Supplemental Figure 1. Retrograde transport of TGN38 is similarly affected by other two different FIP1/RCP siRNAs.
(A) HeLa-Tac-TGN38 cells were either mock treated or transfected with two different FIP1/RCP siRNAs (c and d). Cells were then fixed and Tac-TGN38 TGN localization quantitated. 'n' is the number of cells analyzed.
(B) The efficiency of FIP1/RCP depletion was confirmed by immunoblotting with anti-FIP1/RCP and anti-Synatxin 6 antibodies. Please note that while siRNAs caused the knock-down of FIP1/RCP both splice isoforms, in these immunoblots we only show the predominant ~70kDa splice isoform.

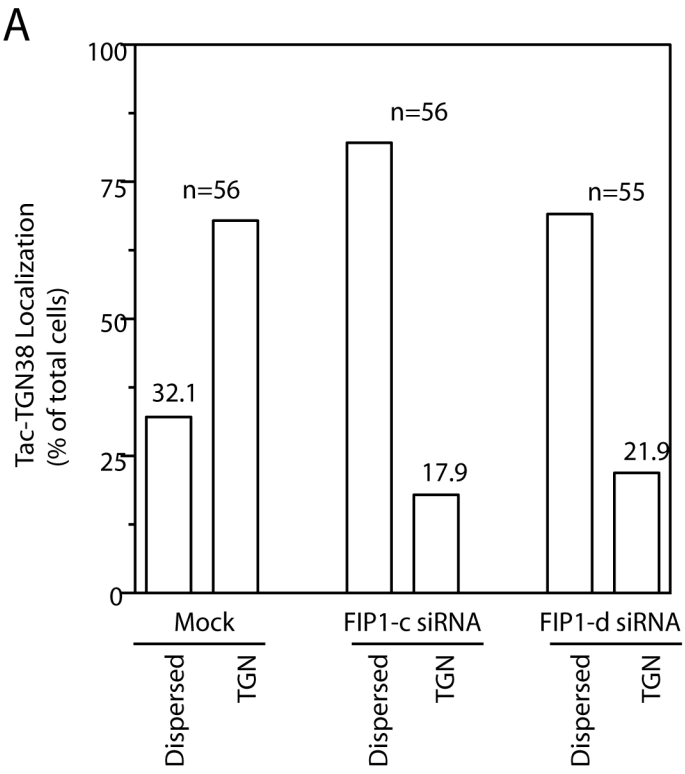
Supplemental Figure 2. FIP1/RCP knock-down has no effect on Golgi and TGN organization.
(A-I) Mock (A-C), Rab11a/b (D-F) or FIP1/RCP (G-I) siRNA-treated HeLa cells were fixed and stained with anti-FIP1/RCP (B, E and H) and anti-Golgin-97 (A, D and G) antibodies.
(J) Lysates from mock or FIP1/RCP siRNA-treated cells were analyzed by immunoblotting for the presence of FIP1/RCP and Golgin-97. Please note that while siRNAs caused the knock-down of both FIP1/RCP splice isoforms, in these immunoblots we only show the predominant ~70kDa splice isoform.

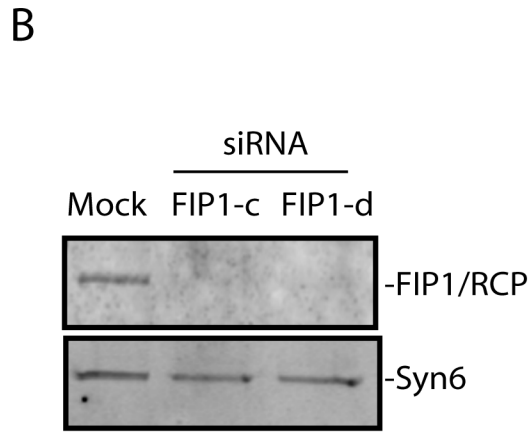
Supplemental Figure 3. FIP1/RCP specifically binds to Rab11 and Rab25.
(A) The constitutively active Rab mutants were cloned into the pGKBKT7 vector. Full-length FIP1/RCP was cloned into the pGAD vector. The interaction between FIP1/RCP and Rab GTPases was analyzed by yeast two-hybrid assay. DDO stands for double (-Leu, -Trp) dropout media. QDO stands for quadruple (-Leu, -Trp,-Ade,-His) dropout media.
(B) Glutathione beads coated with GTP or GDP-loaded GST-Rab11a or GST-Rab14 were incubated with either recombinant purified 6His-FIP1/RCP or 6His-FIP5/Rip11. Beads were then washed and amount of bound 6His-FIP1/RCP or 6His-FIP5/Rip11 analyzed by immunoblotting with anti-6His antibodies.

