Supplementary Information

FFAR2/3 dysfunction aggravates IL-33-induced eosinophilic airway inflammation via $ST2^+CD4^+$ T cell activation

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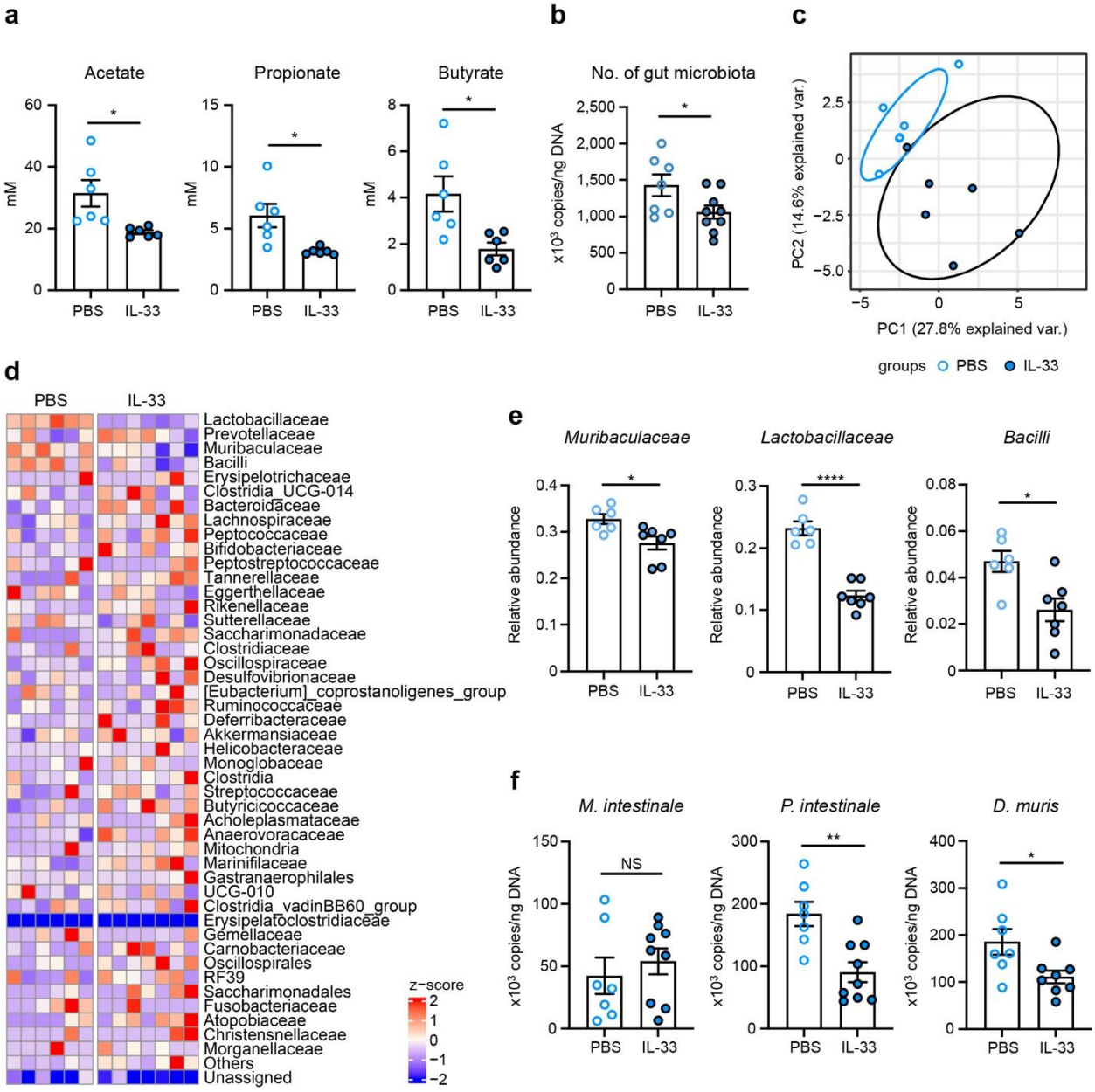
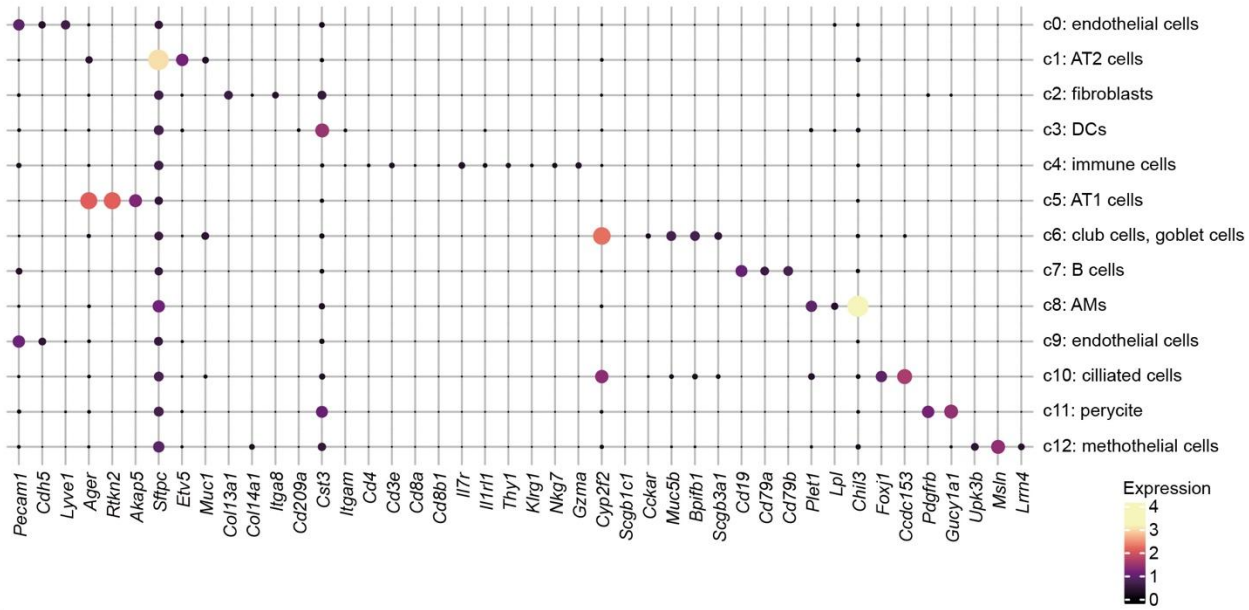


