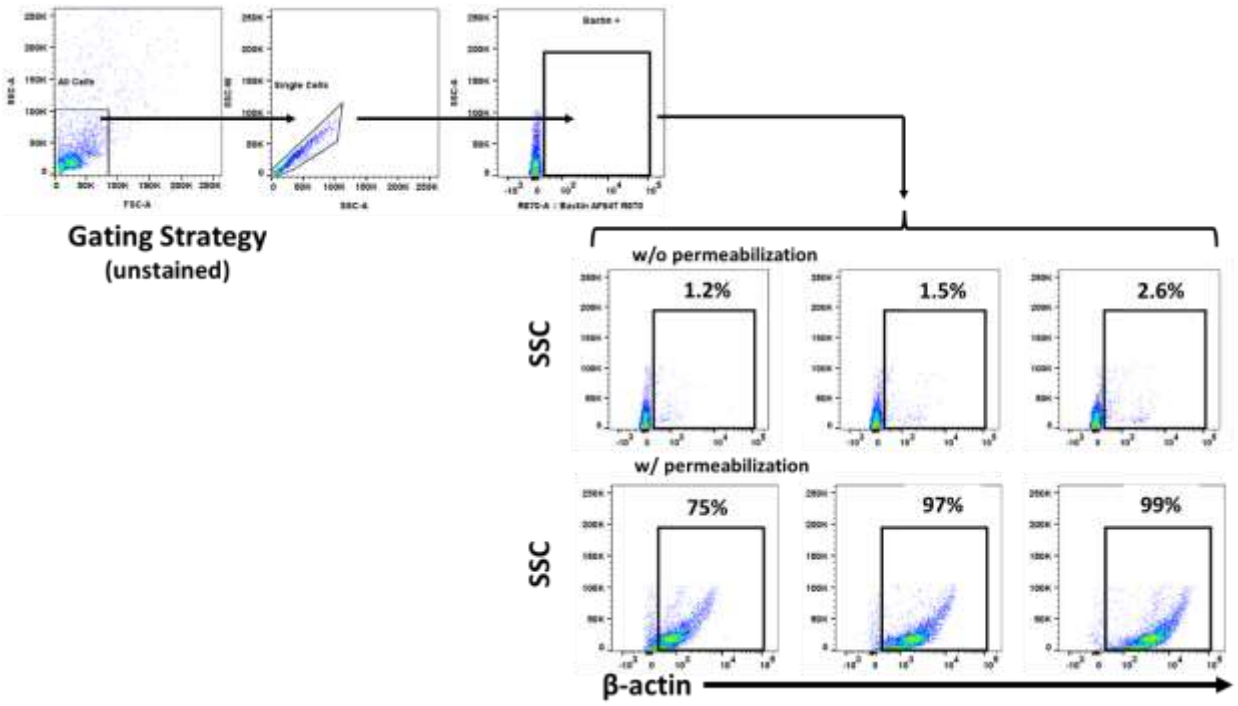
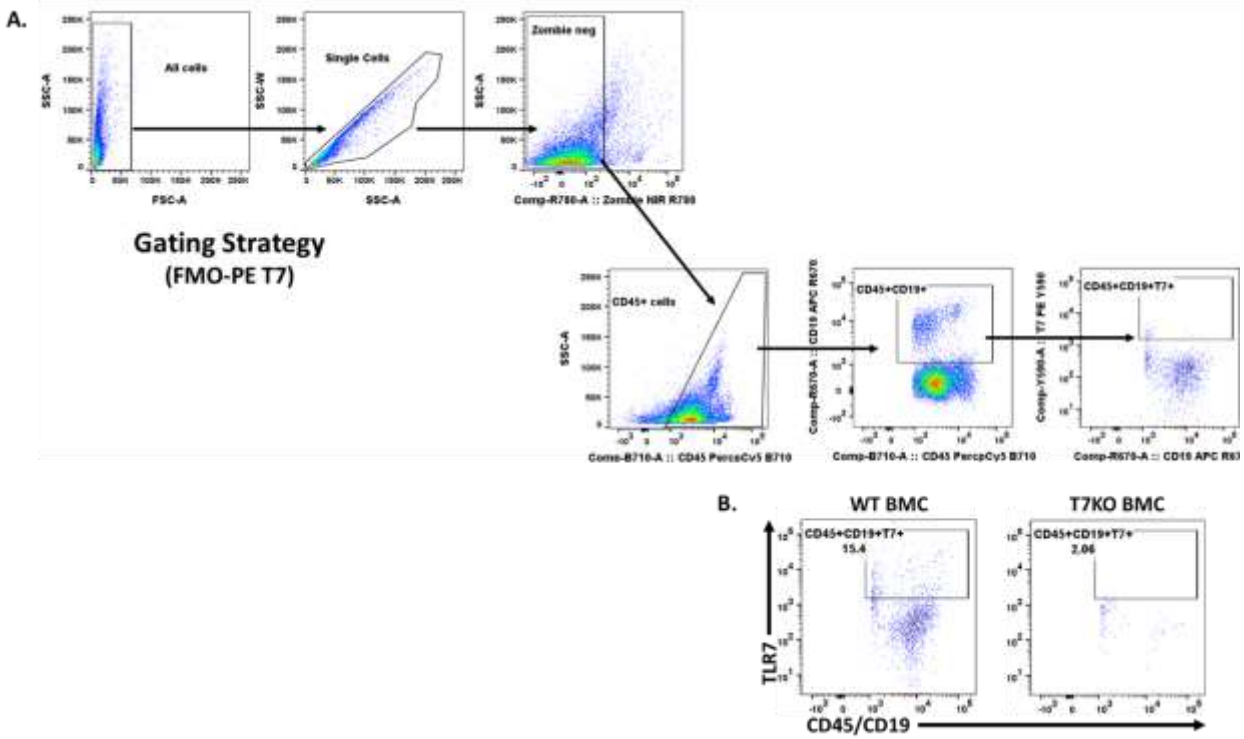
Supplemental Figures

