Supplemental Materials

Reelin amplifies GPVI activation and alphaIIb beta3 integrin outside-in signaling via PLC gamma 2 and Rho GTPases

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Expanded Materials & Methods

Murine Platelet Preparation and Cell Count

Blood was drawn from anaesthetized mice of both sexes via retroorbital plexus and collected in 300 µl heparin (20 U/ml) or 100 µl acid-citrate-dextrose. The blood was centrifuged at 250 g at 22°C for 5 min. The resulting supernatant was centrifuged at 50 g for 6 min to obtain platelet-rich-plasma (PRP). PRP was centrifuged at 650 g using apyrase and prostaglandin I2 for 5 min. The remaining pellet was resuspended in murine Tyrode's buffer (136 mM NaCl, 0.4 mM Na_2HPO4, 2.7 mM KCl, 12 mM $NaHCO_3$, 0.1% glucose, 0.35% bovine serum albumin, pH 7.35), apyrase (0.02 U/ml) and prostacyclin (0.5 µM) and centrifuged at 650 g for 5 min. Depending on the following experiment platelets were either resuspended in Tyrode's buffer (pH 7.35) or Tyrode's buffer (pH 7.35) added $CaCl_2$ (2 mM).

Flow cytometry

Flow cytometry analysis was performed as described elsewhere.[1] A two-colour analysis of murine platelet activation was performed using fluorophore-labelled antibodies for P-selectin expression (Wug.E9-FITC, Emfret Analytics) and active alphaIIb beta3 integrin (JON/A-PE, Emfret Analytics). Heparinized blood was diluted in 500µl Tyrode's buffer (pH 7.35) and washed twice via centrifugation at 650 at 22°C for 5 min. The remaining pellet was resuspended in 500 µl Tyrode's buffer (pH 7.35) supplemented with $CaCl_2$ (2 mM). Platelets were treated with the indicated agonists at 22°C for 15 min. The reaction was stopped by the addition of PBS and samples were analyzed by use of FACSCalibur flow cytometer (BD Biosciences).

For the detection of phosphatidylserine exposure Cy™5 AnnexinV (BD Biosciences) – staining was performed while binding buffer (10mM Hepes, 140 mM NaCl, 2.5 mM $CaCl_2$, pH 7.4)

instead of PBS was used. CD42 (emfret Analytics, M040-1) served as a platelet specific marker.
To analyze the glycoprotein surface expression blood samples were mixed with the following antibodies: FITC-labelled rat anti-mouse GPVI, (emfret Analytics, M011-1), FITC-labelled Rat anti-mouse/human integrin alpha5 chain (CD49e) (emfret Analytics, M080-1), FITC-labelled rat anti-mouse/human integrin beta3 (GPIIIa, CD61) (emfret Analytics, M031-1) and PE-labeled rat anti-mouse GPIbα (CD42b). This was then incubated at 22°C for 15 min before measurement.

Western Blot analysis
Western blot analysis was performed as described earlier to detect total of RAC1, RhoA and CDC42 serving as controls for the determined amount of active Rho-GTPases in the G-LISA® assays.
The lysates were prepared as recommended in the respective protocol of the G-LISA® kits from $80*10^6$ platelets per test sample. Samples were separated with sodium dodecyl sulfate polyacrylamide gelelectrophoresis and transferred onto a nitrocellulose membrane. The membrane was blocked using 5 % powdered skim milk in tris-buffered saline with 0.1 % Tween 20 and probed with antibodies: RAC1, RhoA, CDC42 antibody (RAC1: BD Transduction Lab., 610650; RhoA: ThermoFisher Scientific, 1B8-1C7; CDC42: BD Transduction Lab., 610929).
To perform phosphorylation studies we used $40*10^6$ platelets per treatment sample. The platelets were treated with 5 µg/ml CRP and recombinant reelin (5 nM) for the indicated time points and lysed. After sodium dodecyl sulfate polyacrylamide gelelectrophoresis the samples were probed with Phospho-PLC gamma 2 antibody (3874), total PLC gamma 2 antibody (3872), phospho-Syk (Tyr525/526) antibody (2711), total Syk antibody (2712), phospho-PAK1 (Thr423)/ 2 (Thr402), PAK1 antibody (2602) (all Cell Signaling Technology).
To observe overall phosphorylation of platelets, an anti-phosphotyrosine antibody, clone 4G10® (Merck Millipore KgA, 05-321) was used. Xcell SureLock Mini-Cell and the Novex™ WedgeWell™ 4-12% Tris-Glycine gel (Thermo Fisher Scientific Inc.) were used as described in the manufacturer's protocol. Antibodies against β-Actin (4979, Cell Signaling Tech.) or α-Tubulin (2144, Cell Signaling Tech) served as loading controls.
The primary antibody incubation and the incubation with HRP-conjugated anti-rabbit and anti-mouse and anti-sheep IgGs (GE Healthcare, Code: NA9340, 1:2500) were performed following the manufacturer's manuals. For visualizing protein bands Immobilon Western Chemiluminescent HRP substrate solution (BioRad) and the Vilber Fusion-FX6-EDGE V.070 system and for quantification of the chemiluminescent signals Evolution-Capt EDGE software (Version 18, 02) was used.

Platelet adhesion and spreading
Cover slips (24 x 60 mm) were either coated with recombinant reelin according to established protocols,[2] 100 µg/ml fibrinogen (200 µg/mL type I collagen at a defined area (10 x 10 mm) at 4°C over night. Afterwards they were blocked with 1% bovine serum albumin for 60 min. 8 x 10^4 isolated platelets, which were resuspended in 70 µl Tyrode's buffer (pH 7.35) supplemented with $CaCl_2$ (2 mM), applicated on the prepared cover slips and incubated at room temperature for indicated time points.
Experiments were performed as described previously.[3, 4] In case of the static adhesion assays with the GPVI inhibitor JAQ1, platelets were preincubated with 2 µg/mL JAQ1 (Emfret Analytics, M011-0) for 15 min prior to the application on the cover slips. Non-adherent platelets were carefully removed by rinsing 2 x with PBS. The preparation was fixed by 4 % phosphate buffered formaldehyde (pH 7.0) at 4°C for 10 min, subsequently rinsed again carefully with PBS and inundated with Aquatex (Merck KgA). Platelet adhesion was documented after drying at 4°C over night with Microscope Axio Observer.D1 (Carl Zeiss Microscopy GmbH). The resulting pictures were analyzed using ImageJ-win64 and ZEN 2.6 (blue edition).