Supplementary Fig. 1. Fecal short-chain fatty acid concentration and gut microbiome analysis of IL-33-induced eosinophilic airway inflammation model. a Concentration of short-chain fatty acids in feces. **b** Total bacterial load. **c** Principal coordinates analysis. **d** Heatmap showing the gut microbial composition at the family level. **e** The abundance of *Muribaculaceae*, *Lactobacillaceae*, and *Bacilli* in the fecal samples. **f** The abundances of *Muribaculaceae* species.

Mean ± s.e.m., n = 6–9 mice per group. *P < 0.05, **P < 0.01, ****P < 0.0001 (Student's t-test).

a

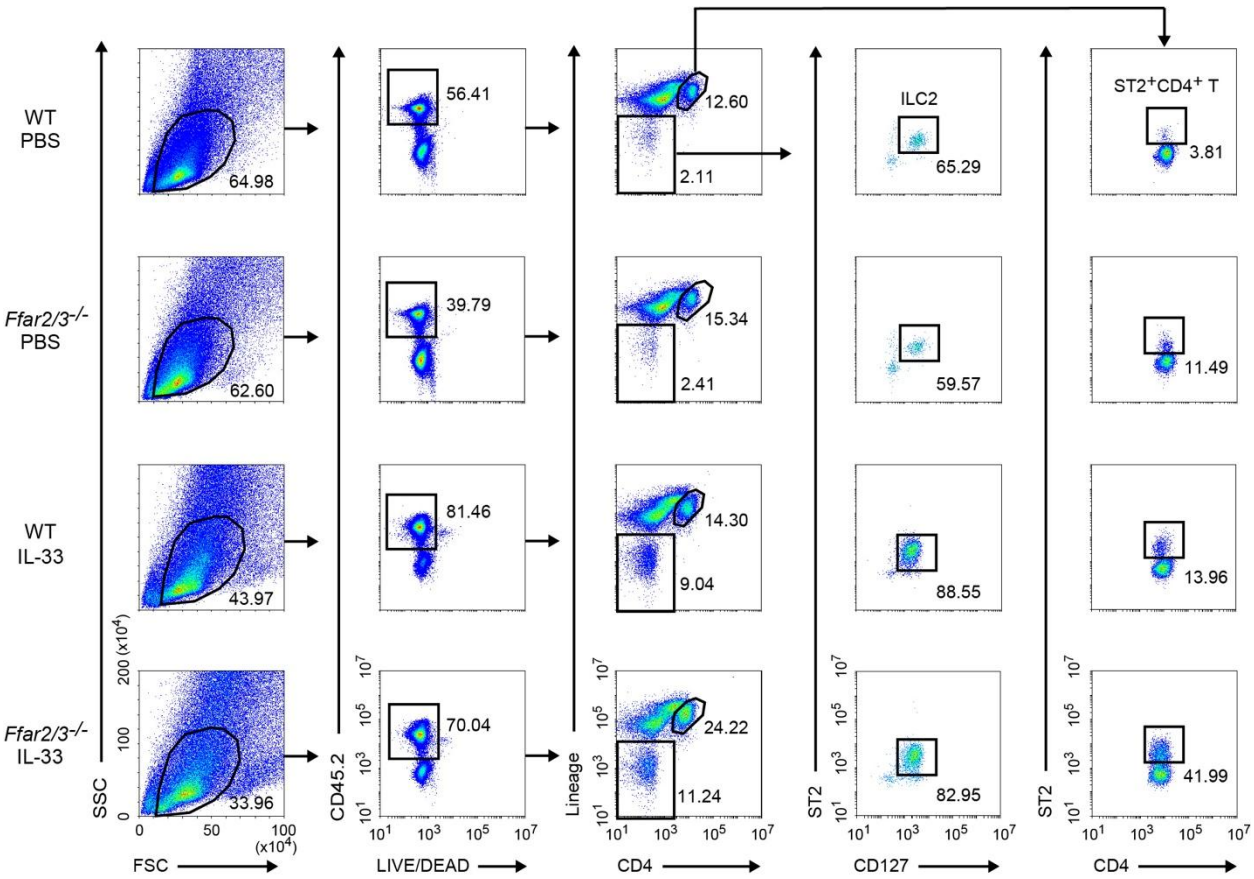


b

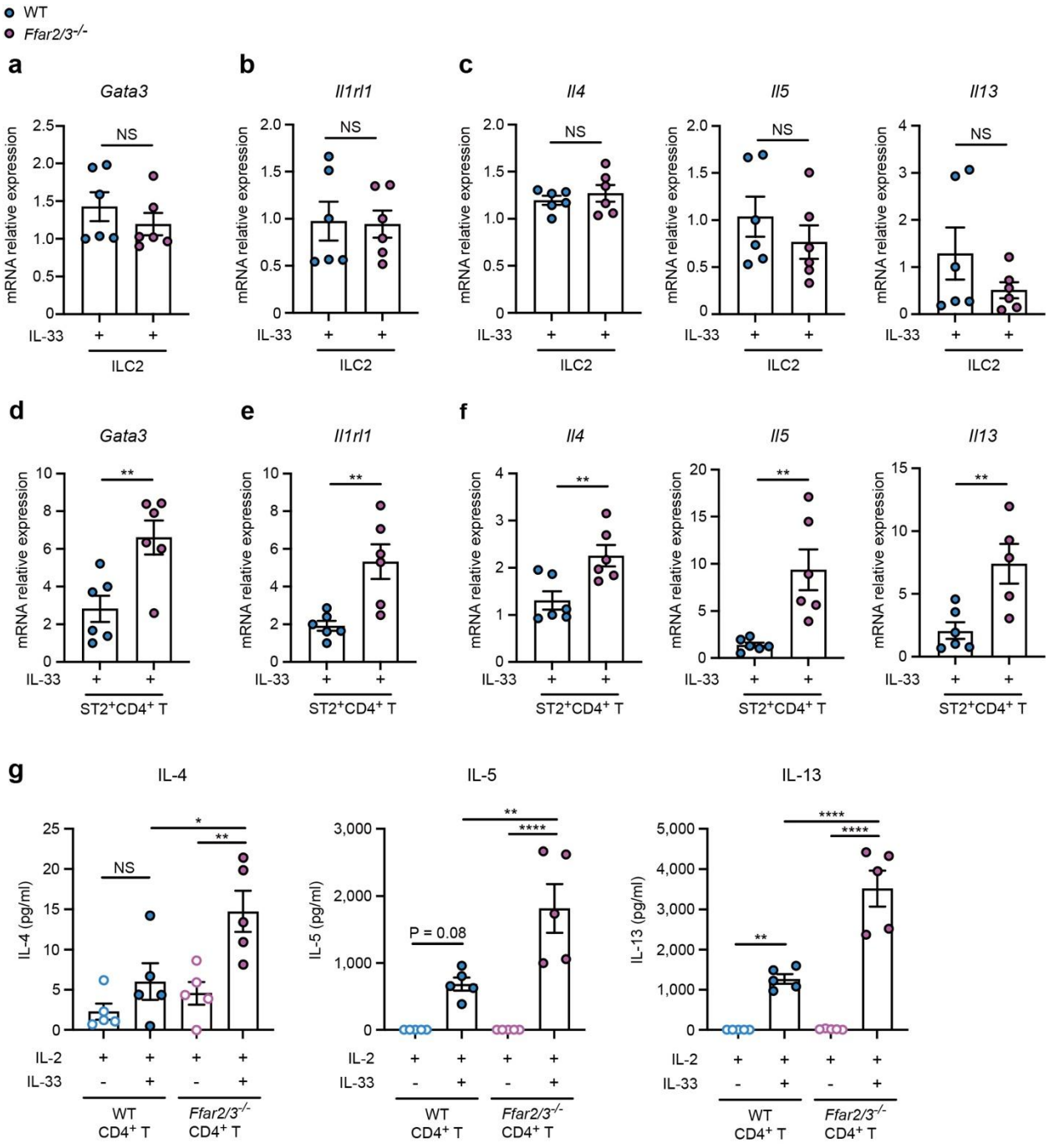
	Subcluster 0	Subcluster 1	Subcluster 2	Subcluster 3	Subcluster 4	Subcluster 5	Subcluster 6	Subcluster 7	Subcluster 8
	Naive T cells	ILC2s	$CD4^+$ T cells	NK cells	B cells	Proliferated cells	γδt T cells	Endothelial cells	Plasma cells
marker1	*Dgka*	*Stab2*	*Cxcr3*	*Gzma*	*Ncf1*	*Ankle1*	*Rorc*	*Arhgef15*	*Jchain*
