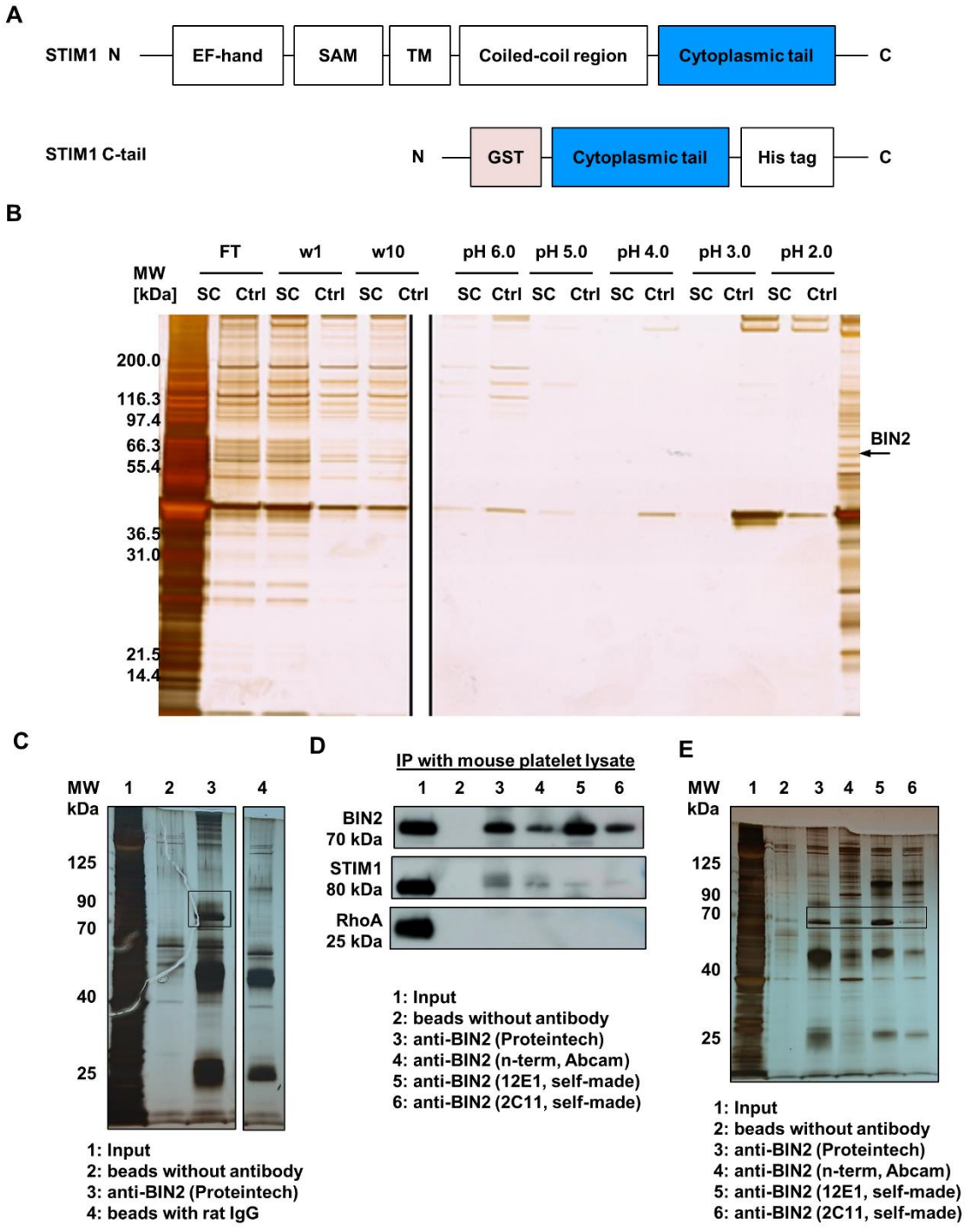
Suppl. Figure 1

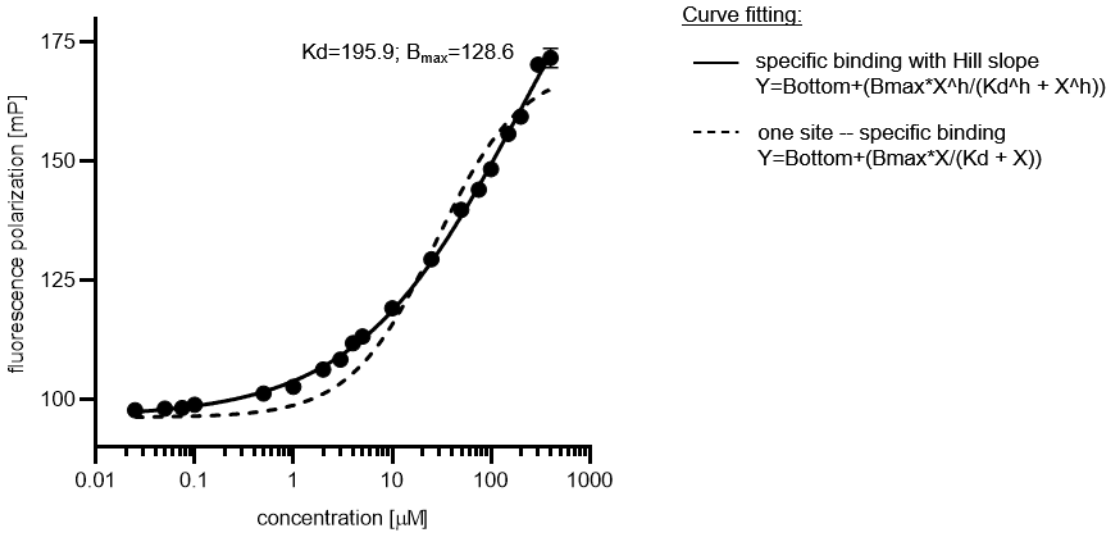


Supplemental Figure 1: Precipitation of STIM1-binding partners using the STIM1 C-tail construct

(A) Domain structure of STIM1 protein and the C-tail construct (aa 484-685) used for the immunoprecipitation. **(B)** 1D-SDS-PAGE of the flow through (FT), the wash (w) and the elution fractions of the STIM1 C-tail IP with resting human lysates. One representative of three independent experiments is shown. **(C-E)** Immunoprecipitation of BIN2 from human (C) and

mouse (D,E) platelet lysates using different BIN2 antibodies. The samples were separated in an SDS page and the proteins were visualized using (C,E) silver staining or (D) analyzed by Western blotting using the indicated antibodies. Boxed area marks the precipitated BIN2 protein. In (C) Lane 4 was run on the same gel as 1-3 but the lanes were noncontiguous.

