

**A comprehensive map of CNS transduction by eight recombinant
adeno-associated virus serotypes upon cerebrospinal fluid
administration in pigs**

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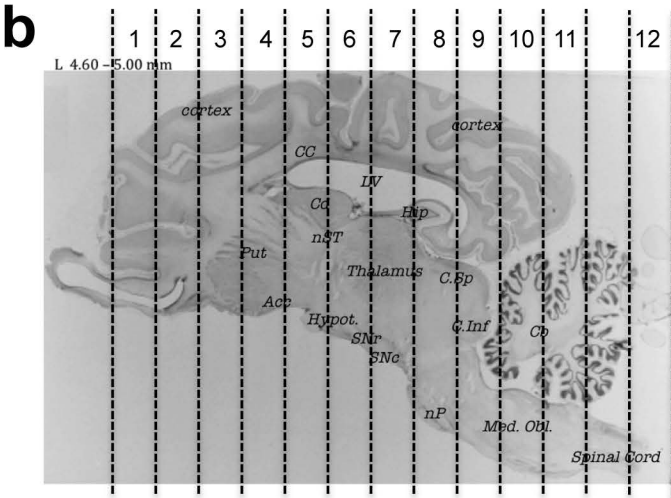
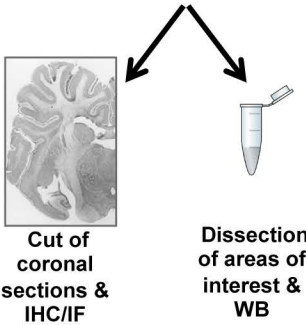
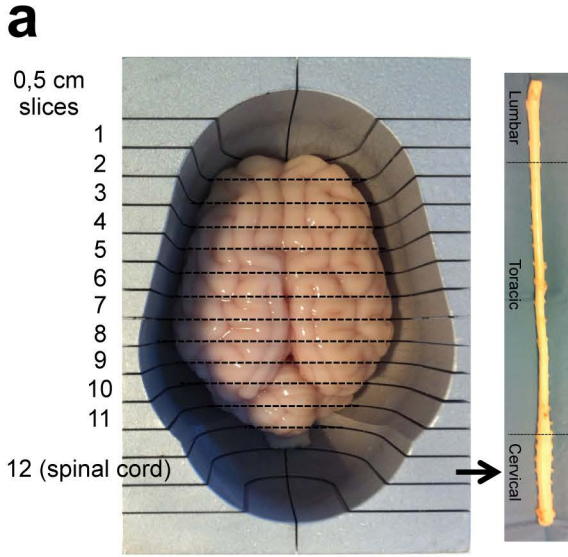
Supplementary Materials:

