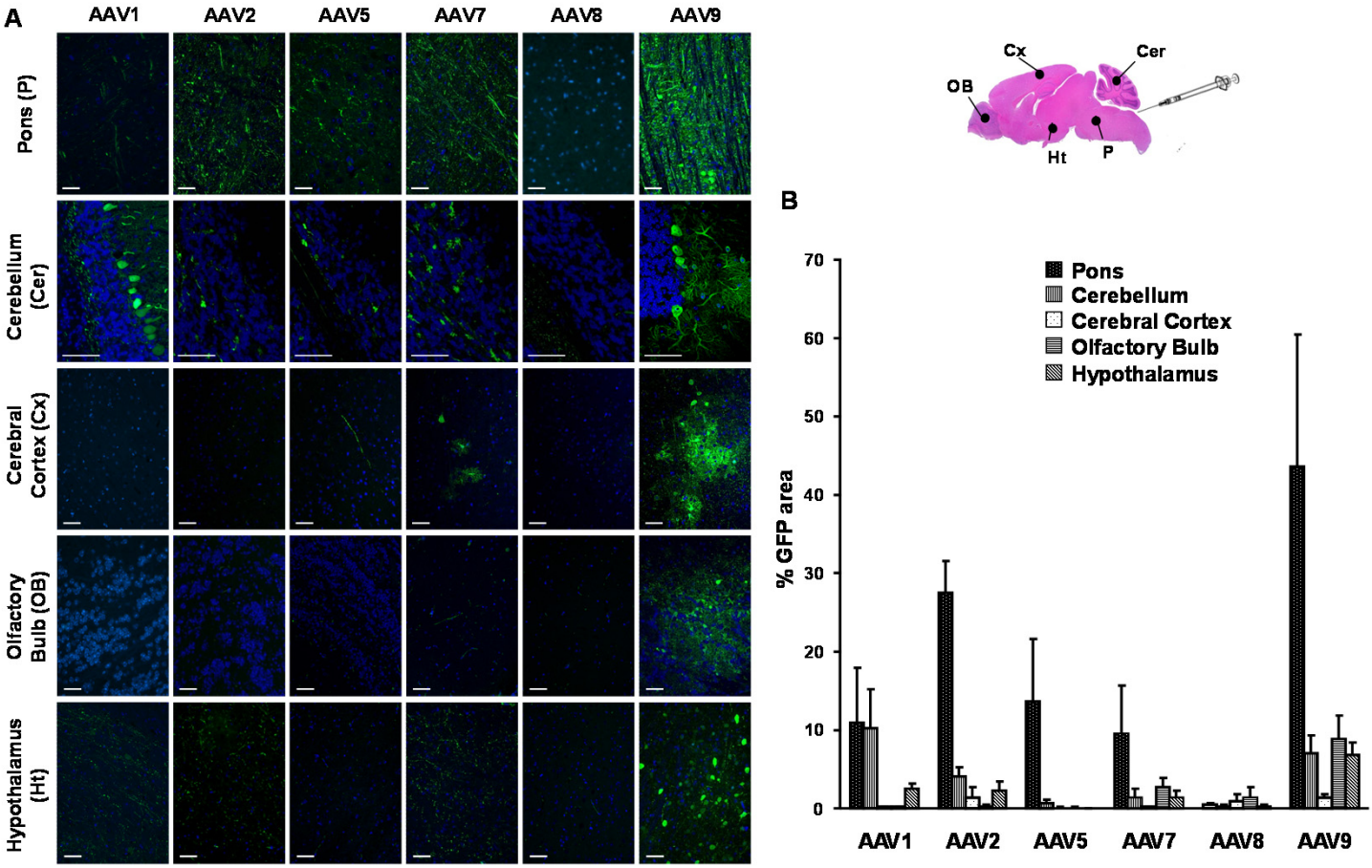
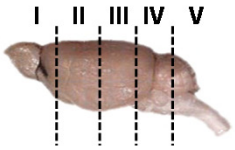
Supplemental Figure 1



Supplemental Figure 1. Widespread CNS transduction after intra-CSF delivery of AAV9. To identify the AAV serotype with the broadest brain biodistribution after direct-CSF administration through the cisterna magna, we injected healthy adult mice with $5x10^{10}$vg of vectors expressing the reporter Green Fluorescent Protein (GFP) under the control of a ubiquitous promoter (CAG). **(A)** Transduction efficiency was analyzed by GFP immunofluorescence 2 weeks after vector administration. All serotypes efficiently transduced cells of the pons (P) and cerebellum (Cer), both easily accessible from the point of injection due to their localization near the cisterna magna (see diagram). The pons was transduced by all serotypes, with lowest and highest efficacy for AAV8 and AAV9, respectively. AAV1 and 9 showed a striking ability to transduce Purkinje cells, which were targeted to a lesser extent by all serotypes with the exception of AAV8. Greater differences among serotypes were observed in deeper and more distant brain regions. Many cells were transduced in the hippocampus (data not shown), cerebral cortex and in the olfactory bulb in the AAV9-injected group, and to a lesser extend in the AAV7 group, whereas no GFP-positive cell bodies were observed with AAV1, 2, 5 or 8. In the hypothalamus, AAV9 showed the greatest efficiency of transduction. Occasional GFP-positive axons could be observed throughout the whole brain in all groups, even in areas with overall low transduction (see image corresponding to cerebral cortex-AAV5), suggesting they projected from neurons transduced in other areas. Scale bars: 100 μm. **(B)** Quantification of the fluorescence signal intensity obtained for each serotype in each CNS area (4-10 images per area) depicted in (A). Results are shown as mean ± SEM; n=3-5 animals per group.

