

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

(19) World Intellectual Property
Organization
International Bureau

(43) International Publication Date
11 April 2024 (11.04.2024)



(10) International Publication Number
WO 2024/075012 A1

(51) International Patent Classification:

C12N 15/86 (2006.01) C12N 15/63 (2006.01)

SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

(21) International Application Number:

PCT/IB2023/059919

(22) International Filing Date:

03 October 2023 (03.10.2023)

(25) Filing Language:

English

(26) Publication Language:

English

(30) Priority Data:

63/378,602 06 October 2022 (06.10.2022) US
63/583,381 18 September 2023 (18.09.2023) US

Declarations under Rule 4.17:

- as to applicant's entitlement to apply for and be granted a patent (Rule 4.17(ii))
- as to the applicant's entitlement to claim the priority of the earlier application (Rule 4.17(iii))

Published:

- with international search report (Art. 21(3))
- in black and white; the international application as filed contained color or greyscale and is available for download from PATENTSCOPE

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

(84) Designated States (unless otherwise indicated, for every kind of regional protection available): ARIPO (BW, CV, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SC, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, ME, MK, MT, NL, NO, PL, PT, RO, RS, SE,

(54) Title: IMPROVED HOST CELLS FOR AAV VECTOR PRODUCTION

(57) Abstract: The disclosure provides improved host cells for producing recombinant adeno-associated virus (AAV) vectors, methods of producing AAV vectors using such improved host cells, and AAV vectors produced thereby.

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IMPROVED HOST CELLS FOR AAV VECTOR PRODUCTION

BACKGROUND OF THE INVENTION

[0001] Adeno-associated virus (AAV) vectors have emerged as an important new modality for treating rare monogenic diseases and disorders where the mutated gene resulting in the loss of function phenotype is known, classic examples being hemophilia A and B, and Duchenne muscular dystrophy. As the field matures, however, there is growing interest in applying the AAV system to treat more complex multigenic disorders, as well as more common diseases with underlying genetic causes. Achieving these goals will, however, require the ability to economically manufacture to GMP standards ever increasing amounts of vector.

[0002] AAV vectors are complex therapeutics which incorporate certain structural and functional features of AAV virions. AAV vectors, like AAV virions, possess a protein shell, the capsid, which encloses a genome. The capsid is responsible for specifically binding surface receptors on target cells and protecting the genome as the vector penetrates the cell by endocytosis, a process called transduction which is akin to viral infection. The genome, in turn, is modified to prevent vector replication in target cells, and further contains genetic information required to express a therapeutic protein of interest in the target cells once transduced. Different strategies have been developed for producing AAV vectors at lab and commercial scales, but all involve growing host cells, introducing various genes required to direct the biosynthetic machinery of the cells to replicate and package vector genomes into capsids, and then purify the resulting vectors made by the cells after a period of incubation.

[0003] Two major approaches have been adopted for introducing into host cells the exogenous genetic material required for vector production. One approach relies on transfecting DNA plasmids into usually mammalian host cells. Often, three plasmids are used, variously containing genes for expressing proteins forming the capsid and which are involved in genome replication and packaging (i.e., AAV cap and rep genes), genes to express helper RNA and proteins from another virus (usually adenovirus or herpes virus) also required for vector replication, and the vector genome sequence itself. The other approach relies on infecting insect host cells with baculovirus engineered to express the genes required for vector production. With either approach, cellular factors may exist which hinder the rate and/or extent at which vector components are synthesized and intact vectors made. For example, it is known that cellular defense mechanisms have evolved to protect cells from virus infection or replication.

[0004] Because host cells are central and essential to the vector manufacturing process, the existence of cellular bottlenecks are a major impediment to efficient and economical vector production at commercial scale. Accordingly, there exists a need in the art for improved host cells in which cellular factors which interfere with vector biosynthesis are deleted or repressed.

SUMMARY OF THE INVENTION

[0005] To address the need in the art, the present disclosure provides modified host cells in which cellular factors upregulated after the introduction of exogenous genetic material required for vector biosynthesis are deleted or repressed, resulting in improvements in vector productivity and quality attributes, such as the proportion of capsids containing intact genomes. The disclosure also provides improved methods for using such host cells to produce AAV vectors, as well as AAV vectors produced using such host cells and methods.

[0006] Certain enumerated non-limiting embodiments (E) of the inventions of the disclosure are set forth below. These and related embodiments are described in further detail in the Detailed Description, including the Examples and Drawings. Those skilled in the art will recognize or will be able to ascertain, using no more than routine experimentation, equivalents to the specific embodiments described herein.

E1. An animal cell modified to reduce expression of at least one gene upregulated in an otherwise unmodified animal cell of the same type in response to infection by an adeno-associated virus (AAV), or to biosynthesis of recombinant AAV (rAAV) vector directed by exogenously introduced nucleic acid.

E2. The animal cell of E1, wherein said upregulated gene is associated with a reduction in the amount of intact AAV vector produced in said unmodified animal cell of the same type relative to the amount of intact AAV vector produced in said modified animal cell.

E3. The animal cell of E1 or E2, wherein the exogenously introduced nucleic acid is introduced into said otherwise unmodified animal cell of the same type by infection with a virus containing such nucleic acid, or by transfection.

E4. The animal cell of E1 to E3, wherein said animal cell is an insect cell and the exogenously introduced nucleic acid is introduced into an otherwise unmodified insect cell of the same type by infection with a baculovirus.

- E5.** The animal cell of E1 to E3, wherein said animal cell is a mammalian cell and the exogenously introduced nucleic acid is introduced into an otherwise unmodified mammalian cell of the same type by transfection.
- E6.** The mammalian cell of E5, wherein said mammalian cell is selected from the group consisting of: HeLa cells, Cos cells, HEK293 cells, A549 cells, BHK cells, Vero cells, NIH 3T3 cells, HT-1080 cells, Sp2/0 cells, NS0 cells, C127 cells, AGE1.HN cells, CAP cells, HKB-11 cells, WI-38 cells, MRC-5 cells, and PER.C6 cells.
- E7.** The mammalian cell of E6, wherein said mammalian cell is a HEK293 cell, or derivative or variant thereof.
- E8.** The mammalian cell of E7, wherein said mammalian cell is a derivative or variant of HEK293 cells selected from the group consisting of: HEK293E, HEK293F, HEK293FT, HEK293G, HEK293H, HEK293S, HEK293SG, and HEK293T cells.
- E9.** The mammalian cell of E5 to E8, wherein said exogenously introduced nucleic acid is introduced into an otherwise unmodified mammalian cell of the same type by transient transfection of one or more DNA plasmids.
- E10.** The mammalian cell of E9, wherein said gene is upregulated at least 3 hours after transient transfection.
- E11.** The mammalian cell of E9, wherein said gene is upregulated at least 6 hours after transient transfection.
- E12.** The mammalian cell of E9, wherein said gene is upregulated at least 12 hours after transient transfection.
- E13.** The mammalian cell of E9, wherein said gene is upregulated at least 24 hours after transient transfection.
- E14.** The mammalian cell of E9, wherein said gene is upregulated at least 36 hours after transient transfection.
- E15.** The mammalian cell of E9, wherein said gene is upregulated at least 48 hours after transient transfection.
- E16.** The mammalian cell of E9, wherein said gene is upregulated at least 72 hours after transient transfection.

E17. The animal cell of E1 to E16, wherein said upregulated gene is a member of a gene ontology category selected from the group consisting of: rDNA Heterochromatin Assembly Gene Ontology (GO:0000183), Inactivation of MAPK Activity Gene Ontology (GO:0000188), mRNA Splicing, Via Spliceosome Gene Ontology (GO:0000398), Innate Immune Response in Mucosa Gene Ontology (GO:0002227), Nucleosome Assembly Gene Ontology (GO:0006334), DNA Replication-Dependent Nucleosome Assembly Gene Ontology (GO:0006335), DNA Replication-Independent Nucleosome Assembly Gene Ontology (GO:0006336), DNA-Templated Transcription, Initiation Gene Ontology (GO:0006352), Inflammatory Response Gene Ontology (GO:0006954), Immune Response Gene Ontology (GO:0006955), Regulation of Transcription by RNA Polymerase II Gene Ontology (GO:0006357), Female Pregnancy Gene Ontology (GO:0007565), Response to Virus Gene Ontology (GO:0009615), Telomere Capping Gene Ontology (GO:0016233), Antibacterial Humoral Response Gene Ontology (GO:0019731), Endoplasmic Reticulum Unfolded Protein Response Gene Ontology (GO:0030968), Telomere Organization Gene Ontology (GO:0032200), CENP-A Containing Nucleosome Assembly Gene Ontology (GO:0034080), PERK-Mediated Unfolded Protein Response Gene Ontology (GO:0036499), Cellular Protein Metabolic Process Gene Ontology (GO:0044267), Negative Regulation of Viral Genome Replication Gene Ontology (GO:0045071), Regulation of Megakaryocyte Differentiation Gene Ontology (GO:0045652), Negative Regulation of Megakaryocyte Differentiation Gene Ontology (GO:0045653), Negative Regulation of Gene Expression, Epigenetic Gene Ontology (GO:0045814), Detection of Chemical Stimulus Involved in Sensory Perception of Smell Gene Ontology (GO:0050911), Defense Response to Virus Gene Ontology (GO:0051607), Type I Interferon Signaling Pathway Gene Ontology (GO:0060337), Regulation of Ribonuclease Activity Gene Ontology (GO:0060700), Regulation of Gene silencing by miRNA Gene Ontology (GO:0060964), and Cellular Response to Interleukin-1 Gene Ontology (GO:0071347).

E18. The animal cell of E1 to E16, wherein said upregulated gene is selected from the group of genes consisting of: ABCC2, ABCF1, ACKR2, ACTA1, ARL14, AGO1, ALB, ANG, ANKRD1, ANO8, ANXA1, AOC3, APEX1, APLN, APOA1, APOBEC3B, APOBEC3H, APOE, APP, ARNT2, ASNS, ATF3, ATF4, BDNF, BHLHA15, BLNK, BST2, BUD31, C3, C4A, C4B, C5, C5AR1, CALCR, CALR3, CAMP, CBX5, CCL20, CCL25, CCL26, CCL4, CCL5, CCND1, CCR10, CCR4, CD14, CD8A, CEBPA, CEBPB, CEBPG, CENPQ, CFP, CGAS, CHGB, CHUK, CISH, CLIC5, CNIH1, COL16A1, CREB1, CREBBP, CREBRF, CSF1, CTH, CTSK, CTSW, CX3CR1, CXCL11, CXCL8, CXCR2, CYP1A1, DAXX, DDIT3, DDIT4, DDX58, DDX60, DNMT3B, DOCK8, DTX3L, DUSP1, DUSP10, DUSP14, DUSP16, DUSP4, DUSP5, DUSP6, DUSP8, ECM1, EGF, EGR1, EIF2AK2, ELK1, EP300, EPAS1, EPHB1, ERBB2,

ESRP1, EXOSC5, FAM83A, FAS, FCGR2A, FEV, FGF2, FGF21, FGF23, FOS, FOSB, FOSL1, FOXO1, FOXO3, GBP1, GBP3, GEM, GIP, GNRH1, GPER1, H1-0, H1-2, H1-3, H1-4, H1-5, H2BC11, H2BC12, H2BC13, H2BC14, H2BC15, H2BC17, H2BC18, H2BC21, H2BC4, H2BC5, H2BC6, H2BC7, H2BC8, H2BU1, H2BW2, H3C1, H3C10, H3C12, H3C13, H3C14, H3C15, H3C2, H3C6, H4-16, H4C1, H4C12, H4C14, H4C15, H4C2, H4C3, H4C4, H4C5, H4C8, H4C9, HAS2, HERPUD1, HGF, HIF1A, HLA-DRB1, HMGA1, HRH1, HS3ST4, IFI27, IFI44, IFI44L, IFI6, IFIH1, IFIT1, IFIT2, IFIT3, IFITM1, IFITM3, IFNAR1, IFNB1, IGFBP3, IGFBP5, IGFBP7, IKBKB, IKBKE, IKBKG, IL16, IL17B, IL17F, IL17RE, IL18, IL23A, IL32, IL33, IL34, IL5, IRAK3, IRF1, IRF3, IRF5, IRF7, IRF9, IRGM, ISG15, ISG20, ITGAM, JUN, JUNB, KDM1A, KITLG, KLF2, LAMB1, LGALS1, LGALS9, LMCD1, LST1, LTB4R, LTB4R2, MADCAM1, MAP2K1, MAP2K3, MAP3K1, MAP3K14, MAPK1, MAPK14, MAPK3, MAPK8, MAVS, MEF2C, MEF2D, MEP1B, MIF, MMP13, MMP25, MR1, MSC, MSLN, MYD88, MYLPF, NFIL3, NFKB1, NFKBIA, NGF, NKX3-1, NLRC4, NOVA2, NR1D1, NR4A3, OAS1, OAS2, OAS3, OASL, ODAPH, OR2B6, OR51E2, OVGP1, PAF1, PARP9, PCK1, PDE6G, PIK3CD, PLA2G1B, PLA2G4C, PLP1, PLSCR1, PMAIP1, PML, PNN, PPRC1, PRDM1, PRKCD, PROC, PSG4, PTAFR, PTGS2, PTHLH, PTPRJ, PTX3, PYCARD, RAF1, RAVR1, RBPJ, RC3H1, RELA, RELB, REST, RET, RGS3, RLN1, RPL39, RPS15A, RSAD2, SAFB, SASH1, SCG2, SCG3, SELENOS, SIM1, SIRT1, SLC38A2, SLPI, SMAD3, SMARCA4, SMARCB1, SMPD1, SOCS1, SOCS3, SP100, SP110, SPI1, SRF, STAT1, STAT4, STC2, STING1, TAC1, TAC3, TAPBP, TFRC, TGFB1, TGFB3, TGM2, TICAM1, TICAM2, TLR2, TLR3, TMEM132A, TNC, TNFAIP3, TNFSF12, TP53, TPT1, TRAF3IP2, TRIM22, TRIM24, TRIM5, ULBP1, VEGFA, VGF, VPB3, WFDC2, and XBP1.

E19. The animal cell of E1 to E18, wherein the expression level of said upregulated gene is repressed pre-transcriptionally or post-transcriptionally.

E20. The animal cell of E1 to E19, wherein the expression level of said upregulated gene is repressed by contacting said cells with a compound effective to repress expression of said upregulated gene.

E21. The animal cell of E1 to E19, wherein the expression level of said upregulated gene is repressed by RNase H recruitment, such as by contacting said cell with an antisense oligonucleotide (AON) comprising nucleotide sequence that is complementary to an RNA transcript transcribed from said upregulated gene, such as an mRNA, by RNA interference (RNAi), or by CRISPR interference (CRISPRi).

E22. The animal cell of E21, wherein RNAi is effected transiently or permanently.

E23. The animal cell of E21 or E22, wherein RNAi is effected by contacting said cell with, or by expressing in said cell, a guide strand RNA molecule effective to target an RNA transcript transcribed from said upregulated gene, such as an mRNA.

- E24.** The animal cell of E23, wherein said guide strand RNA molecule is comprised by a siRNA molecule, a shRNA molecule, or a miRNA molecule.
- E25.** The animal cell of E23 or E24, wherein said guide strand RNA molecule, siRNA molecule, shRNA molecule, or miRNA molecule, is expressed from an expression construct introduced into said cell transiently or stably.
- E26.** The animal cell of E19 to E25, wherein the expression level of said upregulated gene is repressed at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, 96%, 97%, 98%, or 99% compared to the expression of level of said upregulated gene in unmodified animal cells of the same type.
- E27.** The animal cell of E1 to E18, wherein said upregulated gene is inactivated.
- E28.** The animal cell of E27, wherein said upregulated gene is inactivated by altering the genome of said cell.
- E29.** The animal cell of E28, wherein the genome of said cell is altered by deleting nucleotides from, adding nucleotides to, or substituting nucleotides within at least one DNA sequence, or portion thereof, that is required for said upregulated gene to be transcribed or translated from one or both alleles of said gene.
- E30.** The animal cell of E29, wherein said DNA sequence required for said upregulated gene to be transcribed or translated is selected from the group consisting of: a silencer sequence, an insulator sequence, a histone binding site, an enhancer sequence, a promoter sequence, a translation initiation sequence, an exon sequence, an intron sequence, a splice donor or acceptor sequence, a stop codon, and a transcription termination signal sequence.
- E31.** The animal cell of E27 to E30, wherein said upregulated gene is inactivated by deleting all the exons of said gene.
- E32.** The animal cell of E27 to E30, wherein said upregulated gene is inactivated by introducing at least one premature stop codon.
- E33.** The animal cell of E28 to E31, wherein the genome of said cell is altered by homologous recombination.
- E34.** The animal cell of E28 to E32, wherein the genome of said cell is altered by gene editing.

- E35.** The animal cell of E34, wherein said gene editing is effected by expression of a zinc-finger nuclease protein, a transcription-activator-like effector nuclease (TALEN), or a CRISPR-Cas complex comprising a Cas enzyme and guide RNA.
- E36.** A method for producing an adeno-associated virus (AAV) vector, comprising introducing into the modified animal cell of E1 to E34 exogenous genetic information required for AAV vector biosynthesis, incubating said cell under conditions and for time sufficient for the biosynthesis of said AAV vector to occur, and purifying from said cell the AAV vector produced thereby.
- E37.** The method of E36, wherein the exogenous genetic information required for AAV vector biosynthesis comprises one or more genes selected from the group consisting of: an AAV cap gene, an AAV rep gene, a viral helper RNA or protein, and an AAV vector genome.
- E38.** The method of E36, wherein the exogenous genetic information required for AAV vector biosynthesis comprises an AAV cap gene, an AAV rep gene, nucleotide sequence encoding a viral helper RNA and protein, and an AAV vector genome.
- E39.** The method of E36 to E38, wherein the exogenous genetic information required for AAV vector biosynthesis is introduced into said modified animal cells in the form of one or more DNA plasmids by transient or stable transfection.
- E40.** The method of E36 to E38, wherein the exogenous genetic information required for AAV vector biosynthesis is introduced into said modified animal cells in the form of one or more recombinant baculoviruses by infection.
- E41.** The method of E39, wherein a first DNA plasmid comprises an AAV cap and an AAV rep gene, a second DNA plasmid comprises sequence for a viral helper RNA and protein, and a third DNA plasmid comprises sequence for an AAV vector genome.
- E42.** The method of E36 to E39, or E40 to E41, wherein said modified animal cell is a modified mammalian cell.
- E43.** The method of E42, wherein said modified mammalian cell is a modified HEK293 cell, or derivative or variant thereof.
- E44.** The method of E43, wherein said modified HEK293 cell derivative or variant is selected from the group consisting of: HEK293E, HEK293F, HEK293FT, HEK293G, HEK293H, HEK293S, HEK293SG, and HEK293T cells.

- E45.** The method of E36 to E44, wherein said method is effective to produce an amount of AAV vector that is at least 50%, 100%, 150%, 200%, 250%, 300%, 350%, 400%, 450%, or 500% greater than the amount of AAV vector which could be produced in unmodified animal cells of the same type under otherwise identical conditions.
- E46.** The method of E45, wherein the amount of AAV vector produced is determined using quantitative PCR.
- E47.** An AAV vector produced according to the method of E36 to E44.
- E48.** An expression vector comprising an expression cassette for expressing in a host cell a miRNA, a shRNA, or other type of non-coding RNA capable of repressing by RNA interference expression of at least one gene upregulated in response to infection by an adeno-associated virus (AAV), or to biosynthesis of recombinant AAV (rAAV) vector directed by exogenously introduced nucleic acid.
- E49.** The expression vector of E48, wherein said expression cassette expresses a miRNA under control of an RNA pol II promoter.
- E50.** The expression vector of E48, wherein said expression cassette expresses a shRNA under control of an RNA pol III promoter.
- E51.** The expression vector of E48 to E50, wherein said gene is selected from the group of genes consisting of: ACTA1, ARL14, BDNF, CALCR, CSF1, CXCL11, DOCK8, EIF2AK2, EP300, EPHB1, ESRP1, FAM83A, FGF2, FOXO1, GNRH1, HGF, HS3ST4, IFI44L, ITGAM, KITLG, MAP2K3, MAPK1, MAPK3, MIF, MSC, MYD88, OAS3, ODAF1, PAF1, PDE6G, PNN, PPRC1, PTX3, RC3H1, SMARCA4, SP110, SPI1, STAT4, TGM2, TICAM1 and/or TRIM24.
- E52.** A method for producing an adeno-associated virus (AAV) vector, comprising introducing into a host cell the expression vector of E48 to E51 and then, contemporaneously therewith or subsequently, introducing into said host cell exogenous genetic information required for AAV vector biosynthesis, incubating said cell under conditions and for time sufficient for the biosynthesis of said AAV vector to occur, and purifying from said host cell the AAV vector produced thereby.
- E53.** The method of E52, wherein the exogenous genetic information required for AAV vector biosynthesis comprises an AAV cap gene, an AAV rep gene, a nucleotide sequence encoding a viral helper RNA and protein, and an AAV vector genome.

E54. The method of E52 to E53, wherein both the expression vector and the exogenous genetic information required for AAV vector biosynthesis is introduced into said host cell in the form of one or more DNA plasmids by transient transfection.

E55. The method of E48 to E54, wherein said host cell is a mammalian cell.

E56. The method of E55, wherein said mammalian cell is a HEK293 cell, or derivative or variant thereof.

E57. The method of E56, wherein said HEK293 cell derivative or variant is selected from the group consisting of: HEK293E, HEK293F, HEK293FT, HEK293G, HEK293H, HEK293S, HEK293SG, and HEK293T cells.

E58. The method of E48 to E57, wherein said method is effective to produce an amount of AAV vector that is at least 50%, 100%, 150%, 200%, 250%, 300%, 350%, 400%, 450%, or 500% greater than the amount of AAV vector which could be produced in a host cell of the same type in the absence of introducing said expression vector comprising said expression cassette.

E59. An AAV vector produced according to the method of E48 to E58.

BRIEF DESCRIPTION OF THE DRAWINGS

[0007] **Fig. 1.** The experimental design and workflow of transcriptome analysis of rAAV-producing HEK293 cells in production bioreactors. Three independent 50L single-use bioreactors (SUBs) were each sampled prior to transfection and at 7 timepoints post-transfection. RNA was prepared from these samples and subjected to RNA-Seq.

[0008] **Figs. 2A-2F.** Kinetics of rAAV production and cell growth in 50L and 2L Bioreactors. (Figs. 2A-2C) For each time point selected for transcriptome analysis, the vector genome (vg) titer, viable cell density (VCD) and percent viable cells were determined for each of the three 50L bioreactors. (Figs. 2D-2F) An additional set of three independent 2L bioreactors were performed and sampled in the same manner, and vg titer, VCD, and percent viability were determined. Viral genome titer was determined by rAAV qPCR described in Material and Methods. VCD and cell viability were measured by Vi-Cell XR cell viability analyzer (Beckman Coulter Life Sciences). Dashed lines in (Fig. 2A) and (Fig. 2D) indicate the limit of quantification (LOQ) for AAV titer using the qPCR method. Error bars indicate standard deviation of measurements among technical replicates.

[0009] **Figs. 3A-3G.** Overview of transcriptional response to transfection and rAAV production. (Fig. 3A) Number of up- and down-regulated DEGs with at a least 2-fold relative to pre-

transfection (0h) baseline. A total of 1,850 DEGs were detected using DESeq2 with FDR < 0.05, as described in Methods and Figure S1B. (Figs. 3B-3C) Significantly enriched pathways were manually curated and categorized into two major biological functions. The x-axis shows the log transformed corrected p-value in pathway analysis. GO terms with corrected p-value < 0.05 (dotted line) were considered significant, based on Fisher's exact test followed by Benjamini-Hochberg multiple test correction. (Figs. 3D-3F) Transcriptional changes at each time point are shown for DEGs associated with gene ontology categories for inflammatory response, defense response to virus and nucleosome assembly, respectively. DEGs were clustered by hierarchical clustering on the y-axis and ordered by time points on the x-axis. Expression fold-changes were calculated compared with pre-transfection time point 0h. The fold changes were indicated in log₂ scale as shown in the lower right legend. (Fig. 3G) DEGs were clustered according to coordinate temporal changes in transcription. The x-axis represents sampling time points. The y-axis represents log₂ fold-change standardized to time zero mean and unit variance across 8 time points and triplicates. Red lines indicate standardized expression level of individual DEGs. The dark blue line represents the mean of clustered genes while the blue shade area is the range of 95% confidence interval. GO terms were significant when corrected p-value < 0.05 using Fisher's exact test followed by Benjamini-Hochberg multiple test correction.

[0010] **Figs. 4A-4I.** The upregulation of selected DEGs is confirmed at protein and mRNA levels. (Fig. 4A) Time-course protein expression of EGR1 in rAAV-producing cells from a 2L bioreactor by immunoblot. The quantification of EGR1 expression was normalized to actin and compared with pre-transfection (0h) sample. EGR1 transcription FC shows the mean fold change of EGR1 transcripts observed in transcriptome data. FC: fold-change. (Figs. 4B-4F) Select DEGs were confirmed by qRT-PCR analysis of samples from 2L bioreactors. Time-course mRNA expression of IFNB1 (Fig. 4B), CCL5 (Fig. 4C), IL-8 (Fig. 4D), IFIT2 (Fig. 4E) and OASL (Fig. 4F) are shown. (Figs. 4G-4I) Secretion of IFN- β (Fig. 4G), CCL5 (Fig. 4H) and IL-8 (Fig. 4I) in spent medium were quantified by ELISA. The spent medium preparation was described in Methods. Lowest linear point of standard curve is shown to indicate the limit of detection (black dashed line).

[0011] **Figs. 5A-5C.** Upstream regulator analysis to predict potential master regulators that may modulate transcriptional changes to AAV production. (Fig. 5A) Schematic of upstream regulator analysis. Using Ingenuity Pathway Analysis software, observed expression changes were compared to known gene regulatory interactions, and potential upstream regulators were identified based on consistency between the predicted regulatory effects of each regulator and

the actual observed changes in the transcriptome. The circles represent distinct genes. Green arrows (upregulated) and red arrows (downregulated) represent the direction of the observed transcriptional changes, while the width of arrow indicates the magnitude of change. (Fig. 5B) The process to select predicted upstream regulators (UR) via IPA analysis based on number of modulated DEGs. The predicted URs were ranked by activation z-score described in IPA tool inferring whether the expression changes of URs in our RNA-seq agreed (positive z-score) or disagreed (negative z-score) with the protein-protein interaction and/or transcription regulation shown in the literature based on the direction of expression changes of observed DEGs. The UR selection process stopped when the subset of ranked URs collectively regulated more than 80% of DEGs at given time point (dashed line). The red curve indicates the number of cumulative DEGs accounted for at each point in the list of URs. The blue curve represented the DEG/UR ratio given the number of selected URs. (Fig. 5C) The hierarchical clustering heatmap of 107 URs that were expressed in AAV-producing HEK293 cells. Expression fold-changes were calculated compared with pre-transfection time point 0h. The fold change is indicated by the intensity (i.e., lightness to darkness) of a grayscale pad using a log₂ scale. Upregulated expression is indicated by progressively darker grayscale without stipple, whereas downregulated expression is indicated by progressively darker grayscale with stipple. White indicates basal, or unchanged, expression.

[0012] **Fig. 6.** Schematic diagram describing the kinetics of the cellular response to rAAV production in transiently transfected HEK293 cells. The acute cellular response to transfection is induced at early time points post transfection, while inflammatory responses and type-I IFN signaling pathway are increasingly upregulated over time during rAAV production.

[0013] **Figs. 7A-7B.** (Fig. 7A) Bioinformatic workflow for the identification of potential mechanisms and corresponding genes that regulated AAV production via plasmid transfection in HEK293 cells. A total of 708,113 Mbases was yield across 24 samples with mean quality score of 35.85. The percentage of sequences with error rate lower than 0.001 was 93.17%. Expression pattern clustering was performed using time-series analysis based on Dirichlet process Gaussian process (DPGP) mixture model (McDowell, I.C., Manandhar, D., Vockley, C.M., Schmid, A.K., Reddy, T.E., and Engelhardt, B.E. (2018). Clustering gene expression time series data using an infinite Gaussian process mixture model. PLoS Comput Biol 14, e1005896. 10.1371/journal.pcbi.1005896). Pathway analysis was conducted using GOATOOLS with Gene Ontology (GO) database (Klopfenstein, D.V., Zhang, L., Pedersen, B.S., Ramirez, F., Warwick Vesztrocy, A., Naldi, A., Mungall, C.J., Yunes, J.M., Botvinnik, O., Weigel, M., Dampier, W., et al.

(2018). GOATOOLS: A Python library for Gene Ontology analyses. *Sci Rep* 8, 10872. 10.1038/s41598-018-28948-z). The upstream regulator analysis was one function in Ingenuity Pathway Analysis (IPA) that identifies upstream transcriptional regulators that coordinate the expressional changes observed in transcriptome. (Fig. 7B) Step-by-step RNA-Seq preprocessing conducted in this study and corresponding criteria to select regulated genes by transcriptional changes. TPM: transcript per million, estimated by kallisto (Bray, N.L., Pimentel, H., Melsted, P., and Pachter, L. (2016). Near-optimal probabilistic RNA-seq quantification. *Nat Biotechnol* 34, 525-527. 10.1038/nbt.3519).

[0014] **Fig. 8.** Transcriptome data showed high reproducibility among replicates within sampling time points and progressing expression profiles in relation to time in principal component analysis (PCA). The dotted line indicates the trendline of transcriptional changes over time on the plane of PC1 and PC2. These two principal components combined can explain 52.8% of variation of transcriptional change in RNA-Seq results.

[0015] **Figs. 9A-9G.** Hierarchical clustering of genes involved in nucleosome assembly and 8 related biological processes significantly enriched in GO enrichment analysis. Heatmap was ordered by time points. All listed genes were DEGs that passed the selection criteria described in Figure 7B. Expression fold-changes were calculated compared with pre-transfection time point 0h. The fold change is indicated by the intensity (i.e., lightness to darkness) of a grayscale pad using a log₂ scale. Upregulated expression is indicated by progressively darker grayscale without stipple, whereas downregulated expression is indicated by progressively darker grayscale with stipple. White indicates basal, or unchanged, expression.

[0016] **Figs. 10A-10C.** Hierarchical clustering of genes involved in response to virus and 6 related biological processes significantly enriched in GO enrichment analysis. Heatmap was ordered by time points. All listed genes were DEGs that passed the selection criteria described in Figure 7B. Expression fold-changes were calculated compared with pre-transfection time point 0h. The fold change is indicated by the intensity (i.e., lightness to darkness) of a grayscale pad using a log₂ scale. Upregulated expression is indicated by progressively darker grayscale without stipple, whereas downregulated expression is indicated by progressively darker grayscale with stipple. White indicates basal, or unchanged, expression.

[0017] **Figs. 11A-11M.** Time series analysis using DPGP mixture model classified 1,850 DEGs into 13 distinct clusters. The x-axis represents sampling time points. The y-axis represents standardized log₂ fold change standardized to zero mean and unit variance across 8 time points

and triplicates. Red lines indicate standardized expression level for individual genes. The dark blue line represents the mean of clustered genes while the blue shade area is the range of ± 2 standard deviation.

[0018] **Figs. 12A-12E.** DPGP identified clusters and the significantly enriched gene ontologies identified from clustered DEGs. The x-axis of the lower graph in each figure shows the adjusted p-value (FDR) after multiple testing correction using Benjamini-Hochberg procedure. GO terms were significant when $FDR < 0.05$, black dashed line.

[0019] **Figs. 13A-13B.** Hierarchical clustering of genes involved in the Early responding cluster and 2 related biological processes significantly enriched in GO enrichment analysis. Heatmap was ordered by time points. All listed genes were DEGs that passed the selection criteria described in Figure 7B. Expression fold-changes were calculated compared with pre-transfection time point 0h. The fold change is indicated by the intensity (i.e., lightness to darkness) of a grayscale pad using a log₂ scale. Upregulated expression is indicated by progressively darker grayscale without stipple, whereas downregulated expression is indicated by progressively darker grayscale with stipple. White indicates basal, or unchanged, expression.

[0020] **Figs. 14A-14O.** Hierarchical clustering of additional genes significantly enriched in GO enrichment analysis. Heatmap was ordered by time points. All listed genes were DEGs that passed the selection criteria described in Figure 7B. Expression fold-changes were calculated compared with pre-transfection time point 0h. The fold change is indicated by the intensity (i.e., lightness to darkness) of a grayscale pad using a log₂ scale. Upregulated expression is indicated by progressively darker grayscale without stipple, whereas downregulated expression is indicated by progressively darker grayscale with stipple. White indicates basal, or unchanged, expression.

[0021] **Fig. 15.** Graph demonstrating effect on AAV titer of repressing expression of two selected DEGs simultaneously in HEK293 cells as a result of siRNA treatment.

[0022] **Figs. 16A-16D.** Graphs demonstrating lack of off-target effect of treating HEK293 cells with siRNA targeting two different selected DEGs.

DETAILED DESCRIPTION OF THE INVENTION

[0023] The following discussion is directed to various embodiments of the invention. The term “invention” is not intended to refer to any particular embodiment or otherwise limit the scope of the disclosure. Although one or more of these embodiments may be preferred, the

embodiments disclosed should not be interpreted, or otherwise used, as limiting the scope of the disclosure, including the claims. In addition, one skilled in the art will understand that the following description has broad application, and the discussion of any embodiment is meant only to be an example of that embodiment, and not intended to intimate that the scope of the disclosure, including the claims, is limited to that embodiment.

Certain Definitions

[0024] As used herein, “**adeno-associated virus vector**” means an adeno-associated virus (AAV) comprising a naturally occurring or non-naturally occurring AAV capsid encapsidating a vector genome. Adeno-associated virus vector may be abbreviated “AAV vector,” and depending on context, may be referred to by synonymous terms, such as “recombinant AAV vector,” “rAAV vector,” “rAAV,” or just “vector.”

[0025] As used herein, “**vector genome**” means an AAV genome modified both to include a heterologous nucleotide sequence and to render any AAV vector containing the vector genome replication incompetent, such as by inactivating or deleting an endogenous AAV *rep* and/or *cap* gene.

[0026] As used herein, “**heterologous nucleotide sequence**” means a nucleotide sequence that is introduced into an organism (including a virus) from a different organism (including an organism). The sequence of a heterologous nucleotide sequence may be the same as one that occurs in nature, or may be a modified version thereof, or even partially or entirely synthetic.

[0027] As used herein, “**expression cassette**” means a nucleotide sequence comprising a transgene operably linked with regulatory regions or elements for controlling the initiation and termination of transcription of the transgene from DNA into RNA.

[0028] As used herein, “**transgene**” means a nucleotide sequence that encodes at least one polypeptide, and/or the nucleotide sequence coding for at least one functional RNA molecule. Transgene may be referred to by the synonymous term “gene of interest.”

[0029] As used herein, the term “**purify**,” and the related terms “purified” and “purification,” when used in connection with an AAV vector, or sample or preparation thereof, indicates a relative increase or improvement in purity compared with a starting material containing the vector, and/or a prior intermediate purification step in some scheme of sequential purification steps intended to purify the biological product, and does not require a particular qualitative or quantitative degree of purity, unless otherwise specified.

[0030] As used herein, “**transduction**” means the introduction into a target cell of the genome of an AAV vector. Transduction is distinguished from infection, the latter term being used to refer to the introduction into a cell of the genome of a replication competent adeno-associated virus.

[0031] As used herein, “**target cell**” means a cell that an AAV vector is designed or intended to transduce, or is experimentally observed to be transduced by an AAV vector, whether *in vitro*, or *in vivo* in a subject.

[0032] As used herein, “**subject**” means an organism to which an AAV vector is administered for purposes of preventing or treating a disease, disorder, or condition.

Modified Host Cells

[0033] Among other embodiments, the disclosure provides host cells modified to reduce or eliminate expression of one or more genes (such as the differentially expressed genes (DEG) described below) upregulated in unmodified host cells of the same type after introduction, such as by transient transfection, of exogenous genetic material, such as in the form of one or more DNA plasmids, required to direct biosynthesis of recombinant AAV vectors, and/or at different times during their biosynthesis after introduction of the exogenous genetic material.

[0034] As used herein, “host cell” means any type of cell suitable for or adapted to producing recombinant AAV vectors. Host cells can be derived from any type of organism, whether single or multi-cellular. In some embodiments, host cells are animal cells, which in some embodiments are mammalian cells, non-limiting examples of which include cells derived from human, non-human primate (such as macaque, rhesus, or cynomolgus, or some other non-human primate), rat, murine, hamster, porcine, bovine, ovine, caprine, or other type of mammal. In some embodiments, host cells are clonal cell lines capable of dividing for multiple generations before senescence stops growth, or may even be immortal. In some embodiments, host cells can be maintained in adherent cell culture, or in suspension cell culture, or can be adapted to growth in suspension cell culture after initially being maintained in adherent cell culture.

[0035] Non-limiting examples of mammalian host cells maintained as cell lines include HeLa cells, Cos cells, HEK293 cells, A549 cells, BHK cells, Vero cells, NIH 3T3 cells, HT-1080 cells, Sp2/0 cells, NS0 cells, C127 cells, AGE1.HN cells, CAP cells, HKB-11 cells, WI-38 cells, MRC-5 cells, or PER.C6 cells, with many others being possible, any of which can be modified to reduce or eliminate expression of one or more genes (such as the DEGs identified herein, or corresponding

non-human orthologs of such DEGs for host cells of other than human origin) upregulated in unmodified host cells of the same type after introduction, such as by transient transfection, of exogenous genetic material, such as in the form of one or more DNA plasmids, required to direct biosynthesis of recombinant AAV vectors, and/or at different times during their biosynthesis after introduction of the exogenous genetic material.

[0036] According to other embodiments, modified host cells are modified insect host cells, non-limiting examples of which include Sf9 cells, ExpiSf9, Sf21 cells, S2 cells, D.Mel2 cells, Tn-368 cells, or BTI-Tn-5B1-4 cells, with many others being possible, any of which can be modified to reduce or eliminate expression of one or more genes (such as the insect orthologs of the DEGs identified herein) upregulated in unmodified host cells of the same type after introduction, such as by infection, of exogenous genetic material, such as in the form of recombinant baculovirus, required to direct biosynthesis of recombinant AAV vectors, and/or at different times during their biosynthesis after introduction of the exogenous genetic material.

[0037] In some embodiments, modified host cells are modified HEK293 cells (or any variant, strain or derivative of HEK293 cells, including, without limitation, HEK293E (or HEK293-EBNA1), HEK293F, HEK293FT, HEK293G, HEK293H, HEK293S, HEK293SG, or HEK293T cells), which are modified to reduce or eliminate expression of one or more genes (such as the DEGs identified herein) upregulated in unmodified HEK293 cells of the same type after introduction, such as by transient transfection, of exogenous genetic material, such as in the form of one or more DNA plasmids, required to direct biosynthesis of recombinant AAV vectors, and/or at different times during their biosynthesis after introduction of the exogenous genetic material.

[0038] In some embodiments, HEK293 cells, and variants, strains or derivatives thereof, can be adapted for growth in suspension cell culture, and/or in the absence of animal-derived serum.

[0039] The identity of a sample of host cells as being the same as that of a previously characterized type of host cell, such as a particular cell line, such as HEK293 cells, or any other type of known cell or cell line, can be authenticated using short tandem repeat (STR) profiling of cells in such sample and comparing the results against STR profiling of a sample of reference cells obtained from a reliable source, such as a cell line repository, such as American Type Culture Collection (ATCC). According to some non-limiting embodiments, a sample of putative HEK293 cells can be authenticated as such by confirming they comprise the following set of human STR genomic loci and specific alleles (denoted within parentheses): Amelogenin (X); CSF1PO (11, 12); D13S317 (12, 14); D16S539 (9, 13); D5S818 (8, 9); D7S820 (11, 12); THO1 (7, 9.3); TPOX (11); and

vWA (16, 19). Derivatives and variants of HEK293 cells may, however, deviate from the aforementioned STR genotype.

[0040] STR profiling for purposes of cell type authentication is described further in such references as American Type Culture Collection Standards Development Organization Workgroup ASN-0002, Nat Revs Cancer, 10:441-8 (2010); Almeida JL, et al., PLoS Biol 14(6):e1002476. doi:10.1371/journal.pbio.1002476 (2016); Tian, F, et al., BioPharm Intl, 30(7):37-41 (2017); ANSI/ATCC ASN-0002-2021 Authentication of Human Cell Lines: Standardization of Short Tandem Repeat (STR) Profiling. Other methods, such as genetic or biochemical methods, for authenticating a sample of cells as being of a particular type, such as of a particular cell line, such as HEK293 cells, are known in the art. A sample of cells can also be authenticated by establishing their provenance, such as by documenting a reliable chain of custody leading from the sample back to the individual or individuals who originally developed the type of cells in question, such as HEK293 cells, or who originally deposited a viable sample of such cells with a reliable biological depository.

[0041] HEK293 cells are described further in such references as Graham, FL, et al., J Gen Virol, 36:59-72 (1977); Thomas, P and Smart, TG, J Pharm Tox Meths 51:187-200 (2005); Dumont, J, et al., Crit Revs Biotech, 36(6):1110-22 (2016). HEK293 cells, and certain variants, strains, and derivatives thereof are available from the American Type Culture Collection (ATCC) (e.g., CRL-1573), among other reliable sources.

Host Cell Genes Upregulated During AAV Vector Production

[0042] As described further in the Examples, the inventors have surprisingly discovered that a variety of genes are upregulated in host cells shortly after introduction of exogenous genetic material for guiding biosynthesis of AAV vectors and then at different time points during production of AAV vectors by such cells. The inventors even more surprisingly discovered that many of the upregulated genes can be grouped into Gene Ontology categories, which provides important clues as to their function, individually and as part of interrelated networks which are active within the host cells at different times as vector production proceeds. More information about Gene Ontologies can be found in sources such as The Gene Ontology Handbook, Eds. Dessimoz, C and Škunca, N, Humana, New York, NY (2017) (ISBN 978-1-4939-3743-1). The inventors furthermore identified upstream regulators which may control the expression kinetics of the upregulated genes.

[0043] Based on function of the upregulated genes (which are sometimes herein identified as differentially expressed genes, or DEGs), predicted from their grouping within the Gene Ontology categories and the kinetics with which their expression increases over the time course of vector production, the inventors hypothesized that certain DEGs operate, alone and/or in concert with others, to limit the rate and/or extent of vector production and that repressing the expression of such DEGs in host cells is effective to increase vector yield. Confirming the hypothesis, modified host cells are provided in which repression or elimination of DEG expression results in improvements in the amount of intact AAV vector that such host cells are able to produce relative to unmodified host cells of the same type in which expression of a respective DEG is not reduced. Further provided are methods of using such modified host cells to produce AAV vectors, and AAV vectors produced using such host cells and methods.

[0044] In some embodiments, the host cells in which the expression of DEGs is repressed or eliminated are host cells of human origin, and the DEGs are human genes. In other embodiments, the host cells are other than of human origin, and the non-human DEGs which are repressed are genes orthologous to human DEGs.

[0045] In some embodiments, host cells are modified to repress or eliminate expression of genes within Gene Ontology (GO) ID No. 0000183, rDNA Heterochromatin Assembly, non-limiting examples of which genes include those identified in **Table 1**. In some embodiments, host cells are modified to repress or eliminate expression of genes within GO ID No. 0000188, Inactivation of MAPK Activity, non-limiting examples of which genes include those identified in **Table 2**. In some embodiments, host cells are modified to repress or eliminate expression of genes within GO ID No. 0000398, mRNA Splicing, via Spliceosome, non-limiting examples of which genes include those identified in **Table 3**. In some embodiments, host cells are modified to repress or eliminate expression of genes within GO ID No. 0002227, Innate Immune Response in Mucosa, non-limiting examples of which genes include those identified in **Table 4**. In some embodiments, host cells are modified to repress or eliminate expression of genes within GO ID No. 0006334, Nucleosome Assembly, non-limiting examples of which genes include those identified in **Table 5**. In some embodiments, host cells are modified to repress or eliminate expression of genes within GO ID No. 0006335, DNA Replication-Dependent Nucleosome Assembly, non-limiting examples of which genes include those identified in **Table 6**. In some embodiments, host cells are modified to repress or eliminate expression of genes within GO ID No. 0006336, DNA Replication-Independent Nucleosome, non-limiting examples of which genes

include those identified in **Table 7**. In some embodiments, host cells are modified to repress or eliminate expression of genes within GO ID No. 0006352, DNA-Templated Transcription, Initiation, non-limiting examples of which genes include those identified in **Table 8**. In some embodiments, host cells are modified to repress or eliminate expression of genes within GO ID No. 0006954, Inflammatory Response, non-limiting examples of which genes include those identified in **Table 9**. In some embodiments, host cells are modified to repress or eliminate expression of genes within GO ID No. 0006955, Immune Response, non-limiting examples of which genes include those identified in **Table 10**. In some embodiments, host cells are modified to repress or eliminate expression of genes within GO ID No. 0006357, Regulation of Transcription by RNA Polymerase II, non-limiting examples of which genes include those identified in **Table 11**. In some embodiments, host cells are modified to repress or eliminate expression of genes within GO ID No. 0007565, Female Pregnancy, non-limiting examples of which genes include those identified in **Table 12**. In some embodiments, host cells are modified to repress or eliminate expression of genes within GO ID No. 0009615, Response to Virus, non-limiting examples of which genes include those identified in **Table 13**. In some embodiments, host cells are modified to repress or eliminate expression of genes within GO ID No. 0016233, Telomere Capping, non-limiting examples of which genes include those identified in **Table 14**. In some embodiments, host cells are modified to repress or eliminate expression of genes within GO ID No. 0019731, Antibacterial Humoral Response, non-limiting examples of which genes include those identified in **Table 15**. In some embodiments, host cells are modified to repress or eliminate expression of genes within GO ID No. 0030968, Endoplasmic Reticulum Unfolded Protein Response, non-limiting examples of which genes include those identified in **Table 16**. In some embodiments, host cells are modified to repress or eliminate expression of genes within GO ID No. 0032200, Telomere Organization, non-limiting examples of which genes include those identified in **Table 17**. In some embodiments, host cells are modified to repress or eliminate expression of genes within GO ID No. 0034080, CENP-A Containing Nucleosome Assembly, non-limiting examples of which genes include those identified in **Table 18**. In some embodiments, host cells are modified to repress or eliminate expression of genes within GO ID No. 0036499, PERK-Mediated Unfolded Protein Response, non-limiting examples of which genes include those identified in **Table 19**. In some embodiments, host cells are modified to repress or eliminate expression of genes within GO ID No. 0044267, Cellular Protein Metabolic Process, non-limiting examples of which genes include those identified in **Table 20**. In some embodiments, host cells are modified to repress or eliminate expression of genes within GO ID No. 0045071, Negative

Regulation of Viral Genome Replication, non-limiting examples of which genes include those identified in **Table 21**. In some embodiments, host cells are modified to repress or eliminate expression of genes within GO ID No. 0045652, Regulation of Megakaryocyte Differentiation, non-limiting examples of which genes include those identified in **Table 22**. In some embodiments, host cells are modified to repress or eliminate expression of genes within GO ID No. 0045653, Negative Regulation of Megakaryocyte Differentiation, non-limiting examples of which genes include those identified in **Table 23**. In some embodiments, host cells are modified to repress or eliminate expression of genes within GO ID No. 0045814, Negative Regulation of Gene Expression, Epigenetic, non-limiting examples of which genes include those identified in **Table 24**. In some embodiments, host cells are modified to repress or eliminate expression of genes within GO ID No. 0050911, Detection of Chemical Stimulus Involved in Sensory Perception of Smell, non-limiting examples of which genes include those identified in **Table 25**. In some embodiments, host cells are modified to repress or eliminate expression of genes within GO ID No. 0051607, Defense Response to Virus, non-limiting examples of which genes include those identified in **Table 26**. In some embodiments, host cells are modified to repress or eliminate expression of genes within GO ID No. 0060337, Type I Interferon Signaling Pathway, non-limiting examples of which genes include those identified in **Table 27**. In some embodiments, host cells are modified to repress or eliminate expression of genes within GO ID No. 0060700, Regulation of Ribonuclease Activity, non-limiting examples of which genes include those identified in **Table 28**. In some embodiments, host cells are modified to repress or eliminate expression of genes within GO ID No. 0060964, Regulation of Gene silencing by miRNA, non-limiting examples of which genes include those identified in **Table 29**. In some embodiments, host cells are modified to repress or eliminate expression of genes within GO ID No. 0071347, Cellular Response to Interleukin-1, non-limiting examples of which genes include those identified in **Table 30**. In some embodiments, host cells are modified to repress or eliminate expression of genes that are upstream regulators of other genes within the Gene Ontology groups described herein, non-limiting examples of which upstream regulatory genes include those identified in **Table 31**. In any of the foregoing embodiments, host cells which are modified to repress or eliminate expression of genes within such Gene Ontology groups can be any host cell known in the art to be suitable for producing AAV vectors, including without limitation, animal cells, such as insect or mammal cells, including without limitation, cells of human origin, such as cell lines of human origin, including without limitation, HEK293 cells, including variants, strains, and derivatives thereof.

