Figure Legends for Supplemental Figures

Supplemental Figure 1. Gigaxonin expression does not alter the levels of Tubulin Binding Cofactor B (TBCB). Immunoblotting of lysates from control and GAN cells induced to express vector (Vec) or FLAG-Wt-gigaxonin (Gig). The lysates were prepared 72 hr after initiating expression and probed with antibodies to vimentin, FLAG, TBCB, tubulin or actin. Representative blots, 3 experiments.

Supplemental Figure 2. **Mutant (S52G) gigaxonin cannot clear vimentin from cells.** (A) Immunoblotting of lysates from BJ5ta cells prepared at 0, 24, 48 and 72 hr following initiation of FLAG-S52G-gigaxonin expression using antibodies to vimentin, FLAG and actin. Note that although the amount of S52G-gigaxonin increases over time, vimentin levels are unchanged. Representative blots, 3 experiments. (B) BJ5ta cells were fixed at 24, 48 and 72 hr following initiation of FLAG-S52G-gigaxonin expression, and were processed for double Immunofluorescence using antibodies to vimentin and FLAG. Representative images, 4 preparations. Scale, 10 μm.

Supplemental Figure 3. The rod domain is required for vimentin clearance by gigaxonin (A) Double immunofluorescence using anti-vimentin and anti-gigaxonin in $vim^{-/-}$ MEFs ($vim^{-/-}$) and $vim^{-/-}$ MEFs expressing FLAG-Full length-vimentin ($vim^{-/-}$ FL-vim) or FLAG-ΔC-vim ($vim^{-/-}$ ΔC-vim) or FLAG-Rod-vim ($vim^{-/-}$ Rod-vim) or FLAG-ΔN-vim ($vim^{-/-}$ ΔN-vim) or FLAG-Head-vim ($vim^{-/-}$ N-vim) or FLAG-Tail-vim ($vim^{-/-}$ C-vim). Representative images, 4

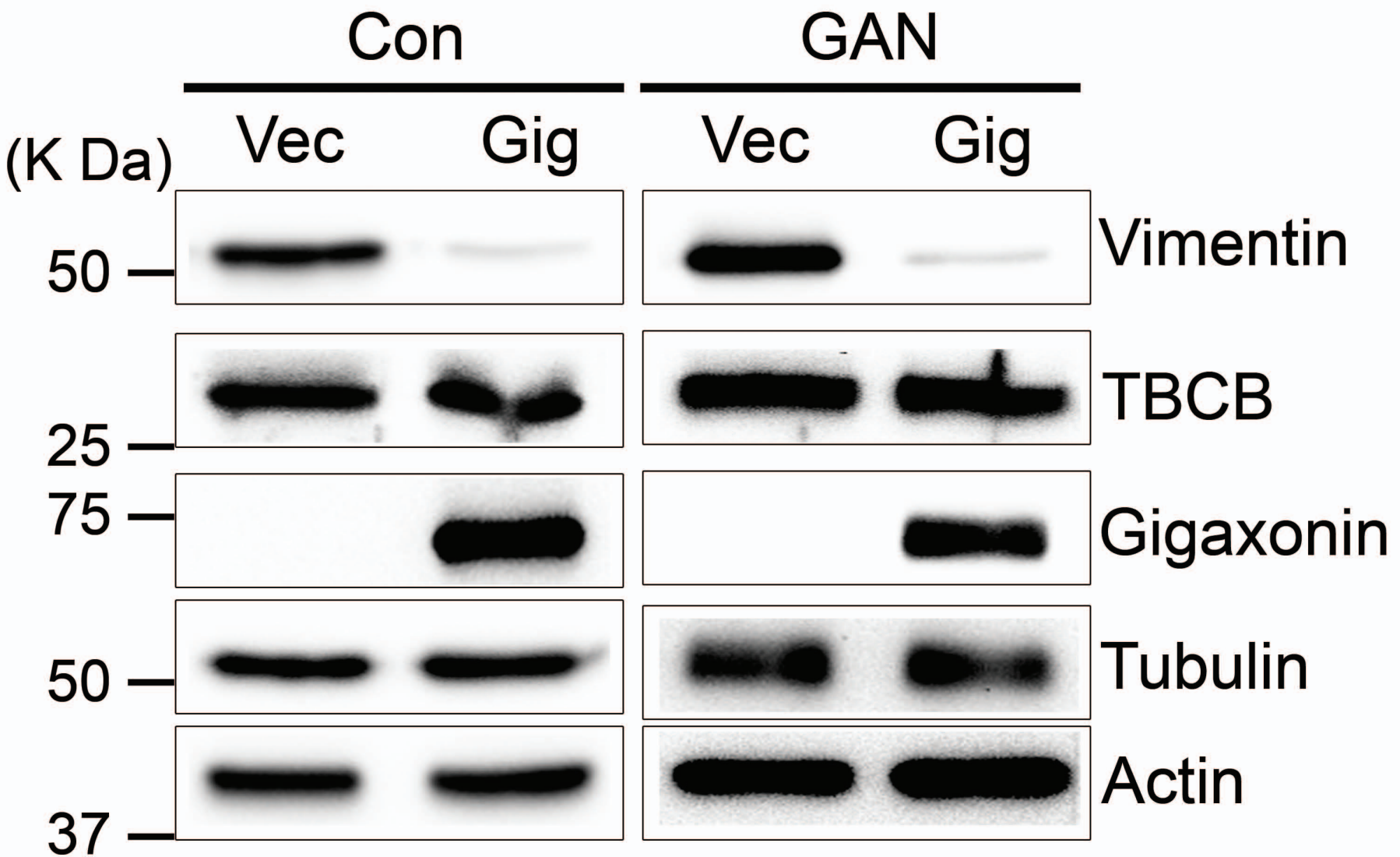
experiments. Scale, 10 μm. (B) Gigaxonin was expressed for 72 hr in the same cell lines used in (A) and labeled with anti-vimentin and anti-gigaxonin. Note that gigaxonin expression caused the clearance of FLAG-FL-vimentin, FLAG-ΔC-vim, FLAG-Rod-vim and FLAG-ΔN-vim. In contrast the FLAG-Head-vim and FLAG-Tail-vim were not cleared. Representative images, 4 experiments. Scale, 10 μm.

