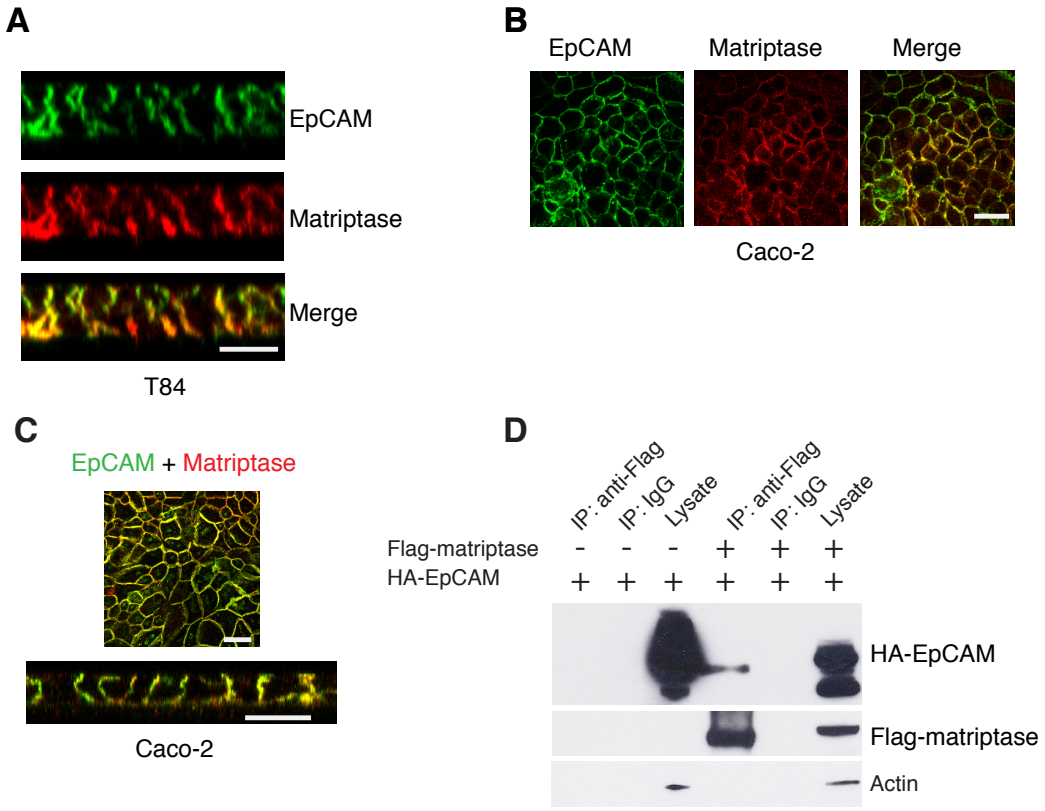
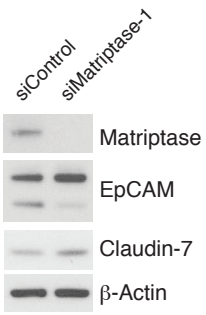
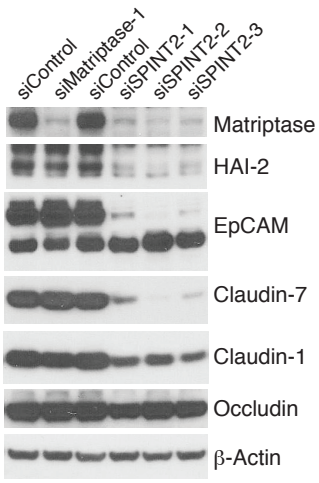


