SUPPLEMENTARY RESULTS

Detection of endogenous Rab14

We have raised rabbit polyclonal antibodies against Rab14 using the recombinant C-terminal region of Rab14 as an immunogen. As shown in supplementary Figure S1A, the affinity purified antibody specifically recognized EGFP-tagged Rab14 expressed in HeLa cells, but none of other Rab proteins tested, including Rab4, Rab5, Rab6, Rab7, Rab8, Rab10, Rab11, and Rab15. A single 24-kDa band was detected with the anti-Rab14 antibody in HeLa cell lysates (supplementary Figure S1A, asterisk); this band was abolished by preincubation of the antibody with full-length Rab14 or the immunogen (data not shown). Likewise, staining of endosomal structures were observed using the anti-Rab14 antibody, and this staining was abrogated by preincubation of the antibody with full-length Rab14 or the immunogen (supplementary Figure S1B), but not with GST, indicating that the purified antibody is specific to Rab14.

Localization of endogenous Rab14

Endogenous Rab14 is partially colocalized with the early endosomal marker, EEA1, and the early endosomal/recycling endosomal marker, TfnR, but not the late endosomal marker, LBPA, or the late endosomal/lysosomal marker, Lamp-1; this is consistent with previous reports (Junutula *et al.*, 2004). Rab14 shows limited colocalization with a TGN marker, golgin-245. Therefore, Rab14 mainly localizes to early endosomes, and to a lesser extent with perinuclear TfnR-positive recycling endosomes, as reported recently (Kelly *et al.*, 2009).

Knockdown of RUFY1

To examine RUFY1 function in the Tfn recycling pathway (Figure 8), we knocked down HeLa cells of RUFY1 by RNAi. Specific and efficient knockdown of RUFY1 in cells transfected with pools of siRNAs for RUFY1 was confirmed by immunoblotting (Supplementary Figure S4A) and immunofluorescence analyses (Supplementary Figure S4B).

SUPPLEMENTARY FIGURE LEGENDS

Figure S1. Specificity of anti-Rab14 antibody

(A) Lysates of HeLa cells transfected with a plasmid encoding the EGFP-tagged Rab protein indicated were subjected to immunoblotting with anti-Rab14 antibody (upper panel) or anti-GFP antibody. Arrows indicate the position of EGFP-tagged Rab14, and an asterisk indicates the position of endogenous Rab14. The affinity purified antibody specifically recognized EGFP-tagged Rab14, but none of other Rab proteins examined. (B) HeLa cells were stained with the anti-Rab14 antibody in the absence (a) or presence of GST (b), full-length of Rab14 (c) or C-terminal region of Rab14 used as an antigen for immunization.

Figure S2. Subcellular localization of endogenous Rab14

HeLa cells were fixed with 3% PFA, permeabilized with saponin, and doubly stained with anti-Rab14 antibody (A-E) and anti-EEA1 (A'), anti-TfnR (B'), anti-Lamp-1 (C'), anti-LBPA (D'), or anti-golgin-245 (E') antibodies. Merged images are shown in A"-E". Insets are magnified images of the boxed areas. Bar, 20 μm.

