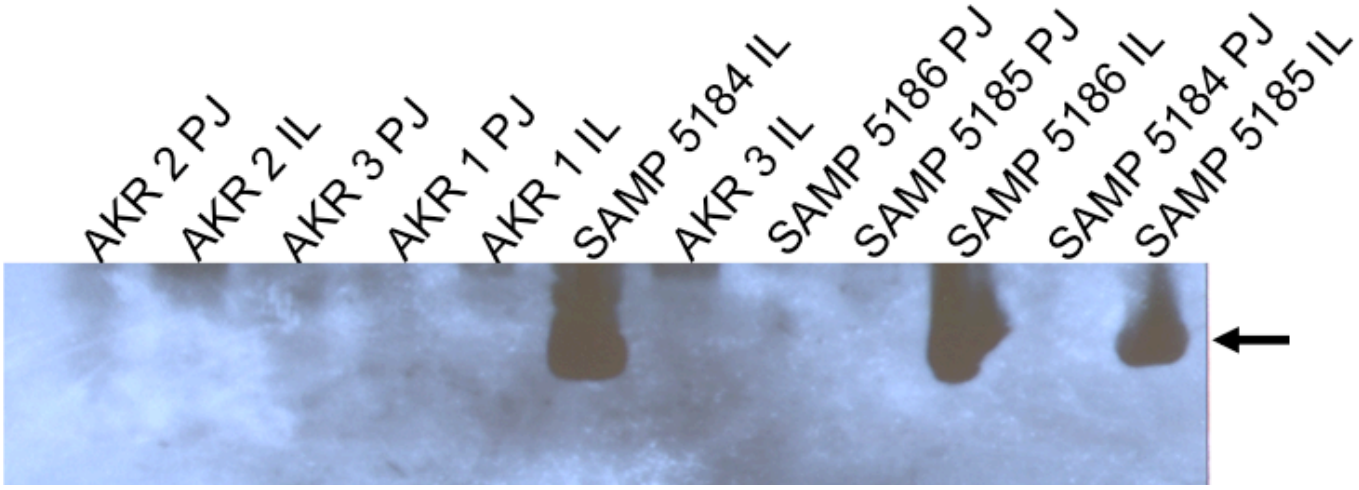
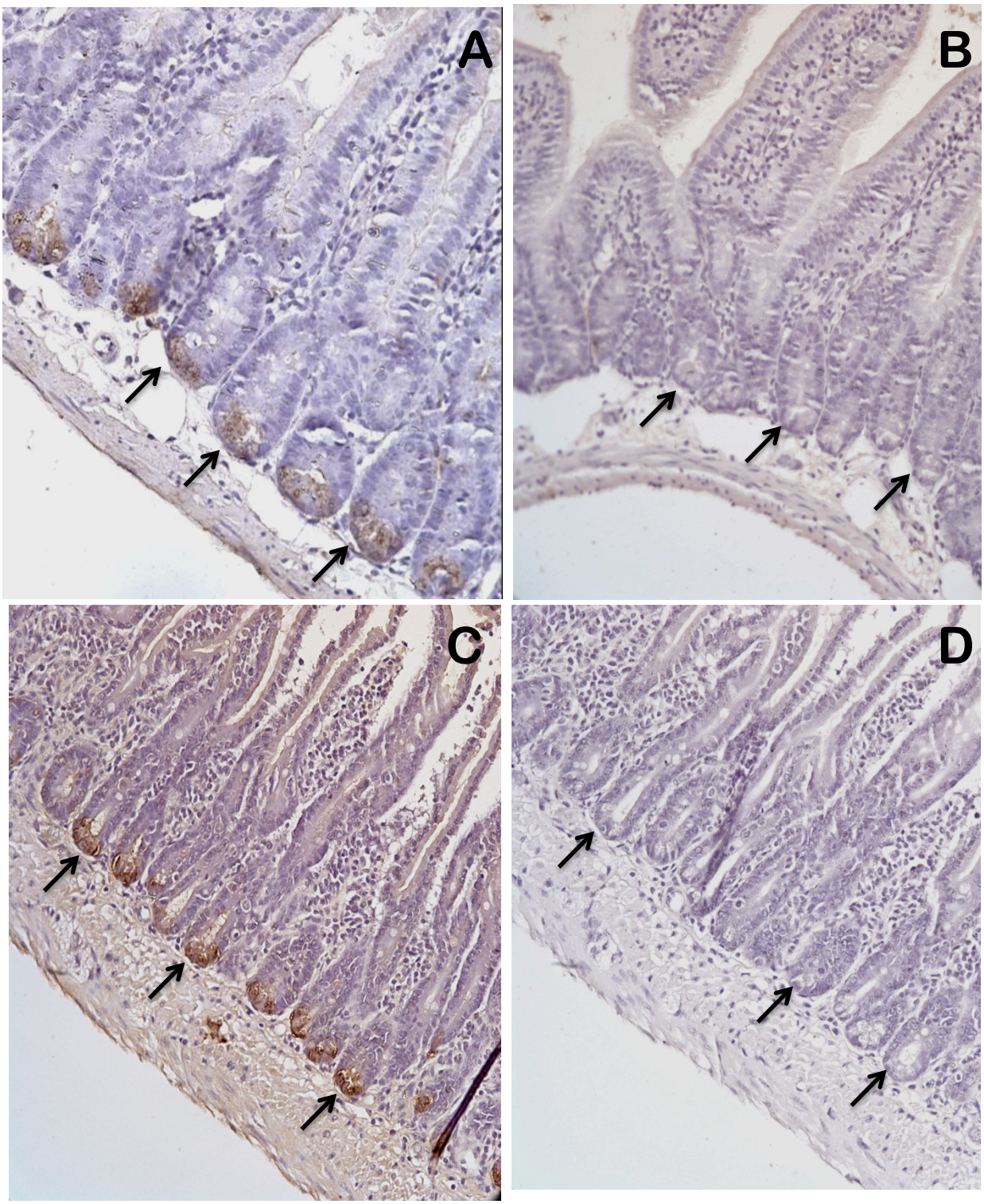

