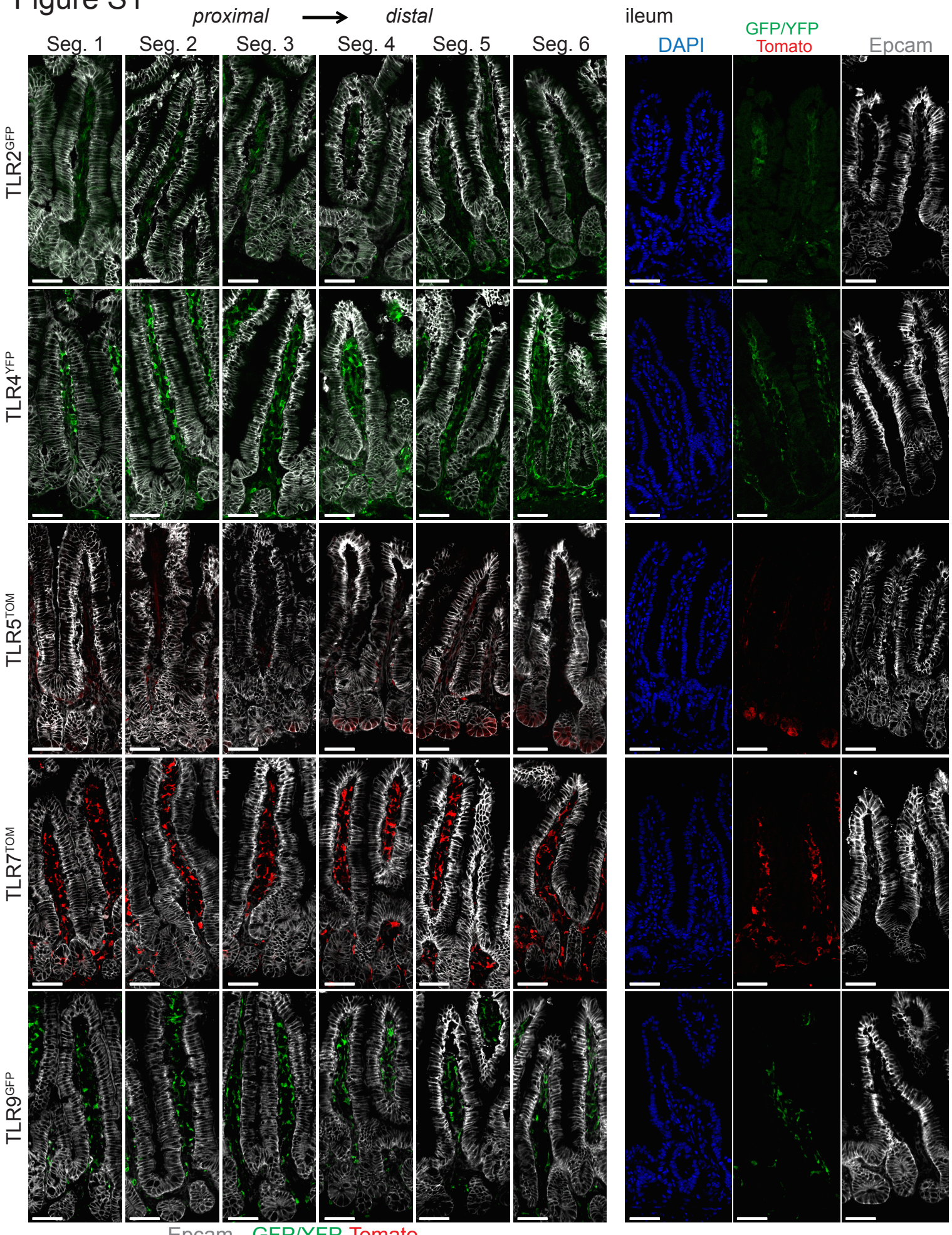
Figure S1
proximal
distal
Seg. 1
Seg. 2
Seg. 3
Seg. 4
Seg. 5
Seg. 6
ileum
DAPI
GFP/YFP
Tomato
Epcam
TLR2GFP
TLR4YFP
TLR5TOM
TLR7TOM
TLR9GFP
Epcam GFP/YFP Tomato

Figure S1: TLR expression throughout the SI, (Related to Figure 1).
SI from TLR reporter mice were divided into six equivalently sized segments and resulting sections were stained with Epcam (epithelial cells) and antibodies to either GFP/YFP or tomato (TLR reporters). Images are numbered and shown from proximal to distal segments of the SI. In the right panels, a single image from the ileum of reporter mice is separated into individual colors to aid in visualization of IEC staining. Images were taken at 20x magnification and exposures were set using staining of WT mouse tissues. Images are representative of at least 2 independent experiments, each including 2-4 mice. Scale bars are 50 microns.

Figure S2

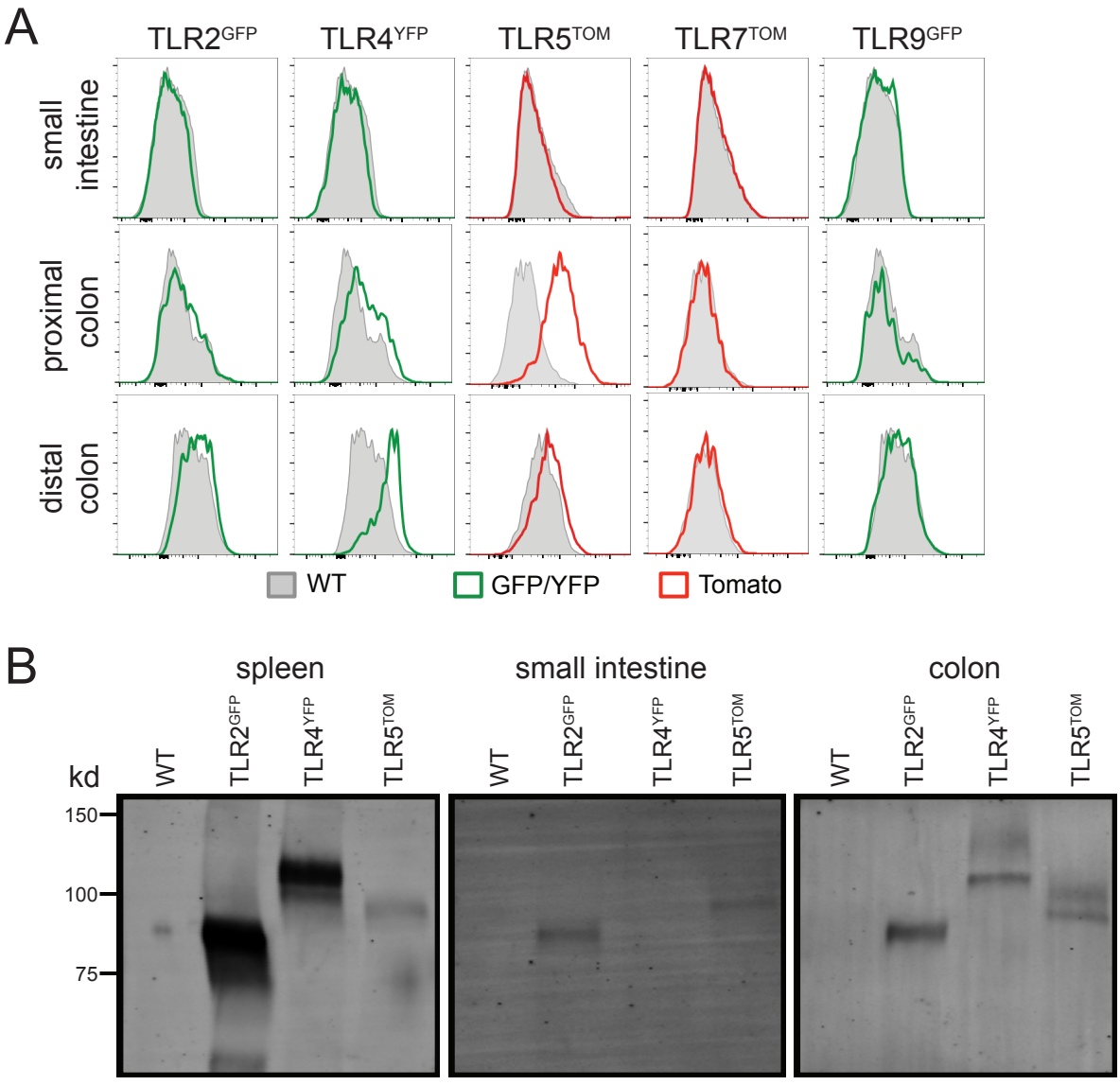

Figure S2: Characterization of TLR reporter mice, (Related to Figure 1).
(A) Flow cytometry of total IECs (live, $CD45^{-}Epcam^{+}$) gated from IEC preparations from TLR reporter mice and WT controls. Flow cytometry plots are representative of 3 independent experiments, each including 2-4 mice of each genotype.
(B) Immunoblots of TLR proteins stained with anti-HA after first being immunoprecipitated from total splenocytes, SI IECs, or colon IECs using the HA tag engineered into the reporter constructs. Experiment was repeated 2 times.

