Supplementary Material to Gremmel et al. “Impact of variables of the P-selectin – P-selectin glycoprotein ligand-1 axis on leukocyte-platelet interactions in cardiovascular disease” (Thromb Haemost 2015; 113.3)

Suppl. Figure 1: Monocyte-platelet aggregates (MPA) and neutrophil-platelet aggregates (NPA) were identified in citrate-anticoagulated blood. In brief, HEPES buffer (for the determination of *in vivo* MPA/NPA formation), thrombin receptor activating peptide (TRAP)-6 (7.1 µM; for the determination of TRAP-6 inducible MPA/NPA formation) or adenosine diphosphate (ADP; 1.5 µM; for the determination of ADP inducible MPA/NPA formation) were added to 5 µl whole blood, diluted in 55 µl HEPES-buffered saline. After 10 min, monoclonal antibodies (anti-CD45-peridinin chlorophyll protein (clone 2D1, Becton Dickinson), anti-CD41-
phycoerythrin, (clone P2, Immunotech), and anti-CD14-allophycocyanin (clone MφP9, BD)), or isotype-matched controls were added. After 15 min, samples were diluted with FACSlysing solution and at least 10000 CD45+ events were acquired immediately. All CD45+ events were selected.

Suppl. Figure 2: Three distinct leukocyte populations were identifiable based on their side scatter versus CD14 characteristics, namely monocytes, granulocytes and lymphocytes.
Suppl. Figure 3: The CD45+CD14+ events were subjected to further analysis for CD14+CD41+ (monocyte-platelet aggregates; A, B) and CD14+CD41− events. Granulocytes, identified based on their side scatter versus CD14 characteristics, were also analysed for CD41+ (neutrophil-platelet aggregates; C, D) and CD41− events. The figure shows these cells without (A, C) and after the exogenous addition of the platelet agonist thrombin receptor activating peptide-6 (B, D).