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Supplemental Information

Development of Intrathecal AAV9 Gene Therapy for Giant Axonal Neuropathy

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Supplementary Material:

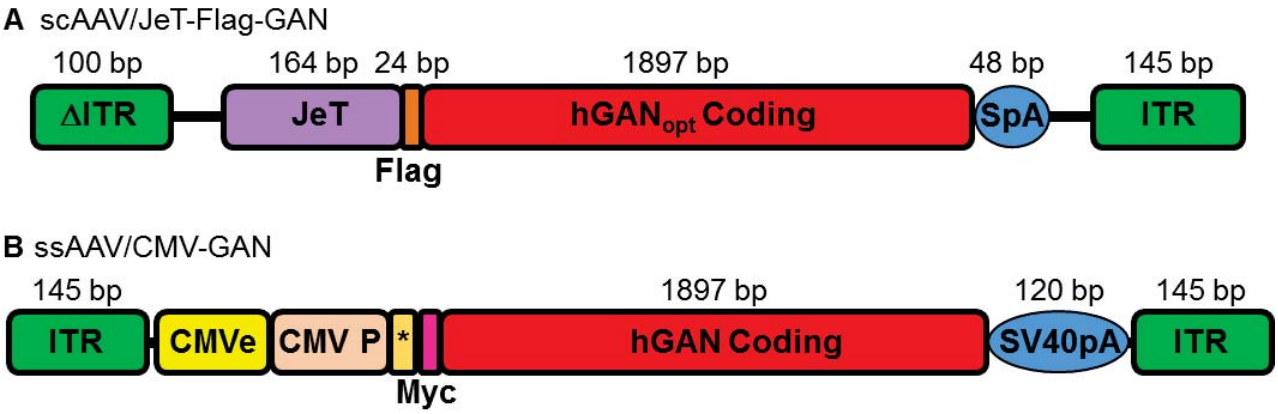

Figure S1. Design of additional vectors used in studies. (A) Schematic diagram of the self-complementary (sc) AAV/JeT-Flag-GAN gene transfer cassette using a JeT promoter, an n-terminal flag tag on full length, codon-optimized human GAN cDNA and the synthetic polyA tail (SpA). (B) Schematic diagram of the single-stranded (ss) AAV/CMV-GAN gene transfer cassette using a CMV enhancer (CMVe) and promoter (CMV P), chimeric intron (*), an N-terminal myc tag on full length, WT human GAN cDNA and the SV40 polyA tail (SV40pA).

