

Supplementary Materials for

Impaired $\alpha_{\text{IIb}}\beta_3$ Integrin Activation and Shear-Dependent Thrombus Formation in Mice Lacking Phospholipase D1

Margitta Elvers, David Stegner, Ina Hagedorn, Christoph Kleinschnitz, Attila Braun, Marijke E. J. Kuijpers, Michael Boesl, Qin Chen, Johan W. M. Heemskerk, Guido Stoll, Michael A. Frohman, Bernhard Nieswandt*

*To whom correspondence should be addressed. E-mail: bernhard.nieswandt@virchow.uni-wuerzburg.de

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	wt	<i>Pld1</i>^{-/-}
Platelets	849 ± 257	889 ± 322
MPV (fl)	5.13 ± 0.25	5.58 ± 0.18
Red blood cells	9.53 ± 1.53	9.14 ± 1.88
White blood cells	7.98 ± 2.76	6.93 ± 1.99
HCT (%)	48.93 ± 7.54	45.78 ± 9.28
GPIb	367 ± 7	373 ± 30
GPV	335 ± 7	358 ± 9
GPIX	521 ± 16	542 ± 22
GPVI	40 ± 4	49 ± 8
α₂	99 ± 5	105 ± 2
β₁	144 ± 3	176 ± 2
α_{IIb}β₃	658 ± 24	691 ± 25
CD9	1491 ± 109	1710 ± 54

Table S1: Blood cell counts and platelet glycoprotein abundance in *Pld1*^{-/-} mice. Platelet and erythrocyte counts per nl, hematocrit and glycoprotein abundance in *Pld1*^{-/-} mice. To analyze glycoprotein abundance, diluted whole blood was stained with fluorophore-labeled antibodies at saturating concentrations for 15 min at room temperature and analyzed directly on a FACScalibur (Becton Dickinson, Heidelberg). Results are given as the mean fluorescence intensity ± SD of at least 9 mice per group. The abbreviations are: mean platelet volume (MPV) and hematocrit (HCT).

