Expanded View Figures

Figure EV1. Analysis of endosomal Rab RNAi screen and the localization of Rabs to REs.

A IFM images of HeLa cells to study the localization of KIF13A-YFP to REs.

B PCR analysis of Rab-knockdown HeLa cells to measure the knockdown efficiency of each shRNA as indicated. DNA band intensities were quantified and indicated on the gels.

C Subcellular fractionation of HeLa cells to study the localization of different Rabs (red box) with respect to STX13 or AP-1.

D, E IFM analysis of HeLa cells that were transfected with different constructs as indicated.

F Live cell imaging of mCherry-Rab22A with respect to GFP-Rab9A in HeLa cells. Arrowheads point to the Rab22A-positive tubular structures. Magnified view of insets (at 0, 16, 40 s) are shown separately. Scale bars: 10 μm.

G IFM images of Rab22A sh, BLOC-1 sh and control sh HeLa cells that were transfected with indicated constructs.

H Graphs represent the measurement of corrected total cell fluorescence (CTCF) in HeLa cells of Fig 2A. n = 6–8 cells. Average CTCF values (AU: arbitrary units) and their respective fold changes (mean ± SEM) are indicated.

Data information: In (A), arrowheads and arrows point to the KIF13A-positive tubular REs and E/SEs, respectively. In (D, E, G), arrowheads point to the STX13- or KIF13A-positive tubular REs or E/SEs. Scale bars: 10 μm.