Figure S3. Specific knockdown of Rab14 by RNAi

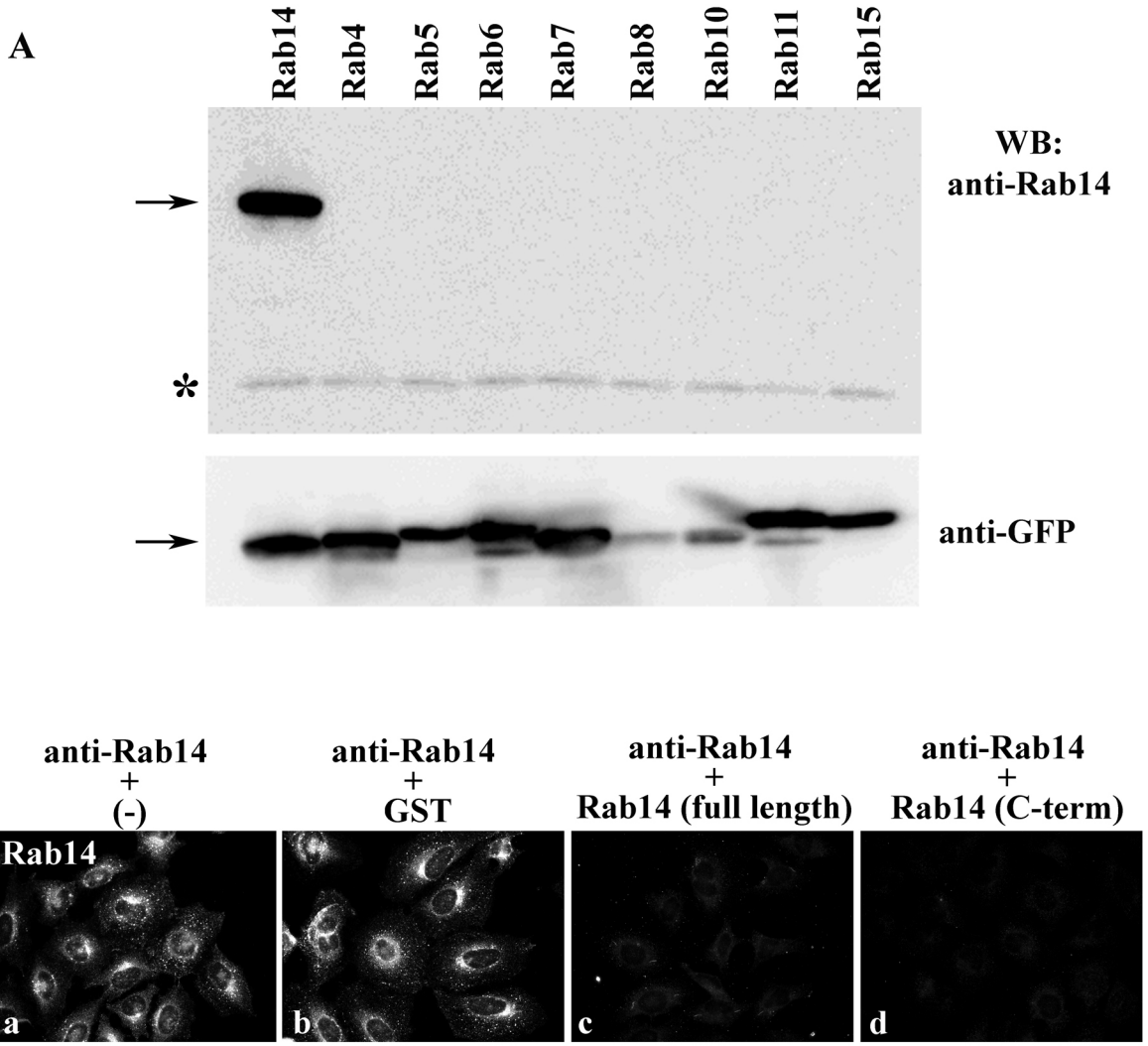
(A and B) HeLa cells were treated with double-stranded oligonucleotide siRNA for Rab14 or control siRNA (A, B), and subjected to immunoblot analysis for the Rab proteins indicated, (A) or immunofluorescence analysis with anti-RUFY1 antibody (B). (C) HeLa cells were treated with a pool of siRNAs to LacZ and Rab14 as in Figure 3, and doubly immunostained for Rab14 (insets) and EEA1, transferrin receptor (TfnR), Lamp-1 or golgin-245.

