Thrombocytopenia-associated mutations in Ser/Thr kinase MASTL deregulate actin cytoskeleton dynamics in platelets

by Begoña Hurtado et al.

SUPPLEMENTARY MATERIAL

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Table S3. KEGG pathways enriched (FDR>0.05) in hypophosphorylated proteins in resting *Mastl*(Δ/Δ) platelets (log_2FC ΔΔ/WT<0.75), considering as statistical background the mouse platelet proteome.

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Table S8. Antibodies used in this work.

SUPPLEMENTARY FIGURES

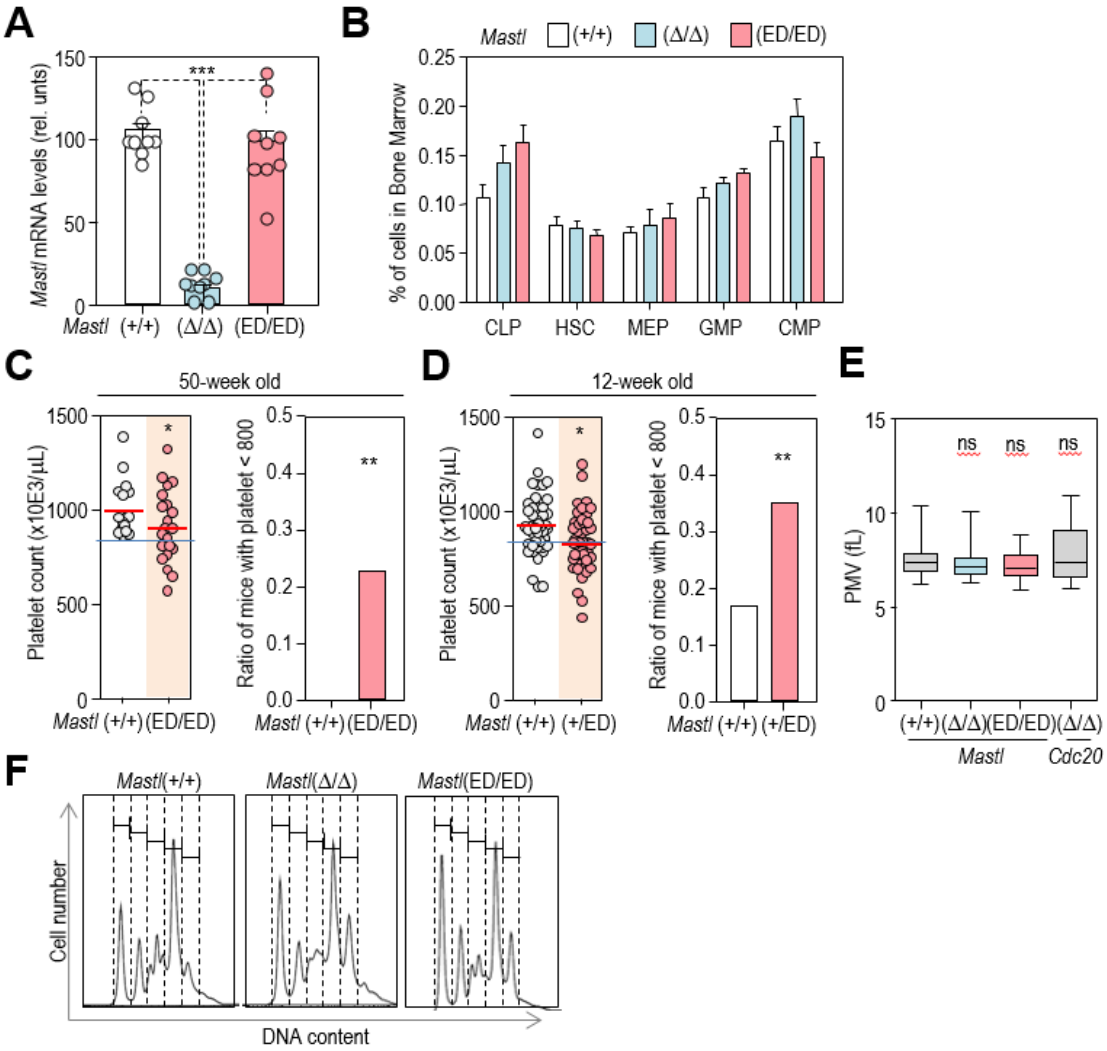


Figure S1. Hematopoietic precursors and megakaryocytes maturation in Mastl mutant mice. A, Levels of *Mastl* mRNA in platelets from mice with the indicated genotypes (N=3 mice per genotype). Mann-Whitney; ***, *P*<0.001. B, Quantification of bone marrow progenitors in bone marrow samples from mice with the indicated genotypes. No significant differences are found in any of the populations analyzed. Data are mean ± SEM. N=5 mice per genotype. C, Platelet counts in peripheral blood from 50-week-old mice with the indicated genotypes. D, Platelet counts in peripheral blood from 12-week-old mice heterozygous for the E166D mutation and control littermates. In panels C,D, *, *P*<0.05; **, *P*<0.01 (Student's t-test with Welch's correction). E, Platelet median volume from mice with the indicated genotypes. The number of mice analyzed correspond to those in Figure 1D. ns, not significant, Student's t-test with Welch's correction. F, Ploidy distribution of CD41+ CD42+ double positive bone marrow cells from mice with the indicated genotype. Data are representative of 4 mice analyzed per genotype.

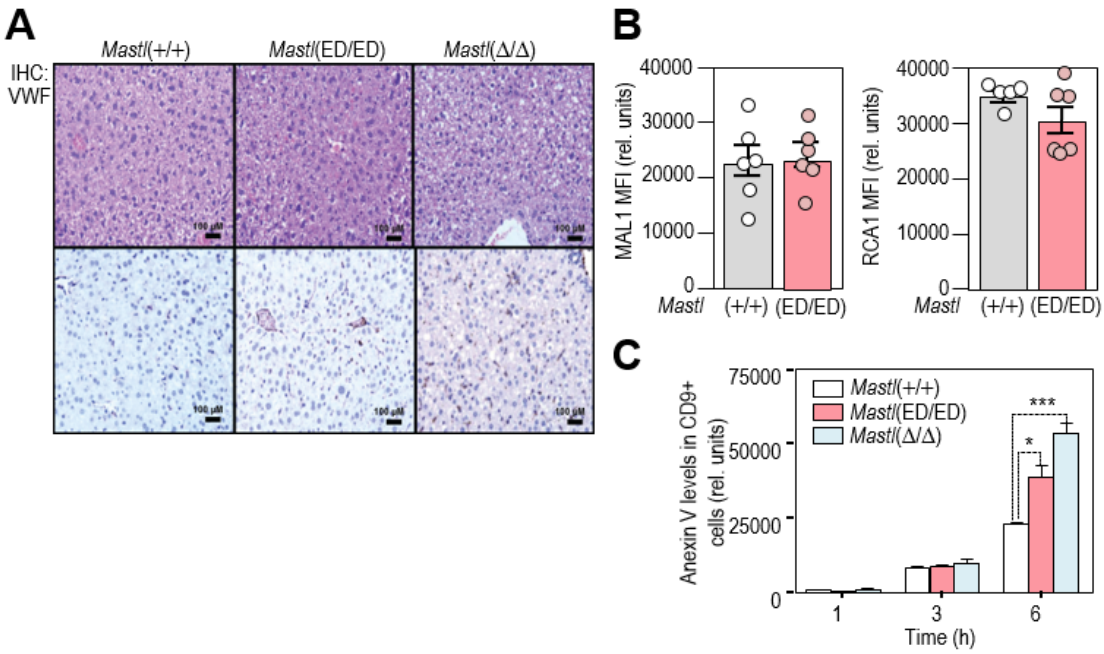


Figure S2. Sialylation and apoptosis profile of Mastl mutant platelets. A, Representative micrographs of liver from mice with the indicated genotypes. H&E, hematoxylin and eosin; immunohistochemistry staining of vWF, von Villebrand Factor. Images are representative of 3 mice analyzed per genotype. Scale bars, 50 μm. B, N-sialylation and galactose exposure was detected with RCA-1 and MAL-II binding, respectively, and measured by flow cytometry in murine platelets from the indicated genotypes. Bars represent mean ± SEM. N=6 mice per genotype. No significant differences were found, Student's t-test. C, Immunostaining for Annexin V in $CD9^+$ cells of the indicated genotypes, 0, 3 and 6 h after isolation. Annexin was detected by flow cytometry using specific antibodies. N=3 mice each genotype. *, $P<0.01$; ***, $P<0.001$; Student's t-test.

