Supplemental Data

Supplemental Figure Legend

Figure S1, related to Figure 1. A. High magnification histological images of proximal stomach of wild type E18 embryo showing squamous epithelium (top) and p63 null E18 embryo showing metaplastic epithelium (middle, bottom). B. Immunofluorescence micrograph of section of stomach of p63 null, E18 embryo showing distribution of villin (green) and Agr2 (red) (top panel) and keratin 8 (green; middle panel). *Lower panel*, histogram of percentage of cells expressing Krt8, Vil1, and Agr2 in metaplastic cells.

Figure S2, related to Figure 2. A. Relative hybridization intensity of Cdx2 probes on expression microarrays probed with RNA from esophagus (ES), proximal stomach (PS), distal stomach (DS), small intestine (SI), and large intestine (LI) from wild type (WT) and p63 null (KO) E18 embryos. B. Heatmaps of p63 null metaplasia and Barrett's esophagus versus esophagus and small intestine. Top, Heatmap of Barrett's esophagus versus normal esophagus and normal human small intestine. Bottom, Heatmap of p63 null proximal stomach versus wild type proximal stomach and small intestine.

Figure S3, related to Figure 2. A. Immunohistochemistry on sections of a human Barrett's esophagus biopsy showing staining with antibodies to markers observed in the p63 null metaplasia of the proximal stomach. The upper left is a histological stain showing a squamous island that is positive for antibodies to p63 (upper right). All other sections show immunohistochemistry using antibodies to the indicated gene products including Krt7, Vil1, Cldn3, Agr2, Muc4, and FCGBP. B. Chart tabulating corresponding data from all 16 Barrett's cases examined. C. Venn diagrams of gene ontology (GO) analysis of datasets from p63 null metaplasia versus Barrett's.

Figure S4, related to Figure 3. A. Tracking Muc4 expression in sections of proximal stomach of p63 null embryos through indicated days of development using antibodies to Muc4 by immunofluorescence. B. Absence of squamous differentiation in p63 null metaplasia. Immunofluorescence micrographs showing the normal differentiation of the squamous epithelium in late embryonic wild type mice marked by loricrin antibodies (red) and the apical distribution of residual embryonic epithelium labeled with keratin 7 antibodies (green). C. Metaplasia in the late embryonic stages of p63 null mice labeled with keratin 7 antibodies (green) and antibodies to loricrin (red).

Figure S5, related to Figure 5. Markers of primitive epithelium at the squamocolumnar junction of wild type mice are expressed in the p63 null metaplasia of E18 embryos. Top frames, anti-Muc4 (green); bottom frames, anti-CEACAM (red).

Figure S6, related to Figure 7. A. Squamocolumnar junction in adult DTA-Krt14-Cre mice with and without Tamoxifen treatment. Histological staining of sections through the squamocolumnar junction of a four-week-old DTA-Krt14-Cre mouse revealing the strict apposition of squamous and columnar epithelia and an overall thinning of the squamous epithelium after one week of Tamoxifen treatment and hyperkeratosis. The deep junction shows cellular disorder and a loss of a cornified layer. Corresponding immunofluorescence with antibodies to Krt6 (red) and Krt7 (green) with and without Tamoxifen treatment over one week. B. Co-localization of CEACAM red) with Krt7-expressing cells (green) at the squamocolumnar junction after treatment with Tamoxifen in DTA-Krt14-CreER mice. C. *Top panel*, Section through the squamocolumnar junction of DTA-Krt14-CreER mice stained with antibodies to loricrin (red) and Krt7 (green). Bottom panel, Section through the squamocolumnar junction of DTA-Krt14-CreER mice treated for seven days with Tamoxifen stained with antibodies to loricrin (red) and Krt7 (green).

Table S1, related to Figure 2. List of genes from two human Barrett's datasets (Barrett's #1 and #2) that overlap with those of p63 null metaplasia (KO proximal stomach vs WT proximal stomach).

Table S2, related to Figure 2. Comparison of top 50 genes overexpressed in p63 null metaplasia with two human datasets derived from Barrett's esophagus. Red indicates that both human datasets showed overexpression of the gene, green only Barrett's 1, blue only Barrett's 2. P values and fold-change are indicated.