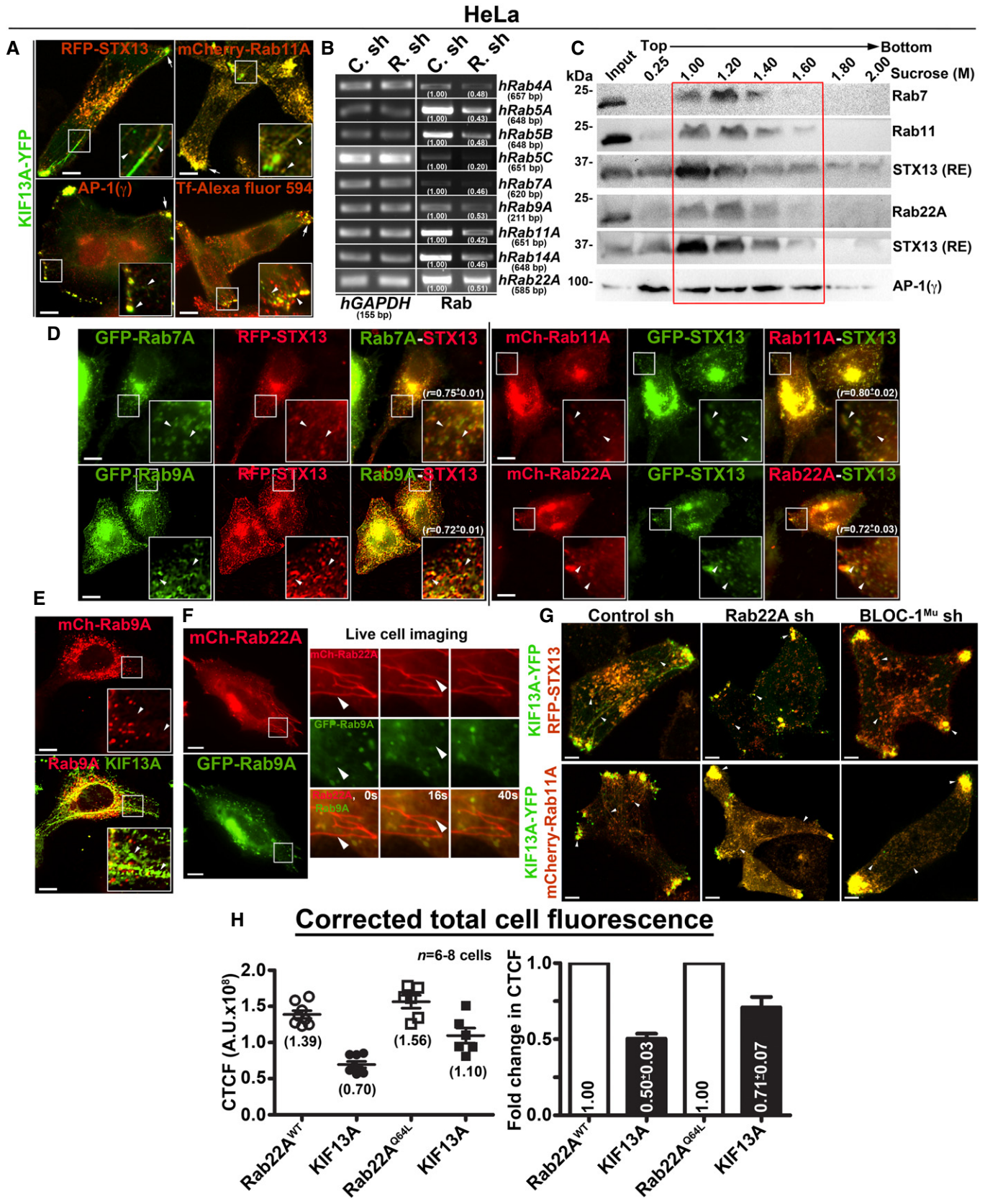

Figure EV1.

Figure EV2. Analysis of mouse melanocytes expressing different constructs of Rab22A.

A Live cell imaging of mCherry-Rab22A with respect to GFP-STX13 in wild-type melanocytes. Arrows point to Rab22A-positive buds or tubular structures arising from STX13-positive E/SEs (arrowheads). Magnified view of insets (at various seconds) is shown separately. Scale bars: 10 μm.

B IFM analysis of mCherry-Rab22A$^{WT/Q64L/S19N}$-transfected melanocytes. Arrowheads point to Rab22A localization, and arrows indicate the melanocyte pigmentation. Nuclei are stained with Hoechst 33258. The colocalization coefficient (*r*, in mean ± SEM) between two markers was indicated separately. Scale bars: 10 μm.

C, D Quantification of melanin content (C, $n = 5$) and the protein levels of TYRP1 (judged by immunoblotting in D, $n = 3$) in melanocytes that were transfected with GFP-Rab22A$^{WT/Q64L/S19N}$. In (D), graph indicates the relative levels (mean ± SEM) of TYRP1 with respect to overexpressed Rab22A. Protein band intensities were quantified and indicated on the gels. $^{**}P \leq 0.01$, $^{***}P \leq 0.001$ and ns = not significant (unpaired Student's *t*-test).

