Supplementary material

Identification of a T follicular helper cell subset that drives anaphylactic IgE

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Fig. S1

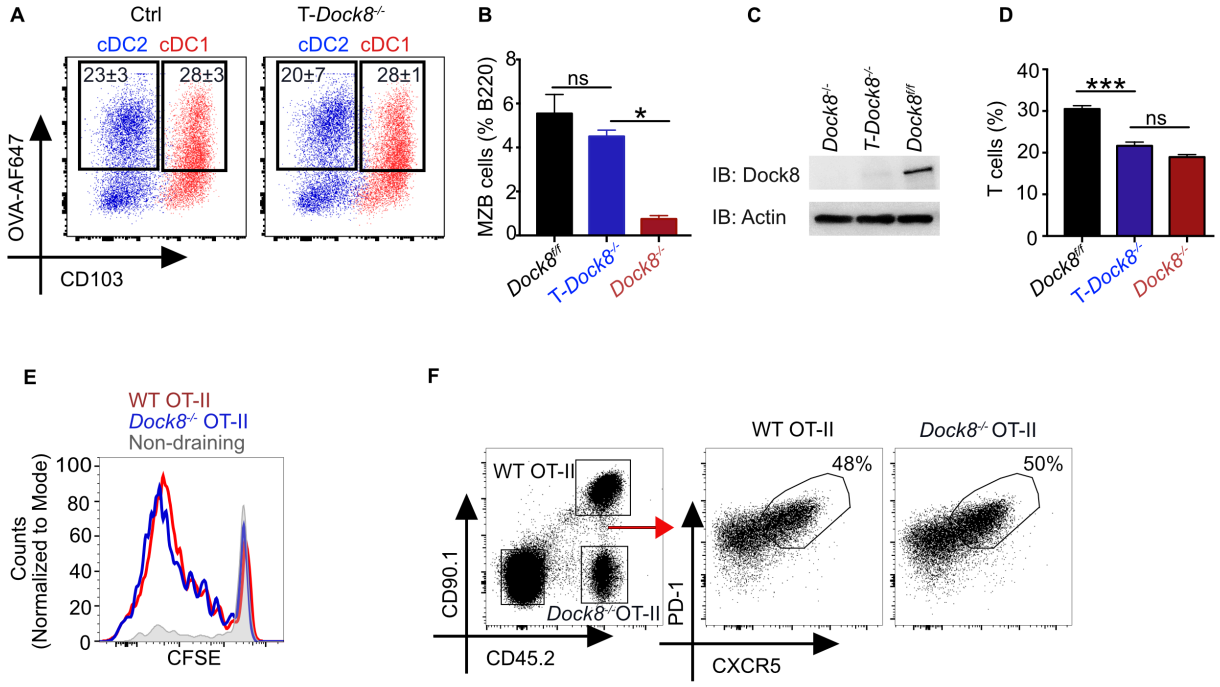
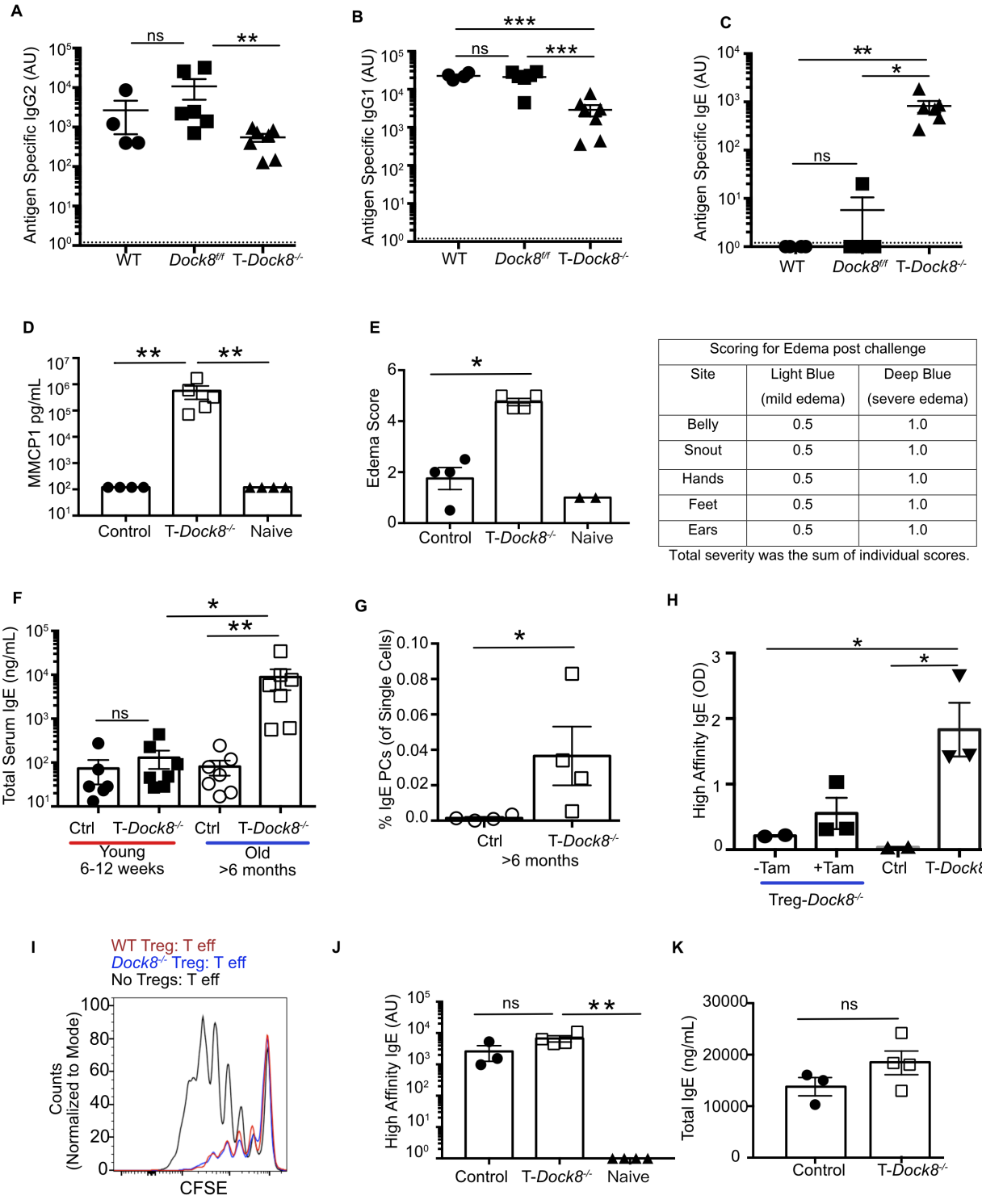

A
Ctrl
T-Dock8-/-
cDC2 cDC1
23±3
28±3
20±7
28±1
OVA-AF647
CD103
B
MZB cells (% B220)
ns
*
Dock8f/f
T-Dock8-/-
Dock8-/-
C
Dock8-/-
T-Dock8-/-
Dock8f/f
IB: Dock8
IB: Actin
D
T cells (%)
***
ns
E
WT OT-II
Dock8-/- OT-II
Non-draining
Counts (Normalized to Mode)
CFSE
F
WT OT-II
Dock8-/- OT-II
48%
50%
CD90.1
CD45.2
PD-1
CXCR5

Fig. S1. T-*Dock8*$^{-/-}$ mice have normal dendritic cell migration and T cell activation

(A) Comparable frequency of OVA^{+} migratory conventional dendritic cells type 1 (cDC1s) and type 2 (cDC2s) in MedLNs of T-*Dock8*$^{-/-}$ or control *Dock8*$^{fl/fl}$ mice 18 hr post intranasal (i.n.) immunization with LPS and OVA-AF647. (B) Splenic marginal zone B cell frequencies in *Dock8*$^{fl/fl}$, T-*Dock8*$^{-/-}$, or *Dock8*$^{-/-}$ mice. (C) DOCK8 immunoblot performed on purified CD4^{+} T cells from *Dock8*$^{-/-}$, T-*Dock8*$^{-/-}$, and control *Dock8*$^{fl/fl}$ mice. (D) Splenic T cell frequencies in *Dock8*$^{fl/fl}$, T-*Dock8*$^{-/-}$, or *Dock8*$^{-/-}$ mice. (E-F) *Dock8*$^{-/-}$ CD4^{+} OVA-specific T cells (OT-II cells) show normal proliferation and Tfh differentiation. (E) Congenically marked *Dock8*^{WT} and *Dock8*$^{-/-}$ OT-II cells were labeled with CFSE and co-transferred into naïve recipients, which were immunized with LPS and NP-OVA i.n. Three days later, proliferation was assessed by CFSE dye dilution. (F) Tfh cell differentiation of *Dock8*^{WT} and *Dock8*$^{-/-}$ OT-II cells was assessed 6 days post immunization. Numbers in flow cytometry plots indicate percentage. Statistical tests: ANOVA (B, D). *P<0.05, ***P<0.001. Data representative of at least two independent experiments with 2-3 mice per group.

Fig. S2



Scoring for Edema post challenge		
Site	Light Blue (mild edema)	Deep Blue (severe edema)
Belly	0.5	1.0
Snout	0.5	1.0
Hands	0.5	1.0
Feet	0.5	1.0
Ears	0.5	1.0

Total severity was the sum of individual scores.

Fig. S2. T-*Dock8*$^{-/-}$ mice have a hyper IgE state

WT, *Dock8*$^{fl/fl}$, or T-*Dock8*$^{-/-}$ mice were immunized i.n. with LPS and NP16-OVA and boosted twice with NP16-OVA. NP16-OVA-specific antibody responses on day 8 post boost were quantitated by ELISA for (A) IgG2c, (B) IgG1, and (C) IgE isotypes. (D) LPS+OVA immunized and boosted T-*Dock8*$^{-/-}$ or control (*Dock8*$^{fl/fl}$) mice were challenged intraperitoneally (i.p.) with NP7-BSA. Plasma was collected thirty minutes post challenge and mouse mast cell protease 1 (MMCP-1) levels were measured by ELISA. (E) LPS and NP16-OVA immunized and boosted mice were challenged i.p. with NP7-BSA in 0.2% Evans blue. Forty minutes later, mice were evaluated for edema as described in the adjacent scoring scheme. (F) Total IgE levels in the sera from young or old naïve T-*Dock8*$^{-/-}$ or control (*Dock8*$^{fl/fl}$) mice. (G) Frequency of CD138^{+} IgE^{+} plasma cells in mesenteric lymph nodes of aged T-*Dock8*$^{-/-}$ or control mice. (H) *Dock8*$^{fl/fl}$ Foxp3-EGFP-cre-ERT2 (Treg-*Dock8*$^{-/-}$), T-*Dock8*$^{-/-}$, or *Dock8*$^{fl/fl}$ (Ctrl) mice were immunized and boosted with LPS and NP16-OVA i.n. as described. Day 8 post boost high-affinity IgE was quantitated by ELISA using NP7-BSA-coated plates. *Dock8* was inducibly deleted in Treg-*Dock8*$^{-/-}$ mice by administering tamoxifen (Tam) (1 mg/day/mouse) in corn oil through oral gavage on three consecutive days before every immunization. (I) Tregs from CD45.2 *Dock8*$^{-/-}$ or WT mice were cultured with naïve CFSE-labeled CD45.1^{+} WT CD4^{+} T cells at a ratio of 1:2 in the presence of α-CD3 and α-CD28 for 4 days. Proliferation was assessed by CFSE dye dilution gated on CD45.1^{+} CD4^{+} T effectors. (J-K) T-Dock8$^{-/-}$ or control (*Dock8*$^{fl/fl}$) mice were immunized with *Alternaria* extract and NP16-Ova (Alt+OVA) and boosted twice as described in the methods. Day 8 sera from boosted mice were analyzed by ELISA for (J) high-affinity IgE using NP7-BSA-coated plates and (K) total IgE. Each symbol indicates an individual mouse. Error bars indicate SEM. Dotted lines in bar graphs represent background readings of sera from naïve mice. Statistical tests: Kruskal–Wallis *H* test (A, C, D, E, F, H, J); ANOVA (B); Student's *t*-test (G, K). *P<0.05,

**P<0.01, ***P<0.001, and ns is not significant. Data representative of two independent experiments with 2-8 mice per group.

