Detergent-insoluble Inclusion Constitutes the First Pathology in PFN1 Transgenic Rats

(supplemental Information)

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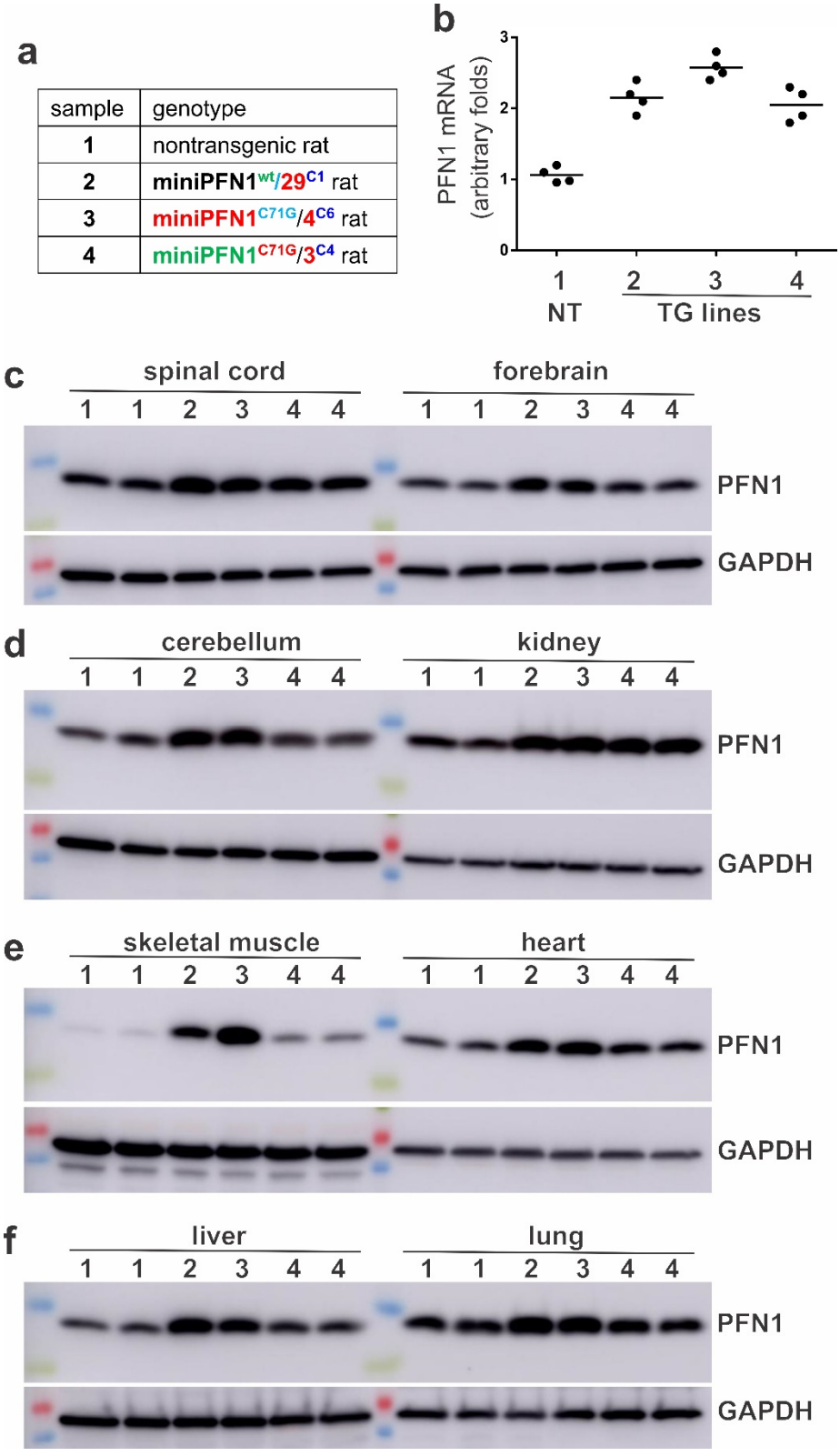

Figure S1: Transgenic rats were created to express human PFN1 with or without pathogenic mutation. **a)** One wild-type (WT) and two mutant (C71G) PFN1 lines of TG rats were established by pronuclear injection of fertilized rat eggs. Copies (C1, C4, or C6) of *PFN1* were determined by quantitative PCR. **b–f)** Expression of PFN1 mRNA (b, n = 4) and proteins (c–f) in non-transgenic (NT) and TG rats were assessed by quantitative PCR (n = 4 male rats) and immunoblotting, respectively. Immunoblotting was repeated twice to confirm the results. Equal loading of total proteins was confirmed by probing the same membranes with an antibody to GAPDH.

