Expanded View Figures

Figure EV1. Effects of RNAi-mediated knock-down of Rho family members on cell morphological features and migration.

A–D Knock-down efficiency controls for Fig 1. (A, B) T-ALL cells; (C) Reh cells; or (D) A375M2 DS cells transfected with Ctrl siRNA or siRNAs against RhoA, RhoB or RhoC; the knock-down efficiency and potential effect on other Rho proteins were detected by immunoblotting.

E, F H1299 cells transfected with Ctrl, RhoA-, RhoB- or RhoC-siRNAs, treated with or without 1 μM nocodazole (noc) for 30 min, were labelled for F-actin. (E) Images show H1299 cells treated without nocodazole (Images of H1299 cells treated with nocodazole are shown in Fig 1H). Scale bars, 20 μm. (F) The fraction of cells forming blebs was quantified. Bars show mean ± SEM (n = 3 experiments). Dunnett's multiple comparison test derived P-values: $^{**}P < 0.01$; $^{***}P < 0.001$. The right half of this graph is also displayed in Fig 1I.

G H1299 cells transfected with different Ctrl, RhoA-, RhoB- or RhoC-siRNAs; the effect of knock-down of individual siRNAs was detected by immunoblotting.

H, I H1299 cells transfected with either RhoB-siRNA alone or RhoB-siRNA together with an siRNA-resistant FLAG-tagged RhoB construct, treated with nocodazole and labelled for F-actin. (H) The fraction of cells forming blebs was quantified. Bars show mean ± SEM (n = 4 experiments). Dunnett's multiple comparison test derived P-values: $^{***}P < 0.001$. (I) The level of RhoB was detected by immunoblotting.

J, K H1299 cells transfected with Ctrl, RhoA-, RhoB- or RhoA+RhoB-siRNAs, treated with or without 1 μM nocodazole (noc) for 30 min and labelled for F-actin. (J) The fraction of cells forming blebs was quantified. Bars show mean ± SEM (n = 3 experiments). Dunnett's multiple comparison test derived P-values: n.s. = non-significant; $^{***}P < 0.001$. (K) The levels of RhoA and RhoB were detected by immunoblotting.

L H1299 cells transfected with FLAG-tagged RhoA, RhoB or RhoC, treated with or without 1 μM nocodazole (noc) for 30 min. Rho GTPases were pulled down with GST-RBD protein, and the GTP-binding activities of RhoA, RhoB and RhoC were detected by immunoblotting with anti-FLAG antibody.

M, N T-ALL cells electroporated with Ctrl, RhoA-, RhoB- or RhoC-siRNA, replated in 2.5 mg/ml 3D-Collagen type I matrix. The cells were imaged, and their migration speed was measured (same data set as in Fig 2A). (M) A representative example of Imaris analysis of T-ALL cell migration with different Rho protein knock-down is displayed. (N) Immunoblotting knock-down efficiency control for Fig 2A.

O, P T-ALL cells electroporated with Ctrl, RhoA-, RhoB- or RhoC-siRNA, replated in 2.5 mg/ml 3D-Collagen type I matrix (same data set as in Fig 2D). (O) Cellular features, that is cell area, roundness, solidity and elongation were analysed. The boxes show the median and quartiles, and the whiskers display 5 and 95 percentiles. Kruskal–Wallis test derived P-values: $^{*}P < 0.05$; $^{**}P < 0.01$; n.s.—non-significant. Data shown are one representative example among three independent experiments. (P) Immunoblotting knock-down efficiency control for Fig 2D.

Q, R T-ALL cells electroporated with Ctrl, RhoA-, RhoB- or RhoC-siRNA, replated on FN. The knock-down efficiency of individual Rho proteins, as well as the levels of phosphorylated and total MLC, was detected by immunoblotting. Bar graph shows quantification of the signal ratio between phospho-MLC and total MLC as mean ± SD (n = 3 experiments). t-test (paired, two-tailed) derived P-values: $^{***}P < 0.001$.

Source data are available online for this figure.

