**SUPPLEMENTAL INFORMATION**

## Supplemental Figure Legends

**Figure S1. Related to Figure 1 and Figure 2.**
(**a**) Pictures of proximal small intestine (left) and colon (right) at 16 weeks of age from co-housed $A20^{wt}$, $A20^{cko}$ and $A20/Myd88^{cko}$ mice. S = stomach, C = cecum. Dashed lines mark pylorus, indicating the transition to proximal small intestine. (**b**) Quantification of A20 and Myd88 genomic DNA in the indicated subsets of SI-LP DCs and macrophages from $A20^{wt}$ or $A20/Myd88^{cko}$ mice. Within each cell type, DNA quantity of $A20/Myd88^{cko}$ mice is relative to that of $A20^{wt}$ mice. Two mice of each genotype are pooled in the experiment. (**c**) Flow cytometry gating strategy to distinguish 3 subsets of SI-LP DCs in $A20^{cko}$ and $A20/Myd88^{cko}$ mice. (**d**) Expression of A20 mRNA within the indicated subsets of SI-LP DCs from $A20^{wt}$, $A20^{cko}$ and $A20/Myd88^{cko}$ mice, relative to *hprt*. Within each DC subset, mRNA expression in $A20^{cko}$ and $A20/Myd88^{cko}$ mice is relative to expression in $A20^{wt}$ mice. Four mice of each genotype are pooled in the experiment. (**e**) WT or A20-deficient bone marrow derived dendritic cells (BMDCs) were generated by culture of bone marrow cells in media containing granulocyte-macrophage colony-stimulating factor for 7 days, after which $CD11c^+$ BMDCs were enriched and stained for surface expression of CD80, CD86 and CD40. Histograms are representative of at least 3 independent experiments.

**Figure S2. Related to Figure 2.**
(**a**) Combined pathological scores of five SI regions evaluated from 10-week-old $A20^{wt}$, $A20^{cko}$ and $A20/Myd88^{cko}$ mice, with or without deficiency in *Rag1*. (**b**) Picture of proximal small intestine from co-housed 16 week-old mice of indicated genotype. S = stomach. Dashed lines mark pylorus, indicating the transition to proximal small intestine. (**c**) Representative histogram of CD80, CD86 or CD40 expression by SI-LP DCs and macrophages from *Rag1*$^{-/-}$ $A20^{wt}$, *Rag1*$^{-/-}$ $A20^{cko}$ and *Rag1*$^{-/-}$ $A20/Myd88^{cko}$ mice. (**d**) Summary of cell surface expression of maturation markers CD80, CD86 and CD40 on SI-LP DCs and macrophages from *Rag1*-/- mice of the indicated genotype ($A20^{wt}$, n = 4; $A20^{cko}$, n = 4; $A20/Myd88^{cko}$, n =5). Cellular expression of maturation markers is represented as mean fluorescence intensity (MFI) relative to that of the same population in *Rag1*-/- $A20^{wt}$ mice. Results are from 2 independent experiments. Each dot represents one mouse. Error bars represent mean ± SEM, *, $P < 0.05$, **, $P < 0.01$, ***, $P < 0.001$, ****, $P < 0.0001$ (unpaired student's *t*-test).

**Figure S3. Related to Figure 3.**
(**a**) Quantification of 16S ribosomal DNA per gram of stool from co-housed mice of the indicated genotype, with or without antibiotics (Abx). Results were relative to average abundance of 16S ribosomal DNA in $A20^{wt}$ mice without antibiotics. (**b,c**) Pathological scores evaluating small intestine (SI) of $A20^{wt}$ and $A20/Myd88^{cko}$ mice at 10 weeks of age, with or without antibiotic treatment. The combined score of all SI regions examined (**b**) or score of each individual region (**c**). Abbreviations: Duodenum (Duo), Jejunum (Jej), Ileum (Ile). Each dot represents one mouse. Error bars represent mean ± SEM.

