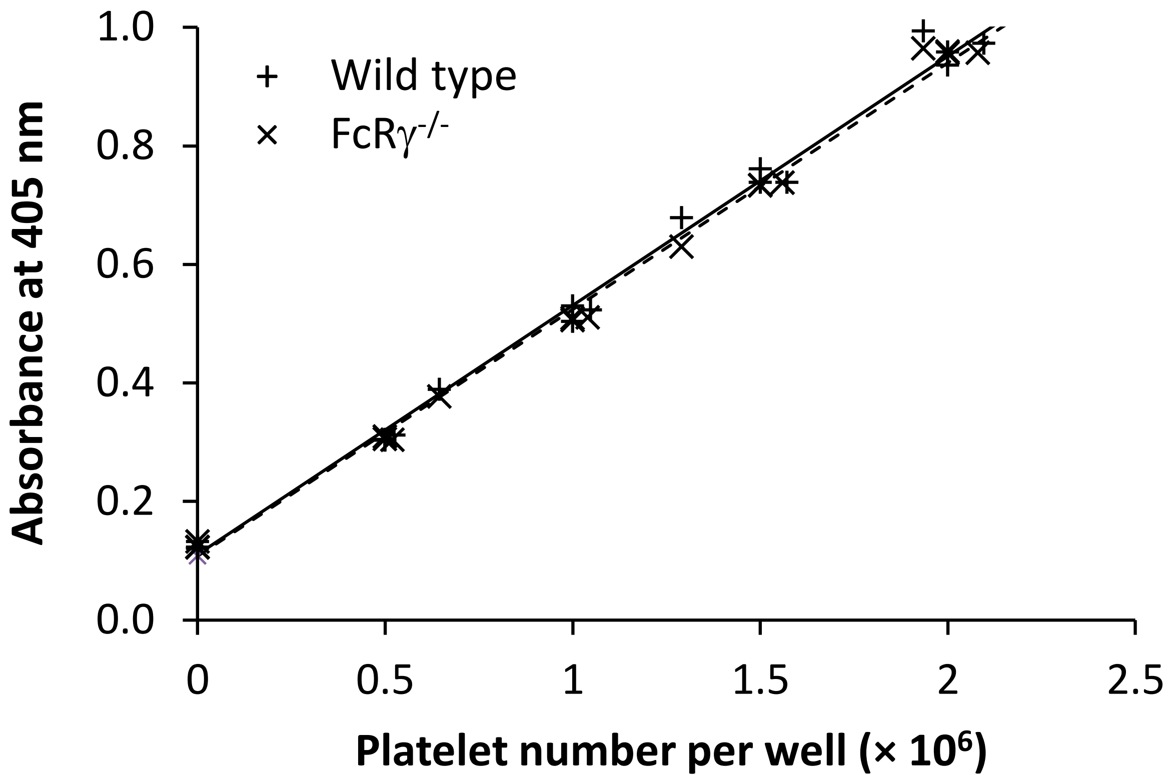
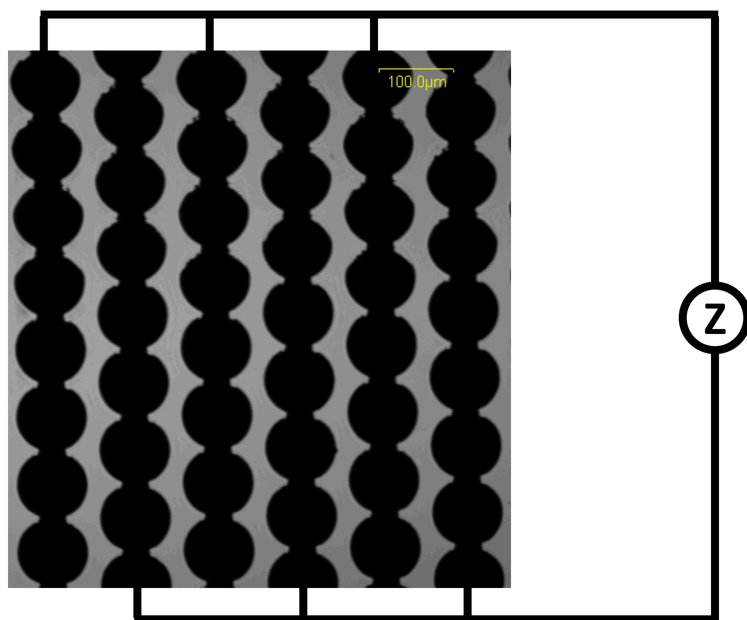


