SUPPLEMENTAL METHODS

Bone Marrow Engraftment Analysis

Four twitcher mice transplanted with bone marrow cells from GFP+ mice along with two uninjected twitcher mice and one GFP+ donor mouse were euthanized two weeks after transplant to evaluate the level of engraftment by flow cytometry analysis and by immunohistochemical analysis. One sagittal half of the brain from these mice was post-fixed for 48 hours in freshly made PBS containing 4% paraformaldehyde and was sectioned at 40 microns using a Leica vibrating microtome. Sections were treated with hydrogen peroxide to remove endogenous peroxidase activity prior to blocking. Samples were incubated for 1 hour at room temperature in blocking solution (10% goat serum, 0.1% triton X-100, 1X PBS), then incubated 14-16 hrs at 4º C in primary antibody solution (3% goat serum, 0.1% triton X-100, 1X PBS, and rabbit anti-GFP (Millipore # AB3080, 1:1000). After incubating with the appropriate secondary antibody for one hour at room temperature, color development was performed using VectaStain ABC Elite Kit (Vector Labs, #PK-6100) with 3,3'-diaminobenzidine tetrachloride (DAB; Polysciences, Inc. #04008) substrate and nickel-cobalt intensification of the reaction product. DAB-processed brain sections were digitized using a Scan-Scope slide scanner (Aperio Technologies, Vista, CA). Virtual slides were viewed using ImageScope software package (v. 10.0; Aperio Technologies) (Supplemental Figure 1). Paraffin embedded spinal cord sections (as described in Methods) were also analyzed for GFP positive donor cells using the same rabbit-anti GFP primary antibody (Supplemental Figure 1).

The remaining half of the brain was processed using the adult brain dissociation kit (MIltenyi, #130-107-677) to prepare single cell suspension and the myelin removal was carried out using the myelin removal beads (Miltenyi, #130-096-7330). Single cell suspensions were prepared from spleen, pooled lymph nodes and blood. The percentage of GFP+ cells in these cell suspensions were determined by flow cytometry (Beckman Coulter CyAn ADP) and expressed as the percentage of total live cells (Supplemental Table 1).

Determination of expression levels of AAV9/GALC constructs in vitro

Lec2 cells, a mutant cell line derived from Chinese Hamster Ovary (CHO) cells amenable for AAV9 transduction (Shen et al., 2011) were cultured in DMEM containing 10% Fetal Bovine Serum and 100 units/mL each of antibiotics penicillin and streptomycin and maintained at 37 ^{0}C and 5% CO_2. For the in vitro assay, $2x10^5$ cells were plated in 12- well tissue culture treated plates and infected with AAV9/WT-mGALC, AAV9/CBA-mGALCopt and AAV9/JeT-mGALCopt vectors at an MOI of $1x10^5$ vector genomes per cell. Cells were collected 48hrs after infection, extracted total RNA and prepared cDNA. Real-time PCR was performed using specific primers for WT-mGALC and mGALCopt. The values were normalized using CHO-GAPDH expression in the cDNA (Supplemental Table 2).

Determination of Psychosine by Tandem Mass Spectrometry

Samples were lysed in $_{dd}H_2O$ and psychosine was extracted in 0.5% acetic acid/methanol solution via shaking/incubation at room temperature for 1 hour. Post incubation, samples were spun at 14,500g for 15 min at 4°C. Supernatant was removed and extracts were further passed through a Shimadzu (Kyoto, Japan) Nexera UHPLC system equipped with a Waters Acquity UPLC BEH amide column. Psychosine was eluted in 85% acetonitrile and 15% 5 mM ammonium formate and 0.2% formic acid and analyzed in a Shimadzu LCMS-8050 triple

quadrupole mass spectrometer equipped with positive ion electrospray. Psychosine was measured using collision-induced dissociation and selected reaction monitoring (SRM). The SRM transition for psychosine was m/z 462 to 282 and the transition for the surrogate standard D-lactosyl-ß1-1'-D-erythro-sphingosine was m/z 624 to 282.

Analysis of sciatic nerve G-ratios

For the quantitative analysis of the demyelination in the sciatic nerve of twitcher mice the HALO™ image analysis platform from Indica labs was used. The algorithm parameters axon quantification module of HALO was optimized for randomly chosen annotation layers across the samples. A minimum of 8 randomly chosen nerve areas were analyzed per mouse and each treatment cohort contained 3 mice except for AAVrh10+BMT treatment (n=2) (Supplemental Figure 4).