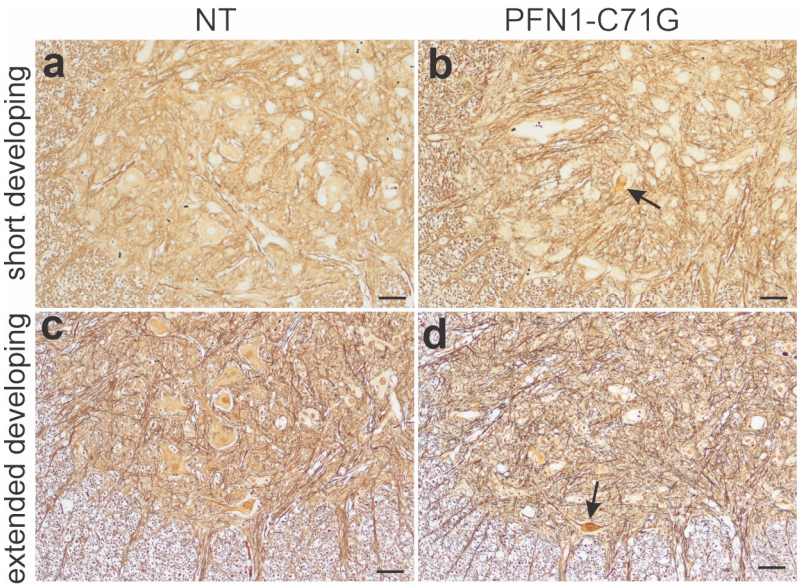

Figure S2: Bielschowski silver staining revealed degenerating neurons in transgenic rats expressing mutant human PFN1. a-d) Lumbar spinal cords were dissected from mutant PFN1 transgenic rats (PFN1-C71G) and non-transgenic littermates (NT) and examined for degenerating neurons in the ventral horns using Bielschowski silver staining. Silver staining was done for two rats (a male and a female) of each genotype. One male and one female PFN1-C71G transgenic rats were examined at paralysis stage (age 280 days) and two paired NT rats were examined at matched ages. Scale bars: 100 µm.

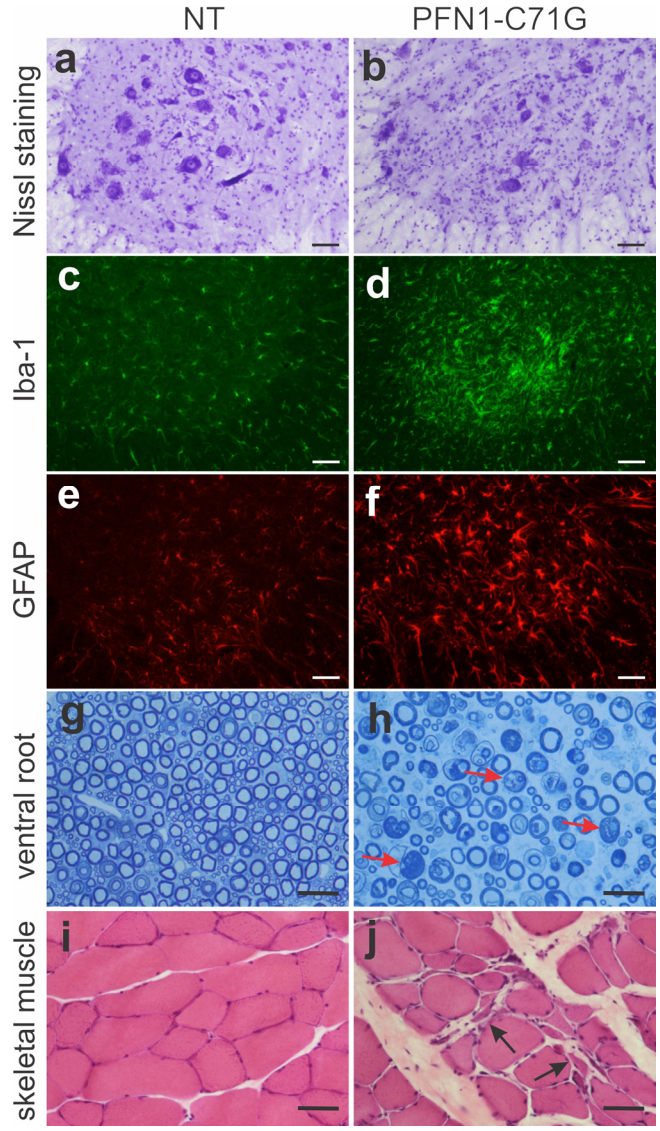

Figure S3: Expression of mutant human PFN1 causes motor neuron loss and denervation muscle atrophy in aged transgenic rats of a low-expressing line (miniPFN1^{C71G}/3^{C4}). a) Nissl staining revealed loss of motor neurons in transgenic (TG) rats (b), but not in non-transgenic (NT) littermates, at 500 days of age. **c–f)** Immunofluorescence staining revealed the activation of microglia (Iba-1) and astrocytes (GFAP) in aged TG rats (d and f), but not in aged NT rats (c and e). **g and h)** Toluidine blue staining revealed degenerating motor axons in TG rats. **i and j)** Hematoxylin and eosin (H&E) staining revealed denervation atrophy in gastrocnemius muscles of aged TG rats compared to aged NT littermates. Four rats of equal sex composition were examined of neuronal loss by Cresyl violet staining on the coronal sections of lumbar spinal cord and were examined of denervation atrophy by HE staining of gastrocnemius muscle. Scale bars: 100 µm (a–f), 30 µm (g and h), and 60 µm (i and j).