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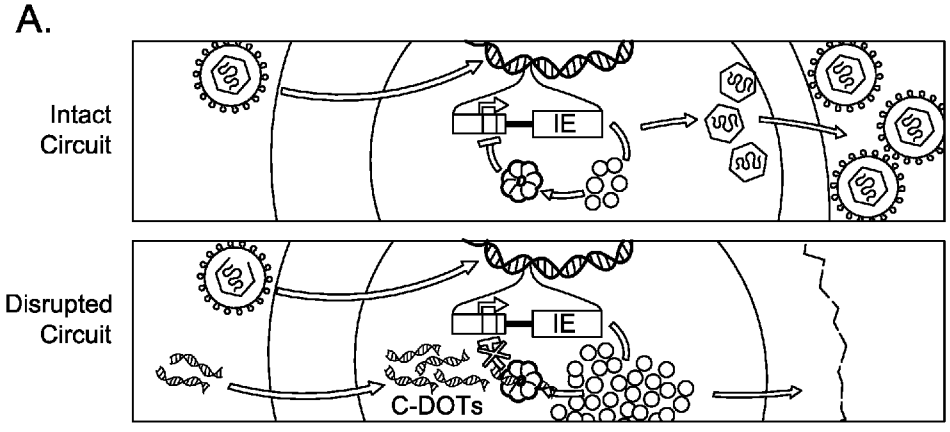

(57) Abstract: The present disclosure provides compositions and methods for inhibiting herpesvirus replication in a cell infected with herpesvirus. The composition includes a double stranded DNA molecule including a sequence of a cis regulatory sequence (crs) of a herpesvirus, wherein the crs is flanked on the 5' end by a first sequence of at least 2 base pairs and on the 3' end by a second sequence of at least 2 base pairs.

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COMPOSITIONS FOR HERPESVIRUS TRANSCRIPTIONAL FEEDBACK CIRCUIT DISRUPTION AND USES THEREOF

INCORPORATION BY REFERENCE OF SEQUENCE LISTING

[0001] A Sequence Listing is provided herewith as a text file, "GLAD-432WO Seq List_ST25" created on June 7, 2019 and having a size of 16 KB. The contents of the text file are incorporated by reference herein in their entirety.

CROSS-REFERENCE TO RELATED APPLICATIONS

[0002] This application claims priority benefit of U.S. provisional application serial no. 62/684,099, filed on June 12, 2018, which application is incorporated herein by reference in its entirety.

INTRODUCTION

[0003] Eight herpesviruses routinely infect humans: herpes simplex virus types 1 and 2, varicella-zoster virus, cytomegalovirus, Epstein-Barr virus, human herpesvirus 6 (variants A and B), human herpesvirus 7, and Kaposi's sarcoma virus or human herpesvirus 8. A simian virus, B virus, occasionally infects humans. All herpesviruses can establish latent infection within specific tissues.

[0004] Herpesviruses are divided into three groups: The α herpesviruses, herpes simplex virus types 1 and 2, and varicella-zoster virus; β herpesviruses, cytomegalovirus, and human herpesviruses 6 and 7; and γ herpesviruses, Epstein-Barr virus and human herpesvirus 8.

[0005] Cytomegalovirus (CMV) is associated with widespread morbidity and mortality. Infection with CMV is common, and it has been estimated that between 50% and 85% of people in the United States have had a CMV infection by the time they are 40 years old. Although CMV infection generally does not produce symptoms in healthy adults, high-risk groups, including immunocompromised organ transplant recipients and HIV-infected individuals, are at risk of developing CMV-associated disease. CMV is a leading cause of birth defects.

SUMMARY

[0006] The present disclosure provides a method for inhibiting herpesvirus replication in a cell infected with herpesvirus, the method including contacting the cell with a double stranded DNA molecule including a sequence of a cis regulatory sequence (crs) of a herpesvirus, wherein the crs is flanked on the 5′ end by a first sequence of at least 2 base

pairs and on the 3′ end by a second sequence of at least 2 base pairs. Compositions including a double stranded DNA molecule including a sequence of a cis regulatory sequence (crs) of a herpesvirus, wherein the crs is flanked on the 5′ end by a first sequence of at least 2 base pairs and on the 3′ end by a second sequence of at least 2 base pairs are also provided herein. The present disclosure further provides a method of treating a herpesvirus infection in an individual, the method comprising administering to the individual an effective amount of a double stranded DNA molecule including a sequence of a cis regulatory sequence (crs) of a herpesvirus, wherein the crs is flanked on the 5′ end by a first sequence of at least 2 base pairs and on the 3′ end by a second sequence of at least 2 base pairs. In some cases, the crs is flanked on the 5′ end by a first sequence of at least 3, 4, 5, 6, or 7 base pairs and on the 3′ end by a second sequence of at least 3, 4, 5, 6, or 7 base pairs. In certain aspects, the first sequence and the second sequence have the same nucleotide sequence. In certain aspects, the first sequence and the second sequence have different nucleotide sequences. In certain aspects, the first sequence and the second sequence have the length. In certain aspects, the first sequence and the second sequence have different lengths. In certain aspects, the nucleotide sequences of the first sequence and/or the second sequence are heterologous to the crs.

[0007] The present disclosure also provides a method for inhibiting replication of a herpesvirus in a cell infected with a herpesvirus, the method including contacting the cell with a double stranded DNA molecule including a sequence of a cis regulatory sequence (crs) of the herpesvirus, wherein the crs is flanked on the 5′ end by a first sequence of at least 2 base pairs and on the 3′ end by a second sequence of at least 2 base pairs. In some cases, the crs is flanked on the 5′ end by a first sequence of at least 7 base pairs and on the 3′ end by a second sequence of at least 7 base pairs. In some cases, one or both of the first sequence of at least 2 base pairs and the second sequence of at least 2 base pairs does not have a DNA sequence of a naturally occurring herpesvirus. In some cases, the herpesvirus is selected from cytomegalovirus (CMV), herpes simplex virus-1 (HSV-1), herpes simplex virus-2 (HSV-2), varicella zoster virus, Epstein-Barr virus (EBV), and Kaposi's sarcoma-associated herpesvirus (KSHV).

[0008] The present disclosure provides a method of treating a herpesvirus infection in an individual, the method comprising administering to the individual an effective amount of a double stranded DNA molecule comprising a sequence comprising a sequence of a cis regulatory sequence (crs) of the herpesvirus, wherein the crs is flanked on the 5′ end by a first sequence of at least 2 base pairs and on the 3′ end by a second sequence of at least 2

base pairs. In some cases, the herpesvirus is CMV. In some cases, the herpesvirus is HSV-1. In some cases, the individual is a human. In some cases, the method includes administering an effective amount of at least two double stranded DNA molecules, each comprising a sequence of a cis regulatory sequence (crs) of a different herpesvirus, wherein the crs is flanked on the 5′ end by a first sequence of at least 2 base pairs and on the 3′ end by a second sequence of at least 2 base pairs. In certain aspects, the first sequence flanking the first DNA molecule and the first sequence flanking the second DNA molecule have the same sequence. In certain aspects, the first sequence flanking the first DNA molecule and the first sequence flanking the second DNA molecule have different sequences. In certain aspects, the second sequence flanking the first DNA molecule and the second sequence flanking the second DNA molecule have the same sequence. In certain aspects, the second sequence flanking the first DNA molecule and the second sequence flanking the second DNA molecule have different sequences.

[0009] In some cases, the method of treating a herpesvirus infection includes administering at least a second therapeutic agent to the individual. In some cases, the second therapeutic agent is ganciclovir, foscarnet, cidofovir, maribavir, or valganciclovir. In some cases, the second therapeutic agent is an HDAC inhibitor.

[0010] In some cases, the individual receiving a treatment for herpesvirus infection is an organ transplant recipient. In some cases, the individual is a bone marrow transplant recipient. In some cases, the individual does not have a herpesvirus infection, and is a prospective organ transplant recipient. In some cases, the individual does not have a herpesvirus infection, and is a prospective bone marrow transplant recipient. In some cases, the individual is a pregnant female. In some cases, the individual is a neonate.

[0011] The present disclosure provides a method of inhibiting herpesvirus replication in an organ or tissue, the method comprising contacting the organ or tissue *in vitro* or *ex vivo* with a double stranded DNA molecule including a sequence including a sequence of a cis regulatory sequence (crs) of the herpesvirus, wherein the crs is flanked on the 5′ end by a first sequence of at least 2 base pairs and on the 3′ end by a second sequence of at least 2 base pairs, as provided herein. In some cases, the herpesvirus is CMV. In some cases, the herpesvirus is HSV-1. In some cases, the organ or tissue is contacted *in vitro* or *ex vivo* with the double stranded DNA molecule including a sequence including a sequence of a cis regulatory sequence (crs) of the herpesvirus for a period of time of from about 15 minutes to about 48 hours, or more than 48 hours, wherein the crs is flanked on the 5′ end by a first sequence of at least 2 base pairs and on the 3′ end by a second sequence of at least 2 base

pairs. In some cases, the organ or tissue is contacted *in vitro* or *ex vivo* with the double stranded DNA molecule in a liquid medium.

[0012] The present disclosure provides a method of reducing the likelihood that a transplant recipient will become infected with herpesvirus from a donor organ or tissue, the method comprising: a) contacting the organ or tissue *in vitro* or *ex vivo* with a double stranded DNA molecule including a sequence including a sequence of a cis regulatory sequence (crs) of the herpesvirus, wherein the crs is flanked on the 5′ end by a first sequence of at least 2 base pairs and on the 3′ end by a second sequence of at least 2 base pairs, thereby producing a double stranded DNA molecule-treated organ or tissue; and b) transplanting the double stranded DNA molecule-treated organ or tissue into the transplant recipient. In some cases, the herpesvirus is CMV. In some cases, the herpesvirus is HSV-1.

[0013] The present disclosure provides a composition, the composition including a double stranded DNA molecule comprising a sequence of a cis regulatory sequence (crs) of the herpesvirus, wherein the crs is flanked on the 5′ end by a first sequence of at least 2 base pairs and on the 3′ end by a second sequence of at least 2 base pairs, as provided herein. In some cases, the crs is flanked on the 5′ end by a first sequence of at least 7 base pairs and on the 3′ end by a second sequence of at least 7 base pairs. In some cases, one or both of the first sequence of at least 2 base pairs and the second sequence of at least 2 base pairs does not have a DNA sequence of a naturally occurring herpesvirus. In some cases, the herpesvirus is selected from cytomegalovirus (CMV), herpes simplex virus-1 (HSV-1), herpes simplex virus-2 (HSV-2), varicella zoster virus, Epstein-Barr virus (EBV), and Kaposi's sarcoma-associated herpesvirus (KSHV).

[0014] In certain aspects, the DNA molecule may include a modification(s) that increases the half-life, e.g., in vivo half-life of the molecule. In certain aspects, the DNA molecule may include a modified backbone. In certain aspects, the DNA molecule may include a modified backbone comprising internal phosphorothioate bonds. In certain aspects, the DNA molecule may include a backbone comprising a plurality phosphorothioate bonds, such as, at least 2, 3, 4, 5, 6, 7, and up to 14 phosphorothioate bonds. . In certain aspects, the phosphorothioate bonds may be distributed evenly across the DNA molecule.

BRIEF DESCRIPTION OF THE DRAWINGS

[0015] FIG. 1 depicts congenital infection, CMV retinitis, and infection in solid organ transplant as a result of CMV infection. FIG. 1 also provides a schematic depicting the

sequence of viral gene expression in CMV and a schematic depicting functional regions of the CMV Immediate Early (IE2) protein.

[0016] FIG. 2 provides a schematic depicting human cytomegalovirus (HCMV) transcriptional feedback circuits and their potential as escape-resistant drug targets (a); a graph showing an IE2-Oligo competition model (b); and calculations for the 50% emergence time of resistance mutants as a function of the mutation rate μ (c).

[0017] FIG. 3A provides graphs illustrating IE2 oligomerization by a double stranded oligonucleotide (ONT) having the sequence GACAGATCGTTTAGTGAACCGTACACGA (SEQ ID NO: 1).

[0018] FIG. 3B provides an image showing that IE2 oligomerizes as a ring around a double stranded oligonucleotide (ONT) having the sequence GACAGATCGTTTAGTGAACCGTACACGA (SEQ ID NO: 1).

[0019] FIG. 4A provides a schematic illustrating a minimal regulatory circuit of IE2.

[0020] FIG. 4B provides a graph illustrating that a double stranded oligonucleotide (ONT) having the sequence GACAGATCGTTTAGTGAACCGTACACGA(SEQ ID NO: 1) breaks negative feedback in an IE2 minimal circuit.

[0021] FIG. 4C provides a graph illustrating that a double stranded oligonucleotide (ONT) having the sequence GACAGATCGTTTAGTGAACCGTACACGA (SEQ ID NO: 1) breaks IE2 negative feedback leading to cytotoxicity in a dose dependent manner.

[0022] FIG. 5 provides graphs illustrating that transcription regulatory circuit disruptors interfere with Herpesvirus productive infection in cell culture. The ONT including the sequence of the HCMV crs has the sequence GACAGATCGTTTAGTGAACCGTACACGA (SEQ ID NO: 1). The ONT including the sequence of the Mouse CMV (MCMV) crs has the sequence GACAGACCAGCGTCGGTACCGTACACGA (SEQ ID NO: 2). The ONT including the sequence of the HSV-1 crs has the sequence CCGAGGACGCCCCGATCGTCCACACGGAG (SEQ ID NO: 3).

[0023] FIG. 6A. Feedback disruption inhibits viral replication in an *in vivo* model. Schematic of the HSV-1 corneal infection model in mice (a). I images showing that an ICP-4 disruptor, a double stranded oligonucleotide (ONT) having the sequence CCGAGGACGCCCCGATCGTCCACACGGAG (SEQ ID NO: 3), interferes with HSV1 replication in cornea. Bottom rows show magnified versions of the identified areas of the images in the row above (b). Quantification of HSV-1 YFP expressing cells in corneas, as determined from the YFP:DAPI ratio (c). HSV-1 viral titers from HSV-1 infected corneas 2

days after treatment with either 25μM PBS, scrambled dsDNA sequence (C-DOT^{Scram})or C-DOT for HSV-1 (C-DOT^{H}) (d). HSV-1 viral genomic DNA quantification by qPCR 2 days after treatment (E).

[0024] FIG. 6B provides a graph showing that a transcription regulatory circuit disruptor, a double stranded oligonucleotide (ONT) having the sequence CCGAGGACGCCCCGATCGTCCACACGGAG (SEQ ID NO: 3), interferes with Herpesvirus productive infection in mice.

[0025] FIG. 7. Feedback circuit disruption interferes with viral replication even at high MOI and limits the evolution of resistance. (a) Flow cytometry of ARPE-19 cells nucleofected with the 28bp dsDNA that titrates IE86 (C-DOT^{C}) or scrambled dsDNA sequence (C-DOT^{Scram}) and infected with a clinically derived CMV (TB40E) encoding an IE86-YFP (MOI = 0.1) then analyzed at 2 days post infection (dpi). (b) **F**low cytometry of ARPE-19 cells nucleofected with a 29bp DNA to titrate IE175 (C-DOT^{H}) or scrambled dsDNA sequence (C-DOT^{Scram}) and infected with HSV-1 (17syn+ strain) encoding an IE175-YFP (MOI = 0.1) then analyzed at 2 dpi. (c) Single-round viral titering of CMV in the presence of 100μM GCV, PBS, or 25μM C-DOT^{C} (or C-DOT^{Scram}) at 4-days post infection under different initial MOIs. (d) Single-round viral titering of HSV-1 in presence of 100μM ACV or 25μM C-DOT^{H} (or C-DOT^{Scram}) at 4-days post infection under different HSV-1 MOIs. (e) Schematic of the continuous-culture experiment; ARPE-19 cells (+/- C-DOT) were infected with CMV or HSV-1 (0.1 Multiplicity of Infection "MOI") and at 4-day post infection, supernatant was collected and was used to infect naïve ARPE-19 cells +/- C-DOT until day 60. (f) Graph illustrating that disrupting viral transcriptional regulatory circuitry using a double stranded oligonucleotide (ONT) having the sequence GACAGATCGTTTAGTGAACCGTACACGA (SEQ ID NO: 1) constitutes an escape resistant therapeutic strategy in Herpesviruses (HCMV). (g) **G**raph illustrating that disrupting viral transcriptional regulatory circuitry using a double stranded oligonucleotide (ONT) having the sequence CCGAGGACGCCCCGATCGTCCACACGGAG (SEQ ID NO: 3) constitutes an escape resistant therapeutic strategy in Herpesviruses (HSV-1).

[0026] FIG. 8. Schematic of the IE86-protein binding assay (a). FPLC chromatography profiles of IE86 protein fragment input and dsDNA input prior to co-incubation (b). Chromatographs of IE86 fragment incubated with either a sequence-scrambled control dsDNA oligonucleotides or crs-containing dsDNA oligonucleotides of differential lengths (c). Schematic of the minimal IE negative-feedback circuit (MIEP-IE86-IRES-GFP) encoded within the feedback-reporter cell line (left) and flow cytometry of feedback reporter cells 48

h after nucleofection with either a 28bp crs-containing DNA oligonucleotide, a scrambled DNA oligonucleotide (negative control), or mock nucleofection (no DNA oligonucleotide) showing that crs-encoding DNA oligonucleotides disrupt feedback and act as a putative C-DOTs (right) (d). (e)-(f) The 28bp crs-containing DNA oligonucleotide optimally disrupts feedback and induces cytotoxicity in the feedback-reporter cell line

[0027] FIG. 9 provides a dose-response analysis in a 96-well plate format. The IC50 is < 1nM for both CMV and HSV-1 C-DOTs.

[0028] FIG. 10 provides a cell-death pathway analysis (pharmacological inhibitors). The mechanism of C-DOT-induced cell death (in the context of the virus infection) was identified using three separate approaches: (i) RNAseq: to identify misregulated expression patterns and the cell-death pathways these group into (ii) Antibody staining: for specific cell-death pathway markers (iii) Pharmacological inhibitors: of cell-death pathways to test which mitigate C-DOT-induced death. These approaches consistently show that CDOT-induced IE86 and IE175 overexpression, respectively, and cause cytotoxicity through distinct cell-death pathways: CDOT-induced IE86 overexpression causes cell death by apoptosis, whereas CDOT-induced IE175 overexpression causes cell death by ferroptosis (a related regulated death pathway). C-DOT alone caused cell death in CMV and HSV-1. Scramble C-DOT caused minimal cell death in CMV and HSV-1.

[0029] FIG. 11 shows simulations and in-vitro analyses indicating that excess crs DNA oligos competitively bind IE86 and would break negative feedback to increase IE86 levels.

[0030] FIG. 12 shows C-DOTs break IE negative feedback in a dose-dependent manner and do not alter cell permissiveness to viral infection.

[0031] FIG. 13 shows C-DOT nanoparticles enhance cell delivery and C-DOT concatemers enhance IE86 sequestration and feedback disruption.

[0032] FIG. 14 shows C-DOTs can be engineered to interfere with a broad range of species-specific herpesviruses including drug-resistant strains.

[0033] FIG. 15 shows C-DOTs do not activate innate immune responses in cell lines and—unlike fomivirsen and acyclovir—do not select for resistant viral mutants.

[0034] FIG. 16 shows C-DOTs can diffuse into naïve mouse corneal cells and increase IE175 expression in mouse corneas following HSV-1 infection.

[0035] FIG. 17 shows nanoparticle C-DOTs efficiently break transcriptional negative feedback and interfere with virus replication and C-DOTs efficiently inhibit virus replication in a mixed infection setting.

DEFINITIONS

[0036] The terms “polynucleotide” and “nucleic acid,” used interchangeably herein, refer to a polymeric form of nucleotides of any length, either ribonucleotides or deoxyribonucleotides. Thus, this term includes, but is not limited to, single-, double-, or multi-stranded DNA or RNA, genomic DNA, cDNA, DNA-RNA hybrids, or a polymer comprising purine and pyrimidine bases or other natural, chemically or biochemically modified, non-natural, or derivatized nucleotide bases.

[0037] The terms "peptide," "polypeptide," and "protein" are used interchangeably herein, and refer to a polymeric form of amino acids of any length, which can include coded and non-coded amino acids, chemically or biochemically modified or derivatized amino acids, and polypeptides having modified peptide backbones.

[0038] The term “heterologous” refers to two components that are defined by structures derived from different sources. For example, in the context of a polypeptide, a “heterologous” polypeptide may include operably linked amino acid sequences that are derived from different polypeptides (e.g., a first component comprising a recombinant polypeptide and a second component derived from a native GDF15 polypeptide). Similarly, in the context of a polynucleotide encoding a chimeric polypeptide, a “heterologous” polynucleotide may include nucleic acid sequences that includes a first component derived from a first source, e.g., a virus and a second component that has a sequence not present next to the first component in the virus. Other exemplary “heterologous” nucleic acids include expression constructs in which a nucleic acid comprising a coding sequence is operably linked to a regulatory element (e.g., a promoter) that is from a genetic origin different from that of the coding sequence (e.g., to provide for expression in a host cell of interest, which may be of different genetic origin than the promoter, the coding sequence or both). For example, a T7 promoter operably linked to a polynucleotide encoding a GDF15 polypeptide or domain thereof is said to be a heterologous nucleic acid. In the context of recombinant cells, “heterologous” can refer to the presence of a nucleic acid (or gene product, such as a polypeptide) that is of a different genetic origin than the host cell in which it is present. In the context of the DNA molecules disclosed herein the flanking first and second sequences be heterologous to the crs.

[0039] A cell has been “genetically modified” or "transformed" or "transfected" by exogenous DNA, e.g. a recombinant expression vector, when such DNA has been introduced inside the cell. The presence of the exogenous DNA results in permanent or transient genetic change. The transforming DNA may or may not be integrated (covalently linked) into the

genome of the cell. In eukaryotic cells, a stably transformed cell is one in which the transforming DNA has become integrated into a chromosome so that it is inherited by daughter cells through chromosome replication. This stability is demonstrated by the ability of the eukaryotic cell to establish cell lines or clones that comprise a population of daughter cells containing the transforming DNA. A "clone" is a population of cells derived from a single cell or common ancestor by mitosis. A "cell line" is a clone of a primary cell that is capable of stable growth in vitro for many generations.

[0040] The term “herpesvirus” is well understood in the art, and refers to any member of the family Herpesviridae. Herpesviruses include, e.g., cytomegalovirus (CMV), herpes simplex virus-1 (HSV-1), herpes simplex virus-2 (HSV-2), varicella zoster virus, Epstein-Barr virus (EBV), and Kaposi’s sarcoma-associated herpesvirus (KSHV; also known as human herpesvirus-8 or HHV-8).

[0041] The term "cytomegalovirus," also known as CMV, refers to a member of the herpesvirus family in any species, including human. CMV is also referred to as a Betaherpesviridae. CMV is a herpesvirus that infects mononuclear cells and lymphocytes.

[0042] The term "human cytomegalovirus, or HCMV" indicates a member of the CMV family that infects humans. HCMV is a beta human herpesvirus with a genome size of 230 Kbp, coding more than 70 viral proteins. HCMV is also designated as human herpesvirus 5 (HHV-5). Mouse CMV (mCMV) indicates a member of the CMV family that infects mice. Rhesus monkey CMV (rhCMV) indicates a member of the CMV family that infects rhesus monkeys.

[0043] The terms “individual,” “subject,” “host,” and “patient,” used interchangeably herein, refer to a mammal, including, but not limited to, murines (rats, mice), non-human primates, humans, canines, felines, ungulates (e.g., equines, bovines, ovines, porcines, caprines), etc. In some cases, the individual is a human. In some cases, the individual is a non-human primate.

[0044] As used herein, the terms "treatment," "treating," and the like, refer to obtaining a desired pharmacologic and/or physiologic effect. The effect may be prophylactic in terms of completely or partially preventing a disease or symptom thereof and/or may be therapeutic in terms of a partial or complete cure for a disease and/or adverse effect attributable to the disease. "Treatment," as used herein, covers any treatment of a disease in a mammal, particularly in a human, and includes: (a) preventing the disease from occurring in a subject which may be predisposed to the disease but has not yet been diagnosed as having it; (b)

inhibiting the disease, i.e., arresting its development; and (c) relieving the disease, i.e., causing regression of the disease.

[0045] A “therapeutically effective amount” or “efficacious amount” refers to the amount of a compound that, when administered to a mammal or other subject for treating a disease, is sufficient to effect such treatment for the disease. The “therapeutically effective amount” will vary depending on the compound or the cell, the disease and its severity and the age, weight, etc., of the subject to be treated.

[0046] The terms "co-administration" and "in combination with" include the administration of two or more therapeutic agents either simultaneously, concurrently or sequentially within no specific time limits. In one embodiment, the agents are present in the cell or in the subject's body at the same time or exert their biological or therapeutic effect at the same time. In one embodiment, the therapeutic agents are in the same composition or unit dosage form. In other embodiments, the therapeutic agents are in separate compositions or unit dosage forms. In certain embodiments, a first agent can be administered prior to (e.g., minutes, 15 minutes, 30 minutes, 45 minutes, 1 hour, 2 hours, 4 hours, 6 hours, 12 hours, 24 hours, 48 hours, 72 hours, 96 hours, 1 week, 2 weeks, 3 weeks, 4 weeks, 5 weeks, 6 weeks, 8 weeks, or 12 weeks before), concomitantly with, or subsequent to (e.g., 5 minutes, 15 minutes, 30 minutes, 45 minutes, 1 hour, 2 hours, 4 hours, 6 hours, 12 hours, 24 hours, 48 hours, 72 hours, 96 hours, 1 week, 2 weeks, 3 weeks, 4 weeks, 5 weeks, 6 weeks, 8 weeks, or 12 weeks after) the administration of a second therapeutic agent.

[0047] As used herein, a "pharmaceutical composition" is meant to encompass a composition suitable for administration to a subject, such as a mammal, especially a human. In general a “pharmaceutical composition” is sterile, and is free of contaminants that are capable of eliciting an undesirable response within the subject (e.g., the compound(s) in the pharmaceutical composition is pharmaceutical grade). Pharmaceutical compositions can be designed for administration to subjects or patients in need thereof via a number of different routes of administration including oral, buccal, rectal, parenteral, intraperitoneal, intradermal, intratracheal and the like. In some embodiments the composition is suitable for administration by a transdermal route, using a penetration enhancer other than dimethylsulfoxide (DMSO). In other embodiments, the pharmaceutical compositions are suitable for administration by a route other than transdermal administration. A pharmaceutical composition will in some embodiments include a subject compound and a pharmaceutically acceptable excipient. In some embodiments, a pharmaceutically acceptable excipient is other than DMSO.

[0048] Before the present invention is further described, it is to be understood that this invention is not limited to particular embodiments described, as such may, of course, vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only, and is not intended to be limiting, since the scope of the present invention will be limited only by the appended claims.

[0049] Where a range of values is provided, it is understood that each intervening value, to the tenth of the unit of the lower limit unless the context clearly dictates otherwise, between the upper and lower limit of that range and any other stated or intervening value in that stated range, is encompassed within the invention. The upper and lower limits of these smaller ranges may independently be included in the smaller ranges, and are also encompassed within the invention, subject to any specifically excluded limit in the stated range. Where the stated range includes one or both of the limits, ranges excluding either or both of those included limits are also included in the invention.

[0050] Unless defined otherwise, 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. Although any methods and materials similar or equivalent to those described herein can also be used in the practice or testing of the present invention, the preferred methods and materials are now described. All publications mentioned herein are incorporated herein by reference to disclose and describe the methods and/or materials in connection with which the publications are cited.

[0051] The disclosures of U.S. Patent Application Publication Nos. US 2017-0360778 A1 and US 2016-0002668 A1 are incorporated by reference herein in their entirety.

[0052] It must be noted that as used herein and in the appended claims, the singular forms "a," "an," and "the" include plural referents unless the context clearly dictates otherwise. Thus, for example, reference to "a double stranded DNA molecule" includes a plurality of such compounds and reference to "the composition" includes reference to one or more compositions and equivalents thereof known to those skilled in the art, and so forth. It is further noted that the claims may be drafted to exclude any optional element. As such, this statement is intended to serve as antecedent basis for use of such exclusive terminology as "solely," "only" and the like in connection with the recitation of claim elements, or use of a "negative" limitation.

[0053] It is appreciated that certain features of the invention, which are, for clarity, described in the context of separate embodiments, may also be provided in combination in a single

embodiment. Conversely, various features of the invention, which are, for brevity, described in the context of a single embodiment, may also be provided separately or in any suitable sub-combination. All combinations of the embodiments pertaining to the invention are specifically embraced by the present invention and are disclosed herein just as if each and every combination was individually and explicitly disclosed. In addition, all sub-combinations of the various embodiments and elements thereof are also specifically embraced by the present invention and are disclosed herein just as if each and every such sub-combination was individually and explicitly disclosed herein.

[0054] The publications discussed herein are provided solely for their disclosure prior to the filing date of the present application. Nothing herein is to be construed as an admission that the present invention is not entitled to antedate such publication. Further, the dates of publication provided may be different from the actual publication dates which may need to be independently confirmed.

DETAILED DESCRIPTION

[0055] The present disclosure provides a method for inhibiting replication of a herpesvirus in a cell infected with a herpesvirus, the method including contacting the cell with a double stranded DNA molecule including a sequence including a sequence of a cis regulatory sequence (crs) of the herpesvirus, wherein the crs is flanked on the 5′ end by a first sequence of at least 2 base pairs and on the 3′ end by a second sequence of at least 2 base pairs. Compositions including a double stranded DNA molecule including a sequence including a sequence of a cis regulatory sequence (crs) of a herpesvirus, wherein the crs is flanked on the 5′ end by a first sequence of at least 2 base pairs and on the 3′ end by a second sequence of at least 2 base pairs, are also provided herein. The present disclosure further provides a method of treating a herpesvirus infection in an individual, the method comprising administering to the individual an effective amount of a double stranded DNA molecule including a sequence including a sequence of a cis regulatory sequence (crs) of a herpesvirus, wherein the crs is flanked on the 5′ end by a first sequence of at least 2 base pairs and on the 3′ end by a second sequence of at least 2 base pairs.

[0056] In some cases, one or both of the first sequence of at least 2 base pairs and the second sequence of at least 2 base pairs does not have a DNA sequence of a naturally occurring herpesvirus. In some cases, the herpesvirus is selected from cytomegalovirus (CMV), herpes simplex virus-1 (HSV-1), herpes simplex virus-2 (HSV-2), varicella zoster virus, Epstein-Barr virus (EBV), and Kaposi's sarcoma-associated herpesvirus (KSHV).

[0057] In some cases, a double stranded DNA molecule including a sequence of a cis regulatory sequence (crs) of the herpesvirus as described herein inhibits herpesvirus replication by at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, or at least 90%, compared to the level of herpesvirus replication in a herpesvirus-infected cell in the absence of the double stranded DNA molecule. In some cases, a double stranded DNA molecule including a sequence of a cis regulatory sequence (crs) of the herpesvirus as described herein inhibits CMV replication by at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, or at least 90%, compared to the level of CMV replication in a CMV-infected cell in the absence of the double stranded DNA molecule. In some cases, a double stranded DNA molecule including a sequence of a cis regulatory sequence (crs) of the herpesvirus as described herein inhibits HSV-1 replication by at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, or at least 90%, compared to the level of HSV-1 replication in a HSV-1-infected cell in the absence of the double stranded DNA molecule.

[0058] In some cases, an effective amount of a double stranded DNA molecule as described herein is an amount that, when administered to an individual in need thereof in monotherapy or combination therapy in one or more administrations, is effective to reduce herpesvirus viral load (e.g., CMV and/or HSV-1) in the individual by at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, or at least 90%, compared to the viral load in the individual before treatment with the double stranded DNA molecule, or in the absence of treatment with the double stranded DNA molecule.

[0059] In some cases, an effective amount of a double stranded DNA molecule as described herein is an amount that, when administered to an individual in need thereof in monotherapy or combination therapy in one or more administrations, is effective to reduce the number of herpesvirus (e.g., CMV and/or HSV-1) genome copies in the individual by at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, or at least 90%, compared to the number of herpesvirus genome copies in the individual before treatment with the double stranded DNA molecule, or in the absence of treatment with the double stranded DNA molecule.

[0060] In some cases, an effective amount of a double stranded DNA molecule as described herein is an amount that, when administered to an individual in need thereof in monotherapy or combination therapy in one or more administrations, is effective to kill cells infected with herpesvirus (e.g., CMV and/or HSV-1). For example, in some cases, an effective amount of

a double stranded DNA molecule as described herein is an amount that, when administered to an individual in need thereof in monotherapy or combination therapy in one or more doses, is effective to kill at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, or more than 70%, of the herpesvirus-infected (e.g., CMV and/or HSV-1) cells in the individual.

[0061] In some cases, a double stranded DNA molecule as described herein is administered prior to exposure of the individual to herpesvirus. In some cases, a double stranded DNA molecule is administered after exposure of the individual to herpesvirus. In some cases, a double stranded DNA molecule is administered to an individual who has been diagnosed as having a herpesvirus infection.

[0062] In some cases, a double stranded DNA molecule as described herein is administered as monotherapy. In some cases, a double stranded DNA molecule is administered in combination therapy with one or more additional therapeutic agents.

[0063] In some cases, a double stranded DNA molecule as described herein is administered to an individual prior to exposure of the individual to CMV. In some cases, a double stranded DNA molecule as described herein is administered to an individual after exposure of the individual to CMV. In some cases, a double stranded DNA molecule as described herein is administered to an individual who has been diagnosed as having a CMV infection.

[0064] In some cases, a double stranded DNA molecule as described herein is administered to an individual prior to exposure of the individual to HSV-1. In some cases, a double stranded DNA molecule as described herein is administered to an individual after exposure of the individual to HSV-1. In some cases, a double stranded DNA molecule as described herein is administered to an individual who has been diagnosed as having a HSV-1 infection.

