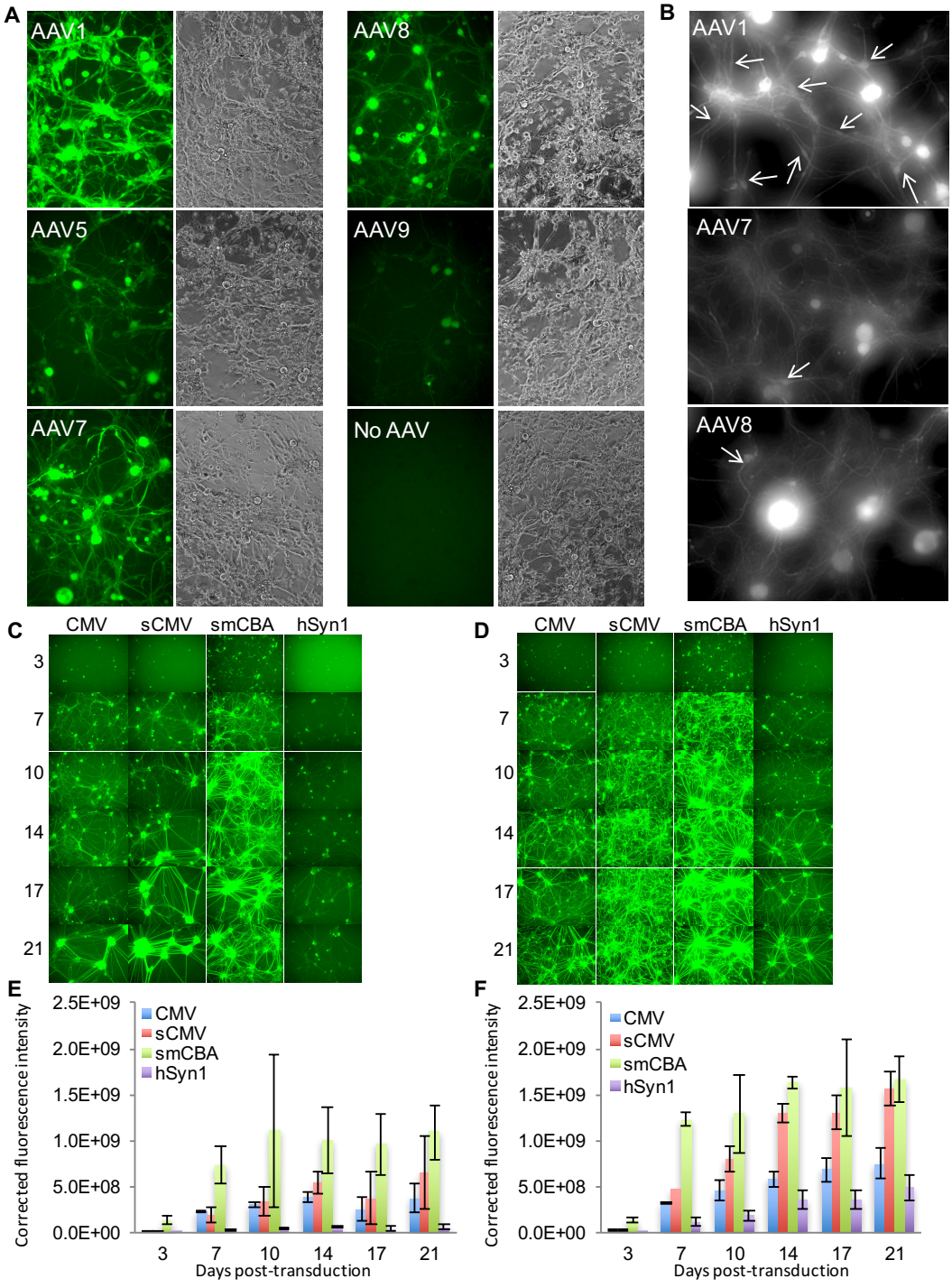
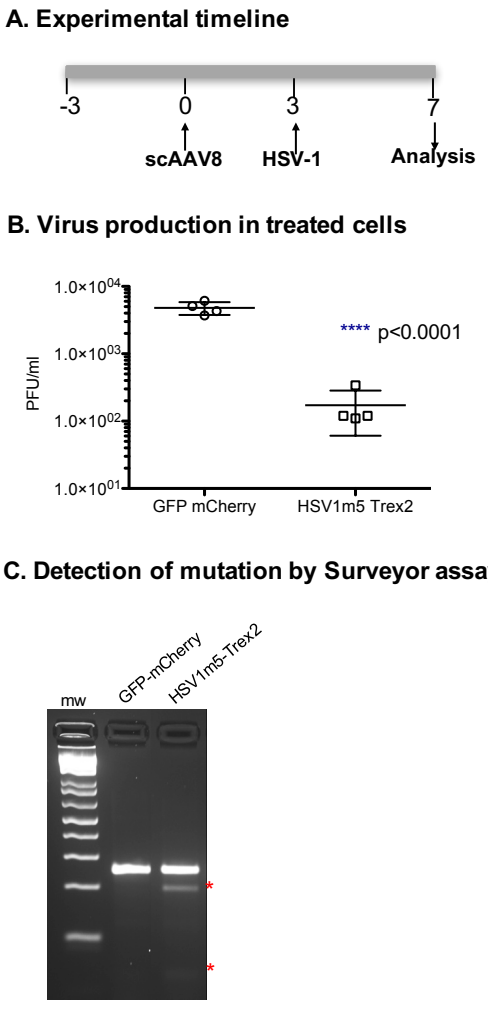


