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RESEARCH ARTICLE

Figures and figure supplements

Guanylate cyclase 1 relies on rhodopsin for intracellular stability and ciliary trafficking

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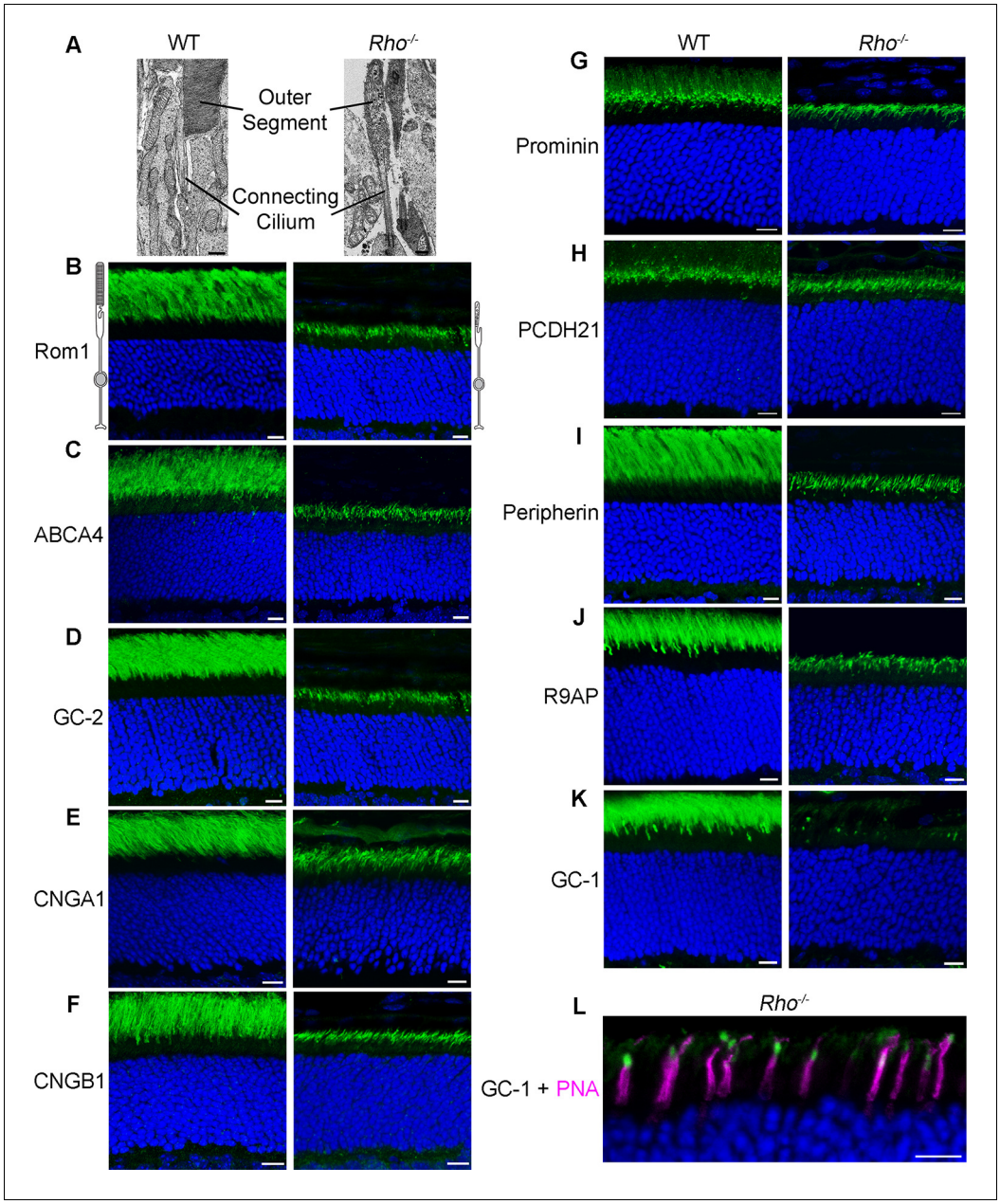

Figure 1. Localization of outer segment membrane proteins in wild-type (WT) and *Rho*$^{-/-}$ retinas. (**A**) Electron micrographs showing the outer segment and connecting cilium in WT and *Rho*$^{-/-}$ rods (scale bar 500 nm). (B–K) Immunofluorescent localization of individual outer segment proteins in WT and *Rho*$^{-/-}$ retinal cross-sections: (**B**) Rom-1; (**C**) ABCA4; (**D**) guanylate cyclase 2 (GC-2); (**E**) cyclic nucleotide gated (CNG) α1; (**F**) CNGβ1; (**G**) prominin; (**H**) protocadherin 21 (PCDH21); (**I**) peripherin; (**J**) R9AP; and (**K**) GC-1. (**L**) Double labeling of GC-1 (green) and the cone maker, PNA (magenta). Here and in the following figures, the identity of antibodies used in each panel is indicated in 'Materials and methods'. Scale bars, 10 μm. Nuclei are stained by Hoechst (blue).