Fig. S3

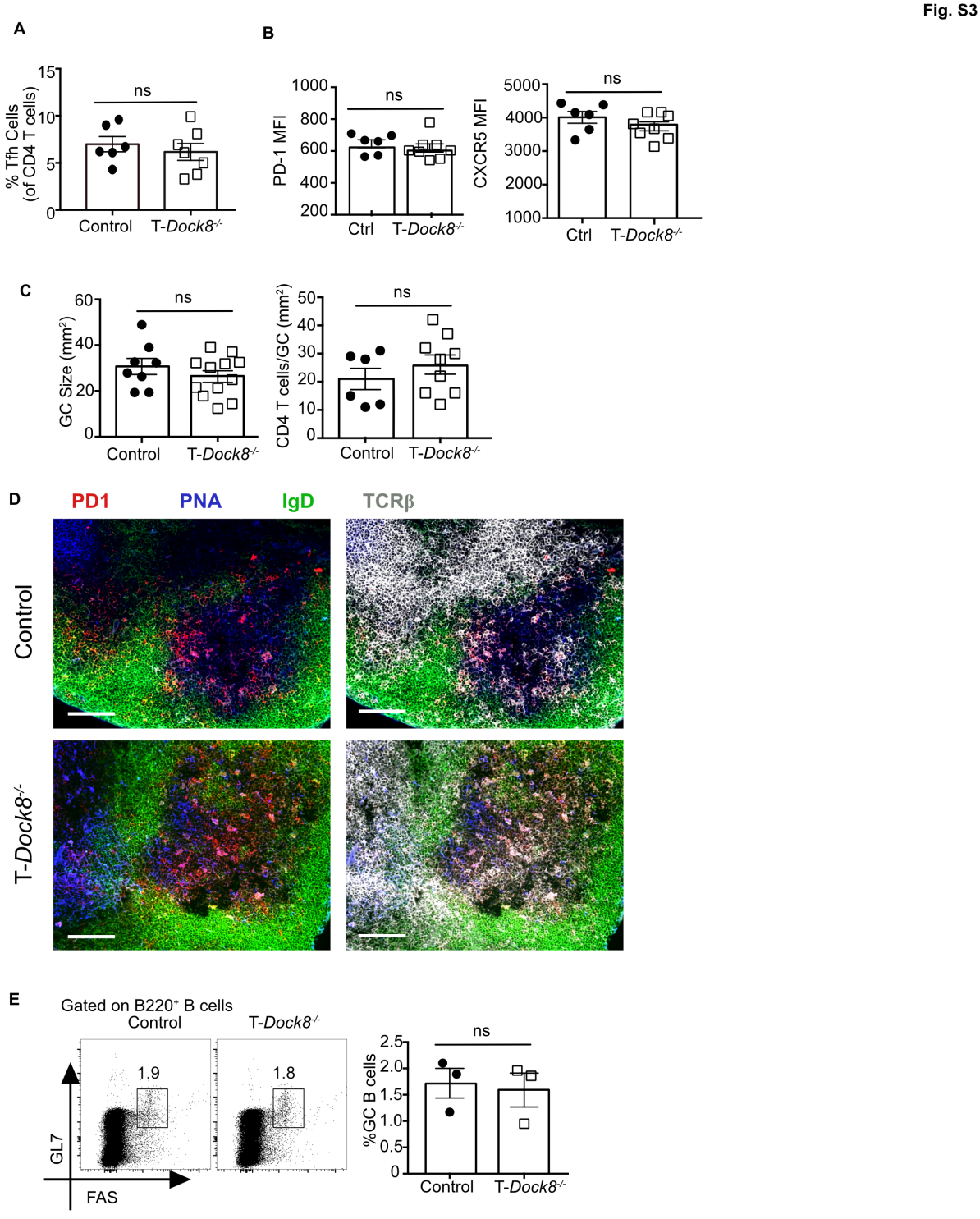

A
% Tfh Cells (of CD4 T cells)
ns
Control
T-Dock8-/-
B
PD-1 MFI
ns
Ctrl
T-Dock8-/-
CXCR5 MFI
ns
Ctrl
T-Dock8-/-
C
GC Size (mm2)
ns
Control
T-Dock8-/-
CD4 T cells/GC (mm2)
ns
Control
T-Dock8-/-
D
PD1
PNA
IgD
TCRβ
Control
T-Dock8-/-
E
Gated on B220+ B cells
Control
T-Dock8-/-
1.9
1.8
GL7
FAS
%GC B cells
ns
Control
T-Dock8-/-

Fig. S3. Tfh cells and germinal centers in T-*Dock8*$^{-/-}$ mice

T-*Dock8*$^{-/-}$ and control (*Dock8*$^{fl/fl}$) mice were immunized i.n. with LPS and NP16-OVA and analyzed for (A) frequency of day 8 Tfh cells and (B) PD-1 and CXCR5 expression on Tfh cells by flow cytometry. (C) Quantification of germinal centers size Tfh cells in GCs as analyzed by confocal microscopy on sections from day 9 MedLN as in Fig 1G. (D) Day 8 immunofluorescent images of GCs from the MedLN of LPS+OVA immunized T-*Dock8*$^{-/-}$ and *Dock8*$^{fl/fl}$ (control) mice. PD-1 (red), PNA (blue), IgD (green), TCRβ (white). Scale bar: 100 μM. (E) Frequency of day 8 GC B cells by flow cytometry. Each symbol indicates an individual mouse. Statistical tests: Student's *t*-test. Error bars indicate SEM. ns is not significant. Data representative of at least two independent experiments with 3-7 mice per group.

Fig. S4

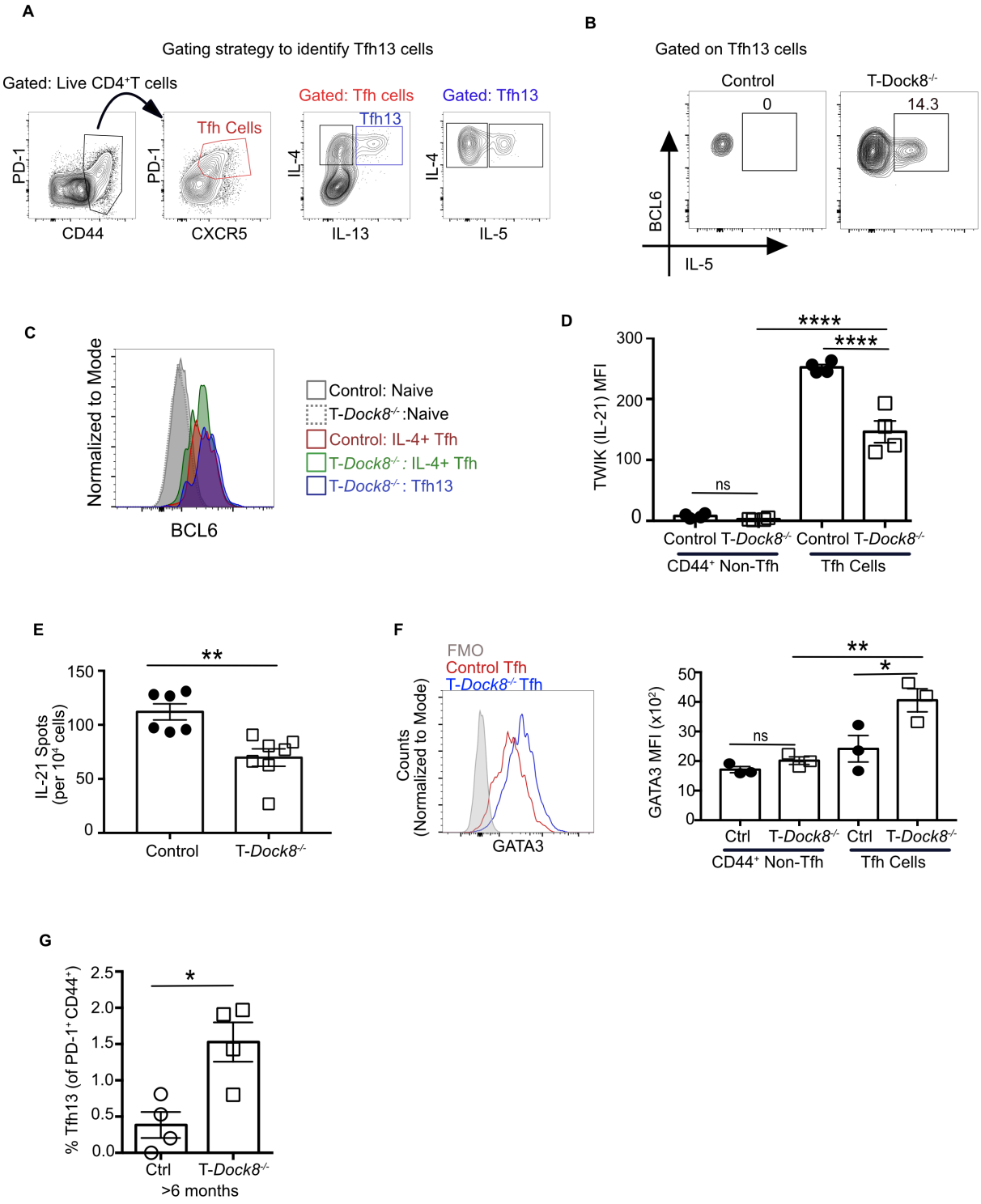

A
Gating strategy to identify Tfh13 cells
Gated: Live CD4+T cells
PD-1
CD44
Tfh Cells
CXCR5
Gated: Tfh cells
Tfh13
IL-4
IL-13
Gated: Tfh13
IL-5
B
Gated on Tfh13 cells
Control
T-Dock8-/-
0
14.3
BCL6
IL-5
C
Normalized to Mode
BCL6
Control: Naive
T-Dock8-/- :Naive
Control: IL-4+ Tfh
T-Dock8-/- : IL-4+ Tfh
T-Dock8-/- : Tfh13
D
TWIK (IL-21) MFI
300
200
100
0
****
****
ns
Control T-Dock8-/- Control T-Dock8-/-
CD44+ Non-Tfh
Tfh Cells
E
IL-21 Spots (per 10^4 cells)
150
100
50
0
**
Control
T-Dock8-/-
F
FMO
Control Tfh
T-Dock8-/- Tfh
Counts (Normalized to Mode)
GATA3
GATA3 MFI (x10^2)
50
40
30
20
10
0
**
*
ns
Ctrl T-Dock8-/- Ctrl T-Dock8-/-
CD44+ Non-Tfh
Tfh Cells
G
% Tfh13 (of PD-1+ CD44+)
2.5
2.0
1.5
1.0
0.5
0.0
*
Ctrl
T-Dock8-/-
>6 months