Supplemental Figure 1. AAV capsid serotype and promoter screening for optimal transduction of primary TG neurons. (**A**) Representative GFP fluorescence (left panels) and phase (right panels) images of neuronal cultures transduced with scAAV-smCBA-GFP vectors with the indicated capsid serotypes at a multiplicity of infection (MOI) of 10^6 vgs/neuron. Uninfected control cultures with no AAV are shown (No AAV). Magnification x100. (**B**) Representative GFP fluorescence images of neuronal

cultures transduced with scAAV-smCBA-GFP vectors with the indicated capsid serotypes at an MOI of 10^6 vgs/neuron. Arrows indicate GFP-positive non-neuronal cells identified based on cell morphology. Magnification x200.
(**C-D**) Representative GFP fluorescence images at 7, 10, 14, 17 and 21 days post transduction of neuronal cultures with scAAV-GFP vectors. AAV8 (**C**) and AAV1 (**D**) reporter vectors were used at a MOI of 10^6 vg/neuron. Magnification 100x. GFP was expressed from the human cytomegalovirus immediate-early (CMV), short CMV (sCMV), small hybrid CMV/chicken beta actin (smCBA) or human synapsin (hSyn) promoters. Quantification of GFP fluorescence in AAV8 (e) and AAV1 (f) vector transduced neuronal cultures at 7, 10, 14, 17 and 21 days post transduction.



Supplemental Figure 2. **Impact of HSV1m5/Trex2 exposure on HSV replication in neuronal cultures**. (**A**) Schematic of the experimental timeline. Neuronal cultures were established in quadruplicate from dissociated murine TGs, and then co-transduced with scAAV8-sCMV-HSV1m5 + scAAV8-sCMV-Trex2, or scAAV8-sCMV-GFP + scAAV8-sCMV-mCherry at a MOI of 10^6 vg/AAV/neuron for 3 days prior to being infected with HSV-1 FΔ*Us5* at a MOI of 1 PFU/neuron for 4 further days. (**B**) Virion release in culture media was quantified by plaque assay. The mean +SD of four wells is indicated. The "p" value was calculated using a one-sided *t-test.* (**C**) Mutagenic event detection by T7E1 assay. The HSV regions containing the target site were PCR amplified from total genomic DNA obtained from four pooled wells, subjected to T7E1 digestion, and separated on a 3% agarose gel. mw: molecular weight size ladder, red asterisks indicate cleavage products.

Supplemental Table 1. NGS analysis for experiment 1 (Figure 3)

Mouse	Treatment	Total mutations	Total in target mutations	
			Insertions	Deletions
1	PBS	0.05%	0.01%	0.04%
6	NV1/Trex2	0.03%	0.01%	0.02%
8	HSV1m5/Trex2	2.05%	0.01%	2.05%
9	HSV1m5/Trex2	0.08%	0.01%	0.07%
10	HSV1m5/Trex2	3.92%	0.00%	3.92%
11	HSV1m5/Trex2	2.03%	0.00%	2.03%

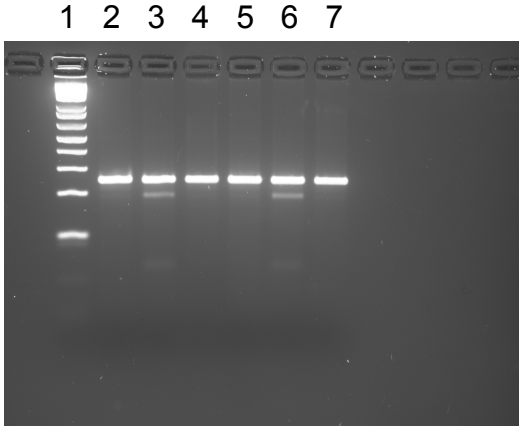
Supplemental Table 2. Sequence of the target site (ON) and off-target sites (OT) for HSV1m5, HSV1m8 and NV1.

