Molecular pharmacology

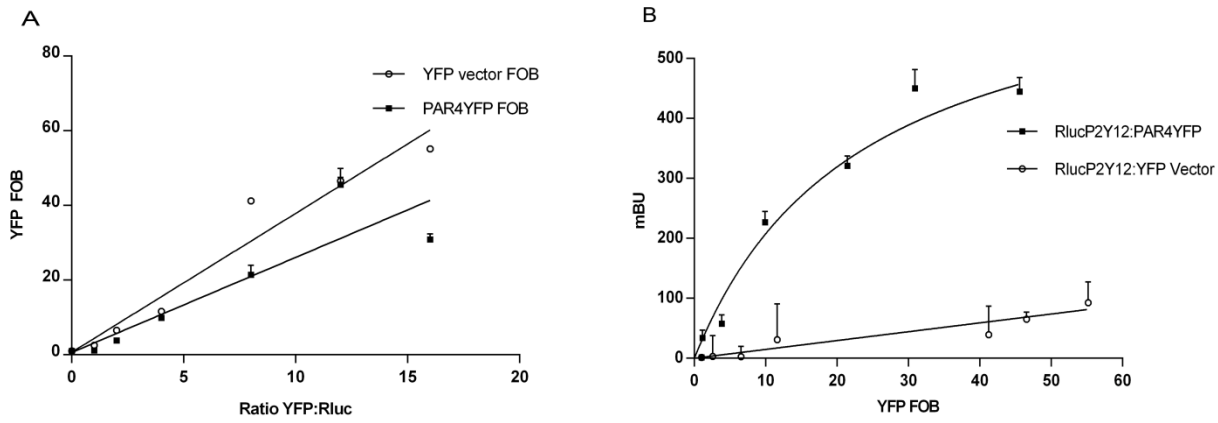
Supplemental Material

The physical association of the P2Y12 receptor with PAR4 regulates arrestin-mediated Akt activation

Aasma Khan, Dongjun Li, Salam Ibrahim, Emer Smyth, and Donna S. Woulfe

Department of Biological Sciences, University of Delaware, Newark, DE, USA. (A.K., D.L., D.W.)
Institute for Translational Medicine and Pharmacology, University of Pennsylvania, Philadelphia, PA, USA. (S.I., E.S.)

Supplemental Figure 1



Supplemental Figure1: HEK293T cells were transiently co-transfected with 0.36µg of Rluc-P2Y12 and varying amounts (0.36-5.76 µg) of PAR4-YFP, or YFP-expressing vector control to achieve YFP: Rluc ratios from 1:1-16:1. 48 hours post transfection, cells were used to measure YFP expression (A) and Saturation BRET (B) as described in methods. A) Fold over basal (FOB) YFP emission increases linearly with increasing ratio of YFP:Rluc : Total YFP emission was measured at 535nm and fold over basal values (FOB) were calculated as fold increase over YFP emission in control cells expressing RlucP2Y12 alone. Linear regression curve was generated using Graph Pad Prism. YFP Vector and PAR4YFP show a linear increase in YFP expression from 1:1 to 16:1 ratio of YFP:Rluc with equations: YFP vector FOB: y= 3.720(x)+0.6816 and R^2= 0.9487 and PAR4YFP FOB: y=2.547(x)+0.6228 and R^2=0.8149 B) Saturation BRET of PAR4 and P2Y12: PAR4 and P2Y12 show a saturable interaction when milli BRET units (mBU) were calculated as described in methods and plotted as a function of YFP FOB. However YFP vector did not show any specific interaction with Rluc P2Y12 even with higher YFP FOB expression values (A). Plotting BRET ouput as mBU against YFP FOB or % maximal BRET against Ratio YFP: Rluc showed no difference in saturation curves and hence %maximal BRET values were plotted against Ratio YFP:Rluc in Figures 1, 2, 3. Shown is a representative saturation BRET experiment with error bars showing ±SEM of 6 technical replicates.

Figure 2

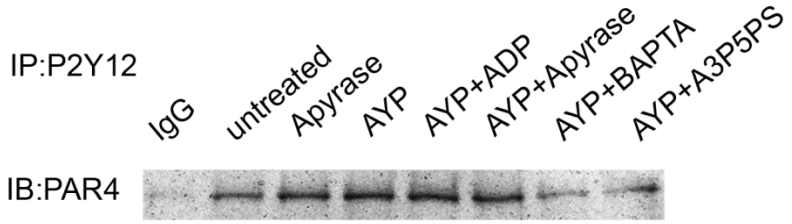


Figure 2: Co-immunoprecipitation of PAR4 and P2Y12 in presence and absence of Apyrase, BAPTA and P2Y1 inhibitor (A3P5PS): HEK293T cells were co-transfected with V5-PAR4-GFP and Flag-P2Y12. 48 hours post transfection cells were left untreated or stimulated with AYPGKF (200μM) 10min at 37°C with and without MeSAMP (150μM) , ADP (20 μM), Apyrase (2U/ml), BAPTA (50μM), A3P5PS (200μM)10min. Cell were lysed, immunoprecipiated with antibodies to Flag (2μg/ml) and immunoblotted with anti V5 (1:1000) (Santa Cruz).