Determination of receptor binding sites on human platelets
Platelets in diluted citrated whole blood from healthy volunteers (local Ethics Committee of the University Medical Center Mainz, Germany: 837.302.12 (8403-F); 2018-13290 1) were

incubated with anti-GPIbalpha-FITC (Beckmann Coulter) or anti-human GPVI-eFluor-660 clone HY101 (eBioscience) for 20 min at room temperature. Using Quantum Simply Cellular anti-mouse IgG, absolute numbers of antigen binding sites per platelet were calculated by flow cytometry (FACS Canto II flow cytometer with FACS Diva software) according to the manufacturer's instructions.

References

1. Jarre A, Gowert NS, Donner L, Munzer P, Klier M, Borst O, et al. Pre-activated blood platelets and a pro-thrombotic phenotype in APP23 mice modeling Alzheimer's disease. Cell Signal. 2014;26(9):2040-50.

2. Leemhuis J, Bouché E, Frotscher M, Henle F, Hein L, Herz J, et al. Reelin signals through apolipoprotein E receptor 2 and Cdc42 to increase growth cone motility and filopodia formation. The Journal of neuroscience : the official journal of the Society for Neuroscience. 2010;30(44):14759-72.

3. Elvers M, Pozgaj R, Pleines I, May F, Kuijpers MJ, Heemskerk JM, et al. Platelet hyperreactivity and a prothrombotic phenotype in mice with a gain-of-function mutation in phospholipase Cgamma2. Journal of thrombosis and haemostasis : JTH. 2010;8(6):1353-63.

4. Gowert NS, Klier M, Reich M, Reusswig F, Donner L, Keitel V, et al. Defective Platelet Activation and Bleeding Complications upon Cholestasis in Mice. Cellular physiology and biochemistry : international journal of experimental cellular physiology, biochemistry, and pharmacology. 2017;41(6):2133-49.

Major Resource Table

Animals (in vivo studies)

Species	Vendor or Source	Background Strain	Sex
mice	Prof. Frotscher, Hamburg	B6C3Fe-a/a-Relnrl/+_TgH(Reeler)	female and male
mice	Prof. Watson	C57BL/6J;129-Gp6^{tm1ware}	female and male

Animal breeding

	Species	Vendor or Source	Background Strain	Other Information
Parent - Male	Rln$^{-/-}$	own breeding strain in the animal facility Düsseldorf	B6C3Fe-a/a-Relnrl/+_TgH(Reeler)	Rln$^{-/-}$ male + Rln$^{-/+}$ female
Parent - Female	Rln$^{-/+}$	own breeding strain in the animal facility Düsseldorf	B6C3Fe-a/a-Relnrl/+_TgH(Reeler)	Rln$^{-/-}$ male + Rln$^{-/+}$ female
Parent - Male	Rln$^{+/+}$	own breeding strain in the animal facility Düsseldorf	B6C3Fe-a/a-Relnrl/+_TgH(Reeler)	wt male + wt female; backcrossing not allowed as of higher burden for the animals, but every 6 generation Rln$^{-/+}$ animals are used to prevent the wt and ko strains drift too far from each other genetically
Parent - Female	Rln$^{+/+}$	own breeding strain in the animal facility Düsseldorf	B6C3Fe-a/a-Relnrl/+_TgH(Reeler)	wt male + wt female; backcrossing not allowed as of higher burden for the animals, but every 6 generation Rln$^{-/+}$ animals are used to prevent the wt and ko strains drift too far from each other genetically
Parent - Male	GPVI$^{-/-}$	own breeding strain in the animal facility Düsseldorf	C57BL/6J;129-Gp6^{tm1ware}	GPVI$^{-/-}$ male + GPVI$^{-/-}$ female

Parent - Female	GPVI$^{-/-}$	own breeding strain in the animal facility Düsseldorf	C57BL/6J;129-Gp6^{tm1ware}	GPVI$^{-/-}$ male + GPVI$^{-/-}$ female
Parent - Male	GPVI$^{+/+}$	own breeding strain in the animal facility Düsseldorf	C57BL/6J;129-Gp6^{tm1ware}	GPVI$^{+/+}$ male + GPVI$^{+/+}$ female
Parent - Female	GPVI$^{+/+}$	own breeding strain in the animal facility Düsseldorf	C57BL/6J;129-Gp6^{tm1ware}	GPVI$^{+/+}$ male + GPVI$^{+/+}$ female
Parent - Male	APP$^{-/-}$	own breeding strain in the animal facility Düsseldorf	C57BL/6-TgH(APP$^{-/-}$)	APP$^{-/-}$ male + APP$^{-/-}$ female
Parent - Female	APP$^{-/-}$	own breeding strain in the animal facility Düsseldorf	C57BL/6-TgH(APP$^{-/-}$)	APP$^{-/-}$ male + APP$^{-/-}$ female

Antibodies

Target antigen	Vendor or Source	Catalog #	Working concentration	Lot # (preferred but not required)
GPVI (JAQ1-FITC)	emfret Analytics	M011-1	5 µl/0.5-1*10^6 platelets in 25 µl	0111-C
Integrin α5 chain (CD49e) (Tap.A12-FITC)	emfret Analytics	M080-1	5 µl/0.5-1*10^6 platelets in 25 µl	0801-B
Integrin β3 (GPIIIa, CD61) (GPIIIa, CD61-FITC)	emfret Analytics	M031-1	5 µl/0.5-1*10^6 platelets in 25 µl	0311-C
GPIbα (CD42b) (Xia.G5-PE)	emfret Analytics	M040-1	5 µl/0.5-1*10^6 platelets in 25 µl	0402-C
P-selectin (-FITC)	emfret Analytics	D200	5µl 0.5-1*10^6 platelets in 25 µl	FE
Integrin β3 (-PE)	emfret Analytics	D200	5 µl/0.5-1*10^6 platelets in 25 µl	FE
RAC1	BD Transduction Laboratories	610650	312,5 ng/ml	5107647
RhoA	ThermoFisher Scientific	1B8-1C7	2µg/ml	SB243276
CDC42	BD Transduction Laboratories	610929	500 ng/ml	7166624
p-PLCγ2 (Tyr759)	Cell Signaling Technologies	3874	82 ng/ml	3874S
PLCγ2	Cell Signaling Technologies	3872	115,5 ng/ml	3872S
p-Syk (Tyr525/526)	Cell Signaling Technologies	2711	804 ng/ml	2711S
Syk	Cell Signaling Technologies	2712	73 ng/ml	2712S

