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Supplementary Materials for

Rab7-harboring vesicles are carriers of the transferrin receptor through the biosynthetic secretory pathway

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The PDF file includes:

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Other Supplementary Material for this manuscript includes the following:

(available at advances.sciencemag.org/cgi/content/full/7/2/eaba7803/DC1)

Tables S1 to S3
Movies S1 to S5

Supplemental information

Supplemental Figures

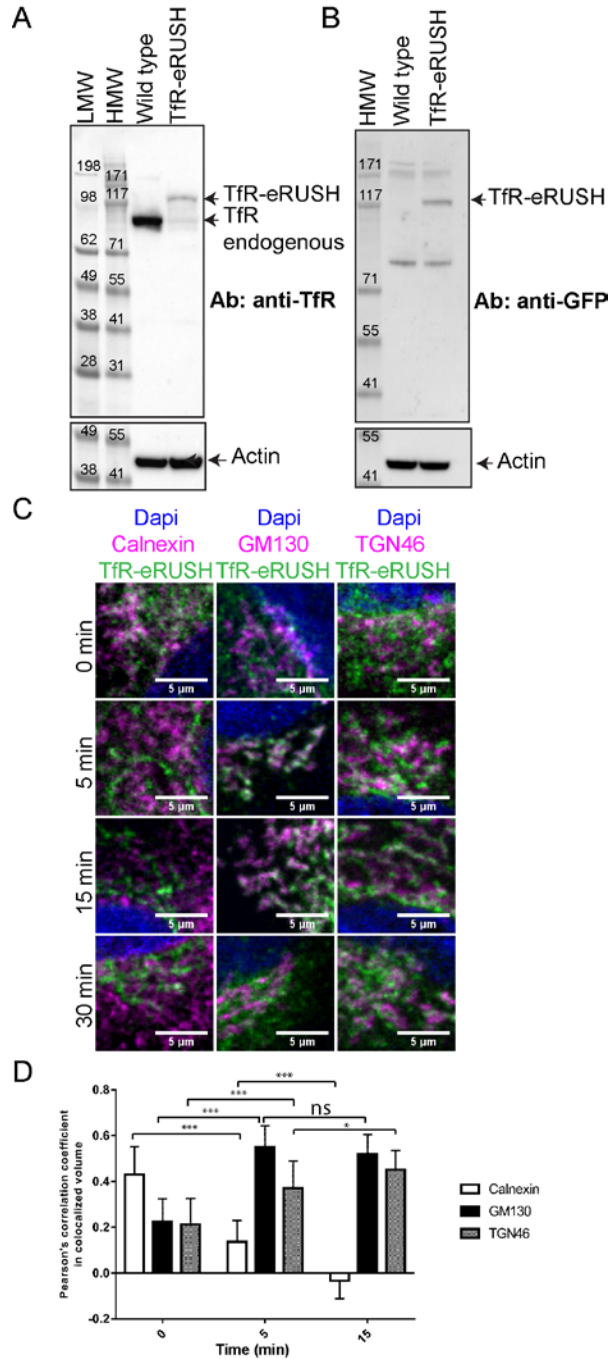


Fig. S1. Characterization and distribution of TfR-eRUSH over time post-biotin addition. (A-B) The complete western blot from Figure 1C represents TfR expression in edited cells (TfR-eRUSH) compared to non-edited cells (wild type). Two molecular weight markers were used LMW (low molecular weight) and HMW (high molecular weight) to confirm TfR molecular size. Actin was used as a loading control. Anti-GFP was used in (A) and Anti-TfR in (B). (C) TfR-eRUSH cells were incubated for indicated times with biotin. The snapshots of cropped merged images from a single plane show TfR-eRUSH (green), Dapi staining (blue) and the indicated organelle marker (magenta). (D) Quantification of TfR-eRUSH colocalization with calnexin, GM130 or TGN46 at indicated times post-biotin addition. The Pearson's correlation coefficient in the total volume of the cell was measured using the Imaris software. The graph shows mean Pearson's correlation coefficient +/- SD and $n = 17-20$ cells from 2 independent experiments. Statistics were measured using unpaired t-test. * p value < 0.05 , ** p value < 0.01 , *** p value < 0.001 . ns = non-significant.

