Supplementary Materials

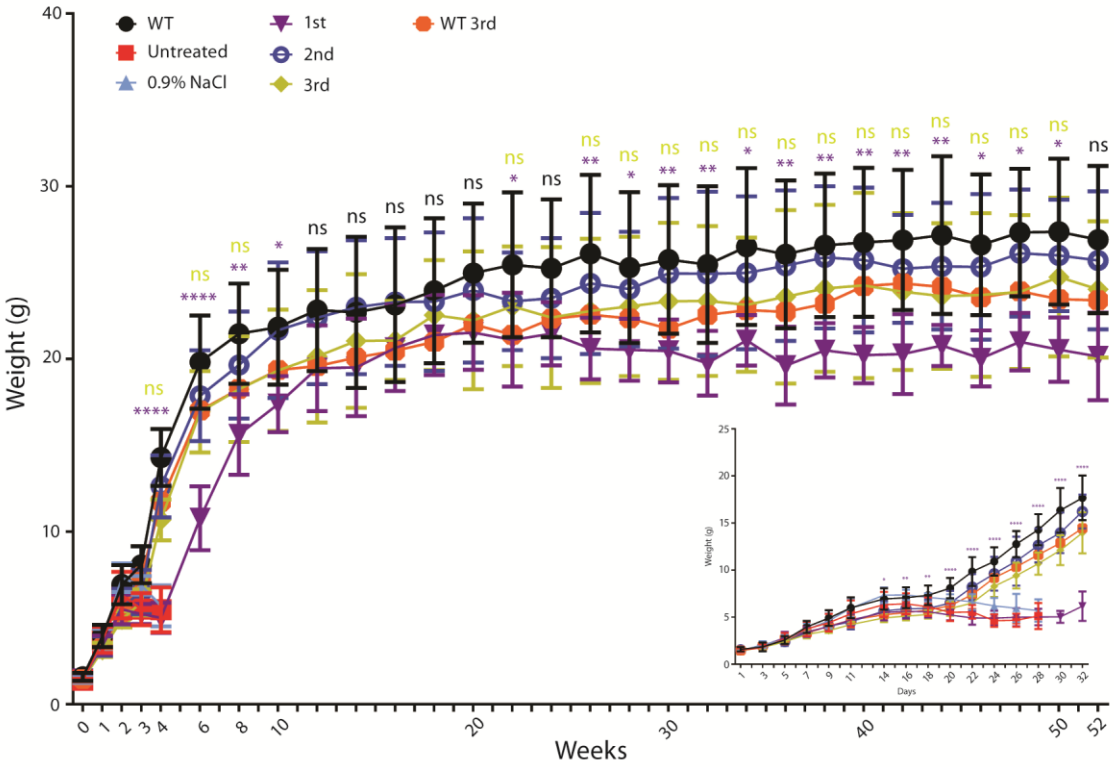

Fig. S1. **Weights of full-dose treatment groups comparing 1st, 2nd, and 3rd generation gene replacement therapy.** Mice were treated at p1 with $4x10^{11}$ GC of the three different generation vectors. Weights were taken every other day for the first 32 days and biweekly afterwards (n=10 each). Error bars indicate mean ± SD; * $p < 0.05$; ** $p < 0.01$; ns = non-significant.

