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Supplemental Information

AAVrh10 Vector Corrects Disease Pathology in MPS IIIA Mice and Achieves Widespread Distribution of SGSH in Large Animal Brains

Michaël Hocquemiller, Kim M. Hemsley, Meghan L. Douglass, Sarah J. Tamang, Daniel Neumann, Barbara M. King, Helen Beard, Paul J. Trim, Leanne K. Winner, Adeline A. Lau, Marten F. Snel, Cathy Gomila, Jérôme Ausseil, Xin Mei, Laura Giersch, Mark Plavsic, and Ralph Laufer

Supplemental table

Table S1: Details of mice that were found dead/required euthanasia

ID# found dead	ID# euthanazed	Age	Gender	Genotype	Treatment	Pathology findings
#498		9 weeks	male	MPS IIIA	medium	NA (mice cannibalized)
#215		10 weeks	male	MPS IIIA	vehicle	The cause of death could not be determined by pathological examination
#429		9 weeks	female	MPS IIIA	medium	The cause of death could not be determined by pathological examination
#453		9 weeks	female	MPS IIIA	high	The cause of death could not be determined by pathological examination
#497		10 weeks	male	MPS IIIA	low	NA (mice cannibalized)
#472		10 weeks	female	MPS IIIA	medium	The cause of death could not be determined by pathological examination
	#319	11 weeks	male	MPS IIIA	low	Acute suppurative bacterial meningoencephalitis
#427		18 weeks	male	MPS IIIA	high	NA (mice cannibalized)
#395		19 weeks	female	MPS IIIA	medium	The cause of death could not be determined by pathological examination
	#424	20 weeks	male	MPS IIIA	high	The cause of death could not be determined by pathological examination
	#325	22 weeks	male	MPS IIIA	vehicle	Acute keratitis with corneal hyperplasia and focal intraepidermal microabscess formation with aggregated neutrophils
#341		23 weeks	male	MPS IIIA	high	NA (mice cannibalized)
#469		25 weeks	male	MPS IIIA	medium	NA (mice cannibalized)
	#466	27 weeks	female	MPS IIIA	high	The cause of death could not be determined by pathological examination