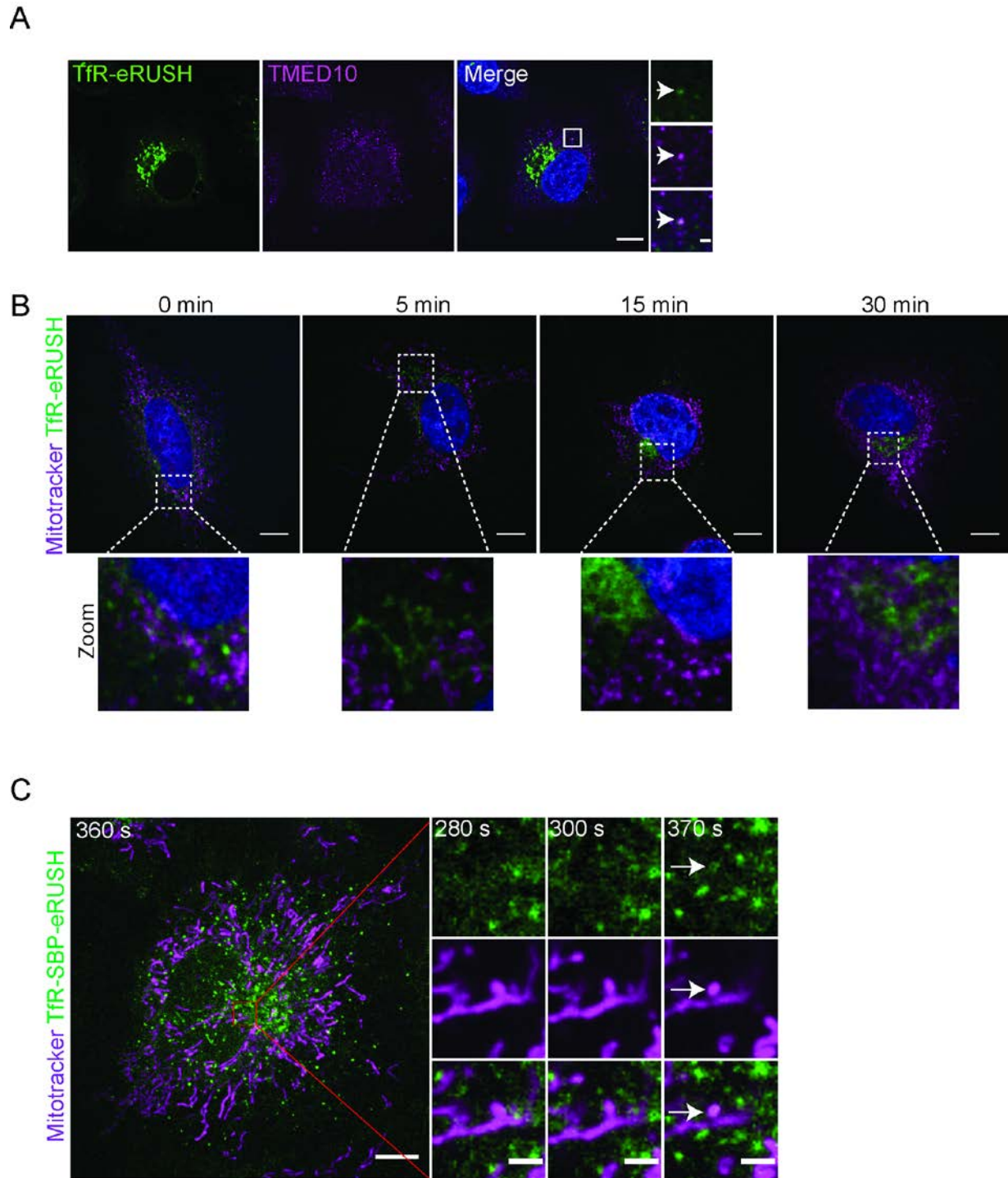


Fig. S2. TfR-eRUSH co-distributes with TMED10 but does not transit through mitochondria. (A) Representative images from a single z-stack indicate the localization of TfR-eRUSH relative to the endogenous TMED10 protein. TfR-eRUSH cells were treated for 12 min with biotin and images were acquired with a spinning disk confocal microscope. Representative images from a single plane show TfR-eRUSH (green), TMED10 (magenta) and nucleus (blue). Scale bar = 10 μ m. Zoomed regions from white

squares indicate a vesicle with TfR-eRUSH co-distributing with TMED10. Scale bar = 1 μm . (B) TfR-eRUSH cells were incubated for indicated times with biotin and mitochondria were visualized with Mitotracker. Representative images from a single plane show TfR-eRUSH (green), mitochondria (magenta) and nucleus (blue). White dashed squares represent zoomed regions (lower panel). (C) Live imaging of TfR-eRUSH cells incubated with MitoTracker and biotin was performed at 30 s per frame using a spinning disk confocal microscope. An isolated event of TfR-mitochondria colocalization was observed 6 min post-biotin addition. The red square represented zoomed regions (left panel).