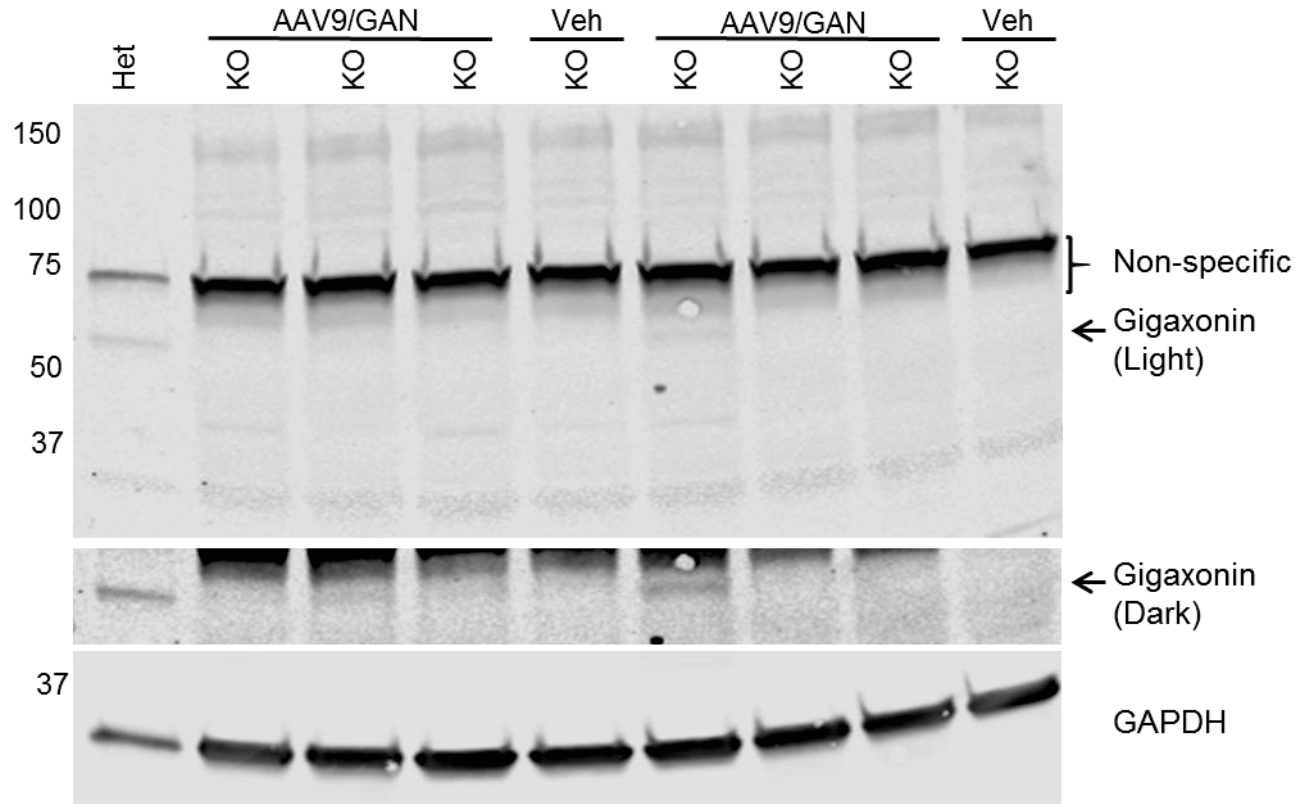

Figure S2. Gigaxonin expression in the lumbar cord of AAV9/JeT-GAN treated GAN/J KO mice. Gigaxonin protein is detected in the lumbar cord of GAN/J KO mice ~ 10 months after a single IT injection of AAV9/JeT-GAN and absent in vehicle injected littermates (cohort 3). Western blots of gigaxonin and GAPDH in heterozygous (het), and either vehicle (Veh) or AAV9/GAN injected KO mice. Top panel = light exposure of gigaxonin expression; middle panel = dark exposure of gigaxonin expression; and bottom panel = GAPDH expression.