Supplemental Figure 2

A

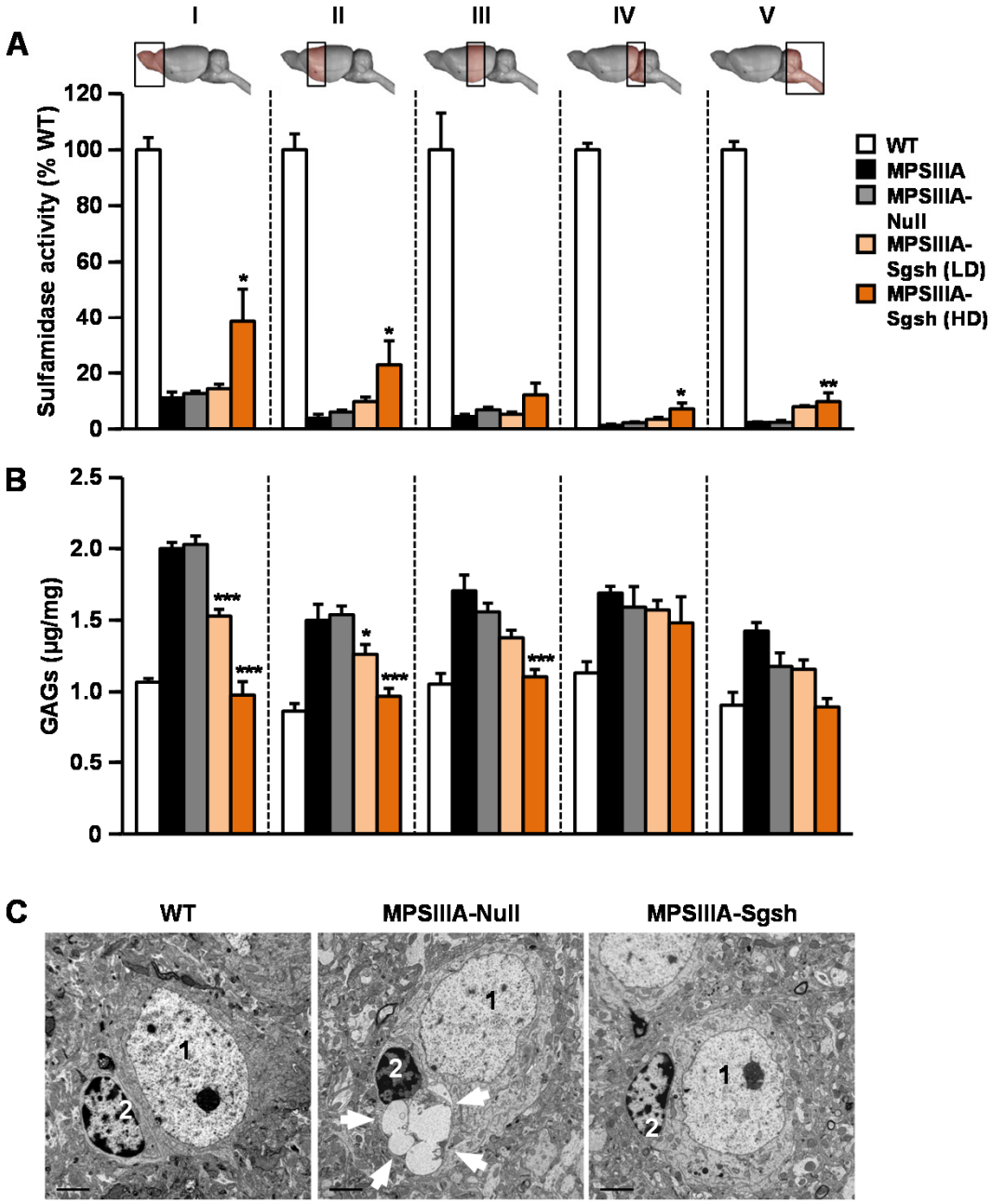
Brain Section 	Vector genomes /diploid genome	
	Males	***Females***
I	0.26 ± 0.04	0.21 ± 0.06
II	0.11 ± 0.03	0.14 ± 0.09
III	0.18 ± 0.07	0.45 ± 0.18
IV	0.11 ± 0.03	0.25 ± 0.10
V	0.45 ± 0.35	0.31 ± 0.09

B

Tissue	Vector genomes /diploid genome	
	Males	***Females***
Liver	1.56 ± 0.33	1.05 ± 0.07
Pancreas	*ND*	*ND*
Intestine	*ND*	0.01 ± 0.00
Kidney	0.02 ± 0.01	0.05 ± 0.00
Urinary bladder	0.01 ± 0.00	*ND*
Gonads	0.01 ± 0.00	0.01 ± 0.00
Spleen	0.17 ± 0.06	0.17 ± 0.02
Lung	0.03 ± 0.01	0.04 ± 0.01
Heart	0.03 ± 0.01	0.05 ± 0.01
Skeletal muscle	0.01 ± 0.00	0.01 ± 0.00

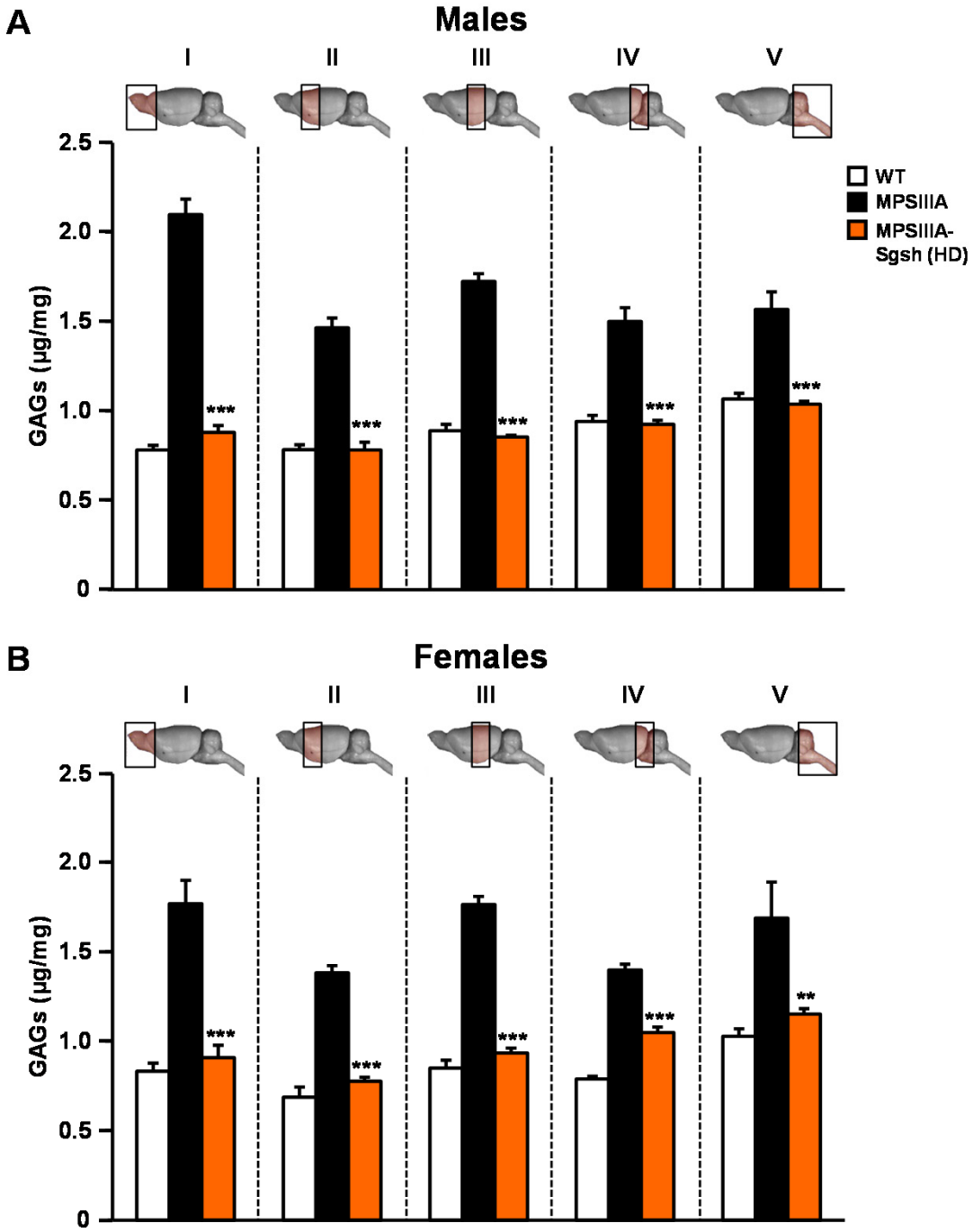
Supplemental Figure 2. AAV9 vector biodistribution following intra-CSF delivery. Adult healthy male and female mice received an intracisternal injection of $5x10^{10}$ vg of AAV9-CAG-GFP. Three weeks after vector administration, gene copy numbers were determined in DNA isolated from several tissues by quantitative PCR with primers specific for GFP. **(A)** The brain was sliced in 5 coronal sections (I-V). Similar gene copy numbers were observed throughout the brain in both genders. **(B)** Vector gene copy numbers in somatic tissues; liver showed the highest transduction levels. Gene copy numbers were very low in all other tissues except for spleen. Results are shown as mean ± SEM; n=3 animals per group. ND, non-detectable.

