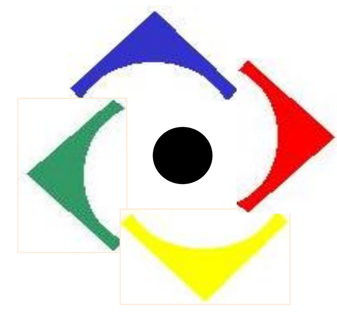

Retinitis Pigmentosa 2 protein regulates transport of isoprenylated proteins to photoreceptor outer segments

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Purpose: X-linked retinitis pigmentosa (XLRP) is a devastating form of retinal degeneration, manifesting early in life with symptoms of night blindness, visual field defects, and decreased visual function. In-vitro, RP2 functions as a GAP for the small GTPase ARL3, a GDI displacement factor (GDF). Mutations in the Rp2 gene account for approximately one quarter of all XLRPs. The purpose of this study was to investigate the consequences of RP2 deletion and identify mechanisms causative of XLRP.
Methods: Intracellular localization of RP2 in photoreceptors was determined by neonatal electroporation of an RP2-EGFP expression vector. An Rp2 knockout mouse was generated using a EUCOMM ES cell line containing a gene trap in intron 1. The knockout mice were characterized by Western blot, immunocytochemistry, and electroretinography (ERG).
Results: RP2-eGFP was localized to the plasma membrane of inner segments, axons and synaptic termini in photoreceptors, but not in outer segments. The Rp2 gene knockout mice were viable and developed normally. Ablation of Rp2 gene expression led to slowly progressing degeneration of cone and rod photoreceptors as indicated by ERG recordings. Scotopic a-wave and photopic b wave amplitudes were reduced as early as one month of age in the knockout mice. The Rp2Y/- ERG amplitudes were further reduced at 6 months of age. Trafficking of transmembrane phototransduction proteins, including cone opsins, to Rp2Y/- photoreceptors outer segments was normal up to 14 months of age. While targeting of transducin α and βγ to the Rp2Y/- outer segments was not affected in the knockout, transport of rod and cone PDE6 as well as GRK1 to outer segments was impeded.
Conclusions: RP2 is distributed to plasma membrane of inner segments and synaptic termini in photoreceptors. RP2 is not essential for trafficking cone opsins and transducin to photoreceptor outer segments, but regulates transport of isoprenylated proteins to photoreceptor outer segments. Our results suggest that RP2/ARL3 may allosterically release prenylated proteins from their soluble complex with PDE6D and unload them to donor membranes (e.g., TGN vesicles).). In the Rp2 knockout, this process is impeded.

