Supplemental Data

Talin1 is the Principal Platelet Rap1 Effector of Integrin Activation

Supplemental Methods

Tln1 mutagenesis using CRISPR-Cas9

The sgRNA and the ssODN were from IDT. Cas9 mRNA/sgRNA/tracRNA (3 μM) was mixed with ssODN (5 ng/μl) and injected into the pronuclei of B6SJLF2 embryos that were obtained through in vitro fertilization of wildtype oocytes by sperm from *Tln1*$^{WT/R35E}$ donor mice. Surviving embryos were implanted into ICR pseudo-pregnant females and pregnancies went to full term. Tissue biopsies for genomic DNA were taken from pups between 7-10 days. Mice were genotyped by PCR using forward primers 5'-CACTTCGGTGAGGGTGTATTCTA-3' and reverse primers 5'-GTGATACCTGGGGAAAAGG GATG-3' followed by Sanger sequencing using forward primers 5'-CGGTGAGGGTGTATTCTAATG-3' for R35E mutation and 5'-CATGCTCTATGTGCTGTCTC-3' for R118E mutation.

Reagents and antibodies

Prostacylin (PGI_2) and ADP were from Sigma-Aldrich. Protease-activated receptor 4-activating peptide (PAR4-AP, AYPGKF-NH_2) was from GenScript. Convulxin was from Enzo Life Sciences. Fibrillar collagen type I was from Chrono-log. Monoclonal antibodies directed against GPIX (Xia.B4) or activated murine αIIbβ3 (JonA/PE) were from Emfret Analytics. 9EG7 antibody was coupled with Alexa Fluor 488 using Alexa Fluor 488 Antibody Labeling kit (Life Technologies). PE-conjugated antibodies against CD41 (MWReg30; control isotype RTK2071), CD61 (2C9.G2; control isotype HTK888), CD29 (HMβ1-1; control isotype HTK888), CD49b (HMα2; control isotype HTK888) and CD49e (HMα5-1; control isotype HTK888) were from Biolegend. FITC-

conjugated antibody against P-selectin (CD62P, RB40.34; control isotype A110-1) was from BD Biosciences.

