Supplementary Fig. S1 Gq inhibitor YM-254890 50 nM is able to block human platelet shape change and aggregation induced by high concentration of agonists. Platelet aggregation induced by 500 μM AYPGKF, 100 μM SFLRN and 100 μM ADP was also abolished.

Supplementary Fig. S2 Human platelet aggregation downstream of combined Gi and G12/13 signaling activation depends on agonist concentrations. (A) Only higher concentration of agonist induces human platelet aggregation downstream of combined Gi and G12/13 coactivation. (B,C) cAMP decrease and RhoA activation demonstrate Gi and G12/13 activation in platelets stimulated with ADP and different concentrations of U46619 in the presence of Gq inhibitor YM-254890. Panel A and C are typical results of 3 experiments using platelets from 3 different donors, and panel B is expressed as mean ± SEM (n = 3).
Supplementary Fig. S3 Human platelet aggregation, the level of cAMP and Western blot with 60 μM YFLLRNP alone and in combination with low and high epinephrine concentrations. (A) Aspirin-treated, washed human platelets were stimulated with 60 μM YFLLRNP alone and in combination with low and high epinephrine concentrations. Only higher concentration of epinephrine in the presence of 60 μM YFLLRNP induced human platelet aggregation. Tracings shown are representative of 3 independent experiments using platelets from different donors. (B) cAMP decrease demonstrated Gi activation in platelets stimulated with different concentrations of epinephrine in the absence or presence of 60 μM YFLLRNP. The summary of 5 experiments using platelets from different donors are represented. (C) Platelet membrane and total protein were separated by SDS-PAGE and Western blotted using p-Akt (Ser473), p-Akt (Thr308) and p-PAK antibodies. Typical results are representative of 5 independent experiments using platelets from different donors.

Supplementary Fig. S4 Human platelet membrane Akt and PAK phosphorylation downstream of Gi and G12/13 pathways occurs only at high agonist concentration. Human platelets preincubated with DMSO or 50 nM YM-254890 were stimulated at 37 °C for 3 minutes with different concentrations of ADP and U46619. Platelet membrane was extracted. Membrane and total protein were separated by SDS-PAGE and Western blotted using p-Akt (Ser473), p-Akt (Thr308) and p-PAK antibodies. Typical results are representative of 3 independent experiments using platelets from different donors.
Supplementary Fig. S5 Human platelet membrane Akt (Thr308) is phosphorylated downstream of Gi and G12/13 Pathways only at high agonist concentration. Platelets were stimulated and platelet membrane was separated as in Figure 5A, and pAkt (Thr308) were detected and analyzed. Representative blot and the summary of 4 experiments using platelets from different donors are represented.

Supplementary Fig. S6 PI3K inhibitor, Akt inhibitor and PAK inhibitor abolish platelet Akt (Thr308) phosphorylation downstream of combined Gi and G12/13 stimulation. Platelets were processed as in Figure 6B, and pAkt (Thr308) was detected.

Supplementary Fig. S7 PAK inhibitor IPA-3 inhibits Akt phosphorylation in human platelet membrane without influence on Akt membrane translocation. Platelets were preincubated with the PAK inhibitor 10 μM IPA-3 and 50 nM YM-254890 for 3 minutes at 37 °C, and platelet aggregation was induced by combination of ADP and AYPGKF. Platelet membrane protein was extracted and western blotted using Akt, p-Akt (Ser473) and p-Akt (Thr308) antibodies. Typical results are representative of 3 independent experiments using platelets from different donors.