**Table S1. Mendelian frequencies of Shp1 conditional KO mice**

| Genotype | *Total number of mice* | *Expected frequency* | *Actual frequency* |
|---|---|---|---|
| *Ptpn6$^{fl/fl}$;PF4-Cre$^{+}$* | 69 | 25% | 22% |
| *Ptpn6$^{fl/fl}$;PF4-Cre$^{-}$* | 105 | 25% | 34% |
| *Ptpn6$^{+/fl}$;PF4-Cre$^{+}$* | 73 | 25% | 24% |
| *Ptpn6$^{+/fl}$;PF4-Cre$^{-}$* | 61 | 25% | 20% |

Data was collected from 12 breeding pairs (*Ptpn6$^{fl/+}$;PF4-Cre$^{+}$* × *Ptpn6$^{fl/fl}$;PF4-Cre$^{-}$*) and analysed using the $\chi^2$ test ($P < 0.01$).

**Table S2. Mendelian frequencies of Shp2 conditional KO mice**

| Genotype | *Total number of mice* | *Expected frequency* | *Actual frequency* |
|---|---|---|---|
| *Ptpn11$^{fl/fl}$;PF4-Cre$^{+}$* | 123 | 50% | 51% |
| *Ptpn11$^{fl/fl}$;PF4-Cre$^{-}$* | 116 | 50% | 49% |

Data was collected from 10 breeding pairs (*Ptpn11$^{fl/fl}$;PF4-Cre$^{+}$* × *Ptpn11$^{fl/fl}$;PF4-Cre$^{-}$*).

**Table S3. Hematology of Shp1 conditional KO mice**

| Hematological parameters | *Ptpn6$^{fl/fl}$;PF4-Cre$^{-}$* (mean ± SEM; n = 36) | *Ptpn6$^{fl/fl}$;PF4-Cre$^{+}$* (mean ± SEM; n = 37) |
|---|---|---|
| PLT ($10^3/\mu L$) | 888 ± 25 | 833 ± 27 |
| MPV (fL) | 5.70 ± 0.04 | 6.03 ± 0.08** |
| RBC ($10^6/\mu L$) | 6.66 ± 0.10 | 5.44 ± 0.21*** |
| HCT (%) | 31.37 ± 0.56 | 26.78 ± 0.60*** |
| WBC ($10^3/\mu L$) | 3.34 ± 0.23 | 3.94 ± 0.27 |
| LYM ($10^3/\mu L$) | 2.67 ± 0.18 | 2.81 ± 0.21 |
| MON ($10^3/\mu L$) | 0.33 ± 0.04 | 0.71 ± 0.037*** |
| NEU ($10^3/\mu L$) | 0.34 ± 0.05 | 0.22 ± 0.02* |
| EOS ($10^3/\mu L$) | 0.07 ± 0.03 | 0.02 ± 0.004 |
| BAS ($10^3/\mu L$) | 0.04 ± 0.02 | 0.09 ± 0.02 |

**Table S4. Hematology of Shp2 conditional KO mice**

| Hematological Parameters | *Ptpn11$^{fl/fl}$;PF4-Cre$^{-}$* (mean ± SEM; n = 39) | *Ptpn11$^{fl/fl}$;PF4-Cre$^{+}$* (mean ± SEM; n = 43) |
|---|---|---|
| PLT ($10^3/\mu L$) | 853 ± 20 | 596 ± 18*** |
| MPV (fL) | 5.70 ± 0.05 | 6.63 ± 0.09*** |
| RBC ($10^6/\mu L$) | 6.88 ± 0.08 | 6.40 ± 0.13** |
| HCT (%) | 31.78 ± 0.48 | 29.23 ± 0.64** |
| WBC ($10^3/\mu L$) | 3.53 ± 0.28 | 4.09 ± 0.25 |
| LYM ($10^3/\mu L$) | 2.96 ± 0.24 | 3.37 ± 0.23 |
| MON ($10^3/\mu L$) | 0.23 ± 0.03 | 0.30 ± 0.05 |
| NEU ($10^3/\mu L$) | 0.25 ± 0.03 | 0.28 ± 0.02 |
| EOS ($10^3/\mu L$) | 0.07 ± 0.03 | 0.08 ± 0.03 |
| BAS ($10^3/\mu L$) | 0.015 ± 0.005 | 0.012 ± 0.003 |

**Table S5. Hematology of Shp1/2 conditional DKO mice**

| Hematological parameters | *Ptpn6*$^{fl/fl}$;*Ptpn11*$^{fl/fl}$; *PF4-Cre*$^{-}$ (mean ± SEM; n = 7) | *Ptpn6*$^{fl/fl}$;*Ptpn11*$^{fl/fl}$; *PF4-Cre*$^{+}$ (mean ± SEM; n = 11) |
|---|---|---|
| PLT ($10^3/\mu L$) | 849 ± 40 | 62 ± 18*** |
| MPV (fL) | 5.8 ± 0.23 | 9.1 ± 0.33*** |
| RBC ($10^6/\mu L$) | 6.73 ± 0.10 | 4.95 ± 0.23*** |
| HCT (%) | 32.7 ± 0.6 | 25 ± 1.03*** |
| WBC ($10^3/\mu L$) | 2.67± 0.39 | 11.31 ± 1.22*** |
| LYM ($10^3/\mu L$) | 2.33 ± 0.34 | 8.72 ± 1.38** |
| MON ($10^3/\mu L$) | 0.23 ± 0.06 | 1.13 ± 0.23** |
| NEU ($10^3/\mu L$) | 0.16 ± 0.03 | 0.27 ± 0.04 |
| EOS ($10^3/\mu L$) | 0.06 ± 0.002 | 0.06 ± 0.002 |
| BAS ($10^3/\mu L$) | 0.02 ± 0.01 | 0.03 ± 0.008 |

**Table S6. Platelet surface glycoprotein expression in whole blood of Shp1 KO mice**

| Surface glycoproteins | *Ptpn6*$^{fl/fl}$;*PF4-Cre*$^{-}$ (mean ± SEM; n = 7) | *Ptpn6*$^{fl/fl}$;*PF4-Cre*$^{+}$ (mean ± SEM; n = 9) |
|---|---|---|
| GPVI | 39.90 ± 2.06 | 28.90 ± 1.97** |
| Integrin α2 | 34.46 ±1.10 | 29.08 ± 1.45* |
| Integrin αIIbβ3 | 383 ± 32.50 | 340 ± 26.27 |
| GPIbα | 66.51 ± 9.51 | 96.50 ± 7.34* |
| CLEC-2 | 13.52 ±1.56 | 15.20 ± 1.65 |
| G6b-B | 16.10 ± 0.57 | 17.30 ± 0.92 |
| ADAM10 | 18.50 ± 0.32 | 20 ± 1.26 |

**Table S7. Platelet surface glycoprotein expression in whole blood of Shp2 KO mice**

| Surface glycoproteins | *Ptpn11*$^{fl/fl}$;*PF4-Cre*$^{-}$ (mean ± SEM; n = 4-6) | *Ptpn11*$^{fl/fl}$;*PF4-Cre*$^{+}$ (mean ± SEM; n = 4-9) |
|---|---|---|
| GPVI | 38.32 ± 2.44 | 35.28± 1.86 |
| Integrin α2 | 31.87 ±1.11 | 32.98 ± 0.68 |
| Integrin αIIbβ3 | 390 ± 47.39 | 364 ± 12.84 |
| GPIbα | 62 ± 14.14 | 66.70 ± 12.67 |
| CLEC-2 | 14.39 ± 0.69 | 18.65 ± 3.01 |
| G6b-B | 14.32 ± 2.24 | 13.68 ± 2.94 |
| ADAM10 | 18.69 ± 2.53 | 18.10 ± 1.69 |

