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Supplemental information

Transcription factor TCF-1 regulates the functions, but not the development, of lymphoid tissue inducer subsets in different tissues

Mingzhu Zheng, Chen Yao, Gang Ren, Kairui Mao, Hyunwoo Chung, Xi Chen, Gangqing Hu, Lei Wang, Xuemei Luan, Difeng Fang, Dan Li, Chao Zhong, Xiaoxiao Lu, Nikki Cannon, Mingxu Zhang, Avinash Bhandoola, Keji Zhao, John J. O'Shea, and Jinfang Zhu

Figure S1

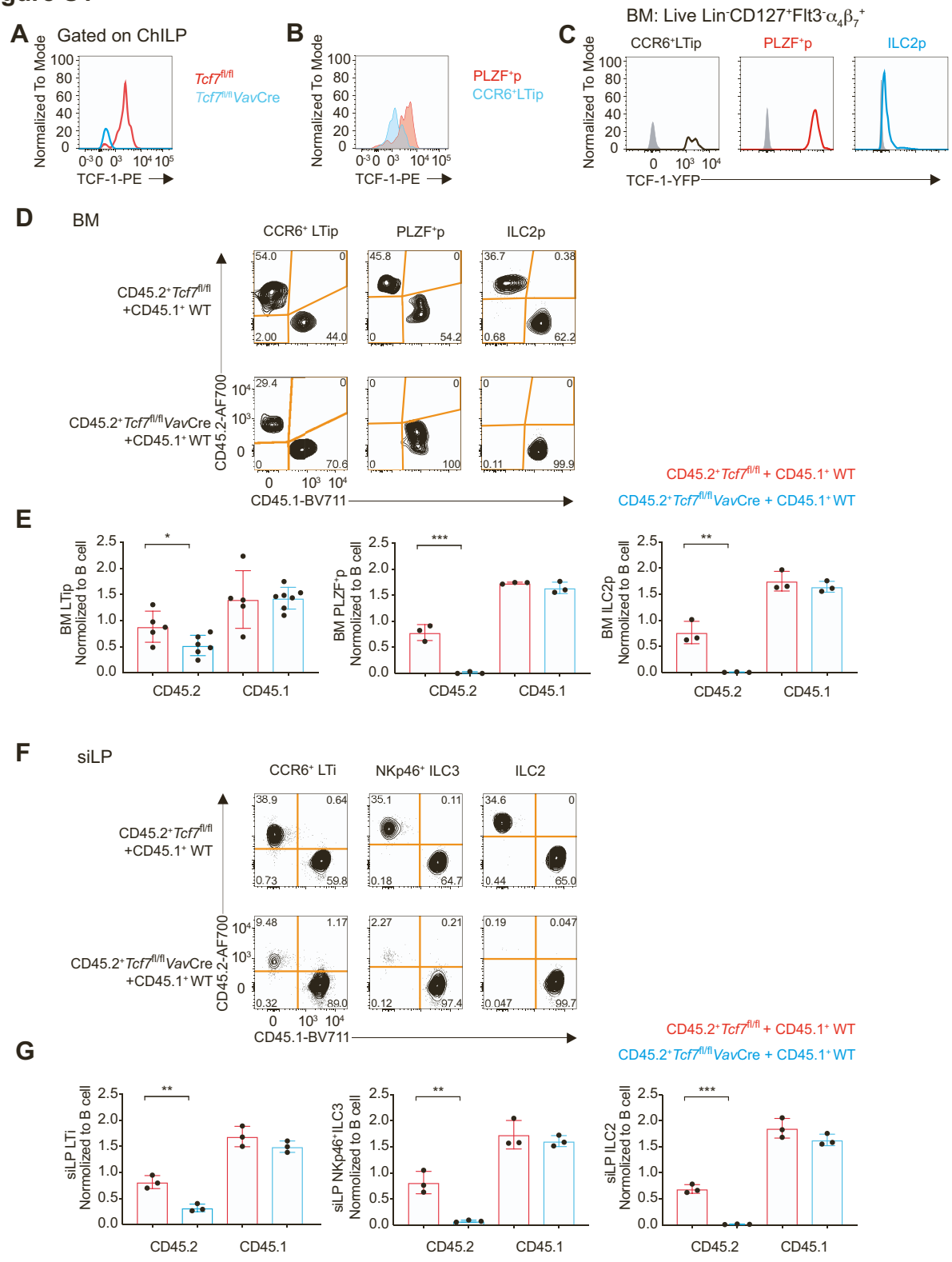

Figure S1, related to Figure 1. Cell intrinsic effect of TCF-1 on the development of ILC subsets.

