Immobilized fibrinogen activates human platelets through glycoprotein VI

Pierre H Mangin,[1] Marie-Blanche Onselaer,[2] Nicolas Receveur,[1] Nicolas Le Lay,[3] Alexander T Hardy,[2] Clare Wilson,[4] Ximena Sanchez,[5] Stéphane Loyau,[3] Arnaud Dupuis,[1] Amir K Babar,[6] Jeanette LC Miller,[6] Helen Philippou,[4] Craig E Hughes,[2,8] Andrew B Herr,[6] Robert AS Ariëns,[4] Diego Mezzano,[5] Martine Jandrot-Perrus,[3,7] Christian Gachet[1] and Steve P. Watson[2,9]

[1]Université de Strasbourg, INSERM, EFS Grand-Est, BPPS UMR-S 1255, FMTS, France; [2]Institute of Cardiovascular Sciences, IBR Building, College of Medical and Dental Sciences, University of Birmingham, UK; [3]Université de Paris Diderot, INSERM UMR_S1148, Hôpital Bichat, Paris, France ; [4]Thrombosis and Tissue Repair Group, Institute of Cardiovascular and Metabolic Medicine, University of Leeds, UK; [5]Laboratorio de Hemostasia, Pontificia Universidad Catolica de Chile, Santiago, Chile; [6]Division of Immunobiology, Center for Systems Immunology & Division of Infectious Diseases, Cincinnati, OH, USA; [7]Acticor Biotech, Hôpital Bichat, INSERM, UMR-S 1148, Paris, France; [8]Institute for Cardiovascular and Metabolic Research, Harborne Building, University of Reading, UK and [9]Centre of Membrane Proteins and Receptors (COMPARE), Universities of Birmingham and Nottingham, Midlands, UK

 doi:10.3324/haematol.2017.182972

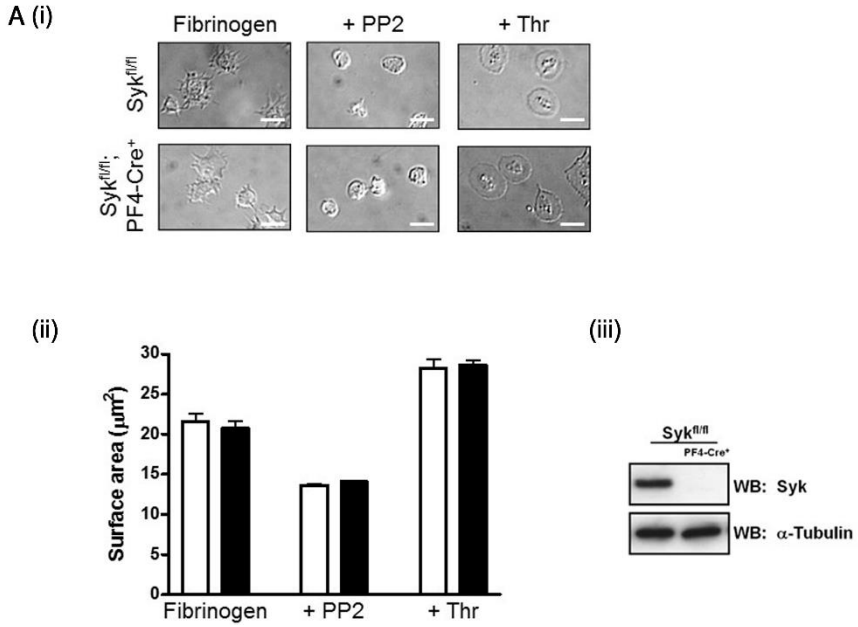
Received: October 20, 2017.

Accepted: February 13, 2018.

Pre-published: February 22, 2018.

Correspondence: pierre.mangin@efs.sante.fr or s.p.watson@bham.ac.uk

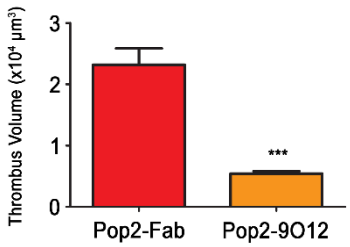
Supplemental Figure 1



Supplemental Figure 1. Syk-deficient platelets have no defect in cytoskeletal reorganization after adhesion to fibrinogen. Washed platelets from wild-type mice ($Syk^{fl/fl}$) and Syk-deficient mice ($Syk^{fl/fl}$;PF4-Cre+) were allowed to adhere to fibrinogen in the presence of either the Src inhibitor PP2 (20 µmol/L), thrombin (0.1 U/mL), or vehicle control, for 45 min at 37°C followed by washing to remove non-adherent platelets, and fixation with PFA. (A)(i). Representative DIC images of platelets adhering to fibrinogen. Scale bars represent 5 µm. (A)(ii). Bar graphs represents the surface area of platelets spreading on fibrinogen. Spreading is expressed as the mean±SEM in 5 or more random fields, in 3 separate experiments. (A)(iii). Representative Western blot from platelets following adhesion to fibrinogen. Following platelet lysis, proteins were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and Western blot for phosphotyrosine. Membranes were then stripped and reprobed for α-Tubulin to confirm equal protein loading. Blots are representative of 3 separate experiments.

Supplemental Figure 2

A



Supplemental Figure 2. Blockade of human GPVI limits platelet accumulation to a growing aggregate. Hirudinated human whole blood labeled with $DIOC_6$ (1 µmol/L) was perfused over immobilized collagen (200 µg/mL) to preform aggregates for 2 min 30, before perfusing the same blood in the presence of the Alexa Fluor 647-conjugated monoclonal antibody against GPIbβ (5 µg/mL) and with a Fab control (Control) or the blocking anti-GPVI antibody 9O12 (50 µg/mL). (A). Bar graphs represent only the volume of the platelet aggregates formed directly over the preformed aggregates (mean±SEM) in 8 random fields, in 6 separate experiments performed with different blood donors. This volume was determined by confocal microscopy by measuring exclusively the volumes formed on the area of the preformed aggregates, thereby avoiding the measure of the adhesion of circulating platelets on the collagen fibers.