TABLE 1

rDNA Heterochromatin Assembly Gene Ontology (GO:0000183)

	SYMBOL	ENSEMBL	UniProt
10163	H4C8	ENSG00000158406	P62805
15317	H3C13	ENSG00000183598	Q71DI3
17007	H4C3	ENSG00000197061	
17038	H3C12	ENSG00000197153	P68431
17227	H4-16	ENSG00000197837	
17897	H3C14	ENSG00000203811	
17902	H3C15	ENSG00000203852	
32826	H4C15	ENSG00000270276	
33133	H4C14	ENSG00000270882	
33715	H4C12	ENSG00000273542	
33944	H3C6	ENSG00000274750	
34112	H3C1	ENSG00000275714	
34196	H4C9	ENSG00000276180	
34331	H4C5	ENSG00000276966	
34366	H4C4	ENSG00000277157	
34643	H4C1	ENSG00000278637	
34665	H4C2	ENSG00000278705	
34683	H3C10	ENSG00000278828	
35542	H3C2	ENSG00000286522	

TABLE 2

Inactivation of MAPK Activity Gene Ontology (GO:0000188)

	SYMBOL	ENSEMBL	UniProt
3867	DUSP16	ENSG00000111266	Q9BY84
5002	DUSP1	ENSG00000120129	P28562
5098	DUSP4	ENSG00000120875	Q13115
7594	DUSP5	ENSG00000138166	Q16690
7723	RGS3	ENSG00000138835	P49796
7776	DUSP6	ENSG00000139318	Q16828
8382	DUSP10	ENSG00000143507	Q9Y6W6
15545	DUSP8	ENSG00000184545	Q13202
34169	DUSP14	ENSG00000276023	O95147

TABLE 3

mRNA Splicing, Via Spliceosome Gene Ontology (GO:0000398)

	SYMBOL	ENSEMBL	UniProt
3010	NOVA2	ENSG00000104967	Q9UNW9
3269	BUD31	ENSG00000106245	P41223
10594	RAVER1	ENSG00000161847	Q8IY67

TABLE 4

Innate Immune Response in Mucosa Gene Ontology (GO:0002227)

SYMBOL	ENSEMBL	UniProt
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5549	H2BC11	ENSG00000124635	P06899
11161	CAMP	ENSG00000164047	
12854	PLA2G1B	ENSG00000170890	P04054
14720	H2BC4	ENSG00000180596	P62807
15575	H2BC21	ENSG00000184678	Q16778
17252	H2BC12	ENSG00000197903	O60814
17612	RPL39	ENSG00000198918	P62891
33768	H2BC8	ENSG00000273802	
33848	H2BC6	ENSG00000274290	
34378	H2BC7	ENSG00000277224	

TABLE 5

Nucleosome Assembly Gene Ontology (GO:0006334)

	SYMBOL	ENSEMBL	UniProt
2569	H2BW2	ENSG00000101812	P0C1H6
5538	H1-3	ENSG00000124575	P16402
5549	H2BC11	ENSG00000124635	P06899
10161	H2BC5	ENSG00000158373	P58876
10163	H4C8	ENSG00000158406	P62805
12265	H1-4	ENSG00000168298	P10412
14720	H2BC4	ENSG00000180596	P62807
15317	H3C13	ENSG00000183598	Q71DI3
15502	H1-5	ENSG00000184357	P16401
15575	H2BC21	ENSG00000184678	Q16778
15684	H2BC13	ENSG00000185130	Q99880
16310	H1-2	ENSG00000187837	P16403
16614	H1-0	ENSG00000189060	P07305
16959	H2BU1	ENSG00000196890	Q8N257
17007	H4C3	ENSG00000197061	
17038	H3C12	ENSG00000197153	P68431
17227	H4-16	ENSG00000197837	
17252	H2BC12	ENSG00000197903	O60814
17897	H3C14	ENSG00000203811	
17899	H2BC18	ENSG00000203814	Q5QNW6
17902	H3C15	ENSG00000203852	
17990	DAXX	ENSG00000204209	Q9UER7
24685	H2BC15	ENSG00000233822	Q99877
32826	H4C15	ENSG00000270276	
33133	H4C14	ENSG00000270882	
33715	H4C12	ENSG00000273542	
33745	H2BC14	ENSG00000273703	Q99879
33768	H2BC8	ENSG00000273802	
33848	H2BC6	ENSG00000274290	
33927	H2BC17	ENSG00000274641	P23527
33944	H3C6	ENSG00000274750	
34112	H3C1	ENSG00000275714	
34196	H4C9	ENSG00000276180	

34331	H4C5	ENSG00000276966	
34366	H4C4	ENSG00000277157	
34378	H2BC7	ENSG00000277224	
34643	H4C1	ENSG00000278637	
34665	H4C2	ENSG00000278705	
34683	H3C10	ENSG00000278828	
35542	H3C2	ENSG00000286522	

TABLE 6

DNA Replication-Dependent Nucleosome Assembly Gene Ontology (GO:0006335)

	SYMBOL	ENSEMBL	UniProt
10163	H4C8	ENSG00000158406	P62805
17007	H4C3	ENSG00000197061	
17038	H3C12	ENSG00000197153	P68431
17227	H4-16	ENSG00000197837	
32826	H4C15	ENSG00000270276	
33133	H4C14	ENSG00000270882	
33715	H4C12	ENSG00000273542	
33944	H3C6	ENSG00000274750	
34112	H3C1	ENSG00000275714	
34196	H4C9	ENSG00000276180	
34331	H4C5	ENSG00000276966	
34366	H4C4	ENSG00000277157	
34643	H4C1	ENSG00000278637	
34665	H4C2	ENSG00000278705	
34683	H3C10	ENSG00000278828	
35542	H3C2	ENSG00000286522	

TABLE 7

DNA Replication-Independent Nucleosome Assembly Gene Ontology (GO:0006336)

	SYMBOL	ENSEMBL	UniProt
10163	H4C8	ENSG00000158406	P62805
17007	H4C3	ENSG00000197061	
17227	H4-16	ENSG00000197837	
32826	H4C15	ENSG00000270276	
33133	H4C14	ENSG00000270882	
33715	H4C12	ENSG00000273542	
34196	H4C9	ENSG00000276180	
34331	H4C5	ENSG00000276966	
34366	H4C4	ENSG00000277157	
34643	H4C1	ENSG00000278637	
34665	H4C2	ENSG00000278705	

TABLE 8

DNA-Templated Transcription, Initiation Gene Ontology (GO:0006352)

	SYMBOL	ENSEMBL	UniProt
10163	H4C8	ENSG00000158406	P62805

17007	H4C3	ENSG00000197061	
17227	H4-16	ENSG00000197837	
32826	H4C15	ENSG00000270276	
33133	H4C14	ENSG00000270882	
33715	H4C12	ENSG00000273542	
34196	H4C9	ENSG00000276180	
34331	H4C5	ENSG00000276966	
34366	H4C4	ENSG00000277157	
34643	H4C1	ENSG00000278637	
34665	H4C2	ENSG00000278705	

TABLE 9

Inflammatory Response Gene Ontology (GO:0006954)

	SYMBOL	ENSEMBL	UniProt
126	TAC1	ENSG00000006128	P20366
142	CCL26	ENSG00000006606	Q9Y258
222	MMP25	ENSG00000008516	Q9NPA2
1240	PTGS2	ENSG00000073756	P35354
1910	NLRC4	ENSG00000091106	Q9NPP4
2032	BLNK	ENSG00000095585	Q8WV28
2820	PYCARD	ENSG00000103490	Q9ULZ3
2975	RELB	ENSG00000104856	Q01201
3116	PLA2G4C	ENSG00000105499	Q9UP65
3361	C5	ENSG00000106804	P01031
3829	IL23A	ENSG00000110944	Q9NPF7
3999	IL17F	ENSG00000112116	Q96PD4
4349	CCL20	ENSG00000115009	P78556
4809	TNFAIP3	ENSG00000118503	P21580
5387	PLP1	ENSG00000123560	P60201
5680	C3	ENSG00000125730	P01024
5931	IL17B	ENSG00000127743	Q9UHF5
6385	CCL25	ENSG00000131142	O15444
6432	AOC3	ENSG00000131471	Q16853
6972	ANXA1	ENSG00000135046	P04083
8075	MEP1B	ENSG00000141434	Q16820
8344	ECM1	ENSG00000143369	Q16610
8564	ACKR2	ENSG00000144648	O00590
9296	IL18	ENSG00000150782	Q14116
10049	IL34	ENSG00000157368	Q6ZMJ4
11040	PTX3	ENSG00000163661	P26022
11052	IL17RE	ENSG00000163701	Q8NFR9
12418	LGALS9	ENSG00000168961	O00182
12491	CXCL11	ENSG00000169248	O14625
12524	PTAFR	ENSG00000169403	P25105
12529	CXCL8	ENSG00000169429	P10145
12729	FOS	ENSG00000170345	P01100
12760	CD14	ENSG00000170458	P08571

13034	PIK3CD	ENSG00000171608	O00329
13113	SCG2	ENSG00000171951	P13521
13175	CEBPB	ENSG00000172216	P17676
14766	CXCR2	ENSG00000180871	P25025
15384	CCR4	ENSG00000183813	P51679
16892	HRH1	ENSG00000196639	P35367
17102	C5AR1	ENSG00000197405	P21730
18118	ABCF1	ENSG00000204574	Q8NE71
19344	LTB4R	ENSG00000213903	Q15722
19345	LTB4R2	ENSG00000213906	Q9NPC1
21055	C4B	ENSG00000224389	P0COL5
26163	IRGM	ENSG00000237693	A1A4Y4
27439	TICAM2	ENSG00000243414	Q86XR7
27790	C4A	ENSG00000244731	P0COL4
33460	CCL5	ENSG00000271503	P13501
34038	CCL4	ENSG00000275302	P13236

TABLE 10

Immune Response Gene Ontology (GO:0006955)

	SYMBOL	ENSEMBL	UniProt
121	MAP3K14	ENSG00000006062	Q99558
223	IL32	ENSG00000008517	P24001
455	FAS	ENSG00000026103	P25445
2125	MADCAM1	ENSG00000099866	Q13477
2219	XBP1	ENSG00000100219	P17861
2330	CNIH1	ENSG00000100528	O95406
3983	ULBP1	ENSG00000111981	Q9BZM6
4349	CCL20	ENSG00000115009	P78556
5442	SLPI	ENSG00000124107	P03973
5680	C3	ENSG00000125730	P01024
5825	IFI6	ENSG00000126709	P09912
5831	CFP	ENSG00000126759	P27918
5931	IL17B	ENSG00000127743	Q9UHF5
5974	VPREB3	ENSG00000128218	Q9UKI3
6385	CCL25	ENSG00000131142	O15444
6529	TRIM22	ENSG00000132274	Q8IYM9
7542	IFI44L	ENSG00000137959	Q53G44
7545	IFI44	ENSG00000137965	Q8TCB0
8165	IFITM3	ENSG00000142089	Q01628
8352	CTSK	ENSG00000143387	P43235
8564	ACKR2	ENSG00000144648	O00590
9567	MR1	ENSG00000153029	Q95460
9626	CD8A	ENSG00000153563	P01732
9646	CEBPG	ENSG00000153879	P53567
11433	GEM	ENSG00000164949	P55040
11451	NFIL3	ENSG00000165030	Q16649
12273	CX3CR1	ENSG00000168329	P49238

12491	CXCL11	ENSG00000169248	O14625
12524	PTAFR	ENSG00000169403	P25105
12529	CXCL8	ENSG00000169429	P10145
12969	APLN	ENSG00000171388	Q9ULZ1
13175	CEBPB	ENSG00000172216	P17676
13211	IL16	ENSG00000172349	Q14005
13259	CTSW	ENSG00000172543	P56202
14655	MYLPF	ENSG00000180209	Q96A32
14766	CXCR2	ENSG00000180871	P25025
15384	CCR4	ENSG00000183813	P51679
15523	CCR10	ENSG00000184451	P46092
16718	HLA-DRB1	ENSG00000196126	P01911
17102	C5AR1	ENSG00000197405	P21730
18086	LST1	ENSG00000204482	O00453
19344	LTB4R	ENSG00000213903	Q15722
23940	TAPBP	ENSG00000231925	O15533
26532	TNFSF12	ENSG00000239697	O43508
34038	CCL4	ENSG00000275302	P13236

TABLE 11

Regulation of Transcription by RNA Polymerase II Gene Ontology (GO:0006357)

	SYMBOL	ENSEMBL	UniProt
997	KLF6	ENSG00000067082	Q99612
4866	KLF9	ENSG00000119138	Q13886
5084	EGR1	ENSG00000120738	P18146
5685	FOSB	ENSG00000125740	P53539
8830	CREB5	ENSG00000146592	Q02930
12942	JUNB	ENSG00000171223	P17275
14208	JUN	ENSG00000177606	P05412
16825	TSC22D2	ENSG00000196428	O75157
16946	ARID5A	ENSG00000196843	Q03989
18283	ZBTB10	ENSG00000205189	Q96DT7

TABLE 12

Female Pregnancy Gene Ontology (GO:0007565)

	SYMBOL	ENSEMBL	UniProt
435	ABCC2	ENSG00000023839	Q92887
1631	COL16A1	ENSG00000084636	Q07092
1657	OVGP1	ENSG00000085465	Q12889
1747	PTHLH	ENSG00000087494	P12272
3376	RLN1	ENSG00000107014	
3377	RLN1	ENSG00000107018	P04808
4104	CLIC5	ENSG00000112782	Q9NZA1
4427	IGFBP5	ENSG00000115461	P24593
4936	TGFB3	ENSG00000119699	P10600
5685	FOSB	ENSG00000125740	P53539
6849	SLC38A2	ENSG00000134294	Q96QD8

8936	GNRH1	ENSG00000147437	P01148
10276	GIP	ENSG00000159224	P09681
11902	TAC3	ENSG00000166863	Q9UHF0
12418	LGALS9	ENSG00000168961	O00182
12729	FOS	ENSG00000170345	P01100
13829	FOSL1	ENSG00000175592	P15407
27383	PSG4	ENSG00000243137	Q00888

TABLE 13

Response to Virus Gene Ontology (GO:0009615)

	SYMBOL	ENSEMBL	UniProt
1819	OAS1	ENSG00000089127	P00973
1873	IRAK3	ENSG00000090376	Q9Y616
3395	DDX58	ENSG00000107201	O95786
3879	OAS3	ENSG00000111331	Q9Y6K5
3880	OAS2	ENSG00000111335	P29728
4389	IFIH1	ENSG00000115267	Q9BYX4
4971	IFIT3	ENSG00000119917	O14879
4973	IFIT2	ENSG00000119922	P09913
6222	BST2	ENSG00000130303	Q10589
6529	TRIM22	ENSG00000132274	Q8IYM9
6691	TPT1	ENSG00000133112	P13693
6855	RSAD2	ENSG00000134321	Q8WYG1
6872	RPS15A	ENSG00000134419	P62244
6990	OASL	ENSG00000135114	Q15646
7469	DDX60	ENSG00000137628	Q8IY21
7545	IFI44	ENSG00000137965	Q8TCB0
7939	CYP1A1	ENSG00000140465	P04798
8165	IFITM3	ENSG00000142089	Q01628
13090	IFNB1	ENSG00000171855	P01574
13166	ISG20	ENSG00000172183	Q96AZ6
13829	FOSL1	ENSG00000175592	P15407
15769	IRF7	ENSG00000185507	Q92985
15829	IFIT1	ENSG00000185745	P09914
15862	IFITM1	ENSG00000185885	P13164
33460	CCL5	ENSG00000271503	P13501
34038	CCL4	ENSG00000275302	P13236

TABLE 14

Telomere Capping Gene Ontology (GO:0016233)

	SYMBOL	ENSEMBL	UniProt
10163	H4C8	ENSG00000158406	P62805
17007	H4C3	ENSG00000197061	
17227	H4-16	ENSG00000197837	
32826	H4C15	ENSG00000270276	
33133	H4C14	ENSG00000270882	
33715	H4C12	ENSG00000273542	

34196	H4C9	ENSG00000276180	
34331	H4C5	ENSG00000276966	
34366	H4C4	ENSG00000277157	
34643	H4C1	ENSG00000278637	
34665	H4C2	ENSG00000278705	

TABLE 15

Antibacterial Humoral Response Gene Ontology (GO:0019731)

	SYMBOL	ENSEMBL	UniProt
2528	WFDC2	ENSG00000101443	Q14508
5442	SLPI	ENSG00000124107	P03973
5549	H2BC11	ENSG00000124635	P06899
11161	CAMP	ENSG00000164047	
12854	PLA2G1B	ENSG00000170890	P04054
14720	H2BC4	ENSG00000180596	P62807
15575	H2BC21	ENSG00000184678	Q16778
17252	H2BC12	ENSG00000197903	O60814
17612	RPL39	ENSG00000198918	P62891
19498	ANG	ENSG00000214274	P03950
33768	H2BC8	ENSG00000273802	
33848	H2BC6	ENSG00000274290	
34378	H2BC7	ENSG00000277224	

TABLE 16

Endoplasmic Reticulum Unfolded Protein Response Gene Ontology (GO:0030968)

	SYMBOL	ENSEMBL	UniProt
689	HERPUD1	ENSG00000051108	Q15011
2219	XBP1	ENSG00000100219	P17861
3128	FGF21	ENSG00000105550	Q9NSA1
3743	CCND1	ENSG00000110092	P24385
4219	STC2	ENSG00000113739	O76061
4612	CTH	ENSG00000116761	P32929
5984	ATF4	ENSG00000128272	P18848
6484	SELENOS	ENSG00000131871	Q9BQE4
11306	CREBRF	ENSG00000164463	Q8IUR6
13751	DDIT3	ENSG00000175197	P35638
14712	BHLHA15	ENSG00000180535	Q7RTS1
32648	CALR3	ENSG00000269058	Q96L12

TABLE 17

Telomere Organization Gene Ontology (GO:0032200)

	SYMBOL	ENSEMBL	UniProt
10163	H4C8	ENSG00000158406	P62805
17007	H4C3	ENSG00000197061	
17038	H3C12	ENSG00000197153	P68431
17227	H4-16	ENSG00000197837	
32826	H4C15	ENSG00000270276	

33133	H4C14	ENSG00000270882	
33715	H4C12	ENSG00000273542	
33944	H3C6	ENSG00000274750	
34112	H3C1	ENSG00000275714	
34196	H4C9	ENSG00000276180	
34331	H4C5	ENSG00000276966	
34366	H4C4	ENSG00000277157	
34643	H4C1	ENSG00000278637	
34665	H4C2	ENSG00000278705	
34683	H3C10	ENSG00000278828	
35542	H3C2	ENSG00000286522	

TABLE 18

CENP-A Containing Nucleosome Assembly Gene Ontology (GO:0034080)

	SYMBOL	ENSEMBL	UniProt
490	CENPQ	ENSG00000031691	Q7L2Z9
10163	H4C8	ENSG00000158406	P62805
17007	H4C3	ENSG00000197061	
17227	H4-16	ENSG00000197837	
32826	H4C15	ENSG00000270276	
33133	H4C14	ENSG00000270882	
33715	H4C12	ENSG00000273542	
34196	H4C9	ENSG00000276180	
34331	H4C5	ENSG00000276966	
34366	H4C4	ENSG00000277157	
34643	H4C1	ENSG00000278637	
34665	H4C2	ENSG00000278705	

TABLE 19

PERK-Mediated Unfolded Protein Response Gene Ontology (GO:0036499)

	SYMBOL	ENSEMBL	UniProt
689	HERPUD1	ENSG00000051108	Q15011
1122	ASNS	ENSG00000070669	P08243
5984	ATF4	ENSG00000128272	P18848
10784	ATF3	ENSG00000162772	P18847
12529	CXCL8	ENSG00000169429	P10145
13751	DDIT3	ENSG00000175197	P35638

TABLE 20

Cellular Protein Metabolic Process Gene Ontology (GO:0044267)

	SYMBOL	ENSEMBL	UniProt
124	TMEM132A	ENSG00000006118	Q24JP5
579	TNC	ENSG00000041982	P24821
1153	LMCD1	ENSG00000071282	Q9NZU5
1279	ANO8	ENSG00000074855	Q9HCE9
1827	CHGB	ENSG00000089199	P05060
1914	LAMB1	ENSG00000091136	P07942

2183	LGALS1	ENSG00000100097	P09382
2706	MSLN	ENSG00000102854	Q13421
2871	SCG3	ENSG00000104112	Q8WXD2
4219	STC2	ENSG00000113739	O76061
4427	IGFBP5	ENSG00000115461	P24593
4471	PROC	ENSG00000115718	P04070
4769	APOA1	ENSG00000118137	P02647
4854	FGF23	ENSG00000118972	Q9GZV9
5680	C3	ENSG00000125730	P01024
6017	VGf	ENSG00000128564	O15240
6205	APOE	ENSG00000130203	P02649
7492	MMP13	ENSG00000137745	P45452
8834	IGFBP3	ENSG00000146674	P17936
10163	H4C8	ENSG00000158406	P62805
10957	IGFBP7	ENSG00000163453	Q16270
11026	ALB	ENSG00000163631	P02768
13113	SCG2	ENSG00000171951	P13521
15317	H3C13	ENSG00000183598	Q71DI3
17007	H4C3	ENSG00000197061	
17038	H3C12	ENSG00000197153	P68431
17227	H4-16	ENSG00000197837	
17897	H3C14	ENSG00000203811	
17902	H3C15	ENSG00000203852	
27790	C4A	ENSG00000244731	P0COL4
32826	H4C15	ENSG00000270276	
33133	H4C14	ENSG00000270882	
33715	H4C12	ENSG00000273542	
33944	H3C6	ENSG00000274750	
34112	H3C1	ENSG00000275714	
34196	H4C9	ENSG00000276180	
34331	H4C5	ENSG00000276966	
34366	H4C4	ENSG00000277157	
34643	H4C1	ENSG00000278637	
34665	H4C2	ENSG00000278705	
34683	H3C10	ENSG00000278828	
35542	H3C2	ENSG00000286522	

TABLE 21

Negative Regulation of Viral Genome Replication Gene Ontology (GO:0045071)

	SYMBOL	ENSEMBL	UniProt
1819	OAS1	ENSG00000089127	P00973
3879	OAS3	ENSG00000111331	Q9Y6K5
3880	OAS2	ENSG00000111335	P29728
5442	SLPI	ENSG00000124107	P03973
6222	BST2	ENSG00000130303	Q10589
6855	RSAD2	ENSG00000134321	Q8WYG1
6990	OASL	ENSG00000135114	Q15646

8165	IFITM3	ENSG00000142089	Q01628
13090	IFNB1	ENSG00000171855	P01574
13166	ISG20	ENSG00000172183	Q96AZ6
15829	IFIT1	ENSG00000185745	P09914
15862	IFITM1	ENSG00000185885	P13164
16246	ISG15	ENSG00000187608	P05161
16422	PLSCR1	ENSG00000188313	O15162
33460	CCL5	ENSG00000271503	P13501

TABLE 22

Regulation of Megakaryocyte Differentiation Gene Ontology (GO:0045652)

	SYMBOL	ENSEMBL	UniProt
1982	AGO1	ENSG00000092847	Q9UL18
4901	NR4A3	ENSG00000119508	Q92570
10163	H4C8	ENSG00000158406	P62805
15317	H3C13	ENSG00000183598	Q71DI3
17007	H4C3	ENSG00000197061	
17038	H3C12	ENSG00000197153	P68431
17227	H4-16	ENSG00000197837	
17897	H3C14	ENSG00000203811	
17902	H3C15	ENSG00000203852	
32826	H4C15	ENSG00000270276	
33133	H4C14	ENSG00000270882	
33715	H4C12	ENSG00000273542	
33944	H3C6	ENSG00000274750	
34112	H3C1	ENSG00000275714	
34196	H4C9	ENSG00000276180	
34331	H4C5	ENSG00000276966	
34366	H4C4	ENSG00000277157	
34643	H4C1	ENSG00000278637	
34665	H4C2	ENSG00000278705	
34683	H3C10	ENSG00000278828	
35542	H3C2	ENSG00000286522	

TABLE 23

Negative Regulation of Megakaryocyte Differentiation Gene Ontology (GO:0045653)

	SYMBOL	ENSEMBL	UniProt
10163	H4C8	ENSG00000158406	P62805
17007	H4C3	ENSG00000197061	
17227	H4-16	ENSG00000197837	
32826	H4C15	ENSG00000270276	
33133	H4C14	ENSG00000270882	
33715	H4C12	ENSG00000273542	
34196	H4C9	ENSG00000276180	
34331	H4C5	ENSG00000276966	
34366	H4C4	ENSG00000277157	
34643	H4C1	ENSG00000278637	

34665	H4C2	ENSG00000278705	
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TABLE 24

Negative Regulation of Gene Expression, Epigenetic Gene Ontology (GO:0045814)

	SYMBOL	ENSEMBL	UniProt
1767	DNMT3B	ENSG00000088305	Q9UBC3
10163	H4C8	ENSG00000158406	P62805
15317	H3C13	ENSG00000183598	Q71DI3
17007	H4C3	ENSG00000197061	
17038	H3C12	ENSG00000197153	P68431
17227	H4-16	ENSG00000197837	
17897	H3C14	ENSG00000203811	
17902	H3C15	ENSG00000203852	
32826	H4C15	ENSG00000270276	
33133	H4C14	ENSG00000270882	
33715	H4C12	ENSG00000273542	
33944	H3C6	ENSG00000274750	
34112	H3C1	ENSG00000275714	
34196	H4C9	ENSG00000276180	
34331	H4C5	ENSG00000276966	
34366	H4C4	ENSG00000277157	
34643	H4C1	ENSG00000278637	
34665	H4C2	ENSG00000278705	
34683	H3C10	ENSG00000278828	
35542	H3C2	ENSG00000286522	

TABLE 25

Detection of Chemical Stimulus Involved in Sensory Perception of Smell Gene Ontology (GO:0050911)

	SYMBOL	ENSEMBL	UniProt
5551	OR2B6	ENSG00000124657	P58173
12013	OR51E2	ENSG00000167332	Q9H255

TABLE 26

Defense Response to Virus Gene Ontology (GO:0051607)

	SYMBOL	ENSEMBL	UniProt
121	MAP3K14	ENSG00000006062	Q99558
1382	EXOSC5	ENSG00000077348	Q9NQT4
1819	OAS1	ENSG00000089127	P00973
2249	APOBEC3H	ENSG00000100298	Q6NTF7
2820	PYCARD	ENSG00000103490	Q9ULZ3
3395	DDX58	ENSG00000107201	O95786
3829	IL23A	ENSG00000110944	Q9NPF7
3879	OAS3	ENSG00000111331	Q9Y6K5
3880	OAS2	ENSG00000111335	P29728
4389	IFIH1	ENSG00000115267	Q9BYX4
4675	GBP3	ENSG00000117226	Q9HOR5

4676	GBP1	ENSG00000117228	P32455
4971	IFIT3	ENSG00000119917	O14879
4973	IFIT2	ENSG00000119922	P09913
5825	IFI6	ENSG00000126709	P09912
6222	BST2	ENSG00000130303	Q10589
6527	TRIM5	ENSG00000132256	Q9C035
6529	TRIM22	ENSG00000132274	Q8IYM9
6855	RSAD2	ENSG00000134321	Q8WXXG1
6990	OASL	ENSG00000135114	Q15646
7469	DDX60	ENSG00000137628	Q8IY21
7542	IFI44L	ENSG00000137959	Q53G44
7649	PARP9	ENSG00000138496	Q8IXQ6
8128	PMAIP1	ENSG00000141682	Q13794
8165	IFITM3	ENSG00000142089	Q01628
11099	DTX3L	ENSG00000163840	Q8TDB6
11299	CGAS	ENSG00000164430	Q8N884
11667	IFI27	ENSG00000165949	P40305
12239	DDIT4	ENSG00000168209	Q9NX09
13090	IFNB1	ENSG00000171855	P01574
13166	ISG20	ENSG00000172183	Q96AZ6
14569	APOBEC3B	ENSG00000179750	Q9UJH17
15769	IRF7	ENSG00000185507	Q92985
15829	IFIT1	ENSG00000185745	P09914
15862	IFITM1	ENSG00000185885	P13164
16246	ISG15	ENSG00000187608	P05161
16422	PLSCR1	ENSG00000188313	O15162
19358	IRF9	ENSG00000213928	Q00978

TABLE 27

Type I Interferon Signaling Pathway Gene Ontology (GO:0060337)

	SYMBOL	ENSEMBL	UniProt
996	SP100	ENSG00000067066	P23497
1819	OAS1	ENSG00000089127	P00973
3879	OAS3	ENSG00000111331	Q9Y6K5
3880	OAS2	ENSG00000111335	P29728
4971	IFIT3	ENSG00000119917	O14879
4973	IFIT2	ENSG00000119922	P09913
5084	EGR1	ENSG00000120738	P18146
5825	IFI6	ENSG00000126709	P09912
6222	BST2	ENSG00000130303	Q10589
6855	RSAD2	ENSG00000134321	Q8WXXG1
6990	OASL	ENSG00000135114	Q15646
8165	IFITM3	ENSG00000142089	Q01628
11667	IFI27	ENSG00000165949	P40305
13090	IFNB1	ENSG00000171855	P01574
13166	ISG20	ENSG00000172183	Q96AZ6
15769	IRF7	ENSG00000185507	Q92985

15829	IFIT1	ENSG000000185745	P09914
15862	IFITM1	ENSG000000185885	P13164
16246	ISG15	ENSG000000187608	P05161
19358	IRF9	ENSG000000213928	Q00978

TABLE 28

Regulation of Ribonuclease Activity Gene Ontology (GO:0060700)

	SYMBOL	ENSEMBL	UniProt
1819	OAS1	ENSG000000089127	P00973
3879	OAS3	ENSG000000111331	Q9Y6K5
3880	OAS2	ENSG000000111335	P29728
6990	OASL	ENSG000000135114	Q15646

TABLE 29

Regulation of Gene silencing by miRNA Gene Ontology (GO:0060964)

	SYMBOL	ENSEMBL	UniProt
1982	AGO1	ENSG000000092847	Q9UL18
10163	H4C8	ENSG000000158406	P62805
15317	H3C13	ENSG000000183598	Q71DI3
17007	H4C3	ENSG000000197061	
17038	H3C12	ENSG000000197153	P68431
17227	H4-16	ENSG000000197837	
17897	H3C14	ENSG000000203811	
17902	H3C15	ENSG000000203852	
32826	H4C15	ENSG000000270276	
33133	H4C14	ENSG000000270882	
33715	H4C12	ENSG000000273542	
33944	H3C6	ENSG000000274750	
34112	H3C1	ENSG000000275714	
34196	H4C9	ENSG000000276180	
34331	H4C5	ENSG000000276966	
34366	H4C4	ENSG000000277157	
34643	H4C1	ENSG000000278637	
34665	H4C2	ENSG000000278705	
34683	H3C10	ENSG000000278828	
35542	H3C2	ENSG000000286522	

TABLE 30

Cellular Response to Interleukin-1 Gene Ontology (GO:0071347)

	SYMBOL	ENSEMBL	UniProt
142	CCL26	ENSG000000006606	Q9Y258
435	ABCC2	ENSG000000023839	Q92887
2820	PYCARD	ENSG000000103490	Q9ULZ3
4349	CCL20	ENSG000000115009	P78556
4675	GBP3	ENSG000000117226	Q9H0R5
4676	GBP1	ENSG000000117228	P32455
5488	PCK1	ENSG000000124253	P35558

5799	NR1D1	ENSG00000126368	P20393
5912	KLF2	ENSG00000127528	Q9Y5W3
6385	CCL25	ENSG00000131142	O15444
9083	ANKRD1	ENSG00000148677	Q15327
11948	NKX3-1	ENSG00000167034	Q99801
12529	CXCL8	ENSG00000169429	P10145
12880	HAS2	ENSG00000170961	Q92819
13175	CEBPB	ENSG00000172216	P17676
33460	CCL5	ENSG00000271503	P13501
34038	CCL4	ENSG00000275302	P13236

TABLE 31

Upstream Regulators

SYMBOL	ENSEMBL	UniProt
KDM1A	ENSG00000004487	O60341
CREBBP	ENSG00000005339	Q92793
PAF1	ENSG00000006712	Q8N7H5
HGF	ENSG00000019991	P14210
MAP2K3	ENSG00000034152	P46734
KITLG	ENSG00000049130	P21583
EIF2AK2	ENSG00000055332	P19525
TRAF3IP2	ENSG00000056972	O43734
PRDM1	ENSG00000057657	O75626
SPI1	ENSG00000066336	P17947
TFRC	ENSG00000072274	P02786
MEF2C	ENSG00000081189	Q06413
REST	ENSG00000084093	Q13127
MAVS	ENSG00000088888	Q7Z434
CBX5	ENSG00000094916	P45973
MAP3K1	ENSG00000095015	Q13233
SIRT1	ENSG00000096717	Q96EB6
SMARCB1	ENSG00000099956	Q12824
MAPK1	ENSG00000100030	P28482
EP300	ENSG00000100393	Q09472
HIF1A	ENSG00000100644	Q16665
APEX1	ENSG00000100823	P27695
NFKBIA	ENSG00000100906	P25963
MAPK3	ENSG00000102882	P27361
IKBKB	ENSG00000104365	O14920
TGFB1	ENSG00000105329	P01137
C5	ENSG00000106804	P01031
DOCK8	ENSG00000107099	Q8NF50
DDX58	ENSG00000107201	O95786
MAPK8	ENSG00000107643	P45983
NFKB1	ENSG00000109320	P19838
SASH1	ENSG00000111961	O94885
MAPK14	ENSG00000112062	Q16539

SIM1	ENSG00000112246	P81133
SRF	ENSG00000112658	P11831
VEGFA	ENSG00000112715	P15692
IL5	ENSG00000113525	P05113
CISH	ENSG00000114737	Q9NSE2
STAT1	ENSG00000115415	P42224
EPAS1	ENSG00000116016	Q99814
MEF2D	ENSG00000116604	Q14814
CREB1	ENSG00000118260	P16220
FOXO3	ENSG00000118689	O43524
EGR1	ENSG00000120738	P18146
IRF1	ENSG00000125347	P10914
IRF3	ENSG00000126456	Q14653
ELK1	ENSG00000126767	P19419
SMARCA4	ENSG00000127616	P51532
TICAM1	ENSG00000127666	Q8IUC6
ATF4	ENSG00000128272	P18848
IRF5	ENSG00000128604	Q13568
RAF1	ENSG00000132155	P04049
NGF	ENSG00000134259	P01138
RC3H1	ENSG00000135870	Q5TC82
SP110	ENSG00000135899	Q9HB58
IL33	ENSG00000137033	O95760
HMGA1	ENSG00000137309	P17096
TLR2	ENSG00000137462	O60603
STAT4	ENSG00000138378	Q14765
FGF2	ENSG00000138685	P09038
EGF	ENSG00000138798	P01133
PML	ENSG00000140464	P29590
TP53	ENSG00000141510	P04637
ERBB2	ENSG00000141736	P04626
IFNAR1	ENSG00000142166	P17181
APP	ENSG00000142192	P05067
FCGR2A	ENSG00000143226	P12318
ACKR2	ENSG00000144648	O00590
GNRH1	ENSG00000147437	P01148
PPRC1	ENSG00000148840	Q5VV67
PTPRJ	ENSG00000149177	Q12913
IL18	ENSG00000150782	Q14116
FOXO1	ENSG00000150907	Q12778
EPHB1	ENSG00000154928	P54762
SAFB	ENSG00000160633	Q15424
FEV	ENSG00000163497	Q99581
PTX3	ENSG00000163661	P26022
PRKCD	ENSG00000163932	Q05655
CAMP	ENSG00000164047	P49913
TLR3	ENSG00000164342	O15455
CGAS	ENSG00000164430	Q8N884

GPB1	ENSG00000164850	Q99527
RET	ENSG00000165731	P07949
SMPD1	ENSG00000166311	P17405
SMAD3	ENSG00000166949	P84022
RBPJ	ENSG00000168214	Q06330
MAP2K1	ENSG00000169032	Q02750
JUNB	ENSG00000171223	P17275
IFNB1	ENSG00000171855	P01574
CEBPB	ENSG00000172216	P17676
ARNT2	ENSG00000172379	Q9HBZ2
MYD88	ENSG00000172936	Q99836
RELA	ENSG00000173039	Q04206
BDNF	ENSG00000176697	P23560
JUN	ENSG00000177606	P05412
MSC	ENSG00000178860	O60682
CSF1	ENSG00000184371	P09603
SOCS3	ENSG00000184557	O14543
STING1	ENSG00000184584	Q86WV6
SOCS1	ENSG00000185338	O15524
IRF7	ENSG00000185507	Q92985
TGM2	ENSG00000198959	P21980
CHUK	ENSG00000213341	O15111
MIF	ENSG00000240972	P14174
CEBPA	ENSG00000245848	P49715
IKBKE	ENSG00000263528	Q14164
IKBKG	ENSG00000269335	Q9Y6K9

[0046] In some non-limiting embodiments, modified host cells are provided which are modified to repress or eliminate expression of any one or more of the following human differentially expressed genes (DEGs)(or respective orthologous non-human genes of host cells of non-human origin): ABCC2, ABCF1, ACKR2, ACTA1 (Entrez Gene ID 58; UniProt Accession P68133), AGO1, ALB, ANG, ANKRD1, ANO8, ANXA1, AOC3, APEX1, APLN, APOA1, APOBEC3B, APOBEC3H, APOE, APP, ARL14 (Entrez Gene ID 80117; UniProt Accession Q8N4G2), ARNT2, ASNS, ATF3, ATF4, BDNF, BHLHA15, BLNK, BST2, BUD31, C3, C4A, C4B, C5, C5AR1, CALCR (Entrez Gene ID 799; UniProt Accession P30988), CALR3, CAMP, CBX5, CCL20, CCL25, CCL26, CCL4, CCL5, CCND1, CCR10, CCR4, CD14, CD8A, CEBPA, CEBPB, CEBPG, CENPQ, CFP, CGAS, CHGB, CHUK, CISH, CLIC5, CNIH1, COL16A1, CREB1, CREBBP, CREBRF, CSF1, CTH, CTSK, CTSW, CX3CR1, CXCL11, CXCL8, CXCR2, CYP1A1, DAXX, DDIT3, DDIT4, DDX58, DDX60, DNMT3B, DOCK8, DTX3L, DUSP1, DUSP10, DUSP14, DUSP16, DUSP4, DUSP5, DUSP6, DUSP8, ECM1, EGF, EGR1, EIF2AK2, ELK1, EP300, EPAS1, EPHB1, ERBB2, ESRP1 (Entrez Gene ID 54845; UniProt Accession Q6NXG1), EXOSC5, FAM83A (Entrez Gene ID 84985; UniProt Accession Q86UY5), FAS, FCGR2A, FEV, FGF2,

FGF21, FGF23, FOS, FOSB, FOSL1, FOXO1, FOXO3, GBP1, GBP3, GEM, GIP, GNRH1, GPER1, H1-0, H1-2, H1-3, H1-4, H1-5, H2BC11, H2BC12, H2BC13, H2BC14, H2BC15, H2BC17, H2BC18, H2BC21, H2BC4, H2BC5, H2BC6, H2BC7, H2BC8, H2BU1, H2BW2, H3C1, H3C10, H3C12, H3C13, H3C14, H3C15, H3C2, H3C6, H4-16, H4C1, H4C12, H4C14, H4C15, H4C2, H4C3, H4C4, H4C5, H4C8, H4C9, HAS2, HERPUD1, HGF, HIF1A, HLA-DRB1, HMGA1, HRH1, HS3ST4 (Entrez Gene ID 9951; UniProt Accession Q9Y661), IFI27, IFI44, IFI44L, IFI6, IFIH1, IFIT1, IFIT2, IFIT3, IFITM1, IFITM3, IFNAR1, IFNB1, IGFBP3, IGFBP5, IGFBP7, IKBKB, IKBKE, IKBKG, IL16, IL17B, IL17F, IL17RE, IL18, IL23A, IL32, IL33, IL34, IL5, IRAK3, IRF1, IRF3, IRF5, IRF7, IRF9, IRGM, ISG15, ISG20, ITGAM (Entrez Gene ID 3684; UniProt Accession P11215), JUN, JUNB, KDM1A, KITLG, KLF2, LAMB1, LGALS1, LGALS9, LMCD1, LST1, LTB4R, LTB4R2, MADCAM1, MAP2K1, MAP2K3, MAP3K1, MAP3K14, MAPK1, MAPK14, MAPK3, MAPK8, MAVS, MEF2C, MEF2D, MEP1B, MIF, MMP13, MMP25, MR1, MSC, MSLN, MYD88, MYLPF, NFIL3, NFKB1, NFKBIA, NGF, NKX3-1, NLRC4, NOVA2, NR1D1, NR4A3, OAS1, OAS2, OAS3, OASL, ODAPH (Entrez Gene ID 152816; UniProt Accession Q17RF5), OR2B6, OR51E2, OVGP1, PAF1, PARP9, PCK1, PDE6G (Entrez Gene ID 5148; UniProt Accession P18545), PIK3CD, PLA2G1B, PLA2G4C, PLP1, PLSCR1, PMAIP1, PML, PNN (Entrez Gene ID 5411; UniProt Accession Q9H307), PPRC1, PRDM1, PRKCD, PROC, PSG4, PTAFR, PTGS2, PTHLH, PTPRJ, PTX3, PYCARD, RAF1, RAVR1, RBPJ, RC3H1, RELA, RELB, REST, RET, RGS3, RLN1, RPL39, RPS15A, RSAD2, SAFB, SASH1, SCG2, SCG3, SELENOS, SIM1, SIRT1, SLC38A2, SLPI, SMAD3, SMARCA4, SMARCB1, SMPD1, SOCS1, SOCS3, SP100, SP110, SPI1, SRF, STAT1, STAT4, STC2, STING1, TAC1, TAC3, TAPBP, TFRC, TGFB1, TGFB3, TGM2, TICAM1, TICAM2, TLR2, TLR3, TMEM132A, TNC, TNFAIP3, TNFSF12, TP53, TPT1, TRAF3IP2, TRIM22, TRIM24 (Entrez Gene ID 8805; UniProt Accession O15164), TRIM5, ULBP1, VEGFA, VGF, VPREB3, WFDC2, and/or XBP1. In any of the foregoing embodiments, host cells which are modified to repress or eliminate expression of such genes can be any host cell known in the art to be suitable for producing AAV vectors, including without limitation, animal cells, such as insect or mammal cells, including without limitation, cells of human origin, such as cell lines of human origin, including without limitation, HEK293 cells, including variants, strains, and derivatives thereof.

[0047] In some non-limiting embodiments, modified host cells are provided which are modified to repress or eliminate expression of any one or more of the following human DEGs (or respective orthologous non-human genes of host cells of non-human origin): ACTA1, ARL14, BDNF, CALCR, CSF1, CXCL11, DOCK8, EIF2AK2, EP300, EPHB1, ESRP1, FAM83A, FGF2, FOXO1, GNRH1, HGF, HS3ST4, IFI44L, ITGAM, KITLG, MAP2K3, MAPK1, MAPK3, MIF, MSC, MYD88, OAS3, ODAPH, PAF1, PDE6G, PNN, PPRC1, PTX3, RC3H1, SMARCA4, SP110, SPI1, STAT4, TGM2, TICAM1

and/or TRIM24. In any of the foregoing embodiments, modified host cells which are modified to repress or eliminate expression of such genes can be any host cell known in the art to be suitable for producing AAV vectors, including without limitation, animal cells, such as insect or mammal cells, including without limitation, cells of human origin, such as cell lines of human origin, including without limitation, HEK293 cells, including variants, strains, and derivatives thereof.

[0048] In some of these embodiments, the modified host cells are modified to repress or eliminate expression of any of the foregoing genes singly, or in combination with one or more others of the listed genes. Thus, for example, in some non-limiting embodiments modified host cells are provided which are modified to repress or eliminate expression of ACTA1 alone, or in combination with one or more of ARL14, BDNF, CALCR, CSF1, CXCL11, DOCK8, EIF2AK2, EP300, EPHB1, ESRP1, FAM83A, FGF2, FOXO1, GNRH1, HGF, HS3ST4, IFI44L, ITGAM, KITLG, MAP2K3, MAPK1, MAPK3, MIF, MSC, MYD88, OAS3, ODAPH, PAF1, PDE6G, PNN, PPRC1, PTX3, RC3H1, SMARCA4, SP110, SPI1, STAT4, TGM2, TICAM1 and/or TRIM24; or ARL14 alone, or in combination with one or more of ACTA1, BDNF, CALCR, CSF1, CXCL11, DOCK8, EIF2AK2, EP300, EPHB1, ESRP1, FAM83A, FGF2, FOXO1, GNRH1, HGF, HS3ST4, IFI44L, ITGAM, KITLG, MAP2K3, MAPK1, MAPK3, MIF, MSC, MYD88, OAS3, ODAPH, PAF1, PDE6G, PNN, PPRC1, PTX3, RC3H1, SMARCA4, SP110, SPI1, STAT4, TGM2, TICAM1 and/or TRIM24; or BDNF alone, or in combination with one or more of ACTA1, ARL14, CALCR, CSF1, CXCL11, DOCK8, EIF2AK2, EP300, EPHB1, ESRP1, FAM83A, FGF2, FOXO1, GNRH1, HGF, HS3ST4, IFI44L, ITGAM, KITLG, MAP2K3, MAPK1, MAPK3, MIF, MSC, MYD88, OAS3, ODAPH, PAF1, PDE6G, PNN, PPRC1, PTX3, RC3H1, SMARCA4, SP110, SPI1, STAT4, TGM2, TICAM1 and/or TRIM24; or CALCR alone, or in combination with one or more of ACTA1, ARL14, BDNF, CSF1, CXCL11, DOCK8, EIF2AK2, EP300, EPHB1, ESRP1, FAM83A, FGF2, FOXO1, GNRH1, HGF, HS3ST4, IFI44L, ITGAM, KITLG, MAP2K3, MAPK1, MAPK3, MIF, MSC, MYD88, OAS3, ODAPH, PAF1, PDE6G, PNN, PPRC1, PTX3, RC3H1, SMARCA4, SP110, SPI1, STAT4, TGM2, TICAM1 and/or TRIM24; or CSF1 alone, or in combination with one or more of ACTA1, ARL14, BDNF, CALCR, CXCL11, DOCK8, EIF2AK2, EP300, EPHB1, ESRP1, FAM83A, FGF2, FOXO1, GNRH1, HGF, HS3ST4, IFI44L, ITGAM, KITLG, MAP2K3, MAPK1, MAPK3, MIF, MSC, MYD88, OAS3, ODAPH, PAF1, PDE6G, PNN, PPRC1, PTX3, RC3H1, SMARCA4, SP110, SPI1, STAT4, TGM2, TICAM1 and/or TRIM24; or CXCL11 alone, or in combination with one or more of ACTA1, ARL14, BDNF, CALCR, CSF1, DOCK8, EIF2AK2, EP300, EPHB1, ESRP1, FAM83A, FGF2, FOXO1, GNRH1, HGF, HS3ST4, IFI44L, ITGAM, KITLG, MAP2K3, MAPK1, MAPK3, MIF, MSC, MYD88, OAS3, ODAPH, PAF1, PDE6G, PNN, PPRC1, PTX3, RC3H1, SMARCA4, SP110, SPI1, STAT4, TGM2, TICAM1 and/or TRIM24; or

DOCK8 alone, or in combination with one or more of ACTA1, ARL14, BDNF, CALCR, CSF1, CXCL11, EIF2AK2, EP300, EPHB1, ESRP1, FAM83A, FGF2, FOXO1, GNRH1, HGF, HS3ST4, IFI44L, ITGAM, KITLG, MAP2K3, MAPK1, MAPK3, MIF, MSC, MYD88, OAS3, ODAPH, PAF1, PDE6G, PNN, PPRC1, PTX3, RC3H1, SMARCA4, SP110, SPI1, STAT4, TGM2, TICAM1 and/or TRIM24; or EIF2AK2 alone, or in combination with one or more of ACTA1, ARL14, BDNF, CALCR, CSF1, CXCL11, DOCK8, EP300, EPHB1, ESRP1, FAM83A, FGF2, FOXO1, GNRH1, HGF, HS3ST4, IFI44L, ITGAM, KITLG, MAP2K3, MAPK1, MAPK3, MIF, MSC, MYD88, OAS3, ODAPH, PAF1, PDE6G, PNN, PPRC1, PTX3, RC3H1, SMARCA4, SP110, SPI1, STAT4, TGM2, TICAM1 and/or TRIM24; or EP300 alone, or in combination with one or more of ACTA1, ARL14, BDNF, CALCR, CSF1, CXCL11, DOCK8, EIF2AK2, EPHB1, ESRP1, FAM83A, FGF2, FOXO1, GNRH1, HGF, HS3ST4, IFI44L, ITGAM, KITLG, MAP2K3, MAPK1, MAPK3, MIF, MSC, MYD88, OAS3, ODAPH, PAF1, PDE6G, PNN, PPRC1, PTX3, RC3H1, SMARCA4, SP110, SPI1, STAT4, TGM2, TICAM1 and/or TRIM24; or EPHB1 alone, or in combination with one or more of ACTA1, ARL14, BDNF, CALCR, CSF1, CXCL11, DOCK8, EIF2AK2, EP300, ESRP1, FAM83A, FGF2, FOXO1, GNRH1, HGF, HS3ST4, IFI44L, ITGAM, KITLG, MAP2K3, MAPK1, MAPK3, MIF, MSC, MYD88, OAS3, ODAPH, PAF1, PDE6G, PNN, PPRC1, PTX3, RC3H1, SMARCA4, SP110, SPI1, STAT4, TGM2, TICAM1 and/or TRIM24; or ESRP1 alone, or in combination with one or more of ACTA1, ARL14, BDNF, CALCR, CSF1, CXCL11, DOCK8, EIF2AK2, EP300, EPHB1, FAM83A, FGF2, FOXO1, GNRH1, HGF, HS3ST4, IFI44L, ITGAM, KITLG, MAP2K3, MAPK1, MAPK3, MIF, MSC, MYD88, OAS3, ODAPH, PAF1, PDE6G, PNN, PPRC1, PTX3, RC3H1, SMARCA4, SP110, SPI1, STAT4, TGM2, TICAM1 and/or TRIM24; or FAM83A alone, or in combination with one or more of ACTA1, ARL14, BDNF, CALCR, CSF1, CXCL11, DOCK8, EIF2AK2, EP300, EPHB1, ESRP1, FGF2, FOXO1, GNRH1, HGF, HS3ST4, IFI44L, ITGAM, KITLG, MAP2K3, MAPK1, MAPK3, MIF, MSC, MYD88, OAS3, ODAPH, PAF1, PDE6G, PNN, PPRC1, PTX3, RC3H1, SMARCA4, SP110, SPI1, STAT4, TGM2, TICAM1 and/or TRIM24; or FGF2 alone, or in combination with one or more of ACTA1, ARL14, BDNF, CALCR, CSF1, CXCL11, DOCK8, EIF2AK2, EP300, EPHB1, ESRP1, FAM83A, FOXO1, GNRH1, HGF, HS3ST4, IFI44L, ITGAM, KITLG, MAP2K3, MAPK1, MAPK3, MIF, MSC, MYD88, OAS3, ODAPH, PAF1, PDE6G, PNN, PPRC1, PTX3, RC3H1, SMARCA4, SP110, SPI1, STAT4, TGM2, TICAM1 and/or TRIM24; or FOXO1 alone, or in combination with one or more of ACTA1, ARL14, BDNF, CALCR, CSF1, CXCL11, DOCK8, EIF2AK2, EP300, EPHB1, ESRP1, FAM83A, FGF2, GNRH1, HGF, HS3ST4, IFI44L, ITGAM, KITLG, MAP2K3, MAPK1, MAPK3, MIF, MSC, MYD88, OAS3, ODAPH, PAF1, PDE6G, PNN, PPRC1, PTX3, RC3H1, SMARCA4, SP110, SPI1, STAT4, TGM2, TICAM1 and/or TRIM24; or GNRH1 alone, or in combination with one or more of ACTA1, ARL14, BDNF, CALCR, CSF1, CXCL11, DOCK8, EIF2AK2, EP300, EPHB1, ESRP1, FAM83A, FGF2, FOXO1, HGF, HS3ST4, IFI44L, ITGAM, KITLG, MAP2K3,

MAPK1, MAPK3, MIF, MSC, MYD88, OAS3, ODAPH, PAF1, PDE6G, PNN, PPRC1, PTX3, RC3H1, SMARCA4, SP110, SPI1, STAT4, TGM2, TICAM1 and/or TRIM24; or HGF alone, or in combination with one or more of ACTA1, ARL14, BDNF, CALCR, CSF1, CXCL11, DOCK8, EIF2AK2, EP300, EPHB1, ESRP1, FAM83A, FGF2, FOXO1, GNRH1, HS3ST4, IFI44L, ITGAM, KITLG, MAP2K3, MAPK1, MAPK3, MIF, MSC, MYD88, OAS3, ODAPH, PAF1, PDE6G, PNN, PPRC1, PTX3, RC3H1, SMARCA4, SP110, SPI1, STAT4, TGM2, TICAM1 and/or TRIM24; or HS3ST4 alone, or in combination with one or more of ACTA1, ARL14, BDNF, CALCR, CSF1, CXCL11, DOCK8, EIF2AK2, EP300, EPHB1, ESRP1, FAM83A, FGF2, FOXO1, GNRH1, HGF, HS3ST4, IFI44L, ITGAM, KITLG, MAP2K3, MAPK1, MAPK3, MIF, MSC, MYD88, OAS3, ODAPH, PAF1, PDE6G, PNN, PPRC1, PTX3, RC3H1, SMARCA4, SP110, SPI1, STAT4, TGM2, TICAM1 and/or TRIM24; or IFI44L alone, or in combination with one or more of ACTA1, ARL14, BDNF, CALCR, CSF1, CXCL11, DOCK8, EIF2AK2, EP300, EPHB1, ESRP1, FAM83A, FGF2, FOXO1, GNRH1, HGF, HS3ST4, ITGAM, KITLG, MAP2K3, MAPK1, MAPK3, MIF, MSC, MYD88, OAS3, ODAPH, PAF1, PDE6G, PNN, PPRC1, PTX3, RC3H1, SMARCA4, SP110, SPI1, STAT4, TGM2, TICAM1 and/or TRIM24; or ITGAM alone, or in combination with one or more of ACTA1, ARL14, BDNF, CALCR, CSF1, CXCL11, DOCK8, EIF2AK2, EP300, EPHB1, ESRP1, FAM83A, FGF2, FOXO1, GNRH1, HGF, HS3ST4, IFI44L, KITLG, MAP2K3, MAPK1, MAPK3, MIF, MSC, MYD88, OAS3, ODAPH, PAF1, PDE6G, PNN, PPRC1, PTX3, RC3H1, SMARCA4, SP110, SPI1, STAT4, TGM2, TICAM1 and/or TRIM24; or KITLG alone, or in combination with one or more of ACTA1, ARL14, BDNF, CALCR, CSF1, CXCL11, DOCK8, EIF2AK2, EP300, EPHB1, ESRP1, FAM83A, FGF2, FOXO1, GNRH1, HGF, HS3ST4, IFI44L, ITGAM, MAP2K3, MAPK1, MAPK3, MIF, MSC, MYD88, OAS3, ODAPH, PAF1, PDE6G, PNN, PPRC1, PTX3, RC3H1, SMARCA4, SP110, SPI1, STAT4, TGM2, TICAM1 and/or TRIM24; or MAP2K3 alone, or in combination with one or more of ACTA1, ARL14, BDNF, CALCR, CSF1, CXCL11, DOCK8, EIF2AK2, EP300, EPHB1, ESRP1, FAM83A, FGF2, FOXO1, GNRH1, HGF, HS3ST4, IFI44L, ITGAM, KITLG, MAPK1, MAPK3, MIF, MSC, MYD88, OAS3, ODAPH, PAF1, PDE6G, PNN, PPRC1, PTX3, RC3H1, SMARCA4, SP110, SPI1, STAT4, TGM2, TICAM1 and/or TRIM24; or MAPK1 alone, or in combination with one or more of ACTA1, ARL14, BDNF, CALCR, CSF1, CXCL11, DOCK8, EIF2AK2, EP300, EPHB1, ESRP1, FAM83A, FGF2, FOXO1, GNRH1, HGF, HS3ST4, IFI44L, ITGAM, KITLG, MAP2K3, MAPK3, MIF, MSC, MYD88, OAS3, ODAPH, PAF1, PDE6G, PNN, PPRC1, PTX3, RC3H1, SMARCA4, SP110, SPI1, STAT4, TGM2, TICAM1 and/or TRIM24; or MAPK3 alone, or in combination with one or more of ACTA1, ARL14, BDNF, CALCR, CSF1, CXCL11, DOCK8, EIF2AK2, EP300, EPHB1, ESRP1, FAM83A, FGF2, FOXO1, GNRH1, HGF, HS3ST4, IFI44L, ITGAM, KITLG, MAP2K3, MAPK1, MIF, MSC, MYD88, OAS3, ODAPH, PAF1, PDE6G, PNN, PPRC1, PTX3, RC3H1, SMARCA4, SP110, SPI1, STAT4, TGM2, TICAM1 and/or TRIM24; or MIF alone, or in

combination with one or more of ACTA1, ARL14, BDNF, CALCR, CSF1, CXCL11, DOCK8, EIF2AK2, EP300, EPHB1, ESRP1, FAM83A, FGF2, FOXO1, GNRH1, HGF, HS3ST4, IFI44L, ITGAM, KITLG, MAP2K3, MAPK1, MAPK3, MSC, MYD88, OAS3, ODAPH, PAF1, PDE6G, PNN, PPRC1, PTX3, RC3H1, SMARCA4, SP110, SPI1, STAT4, TGM2, TICAM1 and/or TRIM24; or MSC alone, or in combination with one or more of ACTA1, ARL14, BDNF, CALCR, CSF1, CXCL11, DOCK8, EIF2AK2, EP300, EPHB1, ESRP1, FAM83A, FGF2, FOXO1, GNRH1, HGF, HS3ST4, IFI44L, ITGAM, KITLG, MAP2K3, MAPK1, MAPK3, MIF, MYD88, OAS3, ODAPH, PAF1, PDE6G, PNN, PPRC1, PTX3, RC3H1, SMARCA4, SP110, SPI1, STAT4, TGM2, TICAM1 and/or TRIM24; or MYD88 alone, or in combination with one or more of ACTA1, ARL14, BDNF, CALCR, CSF1, CXCL11, DOCK8, EIF2AK2, EP300, EPHB1, ESRP1, FAM83A, FGF2, FOXO1, GNRH1, HGF, HS3ST4, IFI44L, ITGAM, KITLG, MAP2K3, MAPK1, MAPK3, MIF, MSC, OAS3, ODAPH, PAF1, PDE6G, PNN, PPRC1, PTX3, RC3H1, SMARCA4, SP110, SPI1, STAT4, TGM2, TICAM1 and/or TRIM24; or OAS3 alone, or in combination with one or more of ACTA1, ARL14, BDNF, CALCR, CSF1, CXCL11, DOCK8, EIF2AK2, EP300, EPHB1, ESRP1, FAM83A, FGF2, FOXO1, GNRH1, HGF, HS3ST4, IFI44L, ITGAM, KITLG, MAP2K3, MAPK1, MAPK3, MIF, MSC, MYD88, ODAPH, PAF1, PDE6G, PNN, PPRC1, PTX3, RC3H1, SMARCA4, SP110, SPI1, STAT4, TGM2, TICAM1 and/or TRIM24; or ODAPH alone, or in combination with one or more of ACTA1, ARL14, BDNF, CALCR, CSF1, CXCL11, DOCK8, EIF2AK2, EP300, EPHB1, ESRP1, FAM83A, FGF2, FOXO1, GNRH1, HGF, HS3ST4, IFI44L, ITGAM, KITLG, MAP2K3, MAPK1, MAPK3, MIF, MSC, MYD88, OAS3, PAF1, PDE6G, PNN, PPRC1, PTX3, RC3H1, SMARCA4, SP110, SPI1, STAT4, TGM2, TICAM1 and/or TRIM24; or PAF1 alone, or in combination with one or more of ACTA1, ARL14, BDNF, CALCR, CSF1, CXCL11, DOCK8, EIF2AK2, EP300, EPHB1, ESRP1, FAM83A, FGF2, FOXO1, GNRH1, HGF, HS3ST4, IFI44L, ITGAM, KITLG, MAP2K3, MAPK1, MAPK3, MIF, MSC, MYD88, OAS3, ODAPH, PDE6G, PNN, PPRC1, PTX3, RC3H1, SMARCA4, SP110, SPI1, STAT4, TGM2, TICAM1 and/or TRIM24; or PDE6G alone, or in combination with one or more of ACTA1, ARL14, BDNF, CALCR, CSF1, CXCL11, DOCK8, EIF2AK2, EP300, EPHB1, ESRP1, FAM83A, FGF2, FOXO1, GNRH1, HGF, HS3ST4, IFI44L, ITGAM, KITLG, MAP2K3, MAPK1, MAPK3, MIF, MSC, MYD88, OAS3, ODAPH, PAF1, PNN, PPRC1, PTX3, RC3H1, SMARCA4, SP110, SPI1, STAT4, TGM2, TICAM1 and/or TRIM24; or PNN alone, or in combination with one or more of ACTA1, ARL14, BDNF, CALCR, CSF1, CXCL11, DOCK8, EIF2AK2, EP300, EPHB1, ESRP1, FAM83A, FGF2, FOXO1, GNRH1, HGF, HS3ST4, IFI44L, ITGAM, KITLG, MAP2K3, MAPK1, MAPK3, MIF, MSC, MYD88, OAS3, ODAPH, PAF1, PDE6G, PPRC1, PTX3, RC3H1, SMARCA4, SP110, SPI1, STAT4, TGM2, TICAM1 and/or TRIM24; or PPRC1 alone, or in combination with one or more of ACTA1, ARL14, BDNF, CALCR, CSF1, CXCL11, DOCK8, EIF2AK2, EP300, EPHB1, ESRP1, FAM83A, FGF2, FOXO1, GNRH1, HGF, HS3ST4, IFI44L, ITGAM, KITLG,

MAP2K3, MAPK1, MAPK3, MIF, MSC, MYD88, OAS3, ODAPH, PAF1, PDE6G, PNN, PTX3, RC3H1, SMARCA4, SP110, SPI1, STAT4, TGM2, TICAM1 and/or TRIM24; or PTX3 alone, or in combination with one or more of ACTA1, ARL14, BDNF, CALCR, CSF1, CXCL11, DOCK8, EIF2AK2, EP300, EPHB1, ESRP1, FAM83A, FGF2, FOXO1, GNRH1, HGF, HS3ST4, IFI44L, ITGAM, KITLG, MAP2K3, MAPK1, MAPK3, MIF, MSC, MYD88, OAS3, ODAPH, PAF1, PDE6G, PNN, PPRC1, RC3H1, SMARCA4, SP110, SPI1, STAT4, TGM2, TICAM1 and/or TRIM24; or RC3H1 alone, or in combination with one or more of ACTA1, ARL14, BDNF, CALCR, CSF1, CXCL11, DOCK8, EIF2AK2, EP300, EPHB1, ESRP1, FAM83A, FGF2, FOXO1, GNRH1, HGF, HS3ST4, IFI44L, ITGAM, KITLG, MAP2K3, MAPK1, MAPK3, MIF, MSC, MYD88, OAS3, ODAPH, PAF1, PDE6G, PNN, PPRC1, PTX3, SMARCA4, SP110, SPI1, STAT4, TGM2, TICAM1 and/or TRIM24; or SMARCA4 alone, or in combination with one or more of ACTA1, ARL14, BDNF, CALCR, CSF1, CXCL11, DOCK8, EIF2AK2, EP300, EPHB1, ESRP1, FAM83A, FGF2, FOXO1, GNRH1, HGF, HS3ST4, IFI44L, ITGAM, KITLG, MAP2K3, MAPK1, MAPK3, MIF, MSC, MYD88, OAS3, ODAPH, PAF1, PDE6G, PNN, PPRC1, PTX3, RC3H1, SP110, SPI1, STAT4, TGM2, TICAM1 and/or TRIM24; or SPI1 alone, or in combination with one or more of ACTA1, ARL14, BDNF, CALCR, CSF1, CXCL11, DOCK8, EIF2AK2, EP300, EPHB1, ESRP1, FAM83A, FGF2, FOXO1, GNRH1, HGF, HS3ST4, IFI44L, ITGAM, KITLG, MAP2K3, MAPK1, MAPK3, MIF, MSC, MYD88, OAS3, ODAPH, PAF1, PDE6G, PNN, PPRC1, PTX3, RC3H1, SMARCA4, SP110, STAT4, TGM2, TICAM1 and/or TRIM24; or STAT4 alone, or in combination with one or more of ACTA1, ARL14, BDNF, CALCR, CSF1, CXCL11, DOCK8, EIF2AK2, EP300, EPHB1, ESRP1, FAM83A, FGF2, FOXO1, GNRH1, HGF, HS3ST4, IFI44L, ITGAM, KITLG, MAP2K3, MAPK1, MAPK3, MIF, MSC, MYD88, OAS3, ODAPH, PAF1, PDE6G, PNN, PPRC1, PTX3, RC3H1, SMARCA4, SP110, SPI1, TGM2, TICAM1 and/or TRIM24; or TGM2 alone, or in combination with one or more of ACTA1, ARL14, BDNF, CALCR, CSF1, CXCL11, DOCK8, EIF2AK2, EP300, EPHB1, ESRP1, FAM83A, FGF2, FOXO1, GNRH1, HGF, HS3ST4, IFI44L, ITGAM, KITLG, MAP2K3, MAPK1, MAPK3, MIF, MSC, MYD88, OAS3, ODAPH, PAF1, PDE6G, PNN, PPRC1, PTX3, RC3H1, SMARCA4, SP110, SPI1, STAT4, TICAM1 and/or TRIM24; or TICAM1 alone, or in combination with one or more of ACTA1, ARL14, BDNF, CALCR, CSF1, CXCL11, DOCK8, EIF2AK2, EP300, EPHB1, ESRP1, FAM83A, FGF2, FOXO1, GNRH1, HGF, HS3ST4, IFI44L, ITGAM, KITLG, MAP2K3, MAPK1, MAPK3, MIF, MSC, MYD88, OAS3, ODAPH, PAF1, PDE6G, PNN, PPRC1, PTX3, RC3H1, SMARCA4, SP110, SPI1, STAT4, TGM2, and/or TRIM24; or TRIM24 alone, or in

combination with one or more of ACTA1, ARL14, BDNF, CALCR, CSF1, CXCL11, DOCK8, EIF2AK2, EP300, EPHB1, ESRP1, FAM83A, FGF2, FOXO1, GNRH1, HGF, HS3ST4, IFI44L, ITGAM, KITLG, MAP2K3, MAPK1, MAPK3, MIF, MSC, MYD88, OAS3, ODAPH, PAF1, PDE6G, PNN, PPRC1, PTX3, RC3H1, SMARCA4, SP110, SPI1, STAT4, TGM2, and/or TICAM1.