**Table S8. Platelet surface glycoprotein expression in whole blood of Shp1/2 DKO mice**

| Surface glycoproteins | *Ptpn6*$^{fl/fl}$;*Ptpn11*$^{fl/fl}$; *PF4-Cre*$^{-}$ (mean ± SEM; n = 4) | *Ptpn6*$^{fl/fl}$;*Ptpn11*$^{fl/fl}$; *PF4-Cre*$^{+}$ (mean ± SEM; n = 5) |
|---|---|---|
| GPVI | 49 ± 2.23 | 0.91 ± 2.26*** |
| Integrin α2 | 3.44 ± 0.16 | 0.96 ± 0.08*** |
| Integrin αIIbβ3 | 305 ± 16 | 4.44 ± 2.90*** |
| GPIbα | 61 ± 15 | 4.63 ± 1.50*** |
| CLEC-2 | 28 ± 4 | 1.77 ± 0.70*** |
| G6b-B | 16 ± 1.30 | 2.93 ± 0.70*** |
| ADAM10 | 14 ± 0.31 | 21.17 ± 1.13** |

**Table S9. Surface glycoprotein expression in Shp1 KO megakaryocytes**

| Surface glycoproteins | *Ptpn6*$^{fl/fl}$;*PF4-Cre*$^{-}$ (mean ± SEM; n = 3) | *Ptpn6*$^{fl/fl}$;*PF4-Cre*$^{+}$ (mean ± SEM; n = 3) |
|---|---|---|
| GPVI | 581 ± 36 | 560 ± 42 |
| Integrin α2 | 189 ± 20 | 177 ± 31 |
| Integrin αIIbβ3 | 1131 ± 77 | 734 ± 40** |
| GPIbα | 752 ± 38 | 733 ± 60 |

**Table S10. Surface glycoprotein expression in Shp2 KO megakaryocytes**

| Surface glycoproteins | *Ptpn11*$^{fl/fl}$;*PF4-Cre*$^{-}$ (mean ± SEM; n = 3) | *Ptpn11*$^{fl/fl}$;*PF4-Cre*$^{+}$ (mean ± SEM; n = 3) |
|---|---|---|
| GPVI | 650 ± 21 | 650 ± 33 |
| Integrin α2 | 299 ± 20 | 241 ± 32 |
| Integrin αIIbβ3 | 1026 ± 8 | 623 ± 83** |
| GPIbα | 769 ± 56 | 436 ± 62* |

**Table S11. Mendelian frequencies of G6b conditional KO mice**

| Genotype | *Total number of mice* | *Expected frequency* | *Actual frequency* |
|---|---|---|---|
| *G6b*$^{fl/fl}$;*PF4-Cre*$^{+}$ | 121 | 50% | 54% |
| *G6b*$^{fl/fl}$;*PF4-Cre*$^{-}$ | 105 | 50% | 46% |

Data was collected from 10 breeding pairs (*G6b*$^{fl/fl}$;*PF4-Cre*$^{+}$ × *G6b*$^{fl/fl}$;*PF4-Cre*$^{-}$).

**Table S12. Hematology of G6b conditional KO mice**

| Hematological parameter | *G6b*$^{fl/fl}$;*PF4-Cre*$^{-}$ (mean ± SEM; n = 20) | *G6b*$^{fl/fl}$;*PF4-Cre*$^{+}$ (mean ± SEM; n = 35) |
|---|---|---|
| PLT ($10^3/\mu L$) | 884 ± 45 | 116 ± 9*** |
| MPV (fL) | 5.70 ± 0.06 | 8.75 ± 0.09*** |
| RBC ($10^6/\mu L$) | 6.18 ± 0.27 | 5.98 ± 0.19 |
| HCT (%) | 29.73 ± 0.73 | 28.72 ± 0.47 |
| WBC ($10^3/\mu L$) | 3.43 ± 0.35 | 6.07 ± 0.48 ** |
| LYM ($10^3/\mu L$) | 3.06 ± 0.33 | 5.17 ± 0.40** |
| MON ($10^3/\mu L$) | 0.26 ± 0.05 | 0.45 ± 0.04* |
| NEU ($10^3/\mu L$) | 0.17 ± 0.02 | 0.39 ± 0.05** |
| EOS ($10^3/\mu L$) | 0.009 ±0.003 | 0.013 ± 0.003 |
| BAS ($10^3/\mu L$) | 0.025 ± 0.008 | 0.036 ± 0.014 |

**Table S13. Platelet surface glycoprotein expression in whole blood of G6b KO mice**

| Surface glycoproteins | *G6b*$^{fl/fl}$;*PF4-Cre*$^{-}$ (mean ± SEM; n = 5) | *G6b*$^{fl/fl}$;*PF4-Cre*$^{+}$ (mean ± SEM; n = 5) |
|---|---|---|
| GPVI | 32 ± 4.23 | 1.82 ± 0.26*** |
| Integrin α2 | 3 ± 0.17 | 0.63 ± 0.22*** |
| Integrin αIIbβ3 | 235 ± 18 | 68 ± 38.18** |
| GPIbα | 54 ± 5 | 14 ± 6.22 ** |
| CLEC-2 | 29 ±1.70 | 12 ± 0.60*** |
| G6b-B | 15 ± 1.36 | 1 ± 0.43*** |
| ADAM10 | 15 ± 0.61 | 24 ± 0.38*** |

**Table S14. Surface glycoprotein expression in G6b KO megakaryocytes**

| Surface glycoproteins | *G6b*$^{fl/fl}$;*PF4-Cre*$^{-}$ (mean ± SEM; n = 4) | *G6b*$^{fl/fl}$;*PF4-Cre*$^{+}$ (mean ± SEM; n = 4) |
|---|---|---|
| GPVI | 714 ± 10 | 217 ± 63** |
| Integrin α2 | 274 ± 41 | 143 ± 27* |
| Integrin αIIbβ3 | 1147 ± 78 | 1107 ± 38 |
| GPIbα | 832 ± 10 | 199 ± 42** |

