

SUPPLEMENTAL MATERIALS

Towards the therapeutic use of TSP-1/CD47 targeting TAX2 peptide as an antithrombotic agent

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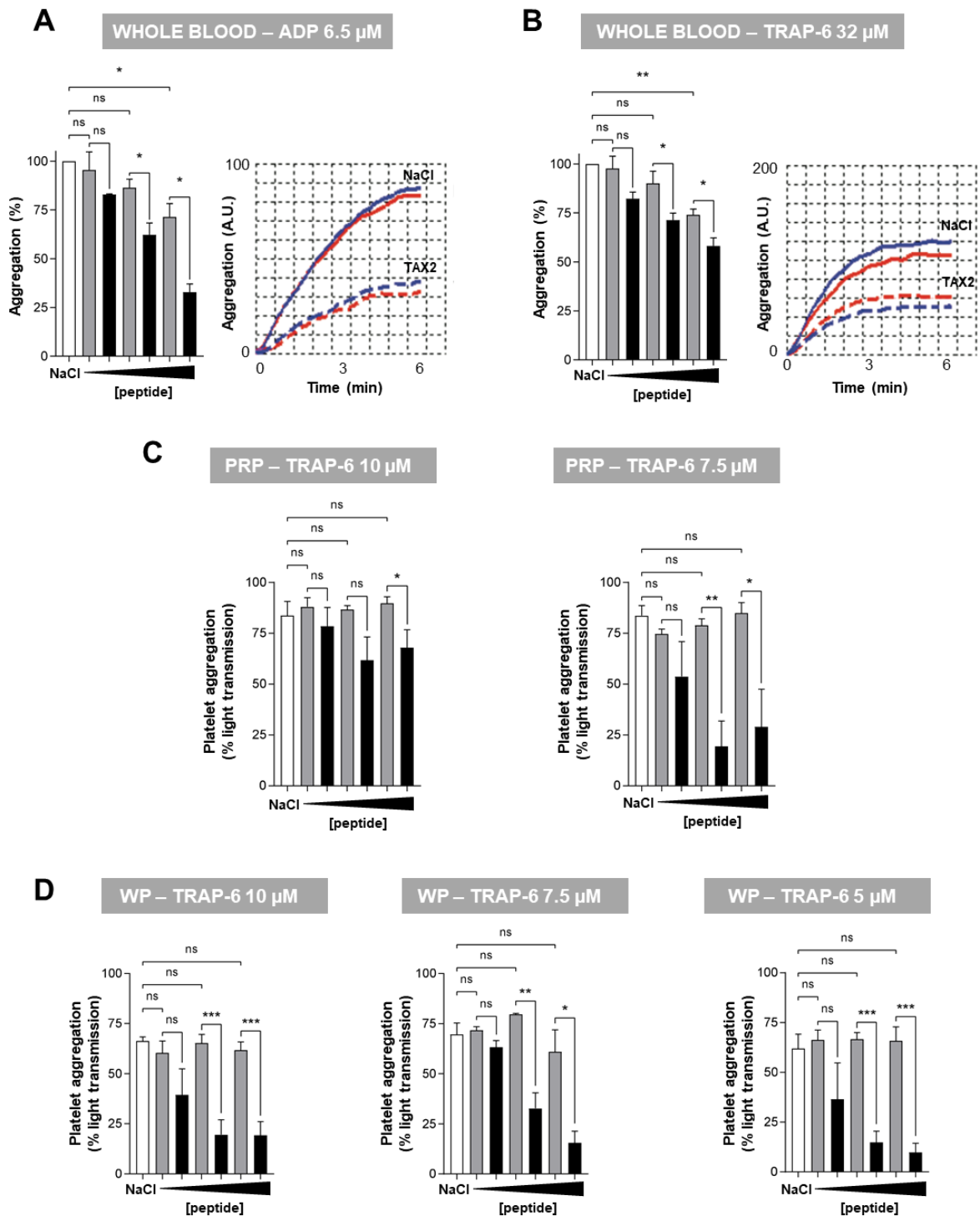
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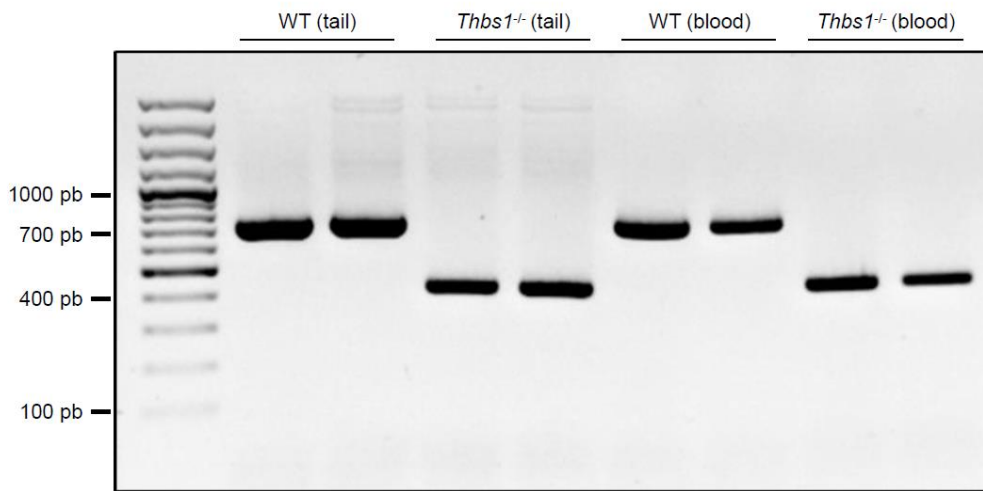
#, § These authors have contributed equally to the work.



