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.

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.

SEQUENCE LISTING

The instant application contains a Sequence Listing which has been submitted electronically in ASCII format and is hereby incorporated by reference in its entirety. Said ASCII copy, created on September 10, 2021, is named 29539-0358WO1_SLL.txt and is 452,609 bytes in size.

TECHNICAL FIELD

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 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.

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 $CaCl_2$ 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 of 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 potently 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 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 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 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 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.

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.

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.

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.

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.

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.

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

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 hPLCδ1 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. 8 discloses SEQ ID NO: 186.

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. 9 discloses SEQ ID NO: 183.

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. 10 discloses SEQ ID NOS 184, 184 and 184, respectively, in order of appearance.

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. 11 discloses SEQ ID NOS 185, 185 and 185, respectively, in order of appearance.

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. 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 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 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 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 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 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.

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 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, βγ-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 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, 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 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 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 expressed 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 a 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 to as type 2 cargo (T2eVLPs), a combination of DNA and proteins will be referred to herein to as type 3 cargo (T3eVLPs), and proteins will be referred to herein to 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 #3and 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). 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, 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 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 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 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 (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 $CaCl_2$ 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 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, 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.

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 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), 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

(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

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 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 0.8μm pore size mixed cellulose esters hydrophilic membrane (Millipore Millex-AA). After filtration, the supernatant can be further purified and 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 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 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

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% CO_2).

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 polyetheylene 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 dH_2O 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 µm cellulose acetate or 0.8 µm 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 µg/mL in cell culture medium; Sigma-Aldrich #TR-1003-G) was supplemented to enhance transduction efficiency, if necessary. Vectofusin-1 (10 µg/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 hPLCδ1 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, hPLCδ1 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, hPLCδ1 (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, hPLCδ1 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, hPLCδ1 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, hPLCδ1 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 hPLCδ1 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, hPLCδ1 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, hPLCδ1 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.

In FIG. 15, hPLCδ1 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 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.

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
lbCas12a	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’-TY<u>C</u>
hAPOBEC3A	5’-T<u>C</u>G
hAPOBEC3B	5'-T<u>C</u>R > T<u>C</u>T
hAPOBEC3C	5’-WY<u>C</u>
hAPOBEC3F	5’-TT<u>C</u>
hAPOBEC3G	5’-CC<u>C</u>
hAPOBEC3H	5’-TT<u>C</u>A ~ TT<u>C</u>T ~ TT<u>C</u>G > AC<u>C</u>CA > TG<u>C</u>A
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

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

TABLE 6 | Exemplary plasma membrane recruitment domains

#	Plasma membrane recruitment domain	Substitution(s)
1.	Pleckstrin homology domain of human phospholipase Cδ1 (hPLCδ1)	
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 (hPDPK1)	
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**
MQTGRTEDDLVPTAPSLGTKEGYLTKQGGLVKTWKTRWFTLHRNELKYFKDQMSPEP
IRILDLTECSAVQFDYSQERVNCFCLVFPFRTFYLCAKTGVEADEWIKILRWKLSQIRKQ
LNQGEGTIR (SEQ ID NO:1)

***Mus musculus*: Pleckstrin homology domain of Mouse Grp1**
PFKIPEDDGNDLTHTFFNPDREGWLLKLGGRVKTWKRRWFILTDNCLYYFEYTTDKEP
RGIIPLENLSIREVEDPRKPNCFELYNPSHKGQVIKACKTEADGRVVEGNHVVYRISAPS
PEEKEEWMKSIKASISRDPFYDMLATRKRRIANKK (SEQ ID NO:2)

***Homo sapiens*: Pleckstrin homology domain of Human Grp1**
NPDREGWLLKLGGGRVKTWKRRWFILTDNCLYYFEYTTDKEPRGIIPLENLSIREVEDP
RKPNCFELYNPSHKGQVIKACKTEADGRVVEGNHVVYRISAPSPEEKEEWMKSIKASIS
(SEQ ID NO:3)

***Homo sapiens*: Pleckstrin homology domain of Human OSBP**
SGSAREGWLFKWTNYIKGYQRRWFVLSNGLLSYYRSKAEMRHTCRGTINLATANITVE
DSCNFIISNGGAQTYHLKASSEVERQRWVTALELAKAKAVK (SEQ ID NO:4)

***Homo sapiens*: Pleckstrin homology domain of Human Btk1**
MAAVILESIFLKRSQQKKKTSPLNFKKRLFLLTVHKLSYYEYDFERGRRGSKKGSIDVEKI
TCVETVVPEKNPPPERQIPRRGEESSEMEQISIIERFPYPFQVVYDEGPLYVFSPTEELR
KRWIHQLKNVIRYNSDLVQKYHPCFWIDGQYLCCSQTAKNAMGCQILENRNGSLKP
(SEQ ID NO:5)

***Homo sapiens*: Pleckstrin homology domain of Human FAPP1**
MEGVLYKWTNYLTGWQPRWFVLDNGILSYYDSQDDVCKGSKGSIKMAVCEIKVHSAD
NTRMELIIPGEQHFYMKAVNAAERQRWLVALGSSKACLTDT (SEQ ID NO:6)

***Homo sapiens*: Pleckstrin homology domain of Human CERT**
MSDNQSWNSSGSEEDPETESGPPVERCGVLSKWTNYIHGWQDRWVVLKNNALSYYK
SEDETEYGCRGSICLSKAVITPHDFDECRFDISVNDSVWYLRAQDPDHRQQWIDAIEQH
KTESGYG (SEQ ID NO:7)

***Homo sapiens*: Pleckstrin homology domain of Human PKD**
TVMKEGWMVHYTSKDTLRKRHYWRLDSKCITLFQNDTGSRYYKEIPLSEILSLEPVKTS
ALIPNGANPHCFEITTANVVYYVGENVVNPSSPSPNNSVLTSGVGADVARMWEIAIQHA
LM (SEQ ID NO:8)

***Homo sapiens*: Pleckstrin homology domain of Human PHLPP1**
RIQLSGMYNVRKGKMQLPVNRWTRRQVILCGTCLIVSSVKDSLTGKMHVLPLIGGKVEE
VKKHQHCLAFSSSGPQSQTYYICFDTFTEYLRWLRQVSKVAS (SEQ ID NO:9)

***Homo sapiens*: Pleckstrin homology domain of Human SWAP70**
DVLKQGYMMKKGHRRKNWTERWFVLKPNIISYYVSEDLKDKKGDILLDENCCVESLPD
KDGKKCLFLVKCFDKTFEISASDKKKKQEWIQAIHSTIH (SEQ ID NO:10)

***Homo sapiens*: Pleckstrin homology domain of Human MAPKAP1**
DMLSSHHYKSFKVSMIHRLRFTTDVQLGISGDKVEIDPVTNQKASTKFWIKQKPISIDSD
LLCACDLAEEKSPSHAIFKLTYLSNHDYKHLYFESDAATVNEIVLKVNYILES (SEQ ID
NO:11)

Baboon Endogenous Retrovirus glycoprotein (BaEVTR)
MGFTTKIIFLYNLVLVYAGFDDPRKAIELVQKRYGRPCDCSGGQVSEPPSDRVSQVTCS
GKTAYLMPDQRWKCKSIPKDTSPSGPLQECPCNSYQSSVHSSCYTSYQQCRSGNKTY
YTATLLKTQTGGTSDVQVLGSTNKLIQSPCNGIKGQSICWSTTAPIHVSDGGGPLDTTRI
KSVQRKLEEIHKALYPELQYHPLAIPKVRDNLMVDAQTLNILNATYNLLLMSNTSLVDDC
WLCLKLGPPTPLAIPNFLLSYVTRSSDNISCLIIPPLLVQPMQFSNSSCLFSPSYNSTEEI
DLGHVAFSNCTSITNVTGPICAVNGSVFLCGNNMAYTYLPTNWTGLCVLATLLPDIDIIP
GDEPVPIPAIDHFIYRPKRAIQFIPLLAGLGITAAFTTGATGLGVSVTQYTKLSNQLISDVQI
LSSTIQDLQDQVDSLAEVVLQNRRGLDLLTAEQGGICLALQEKCCFYVNKSGIVRDKIKT
LQEELERRRKDLASNPLWTGLQGLLPYLLPFLGPLLTLLLLLTIGPCIFNRLVQFVKDRIS
VVQALVLTQQYHQLKPLEYEP (SEQ ID NO:12)

Modified Baboon Endogenous Retrovirus glycoprotein (BaEVTRless)
MGFTTKIIFLYNLVLVYAGFDDPRKAIELVQKRYGRPCDCSGGQVSEPPSDRVSQVTCS
GKTAYLMPDQRWKCKSIPKDTSPSGPLQECPCNSYQSSVHSSCYTSYQQCRSGNKTY
YTATLLKTQTGGTSDVQVLGSTNKLIQSPCNGIKGQSICWSTTAPIHVSDGGGPLDTTRI
KSVQRKLEEIHKALYPELQYHPLAIPKVRDNLMVDAQTLNILNATYNLLLMSNTSLVDDC
WLCLKLGPPTPLAIPNFLLSYVTRSSDNISCLIIPPLLVQPMQFSNSSCLFSPSYNSTEEI
DLGHVAFSNCTSITNVTGPICAVNGSVFLCGNNMAYTYLPTNWTGLCVLATLLPDIDIIP
GDEPVPIPAIDHFIYRPKRAIQFIPLLAGLGITAAFTTGATGLGVSVTQYTKLSNQLISDVQI
LSSTIQDLQDQVDSLAEVVLQNRRGLDLLTAEQGGICLALQEKCCFYVNKSGIVRDKIKT

LQEELERRRKDLASNPLWTGLQGLLPYLLPFLGPLLTLLLLLTIGPCIFNRLTAFINDKLNII
HAM (SEQ ID NO:13)

Fusion protein of Vesicular stomatitis Indiana virus and Rabies virus Glycoproteins (FuG-E)

MVPQVLLFVLLLGFSLCFGKFPIYTIPDELGPWSPIDIHHLSCPNNLVVEDEGCTNLSEFS
YMELKVGYISAIKVNGFTCTGVVTEAETYTNFVGYVTTTFKRKHFRPTPDACRAAYNWK
MAGDPRYEESLHNPYPDYHWLRTVRTTKESLIIISPSVTDLDPYDKSLHSRVFPGGKCS
GITVSSTYCSTNHDYTIWMPENPRPRTPCDIFTNSRGKRASNGNKTCGFVDERGLYKS
LKGACRLKLCGVLGLRLMDGTWVAMQTSDETKWCPPDQLVNLHDFRSDEIEHLVVEE
LVKKREECLDALESIMTTKSVSFRRLSHLRKLVPGFGKAYTIFNKTLMEADAHYKSVRT
WNEIIPSKGCLKVGGRCHPHVNGVFFNGIILGPDDHVLIPEMQSSLLQQHMELLESSVIP
LMHPLADPSTVFKEGDEAEDFVEVHLPKNPIELVEGWFSSWKSSIASFFFIIGLIIGLFLVL
RVGIHLCIKLKHTKKRQIYTDIEMNRLGK (SEQ ID NO:14)

Modified Fusion protein of Vesicular stomatitis Indiana virus and Rabies virus Glycoproteins (FuG-E (P440E))

MVPQVLLFVLLLGFSLCFGKFPIYTIPDELGPWSPIDIHHLSCPNNLVVEDEGCTNLSEFS
YMELKVGYISAIKVNGFTCTGVVTEAETYTNFVGYVTTTFKRKHFRPTPDACRAAYNWK
MAGDPRYEESLHNPYPDYHWLRTVRTTKESLIIISPSVTDLDPYDKSLHSRVFPGGKCS
GITVSSTYCSTNHDYTIWMPENPRPRTPCDIFTNSRGKRASNGNKTCGFVDERGLYKS
LKGACRLKLCGVLGLRLMDGTWVAMQTSDETKWCPPDQLVNLHDFRSDEIEHLVVEE
LVKKREECLDALESIMTTKSVSFRRLSHLRKLVPGFGKAYTIFNKTLMEADAHYKSVRT
WNEIIPSKGCLKVGGRCHPHVNGVFFNGIILGPDDHVLIPEMQSSLLQQHMELLESSVIP
LMHPLADPSTVFKEGDEAEDFVEVHLEKNPIELVEGWFSSWKSSIASFFFIIGLIIGLFLVL
RVGIHLCIKLKHTKKRQIYTDIEMNRLGK (SEQ ID NO:15)

Amphotrophic Murine Leukemia Virus (MLV ENV (amphotropic))

MARSTLSKPPQDKINPWKPLIVMGVLLGVGMAESPHQVFNVTWRVTNLMTGRTANAT
SLLGTVQDAFPKLYFDLCDLVGEEWDPSDQEPYVGYGCKYPAGRQRTRTFDFYVCPG
HTVKSGCGGPGEGYCGKWGCETTGQAYWKPTSSWDLISLKRGNTPWDTGCSKVAC
GPCYDLSKVSNSFQGATRGGRCNPLVLEFTDAGKKANWDGPKSWGLRLYRTGTDPIT
MFSLTRQVLNVGPRVPIGPNPVLPDQRLPSSPIEIVPAPQPPSPLNTSYPPSTTSTPSTS
PTSPSVPQPPPGTGDRLLALVKGAYQALNLTNPDKTQECWLCLVSGPPYYEGVAVVG
TYTNHSTAPANCTATSQHKLTLSEVTGQGLCMGAVPKTHQALCNTTQSAGSGSYYLAA
PAGTMWACSTGLTPCLSTTVLNLTTDYCVLVELWPRVIYHSPDYMYGQLEQRTKYKRE
PVSLTLALLLGGLTMGGIAAGIGTGTTALIKTQQFEQLHAAIQTDLNEVEKSITNLEKSLTS
LSEVVLQNRRGLDLLFLKEGGLCAALKEECCFYADHTGLVRDSMAKLRERLNQRQKLF
ETGQGWFEGLFNRSPWFTTLISTIMGPLIVLLLILLFGPCILNRLVQFVKDRISVVQALVLT
QQYHQLKPIEYEP (SEQ ID NO:16)

Ecotrophic Murine Leukemia Virus (MLV ENV (Ecotropic))

MARSTLSKPLKNKVNPRGPLIPLILLMLRGVSTASPGSSPHQVYNITWEVTNGDRETVW
ATSGNHPLWTWWPDLTPDLCMLAHHGPSYWGLEYQSPFSSPPGPPCCSGGSSPGC
SRDCEEPLTSLTPRCNTAWNRLKLDQTTHKSNEGFYVCPGPHRPRESKSCGGPDSFY
CAYWGCETTGRAYWKPSSSWDFITVNNNLTSDQAVQVCKDNKWCNPLVIRFTDAGRR

VTSWTTGHYWGLRLYVSGQDPGLTFGIRLRYQNLGPRVPIGPNPVLADQQPLSKPKPV
KSPSVTKPPSGTPLSPTQLPPAGTENRLLNLVDGAYQALNLTSPDKTQECWLCLVAGP
PYYEGVAVLGTYSNHTSAPANCSVASQHKLTLSEVTGQGLCIGAVPKTHQALCNTTQT
SSRGSYYLVAPTGTMWACSTGLTPCISTTILNLTTDYCVLVELWPRVTYHSPSYVYGLF
ERSNRHKREPVSLTLALLLGGLTMGGIAAGIGTGTTALMATQQFQQLQAAVQDDLREV
EKSISNLEKSLTSLSEVVLQNRRGLDLLFLKEGGLCAALKEECCFYADHTGLVRDSMAK
LRERLNQRQKLFESTQGWFEGLFNRSPWFTTLISTIMGPLIVLLMILLFGPCILNRLVQFV
KDRISVVQALVLTQQYHQLKPIEYEP (SEQ ID NO:17)

Moloney murine leukemia virus 10A1 strain Glycoprotein (MLV 10A1)
MARSTLSKPLKDKINPWKSLMVMGVLLRVGMAESPHQVFNVTWRVTNLMTGRTANAT
SLLGTVQDAFPRLYFDLCDLVGEEWDPSDQEPYVGYGCKYPGGRKRTRTFDFYVCPG
HTVKSGCGGPREGYCGEWGCETTGQAYWKPTSSWDLISLKRGNTPWDTGCSKMAC
GPCYDLSKVSNSFQGATRGGRCNPLVLEFTDAGKKANWDGPKSWGLRLYRTGTDPIT
MFSLTRQVLNIGPRIPIGPNPVITGQLPPSRPVQIRLPRPPQPPPTGAASIVPETAPPSQ
QPGTGDRLLNLVEGAYRALNLTNPDKTQECWLCLVSGPPYYEGVAVVGTYTNHSTAP
ASCTATSQHKLTLSEVTGQGLCMGAVPKTHQALCNTTQSAGSGSYYLAAPAGTMWAC
STGLTPCLSTTMLNLTTDYCVLVELWPRIIYHSPDYMYGQLEQRTKYKREPVSLTLALLL
GGLTMGGIAAGIGTGTTALIKTQQFEQLHAAIQTDLNEVEKSITNLEKSLTSLSEVVLQNR
RGLDLLFLKEGGLCAALKEECCFYADHTGLVRDSMAKLRERLNQRQKLFESGQGWFE
GLFNRSPWFTTLISTIMGPLIVLLLILLFGPCILNRLVQFVKDRISVVQALVLTQQYHQLKPI
EYEP (SEQ ID NO:18)

***Rattus norvegicus* & synthetic: APOBEC1-XTEN L8-nspCas9-UGI-SV40 NLS**
MSSETGPVAVDPTLRRRIEPHEFEVFFDPRELRKETCLLYEINWGGRHSIWRHTSQNT
NKHVEVNFIEKFTTERYFCPNTRCSITWFLSWSPCGECSRAITEFLSRYPHVTLFIYIARL
YHHADPRNRQGLRDLISSGVTIQIMTEQESGYCWRNFVNYSPSNEAHWPRYPHLWVR
LYVLELYCIILGLPPCLNILRRKQPQLTFFTIALQSCHYQRLPPHILWATGLKSGSETPGT
SESATPESDKKYSIGLAIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGALLFD
SGETAEATRLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFFHRLEESFLVEEDKKH
ERHPIFGNIVDEVAYHEKYPTIYHLRKKLVDSTDKADLRLIYLALAHMIKFRGHFLIEGDLN
PDNSDVDKLFIQLVQTYNQLFEENPINASGVDAKAILSARLSKSRRLENLIAQLPGEKKN
GLFGNLIALSLGLTPNFKSNFDLAEDAKLQLSKDTYDDDLDNLLAQIGDQYADLFLAAKN
LSDAILLSDILRVNTEITKAPLSASMIKRYDEHHQDLTLLKALVRQQLPEKYKEIFFDQSK
NGYAGYIDGGASQEEFYKFIKPILEKMDGTEELLVKLNREDLLRKQRTFDNGSIPHQIHL
GELHAILRRQEDFYPFLKDNREKIEKILTFRIPYYVGPLARGNSRFAWMTRKSEETITPW
NFEEVVDKGASAQSFIERMTNFDKNLPNEKVLPKHSLLYEYFTVYNELTKVKYVTEGMR
KPAFLSGEQKKAIVDLLFKTNRKVTVKQLKEDYFKKIECFDSVEISGVEDRFNASLGTYH
DLLKIIKDKDFLDNEENEDILEDIVLTLTLFEDREMIEERLKTYAHLFDDKVMKQLKRRRY
TGWGRLSRKLINGIRDKQSGKTILDFLKSDGFANRNFMQLIHDDSLTFKEDIQKAQVSG
QGDSLHEHIANLAGSPAIKKGILQTVKVVDELVKVMGRHKPENIVIEMARENQTTQKGQ
KNSRERMKRIEEGIKELGSQILKEHPVENTQLQNEKLYLYYLQNGRDMYVDQELDINRL
SDYDVDHIVPQSFLKDDSIDNKVLTRSDKNRGKSDNVPSEEVVKKMKNYWRQLLNAKLI
TQRKFDNLTKAERGGLSELDKAGFIKRQLVETRQITKHVAQILDSRMNTKYDENDKLIRE