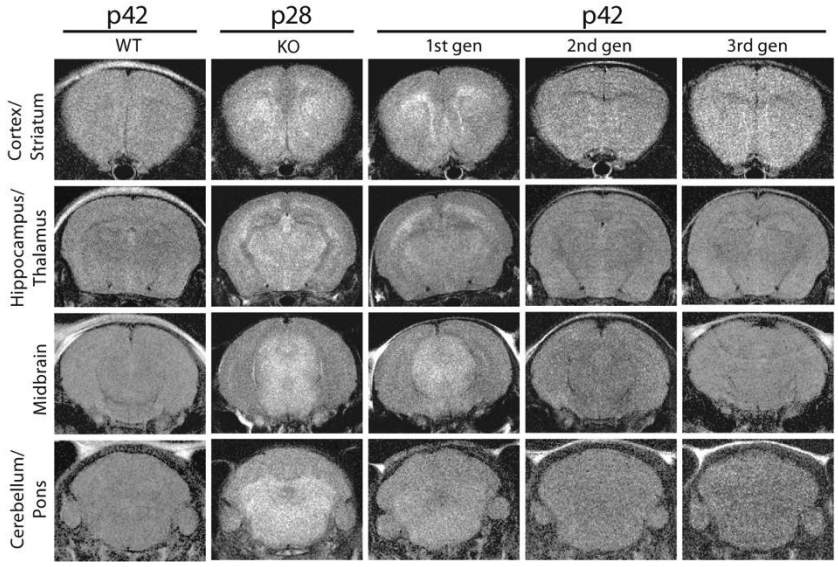

Fig. S2. **Magnetic resonance imaging (MRI) T2 sequence at p28/42.** Mice treated at p1 with either 1st, 2nd, or 3rd generation gene therapy were imaged by MRI (n=3 per group). Shown is the T2 sequence, which emphasizes signals derived from water. Treated and wild-type (WT) mice were imaged at p42. Due to early lethality, untreated (knock-out; KO) mice were imaged at p28. KO mice display strong hyperintensity (white signals) in striatum, cortex, thalamus, midbrain, and cerebellum/pons. A gradual reduction of hyperintense signals can be seen in the different treatment groups. 3rd generation treated mice show the same signal pattern as WT mice, suggesting reversal of MRI T2 pathologic signals.

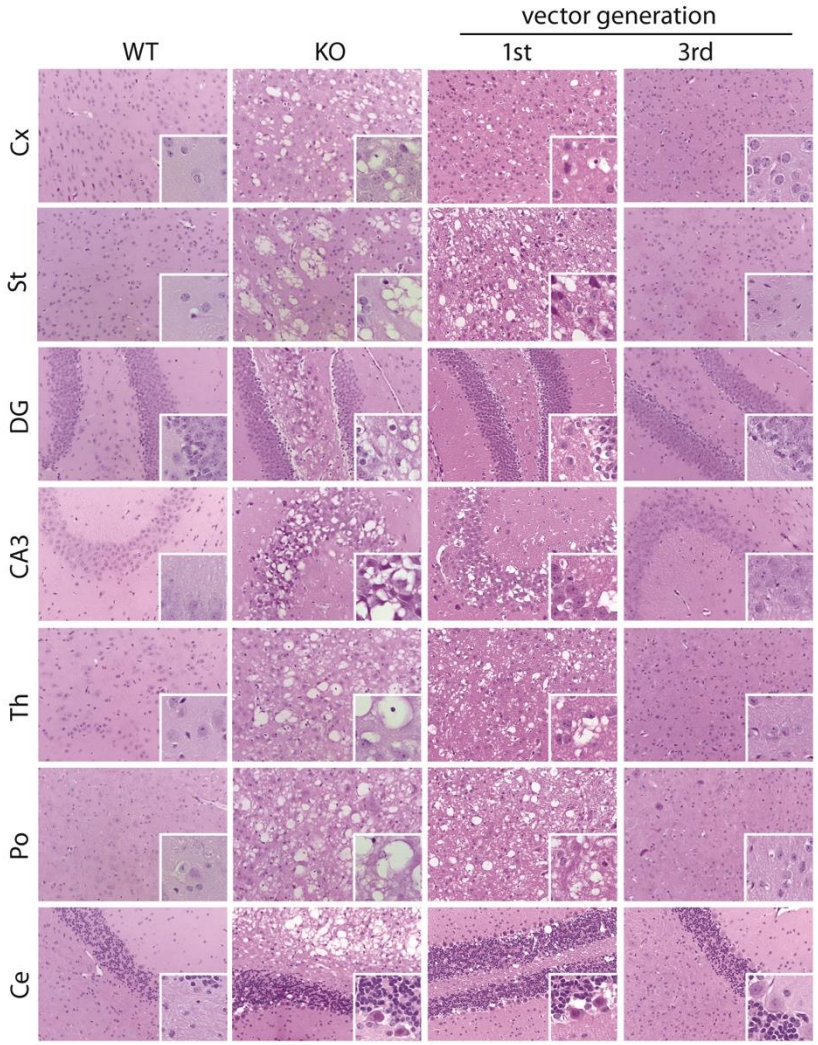

Fig. S3. Treatment of CD KO mice with 3rd generation gene therapy leads to rescue of **neuropathology at p25.** Mice (n=3 per group) were treated at P1 with $4x10^{11}$ GC via facial vein and sacrificed at p25 for neuropathology. Untreated (KO) mice show extensive vacuolization across the brain. 1st generation treated mice show less vacuole formation, but still display substantial defects. 3rd generation treated mice are indistinguishable from wild-type (WT). Cx = cortex; St = striatum; DG = dentate gyrus; CA3 = cornu ammonis 3; Th = thalamus; Po = pons; Ce = cerebellum. Images are x10, insets are x40.