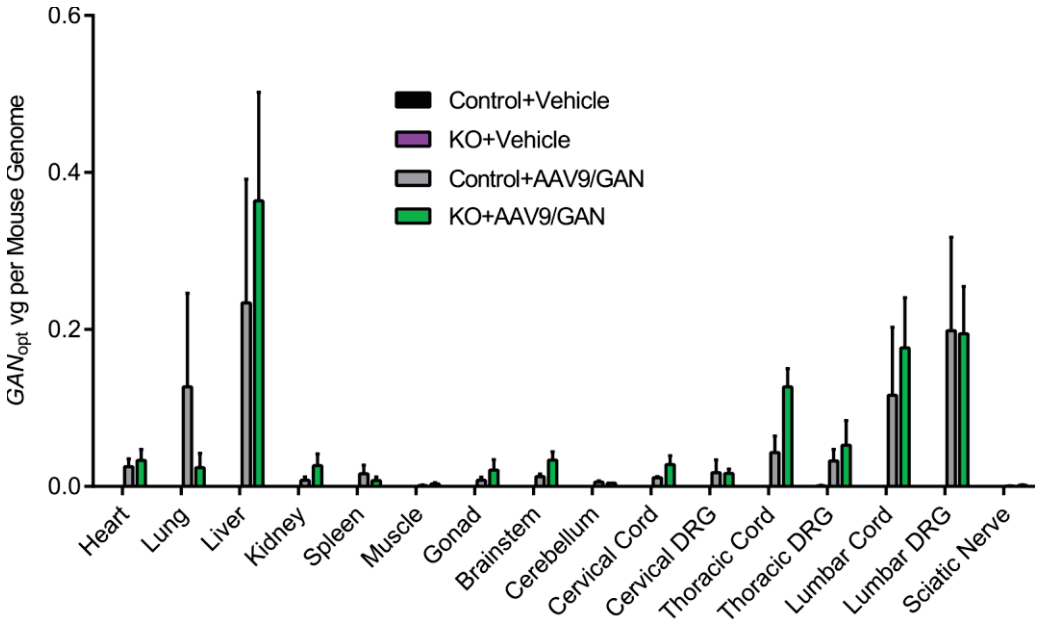

Figure S3. AAV9/JeT-GAN biodistribution in IT injected GAN/J control and KO mice. Vector biodistribution of AAV9/JeT-GAN in GAN/J mice injected at 14-16-months-of-age and harvested at 24-months-of-age (cohort 3) was assessed by measuring the codon-optimized human GAN (GAN_{opt}) vector DNA in each tissue sample. Mouse LaminB2 was measured as an internal control and was detected in all samples. Data are means ± SEM for each group (GAN/J: $n = 3$ per group).