VKVITLKSKLVSDFRKDFQFYKVREINNYHHAHDAYLNAVVGTALIKKYPKLESEFVYGD
YKVYDVRKMIAKSEQEIGKATAKYFFYSNIMNFFKTEITLANGEIRKRPLIETNGETGEIV
WDKGRDFATVRKVLSMPQVNIVKKTEVQTGGFSKESILPKRNSDKLIARKKDWDPKKY
GGFDSPTVAYSVLVVAKVEKGKSKKLKSVKELLGITIMERSSFEKNPIDFLEAKGYKEVK
KDLIIKLPKYSLFELENGRKRMLASAGELQKGNELALPSKYVNFLYLASHYEKLKGSPED
NEQKQLFVEQHKHYLDEIIEQISEFSKRVILADANLDKVLSAYNKHRDKPIREQAENIIHLF
TLTNLGAPAAFKYFDTTIDRKRYTSTKEVLDATLIHQSITGLYETRIDLSQLGGDSGGSTN
LSDIIEKETGKQLVIQESILMLPEEVEEVIGNKPESDILVHTAYDESTDENVMLLTSDAPEY
KPWALVIQDSNGENKIKMLSGGSPKKKRKV (SEQ ID NO:19)

***Homo sapiens*: AID**
MDSLLMNRRKFLYQFKNVRWAKGRRETYLCYVVKRRDSATSFSLDFGYLRNKNGCHV
ELLFLRYISDWDLDPGRCYRVTWFTSWSPCYDCARHVADFLRGNPNLSLRIFTARLYF
CEDRKAEPEGLRRLHRAGVQIAIMTFKDYFYCWNTFVENHERTFKAWEGLHENSVRLS
RQLRRILLPLYEVDDLRDAFRTLGL (SEQ ID NO:20)

***Homo sapiens*:AIDv solubility variant lacking N-terminal RNA-binding region**
LMDPHIFTSNFNNGIGRHKTYLCYEVERLDSATSFSLDFGYLRNKNGCHVELLFLRYISD
WDLDPGRCYRVTWFTSWSPCYDCARHVADFLRGNPNLSLRIFTARLYFCEDRKAEPE
GLRRLHRAGVQIAIMTFKDYFYCWNTFVENHERTFKAWEGLHENSVRLSRQLRRILLPL
YEVDDLRDAFRTLGL (SEQ ID NO:21)

***Homo sapiens*: AIDv solubility variant lacking N-terminal RNA-binding region and the C-terminal poorly structured region**
MDPHIFTSNFNNGIGRHKTYLCYEVERLDSATSFSLDFGYLRNKNGCHVELLFLRYISD
WDLDPGRCYRVTWFTSWSPCYDCARHVADFLRGNPNLSLRIFTARLYFCEDRKAEPE
GLRRLHRAGVQIAIMTFKDYFYCWNTFVENHERTFKAWEGLHENSVRLSRQLRRILLPL
(SEQ ID NO:22)

***Rattus norvegicus*: APOBEC1**
MSSETGPVAVDPTLRRRIEPHEFEVFFDPRELRKETCLLYEINWGGRHSIWRHTSQNT
NKHVEVNFIEKFTTERYFCPNTRCSITWFLSWSPCGECSRAITEFLSRYPHVTLFIYIARL
YHHADPRNRQGLRDLISSGVTIQIMTEQESGYCWRNFVNYSPSNEAHWPRYPHLWVR
LYVLELYCIILGLPPCLNILRRKQPQLTFFTIALQSCHYQRLPPHILWATGLK (SEQ
ID NO:23)

***Mus musculus*: APOBEC3**
MGPFCLGCSHRKCYSPIRNLISQETFKFHFKNLGYAKGRKDTFLCYEVTRKDCDSPVSL
HHGVFKNKDNIHAEICFLYWFHDKVLKVLSPREEFKITWYMSWSPCFECAEQIVRFLAT
HHNLSLDIFSSRLYNVQDPETQQNLCRLVQEGAQVAAMDLYEFKKCWKKFVDNGGRR
FRPWKRLLTNFRYQDSKLQEILRRMDPLSEEEFYSQFYNQRVKHLCYYHRMKPYLCYQ
LEQFNGQAPLKGCLLSEKGKQHAEILFLDKIRSMELSQVTITCYLTWSPCPNCAWQLAA
FKRDRPDLILHIYTSRLYFHWKRPFQKGLCSLWQSGILVDVMDLPQFTDCWTNFVNPK

RPFRPWKGLEIISRRTQRRLRRIKESWGLQDLVNDFGNLQLGPPMSN (SEQ ID NO:24)

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

MGPFCLGCSHRKCYSPIRNLISQETFKFHFKNLGYAKGRKDTFLCYEVTRKDCDSPVSL HHGVFKNKDNIHAEICFLYWFHDKVLKVLSPREEFKITWYMSWSPCFECAEQIVRFLAT HHNLSLDIFSSRLYNVQDPETQQNLCRLVQEGAQVAAMDLYEFKKCWKKFVDNGGRR FRPWKRLLTNFRYQDSKLQEILRR (SEQ ID NO:25)

***Homo sapiens*: APOBEC3A**

MEASPASGPRHLMDPHIFTSNFNNGIGRHKTYLCYEVERLDNGTSVKMDQHRGFLHN QAKNLLCGFYGRHAELRFLDLVPSLQLDPAQIYRVTWFISWSPCFSWGCAGEVRAFLQ ENTHVRLRIFAARIYDYDPLYKEALQMLRDAGAQVSIMTYDEFKHCWDTFVDHQGCPF QPWDGLDEHSQALSGRLRAILQNQGN (SEQ ID NO:26)

***Homo sapiens*: APOBEC3G**

MKPHFRNTVERMYRDTFSYNFYNRPILSRRNTVWLCYEVKTKGPSRPPLDAKIFRGQV YSELKYHPEMRFFHWFSKWRKLHRDQEYEVTWYISWSPCTKCTRDMATFLAEDPKVT LTIFVARLYYFWDPDYQEALRSLCQKRDGPRATMKIMNYDEFQHCWSKFVYSQRELFE PWNNLPKYYILLHIMLGEILRHSMDPPTFTFNFNNEPWVRGRHETYLCYEVERMHNDT WVLLNQRRGFLCNQAPHKHGFLEGRHAELCFLDVIPFWKLDLDQDYRVTCFTSWSPC FSCAQEMAKFISKNKHVSLCIFTARIYDDQGRCQEGLRTLAEAGAKISIMTYSEFKHCW DTFVDHQGCPFQPWDGLDEHSQDLSGRLRAILQNQEN (SEQ ID NO:27)

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

PPTFTFNFNNEPWVRGRHETYLCYEVERMHNDTWVLLNQRRGFLCNQAPHKHGFLE GRHAELCFLDVIPFWKLDLDQDYRVTCFTSWSPCFSCAQEMAKFISKNKHVSLCIFTAR IYDDQGRCQEGLRTLAEAGAKISIMTYSEFKHCWDTFVDHQGCPFQPWDGLDEHSQD LSGRLRAILQNQEN (SEQ ID NO:28)

***Homo sapiens*: APOBEC3H**

MALLTAETFRLQFNNKRRLRRPYYPRKALLCYQLTPQNGSTPTRGYFENKKKCHAEICF INEIKSMGLDETQCYQVTCYLTWSPCSSCAWELVDFIKAHDHLNLGIFASRLYYHWCKP QQKGLRLLCGSQVPVEVMGFPKFADCWENFVDHEKPLSFNPYKMLEELDKNSRAIKR RLERIKIPGVRAQGRYMDILCDAEV (SEQ ID NO:29)

***Homo sapiens*: APOBEC3F**

MKPHFRNTVERMYRDTFSYNFYNRPILSRRNTVWLCYEVKTKGPSRPRLDAKIFRGQV YSQPEHHAEMCFLSWFCGNQLPAYKCFQITWFVSWTPCPDCVAKLAEFLAEHPNVTL TISAARLYYYWERDYRRALCRLSQAGARVKIMDDEEFAYCWENFVYSEGQPFMPWYK FDDNYAFLHRTLKEILRNPMEAMYPHIFYFHFKNLRKAYGRNESWLCFTMEVVKHHSP VSWKRGVFRNQVDPETHCHAERCFLSWFCDDILSPNTNYEVTWYTSWSPCPECAGE VAEFLARHSNVNLTIFTARLYYFWDTDYQEGLRSLSQEGASVEIMGYKDFKYCWENFV YNDDEPFKPWKGLKYNFLFLDSKLQEILE (SEQ ID NO:30)

***Homo sapiens*: APOBEC3F catalytic domain**

KEILRNPMEAMYPHIFYFHFKNLRKAYGRNESWLCFTMEVVKHHSPVSWKRGVFRNQ
VDPETHCHAERCFLSWFCDDILSPNTNYEVTWYTSWSPCPECAGEVAEFLARHSNVN
LTIFTARLYYFWDTDYQEGLRSLSQEGASVEIMGYKDFKYCWENFVYNDDEPFKPWKG
LKYNFLFLDSKLQEILE (SEQ ID NO:31)

Escherichia coli: TadA

MKRTADGSEFESPKKKRKVSEVEFSHEYWMRHALTLAKRAWDEREVPVGAVLVHNN
RVIGEGWNRPIGRHDPTAHAEIMALRQGGLVMQNYRLIDATLYVTLEPCVMCAGAMIH
SRIGRVVFGARDAKTGAAGSLMDVLHHPGMNHRVEITEGILADECAALLSDFFRMRRQ
EIKAQKKAQSSTDSGGSSGGSSGSETPGTSESATPESSGGSSGGSSEVEFSHEYWM
RHALTLAKRARDEREVPVGAVLVLNNRVIGEGWNRAIGLHDPTAHAEIMALRQGGLVM
QNYRLIDATLYVTFEPCVMCAGAMIHSRIGRVVFGVRNAKTGAAGSLMDVLHYPGMNH
RVEITEGILADECAALLCYFFRMPRQVFNAQKKAQSSTD (SEQ ID NO:32)

***Homo sapiens*: Adar1**

MNPRQGYSLSGYYTHPFQGYEHRQLRYQQPGPGSSPSSFLLKQIEFLKGQLPEAPVIG
KQTPSLPPSLPGLRPRFPVLLASSTRGRQVDIRGVPRGVHLGSQGLQRGFQHPSPRG
RSLPQRGVDCLSSHFQELSIYQDQEQRILKFLEELGEGKATTAHDLSGKLGTPKKEINR
VLYSLAKKGKLQKEAGTPPLWKIAVSTQAWNQHSGVVRPDGHSQGAPNSDPSLEPED
RNSTSVSEDLLEPFIAVSAQAWNQHSGVVRPDSHSQGSPNSDPGLEPEDSNSTSALE
DPLEFLDMAEIKEKICDYLFNVSDSSALNLAKNIGLTKARDINAVLIDMERQGDVYRQGT
TPPIWHLTDKKRERMQIKRNTNSVPETAPAAIPETKRNAEFLTCNIPTSNASNNMVTTEK
VENGQEPVIKLENRQEARPEPARLKPPVHYNGPSKAGYVDFENGQWATDDIPDDLNSI
RAAPGEFRAIMEMPSFYSHGLPRCSPYKKLTECQLKNPISGLLEYAQFASQTCEFNMIE
QSGPPHEPRFKFQVVINGREFPPAEAGSKKVAKQDAAMKAMTILLEEAKAKDSGKSEE
SSHYSTEKESEKTAESQTPTPSATSFFSGKSPVTTLLECMHKLGNSCEFRLLSKEGPAH
EPKFQYCVAVGAQTFPSVSAPSKKVAKQMAAEEAMKALHGEATNSMASDNQPEGMIS
ESLDNLESMMPNKVRKIGELVRYLNTNPVGGLLEYARSHGFAAEFKLVDQSGPPHEPK
FVYQAKVGGRWFPAVCAHSKKQGKQEAADAALRVLIGENEKAERMGFTEVTPVTGAS
LRRTMLLLSRSPEAQPKTLPLTGSTFHDQIAMLSHRCFNTLTNSFQPSLLGRKILAAIIMK
KDSEDMGVVVSLGTGNRCVKGDSLSLKGETVNDCHAEIISRRGFIRFLYSELMKYNSQT
AKDSIFEPAKGGEKLQIKKTVSFHLYISTAPCGDGALFDKSCSDRAMESTESRHYPVFE
NPKQGKLRTKVENGEGTIPVESSDIVPTWDGIRLGERLRTMSCSDKILRWNVLGLQGAL
LTHFLQPIYLKSVTLGYLFSQGHLTRAICCRVTRDGSAFEDGLRHPFIVNHPKVGRVSIY
DSKRQSGKTKETSVNWCLADGYDLEILDGTRGTVDGPRNELSRVSKKNIFLLFKKLCSF
RYRRDLLRLSYGEAKKAARDYETAKNYFKKGLKDMGYGNWISKPQEEKNFYLCPV
(SEQ ID NO:33)

***Streptococcus pyogenes*: spCas9 Bipartite NLS**

MDKKYSIGLDIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGALLFDS
GETAEATRLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFFHRLEESFLVEE
DKKHERHPIFGNIVDEVAYHEKYPTIYHLRKKLVDSTDKADLRLIYLALAHMIKFR
GHFLIEGDLNPDNSDVDKLFIQLVQTYNQLFEENPINASGVDAKAILSARLSKSR

RLENLIAQLPGEKKNGLFGNLIALSLGLTPNFKSNFDLAEDAKLQLSKDTYDDDL
DNLLAQIGDQYADLFLAAKNLSDAILLSDILRVNTEITKAPLSASMIKRYDEHHQD
LTLLKALVRQQLPEKYKEIFFDQSKNGYAGYIDGGASQEEFYKFIKPILEKMDGT
EELLVKLNREDLLRKQRTFDNGSIPHQIHLGELHAILRRQEDFYPFLKDNREKIE
KILTFRIPYYVGPLARGNSRFAWMTRKSEETITPWNFEEVVDKGASAQSFIERM
TNFDKNLPNEKVLPKHSLLYEYFTVYNELTKVKYVTEGMRKPAFLSGEQKKAIV
DLLFKTNRKVTVKQLKEDYFKKIECFDSVEISGVEDRFNASLGTYHDLLKIIKDKD
FLDNEENEDILEDIVLTLTLFEDREMIEERLKTYAHLFDDKVMKQLKRRRYTGWG
RLSRKLINGIRDKQSGKTILDFLKSDGFANRNFMQLIHDDSLTFKEDIQKAQVSG
QGDSLHEHIANLAGSPAIKKGILQTVKVVDELVKVMGRHKPENIVIEMARENQTT
QKGQKNSRERMKRIEEGIKELGSQILKEHPVENTQLQNEKLYLYYLQNGRDMY
VDQELDINRLSDYDVDHIVPQSFLKDDSIDNKVLTRSDKNRGKSDNVPSEEVVK
KMKNYWRQLLNAKLITQRKFDNLTKAERGGLSELDKAGFIKRQLVETRQITKHV
AQILDSRMNTKYDENDKLIREVKVITLKSKLVSDFRKDFQFYKVREINNYHHAHD
AYLNAVVGTALIKKYPKLESEFVYGDYKVYDVRKMIAKSEQEIGKATAKYFFYSN
IMNFFKTEITLANGEIRKRPLIETNGETGEIVWDKGRDFATVRKVLSMPQVNIVKK
TEVQTGGFSKESILPKRNSDKLIARKKDWDPKKYGGFDSPTVAYSVLVVAKVEK
GKSKKLKSVKELLGITIMERSSFEKNPIDFLEAKGYKEVKKDLIIKLPKYSLFELEN
GRKRMLASAGELQKGNELALPSKYVNFLYLASHYEKLKGSPEDNEQKQLFVEQ
HKHYLDEIIEQISEFSKRVILADANLDKVLSAYNKHRDKPIREQAENIIHLFTLTNL
GAPAAFKYFDTTIDRKRYTSTKEVLDATLIHQSITGLYETRIDLSQLGGDGSGGG
GSGKRTADGSEFEPKKKRKVSSGGDYKDHDGDYKDHDIDYKDDDDK (SEQ ID
NO:34)

***Staphylococcus aureus: sa*Cas9**

MKRNYILGLDIGITSVGYGIIDYETRDVIDAGVRLFKEANVENNEGRRSKRGARR
LKRRRRHRIQRVKKLLFDYNLLTDHSELSGINPYEARVKGLSQKLSEEEFSAALL
HLAKRRGVHNVNEVEEDTGNELSTKEQISRNSKALEEKYVAELQLERLKKDGE
VRGSINRFKTSDYVKEAKQLLKVQKAYHQLDQSFIDTYIDLLETRRTYYEGPGE
GSPFGWKDIKEWYEMLMGHCTYFPEELRSVKYAYNADLYNALNDLNNLVITRD
ENEKLEYYEKFQIIENVFKQKKKPTLKQIAKEILVNEEDIKGYRVTSTGKPEFTNL
KVYHDIKDITARKEIIENAELLDQIAKILTIYQSSEDIQEELTNLNSELTQEEIEQISN
LKGYTGTHNLSLKAINLILDELWHTNDNQIAIFNRLKLVPKKVDLSQQKEIPTTLV
DDFILSPVVKRSFIQSIKVINAIIKKYGLPNDIIIELAREKNSKDAQKMINEMQKRNR
QTNERIEEIIRTTGKENAKYLIEKIKLHDMQEGKCLYSLEAIPLEDLLNNPFNYEVD
HIIPRSVSFDNSFNNKVLVKQEENSKKGNRTPFQYLSSSDSKISYETFKKHILNL
AKGKGRISKTKKEYLLEERDINRFSVQKDFINRNLVDTRYATRGLMNLLRSYFRV
NNLDVKVKSINGGFTSFLRRKWKFKKERNKGYKHHAEDALIIANADFIFKEWKKL
DKAKKVMENQMFEEKQAESMPEIETEQEYKEIFITPHQIKHIKDFKDYKYSHRVD
KKPNRELINDTLYSTRKDDKGNTLIVNNLNGLYDKDNDKLKKLINKSPEKLLMYH

HDPQTYQKLKLIMEQYGDEKNPLYKYYEETGNYLTKYSKKDNGPVIKKIKYYGN
KLNAHLDITDDYPNSRNKVVKLSLKPYRFDVYLDNGVYKFVTVKNLDVIKKENYY
EVNSKCYEEAKKLKKISNQAEFIASFYNNDLIKINGELYRVIGVNNDLLNRIEVNMI
DITYREYLENMNDKRPPRIIKTIASKTQSIKKYSTDILGNLYEVKSKKHPQIIKKG
(SEQ ID NO:35)