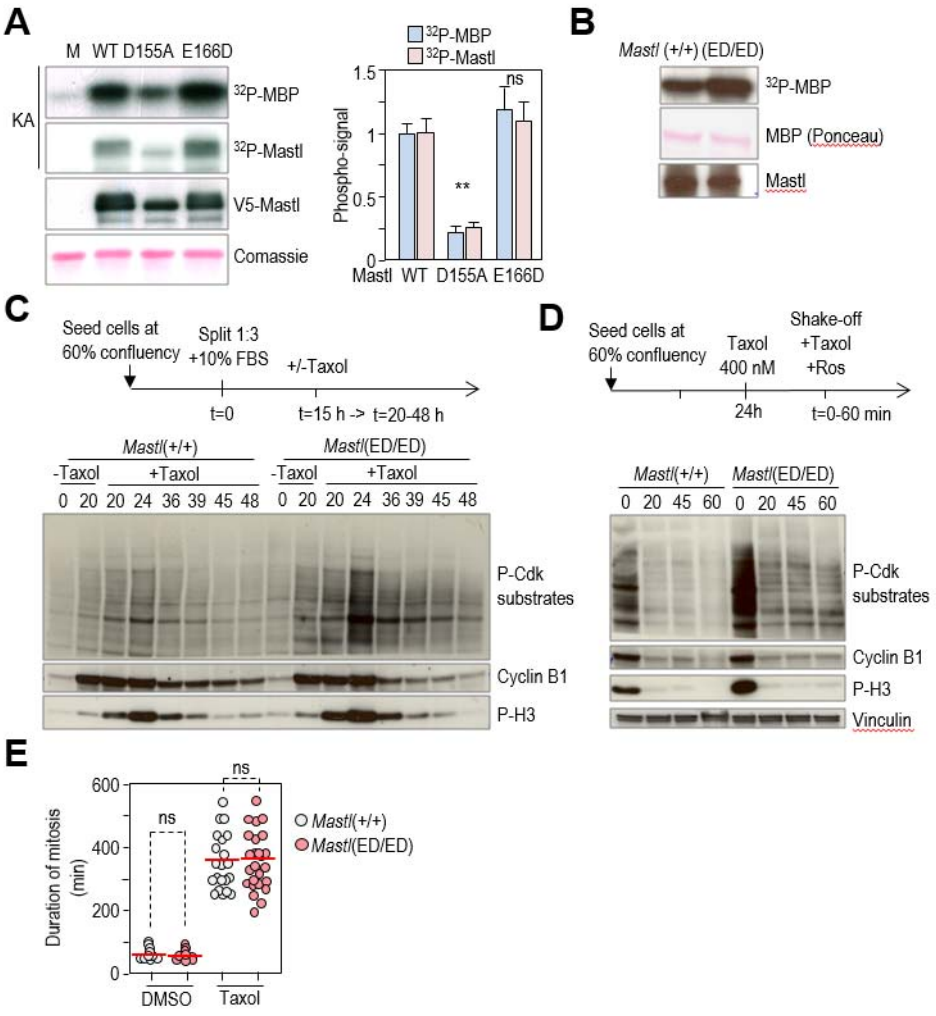


Figure S3. Mastl E166D activity results in increased phosphorylation levels. A, Kinase assay after expression of several V5-Mastl fusion proteins in 293-T cells. Mastl was immunoprecipitated using V5-specific antibodies and kinase activity (KA) was tested using myelin basic protein (MBP) as a substrate. The authophosphorylation of Mastl is also shown. D155A is a kinase-deficient mutant. The histogram shows the mean ± SD from three independent experiments. **, $P<0.01$; ns, not significant, Student's t-test. B, Kinase assays using endogenous Mastl immunoprecipitates from mouse embryonic fibroblasts (MEFs) with the indicated genotypes. MBP was used as a substrate. C, Wild-type or *Mastl*(ED/ED) MEFs were cultured in the presence or absence of taxol and protein lysates were analyzed at different time points as indicated in the top panel to analyze mitotic arrest and slippage in the presence of this microtubule poison. The phosphorylation of Cdk substrates and levels of cyclin B1 were analyzed showing increased phosphor-Cdk substrate signals in the presence of the Mastl ED mutant. D, Wild-type or *Mastl*(ED/ED) MEFs were treated with taxol and mitotic cells were isolated by shaking off. These cells were cultured in the presence of taxol and the Cdk inhibitor roscovitine to induce mitotic exit and the levels of phosphor-Cdk substrates were analyzed at different time points. Mastl ED cells displayed increased phospho-signal in agreement with reduced PP2A/B55 activity during mitotic exit. E, The duration of mitosis in the presence of taxol was tested by time-lapse microscopy showing that the differences in mitotic phospho-signals were not due to delayed mitotic exit. ns, not significant, Student's t-test.

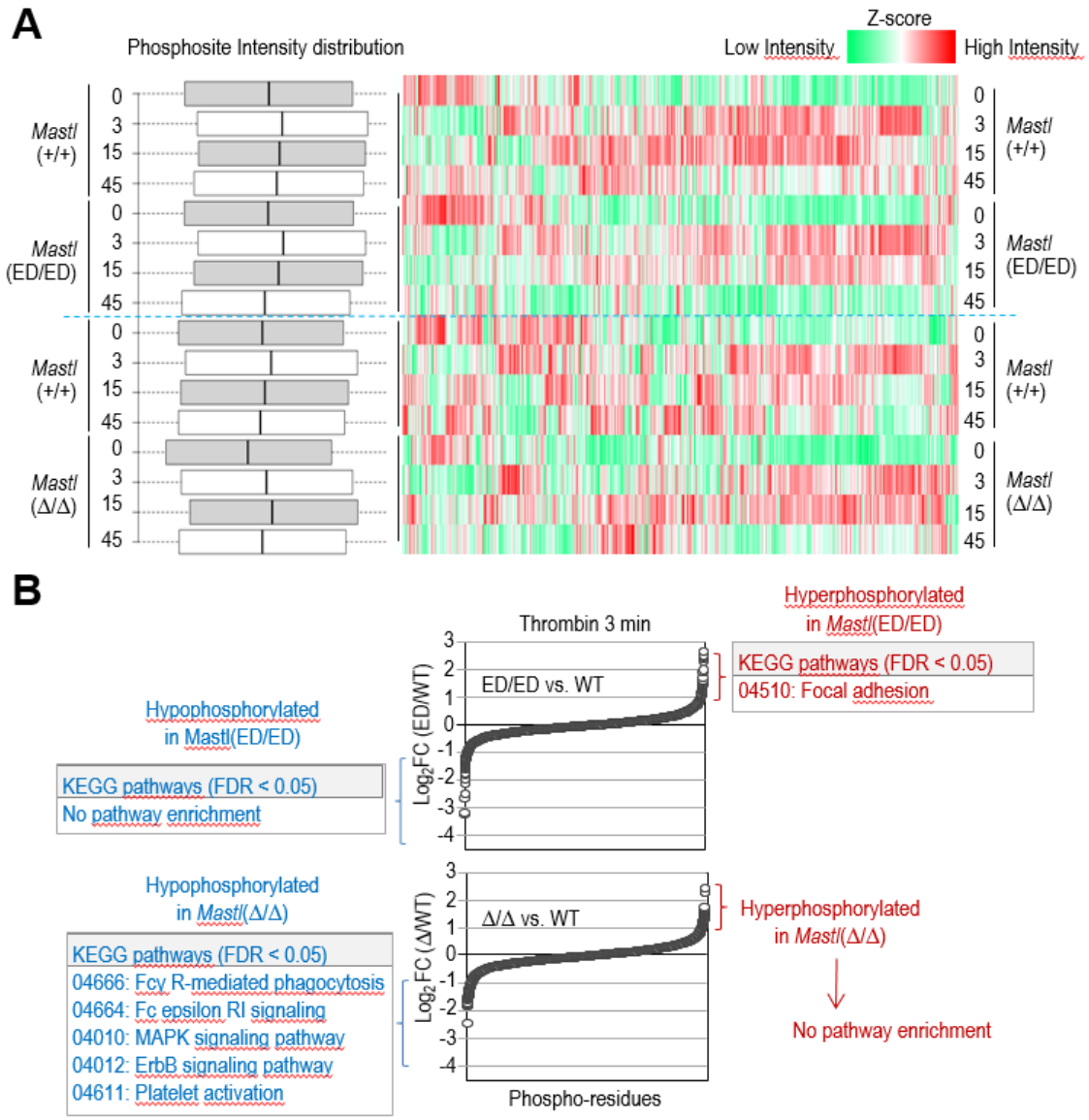


Figure S4. Phospho-proteomic analysis in Mastl mutant platelets after thrombin activation. A, Phosphosite intensity distribution in resting (0 min) and activated (0.05 IU/mL of thrombin for 3, 15 and 45 min) platelets from mice with the indicated genotypes. Left, box-plots of the reporter log_2 intensity distribution of phosphosites; right, heat-map visualizing relative intensity values (Z-score transformed) of all identified phosphosites in their corresponding genotype and time-point. Sites were clustered with Perseus software using Euclidean distance and complete linkage. B, KEGG pathway enrichment in Mastl mutant platelets. Plots represent the fold change (expressed as log2) of the phosphorylation of each single peptide in *Mastl*(ED/ED) (above) or *Mastl*(Δ/Δ) (below) versus *Mastl*(+/+) platelets, activated 3 minutes with 0.05 IU/mL of thrombin. Boxes indicate significant enrichment of KEGG pathways in hyperphosphorylated peptides (Fold change cut-off ≥ 0.75) and hypophosphorylated peptides (Fold change cut-off ≤ -0.75), using the mouse platelet proteome as background. FDR= False discovery rate.

