SUPPLEMENTARY EXPERIMENTAL PROCEDURES

Myosin Light Chain Phosphorylation - MLC phosphorylation in retracting fibrin clots was performed according to a modified method of Daniel et al (1). ^{32}P-labelled washed human platelets were preincubated with vehicle (DMSO) or LY294002 (LY - 25 μM), prior to initiation of the fibrin clot with thrombin (1.0 U/ml) in the presence of 0.5 mg/ml fibrinogen. Clot retraction was terminated at the indicated times by addition of 5x Reducing Buffer (50 mM Hepes, 10% SDS, 25 mM EDTA, 50% glycerol, 0.025% bromophenol blue, 100 mM dithiothreitol) in the presence of protease and phosphatase inhibitors (25 μg/ml aprotinin, 10 μM Leupeptin, 25 μg/ml PMSF, 1 mM benzamadine, 100 nM okadaic acid, 1 mM sodium vanadate, 5 mM EDTA, 25 mM sodium fluoride, and 2.5 mM β-Glycerolphosphate) and further solubilised by sonication. Proteins were separated on 12.5% SDS-PAGE gel, stained with Coomassie blue, and exposed to x-ray film. Band densities were quantified with densitometry using Chemigenius Genesnap™ software (Syngene, Cambridge).

Measurement of Actin Filament Assembly - Filamentous (F)-actin was quantified as described previously (2). Briefly, washed human platelets (5.0 x 10^7 /ml) were pretreated with either vehicle (DMSO) or LY294002 (25 μM), stimulated with 1U/ml thrombin for the indicated times, and fixed by addition of paraformaldehyde (final 2%) for 3 minutes at room temperature. Fixed platelets were permeabilized and F-actin stained using 0.1% Triton X-100 containing FITC-conjugated phalloidin (1 μM) for a further 5 minutes. The level of F-actin was analysed by flow cytometry (FACScan flow cytometer, Beckton-Dickinson). Results are expressed as fold increase over the amount of F-actin in resting platelets.

SUPPLEMENTARY FIGURE LEGENDS

SUPPLEMENTARY FIGURE 1**. Effect of PI3K Inhibition on Thrombin-Induced Platelet Spreading on a High Density Fibrinogen Matrix.** Washed platelets were pretreated with vehicle alone (DMSO) or LY294002 (LY: 25 μM). Platelets (1 x 10^7/ml) were applied to immobilized fibrinogen (100 μg/ml) in the presence of 1 U/ml thrombin (Thr), as described under "Experimental Procedures". Platelet spreading was imaged at the indicated time points using phase contrast microscopy and quantified using MCID computer-based analysis programs. Results represent the mean ± SEM of 3 independent experiments.

SUPPLEMENTARY FIGURE 2**. PI3K p110β is the Major Isoform Regulating Thrombin-Induced Clot Retraction.** Washed platelets derived from (**A**) human blood (3.0 x 10^8/ml) or (**B,C**) from p110$\gamma^{+/+}$, p110$\gamma^{-/-}$, p110$\delta^{+/+}$ or p110$\delta^{-/-}$ mice (2.5 x 10^8/ml), were preincubated with vehicle alone (DMSO), LY294002 (LY: 25 μM) or the PI 3-kinase p110β selective inhibitor TGX221 (221: 0.5 μM), in the presence or absence of ADP/TxA2 antagonists (see "Experimental Procedures"). Platelets were supplemented with 0.5 mg/ml fibrinogen prior to stimulation with 1 U/ml thrombin and the extent of clot retraction quantified as described under "Experimental Procedures". Results represent the mean ± SEM of 4 (**Ai**) and 3 (**Aii, B, C)** independent experiments (^{ns}, p>0.05; **, P<0.01***, *p*<0.001)**.**

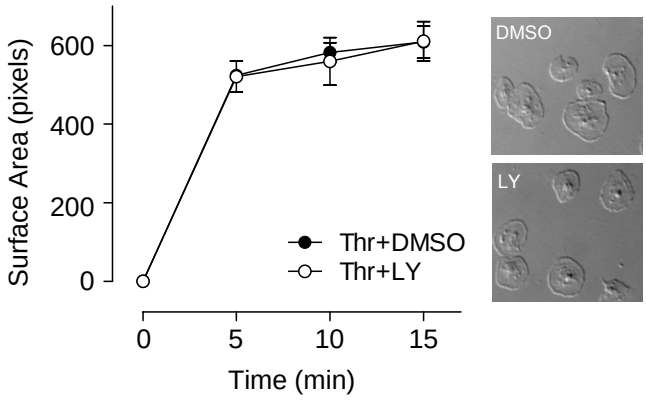
SUPPLEMENTARY FIGURE 3**. PI3K does not Regulate Myosin Light Chain Phosphorylation nor F-Actin Polymerization.** (**A**) ^{32}P-radiolabeled washed platelets pretreated with vehicle alone or LY294002 (LY: 25 μM) were supplemented with 0.5 mg/ml fibrinogen and stimulated with 1U/ml thrombin for indicated time. At each time point, samples were lysed and proteins run on SDS-PAGE under reduced conditions. The level of myosin light chain (MLC) phosphorylation was determined by autoradiography. This image is taken from one representative of 3 independent experiments. (**B**) Washed platelets were pretreated with vehicle alone (DMSO) or LY294002 (LY: 25 μM) prior to stimulation with 1U/ml thrombin. Stimulated samples were lysed at the indicated time points, and F-actin measured by flow cytometry, using FITC-phalloidin, as described under "Supplementary Experimental Procedures".

