(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
30 December 2021 (30.12.2021)

WIPO | PCT

(10) International Publication Number
WO 2021/262788 A3

(51) International Patent Classification:
C12N 15/87 (2006.01) *B01D 69/14* (2006.01)
G01N 15/08 (2006.01)

(21) International Application Number:
PCT/US2021/038588

(22) International Filing Date:
23 June 2021 (23.06.2021)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
63/042,909 23 June 2020 (23.06.2020) US

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(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DJ, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, IT, JO, JP, KE, KG, KH, KN, KP, KR, KW, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, WS, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Declarations under Rule 4.17:
— *as to applicant's entitlement to apply for and be granted a patent (Rule 4.17(ii))*

Published:
— *with international search report (Art. 21(3))*
— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments (Rule 48.2(h))*

(88) Date of publication of the international search report:
03 February 2022 (03.02.2022)

(54) Title: TUNNELING NANOTUBE CELLS AND METHODS OF USE THEREOF FOR DELIVERY OF BIOMOLECULES

(57) Abstract: Tunneling nanotube (TNT) cells, comprising a TNT promoting factor (TPF); and a biomolecule cargo overexpressed by the TNT cell, and methods of use thereof for delivery of the biomolecule cargo from TNT cells to neighboring cells.

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TUNNELING NANOTUBE CELLS AND METHODS OF USE THEREOF FOR DELIVERY OF BIOMOLECULES

CLAIM OF PRIORITY

This application claims the benefit of U.S. Provisional Patent Application Serial No. 63/042,909, filed on June 23, 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.

TECHNICAL FIELD

Described herein are tunneling nanotube (TNT) cells, comprising a mammalian cell that transiently or stably overexpresses cargo and one or more TNT-promoting factors so that the TNT cell is stimulated to form transient tunneling nanotubes with neighboring cells through which cargo can be delivered from the TNT cell to the neighboring cells.

BACKGROUND

Delivery of biomolecules such as proteins and nucleic acids into the cytosol of living cells has been a significant hurdle in the development of biological therapeutics.

SUMMARY

Described herein are compositions and methods for cell-based biomolecule 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 tunneling nanotube (TNT) cells, comprising: a TNT promoting factor (TPF), preferably selected from the group consisting of M-Sec (tumor

necrosis factor, alpha-induced protein 2 (TNFaip2)), Lst1, and RAS like proto-oncogene A (RalA), (e.g., as shown in Table 1) overexpressed in the cell; and a biomolecule cargo overexpressed in the cell in the cytosol or embedded within the phospholipid bilayer.

Also provided herein are methods for delivering a biomolecule to a target cell, e.g., a cell in vivo or in vitro, by contacting the target cell with the TNT cell as described herein comprising the biomolecule as cargo.

Additionally, provided herein are methods for producing a TNT cell comprising a biomolecular cargo, the method comprising: providing a cell overexpressing one or more TPFs (e.g., as shown in Table 1); and maintaining the cell, e.g., in culture, e.g., under optimal survival conditions. In some embodiments, the methods include harvesting and optionally purifying and/or concentrating the produced TNT cells.

Also provided herein are cells overexpressing one or more TPFs (e.g., as shown in Table 1), and a cargo biomolecule.

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

In some embodiments, biomolecule cargo is a gene editing reagent, e.g., 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 riboucleoprotein 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 TNT cell 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 cells are mammalian, e.g., primary or stable mammalian, e.g., human, cell lines.

In some embodiments, the cells are Human Embryonic Kidney (HEK) 293 cells or HEK293 T cells.

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 T2 TNT cell production and RNP/protein delivery. All T2 TNT expression constructs can be stably integrated in the genome of the producer cell. Construct 1 corresponds to the cargo, such as Cas9. Construct 2 corresponds to an optional guide RNA. 1 is translated in the cytosol where it complexes with guide RNA. 3 corresponds to a TPF, such as MSEC. MSEC protein is recruited to the plasma membrane and helps to drive polymerization of actin. These actin polymerizations result in membranous protrusions (tunneling nanotubes) that are able to transiently fuse with target cells and cargo can be delivered to target cells.

FIG 2: Depiction of exemplary T3 TNT cell production and AAV delivery. All T3 TNT expression constructs, including AAV production constructs (construct(s) contain adenoviral genes needed for replication, ITR-flanked DNA cargo, and the rep and cap genes for production of specific AAV replication and capsid proteins) are stably integrated in the genome of the producer cell. Construct 1 corresponds to the ITR-flanked cargo. Construct 2 and 3 correspond to AAV helper and rep/cap constructs. AAV particles form in the cytosol and encapsulate the ITR-flanked DNA cargo. 4 corresponds to a TPF, such as MSEC. MSEC protein is recruited to the plasma membrane and helps to drive polymerization of actin. These actin polymerizations result in membranous protrusions (tunneling nanotubes) that are able to transiently fuse with target cells and AAV particle cargo can be delivered to target cells.

FIG 3: TNT cell-delivered spCas9 genome editing in vitro. Transient transfection was used to create HEK293 eGFP and U2OS eGFP TNT cell lines ("Cell A") that express Cas9 and a TPF (human MSEC). Provided is a graph showing results of flow cytometry analysis of WT cells ("Cell B") expressing eGFP after being mixed with the TNT cells.

FIGs. 4A-B: Exemplary T1 TNT cell-delivered spCas9 genome editing in vitro. 4A, Schematic illustrating generation of HEK293 and U2OS TNT cell lines that express Cas9, sgRNA targeting GFP, and a TPF (human MSEC). 4B, Graph showing results of PCT analysis of CRISPR-mediated editing of a target site in the eGFP in WT cells ("Cell B") mixed with the TNT cells ("Cell A") generated as in FIG 4A.

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 inactivated or deficient 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. Protein delivery of genome editing reagents is the preferred therapeutic delivery modality because proteins and Ribonucleoproteins (RNPs) are transiently present, and elicit the lowest number of off target effects compared to DNA or RNA delivery of ZFNs or RNA guided nucleases (RGNs).[17] Conventional therapeutic monoclonal antibody delivery is successful at utilizing direct injection for proteins. Unfortunately, strategies for direct injection of gene editing proteins 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.

Nanoparticles 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 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 therapeutic.[15]

Recently, virus-like particles (VLPs) have been utilized to deliver mRNA and protein cargo into the cytosol of cells.[2,3,25-30] 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] Lastly, 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.

Currently, the clinical standard vehicles for delivering genome editing therapeutics are adeno-associated virus (AAV). Although AAV can achieve robust

expression of therapeutic cargo, they carry several inherent flaws, including a 4.7 kb size constraint for AAV, varying levels of immunogenicity, neutralization by antibodies, increased off-target effects for DNA delivery compared to protein delivery, low risk of DNA being integrated into the genome, and persistence in non-dividing cells.[21-23]

Tunneling nanotubes are dynamic, actin-driven membrane protrusions that can connect the cytosol of one cell to the cytosol of another cell. Tunneling nanotubes are frequently observed in neuronal cells and immune cells. For example, a single myeloid cell can support up to 75 nanotubes.[1,2,3] Many different types of cells can form tunneling nanotubes if these cells overexpress TNT-promoting factors (TPFs) and this is the foundation of TNT cells. TNT cells overexpress TPFs and cargo and are capable of delivering DNA, RNA and/or protein into neighboring eukaryotic cells through tunneling nanotubes. The TNT cells described herein provide methods for biomolecule delivery that are not achievable with conventional biomolecule delivery systems, such as artificially-derived lipid/gold nanoparticles and viral particle-based delivery systems. TNT cells, like nanoparticles and viral particles, allow the user to specify which type of cargo (DNA, RNA and/or protein) is to be delivered, and cargo is encapsulated. However, unlike nanoparticles and viral particles, TNT cells are producing more cargo while delivering cargo. If TNT cells are transplanted, as an allograft for example, TNT cells can sustain local delivery as long as the allograft remains in the body. Local delivery can be induced by small molecule-inducible promoters, tissue specific promoters, and other types of inducible promoters (i.e, inflammation-inducible promoters). In addition, TNT cells can be equipped with an 'off-switch' that causes the TNT cell to stop delivering cargo.

TNT cells do not have any human-exogenous components exposed on the outside, which minimizes the chances of adverse immune reactions. TNT cells also do not cause permanent cell-cell fusion (syncytia), which can lead to tumorigenesis. The TNT cells transiently fuse with neighboring cells via tunneling nanotubes. TNT cells are entirely comprised of human cellular components, they do not require any virus-derived components to function, and cargo is completely enclosed within TNT cells from the onset of production to the point the cargo is delivered to the target cell. TNT cells could also be delivering TPFs to recipient cells. This could cause recipient cells form TNT and

deliver more cargo to neighboring cells, enhancing delivery deeper into tissues. A variety of different cell types can be converted into TNT cells that can be introduced to patients as autologous/allogenic cell transplant therapies. TNT cells are the first customizable cell-based biomolecule delivery modality, and this modality is also the first cell-based delivery modality for genome editing reagents.

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 genetic diseases, but techniques for delivering genome editing reagents into cells are severely limiting or unsafe for patients. 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] Although adeno-associated viral (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]

Virus-like particles (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. 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. In addition, many VLPs utilize various superfluous viral-components that further limit VLPs as a clinical tool.

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.

Similar to extracellular vesicles, nanoparticles, AAVs and VLPs, TNT cells can achieve transient local delivery of a variety of biomolecules. However, TNT cells are also capable of providing sustained or spatiotemporally inducible local delivery of a variety of biomolecules. Herein we describe methods and compositions for producing and administering TNT cells for *in vitro* and *in vivo* applications 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, this invention provides a novel way of producing and delivering reagents for applications of genome editing, epigenome modulation, transcriptome editing and proteome modulation, thereby increasing the types of therapeutic in vivo genome modifications that are possible.

In an effort to abrogate size constraints, minimize off-target effects, and eliminate prolonged expression, we describe herein tunneling nanotube delivery of biomolecules including genome editing reagents as protein, RNPs, and a variety of specialty DNA

molecules that have different levels of persistence in non-dividing cells using the designer TNT cells described herein.

