*Supporting Information*

# Effects of bacterial Lipopolysaccharides on platelet function: inhibition of weak platelet activation

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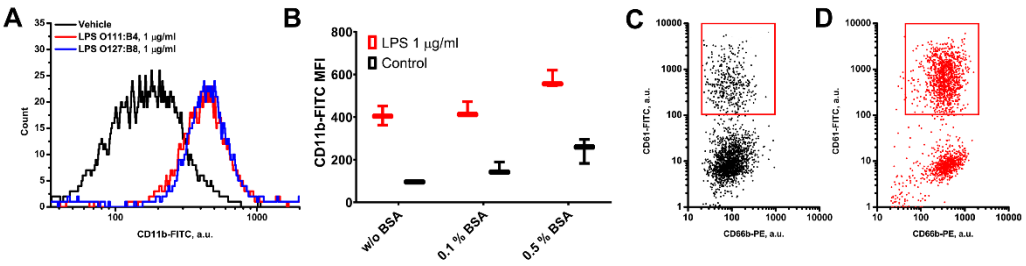


**Figure S1. PMN activation by LPSs.** (A) Preincubation with LPS resulted in the increase in CD11b activation on PMNs. (B) Introduction of BSA did not significantly affect LPS mediated increase in PMN CD11b activation. (C,D) Typical dot-plots of resting platelet-PMN suspension (C) and pre-incubated with LPS (D).

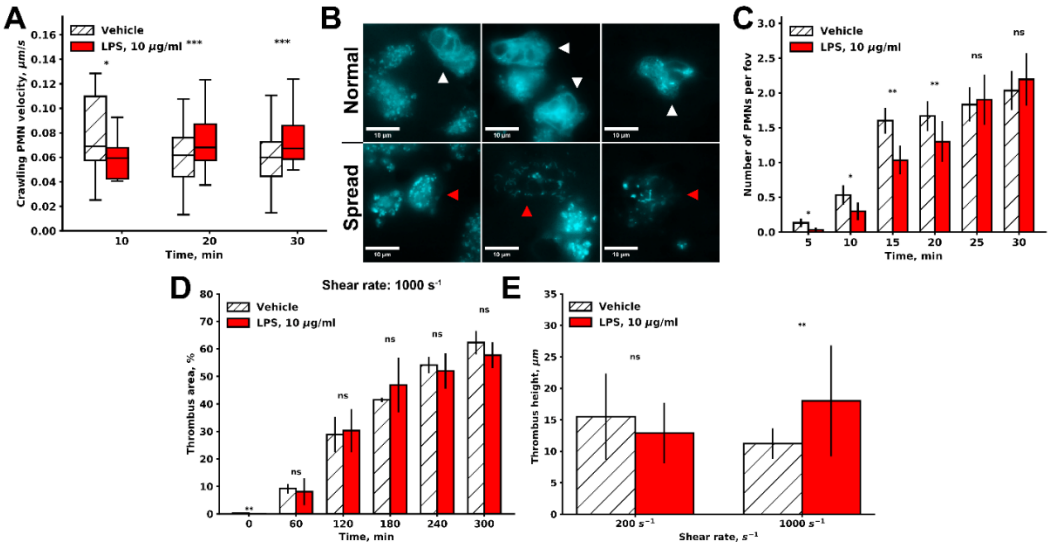


**Figure S2. Parallel-plate flow chamber whole blood experiments.** (A) LPSs slightly increased the velocity of crawling PMNs on fibrillar collagen and significantly increased the amount of highly spread PMNs (B); (C) LPS' effects on the number of PMNs per field of view were limited to a slight decrease at the early stages of thrombus formation; (D) Pre-incubation with LPS did not affected thrombus area at wall shear rate 1000 $s^{-1}$ ; (E) LPSs slightly increased the average height of the growing thrombi at 1000 $s^{-1}$, while there was no significant impact at 200 $s^{-1}$. Bars represent mean, whiskers represent SEM. Significance was calculated by Mann-Witney test, n = 5, * - $p < 0.05$, ** - $p < 0.01$, *** - $p < 0.001$.

