Supplemental Material

In Systemic Lupus Erythematosus anti-dsDNA Antibodies Can Promote Thrombosis through Direct Platelet Activation

Short title: Platelet activation by anti-dsDNA antibodies

Izabella A. Andrianova[a], Anastasiya A. Ponomareva[a,b], Elmira R. Mordakhanova[a], Giang Le Minh[a], Amina G. Daminova[a,b], Tatyana A. Nevzorova[a], Lubica Rauova[c,d], Rustem I. Litvinov[d], John W. Weisel[d,*]

[a]Institute of Fundamental Medicine and Biology, Kazan Federal University, Kazan, Russian Federation

[b]Kazan Institute of Biochemistry and Biophysics, Kazan Scientific Center of the Russian Academy of Sciences, Kazan, Russian Federation

[c]The Children's Hospital of Philadelphia, Philadelphia, Pennsylvania, USA

[d]University of Pennsylvania Perelman School of Medicine, Philadelphia, Pennsylvania, USA

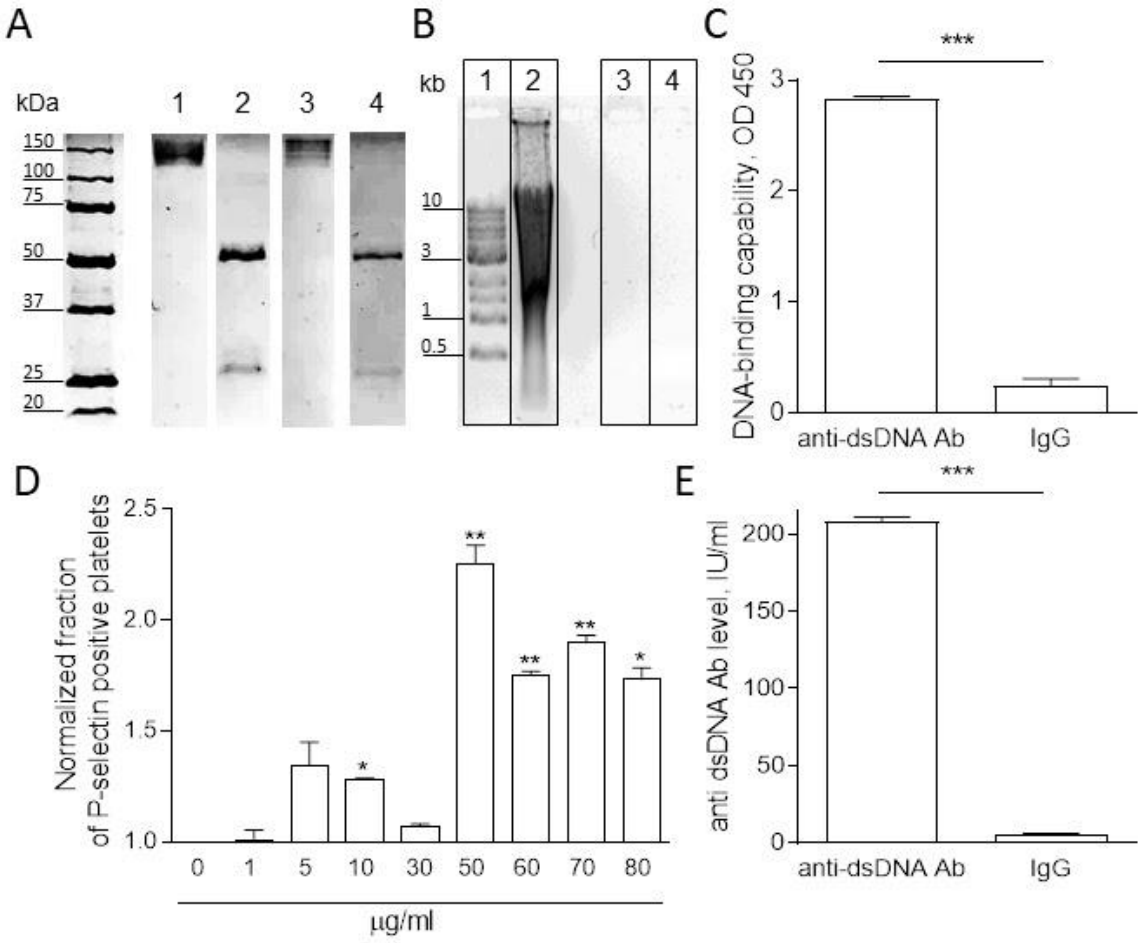

Fig. S1. Purity and dsDNA-binding activity of purified anti-dsDNA Abs. **(A)** SDS-PAGE: lane 1 – control non-dsDNA-binding IgG in non-reducing conditions, lane 2 – control non-dsDNA-binding IgG in reducing conditions, lane 3 – purified anti-dsDNA Abs in non-reducing conditions, lane 4 – purified anti-dsDNA Abs in reducing conditions. Lines 2 and 4 show 2 bands which represent heavy (50 kDa) and light (25 kDa) chains of IgG antibodies. **(B)** Agarose gel electrophoresis showing no detectable DNA contamination in the preparation of purified anti-dsDNA Abs. Lane 1 – DNA markers, lane 2 – calf thymus dsDNA, lane 3 – anti-dsDNA Abs, lane 4 – IgG. **(C)** An ELISA assay of dsDNA-binding activity of purified anti-dsDNA Abs versus equimolar amount of non-dsDNA-binding IgG. Student's *t*-test. ***P<0.001 **(D)** Fraction of P-selectin-positive platelets normalized by control untreated platelets after treatment with anti-dsDNA Abs at various concentrations (0, 10, 30, 50, 60, 70, 80, 90 µg/ml). A Mann-Whitney *U*-test was used to compare with the negative control. *P<0.05, **P<0.01 **(E)** An ELISA assay of dsDNA-binding activity of purified anti-dsDNA Abs versus equimolar amount of non-dsDNA-binding IgG. Student's *t*-test. ***P<0.001