Figure S4. Knockdown of RUFY1

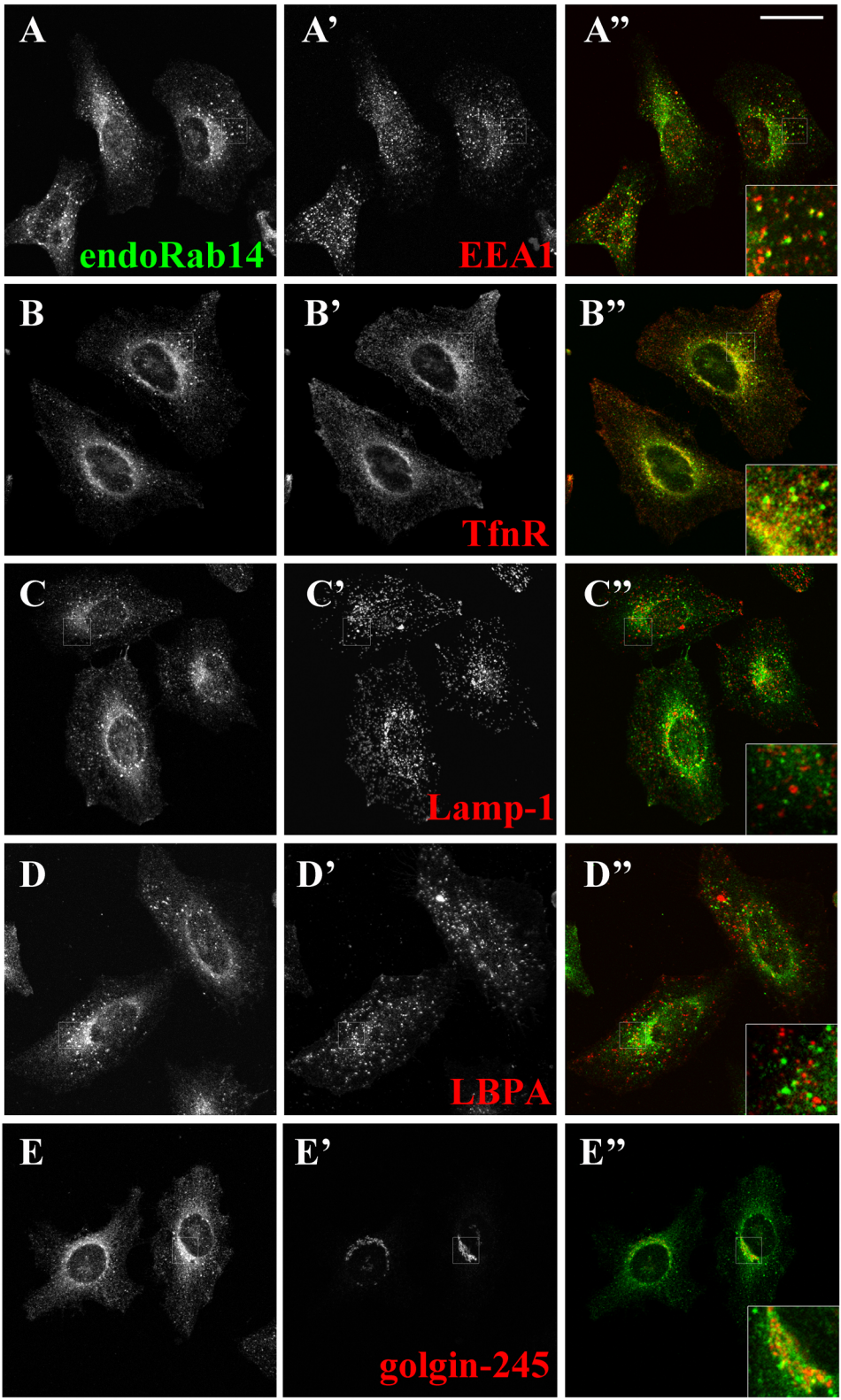
HeLa cells were treated with a pool of siRNAs for LacZ as a control or RUFY1, and subjected to immunoblot analysis using antibody to indicated proteins (A), or immunofluorescence analysis with anti-RUFY1 antibody (B).

Figure S5. Depletion of Rab14 or RUFY1 does not affect surface-bound-Tfn or internalization of Tfn

