Supplementary Figures to Etulain et al. “Acidosis downregulates platelet haemostatic functions and promotes neutrophil proinflammatory responses mediated by platelets” (Thromb Haemost 2012; 107.1)
Suppl. Figure 1: Acidosis decreases platelet adhesion and spreading to fibronectin and collagen. (A) WPs were plated onto fibrinonecin (50 µg/ml) or collagen (20 µg/ml)-coated slides, stained with TRITC-Phalloidin and visualized under fluorescence microscopy. (B) The platelet perimeter was calculated using the ImageJ software on 3 random fields. Results are expressed as percentage of pH 7.4 (n=3). (C) WPs were plated onto fibronectin of collagen-coated wells and, after washing, incubated with p-nitrofenil phosphate. The percentage of adherent platelets relative to pH 7.4 was calculated after reading at 405 nm (n=3). (D) WPs at different pH were stimulated or not with thrombin (0.05 U/ml) for 5 min and then incubated for 60 min with HMEC-1 pretreated with (+) or without (-) TNF-α (20 ng/ml for 24 h before platelet addition). After washing, cells were incubated with p-nitrofenil phosphate and the percentage of adherent platelets was calculated by reading at 405 nm (n=3, *P < 0.05 vs. pH 7.4; #P < 0.05 vs. pH 7.0).
Suppl. Figure 2: The effect of acidosis on platelet function is reversible. WPs were first incubated at different pH for 15 min and then the pH of the acidic samples were restored to physiologic values by the addition of NaOH (1 N). After 10 min of pH neutralization, WPs were stimulated with thrombin (0.05 U/ml) for 5 min. (A) Aggregation was expressed as percentage of pH 7.4 (n=3). (B) Surface expression of P-selectin was detected by flow cytometry (n=3, *P < 0.05 vs. pH 7.4; **P < 0.05 vs. pH 7.0).