Supplemental Figure 4. Gigaxonin expression causes clearance of neuronal specific IF networks. (A) PC12 cells that were expressing vector (upper panel) or FLAG-Wt-gigaxonin (lower panel) for 72 hr were stained with anti-peripherin and anti-FLAG. Scale, 10 μm. (B) Immunofluorescence of PC12 cells that were expressing vector (upper panel) or FLAG-Wt-gigaxonin (lower panel) treated with NGF and stained with anti-FLAG and anti-peripherin. Representative images, 4 preparations. Scale, 20 μm. (C) SH-SY-5Y cells expressing vector (upper panel) or FLAG-Wt-gigaxonin for 72 hr (lower panel) were stained with anti-FLAG and anti-NF-L. Scale, 10 μm. (D) SH-SY-5Y cells that were expressing vector and treated with RA+BDNF (upper panel) or expressing FLAG-Wt-gigaxonin and treated with RA+BDNF (lower panel) were fixed and stained with anti-FLAG and anti-NF-L. Representative images, 4 preparations. Scale, 20 μm.

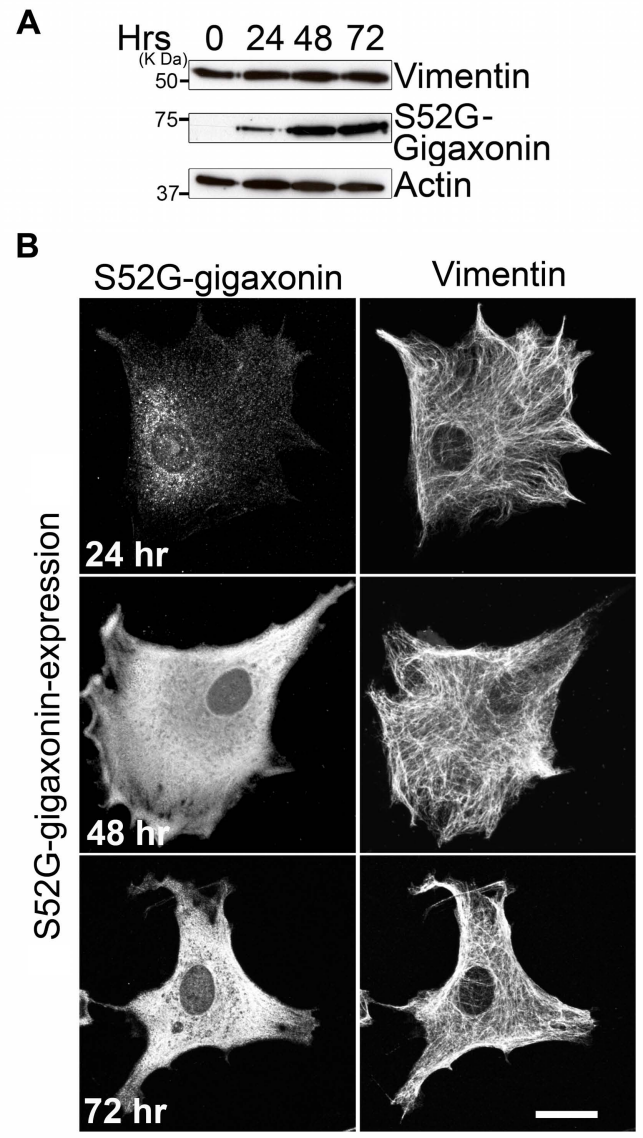
Supplemental Figure 5. Aggregates of peripherin and neurofilaments in DRG neurons of *GAN*$^{-/-}$ mice. Immunofluorescence of NF-M and peripherin in DRG neuron cultures derived from Wt (upper panel) or Gigaxonin-deficient embryos (middle panel) at 3 days *in vitro.* As expected, NF are most concentrated in the cell bodies of both normal and *GAN*$^{-/-}$ neurons, but abnormal