Fig. S4. Characterization of Tfh13 cells in T-*Dock8*$^{-/-}$ mice

(A) Gating strategy to identify Tfh13 cells. (B) T-*Dock8*$^{-/-}$ and control (*Dock8*$^{fl/fl}$) mice were immunized i.n. with LPS and NP16-OVA and analyzed for (B) IL-5 expression by day 8 Tfh13 cells and (C) BCL6 expression in cytokine-producing Tfh cell subsets. (D) IL-21 reporter expression in MedLN Tfh cells from IL-21 TWIK reporter T-*Dock8*$^{-/-}$ or control TWIK *Dock8*$^{fl/fl}$ reporter mice on day 8 post immunization. (E) IL-21 ELISpot of Tfh cells sorted from T-*Dock8*$^{-/-}$ or control (*Dock8*$^{fl/fl}$) mice at day 7 post immunization. (F) GATA3 expression in Tfh cells from T-*Dock8*$^{-/-}$ or control (*Dock8*$^{fl/fl}$) mice post immunization is shown as histogram overlay (left) and as bar graphs (right). (G) Frequency of Tfh13 cells in mesenteric lymph nodes of aged T-*Dock8*$^{-/-}$ or control mice. Each symbol indicates an individual mouse. Error bars indicate SEM. Statistical tests: ANOVA (C, F); Mann–Whitney *U* test (E); Student's *t*-test (G). **P*<0.05, ***P*<0.01, ****P*<0.001, *****P*<0.0001, and ns is not significant. Data representative of at least two independent experiments with 3-7 mice. FMO, fluorescence minus one control for GATA3 staining.

Fig. S5

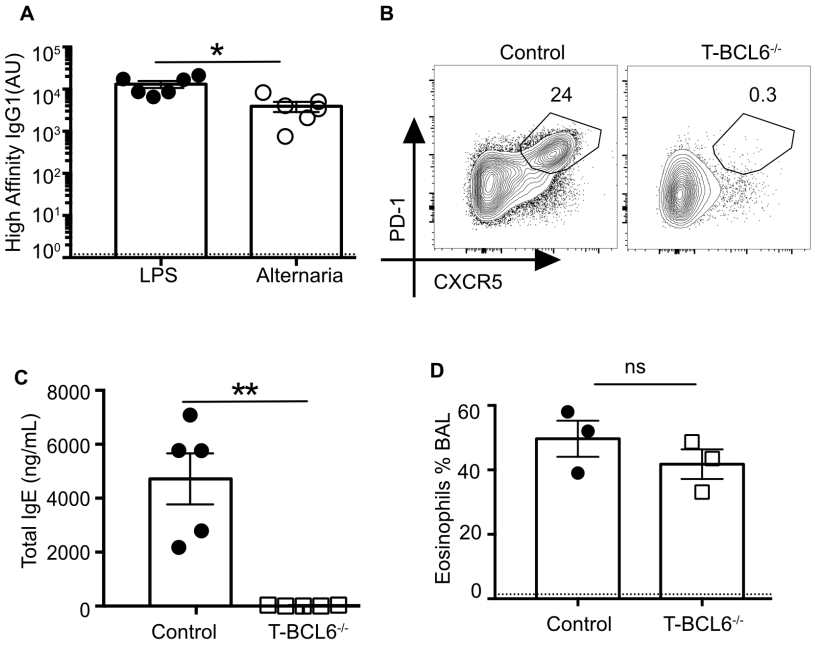

A
High Affinity IgG1(AU)
10^5
10^4
10^3
10^2
10^1
10^0
*
LPS
Alternaria
B
Control
T-BCL6-/-
24
0.3
PD-1
CXCR5
C
Total IgE (ng/mL)
8000
6000
4000
2000
0
**
Control
T-BCL6-/-
D
Eosinophils % BAL
60
40
20
0
ns
Control
T-BCL6-/-

Fig. S5. Characterization of type 2 responses to Alternaria in WT and T-*Bcl6*$^{-/-}$ mice

(A) WT C57BL/6 mice were immunized intranasally (i.n.) with LPS or *Alternaria* extract and NP16-OVA and boosted as described. Day 8 sera from boosted mice were analyzed by ELISA with NP7-BSA-coated plates for high-affinity IgG1. (B-C) Frequency of Tfh cells from *Cd4*^{Cre}*Bcl6*$^{fl/fl}$ (T-*Bcl6*$^{-/-}$) mice or controls (*Bcl6*$^{fl/fl}$) that were immunized with *Alternaria* extract and NP16-OVA. (C) T-*Bcl6*$^{-/-}$ or control (*Bcl6*$^{fl/fl}$) mice were immunized and boosted with *Alternaria* extract and NP16-OVA. Data depict total serum IgE day 8 post-boost. (D) Bronchoalveolar lavage (BAL) eosinophilia in T-*Bcl6*$^{-/-}$ or control (*Bcl6*$^{fl/fl}$) mice that were immunized and challenged with *Alternaria* extract and NP16-OVA. Each symbol indicates an individual mouse. Data representative of 3-6 mice per group. Error bars indicate SEM. Dotted lines in bar graphs represent background readings of sera or BAL eosinophils from naïve mice. Statistical tests: Student's *t*-test (A, D); Mann–Whitney *U* test (C). **P*<0.05, ***P*<0.01, and ns is not significant.

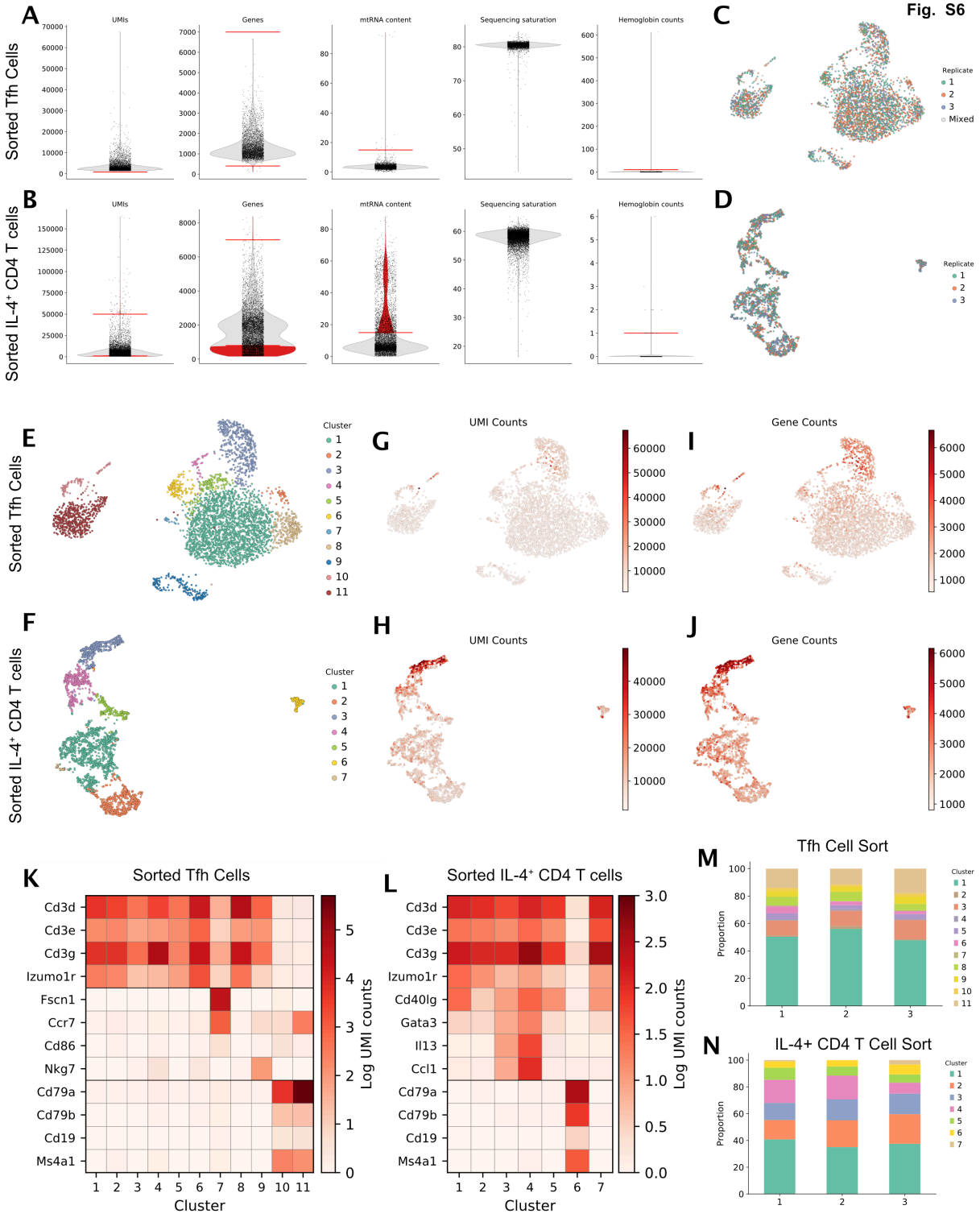


Fig. S6
A
Sorted Tfh Cells
UMIs
Genes
mtRNA content
Sequencing saturation
Hemoglobin counts
B
Sorted IL-4⁺ CD4 T cells
C
D
Replicate
1
2
3
Mixed
E
Sorted Tfh Cells
Cluster
F
Sorted IL-4⁺ CD4 T cells
G
UMI Counts
H
I
Gene Counts
J
K
Sorted Tfh Cells
Cd3d
Cd3e
Cd3g
Izumo1r
Fscn1
Ccr7
Cd86
Nkg7
Cd79a
Cd79b
Cd19
Ms4a1
Cluster
Log UMI counts
L
Sorted IL-4⁺ CD4 T cells
Cd40lg
Gata3
Il13
Ccl1
M
Tfh Cell Sort
Proportion
N
IL-4+ CD4 T Cell Sort

Fig. S6. scRNA-seq QC analysis

(A-B) Violin plots showing 5 per-cell metrics used to gauge the quality of individual single-cells from either sorted Tfh cells or *Il4*-expressing (4get+) activated T cells scRNA-seq runs: UMI counts, gene counts, fraction of the captured transcripts mapped to mtRNA, per-cell sequencing saturation, and cumulative expression of 10 hemoglobin genes. Red horizontal lines show the thresholds above or below which were used to exclude cells for each criterion; sequencing saturation was not used to filter cells. More stringent filtering criteria were used for the second sample. (C-J) 2D UMAP embedding of the single-cell expression profiles from both scRNA-seq runs labeled by (C-D) biological replicate as determined by cell-hashing (*82*), (E-F) cluster assigned by the Leiden community detection algorithm, (G-H) total UMI counts per cell, and (I-J) gene counts per cell. (K-L) Matrix plots showing the log-transformed normalized expression of representative marker genes to identify the T-cell populations of interest and remove B-cells, monocytes, and NK cells. Gene expression is colored relative to the highest expressed gene shown in each matrix. (M-N) Bar plots showing the proportion of cells in each cluster from each biological replicate from each scRNA-seq run, respectively.

