Supplemental Material

	SerpinB2+/+	**SerpinB2-/-**
RBC 10^{12}/L	9.7 ² 0.2	9.5 ² 0.7
HAEMOGLOBIN g/L	149.7 ² 2.1	145.3 ² 7.0
HAEMATOCRIT H L/L	0.8 ² 0.0	0.8 ² 0.1
MCV H fL	85.3 ² 1.2	86.7 ² 1.2
MCH pg	15.3 ² 0.6	15.3 ² 0.6
MCHC L g/L	180.3 ² 2.5	177.7 ² 7.1
PLATELET COUNT x10^9/L	**1165.7 ² 61.6**	**1103.7 ² 151.7**
WCC x10^9/L	8.2 ² 1.6	7.3 ² 1.3
NEUTROPHIL x10^9/L	1.3 ² 0.3	1.3 ² 0.5
LYMPHOCYTE x10^9/L	6.7 ² 1.4	5.8 ² 0.9
PLASMA PROTEIN g/L	74.3 ² 2.1	69.7 ² 4.9
Manual PCV	0.8 ² 0.0	0.73 ² 0.05

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Table S1. Haemotology of blood from SerpinB2$^{-/-}$ and SerpinB2$^{+/+}$ mice (pool of 3 mice for each mouse strain). Platelet count was not different (Bolded).

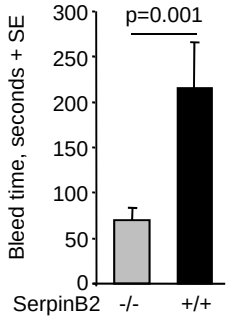


Fig. S1. Bleed time in males. As for Fig. 1A, except male mice were used (n=19-21).

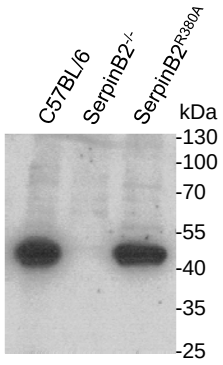


Fig. S2. SerpinB2^{R380A} protein expression. Immunoblotting of resident peritoneal macrophages (that constitutively express SerpinB2) with anti-murine-SerpinB2 antibody illustrating that expression of SerpinB2^{R380A} protein is not affected by this mutation.

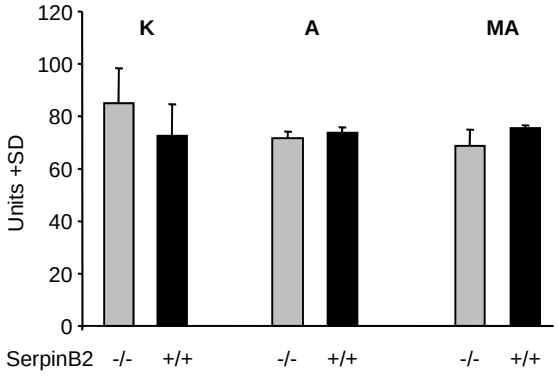


Fig. S3. Thromboelastography. No differences in the Kinetic time, alpha Angle or the Maximum Amplitude in SerpinB2$^{-/-}$ mice (R from this experiment is shown in Fig. 1C). (n=4 mice per group).