**Figure S4. Related to Figure 4.**
Percentage of $IFN\gamma^+IL\text{-}17^+$ T cells (**a**) or $Foxp3^+$ T cells (**b**) among $CD4^+$ T cells were assayed from the same DC–T cell co-cultures as in **Fig. 4**. (**c**) SI-LP macrophages or DCs from $A20^{wt}$, $A20^{cko}$ and $A20/Myd88^{cko}$ mice were co-cultured with naïve OT-II T cells as in **Fig. 4**. For each genotype the macrophages and DCs tested were purified from the same mice. The total number of $IFN\gamma^+$ and $IL\text{-}17^+$ T cells at the end of culture was assayed and normalized to 1,000 input APCs. For reference, a line is drawn through a value of 1. Each dot represents an individual experiment or replicates within one experiment. (**d**) mRNA expression of the indicated cytokine gene in SI-LP macrophages of $A20^{wt}$, $A20^{cko}$ and $A20/Myd88^{cko}$ mice. Expression is relative to *hprt*. (**e-g**) The percentages of $IFNg^+$ or $IL\text{-}17^+$ T cells from co-cultures of $CD103^+CD11b^-$ DCs (**e**) or $CD103^+CD11b^+$ DCs (**f**) from $Myd88^{wt}$ or $Myd88^{cko}$ mice. The percentages of $IFNg^+IL\text{-}17^+$ T cells from $CD103^-CD11b^+$ DC–T cell co-cultures (**g**) were also assayed. Data are from the same experiment described in **Fig. 4j-l**. Error bars represent mean ± SEM. *, $P < 0.05$, **, $P < 0.01$, ***, $P < 0.001$ (unpaired student's *t*-test).

