

Supplement Material

Inorganic polyphosphate amplifies HMGB1-mediated von Willebrand factor release and platelet string formation on endothelial cells

Indranil Biswas, Sumith R. Panicker, Xiaofeng Cai, Padmaja Mehta-D'souza, and Alireza R Rezaie¹

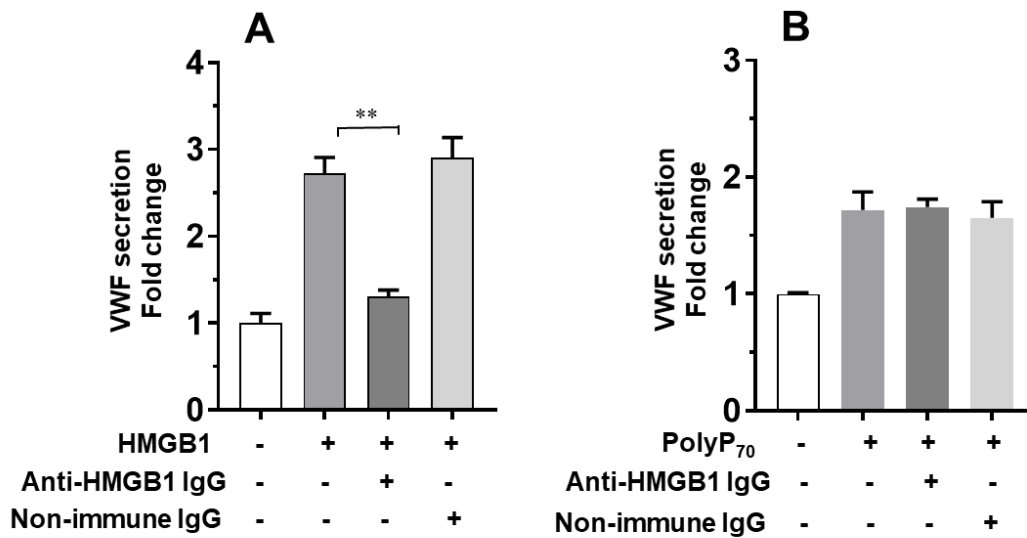


Figure IS- Analysis of polyP₇₀-mediated VWF release by EA.hy926 cells in the absence and presence of an anti-HMGB1 neutralizing antibody. EA.hy926 cells were pretreated with anti-HMGB1 monoclonal antibody (10 μg/mL) or non-immune IgG (10 μg/mL) for 30 min followed by treatment with (A) HMGB1 (40 nM) for 16h. HMGB1-induced VWF release was measured using a Sandwich ELISA as described in Materials and methods. (B) The same as (A) except that cells were pretreated with anti-HMGB1 monoclonal antibody (10 μg/mL) or non-immune IgG (10 μg/mL) for 30 min followed by treatment with polyP₇₀ (50 μM) for 16h. polyP₇₀-induced VWF release was measured using a Sandwich ELISA as described in Materials and methods. All results are shown as means ± SD of three independent experiments. Statistical significance was analyzed by one way ANOVA with Bonferroni post test. **p<0.01.

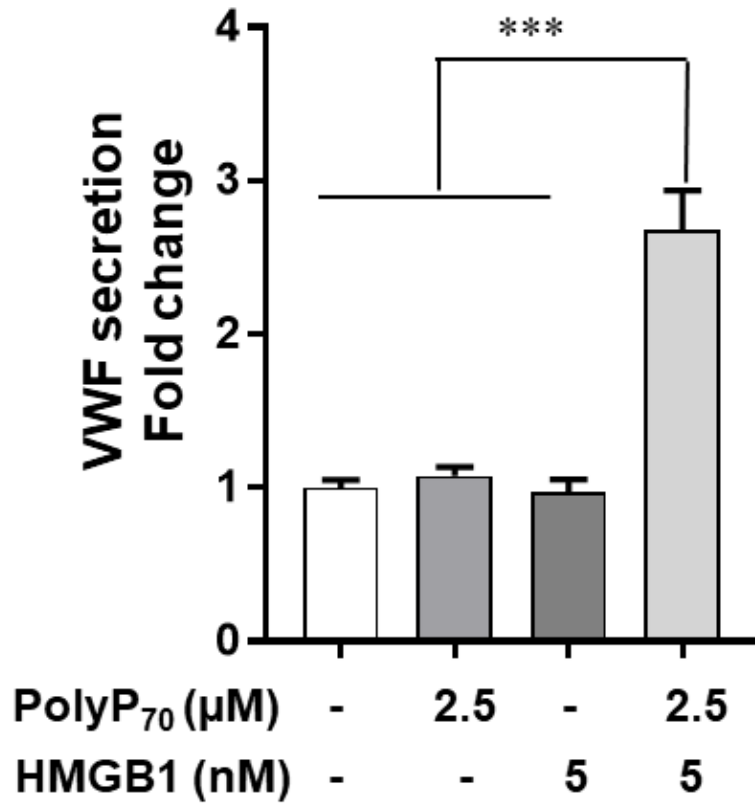


Figure IIS- VWF measurement by Sandwich ELISA- Primary human umbilical vein endothelial cells (HUVECs, Invitrogen) were treated with polyP₇₀ (2.5 μM, 4h) HMGB1 (2.5 nM, 16h) or with their combination (16h) and VWF release was measured using a Sandwich ELISA as described under Materials and methods. All results are shown as means ± SD of three independent experiments. Statistical significance was analyzed by one way ANOVA with Bonferroni post test. ***p<0.005.