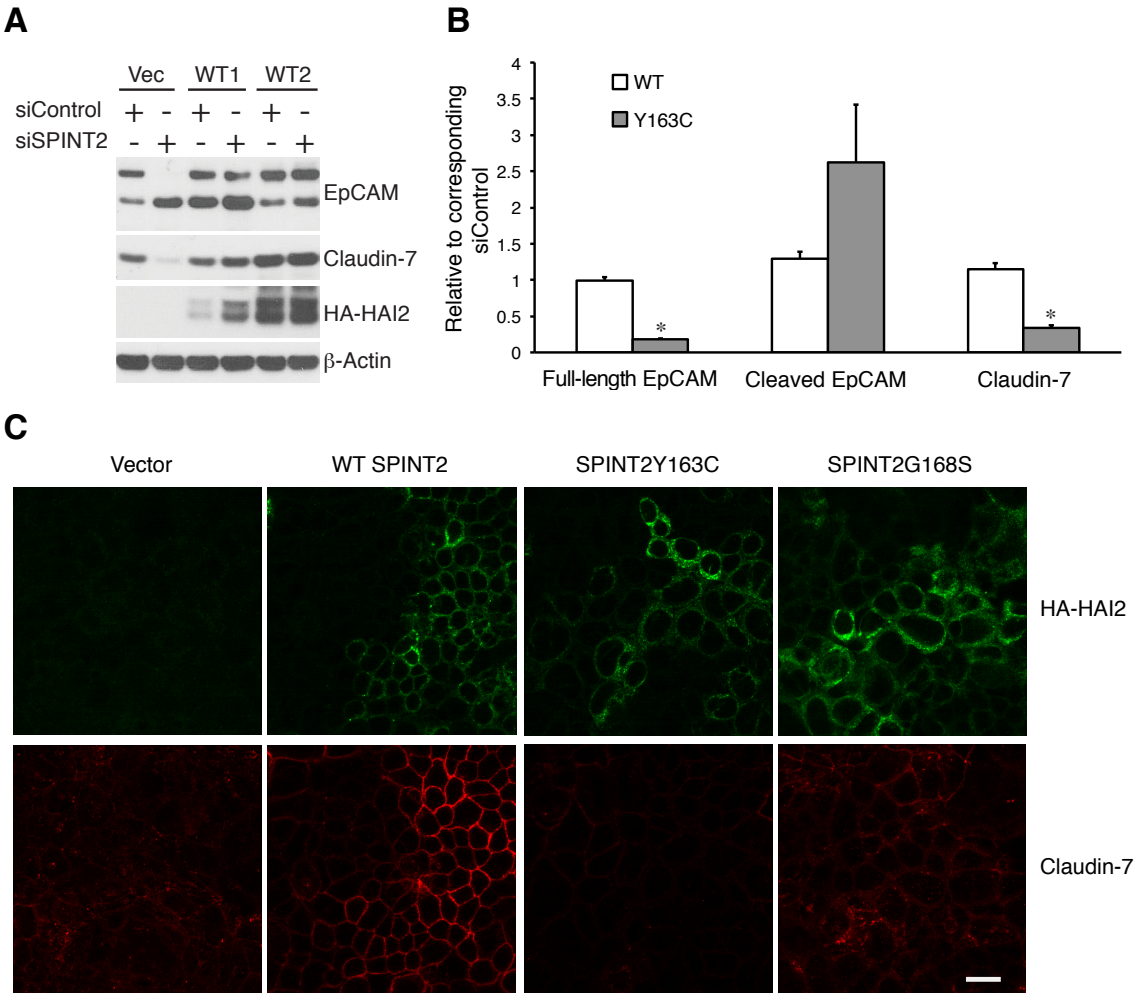
Supplemental Figure 1 (supplemental to Figure 2). **Matriptase/EpCAM co-localization in IEC monolayers and matriptase/EpCAM interaction when co-expressed in 293 cells.** Confluent T84 (**A**) or Caco-2 (**B** and **C**) cell monolayers in Transwells were stained with anti-EpCAM (green) and rabbit anti-matriptase (red) (**A** and **B**) or sheep anti-matriptase (red) (**C**). XZ (**A**) or XY (**B**) or both (**C**) images from confocal immunofluorescence microscopy studies are shown. Scale bars, 10 μm (**A**) or 20 μm (**B** and **C**). (**D**) 293 cells were transfected with plasmid expressing HA-EpCAM with or without plasmid expressing Flag-matriptase. After 48 h, cells were lysed and TX-100 lysates were immunoprecipitated with anti-Flag mAb or control IgG. Immunoprecipitated and lysate proteins were resolved with SDS-PAGE and immunoblotted with anti-HA, anti-Flag or actin as indicated. Representative data from 1 of 3 experiments is shown for all panels.



Supplemental Figure 2 (supplemental to Figure 4). **Maptriptase promotes EpCAM cleavage in T84 cells.** T84 cells were transfected with control siRNA or matriptase siRNA using electroporation. After 72 h, cell lysates were prepared and resolved using SDS-PAGE. EpCAM species and claudin-7 were detected via immunoblotting with anti-EpCAM or anti-claudin-7 Ab. β-actin was used as a loading control. Representative data from 1 of 3 experiments is shown.



Supplemental Figure 3 (supplemental to Figure 6C). **Destabilization of claudin-1 in SPINT2 knockdown Caco-2 cells may be time- and/or cell density-dependent.** Caco-2 cells were transfected with control siRNA, EpCAM siRNA, matriptase siRNA or SPINT2 siRNA via electroporation. Transfected cells were re-plated the next day and cultured for 5 additional days to allow monolayer formation and maturation. Cell lysate proteins were resolved using SDS-PAGE and immunoblotted with anti-matriptase, anti-HAI-2, anti-EpCAM, anti-claudin-7, anti-claudin-1 or anti-occludin. Representative data from 1 of 3 experiments is shown.



Supplemental Figure 4 (supplemental to Figure 7). **SPINT2 siRNA targets only endogenous *SPINT2* expression.** (**A**) Stable vector-transfected or wild type SPINT2-transfected Caco-2 clones were transiently transfected with control siRNA or SPINT2 siRNA that targets non-translated sequences using electroporation. Three days after siRNA transfection, cells were lysed in RIPA buffer, cell lysate protein concentrations were normalized, proteins were resolved using SDS-PAGE, and subsequently immunoblotted with anti-EpCAM, anti-claudin-7 or anti-HAI-2 (anti-HA). Representative data from 1 of 4 experiments is shown. (**B**) Data depicted represent aggregated results obtained with WT HASPINT2 (WT1 and WT2) and mutant HASPINT2 (Y163C1 and Y163C2) as shown in Figure 7C. A two-way ANOVA was performed. P-values related to comparisons between WT HASPINT2 and mutant HASPINT2 transfected cells were determined using a two-tailed t-test without correction for multiple comparisons (*$p<0.001$). (**C**) Single color immunofluorescence images corresponding to the lower panel of Figure 7D lower panel are displayed. Scale bar = 20 μm.