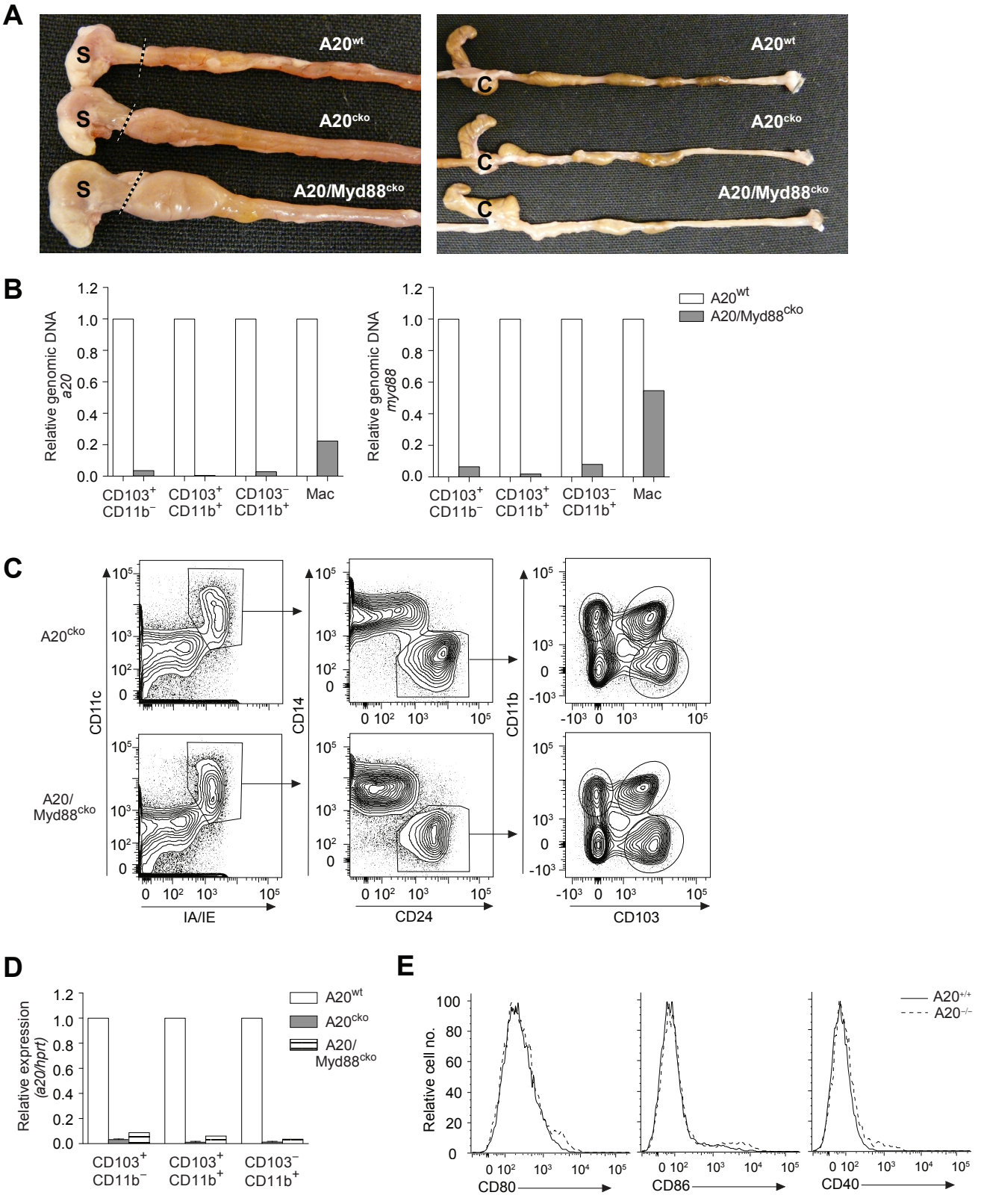
**Figure S5. Related to Figure 5.**

The total number of $CD4^+$ T cells after four days co-culture with SI-LP $CD103^+CD11b^-$ DCs from $A20^{wt}$ and $A20^{cko}$ mice as described in **Fig. 5**, in the presence of CD80/86 blocking antibodies or isotype control (iso). Error bars represent mean ± SEM. *, $P < 0.05$ (unpaired student's *t*-test).

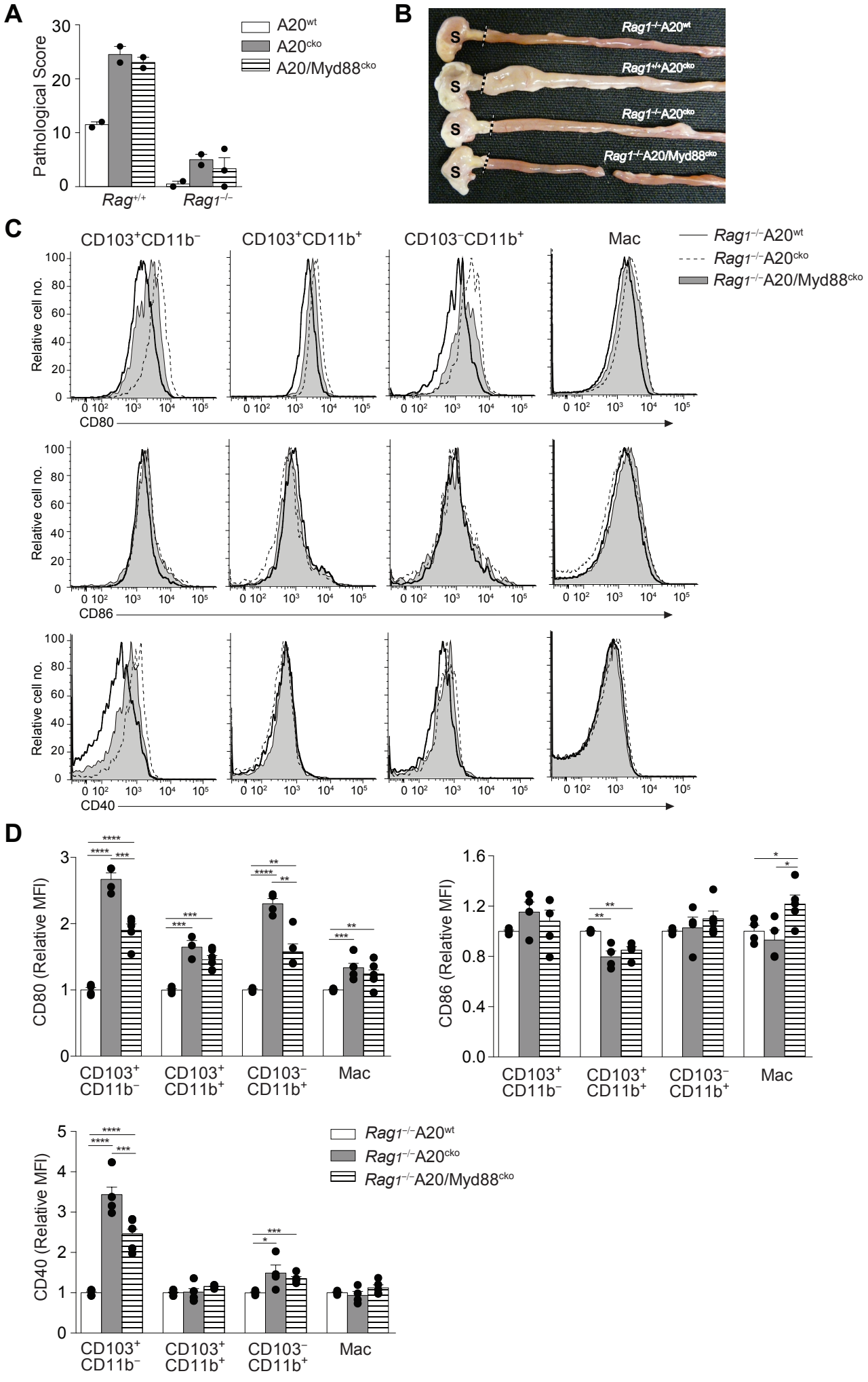
**Figure S6. Related to Figure 4.**
IL-6 protein in supernatant of DC–T cell co-cultures of the indicated DC subset was quantified from the same co-cultures from **Fig. 4**. Error bars represent mean ± SEM, n.s., no statistical significance, *, $P < 0.05$, **, $P < 0.01$, ***, $P < 0.001$ (unpaired student's *t*-test).

