments, for T1 and T3eVLPs, if a single plasmid is used in the transfection, it should comprise sequences encoding one or more virally-derived glycoproteins (e.g., as shown in Table 1), cargo (e.g., a therapeutic protein or a gene editing reagent such as a zinc finger, transcription activator-like effector (TALE), and/or CRISPR-based genome editing/modulating protein

and/or RNP such as those found in Tables 2, 3, 4 & 5), with or without fusion to a plasma membrane recruitment domain (e.g., as shown in Table 6), and a guide RNA, if necessary. Preferably, two to three plasmids are used in the transfection. These two to three plasmids can include the following (any two or more can be combined in a single plasmid):

1. A plasmid comprising sequences encoding a therapeutic protein or a genome editing reagent, with or without a fusion to a plasma membrane recruitment domain.
2. A plasmid comprising one or more virally-derived glycoproteins (e.g., as listed in Table 1).
3. If the genome editing reagent from plasmid 1 requires one or more guide RNAs, a plasmid comprising one or more guide RNAs apposite for the genome editing reagent in plasmid 1.

If it is desired to deliver a type of DNA molecule other than plasmid(s), the above-mentioned transfection can be performed with double-stranded closed-end linear DNA, episome, mini circle, double-stranded oligonucleotide and/or other specialty DNA molecules. Alternatively, for T2 and T4eVLPs, the producer cell line can be made to stably express the constructs (1 through 3) described in the transfection above.

As stated earlier, in some embodiments, the methods include using cells that have or have not been manipulated to express any exogenous proteins except for a viral envelope (e.g., as shown in Table 1), and, if desired, a plasma membrane recruitment domain (e.g., as shown in Table 6). In this embodiment, the “empty” particles that are produced can be loaded with cargo by utilizing nucleofection, lipid, polymer, or CaCl₂ transfection, sonication, freeze thaw, and/or heat shock of purified particles mixed with cargo. In all embodiments, producer cells do not express any gag protein. This type of loading allows for cargo to be unmodified by fusions to plasma membrane recruitment domains and represents a significant advancement from previous VLP technology.

The plasmids, or other types of specialty DNA molecules known in the art or described above, can also preferably include other elements to drive expression or translation of the encoded sequences, e.g., a promoter sequence; an enhancer sequence, e.g., 5' untranslated region (UTR) or a 3' UTR; a polyadenylation site; an insulator

sequence; or another sequence that increases or controls expression (e.g., an inducible promoter element).

Preferably, appropriate producer cell lines are primary or stable human cell lines refractory to the effects of transfection reagents and fusogenic effects due to virally-derived glycoproteins. Examples of appropriate cell lines include Human Embryonic
5 Kidney (HEK) 293 cells, HEK293 T/17 SF cells kidney-derived Phoenix-AMPHO cells, and placenta-derived BeWo cells. For example, such cells could be selected for their ability to grow as adherent cells, or suspension cells. In some embodiments, the producer cells can be cultured in classical DMEM under serum conditions, serum-free conditions,
10 or exosome-free serum conditions. eVLPs, e.g., T1 and T3eVLPs, can be produced from cells that have been derived from patients (autologous eVLPs) and other FDA-approved cell lines (allogenic eVLPs) as long as these cells can be transfected with DNA constructs that encode the aforementioned eVLP production components by various techniques known in the art.

15 In addition, if it is desirable, more than one genome editing reagent can be included in the transfection. The DNA constructs can be designed to overexpress proteins in the producer cell lines. The plasmid backbones, for example, used in the transfection can be familiar to those skilled in the art, such as the pCDNA3 backbone that employs the CMV promoter for RNA polymerase II transcripts or the U6 promoter for RNA
20 polymerase III transcripts. Various techniques known in the art may be employed for introducing nucleic acid molecules into producer cells. Such techniques include chemical-facilitated transfection using compounds such as calcium phosphate, cationic lipids, cationic polymers, liposome-mediated transfection, such as cationic liposome like LIPOFECTAMINE (LIPOFECTAMINE 2000 or 3000 and TransIT-X2),
25 polyethyleneimine, non-chemical methods such as electroporation, particle bombardment, or microinjection.

A human producer cell line that stably expresses the necessary eVLP components in a constitutive and/or inducible fashion can be used for production of T2 and T4eVLPs. T2 and T4eVLPs can be produced from cells that have been derived from patients
30 (autologous eVLPs) and other FDA-approved cell lines (allogenic eVLPs) if these cells

have been converted into stable cell lines that express the aforementioned eVLP components.

Also provided herein are the producer cells themselves.

Production of Cargo-Loaded eVLPs and Compositions

5 Preferably eVLPs are harvested from cell culture medium supernatant 36-48 hours post-transfection, or when eVLPs are at the maximum concentration in the medium of the producer cells (the producer cells are expelling particles into the media and at some point in time, the particle concentration in the media will be optimal for harvesting the particles). Supernatant can be purified by any known methods in the art, such as
10 centrifugation, ultracentrifugation, precipitation, ultrafiltration, and/or chromatography. In some embodiments, the supernatant is first filtered, e.g., to remove particles larger than 1 μm , e.g., through 0.45 pore size polyvinylidene fluoride hydrophilic membrane (Millipore Millex-HV) or 0.8 μm pore size mixed cellulose esters hydrophilic membrane (Millipore Millex-AA). After filtration, the supernatant can be further purified and
15 concentrated, e.g., using ultracentrifugation, e.g., at a speed of 80,000 to 100,000xg at a temperature between 1°C and 5°C for 1 to 2 hours, or at a speed of 8,000 to 15,000 g at a temperature between 1°C and 5°C for 10 to 16 hours. After this centrifugation step, the eVLPs are concentrated in the form of a centrifugate (pellet), which can be resuspended to a desired concentration, mixed with transduction-enhancing reagents, subjected to a
20 buffer exchange, or used as is. In some embodiments, eVLP-containing supernatant can be filtered, precipitated, centrifuged and resuspended to a concentrated solution. For example, polyethylene glycol (PEG), e.g., PEG 8000, or antibody-bead conjugates that bind to eVLP surface proteins or membrane components can be used to precipitate particles. Purified particles are stable and can be stored at 4°C for up to a week or -80°C
25 for years without losing appreciable activity.

 Preferably, eVLPs are resuspended or undergo buffer exchange so that particles are suspended in an appropriate carrier. In some embodiments, buffer exchange can be performed by ultrafiltration (Sartorius Vivaspin 500 MWCO 100,000). An exemplary appropriate carrier for eVLPs to be used for *in vitro* applications would preferably be a
30 cell culture medium that is suitable for the cells that are to be transduced by eVLPs.

Transduction-enhancing reagents that can be mixed into the purified and concentrated eVLP solution for in vitro applications include reagents known by those familiar with the art (Miltenyi Biotec Vectofusin-1, Millipore Polybrene, Takara Retronectin, Sigma Protamine Sulfate, and the like). After eVLPs in an appropriate carrier are applied to the cells to be transduced, transduction efficiency can be further increased by centrifugation. Preferably, the plate containing eVLPs applied to cells can be centrifuged at a speed of 1,150 g at room temperature for 30 minutes. After centrifugation, cells are returned into the appropriate cell culture incubator (humidified incubator at 37°C with 5% CO₂).

An appropriate carrier for eVLPs to be administered to a mammal, especially a human, would preferably be a pharmaceutically acceptable composition. A “pharmaceutically acceptable composition” refers to a non-toxic semisolid, liquid, or aerosolized filler, diluent, encapsulating material, colloidal suspension or formulation auxiliary of any type. Preferably, this composition is suitable for injection. These may be in particular isotonic, sterile, saline solutions (monosodium or disodium phosphate, sodium, potassium, calcium or magnesium chloride and and similar solutions or mixtures of such salts), or dry, especially freeze-dried compositions which upon addition, depending on the case, of sterilized water or physiological saline, permit the constitution of injectable solutions. Another appropriate pharmaceutical form would be aerosolized particles for administration by intranasal inhalation or intratracheal intubation.

The pharmaceutical forms suitable for injectable use include sterile aqueous solutions or suspensions. The solution or suspension may comprise additives which are compatible with eVLPs and do not prevent eVLP entry into target cells. In all cases, the form must be sterile and must be fluid to the extent that the form can be administered with a syringe. It must be stable under the conditions of manufacture and storage and must be preserved against the contaminating action of microorganisms, such as bacteria and fungi. An example of an appropriate solution is a buffer, such as phosphate buffered saline.

Methods of formulating suitable pharmaceutical compositions are known in the art, see, e.g., Remington: The Science and Practice of Pharmacy, 21st ed., 2005; and the books in the series Drugs and the Pharmaceutical Sciences: a Series of Textbooks and Monographs (Dekker, NY). For example, solutions or suspensions used for parenteral,

intradermal, or subcutaneous application can include the following components: a sterile diluent such as water for injection, saline solution, fixed oils, polyethylene glycols, glycerine, propylene glycol or other synthetic solvents; antibacterial agents such as benzyl alcohol or methyl parabens; antioxidants such as ascorbic acid or sodium bisulfite; chelating agents such as ethylenediaminetetraacetic acid; buffers such as acetates, citrates or phosphates and agents for the adjustment of tonicity such as sodium chloride or dextrose. pH can be adjusted with acids or bases, such as hydrochloric acid or sodium hydroxide. The parenteral preparation can be enclosed in ampoules, disposable syringes or multiple dose vials made of glass or plastic.

Pharmaceutical compositions suitable for injectable use can include sterile aqueous solutions (where water soluble) or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersion. For intravenous administration, suitable carriers include physiological saline, bacteriostatic water, Cremophor EL™ (BASF, Parsippany, NJ) or phosphate buffered saline (PBS). In all cases, the composition must be sterile and should be fluid to the extent that easy syringability exists. It should be stable under the conditions of manufacture and storage and must be preserved against the contaminating action of microorganisms such as bacteria and fungi. The carrier can be a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol, and liquid polyethylene glycol, and the like), and suitable mixtures thereof. The proper fluidity can be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. Prevention of the action of microorganisms can be achieved by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, ascorbic acid, thimerosal, and the like. In many cases, it will be preferable to include isotonic agents, for example, sugars, polyalcohols such as mannitol, sorbitol, sodium chloride in the composition. Prolonged absorption of the injectable compositions can be brought about by including in the composition an agent that delays absorption, for example, aluminum monostearate and gelatin.

Sterile injectable solutions can be prepared by incorporating the active compound in the required amount in an appropriate solvent with one or a combination of ingredients

enumerated above, as required, followed by filtered sterilization. Generally, dispersions are prepared by incorporating the active compound into a sterile vehicle, which contains a basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, the preferred methods of preparation are vacuum drying and freeze-drying, which yield a powder of the active ingredient plus any additional desired ingredient from a previously sterile-filtered solution thereof.

The compositions comprising cargo-loaded eVLPs can be included in a container, pack, or dispenser together with instructions for administration.

EXAMPLES

The invention is further described in the following examples, which do not limit the scope of the invention described in the claims.

Methods

The following methods were used in the Examples below. eVLP particles were produced by HEK293T cells using polyethylenimine (PEI) based transfection of plasmids. PEI is Polyethylenimine 25kD linear (Polysciences #23966-2). To make a stock 'PEI MAX' solution, 1g of PEI was added to 1L endotoxin-free dH₂O that was previously heated to ~80°C and cooled to room temperature. This mixture was neutralized to pH 7.1 by addition of 10N NaOH and filter sterilized with 0.22µm polyethersulfone (PES). PEI MAX is stored at -20°C.

HEK293T cells were split to reach a confluency of 70%–90% at time of transfection and are cultured in 10% FBS DMEM media. Cargo vectors, such as one encoding a CMV promoter driving expression of a hPLCδ1 PH fusion to codon optimized Cas9 were co-transfected with a U6 promoter-sgRNA encoding plasmid and the VSV-G envelope plasmid pMD2.G (Addgene #12259). Transfection reactions were assembled in reduced serum media (Opti-MEM; GIBCO #31985-070). For eVLP particle production on 10 cm plates, 7.5 µg PH-Cas9 expressing plasmid, 7.5 µg sgRNA-expression plasmid and 5 µg pMD2.G were mixed in 1 mL Opti-MEM, followed by addition of 27.5µl PEI MAX. After 20-30 min incubation at room temperature, the transfection reactions were dispersed dropwise over the HEK293T cells.

eVLPs were harvested at 48-72 hours post-transfection. eVLP supernatants were filtered using 0.45 μ m cellulose acetate or 0.8 μ m PES membrane filters and transferred to polypropylene Beckman ultracentrifuge tubes that are used with the SW28 rotor (Beckman Coulter #326823). Each ultracentrifuge tube is filled with eVLP-containing supernatant from 3 10 cm plates to reach an approximate final volume of 35-37.5 ml. eVLP supernatant underwent ultracentrifugation at approximately 100,000 xg, or 25,000 rpm, at 4°C for 2 hours. After ultracentrifugation, supernatants were decanted and eVLP pellets resuspended in DMEM 10% FBS media such that they are now approximately 1,000 times more concentrated than they were before ultracentrifugation. eVLPs were added dropwise to cells that were seeded in a 24-well plate 24 hours prior to transduction. Polybrene (5-10 μ g/mL in cell culture medium; Sigma-Aldrich #TR-1003-G) was supplemented to enhance transduction efficiency, if necessary. Vectofusin-1 (10 μ g/mL in cell culture medium, Miltenyi Biotec #130-111-163) was supplemented to enhance transduction efficiency, if necessary. Immediately following the addition of eVLPs, the 24-well plate was centrifuged at 1,150 xg for 30 min at room temperature to enhance transduction efficiency, if necessary.

Example 1.

Cas9 RNP was delivered in VSVG-pseudotyped VLPs with or without a fusion to a PH domain. T2eVLPs containing Cas9 with or without PH fusion and VEGF-targeting sgRNA were applied to HepG2, U2OS, HEK293T, CFPAC1, BeWo, Jurkat, K562, and primary T cells for 48 hours. Gene modification frequencies of the target site within VEGF were obtained by amplicon sequencing. FIG. 3 demonstrates that fusion to PH domains from hPLC δ 1 or hAKT1 significantly enhanced delivery/editing efficiency of Cas9 in T2eVLPs.

Gag fusions to Cas9 or PH fusions to Cas9 with guide RNA targeting GFP were packaged in VLPs or T1eVLPs, respectively. U2OS or HEK293 cell line stably expressing a single copy of GFP were treated with these particles that were previously purified from HEK293T cell culture media (DMEM) 48 hours after transfection of VSVG, Cas9 fusions and guide RNA expressing plasmids. Particle purification and concentration was performed by PVDF filtration and ultracentrifugation at 100,000xg for

2 hours. Gene modification frequencies were determined by T7E1 and flow cytometry. The results are shown in FIGs. 5A-B.

In FIG. 6, hPLC δ 1 PH fusions to codon optimized Cas9 with guide RNA targeting HEK site #3 or VEGF site #2 were packaged in T1eVLPs. U2OS cells were treated with these particles that were previously purified from HEK293T cell culture media (DMEM, 10% FBS) 48 hours after transfection of VSVG, Cas9 fusions and guide RNA expressing plasmids. Particle purification and concentration was performed by filtration and ultracentrifugation at 100,000xg for 2 hours. Gene modification frequencies were determined by amplicon sequencing.

In FIG. 7, hPLC δ 1 (left graph) or hAkt PH (right graph) fusions to codon optimized Cas9 with guide RNA targeting VEGF site #3 were packaged in T1eVLPs. U2OS cells were treated with these particles that were previously purified from HEK293T cell culture media (DMEM, 10% FBS) 48 hours after transfection of VSVG, Cas9 fusions and guide RNA expressing plasmids. Particle purification and concentration was performed by filtration and ultracentrifugation at 100,000xg for 2 hours. Gene modification frequencies were determined by amplicon sequencing.

In FIG. 8, gag fusions to the N or C terminus of Cas9-based base editors (BE3 and BE4) or PH fusions to the N or C terminus of BE3 and BE4 with guide RNA targeting VEGF site #2 were packaged in VLPs and eVLPs, respectively. HEK293T cells were treated with these particles that were previously purified from HEK293T cell culture media (DMEM) 48 hours after transfection of VSVG, base editor fusions and guide RNA expressing plasmids. Particle purification and concentration was performed by filtration and ultracentrifugation at 100,000xg for 2 hours. Gene modification frequencies were determined by amplicon sequencing.

In FIG. 9, hPLC δ 1 fusions to the N terminus of Cas9-based base editors (codon optimized BE4) with guide RNA targeting HEK site #3 were packaged in eVLPs. HEK293T cells were treated with these particles that were previously purified from HEK293T cell culture media (DMEM) 48 hours after transfection of VSVG, base editor fusions and guide RNA expressing plasmids. Particle purification and concentration was performed by filtration and ultracentrifugation at 100,000xg for 2 hours. Gene modification frequencies were determined by amplicon sequencing.

In FIG. 10, hPLC δ 1 fusions to the N terminus of Cas9-based base editors (codon optimized ABE) with guide RNA targeting VEGF site #3 were packaged in eVLPs. HEK293T cells were treated with these particles that were previously purified from HEK293T cell culture media (DMEM) 48 hours after transfection of VSVG, base editor fusions and guide RNA expressing plasmids. Particle purification and concentration was performed by filtration and ultracentrifugation at 100,000xg for 2 hours. Gene modification frequencies were determined by amplicon sequencing.

In FIG. 11, hPLC δ 1 fusions to the N terminus of Cas9-based base editors (codon optimized ABE) with guide RNA targeting HEK site #3 were packaged in eVLPs. HEK293T cells were treated with these particles that were previously purified from HEK293T cell culture media (DMEM) 48 hours after transfection of VSVG, base editor fusions and guide RNA expressing plasmids. Particle purification and concentration was performed by filtration and ultracentrifugation at 100,000xg for 2 hours. Gene modification frequencies were determined by amplicon sequencing.

In FIG. 12, gag fusions to Cas12a or hPLC δ 1 PH fusions to Cas12a with guide RNA targeting FANCF site #1 were packaged in VLPs and eVLPs, respectively. HEK293 cells were treated with these particles that were previously purified from HEK293T cell culture media (DMEM) 48 hours after transfection of VSVG, Cas12a fusions and guide RNA expressing plasmids. Particle purification and concentration was performed by PVDF filtration and ultracentrifugation at 100,000xg for 2 hours. Gene modification frequencies were determined by T7E1.

In FIG. 13, hPLC δ 1 PH fusions to MS2 with MS2-stem loop guide RNA targeting GFP site #1 were packaged in eVLPs with Cas9. HEK293 cells were treated with these particles that were previously purified from HEK293T cell culture media (DMEM) 48 hours after transfection of VSVG, Cas9, PH-MS2 fusions and MS2 stem loop guide RNA expressing plasmids. Particle purification and concentration was performed by PVDF filtration and ultracentrifugation at 100,000xg for 2 hours. Gene modification frequencies were determined by T7E1.

In FIG. 14, hPLC δ 1 PH fusions to DmrC with guide RNA targeting GFP site #1 and Cas9 fused to DmrA repeats were packaged in eVLPs. HEK293 cells were treated with these particles that were previously purified from HEK293T cell culture media

(DMEM) 48 hours after transfection of VSVG, DmrA-Cas9, PH-DmrC fusions and guide RNA expressing plasmids. Particle purification and concentration was performed by PVDF filtration and ultracentrifugation at 100,000xg for 2 hours. Gene modification frequencies were determined by T7E1.

5 In FIG. 15, hPLC81 PH fusions to GCN4 repeats with guide RNA targeting GFP site #1 and Cas9 fused to scFv were packaged in eVLPs. HEK293 cells were treated with these particles that were previously purified from HEK293T cell culture media (DMEM) 48 hours after transfection of VSVG, scFv-Cas9, PH-GCN4 fusions and guide RNA
10 expressing plasmids. Particle purification and concentration was performed by PVDF filtration and ultracentrifugation at 100,000xg for 2 hours. Gene modification frequencies were determined by T7E1.

FIGs. 16-40 show various non-limiting examples of eVLP configurations and possible cargo.

15

TABLE 1 | Exemplary Virally-derived glycoproteins.

Virally-derived glycoproteins
vesicular stomatitis virus glycoprotein (VSVG)
GP64
GP160
RD114
BaEVTR
BaEVTRless
FuG-E
FuG-E (P440E)
MLV ENV (ecotropic)
MLV ENV (amphotropic)
MLV 10A1

TABLE 2 | Exemplary Potential Cas9 and Cas12a orthologs

DNA-binding Cas ortholog	Enzyme class	Nickase mutation	CI mutations
SpCas9	Type II-A	D10A	D10A, H840A
SaCas9	Type II-A	D10A	D10A
CjCas9	Type II-C	D8A	D8A
NmeCas9	Type II-C	D16A	D16A, H588A
asCas12a	Type II-C		D908A, E993A
IbCas12a	Type II-C		D832A, E925A

Nickase mutation residues represents a position of the enzyme either known to be required for catalytic activity of the conserved RuvC nuclease domain or predicted to be required for this catalytic activity based on sequence alignment to CjCas9 where structural information is lacking. All positional information refers to the wild-type protein sequences acquired from uniprot.org.

TABLE 3 | Exemplary Deaminase domains and their substrate sequence preferences.

Deaminase	Nucleotide sequence preference
hAID	5'-WRC
rAPOBEC1*	5'-TC ≥ CC ≥ AC > GC
mAPOBEC3	5'-TYC
hAPOBEC3A	5'-TCG
hAPOBEC3B	5'-TCR > TCT
hAPOBEC3C	5'-WYC
hAPOBEC3F	5'-TTC
hAPOBEC3G	5'-CCC
hAPOBEC3H	5'-TTCA ~ TTCT ~ TTCG > ACCCA > TGCA
ecTadA	
hAdar1	
hAdar2	

Nucleotide positions that are poorly specified or are permissive of two or more nucleotides are annotated according to IUPAC codes, where W = A or T, R = A or G, and Y = C or T.

TABLE 4 | Exemplary Epigenetic modulator domains.