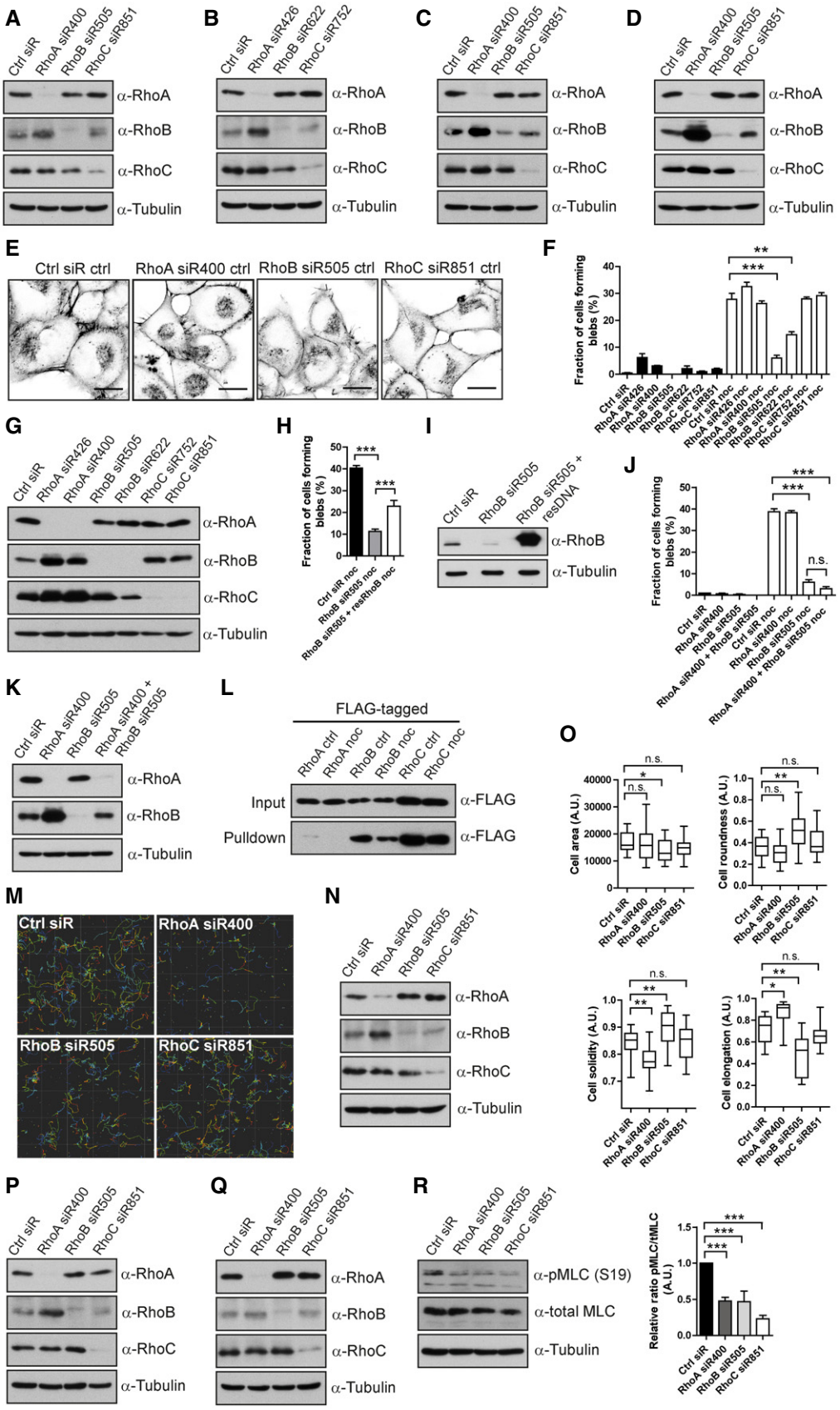
A
Ctrl siR
RhoA siR400
RhoB siR505
RhoC siR851
α-RhoA
α-RhoB
α-RhoC
α-Tubulin
B
Ctrl siR
RhoA siR426
RhoB siR622
RhoC siR752
α-RhoA
α-RhoB
α-RhoC
α-Tubulin
C
Ctrl siR
RhoA siR400
RhoB siR505
RhoC siR851
α-RhoA
α-RhoB
α-RhoC
α-Tubulin
D
Ctrl siR
RhoA siR400
RhoB siR505
RhoC siR851
α-RhoA
α-RhoB
α-RhoC
α-Tubulin
E
Ctrl siR ctrl
RhoA siR400 ctrl
RhoB siR505 ctrl
RhoC siR851 ctrl
F
Fraction of cells forming blebs (%)
Ctrl siR
RhoA siR426
RhoA siR400
RhoB siR505
RhoB siR622
RhoC siR752
RhoC siR851
Ctrl siR noc
RhoA siR426 noc
RhoA siR400 noc
RhoB siR505 noc
RhoB siR622 noc
RhoC siR752 noc
RhoC siR851 noc
G
Ctrl siR
RhoA siR426
RhoA siR400
RhoB siR505
RhoB siR622
RhoC siR752
RhoC siR851
α-RhoA
α-RhoB
α-RhoC
α-Tubulin
H
Fraction of cells forming blebs (%)
Ctrl siR noc
RhoB siR505 noc
RhoB siR505 + resRhoB noc
I
Ctrl siR
RhoB siR505
RhoB siR505 + resDNA
α-RhoB
α-Tubulin
J
Fraction of cells forming blebs (%)
n.s.
Ctrl siR
RhoA siR400
RhoB siR505
RhoA siR400 + RhoB siR505
Ctrl siR noc
RhoA siR400 noc
RhoB siR505 noc
RhoA siR400 + RhoB siR505 noc
K
Ctrl siR
RhoA siR400
RhoB siR505
RhoA siR400 + RhoB siR505
α-RhoA
α-RhoB
α-Tubulin
L
FLAG-tagged
RhoA ctrl
RhoA noc
RhoB ctrl
RhoB noc
RhoC ctrl
RhoC noc
Input
Pulldown
α-FLAG
α-FLAG
M
Ctrl siR
RhoA siR400
RhoB siR505
RhoC siR851
N
Ctrl siR
RhoA siR400
RhoB siR505
RhoC siR851
α-RhoA
α-RhoB
α-RhoC
α-Tubulin
O
Cell area (A.U.)
Cell roundness (A.U.)
Cell solidity (A.U.)
Cell elongation (A.U.)
n.s.
Ctrl siR
RhoA siR400
RhoB siR505
RhoC siR851
P
Ctrl siR
RhoA siR400
RhoB siR505
RhoC siR851
α-RhoA
α-RhoB
α-RhoC
α-Tubulin
Q
Ctrl siR
RhoA siR400
RhoB siR505
RhoC siR851
α-RhoA
α-RhoB
α-RhoC
α-Tubulin
R
Ctrl siR
RhoA siR400
RhoB siR505
RhoC siR851
α-pMLC (S19)
α-total MLC
α-Tubulin
Relative ratio pMLC/tMLC (A.U.)
Ctrl siR
RhoA siR400
RhoB siR505
RhoC siR851

