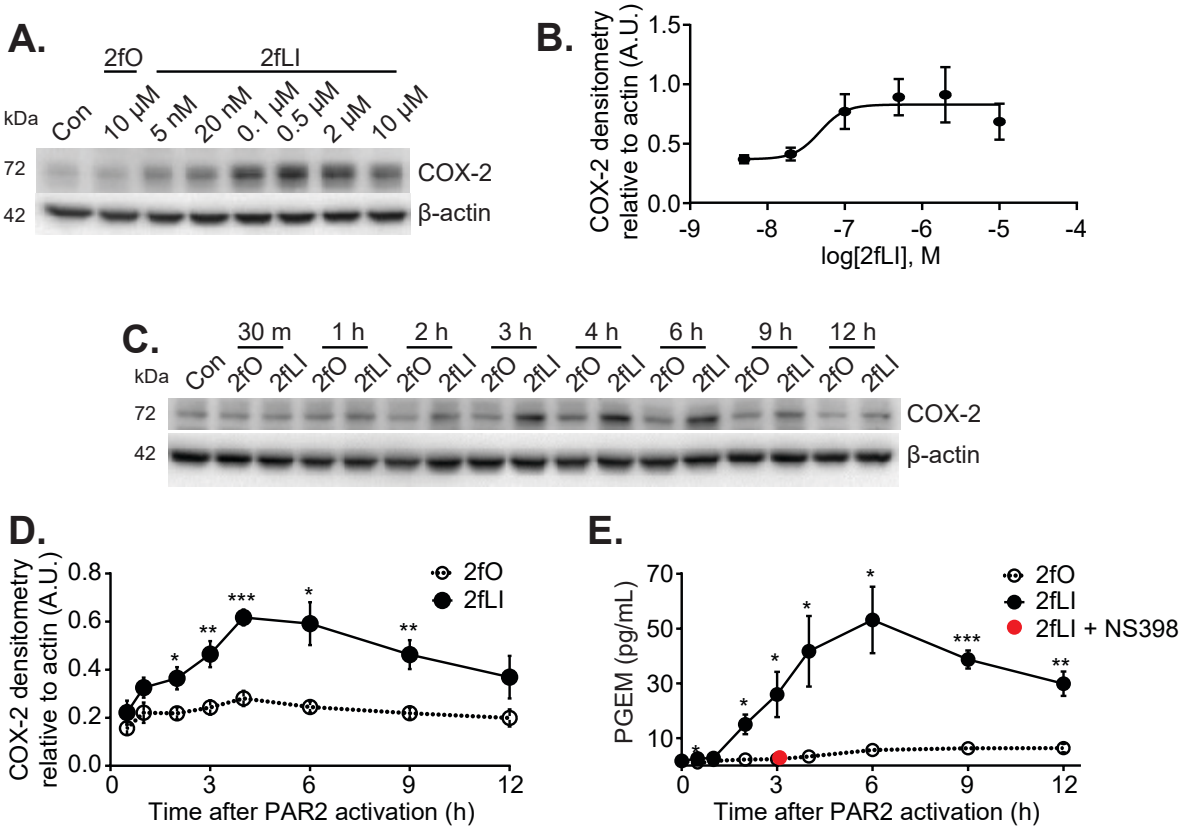
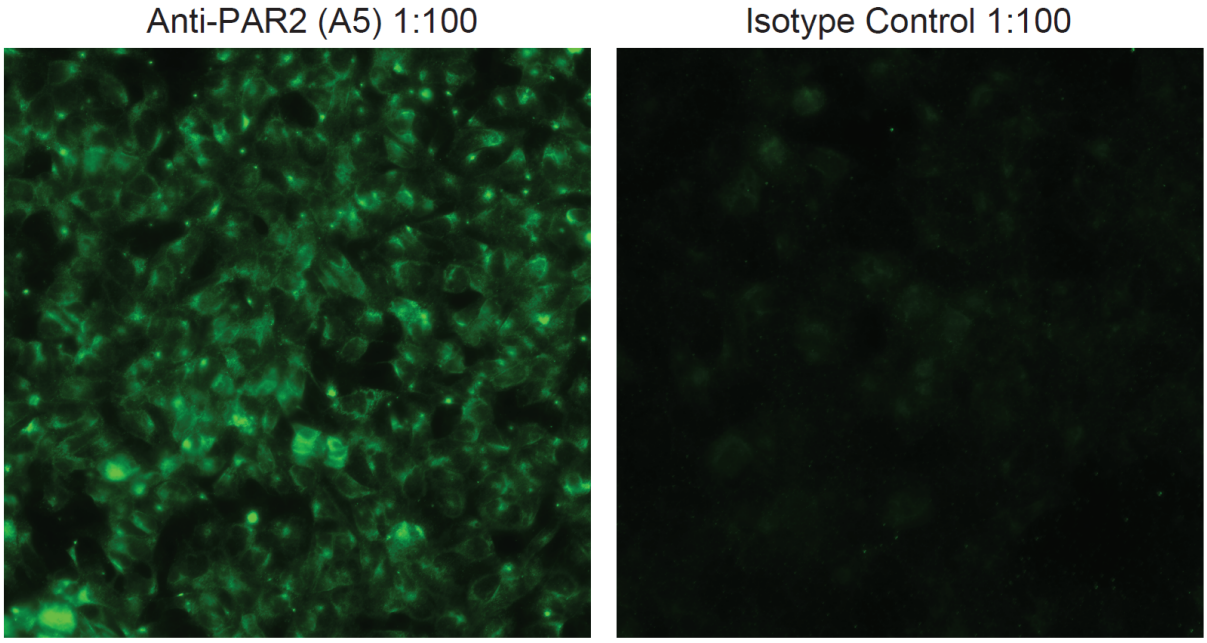
Fernando, Gordon, Beck and MacNaughton
Inhibition of intestinal epithelial wound healing through protease-activated receptor-2 activation in Caco2 cells
Journal of Pharmacology and Experimental Therapeutics