(A) *Tcf7* deletion efficiency in bone marrow ChILP ILC progenitor of WT and *Tcf7*$^{fl/fl}$*Vav*Cre mice was analyzed by flow cytometry. (B) *Tcf7* expression in bone marrow PLZF^{+} ChILP and CCR6^{+} LTip populations of WT mice was analyzed by flow cytometry. (C) *Tcf7* expression in bone marrow CCR6^{+} LTip, PLZF^{+} ChILP and IL2p populations of *Tcf7*-YFP reporter mice was analyzed by flow cytometry. (D-G) Bone marrow (BM) cells were isolated from the WT and *Tcf7*$^{fl/fl}$*Vav*Cre mice bearing a congenic marker (CD45.2) together with CD45.1 marked BM cells at 1:1 ratio and intravenously injected into six-eight-week-old sub-lethally irradiated (550 rad) CD45.1 *Rag2*$^{-/-}$*Il2rg*$^{-/-}$ recipient mice. (D) After six-eight weeks, the generated CCR6^{+} LTip, PLZF^{+} ChILP and IL2p populations were analyzed in the BM of recipients. (E) Quantification of the rate from indicated population in D was normalized to B cells. (F) The generated CCR6^{+} LTi, NKp46^{+} ILC3 and IL2 populations were analyzed in the siLP of recipients. (G) Quantification of the rate from indicated population in F was normalized to B cells. mean ± s.d.; n = 3-5; $*P < 0.05$, $**P<0.01$, $***P<0.001$, Student's *t*-test. Data are representative of at least three independent experiments.