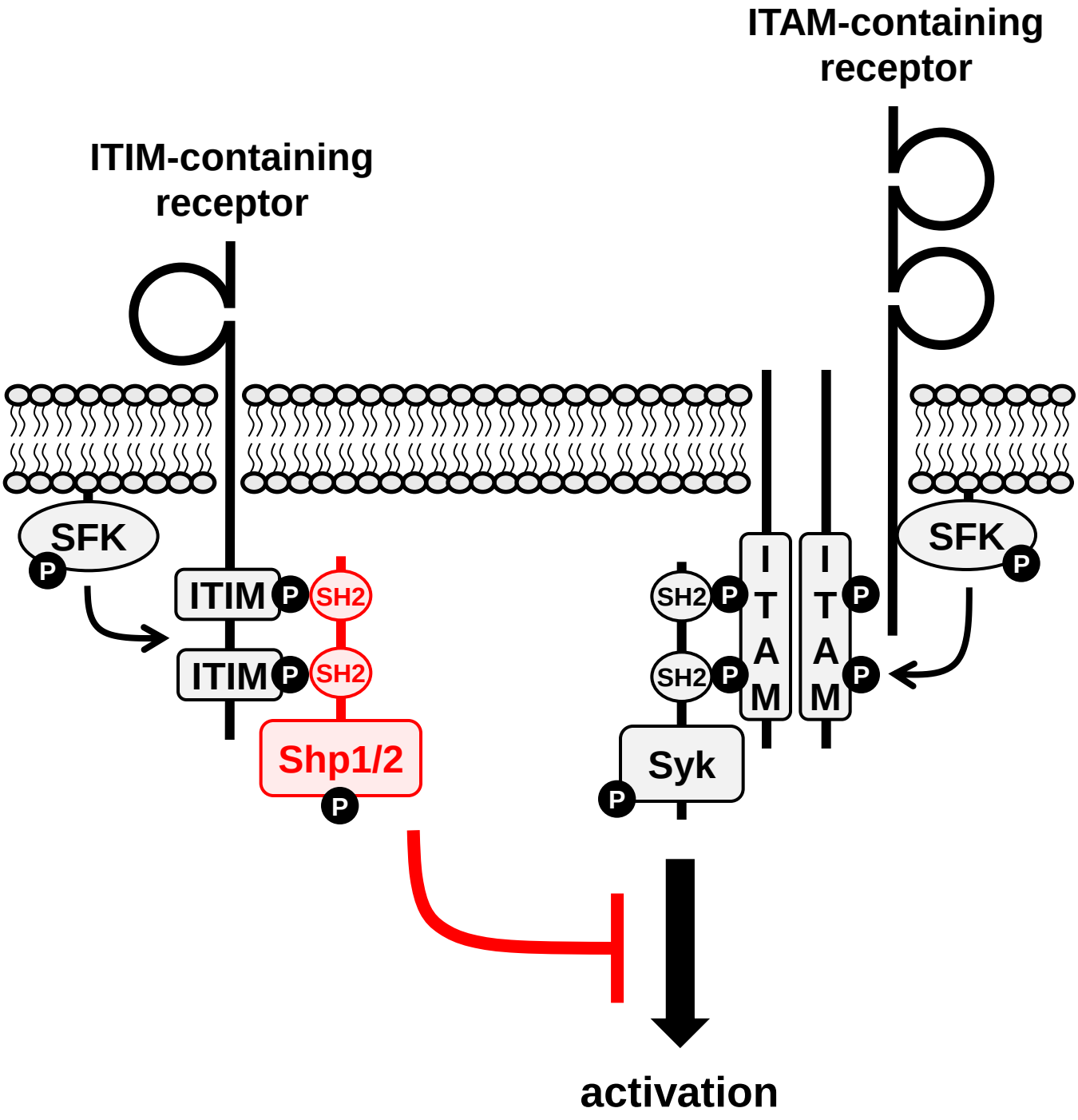


**Figure S1A. Immunoreceptor tyrosine-based inhibition motif (ITIM)-containing receptors negatively regulate immunoreceptor tyrosine-based activation motif (ITAM)-containing receptors.** SFK, Src family kinase.

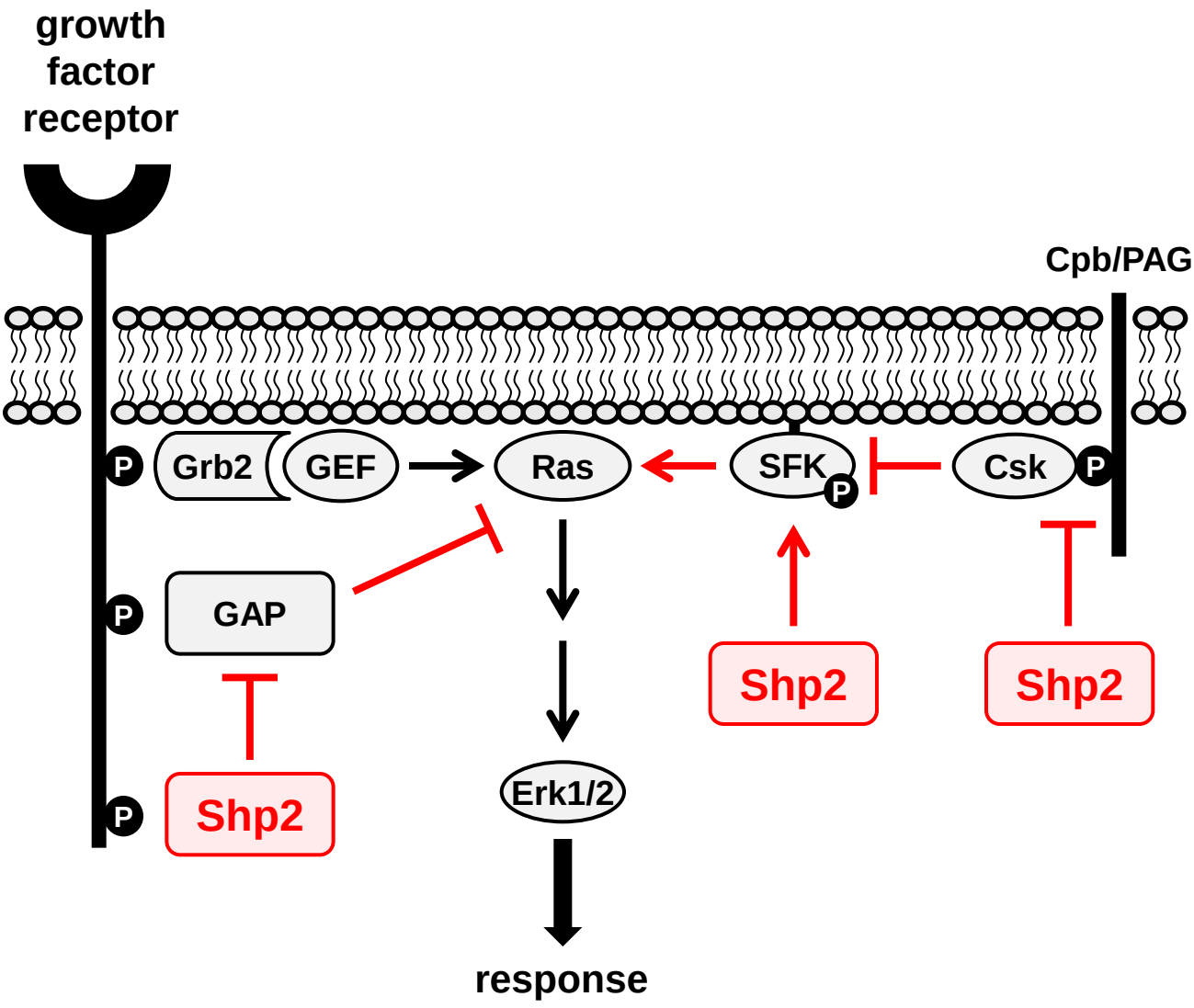


**Figure S1B. Potential mechanisms by which Shp2 positively regulates signaling via the Ras-Erk1/2 pathway.** SFK, Src family kinase; Csk, C-terminal Src kinase; Cpb/PAG, Csk-binding protein/phosphoprotein associated with glycosphingolipid-enriched microdomains; Grb2, Growth factor receptor-bound protein 2; GEF, Guanine nucleotide exchange factor; GAP, GTPase-activating protein.

