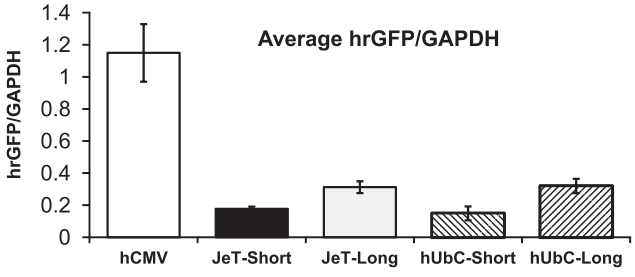
Supplementary Data

SUPPLEMENTARY METHODS

Standard molecular biology and PCR techniques were used to design and construct four mammalian expression plasmids. PCR was used to modify the ends of the DNA fragments, allowing for subcloning into subsequent vectors. Each expression cassette contained a humanized recombinant green fluorescent reporter gene (hrGFP), recovered from the pAAV-hrGFP plasmid (Stratagene, La Jolla, CA). The expression cassettes contained one of two promoters and either a long or short intron sequence. All cassettes contained the SV40 polyadenylation sequence, which was recovered from pUB6/V5-His A (V250-01; Invitrogen, Carlsbad, CA). The human ubiquitin C (hUbC) promoter was recovered from pUB6/V5-His A. The sequence of a ubiquitous synthetic promoter ("JeT") was derived from published literature.[1] The promoter was generated by a PCR-based synthetic gene production strategy.[2] The two introns, long and short, were recovered from pUB6/V5-His A and pCpG-mcs G2 (InvivoGen, San Diego, CA), respectively. Each expression cassette was subcloned into the backbone of the pBLUESCRIPT plasmid (Stratagene). The control chosen for the *in vitro* expression studies was pAAV-hrGFP, which expresses the hrGFP message from the CMV promoter. Large-scale plasmid DNA isolations were completed for each of these plasmids along with the control plasmid. Each of the expression cassettes was confirmed by DNA sequence analysis. Plasmid DNA was quantified and the integrity assessed by restriction endonuclease digestion followed by agarose gel electrophoresis. Plasmid DNA were transfected into HEK293T cells in triplicate with Transit-293 transfection reagent (Mirus, Madison, WI), using the manufacturer's recommended protocol. To normalize for the amount of transfected DNA, and to control for the difference in size of the plasmids, 1.89×10^{11} DNA molecules were transfected into each of the wells. Forty-eight hours after transfection, hrGFP protein expression was visualized by fluorescence microscopy. Quantitative real-time PCR was used to measure the level of hrGFP transcription from each of these constructs. Briefly, total RNA was isolated from the transfected cells, using a mirVana miRNA isolation kit (Applied Biosystems, Foster City, CA), in accordance with the manufacturer's recommended protocol. The transfected cells were homogenized in lysis/binding buffer, using an Omni-Tip homogenization probe (Omni International, Kennesaw, GA). The total RNA was treated with DNase, using a Turbo-DNA-free kit (Applied Biosystems). Random-primed cDNA was prepared from 500 ng of total RNA, using a high-capacity cDNA synthesis kit from Applied Biosystems. The levels of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and hrGFP expression were quantified by real-time PCR. hrGFP expression was normalized to GAPDH expression in each of the wells. The level of expression from each construct was determined by averaging the three independent wells, as shown in Supplementary Fig. S1.



Supplementary Figure S1. Comparison of promoter–intron combinations. A ubiquitous synthesized promoter plus short intron was developed (UsP, patent pending) to allow for scAAV packaging of Hex subunit transgenes. The chart shows the relative GFP expression level of the JeT promoter with the short intron or a long intron compared with the cytomegalovirus (CMV) promoter and the human ubiquitin C (hUbC) promoter with either the same short or long intron. Relative GFP mRNA was assessed in HEK293T cells using real-time PCR normalized to levels of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) ($n=3$; error bars represent the SEM).

REFERENCES

1. Tornøe J, Kusk P, Johansen TE, et al. Generation of a synthetic mammalian promoter library by modification of sequences spacing transcription factor binding sites. Gene 2002;297:21–32.

