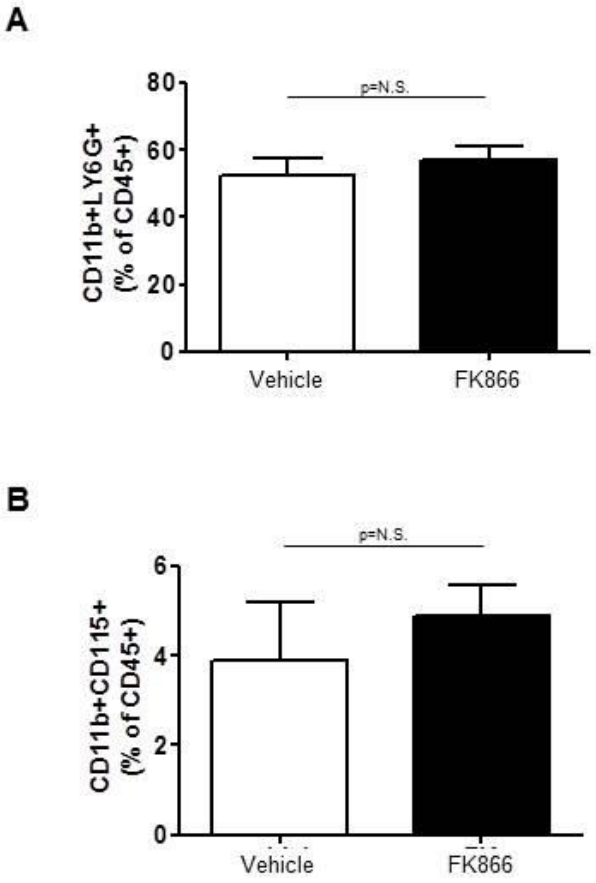
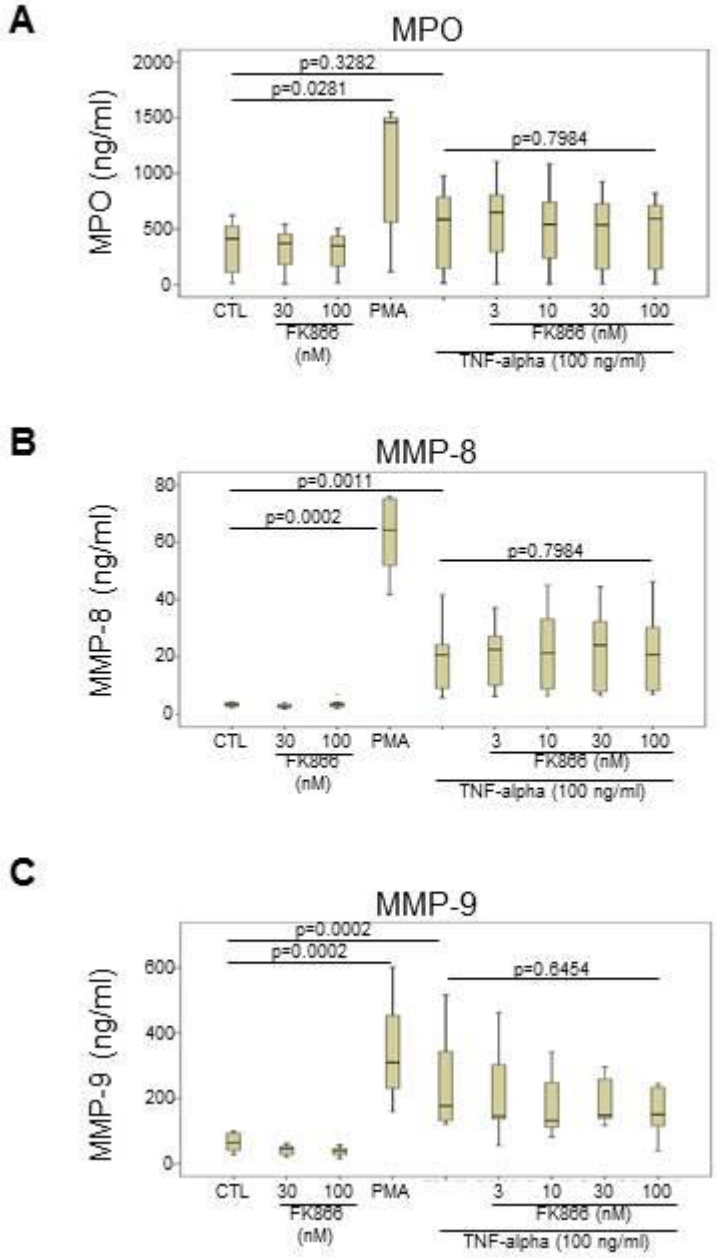
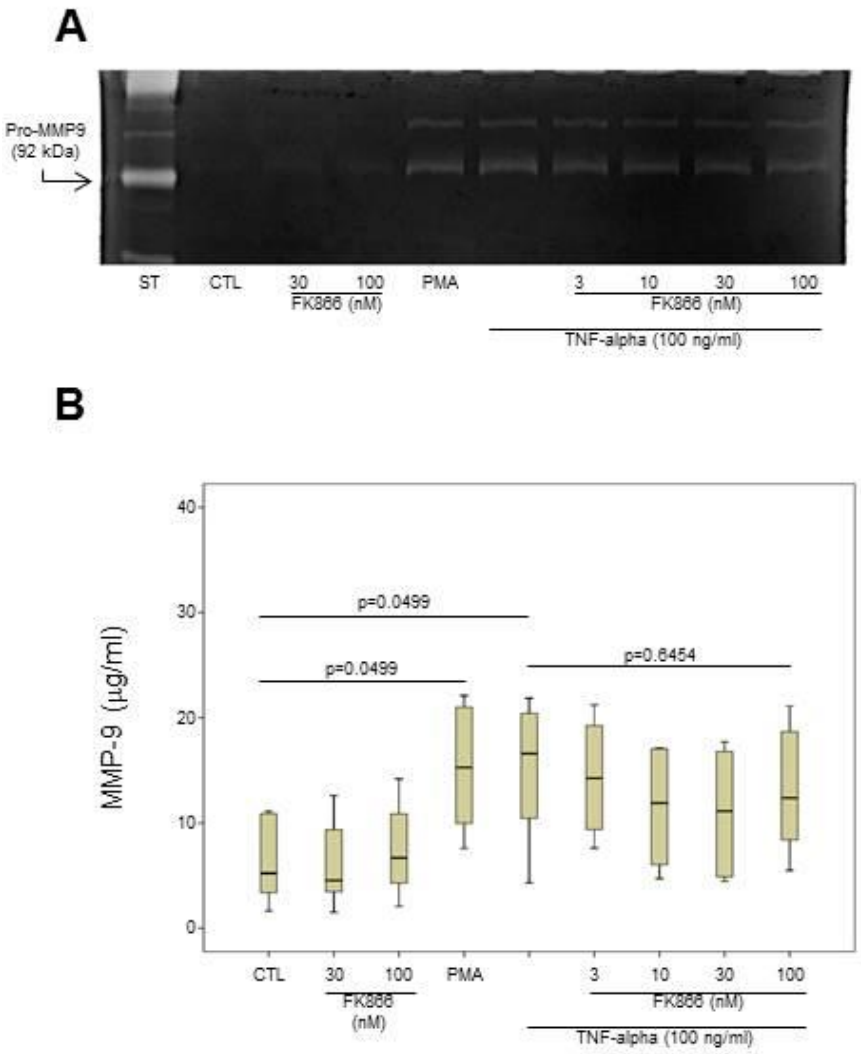
Suppl. Material to Nencioni et al. "Nicotinamide phosphoribosyltransferase inhibition reduces intraplaque CXCL1 production and associated neutrophil infiltration in