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(54) **Title:** RECOMBINANT NUCLEIC ACIDS CONTAINING ALPHAHERPESVIRUS PROMOTER SEQUENCES

(57) **Abstract:** Provided are recombinant nucleic acids (e.g., vectors), and related methods, for expression of a target gene in a host cell. The recombinant nucleic acids comprise a promoter comprising a nucleic acid sequence having at least 70% sequence identity to a sequence selected from SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8 and SEQ ID NO: 9; a cloning site for insertion of a nucleic acid encoding the target gene; and at least one non-promoter regulatory element required for the expression of the target gene in the host cell.

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RECOMBINANT NUCLEIC ACIDS CONTAINING ALPHAHERPESVIRUS PROMOTER SEQUENCES

RELATED APPLICATIONS

[0001] This application claims the benefit of U.S. Provisional Application No. 62/801,524, filed on February 5, 2019, and U.S. Provisional Application No. 62/950,848, filed on December 19, 2019. The entire teachings of the above applications are incorporated herein by reference.

BACKGROUND

[0002] Viruses infect their hosts and introduce their genetic material into cells of the host as part of their replication cycle. This genetic material contains basic "instructions" for producing more copies of these viruses by hijacking the body's normal production machinery to serve the needs of the virus. The host cell will carry out these instructions and produce additional copies of the virus, leading to more and more cells of the host becoming infected. As such, viruses can be used as vehicles to carry genes that may provide therapeutic benefits into a cell.

SUMMARY

[0003] In one aspect, the present invention provides a recombinant vector for expression of a target gene in a host cell, comprising: a promoter comprising a nucleic acid sequence having at least 70% sequence identity to a sequence selected from SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3 and SEQ ID NO: 4; a cloning site for insertion of a nucleic acid encoding the target gene; and at least one non-promoter regulatory element required for the expression of the target gene in the host cell.

[0004] In another aspect, the present invention provides a recombinant vector for expression of a target gene in a host cell, comprising: a promoter comprising a nucleic acid sequence having at least 70% sequence identity to a sequence selected from SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7 and SEQ ID NO: 8; a cloning site for insertion of a nucleic acid encoding the target gene; and at least one non-promoter regulatory element required for the expression of the target gene in the host cell.

[0005] In another aspect, the present invention provides a recombinant vector for expression of a target gene in a host cell, comprising: a promoter comprising a nucleic acid

sequence having at least 70% sequence identity to SEQ ID NO: 9; a cloning site for insertion of a nucleic acid encoding the target gene; and at least one non-promoter regulatory element required for the expression of the target gene in the host cell.

[0006] In some embodiments, the at least one non-promoter regulatory element comprises an enhancer element. In some embodiments, the at least one non-promoter regulatory element comprises an inducer element.

[0007] In some embodiments, the recombinant vector further comprises a selectable marker element.

[0008] In some embodiments, the vector is a viral vector. In certain embodiments, the viral vector is an adeno-associated virus (AAV) vector.

[0009] In some embodiments, the vector is a non-viral vector.

[0010] In some embodiments, the host cell is a mammalian cell. In some embodiments, the host cell is a neuronal cell. In some embodiments, the host cell is a non-neuronal cell (e.g., a liver cells, a kidney cell, a retinal cell).

[0011] In another aspect, the present invention provides a host cell comprising a recombinant vector as described herein.

[0012] In one aspect, the present invention provides recombinant polynucleotide molecules comprising a promoter comprising a nucleic acid sequence having at least 70% sequence identity to a sequence selected from SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, and SEQ ID NO: 4. In another aspect, the present invention provides recombinant polynucleotide molecules comprising a nucleic acid encoding a target gene, wherein the nucleic acid is operably linked to a promoter comprising a nucleic acid sequence having at least 70% sequence identity to a sequence selected from SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, and SEQ ID NO: 4.

[0013] In another aspect, the present invention provides recombinant polynucleotide molecules comprising a promoter comprising a nucleic acid sequence having at least 70% sequence identity to a sequence selected from SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, and SEQ ID NO: 8. In another aspect, the present invention provides recombinant polynucleotide molecules comprising a nucleic acid encoding a target gene, wherein the nucleic acid is operably linked to a promoter comprising a nucleic acid sequence having at least 70% sequence identity to a sequence selected from SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, and SEQ ID NO: 8.

[0014] In another aspect, the present invention provides recombinant polynucleotide molecules comprising a promoter comprising a nucleic acid sequence having at least 70% sequence identity to SEQ ID NO: 9. In another aspect, the present invention provides recombinant polynucleotide molecules comprising a nucleic acid encoding a target gene, wherein the nucleic acid is operably linked to a promoter comprising a nucleic acid sequence having at least 70% sequence identity to SEQ ID NO: 9.

[0015] In another aspect, the present invention provides a recombinant polynucleotide molecule comprising a cloning site for insertion of a nucleic acid encoding a target gene, wherein the cloning site is operably linked to a promoter comprising a nucleic acid having at least 70% sequence identity to a sequence selected from SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, and SEQ ID NO: 4.

[0016] In another aspect, the present invention provides a recombinant polynucleotide molecule comprising a cloning site for insertion of a nucleic acid encoding a target gene, wherein the cloning site is operably linked to a promoter comprising a nucleic acid having at least 70% sequence identity to a sequence selected from SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, and SEQ ID NO: 8.

[0017] In another aspect, the present invention provides a recombinant polynucleotide molecule comprising a cloning site for insertion of a nucleic acid encoding a target gene, wherein the cloning site is operably linked to a promoter comprising a nucleic acid sequence having at least 70% sequence identity to SEQ ID NO: 9.

[0018] In another aspect, the present invention provides methods of expressing a target gene in a host cell, comprising: contacting the host cell with a recombinant polynucleotide molecule such that the recombinant polynucleotide molecule is introduced into the host cell, wherein the recombinant polynucleotide molecule comprises a nucleic acid encoding the target gene and wherein the nucleic acid is operably linked to a promoter comprising a nucleic acid sequence having at least 70% sequence identity to a sequence selected from SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3 and SEQ ID NO: 4, whereby expression of the target gene occurs in the host cell.

[0019] In another aspect, the present invention provides methods of expressing a target gene in a host cell, comprising: contacting the host cell with a recombinant polynucleotide molecule such that the recombinant polynucleotide molecule is introduced into the host cell, wherein the recombinant polynucleotide molecule comprises a nucleic acid encoding the target gene and wherein the nucleic acid is operably linked to a promoter comprising a

nucleic acid sequence having at least 70% sequence identity to a sequence selected from SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, and SEQ ID NO: 8, whereby expression of the target gene occurs in the host cell.

[0020] In another aspect, the present invention provides methods of expressing a target gene in a host cell, comprising: contacting the host cell with a recombinant polynucleotide molecule such that the recombinant polynucleotide molecule is introduced into the host cell, wherein the recombinant polynucleotide molecule comprises a nucleic acid encoding the target gene and wherein the nucleic acid is operably linked to a promoter comprising a nucleic acid sequence having at least 70% sequence identity to SEQ ID NO: 9, whereby expression of the target gene occurs in the host cell.

[0021] In some embodiments, the recombinant polynucleotide molecule is introduced into the host cell by transduction upon contacting the host cell with the recombinant polynucleotide. In another embodiment, the recombinant polynucleotide molecule is introduced into the host cell by transfection upon contacting the host cell with the recombinant polynucleotide.

[0022] Features of the LAP sequences disclosed herein include: i) Smaller size when compared to similar promoters, such as EF1α, CAG, TH, CaMKIIa, among others. This is an advantage in the context of AAV vectors that have a limited genetic payload. ii) Long-term expression that is less prone to repression of transcription. The LAP sequences disclosed herein achieve long-term, chronic transcription of transgenes. This is particularly useful for gene therapy where the therapeutic transgene may need to be expressed for the lifespan of the patient/host.

BRIEF DESCRIPTION OF THE DRAWINGS

[0023] The patent or application file contains at least one drawing executed in color. Copies of this patent or patent application publication with color drawing(s) will be provided by the Office upon request and payment of the necessary fee.

[0024] The foregoing will be apparent from the following more particular description of example embodiments, as illustrated in the accompanying drawings in which like reference characters refer to the same parts throughout the different views. The drawings are not necessarily to scale, emphasis instead being placed upon illustrating embodiments.

[0025] **FIGs. 1A-1D** depict the characterization of PRV latency-associated transcript promoters (LAPs). **FIG. 1A** depicts the complete nucleotide sequence of PRV LAP of 902

base pair (bp), and the sub-regions LAP1 of 498 bp (bold and underlined), LAP2 of 404 bp (underlined), and LAP1_2 of 880 bp. LAP1_2 includes most of the LAP1 and LAP2 sequences, but lacks the first 22 nucleotides of LAP1. The black boxes depict consensus sequences for transcription factors (TFs) including, the GC box: specificity protein 1 and 3 (Sp1 and Sp3); the CCAAT box: nuclear factor Y (NF-Y); and the TATA box: TATA-binding protein (TBP). Colored boxes indicate the coordinates for the binding motif sites of the TFs: 1, green: SRY-Box 10 (SOX10); 2, red: cAMP response element-binding protein (CREB); 3, blue: CCCTC-binding factor (CTCF); 4, brown: oligodendrocyte transcription factor 2 (Olig2); 5, pink: signal transducer and activator of transcription (STAT1). **FIG. 1B** depicts the plasmid maps of the four AAVs designed to transcribe mCherry fluorescent reporter from LAP1, LAP2, LAP1_2 and the EF1α promoter. WPRE of 609 bp is a woodchuck hepatitis virus posttranscriptional enhancer element. All AAVs contain a 479 bp human growth hormone (hGH polyA) poly adenylation sequence, and flanking AAV2 inverted terminal repeats (ITRs) of 141 bp each. The vectors were packaged into the AAV-PhP.eB serotype capsid. The total size of the enhancer-promoter elements and promoter sequence are: AAV-LAP1: 1.87 Kb; AAV-LAP2: 1.77 Kb; AAV-LAP1_2: 2.25 Kb; and AAV-EF1α: 2.63 Kb. **FIG. 1C** depicts the four AAVs used to transduce primary cultures of rat SCG neurons to quantify mCherry expression over a 90-day time-lapse. The relative fluorescence intensity of mCherry expression was measured at 3, 5, 7, 9, 11, 14, 17, 21, 24, 28, 31, 34, 38, 41, 45, 49, 52, 59, 67, 73, 82, and 90 days post infection (dpi) with 3×10^{11} AAV genomes. Scale bar = 100 μm. Data are represented as mean ± SEM; n = 3 SCG culture dishes per group. **FIG. 1D** shows that AAV-driven mCherry expression in SCG cell bodies at 28 days post AAV transduction with LAP1-mCherry, LAP2-mCherry, LAP1_2-mCherry, or EF1α-mCherry.

[0026] **FIGs. 2A-2P** show stable and long-term LAP-mediated transgene expression. **FIG. 2A** is a schematic diagram of the systemic route of AAV administration and CNS tissue processing. Intravenous administrations of AAV vectors were performed by unilateral injections into mouse retro-orbital sinuses (4×10^{11} vg/mouse). Brains and spinal cords were collected at 30 dpi and 190 dpi. The right hemispheres of the brains were processed for iDISCO+ tissue clearing and light-sheet microscopy analysis. The left hemispheres of the brains were sagittally sectioned at 50 μm for immunofluorescence and confocal microscopy analysis. Spinal cords were transversally sliced at 20 μm for immunofluorescence and confocal microscopy analysis. **FIGs. 2B-2P** quantify the densities of mCherry positive cells

per mm^3 across different brain regions in iDISCO+-cleared tissue samples at 30 dpi and 190 dpi. Data are represented as mean ± SEM; n = 2 animals per group, and between five and ten 500 um sections per animal. Data were normalized to those of a vehicle-injected control animal. Significance was determined with Student's t-test (if only two groups were compared), or analysis of variance one-way (ANOVA) followed by Bonferroni post hoc test (if more than two groups were compared). A p value of < 0.05 was statistically significant ($*p < 0.033$; $**p < 0.002$; $***p < 0.001$).

[0027] **FIGs. 3A-3J4** show that all three AAV-LAP sequences drive widespread and long-term transgene expression throughput the brain after retro-orbital injection. Representative immunofluorescence images of sagittal sections show whole-brain distributions of anti-mCherry staining (green) for AAV-LAP1 (**FIG. 3A**), AAV-LAP2 (**FIG. 3B**), AAV-LAP1_2 (**FIG. 3C**), and AAV-EF1α (**FIG. 3D**) at 190 dpi. Cx, cortex; Hip, hippocampus; MRN, midbrain reticular nucleus; Cb, cerebellum; Thal, thalamus; Pn, pons; Hypo, hypothalamus; Str, striatum; OB, olfactory bulb. Scale bar = 1mm. Representative confocal images show anti-mCherry signals (green) in the cortex (**FIG. 3E**), the dentate gyrus (**FIG. 3F**), the striatum (**FIG. 3G**) and the cerebellum (**FIG. 3H**) at 30 dpi and 190 dpi. All images are stack confocal sections. Scale bar = 100 μm. **FIGs. 3I1-3I4** quantify the indirect fluorescence intensities of the anti-mCherry signals driven by the AAV-LAP sequences and AAV-EF1α at 30 dpi and 190 dpi in the cortex, the dentate gyrus, the striatum and the cerebellum. **FIGs. 3J1-3J4** quantify the number of cells expressing mCherry signal per $pixels^2$ by immunohistochemistry (IHC) at 190 dpi in the cortex, the dentate gyrus, the striatum and the cerebellum. Data are represented as mean ± SEM; n = 2 animals and six tissue sections per animal. Data were normalized to those of a vehicle-injected control animal. Significance was determined with Student's t-test (if only two groups were compared), or analysis of variance one-way (ANOVA) followed by Bonferroni post hoc test (if more than two groups were compared). A p value of < 0.05 was statistically significant ($*p < 0.033$; $**p < 0.002$; $***p < 0.001$).

[0028] **FIGs. 4A-4E4** show that LAP2 drives stable and long-term transgene expression in the brain. Representative confocal images show native mCherry fluorescence (red) for AAV-LAP1, AAV-LAP2, AAV-LAP1_2, and AAV-EF1α in the cortex (**FIG. 4A**), the dentate gyrus (**FIG. 4B**), the striatum (**FIG. 4C**) and the cerebellum (**FIG. 4D**) at 30 dpi and 190 dpi. All images are stack confocal sections. Scale bar = 100 μm. **FIGs. 4E1-4E4** quantify the direct fluorescence intensities of native mCherry signal driven by AAV-LAP sequences

and AAV-EF1α at 30 dpi and 190 dpi is shown in the cortex, the dentate gyrus, the striatum and the cerebellum. Data are represented as mean ± SEM; n = 2 animals and six tissue sections per animal. Data were normalized to those of a vehicle-injected control animal. Significance was determined with Student's t-test (if only two groups were compared) or analysis of variance one-way (ANOVA) followed by Bonferroni post hoc test (if more than two groups were compared). A p value of < 0.05 was statistically significant (*$p < 0.033$; **$p < 0.002$; ***$p < 0.001$).

[0029] **FIGs. 5A-5E** show that AAV-LAP2 drives long-term transgene transcription in the CNS. The presence of mCherry mRNA was verified by FISH in brain tissues at 190 dpi using a riboprobe specific to mCherry (green). Nuclei were counterstained with DAPI (blue). **FIGs. 5A-5E** show 20-μm sagittal brain slices for AAV-LAP2 or AAV-EF1α in the cortex, the dentate gyrus, the striatum, the cerebellum and the olfactory bulb, respectively. Panels 2 and 4 depict higher magnification images of the indicated regions (square) in Panels 1 and 3, respectively. Images are stack confocal sections. Scale bars = 100 μm.

[0030] **FIGs. 6A-6F** show that AAV-LAP transgene expression is predominantly in neurons and not oligodendrocytes. Representative confocal images of AAV-mediated mCherry expression (red) in neurons (**FIGs. 6A** and **6B**: green label for the pan-neuronal marker NeuN) or oligodendrocytes (**FIGs. 6C** and **6D**: green label for the oligodendrocyte marker Olig2) in the cortex or the dentate gyrus at 30 dpi. Cells were counterstained with DAPI (blue). NeuN signal can localize with the neuronal cell nucleus as well as the cytoplasm, while the staining for Olig2 signal is mostly nuclear. Arrows depict co-labelling between the cell marker and mCherry. Scale bar = 100 μm. **FIGs. 6E** and **6F** quantify the percentage of mCherry labelled cells corresponding to neurons (NeuN-positive) or oligodendrocytes (Olig2-positive) in the cortex (Cx) or the dentate gyrus (DG) for each promoter. Images are stack confocal sections. Data are represented as mean ± SEM; n = 2 animals and six tissue sections per animal.

[0031] **FIGs. 7A-7D** show that AAV-LAP transgene expression is not detected in microglia and astrocytes. Representative confocal immunofluorescence images of anti-mCherry signal (red), microglia (FIGs. 7A and 7B: green, labeled as Iba1-positive), astrocytes (FIGs. 7C and 7D: green, labeled as S100-positive) in the cortex and dentate gyrus at 30 dpi. The absences of co-labelling between mCherry in astrocytes and microglia are indicated with stars (*). Cells were counterstained with DAPI (blue). Images are stack confocal sections. Scale bar = 100 μm.

[0032] **FIGs. 8A-8F** show that LAP sequences drive widespread and long-term transgene expression in the spinal cord. Spinal cords (lumbar region) were sectioned in a transversal fashion at 20 μm. Representative confocal images at 190 dpi of native AAV-mediated mCherry expression (red, panels 1 and 4), pan-neuronal marker NeuroTrace (green, panels 2 and 5), and merge signal (yellow, panels 3 and 6) for AAV-LAP1, AAV-LAP2, AAV-LAP1_2 and AAV-EF1α. Images are stack confocal sections. DH: dorsal horn; VH ventral horn. Higher magnification images are shown in Panels 4-6. Scale bar = 100 μm.

[0033] **FIGs. 9A-9C** show that LAP sequences drive efficient transgene expression in porcine kidney cells. Representative images of PK15 cells at 48 hours post transfection of a reporter plasmid comprising mCherry driven by LAP1 (**FIG. 9A**), LAP2 (**FIG. 9B**) or LAP1_2 (**FIGs. 9C**). Panel 1: bright field; Panel 2: fluorescence; Panel 3: merge. Scale bar = 50 μm.

[0034] **FIGs. 10A** and **10B** show that LAP2 drives efficient transgene expression in human kidney cells. Representative images of HEK-293 cells at 48 hours post transfection of a reporter plasmid comprising mCherry driven by LAP2 (**FIGs. 10A**) or the EF1α-promoter (**FIGs. 10B**). Panel 1: bright field; Panel 2: fluorescence; Panel 3: merge. Scale bar = 50 μm.

[0035] **FIGs. 11A** and **11B** show that LAP2 drives efficient transgene expression in human liver cells. Representative images of HepG2 cells at 48 hours post transfection of a reporter plasmid comprising mCherry driven by LAP2 (**FIGs. 11A**) or the EF1α-promoter (**FIGs. 11B**). Panel 1: bright field; Panel 2: fluorescence; Panel 3: merge. Scale bar = 50 μm.

[0036] **FIGs. 12A1-12B4** show that LAP2 drives long-term transgene expression throughout the liver. Intravenous administrations of AAV-LAP2 (**FIGs. 12A1-12A4**) or AAV-EF1α-vector (**FIGs. 12B1-12B4**) were performed by unilateral injection into the mouse retro-orbital sinuses (4×10^{11} vg/mouse). The liver was collected at 190 dpi and longitudinally sectioned at 20 μm for IHC and confocal microscopy analysis. Representative confocal images of native mCherry fluorescence (**FIGs. 12A1** and **12B1**: red), anti-mCherry immunostaining (**FIGs. 12A2** and **12B2**: green), DAPI nuclear counterstain (**FIGs. 12A3** and **12B3**: blue), and merged immuno-fluorescence and DAPI images (**FIGs. 12A4** and **12B4**). CV: central vein. Scale bar = 75 μm.

[0037] **FIGs. 13A1-13B4** show that LAP2 drives long-term transgene expression in the mouse kidney. Intravenous administrations of AAV-LAP2 (**FIGs. 13A1-13A4**) or AAV-EF1α-vector (**FIGs. 13B1-13B4**) were performed by unilateral injection into the mouse retro-orbital sinuses (4×10^{11} vg/mouse). The kidneys were collected at 190 dpi and longitudinally

sectioned at 20 μm for IHC and confocal-microscopy analysis. Representative confocal images show native mCherry fluorescence (**FIGs. 13A1** and **13B1**: red), anti-mCherry immunostaining (**FIGs. 13A2** and **13B2**: green), DAPI nuclear counterstain (**FIGs. 13A3** and **13B3**: blue), and merged immune-fluorescence and DAPI images **(FIGs. 13A4** and **13B4**). AV: arcuate vessel. Scale bar = 75 μm.

[0038] **FIGs. 14A1-14B4** show that LAP2 drives long-term transgene expression in the mouse retina. Intravenous administrations of AAV-LAP2 (**FIGs. 14A1-14A4**) or AAV-EF1α-vector (**FIGs. 14B1-14B4**) were performed by unilateral injection into the mouse retro-orbital sinuses (4×10^{11} vg/mouse). The retinae were collected at 190 dpi and transversely cryosectioned at 20 μm for IHC and confocal-microscopy analysis. Representative confocal images show native mCherry fluorescence (**FIGs. 14A1** and **14B1**: red), anti-mCherry immunostaining (**FIGs. 14A2** and **14B2**: green), DAPI nuclear counterstain (**FIGs. 14A3** and **14B3**: blue), and merged immunofluorescence and DAPI images (**FIGs. 14A4** and **14B4**). GCL: ganglion-cell layer; INL: inner nuclear layer; ONL: outer nuclear layer. Scale bar = 75 μm.

DETAILED DESCRIPTION

[0039] A description of example embodiments follows.

[0040] Several aspects of the invention are described below, with reference to examples for illustrative purposes only. It should be understood that numerous specific details, relationships, and methods are set forth to provide a full understanding of the invention. One having ordinary skill in the relevant art, however, will readily recognize that the invention can be practiced without one or more of the specific details or practiced with other methods, protocols, reagents, cell lines and animals. The present invention is not limited by the illustrated ordering of acts or events, as some acts may occur in different orders and/or concurrently with other acts or events. Furthermore, not all illustrated acts, steps or events are required to implement a methodology in accordance with the present invention. Many of the techniques and procedures described, or referenced herein, are well understood and commonly employed using conventional methodology by those skilled in the art.

[0041] Unless otherwise defined, all terms of art, notations and other scientific terms or terminology used herein are intended to have the meanings commonly understood by those of skill in the art to which this invention pertains. In some cases, terms with commonly understood meanings are defined herein for clarity and/or for ready reference, and the

inclusion of such definitions herein should not necessarily be construed to represent a substantial difference over what is generally understood in the art. It will be further understood that terms, such as those defined in commonly-used dictionaries, should be interpreted as having a meaning that is consistent with their meaning in the context of the relevant art and/or as otherwise defined herein.

[0042] The terminology used herein is for the purpose of describing particular embodiments only and is not intended to be limiting. As used herein, the indefinite articles "a," "an" and "the" should be understood to include plural reference unless the context clearly indicates otherwise.

[0043] As used herein, a "cloning site" refers to a short segment of nucleotides in the vector that contain one or more unique restriction sites that allow for insertion of a nucleotide "target gene" or "gene of interest" into the vector.

[0044] As used herein, a "promoter" refers to a region of DNA to which an RNA polymerase binds and initiates transcription (e.g., of a gene).

[0045] As used herein, a "non-promoter regulatory element" refers to non-promoter sequence(s) of a nucleic acid molecule that are capable of increasing or decreasing the expression of specific genes within the recombinant vector. Such non-promoter regulatory elements include, but are not limited to, e.g., enhancer elements, inducer elements, silencer elements, 5' untranslated regions (UTRs), 3'UTRs, terminator elements, CAAT boxes, CCAAT boxes, Pribnow boxes, SECIS elements, polyadenylation signals, A-boxes, Z-boxes, C-boxes, E-boxes, G-boxes, and Cis-regulatory elements (CREs).

[0046] As used herein, the phrase "operably linked" means that the nucleic acid is positioned in the recombinant polynucleotide, e.g., vector, in such a way that enables expression of the nucleic acid under control of the element (e.g., promoter) to which it is linked.

[0047] As used herein, a "selectable marker element" is an element that confers a trait suitable for artificial selection. Examples of selectable marker elements useful in the present invention include, but are not limited to, beta-lactamase, neomycin resistance genes, mutant Fabl genes conferring triclosan resistance, URA3 elements, fluorescent gene products, affinity tags such as GST, His, CBP, MBP, and epitope tags such as Myc HA, FLAG. Selectable marker elements can be negative or positive selection markers.

[0048] The present invention provides, in various embodiments, vectors, such as viral vectors (e.g., adeno-associated viral vectors (AAV)), containing promoter sequences that are

small in size yet able to provide long-lasting transcription of a gene of interest. The promoter sequences have a size that is less than that of many commonly used promoters, and can drive transcription of a transgene in most tissue and cell types (e.g., in mammals). The promoter sequences disclosed herein are less prone to repression or inactivation, making them particularly useful for gene therapies requiring chronic expression of a therapeutic transgene.

[0049] In one aspect, the present invention provides gene delivery vectors expressing a gene of interest. In some embodiments, the gene delivery vector comprises a promoter derived from a genomic region of an alphaherpesvirus genome, e.g., from the pseudorabies virus. In some embodiments, the promoter comprises a latency-associated promoter (LAP) region from an alphaherpesvirus genome.

[0050] In particular embodiments, the LAP region utilized in the vectors of the present invention is selected from SEQ ID NO: 1 (also referred to herein as LAP1), SEQ ID NO: 2 (also referred to herein as LAP2), SEQ ID NO: 3 (also referred to herein as LAP1_2), and SEQ ID NO: 4 (**Table 1**). LAP1_2 (880 bp) includes a 476-bp sequence of LAP1 (498 bp) and the complete LAP2 sequence (404 bp), i.e., LAP1 and LAP2 in tandem but missing the first 22 bp of the LAP1 sequence. SEQ ID NO: 4 (902 bp) comprises the complete sequences of SEQ ID NO: 1 and SEQ ID NO: 2, and is 22 bp longer than SEQ ID NO: 3.

[0051] In some embodiments, the promoter comprises a nucleic acid sequence having at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98% or at least 99% sequence identity to a nucleic acid sequence selected from SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3 and SEQ ID NO: 4. In some embodiments, the promoter comprises a nucleic acid sequence selected from SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3 and SEQ ID NO: 4.

[0052] Without wishing to be bound by theory, it is believed that certain active portions or fragments of a LAP region from an alphaherpesvirus genome can drive expression of a transgene in a variety of cell and tissue types. In some embodiments, the promoter used in the present invention comprises a nucleic acid sequence having at least 70% sequence identity to SEQ ID NO: 9 (**Table 1**). SEQ ID NO: 9 consists of a 232-bp “core” sequence of LAP2 and includes a TATA box sequence. In some embodiments, the promoter comprises a nucleic acid sequence having at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98% or at least 99% sequence identity to SEQ ID NO: 9. In some embodiments, the promoter comprises SEQ ID NO: 9.

[0053] Without wishing to be bound by theory, LAP sequences further comprising SEQ ID NO: 10, SEQ ID NO: 11, and/or SEQ ID NO: 12 (**Table 1**) may have improved transcriptional performance. Accordingly, in some embodiments, the promoter further comprises SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 12, or a combination thereof, e.g., at the 5' or the 3' of SEQ ID NO: 9. SEQ ID NO: 10 (the "CTCF" motif) has been shown to be involved in the epigenetic regulation of herpes virus gene expression, chromatin remodeling and recruitment of transcription factors and proteins (Lang et al., 2017, Lee et al., 2018). SEQ ID NO: 11 (the "alternative CTCF with upstream stabilizer" motif) has been shown to stabilize an alternative CTCF motif and facilitate binding of four more zinc finger domains (Zimmerman et al., 2018). SEQ ID NO: 12 (the "Nuclear transcription factor Y (NF-Y)" motif) is recognized by a transcription factor that binds to the CAAT box located in the promoter regions of several eukaryotes (Chikhirzhina et al., 2008; Fleming et al., 2013).

Table 1. Latency-Associated Promoter (LAP) Nucleic Acid Sequences

SEQ ID NO:	Nucleic Acid Sequence
LAP1 (498 bp) SEQ ID NO: 1	GGGGGAGTCGGGGGGGAGTCGGGGGGGAGTCGGGGGGTCCCAGTTCGGCGCGGTCTCCACAGCGCACAAAGTCCGGCTGCAGT AAATTTACTGCGGGTGCAGTTACCCTGGCGGCCACACCGGCCCGATGGCGCTGCAGTAACTCTACTGCAGCTACAGTTTACTACA GTTGCAGTACCGCGCGCCGCCGCCAAATACTACAGTAGATTTCCTGTGGCCGCCGCGTACTGCAGTTTACCGCGGCTGCAGTAAA CTGCAGTATCGCGCGGTAAATTGCAGTCTGGCAGCCGCGCGTTACTGCAATTAGCGGTGGCTCCCGACACTCTGGCCAATTGGTG CTAATGGGCCGTGATGGTCCATGTGGGGGTGATGTAACCGCCGGGCCCGGTTGGGCACTCAGATGGTGGCCGGGCGCCAGGCCA AAGTGCTGTCTGAGTGCCACTTTATGACTTTGTTTTTCTCAAACAACATCAATTATGGATGCACATCGTGT**ATATA**
LAP2 (404 bp) SEQ ID NO: 2	ATCCCCGGTCCGCGCTCCGCCCACCCATCACAGCAGCCGCGGACGCTGCGCGCCGGAGCGGTCCATCTCGCCAGCCAGCCAACCA GCCGAGCCGCCCAGCCGACCCGAGAGCCCCGAGAGCCAGACTCCCTCAGCCATAGAAGACACCGGGCGGGAGAGACGGACTGA AAAA**ATATA**TCTTTTTTTATTTTGTCTGGGCCTGGAGACCCGCAGCAGGAGCGGAGGTGGGTGCGGGGCCGGGAGCCGGAGCAG GACCGGGAACAGGAACAGGAACAGGAACAGGAACAGGAACAGGAGTGGGGCCGGGAGCAGGAGCAGGAGCGGGAGCCGAAGT GGGGGCAGGAGCGGCGGCGGCCGCAGCAGCAACAGGGTCGCCCCAGTCCGCGGCGAGGAAGAGGGAGCTC
LAP1_2 (880 bp) SEQ ID NO: 3	GGGGGAGTCGGGGGGTCCCAGTTCGGCGCGGTCTCCACAGCGCACAAAGTCCGGCTGCAGTAAATTTACTGCGGGTGCAGTTAC CCTGGCGGCCACACCGGCCCGATGGCGCTGCAGTAACTCTACTGCAGCTACAGTTTACTACAGTTGCAGTACCGCGCGCCGCCGC CAAATACTACAGTAGATTTCCTGTGGCCGCCGCGTACTGCAGTTTACCGCGGCTGCAGTAAACTGCAGTATCGCGCGGTAAATTG CAGTCTGGCAGCCGCGCGTTACTGCAATTAGCGGTGGCTCCCGACACTCTGGCCAATTGGTGCTAATGGGCCGTGATGGTCCATG TGGGGGTGATGTAACCGCCGGGCCCGGTTGGGCACTCAGATGGTGGCCGGGCGCCAGGCCAAAGTGCTGTCTGAGTGCCACTTTA TGACTTTGTTTTTCTCAAACAACATCAATTATGGATGCACATCGTGT**ATATA**ATCCCCGGTCCGCGCTCCGCCCACCCATCACAGC AGCCGCGGACGCTGCGCGCCGGAGCGGTCCATCTCGCCAGCCAGCCAACCAGCCGAGCCGCCCAGCCGACCCGAGAGCCCCGAG AGCCAGACTCCCTCAGCCATAGAAGACACCGGGCGGGAGAGACGGACTGAAAAA**ATATA**TCTTTTTTTATTTTGTCTGGGCCTGG AGACCCGCAGCAGGAGCGGAGGTGGGTGCGGGGCCGGGAGCCGGAGCAGGACCGGGAACAGGAACAGGAACAGGAACAGGAA CAGGAACAGGAGTGGGGCCGGGAGCAGGAGCAGGAGCGGGAGCCGAAGTGGGGGCAGGAGCGGCGGCGGCCGCAGCAGCAAC AGGGTCGCCCCAGTCCGCGGCGAGGAAGAGGGAGCTC
(902 bp) SEQ ID NO: 4	GGGGGAGTCGGGGGGGAGTCGGGGGGGAGTCGGGGGGTCCCAGTTCGGCGCGGTCTCCACAGCGCACAAAGTCCGGCTGCAGT AAATTTACTGCGGGTGCAGTTACCCTGGCGGCCACACCGGCCCGATGGCGCTGCAGTAACTCTACTGCAGCTACAGTTTACTACA GTTGCAGTACCGCGCGCCGCCGCCAAATACTACAGTAGATTTCCTGTGGCCGCCGCGTACTGCAGTTTACCGCGGCTGCAGTAAA CTGCAGTATCGCGCGGTAAATTGCAGTCTGGCAGCCGCGCGTTACTGCAATTAGCGGTGGCTCCCGACACTCTGGCCAATTGGTG CTAATGGGCCGTGATGGTCCATGTGGGGGTGATGTAACCGCCGGGCCCGGTTGGGCACTCAGATGGTGGCCGGGCGCCAGGCCA AAGTGCTGTCTGAGTGCCACTTTATGACTTTGTTTTTCTCAAACAACATCAATTATGGATGCACATCGTGT**ATATA**ATCCCCGGTC CGCGCTCCGCCCACCCATCACAGCAGCCGCGGACGCTGCGCGCCGGAGCGGTCCATCTCGCCAGCCAGCCAACCAGCCGAGCCG CCCAGCCGACCCGAGAGCCCCGAGAGCCAGACTCCCTCAGCCATAGAAGACACCGGGCGGGAGAGACGGACTGAAAAA**ATATA** TCTTTTTTTATTTTGTCTGGGCCTGGAGACCCGCAGCAGGAGCGGAGGTGGGTGCGGGGCCGGGAGCCGGAGCAGGACCGGGAA CAGGAACAGGAACAGGAACAGGAACAGGAACAGGAGTGGGGCCGGGAGCAGGAGCAGGAGCGGGAGCCGAAGTGGGGGCAGG