p-PAK 1 (Thr423) /2 (Thr402)	Cell Signaling Technologies	2601	30 ng/ml	18
PAK1	Cell Signaling Technologies	2602	8 ng/ml	2602S
α-Tubulin	Cell Signaling Technologies	2144	68 ng/ml	2144S
β-Actin (13E5)	Cell Signaling Technologies	4970	61 ng/ml	4970S
phospho-Tyrosine, clone 4G10	Merck Millipore	05-321	0.5-2 µg/ml	2658756
164-496 mreelin, clone G10	Merck Millipore	MAB 5364	2 µg/ml	309957
Anti-Reelin (CR-50) IgG	MBL	D223-3	1 mg/ml	016
GPVI	R&D Systems	AF 6758	1 µg/ml	CFOQ011812A
GPVI	emfret Analytics	M011-0 CUST	1 mg/ml	
Rat IgG2b	ThermoFisher Scientific	02-9688	0.5 mg/ml	TI274976
GPVI	emfret Analytics	M011-F	1 mg/ml	TI274976
Integrin αIIbβ3 (LeoH4)	emfret Analytics	M021-0	1 µg / 10^6 platelets	0210-B
Rabbit IgG (-HRP)	GE Healthcare UK Ltd.	NA934V	1:2500	16963367
Mouse IgG (-HRP)	GE Healthcare UK Ltd.	NA931V	1:2500	14263051
Sheep IgG (-HRP)	R&D Systems	HAF016	1:2500	XDP1218021
anti-human CD42b (clone SZ2)	Beckman Coulter	IM0409	5 µg/ml	
anti-human GPVI-eFluorR-660 clone HY101	eBioscience, Frankfurt	15510147	5 µg/ml	

Cultured Cells

Name	Vendor or Source	Sex (F, M, or unknown)

Other

Description	Source / Repository	Persistent ID / URL

CRP	Richard Farndale, University of Cambridge, UK	
thrombin	Roche Diagnostics	10602400001
recombinant reelin	AG Bock, Clinic of Gastroenterology, Hepatology and Immunology, Heinrich Heine University Medical Center	D11B and D07B
mock (vehicle recombinant reelin)	AG Bock, Clinic of Gastroenterology, Hepatology and Immunology, Heinrich Heine University Medical Center	D11B and D07B
RAC1 G-LISA™ Kit	Cytoskeleton, Inc.	BK 128
RhoA G-LISA™ Kit	Cytoskeleton, Inc.	BK 124
Cdc42 G-LISA™ Kit	Cytoskeleton, Inc.	BK 127
$CaCl_2$	Sigma-Aldrich	C5080
Fibrinogen, human	Sigma-Aldrich	3879
Collagen reagens HORM® suspension	Takeda Austria GmbH	1130630
ADP	Sigma-Aldrich	A2754
U46619	Tocris bioscience	1932
Par4 activating peptide	JPT Peptide Technologies	AYPGKF
paraffin	Roth®	CN48.1
Hematoxylin	Sigma Aldrich	GHS316
$FeCl_3$	Roth®	D8537
PBS	Sigma Aldrich	B207151
Ampuwa dH_2O	Fresenius Kabi	
Pierce® NHS-Activated Magnetic Beads	Thermoscientific	88826
recombinant GPVI	R&D	3627-GP-050
heparin	Braun	12811261969810415
PGI_2	Calbiochem	538925
apyrase	Sigma-Aldrich	A 7646
Complete protease inhibitor cocktail	Roche Diagnostics	04693124001
NaCl	Sigma Aldrich	S3014
Na_2HPO_4	Merck	1.06580.0500
KCl	Merck	9023717
$NaHCO_3$	Merck	0040843
D8+)-glucose	Roth	HN 06.2
BSA	Sigma-Aldrich	A7906
eosin	Roth	3139.2
C_2H_5OH	VWR Chemicals	85033.360
tri-Natriumcitrat Dihydrat	Roth	4088.3
Citric acid	Roth	6490.3

PFA 4%	Roth	P087.5
Aquatex	Merck	1.08562.0050
EHT 1864	Sigma-Aldrich	E1657
DDT	Sigma-Aldrich	D0632
Clarity™Western ECL Substrate	Bio-Rad Lab. Inc.	170-5060
Convulxin	Enzo Lifesciences	ALX-350-100-C050
Collagen Horm®	Takeda Austria	1130630
NEM	Sigma-Aldrich	E3876-5G
Quantum™ Simply Cellular® Mouse IgG Kit	Bio-Rad	FCSC815

