

Supplemental Table 1. List of antibodies used for immunoblots and immunofluorescence studies:

Primary Antibody	Source	Reactivity	Supplier	Reference
Albumin	Monoclonal Rabbit	Human, mouse, rat, cow	Abcam	ab192603
ARF6	Polyclonal Rabbit	Human, mouse, rat	Proteintech	20225-1-AP
Caveolin-1	Polyclonal Rabbit	Human, rat, mouse	Cell Signaling	3238S
Clathrin heavy chain	Monoclonal Mouse	Human, mouse, rat, bovine	Novus Biologicals	NB300-613
Dynamin-2	Polyclonal Rabbit	Human, mouse, rat	Cell Signaling	2342S
PAC1 (FITC-labeled)	Mouse anti- human	Human, mouse	BD Biosciences	340507
Flotillin-1	Polyclonal Rabbit	Human, rat, mouse	Cell Signaling	3253S
IFITM3	Polyclonal Rabbit	Human, mouse, rat	Proteintech	11714-1-AP
Integrin αIIb -FITC labeled clone-HIP8	Mouse anti- human	Human, mouse	BD Biosciences	340929

Integrin αIIb	Monoclonal Mouse	Human, mouse	Santa Cruz Biotechnology	sc-365938
Integrin β3	Monoclonal Rabbit	Human, mouse	Cell Signaling	13166S
IRDye- IgG labeled 680/800 RD Sec. antibody	Donkey	Human, mouse	Li-Cor Biosciences	926-68072
LAMP2 CD107b	Polyclonal Mouse	Human	Biologend	354302
Myosin light chain	Monoclonal Mouse	Human, mouse	Santa Cruz Biotechnology	sc-48414
PF4 (anti-CXCL4)	Monoclonal Human	Human	R&D Systems	IC-7952F
Phosphorylated - myosin light chain	Polyclonal Rabbit	Human, mouse	Cell Signaling	3674S
Rab11A/B	Monoclonal Rabbit	Human, rat, mouse	Cell Signaling	5589
RUNX1	Monoclonal Mouse	Human, rat, mouse	Santa Cruz Biotechnology	sc-365644
β-actin	Polyclonal Mouse	Human, mouse, rat	Santa Cruz Biotechnology	sc-47778

Supplemental Table 2. List of fluorescence labeled conjugated proteins or antibodies used for uptake studies:

PROTEIN	CONJUGATES	SUPPLIER	REFERENCE
Human Albumin	Cy3-ChromePure	Jackson ImmunoResearch Laboratories, Inc	009-160-051
Human Albumin	Alexa Fluor 488	Jackson ImmunoResearch Laboratories, Inc	009-540-051
Fibrinogen from Human plasma	Alexa fluor 546	Invitrogen/Thermo Fisher sci	F13192
Fibrinogen from Human plasma	Alexa Fluor 647	Invitrogen/Thermo Fisher sci	F35200
Human IgG (whole molecule)	Cy3-ChromePure	Jackson ImmunoResearch Laboratories, Inc	009-160-003
Fibrinogen from Human plasma	Cy3- Fibrinogen	ABBY monoclonal	Bsm-1240M-Cy3
Human IgG (whole molecule)	Alexa Fluor 488	Jackson ImmunoResearch Laboratories, Inc	009-000-003