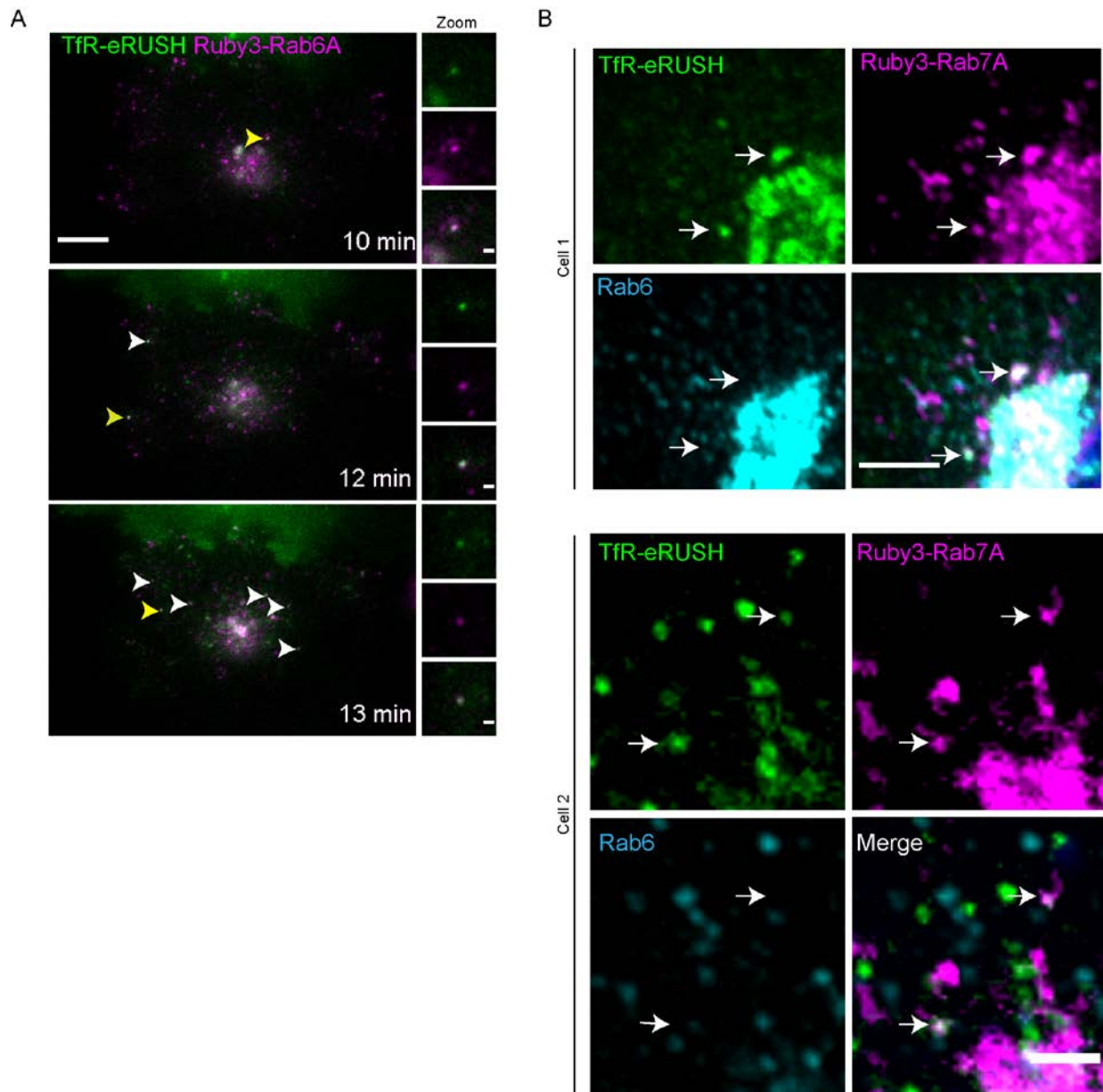
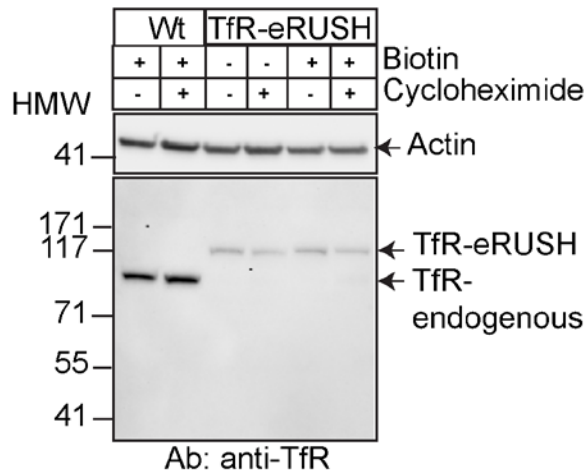


