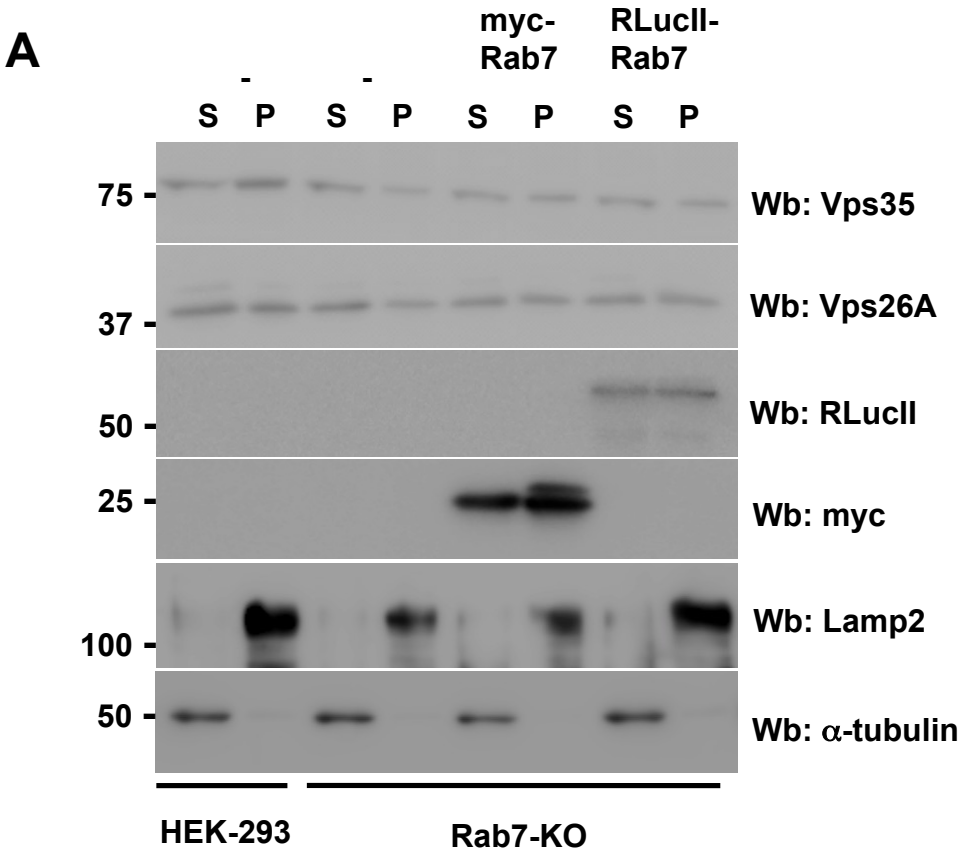

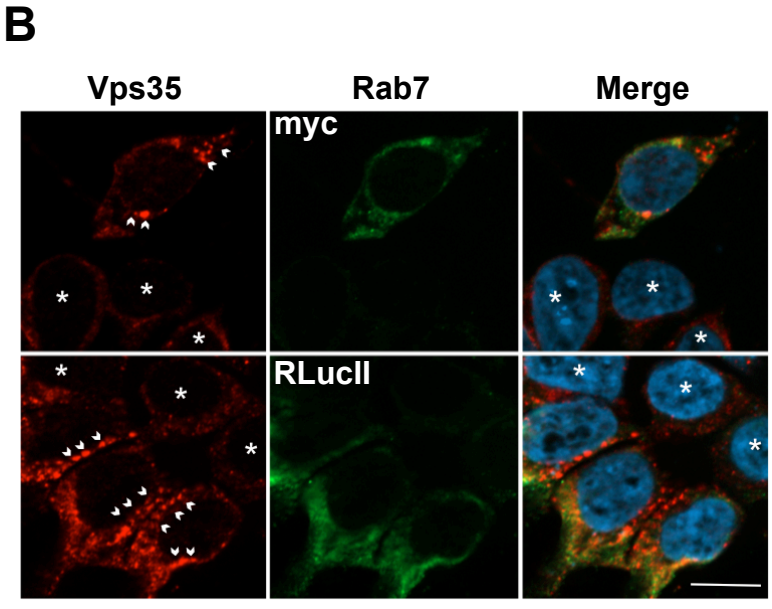

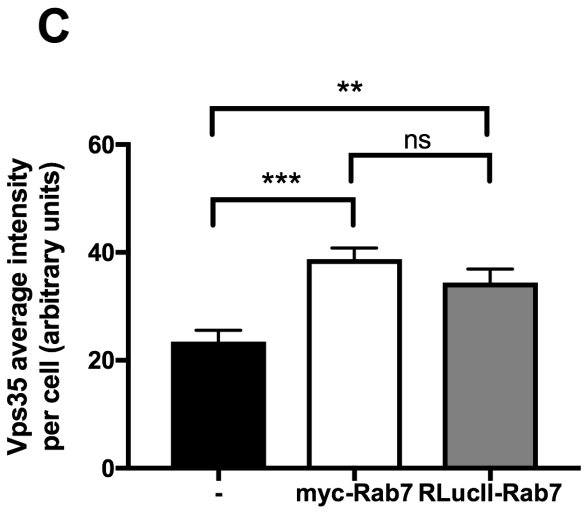

Figure S1. RLucII-Rab7 is properly membrane recruited and functional

(A) HEK-293 or Rab7-KO cells were transfected with myc-Rab7 or RLucII-Rab7. 48 hours post-transfection, a membrane separation assay was performed and samples were subjected to Western blot (Wb) with anti-Vps35, anti-Vps26A, anti-RLucII, anti-myc, anti-Lamp2 antibody (a membrane marker) and anti-α-tubulin (a cytosolic marker). (B) HEK-293 or Rab7-KO cells were transfected with myc-Rab7 or RLucII-Rab7. 48 hours post-transfection, the cells were fixed in 4% paraformaldehyde and immunofluorescence staining was performed with anti-myc (green) or anti-RLucII (green) and anti-Vps26A (red) antibodies. Scale bar = 10 μm. (C) Fluorescence intensity of Vps35 staining was measured in