Supplemental figures

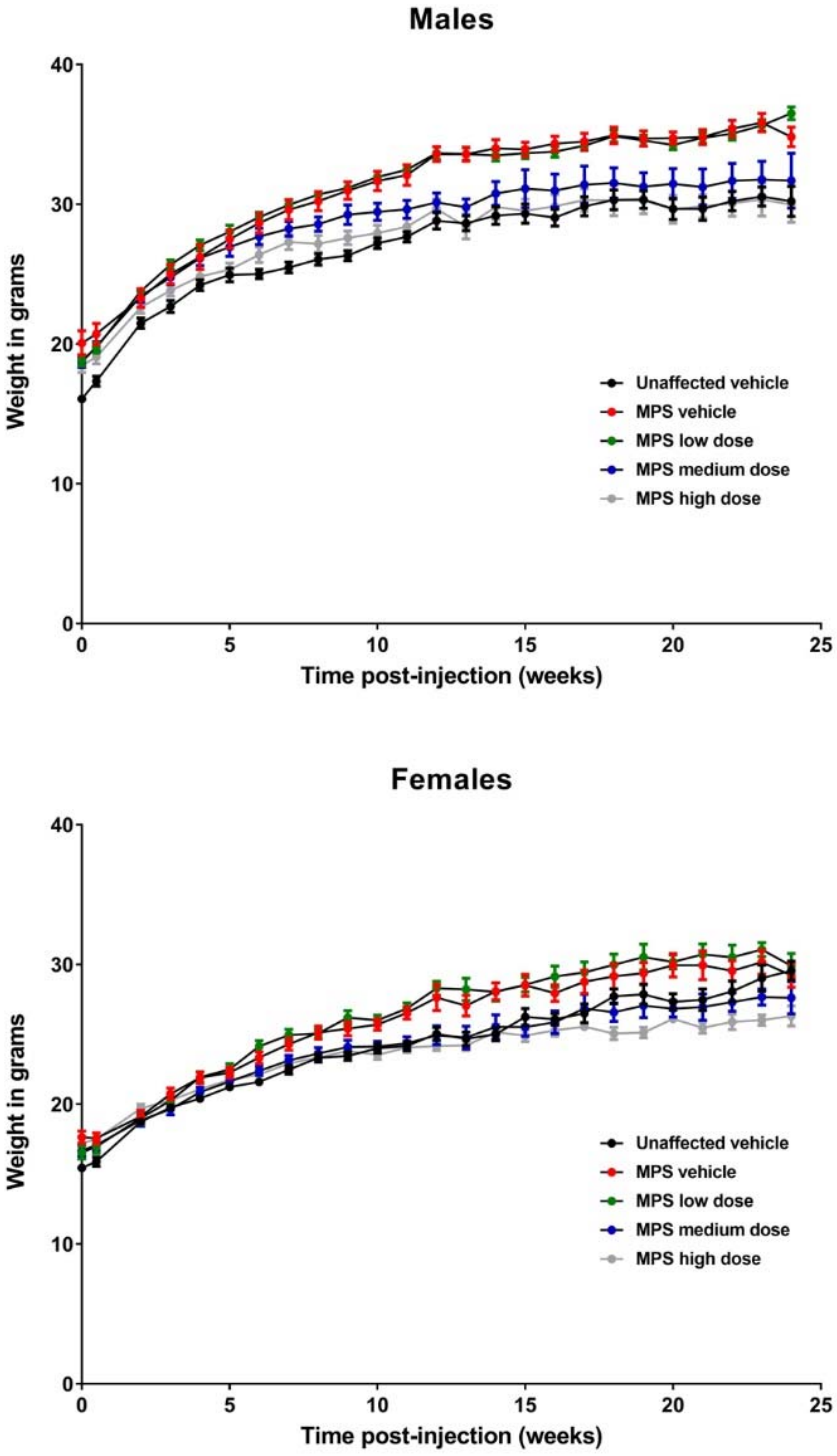

Figure S1. Mouse body weights.

Male and female mice received intra-cranial injections at 5-weeks of age and were weighed at least weekly up to 25-weeks post-injection. N=5-15 mice/group (N=5 mice/group remained at the final time-point). Data are mean ± SEM. MPS vehicle and MPS low dose groups were significantly different from unaffected vehicle mice (Bonferroni test, both $p<0.0001$). The MPS mice that received the medium and high doses were not significantly different from unaffected vehicle mice (Bonferroni test, $p=0.499$ and $p=1.000$ respectively) suggesting that the body weight in these cohorts had been normalised with treatment.

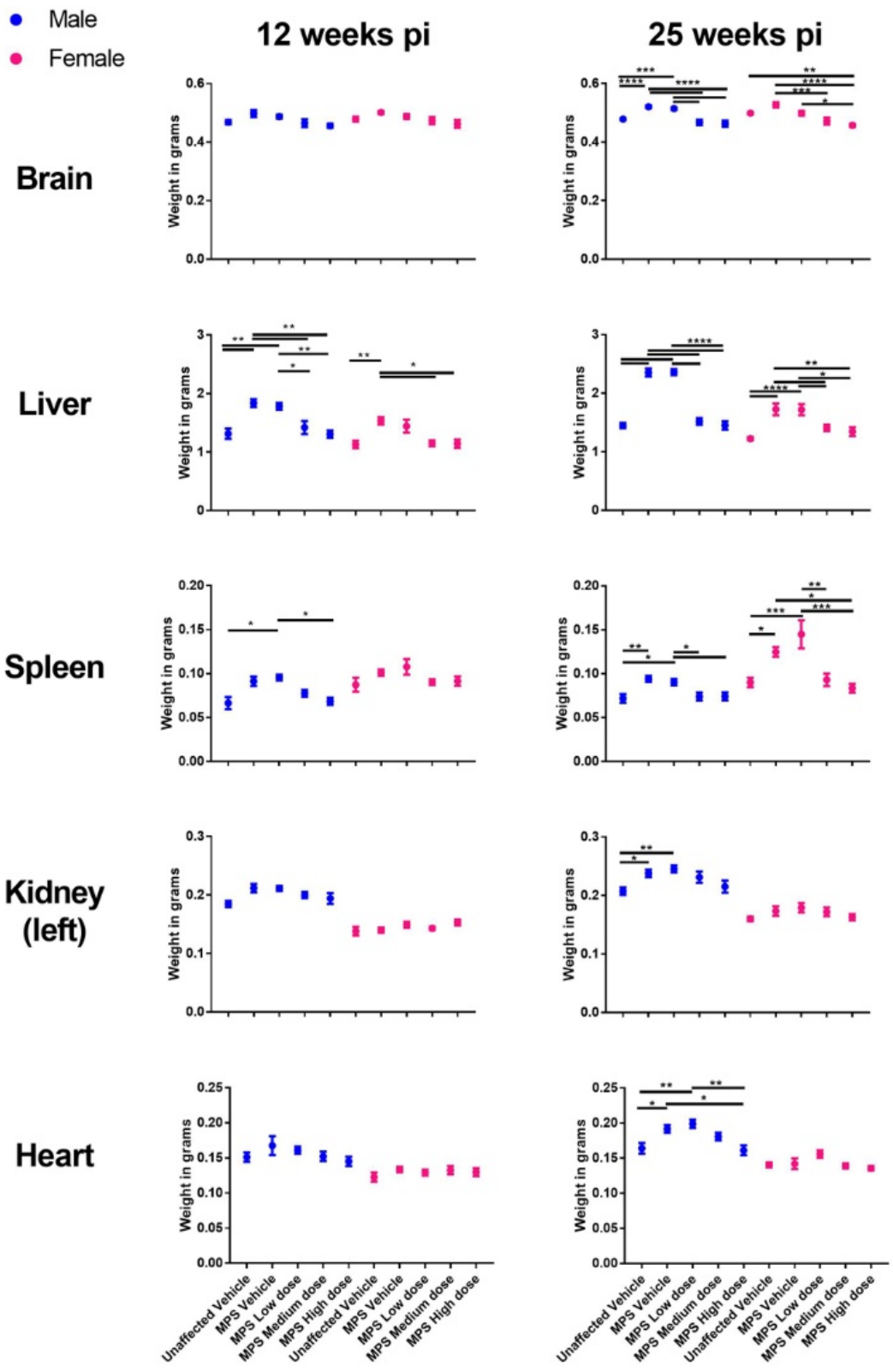

Figure S2. Mouse organ weights at post-mortem.

