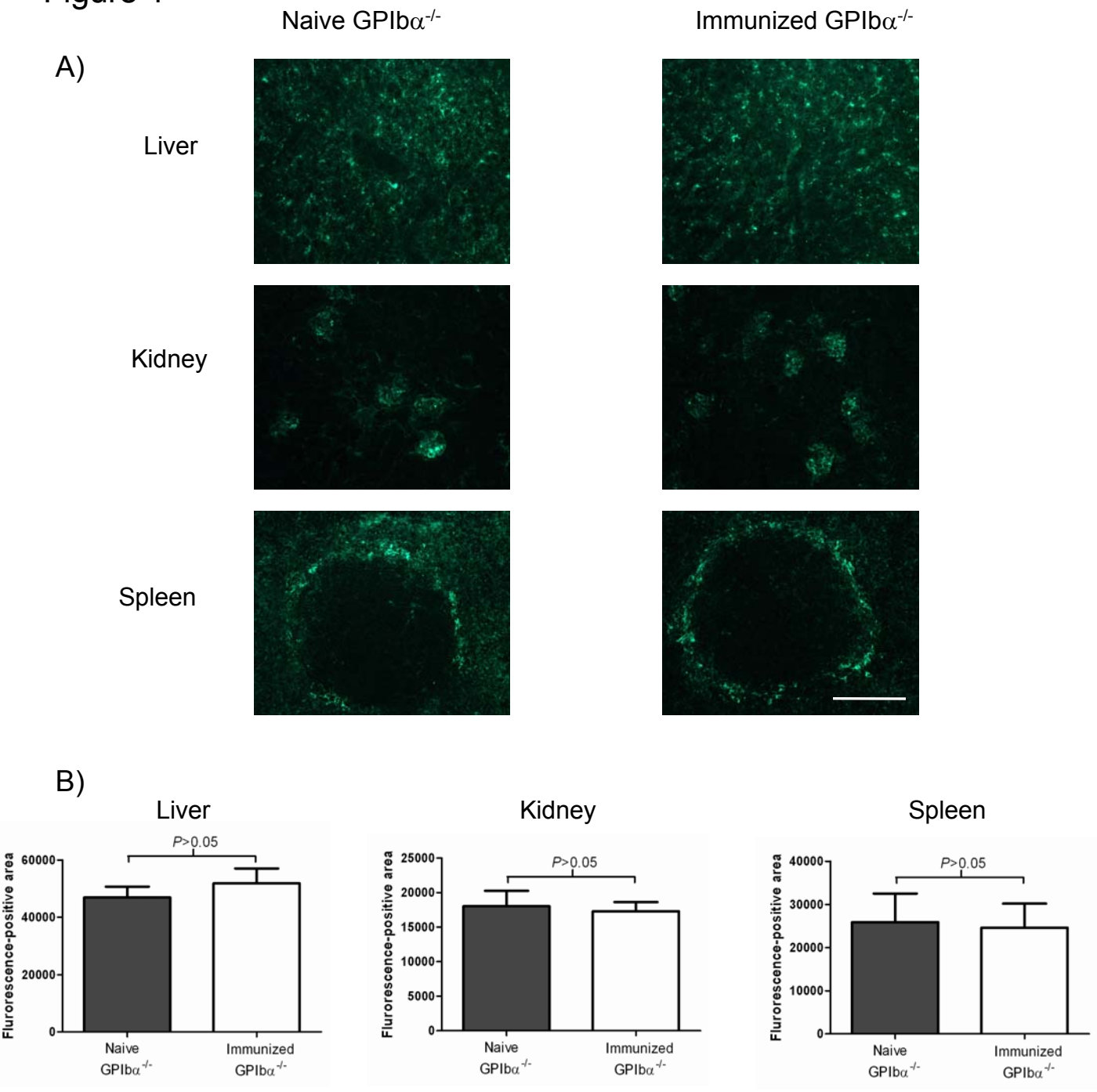
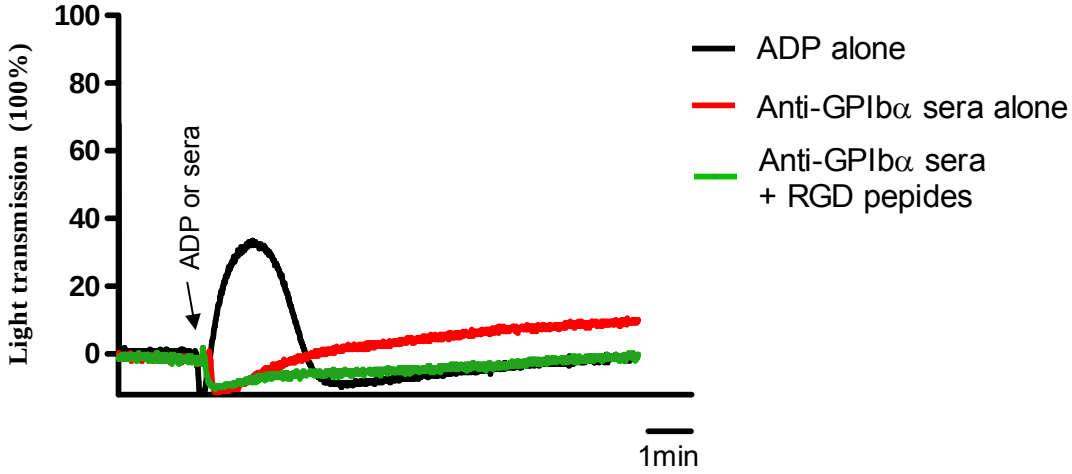
Supplementary Figure 1