SUPPLEMENTAL FIGURES

Figure S1

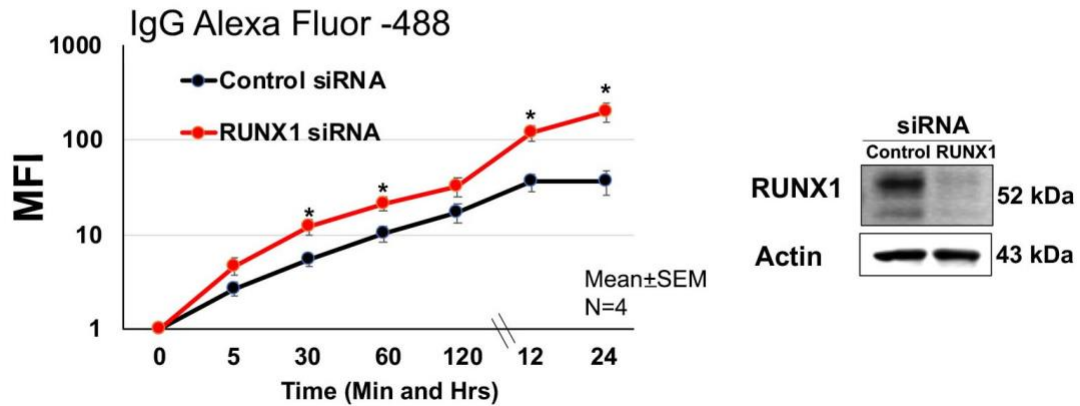
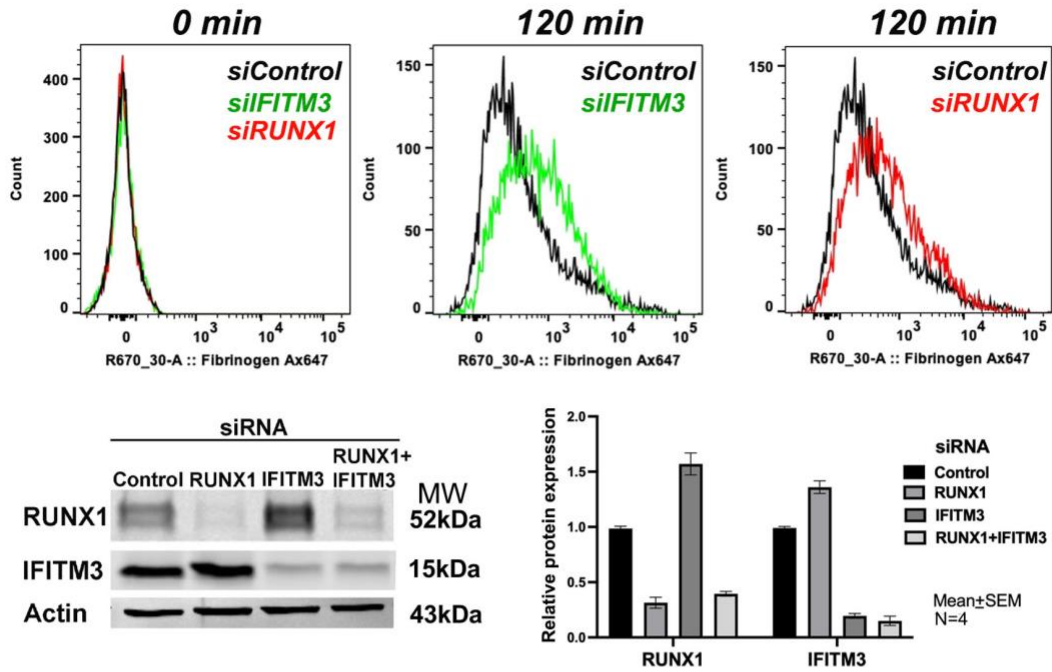


Figure S1. Effect of *RUNX1* knockdown on IgG uptake in HEL cells. HEL cells transfected with control or *RUNX1* siRNA were incubated with 10 μ g/mL IgG-Alexa 488 at 37°C for different time points, washed, fixed with 2% paraformaldehyde and analyzed by flow cytometry to assess IgG uptake. Shown is mean of MFI of two experiments. IgG uptake in *RUNX-1* deficient cells (red) was increased over time as compared to control cells (black). Also shown is a representative immunoblot showing *RUNX-1* expression with actin as loading control.

Figure S2

A.



B.

