

**Supplemental figure 1.** Caco-2 and T-84 monolayers were incubated with CCM from *E. faecalis* V583 (black bars). The trans-epithelial electrical resistance (TEER, a measure of barrier integrity) was measured before and after the addition of CCM. The change in TEER following a 24 hr incubation with CCM from *E. faecalis* V583 was calculated by subtracting the TEER at 24 hr from the TEER at baseline (before CCM was added). An increase in the change in TEER is indicative of the loss of barrier integrity. *E. faecalis* V583 CCM significantly increase a change in TEER following incubation for 24 hr when compared to controls (white bars) for both Caco-2 and T-84 monolayers,  $p < 0.05$ . Data are expressed as mean values  $\pm$  standard error. Comparisons were made using the non-parametric Mann-Whitney test.

**Supplemental figure 2.** T-84 monolayers were incubated with CCM from *E. faecalis* V583 and *E. faecalis*  $\Delta$ gelE. The flux of FITC-dextran across T-84 monolayers was not affected when monolayers were apically cultured with *E. faecalis* V583 CCM for 24 hr compared to the media control or *E. faecalis*  $\Delta$ gelE.

**Supplemental figure 3.** (A) *E. faecalis* V583 mediates monolayer permeability of Caco-2 via PAR2. Monolayers were incubated with or without a PAR2 agonist (FSLLRY-NH<sub>2</sub>) for 24 hr. Monolayers were then incubated with *E. faecalis* V583 CCM or a media control. The flux of FITC-dextran across monolayers was increased when monolayers were cultured with *E. faecalis* V583 CCM for 24 hrs compared to controls. *E. faecalis* V583 CCM-mediated permeability of monolayers was reduced when PAR2 activation was blocked with an antagonist across a range of concentrations as indicated. (B) T-84 monolayers were incubated basally with or without a FSLLRY-NH<sub>2</sub> for 24 hr. Monolayers were then basally incubated with *E. faecalis* V583 CCM or a media control. *E. faecalis* V583 CCM-mediated permeability of T-84 monolayers was not reduced when PAR2 activation was blocked with an antagonist, however the TEER of the monolayers were impacted (C). Data are expressed as mean values  $\pm$  standard error. Comparisons were made using a one-way analysis of variance (ANOVA).

**Supplemental figure 4.** The permeability of the mouse colonic epithelium is not different between WT (n=10) and PAR2<sup>-/-</sup> (n=5) mice. The flux of FITC-dextran across the colonic epithelium (paracellular permeability) over 2 hrs of WT and PAR2<sup>-/-</sup> mice was measured using Ussing chambers. No significant differences in permeability were observed.

**Supplemental figure 5. *E. faecalis* V583 CCM impacts the detection of PAR2 in epithelial cells over time.** *E. faecalis* V583 CCM was incubated with Caco-2 cells for 30 minutes, 1 hr, 2 hr, 4 hr, and 24 hr. The detection of PAR2 in epithelial cells exposed to GeIE decreased over time, with PAR2 detection returning after 24 hr. PAR expression in Caco-2 cells began to decrease following 1 hr.