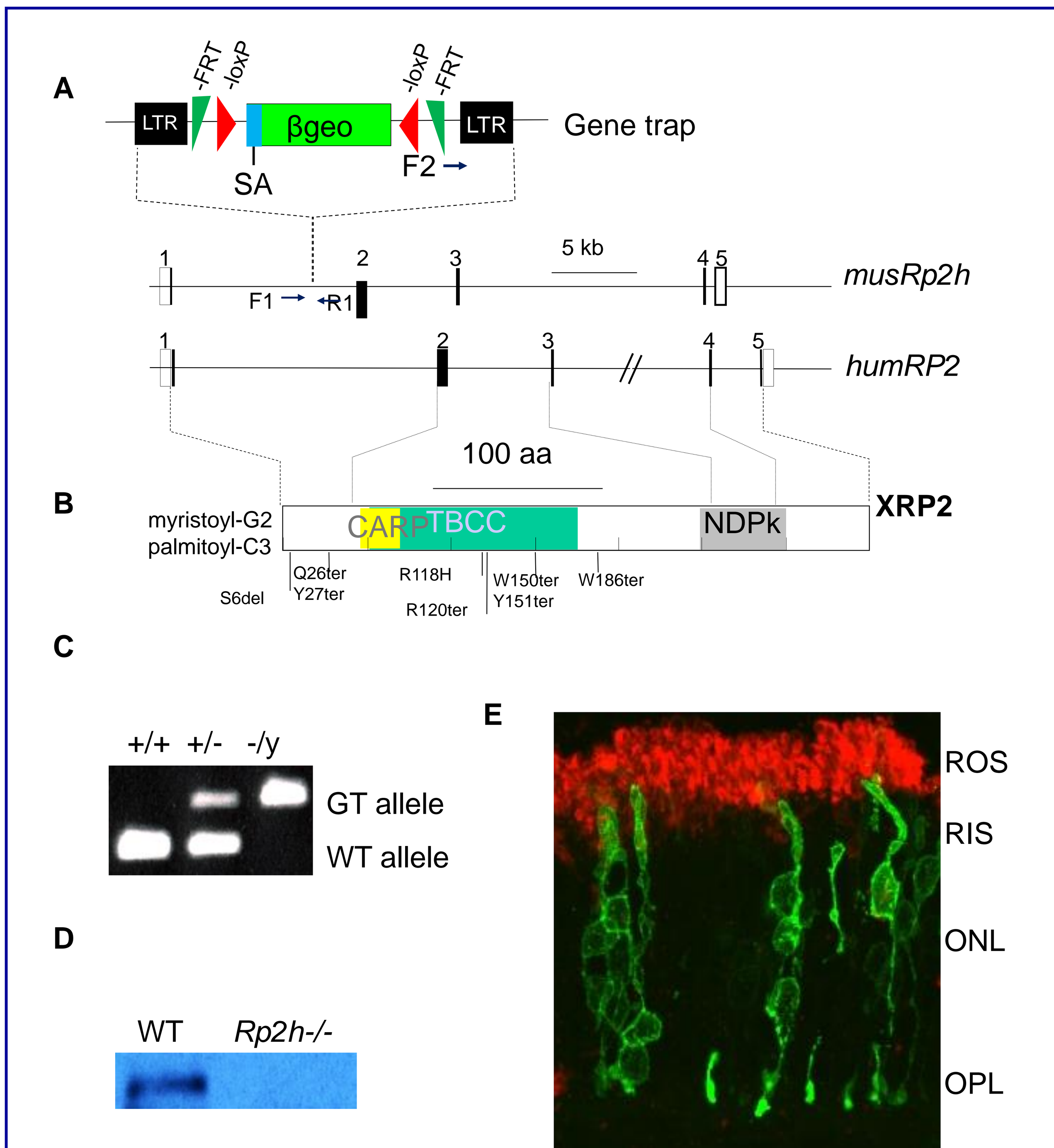

Figure 1. The mouse Rp2h gene knockout. A, the mouse Rp2h gene contains five exons. A gene trap located in intron 1 of mouse Rp2h truncates XRP2 after lysine 32 (K31). The trap contains long terminal repeats (LTR), a splice acceptor (SA), a β-Geo selective cassette containing β-gal and neo genes. The β-Geo cassette is flanked by a pair of FRT and loxP recombination signals in anti-sense orientation. RP2-F1, RP2-F5, and RP2-R are the primers used for genotyping. Underneath, the human RP2 gene; positions of some of the most common RP-associated mutations are shown. B, the human XRP2 polypeptide which is myristoylated and palmitoylated at the N-terminal. CARP, cyclase-associated domain; TBBC, tubulin binding cofactor C domain; NDP, Nucleoside Diphosphate Kinase domain. C, Genotyping. The wild type (WT) allele was genotyped using the primers of RP2-F1 and RP2-R. The mutant Rp2h allele carrying the gene trap was genotyped using the primers of RP2-F5 and RP2-R. D, western blot of WT and knockout retina lysates using an anti-RP2 antibody. E, localization of RP2-eGFP in WT mouse photoreceptors. Mouse photoreceptors expression RP2-eGFP (green) were colabeled with rhodopsin (red). ROS, rod outer segment; RIS, rod inner segment; ONL, outer nuclear lay; OPL, outer plexiform layer.

