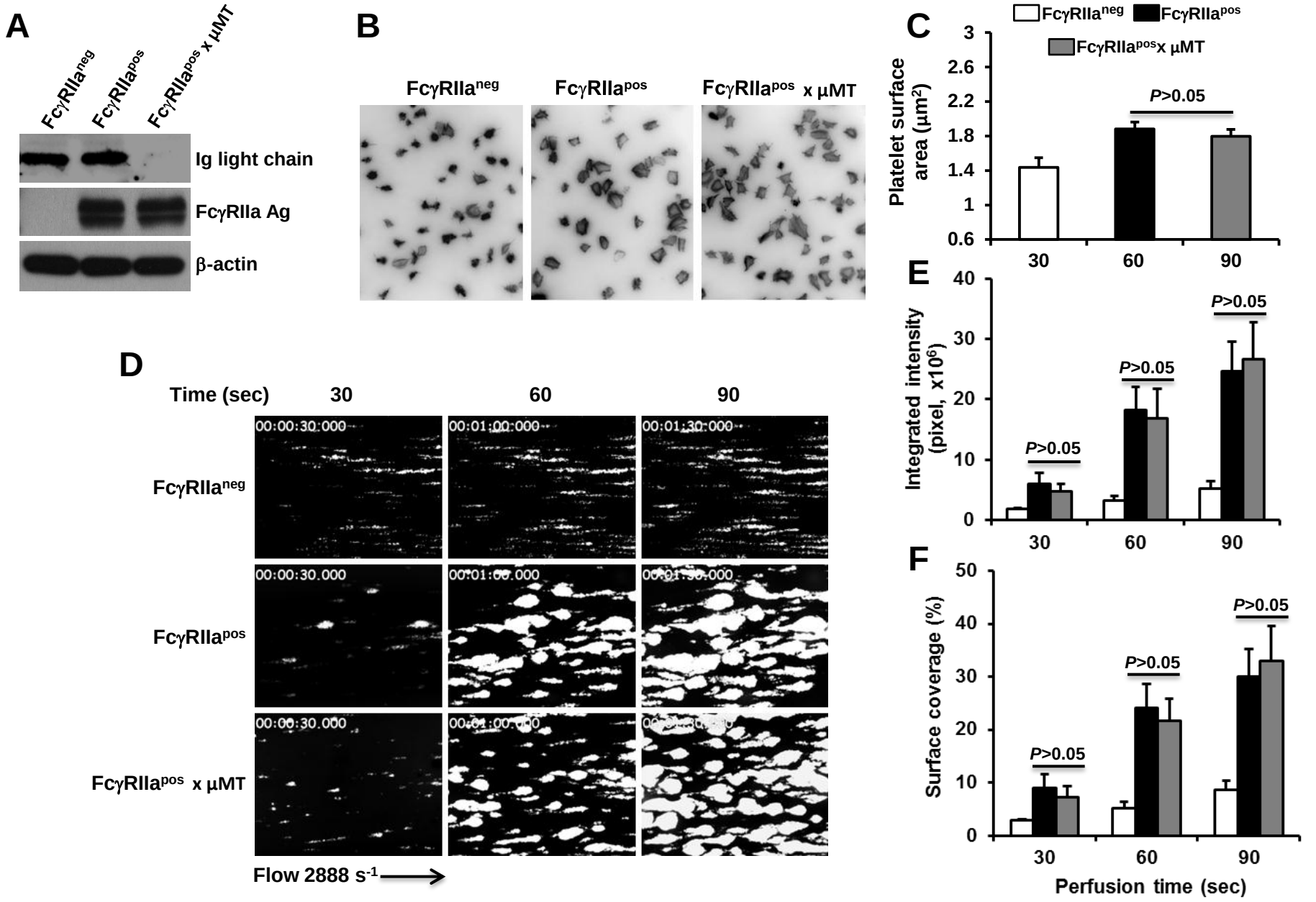
Supplemental Figure 1 – IgG does not contribute to the enhancement conferred by FcγRIIa on αIIbβ3-mediated outside-in signaling. (A) Characteristics of IgG depletion FcγRIIa^{pos} x μMT mice. Western blot analysis of FcγRIIa and mouse Ig protein expression in mice platelet. Note that FcγRIIa^{pos} x μMT mice platelets are depleted of IgG, while expressing comparable level of FcγRIIa as FcγRIIa^{pos} mouse platelets. **(B, C)** The absence of IgG does not affect the enhancement conferred by FcγRIIa on αIIbβ3-mediated outside-in signaling initiated by platelet adhesion to immobilized fibrinogen. Washed platelets from FcγRIIa^{neg}, FcγRIIa^{pos} and FcγRIIa^{pos} x μMT mice were plated on BSA or fibrinogen-coated coverslips in the presence of apyrase (0.25 units/mL) and indomethacin (10 μM) and allowed to spread for 45 minutes. After spreading, platelets were fixed, permeabilized and stained with rhodamine-phalloidin to visualize F-actin. Representative images of 3 independent experiments **(B)**. Quantification of platelet surface area (mean μm^2 ± SEM of at least 200 platelets) upon spreading using Metamorph software **(C)**. Note that the spreading of FcγRIIa^{pos} x μMT platelets was indistinguishable from that of FcγRIIa^{pos} platelets**. (D, E, F)** IgG does not affect the amplifying effect of FcγRIIa on thrombus formation. Laminar flow chambers were coated with 50 μg/mL of type I fibrillar collagen. Whole blood from FcγRIIa^{neg}, FcγRIIa^{pos} and FcγRIIa^{pos} x μMT mice was perfused under conditions of arterial (2888^{s-1}). Images of platelet adhesion and accumulation were acquired using epifluorescence microscopy in real-time at a rate of one frame per second. Representative time-course images of platelet adhesion and accumulation on collagen **(D)**. Quantification of platelet thrombi was performed using MetaMorph. Results are expressed as total integrated fluorescence intensity **(E)** mean±SEM (n=5 per group) or % surface coverage **(F)** and statistically significant differences between the means were determined using Student's t-test. Note that thrombus formation was significantly increased ($P<0.05$) for FcγRIIa^{pos} and FcγRIIa^{pos} x μMT platelets compared to wild-

type FcγRIIa^{neg} murine counterparts, there is no difference between FcγRIIa^{pos} and FcγRIIa^{pos} x μMT (P>0.05).

Supplemental Figure 2 - FcγRIIa does not amplify signaling induced by the simple binding of soluble fibrinogen to aIIbβ3. Washed mouse platelets were stimulated with 3 uM ADP in the presence of 300 ug/ml of soluble fibrinogen, but without stirring. Note that both Src and Syk become tyrosine phosphorylated under conditions of receptor activation and fibrinogen binding. In the absence stirring however, which would promote (1) multivalent interactions of fibrinogen with the platelet surface, (2) αIIbβ3 cross-linking, and (3) transactivation of integrin-associated Src-family kinases, expression of FcγRIIa does not become activated, and therefore is incapable of amplifying signal transduction downstream of ligand binding (compare with **Figure 1C and E**, where platelets are allowed to interact with immobilized fibrinogen).

Supplemental Figure 1



Supplemental Figure 2