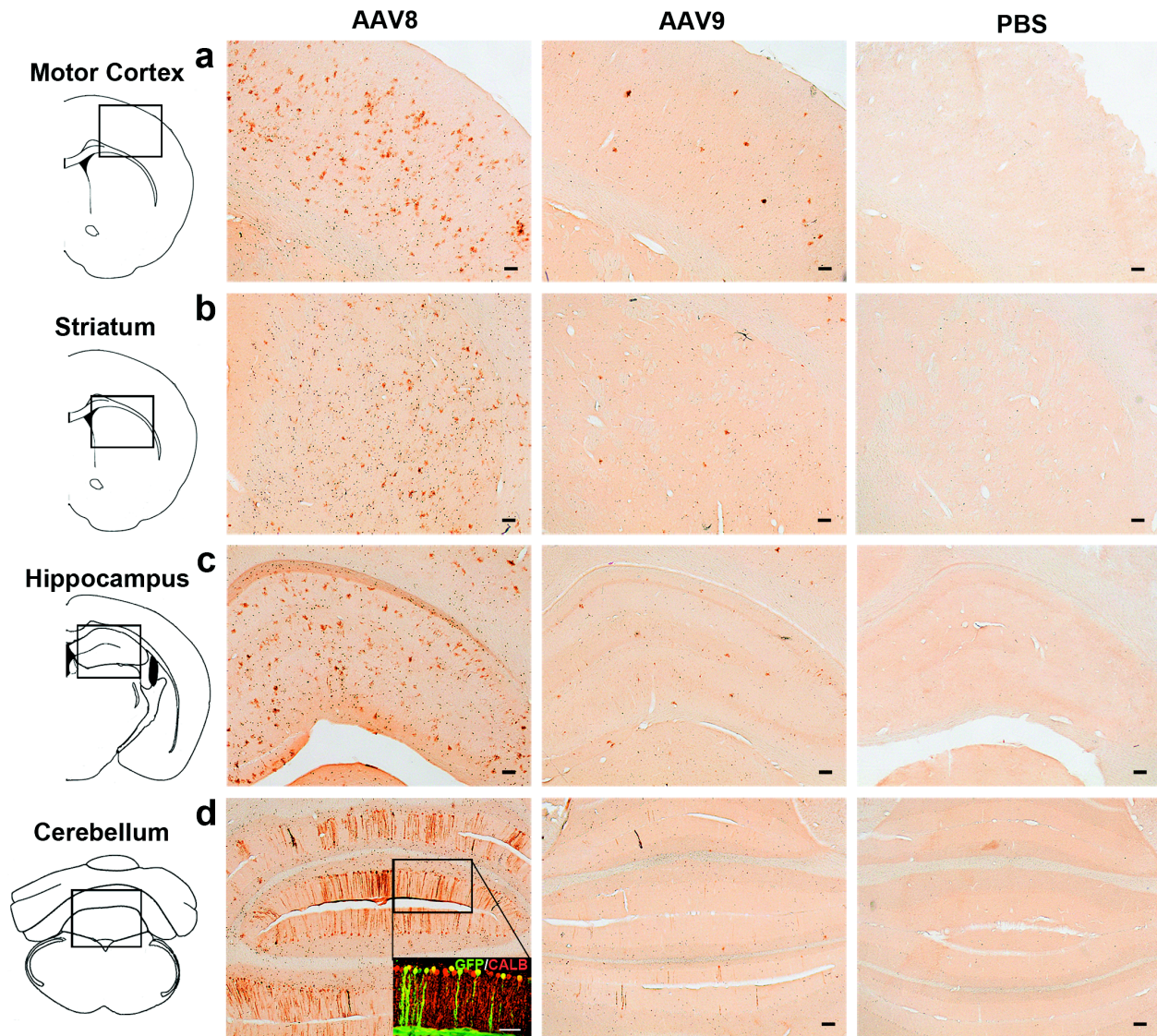
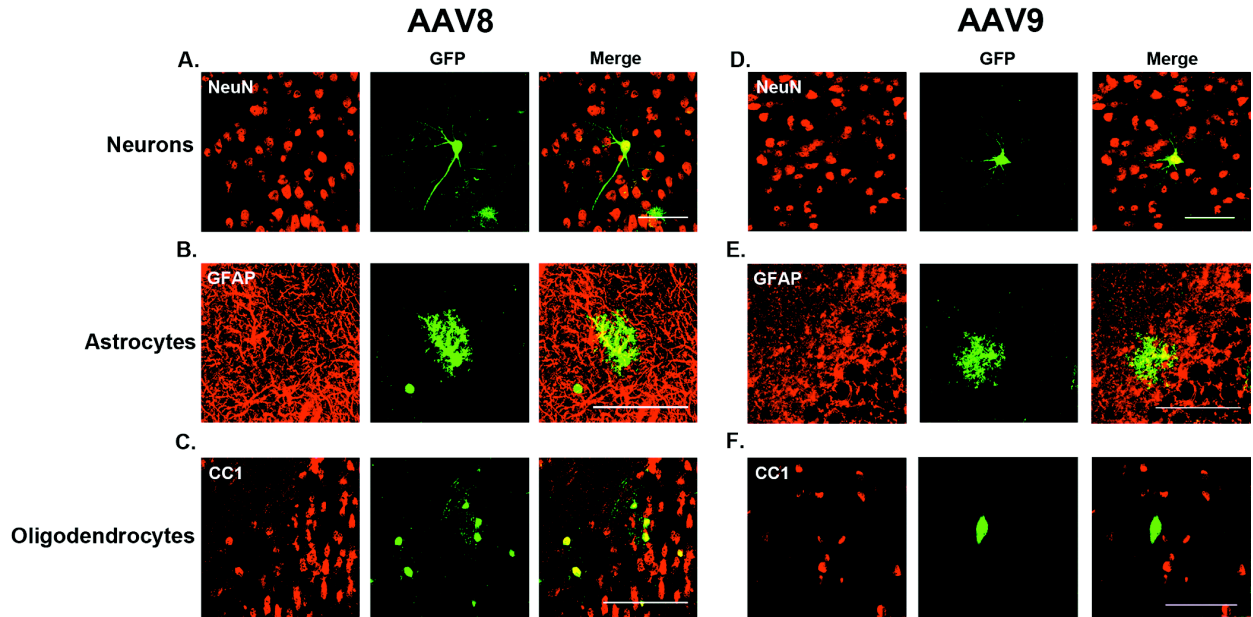