[0049] In some other non-limiting embodiments, modified host cells are provided which are modified to repress or eliminate expression of the human genes (or respective orthologous non-human genes of host cells of non-human origin) MIF and OAS3; OAS3 and PAF1; OAS3 and RC3H1; OAS3 and PPRC1; MIF and PAF1; MIF and RC3H1; MIF and PPRC1; PAF1 and RC3H1; PAF1 and PPRC1; or PPRC1 and RC3H1.

[0050] In some embodiments, repression or elimination in host cells of expression of any gene (such as the DEGs described herein) upregulated in unmodified host cells of the same type after introduction of exogenous genetic material required to direct biosynthesis of recombinant AAV vectors, and/or at different times during their biosynthesis after introduction of the exogenous genetic material, is effective to increase the amount of intact AAV vectors produced in such host cells compared to the amount of AAV vectors produced from unmodified host cells of the same type in which the gene has not been repressed or eliminated. In some embodiments, the amount of a particular AAV vector produced in modified host cells is at least or about 50%, 60%, 70%, 80%, 90%, 100%, 110%, 120%, 130%, 140%, 150%, 175%, 200%, 225%, 250%, 275%, 300%, 325%, 350%, 375%, 400%, 425%, 450%, 475%, or 500% greater than the amount which could be produced in unmodified host cells of the same type under otherwise identical conditions, or a range between and encompassing any of the foregoing specifically enumerated values.

[0051] In some embodiments, repression or elimination in host cells of any gene (such as the DEGs described herein) upregulated in unmodified host cells of the same type after introduction of exogenous genetic material required to direct biosynthesis of recombinant AAV vectors, and/or at different times during their biosynthesis after introduction of the exogenous genetic material, is effective to increase the proportion of AAV vectors produced in such host cells that possess a key quality attribute compared to the proportion of AAV vectors possessing the same key quality attribute produced from unmodified host cells of the same type in which the gene has not been repressed or eliminated. In some embodiments, the proportion of a particular AAV vector produced in modified host cells possessing a key quality attribute is at least or about 50%, 60%, 70%, 80%, 90%, 100%, 110%, 120%, 130%, 140%, 150%, 175%, 200%, 225%, 250%, 275%, 300%, 325%, 350%, 375%, 400%, 425%, 450%, 475%, or 500% greater than the proportion of

AAV vectors possessing the same key quality attribute which could be produced in unmodified host cells of the same type under otherwise identical conditions, or a range between and encompassing any of the foregoing specifically enumerated values. In some embodiments, key quality attributes include the percentage of capsids which contain full length vector genomes relative to the percentage containing less than full length vector genomes, or which do not contain vector genomes. Other key quality attributes include stability, potency, and any other key quality attribute known to those ordinary skill in the art.

Methods of Modifying Host Cells

[0052] Host cells of the disclosure can be modified to repress, reduce or eliminate expression of one or more desired genes (such as the DEGs identified herein) (i.e., silence such genes) using any method known in the art, or yet to be developed, which is effective repress, reduce or eliminate expression of specific genes in similar types of cells. Known approaches include interventions targeting the gene at it exists in the genome, transcription of a gene into RNA, translation of an mRNA into protein, and protein function. Different approaches may be combined, if so desired, to reduce expression of a particular gene, or of multiple genes in the same cell.

[0053] As generally understood, gene transcription gives rise to messenger RNA (mRNA) transcripts which encode proteins, or non-coding RNA transcripts (which may be processed similarly as mRNAs in terms of capping, splicing, and polyadenylation) which have some other function within a cell (although, as known in the art, some protein-encoding transcripts can also contain non-coding functional RNA regions, such as sequences of miRNAs). For convenience, unless otherwise specified, where the disclosure mentions mRNA, such reference is intended to apply to functional non-coding RNA transcripts of genes as well.

[0054] In some embodiments, the genome of a host cell is permanently modified in a manner which is effective to prevent transcription of a desired gene into RNA. In some embodiments, the genome is modified so as to delete ("knock-out") one or more functional DNA sequences essential to gene function, such as deletion of part or all of a transcription control region required to transcribe the gene into RNA, such as a promoter and/or enhancer, or deletion of one or more splice junctions or exons required for a stable mRNA or protein to be expressed. In other embodiments, the entire gene is deleted from the genome. In other embodiments, the genome is modified so as to insert ("knock-in") a DNA sequence which interferes with the proper function of one or more other DNA sequences essential to the expression of the gene, such as insertion

of a DNA sequence which disrupts a transcription control region, or which prevents correct mRNA splicing, or which introduces a premature stop codon.

[0055] Methods for deleting or inserting a desired sequence from or into a genome, respectively, are known in the art including, for example, gene targeting by homologous recombination, or with gene editing methods relying on expression or introduction into cells of zinc-finger nucleases, transcription activator-like effector nucleases (TALEN), or ribonucleoprotein (RNP) editing complexes, such as CRISPR/Cas9, or variants, such as CRISPR/Cas12a (described further in Zhang, L, et al. *Nat Comms*. 12(1):3908 (2021)). Gene editing technologies are described further in Gaj T, et al., *Trends Biotechnol*. 31(7):397-405 (2013); LaFountaine JS, et al., *Int J Pharm*. 494(1):180–194 (2015); Carroll, D, *Gene Genome Edit* (2021) (doi.org/10.1016/j.ggedit.2021.100002); Liu, G, et al., *Mol Cell* 82:333-347 (2022) (doi.org/10.1016/j.molcel.2021.12.002); Nemudryi, AA, et al., *Acta naturae* 6(3):19-40 (2014); Shalem, O, et al., *SCIENCE* 343:84-87 (2014) (DOI: 10.1126/science.1247005); Becker, S and Boch, J, *Gene Genome Edit* (2021) (doi.org/10.1016/j.ggedit.2021.100007); Bogdanove, AJ and Voytas, DF, *SCIENCE* 333:1843-1846 (2011) (DOI:10.1126/science.1204094); Costa, JR, et al, *Genome Editing Using Engineered Nucleases and Their Use in Genomic Screening* (2017) in *Assay Guidance Manual*, Markossian S, et al., Eds.; Guha, TK and Edgell, DR, *Int J Mol Sci* 18:2565 (2017) (doi:10.3390/ijms18122565); Wang, H, et al., *Nature Biotech* 31(6):530-532 (2013) (doi:10.1038/nbt.2595); Bouabe, H and Okkenhaug, K, *Methods Mol Biol*. 1064:315–336 (2013) (doi:10.1007/978-1-62703-601-6_23); Hall, B, et al., *Curr Protoc Cell Biol* 19.12.1-19.12.17 (2009) (doi:10.1002/0471143030.cb1912s44); Hendrie, PC and Russell, DW, *Mol Ther* 12(1):9-17 (2005) (doi:10.1016/j.ymthe.2005.04.006); Zhang, C, et al., *Nuc Acids Res* 33(3):1-7 (2005) (doi:10.1093/nar/gni014); Turakainen, H, et al., *PLOS One* 4(2):1-12 (2009) (doi:10.1371/journal.pone.0004341); Topaloglu, O, et al., *Nuc Acids Res* 33(18):1-7 (2005) (doi:10.1093/nar/gni160).

[0056] In some embodiments, a gene can be silenced by introducing into a host cell a vector (such as a plasmid or viral vector) for expressing a protein effective to repress transcription of the gene, even though the gene itself remains intact. For example, the vector can express a modified transcription factor capable of specifically binding to the transcription control region of a gene, but which carries a repressor domain (such as a KRAB domain) which actively represses transcription of the gene into RNA. In other embodiments of this nature, a target gene can be silenced using CRISPR interference (CRISPRi), in which the expressed protein is a mutated version

of the Cas9 enzyme, termed dead Cas9 (dCas9), in which the catalytic subunits are mutated to prevent their cleavage of DNA. When expressed or otherwise introduced into a host cell (such as by electroporation or lipofection) and targeted to a particular transcription control region by a suitable guide RNA, the dCas9 protein can bind and passively prevent binding by transcription activator proteins, temporarily blocking transcription from the gene. The dCas9 protein can also be further modified by fusing it with a transcriptional repressor domain (such as a KRAB domain), such that after binding a target gene's transcription control region as directed by a guide RNA, transcription of the gene is actively repressed. As understood in the art, expression of the components required for CRISPRi (mutated Cas enzyme and guide RNA) from a vector can be effected transiently, or stably and therefore permanently. CRISPRi and dCas9 are described further in Qi, LS, et al., *Cell* 152(5):1173–1183 (2013), and Gilbert, LA, et al., *Cell* 154(2):442-451 (2013).

[0057] In some embodiments, a gene can be effectively silenced post-transcriptionally by targeting the RNA transcript from the gene, rather than the gene itself (as it is embodied in the genome). A variety of ways of targeting RNA transcripts from genes (such as mRNAs) have been developed, and the particular choice should not be considered limiting. Thus, for example, in some embodiments, host cells can be treated with an expression repression effective amount of one or more types of DNA or RNA anti-sense oligonucleotides (AON) comprising a nucleotide sequence that is complementary (wholly or in part) to a subsequence present in an RNA transcript expressed from a target gene to be silenced. In sufficient amounts, AONs can bind to the transcript through complementary base-pairing and prevent translation from and/or enhance degradation (for example, by RNase H) of the RNA, thereby reducing or eliminating expression of the gene product. Use of AONs for gene silencing is described further in Crooke, ST, et al., *J Biol Chem* 296:100416 (2021) (doi: 10.1016/j.jbc.2021.100416); Crooke, ST, *Nuc Acid Thera* 27(2):70-77 (2017) (DOI:10.1089/nat.2016.0656); Lennox KA, et al., *J Rare Dis Res Treat* 1(3):66–70 (2016); Walder RY and Walder JA, *Proc Natl Acad Sci USA* 85(14):5011–5015 (1988); Dagle JM, et al., *Nucleic Acids Res.* 18(16):4751–4757 (1990); Braasch DA, et al., *Nucleic Acids Res.* 30(23):5160–5167 (2002); Kurreck J, et al., *Nucleic Acids Res.* 30(9):1911-1918 (2002); Grünweller A, et al., *Nucleic Acids Res.* 31(12):3185-3193 (2003).

[0058] In other embodiments, ribozymes designed to target specific RNA transcripts can be expressed in host cells from a vector (such as a plasmid or viral vector). RNA targeting using ribozymes is described further in James, HA and Gibson, I, *Blood* 91(2):371-382 (1998); Trang, P,

et al., *Cellular Micro* 6(6):499-508 (2004) (doi:10.1111/j.1462-5822.2004.00398.x); Kumar, R, et al., *Gene Ther* 12:1486-1492 (2005); Kharma, N, et al., *Nuc Acids Res* 44(4):1-12 (2015) (doi: 10.1093/nar/gkv1111).

[0059] In other embodiments, aspects of the CRISPR/Cas system can be used to target RNA transcripts, rather than DNA genes. For example, RNA nucleases from the Cas13 family can be expressed or otherwise introduced into host cells and targeted to particular RNA transcripts via suitable guide RNAs. RNA targeting with Cas13 enzymes is described further in Abudayyeh, OO, et al., *Nature* 550(7675):280-284 (2017); Cox, DBT, et al., *Science* 358(6366):1019-1027 (2017); and Konermann, S, et al., *Cell* 173(3):665-676 (2018).

[0060] In other embodiments, the RNA transcript of a gene to be silenced can be targeted through mechanisms of RNA interference (RNAi) by introducing into host cells small interfering RNA (siRNA) molecules, short hairpin RNA (shRNA) molecules, microRNA (miRNA) molecules, or any other RNA molecule or RNA mimetic capable of gene silencing by RNAi.

[0061] In some embodiments, gene expression in host cells can be reduced or eliminated by introducing into such cells one or more types of small interfering RNA (siRNA) molecules. Small interfering RNA are short (for example, 21-26 bases long) double-stranded RNA molecules with 2 nucleotide 3' overhangs at each end of the duplex. siRNAs can be designed so that one of the strands of the siRNA duplex serves as a guide strand capable of binding, through complementary base-pairing, to a specific RNA transcript (for example, mRNA). After being introduced into host cells, guide strands of siRNA are loaded by cellular molecular machinery into RNA-induced silencing complexes (RISC) naturally existing within the cells and act to program RISC to target the RNA transcript, thereby preventing translation into protein, and/or enhance degradation by endogenous ribonucleases. The other strand within the shRNA duplex is called the passenger and is not incorporated into RISC.

[0062] siRNA molecules can be prepared in any way that is known in the art. For example, siRNA can be synthesized chemically and then purified, or can be produced biosynthetically as part of a longer double stranded RNA molecule containing concatenated copies of the siRNA sequence which are then cleaved enzymatically to release multiple siRNA from the dsRNA molecule. When produced via chemical synthesis, siRNA may include modified nucleotides which enhance resistance to ribonucleases which might otherwise quickly destroy the molecules. For purposes of gene silencing, siRNA can be introduced into host cells using any method known in the art. For example, siRNA can be mixed with a polycationic polymer, such as

polyethyleneimine (PEI), and then transfected into host cells. Alternatively, long dsRNA molecules containing multiple copies of a siRNA sequence can also be transfected into host cells, and after entering the cytoplasm, can be processed by the cytoplasmic ribonuclease Dicer, to produce multiple copies of siRNA with the characteristic 3' overhangs.

[0063] As noted above, in other embodiments, gene silencing via RNAi can be effected with shRNA and miRNA, which share certain features. MicroRNAs (miRNA or miR) are small (for example 18-24 nucleotide long) double stranded RNA molecules, at least one strand of which contains complementary sequence to that occurring in mRNA transcripts of a variety of genes. miRNA evolved in animals and plants as a post-transcriptional mechanism for regulating gene expression. As they exist in nature, miRNAs are expressed from a host gene, usually by RNA pol II, into a long primary miRNA transcript (pri-miRNA) which may be spliced and processed similarly as mRNA. The sequences of the mature miRNA strands are found in the pri-miRNA as adjacent regions of (often imperfectly) self-complementary sequence, separated by a shorter region of non-complementary nucleotides. Together, these sequences form a hairpin of variable length (for example, 60-90 nucleotides long), with a stem and terminal loop. In the nucleus, the hairpin is recognized by the Microprocessor complex, containing the RNase III enzyme Drosha, which cleaves the pri-miRNA near the base of the stem, releasing the hairpin as a precursor miRNA (pre-miRNA) with a 2 nucleotide 3' overhang. pre-miRNAs are then transported from the nucleus into the cytoplasm where they are further processed by another RNase III enzyme, Dicer, which cleaves the hairpin in two places, releasing the terminal loop sequence, and producing the mature miRNA duplex, including a second 2 nucleotide 3' overhang. One of the strands is then loaded as the guide into RISC, which can then recognize specific mRNAs through complementary base pairing between a subsequence within the guide and complementary target sequence in the mRNA, preventing its translation into protein, and/or enhancing its degradation by endogenous ribonucleases.

[0064] Short hairpin (shRNA) is similar in structure to pre-miRNA, and can also function to silence genes by RNAi, but do not exist naturally. Like pre-miRNA, shRNA molecules possess a stem and terminal loop formed when adjacent self-complementary nucleotide sequences (for example, 19-22 nucleotides long) separated by a shorter region of non-complementary nucleotides (for example, 4-11 nucleotides long) anneal to one another. Also, like pre-miRNA, the hairpin of shRNA can be recognized by Dicer and cleaved to remove the terminal loop, producing a short RNA duplex functionally equivalent to a siRNA molecule, in which one of the

strands serves as guide for programming RISC to target a specific RNA transcript, preventing its translation into protein, and/or enhancing its degradation by endogenous ribonucleases. A characteristic difference between shRNA and miRNA, however, is that while the stem regions of pre-miRNA often contain small regions of non-complementary sequence (bulges), the sequences forming the stem in shRNA hairpins are typically perfectly complementary.

[0065] miRNA or shRNA containing a guide strand sequence designed to target a specific mRNA for repression via RNAi can be produced in host cells using any method known in the art. In some embodiments, an expression vector, such as a plasmid or viral vector, is constructed containing the sequence for an RNA guide strand with the additional sequences required to form miRNA (from a pre-miRNA) or shRNA in the cell. For example, the sequences required to form the stem and loop of a shRNA can be cloned into an expression vector and placed under the control of an RNA pol III promoter, such as a U6 promoter or H1 promoter, and terminated with a short polythymidine tract. In other embodiments, an expression vector containing a miRNA scaffold derived from a naturally occurring pri-mRNA controlled by an RNA pol II promoter and polyA signal sequence can be provided, into which is cloned sequence required to form a mature miRNA duplex (i.e., guide and passenger strands). Such vectors for expressing shRNA or miRNA can be introduced into host cells, transiently or stably, such as by transfection of a plasmid or transduction by a viral vector, after which the RNA transcripts containing the shRNA or pre-miRNA sequences are transcribed, processed by Drosha and/or Dicer, producing siRNA from the shRNA or mature miRNA duplex from the pre-miRNA, and from them guide strands for loading into RISC to target a desired mRNA by RNAi. In some embodiments, the expression vectors for producing shRNA or miRNA in cells can be constructed so that expression of the shRNA or miRNA is constitutive, or inducible in response to an exogenously provided drug. Expression vectors for producing shRNA or miRNA in cells are well known in the art, including vectors available from commercial sources, including the BLOCK-iT shRNA and miRNA vectors sold by ThermoFisher Scientific Inc., which include plasmid, lentiviral, and adenoviral vectors for expressing shRNA and miRNA in cells to silence specific genes by RNAi.

[0066] Host cells modified to repress, reduce or eliminate expression of one or more desired genes, such as the DEGs identified herein, may then be used in the production of AAV vectors using any method known in the art or yet to be discovered.

Adeno-Associated Virus (AAV)

[0067] Modified host cells of the disclosure may be used in the production of vectors created from recombinantly modified adeno-associated virus (AAV). AAV vectors are capable of delivering genes, which may be under the control of transcriptional and other regulatory elements, into targeted cells via transduction. By supplying a functional copy of a gene to a target cell in which the endogenous version is missing or mutated, AAV vectors are useful in gene therapy for a variety of diseases and disorders.

[0068] AAV is a small non-enveloped, apparently non-pathogenic parvovirus that depends on certain other viruses to supply gene products, known as helper factors, essential to its own replication, a quirk of biology that has made AAV well-suited to serve as a recombinant vector. For example, adenovirus (AdV) can serve as a helper virus by providing certain adenoviral factors, such as the E1A, E1B55K, E2A, and E4ORF6 proteins, and the VA RNA, in cells co-infected by adenovirus and AAV. Other helper viruses, such as herpes simplex virus, have been identified as well. The dependence of AAV replication on accessory factors supplied by other viruses led AAV to be characterized as a type of dependovirus. AAV virions have two major structural features, called the capsid and genome, respectively. The capsid is an icosahedral protein shell that encloses and protects (encapsidates) the viral genome, which contains genes and other sequences required for viral replication in infected cells.

[0069] The AAV genome is a single strand of DNA containing two genes called *rep* and *cap*. In AAV2, a naturally occurring AAV that infects humans and is particularly well characterized biologically, the genome is about 4.7 kilobases long. By virtue of alternative splicing of the transcripts from two promoters, the *rep* gene is capable of producing four related multifunctional proteins called Rep (called Rep 78, Rep 68, Rep 52 and Rep 40 in AAV2, named according to their apparent molecular weights) which are involved in viral gene expression, and replication and packaging of genomes. Alternative splicing of the transcript from the single promoter controlling the single *cap* gene produces three related structural proteins, VP1, VP2, and VP3, a total of 60 of which self-assemble to form the virus's icosahedral capsid in a ratio of approximately 1:1:10, respectively. VP1 is longest of the three VP proteins and contains amino acids in its amino terminal region that are absent from VP2, which in turn is longer than VP3 and contains amino acids in its amino terminal region that are absent from VP3. In addition to containing the genome, capsid proteins mediate specific binding interactions with receptors on the surface of target cells, based on which AAV can be restricted in their ability to infect certain animal species, and even tissues within the same type of animal, a phenomenon called tropism.

For example, one type of AAV may preferentially infect liver cells (e.g., hepatocytes) as compared to muscle or neuronal cells.

[0070] In addition to the *rep* and *cap* genes, intact AAV genomes have a relatively short (145 nucleotides in AAV2) sequence element positioned at each of their 5' and 3' ends called an inverted terminal repeat (ITR). ITRs contain nested palindromic sequences that can self-anneal through Watson-Crick base pairing to form a T-shaped, or hairpin, secondary structure. In AAV2, ITRs have been demonstrated to have important functions required for the viral life cycle, including converting the single stranded DNA genome into double stranded form required for gene expression, as well as packaging by Rep proteins of single stranded AAV genomes into capsid assemblies.

[0071] Numerous naturally occurring types of AAV have been discovered in different species. At one time, only six types of primate AAV had been isolated from biological samples (AAV1, AAV2, AAV3, AAV4, AAV5, and AAV6), the first five of which were sufficiently distinct structurally to be classified as different serotypes based on antibody cross reactivity experiments. Later, two novel AAVs, called AAV7 and AAV8 were discovered by PCR amplification of DNA from rhesus monkeys using primers targeting highly conserved regions in the *cap* genes of the previously discovered AAVs. Gao, G, et al., *Novel adeno-associated viruses from rhesus monkeys as vectors for human gene therapy*, *PNAS (USA)* 99(18):11854–11859 (2002). Subsequently, a similar approach was used to clone numerous novel AAVs from human and non-human primate tissues, vastly expanding the scope of known AAV capsid protein sequences. Gao, G, et al., *Clades of Adeno-Associated Viruses Are Widely Disseminated in Human Tissues*, *J Virol.* 78(12):6381–6388 (2004). Many AAV capsid protein sequences are highly similar to each other, or previously identified AAVs, and while often referred to as distinct AAV “serotypes,” not all such capsids would necessarily be expected to be immunologically distinguishable if tested by antibody cross reactivity. AAVs, or AAV capsids, which are not serologically distinguishable from a defined serotype but contain capsid proteins with a different amino acid sequence are better termed variants of the known serotype. Numerous capsids made from naturally and non-naturally occurring capsid proteins have found utility in creating AAV gene therapy vectors.

[0072] As established studying AAV2, after binding its cognate receptor on a cell surface, the AAV viral particle enters the cell via endocytosis. Upon reaching the low pH of lysosomes, capsid proteins undergo a conformational change which allows the capsid to escape into the cytosol and then be transported into the nucleus. Once there, the capsid disassembles, releasing the

genome which is acted on by cellular DNA polymerases to synthesize the second DNA strand starting at the ITR at the 3' end, which functions as a primer after self-annealing. Expression of the *rep* and *cap* genes can then commence, followed by formation and release from the cell of new viral particles.

AAV Vectors

[0073] The relative simplicity of AAV structure and life cycle, and the fact that it is not known to be pathogenic in humans, inspired researchers to engineer AAV and investigate if it could be converted from a virus to a recombinant vector for gene therapy. Briefly, this was done by cloning the entire genome of AAV2, including both ITRs, into a plasmid, removing the *rep* and *cap* genes into a separate plasmid, and replacing them with a heterologous gene expression cassette comprising a promoter controlling a protein encoding transgene. Thus, the only viral genomic sequences retained in the vector genome were the ITRs, due to their critical function in packaging and gene expression, and without which AAV vectors could not be produced or function to express the transgene after transduction of target cells. Finally, to avoid the need for co-infection with a helper virus, necessary for replication of AAV virions, genes for the so-called helper factors (such as, in the case of AdV, the E1A, E1B55K, E2A, E4ORF6, and VA RNA helper factors) were cloned into a third plasmid.

[0074] When the three plasmids (which are sometimes called the transgene, *rep/cap*, and helper plasmids) were transfected together into mammalian host cells, Rep and capsid proteins, and the helper virus factors were expressed from their respective plasmids. These gene products then functioned in the host cells to replicate the vector genome into single stranded DNA from the plasmid on which its sequence resided, assemble capsids, and package the single stranded genomes into the capsids, forming vectors. The vectors could then be purified from the host cells. Because the *rep* and *cap* genes existed in *trans* on a different plasmid, outside their usual context flanked by ITRs, they were not packaged into the vectors. Consequently, while AAV vectors produced this way were able to bind to and convey the expression cassette within their genomes into target cells, they are unable to replicate and create new vector particles.

[0075] If vectors function as intended, after transduction, the expression cassette will be transcriptionally active and produce the gene product encoded by the transgene in the target cell. AAV vectors are highly versatile because vector genomes comprising a variety of transgenes under the control of different functional sequences and regulatory elements in various configurations can be designed and paired with a variety of naturally occurring and engineered

capsids, with different tropisms and other properties. Many types of gene products can therefore be produced, with a degree of control over the types of cells that are transduced and amount of gene product that is made.

[0076] AAV vectors comprise a vector genome encapsidated by an AAV capsid. In some embodiments, the AAV vector genome is a molecule of single-stranded DNA (ssDNA) comprising at least one AAV inverted terminal repeat (ITR) and a heterologous nucleotide sequence with a desired function when present or expressed in a transduced target cell. In some embodiments, the heterologous nucleotide sequence originates from a different type of virus, or an entirely different type of organism, such as an animal, plant, protist, fungus, bacteria, archaea, or other type of organism. In some embodiments, the heterologous nucleotide sequence replaces some or all of the native AAV *rep* and/or *cap* genes so that the vector is incapable of expressing functional Rep or VP proteins in transduced target cells. In some embodiments, the entire sequence of the vector genome consists of heterologous nucleotide sequences except for AAV inverted terminal repeat sequences positioned at the ends of the genome.

Expression Cassettes and Transgenes

[0077] In some embodiments, the heterologous nucleotide sequence comprises or consists of an expression cassette comprising a transgene operably linked with a promoter and optionally one or more enhancers, serving to control transcription initiation of the transgene from DNA into RNA, as well as a transcription termination element, such as a polyadenylation signal sequence, serving to terminate transcription of the transgene into RNA. AAV vectors can comprise more than one transgene, either as part of one transcriptional unit, or each being part of its own transcriptional unit. As described in later sections, expression cassettes can further comprise additional sequence elements designed to influence transcription, transcript stability, translation, or other functions.

[0078] As AAV vectors are typically designed, the structure of the expression cassette, and the genome overall, is limited by the packaging capacity of the capsid, so that the length of the transgene when combined with all other elements in the genome required for vector function, such as the transcriptional control elements and ITRs, does not exceed approximately 5 kilobases in the case of AAV2, although other types of capsids may have greater or smaller packaging limits. Within the size constraints, however, there is great flexibility in choice of transgenes, ITRs, and the other elements required for the vector to function for its intended purpose.

[0079] For purposes of gene therapy, the transgene can be any gene, the product of which would be understood to prevent or treat, although not necessarily cure, any disease, disorder or condition of a subject in need of prevention or treatment. In some embodiments, gene therapy is intended to prevent or treat a disease, disorder or condition characterized by an abnormally low amount or even absence of a product produced by a naturally occurring gene in a subject, such as might occur due to a loss of function mutation. Relating to such embodiments, the transgene can be one intended to compensate for the subject's defective gene by providing to at least some of the subject's cells the same or similar gene product when expressed. A non-limiting example would be a vector designed to express a functional version of clotting factor IX for use in gene therapy of hemophilia B, which is caused by a loss of function mutation in the native factor IX gene. In other embodiments, however, the transgene could be one intended to counteract the effects of a deleterious gain of function mutation in targeted cells. In some embodiments, the transgene can encode a transcriptional activator to increase the activity of an endogenous gene which produces a desirable gene product, or conversely a transcriptional repressor to decrease the activity of an endogenous gene which produces a deleterious gene product.

[0080] In some embodiments, the transgene encodes a polypeptide, examples of which include, but are not limited to, a mini- or micro-dystrophin, a clotting factor (or variants thereof), such as Factor IX or Factor VIII, α 1-antitrypsin, aromatic L-amino acid decarboxylase, arylsulfatase B, cyclic nucleotide-gated channel- β 3, dysferlin, glucose-6-phosphatase catalytic subunit, α -glucosidase, gigaxonin, glial cell line-derived neurotrophic factor, low-density lipoprotein receptor, N- α -acetylglucosaminidase, NADH-ubiquinone oxidoreductase chain 4, neurotrophin 3, ornithine transcarbamylase, RAB escort protein 1, retinaldehyde-binding protein 1, retinal pigment epithelium-specific 65 kDa protein, retinitis pigmentosa GTPase regulator, retinoschisin 1, N-sulfoglucosamine sulfohydrolase, survival of motor neuron protein, UDP glucuronosyltransferase family 1 member A1, and vascular endothelial growth factor, as well as transgenes encoding an antibody, or antigen binding fragment thereof, including modified or engineered antibodies or antigen binding fragments thereof, as well as antigen-binding molecules derived from antibodies, such as scFv, scFv-Fc, minibodies, diabodies, or nanobodies, others being possible. Additional transgenes encoding polypeptides for use in AAV vectors of the disclosure will be familiar to those of ordinary skill in the art. Protein encoding sequences in a transgene can be codon-optimized, and translation start sites (e.g., Kozak sequence) can be modified to increase or decrease their tendency to initiate translation. In some embodiments, a

transgene encoding amino acid sequence can contain one or more open reading frames, and/or contain one or more splice donor and acceptor site pairs to permit alternative splicing of different messages and polypeptide sequences from such messages. Transgenes encoding proteins further comprise one or more stop codons to end translation of the polypeptide chain.

[0081] In some embodiments, the transgene codes for an RNA molecule with a function distinct from encoding protein, such as a regulatory non-coding RNA molecule (e.g., micro-RNA, small interfering RNA, piwi-acting RNA, enhancer RNA, long non-coding RNA, etc.)

[0082] In some embodiments, a vector genome can be designed for purposes of editing or otherwise modifying the genome of a target cell. For example, a vector genome can include an expression cassette or transgene flanked by homology arms intended to promote homologous recombination between the vector genome and the target cell genome. In another example, a vector genome can be designed to carry out CRISPR gene editing by expressing a guide RNA (gRNA) and/or an endonuclease, such as Cas9 or related endonucleases, such as SaCas9, capable of binding the gRNA and cleaving a DNA sequence targeted by the gRNA.

[0083] Selection of any suitable transgene is within the knowledge of the person of ordinary skill in the art based on the intended purpose of gene therapy with the AAV vector, such as prophylaxis and/or therapy of a particular disease, disorder, or condition, existing in a subject for which prevention or treatment is desired.

Transcription Control Regions

[0084] AAV vectors of the disclosure intended to express a transgene in and/or from transduced cells can further comprise, as part of the vector genome, one or more transcription control regions in operable linkage with the transgene. As discussed further below, different types of transcription control regions are known in the art which can be used to control initiation of transcription of the transgene into RNA. As used herein, the term “operable linkage,” and variations such as “operative linkage,” “operably linked,” and “operatively linked,” refers to a functional relationship between the transcription control region and transgene, so that the control region can affect transcription of the transgene (whether positively or negatively), without specifying any particular spatial or structural relationship between them. Thus, for example, a transcription control region could be operably linked with a transgene even though it is positioned 5' or 3' of the transgene, and/or positioned immediately adjacent to or distal from the transgene. Transcription control regions can be constitutively active, active in specific cells or tissues, inducibly active in response to some environmental stimulus, be derived from a

naturally occurring gene (of any suitable species) and can be modified to improve or change its function, or even be entirely synthetic.

[0085] In some embodiments, a transcription control region comprises a promoter region, which comprises the minimal DNA sequence required to initiate transcription by the transcription apparatus in transduced cells (e.g., a TATA box or initiator sequence), often as well as one or more additional proximal elements that act singly or cooperatively to increase the rate of transcription from the basal promoter. Depending on its sequence, a promoter can initiate transcription by RNA polymerase I, II, or III, but promoters from protein encoding genes, which are usually transcribed by RNA pol II, are often used in AAV vectors intended to express a polypeptide product from a transgene in transduced cells.

[0086] In other embodiments, a transcription control region comprises or further comprises at least one enhancer region, which functions to further increase the rate of gene transcription beyond what the basal promoter alone can sustain. In their natural context, enhancers are often positioned distal from the promoter of the gene on which they act, sometimes tens to hundreds or thousands of base pairs upstream (i.e., 5'), but enhancers can also occur elsewhere, such as in introns or downstream (i.e., 3') of the gene on which they act. While promoter regions may contain proximal enhancer elements (subsequences which, if removed, would reduce transcription from the basal promoter), enhancers do not usually contain sequences that can function as a basal promoter. Although in nature enhancer regions are often positioned distally to the promoter of the gene on which they act, enhancer regions, or enhancer elements from within larger enhancer regions (such elements often corresponding to DNA binding sites for transcription factors), can sometimes retain at least some of their transcription enhancing function when removed from their natural context and repositioned much closer to a promoter, whether from the same or even a different gene.

[0087] Enhancer and promoter regions of genes described in the scientific literature may be too large to be accommodated by the packaging capacity of AAV capsids when combined with a transgene and other genomic elements required for vector function. Accordingly, in some embodiments, functional subsequences within longer enhancer or promoter regions can be identified using methods familiar to those of ordinary skill, and the shorter functional subsequences incorporated into transcription control regions for use in the vectors of the disclosure. In this manner, the size of transcription control regions can be reduced while maintaining their desired function. Using this approach functional elements from naturally

occurring enhancers or promoters can be combined in novel ways, such as by modifying their number, spacing and/or arrangement, to create hybrid or synthetic enhancers and or promoters with improved properties. In some embodiments, the enhancer and promoter can each be derived from the same, naturally occurring gene, whereas in other embodiments, the enhancer and promoter can originate from entirely different genes, including genes of different species.

[0088] In some embodiments, from the perspective of a coding strand (i.e., plus strand) single stranded DNA AAV vector genome, a promoter sequence is positioned 5' of a downstream sequence to be transcribed into RNA, such as a transgene encoding a protein. In some embodiments, an enhancer element or region, if present, can be positioned 5' of the promoter sequence, or instead be positioned elsewhere in the genome, such as in a 5' or 3' untranslated region (UTR) adjacent the transgene, in an intron, 3' of a transcription termination signal sequence, or elsewhere. In some embodiments, a vector genome can comprise more than one enhancer region (of same or different types), which can be positioned adjacent to each other, or spaced apart, and/or separated by other functional elements within the genome. In some embodiments, the same enhancer element or region is provided in a tandemly arranged array of repeating units, such as 2, 3, 4, or more.

[0089] In some embodiments, transcription control regions for use in the AAV vectors of the disclosure are non-tissue specific, meaning that they are constitutively active in many different cell types, although not necessarily all. According to some embodiments, non-tissue specific transcription control regions include promoters (which may include enhancer elements proximal to a basal promoter) derived from certain viruses, such as the human cytomegalovirus major immediate early gene (CMV-IE) (*Boshart, M, et al., Cell, 41:521-30 (1985); Yew, NL, et al., Hum Gene Ther, 8:575-84 (1997)*); simian virus 40 (SV40); as well as the retroviral long terminal repeat (LTR) promoters from Rous sarcoma virus (RSV) and Moloney murine leukemia virus (MoMLV). In other embodiments, non-tissue specific transcription control regions include promoters (which may include proximal enhancer elements) can be derived from genes active in many different cell types, including from different types of animals, such as the human polypeptide chain elongation factor (EF1 α) gene; the phosphoglycerate kinase (PGK) gene; the ubiquitin C (UbiC) gene; the chicken beta-actin (CBA) gene; the U1a1 or U1b2 small nuclear RNA promoters (*Bartlett, JS, et al., Proc Natl Acad Sci USA 93: 8852-7 (1996); Wu, Z, et al., Mol Ther, 16(2):280-9 (2008)*); the histone H2 or histone H3 promoters (*Hurt, MM, et al., Mol Cell Biol 11:2929-36 (1991); Wu, Z, et al., Mol Ther, 16(2):280-9 (2008)*).

[0090] Likewise, enhancer regions can be derived from viruses and genes active in different cell types from different types of animals. As noted, in some embodiments, a promoter and enhancer derived from the same gene can be combined to create a transcription control region for use in the vectors of the disclosure, but enhancers and promoters from different genes can be combined to create hybrid transcription control regions. A commonly used example is the 1.6 kb hybrid enh/pro region called CAG (or CAGGS) comprising the CMV immediate-early enhancer, the chicken beta actin (CBA) gene promoter and the CBA intron/exon 1 (*Niwa, H, et al., Gene, 108:193-9 (1991); Ikawa, M, et al., Dev Growth Differ, 37:455-9 (1995)*), and later modifications that reduced its size, including one in which the CBA intron was replaced with a smaller simian virus 40 (SV40) intron (*Wang, Z, et al., Gene Ther, 10:2105-11 (2003)*), and another called CBA hybrid intron (CBh) which replaced the SV40 intron with a hybrid intron composed of a 5' donor splice site from the CBA 5' UTR and a 3' acceptor splice site from the MVM intron (*Gray, SJ, et al., Hum Gene Ther, 22:1143-53 (2011)*).

[0091] In other embodiments, transcription control regions for use in the AAV vectors of the disclosure can be tissue or cell-type specific, meaning that they are more or most active in directing expression of a transgene in tissues or cells of a particular type, compared to other types of tissues or cells. Non-limiting examples include transcription control units that are specifically and more highly active in tissues such as liver (for example, in hepatocytes), brain (for example, in neurons), or skeletal muscle (for example, in myocytes), as compared to other types of tissue or cell existing within a subject organism. Without wishing to be bound by theory, one mechanism by which tissue or cell specificity may occur is the presence in an enhancer and/or promoter of one or more specific binding sites for DNA binding transcriptional activator proteins that are preferentially expressed in certain types of cells as compared to others. Use of a tissue or cell specific transcription control region can be advantageous, in some embodiments, by reducing or even preventing undesired transgene expression in cells of other types that may be transduced by a vector, which can beneficially reduce the risk of off-target effects. Tissue or cell-specific transcription control regions can be derived from genes that are naturally expressed at high levels in tissues or cells of a desired type. For example, in certain non-limiting embodiments, should it be desired to express a particular transgene in liver cells, then a liver tissue specific transcription control region could be obtained from a gene which is expressed at high levels in liver cells but no other types of cells, such as the genes for albumin, transthyretin (prealbumin), alpha 1-antitrypsin, prothrombin, or others.

[0092] Certain tissue or cell-specific genes expressed at high level have both enhancers and promoters, which may be included in transcription control regions. In some embodiments an enhancer and a promoter derived from the same gene may be combined in a tissue or cell-specific transcription control region, whereas in other embodiments, an enhancer from one gene and a promoter from a different gene may be combined in a hybrid tissue or cell-specific transcription control region. When from the same gene, a transcription control region sequence comprising one or more enhancers and promoter may be copied as it exists in the native gene from which it is derived, or engineered to reduce its length, such as by deleting non-transcriptionally active sequences separating the one or more enhancers and the promoter. In some embodiments, enhancers and promoters for use in tissue or cell-specific transcription control regions may derive from genes of different species. In yet other embodiments, the sequence of an enhancer and/or a promoter in a transcription control region can be modified relative to its original sequence by changing, adding or removing nucleotides to improve its function, such as increasing transcription activator binding, reducing transcription repressor binding, or reducing the size of the transcription control region. In some embodiments, it is the enhancer that provides for tissue or cell-specific expression and the promoter is not itself tissue or cell-specific, whereas in other embodiments, it is the promoter that provides for tissue or cell-specific expression and the enhancer, if present, is not itself tissue or cell-specific but is capable of increasing the rate of transcription from the tissue or cell-specific promoter. For example, a strong viral enhancer, such as the human CMV major immediate early gene enhancer, could be paired with a tissue or cell-specific promoter, or a strong tissue or cell-specific enhancer could be paired with a strong viral promoter, such as the SV40 early promoter. In other embodiments, both the enhancer and promoter each are tissue or cell-specific. In some embodiments, different enhancer regions can be combined to form chimeric enhancer regions that are used in transcription control regions in AAV vectors of the disclosure.

Transcription Termination Signal Sequences

[0093] In some embodiments, AAV vectors of the disclosure comprise a vector genome comprising a transcription terminator sequence positioned, from the perspective of a coding (plus) strand single stranded DNA vector genome, 3' of the transgene. In some embodiments, another sequence, such as 3' untranslated region (UTR) sequence, can be positioned between the transgene sequence and the transcription terminator sequence. General information about transcription termination can be found, e.g., in *Proudfoot, NJ, Genes Devel, 25:1770-82 (2011)*;

Kuehner, JN, et al., Nat Rev Mol Cell Biol, 12:283-94 (2011); Porrua, O and Libri, D, Nat Rev Mol Cell Biol, 16:190–202 (2015).

[0094] In some embodiments, particularly when the transgene contains protein encoding sequence (as opposed to the sequence of an RNA with some function other than encoding protein), the transcription terminator sequence can be a polyadenylation signal sequence (abbreviated variously as “polyA,” “pA,” “poly(A)” or “p(A)”). In some embodiments, pA signal sequences can be derived from naturally occurring genes and used in vectors, whereas in other embodiments, pA signals can be modified, such as by shortening them compared to their natural counterparts or altering their sequence to make them more efficient at transcription termination. In other embodiments, pA signals can be hybrid sequences, combining pA sequences from different genes, or synthetic.

[0095] Non-limiting examples of pA signals that may be used in the vectors of the disclosure include the pA signal from the bovine growth hormone gene (bGH pA); human, mouse or rabbit beta-globin gene; SV40 late gene; sNRP1; spA; herpes simplex virus thymidine kinase gene (HSV TK); or adenovirus type 5 L3 polyadenylation site, with many others being possible. In other embodiments, transcription terminators for use in vectors of the disclosure include those that terminate RNA transcripts without directing polyadenylation, such as the histone H4 gene mRNA 3' end processing signal (*Whitelaw, E, et al., Nucleic Acids Res, 14:7059-70 (1986)*).

Other Vector Genomic Elements

[0096] In addition to transcription control regions and transcription termination signal, other sequences, including cis-regulatory elements, can be included in genomes of AAV vectors of the disclosure to improve, control or modulate transgene expression and/or translation in transduced cells, or confer other functions to the vectors. Such elements include, without limitation, untranslated regions from the 5' and/or 3' ends of genes, non-coding exons, introns, splice donor and acceptor sites, lox sites, internal ribosome entry sites (IRES), sequence encoding 2A peptides, elements that stabilize RNA transcripts, binding sites for regulatory miRNAs, micro RNA (miRNA) sequences, elements that enhance nuclear export of mRNAs, including viral post-transcriptional regulatory elements, such as the woodchuck hepatitis virus post-transcriptional regulatory element (WPRE), as well as any other element demonstrated empirically to improve transgene expression, even if the mechanism may be uncertain. In other embodiments, vector genomes can include so-called stuffer or filler sequences, which are intended only to increase the overall length of the vector genome to a desired size, for example, to achieve a length close

to but still under the packaging capacity of a particular capsid, and thereby reduce the likelihood of adventitiously packaging truncated vector genomes or non-vector DNA into capsids.

[0097] Introns can, in some embodiments, be included in vector genomes to increase transgene expression and/or transcript stability. In some embodiments, a protein encoding transgene is provided in which the sequence of exons and intron(s) is the same as in the naturally occurring gene. In a gene possessing multiple exons and introns, however, one or more of the introns can be removed so as to minimize the overall length to facilitate the inclusion of other elements while not exceeding the capsid packaging capacity. In other embodiments, however, an intron can be provided from an entirely different gene than the gene providing the coding sequence for the vector transgene. Whether the intron is from the same or a different gene as the transgene, the intron can be modified from its original sequence, for example by changing certain nucleotides, or removing internal sequences to reduce its overall length while maintaining the splice donor and acceptor sequence motifs required for efficient splicing to occur, or other intronic cis elements important for function (for example, enhancers that may reside in the original unmodified intron sequence). Introns can also be hybrid, where the splice donor portion of the intron from one gene is paired with the splice acceptor portion of the intron from a different gene, or synthetic, with sequence that does not correspond to any known gene's intron. Introns, in some embodiments, can be positioned within, and therefore interrupt, the coding sequence of a transgene (and be provided with the donor and acceptor sites necessary for efficient splicing to occur), whereas in other embodiments an intron is present, but does not interrupt the protein coding sequence, and is instead positioned either 5' or 3' of the coding sequence. Where an intron does not interrupt coding sequence, it may be provided with some exonic sequence carried over from its original genetic context, so long as the exonic sequence does not contain a cryptic translation start signal. In some embodiments, an intron can be positioned 3' of a promoter (from the perspective of plus strand ssDNA vector genome) and 5' of the coding sequence. In other embodiments, an intron can be positioned distally from coding sequence in a vector genome, either upstream or downstream.

[0098] Non-limiting examples of introns that may be used in the AAV vectors of the disclosure include the small intron from the minute virus of mice (MVM) (*Haut, DD and Pintel, DJ, J Virol, 72(3):1834-43 (1998); Haut, DD and Pintel, DJ, Virology, 258:84-94 (1999)*); internally deleted intron 1 from human clotting factor IX (FIXm1 and FIXm2) (*Kurachi, S, et al., J Biol Chem, 270(10):5276-81 (1995)*); chimeric beta globin splice donor and immunoglobulin heavy chain

splice acceptor intron (GenBank U47120.2 nucleotides 890-1022); intron 1 from the mouse alpha globin gene; and the SV40 small t antigen intron which can comprise or consist of base pairs 4644 to 4552 of GenBank record J02400.1, and which can be modified at positions 4582 (g to c), 4580 (g to c), 4578 (a to c), and 4561 (a to t), as reported in *Nathwani, AC, et al., Blood, 107(7):2653-61 (2006)*.

[0099] In some embodiments, post-transcriptional regulatory elements (PRE) can be included in vector genomes to increase transgene expression. Examples of PRE include the woodchuck hepatitis virus post-transcriptional regulatory element (WPRE), the hepatitis B virus post-transcriptional regulatory element (HPRE), and modifications thereof. *Donello, JE, et al., J Virol, 72(6):5085-92 (1998)*; *Loeb, JE, et al., Hum Gene Ther, 10:2295-2305 (1999)*; *Zanta-Boussif, MA, et al., Gene Ther, 16:605-19 (2009)*; *Patricio, MI, et al., Mol Ther Nucleic Acids, 6:198-208 (2017)*; *US Pub. Pat. Appl. 2018-0353620 A1*. In some embodiments, from the perspective of a coding (plus) strand single stranded DNA vector genome, at least one WPRE sequence can be positioned downstream of a transgene (thus, 3' of the stop codon for a transgene encoding a polypeptide) and upstream of the poly(A) signal sequence. In some embodiments, a plurality of PRE can be included, such as 2, 3, or more PRE, of the same or different type, which can be arranged in tandem. In some embodiments,

AAV Inverted Terminal Repeats (ITRs)

[00100] Positioned at the termini of the adeno-associated virus genome are unique nucleotide sequences called inverted terminal repeats (abbreviated, "ITR") which function as origins of viral DNA replication and as priming sites to support the conversion, in infected cells, of the single-stranded (ssDNA) genome into a double-stranded form (dsDNA) competent to support transcription of the *rep* and *cap* protein-encoding genes, also contained in the virus genome. The ITRs also function in packaging of replicated ssDNA genomes into AAV capsids. AAV ITRs contain multipalindromic sequences that fold back on themselves via intrastrand complementary base pairing to form dsDNA T-shaped hairpin secondary structures. To produce AAV vectors, the ITRs are the only AAV viral DNA sequences required to be retained in the vector genome because of their crucial roles in DNA replication and packaging during vector biosynthesis and assembly, and conversion of ssDNA genomes to dsDNA form competent to support transgene transcription after transduction. By contrast, the *rep* and *cap* genes and those encoding the viral helper functions, which are also needed to produce vectors, can be provided in *trans* in a variety of ways known in the art.

[00101] As explained further below, genomes of AAV vectors of the disclosure can include one or more AAV ITRs, such as one, two, three ITRs, or more if desired, which function similarly as they do in unmodified virus. Unless stated otherwise, use of the term “inverted terminal repeat” or “ITR” herein includes intact full-length ITRs, as well as ITRs with modified sequences (such as truncations, internal deletions (such as of a trs or D sequence), additions, and substitutions of one or more nucleotides) that retain one or more of the functions attributable to ITRs (even if less efficiently compared to an intact ITR of the same type), including but not limited to rescue of vector genome from recombinant DNA (such as a plasmid), vector genome replication, and/or packaging of vector genome into assembled AAV capsids.

