McMillan et al., http://www.jcb.org/cgi/content/full/jcb.201604057/DC1

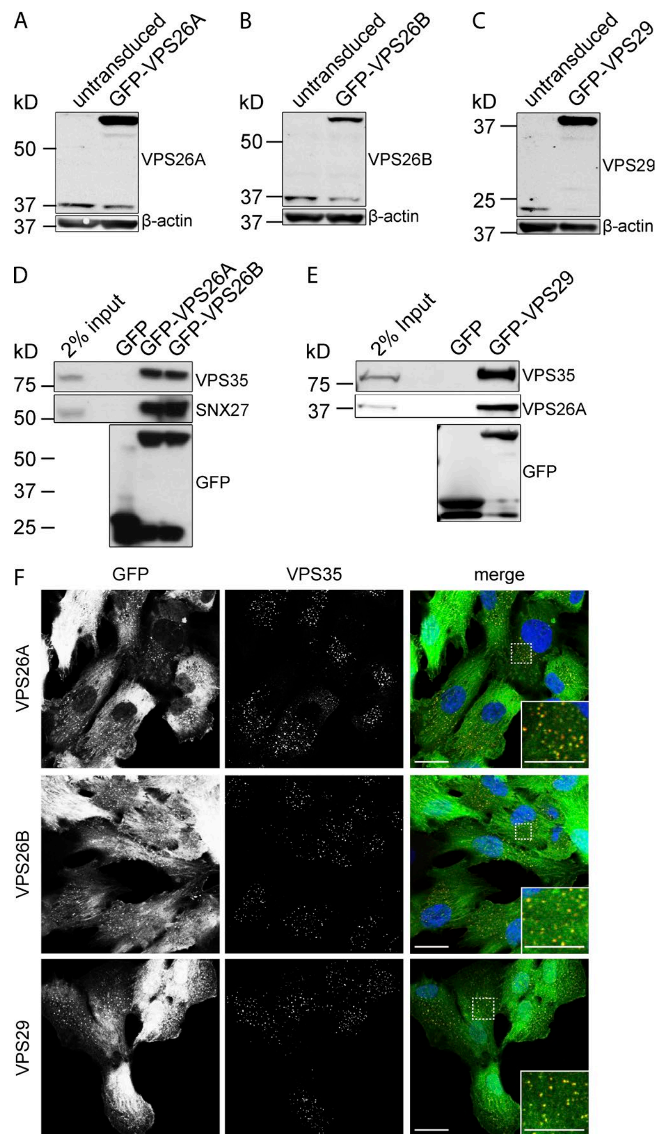

Figure S1. **Expression of GFP-tagged retromer components in lentivirally transduced RPE-1 cells.** (A–C) Fluorescence-based Western blots showing the protein expression levels of GFP-tagged VPS26A, VPS26B, and VPS29. β-Actin as loading control. (D and E) Fluorescence-based Western blots after GFP-Trap immunoprecipitation of GFP-tagged retromer components with the indicated antibodies. The GFP tag does not disrupt incorporation into the retromer heterotrimer. (F) Immunofluorescence staining of VPS35 in RPE-1 cells expressing GFP-tagged retromer components. The GFP tag does not affect association to VPS35 decorated endosomes. DAPI was used as a nuclear stain. Bars: (main) 20 µm; (inset) 10 µm.