Weights of the organs were collected at post-mortem and have been graphed by gender. N=5 mice/group at 12 weeks post-injection. N=7-11 mice/group at 25-weeks post-injection. Data are mean ± SEM. ****p < 0.0001, ***p < 0.001, **p < 0.01, and * p < 0.05 calculated from one-way ANOVA with Bonferroni's multiple comparisons test.

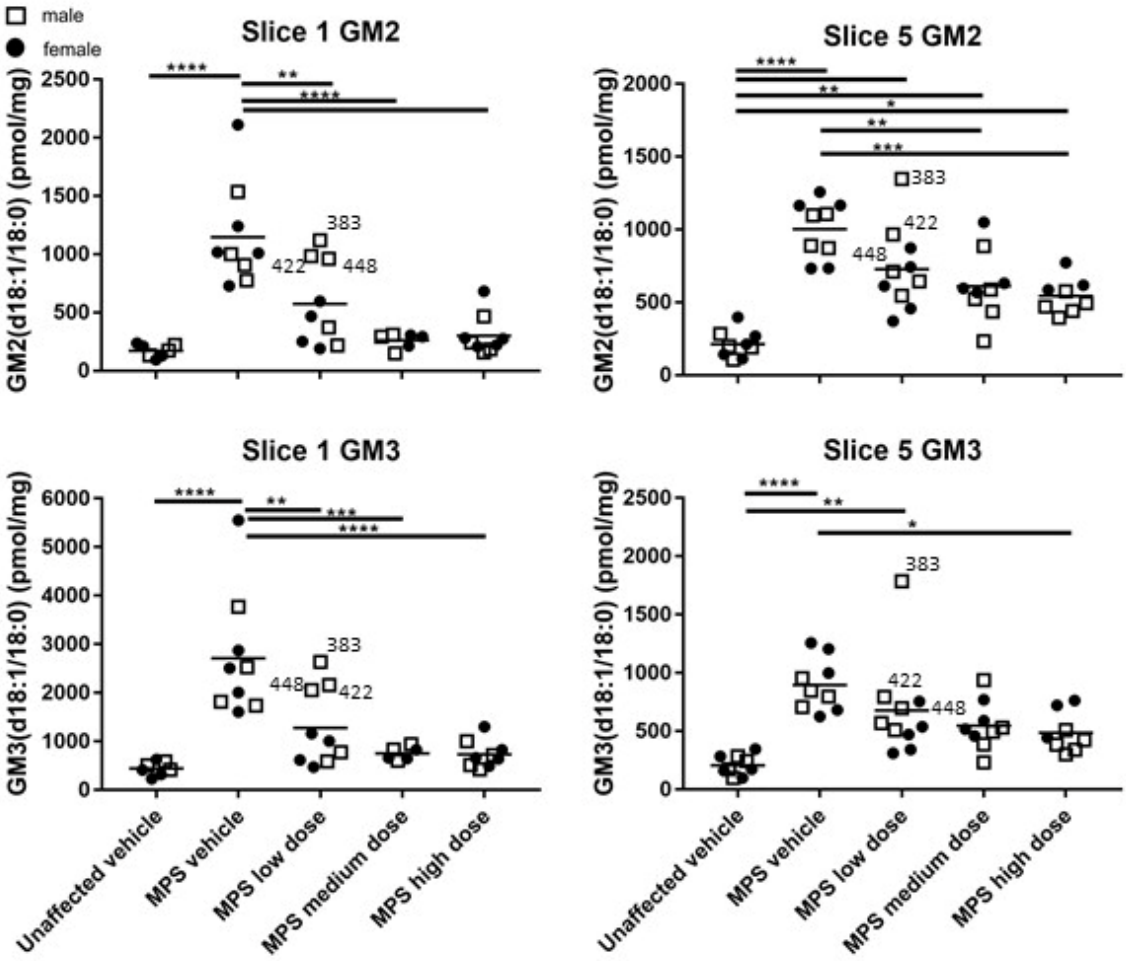

Figure S3. GM2 and GM3 gangliosides at 25 weeks post-injection.

Quantification of GM2 and GM3 gangliosides in mouse brain slices 1 and 5 according to the map given in Figure 1. N=6-10 mice/group. ****$p < 0.0001$, ***$p < 0.001$, **$p < 0.01$, and * $p < 0.05$ calculated from one-way ANOVA with Bonferroni's multiple comparisons test. Male low dose-treated MPS IIIA mice #422, #383 and #448 were outliers, exhibiting no reduction in gangliosides (c.f. MPS IIIA vehicle mice)