**Figure S7. Related to Figure 6.**
mRNA from SI-LP DCs described in **Fig. 6** were analyzed for abundance of IL-12p40 (*il12b*) and IL-12p35 (*il12a*) transcripts in $A20^{wt}$, $A20^{cko}$ and $A20/Myd88^{cko}$ mice. Expression is relative to *hprt*. Error bars represent mean ± SEM. ND = not detected.

# Figure S1


A
A20wt
A20cko
A20/Myd88cko
S
C
B
Relative genomic DNA a20
Relative genomic DNA myd88
A20wt
A20/Myd88cko
CD103+ CD11b−
CD103+ CD11b+
CD103− CD11b+
Mac
C
A20cko
A20/ Myd88cko
CD11c
IA/IE
CD14
CD24
CD11b
CD103
D
Relative expression (a20/hprt)
A20wt
A20cko
A20/ Myd88cko
E
Relative cell no.
A20+/+
A20−/−
CD80
CD86
CD40

# Figure S2


A
Pathological Score
A20wt
A20cko
A20/Myd88cko
Rag+/+
Rag1−/−
B
Rag1−/−A20wt
Rag1+/+A20cko
Rag1−/−A20cko
Rag1−/−A20/Myd88cko
S
C
CD103+CD11b−
CD103+CD11b+
CD103−CD11b+
Mac
Rag1−/−A20wt
Rag1−/−A20cko
Rag1−/−A20/Myd88cko
Relative cell no.
CD80
CD86
CD40
D
CD80 (Relative MFI)
CD86 (Relative MFI)
CD40 (Relative MFI)
CD103+ CD11b−
CD103+ CD11b+
CD103− CD11b+
Mac

