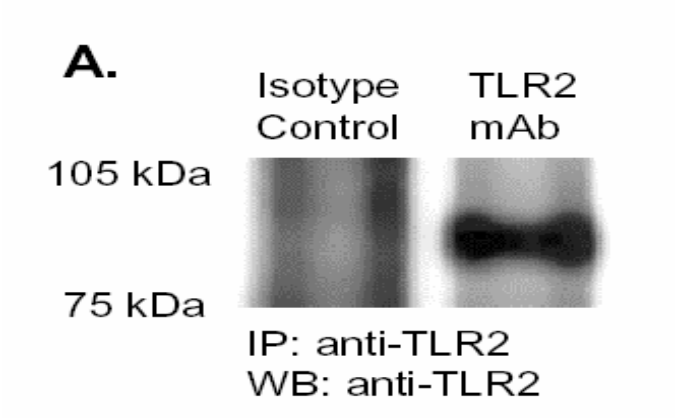
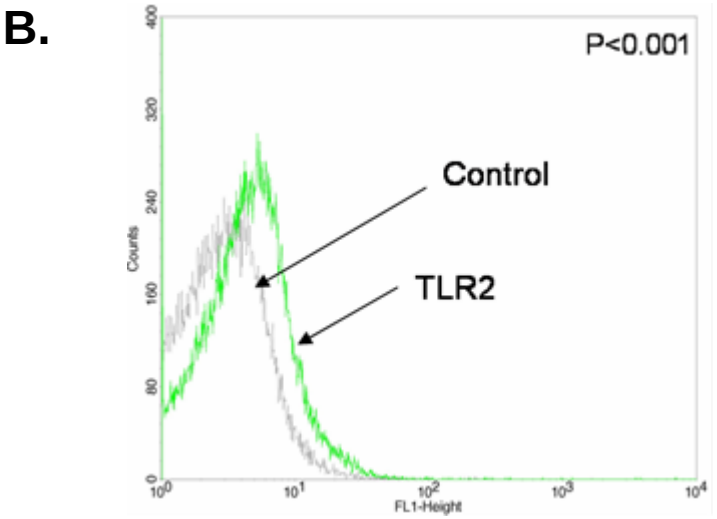
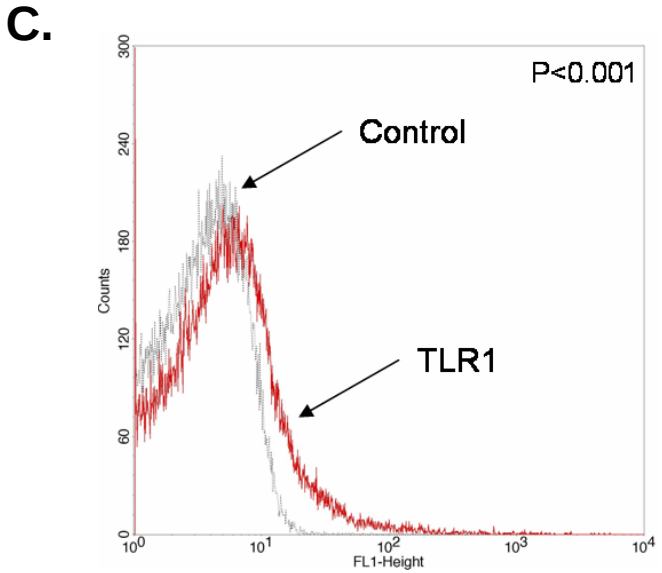
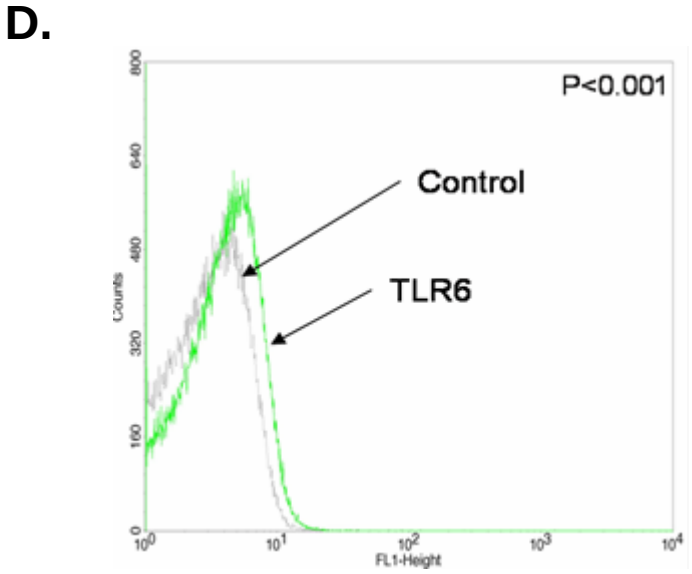
Online Figure I: Human platelets express TLR2, TLR1, and TLR6. (A) Lysed platelets were immunoprecipitated with mouse anti-TLR2 mAb or mouse IgG2a, separated by SDS-PAGE, and immunblotted for TLR2. **(B-D)** Resting platelets were stained with FITC-conjugated anti-TLR2 mAb (green, B), PE-anti-TLR1 mAb (red, C), FITC-anti-TLR6 mAb (green, D), or their respective isotype-matched control antibodies (grey) and analyzed by flow cytometry. For each, a representative histogram of three independent experiments is shown. Quantification of mean fluorescence intensity indicates that TLR2, TLR1, and TLR6 are significantly ($P<0.001$) expressed on the surface of human platelets.

Online Figure I









Online Figure II: Stimulation of platelet TLR2 is sufficient to induce platelet-neutrophil heterotypic aggregates. Isolated platelets and neutrophils were mixed (1 neutrophil:10 platelets) and either left untreated (white) or stimulated with Pam_3CSK4 (10 μg/mL) (black). For other samples, platelets were stimulated with Pam_3CSK4 and mixed with untreated neutrophils (vertical stripes), or Pam_3CSK4-stimulated neutrophils were mixed with untreated platelets (horizontal stripes). Platelets and neutrophils were stained with FITC-anti-CD41 and PE-Cy7-anti-CD14 mAb, respectively, and formation of platelet-neutrophil aggregates was analyzed by flow cytometry. For each group, the percentage of platelet-positive neutrophils (CD14/CD41 positive events) ± SD was quantified. n=5 for all groups; *$P<0.05$ compared to resting mixture.

Online Figure II