

SUPPLEMENTAL INFORMATION

FIGURES

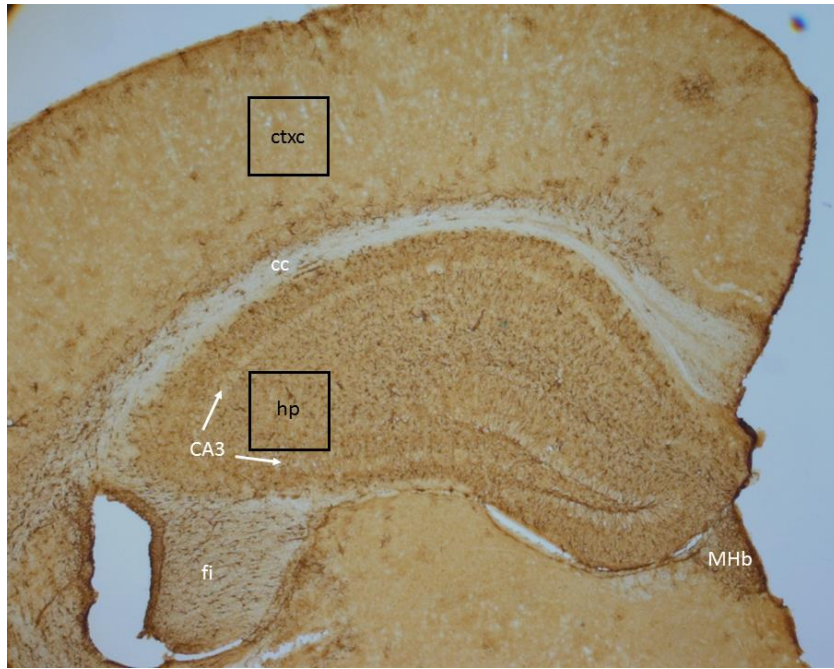


Figure S1. Illustration depicting the areas used for counting inflammatory cells, here demonstrated at low power in a coronal section stained for GFAP. Frames mark areas in cerebral cortex (ctxc) and hippocampus (hp). The areas were selected by applying the 10 x 10 mm frame at first in the hippocampus in the region immediately medially to the CA3 of the hippocampal cortex, while simultaneously containing the medial habenular nucleus (MHb). The microscopic slide was next orientated vertically to contain the ctxc without deviating from the horizontal plane. Fi, fimbria; cc, corpus callosum.

