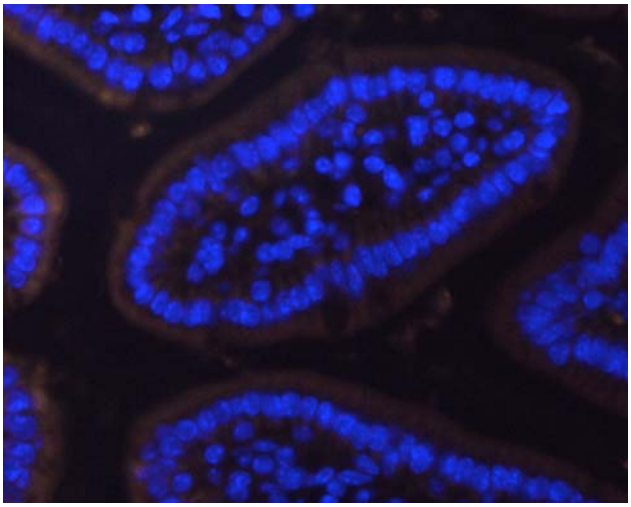
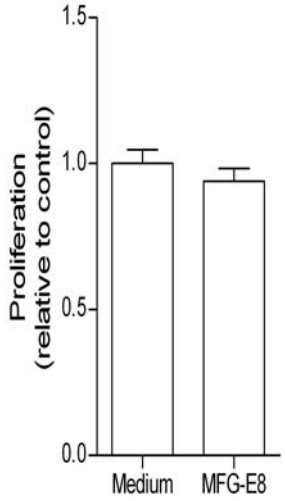


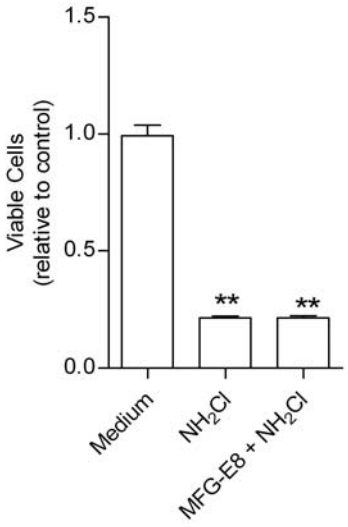
Supplemental Figure 1

Negative control of immunostaining for MFG-E8 and F4/80. The paraffin sections from the small intestine of normal mice were immunostained with a cocktail containing nonimmune hamster IgG and biotin-labeled rat IgG instead of primary antibodies. After washing with PBS, the slide was stained with a secondary antibody cocktail containing Alexa Fluor 546-labeled goat anti-hamster IgG Ab and streptavidin-labeled Alexa Fluor 633 as described in Methods. Nuclei were counterstained with DAPI (blue). Then slides were examined under a fluorescent microscope. A merged staining profile is illustrated. It reveals a background staining in the enterocytes of intestinal villi. Cells in lamina propria were negative for the staining. Original magnification, X 63.



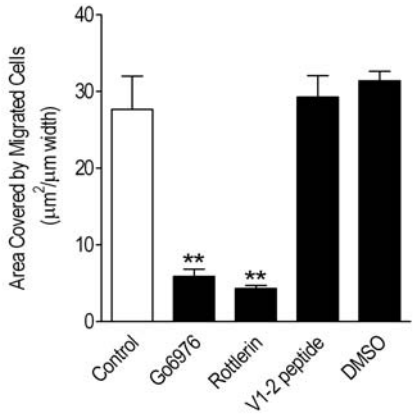
Supplemental Figure 2

Effect of MFG-E8 on cell proliferation. To assay the change in cell number after MFG-E8 treatment, cells were plated in 96-well culture plates at 3 X 10^3/well. At 24 h after plating, cultures were exposed to MFG-E8 (10 nM) or culture medium alone for 24 h. Cells were counted using an MTS-based colorimetric proliferation assay kit (Promega Corp.). Reported values reflect averages of at least 6 replicate wells.