[00102] As they exist in packaged vector genomes, an ITR may be positioned at each of the 5' and/or 3' ends of the ssDNA genome. The 5' ITR can also be referred to as the “left” ITR, and the 3' ITR can also be referred to as the “right” ITR. In the context of a DNA plasmid, however, such as might be used in vector production, the vector genome sequence will exist in complementary double-stranded form, such that there will be two sets each of 5' ITRs and 3' ITRs. To avoid ambiguity therefore, it may be specified on which strand an ITR exists so as to distinguish among them, for example, the 5' or 3' ITR of the plus or sense strand, or the 5' or 3' ITR of the minus or antisense strand, where the plus/sense or minus/antisense strand is in reference to the nucleotide sequence of the transgene.

[00103] As is known in the art, when an ITR renatures to form the hairpin secondary structure, it can fold into one of two configurations, called flip and flop, in which the sequential order of the internal palindromic sequences differs, a phenomenon which is discussed in more detail in *Lusby, E, et al., J Virol, 34:402-9 (1980); Srivastava, A, et al., J Virol, 45(2):555-64 (1983); Samulski, RJ, et al., Cell, 33:135-143 (1983)*. Thus, in some embodiments, the ITRs of genomes of AAV vectors of the disclosure can be in either the flip or flop configuration. Thus, for example, in a genome with two terminal ITRs, one each at the 5' and 3' ends, the 5' and 3' ITRs may both be flip, or both be flop, or the 5' ITR may be flip and the 3' ITR flop, or the 5' ITR may be flop and the 3' ITR flip. When combined with the observation that ssDNA genomes can occur as positive or negative strands, a sample of AAV vectors can comprise eight possible configurations in similar or potentially different proportions. Unless otherwise specified, genomes of AAV vectors of the disclosure are not limited to any of these particular configurations. Rather, any or all could be packaged by such vectors.

[00104] As known in the art, in addition to the palindromic sequences, AAV ITRs contain a so-called D sequence toward the non-terminal end of the ITR which does not have a complementary sequence within the ITR but does have a complementary sequence in the D region of the ITR at the opposite end of the genome (thus, D and D'). As is also known in the art, replication of ssDNA vector genomes ordinarily produces genomes with both positive (plus, or sense) and negative (minus, or anti-sense) strand polarities, and evidence suggests they are packaged into capsids with equal efficiency. Consequently, preparations of AAV vectors, like the viruses from which they are adapted, can contain ssDNA sense and antisense genomes in about equal proportion. ITRs can also be modified, however, by selective removal of the D sequence from one of the two ITRs used to produce AAV vectors, to restrict packaging to either the negative or the positive strand of the vector genome, as described further in *Wang, X-S, et al., J Virol, 70(3):1668-77 (1996)*. Thus, in some embodiments, at least one of the ITRs of genomes of AAV vectors of the disclosure can be modified to remove the D sequence and, in so doing, produce a preparation of AAV vectors in which most or substantially all the vector genomes are either positively stranded or negatively stranded.

[00105] In some embodiments, AAV vector genomes of AAV vectors of the disclosure can contain one or more AAV ITRs originating from different AAV serotypes and variants, have different sequences and lengths, and be positioned at different locations within the genome. The sequence of AAV ITRs for use in the vectors of the disclosure can be wild-type or modified. In other embodiments, AAV ITR sequences can also be included as part of vector genome sequences in plasmids and other types of vectors, such as baculoviruses, used to introduce the vector genome sequence into host cells for purposes of vector production.

[00106] ITRs from AAV2 are frequently used in producing AAV vectors, but alternative embodiments can include using ITRs from any AAV serotype or variant including, for example, ITRs from AAV1, AAV3, AAV3B, AAV4, AAV5, AAV6, AAV7, AAV8, or AAV9, or any other AAV serotype or variant known or yet to be discovered, so long as such ITRs are functional for vector genome replication and packaging, and transgene expression. ITRs could also include modifications to the sequence of a wild-type ITR sequence or be fully synthetic. A modified ITR can be at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to a wild type ITR sequence, such as that from AAV2. A vector genome, in some embodiments, can comprise ITRs from different AAV serotypes or variants (thus, for example, a 5' ITR from one serotype or variant, and a 3' ITR from a different serotype or variant).

[00107] As is known in the art, the double-stranded stem of the hairpin structure formed by palindromic subsequences within the ITR (specifically, the A and A' palindromes) contains a sequence motif, the Rep-binding element (RBE), to which the large AAV Rep proteins bind, after which Rep can introduce a sequence-specific and strand-specific nick in the ITR sequence at a position which is known as the terminal resolution site (trs), a step required for DNA replication of the viral genome to occur. In the wild-type AAV2 ITR, which is 145 nucleotides long, the trs occurs between nucleotides 124 and 125, counting from the 3' end. In some embodiments, an ITR for use in vectors of the disclosure is modified by adding, deleting and/or changing nucleotides in the sequence of the ITR to disrupt the terminal resolution site (trs), which is involved a strategy for producing vectors with self-complementary vector genomes (scAAV), which may have increased transduction efficiency and expression of the vector transgene compared to conventional ssDNA vector genomes.

[00108] In AAV virions and conventional vectors with ssDNA genomes, after uncoating but before viral genes can be transcribed, the genome must first be converted to dsDNA through complementary strand synthesis by cellular DNA polymerase initiating strand elongation at the self-priming 3' ITR, a process which is believed to be slow and inefficient. Mutating or deleting the terminal resolution site from one ITR, such as in the plasmid containing the vector genome sequence used for vector production, has been reported to inhibit or eliminate single-strand nicking at that ITR trs during the vector genome replication cycle, resulting in a dimeric replicative intermediate capable of generating a ssDNA vector genome containing a 5' wild-type ITR, non-ITR genome sequence of one polarity, a central mutated ITR (with no free ends), complementary genome sequence of opposite polarity, and a 3' wild-type ITR. Typically, the self-complementary regions (apart from the ITRs) will comprise an expression cassette to express a heterologous transgene. By containing self-complementary regions of significant length within the same DNA molecule, such scAAV vector genomes are hypothesized to quickly renature after uncoating to form a dsDNA transcriptional template through intramolecular base pairing (hybridization), thereby enhancing transduction efficiency. By designing vector genomes of reduced length so that the ssDNA dimeric inverted repeat genomes, when formed, would not exceed AAV packaging capacity, it is possible to produce vector particles containing self-complementary genomes. While not wishing to be bound by theory, it is likely that scDNA genomes reside within capsids in single-stranded form, similar to conventional non-self-complementary genomes, but then rapidly self-anneal after uncoating to form a dsDNA molecule with a covalently closed ITR at one end and two open ITRs at the other end, resembling the structure of a conventional viral

genome after dsDNA conversion through self-priming. Additional information about AAV genome replication and the design of scAAV can be found in *McCarty, DM, et al., Gene Therapy, 10:2112-18 (2003)*; *McCarty, DM, et al., Gene Therapy, 8:1248-54 (2001)*; *McCarty, DM, Molecular Therapy, 16(10):1648-56 (2008)*; *US Pat. No. 7,790,154*.

[00109] ITR terminal resolution sites can be disrupted in various ways to facilitate production of scAAV vectors. For example, an exogenous sequence can be inserted into the terminal resolution site (trs) sequence itself, or into an adjacent sequence of the ITR, such as between the Rep binding element and the trs. Alternatively, the trs sequence could be deleted partially or in its entirety. In other embodiments, the adjacent D region can be deleted partially or in its entirety. In yet other embodiments, nucleotides within the trs can be substituted with different nucleotides that reduce frequency of trs nicking by Rep. Other ways of rendering ITRs non-resolvable are within the ordinary skill in the art.

[00110] In some embodiments, the ITRs chosen for use in vector production will be from the same serotype or variant as the capsid. For example, AAV2 ITRs can be used in conjunction with AAV2 capsids. In other embodiments, however, vectors can be pseudotyped or hybrid, meaning that a vector genome with ITRs from one serotype or variant can be packaged into a capsid from a different serotype or variant. For example, a vector genome with ITRs from AAV2 could be packaged into a capsid from AAV8 (or any other serotype or variant capsid). This pseudotype is often abbreviated AAV2/8, where the number before the slash indicates the viral origin of the ITRs, and the number after the slash indicates the viral origin of the capsid.

[00111] According to some embodiments, AAV vectors of the disclosure can contain vector genomes with different numbers of ITRs. For example, intact AAV viral genomes usually possess two ITRs, one each positioned at the 5' and 3' termini respectively, and AAV vectors produced using intact ITRs and packaged with ssDNA genomes can also have two ITRs similarly positioned. As discussed, above, however vectors can be designed in such a way that their genomes include three ITRs, two at the ends of the genome as in virus or conventional vectors, but one additional at or near the middle (intact or mutated), as in self-complementary vectors. It has also been observed, however that with some vectors, particularly if the genome length exceeds the average packaging capacity of a capsid, 5' ITRs may be truncated or missing entirely due, it is hypothesized, to premature or variable packaging termination. Although defective interfering particles can result, it is also hypothesized that such particles could nevertheless support transgene expression as a result of complementation of otherwise incomplete genomes

(Kapranov, P, et al., *Hum Gene Ther*, 23:46-55 (2012)). Accordingly, in some embodiments AAV vectors of the disclosure can contain genomes comprising a single functional ITR, such as at the 3' end or the 5', and where at the opposite end is a non-functional truncated ITR, or no ITR sequence. Thus, in some embodiments of AAV vector genomes of AAV vectors of the disclosure, an AAV ITR can be positioned at the 5' end of the genome, or at the 3' end of the genome, or at both the 5' and 3' ends of the genome, as well as in other positions.

[00112] In some embodiments, an AAV vector genome includes an intact full length ITR at each of its 5' and 3' ends. Thus, for example, if both ITRs are from AAV2, such full-length ITRs would be 145 nucleotides long. In other embodiments, however, one or more of the ITRs may be truncated, missing one or more terminal nucleotides relative to the canonical full-length sequence for that type of ITR. Thus, for example, an ITR may lack at least or about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20 nucleotides, or more, from its terminal end relative to the canonical full-length sequence for that type of ITR, such as that of AAV2. Such truncated ITRs can occur in genomes packaged by AAV vectors of the disclosure, but also in vector genome sequences used in the production of AAV vectors, such as in a plasmid. For example, it has been observed that truncated ITRs can still function to produce AAV due to capacity for self-repair if sequences missing from one ITR are retained in the other, which is described further in Wang, X-S, et al., *J Mol Biol*, 250:573-80 (1995); Samulski, RJ, et al., *Cell*, 33:135-43 (1983).

AAV Capsids

[00113] AAV vectors of the disclosure can utilize capsids made from any AAV capsid protein, whether naturally occurring or modified, including those presently known or yet to be discovered or developed, which are suitable for transducing cells in a subject to express GCa case protein from a vector transgene.

[00114] Choice of which capsid protein to use in creating an AAV vector (and the corresponding *cap* gene sequence to be used in its production) can be guided by many considerations and factors. As noted above, by virtue of interacting specifically with certain cell surface receptors, different AAV capsids can have different cell or tissue tropisms, which can be an advantage when it is desired to preferentially transduce certain tissues versus others. For example, to express a transgene product preferentially in muscle, one might produce a vector using a capsid with tropism for muscle over liver. Conversely, to express a transgene product in liver, one might produce a vector using a capsid with tropism for liver over muscle or other tissues.

[00115] Other factors may be important as well. Research has established, for example, that some humans have high neutralizing antibody titers to certain capsids as a result of exposure to naturally occurring AAVs, which can interfere with the ability of AAV vectors with the same or similar capsids to transduce target cells. Thus, in designing a vector for gene therapy, choice of capsid may in some cases be guided by the immunogenicity of the capsid, and/or the seroprevalence of the patients to be treated. Other considerations that may influence capsid choice include manufacturability and stability during storage, with others being known in the art.

[00116] AAV vectors of the disclosure can use capsids made from capsid proteins from naturally occurring AAVs, as well as modified or engineered capsid proteins. For example, naturally occurring capsid proteins can be modified by inserting or deleting amino acids or peptides, or by introducing amino acid substitutions, in the VP1, VP2, and/or VP3 protein sequence intended to improve capsid function in some way, such as tissue tropism, immunogenicity, stability, or manufacturability. Other examples include novel capsids with improved properties created by swapping amino acids or domains from one known capsid to another (mosaic or chimeric capsids) or using DNA shuffling and directed evolution methods.

[00117] In some embodiments, AAV vectors of the disclosure can use capsids from known AAV serotypes and variants, as well as non-naturally occurring capsids, including, without limitation AAV1, AAV2, AAV3A, AAV3B, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAVrh10, AAVrh74, AAV-DJ, AAV-PHP.B, Anc80, AAV2.5, and AAV2i8, with many others being possible.

AAV Vector Production Generally

[00118] Modified host cells of the disclosure can be used in the production of AAV vectors using any method known in the art, or yet to be discovered, which is effective to produce AAV vectors in host cells. As known in the art, AAV vectors can be produced, including at large scale, in a variety of ways. AAV vectors, for example, can be made in mammalian or insect cells and then purified. The traditional approach, which does not rely on coinfection with a helper virus, involves use of three plasmids in the so-called triple transfection method. One plasmid contains genes for helper virus factors, a second contains the AAV genome sequence in double stranded form, and the third contains AAV *rep* and *cap* genes. The *rep/cap* plasmid often contains a *rep* gene from AAV2, although this is not a requirement, and the *cap* gene sequence is chosen based on which AAV capsid protein is desired to constitute the capsid. In practice, the three plasmids are often separately replicated in bacteria, purified, mixed in solution together in predetermined proportions, and then mixed with a transfection agent. The transfection mixture is then used to

transfect suitable mammalian host cells (in adherent or suspension cell culture) which are incubated for sufficient time (e.g., 48 to 72 hours, etc.) and under conditions sufficient for the host cells to express the helper factors and the *rep* and *cap* genes, and for AAV vector genome to be replicated from its plasmid template and packaged into capsids. In some embodiments, the host cells are HEK293 cells, which constitutively express AdV helper factors E1A and E1B, such that the helper plasmid only need contain the AdV E2A, E4ORF6, and VA RNA genes. Use of other mammalian host cells that do not produce AdV or other viral helper factor on their own would necessitate use of a helper plasmid containing whichever helper factors are missing or are otherwise required. Although the so-called triple transfection method described above is commonly employed, there is no requirement that the genes for the helper factors, and *rep* and *cap* genes, be provided on separate plasmids. In principle all these genes could be housed in one plasmid, for example, in which case two plasmids can be used in the transfection.

[00119] Seeking more efficient methods of producing AAV vector at large scale, stable cell lines have been created that contain some but not all the components that would otherwise need to be introduced into cells by transient transfection. Packaging cell lines contain stably integrated AAV *rep* and *cap* genes. Production of AAV in packaging cells requires them to be transiently transfected with a plasmid containing an AAV vector genome and infected with a helper virus. It is also possible to produce AAV vectors in packaging cells without transfection by first infecting them with an AdV (either wild type or in which the E2b gene is deleted) which supplies AdV E1 gene products, which induce *rep* and *cap* expression in the cells, as well as helper factors required for AAV replication, followed by infection with a replication deficient hybrid AdV in which an AAV vector genome replaces the E1 gene in the genome of the hybrid virus.

[00120] In another option, producer cell lines contain stably integrated AAV *rep* and *cap* genes, and also an AAV vector genome. Production of AAV in producer cells requires them to be infected with a helper virus. Packaging and producer cells are described further in, e.g., *Martin, J, et al., Generation and characterization of adeno-associated virus producer cell lines for research and preclinical vector production, Hum. Gene Methods, 24:253–269 (2013)*; *Gao, GP, et al., High-titer adeno-associated viral vectors from a Rep/Cap cell line and hybrid shuttle virus, Hum. Gene Ther., 9:2353–62 (1998)*; *Martin, J, et al., Generation and Characterization of Adeno-Associated Virus Producer Cell Lines for Research and Preclinical Vector Production, Hum. Gene Ther. Meth., 24:253-69 (2013)*; *Clement, N and JC Grieger, Manufacturing of recombinant adeno-associated viral vectors for clinical trials, Mol. Ther. Meth. & Clin. Dev. (2016) 3, 16002*

(doi:10.1038/mtm.2016.2). Other cellular systems for producing AAV vectors in mammalian cells, including at commercial scale, are possible.

[00121] The baculovirus system has also been employed to produce AAV vectors, in which Sf9 insect cells are infected with recombinant baculovirus vectors that variously contain the AAV *rep* and *cap* genes and the AAV genome. The exogenous genes are expressed, followed by genome packaging into vector particles within the cells. In early versions of the system, each component, *rep*, *cap*, and genome, were carried by three separate baculoviruses. Later, modifications were made, such as combining *rep* and *cap* into a single baculovirus, so that only two types of baculovirus were required, as well as producing Sf9 cell lines containing stably integrated AAV *rep* and *cap* genes, which only require infection with a single type of recombinant baculovirus containing an AAV vector genome. Use of the baculovirus system to produce AAV vector is described further in, e.g., *Urabe, M, et al., Insect Cells as a Factory to Produce Adeno-Associated Virus Type 2 Vectors, Hum. Gene Ther., 13:1935–43 (2002); Virag, T, et al., Producing recombinant adeno-associated virus in foster cells: Overcoming production limitations using a baculovirus-insect cell expression strategy, Hum. Gene Ther., 20:807–17 (2009); Smith, RH, et al., A simplified baculovirus-AAV expression vector system coupled with one-step affinity purification yields high-titer rAAV stocks from insect cells, Mol. Ther., 17:1888–96 (2009); Mietzsch, M, et al., OneBac: platform for scalable and high-titer production of adeno-associated virus serotype 1-12 vectors for gene therapy, Hum. Gene Ther. 25(3):212-22 (2014)*. Other cellular systems for producing AAV vectors in insect cells, including at commercial scale, are possible.

Introducing Exogenous Genetic Material into Host Cells for AAV Vector Production

[00122] To produce vectors, exogenous genetic information designed to direct biosynthesis of the various components required for AAV vector assembly (e.g., AAV capsid proteins, Rep proteins, helper virus factors, and vector genomes) can be introduced into host cells transiently or non-transiently. For example, host cells can be transfected with exogenously supplied nucleic acid, such as in the form of one or more DNA plasmids, containing nucleotide sequences coding for the required vector components.

[00123] Various ways are known in the art for transfecting host cells with nucleic acid. These include, without limitation, mixing nucleic acid with certain compounds that can complex with nucleic acids and then be taken up into the cells, including calcium phosphate or cationic organic compounds, such as DEAE-dextran, polyethyleneimine (PEI), polylysine, polyornithine, polybrene, cyclodextrin, cationic lipids, and others known in the art. Transfection can also be performed

non-chemically via electroporation and more exotic technologies, such as biolistic particle delivery. As known in the art, transfection can be transient or stable. With transient transfection, the transfected nucleic acid exists in the cell for a limited period of time and, in the case of DNA, does not integrate into the genome. With stable transfection, DNA introduced into the cell can persist for long periods either as an episomal plasmid or integrated into a chromosome. Usually, to produce stably transfected cells, a plasmid containing a selection marker gene, as well as nucleotide sequence coding for one or more of the required vector components, is transfected into the cells which are then grown and maintained under selective pressure, *i.e.*, conditions that kill non-transfected cells or transfected cells from which the exogenous DNA, including its selection marker, are lost for some reason. For example, plasmids can contain an antibiotic resistance gene and transfected cells can be selected for by adding the antibiotic to the media in which the cells are grown. In some embodiments, the nucleotide sequence coding for one or more of the required vector components introduced into stably transfected host cells is under the control of an inducible promoter and is not expressed, or only at a low level, unless an environmental factor, such as a drug, metal ion, or temperature increase, which induces the promoter, is introduced as the cells are grown.

[00124] In other embodiments, host cell genomes can be modified in a non-transient and targeted fashion using genetic engineering methods, such as knock-in, or gene editing methods, to direct host cells to produce one or more of the required vector components. Examples of such modified host cells include producer cells and packaging cells. In other embodiments, nucleotide sequence coding for one or more of the required vector components can be introduced into host cells for purposes of directing production of AAV vectors via transduction, in which host cells are infected with modified viruses containing such nucleotide sequences. Examples of viral vectors useful for such purposes include adenovirus, retroviruses (including lentiviruses), baculoviruses, vaccinia virus, and herpes simplex virus, with others being possible.

Methods for AAV Vector Production Using Transiently Modified Host Cells

[00125] In some embodiments, the disclosure provides methods for producing AAV vectors in host cells in which one or more of the differentially expressed genes (DEGs) described herein is transiently repressed. In some of these embodiments, the DEG is transiently repressed by transiently expressing in host cells a miRNA, a shRNA, or other type of non-coding RNA which is capable of repressing DEG expression by the mechanism of RNA interference. In some of these embodiments, the miRNA, shRNA, or other type of non-coding RNA capable of repressing DEG

expression by RNAi is produced from an expression vector which is introduced into host cells before or with the exogenous genetic information required to direct biosynthesis of the various components required for AAV vector assembly (e.g., AAV capsid proteins, Rep proteins, helper virus factors, and vector genomes), such as by the classic triple transfection method, or similar methods.

[00126] In some non-limiting embodiments of such methods, a miRNA expression vector can contain an expression cassette comprising a promoter, such as an RNA pol II promoter (such as a CMV promoter, or other type of viral or non-viral RNA pol II promoter), and a sequence coding for a pri-miRNA in which the endogenous miRNA guide and passenger strand subsequences are replaced with engineered guide and passenger strand sequences intended to target the DEG mRNA of interest. In some non-limiting embodiments, a shRNA expression vector can contain an expression cassette comprising a promoter, such as an RNA pol III promoter (such as a U6 promoter, or others), and a sequence coding for a shRNA comprising a target sequence present in the DEG mRNA of interest, a loop sequence, and a guide sequence complementary to the target sequence, followed by a pol III terminator. In some embodiments, the miRNA or shRNA expression cassette is contained within a plasmid, which can be introduced into host cells by transfection, whereas in other embodiments, the miRNA or shRNA expression cassette is contained within viral vector (such as a lentiviral vector, or other type of viral vector), which can be introduced into host cells by viral transduction. Design and production of expression vectors containing a miRNA, a shRNA, or other type of non-coding RNA capable of repressing DEG expression by RNAi for use in the methods of AAV vector production described herein is within the knowledge of those of ordinary skill in the art, including, for example, use of computer algorithms to select the miRNA or shRNA guide, loop, and/or passenger sequences to be included in corresponding expression cassettes to target any particular DEG mRNA of interest. In any of the foregoing embodiments, the DEG targeted for repression is any one or more of ACTA1, ARL14, BDNF, CALCR, CSF1, CXCL11, DOCK8, EIF2AK2, EP300, EPHB1, ESRP1, FAM83A, FGF2, FOXO1, GNRH1, HGF, HS3ST4, IFI44L, ITGAM, KITLG, MAP2K3, MAPK1, MAPK3, MIF, MSC, MYD88, OAS3, ODAF1, PAF1, PDE6G, PNN, PPRC1, PTX3, RC3H1, SMARCA4, SP110, SPI1, STAT4, TGM2, TICAM1 and/or TRIM24.

[00127] In some embodiments, the expression vector containing a miRNA, a shRNA, or other type of non-coding RNA capable of repressing DEG expression by RNAi contains an expression cassette that is bicistronic or multicistronic, and thus is capable of expressing a miRNA, a shRNA,

or other type of non-coding RNA capable of repressing DEG expression by RNAi against two or more DEG of interest. In some other embodiments, the expression vector containing a miRNA, a shRNA, or other type of non-coding RNA capable of repressing DEG expression by RNAi contains two or more expression cassettes, each of which is capable of expressing separately from others a miRNA, a shRNA, or other type of non-coding RNA capable of repressing DEG expression by RNAi against a single distinct DEG of interest. In any of the foregoing embodiments, the DEG targeted for repression is any one or more of ACTA1, ARL14, BDNF, CALCR, CSF1, CXCL11, DOCK8, EIF2AK2, EP300, EPHB1, ESRP1, FAM83A, FGF2, FOXO1, GNRH1, HGF, HS3ST4, IFI44L, ITGAM, KITLG, MAP2K3, MAPK1, MAPK3, MIF, MSC, MYD88, OAS3, ODAPH, PAF1, PDE6G, PNN, PPRC1, PTX3, RC3H1, SMARCA4, SP110, SPI1, STAT4, TGM2, TICAM1 and/or TRIM24.

[00128] In some embodiments, AAV vectors are produced by first transiently repressing a DEG of interest in host cells and thereafter introducing into such host cells the exogenous genetic information required for AAV vector production. In some embodiments of these methods, DEG expression is transiently repressed by introducing into the host cells an expression cassette for producing a miRNA, shRNA, or other type of non-coding RNA capable of repressing DEG expression by RNAi, such as by transfection with a plasmid comprising such expression cassette, or transduction with a viral vector comprising such expression cassette. In some embodiments where it is desired to repress more than one DEG, DEG expression may be transiently repressed by introducing into host cells an expression vector containing a plurality of expression cassettes, each of which is capable of expressing a miRNA, a shRNA, or other type of non-coding RNA capable of repressing DEG expression by RNAi against a single distinct DEG of interest, or an expression vector containing a bicistronic or multicistronic expression cassette, as described above, or by introducing into host cells a plurality of distinct expression vectors, each one of which is capable of expressing one of the DEG of interest desired to be repressed. In any of the foregoing embodiments, the DEG targeted for repression is any one or more of ACTA1, ARL14, BDNF, CALCR, CSF1, CXCL11, DOCK8, EIF2AK2, EP300, EPHB1, ESRP1, FAM83A, FGF2, FOXO1, GNRH1, HGF, HS3ST4, IFI44L, ITGAM, KITLG, MAP2K3, MAPK1, MAPK3, MIF, MSC, MYD88, OAS3, ODAPH, PAF1, PDE6G, PNN, PPRC1, PTX3, RC3H1, SMARCA4, SP110, SPI1, STAT4, TGM2, TICAM1 and/or TRIM24.

[00129] In some embodiments, host cells are first transfected with a plasmid comprising an expression cassette for producing a miRNA, shRNA, or other type of non-coding RNA capable of repressing DEG expression by RNAi. Then, after sufficient time for expression in the host cells of

the miRNA, shRNA, or other type of non-coding RNA capable of repressing DEG expression by RNAi from the transfected plasmids, the exogenous genetic information required for AAV vector production is introduced, such as by further transfection with one or more plasmids comprising such exogenous genetic information, such as by the classic triple transfection method. In some embodiments, the time between transfecting host cells with a plasmid comprising an expression cassette for producing a miRNA, shRNA, or other type of non-coding RNA capable of repressing DEG expression by RNAi and subsequently transfecting the host cells with the exogenous genetic information required for AAV vector production is at least or about 0.5, 0.75, 1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5, 5, 5.5, 6, 6.5, 7, 7.5, 8, 8.5, 9, 9.5, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, or 24 or more hours, or a period of time ranging between any of the foregoing specifically enumerated values. In some embodiments, the amount of mRNA for the DEG which is being targeted for repression by RNAi is reduced at least or about 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or more, compared to the mRNA level prevailing before transfection, or by an amount ranging between any of the foregoing specifically enumerated values. In some embodiments, the amount of AAV vector produced in host cells in which expression of a selected DEG is transiently repressed is increased at least or about 20%, 25%, 30%, 40%, 50%, 60%, 70%, 75%, 80%, 90%, 100%, 125%, 150%, 175%, 200%, 225%, 250%, 275%, 300%, 350%, 400%, 450%, or 500%, or more, compared to the amount of AAV produced in similar host cells in the absence of transiently repressed DEG expression, or by an amount ranging between any of the foregoing specifically enumerated values. In any of the foregoing embodiments, the DEG targeted for repression is any one or more of ACTA1, ARL14, BDNF, CALCR, CSF1, CXCL11, DOCK8, EIF2AK2, EP300, EPHB1, ESRP1, FAM83A, FGF2, FOXO1, GNRH1, HGF, HS3ST4, IFI44L, ITGAM, KITLG, MAP2K3, MAPK1, MAPK3, MIF, MSC, MYD88, OAS3, ODAFPH, PAF1, PDE6G, PNN, PPRC1, PTX3, RC3H1, SMARCA4, SP110, SPI1, STAT4, TGM2, TICAM1 and/or TRIM24.

[00130] In some embodiments, host cells are transfected with a plasmid comprising an expression cassette for producing a miRNA, shRNA, or other type of non-coding RNA capable of repressing DEG expression by RNAi (referred to hereafter for convenience as the RNAi expression cassette) at the same time as introduction into the host cells of the exogenous genetic information required for AAV vector production. In some embodiments, the RNAi expression cassette is contained within its own plasmid, and the exogenous genetic information required for AAV vector production is contained within one or more additional separate plasmids. All four

plasmids are then transfected into host cells at the same time. Thus, for example, in the context of the classic triple transfection technique for AAV production, the RNAi expression cassette can be contained within a first plasmid, the viral helper factors contained within a second plasmid, the AAV *rep* and *cap* genes contained within a third plasmid, and finally the vector genome contained within a fourth plasmid. In some other embodiments, however, the RNAi expression cassette can be contained within one of the plasmids more typically used for AAV production, such as the plasmid comprising the viral helper factors, the plasmid comprising the AAV *rep* and *cap* genes, or the plasmid comprising the vector genome, thereby reducing the total number of plasmids used for AAV production by at least one.

[00131] In some embodiments, the RNAi expression cassette is provided in the same plasmid as the viral helper factors. Thus, for example, according to one non-limiting embodiment, a combination helper-RNAi plasmid can comprise one or more viral helper genes, such as those expressing the adenoviral E2A, E4 and/or VA gene products, as well as an RNAi expression cassette comprising a suitable promoter and transcription terminator, as well as sequence for producing a miRNA, shRNA, or other type of non-coding RNA capable of repressing a desired DEG expression by RNAi. In some methods of AAV vector production employing a combination helper-RNAi plasmid as described herein, the amount of mRNA for the DEG which is being targeted for repression by RNAi is reduced at least or about 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or more, compared to the mRNA level prevailing before transfection, or by an amount ranging between any of the foregoing specifically enumerated values. In some embodiments, the amount of AAV vector produced in host cells in which expression of a selected DEG is transiently repressed by RNAi using a combination helper-RNAi plasmid as described herein is increased at least or about 20%, 25%, 30%, 40%, 50%, 60%, 70%, 75%, 80%, 90%, 100%, 125%, 150%, 175%, 200%, 225%, 250%, 275%, 300%, 350%, 400%, 450%, or 500%, or more, compared to the amount of AAV produced in similar host cells in the absence of transiently repressed DEG expression, or by an amount ranging between any of the foregoing specifically enumerated values. In any of the foregoing embodiments, the DEG targeted for repression is any one or more of ACTA1, ARL14, BDNF, CALCR, CSF1, CXCL11, DOCK8, EIF2AK2, EP300, EPHB1, ESRP1, FAM83A, FGF2, FOXO1, GNRH1, HGF, HS3ST4, IFI44L, ITGAM, KITLG, MAP2K3, MAPK1, MAPK3, MIF, MSC, MYD88, OAS3, ODAPH, PAF1, PDE6G, PNN, PPRC1, PTX3, RC3H1, SMARCA4, SP110, SPI1, STAT4, TGM2, TICAM1 and/or TRIM24.

Host Cell Culture

[00132] For purposes of producing AAV vectors, modified host cells can be grown or maintained in culture under controlled conditions conducive to their growth and vector biosynthesis. For example, modified host cells can be grown in liquid media of defined chemical composition that provides all the nutrients necessary for cell growth and biosynthesis. Exemplary media includes DMEM, DMEM/F12, MEM, RPMI 1640, for mammalian host cells, and Express Five SFM, Sf-900 II SFM, Sf-900 III, or ExpiSf CD, for certain insect cells. Such media may be supplemented with antibiotics, growth factors or cytokines (produced recombinantly or present in animal serum, such as FBS) known to stimulate growth of the particular type of cells in use, as well as other ingredients that may be required for optimal biosynthesis of AAV vectors, but that would otherwise be in limiting supply. Exemplary supplements include essential amino acids, glutamine, vitamin K, insulin, BSA, or transferrin. In addition to the growth media, other culture conditions may be controlled to optimize growth and/or productivity of the cells, such as pH, temperature and CO₂ and oxygen concentration.

[00133] Modified host cells in culture can be grown or maintained in many containers known in the art, such as stirred tank bioreactors, wave bags, spinner flasks, hollow fiber bioreactors, or roller bottle, some of which can be designed and configured for single use or multiple use. Depending on the characteristics of the host cells in question, host cells can be grown in adherent cell culture, where the cells attach to and grow while in contact with a physical substrate, or in suspension cell culture, either where single cells float free in the media that sustains them, or while attached to bead microcarriers, which are suspended in the media. As known in the art, various technologies have been developed and can be used to grow host cells to high cell density, such as perfusion culture, which can increase the overall amount of AAV vector generated per production run.

[00134] Samples of modified host cells can be maintained in frozen cell banks, such as master cell banks and working cell banks, which facilitate production of biological products in many batches over time, while ensuring consistent performance by the host cells. Before a campaign to produce an AAV vector, a frozen sample of host cells from a cell bank would typically be thawed, seeded into a small culture volume, and grown to ever higher densities or numbers in cultures of increasing volume. When host cells have reached a desired cell density and/or volume in culture, exogenous genetic material can be introduced, such as by transfection with plasmid DNA or infection or transduction with viral vectors, to cause them to begin producing

the AAV vector. Alternatively, if using non-transiently modified host cells in which the nucleotide sequence coding for one or more of the required vector components is under inducible control, the environmental factor necessary to induce expression can be introduced. Host cells can then be grown or maintained in culture for time and under conditions sufficient for them to produce the AAV vectors.

AAV Vector Purification

[00135] After biosynthesis in modified host cells, AAV vectors can be purified in a variety of ways known in the art. For example, in some embodiments, host cells can be lysed mechanically or chemically, such with detergent, after which host cell DNA and other components are removed, followed by steps such as density gradient centrifugation, or use of one or more chromatographic separation methods, to achieve a highly purified preparation of AAV vectors for use in research or methods of treatment.

[00136] Chromatography methods useful in the purification of AAV vectors include, without limitation, size exclusion chromatography (SEC); affinity chromatography, using any affinity ligand attached to the chromatography resin or matrix capable of specific binding to a capsid, such as an antibody, lectin, or glycan; immobilized metal chelate chromatography (IMAC); thiophilic adsorption chromatography; hydrophobic interaction chromatography (HIC); multimodal chromatography (MMC); pseudo-affinity chromatography; and ion exchange chromatography (IEX or IEC), such as anion exchange chromatography (AEX) or cation exchange chromatography (CEX).

[00137] In some embodiments, AAV vectors can be purified using antibody-based affinity chromatography in which an antibody, or antibody fragment thereof, is attached to a stationary phase (matrix or resin) loaded into a chromatography column through which a host cell lysate is pumped, followed by washing and eluting of vector which had bound to the antibodies. The antibody bound to the solid phase can be an IgG, or fragment thereof, or a single-chain camelid antibody (such as a heavy chain variable region camelid antibody), other types of antibodies being possible. Non-limiting examples of ligand affinity resins include Sepharose AVB, POROS CaptureSelect AAVX, POROS CaptureSelect AAV8, and POROS CaptureSelect AAV9. See, e.g., *Terova, O, et al., Affinity Chromatography Accelerates Viral Vector Purification for Gene Therapies, BioPharm Intl. eBook pp. 27-35 (2017)*; *Mietzsch, M, et al., Characterization of AAV-Specific Affinity Ligands: Consequences for Vector Purification and Development Strategies, Mol. Ther. Meth. & Clin. Dev., 19:362-73 (2020)*; *Rieser, R, et al., Comparison of Different Liquid*

Chromatography-Based Purification Strategies for Adeno-Associated Virus Vectors, Pharmaceutics 13, 748 (2021) (doi.org/10.3390/pharmaceutics13050748).

[00138] In other embodiments, AAV vectors can be purified using ligand chromatography in which the stationary phase has attached to it the same type of ligand that certain AAVs are known to use when binding to cells, such as a glycan, sialic acid (e.g., an O-linked or N-linked sialic acid), galactose, heparin, heparan sulfate, or a proteoglycan, such as a heparan or heparin sulfate proteoglycan (HSPG). For example, an affinity matrix containing sialic acid residues can be used to purify AAV vectors with capsids that specifically bind to sialic acid (e.g., AAV1, AAV4, AAV5, or AAV6); an affinity matrix containing galactose can be used to purify AAV vectors with capsids that specifically bind to galactose (e.g., AAV9); and an affinity matrix containing heparin, heparan, or HSPG can be used to purify AAV vectors with capsids that specifically bind to HSPG (e.g., AAV2, AAV3A, AAV3B, AAV6, or AAV13).

[00139] Depending on the physicochemical characteristics of the vector, such as the charge on the capsid, AAV vectors can be further purified by performing anion exchange, cation exchange, or hydrophobic interaction chromatography. Other downstream process steps useful for purifying AAV vectors may be used as well, such as, without limitation, desalting and buffer exchange, ultrafiltration, nanofiltration, diafiltration, and tangential flow filtration (TFF). Use of more than one downstream processing step is possible, and a plurality of downstream processing steps can be performed in any order according to the knowledge of those ordinarily skilled in the art.

[00140] Once purified, a preparation of AAV vectors can be titered using any method known in the art, to determine the amount of vector which is present, for example on a volume basis. According to one non-limiting embodiment, the amount of AAV vector in a sample can be determined using quantitative polymerase chain reaction (qPCR) relative to a suitable standard. In some embodiments, a sample of AAV vector is titered using qPCR in which the forward primer, the reverse primer, or probe oligonucleotide are designed to anneal in one of the vector ITR sequences, and/or in some other region within the vector genome, including a sequence unique to the vector, such as a sequence within the transgene. Methods employing qPCR, as well as other methods, to titer the amount of AAV vector in a sample will be familiar to those of ordinary skill in the art.

[00141] Once purified, a preparation of AAV vectors can be assayed to determine the proportion of vector in the sample which possesses a predefined key quality attribute, non-

limiting examples of which include vector potency, stability, and the percentage of capsids which contain full length vector genomes (full capsids) versus the percentage of capsids containing less than full length vector genomes, such as truncated genomes, contaminating plasmid or host cell DNA, or no genomes or detectable nucleic acid material at all (the latter being termed empty capsids). Methods for determining the proportion of AAV vectors in a sample which possesses any desired key quality attribute, such as potency, stability, or the percentage of full versus capsids will be familiar to those of ordinary skill in the art.

AAV Vector Compositions, Formulations, Methods of Administration, Dosages

[00142] In addition to AAV vectors, the present disclosure provides compositions comprising such vectors and as at least one pharmaceutically acceptable excipient, diluent, or carrier. Such vectors may be used, among other things, in methods of prevention and treatment of any desired disease, disorder, or condition affecting a subject.

[00143] Compositions comprising AAV vectors of the disclosure can be provided as aqueous solutions or suspensions, emulsions, and in other forms, such as lyophilized cakes. Vector compositions can be formulated using any suitable diluent and excipients that may be necessary to achieve desired properties, such as pH, ionic strength, tonicity, stability, shelf-life, resistance to freeze-thaw cycles, and ability to be freeze dried, as well as considering the mode of administration. Exemplary diluents and carriers include, without limitation, sterile water for injection, ethanol, and glycerol. Exemplary excipients include, without limitation, salts, buffers, acids, bases, surfactants, saccharides, sugar alcohols, and many others known in the art. Compositions comprising AAV vectors of the disclosure for use in preventing or treating a disease, disorder, or condition in a subject can be packaged in any suitable form, such as vials or pre-filled syringes. In some embodiments, kits are provided with a plurality of vials containing sufficient total amount of vector to achieve a desired total dose to be delivered to a particular subject based on relevant variables, such as such subject's disease severity, body mass, sex, or others.

[00144] Compositions comprising AAV vectors of the disclosure can be administered by any suitable route of administration, non-limiting examples of which include systemic administration, administration directly into a tissue or organ, intravenous administration, intraarterial administration, intralymphatic administration, intraperitoneal administration, intramuscular administration, intraparenchymal administration, intrathecal administration,

intracerebroventricular administration, or intracisternal magna administration, with others being possible.

[00145] Compositions comprising AAV vectors of the disclosure can be administered alone, without any other kinds of therapy, or can be administered simultaneously, contemporaneously, or at any suitable dosing interval with a standard of care treatment, or some other agent, compound, drug, treatment or therapeutic regimen. In some embodiments, compositions comprising AAV vectors of the disclosure can be administered after prophylaxis with immunosuppressive agent, such as a steroid or tacrolimus, or other immunosuppressant drug, or immunosuppressant drugs can be administered afterward to control any humoral and/or cellular immune reaction to the gene therapy.

[00146] Vector compositions can contain any suitable amount of an AAV vector calculated to deliver a prophylactically or therapeutically effective amount of such vector to a subject in a volume that is easily handled or administered to the subject, and/or would not be expected to cause any discomfort or undesirable side effects to the subject.

[00147] In connection with the methods of prevention and treatment provided by the disclosure, AAV vectors and compositions comprising such vectors can be administered in any suitable dose predicted or determined to be effective to achieve the desired degree of prevention or treatment. In some embodiments, doses of an AAV vector of the disclosure for preventing or treating a desired disease, disorder, or condition affecting a subject can be quantified and expressed as vector genomes (vg) per kilogram of subject body weight, abbreviated "vg/kg." In some embodiments, exemplary efficacious doses of an AAV vector of the disclosure include, without limitation, at least or about 1×10^9 vg/kg, 1×10^{10} vg/kg, 1×10^{11} vg/kg, 1×10^{12} vg/kg, 1×10^{13} vg/kg, 1×10^{14} vg/kg, or 1×10^{15} vg/kg, or a range of doses between and including any of the foregoing specifically enumerated doses, other doses being possible.

* * *

[00148] Other objects, features and advantages of the present invention will be apparent from the foregoing detailed description. It should be understood, however, that the detailed description and the specific examples that follow, while indicating specific embodiments of the invention, are given by way of illustration only, since various changes, modifications and equivalents within the spirit and scope of the invention will be apparent from the detailed description and examples to those of ordinary skill in the art and fall within the scope of the appended claims.

[00149] Unless otherwise indicated, use of the term “or” in reference to one or more members of a set of embodiments is equivalent in meaning to “and/or,” and does not require that they be mutually exclusive of each other. Unless otherwise indicated, a plurality of expressly recited numeric ranges also describes a range the lower bound of which is derived from the lower or upper bound of any one of the expressly recited ranges, and the upper bound of which is derived from the lower or upper bound of any other of the expressly recited ranges. Thus, for example, the series of expressly recited ranges “10-20, 20-30, 30-40, 40-50, 100-150, 200-250, 275-300,” also describes the ranges 10-50, 50-100, 100-200, and 150-250, among many others. Unless otherwise indicated, use of the term “about” before a series of numerical values or ranges is intended to modify not only the value or range appearing immediately after it but also each and every value or range appearing thereafter in the same series. Thus, for example, the phrase “about 1, 2, or 3,” is equivalent to “about 1, about 2, or about 3.”

[00150] All publications and references, including but not limited to articles, abstracts, patents, patent applications (whether published or unpublished), and biological sequences (including, but not limited to those identified by specific database reference numbers) cited herein are hereby incorporated herein by reference in their entirety for all purposes to the same extent as if each individual publication or reference were specifically and individually indicated to be so incorporated by reference. Any patent application to which this application claims priority directly or indirectly is also incorporated herein by reference in its entirety.

[00151] Unless otherwise indicated, the examples below describe experiments that were or are performed using standard techniques well known and routine to those of ordinary skill in the art. The examples are illustrative, but do not limit the invention.

* * *

EXAMPLES

[00152] The following examples as well as the figures are included to demonstrate preferred embodiments of the invention. It should be appreciated by those of skill in the art that the techniques disclosed in the examples or figures represent techniques discovered by the inventors to function well in the practice of the invention, and thus can be considered to constitute preferred modes for its practice. However, those of skill in the art should, in light of the present disclosure, appreciate that many changes can be made in the specific embodiments which are disclosed and still obtain a like or similar result without departing from the spirit and scope of the invention.

EXAMPLE 1**Identification of Genes Upregulated After Transfection or During AAV Vector Biosynthesis**

[00153] Experiments were designed and carried out to identify genes upregulated in HEK293 host cells at different time points after transfection and during synthesis of AAV vectors.

Materials and Methods***HEK293 cell line and cell culture***

[00154] For the cell expansion for 50L bioreactor culture, HEK293 cells adapted to growth in suspension were cultured in 1L serum-free FreeStyle F17 Expression Medium supplemented with GlutaMAX Supplement at 10mM (ThermoFisher Scientific, USA) at 37°C, 5% CO₂, 50% humidity and 64 RPM in 3L Corning vent cap flask. Cells were then seeded at 0.3E+6 viable cell density in 5L of F17 medium supplemented with GlutaMAX at 10mM, 0.2% Pluronic F68 and 0.1% FoamAway Irradiated Animal Origin-Free antifoaming agent (ThermoFisher Scientific, USA) in a 10L Wave bag. The Wave bag was rocked at 25 RPM, 37°C, 5% CO₂. Finally, cells were cultured in 50L single-use bioreactor (SUB) (ThermoFisher Scientific, USA) containing 20L of F17 supplemented with 10mM GlutaMAX, 500mL of 10% Pluronic F68 and 25mL of FoamAway and 5L cell culture medium from 10L wave bag described above. The temperature was set at 37°C and the agitator was set at 70 RPM. Forty-eight hours after inoculation, 15L of Expi293 Expression medium (ThermoFisher Scientific, USA) along with 300mL of 10% Pluronic F68 was added to the culture. The culture was brought to cell density of 5E+6 cells/ml before transfection. Viable cell density (VCD) and cell viability were measured by Vi-Cell XR cell viability analyzer (Beckman Coulter Life Sciences). For the culture in 2L bioreactors, the medium and process controls remained the same as 50L SUBs and targeted the final culture volume of 1L with cell density of 5E+6 cells/ml before transfection.

Transfection for rAAV production

[00155] A three-plasmid mix including plasmids encoding AdV helper factors, Rep2/Cap9 genes, and genome containing a mini-dys transgene flanked by AAV2 ITRs was transfected into HEK293 cells using polyethyleneimine (PEI) transfection reagent. One microgram of plasmid mix per million viable cells was used for cell transfection. PEI was added at two times the mass of the three-plasmid mix, seven minutes before delivering the transfection mix to the SUB or 2L bioreactor. Viable cell density and percentage of viable cells were measured using Vi-Cell at the 8 selected sampling time points.

rAAV titer determination

[00156] Cells sampled at 36h, 48h and 72h after transfection were submitted for rAAV titer measurement. The fresh cells were lysed using freeze-thaw lysis method, which allowed rAAV viral particles to remain intact. Unencapsidated DNA in cell lysate was digested using DNase-I (MP Biomedicals, USA) at 263 U/mL incubated at room temperature for 60 minutes followed by DNase-I inactivation and rAAV capsid proteolytic degradation at the same time using 1.3 mM EDTA and 0.53X of Teknova qPCR protease K buffer (1.11 M NaCl, 1.11% Sarkosyl) at 95°C incubation for 10 minutes. FAM-labeled probe and primer pairs specific for the mini-dys transgene were used for TaqMan qPCR protocol on QuantStudio 6 Flex Real-Time PCR system.

RNA-Seq

[00157] Cell pellets at 8 selected sampling time points were submitted for RNA-seq. Messenger RNA enrichment followed by random priming was used for RNA library preparation to exclude rRNA or tRNA. Each RNA library was sequenced three times by Illumina HiSeq 2x150 pair-end configuration. The RNA library preparation and sequencing were conducted by GENEWIZ Inc. Note that each sample was submitted to individual library preparation and independent flow cells without multiplexing, eliminating the likelihood that the clustering was due to artifact of NGS sequencing.

Transcriptome data preprocessing

[00158] Expression levels of 35616 distinct transcripts in the human genome were determined by Kallisto using RNA-Seq data⁵¹. The reference genome sequences for read mapping was GRCh38.99 along with viral transcripts on either HEK293 cells including E1A and E1B or three transfected plasmids including E2A, E4orf6, VAI, VAI1, p5 transcript, p19 transcript and p40 transcript. Three technical repeats from each RNA library were pooled to determine expression level for each transcript. Transcripts with more than 0.5 transcript per million (TPM) at any time points were defined as expressed transcripts. The TPM cutoff was set to observe at least one transcript in each sampled cell assuming two million RNA molecules per cell in average at the time of sampling.

Bioinformatic analysis

[00159] Differentially expressed genes (DEGs) were identified using likelihood ratio test (LRT) in DESeq2 package in R for any expressed transcript that show significant change in expression across 8 time points with false discovery rate (FDR) adjusted p-value less than 0.05 using custom

R script. Time-series analysis was performed using Python program based on Dirichlet process Gaussian process (DPGP) mixture model for expression pattern clustering²⁵. The DPGP mixture model assumes that the expression levels at adjacent time points are dependent, which makes an ideal fit for time-course transcriptome samples. DEGs were submitted to clustering with 200 iterations of cluster assignment using DPGP mixture model. Enriched pathway analysis was conducted using GOATOOLS⁵². The results and figures generated from DP_GP and GOATOOLS Python packages can be reproduced using custom Python code.

RT-qPCR

[00160] Total RNA was extracted using TRIzol reagent (Invitrogen, USA). Reverse transcription was achieved with oligo dT primer using SuperScript IV first-strand synthesis system (Invitrogen, USA) for enrichment of polyA⁺ mRNAs. Expression level of target genes was measured by qPCR using FAM-labeled TaqMan Gene Expression Assay (IFNB1: Hs01077958_s1, CCL5: Hs00982282_m1, CXCL8: Hs00174103_m1, OASL: Hs00984387_m1, IFIT2: Hs00533665_m1) and TaqMan Fast Advanced Master Mix (ThermoFisher Scientific, USA). HPRT1 (Hs02800695_m1) was used as internal control.

Enzyme-linked immunosorbent assay (ELISA)

[00161] The time-course cell culture samples post-transfection were collected from three independent bioreactors. The spent medium was collected from the supernatant of culture after centrifugation at 2000 x g for 10 minutes at 4°C. Spent medium was concentrated 10-fold using disposable ultrafiltration units with a molecular weight cutoff of 3 kDa (ThermoFisher Scientific, USA) before assay. Standard curve was diluted in concentrated medium (F17 and Expi293 in 1:1 ratio) to account for potential matrix effect of concentrated medium selected ELISA kits. The limit of detection was set at the point with lowest concentration on the linear slope of the standard curve and the absorbance obtained using concentrated medium negative control was defined as noise for background subtraction.

Results

[00162] To understand the response of host cells to rAAV vector production via plasmid transfection, samples were collected throughout the course of three 50L production batches and analyzed by bulk RNA sequencing of polyadenylated transcripts (RNA-seq). The rAAV produced in these lots encoded a transgene designed to express a mini-dystrophin protein comprising minimal domains from human dystrophin protein required for muscle function delivered by the

AAV9 capsid, and manufacture was enabled by a helper plasmid encoding the adenovirus E2A, E4, and VA RNA genes⁹. Samples were collected immediately prior to transfection (0h) and at 3, 6, 12, 24, 36, 48, and 72 hours post transfection (HPT) (**Figure 1**). To validate the RNA-seq results via gene-specific assays and to define rAAV replication kinetics, three 2L batches were also conducted under conditions which have been qualified to replicate the 50L process (**Figure 2**). The titer of encapsidated rAAV genome showed that the packaged vectors were predominantly produced between 12 and 24 hpt but continued to increase before harvest (**Figures 2A and 2D**).

[00163] RNA-seq quantification identified 17209 expressed transcripts in at least one of the 24 samples (**Figures 7A-7B**). The result of principal component analysis (PCA) on all expressed transcripts showed clear clustering by sampling time (**Figure 8**), indicating a consistent response to rAAV production via plasmid transfection among biological replicates. Furthermore, the time-series transcriptomic samples were ordered in relation to sampling times on the PCA graph as commonly seen with longitudinal samples. The results from PCA, combined with similar cell growth and viability among replicates (**Figure 2**), suggested that the transcriptomic regulation in transfected cells was highly reproducible between replicates.

[00164] To characterize the cellular transcriptional response to rAAV manufacture, differentially expressed genes (DEGs) were identified in all post-transfection time points relative to pre-transfection cells (0h). In total, 1850 unique DEGs were identified across all time points (**Figure 7B**). The number of upregulated genes increased over time post-transfection (**Figure 3A**).

[00165] To better understand how host cells responded to transfection and rAAV vector production, gene ontology enrichment analysis was performed to identify altered pathways. Significant pathways using 1850 DEGs in gene ontology analysis were involved in DNA/chromatin organization and DNA metabolic processes (**Figure 3B**). Interestingly, most of the DEGs involved in these pathways were histone subunits induced 24 hours post transfection, by which time rAAV production was fully underway (**Figure 3F**). Another subset of significant pathways was related to the innate immune response to virus (**Figure 3C**). Many interferon-stimulated genes (ISGs) were significantly enriched (**Figure 3E**). Expression levels of ISGs generally increased until the time of harvest (72 hours post transfection). The gene ontology category Inflammatory Response was also enriched in the DEG set (**Figure 3D**). A subset of chemokines and chemokine receptors was induced by 12h after transfection. Key components of the NLRC4 inflammasome including NLRC4 and PYCARD (also known as ASC) involved in the activation of inflammatory and apoptotic pathways peaked at 12h post transfection. IL17F, a pro-inflammatory cytokine that

activates expression of other chemokines, was induced between 24h and 36h time points²⁴. These DEGs identified in gene ontologies related to antiviral response suggests an active response to pathogen-associated stimuli in transfected HEK293.