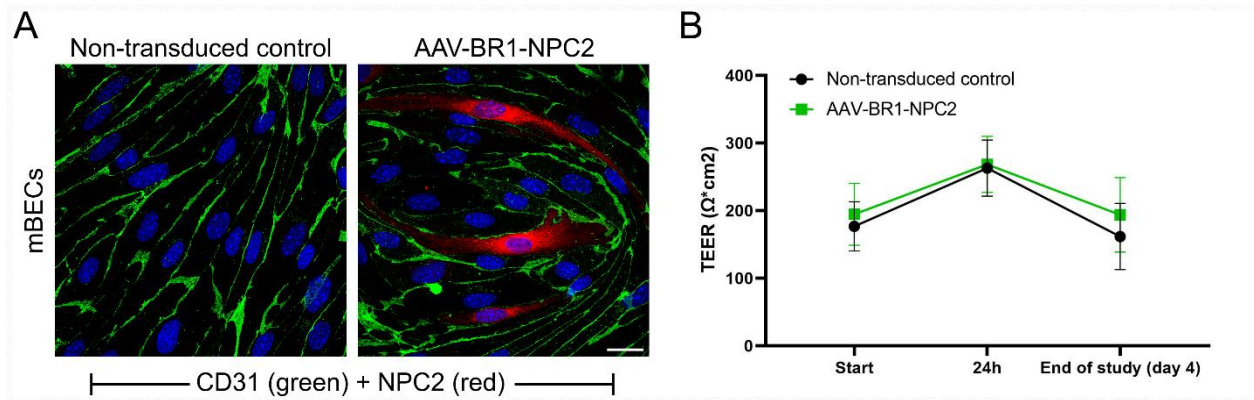


Figure S2. Evaluation of Niemann Pick C2 protein (NPC2) expression and barrier integrity in primary mouse brain endothelial cells (mBECs) after transduction with AAV-BR1-NPC2 *in vitro*. **A)** Images of non-transduced and transduced (AAV-BR1-NPC2) mBECs showing expression of the endothelial cell marker cluster of differentiation 31 (CD31) (green) and NPC2 (red) in the transduced cells. Nuclei are stained with DAPI (4',6-Diamidino-2-phenylindole dihydrochloride) (blue). The scale bar is 20 μm . **B)** The integrity of the *in vitro* blood-brain barrier model is assessed by measuring the transendothelial electrical resistance (TEER). TEER is not affected by transduction with the AAV-BR1-NPC2 vector analyzed with a two-way repeated measure ANOVA ($F[1,6] = 0.430$, $p = 0.5363$). The data are presented as mean $\pm$ SD ($n = 4$ culture inserts/group).

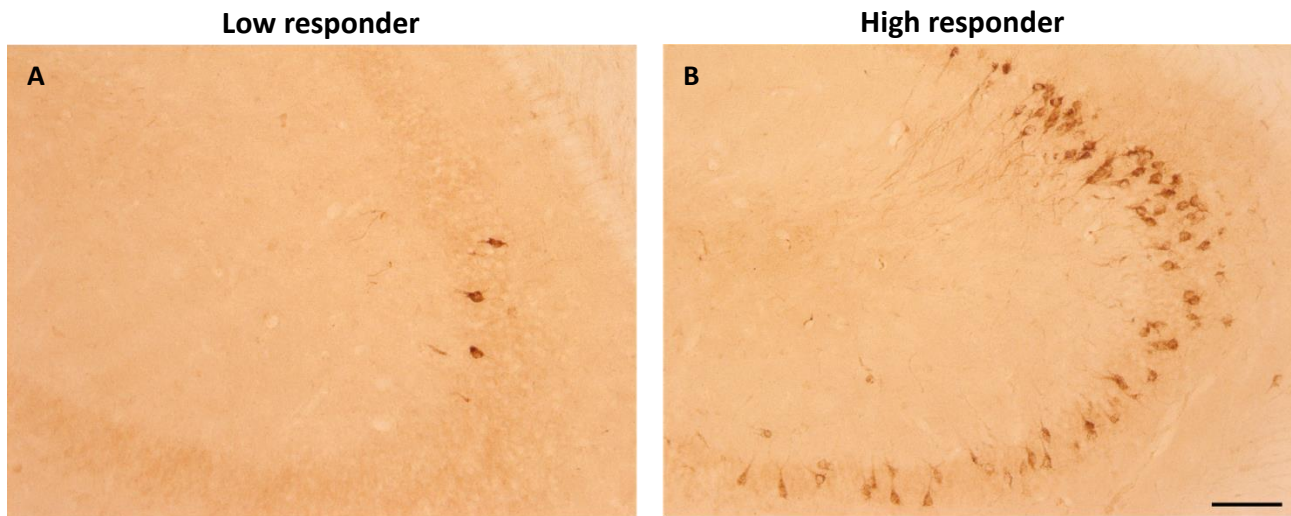


Figure S3. NPC2 expression in the hippocampus of AAV-BR1-NPC2-treated *Npc2*^{-/-} mice. A large variation in the number of NPC2-positive cells was observed between the low (A) and the high responder (B) mice, with almost no NPC2-positive cells observed in the low responder group. The cell morphology of the NPC2-positive cells in the hippocampus resembles that of neurons. The images are representative of n=2 low responders and n=3 high responders. Scale bar = 100 μ m.