Reference

Shen S, Bryant KD, Brown SM, Randell SH, Asokan A. 2011. Terminal N-linked galactose is the primary receptor for adeno-associated virus 9. J Biol Chem 286(15):13532-40.

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Reference

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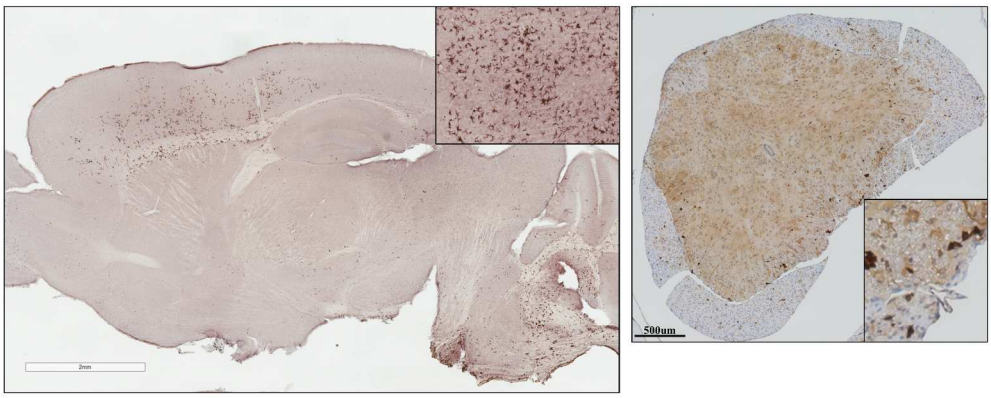


Supplemental Figure 1- IHC image showing engraftment of GFP+ donor cells in the brain and spinal cord of recipient twitcher mouse two weeks after transplantation. The inset shows 4x magnification. The image is representative of three mice.

Tissue	GFP+ cells in the recipient twitcher as % of total live cells (n=3)
Brain	0.3-3.04
Lymph Nodes (Pooled mesenteric, pyloric and inguinal)	2.05-21.65
Spleen	4.5-35.12
Blood (WBCs)	44.5-63.9

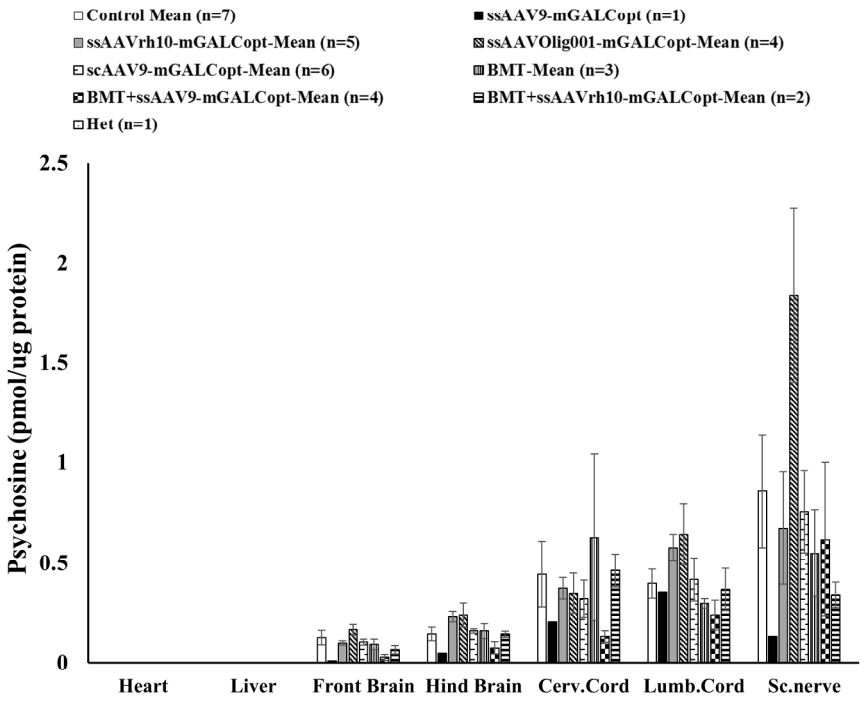
Supplemental Table 1- The engraftment of GFP+ cells in brain, draining lymph nodes (pooled), spleen and blood of recipient twitcher mice expressed as % of the total live cells acquired in flow cytometry analysis.