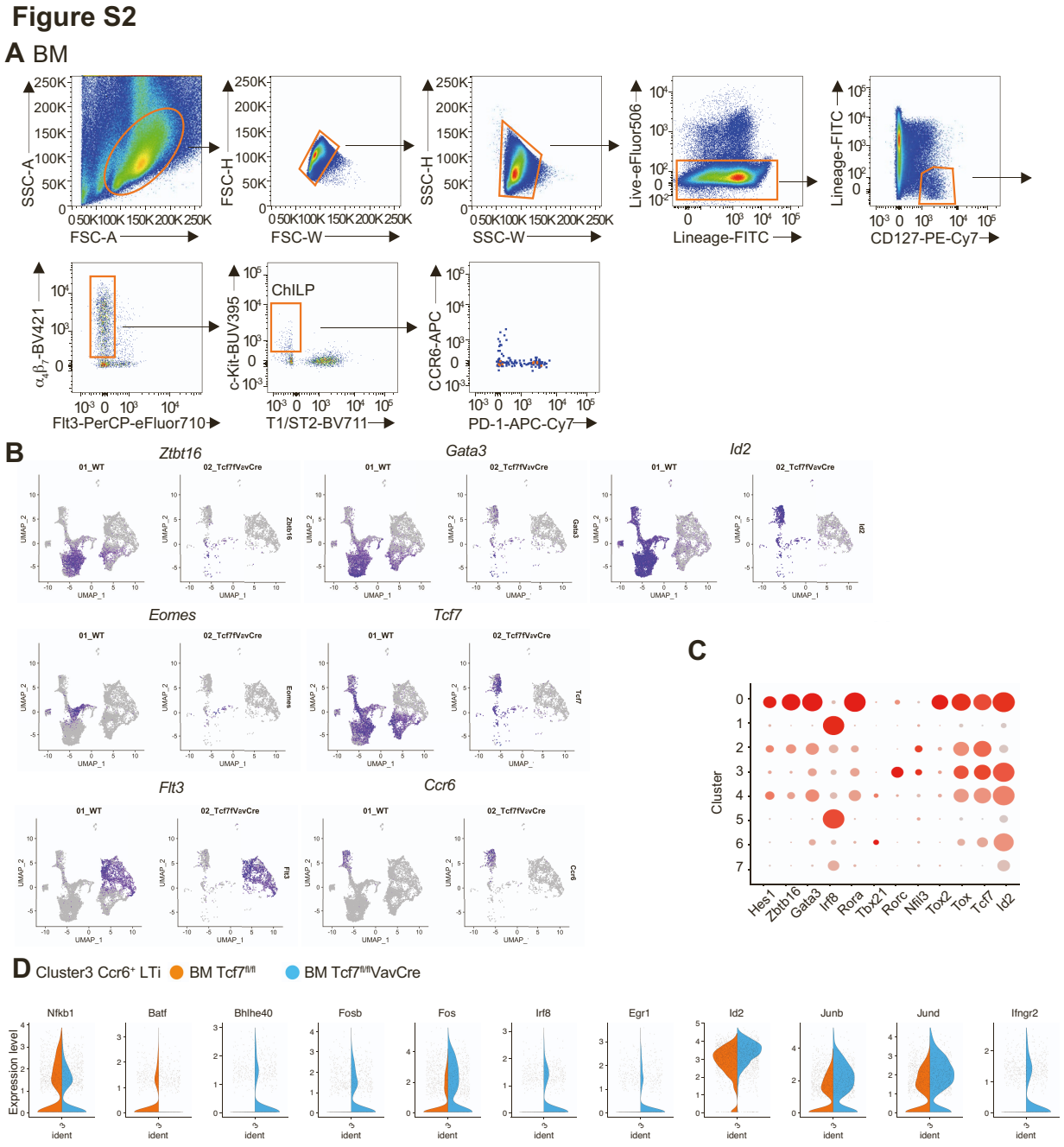

Figure S2, related to Figure 2. scRNA-Seq analysis reveals altered gene expression in the TCF-1-deficient LTi progenitors.

(A) Gating strategy for flow cytometry sorting of ILC progenitors in the BM. (B) Single-cell transcript levels of *Zbtb16, Gata3, Id2, Tcf7, Eomes, Flt3* and *Ccr6* illustrated in the UMAP plots. Transcript levels are color-coded: gray, not expressed; blue, expressed. (C) Dot plots of gene ontology. Each raw

represents one cluster; each cloum represents a key gene. The average expression level is color-coded. The expression percentages are represented by the diameter of the circles. (D) Violin plots of *Nfkb1, Batf, Bhlhe40, Fosb, Fos, Irf8, Egr1, Id2, Junb, Jund* and *Ifngr2* expression in LTi cells from WT and *Tcf7*$^{fl/fl}$*Vav*Cre mice. The violin represents the probability density at each value. Data are representative of two independent experiments.