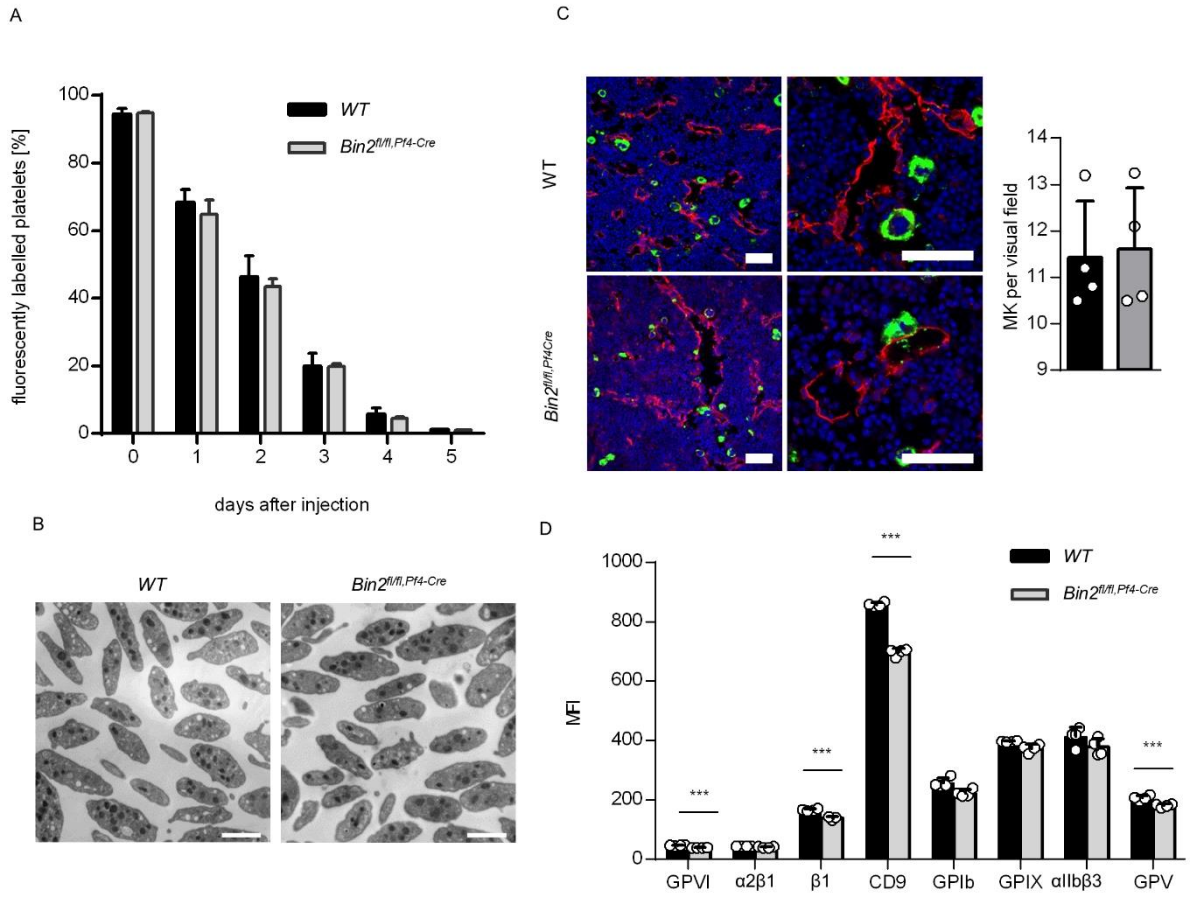
Suppl. Figure 2



Supplemental Figure 2: Determination of binding K_d between BIN2 and the STIM1 cytoplasmic tail.

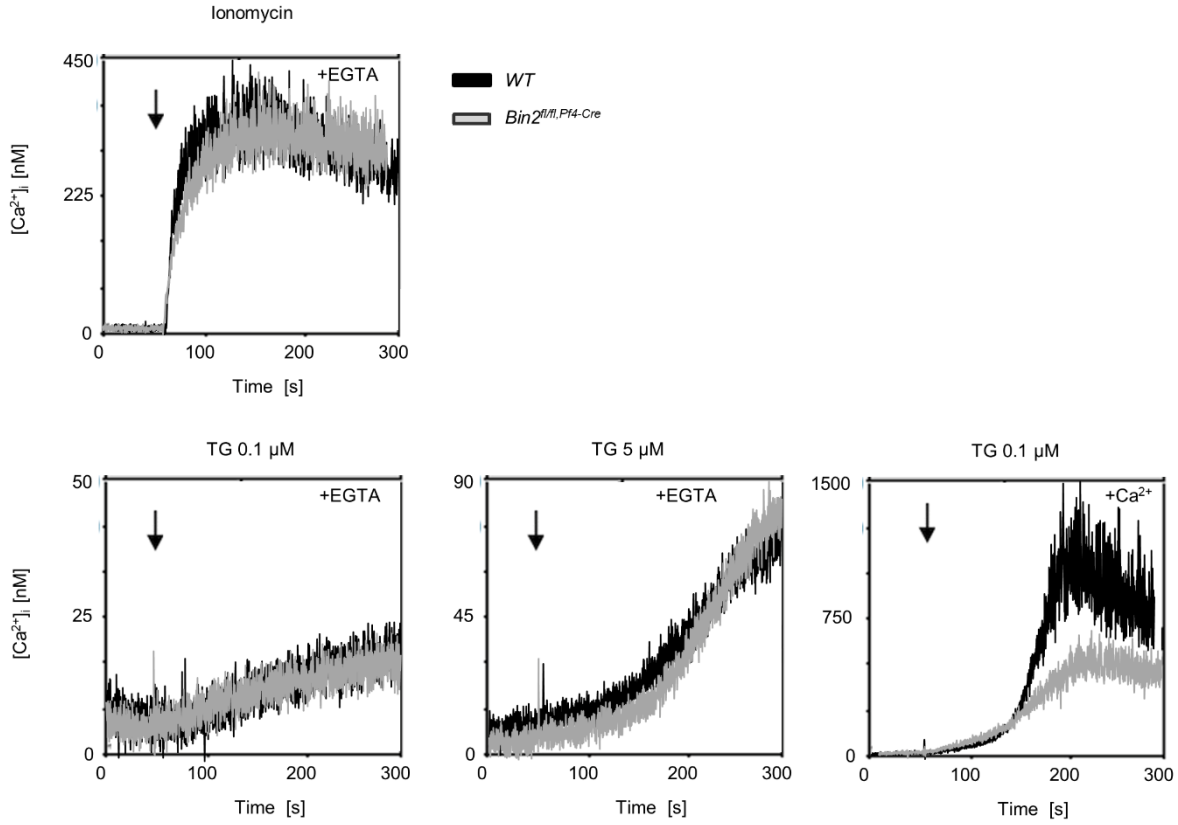
Fluorescence polarisation of 1 µM STIM1-BDP FL in the presence of the indicated concentration recombinant BIN2 was measured (mean + SD of triplicates) and the indicated curve fits performed. Bottom: fluorescence polarisation of free, unbound STIM1 (96.24); Bmax: Top-Bottom; h: Hillslope (0.5232).

Suppl. Figure 3



Supplemental Figure 3: Analysis of *Bin2*$^{fl/fl,Pf4Cre}$ platelets and megakaryocytes
(A) Platelet life span was determined by flow cytometric assessment of the percentage of fluorescently labeled platelets in *WT* and *Bin2*$^{fl/fl,Pf4-Cre}$ mice during a 5-day period after intravenous injection of a Dylight-488 conjugated anti-GPIX derivative [0.5 mg/kg] n=5. Representative of three independent experiments **(B)** Representative transmission electron microscopy (TEM) pictures of resting *WT* and *Bin2*$^{fl/fl,Pf4-Cre}$ platelets. **(C)** Cryosections of bone marrow stained for CD31 (red), GPIX (green), and DAPI (blue) and quantification of the number of megakaryocytes (MKs) per visual field. n=4, 10 visual fields counted per mouse; mean + SD is depicted. Scale bar: 50 µm **(D)** Expression of glycoproteins on the platelet surface was determined by flow cytometry. Diluted whole blood was incubated with FITC-labeled antibodies at saturating concentrations for 15 minutes at room temperature, and platelets were analyzed immediately. Data are expressed as mean fluorescence intensity (MFI) ± SD and P-values were calculated using the Mann-Whitney test with *** $p<0.001$; $n\geq4$. Representative of 3 independent experiments.

