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Supplemental Information

AAV-Mediated Progranulin Delivery to a Mouse Model of Progranulin Deficiency Causes T Cell-Mediated Toxicity

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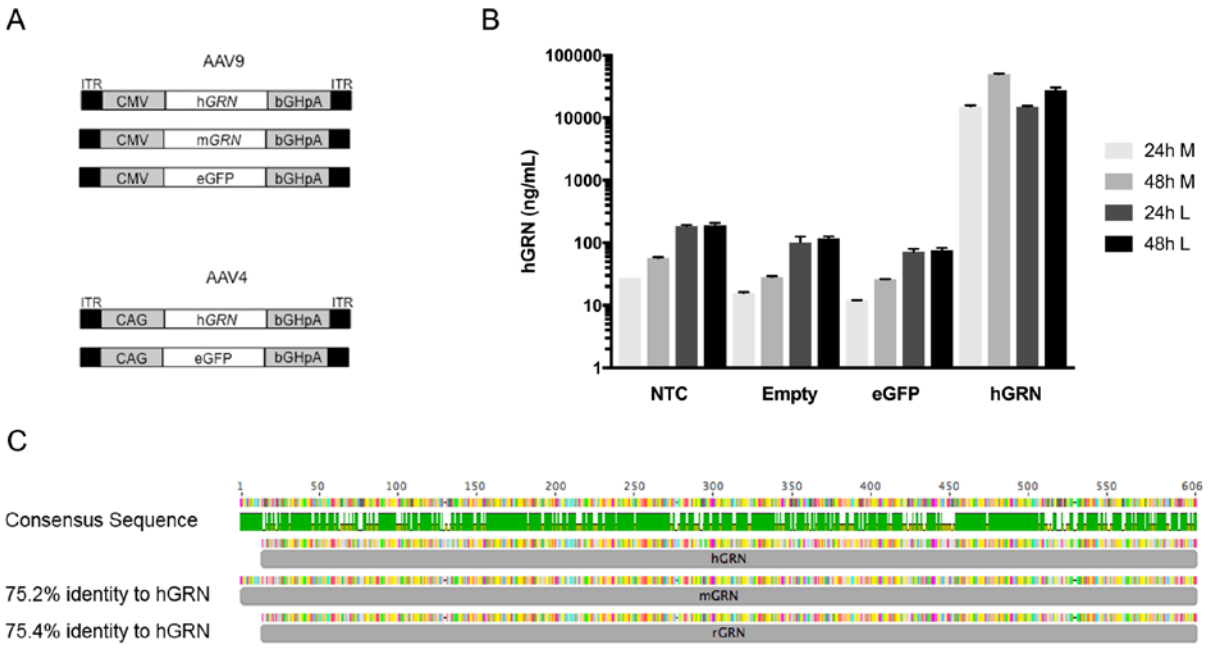


Fig. S1. (A) Schematic of AAV transgene cassettes used in our experiments. For AAV9 vectors, the CMV promoter was used to drive human progranulin (*GRN*), mouse progranulin (*Grn*), or enhanced green fluorescent protein (*eGFP*), followed by the bovine growth hormone polyA (bGHpA), and flanked by AAV2 inverted terminal repeats (ITR). For AAV4 the CAG promoter was used to drive *GRN* or *eGFP*, followed by the bovine growth hormone polyA (bGHpA), flanked by the AAV2 inverted terminal repeats (ITR). (B) Plasmid expression was validated by transfection of HEK293 cells (QBI) with lipofectamine 2000 and measuring hGRN (shown above) or mGRN (data not shown) levels by ELISA in the media or lysate, 24 or 48 hours after transfection as indicated. Our expression plasmids are compared to non-transfected cells (NTC), cells transfected with the empty vector (5/TO) or *eGFP* transfected cells. Levels were similar between hGRN and mGRN. (C) Schematic of hGRN, mGRN, and rat GRN (rGRN) protein consensus and alignments. rGRN and mGRN share 75.4% and 75.2% identity to hGRN respectively. Alignment and figure were generated using Geneious 7.1.7 (https://www.geneious.com).