SUPPLEMENTARY FIGURE 4. **Soluble Agonist Stimulation is Required to Achieve Platelet Spreading on Low Density Immobilized Fibrinogen.** Washed platelets (5 x 10^7/ml) were applied to immobilized fibrinogen (0.2, 1 or 5 μg/ml) in the absence (no agonist) or presence of 1 U/ml thrombin (Thr), as described under "Experimental Procedures". Platelet spreading was imaged in real time and recorded onto DVD. Images were obtained off-line at a 15 minute time point using PowerDVD^{TM}, and are taken from 1 representative of 3 independent experiments.

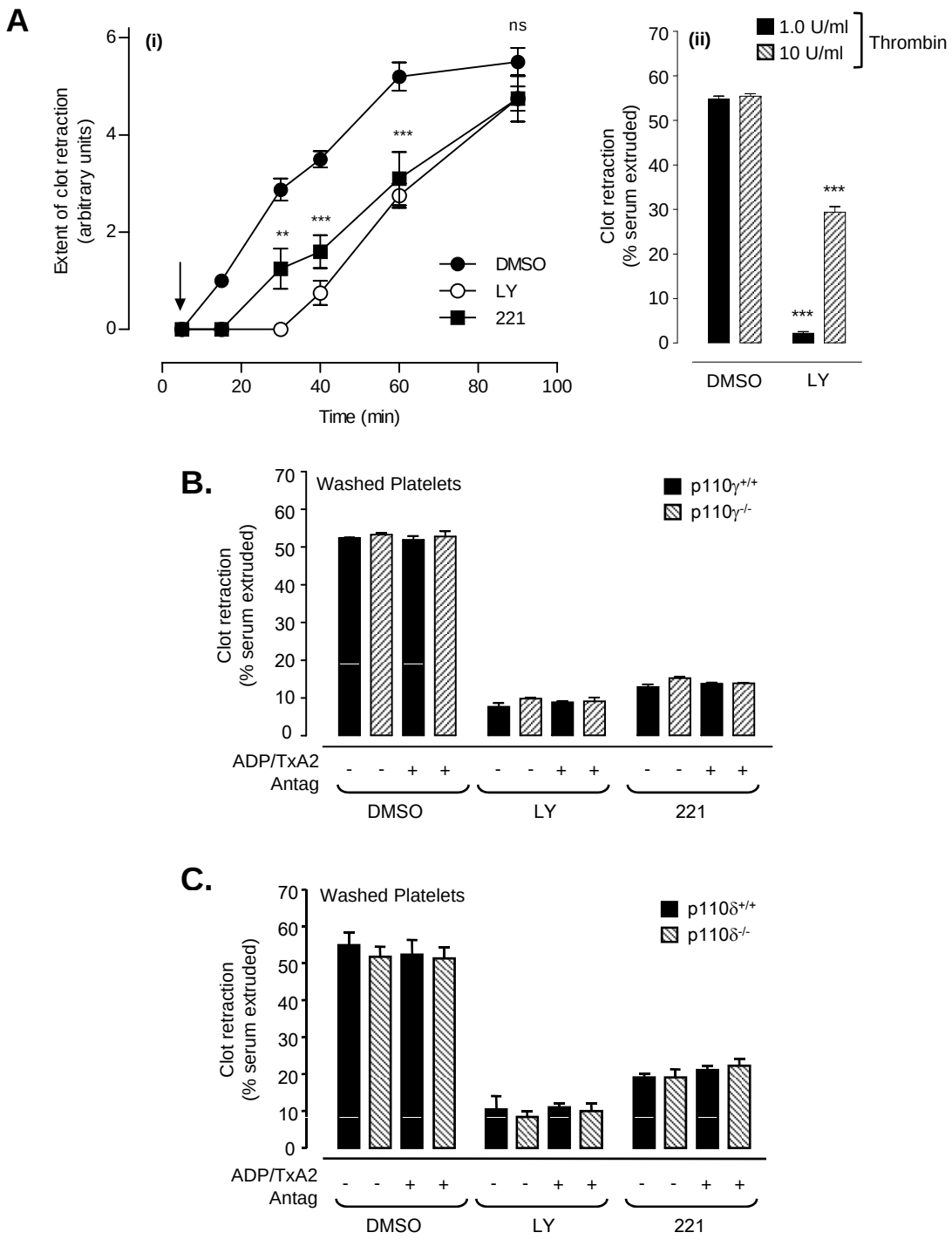
SUPPLEMENTARY VIDEO 1. **PI3K strengthens the interaction of integrin $\alpha_{IIb}\beta_3$ with immobilized fibrinogen.** $DiIC_{12}$ labelled washed platelets pretreated with either vehicle (DMSO) or wortmannin (100 nM) were allowed to adhere and spread on immobilised fibrinogen (5 μg/ml) in the presence of 1 U/ml thrombin. The interaction of platelets with the matrix was observed in real time using TIRF and DIC microscopy as described under "Experimental Procedures". This video is shown at x 5 original speed.