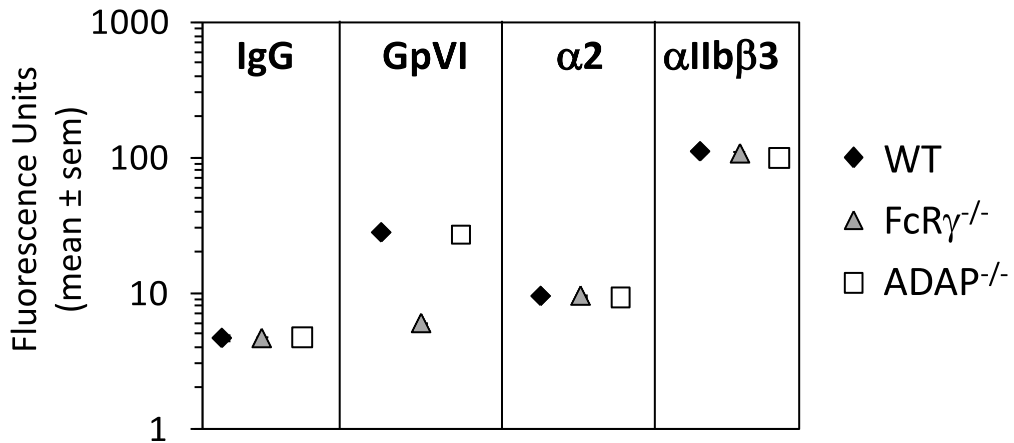
Supporting Fig. 1. Relationship between platelet number and absorbance values obtained in the static adhesion assay. A known number of platelets was added to each well followed by lysis buffer containing para-nitrophenol phosphate for one hour at room temperature (see Materials & Methods). The slopes of the linear regression lines are: Wild Type (solid line) = 0.420 abs. units per 10^6 platelets; FcR$\gamma^{-/-}$ (dashed line) = 0.416 abs. units per 10^6 platelets. Data are n=4 for each genotype.