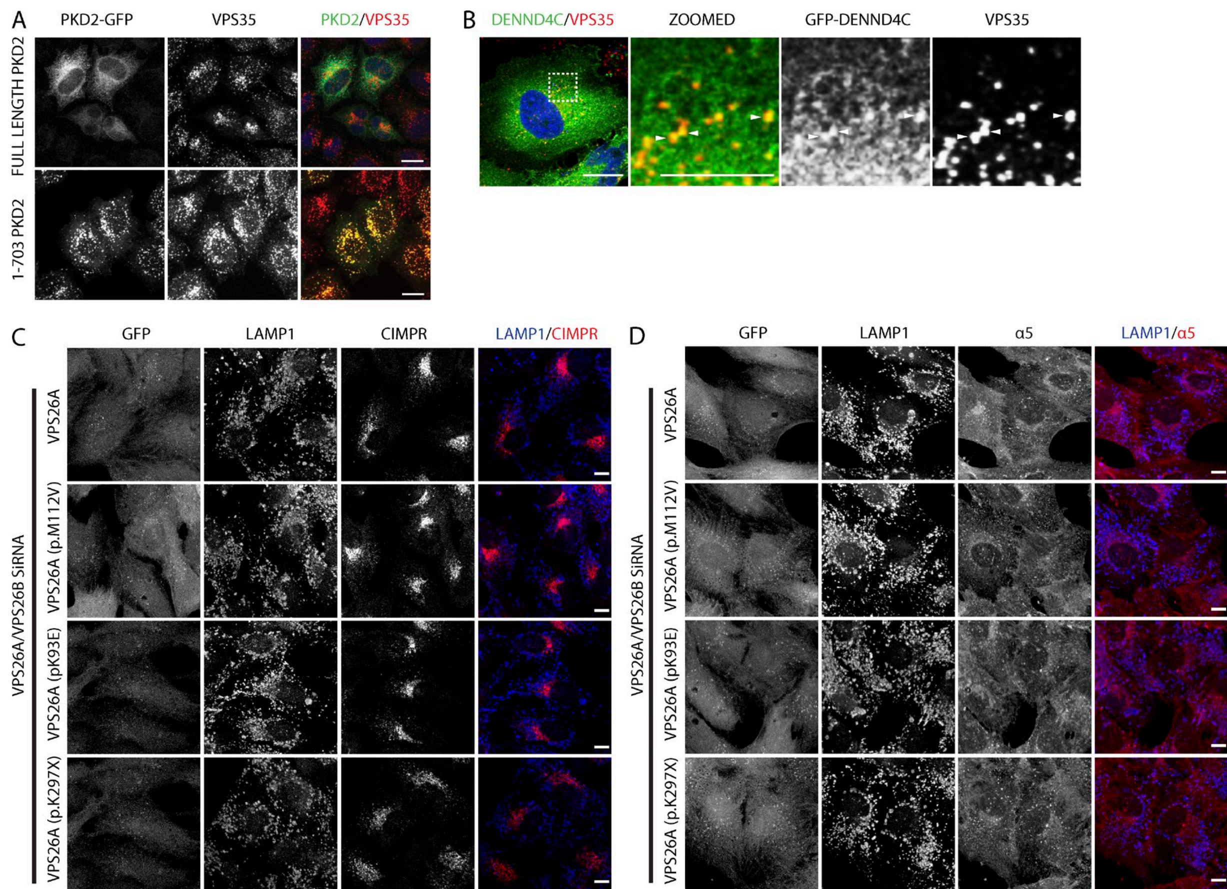

Figure S2. **PKD2 and DENND4C are associated with retromer-decorated endosomes and missense VPS26A mutants do not perturb trafficking of the CI-MPR or α5 integrin.** (A) Immunofluorescence staining of endogenous VPS35 in RPE-1 cells transiently transfect with constructs encoding for GFP-tagged full-length PKD2 or the 1–703 PKD2 truncation mutant. PKD2 is transported from the ER to the somatic plasma membrane from where it can enter the endocytic network (Hoffmeister et al., 2011; Kim et al., 2014). The steady-state distribution of PKD2 is primarily in the ER, but removal of the C-terminal ER retention signal, as in the 1–703 truncation mutant, releases PKD2 to the plasma membrane and the endocytic network. Here it shows extensive co-localization with retromer decorated endosomes. Bars, 10 µm. (B) Consistent with published work (Borner et al., 2012), immunofluorescence staining of endogenous VPS35 in HeLa cells transfected with GFP-DENND4C revealed a predominant cytoplasmic localization with some association of DENND4C on VPS35 positive endosomes. Bars: (left) 10 µm; (zoom) 5 µm. (C) Immunofluorescence costaining of CI-MPR with LAMP1 in RPE-1 cells stably expressing GFP-VPS26A or GFP-VPS26A mutants after RNAi-mediated suppression of VPS26 expression. Bars, 10 µm. (D) Immunofluorescence costaining of α5 integrin with LAMP1 in RPE-1 cells stably expressing GFP-VPS26A or GFP-VPS26A mutants after RNAi-mediated suppression of VPS26 expression. Bars, 10 µm.

Provided online is Table S1, showing SILAC-quantified interactomes of the different retromer subunits in RPE-1 cells. (A) VPS29 interactome raw data. (B) Filtered and combined VPS29 interactome. (C) VPS26 interactome raw data. (D) Filtered and combined VPS26 interactome. (E) VPS26 paralogue-specific interactomes. (F) Filtered and combined VPS35 interactome.

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