Figure S3

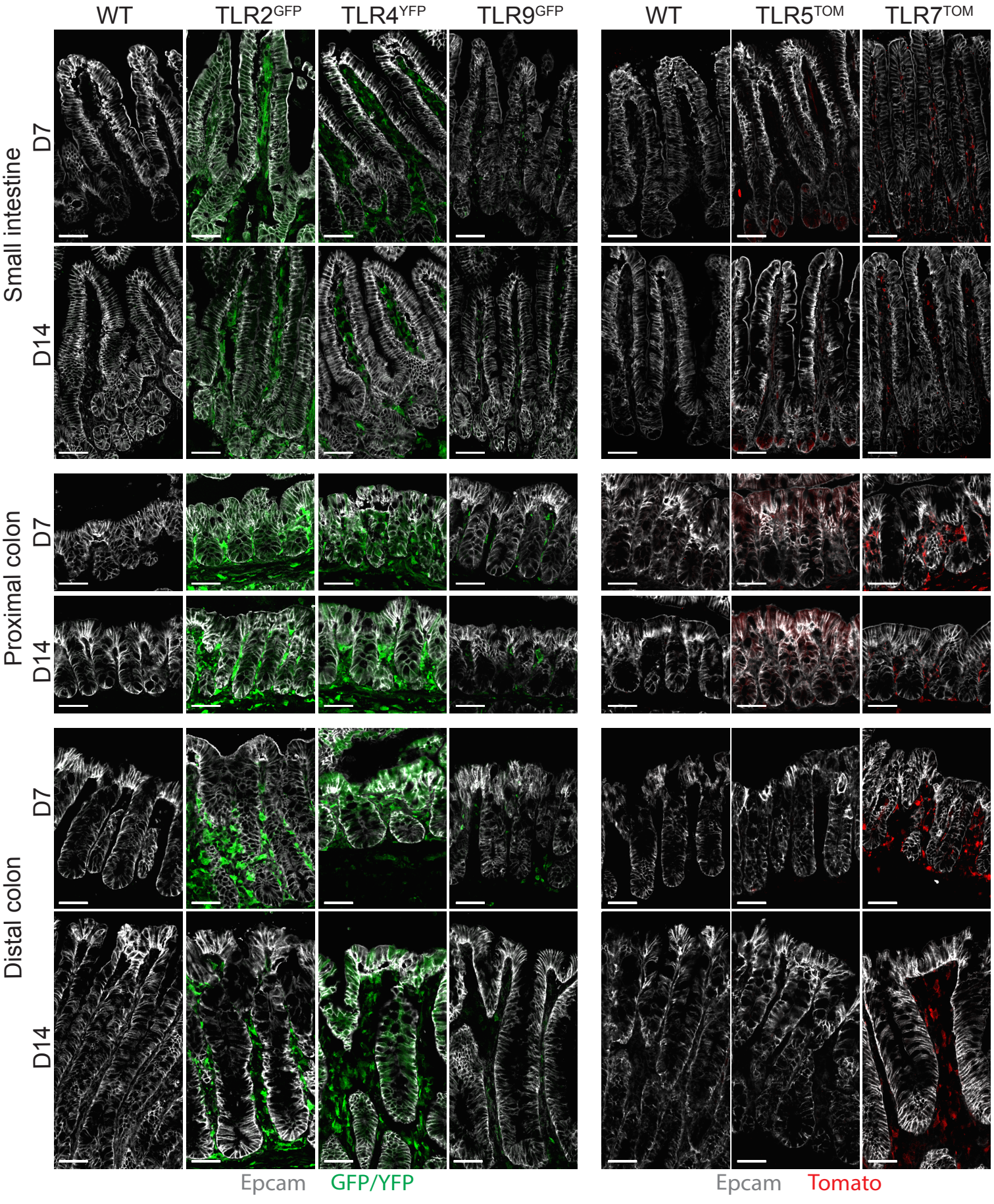
WT
TLR2GFP
TLR4YFP
TLR9GFP
WT
TLR5TOM
TLR7TOM
Small intestine
D7
D14
Proximal colon
D7
D14
Distal colon
D7
D14
Epcam
GFP/YFP
Epcam
Tomato

Figure S3: TLR expression is not altered during DSS-induced colitis, (Related to Figure 1).
Immunostaining of Epcam (epithelial cells) and GFP/YFP or tomato (TLR reporters) in SI and colon sections from indicated TLR reporter mice and WT controls that received DSS in the drinking water for 7 days. Time points at 7 days and 14 days are shown. SI images are localized to the ileum. Images were taken at 20x and exposures were set using staining of WT sections. Images are representative of 2 independent experiments each including 2-4 mice per genotype. Scale bars are 50 microns.

Figure S4

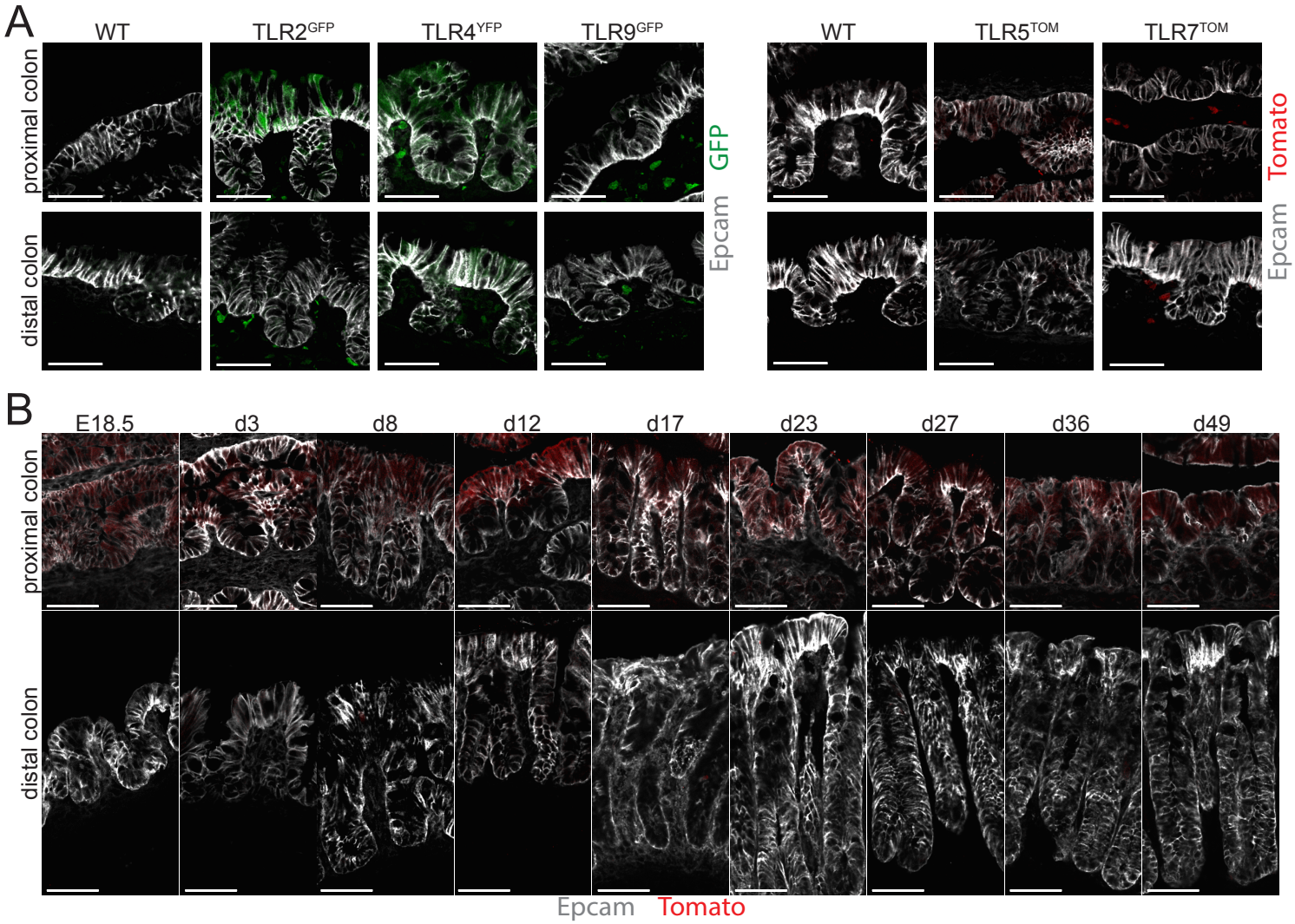
A
WT
TLR2GFP
TLR4YFP
TLR9GFP
WT
TLR5TOM
TLR7TOM
proximal colon
distal colon
GFP
Epcam
Tomato
Epcam
B
E18.5
d3
d8
d12
d17
d23
d27
d36
d49
proximal colon
distal colon
Epcam
Tomato
C
d7
d14
d21
d28
adult
WT colon IECs
TLR5TOM colon IECs
Tomato (TLR5)
1.27
0.75
0.27
0.88
0.53
38.5
41.9
26.3
31.5
28.9
empty channel
D
TLR5TOM
TLR5TOM x MyD88-/- x Trif-/-
WT
Percent of total IECs
60
50
40
30
20
10
0
d7
d14
d21
d28
adult
E
SPF colon IECs
GF colon IECs
Fold over d7 mean value
100
10
1
0.1
0.01
0.001
*
d7
d14
d21
d28
adult
TLR2
TLR4
TLR3
TLR5