[00166] Additional gene ontology categories for which DEGs were highly enriched were also identified by enrichment analysis. These include those identified in **Figs. 9A-9G**, **Figs. 10A-10C**, **Figs. 13A-13B**, and **Figs. 14A-14O**, which also describe the enriched DEGs within each ontology, and the expression fold changes of each DEG over the time course of the experiments (3 hr to 72 hr post transfection). More specifically, gene ontology categories for which DEGs were highly enriched include GO ID No. 0045814, Negative Regulation of Gene Expression, Epigenetic (**Fig. 9A**); GO ID No. 0000183, rDNA Heterochromatin Assembly (**Fig. 9B**); GO ID No. 0006335, DNA Replication-Dependent Nucleosome Assembly (**Fig. 9C**); GO ID No. 0034080, CENP-A Containing Nucleosome Assembly (**Fig. 9D**); GO ID No. 0032200, Telomere Organization (**Fig. 9E**); GO ID No. 0006352, DNA-Templated Transcription, Initiation (**Fig. 9F**); GO ID No. 0006336, DNA Replication-Independent Nucleosome (**Fig. 9G**); GO ID No. 0006955, Immune Response (**Fig. 10A**); GO ID No. 0009615, Response to Virus (**Fig. 10B**); GO ID No. 0045071, Negative Regulation of Viral Genome Replication (**Fig. 10C**); GO ID No. 0000188, Inactivation of MAPK Activity (**Fig. 13A**); GO ID No. 0006357, Regulation of Transcription by RNA Polymerase II (**Fig. 13B**); GO ID No. 0000398, mRNA Splicing, via Spliceosome (**Fig. 14A**); GO ID No. 0002227, Innate Immune Response in Mucosa (**Fig. 14B**); GO ID No. 0007565, Female Pregnancy (**Fig. 14C**); GO ID No. 0016233, Telomere Capping (**Fig. 14D**); GO ID No. 0019731, Antibacterial Humoral Response (**Fig. 14E**); GO ID No. 0030968, Endoplasmic Reticulum Unfolded Protein Response (**Fig. 14F**); GO ID No. 0036499, PERK-Mediated Unfolded Protein Response (**Fig. 14G**); GO ID No. 0044267, Cellular Protein Metabolic Process (**Fig. 14H**); GO ID No. 0045652, Regulation of Megakaryocyte Differentiation (**Fig. 14I**); GO ID No. 0045653, Negative Regulation of Megakaryocyte Differentiation (**Fig. 14J**); GO ID No. 0050911, Detection of Chemical Stimulus Involved in Sensory Perception of Smell (**Fig. 14K**); GO ID No. 0060337, Type I Interferon Signaling Pathway (**Fig. 14L**); GO ID No. 0060700, Regulation of Ribonuclease Activity (**Fig. 14M**); GO ID No. 0060964, Regulation of Gene silencing by miRNA (**Fig. 14N**); and GO ID No. 0071347, Cellular Response to Interleukin-1 (**Fig. 14O**).

[00167] While pathway analysis can discern the general functional categories of gene expression in response to rAAV production, it does not necessarily identify the triggers of these responses. To begin to dissect this mechanistic question, DEGs were clustered based on their expression pattern via time-series analysis followed by gene ontology enrichment analysis. This

time series analysis allows transcriptional changes to be correlated with the processes required for transfection, rAAV replication, and vector assembly. Pattern clustering method based on Dirichlet process Gaussian process (DPGP) was used to categorize similar expression dynamics on time-series data without a pre-specified number of clusters²⁵. By this method 13 clusters, with 142 genes on average per cluster were identified (**Figures 11A-11M**). Clusters containing at least 40 genes were then subjected to gene ontology enrichment analysis, to avoid the high risk of false positive enrichment associated with small gene sets²⁶. Five out of eight clusters had significantly enriched biological processes using gene ontology enrichment analysis (FDR <0.05) (**Figures 12A-12E**). These five clusters covered 62.8% of the DEGs. Based on the time that the normalized transcription reached above the y=0 axis, clusters with significantly enriched gene ontologies were categorized into early, mid-late and late stages.

[00168] Genes in the early cluster were induced at 3 and 6 hpt and reverted to basal expression level by 12 hpt (**Figure 3G**). Notable genes in this cluster included dual specificity phosphatases (DUSPs) 1, 8 and 16, regulate mitogen-activated protein kinase (MAPK) activity. (**Figure 3G, Figures 12A-12E**). The MAPK signaling pathways participate in transcription of immediate-early genes that respond to extracellular stimuli, including Early Growth Response 1 (EGR1), FOS, JUN and MYC (**Figures 12A-12E**)²⁷. The DUSPs feedback to negatively regulate stress kinase activity and are MAPK signaling-inducible genes responding to acute environmental stimuli such as transfection and pathogen recognition receptor signaling²⁸. The protein expression analysis of EGR1 validated the kinetic of EGR1 transcription across time points (**Figure 4A**). Based on the immediate early timing and MAPK pathway involvement, these genes are potentially induced by transfection-associated stress or the earliest stages of rAAV and adenovirus helper gene expression.

[00169] Genes in clusters that showed upregulated transcription in the mid-late and late clusters comprised the majority DEGs, and primarily consisted of nucleosome assembly, inflammatory response and type-I interferon-driven pathways described above (**Figures 3B-3G**). The mid-late cluster included inflammasome-regulated genes and histone subunits and was characterized by significant up-regulation 6h post transfection and sustained up-regulation 12h post transfection (**Figure 3G**). The late responding cluster included DEGs that were not induced until 24h post transfection. Among these, type I interferon induced genes were significantly enriched. Expression of interferon beta (IFNB1) was detected at these time points, albeit at low levels, and correlated with up-regulation of multiple ISGs including IFIT2 and OASL. This

expression pattern was confirmed by RT-qPCR in samples from transfected 2L bioreactors, which showed a consistent trend of mature polyadenylated IFNB1, IFIT2 and OASL mRNAs (**Figures 4B, 4E and 4F**). Interestingly, however, interferon beta protein could not be detected in culture media by ELISA at any timepoint after transfection. This may be due to rapid binding to ubiquitously expressed IFN receptors on HEK cells, maintaining steady state levels of IFNB below the limit of detection. CCL5, a pro-inflammatory ISG that can also be induced by activation of NF- κ B and IRF1^{29,30}, was strongly upregulated in the late cluster. CCL5 transcription was upregulated as early as 6h post transfection, while CCL5 secretion was significantly increased 24h post transfection (**Figures 4C and 4H**). Transcription of another ISG cytokine, IL-8, was also significantly increased followed by increase of IL-8 protein in media (**Figures 4D and 4I**)³¹. These results suggested that transcriptional up-regulation of innate immune response pathways was induced as early as 12h post transfection, with low level expression of type I interferons, which in turn induced cytokine production and the up-regulation of ISGs later in the process. Up-regulation of innate immune response in the cells (**Figure 2D**) correlated with the exponential increase in rAAV titer between 12-24 HPT, suggesting that HEK293 cells detect viral replication and respond with antiviral gene expression.

[00170] To gain insight into what host pathways may be responsible for the observed pattern of antiviral and inflammatory gene expression, we conducted analysis of potential upstream regulators, based on published gene regulatory databases (**Figure 5A**). Using this approach on DEGs identified at 72 HPT, 98 potential upstream regulators were predicted to affect 672 DEGs identified at 72 HPT (**Figure 5B**). This approach was then applied to all seven time points. Note that the upstream regulators were selected regardless of their expression level in the transcriptomic data during the analysis. However, genes that were not detectable prior to or after transfection are unlikely to regulate rAAV production. The union of all seven time points included 107 candidate upstream regulators with expression level higher than 0.5 TPM (**Figure 5C**). The majority of candidate regulators are transcription factors, pathogen recognition receptors, cytokines/cytokine receptors, protein kinases and growth factors. Key signaling nodes of the innate anti-pathogen response were predicted regulators, including interferon alpha/beta receptor subunit 1 (IFNAR1) and interferon regulatory factors (IRF) IRF1, IRF3, IRF5, and IRF7. Notably, toll-like receptors (TLRs) including TLR2 and TLR3, and innate immune signaling transduction adaptor MYD88, as well as both cyclic GMP-AMP synthetase (cGAS) and stimulator of interferon gene (STING1), were detectably expressed in transfected HEK293 and predicted as upstream regulators responsible for observed DEGs. This suggests that the process of plasmid

transfection and rAAV production directly deliver stimuli that induce anti-pathogen responses in HEK293 cells.

Discussion

[00171] Viral vectors are proving remarkably versatile in multiple approaches to gene therapy, both in vivo and ex vivo. However, given their distinct requirements for replication and packaging of the therapeutic genetic payload, viral vectors must be made in living cells. Both the manufacturing processes and the final drug product of viral gene therapy vectors are incompletely understood. Manufacturing highly pure, highly concentrated viral vectors is further complicated by the fact that the mammalian and insect cell lines used to produce them encode multiple pathogen recognition sensors and downstream signaling pathways that evolved to antagonize both exogenous gene expression and viral replication. HEK293 cells have gained broad acceptance as a cellular host for gene therapy vector production, due in part to their ease of transfection. HEK293 express low levels of sensors of exogenous DNA, which may contribute to their utility for viral vector production^{21,32}. However, our results indicate that HEK293 are not agnostic to the non-self-molecules and parasitic gene expression pathways required to produce viral vectors, but instead respond with robust upregulation of, among others, innate immune response genes. In this study, we identified, among others, three cellular responses, including (1) negative regulation of MAPK activity, (2) an inflammatory antiviral response, and (3) transcriptional up-regulation of histone genes.

[00172] Among these induced cellular responses, antiviral genes clearly have the potential to negatively impact rAAV production²⁹. The upregulated DEGs in significantly enriched GOs involving type-I interferon and inflammatory response were ISGs inducible not only directly by interferon but also by the interferon regulatory factor (IRF) family of transcription that are generally activated by pathogen sensors (Figure 3D and 3E)³³. These sensors, also known as pattern recognition receptors (PRR), recognize pathogen-associated molecular patterns (PAMP) from bacterial or viral infection³⁴. In combined with literature-based analysis, IRF1/3/5/7 along with PRRs including TLR2, TLR3, cGAS/STING, RIG-I (also known as DDX58) and PKR (also known as EIF2AK2) were implicated as candidate upstream regulators of ISG expression. An important adaptor of TLR signaling, MyD88, was also identified in the list of potential regulators. The PRRs are constitutively expressed for surveillance of exogenous stimuli and are also inducible by antiviral responses. Many TLRs are cell type specific, and most are not generally thought to be expressed in HEK293 cells. However, our data indicated detectable transcription of TLRs 2, 3, 5

and 6. Our analysis suggests that bacterial, viral DNA, or viral RNA in the cytosol, are being recognized by cognate nucleic acid sensors. While our data do not directly define the pathogen molecules or cellular sensors inducing the gene expression patterns we describe, a recent study demonstrated that rAAV transduction in mouse induced DNA sensor cGAS and antiviral response including TNF- α ³⁵. The potential for endosomal or cytosolic AAV DNA or dsRNA to activate PRRs has not been characterized in rAAV-producing bioreactors. Some HEK293 lines do not express the dominant key adapter of cytoplasmic DNA sensor, STING, while others are reported to²². We found that the HEK293 line used in our experiments do express low but detectable cGAS, which may detect transfected plasmid, or replicating or transduced AAV DNA during bioreactor culture. Multiple additional cytosolic sensors bind transfected plasmid DNA in some cell types, but the relative contribution of these sensors to the effects we observe in HEK293 is not known³⁶. TLR2, another potential regulator identified by our analyses, may be activated by bacterial cell wall components such as triacylated lipoproteins³⁷. The ligands for TLR2 activation could come from the residual bacterial lipoproteins derived from the E. coli plasmid host. Finally, AAV capsid has been reported as a PAMP recognized by TLR2 on the plasma membrane and induced pro-inflammatory cytokines through NF- κ B signaling³⁰. Note that the sensors and adaptors hypothesized above were transcriptionally expressed based on our RNA-seq data. This contradicts previous evidence that TLR family, cGAS, STING or RIG-I were not detectable in HEK293 cells²¹⁻²³. Consistent with our findings, a recent study identified “defense response” as one of significantly enriched pathways in a proteomic analysis between transfected AAV5-producing HEK293 cells and non-transfected control, likely responding to stress including transfection or exogenous components³⁸. Additional investigation may be used to elucidate exactly which sensors cause the induction of the observed antiviral response in HEK293 cells during rAAV production.

[00173] The MAPK family is one of the earliest kinase cascades activated in response to various stressors, including pathogen infection. Previous studies suggested that the internalization of polyethyleneimine (PEI) transfection reagent could induce cell stress and trigger PKC activation¹³. The stress response activates MAPK signaling pathway and transient up-regulation of immediate early genes including EGR1 and FOS/JUN gene families, as well as DUSPs that properly control the MAPK activation^{27,28}. The upregulation of the DUSPs is a negative feedback mechanism to prevent sustained MAPK activation, and several DUSPs have been implicated in downregulating the signaling cascades activated by PRR-PAMP interactions²⁸. While we observed upregulation

of only three DUSP family members, this finding in response to rAAV production is consistent with PRR pathway activation. Confirming this mechanism will require further studies.

[00174] We observed upregulation of 39 histone genes with mid-late kinetics, at 24 HPT. Histone gene transcription is significantly increased during S phase in mammalian cells³⁹. Histone gene transcription is tightly coupled with the progression of DNA synthesis and downregulated as cells exit S phase or by replication inhibitors^{40,41}. Therefore, histone up-regulation is a characteristic of high energy demand tied with active cell proliferation, or in the case of rAAV production, likely with intensive vector genome replication. Previous proteomic analysis on transfected HEK293 cells that generates viral-like particles identified significant up-regulation of histones and mitochondrial components under high metabolic demand conditions, which is consistent with our findings of increased histone expression⁴². The Adenovirus genes E1A and E4orf6 required for effective rAAV production inhibit Cdc2 and promote degradation of cyclin A, allow HEK293 cells stay in S phase⁴³. Therefore, the histone up-regulation could be a consequence of cell cycle regulation induced by helper proteins that facilitate AAV genome replication or vector production.

[00175] Production of biotherapeutics is heavily dependent upon the choice of producer cell line for efficient manufacture. In the case of monoclonal antibodies (mAbs) production, highly efficient CHO cell lines have been developed by adaptation and targeted modification⁴⁴. In contrast to mAbs and other recombinant protein products, the interaction between the host cell and viral replication during the manufacture of viral vectors is significantly more complicated. The AAV Rep proteins and helper genes E2a and E4 are cytotoxic⁴⁵⁻⁴⁷. In addition to producing the protein components of the vector, the cells must also host a robust viral DNA replication process. Viral replication frequently triggers an inhibitory innate immune response, which we demonstrate here is the case for rAAV production following plasmid transfection. The antiviral response has been shown to affect protein synthesis, cellular metabolism, cell proliferation and even promote programmed cell death, and these responses could significantly curtail rAAV production in HEK293 as well as other types of cells⁴⁸. Overexpression of cGAS/STING to activate DNA sensing response in lentiviral vector producing HEK293T cells activated promoters of NF- κ B and interferon- β promoters⁴⁹. The activation of the downstream innate immune response, however, did not strongly impact lentiviral vector production. Nevertheless, existing pathogen sensors that are constitutively expressed may play more significant roles in the regulation of vector production in these producer cell lines. A recent study improved the production of

lentiviral vector in HEK293 cells by 6.7-fold after knocking out constitutively expressed antiviral effectors including PKR and 2'-5'-oligoadenylate synthetase 1 (OAS1), suggesting that host pathways were capable of inhibiting vector production⁵⁰. This is consistent with our finding on the induction of antiviral response during rAAV production. Together, the time-course transcriptomic analysis revealed the cellular response and potential pathways that the host cells use to recognize exogenous stimuli throughout the process of rAAV vector production in suspension HEK293 cells at manufacturing scale for the first time. Our experiments improve our understanding of the state of the host cell during rAAV production, results which led us to strategies for improving vector production platforms better able to meet the growing clinical demand for these therapeutics.

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EXAMPLE 2

Repressing Expression of Upregulated Genes Increases AAV Vector Titer

[00176] A subset of 302 DEGs identified in Example 1 were selected for further study to determine if repressing their expression in host cells individually or in combination through RNA interference would be effective to increase yield of AAV vectors. The selected DEGs included both genes corresponding to upstream regulators (UR) identified using a list of differentially expressed genes and protein networks in the Ingenuity Pathway Analysis (IPA) tool as well as genes the transcriptional change for which was at least 10-fold greater compared with controls at any time point post transfection in the RNA-seq data.

[00177] An optimized screening assay was developed in which synthetic siRNA targeting each of the selected DEGs was transfected into suspension adapted HEK293 cells, after which treated cells were transfected with a mixture of 3 plasmids for production of an AAV vector. Vector yields were then determined relative to controls. Briefly, HEK293 cells grown in suspension culture were added to 96 well plates in 1 mL medium. Cells were then transfected with a mixture of 25nM of siRNA specific for each selected DEG and 1uL of RNAiMAX transfection reagent. After transfection, the cell concentration was 5×10^5 cells/mL. Twenty-four hours after siRNA transfection, cells were transfected with a mixture of 3 plasmids (transgene, helper and rep/cap in a 1:1:2 molar ratio) and polyethyleneimine (PEI) transfection reagent for production of AAV9 vectors containing a genome encoding a mini-dystrophin protein. Seventy-two hours later, titer of AAV vectors produced by the cells was determined by qPCR. As a proxy for the number of viable cells, ATP concentration per well was determined using a Cell-Titer Glo 2.0 Luminescence assay. Two positive and one negative control siRNAs were also tested. The positive control siRNAs included one with a sequence targeting the *cap* gene transcript expressed from the rep/cap plasmid (i3663), which reduces AAV vector titer, and the second targeting the *rep* gene transcript (i782), which increases AAV vector titer by reducing transcripts from the p5 and p19

promoters. The negative control siRNA included sequence targeting no known region of the human genome (iNON) and was therefore expected to have no specific effect on AAV vector titers, and thus reflected the background effect of treatment conditions. Four replicates for each DEG were performed and results analyzed statistically using Dunnetts's T3 multiple comparison test on pairwise comparisons between results for each gene tested and the iNON negative control. Results of the first-round screening assay are shown in **Table 32**, below, which identifies each gene repressed by siRNA transfection, the average AAV titer produced after treatment with DEG-specific siRNA normalized to the AAV titer produced after treatment with the iNON siRNA (i.e., corrected for background), as well as the average AAV titer relative to iNON further normalized to correct for the average amount of ATP per experimental replicate.

Table 32

Gene Symbol	ENTREZ ID	UniProt ID	Mean fold increase in AAV titer relative to iNON	Mean fold increase in AAV titer relative to ATP
CHRM4	1132	P08173	0.70	0.69
ACKR2	1238	O00590	0.71	0.68
ACTA1	58	P68133	1.47	2.44
AHSP	51327	Q9NZD4	0.31	0.34
ALPK2	115701	Q86TB3	0.88	0.83
ANKRD1	27063	Q15327	0.68	1.06
ANLN	54443	Q9NQW6	1.03	1.04
APEX1	328	P27695	0.87	0.98
APP	351	P05067	0.84	0.70
ARID1B	57492	Q8NFD5	0.70	0.76
ARID3B	10620	Q8IVW6	0.81	1.74
ARL14	80117	Q8N4G2	1.41	1.56
ARNT2	9915	Q9HBZ2	1.02	0.88
ASB5	140458	Q8WWX0	0.68	0.59
ATF4	468	P18848	1.01	2.19
BAZ1A	11177	Q9NRL2	0.71	0.81
BAZ2A	11176	Q9UIF9	1.00	0.91
BDNF	627	P23560	1.39	1.06
BICRA	29998	Q9NZM4	0.97	0.84
BOD1L1	259282	Q8NFC6	0.76	0.75
BRD8	10902	Q9H0E9	0.61	0.44
C17orf64	124773	Q86WR6	0.21	0.19
C1orf52	148423	Q8N6N3	1.04	0.85
C2CD6	151254	Q53TS8	1.47	1.50
C5	727	P01031	0.45	0.43
C9orf152	401546	Q5JTZ5	0.97	1.37
CALCR	799	P30988	1.25	1.24

CAMP	820	#N/A	0.40	0.45
CATSPERD	257062	Q86XM0	0.68	0.86
CBX5	23468	P45973	1.03	0.83
CCL4	6351	P13236	0.54	0.36
CCL5	6352	P13501	0.68	0.63
CEBPA	1050	P49715	0.72	0.83
CEBPB	1051	P17676	0.83	0.79
CFDP1	10428	Q9UEE9	0.76	0.63
CGA	1081	P01215	0.47	0.63
CGAS	115004	Q8N884	0.86	0.68
CHD1	1105	O14646	0.60	0.36
CHD7	55636	Q9P2D1	0.61	0.49
CHD8	57680	Q9HCK8	0.84	0.80
CHUK	1147	O15111	1.02	0.88
CIB3	117286	Q96Q77	0.72	0.93
CISH	1154	Q9NSE2	0.59	0.52
CLCA2	9635	Q9UQC9	0.88	0.99
CLDN9	9080	O95484	0.60	0.67
CNTD2	79935	Q9H8S5	0.99	1.11
COL6A5	256076	A8TX70	1.35	1.31
CPA2	1358	P48052	0.91	0.94
CREB1	1385	P16220	0.95	1.77
CREBBP	1387	Q92793	0.69	0.57
CRNKL1	51340	Q9BZJ0	0.32	0.14
CSF1	1435	P09603	1.32	1.53
CSTA	1475	P01040	0.90	0.97
CUX1	1523	P39880	0.31	0.35
CUZD1	50624	Q86UP6	0.79	0.81
CXCL11	6373	O14625	1.53	1.75
CXCL8	3576	P10145	0.82	1.06
CXCR2	3579	P25025	0.60	0.68
DACH1	1602	Q9UI36	1.00	0.97
DDR2	4921	Q16832	0.57	0.64
DDX58	23586	O95786	0.88	0.90
DHRS2	10202	Q13268	0.60	0.58
DOCK8	81704	Q8NF50	1.20	1.47
EGF	1950	P01133	0.82	0.66
EGR1	1958	P18146	0.45	0.50
EGR3	1960	Q06889	0.68	0.79
EIF2AK2	5610	P19525	1.71	1.23
ELAVL3	1995	Q14576	0.52	0.44
ELK1	2002	P19419	1.12	0.70
ELOVL3	83401	Q9HB03	1.16	1.15
ELSPBP1	64100	Q96BH3	1.02	1.14
EMSY	56946	Q7Z589	0.85	1.38
EP300	2033	Q09472	1.43	1.69
EP400	57634	Q96L91	0.56	0.24
EPAS1	2034	Q99814	0.97	0.81

EPHB1	2047	P54762	1.37	1.12
ERBB2	2064	P04626	1.00	1.53
ESRP1	54845	Q6NXG1	1.36	1.85
ETV7	51513	Q9Y603	1.12	1.16
FAM192A	80011	Q9GZU8	0.39	0.19
FAM24B	196792	Q8N5W8	0.69	0.49
FAM83A	84985	Q86UY5	1.27	2.30
FCGR2A	2212	P12318	0.75	0.87
FEV	54738	Q99581	0.82	0.67
FGF2	2247	P09038	2.15	1.60
FGF21	26291	Q9NSA1	0.59	0.59
FNDC11	79025	Q9BVV2	0.76	0.93
FOS	2353	P01100	1.08	1.30
FOSB	2354	P53539	1.08	0.91
FOXJ1	2302	Q92949	1.07	1.11
FOXO1	2308	Q12778	1.54	1.43
FOXO3	2309	O43524	0.75	0.54
FRG2B	441581	Q96QU4	0.94	0.90
FUT6	2528	P51993	0.62	0.67
GAST	2520	P01350	0.58	0.49
GDF15	9518	Q99988	0.50	0.36
GJB5	2709	O95377	0.64	0.61
GNRH1	2796	P01148	1.89	1.49
GPATCH4	54865	#N/A	0.75	0.59
GPBR1	2852	Q99527	1.15	1.17
GSE1	23199	Q14687	0.51	0.41
GSG1L	146395	Q6UXU4	0.62	0.77
GTF2I	2969	P78347	0.79	0.68
GTF3C1	2975	Q12789	0.83	1.37
H1-2	3006	P16403	0.75	0.69
H2AC14	8331	Q99878	1.06	0.93
H2AC15	8330	#N/A	0.62	0.62
H2AC19	723790	#N/A	0.65	0.69
H2AC4	8335	#N/A	0.29	0.31
H2AC6	8334	Q93077	0.70	0.61
H2AC8	3012	P04908	0.38	0.30
H3C1	8350	#N/A	0.43	0.43
H3C2	8358	#N/A	0.63	0.53
H4C4	8360	#N/A	0.44	0.38
H4C5	8367	#N/A	0.48	0.41
HGD	3081	Q93099	0.91	1.07
HGF	3082	P14210	1.45	1.33
HIF1A	3091	Q16665	0.69	0.70
HMGA1	3159	P17096	0.59	0.55
HS3ST4	9951	Q9Y661	1.36	1.14
HTATSF1	27336	O43719	0.71	0.56
IFI27	3429	P40305	0.59	0.69
IFI44	10561	Q8TCB0	0.77	0.77

IFI44L	10964	Q53G44	1.39	1.42
IFIT2	3433	P09913	0.61	0.66
IFIT3	3437	O14879	0.99	1.10
IFNAR1	3454	P17181	1.03	1.09
IFNB1	3456	P01574	1.19	1.33
IKBKB	3551	O14920	0.80	0.73
IKBKE	9641	Q14164	0.41	0.60
IKBKG	8517	Q9Y6K9	0.66	0.73
IL17F	112744	Q96PD4	1.03	1.25
IL18	3606	Q14116	0.87	0.64
IL33	90865	O95760	1.08	0.57
IL5	3567	P05113	1.01	1.12
IRF1	3659	P10914	1.10	1.14
IRF3	3661	Q14653	1.29	1.20
IRF5	3663	Q13568	0.59	0.89
IRF7	3665	Q92985	0.87	0.88
ITGAM	3684	P11215	1.28	1.43
JUN	3725	P05412	0.44	0.69
JUNB	3726	P17275	1.23	1.71
KDM1A	23028	O60341	0.82	0.81
KDM3A	55818	Q9Y4C1	0.73	0.61
KDM3B	51780	Q7LBC6	0.90	1.20
KIF20B	9585	Q96Q89	0.61	0.16
KITLG	4254	P21583	1.33	1.55
KLHDC7B	113730	Q96G42	1.13	0.98
KNL1	57082	Q8NG31	0.69	0.61
KRTAP19-1	337882	Q8IUB9	0.40	0.44
KRTAP19-5	337972	Q3LI72	1.05	0.98
KRTAP5-2	440021	Q701N4	0.91	0.92
LAPTM5	7805	Q13571	1.17	1.22
LRRC3C	100505591	A6NJW4	1.17	1.20
LRRTM2	26045	O43300	0.84	1.11
LST1	7940	O00453	0.70	0.71
MAP2K1	5604	Q02750	1.09	1.05
MAP2K3	5606	P46734	1.51	1.52
MAP3K1	4214	Q13233	1.08	1.50
MAPK1	5594	P28482	1.34	1.53
MAPK14	1432	Q16539	0.72	0.54
MAPK3	5595	P27361	1.17	1.76
MAPK8	5599	P45983	1.06	1.13
MAVS	57506	Q7Z434	1.05	0.81
MC1R	4157	Q01726	1.19	1.04
MED1	5469	Q15648	0.38	0.57
MEF2C	4208	Q06413	0.62	0.58
MEF2D	4209	Q14814	0.48	0.49
MGA	23269	Q8IW/9	0.78	0.56
MIF	4282	P14174	1.39	2.63
MOBP	4336	Q13875	0.81	1.14

MPIG6B	80739	O95866	0.75	0.71
MSANTD2	79684	Q6P1R3	0.92	1.04
MSC	9242	O60682	1.32	0.95
MSH6	2956	P52701	0.26	0.44
MSLN	10232	Q13421	0.46	0.42
MYD88	4615	Q99836	1.25	0.98
NCOR1	9611	O75376	0.36	0.98
NEURL3	93082	Q96EH8	0.60	0.78
NFKB1	4790	P19838	0.55	0.69
NFKBIA	4792	P25963	1.02	1.29
NGF	4803	P01138	1.54	1.33
NR2C1	7181	P13056	1.04	0.81
NUCKS1	64710	Q9H1E3	0.91	0.50
NUMA1	4926	Q14980	0.43	0.45
NUPR1	26471	O60356	0.59	0.63
OAS1	4938	P00973	0.68	0.67
OAS3	4940	Q9Y6K5	2.18	1.54
OASL	8638	Q15646	0.98	0.76
ODAPH	152816	Q17RF5	1.26	1.45
ODF4	146852	Q2M2E3	0.46	0.59
OR2B6	26212	P58173	0.61	1.01
PAF1	54623	Q8N7H5	2.19	2.09
PAPOLG	64895	Q9BWT3	0.51	0.23
PARP1	142	P09874	0.35	0.45
PDE6G	5148	P18545	1.50	1.11
PHF21A	51317	Q96BD5	1.00	0.68
PLA2G1B	5319	P04054	0.61	0.61
PML	5371	P29590	1.08	1.06
PNN	5411	Q9H307	1.47	1.57
POLD1	5424	P28340	0.99	1.01
POLD3	10714	Q15054	0.59	0.57
PPIL4	85313	Q8WUA2	0.34	0.12
PPRC1	23082	Q5VV67	2.97	2.90
PRDM1	639	O75626	0.56	0.45
PRKCD	5580	Q05655	0.96	0.70
PSG4	5672	Q00888	0.65	0.56
PSMD8	5714	P48556	0.06	0.04
PTPRJ	5795	Q12913	0.92	0.82
PTX3	5806	P26022	1.74	2.04
RAB33A	9363	Q14088	0.85	1.00
RAF1	5894	P04049	1.00	1.14
RBM10	8241	P98175	0.87	0.67
RBPJ	3516	Q06330	0.94	0.93
RC3H1	149041	Q5TC82	2.67	4.18
RELA	5970	Q04206	0.98	0.84
REST	5978	Q13127	1.16	1.25
RET	5979	P07949	0.50	0.52
RETN	56729	Q9HD89	0.76	0.70

RFC1	5981	P35251	0.15	0.42
RFPL4A	342931	A6NLU0	0.37	0.38
RFPL4AL1	729974	F8VTS6	0.61	0.82
RIF1	55183	Q5UIP0	0.37	0.46
RNF113A	7737	O15541	0.63	0.27
RPA1	6117	P27694	0.13	0.48
RSAD2	91543	Q8WXG1	0.43	0.35
RSF1	51773	Q96T23	0.52	0.34
S100P	6286	P25815	1.09	1.18
SAFB	6294	Q15424	0.57	0.46
SAP30BP	29115	Q9UHR5	0.64	0.76
SASH1	23328	O94885	0.70	0.76
SBSN	374897	Q6UWP8	0.33	0.31
SCG2	7857	P13521	1.15	1.13
SETD2	29072	Q9BYW2	0.26	0.17
SIM1	6492	P81133	1.09	1.01
SIRT1	23411	Q96EB6	0.62	0.74
SLC22A1	6580	O15245	0.50	0.49
SLC7A4	6545	O43246	0.87	0.94
SLPI	6590	P03973	1.10	1.04
SLU7	10569	O95391	0.08	0.06
SMAD3	4088	P84022	1.31	1.32
SMARCA4	6597	P51532	1.75	1.37
SMARCB1	6598	Q12824	0.95	0.77
SMPD1	6609	P17405	1.58	1.41
SMPX	23676	Q9UHP9	0.92	0.80
SNW1	22938	Q13573	0.30	0.55
SOCS1	8651	O15524	0.82	1.02
SOCS3	9021	O14543	1.07	1.35
SOX30	11063	O94993	0.56	0.68
SP110	3431	Q9HB58	1.86	1.45
SPEN	23013	Q96T58	0.35	1.80
SPI1	6688	P17947	1.31	1.63
SPRY4	81848	Q9C004	0.80	0.81
SRCAP	10847	Q6ZRS2	0.64	0.82
SRF	6722	P11831	0.58	0.48
SRMS	6725	Q9H3Y6	0.86	1.21
STAT1	6772	P42224	0.91	0.78
STAT4	6775	Q14765	1.74	0.99
STING1	340061	Q86WV6	0.69	0.63
STMN4	81551	Q9H169	0.68	0.75
SUGP1	57794	Q8IWZ8	0.74	0.65
TANGO6	79613	Q9C0B7	0.42	0.14
TCEAL7	56849	Q9BRU2	0.40	0.46
TCF20	6942	Q9UGU0	0.86	0.79
TFIP11	24144	Q9UBB9	0.81	0.69
TFRC	7037	P02786	0.53	0.59
TGFB1	7040	P01137	0.30	0.29

TGM2	7052	P21980	1.23	1.18
TICAM1	148022	Q8IUC6	1.94	1.40
TLR2	7097	O60603	1.99	1.11
TLR3	7098	O15455	0.92	0.87
TNFRSF9	3604	Q07011	0.46	0.32
TNNC2	7125	P02585	0.72	0.76
TOP2A	7153	P11388	0.34	0.28
TOP2B	7155	Q02880	0.88	1.07
TP53	7157	P04637	0.66	0.53
TPR	7175	P12270	0.22	0.16
TRAF3IP2	10758	O43734	0.55	0.43
TRANK1	9881	O15050	0.46	0.47
TRIM24	8805	O15164	1.71	1.38
TUBB3	10381	Q13509	0.37	0.31
TYRP1	7306	P17643	0.92	0.77
UBN2	254048	Q6ZU65	0.75	1.08
VEGFA	7422	P15692	0.62	0.69
VRTN	55237	Q9H8Y1	1.05	1.25
WAPL	23063	Q7Z5K2	0.70	0.51
WDHD1	11169	O75717	0.21	0.16
WRNIP1	56897	Q96S55	0.83	0.45
YEATS2	55689	Q9ULM3	0.76	0.75
YIPF7	285525	Q8N8F6	0.55	0.77
YY1	7528	P25490	1.21	1.28
ZBTB11	27107	O95625	0.19	0.17
ZC3H11A	9877	O75152	0.50	1.40
ZDBF2	57683	Q9HCK1	1.01	0.97
ZFHX4	79776	Q86UP3	0.56	0.60
ZHX3	23051	Q9H4I2	0.79	0.91
ZMYM4	9202	Q5VZL5	0.55	0.94
ZNF148	7707	Q9UQR1	0.93	0.90
ZNF281	23528	Q9Y2X9	0.91	0.85
ZNF318	24149	Q5VUA4	0.76	0.78
ZNF451	26036	Q9Y4E5	1.00	0.88
ZSCAN10	84891	Q96SZ4	0.74	0.59

[00178] Based on the results from the first-round screen, a subset of DEGs observed to increase AAV titer by at least 1.4-fold, either relative to background and/or to ATP levels, as well as select other DEGs, were selected for further analysis to confirm that their repression by RNAi in HEK293 host cells led to an increase in AAV titer. A similar experimental format used in the first-round screen was employed, but the effect of siRNA treatment on AAV titer was tested in 3 independent experiments with 4 replicates each. Results from the second-round screen are shown in **Table 33** below, which confirmed that repressing expression of a substantial number

of the genes identified from the first-round screen increased the titer of AAV vectors produced from the treated HEK293 host cells.

Table 33

Gene Symbol	Mean fold increase in AAV titer relative to iNON	Mean fold increase in AAV titer relative to ATP
iNON	1.00	1.00
i3663	0.20	0.15
i782	2.43	2.43
ACTA1	1.70	2.23
ARL14	1.13	1.17
BDNF	1.34	1.13
C2CD6	0.90	0.85
CALCR	1.28	1.19
COL6A5	0.87	0.82
CSF1	1.13	1.24
CXCL11	1.12	1.27
DOCK8	1.30	1.51
EIF2AK2	1.14	1.42
EP300	1.40	1.45
EPHB1	1.31	1.33
ESRP1	1.26	1.71
FAM83A	1.42	1.65
FGF2	1.27	1.95
FOXO1	1.20	1.23
GNRH1	1.17	1.24
HGF	1.08	0.97
HS3ST4	1.47	1.37
IFI44L	1.75	1.53
IFNB1	0.89	0.89
IRF3	0.83	0.91
ITGAM	1.32	1.43
JUNB	0.73	0.92
KITLG	1.08	1.36
MAP2K3	1.70	1.65
MAPK1	1.40	1.25
MAPK3	1.27	1.35
MIF	1.35	1.45
MSC	1.13	1.21
MYD88	1.60	1.52
NGF	0.82	0.97
OAS3	1.55	1.81
ODAPH	1.15	1.15
PAF1	1.96	1.99

PDE6G	1.46	1.03
PNN	1.33	1.75
PPRC1	2.04	2.47
PTX3	1.33	1.63
RC3H1	1.01	1.27
SMAD3	0.79	0.95
SMARCA4	1.01	1.05
SMPD1	0.95	0.95
SP110	1.08	1.39
SPI1	0.86	1.28
STAT4	1.28	1.09
TGM2	1.26	1.59
TICAM1	1.47	1.49
TLR2	0.72	0.78
TRIM24	0.94	1.14
YY1	0.80	1.01

[00179] It was next tested whether repressing expression in of more than one of the genes observed to increase AAV vector titer when repressed singly might further increase AAV vector production in HEK293 host cells. Using an assay format similar those described above, HEK293 cells were transfected with siRNA targeting MIF, OAS3, PAF1, RC3H1, and PPRC1 alone, and in combinations targeting two of these genes simultaneously, as well as in combination with a siRNA targeting expression of the AAV *rep* gene (i782). Twenty-four hours thereafter, treated cells were transfected with the 3-plasmid mixture directing their production of the AAV9 mini-dys vector, and titers determined 72 hours later. The results, shown in **Figure 15**, demonstrated that combined simultaneous repression of two of these genes, or combined simultaneous repression of one of these genes and *rep* expression, often resulted in enhanced AAV vector production by the treated HEK293 host cells. To test for off-target effects of co-transfecting cells with siRNA targeting two different genes simultaneously, total RNA was prepared from selected host cells used in the experiment and analyzed by RT-qPCR to determine the amount of targeted gene expression before and after siRNA treatment. The results, shown in **Figures 16A-16D**, indicate that while the efficiency with which targeted genes were repressed by siRNA treatment varied from gene to gene, the extent of repression was highly consistent even in the presence of other siRNA to target expression of other genes. These results suggested that for the 4 genes examined, off-target effects by the other siRNA affecting expression levels of the gene under examination were minimal or non-existent.

CLAIMS

What is claimed is:

1. A modified animal cell with reduced expression of a gene upregulated in an otherwise unmodified animal cell of the same type in response to infection by an adeno-associated virus (AAV), or to biosynthesis of recombinant AAV (rAAV) vector directed by exogenously introduced nucleic acid, wherein said upregulated gene is associated with a reduction in the amount of intact AAV vector produced in said unmodified animal cell of the same type relative to the amount of intact AAV vector produced in said modified animal cell.
2. The modified animal cell of claim 1, wherein said modified animal cell is an insect cell and the exogenously introduced nucleic acid is introduced into an otherwise unmodified insect cell of the same type by infection with a baculovirus, or wherein said modified animal cell is a mammalian cell and the exogenously introduced nucleic acid is introduced into an otherwise unmodified mammalian cell of the same type by transfection.
3. The modified animal cell of claim 2, wherein said modified animal cell is a mammalian cell selected from the group consisting of: HeLa cells, Cos cells, HEK293 cells A549 cells, BHK cells, Vero cells, NIH 3T3 cells, HT-1080 cells, Sp2/0 cells, NS0 cells, C127 cells, AGE1.HN cells, CAP cells, HKB-11 cells, WI-38 cells, MRC-5 cells, and PER.C6 cells.
4. The modified mammalian cell of claim 3, wherein said mammalian cell is a HEK293 cell, or derivative or variant thereof.
5. The modified mammalian cell of any one of claims 3 to 4, wherein said exogenously introduced nucleic acid is introduced into an otherwise unmodified mammalian cell of the same type by transient transfection of one or more DNA plasmids.
6. The modified animal cell of any one of claims 1 to 5, wherein the expression level of said upregulated gene is reduced pre-transcriptionally or post-transcriptionally.
7. The modified animal cell of any one of claims 1 to 6, wherein the expression level of said upregulated gene is reduced by contacting said cell with a compound effective to repress expression of said upregulated gene transiently or permanently.
8. The modified animal cell of any one of claims 1 to 7, wherein the expression level of said upregulated gene is repressed by RNase H recruitment, by RNA interference (RNAi), or by CRISPR interference (CRISPRi).

9. The modified animal cell of claim 8, wherein RNAi is effected by contacting said cell with, or by expressing in said cell, a guide strand RNA molecule effective to target an RNA transcript transcribed from said upregulated gene, wherein said guide strand RNA molecule is comprised by a siRNA molecule, a shRNA molecule, or a miRNA molecule.
10. The modified animal cell of claim 9, wherein said guide strand RNA molecule, siRNA molecule, shRNA molecule, or miRNA molecule, is expressed from an expression construct introduced into said cell transiently or stably.
11. The modified animal cell of any one of claims 1 to 10, wherein the expression level of said upregulated gene is reduced at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, 96%, 97%, 98%, or 99% compared to the expression of level of said upregulated gene in unmodified animal cells of the same type.
12. The modified animal cell of any one of claims 1 to 6, wherein said upregulated gene is inactivated.
13. The modified animal cell of claim 12, wherein said cell is inactivated by deleting nucleotides from, adding nucleotides to, or substituting nucleotides within at least one DNA sequence, or portion thereof, that is required for said upregulated gene to be transcribed or translated from one or both alleles of said gene.
14. The modified animal cell of claim 13, wherein said DNA sequence required for said upregulated gene to be transcribed or translated is selected from the group consisting of: a silencer sequence, an insulator sequence, a histone binding site, an enhancer sequence, a promoter sequence, a translation initiation sequence, an exon sequence, an intron sequence, a splice donor or acceptor sequence, a stop codon, and a transcription termination signal sequence.
15. The modified animal cell of any one of claims 12 to 14, wherein said upregulated gene is inactivated by deleting all the exons of said gene, or by introducing at least one premature stop codon into the coding sequence of said gene, or by homologous recombination, or by gene editing.
16. The modified animal cell of claim 15, wherein said gene editing is effected by expression of a zinc-finger nuclease protein, a transcription-activator-like effector nuclease (TALEN), or a CRISPR-Cas complex comprising a Cas enzyme and guide RNA.
17. The modified animal cell of any one of claims 1 to 16, wherein said upregulated gene is selected from the group consisting of: ACTA1, ARL14, BDNF, CALCR, CSF1, CXCL11, DOCK8,

EIF2AK2, EP300, EPHB1, ESRP1, FAM83A, FGF2, FOXO1, GNRH1, HGF, HS3ST4, IFI44L, ITGAM, KITLG, MAP2K3, MAPK1, MAPK3, MIF, MSC, MYD88, OAS3, ODAPH, PAF1, PDE6G, PNN, PPRC1, PTX3, RC3H1, SMARCA4, SP110, SPI1, STAT4, TGM2, TICAM1, and TRIM24.

18. A method for producing an adeno-associated virus (AAV) vector, comprising introducing into the modified animal cell of any one of claims 1 to 17 exogenous genetic information required for AAV vector biosynthesis, incubating said cell under conditions and for time sufficient for the biosynthesis of said AAV vector to occur, and purifying from said cell the AAV vector produced thereby.

19. The method of claim 18, wherein the exogenous genetic information required for AAV vector biosynthesis comprises an AAV cap gene, an AAV rep gene, nucleotide sequence encoding a viral helper RNA and protein, and an AAV vector genome.

20. The method of claim 18 or claim 19, wherein the exogenous genetic information required for AAV vector biosynthesis is introduced into said modified animal cell in the form of one or more recombinant baculoviruses by infection, or wherein the exogenous genetic information required for AAV vector biosynthesis is introduced into said modified animal cell in the form of one or more DNA plasmids by transient or stable transfection.

21. The method of any one of claims 18 to 20, wherein said modified animal cell is a modified HEK293 cell, or derivative or variant thereof.

22. The method of any one of claims 18 to 21, wherein said method is effective to produce an amount of AAV vector that is at least 50%, 100%, 150%, 200%, 250%, 300%, 350%, 400%, 450%, or 500% greater than the amount of AAV vector produced in an unmodified animal cell of the same type under otherwise identical conditions.

23. An AAV vector produced according to the method of any one of claims 18 to 21.

24. An expression vector comprising an expression cassette for expressing in a host cell a miRNA, a shRNA, or other type of non-coding RNA capable of repressing by RNA interference expression of at least one gene upregulated in response to infection by an adeno-associated virus (AAV), or to biosynthesis of recombinant AAV (rAAV) vector directed by exogenously introduced nucleic acid.

25. The expression vector of claim 24, wherein said expression cassette expresses a miRNA under control of an RNA pol II promoter, or a shRNA under control of an RNA pol III promoter.

- 26.** The expression vector of claim 24 or claim 25, wherein said gene is selected from the group consisting of: ACTA1, ARL14, BDNF, CALCR, CSF1, CXCL11, DOCK8, EIF2AK2, EP300, EPHB1, ESRP1, FAM83A, FGF2, FOXO1, GNRH1, HGF, HS3ST4, IFI44L, ITGAM, KITLG, MAP2K3, MAPK1, MAPK3, MIF, MSC, MYD88, OAS3, ODAPH, PAF1, PDE6G, PNN, PPRC1, PTX3, RC3H1, SMARCA4, SP110, SPI1, STAT4, TGM2, TICAM1, and TRIM24.
- 27.** A method for producing an adeno-associated virus (AAV) vector, comprising introducing into a host cell the expression vector of any one of claims 24 to 26 and then, contemporaneously therewith or subsequently, introducing into said host cell exogenous genetic information required for AAV vector biosynthesis, incubating said cell under conditions and for time sufficient for the biosynthesis of said AAV vector to occur, and purifying from said host cell the AAV vector produced thereby.
- 28.** The method of claim 27, wherein the exogenous genetic information required for AAV vector biosynthesis comprises an AAV cap gene, an AAV rep gene, a nucleotide sequence encoding a viral helper RNA and protein, and a nucleotide sequence for an AAV vector genome.
- 29.** The method of claim 27 or claim 28, wherein both the expression vector and the exogenous genetic information required for AAV vector biosynthesis is introduced into said host cell in the form of one or more DNA plasmids by transient transfection.
- 30.** The method of claim 29, wherein said host cell is a HEK293 cell, or derivative or variant thereof.
- 31.** The method of any one of claims 27 to 30, wherein said method is effective to produce an amount of AAV vector that is at least 50%, 100%, 150%, 200%, 250%, 300%, 350%, 400%, 450%, or 500% greater than the amount of AAV vector produced in a host cell of the same type in the absence of introducing said expression vector comprising said expression cassette.
- 32.** An AAV vector produced according to the method of any one of claims 27 to 31.

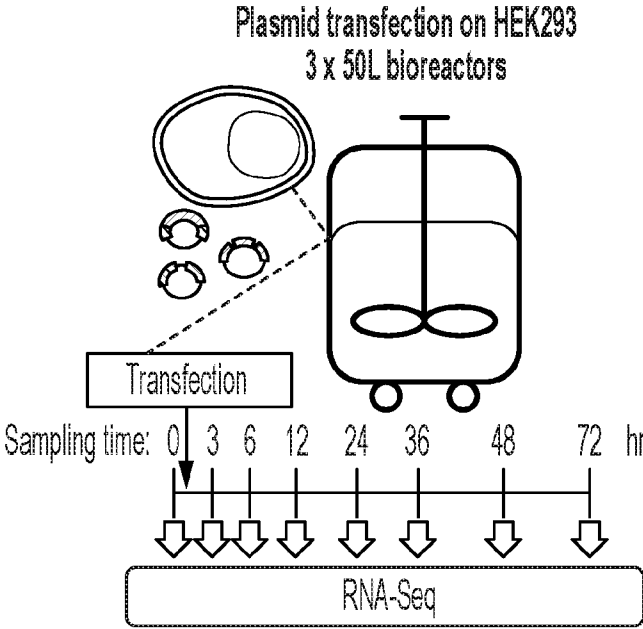
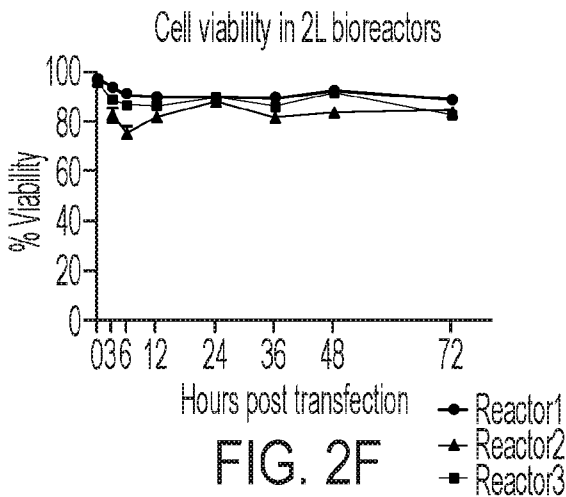
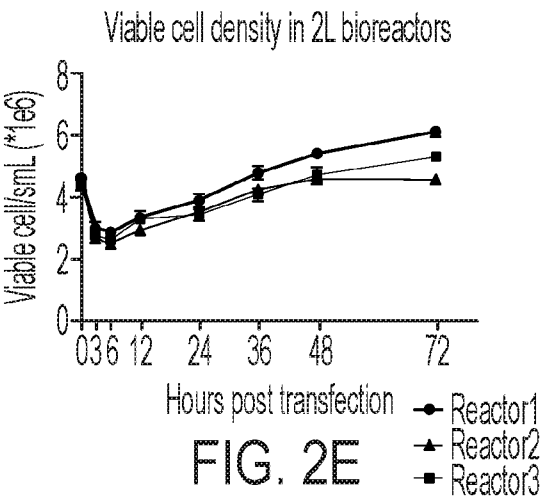
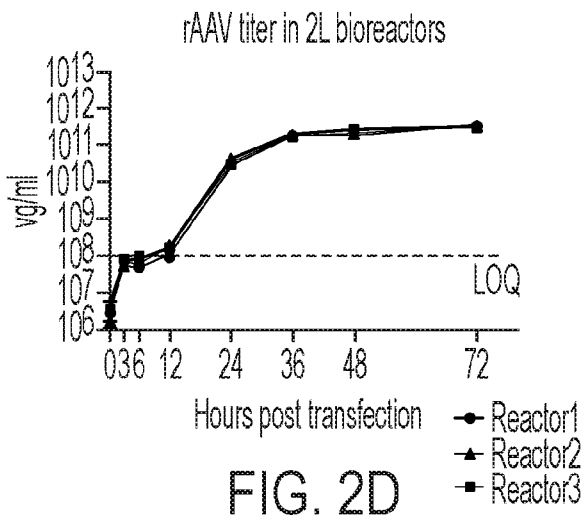
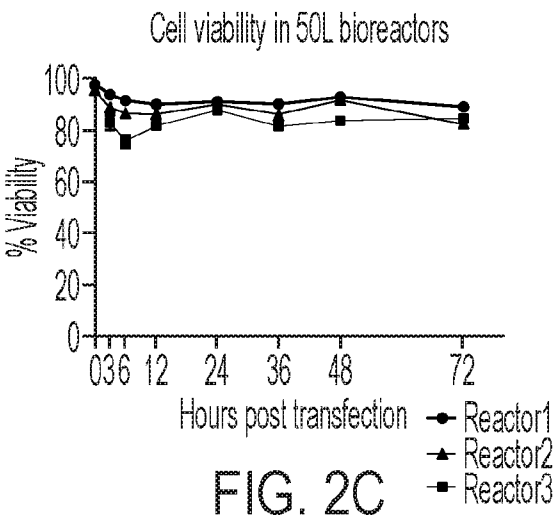
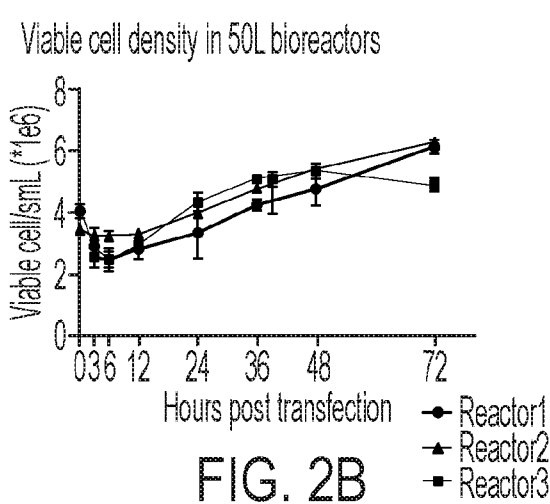
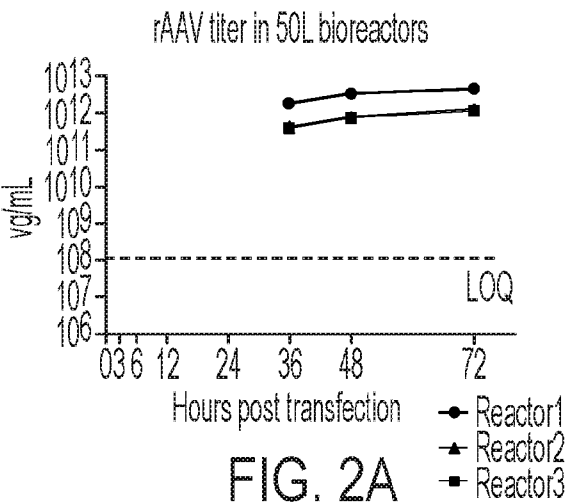