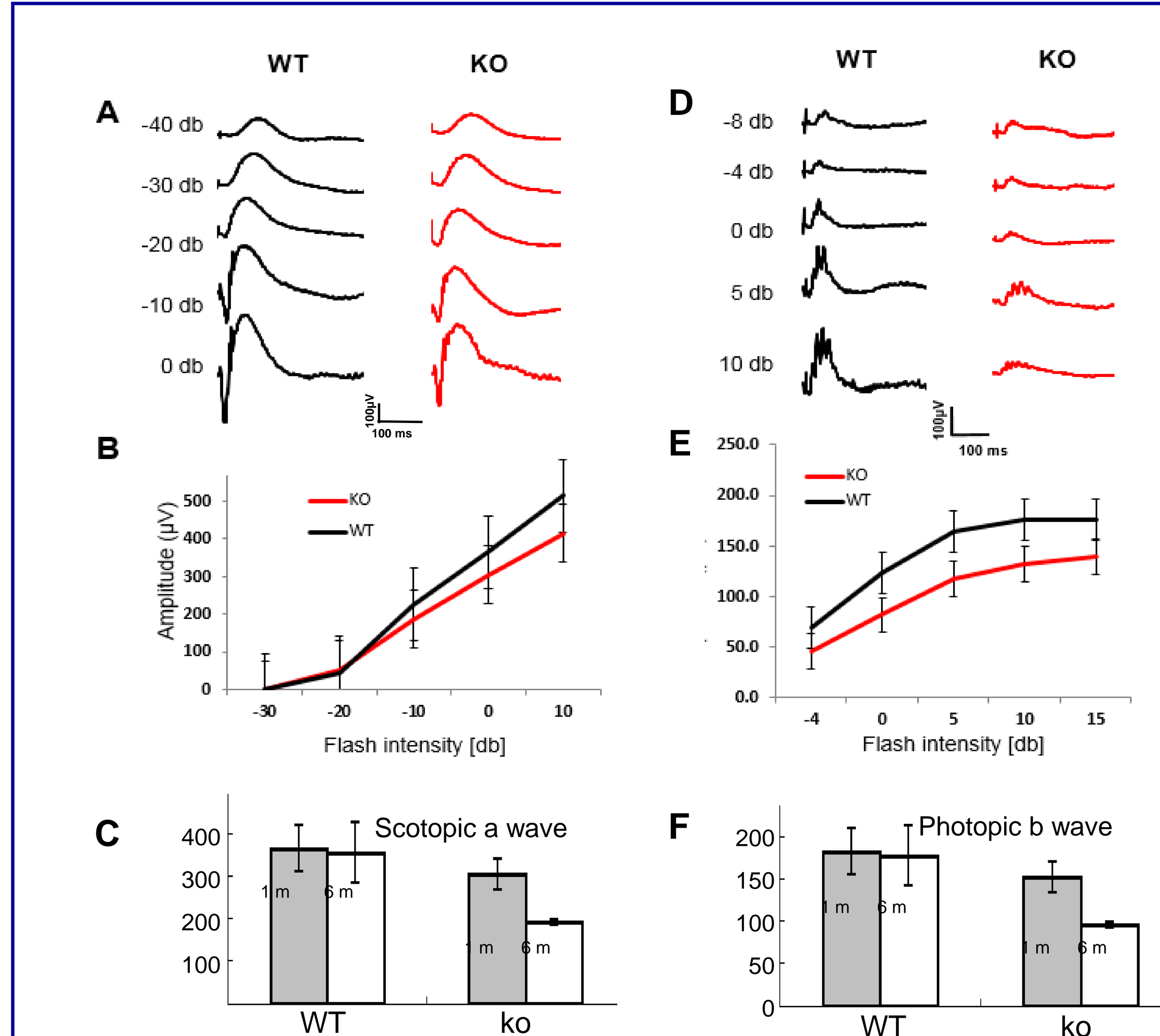

Figure 2. Scotopic and photopic electroretinography. A. Representative scotopic ERG traces recorded from wild-type and Rp2h knockout mice at one month of age. B. Scotopic a wave amplitudes from wild-type (n=3) and Rp2h knockout (n=3) mice at one month of age. Each data point represents mean + SD. C. Single-flash scotopic a wave amplitudes at 0 db for wild type and Rp2h knockout mice at the age of one month (grey bars, average of 3 independent values) and 6 months (white bars, average of 2 independent values). D. Representative photopic ERG traces recorded from the wild-type and Rp2h knockout mice at one month of age. E. Photopic b-wave amplitudes from the wild-type (n=3) and Rp2h knockout (n=3) mice at one month of age. Each data point represents mean + SD. F. Single-flash photopic b wave amplitudes recorded at 10 db for wild type (WT) and Rp2h knockout mice at the age of one month (grey bars, average of 3 independent values) and 6 months (white bars, average of 2 independent values).