marker2	*Gramd3*	*Il17rb*	*Ctla4*	*Adamts14*	*Siglecg*	*Rrm2*	*Blk*	*Myzap*	*Eaf2*
marker3	*Sell*	*Il1rl1*	*Zfp683*	*Klre1*	*Ms4a1*	*Top2a*	*Il23r*	*Cyyr1*	*Derl3*
marker4	*Lef1*	*Arg1*	*Zfp36l2*	*Ccl5*	*Pirb*	*Ncapg*	*Cd163l1*	*Shank3*	*Cacna1h*
marker5	*Satb1*	*Hlf*	*Slamf1*	*Nkg7*	*Cd19*	*Kifc1*	*Il1r1*	*Klf4*	*Igkc*
marker6	*Actn1*	*Grin2d*	*Cd4*	*Tyrobp*	*Tbxas1*	*Pimreg*	*Lingo4*	*Palmd*	*Pou2af1*
marker7	*Klf2*	*Slc7a8*	*Tmem154*	*Klrk1*	*Cd74*	*Ska1*	*Zbtb16*	*Sema3g*	*Irf4*
marker8	*Ccr7*	*Gata3*	*P2rx7*	*Ncr1*	*Cd79b*	*Ncapd2*	*Kcnk1*	*Smad6*	*Mzb1*
marker9	*Pik3ip1*	*Adam8*	*Atp2b4*	*Klrd1*	*Ebf1*	*Hist1h1b*	*Il17re*	*Efnb2*	*Iglc2*
marker10	*Evl*	*Znrf1*	*Trac*	*Cd244a*	*Unc93b1*	*Mxd3*	*Kcnc1*	*Ushbp1*	*Igha*
marker11	*Als2cl*	*Pcsk1*	*Maf*	*Klra8*	*Rassf4*	*Spag5*	*Adgrg5*	*Ecscr*	*Dennd5b*
marker12	*Tdrp*	*Rasgrp4*	*Lck*	*Aoah*	*Ppfibp2*	*Anln*	*Igf1r*	*Meis2*	*Iglc1*