Supplemental Figure 4. FIP1/RCP depletion blocks VAMP4 targeting to TGN.
Mock (A-C) or FIP1/RCP (D-F) siRNA-treated HeLa cells were stained with anti-VAMP4 (A and D, green) or anti-Golgin-97 (B and E, red) antibodies. Yellow in C and F represents the degree of overlap between Golgin-97 and VAMP4. Numbers in panels A and D are the percentage of cells displaying shown phenotype.

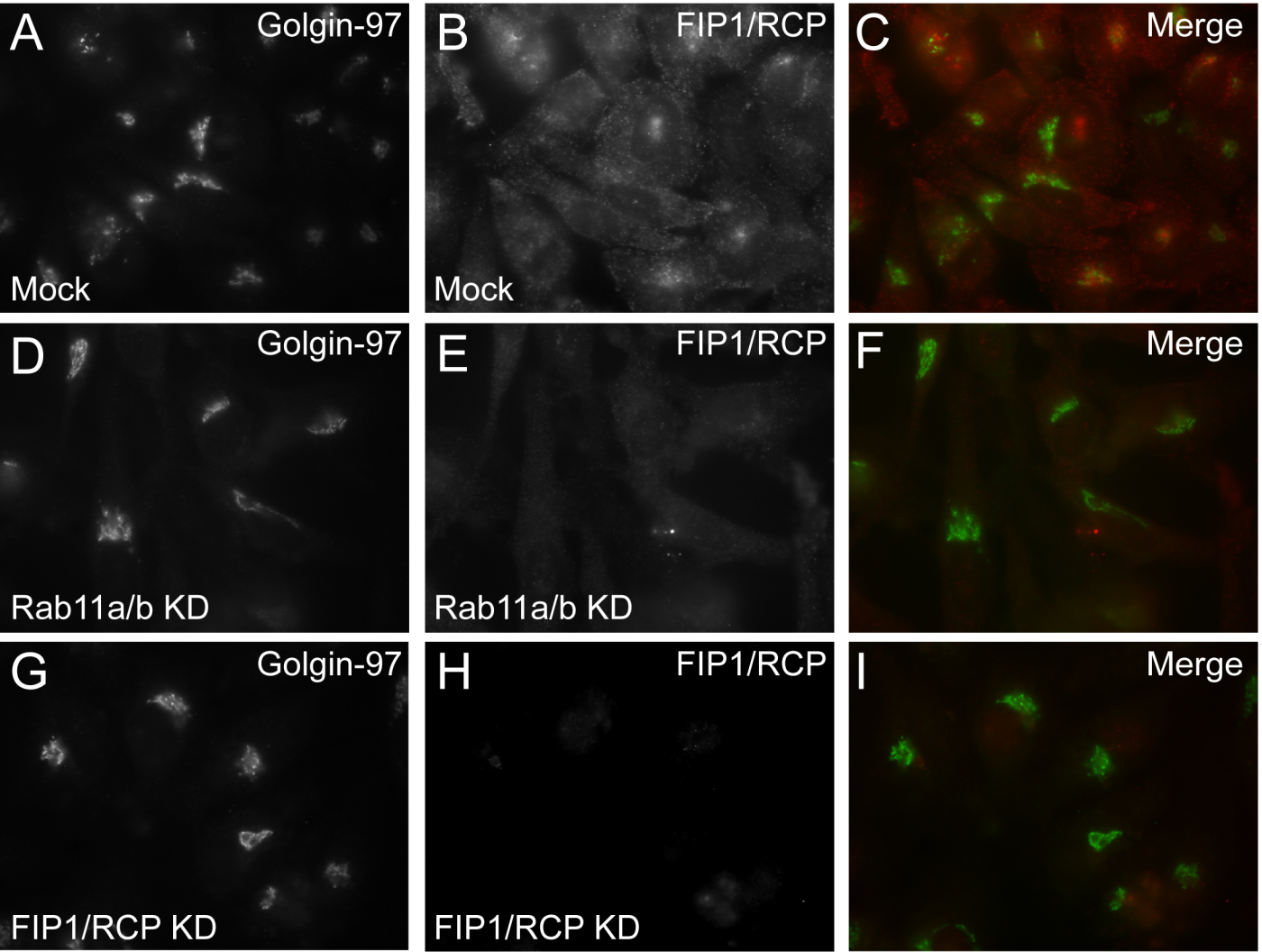
Supplemental Figure 5. FIP1/RCP is not required for CI-MPR6 retrograde transport.
Mock (A-C) or Rab11a/b (D-F) siRNA-treated HeLa cells were stained with anti-Syntaxin 6 (A and D, green) or anti-CI-MPR6 (B and E, red) antibodies. Yellow in C and F represents the degree of overlap between Syntaxin 6 and M6P.