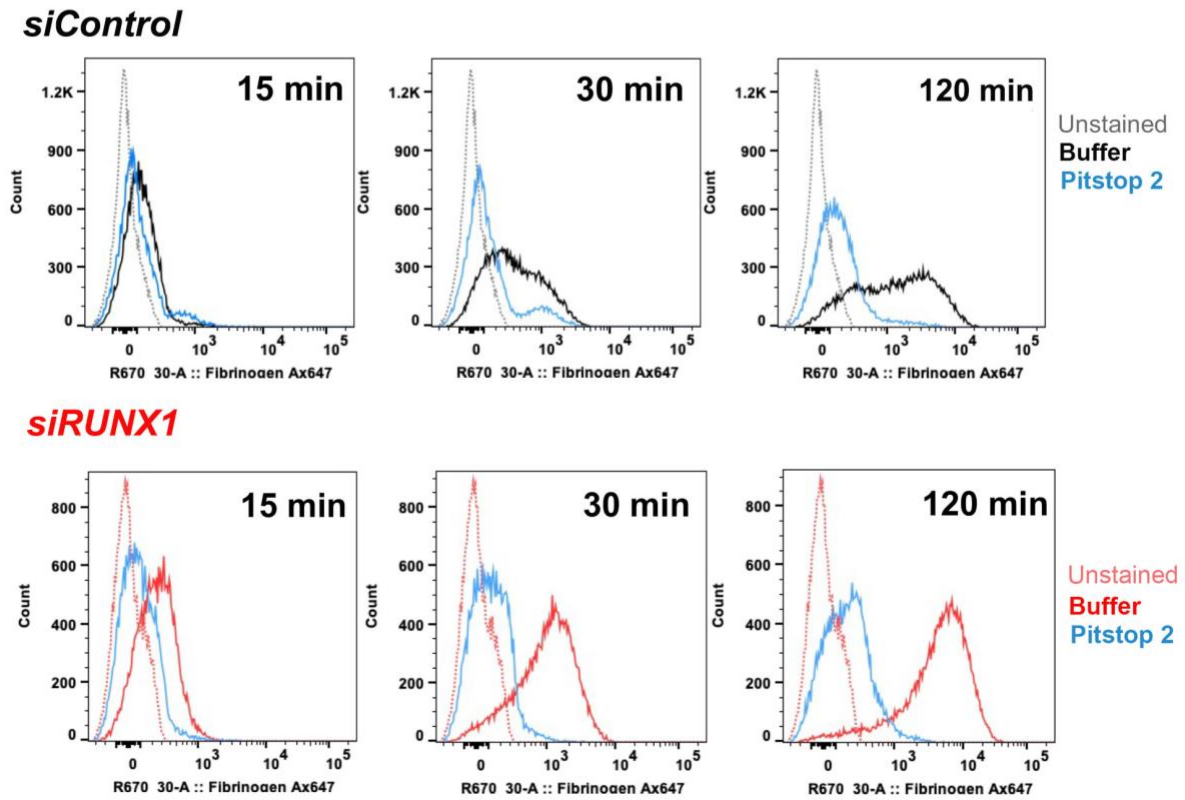


Figure S2. Effect of *IFITM3* knockdown on fibrinogen uptake in HEK cells.

A. Effect of knockdown of *IFITM3* or *RUNX1* on uptake of fibrinogen at 120 minutes by flow cytometry. HEL cells were transfected with siRNAs (100 nM) against *IFITM3* or *RUNX1*. Cells were incubated with 10 μ g/mL Alexa 647-fibrinogen in buffer for 120 minutes at 37°C, fixed and washed with buffer. Fibrinogen uptake was evaluated by flow cytometry. Left panel; 0 minutes. Middle panel: uptake at 120 minutes in *IFITM3 KD* cells (green) compared to control cells (black). The uptake of fibrinogen over 120 min was not inhibited on *IFITM3* knockdown. Right panel: uptake at 120 minutes in *RUNX1*-deficient cells (red) compared to control cells (black). Shown representative of 3 experiments. The immunoblots show the relative protein levels.

B. Effect of Pitstop 2 (inhibitor of clathrin-mediated uptake) on uptake and retention of fibrinogen over 120 min in control cells (top panels) and *RUNX1* siRNA treated HEL cells (lower panels) by flow cytometry. Cells were incubated with Pitstop 2 (30 μ M) for 15 minutes at room temperature. In the top panels: black lines, buffer; blue lines Pitstop 2. In the lower panels: red lines, buffer; blue lines, Pitstop 2. Shown is a representative of 2 experiments.

Figure S3

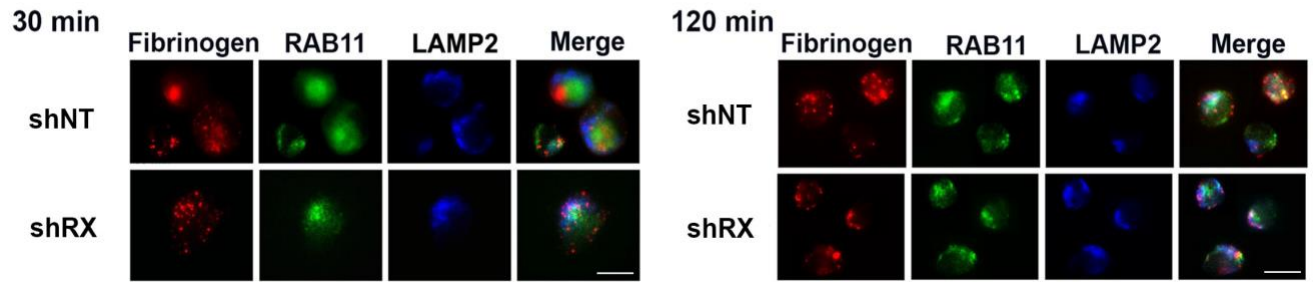


Figure S3. Effect of shRNA RUNX1 knockdown in primary MK on fibrinogen colocalization with RAB11 and LAMP2 (30 and 120 minutes) by

immunofluorescence microscopy. shNT and shRx cells were incubated with Alexa-647 fibrinogen for 30 and 120 min, fixed and immobilized on poly-lysine-coated coverslips. Representative images are shown. Fibrinogen is shown in red fluorescence. Cells were stained with anti-RAB11 (green) or anti-LAMP2 (blue) antibodies to assess colocalization as seen in merged images and evaluated by Nikon E1000 epifluorescence microscope.