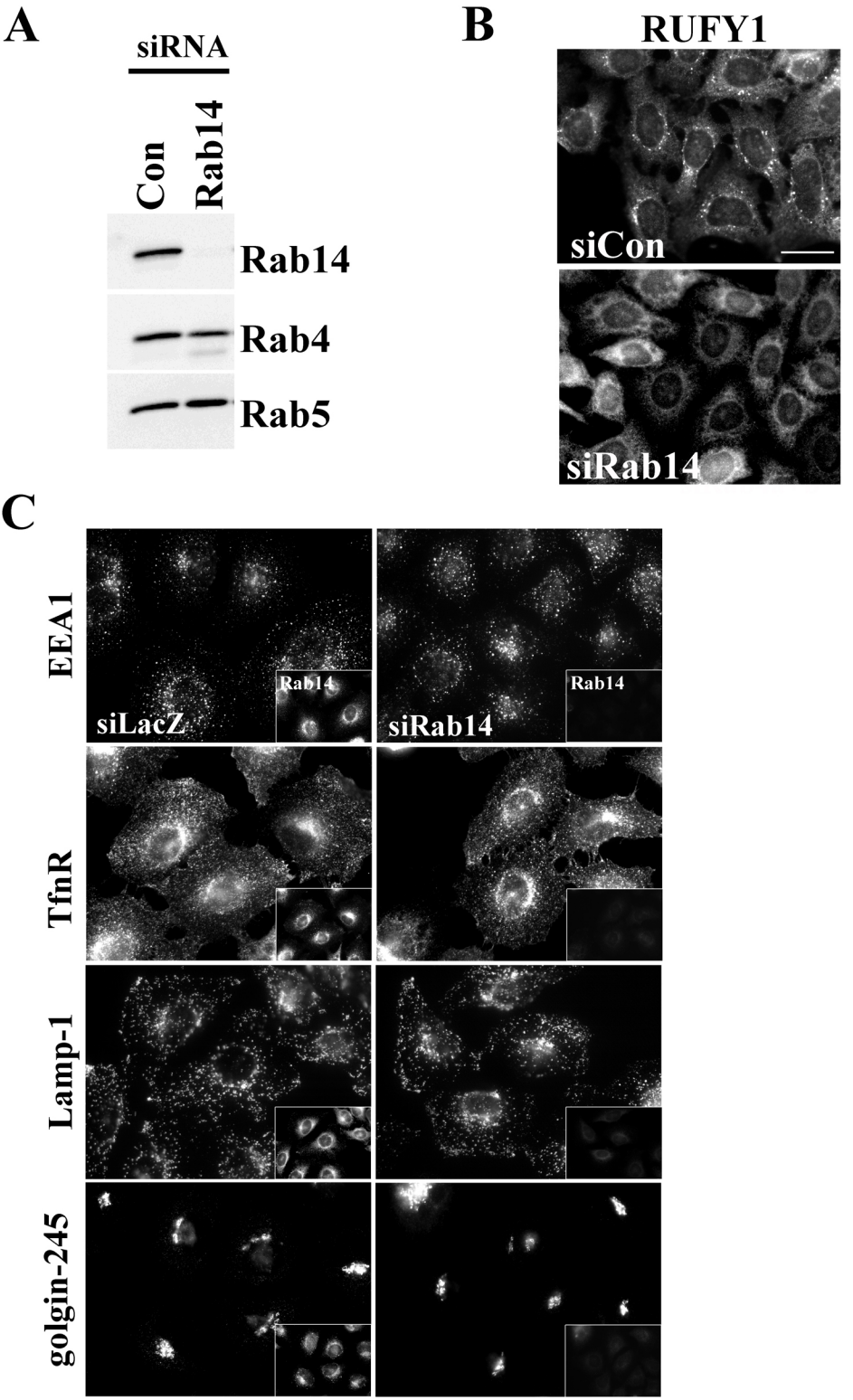
(A) HeLa cells treated with a pool of siRNAs for LacZ, Rab14, both Rab4a and Rab4b, or RUFY1 were incubated with AlexaFluor488-conjugated Tfn at 4°C for 50 min to label the cell surface with Tfn, washed with ice-cold PBS and incubated at 37°C for indicated times to allow internalization. (Insets are longer exposed images) (B) Pixel intensity of AlexaFluor488-Tfn was estimated at the indicated time. The values are the mean ± SD of 50-100 cells at each time point. The graph is a representative of two independent experiments. Bar, 20 μm.