Tunneling nanotubes formed between two cells contain filamentous (F)-actin.[1,2,3] Transient cell-cell membrane fusion occurs to create open-ended tunnels. TPFs include proteins that interact with the exocyst complex, such as M-Sec (TNFaip2), Lst1, and RalA.[39-52] Tunneling nanotubes can deliver contents from one cell to another cell either along the surface or inside of the nanotube. The nanotube does not need to be attached to the substratum. One cell that expresses TPFs can potentially form tunneling nanotubes that connect that cell to other neighboring cells. These tunneling nanotubes can be as long as multiple cell diameters, for example up to several hundred μm, and they have been described as having diameters of 300-800nm. Cell-cell contact for under 5 minutes can be sufficient for tunneling nanotube connection to form between two cells. [39-52]

TNT cells are engineered cells that produce and package proteins, DNAs and/or RNAs of interest and deliver this cargo into the cytosol of cells. TNT cells leverage TPFs that have been shown to be integral to the formation of nanotubes.[1-3] The external side of the TNT cell is composed of plasma membrane and plasma membrane-associated proteins. TNT cells lack virally-derived components and can also be retrofitted with surface molecules that make them capable of semi-specific cell transduction. In addition, TNT cells can be produced from cells derived from a patient or FDA-approved cell lines, then re-introduced into the patient and these 'autologous TNT cells' or 'allogenic TNT cells' can further reduce risks of immunogenicity in similar ways that have been achieved by autologous/allogenic T cell therapies. TNT cells are a safer and more effective option for sustained biomolecule delivery than regular re-administration of VLPs, AAVs and nanoparticles-especially for delivery of genome editing reagents-because TNT cells are composed of all human components whereas the aforementioned viral particles are antigenic and will be recognized and neutralized by antibodies if re-administered in vivo. TNT cells are a delivery vehicle that is producing cargo, and this enables the use of inducible promoters to give spatiotemporal control over production and delivery.

Described herein are compositions and methods for delivering biomolecules including genome editing reagents from TNT cells to target cells for the purposes of

carrying out efficient and site-specific genomic, epigenetic, transcriptomic and proteomic modifications and perturbations in vitro, and ultimately, in vivo for therapeutic purposes.

Section 1: TNT cell production and composition

TNT cells are produced from cells that are either transiently transfected with at least one plasmid or stably expressing construct(s) that have been integrated into the producer cell line genomic DNA. TNT cells can be made from virtually any mammalian cell (i.e. macrophage, osteoclast, fibroblast, mesenchymal stem cells, etc.). Once TNT cell lines are created, TPFs and cargo can be produced in a constitutive or inducible fashion.

In some embodiments, if a single plasmid is used in the transfection, it should comprise sequences encoding one or more TPFs (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), a CRISPR-based genome editing/modulating protein and/or RNP such as those found in Tables 2, 3, 4 & 5, or an AAV that packages DNA encoding the aforementioned therapeutic proteins and/or genome editing agents), 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 an AAV (helper sequences, rep/cap, and an ITR-flanked cargo transfer plasmid) a therapeutic protein or a genome editing reagent.
2. A plasmid comprising one or more TPFs (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 a transient transfection approach is used to create TNT cells, then the composition of the cargo that is to be delivered by TNT cells can be a combination of DNA molecules (from transfection), proteins, RNAs, and/or AAVs with associated AAV DNA cargo. TNT cells will deliver a combination of DNA and RNA if TNT cells are produced via transient transfection of a 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.[53-55] One fraction of the transfected DNA in the nucleus will express components needed to create TNT cells and the other fraction in the cytosol/near the plasma membrane will be transported to neighboring cells through tunneling nanotubes.

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, DNA encoding the aforementioned three components can be stably integrated into the genomic DNA of cells in order to create TNT cells that express TPFs and cargo for a longer period of time than would TNT cells created by a transient transfection approach. The TNT cells produced by stable integration of the aforementioned three components do not deliver plasmid DNA (from transfection approach), but instead deliver proteins, RNAs, and/or AAVs with associated AAV DNA cargo (FIGs. 1 & 2).

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 cells and cell lines for TNT cell production are primary or stable mammalian, e.g., human, cell lines refractory to the effects of transfection techniques known by those in the art. Examples of appropriate cell lines include Human Embryonic Kidney (HEK) 293 cells, HEK293 T/17 SF cells kidney-derived Phoenix-AMPHO cells, placenta-derived BeWo cells, Jurkat T cells, U2OS cells, and HepG2 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. TNT cells e.g., T1 and T3 TNT cells, can be produced from cells that have

been derived from patients (autologous TNT cells) and other FDA-approved cell lines (allogenic TNT cells) as long as these cells can be transfected with DNA constructs that encode the aforementioned TNT cell 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 TNT cell components in a constitutive and/or inducible fashion can be used for production of TNT cells, e.g., T2 and T4 cells. TNT cells, e.g., T2 and T4 TNT cells, can be produced from cells that have been derived from patients (autologous TNT cells) and other FDA-approved cell lines (allogenic TNT cells) if these cells have been converted into stable cell lines that express the aforementioned TNT cell components.

Also provided herein are the TNT cells themselves.

Production of Cargo-producing TNT cells and Compositions

Preferably TNT cells are harvested from 36-48 hours post-transfection/ nucleofection/transduction/other method for transiently or stably introducing TNT cell-encoding components into eukaryotic cells. After centrifugation, the TNT cells can be concentrated in the form of a centrifugate (pellet), which can be resuspended to a desired concentration, mixed with other reagents, subjected to a buffer exchange, or used as is. In some embodiments, TNT cell-containing supernatant can be filtered, precipitated,

centrifuged and resuspended to a concentrated solution. Purified cells can be frozen down in liquid nitrogen and are stable and can be stored at -270°C for years without losing appreciable activity if TNT cell components are stably expressed from the genomic DNA of cells. TNT cells created by transient transfection should be used within a week of initial transfection.

Preferably, TNT cells are resuspended or undergo buffer exchange so that cells are suspended in an appropriate carrier. In some embodiments, buffer exchange can be performed by ultrafiltration or dialysis. An exemplary appropriate carrier for TNT cells to be used for *in vitro* applications would preferably be a cell culture medium that is suitable for the cells that are to be mixed and co-cultured with TNT cells. Cells are co-cultured in the same vessel in an appropriate cell culture incubator (e.g., humidified incubator at 37°C with 5% CO_2).

An appropriate carrier for TNT cells 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. TNT cells could also be administered as an allograft.

The pharmaceutical forms suitable for injectable use include sterile aqueous solutions or suspensions. The solution or suspension may comprise additives that are compatible with TNT 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-producing TNT cells can be included in a container, pack, or dispenser together with instructions for administration.

Section 2: TNT cell cargo and applications

TNT cell "Cargo" can include, e.g., nucleic acids, DNA, RNA, a combination of DNA and RNP, RNP, a combination of DNA and proteins, or proteins, 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 as type 1 cargo (T1), RNP will be referred to as type 2 cargo (T2), a combination of DNA and proteins will be referred to as type 3 cargo (T3), and proteins will be referred to as type 4 cargo (T4). One of skill in the art will appreciate that these are examples and are non-limiting. RNA in this context can be, e.g., a single guide RNA (sgRNA), Clustered Regularly Interspaced Palindromic Repeat (CRISPR) RNA (crRNA), and/or mRNA coding for cargo. Cargo developed for applications of genome editing also includes, e.g., nucleases and base editors. Nucleases include, e.g., 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] sgRNAs complex with genome editing reagents during production within TNT cells, and are co-delivered to neighboring cells that are connected to TNT cells by tunneling nanotubes. To date, this concept has been validated *in vitro* by experiments that demonstrate the T1 and T2 delivery of RGN and CI RGN fused to deaminase and UGI (base editor) as protein for the purposes of site specific editing of a human-exogenous site (FIGs. 3 & 4). For example, T1 TNT cells have been used to deliver Cas9 RNP to U2OS and HEK293 cells for the purposes of editing exogenous GFP (FIGs. 3 & 4).

T3 cargo could refer to AAV (protein capsid and ITR-flanked DNA cargo).

T1-T4 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 (examples shown in 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 (examples shown in Table 5) fused to deaminases (examples shown in Table 3) by one or more variable length polypeptide linkers.

The T1-T4 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, TNT cell 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, TNT cells in the form of an allograft could be engineered to locally deliver shRNA, zinc finger/dCas9 repressors, Cas9, Base editors, and other modulators that inhibit calcineurin and obviate the need for immunosuppressive drugs and suppress allograft rejection. Immunosuppressive drugs lower the risk of allograft rejection, but they increase the risk of opportune infection and cancer. In this context, cargo can be constitutively expressed, or expressed from inducible promoters. Inducible promoters can be induced by addition of small molecule, tissue specific promoters, or inflammation inducible promoters.

In another application, TNT cells locally deliver inducible, programmable, multiplexed epigenetic modifiers.

In another application, TNT cells could be utilized for completely enclosed (never exposed in extracellular space) delivery of AAV particles to neighboring cells. This could help enhance AAV delivery by shielding AAV from antibody neutralization.

In another application, TNT cell 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, TNT cell 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 TNT cell cargo). This example is especially pertinent when combined with TNT cells that are antigen inducible and therefore specific for tumor cells.

In another application, TNT cells could deliver Yamanaka factors Oct3/4, Sox2, Klf4, and c-Myc to human or mouse fibroblasts in order to generate induced pluripotent stem cells.

In another application, TNT cells could deliver dominant-negative forms of proteins in order to elicit a therapeutic effect.

TNT cells that are 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.

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, TNT cells 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.[56]

Unbound steroid receptors reside in the cytosol. After binding to ligands, these receptors will translocate to the nucleus and initiate transcription of response genes. TNT cells 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. [57]

TNT cells can be indicated for treatments that involve targeted disruption of proteins. For example, TNT cells 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. [58-60]

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 TNT cells that can deliver specifically to cancer cells scFvs that bind to and disrupt the function of multiple RAS isoforms. [58-60]

Detailed Methods

T1 TNT cells were produced from cell lines, such as WT HEK293, 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.

WT HEK293 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 codon optimized Cas9 were co-transfected with a U6 promoter-sgRNA (targeting GFP) encoding plasmid, and the human MSEC cDNA encoding plasmid. Transfection reactions were assembled in reduced serum media (Opti-MEM; GIBCO #31985-070). For T1 TNT cell production on 10 cm plates, 7.5 μg Cas9 expression plasmid, 7.5 μg sgRNA-expression plasmid and 5 μg human MSEC expression plasmid 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 WT HEK293 cells.