	AGCGGCGGCGGCCGCAGCAGCAACAGGGTCGCCCCAGTCCGCGGCGAGGAAGAGGGAGCTC
Equine herpes virus SEQ ID NO: 5	GGGCTCGGCGGCGGGGAGCCTCGGTGATGGGCAGCACCAGGGGCTCGGCCTCGGCGTCGGCTCCAGCAGCGCCACCCGGCAGA ACTCGCTCAGCAGCTCGGGGATCAGAAGCTCCGAGAGGCTCCACGGCCCCAGCGCCGCGCCGGCCGCAGGCGAGGTACACGGGGC GCAGCCAGGCCCCCGAGTCCCCATCGGTTGGCCGCGCCGGTGGCTCTGCGCGGCGCCCTCCTCAAAGTCCGGGTCGTGGAACCCGAG GCCCTCGGCCTGGGCCCGCATGTCCTTGCAGCCGTCGTAGTCGGGCAGGACGCGCTGGCGGTACTCCCTCGGAGCCAGGGGAACG CGGGTGCGCTCGCCGGCGCGAGTGTCCACCGTTGGAGGAGCGGCACAGCCTCAGGGGCGCAGAGTCCGGGTAC AGGCGCGCGAACGCGGCCTCGGCCCTCGCGAAACAGTCCGGGCCCGAAGAGGGTGCTGGAGGTGAGGACCGCGCGGCTGAGGTG GCGCTCCCGGGGCCAGCGCACGGCGACCCGCGGAGTCAGGGCGGCCCGCATGTAGATGTGGTACTGGCTGATCGCGGG ACCGTCCTGGGGCCAATCCTCGGTGGAGACCGCGTCCAGCACCAGGAGCTTGCGCCTGGCGGAGCCCAGGCGCAGGCAGAGGTA CTCGACGCAGCCGGTGAAGGCCAGGTCCCCGGTCGACAGCAGGACCCCCTGGGGCGTTGAGGGCCGAGACGTCCGGGGCCCC GGTCCAGTTGCCGGCCCAGGCGTGGGACCGCTTGGTGAGGATGCGGTTCCCCAGGGCCCGCCAGCAGCGCCGAGAGTCCCCCCTTG AGGTCGGACCAGAGGGGCTCGCGCCGGGGCGGGAGGCCGGGAGTCCGCCCAGCAGGTCCTCGTCCTGGAGCGG GGAGTAGAGGACCACCACCTTCACGTCCTCGGGGTCGGGGATCTGGTGCATCCAGGCGGCCCTCCGTCTCAGCGGGCCGCTGGCC GCCAGCTCCCCGAAGCGCGCGCCGTCCCGGGCCGGGGGGCCGCT
bovine herpes virus SEQ ID NO: 6	GCTCCAGTCGACGCCGCCCGGCCGTGCTGCTCCAGGAACGCGTTGGCCTCGGCAAAGTGCGTGCAGTGAACGAAGGGCGCTCG TGACAGCGGCGAGGGGTGGCTGTACGTCAGCACGAGGTGCCGCTTCCCGGCCGCCCCAAAGGCCCGCTGGGCGTGCGCGCCCCA GAGCATGAACACCAGCGGCCGCTCTCCGCGCTCAGGCGGGCCAGTACCGCGTGCACCAGCCGCCCCCCAGCCCAGGCTAGAGTG CGAGCCCCGGCGCCCCCGCTCCGCACGGTGAGCGAGGTGTTAAGCAGCAGCACGCCGCGGCGCGCCCAGTCCTCTAGGCAGCCGTG CGACGGCCGCGGAGCTCCGGGAAAGTTCTTCTGCACGGCAGCGTAGATGTTCTGGAGGCTGGGTGGGACCGGAACGCCGCGCGGTT CACGCTAAAAGCCAAGCCGTGGGCTTGCCCGCGGCTGTGATAGGGGTCCTGGCCCAGAATGACAACCTTGATGTCCTCGGGTGCC GCGTAACGCGTCCACGCGAAGATGTCCGCTTTCGGAGGCAGCACTTGCTCGACGCGGCTCCGGCGCTCGTATTCGCGCAGCGCGT GTCGCGTGTAGGGCATGGCCAGCTCGGGTTCCAGCACGCGCCGCCAGGCTTCGTGGACGCGAATTCAGCGGCGAATGCCCCCCA AGTAATGGCACGCTGGTCTTCTAGCGCTGTTACGCACGGGGAAGTCCATTGGGGCGCCGGCGCTTGGGCGGCGCGGCACCCCGC AAGCGGGGACAAAGACTTTCGGCGCGTTTGGGCCGAAGCCGGGGCCGAAGCCGGGCCGAAGCCGGGCCG AAGCCGGGCCGAAGCCGGGGCCGGGGCCGGGGCCGGGGCATCGCTAGCGGTAGATGCCGTCGCCAGGGCC GCAGGGACCAGTCGTGTGGTATCGGGGCGGCAGGACGCAC
varicella zoster virus SEQ ID NO: 7	CGGGGAGGTGGGGTCGCTTTTTTTTTTCTCTCTCGAGGGGGCCGCGAGAGGGCTGGCCTCCTCTCCCGGGGTCCGCCGGGCGCCC AGAAACCGGGGGGGGGGGTTATTTTCGGGGGGGGGTCCGACCAGCCCGCCCGTCGCCCGCCCGCACAGACAGACACTTTTTT CATAAAAACCGTTCCGCTTTTATTAACAACAAACAGTCCGCGCGCCAGTGGCGCTCACGAGAAAAGGAGGGGACTCCGTCACCC CCGACTCTGCGGGGGGCTCCTCCCCCCGCGCCCTCCCCACACATCGTCCTCGTCCTCGGAGGACGAGGACAACAGCTCC ACCTTGACCGCCGGGCGCAAACCCACCCGGCGGTCTCGCAGCACACCCGGGGCCACCGACACGATGCTCACCCCAAGGATGAC CCCGGTGCGTCCCCGTCGTCCCCGCCCCCTCCTCGCTGTCCCACGCGTCTTCACACCCCACCTCCCAACTCGTCCAGCTCCAAAGC GTGTTCTCTGTCGTCTGCGGTGCCGCCTGGGTTTCTGACGGCCGTTCCGAGCCCCCGTGGTGTCCGAACACGA ACCGTGTTCCGTCGCTCCCCTCCAACACCGTCTCCGCGGCCCCAAAACCGGGCGGCCACATTACTCTGGGGGGGAGGGC ATTCCGAGCCTCGTCCGCCGACGCATACAGCGCCACCGACCGGCCACGGGTGGAAGCACGAGTGGTTCTGCGGCAGGGTC GGGTTCCAGCAGGGCGTGGCGGCAAAACACCCTCGCCCAGGTGGGTACGTCGCCGGCCTCCGGCCCGGCGGCCCCCGGTCTCCGT

	CCCTCGGGAAGGAAGACGGGTCGAAGCGCGGCACCCAGGCCCCATCGGTTTGCTGCGCGGTGGCTATGTGCCGCCTCGTCCACA AAGTCGGCTGCCCCGAGCCCCAGACCCCGAGACTGTCGCGCGAGGTC
Macacine herpes-virus 1 SEQ ID NO: 8	GAGCGCGGCGCGCCAGGCCTCGGGGAAGAGCGGGTGGTCGGTCAGCTCGGCGACGACGCGCGGGGCGCAGTAGGCCTCGAGGG CGGCGGCGGAGGGCGCCGGCGTGTGGCTGGGCCCCGGCGGCACGCGGCGCCAGCCGCCGCGGGGGTCGGCCCGGCGGGCGGT CGCGCCGTCAGCGCGGCGGCCGCGGGCCCCGGGGGCCGGCGCCCCGGGGGCGCGGGCCGCGCGGCCCGGGCCGGCTCTTC TTGCGCGCCGGCGGGCAGGCGGCGGCGGCGCCGCCGGCGAGGAGCTCTTGCGCTTGCGCGCCGGCGGGCGCGCGCG GCGCCGCCCGCGTCTGCGTCGGCCAGCAGGGGGGCGCAGGCTCTGGTTCTGGAAGAGCAGGTCGGCGGCGGCGCCGGCGGAG CGCACCTGGCGCGGCCGCGCCAGCAGCGGCGGCGGCCGGCGACGAGGCTGACGGCGCGCACGGCGGCGACGGCGGC CTCGGCGCCGCCGGCGACGGCGGAGGTCCCCGCGCAGGCGCATGAGCACCAGCGCGTCGCGGTGCGGCGCAGCTCGCGCAGCCA GAGGCGCGGGCGCGGCGGCGGGCCGCGCGGCGCGGCACGGCGCGCGCCGCCAGCCCCGGGCACGGCGGCCA GGTCGCCGCCGTAGCCCTCGGCCAGCGCCTCCAGCACGGCGCGGCAGGCCGTGAGGCACTCGGCGGCGCCGCGGCGT CGTCGTCGTCGTCGTCCTCGTCCTCCCCCGCGGCCCCGGCGCCGGCGCGGGGCCCTCGTCGGAGAGGGC GGCCGCCAGGCGGCGCTGCGGCGATGACGCCCGCGGCGCCGTACCCGGCGGGGAACGGCGGGGACCGCGGCGCTCGGGCT CGGGGGCGGCGGCGGCGGCGCCCGCCGGCGCGGCGTTCTCGCGCCAGCAGGGGCGCGTACGCGCCGCCAGG CTGGTGATGAGGAAGCCC
SEQ ID NO: 9	**ATATA**TCTTTTTTTATTTTGTCTGGGCCTGGAGACCCGCAGCAGGAGCGGAGGTGGGTGCGGGGCCGGAGCCGGAGCAGGACC GGGAACAGGAACAGGAACAGGAACAGGAACAGGAGTGGGGCCGGGAGCAGGAGCGGGAGCCGAAGTGGGG GCAGGAGCGGCGGCCGGCAGCAACAGGGTCGCCCCAGTCCGCGGCGAGGAAGAGGGAGCTC
SEQ ID NO: 10	CCGCGAGGAGGCAG
SEQ ID NO: 11	TGGTGCCCTCTGGTGGTCTTCTAGAGGAATTGCA
SEQ ID NO: 12	TCAGCCAATCAGCGAG
LAP2.1 (232 bp) SEQ ID NO: 13	**ATATA**TCTTTTTTTATTTTGTCTGGGCCTGGAGACCCGCAGCAGGAGCGGAGGTGGGTGCGGGGCCGGAGCCGGAGCAGGACC GGGAACAGGAACAGGAACAGGAACAGGAACAGGAGTGGGGCCGGGAGCAGGAGCGGGAGCCGAAGTGGGG GCAGGAGCGGCGGCCGGCAGCAACAGGGTCGCCCCAGTCCGCGGCGAGGAAGAGGGAGCTC
LAP2.2 (246 bp) SEQ ID NO: 14	**ATATA**TCTTTTTTTATTTTGTCTGGGCCTGGAGACCCGCAGCAGGAGCGGAGGTGGGTGCGGGGCCGGAGCCGGAGCAGGACC GGGAACAGGAACAGGAACAGGAACAGGAACAGGAGTGGGGCCGGGAGCAGGAGCGGGAGCCGAAGTGGGG GCAGGAGCGGCGGCCGGCAGCAACAGGGTCGCCCCAGTCCGCGGCGAGGAAGAGGGAGCTC*CCGCCGAGGAGGCAG*

LAP2.3 (266 bp) SEQ ID NO: 15	**ATATA**TCTTTTTTTATTTTGTCTGGGCCTGGAGACCCGCAGCAGGAGCGGAGGTGGGTGCGGGGCCGGGAGCCGGAGCAGGACC GGGAACAGGAACAGGAACAGGAACAGGAGTGGGGCCGGGAGCAGGAGCGGGAGCCGAAGTGGGG GCAGGAGCGGCGGCGGCCGCAGCAACAGGGTCGCCCCAGTCCGCGGCGAGGAAGAGGGAGCTC*TGGTGCCCTCTGGTGGTC* *TTCTAGAGGAATTGCA*
LAP2.4 (248 bp) SEQ ID NO: 16	*TCAGCCAATCAGCGAG***ATATA**TCTTTTTTTATTTTGTCTGGGCCTGGAGACCCGCAGCAGGAGCGGAGGTGGGTGCGGGGCCGGGA GCCGGAGCAGGACCGGGAACAGGAACAGGAACAGGAGTGGGGCCGGGAGCAGGAGCAGGAGCGG GAGCCGAAGTGGGGGCAGGAGCGGCGGCCGCAGCAACAGGGTCGCCCCAGTCCGCGGCGAGGAAGAGGGAGCTC
LAP2.5 (246 bp) SEQ ID NO: 17	*CCGCGAGGAGGCAG***ATATA**TCTTTTTTTATTTTGTCTGGGCCTGGAGACCCGCAGCAGGAGCGGAGGTGGGTGCGGGGCCGGGAG CCGGAGCAGGACCGGGAACAGGAACAGGAACAGGAACAGGAGTGGGGCCGGGAGCAGGAGCAGGAGCGGG AGCCGAAGTGGGGGCAGGAGCGGCGGCCGCAGCAACAGGGTCGCCCCAGTCCGCGGCGAGGAAGAGGGAGCTC
LAP2.6 (266 bp) SEQ ID NO: 18	*TGGTGCCCTCTGGTGGTCTTCTAGAGGAATTGCA***ATATA**TCTTTTTTTATTTTGTCTGGGCCTGGAGACCCGCAGCAGGAGCGGAGG TGGGGTGCGGGGCCGGGAGCCGGAGCAGGAACAGGAACAGGAACAGGAACAGGAGTGGGGCCGGG AGCAGGAGCAGGAGCGGGAGCCGAAGTGGGGCAGGAGCGGCGGCCGCAGCAACAGGGTCGCCCCAGTCCGCGGCGA GGAAGAGGGAGCTC
LAP2.7 (248 bp) SEQ ID NO: 19	**ATATA**TCTTTTTTTATTTTGTCTGGGCCTGGAGACCCGCAGCAGGAGCGGAGGTGGGTGCGGGGCCGGGAGCCGGAGCAGGACC GGGAACAGGAACAGGAACAGGAACAGGAACAGGAGTGGGGCCGGGAGCAGGAGCGGAGGTGGGG GCAGGAGCGGCGGCCGCAGCAACAGGGTCGCCCCAGTCCGCGGCGAGGAAGAGGGAGCTC*TCAGCCAATCAGCGAG*
LAP2.8 (236 bp) SEQ ID NO: 20	AAAA**ATATA**TCTTTTTTTATTTTGTCTGGGCCTGGAGACCCGCAGCAGGAGCGGAGGTGGGTGCGGGGCCGGGAGCCGGAGCAG GACCGGGAACAGGAACAGGAACAGGAACAGGAACAGGAGTGGGGCCGGGAGCAGGAGCGGGAGCCGAAGT GGGGGCAGGAGCGGCGGCCGCAGCAACAGGGTCGCCCCAGTCCGCGGCGAGGAAGAGGGAGCTC
LAP2.9 (250 bp) SEQ ID NO: 21	AAAA**ATATA**TCTTTTTTTATTTTGTCTGGGCCTGGAGACCCGCAGCAGGAGCGGAGGTGGGTGCGGGGCCGGGAGCCGGAGCAG GACCGGGAACAGGAACAGGAACAGGAACAGGAACAGGAGTGGGGCCGGGAGCAGGAGCGGGAGCCGAAGT GGGGGCAGGAGCGGCGGCCGCAGCAACAGGGTCGCCCCAGTCCGCGGCGAGGAAGAGGGAGCTC*CCGCGAGGAGGCA* *G*
LAP2.10 (270 bp) SEQ ID NO: 22	AAAA**ATATA**TCTTTTTTTATTTTGTCTGGGCCTGGAGACCCGCAGCAGGAGCGGAGGTGGGTGCGGGGCCGGGAGCCGGAGCAG GACCGGGAACAGGAACAGGAACAGGAACAGGAACAGGAGTGGGGCCGGGAGCAGGAGCGGGAGCCGAAGT GGGGGCAGGAGCGGCGGCCGCAGCAACAGGGTCGCCCCAGTCCGCGGCGAGGAAGAGGGAGCTC*TGGTGCCCTCTGGT* *GGTCTTCTAGAGGAATTGCA*

LAP2.11 (252 bp) SEQ ID NO: 23	*TCAGCCAATCAGCGAG*AAAAA**ATATA**TCTTTTTTTTATTTTGTCTGGGCCTGGAGACCCGCAGCAGGAGCGGAGGTGGGTGCGGGGCC GGGAGCCGGAGCAGGACCGGGAACAGGAACAGGAACAGGAACACAGGAGTGGGGCCCGGGAGCAGGAGCAGGA GCGGGAGCCGAAGTGGGGGCAGGAGCGGCCGGCCGCAGCAACACAGGGTCGCCCCAGTCCGCGGCGAGGAAGAGGGAGCT C
LAP2.12 (250 bp) SEQ ID NO: 24	*CCGCGAGGAGGCAG*AAAAA**ATATA**TCTTTTTTTTATTTTGTCTGGGCCTGGAGACCCGCAGCAGGAGCGGAGGTGGGTGCGGGGCCG GGAGCCGGAGCAGGACCGGGAACAGGAACAGGAACAGGAACACAGGAGTGGGGCCCGGGAGCAGGAGCAGGAG CGGGAGCCGAAGTGGGGGCAGGAGCGGCCGGCCGCAGCAACACAGGGTCGCCCCAGTCCGCGGCGAGGAAGAGGGAGCTC
LAP2.13 (270 bp) SEQ ID NO: 25	*TGGTGCCCTCTGGTGGTCTTCTAGAGGAATTGCA*AAAAA**ATATA**TCTTTTTTTTATTTTGTCTGGGCCTGGAGACCCGCAGCAGGAGCG GAGGTGGGTGCGGGGCCGGGAGCAGGACCGGGAACAGGAACAGGAACACAGGAACACAGGAGTGGGGC CGGGAGCAGGAGCAGGAGCGGGAGCCGAAGTGGGGCAGGAGCGGCCGGCGCAGCAACAGGGTCGCCCCAGTCCGCG GCGAGGAAGAGGGAGCTC
LAP2.14 (252 bp) SEQ ID NO: 26	AAAAA**ATATA**TCTTTTTTTTATTTTGTCTGGGCCTGGAGACCCGCAGCAGGAGCGGAGGTGGGTGCGGGGCCGGGAGCCGGAGCAG GACCGGGAACAGGAACAGGAACAGGAACAGGAGTGGGGCCGGGAGCAGGAGCAGGAGCGGGAGCCGAAGT GGGGGCAGGAGCGGCGGCCGCAGCAACAGGGTCGCCCCAGTCCGCGGCGAGGAAGAGGGAGCTC*TCAGCCAATCAGCG AG*
LAP2.15 (329 bp) SEQ ID NO: 27	CAGCCAACCAGCCGAGCCGCCCAGCCGACCCGAGAGCCCCGAGAGCCAGACTCCCTCAGCCATAGAAGACACCGGGCGGGAGA GACGGACTGAAAAAA**ATATA**TCTTTTTTTTATTTTGTCTGGGCCTGGAGACCCGCAGCAGGAGCGGAGGTGGGTGCGGGGCCGGGA GCCGGAGCAGGACCGGGAACAGGAACAGGAACAGGAACAGGAGTGGGGCCGGGAGCAGGAGCAGGAGCGGG GAGCCGAAGTGGGGGCAGGAGCGGCCGGCCGCAGCAACACAGGGTCGCCCCAGTCCGCGGCGAGGAAGAGGGAGCTC
LAP2.16 (383 bp) SEQ ID NO: 28	CACCCATCACAGCAGCCGCGGACGCTGCGCGCCGGAGCGGTCCATCTCGCCAGCCAACCAGCCGAGCCGCCCAGCCGACC CGAGAGCCCCGAGAGCCAGACTCCCTCAGCCATAGAAGACACCGGGCGGGAGAGACGGACTGAAAAAA**ATATA**TCTTTTTTTTATT TTGTCTGGGCCTGGAGACCCGCAGCAGGAGCGGAGGTGGGTGCGGGGCCGGAGCCGGAGCAGGAGCGGCGGCGG ACAGGAACAGGAACAGGAACAGGAGTGGGGCCGGGAGCAGGAGCGGGAGCCGAAGTGGGGGCAGGAGCGGCGGCGG CCGCAGCAGCAACACAGGGTCGCCCCAGTCCGCGGCGAGGAAGAGGGAGCTC
LAP2.17 (397 bp) SEQ ID NO: 29	CACCCATCACAGCAGCCGCGGACGCTGCGCGCCGGAGCGGTCCATCTCGCCAGCCAACCAGCCGAGCCGCCCAGCCGACC CGAGAGCCCCGAGAGCCAGACTCCCTCAGCCATAGAAGACACCGGGCGGGAGAGACGGACTGAAAAAA**ATATA**TCTTTTTTTTATT TTGTCTGGGCCTGGAGACCCGCAGCAGGAGCGGAGGTGGGTGCGGGGCCGGAGCCGGAGCAGGAGCGGCGGCGG ACAGGAACAGGAACAGGAACAGGAGTGGGGCCGGGAGCAGGAGCGGGAGCCGAAGTGGGGGCAGGAGCGGCGGCGG CCGCAGCAGCAACACAGGGTCGCCCCAGTCCGCGGCGAGGAAGAGGGAGCTC*CCGCGAGGAGGCAG*

LAP2.18 (417 bp) SEQ ID NO: 30	CACCCATCACAGCAGCCGCGGACGCTGCGCGCCGGAGCGGTCCATCTCGCCAGCCAACCAGCCGAGCCGCCCAGCCGACC CGAGAGCCCCCGAGAGCCAGACTCCCTCAGCCATAGAAGACACCGGGCGGGAGAGACGGACTGAAAAAA**ATATA**TCTTTTTTTATT TTGTCTGGGCCTGGAGACCCGCAGGAGCGGAAGGTGGGTGCGGGGCCGGGAGCCGGAGCAGGACCGGGAACAGGAGA ACAGGAACAGGAACAGGAGTGGGGCCGGGAGCAGGAGCGGGAGCCGAAGTGGGGGCAGGAGCGGCGGCGGCGG CCGCAGCAGCAACAGGGTCGCCCCAGTCCGCGGCGAGGAAGAGGGAGCTC*TGGTGCCCTCTGGTGGTCTTCTAGAGGAATTGCA*
LAP2.19 (399 bp) SEQ ID NO: 31	*TCAGCCAATCAGCGAG*CACCCATCACAGCAGCCGCGGACGCTGCGGCCGGAGCGGTCCATCTCGCCAGCCAACCAGCCGA GCCGCCCAGCCGACCCCGAGAGCCAGACTCCCTCAGCCATAGAAGACACCGGGCGGGAGAGACGGACTGAAAAAA**A** **TATA**TCTTTTTTTATTTTGTCTGGGCCTGGAGACCCGCAGGAGCGGAAGGTGGGTGCGGGGCCGGGAGCCGGAGCAGGACCG GGAACAGGAACAGGAACAGGAGTGGGGCCGGGAGCAGGAGCGGGAGCCGAAGTGGGGGG CAGGAGCCGGCCGGCCGCAGCAACAGGGTCGCCCCAGTCCGCGGCGAGGAAGAGGGAGCTC
LAP2.20 (397 bp) SEQ ID NO: 32	*CCGCGAGAGAGCAG*CACCCATCACAGCAGCCGCGGACGCTGCGCGCCGGAGCGGTCCATCTCGCCAGCCAACCAGCCGAG CCGCCCAGCCGACCCCGAGAGCCAGACTCCCTCAGCCATAGAAGACACCGGGCGGGAGAGACGGACTGAAAAAA**AT** **ATA**TCTTTTTTTATTTTGTCTGGGCCTGGAGACCCGCAGCAGGAGCGGAAGGTGGGTGCGGGGCCGGGAGCAGGACCGG GAACAGGAACAGGAACAGGAACAGGAGTGGGGCCGGGAGCAGGAGCGGGAGCCGAAGTGGGGGC AGGAGCGGCGGCGGCCGCAGCAACAGGGTCGCCCCAGTCCGCGGCGAGGAAGAGGGAGCTC
LAP2.21 (417 bp) SEQ ID NO: 33	*TGGTGCCCTCTGGTGGTCTTCTAGAGGAATTGCA*CACCCATCACAGCAGCCGCGGACGCTGCGCGCCGGAGCGGTCCATCTCGCCAG CCAGCCAACCAGCCGAGCCGCCCAGCCGACCCCGAGAGCCCCGAGAGCCAGACTCCCTCAGCCATAGAAGACACCGGGCGGGAG AGACGGACTGAAAAAA**ATATA**TCTTTTTTTATTTTGTCTGGGCCTGGAGACCCGCAGCAGGAGCGGAAGGTGGGTGCGGGGCCGGG AGCCGGAGCAGGACCGGGAACAGGAACAGGAACAGGAACAGGAGTGGGGCCGGGAGCAGGAGCGGGAGCCGAAGTGGGGGC GGAGCCGAAGTGGGGGCAGGAGCGGCGGCCGCAGCAACAGGGTCGCCCCAGTCCGCGGCGAGGAAGAGGGAGCTC
LAP2.22 (399 bp) SEQ ID NO: 34	CACCCATCACAGCAGCCGCGGACGCTGCGCGCCGGAGCGGTCCATCTCGCCAGCCAACCAGCCGAGCCGCCCAGCCGACC CGAGAGCCCCCGAGAGCCAGACTCCCTCAGCCATAGAAGACACCGGGCGGGAGAGACGGACTGAAAAAA**ATATA**TCTTTTTTTATT TTGTCTGGGCCTGGAGACCCGCAGCAGGAGCGGAAGGTGGGTGCGGGGCCGGGAGCAGGAGCGGGAGCCGAAGTGGGGGC ACAGGAACAGGAACAGGAACAGGAGTGGGGCCGGGAGCAGGAGCGGGAGCCGAAGTGGGGGCAGGAGCGGCGGCGG CCGCAGCAGCAACAGGGTCGCCCCAGTCCGCGGCGAGGAAGAGGGAGCTC*TCAGCCAATCAGCGAG*
LAP2.23 (386 bp) SEQ ID NO: 35	GCCCACCCATCACAGCAGCCGCGGACGCTGCGCGCCGGAGCGGTCCATCTCGCCAGCCAACCAGCCGAGCCGCCCAGCCG ACCCGAGAGCCCCGAGAGCCAGACTCCCTCAGCCATAGAAGACACCGGGCGGGAGAGACGGACTGAAAAAA**ATATA**TCTTTTTTTT ATTTTGTCTGGGCCTGGAGACCCGCAGCAGGAGCGGAAGGTGGGTGCGGGGCCGGGAGCAGGAGCCGGAGACCGGGAACAGGAACA GGAACAGGAACAGGAACAGGAACAGGAGTGGGGCCGGGAGCAGGAGCGGGAGCCGAAGTGGGGGCAGGAGCGGCGGCGG CGGCCGCAGCAGCAACAGGGTCGCCCCAGTCCGCGGCGAGGAAGAGGGAGCTC
LAP2.24 (387 bp) SEQ ID NO: 36	CGCCCACCCATCACAGCAGCCGCGGACGCTGCGCGCCGGAGCGGTCCATCTCGCCAGCCAACCAGCCGAGCCGCCCAGCC GACCCGAGAGCCCCCGAGAGCCAGACTCCCTCAGCCATAGAAGACACCGGGCGGGAGAGACGGACTGAAAAAA**ATATA**TCTTTTTT TATTTTGTCTGGGCCTGGAGACCCGCAGCAGGAGCGGAAGGTGGGTGCGGGGCCGGGAGCCGGAGACCGGGAACAGGAACA AGGAACAGGAACAGGAACAGGAACAGGAGTGGGGCCGGGAGCAGGAGCGGGAGCCGAAGTGGGGGCAGGAGCGGCGGCG GCGGCCGCAGCAGCAACAGGGTCGCCCCAGTCCGCGGCGAGGAAGAGGGAGCTC

LAP2.25 (388 bp) SEQ ID NO: 37	CCGCCCACCCATCACAGCAGCCGCGGACGCTGCGCGCCGGAGCGGTCCATCTCGCCAGCCAGCCAACCAGCCGAGCCGCCCAGC CGACCCGAGAGCCCCCGAGAGCCAGACTCCCCTCAGCCATAGAAGACACCGGGCGGGAGAGACGGACTGAAAAAA**ATATA**TCTTTTT TTATTTTTGTCTGGGCCTGGAGACCCCGCAGCAGGAGCGGAGGTGGGTGCGGGGGCCGGGAGCCGGAGCAGGACCGGGAACAGGAA CAGGAACAGGAACAGGAACAGGAGTGGGGCCGGGAGCAGGAGCGGGAGCCGAAGTGGGGGCAGGAGCGGC GGCGGCCGCAGCAACAGGGTCGCCCCAGTCCGCGGCGAGGAAGAGGGAGCTC
LAP2.26 (391 bp) SEQ ID NO: 38	GCTCCGCCCACCCATCACAGCAGCCGCGGACGCTGCGCGCCGGAGCGGTCCATCTCGCCAGCCAGCCAACCAGCCGAGCCGCCC AGCCGACCCGAGAGCCCCCGAGAGCCAGACTCCCCTCAGCCATAGAAGACACCGGGCGGGAGAGACGGACTGAAAAAA**ATATA**TCT TTTTTTATTTTGTCTGGGCCTGGAGACCCCGCAGCAGGAGCGGAGGTGGGTGCGGGGGCCGGGAGCCGGAGCAGGACCGGGAACAG GAACAGGAACAGGAACAGGAACAGGAGTGGGGCCGGGAGCAGGAGCGGGAGCCGAAGTGGGGGCAGGAGC GGCGGCCGCAGCAACAGGGTCGCCCCAGTCCGCGGCGAGGAAGAGGGAGCTC
LAP2.27 (358 bp) SEQ ID NO: 39	TGCGCGCCGGAGCGGTCCATCTCGCCAGCCAACCAGCCGAGCCGCCCGACCCGAGAGCCCCGAGAGCCAGACTCCC TCAGCCATAGAAGACACCGGGCGGGAGACGGACTGAAAAAA**ATATA**TCTTTTTTTTATTTTGTCTGGGCCTGGAGACCCGCAGCA GGAGCGGAGGTGGGTGCGGGGCCGGGAGCAGGACCGGGAACAGGAACAGGAACAGGAACAGGAACAGGAG TGGGGCCGGGAGCAGGAGCAGGAGCCGGGAGCCGGAGGCGGCCGGCAGCAACAGGGTCGCCCCA GTCCGCGGCGAGGAAGAGGGAGCTC
LAP2.28 (372 bp) SEQ ID NO: 40	TGCGCGCCGGAGCGGTCCATCTCGCCAGCCAACCAGCCGAGCCGCCCGACCCGAGAGCCCCGAGAGCCAGACTCCC TCAGCCATAGAAGACACCGGGCGGGAGACGGACTGAAAAAA**ATATA**TCTTTTTTTTATTTTGTCTGGGCCTGGAGACCCGCAGCA GGAGCGGAGGTGGGTGCGGGGCCGGGAGCAGGACCGGGAACAGGAACAGGAACAGGAACAGGAACAGGAG TGGGGCCGGGAGCAGGAGCCGGGAGCCGAAGTGGGGGCAGGAGCGGCCGGCAGCAACAGGGTCGCCCCA GTCCGCGGCGAGGAAGAGGGAGCTC*CCGCGAGGAGGCAG*
LAP2.29 (392 bp) SEQ ID NO: 41	TGCGCGCCGGAGCGGTCCATCTCGCCAGCCAACCAGCCGAGCCGCCCGACCCGAGAGCCCCGAGAGCCAGACTCCC TCAGCCATAGAAGACACCGGGCGGGAGACGGACTGAAAAAA**ATATA**TCTTTTTTTTATTTTGTCTGGGCCTGGAGACCCGCAGCA GGAGCGGAGGTGGGTGCGGGGCCGGGAGCAGGACCGGGAACAGGAACAGGAACAGGAACAGGAACAGGAG TGGGGCCGGGAGCAGGAGCAGGAGCCGGGAGCCGGAGGCGGCCGGCAGCAACAGGGTCGCCCCA GTCCGCGGCGAGGAAGAGGGAGCTC*TGGTGCCCTCTGGTGGTCTTCTAGAGGAATTGCA*
LAP2.30 (374 bp) SEQ ID NO: 42	*TCAGCCAATCAGCGAG*TGCGCGCCGGAGCGGTCCATCTCGCCAGCCAACCAGCCGAGCCGCCCAGCCGACCCGAGAGCCCC GAGAGCCAGACTCCCTCAGCCATAGAAGACACCGGGCGGGAGAGACGGACTGAAAAAA**ATATA**TCTTTTTTTTATTTTGTCTGGGCC TGGAGACCCGCAGCAGGAGCGGAGGTGGGTGCGGGGCCGGGAGCCGGAGCAGGAACAGGAACAGGAACAGGAACAG GAACAGGAACAGGAGTGGGGCCGGGAGCAGGAGCGGGAGCCGAAGTGGGGGCAGGAGCGGCCGGCAGCAGC AACAGGGTCGCCCCAGTCCGCGGCGAGGAAGAGGGAGCTC
LAP2.31 (372 bp) SEQ ID NO: 43	*CCGCGAGGAGGCAG*TGCGCGCCGGAGCGGTCCATCTCGCCAGCCAACCAGCCGAGCCGCCCAGCCGACCCGAGAGCCCCG AGAGCCAGACTCCCTCAGCCATAGAAGACACCGGGCGGGAGAGACGGACTGAAAAAA**ATATA**TCTTTTTTTTATTTTGTCTGGGCCT GGAGACCCGCAGCAGGAGCGGAGGTGGGTGCGGGGCCGGGAGCCGGAGCAGGAACAGGAACAGGAACAGGAACAGG AACAGGAACAGGAGTGGGGCCGGGAGCAGGAGCGGGAGCCGAAGTGGGGGCAGGAGCGGCCGGCAGCAGCA ACAGGGTCGCCCCAGTCCGCGGCGAGGAAGAGGGAGCTC

LAP2.32 (392 bp) SEQ ID NO: 44	*TGGTGCCCTCTGGTGGTCTTCTAGAGGAATTGCA*TGCGCGCCGGAGCGGTCCATCTCGCCAGCCAACCAGCCGAGCCGCCCAG CCGACCCGAGAGCCCCGAGAGCCAGACTCCCTCAGCCATAGAAGACACCGGGCGGGAGAGACGGACTGAAAAAA**ATATA**TCTTTT TTTATTTTGTCTGGGCCTGGAGACCCCGCAGCAGGAGCGGAGGTGGGTGCGGGGCCCGGGAGCCCGGGAACCAGGA ACAGGAACAGGAACAGGAACAGGAGTGGGGCCGGGAGCAGGAGCAGGAGCGGGAGCCGAAGTGGGGGCAGGAGCGG CGGCGGCCGCAGCAACAGGGTCGCCCCAGTCCGCGGCGAGGAAGAGGGAGCTC
LAP2.33 (374 bp) SEQ ID NO: 45	TGCGCGCCGGAGCGGTCCATCTCGCCAGCCAACCAGCCGAGCCGCCCAGCCGACCCGAGAGCCCCGAGAGCCAGACTCCC TCAGCCATAGAAGACACCGGGCGGGAGACGGACTGAAAAAA**ATATA**TCTTTTTTTTATTTTGTCTGGGCCTGGAGACCCGCAGCA GGAGCGGAGGTGGGTGCGGGGCCCGGGAGCAGGAGCCGGGAACACAGGAACACAGGAACAGGAACAGGAACAGGAG TGGGGCCGGGAGCAGGAGCGGGAGCCGAAGTGGGGGCAGGAGCGGCGGCCGGCAGCAACAGGGTCGCCCCA GTCCCGCGGCGAGGAAGAGGGAGCTC*TCAGCCAATCAGCGAG*
LAP2.34 (255 bp) SEQ ID NO: 46	GGCGGGAGAGACGGACTGAAAAAA**ATATA**TCTTTTTTTTATTTTGTCTGGGCCTGGAGACCCGCAGCAGGAGCGGAGGTGGGTGCG GGGCCGGGAGCCCGGAGCAGGACCGGGAACACAGGAACACAGGAACACAGGAACAGGAGTGGGGCCCGGGAGCAGGAG CAGGAGCGGGGAGCCCGAAGTGGGGGCAGGAGCGGCGGCCCGCAGCAACAGGGTCGCCCCAGTCCGCGAGGAAGAG GGAGCTC
LAP2.35 (257 bp) SEQ ID NO: 47	CGGGCGGGAGAGACGGACTGAAAAAA**ATATA**TCTTTTTTTTATTTTGTCTGGGCCTGGAGACCCGCAGCAGGAGCGGAGGTGGGTG CGGGGCCGGGAGCCCGGAGCAGGACCGGGAACACAGGAACACAGGAACACAGGAACAGGAGTGGGGCCGGGAGCAGG AGCAGGAGCGGGAGCCGAAGTGGGGGCAGGAGCGGCGGCCGCAGCAACAGGGTCGCCCCAGTCCGCGAGGAAG AGGGAGCTC
LAP2.36 (268 bp) SEQ ID NO: 48	ATAGAAGACACCGGGCGGGAGAGACGGACTGAAAAAA**ATATA**TCTTTTTTTTATTTTGTCTGGGCCTGGAGACCCGCAGCAGGAGC GGAGGTGGGTGCGGGGCCGGGAGCCGGGAGACCGGGAACACAGGAACACAGGAACACAGGAACAGGAGTGGGGG CCGGGAGCAGGAGCAGGAGCGGGAGCCGAAGTGGGGGCAGGAGCGGCGGCCGCAGCAACAGGGTCGCCCCAGTCCGC GGCGAGGAAGAGGGAGCTC
LAP2.37 (313 bp) SEQ ID NO: 49	CCGCCCAGCCGACCCGAGAGCCCCGAGAGCCAGACTCCCTCAGCCATAGAAGACACCGGGCGGGAGAGACGGACTGAAAAAA**AT** **ATA**TCTTTTTTTTATTTTGTCTGGGCCTGGAGACCCGCAGCAGGAGGTGGGTGCGGGGCCGGGAGCCGGGAGCAGGACCGG GAACAGGAACAGGAACAGGAACAGGAGTGGGGCCGGGAGCAGGAGCAGGAGCGGGAGCCGAAGTGGGGGC AGGAGCGGCGGCCGCAGCAACAGGGTCGCCCCAGTCCGCGCGAGGAAGAGGGAGCTC
LAP2.38 (339 bp) SEQ ID NO: 50	TCTCGCCAGCCAGCCAACCAGCCGAGCCGCCCAGCCGACCCGAGAGCCCCGAGAGCCAGACTCCCTCAGCCATAGAAGACACCG GGCGGGAGAGACGGACTGAAAAAA**ATATA**TCTTTTTTTTATTTTGTCTGGGCCTGGAGACCCGCAGCAGGAGCGGAGGTGGGTGCG GGGCCGGGAGAGCCGGAGCAGGACCGGGAACACAGGAACACAGGAACACAGGAACAGGAGTGGGGCCGGGAGCAGGAG CAGGAGCGGGAGCCCGAAGTGGGGGCAGGAGCGGCGGCCGCAGCAACAGGGTCGCCCCAGTCCGCGAGGAAGAG GGAGCTC

