Supplementary Figures

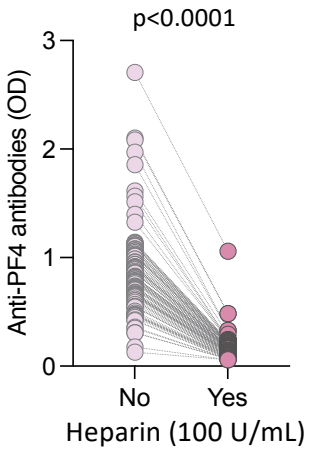

Figure S1. Effect of Heparin at High Concentration on the Detection of Anti-PF4-Polyanion Antibodies in Serum from Hospitalized Covid-19 Patients. To verify the specificity of the anti-PF4 antibody assay, unfractionated porcine heparin (UFH) was added to each well at 100 U/mL. The statistical comparison was performed using paired two-tailed *t*-test.

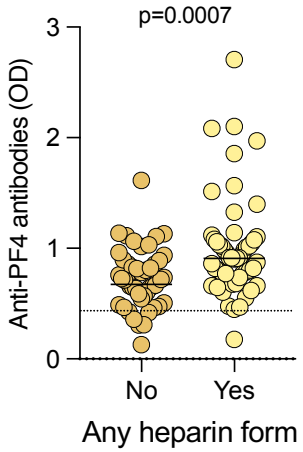

Figure S2. Anti-PF4 Antibody Levels and Prior Treatment with Any Forms of Heparin in Hospitalized Covid-19 Patients. Shown are anti-PF4 antibody levels in hospitalized Covid-19 patients treated with any form of heparin (UFH or LMWH) for at least 6 days at the time of sampling. Statistical comparison was performed using unpaired two-tailed *t*-tests.