Online Figures I-VII

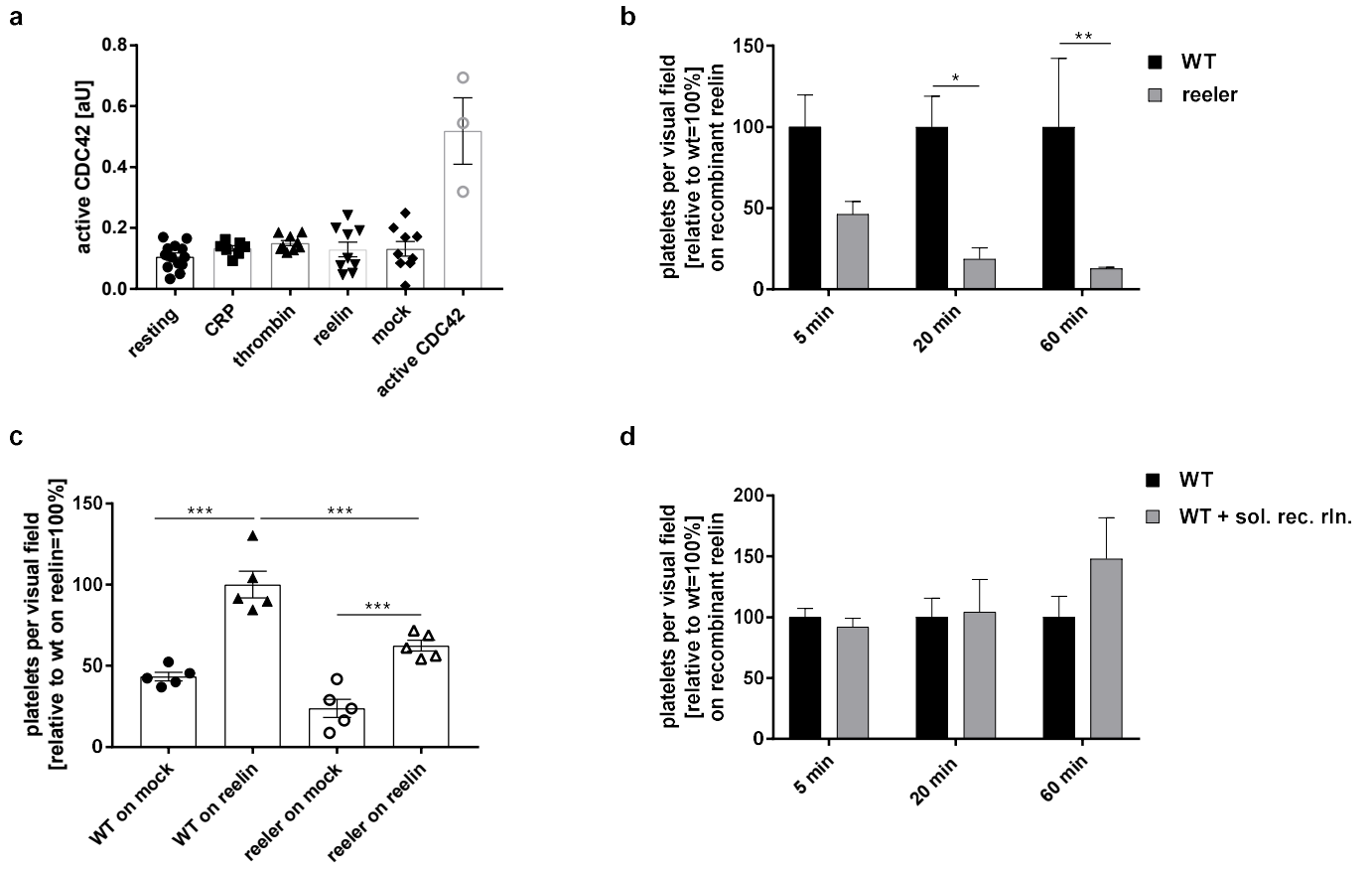

Figure I. Reelin supports platelet adhesion. **(a)** Active CDC42 measurements with positive control, n = 3-13. **(b)** Adhesion of WT and reelin deficient platelets on immobilized reelin after indicated time points, bar graphs depict mean values + SEM, n = 5, * $P < 0.05$, ** $P < 0.01$. **(c)** Reelin supports mice platelet adhesion. Adhesion of WT and reeler platelets on immobilized mock (control) and recombinant reelin (5 nM) for 20 min, bar graphs depict mean values ± SEM, n = 5, *** $P < 0.001$. **(d)** No inhibition of platelet adhesion on immobilized recombinant reelin in the presence of soluble recombinant reelin, bar graphs depict mean values + SEM, n = 5.