Epigenetic modulator	Epigenetic modulation
VP16	transcriptional activation
VP64	transcriptional activation
P65	transcriptional activation
RTA	transcriptional activation
KRAB	transcriptional repression
MeCP2	transcriptional repression
Tet1	Methylation
Dnmt3a	Methylation

5

TABLE 5 | Exemplary CRISPR based RNA-guided RNA binding enzymes

RNA-binding Cas ortholog	Enzyme class
LshCas13a	Type-VI
LwaCas13a	Type-VI
PspCas13b	Type-VI
RfxCas13d	Type-VI

10

TABLE 6 | Exemplary plasma membrane recruitment domains

#	Plasma membrane recruitment domain	Substitution(s)
1.	Pleckstrin homology domain of human phospholipase C δ 1 (hPLC δ 1)	
2.	Pleckstrin homology domain of human Akt1 (hAkt1)	
3.	Mutant Pleckstrin homology domain of human Akt1	E17K
4.	Pleckstrin homology domain of human 3-phosphoinositide-dependent protein kinase 1 (hPDK1)	
5.	Human CD9	
6.	Human CD47	
7.	Human CD63	
8.	Human CD81	
9.	Pleckstrin homology domain of Human Dapp1	
10.	Pleckstrin homology domain of Mouse Grp1	
11.	Pleckstrin homology domain of Human Grp1	

#	Plasma membrane recruitment domain	Substitution(s)
12.	Pleckstrin homology domain of Human OSBP	
13.	Pleckstrin homology domain of Human Btk1	
14.	Pleckstrin homology domain of Human FAPP1	
15.	Pleckstrin homology domain of Human CERT	
16.	Pleckstrin homology domain of Human PKD	
17.	Pleckstrin homology domain of Human PHLPP1	
18.	Pleckstrin homology domain of Human SWAP70	
19.	Pleckstrin homology domain of Human MAPKAP1	

Exemplary Relevant Protein Sequences:

***Homo sapiens*: Pleckstrin homology domain of Human Dapp1**

5 MQTGRTEDDLVTAPSLGTKEGYLTQGGVLVKTWKTRWFTLHRNELKYFKDQMSPEP
IRILDLTECSAVQFDYSQERVNCFCLVFPFRTFYLCAGTGVADWIKLRWKLQIRKQ
LNQGEETIR (SEQ ID NO:1)

***Mus musculus*: Pleckstrin homology domain of Mouse Grp1**

10 PFKIPEDDGNDLTHTFFNPDREGWLLKLGGRVKTWKRRWFILTDNCLYYFEYTTDKEP
RGIIPLENLSIREVEDPRKPNCFELYNPSHKGQVIKACKTEADGRVVEGNHVVYRISAPS
PEEKKEEWMKSIKASISRDPFYDMLATRKRRANKK (SEQ ID NO:2)

***Homo sapiens*: Pleckstrin homology domain of Human Grp1**

15 NPDREGWLLKLGGGRVKTWKRRWFILTDNCLYYFEYTTDKEPRGIIPLENLSIREVEDP
RKNPNCFELYNPSHKGQVIKACKTEADGRVVEGNHVVYRISAPSPREEKEEWMKSIKASIS
(SEQ ID NO:3)

***Homo sapiens*: Pleckstrin homology domain of Human OSBP**

20 SGSAREGWLFKWTNYIKGYQRRWFVLSNGLLSYYRSKAEMRHTCRGTINLATANITVE
DSCNFIISNGGAQTYHLKASSEVERQRWWTALAKAKAVK (SEQ ID NO:4)

***Homo sapiens*: Pleckstrin homology domain of Human Btk1**

25 MAAVILESIFLKRSQQKKTSPLNFKKRLFLTTHVHKLSSYYEYDFERGRGSKKGSIDVEKI
TCVETVVPEKNPPPERQIPRRGEESSEMEQISIIERFPYPFQVVYDEGPLYVFSPTTELR
KRWIHQLKNVIRYNSDLVQKYHPCFWIDGQYLCCSQAKNAMGCQILENRNGSLKP
(SEQ ID NO:5)

***Homo sapiens*: Pleckstrin homology domain of Human FAPP1**

30 MEGVLYKWTNYLTGWQPRWFVLDNGILSYYSQDDVCKGSKGSIKMAVCEIKVHSAD
NTRMELIIPGEQHFYMKAVNAERQRWLVALGSSKACLTDT (SEQ ID NO:6)

Homo sapiens: Pleckstrin homology domain of Human CERT

MSDNQSWNSSGSEEDPETESGPPVERCGVLSKWTNYIHGWQDRWWVLKNNALSYK
SEDETEYGCRGSICLSKAVITPHDFDECRFDISVNDVWYLRAQDPDHRQQWIDAIEQH
KTESGYG (SEQ ID NO:7)

5

Homo sapiens: Pleckstrin homology domain of Human PKD

TVMKEGWMVHYTSKDTLRKRHYWRLDSKCITLFQNDTGSRYKKEIPLSEILSLEPVKTS
ALIPNGANPHCFEITTANVVYVGENVNPSSPSPNNSVLTSGVGADVARMWEIAIQHA
LM (SEQ ID NO:8)

10

Homo sapiens: Pleckstrin homology domain of Human PHLPP1

RIQLSGMYNVRKGMQLPVNRWTRRQVILCGTCLIVSSVKDSLTKMHVLPPLIGGKVEE
VKKHQHCLAFSSSGPQSQTYYICFDTFTEYLRWLRQVSKVAS (SEQ ID NO:9)

15

Homo sapiens: Pleckstrin homology domain of Human SWAP70

DVLKQGYMMKKGHRKNWTERWFLKPNIISSVSEDLDKDKKGDILLDENCCEVSLPD
KDGKKCLFLVKCFDKTFEISASDKKKKQEWIAIHSTIH (SEQ ID NO:10)

Homo sapiens: Pleckstrin homology domain of Human MAPKAP1

20 DMLSSHYYKSKVSMIHLRFTTQVQLGISGDKVEIDPVTNQAQKSTKFWIKQKPIDSD
LLCACDLAEKSPSHAIFKLTYSNHDYKHLVFESDAATVNEIVLKVNYILES (SEQ ID
NO:11)

Baboon Endogenous Retrovirus glycoprotein (BaEVTR)

25 MGFTTKIIFLYNLVLVYAGFDDPRKAIELVQKRYGRPCDCSGGQVSEPPSDRVSQVTCS
GKTAYLMPDQRWKCKSIPKDTSPSGPLQECPCNSYQSSVHSSCYTSYQQCRSGNKTY
YTATLLKTQTGGTSDVQVLGSTNKLQSPCNGIKGQSIWSTTAIPHVSDDGGGGLDTRI
KSVQRKLEEIHKALYPELQYHPLAIPKVRDNLMDAQTLNINATYNLLMSNTSLVDDC
WLCLKLGPPPTLAIPNLLSYVTRSSDNISCLIPLLVQPMQFSNSSCLFSPSYNSTEEI
30 DLGHVAFSNCTSIITNVTGPICAVNGSVFLCGNNMAYTYLPTNWTGLCVLATLLPDIDIIP
GDEPVPIPAIDHFIYRPKRAIQFIPLLAGLGITAAFTTGATGLGVSVTQYTKLSNQLISDVQI
LSSTIQDLQDQVDSLAEVVLQNRRLDLLTAEQGGICLALQEKCCFYVNKSGIVRDKIKT
LQEELERRRKDLASNPLWTGLQGLLPYLLPFLGPLLLTLLLTIGPCIFNRLVQFVKDRIS
VVQALVLTQQYHQLKPLEYEP (SEQ ID NO:12)

35

Modified Baboon Endogenous Retrovirus glycoprotein (BaEVTRless)

MGFTTKIIFLYNLVLVYAGFDDPRKAIELVQKRYGRPCDCSGGQVSEPPSDRVSQVTCS
GKTAYLMPDQRWKCKSIPKDTSPSGPLQECPCNSYQSSVHSSCYTSYQQCRSGNKTY
YTATLLKTQTGGTSDVQVLGSTNKLQSPCNGIKGQSIWSTTAIPHVSDDGGGGLDTRI
40 KSVQRKLEEIHKALYPELQYHPLAIPKVRDNLMDAQTLNINATYNLLMSNTSLVDDC
WLCLKLGPPPTLAIPNLLSYVTRSSDNISCLIPLLVQPMQFSNSSCLFSPSYNSTEEI
DLGHVAFSNCTSIITNVTGPICAVNGSVFLCGNNMAYTYLPTNWTGLCVLATLLPDIDIIP
GDEPVPIPAIDHFIYRPKRAIQFIPLLAGLGITAAFTTGATGLGVSVTQYTKLSNQLISDVQI
LSSTIQDLQDQVDSLAEVVLQNRRLDLLTAEQGGICLALQEKCCFYVNKSGIVRDKIKT

LQEELERRRKDLASNPLWTGLQGLLPYLLPFLGPLLLTLLLLLTIGPCIFNRLTAFINDKLNII
HAM (SEQ ID NO:13)

Fusion protein of Vesicular stomatitis Indiana virus and Rabies virus

Glycoproteins (FuG-E)

5 MVPQVLLFVLLLGFSLCFGKFPIYTIPDELGPWSPIDIHHLSCPNLVEDEGCTNLSEFS
YMEKLVGYISAIKVNNGFTCTGVVTEAETYTNFVGYYVTTTFKRKHFRPTPDACRAAYNWK
MAGDPRIEESLHNPYPDYHWLRTVVRTTKESLIISPSVTDLPYDKSLHSRVFPGGKCS
GITVSSTYCSNHDYTIWMPENPRPRTPCDIFTNSRGKRASNGNKTCGFVDERGLYKS
10 LKGACRLKLCGVLGLRLMDGTWVAMQTSDETKWCPPDQLVNLHDFRSDEIEHLVVEE
LVKKREECLDALESIMTTKSVSFRRLSHLRKLVPGFGKAYTIFNKTLMEDAHYKSVRT
WNEIIPSKGCKLVGGGRCHPHVNGVFFNGIILGPDDHVLIPMQSSLLQQHMELLESSVIP
LMHPLADPSTVFKEGDEAEDFVEVHLPKNPIELVEGWFWSSWKSSIASFFFIIGLIIGLFLVL
RVGIHLCLIKLHHTKKRQIYTDIEMNRLGK (SEQ ID NO:14)

Modified Fusion protein of Vesicular stomatitis Indiana virus and Rabies virus

Glycoproteins (FuG-E (P440E))

15 MVPQVLLFVLLLGFSLCFGKFPIYTIPDELGPWSPIDIHHLSCPNLVEDEGCTNLSEFS
YMEKLVGYISAIKVNNGFTCTGVVTEAETYTNFVGYYVTTTFKRKHFRPTPDACRAAYNWK
20 MAGDPRIEESLHNPYPDYHWLRTVVRTTKESLIISPSVTDLPYDKSLHSRVFPGGKCS
GITVSSTYCSNHDYTIWMPENPRPRTPCDIFTNSRGKRASNGNKTCGFVDERGLYKS
LKGACRLKLCGVLGLRLMDGTWVAMQTSDETKWCPPDQLVNLHDFRSDEIEHLVVEE
LVKKREECLDALESIMTTKSVSFRRLSHLRKLVPGFGKAYTIFNKTLMEDAHYKSVRT
WNEIIPSKGCKLVGGGRCHPHVNGVFFNGIILGPDDHVLIPMQSSLLQQHMELLESSVIP
25 LMHPLADPSTVFKEGDEAEDFVEVHLEKNPIELVEGWFWSSWKSSIASFFFIIGLIIGLFLVL
RVGIHLCLIKLHHTKKRQIYTDIEMNRLGK (SEQ ID NO:15)

Amphotrophic Murine Leukemia Virus (MLV ENV (amphotropic))

MARSTLSKPPQDKINPWKPLIVMGVLLGVGMAESPHQVFNVWTRVTNLMTGRTANAT
30 SLLGTVQDAFPKLYFDLCLDVGEEWDPSDQEPYVGYGCKYPAGRQRTRTFDFYVCPG
HTVKS GCGGPGEGYCGKWGCETTQAYWKPTSSWDLISLKRGNTPWDTGCSKVAC
GPCYDLSKVSNSFQGATRGGRCNPLVLEFTDAGKKANWDGPKSWGLRLYRTGTDPIT
MFSLTRQVLNVGPRVPIGNPVLDPQRLPSSPIEIVPAPQPPSPLNTSYPPSTTSTPSTS
PTSPSPVQPPPGTGDRLLALVKGAYQALNLTNPDKTQECWLCLVSGPPYYEGVAVVG
35 TYTNHSTAPANCTATSQHKLTLEVTGQGLCMGAVPKTHQALCNTTQSAGSGSYLLAA
PAGTMWACSTGLTPCLSTTVLNLTTDYCVLVELWPRVIYHSPDYMYGQLEQRTKYKRE
PVSLTLALLLGGTLMGGIAAGIGTGTTALIKTQQFEQLHAAIQTDLNEVEKSITNLEKSLTS
LSEVVLQNRRLDLLFLKEGGLCAALKECCFYADHTGLVRDSMAKLRLNRQRQKLF
ETGQGWFEGLFNRSPWFITLISTIMGPLIVLLILLFGPCILNRLVQFVKDRISVVQALVLT
40 QQYHQLKPIEYEP (SEQ ID NO:16)

Ecotrophic Murine Leukemia Virus (MLV ENV (Ecotropic))

MARSTLSKPLKNKVNPRGPLIPLILLMLRGVSTASPGSSPHQVYNITWEVTNGDRETVW
ATSGNHPLWTWWPDLTPDLCMLAHGSPSYWGLEYQSPFSSPPGPPCCSGGSSPGC
45 SRDCEEPLTSLTPRCNTAWNRLKLDQTTKHSNEGFYVCPGPHRPRESKSCGGPDSFY
CAYWGCETTGRAYWKPPSSSWDFITVNNNLTSQAVQVCKDNKWCNPLVIRFTDAGR

VTSWTTGHWGLRLYVSGQDPGLTFGIRLRYQNLGPRVPIGPNPVLADQQPLSKPKPV
 KSPSVTKPPSGTPLSPTQLPPAGTENRLLNLVDGAYQALNLTSPDKTQECWLCLVAGP
 PYYEGVAVLGTYSNHTSAPANCSVASQHKLTLEVTGQGLCIGAVPKTHQALCNTTQT
 SSRGSYYLVAPTGTMWACSTGLTPCISTTILNLTDDYCVLVELWPRVYHSPSYVYGLF
 5 ERSNRHKREPVSLLTALLLGGLTMGGIAAGIGTGTTALMATQQFQQQLQAAVQDDLREV
 EKSISNLEKSLTSLSEVVLQNRRLDGLLFLKEGGLCAALKECCFYADHTGLVRDSMAK
 LRERLNQRQKLFESTQGWFEGLFNRSWFTTLLISTIMGPLIVLLMILLFGPCILNRLVQFV
 KDRISVVQALVLTQQYHQLKPIEYEP (SEQ ID NO:17)

10 **Moloney murine leukemia virus 10A1 strain Glycoprotein (MLV 10A1)**
 MARSTLSKPLKDKINPWKSLMVMGVLLRVGMAESPHQVFNVTVRVTNLMTGRTANAT
 SLLGTVQDAFPRLYFDLCLDVGEEWDPDQEPYVGYGCKYPGGRKRTRTFDFYVCPG
 HTVKSGCGGPREGYCGEWGCETTQAYWKPTSSWDLISLKRGNTPWDTGCSKMAC
 GPCYDLSKVSNSFQGATRGGRCNPLVLEFTDAGKKANWDGPKSWGLRLYRTGTDPI
 15 MFSLTRQVLNIGPRIPGNPVITGQLPPSRPVQIRLPRPPQPPTGAASIVPETAPPSQ
 QPGTGDRLLNLVEGAYRALNLTNPDKTQECWLCLVSGPPYYEGVAVVGTYNHSTAP
 ASCTATSQHKLTLEVTGQGLCMGAVPKTHQALCNTTQSAGSGSYLAAPAGTMWAC
 STGLTPCLSTTMLNLTDDYCVLVELWPRIYHSPDYMVGQLEQRTKYKREPVSLLTALLL
 GGLTMGGIAAGIGTGTTALIKTQQFEQLHAAIQTDLNEVEKSITNLEKSLTSLSEVVLQNR
 20 RGLDGLLFLKEGGLCAALKECCFYADHTGLVRDSMAKLRELRNQRQKLFESGQGWFE
 GLFNRSWFTTLLISTIMGPLIVLLILLFGPCILNRLVQFVKDRISVVQALVLTQQYHQLKPI
 EYEP (SEQ ID NO:18)

25 ***Rattus norvegicus* & synthetic: APOBEC1-XTEN L8-nspCas9-UGI-SV40 NLS**
 MSSETGPVAVDPTLRRRIEPHEFEVFFDPRELKTCCLYEINWGGRHSHWRHTSQNT
 NKHVEVNFIKFTTERYFCPNTRCSITWFLSWSPCGECSRAITEFLSRYPHVTLFYIARL
 YHHADPRNRQGLRDLISSGVTIQIMTEQESGYCWRNFVNYSNEAHWPYPHLLWVR
 LYVLELYCIILGLPPCLNLRKQPKLTFFTIALQSCHYQRLPPHILWATGLKSGSETPGT
 SESATPESDKKYSIGLAIGTNSVGWAVITDEYKVPKSKFKVLGNDRHSIKKNLIGALLFD
 30 SGETAEATRLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFHRLSEESFLVEEDKKH
 ERHPIFGNIVDEVAYHEKYPTIYHLRKKLVDDSTDKADRLIYLALAHMIKFRGHFLIEGDLN
 PDNSDVKLFIQLVQTYNQLFEENPINASGVDAKILSARLSKSRLENLIAQLPGEKKN
 GLFGNLIALSLGLTPNFKSNFDLAEDAKLQLSKDTYDDDLNLLAQIGDQYADFLAAKN
 LSDAILLSDILRVNTEITKAPLSASMIKRYDEHHQDLTLLKALVRQQLPEKYKEIFFDQSK
 35 NGYAGYIDGGASQEEFYKFIKPILEKMDGTEELLVKLNREDLLRKQRTFDNGSIPHQIHL
 GELHAILRRQEDFYFPLKDNREKIEKILTFRIPYYVGPLARGNSRFAWMTRKSEETITPW
 NFEEVVDKGASAQSFIERMTNFDKNLPNEKVLPHKSLLEYFTVYNELTKVKYVTEGMR
 KPAFLSGEQKKAIVDLLFKTNRKVTVKQLKEDYFKKIECFDSVEISGVEDRFNASLGTYH
 DLLKIKDKDFLDNEENEDILEDIVLTLLTFEDREMIEERLKYAHLFDDKVMKQLKRRRY
 40 TGWGRLSRKLINGIRDKQSGKTILDFLKSDFANRNFQMQLIHDDSLTFKEDIQKAQVSG
 QGDSLHEHIANLAGSPAIIKKGILQTVKVVDELVKVMGRHKPENIVIAMARENQTTQKGQ
 KNSRERMKRIEIGIKELGSQILKEHPVENTQLQNEKLYLYLQNGRDMYVDQELDINRL
 SDYDVHIVPQSFLKDDSIDNKVLTTRSDKNRGKSDNVPSEEVVKKMKNYWRQLLNAKLI
 TQRKFDNLTKAERGGLSELDKAGFIKRLVETRQITKHVAQILDSRMNTKYDENDKLIRE

VKVITLKSCLVSDFRKDFQFYKVRINNYHHAHDAYLNAVVGTAIIKKYPKLESEFVYGD
 YKVYDVRKMIKSESEQEIGKATAKYFFYSNIMNFFKTEITLANGEIRKRPLIETNGETGEIV
 WDKGRDFATVRKVLSPQVNVKKTEVQTTGGFSKESILPKRNSDKLIARKKDWDPKKY
 GGFDSPTVAYSVLVAKVEKGSKKLKSVKELLGITIMERSSEKPNIDFLEAKGYKEVK
 5 KDLIIKLPKYSLFELENGRKRMLASAGELQKGNELALPSKYVNFYLAHYEKLKGSPE
 NEQKQLFVEQHKHYLDEIIQISEFSKRVLADANLDKVL SAYNKHDKPIREQAENIIHLF
 TLTNLGAPAAFKYFDTTIDRKRYTSTKEVL DATLIHQSTGLYETRIDLSQLGGDSGGSTN
 LSDIIEKETGKQLVIQESILMLPEEVEEVIGNKPESDILVHTAYDESTDENVMMLTSDAPEY
 KPWALVIQDSNGENKIKMLSGGSPKKRKV (SEQ ID NO:19)

10

Homo sapiens: AID

MDSLLMNRRKFLYQFKNVRWAKGRRETYLCYVVKRRDSATSFSLDFGYLRNKNKGCHV
 ELLFLRYISDWLDPGRCYRVTWFTSWSPCYDCARHVADFLRGPNLSLRIFTARLYF
 CEDRKAEPGLRRLHRAGVQIAIMTFKDYFCWNTFVENHERTFKAWEGLHENSVRSL
 15 RQLRRILLPLYEVDDLRLDAFRTLGL (SEQ ID NO:20)

Homo sapiens: AIDv solubility variant lacking N-terminal RNA-binding region

LMDPHIFTSNFNNGIGRHKTYLCYEVERLDSATSFSLDFGYLRNKNKGCHVELLFLRYISD
 WDLDPGRCYRVTWFTSWSPCYDCARHVADFLRGPNLSLRIFTARLYFCEDRKAEP
 20 GLRRLHRAGVQIAIMTFKDYFCWNTFVENHERTFKAWEGLHENSVRSLRQLRRILLPL
 YEVDLRLDAFRTLGL (SEQ ID NO:21)

Homo sapiens: AIDv solubility variant lacking N-terminal RNA-binding region and the C-terminal poorly structured region

MDPHIFTSNFNNGIGRHKTYLCYEVERLDSATSFSLDFGYLRNKNKGCHVELLFLRYISD
 WDLDPGRCYRVTWFTSWSPCYDCARHVADFLRGPNLSLRIFTARLYFCEDRKAEP
 25 GLRRLHRAGVQIAIMTFKDYFCWNTFVENHERTFKAWEGLHENSVRSLRQLRRILLPL
 (SEQ ID NO:22)

Rattus norvegicus: APOBEC1

MSSETGPVAVDPTLRRRIEPHEFEVFFDPRELKTCCLYEINWGGRHSIWRHTSQNT
 NKHVEVNFIEKFTTERYFCPNTRCSITWFLSWSPCGECSRAITEFLSRYPHVTLFYIARL
 YHHADPRNRQGLRDLISSGVTIQIMTEQESGYCWRNFVNYSNEAHWPYPHLLWWR
 30 LYVLELYCIILGLPPCLNLRKQPKLTFTIALQSCHYQRLPPHILWATGLK (SEQ
 ID NO:23)

Mus musculus: APOBEC3

MGPFCGLGCSHRKCYSPIRNLISQETFKFHKNLGYAKGRKDTFLCYEVTRKDCDSPVSL
 HHGVFKNKDNIHAEICFLYWFHDKVLKVLSPREEFKITWYMSWSPCFECAEQIVRFLAT
 40 HHNLSDIFSSRLYNVQDPETQQNLRLVQEGAQVAAMDLYEFKKCWKKFVDNGGRR
 FRPWKRLLTNFRYQDSKLQEILRRMDPLSEEEFYQFYNQVRVHLCYYHRMKPYLCYQ
 LEQFNGQAPLKGCLLSEKQKQAEILFLDKIRSMELSQVTITCYLTWSPCPNCAWQLAA
 FKRDRPDILHIYTSRLYFHWKRPFQKGLCSLWQSGILVDVMDLPQFTDCWTNFFVNP

RPFRPWKGLEIISRRTQRRRLRIKESWGLQDLVNDFGNLQLGPPMSN (SEQ ID NO:24)

***Mus musculus*: APOBEC3 catalytic domain**

5 MGPFCFLGCSHRKCYSPIRNLISQETFKFHFKNLGYAKGRKDTFLCYEVTRKDCDSPVSL
HHGVFKNKDNIHAEICFLYWFHDKVLKVLSPREEFKITWYMSWSPCFECAEQIVRFLAT
HHNLSLDIFSSRLYNVQDPETQQNLRLVQEGAQVAAMDLYEFKKCWKKFVDNGGRR
FRPWKRLLTNFRYQDSKLQEILRR (SEQ ID NO:25)

***Homo sapiens*: APOBEC3A**

10 MEASPASGPRHLMDPHIFTSNFNNGIGRHKTYLCYEVERLDNGTSVKMDQHRGFLHN
QAKNLLCGFYGRHAELRFLDLVPSLQLDPAQIYRVTWFSWSPCFSWGCAGEVRAFLQ
ENTHVRLRIFAARIYDYDPLYKEALQMLRDAGAQSIMTYDEFKHCWDTFVDHQQCPF
QPWDGLDEHSQALSGRLRAILQNQGN (SEQ ID NO:26)