254x190mm (300 x 300 DPI)

	Vector Constructs	Average Quantity of GALC transcripts per GAPDH transcripts
1	ssAAV9-CBA-MS-mGALC	3.51E-03
2	ssAAV9-CBA-mGALCopt	3.16E-03
3	scAAV9-JeT-mGALCopt	7.94E-05

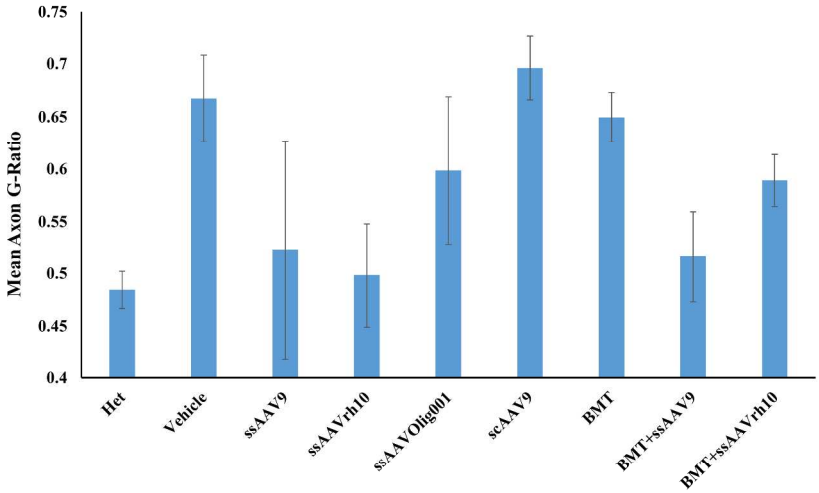
Supplemental Table 2 – The level of GALC mRNA expression in vitro after transduction with AAV9/GALC vectors

338x190mm (300 x 300 DPI)



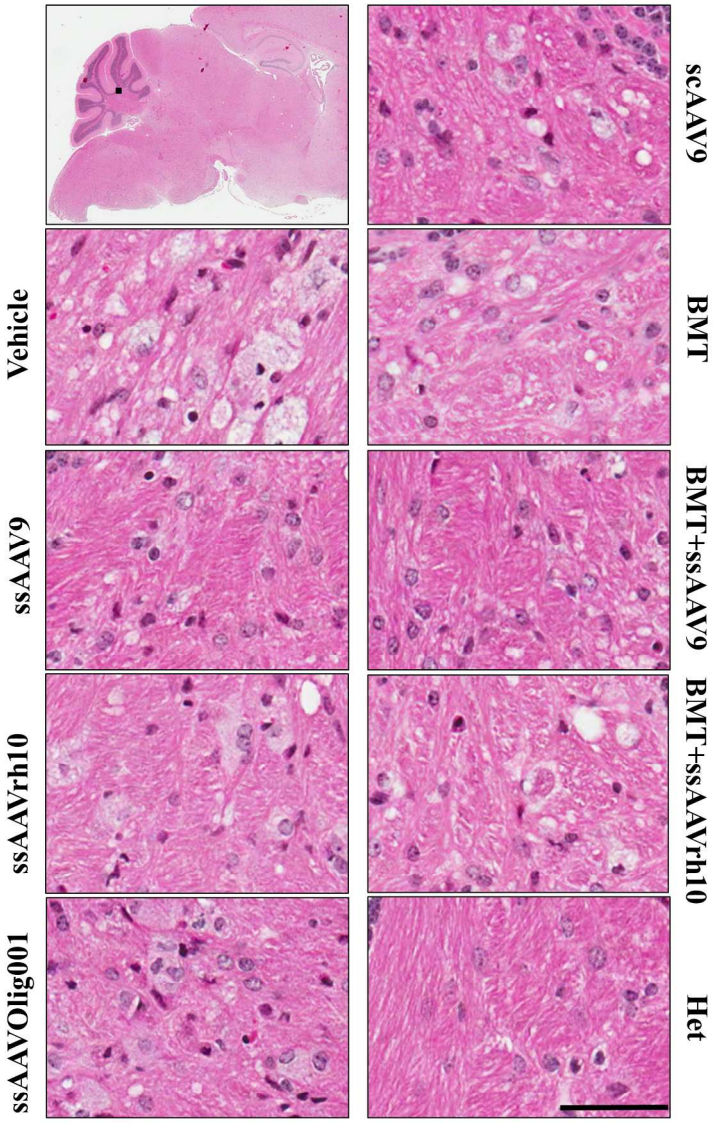
Supplemental Figure-2- Psychosine accumulation in major organs in control and treated twitcher mice. The psychosine levels were below the lower limit of detection in heart and liver of all the mice tested.