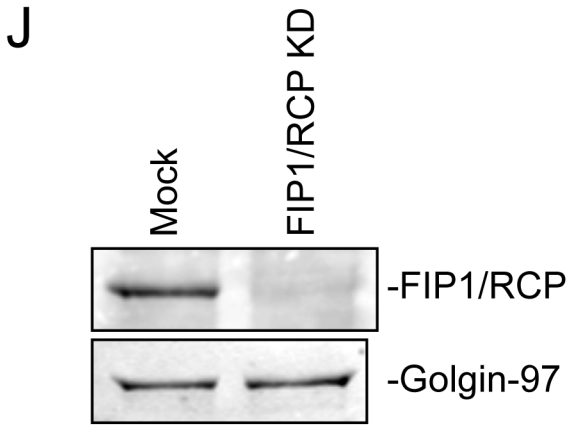
Supplemental Figure 1





Supplemental Figure 2





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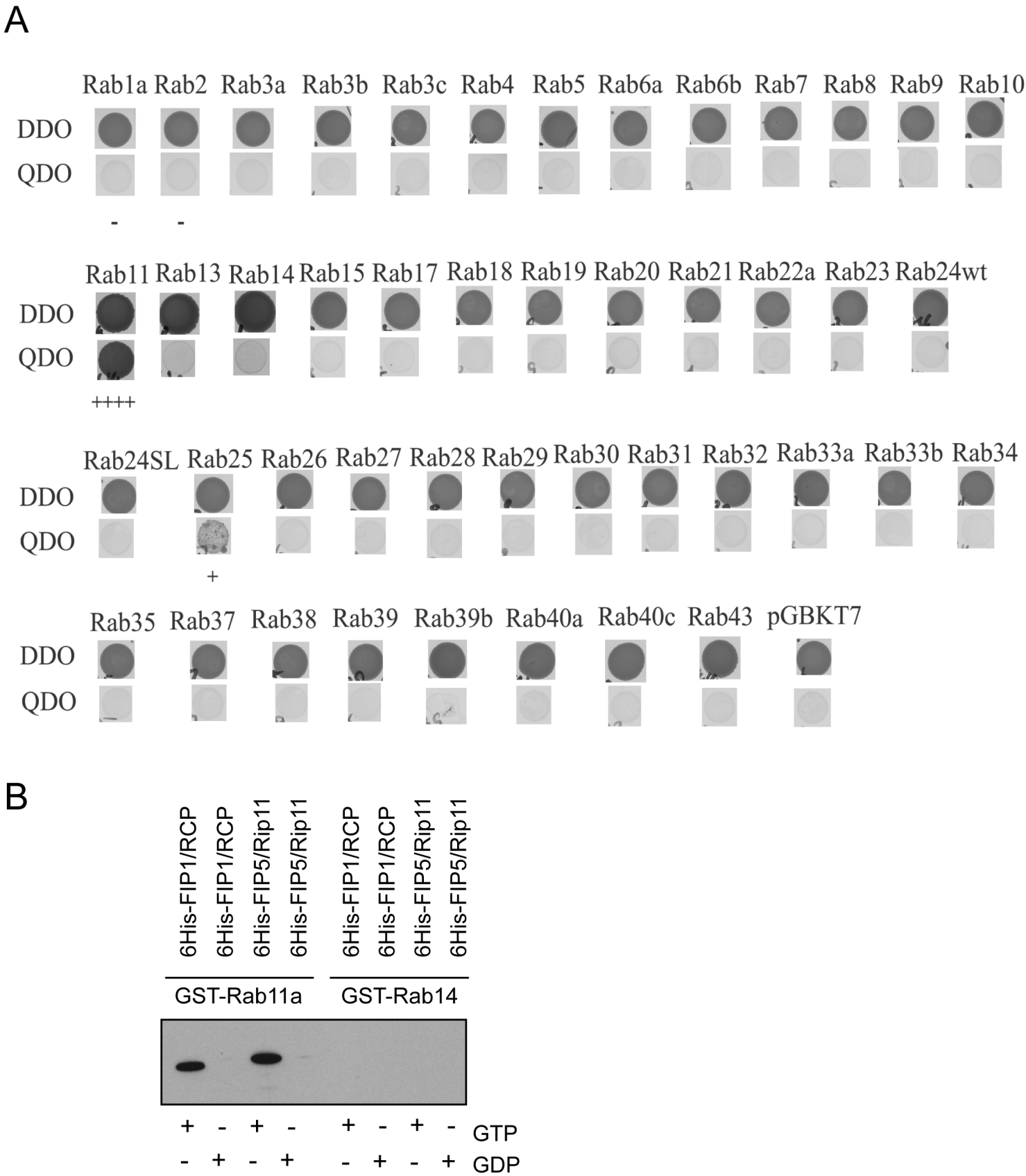
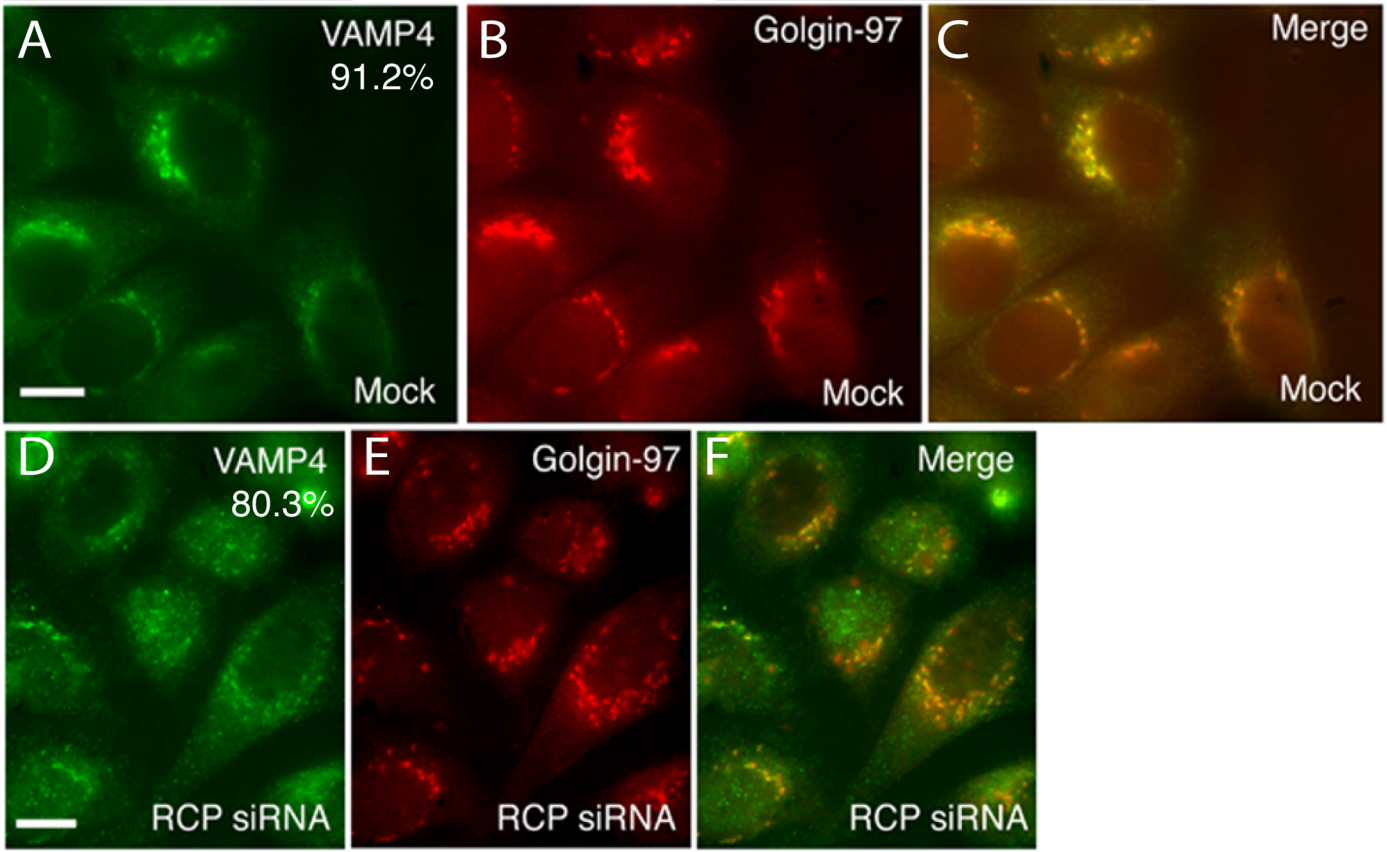


Supplemental Figure 4



Supplemental Figure 5