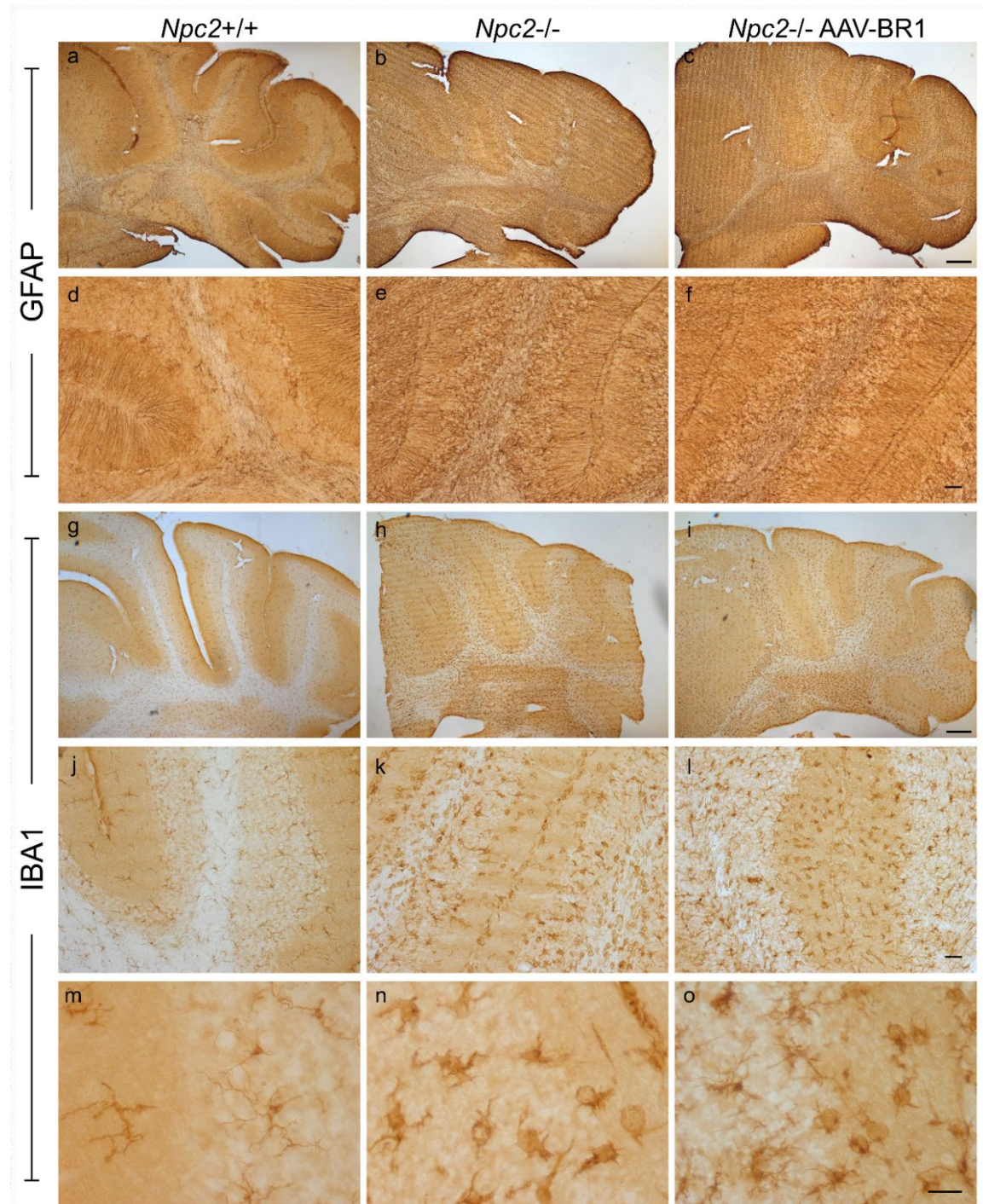


Figure S4. Effect of the AAV-BR1-NPC2 gene therapy on cerebellar inflammation. Cerebellar brain sections evaluated using immunohistochemical staining for the astrocyte marker GFAP (a-f), and microglia marker IBA1 (g-o). The wild-type (*Npc2*^{+/+}) mice exhibit normal expression of astrocytes and microglia in the cerebellum. In contrast, severe and diffuse astrogliosis and reactive microglia are seen in both *Npc2*^{-/-} and AAV-BR1-NPC2-treated *Npc2*^{-/-} mice. Higher magnification of the neuroinflammatory findings in the cerebellum; GFAP (d-f) and IBA1 (j-o). In both untreated and AAV-BR1-NPC2-treated *Npc2*^{-/-} mice severe astrogliosis and reactive microglia with a characteristic amoeboid morphology are present in the cerebellum. Images are representative of *n* = 4 *Npc2*^{+/+} mice, *n* = 4 *Npc2*^{-/-} mice and *n* = 7 AAV-BR1-NPC2 treated *Npc2*^{-/-} mice (both high and low responders). Scale bar = 300 μ m (a-c and g-i), 50 μ m (d-f and j-l), 25 μ m (m-o).