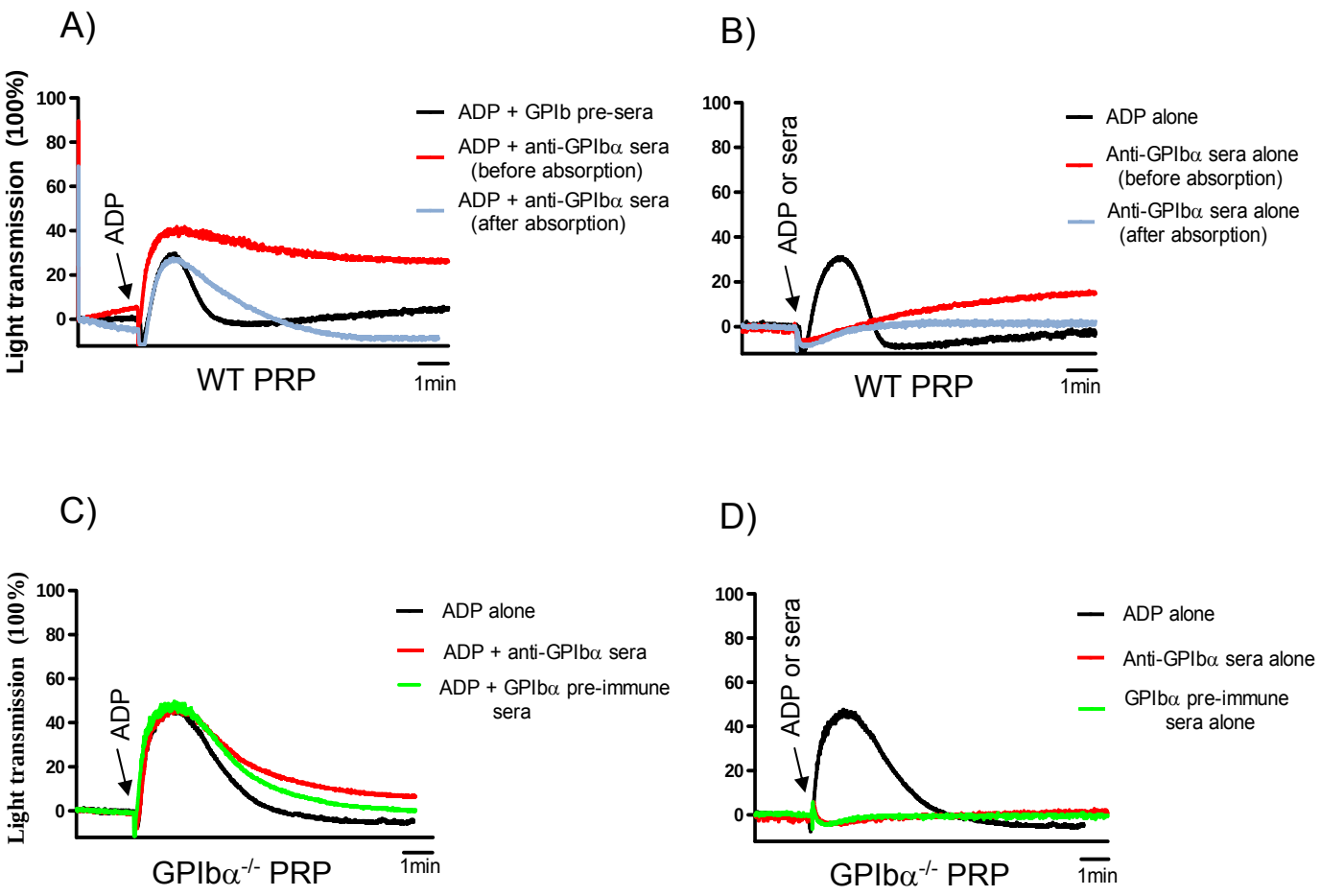
Supplementary Figure 1. Comparable FITC-dextran infusion into liver, kidney and spleen organs were found between naïve and immunized pregnant GPIbα$^{-/-}$ mice. A): FITC-dextran was infused into 15.5 days post-coitum pregnant GPIbα$^{-/-}$ mice. Representative pictures of FITC-dextran infusion into liver, kidney and spleen (scale: 200μm). **B):** Quantitative analysis of the fluorescence-positive area at the liver, kidney and spleen, suggested that the blood supply into these organs was not significantly different between naïve and immunized pregnant GPIbα$^{-/-}$ mice (*P*>0.05, n= 4-6 mice per group).