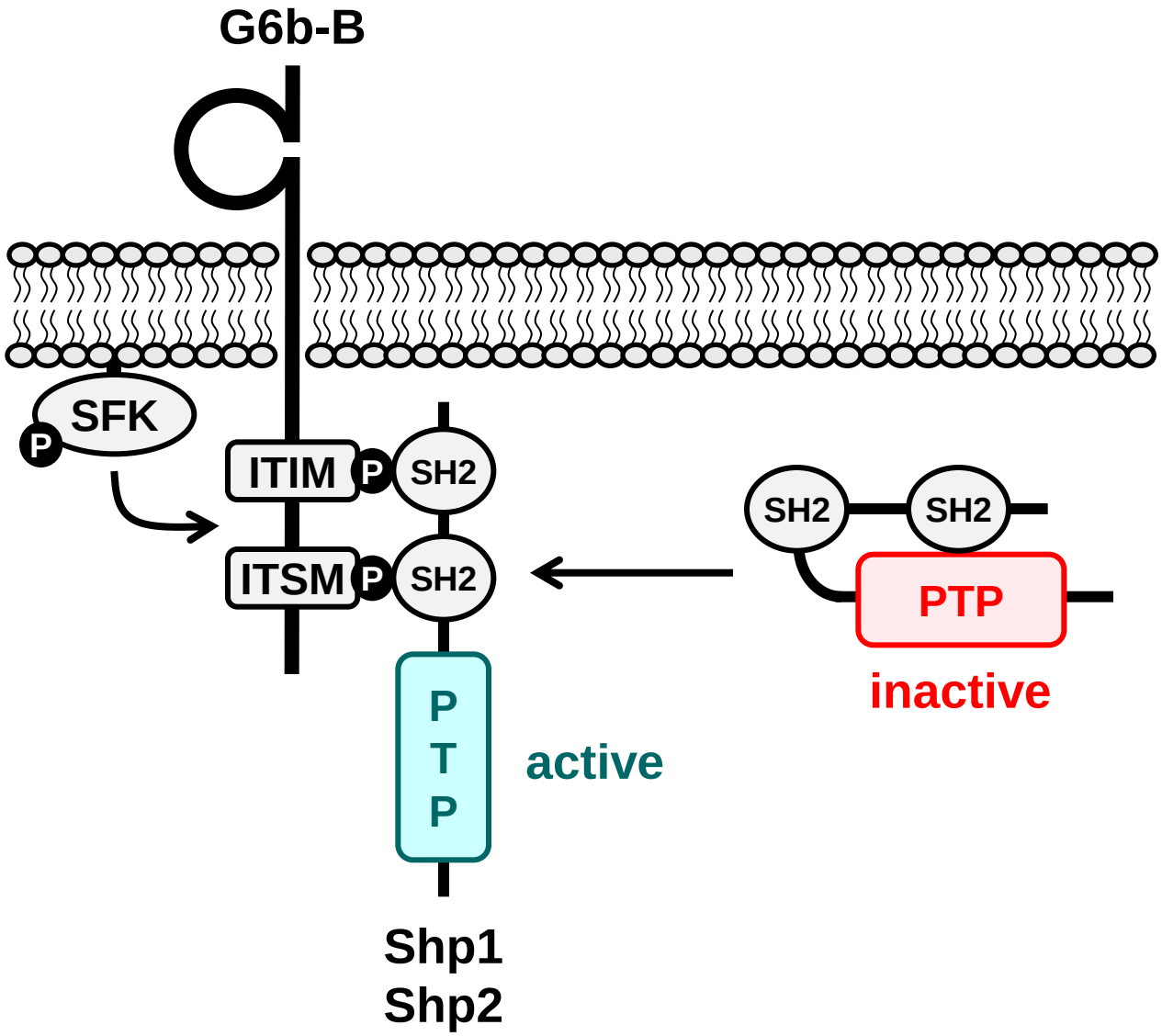


**Figure S1C. G6b-B is constitutively tyrosine phosphorylated by Src family kinases and associated with Shp1 and Shp2.** SFK, Src family kinase; ITIM, immunoreceptor tyrosine-based inhibition motif; ITSM, immunoreceptor tyrosine-based switch motif; PTP, protein-tyrosine phosphatase.

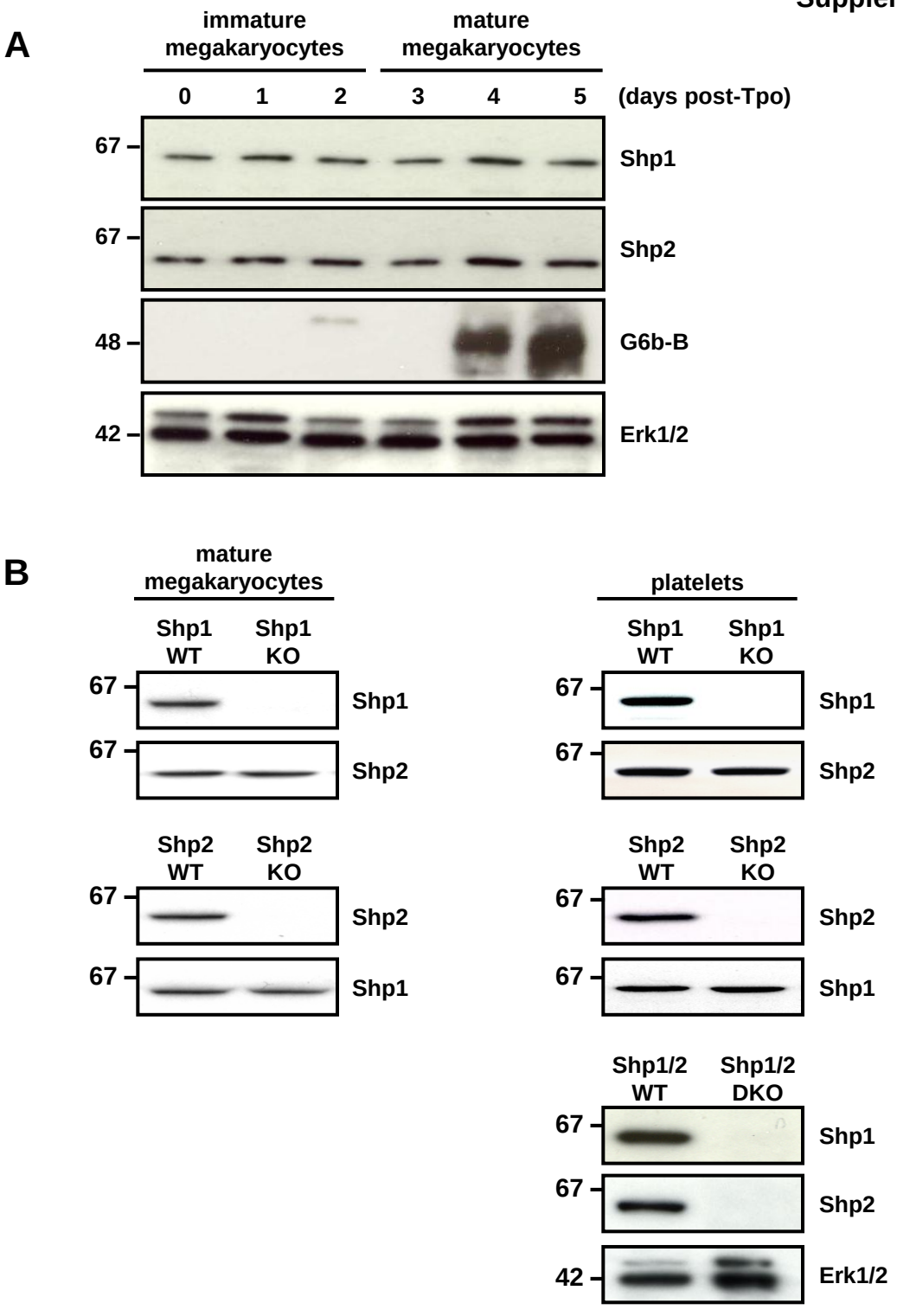


**Figure S2. Shp1 and Shp2 are expressed throughout megakaryocyte development. (A)** Whole cell lysates (WCLs) were prepared of bone marrow (BM) cells from wild-type (WT) mice 0-5 days post culture in the presence of thrombopoietin (Tpo) and western blotted for Shp1, Shp2, G6b-B and Erk1/2. **(B)** WCLs prepared of mature BM-derived megakaryocytes and washed platelets from *Ptpn6*$^{fl/fl}$*;PF4-Cre*$^{+}$ (Shp1 KO), *Ptpn11*$^{fl/fl}$*;PF4-Cre*$^{+}$ (Shp2 KO) and *Ptpn6*$^{fl/fl}$*;Ptpn11*$^{fl/fl}$*;PF4-Cre*$^{+}$ (Shp1/2 DKO) conditional KO mice and litter-matched WT mice (*Ptpn6*$^{fl/fl}$*;PF4-Cre*$^{-}$ *(Shp1 WT), Ptpn11*$^{fl/fl}$*;PF4-Cre*$^{-}$ *(Shp2 WT)* and *Ptpn6*$^{fl/fl}$*;Ptpn11*$^{fl/fl}$*;PF4-Cre*$^{-}$ (Shp1/2 WT)) were western blotted with Shp1, Shp2 and Erk1/2 antibodies. Representative blots from n = 3 independent experiments/genotype.