Figure EV1.

Figure EV2. Effects of RhoB localization on membrane blebbing.

A, B T-ALL cells electroporated with Ctrl or RhoB-siRNA were replated on FN and immunolabelled for RhoB. (A) RhoB intensity quantification. Red bars show the median and quartiles based on pooled data from three independent experiments. Mann–Whitney test derived *P*-value: ***$P < 0.001$. (B) Immunoblotting detecting RhoB knock-down efficiency.

C Reh cells labelled with CellTracker Red Dye (cytoplasmic label) and Hoechst 33342 (nucleus) and immunostained for RhoB. Scale bars, 5 μm. The fluorescent spectra for RhoB and CellTracker labelling are shown below the images. The white arrow indicates the direction for the fluorescence intensity quantification along this line; green and red arrows indicate the RhoB and CellTracker signal, respectively, close to the cell boundaries.

D H1299 cells were transfected with FLAG-RhoB WT or different mutants and labelled for FLAG-tag and F-actin (same data set as in Fig 3C). Cells with different intracellular localization and with blebs were quantified. Bars show mean ± SD ($n = 3$ experiments). Dunnett's multiple comparison test derived *P*-values: **$P < 0.01$; ***$P < 0.001$; n.s. = non-significant.

E Cell viability (relative cell number) of EGFP or EGFP-RhoB stable H1299 cells was analysed by MTT assay. Bars show mean with 95% CI ($n = 3$ experiments).

F Immunoblotting analysed cleaved caspase-3 in EGFP and EGFP-RhoB stable H1299 cells, with puromycin treated cells as positive control.