***Campylobacter jejuni: cj*Cas9**

MARILAFDIGISSIGWAFSENDELKDCGVRIFTKVENPKTGESLALPRRLARSAR
KRLARRKARLNHLKHLIANEFKLNYEDYQSFDESLAKAYKGSLISPYELRFRALN
ELLSKQDFARVILHIAKRRGYDDIKNSDDKEKGAILKAIKQNEEKLANYQSVGEY
LYKEYFQKFKENSKEFTNVRNKKESYERCIAQSFLKDELKLIFKKQREFGFSFSK
KFEEEVLSVAFYKRALKDFSHLVGNCSFFTDEKRAPKNSPLAFMFVALTRIINLL
NNLKNTEGILYTKDDLNALLNEVLKNGTLTYKQTKKLLGLSDDYEFKGEKGTYFI
EFKKYKEFIKALGEHNLSQDDLNEIAKDITLIKDEIKLKKALAKYDLNQNQIDSLSK
LEFKDHLNISFKALKLVTPLMLEGKKYDEACNELNLKVAINEDKKDFLPAFNETY
YKDEVTNPVVLRAIKEYRKVLNALLKKYGKVHKINIELAREVGKNHSQRAKIEKE
QNENYKAKKDAELECEKLGLKINSKNILKLRLFKEQKEFCAYSGEKIKISDLQDE
KMLEIDHIYPYSRSFDDSYMNKVLVFTKQNQEKLNQTPFEAFGNDSAKWQKIEV
LAKNLPTKKQKRILDKNYKDKEQKNFKDRNLNDTRYIARLVLNYTKDYLDFLPLS
DDENTKLNDTQKGSKVHVEAKSGMLTSALRHTWGFSAKDRNNHLHHAIDAVIIA
YANNSIVKAFSDFKKEQESNSAELYAKKISELDYKNKRKFFEPFSGFRQKVLDKI
DEIFVSKPERKKPSGALHEETFRKEEEFYQSYGGKEGVLKALELGKIRKVNGKIV
KNGDMFRVDIFKHKKTNKFYAVPIYTMDFALKVLPNKAVARSKKGEIKDWILMD
ENYEFCFSLYKDSLILIQTKDMQEPEFVYYNAFTSSTVSLIVSKHDNKFETLSKN
QKILFKNANEKEVIAKSIGIQNLKVFEKYIVSALGEVTKAEFRQREDFKK (SEQ
ID NO:36)

***Neisseria meningitidis: nme*Cas9**

MAAFKPNSINYILGLDIGIASVGWAMVEIDEEENPIRLIDLGVRVFERAEVPKTGD
SLAMARRLARSVRRLTRRRAHRLLRTRRLLKREGVLQAANFDENGLIKSLPNTP
WQLRAAALDRKLTPLEWSAVLLHLIKHRGYLSQRKNEGETADKELGALLKGVA
GNAHALQTGDFRTPAELALNKFEKESGHIRNQRSDYSHTFSRKDLQAELILLFE
KQKEFGNPHVSGGLKEGIETLLMTQRPALSGDAVQKMLGHCTFEPAEPKAAKN
TYTAERFIWLTKLNNLRILEQGSERPLTDTERATLMDEPYRKSKLTYAQARKLLG
LEDTAFFKGLRYGKDNAEASTLMEMKAYHAISRALEKEGLKDKKSPLNLSPELQ
DEIGTAFSLFKTDEDITGRLKDRIQPEILEALLKHISFDKFVQISLKALRRIVPLMEQ
GKRYDEACAEIYGDHYGKKNTEEKIYLPPIPADEIRNPVVLRALSQARKVINGVV
RRYGSPARIHIETAREVGKSFKDRKEIEKRQEENRKDREKAAAKFREYFPNFVG
EPKSKDILKLRLYEQQHGKCLYSGKEINLGRLNEKGYVEIDHALPFSRTWDDSF
NNKVLVLGSENQNKGNQTPYEYFNGKDNSREWQEFKARVETSRFPRSKKQRI
LLQKFDEDGFKERNLNDTRYVNRFLCQFVADRMRLTGKGKKRVFASNGQITNL
LRGFWGLRKVRAENDRHHALDAVVVACSTVAMQQKITRFVRYKEMNAFDGKTI

DKETGEVLHQKTHFPQPWEFFAQEVMIRVFGKPDGKPEFEEADTLEKLRTLLA
EKLSSRPEAVHEYVTPLFVSRAPNRKMSGQGHMETVKSAKRLDEGVSVLRVPL
TQLKLKDLEKMVNREREPKLYEALKARLEAHKDDPAKAFAEPFYKYDKAGNRT
QQVKAVRVEQVQKTGVWVRNHNGIADNATMVRVDVFEKGDKYYLVPIYSWQV
AKGILPDRAVVQGKDEEDWQLIDDSFNFKFSLHPNDLVEVITKKARMFGYFASC
HRGTGNINIRIHDLDHKIGKNGILEGIGVKTALSFQKYQIDELGKEIRPCRLKKRP
PVR (SEQ ID NO:37)

***Acidaminococcus sp.: as*Cas12a**

MTQFEGFTNLYQVSKTLRFELIPQGKTLKHIQEQGFIEEDKARNDHYKELKPIID
RIYKTYADQCLQLVQLDWENLSAAIDSYRKEKTEETRNALIEEQATYRNAIHDYF
IGRTDNLTDAINKRHAEIYKGLFKAELFNGKVLKQLGTVTTTEHENALLRSFDKF
TTYFSGFYENRKNVFSAEDISTAIPHRIVQDNFPKFKENCHIFTRLITAVPSLREH
FENVKKAIGIFVSTSIEEVFSFPFYNQLLTQTQIDLYNQLLGGISREAGTEKIKGLN
EVLNLAIQKNDETAHIIASLPHRFIPLFKQILSDRNTLSFILEEFKSDEEVIQSFCKY
KTLLRNENVLETAEALFNELNSIDLTHIFISHKKLETISSALCDHWDTLRNALYER
RISELTGKITKSAKEKVQRSLKHEDINLQEIISAAGKELSEAFKQKTSEILSHAHAA
LDQPLPTTLKKQEEKEILKSQLDSLLGLYHLLDWFAVDESNEVDPEFSARLTGIK
LEMEPSLSFYNKARNYATKKPYSVEKFKLNFQMPTLASGWDVNKEKNNGAILF
VKNGLYYLGIMPKQKGRYKALSFEPTEKTSEGFDKMYYDYFPDAAKMIPKCST
QLKAVTAHFQTHTTPILLSNNFIEPLEITKEIYDLNNPEKEPKKFQTAYAKKTGDQ
KGYREALCKWIDFTRDFLSKYTKTTSIDLSSLRPSSQYKDLGEYYAELNPLLYHI
SFQRIAEKEIMDAVETGKLYLFQIYNKDFAKGHHGKPNLHTLYWTGLFSPENLA
KTSIKLNGQAELFYRPKSRMKRMAHRLGEKMLNKKLKDQKTPIPDTLYQELYDY
VNHRLSHDLSDEARALLPNVITKEVSHEIIKDRRFTSDKFFFHVPITLNYQAANSP
SKFNQRVNAYLKEHPETPIIGIDRGERNLIYITVIDSTGKILEQRSLNTIQQFDYQK
KLDNREKERVAARQAWSVVGTIKDLKQGYLSQVIHEIVDLMIHYQAVVVLENLN
FGFKSKRTGIAEKAVYQQFEKMLIDKLNCLVLKDYPAEKVGGVLNPYQLTDQFT
SFAKMGTQSGFLFYVPAPYTSKIDPLTGFVDPFVWKTIKNHESRKHFLEGFDFL
HYDVKTGDFILHFKMNRNLSFQRGLPGFMPAWDIVFEKNETQFDAKGTPFIAGK
RIVPVIENHRFTGRYRDLYPANELIALLEEKGIVFRDGSNILPKLLENDDSHAIDT
MVALIRSVLQMRNSNAATGEDYINSPVRDLNGVCFDSRFQNPEWPMDADANG
AYHIALKGQLLLNHLKESKDLKLQNGISNQDWLAYIQELRN (SEQ ID NO:38)

***Lachnospiraceae bacterium: lb*Cas12a:**

MSKLEKFTNCYSLSKTLRFKAIPVGKTQENIDNKRLLVEDEKRAEDYKGVKKLLD
RYYLSFINDVLHSIKLKNLNNYISLFRKKTRTEKENKELENLEINLRKEIAKAFKGN
EGYKSLFKKDIIETILPEFLDDKDEIALVNSFNGFTTAFTGFFDNRENMFSEEAKS
TSIAFRCINENLTRYISNMDIFEKVDAIFDKHEVQEIKEKILNSDYDVEDFFEGEFF
NFVLTQEGIDVYNAIIGGFVTESGEKIKGLNEYINLYNQKTKQKLPKFKPLYKQVL
SDRESLSFYGEGYTSDEEVLEVFRNTLNKNSEIFSSIKKLEKLFKNFDEYSSAGI
FVKNGPAISTISKDIFGEWNVIRDKWNAEYDDIHLKKKAVVTEKYEDDRRKSFKK
IGSFSLEQLQEYADADLSVVEKLKEIIIQKVDEIYKVYGSSEKLFDADFVLEKSLKK
NDAVVAIMKDLLDSVKSFENYIKAFFGEGKETNRDESFYGDFVLAYDILLKVDHI
YDAIRNYVTQKPYSKDKFKLYFQNPQFMGGWDKDKETDYRATILRYGSKYYLAI
MDKKYAKCLQKIDKDDVNGNYEKINYKLLPGPNKMLPKVFFSKKWMAYYNPSE

DIQKIYKNGTFKKGDMFNLNDCHKLIDFFKDSISRYPKWSNAYDFNFSETEKYK
DIAGFYREVEEQGYKVSFESASKKEVDKLVEEGKLYMFQIYNKDFSDKSHGTP
NLHTMYFKLLFDENNHGQIRLSGGAELFMRRASLKKEELVVHPANSPIANKNPD
NPKKTTTLSYDVYKDKRFSEDQYELHIPIAINKCPKNIFKINTEVRVLLKHDDNPY
VIGIDRGERNLLYIVVVDGKGNIVEQYSLNEIINNFNGIRIKTDYHSLLDKKEKERF
EARQNWTSIENIKELKAGYISQVVHKICELVEKYDAVIALEDLNSGFKNSRVKVE
KQVYQKFEKMLIDKLNYMVDKKSNPCATGGALKGYQITNKFESFKSMSTQNGFI
FYIPAWLTSKIDPSTGFVNLLKTKYTSIADSKKFISSFDRIMYVPEEDLFEFALDYK
NFSRTDADYIKKWKLYSYGNRIRIFRNPKKNNVFDWEEVCLTSAYKELFNKYGI
NYQQGDIRALLCEQSDKAFYSSFMALMSLMLQMRNSITGRTDVDFLISPVKNSD
GIFYDSRNYEAQENAILPKNADANGAYNIARKVLWAIGQFKKAEDEKLDKVKIAI
SNKEWLEYAQTSVKH (SEQ ID NO:39)

***Leptotrichia shahii*: LshCas13a**

MGNLFGHKRWYEVRDKKDFKIKRKVKVKRNYDGNKYILNINENNNKEKIDNNKFIRKYIN
YKKNDNILKEFTRKFHAGNILFKLKGKEGIIRIENNDDFLETEEVVLYIEAYGKSEKLKALG
ITKKKIIDEAIRQGITKDDKKIEIKRQENEEEIEIDIRDEYTNKTLNDCSIILRIIENDELETKKS
IYEIFKNINMSLYKIIEKIIENETEKVFENRYYEEHLREKLLKDDKIDVILTNFMEIREKIKSNL
EILGFVKFYLNVGGDKKKSKNKKMLVEKILNINVDLTVEDIADFVIKELEFWNITKRIEKVK
KVNNEFLEKRRNRTYIKSYVLLDKHEKFKIERENKKDKIVKFFVENIKNNSIKEKIEKILAE
FKIDELIKKLEKELKKGNCDTEIFGIFKKHYKVNFDSKKFSKKSDEEKELYKIIYRYLKGRI
EKILVNEQKVRLKKMEKIEIEKILNESILSEKILKRVKQYTLEHIMYLGKLRHNDIDMTTVNT
DDFSRLHAKEELDLELITFFASTNMELNKIFSRENININDENIDFFGGDREKNYVLDKKILN
SKIKIIRDLDFIDNKNNITNNFIRKFTKIGTNERNRILHAISKERDLQGTQDDYNKVINIIQNL
KISDEEVSKALNLDVVFKDKKNIITKINDIKISEENNNDIKYLPSFSKVLPEILNLYRNNPKN
EPFDTIETEKIVLNALIYVNKELYKKLILEDDLEENESKNIFLQELKKTLGNIDEIDENIIENY
YKNAQISASKGNNKAIKKYQKKVIECYIGYLRKNYEELFDFSDFKMNIQEIKKQIKDINDN
KTYERITVKTSDKTIVINDDFEYIISIFALLNSNAVINKIRNRFFATSVWLNTSEYQNIIDILD
EIMQLNTLRNECITENWNLNLEEFIQKMKEIEKDFDDFKIQTKKEIFNNYYEDIKNNILTEF
KDDINGCDVLEKKLEKIVIFDDETKFEIDKKSNILQDEQRKLSNINKKDLKKKVDQYIKDK
DQEIKSKILCRIIFNSDFLKKYKKEIDNLIEDMESENENKFQEIYYPKERKNELYIYKKNLFL
NIGNPNFDKIYGLISNDIKMADAKFLFNIDGKNIRKNKISEIDAILKNLNDKLNGYSKEYKE
KYIKKLKENDDFFAKNIQNKNYKSFEKDYNRVSEYKKIRDLVEFNYLNKIESYLIDINWKL
AIQMARFERDMHYIVNGLRELGIIKLSGYNTGISRAYPKRNGSDGFYTTTAYYKFFDEES
YKKFEKICYGFGIDLSENSEINKPENESIRNYISHFYIVRNPFADYSIAEQIDRVSNLLSYS
TRYNNSTYASVFEVFKKDVNLDYDELKKKFKLIGNNDILERLMKPKKVSVLELESYNSDY
IKNLIIELLTKIENTNDTL (SEQ ID NO:40)

***Leptotrichia wadeii:* LwaCas13a**

MKVTKVDGISHKKYIEEGKLVKSTSEENRTSERLSELLSIRLDIYIKNPDNASEEENRIRR
ENLKKFFSNKVLHLKDSVLYLKNRKEKNAVQDKNYSEEDISEYDLKNKNSFSVLKKILLN
EDVNSEELEIFRKDVEAKLNKINSLKYSFEENKANYQKINENNVEKVGGKSKRNIIYDYY
RESAKRNDYINNVQEAFDKLYKKEDIEKLFFLIENSKKHEKYKIREYYHKIIGRKNDKENF
AKIIYEEIQNVNNIKELIEKIPDMSELKKSQVFYKYYLDKEELNDKNIKYAFCHFVEIEMSQ
LLKNYVYKRLSNISNDKIKRIFEYQNLKKLIENKLLNKLDTYVRNCGKYNYYLQVGEIATS
DFIARNRQNEAFLRNIIGVSSVAYFSLRNILETENENGITGRMRGKTVKNNKGEEKYVSG
EVDKIYNENKQNEVKENLKMFYSYDFNMDNKNEIEDFFANIDEAISSIAHGIVHFNLELE
GKDIFAFKNIAPSEISKKMFQNEINEKKLKLKIFKQLNSANVFNYYEKDVIIKYLKNTKFNF

VNKNIPFVPSFTKLYNKIEDLRNTLKFFWSVPKDKEEKDAQIYLLKNIYYGEFLNKFVKNS
KVFFKITNEVIKINKQRNQKTGHYKYQKFENIEKTVPVEYLAIIQSREMINNQDKEEKNTYI
DFIQQIFLKGFIDYLNKNNLKYIESNNNNDNNDIFSKIKIKKDNKEKYDKILKNYEKHNRNK
EIPHEINEFVREIKLGKILKYTENLNMFYLILKLLNHKELTNLKGSLEKYQSANKEETFSDE
LELINLLNLDNNRVTEDFELEANEIGKFLDFNENKIKDRKELKKFDTNKIYFDGENIIKHRA
FYNIKKYGMLNLLEKIADKAKYKISLKELKEYSNKKNEIEKNYTMQQNLHRKYARPKKDE
KFNDEDYKEYEKAIGNIQKYTHLKNKVEFNELNLLQGLLLKILHRLVGYTSIWERDLRFRL
KGEFPENHYIEEIFNFDNSKNVKYKSGQIVEKYINFYKELYKDNVEKRSIYSDKKVKKLK
QEKKDLYIANYIAHFNYIPHAEISLLEVLENLRKLLSYDRKLKNAIMKSIVDILKEYGFVATF
KIGADKKIEIQTLESEKIVHLKNLKKKKLMTDRNSEELCELVKVMFEYKALE (SEQ
ID NO:41)

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

Pleckstrin homology domain of *Homo sapiens* Akt1 (hAkt)
MSDVAIVKEGWLHKRGEYIKTWRPRYFLLKNDGTFIGYKERPQDVDQREAPLN
NFSVAQCQLMKTERPRPNTFIIRCLQWTTVIERTFHVETPEEREEWTTAIQTVA
DGLKKQEEEEMDFRSGSPSDNSGAEEMEVSLAKPKHRVTMNEFEYLKLLGKG
TFGKVDPPV (SEQ ID NO:43)

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

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

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

***Homo sapiens*: P65**
SQYLPDTDDRHRIEEKRKRTYETFKSIMKKSPFSGPTDPRPPPRRIAVPSRSSA
SVPKPAPQPYPFTSSLSTINYDEFPTMVFPSGQISQASALAPAPPQVLPQAPAP
APAPAMVSALAQAPAPVPVLAPGPPQAVAPPAPKPTQAGEGTLSEALLQLQFD
DEDLGALLGNSTDPAVFTDLASVDNSEFQQLLNQGIPVAPHTTEPMLMEYPEAI
TRLVTGAQRPPDPAPAPLGAPGLPNGLLSGDEDFSSIADMDFSALL (SEQ
ID NO:47)

Kaposi's Sarcoma-Associated Herpesvirus Transactivator: RTA
RDSREGMFLPKPEAGSAISDVFEGREVCQPKRIRPFHPPGSPWANRPLPASLA
PTPTGPVHEPVGSLTPAPVPQPLDPAPAVTPEASHLLEDPDEETSQAVKALRE

MADTVIPQKEEAAICGQMDLSHPPPRGHLDELTTTLESMTEDLNLDSPLTPELN
EILDTFLNDECLLHAMHISTGLSIFDTSLF (SEQ ID NO:48)

***Homo sapiens*: KRAB**

MDAKSLTAWSRTLVTFKDVFVDFTREEWKLLDTAQQIVYRNVMLENYKNLVSL
GYQLTKPDVILRLEKGEEP (SEQ ID NO:49)