Supplemental Figure 3



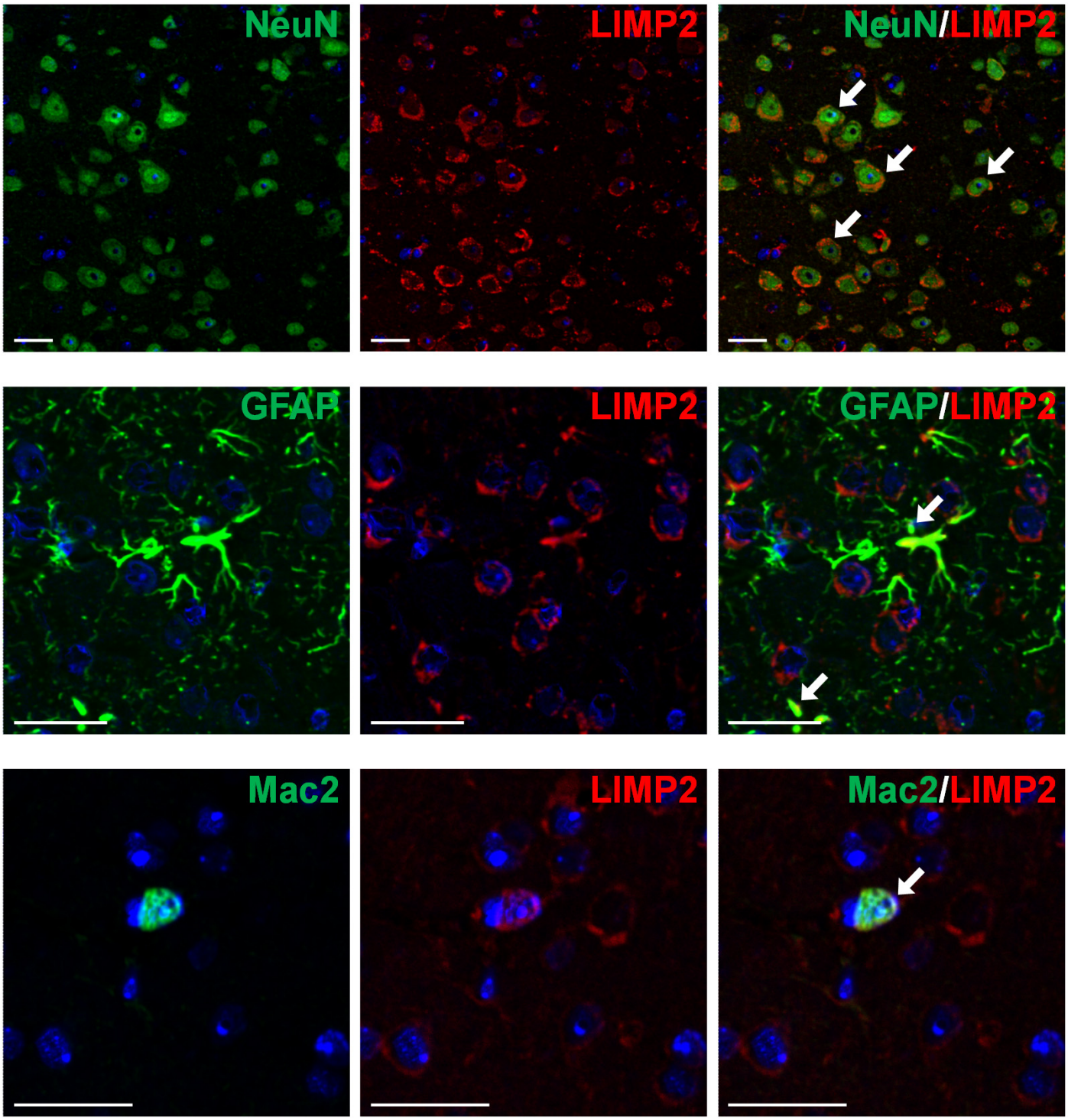
Supplemental Figure 3. Sulfamidase activity and lysosomal pathology after intra-CSF delivery of AAV9-mSgsh to MPSIIIA female mice. MPSIIIA female mice were treated at 2 months of age with $5x10^9$ (LD) or $5x10^{10}$ (HD) vg of AAV9-mSgsh or with $5x10^{10}$ vg of a non-coding (MPSIIIA-Null) vector as control and analyzed 4 months later. **(A)** Percentage of WT sulfamidase activity and **(B)** GAG content in different brain regions (I-V, illustrated in the diagrams above). Results are shown as mean ± SEM; n=4-6 animals per group. WT sulfamidase activity was set to 100%, which corresponded to 3.61 ± 0.09, 3.71 ± 0.43, 4.85 ± 0.34, 4.74 ± 0.18, and 4.73 ± 0.39 nmol/17h/mg protein for sections I-V, respectively. *, $p<0.05$, **, $p<0.01$ and ***, $p<0.001$ *vs.* MPSIIIA-Null. **(C)** Transmission electron microscopy illustrating a striking reduction in lysosomal pathology in cells of the frontal cortex of AAV9-mSgsh-treated females. Large electro-luscent storage vesicles (arrows) were evident in perineuronal glial cells of MPSIIIA females receiving null vector but were absent from perineuronal cells of healthy (WT) or AAV9-mSgsh treated MPSIIIA females. (1) neuron, (2) perineuronal glial cell. Scale bars: 2 µm.