Figure S3

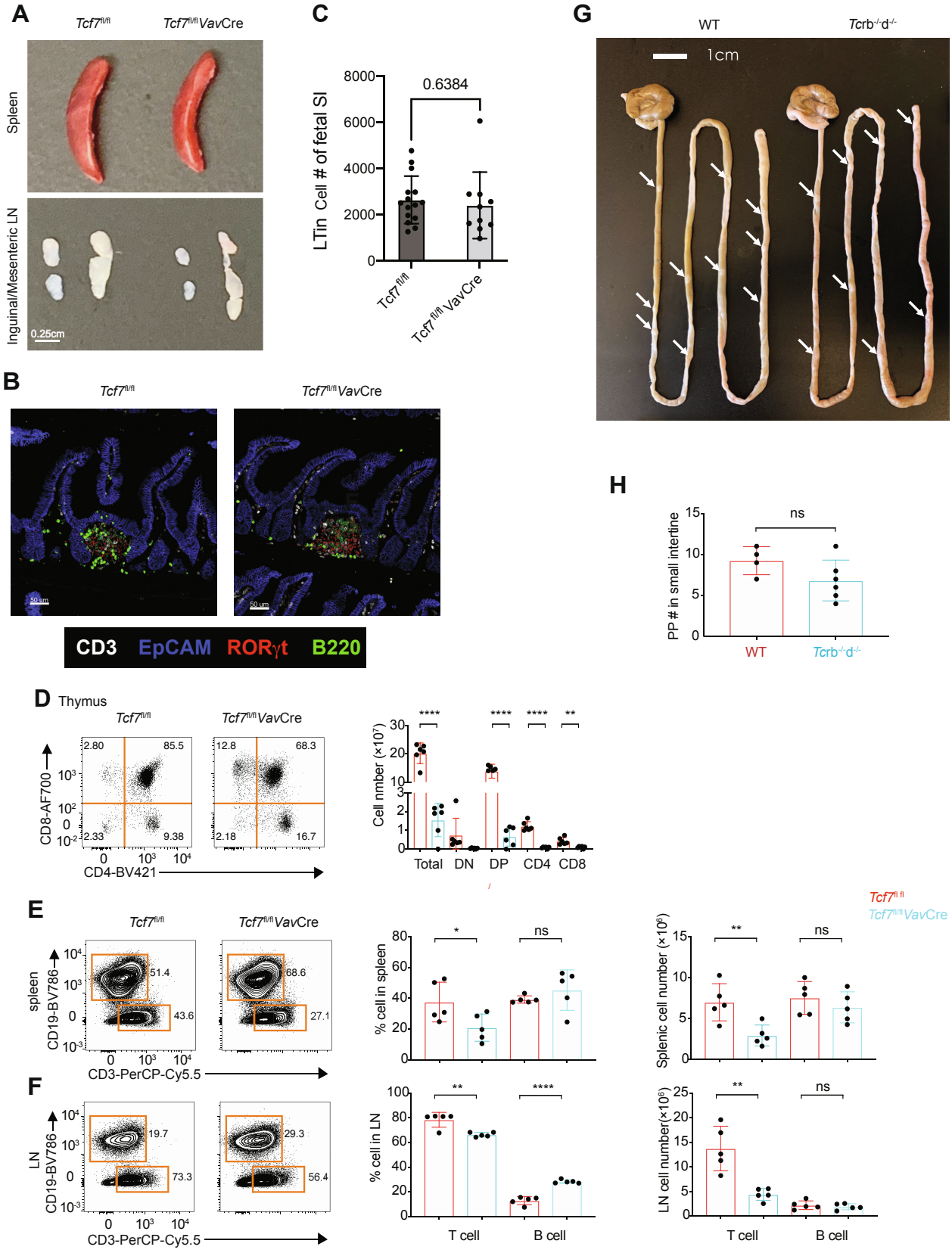
A
Tcf7fl/fl
Tcf7fl/flVavCre
Spleen
Inguinal/Mesenteric LN
0.25cm
C
LTin Cell # of fetal SI
8000
6000
4000
2000
0
0.6384
Tcf7fl/fl
Tcf7fl/flVavCre
G
WT
Tcrb-/-d-/-
1cm
B
Tcf7fl/fl
Tcf7fl/flVavCre
50 um
CD3 EpCAM RORγt B220
H
PP # in small intertine
15
10
5
0
ns
WT
Tcrb-/-d-/-
D Thymus
Tcf7fl/fl
Tcf7fl/flVavCre
CD8-AF700
CD4-BV421
2.80
85.5
2.33
9.38
12.8
68.3
2.18
16.7
Cell nmber (×10⁷)
****
****
****
**
Total
DN
DP
CD4
CD8
E
Tcf7fl/fl
Tcf7fl/flVavCre
spleen
CD19-BV786
CD3-PerCP-Cy5.5
51.4
43.6
68.6
27.1
F
LN
CD19-BV786
CD3-PerCP-Cy5.5
19.7
73.3
29.3
56.4
% cell in spleen
*
ns
% cell in LN
**
****
T cell
B cell
Tcf7fl/fl
Tcf7fl/flVavCre
Splenic cell number (×10⁶)
**
ns
LN cell number(×10⁶)
**
ns
T cell
B cell

Figure S3, related to Figure 3. TCF-1 is not required for the formation of LN and ILFs in the small intestine.