Figure S4: TLR5 expression remains relatively constant in colon IECs, (Related to Figure 3).
(A) Colon sections from d1 WT and TLR reporter mice were stained with antibodies to Epcam (epithelial cells) and GFP/YFP or tomato (TLR reporters). Images were taken at 20x and exposures were set using staining of WT colon sections. Data are representative of 2 independent experiments, each including 2-5 mice of each genotype. Scale bars are 50 microns.
(B) Staining of $TLR5^{TOM}$ proximal and distal colon sections with antibodies to Epcam (epithelial cells) and GFP/YFP or tomato (TLR reporters) at various time points after birth. Data are representative of 2 independent experiments, each including 1-3 mice per time point. Scale bars are 50 microns.
(C) Tomato fluorescence from total IECs isolated from WT or $TLR5^{TOM}$ colons at the indicated time points assessed using flow cytometry. Data are representative of 3 independent experiments, each including 2-5 mice of each genotype.
(D) The percentage of $tomato^{+}$ colon IECs from indicated mice beginning one week after birth, quantified by flow cytometry. Results are pooled from 2-3 independent experiments and each time point includes mice from at least two separate litters. Error bars are mean +/- SEM.
(E) IECs were isolated from the colons of WT mouse from our colony (SPF) and WT mice housed in germ-free isolators (GF) and quantitative RT-PCR was performed to measure TLR expression over time. Values are normalized to expression of beta actin and then expressed as fold over the expression observed in IECs collected at day 7. For SPF mice, data represent 4 mice per time point and the experiment was repeated 2 times. For GF mice, results from 2 separate experiments were pooled to generate results. Error bars are mean +/- SD. Stars denote significant differences between expression values at a particular time point compared to the value at day 7. Significance was calculated using one-way ANOVA. All points without stars are not significant. * $p \leq 0.05$.

Figure S5

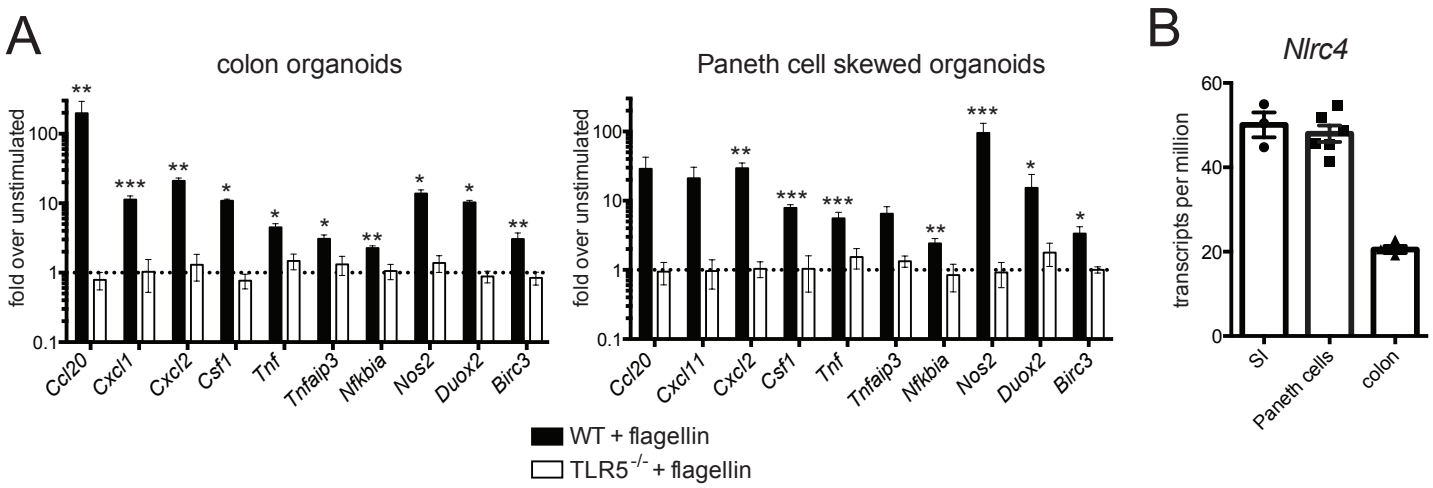

Figure S5: Flagellin-induced gene changes in colon and Paneth cell skewed organoids are TLR5-dependent, (Related to Figure 5).

(A) Quantitative PCR on indicated genes from colon and Paneth cell skewed organoids from WT and TLR5$^{-/-}$ mice stimulated with flagellin for 4 hours. Values were first normalized by determining expression relative to beta actin and then calculated as fold change over the relevant unstimulated condition. Significance was calculated using multiple t-tests. Bars without stars are not significant. Error bars are mean +/- SEM. * $p \leq 0.5$, ** $p \leq .01$, *** $p \leq 0.001$.

(B) *Nlrc4* transcripts in indicated organoids determined by RNA-sequencing.