Supplemental Figure 4



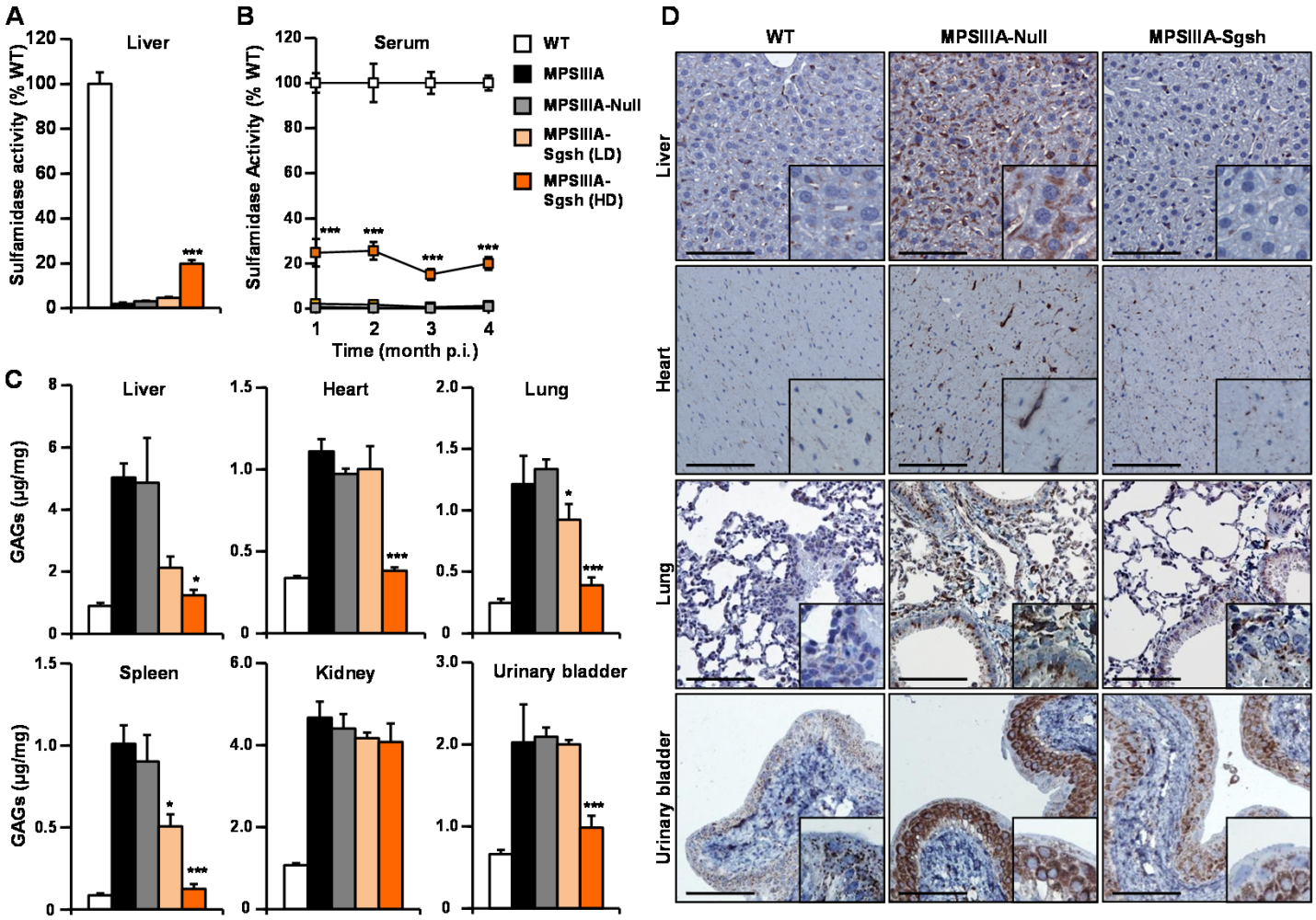
Supplemental Figure 4. Long-term correction of GAG accumulation in the brain of treated MPSIIIA males and females. (A-B) GAG content in different brain regions (I-V, illustrated in the diagrams above) of **(A)** male and **(B)** female MPSIIIA mice measured 10-12 months after a single delivery of $5x10^{10}$ (HD) vg of AAV9-mSgsh. Results are shown as mean ± SEM; n=4-5 animals per group. **, $p<0.01$ and ***, $p<0.001$ *vs.* untreated MPSIIIA mice.