G EGFP-RhoB H1299 cells transfected with Ctrl or EGFP-siRNA, labelled for F-actin. The fraction of cells forming blebs was quantified. Bars show mean ± SEM ($n = 3$ experiments). *t*-test (unpaired, two-tailed) derived *P*-value: ***$P < 0.001$.

H EGFP-RhoB cells treated with DMSO, 1 μM Y27632 or 10 μM Blebbistatin for 30 min, fixed and labelled for F-actin. Scale bars, 10 μm.

I, J COS-7, MCF-7, PC-3, HeLa, NIH3T3 and MDCK cells were transfected with EGFP or EGFP-RhoB and labelled for F-actin (I). Scale bars, 10 μm. (J) Quantification of fractions of cells forming blebs; cell number used for each cell line is shown in the graph.

K EGFP or EGFP-RhoB stable cells were replated in 3D-Collagen type I gels of different densities (0.8, 1.2 and 1.8 mg/ml) and migration straightness analysed (same data set as in Fig 3I and J). Boxes show median and quartiles, and whiskers display the 5 and 95 percentiles based on pooled data from three independent experiments. Kruskal–Wallis test derived *P*-values: n.s. = non-significant.

L Untransfected H1299 cells (left), FLAG-tagged RhoA or RhoC transfected (middle) and EGFP-RhoA or RhoC transfected (right). Cells were immunolabelled for RhoA or RhoC (left), or for FLAG-tag (middle), and stained for F-actin. Scale bars, left and middle: 20 μm, right: 10 μm.

M H1299 cells with or without stable expression of EGFP, EGFP-RhoA, EGFP-RhoB or EGFP-RhoC were labelled for F-actin. Bleb-positive cells were quantified using the F-actin channel. Bars show mean ± SD ($n = 6$ experiments). Dunnett's multiple comparison test derived *P*-values: **$P < 0.01$; ***$P < 0.001$; n.s. = non-significant.

N H1299 cells stably expressing EGFP-tagged RhoA, RhoB or RhoC, or EGFP were pulled down with GST-RBD protein, and the GTP-binding activities were detected by immunoblotting with anti-GFP antibody.

Source data are available online for this figure.