LAP2.39 (359 bp) SEQ ID NO: 51	CTGCGCGCCGGAGCGGTCCATCTCGCCAGCCAACCAGCCGAGCCGCCCAGCCGACCCGAGAGCCCCGAGAGCCAGACTCC CTCAGCCATAGAAGACACCGGGCGGGAGAGACGGACTGAAAAAA**ATATA**TCTTTTTTTATTTTGTCTGGGCCTGGAGACCCGCAGC AGGAGCGGAGGTGGGTGCGGGGCCCGGAGCAGGACCGGGAACACAGGAACAGGAACAGGAACAGGAACAGGA GTGGGGCCGGGAGCAGGAGCCGGGAGCCGAAGTGGGGGCAGGAGCGGCGGCCGCAGCAGCAACAGGGTCGCCCC AGTCCGCGGCGAGGAAGAGGGAGCTC
LAP2.40 (364 bp) SEQ ID NO: 52	GGACGCTGCGCGCCGGAGCGGTCCATCTCGCCAGCCAACCAGCCGAGCCGCCCAGCCGACCCGAGAGCCCCGAGAGCCAG ACTCCCTCAGCCATAGAAGACACCGGGCGGGAGAGACGGACTGAAAAAA**ATATA**TCTTTTTTTATTTTGTCTGGGCCTGGAGACCC GCAGCAGGAGCGGAGGTGGGTGCGGGGCCCGGAGCAGGACCGGGAACACAGGAACAGGAACAGGAACAGGAACAGGAA CAGGAGTGGGGCCGGGAGCAGGAGCCGAAGTGGGGGCAGGAGCGGCGGCCGCAGCAGCAACAGGGTCGCCCCAGTCC GCCCCAGTCCGCGGCGAGGAAGAGGGAGCTC
LAP2.41 (368 bp) SEQ ID NO: 53	CCGCGGACGCTGCGCGCCGGAGCGGTCCATCTCGCCAGCCAACCAGCCGAGCCGCCCAGCCGACCCGAGAGCCCCGAGAG CCAGACTCCCTCAGCCATAGAAGACACCGGGCGGGAGAGACGGACTGAAAAAA**ATATA**TCTTTTTTTATTTTGTCTGGGCCTGGAG ACCCGCAGCAGGAGCGGAGGTGGGTGCGGGGCCGGGAGCAGGACCGGGAACAGGAACAGGAACAGGAACA GGAACAGGAGTGGGGCCGGGAGCAGGAGCGGGAGCCGAAGTGGGGGCAGGAGCGGCGGCCGCAGCAGCAACAG GGTCGCCCCAGTCCGCGGCGAGGAAGAGGGAGCTC
LAP2.42 (376 bp) SEQ ID NO: 54	CACAGCAGCCGCGGACGCTGCGCGCCGGAGCGGTCCATCTCGCCAGCCAGCCGAGCCGCCCAGCCGACCCGAGAGC CCCGAGAGCCAGACTCCCTCAGCCATAGAAGACACCGGGCGGGAGAGACGGACTGAAAAAA**ATATA**TCTTTTTTTATTTTGTCTGG GCCTGGAGACCCGCAGCAGGAGCGGAGGTGGGTGCGGGGCCGGGAGCCGGGACAGGAACAGGAACAGGAAA CAGGAACAGGAACAGGAGTGGGGCCGGGAGCAGGAGCCGAAGTGGGGGCAGGAGCGGCGGCCGCAGCAGC AGCAACAGGGTCGCCCCAGTCCGCGGCGAGGAAGAGGGAGCTC
LAP2.43 (384 bp) SEQ ID NO: 55	CCACCCATCACAGCAGCCGCGGACGCTGCGCGCCGGAGCGGTCCATCTCGCCAGCCAGCCAACCAGCCGAGCCGCCCAGCCGAC CCGAGAGCCCCGAGAGCCAGACTCCCTCAGCCATAGAAGACACCGGGCGGGAGAGACGGACTGAAAAAA**ATATA**TCTTTTTTTAT TTTGTCTGGGCCTGGAGACCCGCAGCAGGAGCGGAGGTGGGTGCGGGGCCGGGAGCCGGGACAGGAACAGGAACAGG AACAGGAACAGGAACAGGAGTGGGGCCGGGAGCAGGAGCGGGAGCCGAAGTGGGGGCAGGAGCGGCGGCG GCCGCAGCAGCAACAGGGTCGCCCCAGTCCGCGGCGAGGAAGAGGGAGCTC
LAP2.44 (385 bp) SEQ ID NO: 56	CCCACCCATCACAGCAGCCGCGGACGCTGCGCGCCGGAGCGGTCCATCTCGCCAGCCAGCCAACCAGCCGAGCCGCCCAGCCGA CCCGAGAGCCCCGAGAGCCAGACTCCCTCAGCCATAGAAGACACCGGGCGGGAGAGACGGACTGAAAAAA**ATATA**TCTTTTTTTA TTTTGTCTGGGCCTGGAGACCCGCAGCAGGAGCGGAGGTGGGTGCGGGGCCGGGAGCCGGGACAGGAACAGGAACAG GAACAGGAACAGGAACAGGAGTGGGGCCGGGAGCAGGAGCCGAAGTGGGGGCAGGAGCGGCGGCCGGC GGCCGCAGCAGCAACAGGGTCGCCCCAGTCCGCGGCGAGGAAGAGGGAGCTC
LAP2.45 (390 bp) SEQ ID NO: 57	CTCCGCCCACCCATCACAGCAGCCGCGGACGCTGCGCGCCGGAGCGGTCCATCTCGCCAGCCAGCCAACCAGCCGAGCCGCCCA GCCGACCCGAGAGCCCCGAGAGCCAGACTCCCTCAGCCATAGAAGACACCGGGCGGGAGAGACGGACTGAAAAAA**ATATA**TCTTT TTTTATTTTGTCTGGGCCTGGAGACCCGCAGCAGGAGCGGAGGTGGGTGCGGGGCCGGGAGCCGGGACAGGAACAGGA ACAGGAACAGGAACAGGAACAGGAGTGGGGCCGGGAGCAGGAGCCGAAGTGGGGGCAGGAGCCGG CGGCGGCCGCAGCAGCAACAGGGTCGCCCCAGTCCGCGGCGAGGAAGAGGGAGCTC

LAP2.46 (396 bp) SEQ ID NO: 58	TCCGCGCTCCGCCCACCCATCACAGCAGCCGCGGACGCTGCGCGCCGGAGCGGTCCATCTCGCCAGCCAGCCAACCAGCCGAGC CGCCCAGCCGACCCGAGAGCCCCGAGAGCCAGACTCCCTCAGCCATAGAAGACACCGGGCGGGAGAGACGGACTGAAAAA**ATA TA**TCTTTTTTTATTTTGTCTGGGCCTGGAGACCCGCAGCAGGAGCGGAGGTGGGTGCGGGGCCGGGAGCCGGAGCAGGACCGGG AACAGGAACAGGAACAGGAACAGGAACAGGAACAGGAGTGGGGCCGGGAGCAGGAGCAGGAGCGGGAGCCGAAGTGGGGGCA GGAGCGGCGGCGGCCGCAGCAGCAACAGGGTCGCCCCAGTCCGCGGCGAGGAAGAGGGAGCTC

The 232-bp “core” sequence (SEQ ID NO: 9) is underlined.

The TATA box DNA sequence (ATATA) is underlined and in bold text.

The “CTCF” motif (SEQ ID NO: 10), the “alternative CTCF with upstream stabilizer” motif (SEQ ID NO: 11), and the “NF-Y” motif are italicized.

[0054] In some embodiments, the promoter comprises a nucleic acid sequence having at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98% or at least 99% sequence identity a nucleic acid sequences selected from SEQ ID NOs: 13-58 (**Table 1**). In some embodiments, the promoter comprises a nucleic acid sequence selected from SEQ ID NOs: 13-58.

[0055] The vectors comprising the LAP sequences disclosed herein can be used for gene delivery (e.g., in mammals, such as a human) and basic and translational research. The host cell may be a cultured cell (i.e., *in vitro*) or reside in a subject (e.g., in an animal model (i.e., *in vivo*)).

[0056] In some embodiments, the vector is a lentivirus gene delivery vector, a retrovirus gene delivery vector, or an adenovirus gene delivery vector. One advantage of the LAP sequences described herein is that they have a considerably smaller size than other commonly used promoters in viral vectors, yet can sustain prolonged transcription in different cell types.

[0057] A relatively small LAP region may be advantageous, given that the DNA payload capacity is highly limited in vectors. For example, the packaging capacity or payload of the AAV vectors is limited to a maximum size of an approximate 5,000 bp. In some embodiments, the LAP region utilized in the vectors of the present invention is between about 200 bp and about 410 bp in length, e.g., about 220-410 bp, about 220-400 bp, about 240-400 bp, about 240-380 bp, about 260-380 bp, about 260-360 bp, about 280-360 bp, about 280-340 bp, about 300-340 bp, or about 320-340 bp in length.

[0058] In particular embodiments, the promoter sequences of the present invention can be used when strong, long-term expression of transgenes is required in cells in culture, in animals or in humans. The promoters are useful in situations where there are genome space constraints but long-lasting promoter transcriptional activity is needed. AAVs are particularly useful for human gene therapy; as such, the LAP sequences of the present invention provide gene therapy applications when utilized with AAVs.

[0059] In some embodiments, a gene of interest is expressed under control of the LAP sequences provided herein for at least about 30 days, about 60 days, about 90 days, about 120 days, about 150, about 180 days, or about 190 days *in vitro*. In some embodiments, a gene of interest is expressed under control of the LAP sequences provided herein for at least about 30

days, about 60 days, about 90 days, about 120 days, about 150, about 180 days, or about 190 days *in vivo* (e.g., in a tissue such as a brain). In some embodiments, a gene of interest is expressed under control of the LAP sequences provided herein for at least about 30 days, about 60 days, about 90 days, about 120 days, about 150, about 180 days, or about 190 days *ex vivo* (e.g., in cultured cells such as neurons).

[0060] In one aspect, the present invention provides a recombinant vector for expression of a target gene in a host cell, comprising a promoter comprising a nucleic acid sequence having at least 70% sequence identity to a sequence selected from SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3 and SEQ ID NO: 4; a cloning site for insertion of a nucleic acid encoding the target gene; and at least one non-promoter regulatory element required for the expression of the target gene in the host cell.

[0061] In some embodiments, the promoter comprises a nucleic acid sequence having at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity to a nucleic acid sequence selected from SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3 and SEQ ID NO: 4. In some embodiments, the promoter comprises a nucleic acid sequence selected from SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3 and SEQ ID NO: 4.

[0062] The LAP sequences described herein can be used in a variety of gene expression vectors, such as a plasmid, a bacterial artificial chromosome (BAC), a cosmid or other non-viral or viral systems to express transgene(s).

[0063] In some embodiments, the vector is a viral vector. In some embodiments, the viral vector is an adeno-associated virus (AAV) vector. Non-limiting examples of viral vectors that can be utilized by the present invention include DNA or RNA viral vectors including but not limited to retroviral vectors, herpes virus vectors, adenovirus vectors, lentivirus vectors, rabies virus vectors, lentiviral vectors, VSV vectors, vaccinia virus vectors, reovirus vectors, semliki forest virus, yellow fever virus, and sindbis virus vectors.

[0064] In some embodiments, the vector is a non-viral vector. Non-viral vectors can be plasmid DNA, liposome-DNA complexes (lipoplexes), and polymer-DNA complexes (polyplexes). Non-viral vectors can be plasmid RNA, liposome-RNA complexes (lipoplexes),

and polymer-RNA complexes (polyplexes). Oligonucleotides and their analogues, either alone or in complexes, are also possible non-viral vector-mediated gene transfer constructs.

[0065] The vectors (e.g., viral vectors) provided herein can be used to express therapeutic transgenes in organs or tissues of animal models and humans, for example, in gene therapies to treat acquired or inherited diseases (e.g., of the nervous system).

[0066] The LAP sequences disclosed herein were derived from the genome of pseudorabies virus (PRV). PRV is an alphaherpesvirus capable of infecting various animals. Although the natural host is the adult pig, PRV can infect a broad range of vertebrates including cattle, sheep, dog, bird, cat, goat, raccoon, chicken, skunk, possum, guinea pig, horse, rabbit, rat, mouse and nonhuman primates (Pomeranz et al., 2005). Cultured human cells are also susceptible to PRV infection and zoonotic PRV infections in humans was reported (Wong et al., 2019).

[0067] Besides PRV being able to infect several different animal species, it is also a pantropic virus. This means that most tissues and organs of an infected host can be susceptible and permissive to PRV infection (Blanchard et al., 2006; Boldogkoi et al., 2000; Fan et al., 2019; Gasparini et al., 2019; Pomeranz et al., 2005). Therefore, the LAP sequences can be potentially used for gene therapies in diverse cell and tissue types.

[0068] In another aspect, the present invention provides a host cell comprising a recombinant vector as described herein. In some embodiments, the host cell is a mammalian cell (e.g., a neuronal cell). In other embodiments, the host cell is selected from a bird cell, a fish cell, an amphibian cell, and a reptile cell. In some embodiments, the host cell comprises an *in vitro* neuronal cell, an *ex vivo* neuronal cell, or both. In some embodiments, the host cell a neuronal cell of a subject. In some embodiments, the neuronal cell resides in a primary motor area, a secondary motor area, a primary somatosensory area, a supplemental somatosensory area, a visual area, a hippocampal formation, striatum, pallidum, thalamus, hypothalamus, a midbrain area, hind brain, an olfactory area or a combination thereof, of the subject. In some embodiments, the midbrain area comprises a motor-related area, a sensory-related area, or both. In some embodiments, the neuronal cell resides in cortex, dentate gyrus, striatum, cerebellum, olfactory bulb or a combination thereof, of the subject. In some embodiments, the neuronal cell comprises a neuron, a microglia cell, an astrocyte, or a combination thereof. In some embodiments, the neuronal cell comprises a neuron.

[0069] In some embodiments, the host cell is selected from the group consisting of a bladder cell, a blood cell, a bone cell, an endothelial cell, an epithelial cell, a fat cell, a heart cell, an intestinal cell, a stomach cell, a kidney cell, a liver cell, a lung cell, a muscle cell, a pancreatic cell, a retina cell, a sex cell, a skin cell, a spleen cell, a stem cell, and a combination thereof. In some embodiments, the host cell is a cancer cell.

[0070] In another aspect, the present invention provides recombinant polynucleotide molecules comprising a nucleic acid encoding a target gene, wherein the nucleic acid is operably linked to a promoter comprising a nucleic acid sequence having at least 70% sequence identity to a sequence selected from SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, and SEQ ID NO: 4. In some embodiments, the promoter comprises a nucleic acid sequence having at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98% or at least 99% sequence identity to a nucleic acid sequence selected from SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3 and SEQ ID NO: 4. In some embodiments, the promoter comprises a nucleic acid sequence selected from SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3 and SEQ ID NO: 4. In some embodiments, the nucleic acid encoding the target gene comprises more than about 4 kb.

[0071] In a further aspect, the present invention provides a recombinant polynucleotide molecule comprising a cloning site for insertion of a nucleic acid encoding a target gene, wherein the cloning site is operably linked to a promoter comprising a nucleic acid having at least 70% sequence identity to a sequence selected from SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, and SEQ ID NO: 4. In some embodiments, the promoter comprises a nucleic acid sequence having at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98% or at least 99% sequence identity to a nucleic acid sequence selected from SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3 and SEQ ID NO: 4. In some embodiments, the promoter comprises a nucleic acid sequence selected from SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3 and SEQ ID NO: 4.

[0072] In another aspect, the present invention provides a recombinant vector for expression of a target gene in a host cell, comprising a promoter comprising a nucleic acid sequence having at least 70% sequence identity to a nucleic acid sequence selected from SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7 and SEQ ID NO: 8; a cloning site for insertion of a nucleic acid encoding the target gene; and at least one non-promoter regulatory element required for the expression of

the target gene in the host cell. SEQ ID NO: 5 comprises a LAP sequence of an equine herpes virus; SEQ ID NO: 6 comprises a LAP sequence of a bovine herpes virus; SEQ ID NO: 7 comprises a LAP sequence of a varicella zoster virus; and SEQ ID NO: 8 comprises a LAP sequence of a Macacine herpesvirus 1 (**Table 1**). In some embodiments, the promoter comprises a nucleic acid sequence having at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98% or at least 99% sequence identity to a nucleic acid sequence selected from SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7 and SEQ ID NO: 8. In some embodiments, the promoter comprises a nucleic acid sequence selected from SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7 and SEQ ID NO: 8.

[0073] In another aspect, the present invention provides recombinant polynucleotide molecules comprising a nucleic acid encoding a target gene, wherein the nucleic acid is operably linked to a promoter comprising a nucleic acid sequence having at least 70% sequence identity to a sequence selected from SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, and SEQ ID NO: 8. In some embodiments, the promoter comprises a nucleic acid sequence having at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98% or at least 99% sequence identity to a nucleic acid sequence selected from SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7 and SEQ ID NO: 8. In some embodiments, the promoter comprises a nucleic acid sequence selected from SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7 and SEQ ID NO: 8. In some embodiments, the nucleic acid encoding the target gene comprises more than about 4 kb.

[0074] In a further aspect, the present invention provides a recombinant polynucleotide molecule comprising a cloning site for insertion of a nucleic acid encoding a target gene, wherein the cloning site is operably linked to a promoter comprising a nucleic acid having at least 70% sequence identity to a sequence selected from SEQ ID NO:5, SEQ ID NO: 6, SEQ ID NO: 7, and SEQ ID NO: 8. In some embodiments, the promoter comprises a nucleic acid sequence having at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98% or at least 99% sequence identity to a nucleic acid sequence selected from SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7 and SEQ ID NO: 8. In some embodiments, the promoter comprises a nucleic acid sequence selected from SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7 and SEQ ID NO: 8.

[0075] In another aspect, the present invention provides a recombinant vector for expression of a target gene in a host cell, comprising a promoter comprising a nucleic acid sequence having at least 70% sequence identity to SEQ ID NO: 9; a cloning site for insertion of a nucleic acid encoding the target gene; and at least one non-promoter regulatory element required for the expression of the target gene in the host cell. In some embodiments, the promoter comprises a nucleic acid sequence having at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98% or at least 99% sequence identity to SEQ ID NO: 9. In some embodiments, the promoter comprises SEQ ID NO: 9. In some embodiments, the promoter further comprises the nucleic acid sequence of SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 12, or a combination thereof. In some embodiments, the promoter comprises a nucleic acid sequence having at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98% or at least 99% sequence identity to a nucleic acid sequence selected from SEQ ID NOs: 13-58. In some embodiments, the promoter comprises a nucleic acid sequence selected from SEQ ID NOs: 13-58.

[0076] In some embodiments, the at least one non-promoter regulatory element comprises an enhancer element. In some embodiments, the at least one non-promoter regulatory element comprises an inducer element. In some embodiments, the recombinant vector further comprises a selectable marker element.

[0077] In another aspect, the present invention provides recombinant polynucleotide molecules comprising a nucleic acid encoding a target gene, wherein the nucleic acid is operably linked to a promoter comprising a nucleic acid sequence having at least 70% sequence identity to SEQ ID NO: 9. In some embodiments, the promoter comprises a nucleic acid sequence having at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98% or at least 99% sequence identity to SEQ ID NO: 9. In some embodiments, the promoter comprises SEQ ID NO: 9. In some embodiments, the promoter further comprises the nucleic acid sequence of SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 12, or a combination thereof. In some embodiments, the promoter comprises a nucleic acid sequence having at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98% or at least 99% sequence identity to a nucleic acid sequence selected from SEQ ID NOs: 13-58. In some embodiments, the promoter comprises a nucleic acid sequence

selected from SEQ ID NOs: 13-58. In some embodiments, the nucleic acid encoding the target gene comprises more than about 4 kb.

[0078] In a further aspect, the present invention provides a recombinant polynucleotide molecule comprising a cloning site for insertion of a nucleic acid encoding a target gene, wherein the cloning site is operably linked to a promoter comprising a nucleic acid sequence having at least 70% sequence identity to SEQ ID NO: 9. In some embodiments, the promoter comprises a nucleic acid sequence having at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98% or at least 99% sequence identity to SEQ ID NO: 9. In some embodiments, the promoter comprises SEQ ID NO: 9. In some embodiments, the promoter further comprises the nucleic acid sequence of SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 12, or a combination thereof. In some embodiments, the promoter comprises a nucleic acid sequence having at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98% or at least 99% sequence identity to a nucleic acid sequence selected from SEQ ID NOs: 13-58. In some embodiments, the promoter comprises a nucleic acid sequence selected from SEQ ID NOs: 13-58.

[0079] In another aspect, the present invention provides methods of expressing a target gene in a host cell, comprising: contacting the host cell with a recombinant polynucleotide molecule such that the recombinant polynucleotide molecule is introduced into the host cell, wherein the recombinant polynucleotide molecule comprises a nucleic acid encoding the target gene and wherein the nucleic acid is operably linked to a promoter comprising a nucleic acid sequence having at least 70% sequence identity to a sequence selected from SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3 and SEQ ID NO: 4, whereby expression of the target gene occurs in the host cell. In some embodiments, the promoter comprises a nucleic acid sequence having at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98% or at least 99% sequence identity to a nucleic acid sequence selected from SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3 and SEQ ID NO: 4. In some embodiments, the promoter comprises a nucleic acid sequence selected from SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3 and SEQ ID NO: 4.

[0080] In another aspect, the present invention provides methods of expressing a target gene in a host cell, comprising: contacting the host cell with a recombinant polynucleotide molecule

such that the recombinant polynucleotide molecule is introduced into the host cell, wherein the recombinant polynucleotide molecule comprises a nucleic acid encoding the target gene and wherein the nucleic acid is operably linked to a promoter comprising a nucleic acid sequence having at least 70% sequence identity to a sequence selected from SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7 and SEQ ID NO: 8, whereby expression of the target gene occurs in the host cell. In some embodiments, the promoter comprises a nucleic acid sequence having at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98% or at least 99% sequence identity to a nucleic acid sequence selected from SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7 and SEQ ID NO: 8. In some embodiments, the promoter comprises a nucleic acid sequence selected from SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7 and SEQ ID NO: 8.

[0081] In another aspect, the present invention provides methods of expressing a target gene in a host cell, comprising: contacting the host cell with a recombinant polynucleotide molecule such that the recombinant polynucleotide molecule is introduced into the host cell, wherein the recombinant polynucleotide molecule comprises a nucleic acid encoding the target gene and wherein the nucleic acid is operably linked to a promoter comprising a nucleic acid sequence having at least 70% sequence identity to SEQ ID NO: 9, whereby expression of the target gene occurs in the host cell. In some embodiments, the promoter comprises a nucleic acid sequence having at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98% or at least 99% sequence identity to SEQ ID NO: 9. In some embodiments, the promoter comprises SEQ ID NO: 9. In some embodiments, the promoter further comprises the nucleic acid sequence of SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 12, or a combination thereof. In some embodiments, the promoter comprises a nucleic acid sequence having at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98% or at least 99% sequence identity to a nucleic acid sequence selected from SEQ ID NOs: 13-58. In some embodiments, the promoter comprises a nucleic acid sequence selected from SEQ ID NOs: 13-58.

[0082] In some embodiments, the neuronal cell resides in the spinal cord of the subject. In some embodiments, the neuronal cell resides in the dorsal horn of the spinal cord. In some

embodiments, the neuronal cell resides in the lumbar spinal cord, thoracic spinal cord, cervical spinal cord or a combination thereof.

[0083] In some embodiments, the recombinant polynucleotide molecule is introduced into the host cell by transduction (e.g., infection by a viral vector) upon contacting the host cell with the recombinant polynucleotide. In another embodiment, the recombinant polynucleotide molecule is introduced into the host cell by transfection upon contacting the host cell with the recombinant polynucleotide. In another embodiment, the recombinant polynucleotide molecule is introduced into the host cell by electroporation upon contacting the host cell with the recombinant polynucleotide.

[0084] Recombinant adeno-associated viral vectors (AAV) are essential neuroscience tools to define connectivity and function of the central nervous system (CNS). Additionally, AAVs are used as gene therapy vectors to treat inherited and acquired CNS diseases. Despite their safety and broad tropism, important issues need to be corrected such as the limited payload capacity and the lack of small gene promoters providing long-term, pan-neuronal transgene expression in the CNS. Commonly used gene promoters are relatively large and can be repressed a few months after viral CNS transduction, risking the long-term performance of single-dose gene therapy applications.

[0085] Recent improvements to recombinant AAVs, including capsid engineering and novel gene promoters to optimize transgene expression have substantially improved gene therapy applications (Mak, Rajapaksha et al. 2017; Hudry and Vandenberghe 2019; Ogden, Kelsic et al. 2019). AAV vectors are widely used in neuroscience and clinical applications given their safety, serotype-dependent broad tropism and transduction efficiency (Aschauer, Kreuz et al. 2013; Samulski and Muzyczka 2014; Mak, Rajapaksha et al. 2017). AAV-9 variant PHP.eB (Chan, Jang et al. 2017), with an enhanced ability to permeate the mouse blood-brain barrier (BBB) and broadly transduce neurons both in the brain and spinal cord after peripheral vascular administration, is one example of recent capsid improvements (Dayton, Grames et al. 2018). A major limitation of recombinant AAVs is their small capsid with limited payload capacity of only ~ 4.9 kb (Russell and Hirata 1998). Accordingly, the discovery of short promoter sequences that sustain strong and long-lived transcription is paramount to expand the transgene payload and achieve chronic therapeutic effect with one viral dose.

[0086] Several strong promoters such as neuron-specific enolase (NSE, 1800 bp) (Peel, Zolotukhin et al. 1997; Delzor, Dufour et al. 2012), calcium/calmodulin-dependent protein kinase II alpha (CaMKIIa, 1300 bp) (Sohal, Zhang et al. 2009) and human elongation factor 1 alpha (EF1α, 1264 bp) (Sohal, Zhang et al. 2009; Qin, Zhang et al. 2010) have been used in systemic AAV delivery (Bedbrook, Deverman et al. 2018). However, the considerable size of these promoter sequences (Bedbrook, Deverman et al. 2018) limit the use of large therapeutic transgenes or multiple small transgenes. Moreover, short promoters such as the human cytomegalovirus immediate-early enhancer and promoter (CMV, 600 bp) (Gray, Foti et al. 2011) or truncated versions of the human synapsin promoter (hSyn, 468 bp) (Jackson, Dayton et al. 2016), have considerably weaker to drive gene transcription and expression, and in some cases, are completely repressed or inactivated only weeks after delivery (Brooks, Harkins et al. 2004; Nathanson, Jappelli et al. 2009; Qin, Zhang et al. 2010; Back, Dossat et al. 2019). Similarly, small ubiquitous promoters like beta glucuronidase (GUSB, 378 bp) (Husain, Passini et al. 2009) or ubiquitin C (UBC, 403 bp) (Qin, Zhang et al. 2010; Powell, Rivera-Soto et al. 2015) have shown weak transcription levels.

[0087] Three alphaherpesvirus latency-associated promoters (LAP), called LAP1 (498 bp), LAP2 (404 bp) and LAP1_2 (880 bp) obtained from the genome of the herpesvirus pseudorabies virus (PRV) are described and validated herein. The *Alphaherpesvirinae* subfamily of the family *Herpesviridae*, includes bovine herpes virus-1 (BHV-1), varicella-zoster virus (VZV), herpes simplex virus (HSV) and PRV. These viruses share genome organization and establish latent infections in sensory ganglia of different mammalian hosts (Koyuncu, Hogue et al. 2013).

[0088] The LAP region of PRV encompasses two independent promoters, LAP1 and LAP2 (Cheung 1989; Cheung and Smith 1999; Jin and Scherba 1999; Jin, Schnitzlein et al. 2000) (**FIG. 1A**). PRV LAP1 contain two GC boxes and three CAAT boxes upstream of the first TATA box. PRV LAP2 containing two GC boxes before the second TATA box (Cheung 1989; Jin and Scherba 1999; Taharaguchi, Kobayashi et al. 2002). It has been proposed that the binding of different transcription factor (TFs) to consensus promoter elements present in LAP, may facilitate escape from nucleosome silencing during the latent infection during the latent infection (Deshmane and Fraser 1989; Leib, Bogard et al. 1989; Devi-Rao, Goodart et al. 1991; Jin, Schnitzlein et al. 2000; Ono, Tomioka et al. 2007). In transgenic mouse lines, PRV LAP

promoted transcription is neuron-specific in the absence of PRV infection (Taharaguchi, Yoshino et al. 2003). However, in transient expression assays, PRV LAP1 and LAP2 promote transcription both in cultured neuronal as well as non-neuronal cells (Cheung and Smith 1999; Taharaguchi, Kobayashi et al. 2002). Furthermore, the activity of tandem LAP1 and LAP2 sequences is significantly increased compared to LAP1 or LAP2 alone (Cheung and Smith 1999).

[0089] Here, a whole-CNS screening approach based on retroorbital systemic delivery of AAV-PHP.eB, iDisco+ tissue-clearing and light-sheet microscopy, was used to identify three small latency-associated promoters (LAP) from the herpesvirus pseudorabies virus (PRV). These promoters are LAP1 (404bp), LAP2 (498bp) and LAP1_2 (880bp). They drive chronic transcription of the virus encoded latency-associated transcript (LAT) during productive and latent phases of PRV infectious cycle. Stable, pan-neuronal transgene transcription and translation were observed from AAV-LAP in brain and spinal cord for six months post AAV transduction. The data suggest that the LAPs are suitable candidates for viral vector-based CNS gene therapies requiring chronic transgene expression after one-time viral-vector administration, PRV LAP1, LAP2 and tandem LAP1_2 promoters are likely suitable for systemic, less invasive, pan-neuronal gene delivery applications that may require stable, chronic transgene expression after a single administration.

[0090] Example 1. Materials and Methods

[0091] Construction of PRV LAP sequences

[0092] The PRV latency-associated transcript promoter (LAP) was PCR amplified from coordinates 95106-96007 of PRV Becker strain genome (GenBank: JF797219.1). The LAP1 region (498bp), was amplified using primer pairs LAP1F (5'- GCA CGC GTA TCT CCG GAA AGA GGA AAT TGA -3') (SEQ ID NO: 59) and LAP1R (5'- GCG GAT CCT ATA TAC ACG ATG TGC ATC CAT AAT -3') (SEQ ID NO: 60). The LAP2 region (404bp), was amplified using primer pairs LAP2F (5'- GCA CGC GTA TCC CCG GTC CGC GCT CCG CCC ACC CA -3') (SEQ ID NO: 61) and LAP2R (5'- GCG GAT CCG AGC TCC CTC TTC CTC GCC GCG GAC TGG -3') (SEQ ID NO: 62). LAP1_2 (902bp) spanning the entire LAP region was amplified using LAP1F and LAP2R (Cheung and Smith 1999). The 5' and 3' regions of these PCR sequences contained the MluI and BamHI restriction sites respectively, used for directional

cloning into vector pAAV-Ef1α-mCherry. pAAV-Ef1α-mCherry was a gift from Karl Deisseroth (Addgene plasmid # 114470). The three AAV-LAP plasmids were constructed by double digestion of vector pAAV-Ef1α-mCherry with MluI and BamHI followed by subcloning of the appropriate LAP fragment upstream of the mCherry reporter gene, flanked by AAV2 inverted terminal repeats (ITRs) and terminated with the SV40 polyA signal.

[0093] Construction of AAV Vectors

[0094] All expression cassettes were packaged into AAV-PhP.eB capsids (gift from Daniela Gradinaru, Addgene plasmid #103005) at the Princeton Neuroscience Institute Viral Core Facility and purified by iodixanol step gradient and column ultrafiltration as previously described (Zolotukhin, Byrne et al. 1999; Chan, Jang et al. 2017). Capsid-protected viral genomes were measured by TaqMan qPCR and reported as genome copies per milliliter (GC/ml).

[0095] Animals

[0096] Animal studies were performed following guidelines and protocols approved by the Institutional Animal Care and Use Committee of Princeton University (protocol # 1943-16 and 1047). Timed-pregnant Sprague-Dawley rats were obtained from Hilltop Labs Inc. (Scottsdale, PA). E17 rat embryos were harvested for isolation of sympathetic SCG neurons. Adult (4 to 6-week-old) wild type C57BL/6J male mice were obtained from Jackson Laboratory (The Jackson Laboratory, Bar Harbor, ME). Mice had at least 48hr of acclimation to the holding facility in the Princeton Neuroscience Institute vivarium before experimental procedures were performed.

[0097] Primary superior cervical ganglia cell culture

[0098] SCG neurons from rat embryos (E17) were cultured in trichambers as previously described (Curanovic, Ch'ng et al. 2009). Briefly, SCG were dissociated with trypsin (2.5 mg/ml, Sigma-Aldrich, The Woodlands, TX) and plated on poly-O-Ornithine and laminin-coated dishes with media containing neurobasal media supplemented with 2% B-27, 100 ng/ml nerve growth factor (NGF), and 1% penicillin-streptomycin-glutamine (Thermo Fisher Scientific, Rockford, IL). Approximately two-thirds of a single ganglia were placed for S (soma) compartment of the trichamber. Three days post seeding, culture medium was treated with 0.1 mM cytosine-D-arabinofuranoside, Ara C (Sigma-Aldrich, The Woodlands, TX) for at least 2 days to eliminate dividing, nonneuronal cells. Culture media was replaced every 5 days, and neurons were incubated at 37°C with 5% CO_2.

[0099] Retro-orbital sinus injection

[00100] Intravenous administration of AAV vectors was performed in mice by unilateral injection into the retro-orbital venous sinus (Yardeni, Eckhaus et al. 2011). Animals were anesthetized using ketamine (80 mg/kg)/xylazine (10 mg/kg) cocktail prior to the procedure. Once unresponsive, animals were placed in lateral recumbence for injection into the medial canthus. Injection volume was 100 µl containing a total of 4×10^{11} viral genomes administered with a 29G1/2 insulin syringe. Animals were placed on regulated heating pads and monitored until ambulant.

[00101] Tissue processing and histological procedures

[00102] Mice were anesthetized with an overdose of ketamine (400 mg/kg)/xylazine (i.p.) and perfused with 4% paraformaldehyde (PFA) at 30 and 190 days post infection (dpi). Brain and spinal cord were post-fixed overnight in 4% PFA at 4°C. After rinsing with phosphate buffered saline (PBS), brains were stored at 4°C in PBS with 0.1% sodium azide. Brains were divided into two parts with the left hemispheres sagittally sectioned at 50 µm using a Leica VT1200 vibratome and at 20 µm using a Leica CM3050 S cryostat. Right hemispheres were used for iDISCO+ tissue clearing protocol (below). Fixed brains and spinal cords were serially incubated in 10% sucrose, 20% sucrose and 30% sucrose at 4°C for cryoprotection. Tissues were embedded in OCT (tissue-Tek, Torrance, CA), frozen in dry ice and stored at -80 °C until sectioning. For cryosectioning, spinal cords were placed in an embedding mold (Sigma-Aldrich, The Woodlands, TX) and filled with ultrapure low melting point agarose (Thermo Fisher Scientific, Rockford, IL) at 37°C. The semi-solid agarose cube was removed and glued (Loctite, Rocky Hill, CT) in the horizontal orientation for transversal slicing at 20 µm using a cryostat.

[00103] Immunostaining

[00104] For immunohistochemistry, free-floating brain sections were washed with PBS and blocked for 1h with 3% bovine serum albumin (BSA), 2% donkey serum and 0.5% Triton X-100 (Sigma-Aldrich, St. Louis, MO). Samples were incubated with primary overnight at 4°C and secondary antibodies for 1h at RT (room temperature) diluted in PBS containing 1% BSA, 1% donkey serum and 0.5% Triton X-100. Cell nuclei were counterstained with 0.5 µg/ml DAPI for 5 minutes (Thermo Fisher Scientific, Rockford, IL). The following primary antibodies were used: rabbit anti-RFP Rockland (1:1000; Limerick, PA), chicken anti-mCherry Abcam (1:500;

Cambridge, MA), mouse anti-NeuN (1:500; Millipore Bioscience Research Reagents, Temecula, CA), rabbit anti-Olig2 (1:500, EMD Millipore, Temecula, CA), rabbit anti-Iba1 (1:1000, Wako Chemical, Richmond, VA) and rabbit anti-S100 (1:5000, Dako, Glostrup, Denmark). The following secondary antibodies were used: Alexa Fluor 488 donkey anti-rabbit IgG, Alexa Fluor 488 donkey anti-mouse IgG, Alexa Fluor 647 donkey anti-rabbit IgG, Alexa Fluor 647 donkey anti-chicken IgG (1:1000, Thermo Fisher Scientific, Rockford, IL). Spinal cord free-floating sections were stained with 1:300 dilution of NeuroTrace 500/525 green fluorescent Nissl stain (Molecular Probes, Eugene, OR) for 1hr. The sections were permeabilized with 0.1% Triton X-100 in PBS at 10 minutes and washed first with PBS followed by PBS with 0.1% Triton X-100 for 10 minutes. Samples were incubated with 0.5 µg/ml DAPI for 5 min and then washed with PBS for 2 hours at room temperature. Fluoromount-G mount medium (Southern Biotech, Birmingham, AL) was applied to brain and spinal cord sections before mounting.

