Supplemental Materials

Molecular Biology of the Cell

Pei et al.

Supplementary information for:

Interferon-gamma-inducible Rab20 regulates endosomal morphology and EGFR degradation in macrophages

Supplementary Figure legends

Figure S1- Characterization of the Rab20 antibody for immunofluorescence

A. Validation the specificity of Rab20 antibody with its immunizing peptide. On the left panel, endogenous Rab20 (Red) in RAW264.7 macrophages was detected with a Rab20 antibody (GeneTex, USA). On the right panel, endogenous Rab20 in RAW264.7 macrophages detected with Rab20 antibody together with the Rab20 immunizing peptide. **B.** Immunostaining with Rab20 antibody in RAW264.7 macrophages expressing EGFP-Rab20. Nuclei were stained with Hoechst 33258 and are shown in blue. The white arrows indicate EGFP-Rab20 positive vacuoles that are also stained by Rab20 antibody. Scale bar: 10 μm.

Figure S2- Localization of Rab20 in resting macrophages

RAW264.7 macrophages were double-stained for Rab20 and the *cis*-Golgi network marker GM130 (A), the *trans*-Golgi network marker Syntaxin-6 (B), the early endosomal marker EEA-1 (C) or the late endosomal marker LAMP-2 (D). Nuclei were stained with Hoechst 33258 and are shown in blue. Insets show regions of interest indicated by the white rectangles. Scale bar: 10 μm.

Figure S3-Acidification of EGFP-Rab20 enlarged endosomes

RAW264.7 macrophages were transfected with EGFP-Rab20WT and after overnight incubation, Lysotracker Red DND-99 (50 nM) was added into the culture. Then time-lapse images were acquired to monitor the dynamics of LysoTracker Red fluorescence association to Rab20-positive/negative endosomes.

Figure S4- Levels of expression of EGFP-Rab20 and Rab20 in macrophages

RAW264264.7 macrophages expressing EGFP or EGF-.Rab20WT were stimulated with 5 ng/ml of IFN-γ for 24 hours. Both endogenous Rab20 and

overexpressed EGFP-Rab20 levels were compared by Western blotting and quantified relative to GAPDH levels.

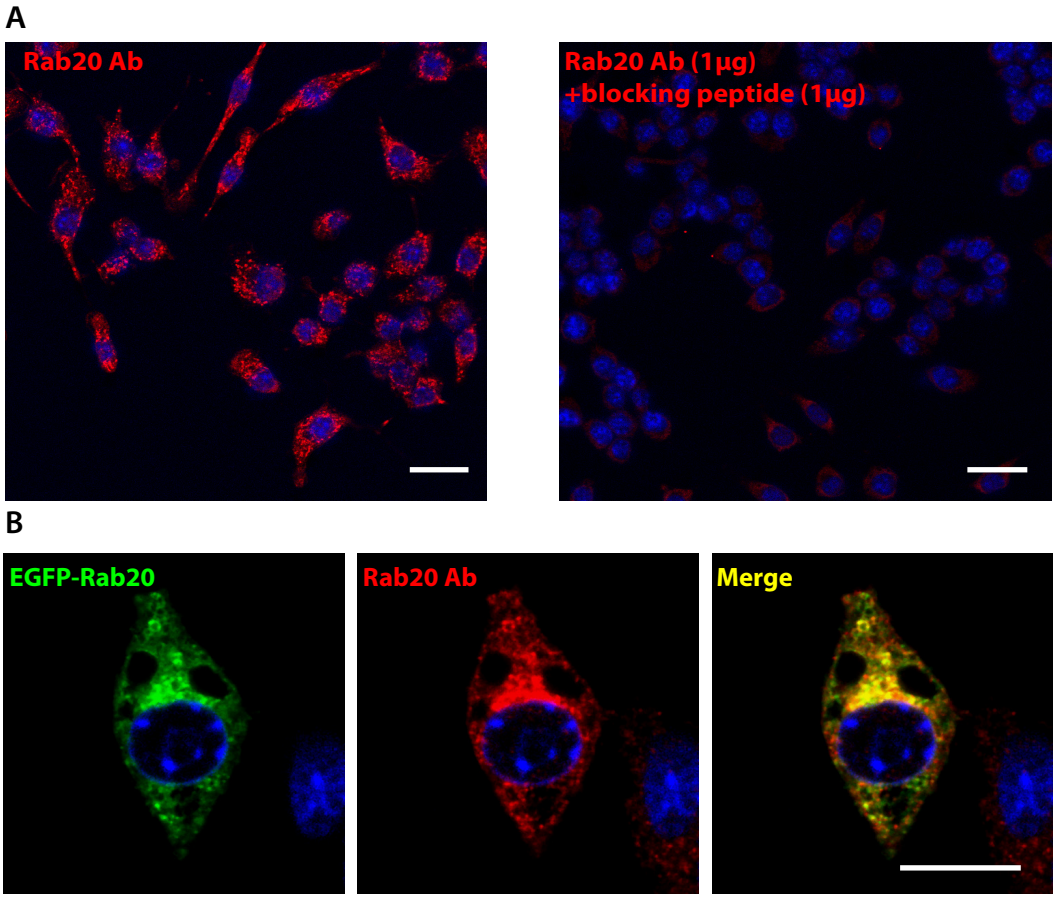
Figure S5- LAMP-1 distribution in resting and IFN-γ activated macrophages at the ultrastructural level