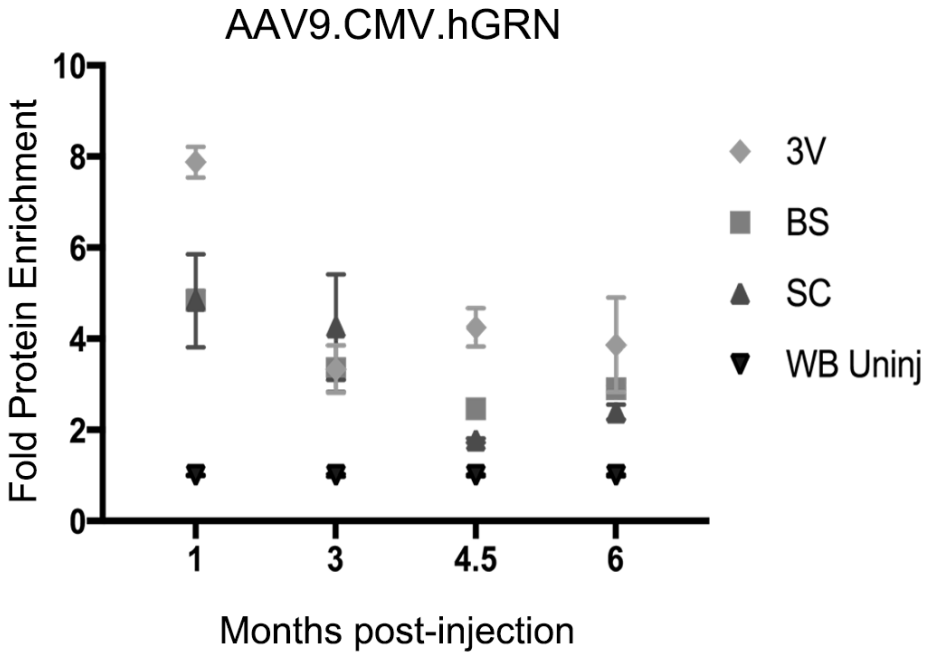


Fig. S2. AAV9 mediates *GRN* expression in *Grn* null mouse brain. *Grn* null mice were injected at 6-7.5 months of age with AAV9.*GRN* in the right lateral ventricle and sacrificed 1- , 3- , 4.5- , or 6 months post-injection. Brains were microdissected and GRN levels measured by ELISA. GRN levels in the third peri-ventricular area (3V), brain stem (BS), and spinal cord (SC) were elevated at all time points compared to uninjected homogenized whole brain (WB). *n* = 3 mice/group at each time point.

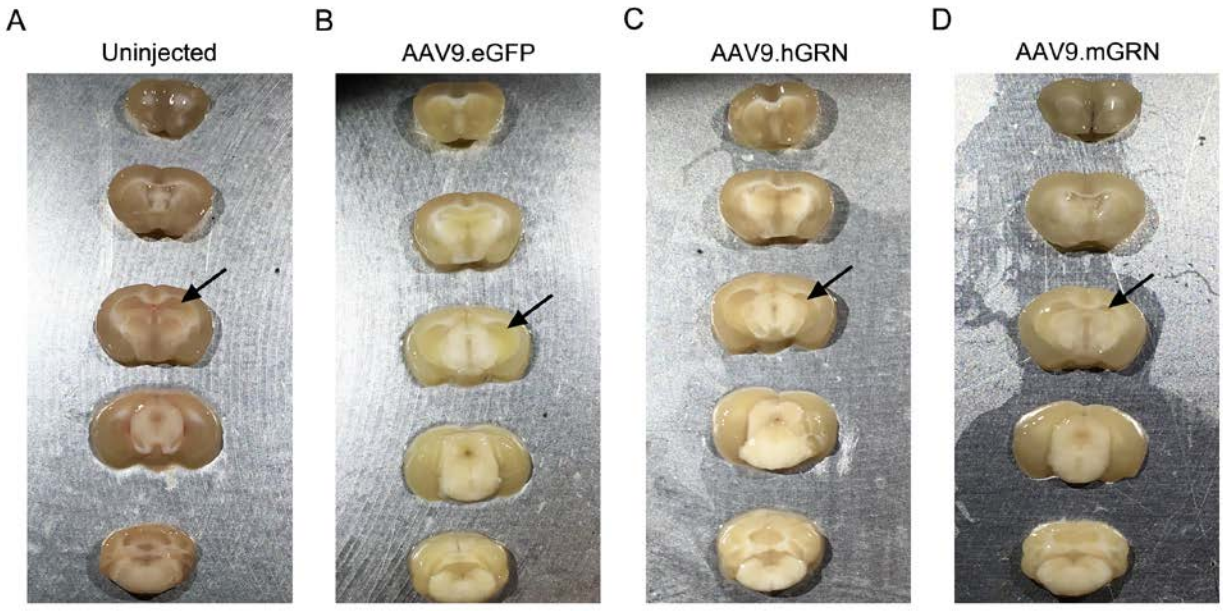


Fig. S3. Gross morphological changes are observed in AAV9.*GRN*- and AAV9.*Grn*-injected *Grn* null mice. Mice were injected with AAV9.*GRN*, AAV9.*Grn*, or AAV9.*eGFP* at 6-7.5 months of age and sacrificed 6 months post-injection. Brains were cut into 2mm sections prior to microdissection. While uninjected (A) and AAV9.*eGFP*-injected (B) mice show no overt morphological differences, the injected (right) hemisphere of mice treated with AAV9.*GRN* (C) and AAV9.*Grn* (D) was noticeably smaller, specifically in the hippocampal region (arrows), with surrounding regions appearing unaffected. n = 10 mice treated with AAV9.*GRN* (6 grossly affected and remainder affected when assessed histologically), n = 3 mice treated with AAV9.*Grn* (3 grossly affected), n = 4 mice treated with AAV9.*eGFP* (0 affected), n = 4 mice uninjected (0 affected).