Supplemental Figure 5



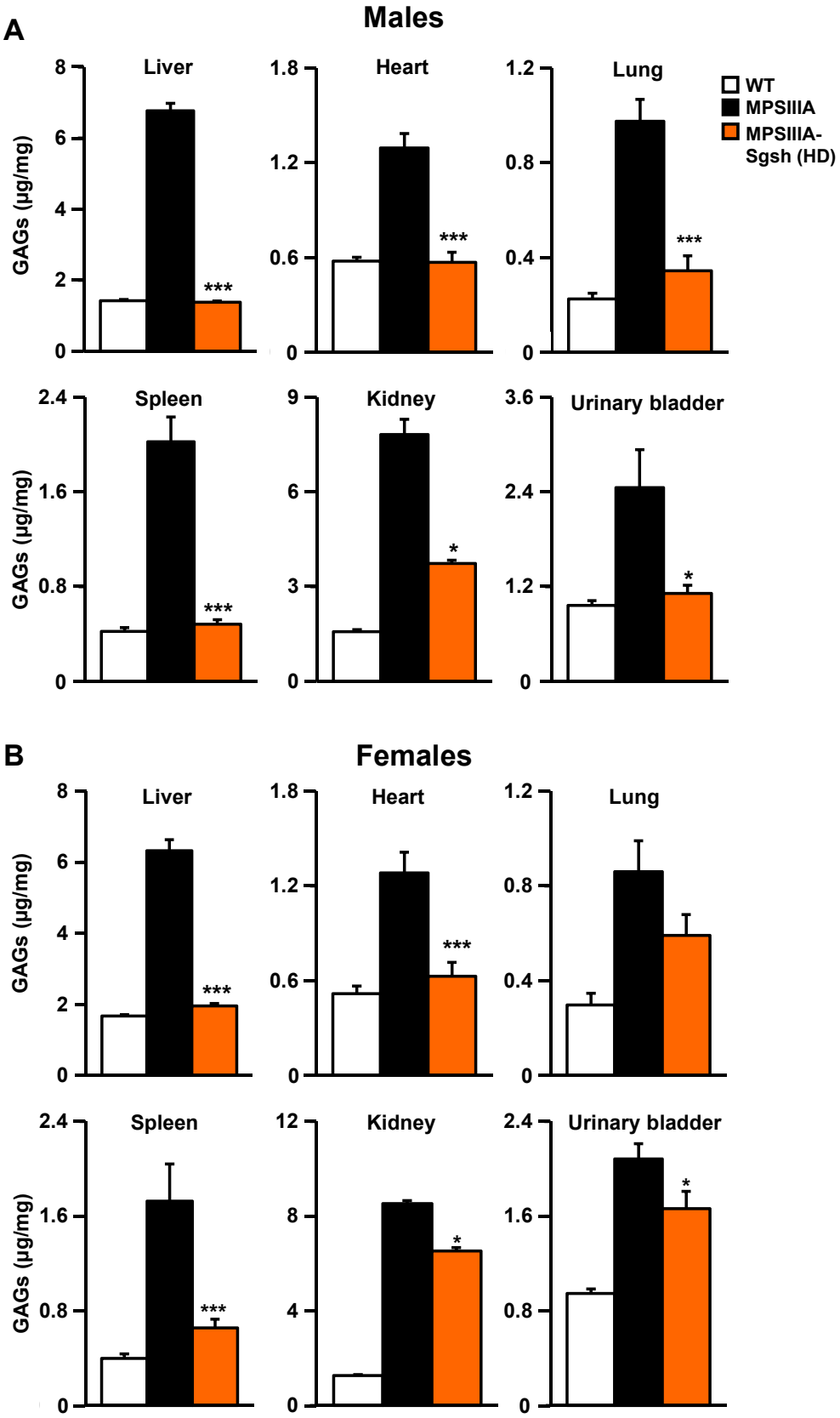
Supplemental Figure 5. Lysosomal pathology in CNS cells of MPSIIIA male mice. Confocal microscopy co-localization studies performed in brain sections from untreated MPSIIIA male mice double-immunostained for LIMP2 (*red*) and specific markers (*green*) for neurons (NeuN, *top panels*), astrocytes (GFAP, *middle panels*) and microglia (Mac2, *bottom panels*). Representative images show that, in untreated MPSIIIA mice, the majority of LIMP2+ cells were neurons. Arrows indicate double-positive cells. Scale bars: 25 μm.

Supplemental Figure 6



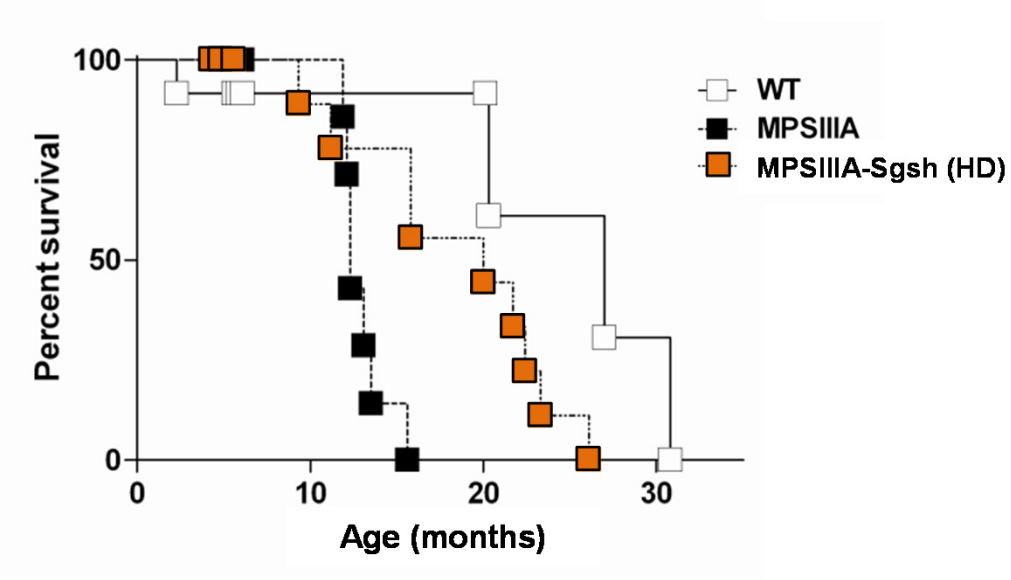
Supplemental Figure 6. Correction of somatic pathology in female MPSIIIA mice following intra-CSF administration of AAV9-mSgsh. (A) Liver sulfamidase activity as % of normal in female MPSIIIA mice four months after intra-CSF delivery of $5x10^9$ (LD) or $5x10^{10}$ (HD) vg of AAV9-mSgsh or $5x10^{10}$ vg of a non-coding (MPSIIIA-Null) vector. WT sulfamidase activity was set to 100% and equaled 12.16 ± 0.31 nmol/17h/mg protein. **(B)** Serum sulfamidase activity was monitored periodically in all experimental groups for 4 months. Activity was practically undetectable in untreated, Null-treated and LD-treated MPSIIIA females and averaged 20% of WT (31.73 ± 1.85 nmol/17h/ml serum) at the high vector dose. p.i., post injection. **(C)** GAG content in liver, heart, lung, spleen, kidney, and urinary bladder. Correction of GAG accumulation was achieved in all tissues with the exception of kidney. Results are shown as mean ± SEM; n=5-8 animals per group. *, $p<0.05$ and ***, $p<0.001$ *vs.* MPSIIIA-Null. **(D)** LAMP1 immunostaining demonstrating correction of lysosomal pathology in MPSIIIA females of the AAV9-mSgsh cohort (HD). Scale bars: 100 µm (20 µm for insets).