Figure S6

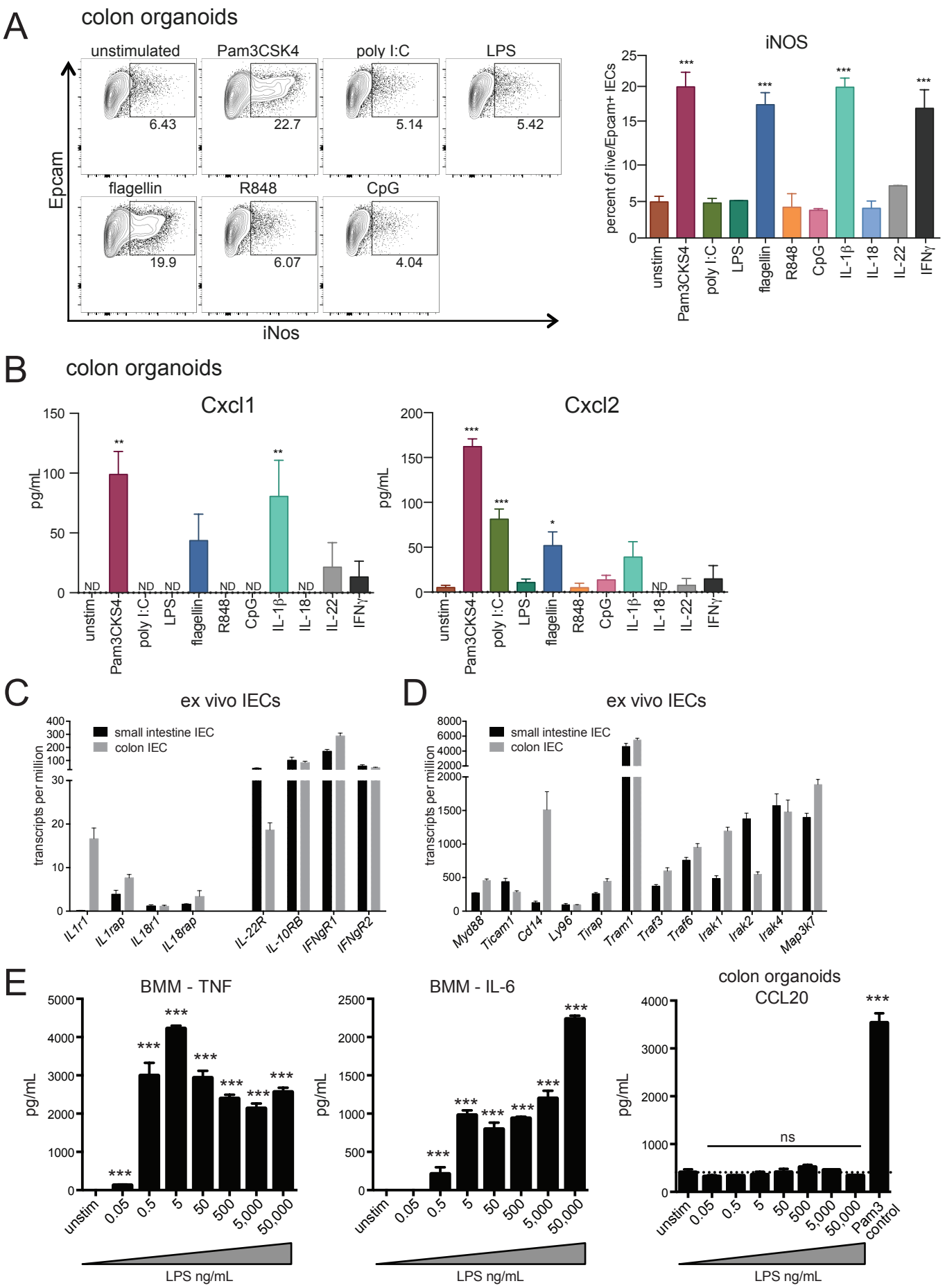
A
colon organoids
unstimulated
Pam3CSK4
poly I:C
LPS
flagellin
R848
CpG
6.43
22.7
5.14
5.42
19.9
6.07
4.04
Epcam
iNos
iNOS
percent of live/Epcam+ IECs
unstim
Pam3CKS4
poly I:C
LPS
flagellin
R848
CpG
IL-1β
IL-18
IL-22
IFNγ
B
colon organoids
Cxcl1
Cxcl2
pg/mL
ND
C
ex vivo IECs
small intestine IEC
colon IEC
transcripts per million
IL1r1
IL1rap
IL18r1
IL18rap
IL-22R
IL-10RB
IFNgR1
IFNgR2
D
ex vivo IECs
Myd88
Ticam1
Cd14
Ly96
Tirap
Tram1
Traf3
Traf6
Irak1
Irak2
Irak4
Map3k7
E
BMM - TNF
BMM - IL-6
colon organoids
CCL20
ns
Pam3 control
LPS ng/mL

Figure S6: Confirmation of TLR-induced genes, (Related to Figure 6).
(A) Flow cytometry of colon organoids stimulated with the indicated ligands using intracellular staining for iNOS. Right panel shows percentages of iNOS-positive cells. Experiment included 3 replicates for each stimulation condition and experiment was repeated twice. Significance was determined by comparing to unstimulated condition using one-way ANOVA. Error bars are mean +/- SEM. * $p \leq 0.5$, ** $p \leq .01$, *** $p \leq 0.001$.
(B) Cxcl1 and Cxcl2 levels measured by ELISA in supernatants of organoids stimulated with indicated ligands or cytokines for 16 hours. Experiment was repeated at least twice. Significance was determined by comparing to unstimulated condition using one-way ANOVA. ND indicates not detected. Error bars are mean +/- SEM. * $p \leq 0.5$, ** $p \leq .01$, *** $p \leq 0.001$.
(C) RNA-sequencing results for indicated cytokine receptor subunits obtained from *ex vivo* IECs.
(D) RNA-sequencing results for indicated TLR signaling components obtained from *ex vivo* IECs. For C-D, IECs were isolated from 4 WT mice from 2 independent litters. Results are transcripts per million. Error bars are mean +/- SEM.
(E) Bone marrow-derived macrophages or colon organoids were stimulated with indicated concentrations of LPS for 16 hours and supernatants were assayed for IL-6 and TNF using cytokine bead array. Ccl20 levels were measured using ELISA. Organoids were stimulated with Pam3CSK4 as a positive control for Ccl20 production. Error bars are mean +/- SEM.

Figure S7

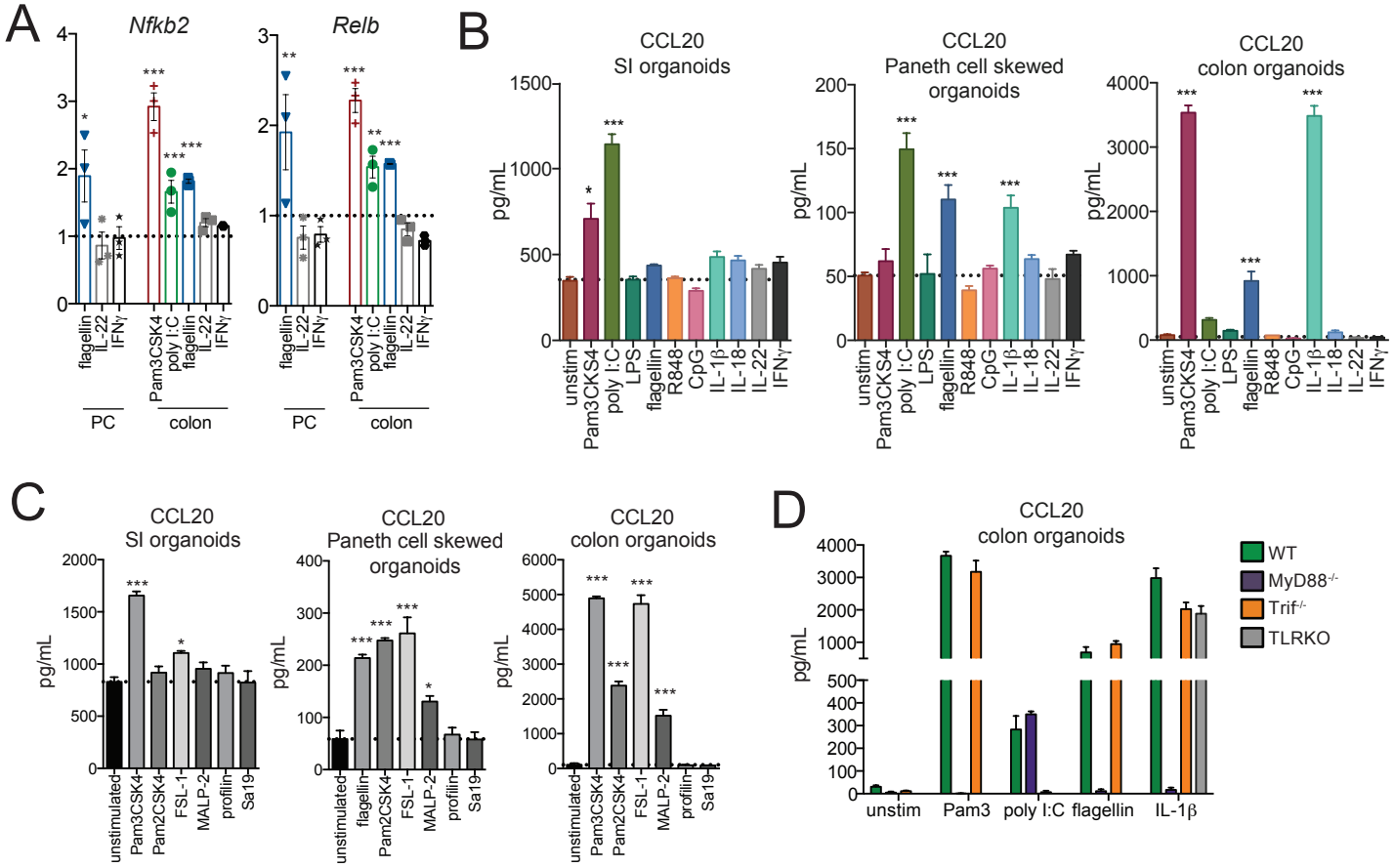

Figure S7: Characterization of the TLR-induced gene program, (Related to Figure 6).

(A) Fold induction of genes significantly induced by TLR ligands but not by IL-22 or IFNγ. TLR ligands that induced the strongest responses in Paneth cell skewed and colon organoids are shown. Each point represents an independent experiment. Error bars are mean +/- SEM. Significance was determined by comparing to unstimulated condition using one-way ANOVA. * $p \leq 0.5$, ** $p \leq .01$, *** $p \leq 0.001$.