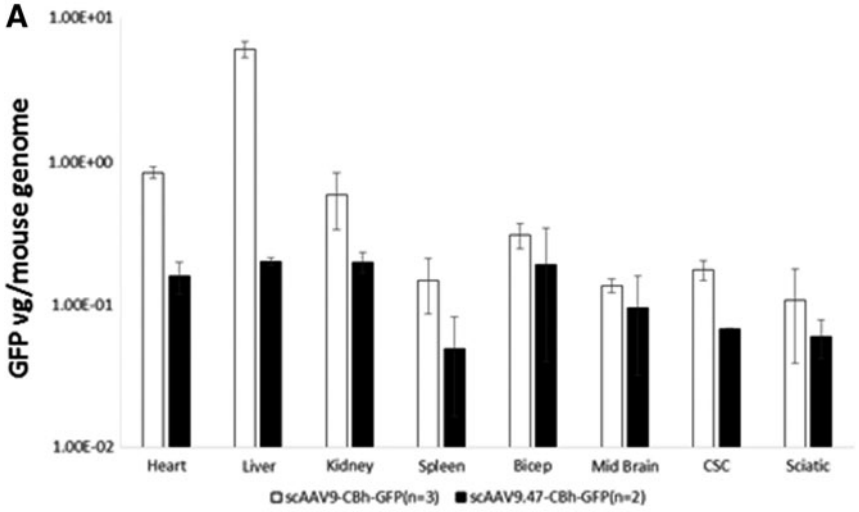
2. Stemmer WP, Crameri A, Ha KD, et al. Single-step assembly of a gene and entire plasmid from large numbers of oligodeoxyribonucleotides. Gene 1995;164:49–53.

Nucleotide sequence of UsP promoter-intron combination

GGGCGGAGTTAGGGCGGAGCCAATCAGCGTGCGCCGTTCCGAAAGTTG
CCTTTTATGGCTGGGCGGAGAATGGGCGGTGAACGCCGATGATTATATAA
GGACGCGCCGGGTGTGGCACAGCTAGTTCCGTCGCAGCCGGGATTTGG
GTCGCGGTTCTTGTTTGTGGATCCCTGTGATCGTCACTTGGTAAGTCACTG
ACTGTCTATGCCTGGGAAAGGGTGGGCAGGAGATGGGGCAGTGCAGGA
AAAGTGGCACTATGAACCCTGCAGCCCTAGGAATGCATCTAGACAATTGTA
CTAACCTTCTTCTCTTTCCTCTCCTGACAGTCCGGAAAGCCACCATG

Supplementary Figure S2. The sequence of the UsP promoter–intron combination as present in the plasmid construct designed for this study. The complete nucleotide sequence, comprising the core JeT promoter sequence (in red), the intron sequence (in green), and restriction sites (in blue), followed by the Kozak consensus sequence with the start codon (in pink), is provided.