Supplemental figure I. TAX2 peptide inhibits ADP- and TRAP-6-induced platelet aggregation in human whole blood, and TRAP-6-induced platelet aggregation in platelet-rich plasma and washed platelets.

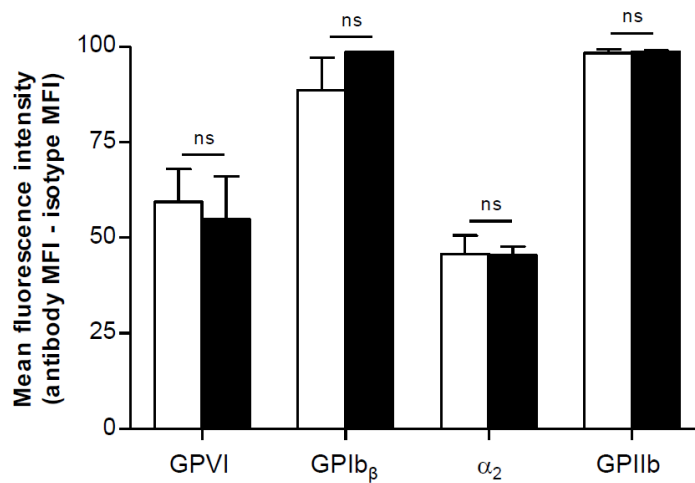
(A, B) TAX2 or the scrambled peptide (100 μ M, 400 μ M, 800 μ M) were incubated with human whole blood 3 min before addition of 6.5 μ M ADP (A) or 32 μ M TRAP-6 (B), and platelet aggregation was measured by the Multiplate[®] Analyzer. Left graph shows the results

expressed as area under the curve (AUC) of arbitrary aggregation units (AU) and normalized to the control (NaCl 0.9%). Right graph shows a representative aggregation curve for each agonist used at the same concentration. AUC is measured over a total time of 6 min and the value is averaged by measurement in duplicate represented by the two curves (blue, red) on the graph. **(C)** TAX2 or the scrambled peptide (100 μ M, 400 μ M, 800 μ M) were incubated 3 min with human platelet-rich plasma (PRP) before addition of 10 or 7.5 μ M TRAP-6, and platelet aggregation was monitored for 3 min by measuring light transmission using a Soderel Aggregometer. **(D)** TAX2 or the scrambled peptide (100 μ M, 400 μ M, 800 μ M) were incubated 3 min human washed platelets (WP) before addition of 10, 7.5 or 5 μ M TRAP-6, and platelet aggregation was monitored for 3 min by measuring light transmission using a Soderel Aggregometer. Data represent the mean \pm SEM of up to 3 **(A, B)**, each performed at least in triplicate), 9 **(C)**, and 9 **(D)** independent experiments (Student's *t* test; *: $p < 0.05$; **: $p < 0.01$; ***: $p < 0.001$, ns: non-significant). White bars, NaCl; grey bars, scrambled peptide; black bars, TAX2