TABLE**Supplemental Table S1**

Group	Number of mice	Sex	High responders
WT	10	5 females 5 males	
<i>Npc2</i> ^{-/-}	9	5 (4) females* 5 (4) males**	
<i>Npc2</i> ^{-/-} AAV- BR1	10	7 females 3 males	3 females 3 males

* A single untreated female *Npc2*^{-/-} mouse was euthanized at 10½ weeks of age due to a 20 % weight loss.

** A single male *Npc2*^{-/-} mouse was excluded at 6 weeks of age due to lower body weight and trouble with coordination and balance compared with cage mates. Subsequently, nine mice were included in this group at the time of treatment.

Supplemental Table S2

Parameter	Score	Description
Grooming	0	Normal, smooth fur
	1	Piloerection
	2	Piloerection and unkempt coat
Body position/ kyphosis	0	Normal body position with a straight spine
	1	Mild kyphosis/ hunched posture
	2	Persistent and severe kyphosis
Tremor	0	No signs of tremor
	1	Mild signs of tremor/tremor are present
	2	Severe signs of tremor making it difficult to walk
Ledge test	0	The mouse walks along the ledge without losing its balance and will lower itself back into the cage using its paws
	1	If the mouse loses its footing while walking along the ledge and does not effectively use its hind legs
	2	If the mouse falls off the ledge, or nearly so while walking or attempting to lower itself, or shakes and refuses to move at all despite encouragement
Gait	0	The mouse moves normally, with its body weight supported on all limbs, with its abdomen not touching the ground, and with both hindlimbs participating evenly
	1	Limp while walking, feet point away from the body during locomotion ("duck feet")
	2	The mouse has difficulty in moving forward and/or falls on a side while walking, and/or drags its abdomen along the ground, has no rearing activity, reluctance to walk/decreased activity
Hindlimb clasping	0	The hindlimbs are consistently splayed outward, away from the abdomen
	1	Both hindlimbs are partially retracted toward the abdomen for more than 50% of the observation time
	2	Hindlimbs are entirely retracted and touching the abdomen for more than 50% of the observation time
Explorative behavior	0	Explore 4 corners in 20 sec
	1	2-3 corners explored in 90 sec
	2	No explorative behavior

Adapted from (31–34).

Supplemental Table S3

Matrix	2.5-DHAP
Solvent	70% EtOH, 30% H2O, 0.1% TFA
Concentration (mg/mL)	5
Dry time(s)	10
Temperature (°C)	80
Number of passes	14
Flow Rate (μL/min)	100
Velocity (mm/min)	1200
Tracking Space (mm)	2
Pressure (psi)	10
Matrix Density (mg/mm ²)	2.9167E+0

MSI spray parameter for matrix application using HTX M3+ sprayer (HTX Technologies)

VIDEO MATERIAL

Video 1

This video demonstrates the performance of a wild-type *Npc2*^{+/+} mouse during the ledge test at 12 weeks of age. This mouse receives a score of 0 (see Supplemental Table I) due to walking along the ledge without losing its balance and both hind limbs participating evenly during walking.

Video 2

This video shows an example of how an untreated *Npc2*^{-/-} mouse performs during the ledge test at 12 weeks of age. This mouse receives a score of 2 (see Supplemental Table I) due to severe trouble with the balance, nearly falling off the ledge, and having difficulties in moving forward.

Video 3

This video shows an example of an untreated *Npc2*^{-/-} mouse at 12 weeks of age. The mouse has piloerection, tremor, and ataxia gait, and stumbles while walking.

Video 4

This video demonstrates the effect of the brain-specific adeno-associated virus (AAV-BR1-NPC2) on the *Npc2*^{-/-} phenotype at 12 weeks of age (three red marks on the tail). Even though presenting tremor, the mouse shows normal activity, smooth fur, and a gait more comparable to the *Npc2*^{+/+} wild-type littermate (two red marks on the tail).

Video 5

This video shows an example of the impact of the AAV-BR1-NPC2 treatment on the performance during the ledge test at 12 weeks of age. The mouse receives a score of 1 (see Supplemental Table I) due to losing its grip while walking and not effectively using its hind legs. Although having some trouble keeping balance, the mouse can still walk along the ledge.