Supplementary Information **Suen et al**

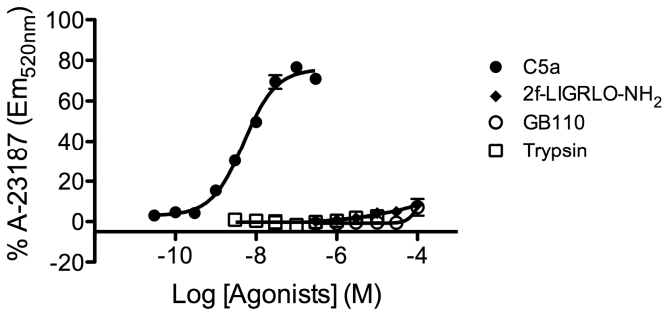

Figure S1. PAR2 agonists do not induce intracellular Ca^{2+} release in U937 cells.

Human U937 cells were treated with C5a (●), 2f-LIGRLO-NH_2 (◆), GB110 (○) and trypsin (□) in the fluorescence intracellular calcium assay. Up to 100μM of 2f-LIGRLO-NH_2 or GB110 and up to 10μM trypsin caused no significant iCa^{2+} release, whereas the hormone C5a did induce iCa^{2+} release at nM concentrations. Error bars represented ± S.E.M. with n≥3.

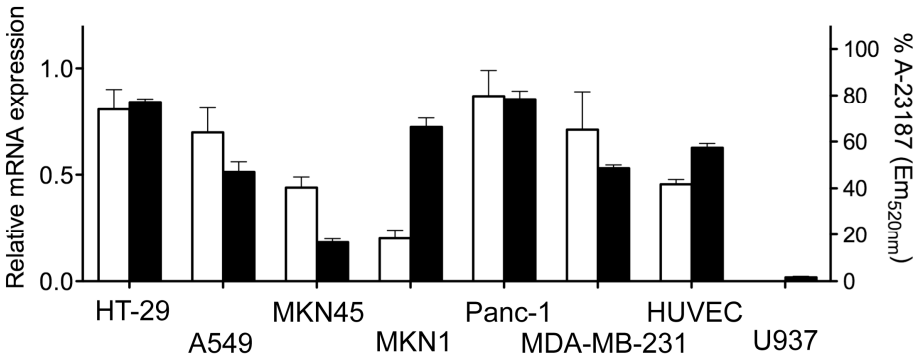

Figure S2. PAR2 mRNA expression correlates with PAR2 surface activity on multiple human cell lines. Quantitative RT-PCR examination of PAR2 mRNA expression in 8 human cell lines is shown in comparison to human housekeeping gene hypoxanthine guanine phosphoribosyl transferase (HPRT). PAR_2 mRNA expression (clear) on each cell line is compared with maximum PAR_2-induced intracellular calcium release (filled). Calcium release is expressed as a percentage of that induced by calcimycin (A-23187) and is used as an indicator of PAR_2 activity on cell surfaces. PAR_2 mRNA levels and its activities were comparable in the cell lines tested. Error bars represented ± S.E.M. with $n \geq 3$.

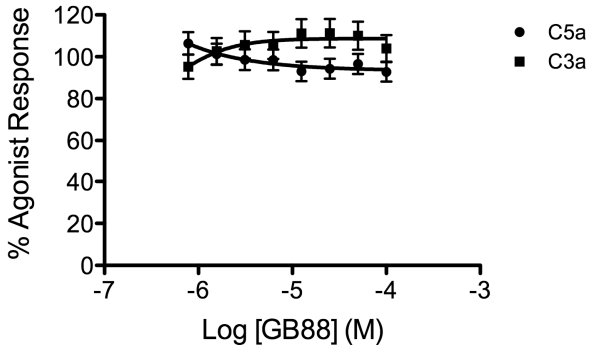

Figure S3. GB88 does not antagonize two other GPCRs. Human U937 cells were treated with either 30nM C5a (●) or 300nM C3a (■) in an intracellular calcium assay. Up to 100μM GB88 showed no significant reduction in iCa^{2+} release in these cells. Error bars represent ± S.E.M. with n≥3.

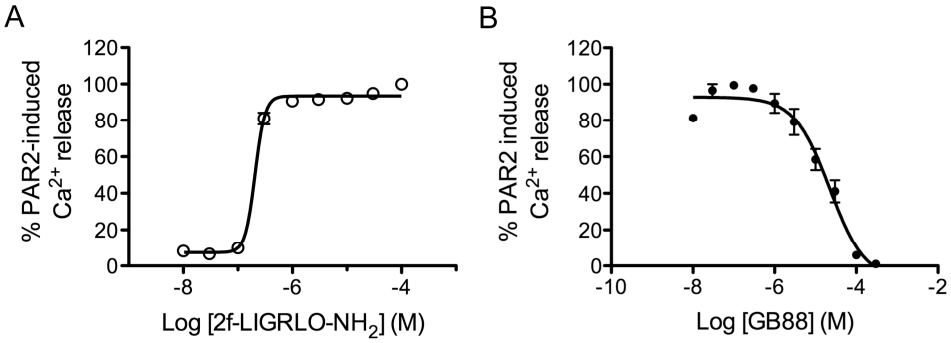

Figure S4. Profile of PAR_2 agonist (2f-LIGRLO-NH_2) and antagonist (GB88) on rat NRK-52e cells. A, concentration-dependent curve for iCa^{2+} mobilization by 2f-LIGRL-NH_2. EC_{50} 210 nM (pEC_{50} 6.7 ± 0.07). B, PAR_2 antagonist GB88 inhibits iCa^{2+} release induced in NRK-52e by 1μM 2f-LIGRLO-NH_2. IC_{50} 20 μM (pIC_{50} 4.7 ± 0.2). Data points = means of 3 experiments in triplicate, bars = S.E.

Method: Quantitative RT-PCR

Cells were plated at 2 x 10^6 cells/well in a 6-well plate for overnight, after which total RNA was isolated using GenElute Mammalian Total RNA Miniprep Kit (Sigma Aldrich) according to manufacturer's instructions. RNA was reverse-transcribed using Superscript III (Invitrogen) and an oligo-dT primer. Relative gene expression was quantitated by real-time PCR using SYBR Green PCR master mix (Applied Biosystems, Foster City, CA, USA) on an Applied Biosystems Prism 7000 sequence detector. Amplification cycle proceded as followed: 50°C for 2 min and 95°C for 10 min, followed by 40 cycles of 95°C for 15 s and 50°C for 1 min. cDNA levels at the linear phase of amplification were compared to hypoxanthine guanine phosphofibosyl transferase (HPRT) levels, and expressed as a relative expression of HPRT. Primer sequences used for HPRT are F- 5' TCAGGCAGTATAATCCAAAGATGGT 3'; R – 5' AGTCTGGCTTATACTCAACACTTCG 3' and for PAR2, F – 5' GGGTTTGCCAAGTAACGGC 3'; R – 5' GGGAACCAGATGACAGAGAGG 3'.