Fig. S7

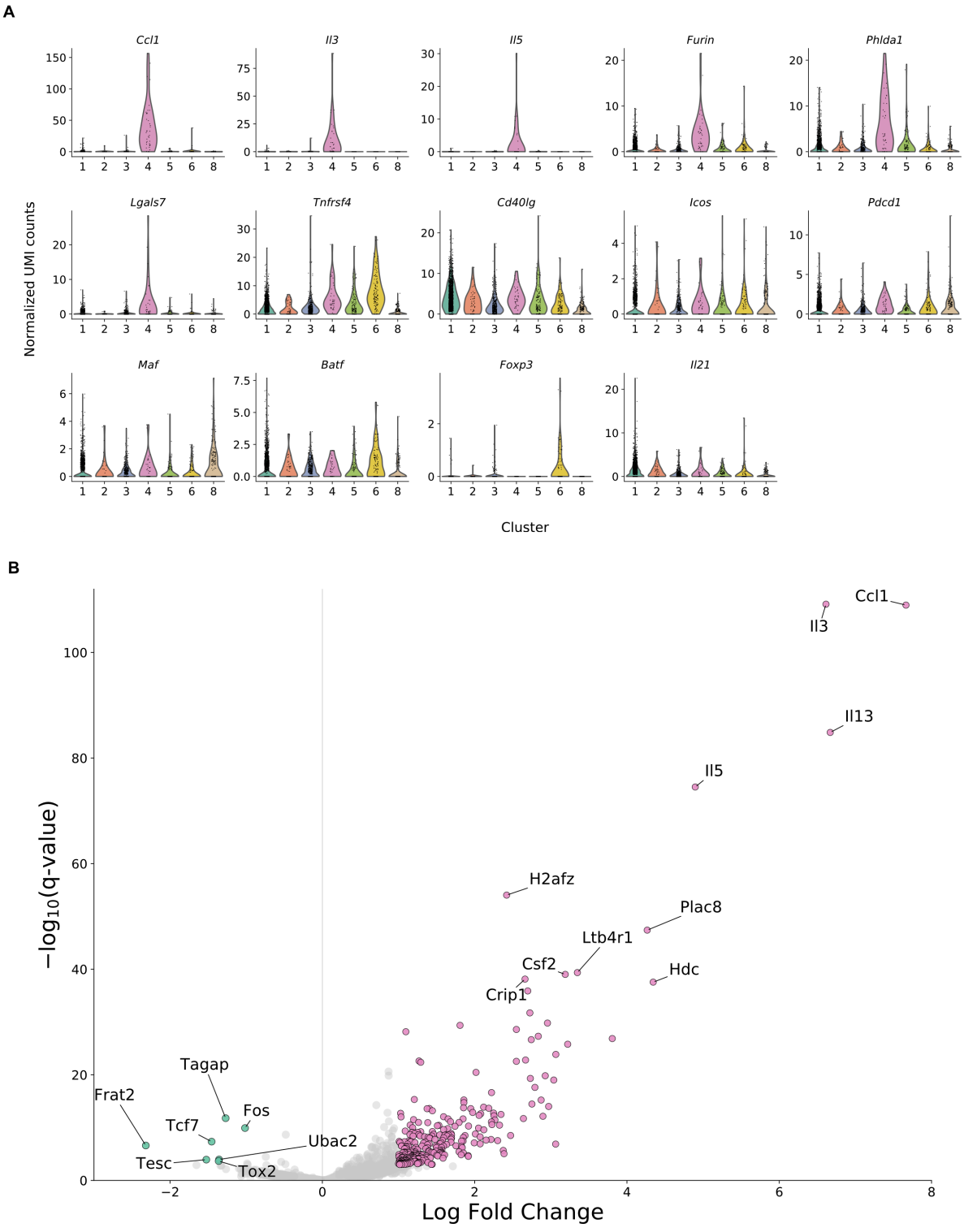

A
Ccl1
Il3
Il5
Furin
Phlda1
Lgals7
Tnfrsf4
Cd40lg
Icos
Pdcd1
Maf
Batf
Foxp3
Il21
Normalized UMI counts
Cluster
B
Ccl1
Il3
Il13
Il5
H2afz
Plac8
Ltb4r1
Csf2
Hdc
Crip1
Tagap
Frat2
Fos
Tcf7
Ubac2
Tesc
Tox2
−log10(q-value)
Log Fold Change

Fig. S7. Differentially expressed genes between Tfh2 and Tfh13 cells

(A) Violin plots showing the expression of *Ccl1, Il3, Il5, Furin, Phlda1, Lgals7, Tnfrsf4* (OX40), *Cd40lg* (CD40L), *Icos*, *Pdcd1* (PD-1), *Maf* (c-Maf), *Batf, Foxp3*, and *Il21* in the seven Tfh cell clusters. (B) Volcano plot showing differentially expressed genes (DEGs) between clusters 1 (Tfh2 cells) and 4 (Tfh13 cells). The horizontal axis shows the $\log_2$-fold change in mean expression between the two clusters and the vertical axis shows the $\log_{10}$ adjusted *P*-value (Benjamini-Hochberg correction) given by a likelihood ratio test. Genes that are more highly expressed in cluster 4 have positive log-fold change. The 7 and 10 most significant DEGs for clusters 1 and 4 are labeled.

Fig. S8

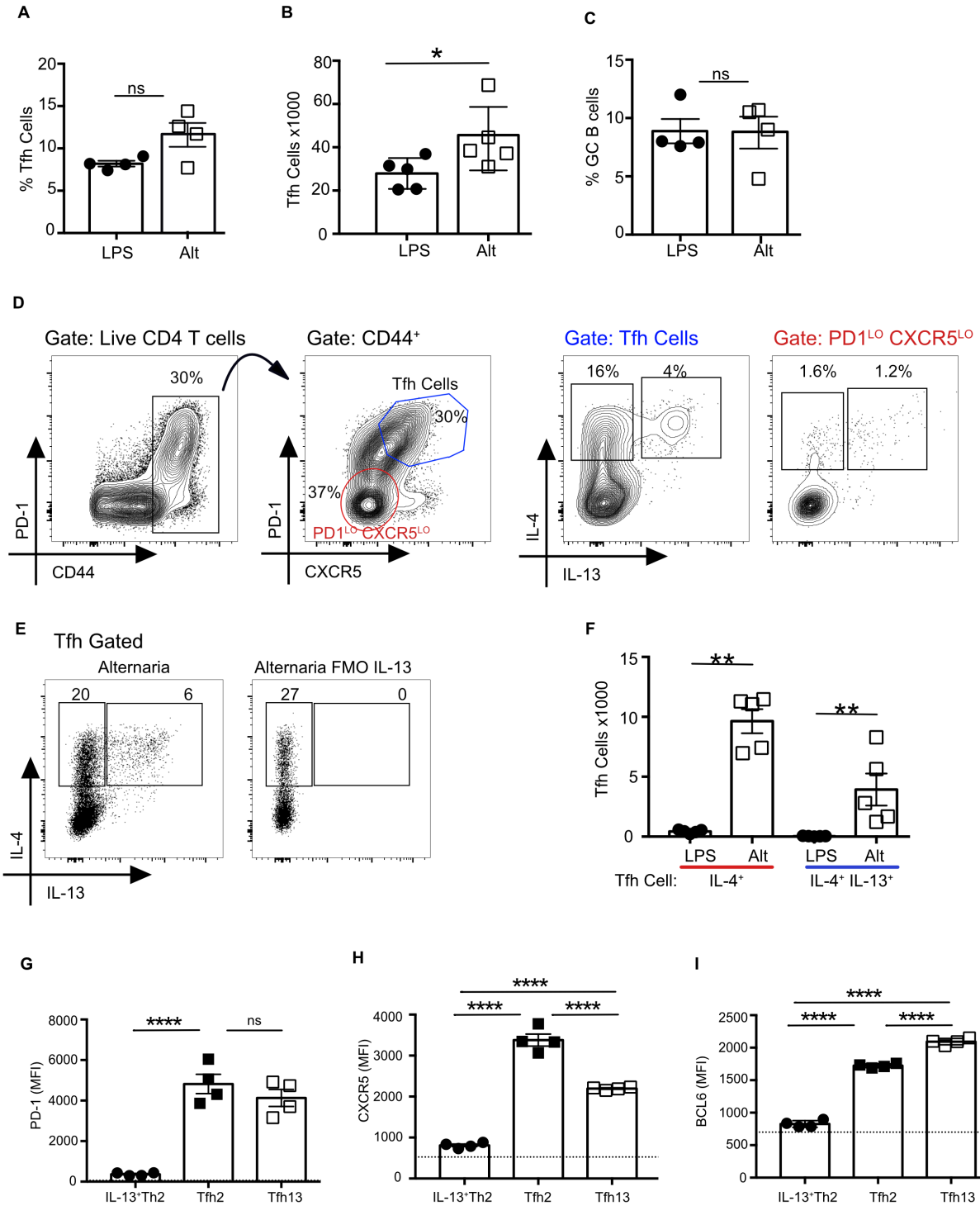

A
% Tfh Cells
ns
LPS
Alt
B
Tfh Cells x1000
*
LPS
Alt
C
% GC B cells
ns
LPS
Alt
D
Gate: Live CD4 T cells
30%
PD-1
CD44
Gate: CD44+
Tfh Cells
30%
37%
PD1LO CXCR5LO
CXCR5
Gate: Tfh Cells
16%
4%
IL-4
IL-13
Gate: PD1LO CXCR5LO
1.6%
1.2%
E
Tfh Gated
Alternaria
20
6
Alternaria FMO IL-13
27
0
IL-4
IL-13
F
Tfh Cells x1000
**
**
LPS
Alt
LPS
Alt
Tfh Cell:
IL-4+
IL-4+ IL-13+
G
PD-1 (MFI)
****
ns
IL-13+Th2
Tfh2
Tfh13
H
CXCR5 (MFI)
****
****
****
IL-13+Th2
Tfh2
Tfh13
I
BCL6 (MFI)
****
****
****
IL-13+Th2
Tfh2
Tfh13

Fig. S8. Characterization of Tfh13 cells induced to Alternaria extract in WT mice

WT C57BL/6 mice were immunized with LPS+OVA or Alt+OVA and Tfh cells were analyzed by flow cytometry 8 days later. (A) Tfh cell frequencies and (B) numbers post immunization. (C) Frequency of GC B cells (PNA^+ IgD^-). (D) Gating strategy to identify Tfh cells ($PD\text{-}1^+$ $CXCR5^+$) and effectors ($PD\text{-}1^{lo}$ $CXCR5^{lo}$) that make cytokines IL-4 and IL-13. Samples were stimulated with PMA+Ionomycin and stained for cytokines as described in methods. (E) Unstained (FMO) control for IL-13 staining. (F) Absolute counts of Tfh2 and Tfh13 cells induced in LPS+OVA or Alt+OVA day 8 post immunization. (G-H) Expression of (G) PD-1, (H) CXCR5 and (I) BCL6 in Tfh2, Tfh13 and IL-13+ Th2 (gated as in D using intracellular cytokine staining). Dotted lines indicate MFI of naïve CD4 T cells. Each symbol indicates an individual mouse. Error bars indicate SEM. Data representative of two to three experiments with 3-5 mice per group. Statistical tests: Student's *t*-test (A-C); Mann–Whitney *U* test (F); ANOVA (G-I). **P*<0.05, ***P*<0.01, ****P*<0.001, *****P*<0.0001, and ns is not significant.

