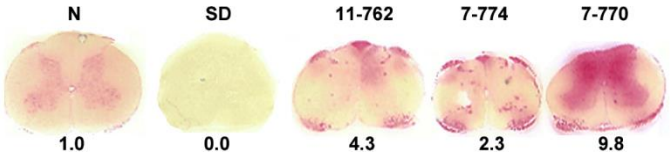

Figure S1. Therapeutic enzyme distribution in the cervical spinal cord of Sandhoff disease (SD) cats 16 weeks after AAV-treatment. SD cats were treated with bilateral thalamus and deep cerebellar nuclei injections of AAV-*fHEXA* and AAV-*fHEXB* (4.4×10^{12} vector genomes total, 1:1 vector ratio) and tissues were collected 16 weeks after treatment. Lysosomal Hex activity (red) detected with a naphthol-based substrate is shown for the cervical intumescence (block K) of AAV-treated SD cats. Corresponding Hex activity against the α-subunit preferred MUGS substrate is shown below each block as fold normal level. Representative control sections for block K are shown from untreated normal cats (N) and untreated SD cats (SD), which express 0.00 ± 0.01 fold normal Hex activity in block K. Specific activity for the normal control block was 17 ± 3.8 nmol 4MU/mg/hr. Abbreviations: MUGS = 4-MU-6-sulfa-2-Acetoamido-2-Deoxy-β-D-glucopyranoside; Hex = hexosaminidase; AAV = adeno-associated virus