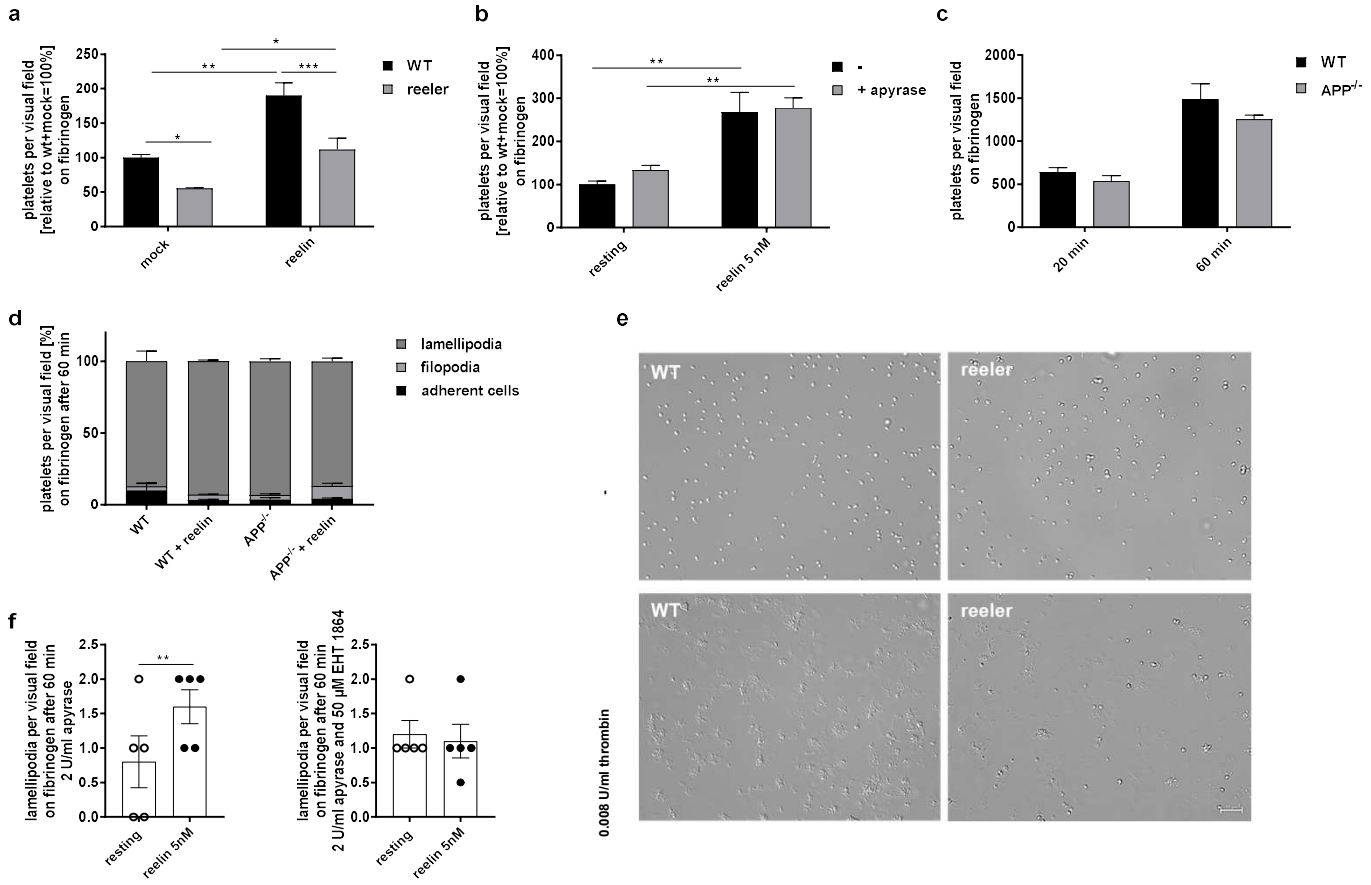

Figure II. Reelin supports outside-in signaling. **(a)** **(a)** Defective adhesion in reelin deficient platelets on fibrinogen can be partially rescued by recombinant reelin. Adhesion of WT and reelin deficient platelets treated with recombinant reelin (5 nM) and vehicle (mock) on fibrinogen for 20 min, bar graphs depict mean values + SEM, n = 5, * P < 0.05, ** P < 0.01, *** P < 0.001. **(b)** No differences of WT platelet adhesion on fibrinogen in the presence of the ADP scavenger apyrase. Platelets untreated or treated with recombinant reelin (5 nM) and apyrase (2 U/ml) were allowed to adhere on fibrinogen for 60 min, bar graphs depict mean values + SEM, n = 5, ** P < 0.01. **(c-d)** No differences in adhesion (c) and spreading (d) of WT and APP-deficient platelets on fibrinogen, bar graphs depict mean values + SEM, n = 5. **(e)** Representative pictures of spread WT and reeler platelets on fibrinogen with thrombin treatment (0.008 U/ml) after 60 min, scale bar 10 µm. (**f)** Lamellipodia formation on fibrinogen after 60 min in the presence of apyrase (2 U/ml) (left and right) and upon RAC1 inhibition (50 µM EHT1864) (right), bar graphs depict mean values ± SEM, n = 5, ** P < 0.01.

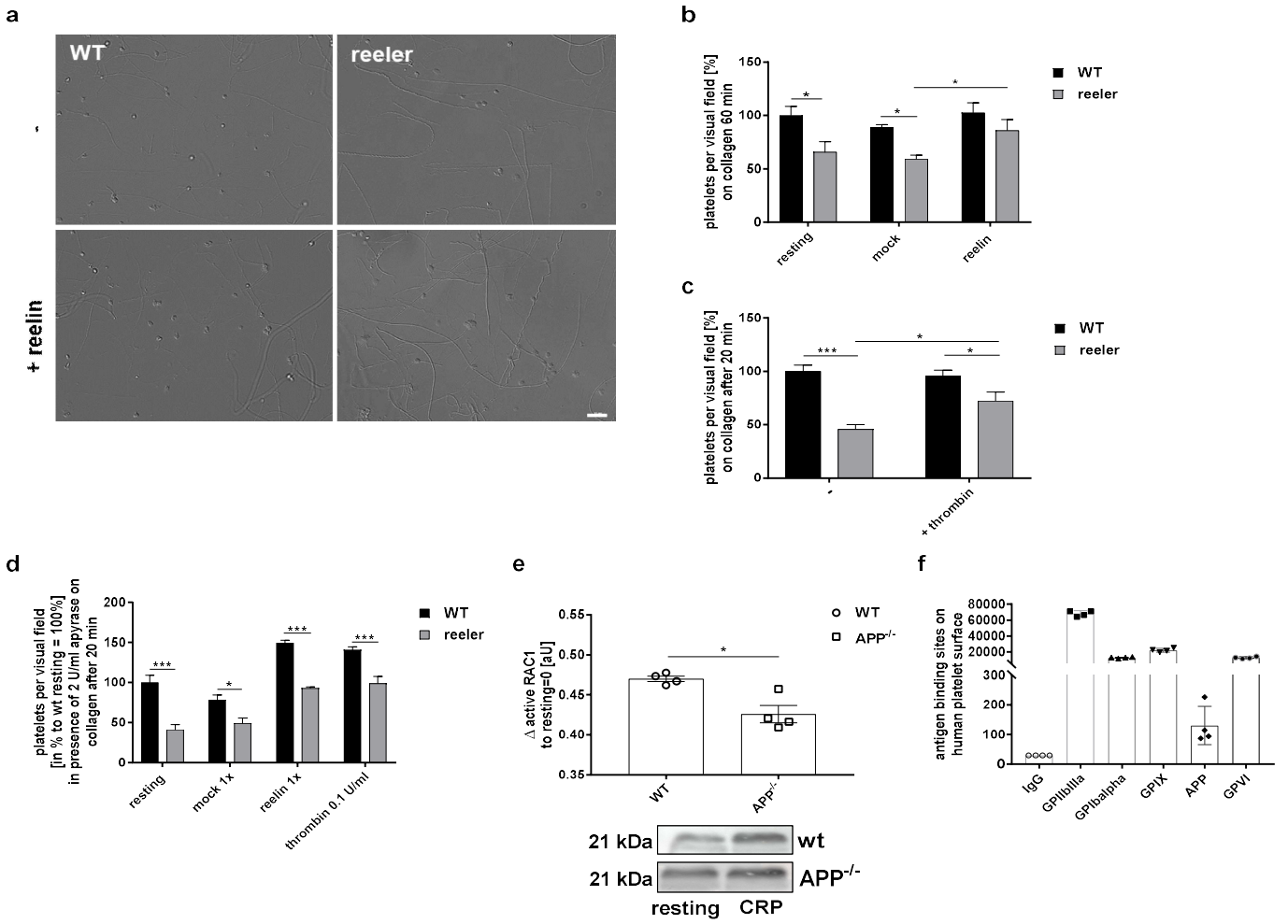

Figure III. Reelin supports adhesion and spreading on collagen via GPVI. (a) Representative pictures of WT and reeler platelets on collagen after 20 min, scale bar 10µm. **(b-d)** Only partial reelin and thrombin rescue of defective reeler adhesion. **(b)** Defective reeler platelet adhesion to collagen is partially rescued by addition of recombinant reelin (5 nM). WT and reeler platelets untreated and treated with recombinant reelin (5 nM) and mock (g) were allowed to adhere on collagen for 60 min, bar graphs mean values + SEM (g), n = 5, * $P < 0.05$. **(c-d)** Comparable phenotype of reelin deficient platelets upon thrombin treatment after 20 min on collagen matrix (c) and in presence of ADP inhibitor (d). Adhesion of WT and reelin deficient platelets with reelin (5 nM), mock, thrombin (0.01 U/ml) and apyrase (2 U/ml) treatment on collagen after 20 min, bar graphs mean values + SEM, n = 5, * $P < 0.05$, *** $P < 0.001$. **(e)** Reduced Rac1 activation in APP deficient platelets after CRP (20 µg/ml) treatment using G-LISA® assay, bar graphs mean values ± SEM, n = 4, * $P < 0.05$ and representative Western blots for total RAC1 as control to active RAC1. **(f)** Small number of APP copies on human platelets, bar graphs mean ± SEM, n = 4.