Table S3, related to Figure 2. Gene Ontology (GO) terms.

Table S4, related to Figure 5. List of 87 genes that overlap between the wild type three-week old squamocolumnar junction (WT junction vs WT proximal stomach), p63 null E18 metaplasia (KO proximal stomach vs WT proximal stomach), and the Barrett's #1 dataset.

Figure S1, related to Figure 1

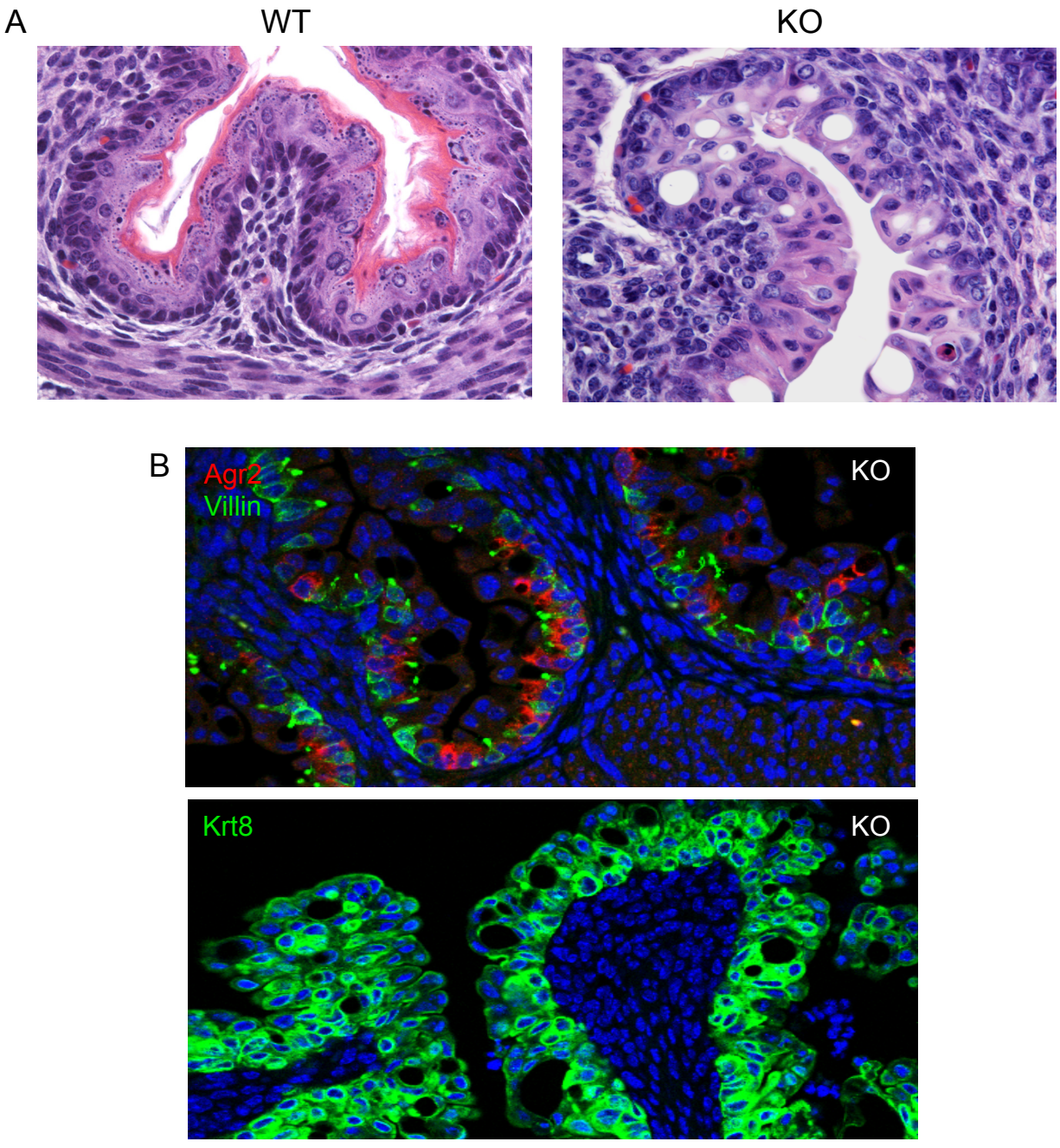
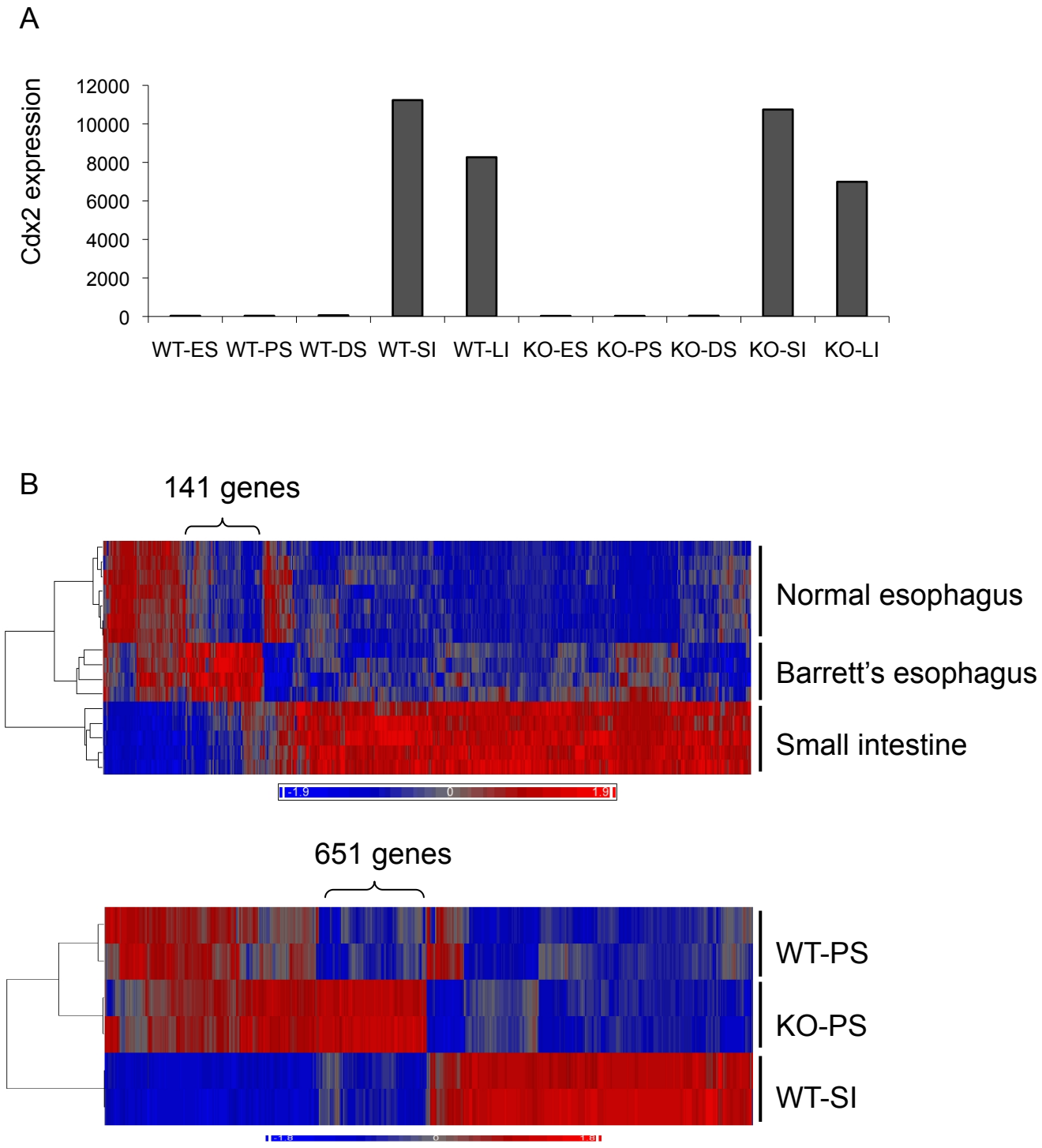