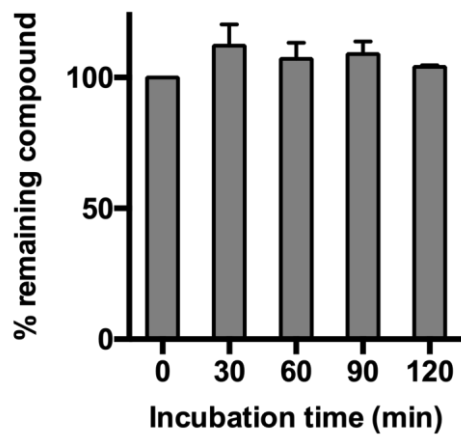
Supplemental figure III. Mice genotyping.

Agarose gel electrophoresis analysis of PCR fragments obtained in genotyping *Thbs1*^{-/-} and wild-type mice. A 700-pb and 400-bp DNA fragment corresponding to the wild-type and the mutated *Thbs1*^{-/-} genotype, respectively, is amplified from tail biopsies and blood samples of wild-type and homozygous *Thbs1*^{-/-} mice. Results of two representative out of three mice in each group are shown.

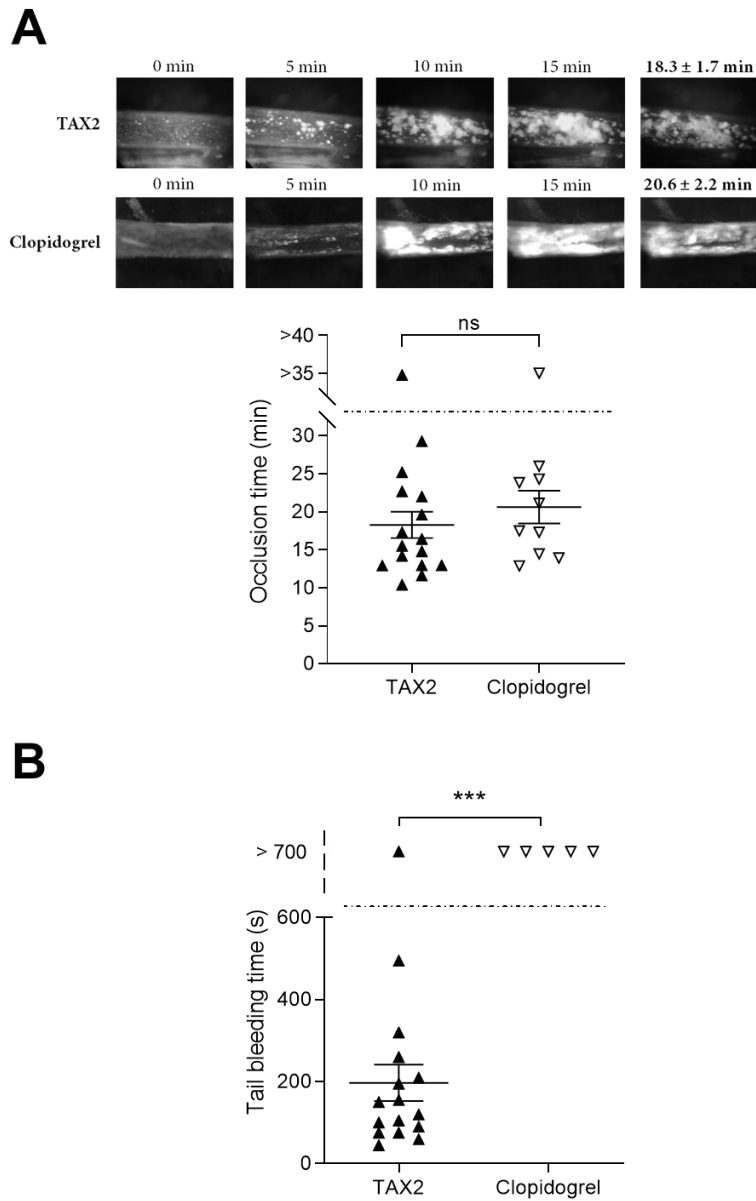


Supplemental figure IV. Comparable surface expression of GPVI, GPIIb β , α_2 and GPIIb in *Thbs1*^{-/-} versus wild-type platelets.