Fig. S9

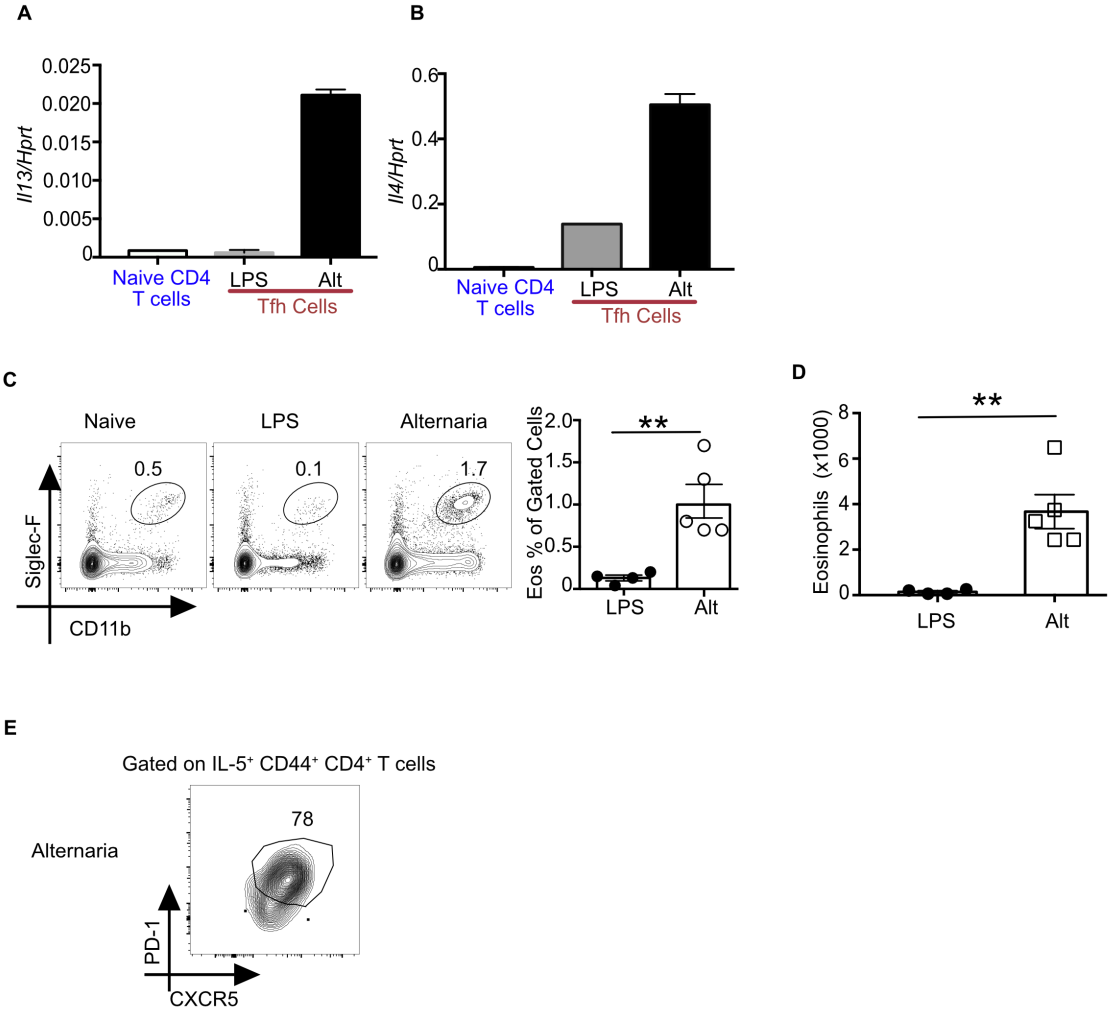

A
Il13/Hprt
0.025
0.020
0.015
0.010
0.005
0
Naive CD4 T cells
LPS
Alt
Tfh Cells
B
Il4/Hprt
0.6
0.4
0.2
0
Naive CD4 T cells
LPS
Alt
Tfh Cells
C
Naive
LPS
Alternaria
0.5
0.1
1.7
Siglec-F
CD11b
Eos % of Gated Cells
2.0
1.5
1.0
0.5
0
**
LPS
Alt
D
Eosinophils (x1000)
8
6
4
2
0
**
LPS
Alt
E
Gated on IL-5+ CD44+ CD4+ T cells
Alternaria
78
PD-1
CXCR5

Fig. S9. Association of Tfh13 cells and LN Eosinophils

Day 7 Tfh cells were sorted from WT C57BL/6 mice (n=4) immunized with Alt+OVA or LPS+OVA. Cells from each immunization were pooled to obtain two biological replicates for each immunization group, and qPCR was performed with technical duplicates for (A) *Il13* and (B*) Il4* transcripts. (C) Frequency and (D) numbers of eosinophils gated on TCRβ$^-$ B220$^-$ MHC-II$^-$ CD11c$^-$ cells in the MedLN at day 8 post immunization with LPS or *Alternaria* and NP-OVA depicted as flow cytometry plots (left) and summary bar graph. (E) Representative flow cytometry plot for PD-1 and CXCR5 expression on IL-5$^+$ CD4 T cell post Alt+OVA immunization. Error bars indicate SEM. Data representative of two experiments with 4-5 mice per group. Statistical tests: Student's *t*-test (C-D). $^{**}P<0.01$.

Fig. S10

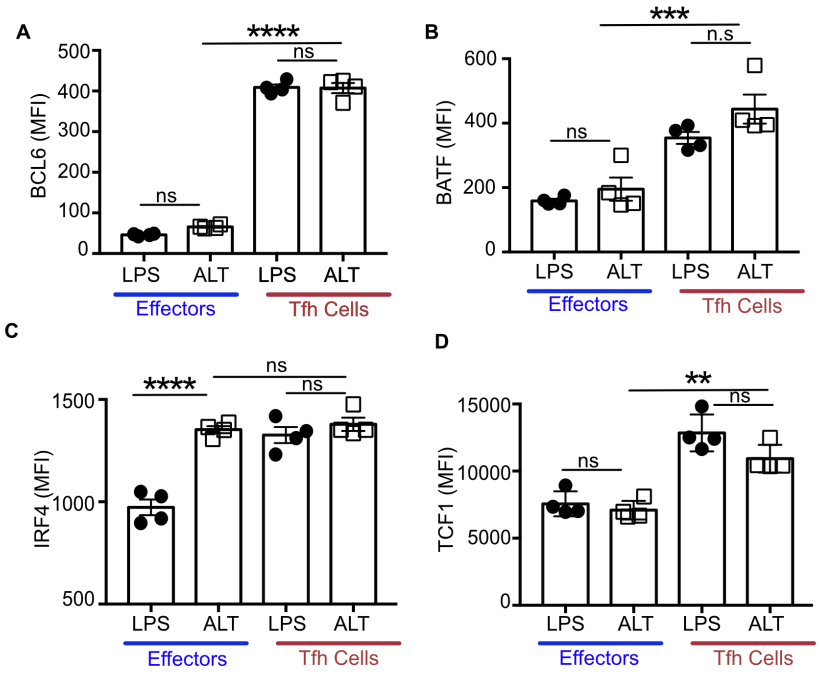

A
BCL6 (MFI)
500
400
300
200
100
0
****
ns
ns
LPS
ALT
LPS
ALT
Effectors
Tfh Cells
B
BATF (MFI)
600
400
200
0
***
n.s
ns
LPS
ALT
LPS
ALT
Effectors
Tfh Cells
C
IRF4 (MFI)
1500
1000
500
****
ns
ns
LPS
ALT
LPS
ALT
Effectors
Tfh Cells
D
TCF1 (MFI)
15000
10000
5000
0
**
ns
ns
LPS
ALT
LPS
ALT
Effectors
Tfh Cells

Fig. S10. Transcription factors in Tfh cells induced to type 1 and 2 immunizations

WT C57BL/6 mice were immunized with Alt+OVA or LPS+OVA and day 7 Tfh cells (PD-1^{+} $CXCR5^{+}$) and effectors (PD-1^{lo} $CXCR5^{lo}$) were analyzed for (A) BCL6, (B) BATF, (C) IRF4, and (D) TCF1. Each symbol indicates an individual mouse. Error bars indicate SEM. ANOVA was performed for (A-D). Data representative of two experiments with 3-4 mice per group. *P<0.05, **P<0.01, ***P<0.001, ****P<0.0001, and ns is not significant.