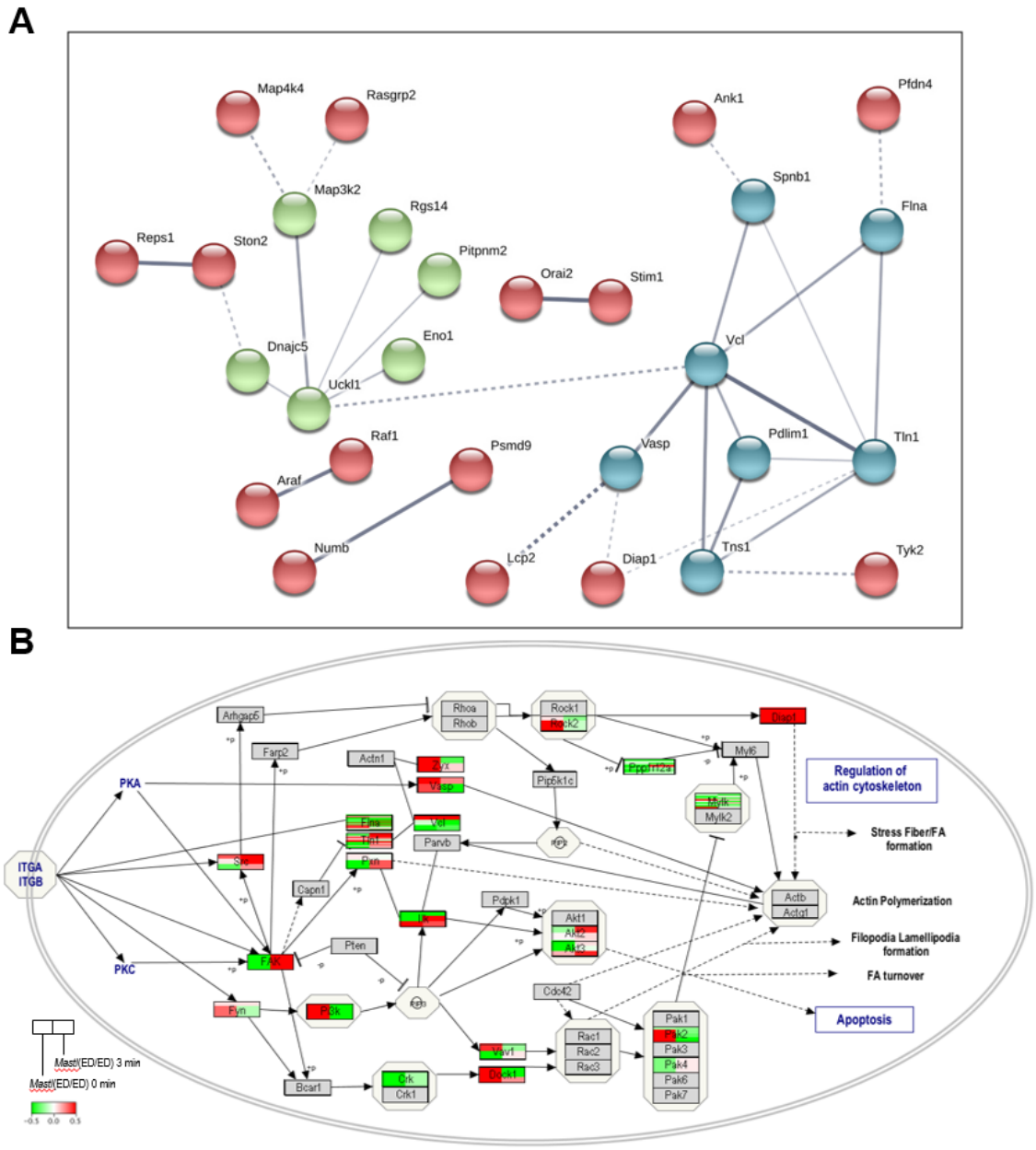


Figure S5. Signaling pathways differentially phosphorylated in Mastl mutant platelets. A, STRING Network analysis of proteins hyperphosphorylated at t=0 and t=3 min in *Mastl*(ED/ED) platelets. Proteins with at least one hyperphosphorylated site ($log_2FC \geq 0.5$) in 0 or 3 min was included. A confidence of 0.7 was used for the network construction and 3 k-means clustering was applied. B, Visualization of the *Mastl*(ED/ED) phosphosites in proteins involved in a simplified focal adhesion pathway (Wikipathway: WP85) using PathVisio. Each rectangle represents one protein and each row inside represents the changes [left half, 0 min (resting); right half, 3 min after activation] in a specific phosphosite.

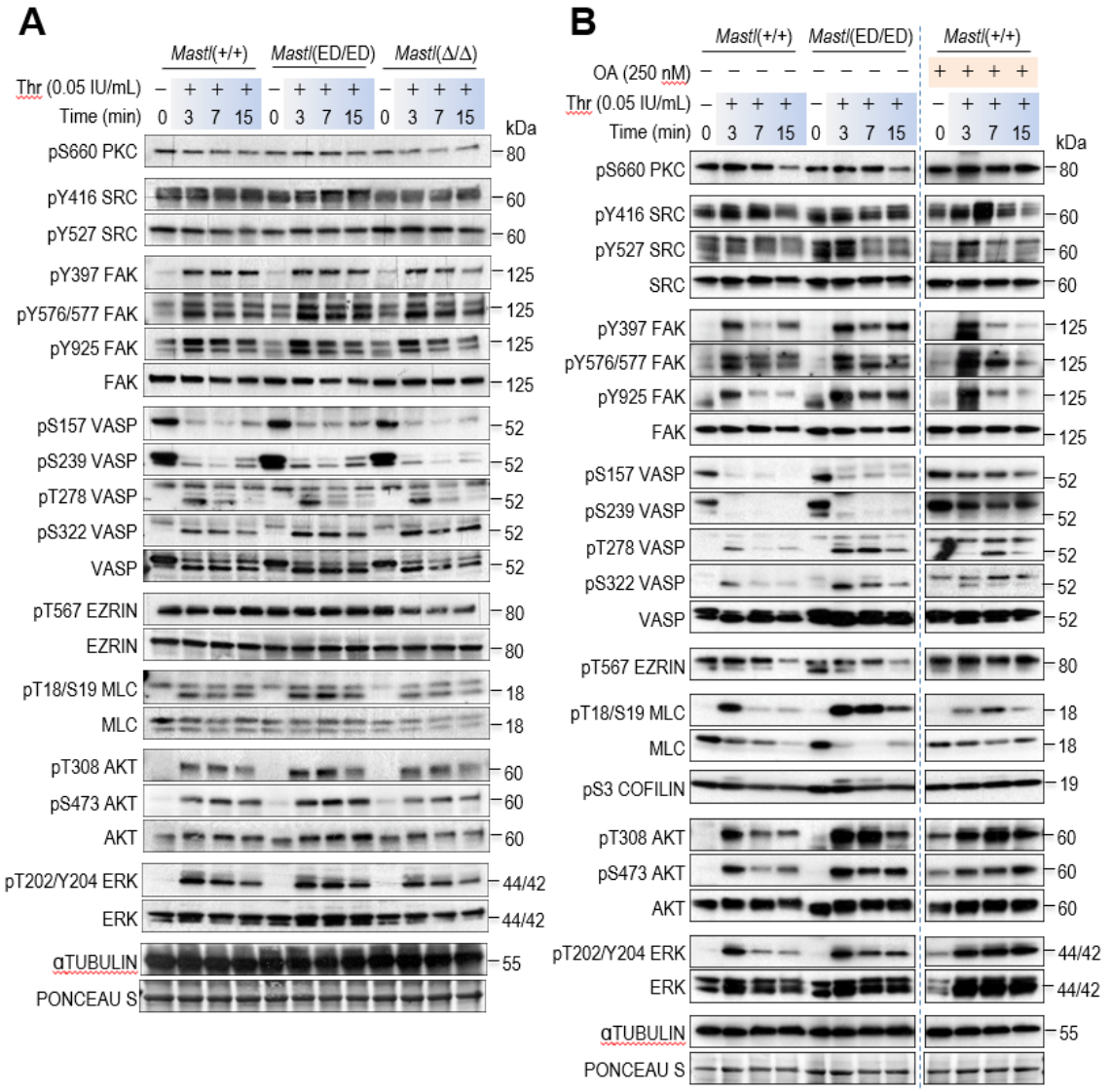
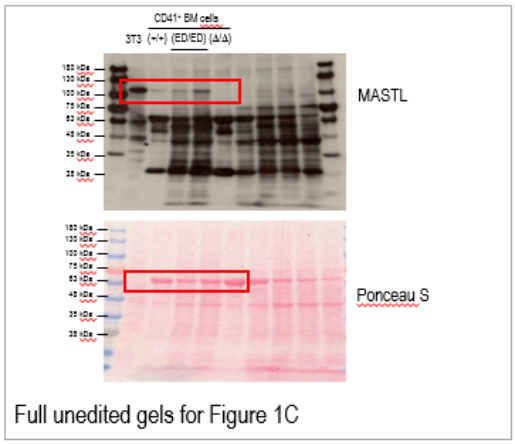