(A) Visualization of spleen and inguinal or mesenteric lymph nodes. (B) Immunofluorescent image of the isolated lymphoid follicles (ILF) in the small intestine. (C) Quantification of LTin cells in fetal small intestine. (D) Flow cytometry analysis and quantification of thymocytes from WT and *Tcf7*$^{fl/fl}$*Vav*Cre mice. (E-F) Flow cytometry analysis of spleen and lymph node cells from WT and *Tcf7*$^{fl/fl}$*Vav*Cre mice. (G) Visualization of Peyer's patches from WT and *Tcrb*$^{-/-}$*d*$^{-/-}$ mice. (H) Quantification of Peyer's patches from the mice in panel G. Each symbol represents an individual mouse. Bar graphs show quantification in percentages and total number. mean ± s.d.; n=10-15 in panel C, n = 5 in panel D-F and 4-6 in panel G; **P<0.05,* ***P<0.01,* *****P* < 0.0001, ns=not significant, Student's *t*-test. Data are representative of at least three independent experiments.

Figure S4 6-8wks

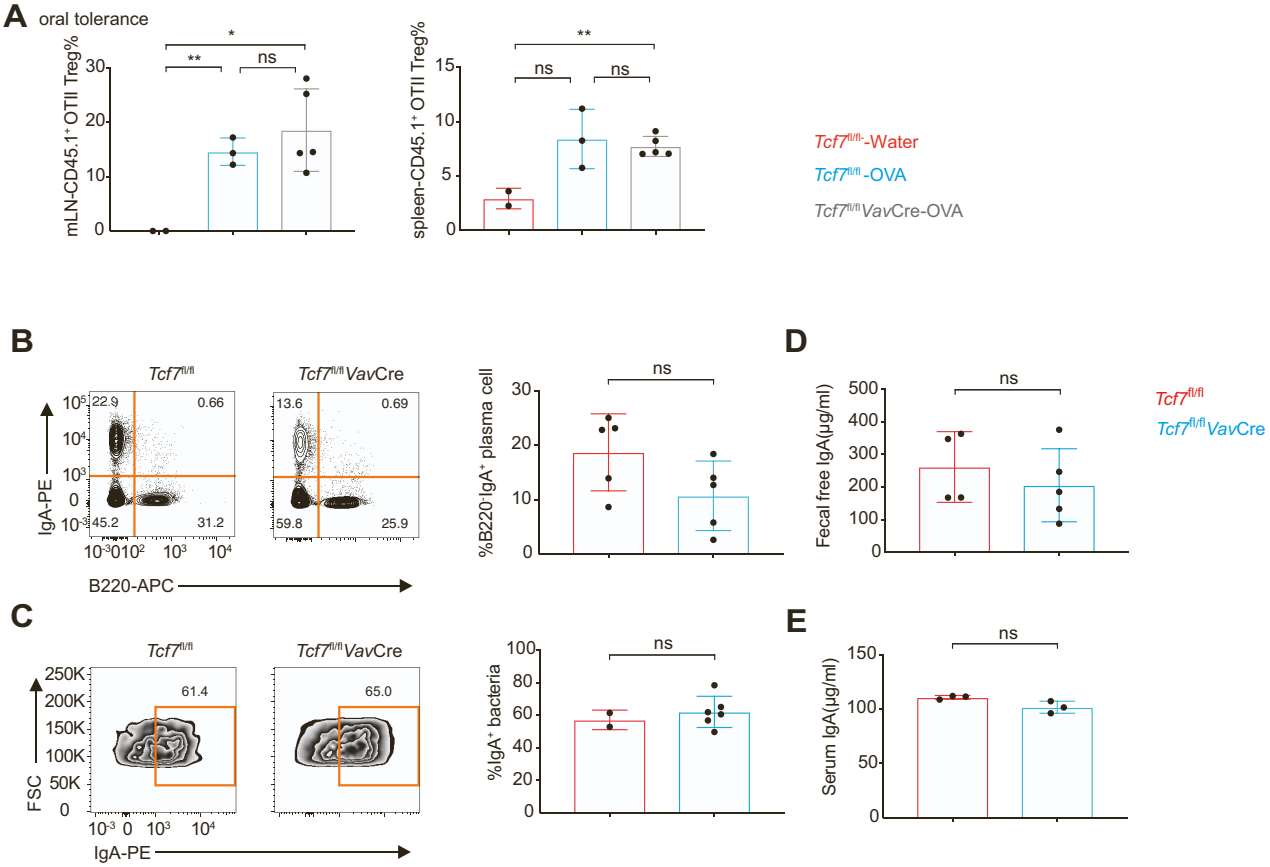

Figure S4, related to Figure 4. Normal IgA production in the small intestine of adult *Tcf7*$^{fl/fl}$*Vav*Cre mice

(A) Quantification of CD45.1$^+$ Treg cells in mLN and spleen of WT and *Tcf7*$^{fl/fl}$*Vav*Cre mice provide with drinking water or drinking water contain OVA. (B) Flow cytometric analysis and quantification of siLP IgA$^+$ B220$^-$ plasma cells in 6-8 weeks old WT and *Tcf7*$^{fl/fl}$*Vav*Cre mice. (C) Flow cytometry analysis and quantification of siLP IgA$^+$ bacteria in mice from B. (D-E) Fecal free and serum IgA in mice from B detected by ELISA assay. mean ± s.d.; n = 2-5; **P* < 0.05, ***P* < 0.01, ns=not significant, Student's t-test. Data are representative of two independent experiments.