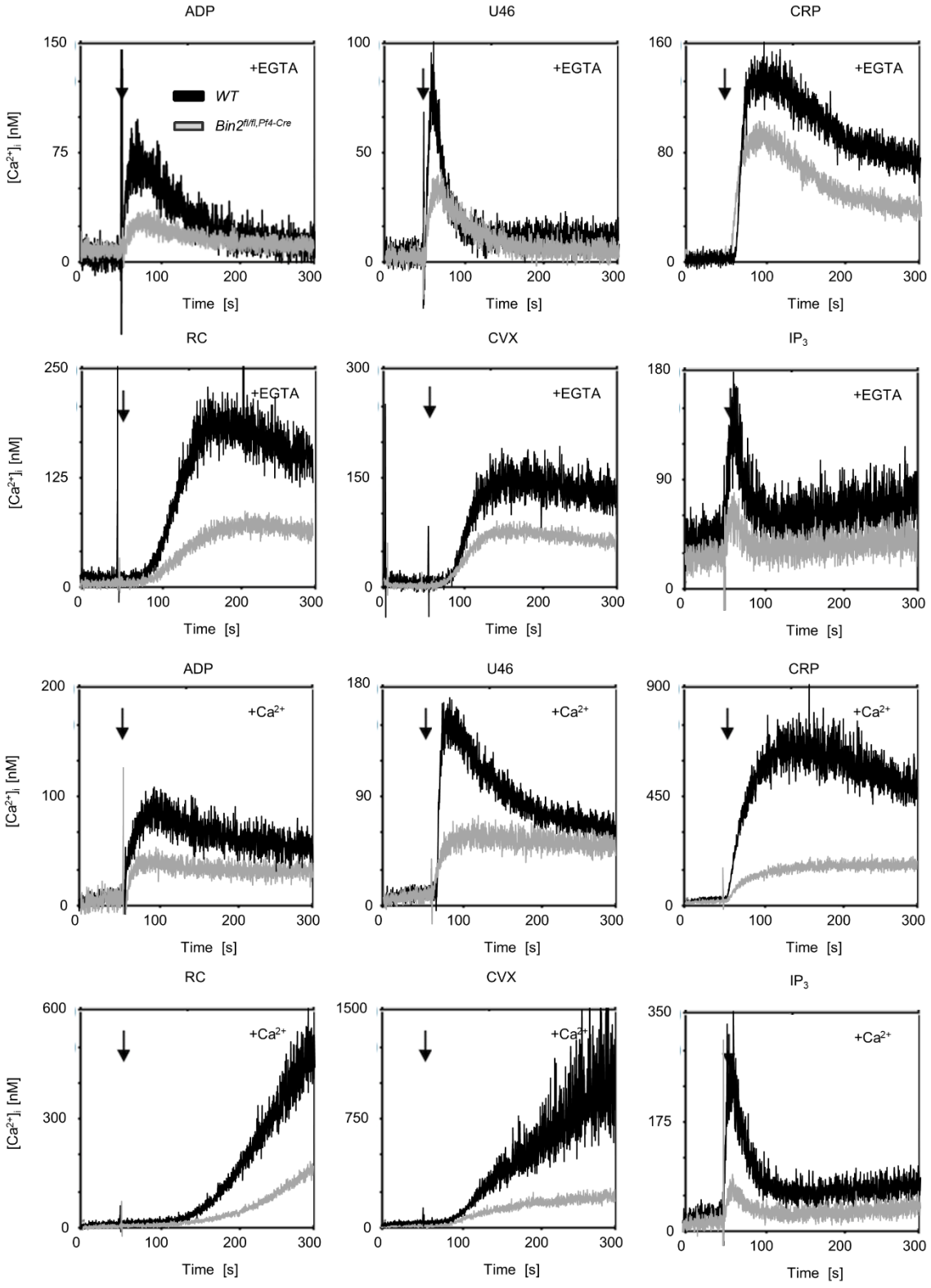
Suppl. Figure 4



Supplemental Figure 4: Representative Ca^{2+} traces

Representative Ca^{2+} traces for the statistical analysis depicted in main figure 2 B-D. Platelets were treated with ionomycin and thapsigargin in the absence (+EGTA) and with thapsigargin in the presence of Ca^{2+}.