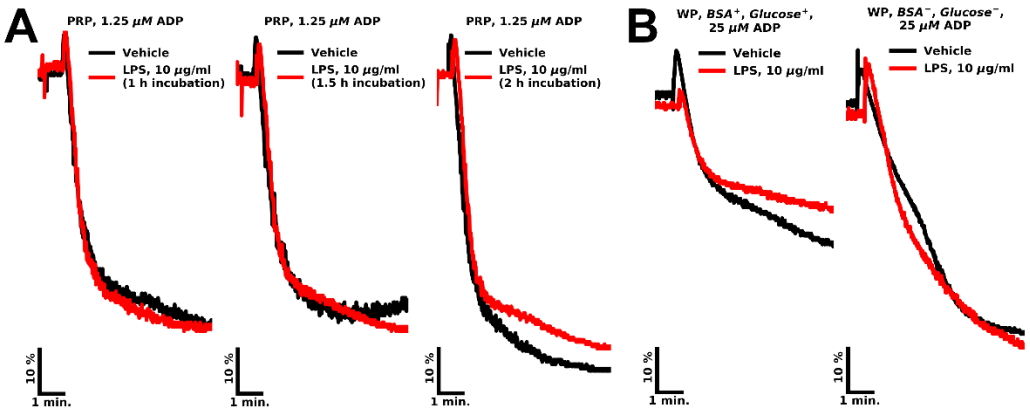


**Figure S3. Preincubation of platelets with LPS did not alter platelet aggregation**. Light transmission aggregometry, typical curves out of n = 10. (A) Platelet rich plasma from citrated blood. (B) Washed platelets in $BSA^+$, $Glucose^+$ and $BSA^-$, $Glucose^-$ Tyrode's buffer.