Supplementary Information

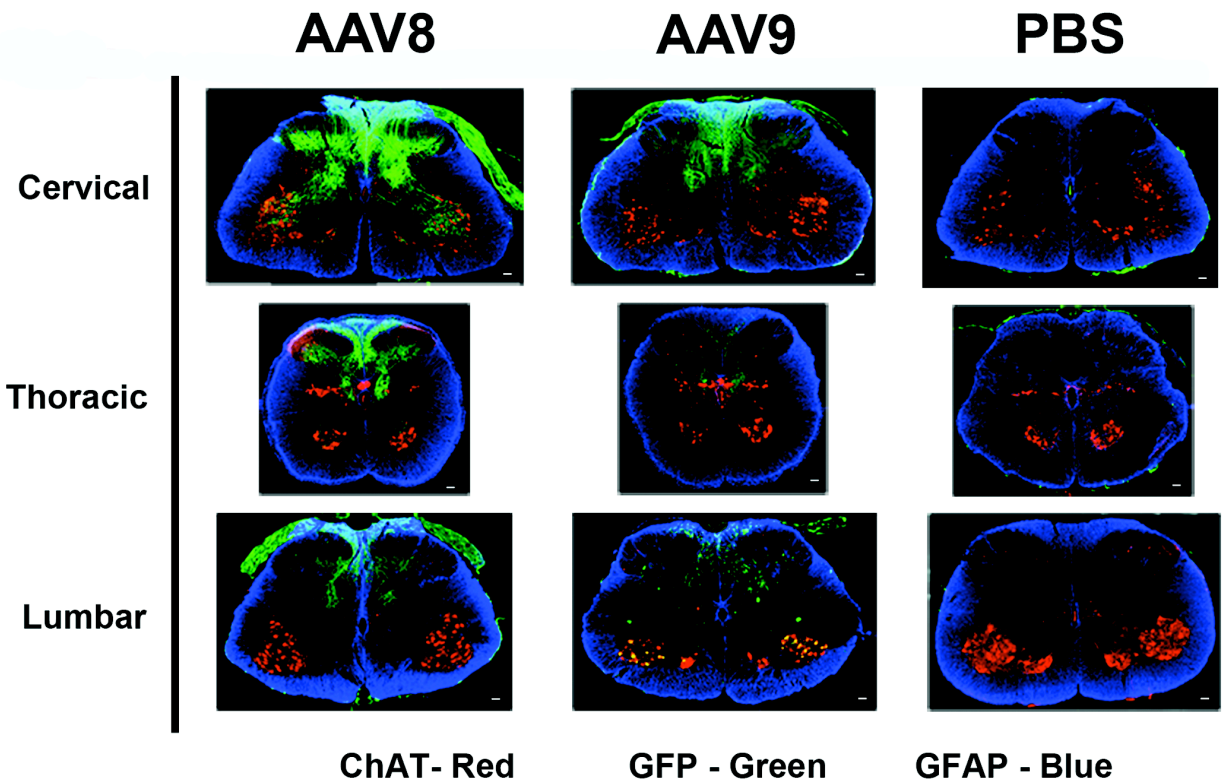


S1 Fig. Representative coronal sections through the brains of AAV treated guinea pigs shows GFP expression throughout. Sections through the motor cortex (a), striatum (b), hippocampus, (c) and cerebellum (d) show GFP expression in AAV8 treated animals and AAV9 treated animals. GFP expression was absent in the PBS treated animal. Co-labeling revealed that GFP expression in the cerebellum was within Purkinje cells due to co-expression with the marker calbindin (d, inset). Scale bars =100µm