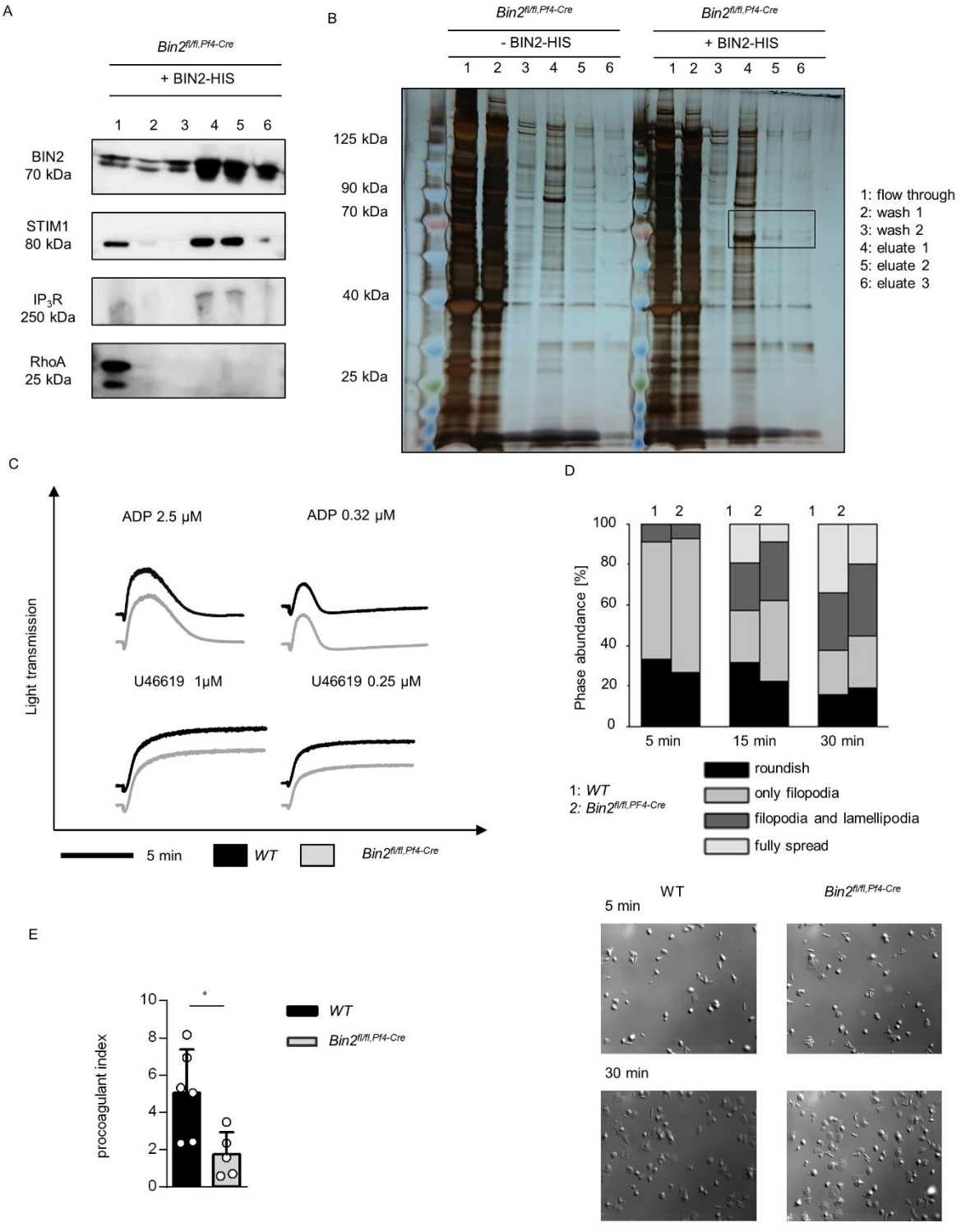
Suppl. Figure 5



Supplemental Figure 5: Representative Ca^{2+} traces

(A;B) Representative traces for the statistical analysis depicted in main figure 2 E-F showing store release (+EGTA) and Ca^{2+} influx (+Ca^{2+}) upon activation of the platelets with the indicated agonists.

Suppl. Figure 6

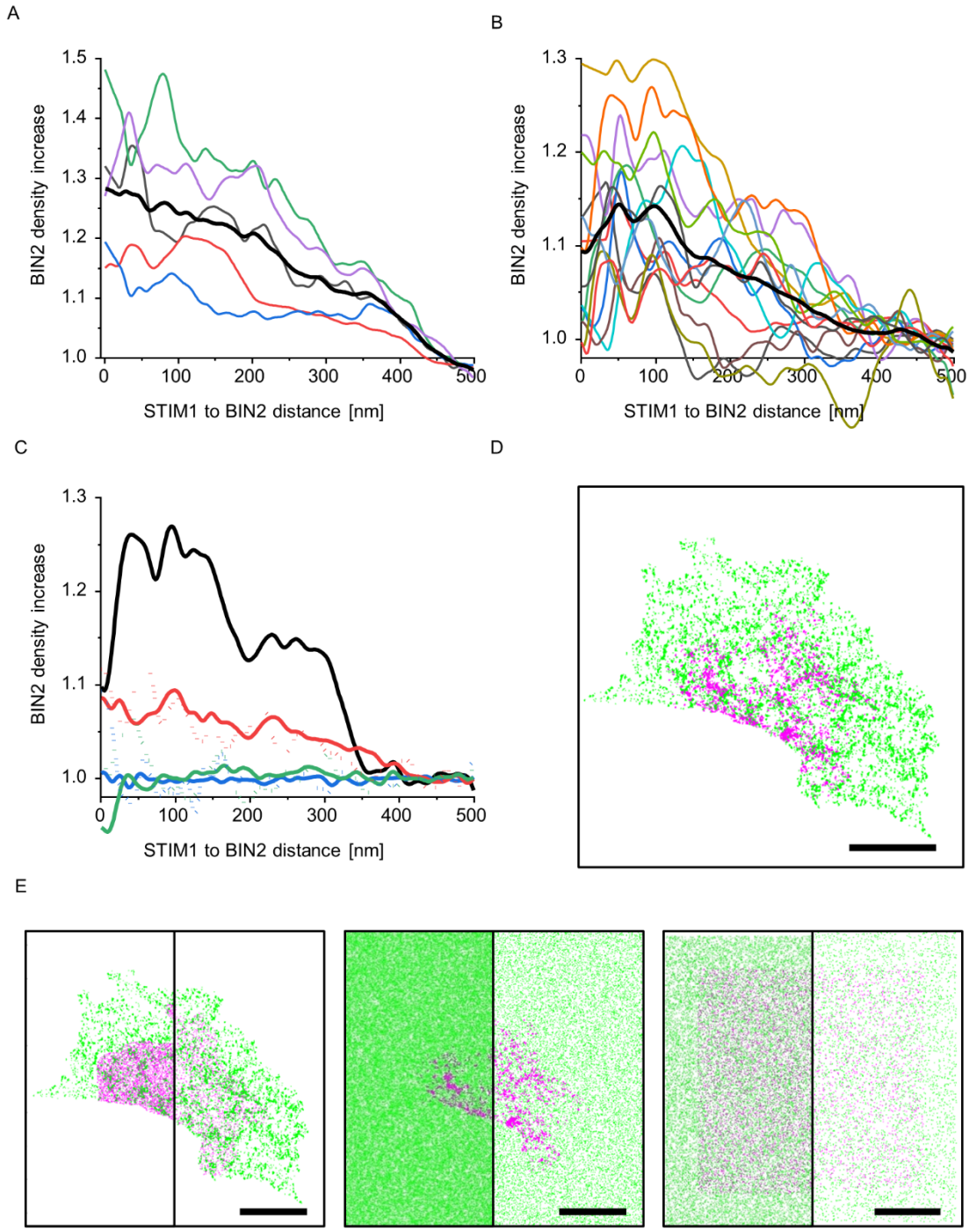


Supplemental Figure 6: Analysis of *Bin2*$^{fl/fl,Pf4Cre}$ platelet function

Bin2$^{fl/fl,Pf4Cre}$ platelet lysates were incubated with recombinant BIN2-HIS protein, followed by a purification step with NI-NTA beads. The different fractions were eluted, separated by SDS-PAGE and analyzed by **(A)** Western blotting using BIN2-, STIM1-, RhoA- and IP_3R-specific antibodies and by **(B)** silver staining. Boxed area marks the precipitated BIN2-His protein. Representative of three independent experiments. **(C)** Washed platelets were stimulated with

the indicated agonists (for ADP PRP was used), and light transmission was recorded using a four channel aggregometer. Representative results of three independent experiments. **(D)** Washed platelets of *WT* and *Bin2*$^{fl/fl,Pf4\text{-}Cre}$ mice were allowed to spread on fibrinogen for up to 30 minutes after stimulation with 0.01 U/mL thrombin. Representative images and statistical evaluation of the percentage of spread platelets at different spreading stages of 3 independent experiments are shown, n=3. **(E)** PS-exposure of platelets adhering on collagen was assessed in a flow adhesion assay at a wall shear rate of 1700 s^{-1} in the presence of 5 U/mL heparin to prevent coagulation. (n=4). Representative example of three independent experiments. Procoagulant index was defined as the ratio of surface coverage of PS-exposing platelets to the total surface covered by platelets. Values are depicted as man ± SD and P-values were calculated using the Mann-Whitney test. *P<0.05.

