

Role of platelet glycoprotein VI and tyrosine kinase Syk in thrombus formation on collagen-like surfaces

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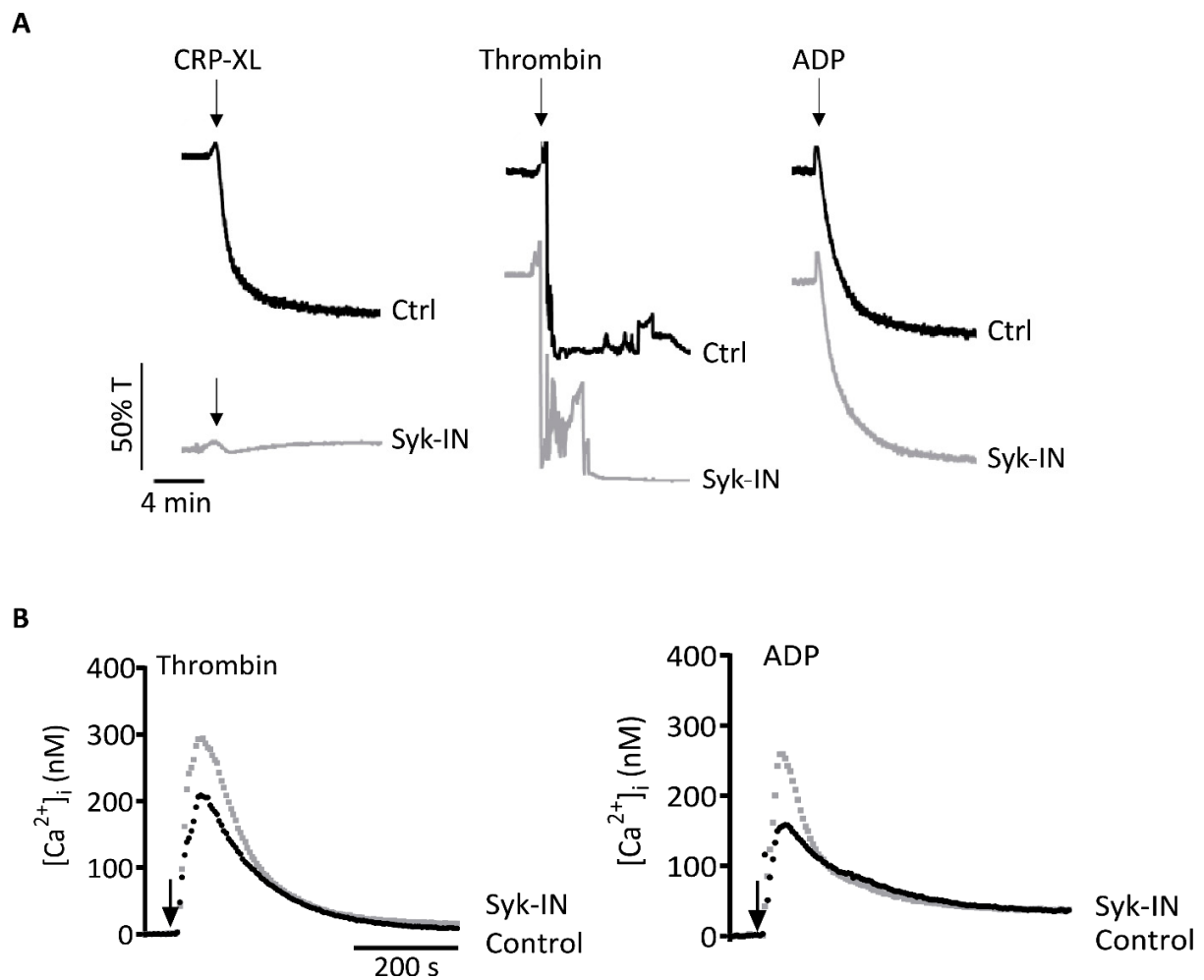