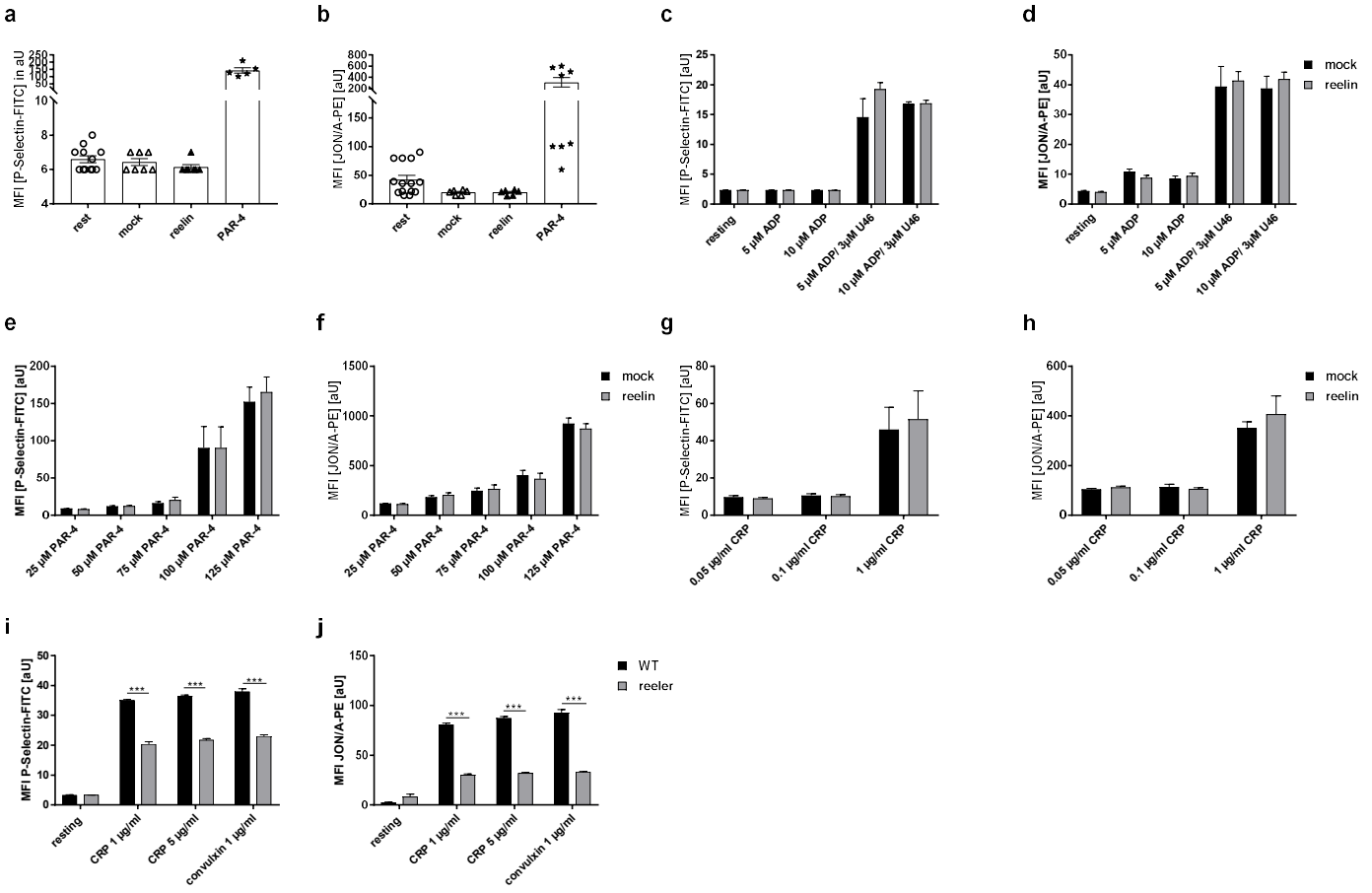

Figure IV. Extracellular reelin does not support platelet activation. (a,b) P-selectin exposure and alphaIIb beta3 integrin activation in reelin, mock and PAR-4 (100 µM) treated platelets, bar graphs depict mean values ± SEM, n = 5-12. **(c,d)** P-selectin and alphaIIb beta3 integrin activation after co-treatment with reelin or mock and ADP or ADP/U46619, bar graphs depict mean values + SEM, n = 5. **(e-f)** P-selectin exposure and alphaIIb beta3 integrin activation after co-treatment with reelin or mock followed by low doses of PAR-4, bar graphs depict mean values + SEM, n = 9. **(g,h)** P-selectin exposure and integrin activation after co-treatment with low doses of CRP following reelin (5 nM) treatment or mock, bar graphs depict mean values + SEM, n = 3-4. **(i,j)** P-selectin exposure and integrin activation of WT and reelin deficient platelets after treatment with CRP at indicated concentrations and convulxin (1 µg/ml), bar graphs depict mean values + SEM, n = 3-4, *** $P < 0.001$.