Suppl. Figure 7



Supplemental Figure 7: Neighbor analysis of STIM1 and BIN2

The STIM1 BIN2 co-localization was verified by localization data neighbor analysis: The STIM1 to BIN2 distance-dependent BIN2 density increase normalized to the average BIN2 density at a distance of 400 to 500 nm for **(A)** 5 individual resting platelets and **(B)** 14 individual activated platelets (colored lines) and the respective averaged BIN2 density profile (black line) show distinct peaks. **(C)** As a control the respective BIN2 density profiles of a single activated platelet (black line, STIM1 and BIN2 localizations in **D**) was compared to three test cases with random

distribution of the localization positions of STIM1 (red lines), random distribution of BIN2 localization positions (green lines) and random distributions for both, STIM1 and BIN2, localization positions (blue lines) at 100% (solid lines, left half of panel in **(E)**) and 25% of the experimentally observed localization densities (dashed lines, right half of panel in **(E)**). A random distribution of one component severely reduces the amplitude or even reduces it to the level of two randomly distributed components while a reduction of the density only leads to increased fluctuations not an increased amplitude of the density distribution. This suggests that the experimentally observed distinct peaks of the BIN2 density have to be attributed to an ordered accumulation of colocalization hotspots of STIM1 and BIN2. The respective localization data of the original data set and the three test cases are shown in **(D)** and **(E)**. **(D)** Scale bar 2 µm. **(E)** Scale bars 2 µm, 1 µm in last panel.

Supplemental Table 1. List of proteins immunoprecipitated with human STIM1 C-tail construct

Individual proteins identified in 14 of the 18 differential protein bands of the STIM1 C-tail affinity column vs. control human platelet samples; summary of LTQ XL and Qtrap 4000 nano-LC-ESI-MS/MS data of three independent biological replicates. Only protein identifications which had been verified by at least two independent corresponding peptides were accepted. Hits are listed according to corresponding protein bands. MW: molecular weight [Da]; Acc.Nr.: UniProtKB/ Swiss-Prot accession number; PB: protein band in Figure 4.14.

Accesion number	Name	Mascot Score	MW	PB
P35579	Myosin heavy chain 9	12,169	226,532	**1**
Q9Y490	Talin 1	1,078	269,767	
P08514	Integrin alpha 2b	1,719	113,377	**2**
P18206	Vinculin	984	123,799	
P06396	Gelsolin	969	85,698	**3**
Q86UX7	Unc-112-related protein 2 (Kindlin 3)	921	75,953	**5**
Q9UBW5-1	Bridging integrator 2 (BIN2), isoform 1	179	61,874	
P06576	ATP synthase subunit beta, precursor	1,482	56,56	**7**
P36542	ATP synthase subunit gamma	1,095	32,996	
P24539	ATP synthase subunit b	920	28,909	
P60709	Beta-actin	4,038	41,737	**8**
P68133	Alpha-actin 1, skeletal muscle	2,077	42,051	
Q9NYL9	Tropomodulin 3	229	39,595	
P68032	Alpha-actin, cardiac muscle	2,077	42,019	**9**
P63261	Gamma-actin	5,165	41,793	**10**
P52907	F-actin capping protein subunit alpha 1	266	32,923	
P67936-2	Tropomyosin alpha 4 chain, isoform 2	197	32,723	
Q15404	Ras suppressor protein 1	691	31,54	**11**
P09493-7	Tropomyosin alpha 1 chain, isoform 7	504	32,678	
Q9H4B7	Tubulin beta 1 chain	97	50,327	
P62258	14-3-3 protein epsilon	770	29,174	**12**
P61224	Ras-related protein Rap-1B, precursor	1,320	20,825	**15**
P19105	Myosin regulatory light chain 2, nonsarcomeric	1,829	19,794	**16**
P24844	Myosin regulatory light chain 2, smooth muscle isoform	1,528	19,827	
P61586	Transforming protein RhoA, precursor	490	21,768	**17**
P23528	Cofilin 1, non-muscle isoform	253	18,502	
P60660	Myosin light polypeptide 6	1,169	16,93	**18**

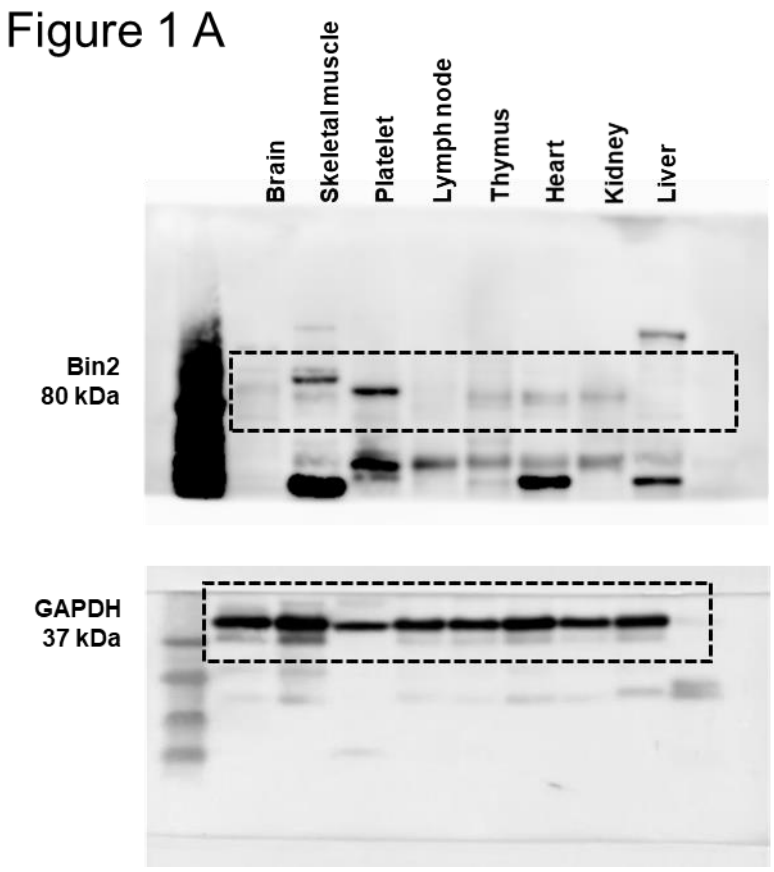
Supplemental Table 2. Basic blood and platelet parameters of *Bin2*$^{-/-}$ mice. Platelet count and size and basic blood parameters of *Bin2*$^{-/-}$ mice against the corresponding *WT* control were analyzed using a blood cell counter (Scil vet). n=4-5, Plt: Platelets; MPV: mean platelet volume; WBC: white blood cells; HCT: hematocrit. Sig.= significance. Test for significance was performed with unpaired Student's t-test; ns: not significant

	WT	***Bin2*$^{-/-}$**	**Sig.**
Plt [x10^3/µl]	928.9 ± 40.01	935.4 ± 96.2	ns
MPV [fl]	5.72 ± 0.1	6.06 ± 0.39	ns
WBC [x10^3/µl]	3.54 ± 1.01	4.08 ± 2.16	ns
RBC [x10^6/µl]	9.67 ± 0.27	9.2 ± 1.28	ns
HGB [g/dl]	14.5 ± 0.41	14.8 ± 0.29	ns
HCT [%]	46.20 ± 1.48	44.46 ± 3.55	ns