Supplementary
Figure 2



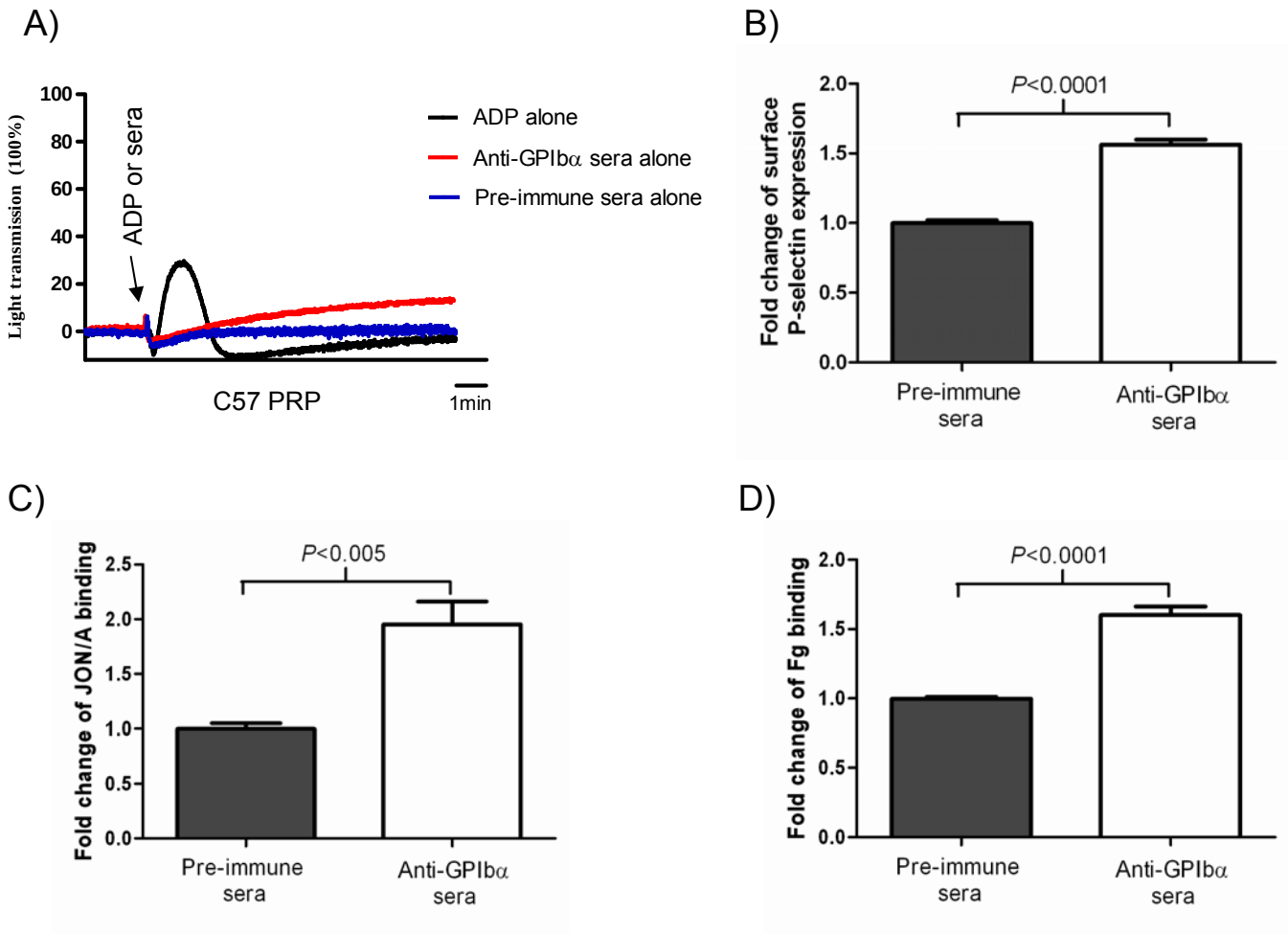
Supplementary Figure 2. RGD peptides inhibited anti-GPIbα induced wild-type platelet aggregation. Wild-type (WT) platelet-rich plasma (PRP) was pre-incubated with or without RGD peptides (final concentration: 2mg/mL) for 10 min, and platelet aggregation was induced by adding anti-GPIbα sera at 2min; ADP (1μM)-induced aggregation was used as control. The WT platelet aggregation induced by anti-GPIbα sera could be inhibited by RGD peptides. The result is representative of three independent experiments.