Figure S5

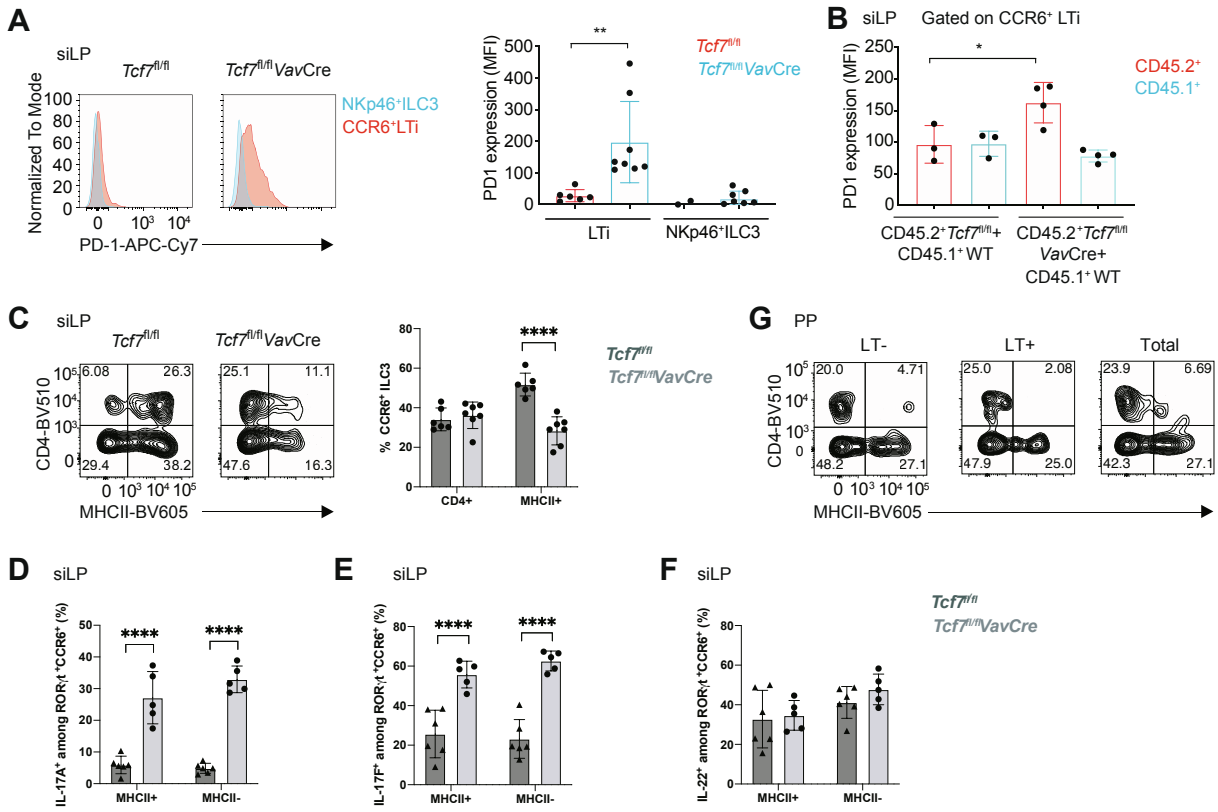

Figure S5, related to Figure 5. Gene expression profiling of the LTi cells from different organs of WT and *Tcf7*$^{fl/fl}$*Vav*Cre mice

(A) Flow cytometric analysis and quantification of PD-1 expression in LTi cells from the siLP of WT and *Tcf7*$^{fl/fl}$*Vav*Cre mice. (B) Flow cytometric analysis and quantification of PD-1 expression in LTi cells from the siLP of mixed BM chimera mice in FigS1F. (C) Flow cytometric analysis and quantification of CD4 and MHCII expression in LTi cells from the siLP of WT and *Tcf7*$^{fl/fl}$*Vav*Cre mice. (D-F) Quantification of IL-17A, IL-17F and IL-22 expression in LTi cells from the siLP of WT and *Tcf7*$^{fl/fl}$*Vav*Cre mice based on MHCII+ and MHCII- population. (G) Flow cytometric analysis and quantification of CD4 and MHCII expression in LTi cells from the PP of WT mice based on LT-, LT+

and total LTi population. mean ± s.d.; n = 3-8; *$P<0.05$, **$P<0.01$, ****$P<0.0001$, Student's *t*-test. Data are representative of two independent experiments.