FIG. 1



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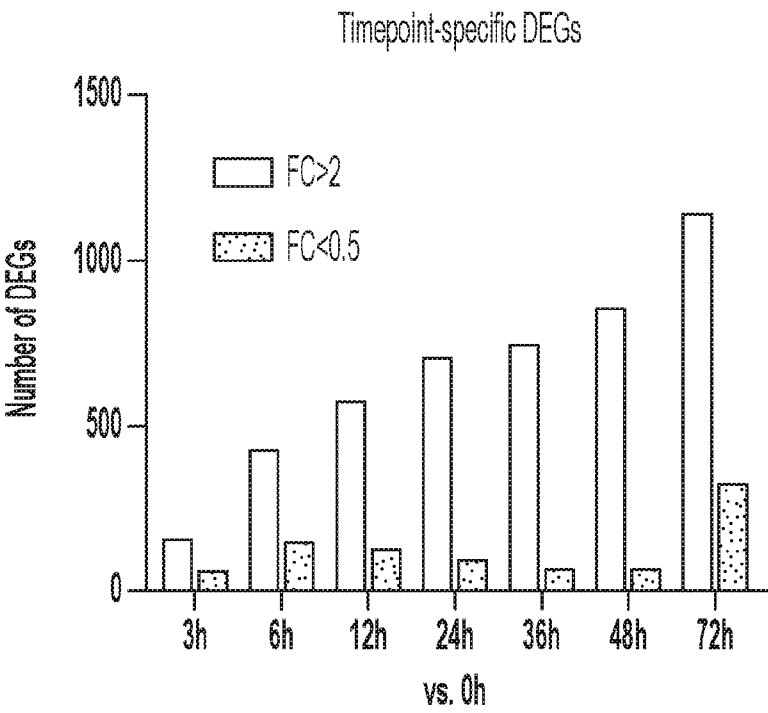
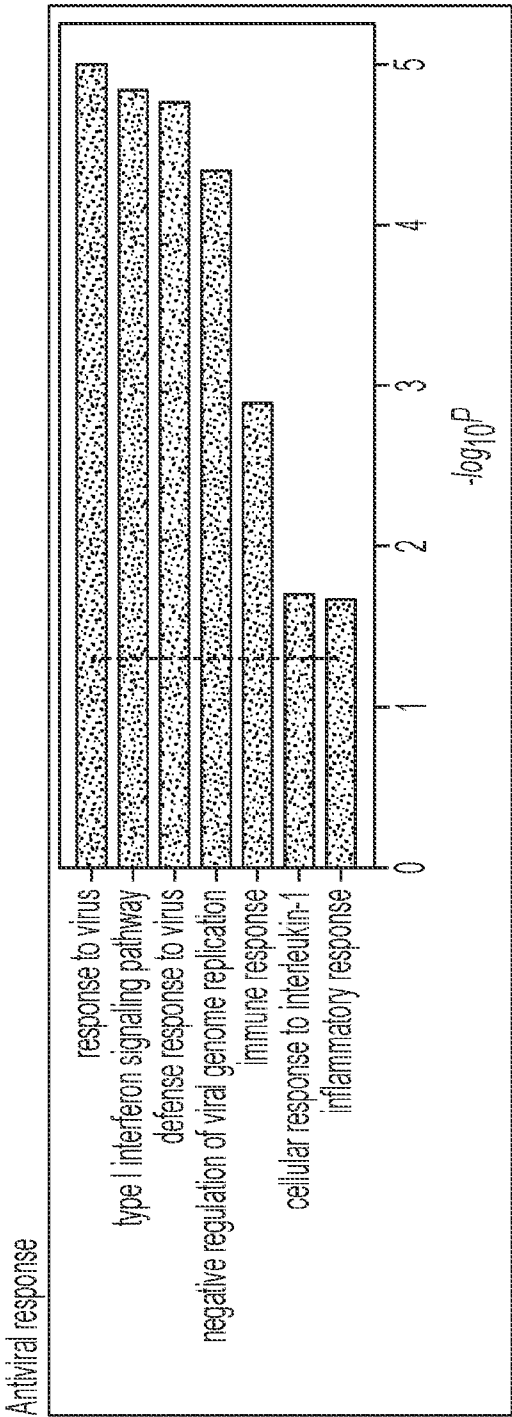
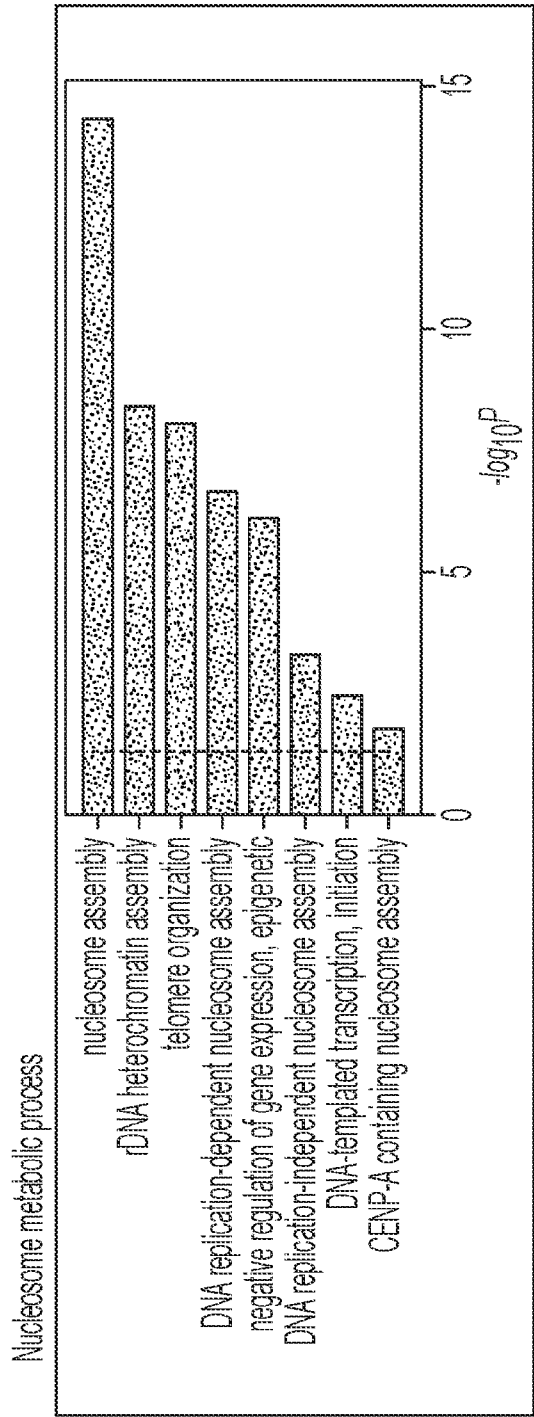
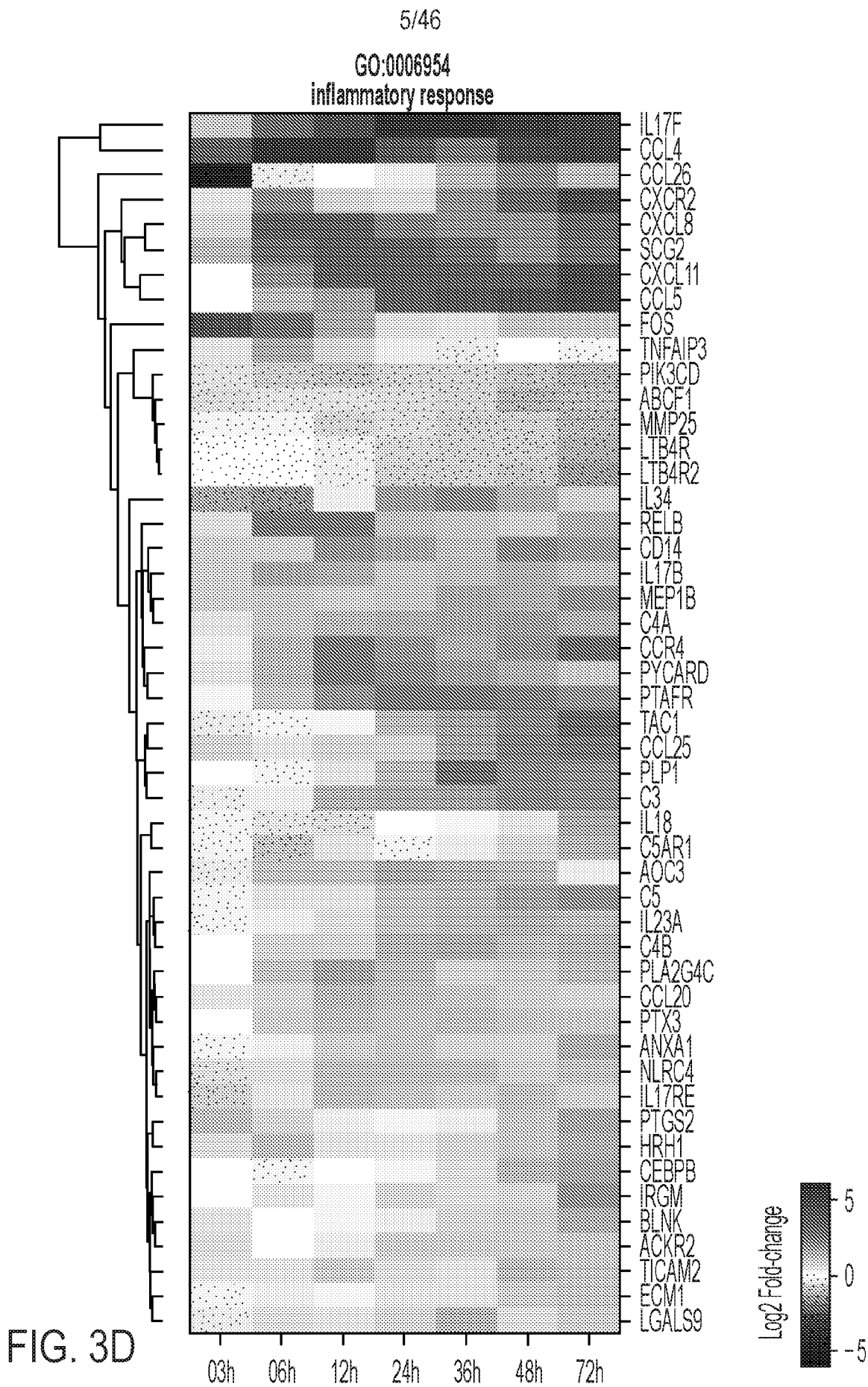
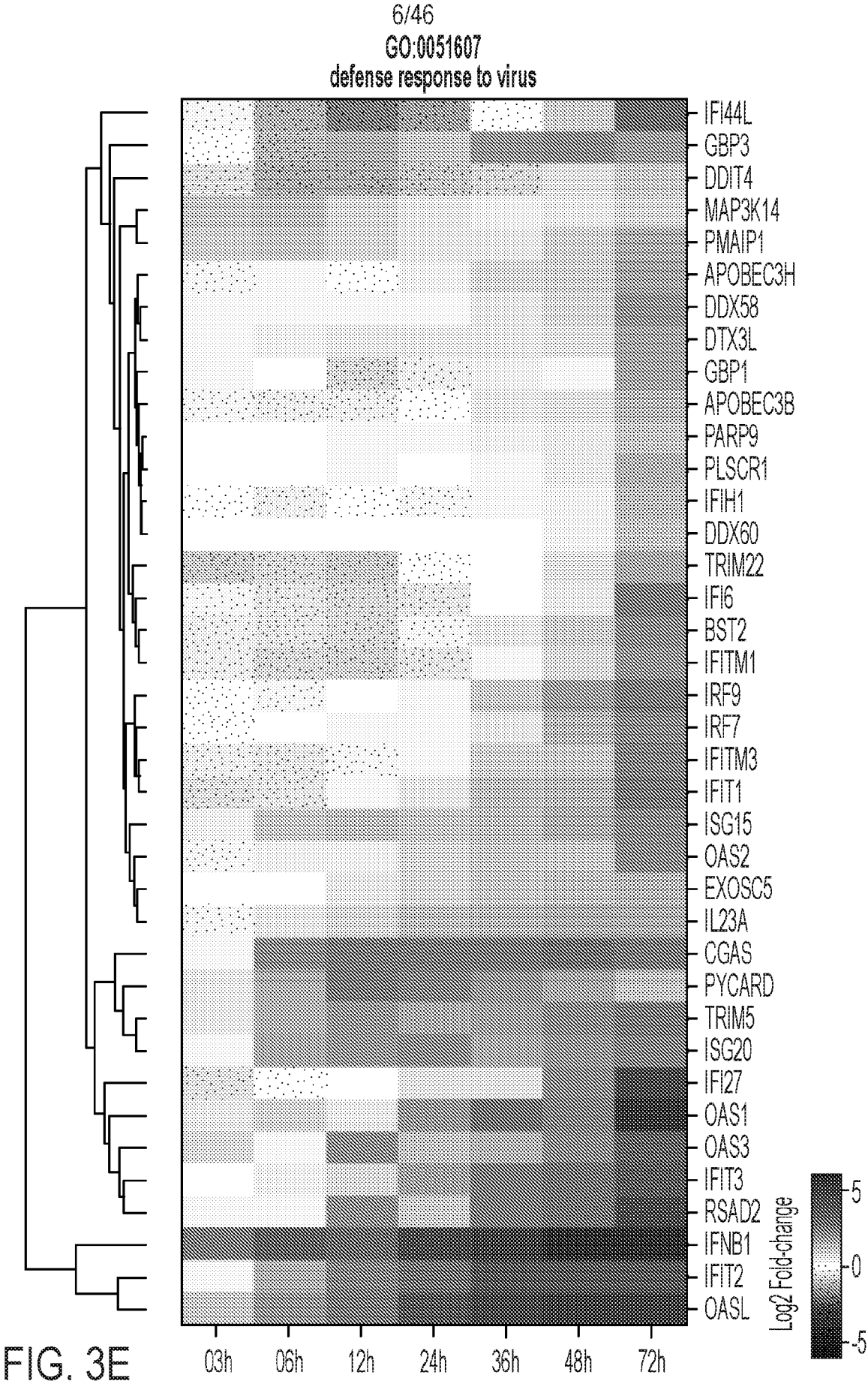


FIG. 3A







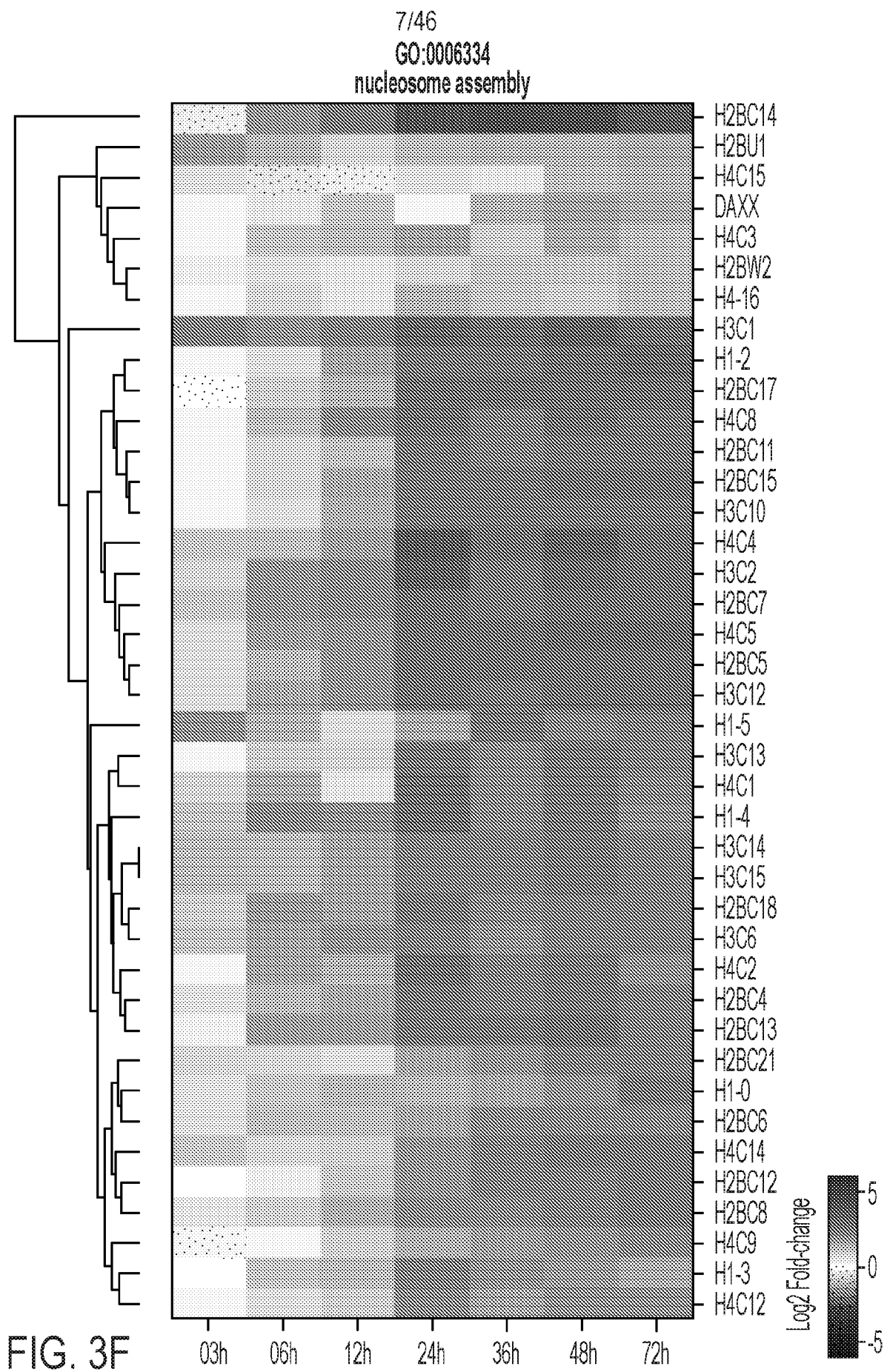
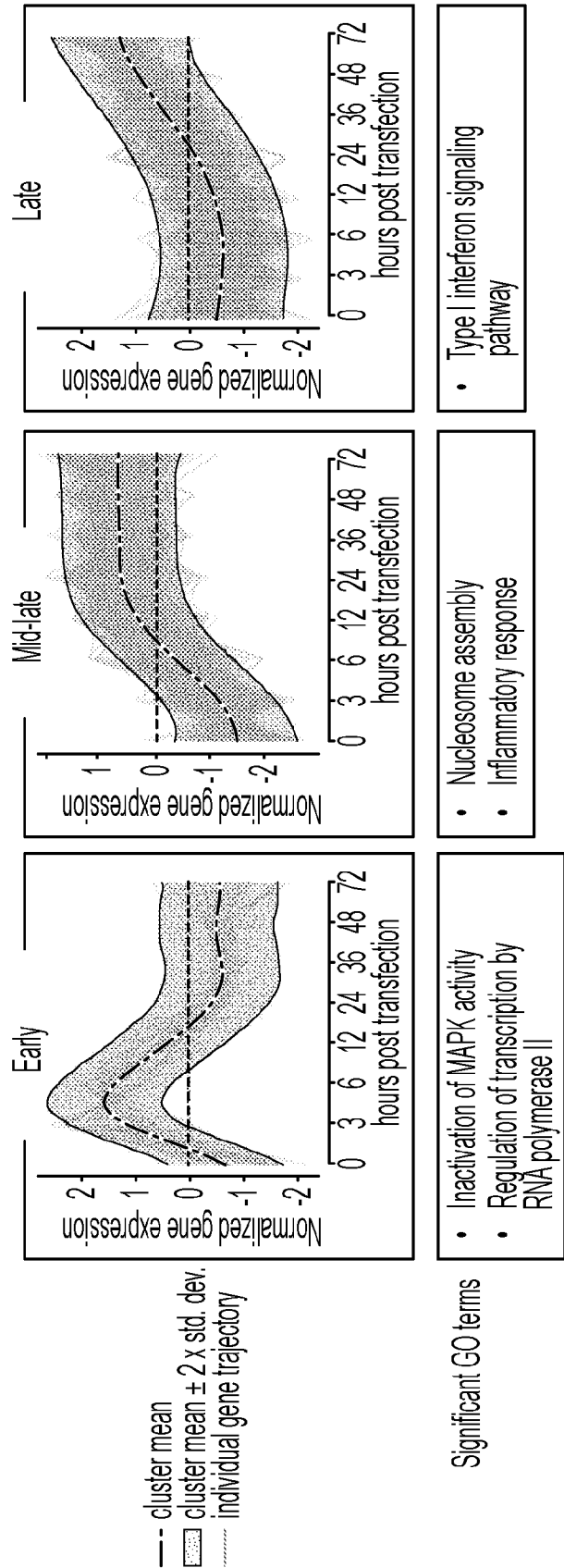


FIG. 3F



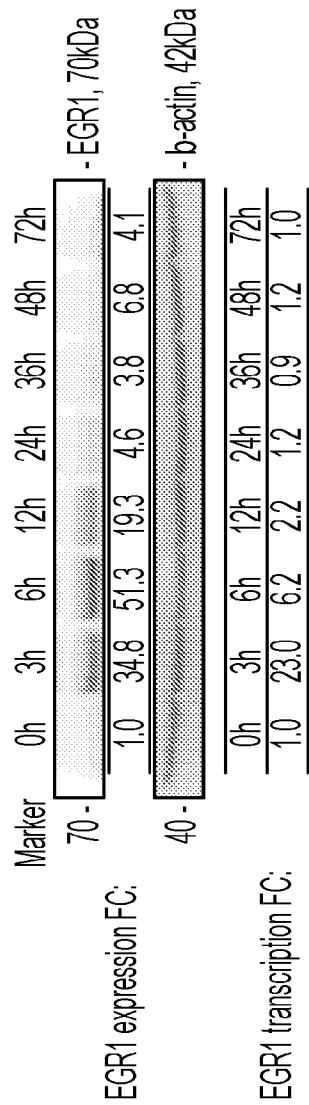


FIG. 4A

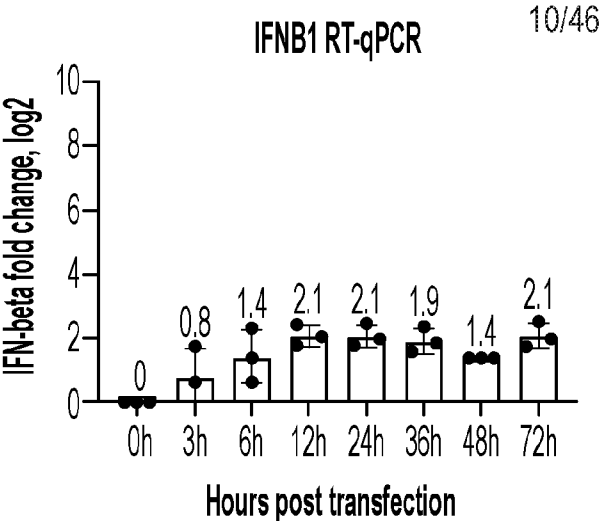


FIG. 4B

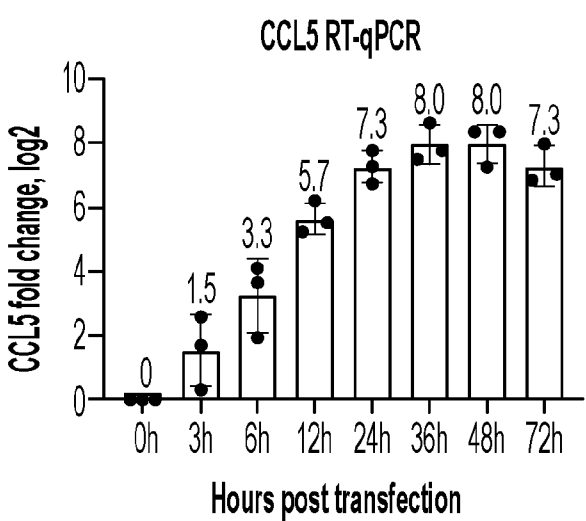


FIG. 4C

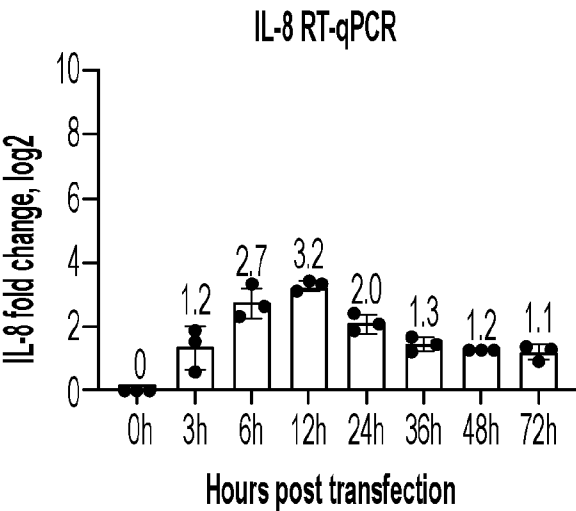


FIG. 4D

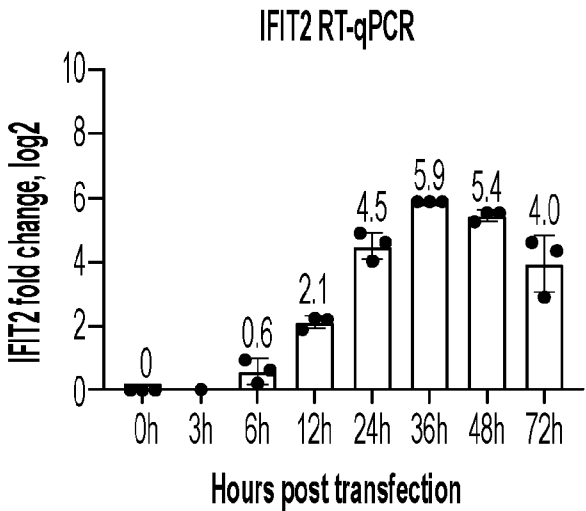


FIG. 4E

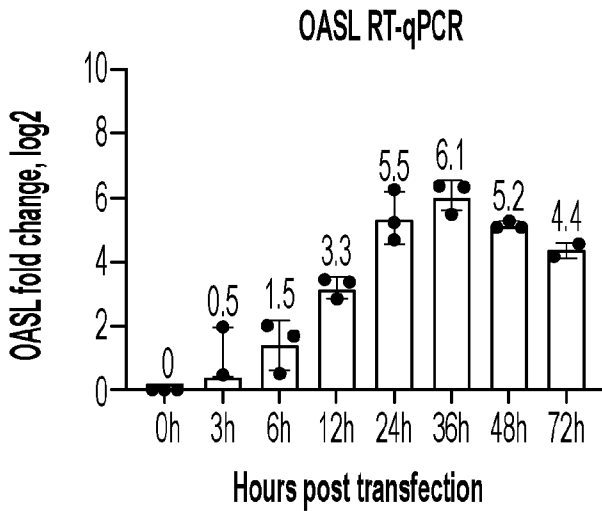
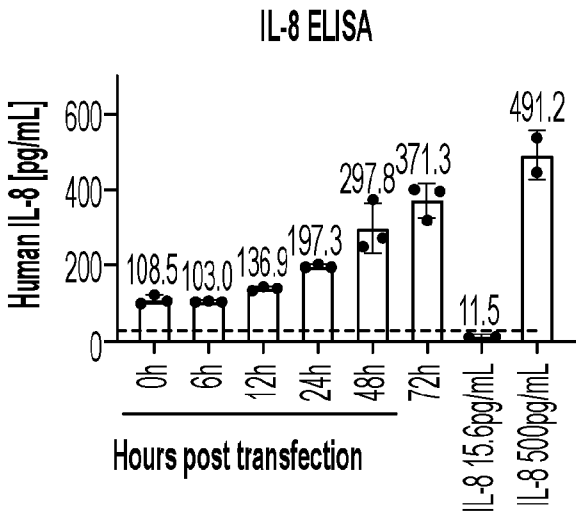
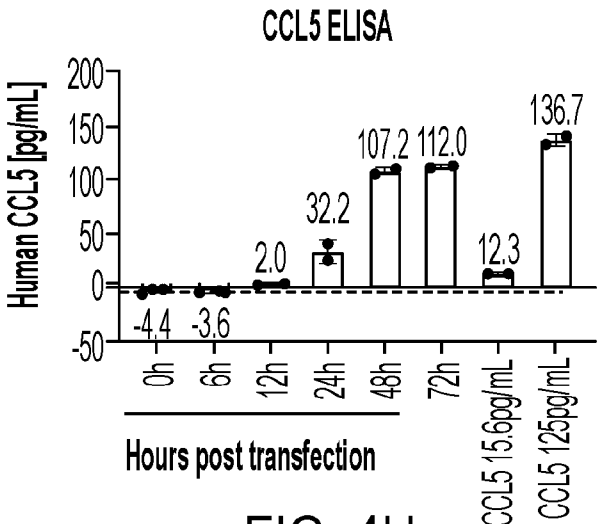
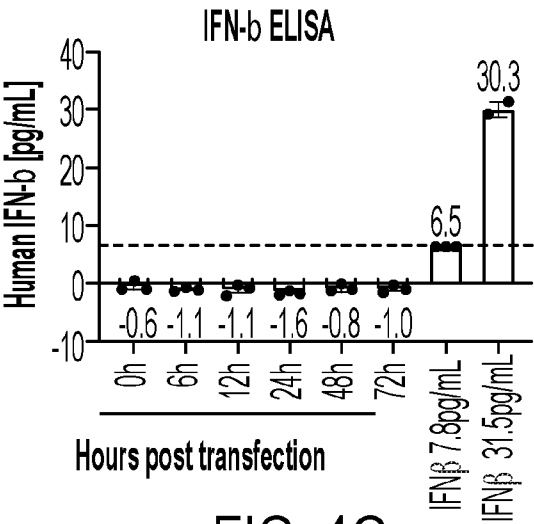


FIG. 4F



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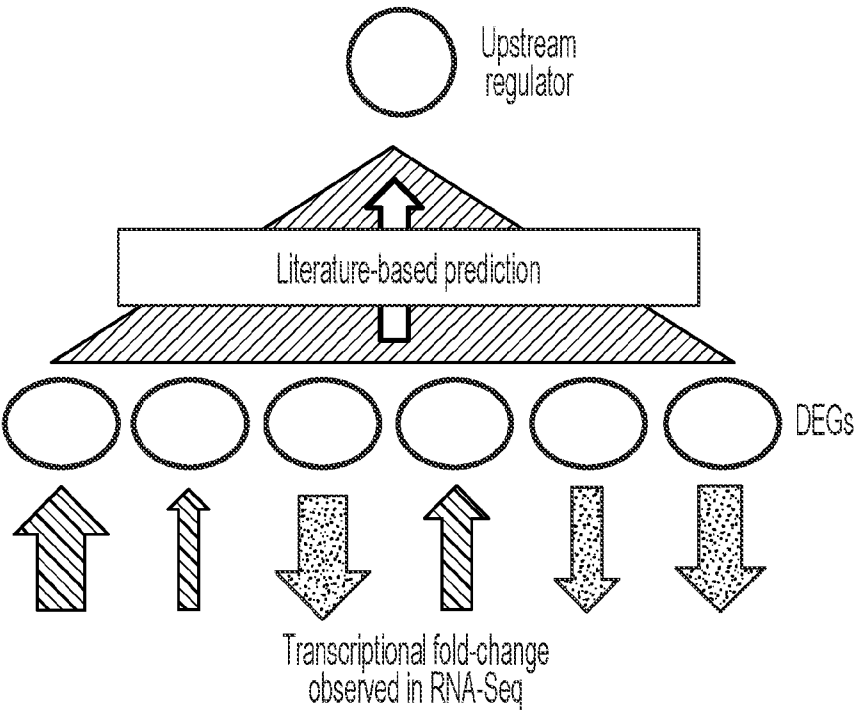


FIG. 5A

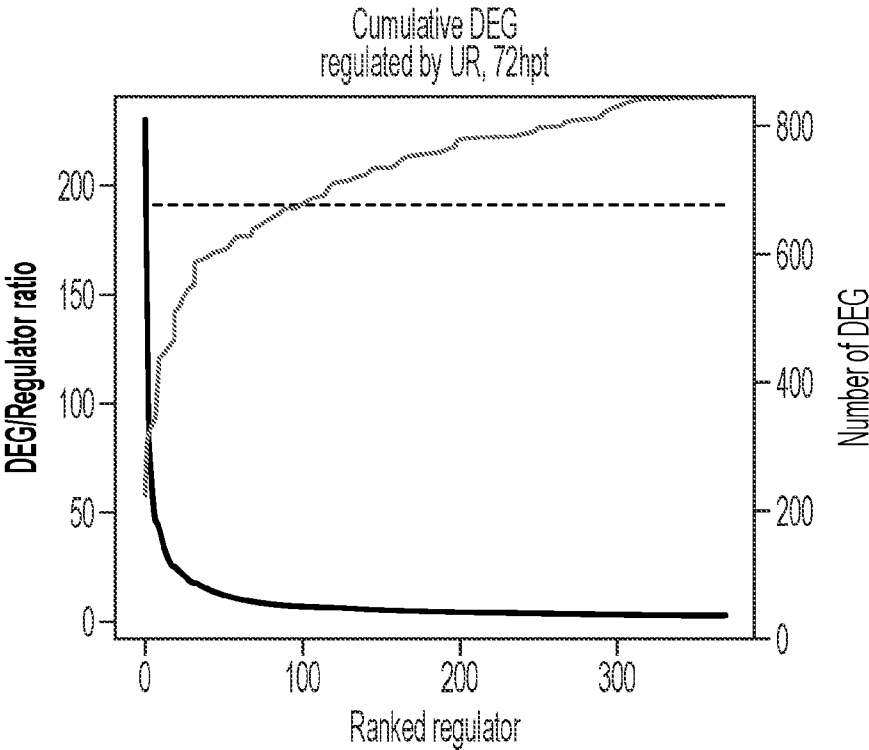


FIG. 5B

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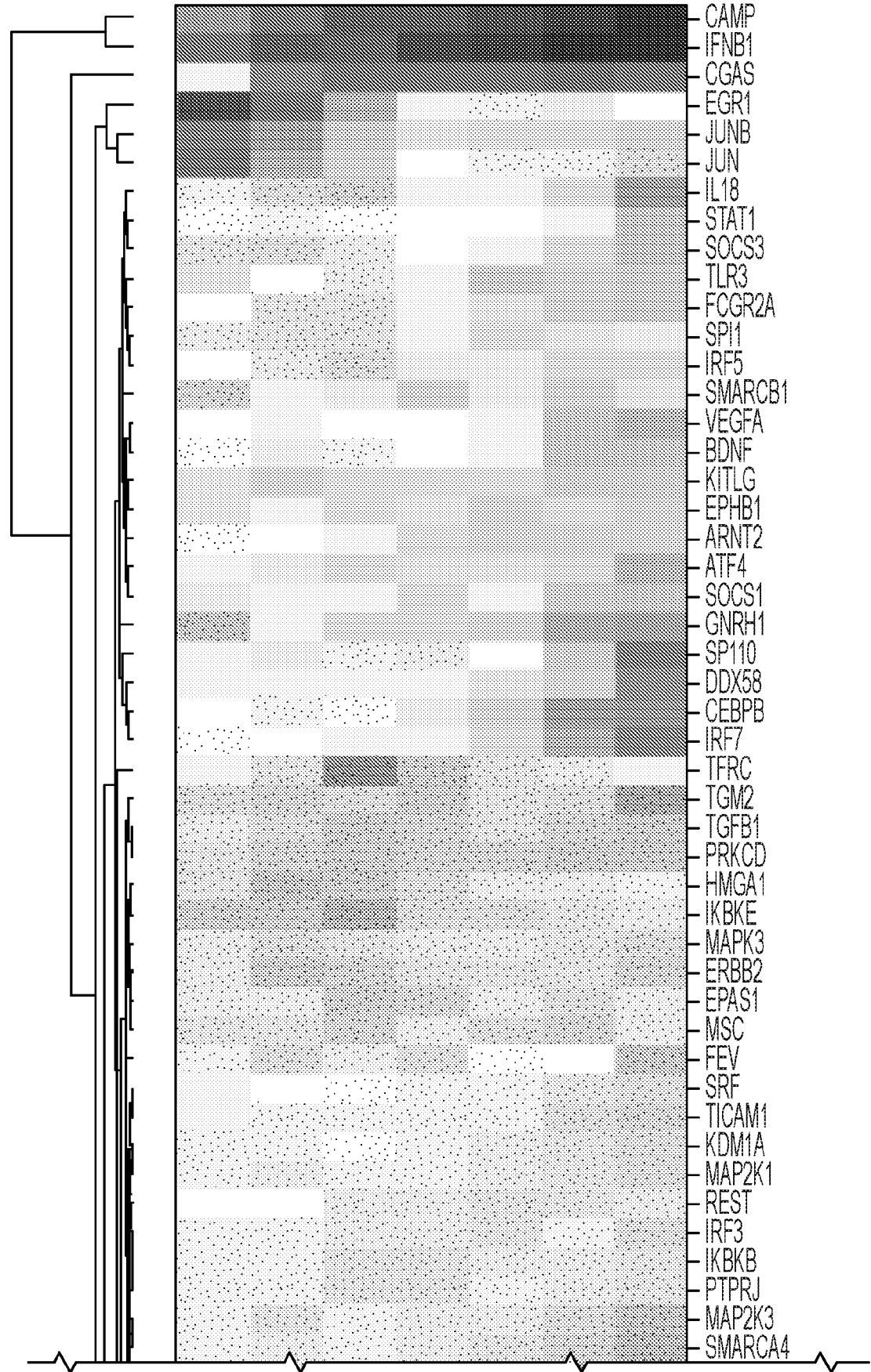


FIG. 5C

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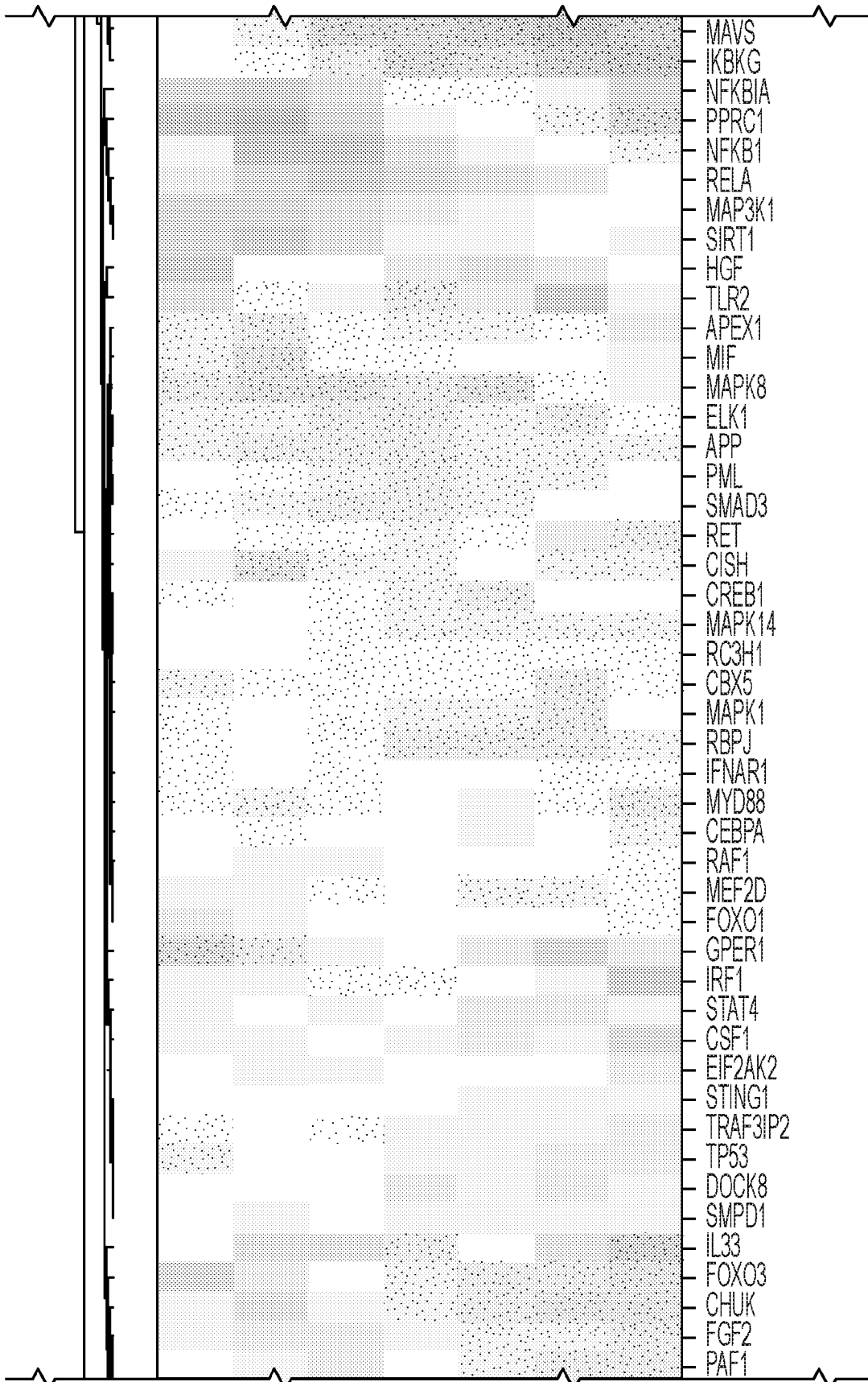


FIG. 5C
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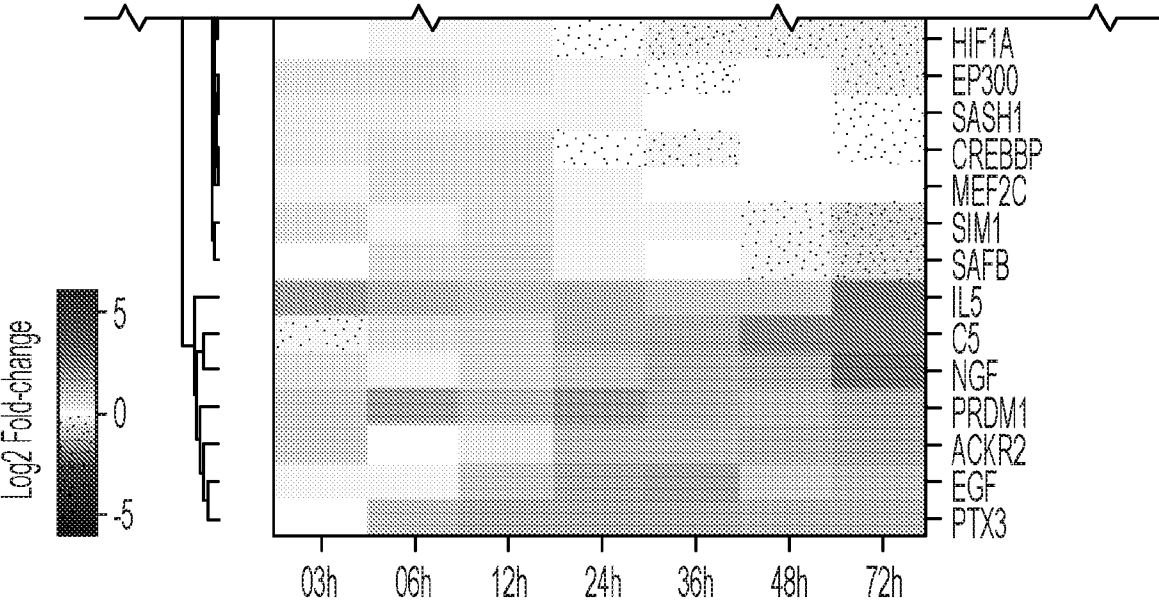


FIG. 5C
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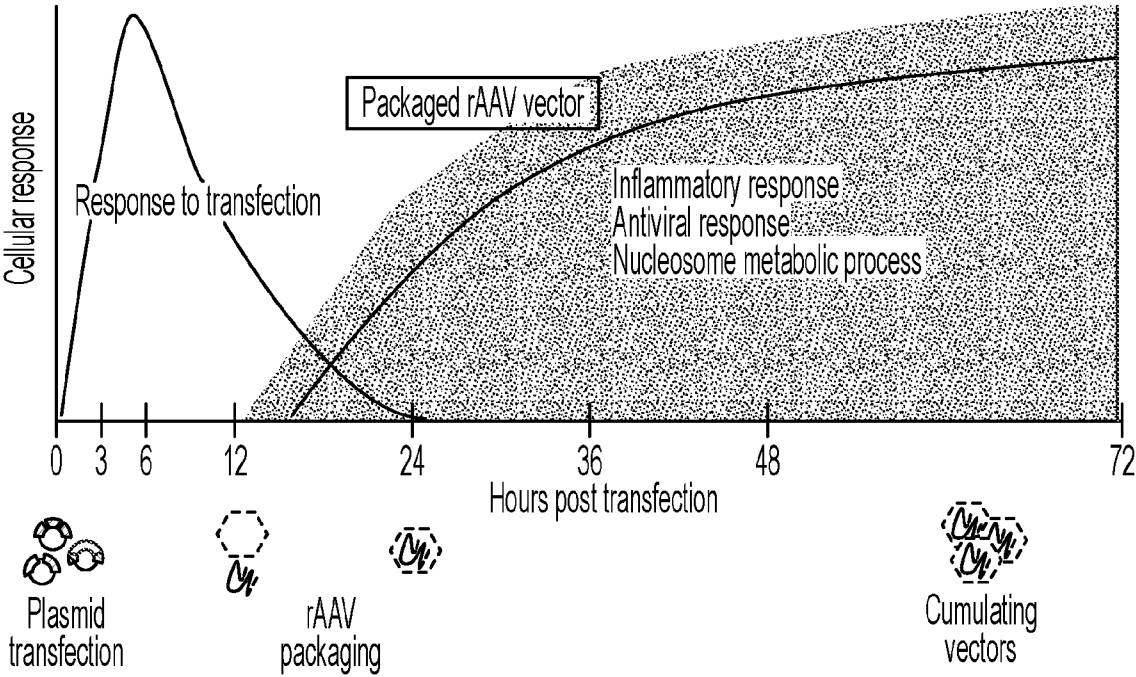


FIG. 6

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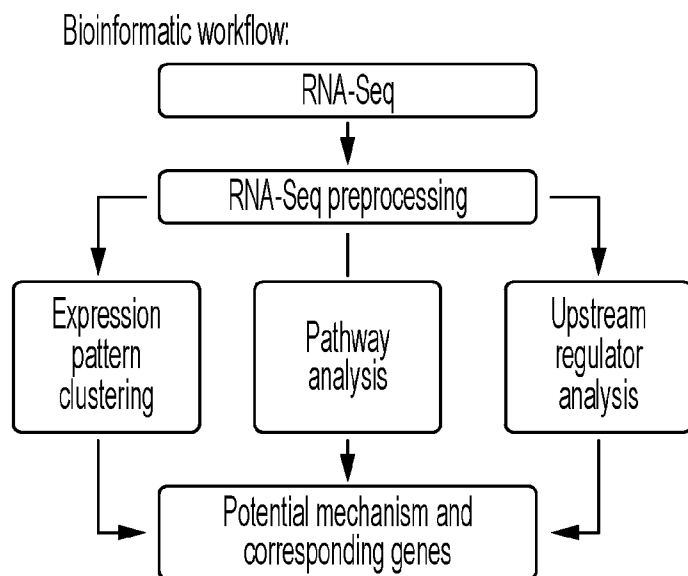


FIG. 7A

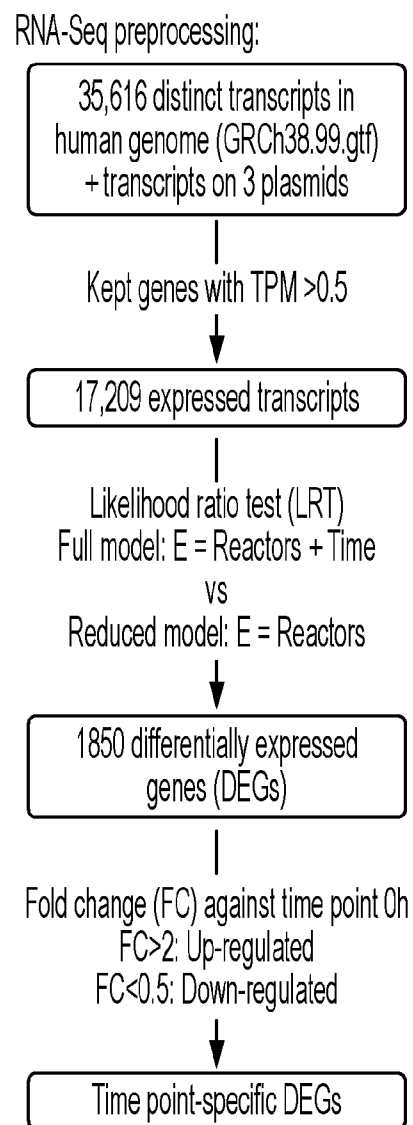


FIG. 7B

PCA plot using 17,209 expressed transcripts

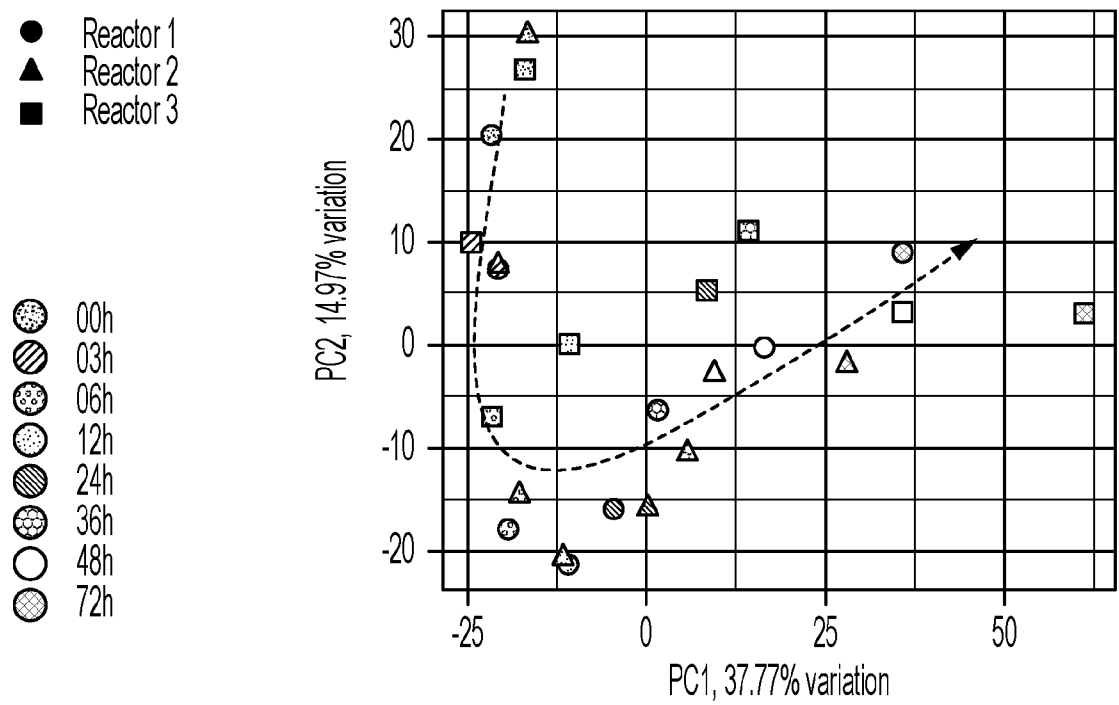


FIG. 8

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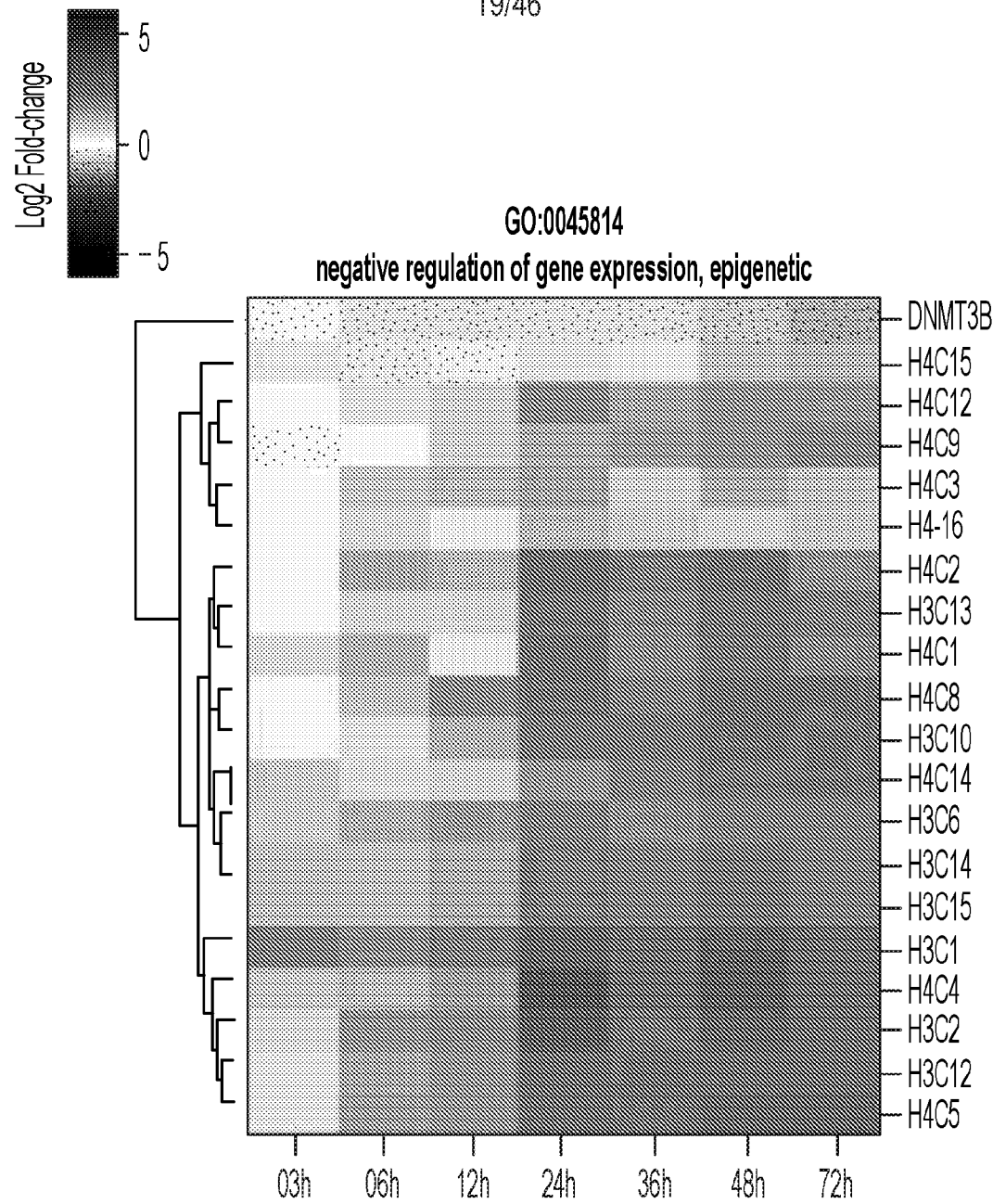