Fig. S3. Rab7A vesicles transporting TfR-eRUSH does not contain Rab6A.

TfR-eRUSH cells transfected with Ruby3-Rab6A were imaged by TIRF microscopy 24 h post-transfection. Representative images were extracted at 10, 12 and 13 min post-biotin addition (scale bar = 10 μm). The white arrows show the Ruby3-Rab6A vesicles co-distributing with TfR-eRUSH. The yellow arrows indicate the zoomed regions of a Ruby3-Rab6A vesicle (magenta) co-distributing with TfR-eRUSH (green). Scale bar = 1 μm . (B) TfR-eRUSH cells were transfected with Ruby3-Rab7A. Biotin was added for 12 min and images were taken with the spinning disk confocal microscope. Two cells were represented as images from a single z-stack. The staining indicates TfR-eRUSH (green), Ruby3-Rab7A (magenta), endogenous Rab6 (cyan), and the merge. Note that Rab7A vesicles contained TfR-eRUSH but no Rab6 (white arrow). Scale bars = 5 μm .

A



B

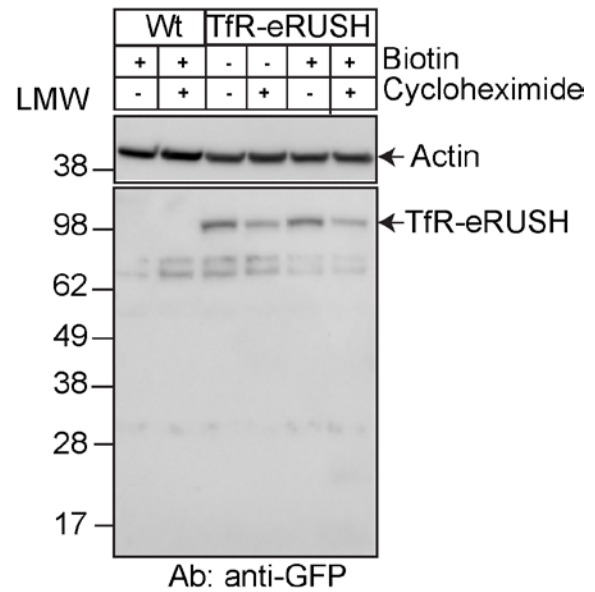
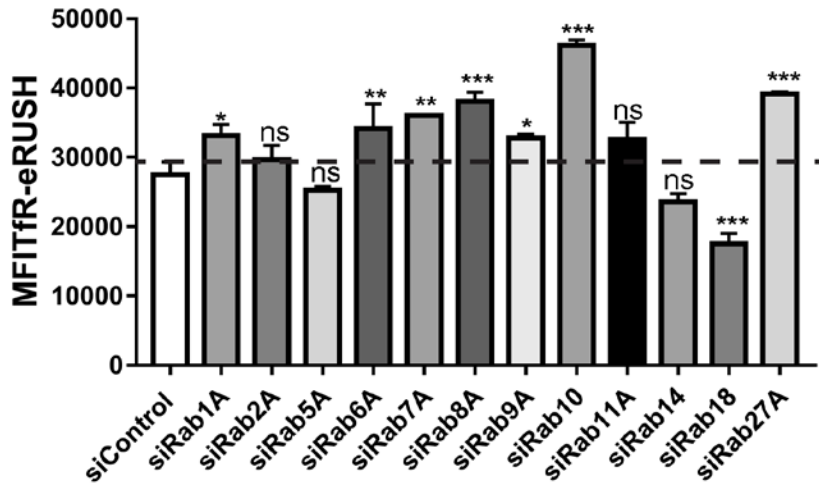