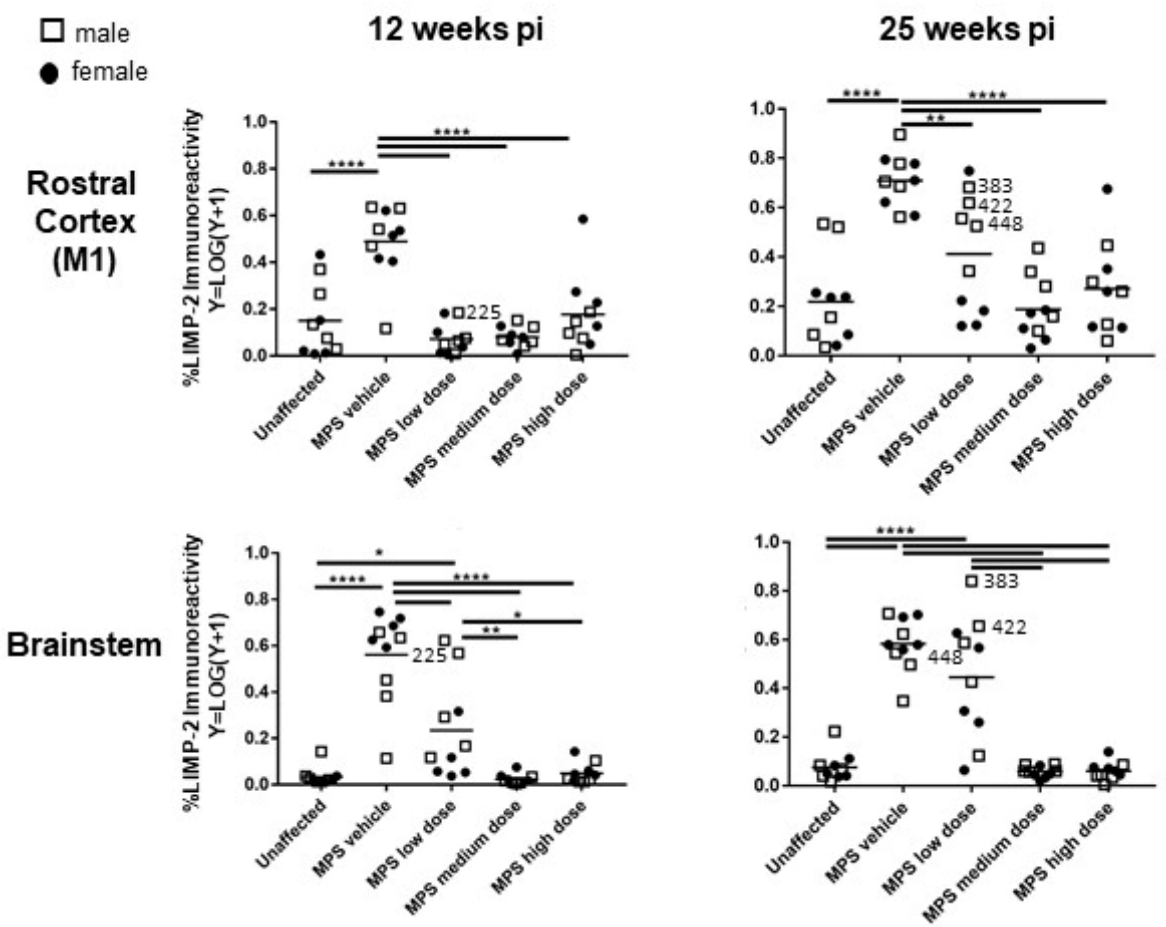

Figure S4. Immunohistochemical staining of LIMP-2 in mouse brain sections at 12- and 25-weeks post-injection.

****p < 0.0001, ***p < 0.001, **p < 0.01, and * p < 0.05 calculated from one-way ANOVA with Bonferroni's multiple comparisons test. Mouse #225 (17-week old male MPS IIIA low dose group) and #422, #383 and #448 (30-week old male MPS IIIA low dose group) were outliers.