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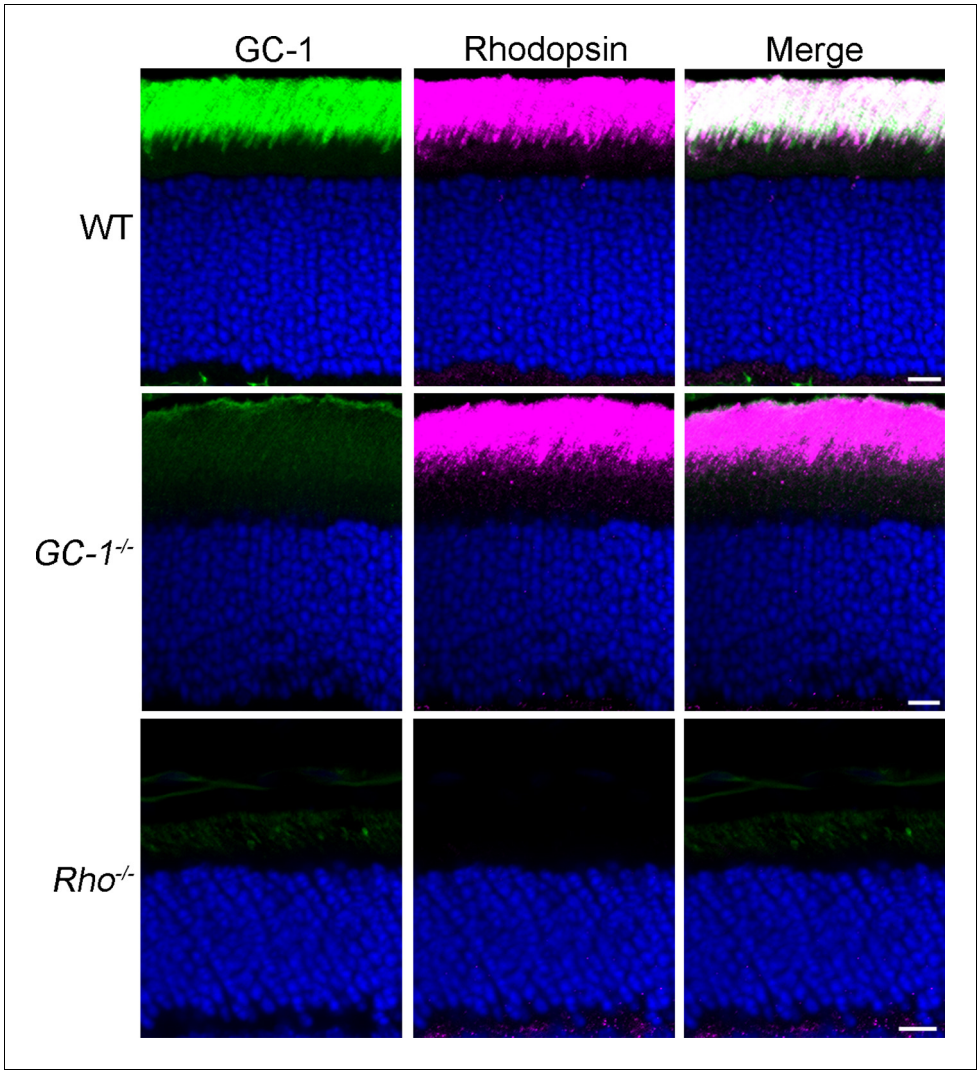

Figure 2. Rhodopsin expression and outer segment localization do not rely on GC-1. Rhodopsin (magenta) and GC-1 (green) were co-immunostained in retinal cross-sections from wild-type (WT), *GC-1*$^{-/-}$, and *Rho*$^{-/-}$ mice. Scale bar, 10 μm. Nuclei stained in blue.