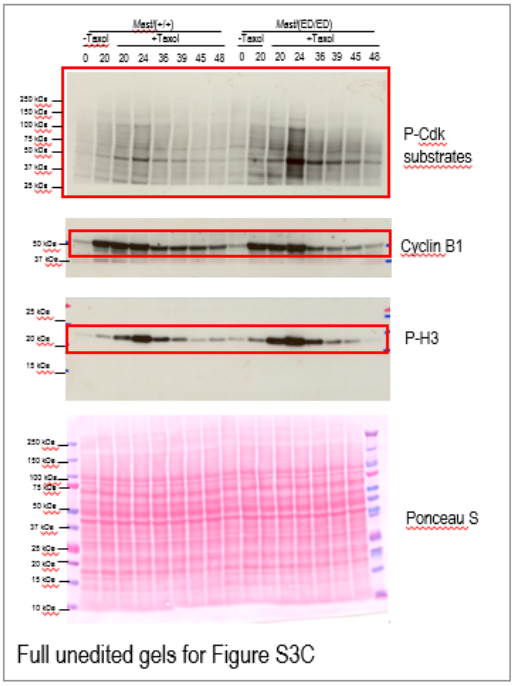
Figure S6. Changes in the phosphorylation status of signaling molecules in Mastl-mutant platelets. A, Validation of phosphoproteomic results in both *Mastl*(ED/ED) and *Mastl*(Δ/Δ) platelets by immunoblot analysis of total protein extracts from washed platelets, in resting (0 min) and activated (3, 7 or 15 min) conditions. B, Phosphorylation of molecules involved in focal adhesion and actin dynamics in *Mastl*(ED/ED) platelets and wild-type platelets after treatment for 15 minutes at 37 ºC with the phosphatase inhibitor okadaic acid before activation with thrombin. Pools of platelets from 3 mice per genotype were used in each condition. Images are representative of three separate experiments. Antibodies are referred to by their commercial name and the phosphoresidues correspond to the human nomenclature.

Figure S7. Full scan of blots shown in the manuscript.