190x254mm (300 x 300 DPI)



Supplemental Figure 3- Average axon G-Ratio in the sciatic nerve for different treatments

254x190mm (300 x 300 DPI)



Supplemental Figure 4- HE images corresponding to the LFB images in Figure 5. The scale bar is 200μm.

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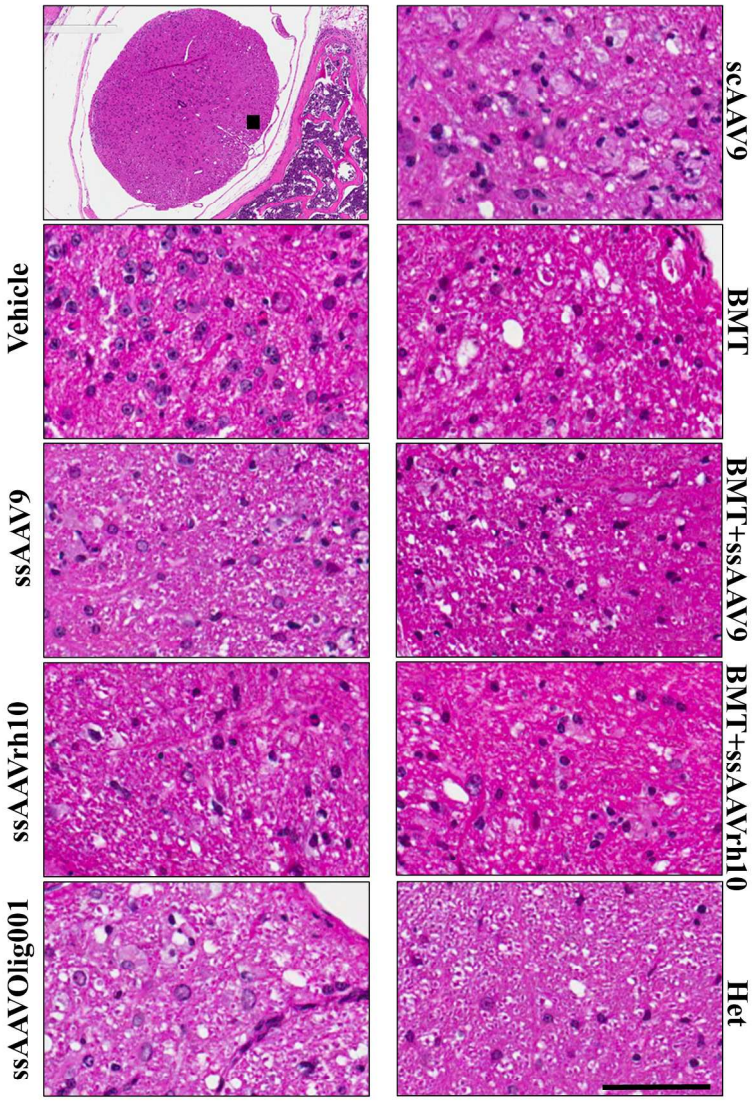

Supplemental Figure 5- HE images corresponding to the LFB images in Figure 6. The scale bar is 200μm.