Supplementary Figure 3



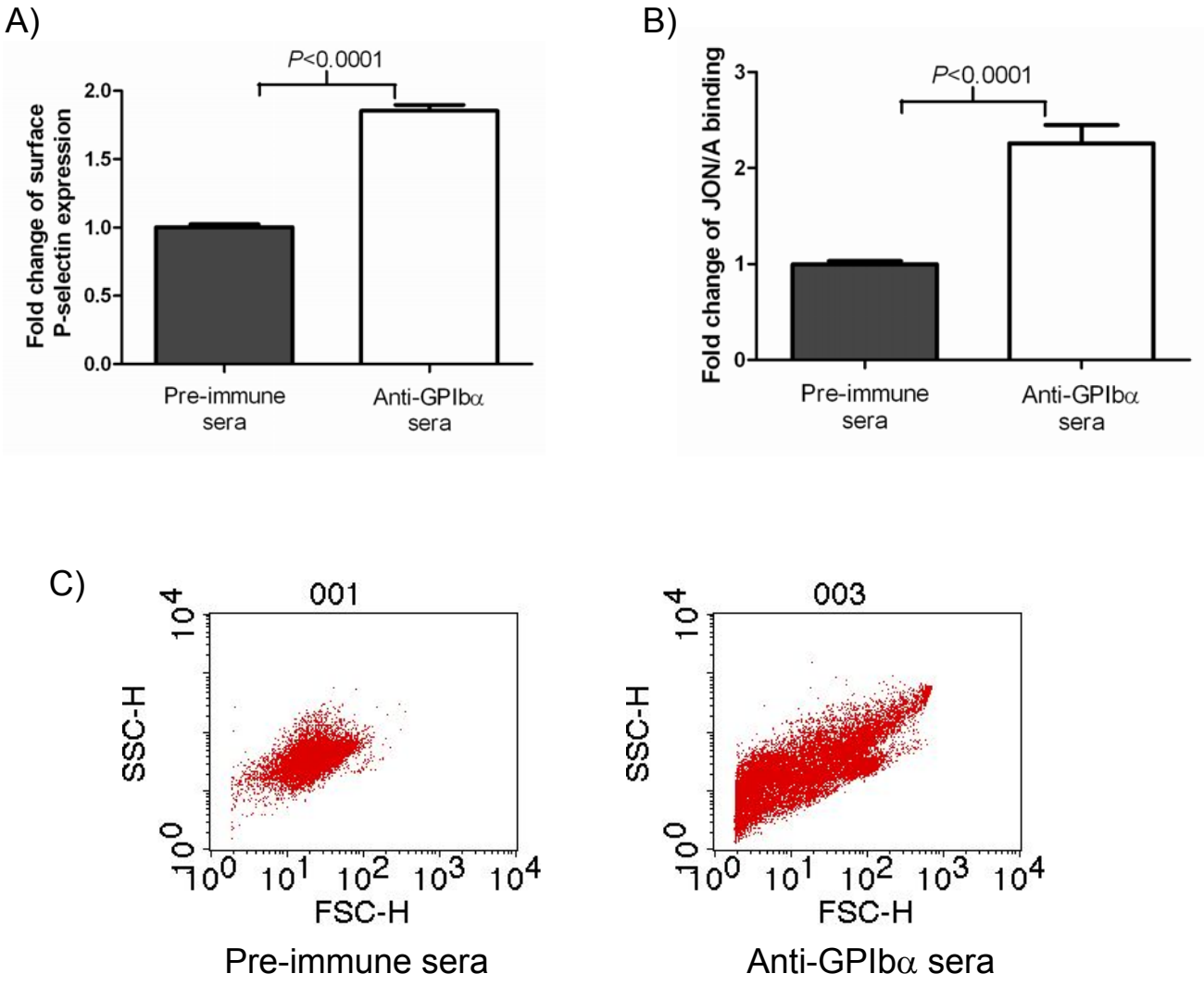
Supplementary Figure 3. Anti-GPIbα antisera specifically caused antigen-positive platelet activation in vitro. To remove the anti-GPIbα antibodies from anti-GPIbα sera, anti-GPIbα sera were incubated with excessive WT platelets. **A):** Anti-GPIbα sera or pre-absorbed anti-GPIbα sera were incubated with WT platelet-rich plasma (PRP) prior to induction of platelet aggregation with ADP (2μM). In contrast to anti-GPIbα sera, the pre-absorbed anti-GPIbα sera failed to enhance ADP-induced platelet aggregation. **B):** Anti-GPIbα or pre-absorbed anti-GPIbα sera alone were added to WT PRP. ADP-induced aggregation was used as control. Contrary to anti-GPIbα sera, the pre-absorbed anti-GPIbα sera failed to induce WT platelet aggregation in the absence of agonist. **C):** Polyclonal anti-GPIbα or pre-immune sera were incubated with GPIbα$^{-/-}$ PRP prior to induction of platelet aggregation with ADP (1μM). Anti-GPIbα sera failed to enhance ADP-induced GPIbα$^{-/-}$ platelet aggregation. **D):** Anti-GPIbα or pre-immune sera alone (i.e. without ADP) were added to GPIbα$^{-/-}$ PRP. ADP-induced aggregation was used as control. Anti-GPIbα sera alone failed to induce GPIbα$^{-/-}$ platelet aggregation.