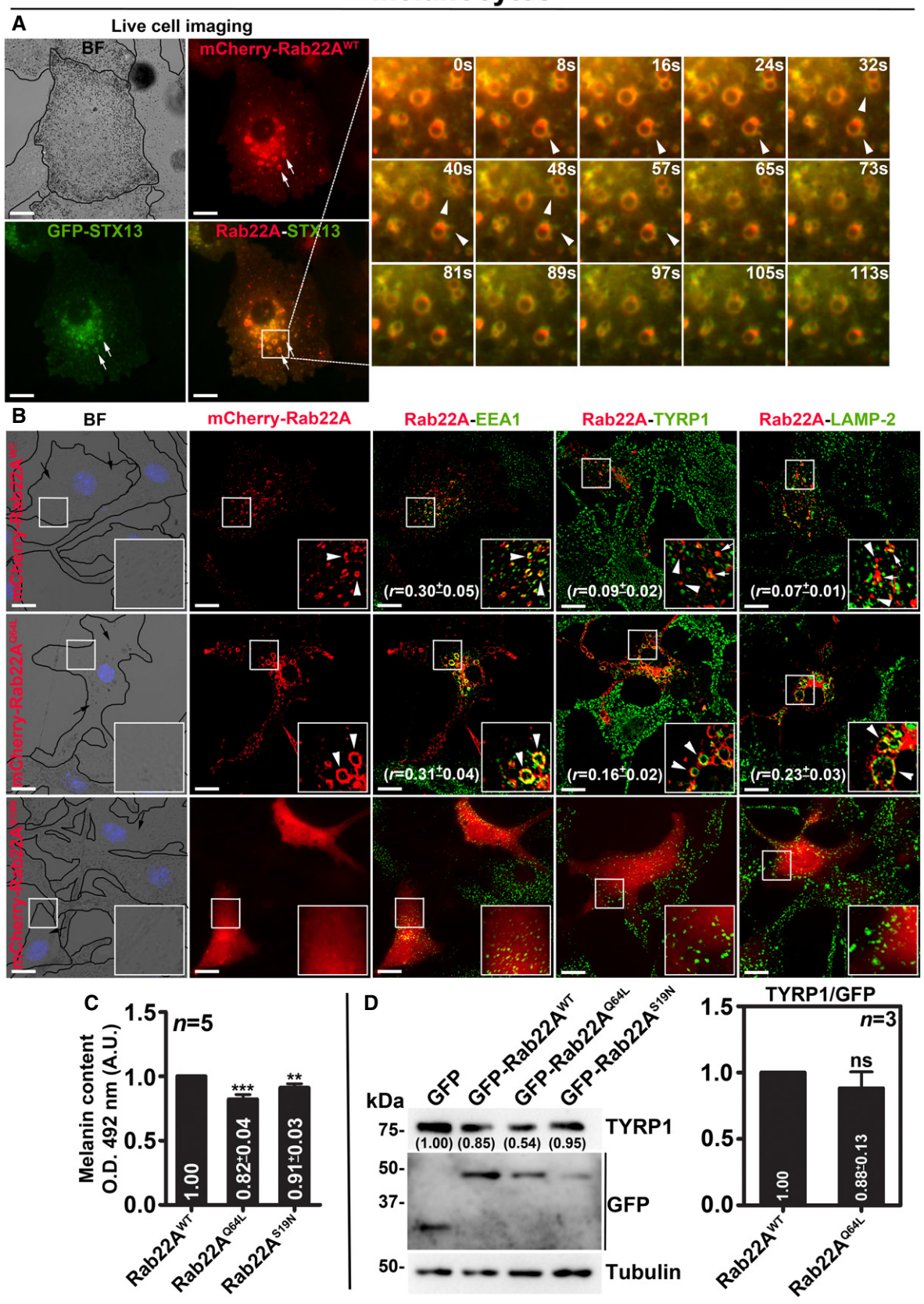

Figure EV2.

Figure EV3. Analysis of cargo recycling in control and Rab22A-, BLOC-1-, BLOC-2-knockdown HeLa cells.

A IFM images of KIF13A-YFP-transfected control and knockdown HeLa cells as indicated. Arrowheads point to the localization of KIF13A to E/SEs or REs.
B Membrane-cytosol fractionation of HeLa cell homogenate for the localization of KIF13A. *, non-specific bands.
C IFM images of control and Rab22A-, BLOC-1-knockdown HeLa cells that were stained with LAMP-2 or internalized with fluorescein–dextran.
D Cell surface levels of LAMP-1 and M6PR in control and Rab22A-, BLOC-1-knockdown HeLa cells measured using flow cytometry. Normalized mean fluorescence intensity (MFI) was calculated (mean ± SEM) and then plotted. $n = 3$.
E IFM images of HeLa cells that were subjected to Tf-Alexa Fluor 594 recycling kinetics.
F Fluorescence intensities Tf in the images of (E) were quantified and plotted (mean ± SEM). $n = 3$. n_c = total number of cells.
G Immunoblotting analysis of Tf receptor in HeLa cells as indicated. Protein band intensities were quantified and indicated on the gels.

Data information: In (A, C, E), arrows point to the localization of cytoskeletal proteins (A) or internalized dextran or lysosomes (C) or accumulation of Tf to the intracellular vesicles (E). Scale bars: 10 μm. In (D, F), $*P \leq 0.05$, $**P \leq 0.01$, $***P \leq 0.001$ and ns = not significant (unpaired Student's *t*-test).