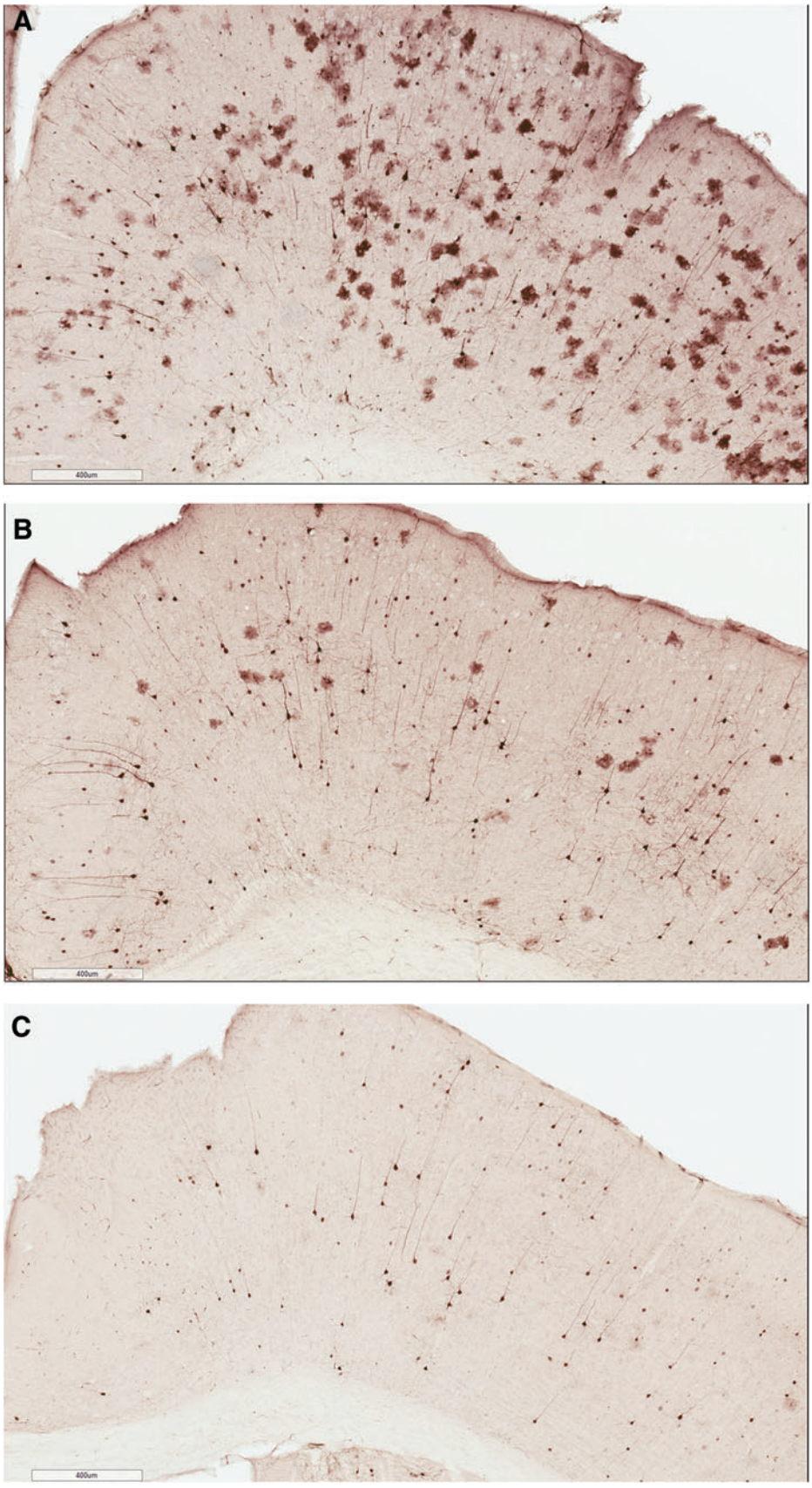
A



B

	Mean number of cells per mm^2		
	Neurons	Astrocytes	Total Cells
scAAV9	30.86	45.80	76.67
scAAV9.47	47.41	11.17	58.58
% change compared to AAV9	⬆ 53.6	⬇ -75.61	⬇ -23.59

Supplementary Figure S3. Biodistribution of GFP **(A)** and average number of neurons and astrocytes per millimeter squared in the brain cortex **(B)** of wild-type mice. Adult wild-type mice were intravenously injected with scAAV9/CBh-*GFP* or scAAV9.47/CBh-*GFP* and analyzed 3 weeks postinjection. **(A)** The average number of GFP vector genomes (vg) in major organs from three mice for scAAV9-CBh-*GFP* vector and two mice for scAAV9.47-CBh-*GFP* vector. CSC, cervical spinal cord. Error bars indicate the SEM. **(B)** The average number of neurons and astrocytes from 40-μm coronal brain sections from these were determined, and the percent change compared with AAV9 was calculated. The count was done by two people independently from three representative images each for each vector, and the data are expressed as average number of cells per millimeter squared.



Supplementary Figure S4. (A) Brain transduction pattern of scAAV9-CBh-*GFP*-BGHpA intravenously injected into wild-type mice and analyzed 3 weeks postinjection. **(B)** Brain transduction pattern of scAAV9.47-CBh-*GFP*-BGHpA intravenously injected into wild-type mice and analyzed 3 weeks postinjection. **(C)** Brain transduction pattern of scAAV9-UsP-*GFP*-SV40pA intravenously injected into wild-type mice and analyzed 3 weeks postinjection.