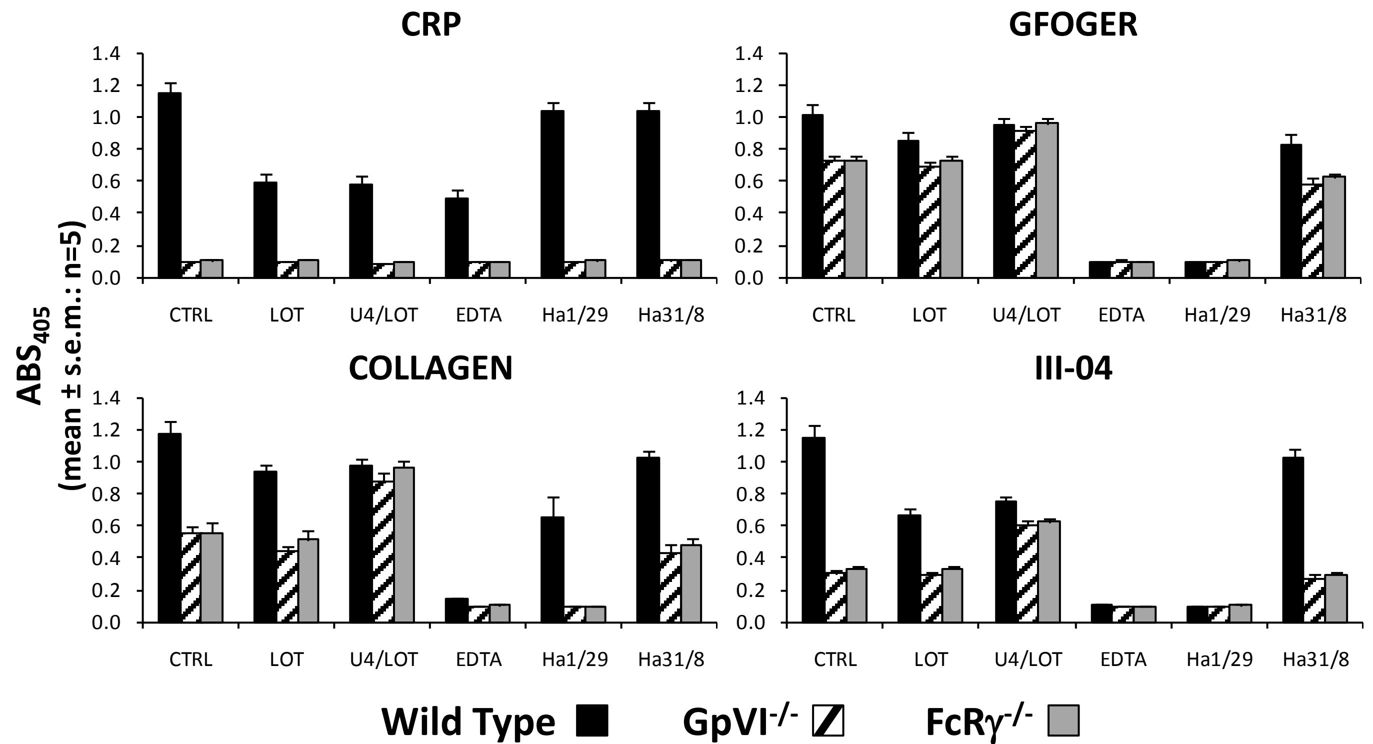
Supporting Fig. 2. Photograph of the microelectrode array on the base of an xCELLigence e-plate well. The microelectrode array comprises two interdigitating electrodes between which electrical impedance (Z) is measured. The gold electrodes cover approximately 80% of the surface of the well. Each of the individual electrode spots are approximately 90 µm in diameter. Non-activated murine platelets are 1-3 µm in diameter. As the platelets settle and spread on the base of the well this increases the impedance (Z). This is reported as a unitless Cell Index value. Firm attachment and spreading of individual platelets will vary depending on the coating ligand. As more platelets settle, attach and spread on the surface of the well this increases the Cell Index value.

Further details about the xCELLigence system and its applications can be found in the following publications:

- Keogh RJ, New Technology for investigating trophoblast function. *Placenta,* 2010;**31**:347-50
- Urcan E *et al.*, Real-time xCELLigence impedance analysis of the cytotoxicity of dental composite components on human gingival fibroblasts. *Dental Materials,* 2010;**26**:51-8

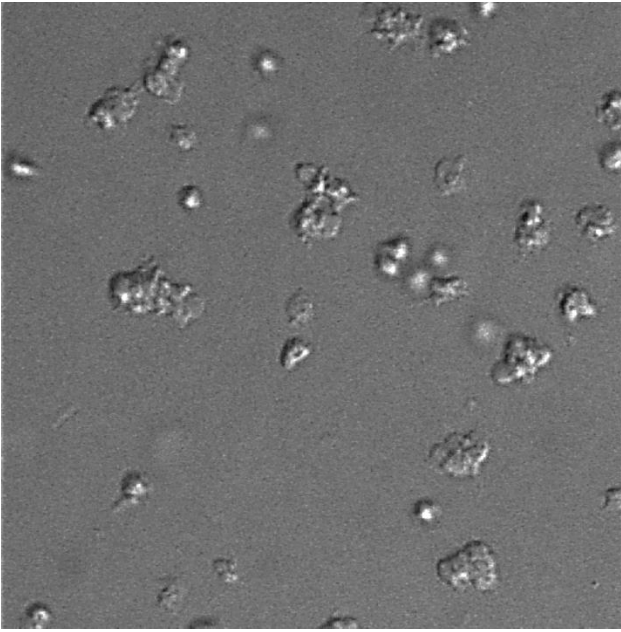


Supporting Fig. 3. Expression levels of GpVI, α2 and αIIbβ3. Levels of glycoproteins on washed platelets from WT, $FcR\gamma^{-/-}$ and $ADAP^{-/-}$ mice were determined using flow cytometry. FITC-labelled primary antibodies were used. IgG is a using a FITC-labelled negative control IgG antibody. Data are the mean ± sem: n=7 (Error bars lie within the symbols).