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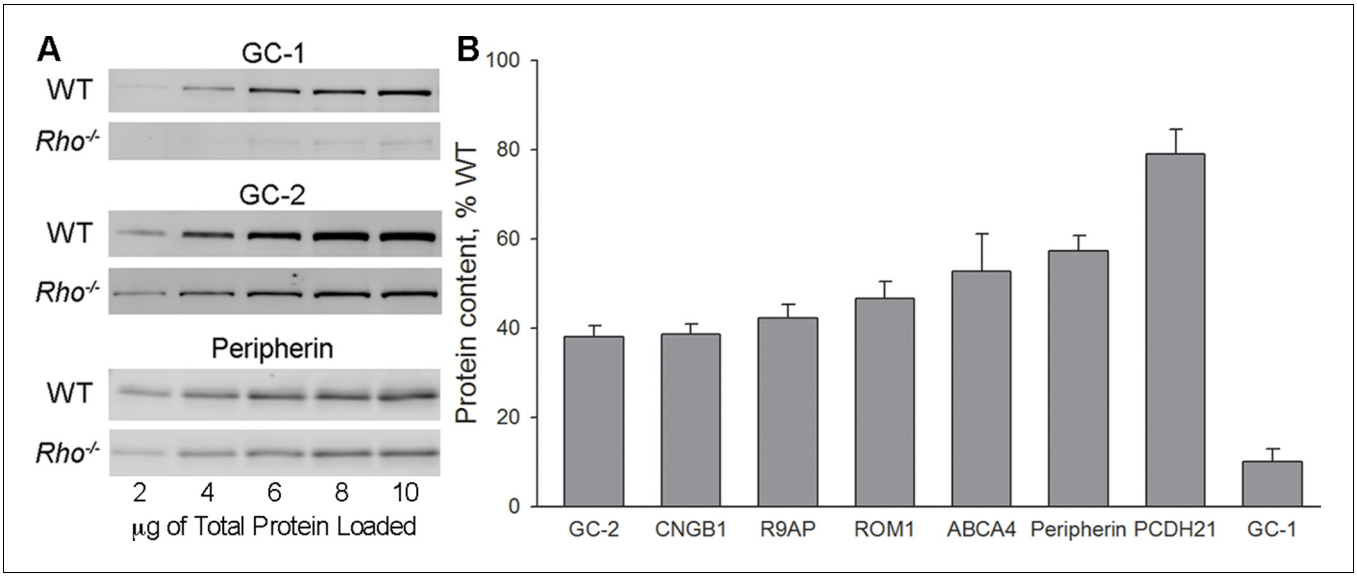

Figure 3. Quantification of outer segment transmembrane proteins in *Rho*$^{-/-}$ retinas at P21. (**A**) Representative Western blots show serial dilutions of wild-type (WT) and *Rho*$^{-/-}$ retinal lysates for three proteins (guanylate cyclase 1 [GC-1], GC-2, and peripherin). The fluorescent signal produced by each band in the serial dilution was plotted and used to calculate the amount of each protein in *Rho*$^{-/-}$ lysate. In these examples, GC-1 was to 10% of its WT content, GC-2 to 38%, and peripherin to 57%. (**B**) Expression levels of outer segment transmembrane proteins in *Rho*$^{-/-}$ retinal lysates calculated as % WT. A minimum of four independent experiments was performed for each protein. Error bars represent SEM.

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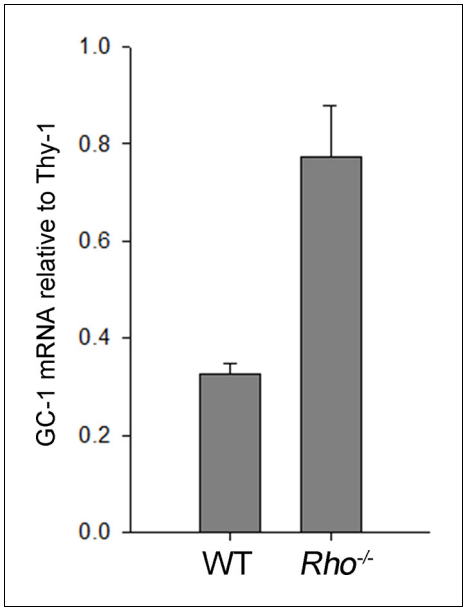