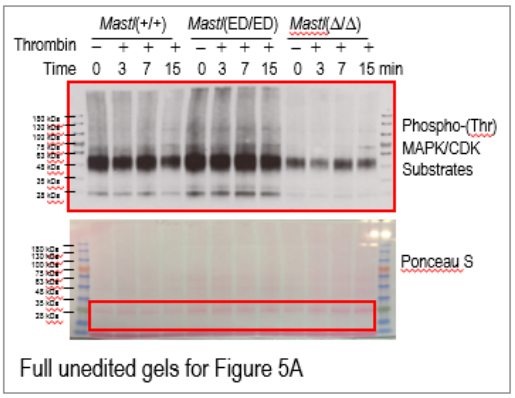
Fig. S7, part 1



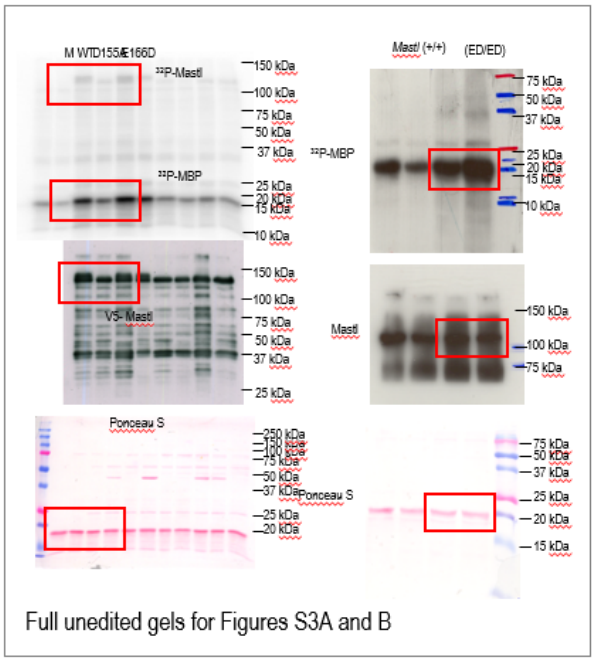
Full unedited gels for Figure 1C



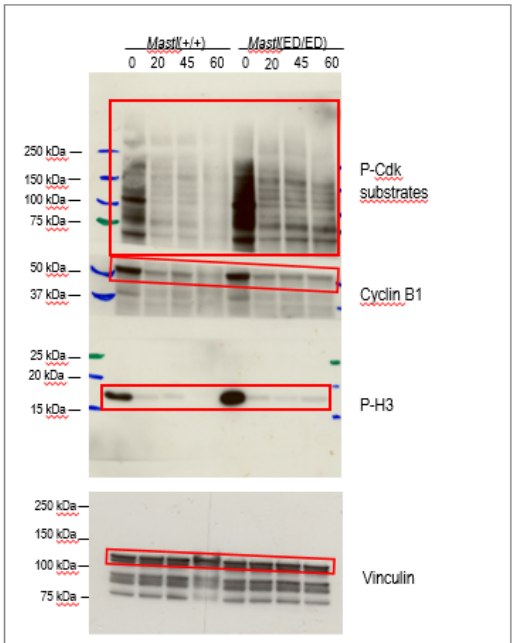
Full unedited gels for Figure S3C



Full unedited gels for Figure 5A

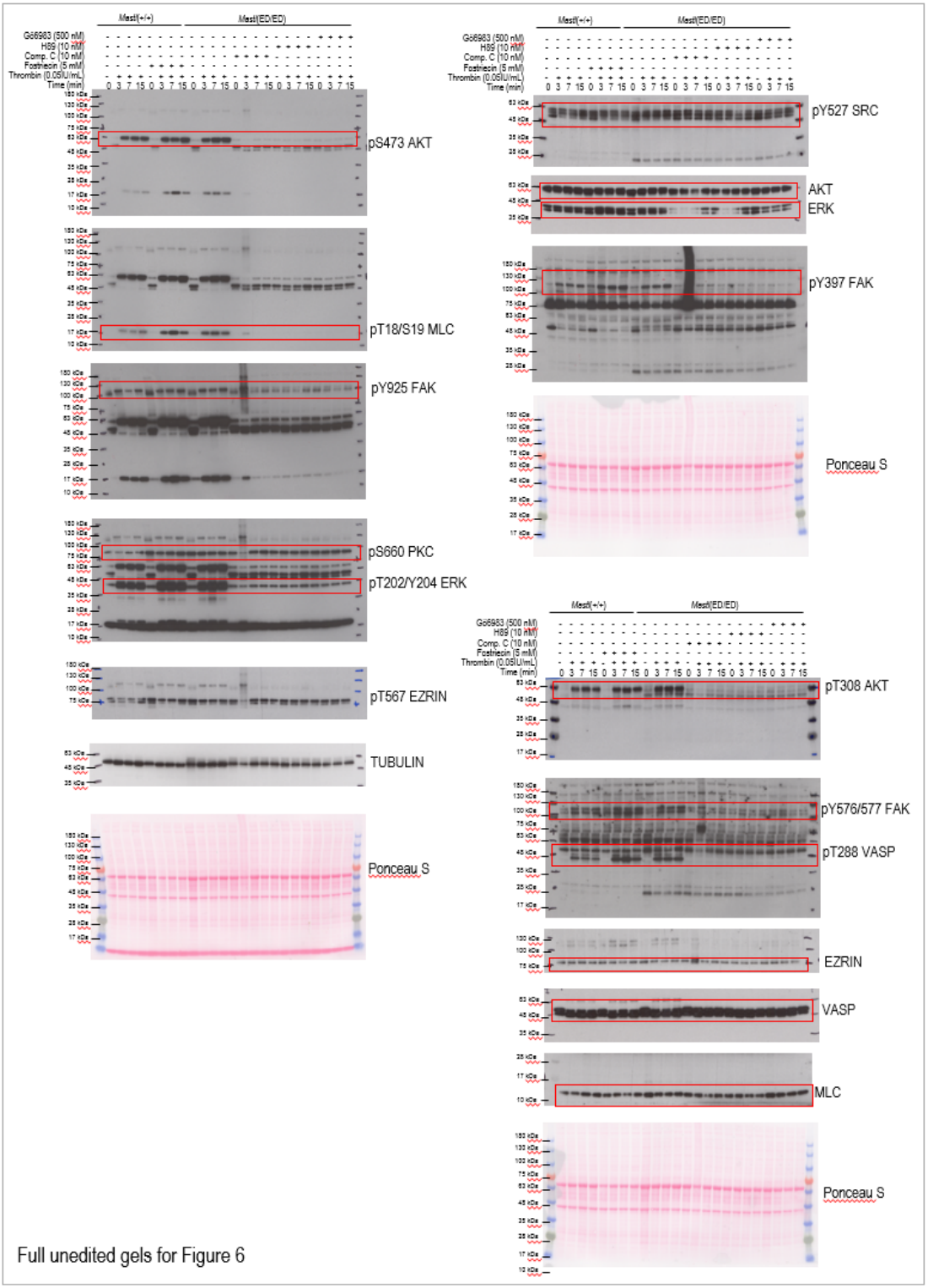


Full unedited gels for Figures S3A and B



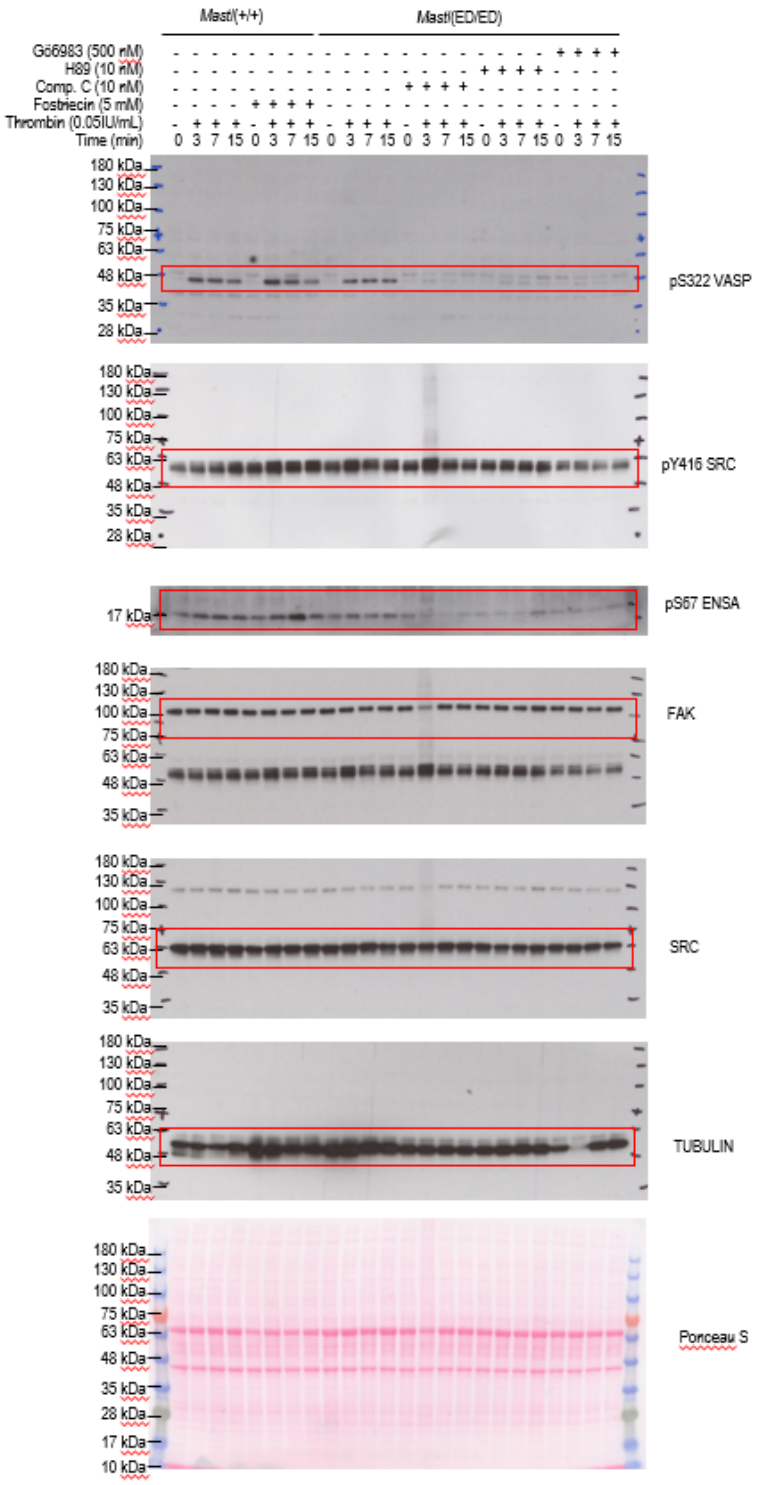
Full unedited gels for Figure S3D

Fig. S7, part 2



Full unedited gels for Figure 6

Fig. S7, part 3



Full unedited gels for Figure 6 (cont.)