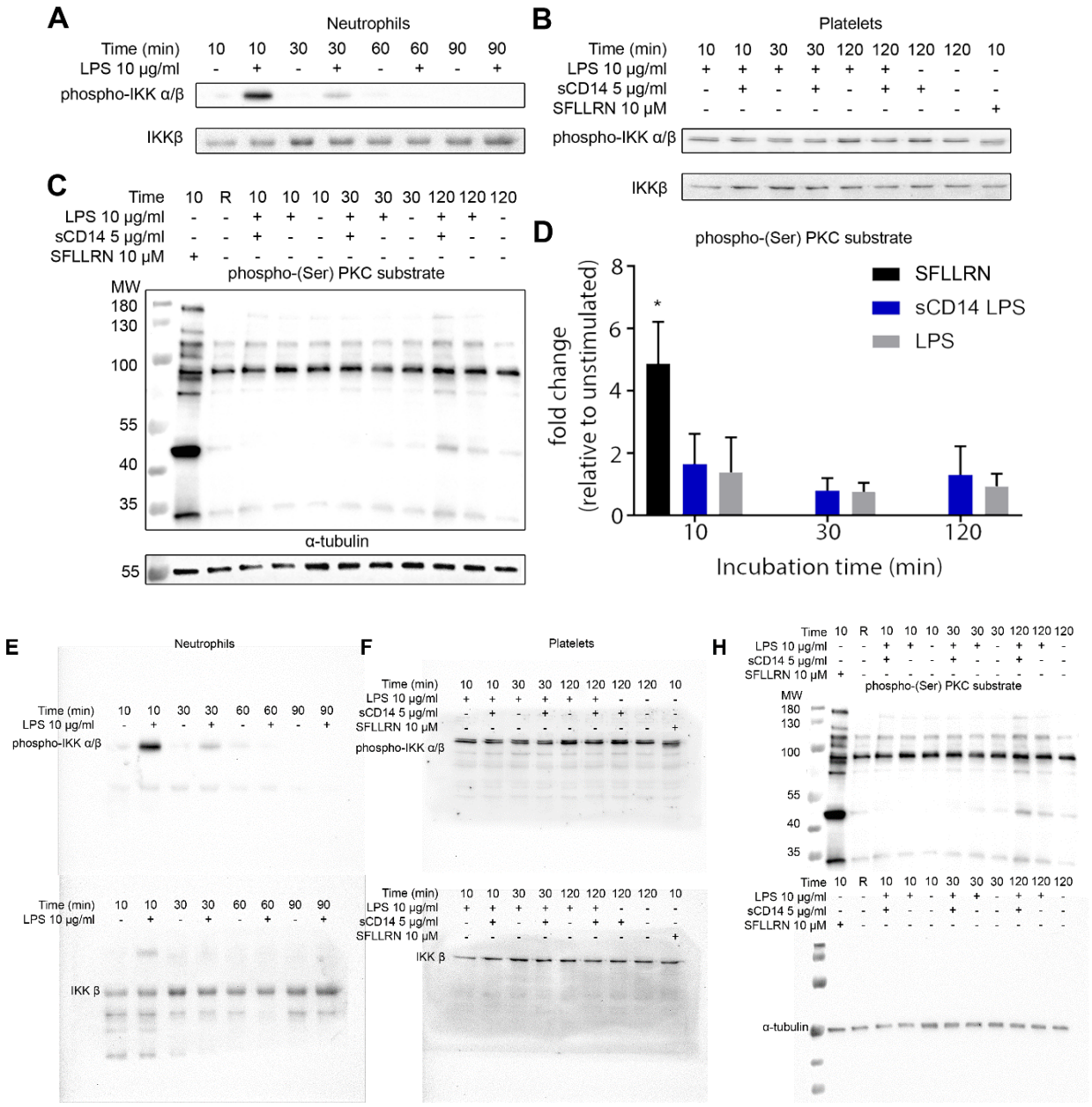


**Figure S4. IKκ phosphorylation in response to LPSs.** (A) 10 minute pre-incubation with 10 µg/ml LPS increased IKκ α/β phosphorylation, which decreased with the flow of time. Loading control was assessed using anti IKκ α/β antibodies after membrane stripping. (B) Neither 10 µM of SFLLRN (TRAP-6) nor 10 µg/ml LPS in the presence and absence of sSCD14 affected IK IKκ α/β phosphorylation in platelets. Loading control was assessed using anti IKκ α/β antibodies after membrane stripping. (C,D) Activation of platelets by 10 µg/ml LPS or 10 µg/ml LPS and sCD14 was non-significant in comparison to 10 µM of SFLLRN (TRAP-6). Full length blots are provided in figure S6. (E-H). Full length western blots. (E) IKκ α/β phosphorylation in neutrophils after incubation with LPS for different time intervals. IKκ β was used as loading control. (G) IKκ α/β phosphorylation in neutrophils after incubation with LPS for different time intervals or activation by 10 µM of SFLLRN (TRAP-6). IKκ β was used as loading control. (H) Ser phosphorylation (PKC substrate) in platelets activated by 10 µM of SFLLRN (TRAP-6) or 10 µg/ml of LPS for different time intervals. α-tubulin was used as loading control.