***Homo sapiens*: APOBEC3G**

15 MKPFRNTVERMYRDTFSYNFYNRPILSRRNTVWLCYEVKTGKPSRPPLDAKIFRGQV
YSELKYHPEMRFFHWFSKWRKLHRDQEYEVWYISWSPCTKCTRDMATFLAEDPKVT
LTIFVARLYYFWDPDYQEALRSLCQKRDGPRATMKIMNYDEFQHCWSKFVYSQRELFE
20 PWNNLPKYIILLHIMLGEILRHSMDDPTFTFNFNNEPWWRGRHETLYCYEVERMHNDT
WLLNQRRGFLCNQAPHKHGFLEGRHAELCFLDVIPFWKLDLDQDYRVTCFTSWSPC
FSCAQEMAKFISKNHVSLCIFTARIYDDQGRCEGLRTLAEAGAKISIMTYSEFKHCW
DTFVDHQQCPFQPWDGLDEHSQDLSGRLRAILQNQEN (SEQ ID NO:27)

***Homo sapiens*: APOBEC3G catalytic domain**

25 PPTFTFNFNNEPWWRGRHETLYCYEVERMHNDTWLLNQRRGFLCNQAPHKHGFLE
GRHAELCFLDVIPFWKLDLDQDYRVTCFTSWSPCFSCAQEMAKFISKNHVSLCIFTAR
IYDDQGRCEGLRTLAEAGAKISIMTYSEFKHCWDTFVDHQQCPFQPWDGLDEHSQD
LSGRLRAILQNQEN (SEQ ID NO:28)

***Homo sapiens*: APOBEC3H**

30 MALLTAETFRLQFNNKRRLRPPYYPRKALLCYQLTPQNGSTPTRGYFENKKKCHAEICF
INEIKSMGLDETQCYQVTCYLTWSPCSCAWELVDFIKAHDHLNLGIFASRLYYHWCKP
QQKGLRLLCGSQVPVEVMGFPAFCDCWENFVDHEKPLSFNPYKMLEELDKNRAIKR
35 RLRIKIPGVRAQGRYMDILCDAEV (SEQ ID NO:29)

***Homo sapiens*: APOBEC3F**

40 MKPFRNTVERMYRDTFSYNFYNRPILSRRNTVWLCYEVKTGKPSRPRLDAKIFRGQV
YSQPEHHAEMCFLSWFCGNQLPAYKCFQITWVSWTPCPDCVAKLAEFLAHPNVTL
TISAARLYYYWERDYRRALCRLSQAGARVKIMDDEEFAYCWENFVYSEGQPFMPWYK
FDDNYAFLHRTLKEILRNPMMEAMYPHIFYHFKNLRKAYGRNESWLCFTMEVVKHHSP
VSWKRGVFRNQVDPETHCHAERCFLSWFCDDILSPNTNYEVTWYTSWSPCECAGE
VAEFLARHSNVNLTIFTARLYYFWDTDYQGLRSLSQEGASVEIMGYKDFKYCWENFV
YNDDEPFKPWKGLKYNFLFLDSKLQEILE (SEQ ID NO:30)

Homo sapiens: APOBEC3F catalytic domain

KEILRNPMEAMYPHIFYFHFKNLRKAYGRNESWLCFTMEVVKHHSPVSWKRGVFRNQ
VDPETHCHAERCFLSWFCDDILSPNTNYEVTWYTSWSPCECAGEVAEFLARHSNVN
5 LTIFTARLYYFWDTDYQEGRLSLSQEGASVEIMGYKDFKYCWENFVYNDDEPFKPKWKG
LKYNFLFLDSKLQEILE (SEQ ID NO:31)

Escherichia coli: TadA

MKRTADGSEFESPKKKRKVSEVEFSHEYWMRHALTLAKRAWDEREVPVGAVLVHNN
10 RVIGEGWNRPIGRHDPTAHAEIMALRQGGLVMQNYRLIDATLYVTLEPCVMCAGAMIH
SRIGRVVFGARDAKTGAAGSLMDVLHHPGMNHRVEITEGILADECAALLSDFFRMRQ
EIKAQKKAQSSTDSSGSSGGSSGSGSETPGTSESATPESSGGSSGSSSEVEFSHEYWM
RHALTLAKRARDEREVPVGAVLVLNNRVIGEGWNRAGLHDPTAHAEIMALRQGGLVM
QNYRLIDATLYVTFEPCVMCAGAMIH SRIGRVVFGVRNAKTGAAGSLMDVLHYPGMNH
15 RVEITEGILADECAALLCYFFRMPRQVFNAQKKAQSSTD (SEQ ID NO:32)

Homo sapiens: Adar1

MNPRQGYSLSGYYTHPFQGYEHRQLRYQQPGPGSSPSSFLKQIEFLKGQLPEAPVIG
KQTPSLPPSLPGLRPRFPVLLASSTRGRQVDIRGVPRGVHLGSQGLQRGFQHPSPRG
20 RSLPQRGVDCLSSHQELSIYQDQEQRILKFLEELGEGKATTAHDLGKLGTPKKEINR
VLYSLAKKGKLQKEAGTPPLWKIAVSTQAWNQHSGVVRPDGHSQGAPNSDPSLEPED
RNSTSVSEDLEPFIAVSAQAWNQHSGVVRPDSSHSQGSPNSDPGLEPEDSNSTSALE
DPLEFLDMAEIKEKICDYLNFVSDSSALNLAKNIGLTKARDINAVLIDMERQGDVYRQGT
TPPIWHLTDKKRERMQIKRNTNSVPETAPAAIPETKRNAEFLTCNIPTSNASNNMVTTEK
25 VENGQEPVIKLENRQEARPEPARLKPPVHYNGPSKAGYVDFENGQWATDDIPDDLNSI
RAAPGEFRAIMEMPSFYSHGLPRCSPYKKLTECQLKNPISGLLEYAQFASQTCEFNMI
QSGPPHEPRFKFQVVINGREFPPAEAGSKKQDAAMKAMTILLEEAKAKDSGKSEE
SSHYTEKESEKTAESQTPTPSATSFSGKSPVTTLLECMHKLGNSCFRLLSKEGPAH
EPKFQYCVAVGAQTFPSVSAPSKKQAKMAAEEAMKALHGEATNSMASDNQPEGMIS
30 ESLDNLESMMPNKVRKIGELVRYLNTNPVGGLLEYARSHGFAAEFKLVDQSGPPHEPK
FVYQAKVGGRWFPVAVCAHKKQKQEAADAALRVLIGENEAERMGFTEVTPVTGAS
LRRTMLLLSRSPAQPKTLPLTGSTFHDQIAMLSHRCFNTLTNSFQPSLLGRKILAAIIMK
KDESDMGVVVSLGTGNRCVKGDSLSLKGETVNDCHAEIISRRGFIRFLYSELMKYNSQT
AKDSIFEPAGGGEKLQIKKTVSFHLYISTAPCGDGFALDKSCSDRAMESTESRHYPVFE
35 NPKQKGLRTKVENEGETIPVESSDIVPTWDGIRLGERLRTMSCSDKILRWNVLGLQGAL
LTHFLQPIYLKSVTLGYLFSQGHLTRAICCRVTRDGSFEDGLRHPFIVNHPKVGRVSIY
DSKRQSGKTKETSVNWCLADGYDLEILDGTRGTVDGPRNELSRVSKKNIFLLFKKLCSF
RYRRDLLRLSYGEAKKAARDYETAKNYFKKGLKDMGYGNWISKPQEEKNFYLCVP
(SEQ ID NO:33)

40

Streptococcus pyogenes: spCas9 Bipartite NLS

MDKKYSIGLDIGTNSVGWAVITDEYKVPSSKKFKVLGNTDRHSIKKNLIGALLFDS
GETAEATRLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFHRLSEESFLVEE
DKKHERHPIFGNIVDEVAYHEKYPTIYHLRKKLVDSTDKADLRILIYLAHAMIKFR
45 GHFLIEGDLNPDNSDVKLFIQLVQTYNQLFEENPINASGVDAKAILSARLSKSR

RLENLIAQLPGEKKNGLFGNLIASLGLTPNFKSNFDLAEDAKLQLSKDITYDDDL
 DNLLAQIGDQYADFLAAKNLSDAILLSDILRVNTEITKAPLSASMIKRYDEHHQD
 LTLLKALVRQQLPKEYKEIFFDQSKNGYAGYIDGGASQEEFYKFIKPILEKMDGT
 EELLVKLNREDLLRKQRTFDNGSIPHQIHLGELHAILRRQEDFYFPLKDNREKIE
 5 KILTRIPYYVGPLARGNSRFAWMTRKSEETITPWNFEVVDKGASAQSFIERM
 TNFDKNLPNEKVLPKHSLLYEYFTVYNELTKVKYVTEGMRKPAFLSGEQKKAIV
 DLLFKTNRKVTVKQLKEDYFKKIECFDSVEISGVEDRFNASLGTYHDLLKIIKDKD
 FLDNEENEDILEDIVLTLTLFEDREMIEERLKYAHLFDDKVMKQLKRRRYTGWG
 RLSRKLINGIRDKQSGKTILDFLKSDGFANRNFMLIHDDSLTFKEDIQKAQVSG
 10 QGDSLHEHIANLAGSPAIIKGLQTVKVDELVKVMGRHKPENIVIAMARENQTT
 QKGQKNSRERMKRIIEGKELGSQILKEHPVENTQLQNEKLYLYYLQNGRDMY
 VDQELDINRLSDYDVHIVPQSFLKDDSIDNKVLTRSDKNRGKSDNVPSEEVVK
 KMKNYWRQLLNAKLITQRKFDNLTKAERGGLSELDKAGFIKRQLVETRQITKHV
 AQILDSRMNTKYDENDKLIREVKVITLKSCLVSDFRKDFQFYKVREINNYHHAHD
 15 AYLNNAVGTALIKKYPKLESEFVYGDYKVYDVRKMIKSEQEIGKATAKYFFYSN
 IMNFFKTEITLANGEIRKRPLIETNGETGEIVWDKGRDFATVRKVLSPQVNVKK
 TEVQTGGFSKESILPKRNSDKLIARKKDWDPKKYGGFDSPTVAYSVLVAKVEK
 GKSKKLKSVKELLGITIMERSSFEKNPIDFLEAKGYKEVKKDLIKLPKYSLELEN
 GRKRMLASAGELQKGNELALPSKYVNFLLASHYEKLGSPEDNEQKQLFVEQ
 20 HKHYLDEIEIQISEFSKRVLADANLDKVL SAYNKHDKPIREQAENIIHLFTLNL
 GAPAAFKYFDTTIDRKRYTSTKEVLDTLIHQSTGLYETRIDLSQLGGDGGSGGG
 GSGKRTADGSEFEPKKRKVSSGGDYKDHDGDYKDHDIDYKDDDDK (SEQ ID
 NO:34)

25 ***Staphylococcus aureus: saCas9***

MKRNYILGLDIGITSVGYGIIDYETRDVIDAGVRLFKEANVENNEGRRSKRGARR
 LKRRRRRHRIQRVKLLFDYNLLTDHSELGINPYEARVKGLSQKLSEEFSAALL
 HLAKRGRGVHNVNEVEEDTGNELSTKEQISRNKALEEKYVAELQLERLKKDGE
 VRGSINRFKTSYVKEAKQLLKVKQAYHQLDQSFIDTYIDLLETRRTYYEGPGE
 30 GSPFGWKDIKEWYEMLMGHCTYFPEELRSVKYAYNADLYNALNDLNNLVITRD
 ENEKLEYEYKFQIIENVFKQKKKPTLKQIAKEILVNEEDIKGYRVTSTGKPEFTNL
 KVVYHDIKDITARKEIENAELLDQIAKILTIYQSSEDIQEELTNLNSLTQEEIEQISN
 LKGYTGTHNLSLKAINLILDELWHTNDNQIAIFNRLKLVPPKVDLSQQKEIPTTLV
 DDFILSPVVKRSFIQSIKVINAIKKYGLPNDIIELAREKNSKDAQKMINEMQKRN
 35 QTNERIEEIIIRTTGKENAKYLIEKIKLHDMQEGKCLYSLEAIPLEDLLNPNFNYEVD
 HIIPRSVSFDNSFNKVLVKQEENSCKGNRTPFQYLSSSDSKISYETFKKHILNL
 AKGKGRISKTKKEYLLEERDINRFSVQKDFINRNLVDTRYATRGLMNLRSYFRV
 NNLDVKVKSINGGFTSFLRRKWFKKERNKGYKHAEDALIINANADFIFKEWKKL
 DKAKKVMENQMFEKQAESMPEIETEYKEIFITPHQIKHIKDFKDYKYSHRVD
 40 KKPNNRELINDTLYSTRKDDKGNTLIVNNLNGLYDKDNDKLLKLINKSPEKLLMYH

HDPQTYQKLKLIMEQYGDEKNPLYKYYEETGNYLTKYSKKDNGPVIKKIKYYGN
KLNAHLDDITDDYPNSRNKVVKLSLKPYRFDVYLDNGVYKFVTVKNLDVIKENYY
EVNSKCYEEAKLKKISNQAEFIASFYNNDLIKINGELYRVIGVNNDLLNRIEVNMI
DITYREYLENMNDKRPPRIIKTIASKTQSIKKYSTDILGNLYEVKSKKHPPQIIKKG
5 (SEQ ID NO:35)

Campylobacter jejuni: cjCas9

MARILAFDIGISSIGWAFSENDELKDCGVRIFTKVENPKTGESLALPRRLARSAR
KRLARRKARLNHLKHLIANEFKLNIEDYQSFDESLAKAYKGLISPYELRFRALN
10 ELLSKQDFARVILHIAKRRGYDDIKNSDDKEKGAILKAIKQNEEKLANYSVGEY
LYKEYFQFKENSKEFTNVRNKKESYERCIASFLKDELKLIFKKQREFGFSFSK
KFEVEVLVSFAFYKRALKDFSHLVGNCSFFTDEKRAPKNSPLAFMFVALTRIINLL
NNLKNTGILYTKDDLNALLNEVLKNGTLTYKQTKLLGLSDDYEFKGEKGTYFI
EFKKYKEFIKALGEHNLSQDDLNEIAKDITLIKDEIKLKKALAKYDLNQNQIDSLSK
15 LEFKDHLNISFKALKLVTPMLLEGKKYDEACNELNLKVAINEDKKDFLPAFNETY
YKDEVTPNPVLRRAIKEYRKVLNALLKKYGVHVKINIELAREVGKNHNSQRAKIEKE
QNENYKAKKDAELECEKLGLKINSKNILKLRLFKEQKEFCAYSGEKIKISDLQDE
KMLEIDHIYPYSRSDSYMNKVLVFTKQNKQEKLNQTPFEAFGNDSAKWQKIEV
LAKNLPTKKQKRILDKNYKDKEQKNFKDRNLNDTRYIARLVNLYTKDYLDLPLS
20 DDENTKLNLTQKGSKVHVEAKSGMLTSALRHTWGFSAKDRNNHLHHAIDAVIIA
YANNSIVKAFSDFKKEQESNSAELYAKKISELDYKNKRKFFEPFSGFRQKVLDKI
DEIFVSKPERKKPSGALHEETFRKEEEFYQSYGGKEGVLKALELGKIRKVNKIV
KNGDMFRVDIFKHKKTNKFYAVPIYTMDFAKVLPNKAVARSKKGEIKDWILMD
ENYEFCSFLYKDSLILIQTKDMQEPEFVYNAFTSSTVSLIVSKHDNKFETLSKN
25 QKILFKNANEKEVIAKSIGIQNLKVFEKYIVSALGEVTKAEFRQREDFKK (SEQ
ID NO:36)

Neisseria meningitidis: nmeCas9

MAAFKPNSINYILGLDIGIASVGWAMVEIDEEENPIRLIDLGVRFERAEVPKTGD
30 SLAMARRLARSVRRLTRRRRAHRLLRTRRLKREGVLQAANFDENGLIKSLPNT
WQLRAAALDRKLTPLWSAVLLHLIKHRGYLSQRKNEGETADKELGALLKGVA
GNAHALQTGDFRTPAELALNKFESGHIRNQSRDYSHTFSRKDLQAEILLFE
KQKEFGNPHVSGGLKEGIETLLMTQRPALSGDAVQKMLGHCTFEPAEPKAAKN
TYTAERFIWLTCLNNLRILEQQSERPLTDTERATLMDEPYRKSCLTYAQARKLLG
35 LEDTAFKGLRYGKDNAEASTLMEMKAYHAISRALEKEGLKDKKSPLNLSPELQ
DEIGTAFSLFKTDEDITGRLKDRIQPEILEALLKHISFDKFVQISLKALRRIVPLMEQ
GKRYDEACAEIYGDHYGKKNTTEKIYLPPIPADEIRNPVLRALSQARKVINGVV
RRYGSPARIHIETAREVGKSFKDRKEIEKRQEENRKDREKAAAKFREYFPNFVG
EPKSKDILKLRLYEQQHGKCLYSGKEINLGRLENEGYVEIDHALPFSRTWDDSF
40 NNVVLVLSSENQNKGNQTPYEYFNGKDNSREWQEFKARVETSRFPRSKKQRI
LLQKFDEDEGFKERNLNDTRYVNRFLCQFVADRMRLTGKGKKRVFASNGQITNL
LRGFWGLRKVRAENDRHHAIDAVVACSTVAMQQKITRFVRYKEMNAFDGKTI

DKETGEVLHQKTHFPQPWEFFAQEVMIRVFGKPDGKPEFEEADTLEKLRTLLA
EKLSSRPEAVHEYVTPLFVSRAPNRKMSGQGHMETVKSARLDEGVSVLRVPL
TQLKLDLEKMNREPERKLYEALKARLEAHKDDPAKAFAPFYKYDKAGNRT
QQVKAVRVEQVQKTGVWVRNHNGIADNATMVRVDVFEGDKYYLVPIYSWQV
5 AKGILPDRAVVQGKDEEDWQLIDDSFNFKFSLHPNDLVEVITKKARMFYGFASC
HRGTGNINIRIHDLDHKIGKNGILEGIGVKTALSFQKYQIDELGKEIRPCRLKKRP
PVR (SEQ ID NO:37)

Acidaminococcus sp.: asCas12a

10 MTQFEGFTNLYQVSKTLRFELIPQGKTLKHIQEQGFIEEDKARNNDHYKELKPIID
RIYKTYADQCLQLVQLDWNLSAAIDSYRKEKTEETRNALIEEQATYRNAIHDF
IGRTDNLTDANKRHAIEYKGLFKAELFNGKVLKQLGTVTTEHENALLRSFDKF
TTYFSGFYENRKNVFSIEDISTAIPIHRIVQDNFPKFENCHIFTRLITAVPSLREH
FENVKKAIGIFVSTSIIEVFSFPFYNQLLTQTQIDLYNQLLGGISREAGTEKIKGLN
15 EVLNLAIQKNDETAHIIASLPHRFIPLFKQLSDRNTLSFILEEFKSDEEVIQSFCKY
KTLLRNENVLETAELFNEINSIDLTHIFISHKKLETISSALCDHWDTLRNALYER
RISELTGKITKSAKEKVQVRSKLEDINLQEIISAAGKELSEAFKQKTSEILSHAHAA
LDQPLPTTLKKQEEKEILKSQDLSLLGLYHLLDWFVAVDESNEVDPEFSARLTGIK
LEMEPSLSFYNKARNYATKKPYSVEKFKNLNFQMPTLASGWDVNKEKNNGAILF
20 VKNGLYYLGIMPKQKGRYKALSFEPTKTEKSEGFDKMYDYDFPDAAKMIPKCS
QLKAVTAHFQTHHTPILLSNNFIEPLEITKEIYDLNNEKEPKKFQTAYAKKTDGQ
KGYREALCKWIDFTRDFLSKYTKTTSIDLSSLRPSSQYKDLGEYYAELNPLLYHI
SFQRIAEKEIMDAVETGKLYLFQIYNKDFAKGHHGKPNLHTLYWTGLFSPENLA
KTSIKLNGQAELFYRPKSRMKRMAHRLGEKMLNKKLKDQKTPIDTLYQELYDY
25 VNHRLSHDLSDEARALLPNVITKEVSHEIKDRRFTSDKFFFHVPITLNYQAANSP
SKFNQVRVNAVYLKEHPETPIIGIDRGERNLIYITVIDSTGKILEQRSLNTIQQFDYQK
KLDNREKERVAAARQAWSVVGTIKDLKQGYLSQVIHEIVDLMIHYQAVVLENLN
FGFKSKRTGIAEKAVYQQFEKMLIDKLNCLVLKDYPAEKVGGVLNPNYQLTDQFT
SFAKMGTSQSGFLFYVPAPYTSKIDPLTGFDVPFVWKTIKNHESRKHFLEGDFDL
30 HYDVKTGDFILHFKMNRNLSFQRGLPGFMPAWDIVFEKNETQFDAQGTPFIAGK
RIVPVIENTHRFTGRYRDLYPANELIALLEEKGIVFRDGSNILPKLLENDSDSHAIT
MVALIRSVLQMRNSNAATGEDYINSPVRDLNGVCFDSRFQNPWPMDADANG
AYHIALKGQLLLNLHKLKESKDLKLQNGISNQDWLAYIQELRN (SEQ ID NO:38)

Lachnospiraceae bacterium: IbCas12a:

35 MSKLEKFTNCYLSKTLRFKAIPVGKTQENIDNKRLLVEDEKRAEDYKGVKKLLD
RYYLSFINDVLHSIKLKNLNNYISLFRKKTRTEKENKELENLEINLRKEIAKAFKGN
EGYKSLFKKDIIETILPEFLDDKDEIALVNSFNGFTTAFTGFFDNRENMFSEEAKS
TSIAFRICINENLTRYISNMDIFEKVDAIFDKHEVQEIKEILNSDYDVEDFFEGEFF
40 NFVLTQEGIDVYNAIIGGFVTESGEKIKGLNEYINLYNQKTKQKLPKFKPLYKQVL
SDRESLSFYGEGYTSDEEVLEVFRNTLNKNSEIFSSIKKLEKLFKNFDEYSSAGI
FVKNQPAISTISKDIFGEWNVIRDKWNAEYDDIHLKKKAVVTEKYEDDRRSFKK
IGSFSLEQLQEYADADLSVVEKLKEIIQKVDEIYKVYGSSEKLFDAFVLEKSLKK
NDAVVAIMKDLLDSVKSFENYIAFFGEGKETNRDESFYGDFVLAYDILLKVDHI
45 YDAIRNYVTQKPYSKDKFKLYFQNPQFMGGWDKDKETDYRATILRYGSKYYLAI
MDKKYAKCLQKIDKDDVNGNYEKINYKLLPGPNKMLPKVFFSKKWMAYYNPSE

DIQKIYKNGTFKKGDMFNLNDCHKLIDFFKDSISRYPKWSNAYDFNFSETEKYK
 DIAGFYREVVEEQGYKVSFESASKKEVDKLVVEEGKLYMFQIYNKDFSDKSHGTP
 NLHTMYFKLLFDENNHGQIRLSGGAELFMRRASLKKEELVVHPANSPIANKNPD
 NPKKTTTTLSYDVYKDKRFSEDQYELHIPIAINKCPKNIFKINTEVRVLLKHDDNPY
 5 VIGIDRGERNLLYIVVVDGKGNIQYSLNEIINNFGIRIKTDYHSLLDKKEKERF
 EARQNWTSIENIKELKAGYISQVVKICELVEKYDAVIALEDLNSGFKNRSRVKVE
 KQVYQKFEKMLIDKLNVMVDKKSNPCATGGALKGYQITNKFESFKSMSTQNGFI
 FYIPAWLTSKIDPSTGFVNLLKTKYTSIADSKKFISSFDRIMYVPEEDLFEFALDYK
 NFSRTDADYIKKWKLYSYGNRIRIFRNPKKNNVFDWEEVCLTSAYKELFNKYGI
 10 NYQQGDIRALLCEQSDKAFYSSFMALMSLMLQMRNSITGRDTDVDFLISPVKNSD
 GIFYDSRNYEAQENAILPKNADANGAYNIARKVLWAIGQFKKAEDEKLDKVKIAI
 SNKEWLEYAQTSVKH (SEQ ID NO:39)