FIGURE S1. Effect of Syk inhibitor PRT-060318 (Syk-IN) on agonist-induced platelet responses. **(A)** Platelets in plasma ($2.5 \times 10^8/\text{ml}$) were pre-incubated with vehicle (DMSO) or Syk-IN ($5 \mu\text{M}$) for 10 min, and then activated with CRP-XL ($10 \mu\text{g}/\text{ml}$), thrombin (8 nM) or stable ADP ($5 \mu\text{M}$), as indicated. Shown are representative traces from light transmission aggregometry. **(B)** Fura-2-loaded platelets in 96-well plates were pre-incubated with Syk-IN ($5 \mu\text{M}$) or left untreated before injection of thrombin (4 nM) or stable ADP ($5 \mu\text{M}$), as in Figure 1. Shown are representative traces of changes in $[Ca^{2+}]_i$ of control (black) and Syk-IN (grey) incubations. Arrows indicate addition indicated of agonists.

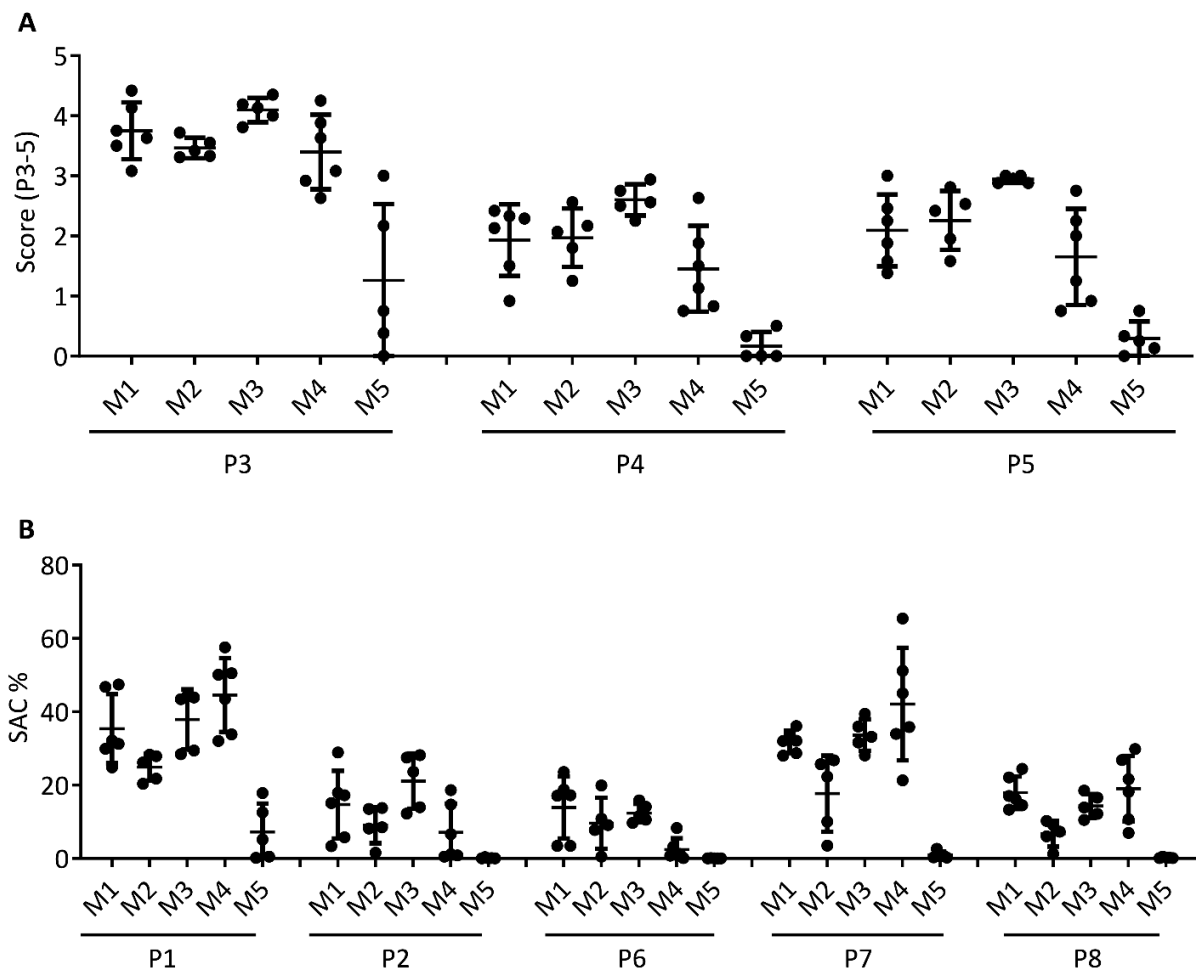


FIGURE S2. Parameters of thrombus formation on immobilised collagen peptides: raw data. Whole-blood was perfused over microspots M1 (GFOGER-GPO + VWF-BP), M2 (CRP-XL + VWF-BP), M3 (GAOGER-GPO + VWF-BP), M4 (GFOGER-GPP + VWF-BP), and M5 (VWF-BP). Microscopic images were analysed for parameters P1-8, as for Figure 2. Shown are raw mean outcome values from individual blood donors. **(A)** Parameters providing surface area coverage (SAC%) information: P1, platelet deposition; P2, platelet aggregate coverage; P6, PS exposure; P7, CD62P expression; P8, fibrinogen binding. **(B)** Score parameters: P3, thrombus morphological score (range 0-5); P4, thrombus multilayer score (range 0-3); P5, thrombus contraction score (range 0-3). Means $\pm$ SD (n=5-7 donors).