Platelet surface expression of integrin GPIIb, GPIIb β , α_2 and GPVI was determined by flow cytometry with FITC-conjugated anti-mouse CD41, GPIIb β , α_2 and GPVI monoclonal antibodies. White bars, wild-type mice; black bars, *Thbs1*^{-/-} mice. Results are expressed as mean fluorescence intensity (MFI) \pm SEM of 3 independent experiments. (Student's *t* test; ns: non-significant).

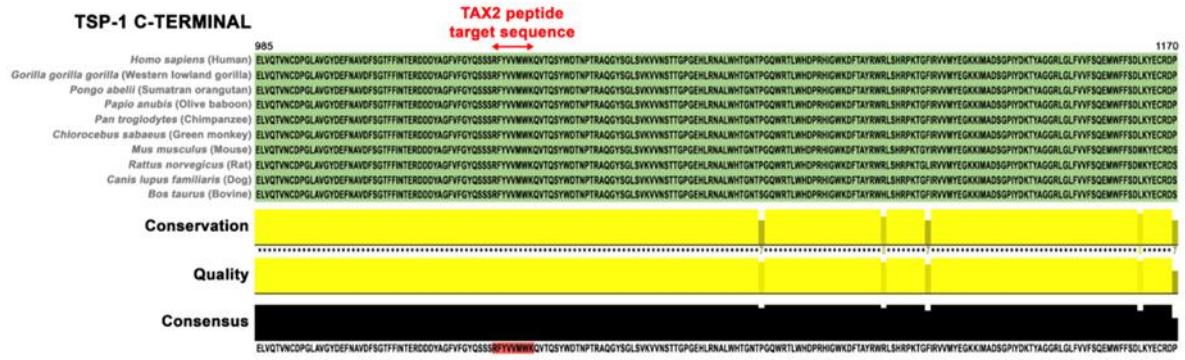


Supplemental figure V. Evaluation of TAX2 peptide stability in human plasma at 37°C. Histogram displaying the time-course of remaining TAX2 compound detected by HPLC-MS/MS (as % of T0) after incubation with human plasma (1 μ M TAX2 solution, n = 2 per time-point).



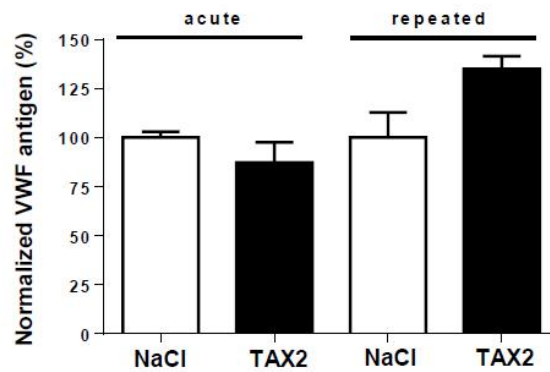
Supplemental figure VI. Comparison between clopidogrel and TAX2 effects on the carotid occlusion and tail bleeding times.

(A) Wild-type mice were intravenously injected with TAX2 (10 mg/kg) or clopidogrel (5 mg/kg) 10 minutes and 4 hours, respectively, before the induction of thrombus formation by 7.5% FeCl₃. Dot plots show times to complete thrombotic occlusion. The results are expressed as mean ± SEM. (Mann-Whitney *U* test; ns: not significant). (B) Tail bleeding times were measured on wild-type mice intravenously injected with TAX2 (10 mg/kg) or clopidogrel (5 mg/kg) 5 minutes and 4 hours, respectively, before tail cutting. The results are expressed as mean ± SEM. (Mann-Whitney *U* test; ***: *p* < 0.001).