HERPESVIRUS

[0065] The present disclosure provides a composition comprising a double stranded DNA molecule comprising a sequence comprising a sequence of a cis regulatory sequence (crs) of the herpesvirus, wherein the crs is flanked on the 5′ end by a first sequence of at least 2 base pairs and on the 3′ end by a second sequence of at least 2 base pairs. The flanking sequences can be any suitable length (e.g., 2-7, 2-10, 3-7, 3-10, 4-7, 4-10, 5-7, or 5-10 base pairs or more), limited only by delivery and/or packaging constraints for the DNA. In some cases, the flanking sequences are 7 base pairs or more in length, e.g., 7-10, 10-20, 20-30, 30-40, 40-50, etc. In some cases, one or both of the flanking sequences have a DNA sequence of a naturally occurring herpesvirus, e.g., the DNA sequences present upstream and downstream

of the crs. In some cases, one or both of the flanking sequences does not have a DNA sequence of a naturally occurring herpesvirus. In some cases, the herpesvirus is selected from cytomegalovirus (CMV), herpes simplex virus-1 (HSV-1), herpes simplex virus-2 (HSV-2), varicella zoster virus, Epstein-Barr virus (EBV), and Kaposi's sarcoma-associated herpesvirus (KSHV). In some cases, one or both of the flanking sequences have an artificial sequence. Any artificial sequence may be selected, e.g., a random sequence of one or more of the bases G, A, T, C may be selected.

[0066] In some embodiments, the herpesvirus is CMV. In some cases, the herpesvirus is HCMV and the sequence of the herpesvirus crs comprises CGTTTAGTGAACCG (SEQ ID NO: 4). In some cases, the sequence of the herpesvirus crs has at least 90% (e.g., 95%, 98%, 99% or 100%) sequence identity to a sequence of the crs of HCMV (e.g., CGTTTAGTGAACCG (SEQ ID NO: 4)). In some cases, the sequence of the herpesvirus crs flanked on the 5′ end by a first sequence of at least 7 base pairs and on the 3′ end by a second sequence of at least 7 base pairs comprises GACAGATCGTTTAGTGAACCGTACACGA (SEQ ID NO: 1). In some cases, the sequence of the herpesvirus crs flanked on the 5′ end by a first sequence of at least 7 base pairs and on the 3′ end by a second sequence of at least 7 base pairs comprises G*ACAGATCGTT*TAGTGAACC*GTACACG*A (SEQ ID NO: 5), wherein * is a phosphorothioated bond.

[0067] In some cases, the herpesvirus is Rhesus CMV (RhCMV). In some cases, the sequence of the herpesvirus crs comprises CGTTTAGGGAACCG (SEQ ID NO: 6). In some cases, the sequence of the herpesvirus crs has at least 90% (e.g., 95%, 98%, 99% or 100%) sequence identity to a sequence of the crs of RhCMV (e.g., CGTTTAGGGAACCG (SEQ ID NO: 6)). In some cases, the sequence of the herpesvirus crs flanked on the 5′ end by a first sequence of at least 7 base pairs and on the 3′ end by a second sequence of at least 7 base pairs comprises GACAGATCGTTTAGGGAACCGTACACGA (SEQ ID NO: 7). In some cases, the sequence of the herpesvirus crs flanked on the 5′ end by a first sequence of at least 7 base pairs and on the 3′ end by a second sequence of at least 7 base pairs comprises G*ACAGATCGT*TTAGGGAAC*CGTACACG*A (SEQ ID NO: 8), wherein * is a phosphorothioated bond.

[0068] In some cases, the herpesvirus is Mouse CMV (MCMV). In some cases, the sequence of the herpesvirus crs comprises CAGCGTCGGTACCG (SEQ ID NO: 9). In some cases, the sequence of the crs has at least 90% (e.g., 95%, 98%, 99% or 100%) sequence identity to a sequence of the crs of MCMV (e.g., CAGCGTCGGTACCG (SEQ ID NO: 9)). In some cases, the sequence of the herpesvirus crs flanked on the 5′ end by a first sequence

of at least 7 base pairs and on the 3′ end by a second sequence of at least 7 base pairs comprises GACAGACCAGCGTCGGTACCGTACACGA (SEQ ID NO: 2). In some cases, the sequence of the herpesvirus crs flanked on the 5′ end by a first sequence of at least 7 base pairs and on the 3′ end by a second sequence of at least 7 base pairs comprises G*ACAGACCA*GCGTCGG*TACCGTACACG*A (SEQ ID NO: 9), wherein * is a phosphorothioated bond.

[0069] In some other cases, the herpesvirus is HSV-1. In some cases, the sequence of the herpesvirus crs comprises CGCCCCGATCGTCCA (SEQ ID NO: 10). In some cases, the sequence of the herpesvirus crs has at least 90% (e.g., 95%, 98%, 99% or 100%) sequence identity to a sequence of the crs of HSV-1 (e.g., CGCCCCGATCGTCCA (SEQ ID NO: 10)). In some cases, the sequence of the herpesvirus crs flanked on the 5′ end by a first sequence of at least 7 base pairs and on the 3′ end by a second sequence of at least 7 base pairs comprises CCGAGGACGCCCCGATCGTCCACACGGAG (SEQ ID NO: 3). In some cases, the sequence of the herpesvirus crs flanked on the 5′ end by a first sequence of at least 7 base pairs and on the 3′ end by a second sequence of at least 7 base pairs comprises CCG*AGGAC*GCCCCGATC*GTCCACACG*GAG (SEQ ID NO: 11), wherein * is a phosphorothioated bond.

REDUCING HERPESVIRUS IN TRANSPLANT ORGANS AND TISSUES

[0070] The present disclosure provides a method of inhibiting herpesvirus replication in an organ or tissue, the method comprising contacting the organ or tissue *in vitro* or *ex vivo* with a double stranded DNA molecule including a sequence comprising a sequence of a cis regulatory sequence (crs) of the herpesvirus, wherein the crs is flanked on the 5′ end by a first sequence of at least 2 base pairs and on the 3′ end by a second sequence of at least 2 base pairs. In some cases, the herpesvirus is CMV. In some cases, the herpesvirus is HSV-1. In some cases, the individual is a human. In some cases, the method includes administering at least a second therapeutic agent. In some cases, the second therapeutic agent is ganciclovir, foscarnet, cidofovir, maribavir, or valganciclovir. In some cases, the second therapeutic agent is an HDAC inhibitor. In some cases, the individual is an organ transplant recipient. In some cases, the individual is a bone marrow transplant recipient. In some cases, the individual does not have a herpesvirus infection, and is a prospective organ transplant recipient. In some cases, the individual does not have a herpesvirus infection, and is a prospective bone marrow transplant recipient. In some cases, the individual is a pregnant female. In some cases, the individual is a neonate

[0071] The present disclosure provides a method of reducing the amount of CMV (e.g., reducing the number of genome copies of CMV) in an organ or tissue, the method comprising contacting the organ or tissue *in vitro* or *ex vivo* with a double stranded DNA molecule including a sequence comprising a sequence of a cis regulatory sequence (crs) of the CMV, wherein the crs is flanked on the 5′ end by a first sequence of at least 2 base pairs and on the 3′ end by a second sequence of at least 2 base pairs. In some cases, the organ or tissue is autologous (the organ or tissue is obtained from a donor, where the donor is also the recipient of the organ or tissue). In some cases, the organ or tissue is allogeneic (the organ or tissue is obtained from a genetically different donor of the same species as the recipient). In some cases, the organ or tissue is xenogeneic (the organ or tissue is obtained from a different species from the recipient).

[0072] Organs and tissues suitable for use in a subject method include, but are not limited to, a kidney, a liver, a pancreas, a heart, a lung, skin, blood tissue (including whole blood; red blood cells; white blood cells; cord blood; and the like, where the blood tissue may comprise an isolated population of blood cells (buffy coat; red blood cells; platelets; lymphocytes; T cells; B cells; or some other population), or where the blood tissue comprises a mixed population of cells), small intestine, an endothelial tissue, a vascular tissue (e.g., a blood vessel), an eye, a stomach, a thymus, bone, bone marrow, cornea, a heart valve, an islet of Langerhans, or a tendon. As used herein, "organ" encompasses a whole organ or a part of an organ. As used herein, "tissue" encompasses a whole tissue or part of a tissue. As used herein, "tissue" encompasses a cell population.

[0073] In some cases, the organ or tissue is contacted *in vitro* or *ex vivo* with the double stranded DNA molecule for a period of time of from about 15 minutes to about 48 hours, or more than 48 hours. For example, in some cases, the organ or tissue is contacted *in vitro* or *ex vivo* with the double stranded DNA molecule for a period of time of from 15 minutes to 1 hour, from 1 hour to 2 hours, from 2 hours to 4 hours, from 4 hours to 8 hours, from 8 hours to 12 hours, from 12 hours to 24 hours, from 24 hours to 48 hours, or more than 48 hours.

[0074] In some cases, the organ or tissue is contacted *in vitro* or *ex vivo* with the double stranded DNA molecule in an amount and for a period of time to reduce the CMV genome copies in the organ or tissue. In some cases, an effective amount of a double stranded DNA molecule is an amount that, when contacted with an organ or tissue, is effective to reduce the number of CMV genome copies in the organ or tissue by at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, or at least 90%, compared to the number of CMV genome copies in the organ or tissue before contacting

with the double stranded DNA molecule, or in the absence of contacting with the double stranded DNA molecule. In some cases, an effective amount of a double stranded DNA molecule is an amount that, when contacted with an organ or tissue for a period of time of from about 2 hours to 48 hours, is effective to reduce the number of CMV genome copies in the organ or tissue by at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, or at least 90%, compared to the number of CMV genome copies in the organ or tissue before contacting with the double stranded DNA molecule, or in the absence of contacting with the double stranded DNA molecule.

[0075] In some cases, the organ or tissue is contacted *in vitro* or *ex vivo* with the double stranded DNA molecule in a liquid medium. The liquid medium can comprise, in addition to the double stranded DNA molecule, one or more of a buffer, a salt, a preservative, etc. In some cases, the liquid medium comprises, in addition to the double stranded DNA molecule, one or both of an HDAC inhibitor, and a transcriptional transactivator.

MODIFIED OLIGONUCLEOTIDES

[0076] The oligonucleotides of the present disclosure may be modified to modulate various properties of the oligonucleotides, e.g., increase half-life and/or to facilitate delivery of the oligonucleotides into cells.

[0077] In some cases, the oligonucleotides of the present disclosure are modified to increase their uptake into a cell, e.g., cell permeability of the oligonucleotides may be increased by the modification(s). The oligonucleotides may be conjugated to any suitable moiety that increases their permeability through a cell membrane, such as, one or more lipid moieties.

[0078] In certain embodiments, the oligonucleotides of the present disclosure are modified to include various nucleic acid analogs without modification to the sequence of the respective oligonucleotides. Suitable analogs include, but are not limited to, peptide nucleic acids (PNAs), locked nucleic acids (LNAs), bridged nucleic acids (BNAs), and the like.

[0079] In some cases, the oligonucleotides of the present disclosure include a modified backbone. In some cases, the oligonucleotides are modified to increase their half-life. Suitable modifications include, e.g., phosphorothioate linkages, phosphorodithioate linkages, methylphosphonate linkages, phosphoramidate linkages, 2'-modifications (e.g., modifications of the 2' position with methyl and methoxyethyl groups), and the like.

[0080] In another aspect, the oligonucleotides of the present disclosure may be modified to facilitate delivery of the oligonucleotides. In some cases, the oligonucleotides are conjugated to or encapsulated by a moiety that facilitates the delivery of the oligonucleotides. Suitable

moieties include delivery vehicles such as, nanoparticles, vesicles, liposomes, micelles, dendrimers, viral particles, microbeads, and the like.

SUBJECTS SUITABLE FOR TREATMENT

[0081] Subjects suitable for treatment with a method of the present disclosure for treating a herpesvirus infection include individuals who have been diagnosed as having a herpesvirus infection. Subjects suitable for treatment with a method of the present disclosure for treating a herpesvirus infection include individuals who have not been diagnosed as having a herpesvirus infection. In some cases, the individual does not have a herpesvirus infection, but is at greater risk than the general population of contracting a herpesvirus infection.

[0082] In some cases, the individual has a herpesvirus infection, and also has an immunodeficiency virus (e.g., human immunodeficiency virus; HIV) infection. In some cases, the individual does not have an immunodeficiency virus (e.g., human immunodeficiency virus; HIV) infection.

[0083] In some cases, the individual is an organ transplant recipient. In some cases, the individual is a liver transplant recipient. In some cases, the individual is a kidney transplant recipient. In some cases, the individual is a liver transplant recipient. In some cases, the individual is a bone marrow transplant recipient. In some cases, the individual is a lung transplant recipient.

[0084] In some cases, the individual does not have a herpesvirus infection; and is a prospective organ transplant recipient. In some cases, the individual does not have a herpesvirus infection; and is a prospective liver transplant recipient. In some cases, the individual does not have a herpesvirus infection; and is a prospective kidney transplant recipient. In some cases, the individual does not have a herpesvirus infection; and is a prospective bone marrow transplant recipient. In some cases, the individual does not have a herpesvirus infection; and is a prospective lung transplant recipient.

[0085] In some cases, the individual is a pregnant female, e.g., a pregnant human female. In some cases, the individual is a neonate, e.g., a human neonate. In some cases, the individual is from 1 hour old to 4 weeks old, e.g., from 1 hour to 4 hours, from 4 hours to 8 hours, from 8 hours to 12 hours, from 12 hours to 1 day, from 1 day to 1 week, or from 1 week to 4 weeks, old. In some cases, the individual is from 4 weeks old to 6 months old.

COMPOSITIONS AND FORMULATIONS

[0086] An active agent is administered to an individual in need thereof in a formulation with a pharmaceutically acceptable excipient(s). A wide variety of pharmaceutically acceptable excipients is known in the art and need not be discussed in detail herein. Pharmaceutically acceptable excipients have been amply described in a variety of publications, including, for example, A. Gennaro (2000) "Remington: The Science and Practice of Pharmacy", 20th edition, Lippincott, Williams, & Wilkins; Pharmaceutical Dosage Forms and Drug Delivery Systems (1999) H. C. Ansel et al., eds 7th ed., Lippincott, Williams, & Wilkins; and Handbook of Pharmaceutical Excipients (2000) A. H. Kibbe et al., eds., 3rd ed. Amer. Pharmaceutical Assoc. For the purposes of the following description of formulations, "active agent" includes an active agent as described above, and optionally one or more additional therapeutic agent.

[0087] In pharmaceutical dosage forms, an active agent may be administered in the form of its pharmaceutically acceptable salts, or it may also be used alone or in appropriate association, as well as in combination, with other pharmaceutically active compounds. The following methods and excipients are merely exemplary and are in no way limiting.

[0088] In some embodiments, an active is formulated in an aqueous buffer. Suitable aqueous buffers include, but are not limited to, acetate, succinate, citrate, and phosphate buffers varying in strengths from about 5 mM to about 100 mM. In some embodiments, the aqueous buffer includes reagents that provide for an isotonic solution. Such reagents include, but are not limited to, sodium chloride; and sugars e.g., mannitol, dextrose, sucrose, and the like. In some embodiments, the aqueous buffer further includes a non-ionic surfactant such as polysorbate 20 or 80. Optionally the formulations may further include a preservative. Suitable preservatives include, but are not limited to, a benzyl alcohol, phenol, chlorobutanol, benzalkonium chloride, and the like. In many cases, the formulation is stored at about 4°C. Formulations may also be lyophilized, in which case they generally include cryoprotectants such as sucrose, trehalose, lactose, maltose, mannitol, and the like. Lyophilized formulations can be stored over extended periods of time, even at ambient temperatures.

[0089] For oral preparations, an active agent can be used alone or in combination with appropriate additives to make tablets, powders, granules or capsules, for example, with conventional additives, such as lactose, mannitol, corn starch or potato starch; with binders, such as crystalline cellulose, cellulose derivatives, acacia, corn starch or gelatins; with

disintegrators, such as corn starch, potato starch or sodium carboxymethylcellulose; with lubricants, such as talc or magnesium stearate; and if desired, with diluents, buffering agents, moistening agents, preservatives and flavoring agents.

[0090] An active agent can be formulated into preparations for injection by dissolving, suspending or emulsifying them in an aqueous or nonaqueous solvent, such as vegetable or other similar oils, synthetic aliphatic acid glycerides, esters of higher aliphatic acids or propylene glycol; and if desired, with conventional additives such as solubilizers, isotonic agents, suspending agents, emulsifying agents, stabilizers and preservatives.

[0091] The term "unit dosage form," as used herein, refers to physically discrete units suitable as unitary dosages for human and animal subjects, each unit containing a predetermined quantity of an active agent, calculated in an amount sufficient to produce the desired effect in association with a pharmaceutically acceptable diluent, carrier or vehicle. The specifications for a given active agent will depend in part on the particular compound employed and the effect to be achieved, and the pharmacodynamics associated with each compound in the host.

[0092] Suitable excipient vehicles are, for example, water, saline, dextrose, glycerol, ethanol, or the like, and combinations thereof. In addition, if desired, the vehicle may contain minor amounts of auxiliary substances such as wetting or emulsifying agents or pH buffering agents. Actual methods of preparing such dosage forms are known, or will be apparent, to those skilled in the art. See, e.g., Remington's Pharmaceutical Sciences, Mack Publishing Company, Easton, Pa., 17th edition, 1985. The composition or formulation to be administered will, in any event, contain a quantity of the agent adequate to achieve the desired state in the subject being treated.

[0093] In certain aspects, the compositions and/or formulations may include at least two, at least three, at least four, or more of the different DNA molecules provided herein. For example, a composition may include a) a first double stranded DNA molecule comprising a HSV crs from a first HSV and b) a second double stranded DNA molecule comprising a HSV crs from a second HSV. The first and second HSVs may be selected independently from HSV-1, HSV-2, and CMV.

[0094] In some cases, a composition may include: a) a first double stranded DNA molecule comprising a HSV crs from a first HSV; b) a second double stranded DNA molecule comprising a HSV crs from a second HSV; and c) a third double stranded DNA molecule comprising a HSV crs from a third HSV. The first, second, and third HSVs may be selected from HSV-1, HSV-2, and CMV.

Dosages

[0095] Those of skill will readily appreciate that dose levels can vary as a function of the specific compound, the severity of the symptoms and the susceptibility of the subject to side effects. Preferred dosages for a given compound are readily determinable by those of skill in the art by a variety of means.

[0096] In some embodiments, a single dose of an active agent is administered. In other embodiments, multiple doses of an active agent are administered. Where multiple doses are administered over a period of time, an active agent is administered twice daily (qid), daily (qd), every other day (qod), every third day, three times per week (tiw), or twice per week (biw) over a period of time. For example, an active agent is administered qid, qd, qod, tiw, or biw over a period of from one day to about 2 years or more. For example, an active agent is administered at any of the aforementioned frequencies for one week, two weeks, one month, two months, six months, one year, or two years, or more, depending on various factors.

[0097] Where two different active agents are administered, a first active agent and a second active agent can be administered in separate formulations. A first active agent and a second active agent can be administered substantially simultaneously, or within about 30 minutes, about 1 hour, about 2 hours, about 4 hours, about 8 hours, about 16 hours, about 24 hours, about 36 hours, about 72 hours, about 4 days, about 7 days, or about 2 weeks of one another.

Routes of Administration

[0098] An active agent is administered to an individual using any available method and route suitable for drug delivery, including *in vivo* and *ex vivo* methods, as well as systemic and localized routes of administration.

[0099] Conventional and pharmaceutically acceptable routes of administration include intranasal, intramuscular, intratracheal, transdermal, subcutaneous, intradermal, topical application, intravenous, vaginal, nasal, and other parenteral routes of administration. An active agent can also be delivered to the subject by enteral administration, e.g., oral administration.

Combination therapy

[00100] In some cases, a method of the present disclosure for treating a herpesvirus infection in an individual comprises administering to the individual, in combined effective amounts: a) a double stranded DNA molecule as described herein; and b) at least a second therapeutic agent. Suitable second therapeutic agents include, but are not limited to, ganciclovir, foscarnet, cidofovir, maribavir, valganciclovir, and intravenous immunoglobulin

(IVIG). Suitable second agents include, but are not limited to, histone deacetylase (HDAC) inhibitors. Suitable second agents include, but are not limited to, transcriptional transactivators.

[00101] Suitable transcriptional transactivators include, e.g., protein kinase C agonists; prostratin; TNF-alpha; 12-deoxyphorbol 13-phenylacetate (DPP); protein-based therapeutic agents that act through related cell-signaling pathways (e.g., the HSV-1 VP16 transactivator); and the like. In some cases, the transcriptional transactivator is prostratin. In some cases, the transcriptional transactivator is TNF-α. In some cases, the transcriptional transactivator is a prostratin analog as described in U.S. Patent No. 8,067,632.

[00102] HDAC inhibitors are known in the art, and any of a variety of HDAC inhibitors can be used. In some cases, the HDAC inhibitor inhibits all Class I HDACs, but does not substantially inhibit any Class II HDAC or any Class III HDAC. In some cases, the HDAC inhibitor specifically inhibits HDAC1 (and does not substantially inhibit other HDAC polypeptides, e.g., does not substantially inhibit HDAC 2, 3, 4, 5, 6, 7, 8, 9, or 10, or any Class III HDAC). In some cases, the HDAC inhibitor specifically inhibits HDAC2 (and does not substantially inhibit other HDAC polypeptides, e.g., does not substantially inhibit HDAC 1, 3, 4, 5, 6, 7, 8, 9, or 10, or any Class III HDAC). In some cases, the HDAC inhibitor inhibits both HDAC1 and HDAC2, but does not substantially inhibit other HDAC polypeptides, e.g., does not substantially inhibit HDAC 3, 4, 5, 6, 7, 8, 9, or 10, or any Class III HDAC.

[00103] Examples of HDAC inhibitors include trichostatin A (TSA) ((R,2E,4E)-7-(4-(dimethylamino)phenyl)-N-hydroxy-4,6-dimethyl-7-oxohepta-- 2,4-dienamide); suberoylanilide hydroxamic acid (SAHA); sulfonamides such as oxamflatin ((E)-N-hydroxy-5-(3-(phenylsulfonamido)phenyl)pent-2-en-4-ynamide); and belinostat (PXD101) ((E)-N-hydroxy-3-(4-(N-phenylsulfamoyl)phenypacrylamide). Other hydroxamic-acid-sulfonamide inhibitors of histone deacetylase are described in: Lavoie et al. (2001) Bioorg. Med. Chem. Lett. 11:2847-50; Bouchain et al. (2003) J. Med. Chem. 846:820-830; Bouchain et al. (2003) Curr. Med. Chem. 10:2359-2372; Marson et al. (2004) Bioorg. Med. Chem. Lett. 14:2477-2481; Finn et al. (2005) Helv. Chim. Acta 88:1630-1657; WO2002030879; WO2003082288; WO20050011661; WO2005108367; WO2006123121; WO2006017214; WO2006017215; and US2005/0234033. Other structural classes of histone deacetylase inhibitors include short chain fatty acids, cyclic peptides, and benzamides. Acharya et al. (2005) Mol. Pharmacol. 68:917-932.

[00104] In some cases, the HDAC inhibitor is a short-chain fatty acid, e.g., a butyrate or a phenylbutyrate. In some cases, the HDAC inhibitor is an epoxyketone-containing cyclic tetrapeptide, e.g. trapoxin. In some cases, the HDAC inhibitor is a non-epoxyketone-containing cyclic tetrapeptide. In some cases, the HDAC inhibitor is a hydroxamic acid, e.g., SAHA. In some cases, the HDAC inhibitor is a benzamide. In some cases, the HDAC inhibitor is valproate. In some cases, the HDAC inhibitor is TSA. In some cases, the HDAC inhibitor is PXD101.

[00105] Further examples of HDAC inhibitors include those disclosed in, e.g., Dokmanovic et al. (2007) Mol. Cancer. Res. 5:981; U.S. Pat. No. 7,642,275; U.S. Pat. No. 7,683,185; U.S. Pat. No. 7,732,475; U.S. Pat. No. 7,737,184; U.S. Pat. No. 7,741,494; U.S. Pat. No. 7,772,245; U.S. Pat. No. 7,795,304; U.S. Pat. No. 7,799,825; U.S. Pat. No. 7,803,800; U.S. Pat. No. 7,842,727; U.S. Pat. No. 7,842,835; U.S. Patent Publication No. 2010/0317739; U.S. Patent Publication No. 2010/0311794; U.S. Patent Publication No. 2010/0310500; U.S. Patent Publication No. 2010/0292320; and U.S. Patent Publication No. 2010/0291003. In some cases, a given HDAC inhibitor or class of HDAC inhibitors is specifically excluded.

[00106] In some cases, a method of the present disclosure for treating a herpesvirus infection in an individual comprises administering to the individual, in combined effective amounts: a) a first double stranded DNA molecule comprising a HSV crs from a first HSV and b) a second double stranded DNA molecule comprising a HSV crs from a second HSV. The first and second HSVs may be selected independently from HSV-1, HSV-2, and CMV.

[00107] In some cases, a method of the present disclosure for treating a herpesvirus infection in an individual comprises administering to the individual, in combined effective amounts: a) a first double stranded DNA molecule comprising a HSV crs from a first HSV; b) a second double stranded DNA molecule comprising a HSV crs from a second HSV; and c) a third double stranded DNA molecule comprising a HSV crs from a third HSV. The first, second, and third HSVs may be selected from HSV-1, HSV-2, and CMV.

Examples

[00108] The following examples are put forth so as to provide those of ordinary skill in the art with a complete disclosure and description of how to make and use the present invention, and are not intended to limit the scope of what the inventors regard as their invention nor are they intended to represent that the experiments below are all or the only experiments performed. Efforts have been made to ensure accuracy with respect to numbers

used (e.g. amounts, temperature, etc.) but some experimental errors and deviations should be accounted for. Unless indicated otherwise, parts are parts by weight, molecular weight is weight average molecular weight, temperature is in degrees Celsius, and pressure is at or near atmospheric. Standard abbreviations may be used, e.g., bp, base pair(s); kb, kilobase(s); pl, picoliter(s); s or sec, second(s); min, minute(s); h or hr, hour(s); aa, amino acid(s); kb, kilobase(s); bp, base pair(s); nt, nucleotide(s); i.m., intramuscular(ly); i.p., intraperitoneal(ly); s.c., subcutaneous(ly); and the like.

Example 1:

[00109] A novel approach that disrupts viral auto-regulatory circuits with a DNA molecule and limits resistance by requiring multiple viral mutations is provided. DNA-based circuit-disruptor oligonucleotide therapies (C-DOTs) that exploit this mechanism by interfering with transcriptional negative feedback in human herpesviruses (CMV and HSV-1) thereby increasing viral transcription factors to cytotoxic levels are disclosed. C-DOTs reduce viral replication >100-fold, prevent emergence of resistant mutants in continuous culture, are effective in high-viremic conditions where existing antivirals are ineffective, and show efficacy in mice. Strikingly, no C-DOT-resistant mutants evolved in >60 days of culture, in contrast to approved herpesvirus antivirals where resistance rapidly evolved. Overall, the results demonstrate that oligonucleotide therapies targeting feedback circuits are escape resistant and could have broad therapeutic applicability to viruses, microbes, and neoplastic cells.

[00110] The time to emergence of drug-resistant 'escape' mutants is typically estimated from the mutation rate, μ, and the effective population size, N[8,9]. Many viruses exhibit large μ such that frequency of mutants ($\mu \times N$) is >1, even for moderate virus population sizes (e.g., if $\mu \sim 10^{-5}$ then for $\mu \times N > 1$ requires only that $N > 10^5$). Herpesviruses, for example, exhibit high mutation rates[10,11], which may explain the substantial antiviral resistance observed in clinical settings[4,5]. In particular, herpes simplex virus type 1 (HSV-1)—a leading cause of blindness—exhibits resistance to acyclovir (ACV) in ~40% of transplant patients[6] while human herpesvirus 5, cytomegalovirus (CMV)—a leading cause of birth defects and transplant failure—exhibits resistance to ganciclovir (GCV) in 30–75% of patients[7]. ACV and GCV resistance arises because their antiviral activity requires herpesvirus thymidine kinase (TK), and single-base mutations destroy TK activity (with a $\mu \sim 10^{-3}$)[10] driving TK escape mutants within a single generation[12], which ultimately led to the development of non-TK drug targets[13,14]. Resistance to these new therapies is still being

clinically evaluated, but, given the generality of resistance to antimicrobials[1-3], is likely unavoidable.

[00111] Combination therapy, wherein multiple drugs simultaneously inhibit different viral targets, is one approach used to limit antiviral resistance. For a two-drug therapy, escape mutants are predicted to arise at a rate $\sim\mu^2$ (i.e., the requirement for mutants to arise becomes $\mu^2 \cdot N > 1$), which requires substantially larger virus populations (i.e., $N > \mu^{-2}$). However, each constituent antiviral must have a distinct molecular target, as well as favorable toxicity, efficacy, bioavailability, and dosing profiles. These criteria can be challenging to satisfy and such targets are still being characterized for herpesviruses. One proposed alternative has been to mimic the evolutionary benefits of combination therapy by inhibiting protein-protein or protein-DNA interactions with a single molecule; this has remained technically challenging but transcriptional auto-regulatory (feedback) circuits present an attractive target for this approach[15]. Both CMV and HSV-1 utilize transcriptional feedback to regulate immediate-early (IE) viral gene expression, which is obligate to transactivate downstream viral genes, ultimately licensing virus maturation[16-19]. In CMV, the 86-kDa immediate early (IE86; a.k.a. IE2) protein, and in HSV-1 the IE175 (a.k.a. ICP4) protein, are indispensable transcriptional transactivators[20,21]. Critically, IE86 and IE175 are cytotoxic when expression is misregulated above tightly auto-regulated homeostatic levels, and both CMV and HSV-1 encode negative-feedback circuits to maintain IE86 and IE175 levels below their respective cytotoxic thresholds[20,22]. These feedback circuits are comprised of a protein-DNA interaction wherein the IE protein binds to a 14–15 bp palindromic cis-repression sequence (crs) within its respective promoter and auto-represses its own transcription (Fig. 2 a). Disrupting this feedback by altering the crs increases IE protein levels to cytotoxic levels, leading to a >100-fold reduction in viral replication[22,23].

[00112] It was hypothesized that oligonucleotides mimicking the palindromic DNA-binding site could titrate IE proteins away from the crs and act as competitive inhibitors to disrupt IE negative feedback (Fig. 2 a). Mathematical modeling predicted that such circuit-disrupting oligonucleotide therapies (C-DOTs) may in fact raise IE protein to cytotoxic levels (Fig. 2 b and Fig. 11 a). Theoretically, to escape C-DOTs and recapitulate a feedback loop, the virus would need to evolve a new IE-protein domain to recognize a new DNA sequence and *simultaneously* evolve a new cognate DNA binding sequence in the IE promoter; these C-DOT escape mutants would evolve on order of μ^2, which would occur substantially slower than observed for ACV/GCV resistance (Fig. 2 c). It is conceivable that single mutants in the IE protein could arise and bind alternate crs-like sequences pre-existing

in the promoter region, thereby circumventing the combinatorial mutation hypothesis. However, there are no comparable palindromes within 500 bp of the MIEP promoter and if such sequences were present, then crs-deletion mutants should strongly select for such IE86 mutants that can recapitulate negative feedback, which does not occur over ~1 month of culturing[22].

[00113] FIG. 2: Theory predicts that antiviral disruption of transcriptional feedback would substantially delay evolution of resistance. (a) Schematics of the herpesvirus IE (IE86 and IE175) transcriptional negative-feedback circuits in the intact wild-type form (upper) and after disruption (lower) by putative circuit-disrupting oligonucleotide therapy (C-DOT). When feedback is intact, IE proteins bind the cis repression DNA sequence in their respective IE promoters (cyan) and downregulate transcriptional activity to prevent IE protein levels from reaching cytotoxic levels. When feedback is disrupted, for example by IE proteins being titrated away by binding free oligonucleotides encoding cis-repression sequences, IE promoter activity is not downregulated and IE proteins reach cytotoxic levels (~1.5-fold above homeostatic levels)[22]. (b) Numerical solutions of an experimentally validated computational model of IE feedback[22,24] showing that C-DOTs effectively break feedback to increase IE protein levels into the cytotoxic regime. See also Fig. 11 a. (c) Analytical calculation for the 50% emergence time of resistance mutants ($\tau_{resistance}$) as a function of the mutation rate μ. The observed emergence of resistance to GCV in the clinic[7] is shown as a point above the measured TK mutation rate ($\mu = 10^{-4}$). Putative C-DOT resistance, predicted to require at least two mutations (one in the protein and one in the promoter), is shown as a line corresponding to measured μ[11]. Inset: representative dynamics of emergence for either a GCV mutant (black) or a putative C-DOT mutant (cyan) that requires only viral two mutations. See Methods for equations.

[00114] To find oligonucleotides that optimally titrate IE proteins, an *in vitro* liquid-chromatography assay was developed to quantify the efficiency of various linear DNA oligonucleotides in catalyzing formation of the IE86 protein-DNA complex (Fig. 8 a). To validate the assay, electrophoretic mobility shift assays (EMSA) were used and it was verified that purified IE86 protein bound double-stranded DNA oligonucleotides in a sequence-specific manner (Fig. 11 b). An array of crs-encoding oligonucleotides of various lengths was tested and it was found that a linear 28 base-pair (bp) DNA most efficiently catalyzed formation of the IE86-DNA complex (Fig. 8 b–c). Shorter or longer crs-encoding DNAs were less efficient at titrating IE86 and promoting protein-DNA complex formation.

[00115] To test if these DNAs disrupted negative feedback, a retinal pigment epithelial cell line stably transduced with a previously described minimal IE86 negative-feedback reporter circuit[22] was used. These cells express the IE86 and GFP under the control of the IE86 promoter-enhancer such that increases in GFP indicate disruption of negative-feedback leading to cell death 48–72 hours later (Fig. 8 d). Cells were nucleofected with the putative C-DOTs, or sequence-scrambled control oligonucleotides, and GFP expression assayed by flow cytometry at 48 h (Fig. 8 d). DNA uptake by cells was verified by cy3 fluorescence (Fig. 11 c) and to enhance oligonucleotide stability, DNAs were modified by internal phosphorothioate bonds (Fig. 11 d). In agreement with the biochemical assays, the 28bp DNA most efficiently increased the GFP+ population (Fig. 8 e) and correspondingly decreased live-cell percentages (Fig. 8 f). Feedback-circuit disruption was dose dependent (Fig. 12 a-b) and was enhanced by concatemerization as well as using nanoparticles to enhance cell uptake (Fig. 13 a-c). Collectively, these results indicated that relatively short (< 30bp) double-stranded DNAs might be viable circuit disruptor oligonucleotides.