Figure 3—figure supplement 1. Transcript levels of GC-1 in the retinas of WT and *Rho*$^{-/-}$ mice. Quantitative RT-PCR of each transcript was performed on four mice of each genotype at P21. The relative mRNA expression level in each sample was normalized to the inner retina marker, Thy1. Error bars represent SEM.
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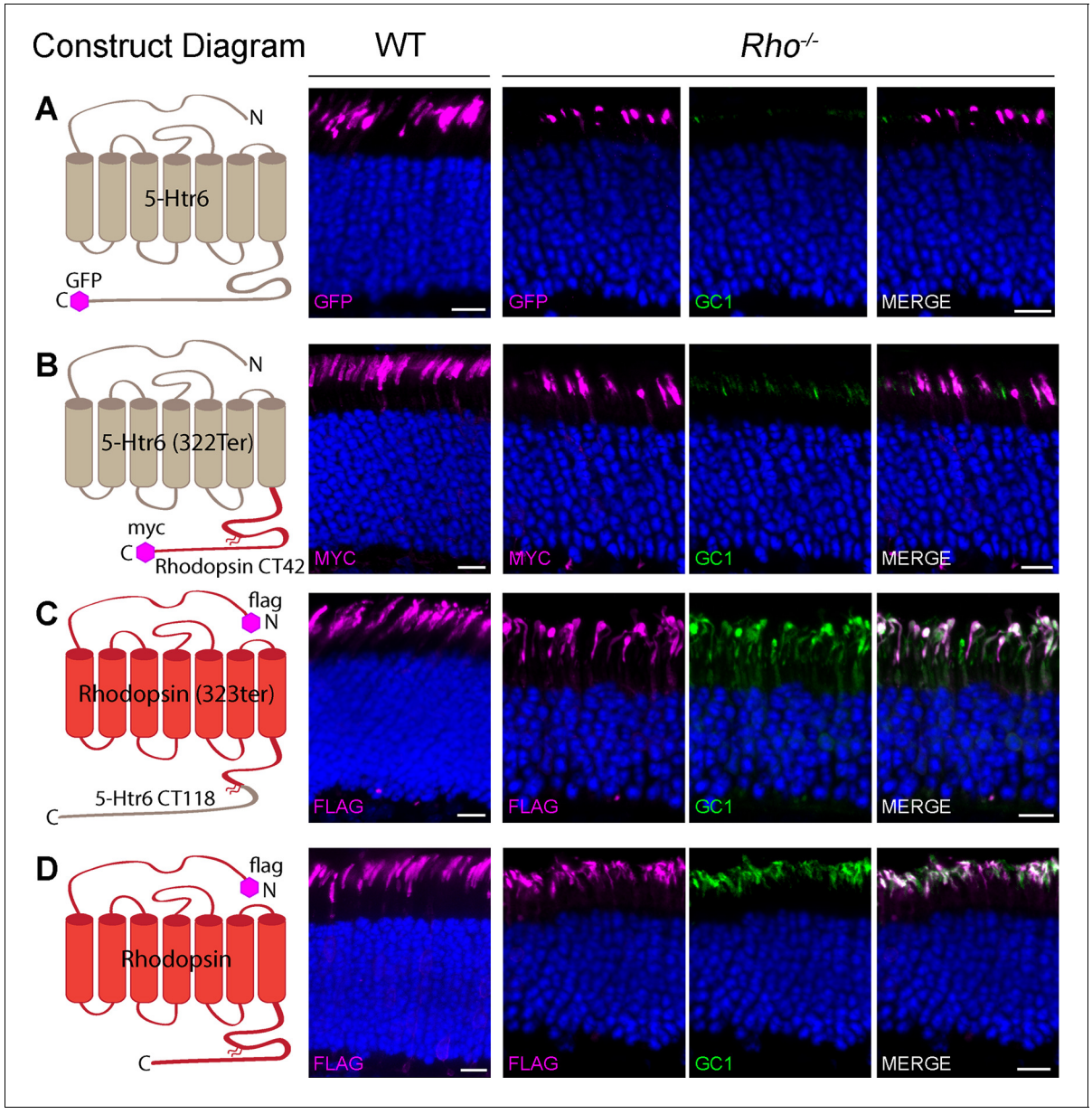

Figure 4. Guanylate cyclase 1 (GC-1) rescue in Rho-/- rods requires the seven-helical core structure of rhodopsin. Wild-type (WT) and *Rho*$^{-/-}$ rods were transfected with (**A**) full-length Htr6, (**B**) seven-helical Htr6 core fused to rhodopsin's C-terminus, (**C**) seven-helical rhodopsin core fused to the C-terminus of Htr6, (**D**) full-length rhodopsin. Sections from WT retinas were stained for each recombinant chimera using anti-green fluorescent protein (GFP), anti-myc, or anti-FLAG antibodies (magenta, each chimera's tag is depicted in the construct diagram). Sections from *Rho*$^{-/-}$ retinas were co-stained for GFP, myc, or FLAG (magenta, left panel) and endogenous GC-1 using anti-GC1 antibodies (green, middle panel). The merged images from *Rho*$^{-/-}$ sections are shown in the right panel. Scale bar, 10 μm. Nuclei are stained by Hoechst (blue).