-Supplementary Figures

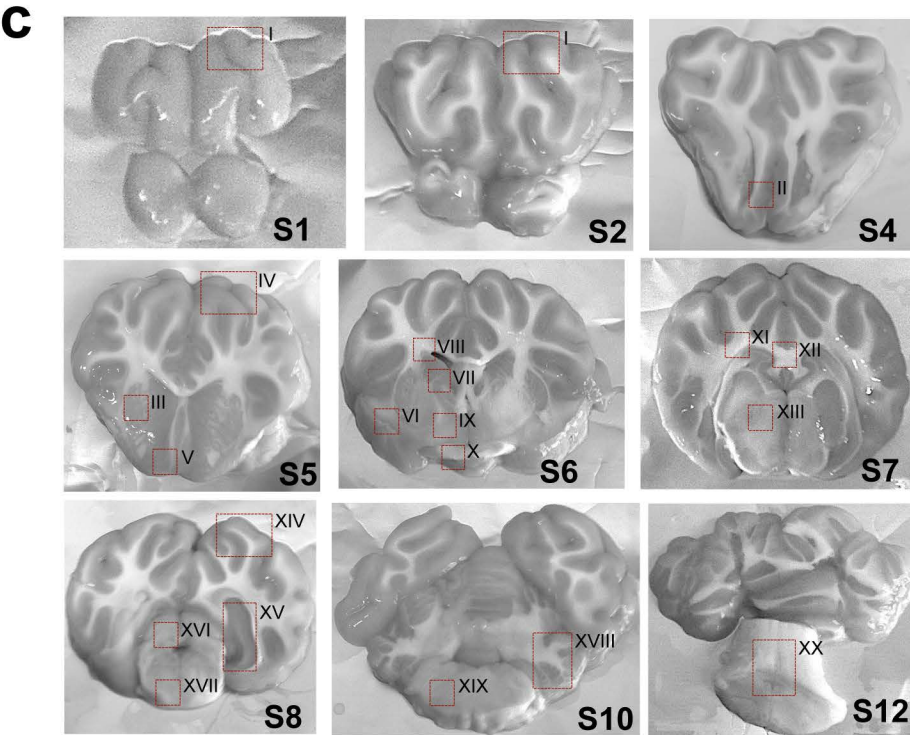
-Supplementary Figure Legends

-Supplementary Tables

SUPPLEMENTARY FIGURE S1



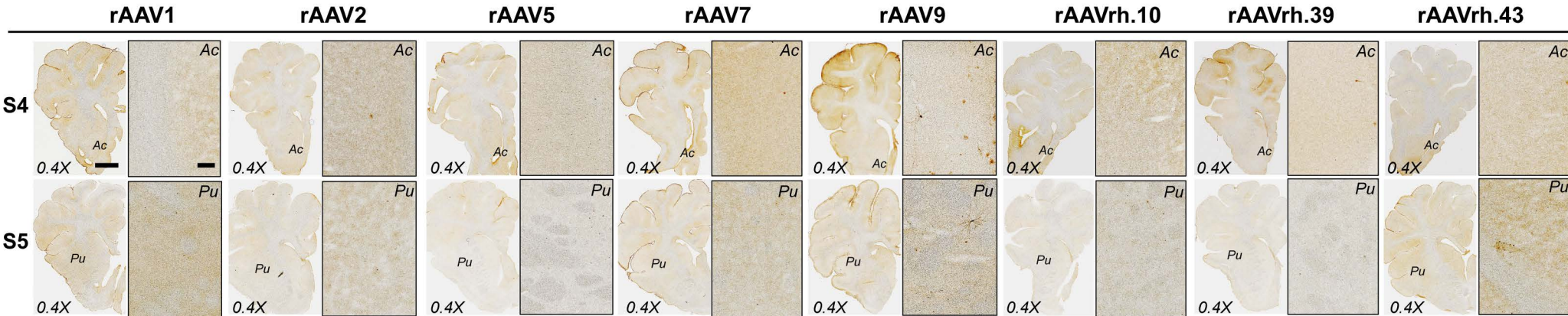
SLICES	AREAS OF INTEREST
Slices 1-3	Frontal cortex (ctx)
Slice 4	Frontal cortex, nucleus accumbens (Acc), putamen (Put)
Slice 5	Parietal cortex, amygdala, hypothalamus (Hypot), nucleus caudatus (Cd).
Slice 6	Parietal cortex, substantia nigra (SN), stria terminalis (ST).
Slice 7	Parietal cortex, hippocampus (Hip.), corpus callosum (CC), thalamus.
Slice 8	Occipital cortex, hippocampus (Hip), nuclei pontis (nP).
Slice 9	Occipital cortex, colliculus superior (CS), colliculus inferior (CInf)
Slice 10	Occipital cortex, cerebellum (Cb)
Slice 11	Medulla oblongata
Slice 12	Spinal cord (cervical region)



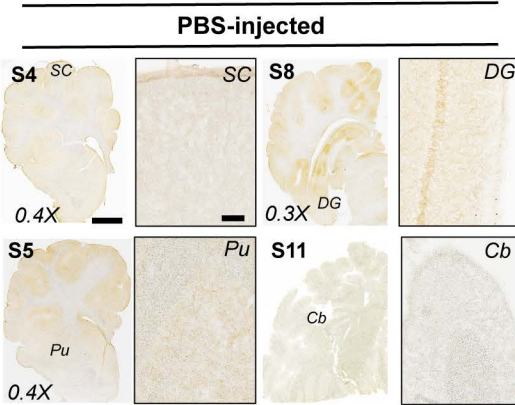
- I. Frontal cortex
- II. Accumbens
- III. Putamen
- IV. Parietal cortex
- V. Hypothalamus
- VI. Amygdala
- VII. N. caudatus
- VIII. Subcallosus
- IX. Sub. nigra
- X. N.pontis
- XI. Stria
- XII. C. callosum
- XIII. Thalamus
- XIV. Occipital cortex
- XV. Hippocampus
- XVI. Colliculi
- XVII. Pons
- XVIII. Cerebellum
- XIX. Med. oblong.
- XX. Spinal cord