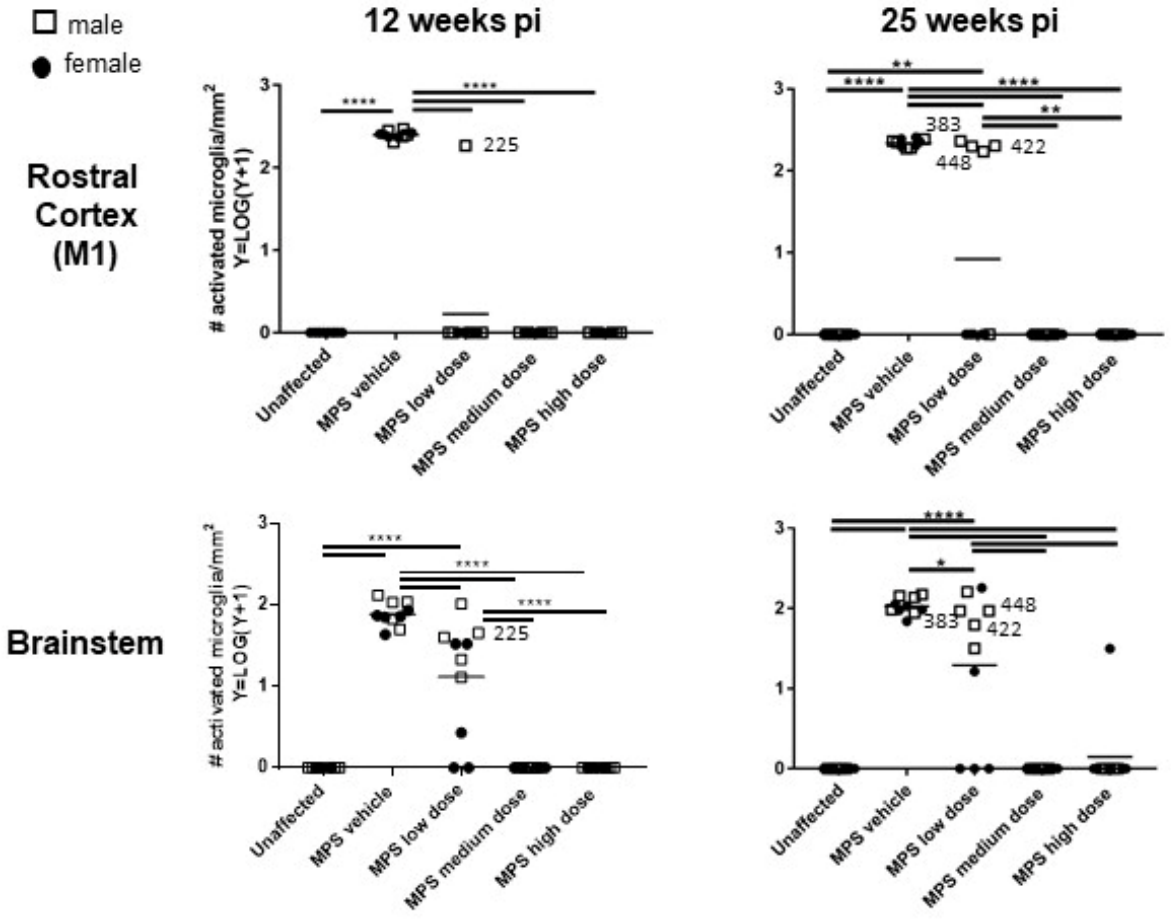

Figure S5. Immunohistochemical staining of ubiquitin in mouse brain sections at 12- and 25-weeks post-injection.

****p < 0.0001, ***p < 0.001, **p < 0.01, and * p < 0.05 calculated from one-way ANOVA with Bonferroni's multiple comparisons test. Mouse #225 (17-week old male MPS IIIA low dose group) and #422, #383 and #448 (30-week old male MPS IIIA low dose group) were outliers.

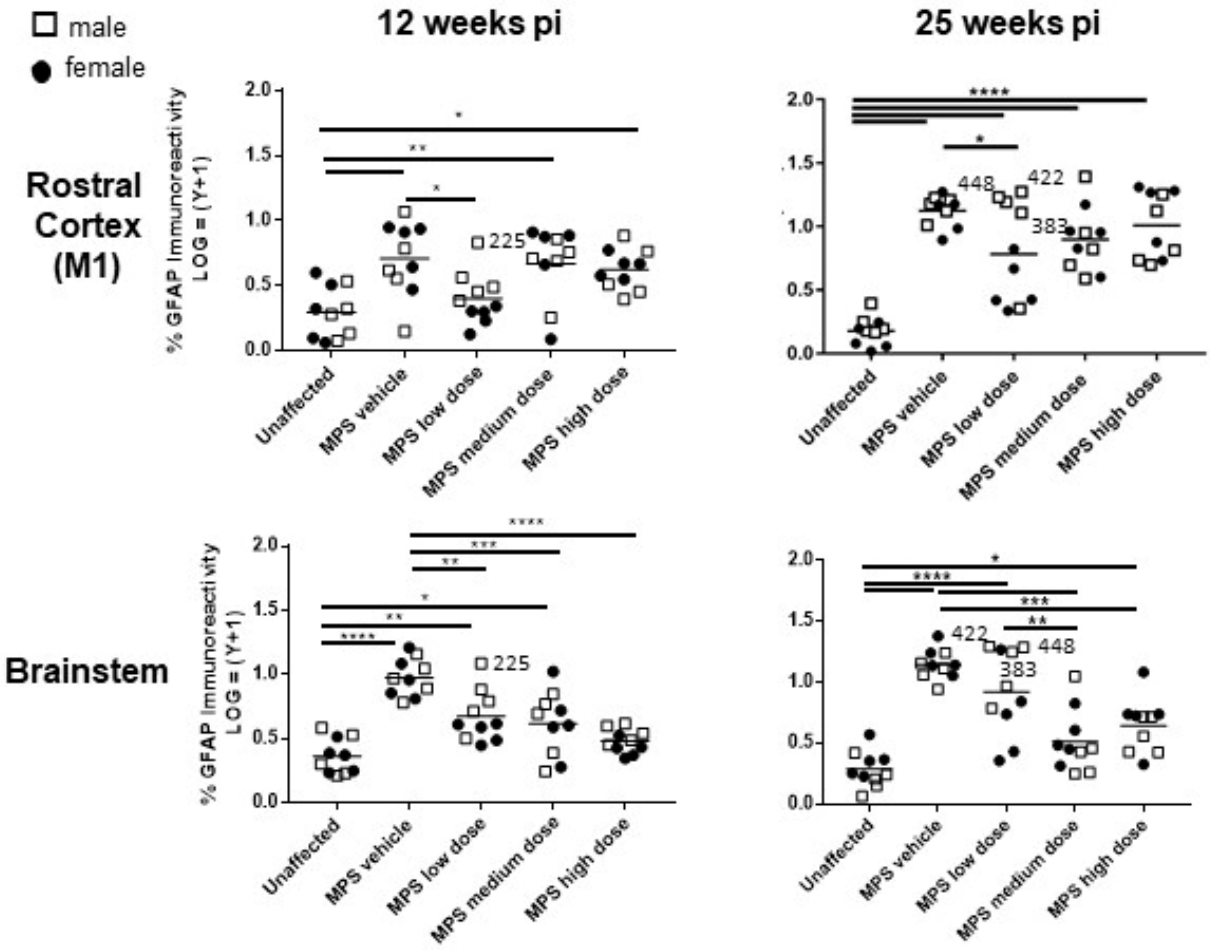

Figure S6. Immunohistochemical staining of GFAP in mouse brain sections at 12- and 25-weeks post-injection.