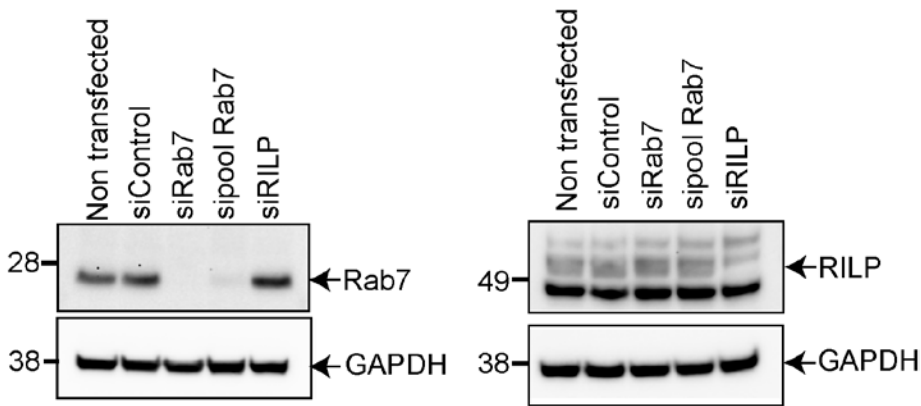
Fig. S4. TfR-eRUSH is not degraded following biotin addition.

(A-B) Western blots representing the absence of degradation of TfR-eRUSH in presence of cycloheximide. Wild type and TfR-eRUSH cells were treated for 4 h with or without 50 $\mu\text{g}/\text{ml}$ of cycloheximide and subsequently treated for 30 min with biotin. Actin was used as a loading control. Anti-TfR antibodies were used for visualization and HMW protein marker was used for molecular weight estimation in (A). Anti-GFP antibodies were used for visualization and LMW protein marker was used for molecular weight estimation in (B).

A



B



C

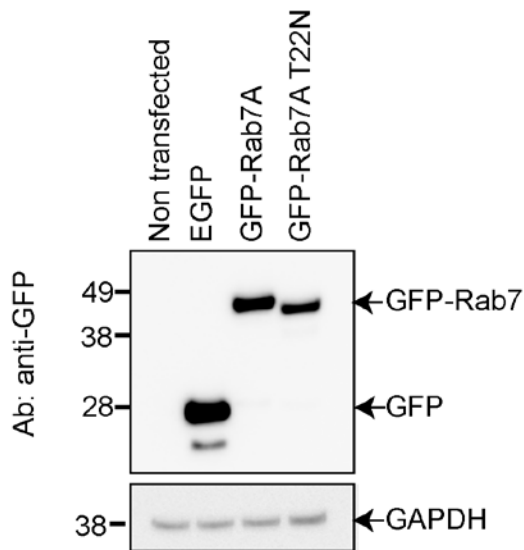


Fig. S5. siRNA screen carried out on TfR-eRUSH cells

(A) Effect of Rab silencing on TfR-eRUSH expression. TfR-eRUSH cells were treated with siRNA sequences targeting 12 different Rab mRNAs and a non-targeting siRNA control. After 48 h post-transfection, the amount of total TfR-eRUSH at time 0 min was monitored by flow cytometry using EGFP fluorescence intensity. Anova test was run to assess for significance (* p value < 0.05, ** p value < 0.01, *** p value < 0.001). (B) Western blot indicating the amount of Rab7 and RILP protein in TfR-eRUSH cells following siRNA Rab7, siRNA pool Rab7 or siRNA RILP depletion. GAPDH is used as a loading control. (C) Western blot indicating the level of expression of GFP, GFP-Rab7 and GFP-Rab7 T22N, transfected for 24h in TfR-eRUSH cells. GAPDH was used as a loading control. Anti-GFP antibody was used for visualization.