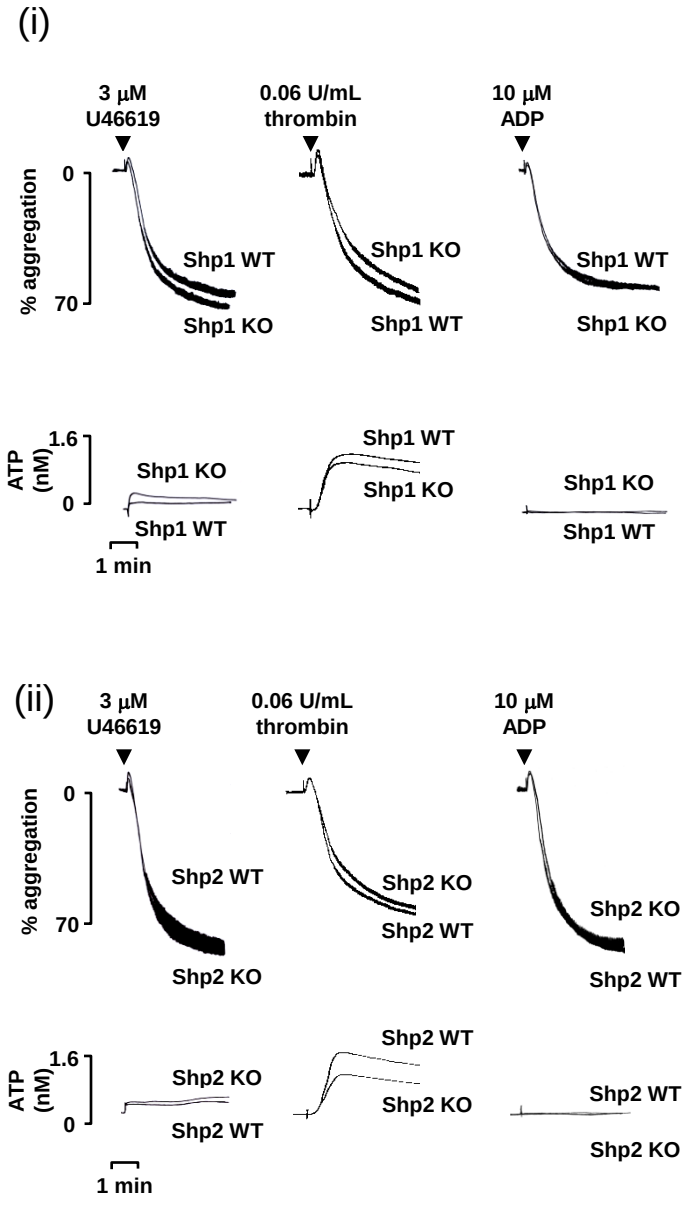


**Figure S3. Normal aggregation/ATP secretion of Shp1 and Shp2-deficient platelets in response to G protein-coupled receptor agonists.** Representative platelet aggregation and ATP secretion traces in response to agonists indicated of **(i)** Shp1 KO, and **(ii)** Shp2 KO platelets; n=4-8 mice/genotype/condition.

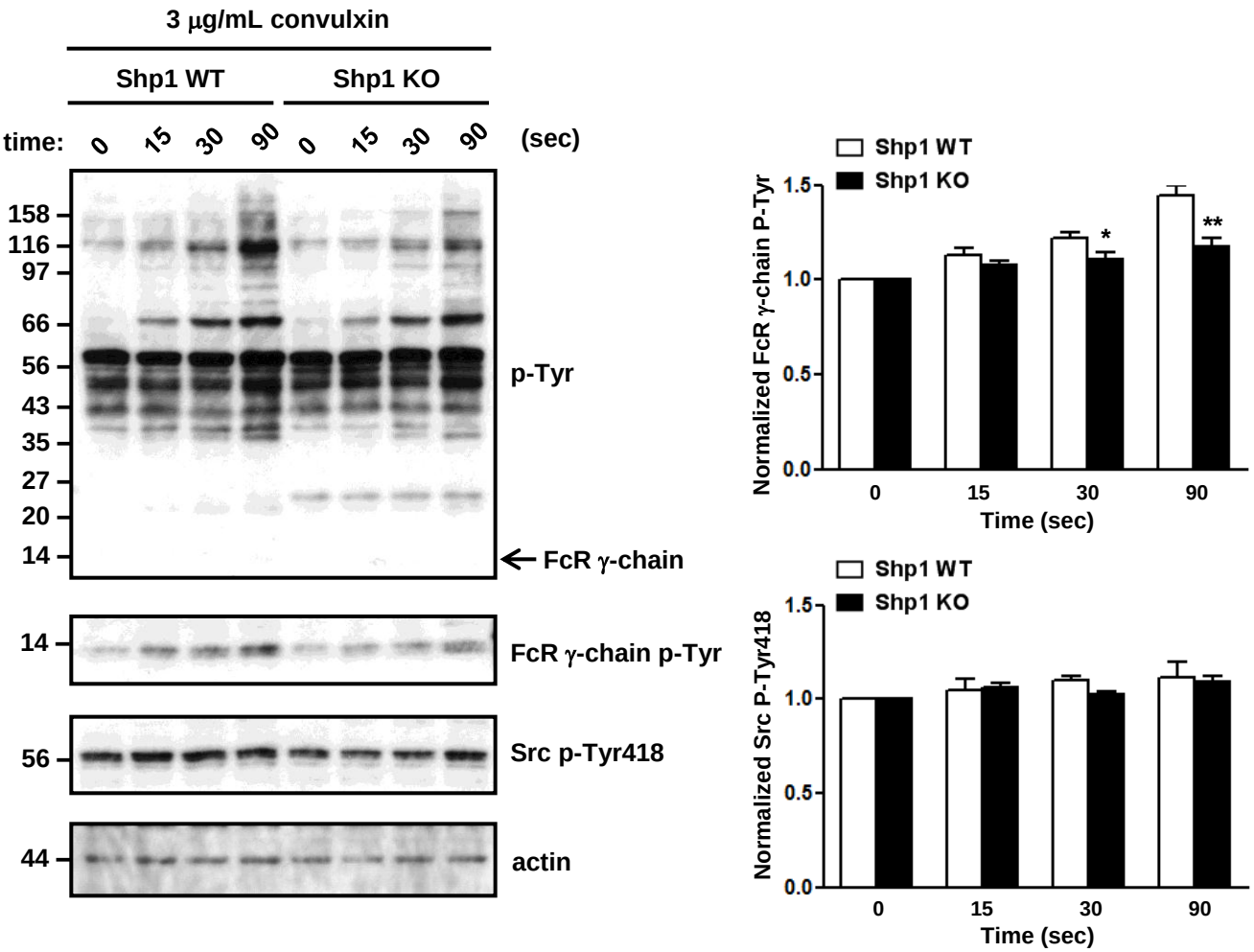


**Figure S4. Reduced GPVI-mediated tyrosine phosphorylation at early time points in Shp1-deficient platelets.** Whole cell lysates (WCLs) of resting and convulxin-stimulated platelets from *Ptpn6*$^{fl/fl}$*;PF4-Cre*$^{+}$ (Shp1 KO) and litter-matched wild-type (*Ptpn6*$^{fl/fl}$*;PF4-Cre*$^{-}$, Shp1 WT) mice were western blotted with anti-phosphotyrosine (p-Tyr), and -Src-pTyr418 antibodies. Membranes were stripped and re-blotted with actin antibody. Representative blots and densitometry quantification from n=3-7 independent experiments/genotype (mean ± SEM; **P* < 0.05, ***P* < 0.01).

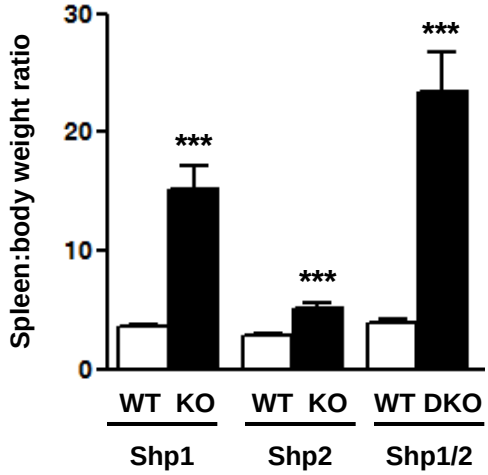


**Figure S5. Splenomegaly in Shp1, Shp2 and Shp1/2 conditional KO mice.** Splenomegaly is observed in megakaryocyte-specific *Ptpn6*$^{fl/fl}$*;PF4-Cre*$^{+}$ (Shp1 KO), *Ptpn11*$^{fl/fl}$*;PF4-Cre*$^{+}$ (Shp2 KO) and *Ptpn6*$^{fl/fl}$*;Ptpn11*$^{fl/fl}$*;PF4-Cre*$^{+}$ (Shp1/2 DKO) mice and corresponding litter matched wild-type (*Ptpn6*$^{fl/fl}$*;PF4-Cre*$^{-}$ (Shp1 WT), *Ptpn11*$^{fl/fl}$*;PF4-Cre*$^{-}$ (Shp2 WT) and *Ptpn6*$^{fl/fl}$*; Ptpn11*$^{fl/fl}$*;PF4-Cre*$^{-}$ (Shp1/2 WT). Data are presented as spleen/body weight (mg/g) ratio (n = 10-20 mice/genotype; ***$P < 0.001$).