## Figure S3

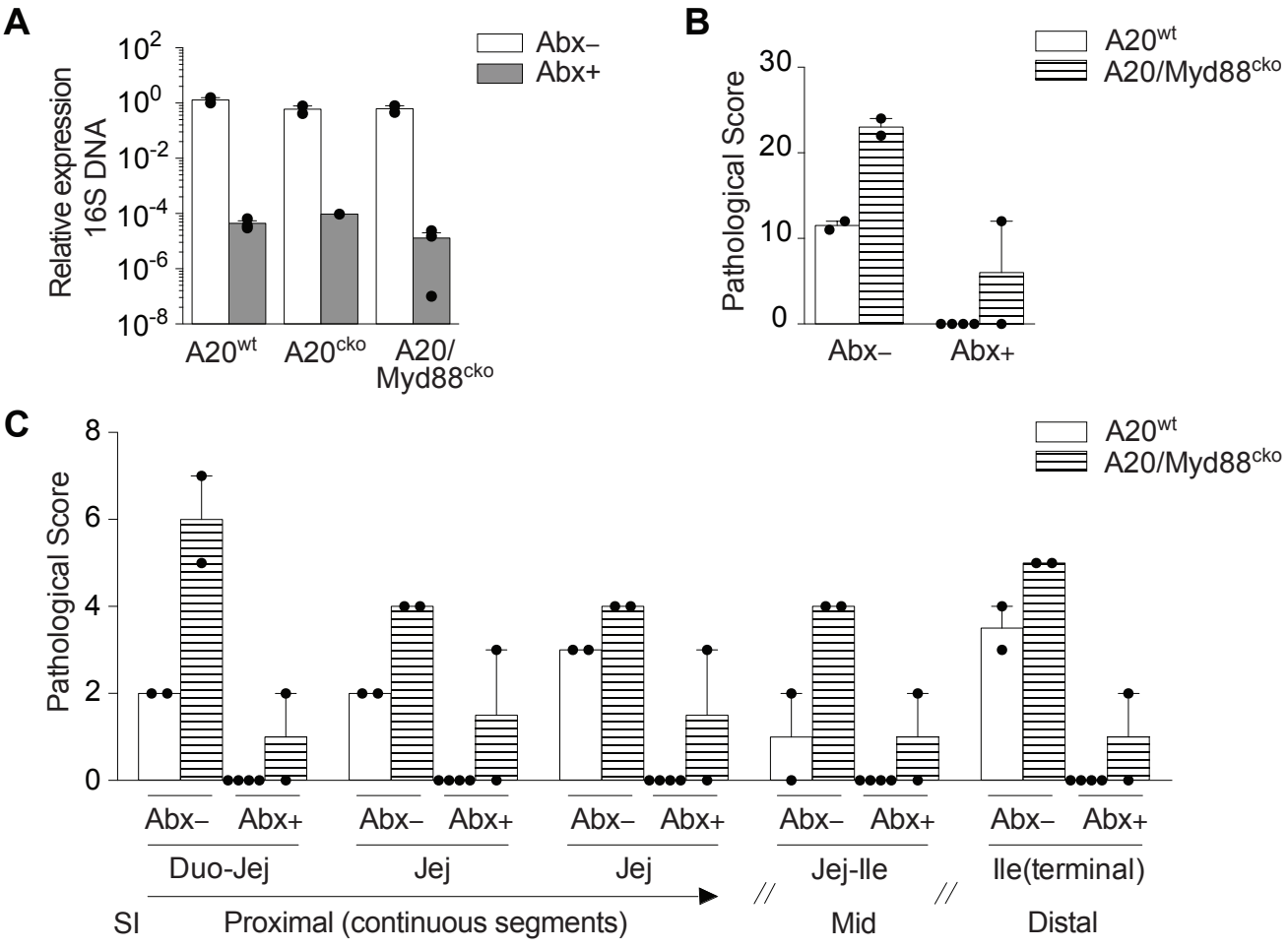

A
Relative expression 16S DNA
$10^{2}$
$10^{0}$
$10^{-2}$
$10^{-4}$
$10^{-6}$
$10^{-8}$
Abx–
Abx+
A20wt
A20cko
A20/ Myd88cko
B
Pathological Score
30
20
10
0
A20wt
A20/Myd88cko
Abx–
Abx+
C
Pathological Score
8
6
4
2
0
A20wt
A20/Myd88cko
Abx–
Abx+
Duo-Jej
Jej
Jej
Jej-Ile
Ile(terminal)
SI
Proximal (continuous segments)
Mid
Distal