[00105] Microscopy

[00106] Neuronal SCG cultures were imaged with a Nikon Ti-E inverted epifluorescence microscope (Nikon Instruments Inc, Tokyo, Japan), containing a Cool Snap ES2 camera (Photometrics, Tucson, AZ) and 4X objective. Tiled images of the entire S compartment were assembled with the Nikon NIS Elements software. To quantify AAV transduction efficacy in various brain regions, brain slices were imaged with a NanoZoomer S60 fluorescent microscope scanner (Hamamatsu, Hamamatsu, Japan). Brain slices were imaged with a Leica STP8000 confocal laser-scanning microscope (Leica Microsystems, Wetzlar, Germany) using 20X and 63X objectives, hybrid (HyD) detectors for sensitive detection, and over a 1024 x 1024 pixels area. The ImageJ software was used to calculate corrected total cell fluorescence as previously reported (Maturana, Aguirre et al. 2017). Cells were selected drawing a region of interest (ROI) and normalized to background intensity from non-fluorescent cells. The calculation of corrected total cell fluorescence was measured as relative fluorescence intensity (RFI) considering area integrated, density and mean gray value of each cell.

[00107] iDISCO+ tissue clearing

[00108] Permeabilization

[00109] Right brain hemispheres were used for iDISCO+ tissue clearing. Brain samples were fixed overnight in 4% PFA prior to tissue clearing as previously described (Renier, Wu et al.

2014). Fixed samples were washed/dehydrated in 20, 40, 60, 80, 100% methanol/water solutions for 1hr each, followed by a 5% hydrogen peroxide/methanol overnight wash (Sigma-Aldrich, St. Louis, MO) and rehydration with a reverse gradient of methanol/water 100, 80, 60, 40, 20% for 1 hour each. Finally, brains were washed with 0.2% Triton X-100 /PBS, followed by 20% DMSO (Thermo Fisher Scientific, Rockford, IL)/0.3M glycine (Sigma-Aldrich, St. Louis, MO)/0.2% Triton X-100/PBS at 37°C for 2 days.

[00110] Immunolabeling

[00111] Samples were incubated in a blocking solution of 10% DMSO/6% donkey serum (EMD Millipore, Temecula, CA)/0.2% Triton X-100/PBS at 37°C for 2-3 days, followed by two 1hr/washes, in PBS/0.2% Tween-20 (Sigma-Aldrich, St. Louis, MO) with 10 μg/ml heparin (solution hereinafter referred to as PTwH, Sigma-Aldrich, St. Louis, MO). Brains were incubated with primary rabbit anti-RFP antibody (1:1000; Rockland, Limerick, PA) in 5% DMSO/3% donkey serum/PTwH at 37°C for 7 days. Next, brains were washed with PTwH 5 times (wash intervals: 10 minutes, 15 minutes, 30 minutes, 1 hour, 2 hours) and incubated at 37°C for 7 days with secondary Alexa Fluor 647 donkey anti-rabbit IgG (1:450, Thermo Fisher Scientific, Rockford, IL) in 3% donkey serum/PTwH and then washed in PTwH for 5 times.

[00112] Tissue clearing

[00113] Brains were sequentially dehydrated in 20, 40, 60, 80, 100% methanol/water for 1 hour each step, followed by 2:1 dichloromethane (DCM; Sigma-Aldrich, St. Louis, MO)/methanol, and 100% DCM washes. Finally, samples were cleared with DBE (Sigma-Aldrich, St. Louis, MO) and stored in the dark at room temperature until imaged.

[00114] Light-sheet microscopy and analysis of cleared tissue

[00115] After immunolabeling and clearing, brain volumes were acquired using a light-sheet Ultramicroscope II (LaVision Biotec, Bielefeld, Germany). Brain halves were glued in the horizontal orientation on a custom-designed 3D-printed holder (Renier, Wu et al. 2014; Renier, Adams et al. 2016) and submerged in DBE. Brains were imaged in the autofluorescent channel for registration purposes with a 488-nm laser diode excitation and a 525-nm maximum emission filter (FF01-525/39-25, Semrock, Rochester, NY), and at 640-nm excitation with a 680-nm maximum emission filter (FF01-680/42-25, Semrock) for cellular imaging of AAV infected cells (anti-RFP). Separate left- and right-sided illumination autofluorescent images were acquired

every 10 micrometers (z-steps size) using a 0.017 excitation-sheet NA and 1.3x magnification. Left and right sided images were sigmoidally blended at the midline. Autofluorescent volumes were registered to the volumetric Allen brain atlas (2015) using affine and b-spline transformations, as described by Renier and colleagues (Renier, Wu et al. 2014; Renier, Adams et al. 2016). To account for movement during acquisition and different imaging parameters between channels, cell signal volumes were registered to autofluorescent volumes with an affine transform. Brain volumes were analyzed with our modified ClearMap software: "ClearMap_cluster" (github.com/PrincetonUniversity/clearmap_cluster), compatible with high performance computing clusters. For all analyzed samples, detected objects on brain edges and ventricles were eroded by 75 μm from the edge of the structure to minimize false positives.

[00116] RNAscope in situ hybridization

[00117] Brain cryosections were mounted on superfrost plus adhesion slides (Thermo Fisher, Waltham, MA) and stored at -80°C. RNA staining was performed using the RNAscope multiplex fluorescent reagent kit (Advanced Cell Diagnostics (ACD), Newark, CA) following the manufacturer's protocol. The mCherry probe ACD# 431201-C2 was used. Slices were pretreated with protease IV for 30 minutes at 40 °C, followed by probe incubation for 2 hours at 40 °C. Then, different amplifier solutions were performed for 30 minutes, 30 minutes, and 15 minutes at 40 °C. Signal was detected with TSA plus fluorescein system (Perkin Elmer, Waltham, MA). Incubation steps were done in the ACD HybEZ hybridization system. Slides were counterstained with DAPI for 30 seconds at room temperature and then washed twice with PBS. Finally, slides were mounted with VECTASHIELD Vibrance antifade mounting medium (Vector Laboratories, Burlingame, CA).

[00118] Statistics

[00119] Statistical data analysis was performed using GraphPad Prism 7 software (GraphPad Software, Inc., La Jolla, CA). Two-tailed Student's test was used to compare between two groups, and non-parametric one-way ANOVA test followed by a Bonferroni multiple comparison post-test to compare among multiple groups. A p value < 0.05 was statistically significant. Data are represented as the mean with SEM.

[00120] Example 2. Small PRV LAP sequences can drive transgene expression in neurons independently of herpesvirus infection.

[00121] The PRV LAP region includes at least two promoter regions defined here as LAP1 and LAP2 (**FIG. 1A**). In the PRV genome, LAP1 and LAP2 are present in tandem as PRV LAP1_2. These sequences, alone or combined, are capable of efficient expression of reporter transgenes in primary sympathetic neurons when used in AAV vectors without PRV infection (**FIGs. 1C** and **1D**). The LAP sequences were analyzed to identify putative regulatory elements using the Jasper (Sandelin, Alkema et al. 2004; Wasserman and Sandelin 2004), the RSAT (Nguyen, Contreras-Moreira et al. 2018) and the CTCFBSDB 2.0 (Ziebarth, Bhattacharya et al. 2013) software. Three cyclic AMP response element-binding protein (CREB) binding motifs were detected upstream of the LAP1 TATA box, and one was detected upstream of the LAP2 TATA box. Moreover, two CCCTC-binding factor (CTCF) binding motifs were detected upstream of the LAP1 TATA box and one was detected downstream of the LAP2 TATA box. Downstream promoter elements (DPE) were identified in LAP2, including three CG boxes and four signal transducer and activator of transcription 1 (STAT1) binding motifs. Additionally, there were lineage-determining TFs (Wang, Pol et al. 2014), such as SRY-Box 10 (SOX10) and oligodendrocyte transcription factor 2 (Olig2), upstream of the LAP1 TATA box and the LAP2 TATA box, respectively (**FIG. 1A**).

[00122] Four AAV recombinants were packaged into serotype PHP.eB capsids by standard methods. Three promoter constructs, LAP1 (498 bp, corresponding to SEQ ID NO: 1), LAP2 (404 bp, corresponding to SEQ ID NO: 2) and LAP1_2 (880 bp, corresponding to SEQ ID NO: 3), were prepared. The ubiquitous EF-1α promoter (1264 bp) was used as a positive control for transgene expression. All four AAV recombinants expressed the fluorescent reporter mCherry (**FIG 1B**). To verify the *in vitro* performance of each promoter, rat primary superior cervical ganglia (SCG) neuronal cultures were transduced with 3×10^{11} genomes of each AAV, and the relative mCherry fluorescence intensity (RFI) was quantified over a 90-day period.

[00123] For neurons transduced with AAV-LAP1, mCherry expression increased abruptly at 11 dpi (64220 RFI) but to a lower level when compared with AAV-LAP2, AAV-LAP1_2 and AAV-EF1α. AAV-EF1α expression increased ~120-fold more at 17 dpi (8.70×10^6; 2.39×10^6; 8.01×10^6 RFI respectively) (**FIG. 1C**). The highest level of expression by all four recombinants was at 28 dpi: LAP1 (291,242 RFI); LAP2 (1.36×10^7 RFI); LAP1_2 (4.36×10^6 RFI) and EF1α (1.40×10^7 RFI) (**FIGs. 1C** and **1D**). LAP2 and EF1 α had the highest mCherry RFI

(LAP2 = EF1α > LAP1_2 > LAP1). Between 38 dpi and 90 dpi, all four AAV recombinants showed a subtle but sustained RFI decrease (**FIG. 1C**), most likely due to the senescence of primary SCG neurons after more than 100 days in culture. Importantly, all three AAV-LAP recombinants showed mCherry transcription in primary neurons for 90 days in the context of AAV transduction and in the absence of PRV infection.

[00124] Example 3. Whole-CNS screening reveals pan-neuronal AAV-LAP transgene expression after six months.

[00125] AAV serotype PHP.eB was used for a promoter screening assay given the enhanced capacity to cross the BBB and transduce C57/BL6 mice CNS after systemic, intravascular delivery (Chan, Jang et al. 2017). AAV-LAP1-mCherry, AAV-LAP2-mCherry, AAV-LAP1_2-mCherry and AAV-EF1α-mCherry were delivered by unilateral retro-orbital venous sinus injection of 4×10^{11} viral genomes/mouse (vg/mouse). As illustrated in **FIG. 2A**, the brains and spinal cords were harvested 30 days and 190 days post-AAV transduction to quantify mCherry transcription and translation. LAP-mCherry expression in the whole intact brain was determined after the tissues were cleared, immunostained with iDISCO+ (immunolabeling-enabled three-dimensional imaging of solvent-cleared organs), and analyzed by light-sheet microscopy and volumetric registration (Renier, Wu et al. 2014; Renier, Adams et al. 2016).

[00126] As demonstrated in **FIGs. 2B-2P**, all four promoters showed stable mCherry expression at both 30 dpi and 190 dpi. The density (number of mCherry positive cells per mm^3 of brain tissue) of LAP2 was higher than those of LAP1 and LAP1_2, and was not significantly different from that of EF1α in different areas of the brain: cortex: primary motor, secondary motor, primary somatosensory and supplemental somatosensory, **FIGs 2B-2E**); hippocampal formation (**FIG. 2G**), pallidum (**FIG. 2I**), hypothalamus (**FIG. 2K**) and olfactory areas (**FIG. 2P**). In cerebellum, LAP2 showed higher mCherry density than LAP1, LAP1_2 and EF1α (**FIG. 2O**). Furthermore, in striatum (**FIG. 2H**); thalamus (**FIG. 2J**); midbrain, motor and sensory areas (**FIGs. 2L** and **2M**), and hindbrain (**FIG. 2N**), LAP2 and LAP1_2 showed significantly higher density than EF1α ($p < 0.05$). Note that the LAP2 nucleotide sequence is 68% shorter than that of EF1α, yet outperforms EF1α in several brain areas.

[00127] To further validate LAP transgene expression in the CNS, mCherry protein expression was assessed by immunohistochemistry (IHC) in sagittal brain sections at 30 dpi and

190 dpi (**FIGs. 3A-3H**). Confocal microscopy analysis showed abundant mCherry staining throughout the cortical somatosensory area (**FIG. 3E**), the dentate gyrus in the hippocampal formation (**FIG. 3F**), the caudoputamen in the striatum (**FIG. 3G**), and the cerebellar cortex (**FIG. 3H**) at 30 dpi. Importantly, mCherry expression was stable for all three LAP sequences at 190 dpi and was similar to that of the larger AAV-EF1α-mCherry promoter (**FIGs. 3E-3H**, Panels 5-8). Next, mCherry expression was quantified at 30 dpi and 190 dpi. The mCherry RFI was similar for all AAV promoters with no significant differences (**FIGs. 3I1-3I4**). The number of mCherry positive cells per pixel^2 after 190 days was subsequently quantified. In the cortex, the number of LAP2 mCherry expressing cells was higher than those observed for LAP1-mCherry and LAP1_2-mCherry [LAP2: 297 ± 19.82 vs. LAP1: 149 ± 5.61 vs LAP1_2: 168 ± 9.22 ($n = 6$, $p < 0.001$) (**FIG. 3J1**). In the dentate gyrus, the striatum and the cerebellum, the number of mCherry positive cells was similar for all LAP variants and EF1α (**FIGs. 3J2-3J4**). Thus, all AAV-LAP variants promote mCherry expression in the brain, further demonstrating that a single administration of AAV-LAP recombinants can be sufficient for driving long term, pan-neuronal transgene expression in the mouse CNS.

[00128] Example 4. The small LAP2 promoter variant drives strong and stable pan-neuronal transgene expression after systemic AAV administration.

[00129] The efficacies of mCherry expression under the control of different PRV LAP sequences were compared. Abundant signal in the cortex (**FIGs. 4A** and **4E1**), dentate gyrus (**FIGs. 4B** and **4E2**), striatum (**FIGs. 4C** and **4E3**) and cerebellum (**FIGs. 4D** and **4E4**) at 30 dpi and 190 dpi were observed. Moreover, the AAV-LAP2 RFI was stable and similar to AAV-EF1α both at 30 dpi and 190 dpi ($p < 0.05$) (**FIGs. 4E1-4E4**; **Table 2**). Although AAV-LAP1 and AAV-LAP1_2 mCherry RFI levels were stable and not significantly different between them at 30 dpi and 190 dpi in cortex, dentate gyrus, striatum and cerebellum (**FIGs. 4E1-4E4**; **Table 2**), both promoters showed significantly less transgene expression compared to LAP2 and EF1α.

Table 2. mCherry Expression in Mouse Brain with Different AAV-PHP.eB-Promoter

Promoter	Brain region	Direct mCherry expression		Indirect IHC mCherry expression	
		30 dpi[a]	190 dpi	30 dpi	190 dpi

		Mean (RFI)[b] ± SEM	Mean (RFI) ± SEM	Mean (RFI) ± SEM	Mean (RFI) ± SEM
LAP1	Cortex	1.05 ± 0.12	1.35 ± 0.14	8.51 ± 0.47	8.46 ± 0.49
	Dentate gyrus	1.36 ± 0.11	0.99 ± 0.04	9.87 ± 0.72	9.00 ± 0.44
	Striatum	1.18 ± 0.13	1.41 ± 0.18	9.14 ± 0.97	9.17 ± 1.41
	Cerebellum	1.31 ± 0.17	1.48 ± 0.15	9.38 ± 0.92	9.75 ± 1.16
LAP2	Cortex	9.66 ± 0.41	9.39 ± 0.69	9.30 ± 0.70	10.18 ± 0.51
	Dentate gyrus	8.61 ± 0.63	9.32 ± 0.63	10.26 ± 0.52	10.01 ± 0.59
	Striatum	9.91 ± 0.51	10.61 ± 0.65	9.92 ± 0.57	11.06 ± 0.66
	Cerebellum	10.66 ± 0.67	10.34 ± 0.84	9.59 ± 0.50	10.26 ± 0.65
LAP1_2	Cortex	5.18 ± 0.43	5.36 ± 0.39	8.01 ± 0.61	8.70 ± 0.36
	Dentate gyrus	3.92 ± 0.70	4.17 ± 0.62	9.20 ± 0.64	10.02 ± 0.72
	Striatum	4.36 ± 0.84	4.67 ± 0.75	10.45 ± 0.48	10.17 ± 0.75
	Cerebellum	4.76 ± 0.54	5.42 ± 0.27	9.48 ± 0.56	9.86 ± 0.54
EF1α	Cortex	8.99 ± 0.62	9.05 ± 0.73	9.87 ± 0.77	10.16 ± 0.62
	Dentate gyrus	9.16 ± 0.57	9.16 ± 0.81	9.40 ± 0.72	9.45 ± 0.64
	Striatum	9.96 ± 0.59	10.93 ± 0.67	11.26 ± 0.61	10.29 ± 0.85
	Cerebellum	10.72 ± 0.49	11.06 ± 0.84	10.92 ± 0.66	10.65 ± 0.89

[a]dpi: days post AAV injection

[b]RFI: relative fluorescence intensity

[00130] Since mRNA half-life is typically shorter than that of the translated protein (Chan, Mugler et al. 2018), mCherry transcripts in AAV-LAP transduced mouse brain slices were measured. AAV-LAP2-mCherry mRNA at 30 dpi (data not shown) and 190 dpi were measured using a mCherry-specific riboprobe. Fluorescent *in situ* hybridization (FISH) showed abundant AAV-LAP2 mCherry RNA in cortex, dentate gyrus, striatum, cerebellum and olfactory bulb (**FIGs. 5A-5E**), further confirming that PRV LAP2 can drive chronic and robust transgene transcription in the CNS.

[00131] Example 5. AAV-LAP transgene expression in the brain is predominant in neurons but not in glial cells.

[00132] The tropism and specificity of AAV transduction and subsequent transgene expression depend on the AAV serotype (Aschauer, Kreuz et al. 2013, Bedbrook; Deverman et al. 2018) and the promoter (von Jonquieres, Frohlich et al. 2016; Hammond, Leek et al. 2017; Dayton, Grames et al. 2018). To characterize which cell-types showed AAV-LAP-mCherry expression after systemic AAV-PHP.eB delivery, co-immunostainings of mCherry protein with markers for neurons (NeuN), oligodencrocytes (Olig2), microglia (Iba1) and astrocytes (S100) were performed in the cortex and dentate gyrus. Co-staining with NeuN and mCherry revealed that over 90% of the neurons imaged expressed mCherry driven by the different AAV-LAP variants in both the cortex and dentate gyrus (**FIGs. 6A**, **6B** and **6E**). Conversely, less than 4% of mCherry-positive oligodendrocytes were detected for all LAP variants (**FIGs. 6C**, **6D** and **6F)**. Moreover, no co-labelling of mCherry with microglia and astrocyte markers for any of the LAP recombinants was observed (**FIGs. 7A-7D**). Overall, these results demonstrate that in the context of systemic brain transduction with AAV-PHP.eB, LAP-mCherry expression is abundant in neurons but not in glial cells.

[00133] Example 6. AAV-LAP constructs exhibit broad, stable and long-term transgene expression throughout the spinal cord.

[00134] In addition to the brain, AAV-LAP performance in spinal cord, where the serotype PHP.eB has shown widespread transduction of gray matter (Chan, Jang et al. 2017; Dayton, Grames et al. 2018), was evaluated.

[00135] Abundant native mCherry expression in both dorsal and ventral horns of the spinal cord at cervical, thoracic (data no shown) and lumbar levels at 190 dpi were observed (**FIGs. 8A-8D**). Quantification of native mCherry fluorescence (RFI) for all promoters was similar, with no statistically significant differences (**FIG. 8E**). However, the LAP2 and LAP1_2 recombinants showed the highest density of mCherry-positive cells per pixel^2, followed by EF1α and LAP1; LAP2 = LAP1_2 > EF1α > LAP1 (**FIG. 8F**). Therefore, all three PRV LAP sequences effectively mediate pan-neuronal, long-term transgene expression in the spinal cord.

[00136] Gene therapy has been used to restore gene function in specific target cells in neurologic and neurodegenerative disorders (Deverman, Ravina et al. 2018). Gene transfer by systemic vector delivery via peripheral vascular transduction can be difficult for efficient expression in a neuron-specific or pan-neuronal fashion in the CNS (Ingusci, Verlengia et al.

2019). Recombinant AAV vectors are among the most efficient vehicles to achieve gene expression in the CNS (Hudry and Vandenberghe 2019, Wang, Tai et al. 2019). Moreover, engineered AAV capsids have shown improved CNS transduction and enhanced capacity to cross the BBB with higher efficiency than naturally-occurring serotypes (Chan, Jang et al. 2017; Bedbrook, Deverman et al. 2018; Hordeaux, Yuan et al. 2019; Qin Huang and Alejandro B. Balazs 2019). Despite these advances, AAV gene therapy is hindered by the small payload size limit of 4.9 Kb for the AAV capsid (Chamberlain, Riyad et al. 2016). For example, CNS therapies for Pompe disease (Colella, Sellier et al. 2019) and Parkinson's disease (Wang, Muramatsu et al. 2002) are based on delivery of relatively large genes such as GAA (2.9 Kb) and GDNF (2.5 Kb). For these and other similar cases, the use of larger promoters or even smaller CMV and hSyn promoters has been shown to be quickly repressed after delivery (Gray, Foti et al. 2011, Jackson, Dayton et al. 2016).

[00137] As described herein, three small pan-neuronal promoters isolated from the genome of the alphaherpesvirus PRV were identified, showing efficient and long-term transgene expression in the mice CNS after systemic AAV PHP.eB delivery. The results presented herein demonstrate that these small PRV LAP sequences can drive long-term expression of a reporter transgene (> 6 months) in brain and spinal cord. PRV LAP uniformly transduced neurons in the cortex, striatum, dentate gyrus and cerebellum. The distribution of mCherry-positive cells was not significantly different between LAP sequences in the dentate gyrus, striatum and cerebellum. However, LAP2 transgene expression was significantly higher in cortex compared to LAP1 and LAP1_2. The whole-brain screening assay demonstrated that the LAP2 variant of only 402 bp can drive stronger mCherry expression than the larger LAP1 and LAP1_2 sequences. Moreover, abundant mCherry mRNA transcribed from LAP2 was detected in every screened brain region at 190 dpi. These results demonstrate both efficient transcription and translation driven from the small PRV LAP2 in CNS after systemic AAV-LAP2 delivery in the absence of PRV infection.

[00138] Although the LAP1-mCherry cell density was significantly lower than that for LAP2, mCherry expression remained stable and long-lasting. Therefore, LAP1 might be useful in cases where low amounts of the therapeutic protein are needed (e.g. enzyme deficiencies) or for cross-correction to non-transduced cells. For example, for lysosomal enzyme deficiency (Husain, Passini et al. 2009) and mucopolysaccharidosis VII diseases (Cearley and Wolfe 2007), where

the enzyme restored by AAV therapy can be secreted from the transduced cell and improve neighboring diseased cells.

[00139] AAV tropism is determined primarily by interactions between the capsid and specific receptors in susceptible and permissive cells (Aschauer, Kreuz et al. 2013; Bedbrook, Deverman et al. 2018). Different AAV serotypes have different tissue tropism. However, the promoter sequence and other sequences included in the vector such as the inverted terminal repeat sequence (ITR), can have a substantial impact on tropism (Haberman and McCown 2002). In addition, the promoter region transcribing the transgene is critically important to optimize AAV vector performance. Efficient transgene expression either in a broad or cell-type specific fashion, requires binding and action of cell-derived TFs to the promoter region (Dayton, Grames et al. 2018; Andersson and Sandelin 2019). Changes in the neuronal environment such as aging or differentiation, also can alter the recruitment of cell-specific regulatory proteins and therefore gene expression in the CNS (Herdegen and Leah 1998). The analysis of the PRV LAP sequence presented herein identified DPE in LAP2, which could control transgene expression onset, duration and cell-type specificity. Additionally, four STAT1 motifs, that in HSV-1 LAP seem to regulate viral reactivation from latency (Kriesel, Jones et al. 2004), were identified. Strikingly, one of these STAT1 motifs in PRV LAP2 was found to co-localize with the TATA box and an Olig2 motif, a known multifaceted TFs promoting neuronal and oligodendrocyte fates (Emery and Lu 2015). The proximity effects associated with these motifs and the transcriptional start site could explain the different levels of CNS transgene expression between LAP2, LAP1 and LAP1_2. Additionally, one CTCF motif was found downstream of the LAP2 TATA box, which could have a role in the resistance to epigenetic silencing during latency, as shown for HSV-1 (Lang, Li et al. 2017; Lee, Raja et al. 2018). Indeed, Zimmerman and colleagues (Zimmerman, Patel et al. 2018) found that insertion of a CTCF motif downstream of the EF1α promoter increased transgene expression significantly compared to native EF1α and CMV promoters. Interestingly, the insertion of a secondary CTCF motif downstream of the CMV TATA box, had no effect on luciferase reporter expression, presumably due to the redundant presence of a native CTCF motif (Zimmerman, Patel et al. 2018). Accordingly, gene expression is susceptible to changes depending of the genetic context and sequence-specific DNA binding proteins. The recruitment of specific TFs from different host cells can modulate transgene transcription by the

same mechanisms regulating resistance to inactivation during latency. Insulator elements like the CTCF-binding factor are independently regulated (Washington, Musarrat et al. 2018) and can protect promoter regions from repression by heterochromatin, maintaining long-lasting transcription (Lang, Li et al. 2017).

[00140] Histological assessment of cell-specific transduction by colocalization of LAP-mCherry, glial and neuronal markers, revealed that LAP sequences express more efficiently in neurons than glia, in cortex and dentate gyrus. LAP-mCherry positive cells colocalized predominantly with neuron-specific markers, and to a lesser extent with oligodendrocytes but not microglia or astrocytes. These findings suggest that PRV LAP sequences have a pan-neuronal promoter profile in the CNS after PHP.eB systemic delivery. This activity has also been found for the HSV LAP sequences due to the presence of a CRE motif upstream of the TATA box (Leib, Nadeau et al. 1991; Kenny, Krebs et al. 1994; Bloom, Stevens et al. 1997). PRV LAP1 and LAP2 are neuron-specific promoters in the absence of other viral proteins and that transgene expression levels vary across different neuronal types (Taharaguchi, Yoshino et al. 2003). Importantly, AAV-PHP.eB transduces neurons predominantly (Chan, Jang et al. 2017), and the combination of this capsid variant with PRV LAP sequences exhibits a strong, long-lasting, pan-neuronal expression profile in the CNS. Thus, PRV LAP sequences may be used not only in the context of recombinant viral vectors (AAV, adenovirus, lentivirus, herpesvirus), but also with non-viral gene delivery platforms. The natural host of PRV is the adult swine, but the virus has an extremely broad tropism and can infect some birds, fish and many types of mammals including some primates (Baskerville and Lloyd 1977). Moreover, human cells in culture are susceptible to PRV infection and there have been some reports of zoonotic infections (Wong, Lu et al. 2019). Therefore, the PRV LAP sequence may be optimized for gene therapy-applications requiring efficient and long-term transgene expression in several different mammals including humans.

[00141] In summary, the data presented herein demonstrated that PRV LAP promoter activity is independent of PRV infection and that small AAV-PHP.eB-LAP sequences express transgenes in a stable and pan-neuronal fashion in brain and spinal cord (CNS). Long-term transgene transcription and translation is paramount for effective and long-lasting single-dose gene therapy

applications. Thus, PRV LAP sequences may be useful for the treatment of genetic CNS diseases.

[00142] Example 7. LAP sequences efficiently drive transgene expression in non-neuronal cells and tissues.

[00143] In the following examples, LAP sequences were demonstrated to efficiently drive transgene expression of a fluorescent reporter protein (mCherry) in several cell and tissue types, including kidney, liver and retina. Furthermore, LAP was found to be compatible with viral vectors (such as AAV) as well as non-viral vectors (such as direct transfection of plasmid DNA).

[00144] As shown in **FIGs. 9A-9C**, the LAP sequences drive efficient transgene expression in porcine kidney cells. Representative images of PK15 cells (porcine kidney epithelial cells, ATCC CCL-33) were obtained at 48 hours post transfection of a reporter plasmid comprising mCherry driven by LAP1 (**FIG. 9A**), LAP2 (**FIG. 9B**), or LAP1_2 (**FIG. 9C**).

[00145] As shown in **FIGs. 10A** and **10B**, LAP2 drives efficient transgene expression in human kidney cells. Representative images of HEK-293 cells (human embryonic kidney epithelial cells, ATCC CRL-1573) were obtained at 48 hours post transfection of a reporter plasmids comprising mCherry driven by LAP2 (**FIG. 10A**) or the EF1α promoter (**FIGs. 10B**).

[00146] As shown in **FIGs. 11A** and **11B**, LAP2 drives efficient transgene expression in human liver cells. Representative images of HepG2 cells (human liver epithelial cells, ATCC HB-8065) were obtained at 48 hours post transfection of a reporter plasmid comprising mCherry driven by LAP2 (**FIG. 11A**) or the EF1α promoter (**FIG. 11B**).

[00147] As shown in **FIGs. 12A1-12B4**, LAP2 drives long-term transgene expression throughout the liver. Intravenous administrations of the AAV-LAP2 (**FIGs. 12A1-12A4**) or the AAV-EF1α-vector (**FIGs. 12B1-12B4**) were performed by unilateral injections into the mouse retro-orbital sinuses (4×10^{11} vg/mouse). The liver was collected at 190 dpi and longitudinally sectioned at 20 μm for subsequent IHC and confocal microscopy analysis. Representative confocal images show native mCherry fluorescence (**FIGs. 12A1** and **12B1**: red), anti-mCherry immunostaining (**FIGs. 12A2** and **12B2**: green), DAPI nuclear counterstain (**FIGs. 12A3** and **12B3**: blue) and merged immunofluorescence and DAPI images (**FIGs. 12A4** and **12B4**).

[00148] As shown in **FIGs. 13A1-13B4**, LAP2 drives long-term transgene expression in the mouse kidney. Intravenous administrations of the AAV-LAP2 (**FIGs. 13A1-13A4**) or the AAV-

EF1α-vector (**FIGs. 13B1-13B4**) were performed by unilateral injections into the mouse retro-orbital sinuses (4 x 10^{11} vg/mouse). The kidneys were collected at 190 dpi and longitudinally sectioned at 20 μm for subsequent IHC and confocal-microscopy analysis. Representative confocal images show native mCherry fluorescence (**FIGs. 13A1** and **13B1**: red), anti-mCherry immunostaining (**FIGs. 13A2** and **13B2**: green), DAPI nuclear counterstain (**FIGs. 13A3** and **13B3**: blue) and merged immunofluorescence and DAPI images (**FIGs. 13A4** and **13B4**).

[00149] As shown in **FIGs. 14A1-14B4**, LAP2 drives long-term transgene expression in the mouse retina. Intravenous administrations of the AAV-LAP2 (**FIGs. 14A1-14A4**) or the AAV-EF1α-vector (**FIGs. 14B1-14B4**) were performed by unilateral injections into the mouse retro-orbital sinuses (4 x 10^{11} vg/mouse). The retinae were collected at 190 dpi and transversely cryosectioned at 20 μm for subsequent IHC and confocal-microscopy analysis. Representative confocal images show native mCherry fluorescence (**FIGs. 14A1** and **14B1**: red), anti-mCherry immunostaining (**FIGs. 14A2** and **14B2**: green), DAPI nuclear counterstain (**FIGs. 14A3** and **14B3**: blue) and merged immunofluorescence and DAPI images (**FIGs. 14A4** and **14B4**).

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[00231] The teachings of all patents, published applications and references cited herein are incorporated by reference in their entirety.

[00232] While example embodiments have been particularly shown and described, it will be understood by those skilled in the art that various changes in form and details may be made therein without departing from the scope of the embodiments encompassed by the appended claims.

CLAIMS

What is claimed is:

1. A recombinant vector for expression of a target gene in a host cell, comprising:

a promoter comprising a nucleic acid sequence having at least 70% sequence identity to a sequence selected from SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3 and SEQ ID NO: 4;

a cloning site for insertion of a nucleic acid encoding the target gene; and

at least one non-promoter regulatory element required for the expression of the target gene in the host cell.

2. The recombinant vector of claim 1, wherein the vector is a viral vector.

3. The recombinant vector of claim 2, wherein the viral vector is an adeno-associated virus (AAV) vector.

4. The recombinant vector of claim 1, wherein the vector is a non-viral vector.

5. The recombinant vector of any of claims 1-4, wherein the host cell is a mammalian cell.

6. The recombinant vector of any one of claims 1-5, wherein the host cell comprises an *in vitro* neuronal cell, an *ex vivo* neuronal cell, or both.

7. The recombinant vector of any one of claims 1-5, wherein the host cell comprises a neuronal cell of a subject.

8. The recombinant vector of claim 7, wherein the neuronal cell resides in a primary motor area, a secondary motor area, a primary somatosensory area, a supplemental somatosensory area, a visual area, a hippocampal formation, striatum, pallidum, thalamus, hypothalamus, a midbrain area, hind brain, an olfactory area or a combination thereof, of the subject.

9. The recombinant vector of claim 8, wherein the midbrain area comprises a motor-related area, a sensory-related area, or both.

10. The recombinant vector of claim 7, wherein the neuronal cell resides in cortex, dentate gyrus, striatum, cerebellum, olfactory bulb or a combination thereof, of the subject.

11. The recombinant vector of any one of claims 6-10, wherein the neuronal cell comprises a neuron, a microglia cell, an astrocyte, or a combination thereof.

12. The recombinant vector of claim 11, wherein the neuronal cell comprises a neuron.

13. The recombinant vector of claim 7, where the neuronal cell resides in the spinal cord of the subject.

14. The recombinant vector of claim 13, where the neuronal cell resides in the dorsal horn of the spinal cord.

15. The recombinant vector of claim 13 or 14, where the neuronal cell resides in the lumbar spinal cord, thoracic spinal cord, cervical spinal cord or a combination thereof.

16. The recombinant vector of any one of claims 1-5, wherein the host cell is selected from the group consisting of a bladder cell, a blood cell, a bone cell, an endothelial cell, an epithelial cell, a fat cell, a heart cell, an intestinal cell, a kidney cell, a liver cell, a lung cell, a muscle cell, a pancreatic cell, a retina cell, a sex cell, a skin cell, a spleen cell, a stem cell, and a combination thereof.

17. The recombinant vector of any one of claims 1-16, wherein the promoter comprises a nucleic acid sequence having at least 90% sequence identity to a nucleic acid sequence selected from the group consisting of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3 and SEQ ID NO: 4.

18. The recombinant vector of claim 17, wherein the promoter comprises a nucleic acid sequence selected from the group consisting of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3 and SEQ ID NO: 4.

19. The recombinant vector of any one of claims 1-18, wherein the at least one non-promoter regulatory element comprises an enhancer element.

20. The recombinant vector of any one of claims 1-19, wherein the at least one non-promoter regulatory element comprises an inducer element.

21. The recombinant vector of any one of claims 1-20, further comprising a selectable marker element.

22. A host cell comprising the recombinant vector of any of claims 1-21.

23. A recombinant polynucleotide molecule comprising a nucleic acid encoding a target gene, wherein the nucleic acid is operably linked to a promoter comprising a nucleic acid sequence having at least 70% sequence identity to a sequence selected from SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3 and SEQ ID NO: 4.

24. The recombinant polynucleotide molecule of claim 23, wherein the promoter comprises a nucleic acid sequence having at least 90% sequence identity to a nucleic acid sequence selected from the group consisting of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3 and SEQ ID NO: 4.

25. The recombinant polynucleotide molecule of claim 24, wherein the promoter comprises a nucleic acid sequence selected from the group consisting of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3 and SEQ ID NO: 4.

26. The recombinant polynucleotide molecule of any one of claims 23-25, wherein the nucleic acid encoding the target gene comprises more than 4 kilobases.

27. A recombinant polynucleotide molecule comprising a cloning site for insertion of a nucleic acid encoding a target gene, wherein the cloning site is operably linked to a promoter comprising a nucleic acid having at least 70% sequence identity to a sequence selected from SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3 and SEQ ID NO: 4.

28. The recombinant polynucleotide molecule of claim 27, wherein the promoter comprises a nucleic acid sequence having at least 90% sequence identity to a nucleic acid sequence selected from the group consisting of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3 and SEQ ID NO: 4.

29. The recombinant polynucleotide molecule of claim 28, wherein the promoter comprises a nucleic acid sequence selected from SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3 and SEQ ID NO: 4.

