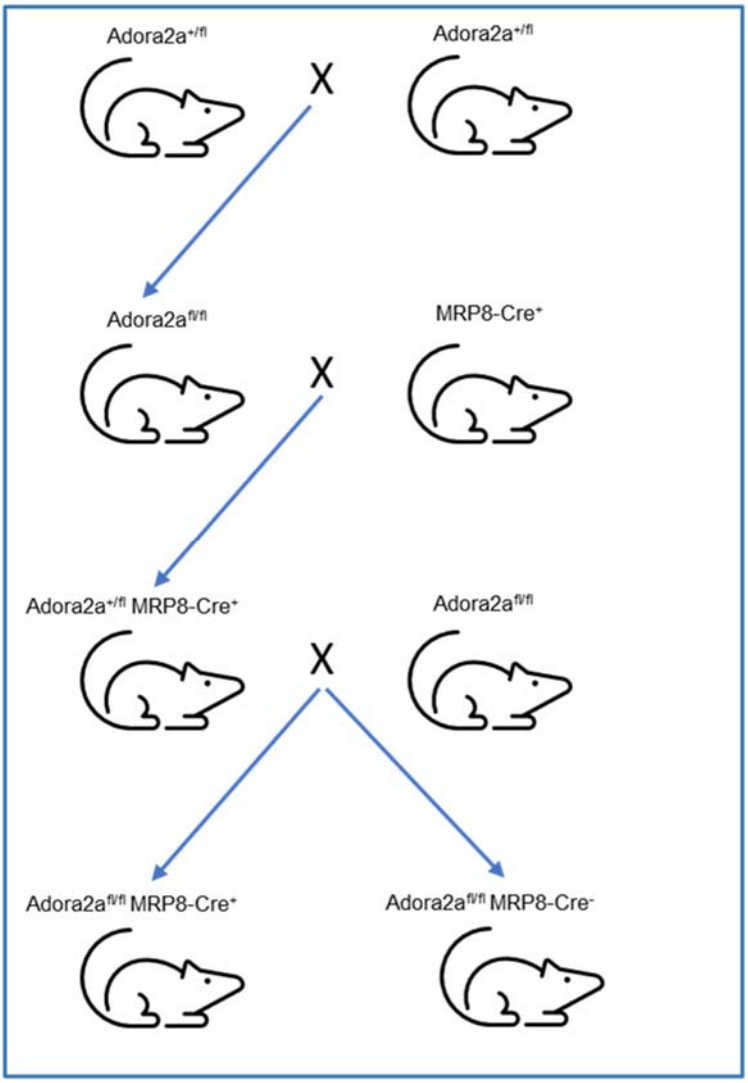
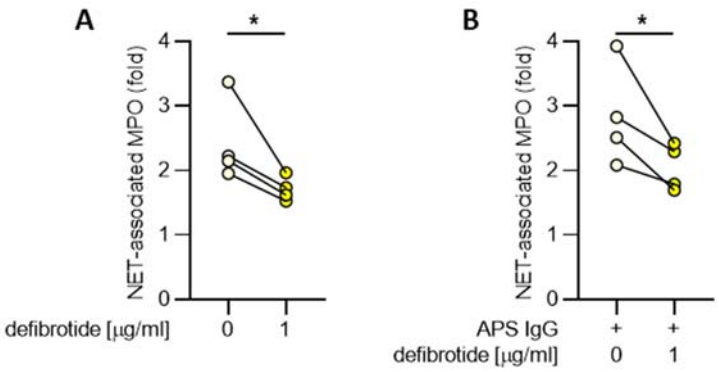
SUPPLEMENATRY INFORMATION

Defibrotide inhibits antiphospholipid antibody-mediated NET formation and venous thrombosis

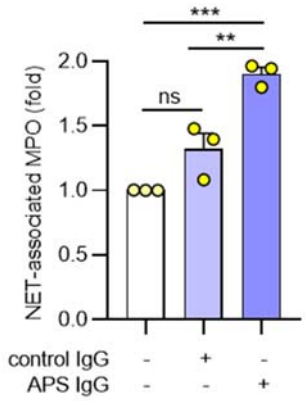
Ali *et al.*



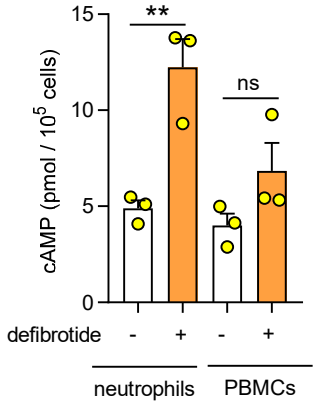
Supplementary Figure 1: Breeding scheme for myeloid-specific knockout of the adenosine A_{2A} receptor as described in Methods.



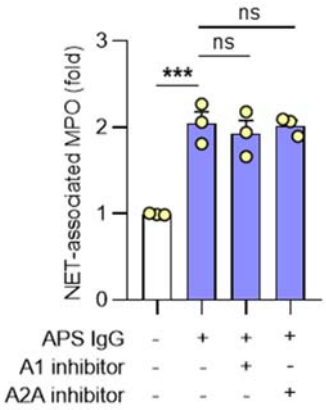
Supplementary Figure 2: Defibrotide suppresses NET formation by neutrophils isolated from patients with triple-positive antiphospholipid antibodies. Neutrophils were isolated from four patients with clinical manifestations of APS who were "triple-positive" for anticardiolipin antibodies, anti-β_2GPI antibodies, and lupus anticoagulant. Neutrophils were cultured for 3 hours in the presence or absence of defibrotide (1 μg ml^{-1}). Some samples were also treated with APS IgG as in Figure 1. Spontaneous NET formation (**A**) and APS IgG-mediated NET formation (**B**) were quantified by measuring the enzymatic activity of nuclease-liberated myeloperoxidase (MPO); **p*<0.05 by paired t test.



Supplementary Figure 3: IgG isolated from heterologous healthy controls does not significantly increase NET formation by control neutrophils. Human neutrophils were isolated from healthy volunteers and then stimulated with healthy control IgG or APS IgG for 3 hours. NET formation was quantified by measuring the enzymatic activity of nuclease-liberated myeloperoxidase (MPO). Mean and standard error of the mean (SEM) are presented for 3 independent experiments; $^{**}p<0.01$, $^{***}p<0.001$, and ns=not significant by one-way ANOVA corrected with Dunnett's method.



Supplementary Figure 4: cAMP levels in neutrophils versus peripheral blood mononuclear cells (PBMCs). Human neutrophils and peripheral blood mononuclear cells were isolated from healthy volunteers and cultured in the presence or absence of 1 µg ml^{-1} defibrotide; cyclic AMP (cAMP) levels were then measured after 30 minutes. Mean and standard error of the mean (SEM) are presented for 3 independent experiments; $^{**}p<0.01$ and ns=not significant by one-way ANOVA corrected with Dunnett's method.



Supplementary Figure 5: Effect of adenosine receptor antagonists on APS IgG-mediated NET formation. Human neutrophils were isolated from healthy volunteers and then treated with APS IgG for 3 hours in the presence or absence of an adenosine A_1 receptor inhibitor (10 μM), or an adenosine A_{2A} receptor inhibitor (10 μM). NET formation was quantified by measuring the enzymatic activity of nuclease-liberated myeloperoxidase (MPO). Mean and standard error of the mean (SEM) are presented for 3 independent experiments; ***p<0.001 and ns=not significant by one-way ANOVA corrected with Dunnett's method.