

Figure S1. The Rac inhibitors EHT 1864 and NSC23766 similarly inhibit platelet lamellipodia formation on fibrinogen. Purified human platelets $(2 \times 10^7 / \text{ml})$ were pretreated with 50 µM EHT 1864 or 300 µM NSC23766 for 10 minutes prior to spreading on fibrinogen coated coverglass (50 µg/ml) in the presence of 2 U/ml apyrase. Representative DIC microscopy images are shown. Scale bar = 10 µm.



Figure S2. Quantification and statistical analyses of western blot data from

Figure 1 and Figure 4. Films from kinase activation western blot experiments in Figures 1 and 4 were digitally scanned and individual bands were quantified for pixel density using Image J software. Western blot densitometry statistical analysis was performed by calculating a paired t-test between the DMSO lane and each test condition for the protein specified (n=3). Shown above are band intensities relative to vehicle control for (**A**) pSyk in Figure 1B, (**B**) pLAT in Figure 1B, (**C**) pFAK in Figure 1B, (**D**) pS6K1 in Figure 1D, (**E**) pSyk in Figure 4B, (**F**) pFAK in Figure 4B and (**G**) pGSK3 β . Asterisk (*) indicates p < 0.05 with respect to vehicle (DMSO).



Figure S3. Anti-TIAM1 sc-872 specifically immunoprecipitates TIAM1 from

human platelet lysates. M-PER-lysed human platelets were incubated at 4°C overnight with 10 µg non-specific rabbit IgGs (IgG), 10 µg anti-TIAM1 (sc-872), or 10 µg anti-TIAM1 in the presence of sc-872P TIAM1 antigen blocking peptide (sc-872 + peptide). Protein A/G Agarose beads were added to immunoprecipitation reactions for 1 hour and then washed 3 times with M-PER buffer. Captured proteins were eluted into Laemelli sample buffer plus DTT and western blotted (WB) for the presence of TIAM1. A 200 kD TIAM1-positive band was detected in whole platelet lysates (input) as well as sc-872 immunoprecipitates. Rabbit IgGs did not immunoprecipitate TIAM1. Inclusion of the TIAM1 antigen blocking peptide sc-872P with sc-872 antisera prevented the immunoprecipitation of TIAM1.



Figure S4. Puromycin does not inhibit platelet lamellipodia formation on

fibrinogen. Purified human platelets $(2 \times 10^7 / \text{ml})$ were pretreated with puromycin (puro, 100 µg/ml, 10 min) or EHT 1864 (50 µM) prior to spreading on fibrinogen coated coverglass (50 µg/ml) in the presence of 2 U/ml apyrase. Representative DIC microscopy images are shown. Scale bar = 10 µm.



Figure S5. Puromycin does not inhibit collagen-induced platelet aggregation

Washed human platelets $(2 \times 10^8 / \text{ml})$ were pretreated with puromycin $(100 \,\mu\text{g/ml}, 10 \,\text{min})$ in the presence of 2 U/ml apyrase before stimulation with collagen $(10 \,\mu\text{g/ml})$ in solution and the change in optical density indicative of platelet aggregation was recorded. EHT 1864 (50 μ M), an efficient inhibitor of platelet aggregation, serves as a positive control.