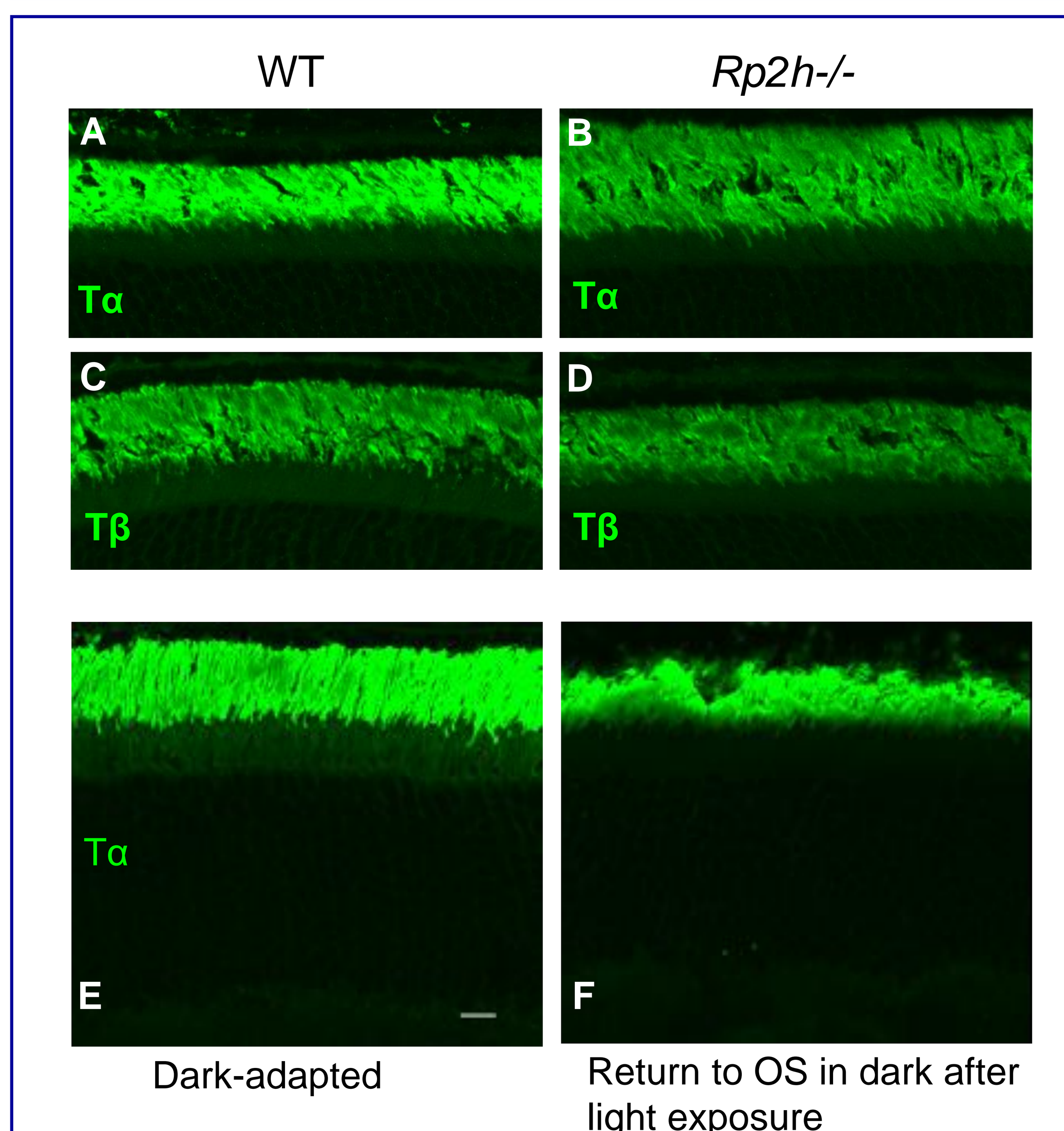

Figure 4. Normal trafficking of transducin in Rp2h -/- photoreceptors and of ODR-3 in mutant C. elegans. A-D. Wild-type and Rp2h -/- retina frozen sections (age of one month) were stained with anti-Tα antibody (A, B), anti-Tβ (C, D). E, F Normal return of transducin α to photoreceptor outer segments during dark-adaptation. WT and Rp2h -/- mice were illuminated with light of 2000 lux for 40 minutes and returned to the dark as described previously (Zhang, Constantine et al., 2011). After 10 hours in the dark, mouse eyeballs were harvested. Frozen sections were stained with anti-Tα antibody.