T1 TNT HEK293 cells were harvested at 48 hours post-transfection. TNT cells were centrifuged at room temperature at 1,500 rpm for 5 minutes. After centrifugation, supernatants were decanted and TNT cell pellets were washed with PBS and centrifuged once more at room temperature at 1,500 rpm for 5 minutes. After centrifugation, supernatants were decanted and TNT cell pellets resuspended in DMEM 10% FBS media. TNT cells were then mixed with HEK293 cells that stably express a single copy of GFP. These two types of cells were seeded in a 24-well plate and co-cultured for 48-72 hours.

EXAMPLES

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

Example 1.

In FIG. 3, transient transfection was used to create HEK293 eGFP and U2OS eGFP TNT cell lines that express Cas9 and a TPF (human MSEC). In separate cell culture vessels, another group of HEK293 eGFP and U2OS eGFP cell lines were transfected with sgRNA targeting GFP. 48 hours post-transfections, TNT cells were mixed with the sgRNA-expressing cells and co-cultured. Flow cytometry was performed after 48 hours of co-culture. In order for GFP knockdown to occur, cells must deliver either sgRNA or Cas9 to each other such that Cas9 complexes with sgRNA within a single cell and this RNP complex is targeted to the GFP gene where indels can be created. HEK293 eGFP and U2OS eGFP each stably express a single copy of GFP. The results,

shown in FIG 3, demonstrated efficient transfer of the gene editing cargo to the WT cells as evidenced by significant reductions in levels of GFP-expressing cells.

In FIG 4A, transient transfection of wild type HEK293 and U2OS cells was used to create TNT cell lines that express Cas9, sgRNA targeting GFP, and a TPF (human MSEC). 48 hours post-transfection, TNT cells were mixed with the GFP expressing cells. Cell lysis was performed after 72 hours of co-culture. GFP-annealing primers were used in PCR to generate GFP amplicons, and amplicon sequencing was performed. In order for GFP knockdown to occur, cells must deliver both sgRNA and Cas9 to neighboring GFP-expressing cells. HEK293 eGFP and U2OS eGFP each stably express a single copy of GFP. The results, shown in FIG 4B, demonstrated efficient transfer of the gene editing cargo to the WT cells as evidenced by the presence of modifications in a target site in the GFP sequence.

TABLE 1 | Exemplary TNT-promoting factors (TPFs).

TNT-promoting factors
Human Msec (SEQ ID NO:25)
Mouse Msec (SEQ ID NO:26)
Human Lst1 isoform 1 (SEQ ID NO:27)
Human Lst1 isoform 4 (SEQ ID NO:28)
Human RalA (SEQ ID NO:29)

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 (* indicates which proteins lack sufficient structural information). All positional information refers to the wild-type protein sequences acquired from uniprot.org.

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

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

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

Exemplary Relevant Protein Sequences:

***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:1)

***Homo sapiens*: AID**
MDSLLMNRRKFLYQFKNVRWAKGRRETYLCYVVKRRDSATSFSLDFGYLRNKNGCHV
ELLFLRYISDWDLDPGRCYRVTWFTSWSPCYDCARHVADFLRGNPNLSLRIFTARLYF
CEDRKAEPEGLRRLHRAGVQIAIMTFKDYFYCWNTFVENHERTFKAWEGLHENSVRLS
RQLRRILLPLYEVDDLRDAFRTLGL (SEQ ID NO:2)

***Homo sapiens*:AIDv solubility variant lacking N-terminal RNA-binding region**
LMDPHIFTSNFNNGIGRHKTYLCYEVERLDSATSFSLDFGYLRNKNGCHVELLFLRYISD
WDLDPGRCYRVTWFTSWSPCYDCARHVADFLRGNPNLSLRIFTARLYFCEDRKAEPE
GLRRLHRAGVQIAIMTFKDYFYCWNTFVENHERTFKAWEGLHENSVRLSRQLRRILLPL
YEVDDLRDAFRTLGL (SEQ ID NO:3)

***Homo sapiens*: AIDv solubility variant lacking N-terminal RNA-binding region and the C-terminal poorly structured region**
MDPHIFTSNFNNGIGRHKTYLCYEVERLDSATSFSLDFGYLRNKNGCHVELLFLRYISD
WDLDPGRCYRVTWFTSWSPCYDCARHVADFLRGNPNLSLRIFTARLYFCEDRKAEPE
GLRRLHRAGVQIAIMTFKDYFYCWNTFVENHERTFKAWEGLHENSVRLSRQLRRILLPL
(SEQ ID NO:4)

***Rattus norvegicus*: APOBEC1**
MSSETGPVAVDPTLRRRIEPHEFEVFFDPRELRKETCLLYEINWGGRHSIWRHTSQNT
NKHVEVNFIEKFTTERYFCPNTRCSITWFLSWSPCGECSRAITEFLSRYPHVTLFIYIARL
YHHADPRNRQGLRDLISSGVTIQIMTEQESGYCWRNFVNYSPSNEAHWPRYPHLWVR
LYVLELYCIILGLPPCLNILRRKQPQLTFFTIALQSCHYQRLPPHILWATGLK (SEQ ID
NO:5)

***Mus musculus*: APOBEC3**
MGPFCLGCSHRKCYSPIRNLISQETFKFHFKNLGYAKGRKDTFLCYEVTRKDCDSPVSL
HHGVFKNKDNIHAEICFLYWFHDKVLKVLSPREEFKITWYMSWSPCFECAEQIVRFLAT
HHNLSLDIFSSRLYNVQDPETQQNLCRLVQEGAQVAAMDLYEFKKCWKKFVDNGGRR
FRPWKRLLTNFRYQDSKLQEILRRMDPLSEEEFYSQFYNQRVKHLCYYHRMKPYLCYQ
LEQFNGQAPLKGCLLSEKGKQHAEILFLDKIRSMELSQVTITCYLTWSPCPNCAWQLAA
FKRDRPDLILHIYTSRLYFHWKRPFQKGLCSLWQSGILVDVMDLPQFTDCWTNFVNPK
RPFRPWKGLEIISRRTQRRLRRIKESWGLQDLVNDFGNLQLGPPMSN (SEQ ID NO:6)

***Mus musculus*: APOBEC3 catalytic domain**
MGPFCLGCSHRKCYSPIRNLISQETFKFHFKNLGYAKGRKDTFLCYEVTRKDCDSPVSL
HHGVFKNKDNIHAEICFLYWFHDKVLKVLSPREEFKITWYMSWSPCFECAEQIVRFLAT
HHNLSLDIFSSRLYNVQDPETQQNLCRLVQEGAQVAAMDLYEFKKCWKKFVDNGGRR
FRPWKRLLTNFRYQDSKLQEILRR (SEQ ID NO:7)

***Homo sapiens*: APOBEC3A**
MEASPASGPRHLMDPHIFTSNFNNGIGRHKTYLCYEVERLDNGTSVKMDQHRGFLHN
QAKNLLCGFYGRHAELRFLDLVPSLQLDPAQIYRVTWFISWSPCFSWGCAGEVRAFLQ

ENTHVRLRIFAARIYDYDPLYKEALQMLRDAGAQVSIMTYDEFKHCWDTFVDHQGCPF
QPWDGLDEHSQALSGRLRAILQNQGN (SEQ ID NO:8)

***Homo sapiens*: APOBEC3G**

MKPHFRNTVERMYRDTFSYNFYNRPILSRRNTVWLCYEVKTKGPSRPPLDAKIFRGQV
YSELKYHPEMRFFHWFSKWRKLHRDQEYEVTWYISWSPCTKCTRDMATFLAEDPKVT
LTIFVARLYYFWDPDYQEALRSLCQKRDGPRATMKIMNYDEFQHCWSKFVYSQRELFE
PWNNLPKYYILLHIMLGEILRHSMDPPTFTFNFNNEPWVRGRHETYLCYEVERMHNDT
WVLLNQRRGFLCNQAPHKHGFLEGRHAELCFLDVIPFWKLDLDQDYRVTCFTSWSPC
FSCAQEMAKFISKNKHVSLCIFTARIYDDQGRCQEGLRTLAEAGAKISIMTYSEFKHCW
DTFVDHQGCPFQPWDGLDEHSQDLSGRLRAILQNQEN (SEQ ID NO:9)

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

PPTFTFNFNNEPWVRGRHETYLCYEVERMHNDTWVLLNQRRGFLCNQAPHKHGFLE
GRHAELCFLDVIPFWKLDLDQDYRVTCFTSWSPCFSCAQEMAKFISKNKHVSLCIFTAR
IYDDQGRCQEGLRTLAEAGAKISIMTYSEFKHCWDTFVDHQGCPFQPWDGLDEHSQD
LSGRLRAILQNQEN (SEQ ID NO:10)

***Homo sapiens*: APOBEC3H**

MALLTAETFRLQFNNKRRLRRPYYPRKALLCYQLTPQNGSTPTRGYFENKKKCHAEICF
INEIKSMGLDETQCYQVTCYLTWSPCSSCAWELVDFIKAHDHLNLGIFASRLYYHWCKP
QQKGLRLLCGSQVPVEVMGFPKFADCWENFVDHEKPLSFNPYKMLEELDKNSRAIKR
RLERIKIPGVRAQGRYMDILCDAEV (SEQ ID NO:11)

***Homo sapiens*: APOBEC3F**

MKPHFRNTVERMYRDTFSYNFYNRPILSRRNTVWLCYEVKTKGPSRPRLDAKIFRGQV
YSQPEHHAEMCFLSWFCGNQLPAYKCFQITWFVSWTPCPDCVAKLAEFLAEHPNVTL
TISAARLYYYWERDYRRALCRLSQAGARVKIMDDEEFAYCWENFVYSEGQPFMPWYK
FDDNYAFLHRTLKEILRNPMEAMYPHIFYFHFKNLRKAYGRNESWLCFTMEVVKHHSP
VSWKRGVFRNQVDPETHCHAERCFLSWFCDDILSPNTNYEVTWYTSWSPCPECAGE
VAEFLARHSNVNLTIFTARLYYFWDTDYQEGLRSLSQEGASVEIMGYKDFKYCWENFV
YNDDEPFKPWKGLKYNFLFLDSKLQEILE (SEQ ID NO:12)