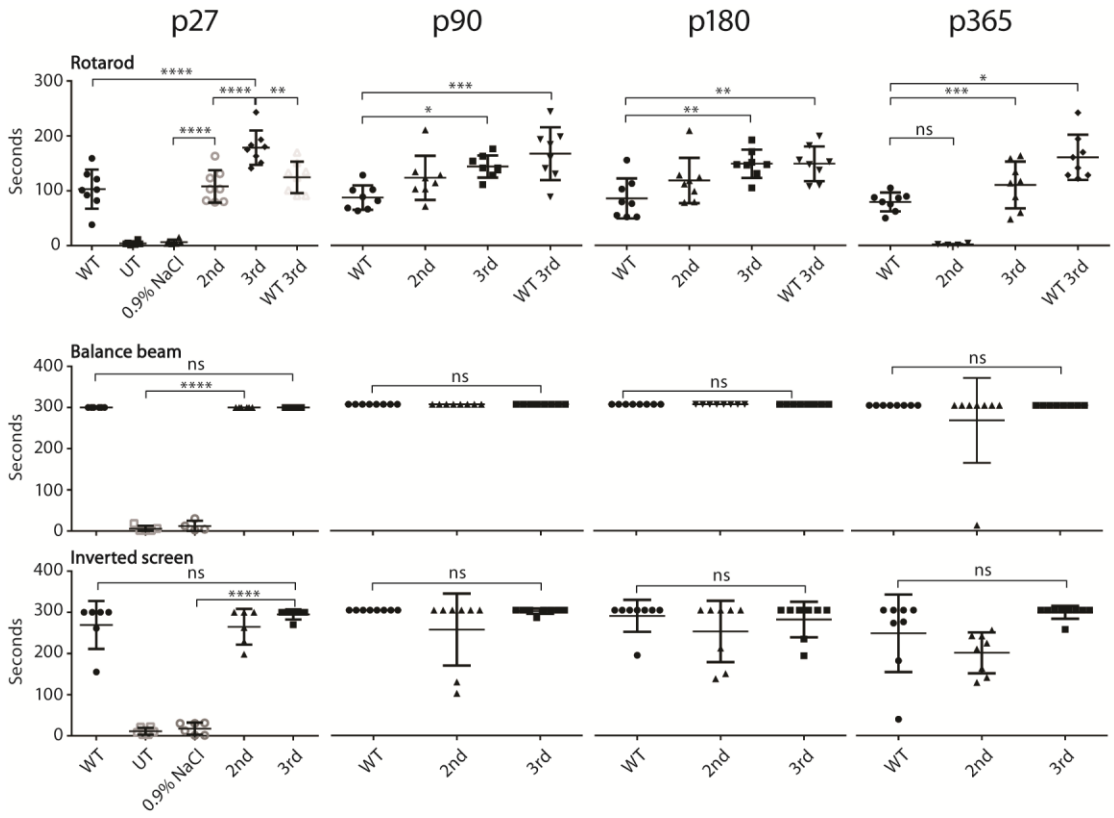

Fig. S4. **Treatment of CD KO mice with 2nd or 3rd generation gene therapy rescues motor function at p27, p90, p180, and p365.** Mice treated at p1 with $4x10^{11}$ GC by facial vein delivery of 2nd or 3rd generation gene therapy were tested on rotarod, balance beam, and inverted screen over the course of 1 year. All four testing time points are shown, demonstrating rescue of the motor function phenotype. Error bars indicate mean ± SD; n=6-8; * p<0.05; ** p<0.01; *** p<0.001; **** p<0.0001; ns = non-significant.

