Supporting Information

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SI Materials and Methods

Microarray Analysis. Total RNA was extracted from isolated colonocytes using the RNeasy midi kit (Qiagen). The RNA was analyzed using an Applied Biosystems platform as recommended by the manufacturer. Two RNA samples were prepared for each of the four sample types ($K8^{+/+}$ untreated, $K8^{+/+}$ antibiotic-treated, $K8^{-/-}$ untreated, and $K8^{-/-}$ antibiotic-treated) for a total of eight samples. Each sample was assayed on duplicate microarrays, and results were quantile normalized per replicate array pair. Following normalization, data were filtered to remove "undetected" microarray probes (detection was defined as a signal-to-noise ratio >3 on both arrays of a replicate pair). The correlation coefficient R of assay signal for each replicate array pair then was calculated to verify high correlation between replicate arrays. Differentially expressed genes were identified using a t test and filtering for $P < 0.01$. Differentially expressed genes were filtered further by applying a false-discovery rate procedure before analysis using the PathArt pathway database from Jubilant Biosys. Normalization, correlation analysis, and t tests were performed using MATLAB (Mathworks). Additional data plotting and visualizations were performed using Spotfire DecisionSite (TIBCO) and GeneSpring (Agilent Technologies).

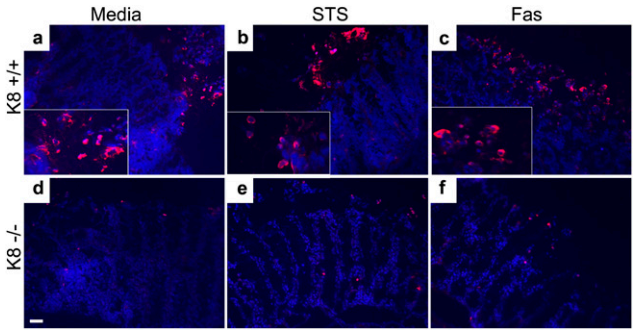


Fig. S1. Resistance of keratin 8-null ($K8^{-/-}$) colon to apoptosis. Colon organ segments from $K8^{+/+}$ and $K8^{-/-}$ mice were cultured in the presence or absence of staurosporine (STS) or Fas for 1 h. The organ cultures then were processed for antibody staining of apoptotic cells (red) using the ApopTag Red In Situ Apoptosis Detection Kit and nuclei (blue). Note the resistance to apoptosis in $K8^{-/-}$ colons (*D–F*). *Insets* show magnified views. (Scale bar: 50 μm.)

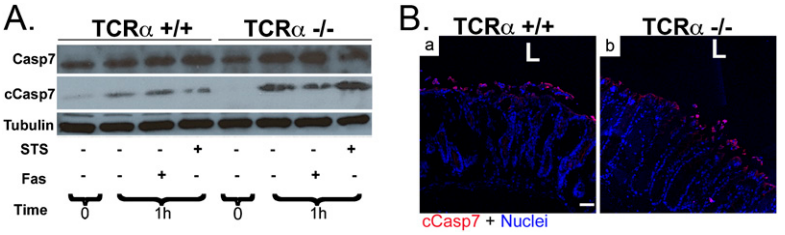


Fig. S2. Colon organ cultures from T-cell receptor α-null ($TCR\alpha^{-/-}$) mice are more susceptible to apoptosis than are parallel cultures from $TCR\alpha^{+/+}$ mice. Colon organ cultures from TCRα WT or $TCR\alpha^{-/-}$ mice were maintained in the presence or absence of staurosporine (STS) or Fas for 1 h. The organ cultures then were processed for immunoblotting (*A*) or immunohistochemistry (*B*) using anti–cleaved caspase-7 (cCasp7) (red) and nuclei (blue). Note the lack of resistance to apoptosis in $TCR\alpha^{-/-}$ colons (*A* and image *b* in *B*). (Scale bar: 50 μm.) Casp, caspase 7; L, lumen.