***Homo sapiens*: MeCP2**

EASVQVKRVLEKSPGKLLVKMPFQASPGGKGEGGGATTSAQVMVIKRPGRKR
KAEADPQAIPKKRGRKPGSVVAAAAAEAKKKAVKESSIRSVQETVLPIKKRKTR
ETVSIEVKEVVKPLLVSTLGEKSGKGLKTCKSPGRKSKESSPKGRSSSASSPPK
KEHHHHHHHHAESPKAPMPLLPPPPPPEPQSSEDPISPPEPQDLSSSICKEEKM
PRAGSLESDGCPKEPAKTQPMVAAAATTTTTTTTTVAEKYKHRGEGERKDIVS
SSMPRPNREEPVDSRTPVTERVS (SEQ ID NO:50)

***Homo sapiens*: Tet1**

LPTCSCLDRVIQKDKGPYYTHLGAGPSVAAVREIMENRYGQKGNAIRIEIVVYTG
KEGKSSHGCPIAKWVLRRSSDEEKVLCLVRQRTGHHCPTAVMVVLIMVWDGIP
LPMADRLYTELTENLKSYNGHPTDRRCTLNENRTCTCQGIDPETCGASFSFGC
SWSMYFNGCKFGRSPSPRRFRIDPSSPLHEKNLEDNLQSLATRLAPIYKQYAP
VAYQNQVEYENVARECRLGSKEGRPFSGVTACLDFCAHPHRDIHNMNNGSTV
VCTLTREDNRSLGVIPQDEQLHVLPLYKLSDTDEFGSKEGMEAKIKSGAIEVLAP
RRKKRTCFTQPVPRSGKKRAAMMTEVLAHKIRAVEKKPIPRIKRKNNSTTTNNS
KPSSLPTLGSNTETVQPEVKSETEPHFILKSSDNTKTYSLMPSAPHPVKEASPG
FSWSPKTASATPAPLKNDATASCGFSERSSTPHCTMPSGRLSGANAAAADGP
GISQLGEVAPLPTLSAPVMEPLINSEPSTGVTEPLTPHQPNHQPSFLTSPQDLA
SSPMEEDEQHSEADEPPSDEPLSDDPLSPAEEKLPHIDEYWSDSEHIFLDANIG
GVAIAPAHGSVLIECARRELHATTPVEHPNRNHPTRLSLVFYQHKNLNKPQHGF
ELNKIKFEAKEAKNKKMKASEQKDQAANEGPEQSSEVNELNQIPSHKALTLTHD
NVVTVSPYALTHVAGPYNHWV (SEQ ID NO:51)

***Homo sapiens*: Dnmt3a**

MPAMPSSGPGDTSSSAAEREEDRKDGEEQEEPRGKEERQEPSTTARKVGRP
GRKRKHPPVESGDTPKDPAVISKSPSMAQDSGASELLPNGDLEKRSEPQPEE
GSPAGGQKGGAPAEGEGAAETLPEASRAVENGCCTPKEGRGAPAEAGKEQK
ETNIESMKMEGSRGRLRGGLGWESSLRQRPMPRLTFQAGDPYYISKRKRDEW
LARWKREAEKKAKVIAGMNAVEENQGPGESQKVEEASPPAVQQPTDPASPTV
ATTPEPVGSDAGDKNATKAGDDEPEYEDGRGFGIGELVWGKLRGFSWWPGRI
VSWWMTGRSRAAEGTRWVMWFGDGKFSVVCVEKLMPLSSFCSAFHQATYN
KQPMYRKAIYEVLQVASSRAGKLFPVCHDSDESDTAKAVEVQNKPMIEWALGG
FQPSGPKGLEPPEEEKNPYKEVYTDMWVEPEAAAYAPPPPAKKPRKSTAEKP
KVKEIIDERTRERLVYEVRQKCRNIEDICISCGSLNVTLEHPLFVGGMCQNCKNC
FLECAYQYDDDGYQSYCTICCGGREVLMCGNNNCCRCFCVECVDLLVGPGAA
QAAIKEDPWNCYMCGHKGTYGLLRRREDWPSRLQMFFANNHDQEFDPPKVY
PPVPAEKRKPIRVLSLFDGIATGLLVLKDLGIQVDRYIASEVCEDSITVGMVRHQ
GKIMYVGDVRSVTQKHIQEWGPFDLVIGGSPCNDLSIVNPARKGLYEGTGRLFF

EFYRLLHDARPKEGDDRPFFWLFENVVAMGVSDKRDISRFLESNPVMIDAKEV
SAAHRARYFWGNLPGMNRPLASTVNDKLELQECLEHGRIAKFSKVRTITTRSN
SIKQGKDQHFPVFMNEKEDILWCTEMERVFGFPVHYTDVSNMSRLARQRLLGR
SWSVPVIRHLFAPLKEYFACV (SEQ ID NO:52)

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

MKCLLYLAFLFIGVNCKFTIVFPHNQKGNWKNVPSNYHYCPSSSDLNWHNDLIG
TALQVKMPKSHKAIQADGWMCHASKWVTTCDFRWYGPKYITHSIRSFTPSVEQ
CKESIEQTKQGTWLNPGFPPQSCGYATVTDAEAVIVQVTPHHVLVDEYTGEWV
DSQFINGKCSNYICPTVHNSTTWHSDYKVKGLCDSNLISMDITFFSEDGELSSL
GKEGTGFRSNYFAYETGGKACKMQYCKHWGVRLPSGVWFEMADKDLFAAAR
FPECPEGSSISAPSQTSVDVSLIQDVERILDYSLCQETWSKIRAGLPISPVDLSYL
APKNPGTGPAFTIINGTLKYFETRYIRVDIAAPILSRMVGMISGTTTERELWDDW
APYEDVEIGPNGVLRTSSGYKFPLYMIGHGMLDSDLHLSSKAQVFEHPHIQDAA
SQLPDDESLFFGDTGLSKNPIELVEGWFSSWKSSIASFFFIIGLIIGLFLVLRVGIH
LCIKLKHTKKRQIYTDIEMNRLGK (SEQ ID NO:53)

Baculovirus envelope glycoprotein GP64

MVSAIVLYVLLAAAAHSAFAAEHCNAQMKTGPYKIKNLDITPPKETLQKDVEITIV
ETDYNENVIIGYKGYYQAYAYNGGSLDPNTRVEETMKTLNVGKEDLLMWSIRQ
QCEVGEELIDRWGSDSDDCFRDNEGRGQWVKGKELVKRQNNNHFAHHTCNK
SWRCGISTSKMYSRLECQDDTDECQVYILDAEGNPINVTVDTVLHRDGVSMILK
QKSTFTTRQIKAACLLIKDDKNNPESVTREHCLIDNDIYDLSKNTWNCKFNRCIK
RKVEHRVKKRPPTWRHNVRAKYTEGDTATKGDLMHIQEELMYENDLLKMNIEL
MHAHINKLNNMLHDLIVSVAKVDERLIGNLMNNSVSSTFLSDDTFLLMPCTNPP
AHTSNCYNNSIYKEGRWVANTDSSQCIDFSNYKELAIDDDVEFWIPTIGNTTYH
DSWKDASGWSFIAQQKSNLITTMENTKFGGVGTSLSDITSMAEGELAAKLTSF
MFGHVVNFVIILIVILFLYCMIRNRNRQY (SEQ ID NO:54)

Human immunodeficiency virus gp160

MRVKEKYQHLWRWGWRWGTMLLGMLMICSATEKLWVTVYYGVPVWKEATTT
LFCASDAKAYDTEVHNVWATHACVPTDPNPQEVVLVNVTENFNMWKNDMVE
QMHEDIISLWDQSLKPCVKLTPLCVSLKCTDLKNDTNTNSSSGRMIMEKGEIKN
CSFNISTSIRGKVQKEYAFFYKLDIIPIDNDTTSYKLTSCNTSVITQACPKVSFEPI
PIHYCAPAGFAILKCNNKTFNGTGPCTNVSTVQCTHGIRPVVSTQLLLNGSLAEE
EVVIRSVNFTDNAKTIIVQLNTSVEINCTRPNNNTRKRIRIQRGPGRAFVTIGKIGN
MRQAHCNISRAKWNNTLKQIASKLREQFGNNKTIIFKQSSGGDPEIVTHSFNCG
GEFFYCNSTQLFNSTWFNSTWSTEGSNNTEGSDTITLPCRIKQIINMWQKVGKA
MYAPPISGQIRCSSNITGLLLTRDGGNSNNESEIFRPGGGDMRDNWRSELYKY
KVVKIEPLGVAPTKAKRRVVQREKRAVGIGALFLGFLGAAGSTMGAASMTLTVQ
ARQLLSGIVQQQNNLLRAIEAQQHLLQLTVWGIKQLQARILAVERYLKDQQLLGI
WGCSGKLICTTAVPWNASWSNKSLEQIWNHTTWMEWDREINNYTSLIHSLIEE
SQNQQEKNEQELLELDKWASLWNWFNITNWLWYIKLFIMIVGGLVGLRIVFAVL
SIVNRVRQGYSPLSFQTHLPTPRGPDRPEGIEEEGGERDRDRSIRLVNGSLALI
WDDLRSLCLFSYHRLRDLLLIVTRIVELLGRRGWEALKYWWNLLQYWSQELKN

SAVSLLNATAIAVAEGTDRVIEVVQGACRAIRHIPRRIRQGLERILL (SEQ ID NO:55)

Endogenous feline virus RD114 ENV

MKLPTGMVILCSLIIVRAGFDDPRKAIALVQKQHGKPCECSGGQVSEAPPNSIQ
QVTCPGKTAYLMTNQKWKCRVTPKISPSGGELQNCPCNTFQDSMHSSCYTEY
RQCRRINKTYYTATLLKIRSGSLNEVQILQNPNQLLQSPCRGSINQPVCWSATA
PIHISDGGGPLDTKRVWTVQKRLEQIHKAMTPELQYHPLALPKVRDDLSLDART
FDILNTTFRLLQMSNFSLAQDCWLCLKLGTPTPLAIPTPSLTYSLADSLANASCQI
IPPLLVQPMQFSNSSCLSSPFINDTEQIDLGAVTFTNCTSVANVSSPLCALNGSV
FLCGNNMAYTYLPQNWTRLCVQASLLPDIDINPGDEPVPIPAIDHYIHRPKRAVQ
FIPLLAGLGITAAFTTGATGLGVSVTQYTKLSHQLISDVQVLSGTIQDLQDQVDSL
AEVVLQNRRGLDLLTAEQGGICLALQEKCCFYANKSGIVRNKIRTLQEELQKRR
ESLATNPLWTGLQGFLPYLLPLLGPLLTLLLILTIGPCVFSRLMAFINDRLNVVHA
MVLAQQYQALKAEEEAQD (SEQ ID NO:56)

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

MSPVKGGTKCIKYLLFGFNFIFWLAGIAVLAIGLWLRFDSQTKSIFEQETNNNNS
SFYTGVYILIGAGALMMLVGFLGCCGAVQESQCMLGLFFGFLLVIFAIEIAAAIWG
YSHKDEVIKEVQEFYKDTYNKLKTKDEPQRETLKAIHYALNCCGLAGGVEQFIS
DICPKKDVLETFTVKSCPDAIKEVFDNKFHIIGAVGIGIAVVMIFGMIFSMILCCAIR
RNREMV (SEQ ID NO:57)

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

MAVEGGMKCVKFLLYVLLLAFCACAVGLIAVGVGAQLVLSQTIIQGATPGSLLPV
VIIAVGVFLFLVAFVGCCGACKENYCLMITFAIFLSLIMLVEVAAAIAGYVFRDKVM
SEFNNNFRQQMENYPKNNHTASILDRMQADFKCCGAANYTDWEKIPSMSKNR
VPDSCCINVTVGCGINFNEKAIHKEGCVEKIGGWLRKNVLVVAAAALGIAFVEVL
GIVFACCLVKSIRSGYEVM (SEQ ID NO:58)

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

MGVEGCTKCIKYLLFVFNFVFWLAGGVILGVALWLRHDPQTTNLLYLELGDKPA
PNTFYVGIYILIAVGAVMMFVGFLGCYGAIQESQCLLGTFFTCLVILFACEVAAGI
WGFVNKDQIAKDVKQFYDQALQQAVVDDDANNAKAVVKTFHETLDCCGSSTL
TALTTSVLKNNLCPSGSNIISNLFKEDCHQKIDDLFSGKLYLIGIAAIVVAVIMIFEM
ILSMVLCCGIRNSSVY (SEQ ID NO:59)

***Homo sapiens*: CD47 "Self Hairpin" 10 Amino Acids**

EVTELTREGE (SEQ ID NO:60)

***Homo sapiens*: CD47 "Self Hairpin" 21 Amino Acids**

GNYTCEVTELTREGETIIELK (SEQ ID NO:61)

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

MWPLVAALLLGSACCGSAQLLFNKTKSVEFTFCNDTVVIPCFVTNMEAQNTTEV
YVKWKFKGRDIYTFDGALNKSTVPTDFSSAKIEVSQLLKGDASLKMDKSDAVSH

TGNYTCEVTELTREGETIIELKYRVVSWFSPNENILIVIFPIFAILLFWGQFGIKTLK
YRSGGMDEKTIALLVAGLVITVIVIVGAILFVPGEYSLKNATGLGLIVTSTGILILLH
YYVFSTAIGLTSFVIAILVIQVIAYILAVVGLSLCIAACIPMHGPLLISGLSILALAQLL
GLVYMKFVE (SEQ ID NO:62)

AAV2: REP52

MELVGWLVDKGITSEKQWIQEDQASYISFNAASNSRSQIKAALDNAGKIMSLTK
TAPDYLVGQQPVEDISSNRIYKILELNGYDPQYAASVFLGWATKKFGKRNTIWL
FGPATTGKTNIAEAIAHTVPFYGCVNWTNENFPFNDCVDKMVIWWEEGKMTAK
VVESAKAILGGSKVRVDQKCKSSAQIDPTPVIVTSNTNMCAVIDGNSTTFEHQQ
PLQDRMFKFELTRRLDHDFGKVTKQEVKDFFRWAKDHVVEVEHEFYVKKGGA
KKRPAPSDADISEPKRVRESVAQPSTSDAEASINYADRYQNKCSRHVGMNLML
FPCRQCERMNQNSNICFTHGQKDCLECFPVSESQPVSVVKKAYQKLCYIHHIM
GKVPDACTACDLVNVDLDDCIFEQ (SEQ ID NO:63)

AAV2: REP78

MPGFYEIVIKVPSDLDEHLPGISDSFVNWVAEKEWELPPDSDMDLNLIEQAPLT
VAEKLQRDFLTEWRRVSKAPEALFFVQFEKGESYFHMHVLVETTGVKSMVLGR
FLSQIREKLIQRIYRGIEPTLPNWFAVTKTRNGAGGGNKVVDECYIPNYLLPKTQ
PELQWAWTNMEQYLSACLNLTERKRLVAQHLTHVSQTQEQNKENQNPNSDAP
VIRSKTSARYMELVGWLVDKGITSEKQWIQEDQASYISFNAASNSRSQIKAALD
NAGKIMSLTKTAPDYLVGQQPVEDISSNRIYKILELNGYDPQYAASVFLGWATKK
FGKRNTIWLFGPATTGKTNIAEAIAHTVPFYGCVNWTNENFPFNDCVDKMVIW
WEEGKMTAKVVESAKAILGGSKVRVDQKCKSSAQIDPTPVIVTSNTNMCAVIDG
NSTTFEHQQPLQDRMFKFELTRRLDHDFGKVTKQEVKDFFRWAKDHVVEVEH
EFYVKKGGAKKRPAPSDADISEPKRVRESVAQPSTSDAEASINYADRYQNKCS
RHVGMNLMLFPCRQCERMNQNSNICFTHGQKDCLECFPVSESQPVSVVKKAY
QKLCYIHHIMGKVPDACTACDLVNVDLDDCIFEQ (SEQ ID NO:64)

AAV2: VP1

MAADGYLPDWLEDTLSEGIRQWWKLKPGPPPPKPAERHKDDSRGLVLPGYKY
LGPFNGLDKGEPVNEADAAALEHDKAYDRQLDSGDNPYLKYNHADAEFQERL
KEDTSFGGNLGRAVFQAKKRVLEPLGLVEEPVKTAPGKKRPVEHSPVEPDSSS
GTGKAGQQPARKRLNFGQTGDADSVPDPQPLGQPPAAPSGLGTNTMATGSG
APMADNNEGADGVGNSSGNWHCDSTWMGDRVITTSTRTWALPTYNNHLYKQ
ISSQSGASNDNHYFGYSTPWGYFDFNRFHCHFSPRDWQRLINNNWGFRPKRL
NFKLFNIQVKEVTQNDGTTTIANNLTSTVQVFTDSEYQLPYVLGSAHQGCLPPF
PADVFMVPQYGYLTLNNGSQAVGRSSFYCLEYFPSQMLRTGNNFTFSYTFEDV
PFHSSYAHSQSLDRLMNPLIDQYLYYLSRTNTPSGTTTQSRLQFSQAGASDIRD
QSRNWLPGPCYRQQRVSKTSADNNNSEYSWTGATKYHLNGRDSLVNPGPAM
ASHKDDEEKFFPQSGVLIFGKQGSEKTNVDIEKVMITDEEEIRTTNPVATEQYGS
VSTNLQRGNRQAATADVNTQGVLPGMVWQDRDVYLQGPIWAKIPHTDGHFHP
SPLMGGFGLKHPPPQILIKNTPVPANPSTTFSAAKFASFITQYSTGQVSVEIEWE
LQKENSKRWNPEIQYTSNYNKSVNVDFTVDTNGVYSEPRPIGTRYLTRNL
(SEQ ID NO:65)

AAV2: VP2

APGKKRPVEHSPVEPDSSSGTGKAGQQPARKRLNFGQTGDADSVPDPQPLG
QPPAAPSGLGTNTMATGSGAPMADNNEGADGVGNSSGNWHCDSTWMGDRV
ITTSTRTWALPTYNNHLYKQISSQSGASNDNHYFGYSTPWGYFDFNRFHCHFS
PRDWQRLINNNWGFRPKRLNFKLFNIQVKEVTQNDGTTTIANNLTSTVQVFTDS
EYQLPYVLGSAHQGCLPPFPADVFMVPQYGYLTLNNGSQAVGRSSFYCLEYFP
SQMLRTGNNFTFSYTFEDVPFHSSYAHSQSLDRLMNPLIDQYLYYLSRTNTPS
GTTTQSRLQFSQAGASDIRDQSRNWLPGPCYRQQRVSKTSADNNNSEYSWT
GATKYHLNGRDSLVNPGPAMASHKDDEEKFFPQSGVLIFGKQGSEKTNVDIEK
VMITDEEEIRTTNPVATEQYGSVSTNLQRGNRQAATADVNTQGVLPGMVWQD
RDVYLQGPIWAKIPHTDGHFHPSPLMGGFGLKHPPPQILIKNTPVPANPSTTFS
AAKFASFITQYSTGQVSVEIEWELQKENSKRWNPEIQYTSNYNKSVNVDFTVDT
NGVYSEPRPIGTRYLTRNL (SEQ ID NO:66)