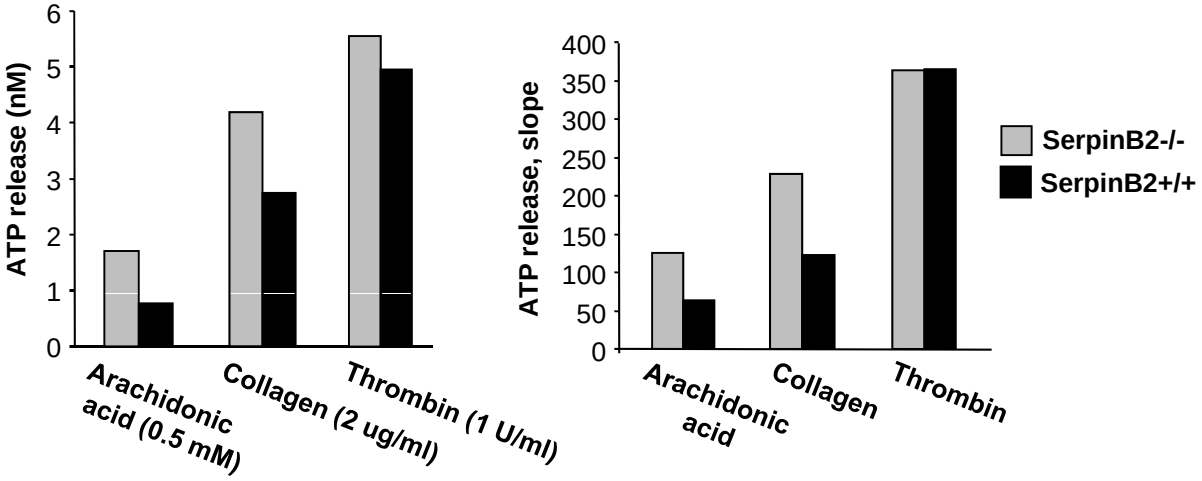


Fig. S4. Whole blood platelet lumi-aggregometery (Chrono-log Model 700, Royal Brisbane Hospital pathology services, Brisbane Qld. Australia). More and faster ATP release by platelets in blood from SerpinB2$^{-/-}$ mice after arachidonic acid and collagen, but not thrombin treatment (pooled blood from 6 mice per mouse strain) .