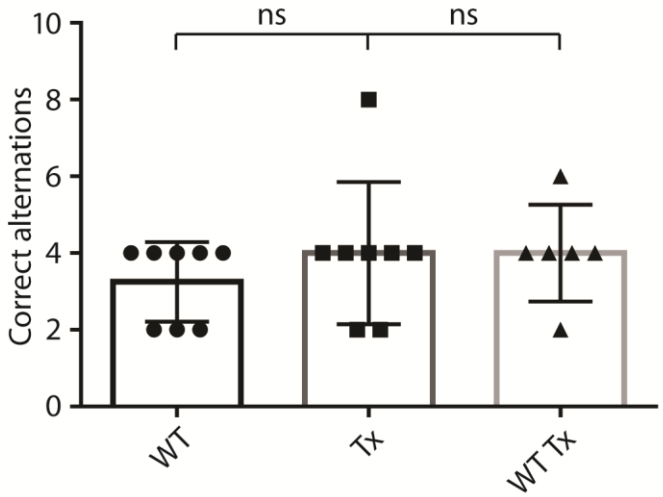

Fig. S5. **T maze at 1 year**. P1 treated CD KO mice and WT mice were tested on T maze for spatial/working memory and compared to untreated WT mice. Each mouse was tested 11 times with 20 seconds retention time between each run. Error bars indicate mean ± SD; n=6-8; ns=not significant.

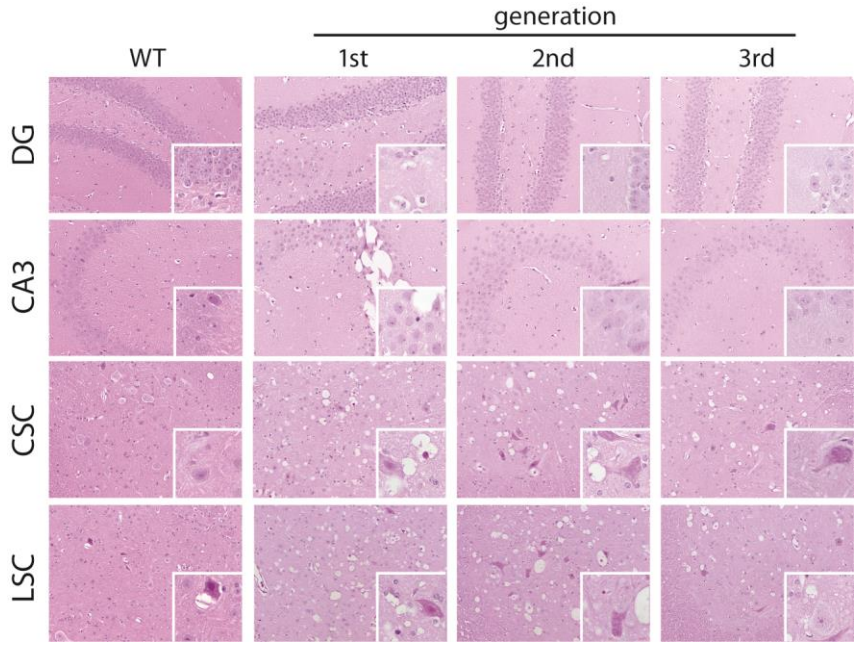

Fig. S6. **Treatment of CD KO mice with 3rd generation gene therapy leads to sustained rescue of neuropathology at 1 year of age.** Mice were treated with $4x10^{11}$ GC of 1st, 2nd, or 3rd generation gene therapy. Mice were sacrificed at 1 year of age and subjected to H&E staining. Shown are 10X images with 40X insets. DG = dentate gyrus, CA3 = cornu ammonis 3, CSC = cervical spinal cord, LSC = lumbar spinal cord.

