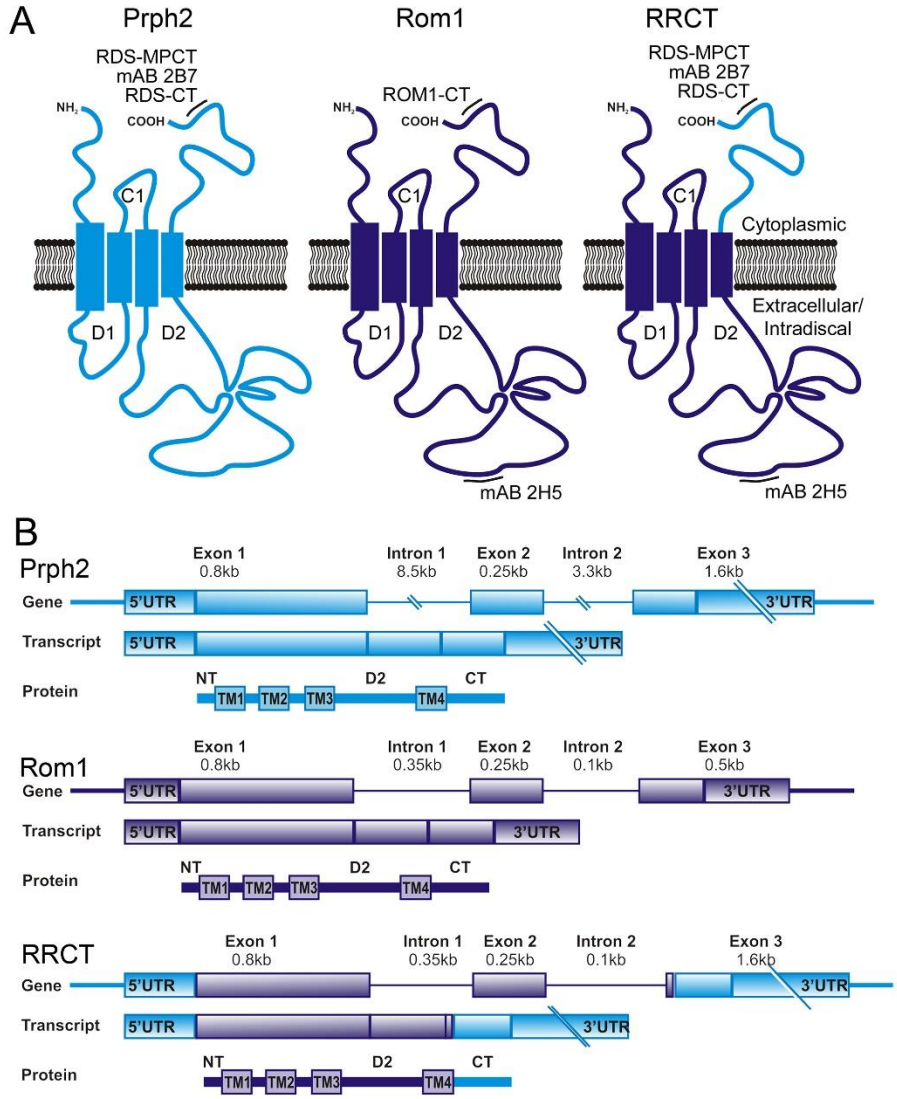
SUPPLEMENTARY FIGURES
Supplementary Figure 1



Supplementary Figure 1. Schematic of the RRCT knockin.
A. Shown are diagrams of Prph2 (blue), Rom1 (purple) and RRCT (blue/purple) proteins in the membrane. Antibody names and epitopes are labeled with small black bars. C1, D1, and D2 refer to the cytoplasmic, first and second intradiscal loops, respectively. **B.** Shown is a schematic for the creation of the RRCT knockin, with endogenous Prph2 (blue) endogenous Rom1 (purple) and the RRCT knockin (blue/purple) on the gene, transcript, and protein level.