RAW264.7 macrophages were treated without (A) or with IFN-γ (B-D), preloaded with BSA-gold 5 nm for 1 hour and finally processed for cryosectioning. LAMP-1 was detected with a rat anti-LAMP-1 antibody followed by protein A-gold (10 nm) labeling. Panels labeled with roman numbers show the regions of interest indicated by white rectangles. Black arrows indicate LAMP-1 labeling on BSA-gold 5 nm pre-loaded organelles. Scale bar: 500 nm.

Figure S6- Rab20DN expression does not affect endocytic or macropinocytic uptake

A. Transferrin endocytosis in macrophages expressing EGFP, EGFP-Rab20 or EGFP-Rab20DN. RAW264.7 macrophages were transfected with EGFP, EGFP-Rab20 or EGFP-Rab20DN (Green) and 25 µg/ml Alexa Fluor® 647-conjugated transferrin (Red) was added for 15 min. The cells were further fixed for immunofluorescence. Nuclei were stained with Hoechst 33258 and are shown in blue. Insets show regions of interest indicated by the white rectangle. Scale bar: 10 µm. **B.** Quantification of the amount of internalized transferrin in macrophages expressing EGFP, EGFP-Rab20 or EGFP-Rab20DN. For EGFP and EGFP-Rab20, at least 50 cells were analyzed. For EGFP-Rab20DN, at least 20 cells were analyzed. Data show mean ± SEM of one representative experiment from three independent experiments. **C.** Dextran 70kDa endocytosis in macrophages expressing EGFP, EGFP-Rab20 or EGFP-Rab20DN. RAW264.7 macrophages were transfected with EGFP, EGFP-Rab20 or EGFP-Rab20DN (Green) and 100 µg/ml Texas Red-conjugated Dextran 70kDa (Red) were added for 1 hour. The cells were further fixed for immunofluorescence. Nuclei were stained with Hoechst 33258 and are shown in blue. Scale bar: 10 µm. D. Quantification of the amount of internalized Dextran 70kDa in macrophages expressing EGFP, EGFP-Rab20 or EGFP-Rab20DN. For each group, at least 50 cells were analyzed. Data show mean ± SEM of one representative experiment from three independent experiments.

Figure S1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+blocking peptide (1μg)
B
EGFP-Rab20
Rab20 Ab
Merge