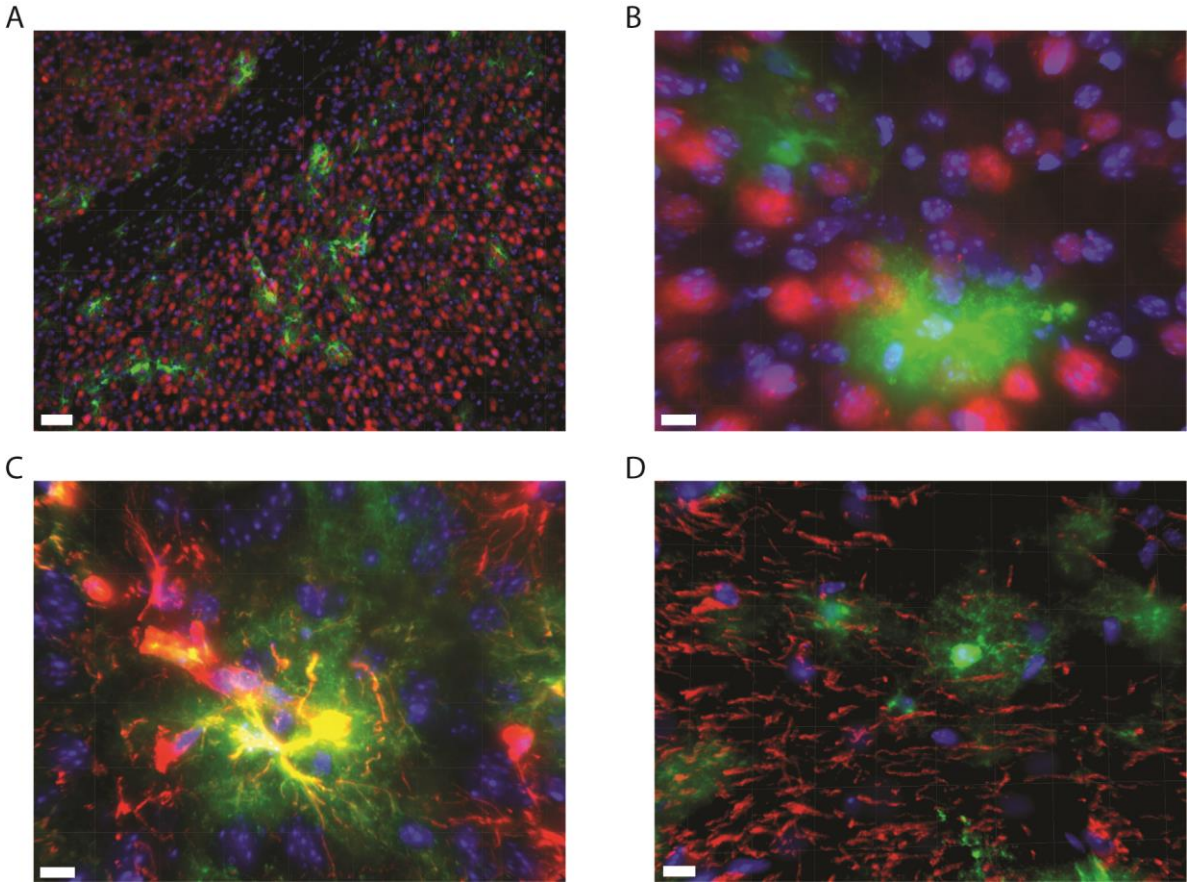

Fig. S7. **phGFAP promoter is specific to GFAP positive cells.** Shown are images of mouse brain stained against the neuronal nucleus marker (NeuN; red, **A** and **B**), glial fibrillary acidic protein (GFAP; red; high magnification of 4A; **C**) and myelin basic protein (MBP; red; high magnification of 4B; **D**) and EGFP experssion (green) driven by a partial human glial fibrillary acidic promoter. Yellow signals indicates co-localization. Scale bar 50µm for A and 10µm for B, C and D.