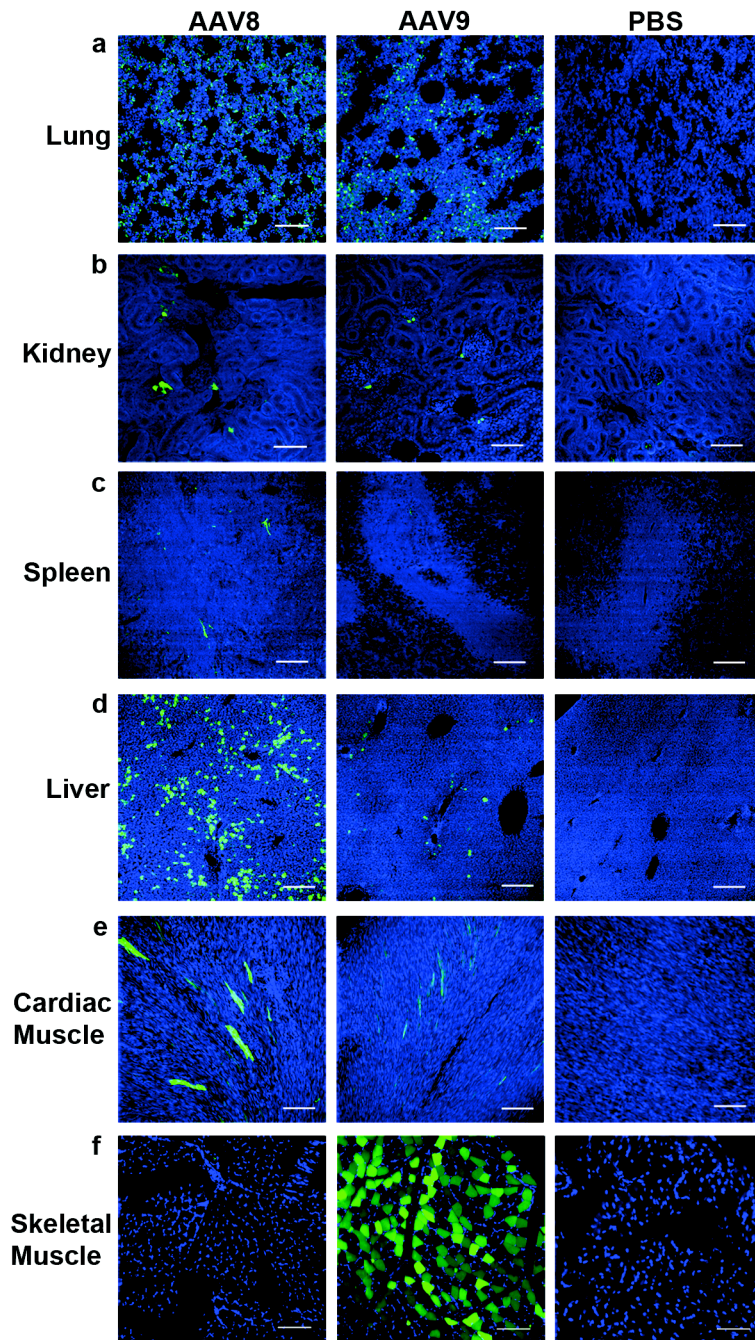


S2 Fig. AAV8 targets neurons, astrocytes and oligodendrocytes in the guinea pig brain.

(a) Co-labeling brain sections (motor cortex) with the neuron mark NeuN (red) indicates GFP expression (green) in neurons. (b) An example of a GFP positive cell is shown co-localizing with the astrocyte marker GFAP (red) (hippocampus). (c) GFP expression was also noted in mature oligodendrocytes as indicated by co-localization with CC1 (red) (striatum). (d-f) show GFP expression in neurons and astrocytes from the brains of AAV9 treated animals. Scale bars =100 μ m



S3 Fig. Spinal cords indicate AAV transduction of dorsal root ganglia and spinal motor neurons. Sections from AAV8 treated spinal cords show labeling of GFP positive fibers in the dorsal roots, dorsal grey matter and dorsomedial white matter indicating DRG neuron transduction. AAV9 treated tissues show a less GFP labeling of DRG fibers, but also show targeting of lower motor neurons (ChAT) in lumbar spinal cord. No GFP is detected in sections from a PBS treated animal. Scale bars =100 μ m



S4 Fig. Sporadic GFP expression is found in multiple AAV treated guinea pig tissues.