SUPPLEMENTARY FIGURE S2

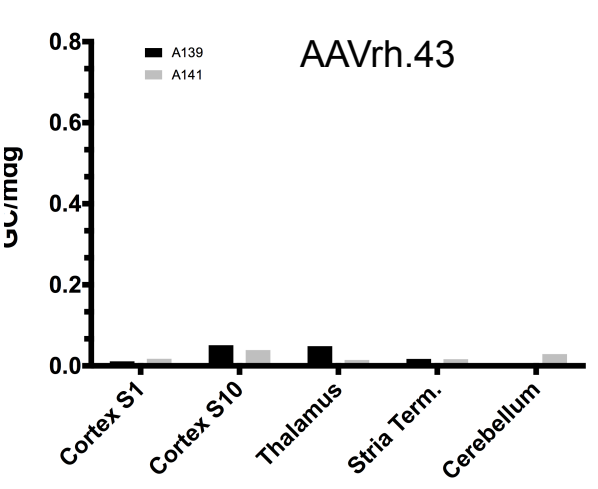
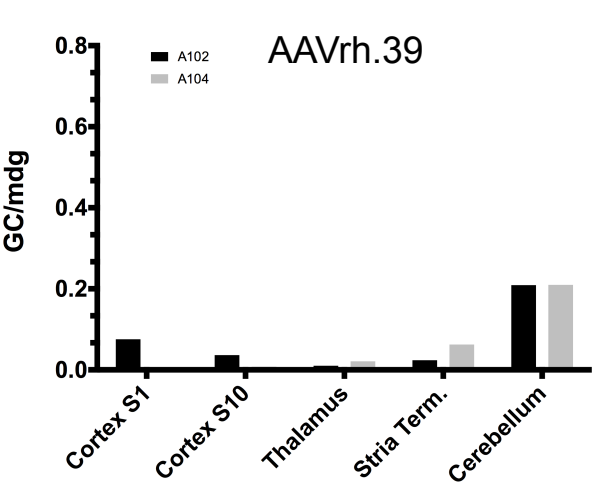
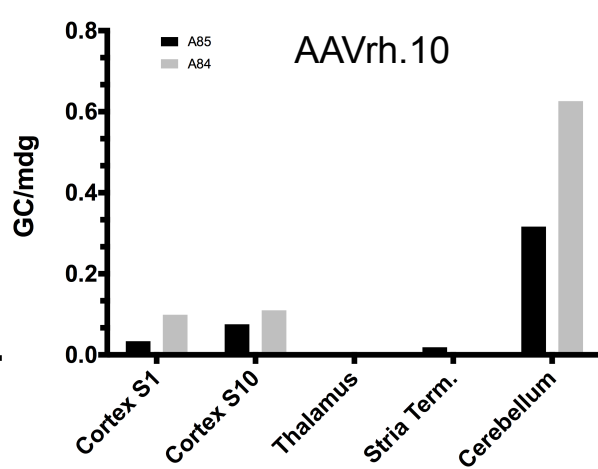
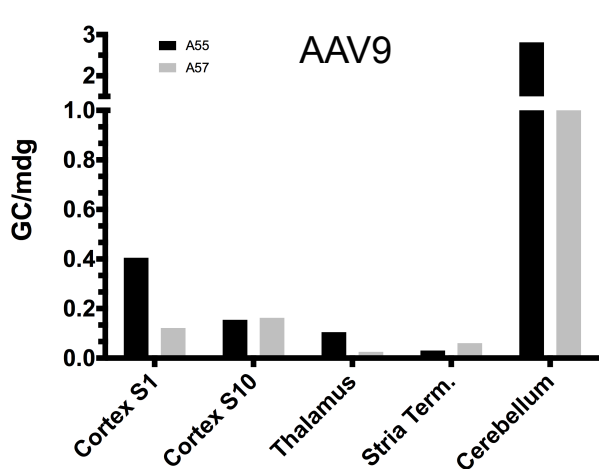
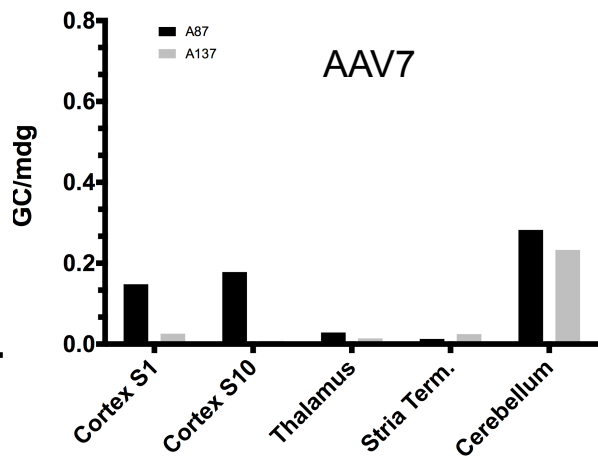