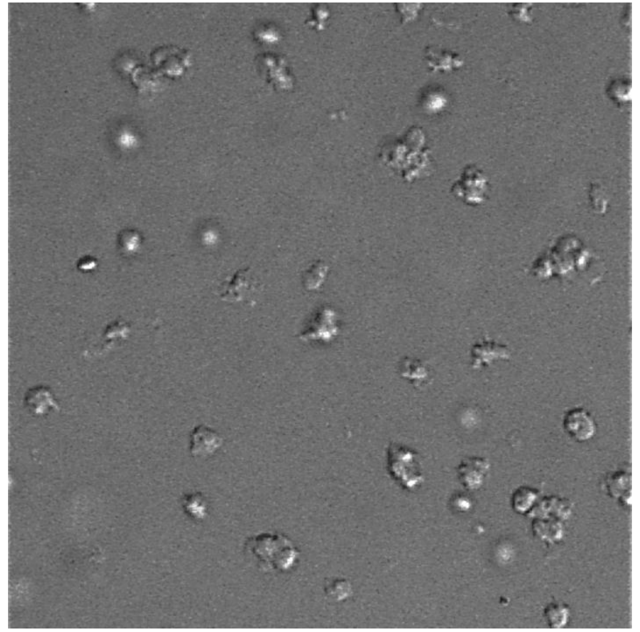


Supporting Fig. 4. Static Adhesion of WT, GpVI$^{-/-}$ and FcR$\gamma^{-/-}$ platelets to CRP, GFOGER, collagen or III-04. Platelets were either untreated (CTRL) or pre-treated with lotrafiban (LOT: 10µM), U46619 (U4: 2µM), EDTA (2mM), Ha1/29 (2µg/ml) and Ha31/8 (2µg/ml). Platelets incubated in wells coated with BSA or GPP showed absorbance levels equal to those with FcR$\gamma^{-/-}$ platelets on CRP.