Figure S2

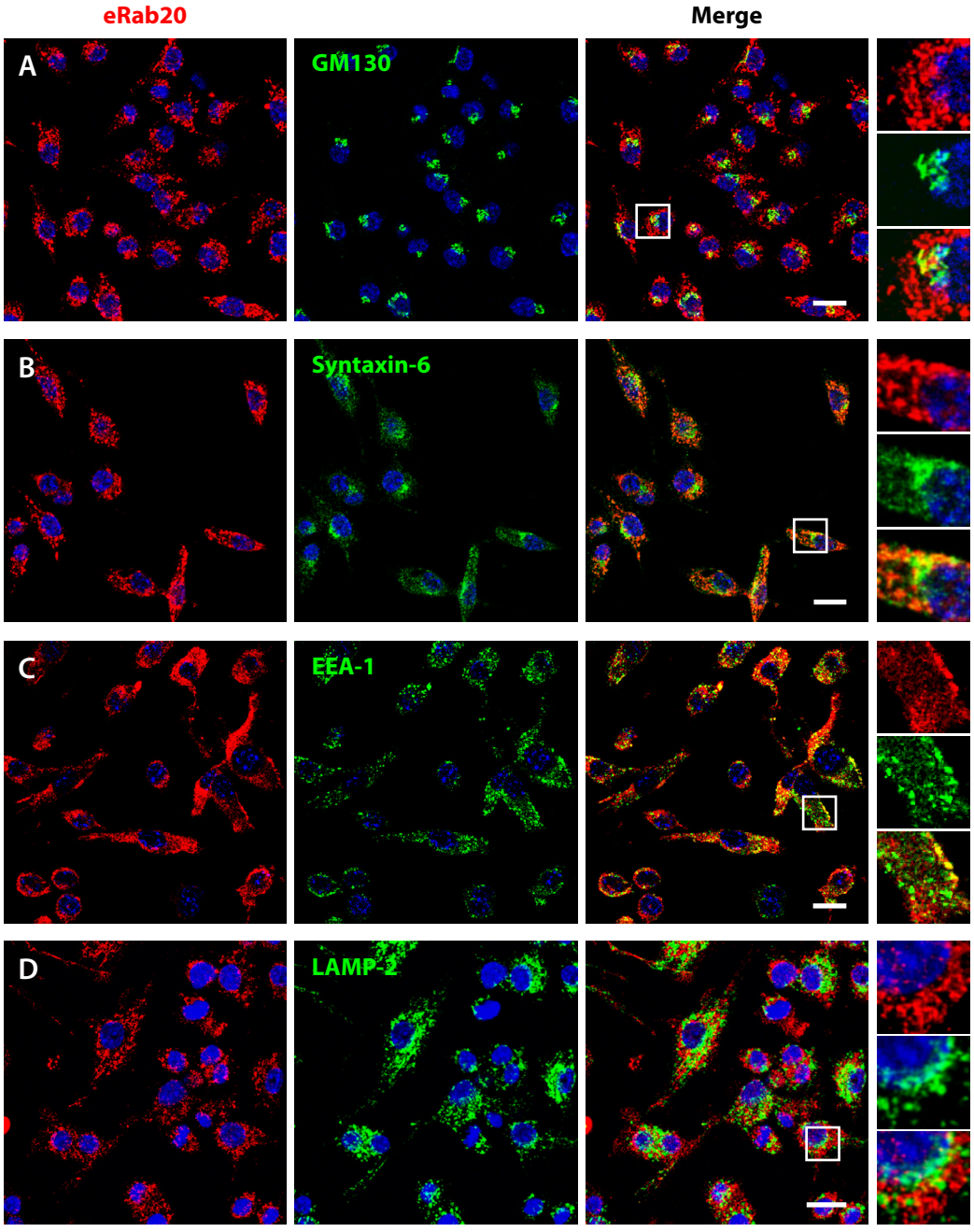
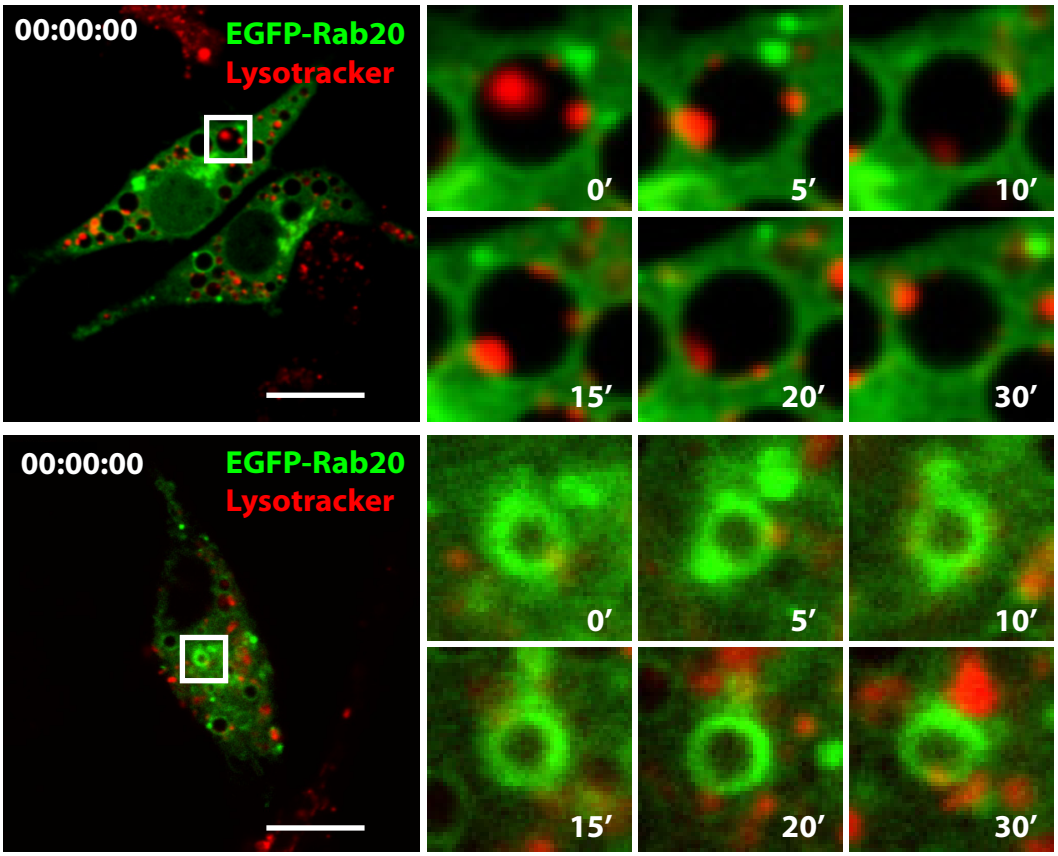