****p < 0.0001, ***p < 0.001, **p < 0.01, and * p < 0.05 calculated from one-way ANOVA with Bonferroni's multiple comparisons test. Mouse #225 (17-week old male MPS IIIA low dose group) and #422, #383 and #448 (30-week old male MPS IIIA low dose group) were outliers.

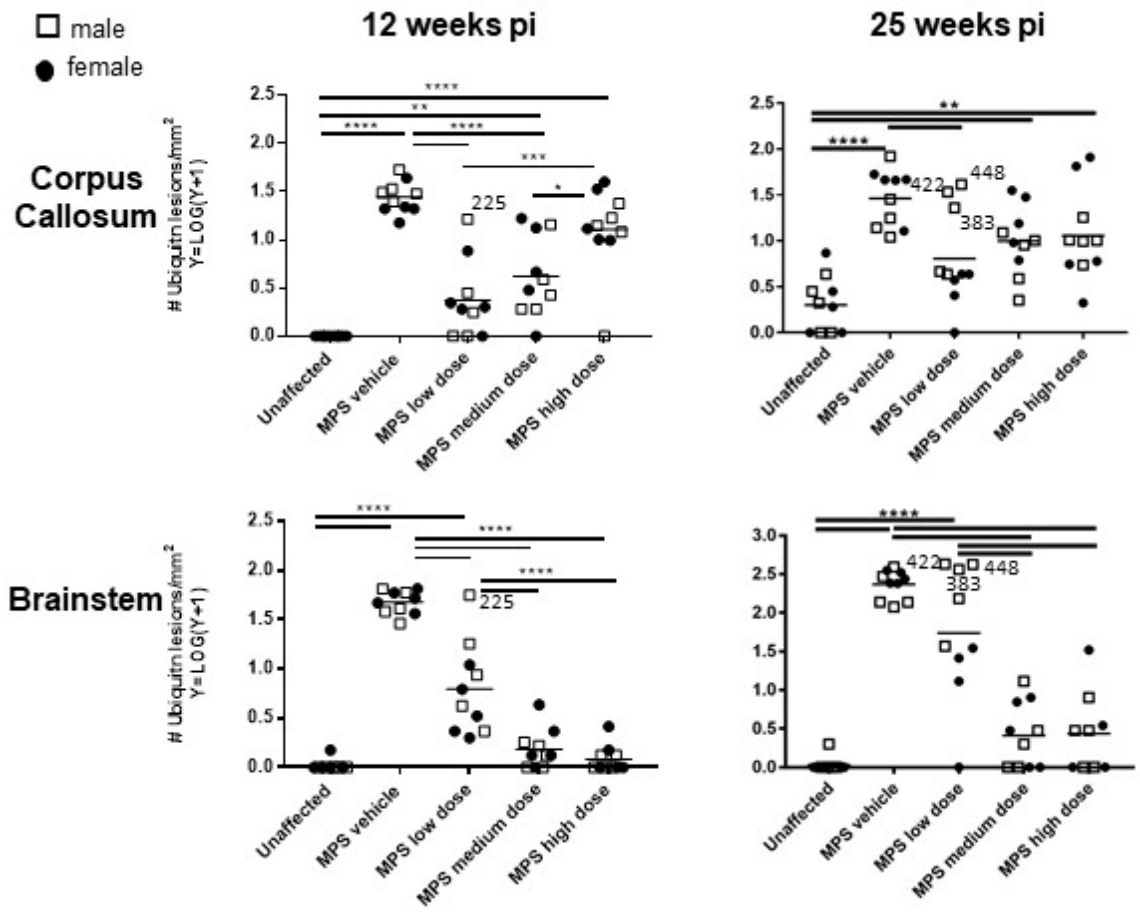

Figure S7. Histochemical staining of ILB4 in mouse brain sections at 12- and 25-weeks post-injection.

****p < 0.0001, ***p < 0.001, **p < 0.01, and * p < 0.05 calculated from one-way ANOVA with Bonferroni's multiple comparisons test. Mouse #225 (17-week old male MPS IIIA low dose group) and #422, #383 and #448 (30-week old male MPS IIIA low dose group) were outliers.