30. A method of expressing a target gene in a host cell, comprising:

contacting the host cell with a recombinant polynucleotide molecule such that the recombinant polynucleotide molecule is introduced into the host cell, wherein the recombinant polynucleotide molecule comprises a nucleic acid encoding the target gene and wherein the nucleic acid is operably linked to a promoter comprising a nucleic acid sequence having at least 70% sequence identity to a sequence selected from SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3 and SEQ ID NO: 4,

whereby expression of the target gene occurs in the host cell.

31. The method of claim 30, wherein the promoter comprises a nucleic acid sequence having at least 90% sequence identity to a nucleic acid sequence selected from the group consisting of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3 and SEQ ID NO: 4.

32. The method of claim 31, wherein the promoter comprises a nucleic acid sequence selected from the group consisting of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3 and SEQ ID NO: 4.

33. The method of any one of claims 30-32, wherein the recombinant polynucleotide molecule is introduced into the host cell by transduction upon contacting the host cell with the recombinant polynucleotide.

34. The method of any one of claims 30-32, wherein the recombinant polynucleotide molecule is introduced into the host cell by transfection upon contacting the host cell with the recombinant polynucleotide.

35. The method of any one of claims 30-34, wherein the host cell is a mammalian cell.

36. The method of any one of claims 30-35, wherein the host cell comprises an *in vitro* neuronal cell, an *ex vivo* neuronal cell, or both.

37. The method of any one of claims 30-35, wherein the host cell comprises a neuronal cell of a subject.

38. The method of claim 37, wherein the neuronal cell resides in a primary motor area, a secondary motor area, a primary somatosensory area, a supplemental somatosensory area, a visual area, a hippocampal formation, striatum, pallidum, thalamus, hypothalamus, a midbrain area, hind brain, an olfactory area or a combination thereof, of the subject.

39. The method of claim 38, wherein the midbrain area comprises a motor-related area, a sensory-related area, or both.

40. The method of claim 37, wherein the neuronal cell resides in cortex, dentate gyrus, striatum, cerebellum, olfactory bulb or a combination thereof, of the subject.

41. The method of any one of claims 36-40, wherein the neuronal cell comprises a neuron, a microglia cell, an astrocyte, or a combination thereof.

42. The method of claim 41, wherein the neuronal cell comprises a neuron.

43. The method of claim 37, where the neuronal cell resides in the spinal cord of the subject.

44. The method of claim 43, where the neuronal cell resides in the dorsal horn of the spinal cord.

45. The method of claim 43 or 44, where the neuronal cell resides in the lumbar spinal cord, thoracic spinal cord, cervical spinal cord or a combination thereof.

46. The method of any one of claims 30-35, wherein the host cell is selected from the group consisting of a bladder cell, a blood cell, a bone cell, an endothelial cell, an epithelial cell, a fat cell, a heart cell, an intestinal cell, a kidney cell, a liver cell, a lung cell, a muscle

cell, a pancreatic cell, a retina cell, a sex cell, a skin cell, a spleen cell, a stem cell, and a combination thereof.

47. A recombinant vector for expression of a target gene in a host cell, comprising:

a promoter comprising a nucleic acid sequence having at least 70% sequence identity to a sequence selected from SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7 and SEQ ID NO: 8;

a cloning site for insertion of a nucleic acid encoding the target gene; and

at least one non-promoter regulatory element required for the expression of the target gene in the host cell.

48. The recombinant vector of claim 47, wherein the vector is a viral vector.

49. The recombinant vector of claim 48, wherein the viral vector is an adeno-associated virus (AAV) vector.

50. The recombinant vector of claim 47, wherein the vector is a non-viral vector.

51. The recombinant vector of any one of claims 47-50, wherein the host cell is a mammalian cell.

52. The recombinant vector of any one of claims 47-51, wherein the host cell comprises an *in vitro* neuronal cell, an *ex vivo* neuronal cell, or both.

53. The recombinant vector of any one of claims 47-51, wherein the host cell comprises a neuronal cell of a subject.

54. The recombinant vector of claim 53, wherein the neuronal cell resides in a primary motor area, a secondary motor area, a primary somatosensory area, a supplemental somatosensory area, a visual area, a hippocampal formation, striatum, pallidum, thalamus, hypothalamus, a midbrain area, hind brain, an olfactory area or a combination thereof, of the subject.

55. The recombinant vector of claim 54, wherein the midbrain area comprises a motor-related area, a sensory-related area, or both.

56. The recombinant vector of claim 53, wherein the neuronal cell resides in cortex, dentate gyrus, striatum, cerebellum, olfactory bulb or a combination thereof, of the subject.

57. The recombinant vector of any one of claims 52-56, wherein the neuronal cell comprises a neuron, a microglia cell, an astrocyte, or a combination thereof.

58. The recombinant vector of claim 57, wherein the neuronal cell comprises a neuron.

59. The recombinant vector of claim 53, where the neuronal cell resides in the spinal cord of the subject.

60. The recombinant vector of claim 59, where the neuronal cell resides in the dorsal horn of the spinal cord.

61. The recombinant vector of claim 59 or 60, where the neuronal cell resides in the lumbar spinal cord, thoracic spinal cord, cervical spinal cord or a combination thereof.

62. The recombinant vector of any one of claims 47-51, wherein the host cell is selected from the group consisting of a bladder cell, a blood cell, a bone cell, an endothelial cell, an epithelial cell, a fat cell, a heart cell, an intestinal cell, a kidney cell, a liver cell, a lung cell, a muscle cell, a pancreatic cell, a retina cell, a sex cell, a skin cell, a spleen cell, a stem cell, and a combination thereof.

63. The recombinant vector of any one of claims 47-62, wherein the promoter comprises a nucleic acid sequence having at least 90% sequence identity to a nucleic acid sequence selected from the group consisting of SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7 and SEQ ID NO: 8.

64. The recombinant polynucleotide molecule of claim 63, wherein the promoter comprises a nucleic acid sequence selected from the group consisting of SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7 and SEQ ID NO: 8.

65. The recombinant vector of any one of claims 47-64, wherein the at least one non-promoter regulatory element comprises an enhancer element.

66. The recombinant vector of any one of claims 47-65, wherein the at least one non-promoter regulatory element comprises an inducer element.

67. The recombinant vector of any one of claims 47-66, further comprising a selectable marker element.

68. A host cell comprising the recombinant vector of any of claims 47-67.

69. A recombinant polynucleotide molecule comprising a nucleic acid encoding a target gene, wherein the nucleic acid is operably linked to a promoter comprising a nucleic acid sequence having at least 70% sequence identity to a sequence selected from the group consisting of SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7 and SEQ ID NO: 8.

70. The recombinant polynucleotide molecule of claim 69, wherein the promoter comprises a nucleic acid sequence having at least 90% sequence identity to a nucleic acid sequence selected from the group consisting of SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7 and SEQ ID NO: 8.

71. The recombinant polynucleotide molecule of claim 70, wherein the promoter comprises a nucleic acid sequence selected from the group consisting of SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7 and SEQ ID NO: 8.

72. The recombinant polynucleotide molecule of any one of claims 69-71, wherein the nucleic acid encoding the target gene comprises more than 4 kilobases.

73. A recombinant polynucleotide molecule comprising a cloning site for insertion of a nucleic acid encoding a target gene, wherein the cloning site is operably linked to a promoter comprising a nucleic acid having at least 70% sequence identity to a sequence selected from the group consisting of SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7 and SEQ ID NO: 8.

74. The recombinant polynucleotide molecule of claim 73, wherein the promoter comprises a nucleic acid sequence having at least 90% sequence identity to a nucleic acid sequence selected from the group consisting of SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7 and SEQ ID NO: 8.

75. The recombinant polynucleotide molecule of claim 74, wherein the promoter comprises a nucleic acid sequence selected from the group consisting of SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7 and SEQ ID NO: 8.

76. A method of expressing a target gene in a host cell, comprising:

contacting the host cell with a recombinant polynucleotide molecule such that the recombinant polynucleotide molecule is introduced into the host cell, wherein the recombinant polynucleotide molecule comprises a nucleic acid encoding the target gene and wherein the nucleic acid is operably linked to a promoter comprising a nucleic acid sequence having at least 70% sequence identity to a sequence selected from SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7 and SEQ ID NO: 8,

whereby expression of the target gene occurs in the host cell.

77. The method of claim 76, wherein the host cell is a mammalian cell.

78. The method of claim 76 or 77, wherein the host cell comprises an *in vitro* neuronal cell, an *ex vivo* neuronal cell, or both.

79. The method of claim 76 or 77, wherein the host cell comprises a neuronal cell of a subject.

80. The method of claim 79, wherein the neuronal cell resides in a primary motor area, a secondary motor area, a primary somatosensory area, a supplemental somatosensory area, a visual area, a hippocampal formation, striatum, pallidum, thalamus, hypothalamus, a midbrain area, hind brain, an olfactory area or a combination thereof, of the subject.

81. The method of claim 80, wherein the midbrain area comprises a motor-related area, a sensory-related area, or both.

82. The method of claim 79, wherein the neuronal cell resides in cortex, dentate gyrus, striatum, cerebellum, olfactory bulb or a combination thereof, of the subject.

83. The method of any one of claims 78-82, wherein the neuronal cell comprises a neuron, a microglia cell, an astrocyte, or a combination thereof.

84. The method of claim 83, wherein the neuronal cell comprises a neuron.

85. The method of claim 79, where the neuronal cell resides in the spinal cord of the subject.

86. The method of claim 85, where the neuronal cell resides in the dorsal horn of the spinal cord.

87. The method of claim 85 or 86, where the neuronal cell resides in the lumbar spinal cord, thoracic spinal cord, cervical spinal cord or a combination thereof.

88. The method of claim 76 or 77, wherein the host cell is selected from the group consisting of a bladder cell, a blood cell, a bone cell, an endothelial cell, an epithelial cell, a fat cell, a heart cell, an intestinal cell, a kidney cell, a liver cell, a lung cell, a muscle cell, a pancreatic cell, a retina cell, a sex cell, a skin cell, a spleen cell, a stem cell, and a combination thereof.

89. The method of any one of claims 76-88, wherein the promoter comprises a nucleic acid sequence having at least 90% sequence identity to a nucleic acid sequence selected from the group consisting of SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7 and SEQ ID NO: 8.

90. The method of claim 89, wherein the promoter comprises a nucleic acid sequence selected from SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7 and SEQ ID NO: 8.

91. The method of any one of claims 76-90, wherein the recombinant polynucleotide molecule is introduced into the host cell by transduction upon contacting the host cell with the recombinant polynucleotide.

92. The method of any one of claims 76-90, wherein the recombinant polynucleotide molecule is introduced into the host cell by transfection upon contacting the host cell with the recombinant polynucleotide.

93. A recombinant vector for expression of a target gene in a host cell, comprising:

a promoter comprising a nucleic acid sequence having at least 70% sequence identity to SEQ ID NO: 9;

a cloning site for insertion of a nucleic acid encoding the target gene; and

at least one non-promoter regulatory element required for the expression of the target gene in the host cell.

94. The recombinant vector of claim 93, wherein the vector is a viral vector.

95. The recombinant vector of claim 94, wherein the viral vector is an adeno-associated virus (AAV) vector.

96. The recombinant vector of claim 93, wherein the vector is a non-viral vector.

97. The recombinant vector of any of claims 93-96, wherein the host cell is a mammalian cell.

98. The recombinant vector of any one of claims 93-97, wherein the host cell comprises an *in vitro* neuronal cell, an *ex vivo* neuronal cell, or both.

99. The recombinant vector of any one of claims 93-97, wherein the host cell comprises a neuronal cell of a subject.

100. The recombinant vector of claim 99, wherein the neuronal cell resides in a primary motor area, a secondary motor area, a primary somatosensory area, a supplemental somatosensory area, a visual area, a hippocampal formation, striatum, pallidum, thalamus, hypothalamus, a midbrain area, hind brain, an olfactory area or a combination thereof, of the subject.

101. The recombinant vector of claim 100, wherein the midbrain area comprises a motor-related area, a sensory-related area, or both.

102. The recombinant vector of claim 99, wherein the neuronal cell resides in cortex, dentate gyrus, striatum, cerebellum, olfactory bulb or a combination thereof, of the subject.

103. The recombinant vector of any one of claims 98-102, wherein the neuronal cell comprises a neuron, a microglia cell, an astrocyte, or a combination thereof.

104. The recombinant vector of claim 103, wherein the neuronal cell comprises a neuron.

105. The recombinant vector of claim 99, where the neuronal cell resides in the spinal cord of the subject.

106. The recombinant vector of claim 105, where the neuronal cell resides in the dorsal horn of the spinal cord.

107. The recombinant vector of claim 105 or 106, where the neuronal cell resides in the lumbar spinal cord, thoracic spinal cord, cervical spinal cord or a combination thereof.

108. The recombinant vector of any one of claims 93-97, wherein the host cell is selected from the group consisting of a bladder cell, a blood cell, a bone cell, an endothelial cell, an epithelial cell, a fat cell, a heart cell, an intestinal cell, a kidney cell, a liver cell, a lung cell, a muscle cell, a pancreatic cell, a retina cell, a sex cell, a skin cell, a spleen cell, a stem cell, and a combination thereof.

109. The recombinant vector of any one of claims 93-108, wherein the promoter comprises a nucleic acid sequence having at least 90% sequence identity to the nucleic acid sequence of SEQ ID NO: 9.

110. The recombinant vector of claim 109, wherein the promoter comprises SEQ ID NO: 9.

111. The recombinant vector of any one of claims 93-110, wherein the promoter further comprises a nucleic acid sequence of SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 12, or a combination thereof.

112. The recombinant vector of any one of claims 93-108, wherein the promoter comprises a nucleic acid sequence having at least 90% sequence identity to a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 13-58.

113. The recombinant vector of claim 112, wherein the promoter comprises a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 13-58.

114. The recombinant vector of any one of claims 93-113, wherein the at least one non-promoter regulatory element comprises an enhancer element.

115. The recombinant vector of any one of claims 93-114, wherein the at least one non-promoter regulatory element comprises an inducer element.

116. The recombinant vector of any one of claims 93-115, further comprising a selectable marker element.

117. A host cell comprising the recombinant vector of any of claims 93-116.

118. A recombinant polynucleotide molecule comprising a nucleic acid encoding a target gene, wherein the nucleic acid is operably linked to a promoter comprising a nucleic acid sequence having at least 70% sequence identity to SEQ ID NO: 9.

119. The recombinant polynucleotide molecule of claim 118, wherein the promoter comprises a nucleic acid sequence having at least 90% sequence identity to the nucleic acid sequence of SEQ ID NO: 9.

120. The recombinant polynucleotide molecule of claim 119, wherein the promoter comprises SEQ ID NO: 9.

121. The recombinant polynucleotide molecule of any one of claims 118-120, wherein the promoter further comprises the nucleic acid sequence of SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 12, or a combination thereof.

122. The recombinant polynucleotide molecule of claim 118, wherein the promoter comprises a nucleic acid sequence having at least 90% sequence identity to a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 13-58.

123. The recombinant polynucleotide molecule of any one of claims 118-122, wherein the nucleic acid encoding the target gene comprises more than 4 kilobases.

124. A recombinant polynucleotide molecule comprising a cloning site for insertion of a nucleic acid encoding a target gene, wherein the cloning site is operably linked to a promoter comprising a nucleic acid sequence having at least 70% sequence identity to SEQ ID NO: 9.

125. The recombinant polynucleotide molecule of claim 124, wherein the promoter comprises a nucleic acid sequence having at least 90% sequence identity to the nucleic acid sequence of SEQ ID NO: 9.

126. The recombinant polynucleotide molecule of claim 125, wherein the promoter comprises SEQ ID NO: 9.

127. The recombinant polynucleotide molecule of any one of claims 124-126, wherein the promoter further comprises the nucleic acid sequence of SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 12, or a combination thereof.

128. The recombinant polynucleotide molecule of claim 124, wherein the promoter comprises a nucleic acid sequence having at least 90% sequence identity to a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 13-58.

129. The recombinant polynucleotide molecule of claim 128, wherein the promoter comprises a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 13-58.

130. A method of expressing a target gene in a host cell, comprising:

contacting the host cell with a recombinant polynucleotide molecule such that the recombinant polynucleotide molecule is introduced into the host cell, wherein the recombinant polynucleotide molecule comprises a nucleic acid encoding the target gene

and wherein the nucleic acid is operably linked to a promoter comprising a nucleic acid sequence having at least 70% sequence identity to SEQ ID NO: 9,

whereby expression of the target gene occurs in the host cell.

131. The method of claim 130, wherein the promoter comprises a nucleic acid sequence having at least 90% sequence identity to the nucleic acid sequence of SEQ ID NO: 9.

132. The method of claim 131, wherein the promoter comprises SEQ ID NO: 9.

133. The method of any one of claims 130-132, wherein the promoter further comprises the nucleic acid sequence of SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 12, or a combination thereof.

134. The method of claim 130, wherein the promoter comprises a nucleic acid sequence having at least 90% sequence identity to a nucleic acid sequence selected from SEQ ID NOs: 13-58.

135. The method of claim 134, wherein the promoter comprises a nucleic acid sequence selected from SEQ ID NOs: 13-58.

136. The method of any one of claims 130-135, wherein the recombinant polynucleotide molecule is introduced into the host cell by transduction upon contacting the host cell with the recombinant polynucleotide.

137. The method of any one of claims 130-135, wherein the recombinant polynucleotide molecule is introduced into the host cell by transfection upon contacting the host cell with the recombinant polynucleotide.

138. The method of any one of claims 130-137, wherein the host cell is a mammalian cell.

139. The method of any one of claims 130-138, wherein the host cell comprises an *in vitro* neuronal cell, an *ex vivo* neuronal cell, or both.

140. The method of any one of claims 130-138, wherein the host cell comprises a neuronal cell of a subject.

141. The method of claim 140, wherein the neuronal cell resides in a primary motor area, a secondary motor area, a primary somatosensory area, a supplemental somatosensory area, a visual area, a hippocampal formation, striatum, pallidum, thalamus, hypothalamus, a midbrain area, hind brain, an olfactory area or a combination thereof, of the subject.

142. The method of claim 141, wherein the midbrain area comprises a motor-related area, a sensory-related area, or both.

143. The method of claim 140, wherein the neuronal cell resides in cortex, dentate gyrus, striatum, cerebellum, olfactory bulb or a combination thereof, of the subject.

144. The method of any one of claims 140-143, wherein the neuronal cell comprises a neuron, a microglia cell, an astrocyte, or a combination thereof.

145. The method of claim 144, wherein the neuronal cell comprises a neuron.

146. The method of claim 140, where the neuronal cell resides in the spinal cord of the subject.

147. The method of claim 146, where the neuronal cell resides in the dorsal horn of the spinal cord.

148. The method of claim 146 or 147, where the neuronal cell resides in the lumbar spinal cord, thoracic spinal cord, cervical spinal cord or a combination thereof.

149. The method of any one of claims 130-138, wherein the host cell is selected from the group consisting of a bladder cell, a blood cell, a bone cell, an endothelial cell, an epithelial cell, a fat cell, a heart cell, an intestinal cell, a kidney cell, a liver cell, a lung cell, a muscle cell, a pancreatic cell, a retina cell, a sex cell, a skin cell, a spleen cell, a stem cell, and a combination thereof.

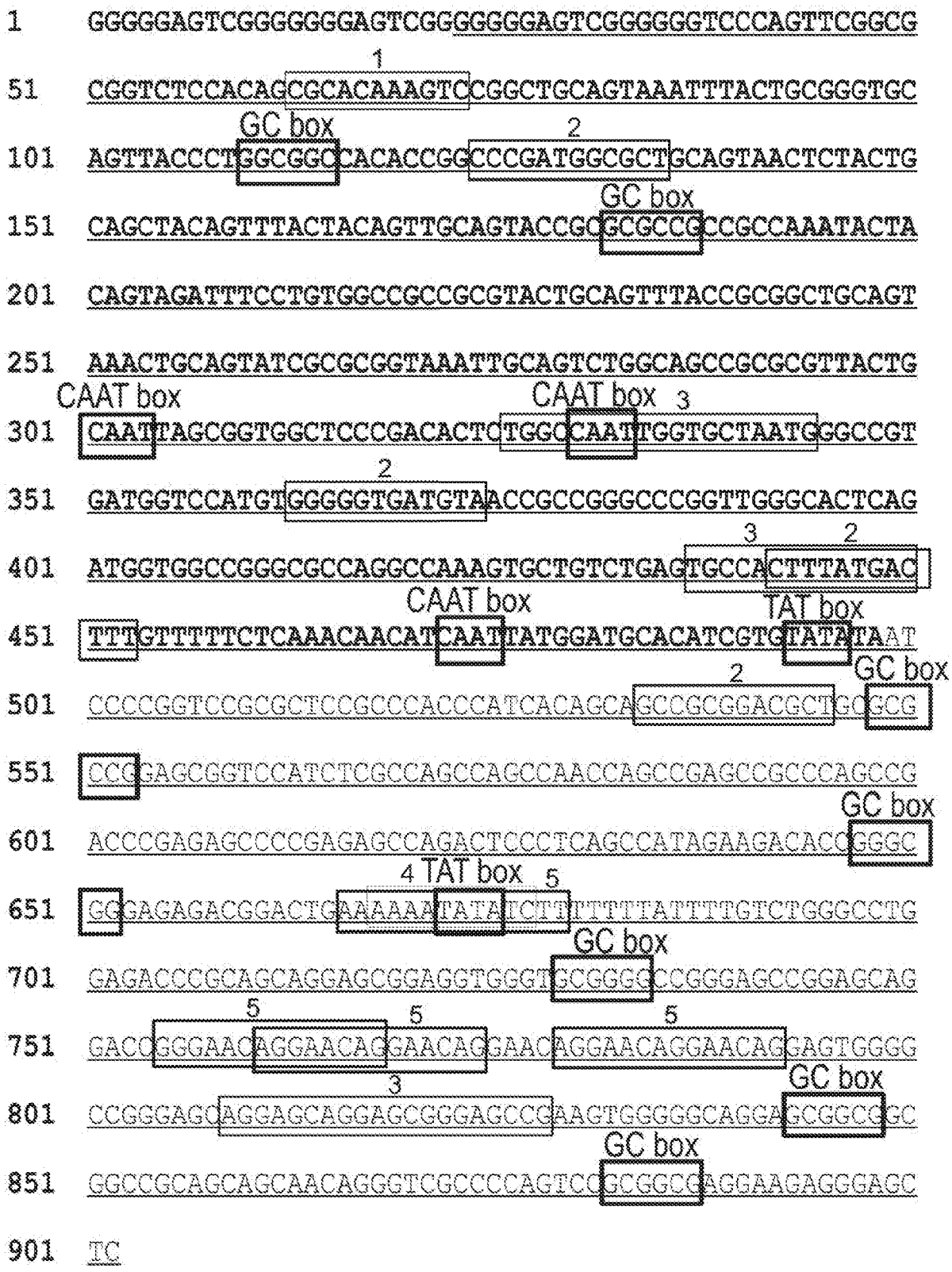

1 GGGGGAGTCGGGGGGGAGTCGGGGGGGAGTCGGGGGGGTCCCAGTTCGGCG
51 CGGTCTCCACAGCGCACAAAGTCCGGCTGCAGTAAATTTACTGCGGGTGC
101 AGTTACCCTGGCGGCCACACCGGCCCGATGGCGCTGCAGTAACTCTACTG
151 CAGCTACAGTTTACTACAGTTGCAGTACCGCGCGCCGCCGCCAAATACTA
201 CAGTAGATTTCCTGTGGCCGCCGCGTACTGCAGTTTACCGCGGCTGCAGT
251 AAACTGCAGTATCGCGCGGTAAATTGCAGTCTGGCAGCCGCGCGTTACTG
301 CAATTAGCGGTGGCTCCCGACACTCTGGCCAATTGGTGCTAATGGGCCGT
351 GATGGTCCATGTGGGGGGTGATGTAACCGCCGGGCCCGGTTGGGCACTCAG
401 ATGGTGGCCGGGCGCCAGGCCAAAGTGCTGTCTGAGTGCCACTTTATGAC
451 TTTGTTTTTCTCAAACAACATCAATTATGGATGCACATCGTGTATATAAT
501 CCCCGGTCCGCGCTCCGCCCACCCATCACAGCAGCCGCGGACGCTGCGCG
551 CCGGAGCGGTCCATCTCGCCAGCCAGCCAACCAGCCGAGCCGCCCAGCCG
601 ACCCGAGAGCCCCGAGAGCCAGACTCCCTCAGCCATAGAAGACACCGGGC
651 GCGAGAGACGGACTGAAAAAATATATCTTTTTTTATTTTGTCTGGGCCTG
701 GAGACCCGCAGCAGGAGCGGAGGTGGGTGCGGGCCCGGGAGCCGGAGCAG
751 GACCGGGAACAGGAACAGGAACAGGAACAGGAACAGGAACAGGAGTGGGG
801 CCGGGAGCAGGAGCAGGAGCGGGAGCCCAAGTGGGGGCAGGAGCGGCGGC
851 GGCCGCAGCAGCAACAGGGTCGCCCCAGTCCGCGGCGAGGAAGAGGGAGC
901 TC
CAAT box
CAAT box
GC box
GC box
GC box
CAAT box
TAT box
GC box
GC box
GC box
TAT box
GC box
GC box
GC box

FIG. 1A

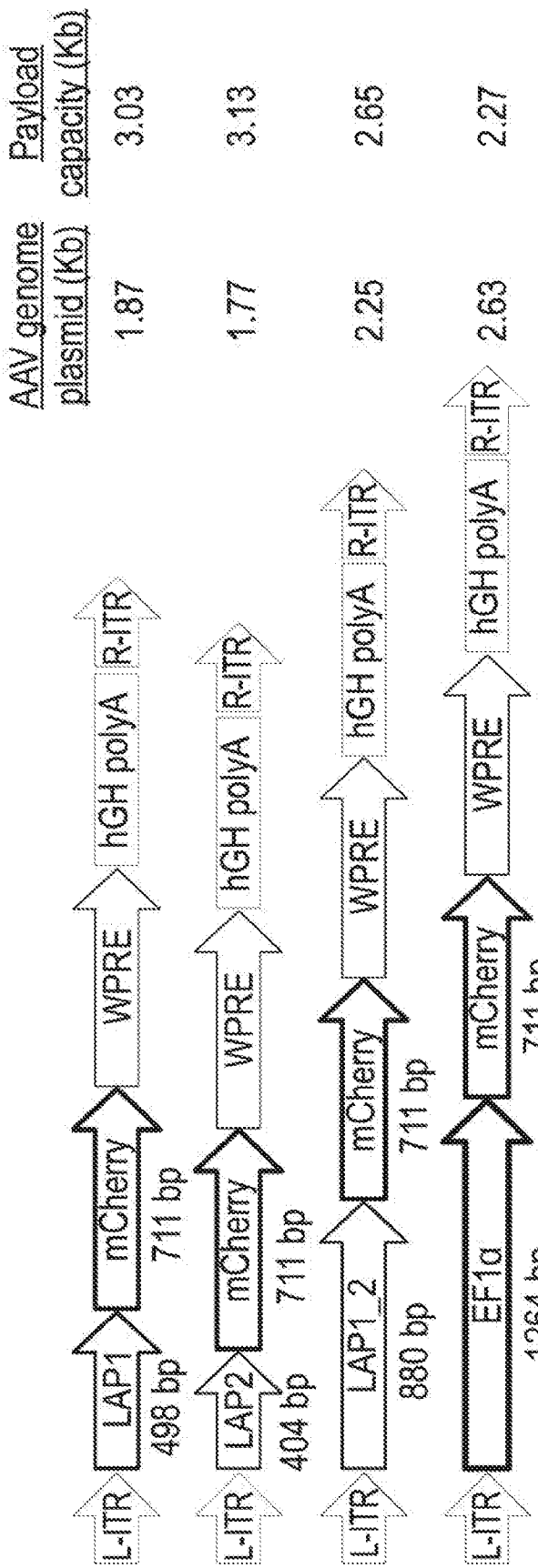

AAV genome plasmid (Kb)
Payload capacity (Kb)
1.87
3.03
1.77
3.13
2.25
2.65
2.63
2.27
L-ITR
LAP1
498 bp
mCherry
711 bp
WPRE
hGH polyA
R-ITR
L-ITR
LAP2
404 bp
mCherry
711 bp
WPRE
hGH polyA
R-ITR
L-ITR
LAP1_2
880 bp
mCherry
711 bp
WPRE
hGH polyA
R-ITR
L-ITR
EF1α
1264 bp
mCherry
711 bp
WPRE
hGH polyA
R-ITR

FIG. 1B

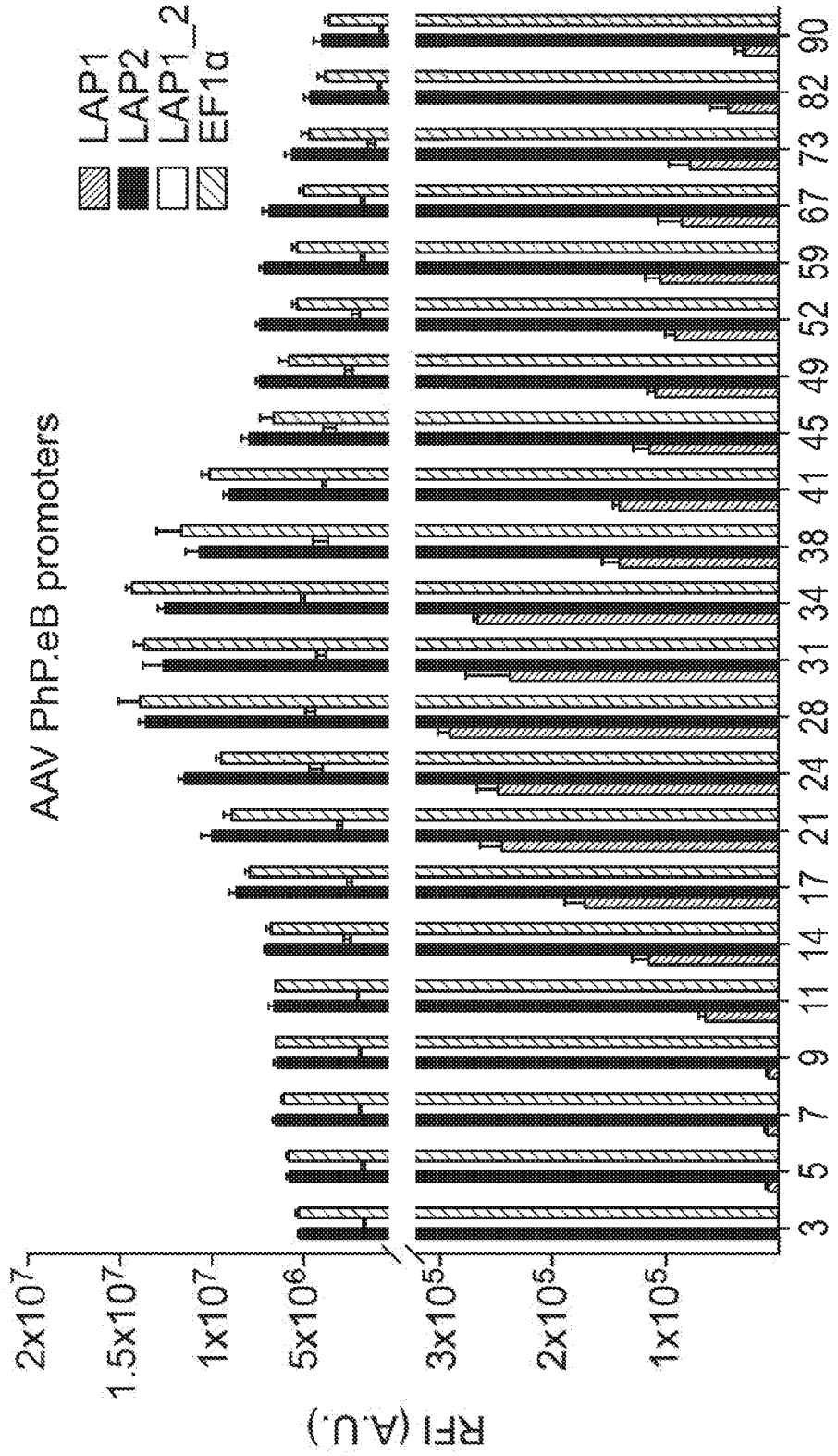

AAV PhP.eB promoters
LAP1
LAP2
LAP1_2
EF1a
RFI (A.U.)
2x10^7
1.5x10^7
1x10^7
5x10^6
3x10^5
2x10^5
1x10^5
Days post infection (dpi)
3 5 7 9 11 14 17 21 24 28 31 34 38 41 45 49 52 59 67 73 82 90

FIG. 1C

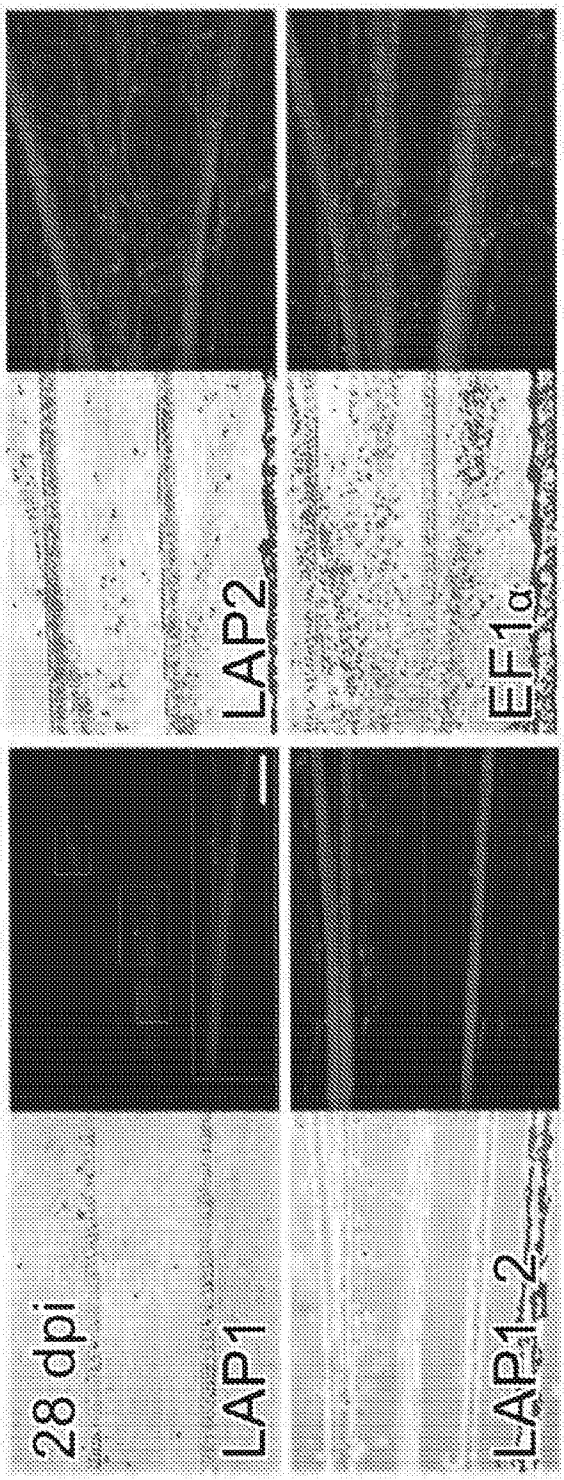


FIG. 1D

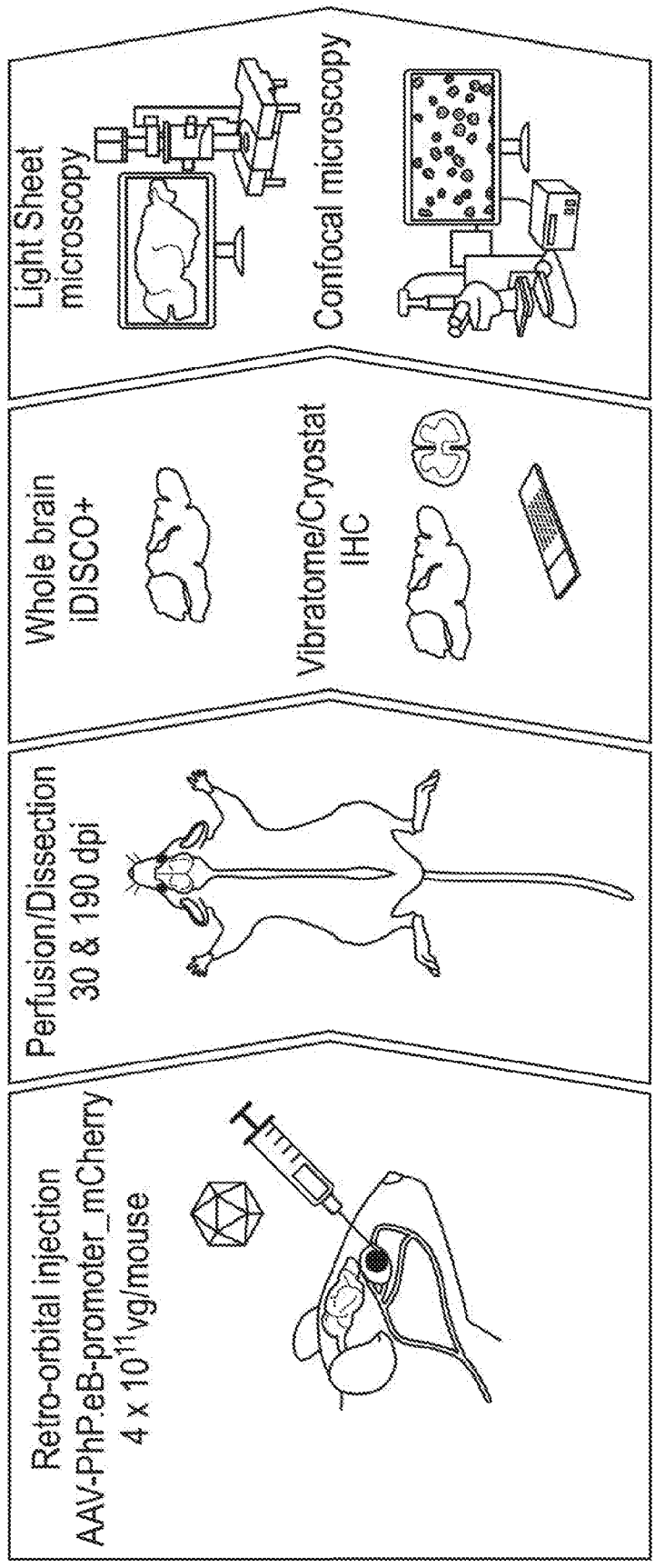

Retro-orbital injection
AAV-PhP.eB-promoter_mCherry
4 x 10^11 vg/mouse
Perfusion/Dissection
30 & 190 dpi
Whole brain
iDISCO+
Vibratome/Cryostat
IHC
Light Sheet
microscopy
Confocal microscopy