Figure S3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5′
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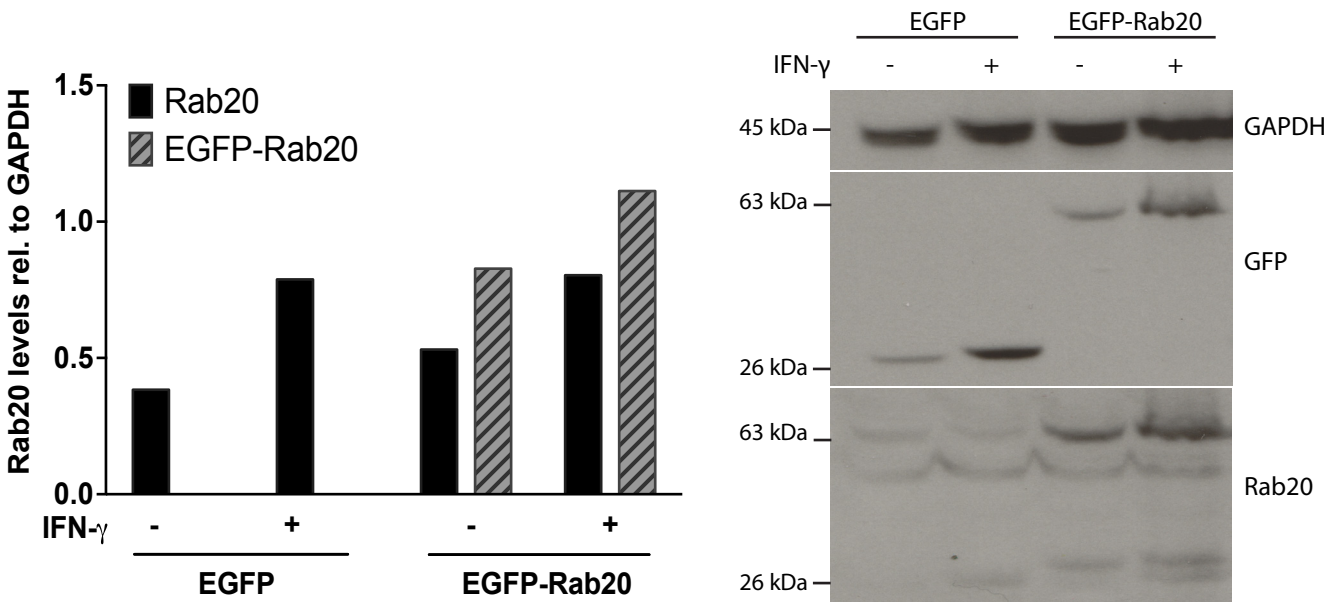
Lysotracker
0′
5′
10′
15′
20′
30′