atherosclerotic mice" (Thromb Haemost 2014; 111.2)



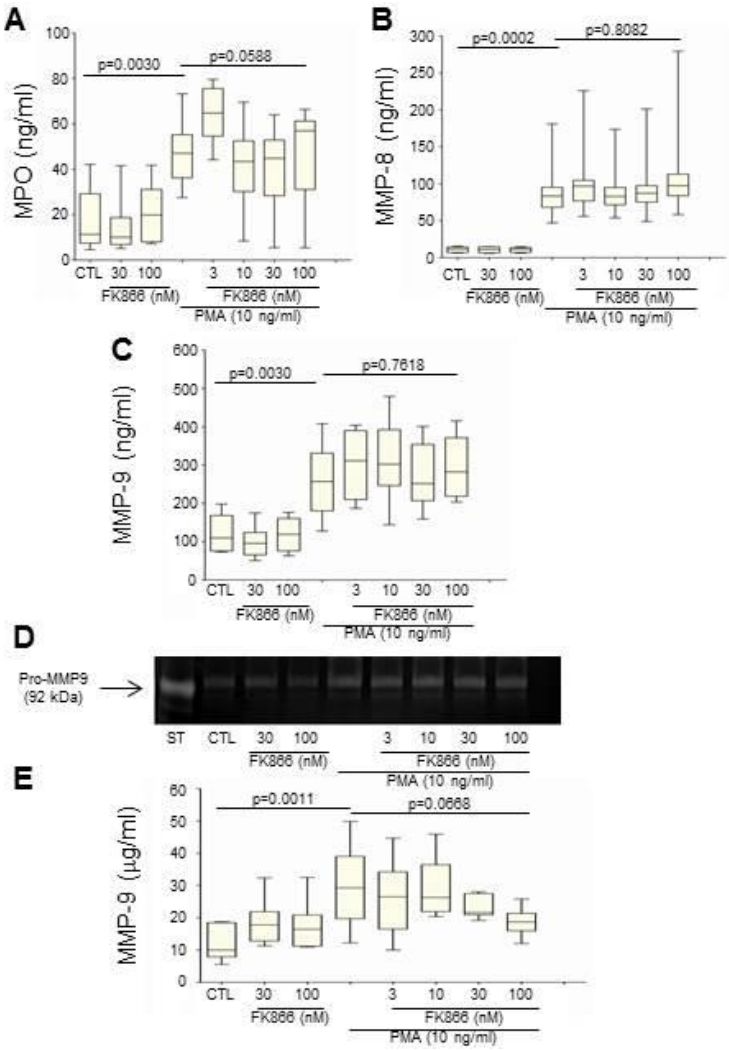
Suppl. Figure 1: FK866 treatment does not affect percentages of circulating neutrophils and monocytes in hypercholesterolaemic mice. A) Circulating neutrophil percentages in FK866 and Vehicle-treated hypercholesterolaemic mice. Neutrophils were quantified as CD45+CD11b+Ly6G+CD115- cells. B) Circulating monocyte percentages in FK866 and Vehicle-treated hypercholesterolaemic mice. Monocytes were quantified as CD45+CD11b+Ly6G-CD115+ cells. Data are expressed as mean±SEM (n=5-6), N.S.= not significant.