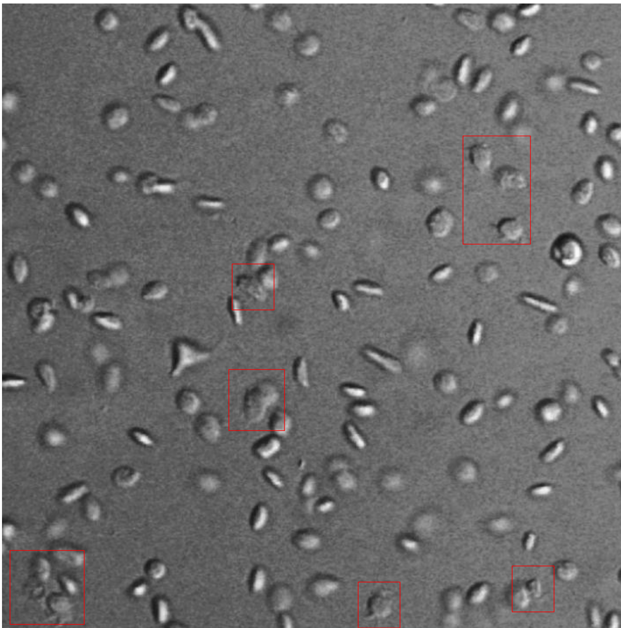
Video 5A: WT & CRP



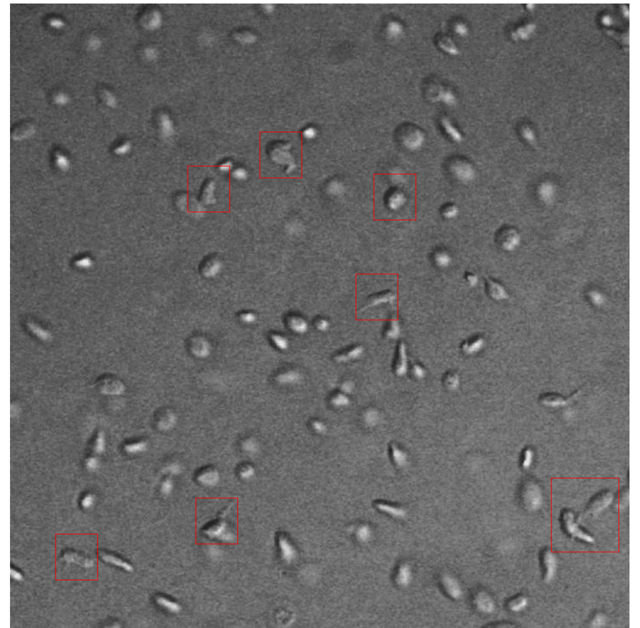
Video 5B: ADAP$^{-/-}$ & CRP



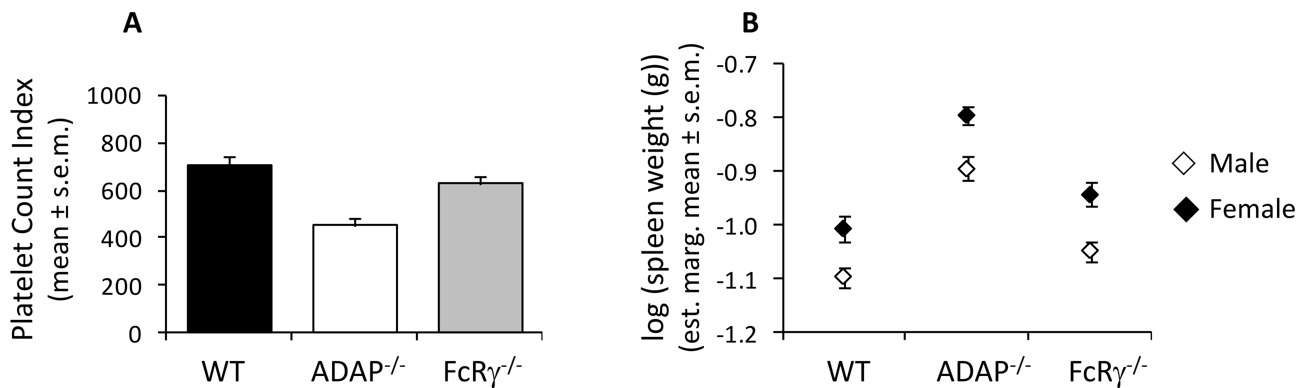
Video 5C: WT & GFOGER



Video 5D: ADAP$^{-/-}$ & GFOGER

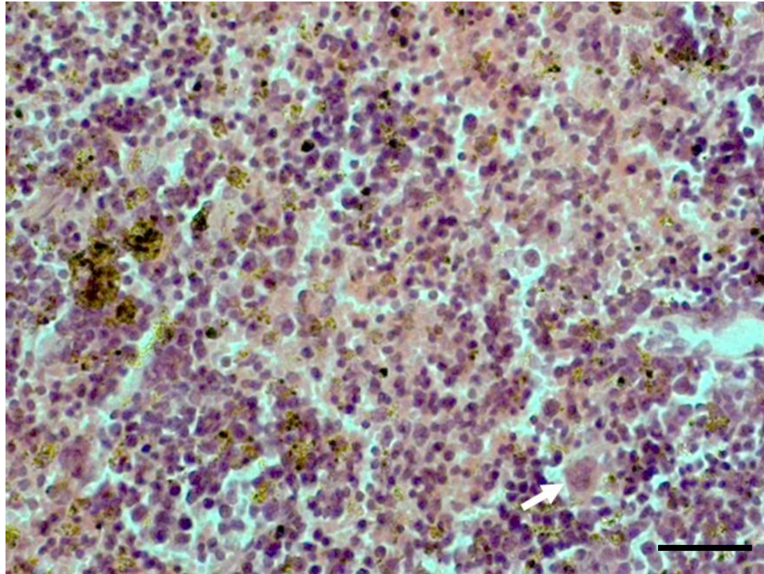


Supporting Fig. 5. Videos of WT (A&C) and ADAP$^{-/-}$ (B&D) platelets adhering to CRP (A&B) and GFOGER (C&D). Initial studies suggested that WT platelets produced more filopodia and were more active on GFOGER than ADAP$^{-/-}$ platelets. No clear difference was observed on CRP. Subsequent studies were blinded. Based on the initial findings, platelets from a further 3 WT and 3 ADAP$^{-/-}$ mice were all correctly identified on GFOGER (1-sided probability = 5%). With CRP, 2/8 samples from 4 WT and 4 ADAP$^{-/-}$ were correctly identified. Platelets were clearly more reactive on CRP than on GFOGER. On CRP they produced mini-aggregates and highly motile lamellipodia. On GFOGER, WT platelets produced more filopodia: their edges were more motile than ADAP$^{-/-}$ platelets. Red boxes indicate areas of particular interest on the videos. Each video represents 15 minutes in real-time. Each field of view is 80 × 80 µm.

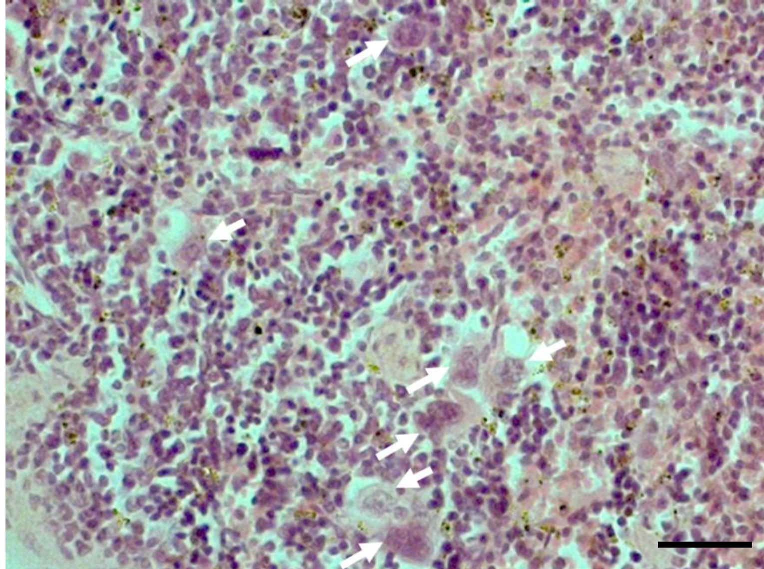