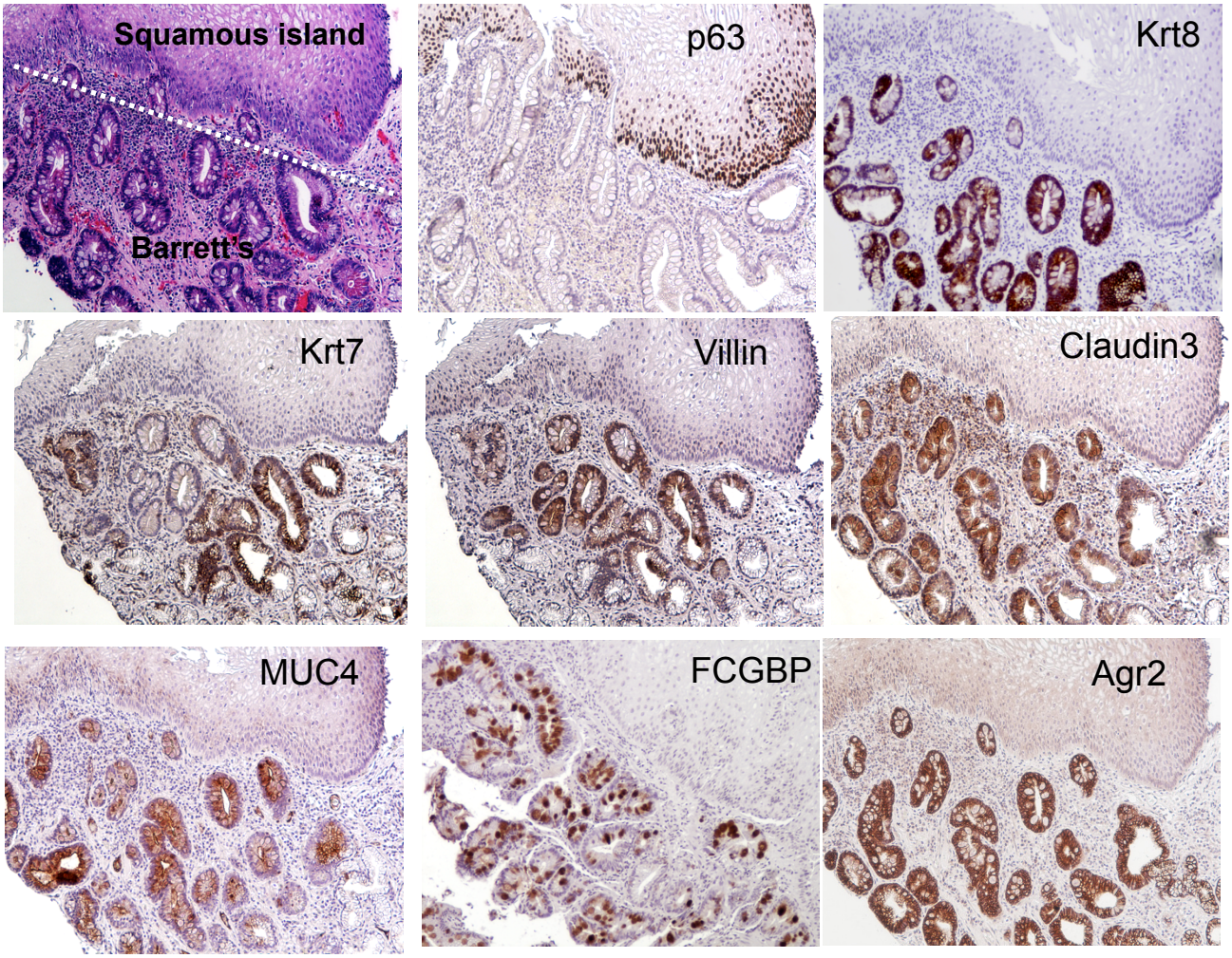
Figure S2, related to Figure 2



Supplemental Figure

Figure S3, related to Figure 2

A



B

Gene symbol	Gene Title	Frequency among BE cases (n=16)
Krt7	Keratin 7	16/16
Muc4	Mucin 4	16/16
Agr2	Anterior gradient 2	16/16
Vil1	Villin 1	14/16
Krt8	Keratin 8	16/16
Cldn3	Claudin 3	16/16
Fcgbp	Fc fragment of IgG Binding protein	16/16
p63	TP63	0/16