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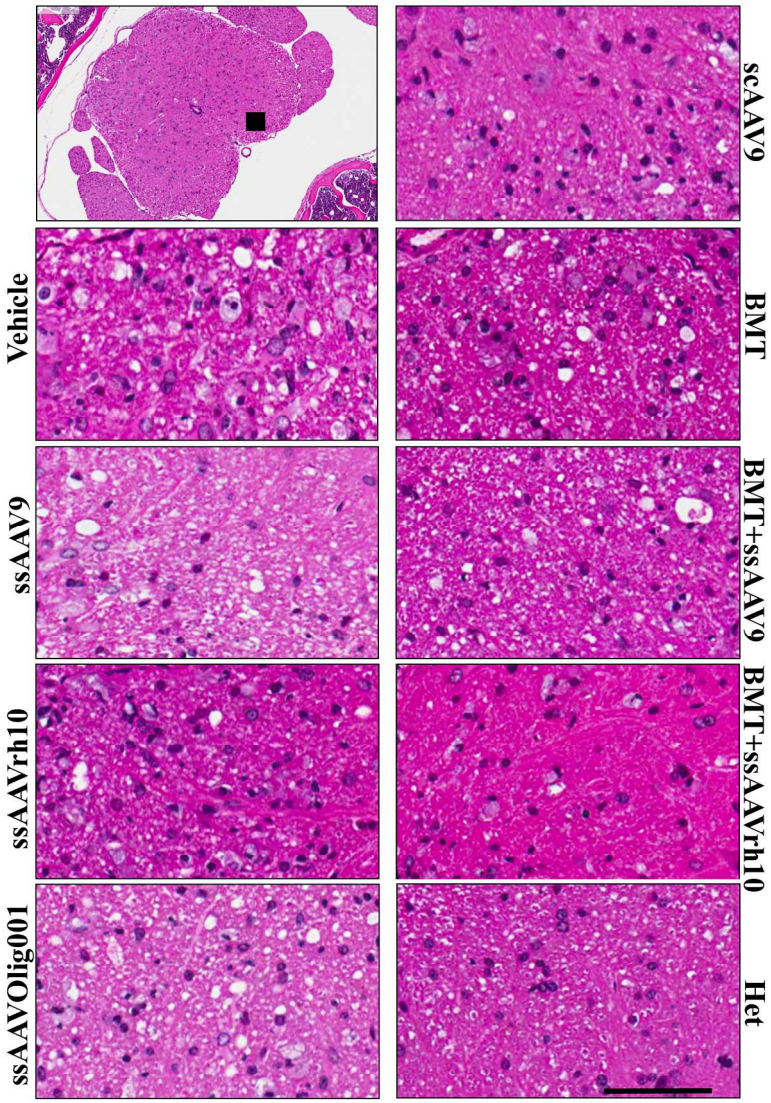

Supplemental Figure 6- HE images corresponding to the LFB images in Figure 7 . The scale bar is 200μm.

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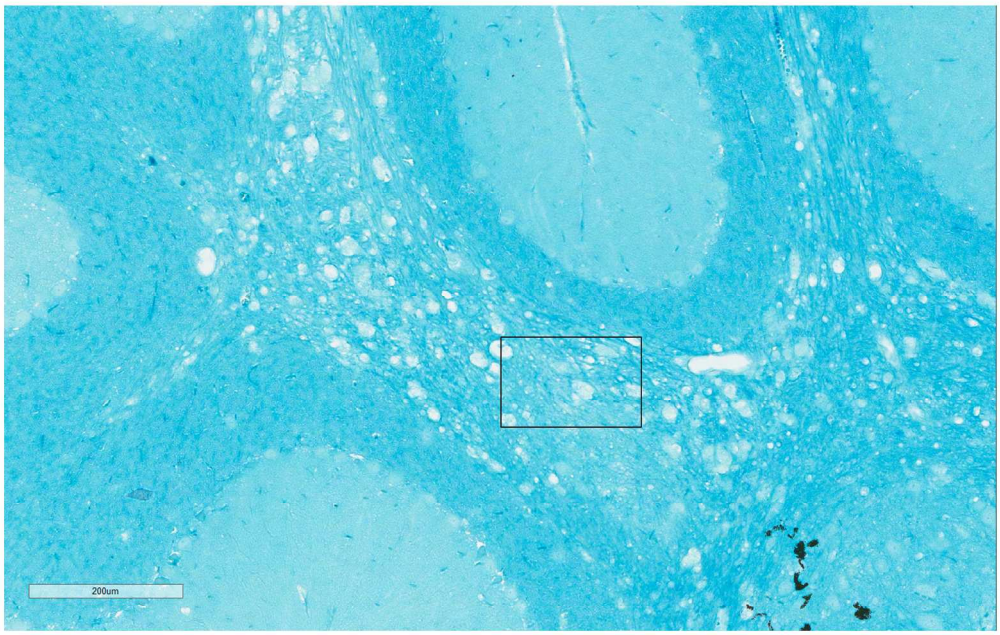
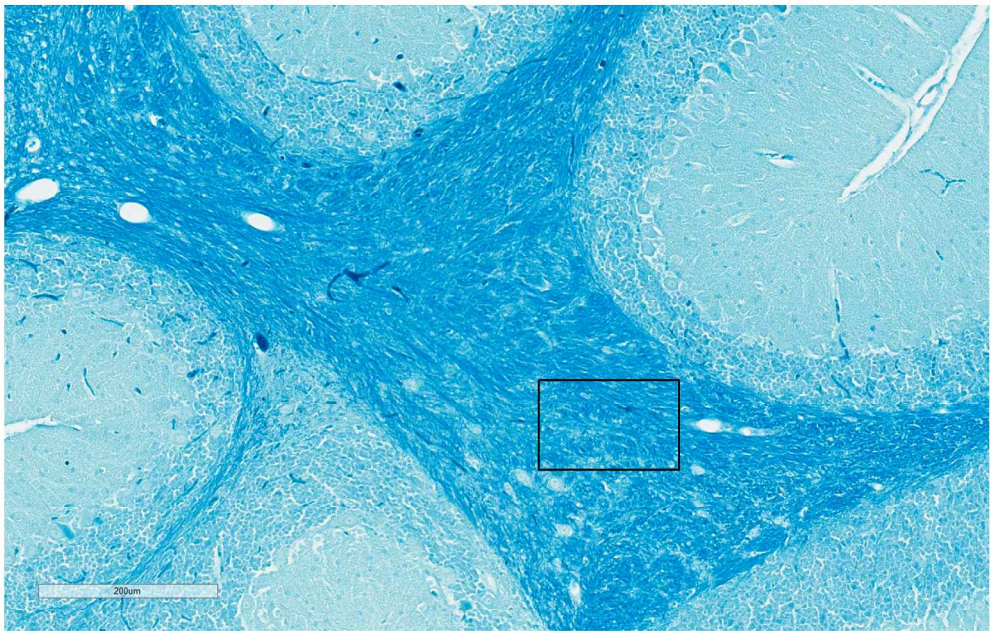