Supplementary Figure 2

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Prph2    1..MA-LLKVKFDQKKRVKLAQGLWLMNWLSVLAGIVLFSLGLFLKIELRKRSEVMNNS-ESH
Rom1     1  MAPVLPVVLPLQPRIRLAQGIWLLSWLLALVGGLTLLCSGHLLVQLGHLGTFLAPSCSFP
RRCT     1  MAPVLPVVLPLQPRIRLAQGIWLLSWLLALVGGLTLLCSGHLLVQLGHLGTFLAPSCSFP
                                      TM1

Prph2   58  FVPNSLIGVGVLSCVFNSLAGKICYDALDPAKYAKWKPWLKPYLAVCIF-FNVILFLVAL
Rom1    61  ALPQTALAAGTVALGTGLGGAGASRASLDAAQYPPWRGVLTPLLAVGTAAGGGLLTLALG
RRCT    61  ALPQTALAAGTVALGTGLGGAGASRASLDAAQYPPWRGVLTPLLAVGTAAGGGLLTLALG
              TM2                                                TM3

Prph2  117  CCFLLRGSLESTLAYGLKNGMKYYRDTDTPGRCFMKKTIDMLQIEFKCCGNNGFRDWFEI
Rom1   121  LALALPVSLNQGLEEGLEAALAHYKDTEVPGRCQAKRLMDELQLRYHCCGRHGYKDWFGV
RRCT   121  LALALPVSLNQGLEEGLEAALAHYKDTEVPGRCQAKRLMDELQLRYHCCGRHGYKDWFGV


Prph2  177  QWISNRYLDFSSKEVKDRIKSNVDGRYLVDGVPFSCCNPSSPRPCIQYQLTNNSAHYSYD
Rom1   181  QWVSNRYLDPSDQDVVDRIQSNVEGLYLIDGVPFSCCNPHSPRPCLQSQLSDPYAHPLFD
RRCT   181  QWVSNRYLDPSDQDVVDRIQSNVEGLYLIDGVPFSCCNPHSPRPCLQSQLSDPYAHPLFD


Prph2  237  HQTEELNLWLRGCRAALLNYYSSLMNSMGVVTLLVWLFEVSITAGLRYLHTALESVSNPE
Rom1   241  PRQPNLNLWAQGCHEVLLEHLQGLSGTLGSILAVTLLLQILVLLGLRYLQTALEGLGGVI
RRCT   241  PRQPNLNLWAQGCHEVLLEHLQGLSGTLGSILAVTLLLQILVLLGLRYLHTALESVSNPE
                                          TM4