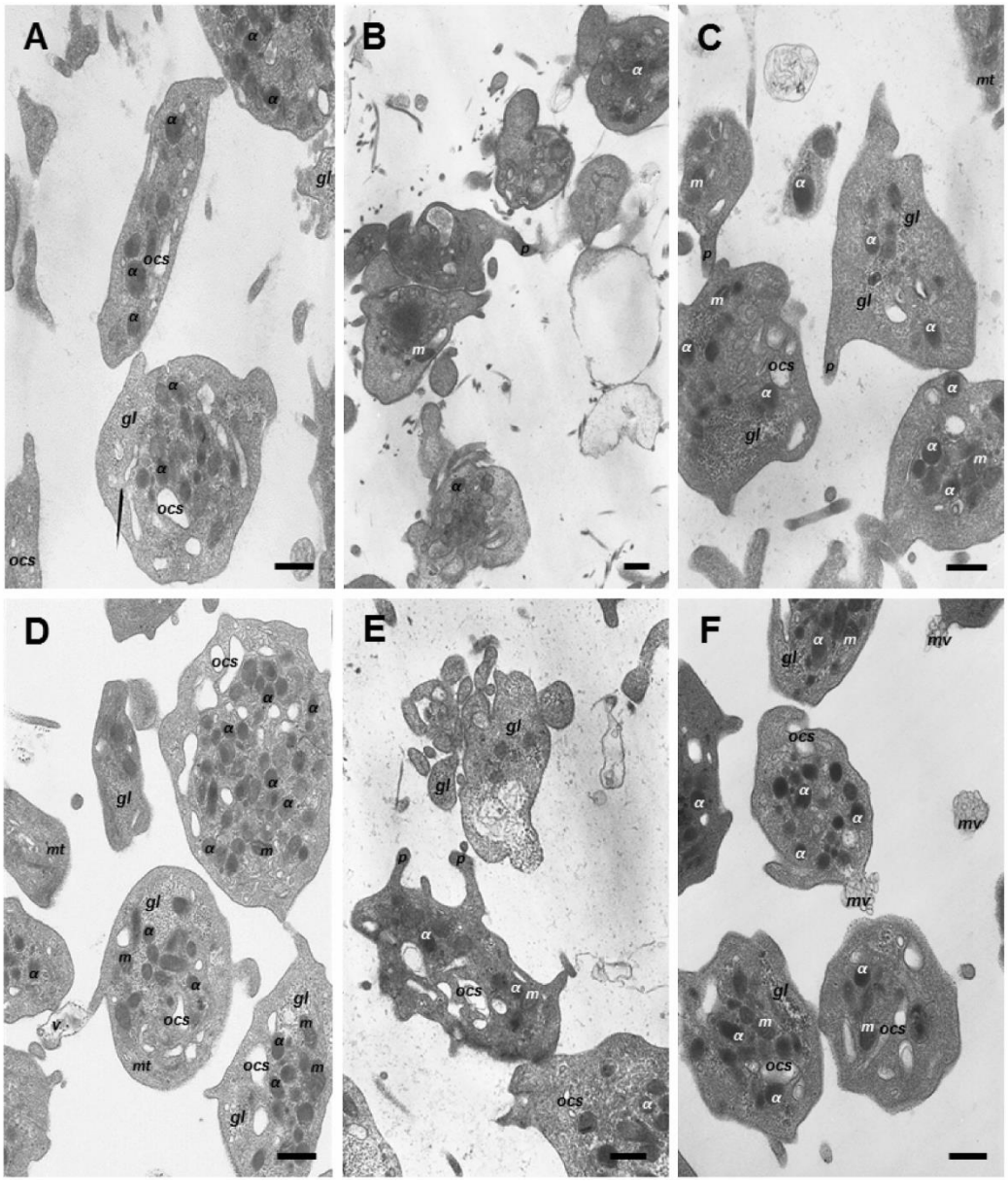

Fig. S2. Representative transmission electron micrographs of isolated normal human platelets under various experimental conditions (see Methods for details). **(A)** Untreated platelets (control); (**B-F**) platelets incubated with: anti-dsDNA Ab/dsDNA complexes **(B)**, anti-dsDNA Ab/dsDNA complexes after pre-treatment with anti-FcγRIIA mAb **(C)**, non-DNA-binding IgG **(D)**, anti-dsDNA Abs **(E)**, and anti-dsDNA Abs after pre-treatment with anti-FcγRIIA mAb **(F)**. In B and E platelets have ultrastructural signs of activation: dramatic shape change, shrinkage, degranulation, membrane protrusions and blebbing, formation of microvesicles. In B: "empty" platelets or platelet-derived structures with low electron density and no organelles inside. Designations: *α*, α-granules; *gl*, glycogen granules; *m*, mitochondria; *mt*, microtubules; *OCS*, open canalicular system; *f*, filopodia; *v*, microvesicle with a single membrane; *mv*, a multivesicular particle. Scale bars = 0.5 µm**.**

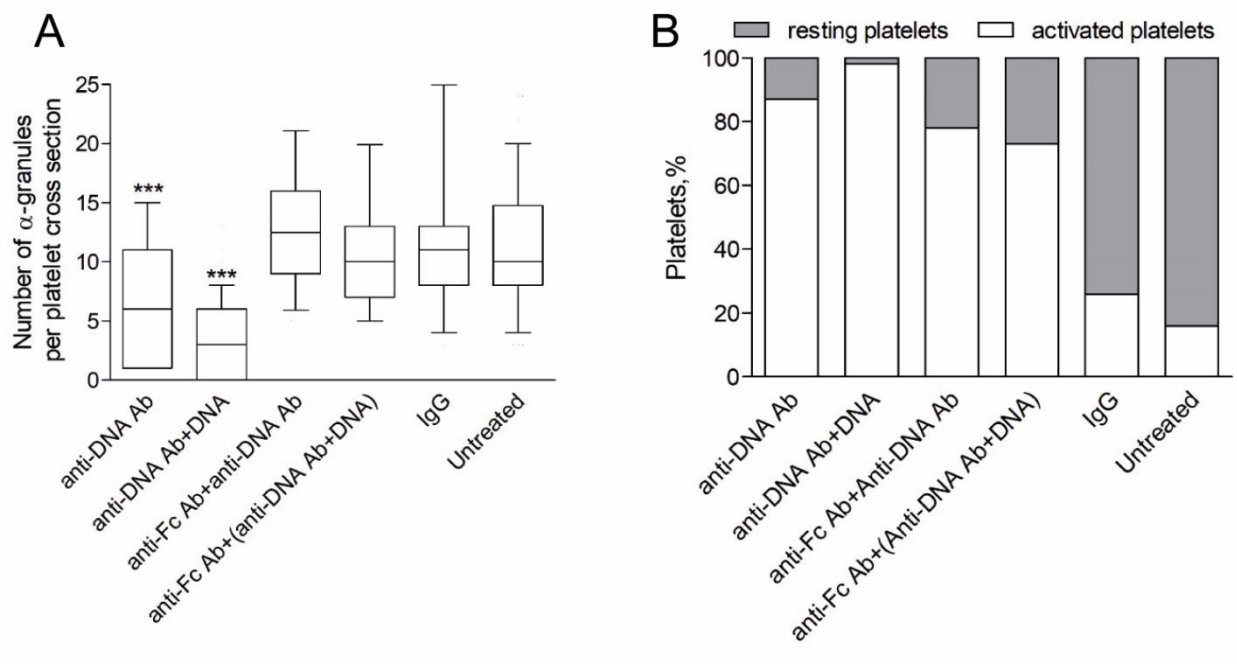