AAV2: VP3

MATGSGAPMADNNEGADGVGNSSGNWHCDSTWMGDRVITTSTRTWALPTYN
NHLYKQISSQSGASNDNHYFGYSTPWGYFDFNRFHCHFSPRDWQRLINNNWG
FRPKRLNFKLFNIQVKEVTQNDGTTTIANNLTSTVQVFTDSEYQLPYVLGSAHQ
GCLPPFPADVFMVPQYGYLTLNNGSQAVGRSSFYCLEYFPSQMLRTGNNFTF
SYTFEDVPFHSSYAHSQSLDRLMNPLIDQYLYYLSRTNTPSGTTTQSRLQFSQA
GASDIRDQSRNWLPGPCYRQQRVSKTSADNNNSEYSWTGATKYHLNGRDSL
VNPGPAMASHKDDEEKFFPQSGVLIFGKQGSEKTNVDIEKVMITDEEEIRTTNP
VATEQYGSVSTNLQRGNRQAATADVNTQGVLPGMVWQDRDVYLQGPIWAKIP
HTDGHFHPSPLMGGFGLKHPPPQILIKNTPVPANPSTTFSAAKFASFITQYSTG
QVSVEIEWELQKENSKRWNPEIQYTSNYNKSVNVDFTVDTNGVYSEPRPIGTR
YLTRNL (SEQ ID NO:67)

Synthetic: **Myc-Tagged Anti CD19 scFv**

EQKLISEEDLDIQMTQTTSSLSASLGDRVTISCRASQDISKYLNWYQQKPDGTV
KLLIYHTSRLHSGVPSRFSGSGSGTDYSLTISNLEQEDIATYFCQQGNTLPYTFG
GGTKLEITGGGGSGGGGSGGGGSEVKLQESGPGLVAPSQSLSVTCTVSGVSL
PDYGVSWIRQPPRKGLEWLGVIWGSETTYYNSALKSRLTIIKDNSKSQVFLKMN
SLQTDDTAIYYCAKHYYYGGSYAMDYWGQGTSVTVSS (SEQ ID NO:68)

Synthetic**: dDZF1**

FKCEHCRILFLDHVMFTIHMGCHGFRDPFKCNMCGEKCDGPVGLFVHMARNA
HGEKPFYCEHCEITFRDVVMYSLHKGYHGFRDPFECNICGYHSQDRYEFSSHI
VRGEH (SEQ ID NO:69)

Synthetic**: dDZF2**

HHCQHCDMYFADNILYTIHMGCHSCDDVFKCNMCGEKCDGPVGLFVHMARNA
HGEKPTKCVHCGIVFLDEVMYALHMSCHGFRDPFECNICGYHSQDRYEFSSHI
VRGEH (SEQ ID NO:70)

Synthetic**: DmrA**

MGRGVQVETISPGDGRTFPKRGQTCVVHYTGMLEDGKKFDSSRDRNKPFKFM
LGKQEVIRGWEEGVAQMSVGQRAKLTISPDYAYGATGHPGIIPPHATLVFDVEL
LKLE (SEQ ID NO:71)

***Synthetic*: DmrB**
MASRGVQVETISPGDGRTFPKRGQTCVVHYTGMLEDGKKVDSSRDRNKPFKF
MLGKQEVIRGWEEGVAQMSVGQRAKLTISPDYAYGATGHPGIIPPHATLVFDVE
LLKLE (SEQ ID NO:72)

***Synthetic*: DmrC**
MGSRILWHEMWHEGLEEASRLYFGERNVKGMFEVLEPLHAMMERGPQTLKET
SFNQAYGRDLMEAQEWCRKYMKSGNVKDLLQAWDLYYHVFRRISK (SEQ ID
NO:73)

***Homo sapiens/Synthetic:* FKBP**
MGVQVETISPGDGRTFPKRGQTCVVHYTGMLEDGKKFDSSRDRNKPFKFMLG
KQEVIRGWEEGVAQMSVGQRAKLTISPDYAYGATGHPGIIPPHATLVFDVELLK
LE (SEQ ID NO:74)

***Homo sapiens/Synthetic:* FRB**
*QGMLEMWHEGLEEASRLYFGERNVKGMFEVLEPLHAMMERGPQTLKETSFN
QAYGRDLMEAQEWCRKYMKSGNVKDLLQAWDLYYHVFRRISK (SEQ ID
NO:75)*

***Synthetic:* Anti-GCN4 scFv**
MGPDIVMTQSPSSLSASVGDRVTITCRSSTGAVTTSNYASWVQEKPGKLFKGLI
GGTNNRAPGVPSRFSGSLIGDKATLTISSLQPEDFATYFCALWYSNHWVFGQG
TKVELKRGGGGSGGGGSGGGGSSGGGSEVKLLESGGGLVQPGGSLKLSCAV
SGFSLTDYGVNWVRQAPGRGLEWIGVIWGDGITDYNSALKDRFIISKDNGKNTV
YLQMSKVRSDDTALYYCVTGLFDYWGQGTLVTVSSYPYDVPDYAGGGGGSG
GGGSGGGGSGGGGS (SEQ ID NO:76)

***Synthetic:* 10x-GCN4 Repeats**
EELLSKNYHLENEVARLKKGSGSGEELLSKNYHLENEVARLKKGSGSGEELLS
KNYHLENEVARLKKGSGSGEELLSKNYHLENEVARLKKGSGSGEELLSKNYHL
ENEVARLKKGSGSGEELLSKNYHLENEVARLKKGSGSGEELLSKNYHLENEVA
RLKKGSGSGEELLSKNYHLENEVARLKKGSGSGEELLSKNYHLENEVARLKKG
SGSGEELLSKNYHLENEVARLKKGS (SEQ ID NO:77)

***Synthetic:* 24x-GCN4 Repeats**
EELLSKNYHLENEVARLKKGSGSGEELLSKNYHLENEVARLKKGSGSGEELLS
KNYHLENEVARLKKGSGSGEELLSKNYHLENEVARLKKGSGSGEELLSKNYHL
ENEVARLKKGSGSGEELLSKNYHLENEVARLKKGSGSGEELLSKNYHLENEVA
RLKKGSGSGEELLSKNYHLENEVARLKKGSGSGEELLSKNYHLENEVARLKKG
SGSGEELLSKNYHLENEVARLKKGSGSGEELLSKNYHLENEVARLKKGSGSGE
ELLSKNYHLENEVARLKKGSGSGEELLSKNYHLENEVARLKKGSGSGEELLSK

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

Synthetic: GFP-targeting Nanobody
VQLVESGGALVQPGGSLRLSCAASGFPVNRYSMRWYRQAPGKEREWVAGMS
SAGDRSSYEDSVKGRFTISRDDARNTVYLQMNSLKPEDTAVYYSNVNVGFEY
WGQGTQVTVSS (SEQ ID NO:79)

Nostoc punctiforme: **Npu DnaE N-terminal Split Intein**
CLSYETEILTVEYGLLPIGKIVEKRIECTVYSVDNNGNIYTQPVAQWHDRGEQEV
FEYCLEDGSLIRATKDHKFMTVDGQMLPIDEIFERELDLMRVDNLPN
(SEQ ID NO:80)

Nostoc punctiforme: **Npu DnaE C-terminal Split Intein**
MIKIATRKYLGKQNVYDIGVERDHNFALKNGFIASNCFN (SEQ ID NO:81)

Synthetic**: Cfa N-Terminal Split Intein**
CLSYDTEILTVEYGFLPIGKIVEERIECTVYTVDKNGFVYTQPIAQWHNRGEQEV
FEYCLEDGSIIRATKDHKFMTTDGQMLPIDEIFERGLDLKQVDGLP (SEQ ID
NO:82)

Synthetic**: Cfa C-Terminal Split Intein**
MVKIISRKSLGTQNVYDIGVEKDHNFLLKNGLVASN (SEQ ID NO:83)

Saccharomyces cerevisiae**: Vma N-terminal Split Intein**
CFAKGTNVLMADGSIECIENIEVGNKVMGKDGRPREVIKLPRGRETMYSVVQKS
QHRAHKSDSSREVPELLKFTCNATHELVVRTPRSVRRLSRTIKGVEYFEVITFE
MGQKKAPDGRIVELVKEVSKSYPISEGPERANELVESYRKASNKAYFEWTIEAR
DLSLLGSHVRKATYQTYAPILY (SEQ ID NO:84)

Saccharomyces cerevisiae**: Vma C-terminal Split Intein**
VLLNVLSKCAGSKKFRPAPAAAFARECRGFYFELQELKEDDYYGITLSDDSDHQ
FLLANQVVVHN (SEQ ID NO:85)

Synechocystis sp. PCC 6803**: Ssp DnaE N-terminal Split Intein**
CLSFGTEILTVEYGPLPIGKIVSEEINCSVYSVDPEGRVYTQAIAQWHDRGEQEV
LEYELEDGSVIRATSDHRFLTTDYQLLAIEEIFARQLDLLTLENIKQTEEALDNHR
LPFPLLDAGTIK (SEQ ID NO:86)

Synechocystis sp. PCC 6803**: Ssp DnaE C-terminal Split Intein**
MVKVIGRRSLGVQRIFDIGLPQDHNFLLANGAIAAN (SEQ ID NO:87)

***Synthetic:* Spy Tag**
VPTIVMVDAYKRYK (SEQ ID NO:88)

***Synthetic:* Spy Catcher**
MVTTLSGLSGEQGPSGDMTTEEDSATHIKFSKRDEDGRELAGATMELRDSSG
KTISTWISDGHVKDFYLYPGKYTFVETAAPDGYEVATAITFTVNEQGQVTVNGE
ATKGDAHTGSSGS (SEQ ID NO:89)

***Bacteriophage MS2:* MS2 RNA Binding Protein**
MASNFTQFVLVDNGGTGDVTVAPSNFANGVAEWISSNSRSQAYKVTCSVRQS
SAQNRKYTIKVEVPKVATQTVGGVELPVAAWRSYLNMELTIPIFATNSDCELIVK
AMQGLLKDGNPIPSAIAANSGIY (SEQ ID NO:90)

***Bacteriophage MS2:* MS2 (N55K) RNA Binding Protein**
MASNFTQFVLVDNGGTGDVTVAPSNFANGVAEWISSNSRSQAYKVTCSVRQS
SAQKRKYTIKVEVPKVATQTVGGVELPVAAWRSYLNMELTIPIFATNSDCELIVK
AMQGLLKDGNPIPSAIAANSGIY (SEQ ID NO:91)

***Bacteriophage MS2:* MS2 (N55K)(V29I) RNA Binding Protein**
MASNFTQFVLVDNGGTGDVTVAPSNFANGIAEWISSNSRSQAYKVTCSVRQSS
AQKRKYTIKVEVPKVATQTVGGVELPVAAWRSYLNMELTIPIFATNSDCELIVKA
MQGLLKDGNPIPSAIAANSGIY (SEQ ID NO:92)

***Bacteriophage PP7*: PP7 RNA Binding Protein**
KTIVLSVGEATRTLTEIQSTADRQIFEEKVGPLVGRLRLTASLRQNGAKTAYRVN
LKLDQADVVDSGLPKVRYTQVWSHDVTIVANSTEASRKSLYDLTKSLVATSQVE
DLVVNLVPLGRS (SEQ ID NO:93)

***Bacteriophage Mu*: COM RNA Binding Protein**
MKSIRCKNCNKLLFKADSFDHIEIRCPRCKRHIIMLNACEHPTEKHCGKREKITH
SDETVRY (SEQ ID NO:94)

***Synthetic:* Zinc Finger ZF6/10**
STRPGERPFQCRICMRNFSIPNHLARHTRTHTGEKPFQCRICMRNFSQSAHLK
RHLRTHTGEKPFQCRICMRNFSQDVSLVRHLKTHLRQKDGERPFQCRICMRNF
SSAQALARHTRTHTGEKPFQCRICMRNFSQGGNLTRHLRTHTGEKPFQCRICM
RNFSQHPNLTRHLKTHLRGS (SEQ ID NO:95)

***Synthetic:* Zinc Finger ZF8/7**
SRPGERPFQCRICMRNFSTMAVLRRHTRTHTGEKPFQCRICMRNFSRREVLEN
HLRTHTGEKPFQCRICMRNFSQTVNLDRHLKTHLRQKDGERPFQCRICMRNFS
KKDHLHRHTRTHTGEKPFQCRICMRNFSQRPHLTNHLRTHTGEKPFQCRICMR
NFSVGASLKRHLKTHLRGS (SEQ ID NO:96)

***Synthetic:* Zinc Finger ZF9**

SRPGERPFQCRICMRNFSDKTKLRVHTRTHTGEKPFQCRICMRNFSVRHNLTR
HLRTHTGEKPFQCRICMRNFSQSTSLQRHLKTHLRGF (SEQ ID NO:97)

Synthetic: **Zinc Finger MK10**

SRPGERPFQCRICMRNFSRRHGLDRHTRTHTGEKPFQCRICMRNFSDHSSLK
RHLRTHTGSQKPFQCRICMRNFSVRHNLTRHLRTHTGEKPFQCRICMRNFSDH
SNLSRHLKTHTGSQKPFQCRICMRNFSQRSSLVRHLRTHTGEKPFQCRICMRN
FSESGHLKRHLRTHLRGS (SEQ ID NO:98)

Synthetic: **Zinc Finger 268**

YACPVESCDRRFSRSDELTRHIRIHTGQKPFQCRICMRNFSRSDHLTTHIRTHT
GEKPFACDICGRKFARSDERKRHTKIHLRQKD (SEQ ID NO:99)

Synthetic: **Zinc Finger NRE**

YACPVESCDRRFSQSHDLTKHIRIHTGQKPFQCRICMRNFSDSSKLSRHIRTHT
GEKPFACDICGRKFARLDNRTAHTKIHLRQKD (SEQ ID NO:100)

Synthetic: **Zinc Finger 268/NRE**

YACPVESCDRRFSRSDELTRHIRIHTGQKPFQCRICMRNFSRSDHLTTHIRTHT
GEKPFACDICGRKFARSDERKRHTKIHLRQKDGERPYACPVESCDRRFSQSHD
LTKHIRIHTGQKPFQCRICMRNFSDSSKLSRHIRTHTGEKPFACDICGRKFARLD
NRTAHTKIHLRQKD (SEQ ID NO:101)

Synthetic: **Zinc Finger 268//NRE**

YACPVESCDRRFSRSDELTRHIRIHTGQKPFQCRICMRNFSRSDHLTTHIRTHT
GEKPFACDICGRKFARSDERKRHTKIHLRQKDGGGSERPYACPVESCDRRFS
QSHDLTKHIRIHTGQKPFQCRICMRNFSDSSKLSRHIRTHTGEKPFACDICGRKF
ARLDNRTAHTKIHLRQKD (SEQ ID NO:102)

Synthetic: **FokI Zinc Finger Nuclease 17-2 Targeting GFP**

SRPGERPFQCRICMRNFSTRQNLDTHTRTHTGEKPFQCRICMRNFSRRDTLER
HLRTHTGEKPFQCRICMRNFSRPDALPRHLKTHLRGSQLVKSELEEKKSELRH
KLKYVPHEYIELIEIARNSTQDRILEMKVMEFFMKVYGYRGKHLGGSRKPDGAIY
TVGSPIDYGVIVDTKAYSGGYNLPIGQADEMQRYVEENQTRNKHINPNEWWKV
YPSSVTEFKFLFVSGHFKGNYKAQLTRLNHITNCNGAVLSVEELLIGGEMIKAGT
LTLEEVRRKFNNGEINF (SEQ ID NO:103)

Synthetic: **FokI Zinc Finger Nuclease 18-2 Targeting GFP**

SRPGERPFQCRICMRNFSSPSKLIRHTRTHTGEKPFQCRICMRNFSDGSNLAR
HLRTHTGEKPFQCRICMRNFSRVDNLPRHLKTHLRGSQLVKSELEEKKSELRH
KLKYVPHEYIELIEIARNSTQDRILEMKVMEFFMKVYGYRGKHLGGSRKPDGAIY
TVGSPIDYGVIVDTKAYSGGYNLPIGQADEMQRYVEENQTRNKHINPNEWWKV
YPSSVTEFKFLFVSGHFKGNYKAQLTRLNHITNCNGAVLSVEELLIGGEMIKAGT
LTLEEVRRKFNNGEINF (SEQ ID NO:104)

Synthetic: **Left FokI Zinc Finger Nuclease Targeting CCR5**

MDYKDHDGDYKDHDIDYKDDDDKMAPKKKRKVGIHGVPAAMAERPFQCRICM
RNFSDRSNLSRHIRTHTGEKPFACDICGRKFAISSNLNSHTKIHTGSQKPFQCRI
CMRNFSRSDNLARHIRTHTGEKPFACDICGRKFATSGNLTRHTKIHLRGSQLVK
SELEEKKSELRHKLKYVPHEYIELIEIARNSTQDRILEMKVMEFFMKVYGYRGKH
LGGSRKPDGAIYTVGSPIDYGVIVDTKAYSGGYNLPIGQADEMQRYVEENQTR
NKHINPNEWWKVYPSSVTEFKFLFVSGHFKGNYKAQLTRLNHITNCNGAVLSV
EELLIGGEMIKAGTLTLEEVRRKFNNGEINF (SEQ ID NO:105)

Synthetic: **Right FokI Zinc Finger Nuclease Targeting CCR5**
MDYKDHDGDYKDHDIDYKDDDDKMAPKKKRKVGIHGVPAAMAERPFQCRICM
RNFSRSDNLSVHIRTHTGEKPFACDICGRKFAQKINLQVHTKIHTGEKPFQCRIC
MRNFSRSDVLSEHIRTHTGEKPFACDICGRKFAQRNHRTTHTKIHLRGSQLVKS
ELEEKKSELRHKLKYVPHEYIELIEIARNSTQDRILEMKVMEFFMKVYGYRGKHL
GGSRKPDGAIYTVGSPIDYGVIVDTKAYSGGYNLPIGQADEMQRYVEENQTRN
KHINPNEWWKVYPSSVTEFKFLFVSGHFKGNYKAQLTRLNHITNCNGAVLSVE
ELLIGGEMIKAGTLTLEEVRRKFNNGEINF(SEQ ID NO:106)

Synthetic**: FokI Nuclease Domain**
QLVKSELEEKKSELRHKLKYVPHEYIELIEIARNSTQDRILEMKVMEFFMKVYGY
RGKHLGGSRKPDGAIYTVGSPIDYGVIVDTKAYSGGYNLPIGQADEMQRYVEE
NQTRNKHINPNEWWKVYPSSVTEFKFLFVSGHFKGNYKAQLTRLNHITNCNGA
VLSVEELLIGGEMIKAGTLTLEEVRRKFNNGEINF (SEQ ID NO:107)

Synthetic**: AcuI Nuclease Domain**
VHDHKLELAKLIRNYETNRKECLNSRYNETLLRSDYLDPFFELLGWDIKNKAGK
PTNEREVVLEEALKASASEHSKKPDYTFRLFSERKFFLEAKKPSVHIESDNETAK
QVRRYGFTAKLKISVLSNFEYLVIYDTSVKVDGDDTFNKARIKKYHYTEYETHFD
EICDLLGRESVYSGNFDKEWLSIENKINHFSVDTL (SEQ ID NO:108)

Synthetic**: Truncated AcuI Nuclease Domain**
YNETLLRSDYLDPFFELLGWDIKNKAGKPTNEREVVLEEALKASASEHSKKPDY
TFRLFSERKFFLEAKKPSVHIESDNETAKQVRRYGFTAKLKISVLSNFEYLVIYDT
SVKVDGDDT (SEQ ID NO:109)