Prph2  297  DPECESEGWLLEKSVPETWKAFLESFKKLGKSNQVEAEGADAGPAPEAG  346
Rom1   301  DGEGEAQGYLFPGGLKDILKTAWLQGGLAHKPAPEEAPPDEEPPKEVLAEA  351
RRCT   301  DPECESEGWLLEKSVPETWKAFLESFKKLGKSNQVEAEGADAGPAPEAG  349
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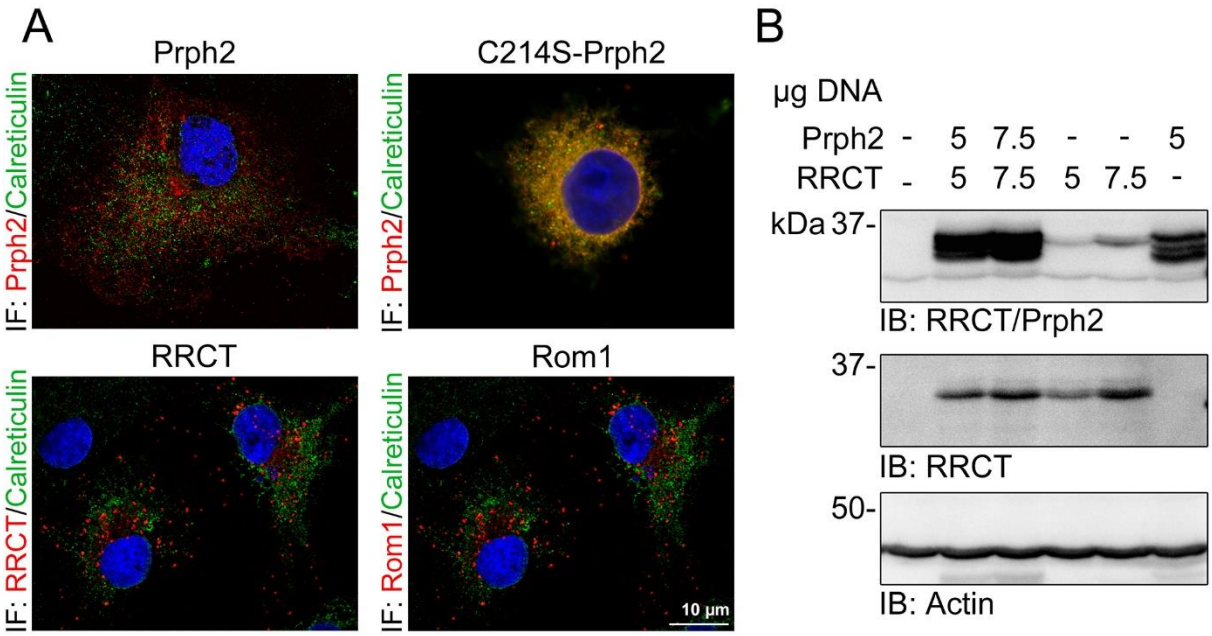
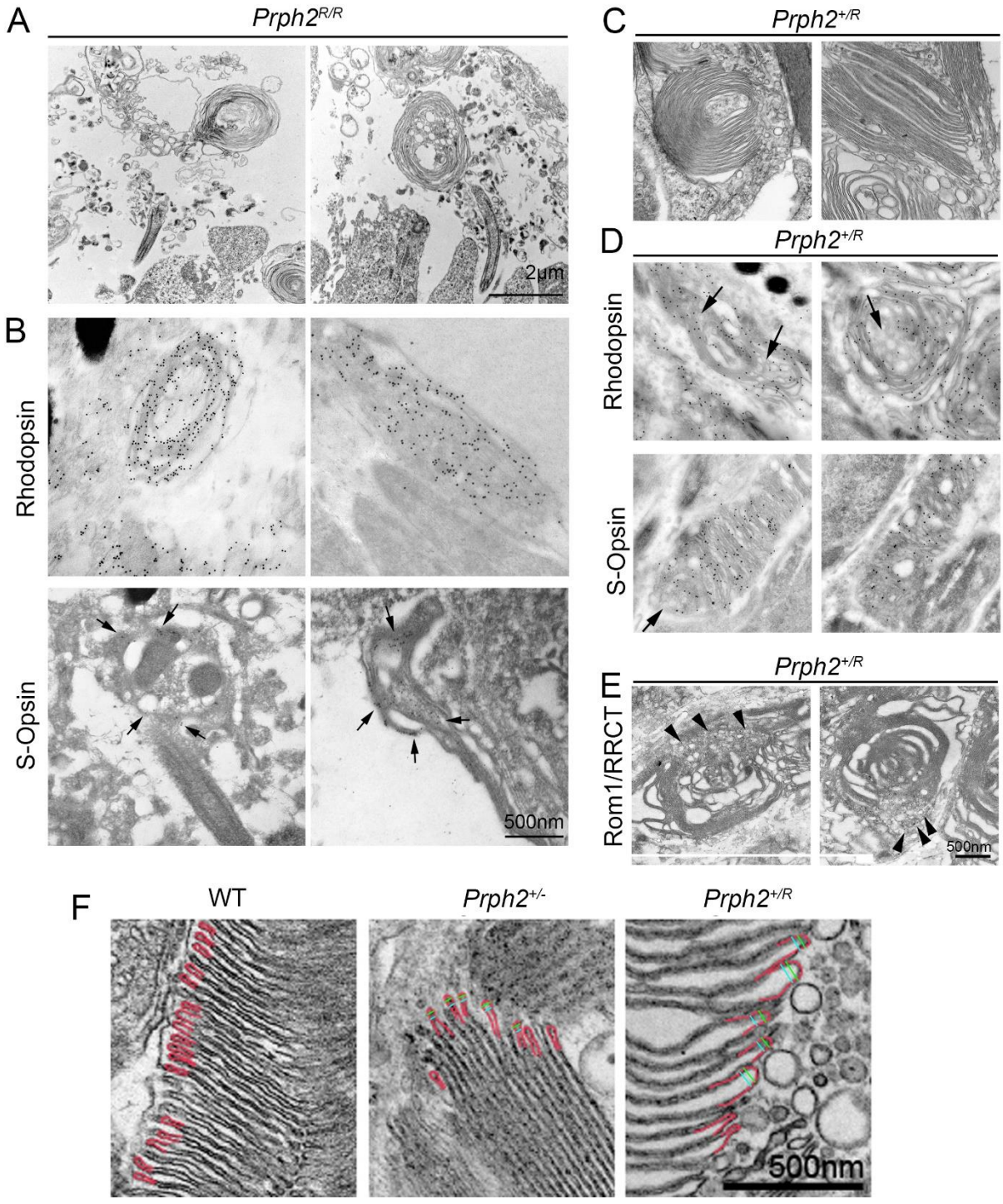
Supplementary Figure 2. Alignment of Prph2, Rom1, and RRCT peptide sequences. Text color indicates changing exon. Underlined Prph2 sequence is replaced with Rom1 sequence in the RRCT allele. TM1-4 indicate the transmembrane regions. Prph2 has a predicted molecular weight of 39.2 kDa (without including glycan), Rom1 has a predicted molecular weight of 37.2 kDa (not glycosylated), and RRCT has a predicted molecular weight of 37.3 kDa (not glycosylated). Yellow is the RDS-D2 epitope. Blue is the hypothetical longer peptide for Rom1 2H5, italics is the hypothetical shorter peptide for 2H5.