Figure S6

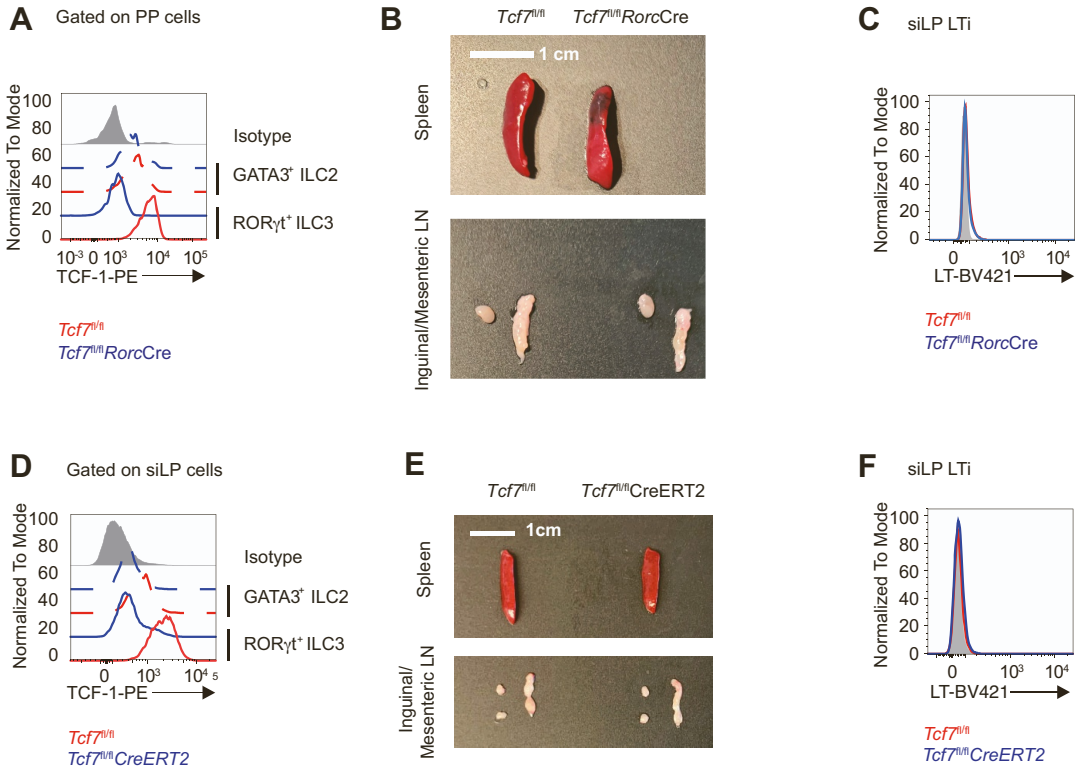

Figure S6, related to Figure 6. The role of TCF-1 in PP formation is developmental stage specific.

(A) *Tcf7* expression in PPs RORγt$^+$ ILC3 and GATA3$^+$ ILC2 populations of WT and *Tcf7*$^{fl/fl}$*Rorc*Cre mice was analyzed by flow cytometry. (B) Visualization of spleen and inguinal or mesenteric lymph nodes. (C) Flow cytometric analysis of LT expression in LTi cells from the siLP of WT and *Tcf7*$^{fl/fl}$*Rorc*Cre mice. (D) *Tcf7* expression in siLP RORγt$^+$ ILC3 and GATA3$^+$ ILC2 populations of TMX-treated