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

KEILRNPMEAMYPHIFYFHFKNLRKAYGRNESWLCFTMEVVKHHSPVSWKRGVFRNQ
VDPETHCHAERCFLSWFCDDILSPNTNYEVTWYTSWSPCPECAGEVAEFLARHSNVN
LTIFTARLYYFWDTDYQEGLRSLSQEGASVEIMGYKDFKYCWENFVYNDDEPFKPWKG
LKYNFLFLDSKLQEILE (SEQ ID NO:13)

Escherichia coli: TadA

MKRTADGSEFESPKKKRKVSEVEFSHEYWMRHALTLAKRAWDEREVPVGAVLVHNN
RVIGEGWNRPIGRHDPTAHAEIMALRQGGLVMQNYRLIDATLYVTLEPCVMCAGAMIH
SRIGRVVFGARDAKTGAAGSLMDVLHHPGMNHRVEITEGILADECAALLSDFFRMRRQ
EIKAQKKAQSSTDSGGSSGGSSGSETPGTSESATPESSGGSSGGSSEVEFSHEYWM
RHALTLAKRARDEREVPVGAVLVLNNRVIGEGWNRAIGLHDPTAHAEIMALRQGGLVM

QNYRLIDATLYVTFEPCVMCAGAMIHSRIGRVVFGVRNAKTGAAGSLMDVLHYPGMNH
RVEITEGILADECAALLCYFFRMPRQVFNAQKKAQSSTD (SEQ ID NO:14)

***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:15)

***Homo sapiens*: Adar2**

MDIEDEENMSSSSTDVKENRNLDNVSPKDGSTPGPGEGSQLSNGGGGGPGRKRPLE
EGSNGHSKYRLKKRRKTPGPVLPKNALMQLNEIKPGLQYTLLSQTGPVHAPLFVMSVE
VNGQVFEGSGPTKKKAKLHAAEKALRSFVQFPNASEAHLAMGRTLSVNTDFTSDQADF
PDTLFNGFETPDKAEPPFYVGSNGDDSFSSSGDLSLSASPVPASLAQPPLPVLPPFPPP
SGKNPVMILNELRPGLKYDFLSESGESHAKSFVMSVVVDGQFFEGSGRNKKLAKARAA
QSALAAIFNLHLDQTPSRQPIPSEGLQLHLPQVLADAVSRLVLGKFGDLTDNFSSPHAR
RKVLAGVVMTTGTDVKDAKVISVSTGTKCINGEYMSDRGLALNDCHAEIISRRSLLRFLY
TQLELYLNNKDDQKRSIFQKSERGGFRLKENVQFHLYISTSPCGDARIFSPHEPILEEPA
DRHPNRKARGQLRTKIESGQGTIPVRSNASIQTWDGVLQGERLLTMSCSDKIARWNVV
GIQGSLLSIFVEPIYFSSIILGSLYHGDHLSRAMYQRISNIEDLPPLYTLNKPLLSGISNAEA
RQPGKAPNFSVNWTVGDSAIEVINATTGKDELGRASRLCKHALYCRWMRVHGKVPSH
LLRSKITKPNVYHESKLAAKEYQAAKARLFTAFIKAGLGAWVEKPTEQDQFSLTP (SEQ
ID NO:16)

***Streptococcus pyogenes*: Cas9 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:17)

***Staphylococcus aureus:* Cas9**

MKRNYILGLDIGITSVGYGIIDYETRDVIDAGVRLFKEANVENNEGRRSKRGARR
LKRRRRHRIQRVKKLLFDYNLLTDHSELSGINPYEARVKGLSQKLSEEEFSAALL
HLAKRRGVHNVNEVEEDTGNELSTKEQISRNSKALEEKYVAELQLERLKKDGE
VRGSINRFKTSDYVKEAKQLLKVQKAYHQLDQSFIDTYIDLLETRRTYYEGPGE
GSPFGWKDIKEWYEMLMGHCTYFPEELRSVKYAYNADLYNALNDLNNLVITRD
ENEKLEYYEKFQIIENVFKQKKKPTLKQIAKEILVNEEDIKGYRVTSTGKPEFTNL
KVYHDIKDITARKEIIENAELLDQIAKILTIYQSSEDIQEELTNLNSELTQEEIEQISN
LKGYTGTHNLSLKAINLILDELWHTNDNQIAIFNRLKLVPKKVDLSQQKEIPTTLV
DDFILSPVVKRSFIQSIKVINAIIKKYGLPNDIIIELAREKNSKDAQKMINEMQKRNR
QTNERIEEIIRTTGKENAKYLIEKIKLHDMQEGKCLYSLEAIPLEDLLNNPFNYEVD

HIIPRSVSFDNSFNNKVLVKQEENSKKGNRTPFQYLSSSDSKISYETFKKHILNL
AKGKGRISKTKKEYLLEERDINRFSVQKDFINRNLVDTRYATRGLMNLLRSYFRV
NNLDVKVKSINGGFTSFLRRKWKFKKERNKGYKHHAEDALIIANADFIFKEWKKL
DKAKKVMENQMFEEKQAESMPEIETEQEYKEIFITPHQIKHIKDFKDYKYSHRVD
KKPNRELINDTLYSTRKDDKGNTLIVNNLNGLYDKDNDKLKKLINKSPEKLLMYH
HDPQTYQKLKLIMEQYGDEKNPLYKYYEETGNYLTKYSKKDNGPVIKKIKYYGN
KLNAHLDITDDYPNSRNKVVKLSLKPYRFDVYLDNGVYKFVTVKNLDVIKKENYY
EVNSKCYEEAKKLKKISNQAEFIASFYNNDLIKINGELYRVIGVNNDLLNRIEVNMI
DITYREYLENMNDKRPPRIIKTIASKTQSIKKYSTDILGNLYEVKSKKHPQIIKKG
(SEQ ID NO:18)

Campylobacter jejuni: **Cas9**

MARILAFDIGISSIGWAFSENDELKDCGVRIFTKVENPKTGESLALPRRLARSAR
KRLARRKARLNHLKHLIANEFKLNYEDYQSFDESLAKAYKGSLISPYELRFRALN
ELLSKQDFARVILHIAKRRGYDDIKNSDDKEKGAILKAIKQNEEKLANYQSVGEY
LYKEYFQKFKENSKEFTNVRNKKESYERCIAQSFLKDELKLIFKKQREFGFSFSK
KFEEEVLSVAFYKRALKDFSHLVGNCSFFTDEKRAPKNSPLAFMFVALTRIINLL
NNLKNTEGILYTKDDLNALLNEVLKNGTLTYKQTKKLLGLSDDYEFKGEKGTYFI
EFKKYKEFIKALGEHNLSQDDLNEIAKDITLIKDEIKLKKALAKYDLNQNQIDSLSK
LEFKDHLNISFKALKLVTPLMLEGKKYDEACNELNLKVAINEDKKDFLPAFNETY
YKDEVTNPVVLRAIKEYRKVLNALLKKYGKVHKINIELAREVGKNHSQRAKIEKE
QNENYKAKKDAELECEKLGLKINSKNILKLRLFKEQKEFCAYSGEKIKISDLQDE
KMLEIDHIYPYSRSFDDSYMNKVLVFTKQNQEKLNQTPFEAFGNDSAKWQKIEV
LAKNLPTKKQKRILDKNYKDKEQKNFKDRNLNDTRYIARLVLNYTKDYLDFLPLS
DDENTKLNDTQKGSKVHVEAKSGMLTSALRHTWGFSAKDRNNHLHHAIDAVIIA
YANNSIVKAFSDFKKEQESNSAELYAKKISELDYKNKRKFFEPFSGFRQKVLDKI
DEIFVSKPERKKPSGALHEETFRKEEEFYQSYGGKEGVLKALELGKIRKVNGKIV
KNGDMFRVDIFKHKKTNKFYAVPIYTMDFALKVLPNKAVARSKKGEIKDWILMD
ENYEFCFSLYKDSLILIQTKDMQEPEFVYYNAFTSSTVSLIVSKHDNKFETLSKN
QKILFKNANEKEVIAKSIGIQNLKVFEKYIVSALGEVTKAEFRQREDFKK (SEQ ID
NO:19)

Neisseria meningitidis: **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:20)

***Acidaminococcus sp.* 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:21)

***Lachnospiraceae bacterium* 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:22)

***Leptotrichia shahii* Cas13a**

MGNLFGHKRWYEVRDKKDFKIKRKVKVKRNYDGNKYILNINENNNKEKIDNNKF
IRKYINYKKNDNILKEFTRKFHAGNILFKLKGKEGIIRIENNDDFLETEEVVLYIEAY
GKSEKLKALGITKKKIIDEAIRQGITKDDKKIEIKRQENEEEIEIDIRDEYTNKTLND
CSIILRIIENDELETKKSIYEIFKNINMSLYKIIEKIIENETEKVFENRYYEEHLREKLL
KDDKIDVILTNFMEIREKIKSNLEILGFVKFYLNVGGDKKKSKNKKMLVEKILNINV
DLTVEDIADFVIKELEFWNITKRIEKVKKVNNEFLEKRRNRTYIKSYVLLDKHEKF
KIERENKKDKIVKFFVENIKNNSIKEKIEKILAEFKIDELIKKLEKELKKGNCDTEIFG
IFKKHYKVNFDSKKFSKKSDEEKELYKIIYRYLKGRIEKILVNEQKVRLKKMEKIEI
EKILNESILSEKILKRVKQYTLEHIMYLGKLRHNDIDMTTVNTDDFSRLHAKEELD
LELITFFASTNMELNKIFSRENINNDENIDFFGGDREKNYVLDKKILNSKIKIIRDLD
FIDNKNNITNNFIRKFTKIGTNERNRILHAISKERDLQGTQDDYNKVINIIQNLKISD
EEVSKALNLDVVFKDKKNIITKINDIKISEENNNDIKYLPSFSKVLPEILNLYRNNPK
NEPFDTIETEKIVLNALIYVNKELYKKLILEDDLEENESKNIFLQELKKTLGNIDEID
ENIIENYYKNAQISASKGNNKAIKKYQKKVIECYIGYLRKNYEELFDFSDFKMNIQ
EIKKQIKDINDNKTYERITVKTSDKTIVINDDFEYIISIFALLNSNAVINKIRNRFFATS
VWLNTSEYQNIIDILDEIMQLNTLRNECITENWNLNLEEFIQKMKEIEKDFDDFKI
QTKKEIFNNYYEDIKNNILTEFKDDINGCDVLEKKLEKIVIFDDETKFEIDKKSNILQ
DEQRKLSNINKKDLKKKVDQYIKDKDQEIKSKILCRIIFNSDFLKKYKKEIDNLIED
MESENENKFQEIYYPKERKNELYIYKKNLFLNIGNPNFDKIYGLISNDIKMADAKF
LFNIDGKNIRKNKISEIDAILKNLNDKLNGYSKEYKEKYIKKLKENDDFFAKNIQNK
NYKSFEKDYNRVSEYKKIRDLVEFNYLNKIESYLIDINWKLAIQMARFERDMHYIV
NGLRELGIIKLSGYNTGISRAYPKRNGSDGFYTTTAYYKFFDEESYKKFEKICYG
FGIDLSENSEINKPENESIRNYISHFYIVRNPFADYSIAEQIDRVSNLLSYSTRYNN
STYASVFEVFKKDVNLDYDELKKKFKLIGNNDILERLMKPKKVSVLELESYNSDY
IKNLIIELLTKIENTNDTL (SEQ ID NO:23)