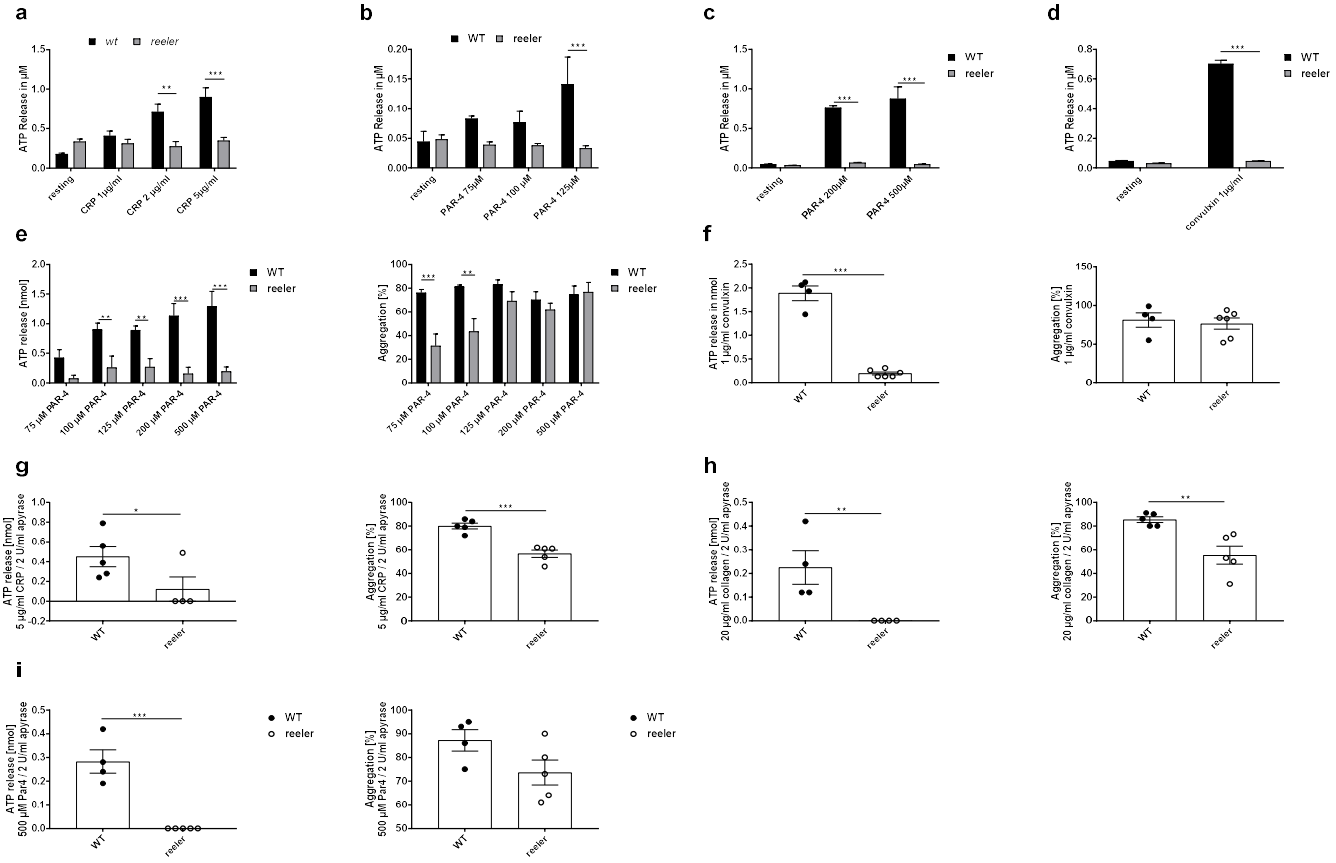

Figure V. Intracellular reelin supports platelet aggregation and ATP release. **(a-d)** ATP release measured by ELISA of WT and reeler platelets after treatment with CRP (a), low and high doses of PAR-4 (b, c) and convulxin (d) at indicated concentrations, bar graphs depict mean values + SEM, n = 4-10 (a), n = 3-4 (b-d), ** P < 0.01, *** P < 0.001. **(e-f)** Aggregation and ATP release as measured by aggregometry of WT and reeler platelets after PAR-4 **(e)** and convulxin **(f)** treatment. Bar graphs depict mean values + SEM, n = 6-8 (e) and mean values ± SEM, n = 4-6 (f) ** P < 0.01, *** P < 0.001. **(g-i)** Aggregometry of WT and reeler platelets in the presence of the ADP inhibitor apyrase (2 U/ml). **(g)** Impaired ATP release and aggregation of reeler platelets after CRP (5 µg/ml) treatment, bar graphs depict mean values ± SEM, n = 4-6, *** P < 0.001. **(h)** Reduced ATP release and aggregation of reeler platelets after stimulation with collagen (20 µg/ml). **(i)** Reduced ATP release but unaltered aggregation of reeler platelets in response to PAR-4 (500 µM). Bar graphs depict mean values ± SEM, n = 4-5, * P < 0.05, ** P < 0.01, *** P < 0.001.