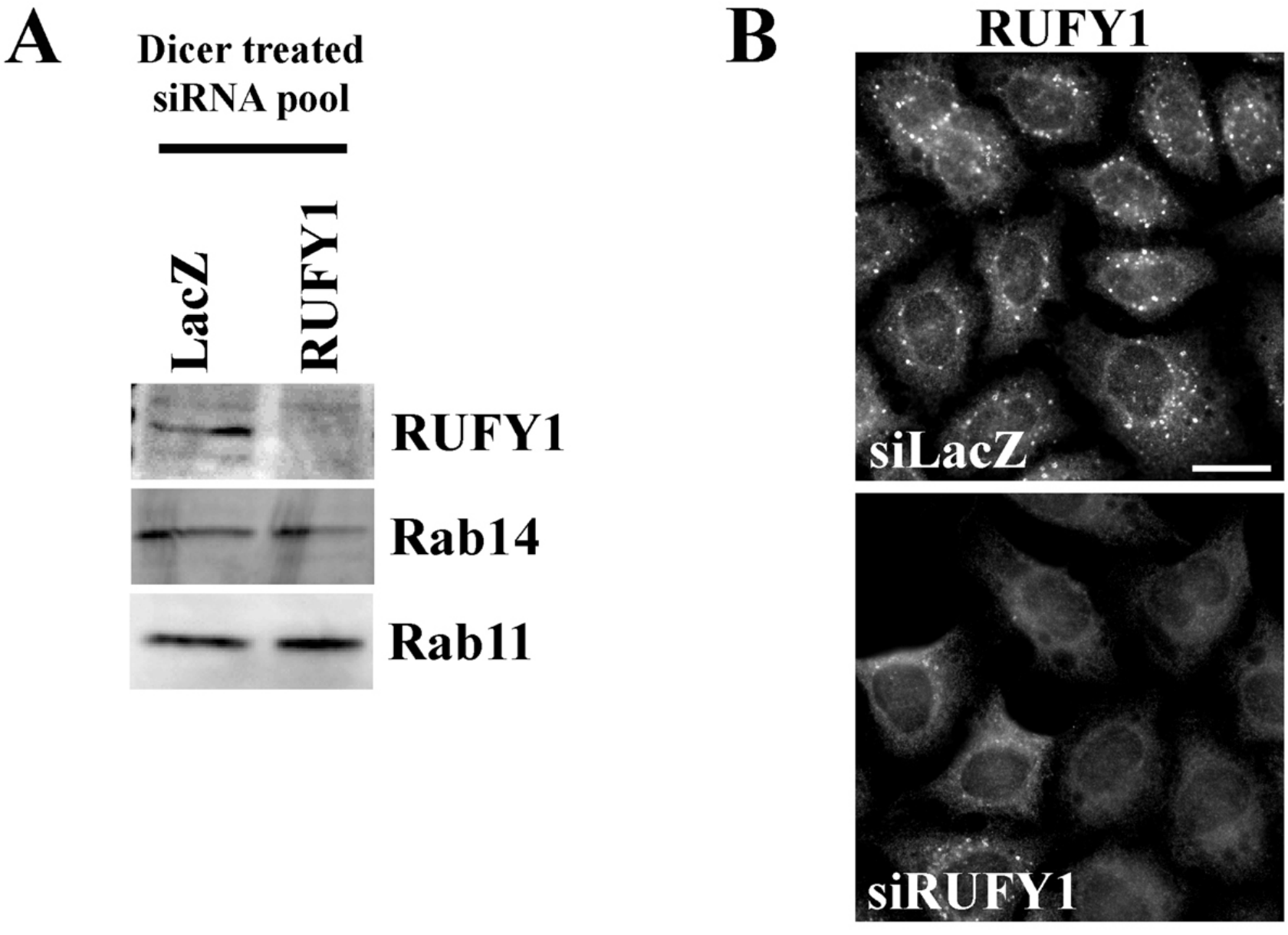
Supplementary Figure S1 (Yamamoto et al.)