***Leptotrichia wadei* Cas13a**

MKVTKVDGISHKKYIEEGKLVKSTSEENRTSERLSELLSIRLDIYIKNPDNASEEE
NRIRRENLKKFFSNKVLHLKDSVLYLKNRKEKNAVQDKNYSEEDISEYDLKNKN
SFSVLKKILLNEDVNSEELEIFRKDVEAKLNKINSLKYSFEENKANYQKINENNVE
KVGGKSKRNIIYDYYRESAKRNDYINNVQEAFDKLYKKEDIEKLFFLIENSKKHEK
YKIREYYHKIIGRKNDKENFAKIIYEEIQNVNNIKELIEKIPDMSELKKSQVFYKYYL
DKEELNDKNIKYAFCHFVEIEMSQLLKNYVYKRLSNISNDKIKRIFEYQNLKKLIE
NKLLNKLDTYVRNCGKYNYYLQVGEIATSDFIARNRQNEAFLRNIIGVSSVAYFS
LRNILETENENGITGRMRGKTVKNNKGEEKYVSGEVDKIYNENKQNEVKENLK
MFYSYDFNMDNKNEIEDFFANIDEAISSIRHGIVHFNLELEGKDIFAFKNIAPSEIS
KKMFQNEINEKKLKLKIFKQLNSANVFNYYEKDVIIKYLKNTKFNFVNKNIPFVPS
FTKLYNKIEDLRNTLKFFWSVPKDKEEKDAQIYLLKNIYYGEFLNKFVKNSKVFF
KITNEVIKINKQRNQKTGHYKYQKFENIEKTVPVEYLAIIQSREMINNQDKEEKNT
YIDFIQQIFLKGFIDYLNKNNLKYIESNNNNDNNDIFSKIKIKKDNKEKYDKILKNYE
KHNRNKEIPHEINEFVREIKLGKILKYTENLNMFYLILKLLNHKELTNLKGSLEKYQ
SANKEETFSDELELINLLNLDNNRVTEDFELEANEIGKFLDFNENKIKDRKELKKF
DTNKIYFDGENIIKHRAFYNIKKYGMLNLLEKIADKAKYKISLKELKEYSNKKNEIE
KNYTMQQNLHRKYARPKKDEKFNDEDYKEYEKAIGNIQKYTHLKNKVEFNELNL
LQGLLLKILHRLVGYTSIWERDLRFRLKGEFPENHYIEEIFNFDNSKNVKYKSGQI
VEKYINFYKELYKDNVEKRSIYSDKKVKKLKQEKKDLYIRNYIAHFNYIPHAEISLL
EVLENLRKLLSYDRKLKNAIMKSIVDILKEYGFVATFKIGADKKIEIQTLESEKIVHL
KNLKKKKLMTDRNSEELCELVKVMFEYKALE (SEQ ID NO:24)

***Homo sapiens*: Msec**

MSEASSEDLVPPLEAGAAPYREEEEAAKKKKEKKKKSKGLANVFCVFTKGKKK
KGQPSSAEPEDAAGSRQGLDGPPPTVEELKAALERGQLEAARPLLALERELAA
AAAAGGVSEEELVRRQSKVEALYELLRDQVLGVLRRPLEAPPERLRQALAVVA
EQEREDRQAAAAGPGTSGLAATRPRRWLQLWRRGVAEAAEERMGQRPAAGA
EVPESVFLHLGRTMKEDLEAVVERLKPLFPAEFGVVAAYAESYHQHFAAHLAAV
AQFELCERDTYMLLLWVQNLYPNDIINSPKLVGELQGMGLGSLLPPRQIRLLEA
TFLSSEAANVRELMDRALELEARRWAEDVPPQRLDGHCHSELAIDIIQITSQAQ
AKAESITLDLGSQIKRVLLVELPAFLRSYQRAFNEFLERGKQLTNYRANVIANINN
CLSFRMSMEQNWQVPQDTLSLLLGPLGELKSHGFDTLLQNLHEDLKPLFKRFT
HTRWAAPVETLENIIATVDTRLPEFSELQGCFREELMEALHLHLVKEYIIQLSKG
RLVLKTAEQQQQLAGYILANADTIQHFCTQHGSPATWLQPALPTLAEIIRLQDPS
AIKIEVATYATCYPDFSKGHLSAILAIKGNLSNSEVKRIRSILDVSMGAQEPSRPL
FSLIKVG (SEQ ID NO:25)

***Mus musculus*: Msec**

MSEASSEDLMPSPEAPDGEEESAKKKEKKSKGLANMFSVFTKGKKKKKDQPR
LSDLEVQPKPRPELDGPLPTVEELKEALEHGRLEVAWQVLALERQLEAAAAAG
GMSNEELVWRQSKVEALYVLLCDQVLGVLRRPLEAAPERLSQALAVVSQEELE
DRRASGGPLAAALEATRPRRWLQRWRGVVAEVAAERLDAQPATAPEGRSEAE
SRFLHMGRTMKEDLEVVVERLKPLFPDEFNVVRTYAESYHYHFASHLCALAQF
ELCERDTYLLLLWVQNLYPNDILNSPKLAQELQGVGLGSLLPPKQIRLLEAMFLS
NEVTSVKQLMARALELESQRWTQDVAPQSLDGHCHSELAIDILQIISQGQTKAE

NITSDVGMQIKQLLLVELAALLRSYQRAFDEFLEKSKLLRNYRVNIMANINNCLFF
WTSVEQKWQISHDSLNRLLEPLKDLKAHGFDTLLQSLFLDLKPLFKKFTQTRWA
NPVETLEEIITTVSSSLPEFSELQDCFREELMETVHLHLVKEYIIRLCKRRLVLKTA
EQQQQLARHILANADAIQGFCTENGSTATWLHRALPMIAEIIRLQDSSAIKIEVAT
YATWYPDFSKGHLNAILAIKGNLPSSEVRSIRNILDINTGVQEPPRPLFSLIKVT
(SEQ ID NO:26)

***Homo sapiens*: Lst1 isoform 1**
MLSRNDDICIYGGLGLGGLLLLAVVLLSACLCWLHRRVKRLERSWHLLSWSQA
QGSSEQELHYASLQRLPVPSSEGPDLRGRDKRGTKEDPRADYACIAENKPT
(SEQ ID NO:27)

***Homo sapiens*: Lst1 isoform 4**
MLSRNDDICIYGGLGLGGLLLLAVVLLSACLCWLHRRVKRLERSWAQGSSEQE
LHYASLQRLPVPSSEGPDLRGRDKRGTKEDPRADYACIAENKPT (SEQ ID
NO:28)

***Homo sapiens*: RalA**
MAANKPKGQNSLALHKVIMVGSGGVGKSALTLQFMYDEFVEDYEPTKADSYRK
KVVLDGEEVQIDILDTAGQEDYAAIRDNYFRSGEGFLCVFSITEMESFAATADFR
EQILRVKEDENVPFLLVGNKSDLEDKRQVSVEEAKNRAEQWNVNYVETSAKTR
ANVDKVFFDLMREIRARKMEDSKEKNGKKKRKSLAKRIRERCCIL (SEQ ID
NO:29)

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

Herpes simplex virus (HSV) type 1 & Synthetic: VP64
GRADALDDFDLDMLGSDALDDFDLDMLGSDALDDFDLDMLGSDALDDFDLDM
L (SEQ ID NO:31)

***Homo sapiens*: P65**
SQYLPDTDDRHRIEEKRKRTYETFKSIMKKSPFSGPTDPRPPPRRIAVPSRSSA
SVPKPAPQPYPFTSSLSTINYDEFPTMVFPSGQISQASALAPAPPQVLPQAPAP
APAPAMVSALAQAPAPVPVLAPGPPQAVAPPAPKPTQAGEGTLSEALLQLQFD
DEDLGALLGNSTDPAVFTDLASVDNSEFQQLLNQGIPVAPHTTEPMLMEYPEAI
TRLVTGAQRPPDPAPAPLGAPGLPNGLLSGDEDFSSIADMDFSALL (SEQ ID
NO:32)

Kaposi's Sarcoma-Associated Herpesvirus Transactivator: RTA
RDSREGMFLPKPEAGSAISDVFEGREVCQPKRIRPFHPPGSPWANRPLPASLA
PTPTGPVHEPVGSLTPAPVPQPLDPAPAVTPEASHLLEDPDEETSQAVKALRE
MADTVIPQKEEAAICGQMDLSHPPPRGHLDELTTTLESMTEDLNLDSPLTPELN
EILDTFLNDECLLHAMHISTGLSIFDTSLF (SEQ ID NO:33)

***Homo sapiens*: KRAB**
MDAKSLTAWSRTLVTFKDVFVDFTREEWKLLDTAQQIVYRNVMLENYKNLVSL
GYQLTKPDVILRLEKGEEP (SEQ ID NO:34)

***Homo sapiens*: MeCP2**
EASVQVKRVLEKSPGKLLVKMPFQASPGGKGEGGGATTSAQVMVIKRPGRKR
KAEADPQAIPKKRGRKPGSVVAAAAAEAKKKAVKESSIRSVQETVLPIKKRKTR
ETVSIEVKEVVKPLLVSTLGEKSGKGLKTCKSPGRKSKESSPKGRSSSASSPPK
KEHHHHHHHHAESPKAPMPLLPPPPPPEPQSSEDPISPPEPQDLSSSICKEEKM
PRAGSLESDGCPKEPAKTQPMVAAAATTTTTTTTTVAEKYKHRGEGERKDIVS
SSMPRPNREEPVDSRTPVTERVS (SEQ ID NO:35)