Supplemental Figure 7 - Larger area image for LFB stained cerebellar region of twitcher treated with vehicle corresponding to the images in Figure 5. The area within the rectangle is included in Figure 5 at a higher (2X) magnification

254x210mm (300 x 300 DPI)



Supplemental Figure 8 - Larger area image for LFB stained cerebellar region of a heterozygous littermate corresponding to the images in Figure 5. The area within the rectangle is included in Figure 5 at a higher (2X) magnification

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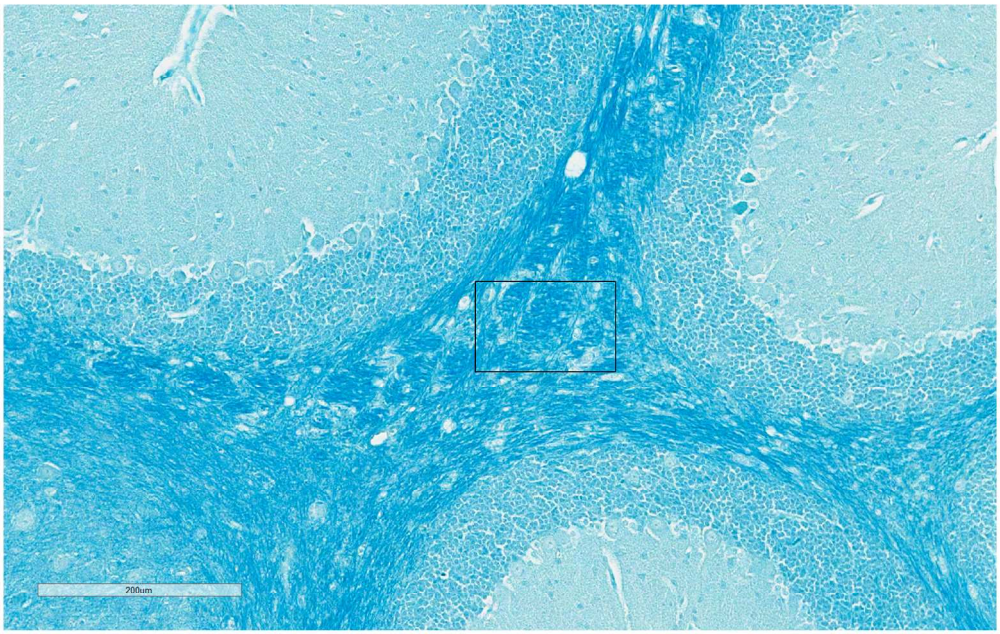

Supplemental Figure 9 - Larger area image for LFB stained cerebellar region of twitcher treated with ssAAV9+BMT combination corresponding to the images in Figure 5. The area within the rectangle is included in Figure 5 at a higher (2X) magnification.

