Online Supplemental Information for Suen et al:

Pathway Selective Antagonism Of Proteinase Activated Receptor 2

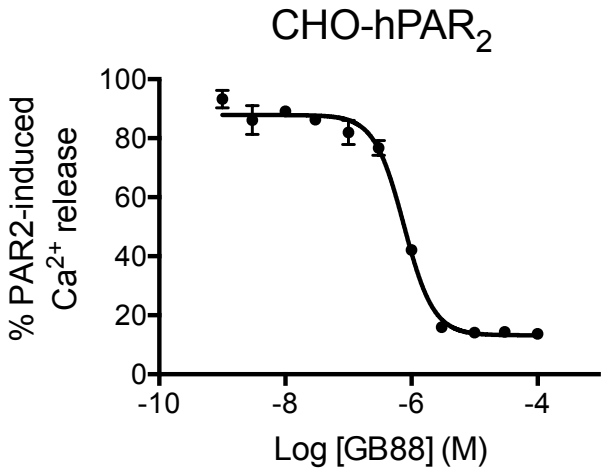

Figure S1. GB88 blocked 2f-LIGRLO-NH_2-induced intracellular calcium release in CHO-hPAR_2. Cells were incubated with various concentrations of GB88 for 15 min, then treated with 2f-LIGRLO-NH_2 (1 μM) and monitored for intracellular calcium release.

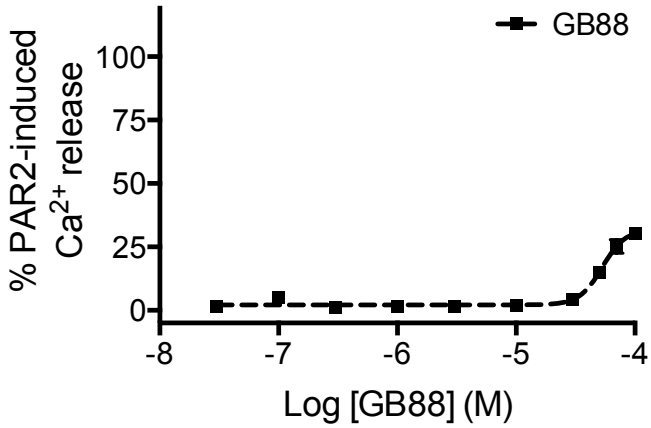

Figure S2. GB88 induced intracellular calcium release at high concentration in HT29.

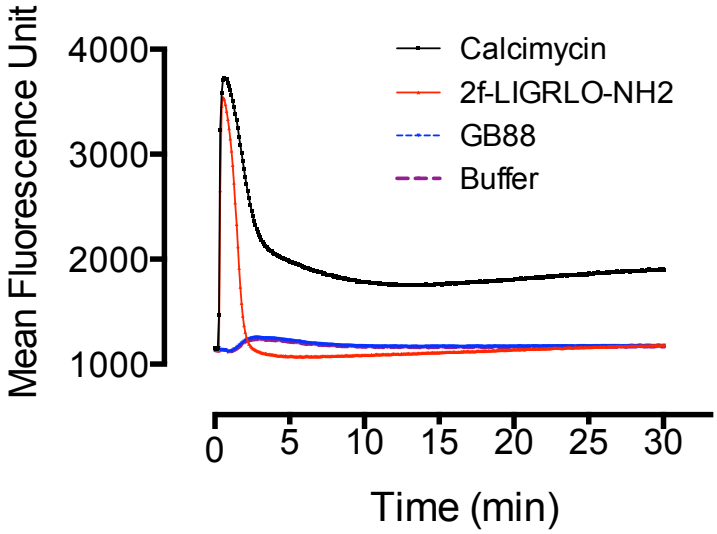

Figure S3. Intracellular calcium release by PAR_2 ligands in HT29 cells. Calcimycin was used a control. Cells were treated with 2f-LIGRLO-NH_2 (1 μM, red) and GB88 (10μM, blue) and

fluorescence measured for 30 min after compound addition. 2f-LIGRLO-NH_2 showed similar maxima as calcimycin and GB88 showed no significant different from buffer (purple) alone.

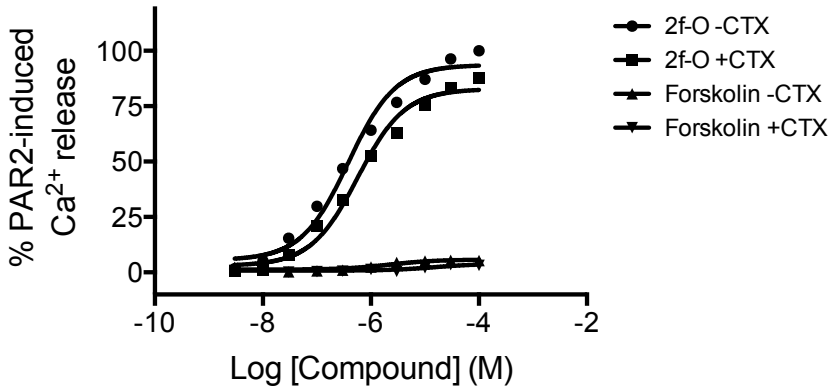

Figure S4. 2f-LIGLRO-NH_2 increased intracellular calcium release independent of Gs in HT29 cells. HT29 cells were treated with cholera toxin (200ng.mL^{-1}, 24 h) and treated with 2f-LIGRLO-NH_2 or forskolin for calcium release. Forskolin failed to induce intracellular calcium release and CTX has no effect on PAR_2-mediated Ca^{2+} response.