(B) Ccl20 levels measured by ELISA in supernatants of organoids stimulated with indicated ligands or cytokines for 16 hours. Significance was determined by comparing to unstimulated condition using one-way ANOVA * $p \leq 0.5$, ** $p \leq .01$, *** $p \leq 0.001$.

(C) Ccl20 levels measured by ELISA in supernatants of organoids stimulated with indicated ligands or cytokines for 16 hours. Significance was determined by comparing to unstimulated condition using one-way ANOVA * $p \leq 0.5$, ** $p \leq .01$, *** $p \leq 0.001$.

(D) Ccl20 levels measured by ELISA in supernatants of organoids generated from WT, MyD88$^{-/-}$Trif$^{-/-}$, or TLRKO (TLR2$^{-/-}$TLR4$^{-/-}$Unc93b$^{3d/3d}$) mice stimulated with the indicated ligands or cytokines for 16 hours. For E-H, results are representative of at least 2 independent experiments. Error bars are mean +/- SEM.

Table S1

Short name	Long name	epitope tag	fluorescent protein	expression in SI IECS	expression in colon IECs	TLR localization in proximal colon
$TLR2^{GFP}$	$TLR2^{HA:GFP}$	HA	GFP	low level of expression throughout	strong expression in proximal colon, no expression in distal colon	apical and basolateral
$TLR4^{YFP}$	$TLR4^{HA:YFP}$	HA	YFP	low level of expression in patches	strong expression in proximal and distal colon	apical, basolateral, and intracellular
$TLR5^{TOM}$	$TLR5^{HA:tdTomato}$	HA	tdTomato	expression in Paneth cells in crypts, increased expression moving from proximal to distal sections of SI	strong expression in proximal colon, no expression in distal colon	apical and basolateral
$TLR7^{TOM}$	$TLR7^{Flag:tdTomato}$	FLAG	tdTomato	no expression	no expression	not determined
$TLR9^{GFP}$	$TLR9^{HA:GFP}$	HA	GFP	no expression	no expression	not detected

Table S1: TLR reporter mouse strains used in this study, (Related to Figure 1).
Features and expression patterns of TLR reporter mice characterized in this study.

Table S2

small intestine

Gene	Pam3CSK4 (TLR2)	poly I:C (TLR3)	LPS (TLR4)	flagellin (TLR5)	R848 (TLR7)	CpG (TLR9)	IL-1β	IL-18	IL-22	IFNγ
CHEMOKINES										
Ccl20	1.8	2.7	0.9	1.2	1.0	1.0	1.1	1.2	0.7	0.4
Ccl28	1.0	0.9	1.0	0.9	0.7	0.8	0.9	0.8	0.7	0.3
Ccl9	1.3	1.2	1.2	1.5	1.0	1.3	1.2	1.2	1.0	0.8
Cxcl1	1.0	1.0	1.0	1.1	1.0	1.0	1.0	1.0	1.3	1.2
Cxcl10	0.9	2.8	0.9	0.9	0.9	0.9	1.1	1.0	0.7	67.4
Cxcl16	1.2	1.1	1.0	1.1	1.0	1.1	0.9	0.9	1.1	1.1
Cxcl2	0.8	1.0	0.7	0.9	0.9	0.4	0.3	0.5	3.9	3.9
CYTOKINES										
Csf1	1.4	1.1	0.9	1.0	0.8	1.1	1.0	0.8	2.5	6.2
Il10	1.0	1.0	1.0	1.1	1.0	1.0	1.0	1.0	1.3	1.2
Il18	1.2	1.2	1.2	1.2	1.1	1.2	1.1	1.2	2.3	1.0
Il1a	1.1	1.8	1.8	1.6	1.3	1.7	1.4	1.0	8.6	13.4
Il1b	0.7	0.7	1.1	0.9	0.6	0.7	0.4	0.6	3.1	2.1
Il6	1.9	2.0	2.3	2.4	1.8	1.9	1.0	0.7	24.0	18.8
Tgfb	1.3	1.1	1.3	1.3	0.8	1.2	1.1	1.3	0.9	0.9
Tnf	18.4	5.7	0.9	3.0	0.9	0.9	0.9	1.2	4.7	64.5
INNATE IMMUNITY										
Casp4	1.5	1.4	1.4	1.5	1.2	1.2	1.1	1.0	2.6	5.0
Gbp6	1.0	1.0	1.0	1.1	1.0	1.0	1.0	1.0	1.3	864.3
Gbp9	0.7	0.7	1.1	0.9	0.5	0.7	0.3	0.6	8.1	8.0
Il22r	1.4	1.4	1.3	1.4	1.2	1.3	1.3	1.3	1.0	1.0
Lcn2	1.6	1.0	1.3	1.1	1.0	0.9	0.7	0.8	15.0	9.0
Nod2	0.9	0.9	0.9	0.9	0.9	0.9	0.9	0.9	1.1	3.0
Pigr	1.1	1.1	1.1	1.1	1.0	1.0	1.0	0.9	1.0	0.8
Tlr2	1.7	1.0	1.0	1.1	1.0	2.6	1.0	1.0	1.3	1.2
CELL DEATH AND SURVIVAL										
Bid	1.3	1.3	1.3	1.3	1.2	1.2	1.1	1.1	1.2	2.2
Birc3	1.2	1.3	1.1	1.2	1.1	1.1	1.1	1.1	1.0	1.0
Bok	1.2	1.3	1.3	1.2	1.1	1.3	1.0	1.0	2.8	2.9
Gsdmd	1.2	1.2	1.1	1.2	1.2	1.2	1.1	1.2	2.9	3.0
NF-KB SIGNALING AND INHIBITION										
Bcl3	1.2	1.3	1.1	1.1	0.9	1.2	0.8	0.9	3.2	4.4
Nfkb1	1.1	1.2	1.2	1.1	1.1	1.1	1.1	1.0	1.0	1.0
Nfkb2	1.2	1.4	1.1	1.2	0.9	1.0	1.0	0.9	1.5	1.4
Nfkbia	1.3	1.4	0.9	1.0	0.9	1.0	1.0	1.0	1.5	0.9
Relb	1.2	1.3	1.0	1.1	1.0	1.1	1.0	1.0	0.9	0.6
Tnfaip3	1.8	2.1	1.2	1.5	1.1	1.3	1.2	1.2	1.3	2.5
Tnip3	0.9	1.1	1.1	1.1	0.9	1.0	1.0	0.9	0.7	0.6
REACTIVE OXYGEN AND NITROGEN										
Cyba	1.2	1.1	1.2	1.1	1.0	1.2	0.9	0.9	0.8	0.8
Duox1	0.9	1.1	1.0	0.9	0.9	0.9	1.2	1.0	0.8	0.8
Duox2	1.6	1.1	0.9	1.4	1.2	1.1	1.2	1.2	10.0	35.1
Duoxa1	1.3	3.2	4.1	2.3	1.0	1.4	0.5	1.7	29.2	21.0
Duoxa2	1.7	1.3	1.4	1.6	1.2	1.3	1.3	1.3	10.0	48.1
Nos2	2.5	1.4	1.1	1.7	0.6	1.0	1.1	1.1	8.8	26.7
Nox1	4.1	1.1	0.9	1.1	0.9	1.0	1.2	1.4	1.5	1.4
Nox3	1.8	1.4	1.6	2.0	2.5	1.7	0.7	0.6	22.7	26.6
Nox4	2.2	1.5	1.8	2.4	2.1	3.3	0.8	0.6	23.5	21.8
Noxa1	1.2	1.1	0.9	1.0	0.9	1.0	1.0	1.0	0.9	0.8
Noxo1	1.2	1.1	0.9	1.0	0.9	1.0	1.0	1.0	1.2	1.1
ANTIMICROBIAL										
Ang4	1.1	1.1	1.2	1.2	1.0	1.2	1.0	1.1	1.0	1.0
Defa17	1.4	1.2	1.2	1.4	1.1	1.4	1.2	1.3	1.3	1.3
Defb1	1.0	1.0	1.0	1.1	1.0	1.0	1.0	1.0	1.3	1.2
Defb14	1.4	1.5	1.5	1.5	1.1	1.5	1.3	1.3	4.0	4.3
Defb3	1.3	1.4	1.3	0.9	0.8	1.1	0.9	0.9	2.4	1.9
Lypd8	0.9	0.9	0.9	0.9	0.9	0.9	0.8	0.9	1.1	1.9
Lyz1	1.0	1.0	1.0	1.1	1.0	1.0	1.0	1.1	0.8	0.8
Mmp7	1.2	1.2	1.3	1.5	1.0	1.3	1.1	1.1	2.2	2.0
Pla2g2a	0.9	1.0	0.9	1.2	1.0	0.8	1.0	1.0	2.1	1.9
Reg3b	1.3	1.0	1.2	1.3	1.1	1.4	0.9	1.1	41.1	39.5
Reg3g	1.4	1.4	1.6	1.3	1.1	1.4	0.6	0.7	38.6	28.1
Retnlb	1.0	1.0	1.0	1.1	1.0	1.0	1.0	1.0	1.3	1.2
S100a7	1.6	0.6	1.8	1.8	1.9	1.1	0.6	0.6	7.5	6.8
S100a8	1.0	1.0	1.0	1.1	1.0	1.0	1.0	1.0	1.3	1.2
S100a9	1.2	1.2	1.3	0.9	1.0	1.2	1.0	1.0	1.7	1.4
EPITHELIAL HOMEOSTASIS										
Muc1	1.0	1.2	1.5	2.5	1.0	1.1	1.0	1.0	4.7	1.2
Muc2	1.0	1.3	1.2	1.4	1.4	1.1	1.1	1.2	1.2	1.0
Muc3	0.8	1.0	0.8	0.8	0.9	0.9	1.0	1.0	3.3	4.4
Muc10	1.2	1.2	1.1	1.0	0.9	1.4	1.1	0.9	2.0	1.9
Muc13	1.1	1.2	1.1	1.2	1.0	1.1	1.0	1.1	2.5	2.0
Tff2	1.2	1.1	1.2	1.1	1.0	1.3	1.2	1.1	1.2	1.4
Tff3	0.9	0.9	0.9	1.0	0.9	1.0	0.9	0.9	0.7	0.7
METABOLIC										
Anpep	1.1	1.1	1.2	1.2	1.1	1.1	0.8	0.9	0.6	0.7
Cyp26b1	0.9	1.1	1.2	1.0	1.0	1.0	1.1	1.1	0.7	0.7
Ghr	1.2	1.2	1.2	1.3	1.1	1.2	1.2	1.2	1.1	1.0
Mdh2	1.2	1.2	1.1	1.2	1.2	1.2	1.2	1.2	1.1	1.0
Rap1gap	1.1	1.1	1.2	1.1	1.3	1.3	1.1	1.1	1.0	0.9
ASSORTED										
Clca3a2	1.4	1.4	1.2	1.2	1.2	1.0	1.2	1.2	1.4	1.7
Clca3b	1.3	1.0	1.0	1.0	0.9	1.0	1.0	1.0	0.7	0.6
Mreg	1.6	1.3	1.1	1.0	1.0	1.2	1.1	1.1	0.6	1.0
Trpv3	0.9	0.8	1.0	0.9	0.9	1.1	0.8	0.9	0.9	1.4
CONTROLS										
Epcam	1.0	1.0	1.1	1.1	1.0	1.0	1.0	1.0	1.0	0.9
Gapdh	1.1	1.1	1.1	1.1	1.2	1.1	1.1	1.2	1.0	1.1
Vil1	1.0	1.0	1.0	1.0	1.0	1.1	0.8	0.9	0.8	1.1