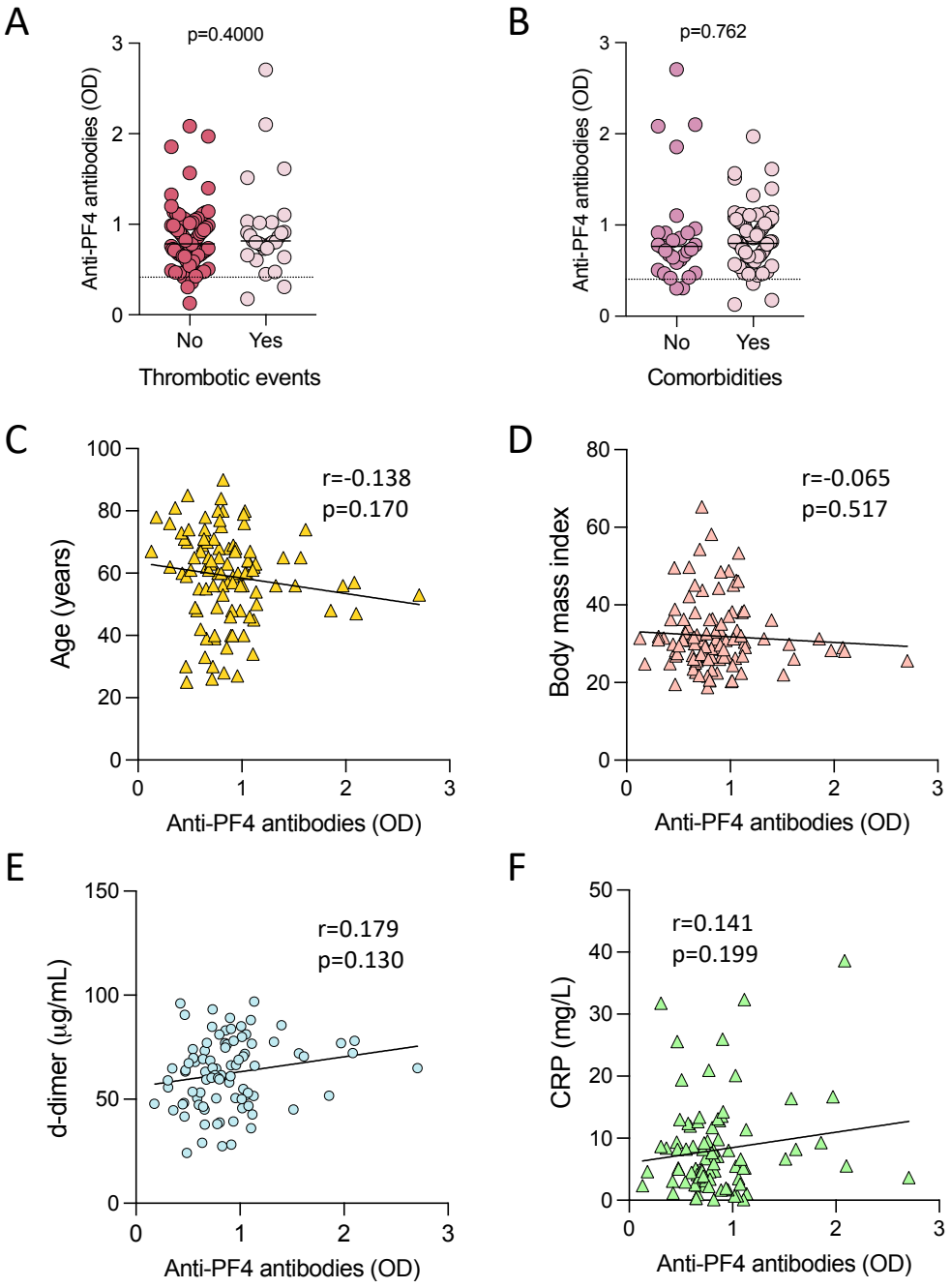

Figure S3. Correlation of Anti-PF4 Antibodies with Demographic, Clinical and Laboratory Parameters in Hospitalized Covid-19 Patients. *(A-B)* Anti-PF4 antibodies in hospitalized Covid-19 patients with and without *(A)* clinically apparent thrombotic events or *(B)* pre-existing comorbidities (i.e., type-I or type-II diabetes, asthma, uncomplicated or complicated hypertension), as assessed by two-tailed by unpaired *t*-test. *(C-F)* Correlation of anti-PF4 antibodies with age *(C),* body mass index *(D), or* plasma levels of d-dimer *(E)* or C-reactive protein (CRP) *(F),* as analyzed by linear regression. The Pearson's correlations and p values are reported.