**Figure S4**

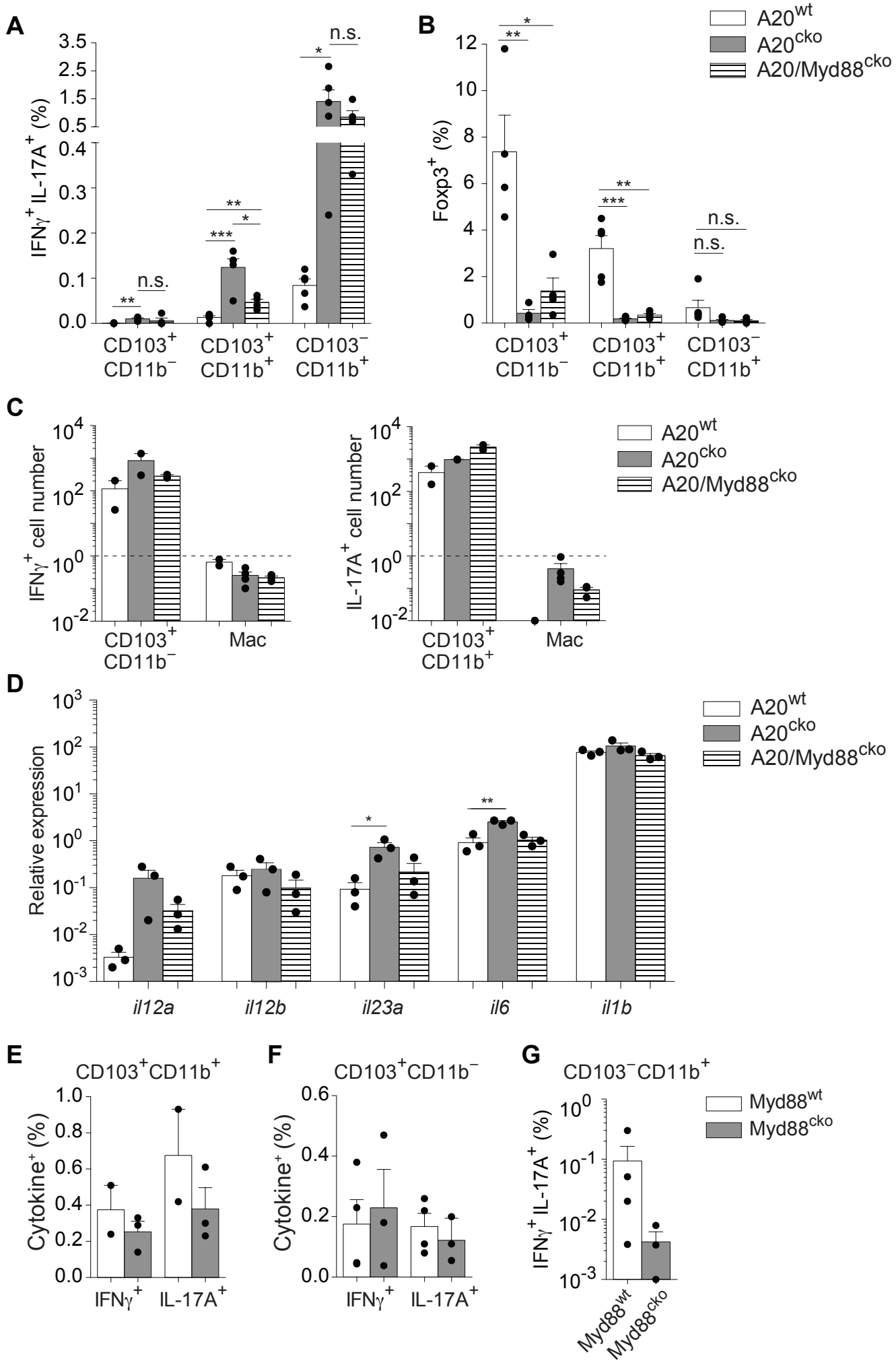

A
B
C
D
E
F
G
A20wt
A20cko
A20/Myd88cko
Myd88wt
Myd88cko
IFNγ+ IL-17A+ (%)
Foxp3+ (%)
IFNγ+ cell number
IL-17A+ cell number
Relative expression
Cytokine+ (%)
CD103+ CD11b−
CD103+ CD11b+
CD103− CD11b+
Mac
il12a
il12b
il23a
il6
il1b
IFNγ+
IL-17A+
n.s.

**Figure S5**

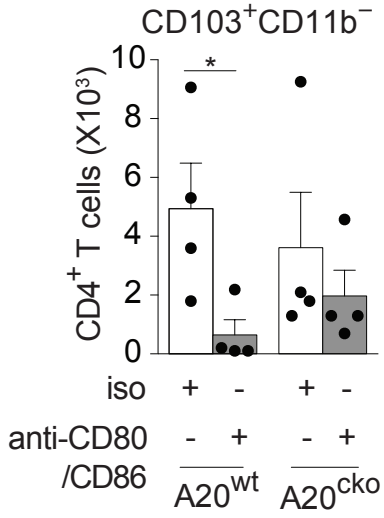

CD103+CD11b−
CD4+ T cells (X10³)
10
8
6
4
2
0
*
iso + - + -
anti-CD80 - + - +
/CD86
A20wt
A20cko

