Supplementary Material to Dai et al. “Integrin-αIIbβ3-mediated outside-in signalling activates a negative feedback pathway to suppress platelet activation” (Thromb Haemost 2016; 116.5)

Suppl. Figure 1. Integrin αIIbβ3 becomes inactivated during platelet disaggregation. (A) Human PRP were incubated with APC-conjugated fibrinogen (APC-Fg) for 10 mins, then stimulated with 4 µM ADP in the presence of fibrinogen (Fg, 200 µg/ml). Platelets were fixed by adding the same volume of 2% formaldehyde. Platelets were fixed at different points in time, such as (a) at stir rest platelets, (b) after changing shape, (c) at full aggregation, and (d) at disaggregation. Single-platelet suspensions were produced by pipetting up and down and subjected to flow cytometry analysis to indicate the level of activated integrin αIIbβ3. (B) Human PRP were incubated with...
APC-conjugated fibrinogen (APC-Fg) or FITC-AP3 for 10 min, then stimulated with low doses of TRAP (0.5 µM). Platelets were fixed by adding the same volume of 2 % formaldehyde. Platelets were fixed at different points in time, such as (a-0'), (b-1') after changing shape, (c-2') at full aggregation, and (d-5') at disaggregation. Single-platelet suspensions were produced by pipetting up and down and subjected to flow cytometry analysis to indicate the level of activated integrin αIIbβ3.
Suppl. Figure 2. Outside-in signaling reduced platelet aggregation through decreased ATP secretion. (A) Washed aspirin-treated human platelets were stimulated with 5 µM TRAP in the presence or absence of apyrase (1U), platelet ATP secretion and aggregation were recorded and quantified at 6 min. (B) Washed aspirin-treated human platelets were stimulated with 0.5 µM TRAP in the presence or absence of RUC2 (33.3 µM), phosphorylation of SHIP1, AKT and total SHIP-1, AKT were evaluated using Western blot analysis. (C) Washed aspirin-treated human platelets were incubated with Fg (200 µg/ml), then stimulated with 5 µM ADP in the presence or absence of 3AC (10 µM). Platelets were lysed at different points in time. Lysates were prepared and immunoprecipitation (IP) was performed with talin followed by immunoblotting with the anti-talin, anti-AKT or anti-SHIP-1 antibody.