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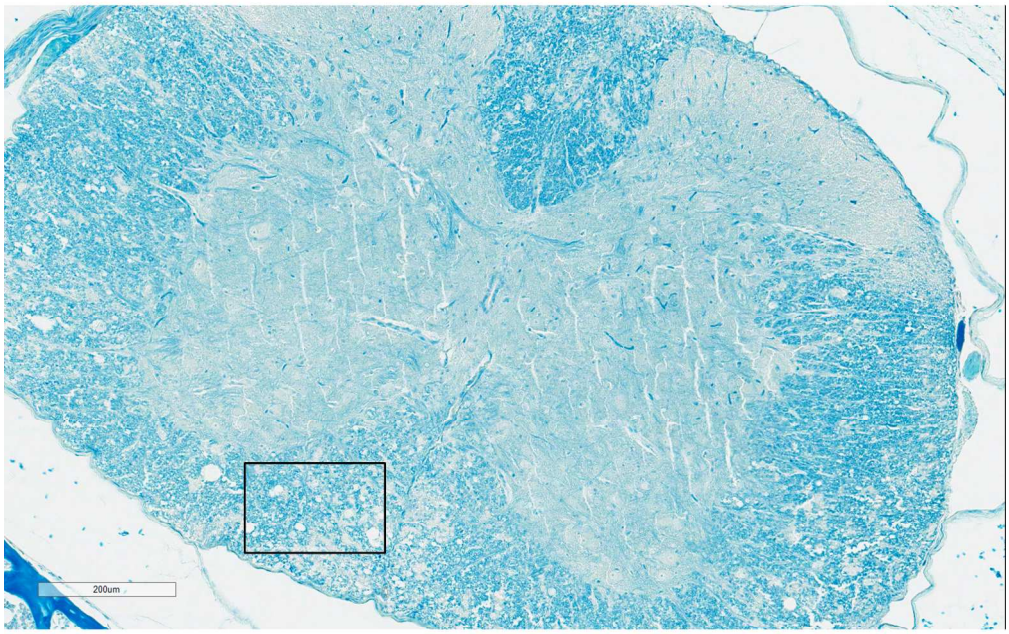

Supplemental Figure 10- Larger area image for LFB stained Cervical cord of twitcher treated with vehicle corresponding to the images in Figure 6. The area within the rectangle is included in Figure 6 at a higher (2X) magnification

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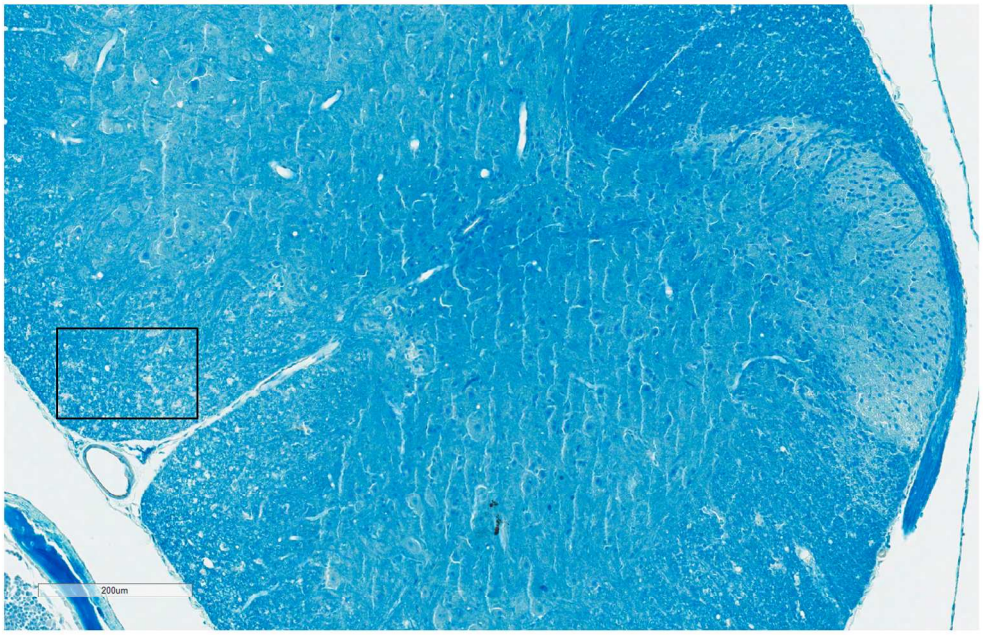
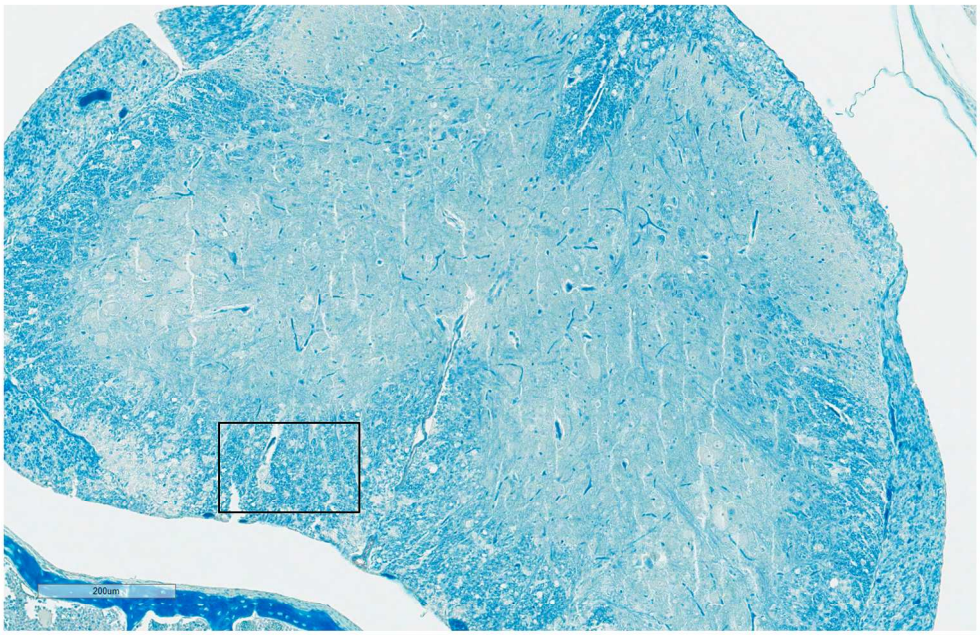
254x209mm (300 x 300 DPI)