Supplemental Figure 1. PAR2 activation induced the expression of COX-2 in a concentration- and time-dependent manner. A-B: Caco2 cells (8 x 10^4 cells/well, 12 well plate) grown for 5 days were serum-starved for 1 h, then treated with varying concentrations of 2fLI (5 nM – 10 μM), or a high concentration of 2fO (10 μM) for 4 h. Control cells were left untreated. A: Whole-cell lysates were analyzed by western blot for COX-2 and β-actin (representative blot of n=6). B: A concentration-response curve was prepared according to the COX-2 densitometry relative to β-actin. C-E: Caco2 cells were treated with 2fO or 2fLI (0.5 μM) for times ranging from 30 min to 12 h. Control cells were left untreated. C: Whole-cell lysates were analyzed by western blot for COX-2 and β-actin (representative blot of n=8). D: Densitometry analysis of COX-2 expression relative to β-actin. E: Supernatants from the same experiments were collected and run on a PGEM EIA (n=6-8). *Statistics: data in D and E were analyzed using an unpaired two-tailed t-test with Welch's correction at each time point. (* p<0.05; ** p<0.01; *** p<0.001).*

Fernando, Gordon, Beck and MacNaughton
Inhibition of intestinal epithelial wound healing through protease-activated receptor-2 activation in Caco2 cells
Journal of Pharmacology and Experimental Therapeutics



Supplemental Figure 2. Expression of PAR2 in confluent CaCo2 cells. A: Caco2 cells grown to confluency on glass chamber slides were fixed with 4% PFA and stained for PAR2 using an in-house PAR2 "A5" antibody, and imaged on a Nikon Ti Eclipse Widefield microscope (40x magnification).

Fernando, Gordon, Beck and MacNaughton
Inhibition of intestinal epithelial wound healing through protease-activated receptor-2 activation in Caco2 cells
Journal of Pharmacology and Experimental Therapeutics

Figure legends for supplemental Excel files

Supplemental File 1. Transcription profiles associated with Gene Ontology "Cell migration [0016477]" pathways. Caco2 cells were either wounded, or treated with 2fLI (10 μM) for 3 h, and used to create four conditions: not wounded control (NW-Con), wounded control (W-Con), not wounded PAR2-activation (NW-PAR2), and wounded PAR2-activated (W-PAR2). Isolated RNA from biological triplicates was sequenced, aligned, and run through CuffDiff to generate differential gene expression lists. Pathway analysis was conducted via the Gene Ontology database, and results for the "Cell migration [0016477]" pathways displayed. Data are expressed as fold change comparisons, and visual heatmaps indicate fold changes down in blue, fold changes up in yellow. Grey indicates genes for which there were no detectable reads.

Supplemental File 2. Transcription profiles associated with Gene Ontology "Regulation of response to wounding [1903034]" pathways. Caco2 cells were either wounded, or treated with 2fLI (10 μM) for 3 h, and used to create four conditions: not wounded control (NW-Con), wounded control (W-Con), not wounded PAR2-activation (NW-PAR2), and wounded PAR2-activated (W-PAR2). Isolated RNA from biological triplicates was sequenced, aligned, and run through CuffDiff to generate differential gene expression lists. Pathway analysis was conducted via the Gene Ontology database, and results for the "regulation of response to wounding [1903034]" pathways displayed. Data are expressed as fold change comparisons, and visual heatmaps indicate fold changes down in blue, fold changes up in yellow. Grey indicates genes for which there were no detectable reads.

Figure legends for supplemental videos

Video S1
Time lapse video of wound healing over 24 h in untreated Caco2 cells.

Video S2
Time lapse video of wound healing over 24 h in Caco2 cells treated at 0 h and 12 h with the inactive peptide, 2fO (10 μM).

Video S3
Time lapse video of wound healing over 24 h in Caco2 cells treated at 0 h and 12 h with the PAR2-activating peptide, 2fLI (10 μM).