FIG. 2A

FIG. 2D

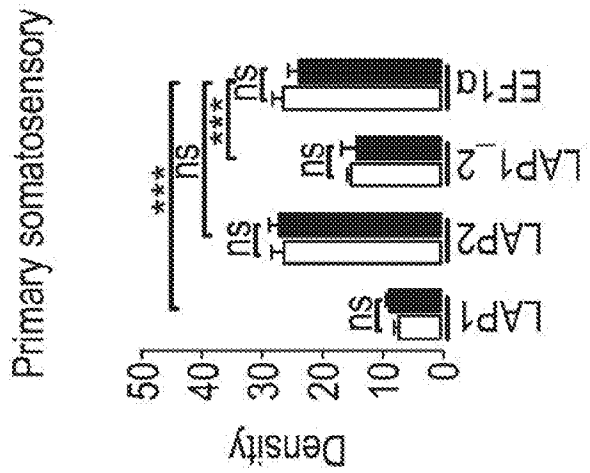


FIG. 2C

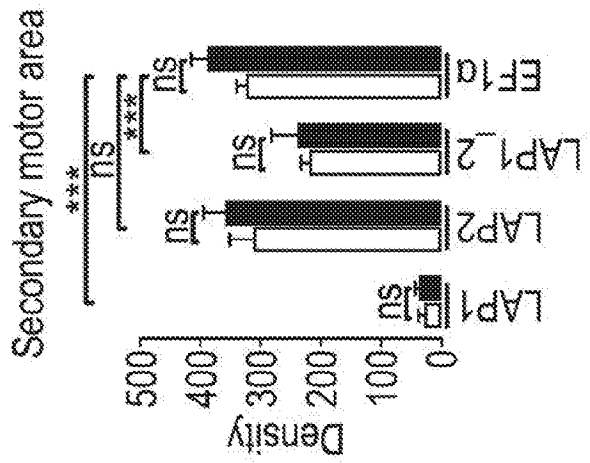


FIG. 2B

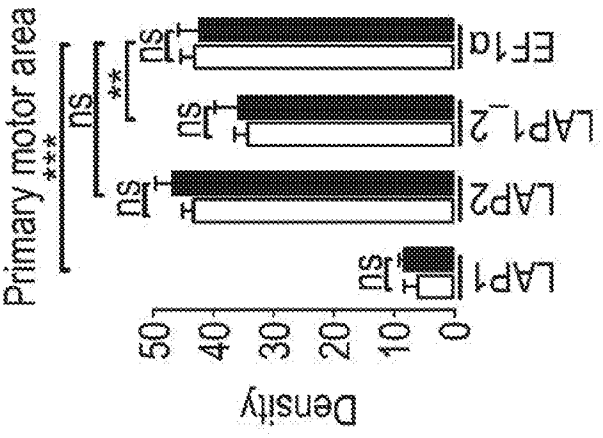


FIG. 2G

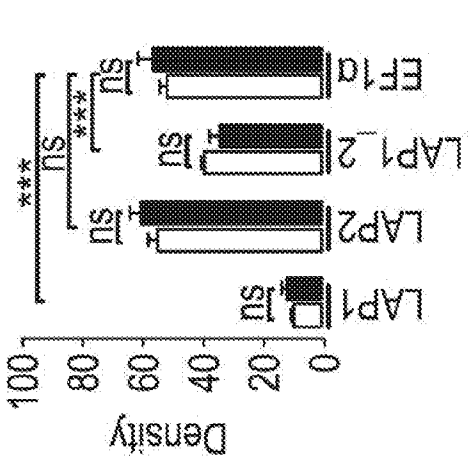


FIG. 2F

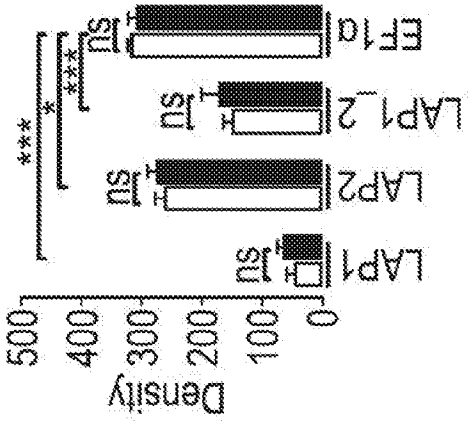


FIG. 2E

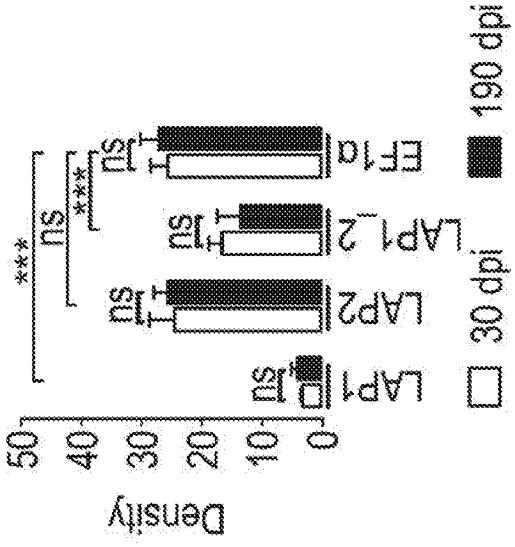


FIG. 2J

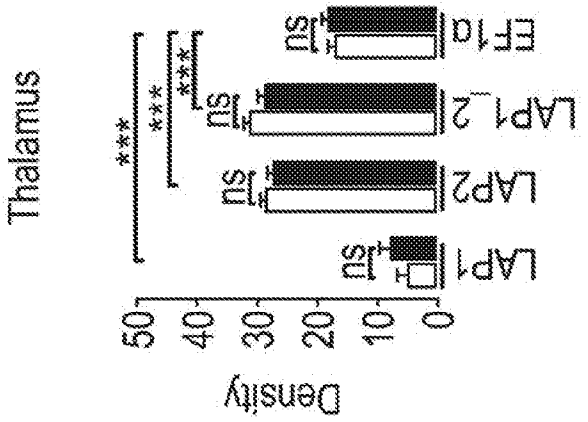


FIG. 2I

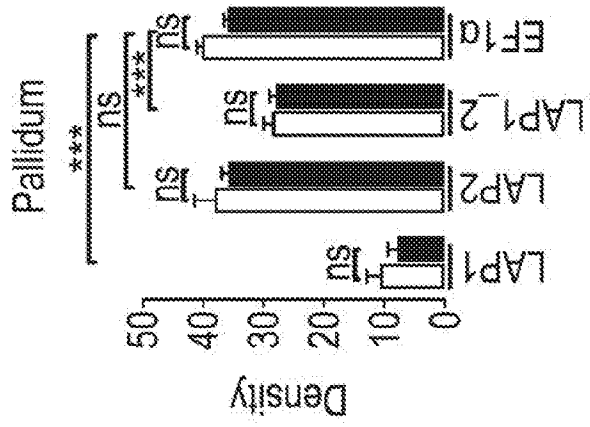


FIG. 2H

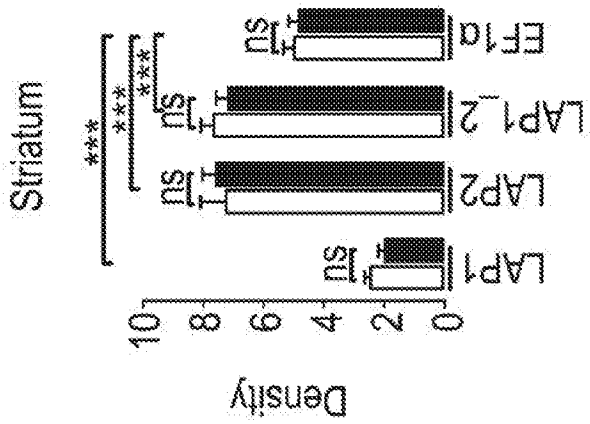


FIG. 2M

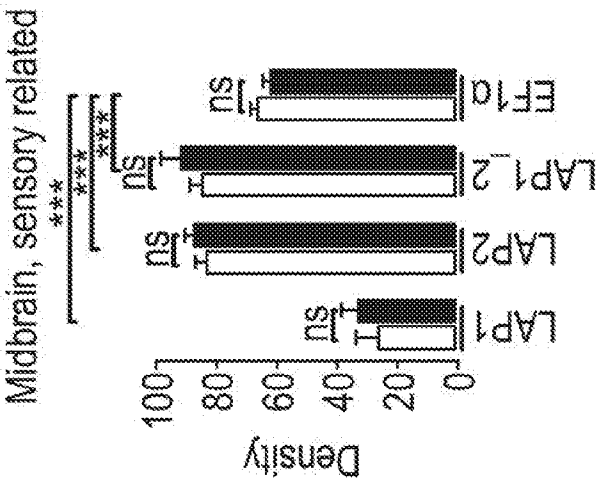


FIG. 2L

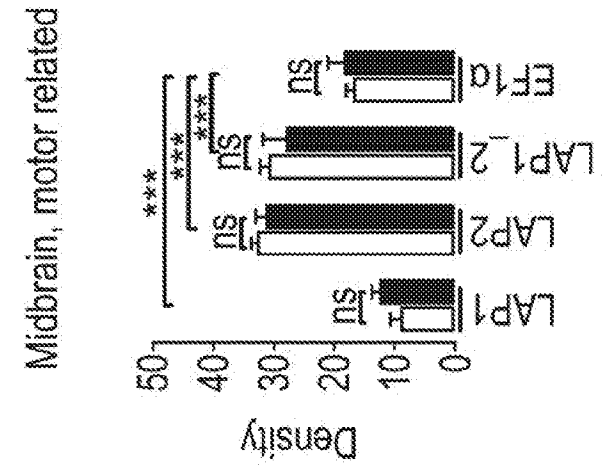


FIG. 2K



FIG. 2N

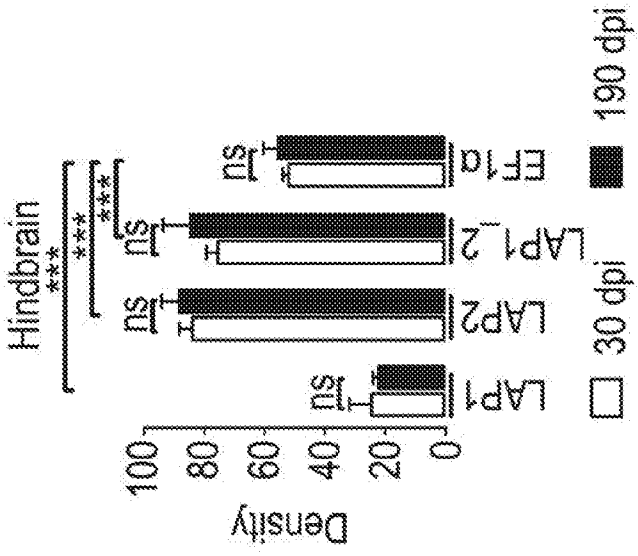


FIG. 2O

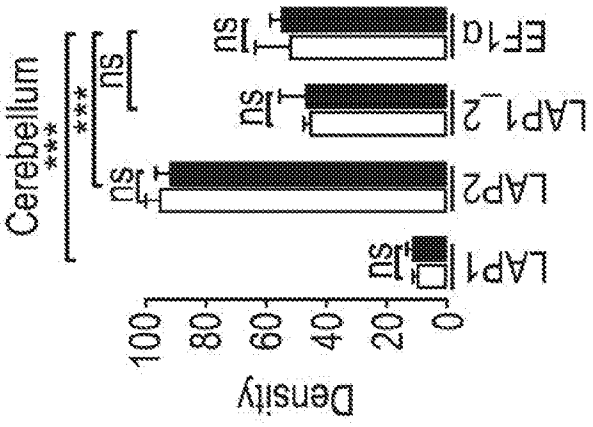
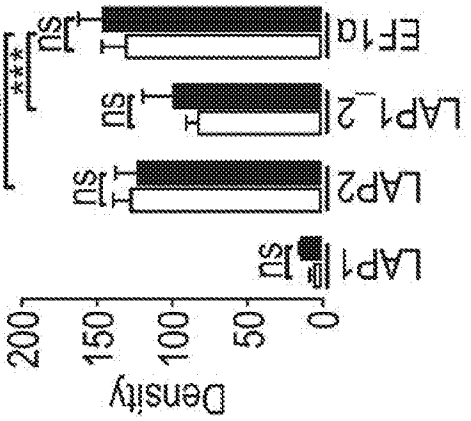
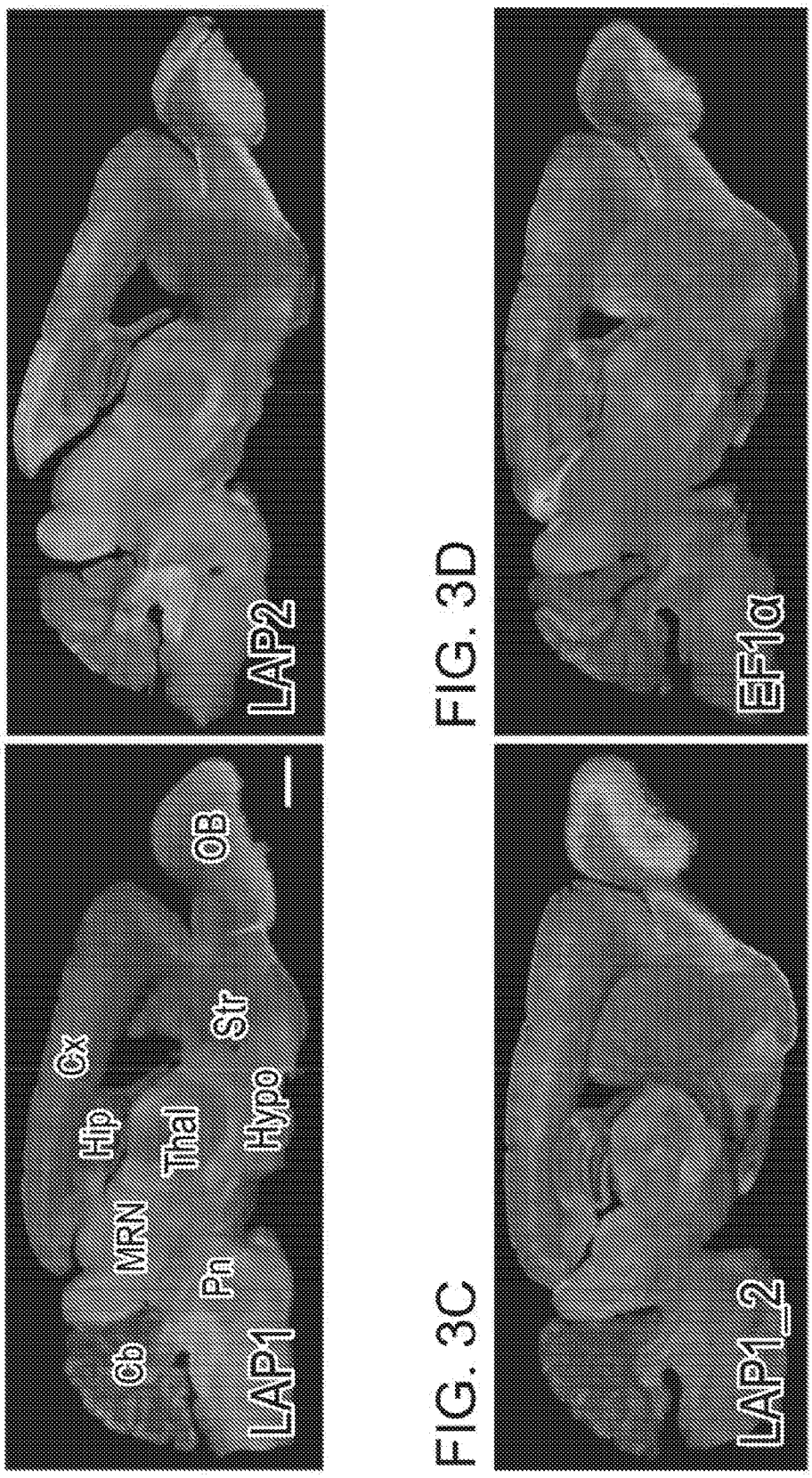


FIG. 2P




FIG. 3A
LAP1
Cb
MRN
Pn
Hip
Thal
Hypo
Str
OB
Cx
FIG. 3B
LAP2
FIG. 3C
LAP1_2
FIG. 3D
EF1α

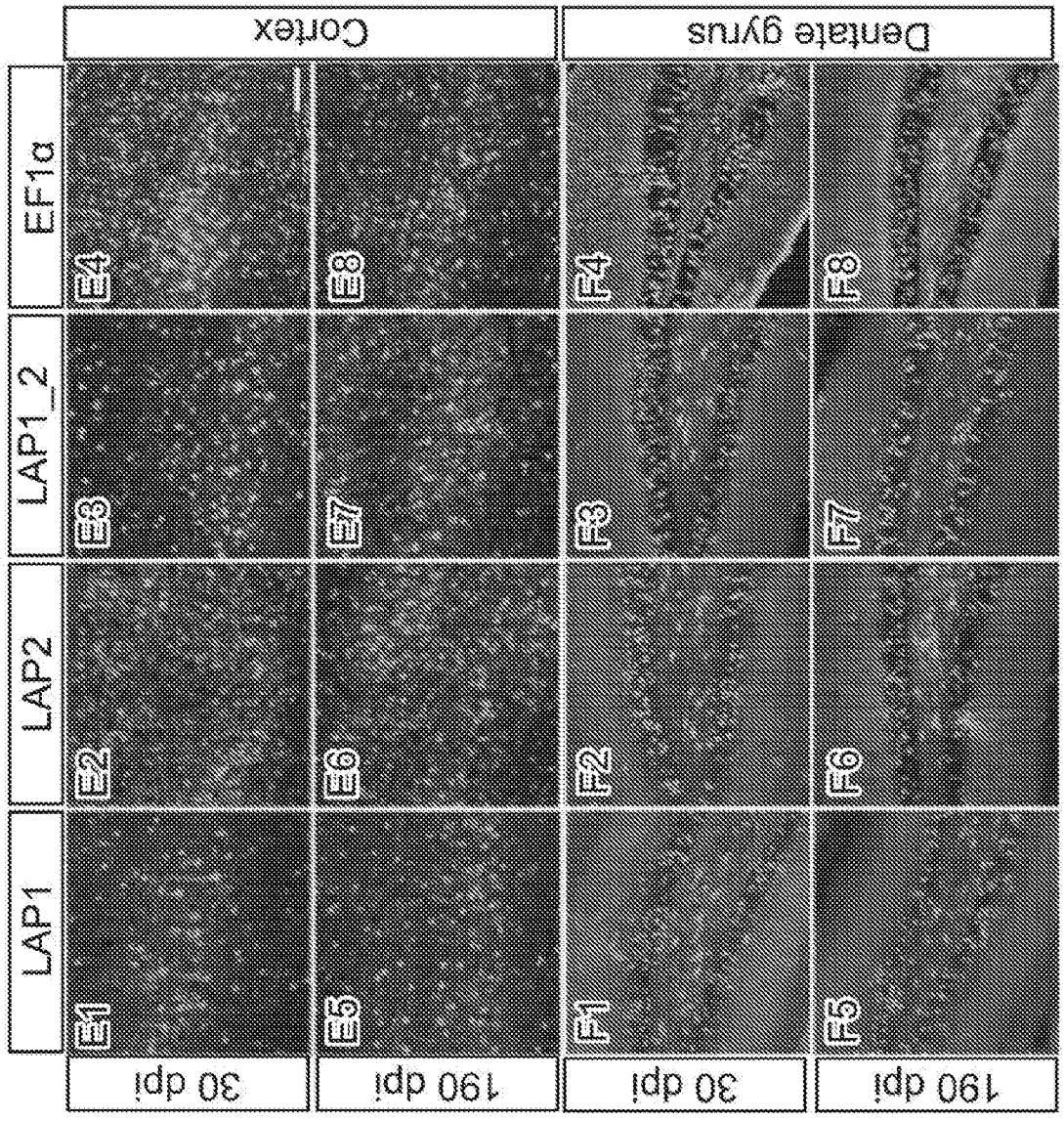

Cortex
Dentate gyrus
EF1α
LAP1_2
LAP2
LAP1
E4
E8
E3
E7
E2
E6
E1
E5
F4
F8
F3
F7
F2
F6
F1
F5
30 dpi
190 dpi
30 dpi
190 dpi

FIG. 3E

FIG. 3F

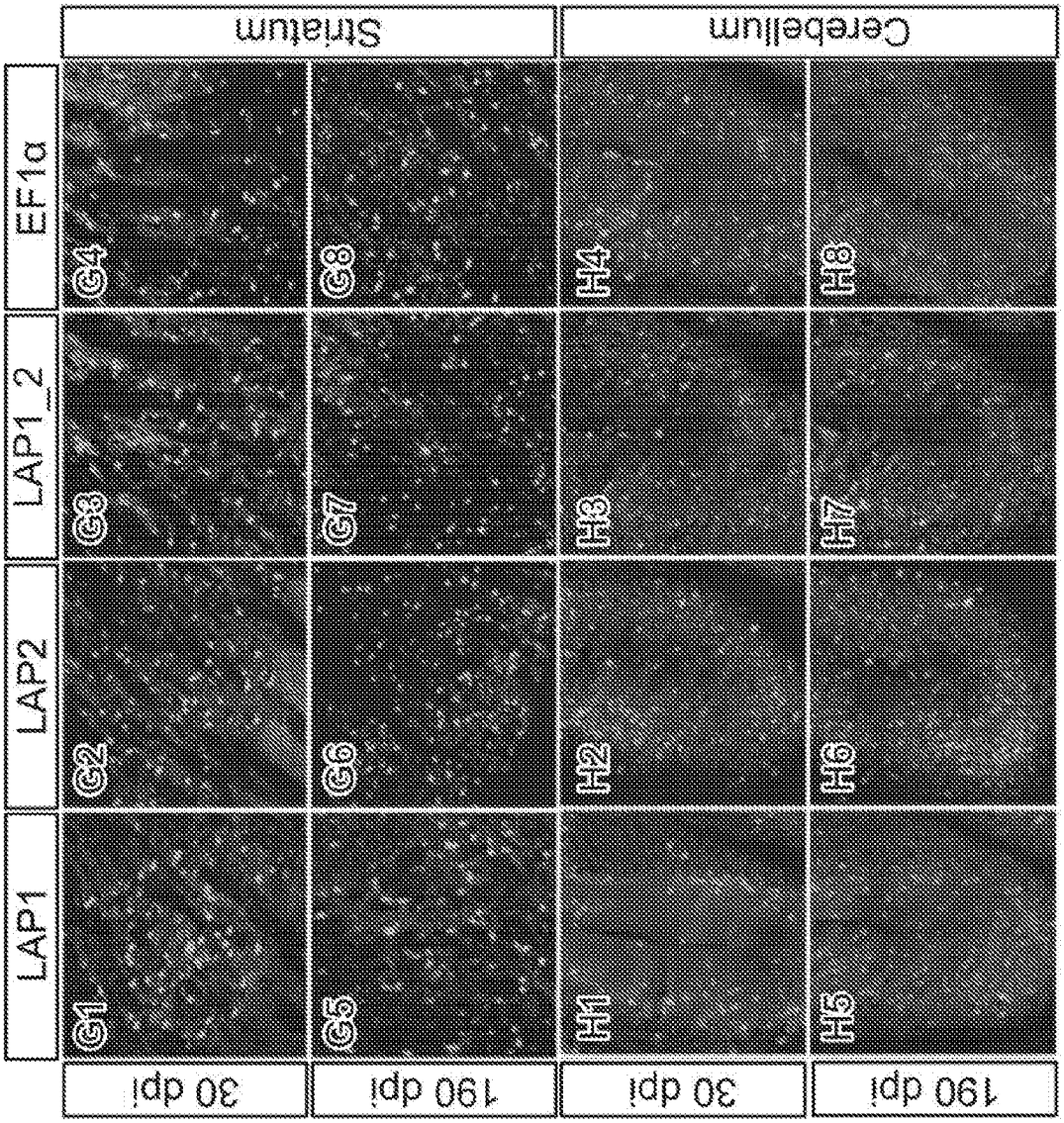

Striatum
Cerebellum
LAP1
LAP2
LAP1_2
EF1α
30 dpi
190 dpi
30 dpi
190 dpi
G1
G2
G3
G4
G5
G6
G7
G8
H1
H2
H3
H4
H5
H6
H7
H8

FIG. 3G

FIG. 3H

FIG. 3I1

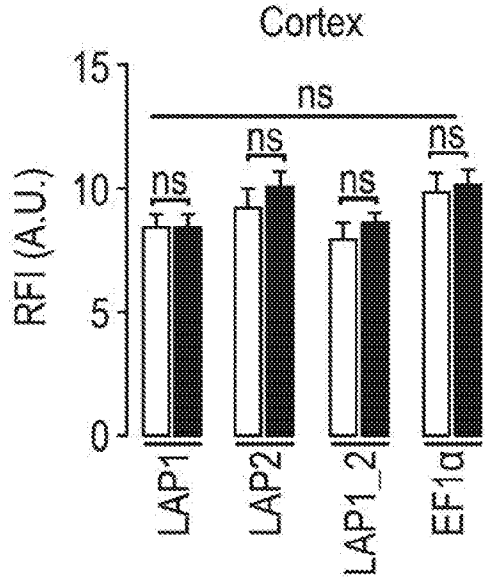


FIG. 3I2

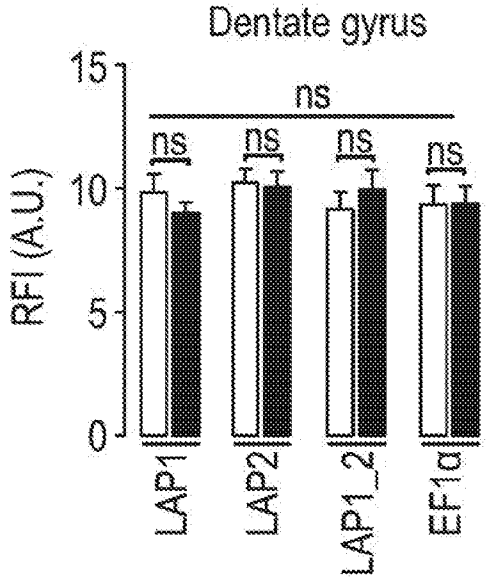


FIG. 3I3

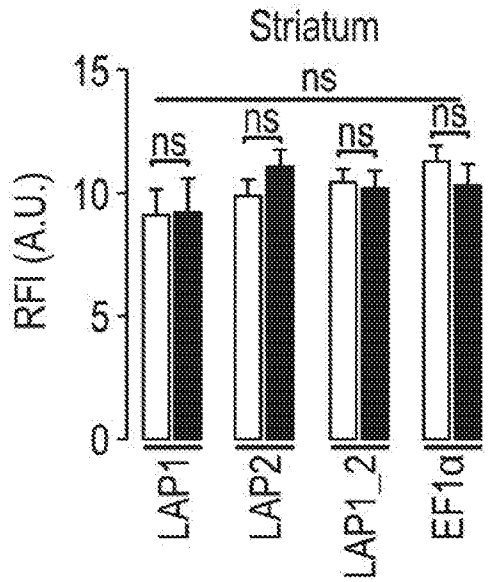


FIG. 3I4

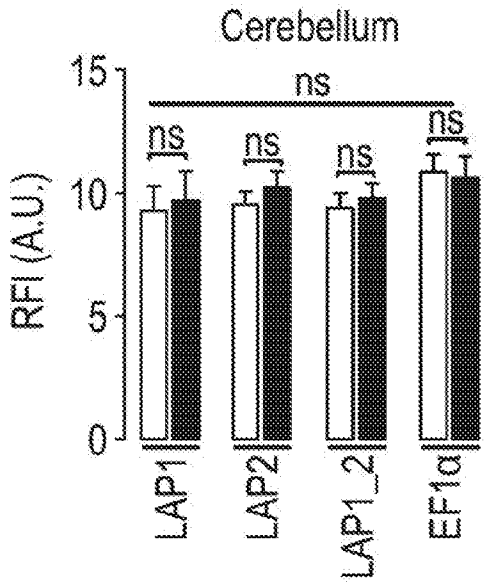




FIG. 3J1

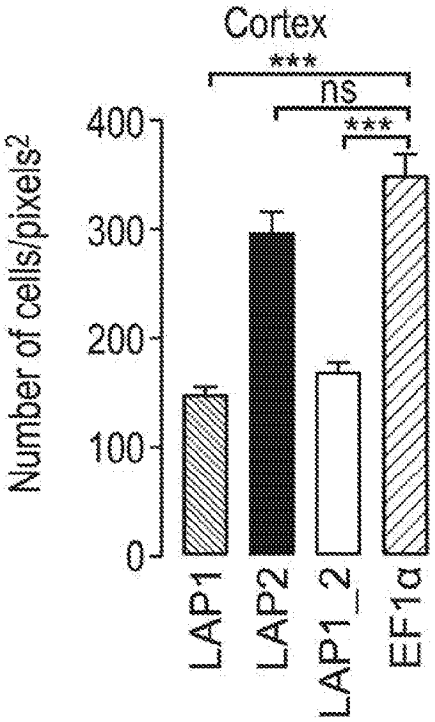


FIG. 3J2

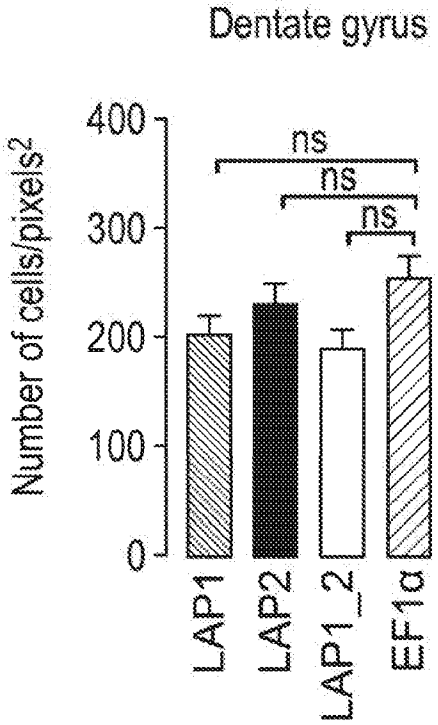


FIG. 3J3

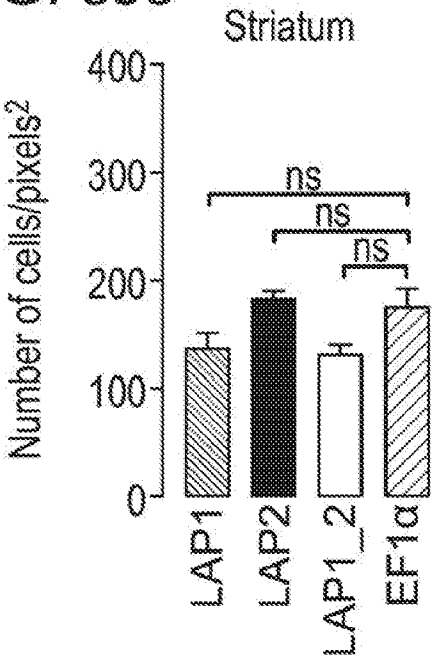
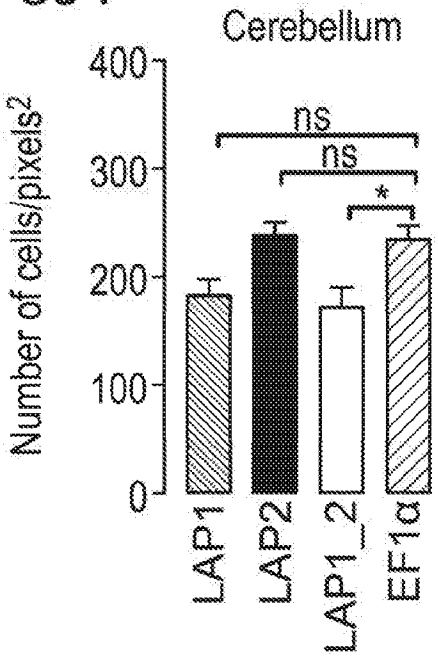


FIG. 3J4



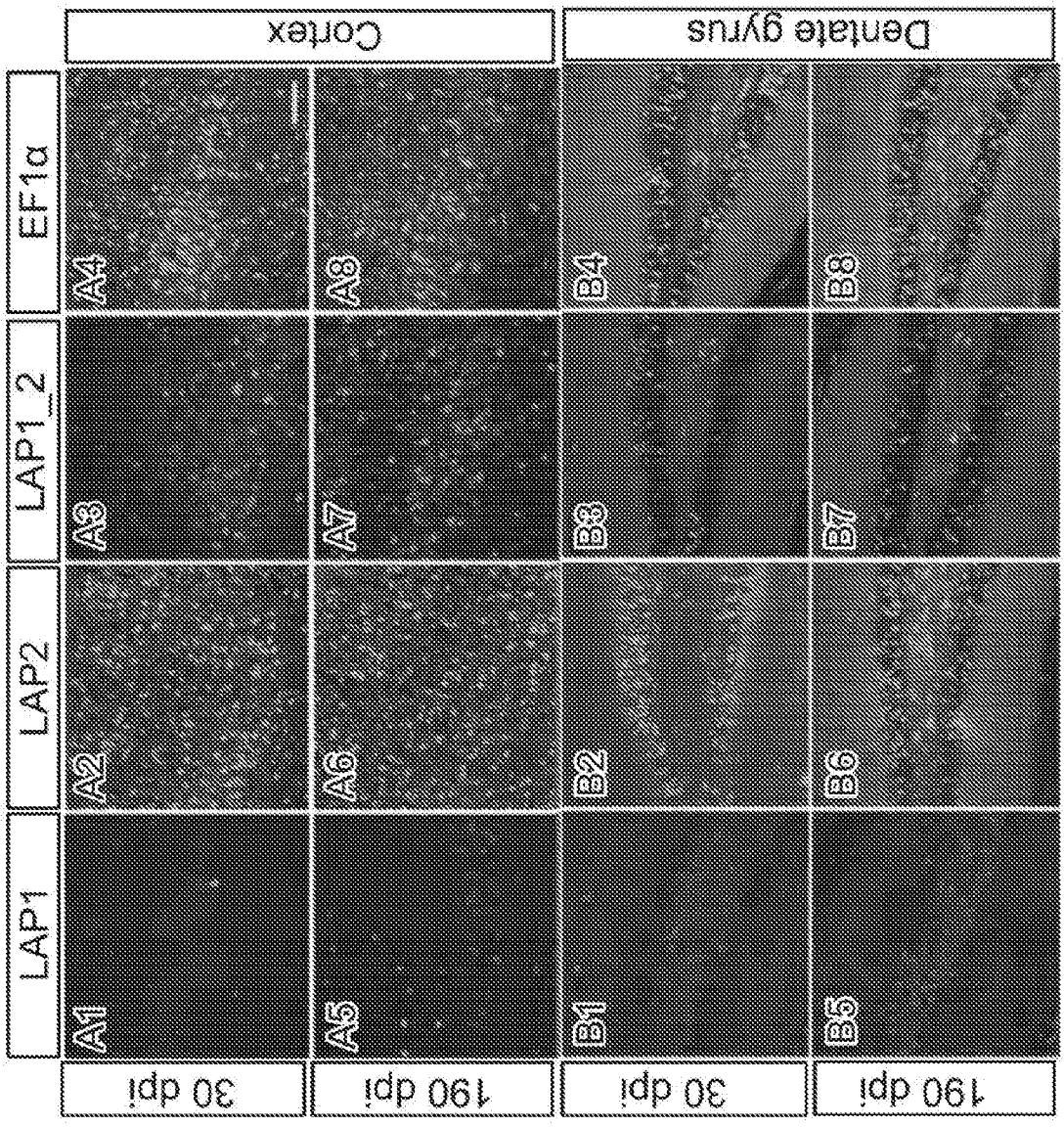

Cortex
Dentate gyrus
EF1α
LAP1_2
LAP2
LAP1
A4
A8
A3
A7
A2
A6
A1
A5
B4
B8
B3
B7
B2
B6
B1
B5
30 dpi
190 dpi
30 dpi
190 dpi