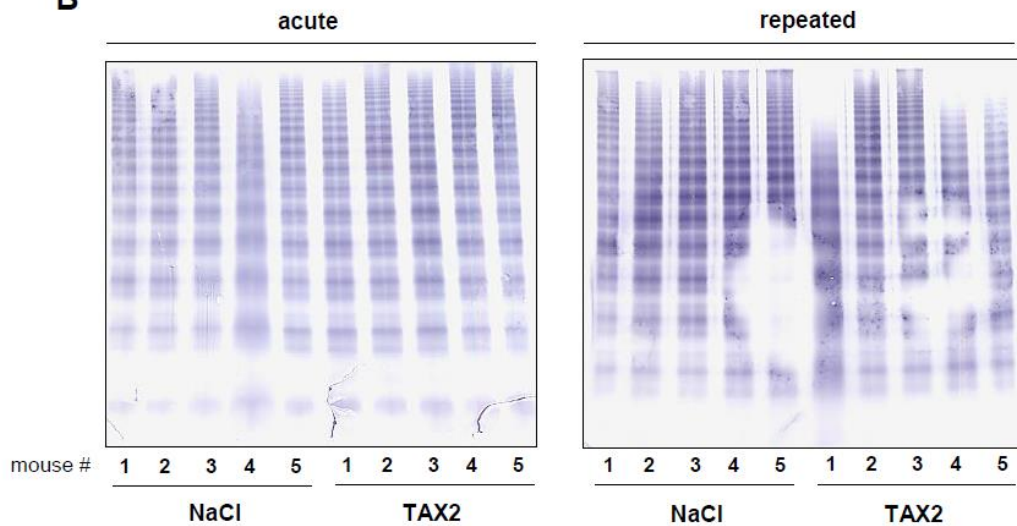


Supplemental figure VII. Multiple sequence alignment of TSP-1 C-terminal region from different species.

A



B



Supplemental figure VIII. Evaluation of TAX2 toxicity on plasma VWF antigen and multimers in mice.

Wild-type mice (8 weeks at study initiation; 5 males per group) were intraperitoneally injected with a single dose of TAX2 (100 mg/kg) or vehicle (NaCl 0.9%) for evaluation of acute toxicity (acute), or daily and for 7 consecutive days for evaluation of repeated toxicity (repeated) on plasma VWF antigen (**A**) and VWF multimers (**B**). **A**, VWF antigen was measured by ELISA and results are expressed as mean \pm SEM. **B**, Multimer composition of circulating VWF protein in mice receiving either acute or repeated administrations of NaCl or TAX2. Gels represent analysis of plasma samples using SDS-agarose electrophoresis.

Major Ressources Table

Animals (*in vivo* studies)

Species	Vendor or Source	Background Strain	Sex	Persistent ID / URL
<i>Mus musculus</i>	Janvier Labs (Saint-Berthevin, France)	C57BL/6Jrj	Female and male	https://www.janvier-labs.com/fiche_produit/souris-c57bl-6jrj/
<i>Rattus norvegicus</i>	Janvier Labs (Saint-Berthevin, France)	Sprague Dawley	Female and male	https://www.janvier-labs.com/fiche_produit/rat_sprague_dawley/

Genetically Modified Animals

	Species	Vendor or Source	Background Strain	Other Information	Persistent ID / URL
Parent - Male	<i>Mus musculus</i>	Charles River Labs (L'Arbresle, France)	C57BL/6J	<i>Thbs1</i> ^{-/-} C57BL/6 (B6.129S2-Thbs1 ^{tm1Hyn} /J)	
Parent - Female	<i>Mus musculus</i>	Charles River Labs (L'Arbresle, France)	C57BL/6J	<i>Thbs1</i> ^{-/-} C57BL/6 (B6.129S2-Thbs1 ^{tm1Hyn} /J)	