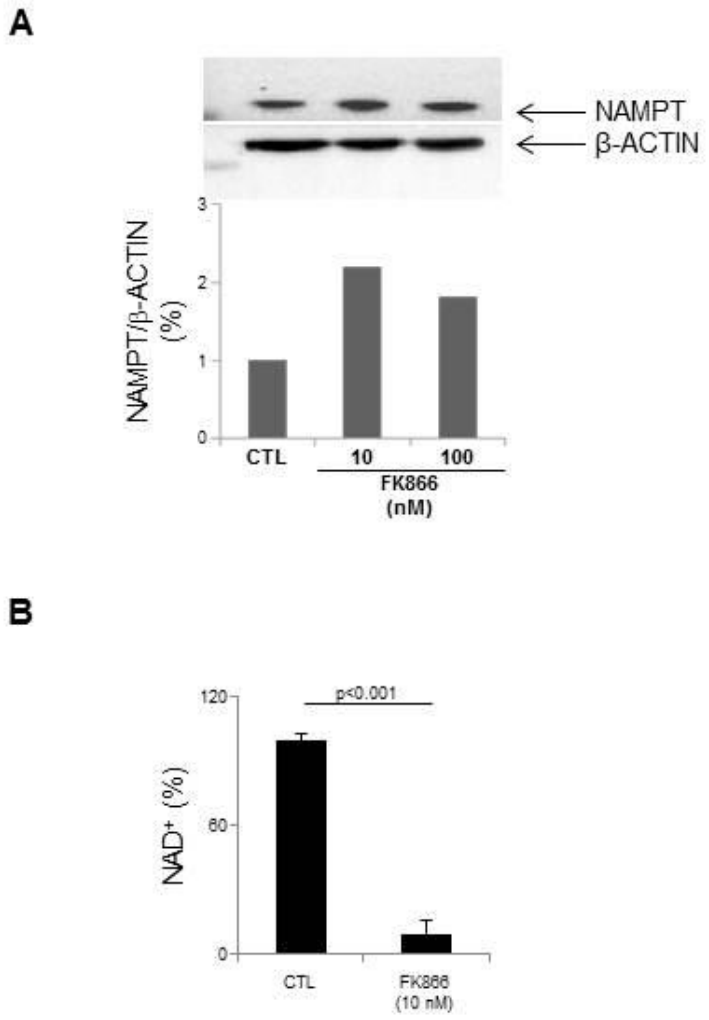
Suppl. Figure 2: Pre-treatment with FK866 does not affect *in vitro* TNF-alpha-induced neutrophil degranulation. MPO (A), MMP-8 (B) and MMP-9 (C) release in supernatants of neutrophils pre-incubated with different concentrations of FK866 and then stimulated in polystyrene dishes in the presence or absence of control medium (CTL), 10 ng/ml phorbol-12-myristate-13-acetate (PMA, positive control), or 100 ng/ml TNF-alpha. Data are expressed as median (interquartile range) (n=8).



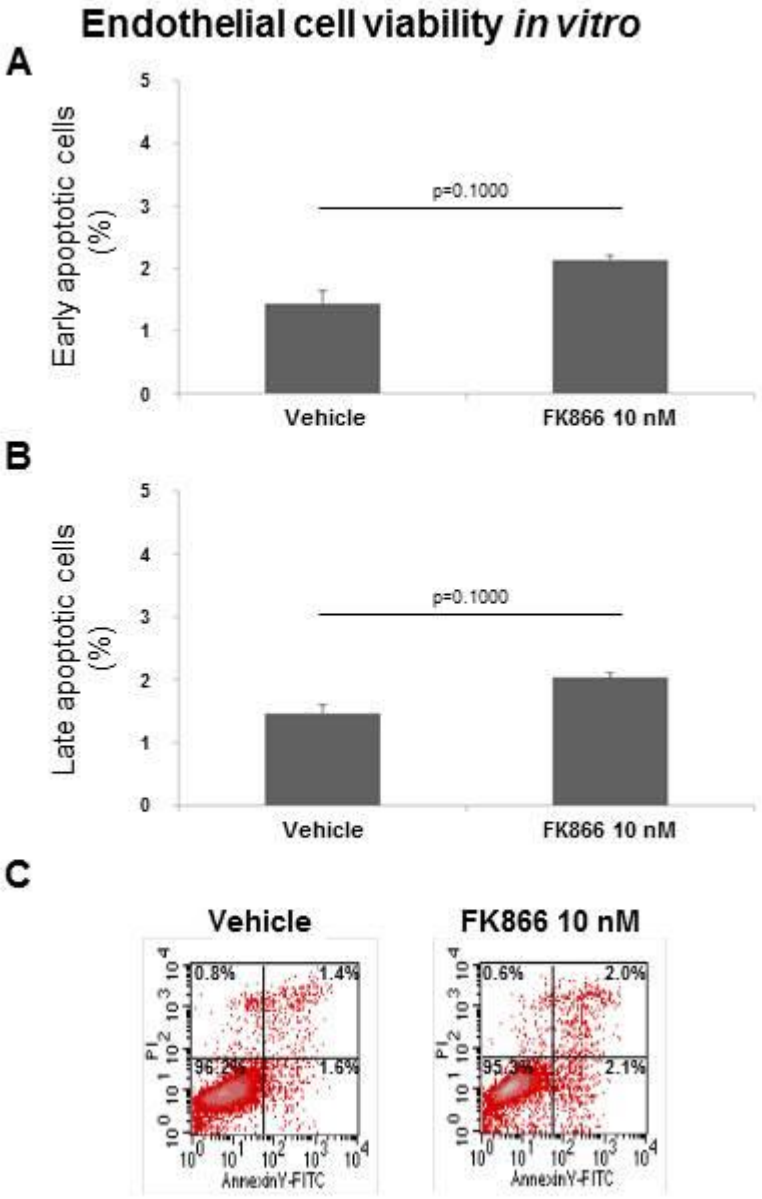
Suppl. Figure 3: Pre-treatment with FK866 does not affect pro-MMP-9 activity in supernatants of TNF-alpha-stimulated neutrophils. A) Representative gel of MMP-9 zymography and results of densitometric quantifications of 8 different experiments performed in polystyrene dishes. White band (arrow) on the gel represents the pro-MMP-9 gelatinolytic activity of standard recombinant pro-MMP-9 (ST), and supernatants of cells treated with control medium (CTL) FK866 alone, 10 ng/ml PMA (positive control) and increasing concentrations of FK866 and concomitant presence of 100 ng/ml TNF-alpha. B) Densitometry of pro-MMP-9 gels. Data are expressed as median (interquartile range) (n=8).



