**Materials** - Human vWf was purified from plasma according to the method of Montgomery and Zimmerman. Human fibrinogen was purified from plasma according to the method of Jakobsen and Kierulf. Heparin (enoxaparin sodium) was from Aventis Pharma (Australia). Hirudin (lepirudin) was from Pharmion (Australia). c7E3 Fab (Abciximab) was from Eli Lilly (The Netherlands). Apyrase, A3P5P, and MRS2179 were from Sigma (USA). ARC69931MX was from AstraZeneca (Australia). Oregon green 488 BAPTA-1, AM, Fura red, AM, DiOC6 and NP-EGTA were from Molecular Probes Inc. All other reagents were from sources previously described.

**Intravital studies** – Approval was gained from the Monash University Animal Ethics Committee for all experiments involving animals. Intravital studies were performed according to a modified method of Denis et al. and Kulkarni et al. Mesenteric arterioles (30-40 μm) of male Wistar rats were injured via photoactivation (550nm, 10-30 sec) of systemically administered Rose-Bengal (5 mg/kg). The dynamics of platelet interactions with the damaged vessel wall and with growing platelet thrombi were viewed by differential interference contrast (DIC) microscopy using a Leica DMIRB microscope (×100 PL APO objective, NA 1.40-0.7) and recorded on video for off-line analysis.

**Preparation of washed platelets and red blood cells** – Blood was collected from healthy volunteers, with the approval of the Monash University Human Ethics Committee. Washed platelets were prepared as described previously. Platelets were suspended at a concentration of 300×10^6/ml in modified Tyrode’s buffer [12 mM NaHCO₃, 10 mM Heps, 137 mM NaCl, 2.7 mM KCl, 5.5 mM D-Glucose, 1 mM CaCl₂, 0.5 mg/ml BSA] containing 0.02 U/ml apyrase. Red blood cells were prepared as described previously. To inhibit residual thrombin and secreted ADP, the packed RBC preparation was supplemented with 200 U/ml hirudin and 0.02 U/ml apyrase.

**In vitro perfusion studies** - Perfusion assays were performed according to a modified method of Cooke et al. Micro capillary tubes (Vitrocom, USA) were coated with either purified vWf (50 μg/ml) or a mixture of vWf and fibrinogen (10 μg/ml vWf + 5, 10, 20 or 50 μg/ml fibrinogen), then blocked with 5% human serum (supplemented with 50 μg/ml phenylmethylsulfonylfluoride).
prior to experimentation. Either whole blood (anticoagulated with 400 U/ml hirudin) or washed platelets (150×10⁶/ml) reconstituted with packed red blood cells (45% hematocrit) were perfused through micro capillary tubes at a shear rate of 600, 1800 or 5000 s⁻¹. Where indicated, platelets were pre-treated with c7E3 Fab (20 μg/ml), ARC69931MX (1 μM), A3P5P (1 mM), MRS2179 (100 μM) or PGE1 (0.5 μg/ml) prior to perfusion. Adherent platelets were visualised by DIC microscopy (×100 PL APO objective, NA 1.40-0.7) on a Leica DMIRB microscope. Adhesion was monitored over a 250 second period and video-recorded for off-line analysis. At 15, 30, 45, 60, 90 and 120 second time points, the number of adherent platelets within an optical field (dimensions 70 μm × 90 μm) was counted. An aggregate was defined as a cluster of at least 5 platelets maintaining physical contact for at least 3 seconds. Aggregate formation was quantitated by counting the number of aggregates present within a field of view, and recording the number of platelets within individual aggregates. An aggregate was classified as ‘unstable’ if it was composed of reversibly-adherent platelets. A ‘stable’ aggregate was classified as one composed of irreversibly adherent platelets.

In some experiments, washed platelets reconstituted with RBCs were perfused over a mixed vWF/fibrinogen matrix for 15 seconds to allow a small number of platelets to adhere (approximately 6-7 platelets per 70 μm × 90 μm field). Modified Tyrode’s buffer was perfused for a further 2 minutes to allow these platelets to adhere irreversibly and begin to spread. Washed platelets pre-treated with either c7E3 Fab (20 μg/ml) or cytochalasin D (5 μM) were reconstituted with RBCs as described above and then perfused over the adherent platelets. DIC microscopy was used to visualize subsequent interactions with the pre-adhered platelets.

**Platelet adhesion to monolayers** - Blood was collected from healthy donors or individuals with Glanzmann’s thrombasthenia (1% αIbβ3). Confluent platelet monolayers (> 90% surface coverage) were obtained by allowing washed platelets (200×10⁶/ml) to spread (30 minutes, 37°C) on glass micro capillary tubes. Anticoagulated whole blood (15 mM trisodium citrate, pH 7.4) was incubated with the fluorescent dye DiOC6 (1 μM, 10 minutes), then perfused over pre-formed platelet monolayers at 1800 s⁻¹ for 1 minute. The interaction of flowing platelets with pre-formed platelet monolayers was viewed in real time using fluorescence microscopy and recorded on video for off-line analysis. The number of platelets tethering to the surface of monolayers was analysed frame by frame (25 frames per second) over the first 5–10 seconds of
flow. In all studies, any cell forming an adhesion contact for more than 40 milliseconds was scored as an adherent platelet.

**Analysis of cytosolic calcium flux under flow conditions** - Changes in intracellular calcium levels were monitored according to previously published methods. Briefly, platelets in PWB ([1]×10^9/ml) were loaded with Oregon Green 488 BAPTA-AM (1 μM, emission wavelength 500-570 nm) and Fura Red-AM (1.25 μM, emission wavelength 600-710 nm) for 30 minutes at 37°C. Calcium dye-loaded platelets were subsequently incubated in PWB with 10 μM NP-EGTA for 30 min at 37°C. NP-EGTA-treated platelets were washed once with PWB and resuspended in modified Tyrode's buffer prior to experimentation. Platelets were combined with red blood cells (to a final concentration of 150×10^6/ml or 20×10^6/ml) for perfusion studies as described above.

To examine changes in calcium flux, sequential images of adherent platelets were captured at a scan rate of 0.586 frames/sec for 200 frames. Real time calcium flux was calculated based on a ratio of signal intensity between the two dye channels, and converted to intracellular calcium concentrations as described previously. NP-EGTA uncaging was carried out following 18 sec of reconstituted blood flow via exposure of platelets to a near UV (300–400-nm) light source generated by a 100-watt mercury lamp directed through the optical path of a Leica DMIRBE confocal microscope, for an interval of 0.6 sec. Control studies were carried out with unloaded control platelets and demonstrated that the brief UV exposure did not lead to photodynamic damage or activation of the platelets under flow.

**Scanning Electron Microscopy (SEM)** - Translocating platelets were fixed and prepared for SEM imaging as described previously. Platelets were imaged on a Hitachi S570 scanning electron microscope (Tokyo, Japan) at 20 kV accelerating voltage, 3mm working distance.

**Statistical Analysis and Computer Programs Used** - Results are presented as mean ± standard error (SEM). Statistical significance was assessed by Student’s t-test. Schematic diagrams in Figures 2 and 4 were drawn using CorelDraw Graphics Suite (Corel Corporation). Video was digitized using Pinnacle Systems DV500 PLUS (Pinnacle Systems, Germany), and edited using Adobe Premiere Pro v1.5 (Adobe Systems Inc).