[00116] **FIG. 8: Biochemical and *in vitro* analyses identify oligonucleotides that disrupt transcriptional feedback circuitry.** (a) Schematic of the IE86-protein binding assay: the C-terminus of the IE86 protein was expressed in *E. coli*, concentrated and incubated with 14–64bp DNA oligonucleotides for 30 minutes at room temperature, then passed through an FPLC column. Protein-DNA complex formation was quantified by optical density (OD 280nm) of FPLC fractions; the IE86 protein (obligate dimer) fragment elutes in the 15mL fraction, free dsDNA oligonucleotides elute in the 18mL fraction, and the multimeric protein-DNA complex elutes in the 13mL fraction. (b) FPLC chromatography profiles of IE86 protein fragment input and dsDNA input prior to co-incubation. (c) Chromatographs of IE86 fragment incubated with either a sequence-scrambled control dsDNA oligonucleotides or crs-containing dsDNA oligonucleotides of differential lengths. The 28bp crs DNA oligonucleotide most efficiently titrates free protein from the 15mL fraction into the 13mL fraction (~98% of protein is found in the 13mL protein-DNA complex fraction when the 28bp crs DNA oligonucleotide is added). (d) Left: Schematic of the minimal IE negative-feedback circuit (MIEP-IE86-IRES-GFP) encoded within the feedback-reporter cell line. Disruption of negative feedback generates increases in GFP fluorescence. Right: Flow cytometry of feedback reporter cells 48 h after nucleofection with either a 28bp crs-containing DNA oligonucleotide, a scrambled DNA oligonucleotide (negative control), or mock nucleofection (no DNA oligonucleotide) showing that crs-

encoding DNA oligonucleotides disrupt feedback and act as a putative C-DOTs. (e, f) The 28bp crs-containing DNA oligonucleotide optimally disrupts feedback and induces cytotoxicity in the feedback-reporter cell line. DNA oligonucleotides (from the FPLC analysis in panel c above) were nucleofected into the reporter cell line and analyzed by flow cytometry after 48 h to determine IE86 expression (GFP; panel e), and cytotoxicity (panel f). (p-value less than 0.05 was considered statistically significant: *<0.05, **<0.01, ***<0.001, ****<0.0001).

[00117] FIG. 11: Simulations and in-vitro analyses indicate that excess crs DNA oligos competitively bind IE86 and would break negative feedback to increase IE86 levels. (a) Numerical solutions of an experimentally validated Ordinary Differential Equation (ODE) model of the Major Immediate Early circuit of CMV[22, 24] modified to include C-DOTs as described in Methods. All parameters are kept the constant and initial levels of oligos at t=0 are varied as indicated. (b) Electrophoresis mobility shift assays (EMSA) verify that the C-terminus of IE86 sequence specifically interacts with crs-containing DNA. Digoxygenin (DIG)-labeled DNA oligo probes of the crs and Δcrs sequences, as described[36], were used for binding and detection. EMSA was performed after incubating DIG-labeled DNA probes with increasing concentrations of IE86 protein (0μM to 20μM) for 30 minutes at room temperature (lanes 1–6). To determine if the IE86-crs interaction was sequence specific, either 20-fold excess of unlabeled crs (*) was added (lane 7) or 20-fold unlabeled Δcrs (**) was added (lanes 7–10). (c) Delivery and stability of C-DOTs in the IE86 minimal-circuit cell line. Fluorescence micrographs of ARPE-19 cells four days post nucleofection with cy3-labeled 28bp DNA oligomer cy3-C-DOT^{C} (left) or free cy3 dye (right). (d) Top: Sequence schematics of the unmodified 28bp C-DOT^{C}(N), the phosphorothioated 28bp C-DOT^{C} (* indicates phosphorothioated bases), and 28bp scrambled DNA (also referred to as C-DOT^{Scram}), which also contains phosphorothioated bases. Bottom: Flow cytometry of ARPE-19 IE86 minimal circuit cell line four days after nucleofection with the various DNA C-DOTs.

[00118] FIG. 13: C-DOT nanoparticles enhance cell delivery and C-DOT concatemers enhance IE86 sequestration and feedback disruption. (a) Cells take up C-DOT^{C}-SNA nanoparticles more efficiently than 'free' C-DOT^{C}. Cy3-tagged C-DOT^{C} DNA oligos were conjugated to 10-nm gold nanoparticles to generate 'spherical nucleic acid' (SNA) nanoparticles. Cells were then incubated in culture with the C-DOTs SNA nanoparticles. 4 days later, cells were assayed for cy3 uptake by microscopy. Micrographs of ARPE-19 cells 4 days after incubation with cy3-tagged C-DOT^{C}-SNA, cy3-tagged 'free'

C-DOT^{C}, or unlabeled 15-nm gold nanoparticles lacking cy3 (as a control) are shown in the cy3 fluorescence channel (left) and the bright field channel (right). (b) FPLC of purified C terminus IE86 protein (N-terminus tagged with maltose binding protein and fractionated for the dimeric form, as in Fig. 2a) then incubated with a C-DOT^{C} containing one crs sequence (C-DOT 1x) of two concatenated crs sequences (C-DOT 2X-concat) for 30 minutes at room temperature (see Extended Data Table 1 for C-DOT sequences). Oligomerized fraction (% absorbance at ~13ml fraction at OD280) was compared for both the samples. (c) Flow cytometry analysis of the IE86 minimal-circuit reporter cell line (ARPE-19 cells) two days after nucleofection with C-DOT (1X), C-DOT (2X)-concat, or the scrambled DNA oligo (C-DOT^{Scram}, negative control).

[00119] Next, the effects of disrupting transcriptional feedback in the context of viral infection were investigated. The 28bp DNA for CMV IE86 was designated as C-DOT^{C} (C-DOT for CMV) and it was found that C-DOT^{C} efficiently disrupted IE86 negative feedback in cells infected with a clinically-derived isolate of CMV[24] (Fig. 7 a). It was next hypothesized that replacing the 14bp crs within C-DOT^{C} with the 15bp repression sequence from IE175's promoter could generate a C-DOT for HSV-1 (now termed C-DOT^{H}). As predicted, C-DOT^{H} disrupted IE175 negative feedback in cells infected with clinically-derived HSV-1[25] (Fig. 7 b). There was minimal difference in %IE positive cells, indicating that C-DOTs did not alter permissiveness of cells to viral infection (Fig. 12 c). Strikingly, the C-DOTs also reduced single-round viral replication titers for both CMV and HSV-1 at multiplicity of infection (MOI) of 0.1 by ~100 fold (Fig. 7 c–d), in agreement with genetic disruption of IE negative feedback[22]. To be sure that the observed antiviral effects were not specific to the virus strain or cell type used, the following were also tested (i) CMV strain AD169 and (ii) various GCV-resistant CMV strains in human foreskin fibroblasts, as well as (iii) murine CMV and (iv) rhesus CMV, in mouse and primate cells respectively, and in all cases found similar 100-fold titer reduction using the corresponding C-DOTs (Fig. 14 a-c).

[00120] **FIG. 12: C-DOTs break IE negative feedback in a dose-dependent manner and do not alter cell permissiveness to viral infection.** (a) Flow cytometry dot plots of the IE86 minimal circuit reporter cell line after nucleofection with increasing doses of the 28bp C-DOT^{C} and 100μM of the 28bp C-DOT^{Scram} (negative control). % GFP+ population was quantified two days post nucleofection (SSC = side scatter). (b) Top: Quantitative (LICOR™) Western blot analysis of IE86 from cell lysates of panel A at two days post C-DOT nucleofection; Bottom: IE86 loading control. (c) Flow cytometry dot plots of naïve or C-DOT-nucleofected ARPE-19 cells after infection with CMV strain TB40/E-

IE86-YFP or HSV-1 strain 17syn+ IE175-YFP. Cells were infected 12h after nucleofection with the indicated C-DOT and analyzed at one-day post infection.

[00121] FIG. 14: C-DOTs can be engineered to interfere with a broad range of species-specific herpesviruses including drug-resistant strains. (a) Sequence homology for the crs of human CMV, rhesus CMV (RhCMV), and murine CMV (MCMV). Green represents sequence homology whereas red represents divergence. (b) C-DOT^{MCMV} interferes with MCMV replication. NIH 3T3 mouse fibroblast cells were nucleofected with either 28bp C-DOT^{MCMV}, or C-DOT^{Scram}, or mock nucleofected 24 hours prior to MCMV infection at MOI = 0.1. 4 days post infection, virus titers were assayed by TCID50. (c) C-DOT^{RhCMV} downregulates RhCMV replication. (Upper) Fluorescent micrographs, 4-day post nucleofection, of Telo-RF cells nucleofected with either a 28bp C-DOT^{RhCMV}, or a C-DOT^{Scram}, or mock nucleofected and then infected with RhCMV (RhCMV 68.1 GFP) at MOI = 0.1. (Lower) Virus titers assayed by TCID-50 at 4 days post infection. (d) C-DOT^{C} interferes with replication of ganciclovir-resistant (GCV^{R}) and foscarnet-resistant (FOS^{R}) CMV strains. MRC-5 cells were nucleofected with C-DOT^{C} or C-DOT^{Scram} then infected 24-hour later with either the parent CMV AD169 (control) or GCV^{R} or FOS^{R} strains (CMV GDGrK17, CMV GDGrP53, CMV 759rD100-1, CMV PFArD100). Virus titers were assayed by TCID-50 at 4 days post infection.

[00122] In stark contrast to ACV and GCV, C-DOT increases virus fold reduction at higher MOI, and, at MOI = 2, C-DOTs exhibited a > 1300x reduction whereas ACV/GCV elicited 35–70x reduction in viral replication (Fig. 7 c–d). Such robustness to high-viremic conditions has not been previously described for an antiviral[26], but is consistent with the putative C-DOT mechanism of action through feedback disruption and IE-mediated cytotoxicity—higher MOIs deliver more genomes, which generates more potentially cytotoxic IE protein.

[00123] Importantly, sequence-scrambled oligonucleotides (C-DOT^{Scram}) did not exhibit antiviral effects suggesting that C-DOTs are not acting via innate-immune mechanisms (e.g., activation of cGAS-STING pathway via TLR9), which is consistent with efficient cGAS pathway activation requiring DNAs of > 300bp[27], whereas C-DOTs are < 30bp. However, to verify that C-DOT activity is independent of cGAS-STING, C-DOTs were tested under conditions of high and low cGAS-STING expression and observed little difference in C-DOT antiviral effects and no effects for C-DOT^{Scram} in either setting (Fig. 15 b). Moreover, C-DOTs did not activate TLR9 expression (Fig. 15 a).

[00124] To examine the rate of emergence of viral escape mutants, the next step involved using a continuous-culture approach where virus was consecutively passaged from infected cells to fresh uninfected cells every 4 days (a typical CMV replication round) until virus was undetectable in presence of C-DOT treatment (~40–60 days; Fig. 7 e). C-DOT^{C} was compared to Fomivirsen[28], the first approved DNA oligonucleotide therapy (an anti-sense DNA for IE86); C-DOT^{H} was compared with ACV. As previously reported[12], it was found that HSV-1 resistance to ACV emerges within two rounds of infection (Fig. 15 f) and that CMV resistance to Fomivirsen[28] arises within 3-4 rounds of infection (Fig. 15 d-e).

[00125] In striking contrast, C-DOT^{C} steadily reduced CMV titers to below the limit of detection by day 52, with no evidence of CMV resistance to the C-DOT^{C} (Fig. 7 f). Subsequent sub-culturing showed that the virus was cleared (Fig. 15 c). Similarly, C-DOT^{H} steadily reduced HSV-1 titers to below detection by day 40, with no evidence of resistance (Fig. 7 g). In agreement with this, sequence analysis indicated that no virus mutations arose in the 500bp-region surrounding the promoter repression sequences and only single-nucleotide transient polymorphisms in the IE-protein regions responsible for DNA binding. Overall, these results indicate that disrupting feedback may be an escape-resistant antiviral strategy.

[00126] **FIG. 7: Feedback circuit disruption interferes with viral replication even at high MOI and limits the evolution of resistance.** (a) Flow cytometry of ARPE-19 cells nucleofected with the 28bp dsDNA that titrates IE86 (C-DOT^{C}) or scrambled dsDNA sequence (C-DOT^{Scram}) and infected with a clinically derived CMV (TB40E) encoding an IE86-YFP (MOI = 0.1) then analyzed at 2 days post infection (dpi). (b) Flow cytometry of ARPE-19 cells nucleofected with a 29bp DNA to titrate IE175 (C-DOT^{H}) or scrambled dsDNA sequence (C-DOT^{Scram}) and infected with HSV-1 (17syn+ strain) encoding an IE175-YFP (MOI = 0.1) then analyzed at 2 dpi. (c) Single-round viral titering of CMV in the presence of 100μM GCV, PBS, or 25μM C-DOT^{C} (or C-DOT^{Scram}) at 4-days post infection under different initial MOIs. (d) Single-round viral titering of HSV-1 in presence of 100μM ACV or 25μM C-DOT^{H} (or C-DOT^{Scram}) at 4-days post infection under different HSV-1 MOIs. (e) Schematic of the continuous-culture experiment; ARPE-19 cells (+/- C-DOT) were infected with CMV or HSV-1 (0.1 MOI) and at 4-day post infection, supernatant was collected and was used to infect naïve ARPE-19 cells +/- C-DOT until day 60. (f) Continuous culture titers for CMV (TB40E-IE86-YFP) in the presence of C-DOT^{C} (red) or mock treatment (black). Fomivirsen resistance (positive slope of the titering dynamics) was observed beginning at day 12 (Fig. 15 d,e). (g) Continuous culture for HSV-1 (17syn+

IE175-YFP virus) in the presence of C-DOT^{H} (red) or mock treatment (black). ACV resistance (positive slope of the titering dynamics) observed beginning at day 4 (Fig. 15 f).

[00127] FIG. 15: C-DOTs do not activate innate immune responses in cell lines and—unlike fomivirsen and acyclovir—do not select for resistant viral mutants. (a) C-DOTs do not activate the TLR9 response. qPCR analysis of TLR9 expression in ARPE-19 cells 4 days after nucleofection of either mock nucleofection, nucleofection with a TLR9-activating oligonucleotide (ODN2216)[44], or nucleofection with C-DOT^{C}. Total RNA was extracted from cells and corresponding cDNA was quantified by qPCR using sequence specific primers for TLR9 (see Extended Data Table 1 for sequences). (b) C-DOTs do not act through the cGAS-STING pathway. ARPE-19 (low cGAS-STING activity) and MRC-5 cells (high cGAS-STING activity) were nucleofected with C-DOT^{C} or mock and infected with TB40E-IE86-YFP or AD169, respectively. Virus titers were assayed by TCID-50 at 4 days post infection. (c) C-DOT^{C} decreases CMV titers below detection by day 60 in the continuous culture setting. CMV titers from the continuous culture experiment followed out to day 60. ARPE-19 cells (+/- 25μM C-DOT) were infected with CMV (TB40E-IE86-YFP; MOI=0.1). (d) CMV and HSV-1 rapidly acquire resistance to approved antivirals (fomivirsen and acyclovir). Viral titers from the continuous culture experiment in the presence or absence of fomivirsen. Mock- or fomivirsen-nucleofected ARPE-19 cells were infected with CMV (TB40E-IE86-YFP; MOI=0.1); at 4 days post infection, supernatants were transferred to infect new naïve ARPE-19 cells (+/- fomivirsen) and transfers repeated every 4 days until day 44. (e) Viral titers from the continuous culture experiment (rounds 3 to 5), +/- 25μM fomivirsen. A positive slope in the titers +fomivirsen (i.e., emergence of resistance) is observed beginning at round 3 of infection. (f) HSV-1 resistance to acyclovir (ACV). Viral titers of ARPE-19 cells infected with HSV-1 (17syn+ IE175-YFP; MOI=1) +/- 100μM ACV and supernatant transferred every 2 days over 3 consecutive rounds of infection. Virus titers were assayed by TCID50 every 2 days post transfer; positive slope in the titers (i.e., resistance) is evident despite 100μM ACV.

[00128] Finally, it was tested if transcriptional feedback could be disrupted *in vivo* using the established model of herpes infection in mice[29]. Briefly, in this model, mice are infected with HSV-1 in the cornea, and interventions are topically applied at the site of infection to test efficacy. Using standard practice, mice were infected with HSV-1 IE175-YFP, then 6 hours later (to avoid interfering with virus uptake), oligonucleotides were applied and after two days, corneas were harvested for imaging and quantification of viral replication by q-PCR and titering (Fig. 6 a). C-DOT uptake by cells was quantified by Cy3

fluorescence (Fig. 16 a) and as predicted, C-DOT treatment first caused an increase in IE175 (Fig. 16 b) followed by a significant reduction in the percentage of HSV-1 infected cells (Fig. 6 b–c). In agreement with these data, C-DOT^{H} treatment reduced viral titer by 150-fold (Fig. 6 d) and significantly reduced viral genome replication (Fig. 6 e). Together, these results demonstrate that feedback disruption reduces viral replication *in vivo*.

[00129] FIG. 6A: Feedback disruption inhibits viral replication in an *in vivo* model. (a) Schematic of the HSV-1 corneal infection model in mice. BL-6 mice, 6-10 weeks old, undergo corneal debridement followed by infection with HSV-1 17syn+ IE175-YFP virus ($1x10^5$ PFU). 6 hours post infection, 25μM C-DOT^{H}, or C-DOT^{Scram}, or PBS, was topically applied to the cornea. Corneas were harvested at 2 days post infection, imaged for YFP, HSV-1 levels quantified by virus titering, and viral genomes quantified by qPCR. (b) Representative YFP-fluorescence images of corneas after harvesting (nuclei stained with DAPI). (c) Quantification of HSV-1 YFP expressing cells in corneas, as determined from the YFP:DAPI ratio. 5 corneas imaged per sample. (d) HSV-1 viral titers from HSV-1 infected corneas 2 days after treatment with either 25μM PBS, C-DOT^{Scram} or C-DOT^{H}. Corneas were dissociated using collagenase, subjected to three freeze-thaw and supernatant was used to titer virus using end time dilution method (TCID50). Each data point represents a pooling of corneas from three mice (i.e., 9 corneas per treatment). (e) HSV-1 viral genomic DNA quantification by qPCR 2 days after treatment. Each data point represents a pooling of corneas from three mice (i.e., 9 corneas per treatment). p-values less than 0.05 were considered statistically significant: *<0.05, **<0.01, ***<0.001.

[00130] FIG. 16: C-DOTs can diffuse into naïve mouse corneal cells and increase IE175 expression in mouse corneas following HSV-1 infection. (a) Fluorescence micrographs of dissected mouse corneas incubated with 25μM cy3-tagged C-DOT^{H} for 1 hour. Corneas were washed three times in PBS and immediately imaged. (b) YFP and DAPI fluorescence micrographs of dissected mouse corneas +/- C-DOT^{H} at one day post HSV-1 infection (17syn+ IE175-YFP).

[00131] Overall, these results indicate that transcriptional feedback could represent a new antiviral target with the potential to substantially delay the emergence of resistance (Fig. 7) and overcome significant treatment barriers including the reduction-in-efficacy at high-viremic loads[26]. In general, oligonucleotide therapies offer specificity with the potential for fewer off target effects over small molecules. While delivery of oligonucleotides remains a major challenge, significant clinical advances have been made with the recent FDA approval of antisense and exon-skipping oligonucleotide therapies delivered via nanoparticles[30-32].

Therefore, C-DOTs using nanoparticle carriers, in the absence of transfection, were also tested and similar antiviral effects were found (Fig. 17 a-b). One could envision nanoparticles harboring combinatorial C-DOTs to treat infections of unknown etiology—a significant problem for ocular infections[33,34]—and the data indicate that such combinatorial 'multiplexed' C-DOTs may be feasible (Fig. 17 c-d).

[00132] FIG. 17: Nanoparticle C-DOTs efficiently break transcriptional negative feedback and interfere with virus replication and C-DOTs efficiently inhibit virus replication in a mixed infection setting. (a) Flow cytometry analysis of IE86-GFP minimal circuit cell line treated with 10-nm gold nanoparticles (control) or 10-nm C-DOT^{C}-SNAs at 4 days post incubation. (b) ARPE-19 cells were treated with gold nanoparticles or C-DOT^{C} SNA, subsequently infected with CMV followed by viral titer at 4 days post infection (by TCID50) (see Extended Data Table 1 for sequences). (c-d) C-DOTs downregulate CMV and HSV-1 infection in a mixed infection setting. (c) Schematic of the mixed infection experiment: ARPE-19 cells nucleofected with equimolar amounts of C-DOT^{C} and C-DOT^{H} or C-DOT^{Scram} were co-infected with CMV (TB40/E-IE86-YFP) and HSV-1 (17syn+ IE175-YFP) at MOI = 0.1. (d) qPCR analysis of CMV and HSV-1 viral genomes in the infected cells at four days post infection using primers specific for CMV and HSV-1 (see Table 1 for sequences).

[00133] From the perspective of resistance, disrupting autoregulatory circuits yields several theoretical advantages and the calculations represent a lower limit for the time to escape from feedback disruptors. Regulatory loci typically have lower genetic variability than enzymes or receptors[10,35] so mutants in the promoter region and reading frame will arise relatively slowly. Second, > 2 mutations will likely be required for generating new DNA-recognition domains in proteins; for herpesviruses even assuming that the regulatory region mutates with average viral frequency[10,35] (it is in fact lower) translates to the escape rate scaling as $\sim 10^{-4n}$ (where $n > 2$). Furthermore, putative escape mutants, if they are generated, would have a relative fitness roughly equivalent to wild type, so their selection coefficient and pre-existing abundance will be low. As such, it is predicted that feedback circuit disruption may be an attractive target in other viruses, microbes, and in neoplastic cells with aberrant auto-regulatory circuits.

MATERIAL AND METHODS

[00134] Mathematical Modeling and Numerical Simulations

[00135] An experimentally validated ODE model of the CMV IE86 negative feedback circuit[22, 24] modified to include state variables for free crs DNA oligomers (*C-DOTs*) and the IE86–C-DOT bound complex (*BC)* was used:

[00136]

$$eq.1 \quad \frac{d[IE86]}{dt} = \alpha_0 + \frac{\alpha_1 k_1^{h_1}}{[IE86]^{h_1} + k_1^{h_1}} + h_1 k_2[BC] - h_1 k_{-2}[IE86]^{h_1}[CDOT] - \gamma_1[IE$$

$$eq.2 \quad \frac{d[CDOT]}{dt} = k_2[BC] - k_{-2}[IE86]^{h_1}[CDOT]$$

$$eq.3 \quad \frac{d[BC]}{dt} = k_{-2}[IE86]^{h_1}[CDOT] - k_2[BC]$$

[00137] where α_0 represents the basal IE86 expression rate (2 unit/hr), α_1 represents the IE86 negative-feedback gain constant (10 unit/hr), h_1 represents the IE86 cooperativity index (hill coefficient), k_1 represents a Michaelis constant (set to 1) for IE86 feedback, γ_1 represents the per-capita IE86 protein degradation rate (0.23 hr-1), k_2 represents the BC dissociation rate (2 hr-1), and k_{-2} represents the BC association rate (1 unit-η1∗hr-1). In this model, the IE86 can oligomerize ($h_1 \sim 6$)22 to bind either the crs in its own promoter (MIEP)—thereby mediating negative feedback and down-regulating its own expression rate—or can bind the crs in C-DOT DNA oligo, thereby sequestering IE86 that might otherwise downregulate its own expression via negative feedback. For both binding events, IE86 can transition between free and oligo-bound states but the negative feedback bound state is not explicitly modeled for parsimony and to not introduce unnecessary parameters. The DNA oligonucleotides were assumed to be stable (i.e., not degrade) over the course of the simulation time (validated below). For Fig. 11, the initial levels of C-DOT at time=0 were varied as indicated, all other initial conditions were zero. The ODEs were numerically solved using Matlab™.

[00138] For calculations of the time to emergence of escape mutants, an analytic approach[7] previously used to calculate fitness differences between viral strains in patients and the time to emergence of GCV-resistant mutants was utilized. Briefly, the equation $s = \frac{1}{t}\ln\left[\frac{q(t)p_0}{p(t)q_0}\right]$ describes the relative fitness, s, of the population and assumes replication occurs in continuous time. The time dynamics of the proportions of most and least fit viral variants (p and q), can be calculated given initial p_0, q_0, and s, (measured at 5.6% for GCV-resistant virus[7]. The mutant arisal time (defined as the time when p(t)=q(t)) can be calculated by algebraic rearrangement $t_{mut} = \frac{1}{s}\ln\left[\frac{1-\mu}{\mu}\right]$. The μ value for TK[8] generates a $t_{mut} \sim 100$

days as observed in the clinic[7]. To predict the time to arisal for a putative C-DOT mutant that has only two single-point mutations (one in IE86 and one in the MIEP region), $t_{mut} = \frac{1}{s} \ln\left[\frac{1-\mu^2}{\mu^2}\right]$, where μ for IE86 and the MIEP region are taken from[9]. The further maximally conservative assumption is made that *s* for C-DOT escape mutants is the same as for TK-escape mutants (in reality, *s* for C-DOT mutants is far lower as it must account for the single mutants that have a 100-fold fitness cost both in presence and absence of C-DOTs).

[00139] Protein expression, EMSA and DNA binding assays

[00140] BL21 competent *E. Coli* cells (New England Biolabs Inc) were transformed with a pMALcXS plasmid encoding the C-terminus part of IE86 fused to the maltose binding protein, as described[36], and induced with isopropyl b-D thiogalactoside (IPTG) in 1L luria broth (Thermofisher Scientific) containing ampicillin (Sigma-Aldrich). Cells were pelleted at 10,000 RPM for 30 minutes at 4°C and resuspended in 40mL lysis buffer (20mM HEPES, pH 7.4, 1M NaCl, 1mM EDTA, 1mM DTT, Roche protease inhibitor cocktail). Lysozyme was added at a final concentration of 1mg/ml and cells were incubated on ice for 30 minutes, followed by addition of 1mM PMSF and sonication on ice (sonicator at 40% amplitude, 10 seconds on, 30 seconds off-6 repeats). 1mM $MgSO_4$, 0.1mg/ml DNAse (Sigma-Aldrich, St Louis, MO) and 25U/ml Benzonase (Sigma- Aldrich) were then added, cells further incubated on ice for 15-30 minutes, centrifuged at 12,000 rpm for 30 minutes at 4°C and the supernatant incubated with amylose resins (Sigma-Aldrich, St Louis, MO) for 2 hours at 4°C with gentle agitation. Batch bound resin was poured into a column and washed with 10 column volumes of lysis buffer, and eluted in elution buffer (20mM HEPES, pH 7.4°C, 250mM NaCl, 10mM Maltose, 1mM EDTA and 1mM DTT). The eluted protein was passed through a Superose 6 column (GE Healthcare Life Sciences) using gel filtration buffer (20mM HEPES, pH 7.4, 250mM NaCl and 1mM DTT) and used for EMSA or for binding assays. Briefly, digoxygenin labeled oligo probe was made using sequences for crs and Δcrs (see Table 1) as described previously[36]. Binding assays were performed by incubating dsDNA oligonucleotides of various lengths with purified MBP-IE86 for 30 minutes at room temperature and running on Superpose-6 column in the presence of gel filtration buffer and monitored oligomerized form of MBP-IE86 (at 13ml column volume on FPLC) in the presence of different sizes of C-DOT[C]. To verify the sequence specific interaction between crs and MBP-IE86, EMSAs were performed using the DIG gel-shift kit, 2nd generation (Sigma-Aldrich) according to the manufacturer's instructions. DNA oligonucleotides were obtained from Integrated DNA Technologies (San Jose, CA) (see

Table 1 for sequences) and resuspended in annealing buffer (100mM Potassium acetate; 30mM HEPES, pH 7.5). Oligonucleotides of complimentary sequences were mixed in equimolar amount, heated to 95°C for 2 minutes, and gradually cooled to 25°C over 45 minutes in a S1000 Thermocycler (Bio-Rad) and stored at –20°C.

[00141] Cell-culture conditions and flow cytometry

[00142] ARPE-19 cells were maintained in a 1:1 mixture of Dulbecco's Modified Eagle's Medium (DMEM)/F-12 (Mediatech Inc.) with 10% fetal bovine serum (FBS) (HyClone) and 50U/ml Penicillin-Streptomycin (Mediatech Inc.) at 37°C and 5% CO_2 in a humified incubator. MRC-5 fibroblasts, NIH 3T3 mouse fibroblast and Telo-RF were maintained in DMEM with 10% FBS and 50U/ml Penicillin and Streptomycin (Mediatech Inc.). Fomivirsen (NIH), Ganciclovir and Acyclovir (Sigma-Aldrich, St Louis, MO) were added to media at the indicated concentrations following virus inoculate removal. The ARPE-19 stably expressing the MIEP-IE86-GFP minimal circuit were previously described[22]. ARPE-19, Telo-RF (telomerase life extended rhesus fibroblast), NIH 3T3 mouse fibroblast and MRC-5 cell lines were obtained from ATCC. Flow cytometry was performed on BD FACS-Calibur (BD, Biosciences) at the Gladstone flow cytomtery core and analyzed suing FlowJo™ (FlowJo, LLC).

[00143] Western blot analysis

[00144] 10^6 ARPE-19 cells were centrifuged at 2,000g for 10 min at 4°C and resuspended in PBS (Sigma- Aldrich, St Louis, MO). Cells were pelleted, resuspended in ice-cold RIPA lysis buffer (Sigma-Aldrich, St Louis, MO) by vortexing and incubated at 4°C for 30 min. The lysed samples were then pelleted at 13,000 rpm for 20 min at 4°C and supernatants were assayed by western blot as previously described[24]. Briefly, 20µg of cell lysate total protein was added to 1x loading buffer (100mM Tris-HCl (pH6.8), 200mM DTT, 4% SDS, 0.1% Bromophenol blue, 20% glycerol), and boiled for 10 minutes at 95°C. The lysed samples and precision plus kaleidoscope prestained protein marker (Bio-Rad) were then loaded on 10% Mini-PROTEAN TGX precast protein gel (Bio-Rad) in duplicate and ran at 90V for 2 hours in Tris-glycine running buffer (925mM Tris, 250mM Glycine and 0.1% SDS). One gel was stained for two hours at room temperature with coomassie blue stain (1g coomassie brilliant blue (Bio-Rad), Methanol (50%[v/v]), Glacial acetic acid (10%[v/v]), final volume to 1L with Milli-Q H_2O, followed by destaining (40% methanol, 7% acetic acid in final volume of 1L in Milli-Q H_2O. The gel was blotted on a PVDF membrane using a semi-dry transfer unit (Trans-Blot Semi-Dry Electrophotetic transfer cell, Bio-Rad) at 25V for 45 minutes. The membrane was blocked with Li-Cor Odyssey™

blocking buffer for 2 hours at room temperature with gentle agitation. IE86 protein was detected by a 2-hour incubation of the membrane with an anti-IE86 antibody (1:100 dilution, Mab 810, Chemicon) in Li-Cor Odyssey™ blocking buffer at room temperature followed by 3 5-minute wash steps (1x PBS + 0.01% Tween-20). The membrane was then incubated with a secondary Li-Cor detection antibody (1:20,000 dilution, goat anti-mouse 800CW) for 1 hour in the dark, washed three times in wash buffer (1x PBS + 0.01% Tween-20) and imaged on an Odyssey system (Li-Cor).

[00145] Viral Replication Kinetics

[00146] The CMV TB40E-IE86-YFP virus was previously described[24] and CMV AD169, GDGrK17, CMV GDGrP53, CMV 759rD100-1, CMV PFArD100 were obtained through the NIH AIDS Reagent Program. MCMV strain K181[37] was kindly provided by Lewis Lanier (UCSF) and Rhesus CMV 68.1 EGFP virus[38] was kindly provided by Peter Barry, UC-Davis. The clinical strain of HSV-1 17syn+ IE175-YFP[39] was kindly provided by Roger Everett, MRC Virology Unit, Glasgow, Scotland that was passaged originally from a clinical isolate[40]. $1x10^6$ ARPE-19 cells were nucleofected with C-DOT^C, C-DOT^H or C-DOT^{Scram} using Amaxa nucleofector (Lonza). 24 hours post-nucleofection cells were infected in triplicate with 0.1 MOI of CMV (TB40E IE86-YFP) or HSV-1 (17syn+ IE175-YFP). Four days post infection, cells were harvested and subjected to three freeze-thaw cycles and used for 10-fold serial dilution titration on naïve ARPE-19 cells. As similar protocol was used to nucleofect MRC-5 cells prior to infection with AD169 and titration on naïve MRC-5 cells. Titration results were converted to PFU/ml using median tissue culture infectious dose (TCID50)[41]. Similarly, corneal tissues were dissociated using collagenase I (Sigma-Aldrich, St Louis, MO) for 30 minutes, subjected to three freeze-thaw cycles and used for TCID50 analysis.

[00147] qPCR, flow cytometry and fluorescent microscopy

[00148] Genomic DNA from CMV or HSV-1 infected ARPE-19 cells or mouse corneal tissue dissociated with collagenase I for 30 minutes was extracted using DNeasy Blood and tissue kit (Qiagen). TLR9 expression analysis was performed by total cell RNA extraction using an RNEASY RNA isolation kit (74104; QIAGEN) and reverse transcription using a QuantiTet Reverse Transcription kit (205311; QIAGEN). Relative quantification of genomic DNA or cDNA was performed on a 7900HT Fast Real-Time PCR System (ThermoFisher Scientific, 4329003) using sequence specific primers (Table 1) and Fast SYBR Green Master Mix (Applied Biosystems, 4385612). To measure the mean fluorescent intensity of YFP, flow cytometry was performed on trypsinized cells using BD FACS

Calibur (BD Biosciences). Fluorescent microscopy of the cornea infected with HSV-1 was performed using Leica Zeiss fluorescent microscope for DAPI (excitation at 345nm) and YFP (excitation at 514 nm). Images were analyzed using Image J software at threshold 100 for DAPI and YFP mask images, followed by particle analysis at size 2 micron^2 for YFP and 10micron^2 for DAPI.