**Figure S6**

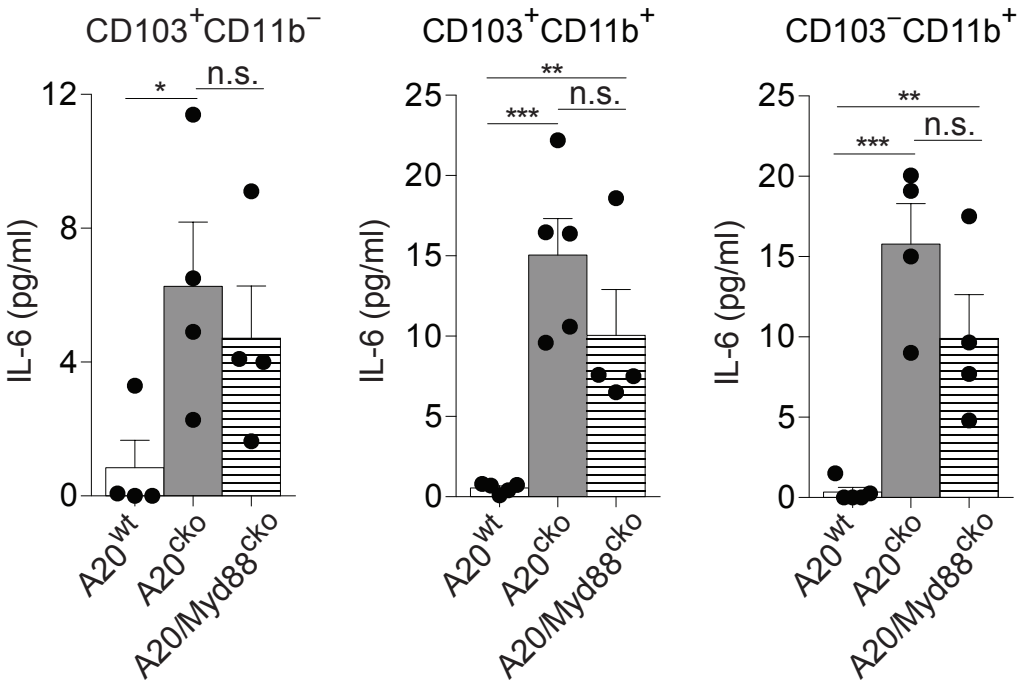
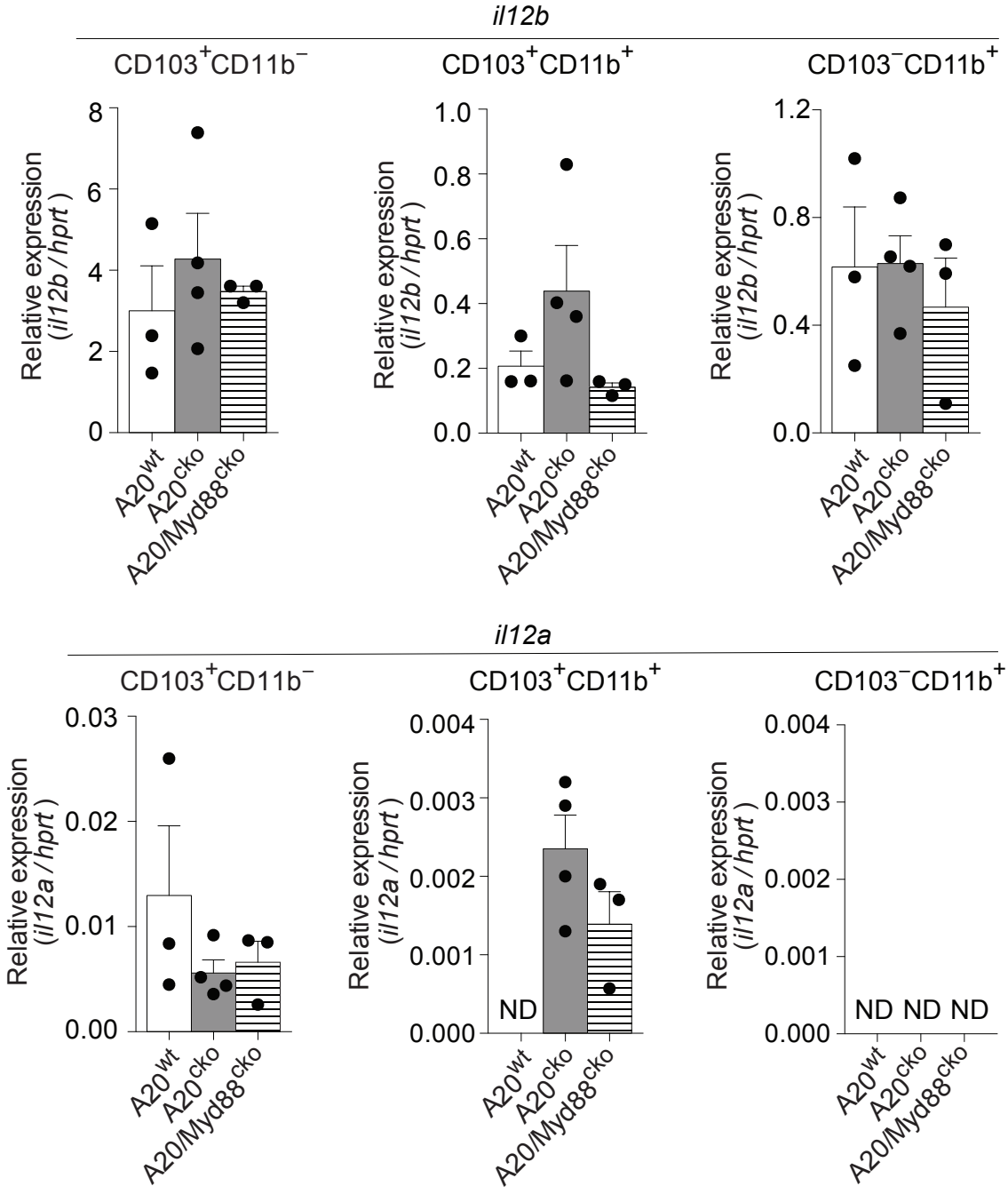