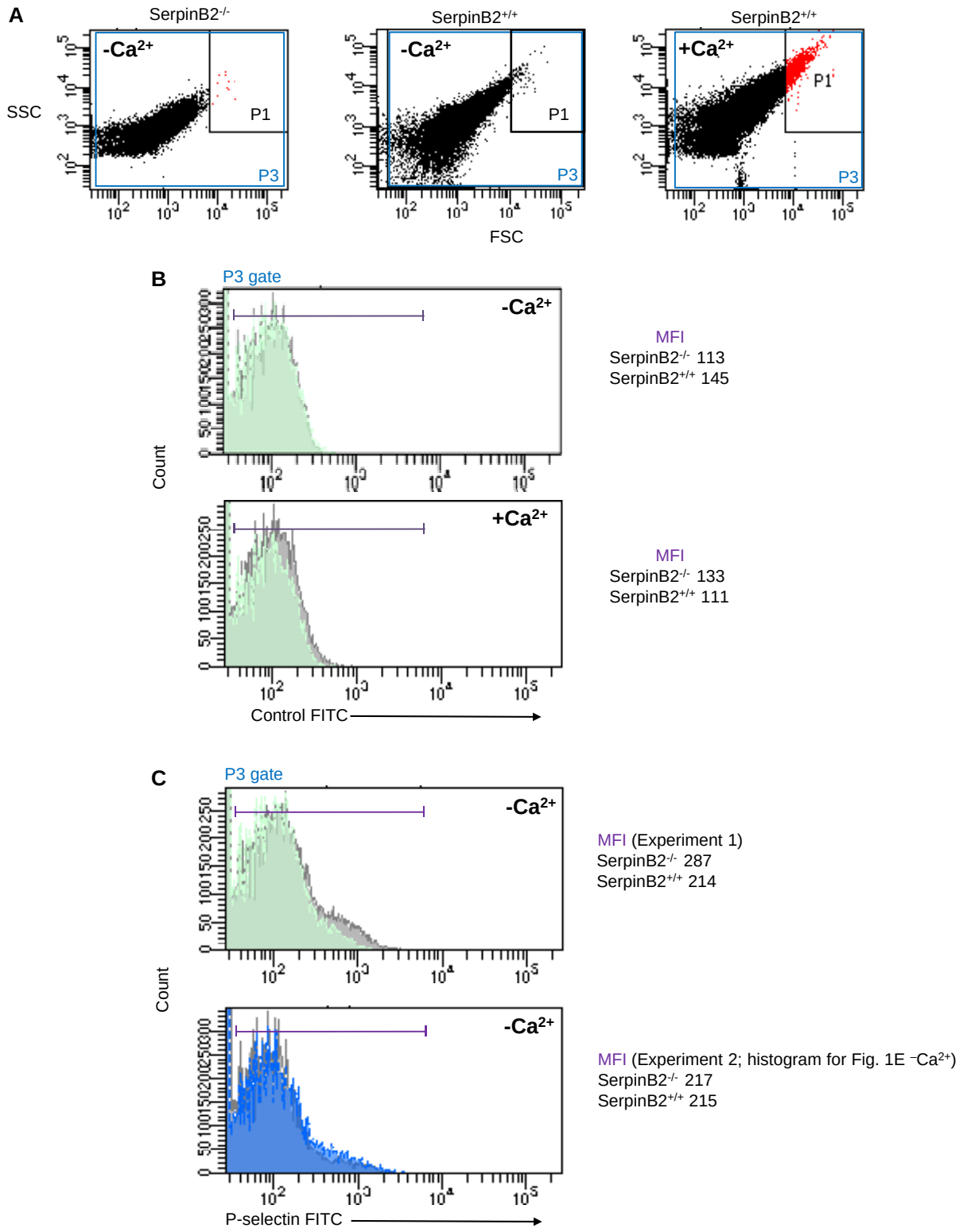


Fig. S5. FACs analysis of platelets from SerpinB2$^{-/-}$ and SerpinB2$^{+/+}$ mice (A) SerpinB2$^{-/-}$; FSC/SSC plot (same as Fig. 1E -Ca^{2+}) but showing P3 gate (blue), all events. SerpinB2$^{+/+}$; same as Fig 1E dot plots but for platelets from SerpinB2$^{+/+}$ mice. (B) Staining of platelets from SerpinB2$^{-/-}$ (grey) and SerpinB2$^{+/+}$ mice (green) with an isotype control FITC labelled antibody before (-Ca^{2+}) and after (+Ca^{2+}) re-calcification. Only events in P3 gate are shown and MFI was obtained from the indicated region (purple bar). (C) Staining of platelets from SerpinB2$^{-/-}$ (grey) and SerpinB2$^{+/+}$ mice (green or blue) with an anti-P selectin antibody before re-calcification (-Ca^{2+}).