[00149] Corneal infection assays in mice

[00150] All experiments were performed with 6- to 10-week old male and female sibling Black 6 mice. Breeding pairs were purchased from Jackson laboratories (Bar Harbor, Maine) and maintained under pathogen-free conditions in the UCSF barrier facility. All animal experiments were conducted in accordance with procedures approved by the UCSF Institutional Animal Care and Use Committee. Corneal epithelial debridement was performed on mice as previously described[42]. Briefly, mice were anesthetized by isofluorane inhalation (Abbott Laboratories, Alameda, CA). The central part of the epithelium was removed down to the basement membrane using an Algerbrush II (Katena Products, Inc., Denville, NJ). 5µl of HSV-1 17syn+ YFP-IE175 (10^5 pfu) were immediately applied to the debrided cornea. 6h later, mice were anesthetized again and 5µl of C-DOT^{H}, C-DOT^{scram} or PBS were applied to the cornea for 5 min. At the indicated time post infection, eyes were enucleated and the corneas were dissected to remove the lens, iris, and retina. Four incisions were made equal distances apart to aid in flattening the corneas. Fresh corneas were counterstained using 0.5µg/ml DAPI, mounted on slides with Fluoro-gel (Electron Microscopy Sciences, Hatfield, PA) and imaged using Leica Zeiss Confocal microscopy. Corneas were dissociated using collagenase I (Sigma-Aldrich, St Louis, MO), total DNA was extracted using DNeasy Blood & Tissue kit (QIAGEN), and subjected to qPCR using Fast SYBR green master mix (4385612; Applied Biosystems), analyzed on a 7900HT Fast Real-Time PCR System (4329003; Thermofisher Scientific). Virus titer was determined as described above.

[00151] Statistical analysis

[00152] Statistical differences were determined by using the two-tailed unpaired Student's *t* test (GraphPad Prism, La Jolla, CA). A p-value less than 0.05 was considered statistically significant: *<0.05, **<0.01, ***<0.001, ****<0.0001, ns: not significant.

[00153] Oligonucleotide preparation, modification, and nanoparticle construction

[00154] C-DOT^{C}, C-DOT^{H}, C-DOT^{MCMV,} C-DOT^{RhCMV}, cy3- C-DOT^{C} were made by annealing sequence specific oligonucleotides (see Table 1 for sequences). Briefly, DNA oligonucleotides were obtained from Integrated DNA Technologies (San Jose, CA) and

resuspended in annealing buffer (100mM Potassium acetate; 30mM HEPES, pH 7.5). Oligonucleotides of complimentary sequences were mixed in equimolar amount, heated to 95°C for 2 minutes, and gradually cooled to 25°C over the period of 45 minutes in a S1000 Thermocycler (Bio-Rad) and stored at –20°C. Spherical nucleic acids (cy3-C-DOT^{C}-SNA) were made using 3' thiol labeled C-DOT^{C} forward strand and 5' cyanine 3- phosphoramidite (cy3) tagged reverse strand oligo obtained from Bioneer, Inc (Alameda, CA) (see Table 1 for sequences). Oligonucleotides were annealed as mentioned above and cy3-C-DOT-SNA were made as described previously[43]. Briefly, annealed oligonucleotides were added to 10nm citrate stabilized gold nanoparticle (Sigma-Aldrich, St Louis, MO) (~3nmol oligonucleotide per 1ml of 10nM colloid). After 20 minutes, 10% SDS (Sigma- Aldrich, St Louis, MO) was added to bring down the concentration of SDS to 0.1% in phosphate buffer (0.01M, pH 7.4). 2.0M NaCl was then added to bring salt concentration to 0.1M. Oligonucleotides were incubated under shaking conditions at room temperature for 30 min, followed by two successive additions of 2.0M NaCl at 30-minute interval to bring the final concentration of NaCl to 0.3M. The final mixture was incubated under shaking conditions to complete the functionalization of oligonucleotides on gold nanoparticles. The cy3-C-DOT-SNA was recovered by three centrifugation steps (13,000 rpm, 20 minutes), resuspended in 1x PBS buffer and stored at 4°C until used.

Table 1. List of oligonucleotides and primers used in the study

Name	**Sequence**
crs	Fw:TAATACGACTCACTATAGGGCGAATTGGAGCTCGTTTAGTGA ACCGTCAGATCTCTAGAAGCTT (SEQ ID NO: 12) Rw:AAGCTTCTAGAGATCTGACGGTTCACTAAACGAGCTCCAATT CGCCCTATAGTGAGTCGTATTA (SEQ ID NO: 13)
Dcrs	Fw:TAATACGACTCACTATAGGGCGAATTGGAGCTCGGCAGGCAT GCAAGCTT (SEQ ID NO: 14) Rw:AAGCTTGCATGCCTGCCGAGCTCCAATTCGCCCTATAGTGAG TCGTATTA (SEQ ID NO: 15)
21bp DNA	Fw: AGATCGTTTAGTGAACCGTAC (SEQ ID NO: 16) Rw: GTACGGTTCACTAAACGATCT (SEQ ID NO: 17)
28bp DNA	Fw: GACAGATCGTTTAGTGAACCGTACACGA (SEQ ID NO: 1) Rw: TCGTGTACGGTTCACTAAACGATCTGTC (SEQ ID NO: 18)
28bp Scramble	Fw: ATACCGCGTAACCAGAGGTATATAGGTC (SEQ ID NO: 19) Rw: GACCTATATACCTCTGGTTACGCGGTAT (SEQ ID NO: 20)
34bp DNA	Fw: CGTTTAGTGAACCGCGCGCGATAGTACATAATCA (SEQ ID NO: 21) Rw: TGATTATGTACTATCGCGCGCGGTTCACTAAACG (SEQ ID NO: 22)
64bp DNA	Fw:TAATACGACTCACTATAGGGCGAATTGGAGCTCGTTTAGTGA ACCGTCAGATCTCTAGAAGCTT (SEQ ID NO: 12)

	Rw:AAGCTTCTAGAGATCTGACGGTTCACTAAACGAGCTCCAATT CGCCCTATAGTGAGTCGTATTA (SEQ ID NO: 13)
C-DOT^{C}	Fw: G*ACAGATCGTT*TAGTGAACC*GTACACG*A (SEQ ID NO: 5) Rw: T*CGTGTAC*GGTTCACTA*AACGATCTGT*C (SEQ ID NO: 39)
C-DOT^{Scram}	Fw: A*TACCGCGTAA*CCAGAGGT*ATATAGGT*C (SEQ ID NO: 40) Rw: G*ACCTATAT*ACCTCTGG*TTACGCGGTA*T (SEQ ID NO: 41)
C-DOT^{RhCMV}	Fw: G*ACAGATCGT*TTAGGGAAC*CGTACACG*A (SEQ ID NO: 8) Rw: T*CGTGTACG*GTTCCCTAA*ACGATCTGT*C (SEQ ID NO: 43)
C-DOT^{MCMV}	Fw: G*ACAGACCA*GCGTCGG*TACCGTACACG*A (SEQ ID NO: 9) Rw: T*CGTGTACGGT*ACCGACGC*TGGTCTGT*C (SEQ ID NO: 42)
cy3-C-DOT^{C}	Fw: /5Cy3/GACAGATCGTTTAGTGAACCGTACACGA (SEQ ID NO: 23) Rw: TCGTGTACGGTTCACTAAACGATCTGTC (SEQ ID NO: 18)
C-DOT^{C} (1x) Concat	Fw: CGTTTAGTGAACCGCGCGCGATAGTACATAATCA (SEQ ID NO: 21) Rw: TGATTATGTACTATCGCGCGCGGTTCACTAAACG (SEQ ID NO: 22)
C-DOT^{C} (2x) Concat	Fw: CGTTTAGTGAACCGCGCGCGCGTTTAGTGAACCG (SEQ ID NO: 24) Rw: CGGTTCACTAAACGCGCGCGCGGTTCACTAAACG (SEQ ID NO: 25)
C-DOT^{H}	Fw: CCG*AGGAC*GCCCCGATC*GTCCACACG*GAG (SEQ ID NO: 11) Rw: CTC*CGTGTGGAC*GATCGGGGC*GTCCT*CGG (SEQ ID NO: 26)
cy3-C-DOT^{H}	Fw: /5Cy3/CCG*AGGAC*GCCCCGATC*GTCCACACG*GAG (SEQ ID NO: 44) Rw: CTC*CGTGTGGAC*GATCGGGGC*GTCCT*CGG (SEQ ID NO: 26)
ODN 2216 FW	Fw: GGGGGACGATCGTCGGGGGG (SEQ ID NO: 27) Rw: CCCCCCGACGATCGTCCCCC (SEQ ID NO: 28)
TLR9-qPCR	Fw: CCGTGACAATTACCTGGCCTTC (SEQ ID NO: 29) Rw: CAGGGCCTTCAGCTGGTTTC (SEQ ID NO: 30)
MIEP 250bp crs	Fw: TTCCTACTTGGCAGTACATCTAC (SEQ ID NO: 31) Rw: CCTATAGGCTAAGCTATACCATC (SEQ ID NO: 32)
IE86 Exon 5	Fw: TGACATCCTCGCCCAGG (SEQ ID NO: 33) Rw: TTACTGAGACTTGTTCCTCAGGT (SEQ ID NO: 34)
IE86-qPCR	Fw: TGACCGAGGATTGCAACGA (SEQ ID NO: 35) Rw: CGGCATGATTGACAGCCTG (SEQ ID NO: 36)
IE175-qPCR	Fw: CCTATAGGCTAAGCTATACCATC (SEQ ID NO: 32) Rw: GTCTGACGGTCTGTCTCTGG (SEQ ID NO: 37)
GAPDH-qPCR	Fw: GTCTGACGGTCTGTCTCTGG (SEQ ID NO: 37) Rw: CAAGAAGATGCGGCTGTCTC (SEQ ID NO: 38)

* Phosphorothioate bond

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[00155] While the present invention has been described with reference to the specific embodiments thereof, it should be understood by those skilled in the art that various changes may be made and equivalents may be substituted without departing from the true spirit and scope of the invention. In addition, many modifications may be made to adapt a particular

situation, material, composition of matter, process, process step or steps, to the objective, spirit and scope of the present invention. All such modifications are intended to be within the scope of the claims appended hereto.

CLAIMS

What is claimed is:

1. A method for inhibiting replication of a herpesvirus in a cell infected with a herpesvirus, the method comprising contacting the cell with a double stranded DNA molecule comprising a sequence of a cis regulatory sequence (crs) of the herpesvirus, wherein the crs is flanked on the 5′ end by a first sequence of at least 2 base pairs and on the 3′ end by a second sequence of at least 2 base pairs.

2. The method of claim 1, wherein the crs is flanked on the 5′ end by a first sequence of at least 7 base pairs and on the 3′ end by a second sequence of at least 7 base pairs.

3. The method of claim 1 or 2, wherein one or both of the first sequence of at least 2 base pairs and the second sequence of at least 2 base pairs does not have a DNA sequence of a naturally occurring herpesvirus.

4. The method of any one of claims 1-3, wherein the herpesvirus is selected from cytomegalovirus (CMV), herpes simplex virus-1 (HSV-1), herpes simplex virus-2 (HSV-2), varicella zoster virus, Epstein-Barr virus (EBV), and Kaposi's sarcoma-associated herpesvirus (KSHV).

5. The method of claim 4, wherein the herpesvirus is CMV.

6. The method of claim 5, wherein the herpesvirus is HCMV and the sequence of the herpesvirus crs comprises CGTTTAGTGAACCG (SEQ ID NO: 4).

7. The method of claim 5, wherein the sequence of the herpesvirus crs has at least 90% sequence identity to the sequence of the herpesvirus crs of HCMV.

8. The method of claim 6, wherein the sequence of the herpesvirus crs flanked on the 5′ end by a first sequence of at least 2 base pairs and on the 3′ end by a second sequence of at least 2 base pairs comprises GACAGATCGTTTAGTGAACCGTACACGA (SEQ ID NO: 1).

9. The method of claim 8, wherein the sequence of the herpesvirus crs flanked on the 5′ end by a first sequence of at least 2 base pairs and on the 3′ end by a second sequence of at least 2 base pairs comprises G*ACAGATCGTT*TAGTGAACC*GTACACG*A (SEQ ID NO: 5), wherein * is a phosphorothioated bond.

10. The method of claim 4, wherein the herpesvirus is HSV-1.

11. The method of claim 10, wherein the sequence of the herpesvirus crs comprises CGCCCCGATCGTCCA (SEQ ID NO: 10).

12. The method of claim 10, wherein the sequence of the herpesvirus crs is at least 90% sequence identity to the sequence of the herpesvirus crs of HSV-1.

13. The method of claim 11, wherein the sequence of the herpesvirus crs flanked on the 5′ end by a first sequence of at least 2 base pairs and on the 3′ end by a second sequence of at least 2 base pairs comprises CCGAGGACGCCCCGATCGTCCACACGGAG (SEQ ID NO: 3).

14. The method of claim 13, the sequence of the herpesvirus crs flanked on the 5′ end by a first sequence of at least 2 base pairs and on the 3′ end by a second sequence of at least 2 base pairs comprises CCG*AGGAC*GCCCCGATC*GTCCACACG*GAG (SEQ ID NO: 11), wherein * is a phosphorothioated bond.

15. The method of any one of claims 1-14, wherein the double stranded DNA molecule comprises a concatamer comprising one or more copies of a sequence comprising the sequence of the herpesvirus crs flanked on the 5′ end by a first sequence of at least 2 base pairs and on the 3′ end by a second sequence of at least 2 base pairs.

16. The method of claim 15, wherein the sequence of the herpesvirus crs is flanked on the 5′ end by a first sequence of at least 7 base pairs and on the 3′ end by a second sequence of at least 7 base pairs.

17. The method of any one of claims 1-16, wherein one or more of the bases of the double stranded DNA molecule comprises a chemical modification.

18. The method of claim 17, wherein the chemical modification is a phosphorothioated bond.

19. A method for inhibiting replication of one or more herpesviruses in a cell infected with one or more herpesviruses, the method comprising contacting the cell with a double stranded DNA molecule comprising a sequence comprising:

a) a sequence of a cis regulatory sequence (crs) of a first herpesvirus, wherein the crs is flanked on the 5′ end by a first sequence of at least 2 base pairs and on the 3′ end by a second sequence of at least 2 base pairs; and

b) a sequence of a crs of a second herpesvirus, wherein the crs is flanked on the 5′ end by a first sequence of at least 2 base pairs and on the 3′ end by a second sequence of at least 2 base pairs.

20. The method of claim 19, wherein the sequence of the crs of the first herpesvirus is flanked on the 5′ end by a first sequence of at least 7 base pairs and on the 3′ end by a second sequence of at least 7 base pairs, and wherein the sequence of the crs of the second herpesvirus is flanked on the 5′ end by a first sequence of at least 7 base pairs and on the 3′ end by a second sequence of at least 7 base pairs.

21. The method of claim 19 or 20, wherein one or both of the first sequence of at least 2 base pairs and the second sequence of at least 2 base pairs flanking the crs of the first herpesvirus does not have a DNA sequence of a naturally occurring herpesvirus, and wherein one or both of the first sequence of at least 2 base pairs and the second sequence of at least 2 base pairs flanking the crs of the second herpesvirus does not have a DNA sequence of a naturally occurring herpesvirus.

22. The method of any one of claims 19-21, wherein the first herpesvirus and second herpesvirus are selected from cytomegalovirus (CMV), herpes simplex virus-1 (HSV-1), herpes simplex virus-2 (HSV-2), varicella zoster virus, Epstein-Barr virus (EBV), and Kaposi's sarcoma-associated herpesvirus (KSHV).

23. The method of claim 19, wherein the first herpesvirus and the second herpesvirus are different.

24. The method of claim 22, wherein the first herpesvirus is CMV.

25. The method of claim 24, wherein the first herpesvirus is HCMV and the sequence of the first herpesvirus crs comprises CGTTTAGTGAACCG (SEQ ID NO: 4).

26. The method of claim 24, wherein the sequence of the first herpesvirus crs has at least 90% sequence identity to the sequence of the herpesvirus crs of HCMV.

27. The method of claim 25, wherein the sequence of the first herpesvirus crs flanked on the 5′ end by a first sequence of at least 2 base pairs and on the 3′ end by a second sequence of at least 2 base pairs comprises GACAGATCGTTTAGTGAACCGTACACGA (SEQ ID NO: 1).

28. The method of claim 27, wherein the sequence of the first herpesvirus crs flanked on the 5′ end by a first sequence of at least 2 base pairs and on the 3′ end by a second sequence of at least 2 base pairs comprises G*ACAGATCGTT*TAGTGAACC*GTACACG*A (SEQ ID NO: 5), wherein * is a phosphorothioated bond.

29. The method of claim 22, wherein the second herpesvirus is HSV-1.

30. The method of claim 29, wherein the sequence of the second herpesvirus crs comprises CGCCCCGATCGTCCA (SEQ ID NO: 10).

31. The method of claim 29, wherein the sequence of the second herpesvirus crs is at least 90% sequence identity to the sequence of the herpesvirus crs of HSV-1.

32. The method of claim 30, wherein the sequence of the second herpesvirus crs flanked on the 5′ end by a first sequence of at least 2 base pairs and on the 3′ end by a second sequence of at least 2 base pairs comprises CCGAGGACGCCCCGATCGTCCACACGGAG (SEQ ID NO: 3).

33. The method of claim 13, the sequence of the second herpesvirus crs flanked on the 5′ end by a first sequence of at least 2 base pairs and on the 3′ end by a second sequence of at least 2 base pairs comprises CCG*AGGAC*GCCCCGATC*GTCCACACG*GAG (SEQ ID NO: 11), wherein * is a phosphorothioated bond.

34. The method of any one of claims 19-33, wherein the double stranded DNA molecule comprises a concatamer comprising one or more copies of a sequence comprising the sequence of the first herpesvirus crs flanked on the 5′ end by a first sequence of at least 2

base pairs and on the 3′ end by a second sequence of at least 2 base pairs; and the sequence of a crs of a second herpesvirus, wherein the crs is flanked on the 5′ end by a first sequence of at least 2 base pairs and on the 3′ end by a second sequence of 2 base pairs.

35. The method of claim 34, wherein the sequence of the first herpesvirus crs is flanked on the 5′ end by a first sequence of at least 7 base pairs and on the 3′ end by a second sequence of at least 7 base pairs, and wherein the sequence of the second herpesvirus crs is flanked on the 5′ end by a first sequence of at least 7 base pairs and on the 3′ end by a second sequence of at least 7 base pairs.

36. The method of any one of claims 19-35, wherein one or more of the bases of the double stranded DNA molecule comprises a chemical modification.

37. The method of claim 36, wherein the chemical modification is a phosphorothioated bond.

38. A method of treating a herpesvirus infection in an individual, the method comprising administering to the individual an effective amount of a double stranded DNA molecule comprising a sequence comprising a sequence of a cis regulatory sequence (crs) of the herpesvirus, wherein the crs is flanked on the 5′ end by a first sequence of 2 base pairs and on the 3′ end by a second sequence of 2 base pairs.

39. The method of claim 38, wherein the crs is flanked on the 5′ end by a first sequence of at least 7 base pairs and on the 3′ end by a second sequence of at least 7 base pairs.

40. The method of claim 38 or 39, wherein one or both of the first sequence of at least 2 base pairs and the second sequence of at least 2 base pairs does not have a DNA sequence of a naturally occurring herpesvirus.

41. The method of any one of claims 38-40, wherein the herpesvirus is selected from cytomegalovirus (CMV), herpes simplex virus-1 (HSV-1), herpes simplex virus-2 (HSV-2), varicella zoster virus, Epstein-Barr virus (EBV), and Kaposi's sarcoma-associated herpesvirus (KSHV).

42. The method of claim 41, wherein the herpesvirus is CMV.

43. The method of claim 42, wherein the herpesvirus is HCMV and the sequence of the herpesvirus crs comprises CGTTTAGTGAACCG (SEQ ID NO: 4).

44. The method of claim 42, wherein the sequence of the herpesvirus crs has at least 90% sequence identity to the sequence of the herpesvirus crs of HCMV.

45. The method of claim 43, wherein the sequence of the herpesvirus crs flanked on the 5′ end by a first sequence of at least 2 base pairs and on the 3′ end by a second sequence of at least 2 base pairs comprises GACAGATCGTTTAGTGAACCGTACACGA (SEQ ID NO: 1).

46. The method of claim 45, wherein the sequence of the herpesvirus crs flanked on the 5′ end by a first sequence of at least 2 base pairs and on the 3′ end by a second sequence of at least 2 base pairs comprises G*ACAGATCGTT*TAGTGAACC*GTACACG*A (SEQ ID NO: 5), wherein * is a phosphorothioated bond.

47. The method of claim 41, wherein the herpesvirus is HSV-1.

48. The method of claim 47, wherein the sequence of the herpesvirus crs comprises CGCCCCGATCGTCCA (SEQ ID NO: 10).

49. The method of claim 47, wherein the sequence of the herpesvirus crs is at least 90% sequence identity to the sequence of the herpesvirus crs of HSV-1.

50. The method of claim 48, wherein the sequence of the herpesvirus crs flanked on the 5′ end by a first sequence of at least 2 base pairs and on the 3′ end by a second sequence of at least 2 base pairs comprises CCGAGGACGCCCCGATCGTCCACACGGAG (SEQ ID NO: 3).

51. The method of claim 50, the sequence of the herpesvirus crs flanked on the 5′ end by a first sequence of at least 2 base pairs and on the 3′ end by a second sequence of at least 2 base pairs comprises CCG*AGGAC*GCCCCGATC*GTCCACACG*GAG (SEQ ID NO: 11), wherein * is a phosphorothioated bond.

52. The method of any one of claims 38-51, wherein the double stranded DNA molecule comprises a concatamer comprising one or more copies of a sequence comprising the

sequence of the herpesvirus crs flanked on the 5′ end by a first sequence of at least 2 base pairs and on the 3′ end by a second sequence of at least 2 base pairs.

53. The method of claim 52, wherein the sequence of the herpesvirus crs is flanked on the 5′ end by a first sequence of at least 7 base pairs and on the 3′ end by a second sequence of at least 7 base pairs.

54. The method of any one of claims 38-53, wherein one or more of the bases of the double stranded DNA molecule comprises a chemical modification.

55. The method of claim 54, wherein the chemical modification is a phosphorothioated bond.

56. The method of any one of claims 38-55, wherein the individual is a human.

57. The method of any one of claims 38-56, wherein the individual is an organ transplant recipient.

58. The method of any one of claims 38-56, wherein the individual is a bone marrow transplant recipient.

59. The method of any one of claims 38-56, wherein the individual does not have a herpesvirus infection, and is a prospective organ transplant recipient.

60. The method of any one of claims 38-56, wherein the individual does not have a herpesvirus infection, and is a prospective bone marrow transplant recipient.

61. The method of any one of claims 38-56, wherein the individual is a pregnant female.

62. The method of any one of claims 38-56, wherein the individual is a neonate.

63. The method of any one of claims 38-62, the method further comprising administering at least a second therapeutic agent.

64. The method of claim 63, wherein the second therapeutic agent is ganciclovir, foscarnet, cidofovir, maribavir, or valganciclovir.

65. The method of claim 63, wherein the second therapeutic agent is a histone deacetylase (HDAC) inhibitor.

66. The method of claim 63, wherein the second therapeutic agent is a transcriptional transactivator.

67. A method of treating a herpesvirus infection in an individual, the method comprising administering to the individual an effective amount of a double stranded DNA molecule comprising a sequence comprising:

a) a sequence of a cis regulatory sequence (crs) of a first herpesvirus, wherein the crs is flanked on the 5′ end by a first sequence of at least 2 base pairs and on the 3′ end by a second sequence of at least 2 base pairs; and

b) a sequence of a crs of a second herpesvirus, wherein the crs is flanked on the 5′ end by a first sequence of at least 2 base pairs and on the 3′ end by a second sequence of at least 2 base pairs.

68. The method of claim 67, wherein the sequence of the crs of the first herpesvirus is flanked on the 5′ end by a first sequence of at least 7 base pairs and on the 3′ end by a second sequence of at least 7 base pairs, and wherein the sequence of the crs of the second herpesvirus is flanked on the 5′ end by a first sequence of at least 7 base pairs and on the 3′ end by a second sequence of at least 7 base pairs.

69. The method of claim 67 or 68, wherein one or both of the first sequence of at least 2 base pairs and the second sequence of at least 2 base pairs flanking the crs of the first herpesvirus does not have a DNA sequence of a naturally occurring herpesvirus, and wherein one or both of the first sequence of at least 2 base pairs and the second sequence of at least 2 base pairs flanking the crs of the second herpesvirus does not have a DNA sequence of a naturally occurring herpesvirus.

70. The method of any one of claims 67-69, wherein the first herpesvirus and second herpesvirus are selected from cytomegalovirus (CMV), herpes simplex virus-1 (HSV-1), herpes simplex virus-2 (HSV-2), varicella zoster virus, Epstein-Barr virus (EBV), and Kaposi's sarcoma-associated herpesvirus (KSHV).

71. The method of claim 67, wherein the first herpesvirus and the second herpesvirus are different.

72. The method of claim 70, wherein the first herpesvirus is CMV.

73. The method of claim 72, wherein the first herpesvirus is HCMV and the sequence of the first herpesvirus crs comprises CGTTTAGTGAACCG (SEQ ID NO: 4).

74. The method of claim 72, wherein the sequence of the first herpesvirus crs has at least 90% sequence identity to the sequence of the herpesvirus crs of HCMV.

75. The method of claim 73, wherein the sequence of the first herpesvirus crs flanked on the 5′ end by a first sequence of at least 2 base pairs and on the 3′ end by a second sequence of at least 2 base pairs comprises GACAGATCGTTTAGTGAACCGTACACGA (SEQ ID NO: 1).

76. The method of claim 75, wherein the sequence of the first herpesvirus crs flanked on the 5′ end by a first sequence of at least 2 base pairs and on the 3′ end by a second sequence of at least 2 base pairs comprises G*ACAGATCGTT*TAGTGAACC*GTACACG*A (SEQ ID NO: 5), wherein * is a phosphorothioated bond.

77. The method of claim 70, wherein the second herpesvirus is HSV-1.

78. The method of claim 77, wherein the sequence of the second herpesvirus crs comprises CGCCCCGATCGTCCA (SEQ ID NO: 10).

79. The method of claim 77, wherein the sequence of the second herpesvirus crs is at least 90% sequence identity to the sequence of the herpesvirus crs of HSV-1.

80. The method of claim 78, wherein the sequence of the second herpesvirus crs flanked on the 5′ end by a first sequence of at least 2 base pairs and on the 3′ end by a second sequence of at least 2 base pairs comprises CCGAGGACGCCCCGATCGTCCACACGGAG (SEQ ID NO: 3).

81. The method of claim 80, the sequence of the second herpesvirus crs flanked on the 5′ end by a first sequence of at least 2 base pairs and on the 3′ end by a second sequence of at

least 2 base pairs comprises CCG*AGGAC*GCCCCGATC*GTCCACACG*GAG (SEQ ID NO: 11), wherein * is a phosphorothioated bond.

82. The method of any one of claims 67-81, wherein the double stranded DNA molecule comprises a concatamer comprising one or more copies of a sequence comprising the sequence of the first herpesvirus crs flanked on the 5′ end by a first sequence of at least 2 base pairs and on the 3′ end by a second sequence of at least 2 base pairs; and the sequence of a crs of a second herpesvirus, wherein the crs is flanked on the 5′ end by a first sequence of at least 2 base pairs and on the 3′ end by a second sequence of 2 base pairs.

83. The method of claim 82, wherein the sequence of the first herpesvirus crs is flanked on the 5′ end by a first sequence of at least 7 base pairs and on the 3′ end by a second sequence of at least 7 base pairs, and wherein the sequence of the second herpesvirus crs is flanked on the 5′ end by a first sequence of at least 7 base pairs and on the 3′ end by a second sequence of at least 7 base pairs.

84. The method of any one of claims 67-83, wherein one or more of the bases of the double stranded DNA molecule comprises a chemical modification.

85. The method of claim 84, wherein the chemical modification is a phosphorothioated bond.

86. The method of any one of claims 67-85, wherein the individual is a human.

87. The method of any one of claims 67-86, wherein the individual is an organ transplant recipient.

88. The method of any one of claims 67-86, wherein the individual is a bone marrow transplant recipient.

89. The method of any one of claims 67-86, wherein the individual does not have a herpesvirus infection, and is a prospective organ transplant recipient.

90. The method of any one of claims 67-86, wherein the individual does not have a herpesvirus infection, and is a prospective bone marrow transplant recipient.

91. The method of any one of claims 67-86, wherein the individual is a pregnant female.

92. The method of any one of claims 67-86, wherein the individual is a neonate.

93. The method of any one of claims 67-92, the method further comprising administering at least a second therapeutic agent.

94. The method of claim 93, wherein the second therapeutic agent is ganciclovir, foscarnet, cidofovir, maribavir, or valganciclovir.

95. The method of claim 93, wherein the second therapeutic agent is a histone deacetylase (HDAC) inhibitor.

96. The method of claim 93, wherein the second therapeutic agent is a transcriptional transactivator.

97. A method of inhibiting herpesvirus replication in an organ or tissue, the method comprising contacting the organ or tissue *in vitro* or *ex vivo* with a double stranded DNA molecule comprising a sequence comprising a sequence of a cis regulatory sequence (crs) of the herpesvirus, wherein the crs is flanked on the 5′ end by a first sequence of at least 2 base pairs and on the 3′ end by a second sequence of at least 2 base pairs.

98. The method of claim 97, wherein the crs is flanked on the 5′ end by a first sequence of at least 7 base pairs and on the 3′ end by a second sequence of at least 7 base pairs.

99. The method of claim 97 or 98, wherein one or both of the first sequence of at least 2 base pairs and the second sequence of at least 2 base pairs does not have a DNA sequence of a naturally occurring herpesvirus.

100. The method of any one of claims 97-99, wherein the herpesvirus is selected from cytomegalovirus (CMV), herpes simplex virus-1 (HSV-1), herpes simplex virus-2 (HSV-2), varicella zoster virus, Epstein-Barr virus (EBV), and Kaposi's sarcoma-associated herpesvirus (KSHV).

101. The method of claim 100, wherein the herpesvirus is CMV.

102. The method of claim 101, wherein the herpesvirus is HCMV and the sequence of the herpesvirus crs comprises CGTTTAGTGAACCG (SEQ ID NO: 4).

103. The method of claim 101, wherein the sequence of the herpesvirus crs has at least 90% sequence identity to the sequence of the herpesvirus crs of HCMV.

104. The method of claim 102, wherein the sequence of the herpesvirus crs flanked on the 5′ end by a first sequence of at least 2 base pairs and on the 3′ end by a second sequence of at least 2 base pairs comprises GACAGATCGTTTAGTGAACCGTACACGA (SEQ ID NO: 1).

105. The method of claim 104, wherein the sequence of the herpesvirus crs flanked on the 5′ end by a first sequence of at least 2 base pairs and on the 3′ end by a second sequence of at least 2 base pairs comprises G*ACAGATCGTT*TAGTGAACC*GTACACG*A (SEQ ID NO: 5), wherein * is a phosphorothioated bond.

106. The method of claim 100, wherein the herpesvirus is HSV-1.

107. The method of claim 106, wherein the sequence of the herpesvirus crs comprises CGCCCCGATCGTCCA (SEQ ID NO: 10).

108. The method of claim 106, wherein the sequence of the herpesvirus crs is at least 90% sequence identity to the sequence of the herpesvirus crs of HSV-1.

109. The method of claim 107, wherein the sequence of the herpesvirus crs flanked on the 5′ end by a first sequence of at least 2 base pairs and on the 3′ end by a second sequence of at least 2 base pairs comprises CCGAGGACGCCCCGATCGTCCACACGGAG (SEQ ID NO: 3).

110. The method of claim 109, the sequence of the herpesvirus crs flanked on the 5′ end by a first sequence of at least 2 base pairs and on the 3′ end by a second sequence of at least 2 base pairs comprises CCG*AGGAC*GCCCCGATC*GTCCACACG*GAG (SEQ ID NO: 11), wherein * is a phosphorothioated bond.

111. The method of any one of claims 97-110, wherein the double stranded DNA molecule comprises a concatamer comprising one or more copies of a sequence comprising the sequence of the herpesvirus crs flanked on the 5′ end by a first sequence of at least 2 base pairs and on the 3′ end by a second sequence of at least 2 base pairs.

112. The method of claim 111, wherein the sequence of the herpesvirus crs is flanked on the 5′ end by a first sequence of at least 7 base pairs and on the 3′ end by a second sequence of at least 7 base pairs.

113. The method of any one of claims 97-112, wherein one or more of the bases of the double stranded DNA molecule comprises a chemical modification.

114. The method of claim 113, wherein the chemical modification is a phosphorothioated bond.

115. The method of claim 97, wherein the organ or tissue is contacted *in vitro* or *ex vivo* with the double stranded DNA molecule comprising the sequence comprising the sequence of the cis regulatory sequence (crs) of the herpesvirus for a period of time of from about 15 minutes to about 48 hours, wherein the crs is flanked on the 5′ end by a first sequence of at least 2 base pairs and on the 3′ end by a second sequence of at least 2 base pairs.

116. The method of claim 115, wherein the organ or tissue is contacted *in vitro* or *ex vivo* with the double stranded DNA molecule in a liquid medium.

117. A method of inhibiting herpesvirus replication in an organ or tissue, the method comprising contacting the organ or tissue *in vitro* or *ex vivo* with a double stranded DNA molecule comprising:

a) a sequence of a cis regulatory sequence (crs) of a first herpesvirus, wherein the crs is flanked on the 5′ end by a first sequence of at least 2 base pairs and on the 3′ end by a second sequence of at least 2 base pairs; and

b) a sequence of a crs of a second herpesvirus, wherein the crs is flanked on the 5′ end by a first sequence of at least 2 base pairs and on the 3′ end by a second sequence of at least 2 base pairs.