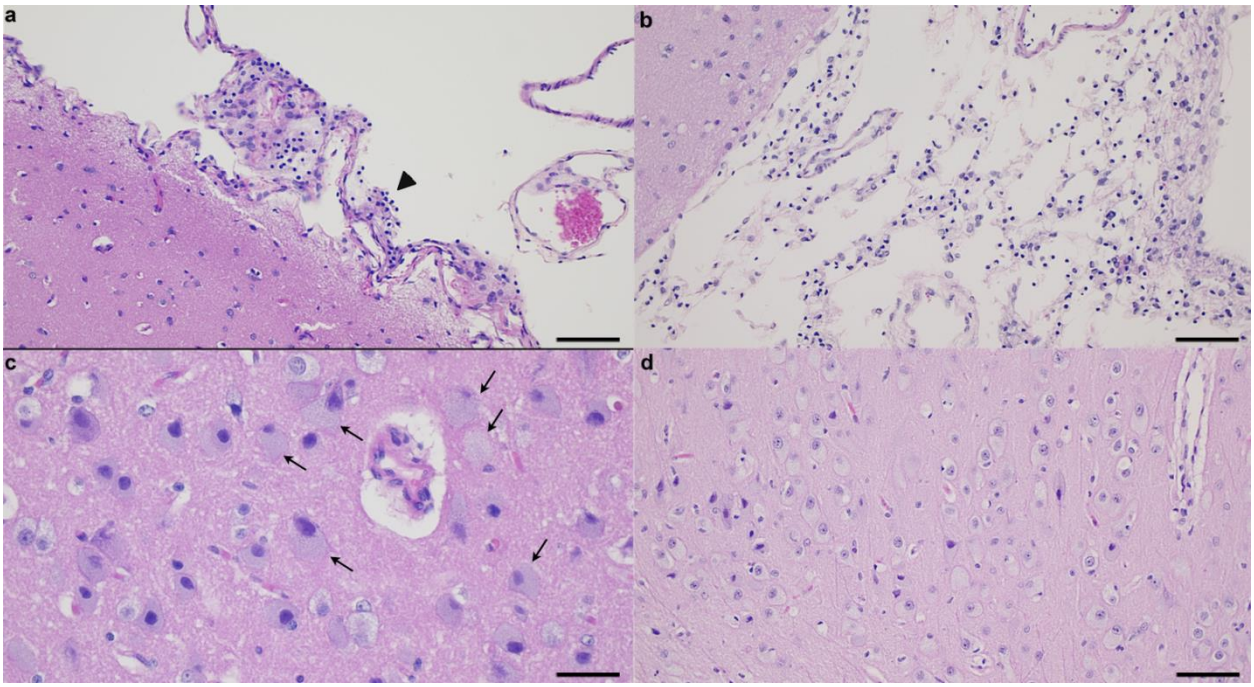

Figure S2. Histological abnormalities in the central nervous system of AAV-treated and untreated Sandhoff disease (SD) cats. SD cats were treated with bilateral thalamus and deep cerebellar nuclei injections of AAV-*fHEXA* and AAV-*fHEXB* (4.4 x 10^{12} vector genomes total, 1:1 vector ratio). Tissues were collected 16 weeks after treatment and analyzed for histological abnormalities. (**a**) Shown is a representative region from cat 7-770, block D. Rarely, the leptomeninges are multifocally expanded by small accumulations of lymphocytes and fibroblasts (black arrow head). Bar = 50 μM. (**b**) The abnormality was more diffusely seen in untreated SD cats. Bar = 50 μM. (**c**) Shown is a representative region from cat 7-770, block E. Mildly swollen neurons with copious, foamy, cytoplasmic vacuoles, which occasionally displace the neuronal nucleus peripherally (black arrows), were seen in discrete locations of deeper cortical laminae and scattered throughout cortical gray matter. Bar = 12.5 μM. (**d**) In untreated SD cats, markedly swollen neurons with copious cytoplasmic vacuoles and a peripherally displaced nucleus were apparent throughout all cerebral cortical laminae and scattered throughout the remaining cerebral gray matter. Bar = 50 μM.

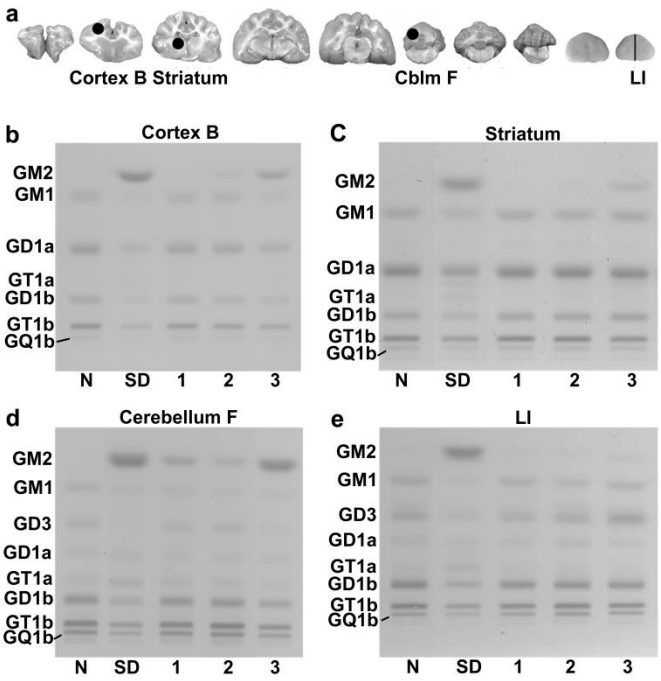

Figure S3. HPTLC of glycosphingolipids in the central nervous system of Sandhoff disease (SD) cats 16 weeks after treatment. SD cats were treated with bilateral thalamus and deep cerebellar nuclei injections of AAV-*fHEXA* and AAV-*fHEXB* (4.4 x 10^{12} vector genomes total, 1:1 vector ratio). Tissues were collected 16 weeks after treatment and ganglioside distribution was compared to that of normal cats and untreated SD cats. (**a**) Shown are locations of the 8 mm punch biopsy samples from representative CNS locations (black circles). The qualitative distribution of gangliosides on chromatograms was visualized in the (**b**) cortex block B, (**c**) striatum (from block C in **Fig. 1a**), (**d**) cerebellum block F, and (**e**) lumbar intumescence (LI, block O in **Fig. 1a**; half of the block was used). The amount of gangliosides spotted per lane was equivalent to 1.5 μg sialic acid. N, representative normal cat; SD, representative untreated SD cat; 1, cat 7-774; 2, cat 7-770; 3, cat 11-762. Abbreviations: Cblm = cerebellum; CNS = central nervous system; HPTLC = high performance thin layer chromatography

