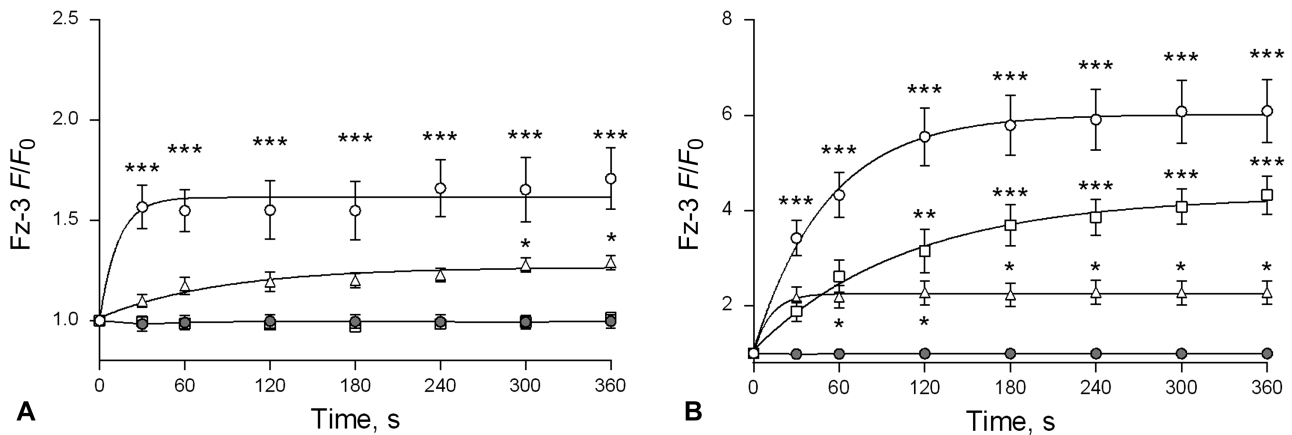


Supplementary Fig. S1 Calibration of fluorophores. Washed Fz-3 (A) or Fluo-4 (B) loaded platelet suspensions were treated for 15 minutes with Zn²⁺ or Ca²⁺ ionophores in the presence of 2 mM CaCl₂ or ZnSO₄, to determine R_{max} values. Fz-3 R_{min} was obtained from platelets pre-treated for 15 minutes with 50 μM TPEN, while R_{max} was obtained following co-incubation with 5 μM pyrithione (Py) with 2 mM ZnSO₄ for 15 minutes. Fluo-4 R_{min} was obtained from platelets pre-treated for 15 minutes with 2 mM EGTA in the absence of extracellular Ca²⁺, while R_{max} was obtained following pre-incubation with 1 μM A23187 in the presence of 2 mM CaCl₂.



Supplementary Fig. S2. Extracellular calcium does not significantly affect agonist- or ionophore-evoked [Zn²⁺]_i fluctuations. Washed, Fz-3 loaded platelets suspended in Tyrode's buffer supplemented with 2 mM CaCl₂ were stimulated with platelet agonists (A); CRP-XL (1 μg/mL, ○), U46619 (10 μM, ◊), thrombin (1 U/mL) or ionophores (B); clioquinol (◻, 300 μM), pyrithione (△, 300 μM), A23187 (○, 300 μM), during which changes in fluorescence were monitored. ● vehicle control (DMSO). Data are mean ± standard error of the mean (SEM) from at least 4 independent experiments. Significance is denoted as ****p* < 0.001, ***p* < 0.01 or **p* < 0.05.