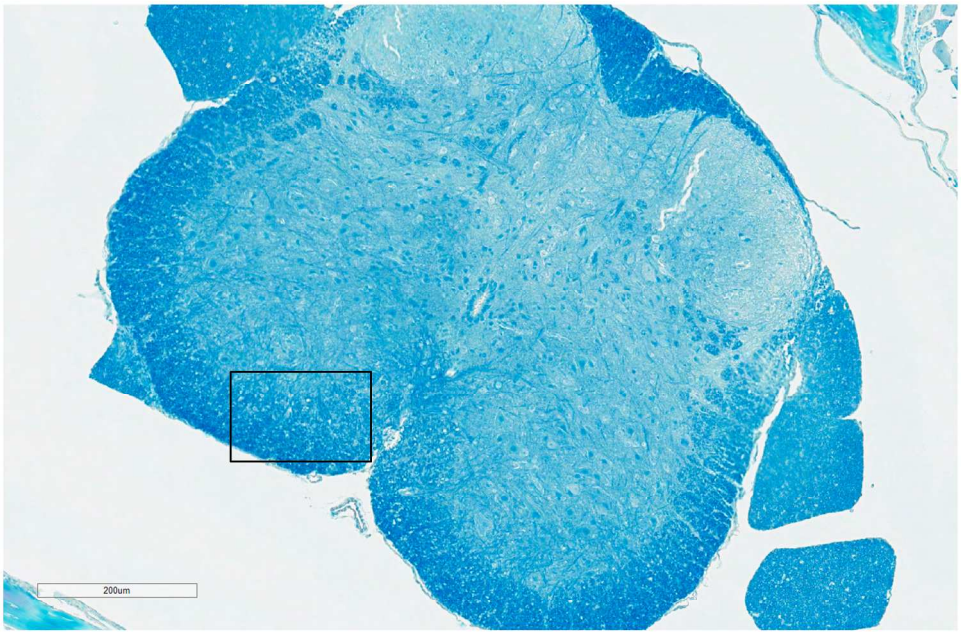
Supplemental Figure 11 - Larger area image for LFB stained cervical spinal cord of twitcher treated with ssAAV9+BMT combination corresponding to the images in Figure 6. The area within the rectangle is included in Figure 6 at a higher (2X) magnification.

254x209mm (300 x 300 DPI)



Supplemental Figure 12 – Larger area image for LFB stained Lumbar spinal cord of twitcher treated with vehicle corresponding to the images in Figure 7. The area within the rectangle is included in Figure 7 at a higher (2X)

254x209mm (300 x 300 DPI)



Supplemental Figure 13 - Larger area image for LFB stained Lumbar spinal cord of twitcher treated with ssAAV9+BMT combination corresponding to the images in Figure 7. The area within the rectangle is included in Figure 7 at a higher (2X) magnification.

254x209mm (300 x 300 DPI)