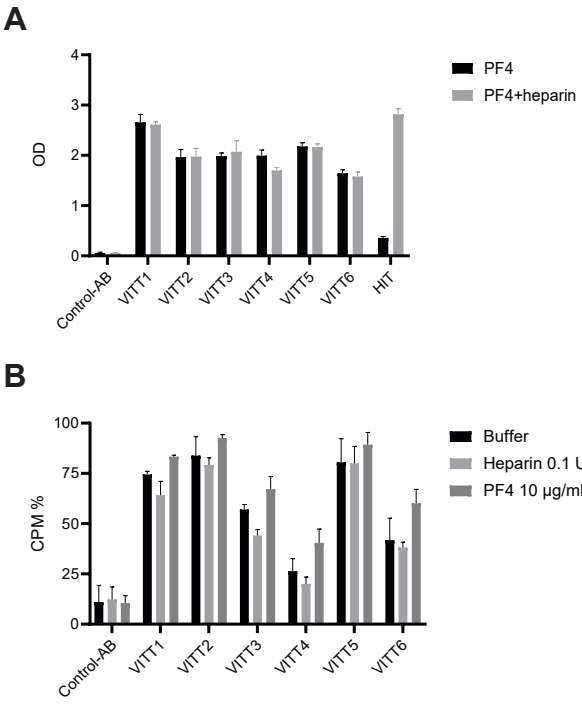
Supplementary Figure 1: Characterising VITT IgG. (A) Presence of anti-PF4 or anti-PF4/heparin antibodies in patient sera was measured using a solid phase PF4 (15 µg/mL) or PF4 (15 µg/mL)/heparin (0.1 U/mL) ELISA. (B) Platelet activation was measured by ^{14}C-SRA in the presence or absence of PF4 (10 µg/mL) or 0.1 U/mL heparin.

Supplementary Figure 2: Negatively charged anticoagulants interfere with PF4 bound to ELISA plates. (A) Addition of argatroban, bivalirudin, or fondaparinux up to 16 ug/mL have no effect on plate-bound PF4, while low doses of UF heparin (from 0.1 U/mL) or danaparoid (from 0.25 U/mL) strip off PF4 from the plate. (B) Co-incubation of PF4 and UF heparin or PF4 and danaparoid on ELISA plates stabilises plate-bound PF4. Dashed lines indicate therapeutic plasma level range. Data shown as mean ± S.D.

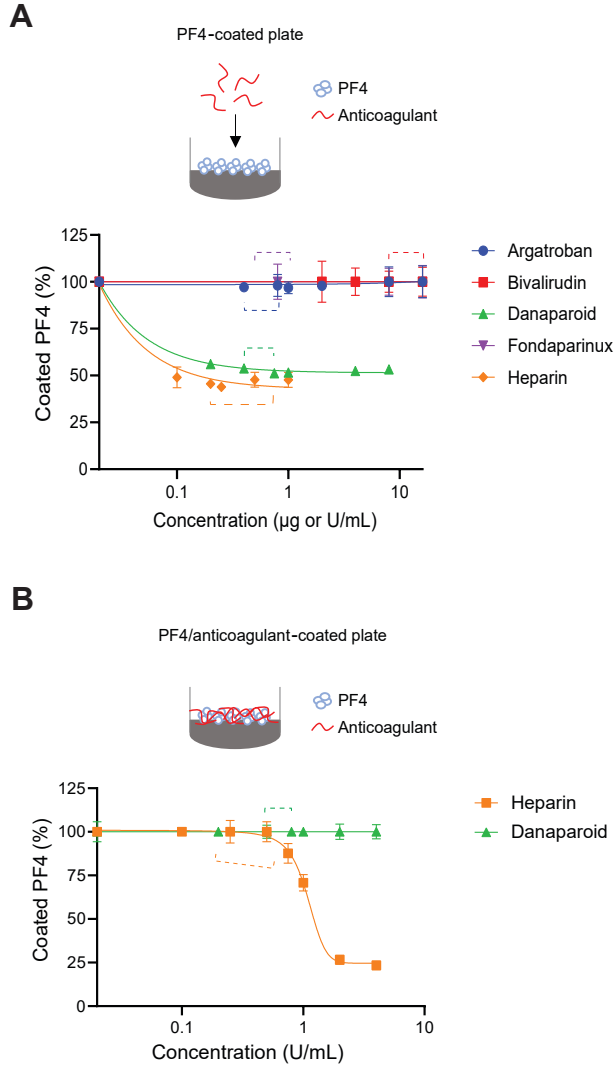
Supplementary Figure 3: Affinity purified anti-PF4 IgG from VITT patient sera. Non-reducing SDS gel showing affinity purified anti PF4 IgG from VITT patient (VITT). Commercial monoclonal IgG (Ctrl) used as a comparison. L indicates ladder.

