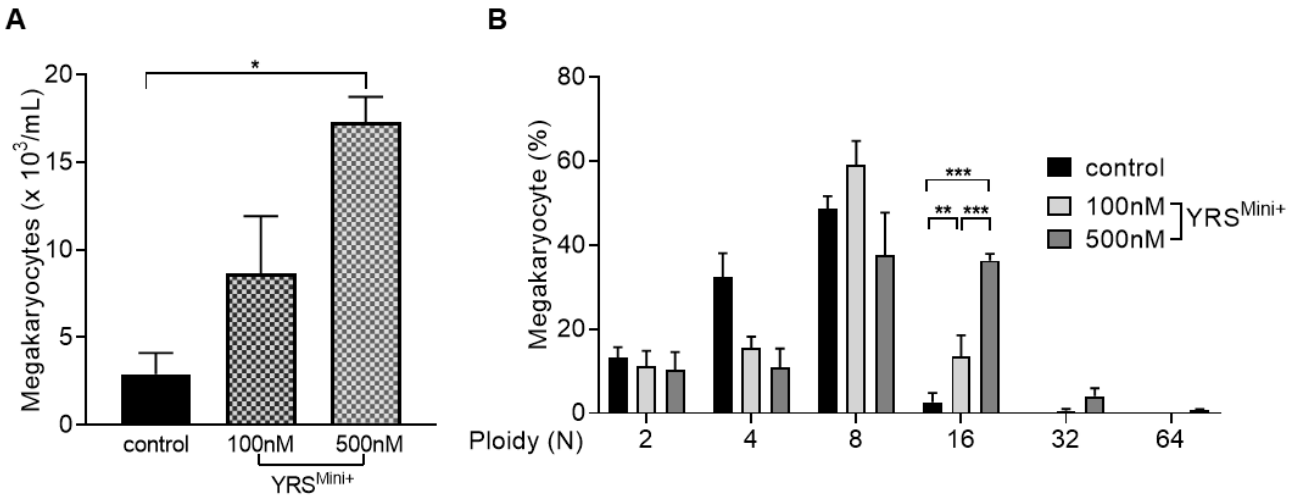
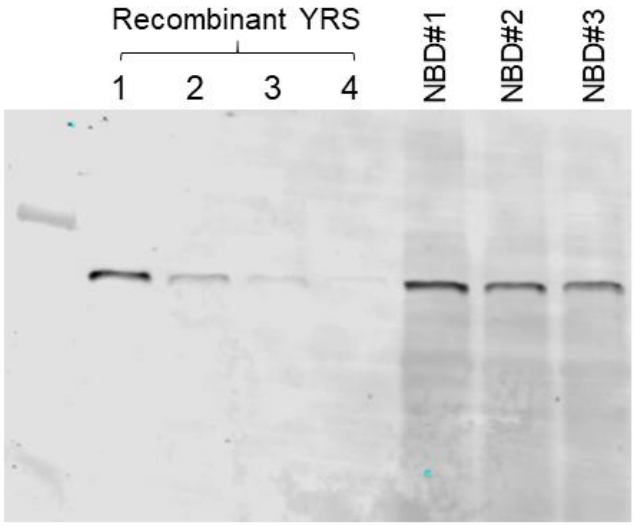
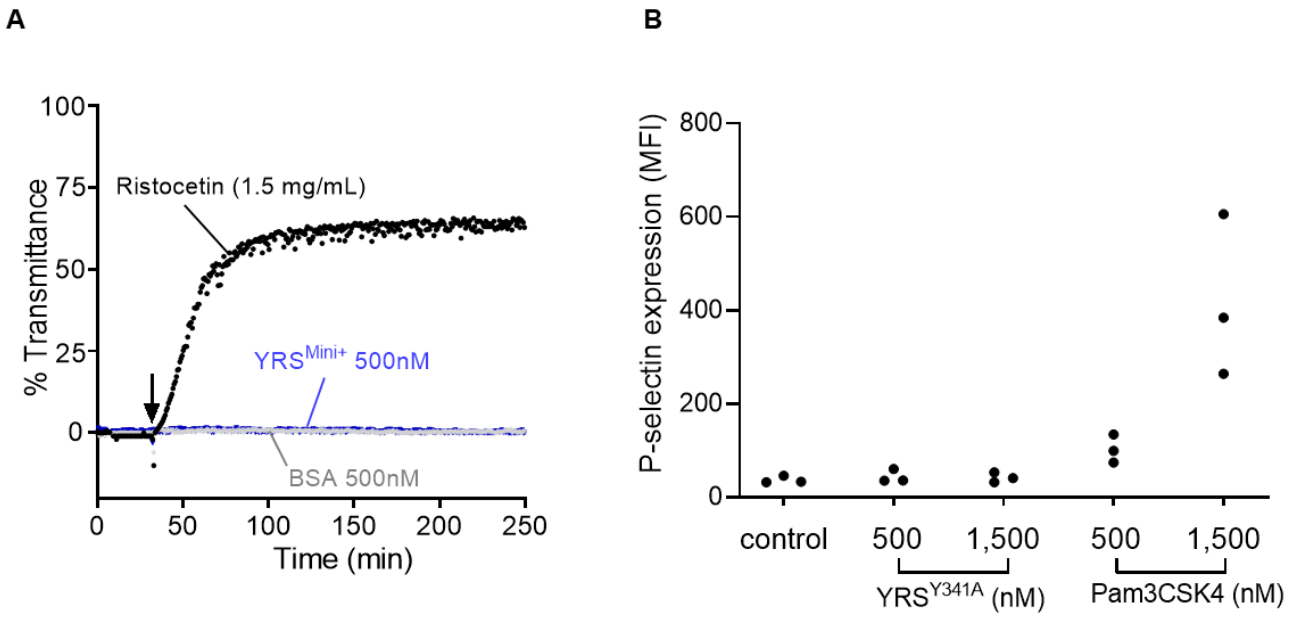
Supporting Information