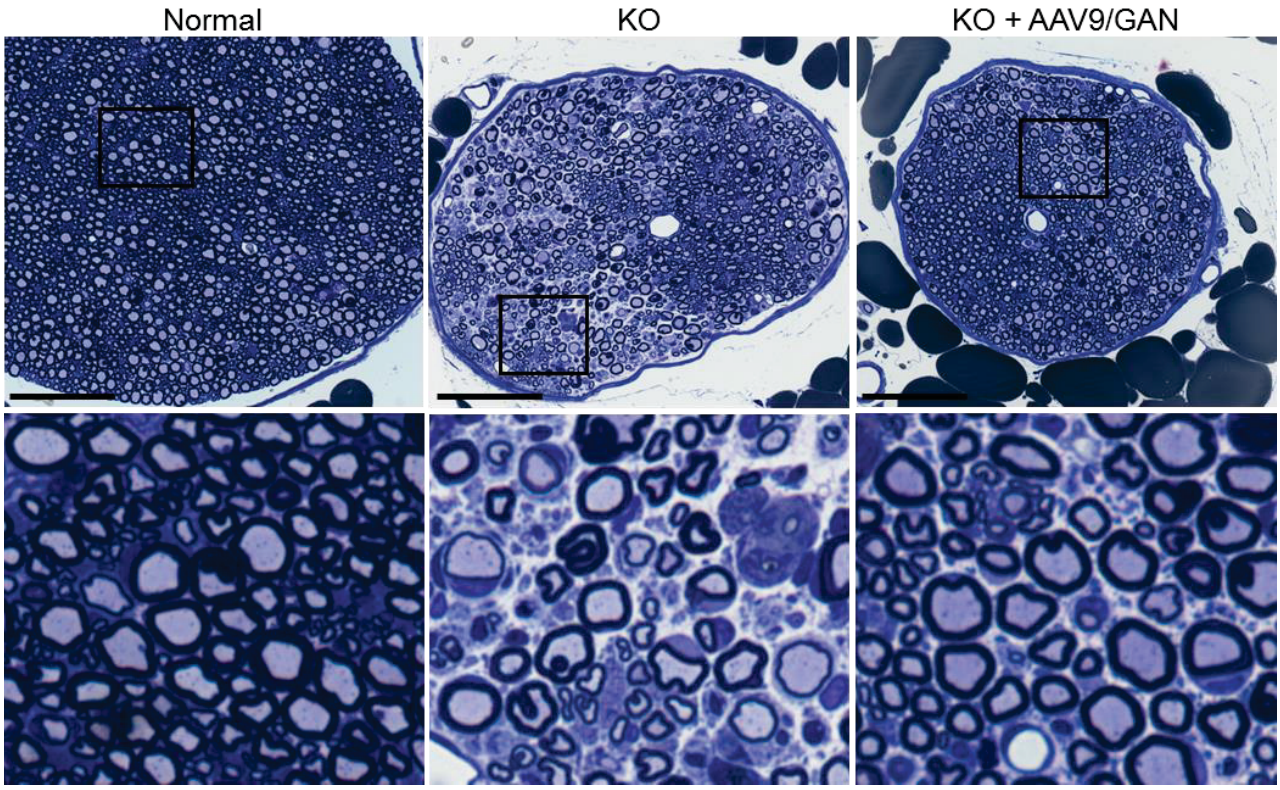

Figure S4. GAN gene therapy improves GAN sciatic nerve pathology. Representative images of 1 μm thick toluidine blue-stained sciatic nerve sections from 24-month-old normal control mice (left), control GAN/Y KO mice (middle) or GAN/Y KO mice injected with AAV9/JeT-Flag-GAN (right) at 18 months of age (cohort 4). Representative images from $n = 3$ per normal control group and 6 per each GAN group. Boxes indicate area magnified in corresponding frame below. Scale bars represent 70 μm.