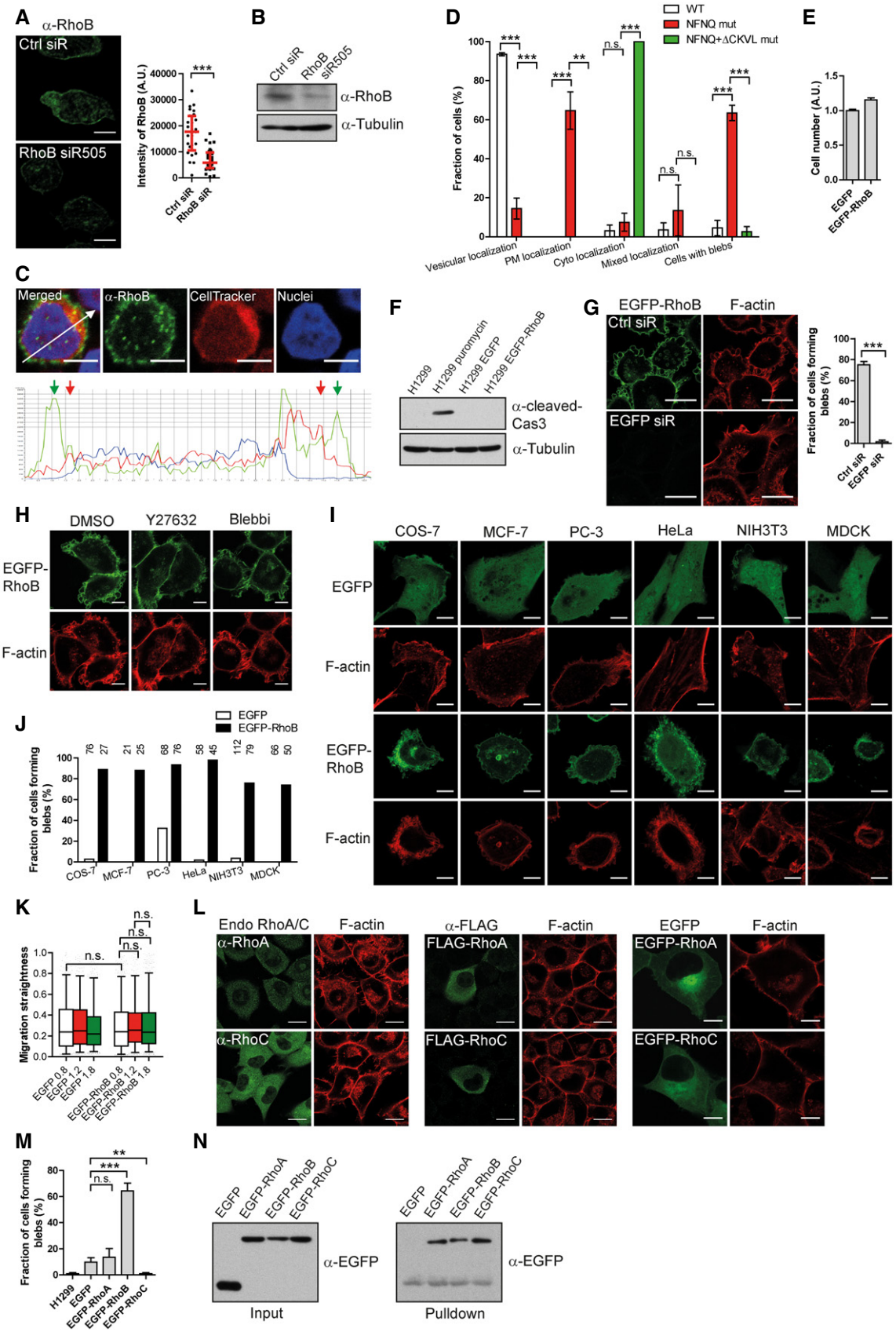
A
α-RhoB
Ctrl siR
RhoB siR505
Intensity of RhoB (A.U.)
Ctrl siR
RhoB siR
B
Ctrl siR
RhoB siR505
α-RhoB
α-Tubulin
D
WT
NFNQ mut
NFNQ+ΔCKVL mut
Fraction of cells (%)
Vesicular localization
PM localization
Cyto localization
Mixed localization
Cells with blebs
E
Cell number (A.U.)
EGFP
EGFP-RhoB
C
Merged
α-RhoB
CellTracker
Nuclei
F
H1299
H1299 puromycin
H1299 EGFP
H1299 EGFP-RhoB
α-cleaved-Cas3
α-Tubulin
G
EGFP-RhoB
F-actin
Ctrl siR
EGFP siR
Fraction of cells forming blebs (%)
H
DMSO
Y27632
Blebbi
EGFP-RhoB
F-actin
I
COS-7
MCF-7
PC-3
HeLa
NIH3T3
MDCK
EGFP
F-actin
EGFP-RhoB
F-actin
J
EGFP
EGFP-RhoB
Fraction of cells forming blebs (%)
76 27 21 25 68 76 58 45 112 79 66 50
COS-7
MCF-7
PC-3
HeLa
NIH3T3
MDCK
K
Migration straightness
n.s.
EGFP 0.8
EGFP 1.2
EGFP 1.8
EGFP-RhoB 0.8
EGFP-RhoB 1.2
EGFP-RhoB 1.8
L
Endo RhoA/C
F-actin
α-RhoA
α-RhoC
α-FLAG
FLAG-RhoA
FLAG-RhoC
EGFP
EGFP-RhoA
EGFP-RhoC
M
Fraction of cells forming blebs (%)
H1299
EGFP
EGFP-RhoA
EGFP-RhoB
EGFP-RhoC
N
EGFP
EGFP-RhoA
EGFP-RhoB
EGFP-RhoC
α-EGFP
Input
Pulldown

Figure EV2.