**Supplementary Figure S1. Sequence Comparisons of Mouse Enteric α-Defensin and CRS4C Gene Products.** The deduced primary structure of the prototypic CRS4C gene CRS4C-1 is shown aligned with the gene of the α-defensin Crp-4 (Crp-4) and grouped by exon. Cysteines are denoted by asterisks. Note that the signal peptide and prosegment of Crp-4 are nearly identical to the first exon-encoded sequence of CRS4C-1. Also note that the sequence of the MMP-7 activated, mature Crp-4 peptide including the canonical α-defensin arrangement of cysteines is unrelated to the second exon-encoded sequence of CRS4C-1. The three MMP-7 cleavage sites identified in Crp-4 (Shirafuji, Y., Tanabe, H., Satchell, D.P., *et al.*, J. Biol. Chem. 278: 7910–7919, 2003) are indicated by black downward arrows. The sequences of corresponding sites in the primary structure of pro-CRS4C-1 are similar to those in Crp-4.

## Supplementary Figure S2




**Supplementary Figure S2. Comparative CRS4C Peptide Levels in SAMP1/YitFc and AKR Mouse Strains.** Relative levels of CRS4C peptide in protein extracts (see Experimental Procedures) of proximal jejunum (PJ) and ileum (IL) was determined for three individual AKR (1-3) and SAMP1/YitFc (SAMP5184, 5185, 5186) mice by western blotting. Samples (500 μg) of total extracted organ protein 10 week-old AKR and SAMP1/YitFc mice were separated by AU-PAGE and blotted onto a nitrocellulose membrane. The blot was subjected to Western analysis using primary goat anti-CRS4C-1 antiserum diluted 1:20 (see Experimental Procedures). Immunopositive bands that comigrate with recombinant CRS4C-1 (not shown) are indicated by the arrow at right. Under these conditions, CRS4C peptides were detected only in ileum of the SAMP1/YitFc mice. We thank Ms. Claire Dubois for performing this western blot analysis.




**Supplementary Figure S3. Immunohistolocalization of CRS4C in C57BL/6 Mouse Small Intestinal Tissue.** Immunohistochemical staining of the intestinal crypts of C57BL/6 mice using anti-CRS4C-1 antiserum (A), anti-Crp-5 antiserum (C) and preimmune sera (B, D) is shown above. Immunopositive Paneth cells are indicated by black arrows. CRS4C peptides are products of Paneth cells in mouse intestinal crypt.

## *Pro-Cryptdin-4*

**MKTLVLLSALVLLAFQVQA DPIQNTDEETKTEEQPGEEDQAVS ISFGGQEGSA LHEKS LRGLLCYCRKGHCKRGERVRGTCGIRFLYCCPRRR**

## *Pro-CRS4C-1*

**MKKLVLLFALVLLAFQVQA DSIQNTDEETKTEEQPGEKDQAVS VSFGDPQGSA LQDAA LGWGRRCPQCPRCPSCPSCPRCPRCPRCKCNPK**

**Supplementary Figure S4. N-Terminal Sequencing Analysis of Recombinant CRS4C-1 Exposed to MMP-7 *in vitro*.** Samples of recombinant pro-Crp-4 and pro-CRS4C-1 incubated overnight with 0.5 mol equivalents of MMP-7 were analyzed by N-terminal peptide sequencing. The cleavage sites disclosed by protein sequencing are noted by downward arrows. MMP-7 mediated cleavage of pro-CRS4C peptides yields the products of the second exons of CRS4C genes.