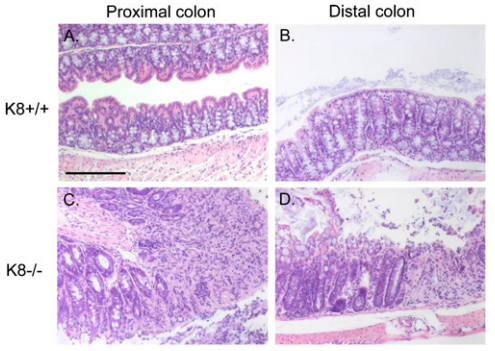
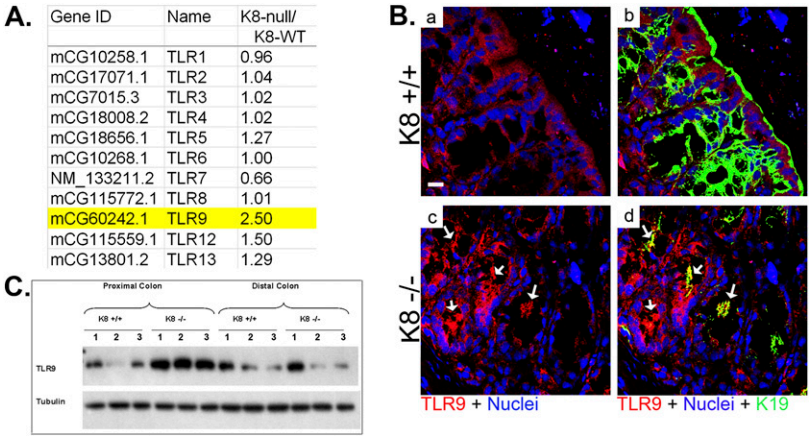


Fig. S3. Inflammation in proximal and distal colon of $K8^{-/-}$ mice. Proximal (*A* and *C*) and distal (*B* and *D*) colons from $K8^{+/+}$ (*A* and *B*) and $K8^{-/-}$ (*C* and *D*) mice were isolated, fixed, and processed for paraffin embedding. Paraffin sections were stained with hematoxylin and eosin, and images were captured on a Zeiss Axiovert 200M microscope. Inflammation is more pronounced in the proximal colon(*C*) than in the distal colon (*D*) of $K8^{-/-}$ mice. The panels are representative of histologic analyses of colons isolated from at least eight $K8^{+/+}$ and eight $K8^{-/-}$ animals. (Scale bar: 200 μm.)



Gene ID	Name	K8-null/ K8-WT
mCG10258.1	TLR1	0.96
mCG17071.1	TLR2	1.04
mCG7015.3	TLR3	1.02
mCG18008.2	TLR4	1.02
mCG18656.1	TLR5	1.27
mCG10268.1	TLR6	1.00
NM_133211.2	TLR7	0.66
mCG115772.1	TLR8	1.01
mCG60242.1	TLR9	2.50
mCG115559.1	TLR12	1.50
mCG13801.2	TLR13	1.29

Fig. S4. Altered TLR9 expression in K8$^{-/-}$ colon. (*A*) Total RNA from freshly isolated K8$^{-/-}$ and K8$^{+/+}$ colonocytes was used for microarray analysis. The table depicts Toll-like receptor (TLR) fold change between K8$^{-/-}$ and K8$^{+/+}$ colonocytes as determined by microarray analysis. (*B*) Frozen colon sections from 3-mo-old K8$^{+/+}$ (*a* and *b*) and K8$^{-/-}$ (*c* and *d*) mice were triple stained for TLR9 (red), K19 (green), and nuclei (blue). Merged images for the indicated triple staining are shown. Note increased colonocyte TLR9 by microarray analysis (*A*), immunohistochemistry (*B*), and immunoblotting (*C*) in K8$^{-/-}$ colons. Numbers in *C* identify individual mice. (Scale bar: 10 μm.)

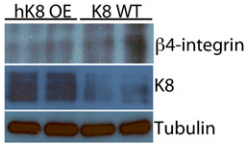


Fig. S5. Altered β4-integrin expression in colons of K8-overexpressing mice. Colon lysates from mice overexpressing human K8 (hK8 OE) and K8 WT mice were blotted with antibodies to β4-integrin, K8, and tubulin. Note lower expression of β4-integrin in hK8OE colons than in K8 WT colons. Because of the strong immune reactivity of the K8 antibody (Troma I), we diluted the tissue homogenate significantly to demonstrate the overexpression of K8. Several K8 species are noted because of proteolytic degradation, which occasionally occurs.

