NON-REDUNDANT ROLES OF PHOSPHOINOSITIDE 3-KINASE ISOFORMS α AND β IN GLYCOPROTEIN VI-INDUCED PLATELET SIGNALING AND THROMBUS FORMATION*

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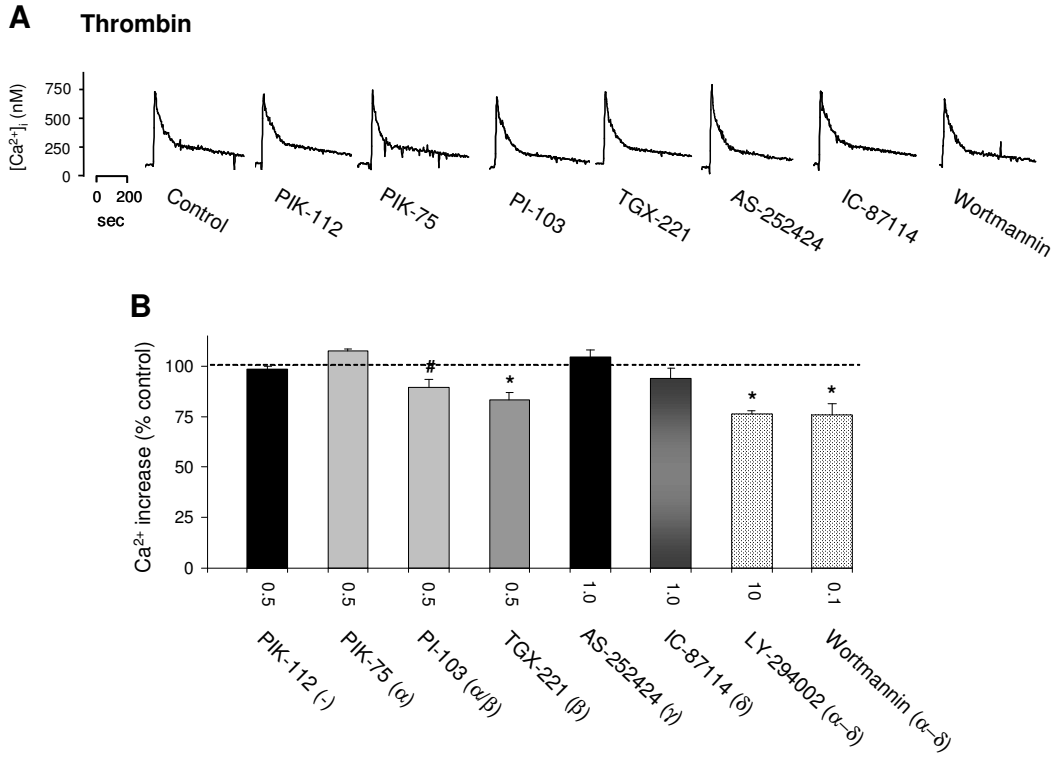
Supplementary data