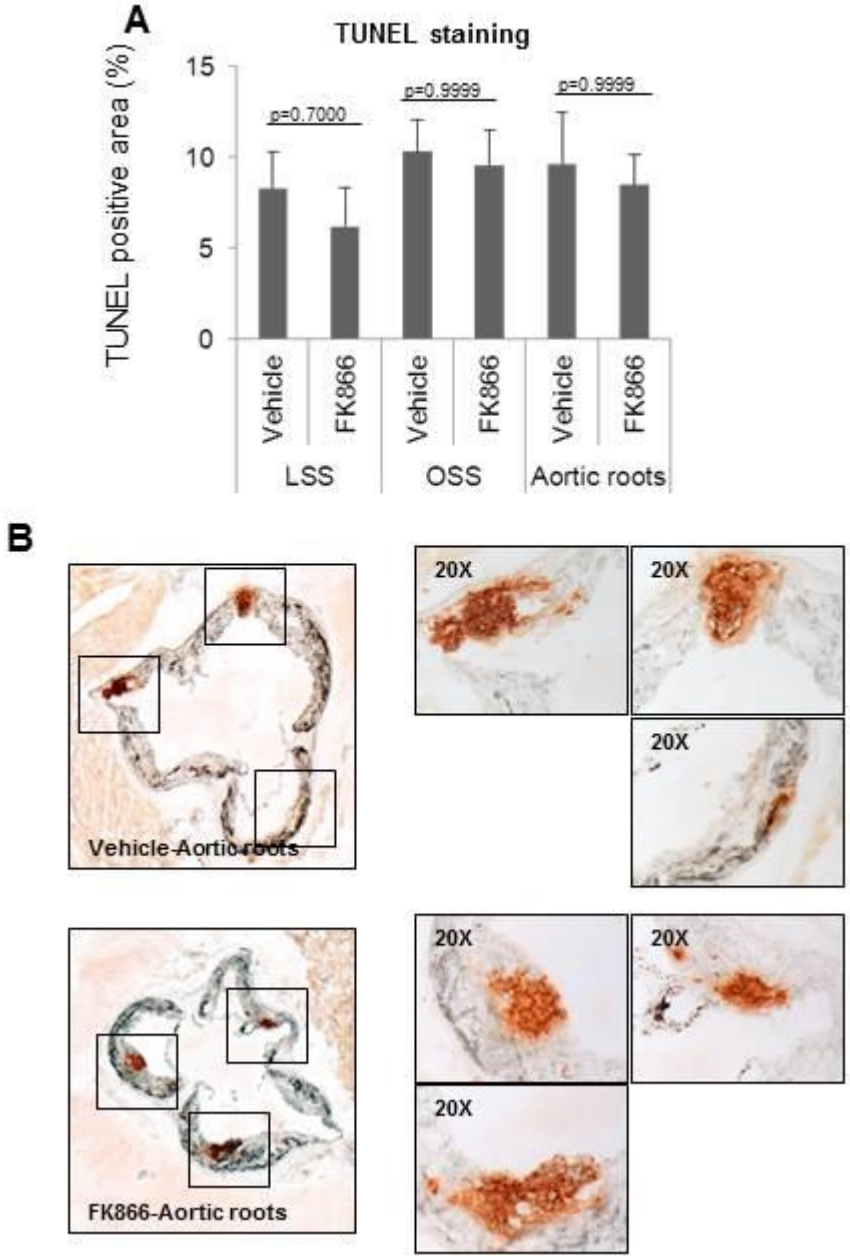
Suppl. Figure 4: Pre-treatment with FK866 does not affect *in vitro* PMA-induced neutrophil degranulation. MPO (A), MMP-8 (B) and MMP-9 (C) release in supernatants of neutrophils pre-incubated with different concentrations of FK866 and then stimulated in polystyrene dishes in the presence or absence of control medium (CTL) or 10 ng/ml PMA. D. Representative gel of MMP-9 zymography and results of densitometric quantifications of 8 different experiments performed in polystyrene dishes. White band (arrow) on the gel represents the pro-MMP-9 gelatinolytic activity of standard recombinant pro-MMP-9 (ST), and supernatants of cells treated with control medium (CTL) FK866 alone, 10 ng/ml PMA alone and with increasing concentrations of FK866. E. Densitometry of pro-MMP-9 gels. Data are expressed as median (interquartile range) (n=8).



Suppl. Figure 5: NAMPT is expressed in HECV cells. HECV cells were incubated for 48 h with control vehicle or FK866 at the indicated concentrations. Thereafter, cells were used for cytosolic extract preparation and NAMPT and β-actin levels were quantified by immunoblotting. A) One representative experiment out of three is presented. Lower panel, Densitometry of the immunoblotting. B) Intracellular NAD^+ determination in endothelial cells incubated in the presence of absence of FK866 (10 nM).



Suppl. Figure 6: FK866 treatment does not affect *in vitro* endothelial cell viability. A) Analysis of apoptosis rate in cultured human endothelial cells in the presence of vehicle (1‰ DMSO) or FK866 (10 nM) for 48h. Results were expressed as mean±SD (n=3 per group). B) Representative panel of flow cytometric analysis of annexin V/PI staining of vehicle- or FK866 treated cells at 48h of incubation.



Suppl. Figure 7: FK866 treatment does not affect *in vivo* intraplaque apoptosis. A) Quantification of apoptotic intraplaque areas in Vehicle- and FK866-treated animals Data are expressed as mean±SEM (n=3-5 per group). B) Representative microphotographs of cryosections from aortic roots of Vehicle- and FK866-treated mice stained for TUNEL (apoptotic cells). On the right panel, higher magnification (20X) images showing intraplaque apoptotic areas are shown.