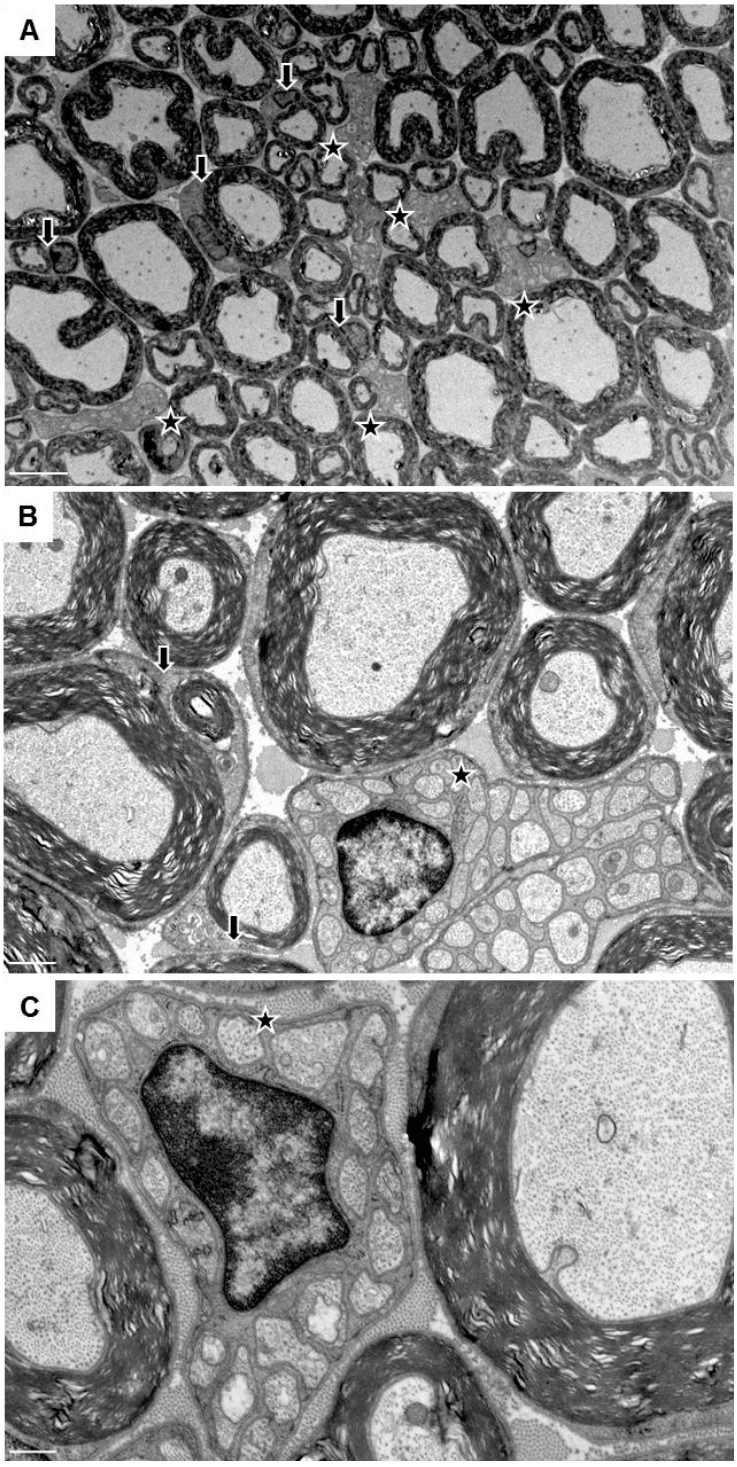

Figure S5. Normal sciatic nerves from control mice. EM examination of sciatic nerves from 24-month-old normal control mice (cohort 4). Stars indicate intact unmyelinated fibers and associated Schwann cells and arrows indicate normal Schwann cell cytoplasm associated with myelinated fibers. Representative images from $n = 3$ mice. Scale bar: 5 μm (A), 1 μm (B) and 0.5 μm (C).