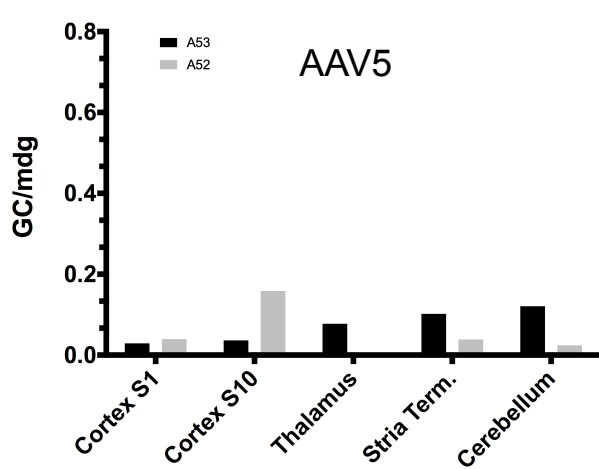
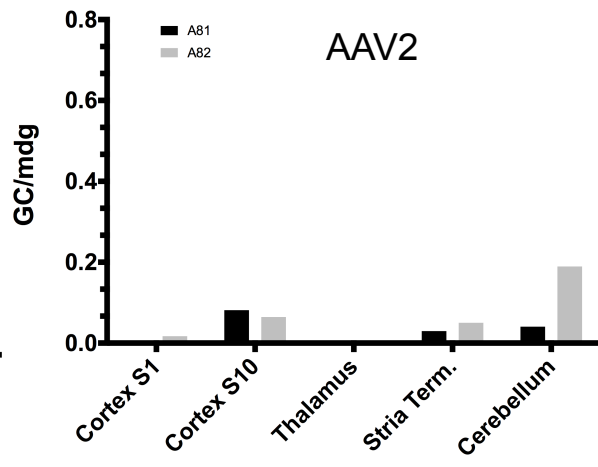
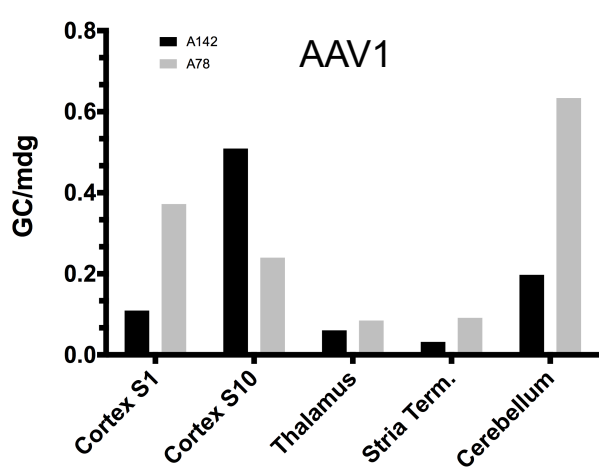
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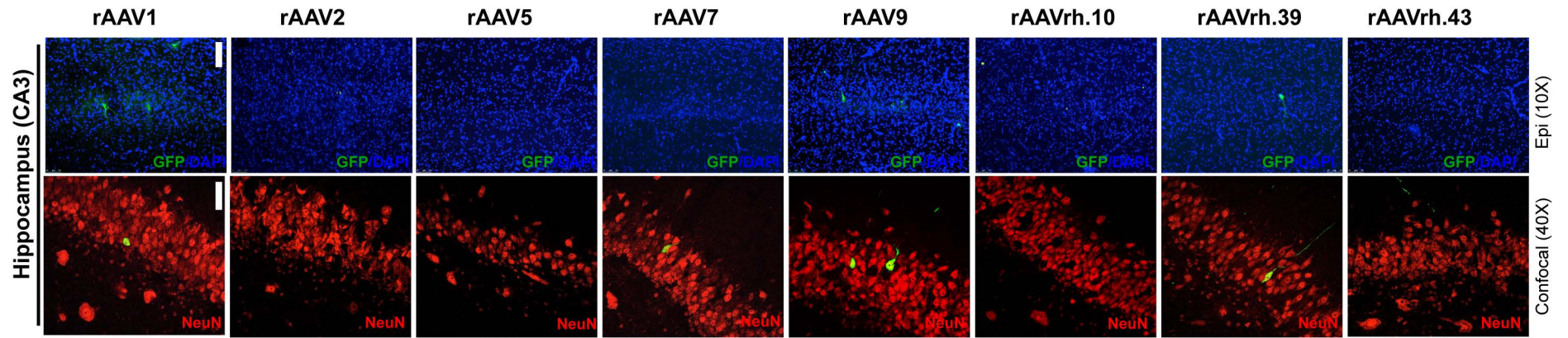
b