SUPPLEMENTARY REFERENCES

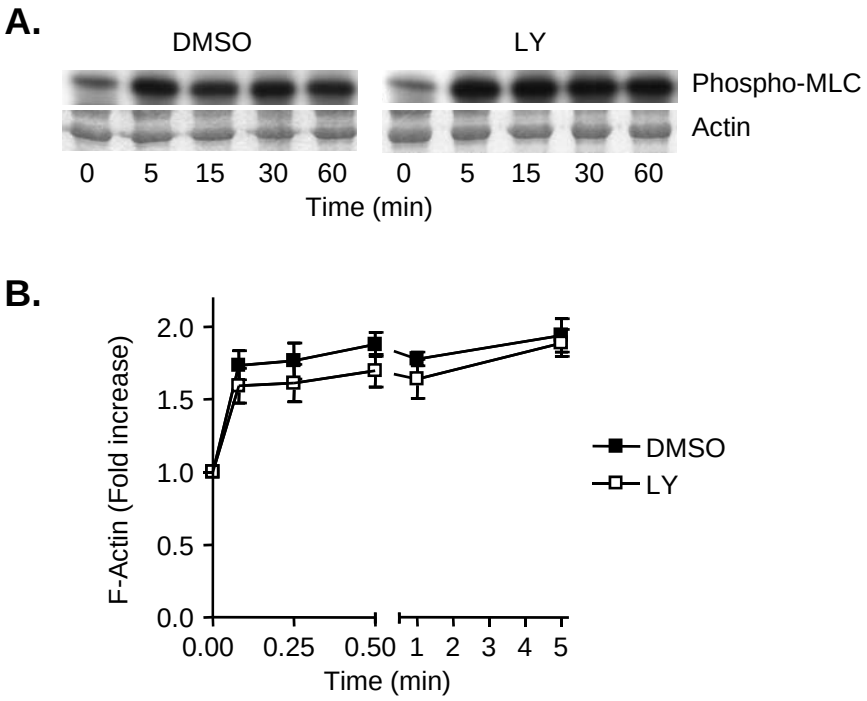
1. Daniel, J. L., Molish, I. R., and Holmsen, H. (1981) *J Biol Chem* **256**, 7510-7514
2. Hartwig, J. H. (1992) *J Cell Biol* **118**, 1421-1442



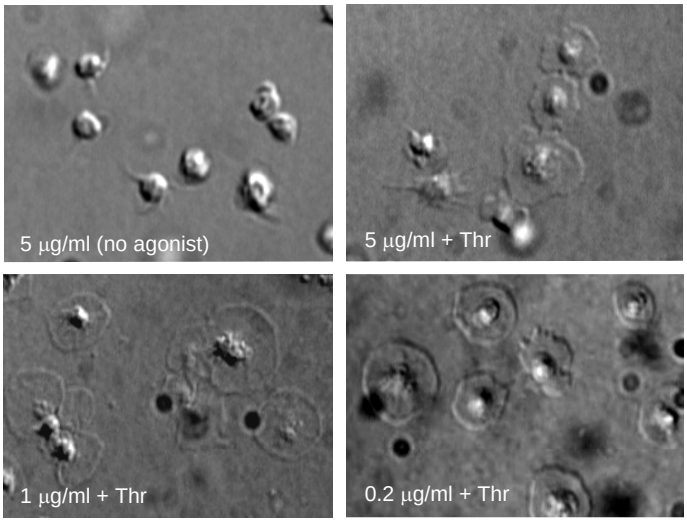
Supplementary Figure 1



Supplementary Figure 2



Supplementary Figure 3



Supplementary Figure 4