Supplementary Material to Krauel, Schulze et al. “Further insights into the anti-PF4/heparin IgM immune response” (Thromb Haemost 2016; 115.3)

Suppl. Figure 1A
Suppl. Figure 1B

Suppl. Figure 1: Experimental setups for the CASP of splenectomised mice and their cellular consequences. (A) CASP was performed on the day of splenectomy (a.) or 6 days after splenectomy (b.) and 14 days after CASP serum was obtained and analysed by fluid-phase ELISA. (B) Following isolation of peritoneal cells from untreated mice (left) and mice 6 days after splenectomy (right) the cells were stained with anti-mouse B220-Pacific Blue, CD3ε-PerCP/Cy5.5, CD23-PE, CD21-FITC, CD5-Alexa Fluor 647 and CD11b-Brilliant Violet 605. Representative dot plots of B220+ B cells show percentages of follicular (Fo) B cells and B1 cells (upper row) as well as B1a and B1b cells (lower row; absolute number of B1a cells: Untreated mice, 3.6x10^5 vs. Splenectomy, 0.7x10^5; absolute number of B1b cells: Untreated mice, 2.2x10^5 vs. Splenectomy, 1.1x10^5).
Suppl. Figure 2A

![Graph showing comparison between wildtype mice and β/δ TCR−/− mice in terms of B220-Pacific Blue and CD3ε-PerCP/Cy5.5 levels for B cells and T cells.]

Suppl. Figure 2B

![Graph showing IgM Ab levels across different conditions: PF4, PF4/heparin, PF4/heparin + heparin, with TCR−/−, CASP conditions indicated.]

ns: not significant

*: significant difference
Suppl. Figure 2: β/δ TCR⁺ mice produce anti-PF4/heparin IgM in the absence of T cells.

(A) Following isolation of peripheral blood cells from wild-type mice (left) and β/δ TCR⁺ mice (right) the cells were stained with anti-mouse B220-Pacific Blue and anti-mouse CD3ε-PerCP/Cy5.5. Representative dot plots are shown. (B) β/δ TCR⁺ mice underwent CASP procedure (n=5) and serum was obtained at day 14. The binding of IgM to murine PF4/heparin complexes (squares) was evaluated by fluid-phase ELISA. Murine PF4 (circles) and murine PF4/heparin complexes in the presence of 100 IU/ml heparin (triangles) were used as controls for specificity. Data are ELISA OD units of individual sera and mean OD.

*p<0.05; ns: not significant