***Homo sapiens*: Tet1**
LPTCSCLDRVIQKDKGPYYTHLGAGPSVAAVREIMENRYGQKGNAIRIEIVVYTG
KEGKSSHGCPIAKWVLRRSSDEEKVLCLVRQRTGHHCPTAVMVVLIMVWDGIP
LPMADRLYTELTENLKSYNGHPTDRRCTLNENRTCTCQGIDPETCGASFSFGC
SWSMYFNGCKFGRSPSPRRFRIDPSSPLHEKNLEDNLQSLATRLAPIYKQYAP
VAYQNQVEYENVARECRLGSKEGRPFSGVTACLDFCAHPHRDIHNMNNGSTV
VCTLTREDNRSLGVIPQDEQLHVLPLYKLSDTDEFGSKEGMEAKIKSGAIEVLAP
RRKKRTCFTQPVPRSGKKRAAMMTEVLAHKIRAVEKKPIPRIKRKNNSTTTNNS
KPSSLPTLGSNTETVQPEVKSETEPHFILKSSDNTKTYSLMPSAPHPVKEASPG
FSWSPKTASATPAPLKNDATASCGFSERSSTPHCTMPSGRLSGANAAAADGP
GISQLGEVAPLPTLSAPVMEPLINSEPSTGVTEPLTPHQPNHQPSFLTSPQDLA
SSPMEEDEQHSEADEPPSDEPLSDDPLSPAEEKLPHIDEYWSDSEHIFLDANIG
GVAIAPAHGSVLIECARRELHATTPVEHPNRNHPTRLSLVFYQHKNLNKPQHGF
ELNKIKFEAKEAKNKKMKASEQKDQAANEGPEQSSEVNELNQIPSHKALTLTHD
NVVTVSPYALTHVAGPYNHWV (SEQ ID NO:36)

***Homo sapiens*: Dnmt3a**
MPAMPSSGPGDTSSSAAEREEDRKDGEEQEEPRGKEERQEPSTTARKVGRP
GRKRKHPPVESGDTPKDPAVISKSPSMAQDSGASELLPNGDLEKRSEPQPEE
GSPAGGQKGGAPAEGEGAAETLPEASRAVENGCCTPKEGRGAPAEAGKEQK
ETNIESMKMEGSRGRLRGGLGWESSLRQRPMPRLTFQAGDPYYISKRKRDEW
LARWKREAEKKAKVIAGMNAVEENQGPGESQKVEEASPPAVQQPTDPASPTV
ATTPEPVGSDAGDKNATKAGDDEPEYEDGRGFGIGELVWGKLRGFSWWPGRI
VSWWMTGRSRAAEGTRWVMWFGDGKFSVVCVEKLMPLSSFCSAFHQATYN
KQPMYRKAIYEVLQVASSRAGKLFPVCHDSDESDTAKAVEVQNKPMIEWALGG
FQPSGPKGLEPPEEEKNPYKEVYTDMWVEPEAAAYAPPPPAKKPRKSTAEKP
KVKEIIDERTRERLVYEVRQKCRNIEDICISCGSLNVTLEHPLFVGGMCQNCKNC
FLECAYQYDDDGYQSYCTICCGGREVLMCGNNNCCRCFCVECVDLLVGPGAA
QAAIKEDPWNCYMCGHKGTYGLLRRREDWPSRLQMFFANNHDQEFDPPKVY
PPVPAEKRKPIRVLSLFDGIATGLLVLKDLGIQVDRYIASEVCEDSITVGMVRHQ
GKIMYVGDVRSVTQKHIQEWGPFDLVIGGSPCNDLSIVNPARKGLYEGTGRLFF
EFYRLLHDARPKEGDDRPFFWLFENVVAMGVSDKRDISRFLESNPVMIDAKEV
SAAHRARYFWGNLPGMNRPLASTVNDKLELQECLEHGRIAKFSKVRTITTRSN

SIKQGKDQHFPVFMNEKEDILWCTEMERVFGFPVHYTDVSNMSRLARQRLLGR
SWSVPVIRHLFAPLKEYFACV (SEQ ID NO:37)

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

ATGGACAAGAAGTACAGCATCGGCCTGGACATCGGCACCAACTCTGTGGG
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TGCTGTTCGACAGCGGCGAAACAGCCGAGGCCACCCGGCTGAAGAGAACC
GCCAGAAGAAGATACACCAGACGGAAGAACCGGATCTGCTATCTGCAAGAG
ATCTTCAGCAACGAGATGGCCAAGGTGGACGACAGCTTCTTCCACAGACTG
GAAGAGTCCTTCCTGGTGGAAGAGGATAAGAAGCACGAGCGGCACCCCAT
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CTACCACCTGAGAAAGAAACTGGTGGACAGCACCGACAAGGCCGACCTGC
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TGATCGAGGGCGACCTGAACCCCGACAACAGCGACGTGGACAAGCTGTTC
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CGACATCCTGAGAGTGAACACCGAGATCACCAAGGCCCCCCTGAGCGCCT
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CTGGCCGACGCTAATCTGGACAAAGTGCTGTCCGCCTACAACAAGCACCGG
GATAAGCCCATCAGAGAGCAGGCCGAGAATATCATCCACCTGTTTACCCTG
ACCAATCTGGGAGCCCCTGCCGCCTTCAAGTACTTTGACACCACCATCGAC
CGGAAGAGGTACACCAGCACCAAAGAGGTGCTGGACGCCACCCTGATCCA
CCAGAGCATCACCGGCCTGTACGAGACACGGATCGACCTGTCTCAGCTGG
GAGGCGACGGATCCCCCAAGAAGAAGAGGAAAGTCTCGAGCGACTACAAA
GACCATGACGGTGATTATAAAGATCATGACATCGATTACAAGGATGACGATG
ACAAGGCTGCAGGATGA (SEQ ID NO:38)

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

ATGGACAAGAAGTACAGCATCGGCCTGGACATCGGCACCAACTCTGTGGG
CTGGGCCGTGATCACCGACGAGTACAAGGTGCCCAGCAAGAAATTCAAGGT
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TGCTGTTCGACAGCGGCGAAACAGCCGAGGCCACCCGGCTGAAGAGAACC
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ACAAAGACCATGACGGTGATTATAAAGATCATGACATCGATTACAAGGATGA
CGATGACAAGTGA (SEQ ID NO:39)

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

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GAAAGGAGACATGCCTGCTGTACGAGATCAACTGGGGAGGCAGGCACTCC
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ATCGAGAAGTTTACCACAGAGCGGTACTTCTGCCCCAATACCAGATGTAGC
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TCTAA (SEQ ID NO:40)

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

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GACCCTGGCAAAGCGAGCATGGGATGAAAGAGAAGTCCCCGTGGGCGCCG
TGCTGGTGCACAACAATAGAGTGATCGGAGAGGGATGGAACAGGCCAATC
GGCCGCCACGACCCTACCGCACACGCAGAGATCATGGCACTGAGGCAGGG
AGGCCTGGTCATGCAGAATTACCGCCTGATCGATGCCACCCTGTATGTGAC
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OTHER EMBODIMENTS

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

WHAT IS CLAIMED IS:

1. A tunneling nanotube (TNT) cell, comprising:
   (i) a TNT promoting factor (TPF), preferably selected from the group consisting of M-Sec, leukocyte-specific transcript 1 (Lst1), and RAS like proto-oncogene A (RalA), overexpressed in the cell; and
   (ii) a biomolecule cargo overexpressed in the cell in the cytosol or embedded within the phospholipid bilayer.

2. The TNT cell of claim 1, wherein the biomolecule cargo is a therapeutic or diagnostic protein or nucleic acid encoding a therapeutic or diagnostic protein.

3. The TNT cell of claim 1, wherein the biomolecule cargo is a gene editing reagent.

4. The TNT cell of claim 1, wherein the gene editing reagent comprises a zinc finger (ZF), transcription activator-like effector (TALE), and/or CRISPR-based genome editing or modulating protein; a nucleic acid encoding a zinc finger (ZF), transcription activator-like effector (TALE), and/or CRISPR-based genome editing or modulating protein; or a riboucleoprotein complex (RNP) comprising a CRISPR-based genome editing or modulating protein.

5. The TNT cell of claim 4, wherein the gene editing reagent is selected from the proteins listed in Tables 2, 3, 4 & 5.

6. The TNT cell of claim 4, wherein the gene editing reagent comprises a CRISPR-based genome editing or modulating protein, and the TNT cell further comprises one or more guide RNAs that bind to and direct the CRISPR-based genome editing or modulating protein to a target sequence.

7. A method of delivering a biomolecule to a target cell, preferably a cell *in vitro* or *in vivo*, the method comprising contacting the target cell with the TNT cell of claim 1 comprising the biomolecule as cargo.

8. A method of producing a TNT cell comprising a biomolecular cargo, the method comprising: providing a cell overexpressing one or more TPFs, preferably selected from the group consisting of M-Sec, leukocyte-specific transcript 1 (Lst1), and maintaining the cell.

9. The method of claim 8, further comprising harvesting and optionally purifying and/or concentrating the produced TNT cells.

10. The method of claim 8, wherein the biomolecule cargo is a therapeutic or diagnostic protein or nucleic acid encoding a therapeutic or diagnostic protein.

11. The method of claim 8, wherein the biomolecule cargo is a gene editing reagent.

12. The method of claim 8, wherein the gene editing reagent comprises a zinc finger (ZF), transcription activator-like effector (TALE), and/or CRISPR-based genome editing or modulating protein; a nucleic acid encoding a zinc finger (ZF), transcription activator-like effector (TALE), and/or CRISPR-based genome editing or modulating protein; or a riboucleoprotein complex (RNP) comprising a CRISPR-based genome editing or modulating protein.

13. The method of claim 12, wherein the gene editing reagent is selected from the proteins listed in Tables 2, 3, 4 & 5.

14. The method of claim 12, wherein the gene editing reagent comprises a CRISPR-based genome editing or modulating protein, and the TNT cell further comprises one or more guide RNAs that bind to and direct the CRISPR-based genome editing or modulating protein to a target sequence.