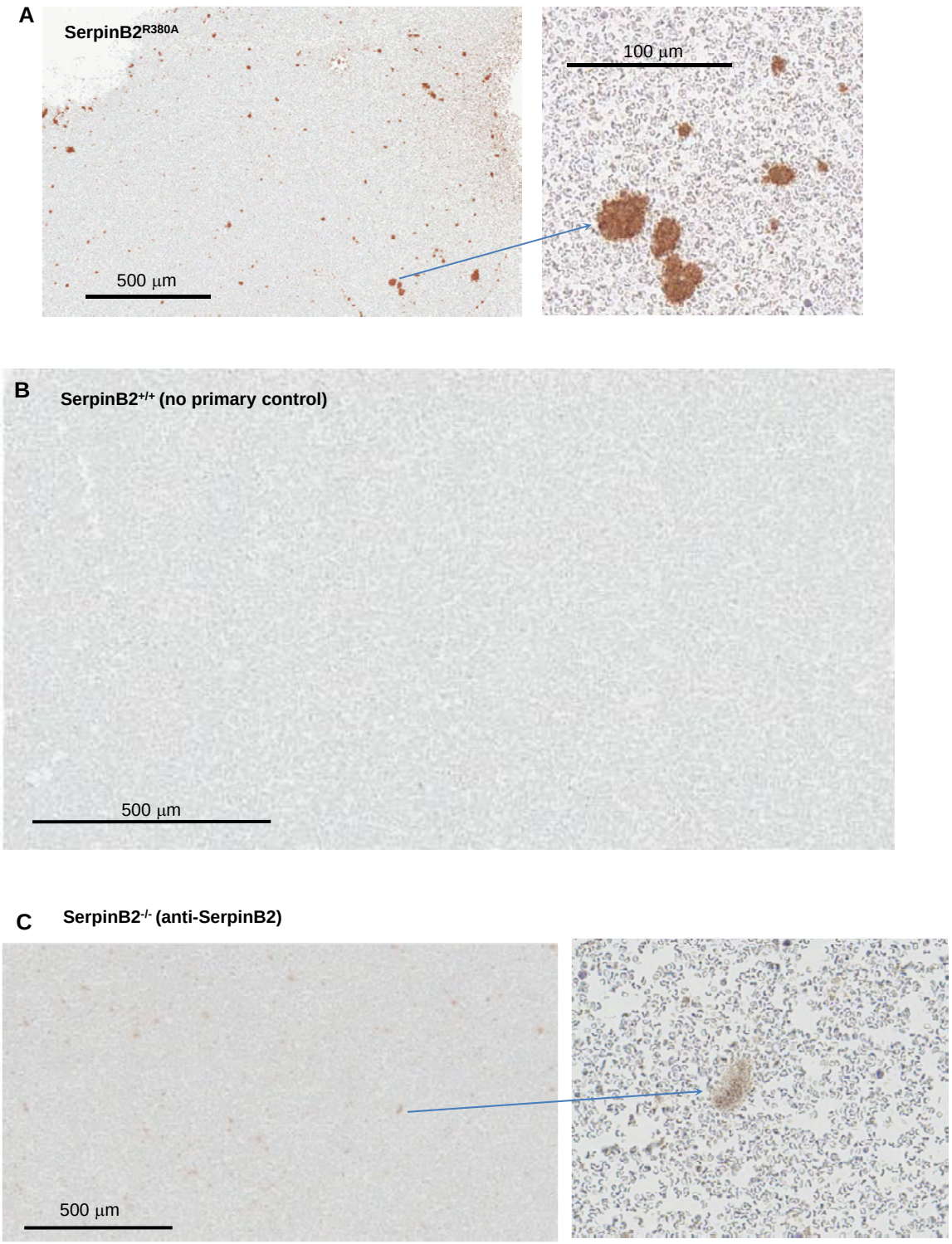
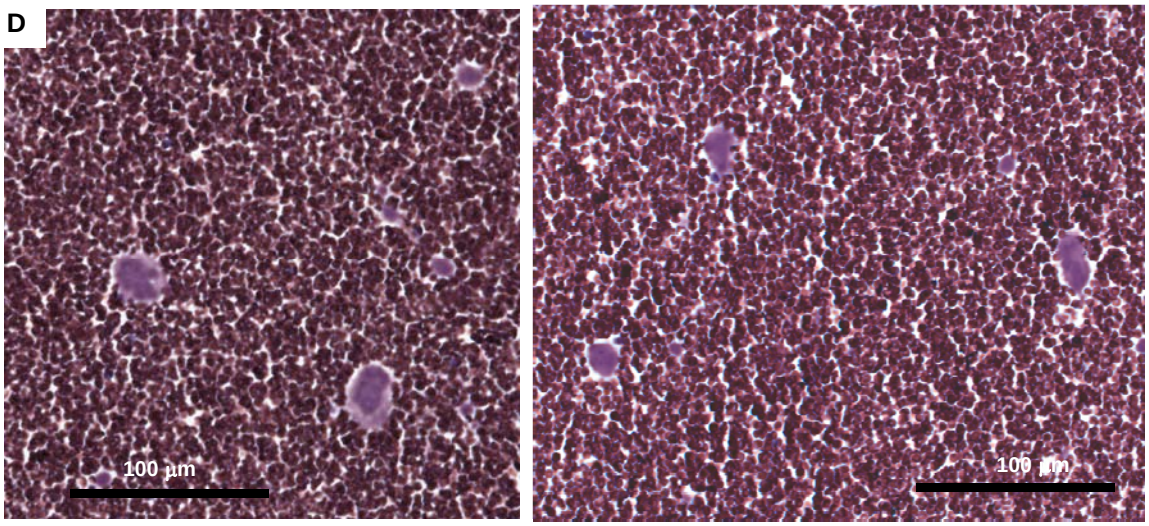


Fig. S6. IHC controls (A) IHC as shown in Fig. 2B top panel for C57BL/6 mice, but for SerpinB2^{R380A} mice. (B) Blood clot from SerpinB2+/+ mouse with no primary antibody. (C) Blood clot from SerpinB2-/- mouse stained as in Fig. 2B.