C

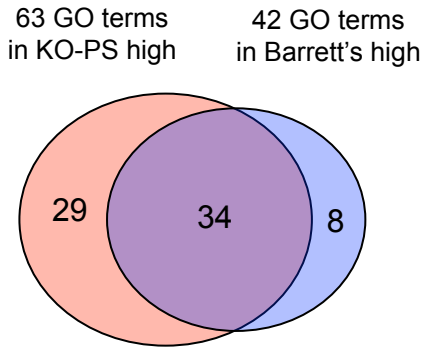


Figure S4, related to Figure 3

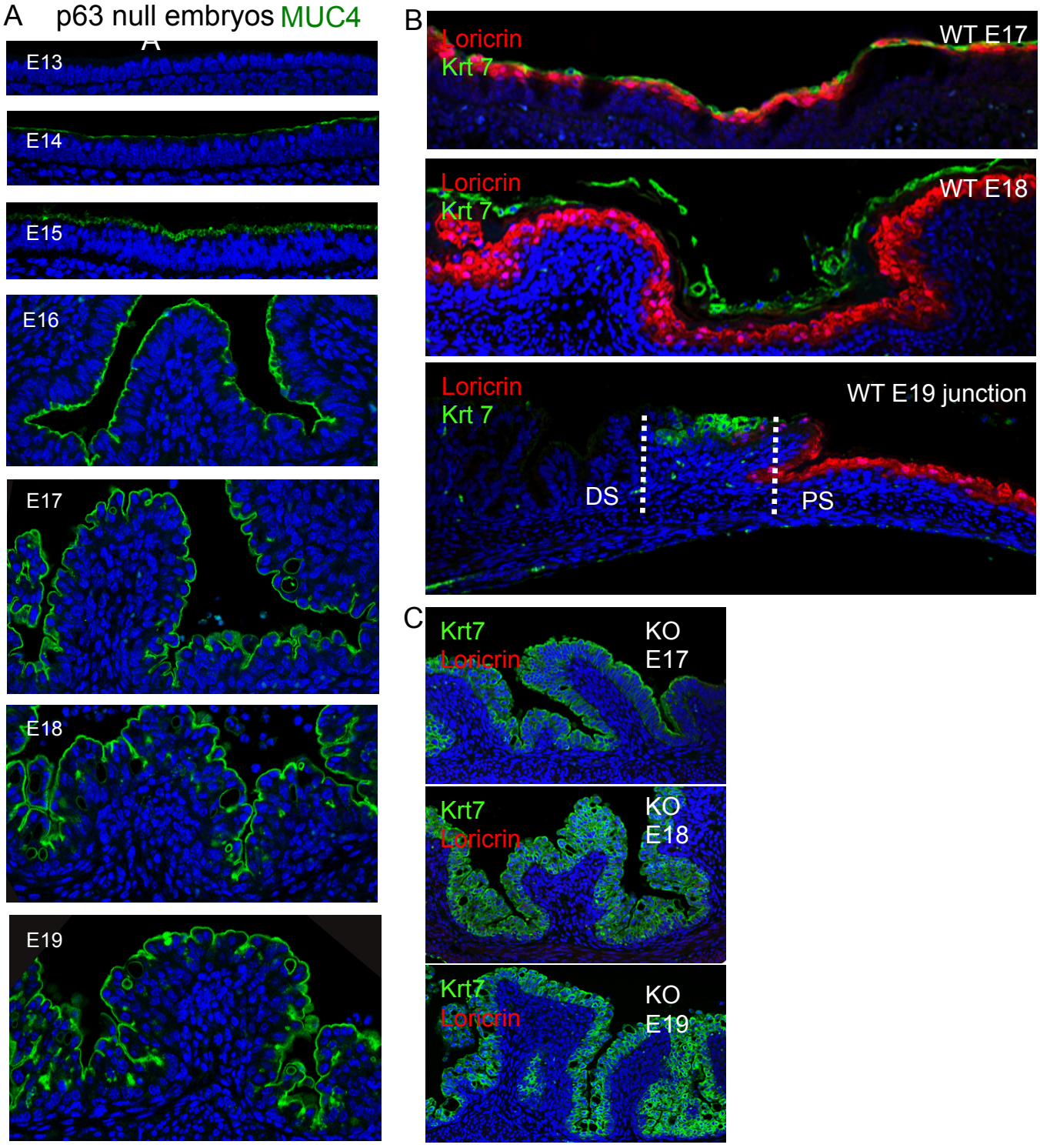