15. A cell overexpressing one or more TPFs, preferably selected from the group consisting of M-Sec, leukocyte-specific transcript 1 (Lst1), and a cargo biomolecule.

16. The cell of claim 15, wherein the biomolecule cargo is a therapeutic or diagnostic protein or nucleic acid encoding a therapeutic or diagnostic protein.

17. The cell of claim 15, wherein the biomolecule cargo is a gene editing reagent.

18. The cell of claim 15, wherein the gene editing reagent comprises a zinc finger (ZF), transcription activator-like effector (TALE), and/or CRISPR-based genome editing or modulating protein; a nucleic acid encoding a zinc finger (ZF), transcription activator-like effector (TALE), and/or CRISPR-based genome editing or modulating protein; or a riboucleoprotein complex (RNP) comprising a CRISPR-based genome editing or modulating protein.

19. The cell of claim 18, wherein the gene editing reagent is selected from the proteins listed in Tables 2, 3, 4 & 5.

20. The cell of claim 18, wherein the gene editing reagent comprises a CRISPR-based genome editing or modulating protein, and the TNT cell further comprises one or more guide RNAs that bind to and direct the CRISPR-based genome editing or modulating protein to a target sequence.

21. The cells of claims 15-20, wherein the cells are primary or stable human cell lines.

22. The cells of claim 21, which are Human Embryonic Kidney (HEK) 293 cells or HEK293 T cells.

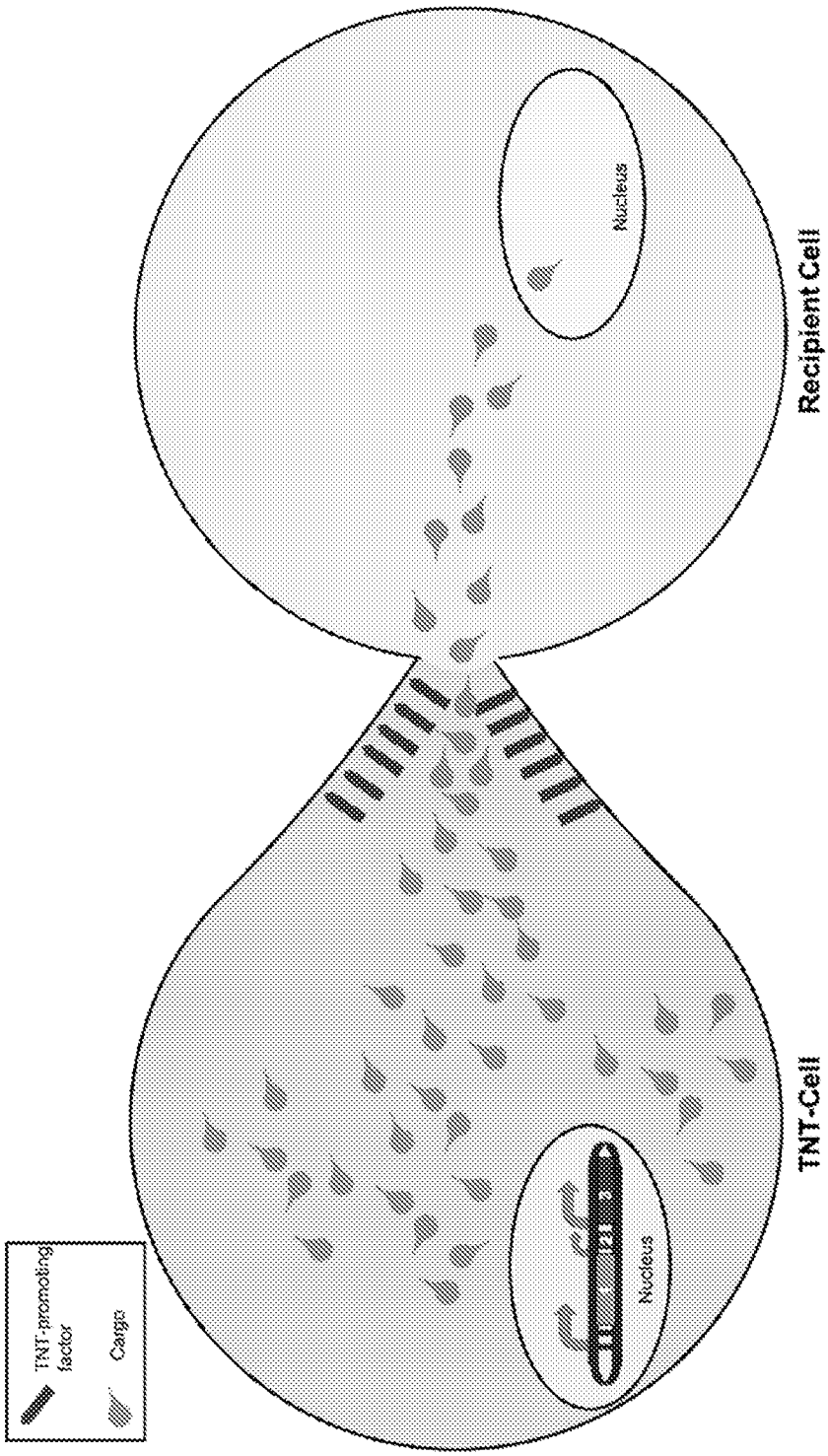

TNT-promoting factor
Cargo
TNT-Cell
Nucleus
Recipient Cell
Nucleus

FIG. 1

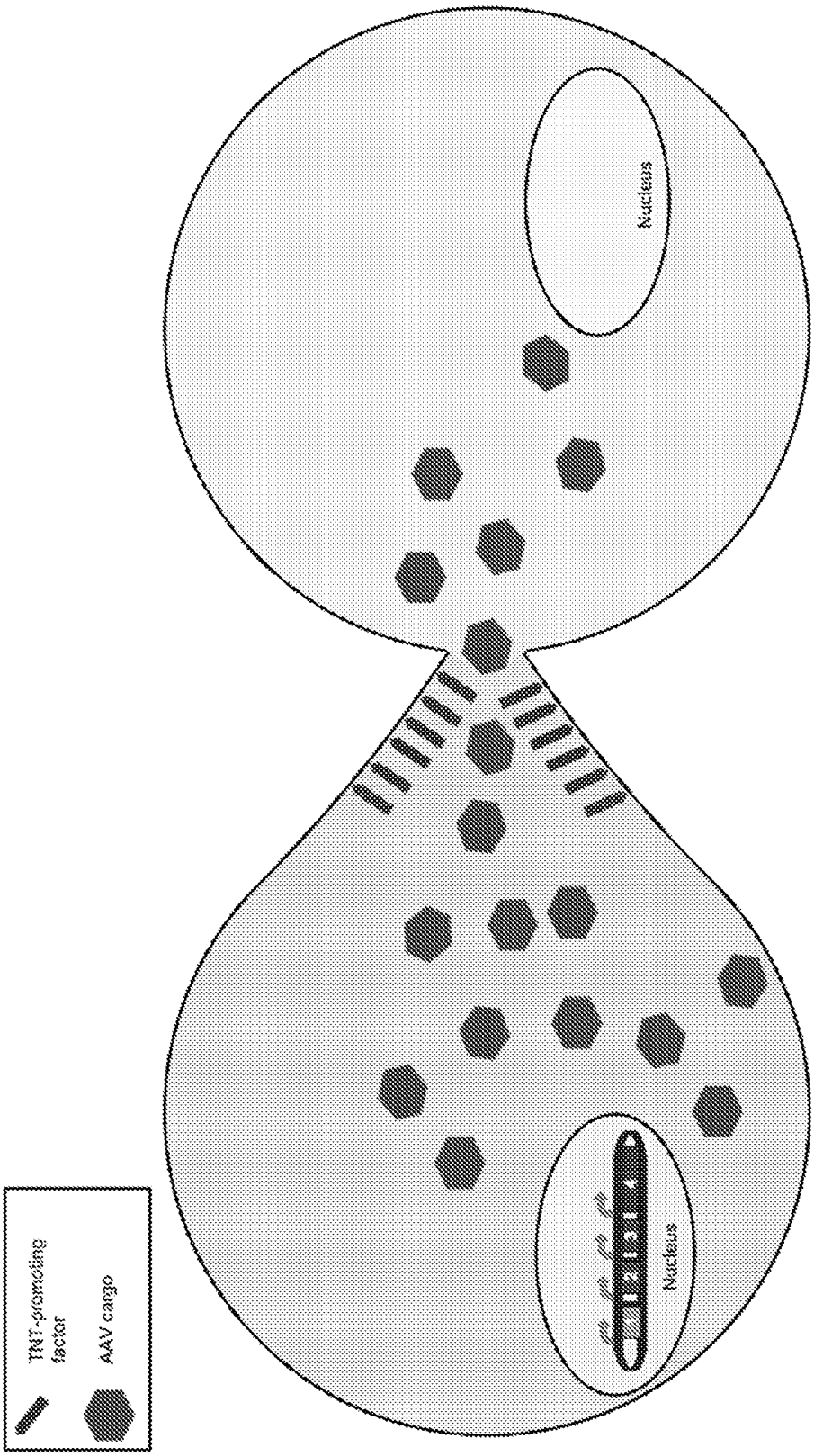


FIG. 2

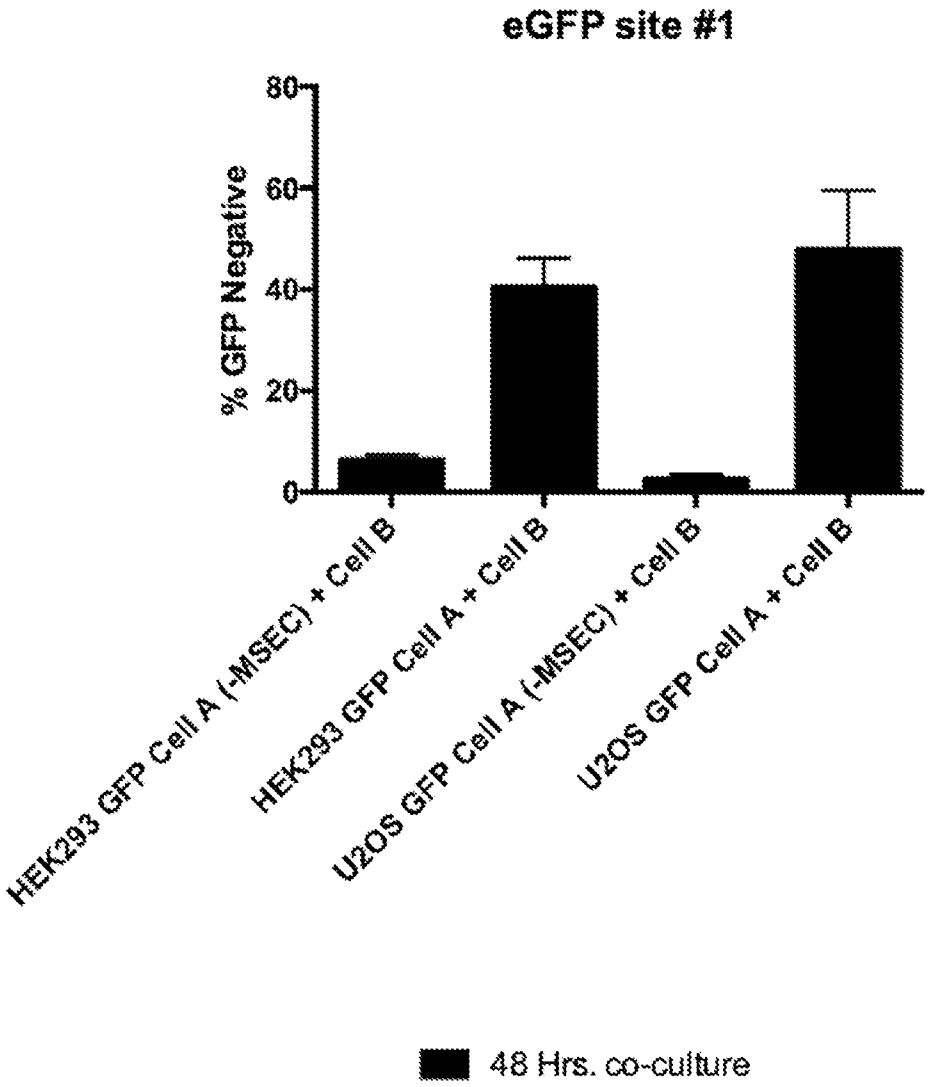

eGFP site #1
% GFP Negative
80
60
40
20
0
HEK293 GFP Cell A (-MSEC) + Cell B
HEK293 GFP Cell A + Cell B
U2OS GFP Cell A (-MSEC) + Cell B
U2OS GFP Cell A + Cell B
48 Hrs. co-culture

FIG. 3

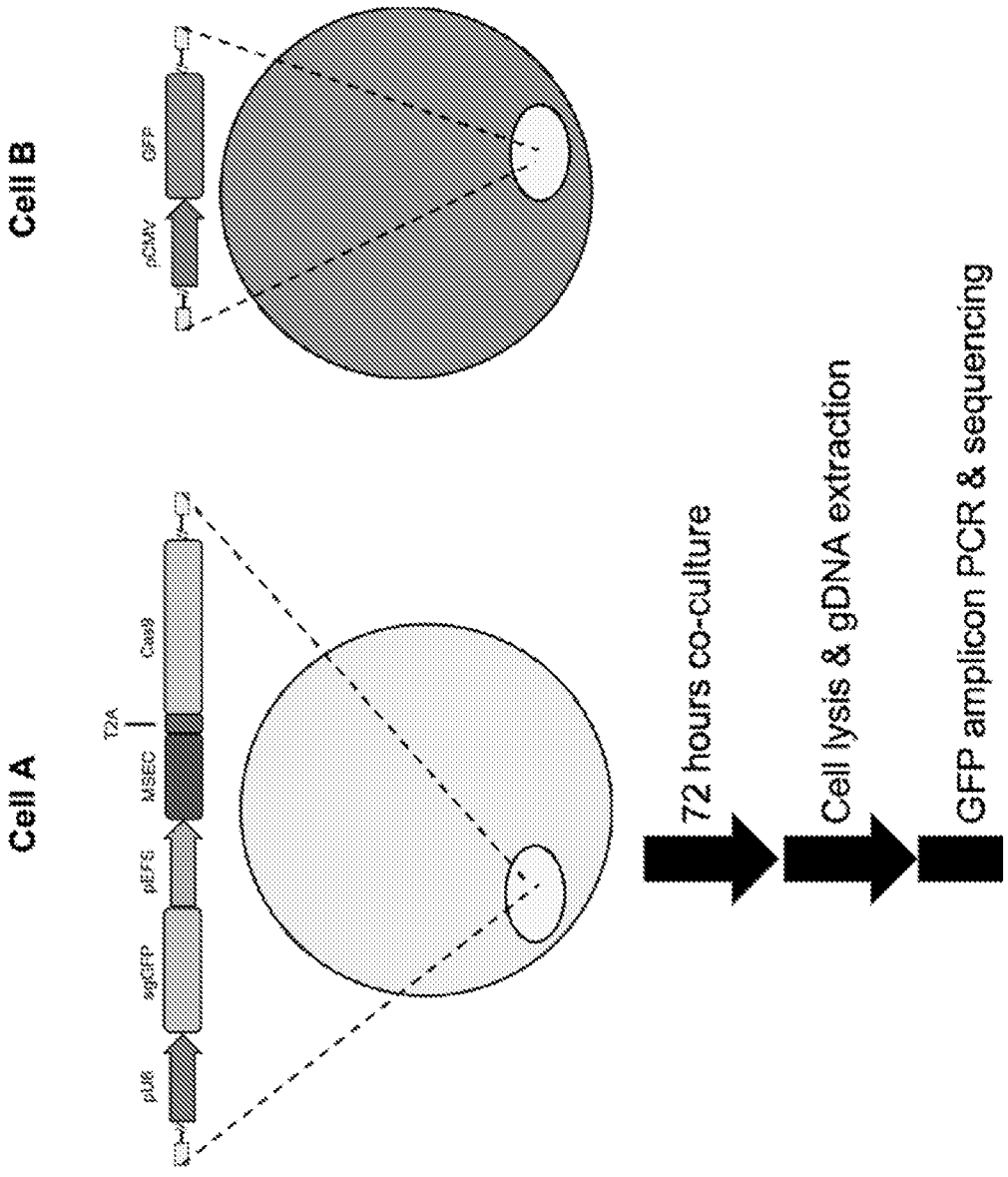

Cell B
pCMV
GFP
Cell A
pU6
sgGFP
pEFS
MSEC
T2A
Cas9
72 hours co-culture
Cell lysis & gDNA extraction
GFP amplicon PCR & sequencing

FIG. 4A

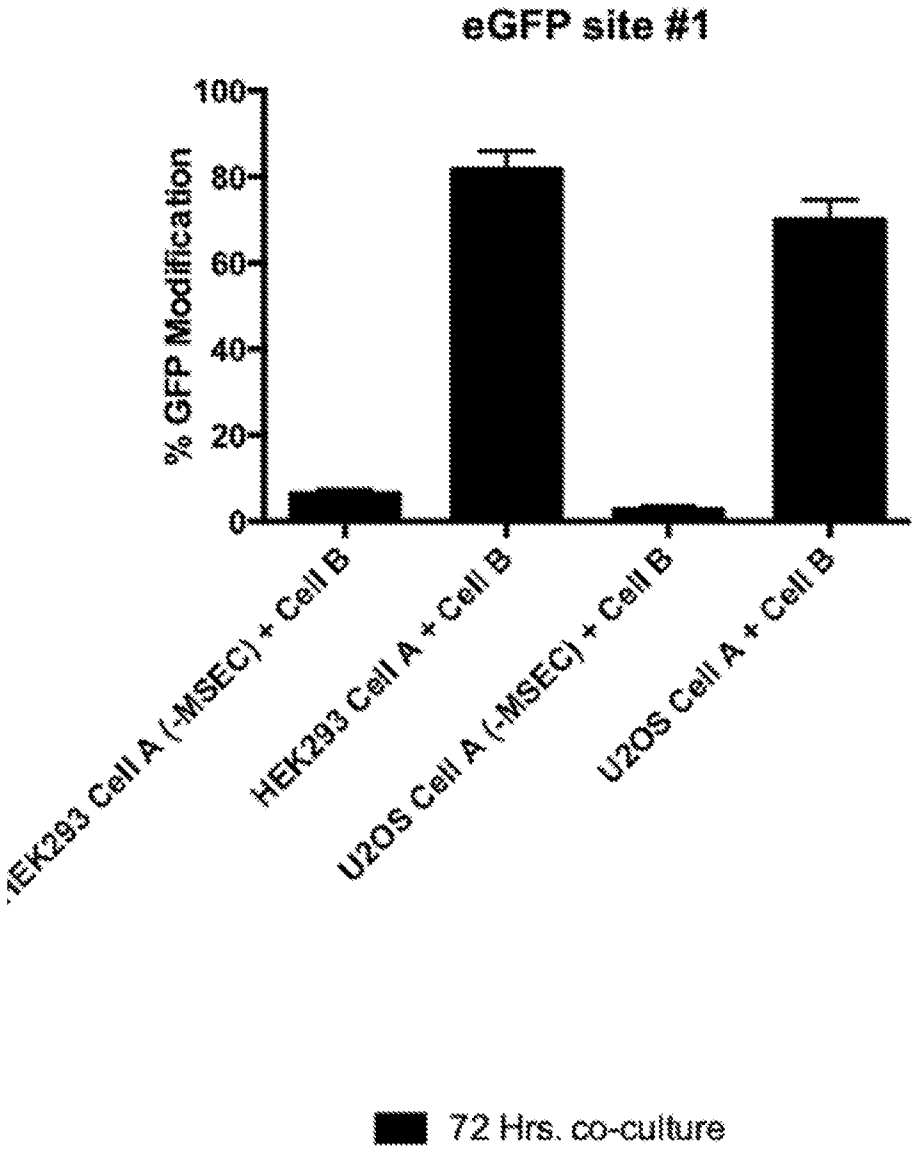

eGFP site #1
% GFP Modification
100
80
60
40
20
0
HEK293 Cell A (-MSEC) + Cell B
HEK293 Cell A + Cell B
U2OS Cell A (-MSEC) + Cell B
U2OS Cell A + Cell B
72 Hrs. co-culture

FIG. 4B