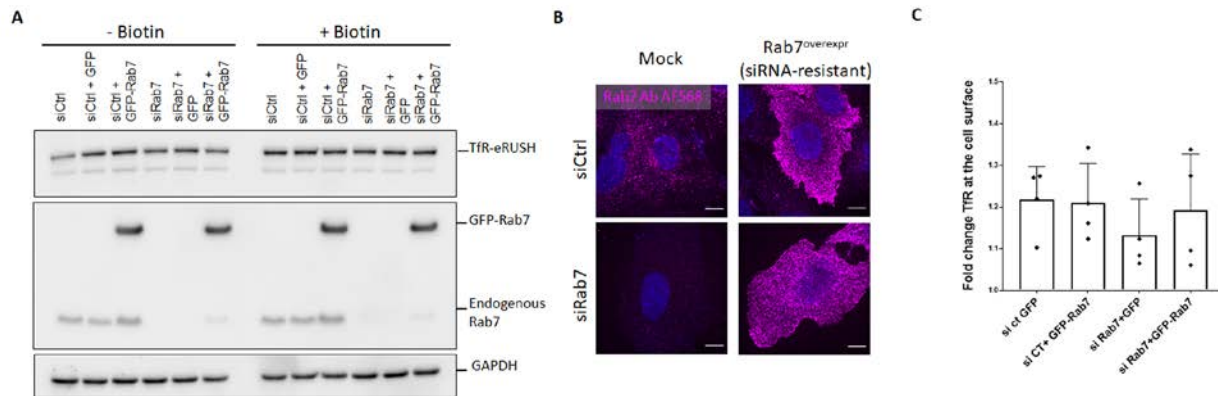


Fig. S6. Rab7 rescue experiment.

TfR-eRUSH cells were incubated in presence of avidin and transfected with control siRNA (siCtrl) or Rab7A siRNA (siRab7) for 24 h. To carry out rescue experiments, cells were further transfected with a GFP or a GFP-Rab7 siRNA-resistant construct for an additional 24 h. the next day, TfR-eRUSH cells were incubated with 40 μ M of biotin for 30 min to induce TfR-eRUSH release from the ER. (A) Western blotting analyses were carried out to observe Rab7A depletion and expression of GFP-Rab7A siRNA-resistant construct. Anti-Rab7 antibody was used to visualize GFP-Rab7A and endogenous Rab7 proteins, anti-TfR antibody was used for TfR-eRUSH and anti-GAPDH was used as a loading control. (B) Representative confocal images showing that GFP-Rab7A is mislocalized in cells treated with control siRNA or Rab7A siRNA. Rabbit anti-Rab7 antibody and anti-rabbit Alexa 647 antibody were used for visualization (magenta), Dapi (blue, nuclear staining). Scale bar = 10 μ m. (C) Flow cytometry analysis as in Figure 5A-C of the amount of Tf-A647 bound at the surface of TfR-eRUSH cells at 15 min post-biotin addition. Data shown are n = 4 individual experiments performed in duplicates (10,000 cells per conditions, +/- SD).