118. The method of claim 117, wherein the sequence of the crs of the first herpesvirus is flanked on the 5′ end by a first sequence of at least 7 base pairs and on the 3′ end by a second sequence of at least 7 base pairs, and wherein the sequence of the crs of the second herpesvirus is flanked on the 5′ end by a first sequence of at least 7 base pairs and on the 3′ end by a second sequence of at least 7 base pairs.

119. The method of claim 117 or 118, wherein one or both of the first sequence of at least 2 base pairs and the second sequence of at least 2 base pairs flanking the crs of the first herpesvirus does not have a DNA sequence of a naturally occurring herpesvirus, and wherein one or both of the first sequence of at least 2 base pairs and the second sequence of at least 2 base pairs flanking the crs of the second herpesvirus does not have a DNA sequence of a naturally occurring herpesvirus.

120. The method of any one of claims 117-119, wherein the first herpesvirus and second herpesvirus are selected from cytomegalovirus (CMV), herpes simplex virus-1 (HSV-1), herpes simplex virus-2 (HSV-2), varicella zoster virus, Epstein-Barr virus (EBV), and Kaposi's sarcoma-associated herpesvirus (KSHV).

121. The method of claim 117, wherein the first herpesvirus and the second herpesvirus are different.

122. The method of claim 120, wherein the first herpesvirus is CMV.

123. The method of claim 122, wherein the first herpesvirus is HCMV and the sequence of the first herpesvirus crs comprises CGTTTAGTGAACCG (SEQ ID NO: 4).

124. The method of claim 122, wherein the sequence of the first herpesvirus crs has at least 90% sequence identity to the sequence of the herpesvirus crs of HCMV.

125. The method of claim 123, wherein the sequence of the first herpesvirus crs flanked on the 5′ end by a first sequence of at least 2 base pairs and on the 3′ end by a second sequence of at least 2 base pairs comprises GACAGATCGTTTAGTGAACCGTACACGA (SEQ ID NO: 1).

126. The method of claim 125, wherein the sequence of the first herpesvirus crs flanked on the 5′ end by a first sequence of at least 2 base pairs and on the 3′ end by a second sequence of at least 2 base pairs comprises G*ACAGATCGTT*TAGTGAACC*GTACACG*A (SEQ ID NO: 5), wherein * is a phosphorothioated bond.

127. The method of claim 120, wherein the second herpesvirus is HSV-1.

128. The method of claim 127, wherein the sequence of the second herpesvirus crs comprises CGCCCCGATCGTCCA (SEQ ID NO: 10).

129. The method of claim 127, wherein the sequence of the second herpesvirus crs is at least 90% sequence identity to the sequence of the herpesvirus crs of HSV-1.

130. The method of claim 128, wherein the sequence of the second herpesvirus crs flanked on the 5′ end by a first sequence of at least 2 base pairs and on the 3′ end by a second sequence of at least 2 base pairs comprises CCGAGGACGCCCCGATCGTCCACACGGAG (SEQ ID NO: 3).

131. The method of claim 130, the sequence of the second herpesvirus crs flanked on the 5′ end by a first sequence of at least 2 base pairs and on the 3′ end by a second sequence of at least 2 base pairs comprises CCG*AGGAC*GCCCCGATC*GTCCACACG*GAG (SEQ ID NO: 11), wherein * is a phosphorothioated bond.

132. The method of any one of claims 117-131, wherein the double stranded DNA molecule comprises a concatamer comprising one or more copies of a sequence comprising the sequence of the first herpesvirus crs flanked on the 5′ end by a first sequence of at least 2 base pairs and on the 3′ end by a second sequence of at least 2 base pairs; and the sequence of a crs of a second herpesvirus, wherein the crs is flanked on the 5′ end by a first sequence of at least 2 base pairs and on the 3′ end by a second sequence of 2 base pairs.

133. The method of claim 132, wherein the sequence of the first herpesvirus crs is flanked on the 5′ end by a first sequence of at least 7 base pairs and on the 3′ end by a second sequence of at least 7 base pairs, and wherein the sequence of the second herpesvirus crs is flanked on the 5′ end by a first sequence of at least 7 base pairs and on the 3′ end by a second sequence of at least 7 base pairs.

134. The method of any one of claims 117-133, wherein one or more of the bases of the double stranded DNA molecule comprises a chemical modification.

135. The method of claim 134, wherein the chemical modification is a phosphorothioated bond.

136. The method of claim 117, wherein the organ or tissue is contacted *in vitro* or *ex vivo* with the double stranded DNA molecule comprising the sequence comprising the sequence of the cis regulatory sequence (crs) of the herpesvirus for a period of time of from about 15 minutes to about 48 hours, wherein the crs is flanked on the 5′ end by a first sequence of at least 2 base pairs and on the 3′ end by a second sequence of at least 2 base pairs.

137. The method of claim 136, wherein the organ or tissue is contacted *in vitro* or *ex vivo* with the double stranded DNA molecule in a liquid medium.

138. A method of reducing the likelihood that a transplant recipient will become infected with herpesvirus from a donor organ or tissue, the method comprising:

a) contacting the organ or tissue *in vitro* or *ex vivo* with a double stranded DNA molecule comprising a sequence comprising a sequence of a cis regulatory sequence (crs) of the herpesvirus, wherein the crs is flanked on the 5′ end by a first sequence of at least 2 base pairs and on the 3′ end by a second sequence of at least 2 base pairs, thereby producing a double stranded DNA molecule-treated organ or tissue; and

b) transplanting the double stranded DNA molecule-treated organ or tissue into the transplant recipient.

139. The method of claim 138, wherein the crs is flanked on the 5′ end by a first sequence of at least 7 base pairs and on the 3′ end by a second sequence of at least 7 base pairs.

140. The method of claim 138 or 139, wherein one or both of the first sequence of at least 2 base pairs and the second sequence of at least 2 base pairs does not have a DNA sequence of a naturally occurring herpesvirus.

141. The method of any one of claims 138-140, wherein the herpesvirus is selected from cytomegalovirus (CMV), herpes simplex virus-1 (HSV-1), herpes simplex virus-2 (HSV-2), varicella zoster virus, Epstein-Barr virus (EBV), and Kaposi's sarcoma-associated herpesvirus (KSHV).

142. The method of claim 141, wherein the herpesvirus is CMV.

143. The method of claim 142, wherein the herpesvirus is HCMV and the sequence of the herpesvirus crs comprises CGTTTAGTGAACCG (SEQ ID NO: 4).

144. The method of claim 142, wherein the sequence of the herpesvirus crs has at least 90% sequence identity to the sequence of the herpesvirus crs of HCMV.

145. The method of claim 143, wherein the sequence of the herpesvirus crs flanked on the 5′ end by a first sequence of at least 2 base pairs and on the 3′ end by a second sequence of at least 2 base pairs comprises GACAGATCGTTTAGTGAACCGTACACGA (SEQ ID NO: 1).

146. The method of claim 145, wherein the sequence of the herpesvirus crs flanked on the 5′ end by a first sequence of at least 2 base pairs and on the 3′ end by a second sequence of at least 2 base pairs comprises G*ACAGATCGTT*TAGTGAACC*GTACACG*A (SEQ ID NO: 5), wherein * is a phosphorothioated bond.

147. The method of claim 141, wherein the herpesvirus is HSV-1.

148. The method of claim 147, wherein the sequence of the herpesvirus crs comprises CGCCCCGATCGTCCA (SEQ ID NO: 10).

149. The method of claim 147, wherein the sequence of the herpesvirus crs is at least 90% sequence identity to the sequence of the herpesvirus crs of HSV-1.

150. The method of claim 148, wherein the sequence of the herpesvirus crs flanked on the 5′ end by a first sequence of at least 2 base pairs and on the 3′ end by a second sequence of at least 2 base pairs comprises CCGAGGACGCCCCGATCGTCCACACGGAG (SEQ ID NO: 3).

151. The method of claim 150, the sequence of the herpesvirus crs flanked on the 5′ end by a first sequence of at least 2 base pairs and on the 3′ end by a second sequence of at least 2 base pairs comprises CCG*AGGAC*GCCCCGATC*GTCCACACG*GAG (SEQ ID NO: 11), wherein * is a phosphorothioated bond.

152. The method of any one of claims 138-151, wherein the double stranded DNA molecule comprises a concatamer comprising one or more copies of a sequence comprising

the sequence of the herpesvirus crs flanked on the 5′ end by a first sequence of at least 2 base pairs and on the 3′ end by a second sequence of at least 2 base pairs.

153. The method of claim 152, wherein the sequence of the herpesvirus crs is flanked on the 5′ end by a first sequence of at least 7 base pairs and on the 3′ end by a second sequence of at least 7 base pairs.

154. The method of any one of claims 138-153, wherein one or more of the bases of the double stranded DNA molecule comprises a chemical modification.

155. The method of claim 154, wherein the chemical modification is a phosphorothioated bond.

156. A method of reducing the likelihood that a transplant recipient will become infected with one or more herpesviruses from a donor organ or tissue, the method comprising:

a) contacting the organ or tissue *in vitro* or *ex vivo* with a double stranded DNA molecule comprising a sequence comprising a sequence of a cis regulatory sequence (crs) of a first herpesvirus, wherein the crs is flanked on the 5′ end by a first sequence of at least 2 base pairs and on the 3′ end by a second sequence of at least 2 base pairs; and a sequence of a crs of a second herpesvirus, wherein the crs is flanked on the 5′ end by a first sequence of at least 2 base pairs and on the 3′ end by a second sequence of at least 2 base pairs, thereby producing a double stranded DNA molecule-treated organ or tissue; and

b) transplanting the double stranded DNA molecule-treated organ or tissue into the transplant recipient.

157. The method of claim 156, wherein the sequence of the crs of the first herpesvirus is flanked on the 5′ end by a first sequence of at least 7 base pairs and on the 3′ end by a second sequence of at least 7 base pairs, and wherein the sequence of the crs of the second herpesvirus is flanked on the 5′ end by a first sequence of at least 7 base pairs and on the 3′ end by a second sequence of at least 7 base pairs.

158. The method of claim 156 or 157, wherein one or both of the first sequence of at least 2 base pairs and the second sequence of at least 2 base pairs flanking the crs of the first herpesvirus does not have a DNA sequence of a naturally occurring herpesvirus, and wherein one or both of the first sequence of at least 2 base pairs and the second sequence of

at least 2 base pairs flanking the crs of the second herpesvirus does not have a DNA sequence of a naturally occurring herpesvirus.

159. The method of any one of claims 156-158, wherein the first herpesvirus and second herpesvirus are selected from cytomegalovirus (CMV), herpes simplex virus-1 (HSV-1), herpes simplex virus-2 (HSV-2), varicella zoster virus, Epstein-Barr virus (EBV), and Kaposi's sarcoma-associated herpesvirus (KSHV).

160. The method of claim 159, wherein the first herpesvirus and the second herpesvirus are different.

161. The method of claim 159, wherein the first herpesvirus is CMV.

162. The method of claim 161, wherein the first herpesvirus is HCMV and the sequence of the first herpesvirus crs comprises CGTTTAGTGAACCG (SEQ ID NO: 4).

163. The method of claim 161, wherein the sequence of the first herpesvirus crs has at least 90% sequence identity to the sequence of the herpesvirus crs of HCMV.

164. The method of claim 162, wherein the sequence of the first herpesvirus crs flanked on the 5′ end by a first sequence of at least 2 base pairs and on the 3′ end by a second sequence of at least 2 base pairs comprises GACAGATCGTTTAGTGAACCGTACACGA (SEQ ID NO: 1).

165. The method of claim 164, wherein the sequence of the first herpesvirus crs flanked on the 5′ end by a first sequence of at least 2 base pairs and on the 3′ end by a second sequence of at least 2 base pairs comprises G*ACAGATCGTT*TAGTGAACC*GTACACG*A (SEQ ID NO: 5), wherein * is a phosphorothioated bond.

166. The method of claim 159, wherein the second herpesvirus is HSV-1.

167. The method of claim 166, wherein the sequence of the second herpesvirus crs comprises CGCCCCGATCGTCCA (SEQ ID NO: 10).

168. The method of claim 166, wherein the sequence of the second herpesvirus crs is at least 90% sequence identity to the sequence of the herpesvirus crs of HSV-1.

169. The method of claim 167, wherein the sequence of the second herpesvirus crs flanked on the 5′ end by a first sequence of at least 2 base pairs and on the 3′ end by a second sequence of at least 2 base pairs comprises CCGAGGACGCCCCGATCGTCCACACGGAG (SEQ ID NO: 3).

170. The method of claim 169, the sequence of the second herpesvirus crs flanked on the 5′ end by a first sequence of at least 2 base pairs and on the 3′ end by a second sequence of at least 2 base pairs comprises CCG*AGGAC*GCCCCGATC*GTCCACACG*GAG (SEQ ID NO: 11), wherein * is a phosphorothioated bond.

171. The method of any one of claims 156-170, wherein the double stranded DNA molecule comprises a concatamer comprising one or more copies of a sequence comprising the sequence of the first herpesvirus crs flanked on the 5′ end by a first sequence of at least 2 base pairs and on the 3′ end by a second sequence of at least 2 base pairs; and the sequence of a crs of a second herpesvirus, wherein the crs is flanked on the 5′ end by a first sequence of at least 2 base pairs and on the 3′ end by a second sequence of 2 base pairs.

172. The method of claim 171, wherein the sequence of the first herpesvirus crs is flanked on the 5′ end by a first sequence of at least 7 base pairs and on the 3′ end by a second sequence of at least 7 base pairs, and wherein the sequence of the second herpesvirus crs is flanked on the 5′ end by a first sequence of at least 7 base pairs and on the 3′ end by a second sequence of at least 7 base pairs.

173. The method of any one of claims 156-172, wherein one or more of the bases of the double stranded DNA molecule comprises a chemical modification.

174. The method of claim 173, wherein the chemical modification is a phosphorothioated bond.

175. A composition comprising a double stranded DNA molecule comprising a sequence comprising a sequence of a cis regulatory sequence (crs) of the herpesvirus, wherein the crs is flanked on the 5′ end by a first sequence of at least 2 base pairs and on the 3′ end by a second sequence of at least 2 base pairs.

176. The composition of claim 175, wherein the crs is flanked on the 5′ end by a first sequence of at least 7 base pairs and on the 3′ end by a second sequence of at least 7 base pairs.

177. The composition of claim 175 or 176, wherein one or both of the first sequence of at least 2 base pairs and the second sequence of at least 2 base pairs does not have a DNA sequence of a naturally occurring herpesvirus.

178. The composition of any one of claims 175-177, wherein the herpesvirus is selected from cytomegalovirus (CMV), herpes simplex virus-1 (HSV-1), herpes simplex virus-2 (HSV-2), varicella zoster virus, Epstein-Barr virus (EBV), and Kaposi's sarcoma-associated herpesvirus (KSHV).

179. The composition of claim 178, wherein the herpesvirus is CMV.

180. The composition of claim 179, wherein the herpesvirus is HCMV and the sequence of the herpesvirus crs comprises CGTTTAGTGAACCG (SEQ ID NO: 4).

181. The composition of claim 179, wherein the sequence of the herpesvirus crs has at least 90% sequence identity to the sequence of the herpesvirus crs of HCMV.

182. The composition of claim 180, wherein the sequence of the herpesvirus crs flanked on the 5′ end by a first sequence of at least 2 base pairs and on the 3′ end by a second sequence of at least 2 base pairs comprises GACAGATCGTTTAGTGAACCGTACACGA (SEQ ID NO: 1).

183. The composition of claim 182, the sequence of the herpesvirus crs flanked on the 5′ end by a first sequence of at least 2 base pairs and on the 3′ end by a second sequence of at least 2 base pairs comprises G*ACAGATCGTT*TAGTGAACC*GTACACG*A (SEQ ID NO: 5), wherein * is a phosphorothioated bond.

184. The composition of claim 178, wherein the herpesvirus is HSV-1.

185. The composition of claim 184, wherein the sequence of the herpesvirus crs comprises CGCCCCGATCGTCCA (SEQ ID NO: 10).

186. The composition of claim 184, wherein the sequence of the herpesvirus crs has at least 90% sequence identity to the sequence of the herpesvirus crs of HSV-1.

187. The composition of claim 185, wherein the sequence of the herpesvirus crs flanked on the 5′ end by a first sequence of at least 2 base pairs and on the 3′ end by a second sequence of at least 2 base pairs comprises CCGAGGACGCCCCGATCGTCCACACGGAG (SEQ ID NO: 3).

188. The composition of claim 187, the sequence of the herpesvirus crs flanked on the 5′ end by a first sequence of at least 2 base pairs and on the 3′ end by a second sequence of at least 2 base pairs comprises CCG*AGGAC*GCCCCGATC*GTCCACACG*GAG (SEQ ID NO: 11), wherein * is a phosphorothioated bond.

189. The composition of any one of claims 175-188, wherein the double stranded DNA molecule comprises a concatamer comprising one or more copies of a sequence comprising the sequence of the herpesvirus crs flanked on the 5′ end by a first sequence of at least 2 base pairs and on the 3′ end by a second sequence of at least 2 base pairs.

190. The composition of claim 189, wherein the sequence of the herpesvirus crs is flanked on the 5′ end by a first sequence of at least 7 base pairs and on the 3′ end by a second sequence of at least 7 base pairs.

191. The composition of any one of claims 175-190, wherein one or more of the bases of the double stranded DNA molecule comprises a chemical modification.

192. The composition of claim 191, wherein the chemical modification is a phosphorothioated bond.

193. The composition of any one of claims 175-192, wherein the composition further comprises a delivery vehicle for the double stranded DNA molecule.

194. The composition of claim 193, wherein the delivery vehicle is a nanoparticle, and wherein the double stranded DNA molecule is positioned in or on the nanoparticle.

195. The composition of claim 193 or 194, wherein the delivery vehicle is viral.

196. The composition of claim 193 or 194, wherein the delivery vehicle is non-viral.

197. A composition comprising:

a) a sequence of a cis regulatory sequence (crs) of a first herpesvirus, wherein the crs is flanked on the 5′ end by a first sequence of at least 2 base pairs and on the 3′ end by a second sequence of at least 2 base pairs; and

b) a sequence of a crs of a second herpesvirus, wherein the crs is flanked on the 5′ end by a first sequence of at least 2 base pairs and on the 3′ end by a second sequence of at least 2 base pairs.

198. The composition of claim 197, wherein the sequence of the crs of the first herpesvirus is flanked on the 5′ end by a first sequence of at least 7 base pairs and on the 3′ end by a second sequence of at least 7 base pairs, and wherein the sequence of the crs of the second herpesvirus is flanked on the 5′ end by a first sequence of at least 7 base pairs and on the 3′ end by a second sequence of at least 7 base pairs.

199. The composition of claim 197 or 198, wherein one or both of the first sequence of at least 2 base pairs and the second sequence of at least 2 base pairs flanking the crs of the first herpesvirus does not have a DNA sequence of a naturally occurring herpesvirus, and wherein one or both of the first sequence of at least 2 base pairs and the second sequence of at least 2 base pairs flanking the crs of the second herpesvirus does not have a DNA sequence of a naturally occurring herpesvirus.

200. The composition of any one of claims 197-199, wherein the first herpesvirus and second herpesvirus are selected from cytomegalovirus (CMV), herpes simplex virus-1 (HSV-1), herpes simplex virus-2 (HSV-2), varicella zoster virus, Epstein-Barr virus (EBV), and Kaposi's sarcoma-associated herpesvirus (KSHV).

201. The composition of claim 200, wherein the first herpesvirus and the second herpesvirus are different.

202. The composition of claim 200, wherein the first herpesvirus is CMV.

203. The composition of claim 202, wherein the first herpesvirus is HCMV and the sequence of the first herpesvirus crs comprises CGTTTAGTGAACCG (SEQ ID NO: 4).

204. The composition of claim 202, wherein the sequence of the first herpesvirus crs has at least 90% sequence identity to the sequence of the herpesvirus crs of HCMV.

205. The composition of claim 203, wherein the sequence of the first herpesvirus crs flanked on the 5′ end by a first sequence of at least 2 base pairs and on the 3′ end by a second sequence of at least 2 base pairs comprises GACAGATCGTTTAGTGAACCGTACACGA (SEQ ID NO: 1).

206. The composition of claim 205, wherein the sequence of the first herpesvirus crs flanked on the 5′ end by a first sequence of at least 2 base pairs and on the 3′ end by a second sequence of at least 2 base pairs comprises G*ACAGATCGTT*TAGTGAACC*GTACACG*A (SEQ ID NO: 5), wherein * is a phosphorothioated bond.

207. The composition of claim 200, wherein the second herpesvirus is HSV-1.

208. The composition of claim 207, wherein the sequence of the second herpesvirus crs comprises CGCCCCGATCGTCCA (SEQ ID NO: 10).

209. The composition of claim 207, wherein the sequence of the second herpesvirus crs is at least 90% sequence identity to the sequence of the herpesvirus crs of HSV-1.

210. The composition of claim 208, wherein the sequence of the second herpesvirus crs flanked on the 5′ end by a first sequence of at least 2 base pairs and on the 3′ end by a second sequence of at least 2 base pairs comprises CCGAGGACGCCCCGATCGTCCACACGGAG (SEQ ID NO: 3).

211. The composition of claim 210, the sequence of the second herpesvirus crs flanked on the 5′ end by a first sequence of at least 2 base pairs and on the 3′ end by a second sequence of at least 2 base pairs comprises CCG*AGGAC*GCCCCGATC*GTCCACACG*GAG (SEQ ID NO: 11), wherein * is a phosphorothioated bond.

212. The composition of any one of claims 197-211, wherein the double stranded DNA molecule comprises a concatamer comprising one or more copies of a sequence comprising the sequence of the first herpesvirus crs flanked on the 5′ end by a first sequence of at least 2 base pairs and on the 3′ end by a second sequence of at least 2 base pairs; and the sequence of a crs of a second herpesvirus, wherein the crs is flanked on the 5′ end by a first sequence of at least 2 base pairs and on the 3′ end by a second sequence of 2 base pairs.

213. The composition of claim 212, wherein the sequence of the first herpesvirus crs is flanked on the 5′ end by a first sequence of at least 7 base pairs and on the 3′ end by a second sequence of at least 7 base pairs, and wherein the sequence of the second herpesvirus crs is flanked on the 5′ end by a first sequence of at least 7 base pairs and on the 3′ end by a second sequence of at least 7 base pairs.

214. The composition of any one of claims 197-213, wherein one or more of the bases of the double stranded DNA molecule comprises a chemical modification.

215. The composition of claim 214, wherein the chemical modification is a phosphorothioated bond.

216. The composition of any one of claims 197-215, wherein the composition further comprises a delivery vehicle for the double stranded DNA molecule.

217. The composition of claim 216, wherein the delivery vehicle is a nanoparticle, and wherein the double stranded DNA molecule is positioned in or on the nanoparticle.

218. The composition of claim 216 or 217, wherein the delivery vehicle is a virus.

219. The composition of claim 216 or 217, wherein the delivery vehicle is non-viral.

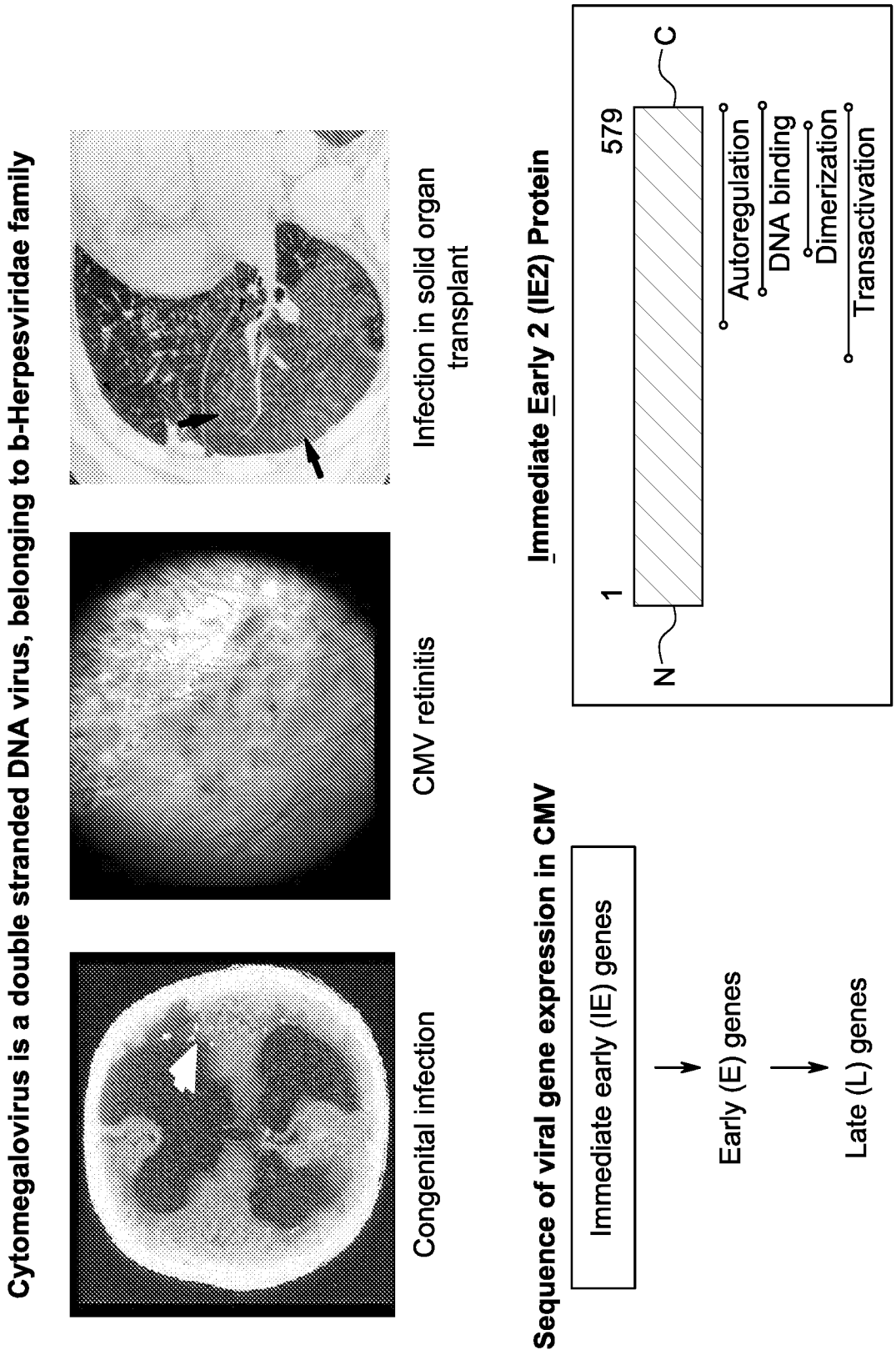

Cytomegalovirus is a double stranded DNA virus, belonging to b-Herpesviridae family
Congenital infection
CMV retinitis
Infection in solid organ transplant
Immediate Early 2 (IE2) Protein
1
579
N
C
Autoregulation
DNA binding
Dimerization
Transactivation
Sequence of viral gene expression in CMV
Immediate early (IE) genes
Early (E) genes
Late (L) genes

FIG. 1

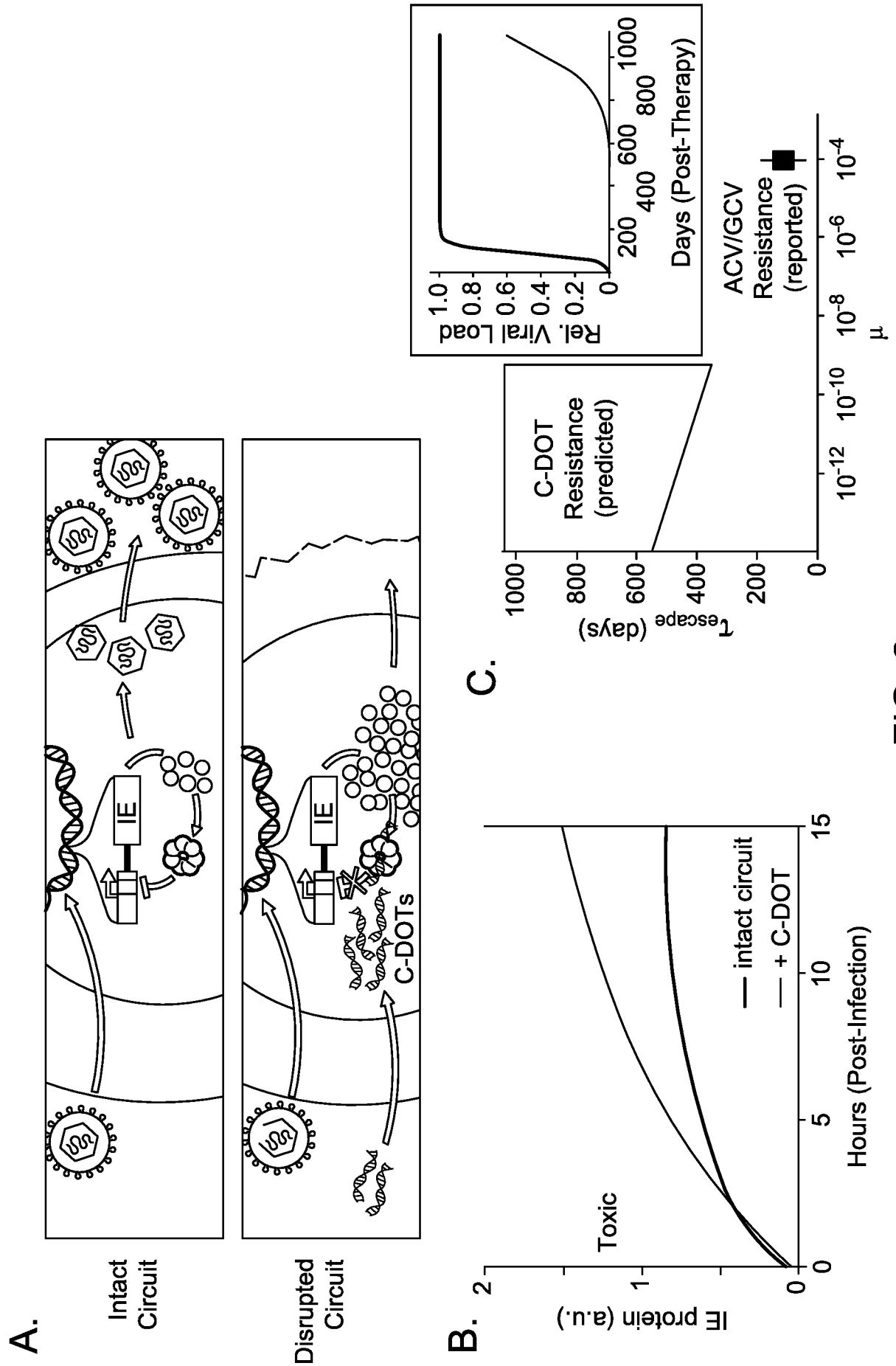

A.
Intact Circuit
Disrupted Circuit
IE
C-DOTs
B.
IE protein (a.u.)
Toxic
intact circuit
+ C-DOT
Hours (Post-Infection)
C.
C-DOT Resistance (predicted)
τescape (days)
ACV/GCV Resistance (reported)
μ
Rel. Viral Load
Days (Post-Therapy)

FIG. 2

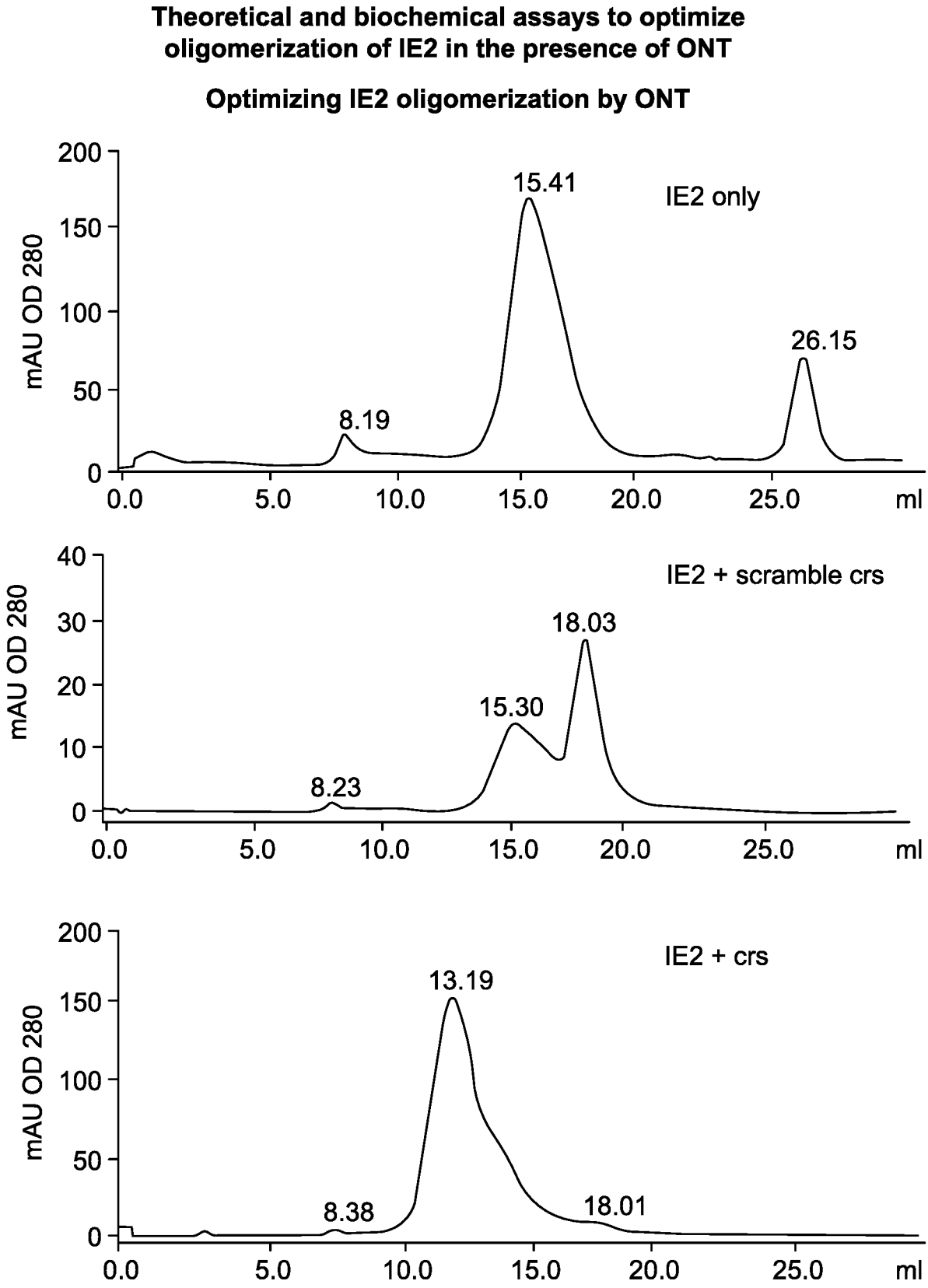

Theoretical and biochemical assays to optimize oligomerization of IE2 in the presence of ONT
Optimizing IE2 oligomerization by ONT
mAU OD 280
200
150
100
50
0
15.41
IE2 only
26.15
8.19
0.0
5.0
10.0
15.0
20.0
25.0
ml
mAU OD 280
40
30
20
10
0
IE2 + scramble crs
18.03
15.30
8.23
0.0
5.0
10.0
15.0
20.0
25.0
ml
mAU OD 280
200
150
100
50
0
IE2 + crs
13.19
8.38
18.01
0.0
5.0
10.0
15.0
20.0
25.0
ml