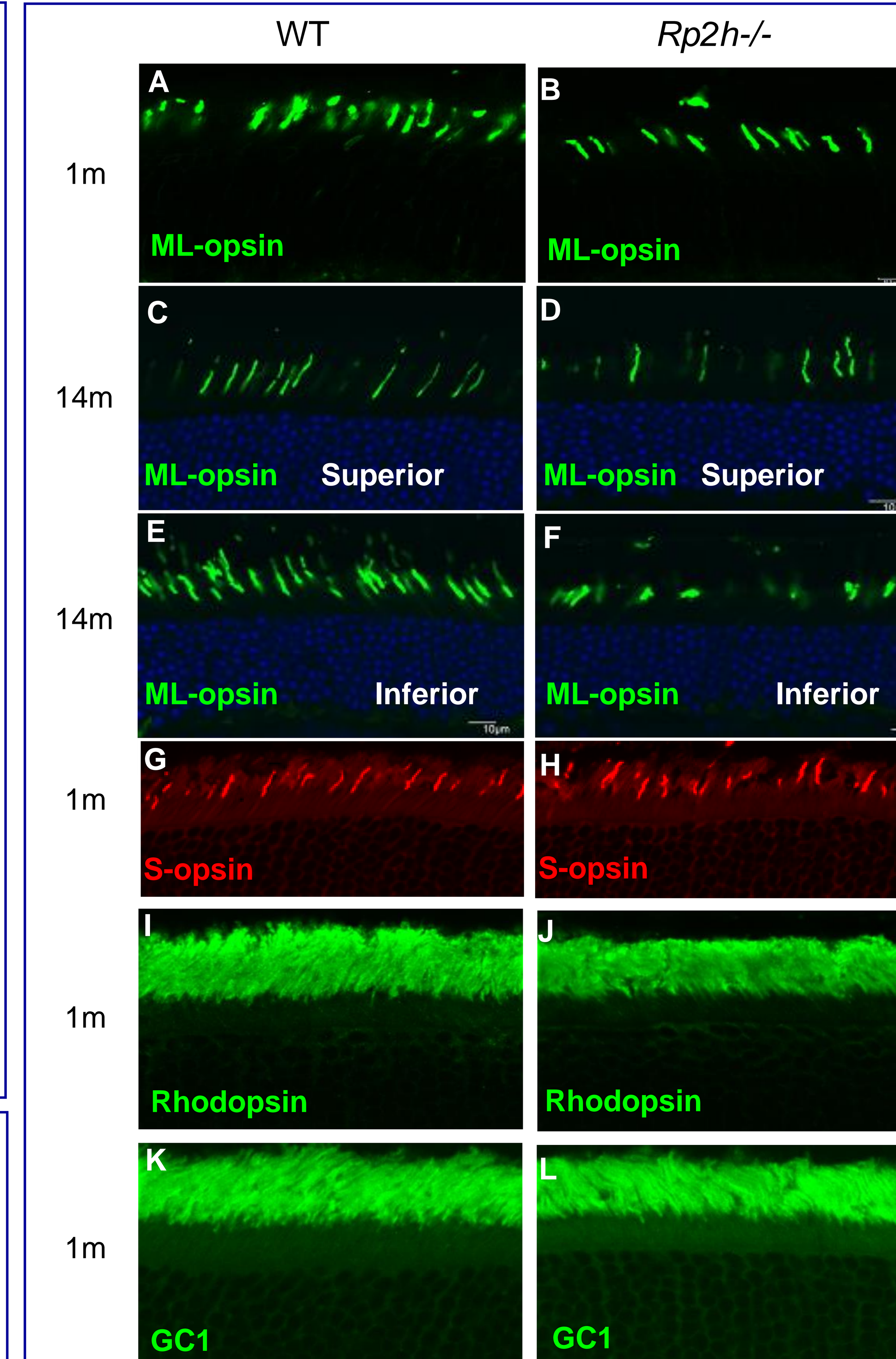

Figure 3. Localization of transmembrane proteins in photoreceptors. A-F. Wild-type and Rp2h knockout mouse retina frozen sections (age of one month, A,B; and age 14 months, C-F) were stained with anti-M/L-opsin antibody (green). The 14 months old retinas C-F were counterstained with DAPI (blue) to contrast the ONL. G, H. Distribution of S-opsin in the retina of the Rp2h -/- mouse. Mouse retina frozen sections at the age of 1 