**Figure S1.** Establishment of embryonic stem cell lines with reduced expression of Filamin A and their differentiation into megakaryocytes and platelets. (A) shRNA sequence used to knock down filamin A, with corresponding immunoblot (panel B) of the ES cell line termed FlnA_{Low}. (C) As shown schematically in Fig. 2A, CD41-positive cells were isolated from embryoid bodies that had been derived from FlnA_{Low} embryonic stem cells and cultured in the presence of TERT-TPO stroma feeder cells for an additional six days. % GFP + FlnA_{Low} cells were reduced from 31 % (EB Day 6 CD41 Day 0) to 10% (EB Day 12 CD41 Day 6). (D) FACS analysis of platelet size in the FlnA_{Low}, GFP-positive versus the FlnA_{Normal}, GFP-negative population.

**Figure S2.** Flow cytometric analysis of size and F-actin content of platelets from wild-type, Tg33, and GPIbα-null mice. After incubation with and FITC-labeled CD41 antibody to mark platelets, whole blood samples were fixed, permeabilized, and stained for F-actin. Relative expression is represented as mean fluorescent intensity, with expression in wild-type platelets arbitrarily set to 100. Note that the increase in F-actin content increases relatively proportional with platelet size.
Figure S1

A

Targeting sequence to knock down only filamin A

Target sequence for FlnA

\[ \text{AGATGCATAAGGTCAAGTGT} \]

mouse filamin A sequence (100%): \[ \text{AGATGCATAAGGTCAAGTGT} \]

mouse filamin B sequence (59%): \[ \text{GGATCCAGCAAGGTCAAGATT} \]

B

Filamin A

Filamin B

HSC70

C

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<tr>
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D

P < 0.05

Relative platelet size

FlnA<sub>Normal</sub> FlnA<sub>Low</sub>
Figure S2

Relative Size (forward scatter)

WT  IL4R-Ilα  IbaKO

n=6  P < .001

Relative F-actin content

WT  IL4R-Ilα  IbaKO

n=6  P < .001