Figure S5, related to Figure 5

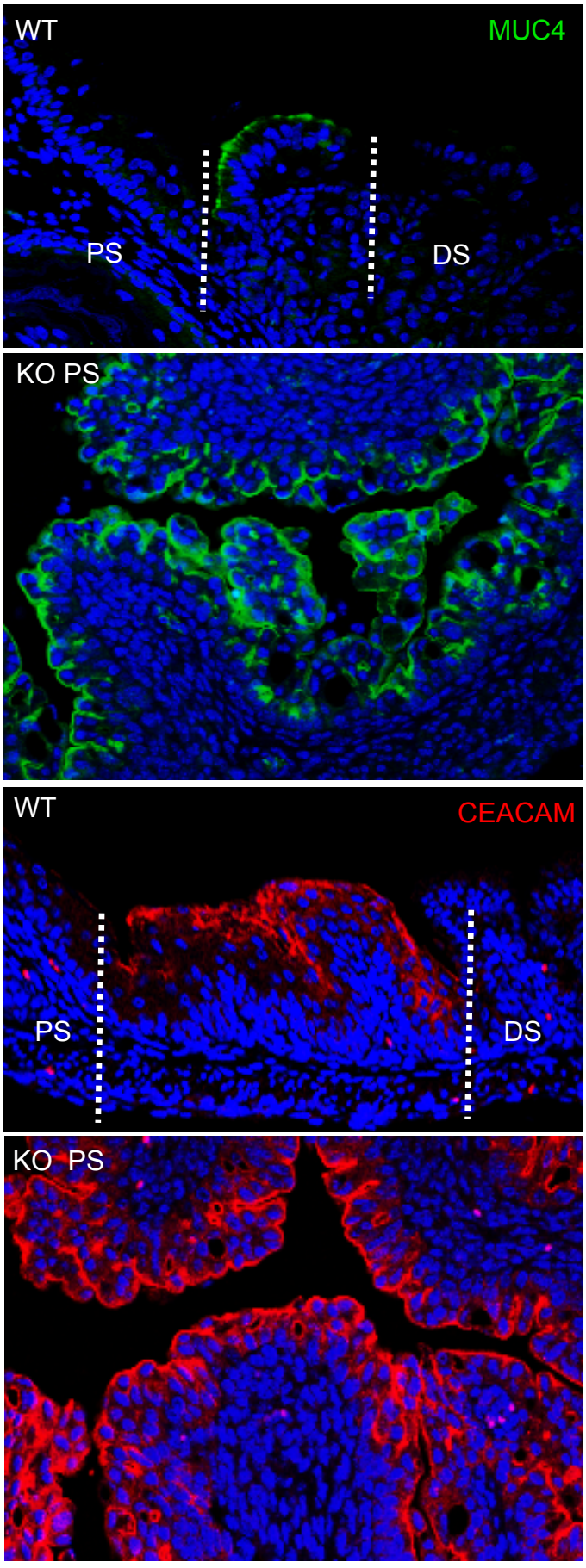


Figure S6, related to Figure 7