Supplemental Table 3. Basic blood and platelet parameters of *Bin2*$^{fl/fl,Pf4Cre}$ mice. Platelet count and size and basic blood parameters of *Bin2*$^{fl/fl,Pf4Cre}$ mice against the corresponding *WT* control were analyzed using a blood cell counter (Scil vet). n=4-5, Plt: Platelets; MPV: mean platelet volume; WBC: white blood cells; HCT: hematocrit. Sig.= significance. Test for significance was performed with unpaired Student's t-test; ns: not significant

	WT	***Bin2*$^{fl/fl,Pf4-Cre}$**	**Sig.**
Plt [x10^3/µl]	830.2 ± 51.44	821.6 ± 116.48	ns
MPV [fl]	6.98 ± 0.08	6.8 ± 0.21	ns
WBC [x10^3/µl]	8.68 ± 1.73	8.26 ± 2.81	ns
RBC [x10^6/µl]	8.032 ± 0.38	8.34 ± 0.71	ns
HGB [g/dl]	15.24 ± 0.71	15.56 ± 1.02	ns
HCT [%]	45.48 ± 2.12	47.6 ± 3.59	ns

Full unedited gel for Figure 1A:



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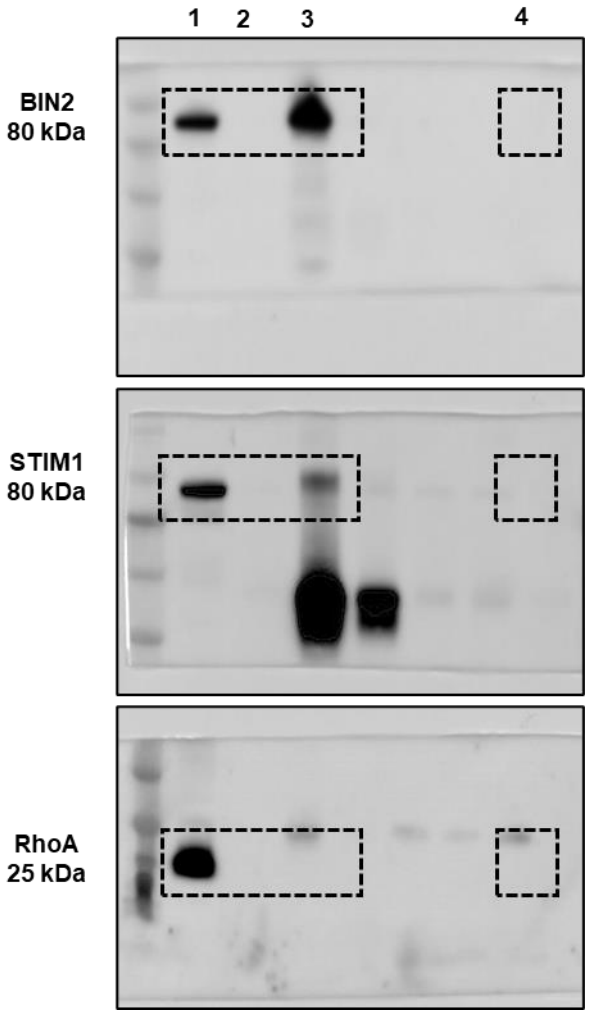
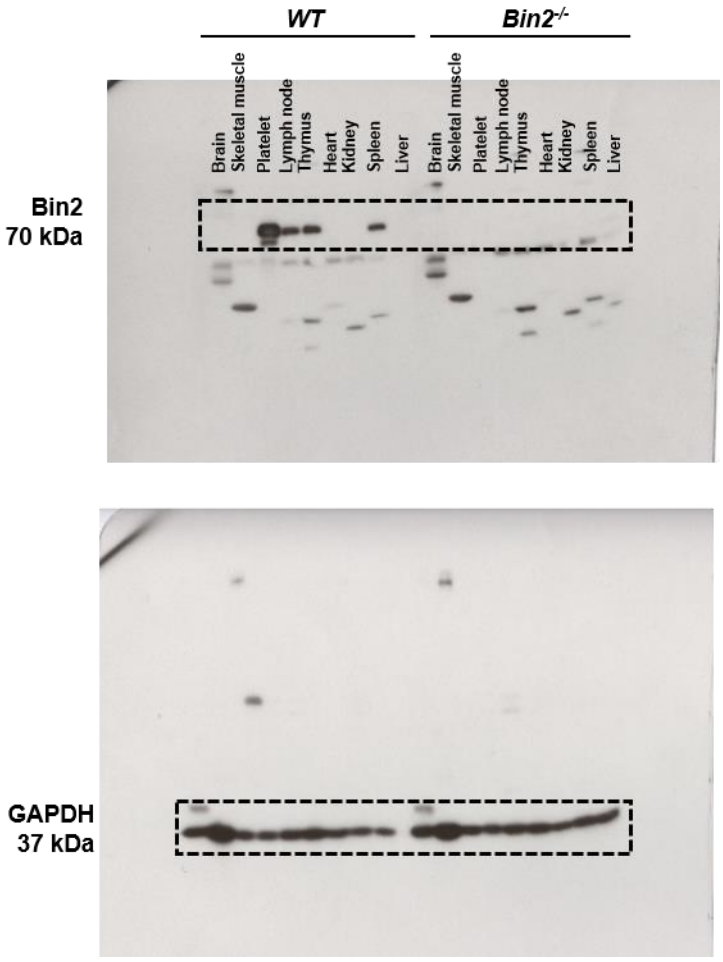


Full unedited gel for Figure 1 D:

Figure 1 D



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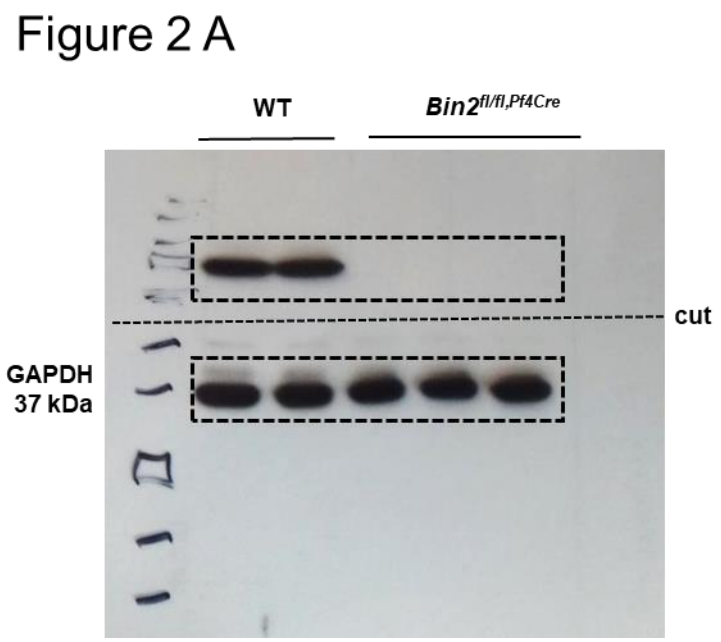