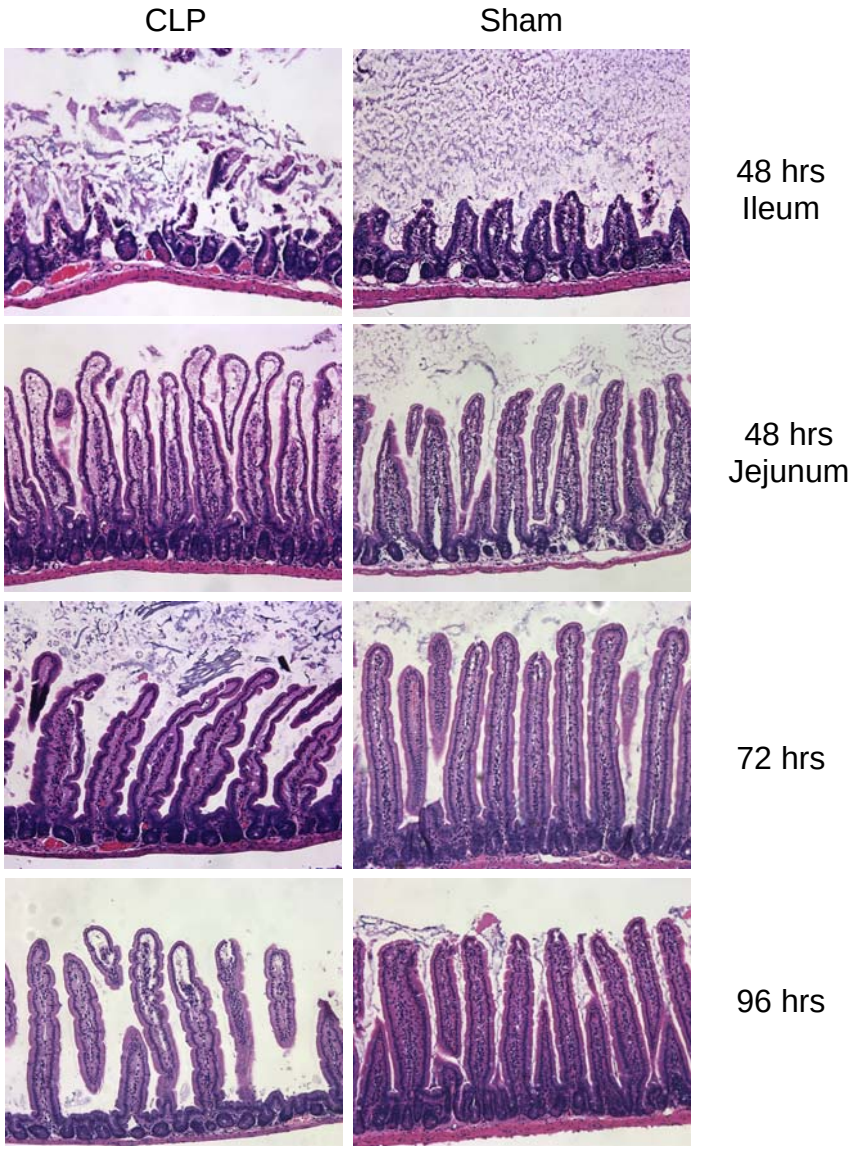
Supplemental Figure 3

Effect of MFG-E8 on cell-permeant reactive oxygen species (ROS)-induced IEC-18 cell injury. IEC-18 cells were plated in 96-well culture plates at 3 X 10^3/well. At 24 h after plating, cultures were exposed to culture medium alone, NH_2Cl (0.3 mM, a cell-permeant ROS), or MFG-E8 (10 nM) + NH_2Cl (0.3 mM) for 4 h. The viable cells were determined using a colorimetric cell viability assay kit (Promega Corp.). Reported values reflect averages of at least 6 replicate wells. NH_2Cl (monochloramine) was prepared using a standard protocol in our laboratory (Zhu et al., Am J Physiol Cell Physiol. 2003. 285:C1294-303). $^{**}P<0.01$, compared with the Medium group.



