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Supplemental Information

TACI Constrains T_H17 Pathogenicity and Protects against Gut Inflammation

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TRANSPARENT METHODS

Mice. TACI$^{-/-}$ mice (Yan et al., 2001) were a kind gift of Vishva Dixit (Genentech). C57BL/6 (Stock No: 000664), B6.SJL-CD45.1 (C57BL/6 congenic; Stock No: 002014), μMT (Stock No: 002288) and RAG1$^{-/-}$ (Stock No: 002216) mice were purchased from The Jackson Laboratory. TACI$^{-/-}$ mice were backcrossed to C57BL/6 background for at least 6 generations before use. TACI$^{-/-}$ and μMT mice were crossed to generate TACI$^{-/-}$ μMT mice. All mice were bred in our animal facilities and maintained under specific pathogen-free conditions. Experiments conducted with TACI$^{-/-}$ and WT (C57BL/6) or TACI$^{-/-}$ μMT and μMT mice used mice of the different genotypes bred separately to generate age-matched mice which were neither littermates nor co-housed together. Experiments with mice were conducted according to guidelines issued by the A*STAR Biological Resource Centre Institutional Animal Care and Use Committee (IACUC).

Cell isolation and flow cytometry. Single cell suspensions of Spl and LN of mice were prepared by standard methods. Single cells were isolated from PP and LP using a previously reported protocol (Couter and Surana, 2016) with some modifications. Before staining with relevant fluorochrome-conjugated Abs, cells were treated with F_c block (Ab against CD16/32, 93; eBioscience). Abs against CD4 (GK1.5), CD62L (MEL-14), CD44 (IM7), TACI (CD267; 8F10), IL-17A (TC11-18H10), IFN-γ (XMG1.2), IL-4 (11B11) and IL-10 (JES5-16E3) were from BD Biosciences. Abs against CD25 (PC61.5), RORγt (B2D) and Foxp3 (FJK-16s) were from eBioscience. Abs against CD3 (145-2C11), TCRβ (H57-597) and BAFF-R (CD268; 7H22-E16) were from Biolegend. Ab against BCMA (CD269; 161616) was from R&D Systems. BAFF tagged with mouse IgG2a Fc (BAF-M5257) was from

ACROBiosystems and APRIL tagged with human IgG Fc (PKSM041367) was from Elabscience. Secondary Abs against mouse IgG2a (m2a-15F8) and human IgG Fc were from eBioscience. Antibody 4',6-diamidino-2-phenylindole (DAPI; Sigma-Aldrich) or Zombie Aqua™ Fixable Viability Dye (BioLegend) was used to exclude dead cells. To perform intracellular staining of cytokines, $CD3^+CD4^+$ T cells were first stimulated with 50 ng/ml PMA and 1 μg/ml ionomycin (Sigma-Aldrich) at 37°C for 4 h and BD GolgiPlug™ (BD Biosciences) was added to the cultures in the last 2 h. Cells were then collected and stained with Abs against appropriate cell surface molecules, fixed and permeabilized with BD Cytofix/Cytoperm Kit (BD Biosciences) before staining with Abs against cytokines. Foxp3 / Transcription Factor Staining Buffer Set (eBioscience) was used for visualizing the expression of transcription factors RORγt and Foxp3 in $CD3^+CD4^+$ T cells *ex vivo*. Samples were acquired on a LSRII cytometer (BD Biosciences) and analyzed with FlowJo software (TreeStar).

Stimulation of $CD4^+$ T cells with cytokines and measurement of cytokine production by ELISA. WT and $TACI^{-/-}$ $CD3^+CD4^+$ T cells were grown in Iscove's Modified Dulbecco's Medium (IMDM) supplemented with 10% fetal bovine serum (FBS), L-glutamine, sodium pyruvate and penicillin/streptomycin (all from Sigma-Aldrich). Cells were activated by 1 μg/ml plate-bound anti-CD3 Ab and 1 μg/ml soluble anti-CD28 Ab and differentiated for 3 days into T_H0 (10 μg/ml anti-IFN-γ + 10 μg/ml anti-IL-4 Abs), T_H1 (10 ng/ml IL-12 + 10 μg/ml anti-IL-4 Ab), T_H2 (10 ng/ml IL-4 + 10 μg/ml anti-IFN-γ Ab), T_H17 (5 ng/ml TGF-β + 20 ng/ml IL-6 + 5 ng/ml IL-23) and T_{reg} (5 ng/ml TGF-β + 100 IU/ml IL-2) lineages *in vitro*. All Abs were obtained from eBioscience and all cytokines were from R&D Systems. In control experiments, B cells enriched from WT spleen were labeled with 1 μM CellTrace™ CFSE

(Invitrogen) and activated with 1 μg/ml LPS (Sigma) in the presence of vehicle or increasing concentrations (0.1, 1, 10, 100 ng/ml) of recombinant mouse BAFF or APRIL (both from R&D Systems) for 4 days, followed by flow cytometric assessment of CFSE dilution. In parallel, vehicle or increasing concentrations (as above) of either cytokine was added to T_H17 or T_{reg} cultures. To examine cytokine secretion by T_H cells differentiated *in vitro*, cells were harvested and counted on day 3. 5×10^5 cells were then re-seeded in fresh media and re-stimulated by 1 μg/ml plate-bound anti-CD3 Ab. 24 h later, culture supernatants were harvested to perform enzyme-linked immunosorbent assay (ELISA) using kits to detect mouse IFN-γ, IL-4 and IL-17A (Thermo FisherScientific). To determine if TACI deficiency affected TCRβ expression in $CD3^+CD4^+$ T cells, TCRβ expression in unstimulated WT and $TACI^{-/-}$ T cells or cells activated by 1 μg/ml plate-bound anti-CD3 Ab and 1 μg/ml soluble anti-CD28 Ab for 24 h were assessed.

Generation of bone marrow chimeras. 6 week-old female C57BL/6 mice were subjected to a dose of 900 cGy in a γ-irradiator at a rate of ~1Gy/min to deplete their hematopoietic compartments. 24 h later, BM cells were harvested from $CD45.1^+$ WT and $CD45.2^+$ $TACI^{-/-}$ mice, mixed in a 1:1 ratio (1×10^6 cells each) and injected intravenously into irradiated mice. The health of recipient mice was closely monitored and flow cytometric assessment of their blood lymphocytes 4 weeks post injection confirmed successful BM reconstitution and chimerism (data not shown). Chimeric mice between 8 to 10 weeks post injection were then sacrificed to analyse the frequencies and numbers of T_H17 and T_{reg} cells in various organs.

RNA isolation and quantitative real-time PCR. Total RNA was isolated using TRIzol (Thermo Fisher Scientific) and precipitated with isopropanol. cDNA was prepared using RevertAid H Minus First-Strand cDNA Synthesis Kit (Fermentas) and real-time PCR performed using SYBR Green Master Mix on the ABI Prism 7500 system (Applied Biosystems). Primer sequences were as follows: TACI forward, 5'-GAG CTC GGG AGA CCA CAG GCC-3'; TACI reverse, 5'-GGC AGA CCC CCA GTG TGC AGT A-3'; IRF4 forward, 5'-GCT GCA TAT CTG CCT GTA TTA CCG-3'; IRF4 reverse, 5'-GTG GTA ACG TGT TCA GGT AAC TCG TAG-3'; c-MAF forward, 5'-AGC AGT TGG TGA CCA TGT CG-3'; c-MAF reverse, 5'-TGG AGA TCT CCT GCT TGA GG-3'; JUNB forward, 5'-TCA CGA CGA CTC TTA CGC AG-3'; JUNB reverse, 5'-CCT TGA GAC CCC GAT AGG GA-3'; FOS forward, 5'-CGG GTT TCA ACG CCG ACT A-3'; FOS reverse, 5'-TTG GCA CTA GAG ACG GAC AGA-3'; EGR2 forward, 5'-CCT CCA CTC ACG CCA CTC TC-3'; EGR2 reverse, 5'-CAC CAC CTC CAC TTG CTC CTG-3'; SLC3A2 forward, 5'- GAA GAT CAA GGT GGC GGA GGA C-3'; SLC3A2 reverse, 5'- CAA GTA CTC CAG ATG GCT CTT CAG ACC-3'; AP-1/JUN forward, 5'-CCA GAA GAT GGT GTG GTG TTT-3'; AP-1/JUN reverse, 5'- CTG ACC CTC TCC CCT TGC-3'; GAPDH forward, 5′-TGT GTC CGT CGT GGA TCT GA-3′, GAPDH reverse, 5′-TTG CTG TTG AAG TCG CAG GAG-3′. Transcript levels of genes were normalized to those of GAPDH.

Western blotting. $CD4^{+}CD62L^{hi}CD44^{lo}CD25^{-}$ T_{naive} cells were FACS-sorted from pooled Spl and LN of $TACI^{-/-}$ or WT mice and stimulated or not for 4 h and 24 h with anti-CD3 and anti-CD28 Abs. In some experiments, T_{naive} cells were cultured for 24 h in T_H17-polarizing conditions with or without 100 ng/ml CsA. Cells were subsequently lysed using NE-PER™ Nuclear and Cytoplasmic Extraction Reagents (Thermo

Fisher Scientific) according to manufacturer's instructions to extract separate cytoplasmic and nuclear protein fractions. Protein concentrations were quantified by the bicinchoninicacid (BCA) assay (Pierce) and read in an Infinite® M1000 PRO microplate reader (TECAN). For Western blot analysis, equal amounts of proteins were separated by sodium dodecyl sulphate- polyacrylamide gel electrophoresis (SDS-PAGE), transferred onto a nitrocellulose membrane and blotted with specific Abs against NFAT1 (#5861), NFAT2 (#8032), NFAT4 (#4998), IRF4 (#4964) and JUNB (#3753) were obtained from Cell signalling. Abs against c-MAF (sc-7866) and HDAC1 (sc-8410) were purchased from Santa Cruz and Ab against RORγt (B2D) was from eBioscience.

Induction of colitis and evaluation of disease severity. To induce acute colitis, 2% (w/v) dextran sodium sulphate (DSS, molecular mass 36–50 kDa; Sigma-Aldrich) was added to drinking water and fed to mice *ad libitum* for up to 15 days. To induce chronic colitis, 5×10^5 $CD4^+CD62L^{hi}CD44^{lo}CD25^-$ T_{naive} or $CD4^+CD62L^{hi}CD44^{lo}CD25^+$ T_{reg} cells were FACS-sorted on a FACSAria III (BD Biosciences) from Spl and LN of $TACI^{-/-}$ or WT mice and T_{naive} cells alone or in conjunction with T_{reg} cells were intraperitoneally injected into age- and gender-matched $RAG1^{-/-}$ mice. For both acute and chronic colitis models, mice were observed for signs of colitis by monitoring weight loss, stool consistency and blood in the stool (data not shown for latter 2 parameters). Any animal showing clinical symptoms of debilitating disease (with weight loss > 30% of original weight) and became moribund were sacrificed following Institutional Animal Care and Use Committee regulations. The weight losses of such mice were not included in the generation of curves depicted in Figures 4A, 5A, 5E and 5G.

Colon histology. Entire colons were removed from mice 0 or 6 days after DSS treatment, cut open lengthwise, gently cleaned with sterile phosphate-buffered saline (PBS) to remove faeces and fixed in 10% neutral buffered formalin (NBF). 4 – 6 μm paraffin-embedded sections were cut and stained with hematoxylin and eosin (H&E), hematoxylin and Ki-67 (Abcam) or hematoxylin and periodic acid Schiff (Abcam). Histomorphological scoring of colon pathology was performed in a blinded fashion based on the criteria of severity and spatial extent of inflammation due to infiltration by immune cells, degree of epithelial cell loss and ulceration, hyperplasia and dysplasia as previously described (Erben et al., 2014).

Microarray analysis. T_{naive} cells were FACS-sorted from Spl and LN of WT and $TACI^{-/-}$ mice (n = 2 each). Total RNA was isolated using TRIzol (Invitrogen) and precipitated with isopropanol, followed by DNase I digestion using RNase-Free DNase Set and purification using RNeasy MinElute Cleanup Kit (both kits from Qiagen). After quantification with NanoDrop ND-2000 spectrophotometer and determination that the RNA integrity number (RIN) of all RNA samples were > 8.5 with Agilent 2100 Bioanalyzer, single-stranded cDNA was then prepared and hybridized on a GeneChip Mouse Gene 2.0 ST Array (Affymetrix). Significantly differentially expressed genes (DEGs) between $TACI^{-/-}$ and WT T_{naive} cells (fold change > 1.5-fold; two-tailed *t*-test with equal variances, $p < 0.05$) were detected using Partek Genomics Suite (PGS) software (Partek Inc.). GSEA was performed to identify the most significantly enriched gene sets corresponding to specific cellular and transcriptional pathways based on DEGs between $TACI^{-/-}$ vs WT T cells.

Calcium flux assay. $CD3^+CD4^+$ T cells were enriched from the Spl of WT and $TACI^{-/-}$ mice using Naive $CD4^+$ T Cell Isolation Kit, mouse as above and incubated with 4 μM Indo-1 acetoxy-methyl ester (Indo-1 AM, Molecular Probes) for 1 h at 37°C in complete media. Cells were then stained with anti-CD4 Ab, their density adjusted to 1×10^6 cells/ml and equilibrated for 10 min at room temperature. An initial 3 min baseline Indo-1 AM fluorescence reading was acquired on the LSRII before 15 μg/ml biotinylated anti-CD3 Ab and 100 μg/ml streptavidin were added. Calcium flux was measured in real time for a further 15 min and 2 μM ionomycin was then added to induce equivalent maximal fluxing from both WT and $TACI^{-/-}$ T cells as a positive control. Intracellular calcium concentration was calculated based on the ratio of fluorescence at 395 and 525 nm.

Statistical analyses. Differences in numerical values between samples were compared by unpaired *t*-test for parametric data sets with 2 variables, Mann-Whitney test for non-parametric data sets, two-way repeated-measures analysis of variance (ANOVA) and log-rank (Mantel-Cox) test respectively for colitis-associated weight loss data and Kaplan-Meier survival curves using Prism (version 8; GraphPad Software). In general, a value of $p < 0.05$ for a given comparison was regarded as statistically significant.

SUPPLEMENTAL REFERENCES

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Yan, M., Wang, H., Chan, B., Roose-Girma, M., Erickson, S., Baker, T., Tumas, D., Grewal, I.S., Dixit, V.M., 2001. Activation and accumulation of B cells in TACI-deficient mice. Nat. Immunol. 2, 638–643. https://doi.org/10.1038/89790

Figure S1. Expression of BAFF-R and BCMA in $CD4^+$ T cells stimulated under various T_H-polarizing conditions. Related to Figure 1. BAFF-R and BCMA expression in WT (blue histogram) and $TACI^{-/-}$ (red histogram) splenic $CD3^+CD4^+$ T cells stimulated under T_H0-, T_H1-, T_H2-, T_H17- or T_{reg}-polarizing conditions for 3 days as assessed by flow cytometry. Shaded histogram represents staining by isotype control Ab. Data are representative of 2 independent experiments.

Figure S2. TACI constrains production of IL-17A by T_H17 cells. Related to Figure 1. WT and $TACI^{-/-}$ $CD4^+$ T cells were differentiated under T_H0-, T_H1-, T_H2- or T_H17-polarizing conditions for 3 days as in Figures 1A, B and C, re-seeded in equal numbers and re-stimulated for further 1 day. The concentrations of IFN-γ **(A)**, IL-4 **(B)** and IL-17A **(C)** secreted in the culture supernatant by the different T_H lineages were then measured by ELISA. Cells were also separately differentiated under T_{reg}-polarizing conditions and their intracellular Foxp3 expression was assessed by flow cytometry **(D)**. Data in **(A to D)** are the mean ± SEM of 3 technical replicates; unpaired parametric *t*-test; **, $p < 0.01$; ****; $p < 0.0001$; ns, not significant. Actual *p*-

values are indicated in parentheses below the asterisks or “ns”. All data are representative of 2 independent experiments; n.d., not detected.

Figure S3. BAFF and APRIL neither bind CD4$^+$ T cells nor affect T_H17 or T_{reg} differentiation. Related to Figure 1. (A) B cells were enriched from WT spleen, labeled with CFSE and stimulated with LPS in the presence of empty vehicle (blue histogram) or increasing concentrations (1, 10 or 100 ng/ml) of BAFF or APRIL (red histograms) for 4 days, following which CFSE dilution was assessed by flow cytometry. Shaded histogram represents CFSE expression of unstimulated, labeled B cells. The mean frequencies of proliferated B cells stimulated with LPS in the presence of each BAFF or APRIL concentration, assessed from gating on diluted CFSE peaks, were compared with that of cells stimulated with LPS alone. **(B)** WT and TACI$^{-/-}$ CD4$^+$ T cells were differentiated under T_H17- or T_{reg}-polarizing conditions in the presence of vehicle or increasing concentrations of BAFF or APRIL as in **(A)** for 3 days, following which their expression of IL-17A (top panel) or Foxp3 (bottom panel) was assessed. **(C)** Flow cytometric assessment of binding of BAFF or APRIL to splenic B or CD4$^+$ T cells (blue histograms) compared with background binding due to secondary Ab alone (shaded histograms). All data are representative of 2 independent experiments. Data for bar plots in **(A)** are the mean ± SEM of at least 3 technical replicates; unpaired parametric *t*-test; **, $p < 0.01$; ns, not significant.

Figure S4. Gating strategies to identify T_H17 or T_{reg} cells in LP of $TACI^{-/-}$ and WT mice and in LP of BM chimeras. Related to Figures 2 and 3. Cells isolated from the LP of $TACI^{-/-}$ and WT mice were stimulated as in Figures 2A and B and assessed for intracellular IL-17A and IL-10 **(A)** or Foxp3 and RORγt **(B)** expression in $CD3^+CD4^+$ T cells by flow cytometry. **(C and D)** Cells isolated from LP of BM chimeric mice were stimulated and assessed as in **(A and B)**. Gating on live cells was followed by $CD45^+CD3^+CD4^+IL\text{-}17^+$ and $IL\text{-}10^+$ **(A)** or $Foxp3^+$ and $ROR\gamma t^+$ **(B)** cells. Similar strategies were applied in BM chimeras to analyze $IL\text{-}17^+$ and $IL\text{-}10^+$ **(C)** or $Foxp3^+$ and $ROR\gamma t^+$ **(D)** cells except that $TACI^{-/-}$ CD3+CD4+ T cells were distinguished by expression of CD45.2 from WT counterparts by expression of CD45.1. $IL\text{-}17^+$ and $Foxp3^+$ cells were used to respectively identify T_H17 and T_{reg} cells due to their more consistent expression between mouse replicates (Figures 2 and 3). Data in **(A and B)** are representative of at least 7 $TACI^{-/-}$ and WT mice and in **(C and D)** of at least 11 BM chimeras.

Figure S5. Gating strategies utilizing relevant no staining controls to identify T_H17 or T_{reg} cells in $TACI^{-/-}$ and WT mice. Related to Figure 2. Cells isolated from various indicated organs of $TACI^{-/-}$ and WT mice were stimulated as in Figures 2A and B and assessed for intracellular IL-17A and IL-10 **(A)** or Foxp3 and RORγt **(B)** expression in $CD3^+CD4^+$ T cells by flow cytometry. Gating on live cells was followed by $CD45^+CD3^+CD4^+IL\text{-}17^+$ and $IL\text{-}10^+$ (A) or $Foxp3^+$ and $ROR\gamma t^+$ (B) cells. Relevant no staining controls for $IL\text{-}17A^+$, $IL\text{-}10^+$, $ROR\gamma t^+$ and $Foxp3^+$ cells amongst $CD3^+CD4^+$ T cells of $TACI^{-/-}$ vs WT mice were included to clearly separate cells which expressed a certain cytokine or transcription factor from those that did not. Data are representative of at least 7 $TACI^{-/-}$ and WT mice.

Figure S6. Gating strategies utilizing relevant no staining controls to identify T_H17 or T_{reg} cells amongst $TACI^{-/-}$ and WT $CD3^+CD4^+$ T cells in BM chimeric mice. Related to Figure 3. Cells isolated from various indicated organs of BM chimeric mice were stimulated as in Figures 2A and B and assessed for intracellular IL-17A and IL-10 **(A)** or Foxp3 and RORγt **(B)** expression amongst $TACI^{-/-}$ ($CD45.2^+$) and WT ($CD45.1^+$) $CD3^+CD4^+$ T cells by flow cytometry. As in Figure S4, relevant no staining controls for $IL\text{-}17A^+$, $IL\text{-}10^+$, $ROR\gamma t^+$ and $Foxp3^+$ cells amongst $TACI^{-/-}$ ($CD45.2^+$) and WT ($CD45.1^+$) $CD3^+CD4^+$ T cells were included to clearly separate cells which expressed a certain cytokine or transcription factor from those that did not. Data are representative of at least 11 BM chimeras.

Figure S7. Normal frequencies and numbers of T_H1 and T_H2 cells in $TACI^{-/-}$ mice. Related to Figure 2. Cells from the Spl and mLN of $TACI^{-/-}$ (filled circles) or WT (open circles) mice were stimulated as in Figures 2A and B. Frequencies and numbers of $CD3^+CD4^+$ T cells expressing IFN-γ **(A and B)** or IL-4 **(C and D)** was assessed by flow cytometry. Data in **(A and C)** are representative of 4 mice of each genotype analyzed. Data in **(B and D)** are based on 4 mice analyzed with each symbol representing one mouse and horizontal bars indicating the mean; Mann-Whitney test, ns, not significant.

Figure S8. RORγt upregulation in WT and $TACI^{-/-}$ T_{naive} cells induced by T_H17-polarizing conditions is attenuated by CsA treatment. Related to Figure 6. Flow cytometry analysis of RORγt expression in WT and $TACI^{-/-}$ T_{naive} cells activated under T_H17-polarizing conditions in the presence or absence of CsA for 24 h as in Figure 6E. Data are representative of 2 independent experiments.

Figure S9. TCRβ expression in TACI$^{-/-}$ and WT CD4^{+} T cells. Related to Figure 6.

TCRβ expression in CD4^{+} T cells from the Spls of WT (blue histogram) and TACI$^{-/-}$ (red histogram) mice left unstimulated or stimulated with plate-bound anti-CD3 and soluble anti-CD28 Abs for 24 h as assessed by flow cytometry. Shaded histogram represents staining by isotype control Ab. Data are representative of 2 independent experiments.

Figure S1

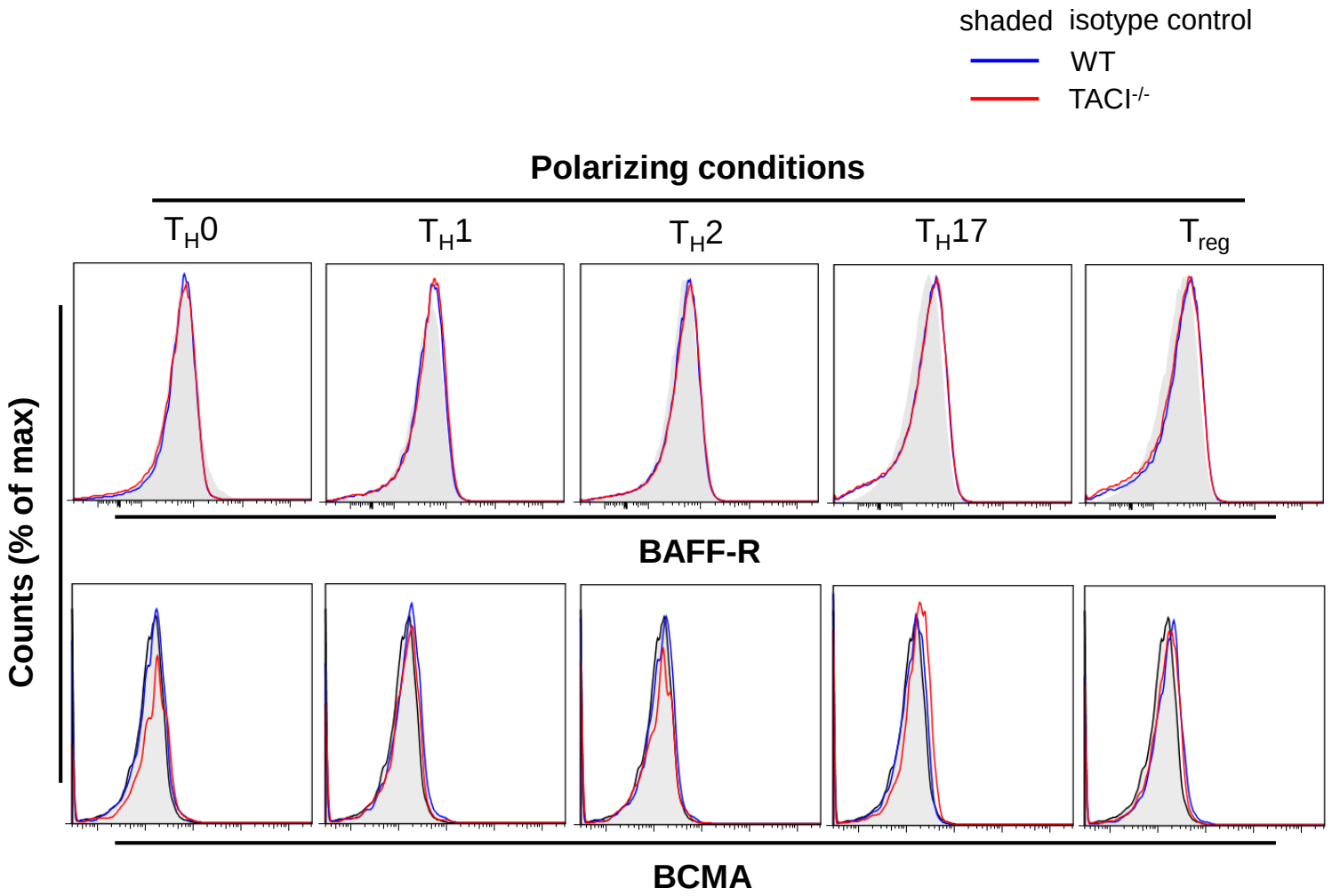

shaded isotype control
WT
TACI-/-
Polarizing conditions
TH0
TH1
TH2
TH17
Treg
Counts (% of max)
BAFF-R
BCMA

Figure S2

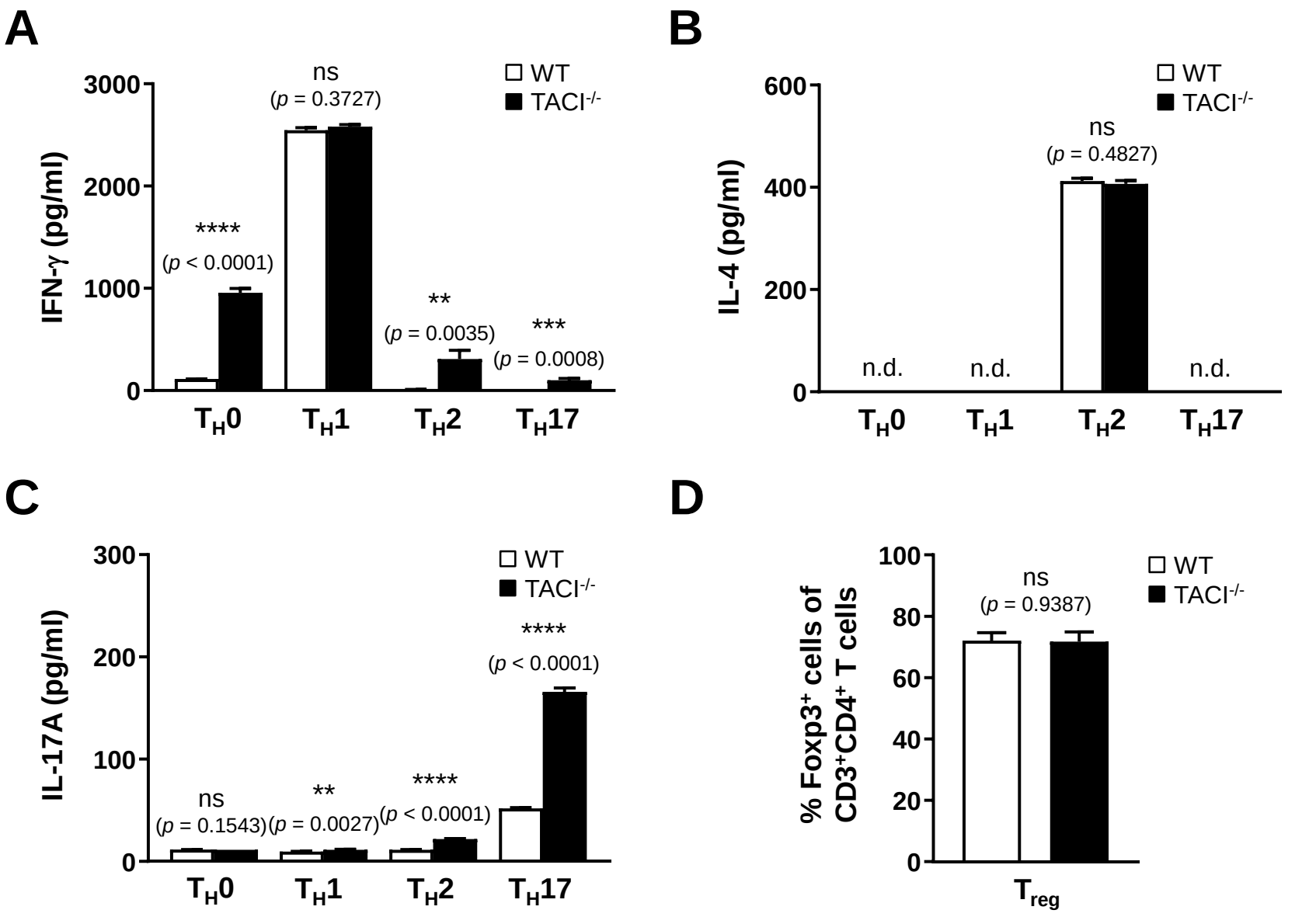

A
IFN-γ (pg/ml)
3000
2000
1000
0
WT
TACI-/-
ns
(p = 0.3727)
****
(p < 0.0001)
**
(p = 0.0035)
***
(p = 0.0008)
TH0
TH1
TH2
TH17
B
IL-4 (pg/ml)
600
400
200
0
WT
TACI-/-
ns
(p = 0.4827)
n.d.
n.d.
n.d.
TH0
TH1
TH2
TH17
C
IL-17A (pg/ml)
300
200
100
0
WT
TACI-/-
ns
(p = 0.1543)
**
(p = 0.0027)
****
(p < 0.0001)
****
(p < 0.0001)
TH0
TH1
TH2
TH17
D
% Foxp3+ cells of CD3+CD4+ T cells
100
80
60
40
20
0
WT
TACI-/-
ns
(p = 0.9387)
Treg

Figure S3

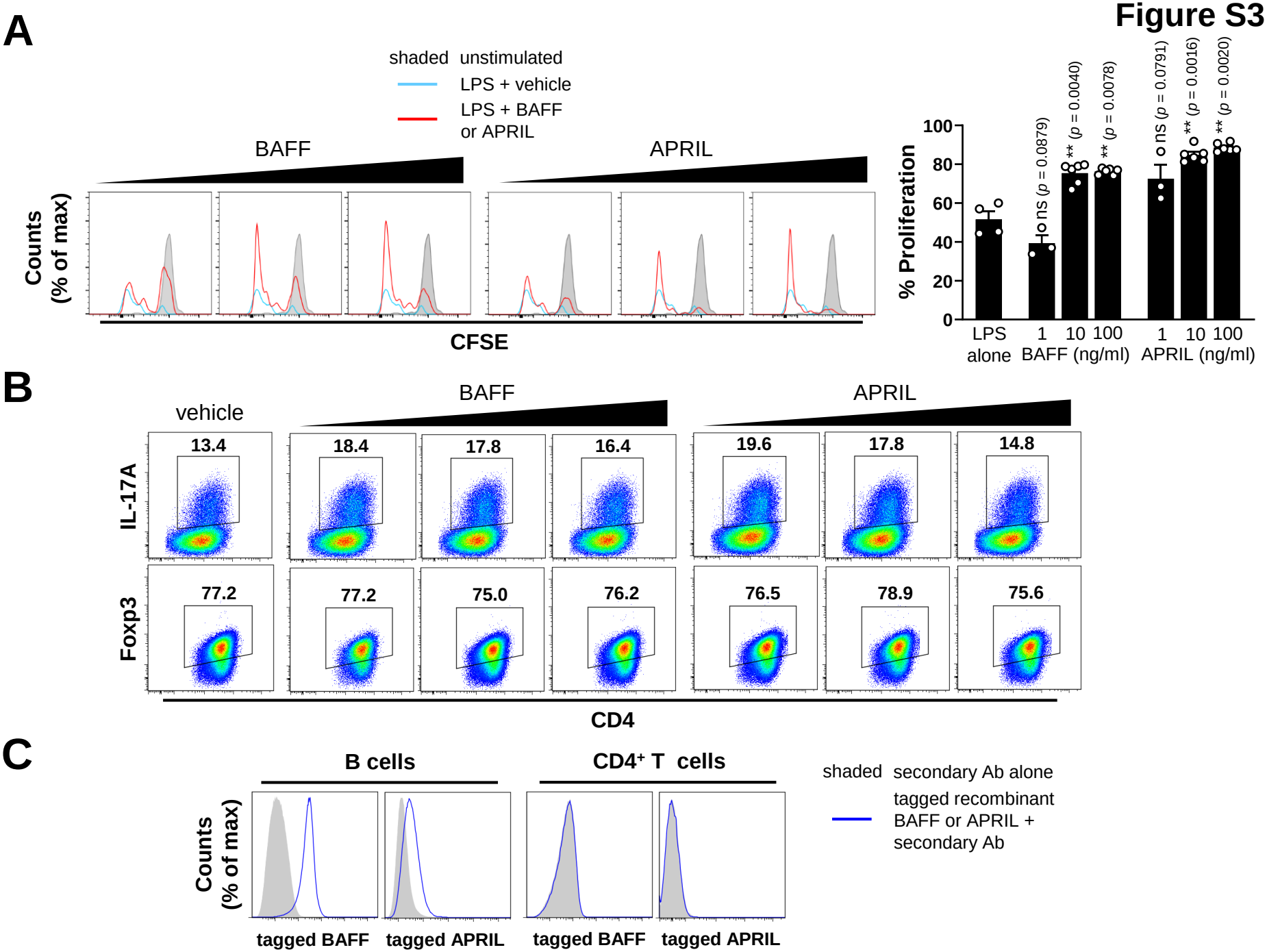

A
shaded unstimulated
LPS + vehicle
LPS + BAFF or APRIL
BAFF
APRIL
Counts (% of max)
CFSE
% Proliferation
ns (p = 0.0879)
** (p = 0.0040)
** (p = 0.0078)
ns (p = 0.0791)
** (p = 0.0016)
** (p = 0.0020)
LPS alone
1
10
100
BAFF (ng/ml)
APRIL (ng/ml)
B
vehicle
BAFF
APRIL
IL-17A
Foxp3
13.4
18.4
17.8
16.4
19.6
17.8
14.8
77.2
77.2
75.0
76.2
76.5
78.9
75.6
CD4
C
B cells
CD4+ T cells
Counts (% of max)
tagged BAFF
tagged APRIL
tagged BAFF
tagged APRIL
shaded secondary Ab alone
tagged recombinant BAFF or APRIL + secondary Ab

Figure S4

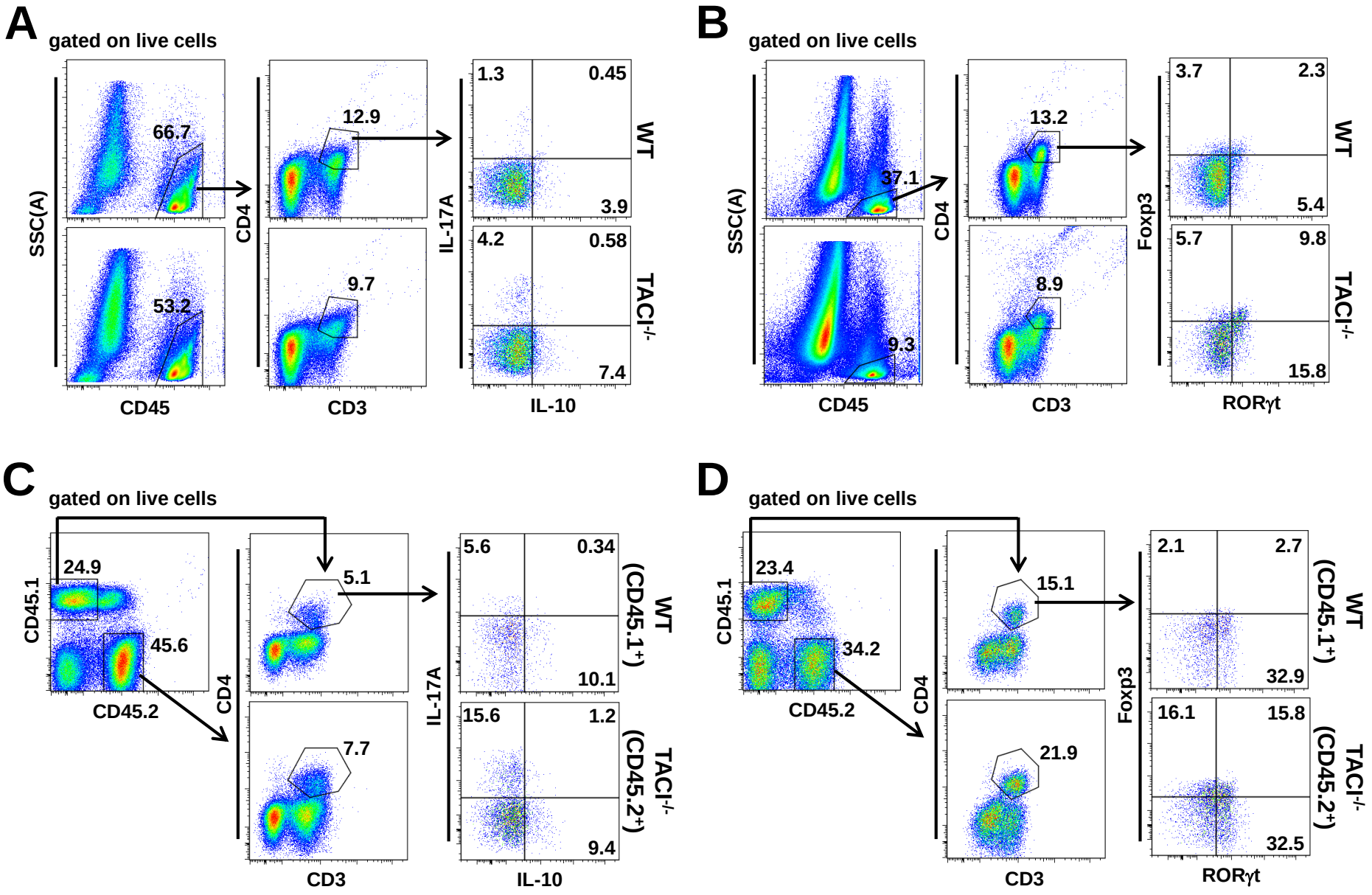

A
gated on live cells
SSC(A)
CD45
66.7
53.2
CD4
CD3
12.9
9.7
IL-17A
IL-10
1.3
0.45
3.9
4.2
0.58
7.4
WT
TACI-/-
B
gated on live cells
SSC(A)
CD45
37.1
9.3
CD4
CD3
13.2
8.9
Foxp3
RORγt
3.7
2.3
5.4
5.7
9.8
15.8
WT
TACI-/-
C
gated on live cells
CD45.1
CD45.2
24.9
45.6
CD4
CD3
5.1
7.7
IL-17A
IL-10
5.6
0.34
10.1
15.6
1.2
9.4
WT (CD45.1+)
TACI-/- (CD45.2+)
D
gated on live cells
CD45.1
CD45.2
23.4
34.2
CD4
CD3
15.1
21.9
Foxp3
RORγt
2.1
2.7
32.9
16.1
15.8
32.5
WT (CD45.1+)
TACI-/- (CD45.2+)

Figure S5

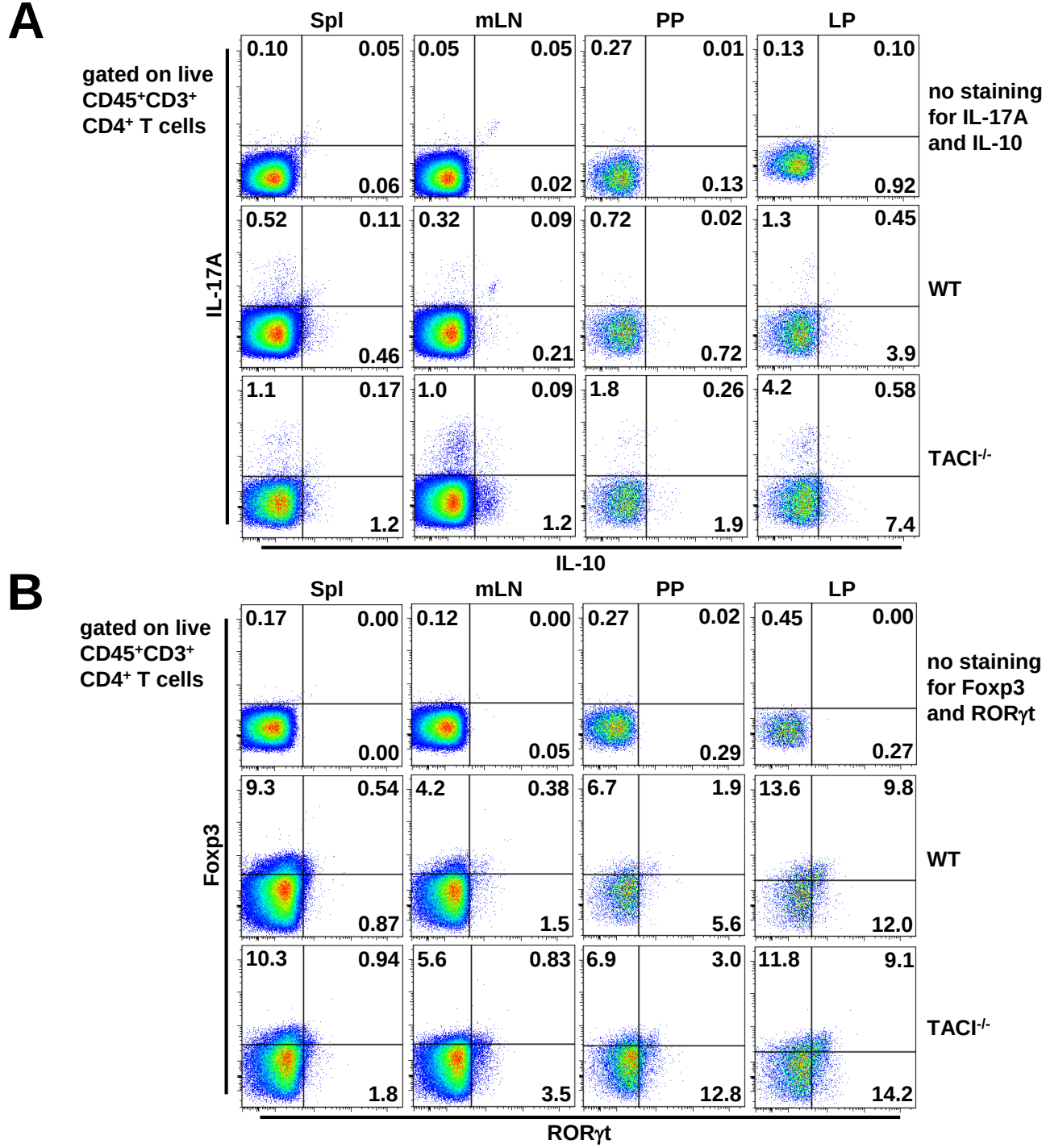

A
Spl
mLN
PP
LP
gated on live
CD45+CD3+
CD4+ T cells
no staining
for IL-17A
and IL-10
WT
TACI-/-
IL-17A
IL-10
0.10
0.05
0.06
0.05
0.05
0.02
0.27
0.01
0.13
0.13
0.10
0.92
0.52
0.11
0.46
0.32
0.09
0.21
0.72
0.02
0.72
1.3
0.45
3.9
1.1
0.17
1.2
1.0
0.09
1.2
1.8
0.26
1.9
4.2
0.58
7.4
B
Spl
mLN
PP
LP
gated on live
CD45+CD3+
CD4+ T cells
no staining
for Foxp3
and RORγt
WT
TACI-/-
Foxp3
RORγt
0.17
0.00
0.00
0.12
0.00
0.05
0.27
0.02
0.29
0.45
0.00
0.27
9.3
0.54
0.87
4.2
0.38
1.5
6.7
1.9
5.6
13.6
9.8
12.0
10.3
0.94
1.8
5.6
0.83
3.5
6.9
3.0
12.8
11.8
9.1
14.2

Figure S6

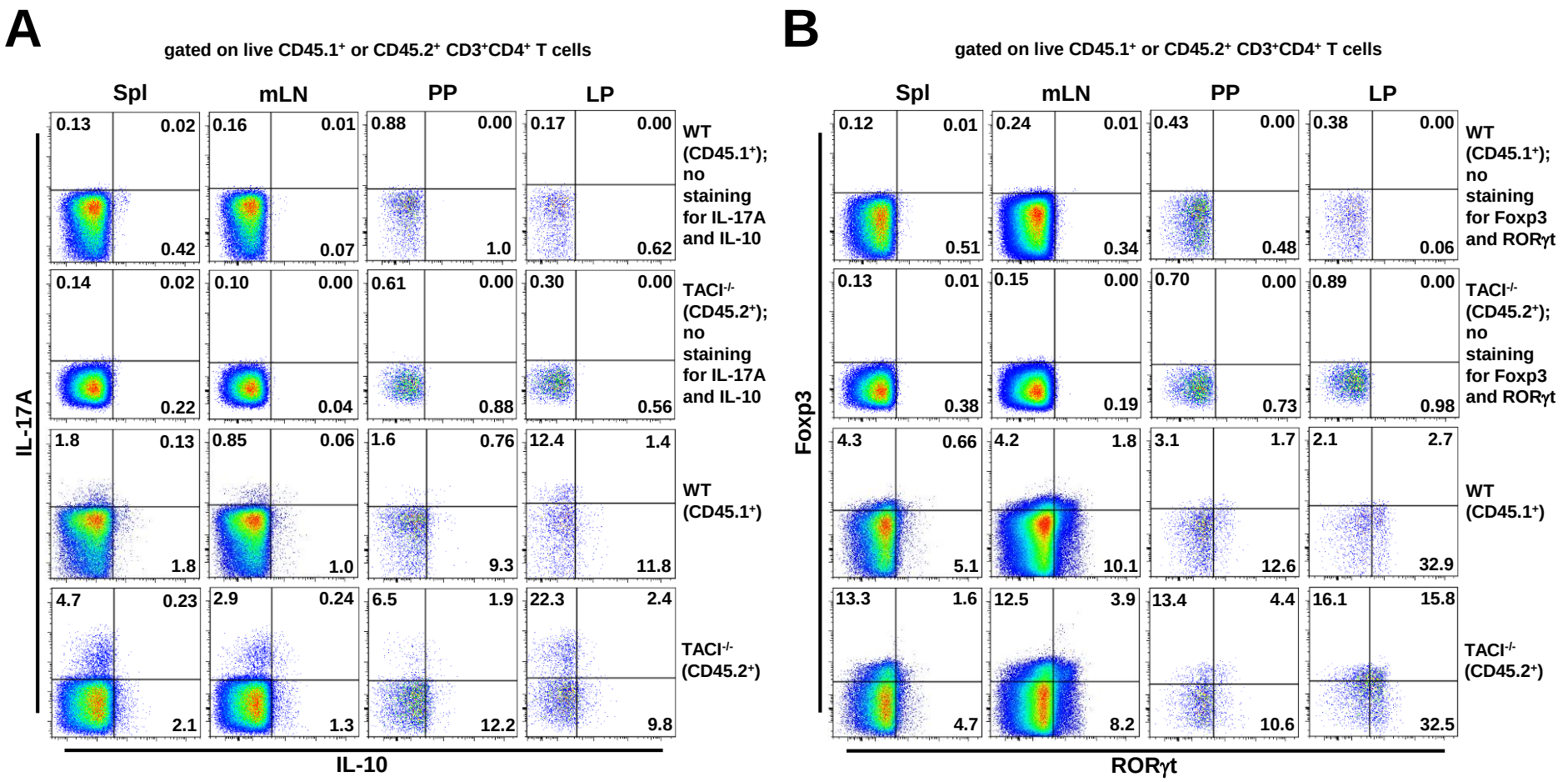

A
gated on live CD45.1+ or CD45.2+ CD3+CD4+ T cells
Spl
mLN
PP
LP
IL-17A
IL-10
0.13 0.02 0.42
0.16 0.01 0.07
0.88 0.00 1.0
0.17 0.00 0.62
WT (CD45.1+); no staining for IL-17A and IL-10
0.14 0.02 0.22
0.10 0.00 0.04
0.61 0.00 0.88
0.30 0.00 0.56
TACI-/- (CD45.2+); no staining for IL-17A and IL-10
1.8 0.13 1.8
0.85 0.06 1.0
1.6 0.76 9.3
12.4 1.4 11.8
WT (CD45.1+)
4.7 0.23 2.1
2.9 0.24 1.3
6.5 1.9 12.2
22.3 2.4 9.8
TACI-/- (CD45.2+)
B
gated on live CD45.1+ or CD45.2+ CD3+CD4+ T cells
Spl
mLN
PP
LP
Foxp3
RORγt
0.12 0.01 0.51
0.24 0.01 0.34
0.43 0.00 0.48
0.38 0.00 0.06
WT (CD45.1+); no staining for Foxp3 and RORγt
0.13 0.01 0.38
0.15 0.00 0.19
0.70 0.00 0.73
0.89 0.00 0.98
TACI-/- (CD45.2+); no staining for Foxp3 and RORγt
4.3 0.66 5.1
4.2 1.8 10.1
3.1 1.7 12.6
2.1 2.7 32.9
WT (CD45.1+)
13.3 1.6 4.7
12.5 3.9 8.2
13.4 4.4 10.6
16.1 15.8 32.5
TACI-/- (CD45.2+)

Figure S7

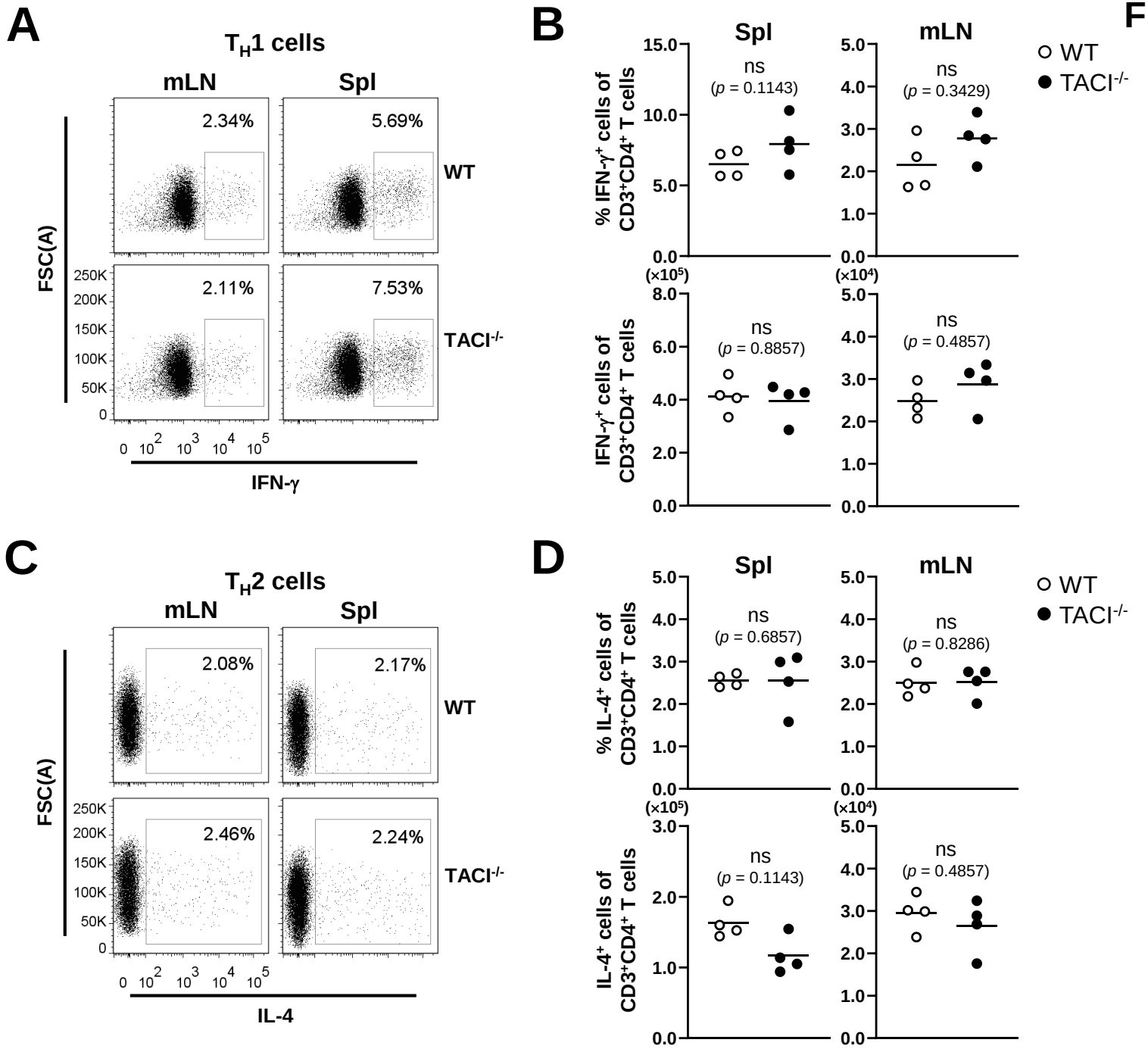

A
T_H1 cells
mLN
Spl
2.34%
5.69%
2.11%
7.53%
WT
TACI-/-
FSC(A)
IFN-γ
B
Spl
mLN
ns
(p = 0.1143)
ns
(p = 0.3429)
ns
(p = 0.8857)
ns
(p = 0.4857)
% IFN-γ+ cells of CD3+CD4+ T cells
IFN-γ+ cells of CD3+CD4+ T cells
(×10⁵)
(×10⁴)
WT
TACI-/-
C
T_H2 cells
2.08%
2.17%
2.46%
2.24%
IL-4
D
ns
(p = 0.6857)
ns
(p = 0.8286)
ns
(p = 0.1143)
ns
(p = 0.4857)
% IL-4+ cells of CD3+CD4+ T cells
IL-4+ cells of CD3+CD4+ T cells

Figure S8

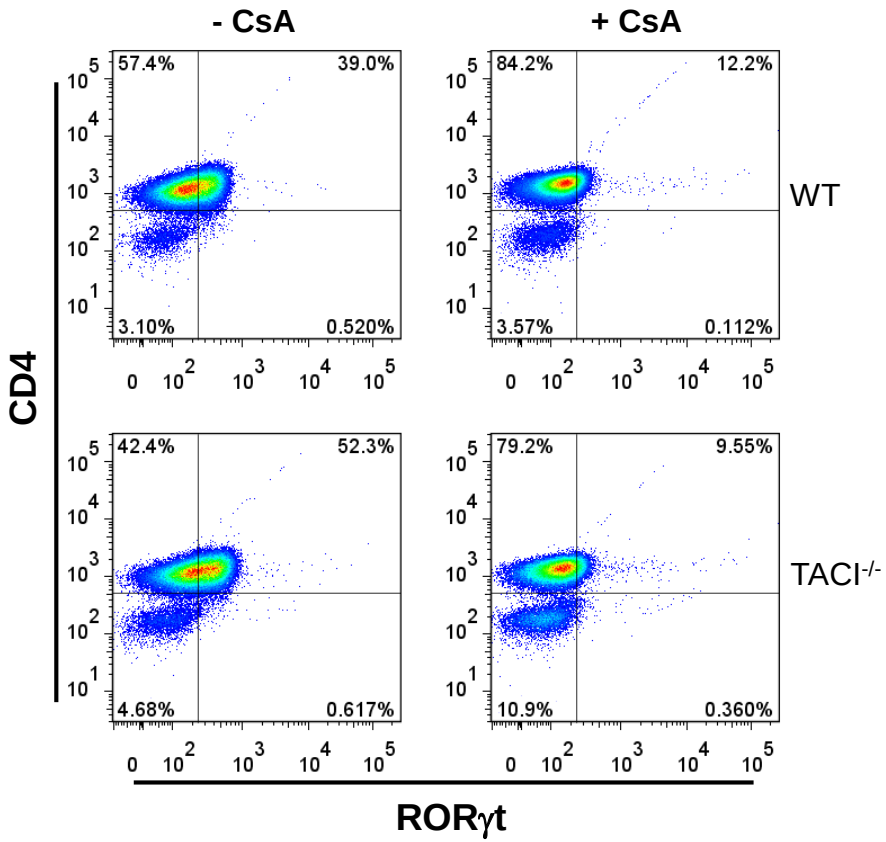
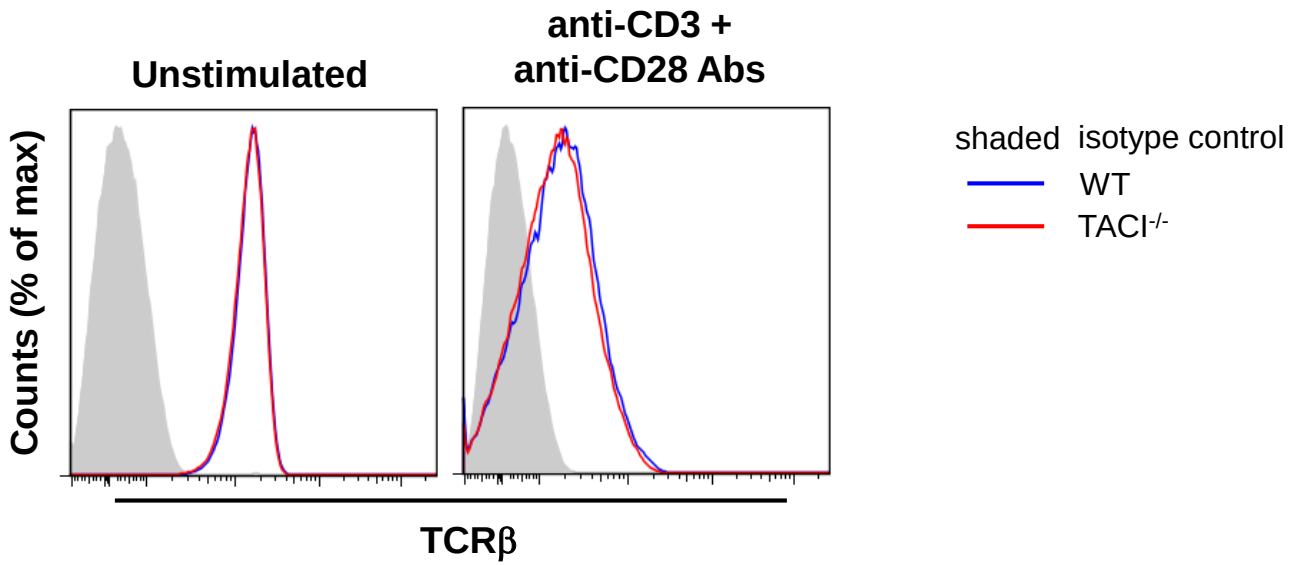

- CsA
+ CsA
57.4%
39.0%
3.10%
0.520%
84.2%
12.2%
3.57%
0.112%
42.4%
52.3%
4.68%
0.617%
79.2%
9.55%
10.9%
0.360%
WT
TACI-/-
CD4
RORγt

Figure S9


Unstimulated
anti-CD3 +
anti-CD28 Abs
Counts (% of max)
TCRβ
shaded isotype control
WT
TACI-/-