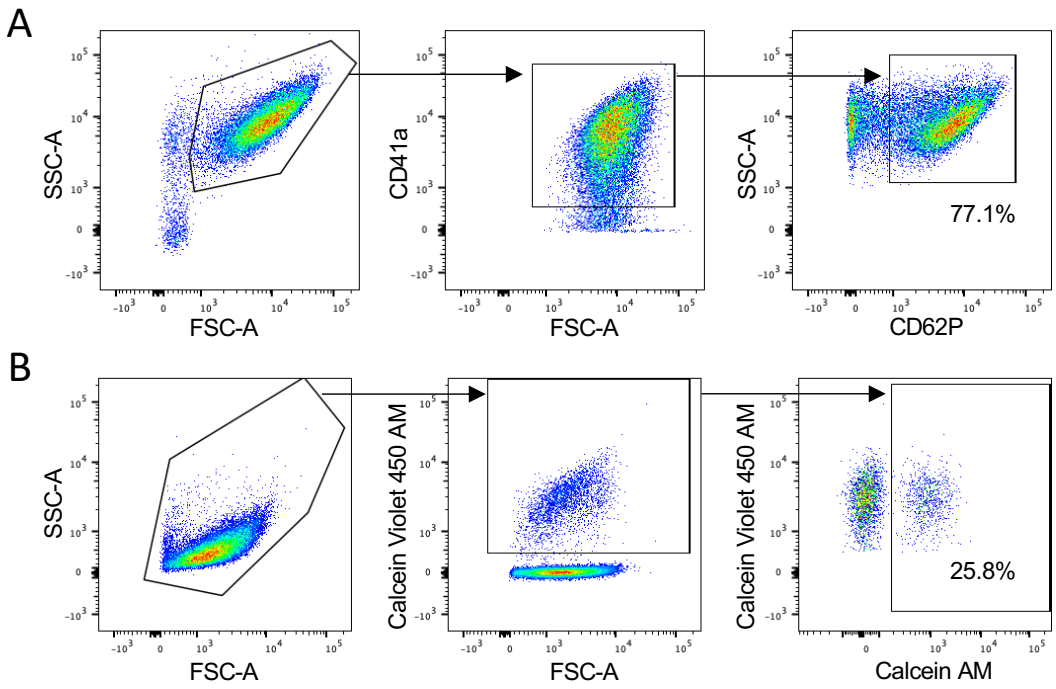

Figure S4. Representative flow-cytometry plots of platelet activation and aggregation. (A) Platelet activation. Platelets from healthy donors were treated with Covid-19 patient sera, and activated platelets were identified as $CD41a^{+}CD62P^{+}$ events. (B) Platelet aggregation. Platelets from healthy donors were separately stained either with Calcein Violet 450 AM Viability Dye (V450) or with Calcein AM Viability Dye (AM). The two types of stained platelets were mixed and treated with Covid-19 patient sera. Aggregated platelets were identified as double-positive events for both dyes.

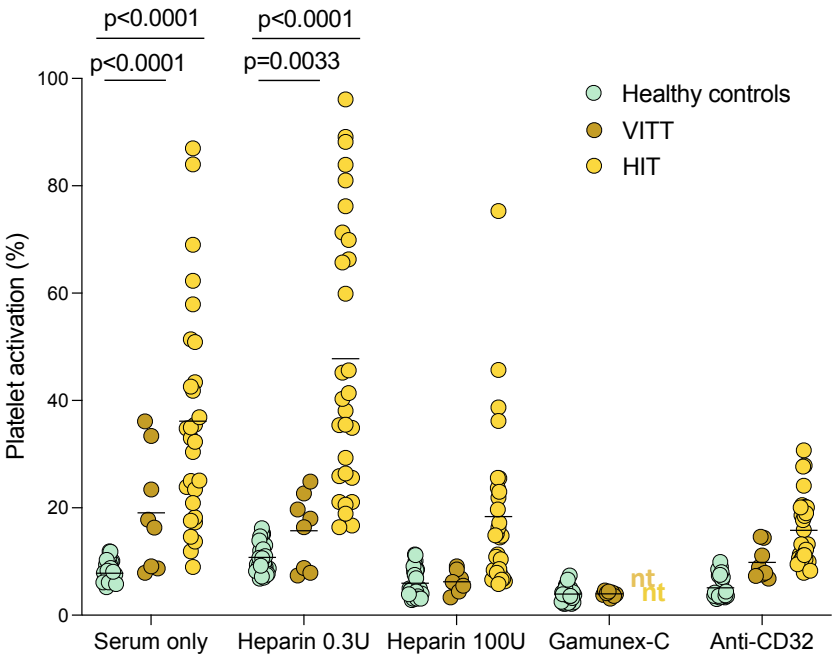

Figure S5. Platelet Activation Induced by Serum from HIT and VITT Patients. Shown are platelet activation levels induced by sera from 29 patients with HIT and 8 patients with VITT syndrome versus sera from healthy blood donors, as measured by surface expression of P-selectin (CD62P) on freshly drawn platelets from healthy blood donors. The tests were performed in the presence or absence of low-dose heparin (0.3 U/mL) as a stimulant, or high-dose heparin (100 U/mL), concentrated human immunoglobulins (Gamunex-C) or an anti-CD32 blocking antibody as inhibitors. Statistical differences for the indicated comparisons were calculated by unpaired, two-tailed *t*-test. nt = not tested.