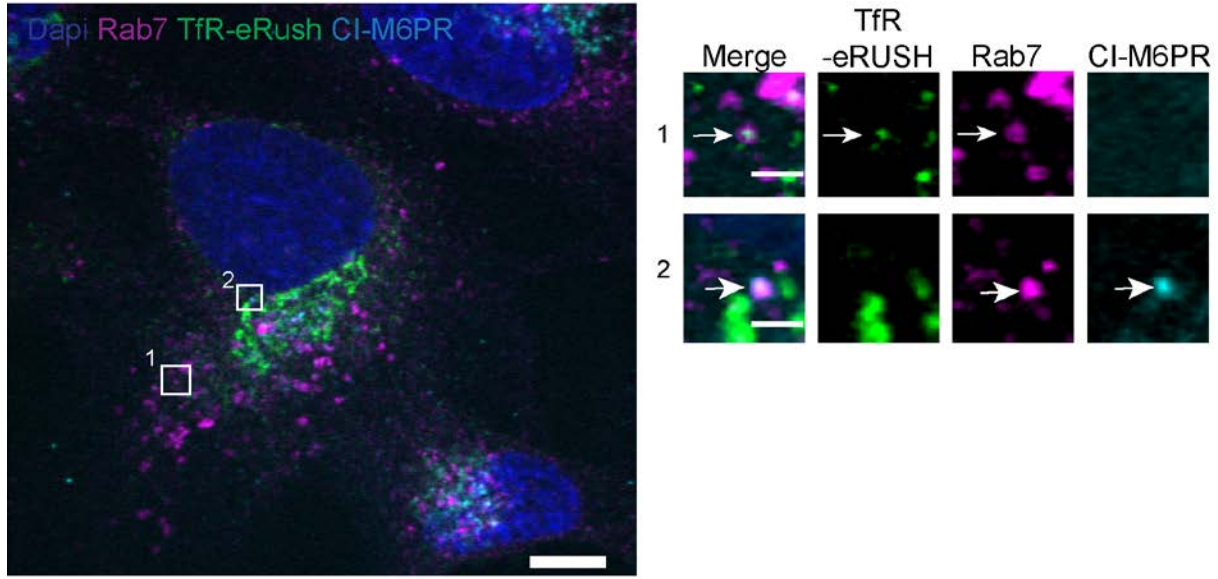


Fig. S7. Nature of the TfR-Rab7 vesicles

TfR-eRUSH distribution relative to CI-M6PR after 10 min of biotin incubation. Images were acquired with spinning disk confocal microscopy and represented as a single z-plan. White squares indicated zoomed region with single staining and the merge. Note that TfR-eRUSH vesicles labeled with Rab7 (magenta) do not contain CI-M6PR (cyan) (square 1) while CI-M6PR can be localized in Rab7⁺ vesicles (square 2).

Supplemental Tables

Table S1. Pathway enrichment analysis of proteins binding to TfR-eRUSH membranes at T0-T15.

LC-MS/MS identification of the proteins associated to TfR-eRUSH membranes enriched > 1.5-fold at T15 compared to T0 and their associated ontology pathways.

Table S2. Analysis of the Rab family members identified by mass spectrometry.

LC-MS/MS identification of the proteins from the Rab GTPase family associated to TfR-eRUSH membranes. Fold change is shown for all detected Rabs. The red Rabs indicate that they are significantly enriched at T15 compared to T0.

Table S3. List of the target sequences used in the siRNA screen.

Oligonucleotides contained in the custom-made siGenome Smart pool cherry-pick library and used for the siRNA screen.

Supplemental Movies

Movie S1. Transport of neosynthesized TfR toward the PM.

TfR-eRUSH cells were incubated with biotin and imaged using 3D spinning disk confocal microscopy. The images correspond to a z-stack spaced by 0.6 μm acquired every 30 s for 40 min. Three-dimensional reconstruction was performed using Imaris.

Movie S2. TfR-eRUSH trafficking is mostly independent of the mitochondrial distribution.

TfR-eRUSH cells were incubated with biotin and MitoTracker then imaged using 3D spinning disk confocal microscopy. The movie corresponds to a single plane from a z-stack spaced by 0.5 μm acquired every 10 s for 10 min. TfR-eRUSH is shown in green and MitoTracker in magenta. Scale bar = 2 μm .

Movie S3. A subset of neosynthesized TfR-eRUSH traffics through Rab7-positive vesicles.

TfR-eRUSH cells were transfected with Ruby3-Rab7A for 24 h and imaged in the presence of biotin using 3D spinning disk confocal microscopy. The movie corresponds to a single plane from a z-stack spaced by 0.3 μm acquired every 10 s from 7 min to 24 min. TfR-eRUSH is shown in green and Ruby3-Rab7A in magenta. Scale bar = 2 μm .

Movie S4. TfR-eRUSH vesicles transiently interact with Rab7A.

TfR-eRUSH cells were transfected with Ruby3-Rab7A for 24 h and imaged in the presence of biotin using TIRF microscopy. The movie represents a cropped vesicle starting from 706 s to 748 s post-biotin addition. A single evanescent field is acquired in TIRF mode every 2 s. TfR-eRUSH is shown in green and Ruby3-Rab7A in magenta. Scale bar = 2 μm .

Movie S5. TfR-eRUSH vesicles interact with Rab6A.

TfR-eRUSH cells were transfected with Ruby3-Rab6A for 24 h and imaged in the presence of biotin using TIRF microscopy. The movie represents a cropped vesicle

starting from 838 s to 878 s post-biotin addition. A single evanescent field is acquired in TIRF mode every 2 s. TfR-eRUSH is shown in green and Ruby3-Rab6A in magenta. Scale bar = 2 μm .