***Leptotrichia shahii*: LshCas13a**

MGNLFGHKRWYEVDRDKDFIKRKVKVKNRYDGNKYILNINENNNKEKIDNNKFIRKYIN
 YKKNDNILKEFTRKFHAGNIFKLKGKEGIIRIENNDDFLETEEVVLYIEAYGKSEKLKALG
 ITKKKIIDEAIRQGITKDDKKIEIKRQENEEIEIDIRDEYTNKTLNDCSIIIRIENDELETKKS
 IYEIFKNINMSLYKIEKIIENETEKVFENRYEEHLREKLLKDDKIDVILTNFMEIREKIKSNL
 EILGFVKFYLNVGDKKSKNKKMLVEKILNINVDLTVEDIADFVIKELEFWNITKRIEKVK
 20 KVNNEFLEKRRNRITYIKSYVLLDKHEKFKIERENKKDKIVKFFVENIKNNSIKEKIEKILAE
 FKIDELIKKLEKELKKGNCDTEIFGIFKKHYKVNFDSSKKFSKKSDEEKELYKIIYRYLKGRI
 EKILVNEQKVRLKKMEKIEIEKILNESILSEKILKRVKQYTLHEIMYLGKLRHNDIDMTTVNT
 DDFSRLHAKEELDLELITFFASTNMELNKIFSRENINNDENIDFFGGDREKNYVLDKKILN
 SKIKIIRDLDFIDNKNITNNFIRKFTKIGTNERNRILHAISKERDLQGTQDDYNKVINIIQNL
 25 KISDEEVSKALNLDVVFCDKKNITKINDIKISEENNNDIKYLPFSKVLPEILNLYRNNPKN
 EPFDTIETEKIVLNALIYVNKELYKKLILEDDEENESKNIFLQELKKT LGNIDEIDENIENY
 YKNAQISASKGNNKAIKKYQKKVIECYIGYLRKNYEELDFDSDFKMNIQEIKKQIKDINDN
 KTYERITVKTSDKTIVINDDFEYIISIFALLNSNAVINKIRNRFFATSVWLNTSEYQNIIDILD
 EIMQLNTRLNECITENWNLNLEEFIQKMKIEIEKDFDDFKIQTKKEIFNNYYEDIKNNILTEF
 30 KDDINGCDVLEKKLEKIVIFDDETKFEIDKKSNIHQDEQRKLSNINKKDLKKKVDQYIKDK
 DQEIKSKILCRIIFNSDFLKKYKKEIDNLIEDMESEENENKFQEIYYPKERKNELYIYKKNLFL
 NIGNPNFDKIYGLISNDIKMADAKFLFNIDGKNIRKNKISEIDAILKNLNDKLNKYSKEYKE
 KYIKKLKENDDDFAKNIQNKNYKSFEKDYNRVSEYKKIRDLVEFNLYLNKIESYLIDINWKL
 AIQMARFERDMHYIVNGLRELGIKLSGYNTGISRAYPKRNGSDGFYTTTAYYKFFDEES
 35 YKKFEKICYGFGIDLSENSEINKPENESIRNYISHFYIVRNPFADYSIAEQIDRVSNLLSYS
 TRYNNSTYASVFEVFKDVLNDYDELKKKFKLIGNNDILERLMKPKKVSVLELESYNSDY
 IKNLIELLT KIENTNDTL (SEQ ID NO:40)

***Leptotrichia wadeii*: LwaCas13a**

MKVTKVDGISHKKYIEEGKLVKSTSEENRTSERLSELLSIRLDIYIKNPDNASEEENRIRR
 ENLKKFFSNKVLHLKDSVLYLKNRKEKNAVQDKNYSEEDISEYDLKNKNSFSVLKILLN
 EDVNSEEEIFRKDVEAKLNKINSKYSEENKANYQKINENNVEKVGGKSKRNIIYDYY
 RESAKRNDYINNVEAFDKLYKKEDIEKLFFLIENSKKHEKYKIREYYHKIIGRKNDKENF
 AKIYEEIQNVNNIKELIEKIPDMSELKKSQVFYKYLDKEELNDKNIKYAFCHFVEIEMSQ
 45 LLKNYVYKRLSNISNDKIRIFEYQNLKKLIENKLLNKLDYVRNCGKYNYYLQVGEIATS
 DFIARNRQNEAFLRNIIGVSSVAYFSLRNILETENENGITGRMRGKTVKNNKGEEKYVSG
 EVDKIYNENKQNEVKENLKMFSYDFNMDNKNIEIDFFANIDEAIISSIAHGIVHFNLELE
 GKDIFAFKNIAPSEISKMFQNEINEKKLKLKIFKQLNSANVFNYEYKDVIIKYLKNTKFNF

VNKNIPFVPSFTKLYNKIEDLRNTLKFFWSVPKDKEEKDAQIYLLKNIYYGEFLNKFVKNS
KVFFKITNEVIKINKQRNQKTGHYKYQKFENIEKTVPEYLAIQSREMINNQDKEEKNTYI
DFIQQIFLKGFDYLNKNLKYIESNNNNNDNNDIFS KIKIKKDNKEKYDKILKNYEKHNRNK
EIPHEINEFVREIKLGKILKYTENLNMFYLLKLNHKELTNLKGSLEKYQSANKEETFSD
5 LELINLLNLDNNRVTEDFEANEIGKFLDFNENKIKDRKELKKFDTNKIYFDGENIIKHRA
FYNIKKYGMLNLEKIADKAKYKISLKEKEYSNKKNEIEKNYTMQQNLHRKYARPKKDE
KFNDEYKEYEKAIGNIQKYTHLKNKVEFNELNLLQGILLKILHRLVGYTSIWERDLRFR
KGEFPENHYIEEIFNFDNSKNVYKSGQIVEKYINFYKELYKDNVEKRSISDKKVKLLK
QEKKDLYIANIAHFNYIPHAESLLEVLENLRKLLSYDRKLKNAIMKSIVDILKEYGFVATF
10 KIGADKKIEIQTLESEKIVHLKNLKKKKLMTDRNSEELCELVKVMFEYKALE (SEQ
ID NO:41)

Pleckstrin homology domain of *Homo sapiens* phospholipase Cδ1 (hPLCδ1)
MDSGRDFLTLHGLQDDEDLQALLKGSQLLKVKSSSWRRERFYKLQEDCKTIW
15 QESRKVMRTPESQLFSIEDIQEVRMGHRTEGLEKFARDVPEDRCFSIVFKDQR
NTLDLIAPSPADAQHWVLGLHKIIHSGSMDQRQKLQHWIHSCLRKADKNKDN
KMSFKELQNFLKELNIQ (SEQ ID NO:42)

Pleckstrin homology domain of *Homo sapiens* Akt1 (hAkt)
20 MSDVAIVKEGWLHHRGEYIKTWRPRYFLLKNDGTFIGYKERPQDQVDQREAPLN
NFSVAQCQLMKTERPRPNTFIIRCLQWTTVIERTFHVETPEEREETWTTAIQTV
DGLKKQEEEEEMDFRSGSPSDNSGAEEMEVS LAKPKHRVTMNEFEYLKLLGKG
TFGKVDPPV (SEQ ID NO:43)

Pleckstrin homology domain of *Homo sapiens* PDPK1 (hPDPK1)
25 KMGPVDKRKGLFARRRQLLLTEGPHLYYVDPVNVKVLKGEIPWSQELRPEAKNF
KTFFVHTPNRTYYLMDPSGNAHKWCRKIQEVWRQRYQSH (SEQ ID
NO:44)

Herpes simplex virus (HSV) type 1: VP16 Transcription Activation Domain
30 PTDALDDFDLDMPLADALDDFDLDMPLADALDDFDLDM (SEQ ID NO:45)

Herpes simplex virus (HSV) type 1 & Synthetic: VP64
35 GRADALDDFDLDMLGSDALDDFDLDMLGSDALDDFDLDMLGSDALDDFDLDM
L (SEQ ID NO:46)

***Homo sapiens*: P65**
SQYLPDTDDRHRIEEKRKRTYETFKSIMKKSPFSGPTDPRPPPRRIAVPSRSSA
40 SVPKPAPQYPFTSSLSTINYDEFPTMVFPSPGQISQASALAPAPPQVLPQAPAP
APAPAMVSALAQAPAPVPVLAPGPPQAVAPPAPKPTQAGEGTLSEALLQLQFD
DEDLGALLGNSTDPVFTDLASVDNSEFQQLLNQGIPVAPHTTEPMLMEYPEAI
TRLVTGAQRPPDPAPAPLGAPGLPNGLLSGDEDFSSIADMDFSALL (SEQ
ID NO:47)

Kaposi's Sarcoma-Associated Herpesvirus Transactivator: RTA
45 RDSREGMFLPKPEAGSAISDVFE GREVCQPKRIRPFHPPGSPWANRPLPASLA
PTPTGPVHEPVGSLTPAPVPQPLDPAPAVTPEASHLLEDPEETSQAVKALRE

MADTVIPQKEEAAICGQMDLSHPPPRGHLDELTTTLESMTEDLNLDSPLTPELN
EILDFTLNDECLLHAMHISTGLSIFDTSLF (SEQ ID NO:48)

Homo sapiens: KRAB

5 MDAKSLTAWSRTLVTFKDVFDFTREEWKLLDTAQQIVYRNVMLENYKNLVSL
GYQLTKPDVILRLEKGEEP (SEQ ID NO:49)

Homo sapiens: MeCP2

10 EASVQVKRVLEKSPGKLLVKMPFQASPGGKGEGGGATTSAQVMVIKRPGRKR
KAEADPQAIPKKRGRKPGSVVAAAAEAKKKAVKESSIRSVQETVLPKKRKR
ETVSIEVKEVVKPLLSTLGEKSGKGLTKCKSPGRKSKESSPKGRSSSASSPPK
KEHHHHHHHAESPKAPMPLPPPPPEPQSSSEDPIPPPEPQDLSSSICKEEKM
PRAGSLESDGCPKEPAKTQPMVAAAATTTTTTTTTTVAEKYKHRGEGERKDVS
SSMPRPNREEPVDSRTPVTERVS (SEQ ID NO:50)

Homo sapiens: Tet1

15 LPTCSCLDRIQKDKGPYYTHLGAGPSVAAVREIMENRYGQKGNIRIEIVVYT
KEGKSSHGCPKIAKWLLRRSSDEEKVLCLVRQRTGHHCPATAVMVVLIMVWDGIP
LPMADRLYTELTKSYNGHPTDRRCTLNENRTCTCQGIDPETCGASFSGC
20 SWSMYFNGCKFGRSPSPRRFRIDPSSPLHEKNLEDNLQSLATRLAPIYKQYAP
VAYQNQVEYENVARECRLGSKEGRPFSGVTACLDCAHPHRDIHNMNNGSTV
VCTLTREDNRSLGVIPQDEQLHVLPLYKLSDTDEFGSKEGMEAKIKSGAIEVLAP
RRKKRTCTQPVPRSGKKRAAMMTEVLAKHRAVEKKPIPRIKRKNNSTTTNNS
KPSSLPTLGSNTETVQPEVKSETEPHFILKSSDNTKTYSLMPSAPHPVKEASPG
25 FSWSPKTASATPAPLKNDATASCGFSERSSTPHCTMPSGRLSGANAAAADGP
GISQLGEVAPLPTLSAPVMEPLINSEPSTGVTEPLTPHQPNHQPSFLTSPQDLA
SSPMEEDEQHSEADEPPSDEPLSDDPLSPAEEKLPHIDEYWSDSEHIFLDANIG
GVAIAPAHGSLVIECARRELHATTPVEHPNRNHPTRLSLVFYQHKNLNKPQHGF
ELNLIKFEAKEAKNKKMKASEQKDQAANEGPEQSSEVNELNQIPSHKALTTHD
30 NVVTVSPYALTHVAGPYNHWW (SEQ ID NO:51)

Homo sapiens: Dnmt3a

MPAMPSSGPGDTSSSAAEREEDRKDGEEQEEPRGKEERQEPSTTARKVGRP
GRKRKHPPVESGDTPKDPAVISKSPSMAQDSGASELLPNGDLEKRSEPQPEE
35 GSPAGGQKGGAPAEEGEGAAETLPEASRAVENGCTPKEGRGAPAEAGKEQK
ETNIESMKMEGSRGRLRGGLGWESSLRQRPMPRLTFQAGDPYYISKRRDEW
LARWKREAEKKAKVIAGMNAVEENQGPGESQKVEEASPPAVQQPTDPASPTV
ATTPEPVGSDAGDKNATKAGDDEPEYEDGRGFGIGELVWGKLRGFSWWPGRI
VSWWMTGRSRAAEGTRWWMWFGDGKFSVVCVEKLMPLSSFCSAFHQATYN
40 KQPMYRKAIYEVQLVASSRAGKLPVCHDSDESATAKAVEVQNKPMIEWALGG
FQPSGPKGLEPPEEEKNPYKEVYTDMWVEPEAAAYAPPPPAKKPRKSTAEKP
KVKEIIDERTRELRVYEVQRQCRNIEDICISCGSLNVTLEHPLFVGGMCMQCNCKNC
FLECAYYQYDDDGYSYCTICCGGREVLNCGNNCCRCFCVECDLLVGPAA
QAAIKEDPWNCYMGHKGTYGLLRREDWPSRLQMFFANNHDQEFDPKVVY
45 PPVPAEKRKPIRVLSLFDGIATGLLVKDLGIQVDRYIASEVCEDSITVGMVRHQ
GKIMYVGDVRSVTQKHIQEWGPFDLVIGGSPCNDLSIVNPARKGLYEGTGRLFF

EFYRLLHDARPKEGDDRPFFWLFENVVAMGVSDKRDISRFLSNPVMIDAKEV
SAAHRARYFWGNLPGMNRPLASTVNDKLELQECLEHGRIAKFSKVRTITTRSN
SIKQKGDQHFPVFMNEKEDILWCTEMERVFGFPVHYTDVSNMSRLARQRLLGR
SWSVPVIRHLFAPLKEYFACV (SEQ ID NO:52)

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Indiana vesiculovirus, formerly Vesicular stomatitis Indiana virus G**Protein: VSVG**

MKCLLYLAFLFIGVNCKFTIVFPHNQKGNWKNVPSNYHYCPSSSDLNWHNDLIG
TALQVKMPKSHKAIQADGWMCHASKWTTTCDFRWYGPKYITHSIRSFTPSVEQ
CKESIEQTKQGTWLNPGFPPQSCGYATVTDAAVIVQVTPHHVLVDEYTGGEW
DSQFINGKCSNYICPTVHNSTTWHSDYKVKGLCDSNLISMDITFFSEDGELSSL
GKEGTGFRSNFYAYETGGKACKMQYCKHWGVRLPSGVWFEMADKDLFAAAR
FPECPEGSSISAPSQTSVDVSLIQDVERILDYSLCQETWSKIRAGLPISPVDLSYL
APKNPGTGPAFTIINGTLKYFETRYIRVDIAAPILSRMVGMISGTTTERELWDDW
APYEDVEIGPNGVLRTSSGYKFPLYMIGHGMLDSDLHLSSKAQVFEHPHIQDAA
SQLPDDESLLFFGDTGLSKNPIELVEGWFWSSWKSSIASFFFFIIGLIIGLFLVLRVGIH
LCIKLKHTKKRQIYTDIEMNRLGK (SEQ ID NO:53)

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Baculovirus envelope glycoprotein GP64

MVSAIVLYVLLAAAAHSAFAAEHCNAQMKTGPYKIKNLDITPPKETLQKDVEITIV
ETDYNENVIIGYKGYQAYAYNGGSLDPNTRVEETMKTNLVNGKEDLLMWSIRQ
QCEVGEELIDRWGSDSDDCFRDNEGRGQWVKGKELVKRQNNNHFAHHTCNK
SWRCGISTSKMYSRLECQDDTDECQVYILDAEGNPINVTVDTVLHRDGVSMILK
QKSTFTTRQIKAACLLIKDDKNNPESVTREHCLIDNDIYDLSKNTWNCKFNRCIK
RKVEHRVKKRPPTWRHNVRAKYTEGDTATKGDLMHIQEELMYENDLLKMNIEL
MHAHINKLNNMLHDLIVSAKVDERLIGNLMNNSVSSTFLSDDTFLMPCNTNP
AHTSNCYNNSIYKEGRWVANTDSSQCIDFSNYKELAIDDDEFWIPTIGNTTYH
DSWKDASGWSFIAQQKSNLITTMENKFGGVGTSLSDITSMAEGELAAKLTSF
MFGHVNVFVILVILFLYCMIRNRNRQY (SEQ ID NO:54)

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Human immunodeficiency virus gp160

MRVKEKYQHLWRWGWRWGTMLLGMLMICSATEKLWTVYYGVPVWKEATTT
LFCASDAKAYDTEVHNWVATHACVPTDPNPQEVVLNVNVTENFNMWKNDMVE
QMHEDIISLWDQSLKPCVKLTPLCVSLKCTDLKNDTNTNSSSGRMIMEKGEIKN
CSFNISTSIRGKVQKEYAFFYKLDIIPIDNDTTSYKLTSCNTSVITQACPKVSFEPI
PIHYCAPAGFAILKCNNKTFNGTGPCTNVSTVQCTHGIRPVVSTQLLNGLSLAEE
EVSIRSVNFTDNAKTIIVQLNTSVEINCTRPNNNTRKRIRIQRGPGRAFVTIGKIGN
MRQAHCNISRAKWNNTLKQIASKLREQFGNNKTIIFKQSSGGDPEIVTHSFNCG
GEFFYCNSTQLFNSTWFNSTWSTEGSNNTSGSDTITLPCRIKQIINMWQKVGKA
MYAPPISGQIRCSSNITGLLLTRDGGNSNNESEIFRPGGGDMRDNWRSELYKY
KVVKIEPLGVAPTKAKRRVVQREKRAVGIGALFLGFLGAAGSTMGAASMTLTVQ
ARQLLSGIVQQQNNLLRAIEAQQHLLQLTVWGIKQLQARILAVERYLKDQQLLGI
WGCSGKLICTTAVPWNASWSNKSLEQIWNHTTWMEWDREINNYTSLIHSLIEE
SQNQKEKNEQELLELDKWASLWNWFNITNWLWYIKLFIMIVGGLVGLRIVFAVL
SIVNRVRQGYSPLSFQTHLPTPRGPDRPEGIEEEGGERDRDRSIRLVNGSLALI
WDDLRLSLCLFSYHRLRDLILLIVTRIVELLGRRGWEALKYWWNLLQYWSQELKN

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SAVSLLNATAIAVAEGTDRVIEVVQGACRAIRHIPRRIRQGLERILL (SEQ ID NO:55)

Endogenous feline virus RD114 ENV

5 MKLPTGMVILCSLIIVRAGFDDPRKAIALVQKQHGKPCCECSGGQVSEAPPNSIQ
QVTCPGKTAYLMTNQKWKCRVTPKISPSGGELQNCPCNTFQDSMHSSCYTEY
RQCRRINKTYTATLLKIRSGSLNEVQILQNPQNLLQSPCRGSINQPVCSATA
PIHISDGGGPLDTRVWTVQKRLEQIHKAMTPELQYHPLALPKVRDDLSDART
FDILNTTFRLLQMSNFSLAQDCWLCCLKGTPTPLAIPTPSLTYSLADSLANASCQI
10 IPPLLQPMQFSNSSCLSSPFINDTEQIDLGAVTFTNCTSVANVSSPLCALNGSV
FLCGNNMAYTYLPQNWTRLCVQASLLPDIDINPGDEPVPIPAIDHYIHRPKRAVQ
FIPLLAGLGITAAFTTGATGLGVSVTQYTKLSHQLISDVQVLSGTIQDLQDQVDSL
AEVVLQNRRLDLLTAEQGGICLALQEKCCFYANKSGIVRNKIRTLQEELQKRR
ESLATNPLWTGLQGFLPYLLPLLGPLLTLILLITIGPCVFSRLMAFINDRLNVVHA
15 MVLAQQYQALKAEEEEAQD (SEQ ID NO:56)

***Homo sapiens*: CD9 Complete Protein**

MSPVKGGTKCIKYLLFGFNFIWLAGIIVLAIGLWLRFDSSQTKSIFEQETNNNNS
SFYTGVIYILIGAGALMMLVGFLGCCGAVQESQCMLGLFFGFLLVIFAIEIAAAIWG
20 YSHKDEVIKEVQEFYKDTYNKLKTKDEPQRETLKAIHYALNCCGLAGGVEQFIS
DICPKKDVLETFTVKSCPDAIKEVFDNKFHIIIGAVGIGIAVVMIFGMIFSMILCCAIR
RNREMV (SEQ ID NO:57)

***Homo sapiens*: CD63 Complete Protein**

25 MAVEGGMKCVKFLLYVLLAFACAVGLIAGVGVAQLVLSQTIIQGATPGSLLPV
VIAVGVFLFLVAFVGCCGACKENYCLMITFAIFLSLIMLVEVAAAAGYVFRDKVM
SEFNNNFRQQMENYPKNNHTASILDRMQADFKCCGAANYTDWEKIPSMKSNR
VPDSCCINVTVGCGINFNEKAIHKEGCVKEIGGWLRKNVLVAAAALGIAFVEVL
GIVFACCLVKSIRSGYEV (SEQ ID NO:58)

***Homo sapiens*: CD81 Complete Protein**

30 MGVEGCTKCIKYLLFVFNFVFWLAGGVILGVALWLRHDPQTTNLLYLELGDKPA
PNTFYVGIYILIAVGAVMMFVGFLGCGYGAIQESQCLLGTFFTCLVILFACEVAAGI
WGFVNKDQIAKDVKQFYDQALQQA VVDDANNAKAVVKTFFHETLDCCGSSTL
35 TALTTSVLKNNLCPSGSNIISNLFKEDCHQKIDDLFSGKLYLIGIAAIVVAVIMIFEM
ILSMVLCCGIRNSSVY (SEQ ID NO:59)

***Homo sapiens*: CD47 “Self Hairpin” 10 Amino Acids**

40 EVTELTREGE (SEQ ID NO:60)

***Homo sapiens*: CD47 “Self Hairpin” 21 Amino Acids**

GNYTCEVTELTREGETIIEELK (SEQ ID NO:61)

***Homo sapiens*: CD47 Complete Protein**

45 MWPLVAALLLGSACCGSAQLLFNKTKSVEFTFCNDTVVIPCFVTNMEAQNTTEV
YVKWKFKGRDIYTFD GALNKSTVPTDFSSAKIEVSQLLKGDASLKMDKSDAVSH

TGNYTCEVTELTREGETIIEELKYRVVSWFSPNENILIVIFPIFAILLFWGQFGIKTLK
YRSGGMDEKTIALLVAGLVITVIVIVGAILFVPGEYSLKNATGLGLIVTSTGILILLH
YYVFSTAIGLTSFVIAILVIQVIAYILAVVGLSLCIAACIPMHGPLLISGLSILALAQLL
GLVYMKFVE (SEQ ID NO:62)

