Plasmid Design

To make single stranded AAV2 compatible plasmids containing constructs compatible for CEM control, a parent single stranded AAV2 plasmid containing a CBA promoter upstream of Firefly luciferase was used. SpeI (New England Biolabs, R3133S) and NcoI (New England Biolabs, R3193S) restriction enzymes were used to remove most of the CBA promoter. In-Fusion HD Enzyme Premix (Takara Bio) was used to insert the cassette containing 12 ZFHD1-specific zinc finger DNA binding domains upstream of a JET promoter. This cassette is inserted in front of the Firefly luciferase gene and cleavage of the T2a peptide would ensure that the ZFHD1 and luciferase proteins are separated inside the cell. In-Fusion HD Cloning Plus was also used to insert a cassette containing a JET promoter upstream of the Firefly luciferase gene.

To make a lentivirus compatible plasmid containing Renilla luciferase, a parent plasmid containing a EF1α promoter was cut with NotI (New England Biolabs, R3189S). Addgene plasmid #74444 containing Renilla luciferase was used as a template to create a cassette containing Renilla luciferase that was inserted into parent plasmid using In-Fusion HD Cloning Plus. All plasmid sequences were verified with sanger sequencing. Single stranded AAV2 plasmids had their ITRs (Inverted Terminal Repeat) verified through restriction enzyme digestion using either XmaI (New England Biolabs, R0180L) or SrfI (New England Biolabs, R0629S). Plasmid constructs were adapted from Hirsch et al.[29], Hathaway et al.[27], and Toyama et al.[28]

AAV2 Production

For viruses that were packaged by the Hirsch Lab and Hathaway Lab:

All viruses were purified through CsCl gradient ultracentrifugation. Fractions containing the functional viruses were determined by adding 1 uL of the virus in CsCl to HEK293 cells in a 24 well plate and then running a luciferase assay to determine relative luminescence per well. Fractions thought to contain functional virus were then confirmed with qPCR. Titer of the functional virus with qPCR was then determined post virus fraction dilation.

Viral Titers:

AAV2 +ZF-FKBP: 1.7E+13 vg/mL
AAV2 -ZF-FKBP: 2.0E+10 vg/mL
AAV2 +ZF-FKBP_GFP: 7.9E+12 vg/mL
AAV8 +ZF-FKBP: 1.9E+13 vg/mL.

qPCR Primers for AAV2 Titering.

Primer Description	**Primer Sequence 5'-3'**
Firefly Luciferase Forward Primer	TGAGTACTTCGAAATGTCCGTTC
Firefly Luciferase Reverse Primer	GTATTCAGCCCATATCGTTTCAT

Statistics

ANOVA calculations through GraphPad Prism v.9 were used to determine p values. Comparisons were made between treatment groups and groups that received 0 nM CEM87. Statistical significance was determined based on the follow p value parameters: * = $P \leq 0.05$, ** = $P \leq 0.01$, *** = $P \leq 0.001$, **** = $P \leq 0.0001$