Escherichia coli**: Ferritin**
MLKPEMIEKLNEQMNLELYSSLLYQQMSAWCSYHTFEGAAAFLRRHAQEEMT
HMQRLFDYLTDTGNLPRINTVESPFAEYSSLDELFQETYKHEQLITQKINELAHA
AMTNQDYPTFNFLQWYVSEQHEEEKLFKSIIDKLSLAGKSGEGLYFIDKELSTLD
TQN (SEQ ID NO:110)

Escherichia coli**: Ferritin (H34L)(T64I)**
MLKPEMIEKLNEQMNLELYSSLLYQQMSAWCSYLTFEGAAAFLRRHAQEEMTH
MQRLFDYLTDIGNLPRINTVESPFAEYSSLDELFQETYKHEQLITQKINELAHAA
MTNQDYPTFNFLQWYVSEQHEEEKLFKSIIDKLSLAGKSGEGLYFIDKELSTLDT
QN (SEQ ID NO:111)

***Mus musculus & Synthetic*: Light & Heavy Chain Ferritin Chimera**
MTSQIRQNYSTEVEAAVNRLVNLHLRASYTYLSLGFFFDRDDVALEGVGHFFRE
LAEEKREGAERLLEFQNDRGGRALFQDVQKPSQDEWGKTQEAMEAALAMEK
NLNQALLDLHALGSARADPHLCDFLESHYLDKEVKLIKKMGNHLTNLRRVAGPQ
PAQTGAPQGSLGEYLFERLTLKHDARGGGGSDYKDDDDKGGGGSRVMTTAS
PSQVRQNYHQDAEAAINRQINLELYASYVYLSMSCYFDRDDVALKNFAKYFLH
QSHEEREHAEKLMKLQNQRGGRIFLQDIKKPDRDDWESGLNAMECALHLEKS
VNQSLLELHKLATDKNDPHLCDFIETYYLSEQVKSIKELGDHVTNLRKMGAPEA
GMAEYLFDKHTLGHGDESTR (SEQ ID NO:112)

***Homo sapiens & Synthetic*: Light & Heavy Chain Ferritin Chimera**
SQIRQNYSTDVEAAVNSLVNLYLQASYTYLSLGFYFDRDDVALEGVSHFFRELA
EEKREGYERLLKMQNQRGGRALFQDIKKPAEDEWGKTPDAMKAAMALEKKLN
QALLDLHALGSARTDPHLCDFLETHFLDEEVKLIKKMGDHLTNLHRLGGPEAGL
GEYLFERLTLKHDARGGGGSDYKDDDDKGGGGSRVMTTASTSQVRQNYHQD
SEAAINRQINLELYASYVYLSMSYYFDRDDVALKNFAKYFLHQSHEEREHAEKL
MKLQNQRGGRIFLQDIKKPDCDDWESGLNAMECALHLEKNVNQSLLELHKLAT
DKNDPHLCDFIETHYLNEQVKAIKELGDHVTNLRKMGAPESGLAEYLFDKHTLG
DSDNES (SEQ ID NO:113)

***Arabidopsis thaliana*: Cry2**
MKMDKKTIVWFRRDLRIEDNPALAAAAHEGSVFPVFIWCPEEEGQFYPGRASR
WWMKQSLAHLSQSLKALGSDLTLIKTHNTISAILDCIRVTGATKVVFNHLYDPVS
LVRDHTVKEKLVERGISVQSYNGDLLYEPWEIYCEKGKPFTSFNSYWKKCLDM
SIESVMLPPPWRLMPITAAAEAIWACSIEELGLENEAEKPSNALLTRAWSPGWS
NADKLLNEFIEKQLIDYAKNSKKVVGNSTSLLSPYLHFGEISVRHVFQCARMKQII
WARDKNSEGEESADLFLRGIGLREYSRYICFNFPFTHEQSLLSHLRFFPWDADV
DKFKAWRQGRTGYPLVDAGMRELWATGWMHNRIRVIVSSFAVKFLLLPWKWG
MKYFWDTLLDADLECDILGWQYISGSIPDGHELDRLDNPALQGAKYDPEGEYIR
QWLPELARLPTEWIHHPWDAPLTVLKASGVELGTNYAKPIVDIDTARELLAKAIS
RTREAQIMIGAAPDEIVADSFEALGANTIKEPGLCPSVSSNDQQVPSAVRYNGS
AAVKPEEEEERDMKKSRGFDERELFSTAESSSSSSVFFVSQSCSLASEGKNLE
GIQDSSDQITTSLGKNGCK (SEQ ID NO:114)

***Arabidopsis thaliana*: CIBN**
MNGAIGGDLLLNFPDMSVLERQRAHLKYLNPTFDSPLAGFFADSSMITGGEMD
SYLSTAGLNLPMMYGETTVEGDSRLSISPETTLGTGNFKAAKFDTETKDCNEAA
KKMTMNRDDLVEEGEEEKSKITEQNNGSTKSIKKMKHKAKKEENNFSNDSSKV
TKELEKTDYI (SEQ ID NO:115)

***Synthetic:* LoV2-Ja**
SLATTLERIEKNFVITDPRLPDNPIIFASDSFLQLTEYSREEILGRNCRFLQGPETD
RATVRKIRDAIDNQTEVTVQLINYTKSGKKFWNLFHLQPMRDQKGDVQYFIGVQ
LDGTEHVRDAAEREGVMLIKKTAENIDEAAKEL (SEQ ID NO:116)

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

DIEDEENMSSSSTDVKENRNLDNVSPKDGSTPGPGEGSQLSNGGGGGPGRK
RPLEEGSNGHSKYRLKKRRKTPGPVLPKNALMQLNEIKPGLQYTLLSQTGPVH
APLFVMSVEVNGQVFEGSGPTKKKAKLHAAEKALRSFVQFPNASEAHLAMGRT
LSVNTDFTSDQADFPDTLFNGFETPDKAEPPFYVGSNGDDSFSSSGDLSLSAS
PVPASLAQPPLPVLPPFPPPSGKNPVMILNELRPGLKYDFLSESGESHAKSFVM
SVVVDGQFFEGSGRNKKLAKARAAQSALAAIFNLHLDQTPSRQPIPSEGLQLHL
PQVLADAVSRLVLGKFGDLTDNFSSPHARRKVLAGVVMTTGTDVKDAKVISVST
GTKCINGEYMSDRGLALNDCHAEIISRRSLLRFLYTQLELYLNNKDDQKRSIFQK
SERGGFRLKENVQFHLYISTSPCGDARIFSPHEPILEGSRSYTQAGVQWCNHG
SLQPRPPGLLSDPSTSTFQGAGTTEPADRHPNRKARGQLRTKIESGEGTIPVRS
NASIQTWDGVLQGERLLTMSCSDKIARWNVVGIQGSLLSIFVEPIYFSSIILGSLY
HGDHLSRAMYQRISNIEDLPPLYTLNKPLLSGISNAEARQPGKAPNFSVNWTVG
DSAIEVINATTGKDELGRASRLCKHALYCRWMRVHGKVPSHLLRSKITKPNVYH
ESKLAAKEYQAAKARLFTAFIKAGLGAWVEKPTEQDQFSLTP (SEQ ID
NO:117)

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

DIEDEENMSSSSTDVKENRNLDNVSPKDGSTPGPGEGSQLSNGGGGGPGRK
RPLEEGSNGHSKYRLKKRRKTPGPVLPKNALMQLNEIKPGLQYTLLSQTGPVH
APLFVMSVEVNGQVFEGSGPTKKKAKLHAAEKALRSFVQFPNASEAHLAMGRT
LSVNTDFTSDQADFPDTLFNGFETPDKAEPPFYVGSNGDDSFSSSGDLSLSAS
PVPASLAQPPLPVLPPFPPPSGKNPVMILNELRPGLKYDFLSESGESHAKSFVM
SVVVDGQFFEGSGRNKKLAKARAAQSALAAIFNLHLDQTPSRQPIPSEGLQLHL
PQVLADAVSRLVLGKFGDLTDNFSSPHARRKVLAGVVMTTGTDVKDAKVISVST
GTKCINGEYMSDRGLALNDCHAEIISRRSLLRFLYTQLELYLNNKDDQKRSIFQK
SERGGFRLKENVQFHLYISTSPCGDARIFSPHEPILEGSRSYTQAGVQWCNHG
SLQPRPPGLLSDPSTSTFQGAGTTEPADRHPNRKARGQLRTKIESGQGTIPVR
SNASIQTWDGVLQGERLLTMSCSDKIARWNVVGIQGSLLSIFVEPIYFSSIILGSL
YHGDHLSRAMYQRISNIEDLPPLYTLNKPLLSGISNAEARQPGKAPNFSVNWTV
GDSAIEVINATTGKDELGRASRLCKHALYCRWMRVHGKVPSHLLRSKITKPNVY
HESKLAAKEYQAAKARLFTAFIKAGLGAWVEKPTEQDQFSLTP (SEQ ID
NO:118)

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

VLADAVSRLVLGKFGDLTDNFSSPHARRKVLAGVVMTTGTDVKDAKVISVSTGT
KCINGEYMSDRGLALNDCHAEIISRRSLLRFLYTQLELYLNNKDDQKRSIFQKSE
RGGFRLKENVQFHLYISTSPCGDARIFSPHEPILEGSRSYTQAGVQWCNHGSL
QPRPPGLLSDPSTSTFQGAGTTEPADRHPNRKARGQLRTKIESGEGTIPVRSN
ASIQTWDGVLQGERLLTMSCSDKIARWNVVGIQGSLLSIFVEPIYFSSIILGSLYH
GDHLSRAMYQRISNIEDLPPLYTLNKPLLSGISNAEARQPGKAPNFSVNWTVGD
SAIEVINATTGKDELGRASRLCKHALYCRWMRVHGKVPSHLLRSKITKPNVYHE
SKLAAKEYQAAKARLFTAFIKAGLGAWVEKPTEQDQFSLTP (SEQ ID
NO:119)

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

VLADAVSRLVLGKFGDLTDNFSSPHARRKVLAGVVMTTGTDVKDAKVISVSTGT
KCINGEYMSDRGLALNDCHAEIISRRSLLRFLYTQLELYLNNKDDQKRSIFQKSE
RGGFRLKENVQFHLYISTSPCGDARIFSPHEPILEGSRSYTQAGVQWCNHGSL
QPRPPGLLSDPSTSTFQGAGTTEPADRHPNRKARGQLRTKIESGQGTIPVRSN
ASIQTWDGVLQGERLLTMSCSDKIARWNVVGIQGSLLSIFVEPIYFSSIILGSLYH
GDHLSRAMYQRISNIEDLPPLYTLNKPLLSGISNAEARQPGKAPNFSVNWTVGD
SAIEVINATTGKDELGRASRLCKHALYCRWMRVHGKVPSHLLRSKITKPNVYHE
SKLAAKEYQAAKARLFTAFIKAGLGAWVEKPTEQDQFSLTP (SEQ ID NO:120)

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

MASNFTQFVLVDNGGTGDVTVAPSNFANGIAEWISSNSRSQAYKVTCSVRQSS
AQNRKYTIKVEVPKGAWRSYLNMELTIPIFATNSDCELIVKAMQGLLKDGNPIPS
AIAANSGIYGGSGSGAGSGSPAGGGAPGSGGGSKAERMGFTEVTPVTGASLR
RTMLLLSRSPEAQPKTLPLTGSTFHDQIAMLSHRCFNTLTNSFQPSLLGRKILAA
IIMKKDSEDMGVVVSLGTGNRCVKGDSLSLKGETVNDCHAEIISRRGFIRFLYSE
LMKYNSQTAKDSIFEPAKGGEKLQIKKTVSFHLYISTAPCGDGALFDKSCSDRA
MESTESRHYPVFENPKQGKLRTKVENGEGTIPVESSDIVPTWDGIRLGERLRT
MSCSDKILRWNVLGLQGALLTHFLQPIYLKSVTLGYLFSQGHLTRAICCRVTRD
GSAFEDGLRHPFIVNHPKVGRVSIYDSKRQSGKTKETSVNWCLADGYDLEILDG
TRGTVDGPRNELSRVSKKNIFLLFKKLCSFRYRRDLLRLSYGEAKKAARDYETA
KNYFKKGLKDMGYGNWISKPQEEKNFYLCPVGSGSGSLPPLERLT (SEQ ID NO:121)

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

MASNFTQFVLVDNGGTGDVTVAPSNFANGIAEWISSNSRSQAYKVTCSVRQSS
AQNRKYTIKVEVPKGAWRSYLNMELTIPIFATNSDCELIVKAMQGLLKDGNPIPS
AIAANSGIYGGSGSGAGSGSPAGGGAPGSGGGSKAERMGFTEVTPVTGASLR
RTMLLLSRSPEAQPKTLPLTGSTFHDQIAMLSHRCFNTLTNSFQPSLLGRKILAA
IIMKKDSEDMGVVVSLGTGNRCVKGDSLSLKGETVNDCHAEIISRRGFIRFLYSE
LMKYNSQTAKDSIFEPAKGGEKLQIKKTVSFHLYISTAPCGDGALFDKSCSDRA
MESTESRHYPVFENPKQGKLRTKVENGQGTIPVESSDIVPTWDGIRLGERLRT
MSCSDKILRWNVLGLQGALLTHFLQPIYLKSVTLGYLFSQGHLTRAICCRVTRD
GSAFEDGLRHPFIVNHPKVGRVSIYDSKRQSGKTKETSVNWCLADGYDLEILDG
TRGTVDGPRNELSRVSKKNIFLLFKKLCSFRYRRDLLRLSYGEAKKAARDYETA
KNYFKKGLKDMGYGNWISKPQEEKNFYLCPVGSGSGSLPPLERLTL (SEQ ID NO:122)

***Ruminococcus flavefaciens*: RfxCas13d (CasRx)**

EASIEKKKSFAKGMGVKSTLVSGSKVYMTTFAEGSDARLEKIVEGDSIRSVNEG
EAFSAEMADKNAGYKIGNAKFSHPKGYAVVANNPLYTGPVQQDMLGLKETLEK
RYFGESADGNDNICIQVIHNILDIEKILAEYITNAAYAVNNISGLDKDIIGFGKFSTV
YTYDEFKDPEHHRAAFNNNDKLINAIKAQYDEFDNFLDNPRLGYFGQAFFSKEG
RNYIINYGNECYDILALLSGLRHWVVHNNEEESRISRTWLYNLDKNLDNEYISTL

NYLYDRITNELTNSFSKNSAANVNYIAETLGINPAEFAEQYFRFSIMKEQKNLGF
NITKLREVMLDRKDMSEIRKNHKVFDSIRTKVYTMMDFVIYRYYIEEDAKVAAAN
KSLPDNEKSLSEKDIFVINLRGSFNDDQKDALYYDEANRIWRKLENIMHNIKEFR
GNKTREYKKKDAPRLPRILPAGRDVSAFSKLMYALTMFLDGKEINDLLTTLINKF
DNIQSFLKVMPLIGVNAKFVEEYAFFKDSAKIADELRLIKSFARMGEPIADARRA
MYIDAIRILGTNLSYDELKALADTFSLDENGNKLKKGKHGMRNFIINNVISNKRFH
YLIRYGDPAHLHEIAKNEAVVKFVLGRIADIQKKQGQNGKNQIDRYYETCIGKDK
GKSVSEKVDALTKIITGMNYDQFDKKRSVIEDTGRENAEREKFKKIISLYLTVIYHI
LKNIVNINARYVIGFHCVERDAQLYKEKGYDINLKKLEEKGFSSVTKLCAGIDETA
PDKRKDVEKEMAERAKESIDSLESANPKLYANYIKYSDEKKAEEFTRQINREKA
KTALNAYLRNTKWNVIIREDLLRIDNKTCTLFRNKAVHLEVARYVHAYINDIAEVN
SYFQLYHYIMQRIIMNERYEKSSGKVSEYFDAVNDEKKYNDRLLKLLCVPFGYCI
PRFKNLSIEALFDRNEAAKFDKEKKKVSGNSGSG (SEQ ID NO:123)

***Ruminococcus flavefaciens* & *Synthetic*: dead RfxCas13d (dCasRx)**
EASIEKKKSFAKGMGVKSTLVSGSKVYMTTFAEGSDARLEKIVEGDSIRSVNEG
EAFSAEMADKNAGYKIGNAKFSHPKGYAVVANNPLYTGPVQQDMLGLKETLEK
RYFGESADGNDNICIQVIHNILDIEKILAEYITNAAYAVNNISGLDKDIIGFGKFSTV
YTYDEFKDPEHHRAAFNNNDKLINAIKAQYDEFDNFLDNPRLGYFGQAFFSKEG
RNYIINYGNECYDILALLSGLAHWVVANNEEESRISRTWLYNLDKNLDNEYISTL
NYLYDRITNELTNSFSKNSAANVNYIAETLGINPAEFAEQYFRFSIMKEQKNLGF
NITKLREVMLDRKDMSEIRKNHKVFDSIRTKVYTMMDFVIYRYYIEEDAKVAAAN
KSLPDNEKSLSEKDIFVINLRGSFNDDQKDALYYDEANRIWRKLENIMHNIKEFR
GNKTREYKKKDAPRLPRILPAGRDVSAFSKLMYALTMFLDGKEINDLLTTLINKF
DNIQSFLKVMPLIGVNAKFVEEYAFFKDSAKIADELRLIKSFARMGEPIADARRA
MYIDAIRILGTNLSYDELKALADTFSLDENGNKLKKGKHGMRNFIINNVISNKRFH
YLIRYGDPAHLHEIAKNEAVVKFVLGRIADIQKKQGQNGKNQIDRYYETCIGKDK
GKSVSEKVDALTKIITGMNYDQFDKKRSVIEDTGRENAEREKFKKIISLYLTVIYHI
LKNIVNINARYVIGFHCVERDAQLYKEKGYDINLKKLEEKGFSSVTKLCAGIDETA
PDKRKDVEKEMAERAKESIDSLESANPKLYANYIKYSDEKKAEEFTRQINREKA
KTALNAYLRNTKWNVIIREDLLRIDNKTCTLFANKAVALEVARYVHAYINDIAEVN
SYFQLYHYIMQRIIMNERYEKSSGKVSEYFDAVNDEKKYNDRLLKLLCVPFGYCI
PRFKNLSIEALFDRNEAAKFDKEKKKVSGNSGSGPKKKRKVAAAYPYDVPDYA
(SEQ ID NO:124)

***Prevotella sp. P5-125:* PspCas13b**
MNIPALVENQKKYFGTYSVMAMLNAQTVLDHIQKVADIEGEQNENNENLWFHP
VMSHLYNAKNGYDKQPEKTMFIIERLQSYFPFLKIMAENQREYSNGKYKQNRV
EVNSNDIFEVLKRAFGVLKMYRDLTNHYKTYEEKLNDGCEFLTSTEQPLSGMIN
NYYTVALRNMNERYGYKTEDLAFIQDKRFKFVKDAYGKKKSQVNTGFFLSLQD
YNGDTQKKLHLSGVGIALLICLFLDKQYINIFLSRLPIFSSYNAQSEERRIIIRSFGI
NSIKLPKDRIHSEKSNKSVAMDMLNEVKRCPDELFTTLSAEKQSRFRIISDDHNE
VLMKRSSDRFVPLLLQYIDYGKLFDHIRFHVNMGKLRYLLKADKTCIDGQTRVR
VIEQPLNGFGRLEEAETMRKQENGTFGNSGIRIRDFENMKRDDANPANYPYIVD
TYTHYILENNKVEMFINDKEDSAPLLPVIEDDRYVVKTIPSCRMSTLEIPAMAFHM
FLFGSKKTEKLIVDVHNRYKRLFQAMQKEEVTAENIASFGIAESDLPQKILDLISG