FIG. 3A

Theoretical and biochemical assays to optimize oligomerization of IE2 in the presence of ONT

IE2 oligomerize as a ring around ONT

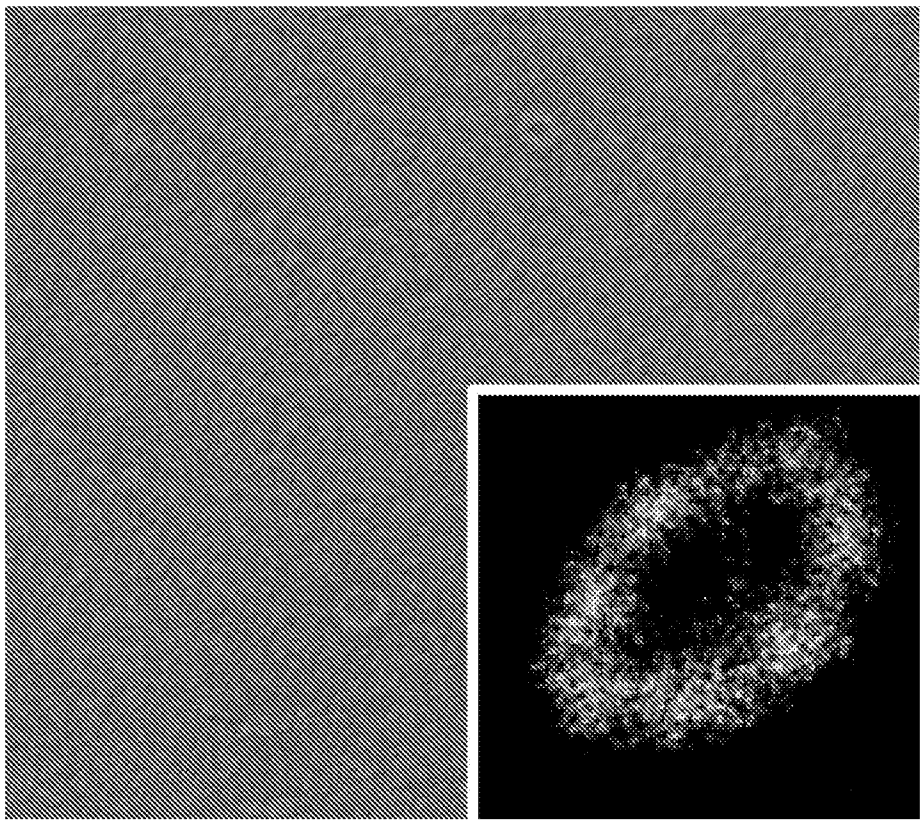


FIG. 3B

In-vitro assays to optimize oligomerization of IE2 in the presence of ONT

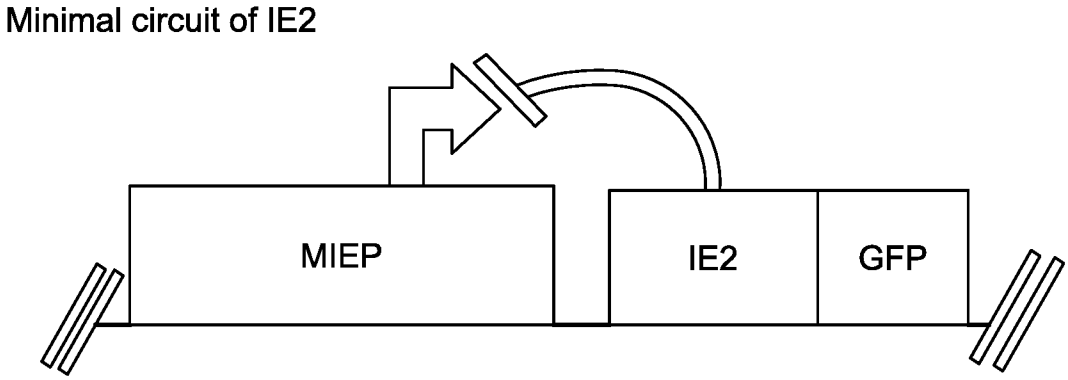


FIG. 4A

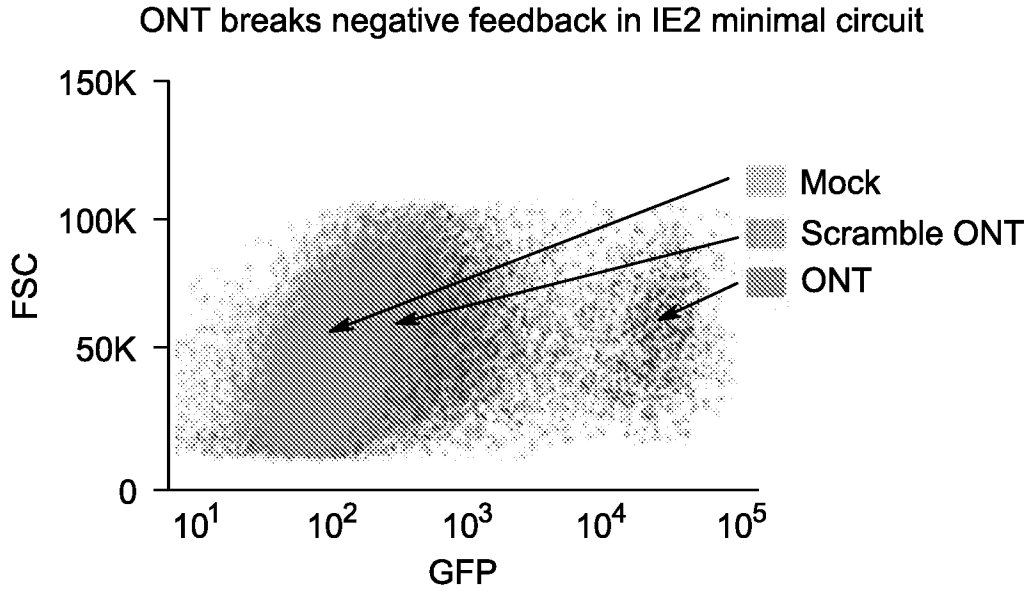


FIG. 4B

In-vitro assays to optimize oligomerization of IE2 in the presence of ONT

ONT breaks IE2 negative feedback leading to cytotoxicity in a dose dependent manner

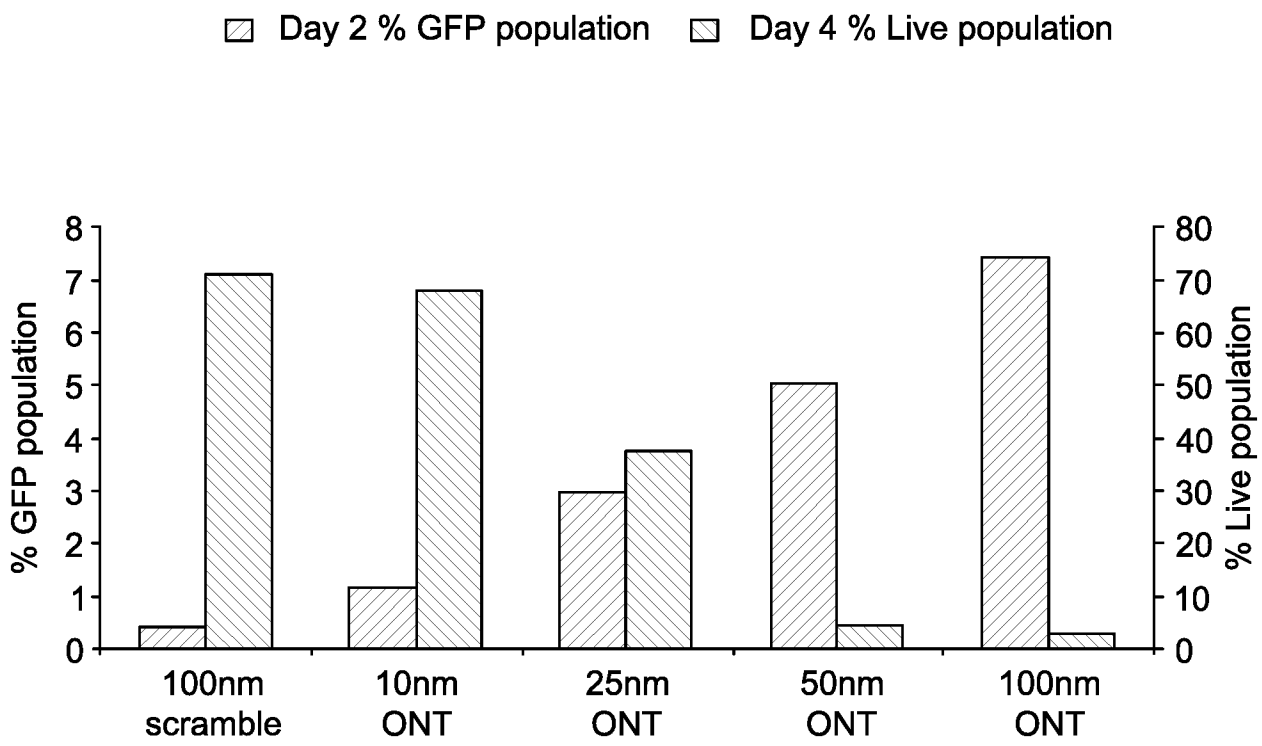
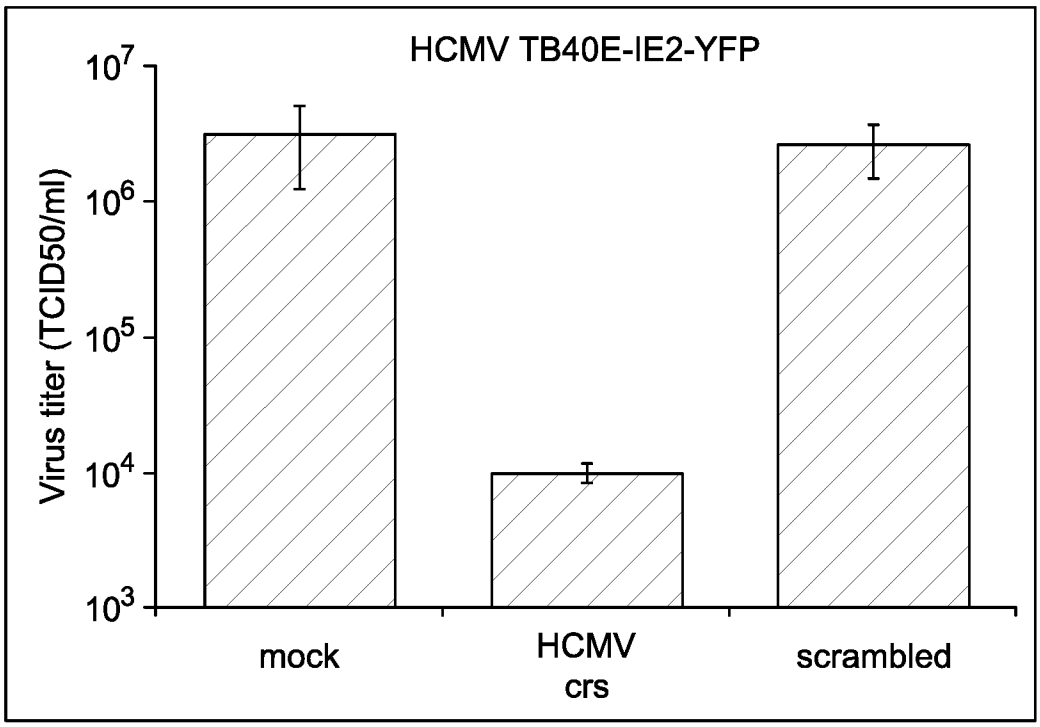


FIG. 4C

Transcription regulatory circuit disruptor interferes with Herpesvirus productive infection in cell culture



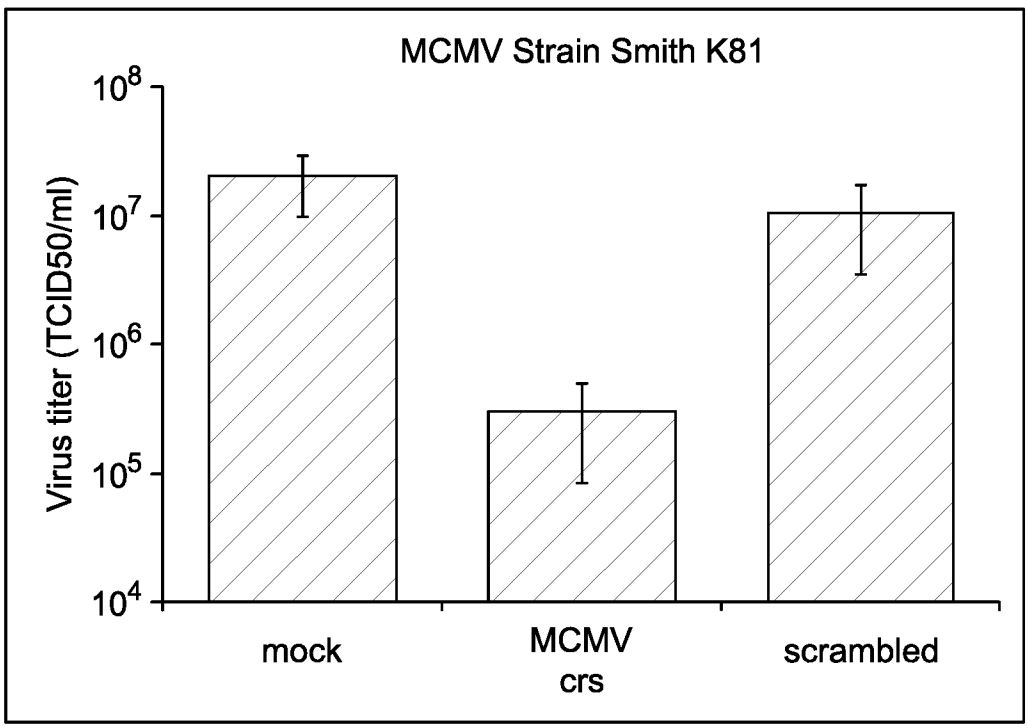


FIG. 5

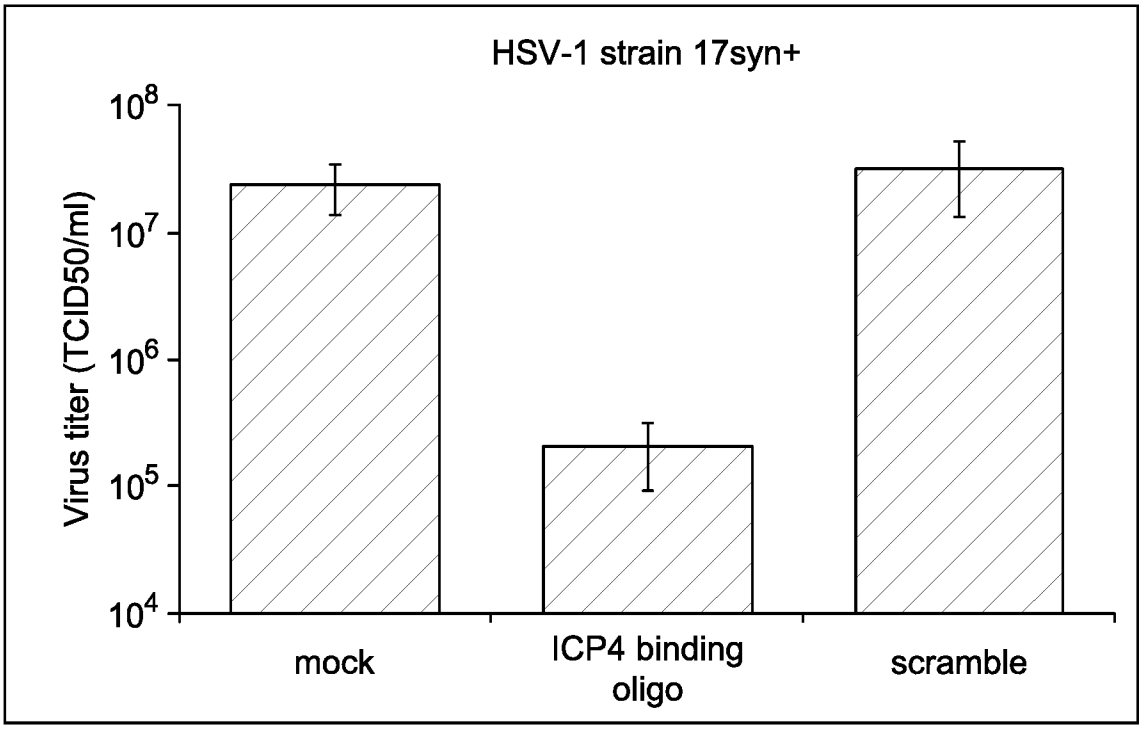

HSV-1 strain 17syn+
Virus titer (TCID50/ml)
10^8
10^7
10^6
10^5
10^4
mock
ICP4 binding oligo
scramble

FIG. 5 (Cont`d)

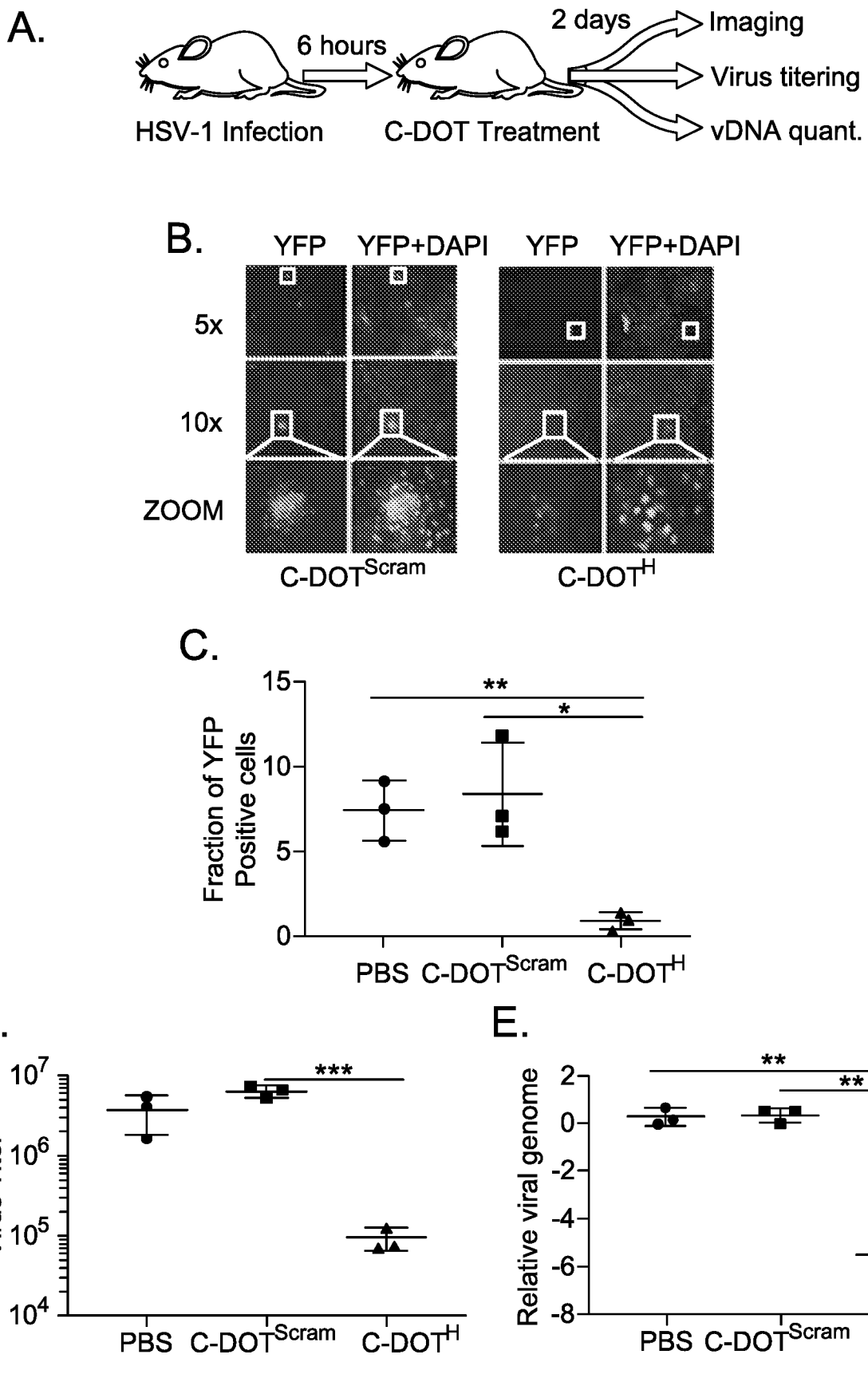

A.
6 hours
2 days
Imaging
Virus titering
vDNA quant.
HSV-1 Infection
C-DOT Treatment
B.
YFP
YFP+DAPI
YFP
YFP+DAPI
5x
10x
ZOOM
C-DOTScram
C-DOTH
C.
Fraction of YFP Positive cells
15
10
5
0
**
*
PBS
C-DOTScram
C-DOTH
D.
Virus Titer
10^7
10^6
10^5
10^4
***
PBS
C-DOTScram
C-DOTH
E.
Relative viral genome
2
0
-2
-4
-6
-8
**
**
PBS
C-DOTScram
C-DOTH

FIG. 6A

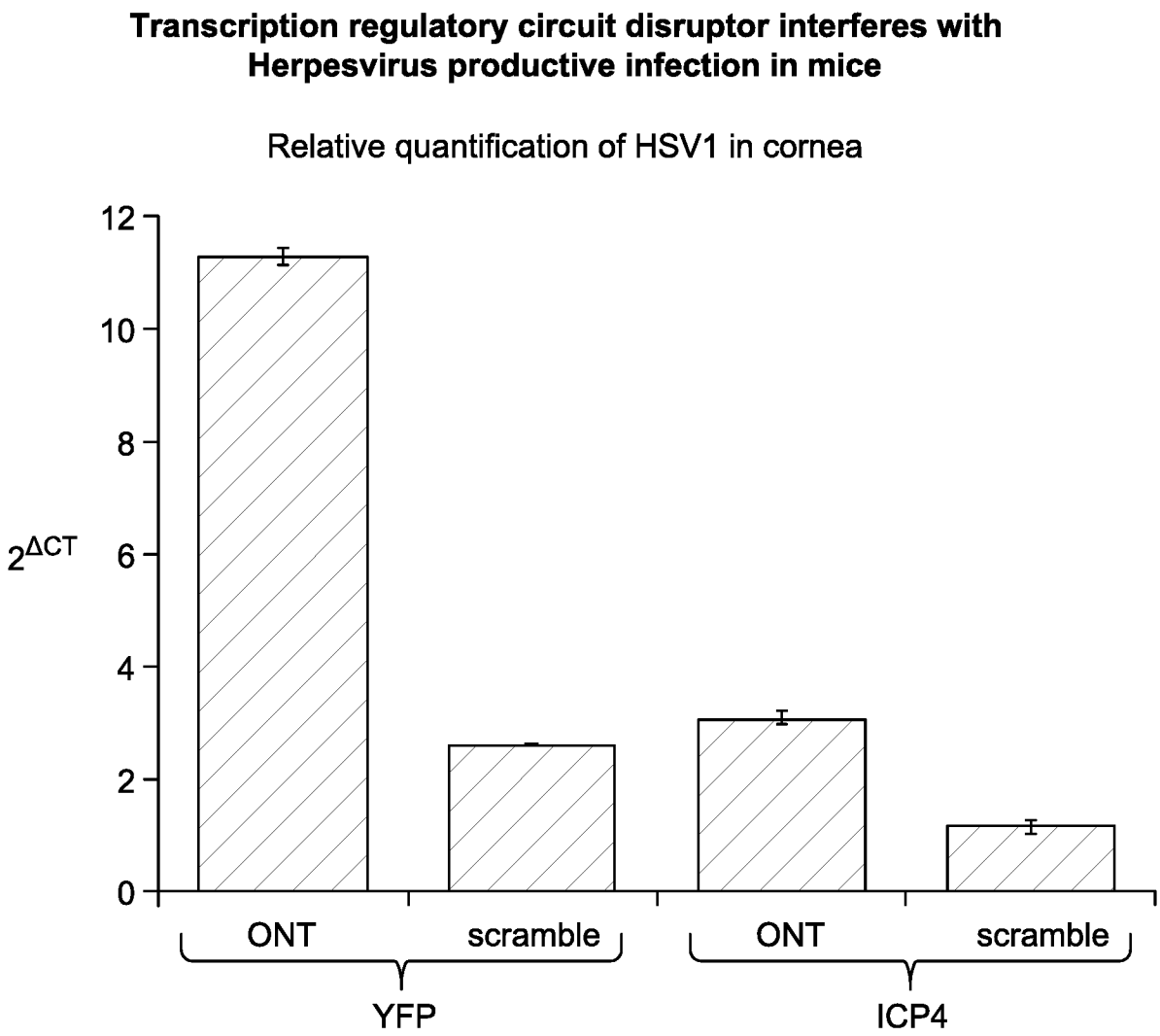

Transcription regulatory circuit disruptor interferes with Herpesvirus productive infection in mice
Relative quantification of HSV1 in cornea
$2^{\Delta CT}$
12
10
8
6
4
2
0
ONT
scramble
ONT
scramble
YFP
ICP4

FIG. 6B

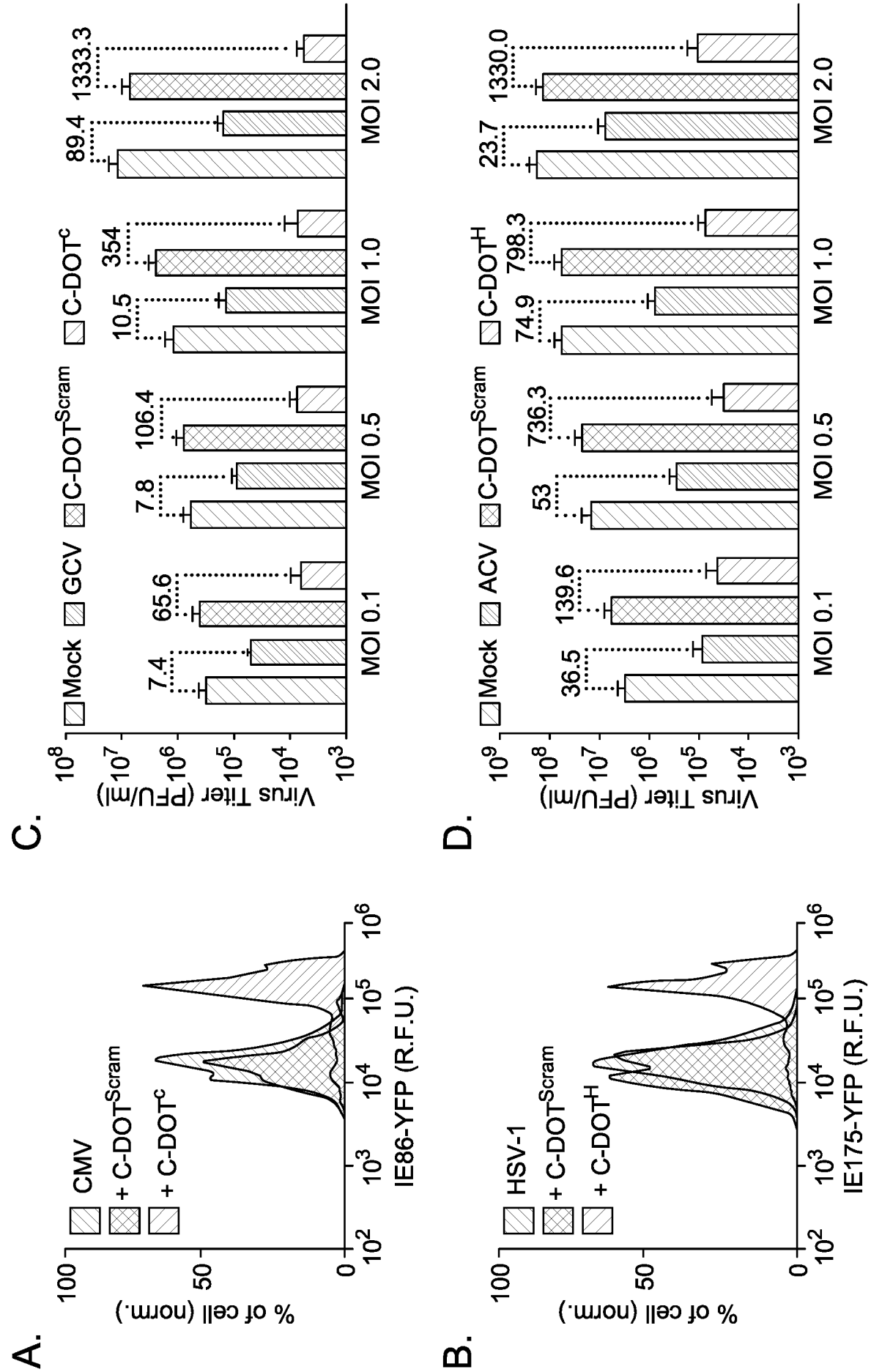

A.
CMV
+ C-DOT^Scram
+ C-DOT^c
% of cell (norm.)
IE86-YFP (R.F.U.)
B.
HSV-1
+ C-DOT^Scram
+ C-DOT^H
% of cell (norm.)
IE175-YFP (R.F.U.)
C.
Mock
GCV
C-DOT^Scram
C-DOT^c
Virus Titer (PFU/ml)
7.4
65.6
7.8
106.4
10.5
354
89.4
1333.3
MOI 0.1
MOI 0.5
MOI 1.0
MOI 2.0
D.
Mock
ACV
C-DOT^Scram
C-DOT^H
Virus Titer (PFU/ml)
36.5
139.6
53
736.3
74.9
798.3
23.7
1330.0
MOI 0.1
MOI 0.5
MOI 1.0
MOI 2.0

FIG. 7

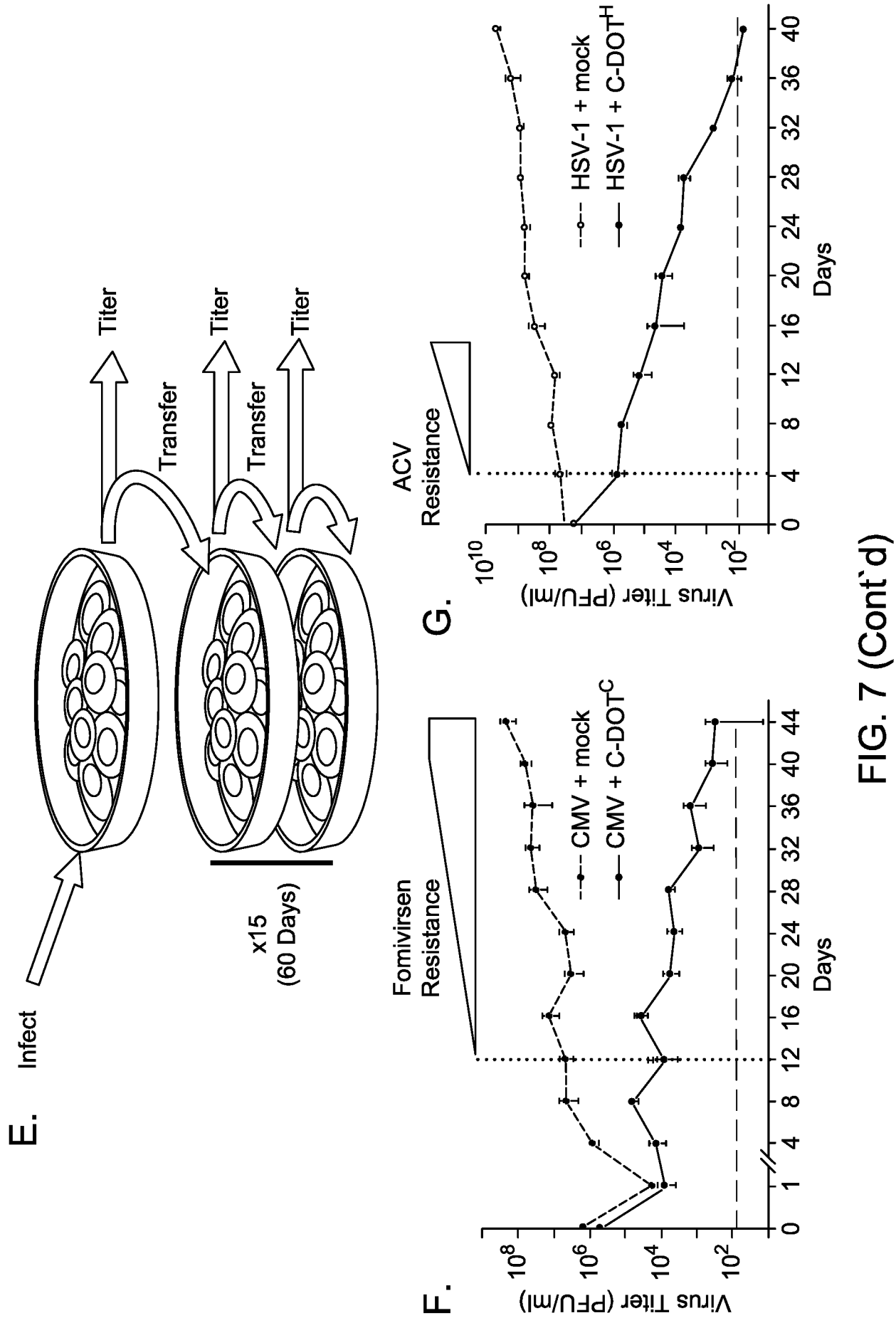

E.
Infect
Titer
Transfer
Titer
Transfer
Titer
x15
(60 Days)
F.
Fomivirsen
Resistance
CMV + mock
CMV + C-DOT^C
Virus Titer (PFU/ml)
Days
G.
ACV
Resistance
HSV-1 + mock
HSV-1 + C-DOT^H
Virus Titer (PFU/ml)
Days