WT and *Tcf7*$^{fl/fl}$CreERT2 mice was analyzed by flow cytometry at day 7 after TMX injection. (E) Visualization of spleen and inguinal or mesenteric lymph nodes. (F) Flow cytometric analysis of LT expression in LTi cells from the siLP of TMX treated WT and *Tcf7*$^{fl/fl}$CreERT2 mice. Data are representative of two independent experiments.

Figure S7

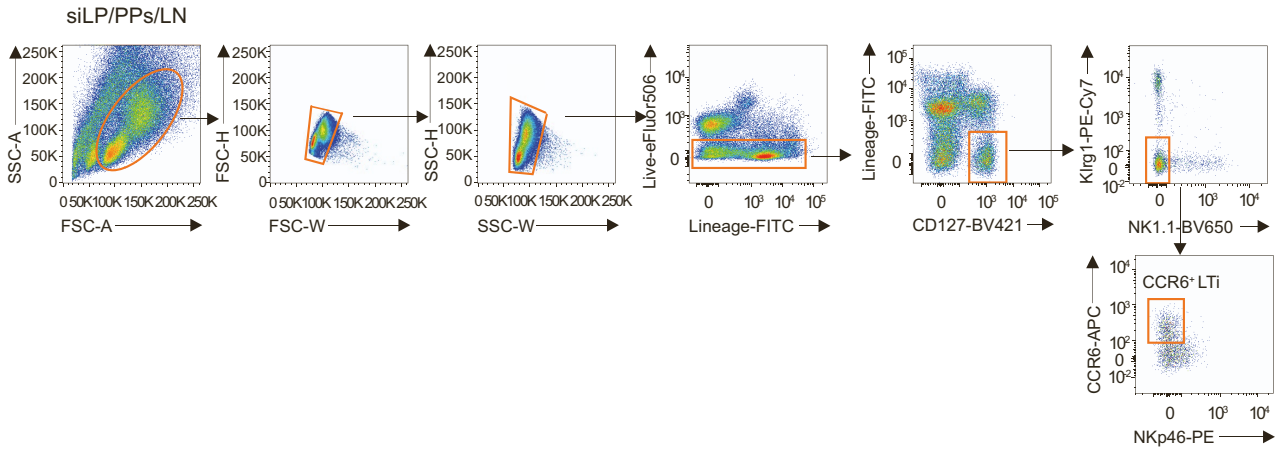

Figure S7, related to Figure 7. LTi cells were collected by FACS sorting.

Gating strategy for flow cytometry sorting of CCR6^{+} LTi cells in the siLP, PPs and LN.