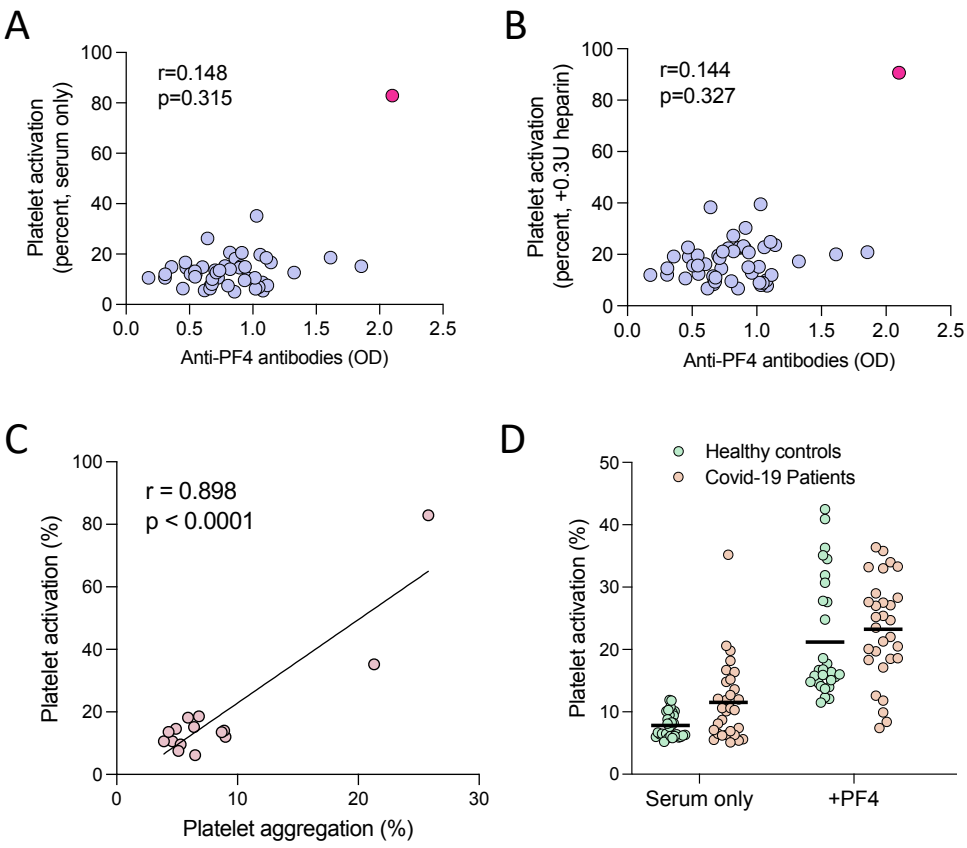

Figure S6. Platelet Activation Induced by Serum from Covid-19 Patients and Correlation with Anti-PF4 Antibodies or Platelet Aggregation. *(A-B)* Correlation of platelet activation induced by serum from hospitalized Covid-19 patients assayed in the absence *(A)* or presence *(B)* of low-dose heparin (0.3 U/mL). A single outlier (purple) was excluded from the statistical analyses. *(C)* Correlation of platelet activation with platelet aggregation induced by sera from a selected group of hospitalized Covid-19 patients (n=15). Statistical associations were evaluated using linear regression. The Pearson's correlations and p value are reported. *(D)* Effect of exogenous PF4 (50 µg/mL) on platelet activation induced by serum from hospitalized covid-19 patients and healthy blood donors. In Covid-19 patients, the mean level of platelet activation was 11.5±6.3 without PF4 and 23.2±8.0 in the presence of PF4 (a 201.6% increase); in healthy controls, it was 7.8±1.9 without PF4 and 21.2±9.2 in the presence of PF4 (a 271.1% increase).