Figure S4

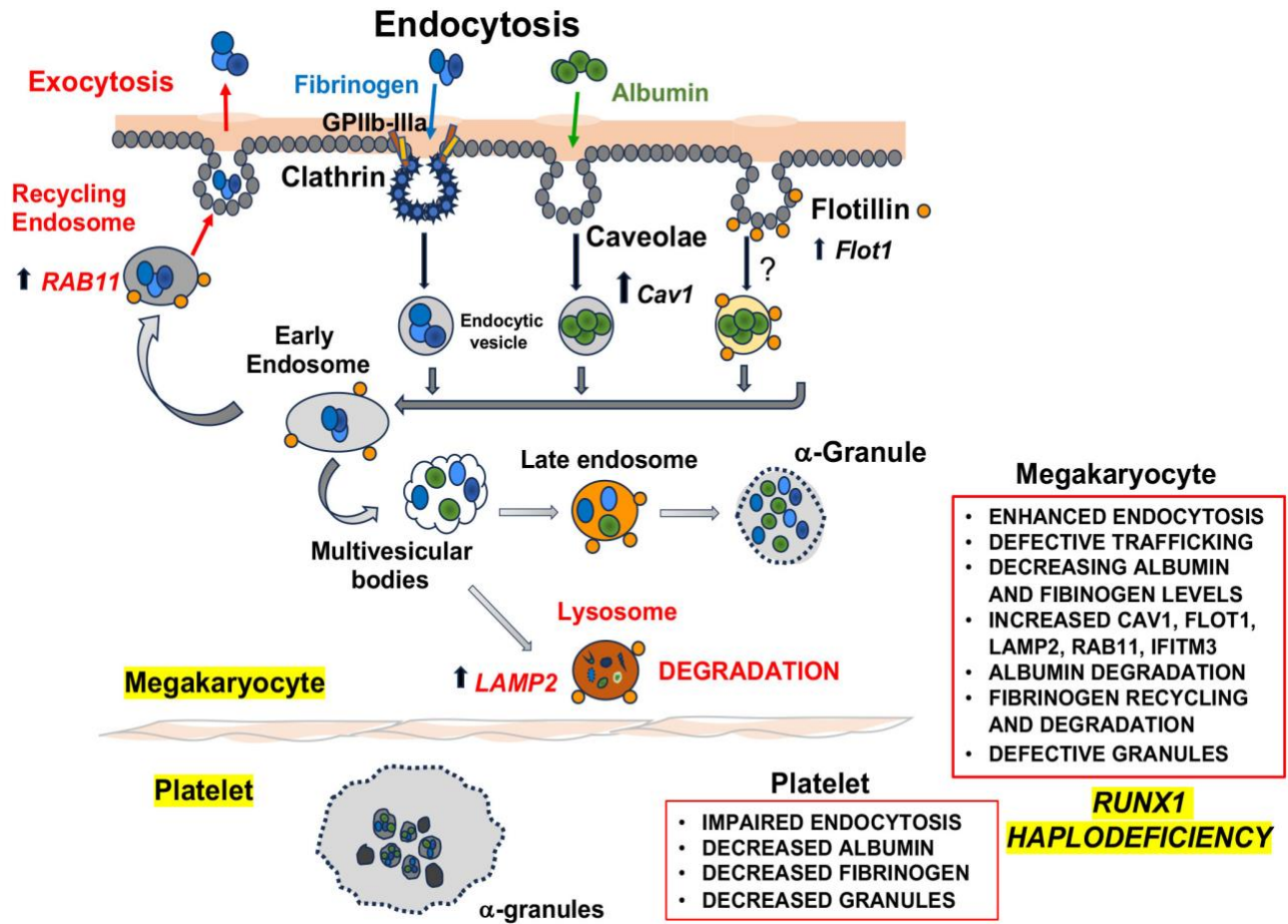


Figure S4 Schema showing the endocytic pathways and alterations in megakaryocytes and platelets in *RUNX1* haplodeficiency.