Supporting Fig. 6. Thrombocytopenia and splenomegaly in ADAP$^{-/-}$ mice. (A) For each preparation of washed platelets, a Platelet Count Index (PCI = (Platelet count (10^6/ml) × Volume (ml))/number of mice), indicating the number of platelets drawn from the mice, was calculated. PCIs from WT and FcR$\gamma^{-/-}$ were not significantly different. The ADAP$^{-/-}$ PCI was 60-70% of that observed in WT and FcR$\gamma^{-/-}$ mice. This difference was statistically significant (ANOVA, Waller-Duncan post-hoc test; N values: WT=32, ADAP$^{-/-}$=32, FcR$\gamma^{-/-}$=25). (B) Spleens were collected from mice over 10 weeks old of known sex, age, genotype and weight. The log(spleen weight (g)) was analysed using ANCOVA. Age and weight were treated as covariates. Sex and genotype were fixed factors. Female spleens were 26% heavier than male (P<0.0001). ADAP$^{-/-}$ spleens were 61% heavier than WT (P<0.0001) and 42% heavier than FcR$\gamma^{-/-}$ spleens (P<0.0001). FcR$\gamma^{-/-}$ spleens were 14% heavier than WT (P=0.011). Mouse weight was not a significant covariate (P=0.076); however, age was: spleen weight increased with age (P<0.0001). Values are the estimated marginal mean ± sem derived in SPSS. These represent predicted means with covariates weight and age held at their mean values. (N values: WT male=32; WT female=30; ADAP$^{-/-}$ male=30; ADAP$^{-/-}$ female=36; FcR$\gamma^{-/-}$ male=31; FcR$\gamma^{-/-}$ female=29.)