Fig. S11

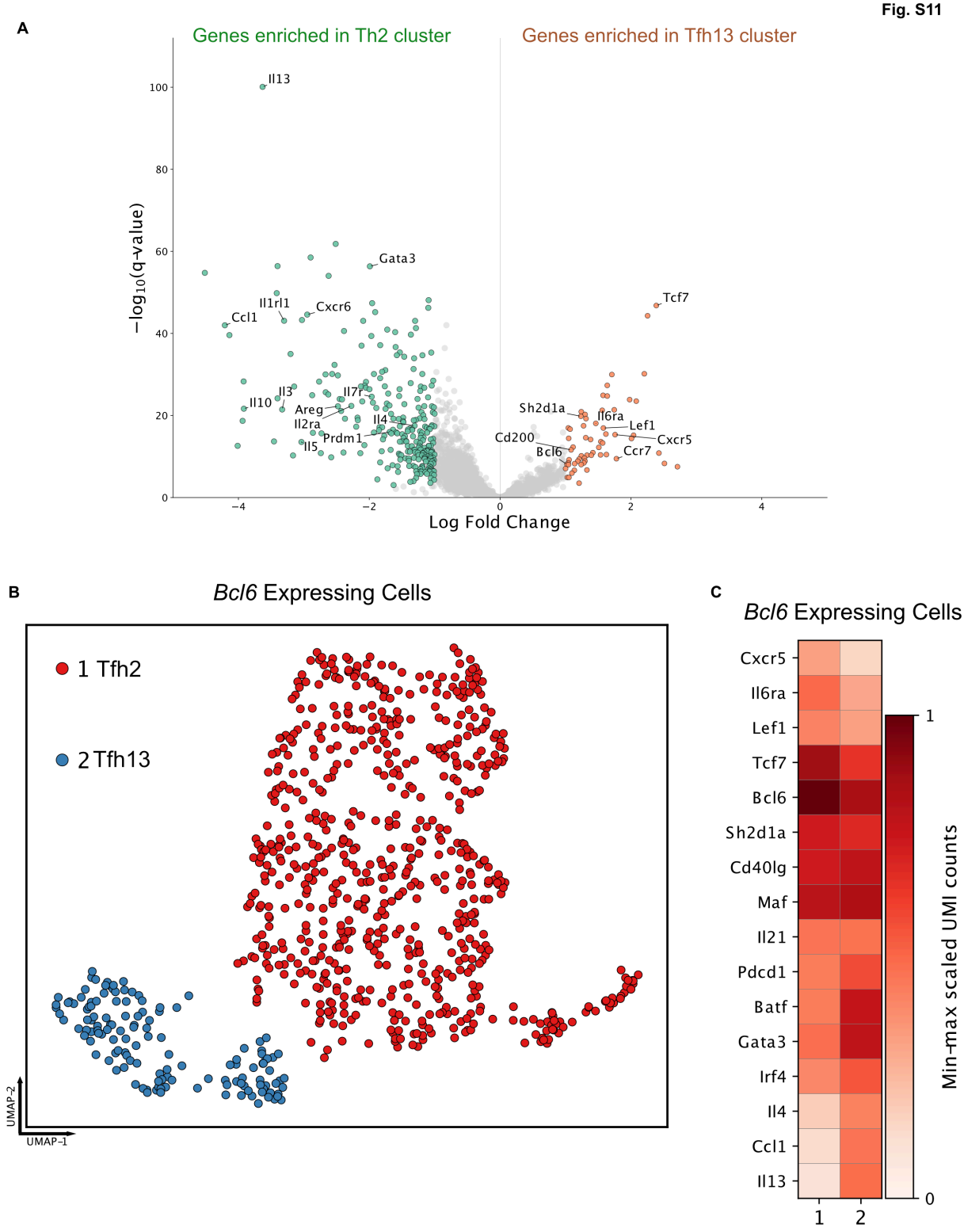

A
Genes enriched in Th2 cluster
Genes enriched in Tfh13 cluster
−log10(q-value)
Log Fold Change
Il13
Gata3
Il1rl1
Cxcr6
Ccl1
Il3
Il7r
Il10
Areg
Il2ra
Il4
Prdm1
Il5
Tcf7
Sh2d1a
Il6ra
Lef1
Cd200
Cxcr5
Bcl6
Ccr7
B
Bcl6 Expressing Cells
1 Tfh2
2 Tfh13
UMAP-2
UMAP-1
C
Bcl6 Expressing Cells
Cxcr5
Il6ra
Lef1
Tcf7
Bcl6
Sh2d1a
Cd40lg
Maf
Il21
Pdcd1
Batf
Gata3
Irf4
Il4
Ccl1
Il13
Min-max scaled UMI counts

Fig. S11. Tfh13 cells are a distinct T cell subset

4get/*Il4*-reporter mice were immunized intranasally (i.n.) with *Alternaria* extract and NP19-OVA. 4get$^+$ CD44$^+$ CD4 T cells were sorted and single-cell RNA sequencing analysis was performed. (A) Volcano plot showing differentially expressed genes (DEGs) between clusters 1 (Th2 cells, n=405) and 2 (Tfh13 cells, n=340) from the *Il13*$^+$ selected cells. The horizontal axis shows the $\log_2$ fold change in mean expression between the two clusters and the vertical axis shows the $\log_{10}$ adjusted p-value (Benjamini-Hochberg correction) given by a likelihood ratio test. Genes that are more highly expressed in cluster 2 have positive log fold change. Selected statistically significant DEGs for clusters 1 and 2 are labeled. (B-C) *Bcl6*-expressing cell analyses: cells expressing 1 or more transcripts of *Bcl6* were isolated and subjected to the dimensionality reduction and clustering strategy outlined in the Materials and Methods. (B) 2D UMAP embedding of the single-cell expression profiles of n=805 *Bcl6*$^+$ T cells. Low resolution (0.1) Leiden community detection on the cell-cell k=10 nearest neighbor graph segregates the cells into two general clusters; 1: Tfh2 cells (n=668); 2: Th13 cells (n=137). (C) A matrix plot showing the expression of key marker genes that are similarly and differentially expressed between Tfh2 and Th13 cells. The color scale represents the log-transformed normalized UMI counts that have been scaled between 0 and 1 separately for each gene.

Fig. S12

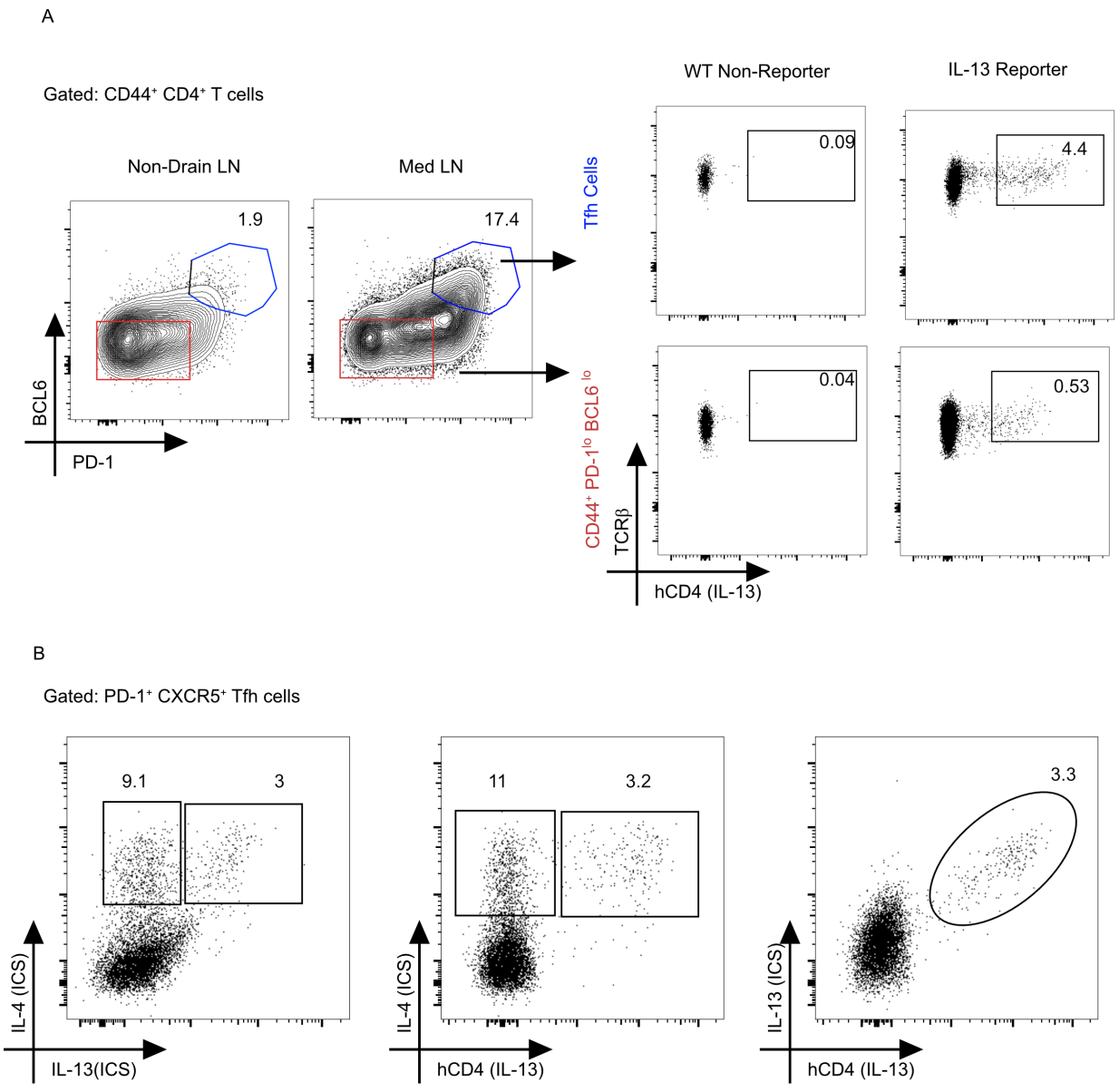

A
Gated: CD44⁺ CD4⁺ T cells
Non-Drain LN
Med LN
1.9
17.4
BCL6
PD-1
Tfh Cells
WT Non-Reporter
IL-13 Reporter
0.09
4.4
0.04
0.53
CD44⁺ PD-1ˡᵒ BCL6ˡᵒ
TCRβ
hCD4 (IL-13)
B
Gated: PD-1⁺ CXCR5⁺ Tfh cells
9.1
3
IL-4 (ICS)
IL-13(ICS)
11
3.2
IL-4 (ICS)
hCD4 (IL-13)
3.3
IL-13 (ICS)
hCD4 (IL-13)

Fig. S12. Smart13/IL-13 reporter mice validation

Smart13/IL-13 reporter mice were immunized with *Alternaria* extract and NP19-OVA. MedLN cells were stained for Tfh markers and human CD4 (hCD4) indicative of IL-13 expression. (A) IL-13 reporter expression in Tfh cells and effectors day 3 post boost. Non-reporter mice were used as controls for the stain. (B) Comparison of hCD4 expression on day 8 Tfh cells that make IL-13 protein as measured by intracellular staining (ICS).

