Supplementary Material to Tunjungputri et al. “Human recombinant alkaline phosphatase inhibits ex vivo platelet activation in humans” (Thromb Haemost 2016; 116.6)

Suppl. Figure 1. Flow cytometry gating strategy for determination of P-selectin expression on platelets and platelet-fibrinogen binding. Platelets are gated based on forward and side scatter characteristics (A) followed by positivity for the platelet marker CD61 (B). The median fluorescence intensity (MFI) of P-selectin and fibrinogen, after stimulation with ADP (31 µM) and CRP-XL (625 µM), was determined from the gated platelet population (C).
Suppl. Figure 2. The platelet-inhibiting effect of recAP in whole blood is dose- and time-dependent. (A) Whole blood from healthy volunteers was pre-treated with different concentrations of recAP for 45 minutes prior to stimulation using low and high concentrations of ADP or CRP-XL. (B) Whole blood from healthy volunteers was pre-treated with recAP (40 U/ml) with different incubation times prior to stimulation using ADP and CRP-XL. The median fluorescence intensity (MFI) of the platelet surface expression of P-selectin and platelet-
fibrinogen binding was measured by using flow cytometry. Data are presented as medians with IQR from 6 healthy donors. * $P < 0.05$, ** $P < 0.01$.

Suppl. Figure 3. The platelet-inhibiting effect of recAP on Pam3CSK4-stimulated whole blood. (A) Whole blood was pre-treated for 45 minutes with medium or recAP (40 U/ml) prior to stimulation with Pam3CSK4. (B) Platelet-monocyte complex formation after exposure to Pam3CSK4 was determined by quantifying the MFI of the platelet marker CD61 on CD14+ cells. Data are presented as medians with IQR from 6 healthy donors. ** $P < 0.01$. 
Suppl. Figure 4. The direct platelet-inhibiting effect of recAP in platelet-rich plasma (PRP) is dose- and time-dependent. (A) PRP from healthy volunteers was pre-treated with different concentrations of recAP for 45 minutes prior to stimulation using low and high concentrations of ADP or CRP-XL. (B) PRP from healthy volunteers was pre-treated with recAP (40 U/ml) with different incubation times prior to stimulation using ADP and CRP-XL. The median fluorescence intensity (MFI) of the platelet surface expression of P-selectin and platelet-fibrinogen binding was measured by using flow cytometry. Data are presented as medians with IQR from 6 healthy donors. * $P < 0.05$, ** $P < 0.01$. 
Suppl. Figure 5. Measurement of purine content in CRP-XL-induced PRP. Purine levels were determined in the supernatant of PRP pre-treated with recAP (40 U/ml) or inactive recAP (in equivalent protein content) and subsequently stimulated with CRP-XL (39 uM). Presented data are medians with IQR from 4 healthy donors.