Antibodies

Target antigen	Vendor or Source	Catalog #	Working concentration	Persistent ID / URL
CD47	BD Pharmingen (Le Pont-de-Claix, France)	Clone B6H12, catalog no. 556044	0.5 µg/mL (1:1000)	https://wwwbdbiosciences.com/us/applications/research/stem-cell-research/cancer-research/human/purified-mouse-anti-human-cd47-b6h12/p/556044
CD47	BD Pharmingen (Le Pont-de-Claix, France)	Clone MIAP301, catalog no. 555297	0.5 µg/mL (1:1000)	https://wwwbdbiosciences.com/us/applications/research/stem-cell-research/cancer-research/mouse/purified-rat-anti-mouse-cd47-miap301/p/555297
TSP-1	Abcam (Paris, France)	Clone A6.1, catalog no. ab1823	1:1000	https://www.abcam.com/thrombospondin-antibody-a61-ab1823.html

pTyr	Merck Millipore (Darmstadt, Germany)	Clone 4G10, catalog no. 05-1050-M	1:1000	https://www.sigmaaldrich.com/catalog/product/mm/051050m?lang=fr&region=FR
β-actin	Santa Cruz Biotechnology	Clone C4, catalog no. sc-47778	0.2 µg/mL (1:1000)	https://www.scbt.com/fr/p/beta-actin-antibody-c4
GPIIb	Abcam (Paris, France)	Clone [EPR4330], catalog no. ab134131	0.721 µg/mL (1:1000)	https://www.abcam.com/cd41-antibody-epr4330-ab134131.html
GPIIIa	Abcam (Paris, France)	Clone [EPR2342], catalog no. ab119992	0.03 µg/mL (1:1000)	https://www.abcam.com/integrin-beta-3-antibody-epr2342-ab119992.html
GPIb	Abcam (Paris, France)	Clone [EPR6995], catalog no. ab134087	0.119 µg/mL (1:1000)	https://www.abcam.com/cd42b-antibody-epr6995-ab134087.html
CD36	Abcam (Paris, France)	Clone [EPR6573], catalog no. ab133625	1.685 µg/mL (1:1000)	https://www.abcam.com/cd36-antibody-epr6573-ab133625.html
CD36	Stemcell	Clone FA6-152	2 µg/mL (1:500)	https://www.stemcell.com/anti-human-cd36-antibody-clone-fa6-152.html
Von Willebrand Factor	Dako Agilent (USA)	Catalog no. A0082	5 µg/mL	
Normal mouse IgG	Santa Cruz Biotechnology	Catalog no. sc-2025	10 µg	https://www.scbt.com/fr/p/normal-mouse-igg
Normal rat IgG	Santa Cruz Biotechnology	Catalog no. sc-2026	10 µg	https://www.scbt.com/fr/p/normal-rat-igg
HRP-linked secondary antibodies anti-mouse IgG	Cell Signaling Technology	Catalog no. 7076S	1:5000-10000	https://www.cellsignal.com/products/secondary-antibodies/anti-mouse-igg-hrp-linked-antibody/7076
HRP-linked secondary antibodies anti-rat IgG	Cell Signaling Technology	Catalog no. 7077S	1:7500-10000	https://www.cellsignal.com/products/secondary-antibodies/anti-rat-igg-hrp-linked-antibody/7077
HRP-linked secondary antibodies anti-rabbit IgG	Cell Signaling Technology	Catalog no. 7074S	1:7500-10000	https://www.cellsignal.com/products/secondary-antibodies/anti-rabbit-igg-hrp-linked-antibody/7074
HRP-linked anti-Von Willebrand Factor	Dako Agilent (USA)	Catalog no. P0226	1:6000	

FITC-conjugated anti-mouse GPIb β	Emfret Analytics (Eibelstadt, Germany)	Clone Xia.C3, catalog no. M050-1	1:20	https://www.emfret.com/uploads/tx_be_products/M050-1_XiaC3.pdf
FITC-conjugated anti-mouse GPVI	Emfret Analytics (Eibelstadt, Germany)	Clone JAQ1, catalog no. M011-1	1:20	https://www.emfret.com/uploads/tx_be_products/M011-1_JAQ1.pdf
FITC-conjugated anti-mouse α 2	Emfret Analytics (Eibelstadt, Germany)	Clone Sam.G4, catalog no. M070-1	1:20	https://www.emfret.com/uploads/tx_be_products/M070-1_SamG4.pdf
FITC-conjugated anti-mouse α IIb	BD Biosciences (Le Pont de Claix, France)	Clone MWReg30, catalog no. 553848	62.5 μ g/mL (1:8)	https://www.bdbiosciences.com/eu/applications/research/b-cell-research/surface-markers/mouse/fitc-rat-anti-mouse-cd41-mwreg30/p/553848