

Figure S1. Tff2 expression in mouse tissues. (A) *Tff2* expression in the small intestine served as a control for relative quantification. *Tff2* expression was significantly upregulated in small intestinal polyps and the normal stomach compared to the small intestine (** $P < 0.0001$; one-way analysis of variance and Dunnett's multiple comparisons test, $n=3$). (B) Western blot analysis of mouse tissue extracts; (a) Tff2 antibody and (b) Gapdh antibody; each lane was loaded with 50 μg of protein extracted from the respective tissues, including the small intestine, colon, stomach, and intestinal polyps. Three mice were used in this study. Tff2, trefoil factor family member 2; Gapdh, glyceraldehyde-3-phosphate dehydrogenase.

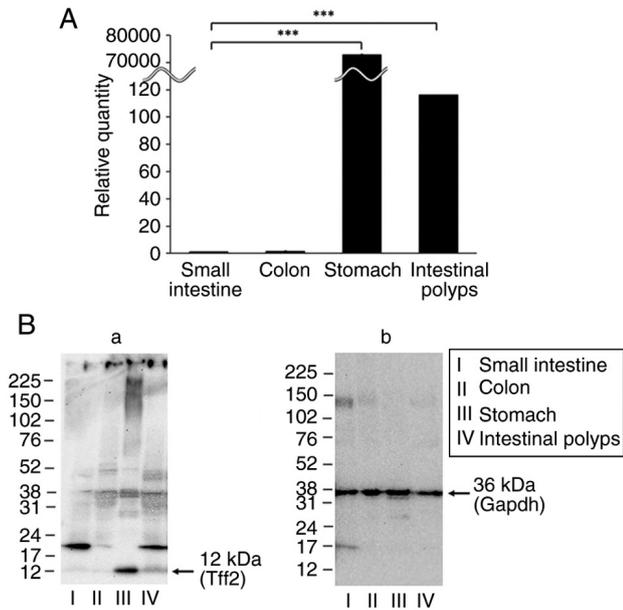


Figure S2. Validation of immunostaining with the TFF2 antibody was performed by transiently transfecting DLD-1 cells with *pcDNA 3.1/c-(K)-DYK-TFF2* (human *TFF2*) at pH 7.4. Additionally, mock cells were prepared by transiently transfecting DLD-1 cells with *pcDNA 3.1/c-(K)-DYK* (empty vector). P-values were calculated using relative quantification values via the nonparametric Mann-Whitney U test. (A) Relative comparisons using reverse transcription-quantitative polymerase chain reaction; *TFF2* expression in untransfected DLD-1 cells served as a control for relative quantification, with *GAPDH* as the reference gene. *TFF2* expression exhibited significant differences between mock or *hTFF2* transfected cells and untransfected cells as a control (** $P < 0.0001$). Each assay was conducted in quadruplicate, and the bar and error bar represent the mean and standard error (SE), respectively. (B) Immunohistochemistry against TFF2; (a) to mitigate false positives or nonspecific binding, we omitted the primary antibodies and considered them as negative controls. (b) Images of DLD-1 cells in the presence of the TFF2 primary antibody. Cells were observed under x100 magnification (scale bar=10 μm). (C) Quantification of TFF2-specific DAB staining. (a) and (b) refer to those shown in (B). Quantification of DAB staining was performed using the ImageJ software, utilizing images from four different fields of view ($P < 0.05$). TFF2, trefoil factor family member 2.