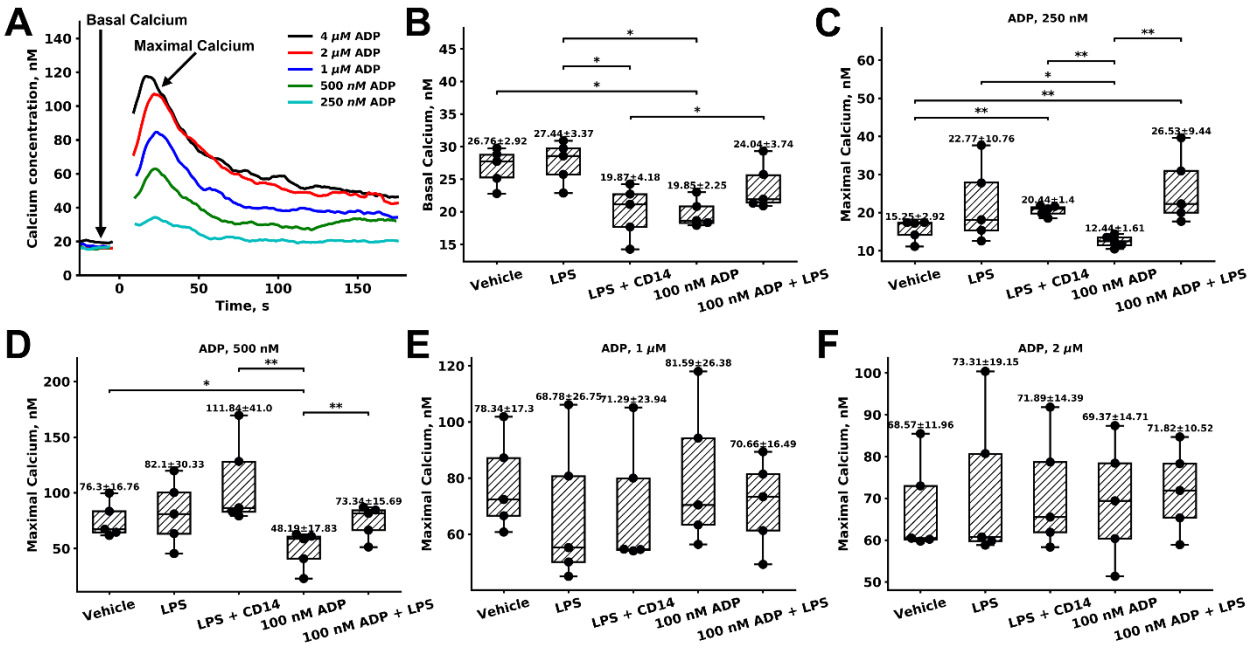


**Figure S5. LPS effects on platelet calcium signalling.** (A) Typical platelet suspension calcium response to ADP at different concentrations. (B) Basal calcium concentration in resting platelets upon pre-incubation with vehicle, 10 µg/ml LPS, 10 µg/ml LPS and 5 µg/ml sCD14, 100 nM ADP, 100 nM ADP and 10 µg/ml LPS for 30 minutes. (C-F) Calcium mobilization (Maximal calcium concentration minus basal calcium concentration) for platelets stimulated by 250 nM (C), 500 nM (D), 1 µM (E) and 2 µM (F) of ADP. Significance was calculated by Mann-Witney test (* - $p < 0.05$; ** - $p < 0.01$; *** - $p < 0.001$; n = 5).

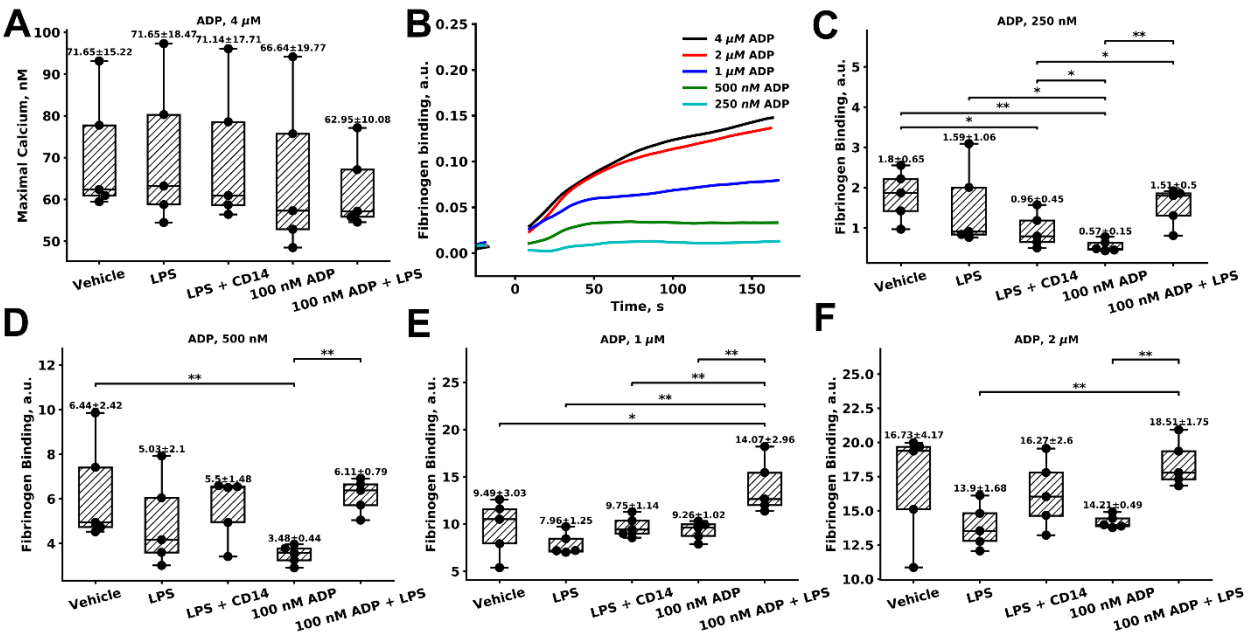


**Figure S6.** (A) Maximal calcium concentration upon stimulation by 4 µM of ADP. (B) Characteristic fibrinogen binding changes upon stimulation of platelets by different concentrations of ADP in continuous flow cytometry experiments. (C-F) Fibrinogen binding upon pre-incubation of platelets with vehicle, 10 µg/ml LPS, 10 µg/ml LPS and 5 µg/ml sCD14, 100 nM ADP, 100 nM ADP and 10 µg/ml LPS activated by 250 nM (C), 500 nM (D), 1 µM (E) and 2 µM (F) of ADP. Significance was calculated by Mann-Witney test (* - $p < 0.05$; ** - $p < 0.01$; *** - $p < 0.001$; n = 5).