aggregates can be detected in the neurites derived from *GAN*$^{-/-}$mice (asterisks). These aggregates are reminiscent of those associated with the axonal swellings detected in GAN patients' neurons. Scale 50 μm. Enlarged images of an aggregate in GAN$^{-/-}$ mouse DRG (indicated by a white rectangle) are shown in the bottom panel.

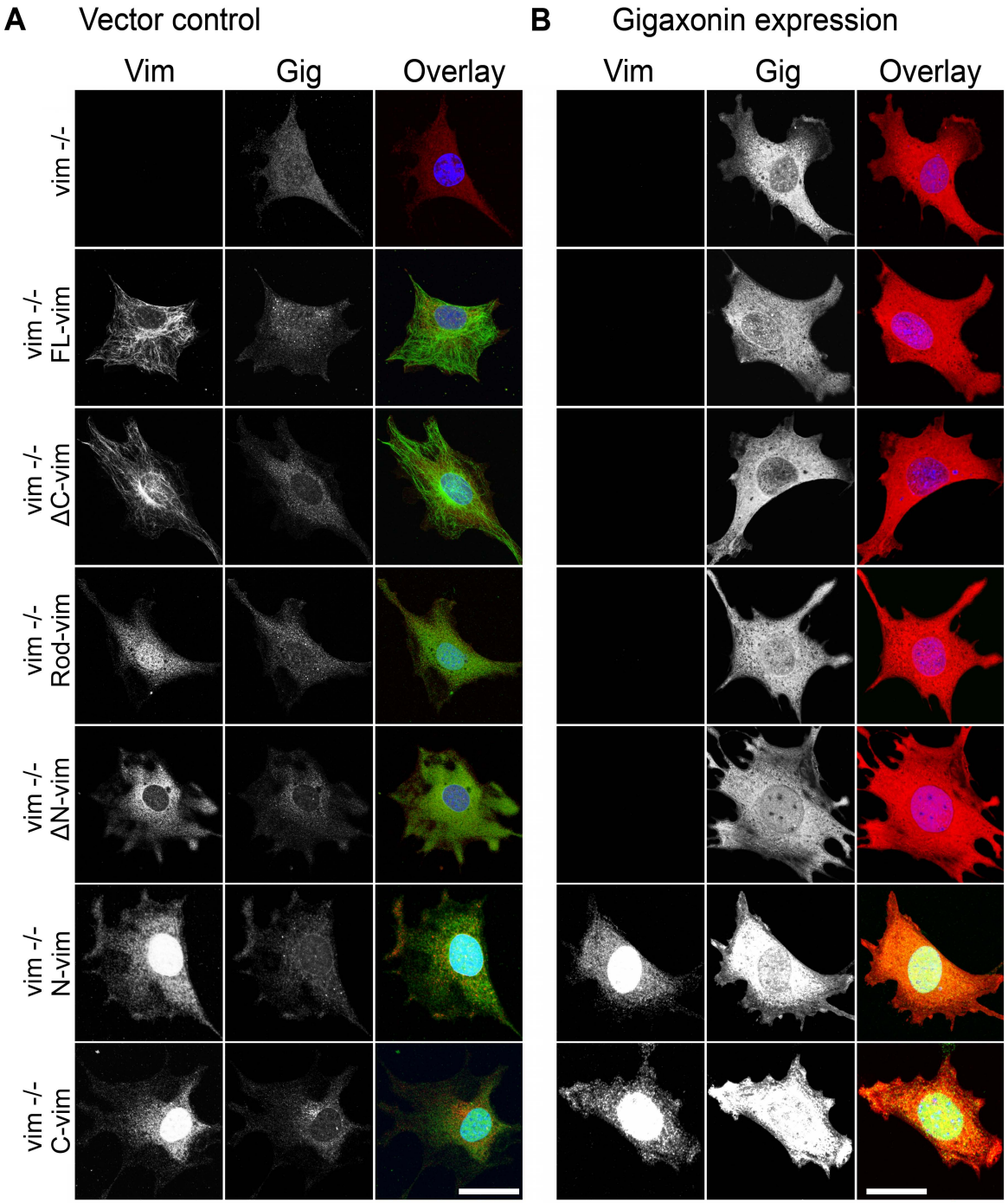
SUPPLEMENTAL FIGURE 1



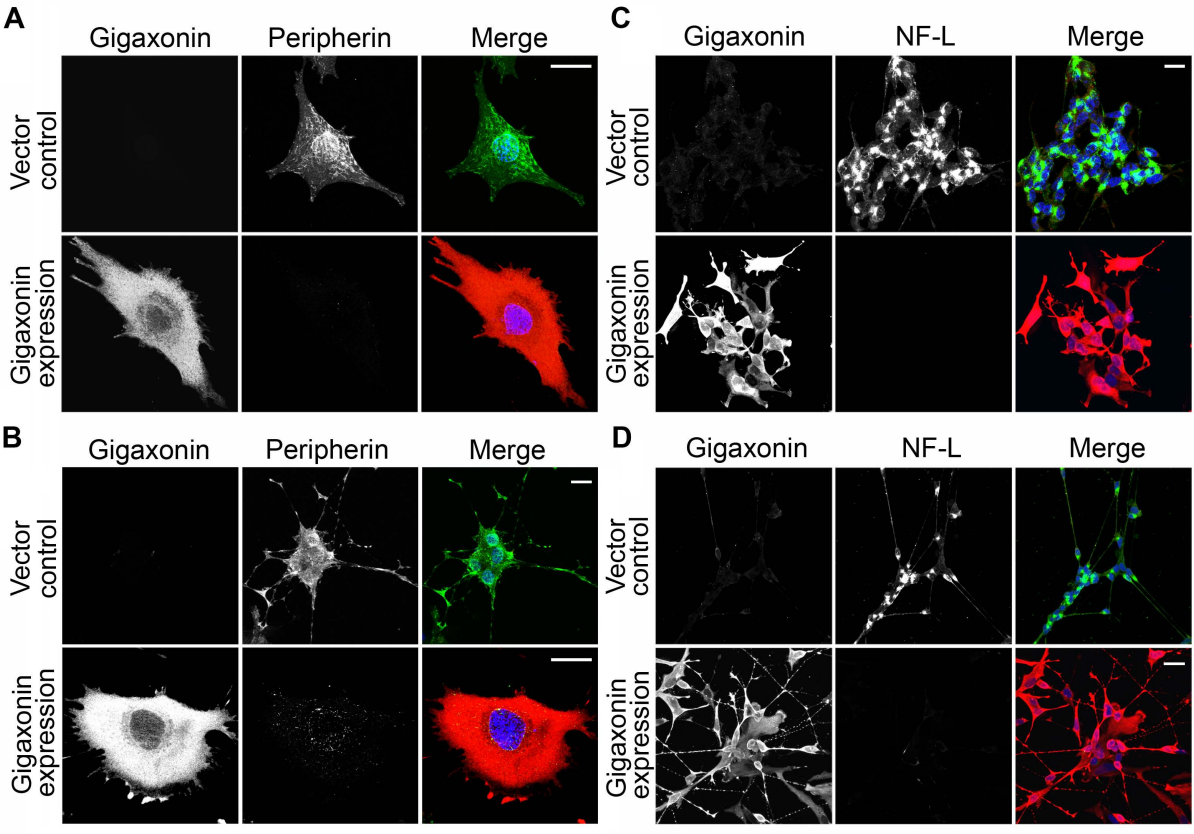
SUPPLEMENTAL FIGURE 2



SUPPLEMENTAL FIGURE 3



SUPPLEMENTAL FIGURE 4



SUPPLEMENTAL FIGURE 5