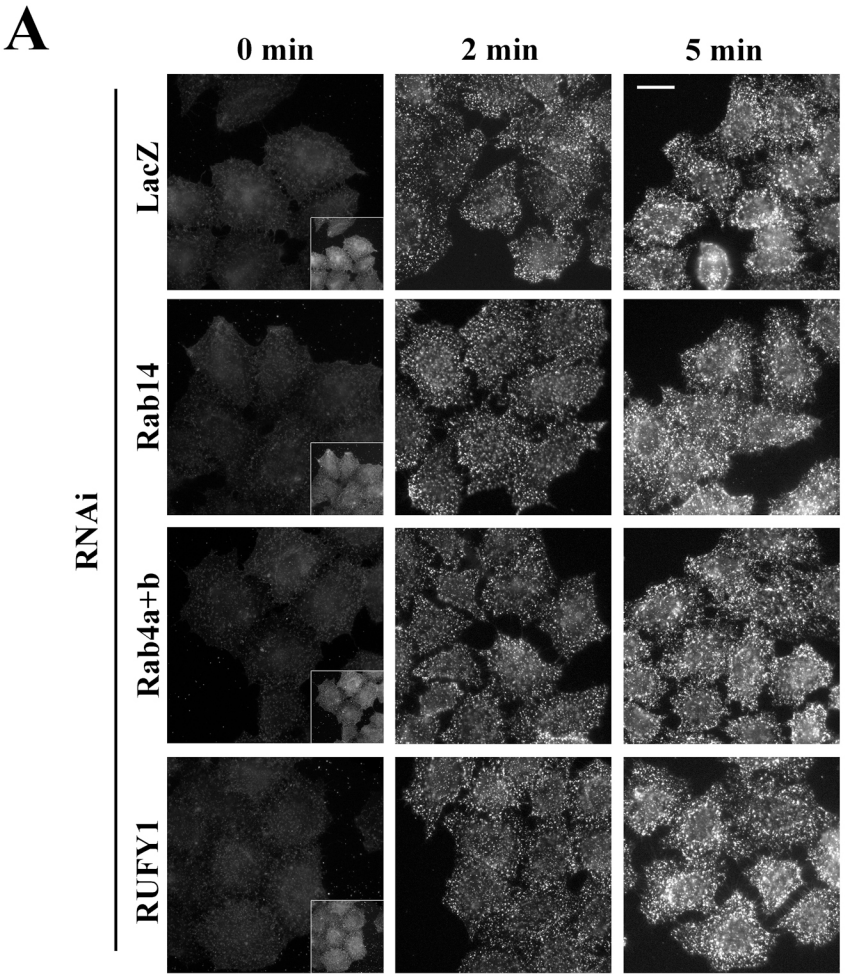
Supplementary Figure S2 (Yamamoto et al.)

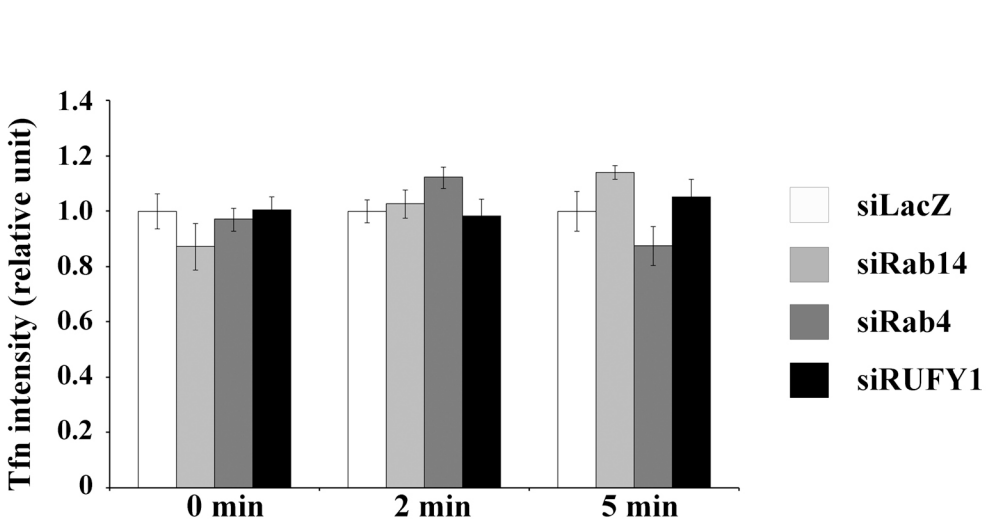


Supplementary Figure S3A, B and C (Yamamoto et al.)



Supplementary figure S4 (Yamamoto et al.)





Supplementary Figure S5A and B (Yamamoto et al.)