Blood counts

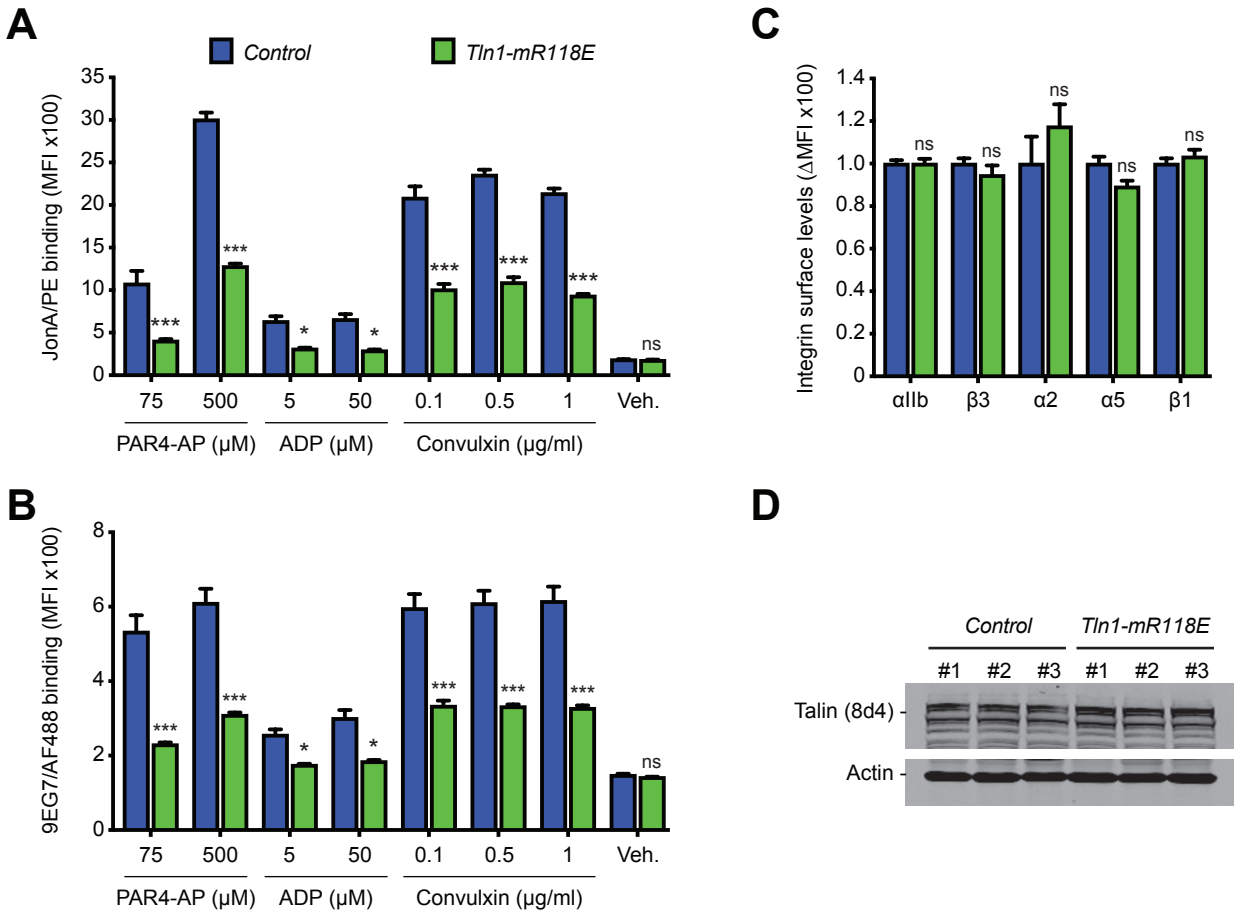
Peripheral blood was collected from the retro-orbital plexus and transferred to tubes containing K^+.EDTA. Cell counts were performed using a Hemavet 950FS Hematology System programmed with mouse-specific settings (Drew Scientific). All samples were tested in duplicate, and the mean for each animal was plotted.