month were stained with anti-S-opsin (red). I-L. One-month old retina sections stained with anti-rhodopsin (I, J), and anti-GC1 (K, L). Scale bar: 10 µm.

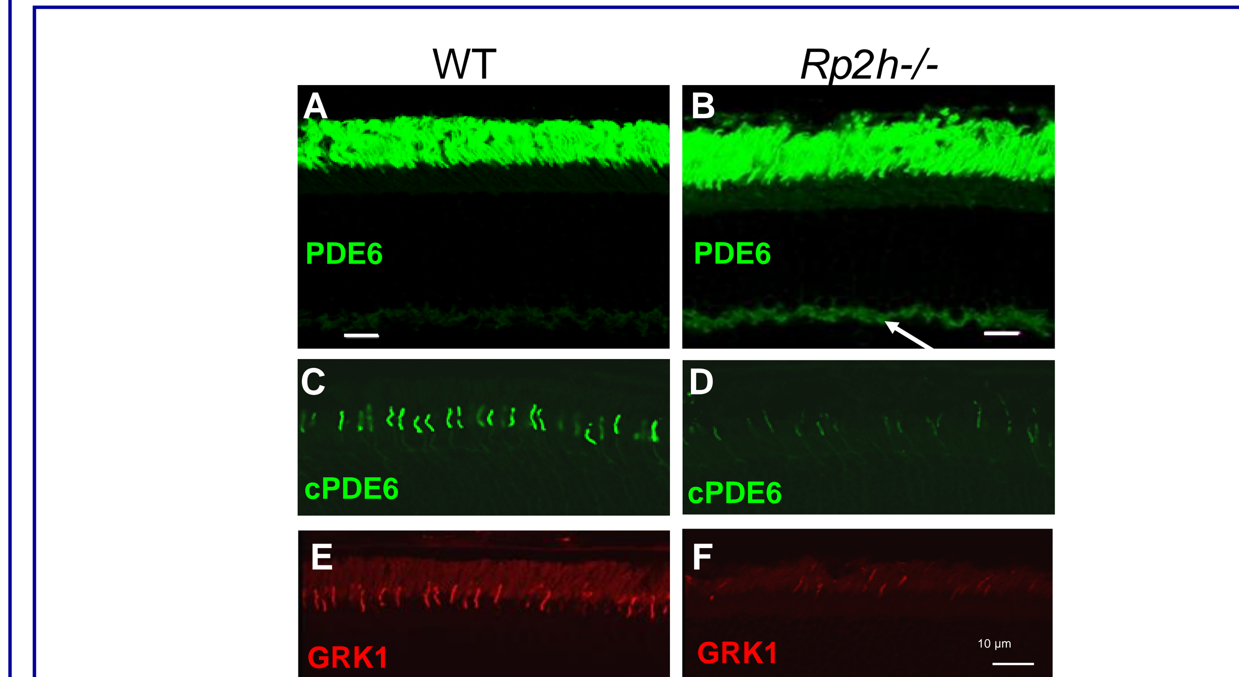

Figure 5. Trafficking defects of peripheral membrane proteins in RP2h -/- photoreceptors. A-F. Frozen sections were Labeled with anti-PDE6 (A, B), anti-cone PDE6α (C, D), and anti-GRK1 (E, F). Scale bar: 10 µm. Arrow in $F points ate mislocalized PDE6 in the synaptic region.