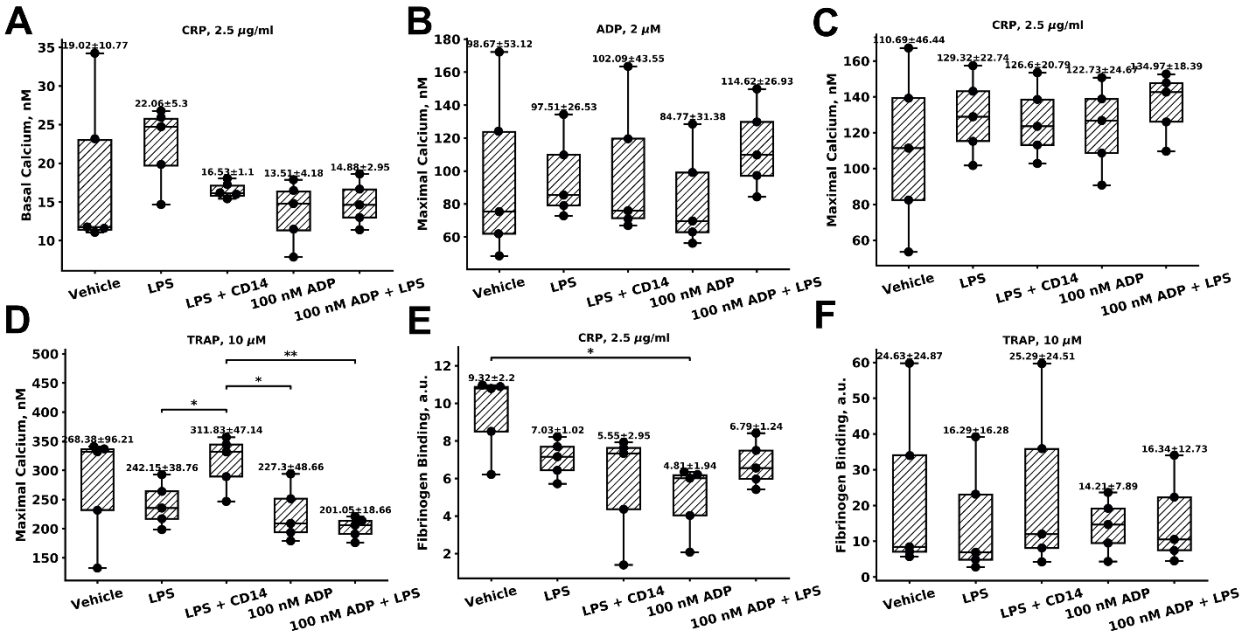


**Figure S7.** (A) Basal calcium concentration in resting platelets upon pre-incubation with vehicle, 10 μg/ml LPS, 10 μg/ml LPS and 5 μg/ml sCD14, 100 nM ADP, 100 nM ADP and 10 μg/ml LPS for 2 h. (B-D) Changes in calcium concentration (Maximal Calcium – Basal Calcium) for platelets stimulated by 2 μM of ADP (B), 2.5 μg/ml of CRP (C) and 10 μM of SFLLRN (D) at different conditions in the continuous regime. (E,F) Maximal fibrinogen binding for platelets stimulated by 2.5 μg/ml of CRP (E) and 10 μM of SFLLRN (F) at different conditions in the continuous regime. Significance was calculated by Mann-Witney test (* - $p < 0.05$; ** - $p < 0.01$; *** - $p < 0.001$, n = 5).