Fig. S7, part 4

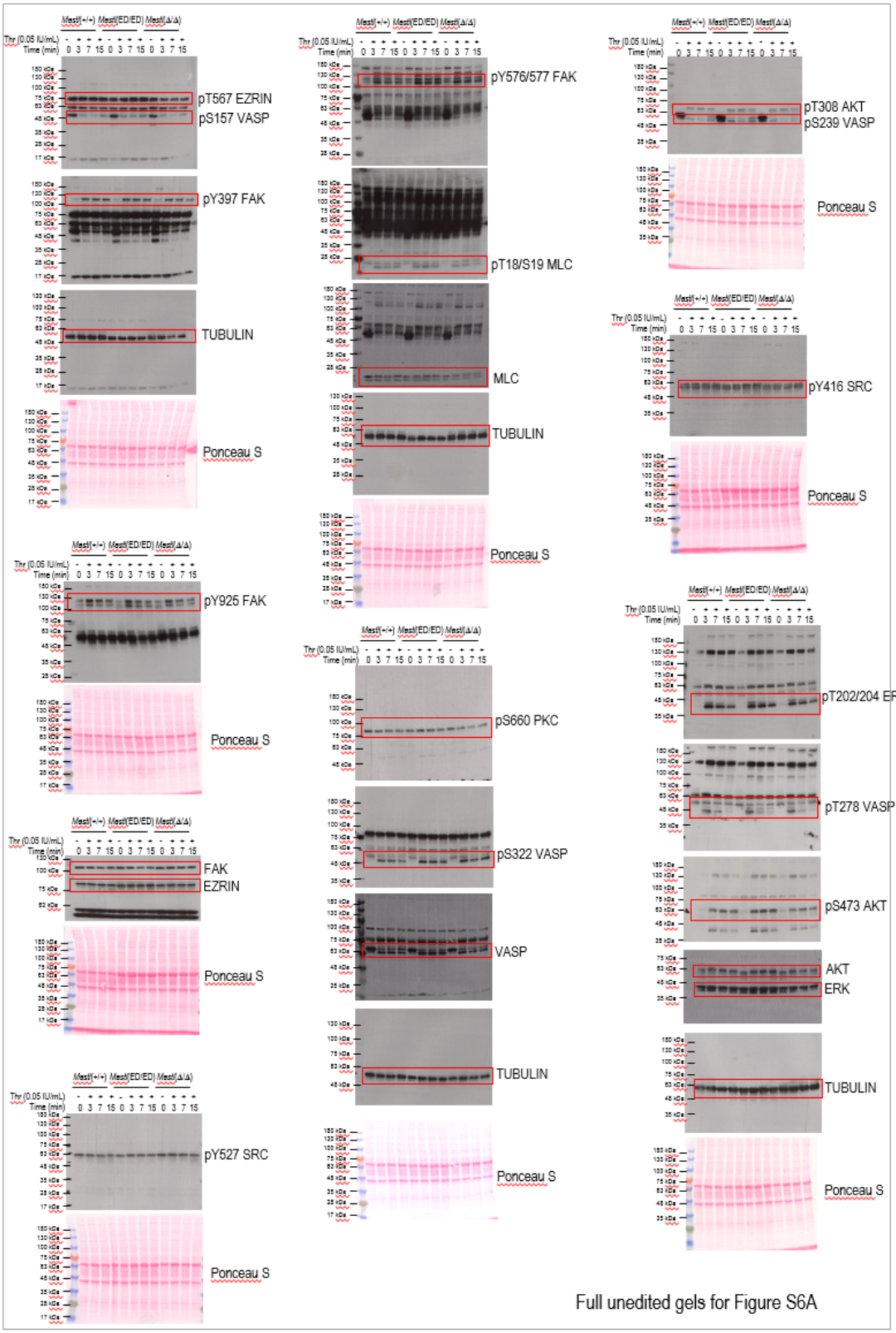
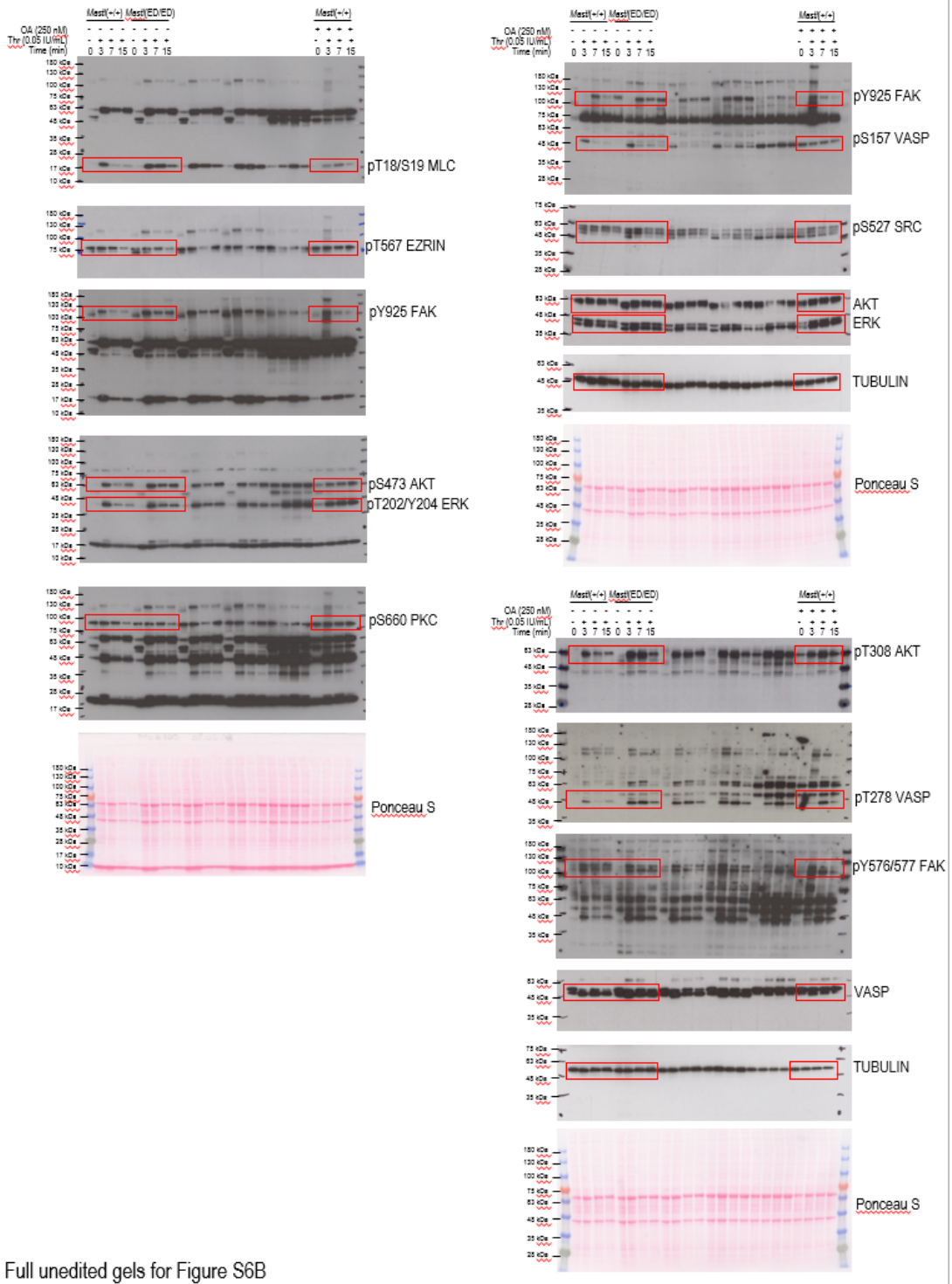
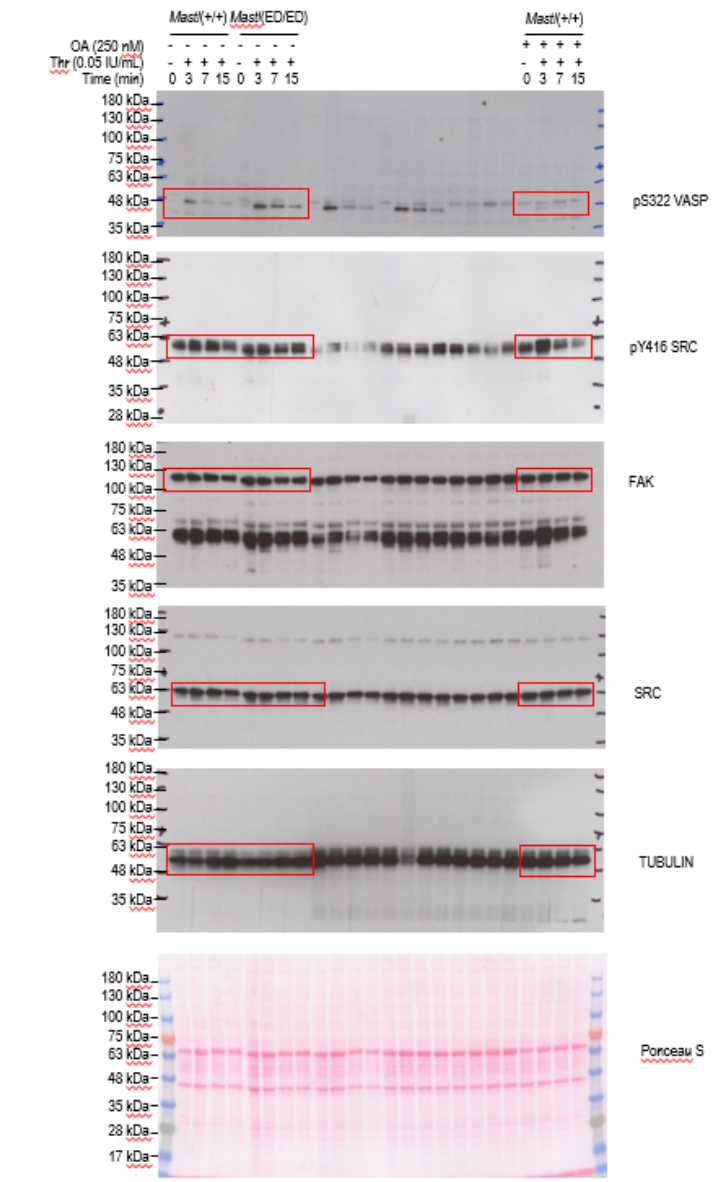

Mast(+/+) Mast(ED/ED) Mast(Δ/Δ)
Thr (0.05 IU/mL)
Time (min)
0 3 7 15 0 3 7 15 0 3 7 15
pT567 EZRIN
pS157 VASP
pY397 FAK
TUBULIN
Ponceau S
pY576/577 FAK
pT18/S19 MLC
MLC
TUBULIN
Ponceau S
pT308 AKT
pS239 VASP
Ponceau S
pY416 SRC
Ponceau S
pY925 FAK
Ponceau S
pS660 PKC
pS322 VASP
VASP
TUBULIN
Ponceau S
pT202/204 ERK
pT278 VASP
pS473 AKT
AKT
ERK
TUBULIN
Ponceau S
FAK
EZRIN
Ponceau S
pY527 SRC
Ponceau S
Full unedited gels for Figure S6A

Fig. S7, part 5



Full unedited gels for Figure S6B

Fig. S7, part 6



Full unedited gels for Figure S6B (cont)

SUPPLEMENTARY TABLES

Supplementary Table 1. Full data from phospho-proteomic studies.