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AAV2: REP52

MELVGWLVDKGITSEKQWQEDQASYISFNAASNSRSQIKAALDNAGKIMSLTK
TAPDYLVGQQPVEDISSNRIYKILELNGYDPQYAASVFLGWATKKFGKRNTIWL
FGPATTGKTNIAEIAHTVPFYGCVNWTNENFPFNDKVDKMVIWWEEGKMTAK
VVESAKAILGGSKVRVDQKCKSSAQIDPTPVIVTSNTNMCAVIDGNSTTFEHQQ
PLQDRMFKFELTRRLDHDGKVTQKQEVKDFFRWAKDHVVEVEHEFYVKKGGA
KKRPAPSDADISEPKRVRESVAQPSTSDAEASINYADRYQNKCSRHVGMNML
FPCRQCERMNQNSNICFTHGQKDCLECFVSESQPVSVVKKAYQKLCYIHHIM
GKVPDACTACDLNVNVDLDDCIFEQ (SEQ ID NO:63)

15

AAV2: REP78

MPGFYEIVIKVPSDLDEHLPGISDSFVNWVAEKEWELPPDSDMDLNLIEQAPLT
VAEKLQRDFLTEWRRVSKAPEALFFVQFEKGESYFHMHVLEVTTGVKSMVLGR
FLSQIREKLIQRIYRGIEPTLPNWFVAVTKTRNGAGGGNKVVDECYIPNYLLPKTQ
PELQWAWWTNMEQYLSACLNLTERKRLVAQHLTHVSQTQEQNKENQNPNSDAP
VIRSKTSARYMELVGWLVDKGITSEKQWQEDQASYISFNAASNSRSQIKAALD
NAGKIMSLTKTAPDYLVGQQPVEDISSNRIYKILELNGYDPQYAASVFLGWATKK
FGKRNTIWLFGPATTGKTNIAEIAHTVPFYGCVNWTNENFPFNDKVDKMVIW
WEEGKMTAKVVESAKAILGGSKVRVDQKCKSSAQIDPTPVIVTSNTNMCAVIDG
NSTTFEHQQPLQDRMFKFELTRRLDHDGKVTQKQEVKDFFRWAKDHVVEVEH
EFYVKKGGAKKRPAPSDADISEPKRVRESVAQPSTSDAEASINYADRYQNKCS
RHVGMNMLMLFPCRQCERMNQNSNICFTHGQKDCLECFVSESQPVSVVKKAY
QKLCYIHHIMGKVPDACTACDLNVNVDLDDCIFEQ (SEQ ID NO:64)

30

AAV2: VP1

MAADGYLPDWLEDTLSEGIRQWWKLKPGPPPPKPAERHKDDSRGLVLPGYKY
LGPFNGLDKGEPVNEADAAALEHDKAYDRQLDSGDNPYLKYNHADADEFQERL
KEDTSFGGNLGRAVFQAKKRVLEPLGLVEEPVKTA PGKKRPVEHSPVEPDSSS
GTGKAGQQPARKRLNFGQTGDADSVDPQPLGQPPAAPSGLGTNTMATGSG
APMADNNEGADGVGNSSGNWHCDSTWMGDRVITTSTRTWALPTYNNHLYKQ
ISSQSGASNDNHYFGYSTPWGYFDFNRFHCHFSPRDWQRLINNNWGFPRPKRL
NFKLFNIQVKEVTQNDGTTTIANNLTSTVQVFTDSEYQLPYVLGSAHQGCLPPF
PADVFMVPQYGYLTLNNGSQAVGRSSFYCLEYFPSQMLRTGNNFTFSYTFEDV
PFHSSYAHSQSLDRLMNPLIDQYLYLSRTNTPSGTTTQSRLQFSQAGASDIRD
QSRNWLPGPCYRQQRVSKTSADNNNSEYSWTGATKYHLNGRDSLVPNGPAM
ASHKDDEEKFFPQSGVLIFGKQGSEKTNVDIEKVMITDEEEIRTTNPVATEQYGS
VSTNLQRGNRQAATADVNTQGVLPGMVWQDRDVYLQGPIWAKIPHTDGHFHP
SPLMGGFGLKHPPPQILIKNTPVPANPSTTFSAAKFASFITQYSTGQVSVEIEWE
LQKENS KRWNPEIQYTSNYNKS VNVDFTVDTNGVYSEPRPIGTRYLTRNL
(SEQ ID NO:65)

45

AAV2: VP2

APGKKRPVEHSPVEPDSSSGTGKAGQQPARKRLNFGQTGDADSVDPDPQLG
 QPPAAPSGGLGTNTMATGSGAPMADNNEGADGVGNSSGNWHCDSTWMGDRV
 ITTSTRTWALPTYNNHLYKQISSQSGASNDNHYFGYSTPWGYFDFNRFHCHFS
 5 PRDWQRLINNNWGFPRKRLNFKLFNIQVKEVTQNDGTTTIANNLTSTVQVFTDS
 EYQLPYVLGSAHQGCLPPFPADVFMVPQYGYLTLNNGSQAVGRSSFYCLEYFP
 SQMLRTGNNFTFSYTFEDVPFHSSYAHSQSLDRLMNPLIDQYLYLSRTNTPS
 GTTTQSRLQFSQAGASDIRDQSRNWLPGPCYRQQRVSKTSADNNNSEYSWT
 GATKYHLNGRDSLVPNGPAMASHKDDEEKFFPQSGVLIFGKQGSEKTNVDIEK
 10 VMITDEEEIIRTTNPVATEQYGSVSTNLQRGNRQAATADVNTQGVLPGMVWQD
 RDVYLQGPWAKIPHTDGHFHPSPLMGGFGLKHPPPQILIKNTPVPANPSTTFS
 AAKFASFITQYSTGQVSVEIEWELQKENSkrwnPEIQYTSNYNKSvNVDFTVDT
 NGVYSEPRPIGTRYLTRNL (SEQ ID NO:66)

AAV2: VP3

MATGSGAPMADNNEGADGVGNSSGNWHCDSTWMGDRVITTSTRTWALPTYN
 NHLYKQISSQSGASNDNHYFGYSTPWGYFDFNRFHCHFS PRDWQRLINNNWG
 FRPKRLNFKLFNIQVKEVTQNDGTTTIANNLTSTVQVFTDSEYQLPYVLGSAHQ
 GCLPPFPADVFMVPQYGYLTLNNGSQAVGRSSFYCLEYFPSQMLRTGNNFTF
 20 SYTFEDVPFHSSYAHSQSLDRLMNPLIDQYLYLSRTNTPSGTTTQSRLQFSQA
 GASDIRDQSRNWLPGPCYRQQRVSKTSADNNNSEYSWTGATKYHLNGRDSL
 VNPNGPAMASHKDDEEKFFPQSGVLIFGKQGSEKTNVDIEKVMITDEEEIIRTTNP
 VATEQYGSVSTNLQRGNRQAATADVNTQGVLPGMVWQDRDVYLQGPWAKIP
 HTDGHFHPSPLMGGFGLKHPPPQILIKNTPVPANPSTTFSAAKFASFITQYSTG
 25 QVSVEIEWELQKENSkrwnPEIQYTSNYNKSvNVDFTVDTNGVYSEPRPIGTR
 YLTRNL (SEQ ID NO:67)

Synthetic: Myc-Tagged Anti CD19 scFv

EQKLISEEDLDIQMTQTSSLSASLGDRVTISCRASQDISKYLNWYQQKPDGTV
 30 KLLIYHTSRLHSGVPSRFSGSGSGTDYSLTISNLEQEDIATYFCQQGNTLPYTFG
 GGTGLEITGGGGSGGGGGSGGGGSEVKLQESGPGLVAPSQSLSVTCTVSGVSL
 PDYGVSWIRQPPRKGLEWLGVIWGSETTYNSALKSRLTIKDNSKSQVFLKMN
 SLQTD DTAIYYCAKHYYYGGSYAMDYWGQGTSVTVSS (SEQ ID NO:68)

Synthetic: dDZF1

FKCEHCRILFLDHVMFTIHMGCCHGFRDPFKCNMCGEKCDGPVGLFVHMARNA
 HGEKPFYCEHCEITFRDVVMYSLHKG YHGFRDPFECNICGYHSQDRYEFSSHI
 VRGEH (SEQ ID NO:69)

Synthetic: dDZF2

HHCQHCDMYFADNILYTIHMGCCHSCDDVFKCNMCGEKCDGPVGLFVHMARNA
 HGEKPTKCVHCGIVFLDEV MYALHMSCHGFRDPFECNICGYHSQDRYEFSSHI
 VRGEH (SEQ ID NO:70)

Synthetic: DmrA

45

MGRGVQVETISPGDGRTFPKRGQTCVVHYTGMLEDGKKFDSSRDRNKPFFKM
LGKQEVIRGWEEGVAQMSVGQRAKLTISPDYAYGATGHPGIIPPHATLVFDVEL
LKLE (SEQ ID NO:71)

5 **Synthetic: DmrB**

MASRGVQVETISPGDGRTFPKRGQTCVVHYTGMLEDGKKVDSSRDRNKPFFK
MLGKQEVIRGWEEGVAQMSVGQRAKLTISPDYAYGATGHPGIIPPHATLVFDVE
LLKLE (SEQ ID NO:72)

10 **Synthetic: DmrC**

MGSRLWHEMWHEGLEEASRLYFGERNVKGMFEVLEPLHAMMERGPQTLKET
SFNQAYGRDLMEAQEWCRKYMKSGNVKDLLQAWDLYYHVFRISK (SEQ ID
NO:73)

15 **Homo sapiens/Synthetic: FKBP**

MGVQVETISPGDGRTFPKRGQTCVVHYTGMLEDGKKFDSSRDRNKPFFKMLG
KQEVIRGWEEGVAQMSVGQRAKLTISPDYAYGATGHPGIIPPHATLVFDVELLK
LE (SEQ ID NO:74)

20 **Homo sapiens/Synthetic: FRB**

QGMLEMWHEGLEEASRLYFGERNVKGMFEVLEPLHAMMERGPQTLKETSFN
QAYGRDLMEAQEWCRKYMKSGNVKDLLQAWDLYYHVFRISK (SEQ ID
NO:75)

25 **Synthetic: Anti-GCN4 scFv**

MGPDIVMTQSPSSLSASVGDRVITCRSSTGAVTTSNYASWWQEKPGKLFKGLI
GGTNNRAPGVPSRFSGSLIGDKATLTISLQPEDFATYFCALWYSNHWVFGQG
TKVELKRGGGGSGGGGSGGGGSGGGGSEVKLLESGGGLVQPGGSLKLSCAV
SGFSLTDYGVNWWRQAPGRGLEWIGVIWGDGITDYNALKDRFIISKDNGKNTV
30 YLQMSKVRSDDTALYYCVTGLFDYWGQGTLLTVSSYPYDVPDYAGGGGGSG
GGGSGGGGSGGGGS (SEQ ID NO:76)

Synthetic: 10x-GCN4 Repeats

35 EELLSKNYHLENEVARLKKGSGSGEELLSKNYHLENEVARLKKGSGSGEELLS
KNYHLENEVARLKKGSGSGEELLSKNYHLENEVARLKKGSGSGEELLSKNYHL
ENEVARLKKGSGSGEELLSKNYHLENEVARLKKGSGSGEELLSKNYHLENEVA
RLKKGSGSGEELLSKNYHLENEVARLKKGSGSGEELLSKNYHLENEVARLKKG
SGSGEELLSKNYHLENEVARLKKGS (SEQ ID NO:77)

40 **Synthetic: 24x-GCN4 Repeats**

EELLSKNYHLENEVARLKKGSGSGEELLSKNYHLENEVARLKKGSGSGEELLS
KNYHLENEVARLKKGSGSGEELLSKNYHLENEVARLKKGSGSGEELLSKNYHL
ENEVARLKKGSGSGEELLSKNYHLENEVARLKKGSGSGEELLSKNYHLENEVA
RLKKGSGSGEELLSKNYHLENEVARLKKGSGSGEELLSKNYHLENEVARLKKG
45 SGSGEELLSKNYHLENEVARLKKGSGSGEELLSKNYHLENEVARLKKGSGSGE
ELLSKNYHLENEVARLKKGSGSGEELLSKNYHLENEVARLKKGSGSGEELLSK

5 NYHLENEVARLKKGSGSGEELLSKNYHLENEVARLKKGSGSGEELLSKNYHLE
NEVARLKKGSGSGEELLSKNYHLENEVARLKKGSGSGEELLSKNYHLENEVAR
LKKGSGSGEELLSKNYHLENEVARLKKGSGSGEELLSKNYHLENEVARLKKGS
GSGEELLSKNYHLENEVARLKKGSGSGEELLSKNYHLENEVARLKKGSGSGEE
LLSKDYHLENEVARLKKGSGSGEELLSKNYHLENEVARLKKGS (SEQ ID
NO:78)

Synthetic: GFP-targeting Nanobody

10 VQLVESGGALVQPGGSLRLSCAASGFPVNRYSMRWYRQAPGKEREWAGMS
SAGDRSSYEDSVKGRFTISRDDARNTVYLQMNSLKPEDTAVYYSNVNVGFEY
WGQGTQVTVSS (SEQ ID NO:79)

Nostoc punctiforme: Npu DnaE N-terminal Split Intein

15 CLSYETEILTVEYGLLPIGKIVEKRIECTVYSVDNNGNIYTQPVAQWHDRGEQEV
FEYCLEDDGSLIRATKDHKFMTVDGQMLPIDEIFERELDLMRVDNLPN
(SEQ ID NO:80)

Nostoc punctiforme: Npu DnaE C-terminal Split Intein

20 MIKIATRKYLGKQNVYDIGVERDHNFKNGFIASNCFN (SEQ ID NO:81)

Synthetic: Cfa N-Terminal Split Intein

25 CLSYDTEILTVEYGFLPIGKIVEERIECTVYTVDKNGFVYTQPIAQWHNRGEQEV
FEYCLEDDGSIIRATKDHKFMTTDGQMLPIDEIFERGLDLKQVDGLP (SEQ ID
NO:82)

Synthetic: Cfa C-Terminal Split Intein

MVKIISRKSLGTQNVYDIGVEKDHNFLKNGLVASN (SEQ ID NO:83)

Saccharomyces cerevisiae: Vma N-terminal Split Intein

30 CFAKGTNVLMADGSIECIENIEVGKVMGKDGRPREVIKLPRGRETMYSVVQKS
QHRAHKSDSSREVPELLKFTCNATHELVVRTPRSVRRLSRTIKGVEYFEVITFE
MGQKKAPDGRIVELVKEVSKSYPISEGPERANELVESYRKASNKAYFEWTIEAR
DLSLLGSHVRKATYQTYAPILY (SEQ ID NO:84)

Saccharomyces cerevisiae: Vma C-terminal Split Intein

35 VLLNVLSKCAGSKKFRPAPAAAFARECRGFYFELQELKEDDYGITLSDDSDHQ
FLLANQVVVHN (SEQ ID NO:85)

Synechocystis sp. PCC 6803: Ssp DnaE N-terminal Split Intein

40 CLSFGTEILTVEYGPLPIGKIVSEEINCSVYSVDPEGRVYTQAIQWHDRGEQEV
LEYELEDGSGVIRATSDHRFLTDDYQLLAIEEIFARQLDLLTLENIKQTEEALDNHR
LPFPLLDAGTIK (SEQ ID NO:86)

Synechocystis sp. PCC 6803: Ssp DnaE C-terminal Split Intein

45 MVKVIGRRSLGVQRIFDIGLPQDHNFLLANGAIAAN (SEQ ID NO:87)

Synthetic: Spy Tag

VPTIVMVDAYKRYK (SEQ ID NO:88)

Synthetic: Spy Catcher

5 MVTTL SGLSGEQGPSGDMTTEEDSATHIKFSKRDEDEGRELAGATMELRDSSG
 KTISTWISDGHVKDFYLYPGKYTFVETAAPDGYEVATAITFTVNEQGQVTVNGE
 ATKGDAHTGSSGS (SEQ ID NO:89)

Bacteriophage MS2: MS2 RNA Binding Protein

10 MASNFTQFVLVDNNGGTGDVT VAPSNFANGVAEWISSNSRSQAYKVTCSVRQS
 SAQNRKYTIKVEVPKVATQTVGGVELPVAAWRSYLNMEITPIFATNSDCELVK
 AMQGLLKDGNIPIPSAIAANSIY (SEQ ID NO:90)

Bacteriophage MS2: MS2 (N55K) RNA Binding Protein

15 MASNFTQFVLVDNNGGTGDVT VAPSNFANGVAEWISSNSRSQAYKVTCSVRQS
 SAQKRKYTIKVEVPKVATQTVGGVELPVAAWRSYLNMEITPIFATNSDCELVK
 AMQGLLKDGNIPIPSAIAANSIY (SEQ ID NO:91)

Bacteriophage MS2: MS2 (N55K)(V29I) RNA Binding Protein

20 MASNFTQFVLVDNNGGTGDVT VAPSNFANGIAEWISSNSRSQAYKVTCSVRQSS
 AQKRKYTIKVEVPKVATQTVGGVELPVAAWRSYLNMEITPIFATNSDCELVKA
 MQGLLKDGNIPIPSAIAANSIY (SEQ ID NO:92)

Bacteriophage PP7: PP7 RNA Binding Protein

25 KTI VLSVGEATR TLTEIQSTADRQIFEEKVGPLVGRRLTASLRQNGAKTAYRVN
 LKLDQADVVD SGLPKVRYTQVWSHDVTIVANSTEASRKS LYDLTKSLVATSQVE
 DLVVNLVPLGRS (SEQ ID NO:93)

Bacteriophage Mu: COM RNA Binding Protein

30 MKSIRCKNCKNLLFKADSF DHIEIRCP RCKRHIIMLNACEHPTEKHCGKREKITH
 SDETVRY (SEQ ID NO:94)

Synthetic: Zinc Finger ZF6/10

35 STRPGERPFQCRICMRNFSIPNHLARHTRTHTGEKPFQCRICMRNFSQSAHLK
 RHLRTH TGEKPFQCRICMRNFSQDVSLVRHLKTHLRQKDGERPFQCRICMRNF
 SSAQALARHTRTHTGEKPFQCRICMRNFSQGGNLTRHLRTH TGEKPFQCRICM
 RNFSQH PNLTRHLKTHLRGS (SEQ ID NO:95)

Synthetic: Zinc Finger ZF8/7

40 SRPGERPFQCRICMRNFSTMAVLRHTRTHTGEKPFQCRICMRNFSRREVLEN
 HLRTH TGEKPFQCRICMRNFSQTVNLDRHLKTHLRQKDGERPFQCRICMRNFS
 KKDHLHRHTRTHTGEKPFQCRICMRNFSQRPHLTNHLRTH TGEKPFQCRICMR
 NFSVGASLKRHLKTHLRGS (SEQ ID NO:96)

Synthetic: Zinc Finger ZF9

45

SRPGERPFQCRICMRNFSDKTKLRVHTRTHTGEKPFQCRICMRNFSVRHNLTR
HLRTHHTGEKPFQCRICMRNFSQSTSLQRHLKTHLRGF (SEQ ID NO:97)

Synthetic: Zinc Finger MK10

5 SRPGERPFQCRICMRNFSRRHGLDRHTRTHTGEKPFQCRICMRNFSDHSSLK
RHLRTHHTGSQKPFQCRICMRNFSVRHNLTRHLRTHHTGEKPFQCRICMRNFSDH
SNLSRHLKTHHTGSQKPFQCRICMRNFSQRSSLVRHLRTHHTGEKPFQCRICMRN
FSESGHLKRHLRTHLRGS (SEQ ID NO:98)

Synthetic: Zinc Finger 268

10 YACPVESCDRRFSRSDDELTRHIRIHTGQKPFQCRICMRNFSRSDHLTTHIRTHHT
GEKPFACDICGRKFARSDEKRHTKIHLRQKD (SEQ ID NO:99)

Synthetic: Zinc Finger NRE

15 YACPVESCDRRFSQSHDLTKHIRIHTGQKPFQCRICMRNFSQSSKLSRHIRTHHT
GEKPFACDICGRKFARLDNRHTAHTKIHLRQKD (SEQ ID NO:100)

Synthetic: Zinc Finger 268/NRE

20 YACPVESCDRRFSRSDDELTRHIRIHTGQKPFQCRICMRNFSRSDHLTTHIRTHHT
GEKPFACDICGRKFARSDEKRHTKIHLRQKDGGERPYACPVESCDRRFSQSHD
LTKHIRIHTGQKPFQCRICMRNFSQSSKLSRHIRTHHTGEKPFACDICGRKFARLD
NRHTAHTKIHLRQKD (SEQ ID NO:101)

Synthetic: Zinc Finger 268/NRE

25 YACPVESCDRRFSRSDDELTRHIRIHTGQKPFQCRICMRNFSRSDHLTTHIRTHHT
GEKPFACDICGRKFARSDEKRHTKIHLRQKDGGSERPYACPVESCDRRFS
QSHDLTKHIRIHTGQKPFQCRICMRNFSQSSKLSRHIRTHHTGEKPFACDICGRKF
ARLDNRHTAHTKIHLRQKD (SEQ ID NO:102)

Synthetic: FokI Zinc Finger Nuclease 17-2 Targeting GFP

30 SRPGERPFQCRICMRNFSTRQNLDTHTTRTHTGEKPFQCRICMRNFSRRDTLER
HLRTHHTGEKPFQCRICMRNFSRDPALPRHLKTHLRGSQLVKSELEKKSELRH
KLKYVPHEYIELIEIARNSTQDRILEMKVMEFFMKVYGYRGKHLGGSRKPDGAIY
TVGSPIDYGVIVDTKAYSGGYNLPIGQADEMQRYVEENQTRNKHINPNEWWKV
35 YPSSVTEFKFLFVSGHFKGNYKAQLTRLNHITNCNGAVLSVEELLIGGEMIKAGT
LTLEEVRRKFNNGEINF (SEQ ID NO:103)

Synthetic: FokI Zinc Finger Nuclease 18-2 Targeting GFP

40 SRPGERPFQCRICMRNFSSPSKLIHTRTHTGEKPFQCRICMRNFSDGSNLAR
HLRTHHTGEKPFQCRICMRNFSRVDNLPRHLKTHLRGSQLVKSELEKKSELRH
KLKYVPHEYIELIEIARNSTQDRILEMKVMEFFMKVYGYRGKHLGGSRKPDGAIY
TVGSPIDYGVIVDTKAYSGGYNLPIGQADEMQRYVEENQTRNKHINPNEWWKV
YPSSVTEFKFLFVSGHFKGNYKAQLTRLNHITNCNGAVLSVEELLIGGEMIKAGT
45 LTLEEVRRKFNNGEINF (SEQ ID NO:104)

Synthetic: Left FokI Zinc Finger Nuclease Targeting CCR5

MDYKDHDGDYKDHDIDYKDDDDKMAPKKRKVGIVPAAMAERPFQCRICM
 RNFSDRSNLSRHIRTHTGEKPFACDICGRKFAISSNLNSHTKIHTGSQKPFQCRI
 CMRNFSRSDNLARHIRTHTGEKPFACDICGRKFATSGNLTRHTKIHLRGSQVLK
 SELEEKKSELRHKLKYVPHEYIELIEIARNSTQDRILEMKVMEFFMKVYGYRGKH
 5 LGGSRKPDGAIYTVGSPIDYGVIVDTKAYSGGYNLPIGQADEMQRYVEENQTR
 NKHINPNEWVKVYPSSVTEFKFLFVSGHFKGNYKAQLTRLNHITNCNGAVLSV
 EELLIGGEMIKAGTLTLEEVRKFNNGEINF (SEQ ID NO:105)