Rab7-KO cells (black bar), Rab7-KO cells expressing myc-Rab7 (white bar) or RLucII-Rab7 (grey bar). Data is represented as mean Vps35 intensity ± SEM from 31, 14, and 16 cells per condition. *** $P < 0.001$, ** $P < 0.01$, ns, not significant, One-way ANOVA with Tukey's post-hoc test.

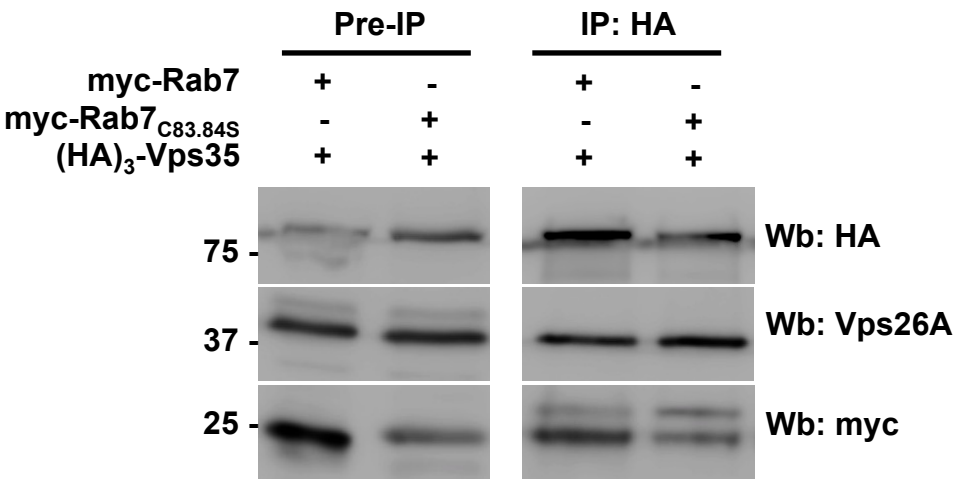

Figure S2. Palmitoylation does not affect the Rab7/retromer interaction *per se*. HEK-293 were transfected with myc-Rab7, myc-Rab7$_{C83,84S}$ or (HA)$_3$-Vps35. 24 hours after transfections, equal amounts of cell lysates from myc-Rab7 or myc-Rab7$_{C83,84S}$ transfected cells were mixed with lysate from HEK-293 expressing (HA)$_3$-Vps35, followed by immunoprecipitation with anti-HA antibody. Eluted samples were loaded onto a 12% polyacrylamide gel and subjected to Western blot (Wb) analysis with anti-myc, anti-Vps26A and anti-HA antibodies.