Western blotting

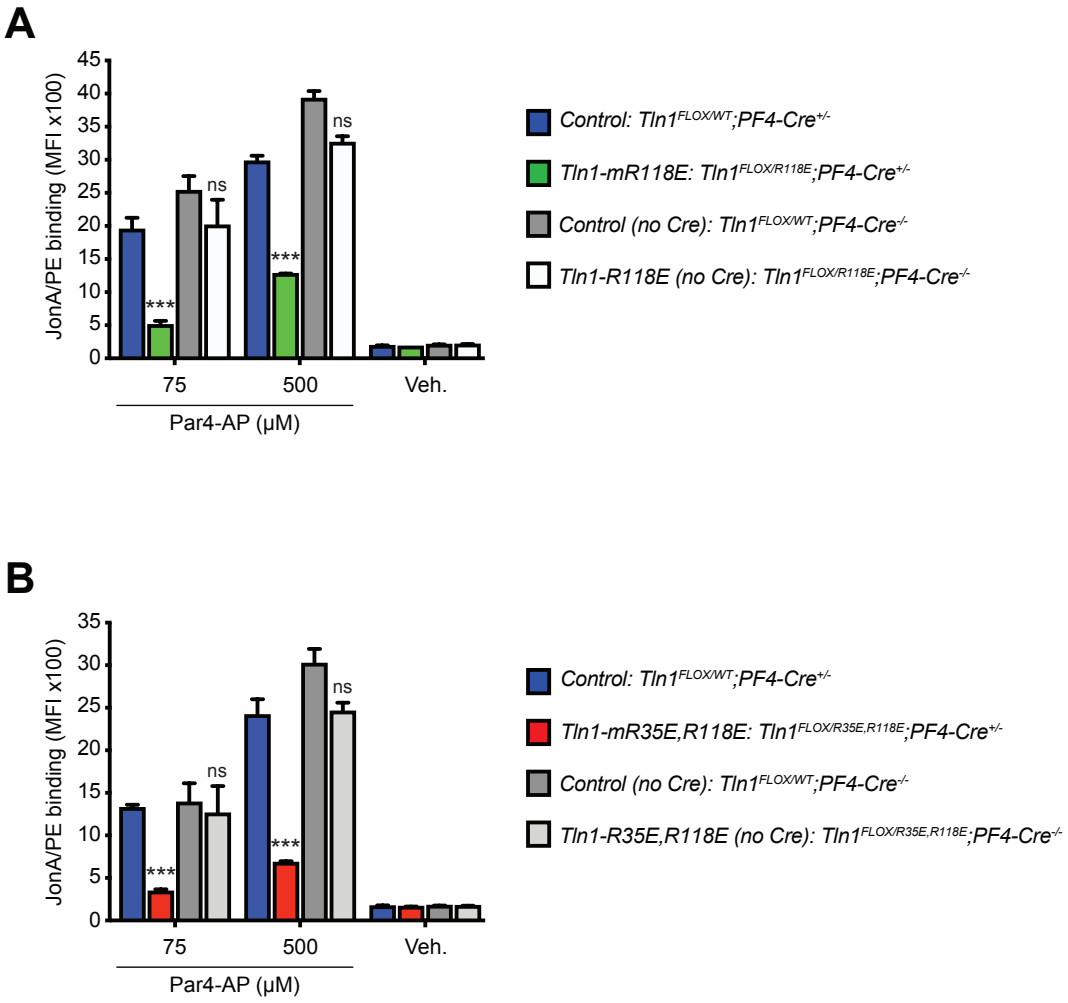
Washed platelets were pelleted by centrifugation at 700 g for 5 min at room temperature and then lysed in Laemmli sample buffer. Lysates were subjected to a 4-20% gradient SDS-PAGE. Antibody directed against talin1 (8d4) was from Novus Biologicals. Antibody against β-actin (AC-15) was from Sigma-Aldrich. The appropriate IRDye/Alexa Fluor-coupled secondary antibodies were from LI-COR. Nitrocellulose membranes were scanned using an Odyssey CLx infrared imaging system (LI-COR) and blots were processed using Image Studio Lite software (LI-COR).