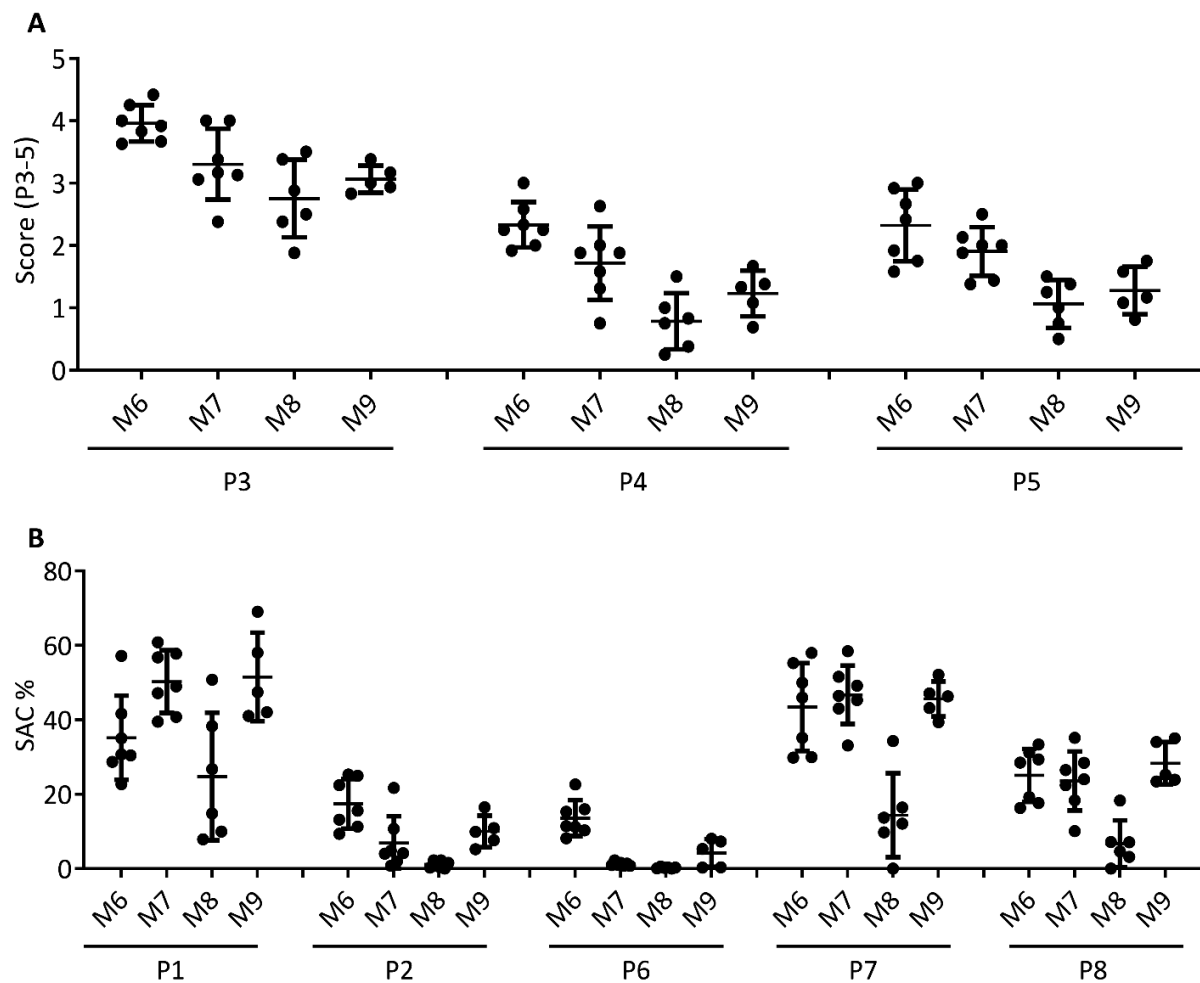


FIGURE S3. Parameters of thrombus formation on immobilised collagens: raw data. Whole-blood was perfused over microspots M6 (collagen-H), M7 (fibrillar collagen-I), M8 (monomeric collagen-I), M9 (collagen-III). Microscopic images were captured and analysed for parameters P1-8, as for Figure 5. Shown are raw mean outcome values from individual blood donors. **(A)** Parameters providing surface area coverage (SAC%) information. **(B)** Score parameters. See further Suppl. Figure S2. Means $\pm$ SD (n=5-7 donors).