Fig. S1



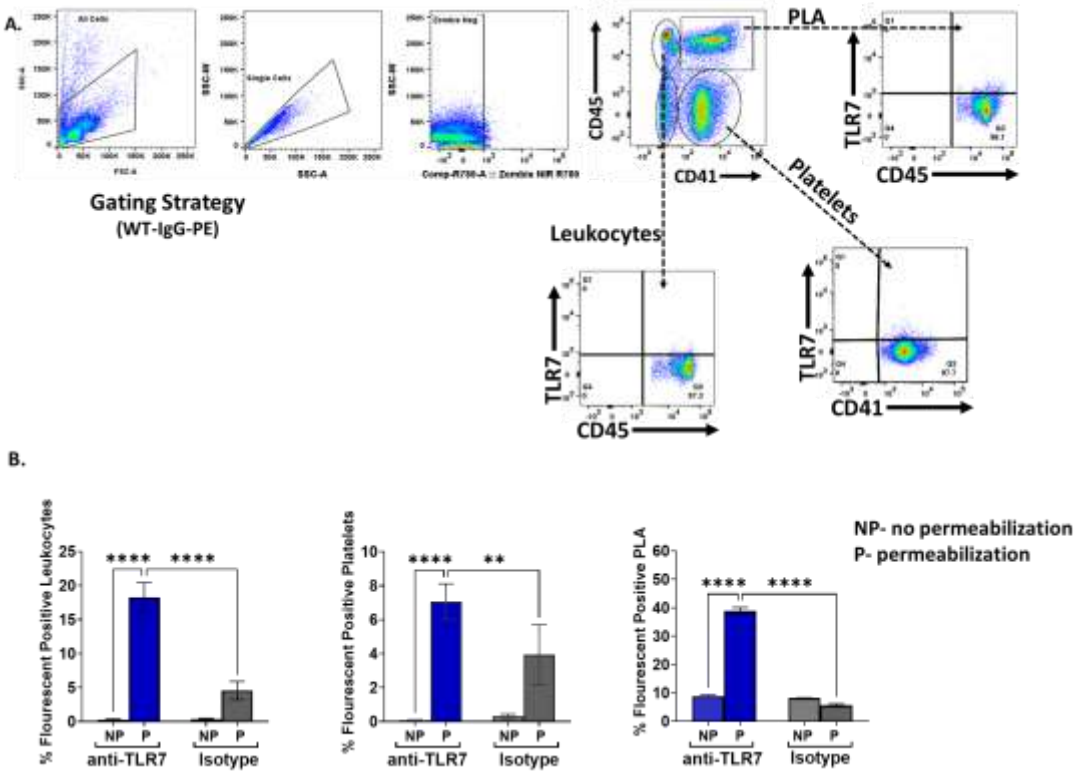
Supplemental Figure 1 (S1). Efficiency of permeabilization of peripheral blood cells using constitutively expressed cytoskeletal protein beta actin. Whole blood from WT mice was collected, and red blood cells were lysed with RBC lysis buffer prior to viability dye staining and treatment with a Fc receptor blocking agent. Cells then either underwent staining with AF647 anti-mouse beta actin antibody with or without permeabilization. An unstained sample was used to set gate; cells were gated for FSC, and SSC properties followed by positivity for beta actin. Cells that underwent permeabilization clearly demonstrated higher percentage of cells positive for beta actin indicating effectiveness of the permeabilization technique.