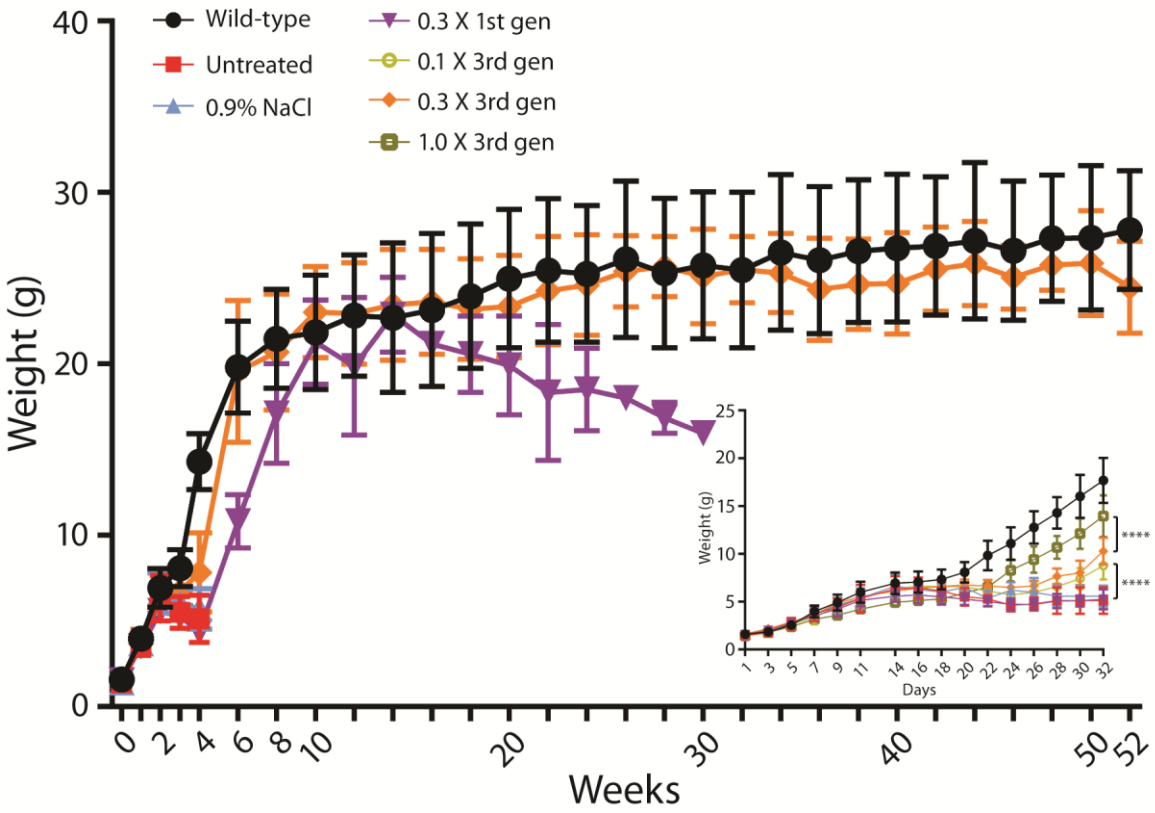

Fig. S8. **rAAV dose-dependent weights.** Shown are weights by day (inset) and week of CD KO mice treated at p1 with either full, 3- or 10-fold lower dose of the 3rd generation gene therapy construct. For comparison, CD KO mice were treated with the 1st generation gene therapy at 3-fold lower dose. Mice treated with 10-fold lower dose are only shown for the first four weeks. For weights by week of CD KO mice treated with full dose 3rd generation, see Fig. S1. Error bars indicate mean ± SD; n=8-10, except for 0.3X 1st gen after 24 weeks because animals started to die; **** $p<0.0001$.

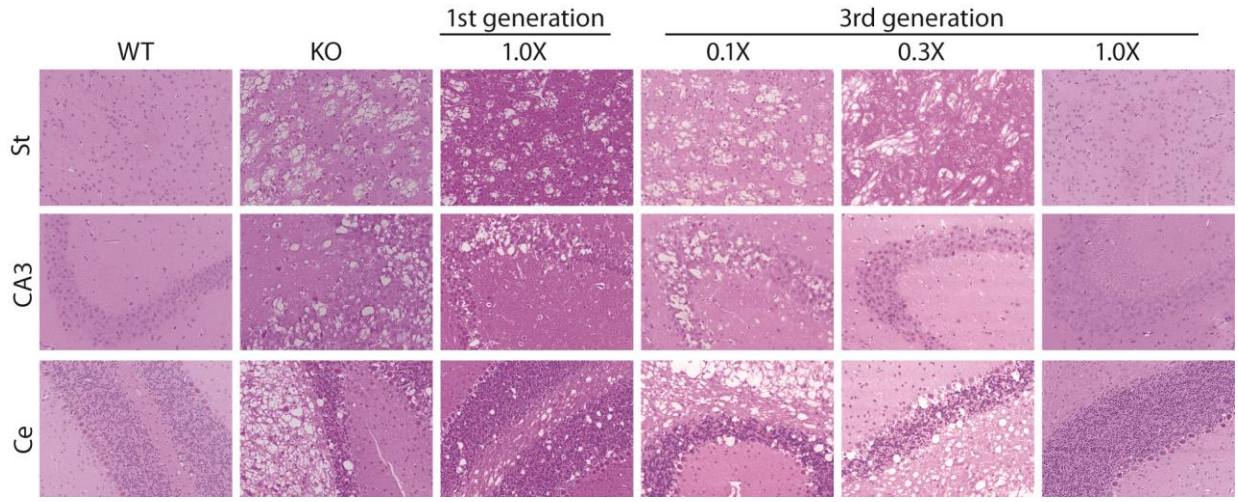

Fig. S9. **Dose-dependent neuropathology.** CD KO pups were treated intravenously at p1 with either $4x10^{11}$, $1.33x10^{11}$, or $4x10^{10}$ GC of 3rd generation gene therapy. Mice were sacrificed at p25 and subjected to H&E staining and microscopy. Shown are 10X magnification of 7 different brain regions. Wild-type (WT) and untreated (KO) mice were used as controls. See Fig. S3 for legend.