Supplementary Figure 4: Effect of increasing doses of PF4 on platelet activation. PF4-mediated platelet activation across a range of PF4 concentrations from 0 to 50 µg/mL, as measured by anti-CD62p by flow cytometry.

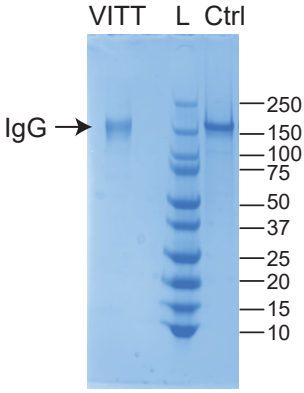
Supplementary Figure 5: Effect of heparin treatment on HIT mice. The HIT condition was recreated by intravenous injection of HIT IgG or HIT-like monoclonal antibody (KKO) and intraperitoneal injection of heparin (1 U/g) into FcγRIIa^{+}/hPF4^{+} mice[16,18,20]. (A) Platelet counts, (B) platelet lung fluorescence, and (C) body temperature of mice following treatment with control IgG or HIT IgG in the absence or presence of heparin (at 1U/g), with or without heparin infusion via minipump (18 U/kg/h). * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$, relative to HIT IgG with heparin. Data shown as mean ± S.D.



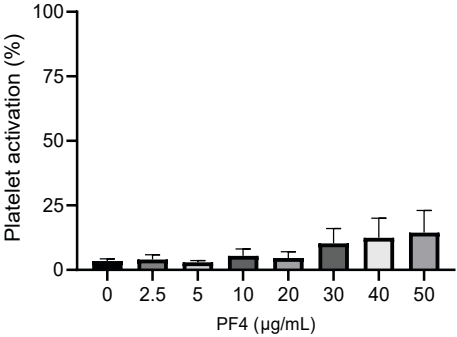
Supplementary Figure 1



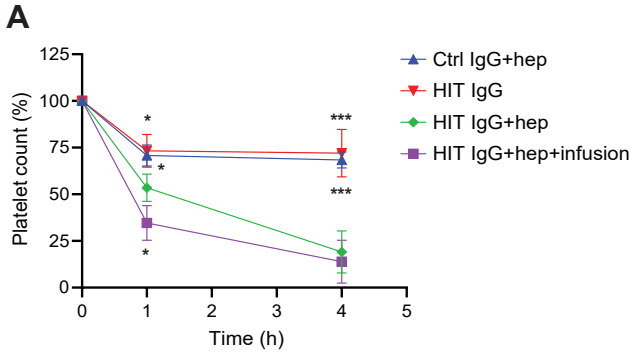
Supplementary Figure 2

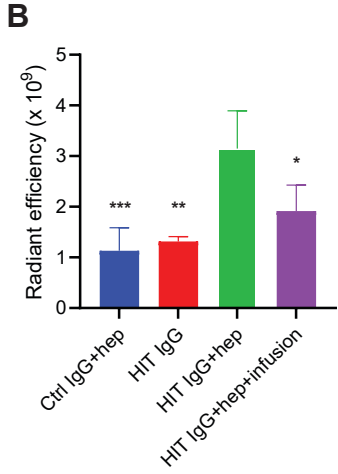


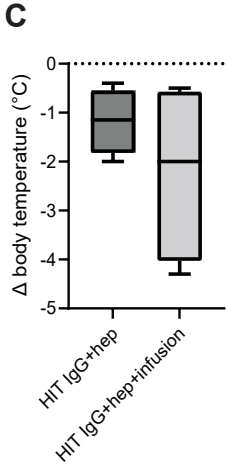
Supplementary Figure 3



Supplementary Figure 4







Supplementary Figure 5