Fig. S2



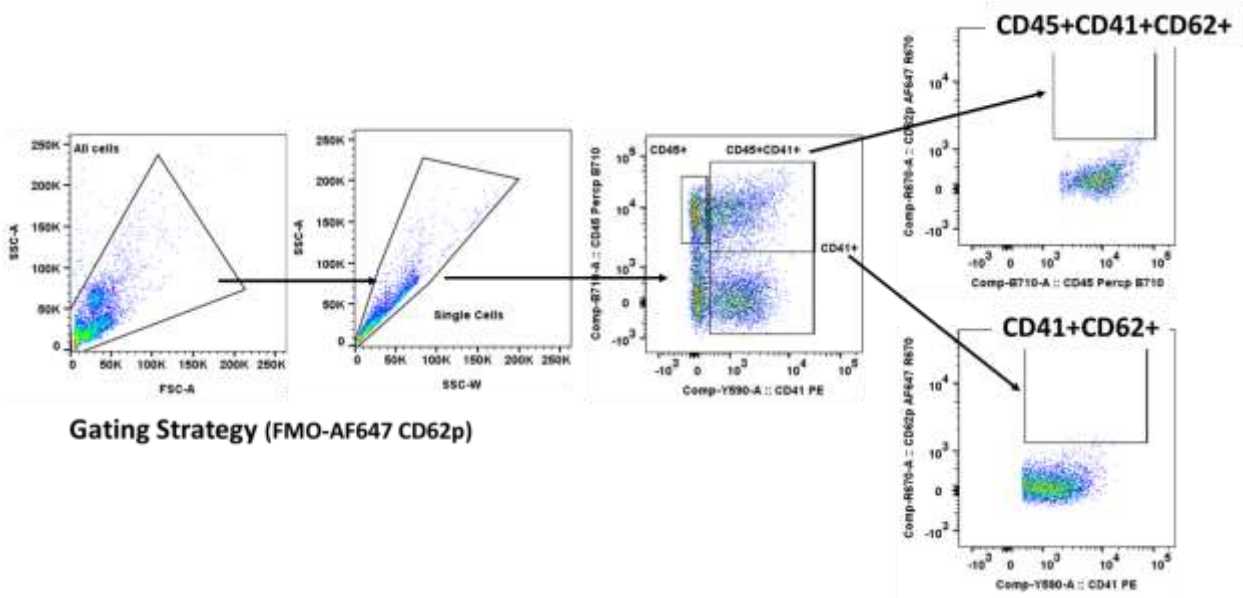
Supplemental Figure 2 (S2). Mouse bone marrow cells express TLR7 protein. Mouse bone marrow cells were collected from age-matched WT and TLR7$^{-/-}$ male mice. Red blood cells were lysed with RBC lysis buffer prior to viability dye staining, treatment with a Fc receptor blocking agent, and surface staining with PerCP Cy5.5-anti-mouse CD45 and APC-anti-mouse CD19, pan-leukocyte and B cell marker, respectively. Cells were then permeabilized and stained with PE-anti-mouse TLR7 mAB. **(A)** A FMO (fluorescence minus one)-PE-TLR7 sample was used to set the gates; cells were gated for FSC, and SSC followed by double positive CD45/CD19 events and percent of TLR7 positive cells were determined from the preceding population. **(B)** Bone marrow cells from WT mice demonstrated higher percentage of cells positive for TLR7 protein (15%) compared to TLR7 deficient mice. Cells from TLR7 deficient mice showed minimal levels of non-specific binding (2%), indicating antibody specificity for TLR7 protein.