NAHGKDVDAFIRLTVDDMLTDTERRIKRFKDDRKSIRSADNKMGKRGFKQISTG
KLADFLAKDIVLFQPSVNDGENKITGLNYRIMQSAIAVYDSGDDYEAKQQFKLMF
EKARLIGKGTTEPHPFLYKVFARSIPANAVEFYERYLIERKFYLTGLSNEIKKGNR
VDVPFIRRDQNKWKTPAMKTLGRIYSEDLPVELPRQMFDNEIKSHLKSLPQME
GIDFNNANVTYLIAEYMKRVLDDDFQTFYQWNRNYRYMDMLKGEYDRKGSLQ
HCFTSVEEREGLWKERASRTERYRKQASNKIRSNRQMRNASSEEIETILDKRLS
NSRNEYQKSEKVIRRYRVQDALLFLLAKKTLTELADFDGERFKLKEIMPDAEKGI
LSEIMPMSFTFEKGGKKYTITSEGMKLKNYGDFFVLASDKRIGNLLELVGSDIVS
KEDIMEEFNKYDQCRPEISSIVFNLEKWAFDTYPELSARVDREEKVDFKSILKILL
NNKNINKEQSDILRKIRNAFDHNNYPDKGVVEIKALPEIAMSIKKAFGEYAIMKGS
LQ (SEQ ID NO:125)

***Synthetic*: L17E**
IWLTALKFLGKHAAKHEAKQQLSKL (SEQ ID NO:126)

***Synthetic*: L17E-Transmembrane**
IWLTALKFLGKHAAKHEAKQQLSKLNAVGQDTQEVIVVPHSLPFKVVVISAILALV
VLTIISLIILIMLWQKKPR (SEQ ID NO:127)

***Synthetic*: KALA**
WEAKLAKALAKALAKHLAKALAKALKACEA (SEQ ID NO:128)

***Synthetic*: KALA-Transmembrane**
WEAKLAKALAKALAKHLAKALAKALKACEANAVGQDTQEVIVVPHSLPFKVVVIS
AILALVVLTIISLIILIMLWQKKPR (SEQ ID NO:129)

***Synthetic*: Vectofusin**
KKALLHAALAHLLALAHHLLALLKKA (SEQ ID NO:130)

***Synthetic*: Vectofusin-Transmembrane**
KKALLHAALAHLLALAHHLLALLKKANAVGQDTQEVIVVPHSLPFKVVVISAILAL
VVLTIISLIILIMLWQKKPR (SEQ ID NO:131)

***Synthetic*: Transmembrane Domain**
NAVGQDTQEVIVVPHSLPFKVVVISAILALVVLTIISLIILIMLWQKKPR (SEQ ID
NO:132)

Lactococcus lactis: Nisin A
ITSISLCTPGCKTGALMGCNMKTATCHCSIHVSK (SEQ ID NO:133)

Lactococcus lactis NIZO 22186: Nisin Z
ITSISLCTPGCKTGALMGCNMKTATCNCSIHVSK (SEQ ID NO:134)

Lactococcus lactis subsp. lactis F10: Nisin F
ITSISLCTPGCKTGALMGCNMKTATCNCSVHVSK (SEQ ID NO:135)

Lactococcus lactis 61-14: Nisin Q
ITSISLCTPGCKTGVLMGCNLKTATCNCSVHVSK (SEQ ID NO:136)

Streptococcus hyointestinalis: Nisin H
FTSISMCTPGCKTGALMTCNYKTATCHCSIKVSK (SEQ ID NO:137)

Streptococcus uberis: Nisin U
ITSKSLCTPGCKTGILMTCPLKTATCGCHFG (SEQ ID NO:138)

Streptococcus uberis: Nisin U2
VTSKSLCTPGCKTGILMTCPLKTATCGCHFG (SEQ ID NO:139)

Streptococcus galloyticus subsp. pasteurianus: Nisin P
VTSKSLCTPGCKTGILMTCAIKTATCGCHFG (SEQ ID NO:140)

***L. lactis NZ9800*: Nisin A S29A**
ITSISLCTPGCKTGALMGCNMKTATCHCAIHVSK (SEQ ID NO:141)

***L. lactis NZ9800*: Nisin A S29D**
ITSISLCTPGCKTGALMGCNMKTATCHCDIHVSK (SEQ ID NO:142)

L. lactis NZ9800: Nisin A S29E
ITSISLCTPGCKTGALMGCNMKTATCHCEIHVSK (SEQ ID NO:143)

L. lactis NZ9800: Nisin A S29G
ITSISLCTPGCKTGALMGCNMKTATCHCGIHVSK (SEQ ID NO:144)

***L. lactis NZ9800:* Nisin A K22T**
ITSISLCTPGCKTGALMGCNMTTATCHCSIHVSK (SEQ ID NO:145)

L. lactis NZ9800: Nisin A N20P
ITSISLCTPGCKTGALMGCPMKTATCHCSIHVSK (SEQ ID NO:146)

L. lactis NZ9800: Nisin A M21V
ITSISLCTPGCKTGALMGCNVKTATCHCSIHVSK (SEQ ID NO:147)

L. lactis NZ9800: Nisin A K22S
ITSISLCTPGCKTGALMGCNMSTATCHCSIHVSK (SEQ ID NO:148)

L. lactis NZ9800: Nisin Z N20K
ITSISLCTPGCKTGALMGCKMKTATCNCSIHVSK (SEQ ID NO:149)

L. lactis NZ9800: Nisin Z M21K
ITSISLCTPGCKTGALMGCNKKTATCNCSIHVSK (SEQ ID NO:150)

Relevant RNA Sequences (5'-3')

***Synthetic*: MS2 Stem Loop spCas9 Scaffold RNA for sgRNA with Terminator Example 1**

GUUUUAGAGCUAGGCCAACAUGAGGAUCACCCAUGUCUGCAGGGCCUAG CAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGGCCAACAUGAGGAUCA CCCAUGUCUGCAGGGCCAAGUGGCACCGAGUCGGUGCUUUUUUU (SEQ ID NO:151)

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

GUUUUAGAGCUAGGCCAACAUGAGGAUCACCCAUGUCUGCAGGGCCUAG CAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGGCCAACAUGAGGAUCA CCCAUGUCUGCAGGGCCAAGUGGCACCGAGUCGGUGCGGGAGCACAUGA GGAUCACCCAUGUGCGACUCCCACAGUCACUGGGGAGUCUUCCCUUUUU UU (SEQ ID NO:152)

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

GUUUAAGAGCUAUGCUGGAAACAGCAUAGCAAGUUUAAAUAAGGCUAGUC CGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGCGGGAGCACAUGAG GAUCACCCAUGUGCGACUCCCACAGUCACUGGGGAGUCUUCCCUUUUUU U (SEQ ID NO:153)

***Synthetic*: 4xMS2 Stem Loop RNA Scaffold Example**

UUCUAGAUCAUCGAAACAUGAGGAUCACCCAUAUCUGCAGUCGACAUCGA AACAUGAGGAUCACCCAUGUCUGCAGUCGACAUCGAAACAUGAGGAUCAC CCAUGUCUGCAGUCGACAUCGAAACAUGAGGAUCACCCAUGUCUGCAGU CGACAUCGAAAUCGAUAAGCUUCAGAUCAGAUCCUAG (SEQ ID NO:154)

***Synthetic*: MS2 Stem Loop Example 1**

ACAUGAGGAUCACCCAUGU (SEQ ID NO:155)

***Synthetic*: MS2 Stem Loop Example 2**

ACAUGAGGAUCACCCAUAU (SEQ ID NO:156)

***Synthetic*: MS2 Stem Loop Example 3**

CCACAGUCACUGGG (SEQ ID NO:157)

***Synthetic*: 2xMS2 Stem Loop Example**

ACAUGAGGAUCACCCAUGUCUGCAGGGCCUAGCAAGUUAAAAUAAGGCUA GUCCGUUAUCAACUUGGCCAACAUGAGGAUCACCCAUGU (SEQ ID NO:158)

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

GUUUUAGAGCUAGGCCGGAGCAGACGAUAUGGCGUCGCUCCGGCCUAGC
AAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGGCCGGAGCAGACGAUAU
GGCGUCGCUCCGGCCAAGUGGCACCGAGUCGGUGCUUUUUUU (SEQ ID
NO:159)

***Synthetic*: PP7 Stem Loop Example**
GCCGGAGCAGACGAUAUGGCGUCGCUCCGGCC (SEQ ID NO:160)

***Synthetic*: COM Stem Loop spCas9 Scaffold RNA for sgRNA with Terminator Example**
GUUUAAGAGCUAUGCUGGAAACAGCAUAGCAAGUUUAAAUAAGGCUAGUC
CGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGCCUGAAUGCCUGCG
AGCAUCUUUUUUU (SEQ ID NO:161)

***Synthetic*: COM Stem Loop Example**
CUGAAUGCCUGCGAGCAUC (SEQ ID NO:162)

***Synthetic ZKSCAN1 Circular Splice RNA:* Upstream Intron**
AGUGACAGUGGAGAUUGUACAGUUUUUUCCUCGAUUUGUCAGGAUUUUU
UUUUUUUGACGGAGUUUAACUUCUUGUCUCCCAGGUAGGAAGUGCAGUG
GCGUAAUCUCGGCUCACUACAACCUCCACCUCCUGGGUUCAAGCGUUUC
UCCUGCCUCAGCUUUCCGAGUAGCUGGGAUUACAGGCGCCUGCCACCAU
GCCCUGCUGACUUUUGUAUUUUUAGUAGAGACGGGGUUUCACCAUGUUG
GCCAGGCUGGUCUUGAACUCCUGACCGCAGGCGAUUGGCCUGCCUCGGC
CUCCCAAAGUGCUGAGAUUACAGGCGUGAGCCACCACCCCCGGCCUCAG
GAGCGUUCUGAUAGUGCCUCGAUGUGCUGCCUCCUAUAAAGUGUUAGCA
GCACAGAUCACUUUUUGUAAAGGUACGUACUAAUGACUUUUUUUUUAUAC
UUCAGG (SEQ ID NO:163)

***Synthetic ZKSCAN1 Circular Splice RNA:* Downstream Intron**
UAAGAAGCAAGGUUUCAUUUAGGGGAAGGGAAAUGAUUCAGGACGAGAG
UCUUUGUGCUGCUGAGUGCCUGUGAUGAAGAAGCAUGUUAGUCCUGGGC
AACGUAGCGAGACCCCAUCUCUACAAAAAAUAGAAAAAUUAGCCAGGUAU
AGUGGCGCACACCUGUGAUUCCAGCUACGCAGGAGGCUGAGGUGGGAGG
AUUGCUUGAGCCCAGGAGGUUGAGGCUGCAGUGAGCUGUAAUCAUGCCA
CUACUCCAACCUGGGCAACACAGCAAGGACCCUGUCUCAAAAGCUACUUA
CAGAAAAGAAUUAGGCUCGGCACGGUAGCUCACACCUGUAAUCCCAGCAC
UUUGGGAGGCUGAGGCGGGCAGAUCACUUGAGGUCAGGAGUUUGAGACC
AGCCUGGCCAACAUGGUGAAACCUUGUCUCUACUAAAAAUAUGAAAAUUA
GCCAGGCAUGGUGGCACAUUCCUGUAAUCCCAGCUACUCGGGAGGCUGA
GGCAGGAGAAUCACUUGAACCCAGGAGGUGGAGGUUGCAGUAAGCCGAG
AUCGUACCACUGUGCUCUAGCCUUGGUGACAGAGCGAGACUGUCUUAAA
AAAAAAAAAAAAAAAAAAAGAAUUAAUUAAAAAUUUAAAAAAAAAAUGAAAAA
AAGCUGCAUGCUUGUUUUUUGUUUUUAGUUAUUCUACAUUGUUGUCAUU
AUUACCAAAUAUUGGGGAAAAUACAACUUACAGACCAAUCUCAGGAGUUA

AAUGUUACUACGAAGGCAAAUGAACUAUGCGUAAUGAACCUGGUAGGCAU
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**
AAGGGTTCAGTGCGTGGGCG (SEQ ID NO:176)

Synthetic: **FokI Zinc Finger Nuclease 17-2 & 18-2 Binding Site in GFP**
GATCCGCCACAACATCGAGGACGGCA (SEQ ID NO:177)

Human codon optimized *Streptococcus pyogenes* Cas9 (spCas9) NLS
ATGGACAAGAAGTACAGCATCGGCCTGGACATCGGCACCAACTCTGTGGG
CTGGGCCGTGATCACCGACGAGTACAAGGTGCCCAGCAAGAAATTCAAGGT
GCTGGGCAACACCGACCGGCACAGCATCAAGAAGAACCTGATCGGAGCCC
TGCTGTTCGACAGCGGCGAAACAGCCGAGGCCACCCGGCTGAAGAGAACC
GCCAGAAGAAGATACACCAGACGGAAGAACCGGATCTGCTATCTGCAAGAG
ATCTTCAGCAACGAGATGGCCAAGGTGGACGACAGCTTCTTCCACAGACTG
GAAGAGTCCTTCCTGGTGGAAGAGGATAAGAAGCACGAGCGGCACCCCAT
CTTCGGCAACATCGTGGACGAGGTGGCCTACCACGAGAAGTACCCCACCAT
CTACCACCTGAGAAAGAAACTGGTGGACAGCACCGACAAGGCCGACCTGC
GGCTGATCTATCTGGCCCTGGCCCACATGATCAAGTTCCGGGGCCACTTCC
TGATCGAGGGCGACCTGAACCCCGACAACAGCGACGTGGACAAGCTGTTC
ATCCAGCTGGTGCAGACCTACAACCAGCTGTTCGAGGAAAACCCCATCAAC
GCCAGCGGCGTGGACGCCAAGGCCATCCTGTCTGCCAGACTGAGCAAGAG
CAGACGGCTGGAAAATCTGATCGCCCAGCTGCCCGGCGAGAAGAAGAATG
GCCTGTTCGGAAACCTGATTGCCCTGAGCCTGGGCCTGACCCCCAACTTCA
AGAGCAACTTCGACCTGGCCGAGGATGCCAAACTGCAGCTGAGCAAGGAC
ACCTACGACGACGACCTGGACAACCTGCTGGCCCAGATCGGCGACCAGTA
CGCCGACCTGTTTCTGGCCGCCAAGAACCTGTCCGACGCCATCCTGCTGAG
CGACATCCTGAGAGTGAACACCGAGATCACCAAGGCCCCCCTGAGCGCCT
CTATGATCAAGAGATACGACGAGCACCACCAGGACCTGACCCTGCTGAAAG
CTCTCGTGCGGCAGCAGCTGCCTGAGAAGTACAAAGAGATTTTCTTCGACC
AGAGCAAGAACGGCTACGCCGGCTACATTGACGGCGGAGCCAGCCAGGAA
GAGTTCTACAAGTTCATCAAGCCCATCCTGGAAAAGATGGACGGCACCGAG
GAACTGCTCGTGAAGCTGAACAGAGAGGACCTGCTGCGGAAGCAGCGGAC
CTTCGACAACGGCAGCATCCCCCACCAGATCCACCTGGGAGAGCTGCACG
CCATTCTGCGGCGGCAGGAAGATTTTTACCCATTCCTGAAGGACAACCGGG
AAAAGATCGAGAAGATCCTGACCTTCCGCATCCCCTACTACGTGGGCCCTC
TGGCCAGGGGAAACAGCAGATTCGCCTGGATGACCAGAAAGAGCGAGGAA
ACCATCACCCCCTGGAACTTCGAGGAAGTGGTGGACAAGGGCGCTTCCGC
CCAGAGCTTCATCGAGCGGATGACCAACTTCGATAAGAACCTGCCCAACGA
GAAGGTGCTGCCCAAGCACAGCCTGCTGTACGAGTACTTCACCGTGTATAA
CGAGCTGACCAAAGTGAAATACGTGACCGAGGGAATGAGAAAGCCCGCCTT
CCTGAGCGGCGAGCAGAAAAAGGCCATCGTGGACCTGCTGTTCAAGACCA
ACCGGAAAGTGACCGTGAAGCAGCTGAAAGAGGACTACTTCAAGAAAATCG
AGTGCTTCGACTCCGTGGAAATCTCCGGCGTGGAAGATCGGTTCAACGCCT
CCCTGGGCACATACCACGATCTGCTGAAAATTATCAAGGACAAGGACTTCC