GFP expression was most abundant in lungs (a) and liver (d) from AAV8 treated animals and from skeletal muscle of AAV9 treated animals (f). GFP is shown in green and the nuclear marker DAPI is shown in blue. Scale bars =100 μ m

S1 Table. Antibodies used in this study

Antibody	Source	Catalog #	Dilution		
			Gut	CNS	Periphery
Primary Antibodies					
Goat anti-Calretinin	Swant, Marly, Switzerland	CG1	1:200		
Rabbit anti-VIP (Vasoactive Intestinal Peptide)	Abcam, Cambridge, MA, USA	ab43841	1:200		
Goat anti-nNOS (neuronal Nitric Oxide Synthase)	Abcam, Cambridge, MA, USA	ab1376	1:200		
Mouse anti HuD/C	Thermo Fisher, Waltham, MA, USA	A-21271	1:50		
Rabbit anti-Calbindin D-28K	Millipore, Temecula, CA, USA	ab1178	1:200	1:400	
Goat anti-ChAT (Choline Acetyltransferase)	Millipore, Temecula, CA, USA	ab144P	1:50	1:50	
Rabbit anti-S100 Ab-2	Thermo Fisher, Waltham, MA, USA	RB-044-A0	1:200	1:500	
Chicken anti-GFAP (Glia Fibrillary Acidic Protein)	Abcam, Cambridge, MA, USA	ab4674		1:500	
Mouse anti-NeuN (neuronal nuclei)	Millipore, Temecula, CA, USA	MAB377		1:50	
Mouse anti-CC-1 (APC)	Novus Biologicals, Littleton, CO, USA	NB600-1021		1:400	
Chicken anti-GFP (Green Fluorescent Protein)	Abcam, Cambridge, MA, USA	ab13970	1:200	1:500	1:500
Secondary Antibodies					
Donkey anti-chicken Alexa Fluor 488	Jackson ImmunoResearch, West Grove, PA, USA	703-545-155	1:200	1:500	1:500
Donkey anti-chicken Cy3-conjugated	Jackson ImmunoResearch, West Grove, PA, USA	703-165-155	1:200		
Donkey anti-goat Cy3-conjugated	Jackson ImmunoResearch, West Grove, PA, USA	705-165-147	1:200		
Donkey anti-goat Alexa Fluor 647	Jackson ImmunoResearch, West Grove, PA, USA	705-605-147	1:200		
Donkey anti-mouse Alexa Fluor 647	Jackson ImmunoResearch, West Grove, PA, USA	715-605-150	1:200		
Donkey anti-mouse Cy3-conjugated	Jackson ImmunoResearch, West Grove, PA, USA	715-165-150	1:200	1:500	
Donkey anti-rabbit Alexa Fluor 647	Jackson ImmunoResearch, West Grove, PA, USA	711-605-152	1:200	1:500	
Donkey anti-rabbit Cy3-conjugated	Jackson ImmunoResearch, West Grove, PA, USA	711-165-152	1:200		
Donkey anti-chicken Biotin-SP-conjugated (chromagen)	Jackson ImmunoResearch, West Grove, PA, USA	703-065-155		1:500	

Supplementary Table 2) Guinea pig ENS cell counts

Structure	Region	Total HuD+ Neurons	Total GFP+ Neurons	Number of animals	Range of GFP+ cells/animal
<i>Myenteric Plexus</i>					
AAV8	Stomach	618	53	2	2-51
	Duodenum	641	102	2	38-64
	Jejunum	660	70	2	27-43
	Ileum	623	40	2	16-24
	Cecum	848	26	2	13
	Colon	739	49	2	11-38
AAV9	Stomach	624	11	2	3-8
	Duodenum	637	35	2	15-20
	Jejunum	642	23	2	8-15
	Ileum	711	11	2	5-6
	Cecum	625	18	2	8-10
	Colon	667	25	2	11-14
<i>Submucosal Plexus</i>					
AAV8	Duodenum	450	62	2	19-43
	Jejunum	426	121	2	48-73
	Ileum	401	95	2	15-80
	Cecum	494	66	2	3-63
	Colon	482	111	2	50-61
AAV9	Duodenum	319	17	2	7-10
	Jejunum	387	3	2	1-2
	Ileum	555	11	2	5-6
	Cecum	465	13	2	3-1
	Colon	536	15	2	6-9