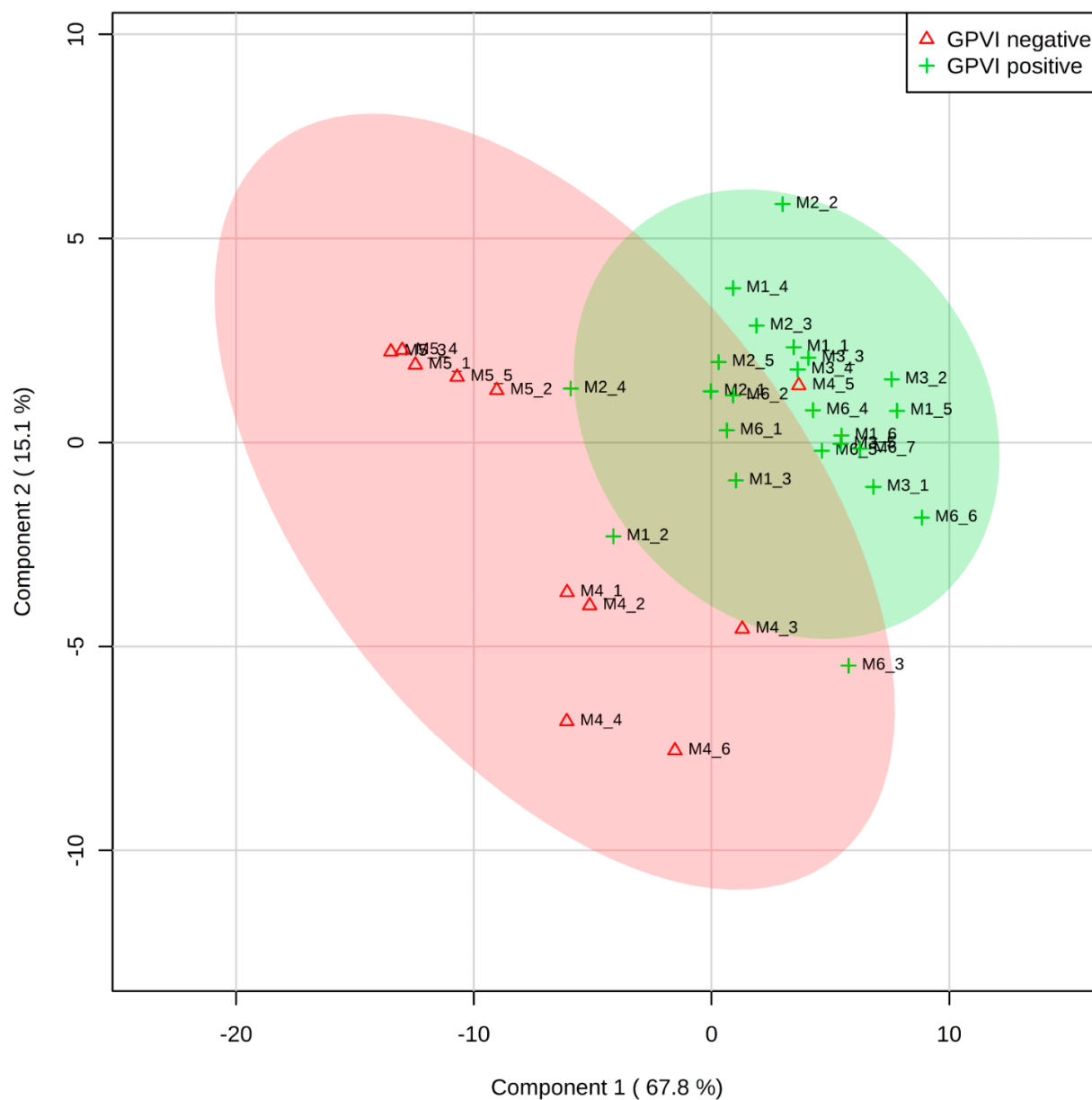


FIGURE S4. Partial Least Squares with components 1 and 2, indicating distribution of thrombus formation parameters at microspots M1-6 for 5-7 individual blood samples per microspot. Note, the clustering (green area) of flow runs over (GPO)_n containing surfaces M1-3 and M6, whereas flow runs with other surfaces M4 and M5 out-clustered with more negative contributions to component 2 or 1, respectively (red area). Red triangles indicate assumed negative GPVI contribution, green plusses indicate positive contribution.

TABLE S1. Scaled subtracted parameter values of thrombus formation (means), indicating effects of Syk-IN, for microspots *M1-9* and parameters *P1-8*.

	<i>P1</i>	<i>P2</i>	<i>P3</i>	<i>P4</i>	<i>P5</i>	<i>P6</i>	<i>P7</i>	<i>P8</i>