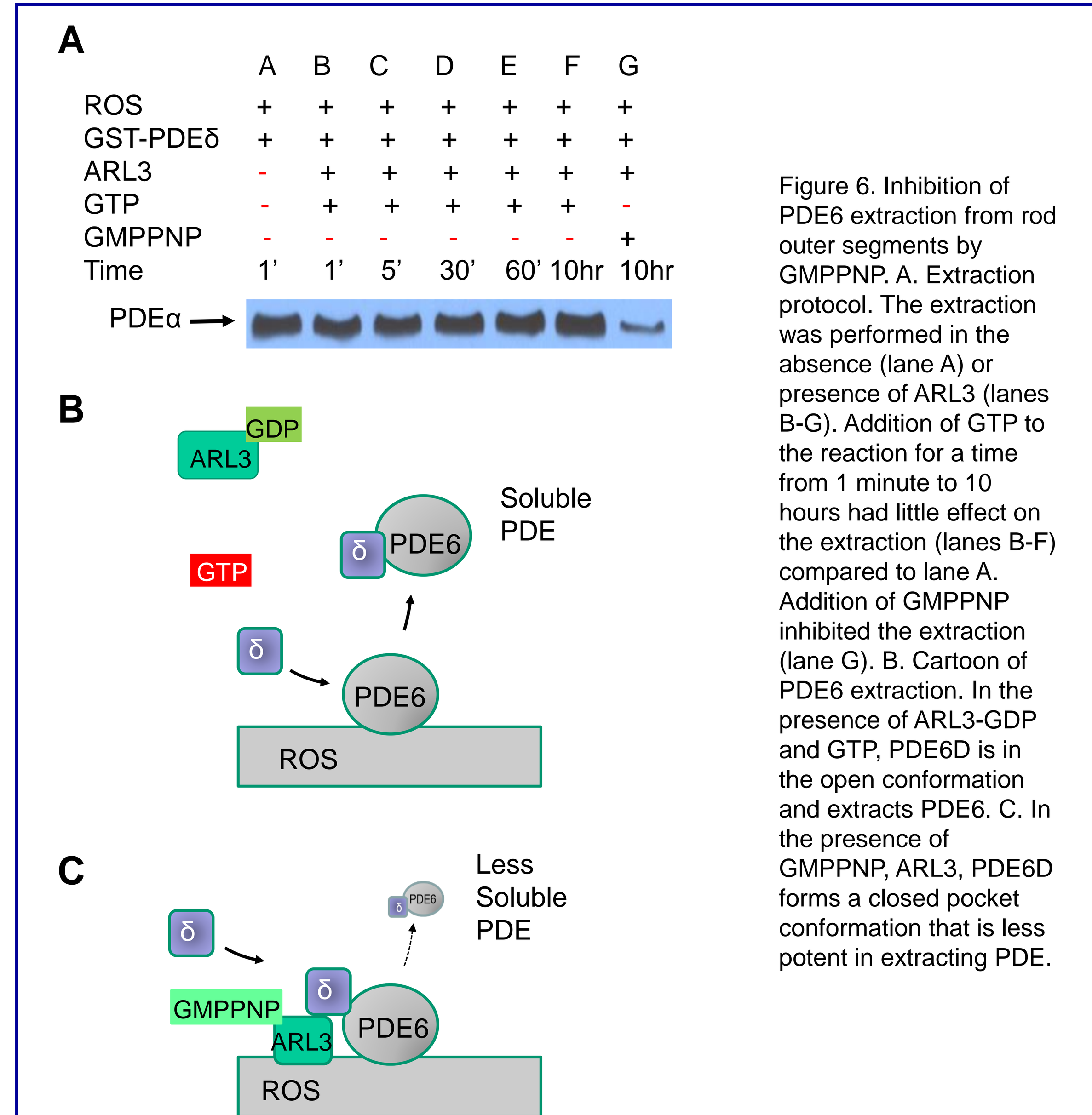

Figure 6. Inhibition of PDE6 extraction from rod outer segments by GMPPNP. A. Extraction protocol. The extraction was performed in the absence (lane A) or presence of ARL3 (lanes B-G). Addition of GTP to the reaction for a time from 1 minute to 10 hours had little effect on the extraction (lanes B-F) compared to lane A. Addition of GMPPNP inhibited the extraction (lane G). B. Cartoon of PDE6 extraction. In the presence of ARL3-GDP and GTP, PDE6D is in the open conformation and extracts PDE6. C. In the presence of GMPPNP, ARL3, PDE6D forms a closed pocket conformation that is less potent in extracting PDE.