Synthetic: Right FokI Zinc Finger Nuclease Targeting CCR5
 10 MDYKDHDGDYKDHDIDYKDDDDKMAPKKRKVGIVPAAMAERPFQCRICM
 RNFSRSDNLSVHIRTHTGEKPFACDICGRKFAQKINLQVHTKIHTGEKPFQCRI
 MRNFSRSDVLSEHIRTHTGEKPFACDICGRKFAQRNHRRTHTKIHLRGSQVLKS
 ELEEKKSELRHKLKYVPHEYIELIEIARNSTQDRILEMKVMEFFMKVYGYRGKHL
 GGSRKPDGAIYTVGSPIDYGVIVDTKAYSGGYNLPIGQADEMQRYVEENQTRN
 15 KHINPNEWVKVYPSSVTEFKFLFVSGHFKGNYKAQLTRLNHITNCNGAVLSVE
 ELLIGGEMIKAGTLTLEEVRKFNNGEINF (SEQ ID NO:106)

Synthetic: FokI Nuclease Domain
 20 QLVKSELEEKKSELRHKLKYVPHEYIELIEIARNSTQDRILEMKVMEFFMKVYGY
 RGKHLGGSRKPDGAIYTVGSPIDYGVIVDTKAYSGGYNLPIGQADEMQRYVEE
 NQTRNKHINPNEWVKVYPSSVTEFKFLFVSGHFKGNYKAQLTRLNHITNCNGA
 VLSVEELLIGGEMIKAGTLTLEEVRKFNNGEINF (SEQ ID NO:107)

Synthetic: Acul Nuclease Domain
 25 VHDHKLELAKLIRNYETNRKECLNSRYNETLLRSDYLDPPFELLGWDIKNKAGK
 PTNEREVVLEEALCASASEHSKKPDYTFRLFSEKFFLEAKKPSVHIESDNETAK
 QVRRYGFTAKLKISVLSNFEYLVYDTSVKVDGDDTFNKARIKKYHYTEYETHFD
 EICDLLGRESVYSGNFDKEWLSIENKINHFSVDTL (SEQ ID NO:108)

Synthetic: Truncated Acul Nuclease Domain
 30 YNETLLRSDYLDPPFELLGWDIKNKAGKPTNEREVVLEEALCASASEHSKKPDY
 TRLFSEKFFLEAKKPSVHIESDNETAKQVRRYGFTAKLKISVLSNFEYLVYDT
 SVKVDGDDT (SEQ ID NO:109)

Escherichia coli: Ferritin
 35 MLKPEMIEKLNEQMNLELYSSLLYQQMSAWCSYHTFEGAAFLRRHAQEEMT
 HMQRFLDYLDTDGNLPRINTVESPFAYSSLDLDFQETYKHEQLITQKINELAHA
 AMTNQDYPTFNFLQWYVSEQHEEEKLFKSIIDKLSLAGKSGEGLYFIDKELSTLD
 TQN (SEQ ID NO:110)

Escherichia coli: Ferritin (H34L)(T64I)
 40 MLKPEMIEKLNEQMNLELYSSLLYQQMSAWCSYLTTFEGAAFLRRHAQEEMTH
 MQRFLDYLDIGNLPRINTVESPFAYSSLDLDFQETYKHEQLITQKINELAHAA
 MTNQDYPTFNFLQWYVSEQHEEEKLFKSIIDKLSLAGKSGEGLYFIDKELSTLDT
 45 QN (SEQ ID NO:111)

***Mus musculus* & Synthetic: Light & Heavy Chain Ferritin Chimera**

MTSQIRQNYSTEVEAAVNRLVNLHLRASYYTSLGFFFFDRDDVALEGVGHFFRE
LAEKREGAERLLEFQNDRGGRALFQDVQKPSQDEWGKTQEAMEAALAMEK
NLNQALLDLHALGSARADPHLCDFLESHYLDKEVKLIKKMGNHLTNLRVAGPQ
5 PAQTGAPQGSGLGEYLFERLTLKHDARGGGGSDYKDDDDKGGGGSRVMTTAS
PSQVRQNYHQDAEAAINRQINLELYASYVYLSMSCYFDRDDVALKNFAKYFLH
QSHEEREHA EKLMKLQNQRGGRIFLQDIKKPDRDDWESGLNAMECALHLEKS
VNQSLLELHKLATDKNDPHLCDFIETYYLSEQVKS IKELGDHVTNLRKMGAPEA
GMAEYLFDKHTLGHGDESTR (SEQ ID NO:112)

***Homo sapiens* & Synthetic: Light & Heavy Chain Ferritin Chimera**

SQIRQNYSTDVEAAVNSLVNLYLQASYTYLSLGFYFDRDDVALEGVSHFFRELA
EEKREGYERLLKMQNQRGGRALFQDIKKPAEDEWGKTPDAMKAAMALEKKLN
QALLDLHALGSARTDPHLCDFLETHFLDEEVKLIKKMGDHLTNLHRLGGPEAGL
15 GEYLFERLTLKHDARGGGGSDYKDDDDKGGGGSRVMTTASTSQVRQNYHQD
SEAAINRQINLELYASYVYLSMSYFDRDDVALKNFAKYFLHQSHEEREHA EK
MKLQNQRGGRIFLQDIKKPDCDDWESGLNAMECALHLEKNVNQSLLELHKLAT
DKNDPHLCDFIETHYLNQVKA IKELGDHVTNLRKMGAPESGLAEYLFDKHTLG
DSDNES (SEQ ID NO:113)

***Arabidopsis thaliana*: Cry2**

MKMDKKTIVWFRRDLRIEDNPALAAAAHEGSVFPVFIWCPEEEGQFYPPGRASR
VWMKQSLAHLSSQLKALGSDLTLIKTHNTISAILDCIRVTGATKVVFNHLYPDVS
LVRDHTVKEKLVERGISVQSYNGDLLYEPWEIYCEKGKPFSTFNSYWKCLDM
25 SIESVMLPPPWRMLPITAAAEI WACSIEELGLENEAEKPSNALLTRAWSPGWS
NADKLLNEFIEKQLIDYAKNSKKVVG NSTSLLSPYLHFGEISVRHVFCARMKQII
WARDKNSEGEESADLFLRGIGLREYSRYICFNFPFTHEQSLLSHLRFFPWDADV
DKFKAWRQGRGTGYPLVDAGMRELWATGWMHNRIRVIVSSFAVKFLLPWKWG
MKYFWDTLDDADLECDILGWQYISGSIPDGHELDRLDNPALQGAKYDPEGEYIR
30 QWLPELARLPTEWIIHPWDAPLTVLKASGVELGTNYAKPIVDIDTARELLAKAIS
RTREAQIMIGAAPDEIVADSFEALGANTIKEPGLCPVSSNDQQVPSAVRYNGS
AAVKPEEEEEERDMKKS RGFDERELFSTAESSSSSSVFFVSQSCSLASEGKNLE
GIQDSSDQITTS LGKNGCK (SEQ ID NO:114)

***Arabidopsis thaliana*: CIBN**

MNGAIGGDLLL NFPDMSVLERQRAHLKYLNP TFDSPLAGFFADSSMITGGEMD
SYLSTAGLNLPM MYGETTVEGDSRLSISPETT LGTGNFKAAKFD TETKDCNEAA
KKMTMNRDDLVEEGEEEEKSKITEQNNGSTKSIKKMKHKAKKEENNFSNDSSKV
40 TKELEKTDYI (SEQ ID NO:115)

Synthetic: LoV2-Ja

SLATT LERIEKNFVITDPRLPDNPIIFASDSFLQLTEYSREEILGRNCRFLQGPETD
RATVRKIRDAIDNQTEVTVQLINYTKSGKKFWNL FHLQPMRDQKGDVQYFIGVQ
LDGTEHV RDAAEREGVMLIKKTAENIDEAAKEL (SEQ ID NO:116)

***Homo sapiens*: Full Length WT ADAR2**

DIEDEENMSSSSSTDVKENRNLDNVSPKDGSTPGPGEGSQLSNGGGGGPGRK
 RPLEEGSNHGSKYRLKKRRKTPGPVLPKNALMQLNEIKPGLQYTLLSQTGPVH
 APLFVMSVEVNGQVFEGSGPTKKKAKLHAAEKALRSFVQFPNASEAHLAMGRT
 LSVNTDFTSDQADFPDTLNFNGFETPDKAEPFFYVGSNGDDSFSSSGDLSLSAS
 5 PVPASLAQPPLPVLPPFPPPSGKNPVMILNELRPGLKYDFLSESGESHAKSFVM
 SVVVDGQFFEFGSGRNKKLAKARAAQSALAAIFNLHLDQTPSRQPIPSEGLQLHL
 PQVLADAVSRLVLGKFGDLTDNFSSPHARRKVLAVVMTTGTVDKDAKVISVST
 GTKCINGEYMSDRGLALNDCHAEIISRRSLLRFLYTQLELYLNNKDDQKRSIFQK
 SERGGFRLKENVQFHLYISTSPCGDARIFSPHEPILEGSRSYTQAGVQWCNHG
 10 SLQPRPPGLLSDPSTSTFQAGTTEPADRHPNRKARGQLRTKIESGEGTIPVRS
 NASIQTWGVLQGERLLTMSCSDKIARWNVVGIGSLLSIFVEPIYFSSIILGSLY
 HGDHLSRAMYQRISNIEDLPPLYTLNKPLLSGISNAEARQPGKAPNFSVNWTVG
 DSAIEVINATTGKDELGRASRLCKHALYCRWMMRVHGKVP SHLLRSKITKPNVYH
 15 ESKLAAKEYQAAKARLFTAFIKAGLGAWVEKPTEQDQFSLTP (SEQ ID
 NO:117)

***Homo sapiens*: Full Length WT ADAR2 (E488Q)**

DIEDEENMSSSSSTDVKENRNLDNVSPKDGSTPGPGEGSQLSNGGGGGPGRK
 RPLEEGSNHGSKYRLKKRRKTPGPVLPKNALMQLNEIKPGLQYTLLSQTGPVH
 20 APLFVMSVEVNGQVFEGSGPTKKKAKLHAAEKALRSFVQFPNASEAHLAMGRT
 LSVNTDFTSDQADFPDTLNFNGFETPDKAEPFFYVGSNGDDSFSSSGDLSLSAS
 PVPASLAQPPLPVLPPFPPPSGKNPVMILNELRPGLKYDFLSESGESHAKSFVM
 SVVVDGQFFEFGSGRNKKLAKARAAQSALAAIFNLHLDQTPSRQPIPSEGLQLHL
 25 PQVLADAVSRLVLGKFGDLTDNFSSPHARRKVLAVVMTTGTVDKDAKVISVST
 GTKCINGEYMSDRGLALNDCHAEIISRRSLLRFLYTQLELYLNNKDDQKRSIFQK
 SERGGFRLKENVQFHLYISTSPCGDARIFSPHEPILEGSRSYTQAGVQWCNHG
 SLQPRPPGLLSDPSTSTFQAGTTEPADRHPNRKARGQLRTKIESGQGTIPVR
 SNASIQTWGVLQGERLLTMSCSDKIARWNVVGIGSLLSIFVEPIYFSSIILGSL
 30 YHGDHLSRAMYQRISNIEDLPPLYTLNKPLLSGISNAEARQPGKAPNFSVNWTV
 GDSAIEVINATTGKDELGRASRLCKHALYCRWMMRVHGKVP SHLLRSKITKPNVY
 HESKLAAKEYQAAKARLFTAFIKAGLGAWVEKPTEQDQFSLTP (SEQ ID
 NO:118)

***Homo sapiens*: Truncated WT ADAR2**

35 VLADAVSRLVLGKFGDLTDNFSSPHARRKVLAVVMTTGTVDKDAKVISVSTGT
 KCINGEYMSDRGLALNDCHAEIISRRSLLRFLYTQLELYLNNKDDQKRSIFQKSE
 RGGFRLKENVQFHLYISTSPCGDARIFSPHEPILEGSRSYTQAGVQWCNHGSL
 QPRPPGLLSDPSTSTFQAGTTEPADRHPNRKARGQLRTKIESGEGTIPVRSN
 ASIQTWGVLQGERLLTMSCSDKIARWNVVGIGSLLSIFVEPIYFSSIILGSLYH
 40 GDHLSRAMYQRISNIEDLPPLYTLNKPLLSGISNAEARQPGKAPNFSVNWTVD
 SAIEVINATTGKDELGRASRLCKHALYCRWMMRVHGKVP SHLLRSKITKPNVYHE
 SKLAAKEYQAAKARLFTAFIKAGLGAWVEKPTEQDQFSLTP (SEQ ID
 NO:119)

***Homo sapiens*: Truncated WT ADAR2 (E488Q)**

45

VLADAVSRLVLGKFGDLTDNFSSPHARRKVLAVVMTTGTVDKDAKVISVSTGT
 KCINGEYMSDRGLALNDCHAEIISRRSLLRFLYTQLELYLNNKDDQKRSIFQKSE
 RGGFRLKENVQFHLYISTSPCGDARIFSPHEPILEGSRSYTQAGVQWCNHGSL
 QPRPPGLLSDPSTSTFQAGTTEPADRHPNRKARGQLRTKIESGQGTIPVRSN
 5 ASIQTWDGVLQGERLLTMSCSDKIARWNVVGIQGSLLSIFVEPIYFSSIIIGSLYH
 GDHLSTRAMYQRISNIEDLPPLYTLNKPLLSGISNAEARQPGKAPNFSVNWTVDG
 SAIEVINATTGKDELGRASRLCKHALYCRWMRVHGKVP SHLLRSKITKPNVYHE
 SKLAAKEYQAAKARLFTAFIKAGLGAWWEKPTEQDQFSLTP (SEQ ID
 NO:120)

***Homo sapiens & Synthetic: MS2-ADAR1 Deaminase Domain-Nuclear
 Exclusion Signal***

MASNFTQFVLVDNNGGTGDVTVAPSNFANGIAEWISSNSRSQAYKVTCSVRQSS
 AQNRKYTIKVEVPKGAWRSYLNMEITPIFATNSDCELVKAMQGLLKDGNPIS
 15 AIAANSYIYGGSGSGAGSGSPAGGGAPGSGGGSKAERMGTFTVPTGASLR
 RTMILLRSPEAQPKTLPLTGSTFHDQIAMLSHRCFNTLTNSFQPSLLGRKILAA
 IIMKKDSEDMGVVSLGTGNRCVKGDSLSLKGETVNDCHAEIISRRGFIRFLYSE
 LMKYNSQTAKDSIFEPAGGGEKLQIKKTVSFHLYISTAPCGDGALFDKSCSDRA
 MESTESRHYPVFENPKQGKLRTKVENGEGETIPVESSDIVPTWDGIRLGERLRT
 20 MSCSDKILRWNVLGLQGALLTHFLQPIYLKSVTLGYLFSQGHLLTRAICCRVTRD
 GSAFEDGLRHPFIVNHPKVGRVSIYDSKRQSGTKETSVNWCLADGYDLEILDG
 TRGTVDGPRNELSRVSKKNIFLLFKKLCSFRYRRDLLRLSYGEAKKAARDYETA
 KNYFKKGLKDMGYGNWISKPQEEKNFYLCVPVSGSGSLPPLERLT (SEQ ID
 NO:121)

***Homo sapiens & Synthetic: MS2-ADAR1 Deaminase Domain (E1008Q)-
 Nuclear Exclusion Signal***

MASNFTQFVLVDNNGGTGDVTVAPSNFANGIAEWISSNSRSQAYKVTCSVRQSS
 AQNRKYTIKVEVPKGAWRSYLNMEITPIFATNSDCELVKAMQGLLKDGNPIS
 30 AIAANSYIYGGSGSGAGSGSPAGGGAPGSGGGSKAERMGTFTVPTGASLR
 RTMILLRSPEAQPKTLPLTGSTFHDQIAMLSHRCFNTLTNSFQPSLLGRKILAA
 IIMKKDSEDMGVVSLGTGNRCVKGDSLSLKGETVNDCHAEIISRRGFIRFLYSE
 LMKYNSQTAKDSIFEPAGGGEKLQIKKTVSFHLYISTAPCGDGALFDKSCSDRA
 MESTESRHYPVFENPKQGKLRTKVENGGGTIPVESSDIVPTWDGIRLGERLRT
 35 MSCSDKILRWNVLGLQGALLTHFLQPIYLKSVTLGYLFSQGHLLTRAICCRVTRD
 GSAFEDGLRHPFIVNHPKVGRVSIYDSKRQSGTKETSVNWCLADGYDLEILDG
 TRGTVDGPRNELSRVSKKNIFLLFKKLCSFRYRRDLLRLSYGEAKKAARDYETA
 KNYFKKGLKDMGYGNWISKPQEEKNFYLCVPVSGSGSLPPLERLT (SEQ ID
 NO:122)

Ruminococcus flavefaciens: RfxCas13d (CasRx)

EASIEKKKSFAKGMGVKSTLVSGSKVYMTTFAEGSDARLEKIVEGDSIRSVNEG
 EAFSAEMADKNAGYKIGNAKFSHPKGYAVVANNPLYTGVPVQDMLGLKETLEK
 RYFGESADGNDNICIQVIHNILDEIKILAEYITNAAAYAVNNISGLDKDIIGFGKFSTV
 45 YTYDEFKDPHEHRAAFNNNDKLINAIAKAYDEFDNFLDNPRLG YFGQAFFSKEG
 RNYIINYGNECYDILALLSGLRHVVHNNEEESRISRTWLYNLDKNLNEYISTL

NYLYDRITNELTNSFSKNSAANVNYIAETLGINPAEFAEQYFRFSIMKEQKNLGF
 NITKLREVMLEDRKDMSEIRKNHKVFDSIRTKVYTMMDFVIYRYIEEDAKVAAAN
 KSLPDNEKSLSEKDIFVINLRGSFNDDQKDALLYDEANRIWRKLENIMHNIKEFR
 GNKTREYKKKDAPRLPRILPAGRDVSAFSKLMYALTMFLDGKEINDLLTTLINKF
 5 DNIQSFLKVMPLIGVNAKFVEEYAFFKDSAKIADELRLIKSFARMGEPIADARRA
 MYIDAIRILGTNLSYDELKALADTFSLDENGKLLKKGKHGMRNFIINNVISNKRFB
 YLIRYGDPAHLHEIAKNEAVVKFVLGRIADIQKKQGQNGKNQIDRYETCIGKDK
 GKSVEKVDALTKIITGMNYDQFDKKRSVIEDTGRENAEREKFKKIISLYLTVIYHI
 LKNIVNINARVIGFHCVERDAQLYKEKGYDINLKKLEEKGFSSVTCLCAGIDETA
 10 PDKRKDVEKEMAERAKESIDSLESANPKLYANYIKYSDEKKAEEFTRQINREKA
 KTALNAYLRNTKWNVIREDLLRIDNKTCTLFNRKAVHLEVARYVHAYINDIAEVN
 SYFQLYHYIMQRIIMNERYEKSSGKVSEYFDAVNDEKKYNDRLKLLCVPFGYCI
 PRFKNLSIEALFDRNEAAKFDKEKKKVSGNSGSG (SEQ ID NO:123)

***Ruminococcus flavefaciens* & Synthetic: dead RfxCas13d (dCasRx)**

EASIEKKKSFAKGMGVKSTLVSGSKVYMTTFAEGSDARLEKIVEGDSIRSVNEG
 EAFSAEMADKNAGYKIGNAKFSPKGYAVVANNPLYTGPVQQDMLGLKETLEK
 RYFGESADGNDNICIQVIHNILDIEKILAEYITNAAAYAVNNISGLDKDIIGFGKFSTV
 YTYDEFKDPHEHRAAFNNNDKLINAIKAQYDEFDNFLDNPRLYGFGAFFSKEG
 20 RNYIINYGNECYDILALLSGLAHVWVANNEEESRISRTWLYNLDKNLDNEYISTL
 NYLYDRITNELTNSFSKNSAANVNYIAETLGINPAEFAEQYFRFSIMKEQKNLGF
 NITKLREVMLEDRKDMSEIRKNHKVFDSIRTKVYTMMDFVIYRYIEEDAKVAAAN
 KSLPDNEKSLSEKDIFVINLRGSFNDDQKDALLYDEANRIWRKLENIMHNIKEFR
 GNKTREYKKKDAPRLPRILPAGRDVSAFSKLMYALTMFLDGKEINDLLTTLINKF
 25 DNIQSFLKVMPLIGVNAKFVEEYAFFKDSAKIADELRLIKSFARMGEPIADARRA
 MYIDAIRILGTNLSYDELKALADTFSLDENGKLLKKGKHGMRNFIINNVISNKRFB
 YLIRYGDPAHLHEIAKNEAVVKFVLGRIADIQKKQGQNGKNQIDRYETCIGKDK
 GKSVEKVDALTKIITGMNYDQFDKKRSVIEDTGRENAEREKFKKIISLYLTVIYHI
 LKNIVNINARVIGFHCVERDAQLYKEKGYDINLKKLEEKGFSSVTCLCAGIDETA
 30 PDKRKDVEKEMAERAKESIDSLESANPKLYANYIKYSDEKKAEEFTRQINREKA
 KTALNAYLRNTKWNVIREDLLRIDNKTCTLFANKAVALEVARYVHAYINDIAEVN
 SYFQLYHYIMQRIIMNERYEKSSGKVSEYFDAVNDEKKYNDRLKLLCVPFGYCI
 PRFKNLSIEALFDRNEAAKFDKEKKKVSGNSGSGPKKKRKVAAAYPYDVPDYA
 (SEQ ID NO:124)

Prevotella sp. P5-125: PspCas13b

MNIPALVENQKKYFGTYSVMAMLNQTVLDHIQKVADIEGEQNENNENLWFHP
 VMSHLYNAKNGYDKQPEKTMFIERLQSYFPFLKIMAENQREYSNGKYKQNRV
 EVNSNDIFEVLKRAFGVLKMYRDLTNHYKTYEEKLNDGCEFLTSTEQPLSGMIN
 40 NYYTVALRNMNERYGYKTEDLAFIQDKRFKFKVDAYGKKKSQVNTGFFLSLQD
 YNGDTQKKLHLSGVGIALICLFLDKQYINIFLSRLPIFSSYNAQSEERRIIRSFGI
 NSIKLPKDRIHSEKSNKSVAMDMLNEVKRCPDELFTTLSAEKQSRFRIISDDHNE
 VLMKRSSDRFVPLLLQYIDYGKLFDFHVFHVNMGKLRLLKADKTCIDGQTRVR
 VIEQPLNGFGRLEEAETMRKQENGTFGNSGIRIRDFENMKRDDANPANYPIYVD
 45 TYTHYLENNKVEMFINDKEDSAPLLPVIEDDRYVVKTIPTSCRMSTLEIPAMAFHM
 FLFGSKKTEKLIVDVHNRKRLFQAMQKEEVTAENIASFGIAESDLPQKILDLSG

NAHGKDVDAFIRLTVDDMLTDTERRIKRFKDDRKSIRSADNKMGRGFKQISTG
 KLADFLAKDIVLFQPSVNDGENKITGLNYRIMQSAIAVYDSGDDYEAKQQFKLMF
 EKARLIGKGTTEPHFPLYKVFARSIPANAVEFYERYLIERKFYLTGLSNEIKKGNR
 5 VDVPFIRRDQNKWKTPAMKTLGRIYSEDLPVELPRQMFMDNEIKSHLKSPLQME
 GIDFNNANVTYLIAEYMKRVLDDDFQTFYQWNRNYRYMDMLKGEYDRKGSLQ
 HCFTSVEEREGLWKERASRTERYRKQASNKIRSNRQMRNASSEETILDKRLS
 NSRNEYQKSEKVIIRYRVQDALLFLLAKKTLTELADFDGERFKLKEIMPDAEKGI
 LSEIMPMSFTFEKGGKKYTITSEGMKLKNYGDFVFLASDKRIGNLLELVGSDIVS
 10 KEDIMEEFNKYDQCRPEISSIVFNLEKWAFDTYPELSARVDREKVDKFSILKILL
 NNKNINKEQSDILRKIRNAFDHNNYPDKGVVEIKALPEIAMSIIKAFGEYAIMKGS
 LQ (SEQ ID NO:125)