FIG. 9A

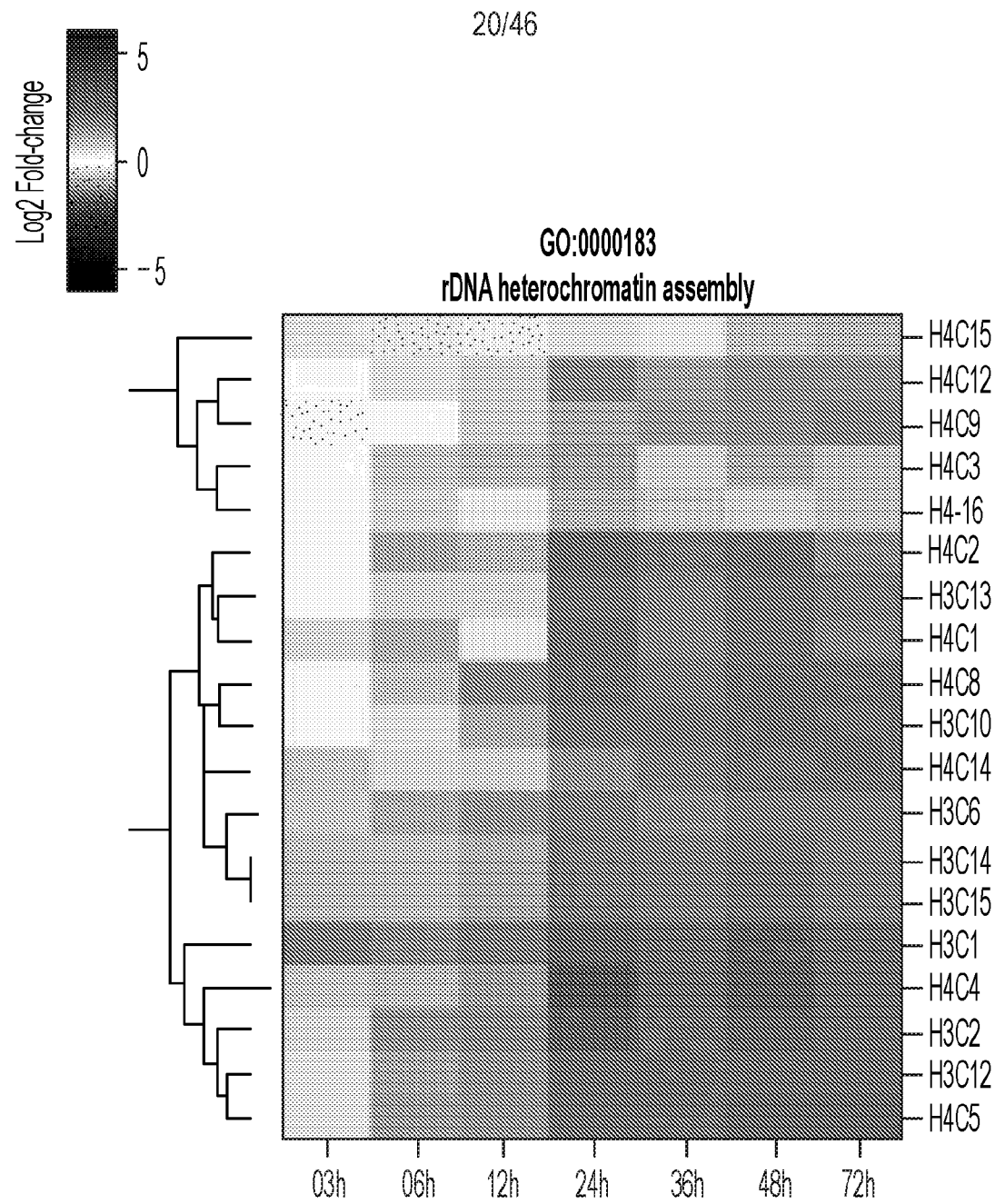


FIG. 9B

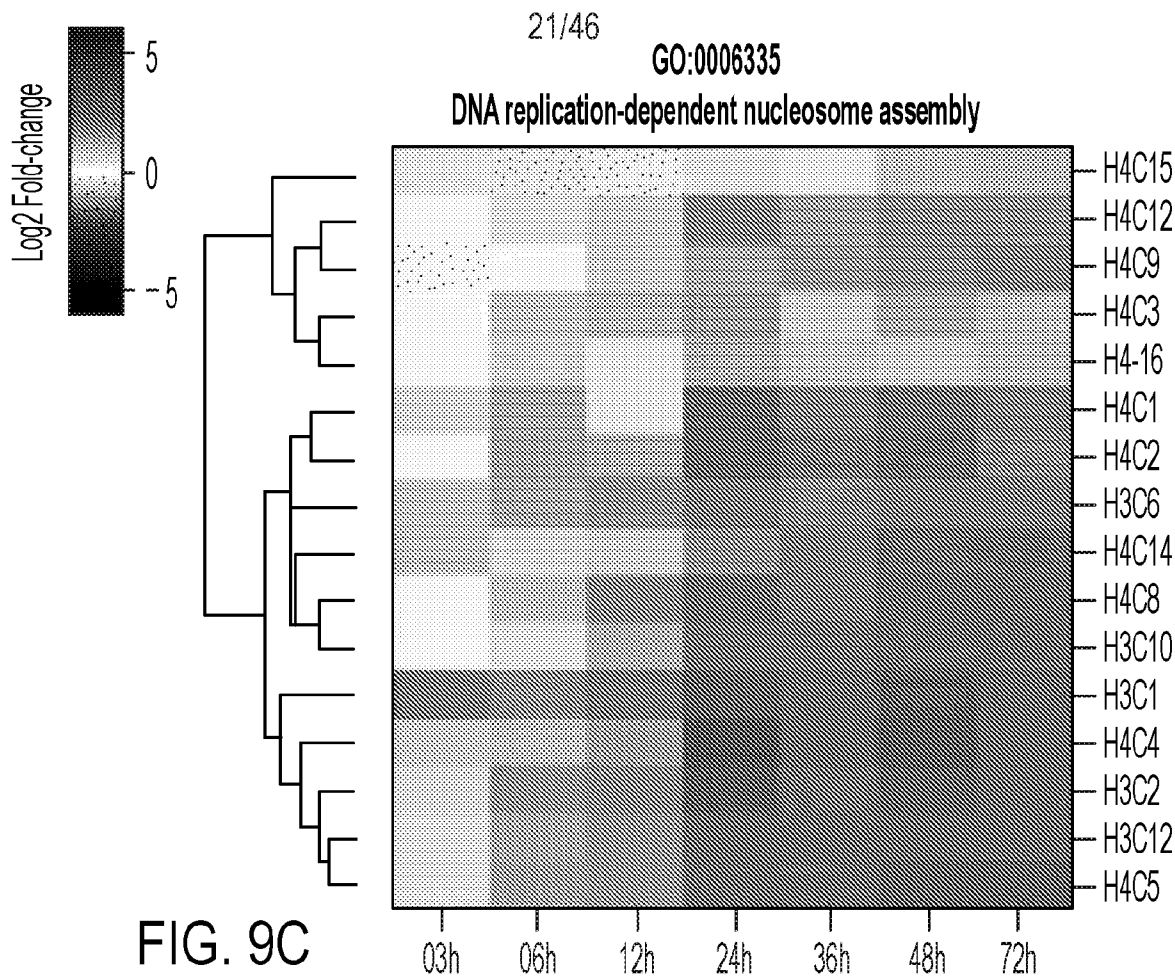


FIG. 9C

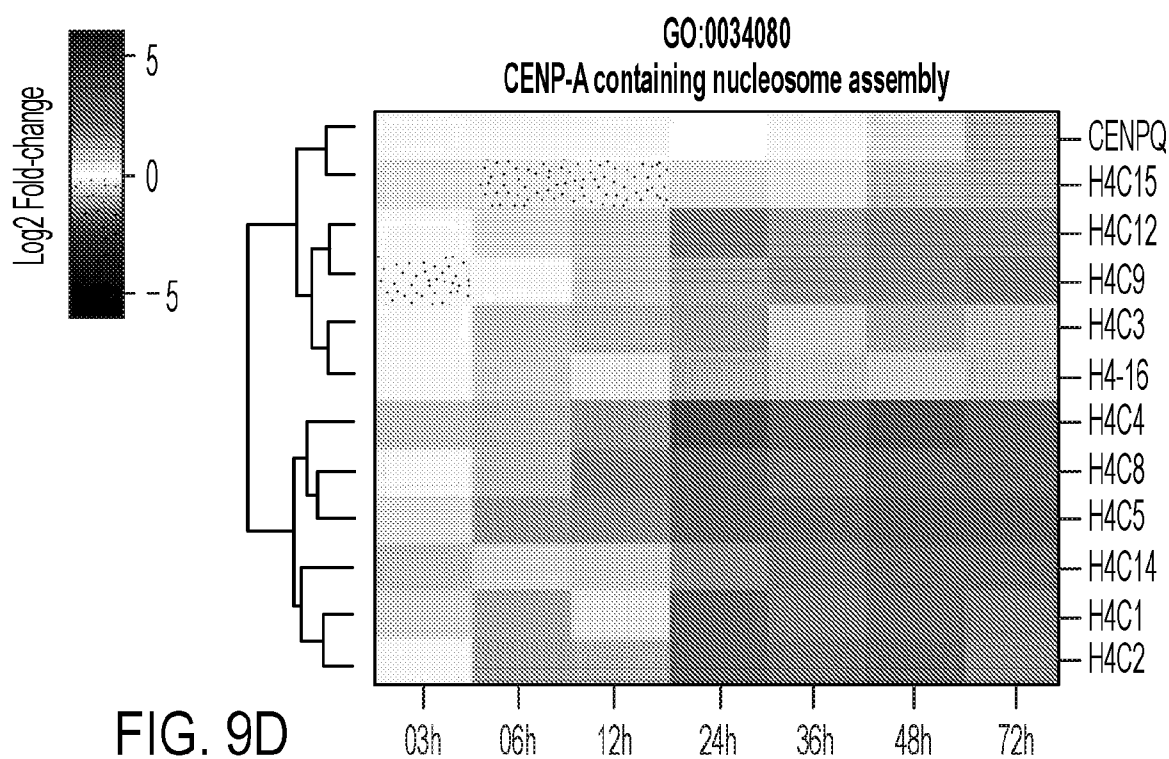


FIG. 9D

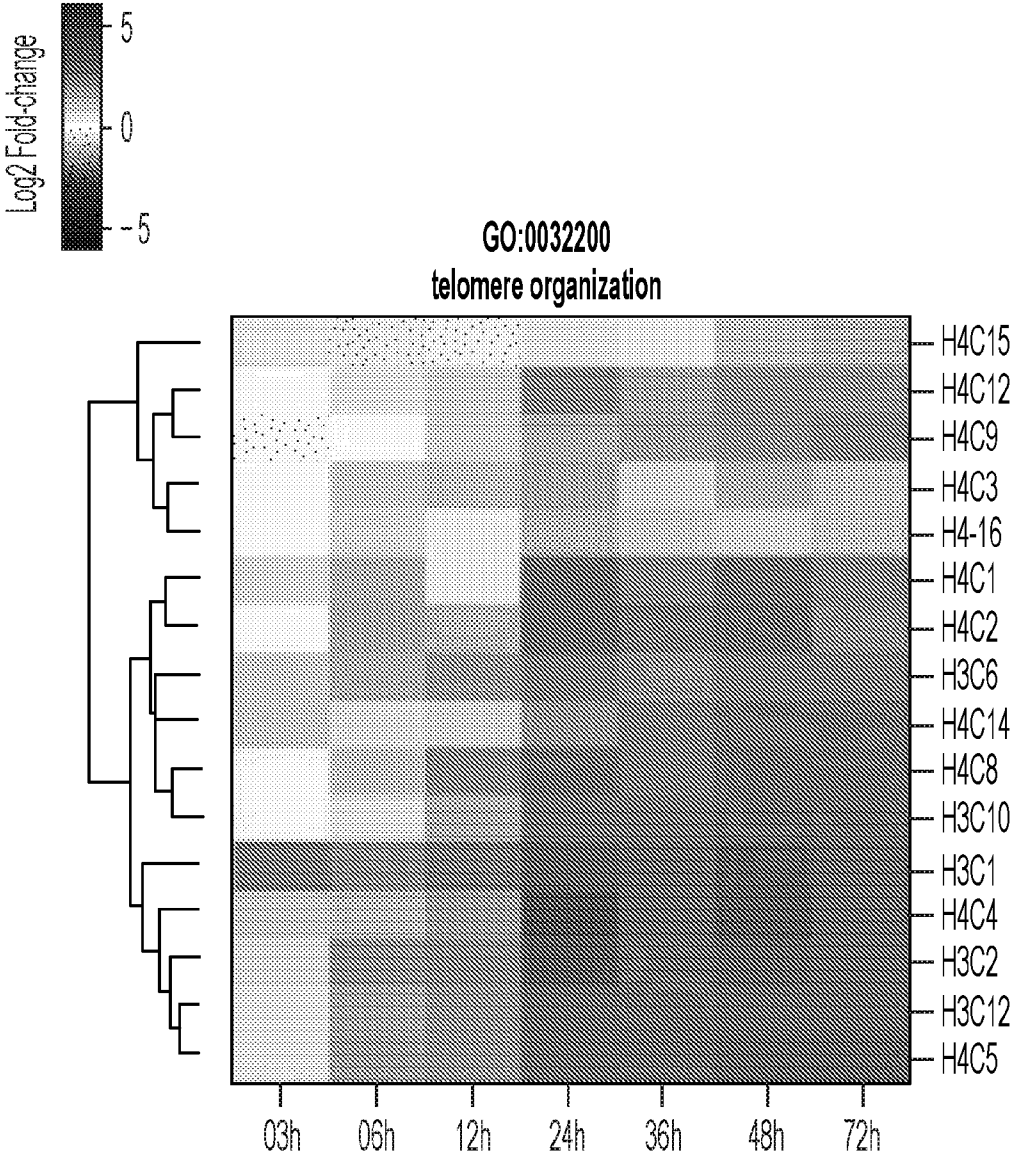


FIG. 9E

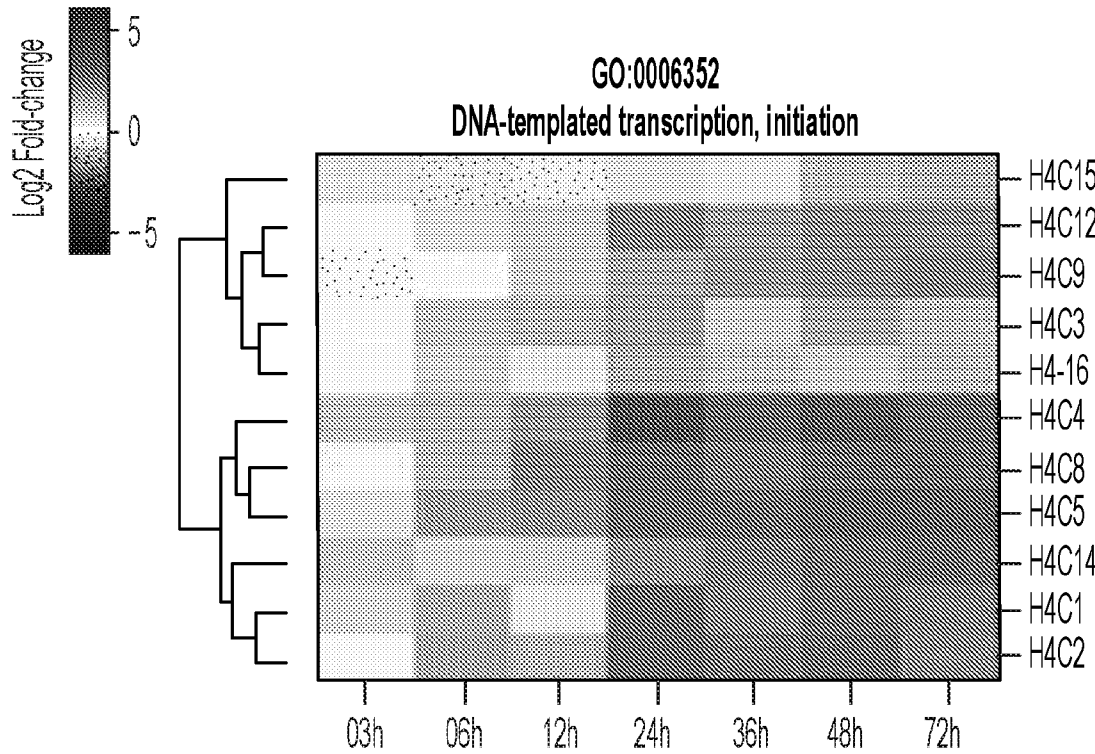


FIG. 9F

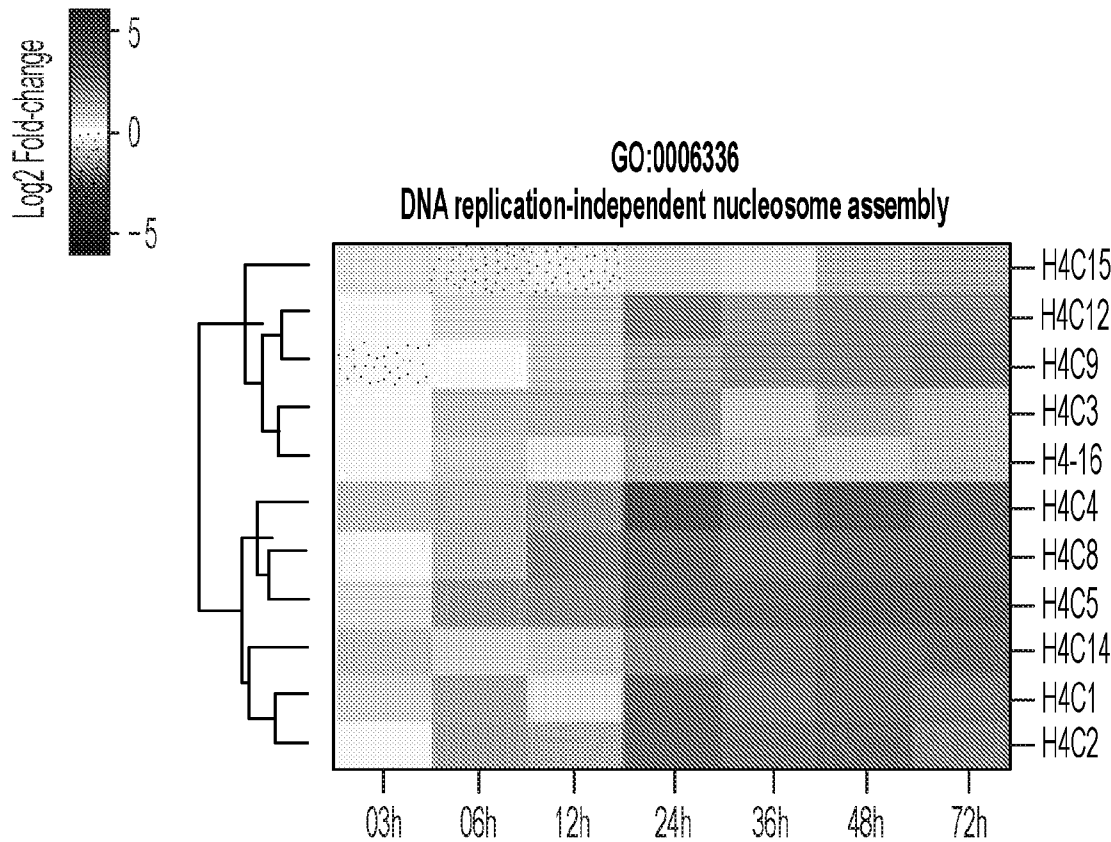


FIG. 9G

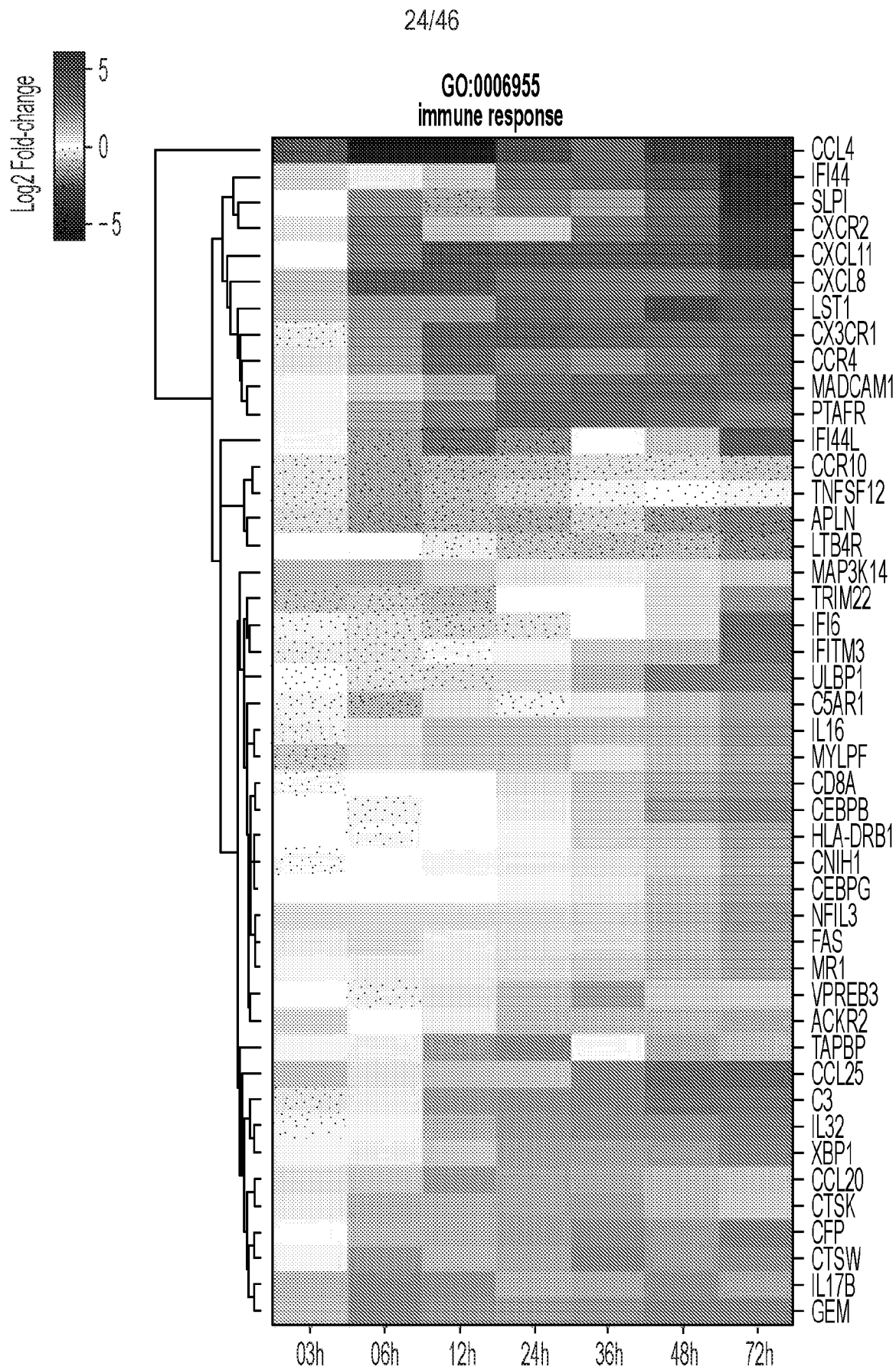


FIG. 10A

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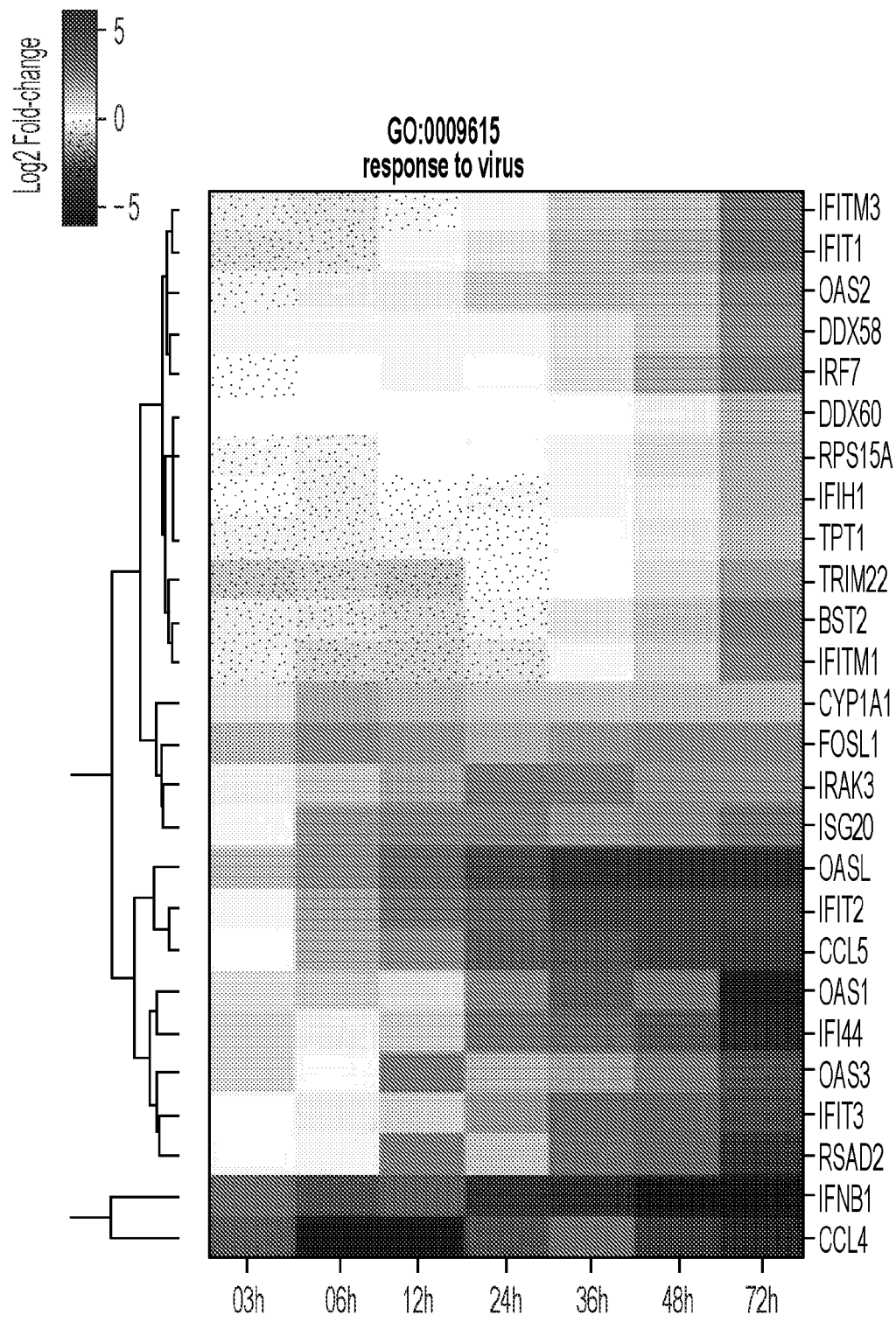


FIG. 10B

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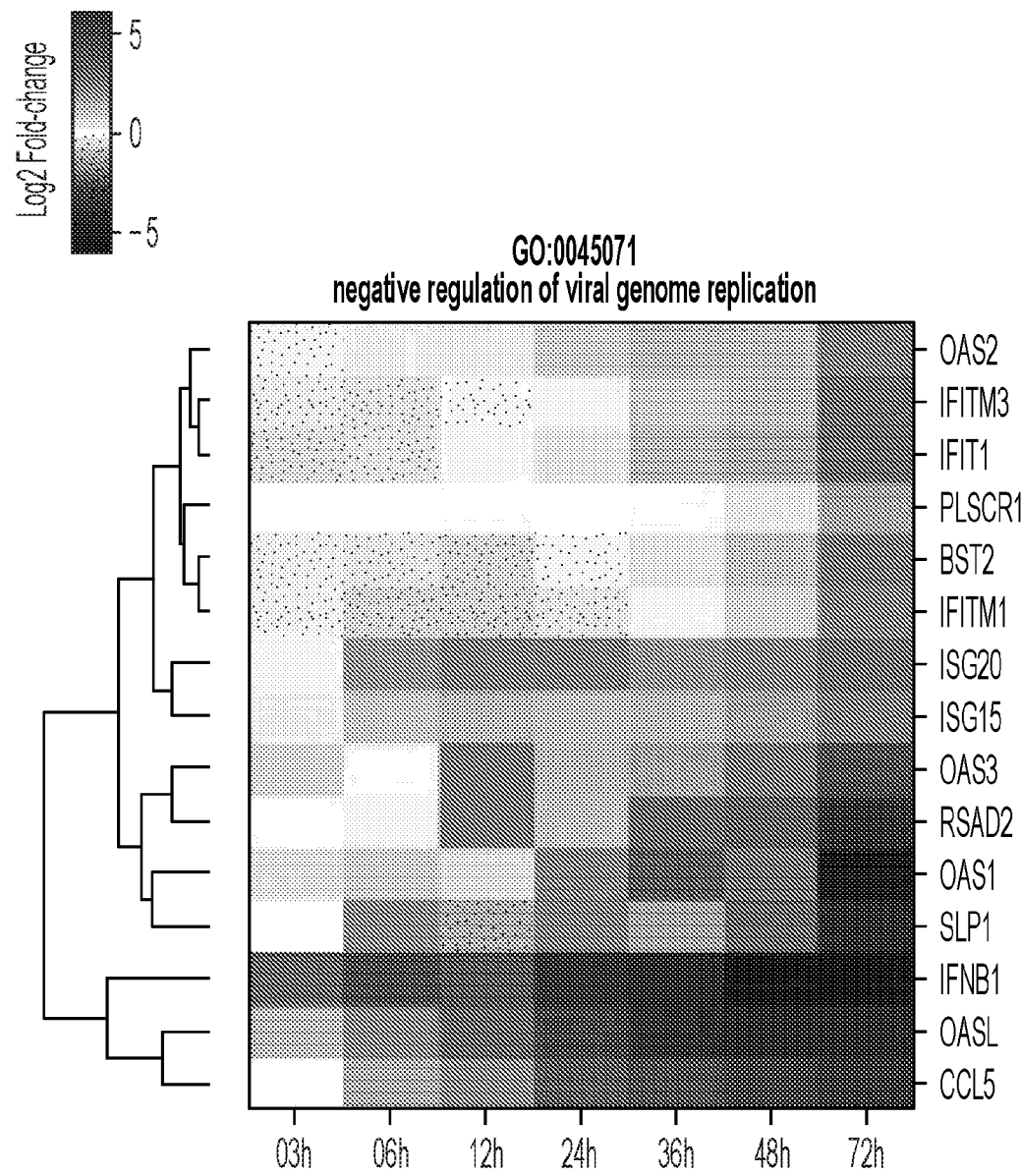


FIG. 10C

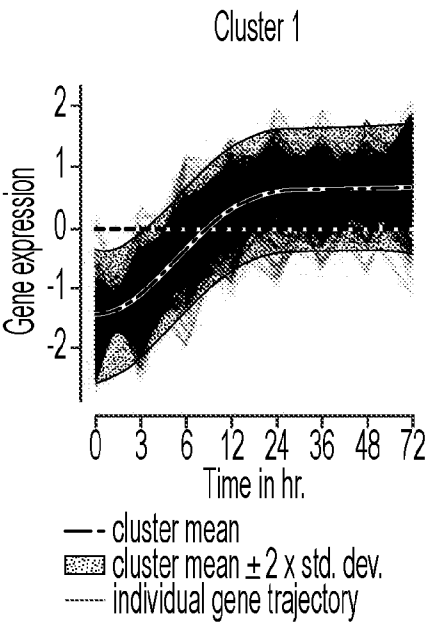


FIG. 11A

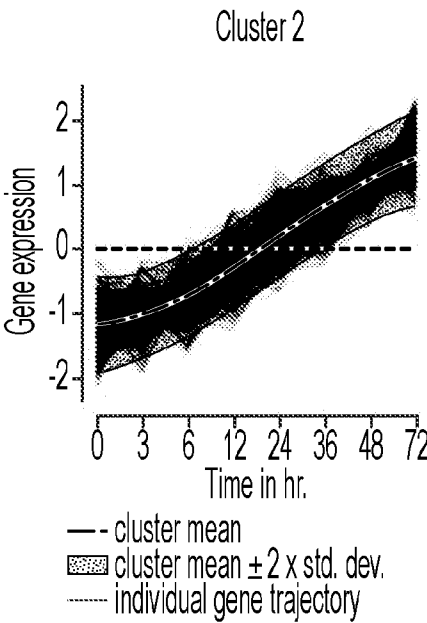


FIG. 11B

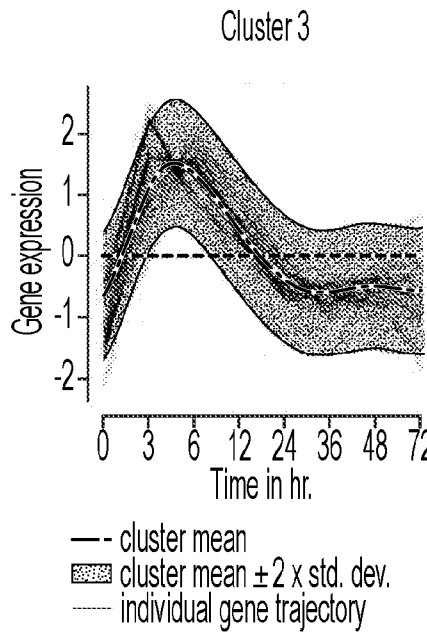


FIG. 11C

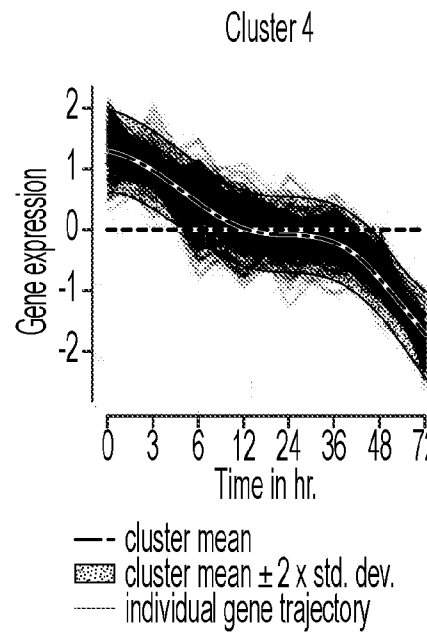


FIG. 11D

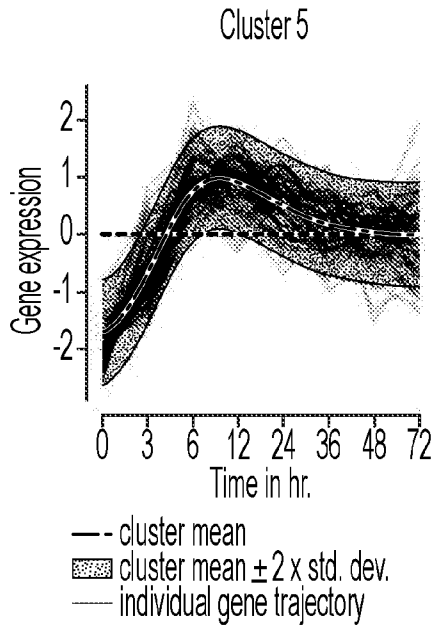


FIG. 11E

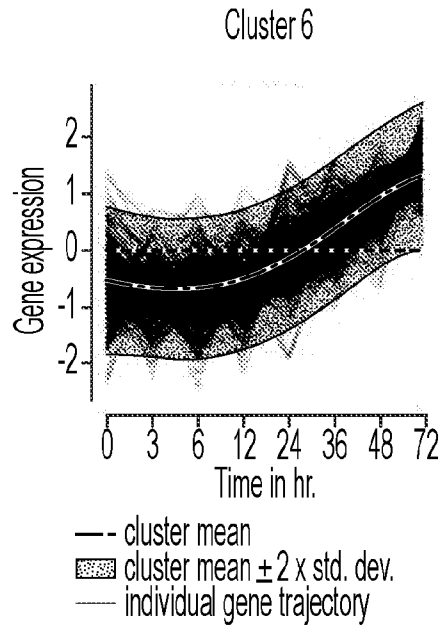


FIG. 11F

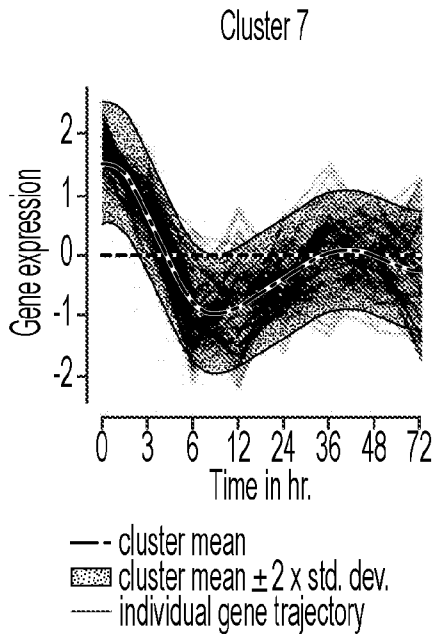


FIG. 11G

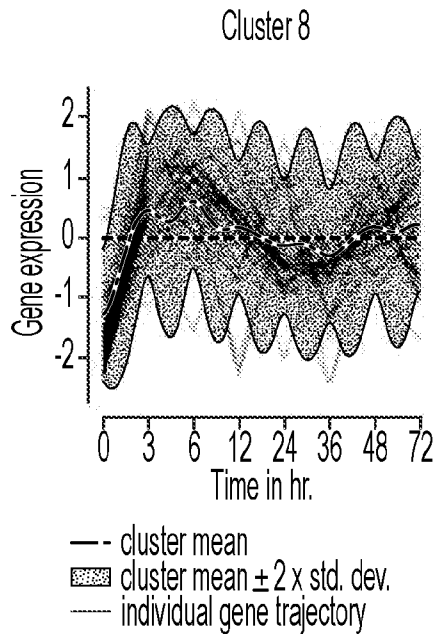


FIG. 11H

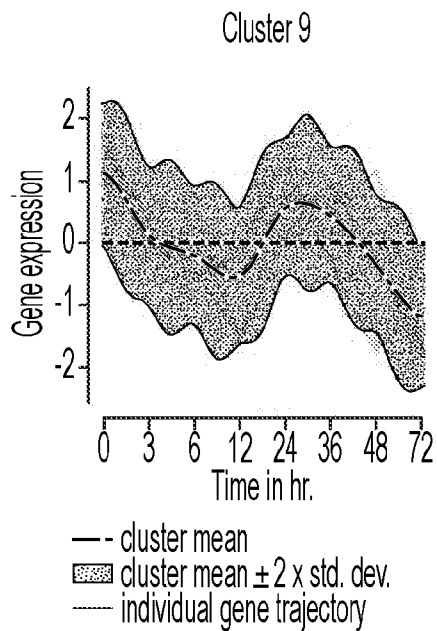


FIG. 11I

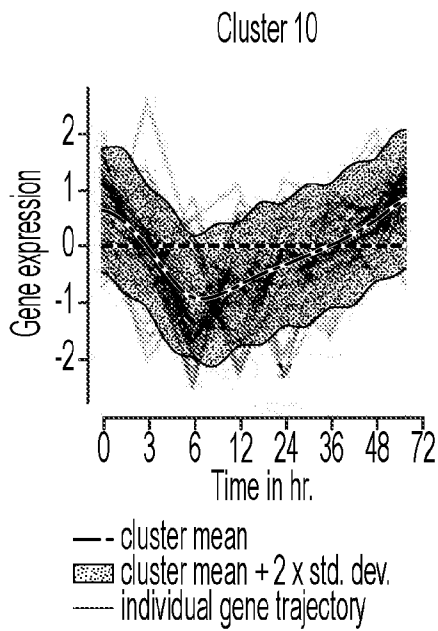


FIG. 11J

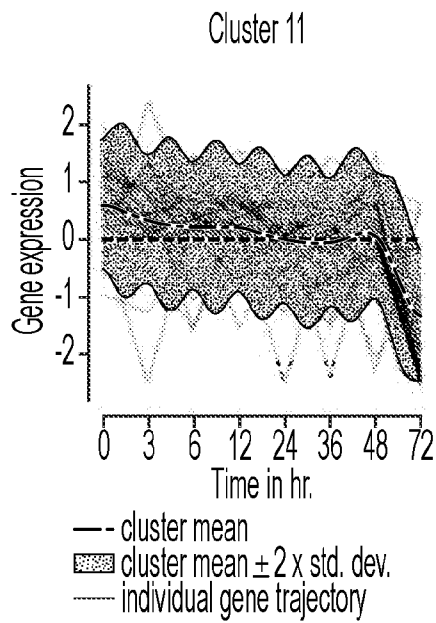


FIG. 11K

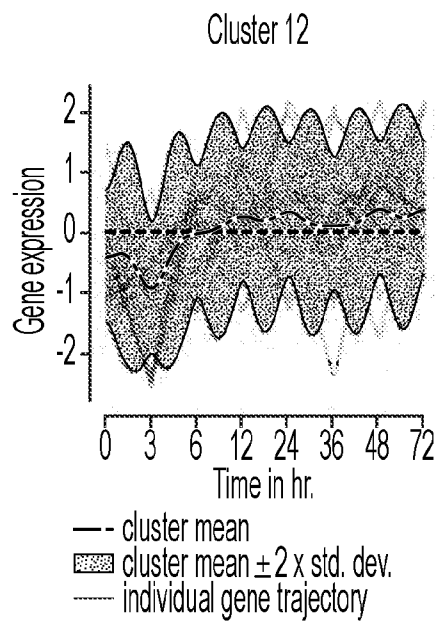


FIG. 11L

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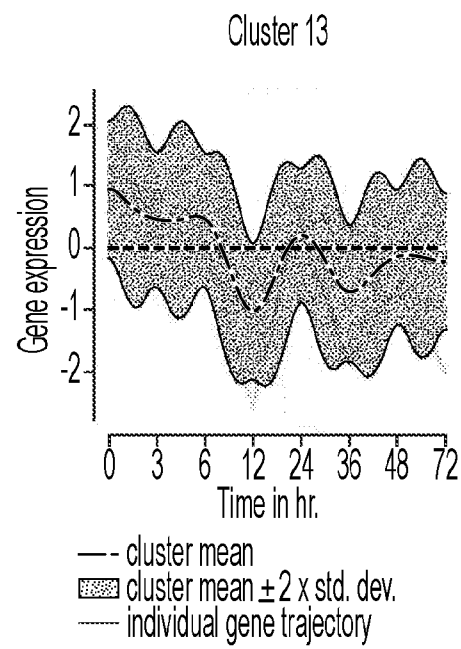
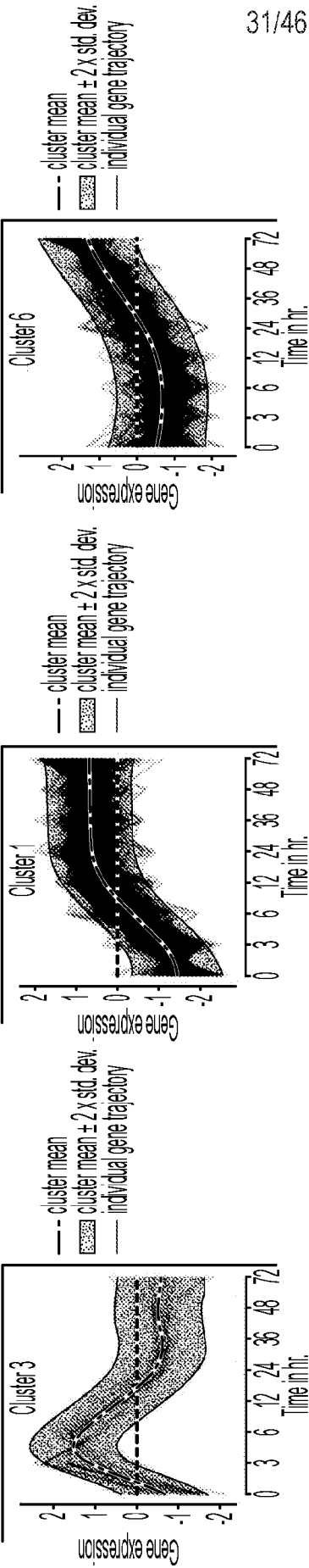


FIG. 11M



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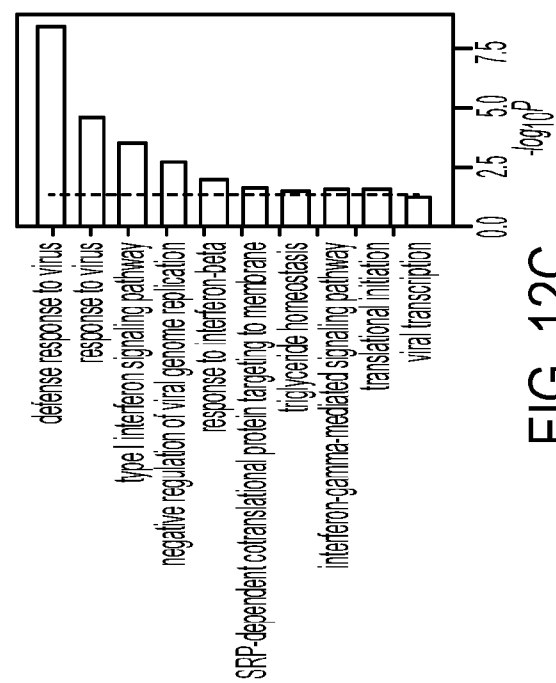


FIG. 12C

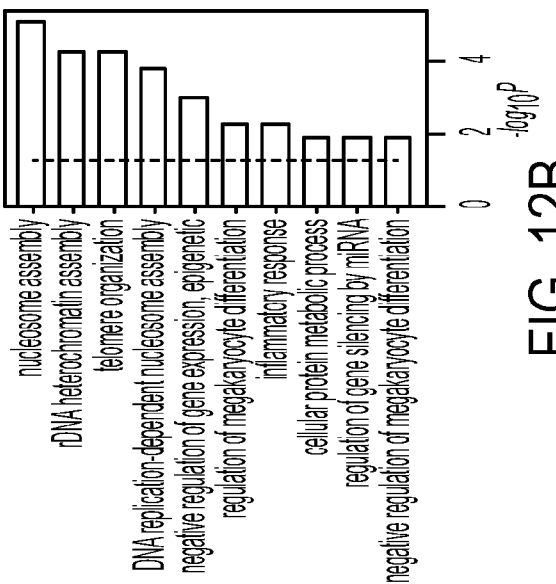


FIG. 12B

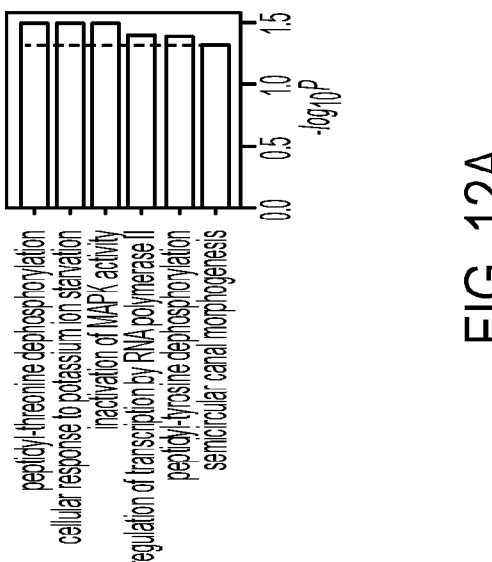


FIG. 12A

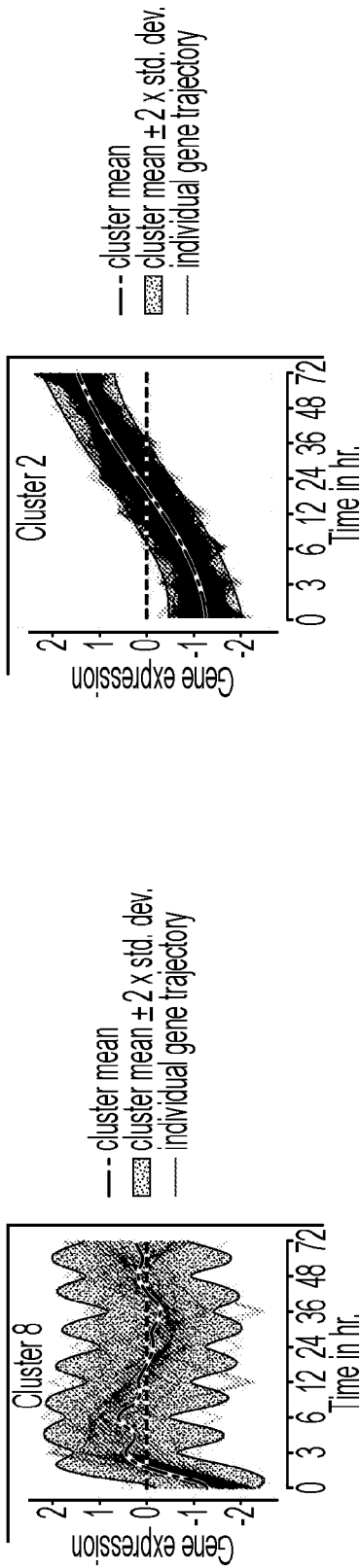


FIG. 12D

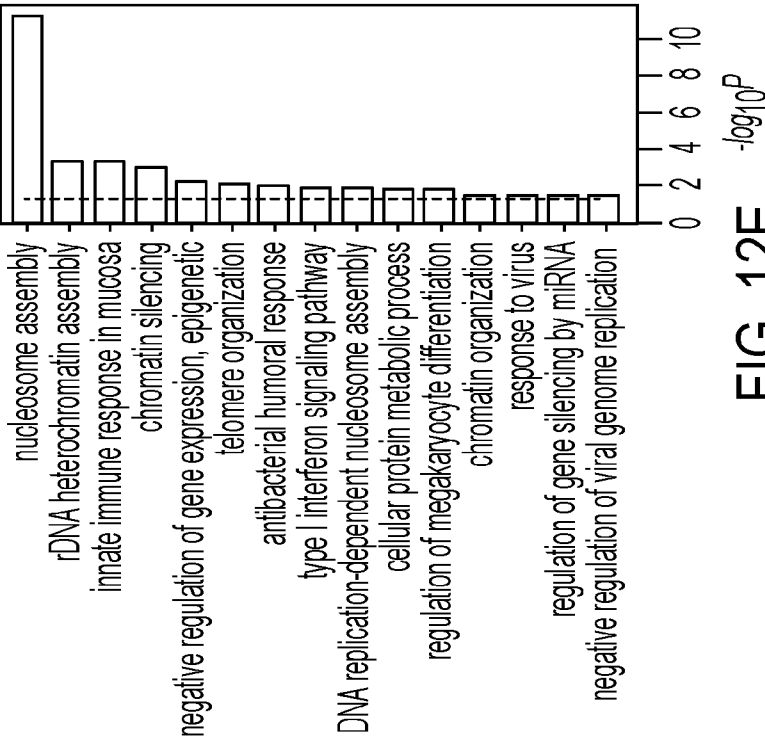


FIG. 12E

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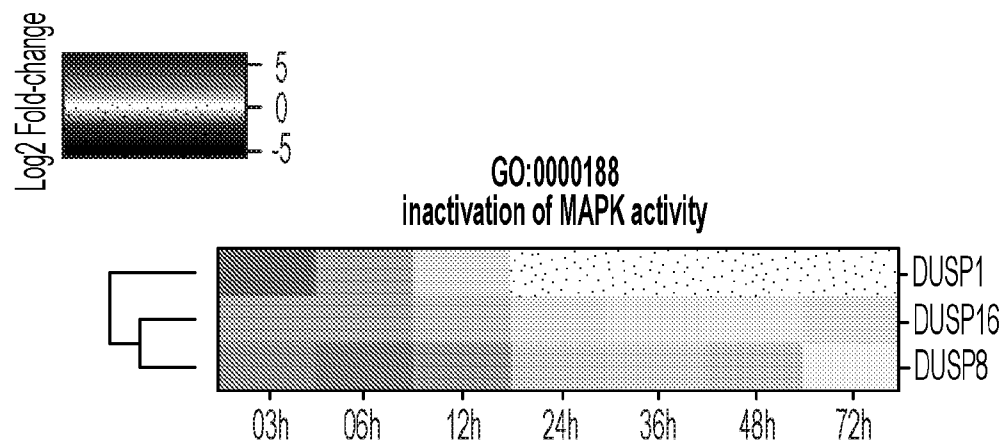


FIG. 13A

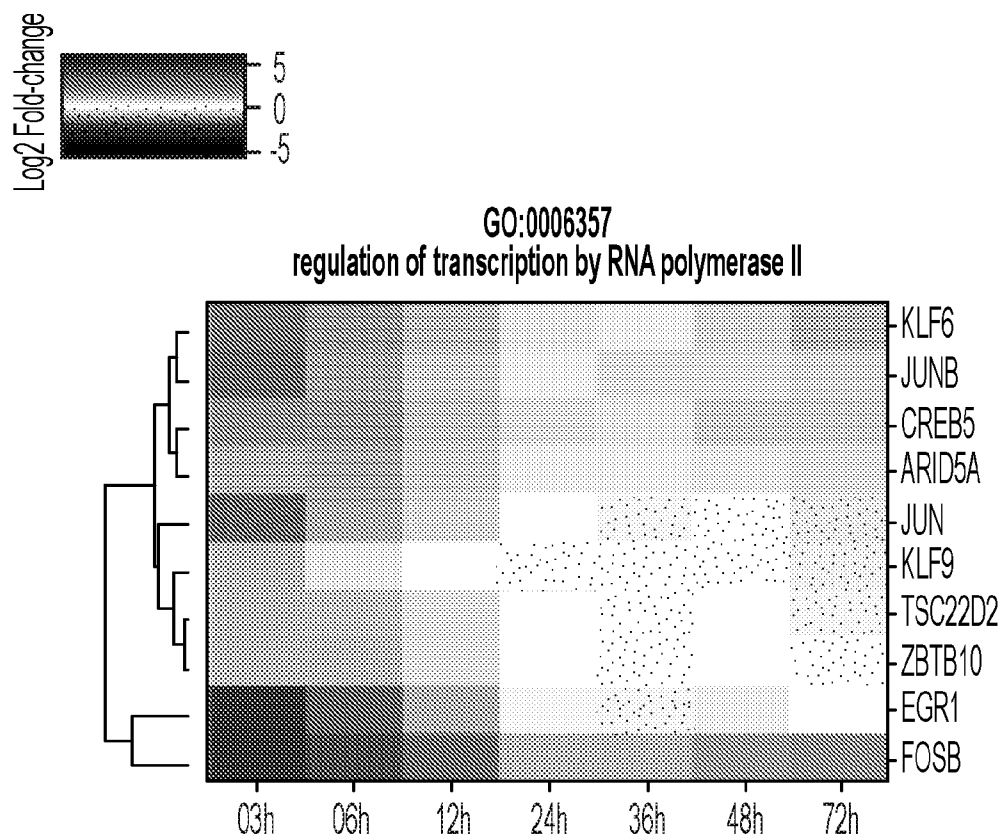


FIG. 13B

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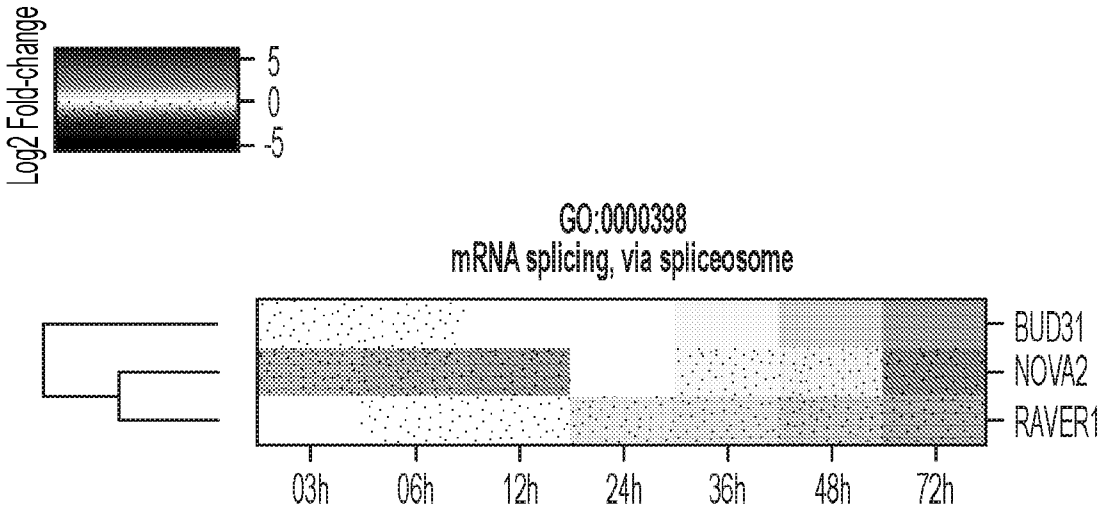