Supplementary
Figure 4



Supplementary Figure 4. Anti-GPIbα antisera induce C57BL/6J WT platelet aggregation and P-selectin expression, and enhance JON/A binding and fibrinogen binding. A): Polyclonal anti-GPIbα or pre-immune sera alone (i.e. without ADP) were added to C57BL/6J WT PRP; ADP (0.5μM)-induced aggregation was used as control. Anti-GPIbα, but not pre-immune, sera were able to induce platelet aggregation in C57BL/6J WT PRP in the absence of soluble agonist. **B-D):** Polyclonal anti-GPIbα or pre-immune sera were incubated with the gel-filtered C57BL/6J WT platelets, and then stained by FITC-labeled anti-P-selectin antibody, PE-labeled JON/A antibody (specifically recognizing the active form of β3 integrin), or Alexa Fluor 488-labeled fibrinogen (Fg), respectively. Compared to pre-immune sera, anti-GPIbα polyclonal sera-treated C57BL/6J WT platelets exhibited significantly more P-selectin expression, JON/A binding and Fg binding (P<0.0001) (n= 3-4 mice per group).

Supplementary
Figure 5



Supplementary Figure 5. Platelets from anti-GPIbα-injected mice exhibited significantly higher P-selectin expression and JON/A binding, and tended to form micro-aggregates. Polyclonal anti-GPIbα or pre-immune sera (100μl per 20g mouse) were injected into wild-type (WT) mice. Platelets were then purified from these injected mice twenty-four hours later. **A-B):** The purified platelets were stained by FITC-labeled anti-P-selectin antibody, or PE-labeled JON/A antibody (specifically recognizing the active form of β3 integrin), respectively. Compared to pre-immune sera injection, WT platelets from anti-GPIbα-injected mice exhibited significantly more P-selectin expression and JON/A binding (P<0.0001) (n=3-4 mice per group). **C):** Representative flow cytometry dot plot diagram of purified platelets from anti-GPIbα or pre-immune sera injected WT mice. The platelets from anti-GPIbα-injected mice tend to form micro-aggregates. Results of **A)-C)** are representative of three independent experiments.