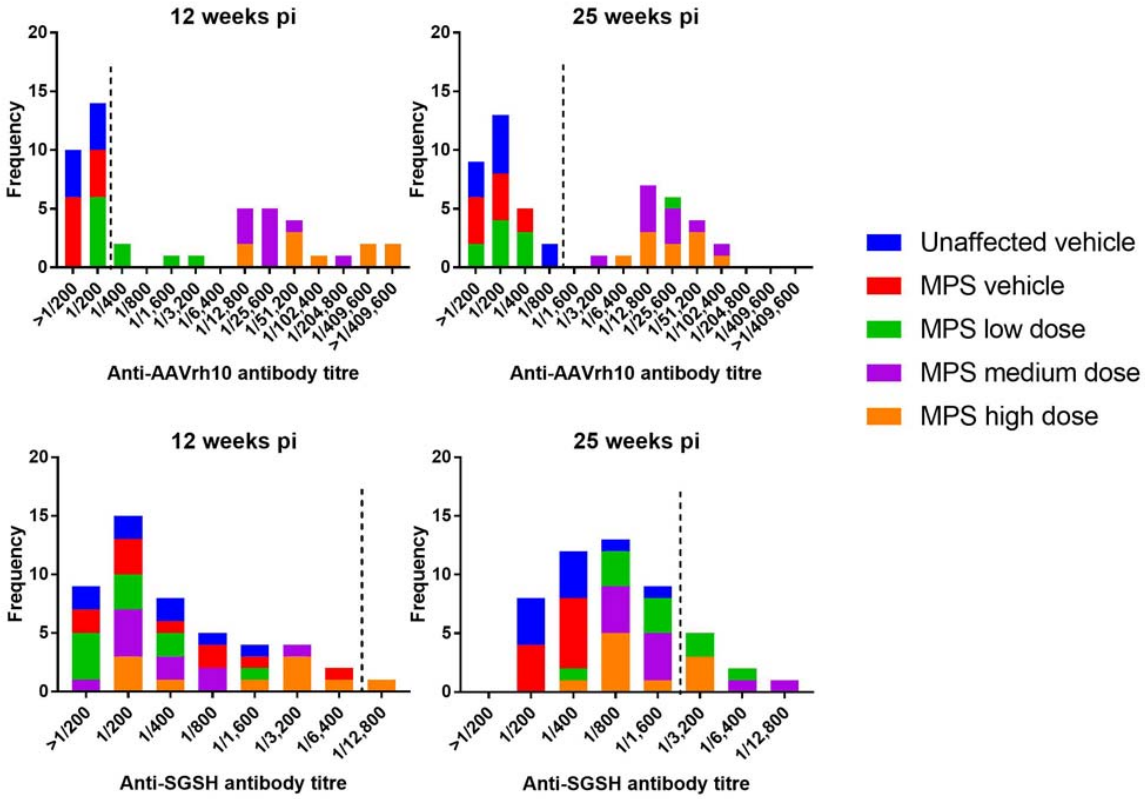

Figure S8. Antibody Titres in mouse sera at 12- and 25-weeks post-injection.

The dotted line indicates the highest dilution of serum from vehicle-treated animals that is 2SD above the blank.

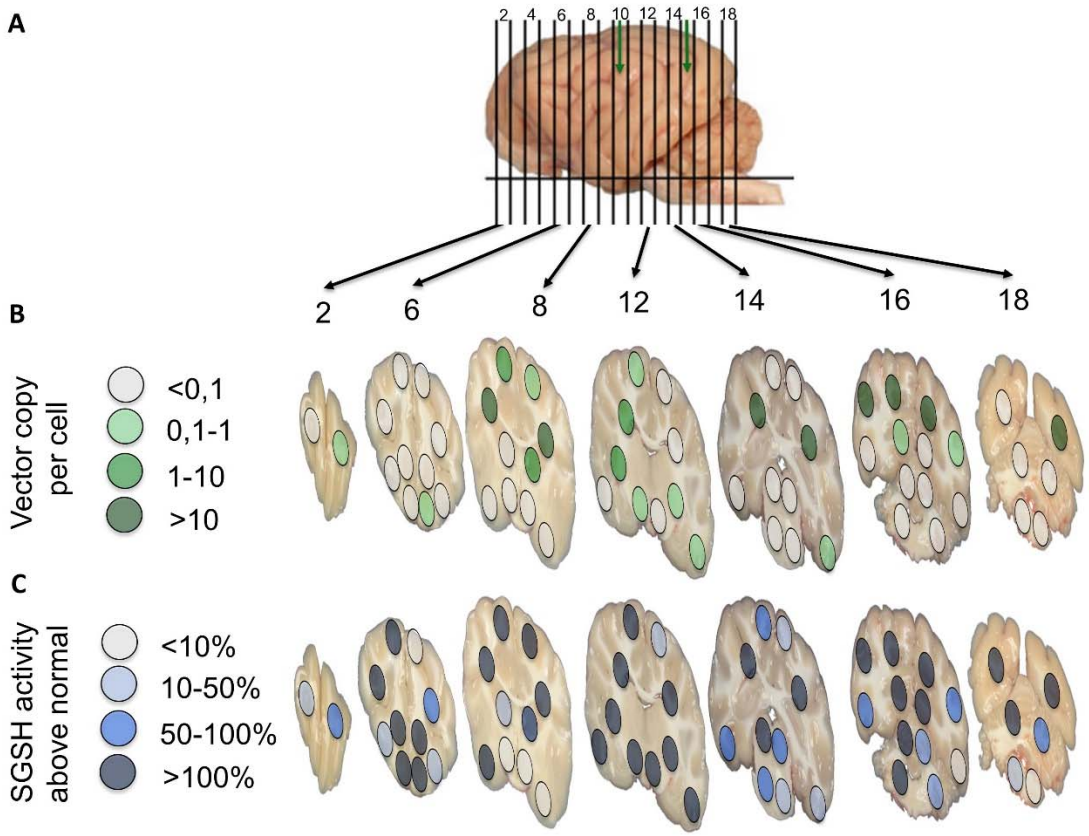

Figure S9. Vector and enzyme activity biodistribution in dog brain.

