SUPPLEMENTARY DATA

**Figure S1.** Generation and purification of recombinant C-terminal domains. Negative affinity purification of (A) CTER, (B) romCTER, and (C) E321L, K324 CTER proteins. Coomassie-blue stained TRIS-Tricine gels showing: starting material (lanes 1), cleaved (lanes 2), purified (lanes 3), and concentrated (lanes 4) samples. Helical wheel representations illustrate a lack of amphipathicity in the corresponding region of the rom-1 C-terminus and the retention of amphipathicity in the E321L, K324 CTER mutant, which retains a well-developed hydrophobic face, net positive charge, and increased hydrophobicity. Yellow, hydrophobic residues; purple, serine and threonine; blue, basic residues; red, acidic residues; green, proline; gray, other residues. Arrows indicate relative hydrophobic moments. Helical wheel representations and helix properties, including hydrophobic moments ($<\mu H>$), hydrophobicities ($<H>$), and net charges ($z$), were calculated using the HELIQUEST server (Gautier et al., 2008).

**Figure S2.** CTER association with membranes does not compromise bilayer integrity. Relief of calcein fluorescence self-quenching in liposomes was assayed (as described in Methods) to determine whether CTER induced leakiness. CTER was added at RT to calcein-loaded dioleoyl disk-mix liposomes at a P:L molar ratio of 1:10 (a ratio of ~8000 CTER molecules per liposome). After an initial small efflux (~1-3% of total releasable signal), the vesicles remained impermeable to further leakage. In conjunction with the data presented in Figures 2 and 3, these results suggest that the membrane remodeling induced by CTER association with vesicles does not disrupt bilayer integrity, a finding consistent with AH wedging into a single leaflet.

**Figure S3.** P/rds expressed in AD293 cells assembles into tetrameric complexes that are incorporated into disulfide-mediated oligomers. A) Transfected AD293 cells were treated with N-ethylmaleimide and extracted with Triton X-100, and post-nuclear supernatants were assayed by Western blot analysis. Akin to expression in vertebrate photoreceptors (Molday et al., 1987) and COS-1 cells (Goldberg et al., 1995), a substantial portion (~50%) of the AD293 cell expressed P/rds was present as disulfide-dependent dimers. In contrast, rom-1 expressed in AD293 cells, (like that in COS-1 cells) did not form disulfide-linked dimers. B) Sedimentation of AD293 cell expressed P/rds extracted under reducing conditions as described in Methods. Akin to expression in vertebrate photoreceptors and COS-1 cells (Goldberg et al., 1995; Goldberg and Molday, 1996), Triton X-100 solubilized P/rds generated a single major peak with a mobility characteristic of a tetramer. No significant reactivity was present in the particulate (P) fraction. Data from a representative experiment (of four performed) is shown. C) P/rds co-expressed with rom-1 likewise sedimented under reducing conditions as a single major peak indicative of a tetrameric stoichiometry, like that in co-transfected COS-1 cells and native outer segment membranes (Clarke et al., 2000; Goldberg et al., 1995). P/rds, solid line/filled circles; rom-1 dashed line/open circles. No significant reactivity was present in the particulate (P) fraction. Data from a representative
experiment (of two performed) is shown. D) In contrast, P/rds extracted from AD293 under non-reducing conditions, like that from transfected COS-1 cells and native outer segment membranes (Loewen and Molday, 2000), sedimented as heterogeneous heavier species, consistent with the assembly of P/rds tetramers into disulfide-linked polymers. Significant reactivity was present in the particulate (P) fraction. Data from a representative experiment (of two performed) is shown.

**Figure S4.** Small diameter tubulovesicular membranes induced by P/rds expression in AD293 cells. (A) TEM imaging of a fixed and enbloc stained AD293 cell showing multiple foci of clustered membranes (dashed box and ellipse); scale bar, 500 nm. (B) Higher magnification view of area boxed in (A); membrane-containing foci (similar to those presented in Fig. 6) are observed adjacent to areas of rough endoplasmic reticulum (RER); scale bar, 100 nm. Postembedding immunogold labeling analyses (C-E) of P/rds distribution in expressing (C) and non-expressing (E) AD293 cells; scale bars, 2 µm. P/rds distribution at the ultrastructural level was consistent with LSCM analyses; gold labeling was largely internal and particles were rarely found at the plasma membrane (C, arrows). (D) Higher magnification view of the area boxed in (C) shows a gold particle cluster associated with a circular array of small diameter vesiculated tubules; scale bar, 100 nm.

**Supplemental movie 1.** Localization of P/rds (red) in a cell containing a moderate level of protein, relative to an anti-KDEL antibody, which marks the endoplasmic reticulum (green). Hoechst-stained nuclei appear blue. A 2x zoom factor has been applied to the Z-axis. The lack of overlapping signals demonstrates that P/rds is efficiently exported from the endoplasmic reticulum.

**Supplemental movie 2.** Localization of P/rds (red) in a cell containing a moderate level of protein, relative to an anti-p58/ERGIC-53 antibody, which marks the ERGIC (green). Hoechst-stained nuclei appear blue. A 2x zoom factor has been applied to the Z-axis. The lack of co-localized signals demonstrates that little P/rds is present in the ERGIC.

**Supplemental movie 3.** Localization of P/rds (red) in a cell containing a moderate level of protein, relative to an anti-Giantin antibody, which marks the Golgi (green). Hoechst-stained nuclei appear blue. A 2x zoom factor has been applied to the Z-axis. The separation of red and green signals demonstrates that the vast majority of the P/rds present is not retained in the Golgi.

**Supplemental movie 4.** Localization of P/rds (red) in a cell containing a moderate level of protein, relative to an anti-Rab 11 antibody, which marks the endosomal recycling compartment (green). Hoechst-stained nuclei appear blue. A 2x zoom factor has been applied to the Z-axis. The lack of overlapping signals demonstrates that P/rds accumulates in membranes other than those associated with the ERC.
Supplementary References


Figure S1

Generation and purification of recombinant C-terminal domains

A

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CTER --

MW (kDa)

17 14.4 12.1 9 6.5 4.3 3.6

<μH> 0.618

H> 0.243

z 3

B

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romCTER --

MW (kDa)

17 14.4 12.1 9 6.5 4.3 3.6

<μH> 0.254

H> 0.368

z 2

C

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E321L, K324A --

MW (kDa)

17 14.4 12.1 9 6.5 4.3 3.6

<μH> 0.477

H> 0.425

z 3
Membrane integrity is retained in the presence of CTER

Figure S2
Figure S3

Tetramerization and disulfide-mediated self-assembly of P/rds in AD293
Figure S4

Small diameter tubulovesicular membranes induced by P/rds expression