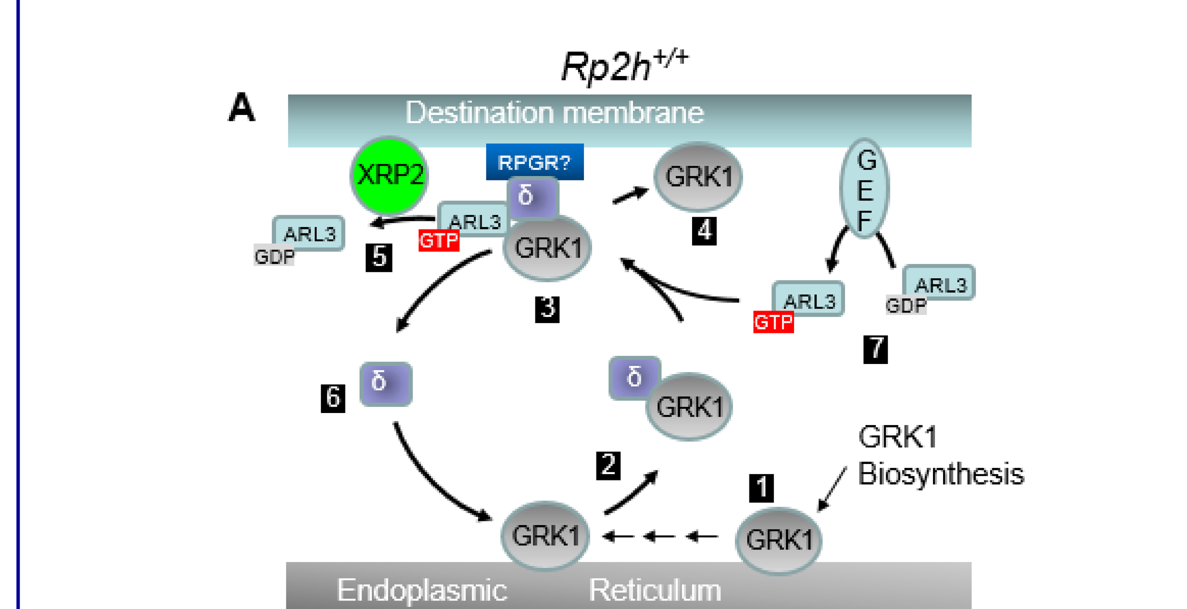

Figure 7. GRK1 trafficking from the ER to the destination membrane. A, model of ARL3/XRP2 dependent trafficking in WT photoreceptors. After biosynthesis and prenylation in the cytosol, GRK1 and other prenylated proteins dock to the ER membrane (1). Following CAAX processing (AAX cleavage, Cys-carboxymethylation), PDE6D extracts GRK1 forming a soluble complex (2). PDE6D-GRK1 then combine with ARL3-GTP to form a ternary complex which docks to the membrane. RPGR (3). ARL3-GTP alters the open conformation of PDE6D to a closed conformation, evicting cargo (GRK1) to bind to membranes (4). ARL3-GTP dissociates from PDE6D and binds to XRP2 accelerating GTP hydrolysis (5). PDE6D is released to begin another round transport (6). GDP/GTP exchange catalyzed by a guanine nucleotide exchange factor (GEF) regenerates ARL3-GTP (7).

Summary of Results

1. An RP2 gene mouse line was generated. .
2. RP2 is primarily localized to plasma membrane of photoreceptor inner segments.
3. RP2Y/- mice display a cone-rod dystrophy as early as one month of age and the
4. Rhodopsin and GC1 traffics normally to *Rp2*$^{Y/-}$ ROS Cones develop, and form short COS,
5. Distribution of transducin α and β is normal and return of transducin to ROS during dark-adaption after light-adaption is not affected in the RP2 knockout mice.
6. transport of rod and cone PDE6 as well as GRK1 to outer segments was impeded in the *Rp2*$^{Y/-}$ retina.