Supplementary Figure 3



Supplementary Figure 3. Expression of RRCT in transfected cells.
A. COS-7 cells were transiently transfected with Prph2 constructs (either WT or C214S), RRCT, or Rom1 as indicated above each panel. Cells were labeled for Prph2/RRCT (RDS-CT) or Rom1 in red, calreticulin in green, and DAPI in blue. Images captured at 100x, scale bar: 10 µm. **B.** COS-7 cells were transiently transfected with Prph2 and/or RRCT in amounts listed above the blots. Cell lysates were collected and separated on reducing SDS-PAGE. Resultant blots were probed for Prph2/RRCT (RDS-CT) or RRCT (mAB 2H5), with actin as a control.

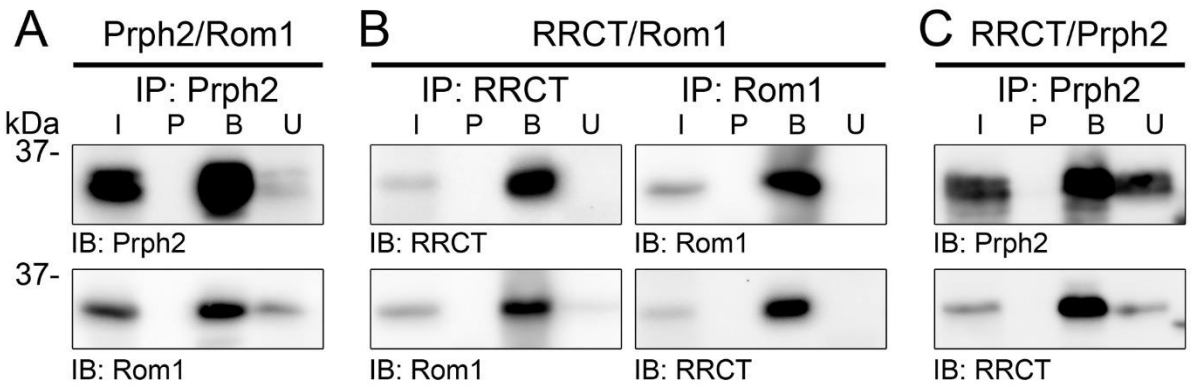
Supplementary Figure 4



Supplementary Figure 4. RRCT alone cannot support OS formation while expression of RRCT and WT Prph2 results in accumulation of abnormal vesicular structures.
A. Shown are representative P30 EM images collected at 15,000x. Scale bars 2µm **B.** Rod and cone OSs were identified by immunogold labeling with rhodopsin (Fliesler polyclonal), and S-

opsin (Craft polyclonal) antibodies, respectively (captured at 50,000x). Arrows highlight immunogold particles using the S-opsin antibodies. Scale bars 500nm **C.** Shown are representative P30 EM images collected at 40,000x. Scale bars 500nm **D.** Rod OSs were identified by immunogold labeling with rhodopsin (Fliesler polyclonal) while cone OSs were identified by labeling with S-opsin (Craft polyclonal) antibodies (captured at 25,000x or 30,000x). Arrows indicate vesicular structures largely lacking labeling for rhodopsin and S-opsin. Scale bars 500nm **E.** Rom1/RRCT was largely localized to vesicular structures (arrowheads), identified using mAB 2H5. Images captured at 50,000x. Scale bars 500nm. **F.** Shown are representative EM images captured at 40,000x (images are the same as those presented in Figure 6 for illustrative purposes). A selection of rims has been traced in red to show variations in pinching seen both between and within genotypes. A selection of outer rim diameters have been labeled in green with inner rim diameters labeled in blue. Scale bar 500 nm.

Supplementary Figure 5



Supplementary Figure 5. RRCT protein retains the ability to bind Prph2 and Rom1 *in vitro*. COS7 cells were double transfected with the constructs indicated at the top of each section. **A.** IP was performed for Prph2 (RDS-D2), and blots were probed for Prph2 (mAB 2B7) and Rom1 (mAB 2H5). **B.** IP was performed for RRCT (RDS-CT), and blots were probed for RRCT (mAB 2B7) and Rom1 (ROM1-CT). **C.** IP was performed for Prph2 (RDS-D2) and blots were probed for Prph2 (RDS-D2) and RRCT (mAB 2H5). I: input, P: preclear, B: bound, U: unbound.