FIG. 4A

FIG. 4B

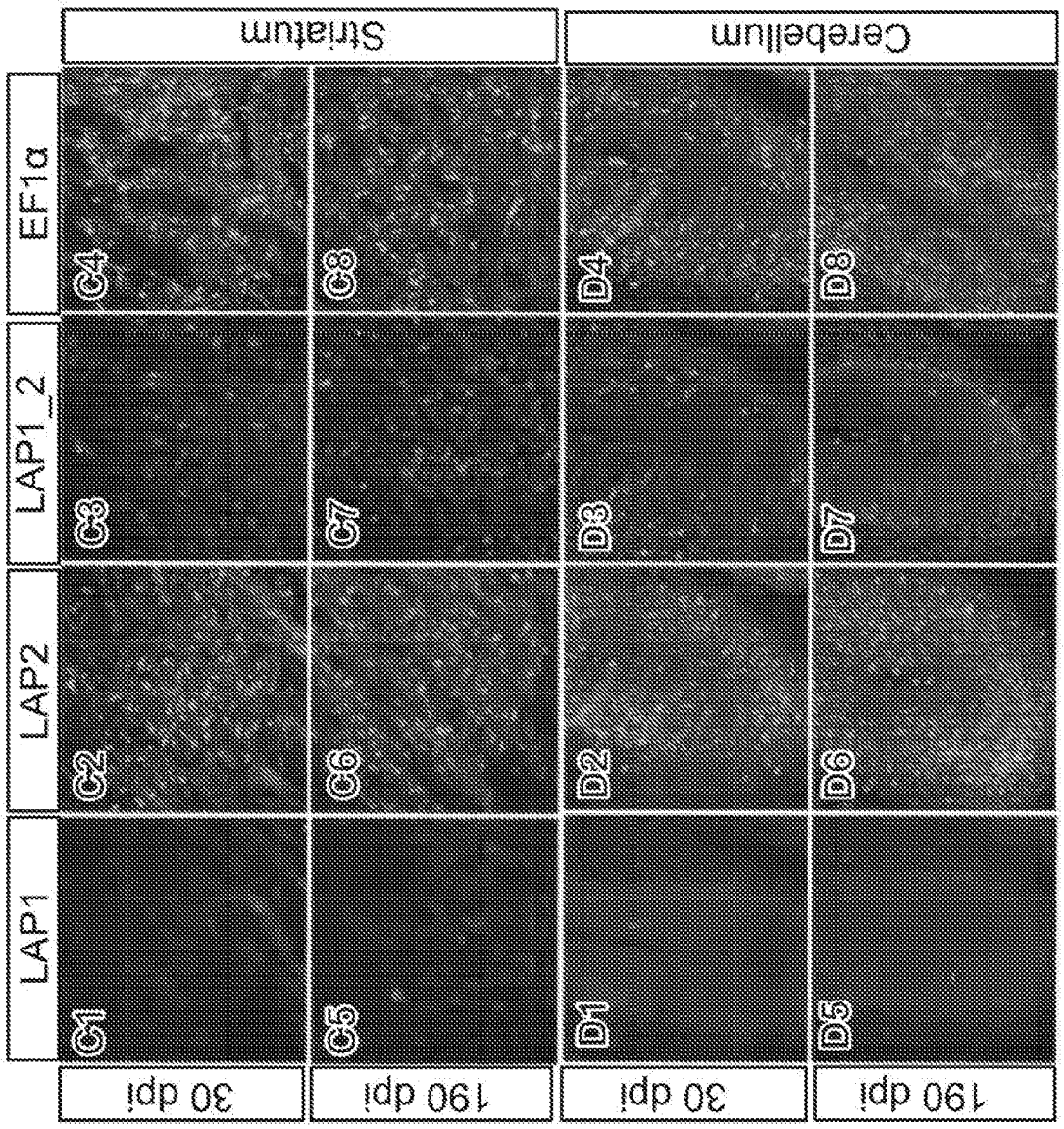

Striatum
Cerebellum
EF1α
LAP1_2
LAP2
LAP1
C4
C8
D4
D8
C3
C7
D3
D7
C2
C6
D2
D6
C1
C5
D1
D5
30 dpi
190 dpi
30 dpi
190 dpi

FIG. 4C

FIG. 4D

FIG. 4E1

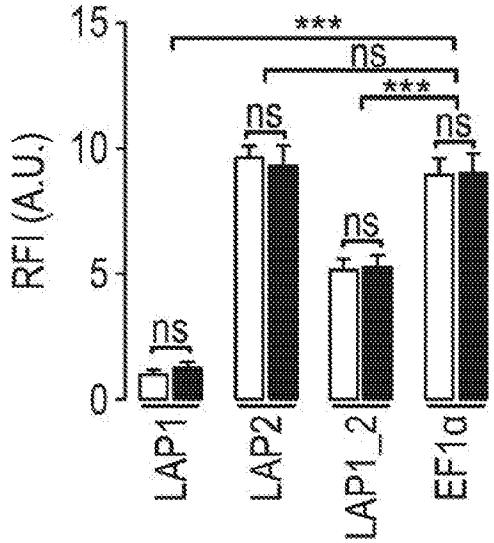


FIG. 4E3

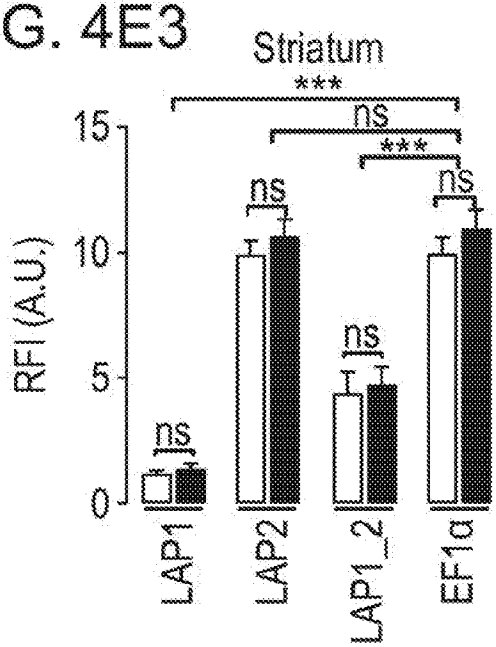


FIG. 4E2

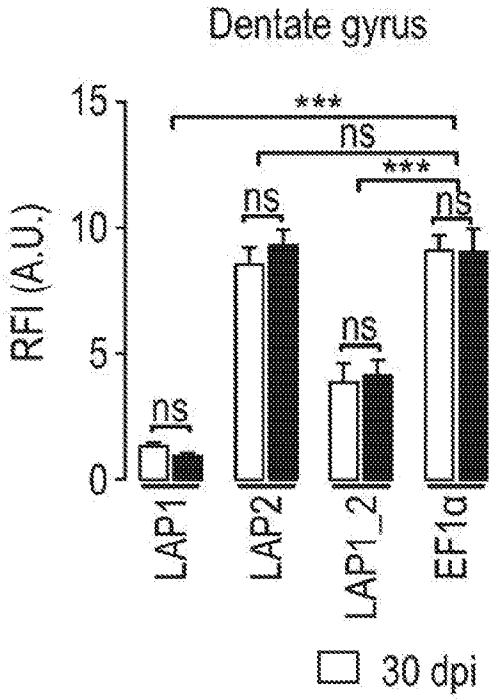
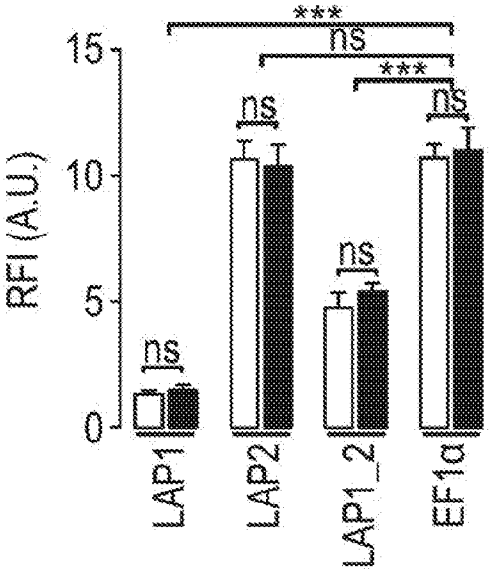


FIG. 4E4



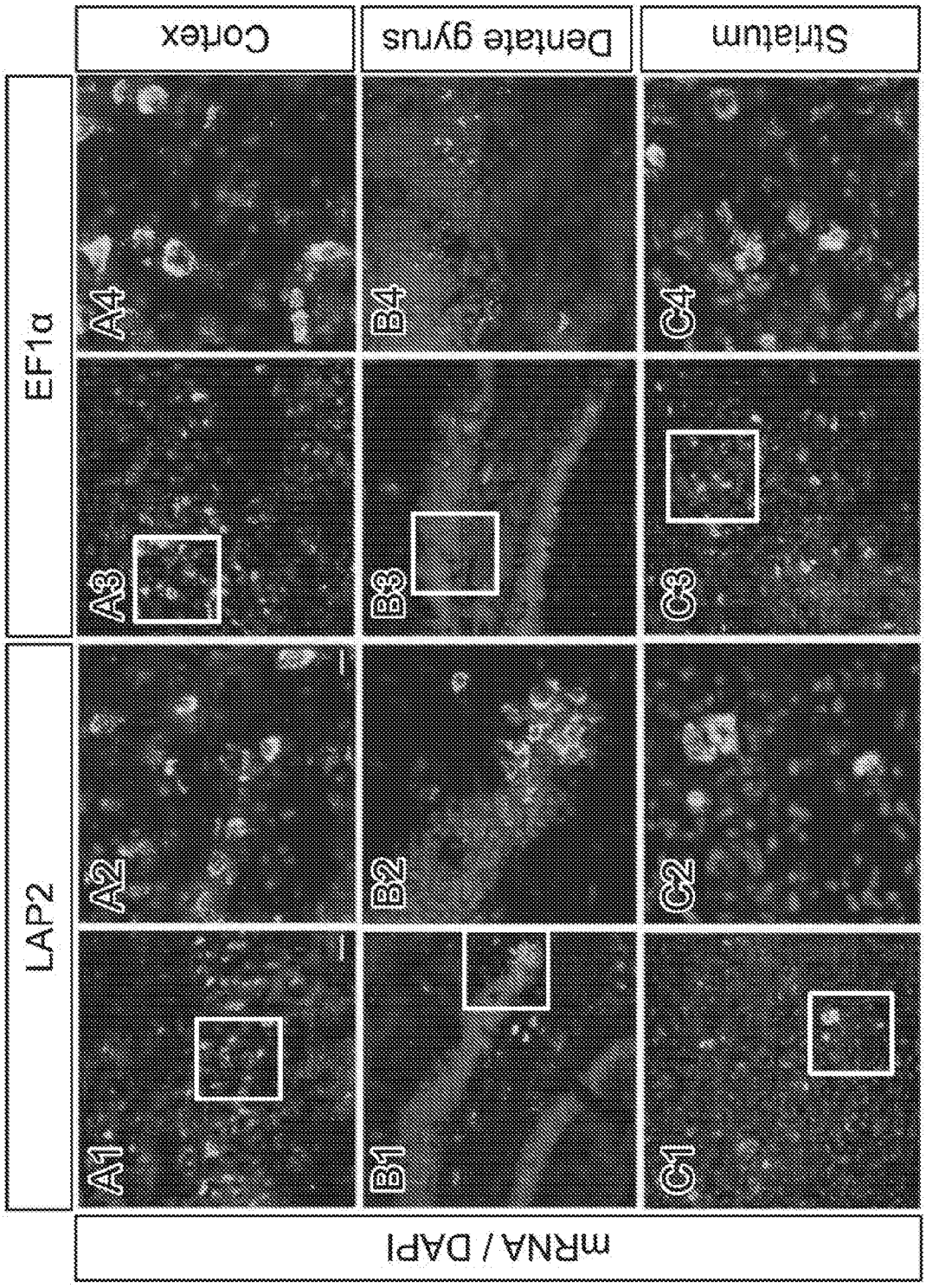


FIG. 5A

FIG. 5B

FIG. 5C

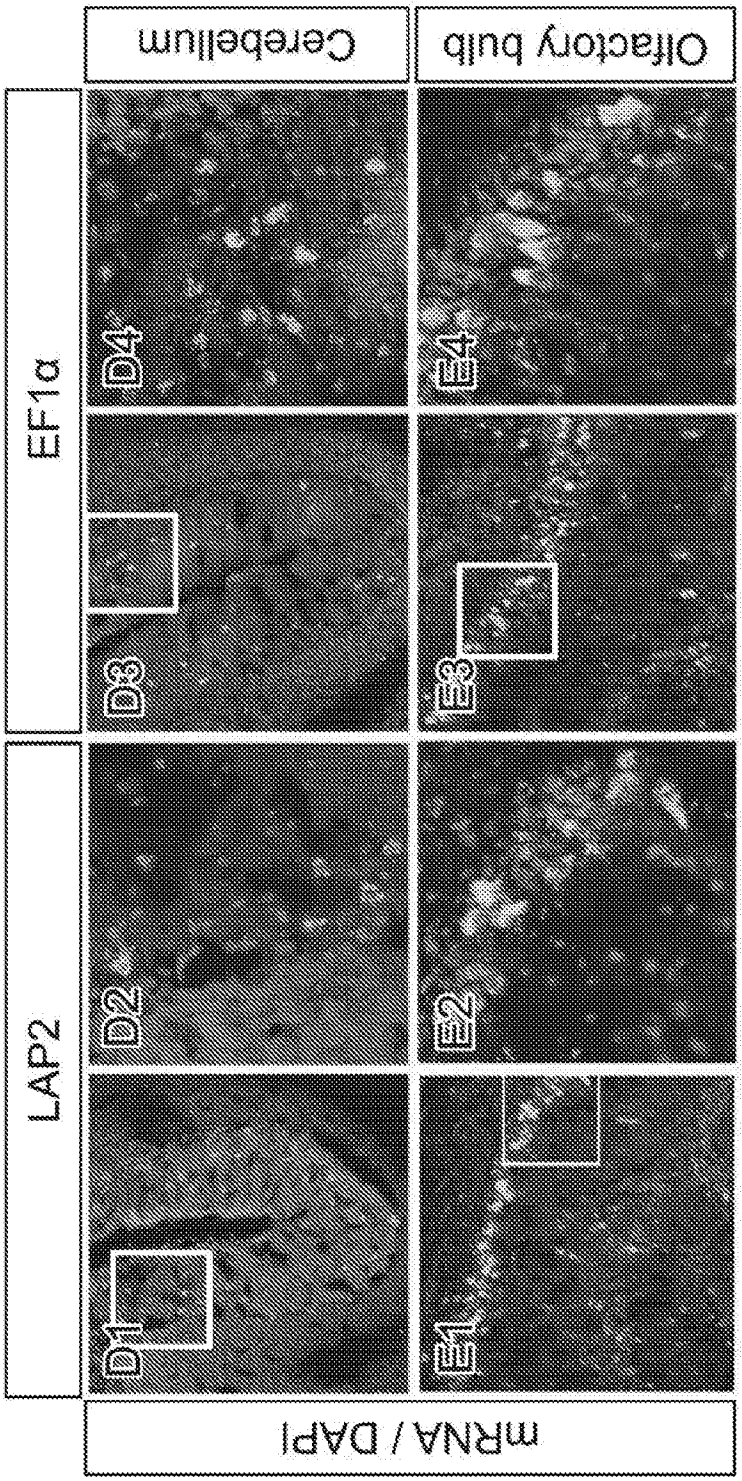

Cerebellum
Olfactory bulb
EF1α
LAP2
mRNA / DAPI
D1
D2
D3
D4
E1
E2
E3
E4

FIG. 5D

FIG. 5E

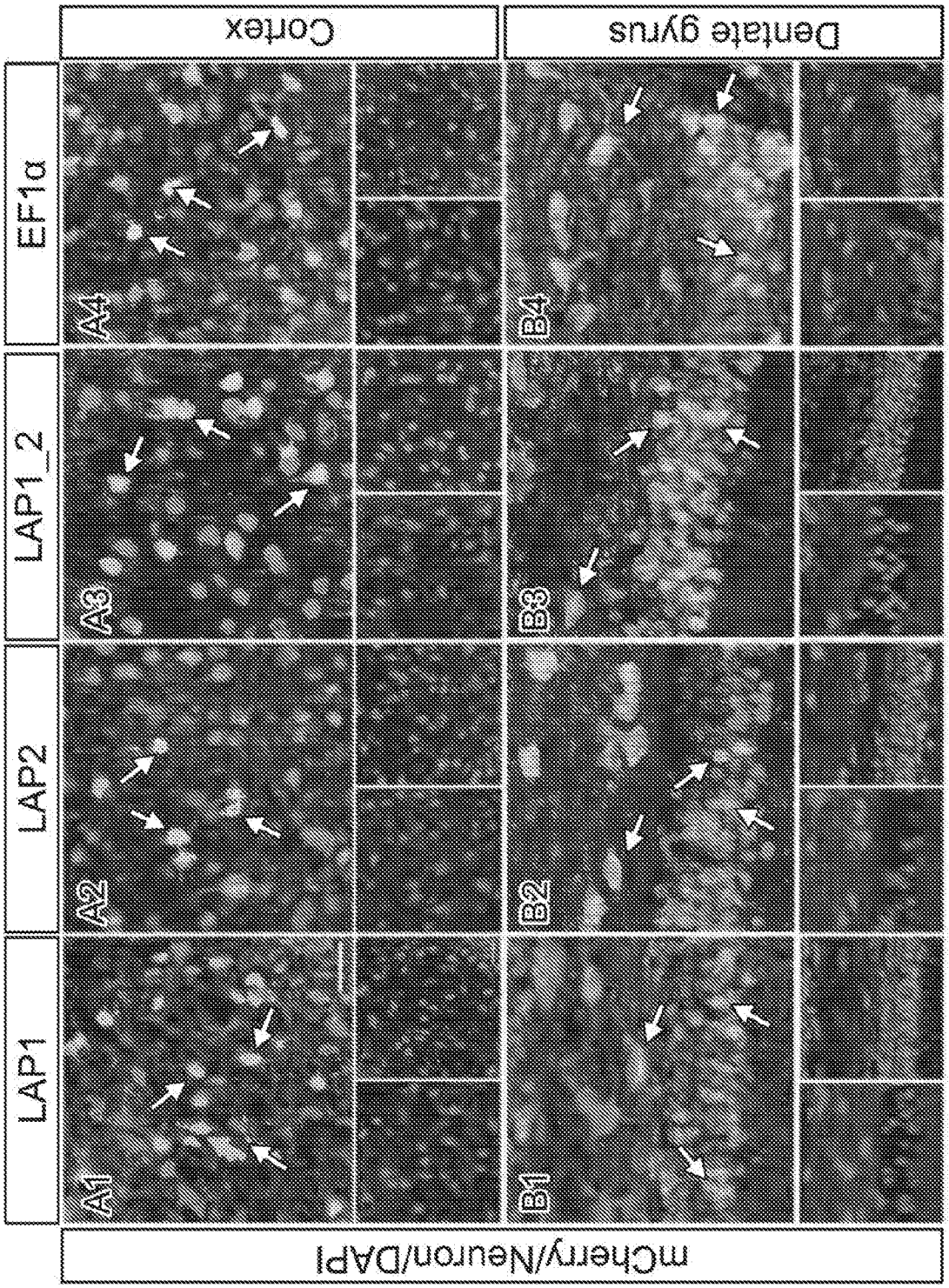

Cortex
Dentate gyrus
EF1α
LAP1_2
LAP2
LAP1
mCherry/Neuron/DAPI
A4
A3
A2
A1
B4
B3
B2
B1

FIG. 6A

FIG. 6B

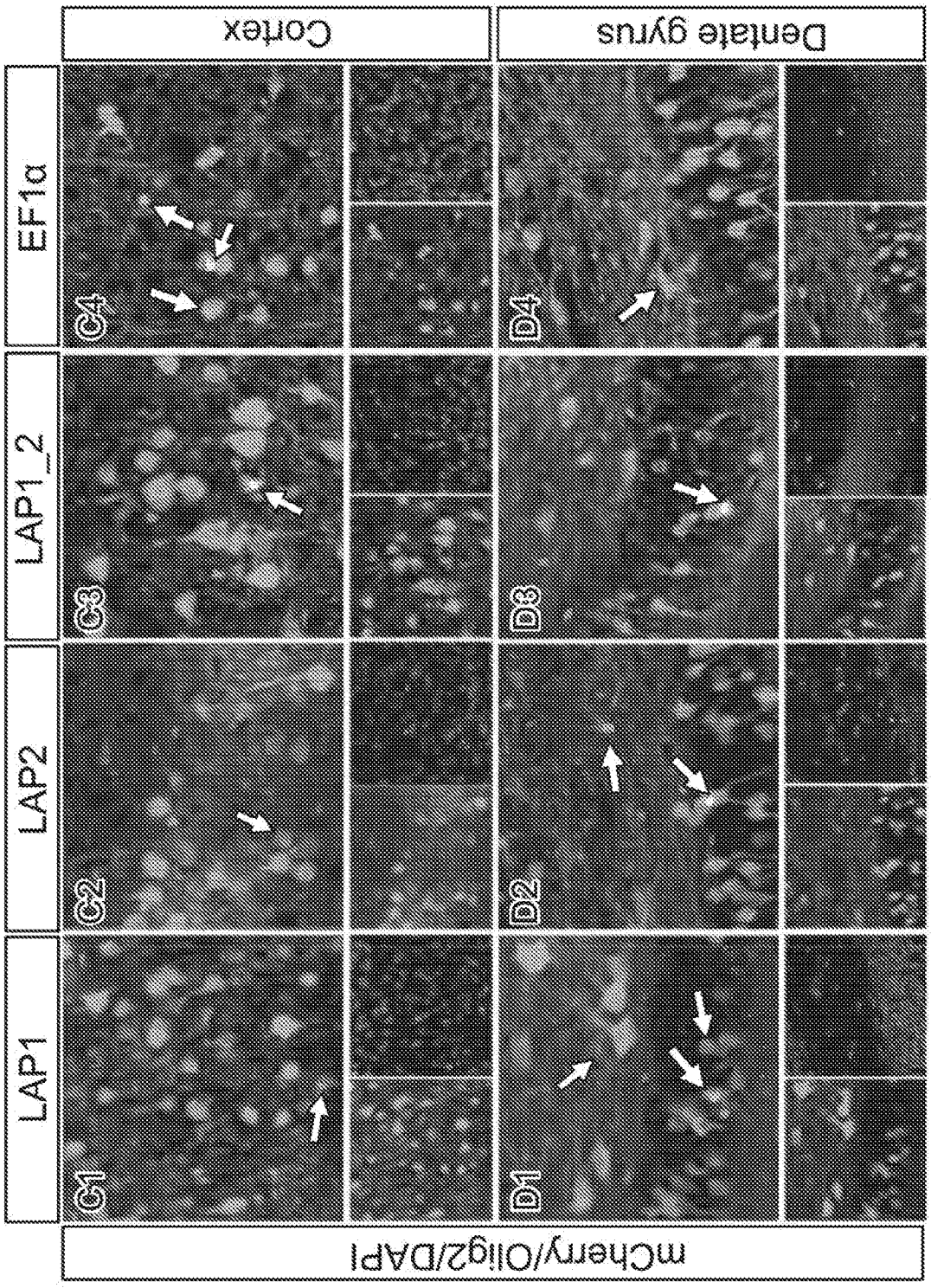

Cortex
Dentate gyrus
EF1α
LAP1_2
LAP2
LAP1
mCherry/Olig2/DAPI
C4
C3
C2
C1
D4
D3
D2
D1

FIG. 6C

FIG. 6D

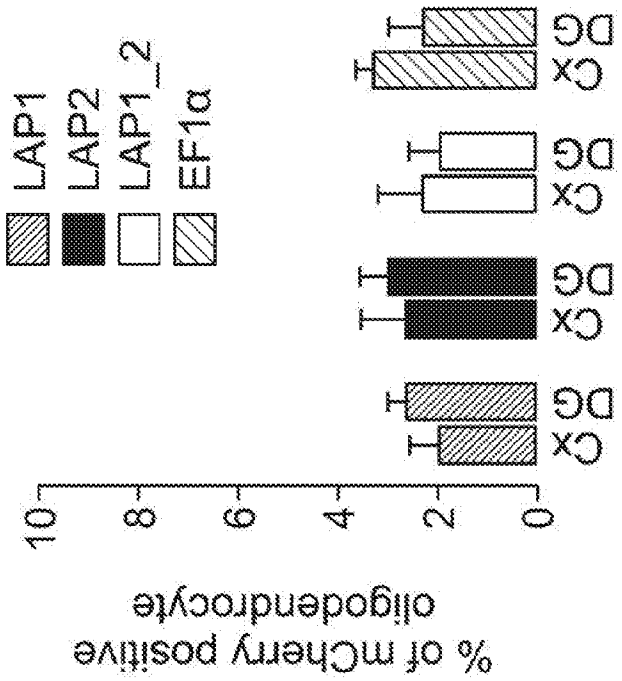

LAP1
LAP2
LAP1_2
EF1α
10
8
6
4
2
0
% of mCherry positive
oligodendrocyte
CX
DG
CX
DG
CX
DG
CX
DG

FIG. 6F

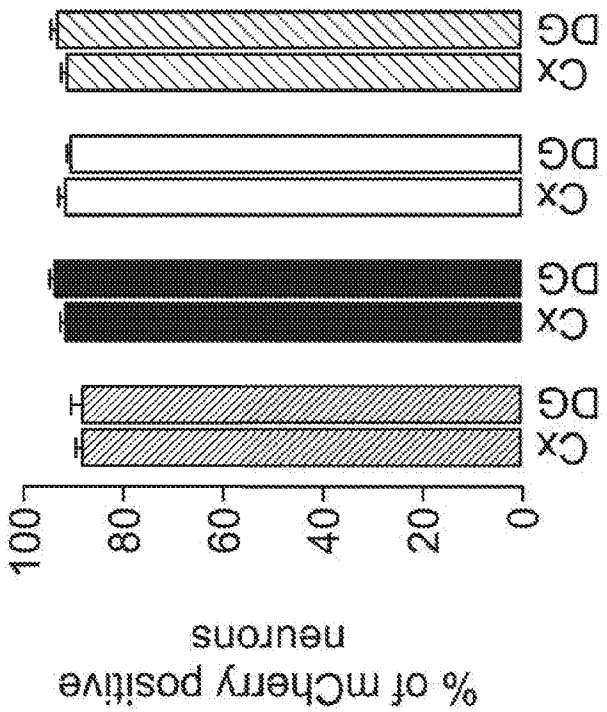

100
80
60
40
20
0
% of mCherry positive
neurons
CX
DG
CX
DG
CX
DG
CX
DG

FIG. 6E

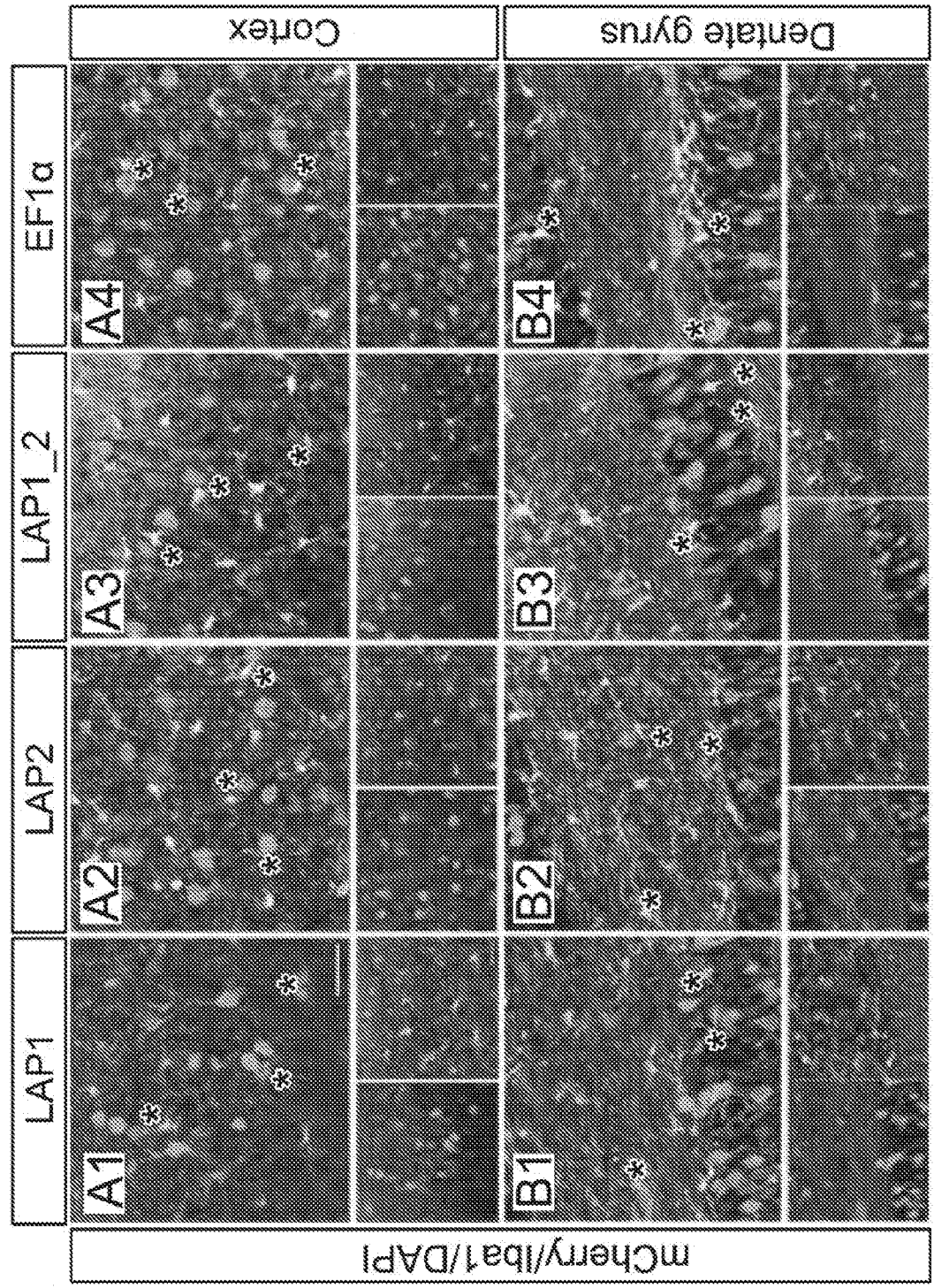

Cortex
Dentate gyrus
EF1α
LAP1_2
LAP2
LAP1
mCherry/Iba1/DAPI
A4
A3
A2
A1
B4
B3
B2
B1

FIG. 7A

FIG. 7B

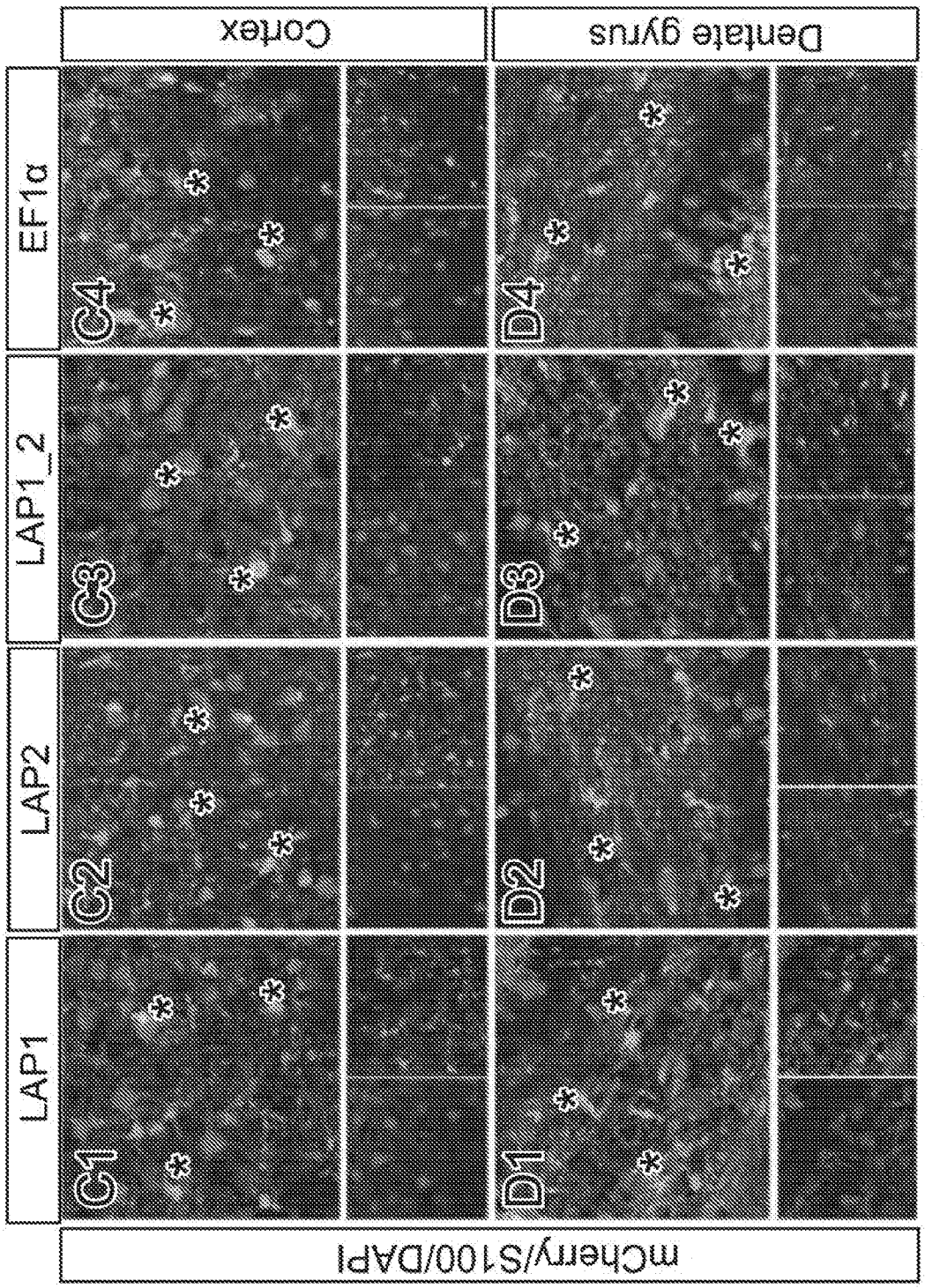

Cortex
Dentate gyrus
EF1α
LAP1_2
LAP2
LAP1
mCherry/S100/DAPI
C4
C3
C2
C1
D4
D3
D2
D1