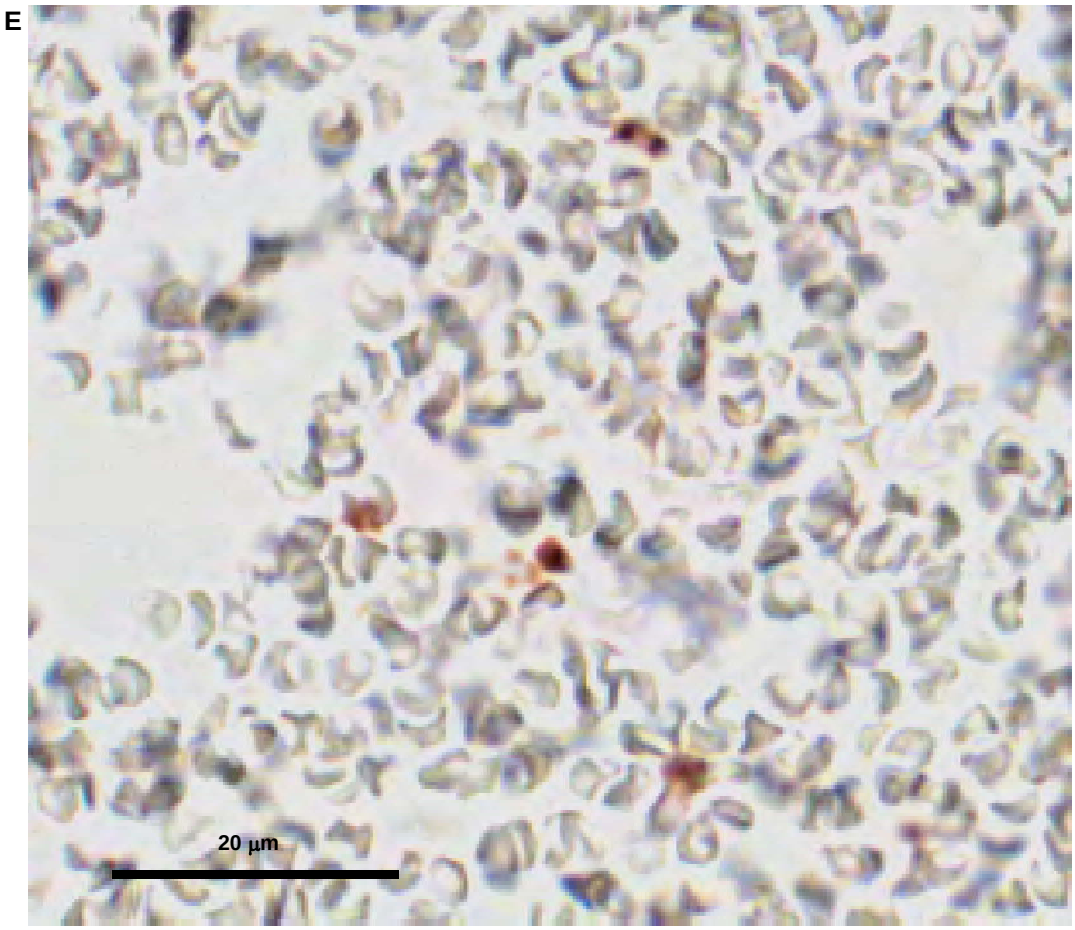


Fig. S6 continued (D) Wright-Giemsa staining of parrafin sections described in Fig. 2B. Platelets characteristically stain violet to purple, and red blood cells dark crimson. (E) IHC as in Fig 2B of blood from SerpinB2$^{+/+}$ mice using blood straight from a tail bleed into formaldehyde.