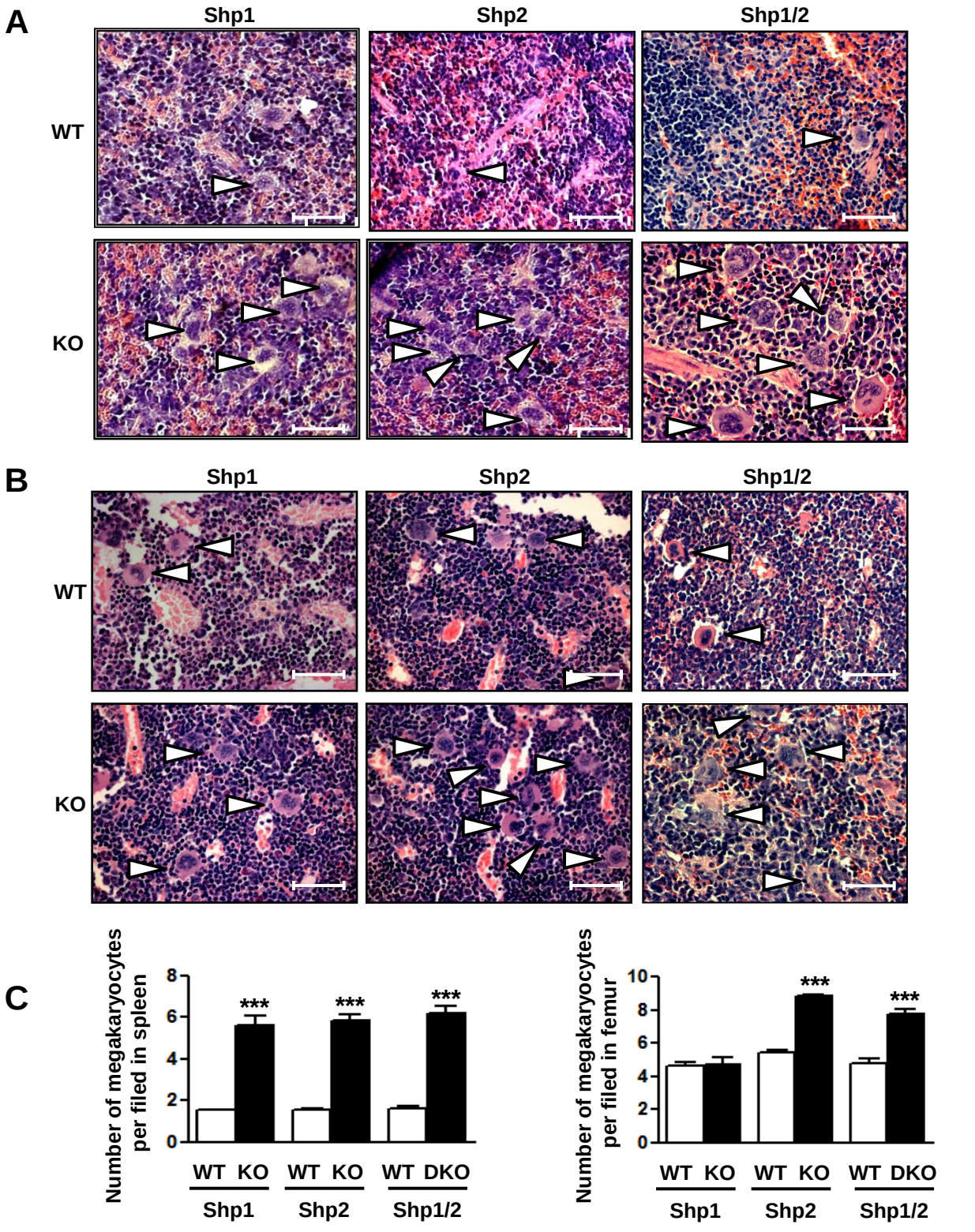


**Figure S6. Extramedullary hematopoiesis in Shp1, Shp2 and Shp1/2 conditional KO mice.** H&E-stained of **(A)** spleen and **(B)** femur sections from *Ptpn6*$^{fl/fl}$*;PF4-Cre*$^{+}$ (Shp1 KO), *Ptpn11*$^{fl/fl}$*;PF4-Cre*$^{+}$ (Shp2 KO), *Ptpn6*$^{fl/fl}$*;Ptpn11*$^{fl/fl}$*;PF4-Cre*$^{+}$ (Shp1/2 DKO) and corresponding litter matched wild-type (*Ptpn6*$^{fl/fl}$*;PF4-Cre*$^{-}$ (Shp1 WT), *Ptpn11*$^{fl/fl}$*;PF4-Cre*$^{-}$ (Shp2 WT), *Ptpn6*$^{fl/fl}$*; Ptpn11*$^{fl/fl}$*;PF4-Cre*$^{-}$ (Shp1/2 WT). Representative images from n = 4 mice/genotype, 8-10 fields of view per tissue sample, through five marrow sections. Bright field images were obtained using a Zeiss Axiovert 200 inverted high-end microscope (Welwyn Garden City, UK) with a 20× objective. Arrowheads indicate megakaryocytes. (scale bar = 50 μm). **(C)** Quantification of the number of megakaryocytes per field, ***$P < 0.001$.