Paneth cell skewed

Gene	Pam3CSK4 (TLR2)	poly I:C (TLR3)	LPS (TLR4)	flagellin (TLR5)	R848 (TLR7)	CpG (TLR9)	IL-1β	IL-18	IL-22	IFNγ
CHEMOKINES										
Ccl20	1.9	2.6	1.4	5.9	1.1	1.6	1.8	0.9	0.9	0.6
Ccl28	0.3	1.6	1.2	2.3	0.6	0.8	1.2	1.1	0.2	0.2
Ccl9	1.3	1.6	1.6	1.8	1.3	1.8	1.6	1.7	0.7	0.8
Cxcl1	1.2	1.2	1.2	1.3	1.2	1.3	1.4	1.2	0.9	1.2
Cxcl10	0.8	23.0	0.6	3.1	1.2	0.9	1.7	1.4	0.5	935.3
Cxcl16	1.3	1.4	1.5	2.1	1.4	1.7	1.7	1.7	1.3	1.8
Cxcl2	0.8	0.9	1.2	1.5	0.9	0.9	1.8	1.0	0.9	0.7
CYTOKINES										
Csf1	1.0	1.0	1.0	1.2	0.8	0.9	1.6	1.0	0.7	1.5
Il10	1.2	1.2	1.2	1.1	1.2	1.3	1.4	1.2	0.9	1.2
Il18	1.2	1.4	1.1	1.3	1.3	2.0	1.5	1.6	0.8	1.2
Il1a	1.3	1.3	1.4	1.5	1.1	1.2	1.4	1.1	0.7	0.4
Il1b	1.2	1.2	1.3	1.4	0.8	1.1	1.3	1.1	0.9	1.0
Il6	1.0	1.4	1.1	1.0	0.8	1.3	1.2	1.0	1.2	1.0
Tgfb	1.1	1.5	1.4	2.7	1.1	1.4	1.9	1.3	0.5	0.5
Tnf	1.9	2.1	1.1	17.8	0.9	1.3	3.1	1.8	3.7	14.7
INNATE IMMUNITY										
Casp4	1.2	1.4	1.6	3.9	1.2	1.7	2.2	1.5	2.6	8.5
Gbp6	1.2	1.2	1.2	1.5	1.2	1.3	5.8	1.5	0.9	1240
Gbp9	0.8	1.2	1.1	1.1	0.9	1.1	1.1	1.0	1.2	10.0
Il22r	1.0	1.4	1.3	1.5	1.3	1.5	1.6	1.4	0.6	0.6
Lcn2	1.2	1.2	1.2	1.4	3.5	2.4	1.4	1.2	0.9	1.2
Nod2	1.2	1.2	2.1	1.1	1.2	3.0	1.4	1.2	0.9	11.9
Pigr	1.0	1.2	1.2	1.7	1.1	1.2	1.4	1.2	1.1	1.3
Tlr2	3.2	1.2	1.2	8.7	1.2	1.8	2.3	2.8	0.9	1.2
CELL DEATH AND SURVIVAL										
Bid	0.9	1.4	1.5	1.7	1.3	1.3	1.6	1.5	1.0	2.9
Birc3	1.1	1.6	1.4	4.2	1.4	1.3	2.5	1.3	1.4	1.6
Bok	1.3	1.5	1.3	1.9	1.2	1.3	2.0	1.3	1.5	1.5
Gsdmd	1.1	1.2	1.2	1.6	1.1	1.2	1.4	1.2	1.3	1.9
NF-KB SIGNALING AND INHIBITION										
Bcl3	1.1	1.2	1.2	1.2	1.1	1.2	1.5	1.2	1.7	1.6
Nfkb1	0.9	1.4	1.2	1.3	1.2	1.2	1.5	1.3	1.0	1.3
Nfkb2	1.0	1.3	1.2	1.9	1.0	1.2	1.6	1.1	0.9	1.0
Nfkbia	1.3	1.6	1.3	2.0	1.1	1.5	1.8	1.4	1.1	1.2
Relb	1.1	1.3	1.0	1.9	1.0	1.1	1.6	1.0	0.8	0.8
Tnfaip3	1.2	2.2	1.9	3.7	1.3	1.6	2.6	1.5	1.2	1.2
Tnip3	3.2	5.6	8.4	7.1	4.9	2.4	1.8	1.2	3.1	6.6
REACTIVE OXYGEN AND NITROGEN										
Cyba	1.2	1.3	1.2	3.8	1.1	1.1	1.7	1.2	0.7	0.8
Duox1	0.8	1.1	1.8	1.5	0.8	0.9	0.9	1.6	0.6	1.4
Duox2	1.1	1.2	1.4	5.3	1.2	1.3	1.5	1.6	1.8	2.8
Duoxa1	0.5	0.4	0.7	0.7	0.6	0.9	0.5	0.7	1.0	0.4
Duoxa2	1.2	1.3	1.7	4.1	1.3	1.6	1.8	1.7	1.5	2.8
Nos2	1.5	0.9	1.4	4.2	0.7	1.0	2.2	1.1	1.4	2.5
Nox1	1.2	1.5	1.6	1.5	1.3	1.7	1.6	1.6	1.0	1.3
Nox3	1.0	0.4	1.1	0.9	0.7	0.3	1.1	0.9	2.7	2.1
Nox4	0.9	1.2	1.2	1.0	1.0	1.1	1.3	1.5	1.5	1.2
Noxa1	0.7	1.2	0.9	2.5	1.8	1.8	2.0	1.4	1.5	1.2
Noxo1	1.1	1.3	1.4	1.7	1.2	1.4	1.5	1.4	1.0	1.1
ANTIMICROBIAL										
Ang4	1.0	1.1	1.1	1.0	1.1	1.2	1.2	1.2	0.7	0.8
Defa17	1.3	1.3	1.5	1.2	1.4	1.6	1.6	1.5	1.0	1.2
Defb1	0.8	1.0	0.6	1.3	0.7	1.7	0.7	0.6	0.2	0.5
Defb14	1.3	1.5	1.5	1.4	1.5	1.9	1.8	1.5	1.4	1.4
Defb3	1.4	1.4	1.6	1.4	1.1	1.8	1.8	1.8	1.0	0.9
Lypd8	1.0	1.2	1.1	1.0	1.1	1.0	1.2	1.1	1.0	3.3
Lyz1	0.9	0.9	1.0	0.9	1.0	1.1	1.0	1.0	0.7	0.8
Mmp7	1.1	1.3	1.4	1.3	1.3	1.4	1.4	1.4	1.3	1.6
Pla2g2a	1.2	1.4	1.5	1.7	1.3	1.2	1.5	1.4	2.5	2.7
Reg3b	1.2	1.0	1.4	1.3	1.4	1.5	1.3	1.4	56.8	31.9
Reg3g	1.4	1.6	2.1	2.8	1.6	1.8	2.0	1.9	74.7	41.6
Retnlb	1.2	1.2	1.2	1.1	1.2	1.3	1.4	1.2	0.9	1.2
S100a7	0.5	0.4	0.4	1.2	0.9	0.7	0.6	0.8	0.5	0.6
S100a8	0.3	0.3	0.6	0.3	0.3	0.3	0.4	0.3	0.2	0.3
S100a9	1.3	1.4	1.4	1.3	1.4	1.7	1.7	1.7	0.9	1.1
EPITHELIAL HOMEOSTASIS										
Muc1	0.5	2.7	1.8	2.4	0.6	0.6	0.9	0.7	1.2	2.5
Muc2	0.9	1.4	1.3	1.0	1.4	1.5	1.2	1.5	1.0	1.0
Muc3	1.0	1.2	1.3	1.2	1.2	1.4	1.2	1.3	1.7	2.2
Muc10	1.0	1.3	1.2	1.2	1.2	1.6	1.6	1.6	1.0	1.0
Muc13	1.0	1.3	1.4	1.3	1.3	1.6	1.5	1.5	1.8	1.9
Tff2	1.3	1.4	1.4	1.2	1.4	1.6	1.6	1.4	1.0	1.1
Tff3	1.0	0.9	0.9	0.9	0.9	0.8	0.9	0.8	0.6	0.7
METABOLIC										
Anpep	0.9	1.0	1.0	0.9	1.0	1.0	1.0	1.0	0.5	0.9
Cyp26b1	1.4	0.6	1.2	0.9	0.6	1.1	0.6	0.7	0.1	1.3
Ghr	1.2	1.3	1.4	1.0	1.3	1.6	1.5	1.5	0.7	0.8
Mdh2	1.3	1.3	1.4	1.2	1.4	1.6	1.4	1.4	1.0	1.1
Rap1gap	1.0	1.4	1.2	0.8	1.3	1.4	1.4	1.5	0.8	0.7
ASSORTED										
Clca3a2	1.2	1.3	1.2	2.6	1.2	1.4	1.8	1.3	2.5	4.3
Clca3b	1.3	1.4	1.6	3.3	1.5	1.7	1.9	1.7	1.8	1.3
Mreg	1.3	1.6	1.5	2.3	1.6	1.7	2.1	2.0	0.7	2.4
Trpv3	1.0	1.1	1.2	0.9	1.3	1.2	1.4	1.2	0.9	1.1
CONTROLS										
Epcam	1.2	1.3	1.4	1.1	1.3	1.6	1.5	1.5	1.2	1.3
Gapdh	1.1	1.0	1.0	1.1	1.0	1.1	1.0	1.0	1.0	1.1
Vil1	0.8	0.9	0.9	0.9	0.9	0.9	0.9	0.8	0.8	1.3