SUPPLEMENTARY FIGURE S3



SUPPLEMENTARY FIGURE S4



Supplementary Figure Legends

Supplementary Figure S1: Scheme of pig brain and spinal cord dissection. (a) Pig brain and spinal cord cervical region were divided in 12 coronal slices (0,5 cm) from the rostral to the caudal part to the central nervous system (CNS). Different areas were dissected from one half of each slice for biochemical analysis (western blot). The other half of each slice was used for histological analysis. (b) Sagittal representation of sliced pig brain (adapted figure derived from³¹) in which the main areas of interest are shown. In the table are listed the main areas of interest associated with each of the twelve coronal slices. (c) The CNS areas dissected for western blot analysis were shown.

Supplementary Figure S2: IHC analysis of GFP distribution pattern in basal ganglia of pig brain upon ICM delivery of rAAV1, 2, 5, 7, 9, rh.10, rh.39, and rh.43.

(a) IHC GFP expression in the brain of rAAV-injected pigs in basal ganglia (Putamen and accumbens) are shown in the enlarged (4X) images taken from the 40 µm coronal cryosections (slice S4 and S5). (b) IHC GFP in different CNS areas of control PBS-injected pigs is also shown.

Pu: Putamen; Ac:Accumbens; SC: frontal superficial cerebral cortex (layers I-III); DG: Dentate gyrus of hippocampus; Cb: Cerebellum.

Scale bar for 0,4X images: 4mm. Scale bar of 4X images: 200 µm.

Supplementary Figure S3: Distribution of rAAV vector genomes following ICM injection in pig.

Five different regions of pig brain were isolated from two animals for each serotype injected. The number of viral genome within each region was analyzed by using a LightCycler SYBR green I system. The abundance of vector genomes is showed as copies per diploid cell.

GC: genome copies; mdg: molecules of diploid genome.

Supplementary Figure S4. Cell type tropism of rAAV serotypes in the CA3 area of the hippocampus of injected pigs. Epi-fluorescent images (10X) showed GFP expressing cells co-stained with DAPI in the CA3 area of the hippocampus of rAAV-injected pigs. Confocal images (40X) showed GFP co-localization with NeuN marker in the same area.

Scale bar for epifluorescent images: 75 μm . Scale bar for confocal images: 50 μm .

Supplementary Table S1. Summary of injections in P30 pigs

	Subjects	Weight BI (Kg)	Weight PI (Kg)	Dose (E+12 GC/Kg)	Volume (mL)
rAAV1	A78	7.3	10.7	1.5	0.95
	A79	3.6	5.8	1.5	0.5
	A142	9.5	13.5	1.5	1.3
rAAV2	A81	6.5	10.8	1.5	2.2
	A82	6.4	13	1.5	2.2
	A83	4.9	8.3	1.5	1.8
rAAV5	A52	8.9	14	1.5	2.8
	A53	7.3	13.2	1.5	2.4
	A54	6.2	10.7	1.5	2
rAAV7	A87	3.7	6.5	1.5	1.5
	A137	6.5	11.3	1.5	2.3
	A138	8.3	13.1	1.5	2.9
rAAV9	A55	6.1	11	1.5	2
	A56	7	12	1.5	2.3
	A57	5.7	11.3	1.5	2.2
rAAV rh.10	A84	4.8	7.3	1.5	1.7
	A85	5.3	10.5	1.5	1.8
	A88	6.9	11.4	1.5	2.3
rAAV rh.39	A102	7.8	14.1	1.5	2.5
	A103	9	13.9	1.5	3
	A104	8.3	12.8	1.5	2.7
rAAV rh.43	A139	7.3	13.3	1.5	2.3
	A141	7.9	13	1.5	2.5
	A143	7.8	11.1	1.5	2.5
CTRL	A86	8.5	12	-	2
	A89	6.2	10.6	-	2
	A133	9	12.1	-	2

Abbreviations: GC, genome copies; CTRL, Control-PBS injected pigs.
BI: before injection; PI: post injection.

Supplementary Table S2. Neutralizing anti-AAV antibody levels

AAV vector	Subject	Serum Anti-AAV		CSF Anti-AAV	
		Pre inj.	1 month Post inj.	Pre inj.	1 month Post Inj.
<i>rAAV1</i>	#A78	-	+	-	+
	#A79	-	-	-	-
	#A135	-	-	-	-
<i>rAAV2</i>	#A81	-	+	-	+
	#A82	-	+	-	+
	#A83	-	+	-	+
<i>rAAV5</i>	#A52	-	-	-	+
	#A53	-	+	-	+
	#A54	-	+	-	+
<i>rAAV7</i>	#A87	-	-	-	-
	#A137	-	+	-	-
	#A138	-	+	-	-
<i>rAAV9</i>	#A55	-	-	-	-
	#A56	-	-	-	-
	#A57	-	-	-	-
<i>rAAVrh.10</i>	#A84	-	-	-	-
	#A85	-	+	-	-
	#A88	-	-	-	-
<i>rAAVrh.39</i>	#A102	-	-	-	-
	#A103	-	-	-	-
	#A104	-	-	-	-
<i>rAAVrh.43</i>	#A139	-	-	-	-
	#A141	-	-	-	-
	#A143	-	-	-	-

Abbreviations: CSF, cerebrospinal fluid; AAV, Adeno-Associated Viral vector; inj., injection.
 “-“ Indicates undetectable Nab response. “+” indicates the presence of anti-AAV neutralizing antibodies in the sample

Supplementary Table S3. Biochemical and hematological parameters

Target	Variable unit	Control group		rAAV1		rAAV2		rAAV5		rAAV7		rAAV9		rAAVrh.10		rAAVrh.39		rAAVrh.43	
		Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post
Renal Damage	Creatinine mg/dL	1.06 ±0.08	0.99 ±0.04	0.99 ±0.14	0.93 ±0.08	0.96 ±0.07	0.95 ±0.06	1.11 ±0.14	1.00 ±0.05	0.92 ±0.07	0.82 ±0.02	1.09 ±0.08	0.99 ±0.06	0.91 ±0.03	0.82 ±0.08	0.99 ±0.03	0.88 ±0.07	1.11 ±0.04	0.89 ±0.03
	Urea mg/dL	11.63 ±2.31	20.24 ±4.56	10.30 ±3.40	19.19 ±2.95	12.81 ±3.18	15.11 ±2.26	7.53 ±2.18	10.22 ±1.09	13.28 ±6.21	13.01 ±0.94	10.73 ±5.17	18.28 ±2.73	17.40 ±5.62	18.74 ±2.58	13.40 ±2.63	16.80 ±4.26	8.57 ±2.77	17.64 ±3.06
Liver Damage	AST U/L	27.67 ±3.17	43.60 ±7.54	30.75 ±3.47	45.00 ±4.63	47.20 ±6.16	50.00 ±7.78	41.00 ±9.99	40.25 ±7.09	33.33 ±9.40	48.50 ±6.50	34.75 ±2.95	43.00 ±3.69	46.00 ±4.80	46.75 ±4.31	60.50 ±7.01	52.00 ±9.11	31.67 ±3.84	51.07 ±9.95
	ALT U/L	39.50 ±5.68	52.80 ±9.26	41.50 ±8.29	49.33 ±7.40	36.80 ±3.61	49.40 ±8.28	45.50 ±2.50	40.50 ±8.10	39.33 ±5.61	62.50 ±4.50	35.00 ±1.08	47.43 ±4.91	49.00 ±6.77	53.50 ±0.65	50.75 ±6.56	50.25 ±9.88	36.00 ±1.53	58.10 ±6.00
Inflammation Acute Phase Response	Albumin g/dL	3.01 ±0.17	2.35 ±0.28	2.61 ±0.21	2.10 ±0.19	2.33 ±0.22	2.04 ±0.26	2.98 ±0.44	2.08 ±0.10	2.59 ±0.65	2.06 ±0.52	2.96 ±0.09	2.50 ±0.13	2.08 ±0.37	1.75 ±0.26	2.80 ±0.18	2.49 ±0.35	2.80 ±0.07	1.80 ±0.45
	Albumin/globulin Ratio	1.75 ±0.14	0.99 ±0.20	1.58 ±0.18	0.95 ±0.17	1.50 ±0.21	0.85 ±0.18	2.27 ±0.48	1.18 ±0.13	1.74 ±0.64	0.85 ±0.26	1.70 ±0.08	1.14 ±0.10	0.90 ±0.30	0.66 ±0.13	1.18 ±0.17	0.94 ±0.25	1.90 ±0.05	0.68 ±0.12
	Total Protein g/dL	4.77 ±0.17	4.88 ±0.13	4.29 ±0.24	4.51 ±0.10	3.95 ±0.16	4.72 ±0.31	4.31 ±0.35	3.90 ±0.16	4.49 ±0.35	4.52 ±0.39	4.73 ±0.12	4.78 ±0.20	4.67 ±0.10	4.50 ±0.18	5.39 ±0.48	5.89 ±0.70	4.28 ±0.09	4.88 ±0.19
	WBC x 10 ³ /μL	10.41 ±0.34	17.27 ±1.91	9.28 ±0.71	17.27 ±2.65	11.33 ±0.76	18.69 ±2.01	12.20 ±2.72	18.29 ±2.18	11.72 ±2.35	18.17 ±3.59	8.64 ±1.47	15.2 ±1.72	18.53 ±8.53	19.51 ±2.15	16.38 ±2.60	17.48 ±2.49	10.60 ±1.54	17.57 ±3.14
	Hb g/dL	8.80 ±0.39	8.98 ±0.34	8.97 ±0.42	8.85 ±0.46	9.70 ±0.79	8.54 ±0.76	9.80 ±0.50	9.56 ±0.52	8.67 ±0.74	7.37 ±1.02	11.77 ±0.94	10.50 ±0.50	10.10 ±0.81	8.00 ±0.91	10.18 ±1.30	10.28 ±0.97	8.90 ±0.38	9.33 ±0.33
Inflammation Immunodeficiency	Lymphocytes x 10 ³ /μL	5.31 ±0.64	9.14 ±1.42	5.33 ±0.57	9.13 ±0.86	6.33 ±1.04	9.81 ±0.88	7.90 ±3.53	9.43 ±0.90	5.41 ±0.74	7.37 ±1.62	4.17 ±0.85	7.33 ±0.87	12.00 ±5.75	8.93 ±0.99	6.20 ±1.37	8.31 ±0.84	5.94 ±1.19	9.03 ±1.45
	Monocytes x 10 ³ /μL	0.38 ±0.07	0.74 ±0.13	0.43 ±0.07	0.74 ±0.09	0.45 ±0.05	0.79 ±0.31	0.67 ±0.29	0.83 ±0.12	0.36± 0.09	0.74 ±0.20	0.43± 0.03	0.67 ±0.11	1.18 ±0.82	0.73 ±0.14	0.59 ±0.21	0.63 ±0.14	0.51 ±0.07	0.92 ±0.21
	Neutrophils x 10 ³ /μL	4.44 ±0.76	6.72 ±0.63	3.24 ±0.21	6.77 ±1.90	4.28 ±0.48	7.33 ±1.24	3.38 ±1.05	7.43 ±1.69	5.81 ±1.91	9.29 ±3.23	3.73 ±1.02	6.71 ±1.10	4.78 ±1.73	9.12 ±1.51	9.31 ±1.10	7.80 ±1.55	3.85 ±0.48	7.09 ±2.13
	Eosinophils x 10 ³ /μL	0.13± 0.08	0.44 ±0.01	0.09 ±0.01	0.32 ±0.07	0.07 ±0.01	0.50 ±0.23	0.09 ±0.05	0.28 ±0.03	0.05 ±0.01	0.64 ±0.26	0.20 ±0.13	0.30 ±0.04	0.06 ±0.02	0.52 ±0.17	0.08 ±0.01	0.56 ±0.26	0.15 ±0.05	0.34 ±0.11

Pre- and post-injection biochemical and hematological markers. Data are reported as mean ± standard error (SEM). Abbreviations: AST, aspartate aminotransferase; ALT, alanine aminotransferase; WBC, white blood cells; Hb, hemoglobin.

Supplementary Table S4. Summary of GFP transduction

Brain areas	WB _{n=3}								IF/IHC _{n=2}							
	rAAV1	rAAV2	rAAV5	rAAV7	rAAV9	rAAV rh.10	rAAV rh.39	rAAV rh.43	rAAV1	rAAV2	rAAV5	rAAV7	rAAV9	rAAV rh.10	rAAV rh.39	rAAV rh.43
Cortex	++	n.d.	+	++	++++	n.d.	n.d.	n.d.	++	++	½ +	++	++++	½ +	n.d.	+
Putamen	+	n.d.	+	+	+	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	++	n.d.	n.d.	n.d.
N.caudatus	n.d.	n.d.	++	½ +	++	n.d.	n.d.	n.d.	½ + <small>n=2</small>	n.d.	n.d.	n.d.	+	n.d.	n.d.	n.d.
Accumbens	½ +	n.d.	+	½ +	+++	n.d.	n.d.	n.d.	½ +	n.d.	n.d.	n.d.	++	n.d.	n.d.	n.d.
Corpus callosum	n.d.	n.d.	+	½ +	++	n.d.	n.d.	n.d.	+	n.d.	+	n.d.	+	n.d.	n.d.	n.d.
Thalamus	½ +	n.d.	+	½ +	++	n.d.	n.d.	n.d.	n.d.	n.d.	½ +	n.d.	+	n.d.	n.d.	n.d.
Hypothalamus	½ +	n.d.	+	+	++	n.d.	n.d.	½ +	n.d.	n.d.	½ +	n.d.	½ +	n.d.	n.d.	n.d.
Stria terminalis	½ +	n.d.	+	½ +	++	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	½ +	+	n.d.	n.d.	n.d.
Sub. Nigra	½ +	n.d.	+	½ +	++	n.d.	n.d.	n.d.	n.d.	n.d.	½ +	n.d.	+	n.d.	n.d.	n.d.
Hippocampus	+	n.d.	+	½ +	++	n.d.	n.d.	n.d.	+	n.d.	½ +	½ +	++	n.d.	n.d.	n.d.
N. Pontis	½ +	n.d.	n.d.	n.d.	+	n.d.	n.d.	n.d.	½ +	n.d.	½ +	½ +	++	n.d.	n.d.	n.d.
Colliculi	½ +	n.d.	n.d.	n.d.	+	n.d.	n.d.	½ +	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Cerebellum	+	n.d.	n.d.	++	+++	+	½ +	n.d.	+	½ +	½ +	+++	++++	½ +	+	½ +
Medulla	½ +	n.d.	n.d.	n.d.	+	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	++	n.d.	n.d.	n.d.
Ablongata	½ +	n.d.	n.d.	n.d.	+	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	++	n.d.	n.d.	n.d.
Spinal cord	+	n.d.	n.d.	½ +	++	n.d.	+	n.d.	++	n.d.	½ +	+	+++	n.d.	½ +	½ +

Abbreviations: AAV, adeno-associated virus; WB, western blotting; IHC, immunohistochemistry; IF, immunofluorescence; n, the number of animals analyzed Scoring: maximum GFP signal detected was scored with +++++; minimum GFP signal detected was scored with ½+; n.d. not detected.