Supplementary Material to Mojica Muñoz et al. “Recombinant GPVI-Fc added to single or dual antiplatelet therapy in vitro prevents plaque-induced platelet thrombus formation” (https://doi.org/10.1160/TH16-11-0856)

Suppl. Figure 1: Concentration dependency of the effect of GPVI-Fc addition to blood on platelet aggregation onto human atherosclerotic plaque under arterial flow. Blood samples were pre-incubated for 10 min at 37 °C with DIOC6 (1µM; for platelet staining). GPVI-Fc was added to blood in different concentrations (10µg/ml, 20µg/ml or 50µg/ml) or not (control) before blood perfusion over plaque homogenate at a shear rate of 600/sec. The effect of GPVI-Fc on the kinetics of platelet deposition onto plaque from flowing blood was recorded (1frame/sec) for 4.5 min and analyzed as described in methods. Mean + SEM; n=9.
Suppl. Figure 2: Inhibition of ADP- and arachidonic acid (AA)-induced static platelet aggregation in blood by ticagrelor or ASA, respectively. Blood samples were untreated, contained ASA (1mM), ethanol (0.1 %) or ticagrelor (1µM or 5µM) and were pre-incubated at 37 °C for 3 min in the absence of stirring. ADP (5µM), or AA (0.6mM) respectively, was added, stirring was started and the increase in electrical impedance was recorded continuously for 5 min. Values are expressed in aggregation units over the time period (AU* min). For further details see methods. Mean + SD; n=6. **: p < 0.01, ***: p < 0.001 for secondary pair-wise comparisons. AA = arachidonic acid; ADP = adenosine diphosphate; ASA = acetylsalicylic acid; EtOH = ethanol; tica = ticagrelor.
Suppl. Figure 3: Effect of ASA and ticagrelor alone and in combination on platelet deposition onto human atherosclerotic plaque under arterial flow. Blood was incubated with ticagrelor (1µM or 5µM) or contained ASA (1mM) as described in legend of Fig. 2 before perfusion over plaque homogenate at a shear rate of 600/sec for 5 min. Platelet fluorescence images were analyzed 200sec after start of flow. Mean ± SD; n=6. *p < 0.05; **p < 0.01 for secondary pair-wise comparisons. Abbreviations as in Figure 2.
Suppl. Figure 4: Effect of ethanol (0.1 %) on platelet aggregation and adhesion kinetics onto human atherosclerotic plaque under arterial flow. Blood contained either ethanol (0.1 %) used to dissolve ticagrelor or not (control) before perfusion over plaque homogenate at a shear rate of 600/sec. In the adhesion experiments blood contained abciximab. Other details see in methods. Mean + SD; n=6.
Suppl. Figure 5: Effect of the anti-α2-integrin antibody P1E6 added to blood alone or on top of GPVI-Fc on PFA-200 closure time. The effect of the anti-α2-integrin-antibody P1E6 (5µg/ml), GPVI-Fc (10µg/ml; 100µg/ml) alone or in combination on (A) collagen/epinephrine closure time and (B) collagen/ADP closure time measured with the PFA-200 device. For further details see methods. Mean ± SD; n=8; The grey area represents the normal range of the method.