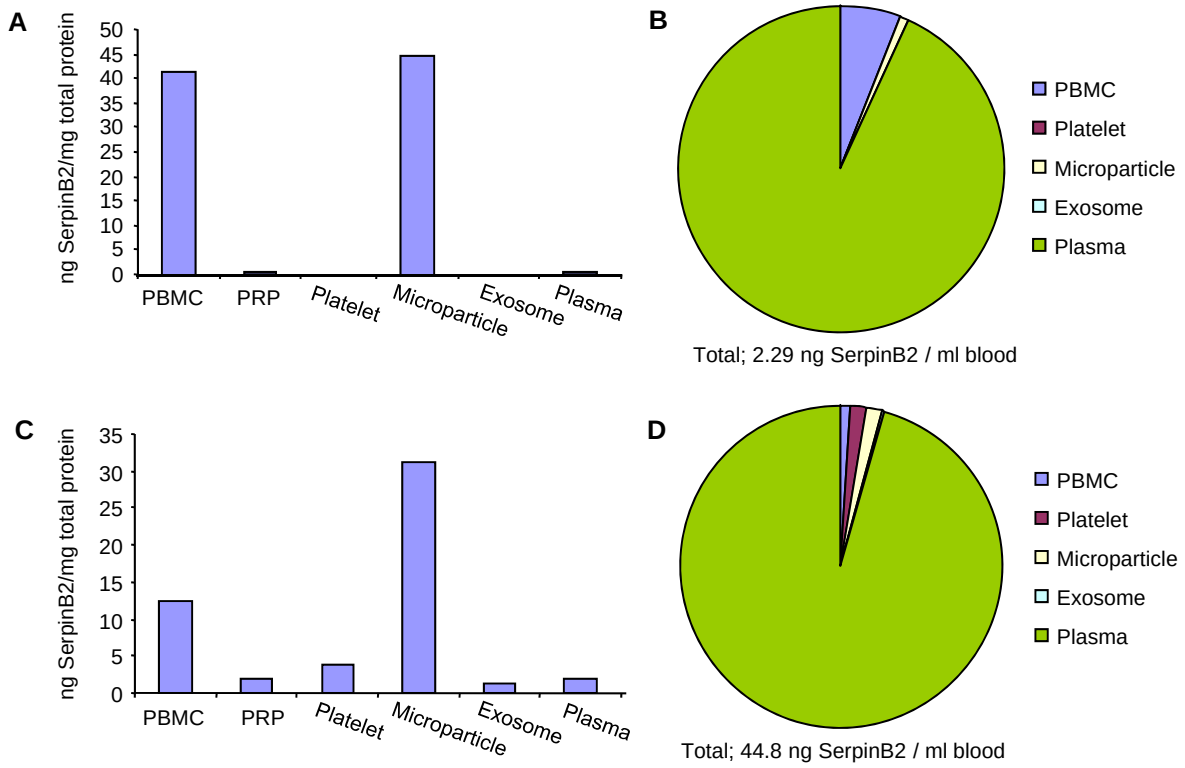


Fig. S7. SerpinB2 protein levels in human blood fractions as determined by quantitative ELISA (LifeSpan BioSciences Inc). (A) SerpinB2 levels in blood fractions from a deidentified healthy male expressed as ng of SerpinB2 protein per mg of total protein (Bradford Protein Assays, ThermoFisher). Plasma samples are taken after removal of all other fractions. (B) The same data as in A expressed as a percentage of total SerpinB2 levels in whole blood present in each fractions. (C, D) As for A and B, respectively, using blood from a deidentified pregnant woman. (Ethics approval HREC/08/QRBW/16; modification approval 21/3/2012; Human Research Ethics Committee, Royal Brisbane & Women's Hospital, Brisbane Australia).

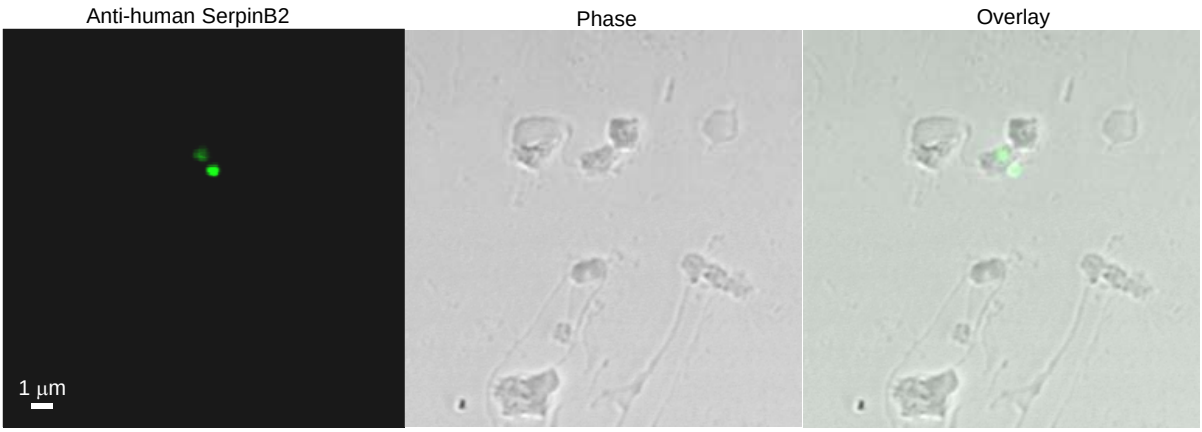


Fig. S8. Immunofluorescent microscopy of smears of platelet fractions from Fig. S7A fixed in 100% cold methanol and stained as described (Schroder et al 2014) using a mouse anti-human SerpinB2 antibody (American Diagnostica, #3750) and fluorescein isothiocyanate (FITC)-labelled anti-mouse secondary antibody (Chemicon).