Supplemental Material to Guidetti et al. “Novel pharmacological inhibitors demonstrate the role of the tyrosine kinase Pyk2 in adhesion and aggregation of human platelets” (Thromb Haemost 2016; 116.4)

Suppl. Figure 1: Structures of the three inhibitors PF-431396, PF-4594755, PF-4520440.
Suppl. Figure 2: The anti-phosphoPyk2(Tyr402) antibody in total lysates from stimulated mouse platelet recognizes two major bands with slightly different molecular mass between 100 and 150 kDa. Only the lower band corresponds to phospho-Pyk2, because it has a electrophoretic mobility identical to that of genuine Pyk2, as revealed by immunoblotting with anti-Pyk2 antibody (lower panel), and it is not detected in thrombin-stimulated platelets from Pyk2 KO mice (top panel).
Suppl. Figure 3: Analysis of platelet adhesion and spreading on collagen. Human platelets were incubated for 30 minutes with 10 μM of the reported inhibitors and then were allowed to adhere to immobilized collagen type I for 30 minutes. Adherent cells, stained with TRITC-phalloidin, were visualized by fluorescence microscopy at 100x magnification (A). Platelet adhesion (as number of cells/field) and spreading (as average cell area) are reported in B and C, respectively.