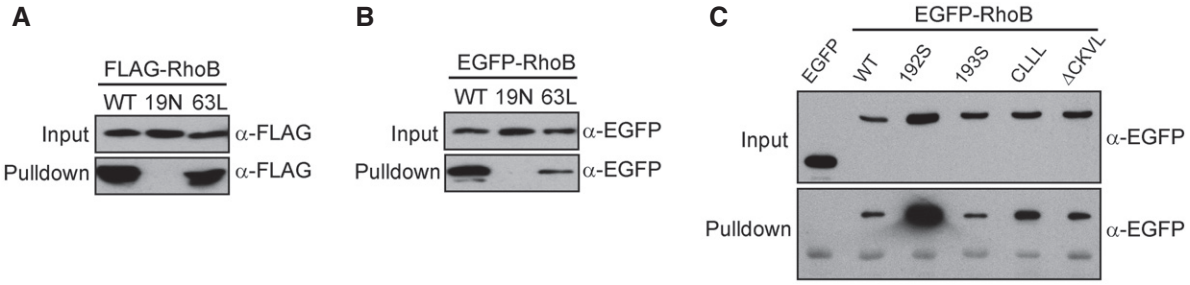

Figure EV3. GTP-binding activities of RhoB and its mutants.

A H1299 cells transfected with FLAG-tagged RhoB wild-type (WT), a dominant-negative mutant (19N) or a constitutively active mutant (63L). Rho GTPases were pulled down with GST-RBD protein, and the GTP-binding activities were detected by immunoblotting with an anti-FLAG antibody.

B H1299 cells transfected with EGFP-tagged RhoB (WT), (19N) or (63L). Rho GTPases were pulled down with GST-RBD protein, and the GTP-binding activities were detected by immunoblotting with an anti-GFP antibody.

C H1299 cells stably expressing EGFP-tagged RhoB (WT), (192S), (193S), (CLLL) or (ΔCKVL), or EGFP were pulled down with GST-RBD protein, and the GTP-binding activities were detected by immunoblotting with an anti-GFP antibody.

Source data are available online for this figure.

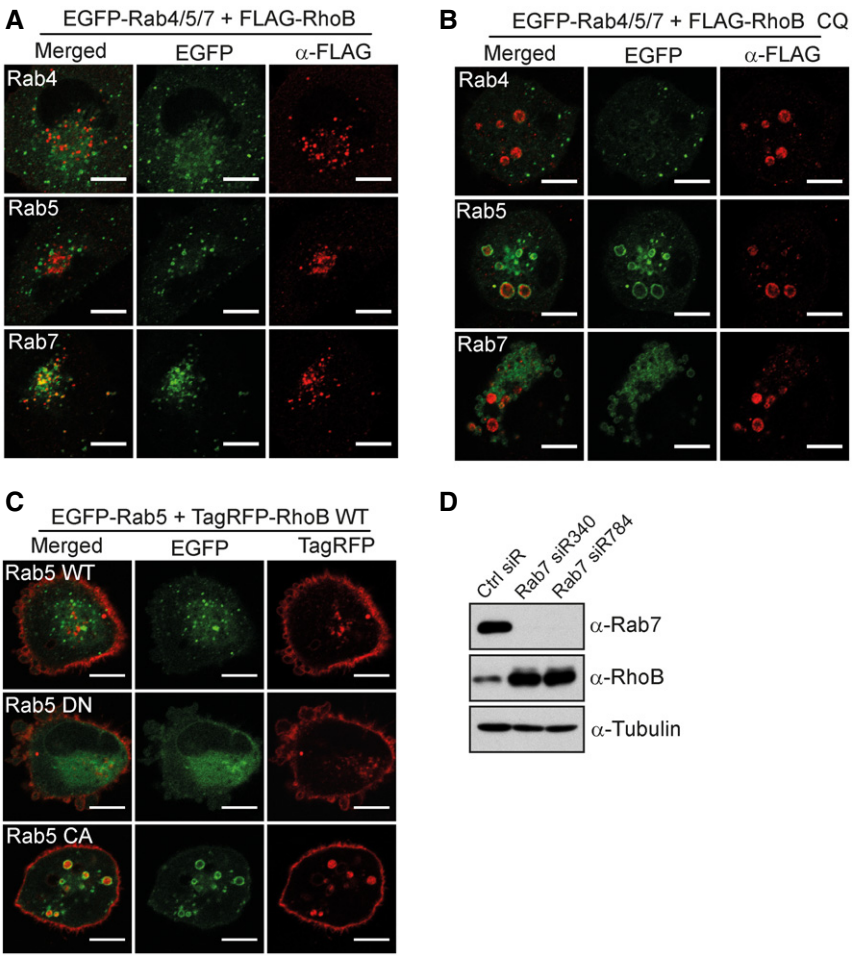

Figure EV4. Internalization and intracellular trafficking of RhoB.