A

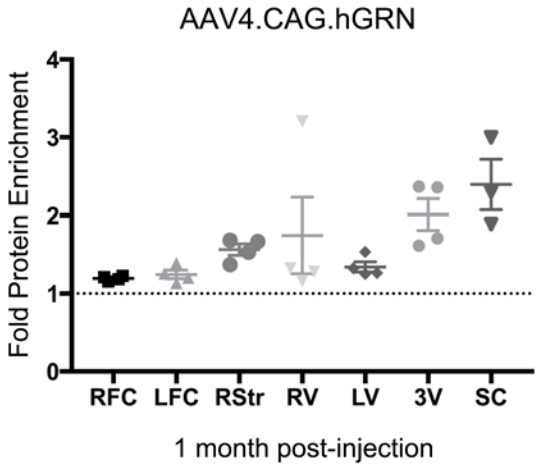
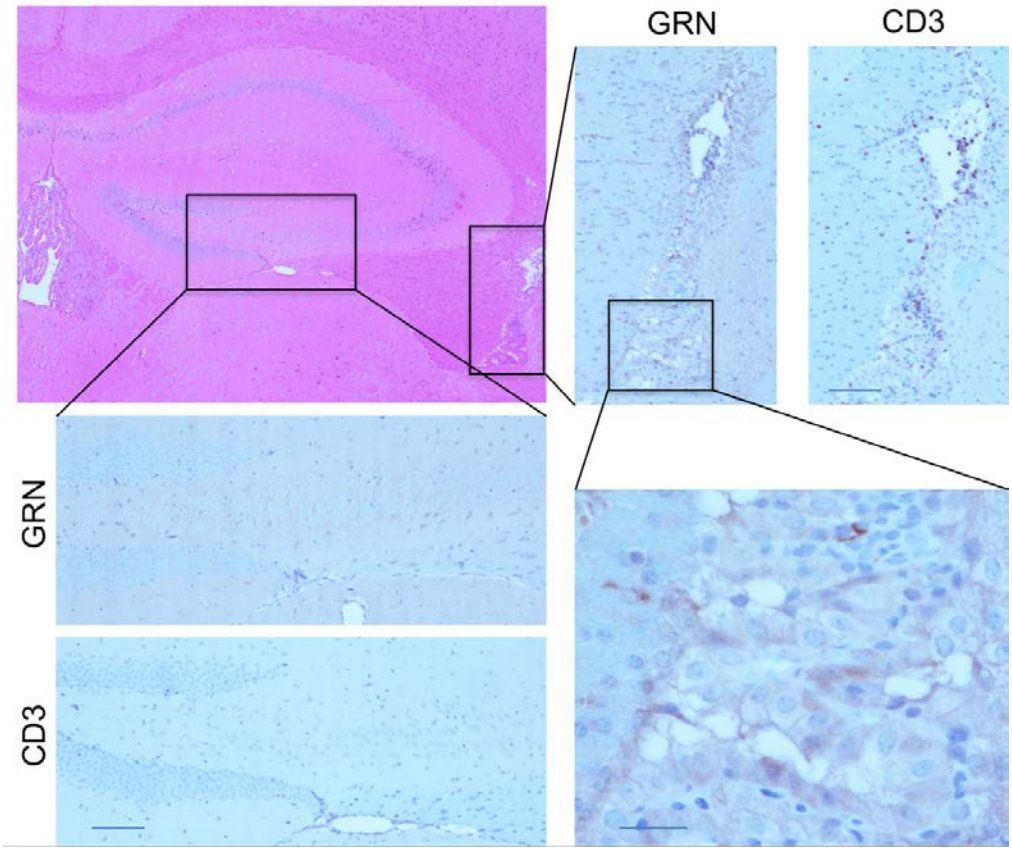


Fig. S4. AAV4 mediates *GRN* expression and toxicity in ependyma-rich areas of *Grn* null mouse brain. *Grn* null mice were injected at 6-9 months of age with AAV4.*GRN* in the right lateral ventricle. (A) Brains were microdissected 1 month post-injection and GRN levels were measured by ELISA. GRN levels in the right and left frontal cortex (RFC, LFC), right striatum (RStr), right and left peri-ventricular area (RV, LV), third ventricle (3V), and spinal cord (SC) were measured, with uninjected whole brain represented by a dotted line. A small increase in expression was seen that was most pronounced in regions with a high ependymal content, such as RV, 3V and SC. (*n* = 3 mice/group.) (B) Mice were sacrificed 3 months post-injection and analyzed by immunohistochemistry. In the ependyma and choroid of the lateral ventricle (side panels, scale bar: 100 µm), GRN levels were increased (inset, scale bar: 25 µm) with a T cell infiltrate (CD3) and hypertrophy, while in hippocampal parenchyma (bottom panels, scale bars: 100 µm), no GRN expression or infiltrate was seen and tissue morphology was intact (*n* = 3.)