See accompanying Excel file.

Supplementary Table 2. KEGG pathways enriched (FDR>0.05) in hyperphosphorylated proteins in resting *Mastl*(ED/ED) platelets (log_2FC ED/WT>0.75), considering as statistical background the mouse platelet proteome.

Pathway	Description	OGC[1]	FDR[2]	Matching proteins
4012	ErbB signaling pathway	8	0.00132	Cbl,Map2k2,Pak1,Pak2,Pak3,Pik3r1,Prkca,Raf1
4510	Focal adhesion	11	0.00132	Diap1,Dock1,Flna,Pak1,Pak2,Pak3,Pik3r1,Prkca,Raf1,Tln1,Zyx
5211	Renal cell carcinoma	7	0.00132	Map2k2,Pak1,Pak2,Pak3,Pik3r1,Ptpn11,Raf1
4270	Vascular smooth muscle contraction	7	0.00546	Adcy6,Adcy9,Cald1,Map2k2,Mrvi1,Prkca,Raf1
4650	Natural killer cell mediated cytotoxicity	7	0.00546	Map2k2,Pak1,Pik3r1,Prkca,Ptpn11,Raf1,Syk
4810	Regulation of actin cytoskeleton	10	0.00546	Diap1,Dock1,Map2k2,Myh9,Pak1,Pak2,Pak3,Pik3r1,Raf1,Wasf2
4660	T cell receptor signaling pathway	7	0.0063	Cbl,Map2k2,Pak1,Pak2,Pak3,Pik3r1,Raf1
4010	MAPK signaling pathway	9	0.00689	Arrb1,Flna,Map2k2,Map4k1,Map4k4,Pak1,Pak2,Prkca,Raf1
5205	Proteoglycans in cancer	9	0.00689	Cbl,Eif4b,Flna,Map2k2,Pak1,Pik3r1,Prkca,Ptpn11,Raf1
4611	Platelet activation	8	0.00978	Adcy6,Adcy9,Fermt3,Fga,Pik3r1,Snap23,Syk,Tln1
4916	Melanogenesis	5	0.00978	Adcy6,Adcy9,Map2k2,Prkca,Raf1
4910	Insulin signaling pathway	8	0.0101	Cbl,Fasn,Map2k2,Pik3r1,Prkaa1,Prkar2b,Pygm,Raf1
5223	Non-small cell lung cancer	5	0.0101	Foxo3,Map2k2,Pik3r1,Prkca,Raf1
4022	cGMP-PKG signaling pathway	7	0.0131	Adcy6,Adcy9,Map2k2,Mrvi1,Pde5a,Pik3r1,Raf1
4921	Oxytocin signaling pathway	7	0.0141	Adcy6,Adcy9,Map2k2,Pik3r1,Prkaa1,Prkca,Raf1

4014	Ras signaling pathway	8	0.0162	Map2k2,Pak1,Pak2,Pak3,Pik3r1,Prkca,Ptpn11,Raf1
4151	PI3K-Akt signaling pathway	9	0.0168	Eif4b,Foxo3,Map2k2,Pik3r1,Pkn1,Prkaa1,Prkca,Raf1,Syk
4068	FoxO signaling pathway	6	0.0197	Foxo3,Homer3,Map2k2,Pik3r1,Prkaa1,Raf1
4015	Rap1 signaling pathway	7	0.0261	Adcy6,Adcy9,Map2k2,Pik3r1,Prkca,Raf1,Tln1
4062	Chemokine signaling pathway	7	0.0261	Adcy6,Adcy9,Arrb1,Foxo3,Pak1,Pik3r1,Raf1
4664	Fc epsilon RI signaling pathway	5	0.0261	Map2k2,Pik3r1,Prkca,Raf1,Syk
4666	Fc gamma R-mediated phagocytosis	6	0.0261	Pak1,Pik3r1,Prkca,Raf1,Syk,Wasf2
4750	Inflammatory mediator regulation of TRP channels	5	0.0261	Adcy6,Adcy9,Alox12,Pik3r1,Prkca
4912	GnRH signaling pathway	5	0.0261	Adcy6,Adcy9,Map2k2,Prkca,Raf1
5213	Endometrial cancer	4	0.0261	Foxo3,Map2k2,Pik3r1,Raf1
5220	Chronic myeloid leukemia	5	0.0261	Cbl,Map2k2,Pik3r1,Ptpn11,Raf1
4961	Endocrine and other factor-regulated calcium reabsorption	4	0.0277	Adcy6,Adcy9,Cltc,Prkca
5100	Bacterial invasion of epithelial cells	5	0.0389	Cbl,Cltc,Dock1,Pik3r1,Wasf2
4915	Estrogen signaling pathway	5	0.0419	Adcy6,Adcy9,Map2k2,Pik3r1,Raf1
4917	Prolactin signaling pathway	4	0.0419	Foxo3,Map2k2,Pik3r1,Raf1
4540	Gap junction	5	0.0432	Adcy6,Adcy9,Map2k2,Prkca,Raf1
5032	Morphine addiction	4	0.0432	Adcy6,Adcy9,Arrb1,Prkca

[1]OGC, observed gene count
[2]FDR, False discovery rate.

Supplementary Table 3. KEGG pathways enriched (FDR>0.05) in hypophosphorylated proteins in resting *Mastl*(Δ/Δ) platelets (log_2FC ΔΔ/WT<0.75), considering as statistical background the mouse platelet proteome.

Pathway	Description	OGC[1]	FDR[2]	Matching proteins
4012	ErbB signaling pathway	8	0.00132	Cbl,Map2k2,Pak1,Pak2,Pak3,Pik3r1,Prkca,Raf1
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4921	Oxytocin signaling pathway	7	0.0141	Adcy6,Adcy9,Map2k2,Pik3r1,Prkaa1,Prkca,Raf1
4014	Ras signaling pathway	8	0.0162	Map2k2,Pak1,Pak2,Pak3,Pik3r1,Prkca,Ptpn11,Raf1
4151	PI3K-Akt signaling pathway	9	0.0168	Eif4b,Foxo3,Map2k2,Pik3r1,Pkn1,Prkaa1,Prkca,Raf1,Syk
4068	FoxO signaling pathway	6	0.0197	Foxo3,Homer3,Map2k2,Pik3r1,Prkaa1,Raf1
4015	Rap1 signaling pathway	7	0.0261	Adcy6,Adcy9,Map2k2,Pik3r1,Prkca,Raf1,Tln1
4062	Chemokine signaling pathway	7	0.0261	Adcy6,Adcy9,Arrb1,Foxo3,Pak1,Pik3r1,Raf1
4664	Fc epsilon RI signaling pathway	5	0.0261	Map2k2,Pik3r1,Prkca,Raf1,Syk

4666	Fc gamma R-mediated phagocytosis	6	0.0261	Pak1,Pik3r1,Prkca,Raf1,Syk,Wasf2
4750	Inflammatory mediator regulation of TRP channels	5	0.0261	Adcy6,Adcy9,Alox12,Pik3r1,Prkca
4912	GnRH signaling pathway	5	0.0261	Adcy6,Adcy9,Map2k2,Prkca,Raf1
5213	Endometrial cancer	4	0.0261	Foxo3,Map2k2,Pik3r1,Raf1
5220	Chronic myeloid leukemia	5	0.0261	Cbl,Map2k2,Pik3r1,Ptpn11,Raf1
4961	Endocrine and other factor-regulated calcium reabsorption	4	0.0277	Adcy6,Adcy9,Cltc,Prkca
5100	Bacterial invasion of epithelial cells	5	0.0389	Cbl,Cltc,Dock1,Pik3r1,Wasf2
4915	Estrogen signaling pathway	5	0.0419	Adcy6,Adcy9,Map2k2,Pik3r1,Raf1
4917	Prolactin signaling pathway	4	0.0419	Foxo3,Map2k2,Pik3r1,Raf1
4540	Gap junction	5	0.0432	Adcy6,Adcy9,Map2k2,Prkca,Raf1
5032	Morphine addiction	4	0.0432	Adcy6,Adcy9,Arrb1,Prkca

[1]OGC, observed gene count
[2]FDR, False discovery rate.

Supplementary Table 4. KEGG pathways enriched (FDR>0.01) in hyperphosphorylated proteins in *Mastl*(ED/ED) platelets 3 minutes after stimulation with thrombin (log_2FC ED/WT>0.75).