A, B H1299 cells co-transfected with EGFP-tagged Rab4, Rab5 or Rab7, together with FLAG-tagged RhoB. Cells were treated without (A) or with (B) 50 μM CQ for 6 h, fixed and immunolabelled for FLAG-tag. Scale bars, 10 μm.

C H1299 cells co-transfected with EGFP-Rab5 wild-type (WT), a dominant-negative (DN) Rab5 mutant, or a constitutively active (CA) Rab5 mutant, together with TagRFP-RhoB (WT). Scale bars, 10 μm.

D H1299 cells transfected with Ctrl or Rab7-siRNAs. The levels of Rab7 and RhoB were detected by immunoblotting as a control for Fig 5D.

Source data are available online for this figure.

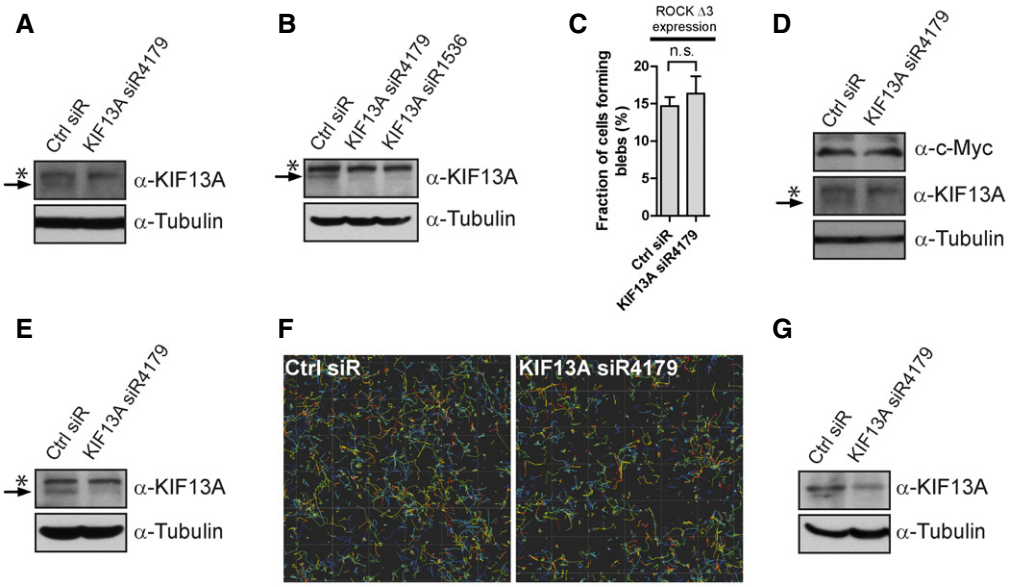

Figure EV5. Effects of KIF13A knock-down on RhoB localization and membrane blebbing.

A Knock-down efficiency control for Fig 6C. H1299 cells transfected with Ctrl or KIF13A-siRNA. The knock-down efficiency was analysed by immunoblotting. Arrow indicates the KIF13A bands and asterisk indicates unspecific bands.

B Knock-down efficiency control for Fig 6D. H1299 cells transfected with Ctrl or KIF13A-siRNAs. The knock-down efficiency was analysed by immunoblotting. Arrow indicates the KIF13A bands, and asterisk indicates unspecific bands.

C, D H1299 cells transfected with Ctrl or KIF13A-siRNA, together with a myc-tagged active ROCK mutant (ROCK-Δ3). The fraction of cells forming blebs was quantified (C), and the levels of myc-ROCK-Δ3 and KIF13A were detected by immunoblotting (D). Bars show mean ± SEM (n = 3 experiments). t-test (unpaired, two-tailed) derived P-value: n.s. = non-significant.

E Knock-down efficiency control for Fig 6F. T-ALL cells electroporated with Ctrl or KIF13A-siRNA. The knock-down efficiency was analysed by immunoblotting. Arrow indicates the KIF13A bands, and asterisk indicates unspecific bands.

F, G T-ALL cells electroporated with Ctrl or KIF13A-siRNA and replated in 2.5 mg/ml 3D-Collagen type I gel. A representative example of Imaris tracking analysis is displayed (F). The knock-down efficiency was analysed by immunoblotting (G). Corresponds to Fig 6H.

Source data are available online for this figure.