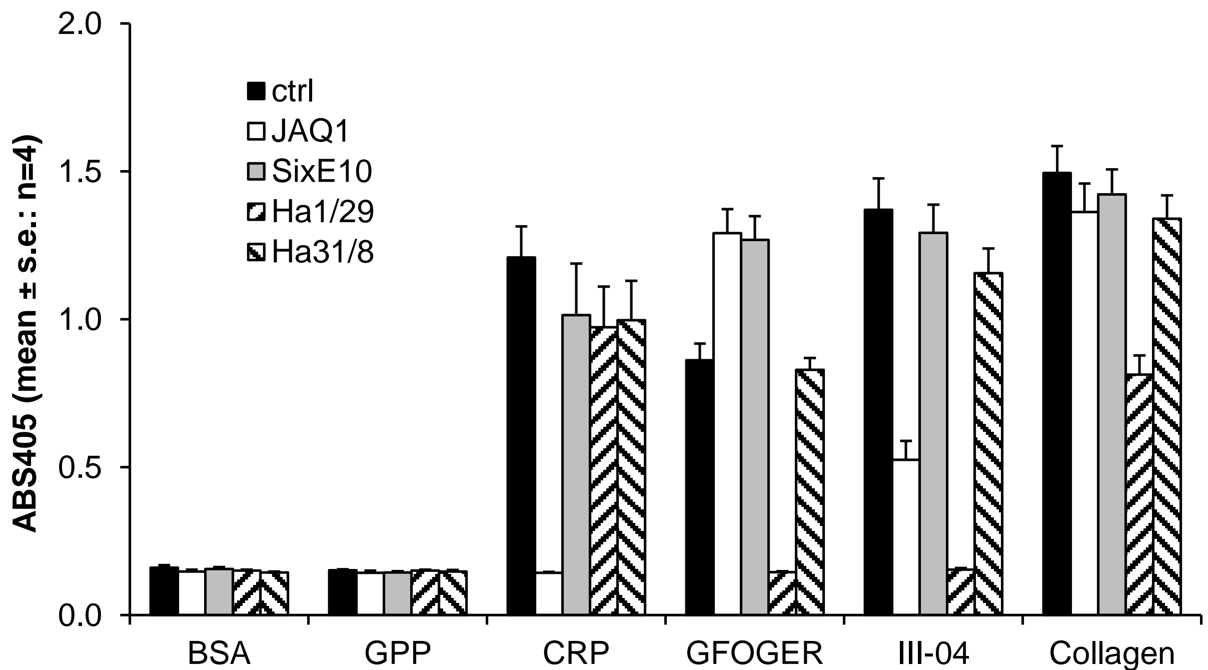
A: WT



B: ADAP



Supporting Fig. 7. Histological sections of WT and ADAP$^{-/-}$ spleens. WT (A) and ADAP$^{-/-}$ (B) spleens were fixed with 3.6% formaldehyde, sectioned, mounted and stained with haematoxylin and eosin. Images were obtained with a 40x objective (scale bar = 20µm). Megakaryocytes are indicated by white arrows. Histological examination revealed that WT spleens had 5.5 ± 0.6 (mean ± sem; n = 4) megakaryocytes per 10 high-power fields (0.0225 mm^2) whereas ADAP$^{-/-}$ spleens had 25.3 ± 5.6 (mean ± sem: n = 4), (P=0.038, Student's *t*-test).



Supporting Fig. 8. Effect of anti-murine GpVI antibodies JAQ1 (10 µg/ml) and Six.E10 (10 µg/ml) on adhesion of wild type mouse platelets to collagen peptides. JAQ1 abolished adhesion to CRP whereas Six.E10, which does not bind to the collagen-binding locus on GpVI, did not. JAQ1 had a partial inhibitory effect on adhesion to III-04 which was less than that observed in FcR$\gamma^{-/-}$ platelets (see main manuscript Fig. 4). Both JAQ1 and Six.E10 increased adhesion to GFOGER. This is likely to be due to a low level of activation of GpVI by the limited cross-linking of the receptor by these antibodies. This is sufficient to maximise binding to GFOGER via $\alpha2\beta1$.