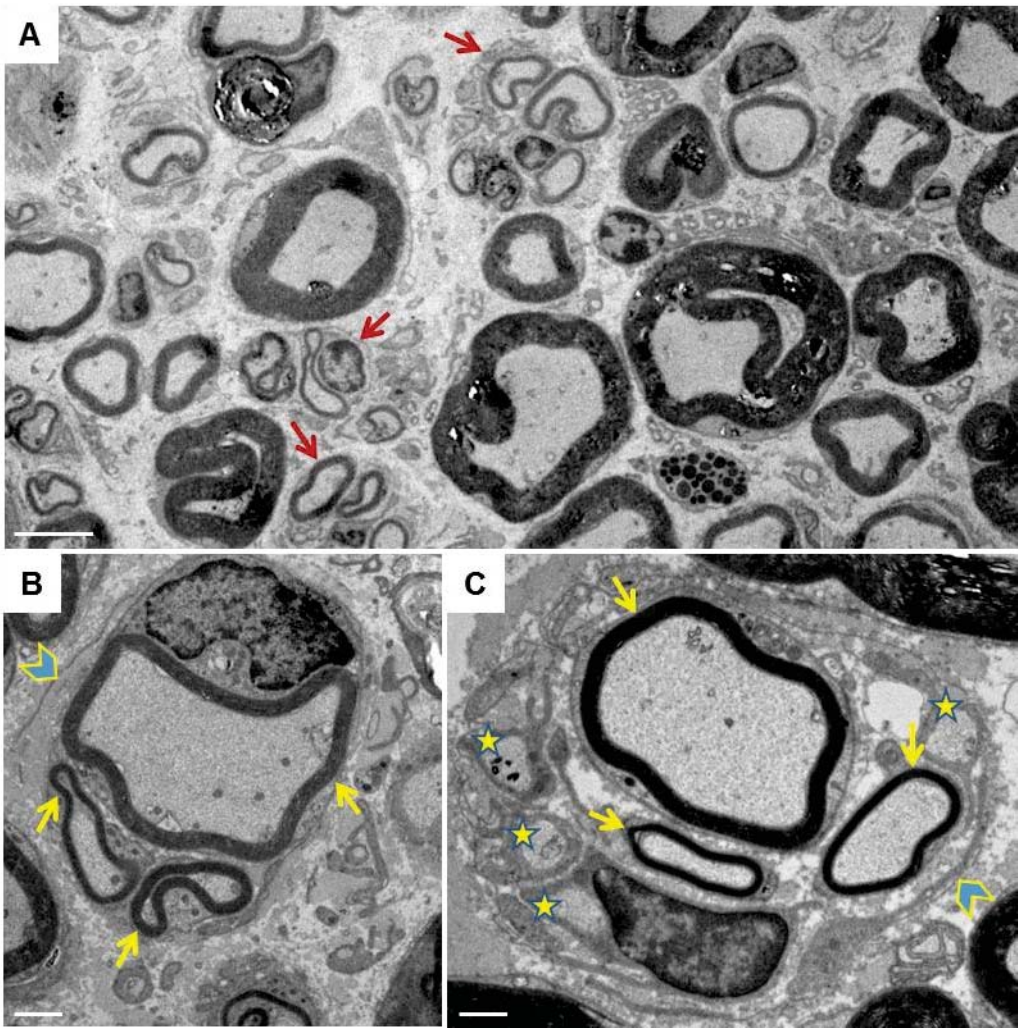

Figure S6. AAV9/JeT-GAN gene therapy reveals axonal regeneration in GAN nerves. EM examination of sciatic nerves from AAV9/JeT-GAN injected GAN/Y KO mice at 6 months post IT injection (cohort 4) (A-C). (A) Arrows indicate regenerating clusters. (B, C) Arrows indicate closely apposed conFigureurations of regenerating myelinated fibers, stars indicate regenerating unmyelinated fibers and arrowheads indicate discontinuous basal lamina belonging to the original degenerated fiber and surrounding the regenerating clusters. Representative images from $n = 6$. Scale bars: 5 µm (A), 1 µm (B, C).

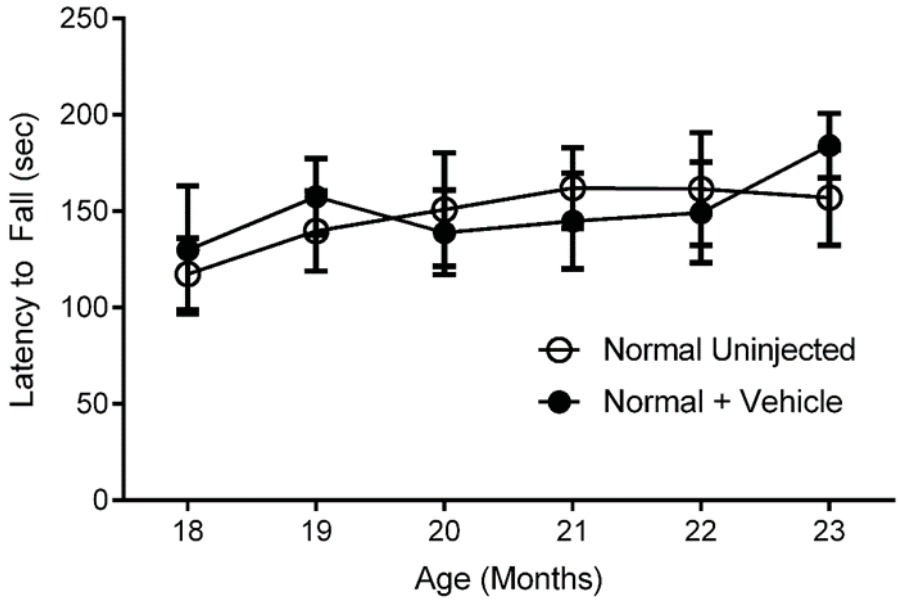

Figure S7. Intrathecal injection does not affect rotarod performance. Using the accelerating rotarod, no difference in rotarod performance was found in aged uninjected mice and mice that received a vehicle IT injection (cohort 2). Data shown are means ± SEM for each group (n = 8-10).

Table S1. Use of GAN viral vectors discussed in text.

Vector	In vitro Application	In vivo Application
scAAV/JeT-GAN	Expression studies and IF clearance in patient fibroblasts	IT injection studies in GAN KO mice for pathological and functional treatment studies
scAAV/JeT-Flag-GAN	Expression studies and IF clearance in patient fibroblasts	IT injection in GAN KO mice for sciatic nerve rescue and direct brain injection studies to assess NF clearance.
ssAAV/CMV-GAN	IF clearance in patient fibroblasts	