FIG. 7 (Cont`d)

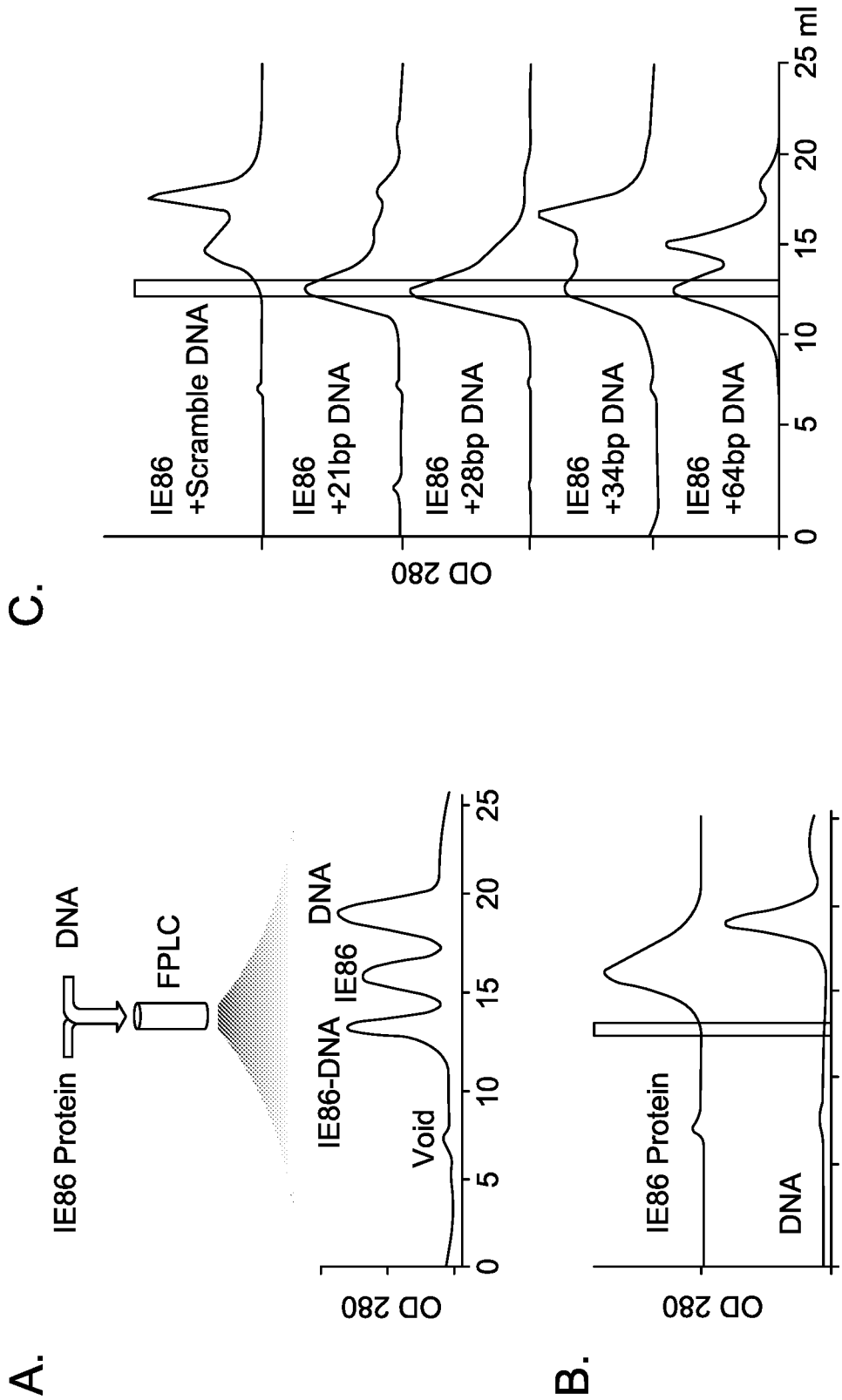

A.
IE86 Protein
DNA
FPLC
IE86-DNA
IE86
DNA
Void
OD 280
0
5
10
15
20
25
B.
IE86 Protein
DNA
OD 280
0
5
10
15
20
25 ml
C.
IE86
+Scramble DNA
IE86
+21bp DNA
IE86
+28bp DNA
IE86
+34bp DNA
IE86
+64bp DNA
OD 280
0
5
10
15
20
25 ml

FIG. 8

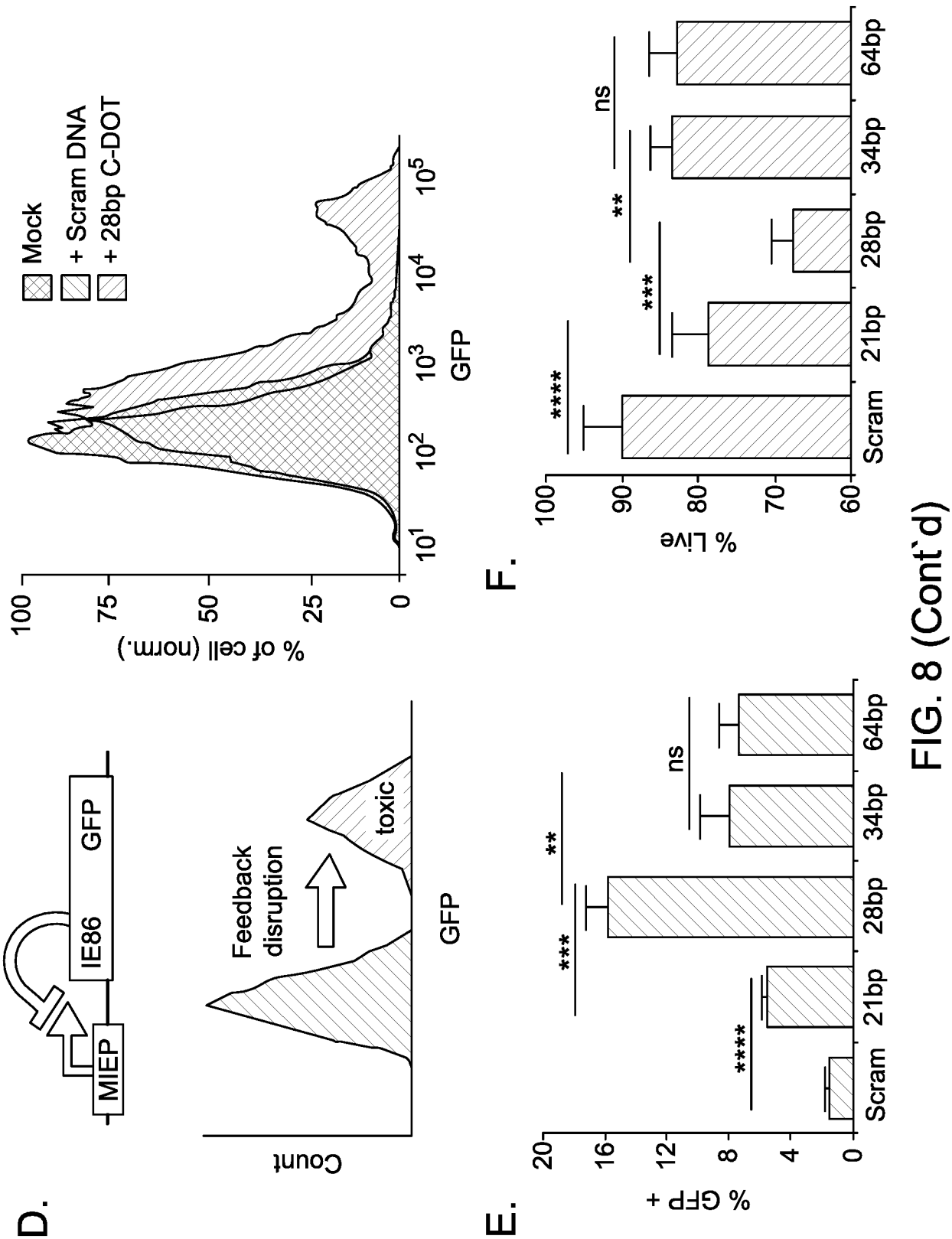

D.
MIEP
IE86
GFP
Feedback disruption
toxic
Count
GFP
% of cell (norm.)
Mock
+ Scram DNA
+ 28bp C-DOT
F.
% Live
Scram
21bp
28bp
34bp
64bp
****
***
**
ns
E.
% GFP +
Scram
21bp
28bp
34bp
64bp
***
**
****
ns

FIG. 8 (Cont`d)

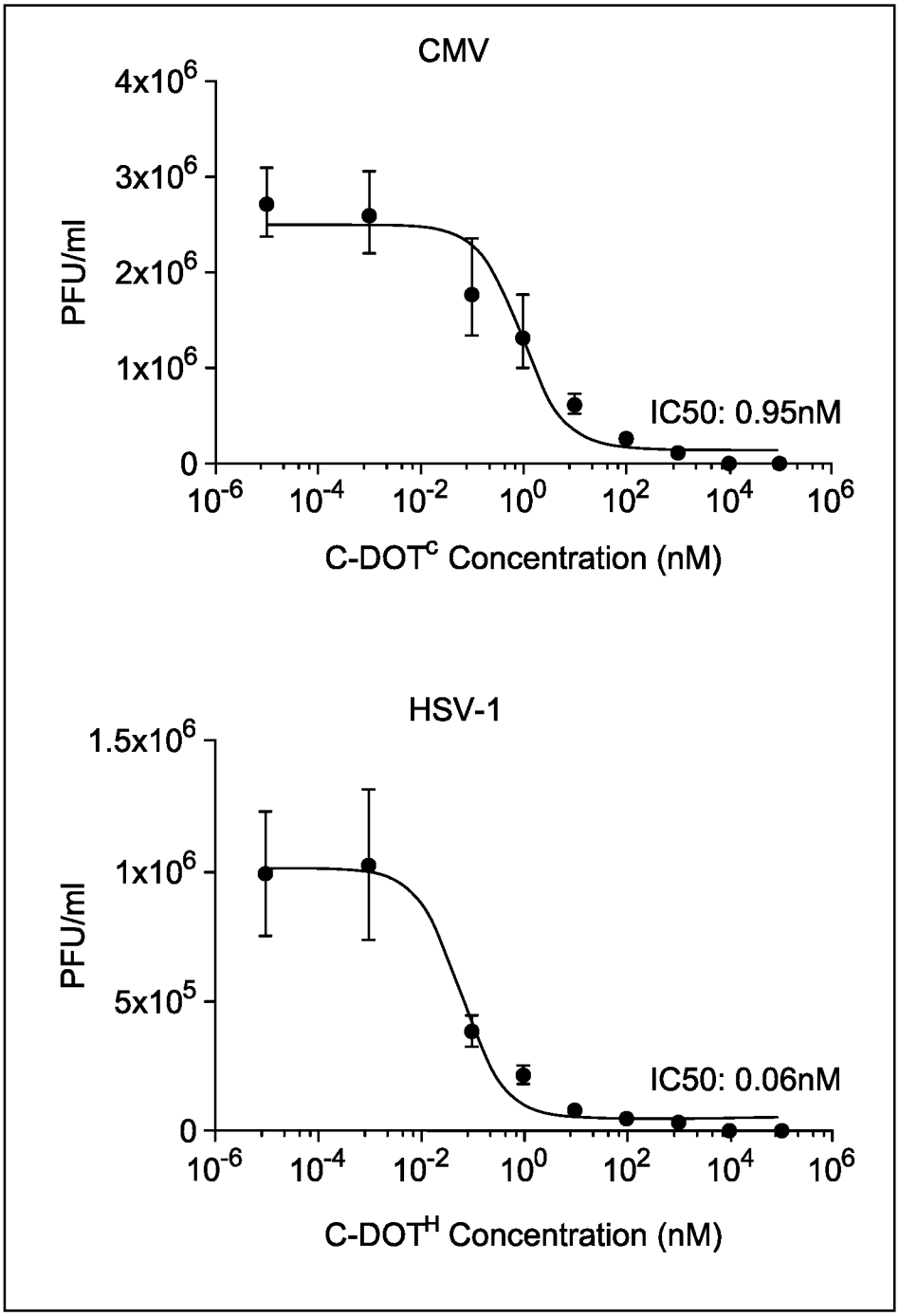

CMV
4x10^6
3x10^6
2x10^6
1x10^6
0
PFU/ml
IC50: 0.95nM
10^-6
10^-4
10^-2
10^0
10^2
10^4
10^6
C-DOT^C Concentration (nM)
HSV-1
1.5x10^6
1x10^6
5x10^5
0
PFU/ml
IC50: 0.06nM
10^-6
10^-4
10^-2
10^0
10^2
10^4
10^6
C-DOT^H Concentration (nM)

FIG. 9

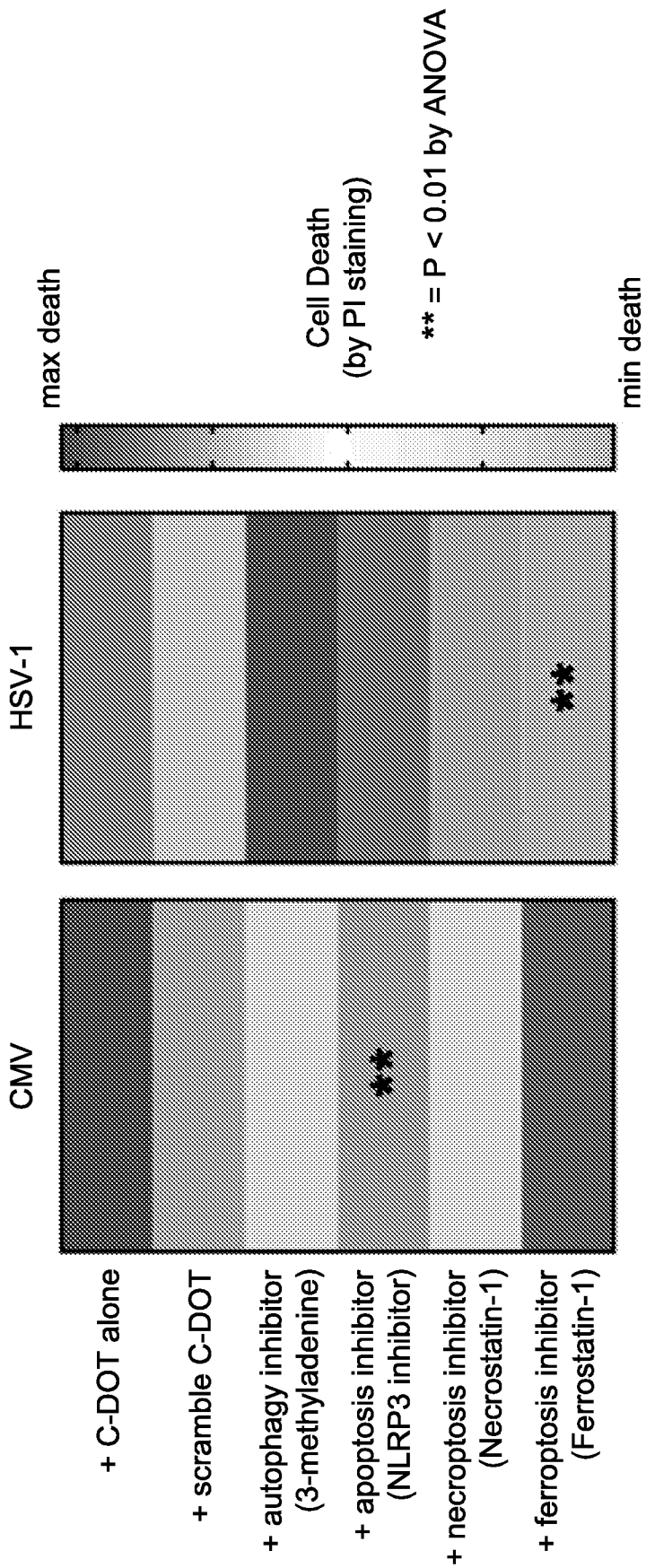

max death
Cell Death
(by PI staining)
** = P < 0.01 by ANOVA
min death
HSV-1
CMV
**
**
+ C-DOT alone
+ scramble C-DOT
+ autophagy inhibitor
(3-methyladenine)
+ apoptosis inhibitor
(NLRP3 inhibitor)
+ necroptosis inhibitor
(Necrostatin-1)
+ ferroptosis inhibitor
(Ferrostatin-1)

FIG. 10

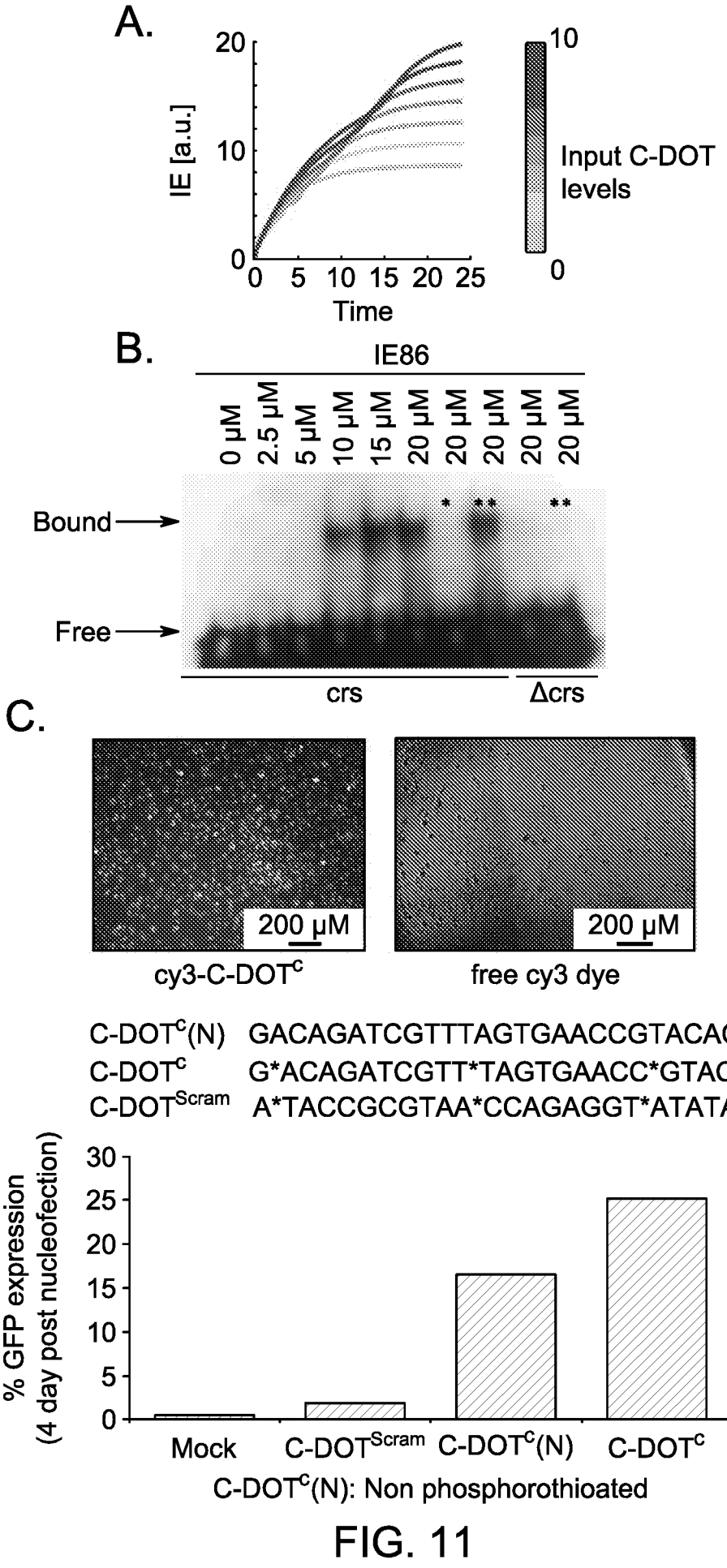

A.
IE [a.u.]
20
10
0
0 5 10 15 20 25
Time
10
Input C-DOT levels
0
B.
IE86
0 μM
2.5 μM
5 μM
10 μM
15 μM
20 μM
20 μM
20 μM
20 μM
20 μM
Bound
Free
*
**
**
crs
Δcrs
C.
200 μM
200 μM
cy3-C-DOTc
free cy3 dye
D.
C-DOTc(N) GACAGATCGTTTAGTGAACCGTACACGA
C-DOTc G*ACAGATCGTT*TAGTGAACC*GTACACG*A
C-DOTScram A*TACCGCGTAA*CCAGAGGT*ATATAGGT*C
% GFP expression (4 day post nucleofection)
30
25
20
15
10
5
0
Mock
C-DOTScram
C-DOTc(N)
C-DOTc
C-DOTc(N): Non phosphorothioated

FIG. 11

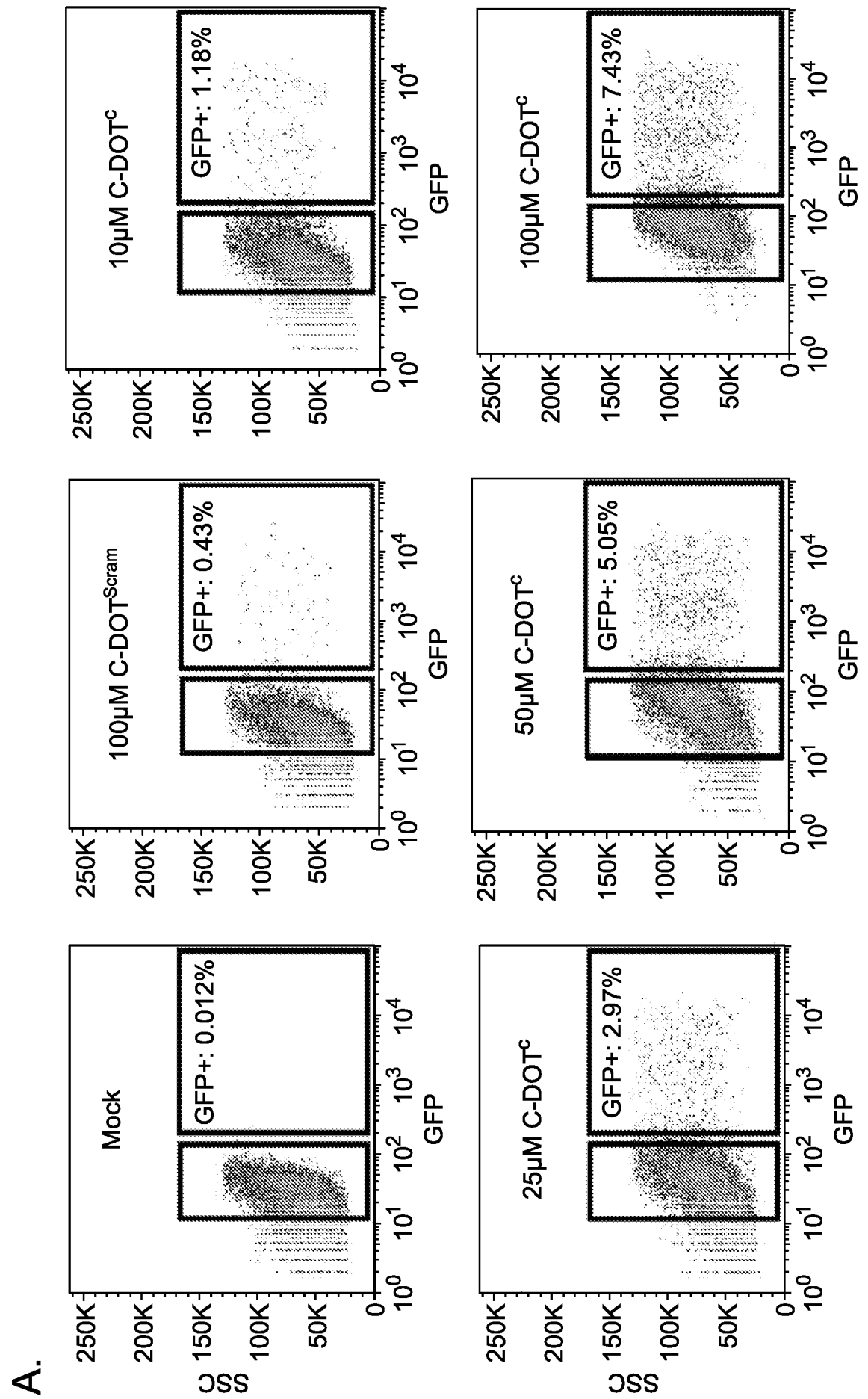

A.
Mock
GFP+: 0.012%
100μM C-DOTScram
GFP+: 0.43%
10μM C-DOTc
GFP+: 1.18%
25μM C-DOTc
GFP+: 2.97%
50μM C-DOTc
GFP+: 5.05%
100μM C-DOTc
GFP+: 7.43%
SSC
GFP
250K
200K
150K
100K
50K
0
10^0
10^1
10^2
10^3
10^4

FIG. 12

B.

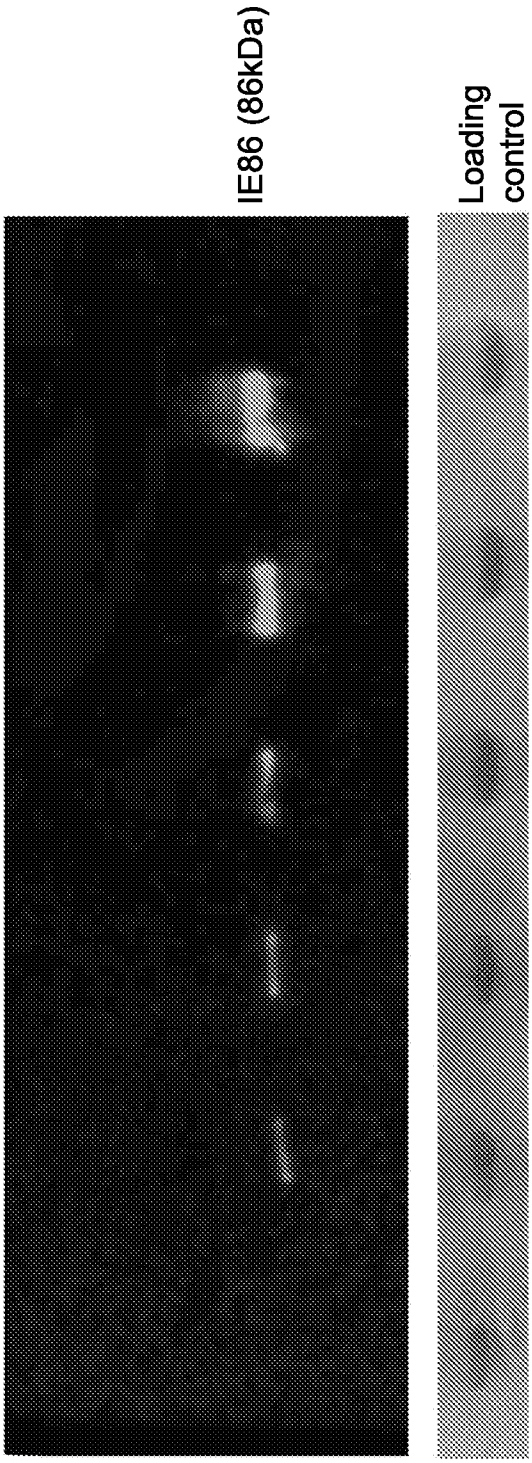


FIG. 12 (Cont'd)

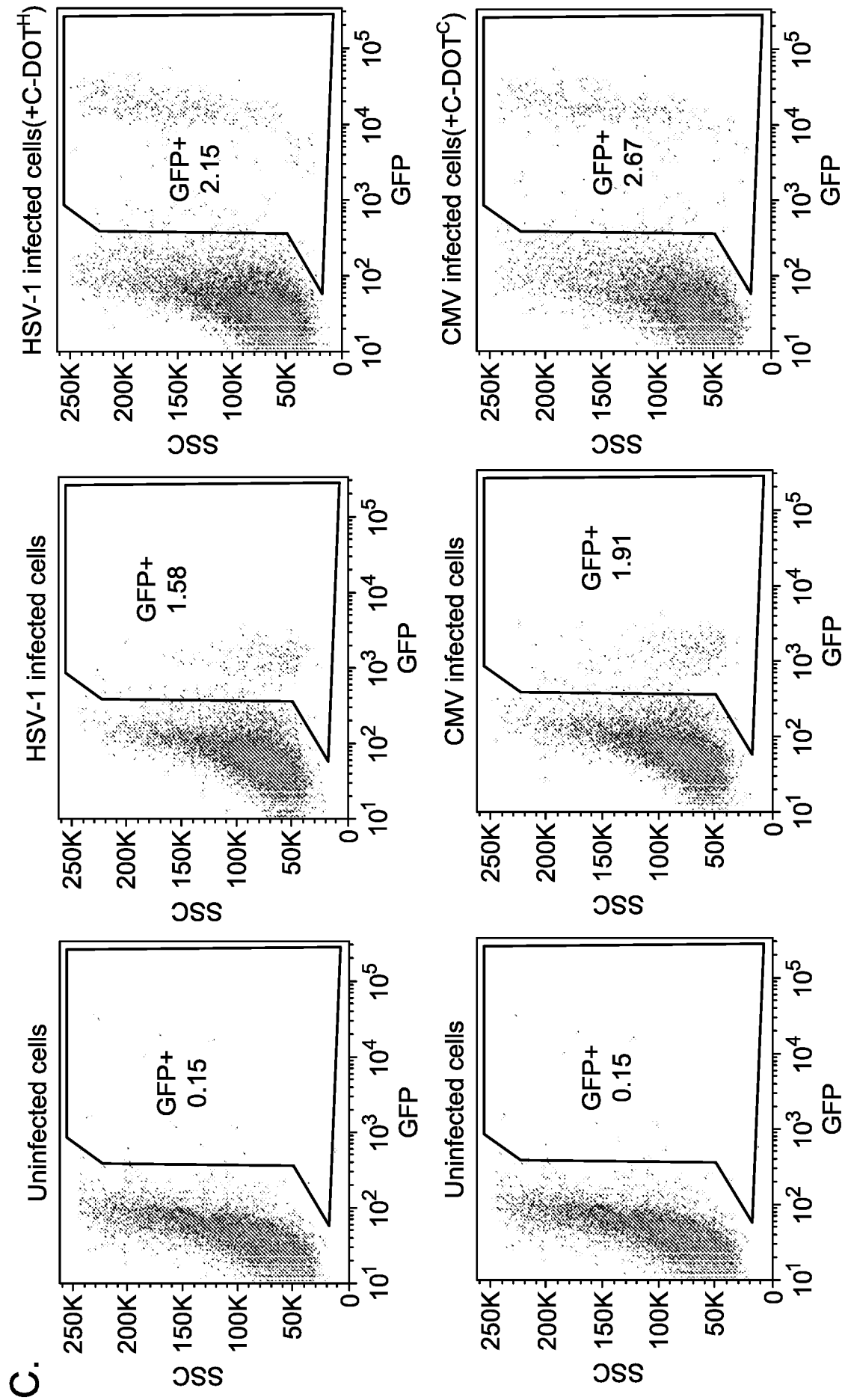
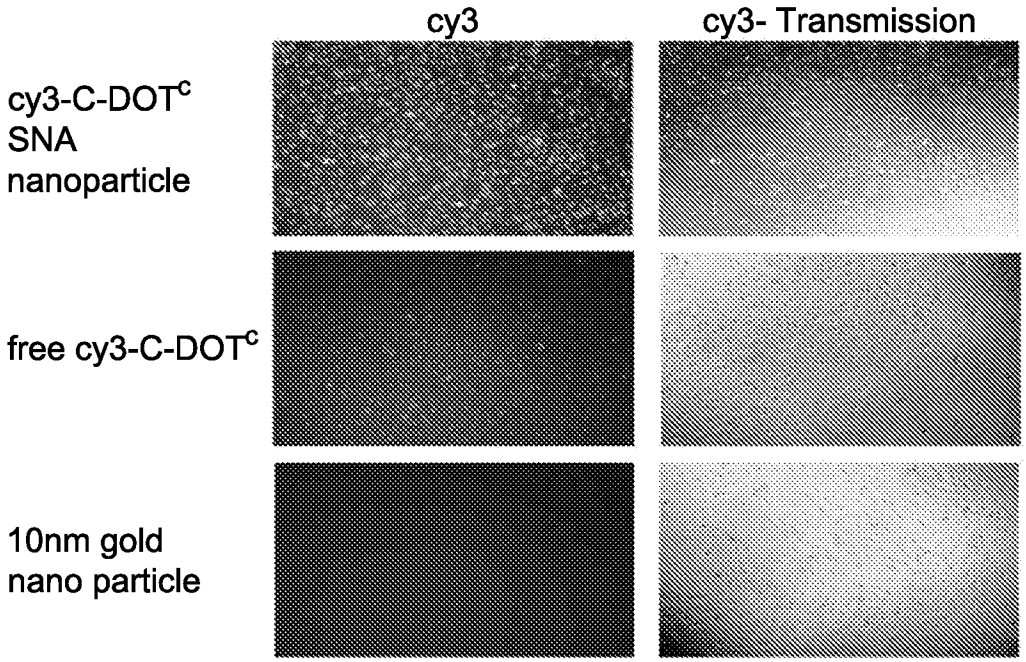

C.
Uninfected cells
GFP+
0.15
HSV-1 infected cells
GFP+
1.58
HSV-1 infected cells(+C-DOT^H)
GFP+
2.15
Uninfected cells
GFP+
0.15
CMV infected cells
GFP+
1.91
CMV infected cells(+C-DOT^C)
GFP+
2.67
SSC
GFP
250K
200K
150K
100K
50K
0
10^1
10^2
10^3
10^4
10^5

FIG. 12 (Cont'd)

A.


cy3
cy3- Transmission
cy3-C-DOT^c SNA nanoparticle
free cy3-C-DOT^c
10nm gold nano particle

B.