Fig. S13

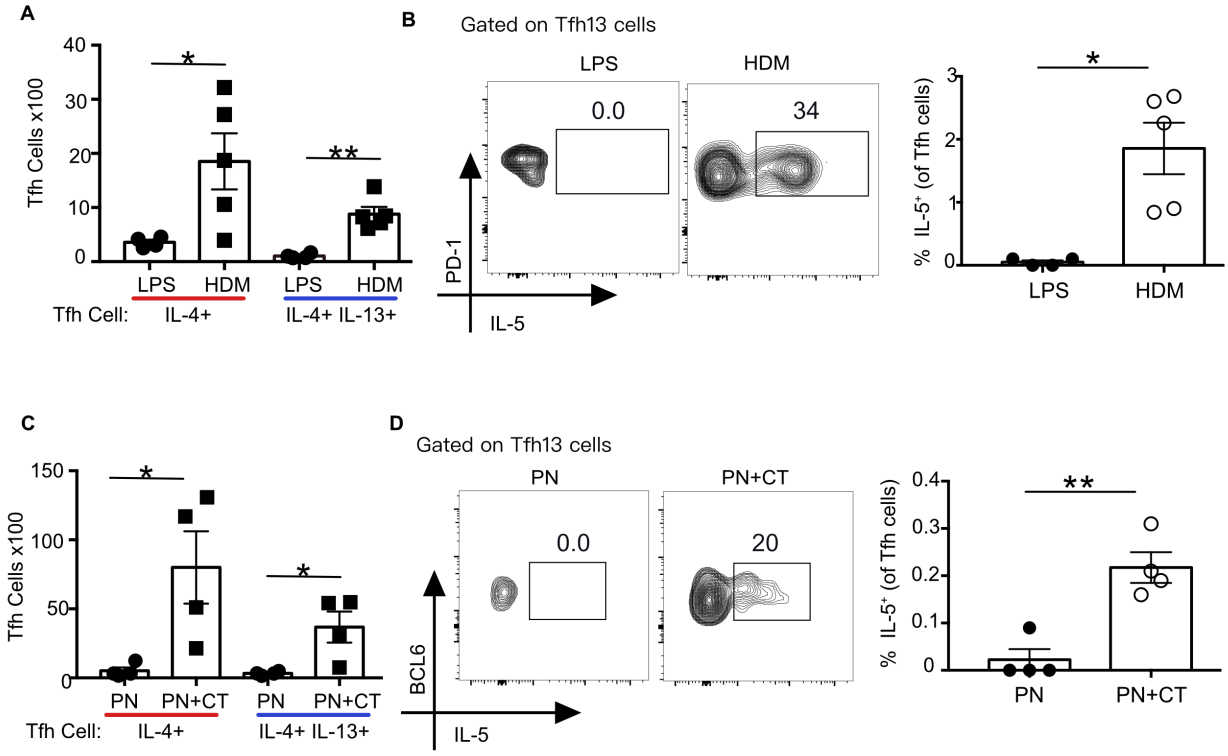

A
Tfh Cells x100
40
30
20
10
0
*
**
LPS
HDM
LPS
HDM
Tfh Cell:
IL-4+
IL-4+ IL-13+
B
Gated on Tfh13 cells
LPS
HDM
0.0
34
PD-1
IL-5
% IL-5+ (of Tfh cells)
3
2
1
0
*
LPS
HDM
C
Tfh Cells x100
150
100
50
0
*
*
PN
PN+CT
PN
PN+CT
Tfh Cell:
IL-4+
IL-4+ IL-13+
D
Gated on Tfh13 cells
PN
PN+CT
0.0
20
BCL6
IL-5
% IL-5+ (of Tfh cells)
0.4
0.3
0.2
0.1
0
**
PN
PN+CT

Fig. S13. Tfh13 cells are induced to multiple allergens

(A, B) WT C57BL/6 mice were immunized intranasally (i.n.) with LPS or HDM and NP19-OVA and intracellular expression of (A) IL-4 and IL-13 from day 8 MedLN Tfh cells induced after primary immunization is depicted as absolute counts. (B) IL-5 expression in Tfh13 cells. (C, D) WT C57BL/6 mice were intragastrically immunized with ground peanut (PN) ± cholera toxin. (C) IL-4 and IL-13 from day 8 MesLN Tfh cells induced after primary immunization is depicted as absolute counts. (D) IL-5 expression in Tfh13 cells. Each symbol indicates an individual mouse. Error bars indicate SEM. Statistical tests: Student's *t*-test (A, D); Mann–Whitney *U* test (B, C). **P*<0.05, ***P*<0.01. Data representative of at least two independent experiments with 4-5 mice per group.

Fig. S14

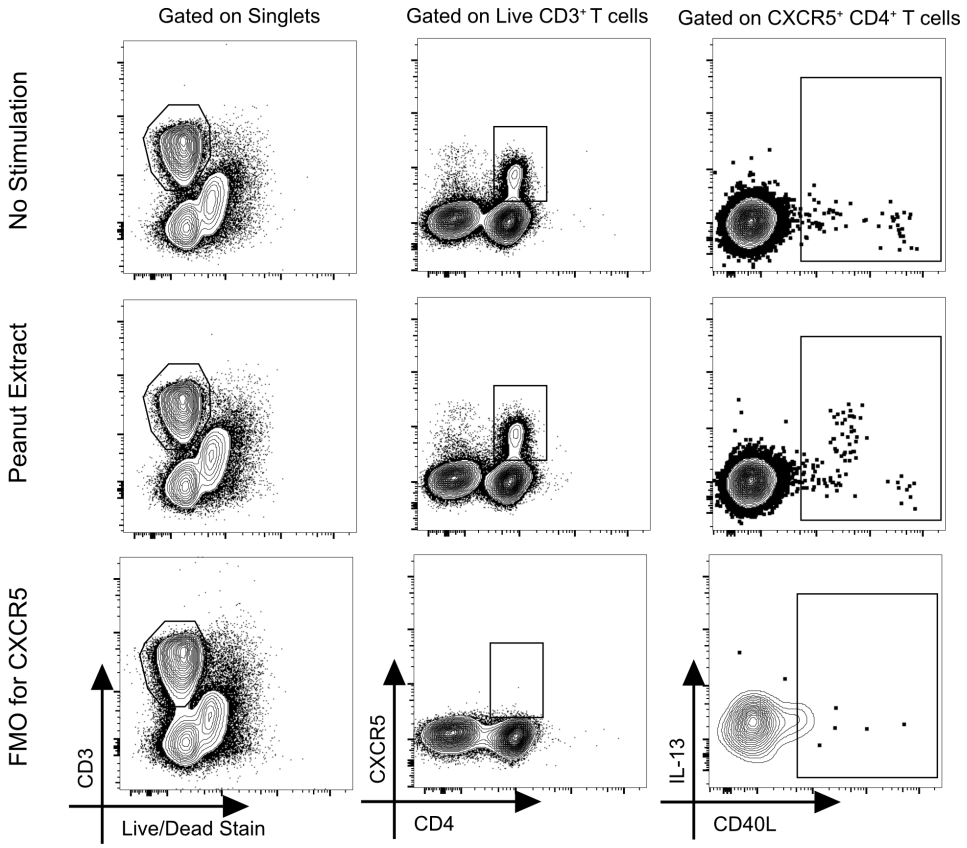
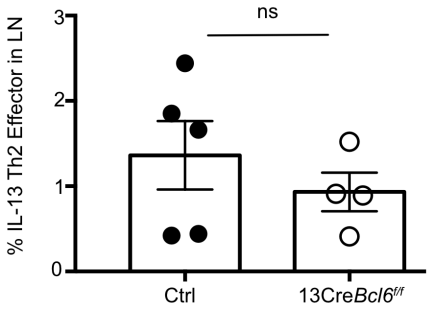

Gated on Singlets
Gated on Live CD3+ T cells
Gated on CXCR5+ CD4+ T cells
No Stimulation
Peanut Extract
FMO for CXCR5
CD3
Live/Dead Stain
CXCR5
CD4
IL-13
CD40L

Fig. S14. Gating strategy for peanut-specific circulating Tfh (cTfh) cells

Representative flow plot of PBMCs from a peanut-allergic patient shown that were stimulated or not with crude peanut extract, and cTfh cells were gated as depicted. FMO, fluorescence minus one for CXCR5 demonstrates the specificity of CXCR5 staining and identification of cTfh cells.

Fig. S15

A


ns
% IL-13 Th2 Effector in LN
3
2
1
0
Ctrl
13Cre*Bcl6*$^{f/f}$

B

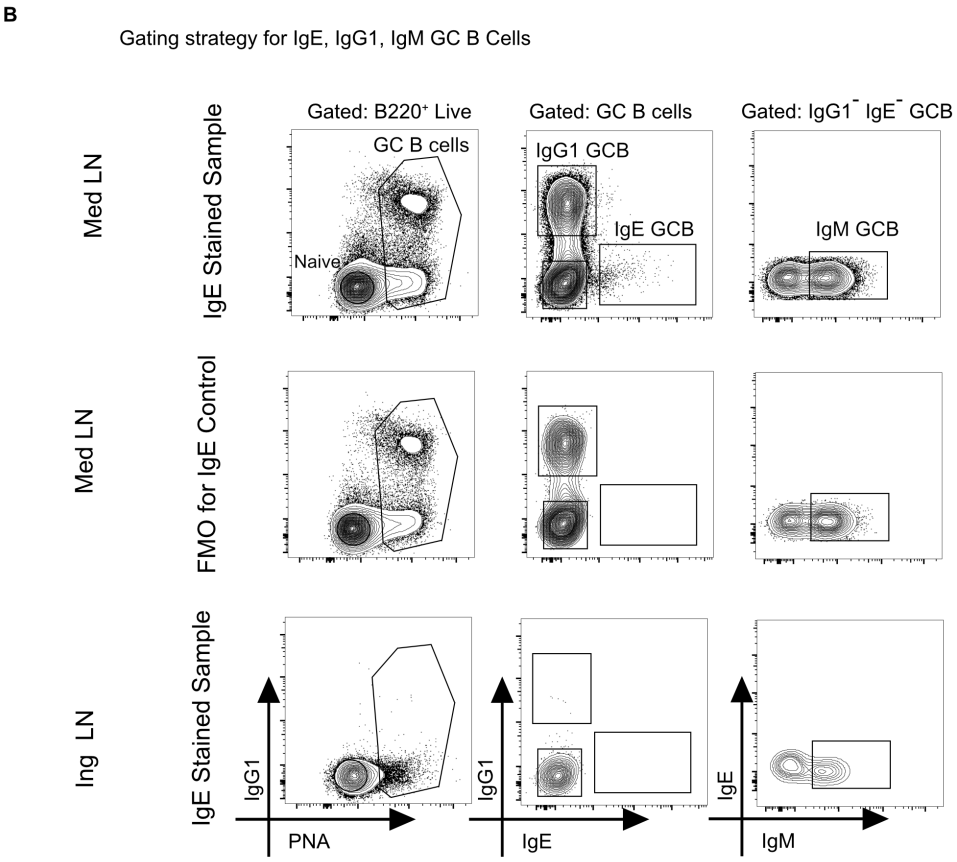

Gating strategy for IgE, IgG1, IgM GC B Cells
Med LN
IgE Stained Sample
Gated: B220^{+} Live
Gated: GC B cells
Gated: IgG1^{-} IgE^{-} GCB
GC B cells
Naive
IgG1 GCB
IgE GCB
IgM GCB
Med LN
FMO for IgE Control
Ing LN
IgE Stained Sample
IgG1
PNA
IgG1
IgE
IgE
IgM

Fig. S15. Loss of Tfh13 cells abrogates production of high-affinity IgE

(A) Frequencies of Th2 (PD-1^{lo} CXCR5^{lo} IL-13^{+} CD4 T cells) from 13Cre*Bcl6*$^{fl/fl}$ or control *Bcl6*$^{fl/fl}$ mice that were immunized with ALT+OVA on day 8 post immunization. Data representative of two experiments with 3-5 mice per group. Statistical tests: Student's *t*-test**.** (B) WT C57BL/6 mice were immunized intranasally (i.n.) with Alt+OVA and day 9 MedLN cells were stained for IgE, IgG1 GC B cells. Non-draining inguinal LN shown to demonstrate an unimmunized site.