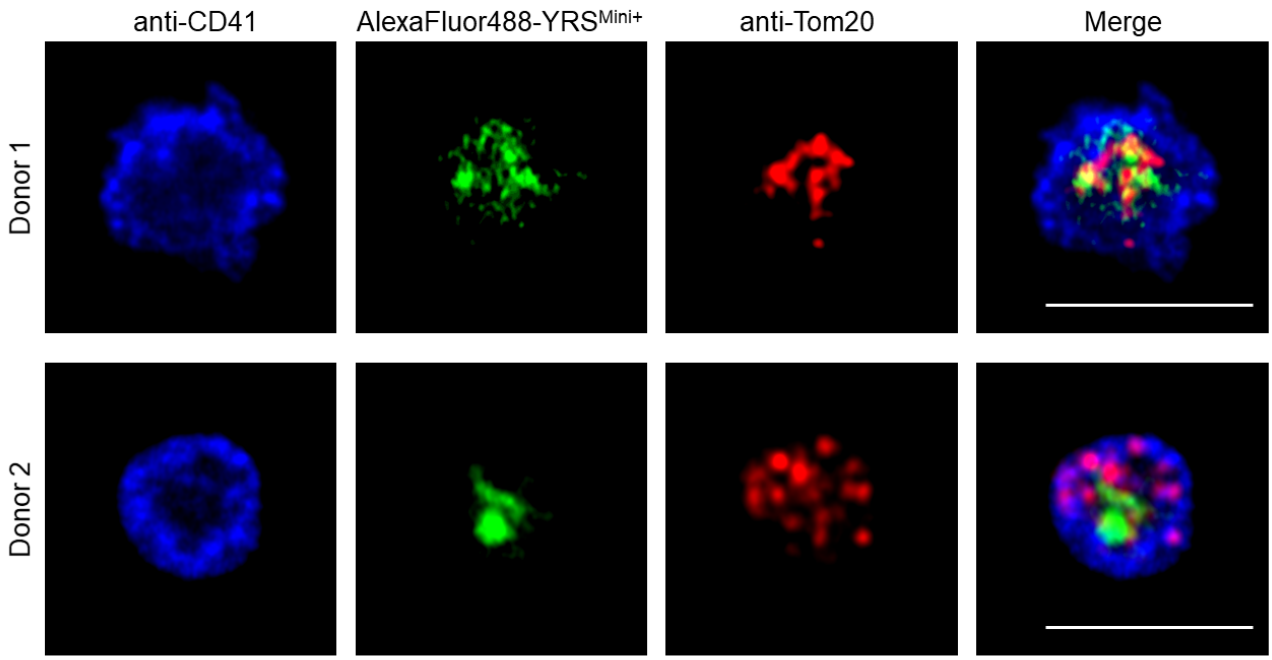
Supplemental Figure 1. YRS^{Mini+} has an activity to enhance megakaryopoiesis. Mouse bone marrow cells were cultured with indicated concentration of YRS^{Mini+} for three days. (A) Megakaryocyte number was determined by flow cytometry. *P < .05 by one-way ANOVA with Dunn's multiple comparison test. (B) YRS^{Mini+} increased megakaryocytes with higher ploidy (ploidy >16N) compared to control. **P < .01, ***P < 0.001 by two-way ANOVA with Tukey's multiple comparison test.



Supplemental Figure 2. Semi-quantitative Western blot analysis of platelet lysates. Platelets from healthy donors were washed and lysed to be loaded onto SDS-PAGE under reduced conditions, along with purified recombinant YRS protein of known concentration (1: 62.5 ng, 2: 31.3 ng, 3: 15.6 ng. 4: 7.8 ng). The membrane was probed with rabbit anti-YRS polyclonal antibody and then, incubated with IRDye800CW goat anti-rabbit IgG. The signal intensity was quantified using Licor ImageStudio, and the amount of YRS detected in each lysate was normalized by count of platelet measured before lysis. The amount of YRS per platelet was then, multiplied by platelet count in the whole blood of each donor to estimate the amount of platelet-stored YRS in whole blood calculated as 26.8 ± 10.5 nM (n = 3). This experiment was repeated 3 times and representative result is shown.



Supplemental Figure 3. Testing the effect of YRS on platelet activation. (A) Platelet-rich plasma was prepared from healthy donors and aggregation assay was performed with platelets at 3 X 10^{11}/L at 37°C using Model 440 aggregometer (Chrono-Log). (B) Whole blood samples collected from C57BL/6J mouse were treated with YRSY341A or synthetic ligand of TLR12/TLR1 (Pam3CSK4) at the concentrations indicated. Platelets were gated by staining with anti-CD41 antibody and the surface expression of P-selectin was measured by flow cytometry.



Supplemental Figure 4. Confocal analysis of platelets uploaded with YRS^{Mini+}. Platelet-rich plasma of healthy donors were pre-incubated with AlexaFluor488 labeled YRS^{Mini+} (500 nM) at 37ºC for 17 hours. Platelets with pre-loaded YRS^{Mini+} were mixed with red blood cells and perfused on the surface coated with VWF A1 at low shear rate (100 s^{-1}). After perfusion, adhered platelets were fixed with 2% paraformaldehyde, permeabilized with 0.1% Triton X, and stained with anti-Tom20 antibody (mitochondrial marker) followed by AlexaFluor555 anti-mouse IgG and Brilliant Violet 421 anti-human CD41 antibody. Images were taken using confocal microscope (LSM 880; Carl Zeiss). Scale bar = 5 μm