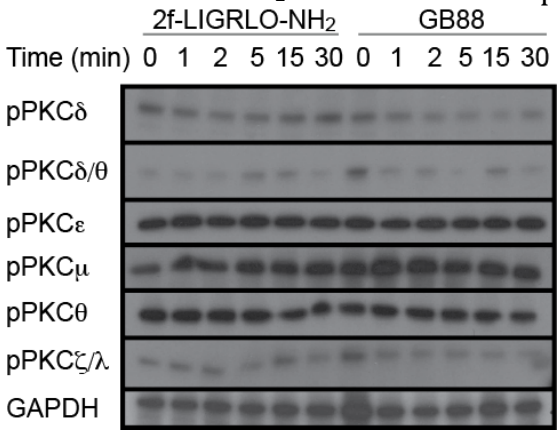

Figure S5. 2f-LIGRLO-NH_2 and GB88 failed to significantly increase phosphorylation of PKC subtypes in HT29 cells. Cells were treated with 2f-LIGRLO-NH_2 (1 μM) or GB88 (10 μM) for various durations and examined by western blot using phosphor-specific antibodies.

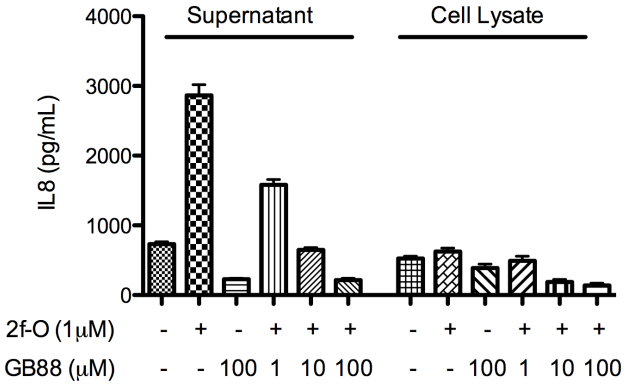

Figure S6. GB88 blocks IL-8 secretion in HT29 cells. Cells were treated with 2f-LIGRLO-NH_2 (1 μM, 24 h) with various concentration of GB88. Supernatants or cell lysates were collected and analyzed by ELISA. GB88 was able to block 2f-LIGRLO-NH_2-induced IL-8 secretion and no significant changes in level of IL-8 in cells.

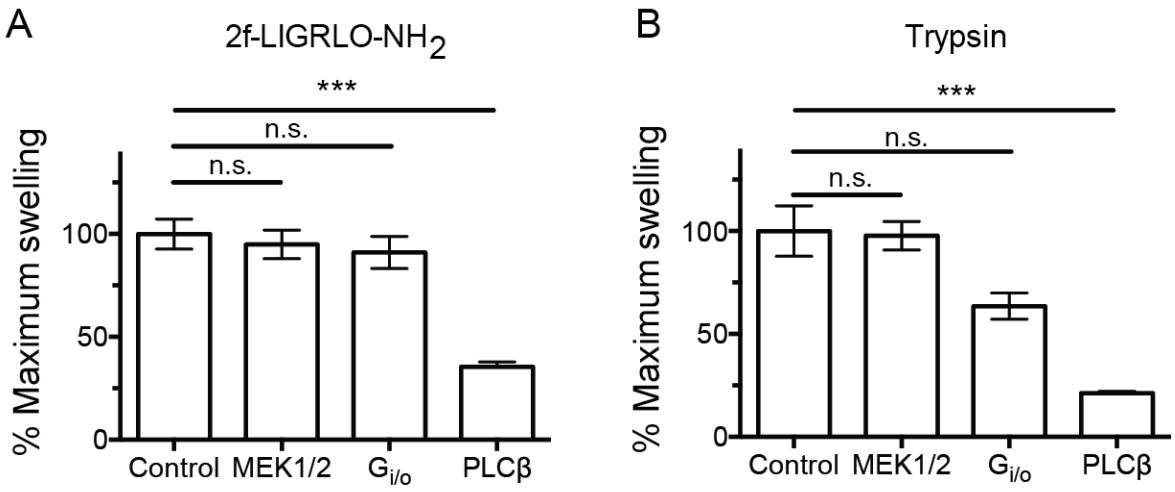

Figure S7. Effects of inhibitors on PAR2-induced paw oedema in rat. PLCb (100 μM, 30 min) significantly inhibited Wistar rat paw oedema induced by intraplana injection of PAR2 agonist 2f-LIGRLO-NH_2 (A, 350 μg) or trypsin (B, 20 μg). Other inhibitors (MEK1/2 (U0126, 10 μM) or Gi/o (PTX, 5 μg) failed to cause any significant changes. N = 3, *** $p<0.001$.