B


GRN
CD3
GRN
CD3

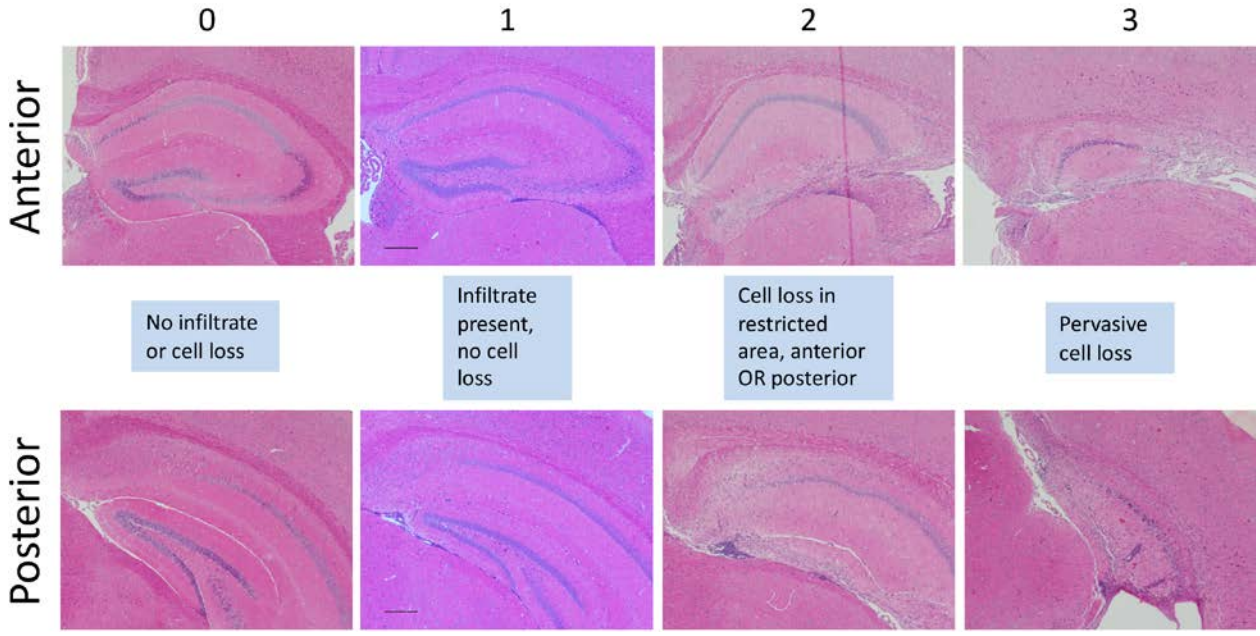

0
1
2
3
Anterior
Posterior
No infiltrate or cell loss
Infiltrate present, no cell loss
Cell loss in restricted area, anterior OR posterior
Pervasive cell loss

Fig. S5. Hippocampal pathology for AAV9.*GRN* or AAV9.*eGFP*-treated mice was assessed using a rating scale. A score of 0 was given if no pathology was seen. A score of 1 indicated that a hypercellular infiltrate was present but there was no cell loss. A score of 2 indicated cellular loss but in a restricted area, whether anterior only, posterior only, or throughout but affecting a small portion of the hippocampus. A score of 3 indicated pervasive cell loss affecting a large portion of the hippocampus. Reviewers were first trained using this scale and then presented with an anterior and a posterior image for each mouse. Each reviewer assigned a score based on observed pathology and was blinded with regards to treatment or time post-injection.

AAV9	1 month	3 months	6 months	9 months	AAV4	1 month	3 months
AAV9.*hGRN*	3/3	2/2	10/10	2/2	**AAV4.*hGRN***	3/3	3/3
AAV9.*eGFP*	0/3	0/6	0/5	0/4	**AAV4.*eGFP***	0/2	0/1

Table S1. Overview of studies performed in GRN null mice. Numerator represents number of affected mice. Denominator represents number of treated mice at each time point. All *GRN*-treated mice at all time points were affected, while no *eGFP*-treated mice were affected.