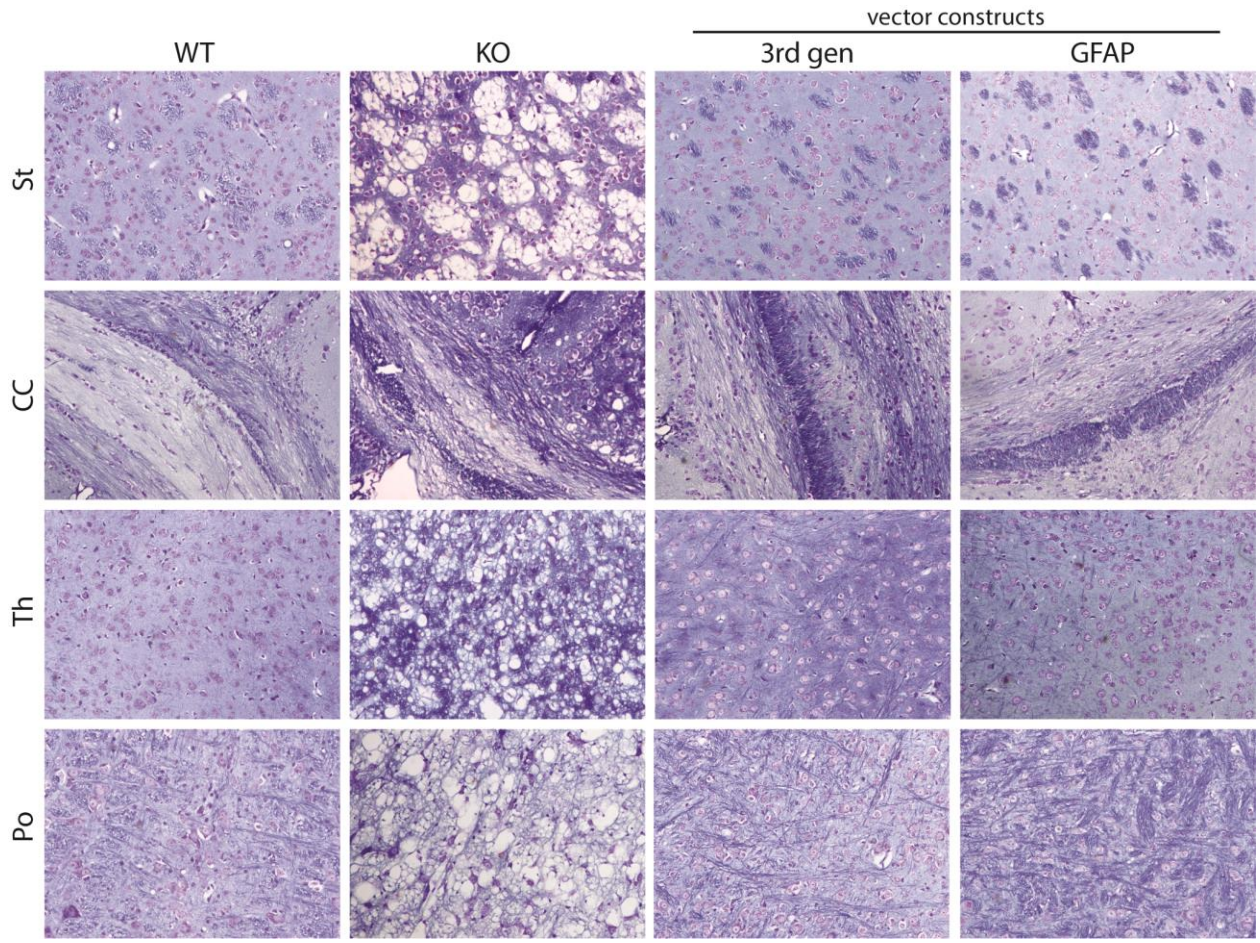

Fig. S10. **Luxol fast blue staining and astrocytic ASPA expression**. CD KO mice were treated at p1 via facial vein with either $4x10^{11}$ GC of 3rd generation gene therapy or GFAP-hASPA-Opt in rAAV9. Shown are 4 brain regions of p25 mice vs. wild-type (WT) and untreated (KO) control mice. Shown are 10X magnifications. See Fig. S3 for legend.