colon

Gene	Pam3CSK4 (TLR2)	poly I:C (TLR3)	LPS (TLR4)	flagellin (TLR5)	R848 (TLR7)	CpG (TLR9)	IL-1β	IL-18	IL-22	IFNγ
CHEMOKINES										
Ccl20	490.2	12.4	3.5	72.4	1.7	2.7	204.1	1.2	0.5	0.1
Ccl28	1.5	1.0	1.0	1.4	0.8	0.9	1.5	0.9	0.5	0.3
Ccl9	1.6	1.3	1.2	1.5	1.0	1.3	1.6	1.1	1.2	1.3
Cxcl1	69.6	1.0	1.0	2.2	1.1	1.0	26.2	1.0	10.5	4.4
Cxcl10	5.8	18.1	1.2	1.8	1.0	1.2	2.8	1.0	0.8	219.3
Cxcl16	2.3	1.0	1.0	1.5	0.9	1.1	1.8	1.0	0.7	0.9
Cxcl2	20.5	0.8	1.4	7.3	1.8	1.6	9.1	1.0	1.1	1.2
CYTOKINES										
Csf1	20.1	2.2	2.7	7.8	1.5	1.8	14.5	1.6	1.8	11.0
Il10	1.0	1.0	1.0	1.0	1.1	1.0	1.0	1.0	1.0	1.2
Il18	1.0	1.2	1.2	1.0	1.1	1.2	1.0	1.1	1.7	0.8
Il1a	0.6	0.5	1.0	1.5	0.4	0.3	0.6	0.4	0.3	1.1
Il1b	1.2	1.6	2.0	1.6	1.3	2.2	2.1	1.9	1.5	0.8
Il6	3.4	2.5	5.1	3.5	1.9	1.3	3.1	1.6	0.8	0.9
Tgfb	1.9	1.0	1.1	1.6	1.0	1.1	2.1	0.9	0.8	0.9
Tnf	9.9	4.1	2.2	3.8	0.9	1.5	8.0	1.3	2.0	6.6
INNATE IMMUNITY										
Casp4	2.8	1.5	1.2	2.1	1.0	1.3	2.5	1.0	1.8	4.6
Gbp6	9.8	2.9	1.0	12.7	1.1	1.0	10.9	1.0	1.0	2778
Gbp9	6.1	0.8	3.9	6.2	0.6	1.2	2.8	1.4	0.9	121.7
Il22r	1.9	1.3	1.3	1.4	1.2	1.3	2.1	1.4	1.1	1.0
Lcn2	3.5	0.9	0.9	1.3	0.7	0.8	3.2	0.8	1.3	0.9
Nod2	57.5	4.0	4.3	18.2	1.1	1.8	56.6	1.7	1.0	12.9
Pigr	1.2	1.1	1.1	1.2	1.0	1.1	1.3	1.0	0.9	0.8
Tlr2	9.2	1.5	1.4	2.5	1.2	1.3	4.8	1.3	1.4	1.6
CELL DEATH AND SURVIVAL										
Bid	2.5	1.3	1.3	1.7	1.2	1.3	2.4	1.2	1.1	3.1
Birc3	2.8	1.6	1.2	1.8	1.1	1.1	2.8	1.2	1.1	1.1
Bok	1.6	1.1	1.1	1.4	1.1	1.3	1.6	1.1	1.4	1.5
Gsdmd	1.6	1.1	1.0	1.4	1.1	1.1	1.7	1.1	1.4	1.8
NF-KB SIGNALING AND INHIBITION										
Bcl3	2.2	1.5	1.2	1.4	1.1	1.1	2.0	1.2	2.9	3.8
Nfkb1	1.4	1.3	1.2	1.3	1.1	1.2	1.4	1.1	1.0	1.3
Nfkb2	2.9	1.7	1.3	1.8	0.9	1.3	2.5	1.2	1.2	1.2
Nfkbia	3.9	1.8	1.1	1.6	0.9	1.0	2.6	0.9	1.2	1.4
Relb	2.3	1.5	1.2	1.6	1.0	1.2	2.1	1.2	0.8	0.7
Tnfaip3	5.5	2.9	1.5	2.3	1.1	1.3	4.0	1.2	1.1	2.3
Tnip3	2.8	1.5	1.2	1.8	1.0	1.1	2.3	1.0	1.7	1.7
REACTIVE OXYGEN AND NITROGEN										
Cyba	2.2	1.3	1.1	1.7	1.1	1.2	2.2	1.2	1.0	1.0
Duox1	0.7	0.9	1.0	1.1	0.9	1.0	1.0	1.1	1.0	0.9
Duox2	20.0	1.4	1.6	10.6	1.2	1.2	28.2	1.4	3.7	5.3
Duoxa1	1.3	1.4	0.7	1.3	1.8	1.7	2.0	1.8	0.8	0.6
Duoxa2	29.3	1.0	1.4	10.8	1.1	1.2	22.6	1.0	2.9	6.6
Nos2	46.6	2.7	1.6	10.9	1.2	1.3	32.5	1.4	4.5	19.5
Nox1	5.1	1.3	1.4	3.2	1.2	1.3	4.8	1.2	0.9	0.8
Nox3	4.1	2.6	2.5	2.4	3.2	1.1	1.9	1.1	0.8	1.4
Nox4	2.1	2.9	2.0	2.9	2.2	1.9	1.2	1.4	1.0	1.2
Noxa1	2.1	1.2	1.3	1.6	1.1	1.1	2.3	1.1	1.0	1.0
Noxo1	2.4	1.3	1.2	1.7	1.1	1.3	2.6	1.3	1.1	1.0
ANTIMICROBIAL										
Ang4	1.3	1.2	1.3	1.4	1.1	1.3	1.4	1.2	1.1	1.1
Defa17	0.9	0.8	0.9	0.8	0.8	0.8	0.9	0.6	1.3	1.0
Defb1	1.1	1.2	0.9	1.2	1.0	1.3	0.9	1.3	2.1	1.7
Defb14	0.9	0.8	1.4	1.3	1.2	1.2	1.5	0.8	1.7	2.0
Defb3	1.0	2.0	1.6	2.1	1.1	1.5	1.7	1.6	4.4	2.6
Lypd8	1.0	0.9	0.8	0.9	0.9	0.9	1.0	0.9	0.8	1.2
Lyz1	1.3	1.3	1.2	1.0	1.1	1.2	1.1	1.1	1.0	1.5
Mmp7	1.7	1.2	0.9	1.4	0.7	1.0	0.8	1.1	1.3	1.0
Pla2g2a	1.9	1.4	1.3	1.8	1.4	1.4	1.9	1.3	3.6	2.7
Reg3b	1.3	1.2	1.5	1.5	1.3	1.3	1.9	1.4	16.8	49.1
Reg3g	3.8	1.7	1.9	2.6	1.7	1.8	3.1	1.5	27.4	44.5
Retnlb	1.0	1.0	1.0	1.0	1.1	1.0	1.0	1.0	11.5	66.2
S100a7	1.4	2.5	2.4	2.7	1.9	1.2	1.9	2.2	1.5	2.1
S100a8	1.0	1.0	1.0	1.0	1.1	1.0	1.0	1.0	2.3	1.2
S100a9	1.1	1.1	1.2	1.5	1.0	1.0	1.6	1.3	2.4	2.1
EPITHELIAL HOMEOSTASIS										