Pathway	Description	OGC[1]	FDR[2]	Matching proteins
4510	Focal adhesion	9	0.02	Dock1,Flna,Ilk,Pak1,Pak2,Pak3,Thbs1,Tln1,Vasp

[1]OGC, observed gene count
[2]FDR, False discovery rate.

Supplementary Table 5. KEGG pathways enriched (FDR>0.01) in hyperphosphorylated proteins in both resting and 3-min-activated *Mastl*(ED/ED) platelets (log_2FC ED/WT>0.5), considering as statistical background the mouse platelet proteome.

Pathway	Description	OGC[1]	FDR[2]	Matching proteins
4611	Platelet activation	5	0.00121	Lcp2,Rasgrp2,Stim1,Tln1,Vasp
4510	Focal adhesion	6	0.00121	Diap1,Flna,Raf1,Tln1,Vasp,Vcl
4015	Rap1 signaling pathway	6	0.00121	Lcp2,Raf1,Rasgrp2,Rgs14,Tln1,Vasp

[1]OGC, observed gene count
[2]FDR, False discovery rate.

Supplementary Table 6. KEGG pathways enriched (FDR>0.05) in hyperphosphorylated proteins in *Mastl*(ED/ED) platelets 15 minutes after stimulation with thrombin (log_2FC ED/WT>1.0), considering as statistical background the mouse platelet proteome.

Pathway	Description	OGC[1]	FDR[2]	Matching proteins
4012	ErbB signaling pathway	9	8.07E-07	Camk2g,Cbl,Gab1,Nck2,Pak1,Pak2,Pak3,Ptk2,Src
5205	Proteoglycans in cancer	11	1.99E-05	Ank1,Arhgef12,Camk2g,Cbl,Flna,Gab1,Itpr1,Pak1,Ppp1r12c,Ptk2,Src
4810	Regulation of actin cytoskeleton	10	9.29E-05	Arhgef12,Arpc1a,Pak1,Pak2,Pak3,Ppp1r12c,Ptk2,Src,Ssh3,Wasf2
4611	Platelet activation	8	0.000111	Adcy5,Arhgef12,Fermt3,Fga,Itpr1,Rasgrp2,Src,Vasp
4660	T cell receptor signaling pathway	7	0.000237	Cbl,Nck2,Pak1,Pak2,Pak3,Prkcq,Tec
5100	Bacterial invasion of epithelial cells	6	0.000363	Arpc1a,Cbl,Gab1,Ptk2,Src,Wasf2
4062	Chemokine signaling pathway	8	0.00066	Adcy5,Arrb2,Fgr,Pak1,Ptk2,Ptk2b,Rasgrp2,Src
4510	Focal adhesion	8	0.00165	Flna,Pak1,Pak2,Pak3,Ppp1r12c,Ptk2,Src,Vasp
4270	Vascular smooth muscle contraction	6	0.00314	Adcy5,Arhgef12,Cald1,Itpr1,Ppp1r12c,Prkcq
4750	Inflammatory mediator regulation of TRP channels	6	0.00314	Adcy5,Alox12,Camk2g,Itpr1,Prkcq,Src
4020	Calcium signaling pathway	7	0.00333	Atp2a3,Camk2g,Itpkb,Itpr1,Orai2,P2rx5,Ptk2b
4360	Axon guidance	6	0.00373	Arhgef12,Nck2,Pak1,Pak2,Pak3,Ptk2
4912	GnRH signaling pathway	5	0.00431	Adcy5,Camk2g,Itpr1,Ptk2b,Src
4144	Endocytosis	7	0.00744	Arfgap3,Arrb2,Cbl,Fam125b,Nedd4,Rabep1,Src

4921	Oxytocin signaling pathway	6	0.00808	Adcy5,Camk2g,Itpr1,Ppp1r12c,Prkaa1,Src
4014	Ras signaling pathway	7	0.00883	Brap,Gab1,Pak1,Pak2,Pak3,Rasa3,Rasgrp2

[1]OGC, observed gene count
[2]FDR, False discovery rate.

Supplementary Table 7. KEGG pathways enriched (FDR>0.01) in hyperphosphorylated proteins in *Mastl*(ED/ED) platelets 45 minutes after stimulation with thrombin (log_2FC ED/WT>1.0), considering as statistical background the mouse platelet proteome.

Pathway	Description	OGC[1]	FDR[2]	Matching proteins
4540	Gap junction	6	0.000116	Src,Tuba4a,Tubb2a,Tubb2b,Tubb4a,Tubb5
4145	Phagosome	7	0.00015	Dync1li1,Thbs1,Tuba4a,Tubb2a,Tubb2b,Tubb4a,Tubb5
4510	Focal adhesion	6	0.00577	Akt2,Dock1,Itga6,Src,Thbs1,Tln1

[1]OGC, observed gene count
[2]FDR, False discovery rate.

Supplementary Table 8. Antibodies used in this work.

Antigen-conjugate* [clone]	Application**	Catalog Nº	Source
α-tubulin (DM1A)	IF; WB	T9026	Sigma Aldrich
AKT	WB	9272	Cell signalling
CD117- APC-H7 [2B8]	FC	560250	BD Biosciences
CD127(IL-7R)-AlexaFluor488 [RUO]	FC	561533	BD Biosciences
CD16/CD32-PE-Cy7 [93]	FC	25-0161-81	eBioscience
CD34- PE [RAM34]	FC	551387	BD Biosciences
CD41-FITC [MWReg30]	FC	553848	BD Biosciences
CD41a-PE [JON/A]	FC	D200	Emfret Analytics
CD42c (GPIbb)-DyeLight488	i.v.; FC	X488	Emfret Analytics
CD42d-APC [1C2]	FC	17-0421-80	eBioscience
CD9-FITC [EM-04]	FC	MA1-10311	ThermoFisher
Cyclin B1	WB	sc-752	Santa Cruz
Ezrin / Radixin / Moesin	WB	3142	Cell signalling
FAK [D2R2E]	WB	13009T	Cell signalling

FVIII (Von Willebrand Factor)	IHC	A0082	DAKO
Lineage Isotype control Cocktail (Lin)-APC	FC	558074	BD Biosciences
Ly-6A/E (Sca-1) PerCP-Cy5.5 [D7]	FC	45-5981-80	eBioscience
Mastl	WB	AP14289c	AbGent
Myosin light chain	WB	3672	Cell signalling
p44/42 MAPK (Erk1/2)	WB	9102	Cell signalling
Phospho-(Thr) MAPK/CDK Substrate	WB	2321	Cell signalling
Phospho-AKT (Ser473) [D9E]	WB	4060	Cell signalling
Phospho-AKT (THR308)[C31E5E]	WB	2965S	Cell signalling
Phospho-ENSA (ser67)/ARPP19 (ser62)	WB	5240	Cell signalling
Phospho-Ezrin(Thr567)/ Radizin(Thr564) / Moesin	WB	3149	Cell signalling
Phospho-FAK (Tyr397) [D20B1]	WB	8556P	Cell signalling
Phospho-FAK (Tyr576/577)	WB	3281T	Cell signalling
Phospho-FAK (Tyr925)	WB	3284T	Cell signalling
Phospho-histone H3 (S10)	WB	06-570	Millipore
Phospho-Myosin Light Chain 2 (Thr18/Ser19)	WB	3674	Cell signalling
Phospho-p44/42 MAPK (Erk1/2) (Thr202/Tyr204)	WB	9101	Cell signalling
Phospho-PKC (pan) (βII Ser660)	WB	9371S	Cell signalling
Phospho-Ser CDK substrates	WB	2324	Cell Signaling
Phospho-SRC (Tyr527)	WB	2105	Cell signalling
Phospho-SRC Family (Tyr416) [D49G4]	WB	6943	Cell signalling
Phospho-VASP (Ser157)	WB	3111	Cell signalling
Phospho-VASP (Ser239)	WB	3114	Cell signalling
Phospho-VASP (Ser322)	WB		Dr. Peter Storz$
Phospho-VASP (Thr278)	WB	VP2781	ECM Biosciences
SRC [32G6]	WB	2123	Cell signalling
VASP [9A2]	WB	3132	Cell signalling

* APC: allophycocyanin; FITC: Fluorescein-5-isothiocyanate; PE: phycoerythrin; PerCP-Cy5.5: peridinin chlorophyll Protein Cyanin 5.5; APC-H7: allophycocyanin-H7; PE-Cy7: phycoerythrin-cyanin 7

** FC: Flow Cytometry; i.v.: in vivo; WB: Western Blot; IHC: Immunohostochemistry

$ Mayo Foundation for Medical Education and Research (MFMER), Jacksonville, Florida.