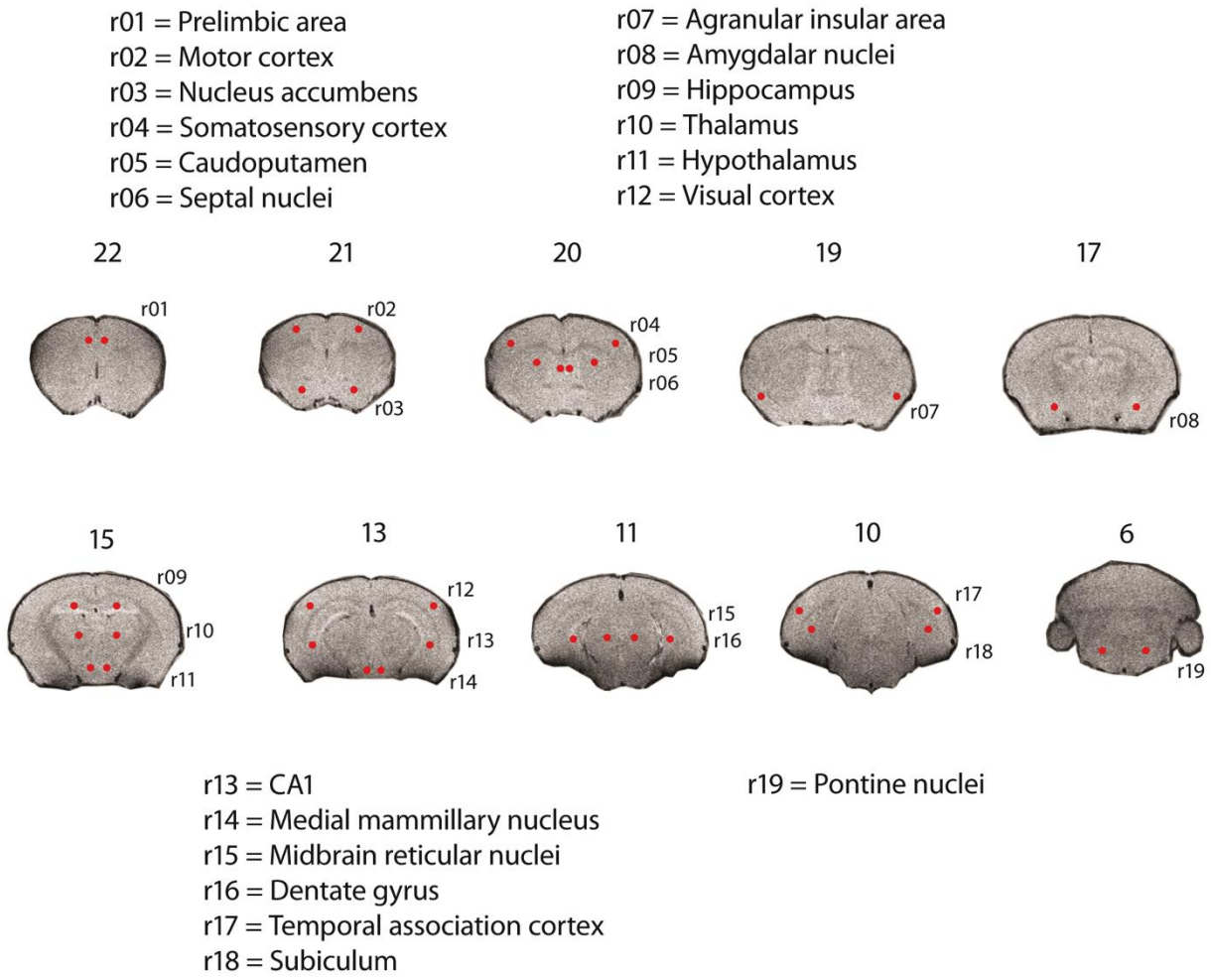

Fig. S11. **Resting state functional magnet resonance imaging (rs-fMRI) regions of interest**. 19 regions of interest (ROI) were selected to represent regions of motor and cognitive function. N=9-10 mice per group were imaged while anesthetized to acquire rs-fMRI.

Video S1: **Rotarod 27 day post-treatment.** Shown are untreated CD KO, 2nd and 3rd generation treated and wild-type control mice performing on rotarod.

Video S2: **Rotarod 1 year post-treatment**. Shown are 1st generation treated, wild-type control and 3rd generation treated mice performing on rotarod.