Fig. S16

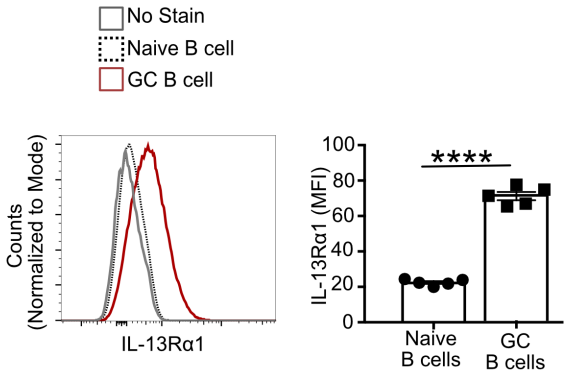

No Stain
Naive B cell
GC B cell
Counts
(Normalized to Mode)
IL-13Rα1
IL-13Rα1 (MFI)
100
80
60
40
20
0
****
Naive
B cells
GC
B cells

Fig. S16. GC B cells express IL-13Rα1

WT C57BL/6 mice were immunized intranasally (i.n.) with Alt+OVA. Expression of IL-13Rα1 on MedLN B cells at day 8 post immunization with is shown as histogram overlay (left) and as summary bar graphs (right). Data representative of two independent experiments with 3-5 mice. Statistical test: Student's *t*-test. ****$P<0.0001$

Supplementary Tables

Table S1: 10X Genomics scRNA-seq sequencing metrics

Run	10X Chemistry	Number of Reads	Cells Captured	Median UMI Counts per Cell	Mean Reads per Cell	Median Genes per Cell	Total Genes Detected	Valid Barcodes	Sequencing Saturation	Fraction Reads in Cells
Tfh Sort	V2	158,232,094	3,956	2,708	39,998	1,180	14,966	96.80%	79.30%	74.90%
IL-4+ Sort	V3	281,768,394	7,674	3,961	36,717	1,514	17,743	97.00%	57.60%	69.40%

Table S2: 10X Genomics scRNA-seq mapping metrics

	% Q30 Bases in				% Reads Mapped to			% Reads Mapped Confidently to			
Run	Barcode	RNA Read	Sample Index	UMI	Genome	Antisense Gene	Genome	Intergenic Regions	Intronic Regions	Exonic Regions	Transcriptome
Tfh Sort	98.10	81.40	97.40	98.30	94.70	1.60	90.10	2.70	17.90	69.60	66.30
IL-4+ Sort	93.20	91.50	87.40	93.00	94.10	1.70	90.00	5.30	18.80	65.90	62.60

Table S3: Single Cell exclusion criteria

				Cells excluded by				
Run	Total Cells	Cells passing QC	T-Cells recovered	HTO multiplet[a]	UMI count	Gene count	mtRNA content	Hemoglobin UMI count
Tfh Sort	3,956	3,811	3,002	126	8	0	11	1
IL-4+ Sort	7,674	4,096	3,863	1,059	66	1,770	660	23

[a]HTO multiplets denote cells classified as either belonging to multiple HTOs or those with too few tags to be confidently classified.

Table S4: Clinical Characteristics of Aeroallergen-sensitized Patients (for Figure 4)			
	Allergic N=13	Control N=16	P value
Age-Years mean (SD)	10 (2.6)	11.2 (2.6)	0.23
Sex Female N (%)	5 (38.5)	7 (44)	0.77
Total IgE (kU/L) mean (SD)	610 (631)	62 (77)	<0.05
Total eosinophils (cells/uL) mean (SD)	337 (387)	119.4 (92.5)	<0.05
Allergen N (%)*			
Dermatophagoides farina	6 (46)		
Dermatophagoides pteronyssis	6 (46)		
Dog	6 (46)		
Cat	6 (46)		
Cockroach	3 (23)		
Mouse	2 (15)		
Rat	2 (15)		
Mugwort	1 (8)		
Alternaria alternata	2 (15)		
Cladosporium herbarum	0 (0)		

*Percentages add to greater than 100% as some individuals were allergic to more than one allergen.

Table S5: Antibody Clones and Dilutions

All targets are mouse proteins except where indicated.

Target (Clone)	Species	Fluorochrome/conjugate	Dilution	Source
TCRβ (H57-597)	Armenian Hamster IgG	Percp/cy5.5 PE/Cy7	1:300 1:300	Biolegend
CD4 (GK1.5) (RM4-5)	Rat IgG_{2b}, Rat IgG_{2a}	Percp/cy5.5 PE/Cy7 APC-Fire™750 PE-CF594	1:400 1:400 1:400 1:400	Biolegend BD Bioscience
Human CD4 (RPA-T4)	Mouse IgG_1	AF647 BV605 APC-Cy7	5ul/test 5ul/test 3:100	Biolegend Tonbo Biosciences
CD44 (IM7)	Rat IgG_{2b}	FITC BV605	1:600 1:600	Biolegend
CXCR5 (L138D7) (2G8)	Rat IgG_{2b}, Rat IgG_{2a}	Biotin Biotin	1:200 1:150	Biolegend, BD Bioscience
PD-1 (29F.1A12) (RMP1-30)	Rat IgG_{2a}, Rat IgG_{2b}	APC-Fire™750 BV421 PE	1:200 1:200 1:200	Biolegend
IL-4 (11B11)	Rat IgG_1	PE APC	1:100 1:100	Biolegend
IL-5 (TRFK5)	Rat IgG_1	APC	1:150	Biolegend
IL-13 (eBio13A)	Rat / IgG_1	PE/Cy7 FITC	1:100 1:100	eBioscience
B220 (RA3-6B2)	Rat IgG_{2a}	Percp/cy5.5 APC-Fire™750	1:400	Biolegend
CD19 (6D5)	Rat IgG_{2a}	PE/Cy7 FITC Percp/cy5.5	1:400	Biolegend
GL7	Rat IgM	FITC Percp/cy5.5	1:600	Biolegend

CD95 (Jo2)	Armenian Hamster IgG$_2$	PE APC	1:300	BD Bioscience
CD23 (B3B4)	Rat IgG$_{2a}$	PE	1:400	Biolegend
CD21/35 (7E9)	Rat IgG$_{2a}$	APC	1:200	Biolegend
CD11c (N418)	Armenian Hamster IgG	Pacific Blue APC/Cy7	1:300	Biolegend
MHC II (M5/114.15.2)	Rat IgG$_{2b}$	BV510 BV412	1:800 1:800	Biolegend
CD11b (M1/70)	Rat IgG$_{2b}$	PE/Cy7	1:500	Biolegend
Va2 (B20.1)	Rat IgG$_{2a}$	PB PE	1:400 1:400	Biolegend
CD103 (2E7)	Armenian Hamster IgG	PE	1:300	Biolegend
CD24 (M1/69)	Rat IgG$_{2b}$	PeCy7	1:400	Biolegend
CD45.1 (A20)	Mouse IgG$_{2a}$	PE, APC	1:200	Biolegend
CD45.2 (104)	Mouse (SJL) IgG$_{2a}$	PE, APC, PB	1:200	Biolegend
Siglec-F (E50-2440)	Rat IgG$_{2a}$	PE	1:300	BD Bioscience
GR-1 (RB6-8C5)	Rat IgG$_{2b}$	APC	1:000	Biolegend
ICOS (15F9) (C398.4A)	Syriyan Hamster IgG ArmenianHamster IgG	PE FITC	1:200 1:200	Biolegend BD Bioscience
GATA-3 (L50-823) (TWAJ)	 Mouse IgG$_1$ Rat IgG$_{2b}$	 Pacific Blue PeCy7	 1:50 1:30	 BD Bioscience eBioscience
Foxp3 (FJK-16s)	Rat IgG$_{2a}$	Pacific Blue	1:100	eBioscience
IRF4 (IRF4.3E4)	Rat IgG$_1$	PE	1:300	Biolegend
BCL-6 (K112-91)	Mouse IgG$_1$	FITC AF647	1:50 1:50	BD Biosciences
TCF-1(C63D9)	Rabbit IgG	Purified	1:200	Cell Signaling
BATF (S39-1060)	Mouse IgG$_1$	PE	1:100	BD Biosciences
ICOS (15F9) (C398.4A)	Syriyan Hamster IgG ArmenianHamster IgG	PE FITC	1:200 1:200	Biolegend BD Bioscience
IgD (11-26c.2a)	Rat IgG$_{2a}$	AF647	1:400	Biolegend

		Percp/cy5.5 AF488	1:300 1:300	
IgG1 (A85-1)	Rat IgG_1	V450	1:500	BD Bioscience
IgE (RME-1)	Rat IgG_1	FITC Purified	1:400 1:30	Biolegend
CD16/CD32 (BD Fc Block™)	Rat IgG_{2b}	Purified	1:50	BD Bioscience
CD138 (281-2)	Rat IgG_{2a}	PeCy7	1:300	Biolegend
IL-13Rα (13MOKA)	Rat IgG_{2a}	PE	1:100	Biolegend
Peanut Agglutinin		Biotin	1:800	Vector Labs
Viability dye aqua		510nm (Amcyan channel)	1:1000	Life Technologies
Human CD3 (UCHT1)	Mouse IgG_1	PerCP-Cy5.5	4.25:100	Tonbo Biosciences
Human CD45RA (HI100)	Mouse IgG_{2b}	V450	2.75:100	Tonbo Biosciences
Human CXCR5 (RF8B2)	Rat IgG_{2b}	BB515	5:100	BD Bioscience
Human ICOS (DX29)	Mouse IgG_1	BV711	2.4:100	BD Bioscience
Human PD-1 (EH12.2H7)	Mouse IgG_1	BV605	2:100	Biolegend
Human IL-4 (MP4-25D2)	Rat IgG_1	PE-Cy7	5:100	Biolegend
Human IL-13 (JES10-5A2)	Rat IgG_1	APC	5:100	Biolegend
Human IFNγ (4S.B3)	Mouse IgG_1	AF700	2:100	Biolegend
IgE (R35-72) Elisa:capture	Rat IgG_1	Purified	1:250	BD Bioscience
IgE Elisa:detection	Goat anti-mouse IgE	HRP	1:4000	Southern Biotech
IgG1 Elisa:detection	Goat anti-mouse IgG1	HRP	1:6000	Southern Biotech
IgG2c Elisa:detection	Goat anti-mouse IgG2c	HRP	1:5000	Southern Biotech
DOCK8	Rabbit IgG	Purified	1:1000	Clontech/Takara

Rabbit IgG	Goat anti-Rabbit IgG (H+L)	HRP	1:5000	Invitrogen