Supplemental Figure 7



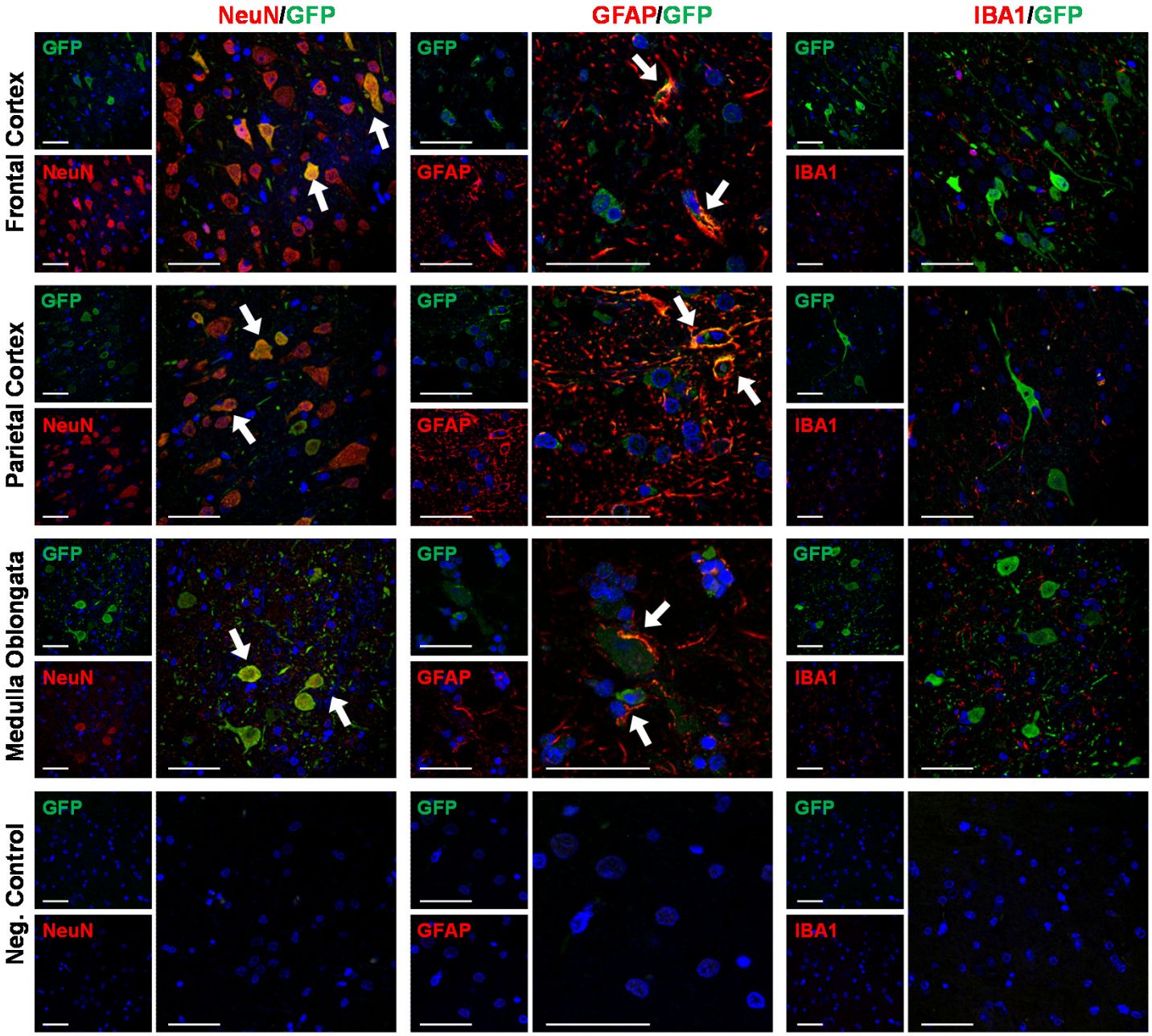
Supplemental Figure 7. Long-term correction of GAG accumulation in somatic organs of treated MPSIIIA males and females. (A-B) GAG content in different somatic organs of **(A)** male and **(B)** female MPSIIIA mice measured 10-12 months after a single delivery of $5x10^{10}$ (HD) vg of AAV9-mSgsh. Results are shown as mean ± SEM; n=4-5 animals per group. *, $p<0.05$ and ***, $p<0.001$ *vs.* untreated MPSIIIA.

Supplemental Figure 8



Supplemental Figure 8. Prolonged survival of MPSIIIA females after treatment with AAV9-mSgsh. Kaplan–Meier survival analysis in WT (n=12), MPSIIIA (n=12) and AAV9 HD-treated (n=13) MPSIIIA females. $p = 0.0085$ for comparison between untreated and AAV9-treated MPSIIIA.