TGGACAATGAGGAAAACGAGGACATTCTGGAAGATATCGTGCTGACCCTGA
CACTGTTTGAGGACAGAGAGATGATCGAGGAACGGCTGAAAACCTATGCCC
ACCTGTTCGACGACAAAGTGATGAAGCAGCTGAAGCGGCGGAGATACACC
GGCTGGGGCAGGCTGAGCCGGAAGCTGATCAACGGCATCCGGGACAAGC
AGTCCGGCAAGACAATCCTGGATTTCCTGAAGTCCGACGGCTTCGCCAACA
GAAACTTCATGCAGCTGATCCACGACGACAGCCTGACCTTTAAAGAGGACA
TCCAGAAAGCCCAGGTGTCCGGCCAGGGCGATAGCCTGCACGAGCACATT
GCCAATCTGGCCGGCAGCCCCGCCATTAAGAAGGGCATCCTGCAGACAGT
GAAGGTGGTGGACGAGCTCGTGAAAGTGATGGGCCGGCACAAGCCCGAGA
ACATCGTGATCGAAATGGCCAGAGAGAACCAGACCACCCAGAAGGGACAG
AAGAACAGCCGCGAGAGAATGAAGCGGATCGAAGAGGGCATCAAAGAGCT
GGGCAGCCAGATCCTGAAAGAACACCCCGTGGAAAACACCCAGCTGCAGA
ACGAGAAGCTGTACCTGTACTACCTGCAGAATGGGCGGGATATGTACGTGG
ACCAGGAACTGGACATCAACCGGCTGTCCGACTACGATGTGGACCATATCG
TGCCTCAGAGCTTTCTGAAGGACGACTCCATCGACAACAAGGTGCTGACCA
GAAGCGACAAGAACCGGGGCAAGAGCGACAACGTGCCCTCCGAAGAGGTC
GTGAAGAAGATGAAGAACTACTGGCGGCAGCTGCTGAACGCCAAGCTGATT
ACCCAGAGAAAGTTCGACAATCTGACCAAGGCCGAGAGAGGCGGCCTGAG
CGAACTGGATAAGGCCGGCTTCATCAAGAGACAGCTGGTGGAAACCCGGC
AGATCACAAAGCACGTGGCACAGATCCTGGACTCCCGGATGAACACTAAGT
ACGACGAGAATGACAAGCTGATCCGGGAAGTGAAAGTGATCACCCTGAAGT
CCAAGCTGGTGTCCGATTTCCGGAAGGATTTCCAGTTTTACAAAGTGCGCG
AGATCAACAACTACCACCACGCCCACGACGCCTACCTGAACGCCGTCGTGG
GAACCGCCCTGATCAAAAAGTACCCTAAGCTGGAAAGCGAGTTCGTGTACG
GCGACTACAAGGTGTACGACGTGCGGAAGATGATCGCCAAGAGCGAGCAG
GAAATCGGCAAGGCTACCGCCAAGTACTTCTTCTACAGCAACATCATGAACT
TTTTCAAGACCGAGATTACCCTGGCCAACGGCGAGATCCGGAAGCGGCCTC
TGATCGAGACAAACGGCGAAACCGGGGAGATCGTGTGGGATAAGGGCCGG
GATTTTGCCACCGTGCGGAAAGTGCTGAGCATGCCCCAAGTGAATATCGTG
AAAAAGACCGAGGTGCAGACAGGCGGCTTCAGCAAAGAGTCTATCCTGCCC
AAGAGGAACAGCGATAAGCTGATCGCCAGAAAGAAGGACTGGGACCCTAA
GAAGTACGGCGGCTTCGACAGCCCCACCGTGGCCTATTCTGTGCTGGTGG
TGGCCAAAGTGGAAAAGGGCAAGTCCAAGAAACTGAAGAGTGTGAAAGAGC
TGCTGGGGATCACCATCATGGAAAGAAGCAGCTTCGAGAAGAATCCCATCG
ACTTTCTGGAAGCCAAGGGCTACAAAGAAGTGAAAAAGGACCTGATCATCA
AGCTGCCTAAGTACTCCCTGTTCGAGCTGGAAAACGGCCGGAAGAGAATGC
TGGCCTCTGCCGGCGAACTGCAGAAGGGAAACGAACTGGCCCTGCCCTCC
AAATATGTGAACTTCCTGTACCTGGCCAGCCACTATGAGAAGCTGAAGGGC
TCCCCCGAGGATAATGAGCAGAAACAGCTGTTTGTGGAACAGCACAAGCAC
TACCTGGACGAGATCATCGAGCAGATCAGCGAGTTCTCCAAGAGAGTGATC
CTGGCCGACGCTAATCTGGACAAAGTGCTGTCCGCCTACAACAAGCACCGG
GATAAGCCCATCAGAGAGCAGGCCGAGAATATCATCCACCTGTTTACCCTG
ACCAATCTGGGAGCCCCTGCCGCCTTCAAGTACTTTGACACCACCATCGAC
CGGAAGAGGTACACCAGCACCAAAGAGGTGCTGGACGCCACCCTGATCCA
CCAGAGCATCACCGGCCTGTACGAGACACGGATCGACCTGTCTCAGCTGG
GAGGCGACGGATCCCCCAAGAAGAAGAGGAAAGTCTCGAGCGACTACAAA

GACCATGACGGTGATTATAAAGATCATGACATCGATTACAAGGATGACGATG
ACAAGGCTGCAGGATGA (SEQ ID NO:178)

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

ATGGACAAGAAGTACAGCATCGGCCTGGACATCGGCACCAACTCTGTGGG
CTGGGCCGTGATCACCGACGAGTACAAGGTGCCCAGCAAGAAATTCAAGGT
GCTGGGCAACACCGACCGGCACAGCATCAAGAAGAACCTGATCGGAGCCC
TGCTGTTCGACAGCGGCGAAACAGCCGAGGCCACCCGGCTGAAGAGAACC
GCCAGAAGAAGATACACCAGACGGAAGAACCGGATCTGCTATCTGCAAGAG
ATCTTCAGCAACGAGATGGCCAAGGTGGACGACAGCTTCTTCCACAGACTG
GAAGAGTCCTTCCTGGTGGAAGAGGATAAGAAGCACGAGCGGCACCCCAT
CTTCGGCAACATCGTGGACGAGGTGGCCTACCACGAGAAGTACCCCACCAT
CTACCACCTGAGAAAGAAACTGGTGGACAGCACCGACAAGGCCGACCTGC
GGCTGATCTATCTGGCCCTGGCCCACATGATCAAGTTCCGGGGCCACTTCC
TGATCGAGGGCGACCTGAACCCCGACAACAGCGACGTGGACAAGCTGTTC
ATCCAGCTGGTGCAGACCTACAACCAGCTGTTCGAGGAAAACCCCATCAAC
GCCAGCGGCGTGGACGCCAAGGCCATCCTGTCTGCCAGACTGAGCAAGAG
CAGACGGCTGGAAAATCTGATCGCCCAGCTGCCCGGCGAGAAGAAGAATG
GCCTGTTCGGAAACCTGATTGCCCTGAGCCTGGGCCTGACCCCCAACTTCA
AGAGCAACTTCGACCTGGCCGAGGATGCCAAACTGCAGCTGAGCAAGGAC
ACCTACGACGACGACCTGGACAACCTGCTGGCCCAGATCGGCGACCAGTA
CGCCGACCTGTTTCTGGCCGCCAAGAACCTGTCCGACGCCATCCTGCTGAG
CGACATCCTGAGAGTGAACACCGAGATCACCAAGGCCCCCCTGAGCGCCT
CTATGATCAAGAGATACGACGAGCACCACCAGGACCTGACCCTGCTGAAAG
CTCTCGTGCGGCAGCAGCTGCCTGAGAAGTACAAAGAGATTTTCTTCGACC
AGAGCAAGAACGGCTACGCCGGCTACATTGACGGCGGAGCCAGCCAGGAA
GAGTTCTACAAGTTCATCAAGCCCATCCTGGAAAAGATGGACGGCACCGAG
GAACTGCTCGTGAAGCTGAACAGAGAGGACCTGCTGCGGAAGCAGCGGAC
CTTCGACAACGGCAGCATCCCCCACCAGATCCACCTGGGAGAGCTGCACG
CCATTCTGCGGCGGCAGGAAGATTTTTACCCATTCCTGAAGGACAACCGGG
AAAAGATCGAGAAGATCCTGACCTTCCGCATCCCCTACTACGTGGGCCCTC
TGGCCAGGGGAAACAGCAGATTCGCCTGGATGACCAGAAAGAGCGAGGAA
ACCATCACCCCCTGGAACTTCGAGGAAGTGGTGGACAAGGGCGCTTCCGC
CCAGAGCTTCATCGAGCGGATGACCAACTTCGATAAGAACCTGCCCAACGA
GAAGGTGCTGCCCAAGCACAGCCTGCTGTACGAGTACTTCACCGTGTATAA
CGAGCTGACCAAAGTGAAATACGTGACCGAGGGAATGAGAAAGCCCGCCTT
CCTGAGCGGCGAGCAGAAAAAGGCCATCGTGGACCTGCTGTTCAAGACCA
ACCGGAAAGTGACCGTGAAGCAGCTGAAAGAGGACTACTTCAAGAAAATCG
AGTGCTTCGACTCCGTGGAAATCTCCGGCGTGGAAGATCGGTTCAACGCCT
CCCTGGGCACATACCACGATCTGCTGAAAATTATCAAGGACAAGGACTTCC
TGGACAATGAGGAAAACGAGGACATTCTGGAAGATATCGTGCTGACCCTGA
CACTGTTTGAGGACAGAGAGATGATCGAGGAACGGCTGAAAACCTATGCCC
ACCTGTTCGACGACAAAGTGATGAAGCAGCTGAAGCGGCGGAGATACACC
GGCTGGGGCAGGCTGAGCCGGAAGCTGATCAACGGCATCCGGGACAAGC
AGTCCGGCAAGACAATCCTGGATTTCCTGAAGTCCGACGGCTTCGCCAACA

GAAACTTCATGCAGCTGATCCACGACGACAGCCTGACCTTTAAAGAGGACA
TCCAGAAAGCCCAGGTGTCCGGCCAGGGCGATAGCCTGCACGAGCACATT
GCCAATCTGGCCGGCAGCCCCGCCATTAAGAAGGGCATCCTGCAGACAGT
GAAGGTGGTGGACGAGCTCGTGAAAGTGATGGGCCGGCACAAGCCCGAGA
ACATCGTGATCGAAATGGCCAGAGAGAACCAGACCACCCAGAAGGGACAG
AAGAACAGCCGCGAGAGAATGAAGCGGATCGAAGAGGGCATCAAAGAGCT
GGGCAGCCAGATCCTGAAAGAACACCCCGTGGAAAACACCCAGCTGCAGA
ACGAGAAGCTGTACCTGTACTACCTGCAGAATGGGCGGGATATGTACGTGG
ACCAGGAACTGGACATCAACCGGCTGTCCGACTACGATGTGGACCATATCG
TGCCTCAGAGCTTTCTGAAGGACGACTCCATCGACAACAAGGTGCTGACCA
GAAGCGACAAGAACCGGGGCAAGAGCGACAACGTGCCCTCCGAAGAGGTC
GTGAAGAAGATGAAGAACTACTGGCGGCAGCTGCTGAACGCCAAGCTGATT
ACCCAGAGAAAGTTCGACAATCTGACCAAGGCCGAGAGAGGCGGCCTGAG
CGAACTGGATAAGGCCGGCTTCATCAAGAGACAGCTGGTGGAAACCCGGC
AGATCACAAAGCACGTGGCACAGATCCTGGACTCCCGGATGAACACTAAGT
ACGACGAGAATGACAAGCTGATCCGGGAAGTGAAAGTGATCACCCTGAAGT
CCAAGCTGGTGTCCGATTTCCGGAAGGATTTCCAGTTTTACAAAGTGCGCG
AGATCAACAACTACCACCACGCCCACGACGCCTACCTGAACGCCGTCGTGG
GAACCGCCCTGATCAAAAAGTACCCTAAGCTGGAAAGCGAGTTCGTGTACG
GCGACTACAAGGTGTACGACGTGCGGAAGATGATCGCCAAGAGCGAGCAG
GAAATCGGCAAGGCTACCGCCAAGTACTTCTTCTACAGCAACATCATGAACT
TTTTCAAGACCGAGATTACCCTGGCCAACGGCGAGATCCGGAAGCGGCCTC
TGATCGAGACAAACGGCGAAACCGGGGAGATCGTGTGGGATAAGGGCCGG
GATTTTGCCACCGTGCGGAAAGTGCTGAGCATGCCCCAAGTGAATATCGTG
AAAAAGACCGAGGTGCAGACAGGCGGCTTCAGCAAAGAGTCTATCCTGCCC
AAGAGGAACAGCGATAAGCTGATCGCCAGAAAGAAGGACTGGGACCCTAA
GAAGTACGGCGGCTTCGACAGCCCCACCGTGGCCTATTCTGTGCTGGTGG
TGGCCAAAGTGGAAAAGGGCAAGTCCAAGAAACTGAAGAGTGTGAAAGAGC
TGCTGGGGATCACCATCATGGAAAGAAGCAGCTTCGAGAAGAATCCCATCG
ACTTTCTGGAAGCCAAGGGCTACAAAGAAGTGAAAAAGGACCTGATCATCA
AGCTGCCTAAGTACTCCCTGTTCGAGCTGGAAAACGGCCGGAAGAGAATGC
TGGCCTCTGCCGGCGAACTGCAGAAGGGAAACGAACTGGCCCTGCCCTCC
AAATATGTGAACTTCCTGTACCTGGCCAGCCACTATGAGAAGCTGAAGGGC
TCCCCCGAGGATAATGAGCAGAAACAGCTGTTTGTGGAACAGCACAAGCAC
TACCTGGACGAGATCATCGAGCAGATCAGCGAGTTCTCCAAGAGAGTGATC
CTGGCCGACGCTAATCTGGACAAAGTGCTGTCCGCCTACAACAAGCACCGG
GATAAGCCCATCAGAGAGCAGGCCGAGAATATCATCCACCTGTTTACCCTG
ACCAATCTGGGAGCCCCTGCCGCCTTCAAGTACTTTGACACCACCATCGAC
CGGAAGAGGTACACCAGCACCAAAGAGGTGCTGGACGCCACCCTGATCCA
CCAGAGCATCACCGGCCTGTACGAGACACGGATCGACCTGTCTCAGCTGG
GAGGCGACGGATCCGGCGGAGGCGGAAGCGGGAAAAGAACCGCCGACGG
CAGCGAATTCGAGCCCAAGAAGAAGAGGAAAGTCTCGAGCGGAGGCGACT
ACAAAGACCATGACGGTGATTATAAAGATCATGACATCGATTACAAGGATGA
CGATGACAAGTGA (SEQ ID NO:179)

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

ATGAAACGGACAGCCGACGGAAGCGAGTTCGAGTCACCAAAGAAGAAGCG
GAAAGTCTCCTCAGAGACTGGGCCTGTCGCCGTCGATCCAACCCTGCGCC
GCCGGATTGAACCTCACGAGTTTGAAGTGTTCTTTGACCCCCGGGAGCTGA
GAAAGGAGACATGCCTGCTGTACGAGATCAACTGGGGAGGCAGGCACTCC
ATCTGGAGGCACACCTCTCAGAACACAAATAAGCACGTGGAGGTGAACTTC
ATCGAGAAGTTTACCACAGAGCGGTACTTCTGCCCCAATACCAGATGTAGC
ATCACATGGTTTCTGAGCTGGTCCCCTTGCGGAGAGTGTAGCAGGGCCATC
ACCGAGTTCCTGTCCAGATATCCACACGTGACACTGTTTATCTACATCGCCA
GGCTGTATCACCACGCAGACCCAAGGAATAGGCAGGGCCTGCGCGATCTG
ATCAGCTCCGGCGTGACCATCCAGATCATGACAGAGCAGGAGTCCGGCTA
CTGCTGGCGGAACTTCGTGAATTATTCTCCTAGCAACGAGGCCCACTGGCC
TAGGTACCCACACCTGTGGGTGCGCCTGTACGTGCTGGAGCTGTATTGCAT
CATCCTGGGCCTGCCCCCTTGTCTGAATATCCTGCGGAGAAAGCAGCCCCA
GCTGACCTTCTTTACAATCGCCCTGCAGTCTTGTCACTATCAGAGGCTGCCA
CCCCACATCCTGTGGGCCACAGGCCTGAAGTCTGGAGGATCTAGCGGAGG
ATCCTCTGGCAGCGAGACACCAGGAACAAGCGAGTCAGCAACACCAGAGA
GCAGTGGCGGCAGCAGCGGCGGCAGCGACAAGAAGTACAGCATCGGCCT
GGACATCGGCACCAACTCTGTGGGCTGGGCCGTGATCACCGACGAGTACA
AGGTGCCCAGCAAGAAATTCAAGGTGCTGGGCAACACCGACCGGCACAGC
ATCAAGAAGAACCTGATCGGAGCCCTGCTGTTCGACAGCGGCGAAACAGC
CGAGGCCACCCGGCTGAAGAGAACCGCCAGAAGAAGATACACCAGACGGA
AGAACCGGATCTGCTATCTGCAAGAGATCTTCAGCAACGAGATGGCCAAGG
TGGACGACAGCTTCTTCCACAGACTGGAAGAGTCCTTCCTGGTGGAAGAGG
ATAAGAAGCACGAGCGGCACCCCATCTTCGGCAACATCGTGGACGAGGTG
GCCTACCACGAGAAGTACCCCACCATCTACCACCTGAGAAAGAAACTGGTG
GACAGCACCGACAAGGCCGACCTGCGGCTGATCTATCTGGCCCTGGCCCA
CATGATCAAGTTCCGGGGCCACTTCCTGATCGAGGGCGACCTGAACCCCG
ACAACAGCGACGTGGACAAGCTGTTCATCCAGCTGGTGCAGACCTACAACC
AGCTGTTCGAGGAAAACCCCATCAACGCCAGCGGCGTGGACGCCAAGGCC
ATCCTGTCTGCCAGACTGAGCAAGAGCAGACGGCTGGAAAATCTGATCGCC
CAGCTGCCCGGCGAGAAGAAGAATGGCCTGTTCGGAAACCTGATTGCCCT
GAGCCTGGGCCTGACCCCCAACTTCAAGAGCAACTTCGACCTGGCCGAGG
ATGCCAAACTGCAGCTGAGCAAGGACACCTACGACGACGACCTGGACAAC
CTGCTGGCCCAGATCGGCGACCAGTACGCCGACCTGTTTCTGGCCGCCAA
GAACCTGTCCGACGCCATCCTGCTGAGCGACATCCTGAGAGTGAACACCGA
GATCACCAAGGCCCCCCTGAGCGCCTCTATGATCAAGAGATACGACGAGCA
CCACCAGGACCTGACCCTGCTGAAAGCTCTCGTGCGGCAGCAGCTGCCTG
AGAAGTACAAAGAGATTTTCTTCGACCAGAGCAAGAACGGCTACGCCGGCT
ACATTGACGGCGGAGCCAGCCAGGAAGAGTTCTACAAGTTCATCAAGCCCA
TCCTGGAAAAGATGGACGGCACCGAGGAACTGCTCGTGAAGCTGAACAGA
GAGGACCTGCTGCGGAAGCAGCGGACCTTCGACAACGGCAGCATCCCCCA
CCAGATCCACCTGGGAGAGCTGCACGCCATTCTGCGGCGGCAGGAAGATT
TTTACCCATTCCTGAAGGACAACCGGGAAAAGATCGAGAAGATCCTGACCT
TCCGCATCCCCTACTACGTGGGCCCTCTGGCCAGGGGAAACAGCAGATTC
GCCTGGATGACCAGAAAGAGCGAGGAAACCATCACCCCCTGGAACTTCGA

GGAAGTGGTGGACAAGGGCGCTTCCGCCCAGAGCTTCATCGAGCGGATGA
CCAACTTCGATAAGAACCTGCCCAACGAGAAGGTGCTGCCCAAGCACAGCC
TGCTGTACGAGTACTTCACCGTGTATAACGAGCTGACCAAAGTGAAATACGT
GACCGAGGGAATGAGAAAGCCCGCCTTCCTGAGCGGCGAGCAGAAAAAGG
CCATCGTGGACCTGCTGTTCAAGACCAACCGGAAAGTGACCGTGAAGCAGC
TGAAAGAGGACTACTTCAAGAAAATCGAGTGCTTCGACTCCGTGGAAATCTC
CGGCGTGGAAGATCGGTTCAACGCCTCCCTGGGCACATACCACGATCTGCT
GAAAATTATCAAGGACAAGGACTTCCTGGACAATGAGGAAAACGAGGACAT
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CGAGGAACGGCTGAAAACCTATGCCCACCTGTTCGACGACAAAGTGATGAA
GCAGCTGAAGCGGCGGAGATACACCGGCTGGGGCAGGCTGAGCCGGAAG
CTGATCAACGGCATCCGGGACAAGCAGTCCGGCAAGACAATCCTGGATTTC
CTGAAGTCCGACGGCTTCGCCAACAGAAACTTCATGCAGCTGATCCACGAC
GACAGCCTGACCTTTAAAGAGGACATCCAGAAAGCCCAGGTGTCCGGCCA
GGGCGATAGCCTGCACGAGCACATTGCCAATCTGGCCGGCAGCCCCGCCA
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Human codon optimized *Streptococcus pyogenes* Cas9 (spCas9) ABE

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Human codon optimized VSVG

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TGAAGCACACCAAAAAGAGACAGATCTACACCGACATCGAGATGAACCGGC
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.