Table S1. Hex activity against MUG substrate in brain, spinal cord, cerebrospinal fluid, sciatic nerve, and pituitary of AAV-treated and untreated Sandhoff disease (SD) cats

		Fold normal MUG activity[1, 2]				
		SD + AAV				**SD no tx**
Region	Block	11-762	7-770	7-774	Mean, (s.d)	Mean, (s.d)
Cerebrum	A	3.2	1.4	2.0	**2.2** (0.87)	**0.00** (0.00)
	B	2.6	2.3	1.9	**2.3** (0.35)	**0.00** (0.00)
	C	3.6	4.8	2.7	**3.7** (1.1)	**0.00** (0.00)
	D	5.4	23	29	**19** (12)	**0.00** (0.00)
	E	79	23	23	**42** (33)	**0.00** (0.00)
Cerebellum	F	4.3	5.4	7.8	**5.8** (1.8)	**0.00** (0.00)
	G	19	25	66	**36** (25)	**0.01** (0.01)
	H	22	40	2.5	**22** (19)	**0.00** (0.00)
Spinal cord	I	4.9	7.9	1.7	**4.8** (3.1)	**0.00** (0.00)
	J	2.6	7.0	1.8	**3.8** (2.8)	**0.00** (0.00)
	K	4.4	9.0	2.1	**5.2** (3.5)	**0.00** (0.01)
	L	29	7.8	3.2	**13** (14)	**0.01** (0.02)
	M	7.5	13	3.8	**8.1** (4.6)	**0.00** (0.00)
	N	7.1	9.4	3.4	**6.6** (3.0)	**0.01** (0.01)
	O	18	15	4.1	**12** (7.2)	**0.00** (0.01)
CSF	N/A	15	4.9	8.5	**9.5** (5.1)	**0.03** (0.04)
Sciatic N.	N/A	4.4	4.5	2.0	**3.6** (1.4)	**0.01** (0.01)
Pituitary	N/A	0.08	0.06	0.11	**0.08** (0.03)	**0.01** (0.00)

[1] Hex activity against the β-subunit specific substrate (MUG) was significantly higher in AAV-treated SD cats (n = 3) than in untreated SD cats (n = 5) in **A-H** ($P \leq 0.018$ for each block), **I-O** ($P \leq 0.013$ for each block), **CSF** ($P = 0.025$), **sciatic nerve** ($P = 0.018$), and **pituitary** ($P = 0.018$).

[2] Hex activity against the β-subunit specific substrate (MUG) was significantly higher in AAV-treated SD cats (n = 3) than in normal cats (n = 4) in **A-H** ($P \leq 0.026$ for each block), **J-O** ($P \leq$

0.026 for each block), **CSF** (P = 0.026), and **sciatic nerve** (P = 0.018), but not in block **I** (P = 0.056), and was significantly lower than in normal cats in **pituitary** (P = 0.026).

