Supplemental Information

Methods

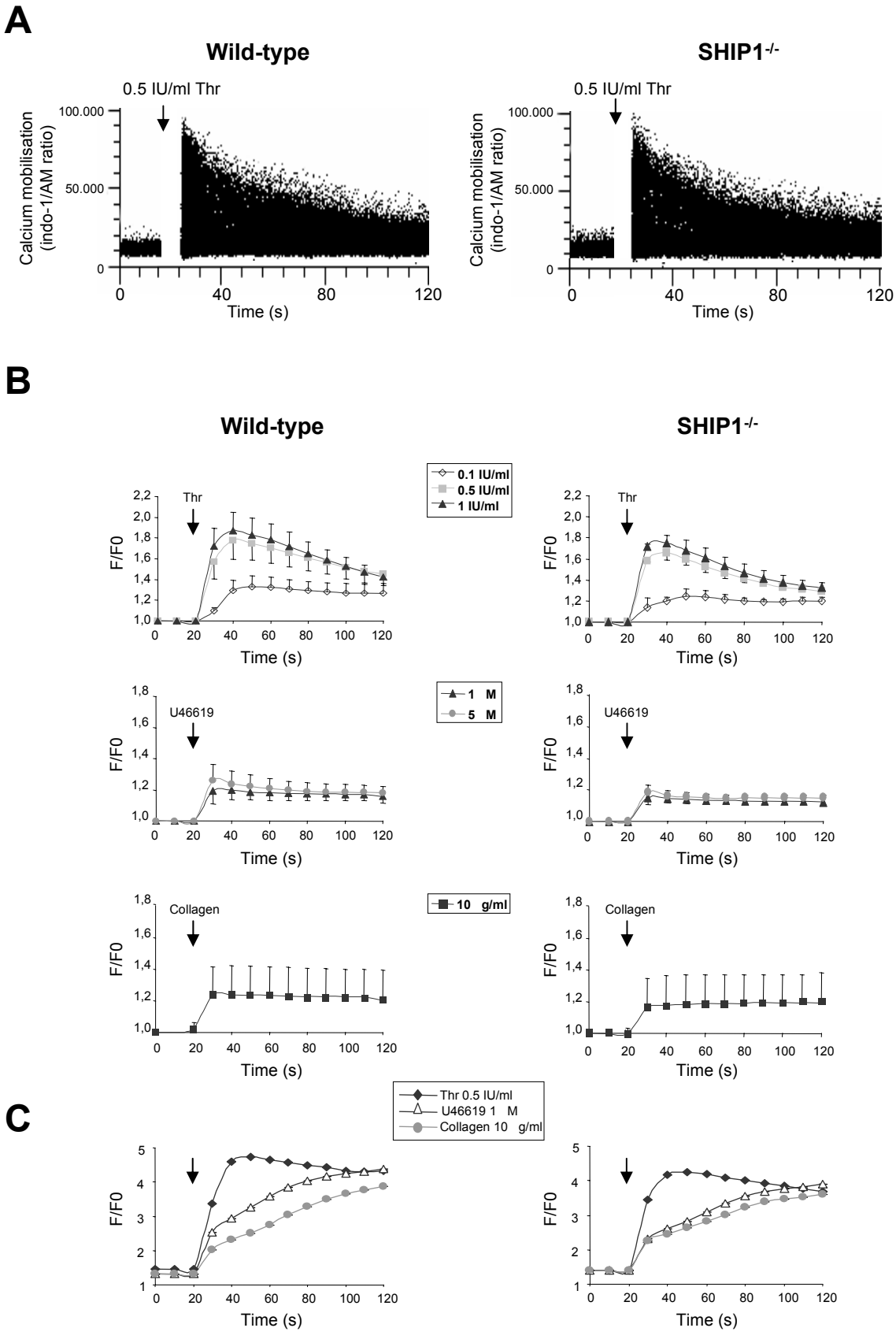
Measurement of intracellular Ca^{2+}. Platelets isolated from platelet-rich plasma were resuspended in Tyrode's buffer containing 0.35 % human serum albumin, PGI2 (500 nM) and apyrase (0.02 unit/ml) at a density of 7.5×10^8 platelets/ml and then incubated with 15 M indo-1/AM in the presence of pluronic F-127 (v/v) for 45 minutes in the dark at room temperature. Following centrifugation, platelets were resuspended at a density of 3×10^8 platelets/ml in Tyrode's buffer containing 0.1 % human serum albumin. The final platelet suspension was stored at room temperature and each sample (6×10^7 platelet/ml at final concentration) is prewarmed at 37°C for 2 minutes before starting the calcium measurements. When indicated, the overall pattern of cytosolic calcium was measured in presence of 1 mM of extracellular calcium. Flow cytometric analysis of indo-1/AM fluorescence was performed using a LSRII apparatus (BD Biosciences). The indo-1/AM fluorescence ratio was collected in real time during 2 minutes at a rate of 1000 cells/second.

Supplemental figure

Figure S1

Cytosolic calcium response in SHIP1-deficient mice. Platelets were loaded with indo-1/AM and the bound-free calcium ratio was recorded before and after stimulation by thrombin, collagen or the thomboxane A2 analogue U46619. **(A)** Typical time course of indo-1/AM ratio in 0.5 IU/ml thrombin stimulated platelets in the absence of extracellular calcium. **(B)** Increases of intracellular calcium are expressed as the ratio of fluorescence intensity of indo-1/AM over baseline (F/F0) in the absence of extracellular calcium over time. Values are means ± SEM of three independent determinations. **(C)** A representative example of two

independent experiments of intracellular calcium measurement in the presence of extracellular calcium over time is shown.



Séverin et *al., Suppl. Figure 1*