Enzyme	site	Sequence
HSV1m5	ON	ATAAACTCACACACGGCGTCCTGG
HSV1m5	OT-1	ATA**g**ACTCACACA**t**G**c**CGTCCTG**t**
HSV1m5	OT-2	ATAAAC**a**CACACA**a**G**c**a**G**TCCTGG
HSV1m5	OT-3	AT**c**AACTCACAC**c**CG**t**C**c**TCCTGG
HSV1m5	OT-4	A**a**AAACTCA**a**ACA**t**GGCGTCC**a**G**t**
HSV1m5	OT-5	AT**gc**ACTCA**a**ACAC**t**G**t**GTCCTGG
HSV1m5	OT-6	**t**TAAA**t**TCA**a**AC**t**CGGCGTC**t**TGG
HSV1m5	OT-7	**ccc**AACTCACACAC**t**GC**t**TCCTGG
HSv1m8	ON	CCGCTCTGTTTTACCGCGTCTACG
HSV1m8	OT-1	CC**c**CTCTGTTTT**t**C**t**G**g**GTCTACG
HSV1m8	OT-2	C**g**GCT**t**TGTTTTA**t**CGC**a**TCT**t**CG
HSV1m8	OT-3	CCGC**a**CTGTTT**g**ACCGC**c**TC**c**AC**t**
HSV1m8	OT-4	CC**a**CTCTGT**a**TTACC**at**GTCTAC**t**
HSV1m8	OT-5	**t**CGCTCTGT**c**TT**g**C**t**GC**c**TCTACG
HSV1m8	OT-6	C**a**GCTCT**t**TTTT**c**CC**tt**GTCTACG
HSV1m8	OT-7	C**t**GCTCTGTTT**c**ACC**tg**GTCTAC**t**
HSV1m8	OT-18	CCG**g**T**t**TGTTTT**g**CC**a**CGTCTAC**t**
HSV1m8	OT-10	**t**C**c**CTCT**c**TTTTAC**a**G**aa**TCTACG
HSV1m8	OT-11	**t**C**c**CTCTGTTT**gt**CC**t**CGT**g**TACG
NV-1	ON	TTGTTCTCAGGTA**C**CTCGGCCAA
NV-1	OT-1	TTGTTCTCAGGTACCTC**t**G**a**CA**a**
NV-1	OT-2	CTGGCTGAGGT**c**CCT**c**AGA**g**CAA
NV-1	OT-3	C**g**GGCT**c**AGG**c**ACCTGAGAACAA
NV-1	OT-4	C**a**GGCTG**g**GGTACCTGAGAACA**t**
NV-1	OT-5	**t**TGGCT**a**AG**c**TACCTG**g**GAACAA

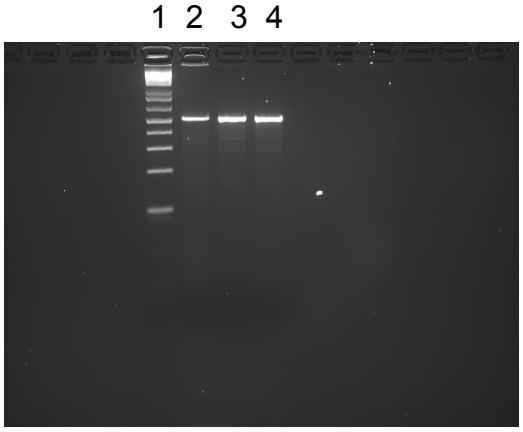
Supplemental Table 3. Primers used for PCR amplification of the regions containing the off-target (OT) sites in the mouse genome.