Table S1. The 20 genes most highly up-regulated in the K8$^{-/-}$ colon crypts

Gene ID	Gene name	Relative up-regulation*
mCG8607.1	Mast cell protease 2	149.0
mCG8610	Mast cell protease 1	145.3
mCG133706	Mast cell protease 9	99.1
mCG5445.2	Secretory leukocyte protease inhibitor	84.2
mCG130832.1	Mast cell protease 4	58.7
mCG21886.2	Carboxypeptidase A3, mast cell	27.8
mCG14532.1	Phospholipase A2, group IIA	27.0
mCG17632.2	Mast cell protease 7	25.2
mCG65051.3	T-cell–specific GTPase	21.3
mCG6027.2	Ia-associated invariant chain	16.4
mCG4516.2	Granzyme A	15.8
mCG14391.2	Glial cells missing homolog 1	15.6
mCG17997.2	Indoleamine-pyrrole 2,3 dioxygenase	15.5
mCG125443.1	Regenerating islet-derived 3-γ	15.4
mCG7888.2	Calbindin 3	14.6
mCG130827	Granzyme B	14.0
mCG20526.2	Keratin complex 1, acidic, gene 13	13.6
mCG8608.1	Mast cell protease 5	13.5
mCG52384.1	Chemokine (C-C motif) ligand 7	13.2
mCG11880.2	Malic enzyme	11.7

*Genes up-regulated in K8 $^{-/-}$ compared with K8 $^{+/+}$ colonocytes.

Table S2. Ribosomal DNA (rDNA) gene copies per mg wet stool

	Control	Antibiotic treated	*P* value
K8 $^{+/+}$	$(1.33 \pm 0.03) \times 10^8$	$(1.51 \pm 0.42) \times 10^5$	0.002
K8 $^{-/-}$	$(5.69 \pm 0.59) \times 10^6$	$(4.78 \pm 0.10) \times 10^4$	0.017

Stools were collected from three pairs of K8 $^{+/+}$ and K8 $^{-/-}$ mice treated with or without antibiotics. Results are based on TaqMan real-time PCR with universal bacterial primers. To estimate bacterial density, a conversion factor of five rDNA gene copies per genome (i.e., per one bacterium) was used as suggested for the gut microbiota (1).

1. Palmer C, Bik EM, DiGiulio DB, Relman DA, Brown PO (2007) Development of the human infant intestinal microbiota. *PLoS Biol* 5:e177.

Table S3. Fold change in expression of selected genes in K8$^{-/-}$ and K8$^{+/+}$ colonocytes as determined by microarray analysis and real-time PCR

Gene ID	Product	Primer	Microarray*	Real-time PCR
mCG19311.2	Survivin	5′-AGCATAGAAAGCACTCCCCT 5′-CAATTGACTGACGGGTAGTC	+3.59	+5.68
mCG19474.1	TRAF5	5′-CAGCGTGAAACAGAGGATCA 5′-TGCTTAAACCGCTCTTCGTT	+1.84	+1.69
mCG1848.2	Caspase 3	5′-ATGGGAGCAAGTCAGTGGAC 5′-CGTACCAGAGCGAGATGACA	+1.04	−1.61
mCG19990.2	Caspase 9	5′-TTCCCAGGTTTTGTCTCCTG 5′-GGGACTGCAGGTCTTCAGAG	−1.35	−1.44
mCG21273.2	TRAF1	5′-GATGGCTCAGGCAAGAAGAC 5′-AGCATGCTCTCGGTTGTTCT	−3.13	−6.36
mCG5961.2	Caspase 14	5′-GATGAGGTTGCTGTGCTCAA 5′-GAACACATCCGTCAGGGTCT	−3.16	−2.45
mCG9402.1	Peroxiredoxin 6	5′-TTGATGATAAGGGCAGGGAC 5′-TTGATGATAAGGGCAGGGAC	−6.44	−2.21
mCG2515.1	Clusterin	5′-AGAAAAAAACCAACGCAGAGC 5′-ATGATGCCAGATGCCCGAGC	−8.93	−3.39
mCG9908.1	Transglutaminase 3	5′-TGGCAGTAGGCAAAGAAGTC 5′-ACGTTCACAGGCTTCCGCAC	−15.60	−14.42

*Positive and negative numbers indicate genes up- and down-regulated, respectively, in K8$^{-/-}$ compared with K8$^{+/+}$ colonocytes. TRAF, TNF receptor-associated factor.

Table S4. Microarray data analysis using Jubilant PathArt to determine the most significant overrepresented pathway between K8$^{+/+}$ and K8 $^{-/-}$ colonocytes

Jubilant physiology or disease	Overlapping genes	Total number of genes in pathway	Overlap *P* value
Apoptosis	46	160	3.1×10^{-5}
Growth and differentiation	39	135	1.1×10^{-4}
Breast cancer	8	12	1.1×10^{-4}
Cell cycle	20	57	3.3×10^{-4}
Diabetes type II	36	129	4.0×10^{-4}
Parkinson disease	20	58	4.2×10^{-4}
Atherosclerosis	29	110	3.5×10^{-3}