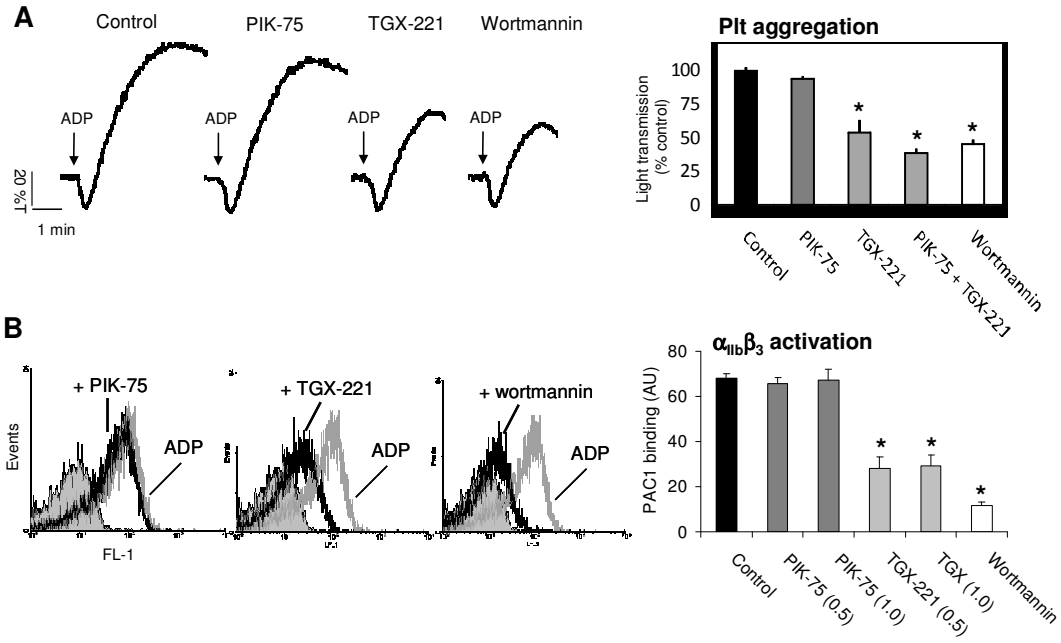
SUPPLEMENTARY TABLE I

Inhibition of PI3K lipid kinase activity for purified enzymes *in vitro*. Listed are IC_{50} values for the indicated compounds, regarding inhibition of the catalytic activity of recombinant class I PI3K isoforms in cell-free system. Data were drawn from appropriate patent and publication information, see Refs. (31,41).

Compound	Relative specificity	IC_{50} (μM)			
		α	β	γ	δ
PIK-112	none	>100	>100	>100	>100
PIK-75	α	0.008	0.34	0.9	>20
PI-103	α/β	0.004	0.018	>500	>20
YM-024	α/δ	0.30	2.65	9.1	0.33
TGX-221	β	7.0	0.008	3.5	0.2
AS-252424	γ	1.1	>20	0.035	>20
IC-87114	δ	>100	75	29	0.1
LY-294002	α–δ	0.50	1.0	n.d.	0.57
Wortmannin	α–δ	0.001	0.003	n.d.	0.001



Supplementary fig. 1. **Inhibition of platelet PI3K isoforms moderately affects thrombin receptor-induced Ca^{2+} responses.** Human Fura-2-loaded platelets were treated with aspirin, preincubated with ADP receptor blockers, and treated with PI3K inhibitor, as described for Fig. 2. Shown are Ca^{2+} responses upon stimulation with thrombin (10 nM). *A.* Representative $[Ca^{2+}]_i$ traces; *B.* inhibitor effects expressed as percentages of time-$[Ca^{2+}]_i$ integrals relative to the control condition. Note that at a low dose of thrombin (1 nM), PIK-75, TGX-221 or the combination of both (0.5 μM) suppressed the Ca^{2+} rise to 107±3%, 87±2% or 101±5% of control, respectively. Data are means ± S.E. (n=3-5); *p<0.05 compared to control.



Supplementary fig. 2. **Specificity of PI3Kβ inhibitor TGX-221 for affecting ADP-induced platelet activation.** Human PRP was preincubated with Me_2SO vehicle (control), TGX-221 (0.5-1 μM), PIK-75 (0.5-1 μM) or wortmannin (1 μM), prior to stimulation with ADP. *A.* Representative light transmission aggregation traces induced by 5 μM ADP, and quantitative effect of inhibitors on ADP-induced platelet aggregation. *B.* Binding of FITC-PAC1 to platelets stimulated with 20 μM ADP (10 min). Fluorescence histograms are shown of unstimulated platelets (filled grey), vehicle controls (grey lines) and samples containing indicated inhibitor (black overlays). Means ± S.E. (n=4); *p<0.05 compared to control.