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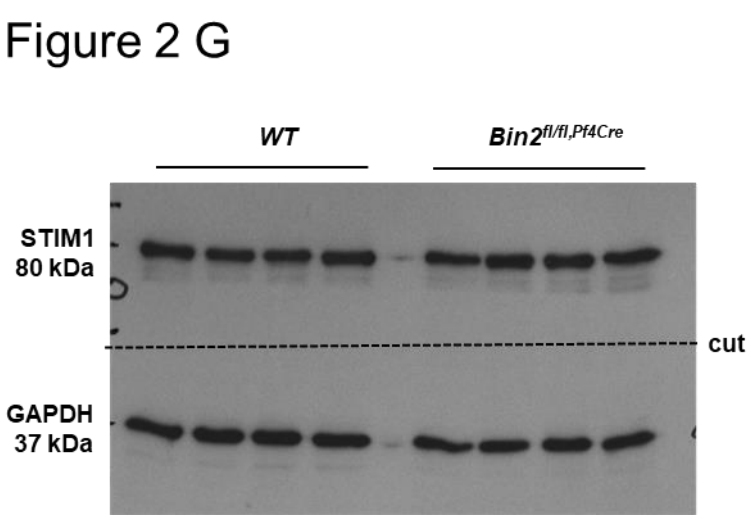

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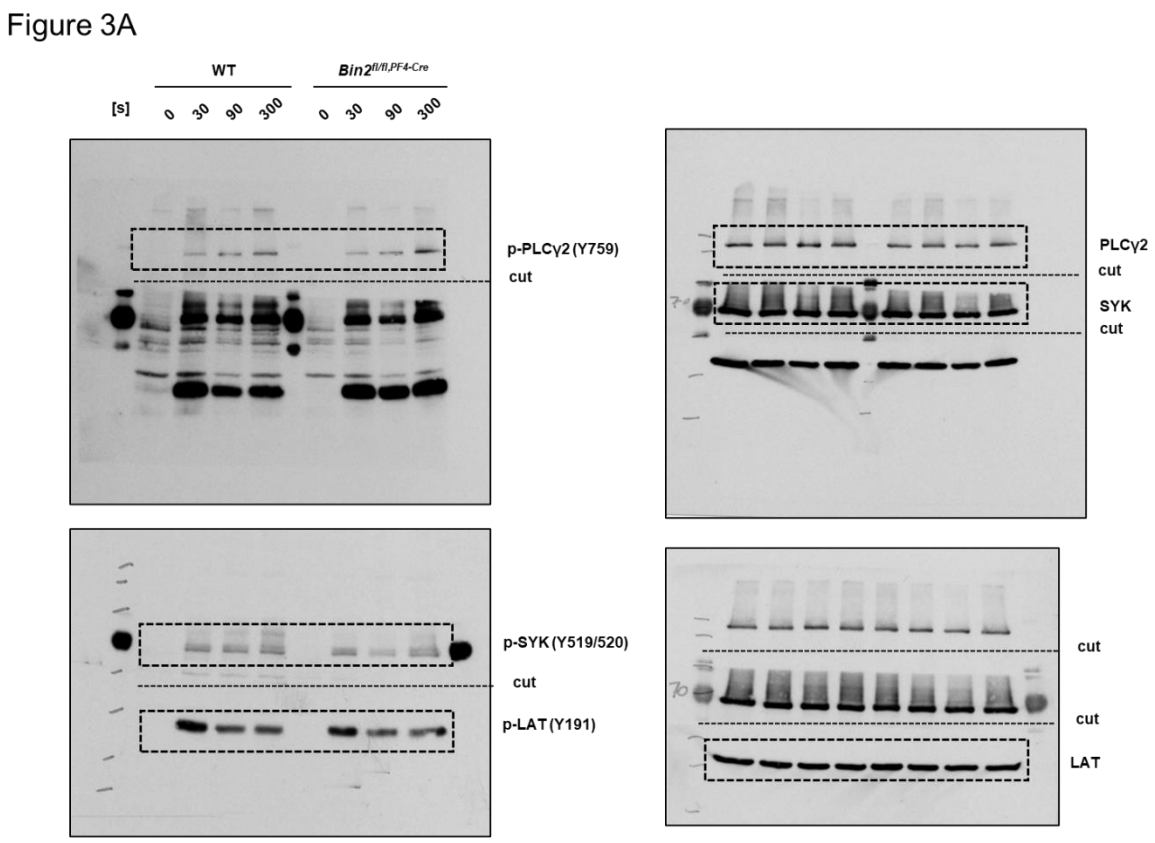

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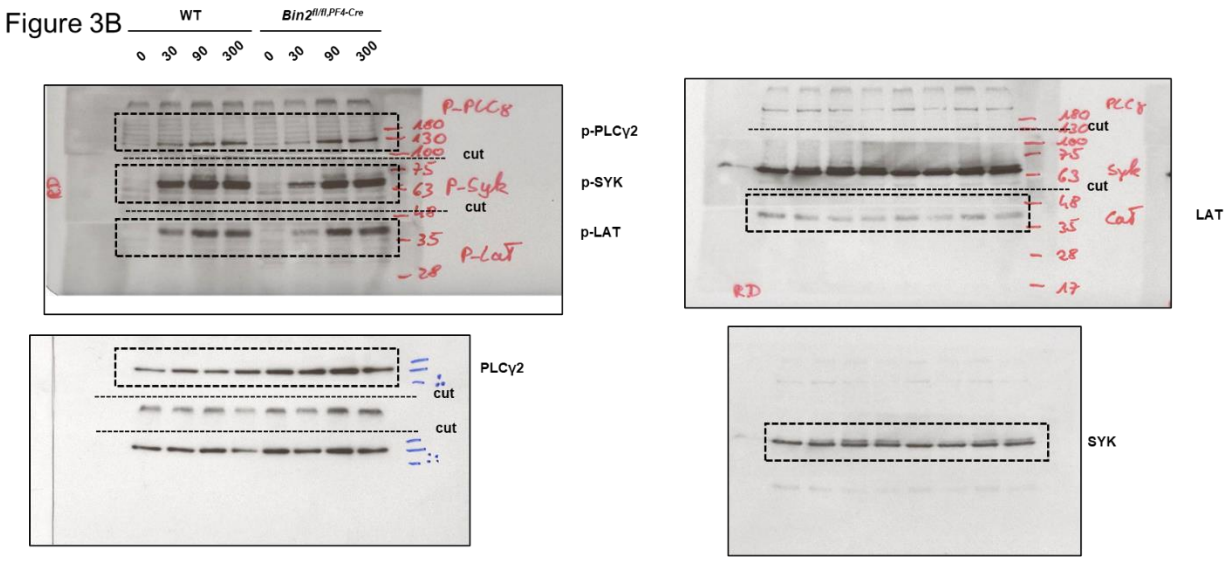

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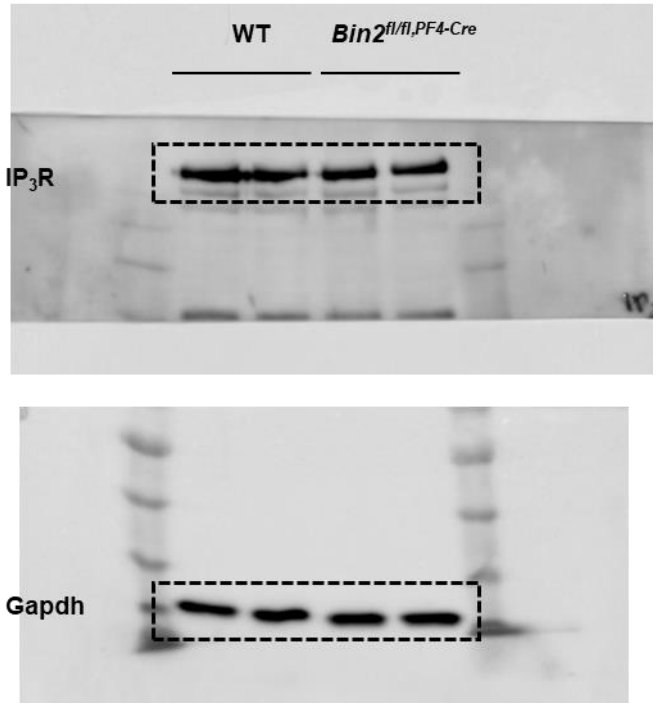
Figure 3D