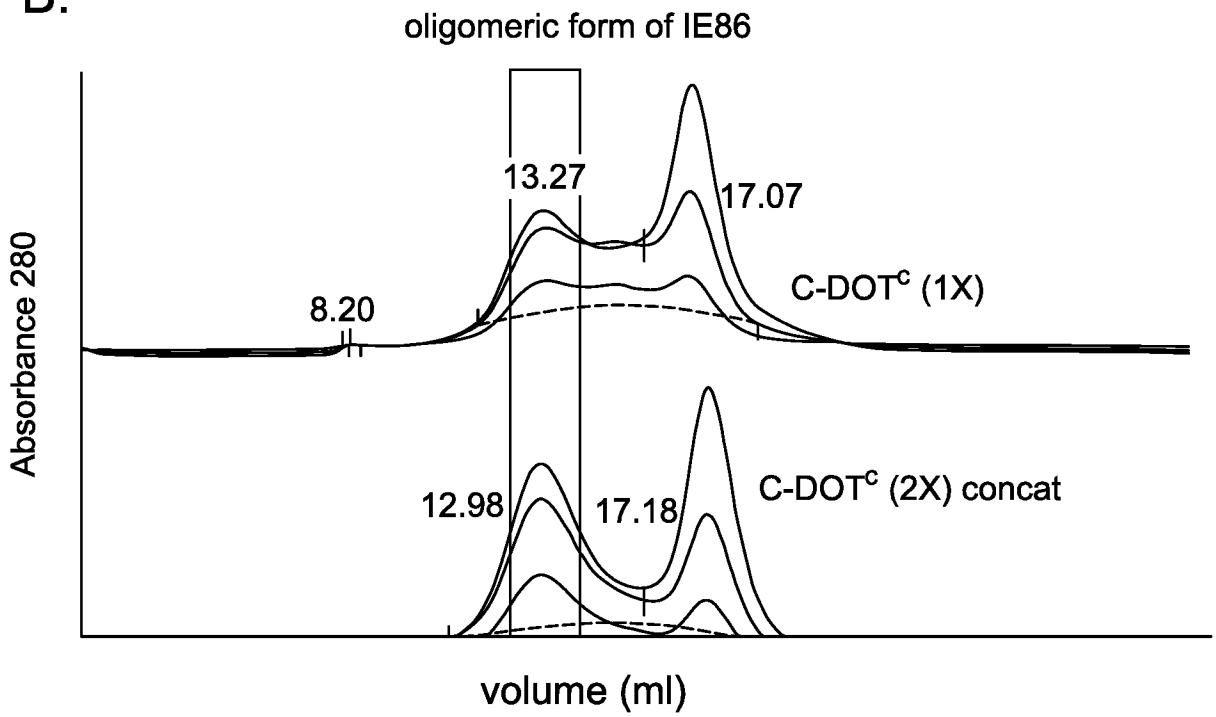

oligomeric form of IE86
13.27
17.07
8.20
C-DOT^c (1X)
Absorbance 280
12.98
17.18
C-DOT^c (2X) concat
volume (ml)

FIG. 13

C.

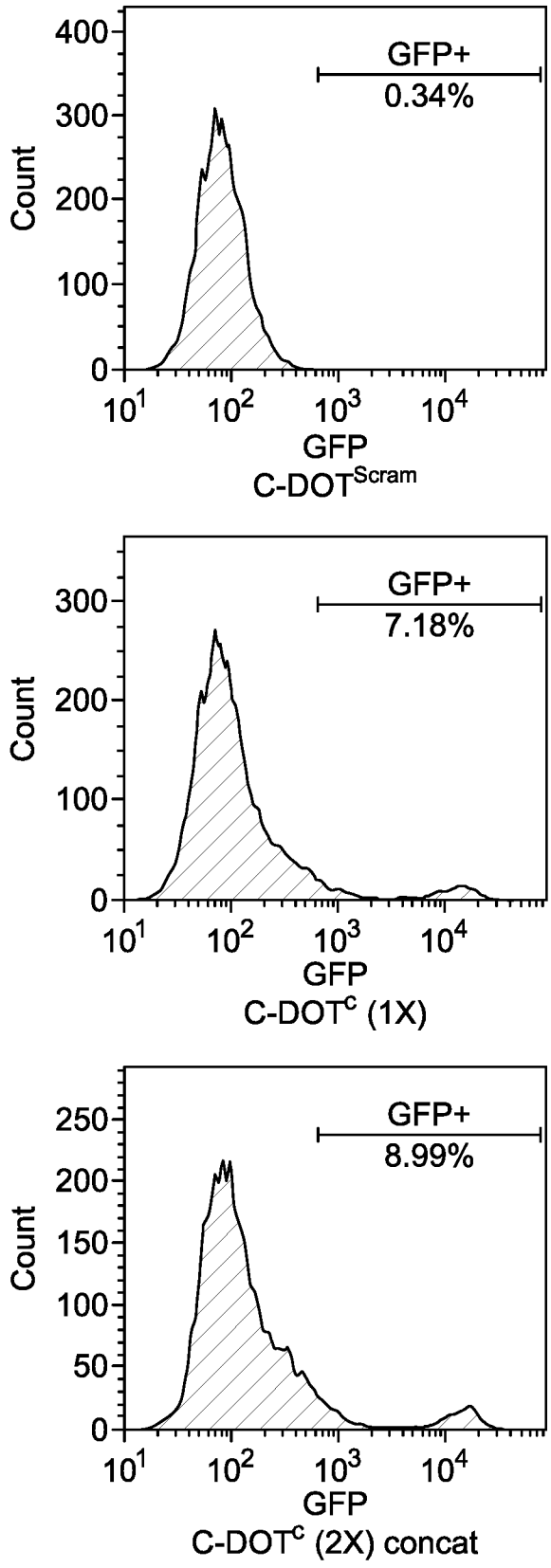
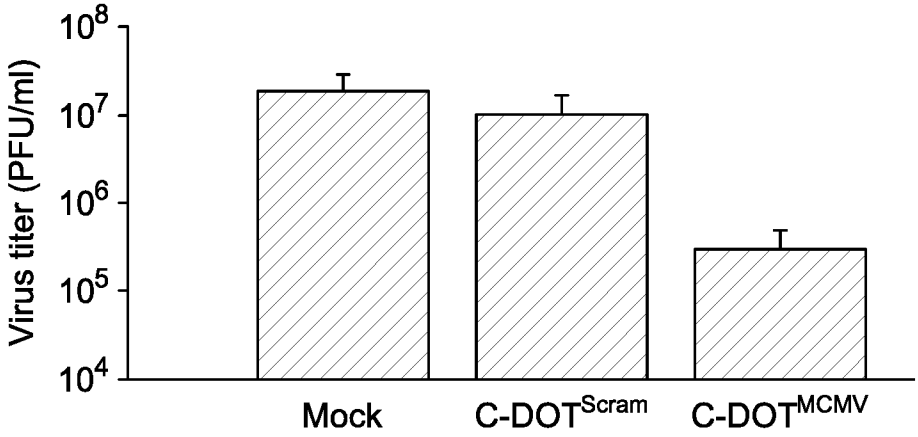


FIG. 13 (Cont'd)

A.

CMV	T	C	G	T	T	T	A	G	T	G	A	A	C	C
RhCMV	T	C	G	T	T	T	A	G	G	G	A	A	C	C
MCMV	C	C	A	G	C	G	T	C	G	G	T	A	C	C

B.



C.

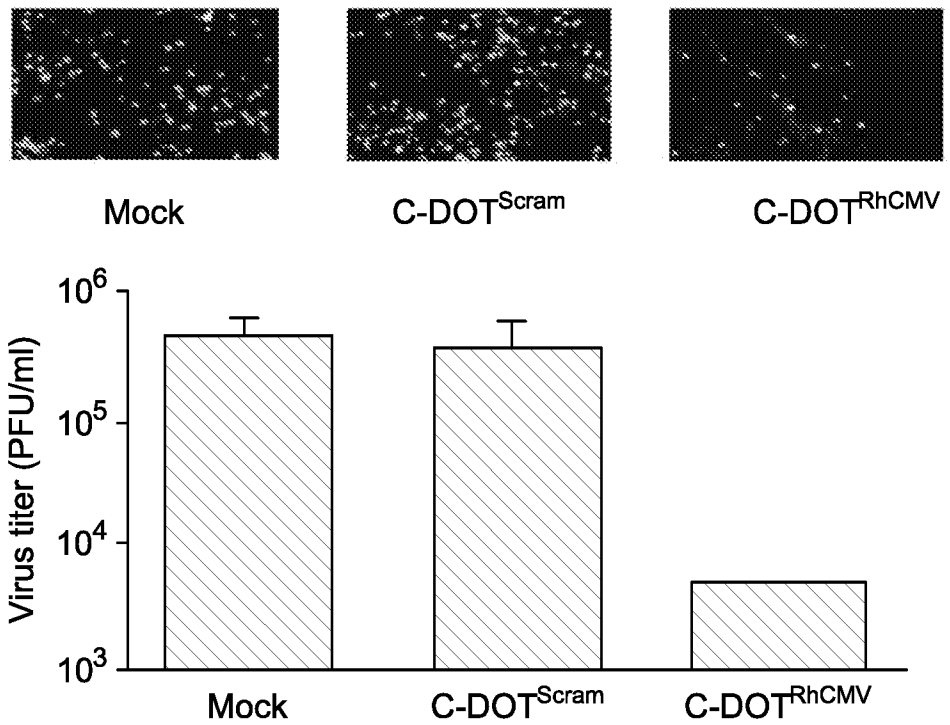


FIG. 14

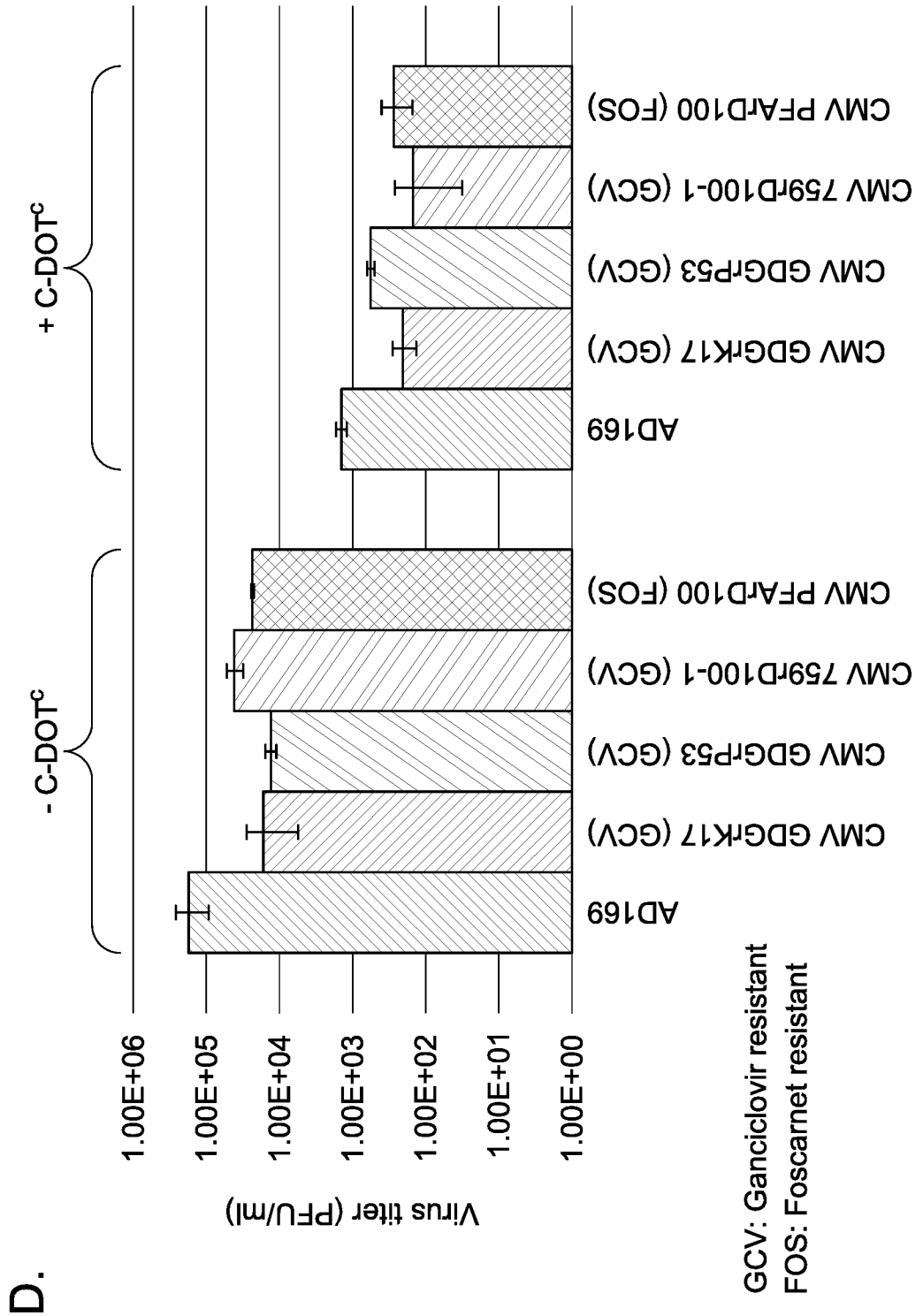

D.
Virus titer (PFU/ml)
1.00E+06
1.00E+05
1.00E+04
1.00E+03
1.00E+02
1.00E+01
1.00E+00
- C-DOT^c
+ C-DOT^c
AD169
CMV GDGrK17 (GCV)
CMV GDGrP53 (GCV)
CMV 759rD100-1 (GCV)
CMV PFArD100 (FOS)
AD169
CMV GDGrK17 (GCV)
CMV GDGrP53 (GCV)
CMV 759rD100-1 (GCV)
CMV PFArD100 (FOS)
GCV: Ganciclovir resistant
FOS: Foscarnet resistant

FIG. 14 (Cont'd)

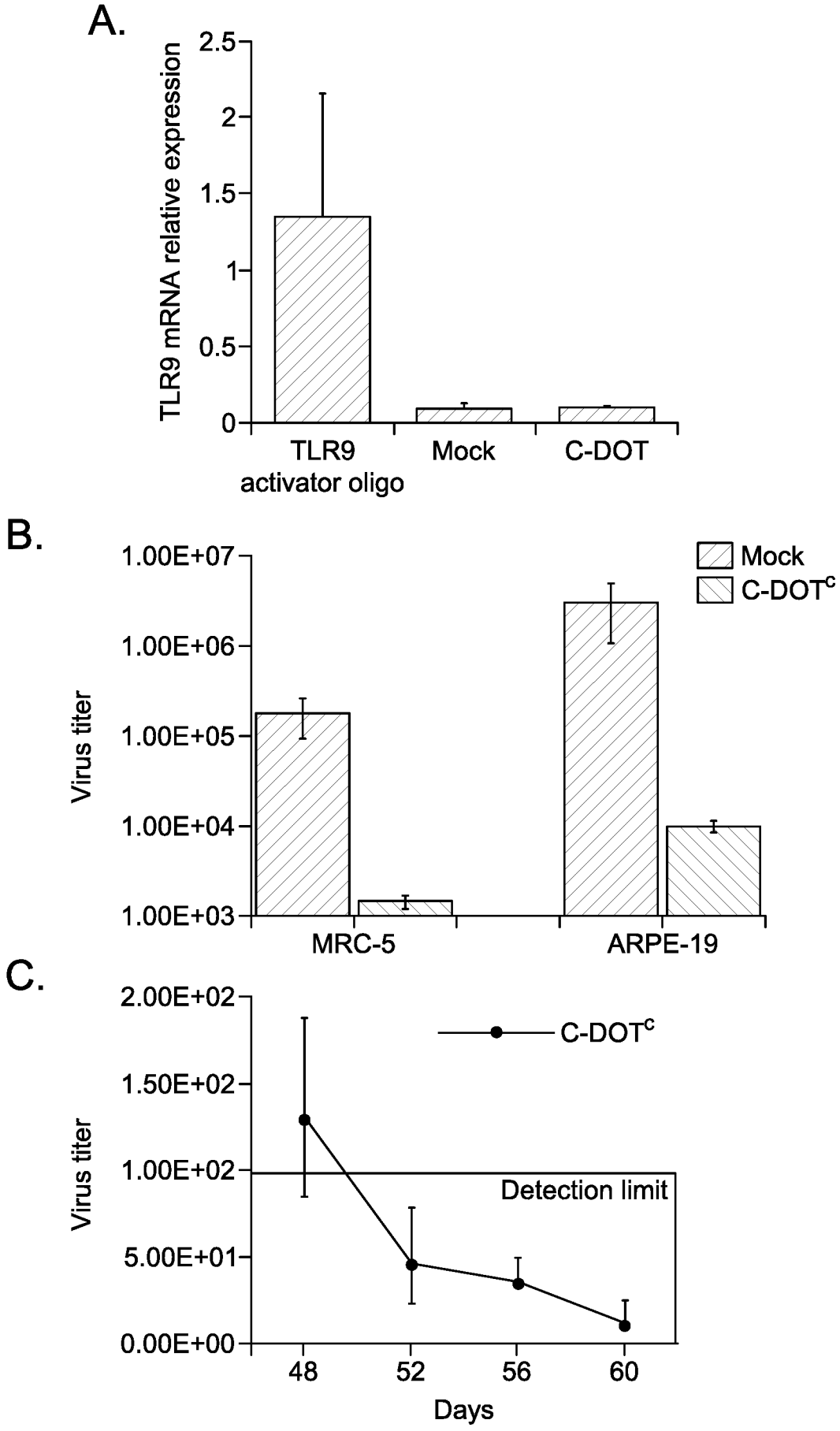

A.
TLR9 mRNA relative expression
2.5
2
1.5
1
0.5
0
TLR9 activator oligo
Mock
C-DOT
B.
Virus titer
1.00E+07
1.00E+06
1.00E+05
1.00E+04
1.00E+03
Mock
C-DOTc
MRC-5
ARPE-19
C.
Virus titer
2.00E+02
1.50E+02
1.00E+02
5.00E+01
0.00E+00
C-DOTc
Detection limit
48
52
56
60
Days

FIG. 15

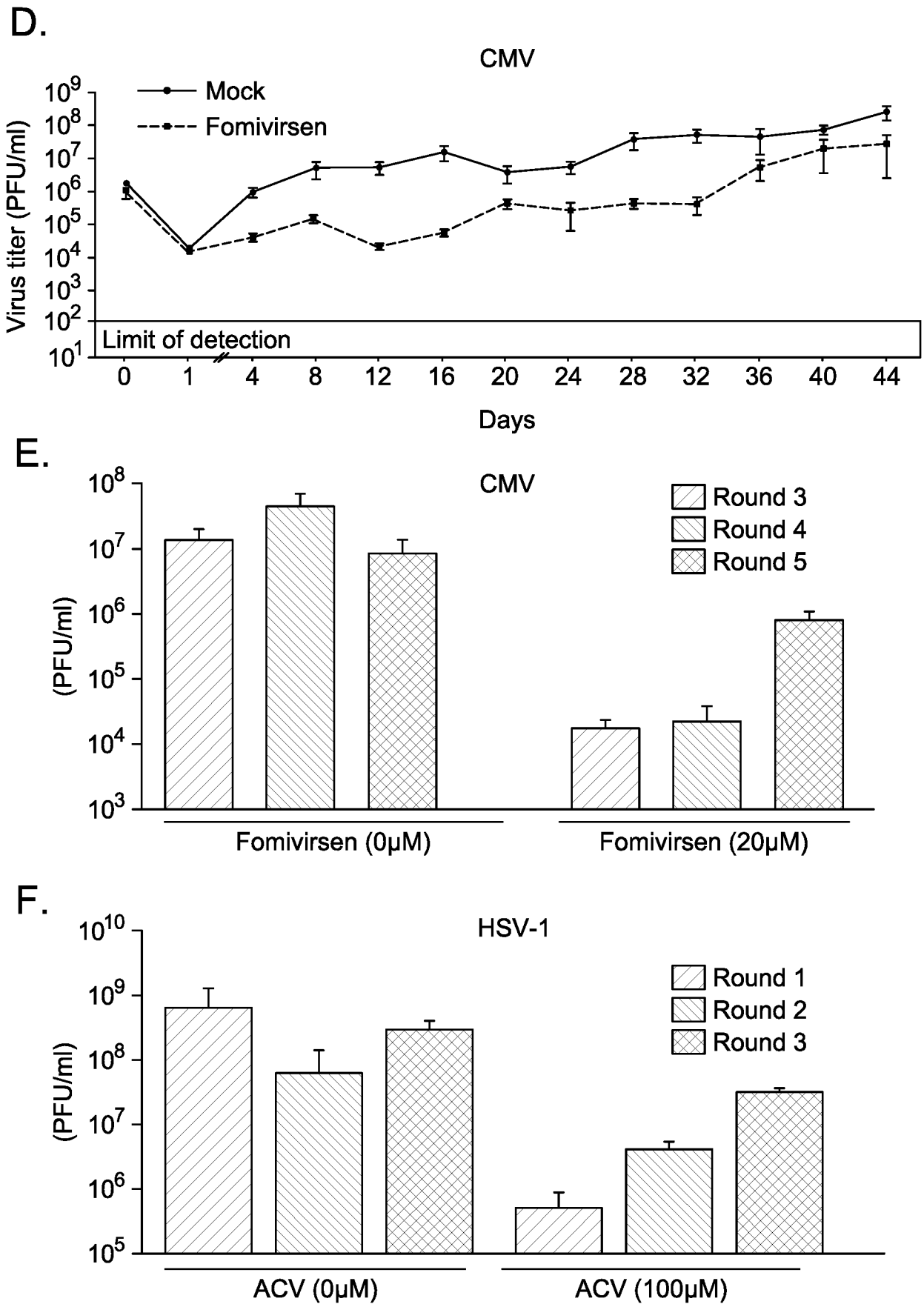
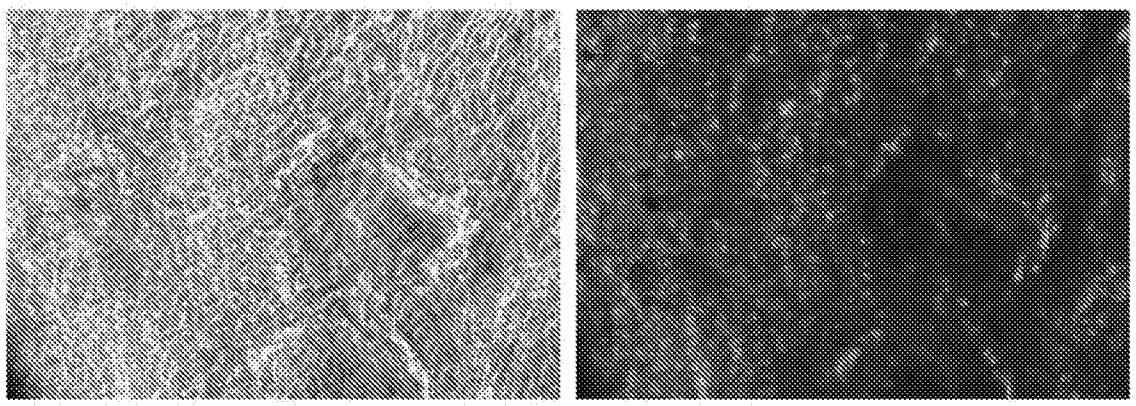

D.
CMV
Mock
Fomivirsen
Virus titer (PFU/ml)
Limit of detection
Days
E.
CMV
Round 3
Round 4
Round 5
(PFU/ml)
Fomivirsen (0μM)
Fomivirsen (20μM)
F.
HSV-1
Round 1
Round 2
Round 3
(PFU/ml)
ACV (0μM)
ACV (100μM)

FIG. 15 (Cont'd)

A.



B.

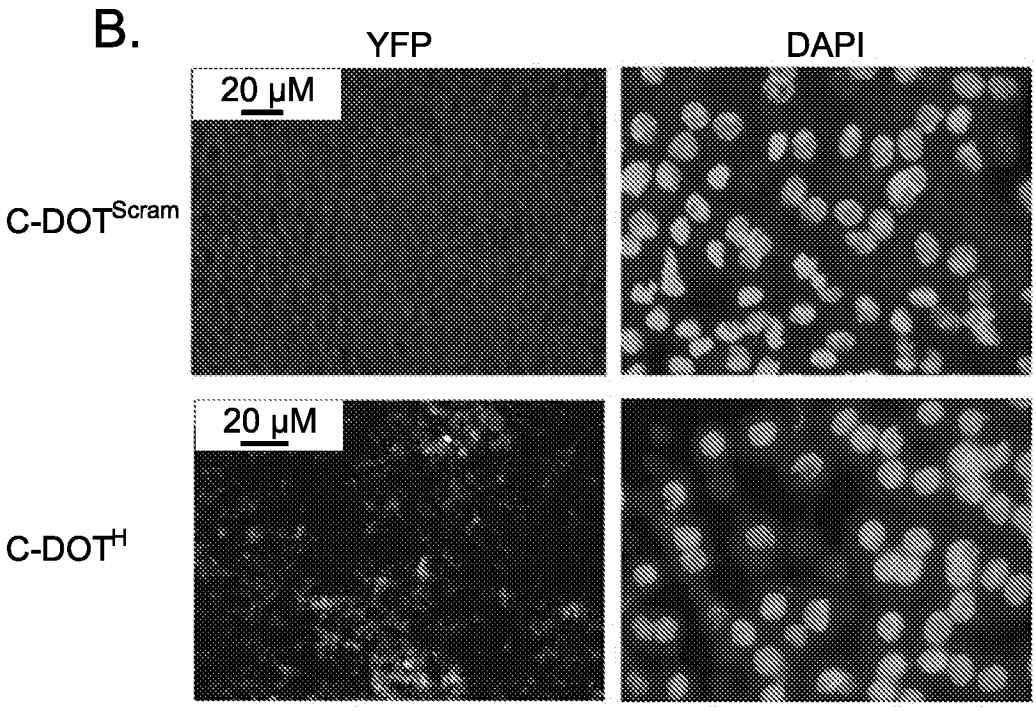


FIG. 16

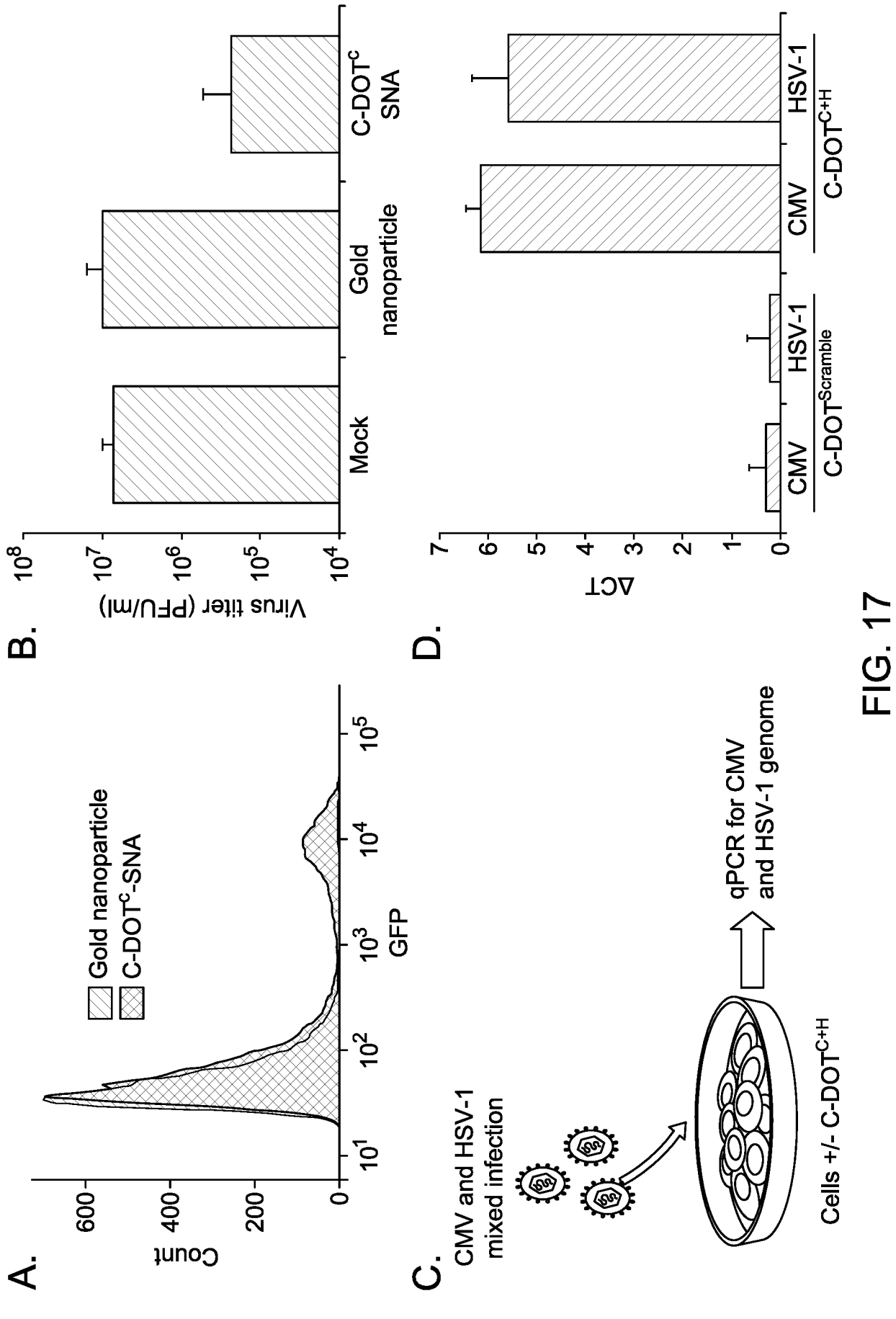

A.
Count
600
400
200
0
10^1
10^2
10^3
10^4
10^5
GFP
Gold nanoparticle
C-DOT^c-SNA
B.
Virus titer (PFU/ml)
10^8
10^7
10^6
10^5
10^4
Mock
Gold nanoparticle
C-DOT^c SNA
C.
CMV and HSV-1 mixed infection
qPCR for CMV and HSV-1 genome
Cells +/- C-DOT^{C+H}
D.
ΔCT
7
6
5
4
3
2
1
0
CMV
HSV-1
C-DOT^{Scramble}
CMV
HSV-1
C-DOT^{C+H}

FIG. 17

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US19/36841

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing:

   a. ☒ forming part of the international application as filed:

      ☒ in the form of an Annex C/ST.25 text file.

      ☐ on paper or in the form of an image file.

   b. ☐ furnished together with the international application under PCT Rule 13*ter*.1(a) for the purposes of international search only in the form of an Annex C/ST.25 text file.

   c. ☐ furnished subsequent to the international filing date for the purposes of international search only:

      ☐ in the form of an Annex C/ST.25 text file (Rule 13*ter*.1(a)).

      ☐ on paper or in the form of an image file (Rule 13*ter*.1(b) and Administrative Instructions, Section 713).

2. ☐ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that forming part of the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US19/36841

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☒ Claims Nos.: 4-18, 22-37, 41-66, 70, 72-96, 100-114, 120, 122-135, 141-155, 159-174, 178-196, 200-219
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US19/36841

A. CLASSIFICATION OF SUBJECT MATTER

IPC - A61K 31/711, 31/713, 39/12, 39/245; C07H 21/04; C12Q 1/68, 1/70, 1/705 (2019.01)

CPC - A61K 31/711, 31/713, 39/12, 39/245; C07H 21/04; C12Q 1/68, 1/70, 1/705; C12P 19/34; C12N 5/16, 15/113, 15/1131, 15/1133; C07K 14/005, 14/03, 14/035, 14/045

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
See Search History document

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
See Search History document

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
See Search History document

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X -- Y	US 2004/0023206 A1 (POLANSKY, H) 5 February 2004; paragraphs [0004], [0159], [0244], [0361], [0388]-[0389], [0395], [0726], [0782], [0822], [0839], [0860], [0880]	1-2, 3/1-2, 19-20, 21/19-20, 38-39, 40/38-39, 67-68, 69/67-68, 71, 175-176, 177/175-176, 197-198, 199/197-198 ---------- 97-98, 99/97-98, 115-118, 119/117-118, 121, 136-137, 138-139, 140/138-139, 156-157, 158/156-157
Y	WO 2018/075980 A1 (MODERNATX, INC.) 26 April 2018; page 3, lines 25-27; page 101, lines 30-31; page 102, line 19, page 108, lines 28-30	97-98, 99/97-98, 115-118, 119/117-118, 121, 136-137, 138-139, 140/138-139, 156-157, 158/156-157

☒ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"D" document cited by the applicant in the international application
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
6 September 2019 (06.09.2019)	01 OCT 2019
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-8300	Authorized officer Shane Thomas Telephone No. PCT Helpdesk: 571-272-4300

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US19/36841

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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