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Antigen	Clone	Source	Reference
Mouse P-selectin	WUG1.9	In-house generated	1
Mouse GPIIb/IIIa	JON/A-PE	In-house generated	2
Mouse GPIX	p0p6	In-house generated	3
Mouse GPV	DOM/B	In-house generated	this study
Mouse GPV	DOM/C	In-house generated	this study
Mouse GPV	DOM3	In-house generated	4
Human GPV	LUM/B	In-house generated	this study
Human GPV	LUM1	In-house generated	this study
Human GPV	LUM2	In-house generated	this study
Human GPV	LUM3	In-house generated	this study
Human GPV	LUM4	In-house generated	this study
Human GPV	LUM5	In-house generated	this study
Mouse a2 integrin	LEN/B	In-house generated	5
Mouse/human GPVI	JAQ1	In-house generated	6
Mouse GPIIb/IIIA	JON1	In-house generated	4
Mouse GPIIIa	EDL1	In-house generated	4
Mouse Integrin α2	LEN1	In-house generated	7
Mouse Integrin α5	BAR1	In-house generated	8
Mouse CD9	ULF1	In-house generated	9
Mouse CD84	JER-1	In-house generated	10

Supplementary Table 1: in-house generated antibodies

Mouse GPIba	p0p/B	In-house generated	11
Mouse GPIba	p0p4	In-house generated	12
Mouse/human GPIbβ	p0p1	In-house generated	13

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The novel antibodies were generated by hybridoma technology following immunisation of *Gp5^{-/-}* mice or Wistar rats with recombinant hGPV protein or GPV immunoprecipitated from mouse or human platelet lysates.

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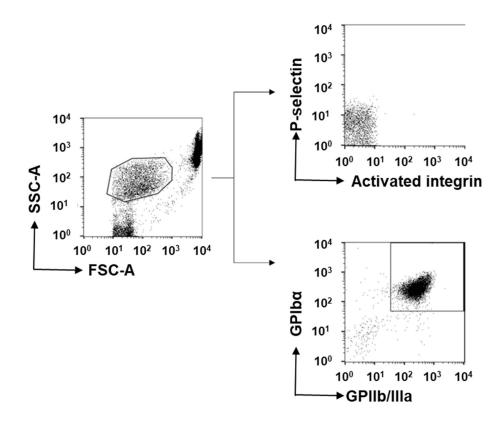
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а Aim b How much GPV signal inside & outside main structures? Fibrin Platelets (GPIX) GPV Complete GPV signal GPV signal within GPV signal outside GPV signal inside GPV signal outside Fibrin-rich region Fibrin-rich region Thrombus Thrombus C How much GPV signal inside Fibrin but outside Thrombus? Subtract Thrombus area from Fibrin-rich Add negative thrombus area to negative of \rightarrow region fibrin Only consider GPV present within this ÷ GPV signal outside of Thrombus and Fibrin region Background

8 Supplementary Fig. 1. Cleaved GPV preferentially localises to fibrin adjacent to thrombus. 9 (a) Image analysis pipeline to quantify GPV intensities (stained with AF546-labeled DOM/C) 10 inside fibrin fibres (Fibrin(ogen) AF488) and outside the thrombus (platelets labelled with anti-11 GPIX derivative AF405). (b) First, GPV signal was analysed inside and outside the 12 thrombus/fibrin. (c) GPV intensities was calculated inside fibrin but outside GPIX-positive area.

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16 Supplementary Fig. 2. Exemplified gating strategy based on FSC/SSC characteristics.

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19 **References**

- 201Schulte, V. et al. Targeting of the collagen-binding site on glycoprotein VI is not essential21for in vivo depletion of the receptor. Blood 101, 3948-3952, doi:10.1182/blood-2002-10-223242 (2003).
- 23 2 Bergmeier, W. *et al.* Flow cytometric detection of activated mouse integrin alphaIIbbeta3 24 with a novel monoclonal antibody. *Cytometry* **48**, 80-86, doi:10.1002/cyto.10114 (2002).
- Stegner, D. *et al.* Thrombopoiesis is spatially regulated by the bone marrow vasculature.
 Nature communications 8, 127, doi:10.1038/s41467-017-00201-7 (2017).
- 4 Nieswandt, B., Bergmeier, W., Rackebrandt, K., Gessner, J. E. & Zirngibl, H.
 Identification of critical antigen-specific mechanisms in the development of immune
 thrombocytopenic purpura in mice. *Blood* 96, 2520-2527 (2000).
- 305Shida, Y. *et al.* Analysis of the role of von Willebrand factor, platelet glycoprotein VI-,31and $\alpha 2\beta$ 1-mediated collagen binding in thrombus formation. *Blood* **124**, 1799-1807,32doi:10.1182/blood-2013-09-521484 (2014).
- 336Nieswandt, B. *et al.* Long-term antithrombotic protection by in vivo depletion of platelet34glycoprotein VI in mice. J. Exp. Med. 193, 459-469, doi:10.1084/jem.193.4.459 (2001).
- Nieswandt, B. *et al.* Expression and function of the mouse collagen receptor glycoprotein
 VI is strictly dependent on its association with the FcRgamma chain. *J. Biol. Chem.* 275,
 23998-24002, doi:10.1074/jbc.M003803200 (2000).
- 388Gruner, S. et al. Multiple integrin-ligand interactions synergize in shear-resistant platelet39adhesion at sites of arterial injury in vivo. Blood 102, 4021-4027, doi:10.1182/blood-2003-4005-1391 (2003).
- 41 9 Nieswandt, B. *et al.* Glycoprotein VI but not $\alpha 2\beta 1$ integrin is essential for platelet 42 interaction with collagen. *The EMBO Journal* **20**, 2120-2130, 43 doi:10.1093/emboj/20.9.2120 (2001).
- Hofmann, S., VÖGtle, T., Bender, M., Rose-John, S. & Nieswandt, B. The SLAM family
 member CD84 is regulated by ADAM10 and calpain in platelets. *J. Thromb. Haemost.* 10,
 2581-2592, doi:10.1111/jth.12013 (2012).
- 47 11 Massberg, S. *et al.* A crucial role of glycoprotein VI for platelet recruitment to the injured
 48 arterial wall in vivo. *J. Exp. Med.* **197**, 41-49 (2003).
- Bergmeier, W., Rackebrandt, K., Schroder, W., Zirngibl, H. & Nieswandt, B. Structural
 and functional characterization of the mouse von Willebrand factor receptor GPIb-IX with
 novel monoclonal antibodies. *Blood* 95, 886-893 (2000).
- Nieswandt, B. *et al.* Acute systemic reaction and lung alterations induced by an antiplatelet
 integrin GPIIb/IIIa antibody in mice. *Blood* 94, 684-693 (1999).
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