Fig. S3



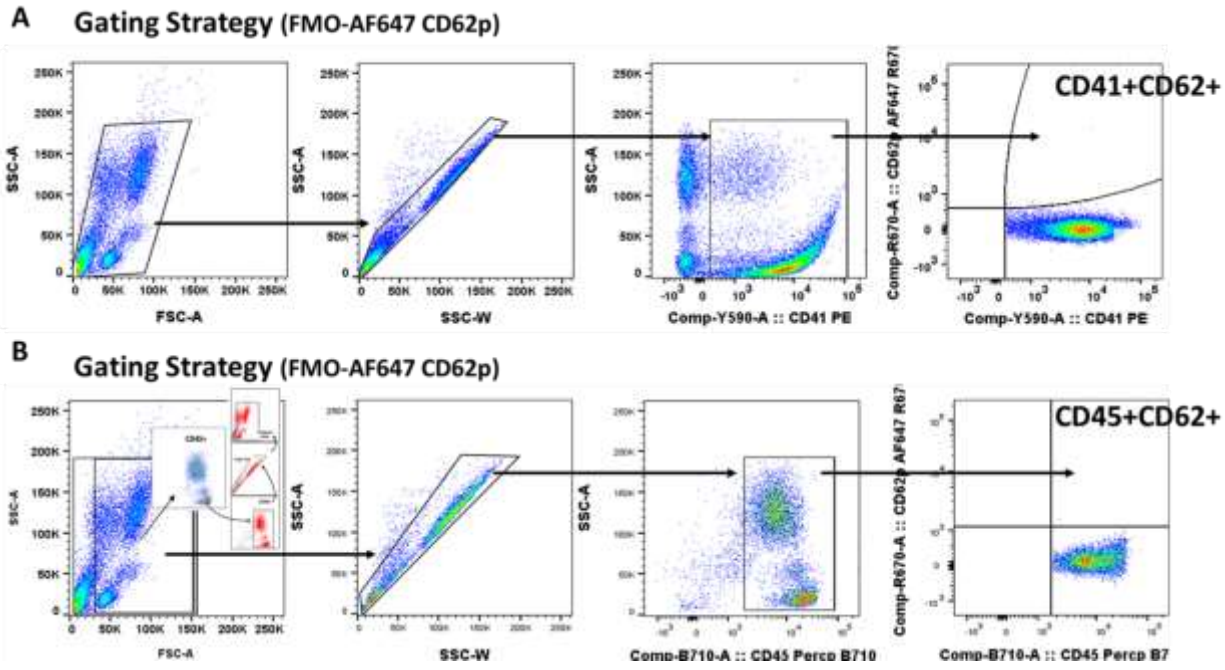
Supplemental Figure 3 (S3). TLR7 expression in mouse platelets and leukocytes. Wild type mouse whole blood was collected, and red blood cells were lysed with RBC lysis buffer prior to viability dye staining and treatment with a Fc receptor blocking agent. Cells were then stained with PerCP Cy5.5-anti-mouse CD45 and FITC-anti-mouse CD41 followed with or without permeabilization and staining with PE-anti-mouse TLR7 or PE mouse IgG1, κ isotype control. Cells were gated for FSC, and SSC properties followed by CD41+ cells (platelets), or CD45+ cells (leukocytes), or double positive CD41+CD45+ (PLA). The percentage of cells positive for TLR7 were then quantified in each population. (A) Gating strategy. (B) There was very minimal staining in non-permeabilized (NP) leukocytes, platelets, and PLA compared to permeabilized (P) cells. Permeabilized platelets, leukocytes, and PLA stained with anti-TLR7 antibody also showed significantly higher positive TLR7 signal than the isotype control. (n=2 mice per group w/and w/o permeabilization). T7=TLR7