Synthetic: L17E

15 IWLTALKFLGKHAAKHEAKQQLSKL (SEQ ID NO:126)

Synthetic: L17E-Transmembrane

IWLTALKFLGKHAAKHEAKQQLSKLNAVGGQDTQEIVVPHSLPFGKVVVISAILALV
 VLTIIISLIILIMLWQKKPR (SEQ ID NO:127)

Synthetic: KALA

20 WEAKLAKALAKALAKHLAKALAKALKACEA (SEQ ID NO:128)

Synthetic: KALA-Transmembrane

25 WEAKLAKALAKALAKHLAKALAKALKACEANAVGGQDTQEIVVPHSLPFGKVVVIS
 AILALVLTIIISLIILIMLWQKKPR (SEQ ID NO:129)

Synthetic: Vectofusin

KKALLHAALAHLLALAHLLALLKKA (SEQ ID NO:130)

Synthetic: Vectofusin-Transmembrane

30 KKALLHAALAHLLALAHLLALLKKANAVGGQDTQEIVVPHSLPFGKVVVISAILAL
 VLTIIISLIILIMLWQKKPR (SEQ ID NO:131)

Synthetic: Transmembrane Domain

35 NAVGGQDTQEIVVPHSLPFGKVVVISAILALVLTIIISLIILIMLWQKKPR (SEQ ID
 NO:132)

Lactococcus lactis: Nisin A

40 ITSISLCTPGCKTGALMGCNMKTATCHCSIHVSK (SEQ ID NO:133)

Lactococcus lactis NIZO 22186: Nisin Z

ITSISLCTPGCKTGALMGCNMKTATCNCSTIHVSK (SEQ ID NO:134)

Lactococcus lactis subsp. lactis F10: Nisin F

45 ITSISLCTPGCKTGALMGCNMKTATCNCSTVHVSK (SEQ ID NO:135)

Lactococcus lactis 61-14: Nisin Q

ITSISLCTPGCKTGVLGMCNLKTATCNCVHVSK (SEQ ID NO:136)

Streptococcus hyointestinalis: Nisin H

5 FTSISMCTPGCKTGALMTCNYKTATCHCSIKVSK (SEQ ID NO:137)

Streptococcus uberis: Nisin U

ITSKSLCTPGCKTGILMTCPLKTATCGCHFG (SEQ ID NO:138)

Streptococcus uberis: Nisin U2

10 VTSKSLCTPGCKTGILMTCPLKTATCGCHFG (SEQ ID NO:139)

Streptococcus galloyticus subsp. pasteurianus: Nisin P

15 VTSKSLCTPGCKTGILMTCALKTATCGCHFG (SEQ ID NO:140)

***L. lactis* NZ9800: Nisin A S29A**

ITSISLCTPGCKTGALMGCMKTATCHCAIHVSK (SEQ ID NO:141)

***L. lactis* NZ9800: Nisin A S29D**

20 ITSISLCTPGCKTGALMGCMKTATCHCDIHVSK (SEQ ID NO:142)

***L. lactis* NZ9800: Nisin A S29E**

ITSISLCTPGCKTGALMGCMKTATCHCEIHVSK (SEQ ID NO:143)

***L. lactis* NZ9800: Nisin A S29G**

25 ITSISLCTPGCKTGALMGCMKTATCHCGIHVSK (SEQ ID NO:144)

***L. lactis* NZ9800: Nisin A K22T**

30 ITSISLCTPGCKTGALMGCMTTATCHCSIHVSK (SEQ ID NO:145)

***L. lactis* NZ9800: Nisin A N20P**

ITSISLCTPGCKTGALMGCPMKTATCHCSIHVSK (SEQ ID NO:146)

***L. lactis* NZ9800: Nisin A M21V**

35 ITSISLCTPGCKTGALMGCVKTATCHCSIHVSK (SEQ ID NO:147)

***L. lactis* NZ9800: Nisin A K22S**

ITSISLCTPGCKTGALMGCMSTATATCHCSIHVSK (SEQ ID NO:148)

***L. lactis* NZ9800: Nisin Z N20K**

40 ITSISLCTPGCKTGALMGCKMKTATCNCVSIHVSK (SEQ ID NO:149)

***L. lactis* NZ9800: Nisin Z M21K**

45 ITSISLCTPGCKTGALMGCKMKTATCNCVSIHVSK (SEQ ID NO:150)

Relevant RNA Sequences (5'-3')**Synthetic: MS2 Stem Loop spCas9 Scaffold RNA for sgRNA with Terminator Example 1**

5 GUUUUAGAGCUAGGCCAACAUAGAGGAUCACCCAUGUCUGCAGGGCCUAG
 CAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGGCCAACAUAGAGGAUCA
 CCAUGUCUGCAGGGCCAAGUGGCACCGAGUCGGUGCUUUUUUUU (SEQ
 ID NO:151)

Synthetic: MS2 Stem Loop spCas9 Scaffold RNA for sgRNA with Terminator Example 2

10 GUUUUAGAGCUAGGCCAACAUAGAGGAUCACCCAUGUCUGCAGGGCCUAG
 CAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGGCCAACAUAGAGGAUCA
 CCAUGUCUGCAGGGCCAAGUGGCACCGAGUCGGUGCGGGAGCACAUGA
 15 GAUCACCCAUGUGCGACUCCACAGUCACUGGGGAGUCUUCCCUUUUU
 UU (SEQ ID NO:152)

Synthetic: MS2 Stem Loop spCas9 Scaffold RNA for sgRNA with Terminator Example 3

20 GUUUUAGAGCUAUGCUGGAAACAGCAUAGCAAGUUUAAAAUAAGGCUAGUC
 CGUUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGCGGGAGCACAUGAG
 GAUCACCCAUGUGCGACUCCACAGUCACUGGGGAGUCUUCCCUUUUUU
 U (SEQ ID NO:153)

Synthetic: 4xMS2 Stem Loop RNA Scaffold Example

25 UUCUAGAUAUCGAAACAUGAGGAUCACCCAUAUCUGCAGUCGACAUCGA
 AACAUAGAGGAUCACCCAUGUCUGCAGUCGACAUCGAAACAUGAGGAUCAC
 CCAUGUCUGCAGUCGACAUCGAAACAUGAGGAUCACCCAUGUCUGCAGU
 CGACAUCGAAAUCAUAAGCUUCAGAUCAUAUCCUAG (SEQ ID NO:154)

Synthetic: MS2 Stem Loop Example 1

30 ACAUGAGGAUCACCCAUGU (SEQ ID NO:155)

Synthetic: MS2 Stem Loop Example 2

35 ACAUGAGGAUCACCCAUAU (SEQ ID NO:156)

Synthetic: MS2 Stem Loop Example 3

CCACAGUCACUGGG (SEQ ID NO:157)

Synthetic: 2xMS2 Stem Loop Example

40 ACAUGAGGAUCACCCAUGUCUGCAGGGCCUAGCAAGUUAAAAUAAGGCUA
 GUCCGUUAUCAACUUGGCCAACAUAGAGGAUCACCCAUGU (SEQ ID
 NO:158)

Synthetic: 2xPP7 Stem Loop spCas9 Scaffold RNA for sgRNA with Terminator Example

GUUUUAGAGCUAGGCCGGAGCAGACGAUAUGGCGUCGCUCCGGCCUAGC
AAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGGCCGGAGCAGACGAUAU
GGCGUCGCUCCGGCCAAGUGGCACCGAGUCGGUGCUUUUUUU (SEQ ID
NO:159)

5

Synthetic: PP7 Stem Loop Example

GCCGGAGCAGACGAUAUGGCGUCGCUCCGGCC (SEQ ID NO:160)

Synthetic: COM Stem Loop spCas9 Scaffold RNA for sgRNA with Terminator Example

10

GUUUAAAGAGCUAUGCUGGAAACAGCAUAGCAAGUUUAAAUAAGGCUAGUC
CGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGCCUGAAUGCCUGCG
AGCAUCUUUUUUU (SEQ ID NO:161)

15

Synthetic: COM Stem Loop Example

CUGAAUGCCUGCGAGCAUC (SEQ ID NO:162)

Synthetic ZKSCAN1 Circular Splice RNA: Upstream Intron

20

AGUGACAGUGGAGAUUGUACAGUUUUUUUCCUCGAUUUGUCAGGAUUUUU
UUUUUUUUGACGGAGUUUAACUUCUUGUCUCCAGGUAGGAAGUGCAGUG
GCGUAAUCUCGGCUCACUACAACCUCCACCUCUGGGUUAAGCGUUUC
UCCUGCCUCAGCUUUUCCGAGUAGCUGGGAUUACAGGCGCCUGCCACCAU
GCCCUGCUGACUUUUUGAUUUUUUAGUAGAGACGGGGUUUACCAUGUUG
GCCAGGCUGGUCUUGAACUCCUGACCGCAGGCGAUUUGGCCUGCCUCGGC
CUCCCAAAGUGCUGAGAUUACAGGCGUGAGCCACCACCCCGGCCUCAG
GAGCGUUCUGAUAGUGCCUCGAUGUGCUGCCUCCUAUAAAGUGUUAGCA
GCACAGAUACAUUUUUUGUAAAGGUACGUACUAAUGACUUUUUUUUUAUAC
UUCAGG (SEQ ID NO:163)

25

Synthetic ZKSCAN1 Circular Splice RNA: Downstream Intron

30

UAAGAAGCAAGGUUUCAUUUAGGGGAAGGGAAAUGAUUCAGGACGAGAG
UCUUUGUGCUGCUGAGUGCCUGUGAUGAAGAAGCAUGUUAGUCCUGGGC
AACGUAGCGAGACCCCAUCUCUACAAAAAAUAGAAAAUUAGCCAGGUUU
AGUGGGCGACACCUGUGAUUCCAGCUACGCAGGAGGCUGAGGUGGGAGG
AUUGCUUGAGCCCAGGAGGUUGAGGCUGCAGUGAGCUGUAAUCAUGCCA
CUACUCCAACCUGGGCAACACAGCAAGGACCCUGUCUAAAAGCUACUUA
CAGAAAAGAAUUAGGCUCGGCACGGUAGCUCACACCUGUAAUCCCAGCAC
UUUGGGAGGCUGAGGCGGGCAGAUACAUUGAGGUCAGGAGUUUGAGACC
AGCCUGGCCAACAUUGGUGAAACCUUGUCUCUACUAAAAUUAUGAAAAUUA
GCCAGGCAUGGUGGCACAUUCCUGUAAUCCCAGCUACUCGGGAGGCUGA
GGCAGGAGAAUCACUUGAACCCAGGAGGUGGAGGUUGCAGUAAGCCGAG
AUCGUACCACUGUGCUCUAGCCUUGGUGACAGAGCGAGACUGUCUAAA
AAAAAAAAAAAAAAAAAAGAAUUAUUAAAAUUAAAAAAAAAUGAAAA
AAGCUGCAUGCUUGUUUUUUUGUUUUUAGUUUUUACAUUGUUGUCAUU
AUUACCAAUAUUGGGGAAAAUACAACUUACAGACCAAUCUCAGGAGUUA

45

AAUGUUACUACGAAGGCAAUGAACUAUGCGUAAUGAACCUAGGUAGGCAU
UAG (SEQ ID NO:164)

***Homo sapiens Beta-globin and Immunoglobulin Heavy Chain Genes: Linear
Splice RNA Intron***

GUAAGUAUCAAGGUUACAAGACAGGUUUAAGGAGACCAAUAGAAACUGGG
CUUGUCGAGACAGAGAAGACUCUUGCGUUUCUGAUAGGCACCUAUUGGU
CUUACUGACAUCCACUUUGCCUUUCUCUCCACAG (SEQ ID NO:165)

Relevant DNA Sequences (5'-3')

Synthetic: Zinc Finger ZF6/10 Binding Site

GAAGAAGCTGCAGGAGGT (SEQ ID NO:166)

Synthetic: Zinc Finger ZF8/7 Binding Site

GCTGGAGGGGAAGTGGTC (SEQ ID NO:167)

Synthetic: Zinc Finger ZF6/10 & ZF8/7 Binding Site

GAAGAAGCTGCAGGAGGTGCTGGAGGGGAAGTGGTC (SEQ ID NO:168)

Synthetic: Zinc Finger ZF6/10 & ZF8/7 Binding Site 8x Repeat Example

TGAAGAAGCTGCAGGAGGTGCTGGAGGGGAAGTGGTCCGGATCTTGAAGA
AGCTGCAGGAGGTGCTGGAGGGGAAGTGGTCCGGATCTTGAAGAAGCTGC
AGGAGGTGCTGGAGGGGAAGTGGTCCGGATCTTGAAGAAGCTGCAGGAGG
TGCTGGAGGGGAAGTGGTCCGGATCTTGAAGAAGCTGCAGGAGGTGCTGG
AGGGGAAGTGGTCCGGATCTTGAAGAAGCTGCAGGAGGTGCTGGAGGGGA
AGTGGTCCGGATCTTGAAGAAGCTGCAGGAGGTGCTGGAGGGGAAGTGGT
CCGGATCTTGAAGAAGCTGCAGGAGGTGCTGGAGGGGAAGTGGTCC (SEQ
ID NO:169)

Synthetic: Zinc Finger ZF9 Binding Site

GTAGATGGA (SEQ ID NO:170)

Synthetic: Zinc Finger MK10 Binding Site

CGGCGTAGCCGATGTCGCGC (SEQ ID NO:171)

Synthetic: Zinc Finger 268 Binding Site

AAGGGTTCA (SEQ ID NO:172)

Synthetic: Zinc Finger NRE Binding Site

GCGTGGGCG (SEQ ID NO:173)

Synthetic: Zinc Finger 268/NRE or 268//NRE Binding Site Example 1

AAGGGTTCAGCGTGGGCG (SEQ ID NO:174)

Synthetic: Zinc Finger 268/NRE or 268//NRE Binding Site Example 2

AAGGGTTCAGGCGTGGGCG (SEQ ID NO:175)

Synthetic: Zinc Finger 268/NRE or 268//NRE Binding Site Example 3

5 AAGGGTTCAGTGCCTGGGCG (SEQ ID NO:176)

Synthetic: FokI Zinc Finger Nuclease 17-2 & 18-2 Binding Site in GFP

GATCCGCCACAACATCGAGGACGGCA (SEQ ID NO:177)

10 **Human codon optimized *Streptococcus pyogenes* Cas9 (spCas9) NLS**
ATGGACAAGAAGTACAGCATCGGCCTGGACATCGGCACCAACTCTGTGGG
CTGGGCGCGTGATCACCGACGAGTACAAGGTGCCAGCAAGAAATTCAGGT
GCTGGGCAACACCGACCGGCACAGCATCAAGAAGAACCTGATCGGAGCCC
TGCTGTTTCGACAGCGGCGAAACAGCCGAGGCCACCCGGCTGAAGAGAACC
15 GCCAGAAGAAGATACACCAGACGGAAGAACC GGATCTGCTATCTGCAAGAG
ATCTTCAGCAACGAGATGGCCAAGGTGGACGACAGCTTCTTCACAGACTG
GAAGAGTCCTTCTGGTGGAAGAGGATAAGAAGCACGAGCGGCACCCCAT
CTTCGGCAACATCGTGGACGAGGTGGCCTACCACGAGAAGTACCCCAACAT
CTACCACTGAGAAAAGAACTGGTGGACAGCACCAGACAAGGCCGACCTGC
20 GGCTGATCTATCTGGCCCTGGCCACATGATCAAGTTCCGGGGCACTTCC
TGATCGAGGGCGACCTGAACCCCGACAACAGCGACGTGGACAAGCTGTTT
ATCCAGCTGGTGCAGACCTACAACCAGCTGTTTCGAGGAAAACCCCATCAAC
GCCAGCGGCGTGGACGCCAAGGCCATCCTGTCTGCCAGACTGAGCAAGAG
CAGACGGCTGGAAAATCTGATCGCCAGCTGCCCGGCGAGAAGAAGAATG
25 GCCTGTTTCGAAACCTGATTGCCCTGAGCCTGGGCCTGACCCCAACTTCA
AGAGCAACTTCGACCTGGCCGAGGATGCCAACTGCAGCTGAGCAAGGAC
ACCTACGACGACGACCTGGACAACCTGCTGGCCAGATCGGCGACCAAGTA
CGCCGACCTGTTTCTGGCCGCCAAGAACCTGTCCGACGCCATCCTGCTGAG
CGACATCCTGAGAGTGAACACCGAGATCACCAAGGCCCCCTGAGCGCCT
30 CTATGATCAAGAGATACGACGAGCACCACAGGACCTGACCCTGCTGAAAG
CTCTCGTGCGGCAGCAGCTGCCTGAGAAGTACAAAGAGATTTTCTTCGACC
AGAGCAAGAACGGCTACGCCGGCTACATTGACGGCGGAGCCAGCCAGGAA
GAGTTCTACAAGTTCATCAAGCCCATCCTGGAAAAGATGGACGGCACCGAG
GAACTGCTCGTGAAGCTGAACAGAGAGGACCTGCTGCGGAAGCAGCGGAC
35 CTTGACAACGGCAGCATCCCCACCAGATCCACCTGGGAGAGCTGCACG
CCATTCTGCGGCGGCAGGAAGATTTTACCCATTCTGAAGGACAACCGGG
AAAAGATCGAGAAGATCCTGACCTTCCGCATCCCCTACTACGTGGGCCCTC
TGCCAGGGGAAACAGCAGATTGCTGATGACCAGAAAGAGCGAGGAA
ACCATCACCCCTGGAACCTCGAGGAAGTGGTGGACAAGGGCGCTTCCGC
40 CCAGAGCTTCATCGAGCGGATGACCAACTTCGATAAGAACCTGCCAACGA
GAAGGTGCTGCCAAGCACAGCCTGCTGTACGAGTACTTCACCGTGTATAA
CGAGCTGACCAAAGTGAAATACGTGACCGAGGGAATGAGAAAGCCCGCCTT
CCTGAGCGGCGAGCAGAAAAAGGCCATCGTGGACCTGCTGTTCAAGACCA
ACCGGAAAGTGACCGTGAAGCAGCTGAAAGAGGACTACTTCAAGAAAATCG
45 AGTGCTTCGACTCCGTGGAATCTCCGGCGTGGAAGATCGGTTCAACGCCT
CCCTGGGCACATACCACGATCTGCTGAAAATTATCAAGGACAAGGACTTCC

TGGACAATGAGGAAAACGAGGACATTCTGGAAGATATCGTGCTGACCCTGA
CACTGTTTGAGGACAGAGAGATGATCGAGGAACGGCTGAAAACCTATGCCC
ACCTGTTTCGACGACAAAGTGATGAAGCAGCTGAAGCGGCGGAGATACACC
GGCTGGGGCAGGCTGAGCCGGAAGCTGATCAACGGCATCCGGGACAAGC
5 AGTCCGGCAAGACAATCCTGGATTTCTGAAGTCCGACGGCTTCGCCAACA
GAACTTCATGCAGCTGATCCACGACGACAGCCTGACCTTTAAAGAGGACA
TCCAGAAAGCCCAGGTGTCCGGCCAGGGCGATAGCCTGCACGAGCACATT
GCCAATCTGGCCGGCAGCCCCGCCATTAAGAAGGGCATCCTGCAGACAGT
GAAGGTGGTGGACGAGCTCGTGAAAGTGATGGGCCGGCACAAGCCCCGAGA
10 ACATCGTGATCGAAATGGCCAGAGAGAACCAGACCACCCAGAAGGGACAG
AAGAACAGCCGCGAGAGAAATGAAGCGGATCGAAGAGGGCATCAAAGAGCT
GGGCAGCCAGATCCTGAAAGAACACCCCGTGGAACACCCAGCTGCAGA
ACGAGAAGCTGTACCTGTACTACCTGCAGAATGGGCGGGATATGTACGTGG
ACCAGGAAGTGGACATCAACCGGCTGTCCGACTACGATGTGGACCATATCG
15 TGCCTCAGAGCTTTCTGAAGGACGACTCCATCGACAACAAGGTGCTGACCA
GAAGCGACAAGAACCGGGGCAAGAGCGACAACGTGCCCTCCGAAGAGGTC
GTGAAGAAGATGAAGAACTACTGGCGGCAGCTGCTGAACGCCAAGCTGATT
ACCCAGAGAAAGTTTCGACAATCTGACCAAGGCCGAGAGAGGCGGCCTGAG
CGAACTGGATAAGGCCGGCTTCATCAAGAGACAGCTGGTGGAAACCCGGC
20 AGATCACAAAGCACGTGGCACAGATCCTGGACTCCCGGATGAACACTAAGT
ACGACGAGAATGACAAGCTGATCCGGGAAGTGAAAGTGATCACCTGAAGT
CCAAGCTGGTGTCCGATTTCCGGAAGGATTTCCAGTTTTACAAAGTGCGCG
AGATCAACAACCTACCACCACGCCACGACGCCTACCTGAACGCCGTCTGTG
GAACCGCCCTGATCAAAAAGTACCCTAAGCTGGAAAGCGAGTTCGTGTACG
25 GCGACTACAAGGTGTACGACGTGCGGAAGATGATCGCCAAGAGCGAGCAG
GAAATCGGCAAGGCTACCGCCAAGTACTTCTTCTACAGCAACATCATGAACT
TTTTCAAGACCGAGATTACCCTGGCCAACGGCGAGATCCGGAAGCGGCCTC
TGATCGAGACAAACGGCGAAACCGGGGAGATCGTGTGGGATAAGGGCCGG
GATTTTGCCACCGTGCGGAAAGTGCTGAGCATGCCCCAAGTGAATATCGTG
30 AAAAAAGACCGAGGTGCAGACAGGCGGCTTCAGCAAAGAGTCTATCCTGCCC
AAGAGGAACAGCGATAAGCTGATCGCCAGAAAGAAGGACTGGGACCCTAA
GAAGTACGGCGGCTTCGACAGCCCCACCGTGGCCTATTCTGTGCTGGTGG
TGGCCAAAGTGGAAGGGCAAGTCCAAGAACTGAAGAGTGTGAAAGAGC
TGCTGGGGATCACCATCATGGAAAGAAGCAGCTTCGAGAAGAATCCCATCG
35 ACTTTCTGGAAGCCAAGGGCTACAAAGAAGTGAAAAAGGACCTGATCATCA
AGCTGCCTAAGTACTCCCTGTTTCGAGCTGGAAAACGGCCGGAAGAGAATGC
TGGCCTCTGCCGGCGAACTGCAGAAGGGAAACGAACTGGCCCTGCCCTCC
AAATATGTGAACTTCCTGTACCTGGCCAGCCACTATGAGAAGCTGAAGGGC
TCCCCCGAGGATAATGAGCAGAAACAGCTGTTTGTGGAACAGCACAAGCAC
40 TACCTGGACGAGATCATCGAGCAGATCAGCGAGTTCTCCAAGAGAGTGATC
CTGGCCGACGCTAATCTGGACAAAGTGCTGTCCGCCTACAACAAGCACCCG
GATAAGCCCATCAGAGAGCAGGCCGAGAATATCATCCACCTGTTTACCCTG
ACCAATCTGGGAGCCCCTGCCGCCTTCAAGTACTTTGACACCACCATCGAC
CGGAAGAGGTACACCAGCACCAAAGAGGTGCTGGACGCCACCCTGATCCA
45 CCAGAGCATCACCGGCCTGTACGAGACACGGATCGACCTGTCTCAGCTGG
GAGGCGACGGATCCCCCAAGAAGAAGAGGAAAGTCTCGAGCGACTACAAA