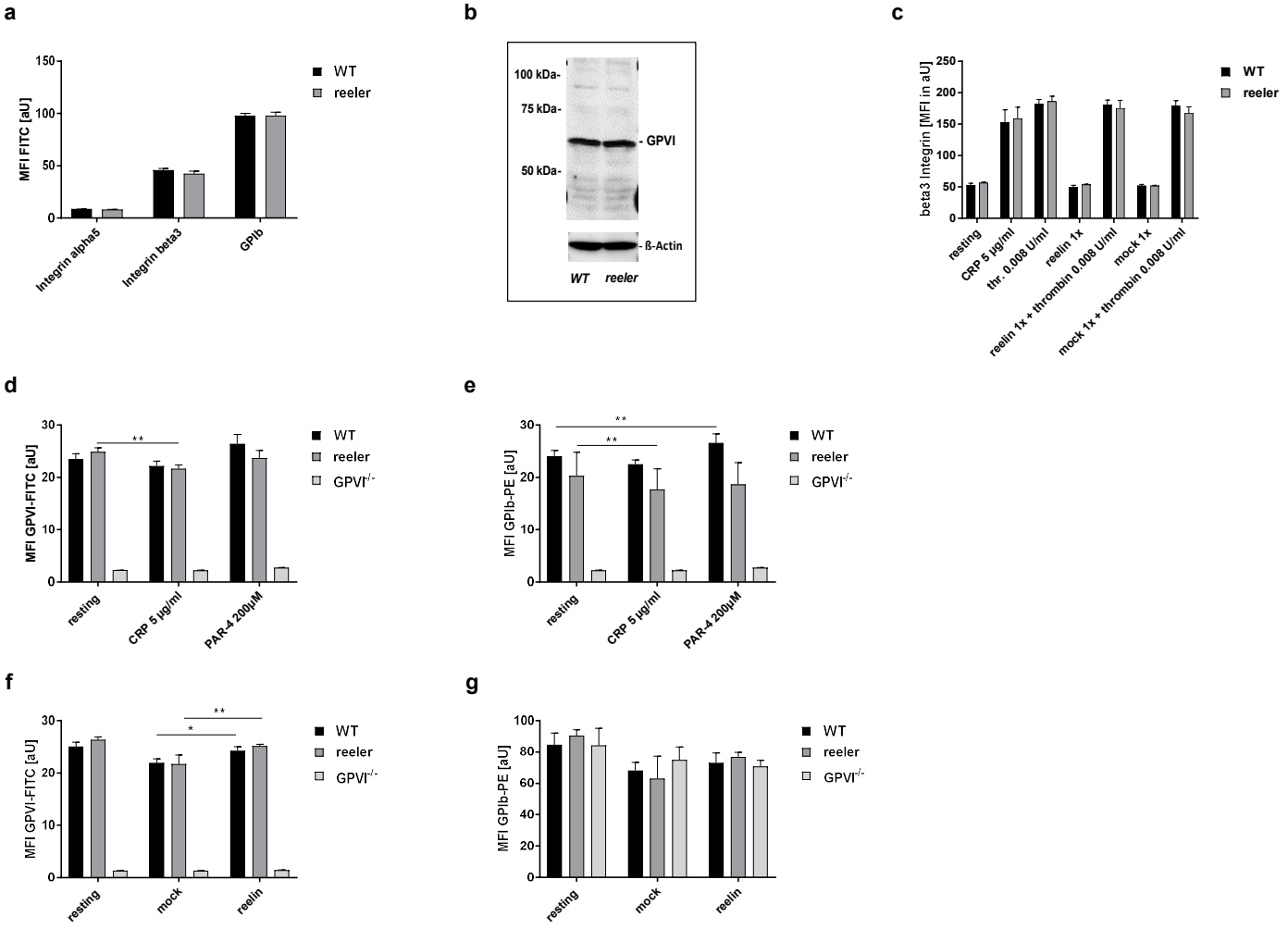

Figure VI. Glycoprotein expression of reelin-deficient platelets. (a,b) Glycoprotein expression of WT and reeler platelets under resting conditions. **(a)** Flow cytometric analysis of glycoproteins and **(b)** representative Western blot of GPVI in reeler platelets, bar graphs depict mean values + SEM, n = 3. **(c)** No differences in beta3 integrin expression of WT and reeler platelets after treatment with indicated agonists, bar graphs depict mean values + SEM, n = 5. **(d-e)** GPVI (d) and GPIb (e) exposure at the platelet surface after GPVI stimulation as determined by flow cytometry, GPVI deficient platelets serve as negative control, bar graphs depict mean values + SEM, n = 5, ** $P < 0.01$. **(f-g)** GPVI (f) and GPIb (g) exposure at the platelet surface after reelin treatment, GPVI deficient platelets serve as negative control, bar graphs depict mean values + SEM, n = 5, * $P < 0.05$, ** $P < 0.01$.

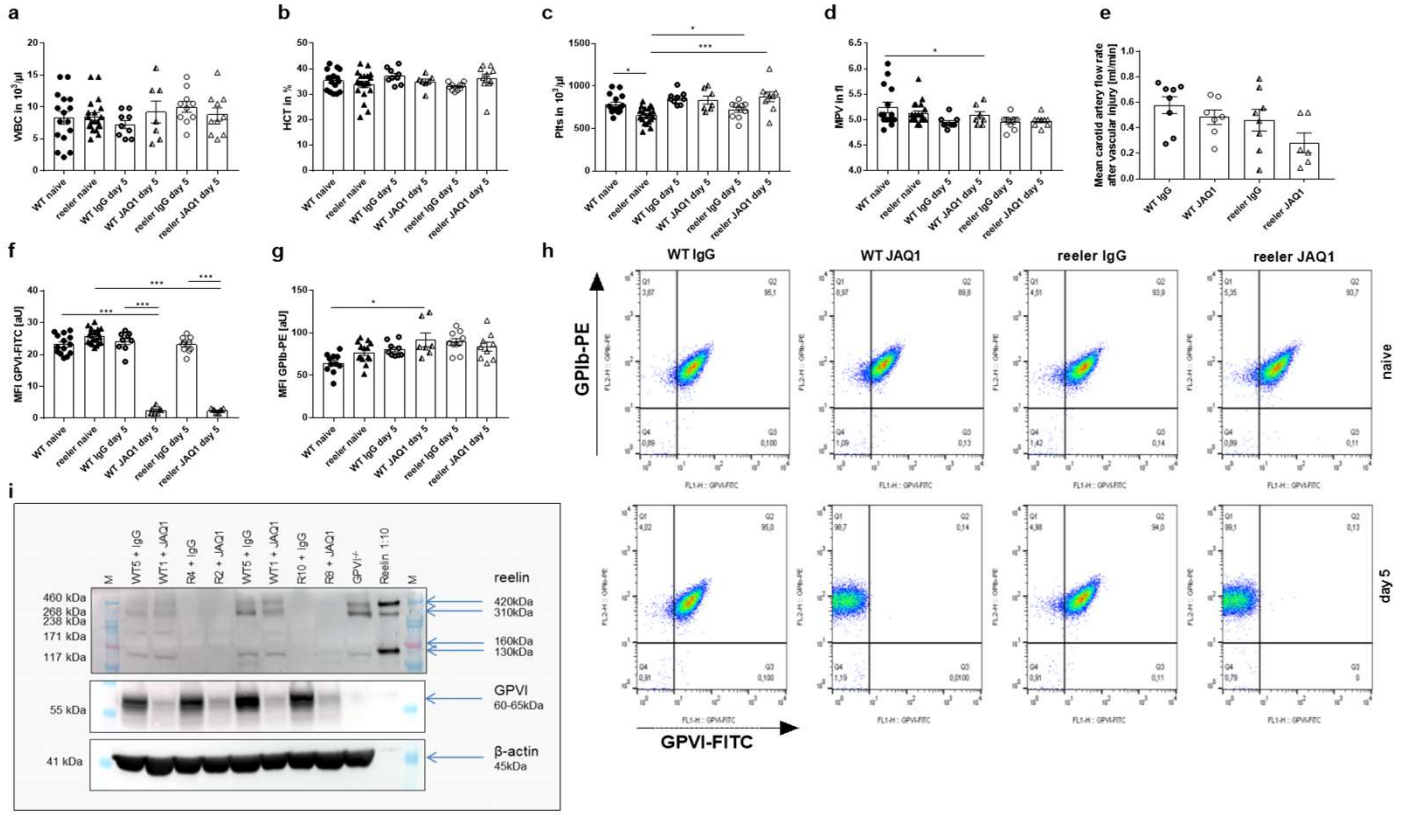

Figure VII. FeCl3 induced injury of the carotid artery in GPVI depleted mice. **(a-d)** Blood cell counts were measured in naive and JAQ1 or IgG treated animals before injury. Bar graphs depict mean values ± SEM, n = 12-16 (naive) n = 5-10 (treated). **(e)** Mean flow rate in the carotid artery 30 min after vascular injury using 10% $FeCl_3$, bar graphs depict mean values ± SEM, n = 4-6. **(f-h)** Flow cytometric analysis confirmed GPVI depletion in mice before and after vascular injury. (**f**) Mean fluorescence intensity of anti-GPVI-FITC, bar graphs depict mean values ± SEM, n = 12-16 (naive) and n = 5-9 (treated), *** P < 0.001. **(g)** Mean fluorescence intensity of anti-GPIb-PE, bar graphs depict mean values ± SEM, n = 7-10 (naive) and n = 5-9 (treated) * P < 0.05. (**h**) Representative flow cytometric dot plots of platelets from mice treated with JAQ1 or IgG. Cells were gated for GPVI (x-axis) and GPIb (y-axis). **(i)** Western Blot analysis was performed to confirm GPVI deletion and reelin expression in platelets post vascular injury.