FIG. 7C

FIG. 7D

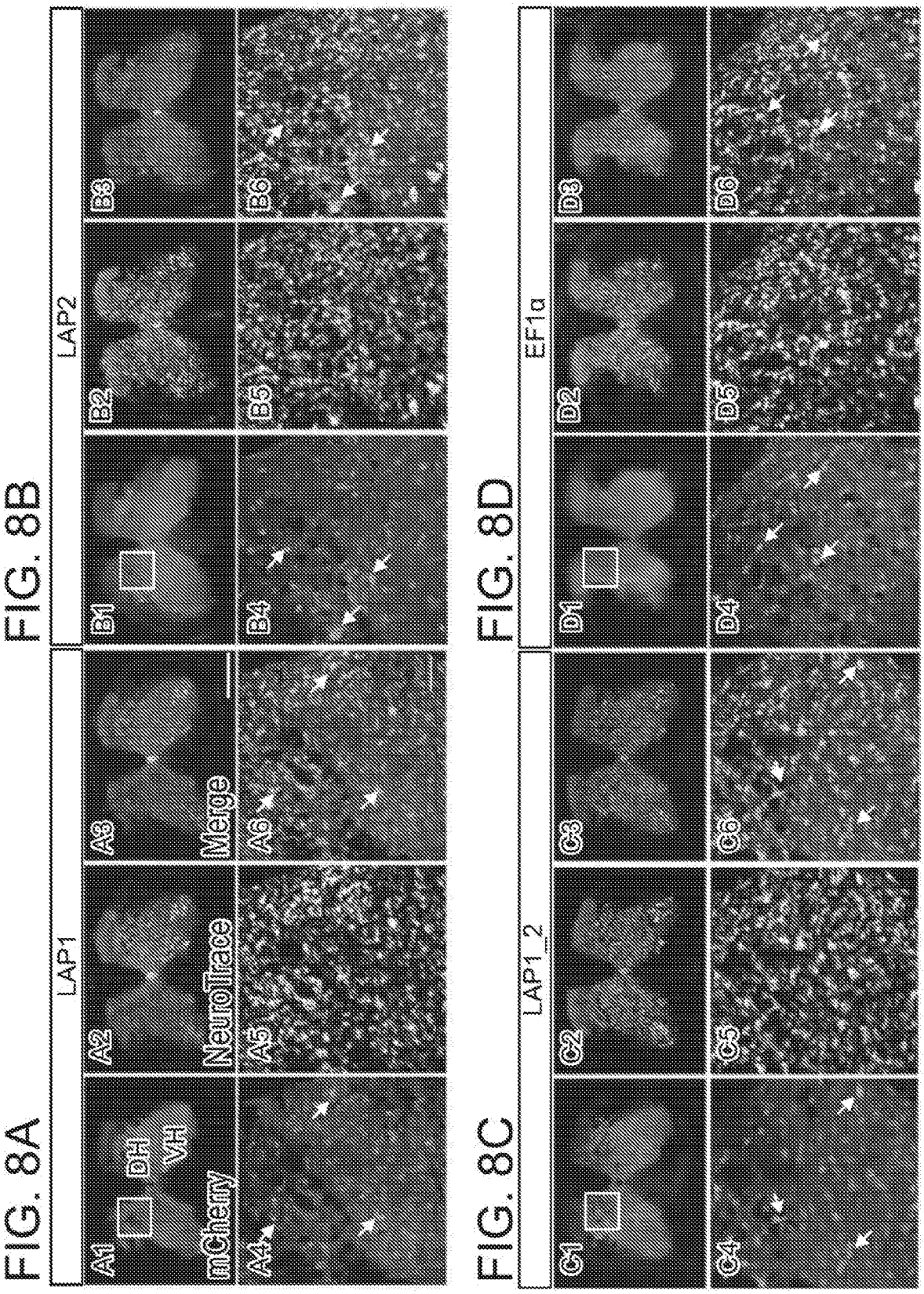

FIG. 8A
LAP1
LAP2
A1
DH
VH
mCherry
A2
NeuroTrace
A3
Merge
A4
A5
A6
B1
B2
B3
B4
B5
B6
FIG. 8B
FIG. 8C
LAP1_2
EF1α
C1
C2
C3
C4
C5
C6
D1
D2
D3
D4
D5
D6
FIG. 8D

FIG. 8F

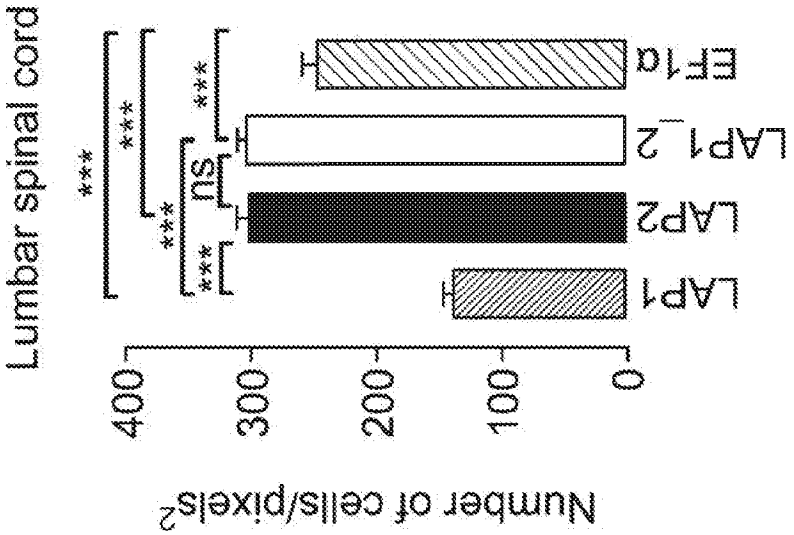
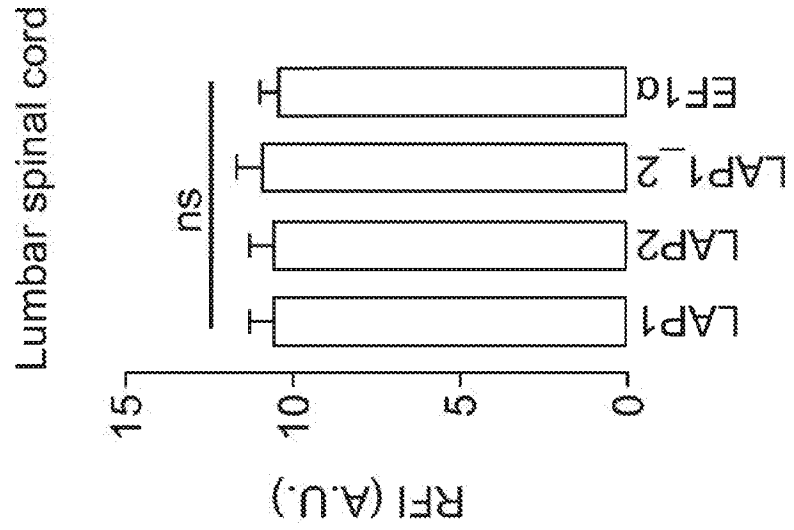

Lumbar spinal cord
Number of cells/pixels²
400
300
200
100
0
LAP1
LAP2
LAP1_2
EF1α
***
***
***
ns
***

FIG. 8E


Lumbar spinal cord
RFI (A.U.)
15
10
5
0
LAP1
LAP2
LAP1_2
EF1α
ns

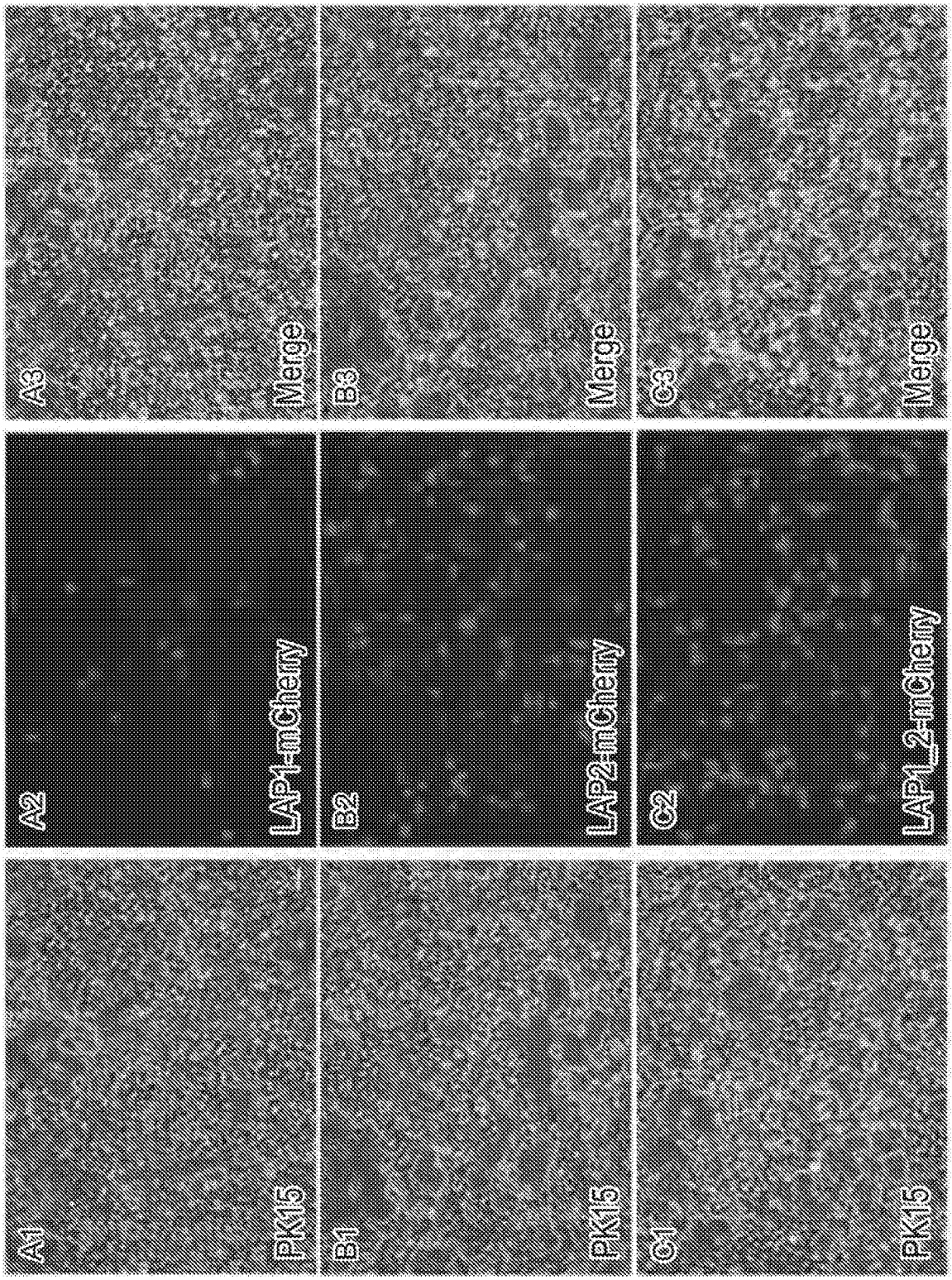


FIG. 9A

FIG. 9B

FIG. 9C

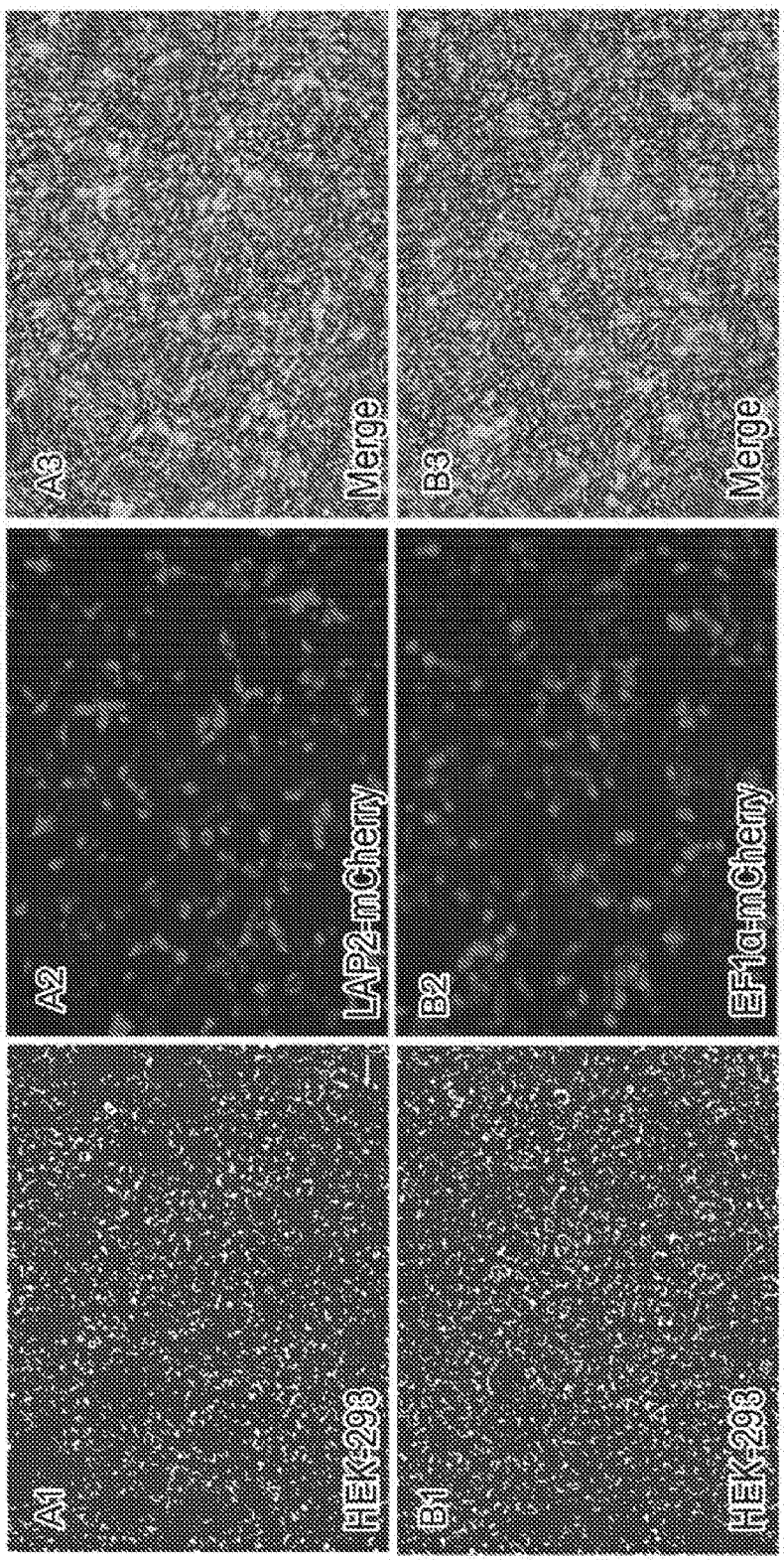


FIG. 10A

FIG. 10B

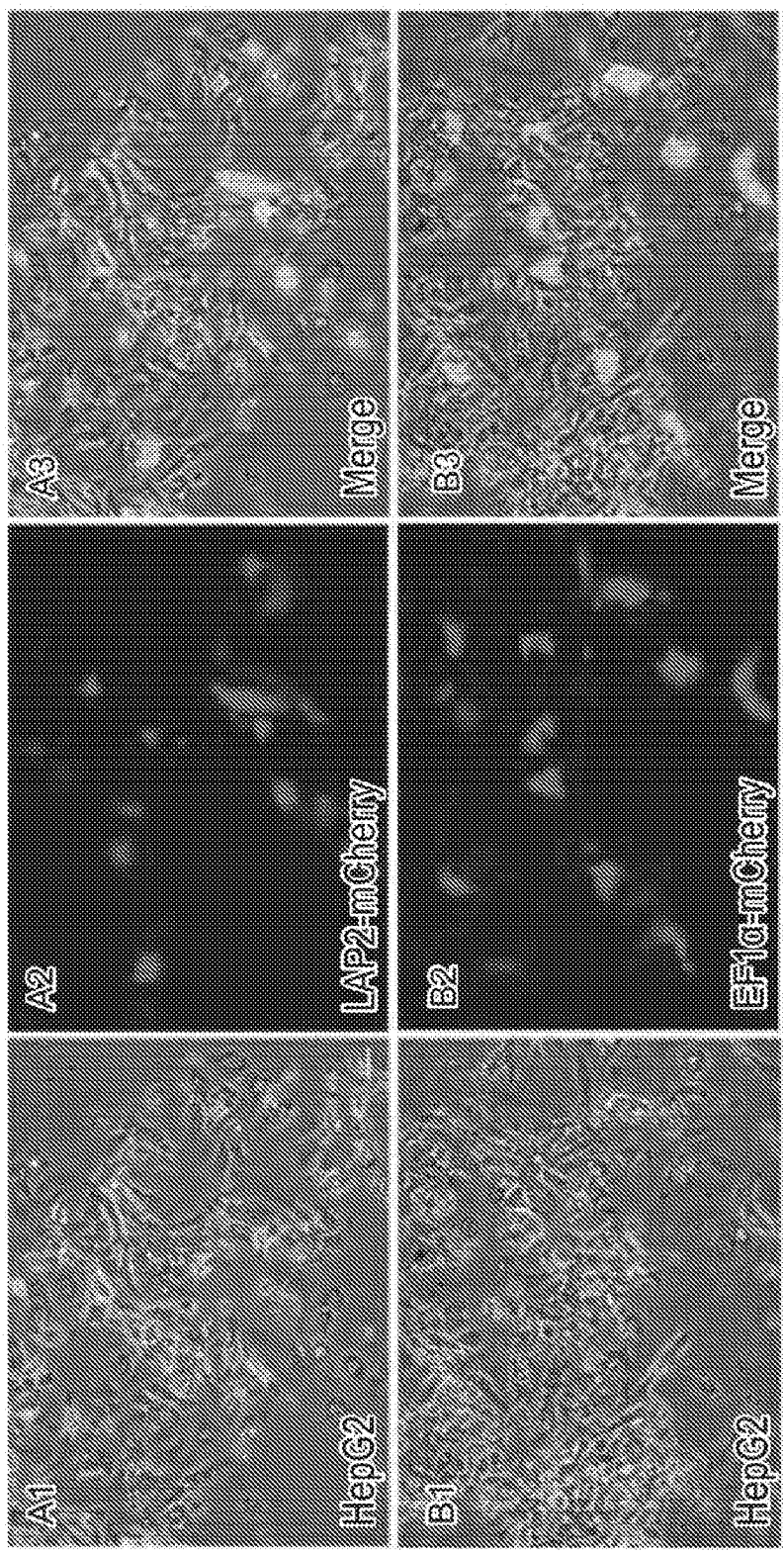
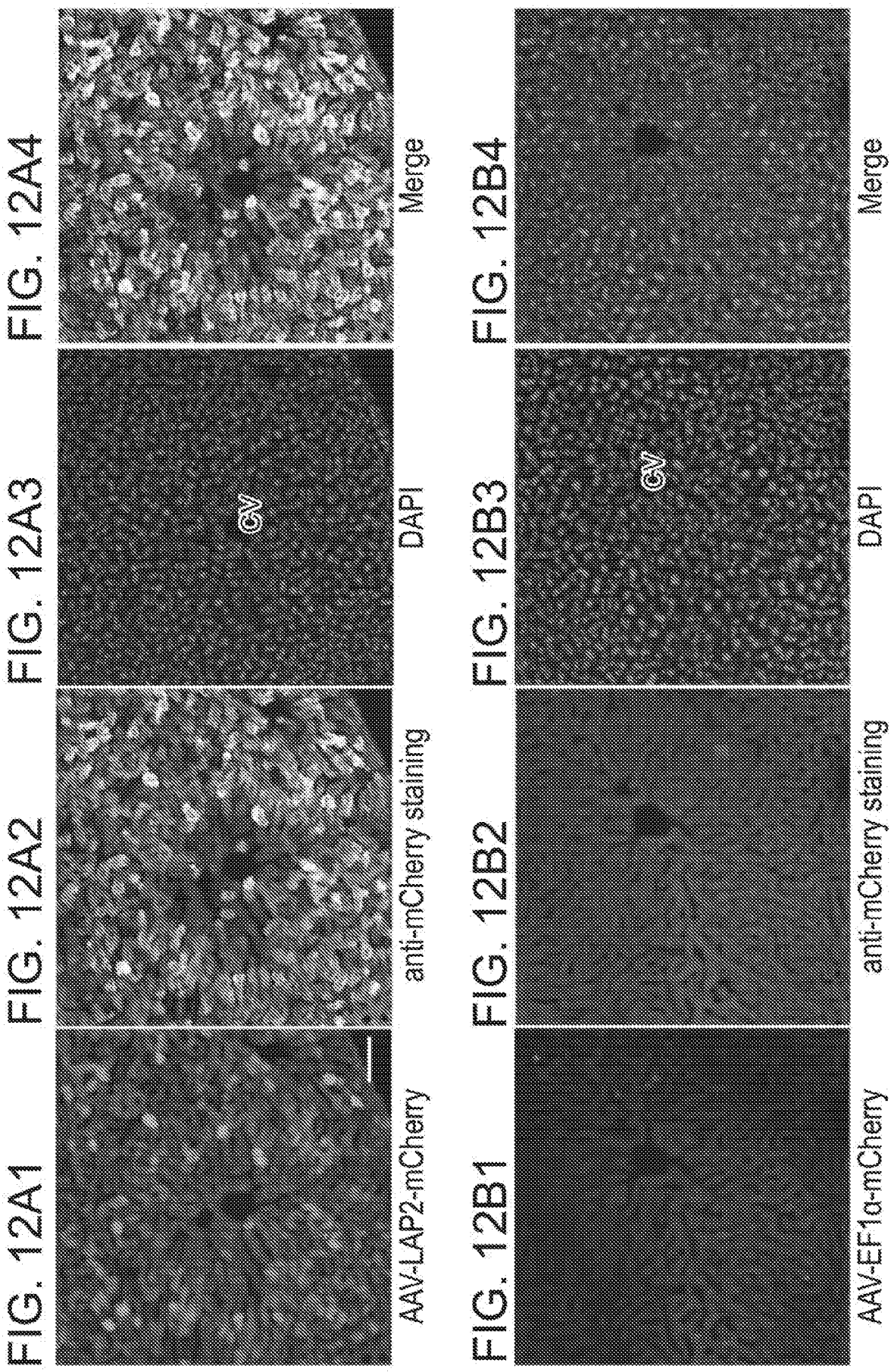
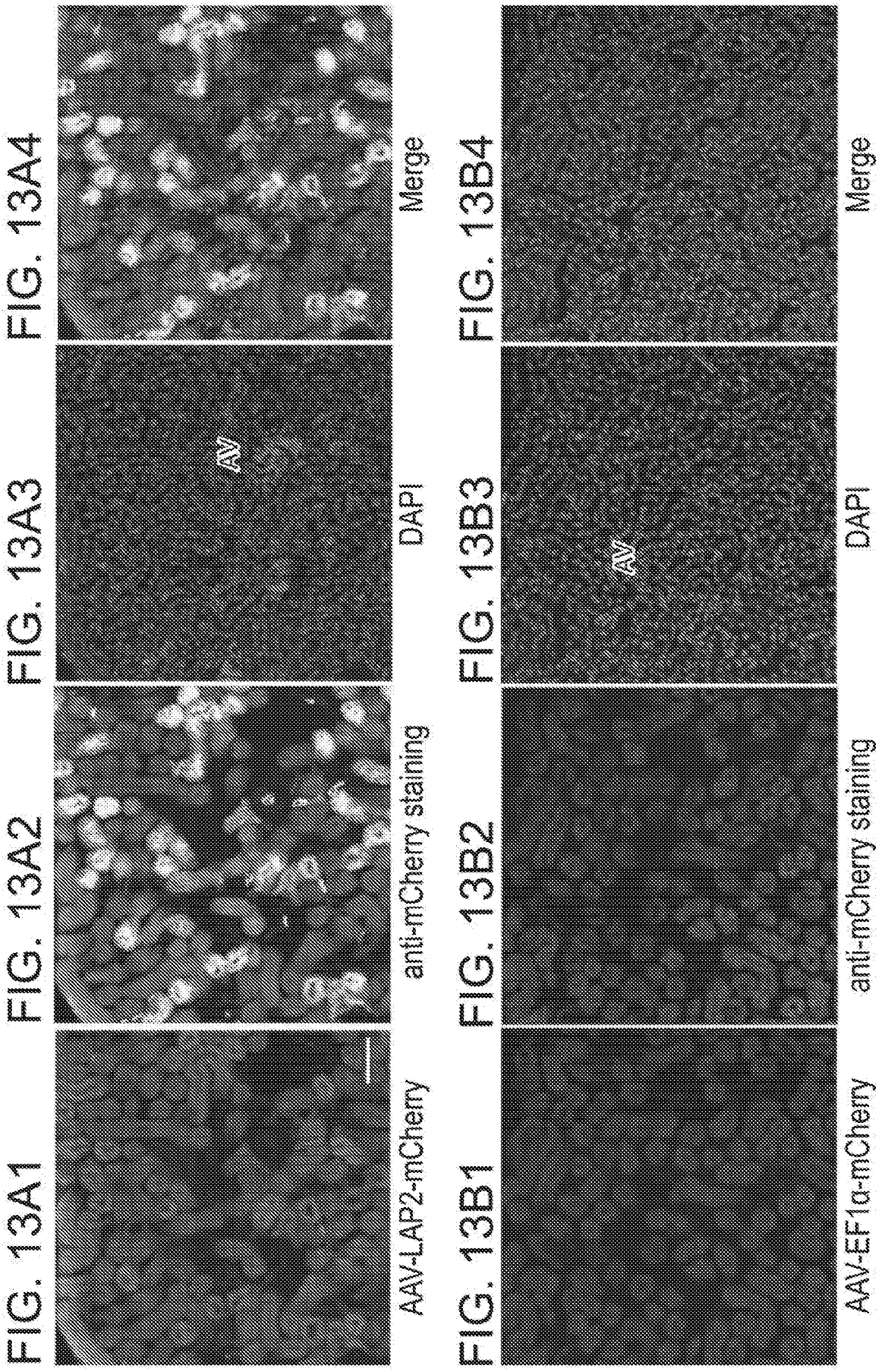
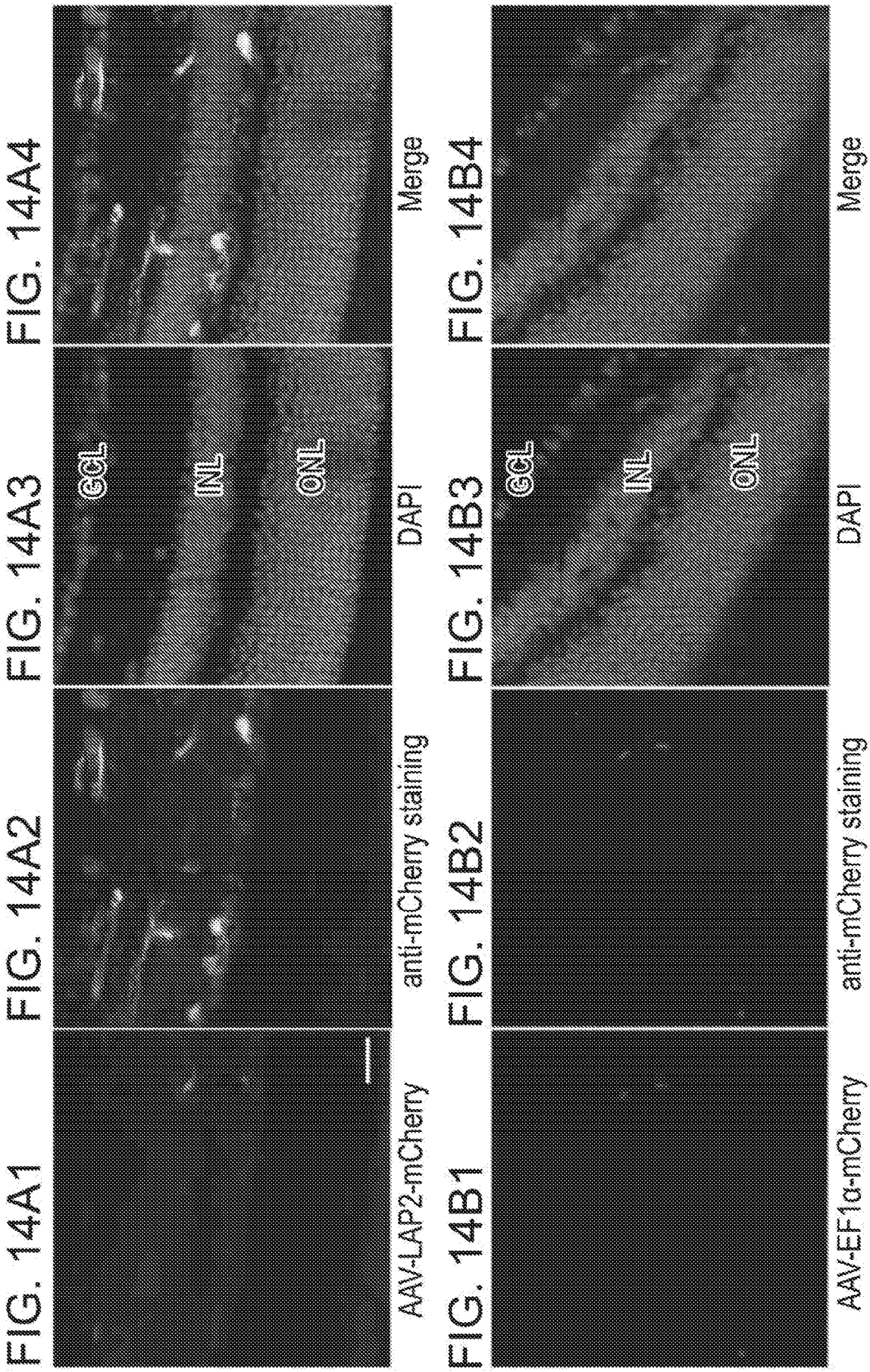


FIG. 11A

FIG. 11B


FIG. 12A1
FIG. 12A2
FIG. 12A3
FIG. 12A4
FIG. 12B1
FIG. 12B2
FIG. 12B3
FIG. 12B4
AAV-LAP2-mCherry
anti-mCherry staining
DAPI
Merge
AAV-EF1α-mCherry
anti-mCherry staining
DAPI
Merge
CV
CV


FIG. 13A1
FIG. 13A2
FIG. 13A3
FIG. 13A4
AAV-LAP2-mCherry
anti-mCherry staining
DAPI
Merge
AV
FIG. 13B1
FIG. 13B2
FIG. 13B3
FIG. 13B4
AAV-EF1α-mCherry
anti-mCherry staining
DAPI
Merge
AV


FIG. 14A1
FIG. 14A2
FIG. 14A3
FIG. 14A4
GCL
INL
ONL
AAV-LAP2-mCherry
anti-mCherry staining
DAPI
Merge
FIG. 14B1
FIG. 14B2
FIG. 14B3
FIG. 14B4
GCL
INL
ONL
AAV-EF1α-mCherry
anti-mCherry staining
DAPI
Merge

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 20/16787

A. CLASSIFICATION OF SUBJECT MATTER

IPC - C12N 15/86, C12Q 1/6897, C12Q 1/70 (2020.01)

CPC - C12N 15/861, C12N 15/869, C12N 2750/14143; C12N 2830/002, C12N 2799/026

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

B. FIELDS SEARCHED

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

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

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

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	TAHARAGUCHI et al. The latency-associated transcript promoter of pseudorabies virus directs neuron-specific expression in trigeminal ganglia of transgenic mice. J Gen. Virol. 1 August 2003, Vol 84, No. 8, pp 2015-2022	1-5, 23-29
Y	DEAN et al. A 3' Coterminal Gene Cluster in Pseudorabies Virus Contains Herpes Simplex Virus UL1, UL2, and UL3 Gene Homologs and a Unique UL3.5 Open Reading Frame. J Virol., October 1993, Vol 67, No. 10. pp 5955-5961	1-5, 23-29
Y	GenBank submission L13855, 25 February 1994 [online]. [Retrieved on 25 May 2020]. Retrieved from the internet <URL: https://www.ncbi.nlm.nih.gov/nuccore/L13855 > entire document	1-5, 23-29
Y	US 2010/0240739 A1 (BARKATS) 23 September 2010 (23.09.2010) abstract, para [0006], [0008], [0044]	2-3, 5/(2-3)
Y	GenBank submission JF797219, 2 November 2011 [online]. [Retrieved on 25 May 2020]. Retrieved from the internet <URL: https://www.ncbi.nlm.nih.gov/nuccore/JF797219 > entire document	25, 26/25, 29

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

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

Date of the actual completion of the international search	Date of mailing of the international search report
25 May 2020	16 JUL 2020
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-8300	Authorized officer Lee Young Telephone No. PCT Helpdesk: 571-272-4300

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 20/16787

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

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

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

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

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

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

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

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

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

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

3. Additional comments:

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 20/16787

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

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

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

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

3. ☒ Claims Nos.: 6- 22, 35-46, 52-68, 83-84, 87, 89-92, 98-117, 123, 136-149
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

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

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

---------- see extra sheet ----------

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

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

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

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
1-5 and 23-29, limited to a nucleic acid sequence having at least 70% sequence identity to SEQ ID NO: 1

Remark on Protest

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

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

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.
PCT/US 20/16787

Continuation of Box No. III, Observations where unity of invention is lacking:

This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be searched, the appropriate additional search fees must be paid.

Group I+: Claims 1-5, 23-29, 47-51, 69-75, 93-97, 118-122, 124-129 directed to a recombinant vector for expression of a target gene in a host cell, comprising: a promoter, a cloning site for insertion of a nucleic acid encoding the target gene, and at least one non-promoter regulatory element required for the expression of the target gene in the host cell. The recombinant expression vector will be searched to the extent that the promoter comprises a nucleic acid sequence having at least 70% sequence identity to SEQ ID NO: 1. It is believed that claims 1-5, 23-29 encompass this first named invention, and thus these claims will be searched without fee to the extent that they encompass a recombinant expression vector comprising a promoter comprising a nucleic acid sequence having at least 70% sequence identity to SEQ ID NO: 1. Additional recombinant expression vectors will be searched upon the payment of additional fees. Applicants must specify the claims that encompass any additionally elected recombinant expression vectors. Applicants must further indicate, if applicable, the claims which encompass the first named invention, if different than what was indicated above for this group. Failure to clearly identify how any paid additional invention fees are to be applied to the "+" group(s) will result in only the first claimed invention to be searched. An exemplary election would be a recombinant expression vector comprising a promoter comprising a nucleic acid sequence having at least 70% sequence identity to SEQ ID NO: 5 (claims 47-51, 69-75). Another exemplary election would be a recombinant expression vector comprising a promoter comprising a nucleic acid sequence having at least 70% sequence identity to SEQ ID NO: 9 (claims 93-97, 118-122, 124-127).

Group II+: Claims 30-34, 76-82, 85-86, 88, and 130-135 directed to a method of expressing a target gene in a host cell, comprising contacting the host cell with a recombinant polynucleotide molecule that is introduced into the host cell, wherein the polynucleotide molecule comprises a nucleic acid encoding the target gene and wherein the nucleic acid is operably linked to a promoter. Group II+ will be searched upon payment of additional fees. The method may be searched, for example, to the extent that the promoter has at least 70% sequence identity to SEQ ID NO: 1. It is believed that claims 30-34 limited to a method of expressing a target gene with a polynucleotide comprising a recombinant polynucleotide molecule at least 70% sequence identity to SEQ ID NO: 1 read on this exemplary invention. Additional promoter driven target genes will be searched upon the payment of additional fees. Applicants must specify the claims that encompass any additionally elected. Failure to clearly identify how any paid additional invention fees are to be applied to the "+" group(s) will result in only the first claimed invention to be searched. An exemplary election would be a method of expressing a target gene in a host cell, comprising: contacting the host cell with a recombinant polynucleotide molecule, wherein the recombinant polynucleotide molecule comprises a nucleic acid encoding the target gene operably linked to a promoter comprising a nucleic acid sequence having at least 70% sequence identity to SEQ ID NO: 9 (130-133).

The inventions listed as Groups I+ and II+ do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

Special Technical Features

No technical features are shared between the nucleic acid promoter sequences of Group I+ and the nucleic acid promoter sequences Group II+, accordingly, these groups lack unity a priori.

Additionally, even if the inventions listed as Group I+ and or Group II+ were considered to share technical features, these shared technical features are previously disclosed by the prior art, as further discussed below.

Groups I+ requires an isolated recombinant nucleic acid with a promoter driven target gene, not required by Group II+.

Group II+ requires method of expressing a target gene in a host cell, not required by Group I+.

Common Technical Features

The inventions of Group I+ and II+ share the technical feature of a recombinant polynucleotide molecule, comprising a nucleic acid sequence encoding a target gene, wherein the nucleic acid is operably linked to a nucleic acid encoding a promoter operable for expression in a host cell.

However, this shared technical feature does not represent a contribution over prior art, because the shared technical feature is made obvious by US 2019/0032082 A1 to Sirion Biotech GMBH (hereinafter 'Sirion'). Sirion discloses said recombinant nucleic acid comprising a promoter operably linked to a target gene (transgene) (para [0008] "cell comprising ... a nucleic acid comprising in 5' to 3' direction ... a promoter ... a transgenic coding sequence under the control of said promoter", para [0034] "Preferred transgenic coding ... which, upon expression, produce a transgenic protein", para [0027] "the cell of the invention provides a functioning ... expression system").

As the technical feature was known in the art at the time of the invention, this cannot be considered a special technical feature that would otherwise unify the inventions.

Groups I+ and II therefore lack unity under PCT Rule 13 because they do not share a same or corresponding special technical feature.