FIG. 14A

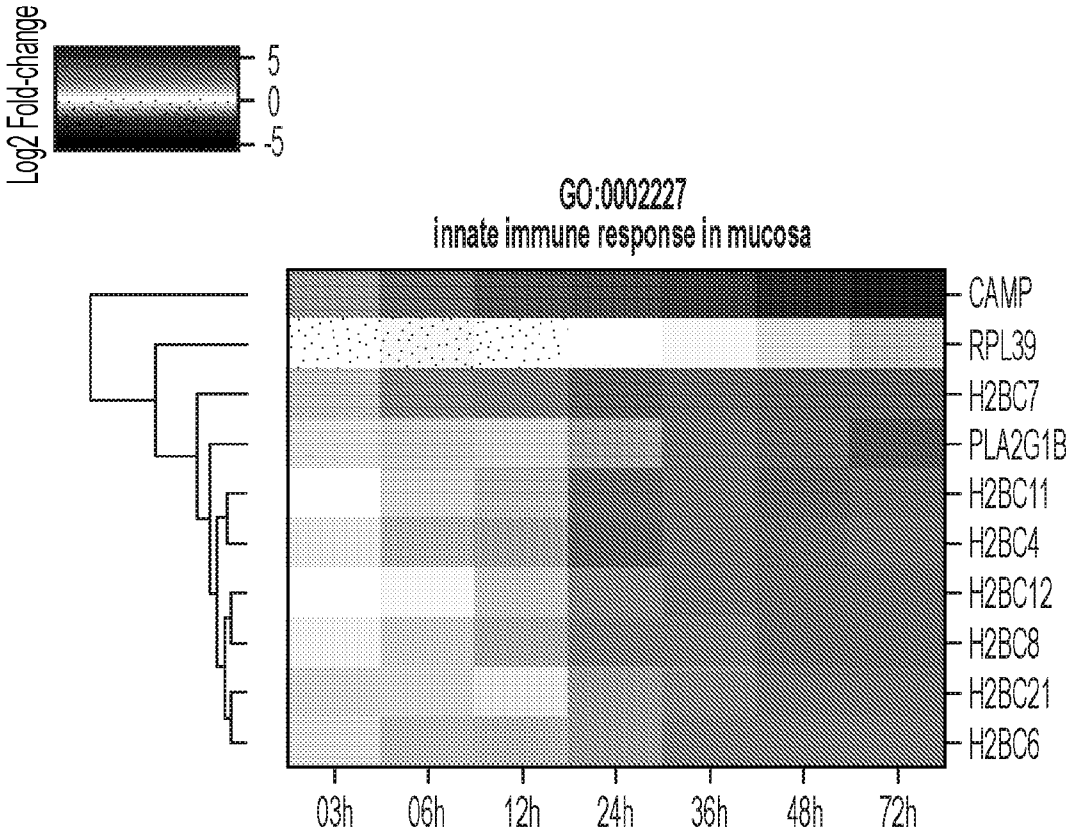


FIG. 14B

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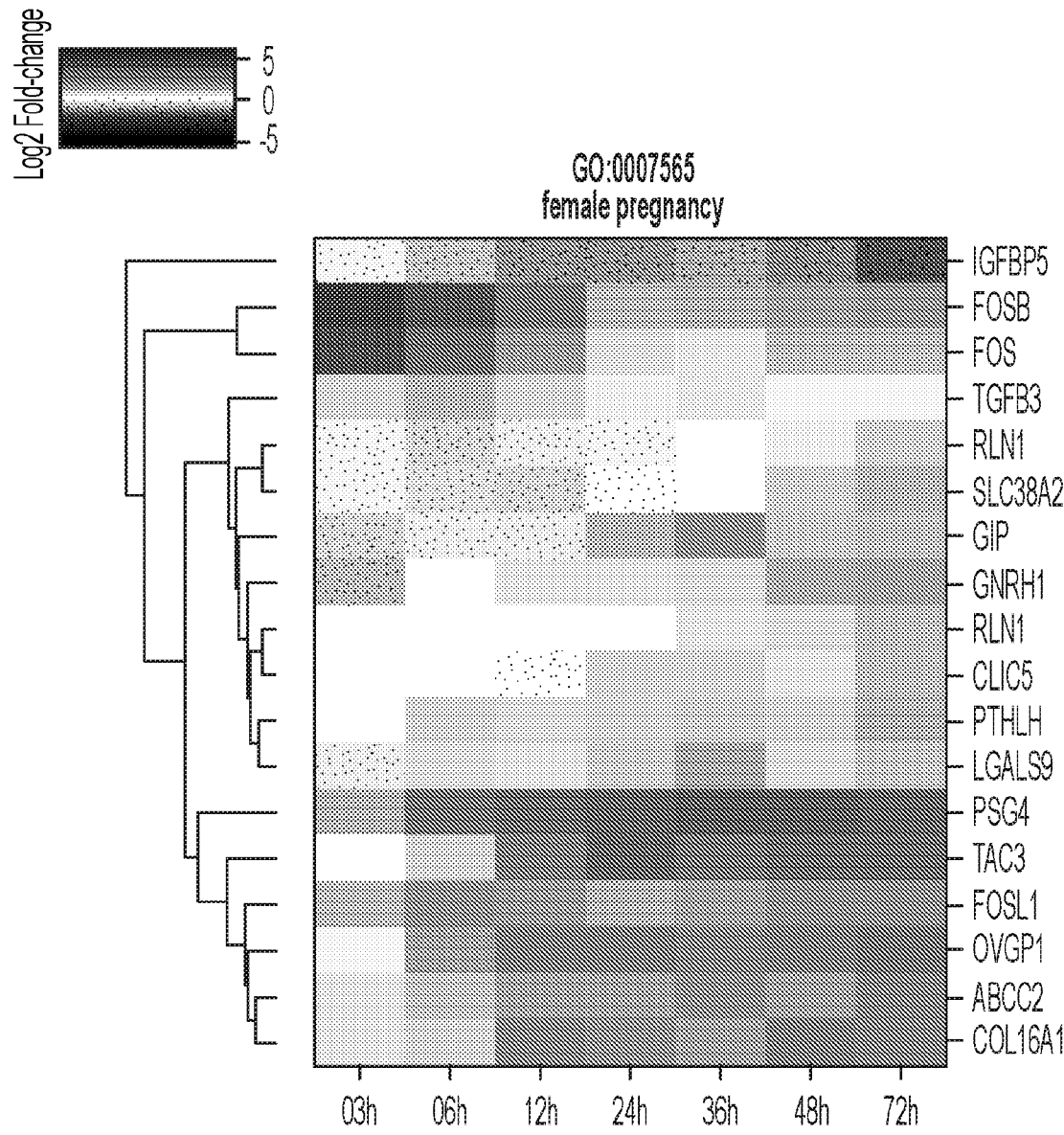


FIG. 14C

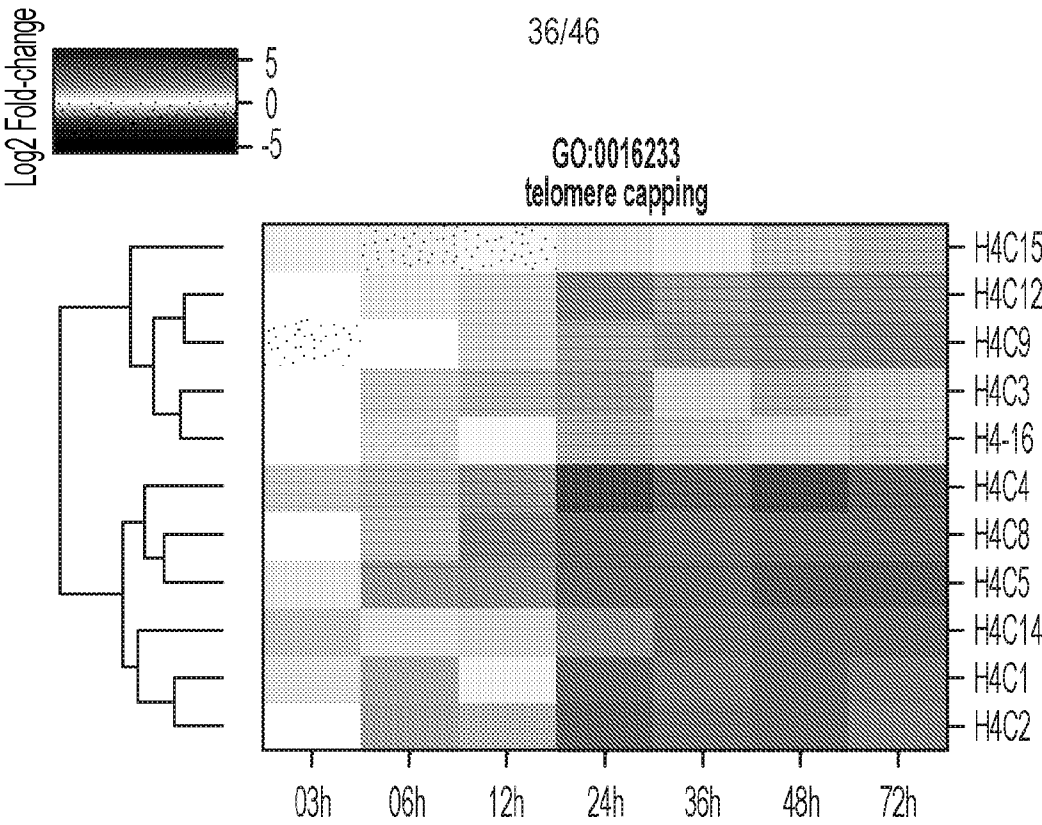


FIG. 14D

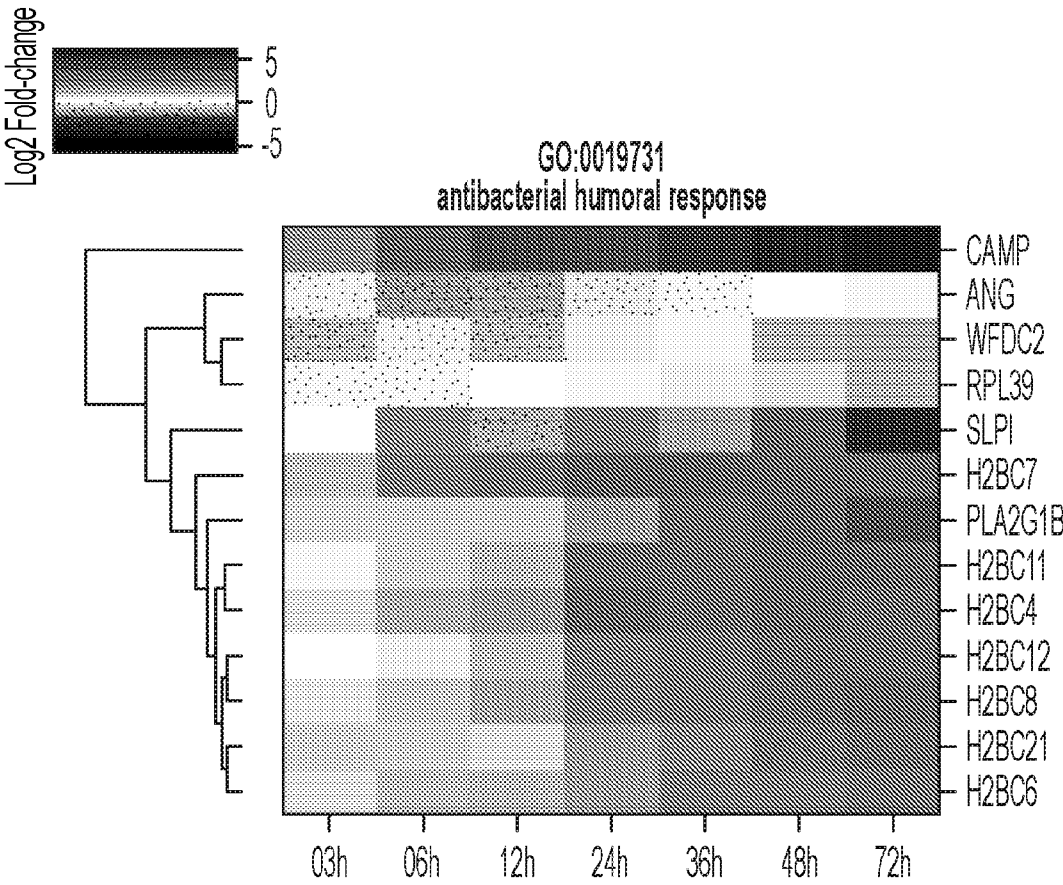


FIG. 14E

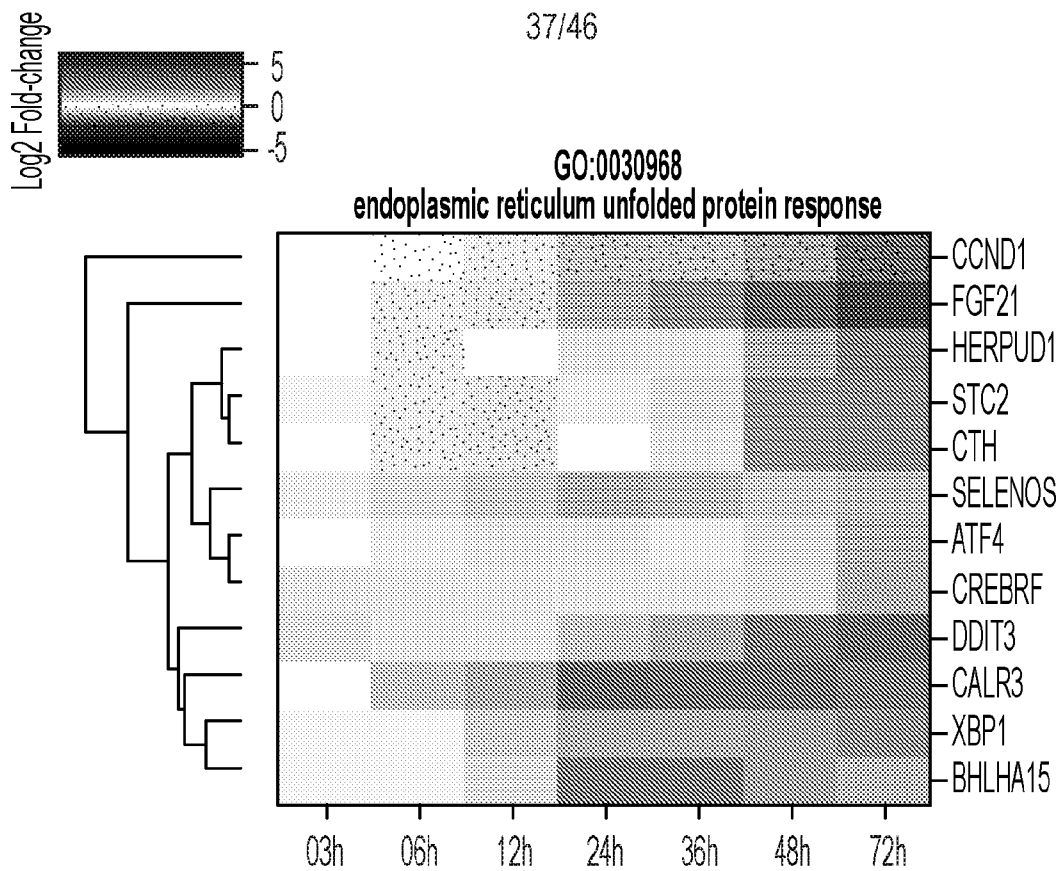


FIG. 14F

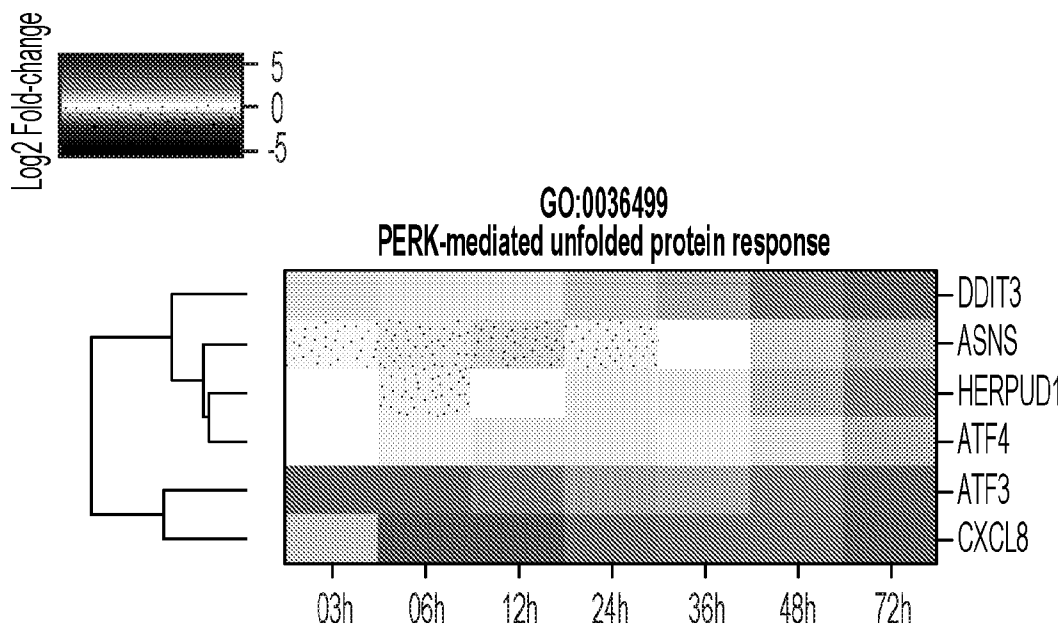
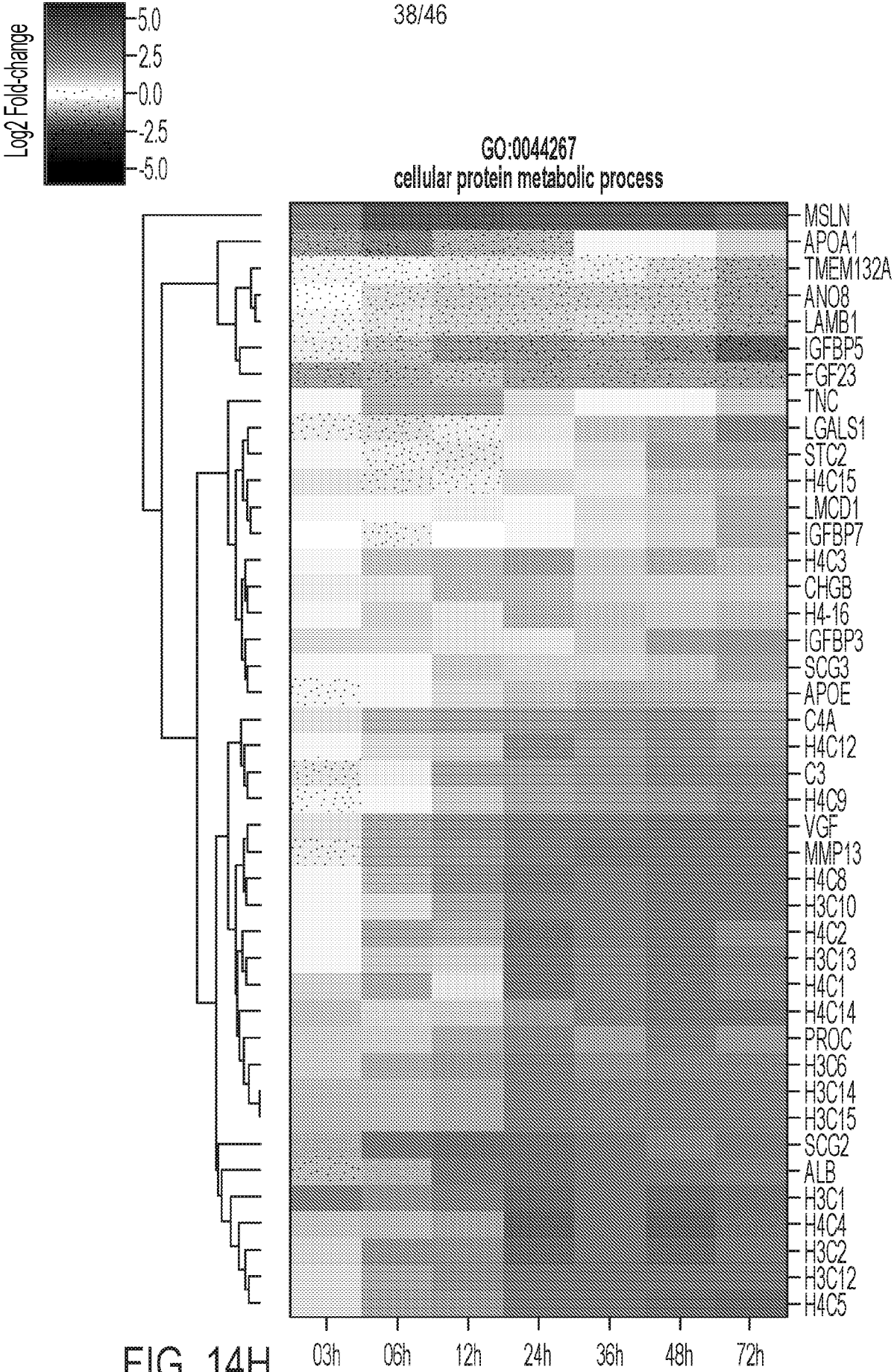


FIG. 14G



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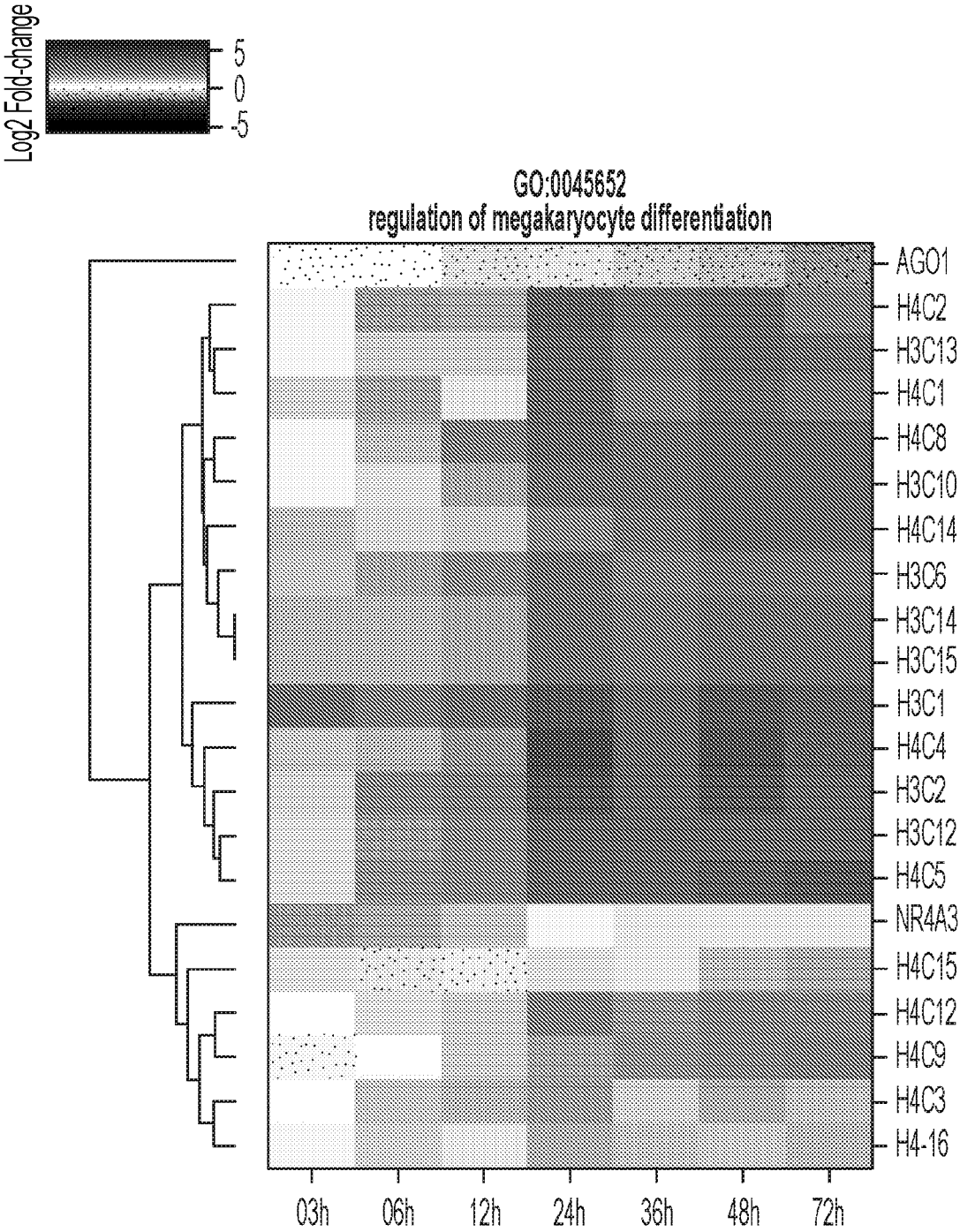


FIG. 14I

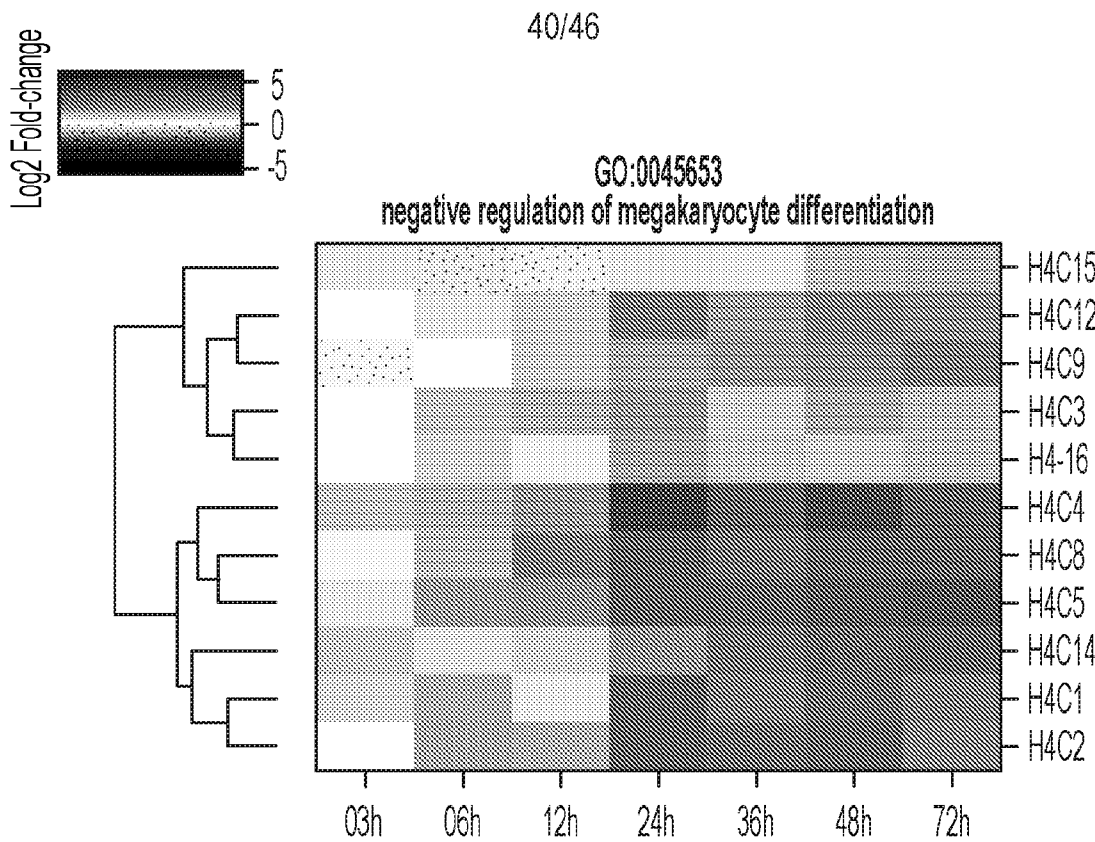


FIG. 14J

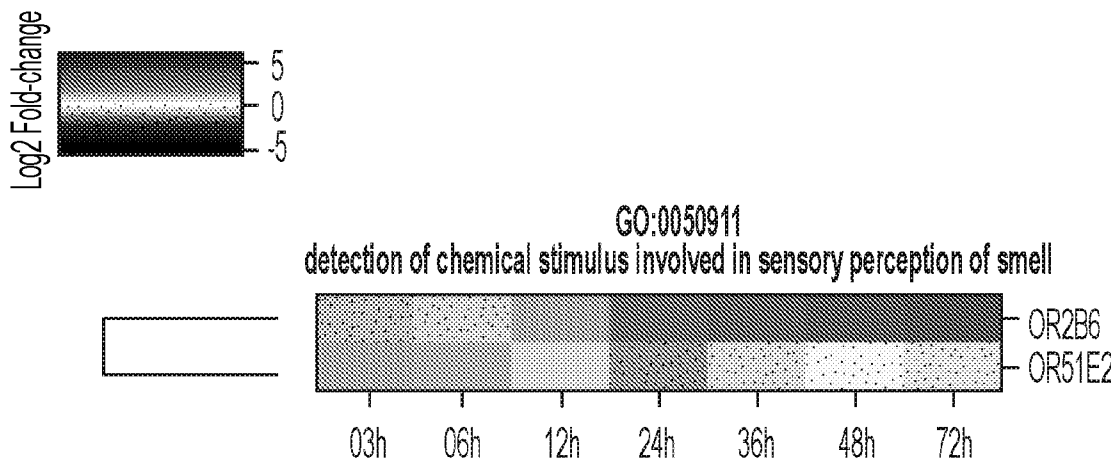


FIG. 14K

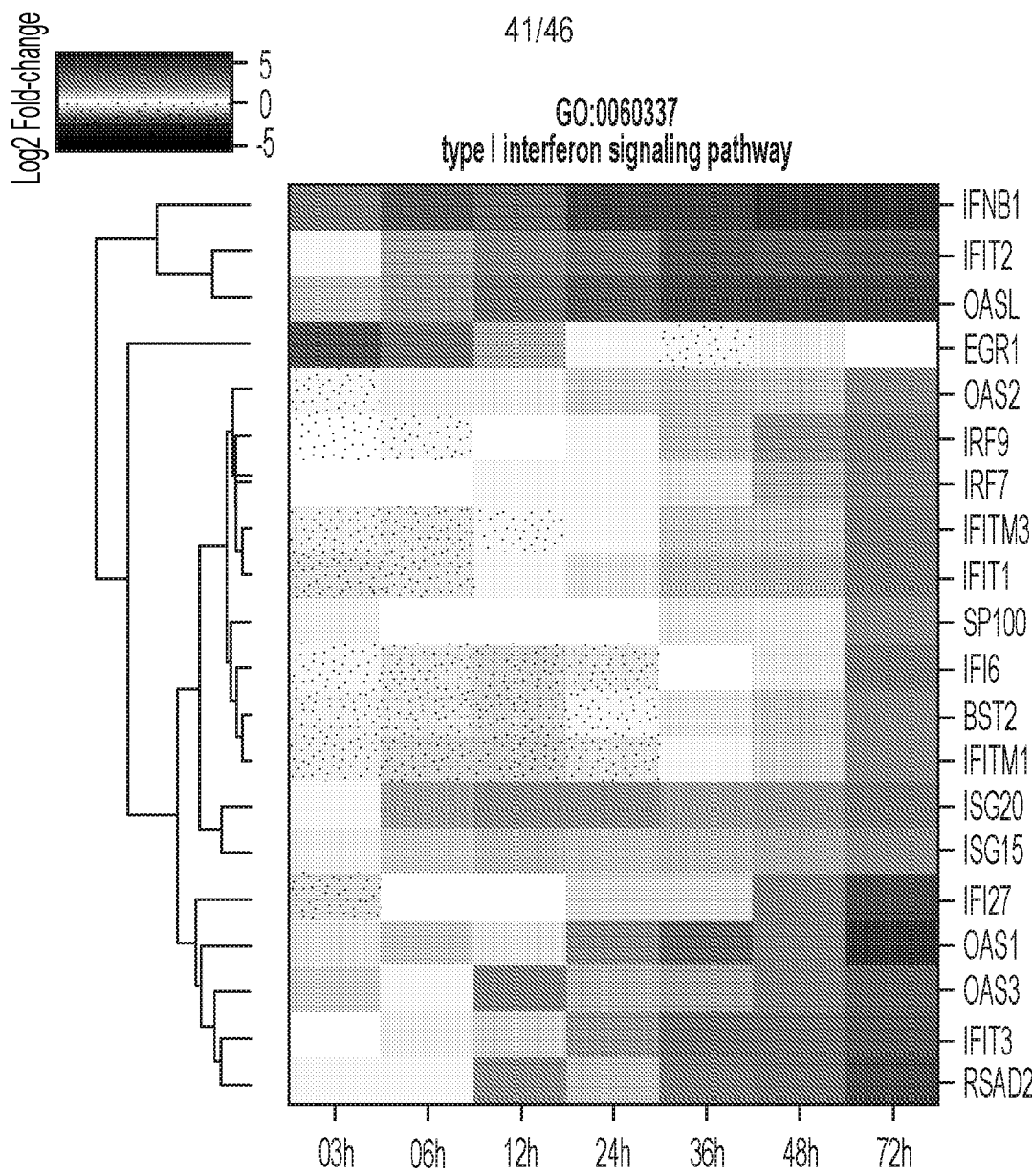


FIG. 14L

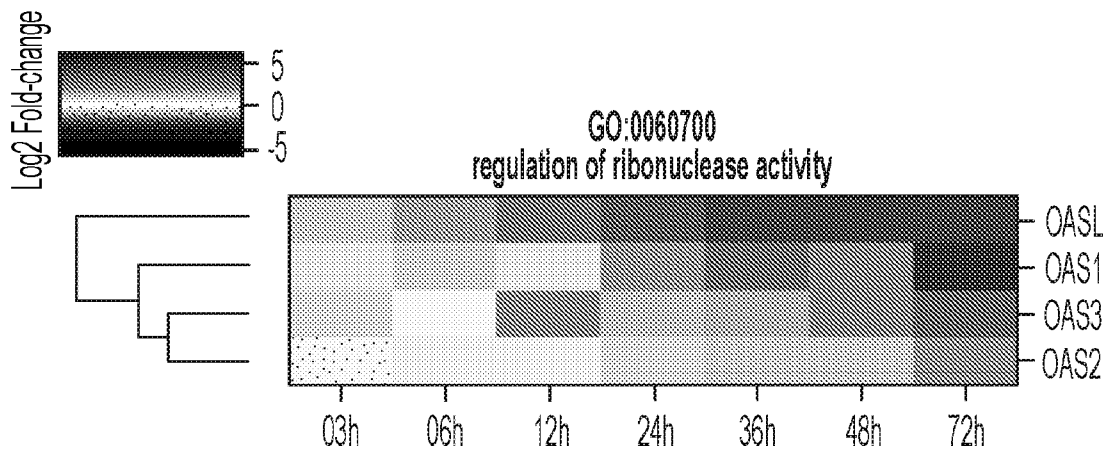


FIG. 14M

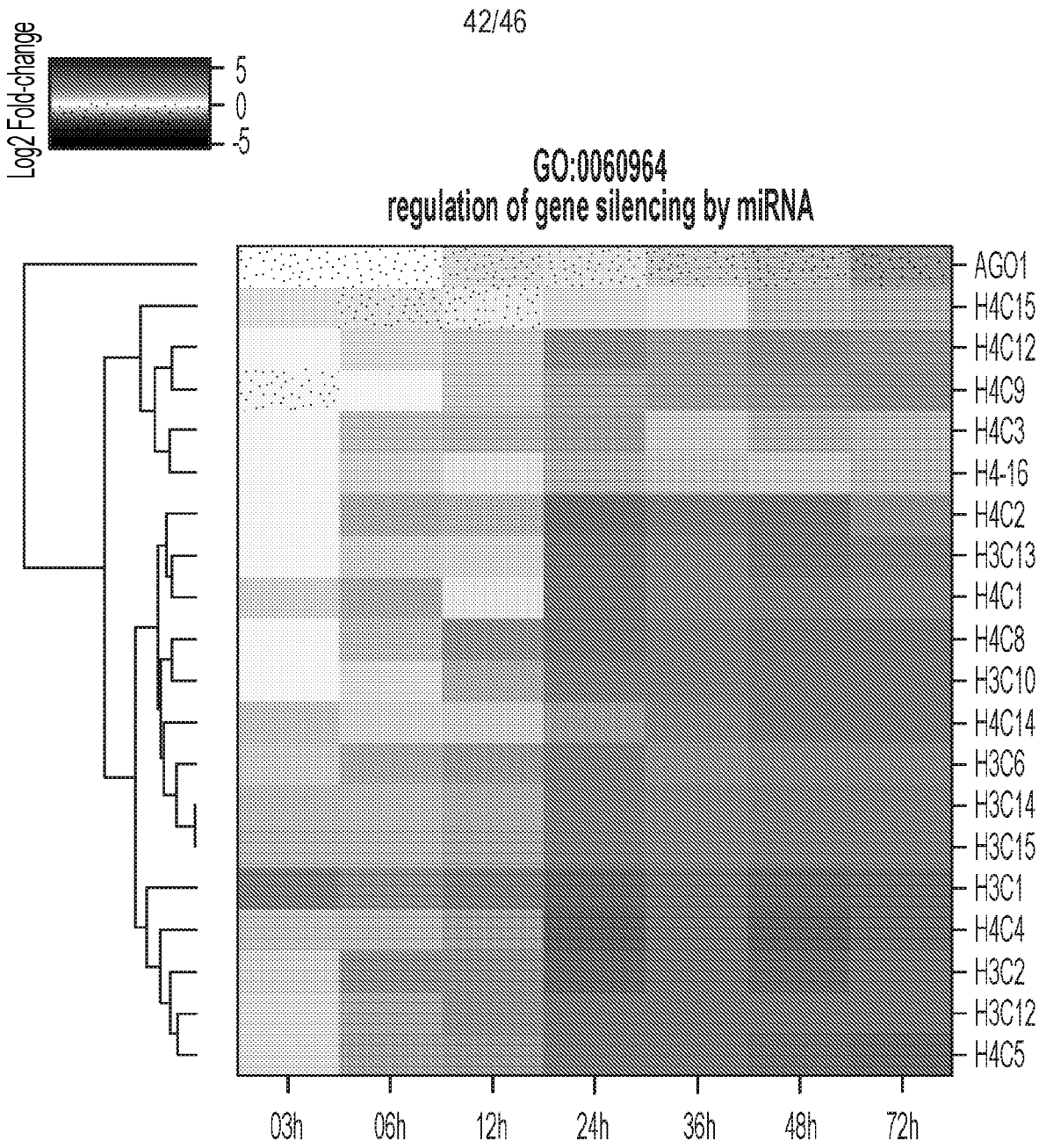


FIG. 14N

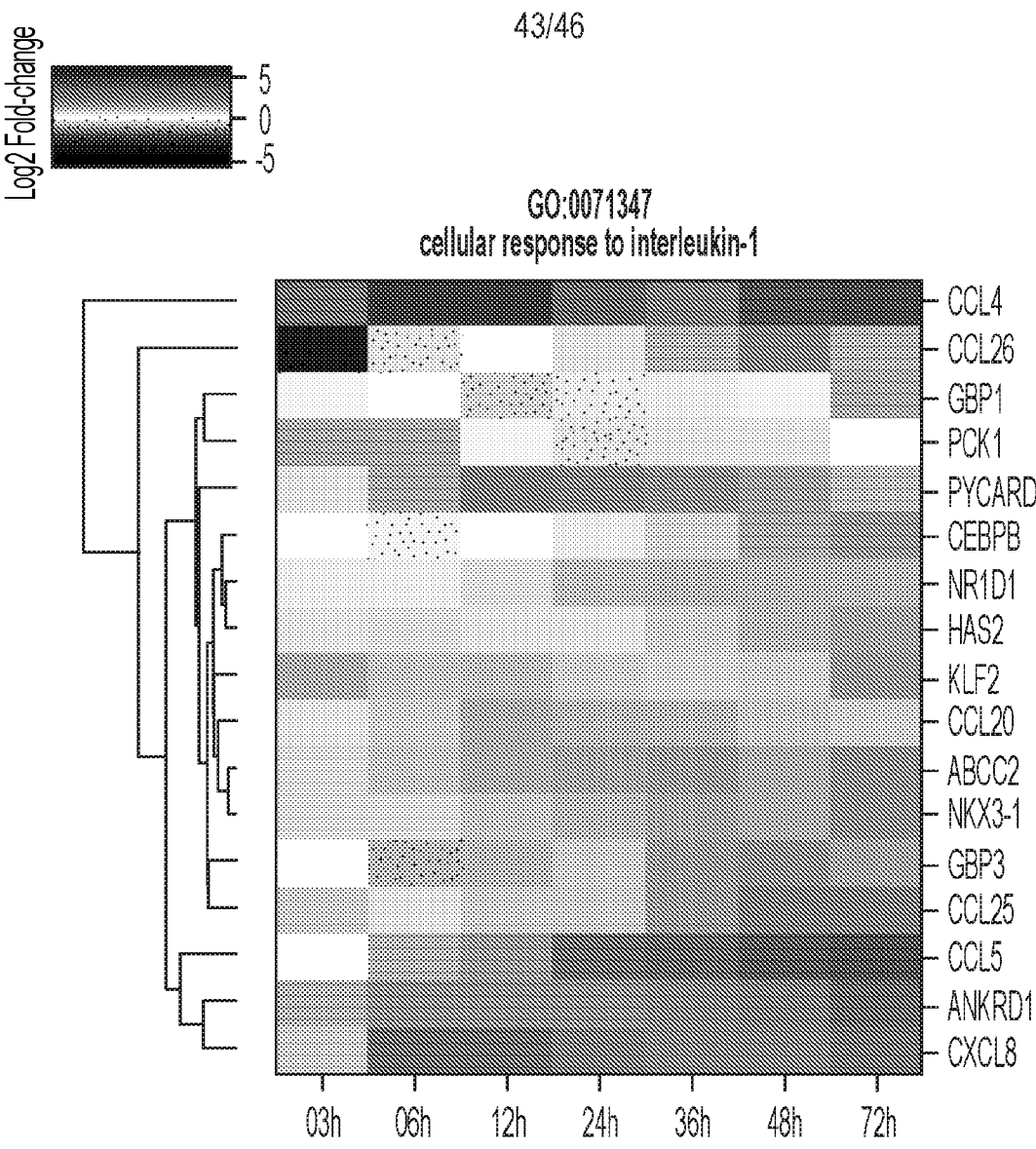


FIG. 14O

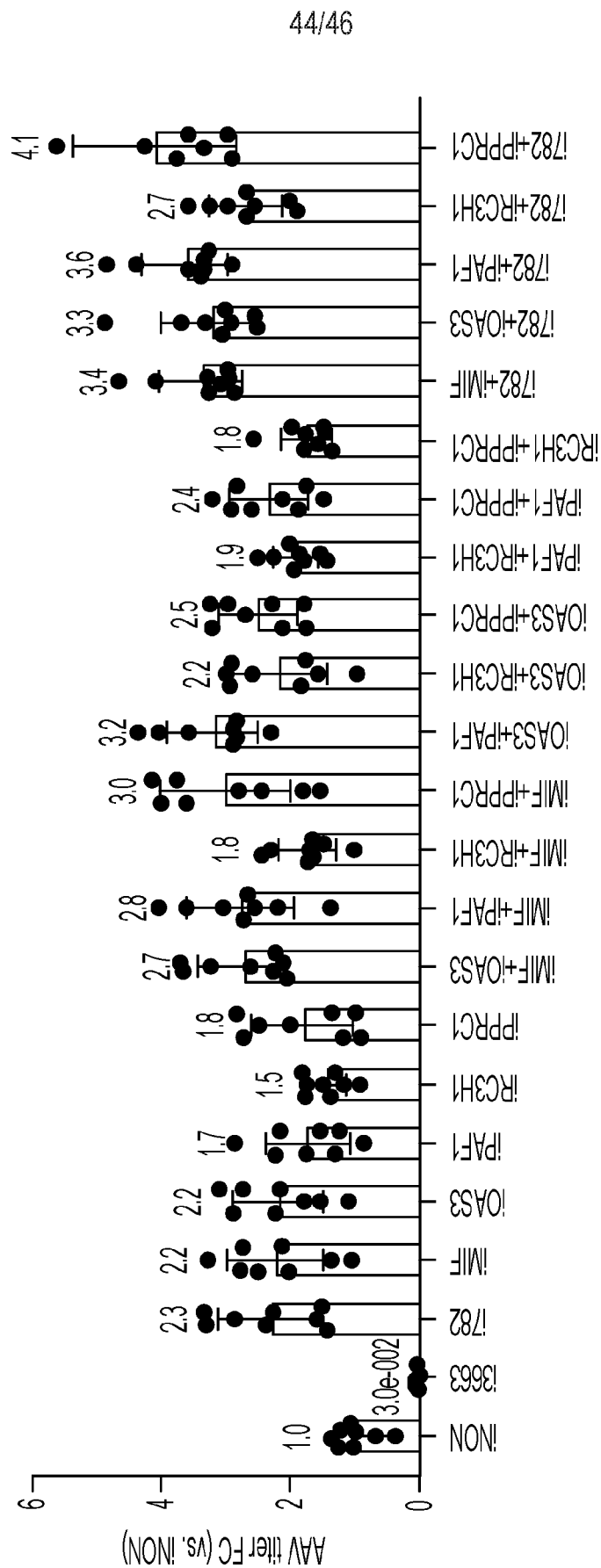


FIG. 15

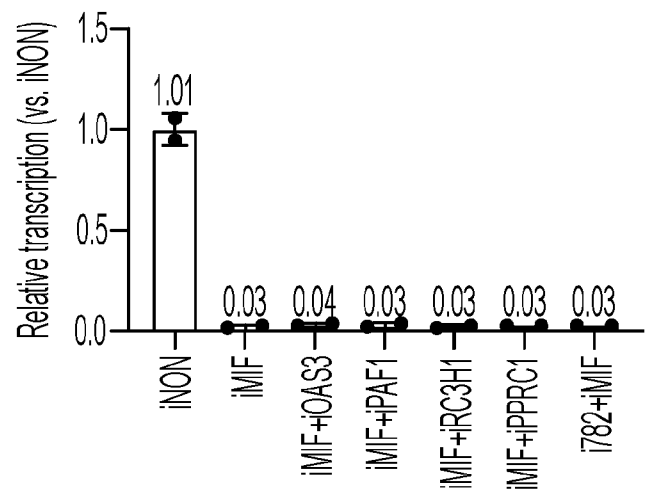


FIG. 16A

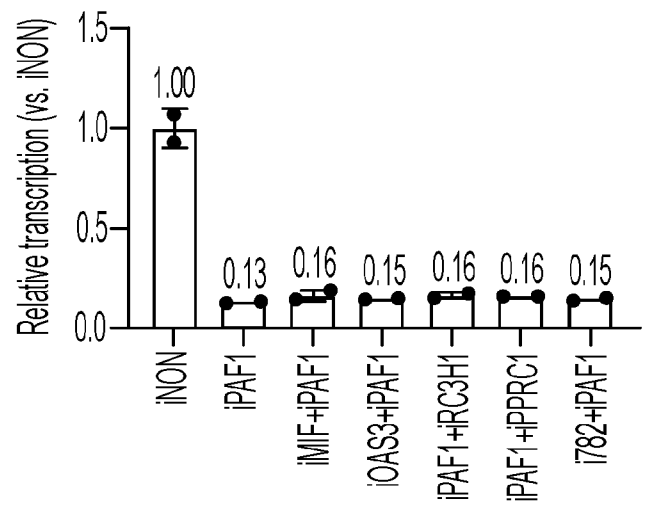


FIG. 16B

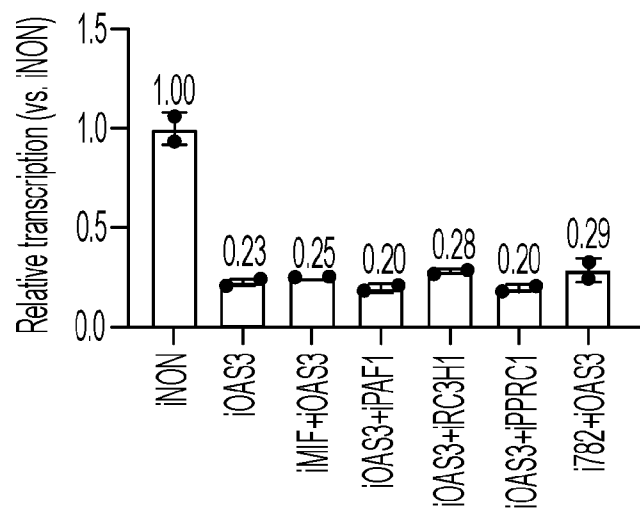


FIG. 16C

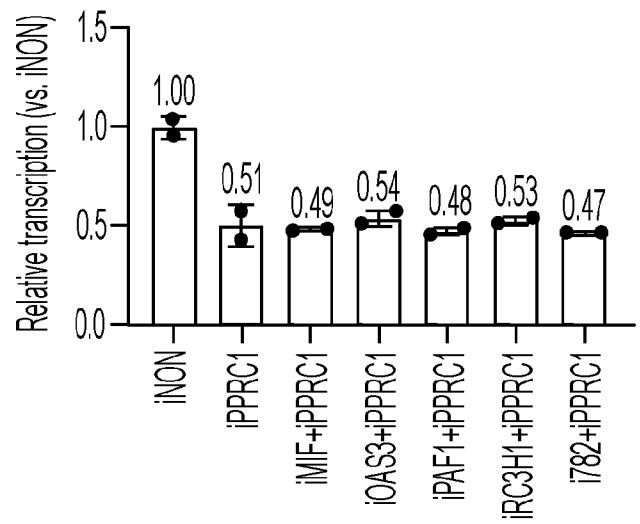


FIG. 16D

INTERNATIONAL SEARCH REPORT

International application No
PCT/IB2023/059919**A. CLASSIFICATION OF SUBJECT MATTER****INV. C12N15/86****ADD. C12N15/63**

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)

C12N C40B

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

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

EPO-Internal**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	MANO MIGUEL ET AL: "Genome-wide RNAi screening identifies host restriction factors critical for in vivo AAV transduction", PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES, vol. 112, no. 36, 24 August 2015 (2015-08-24), pages 11276-11281, XP093113502, ISSN: 0027-8424, DOI: 10.1073/pnas.1503607112 figures 1-5 ----- -/--	1-32



Further documents are listed in the continuation of Box C.



See patent family annex.

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"P" document published prior to the international filing date but later than the priority date claimed

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"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

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Date of the actual completion of the international search

8 January 2024

Date of mailing of the international search report

22/01/2024

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Fax: (+31-70) 340-3016

Authorized officer

Merk1, Philipp

INTERNATIONAL SEARCH REPORT

International application No

PCT/IB2023/059919

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	STRASSER LISA ET AL: "Proteomic Landscape of Adeno-Associated Virus (AAV)-Producing HEK293 Cells", INTERNATIONAL JOURNAL OF MOLECULAR SCIENCES, vol. 22, no. 21, 25 October 2021 (2021-10-25), page 11499, XP093113486, Basel, CH ISSN: 1422-0067, DOI: 10.3390/ijms222111499 Retrieved from the Internet: URL:https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8584267/pdf/ijms-22-11499.pdf> abstract, introduction -----	1-32
A,P	VIRGOLINI NIKOLAUS ET AL: "Transcriptome analysis of Sf9 insect cells during production of recombinant Adeno-associated virus", BIOTECHNOLOGY JOURNAL, vol. 18, no. 2, 9 December 2022 (2022-12-09), XP093113550, DE ISSN: 1860-6768, DOI: 10.1002/biot.202200466 Retrieved from the Internet: URL:https://onlinelibrary.wiley.com/doi/full-xml/10.1002/biot.202200466> Table 1, 2, Fig 5 -----	1-32
T	CHUNG CHENG-HAN ET AL: "Production of rAAV by plasmid transfection induces antiviral and inflammatory responses in suspension HEK293 cells", MOLECULAR THERAPY- METHODS & CLINICAL DEVELOPMENT, vol. 28, 1 March 2023 (2023-03-01), pages 272-283, XP093113170, GB ISSN: 2329-0501, DOI: 10.1016/j.omtm.2023.01.002 the whole document -----	
X,P	WO 2023/077078 A1 (ULTRAGENYX PHARMACEUTICAL INC [US]) 4 May 2023 (2023-05-04) 0006; claims 1, 53 ----- -/--	1-4, 6-10, 17-32

INTERNATIONAL SEARCH REPORT

International application No

PCT/IB2023/059919

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X,P	WANG YONGDAN ET AL: "Transcriptomic Features Reveal Molecular Signatures Associated with Recombinant Adeno-associated Virus Production in HEK293 Cells", BIOTECHNOLOGY PROGRESS, vol. 39, no. 4, 2 May 2023 (2023-05-02), XP093113228, Hoboken, USA ISSN: 8756-7938, DOI: 10.1002/btpr.3346 Retrieved from the Internet: URL:https://aiche.onlinelibrary.wiley.com/doi/pdf/10.1002/btpr.3346> introduction, section 3.4 in general, page 9, right column, last para of 3.4.4 -----	1-32
X,P	Wang Yongdan ET AL: "Supplementary Information Transcriptomic Features Reveal Molecular Signatures Associated with Recombinant Adeno-associated Virus Production in HEK293 Cells", Biotechnology Progress, 2 May 2023 (2023-05-02), pages 1-17, XP093113266, Retrieved from the Internet: URL:https://aiche.onlinelibrary.wiley.com/doi/full/10.1002/btpr.3346 [retrieved on 2023-12-18] the whole document -----	1-32

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/IB2023/059919

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2023077078	A1	04-05-2023	NONE