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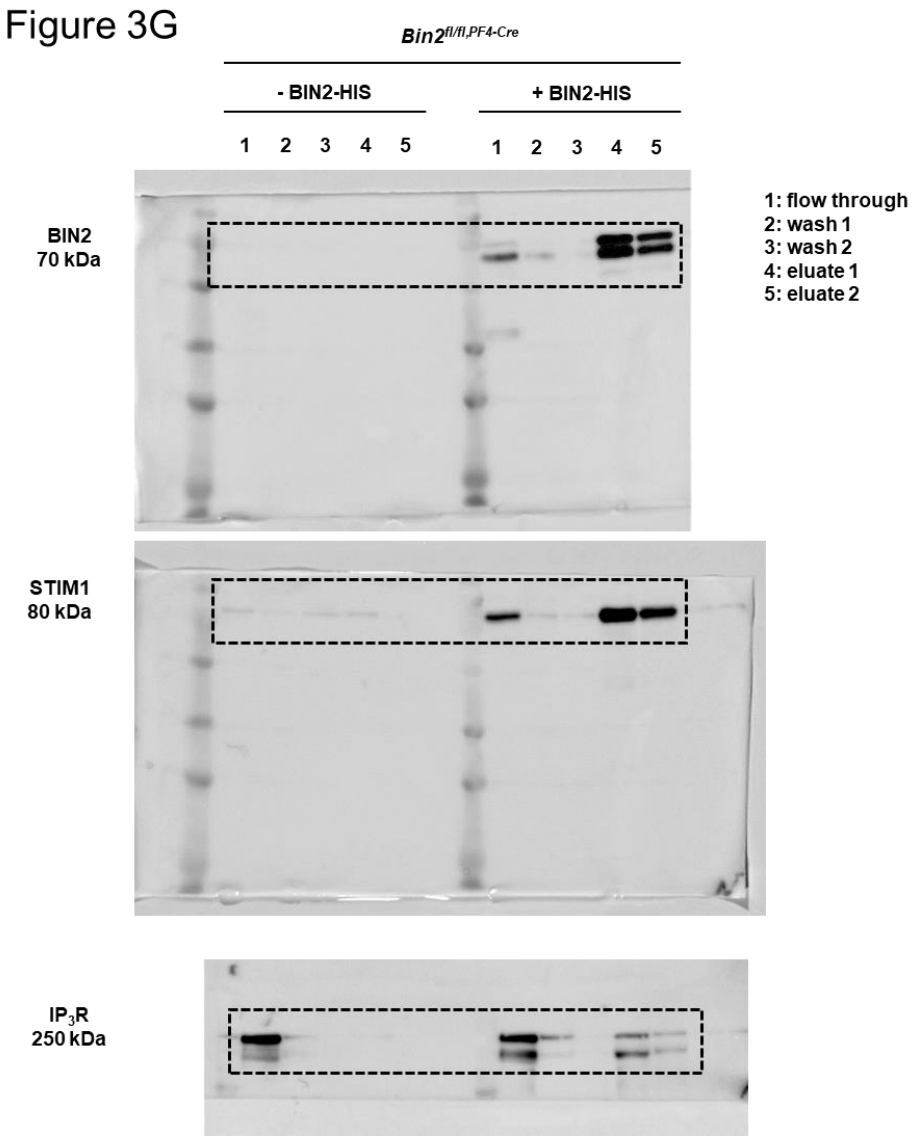
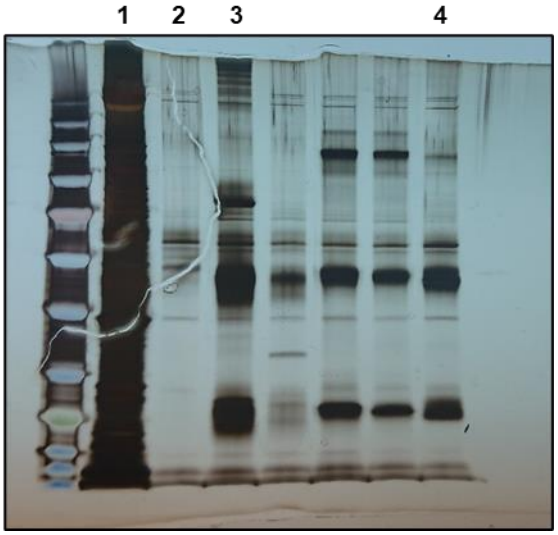


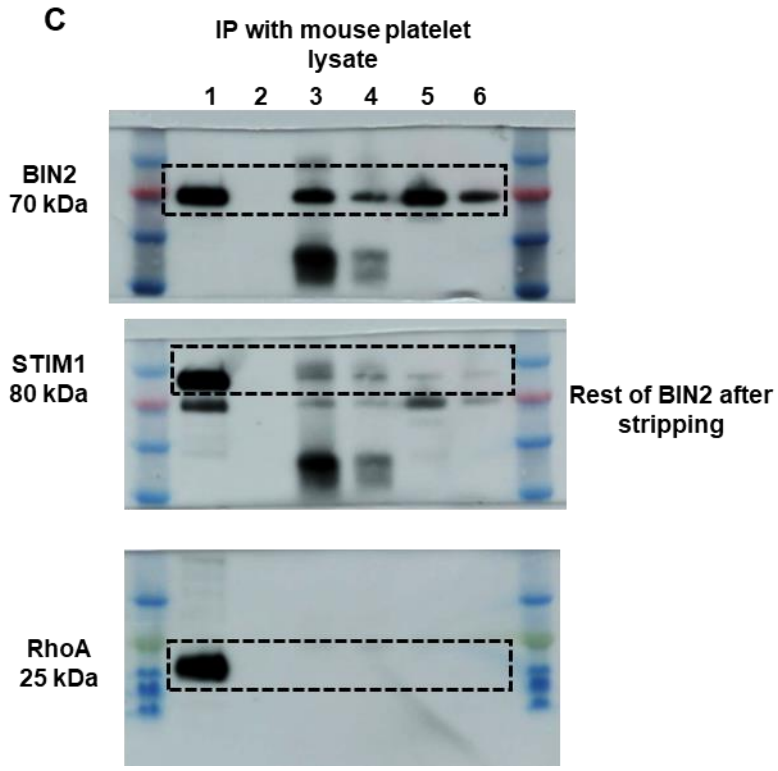
Full unedited gel for Supplemental Figure 1 C:

Suppl Figure 1C



Full unedited gel for Supplemental Figure 1 D

Supl Figure 1D



Full unedited gel for Supplemental Figure 5 A: