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Supporting Online Material for

Activation of β-Catenin in Dendritic Cells Regulates Immunity Versus Tolerance in the Intestine

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This PDF file includes:

Supporting Online Material
Materials and Methods
Supplementary Text
Figures. S1 to S14
Figure Legends S1 to S14
Table S1
References

Materials and Methods

Mice. C57BL/6 male mice 6-12 weeks of age were obtained from Jackson Laboratories. OT-II, TCF/LEF-reporter mice (B6.Cg-Tg(BAT-lacZ)3Picc/J)(*1*), β-catenin floxed mice (B6.129-Ctnnb1<tm2Kem>/KnwJ) (*2*) were originally obtained from Jackson Laboratories, and bred on-site. CD11-cre mice (*3*) mice were backcrossed to C57BL/6 mice for 5 generations. To specifically knock-down β-catenin in DCs, we have crossed floxed β-catenin allele mice (β-cat$^{fl/fl}$) (*2*) with transgenic mice (DC-cre) expressing cre enzyme under the control of CD11cpromoter (3), to generate β-cat$^{DC-/-}$ mice. Successful cre-mediated deletion was confirmed by PCR and protein expression. Mice were maintained under specific pathogen-free conditions in the Emory Vaccine Center or Emory Whitehead Biomedical Research Building vivariums. All animal protocols were reviewed and approved by the Institute Animal Care and Use Committee of Emory University.

Isolation of LP cells. Small/Large Intestine: Briefly, intestines from littermates of β-cat$^{fl/fl}$ and β-cat$^{DC-/-}$ mice were removed and carefully cleaned of their mesentery, Peyer's patches excised (small intestine), and the intestines opened longitudinally, and washed of fecal contents. Intestines were then cut into 0.5cm pieces, transferred into 50ml conical tubes, and shaken at 250 rpm for 20 min at 37°C in HBSS medium (Life Technologies), supplemented with 5% FBS (CellGro), containing 2mM EDTA. This process was repeated two additional times. The cell suspensions were passed through a strainer and remaining intestinal tissue was washed and then minced, transferred to a 50-ml conical tube, and shaken for 30 min at 37°C in RPMI + 5% FBS containing Type VIII collagenase at 200 U/ml (Sigma). The cell suspension was collected and passed through a strainer, and pelleted by centrifugation at 1200 rpm. CD11b^{+} or CD11c^{+} cells were enriched by positive selection with CD11b and CD11c microbeads (Miltenyi Biotech), respectively. For FACS-sorting experiments intestinal CD11b/c-enriched cells were stained with PE-conjugated anti-CD11b (clone M1/70), Per-CP-conjugated anti-CD45 (clone 30-F11; Pharmingen), APC conjugated anti- CD11c (clone HL3) and AlexaFluor 700 conjugated anti-IA^{b} (clone M5/114) all from eBioscience unless otherwise noted. Stained cells were sorted to purify indicated populations on a FACS Aria at The Emory Vaccine Center Flow Cytometry Core Facility.

Purification of splenic dendritic cells. In brief, spleens isolated from mice were into small fragments and then digested the fragments with collagenase type 4 (1 mg ml^{-1}) in complete DMEM plus 2% FBS for 30 min at 37 °C. We washed the cells twice and enriched CD11c^{+} DCs with the CD11c-specific microbeads from Miltenyi Biotech. The resulting purity of CD11c^{+} DCs was approximately 95%.

Flow cytometry. Isolated splenocytes or small intestinal LP cells were resuspended in PBS containing 5% FBS. After incubation for 15 min at 4°C with the blocking antibody 2.4G2 (anti-FcγRIII/I), the cells were stained at 4°C for 30 min with the appropriate labeled antibodies. Samples were then washed two times in PBS containing 5% FBS. The samples were immediately analyzed at this point, or fixed in PBS containing 2% paraformaldehyde and stored at 4°C. Antibodies used for analysis were from eBioscience unless otherwise noted: FITC-labeled anti mouse CD11c, PE-labeled anti mouse CD103 (BD Pharmingen), PerCP-labeled anti mouse CD45 (BD Pharmingen), PE-Cy7-labeled anti mouse F4/80, APC-labeled anti mouse CD11b, Alexa Fluor-labeled anti mouse IA^b. Intracellular staining for Raldh, β-catenin and β-galactosidase were performed using goat polyclonal Raldh antibody (Abcam), rabbit monoclonal β-catenin antibody (Cell signaling Technology), rabbit monoclonal β-galactosidase antibody (Abcam) or with appropriate isotype control in TBS containing 1% bovine serum albumin, followed by incubation with Alexa Fluor 488-conjugated rabbit-anti-goat immunoglobulin or goat anti-rabbit immunoglobulin G (IgG; Molecular Probes, Eugene, OR). Intracellular IL-17 or Foxp3 staining was performed using PE-labeled anti-mouse IL-17 or Foxp3 (eBioscience). Flow cytometric analysis was performed on a Becton Dickinson FACSCaliber or LSR II flow cytometer at the Emory Vaccine Center.

Real-time PCR. Total RNA was isolated from purified LP antigen presenting cells using the Qiagen RNeasy Mini Kit, according to the manufacturer's protocol, with on-column DNase digestion using the RNase-Free DNase set. cDNA was generated using the Superscript First-Strand Synthesis System for RT-PCR and oligo dT primers (Invitrogen), according to the manufacturer's protocol. cDNA was used as a template for quantitative real-time PCR using SYBR Green Master Mix (BioRad) and gene specific primers (Tabel 1). PCR and analysis was performed using a MyiQ ICycler (BioRad). Gene expression was calculated relative to *Gapdh*.

Stimulation of lymphocytes. Purified lamina propria APC subsets ($1x10^5$) were co-cultured with naïve $CD4^+CD62L^+$ OT-II $CD4^+$ T cells ($1x10^5$) and OVA peptide (ISQVHAAHAEINEAGR; 5 μg/ml) in 200 ml RPMI complete medium in 96-well round bottom plates. Supernatants were analyzed after 90 h and cells were harvested and analyzed directly, or restimulated. For restimulation cells were collected after 90 h of primary culture, and then restimulated for 6 h with plate-bound antibodies against CD3 (10 μg/ml; 145.2C11 from Becton Dickinson) and CD28 (2 μg/ml; 37.51 from Becton Dickinson), in the presence of brefeldin A (Becton Dickinson) for intracellular cytokine detection. Lymphocytes from the small or large intestine LP were stimulated with PMA (50ng/ml) and ionomycin (500ng/ml) for 6 h in the presence of GolgiPlug.

Cytokine detection. IL-17, IL-6, IL-12(p40), IL-12(p70), IL-10, TNF-α, and IFN-γ, IL- 1β in culture supernatants were quantitated by ELISA kits from BD Biosciences.IL-23 was measured by an ELISA kit from eBioscience. For intracellular cytokine analysis, T cells were stimulated with PMA and ionomycin 6 h in the presence of GolgiPlug (PharMingen) and subsequently incubated with blocking 2.4G2 anti-FcγRIII/II (PharMingen) and FITC conjugated CD4, PerCP-conjugated CD45. Cells were permeabilized using Cytofix/Cytoperm and stained using PE-conjugated IL-17A, APC-conjugated IFN-γ or FoxP3 antibodies according to the manufacturer's protocol (PharMingen).

Antibiotic-treatment of mice: TCF/LEF reporter mice, or β-cat$^{fl/fl}$ and β-cat$^{DC-/-}$ mice were fed with an antibiotic cocktail (ampicilin-1g/L, neomycin sulfate-1g/L and vancomycin 0.5g/L) in drinking water for six weeks to sterilize the intestinal microbiota . All antibiotics were purchased from Sigma-Aldrich. After 6 weeks of treatment, β-galactosidase expression in the intestinal DC and macrophages subsets in TCF-reporter mice was compared to untreated mice. Similarly, lamina propria T lymphocytes were isolated from β-cat$^{fl/fl}$ and β-cat$^{DC-/-}$ mice and stimulated *in vitro* with plate bound anti-CD3/CD28 antibody, then analyzed for intracellular cytokine responses as described above.

Induction of DSS-induced colitis. β-cat$^{fl/fl}$ and β-cat$^{DC-/-}$ mice were fed 2% (w/v) DSS in their drinking water for 7 days, and sacrificed at day 8. Each group consisted of five mice and was compared with a control group, which received only water. Body weight was measured every day. At day 8, total lamina propria T lymphocytes were isolated and stimulated *in vitro* with plate bound anti-CD3/CD28 antibody, and then analyzed for intracellular cytokine responses as described above. When the mice were sacrificed, different parts of the colon were isolated for histology.

Histopathology. Mice were sacrificed and spleens or small intestines were snap frozen in tissue-freezing medium (Triangle Biomedical Sciences). Tissue sections (6-μm) were air dried and fixed with a 1:1 acetone methanol mixture at –20°C for 10 min. The tissues were rehydrated, and stained with hematoxylin and eosin to study histological changes after DSS-induced damage.

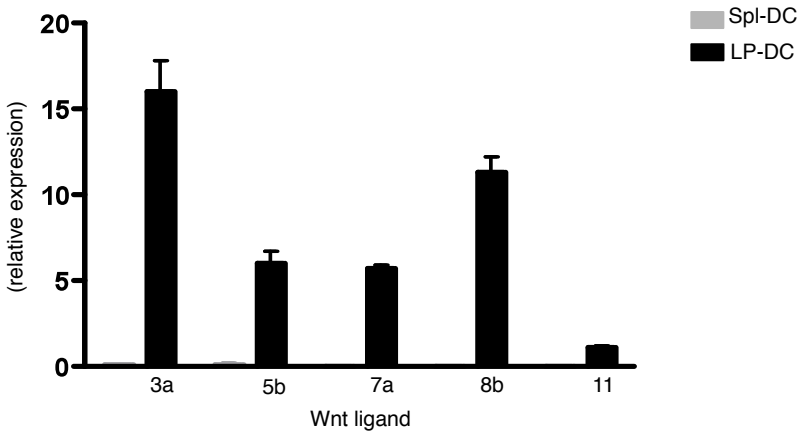
Statistics. Statistical significance of differences in means ± SEM of cytokines released by cells from different groups was calculated using the Student's t test (one-tailed). P values less than 0.05 were considered statistically significant.

Supplementary Text

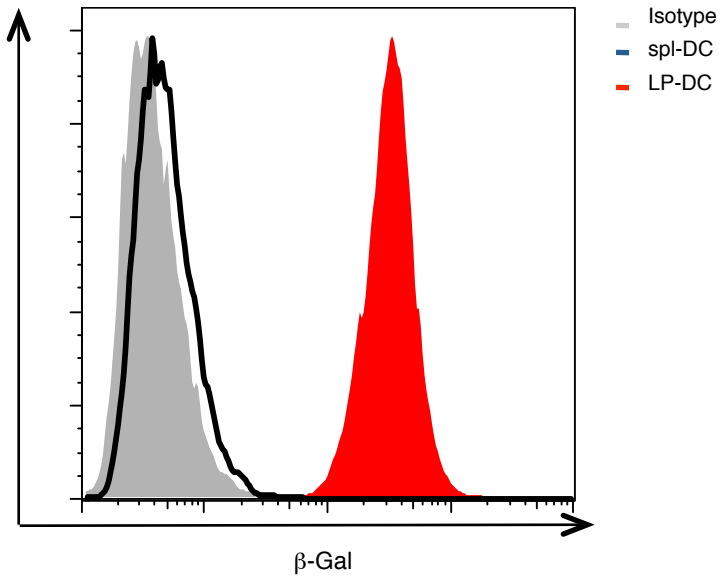
This text adds information regarding Figs. S8A, S8B and S9

The ability of LP-DCs to induce Tregs is chiefly mediated by a specialized subset of CD103$^+$ DCs (*4, 5*). Thus, we analyzed the frequencies of this DC subset in β-cat$^{fl/fl}$ and β-cat$^{DC-/-}$ mice. Both the CD11c$^+$ CD11b$^-$ and CD11c$^+$ CD11b$^+$ subsets contained sub-populations of CD103expressing DCs (fig S8B), although the proportions were markedly different: nearly 60% of CD11c$^+$CD11b$^-$ DCs were CD103$^+$, whereas only 30% of CD11c$^+$CD11b$^+$ DCs were CD103$^+$ in the LP of β-cat$^{fl/fl}$ (wild type mice) (fig S8B). Consistent with this, the CD11c$^+$CD11b$^+$ DC subset induced Tregs less efficiently than the CD11c$^+$CD11b$^-$ DCs, in the absence of exogenous TGF-β (fig S6). We also observed slightly reduced frequencies of CD11c$^+$CD11b$^-$CD103$^+$ and CD11c$^+$CD11b$^+$CD103$^+$ DCs in β-cat$^{DC-/-}$ mice (fig S8B). Additionally, β-catenin activity was increased in the CD103$^+$ DCs compared to the CD103$^-$ DCs within the CD11c$^+$CD11b$^-$ subset in the TCF/LEF reporter mice (fig S9), consistent with the enhanced capacity of CD103$^+$ DCs to induce Tregs (*4, 5*).

S1


Spl-DC
LP-DC
20
15
10
5
0
(relative expression)
3a
5b
7a
8b
11
Wnt ligand

S2



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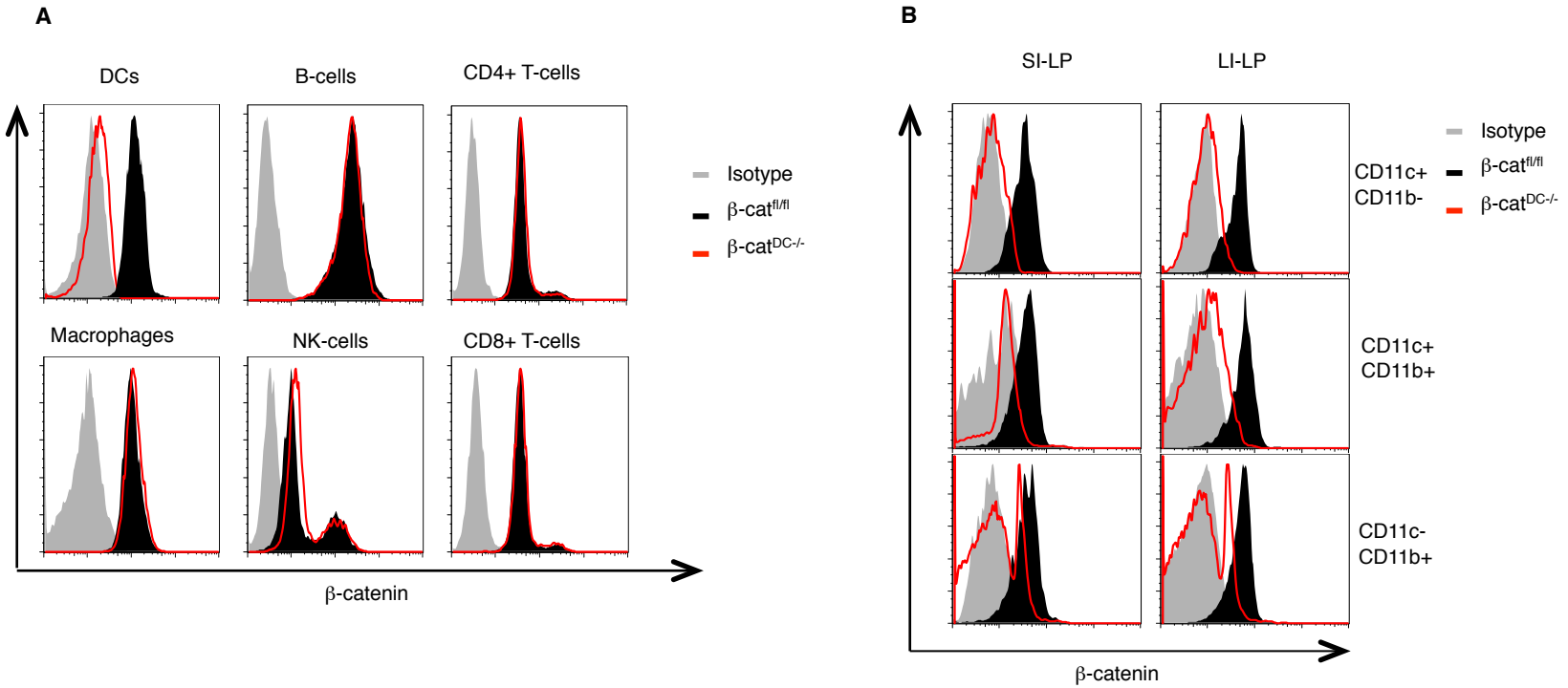
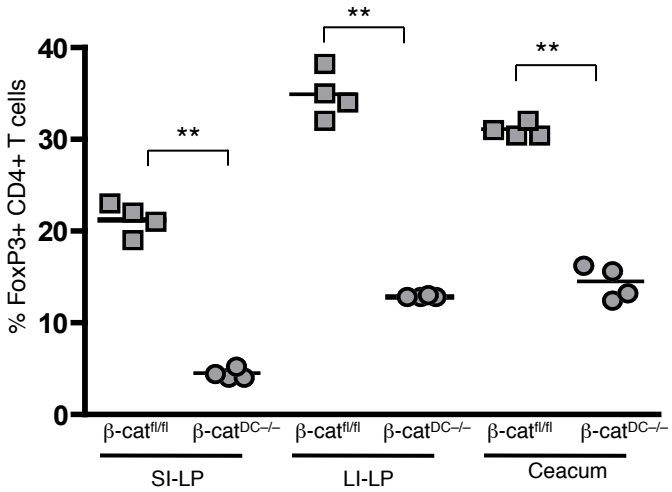
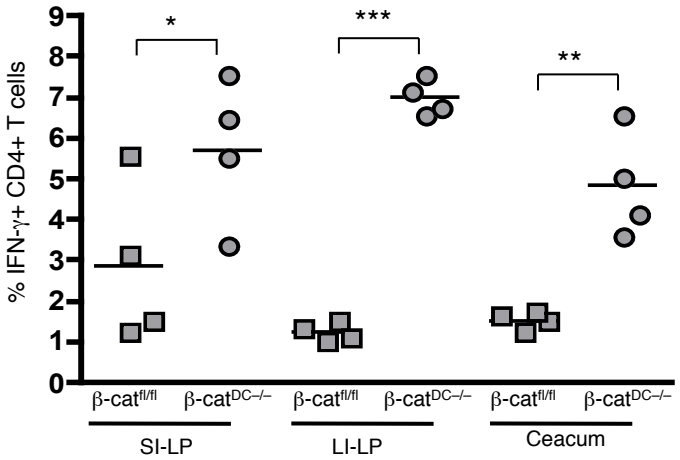

A
DCs
B-cells
CD4+ T-cells
Macrophages
NK-cells
CD8+ T-cells
β-catenin
Isotype
β-cat^fl/fl
β-cat^DC-/-
B
SI-LP
LI-LP
CD11c+ CD11b-
CD11c+ CD11b+
CD11c- CD11b+
β-catenin
Isotype
β-cat^fl/fl
β-cat^DC-/-

S4

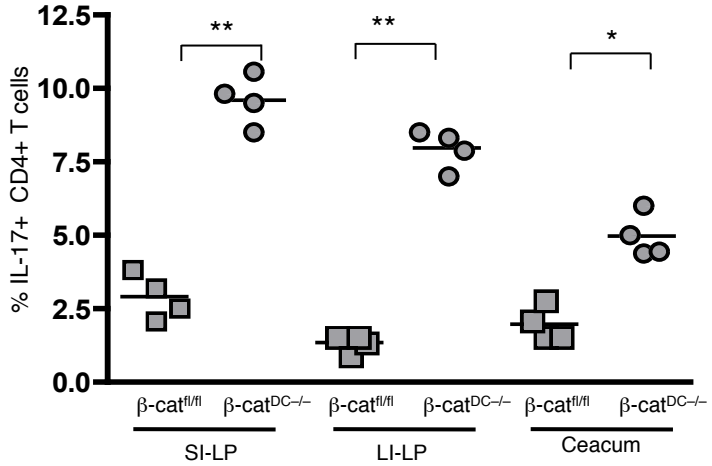
A


% FoxP3+ CD4+ T cells
40
30
20
10
0
**
**
**
β-catfl/fl
β-catDC–/–
β-catfl/fl
β-catDC–/–
β-catfl/fl
β-catDC–/–
SI-LP
LI-LP
Ceacum

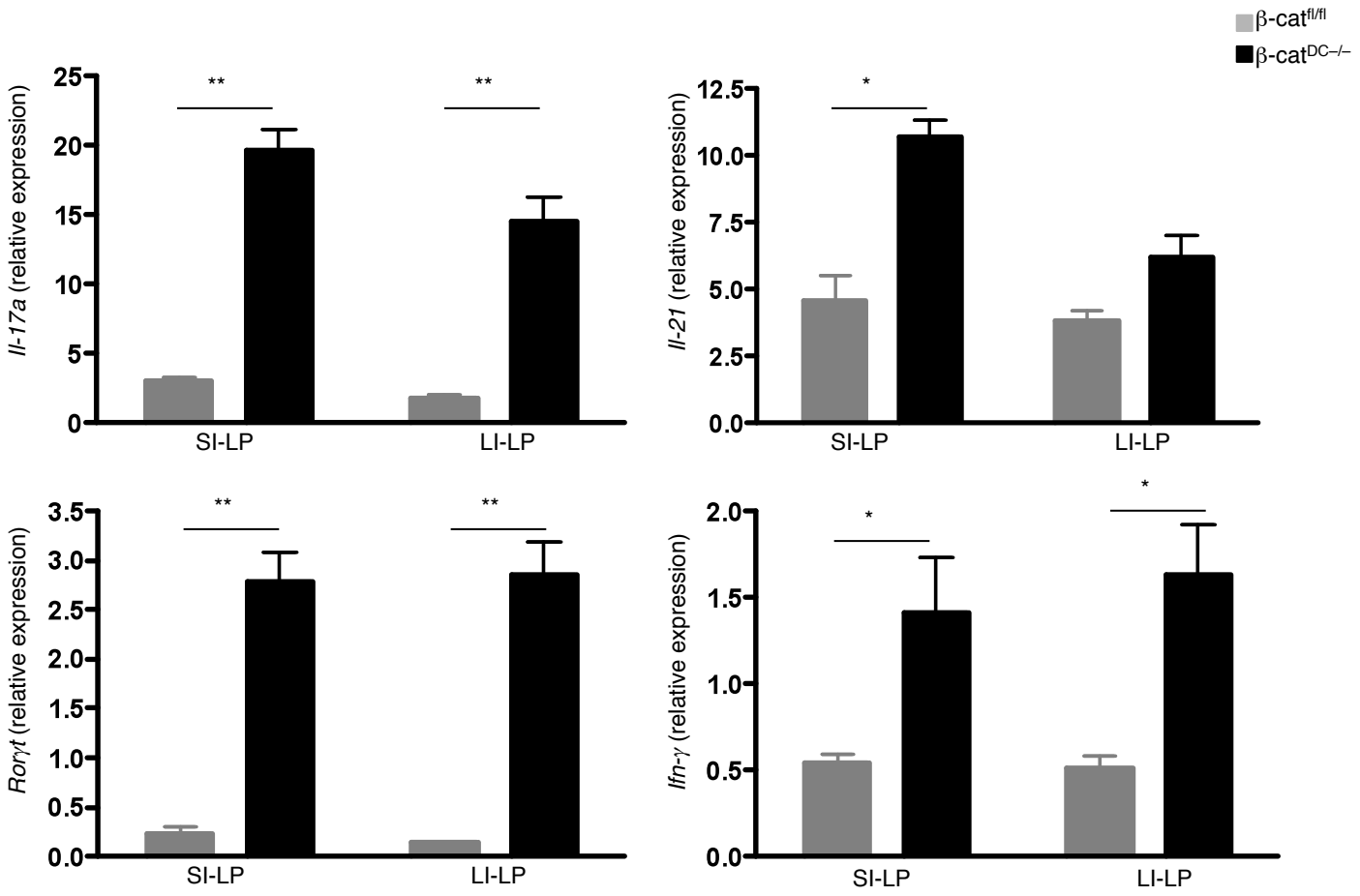
B


% IFN-γ+ CD4+ T cells
9
8
7
6
5
4
3
2
1
0
*
***
**
β-catfl/fl
β-catDC–/–
β-catfl/fl
β-catDC–/–
β-catfl/fl
β-catDC–/–
SI-LP
LI-LP
Ceacum

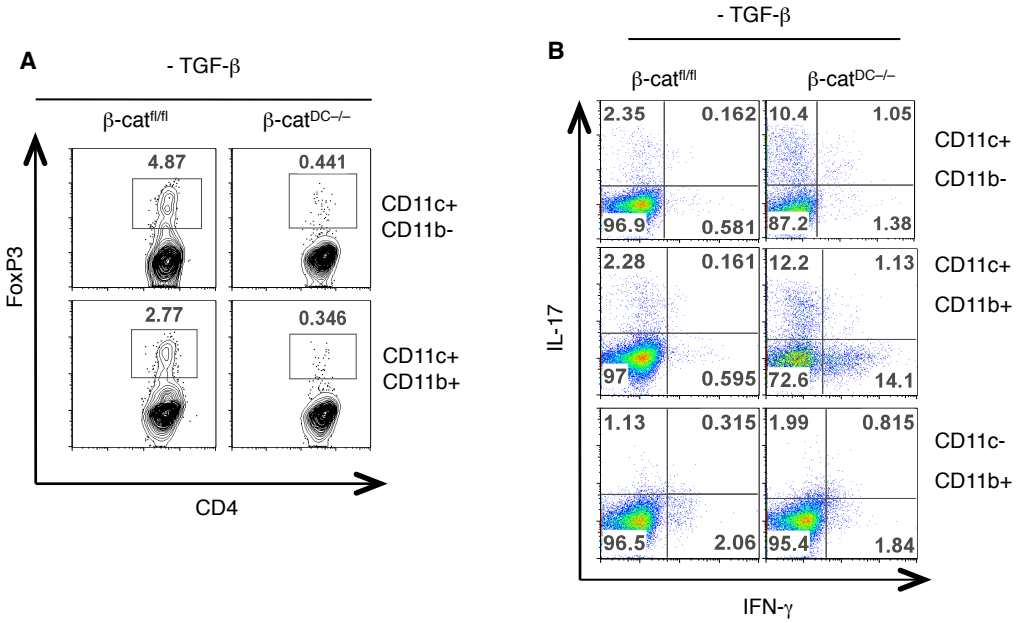
C


% IL-17+ CD4+ T cells
12.5
10.0
7.5
5.0
2.5
0.0
**
**
*
β-catfl/fl
β-catDC–/–
β-catfl/fl
β-catDC–/–
β-catfl/fl
β-catDC–/–
SI-LP
LI-LP
Ceacum

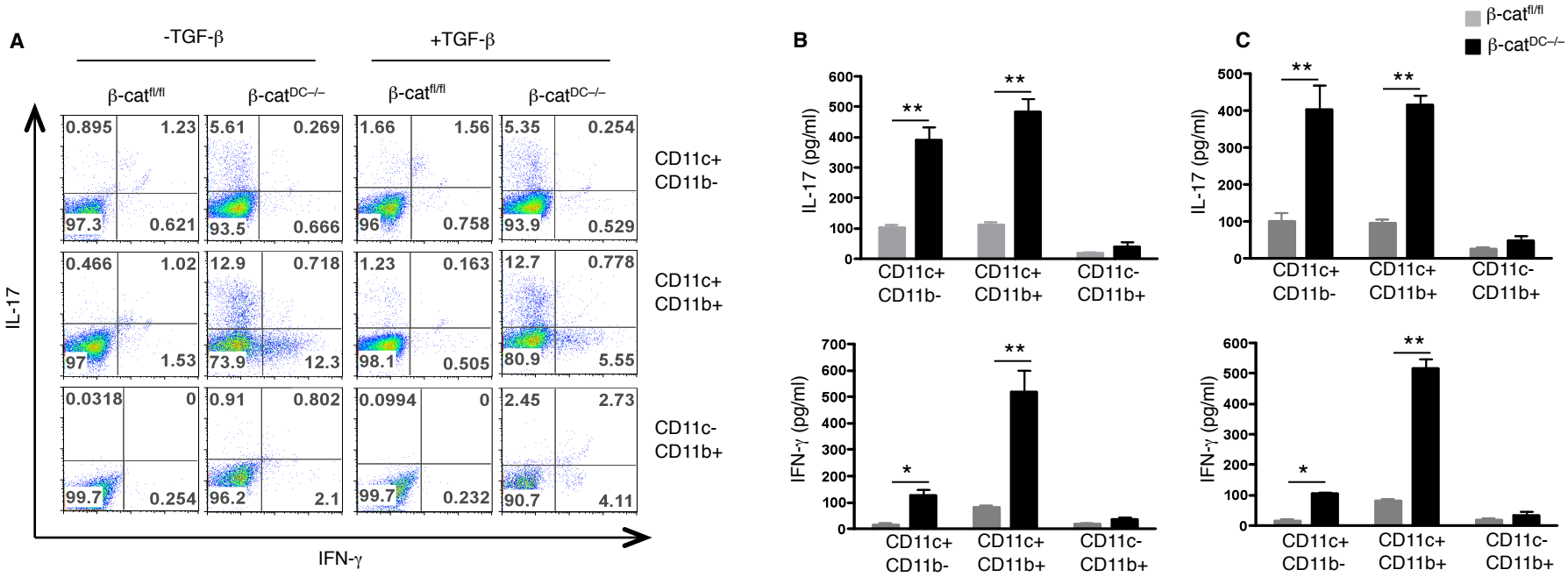
S5


β-cat^fl/fl
β-cat^DC–/–
Il-17a (relative expression)
Il-21 (relative expression)
Rorγt (relative expression)
Ifn-γ (relative expression)
SI-LP
LI-LP
**
*

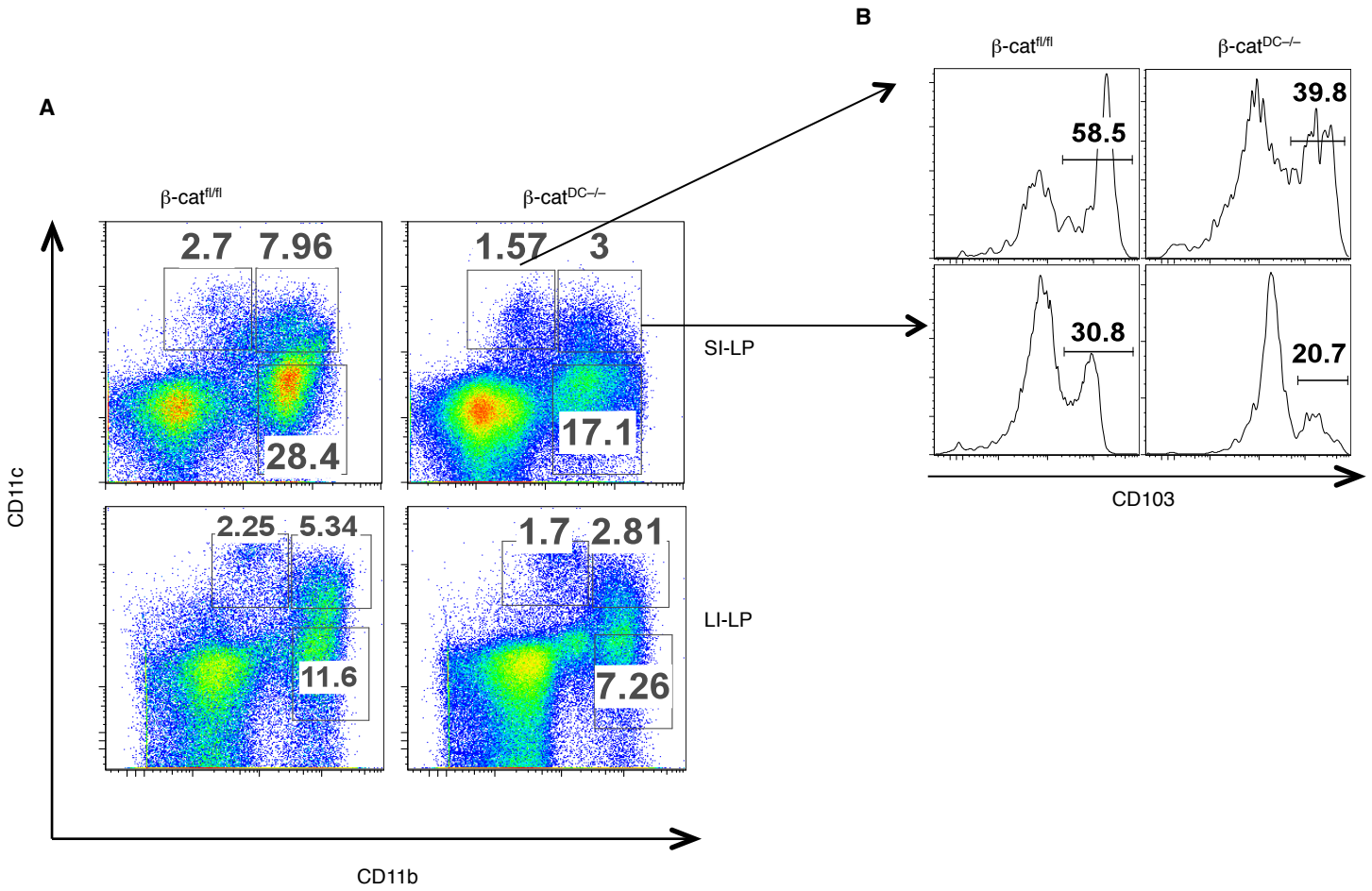
S6

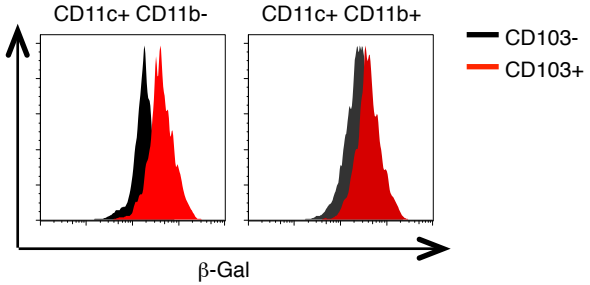

A
- TGF-β
β-cat^fl/fl
β-cat^DC–/–
4.87
0.441
2.77
0.346
CD11c+
CD11b-
CD11c+
CD11b+
FoxP3
CD4
B
- TGF-β
β-cat^fl/fl
β-cat^DC–/–
2.35
0.162
96.9
0.581
10.4
1.05
87.2
1.38
CD11c+
CD11b-
2.28
0.161
97
0.595
12.2
1.13
72.6
14.1
CD11c+
CD11b+
1.13
0.315
96.5
2.06
1.99
0.815
95.4
1.84
CD11c-
CD11b+
IL-17
IFN-γ

S7

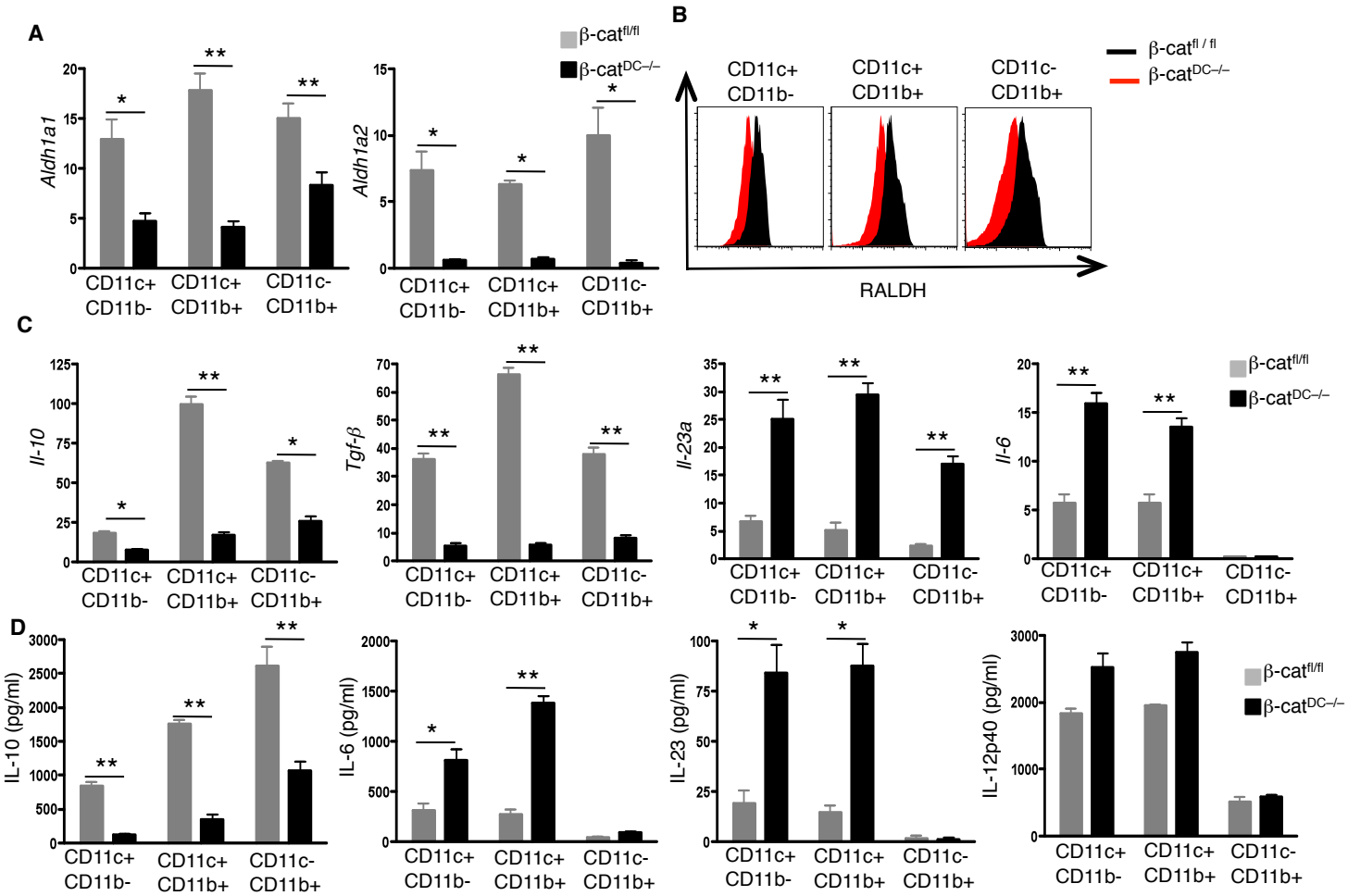

A
-TGF-β
+TGF-β
β-catfl/fl
β-catDC–/–
β-catfl/fl
β-catDC–/–
0.895
1.23
97.3
0.621
5.61
0.269
93.5
0.666
1.66
1.56
96
0.758
5.35
0.254
93.9
0.529
CD11c+ CD11b-
0.466
1.02
97
1.53
12.9
0.718
73.9
12.3
1.23
0.163
98.1
0.505
12.7
0.778
80.9
5.55
CD11c+ CD11b+
0.0318
0
99.7
0.254
0.91
0.802
96.2
2.1
0.0994
0
99.7
0.232
2.45
2.73
90.7
4.11
CD11c- CD11b+
IL-17
IFN-γ
B
IL-17 (pg/ml)
IFN-γ (pg/ml)
CD11c+ CD11b-
CD11c+ CD11b+
CD11c- CD11b+
C
β-catfl/fl
β-catDC–/–
IL-17 (pg/ml)
IFN-γ (pg/ml)
CD11c+ CD11b-
CD11c+ CD11b+
CD11c- CD11b+

S8

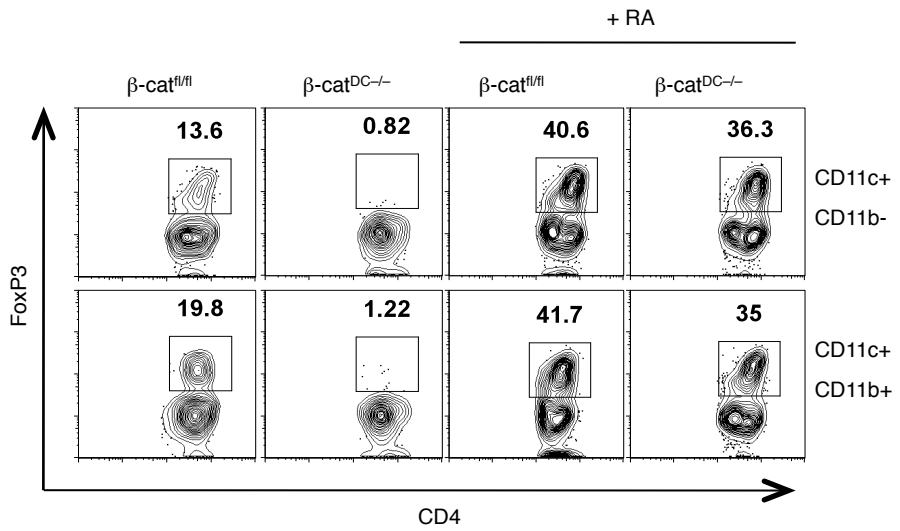

A
β-cat^fl/fl
β-cat^DC–/–
2.7
7.96
28.4
1.57
3
17.1
SI-LP
2.25
5.34
11.6
1.7
2.81
7.26
LI-LP
CD11c
CD11b
B
β-cat^fl/fl
β-cat^DC–/–
58.5
39.8
30.8
20.7
CD103


CD11c+ CD11b-
CD11c+ CD11b+
CD103-
CD103+
β-Gal

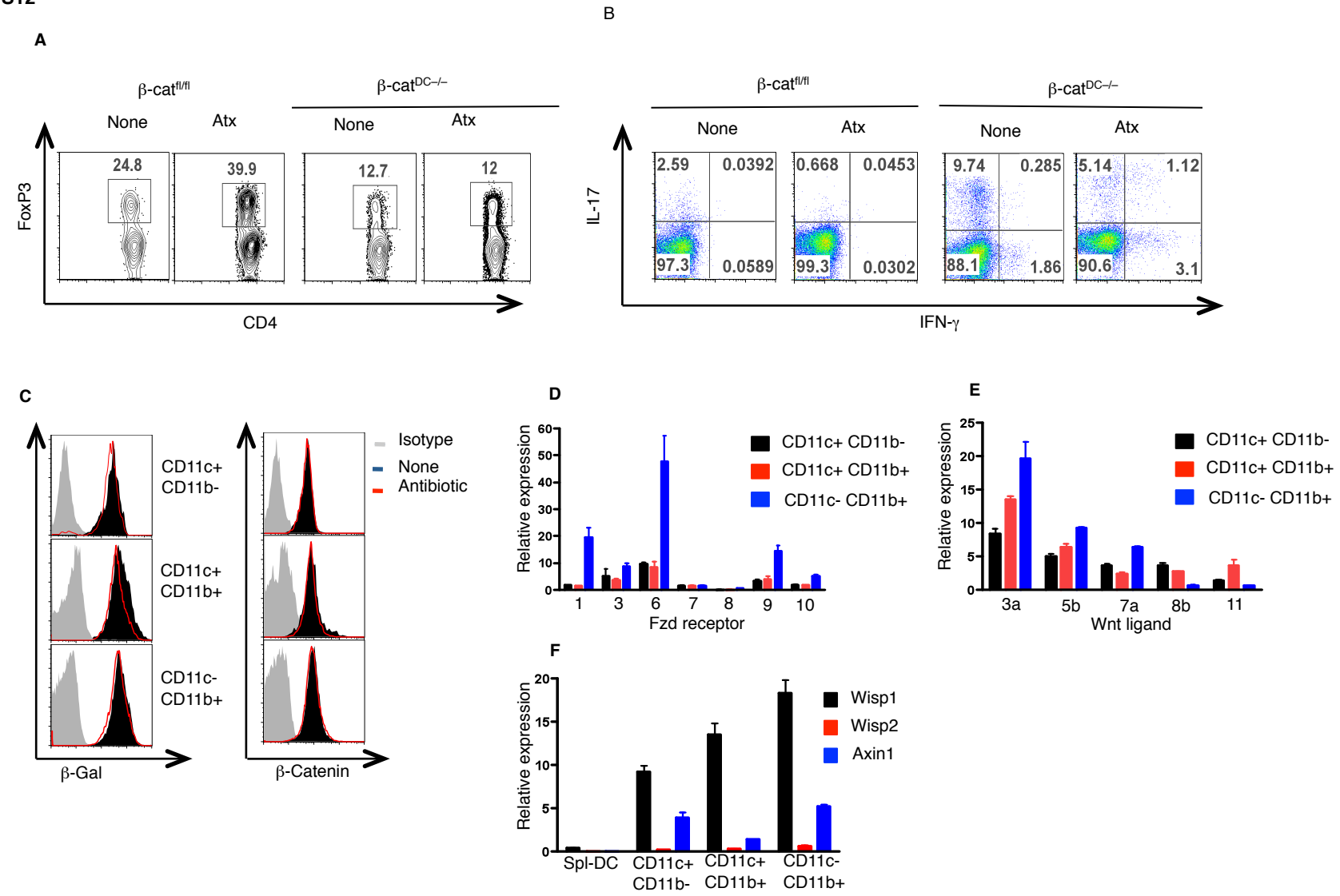
S10

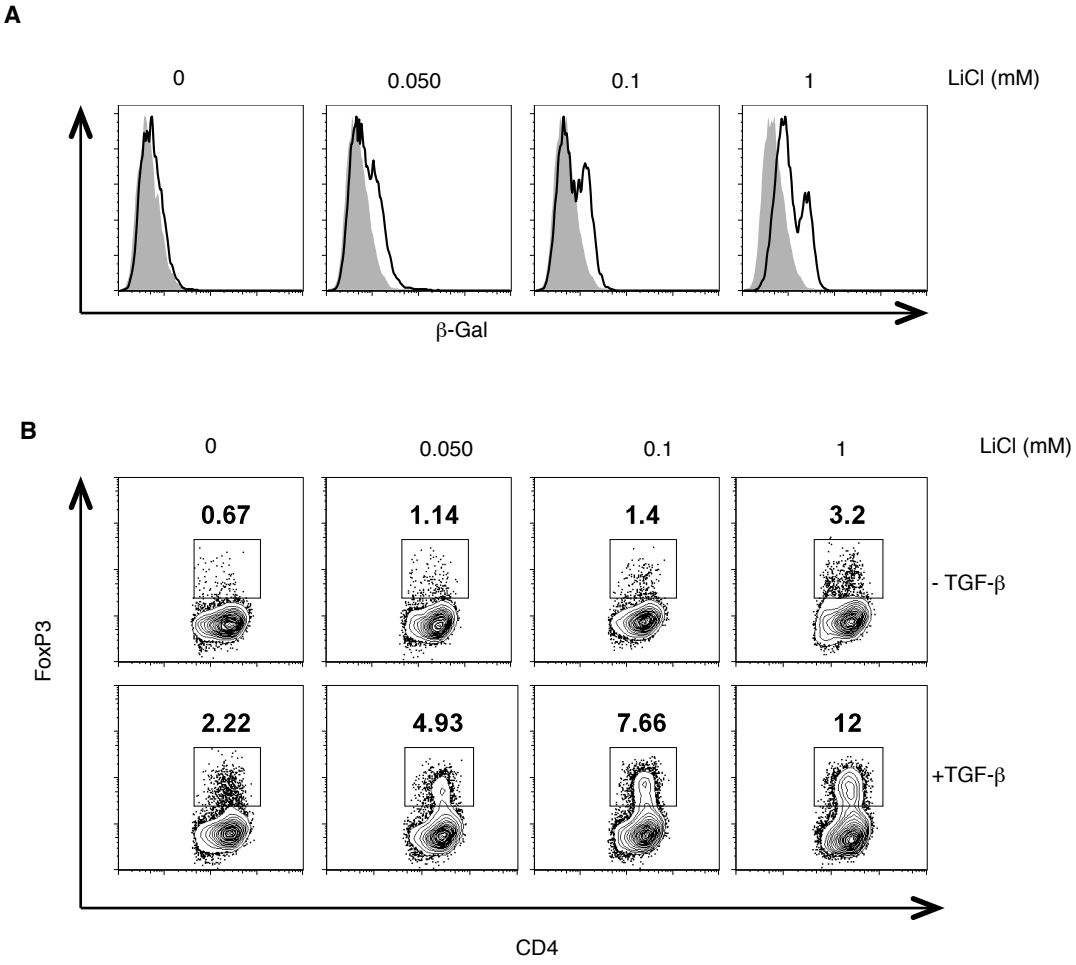

A
Aldh1a1
Aldh1a2
β-catfl/fl
β-catDC–/–
CD11c+ CD11b-
CD11c+ CD11b+
CD11c- CD11b+
B
β-catfl / fl
β-catDC–/–
CD11c+ CD11b-
CD11c+ CD11b+
CD11c- CD11b+
RALDH
C
Il-10
Tgf-β
Il-23a
Il-6
D
IL-10 (pg/ml)
IL-6 (pg/ml)
IL-23 (pg/ml)
IL-12p40 (pg/ml)

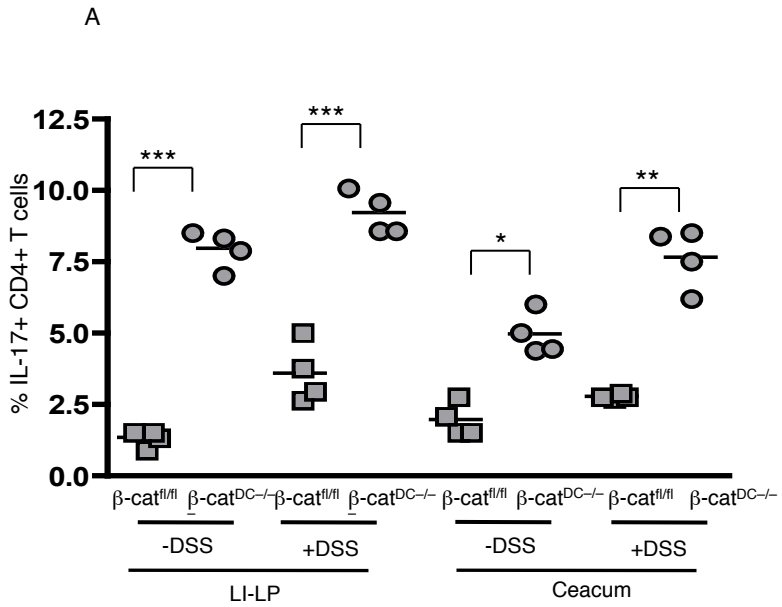
S11

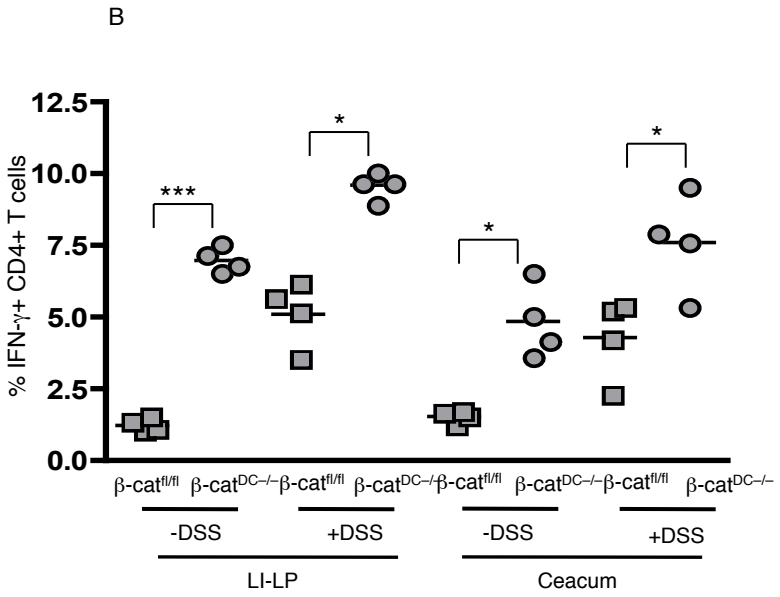

+ RA
β-cat^fl/fl
β-cat^DC–/–
β-cat^fl/fl
β-cat^DC–/–
13.6
0.82
40.6
36.3
CD11c+
CD11b-
19.8
1.22
41.7
35
CD11c+
CD11b+
FoxP3
CD4

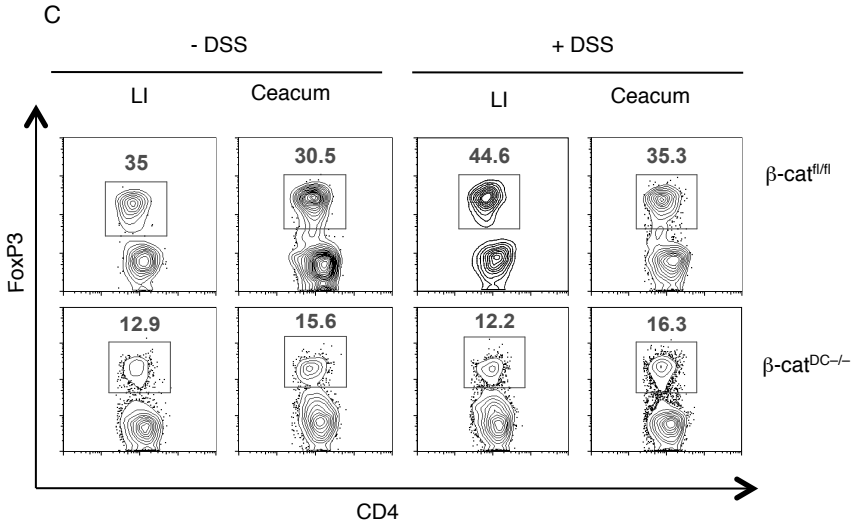
S12

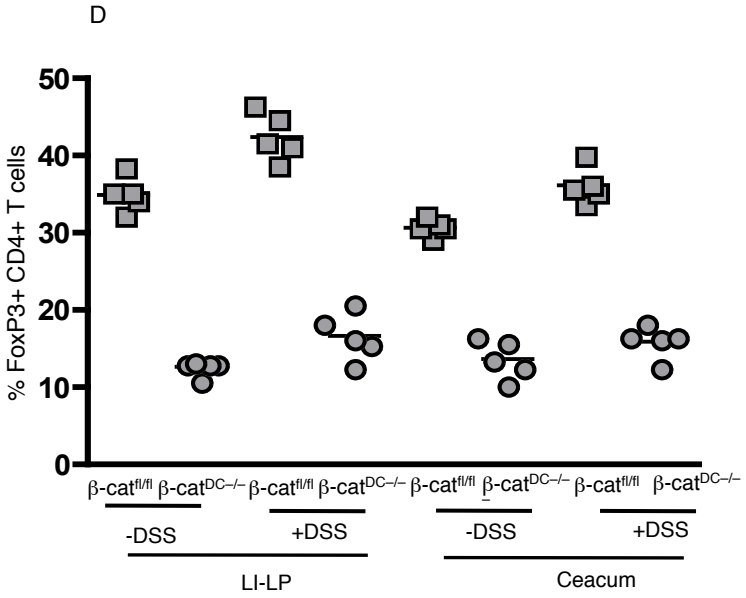

A
β-cat fl/fl
β-cat DC–/–
None
Atx
None
Atx
24.8
39.9
12.7
12
FoxP3
CD4
B
β-cat fl/fl
β-cat DC–/–
None
Atx
None
Atx
2.59
0.0392
97.3
0.0589
0.668
0.0453
99.3
0.0302
9.74
0.285
88.1
1.86
5.14
1.12
90.6
3.1
IL-17
IFN-γ
C
CD11c+ CD11b-
CD11c+ CD11b+
CD11c- CD11b+
β-Gal
β-Catenin
Isotype
None
Antibiotic
D
Relative expression
CD11c+ CD11b-
CD11c+ CD11b+
CD11c- CD11b+
1
3
6
7
8
9
10
Fzd receptor
E
Relative expression
CD11c+ CD11b-
CD11c+ CD11b+
CD11c- CD11b+
3a
5b
7a
8b
11
Wnt ligand
F
Relative expression
Wisp1
Wisp2
Axin1
Spl-DC
CD11c+ CD11b-
CD11c+ CD11b+
CD11c- CD11b+


A
0
0.050
0.1
1
LiCl (mM)
β-Gal
B
0
0.050
0.1
1
LiCl (mM)
0.67
1.14
1.4
3.2
- TGF-β
2.22
4.93
7.66
12
+TGF-β
FoxP3
CD4


A
% IL-17+ CD4+ T cells
12.5
10.0
7.5
5.0
2.5
0.0
***
***
*
**
β-catfl/fl
β-catDC–/–
β-catfl/fl
β-catDC–/–
β-catfl/fl
β-catDC–/–
β-catfl/fl
β-catDC–/–
-DSS
+DSS
-DSS
+DSS
LI-LP
Ceacum


B
% IFN-γ+ CD4+ T cells
12.5
10.0
7.5
5.0
2.5
0.0
***
*
*
*
β-catfl/fl
β-catDC–/–
β-catfl/fl
β-catDC–/–
β-catfl/fl
β-catDC–/–
β-catfl/fl
β-catDC–/–
-DSS
+DSS
-DSS
+DSS
LI-LP
Ceacum


C
- DSS
+ DSS
LI
Ceacum
LI
Ceacum
35
30.5
44.6
35.3
12.9
15.6
12.2
16.3
β-catfl/fl
β-catDC–/–
FoxP3
CD4


D
% FoxP3+ CD4+ T cells
50
40
30
20
10
0
β-catfl/fl
β-catDC–/–
β-catfl/fl
β-catDC–/–
β-catfl/fl
β-catDC–/–
β-catfl/fl
β-catDC–/–
-DSS
+DSS
-DSS
+DSS
LI-LP
Ceacum

Supplementary Figure Legends

Fig. S1. Expression levels of Wnt-ligand mRNA in splenic DCs and LP-DCs.
mRNA expression levels of Wnt-ligands in $CD11c^+$ DCs isolated from spleen and SI-LP of β-$cat^{fl/fl}$ mice. Error bars indicate mean ± SEM. Data are from representative of three experiments.

S2. β-catenin activitation in splenic DCs and LP-DCs.
FACS plot showing the intracellular expression levels of β-galactosidase in $CD11c^+$ DCs isolated from spleen and SI-LP of TCF/LEF-reporter mice. Data are from one experiment representative of three.

S3. β-catenin expression levels in splenic DCs and LP-DCs.
(A) FACS plot showing the intracellular expression levels of β-catenin in DCs, macrophages, T- and B-cells isolated from spleen of β-$cat^{fl/fl}$ or β-$cat^{DC-/-}$ mice. (B) FACS plot showing the intracellular expression levels of β-catenin in LP-DCs and LP-macrophages isolated from intestine of β-$cat^{fl/fl}$ or β-$cat^{DC-/-}$ mice. Data are from one experiment representative of three.

S4. β-$cat^{DC-/-}$ mice have reduced frequencies of $FoxP3^+$ $CD4^+$ Tregs and increased frequencies of Th17 or Th1 cells in the intestine.
(A - C) Percentage of $CD4^+$ T cells positive for FoxP3, IL-17, IFN-γ isolated from SI-LP, LI-LP and ceacum of β-$cat^{fl/fl}$ (square) or β-$cat^{DC-/-}$ (circle) mice, as assessed by intracellular staining and flow cytometry. Each square or circle represents one mouse (*P < 0.05; **P< 0.001; ***P < 0.0001). Error bars indicate mean ± SEM.

S5. β-$cat^{DC-/-}$ mice have elevated levels of *Il-17a, Il-21, Rorγt and Ifn-γ* mRNA.
Expression levels of *Ifn-γ, Il-17a, Il-21 and Rorγt* mRNA in $CD4^+$ T cells isolated from SI-LP and LI-LP of β-$cat^{fl/fl}$ or β-$cat^{DC-/-}$ mice (*P < 0.05; **P < 0.005). Error bars indicate mean ± SEM. Data are from representative of three experiments.

S6. Induction of Tregs by LP-DC subsets in the absence of TGF-β.
(A) Intracellular expression of FoxP3 and (B) IFN-γ and IL-17 in naïve $CD4^+$ T cells stimulated by intestinal APCs from β-$cat^{fl/fl}$ or β-$cat^{DC-/-}$ mice as assessed by intracellular staining and flow cytometry. $CD11c^+$ $CD11b^-$ and $CD11c^+$ $CD11b^+$ DCs were isolated from SI-LP or LI-LP of β-$cat^{fl/fl}$ and β-$cat^{DC-/-}$ mice and co-incubated with naive $CD4^+$ OT-II T cells with OVA in the absence of TGF-β. Numbers in FACS plots represent percentage of cells positive for the indicated protein. Data are from one experiment representative of three.

S7. β-$cat^{DC-/-}$ LP-DCs from large intestine promote higher levels of Th17 and Th1 cells.
(A) Intracellular expression of IL-17 and IFN−γ in naïve $CD4^+$ T cells stimulated by intestinal APCs from β-$cat^{fl/fl}$ or β-$cat^{DC-/-}$ mice as assessed by intracellular staining and flow cytometry. $CD11c^+CD11b^-$ and $CD11c^+$ $CD11b^+$ DCs, and $CD11c^-$ $CD11b^+$ macrophages isolated from LI-LP of β-$cat^{fl/fl}$ and β-$cat^{DC-/-}$ mice were co-incubated with naive $CD4^+$ OT-II T cells with OVA in

the presence of TGF-β (1 ng/ml). After 4 d, OT-II cells were re-stimulated for 6 h with plated bound antibodies to CD3 and CD28. Numbers in FACS plots represent percentage of cells positive for the indicated protein. (B, C) Cytokine concentrations in the supernatants of LP-DCs or LP-macrophages of SI and LI isolated from β-cat$^{fl/fl}$ or β-cat$^{DC-/-}$ mice and co-incubated with OT-II T cells for 4 days and analyzed by ELISA (*P < 0.05; **P < 0.005). Error bars indicate means ± SEM. Data are from representative of three experiments

S8. DC subsets in β-cat$^{fl/fl}$ and β-cat$^{DC-/-}$ mice. (A) FACS plot showing the percentages of CD11c^{+} CD11b^{-} and CD11c^{+} CD11b^{+} DCs, and CD11c^{-} CD11b^{+} macrophages isolated from SI-LP and LI-LP of β-cat$^{fl/fl}$ or β-cat$^{DC-/-}$ mice. (B) FACS plot showing the percentage of CD103+ cells contained within the CD11c^{+}CD11b^{-} and CD11c^{+} CD11b^{+} DC subsets isolated from LP β-β-cat$^{fl/fl}$ or β-cat$^{DC-/-}$ mice. Numbers in FACS plots represent percentage of cells positive for CD103 expression. Data are from one experiment representative of three.