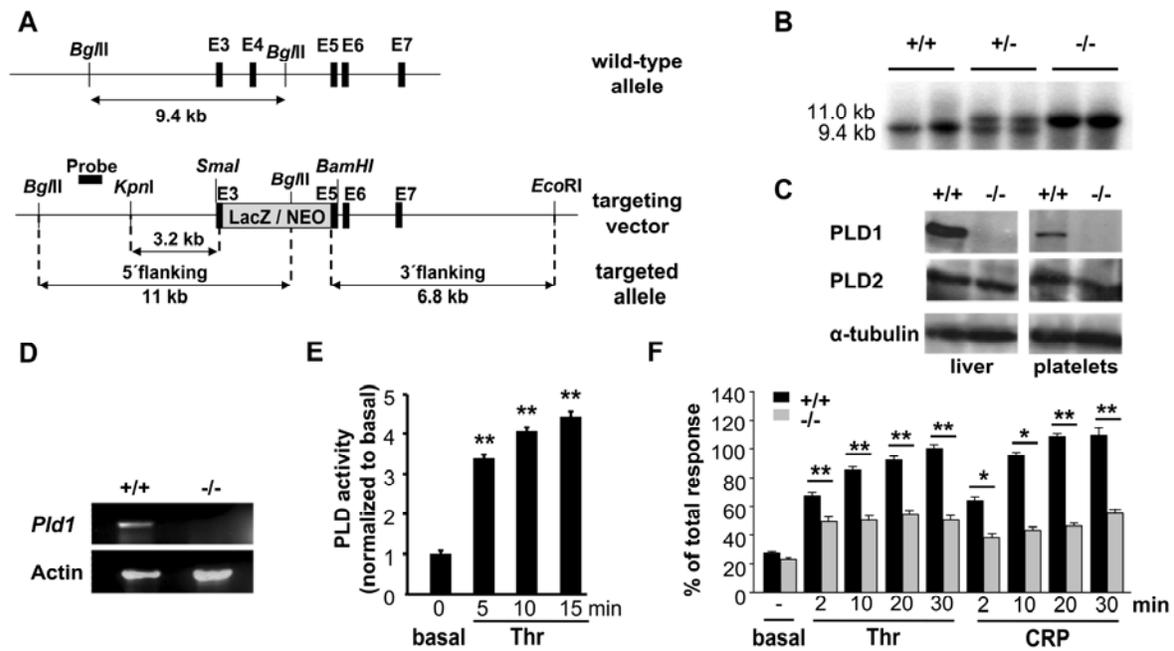


Fig. S1: Generation of PLD1-deficient mice. (A) Targeting strategy. Exons 3 to 5 were targeted for heterologous recombination. Neo-LacZ: neomycin resistance and LacZ cDNA. (B) Southern Blot of *Bgl*II-digested genomic DNA of wild-type (+/+), heterozygous (+/-), or *Pld1*^{-/-} (-/-) mice labeled with the external probe indicated in A. (C) Protein lysates of the indicated organs immunoblotted using PLD1 (Cell Signaling), PLD2 (Acris), and tubulin antibodies. (D) RT-PCR of platelet cDNA from wild-type (+/+) and *Pld1*^{-/-} (-/-) platelets. (E) Activation of PLD in platelets in response to 0.5 U/ml thrombin was measured using a live cell assay that requires in vitro culture with ³H-palmitate to label phospholipids. (F) PLD activity of wild-type and *Pld1*^{-/-} platelets was measured using a non-radioactive, non-culture requiring assay under resting conditions (basal) or upon stimulation with thrombin (Thr) or CRP. PLD activity of wild-type platelets upon thrombin stimulation was set as 100%.

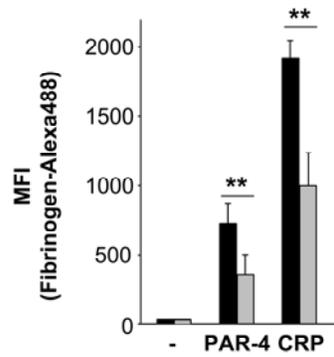


Fig. S2: Impaired $\alpha_{IIb}\beta_3$ activation in *Pld1*^{-/-} platelets under stirring conditions.

Washed platelets were incubated with 50 $\mu\text{g/ml}$ human Alexa-488 labeled fibrinogen and stimulated with 2 mM PAR-4 peptide ($\text{NH}_2\text{-AYPGKF}$) or 1 $\mu\text{g/ml}$ CRP under stirring conditions in an aggregometry tube. Data shown are MFI \pm SD ($n \geq 4$ sets of platelets per group).

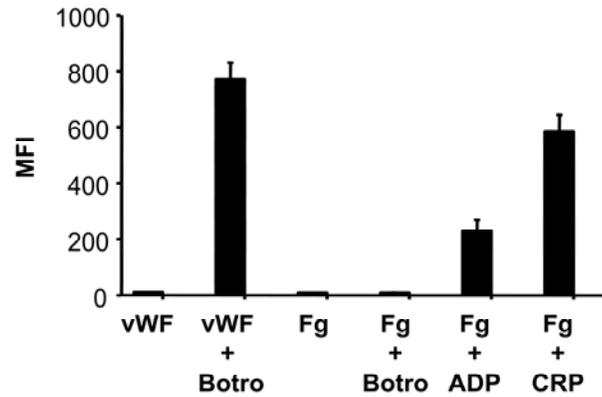


Fig. S3: Botrocetin induces vWF binding, but not integrin activation, in platelets. Washed wild-type platelets were incubated with vWF and fibrinogen (Fg) at 20 $\mu\text{g}/\text{ml}$ and stimulated with 20 $\mu\text{g}/\text{ml}$ botrocetin (botro), 3 μM ADP, or 1 $\mu\text{g}/\text{ml}$ CRP. Bound vWF was detected by FITC-conjugated anti-vWF (Dako). Data shown are MFI \pm SD from one representative experiment (with n = 4 sets of platelets).