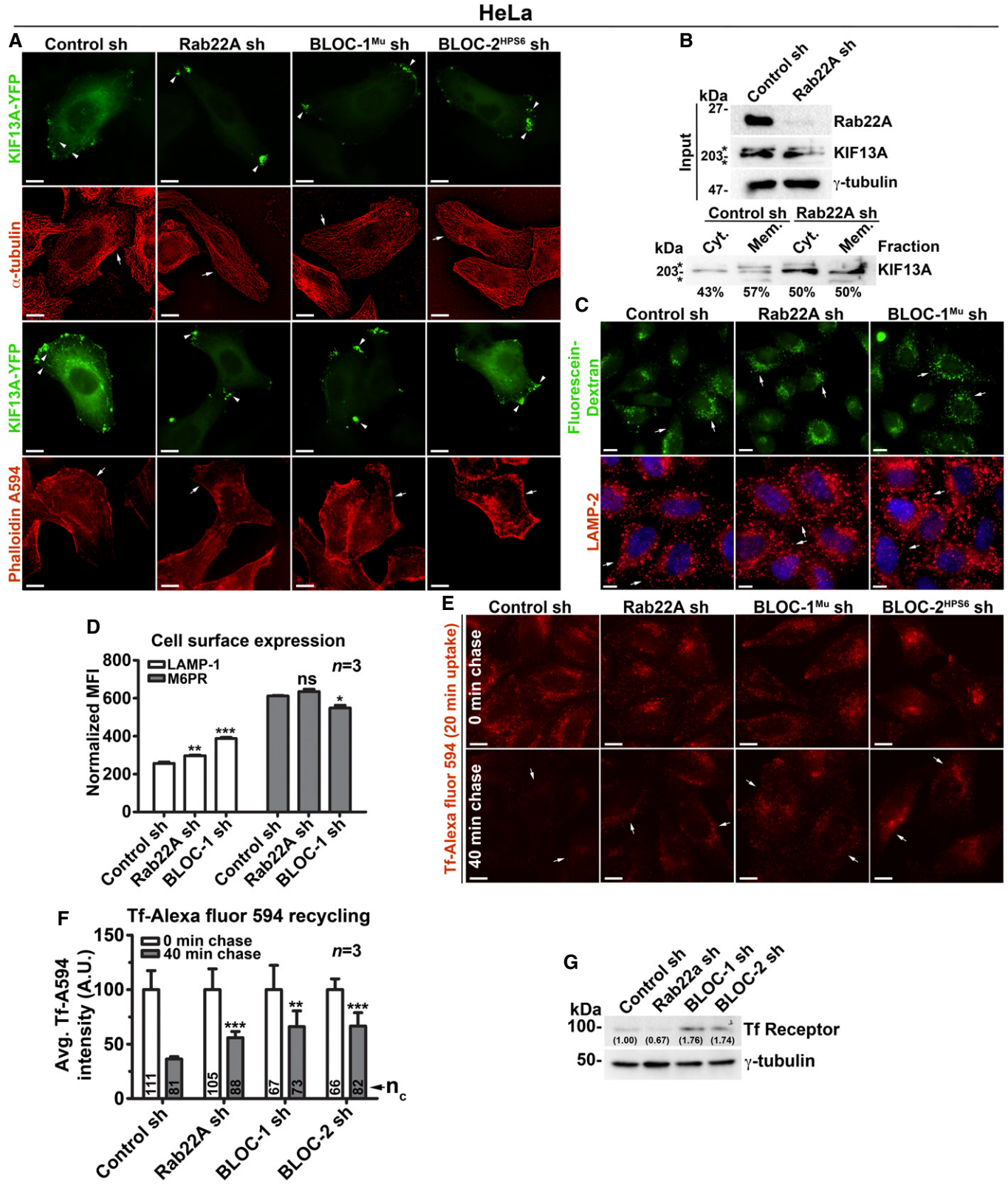
HeLa
A
Control sh
Rab22A sh
BLOC-1Mu sh
BLOC-2HPS6 sh
KIF13A-YFP
α-tubulin
KIF13A-YFP
Phalloidin A594
B
Control sh
Rab22A sh
kDa
Input
27-
203*
47-
Rab22A
KIF13A
γ-tubulin
Control sh
Rab22A sh
kDa
Cyt.
Mem.
Cyt.
Mem.
Fraction
203*
KIF13A
43%
57%
50%
50%
C
Control sh
Rab22A sh
BLOC-1Mu sh
Fluorescein-Dextran
LAMP-2
D
Cell surface expression
Normalized MFI
800
600
400
200
0
LAMP-1
M6PR
n=3
ns
*
**
***
Control sh
Rab22A sh
BLOC-1 sh
Control sh
Rab22A sh
BLOC-1 sh
E
Control sh
Rab22A sh
BLOC-1Mu sh
BLOC-2HPS6 sh
Tf-Alexa fluor 594 (20 min uptake)
0 min chase
40 min chase
F
Tf-Alexa fluor 594 recycling
Avg. Tf-A594 intensity (A.U.)
150
100
50
0
0 min chase
40 min chase
n=3
111
81
105
88
67
73
66
82
nc
Control sh
Rab22A sh
BLOC-1 sh
BLOC-2 sh
G
Control sh
Rab22a sh
BLOC-1 sh
BLOC-2 sh
kDa
100-
50-
(1.00)
(0.67)
(1.76)
(1.74)
Tf Receptor
γ-tubulin

Figure EV3.