Abbreviations: no tx = no treatment; N/A = not applicable; Sciatic N. = sciatic nerve; CSF = cerebrospinal fluid, SD = Sandhoff disease; AAV = adeno associated virus; Hex = hexosaminidase; MUG = 4-MU-N-acetyl-β-D-glucosaminide

Table S2. Vector copy number in brain, spinal cord, sciatic nerve, and pituitary of AAV-treated Sandhoff disease (SD) cats

		Vector copy number per µg genomic DNA			
Region	Block	11-762	7-770	7-774	Mean, (s.d)
Cerebrum	A	12 000	7 300	27 000	**15 000** (10 000)
	B	7 600	6 500	11 000	**8 400** (2 300)
	C	17 000	12 000	9 900	**13 000** (3 600)
	D	35 000	36 000	54 000	**42 000** (11 000)
	E	79 000	25 000	36 000	**47 000** (29 000)
Cerebellum	F	1 900	6 300	5 200	**4 500** (2 300)
	G	71 000	4 900	67 000	**48 000** (37 000)
	H	nd	8 000	nd	N/A
Spinal cord	I	2 100	1 300	3 900	**2 400** (1 300)
	J	3 600	950	6 800	**3 800** (2 900)
	K	2 700	1 500	4 500	**2 900** (1 500)
	L	4 500	3 200	6 000	**4 600** (1 400)
	M	2 200	1 200	2 900	**2 100** (850)
	N	2 600	850	2 600	**2 000** (1 000)
	O	2 600	2 100	3 500	**2 700** (710)
Sciatic nerve	N/A	7 400	1 900	2 700	**4 000** (3 000)
Pituitary	N/A	1 125	2 800	600	**1 500** (1 100)

Block lettering corresponds to **Fig. 1a**.

Abbreviations: CNS = central nervous system; nd = not determined as no sample available for quantitative PCR analysis; N/A = not applicable

Table S3. Quantification of ganglioside storage in the central nervous system of Sandhoff disease (SD) cats 16 weeks after treatment

Sample[1]	Tx group	N value	Total sialic acid	GM2	GA2
			(μg/100 mg dry weight, mean (s.d.))		
Cortex B	Normal no tx	2	**550** (130)	**0.0** (0.0)	**0.0** (0.0)
	SD no tx	4	**1 200** (620)	**570** (310)	**1 200** (330)
	SD + AAV	3	**550** (54)*	**61** (73)*	**120** (200)*
Striatum	N no tx	2	**380** (40)	**0.0** (0.0)	**0.0** (0.0)
	SD no tx	3	**1 100** (270)	**440** (100)	**1 200** (410)
	SD + AAV	3	**470** (70)**	**14** (17)**	**4.0** (4.6)**
Cerebellum F	Normal no tx	2	**430** (28)	**0.0** (0.0)	**0.0** (0.0)
	SD no tx	2	**930** (14)	**510** (24)	**1 800** (40)
	SD + AAV	3	**490** (120)	**95** (85)	**340** (270)
LI	Normal no tx	2	**140** (13)	**1.5** (2.1)	**1.5** (2.1)
	SD no tx	4	**470** (160)	**240** (120)	**490** (150)
	SD + AAV	3	**140** (14)*	**4.0** (0.0)*	**2.0** (1.7)*

[1] Ganglioside storage was quantified in 8 mm punch biopsy samples shown in **Fig. S3a** for comparison to that of normal cats and untreated SD cats.

*, concentration was significantly lower than in untreated SD cats, $P \leq 0.026$; **, concentration was significantly lower than in untreated SD cats, $P = 0.040$

Abbreviations: Tx = treatment; LI = lumbar intumescence

Video S1. Untreated Sandhoff disease (SD) cat at 3.2 months old. An untreated SD cat demonstrates severe overt whole body tremors, instability and limited ambulation that warrants a clinical rating score of 5 (on a scale of 10; see **Fig. 7a**). This animal reached the humane endpoint (inability to stand on 2 consecutive days) at 4.6 months old. Untreated animals do not show signs of pain during the disease process.

Video S2. AAV-treated Sandhoff disease (SD) cat with a wide based stance near the study endpoint. An AAV-treated SD cat (11-762) at 4.8 months old demonstrates hind limb weakness and a wide based stance that warrants a clinical rating score of 8 (on a scale of 10; see **Fig. 7a**). This animal also displays subtle tremors, but overt whole body tremors are absent.