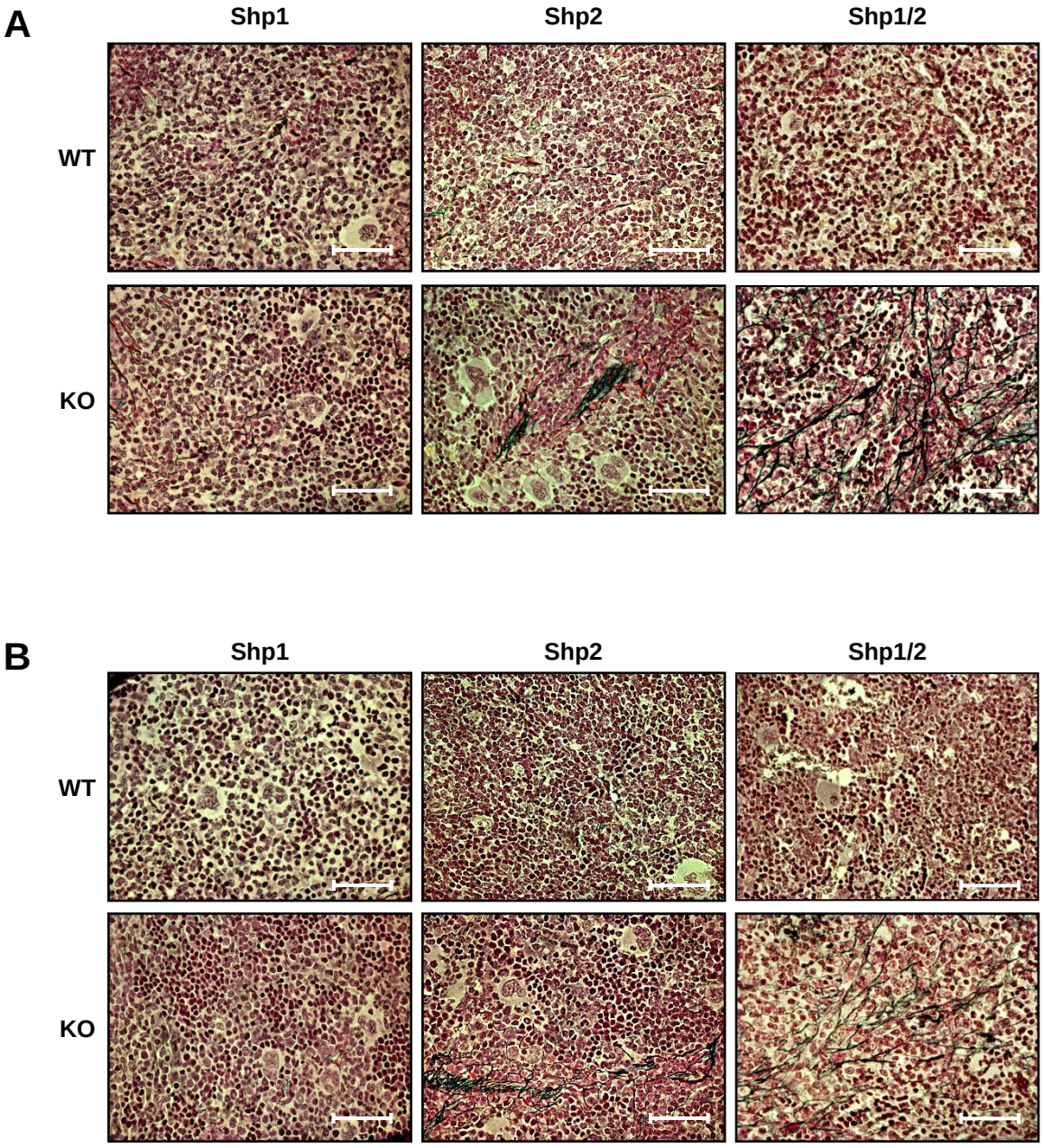


**Figure S7. Myelofibrosis in Shp2 and Shp1/2 conditional KO mice.** Reticulin-stained **(A)** spleen and **(B)** femur sections from *Ptpn6*$^{fl/fl}$*;PF4-Cre*$^{+}$ (Shp1 KO), *Ptpn11*$^{fl/fl}$*;PF4-Cre*$^{+}$ (Shp2 KO), *Ptpn6*$^{fl/fl}$*;Ptpn11*$^{fl/fl}$*;PF4-Cre*$^{+}$ (Shp1/2 DKO) and corresponding litter matched wild-type (*Ptpn6*$^{fl/fl}$*;PF4-Cre*$^{-}$ (Shp1 WT), *Ptpn11*$^{fl/fl}$*;PF4-Cre*$^{-}$ (Shp2 WT), *Ptpn6*$^{fl/fl}$*; Ptpn11*$^{fl/fl}$*;PF4-Cre*$^{-}$ (Shp1/2 WT). Representative images from n = 4 mice/genotype, 8-10 fields of view per tissue sample, through five marrow sections. Bright field images were obtained using a Zeiss Axiovert 200 inverted high-end microscope (Welwyn Garden City, UK) with a 20× objective (scale bar = 50 μm).

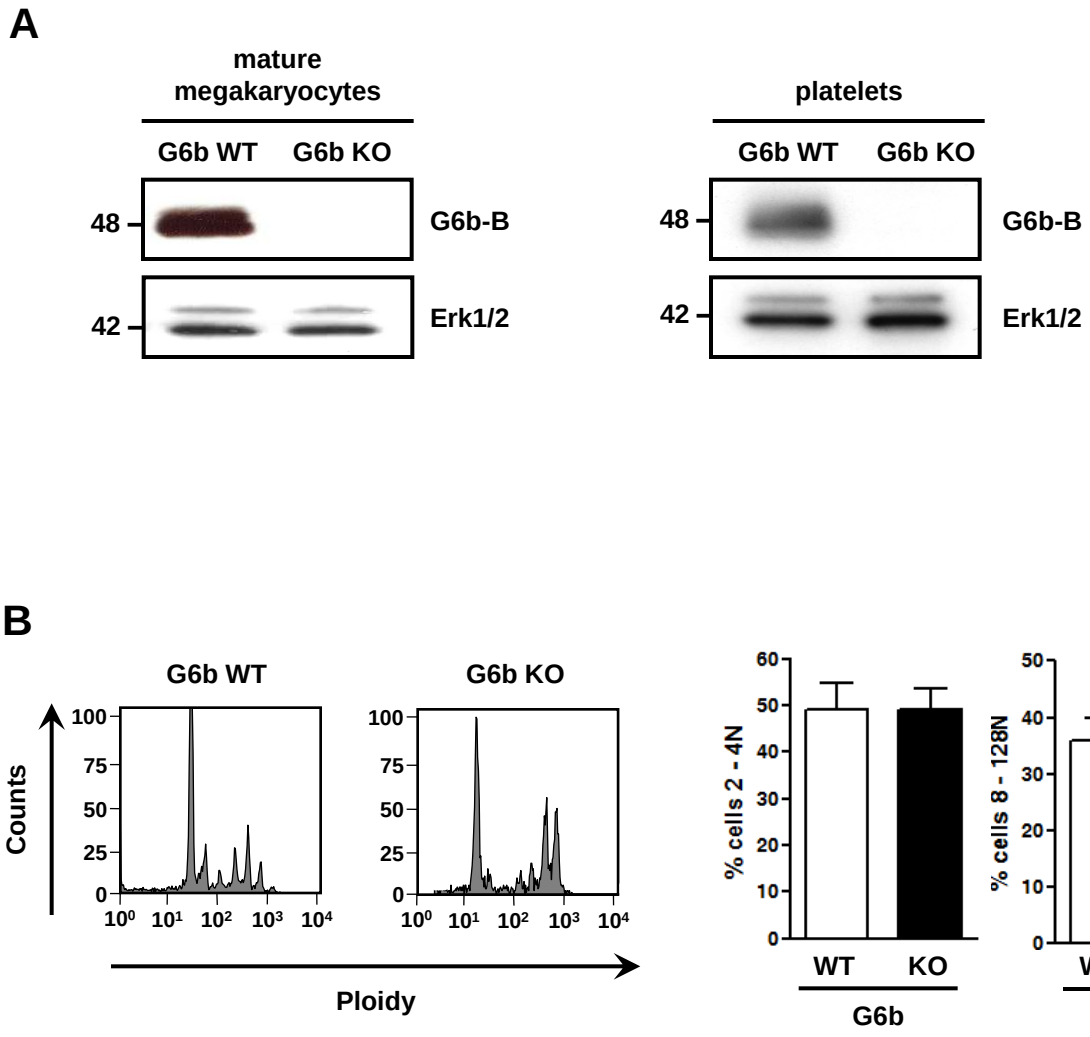


**Figure S8. G6b conditional KO mice partially phenocopy Shp1/2 conditional DKO mice. (A)** Whole cell lysates (WCLs) of washed platelets and cultured mature bone marrow (BM)-derived megakaryocytes from *G6b$^{fl/fl}$;PF4-Cre$^{+}$* (G6b KO) and litter-matched wild-type (*G6b$^{fl/fl}$;PF4-Cre$^{-}$*, G6b WT) mice were western blotted with anti-G6b-B and -Erk1/2 antibodies. **(B)** Ploidy of mature BM-derived megakaryocytes were quantified by propidium iodide staining and flow cytometry. Representative profiles are shown; n = 4-6 mice/genotype. The percentage of 2-4N and 8-128N ploidy cells was quantified. Mean ± SEM; n = 4-6 mice/genotype.