Enzyme	OT site	Forward primer	Reverse primer
HSV1m5	OT-1	CTGTGTGTAGAAGACTCTAGAGGG	CTGTGTGTAGAAGACTCTAGAGGG
HSV1m5	OT-2	AAAGGGCAAGCCTGCCTTCC	CTCACTCTACCCTTAGACCCC
HSV1m5	OT-3	GCCAGAAGTTCCAGCTCAATGTC	AGGGACTTGTCAACATGAGCGG
HSV1m5	OT-4	CCAAGACAGAAGCTCCTTACGTCA	CATTGACCTGAACCTGGGTCC
HSV1m5	OT-5	CAGGTTTGCTCCTGTGATTGGTG	TGTGTCCACAACAGACCGCAG
HSV1m5	OT-6	TGTGGTGTGGTGTGGTGCCT	GGGGCAAATTCTGCACCTCTTGT
HSV1m5	OT-7	CCACAAAACTGTGTCTCTAGCTGC	CCACAAAACTGTGTCTCTAGCTGC
HSV1m8	OT-1	CAGGTAAGAGCCAGAACAATGGTC	CATTGTCCACTTGAAGTGTGGCAG
HSV1m8	OT-2	GAAAGCAAGCTTCCTCCCTCG	GGTGGGAGATTCCACCTTCTTTG
HSV1m8	OT-3	CAGGACCTCTGGATCACTTGATTC	CTCTCAAAGCTGTGCAGGACTAAC
HSV1m8	OT-4	GGTTGAGAGGGTGGTTGGAAAAAG	CAGAGGGGGTAGGTGAAAGATTG
HSV1m8	OT-5	GGGTAGATGGCTGAGAAGGAAC	GACTCAGGTCTTGAGTGTGAAGTG
HSV1m8	OT-6	CTCCAGCCAACATGTGGAGAAG	ACTGTCTGGGAACGGGGCAA
HSV1m8	OT-7	GGCCTTAAGGCTTGATAGACAACC	CCTTGTCTGTCACCAGTCTCTC
HSV1m8	OT-8	GATCAAACACCCTAGCCAAGGCA	CCAAACAGGAAAACCTGAGATGGC
HSV1m8	OT-9	GATGCATTTGCACAGAGGGTCC	GAGAATAGTTTAGATGGGAGGGGG
HSV1m8	OT-10	GCTCACCTGCATATGCGTATGCT	CTTTCCAGCTGATTTCAGAAGCCC
NV-1	OT-1	AAGCTGCCTCACCGGTCTCA	GCAGAAGACAGTGGTATTGAGGAG
NV-1	OT-2	GGAGTAGGGATCTGAGACTTCG	GAATCCAAACCCATTGCTCGCC
NV-1	OT-3	GCCTCCAACAAGACCTGGAAC	CACCAAGCACAGGTTAGACAGTAC
NV-1	OT-4	GGTTGGATCCTCTGGGCTTG	GTGGCATTAGGAAGTGACATTCGC
NV-1	OT-5	GTGCCTTTTCAAAGGATCTCACCC	GGAAAGGATTGGCCATTTTGGCTG

Full unedited gels for Figure 1

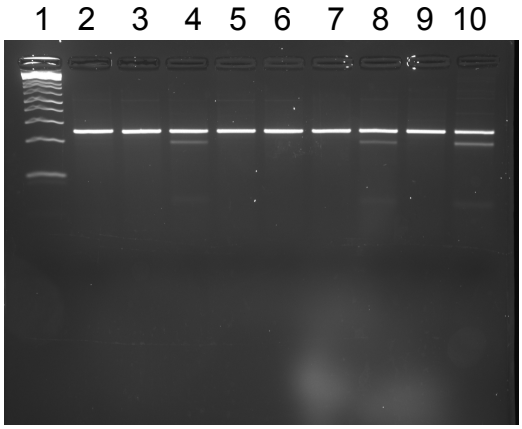


Full unedited gel for Figure 1, left panel. Lanes 1-4 correspond to the lanes shown in the manuscript cropped figure.

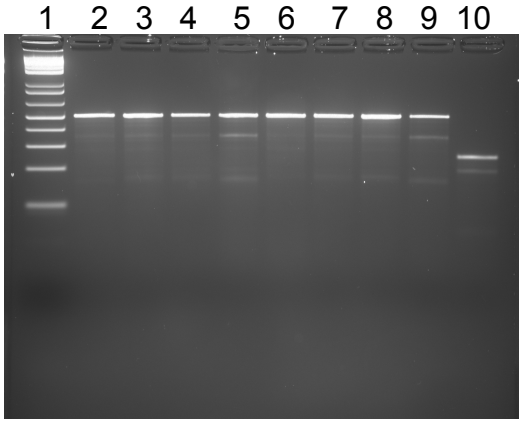


Full unedited gels for Figure 1, right panel. Lanes 1-4 correspond to the lanes shown in the manuscript cropped figure.

Full unedited gels for Figure 2

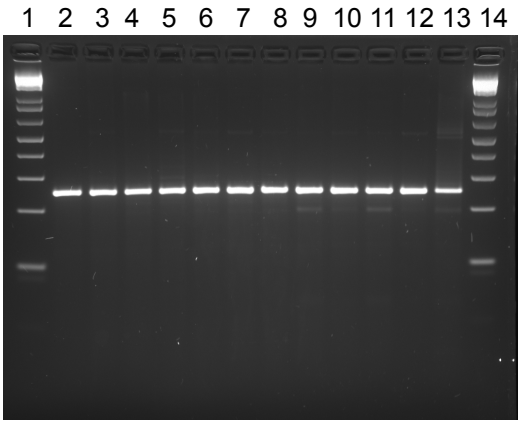


Full unedited gel for Figure 2, top panel. Lanes 1-10 correspond to the lanes shown in the manuscript cropped figure.



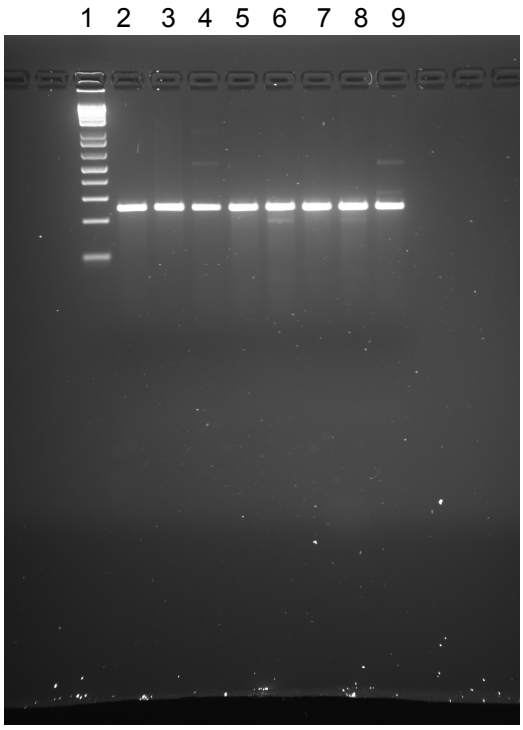
Full unedited gels for Figure 2, bottom panel. Lanes 1-10 correspond to the lanes shown in the manuscript cropped figure.

Full unedited gels for Figure 3

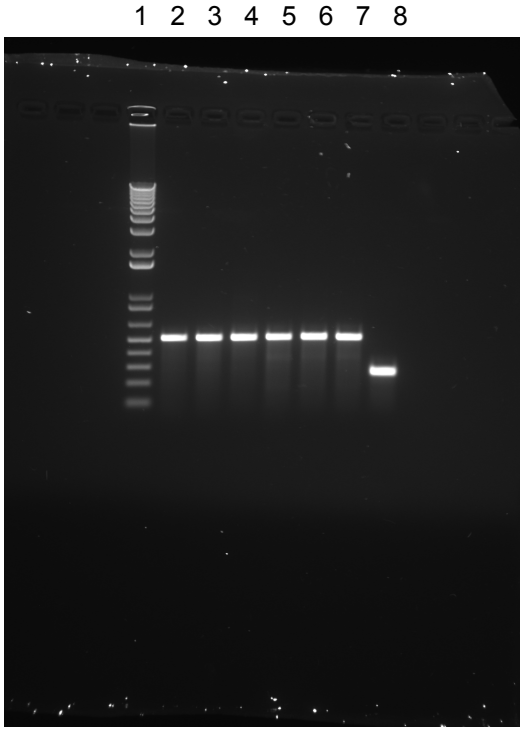


Full unedited gel for Figure 3, top panel. Lanes 1-13 correspond to the lanes shown in the manuscript cropped figure.

Full unedited gels for Figure 5



Full unedited gel for Figure 5, top panel. Lanes 1-8 correspond to the lanes shown in the manuscript cropped figure.



Full unedited gels for Figure 5, bottom panel. Lanes 1-7 correspond to the lanes shown in the manuscript cropped figure.

Full unedited gels for Supplemental Figure 2

Full unedited gel for Figure 2c, .
Lanes 1, 2, and 4 correspond to the lanes shown in the supplemental figure 2.