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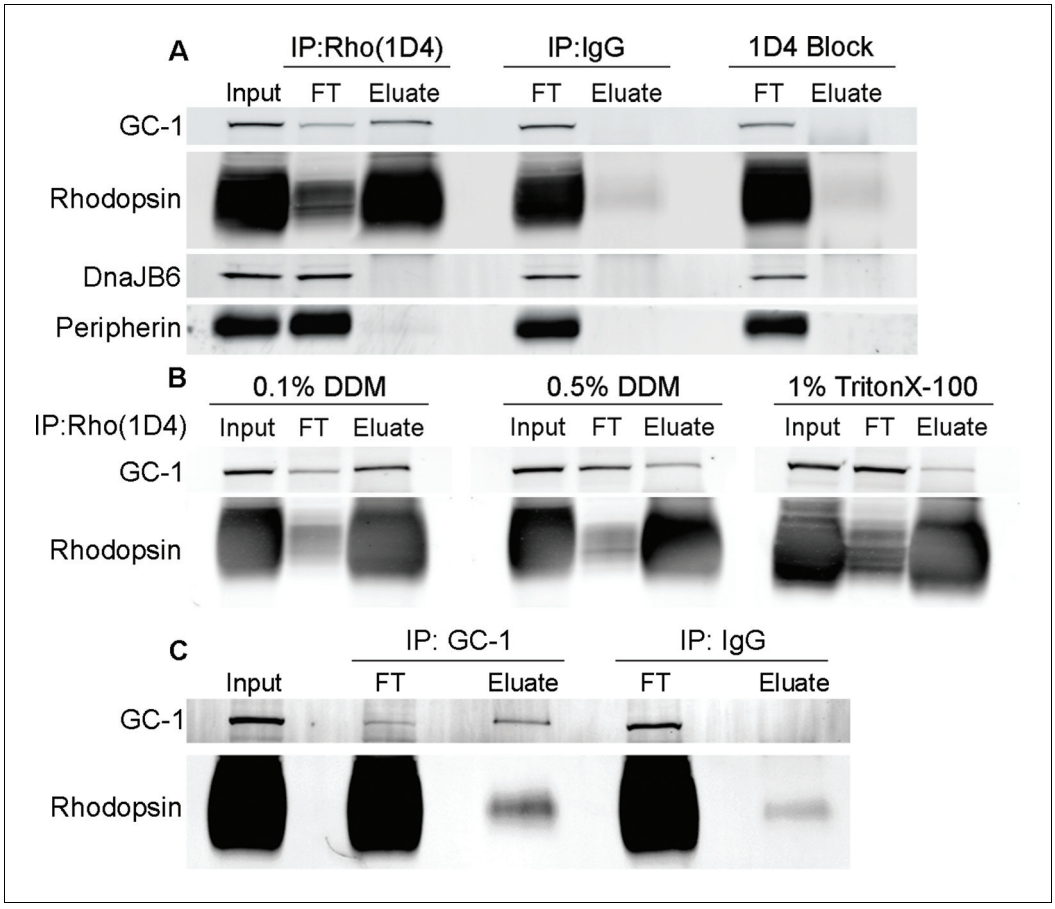

Figure 5. Guanylate cyclase 1 (GC-1) co-precipitation with rhodopsin from mouse retinal lysate. (**A**) GC-1 and rhodopsin co-precipitation by monoclonal anti-rhodopsin antibody 1D4. Wild-type (WT) mouse retinal lysate (Input) was incubated with 1D4 antibody and then bound to protein A/G beads. After the unbound material in flow through (FT) was removed, the beads were washed and bound proteins were eluted (Eluate) and analyzed by Western blotting for GC-1, rhodopsin, DnaJB6, and peripherin. Non-specific protein binding was probed using either non-immune mouse IgG or 1D4 antibody treated with its epitope blocking peptide. (**B**) Co-precipitation of GC-1 and rhodopsin by the 1D4 antibody from retinal membranes solubilized under different detergent conditions. (**C**) Rhodopsin and GC-1 co-precipitation by monoclonal anti-GC-1 antibody 1S4. *Rho*$^{+/-}$ mouse retinal lysate (Input) was incubated with 1S4 antibody bound to protein A/G beads. After the unbound material in flow through (FT) was removed, bound proteins were eluted from the beads (Eluate) and analyzed by Western blotting for GC-1 and rhodopsin. Non-specific rhodopsin binding was probed using non-immune mouse IgG. Protein loading on each lane was normalized to input in all panels.

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