	Off-target sequences	Position	Location	CFD scores	MIT scores	PCR oligo sequences 5' > 3'
gRNA1:	**ATGACCATTTGTGCCCGAAT / TGG**	**chr4:43555934-43555856:+**	**Exon:Tln1**	**97**	**95**	
	AAGACCCTCTGTGCCCAAAT / CGG	chr2:174822060-174822082:+	Intergenic:Edn3-Gm14617	0.318935	0.25	F: ATGGTTGGTGCGAATAGGAAGTAGG R: GAATTAGCAGAAGTCGGCAGCTAGG
	ATGAACATTCATGCCCAAAT / CGG	chr17:69237876-69237898:+	Intron:Epb4.1l3	0.284444	0.30	F: GTGGCTTAGTTGTGGGTGAATCTCT R: GTTGAAACTGGTCAGTGAGTGTCAG
	GTGACCATTTCTGCCCAATT / AGG	chr2:119692094-119692116:+	Exon:Rtf1	0.215385	0.11	F: CATGAAGCAGATTCCCTTTGCATCC R: ACTAACGCTTGGCCCTTCTAAAACA
	ATGAGGGTTTGTGCCAGAAT / GGG	chr10:60359473-60359495:+	Intron:4632428N05Rik	0.211765	0.10	F: CCCAGAGAGAAACCTTATGGGTGAG R: AGAAATAGCAGTTTGCCGAGACAGG
	ATGCCCACTGGTGCCAGAAT / AGG	chr4:29493944-29493966:-	Intergenic:Gm11923-Gm11925	0.176471	0.24	F: GCTTTTACGGTGATACAGAAGAGCG R: CCATCCCAAATTGACTTTCCAAATGA
gRNA2:	**CTTACCAATTCGGGCACAAA / TGG**	**chr4:43555938-43555860:-**	**Exon:Tln1**	**97**	**90**	
	TTTAGAAATTCGGACACAAA / TGG	chr17:65378775-65378797:+	Intron:Tmem232	0.417857	0.14	F: CAGCTGTGAACCCTTATGCAAGTCA R: GGGAGGGTGCTTCTGAGTTAGTGGA
	GTTACTTATTCAGGCACAAA / TGG	chr1:61804468-61804490:-	Intron:4930448K12Rik	0.346196	0.29	F: TGCGTGACCTCTGTCATCACTGAAT R: TATGTCTGTAAGACTGCCCCCTCGT
	ATTACCAATTTGGGCACAAA / AGG	chr13:55268370-55268392:+	Intron:Nsd1	0.307692	4.79	F: CAGTCAGTGTAGTCCTTGGTTTTTGT R: GGAACTGATTTTACAACTGGCCCAA
	GTTAGCAAGTAGGGCACAAA / TGG	chr7:67494388-67494410:-	Intergenic:Mef2a-Lrrc28	0.254348	0.49	F: AAAGCCTCAACGATCCTCTTCTCTG R: GTCCTAAAGCACTGTCATCTGGTCG
	CTTACCAGTTAGGACACTAA / AGG	chr14:83619776-83619798:+	Intergenic:Gm24774-Pcdh17	0.20625	0.02	F: AGCAACAATGGCAAAATCAACATCCT R: ATCTCACTTCTGATCCCGGGTGTTA

Supplemental Table 1. List of potential CRISPR-Cas9 off-target sites. Top five off-target sequences for each gRNA utilized to generate both Tln1-R118E and Tln1-R35E,R118E mice. Matches are ranked by CFD (Cutting Frequency Determination, from *Doench et al. Nature Biotechnology 34, 184-191 (2016)*) off-target score from most to least likely. The higher the CFD specificity score, the lower are off-target effects in the genome. Mismatches between off-target sites and gRNA sequences are indicated in red. DNA oligos used for PCR and Sanger sequencing are listed for each off-target sequence.



Supplemental Figure 1. Characterization of integrin activation, integrin surface levels and talin1 expression in Tln1-mR118E platelets. (A-B) Impaired αIIbβ3 integrin activation in Tln1-mR118E platelets. Flow cytometry assay to measure binding of GPIX-labeled platelets in whole blood to (A) JonA/PE antibody or (B) Alexa-Fluor 488 coupled 9EG7 antibody in response to agonist stimulation. Bar graphs represent MFI ± SEM (n = 6 mice, representative of ≥ 3 independent experiments). Two-way ANOVA with Tukey post-test; ns, not significant; *, $p<0.05$; ***, $p<0.001$. (C) Surface expression of αIIb, β3, α2, α5 and β1 integrins in Tln1-mR35E,R118E platelets was measured by flow cytometry. Bar graph represents MFI ± SEM (n = 6 mice). Two tailed *t*-test. ns, not significant. (D) Expression of talin1 mutant in Tln1-mR118E platelets was assayed by Western blotting. Results are representative of 3 independent experiments, n = 3 mice each time.



Supplemental Figure 2. Functional analysis to rule out potential CRISPR/Cas9 off-target effects on integrin activation in Tln1-mR118E and Tln1-mR35E,R118E platelets. Flow cytometry assay to measure binding of GPIX-labeled platelets in whole blood to JonA/PE antibody in response to PAR4-AP stimulation. Reduction in αIIbβ3 integrin activation in (A) Tln1-mR118E and (B) Tln1-mR35E,R118E platelets was only observed in the presence of PF4-Cre. Bar graphs represent MFI ± SEM (n = 6 mice, representative of ≥ 3 independent experiments). Two-way ANOVA with Tukey post-test. ns, not significant; ***, $p<0.001$.