Muc1	1.8	0.8	1.0	1.7	1.0	1.0	1.9	1.0	1.3	1.2
Muc2	1.0	1.2	1.2	1.1	1.3	1.2	1.2	1.2	1.0	0.9
Muc3	0.7	0.9	0.9	0.8	0.9	0.9	0.9	1.0	1.0	1.1
Muc10	1.1	1.5	0.9	1.7	1.0	1.4	2.1	1.0	3.1	2.7
Muc13	1.4	1.0	0.9	1.3	0.9	1.0	1.5	1.0	2.1	2.0
Tff2	0.5	0.7	0.9	1.2	0.6	0.7	0.9	0.7	1.2	1.4
Tff3	1.2	1.1	1.1	1.0	1.1	1.1	1.0	1.0	0.9	0.9
METABOLIC										
Anpep	0.8	1.1	0.8	1.0	0.8	0.9	0.9	0.7	1.4	1.1
Cyp26b1	0.5	1.1	1.0	0.9	0.8	0.7	0.6	0.9	0.5	0.3
Ghr	0.8	1.1	1.2	0.9	1.0	1.0	0.8	0.9	0.8	0.7
Mdh2	1.0	1.1	1.1	1.1	1.1	1.2	1.1	1.1	1.1	1.1
Rap1gap	0.9	1.2	1.3	1.1	1.3	1.3	1.0	1.4	1.0	0.7
ASSORTED										
Clca3a2	5.3	2.0	2.3	3.4	1.7	1.6	6.5	1.6	1.0	0.3
Clca3b	2.5	1.1	1.2	1.7	1.0	1.1	2.4	1.0	0.9	0.8
Mreg	1.1	1.5	1.1	0.8	1.8	1.0	0.6	1.6	0.4	0.4
Trpv3	1.1	1.0	1.1	1.2	1.3	1.0	1.6	1.2	1.0	1.0
CONTROLS										
Epcam	0.9	0.9	0.9	0.9	0.9	0.9	0.9	0.9	0.9	0.9
Gapdh	0.9	0.9	1.0	1.0	1.0	1.0	0.9	1.0	0.9	0.8
Vil1	0.9	0.9	0.9	0.9	0.9	1.0	1.0	1.0	0.9	1.0

Table S2: Gene expression in organoids after stimulation with TLR ligands or cytokines, (Related to Figure 6).

Nanostring analysis of gene expression changes induced by stimulation with TLR ligands or cytokines for 4 hours. Genes are grouped into functional categories. Numbers are fold change of normalized transcript counts in stimulated over unstimulated organoids (averaged between three independent experiments). Significantly increased genes ($p \leq 0.05$) are shown in shades of red, with darker red indicating increased levels of significance. Significantly decreased genes ($p \leq 0.05$) are shown in shades of blue, with darker blue indicating increased levels of significance. Significance was calculated by comparing normalized transcript counts in stimulated vs. unstimulated organoids using one-way ANOVA.

Table S3

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Oligonucleotides		
Ccl20 F primer: GCCTCTCGTACATACAGACGC	Primer Bank	N/A
Ccl20 R primer: CCAGTTCTGCTTTGGATCAGC	Primer Bank	N/A
Cxcl1 F primer: CTGGGATTCACCTCAAGAACATC	Primer Bank	N/A
Cxcl1 R primer: CAGGGTCAAGGCAAGCCTC	Primer Bank	N/A
Cxcl2 F primer: CCAACCACCAGGCTACAGG	Primer Bank	N/A
Cxcl2 R primer: GCGTCACACTCAAGCTCTG	Primer Bank	N/A
Csf1 F primer: GACTTCATGCCAGATTGCC	Primer Bank	N/A
Csf1 R primer: GGTGGCTTTAGGGTACAGG	Primer Bank	N/A
Tnf F primer: CCTGTAGCCCACGTCGTAG	Primer Bank	N/A
Tnf R primer: GGGAGTAGACAAGGTACAACCC	Primer Bank	N/A
Tnfaip3 F primer: CTGGATGTCAATCAACAATGGGA	Primer Bank	N/A
Tnfaip3 R primer: ACTAGGGTGTGAGTGTTTTCTGT	Primer Bank	N/A
Nfkbia F primer: GAAGCCGCTGACCATGGAA	Primer Bank	N/A
Nfkbia R primer: GATCACAGCCAAGTGGAGTGGA	Primer Bank	N/A
Nos2 F primer: GTTCTCAGCCCAACAATACAAGA	Primer Bank	N/A
Nos2 R primer: GTGGACGGGTCGATGTCAC	Primer Bank	N/A
Duox2 F primer: AAGTTCAAGCAGTACAAGCGAT	Primer Bank	N/A
Duox2 R primer: TAGGCACGGTCTGCAAACAG	Primer Bank	N/A
Birc3 F primer: ACGCAGCAATCGTGCATTTTG	Primer Bank	N/A
Birc3 R primer: CCTATAACGAGGTCACTGACGG	Primer Bank	N/A
Beta actin F primer: GGCTGTATTCCCCTCCATCG	Primer Bank	N/A
Beta actin R primer: CCAGTTGGTAACAATGCCATGT	Primer Bank	N/A

Table S3: Quantitative RT-PCR primers, (Related to STAR methods).
Oligonucleotide primers used for measuring gene expression in stimulated organoids.