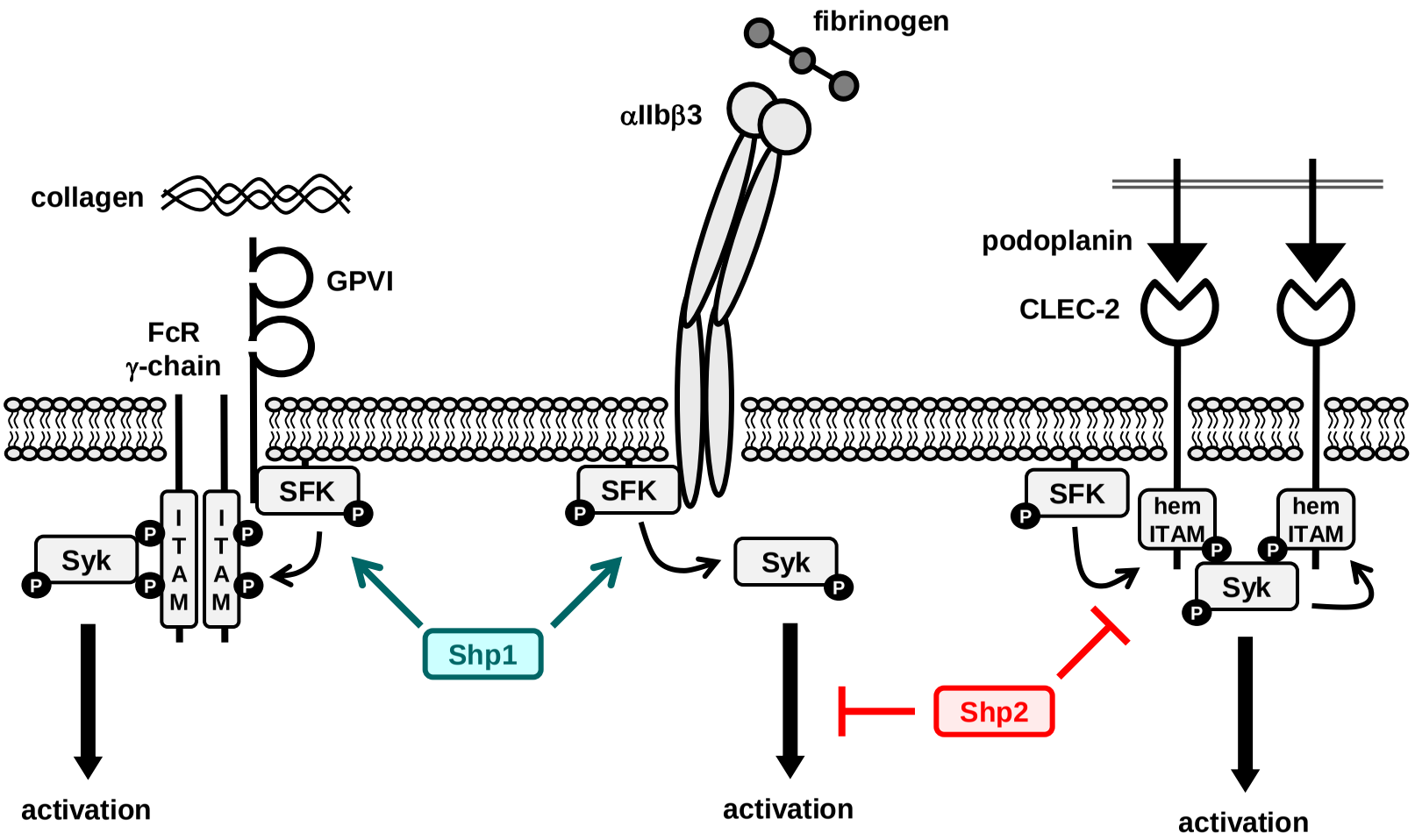


**Figure S9. Distinct functions of Shp1 and Shp2 in platelets.** SFK, Src family kinase; ITAM, immunoreceptor tyrosine-based activation motif.

Supplemental data

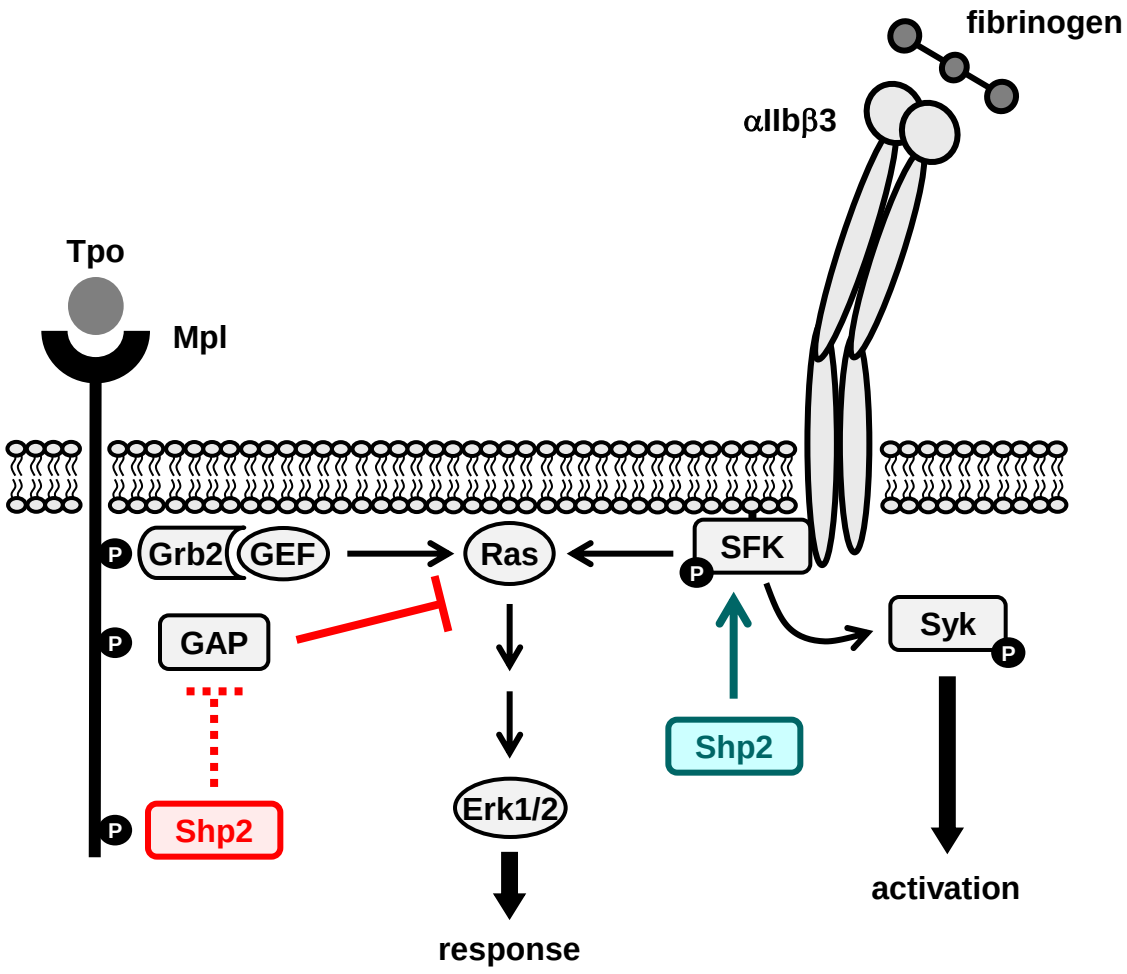


**Figure S10. Shp2 is a positive regulator of Mpl and integrin αIIbβ3 signaling in megakaryocytes.** SFK, Src family kinase; ITAM, immunoreceptor tyrosine-based activation motif; Tpo, thrombopoietin; Grb2, Growth factor receptor-bound protein 2; GEF, Guanine nucleotide exchange factor; GAP, GTPase-activating protein.

**Supplementary Videos**

**Fig2video1. Laser-induced thrombus formation in a *Ptpn6$^{fl/fl}$;PF4-Cre$^{-}$* (Shp1 WT) mouse.** Representative video of fluorescently-labelled platelets (green) accumulating at the site of laser-induced injury in an arteriole in the cremaster muscle of a Shp1 WT mouse. A timer is shown in the top left corner and a 10 μm scale bar in the bottom left corner.

**Fig2video2. Laser-induced thrombus formation in a *Ptpn6$^{fl/fl}$;PF4-Cre$^{+}$* (Shp1 KO) mouse.** Representative video of fluorescently-labelled platelets (green) accumulating at the site of laser-induced injury in an arteriole in the cremaster muscle of a Shp1 KO mouse. A timer is shown in the top left corner and a 10 μm scale bar in the bottom left corner.

**Fig2video3. Laser-induced thrombus formation in a *Ptpn11$^{fl/fl}$;PF4-Cre$^{-}$* (Shp2 WT) mouse.** Representative video of fluorescently-labelled platelets (green) accumulating at the site of laser-induced injury in an arteriole in the cremaster muscle of a Shp2 WT mouse. A timer is shown in the top left corner and a 10 μm scale bar in the bottom left corner.

**Fig2video4. Laser-induced thrombus formation in a *Ptpn11$^{fl/fl}$;PF4-Cre$^{+}$* (Shp2 KO) mouse.** Representative video of fluorescently-labelled platelets (green) accumulating at the site of laser-induced injury in an arteriole in the cremaster muscle of a Shp2 KO mouse. A timer is shown in the top left corner and a 10 μm scale bar in the bottom left corner.