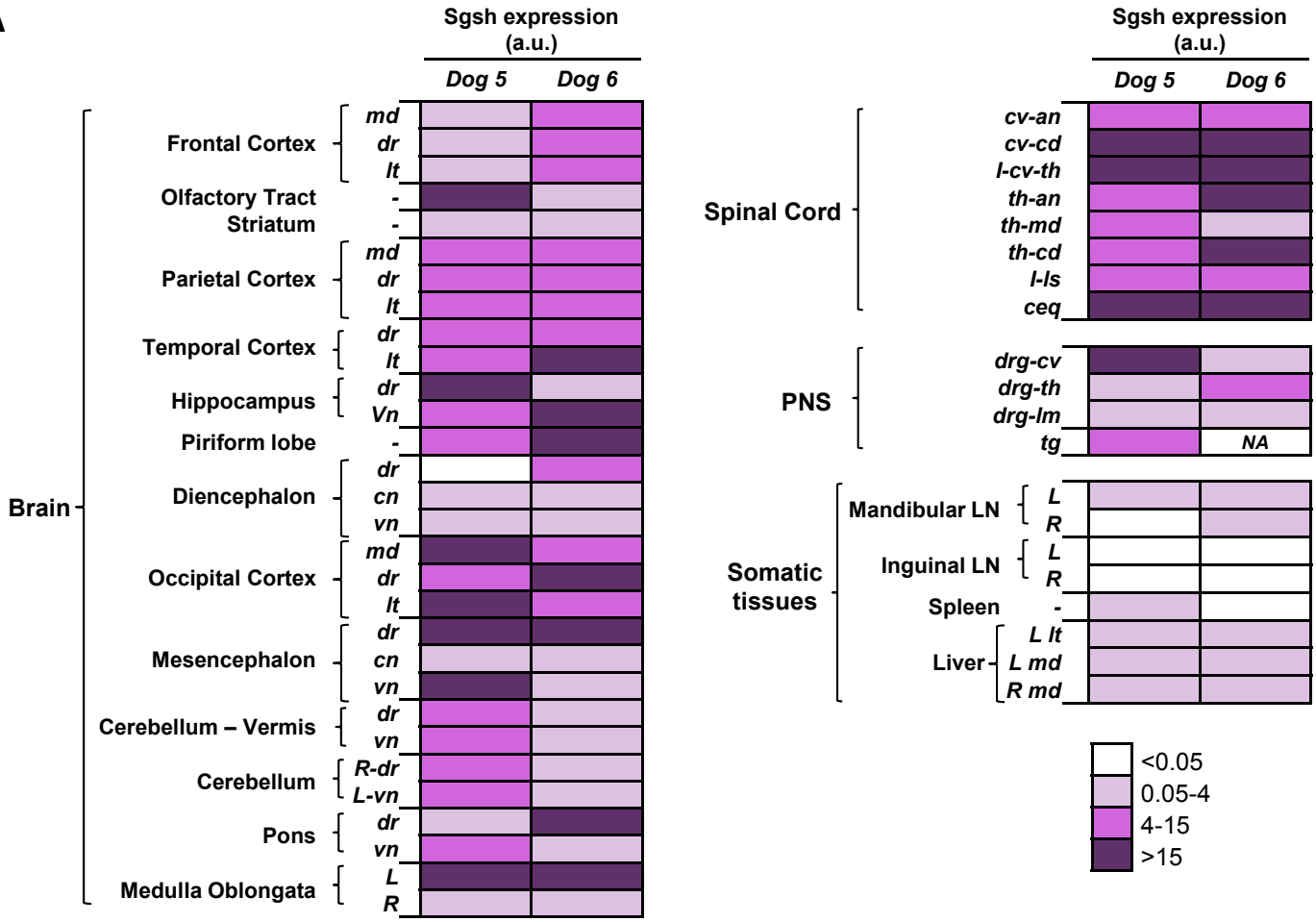
Supplemental Figure 9



Supplemental Figure 9. Efficient transduction of neurons after intra-CSF AAV9 delivery. Confocal microcopy co-localization study on brain sections from healthy Beagle dogs that received an intracisternal administration of $2x10^{13}$ vg of AAV9-GFP. Sections from different brain areas were double-immunostained for GFP (*green*) and specific markers (*red*) for neurons (NeuN, *left panels*), astrocytes (GFAP, *central panels*) or microglia (IBA1, *right panels*). Nuclei were counterstained with TO-PRO-3®. Representative images showing efficient transduction of neurons, scarce transduction of astrocytes and no transduction of microglia after intra-CSF AAV9 delivery to dogs. Arrows indicate double-positive cells. Scale bars: 50 µm.

Supplemental Figure 10

A

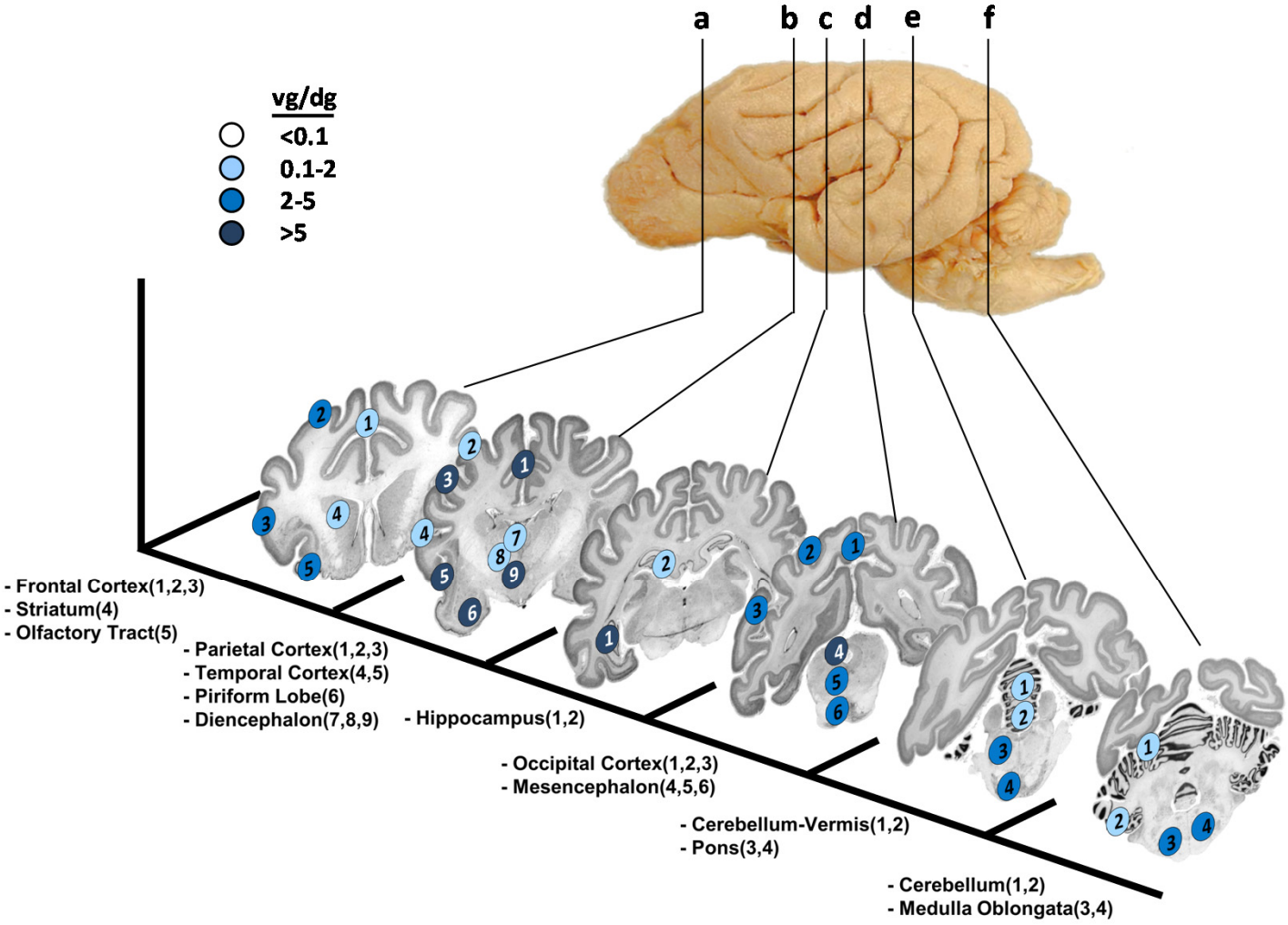


B

Dog ID	Sample	Days post-injection						
		0	8	15	21	30	60	90
5	Serum	<1:1	*NA*	>1:1000	*NA*	>1:1000	>1:1000	>1:1000
	CSF	1:1-1:3.1	*NA*	1:100-1:1000	*NA*	1:100-1:1000	1:100-1:1000	1:100-1:1000
6	Serum	<1:1	>1:1000	>1:1000	>1:1000	>1:1000	>1:1000	>1:1000
	CSF	1:1-1:3.1	1:1-1:3.1	>1:1000	1:100-1:1000	1:10-1:100	1:100-1:1000	1:10-1:100

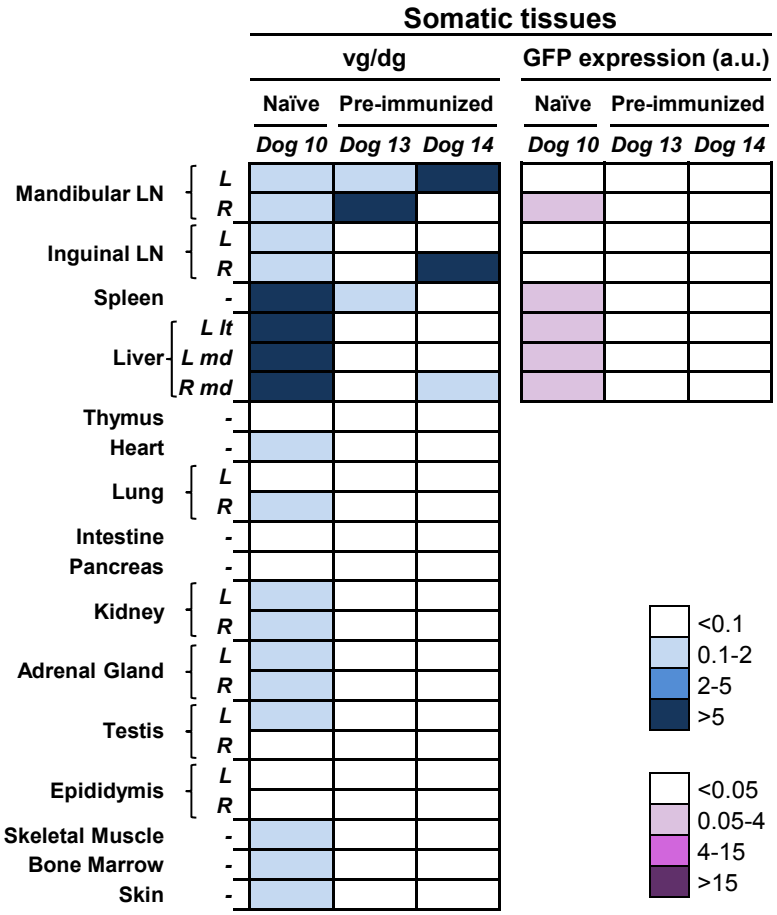
Supplemental Figure 10. Persistence of human sulfamidase expression after intra-CSF AAV9h-Sgsh delivery. (**A**) Human sulfamidase mRNA expression in CNS, PNS and somatic organs 3 months post intra-CSF delivery of $2x10^{13}$ vg of AAV9-hSgsh vectors to healthy Beagle dogs (Dogs 5 and 6). md, medial; dr, dorsal; lt, lateral; vn, ventral; cn, central; R, Right; L, Left; cv-an, cervical anterior; cv-cd, cervicocaudal; I, intumescence; cv-th, cervicothoracic; th-an, thoracic anterior; th-md, thoracic medial; th-cd, thoracic caudal; ls, lumbosacral; ceq, cauda equina; drg, dorsal root ganglia; cv, cervical; th, thoracic; lm, lumbar; LN, lymph node. a.u, arbitrary units. (**B**) Follow-up of neutralizing antibody titers in paired CSF and serum samples obtained before and after intra-CSF delivery of AAV9-hSgsh vectors to Dogs 5 and 6. NA, non-available.

Supplemental Figure 11



Supplemental Figure 11. Widespread vector distribution following AAV9 ICV-delivery to the CNS. Color-coded schematic representation of gene copy numbers quantified in samples from an ICV AAV9-GFP-injected dog (Dog 11) depicted in Figure 10. Sampled areas are numerically indicated on the left hemisphere of six representative sections (a-f) spanning the whole dog encephalon.

Supplemental Figure 12



Supplemental Figure 12. Lack of transduction of peripheral organs following intra-CSF AAV9 delivery to pre-immunized dogs. Vector gene copy number and GFP mRNA expression in peripheral organs following intra-CSF delivery of $2x10^{13}$ vg of AAV9-GFP vectors to naïve (Dog 10) or pre-immunized (Dogs 13 and 14) dogs. md, medial; lt, lateral; R, Right; L, Left; LN, lymph node; vg/dg, vector genome/diploid genome; a.u, arbitrary units.

Supplemental Table 1

Dog ID	Measure	Days post-injection						
		0	**8**	**15**	**21**	**30**	**60**	**90**
1	RBC	*NA*	0	-	-	-	-	-
	WBC	*NA*	0	-	-	-	-	-
	TP	*NA*	23.3	-	-	-	-	-
2	RBC	0	0	-	-	-	-	-
	WBC	0	0	-	-	-	-	-
	TP	18	18	-	-	-	-	-
3	RBC	4*	0	-	-	-	-	-
	WBC	3200*	0	-	-	-	-	-
	TP	31.6*	23.4	-	-	-	-	-
4	RBC	0	0	-	-	-	-	-
	WBC	0	0	-	-	-	-	-
	TP	20.2	17.9	-	-	-	-	-
5	RBC	0	*NA*	0	*NA*	1470*	0	0
	WBC	0	*NA*	183	*NA*	97*	11	9
	TP	21	*NA*	60.9	*NA*	97*	51.2	46.9
6	RBC	0	0	0	0	0	0	0
	WBC	1	3	50	54	27	6	4
	TP	18.9	16.6	22.2	39.1	35.6	20.8	25
7	RBC	0	0	0	0	0	235*	0
	WBC	1	0	1	0	2	4*	5
	TP	17.6	17.1	14.9	17.4	25.1	22.7*	21
8	RBC	0	251*	0	0	0	0	0
	WBC	1	3*	1	1	0	2	3
	TP	17.9	19.6*	16.9	17.1	20.3	29.4	18.9
9	RBC	0	0	0	0	0	0	0
	WBC	0	2	1	1	1	0	4
	TP	13.4	14.8	11.6	11.9	15.2	18	18.9
10	0	0	71	-	-	-	-	-
	WBC	1	2	-	-	-	-	-
	TP	16.4	16.4	-	-	-	-	-
11	RBC	*NA*	5	-	-	-	-	-
	WBC	*NA*	2	-	-	-	-	-
	TP	*NA*	18.4	-	-	-	-	-
12	RBC	0	1	-	-	-	-	-
	WBC	3	1	-	-	-	-	-
	TP	16.3	16.3	-	-	-	-	-
13**	RBC	0	0	-	-	-	-	-
	WBC	0	4	-	-	-	-	-
	TP	18.7	19.3	-	-	-	-	-
14**	RBC	0	0	-	-	-	-	-
	WBC	0	3	-	-	-	-	-
	TP	19.1	15.7	-	-	-	-	-

All dogs received an intra-CSF dose of $2x10^{13}$ vg of AAV9 vectors.
Values for normal CFS are: Red blood cell count (RBC) = 0, White Blood Cell Count (WBC) < 5 cells/µl and Total Protein (TP) <25 mg/dl.
*= sample contaminated with blood.
** For pre-immunized dogs, the "0" time-point corresponds to samples obtained before intracisternal administration, i.e. 30 days after pre-immunization with intravenous delivery of AAV9-null vector
NA, sample not available.