GACCATGACGGTGATTATAAAGATCATGACATCGATTACAAGGATGACGATG
ACAAGGCTGCAGGATGA (SEQ ID NO:178)

Human codon optimized *Streptococcus pyogenes* Cas9 (spCas9) Bipartite (BP) NLS

5 ATGGACAAGAAGTACAGCATCGGCCTGGACATCGGCACCAACTCTGTGGG
CTGGGCGCGTGATCACCGACGAGTACAAGGTGCCAGCAAGAAATTCAGGT
GCTGGGCAACACCGACCGGCACAGCATCAAGAAGAACCTGATCGGAGCCC
10 TGCTGTTTCGACAGCGGCGAAACAGCCGAGGCCACCCGGCTGAAGAGAACC
GCCAGAAGAAGATACACCAGACGGAAGAACCAGGATCTGCTATCTGCAAGAG
ATCTTCAGCAACGAGATGGCCAAGGTGGACGACAGCTTCTTCCACAGACTG
GAAGAGTCCTTCTGGTGGAAGAGGATAAGAAGCACGAGCGGCACCCCAT
CTTCGGCAACATCGTGGACGAGGTGGCCTACCACGAGAAGTACCCCAACAT
15 CTACCACCTGAGAAAGAACTGGTGGACAGCACCGACAAGGCCGACCTGC
GGCTGATCTATCTGGCCCTGGCCACATGATCAAGTTCCGGGGCCACTTCC
TGATCGAGGGCGACCTGAACCCCGACAACAGCGACGTGGACAAGCTGTTT
ATCCAGCTGGTGCAGACCTACAACCAGCTGTTTCGAGGAAAACCCCATCAAC
GCCAGCGGCGTGGACGCCAAGGCCATCCTGTCTGCCAGACTGAGCAAGAG
CAGACGGCTGGAAAATCTGATCGCCAGCTGCCCGGCGAGAAGAAGAATG
20 GCCTGTTTCGAAACCTGATTGCCCTGAGCCTGGGCCTGACCCCAACTTCA
AGAGCAACTTCGACCTGGCCGAGGATGCCAACTGCAGCTGAGCAAGGAC
ACCTACGACGACGACCTGGACAACCTGCTGGCCAGATCGGCGACCAAGTA
CGCCGACCTGTTTCTGGCCGCAAGAACCTGTCCGACGCCATCCTGCTGAG
CGACATCCTGAGAGTGAACACCGAGATCAACCAAGGCCCCCTGAGCGCCT
25 CTATGATCAAGAGATACGACGAGCACCACAGGACCTGACCCTGCTGAAAG
CTCTCGTGCGGCAGCAGCTGCCTGAGAAGTACAAAGAGATTTTCTTCGACC
AGAGCAAGAACGGCTACGCCGGCTACATTGACGGCGGAGCCAGCCAGGAA
GAGTTCTACAAGTTCATCAAGCCCATCCTGGAAAAGATGGACGGCACCGAG
GAACTGCTCGTGAAGCTGAACAGAGAGGACCTGCTGCGGAAGCAGCGGAC
30 CTTGACAACGGCAGCATCCCCACCAGATCCACCTGGGAGAGCTGCACG
CCATTCTGCGGCGGCAGGAAGATTTTACCCATTCTGAAGGACAACCGGG
AAAAGATCGAGAAGATCCTGACCTTCCGCATCCCCTACTACGTGGGCCCTC
TGGCCAGGGGAAACAGCAGATTTCGCTGATGACCAGAAAGAGCGAGGAA
ACCATCACCCCTGGAACTTCGAGGAAGTGGTGGACAAGGGCGCTTCCGC
35 CCAGAGCTTCATCGAGCGGATGACCAACTTCGATAAGAACCTGCCCAACGA
GAAGGTGCTGCCAAGCACAGCCTGCTGTACGAGTACTTCACCGTGTATAA
CGAGCTGACCAAAGTGAAATACGTGACCGAGGGAATGAGAAAGCCCGCCTT
CCTGAGCGGCGAGCAGAAAAAGGCCATCGTGGACCTGCTGTTCAAGACCA
ACCGGAAAGTGACCGTGAAGCAGCTGAAAGAGGACTACTTCAAGAAAATCG
40 AGTGCTTCGACTCCGTGGAAATCTCCGGCGTGAAGATCGGTTCAACGCCT
CCCTGGGCACATACCACGATCTGCTGAAAATTATCAAGGACAAGGACTTCC
TGGACAATGAGGAAAACGAGGACATTCTGGAAGATATCGTGCTGACCCTGA
CACTGTTTGAGGACAGAGAGATGATCGAGGAACGGCTGAAAACCTATGCCC
ACCTGTTTCGACGACAAAGTGATGAAGCAGCTGAAGCGGCGGAGATACACC
45 GGCTGGGGCAGGCTGAGCCGGAAGCTGATCAACGGCATCCGGGACAAGC
AGTCCGGCAAGACAATCCTGGATTTCTGAAGTCCGACGGCTTCGCCAACA

GAAACTTCATGCAGCTGATCCACGACGACAGCCTGACCTTTAAAGAGGACA
TCCAGAAAGCCCAGGTGTCCGGCCAGGGCGATAGCCTGCACGAGCACATT
GCCAATCTGGCCGGCAGCCCCGCCATTAAGAAGGGGCATCCTGCAGACAGT
5 GAAGGTGGTGGACGAGCTCGTGAAAGTGATGGGCCGGCACAAGCCCCGAGA
ACATCGTGATCGAAATGGCCAGAGAGAACCAGACCACCCAGAAGGGACAG
AAGAACAGCCGCGAGAGAATGAAGCGGATCGAAGAGGGGCATCAAAGAGCT
GGGCAGCCAGATCCTGAAAGAACACCCCGTGGAAAACACCCAGCTGCAGA
ACGAGAAGCTGTACCTGTACTACCTGCAGAATGGGCGGGATATGTACGTGG
10 ACCAGGAACTGGACATCAACCGGCTGTCCGACTACGATGTGGACCATATCG
TGCCTCAGAGCTTTCTGAAGGACGACTCCATCGACAACAAGGTGCTGACCA
GAAGCGACAAGAACCGGGGCAAGAGCGACAACGTGCCCTCCGAAGAGGTC
GTGAAGAAGATGAAGAACTACTGGCGGCAGCTGCTGAACGCCAAGCTGATT
ACCCAGAGAAAGTTTCGACAATCTGACCAAGGCCGAGAGAGGGCGGCCTGAG
CGAACTGGATAAGGCCGGCTTCATCAAGAGACAGCTGGTGGAAACCCGGC
15 AGATCACAAAGCACGTGGCACAGATCCTGGACTCCCGGATGAACACTAAGT
ACGACGAGAATGACAAGCTGATCCGGGAAGTGAAAGTGATCACCTGAAGT
CCAAGCTGGTGTCCGATTTCCGGAAGGATTTCCAGTTTTACAAAGTGCGCG
AGATCAACAACCTACCACCACGCCACGACGCCTACCTGAACGCCGTCTGTGG
GAACCGCCCTGATCAAAAAGTACCCTAAGCTGGAAAGCGAGTTCTGTGTACG
20 GCGACTCAAGGTGTACGACGTGCGGAAGATGATCGCCAAGAGCGCAG
GAAATCGGCAAGGCTACCGCCAAGTACTTCTTCTACAGCAACATCATGA
TTTTCAAGACCGAGATTACCCTGGCCAACGGCGAGATCCGGAAGCGGCCTC
TGATCGAGACAAACGGCGAAACCGGGGAGATCGTGTGGGATAAGGGCCGG
GATTTTGCCACCGTGCGGAAAGTGCTGAGCATGCCCAAGTGAATATCGTG
25 AAAAAAGACCGAGGTGCAGACAGGCGGCTTCAGCAAAGAGTCTATCCTGCC
AAGAGGAACAGCGATAAGCTGATCGCCAGAAAGAAGGACTGGGACCCTAA
GAAGTACGGCGGCTTCGACAGCCCCACCGTGGCCTATTCTGTGCTGGTGG
TGGCCAAAGTGGAAGAGGGCAAGTCCAAGAACTGAAGAGTGTAAGAGC
TGCTGGGGATCACCATCATGGAAAGAAGCAGCTTCGAGAAGAATCCCATCG
30 ACTTTCTGGAAGCCAAGGGCTACAAAGAAGTGAAAAAGGACCTGATCATCA
AGCTGCCTAAGTACTCCCTGTTTCGAGCTGGAAAACGGCCGGAAGAGAATGC
TGGCCTCTGCCGGCGAACTGCAGAAGGGGAAACGAACTGGCCCTGCCCTCC
AAATATGTGAACTTCCTGTACCTGGCCAGCCACTATGAGAAGCTGAAGGGC
TCCCCCGAGGATAATGAGCAGAAACAGCTGTTTGTGGAACAGCACAAGCAC
35 TACCTGGACGAGATCATCGAGCAGATCAGCGAGTTCTCCAAGAGAGTGATC
CTGGCCGACGCTAATCTGGACAAAGTGCTGTCCGCCTACAACAAGCACCCG
GATAAGCCCATCAGAGAGCAGGCCGAGAATATCATCCACCTGTTTACCCTG
ACCAATCTGGGAGCCCCTGCCGCCTTCAAGTACTTTGACACCACCATCGAC
CGGAAGAGGTACACCAGCACCAAGAGGTGCTGGACGCCACCCTGATCCA
40 CCAGAGCATCACCGGCCTGTACGAGACACGGATCGACCTGTCTCAGCTGG
GAGGCGACGGATCCGGCGGAGGCGGAAGCGGGAAAAGAACCGCCGACGG
CAGCGAATTCGAGCCCAAGAAGAAGAGGAAAAGTCTCGAGCGGAGGCGACT
ACAAAGACCATGACGGTGATTATAAAGATCATGACATCGATTACAAGGATGA
CGATGACAAGTGA (SEQ ID NO:179)

45

Human codon optimized *Streptococcus pyogenes* Cas9 (spCas9) BE4

ATGAAACGGACAGCCGACGGAAGCGAGTTCGAGTCACCAAAGAAGAAGCG
GAAAGTCTCCTCAGAGACTGGGCCTGTCGCCGTCGATCCAACCCTGCGCC
GCCGGATTGAACCTCACGAGTTTGAAGTGTTCTTTGACCCCCGGGAGCTGA
5 GAAAGGAGACATGCCTGCTGTACGAGATCAACTGGGGAGGCAGGCACTCC
ATCTGGAGGCACACCTCTCAGAACACAAATAAGCACGTGGAGGTGAACTTC
ATCGAGAAGTTTACCACAGAGCGGTACTTCTGCCCCAATACCAGATGTAGC
ATCACATGGTTTCTGAGCTGGTCCCCTTGCGGAGAGTGTAGCAGGGCCATC
ACCGAGTTCCTGTCCAGATATCCACACGTGACACTGTTTATCTACATCGCCA
10 GGCTGTATCACCACGCAGACCCAAGGAATAGGCAGGGCCTGCGCGATCTG
ATCAGCTCCGGCGTGACCATCCAGATCATGACAGAGCAGGAGTCCGGCTA
CTGCTGGCGGAACCTTCGTGAATTATTCTCCTAGCAACGAGGCCCACTGGCC
TAGGTACCCACACCTGTGGGTGCGCCTGTACGTGCTGGAGCTGTATTGCAT
CATCCTGGGCCTGCCCCCTTGTCTGAATATCCTGCGGAGAAAGCAGCCCCA
15 GCTGACCTTCTTTACAATCGCCCTGCAGTCTTGTCACTATCAGAGGCTGCCA
CCCCACATCCTGTGGGCCACAGGCCTGAAGTCTGGAGGATCTAGCGGAGG
ATCCTCTGGCAGCGAGACACCAGGAACAAGCGAGTCAGCAACACCAGAGA
GCAGTGGCGGCAGCAGCGGCGGAGCGACAAGAAGTACAGCATCGGCCT
GGACATCGGCACCAACTCTGTGGGTGGGCCGTGATCACCAGCAGGTACA
20 AGGTGCCCAGCAAGAAATTCAAGGTGCTGGGCAACACCGACCGGCACAGC
ATCAAGAAGAACCTGATCGGAGCCCTGCTGTTTCGACAGCGGCGAAACAGC
CGAGGCCACCCGGCTGAAGAGAACCGCCAGAAAGAAGATACACCAGACGGA
AGAACCGGATCTGCTATCTGCAAGAGATCTTCAGCAACGAGATGGCCAAGG
TGGACGACAGCTTCTTCCACAGACTGGAAGAGTCCTTCTGGTGGAAAGAGG
25 ATAAGAAGCACGAGCGGCACCCCATCTTCGGCAACATCGTGGACGAGGTG
GCCTACCACGAGAAGTACCCACCATCTACCACCTGAGAAAGAACTGGTG
GACAGCACCGACAAGGCCGACCTGCGGCTGATCTATCTGGCCCTGGCCCA
CATGATCAAGTTCCGGGGCCACTTCTGATCGAGGGCGACCTGAACCCCG
ACAACAGCGACGTGGACAAGCTGTTTCATCCAGCTGGTGCAGACCTACAACC
30 AGCTGTTTCGAGGAAAACCCCATCAACGCCAGCGGCGTGGACGCCAAGGCC
ATCCTGTCTGCCAGACTGAGCAAGAGCAGACGGCTGGAAAATCTGATCGCC
CAGCTGCCCGGCGAGAAGAAGAATGGCCTGTTTCGGAACCTGATTGCCCT
GAGCCTGGGCCTGACCCCCAACTTCAAGAGCAACTTCGACCTGGCCGAGG
ATGCCAAACTGCAGCTGAGCAAGGACACCTACGACGACGACCTGGACAAC
35 CTGCTGGCCCAGATCGGCGACCAAGTACGCCGACCTGTTTCTGGCCGCCAA
GAACCTGTCCGACGCCATCCTGCTGAGCGACATCCTGAGAGTGAACACCGA
GATACCAAGGCCCCCCCTGAGCGCCTCTATGATCAAGAGATACGACGAGCA
CCACCAGGACCTGACCCTGCTGAAAGCTCTCGTGCGGCAGCAGCTGCCTG
AGAAGTACAAAGAGATTTTCTTCGACCAGAGCAAGAACGGCTACGCCGGCT
40 ACATTGACGGCGGAGCCAGCCAGGAAGAGTTCTACAAGTTCATCAAGCCCA
TCCTGGAAAAGATGGACGGCACCGAGGAACTGCTCGTGAAGCTGAACAGA
GAGGACCTGCTGCGGAAGCAGCGGACCTTCGACAACGGCAGCATCCCCCA
CCAGATCCACCTGGGAGAGCTGCACGCCATTCTGCGGCGGCAGGAAGATT
TTTACCCATTCTGAAGGACAACCGGGAAAAGATCGAGAAGATCCTGACCT
45 TCCGCATCCCCTACTACGTGGGCCCTCTGGCCAGGGGAAACAGCAGATTC
GCCTGGATGACCAGAAAGAGCGAGGAAACCATCACCCCTGGAACCTTCGA

GGAAGTGGTGGACAAGGGCGCTTCCGCCCAGAGCTTCATCGAGCGGATGA
CCAACTTCGATAAGAACCTGCCCAACGAGAAGGTGCTGCCCAAGCACAGCC
TGCTGTACGAGTACTTCACCGTGTATAACGAGCTGACCAAAGTGAAATACGT
GACCGAGGGAATGAGAAAGCCCGCCTTCTGAGCGGCGAGCAGAAAAAGG
5 CCATCGTGGACCTGCTGTTCAAGACCAACCGGAAAGTGACCGTGAAGCAGC
TGAAAGAGGACTACTTCAAGAAAATCGAGTGCTTCGACTCCGTGGAAATCTC
CGGCGTGGAAAGATCGGTTCAACGCCTCCCTGGGCACATACCACGATCTGCT
GAAAATTATCAAGGACAAGGACTTCTGGACAATGAGGAAAACGAGGACAT
TCTGGAAGATATCGTGCTGACCCTGACACTGTTTGAGGACAGAGAGATGAT
10 CGAGGAACGGCTGAAAACCTATGCCACCTGTTTCGACGACAAAAGTGATGAA
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15 GGGCGATAGCCTGCACGAGCACATTGCCAATCTGGCCGGCAGCCCCGCCA
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 20 TCTAA (SEQ ID NO:180)

Human codon optimized *Streptococcus pyogenes* Cas9 (spCas9) ABE

ATGAAACGGACAGCCGACGGAAGCGAGTTCGAGTCACCAAAGAAGAAGCG
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 25 GACCCTGGCAAAGCGAGCATGGGATGAAAGAGAAGTCCCCGTGGGCGCCG
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 CAGAGGGAATCCTGGCAGACGAGTGCGCCGCCCTGCTGAGCGATTTCTTTA
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5 ACATCAACCGGCTGTCCGACTACGATGTGGACCATATCGTGCCTCAGAGCT
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GCTAATCTGGACAAAAGTGCTGTCCGCCTACAACAAGCACCGGGATAAGCCC
ATCAGAGAGCAGGCCGAGAATATCATCCACCTGTTTACCCTGACCAATCTG
GGAGCCCCTGCCGCCTTCAAGTACTTTGACACCACCATCGACCGGAAGAG
35 GTACACCAGCACCAAGAGGTGCTGGACGCCACCCTGATCCACCAGAGCA
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40 **Human codon optimized VSVG**

ATGAAATGTCTGCTGTACCTGGCTTTCCTGTTTCATCGGCGTGAAGTGAAGT
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45 AGGCCGACGGATGGATGTGCCACGCCAGCAAGTGGGTCAACCTGTGAC
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 GGCTGAACCCCGGCTTCCCCCCCCAGAGCTGCGGCTACGCCACCGTTACC
 GATGCCGAGGCCGTGATCGTGCAGGTGACACCTCACCACGTGCTGGTCTGA
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 5 CAATTACATCTGCCCCACCGTGCACAACAGCACCACTGGCACAGCGATTA
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 10 ATGGCCGATAAGGACCTGTTGCGCGCTGCCAGATTCCCAGAGTGCCCTGA
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 15 GAAATACTTCGAGACCAGATACATCCGGGTGGACATCGCCGCTCCTATCCT
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 20 CTCATATCCAAGACGCGCCAGCCAGCTGCCTGATGACGAGAGCCTGTTTT
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 TCATCGGACTGTTCTGCTGAGAGTGGGGATCCACCTGTGCATCAAGC
 TGAAGCACACCAAAAAGAGACAGATCTACACCGACATCGAGATGAACCGGC
 25 TGGGGAAGTGA (SEQ ID NO:182)

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OTHER EMBODIMENTS

It is to be understood that while the invention has been described in conjunction with the detailed description thereof, the foregoing description is intended to illustrate and not limit the scope of the invention, which is defined by the scope of the appended claims. Other aspects, advantages, and modifications are within the scope of the following claims.

WHAT IS CLAIMED IS:

1. An enhanced virus-like particle (eVLP), comprising:
a membrane comprising a phospholipid bilayer with one or more virally-derived glycoproteins, optionally a glycoprotein as shown in Table 1, on the external side; and
a cargo disposed in the core of the eVLP on the inside of the membrane,
wherein the eVLP does not comprise an exogenous gag and/or pol protein.
2. The eVLP of claim 1, wherein the cargo is a therapeutic or diagnostic protein or nucleic acid encoding a therapeutic or diagnostic protein, or a chemical, optionally a small molecule therapeutic or diagnostic.
3. The eVLP of claim 1, wherein the cargo is a gene editing reagent.
4. The eVLP of claim 1, wherein the gene editing reagent comprises a zinc finger (ZF), transcription activator-like effector (TALE), and/or CRISPR-based genome editing or modulating protein; a nucleic acid encoding a zinc finger (ZF), transcription activator-like effector (TALE), and/or CRISPR-based genome editing or modulating protein; or a ribonucleoprotein complex (RNP) comprising a CRISPR-based genome editing or modulating protein.
5. The eVLP of claim 4, wherein the gene editing reagent is selected from the proteins listed in Tables 2, 3, 4 & 5.
6. The eVLP of claim 4, wherein the gene editing reagent comprises a CRISPR-based genome editing or modulating protein, and the eVLP further comprises one or more guide RNAs that bind to and direct the CRISPR-based genome editing or modulating protein to a target sequence.
7. The eVLP of claims 1-6, wherein the cargo comprises a fusion to a plasma membrane recruitment domain, preferably as shown in Table 6.

8. A method of delivering a cargo to a target cell, optionally a cell *in vivo* or *in vitro*, the method comprising contacting the cell with the eVLP of claims 1-7 comprising the cargo.
9. A method of producing an eVLP comprising a cargo, the method comprising:
providing a cell expressing one or more virally-derived glycoproteins (optionally as shown in Table 1), and a cargo, wherein the cell does not express an exogenous gag and/or pol protein; and
maintaining the cell under conditions such that the cells produce eVLPs.
10. The method of claim 9, further comprising harvesting and optionally purifying and/or concentrating the produced eVLPs.
11. The method of claim 9, wherein the cargo is a therapeutic or diagnostic protein or nucleic acid encoding a therapeutic or diagnostic protein, or a small molecule, optionally a therapeutic or diagnostic small molecule.
12. The method of claim 9, wherein the cargo is a gene editing reagent.
13. The method of claim 9, wherein the gene editing reagent comprises a zinc finger (ZF), transcription activator-like effector (TALE), and/or CRISPR-based genome editing or modulating protein; a nucleic acid encoding a zinc finger (ZF), transcription activator-like effector (TALE), and/or CRISPR-based genome editing or modulating protein; or a ribonucleoprotein complex (RNP) comprising a CRISPR-based genome editing or modulating protein.
14. The method of claim 13, wherein the gene editing reagent is selected from the proteins listed in Tables 2, 3, 4 & 5.
15. The method of claim 13, wherein the gene editing reagent comprises a CRISPR-based genome editing or modulating protein, and the eVLP further comprises one or more guide RNAs that bind to and direct the CRISPR-based genome editing or modulating protein to a target sequence.

16. The method of claim 9-15, wherein the cargo comprises a fusion to a plasma membrane recruitment domain, preferably as shown in Table 6.
17. A cell expressing one or more virally-derived glycoproteins (optionally as shown in Table 1), and a cargo, wherein the cell does not express an exogenous gag protein.
18. The cell of claim 17, wherein the cargo is a therapeutic or diagnostic protein or nucleic acid encoding a therapeutic or diagnostic protein, or a small molecule, optionally a therapeutic or diagnostic small molecule.
19. The cell of claim 17, wherein the cargo is a gene editing reagent.
20. The cell of claim 17, wherein the gene editing reagent comprises a zinc finger (ZF), transcription activator-like effector (TALE), and/or CRISPR-based genome editing or modulating protein; a nucleic acid encoding a zinc finger (ZF), transcription activator-like effector (TALE), and/or CRISPR-based genome editing or modulating protein; or a ribonucleoprotein complex (RNP) comprising a CRISPR-based genome editing or modulating protein.
21. The cell of claim 20, wherein the gene editing reagent is selected from the proteins listed in Tables 2, 3, 4 & 5.
22. The cell of claim 20, wherein the gene editing reagent comprises a CRISPR-based genome editing or modulating protein, and the eVLP further comprises one or more guide RNAs that bind to and direct the CRISPR-based genome editing or modulating protein to a target sequence.
23. The cells of claims 17-22, wherein the cargo comprises a fusion to a plasma membrane recruitment domain, preferably as shown in Table 6.
24. The cells of claims 17-23, wherein the cells are primary or stable human cell lines.
25. The cells of claim 24, which are Human Embryonic Kidney (HEK) 293 cells or HEK293 T cells.