<i>M1</i>	1.18±0.46	0.10±0.14	0.51±0.17	0.15±0.16	0.21±0.20	0.03±0.05	0.71±0.49	0.62±0.66
<i>M2</i>	1.15±0.24	0.16±0.13	0.77±0.15	0.30±0.20	0.30±0.15	0.04±0.06	0.69±0.39	0.31±0.28
<i>M3</i>	0.94±0.24	0.10±0.07	0.71±0.17	0.37±0.23	0.34±0.12	0.01±0.00	0.47±0.27	0.11±0.08
<i>M4</i>	0.93±0.29	0.17±0.29	0.73±0.09	0.34±0.34	0.37±0.40	0.13±0.16	0.25±0.18	0.04±0.03
<i>M5</i>	0.97±1.18	1.27±2.13	0.13±0.12	0.00±0.00	0.00±0.00	0.63±0.79	1.40±2.65	0.36±0.43
<i>M6</i>	1.12±0.33	0.19±0.10	0.61±0.15	0.22±0.17	0.28±0.18	0.02±0.02	0.65±0.35	0.42±0.44
<i>M7</i>	0.88±0.21	0.03±0.06	0.60±0.20	0.10±0.11	0.17±0.12	0.03±0.02	0.13±0.08	0.02±0.02
<i>M8</i>	0.87±0.56	2.91±6.23	0.61±0.35	0.64±0.74	0.42±0.28	0.16±0.15	0.25±0.16	0.12±0.17
<i>M9</i>	0.79±0.20	0.07±0.07	0.49±0.06	0.13±0.23	0.11±0.13	0.03±0.03	0.14±0.12	0.05±0.05