Fig. S3. Quantification of the ultrastructural signs of platelet activation under various experimental conditions. (A) Number of α-granules per platelet cross-section based on the quantification of transmission electron micrographs like those presented in Fig. S2. The boxes shown from left to right represent the following samples: platelets incubated with anti-DNA Abs (n=47); anti-DNA Ab/dsDNA complexes (n=100); anti-DNA Abs after pre-treatment with anti-FcγRIIA mAb (n=38); anti-DNA Ab/dsDNA complexes after pre-treatment with anti-FcγRIIA mAb (n=100), non-DNA-binding IgG (n=27) or without treatment (untreated, n=100). The bars show 10 and 90 percentiles. A Kruskal-Wallis test was used for statistical analysis. ***$p<0.001$ compared with Control. **(B)** Percentage of activated platelets based on quantification of the transmission electron micrographs obtained under various experimental conditions and represented on Fig. S2. The bars shown from left to right represent the following samples: platelets incubated with anti-dsDNA Abs (n=131); anti-dsDNA Ab/dsDNA complexes (n=164); anti-dsDNA Abs after pre-treatment with anti-FcγRIIA mAb (n=37); anti-dsDNA Ab/dsDNA complexes after pre-treatment with anti-FcγRIIA mAb (n=107); non-DNA-binding IgG (n=27) or without treatment (untreated, n=262). *n* is the number of individual platelets analyzed on the micrographs.