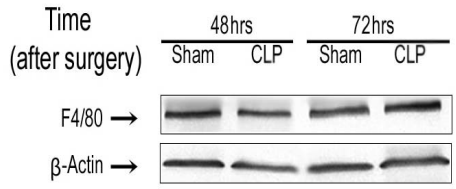
Supplemental Figure 4

Effect of selective inhibitors for PKCα, PKCδ, and PKCε on spontaneous IEC-18 cell migration. Confluent IEC-18 monolayers were divided into groups as indicated in the figure. Cells in PKC inhibitor groups were pretreated with Gö6976 (5 μM, PKCα inhibitor), Rottlerin (10 μM, PKCδ inhibitor), or V1-2 (150 μg/ml, PKCε translocation inhibitor peptide), or DMSO (5 μl/ml, solvent for dissolving Gö6976 and Rottlerin) for 90 min. Afterwards, wounding was performed as described in Methods. Wounded monolayers were cultured for 5 h after addition of serum-free culture medium with or without test-compounds. Cell migration was quantitatively analyzed as described Methods. n = 3 per group. Results are expressed as means $\pm$ S.E.M. ** $P<0.01$ compared with the control group.



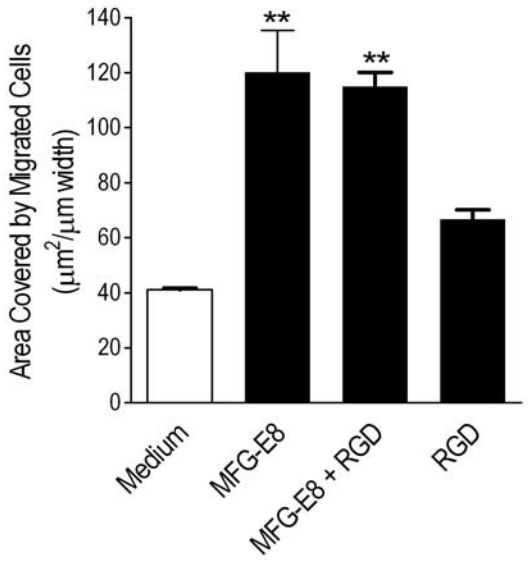
Supplemental Figure 5

CLP induces the injury in the murine small intestine. Mice were subjected to CLP or sham operation as described in Methods. Animals were sacrificed at indicated time intervals. Small intestinal tissues were processed for routine histology. Paraffin-embedded sections were stained with hematoxylin and eosin for light microscopy. Original magnification, X 10



Supplemental Figure 6

F4/80 expression after CLP. Mice were subjected to CLP or sham operation as described in Methods. The expression of F4/80 in the small intestine was examined at the indicated intervals of post-surgery with western blotting. The same blot was stripped and reprobed with mAb against β-actin following a standard procedure. Anti-F4/80 mAb (1:500, Serotec Ltd). Anti-β-actin mAb (1:10,000, Sigma Chemical).



Supplemental Figure 7

RGD peptide, a blockade for αvβ3/5 integrin, does not block MFG-E8-induced IEC-18 cell migration. Serum-starved confluent IEC-18 monolayers were left untreated or were treated with RGD peptide (1 mM, Bachem) for 90 min, as indicated, prior to the induction of wounding. This dose was in excess of that used by Croyle et al. (Croyle, M.A., Walter, E., Janich, S., Roessler, B.J., and Amidon, G.L. 1998. *Hum. Gene Ther.* **9**:561-573.). Wounded monolayers were treated with MFG-E8 (10 nM) for 5 h, where mentioned, in the presence or absence of RGD peptide. Cell migration was quantitatively analyzed as described in Methods. n = 6. ** $P<0.01$ versus control.