Fig. S4



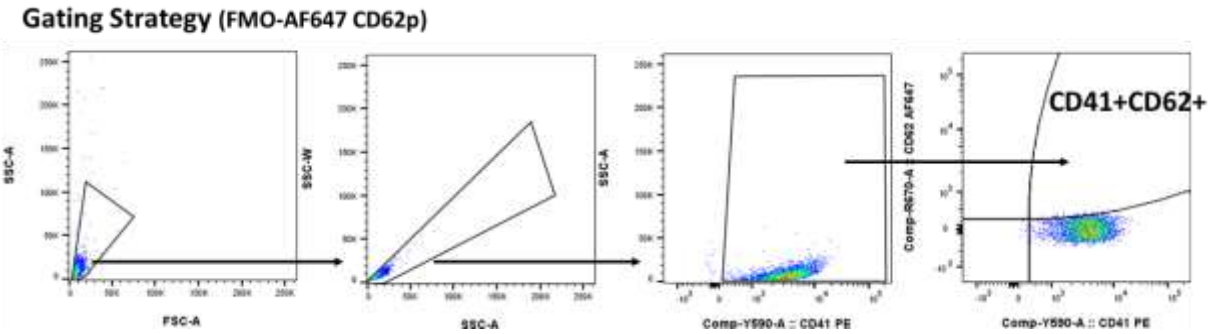
Supplemental Figure 4 (S4). Gating strategy for in vitro platelet activation and PLA formation. Whole blood was collected and following treatment, cells were stained with PerCP Cy5.5-anti-mouse CD45, PE-anti-mouse CD41, and AF647-anti-mouse CD62p. FMO-CD62P sample was used to set gates. Cells were gated for FSC, and SSC properties followed by $CD41^+$ cells (platelets) or double positive $CD45^+CD41^+$ (platelet leukocyte aggregates). The percentage of cells positive for CD62P were then quantified in each population.

Fig. S5



Supplemental Figure 5 (S5). Gating strategy for in vivo platelet activation and PLA formation. Four- or 24-hours following sham or CLP procedure, whole blood was collected, and cells were stained with PerCP Cy5.5-anti-mouse CD45, PE-anti-mouse CD41, and AF647-anti-mouse CD62P. Fluorescence minus one (FMO)-CD62P sample was used to set gates. The percentage of cells positive for CD62P were then quantified in each population. **(A)** Cells gated for all $CD41^+$ cells (platelets) followed by double positive $CD41^+/CD62P^+$. **(B)** Cells gated based on FSC and SSC properties, followed by $CD45^+$ cells (leukocytes), and then double positive $CD45^+CD62P^+$ (activated PLA). Insert shows back-gating to verify $CD45^+$ population.

Fig. S6



Supplemental Figure 6 (S6). Gating strategy for in vitro/ex vivo platelet activation using isolated platelets. 24-hours following sham or CLP procedure, whole blood was collected, and processed to plasma. Isolated platelets from WT or T7KO mice were treated with 10% or 40% plasma or for WT mice with 1 mM loxoribine and then stained with PE-anti-mouse CD41 and AF647-anti-mouse CD62P. Fluorescence minus one (FMO)-CD62p sample was used in each individual experiment to set gates. The percentage of cells positive for CD62P were then quantified from the $CD41^+$ platelet population. Figure shows cells gated for all $CD41^+$ cells (platelets) followed by double positive $CD41^+/CD62P^+$.