Figure S 4


Rab20 levels rel. to GAPDH
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1.0
0.5
0.0
Rab20
EGFP-Rab20
IFN-γ
-
+
-
+
EGFP
EGFP-Rab20
EGFP
EGFP-Rab20
IFN-γ
-
+
-
+
45 kDa
63 kDa
26 kDa
63 kDa
26 kDa
GAPDH
GFP
Rab20

Figure S5

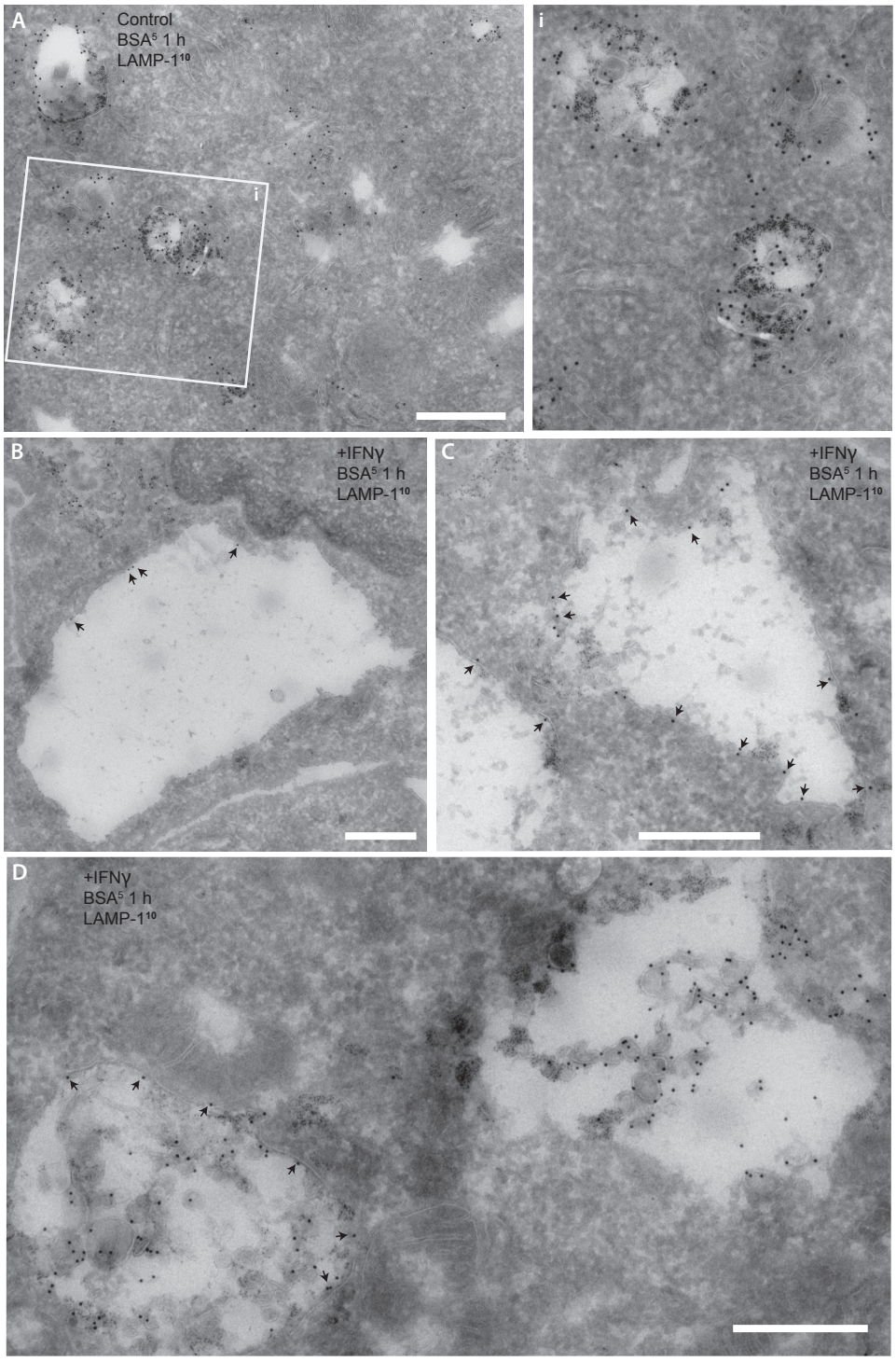
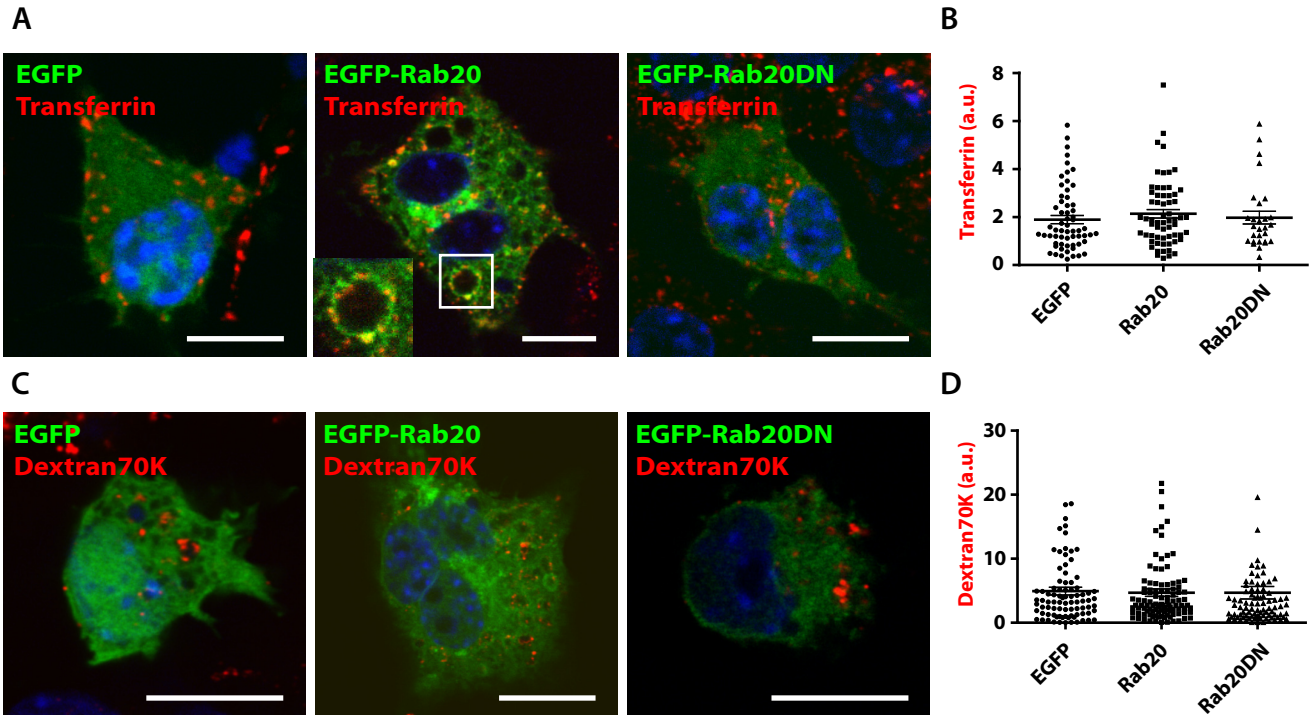

A
Control
BSA5 1 h
LAMP-110
i
i
B
+IFNγ
BSA5 1 h
LAMP-110
C
+IFNγ
BSA5 1 h
LAMP-110
D
+IFNγ
BSA5 1 h
LAMP-110

Figure S6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Transferrin
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Transferrin
EGFP-Rab20DN
Transferrin
B
Transferrin (a.u.)
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0
EGFP
Rab20
Rab20DN
C
EGFP
Dextran70K
EGFP-Rab20
Dextran70K
EGFP-Rab20DN
Dextran70K
D
Dextran70K (a.u.)
30
20
10
0
EGFP
Rab20
Rab20DN