**Fig. S1. EPCAM and TROP2 are co-expressed in developing mouse epithelia. (A-H').** Immunohistochemical analysis of EPCAM (A-H) and TROP2 (A'-H') protein expression in tongue (A,A'), palate (B,B'), incisors (C,C'), nasal cavity (D,D'), forestomach (E,E'), glandular stomach (F,F'), thymus (G,G'), and lungs (H,H') from newborn wildtype mice. Insets show corresponding tissues from EPCAM-deficient or TROP2-deficient mice used as controls for antibody specificity. Expression of EPCAM and TROP2 is indicated by arrowheads. Co-expression of EPCAM and TROP2 was detected in stratified epithelia within oral cavity and forestomach (A-B', E,E'), pseudostratified epithelium in nasal cavity (D,D'), as well as in epithelial cells of stratum intermedium in developing tooth (C,C'), thymus medulla (G,G'), bronchiole (H,H', arrowheads) and alveole (H,H', arrows). EPCAM expression in glandular stomach could not be evaluated due to apparent non-specific staining (F,F', open arrowheads). Size bars: (A-B', E-G') 50 μm, (C-D') 75 μm.
**Fig. S2. Loss of EPCAM triggers intestinal inflammation.** (A). Schematics of CRISPR/Cas9-mediated targeting leading to a 20 bp deletion within exon 4 of mouse *Epcam* (left), and a 61 bp deletion within the single-exon *Tacstd2* (right) gene. The deleted parts of the open reading frames and the corresponding regions in the encoding proteins are indicated in red. Only the portion of the protein N-terminal to the mutation site can be correctly translated. Abbreviations in (A): SP, signal peptide; m1, EPCAM motif 1/cysteine rich domain; TY, thyroglobulin 1A domain; CPD, cysteine poor domain; TM, transmembrane domain; ICD, intracellular domain. (B). Representative chromatogram results of DNA sequencing of wildtype (top) and (bottom) *Epcam*<sup>-/-</sup> (left) and *Tacstd2*<sup>-/-</sup> (right) mice, confirming successful targeting. (C). Immunohistochimical analysis of CD45 protein expression in small intestines (top) and colon (bottom) from 21 days old control (left) and *Epcam*<sup>-/-</sup> (right) mice. Examples of CD45-positive cells are indicated by arrowheads. Size bars: 50 μm.
**Supplementary figure 3.**

**Fig. S3. Loss of EPCAM affects production of pancreatic enzymes.** Immunohistochemical detection of trypsin (A-A"), chymotrypsin (B-B"), and pancreatic elastase (C-C") in pancreas from 21 days old control (A-C), *Epcam"* (A'-C'), and *Tacstd2"* (A"-C") mice. EPCAM-deficiency leads to an increased intracellular content of digestive enzymes in exocrine pancreas. Size bars: 50 μm.
Fig. S4. Loss of TROP2 does not affect development of intestines and pancreas in EPCAM-deficient mice. (A-C‴). H&E stain of small intestines (jejunum, A-A‴), colon (B-B‴) and pancreas (C-C‴) from 14 days old control (A-C), and their littermate Epcam⁻/⁻ (A′-C′) and Epcam⁻/⁻;Tacstd2⁻/⁻ (A″-C″) mice. Defects resulting from the loss of EPCAM in these tissues were not further affected by the absence of TROP2. Size bars: (A-C‴) 75 μm.
**Fig. S5. Expression of epithelial markers in mice lacking EPCAM and/or TROP2.** Quantification of Western blot signal shown in Figure 5, representing expression of claudin-7 (top), claudin-1 (middle), and E-cadherin (bottom) in tissues from 14 days old wildtype (Control), *Epcam*^-/-^, *Tacstd2*^-/-^, and *Epcam*^-/-^;*Tacstd2*^-/-^ double-deficient mice. Each bar represents average value from two mice per genotype and tissue, relative to GAPDH (see Figure 5).
Table S1. Sequences of guide RNAs and genotyping primers used in this study.

| Gene       | Guide target/Primer sequences                                      |
|------------|--------------------------------------------------------------------|
| Gene targeting |                                                                 |
| **Epcam** | Guide 1: 5’-gaggaggataaagccgag-3’                                   |
|            | Guide 2: 5’-tgactcacagcaagtctggg-3’                                 |
| **Tacstd2** | Guide 1: 5’-gatggcgacccgcttttgca-3’                                |
|            | Guide 2: 5’-gtcgcagaccgtcatcttgt-3’                                 |
| Screening and genotyping |                                                          |
| **Epcam** | 5’-aatccttggcctggatggc-3’                                           |
|            | 5’-ggaaagctcactcactagg-3’                                           |
| **Tacstd2** | 5’-tctggtctgtagttggagg-3’                                           |
|            | 5’-agcagcaggcacttggaag-3’                                           |

Table S2. List of antibodies used in the study

**Immunohistochemistry**

| Antigen | Manufacturer                      | Cat. No. | Concentration |
|---------|-----------------------------------|----------|---------------|
| EpCAM   | R&D Systems, Minneapolis, MN      | AF960    | 5 ug/ml       |
| Claudin-7 | Life Technologies, Rockford, IL | 34-9100  | 2.5 ug/ml     |
| Trop2   | Abcam, Cambridge, MA             | ab214488 | 5 ug/ml       |
| CD45    | Abcam, Cambridge, MA             | ab23910  | 2.5 ug/ml     |
| Trypsin | R&D Systems, Minneapolis, MN     | AF3848   | 5 ug/ml       |
| Chymotrypsin | R&D Systems, Minneapolis, MN | AF6907   | 5 ug/ml       |
| Elastase | Invitrogen                       | PAS2-8155| 5 ug/ml       |

| Antigen | Manufacturer                      | Cat. No. | Concentration |
|---------|-----------------------------------|----------|---------------|
| Anti-rabbit | Vector Laboratories, Burlingame, CA | BA-1000 | 2.5 ug/ml     |
| Anti-goat  | Vector Laboratories, Burlingame, CA | BA-9500 | 2.5 ug/ml     |
| Anti-rat   | Vector Laboratories, Burlingame, CA | BA-9400 | 2.5 ug/ml     |
| Anti-sheep | Vector Laboratories, Burlingame, CA | BA-6000 | 2.5 ug/ml     |

**Western Blot**

| Antigen | Manufacturer                      | Cat. No. | Concentration |
|---------|-----------------------------------|----------|---------------|
| EpCAM   | R&D Systems, Minneapolis, MN      | AF960    | 1 ug/ml       |
| Claudin-7 | Life Technologies, Rockford, IL | 34-9100  | 0.5 ug/ml     |
| Trop2   | Abcam, Cambridge, MA             | ab214488 | 1 ug/ml       |
| GAPDH   | Abcam, Cambridge, MA             | ab9485   | 0.5 ug/ml     |

| Antigen | Manufacturer                      | Cat. No. | Concentration |
|---------|-----------------------------------|----------|---------------|
| Anti-rabbit | Dako Cytomation, Carpinteria, CA | D0487    | 1 ug/ml       |
| Anti-goat  | Sigma, St.Louis, MO              | A4187    | 1 ug/ml       |