CD103+CD11b−
CD103+CD11b+
CD103−CD11b+
IL-6 (pg/ml)
12
8
4
0
25
20
15
10
5
*
n.s.
**
***
A20wt
A20cko
A20/Myd88cko

**Figure S7**


il12b
CD103+CD11b−
CD103+CD11b+
CD103−CD11b+
Relative expression (il12b / hprt)
8
6
4
2
0
1.0
0.8
0.6
0.4
0.2
0.0
1.2
0.8
0.4
0.0
A20wt
A20cko
A20/Myd88cko
il12a
CD103+CD11b−
CD103+CD11b+
CD103−CD11b+
Relative expression (il12a / hprt)
0.03
0.02
0.01
0.00
0.004
0.003
0.002
0.001
0.000
ND
ND ND ND
A20wt
A20cko
A20/Myd88cko

**Supplemental Experimental Procedures**

## Intestinal lamina propria preparation

Small intestine was flushed with HBSS/10mM HEPES followed by removal of Peyer's patches. Small intestine was cut longitudinally and washed thoroughly with HBSS/10mM HEPES to remove intestinal contents. Organ weight was recorded after brief wicking of liquid. Small intestine was then cut into 0.5cm pieces, washed twice for 10 min in HBSS/10mM HEPES/5mM EDTA/1.25% BSA/1mM DTT, followed by another 10 min wash in HBSS/10mM HEPES/1.25% BSA. Tissue was digested in C-tubes (Miltenyi) with enzymes provided in the lamina propria dissociation kit. Digestion volumes were adjusted based upon organ weight. Following digestion, tissues were dissociated using the GentleMACS™ Dissociator (Miltenyi). The resulting cellular suspension was filtered through a 100m. mesh, centrifuged, and filtered again through a 40μm mesh prior to RBC lysis and analysis.

## Quantitative PCR

Cells were sorted into Trizol® (Thermo Fisher) and cDNA prepared using QuantiTect Reverse Transcription Kit (Qiagen). 10 to 30ng cDNA was used for each reaction using the following Taqman probe sets: *il6* (Mm00446190_m1), *il23a* (Mm00518984_m1), *il12a* (Mm00434165_m1), *il12b* (Mm00434174_m1), A20 (*tnfaip3;* Mm00437121_m1), *myd88* (Mm00440338_m1), and *il1b* (Mm00434228_m1). Genomic deletion of A20 exon 2 and Myd88 exon 3 was quantified as previously described (Hou et al., 2008; Tavares et al., 2010).

## Histological Analysis

Each segment was scored for inflammation (I, score 0 – 4), for extent/percent of tissue involving any inflammatory changes (E1, score 0 – 4), and for extent of the most severe changes (E2, score 0 – 4), using a modification of a previously described scoring system (Hale et al., 2005); architectural distortion was generally not present, so scoring for mucosal architectural changes was not performed. Segment pathological score = I + E1 + E2 (maximum score for each mouse = 60). An inflammation score of 1 (mild inflammation, limited to the mucosa) required the presence of 4 or more neutrophils. Since large numbers of lymphocytes and macrophages are normally present in the small intestine, increased numbers of mononuclear cells did not trigger a score for inflammation in the absence of neutrophils. Foci of granulomatous inflammation, with or without giant cells, were counted in the extent of inflammation score if sufficient neutrophils were also present to trigger an inflammation score greater than 0.