(A) Representation of brain slicing of a dog injected with 500µl in two sites per hemisphere (green arrows) for a total dose of 2E+12 vector genomes. (B) qPCR analysis were performed using PCR TaqMan method using primers and probe specific of the transgene and results (green dots) are expressed as mean vector copy per cell. (C) Enzyme activity analysis were performed using the 2 steps method resulting in the release of measurable 4-MU fluorescent substance. Results (blue dots) were normalized as % of endogenous activity determined as mean value of 80 punches from 2 PBS injected hemispheres of 2 distinct dogs.

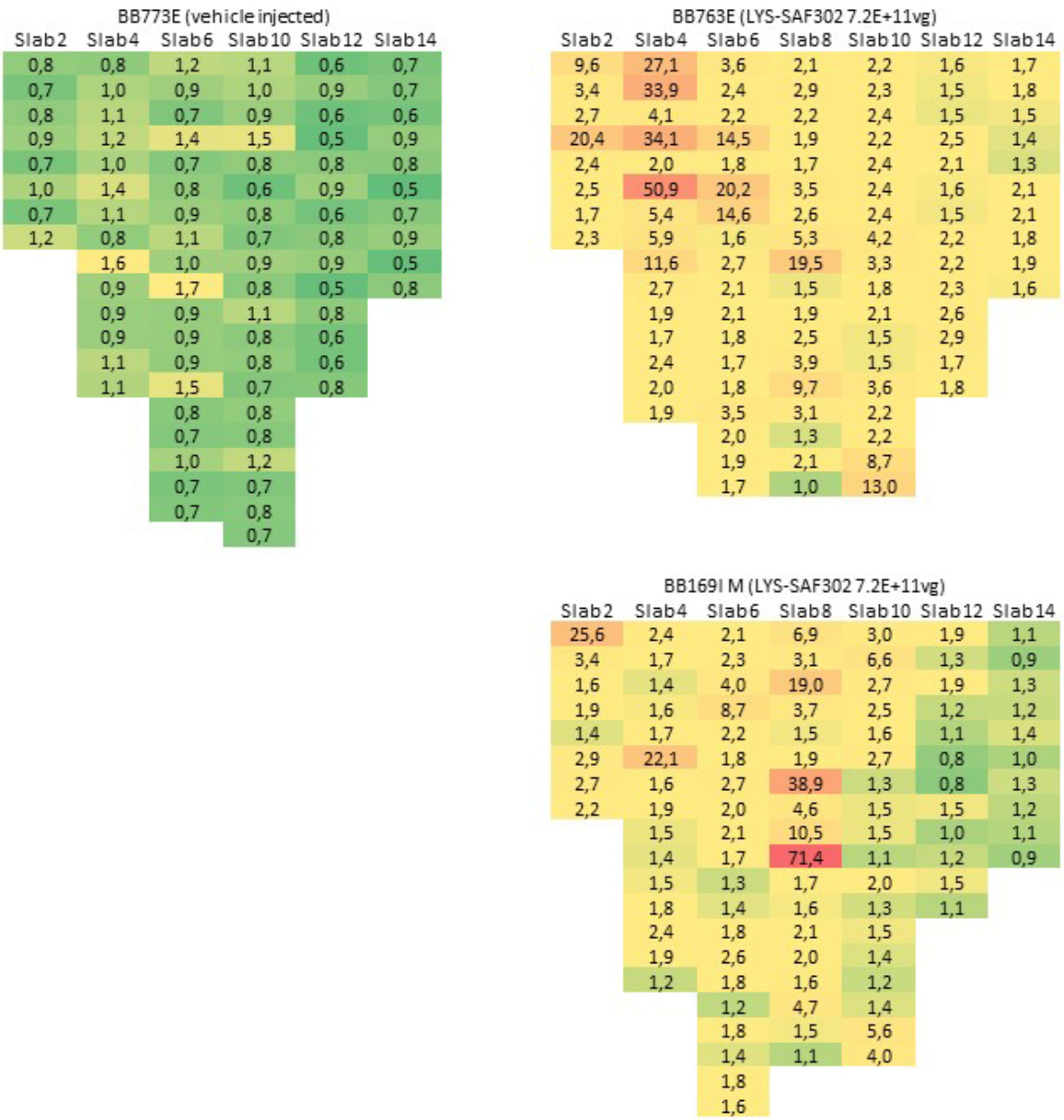

Figure S10: Numerical data of SGSH activity in NHP brain.

Representation of SGSH activity data in the brain samples of the vehicle injected animal (NHP#BB773E) and two NHP that received four injections of 50 µL (two per hemisphere) of LYS-SAF302 into the white matter at 5 µL/min at a total dose 7.2E+11vg (NHP#BB763E and NHP#BB169I). Results of each analysed brain punches from each brain slabs (see Figure 6) are expressed as nmol/h/mg with color code reflecting relative values (lowest activity in green and highest activity in red).