S9. CD103^{+} DCs within the CD11c^{+}CD11b^{-} DC subset show higher β-catenin activity than CD103^{-} DCs. FACS plot showing the intracellular expression levels of β-galactosidase in CD103^{+} or CD103^{-} DC subsets isolated from SI-LP of TCF/LEF-reporter mice. Data are from one experiment representative of three.

S10. β-catenin signaling in intestinal DCs promotes the expression of Raldh and suppresses the expression of proinflammatory cytokines. (A) Expression of *Aldh1a1* and *Aldh1a2* mRNA in CD11c^{+} CD11b^{-} and CD11c^{+} CD11b^{+} DCs, and CD11c^{-} CD11b^{+} macrophages isolated from LI-LP of β-cat$^{fl/fl}$ or β-cat$^{DC-/-}$ mice. (B) Expression of Raldh protein by CD11c^{+} and CD11c^{+} CD11b^{+} DCs, and CD11c^{-} CD11b^{+} macrophages isolated from LI-LP of β-cat$^{fl/fl}$ or β-cat$^{DC-/-}$ mice, as assessed by intracellular staining and flow cytometry. (C) Expression of *Il-10*, *Tgf-β1*, *Il-23a* and *Il-6* mRNAs in CD11c^{+}CD11b^{-} and CD11c^{+} CD11b^{+} DCs, and CD11c^{-} CD11b^{+} macrophages isolated from LI-LP of β-cat$^{fl/fl}$ or β-cat$^{DC-/-}$ mice. (D) Cytokine concentrations in the supernatants of CD11c^{+} CD11b^{-} and CD11c^{+} CD11b^{+} DCs, and CD11c^{-} CD11b^{+} macrophages isolated from LI-LP of β-cat$^{fl/fl}$ or β-cat$^{DC-/-}$ mice after 24 h as analyzed by ELISA. *P < 0.05; **P < 0.005. Error bars indicate mean ± SEM.

S11. Addition of exogenous RA can rescue Treg promoting defect in β-cat$^{DC-/-}$ LP-DCs Intracellular expression of FoxP3 in naïve CD4^{+} OT-II T cells stimulated by LP-DC subsets from β-cat$^{fl/fl}$ or β-cat$^{DC-/-}$ mice. CD11c^{+} CD11b^{-} and CD11c^{+} CD11b^{+} DCs were isolated from SI-LP or LI-LP of β-cat$^{fl/fl}$ and β-cat$^{DC-/-}$ mice, and co-incubated with naïve OT-II T cells with OVA + TGF-β (1ng/ml) in the presence or absence of (0.5 nM) RA. Numbers in FACS plots represent percentage of cells positive for the indicated protein. Data are from one experiment representative of two.

S12. β-catenin activation in intestinal DCs is independent of commensals and induced by Wnt-signaling. (A, B) FACS plots representing the percentage of CD4+ T cells that express FoxP3 (A), IL-17 and IFN-γ (B) isolated from LI-LP of β-cat$^{fl/fl}$ or β-cat$^{DC-/-}$ mice treated with (Atx) or without (None) antibiotics. (C) FACS plots showing the intracellular expression levels of β-galactosidase or β-catenin protein in CD11c^{+} CD11b^{-} and CD11c^{+} CD11b^{+} DCs, and CD11c^{-} CD11b^{+} macrophages isolated from LI-LP of TCF-reporter mice treated with or without antibiotics. (D, E) mRNA expression of Fzd receptors (D) and Wnt ligands (E) in

$CD11c^+CD11b^-$ and $CD11c^+$ $CD11b^+$ DCs, and $CD11c^-$ $CD11b^+$ macrophages isolated from LI-LP of β-$cat^{fl/fl}$ mice. (F) mRNA expression levels of Wnt- β-catenin target genes Wisp1, Wisp2 and Axin1 in $CD11c^+CD11b^-$ and $CD11c^+$ $CD11b^+$ DCs, and $CD11c^-$ $CD11b^+$ macrophages isolated from LI-LP of β-$cat^{fl/fl}$ mice. Error bars indicate mean ± SEM. Data are from one experiment representative of three.

S13. Activation of the β-catenin pathway is sufficient to promote Treg differentiation. (A) Expression of β-galactosidase by spl-DCs isolated from TCF-reporter mice treated with various concentrations of lithium chloride for 2hrs and analyzed by flow cytometry after 12h culture. (B) spl-DCs ($1x10^5$) were treated with various concentrations of lithium chloride for 2 hrs and then washed, and co-incubated with naïve $CD4^+$ OT-II T cells ($1x10^5$),and OVA peptide in the presence or absence of TGF-β (1ng/ml) for 4d. Intracellular expression of FoxP3 in naïve $CD4^+$ T cells stimulated by LiCl treated DCs as assessed by intracellular staining and flow cytometry. Numbers in FACS plots represent percentage of cells positive for the indicated protein. Data are from one experiment representative of two.

S14. β-$cat^{DC-/-}$ mice have increased frequencies of IFN-γ^+ and IL-17^+ $CD4^+$ effector T cells in the intestine upon DSS treatment. (A -D) Percentage of $CD4^+$ cells positive for FoxP3, IL17 and IFN-γ isolated from LI-LP and ceacum of β-$cat^{fl/fl}$ (square) or β-$cat^{DC-/-}$ (circle) mice on day 8 treated with or without 2% DSS for 7 days, as assessed by intracellular staining and flow cytometry. Each square or circle represents one mouse (*$P < 0.05$; **$P < 0.001$; ***$P < 0.0001$). Error bars indicate mean ± SEM.

Table S1 RT-PCR primers used

Gene	Forward	Reverse
Il17a	TTTAACTCCCTTGGCGCAAAA	CTTTCCCTCCGCATTGACAC
Ifng	GAACTGGCAAAAGGATGGTGA	TGTGGGTTGTTGACCTCAAAC
Il6	GAGGATACCACTCCCAACAGACC	AAGTGCATCATCGTTGTTCATACA
Il23a	TGCTGGATTGCAGAGCAGTTAA	GCATGCAGAATTCCGAAGA
Aldh1a1	ATGGTTTAGCAGCAGGACTCTTC	CCAGACATCTTGAATCCACCGAA
Aldh1a2	GACTTGTAGCAGCTGTCTTCACT	TCACCCATTTCTCTCCCATTTCC
Tgf-b	TGACGTCACTGGAGTTGTACGG	GGTTCATGTCATGGATGGTGC
Rorgt	CAGCCAACATGTGGAAAAGCT	GGGAAGGCGGCTTGGA
Il21	AAGATTCCTGAGGATCCGAGAAG	TGCATTCGTGAGCGTCTATAGTG
Fzd1	TGCCCAGTGTCTTTCTCCTT	TCTCTTTAGCCTCTCCCAACC
Fzd2	ATCTGGAAACCTCCCAATCC	CGTTTTGTTGCCCATTCTCT
Fzd3	GCAGTTACCGAGGAATGGAG	GCTGGTGCCATGTGTAATGT
Fzd4	CTATTCCCGCAGTGAAAACC	GGCCATGCCAAAGAAATAGA
Fzd5	TCTTGTCTGCGTGCTACCTG	AAGAGAGGAGCCACCACAAA
Fzd6	AGCCACCACACTCAGCTTTT	CTACACTCTCCCTGCCCAAC
Fzd7	AGAGACAAAGCGGGAAACAA	TGTGCCTGAATGGGTATGAA
Fzd8	TCCGTTCAGTCATCAAGCAG	ATAGAAAAGGCAGGCGACAA
Fzd9	GAAGCTGGAGAAGCTGATGG	AAGTCCATGTTGAGGCGTTC
Fzd10	GCTGCCCACATAACACACAC	TCCTCACCCTCACTTGGTTC

Wnt1 ATTTTGCGCTGTGACCTCTT AGCAACCTCCTTTCCCACTT
Wnt2 GGTCAGCTCTTCATGGTGGT GGAACTGGTGTTGGCACTCT
Wnt3a TCGGAGATGGTGGTAGAGAAA CGCAGAAGTTGGGTGAGG
Wnt4 AAGAGGAGACGTGCGAGA AA CACCACCTTCCCAAAGACAG
Wnt5a CTCTCCGAAGTCCATGTCGT GGACGATACTCCAGGCAGAG
Wnt5b TCTCCGCCTCACAAAAGTCT CACAGACACTCTCAAGCCCA
Wnt7a GACAAATACAACGAGGCCGT GGCTGTCTTATTGCAGGCTC
Wnt8b CCAGAGTTCCGGGAGGTAG GAGATGGAGCGGAAGGTGT
Wnt11 TGCTTGACCTGGAGAGAGGT AGCCCGTAGCTGAGGTTGT
Wisp1 CCCCTACAAGTCCAAGACCA TTTACCCTGAGCCACACACC
Wisp2 GTTTTGTGCCGCTGTGATG CTGAGGAGGGCTGGATTG
Axin2 AACCTATGCCCGTTTCCTCT CCACACATTTCTCCCTCTCC
Gapdh CCAGGTTGTCTCCTGCGACTT CCTGTTGCTGTAGCCGTATTCA

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