SUPPLEMENTAL INFORMATION

Differential role of the RAC1-binding proteins FAM49b (CYRI-B) and CYFIP1 in platelets

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Supplemental Table S1. Glycoprotein expression in *Cyfip1*$^{-/-}$, *Fam49b*$^{-/-}$ and *Cyfip1/ Fam49b* $^{-/-}$ platelets. Platelet glycoprotein expression was determined via flow cytometry (n = 6). Values are presented as mean ± standard deviation. **P* < .05, ***P* < .01.

	Cyfip1$^{+/+}$	*Cyfip1*$^{-/-}$	significance
β1	7044 ± 200,6	7125 ± 131,5	ns
GPIb	16577 ± 789,7	15988 ± 794,3	ns
GPV	11152 ± 337,5	10791 ± 353,8	ns
GPIX	20852 ± 440,2	19843 ± 875,1	*
αIIbβ3	28755 ± 1116	27538 ± 1012	ns
α2	1934 ± 61,17	1763 ± 238,1	*
GPVI	1973 ± 61,02	1918 ± 145	ns
CLEC-2	5024 ± 327	4732 ± 502,2	ns

	Fam49b$^{+/+}$	*Fam49b*$^{-/-}$	significance
β1	7552 ± 151,7	7245 ± 179	**
GPIb	16033 ± 1886	17670 ± 979,7	ns
GPV	11086 ± 327,6	10993 ± 288,1	ns
GPIX	21730 ± 461,3	21062 ± 624,7	ns
αIIbβ3	30189 ± 1816	27808 ± 1192	ns
α2	2257 ± 95,67	2137 ± 116,5	ns
GPVI	2090 ± 95,9	2091 ± 47,71	ns
CLEC-2	5503 ± 168,6	5461 ± 228,5	ns

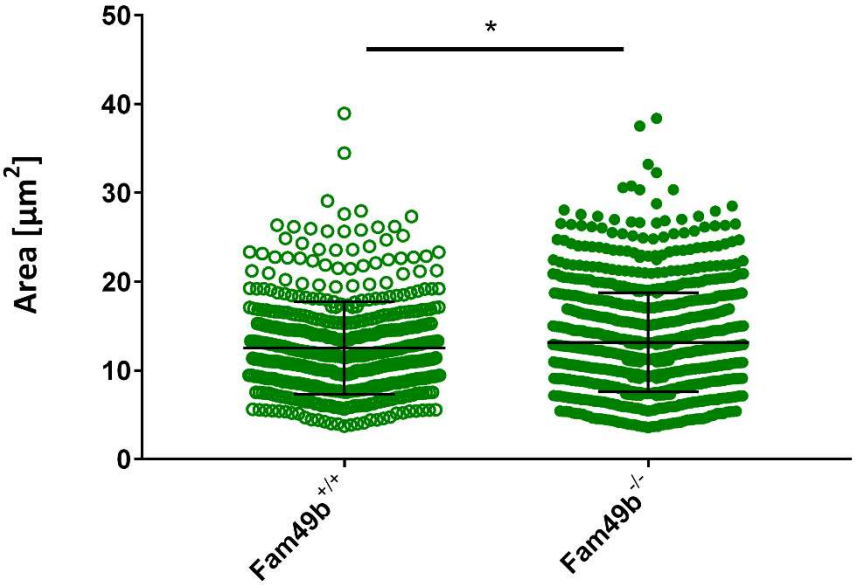
	Cyfip1/Fam49b$^{+/+}$	*Cyfip1/Fam49b*$^{-/-}$	significance
β1	6941 ± 163	7023 ± 143	ns
GPIb	14478 ± 466	14004 ± 490	ns
GPV	10877 ± 323	10487 ± 357	ns
GPIX	17821 ± 247	17172 ± 509	*
αIIbβ3	18368 ± 322	17698 ± 610	ns
α2	2668 ± 127	2689 ± 20,8	ns
GPVI	2486 ± 28,4	2474 ± 89,4	ns
CLEC-2	5278 ± 213	5065 ± 223	ns

Supplemental Methods

Platelet spreading upon ADP-stimulation

For platelet spreading experiments, coverslips were incubated with human fibrinogen (100 µg/ml; Sigma) overnight at 4°C and blocked with BSA (1%) at 37°C for one hour. The coated slides were washed with Tyrode's buffer before preactivated platelets (10 µM ADP (Sigma)), were allowed to spread on the coverslips. After 30 minutes, platelets were fixed with 4% paraformaldehyde (PFA) in PBS containing 0.1% Triton-X100 for 10 min, washed with PBS and blocked with 5% BSA in PBS for one hour. Subsequently, the samples were stained with AlexaFluor 647-conjugated phalloidin for 1 hour at RT, washed with PBS and imbedded in mounting medium. Spread platelets were visualized with a Leica TCS SP8 inverted confocal microscope (100x/1.4 oil objective, Leica, Germany). Images were analyzed using ImageJ.

Supplemental Figures



Supplemental Figure S1. Quantification of the projected cell area of ADP-stimulated *Fam49b*$^{+/+}$- and *Fam49b*$^{-/-}$-platelets after 30 minutes of spreading on fibrinogen-coated surfaces. The area of mutated cells was increased. For each condition, at least 500 cells were analyzed. Values are mean plus or minus SD. *$P < 0.05$.