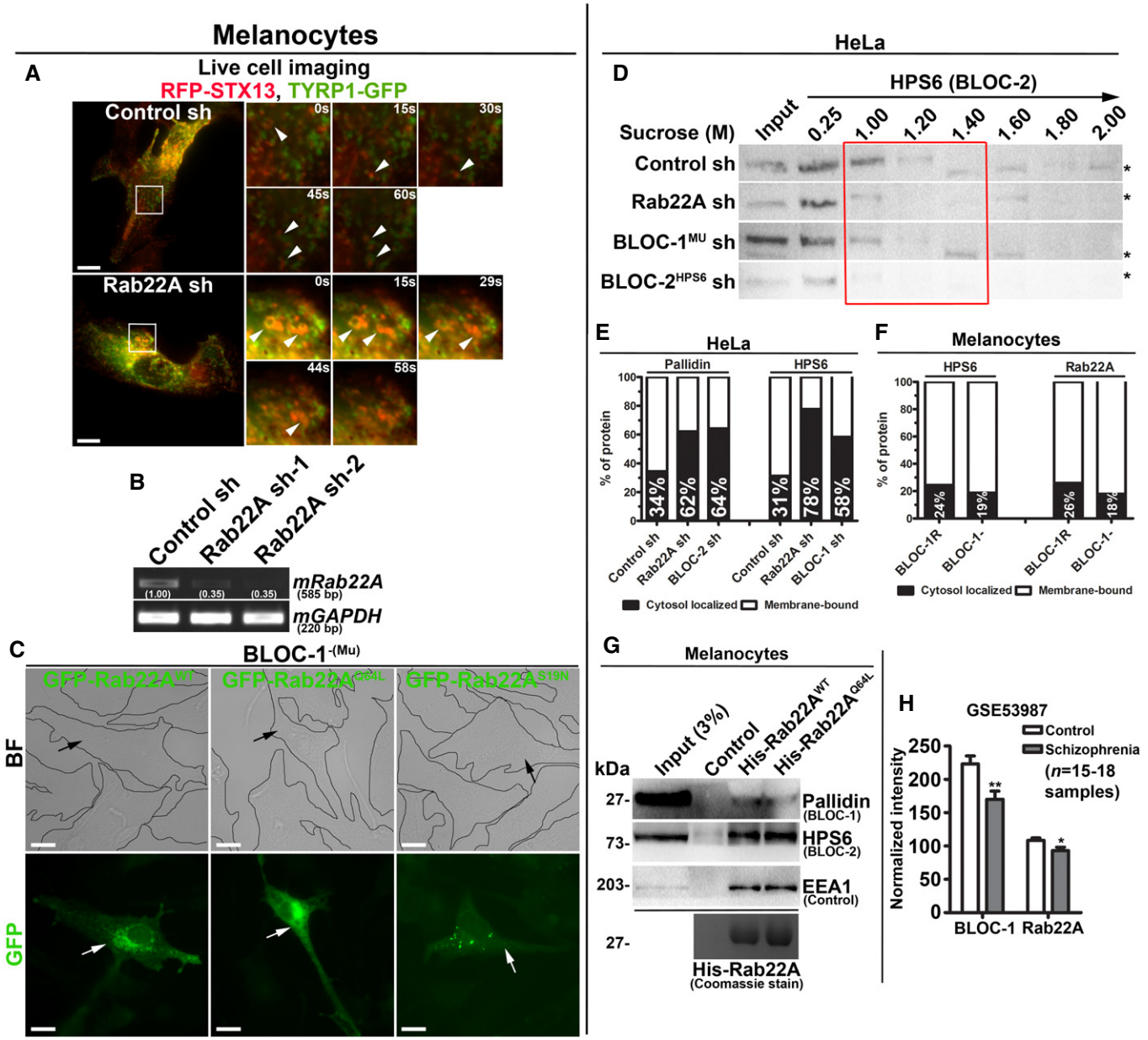

Figure EV4. Analysis of Rab22A-knockdown and BLOC-1-deficient melanocytes expressing different constructs and membrane association and interaction studies of Rab22A. Rab22A as possible susceptible gene for schizophrenia.

A Live cell imaging of TYRP1-GFP with respect to RFP-STX13 in control and Rab22A-depleted melanocytes. Arrowheads point to TYRP1-positive structures. Magnified view of insets (at various seconds) is shown separately. Scale bars: 10 μm.

B PCR analysis of Rab22A-depleted melanocytes. DNA band intensities were quantified and indicated on the gels.

C BF and IFM images of GFP-Rab22A$^{WT/Q64L/S19N}$-transfected BLOC-1-deficient melanocytes. Black and white arrows indicate the melanocyte pigmentation and GFP-Rab22A expression, respectively. Scale bars: 10 μm.

D Subcellular membrane fractionation of control and knockdown HeLa cells. The fractions were probed for HPS6 (red box).

E, F Graphs represent the percentage of membrane association of pallidin or HPS6 or Rab22A in the indicated cell types and derived from Fig 5B and C, respectively. $n = 1$.

G Pull-down of His-Rab22A$^{WT/Q64L}$ using melanocyte lysate. The bead-bound His-Rab22A was shown as coomassie-stained gel separately.

H Transcript levels of Rab22A and pallidin (BLOC-1) in control and schizophrenia patients. Analysed from data set GSE53987 and then plotted (mean ± SEM). n = 15–18 samples. *$P \leq 0.05$ and **$P \leq 0.01$ (unpaired Student's t-test).