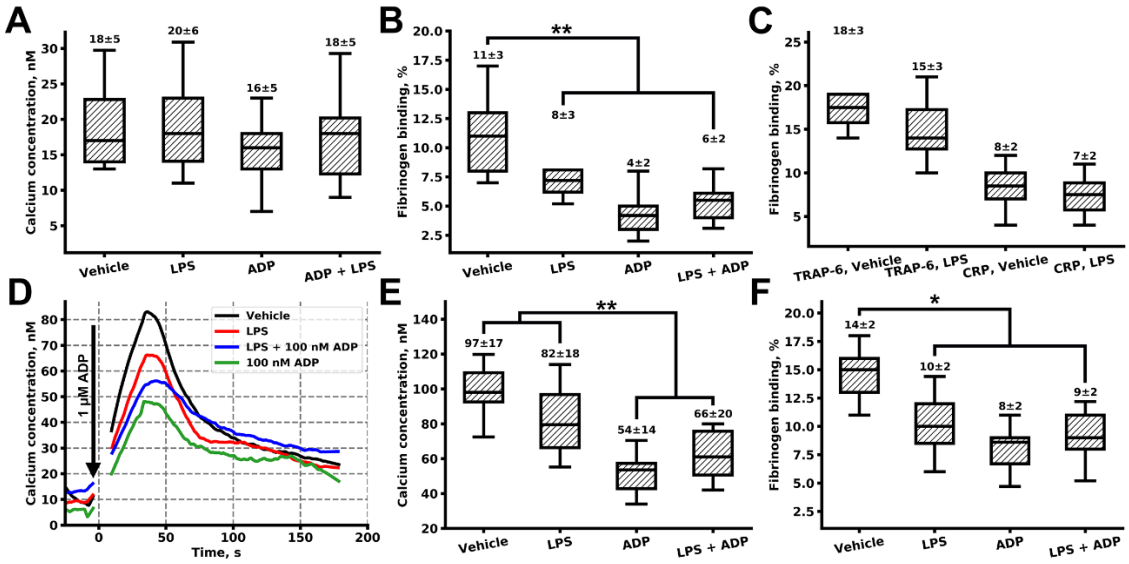


**Figure S8.** (A) Basal calcium concentration in resting platelets upon pre-incubation with vehicle, 100 μg/ml LPS, 100 nM ADP or 100 nM ADP and 100 μg/ml LPS. (B) Fibrinogen binding by platelets upon pre-incubation with vehicle, 100 μg/ml LPS, 100 nM ADP or 100 nM ADP and 100 μg/ml LPS and activation by 500 nM ADP. (C) Fibrinogen binding by platelets upon pre-incubation with vehicle or 100 μg/ml LPS and activation by 1 μM TRAP-6 or 1 μg/ml CRP. (D) Typical cytosolic calcium concentration curves upon activation by 1 μM ADP after pre-incubation with 100 μg/ml LPS, 100 μg/ml LPS and 100 nM ADP, 100 nM ADP or vehicle. (E,F) Pretreatment with LPS and ADP or sole ADP resulted in a significant decrease in a calcium (E) and fibrinogen (F) responses to 1 μM ADP (n = 15 donors, significance was calculated by Mann-Whitney test, * - $p < 0.05$; ** - $p < 0.01$; *** - $p < 0.001$).

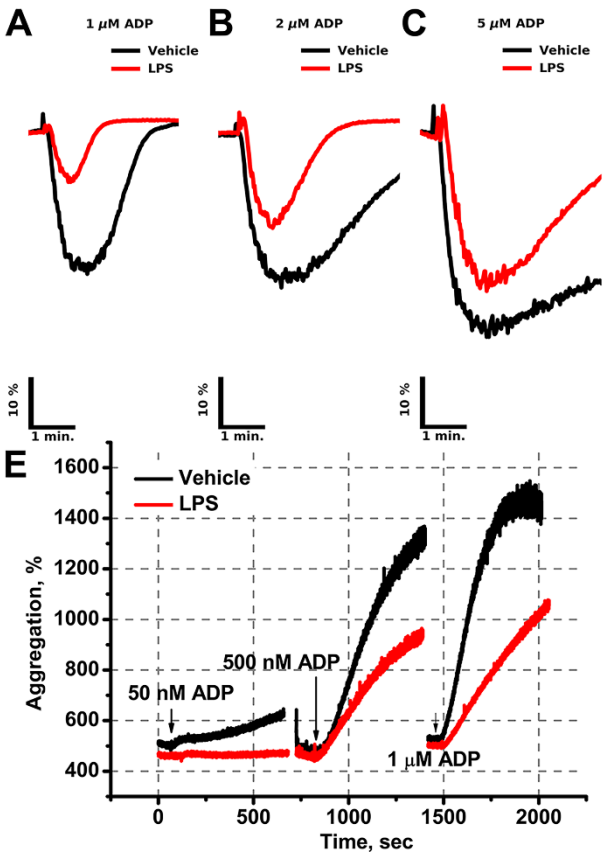


**Figure S9. LPS mediated alterations in platelet aggregation.** Pre-treatment with 100 µg/ml of LPS resulted in a decrease in platelet aggregation in response to 1 µM (A), 2 µM (B) and 5 µM of ADP (C). Platelet di-aggregate formation was impaired by LPS upon stimulation by all concentrations of ADP tested (E).