marker13	*Nsg2*	*Aff4*	*Hnrnpll*	*Prf1*	*H2-Eb1*	*Tpx2*	*Smox*	*Cldn5*	*Txndc5*
marker14	*Ikbke*	*Syne1*	*Fgl2*	*Samd3*	*H2-Ab1*	*Ncapg2*	*Lmo4*	*Ccm2l*	*Rhobtb1*
marker15	*Slamf6*	*Ern1*	*Sntb1*	*Gsap*	*Sftpd*	*Ndc80*	*Il18r1*	*Cd36*	*Tnfrsf13b*
marker16	*Gimap4*	*Ccr8*	*Foxp3*	*Zeb2*	*Snhg11*	*Clspn*	*8-Sep*	*Ace*	*Tnfrsf17*
marker17	*Atp1b3*	*Il5*	*Cd3e*	*Itgam*	*Ptprj*	*Hmmr*	*Trdc*	*Sema3c*	*Prg2*
marker18	*Tcf7*	*Ccr2*	*Crybg2*	*Gzmb*	*Rilp*	*Dlgap5*	*Sox13*	*Egfl7*	*Txndc11*
marker19	*Usp28*	*Itm2b*	*Il21r*	*Klrb1f*	*Map3k9*	*Kif23*	*Capg*	*Eng*	*Ckap4*
marker20	*Rflnb*	*Furin*	*Il12rb1*	*Fcer1g*	*Il33*	*Mki67*	*Mmp25*	*Tie1*	*Ell2*

Supplementary Fig. 2. Single-cell RNA-seq analysis of the lung tissue from IL-33-treated wild-type and *Ffar2/Ffar3*-deficient mice. a Cell-type annotation of each cluster in single-cell

RNA-seq analysis based on gene expression pattern. **b** The list of top 20 genes in each subcluster population within Cluster 4 (immune cells).

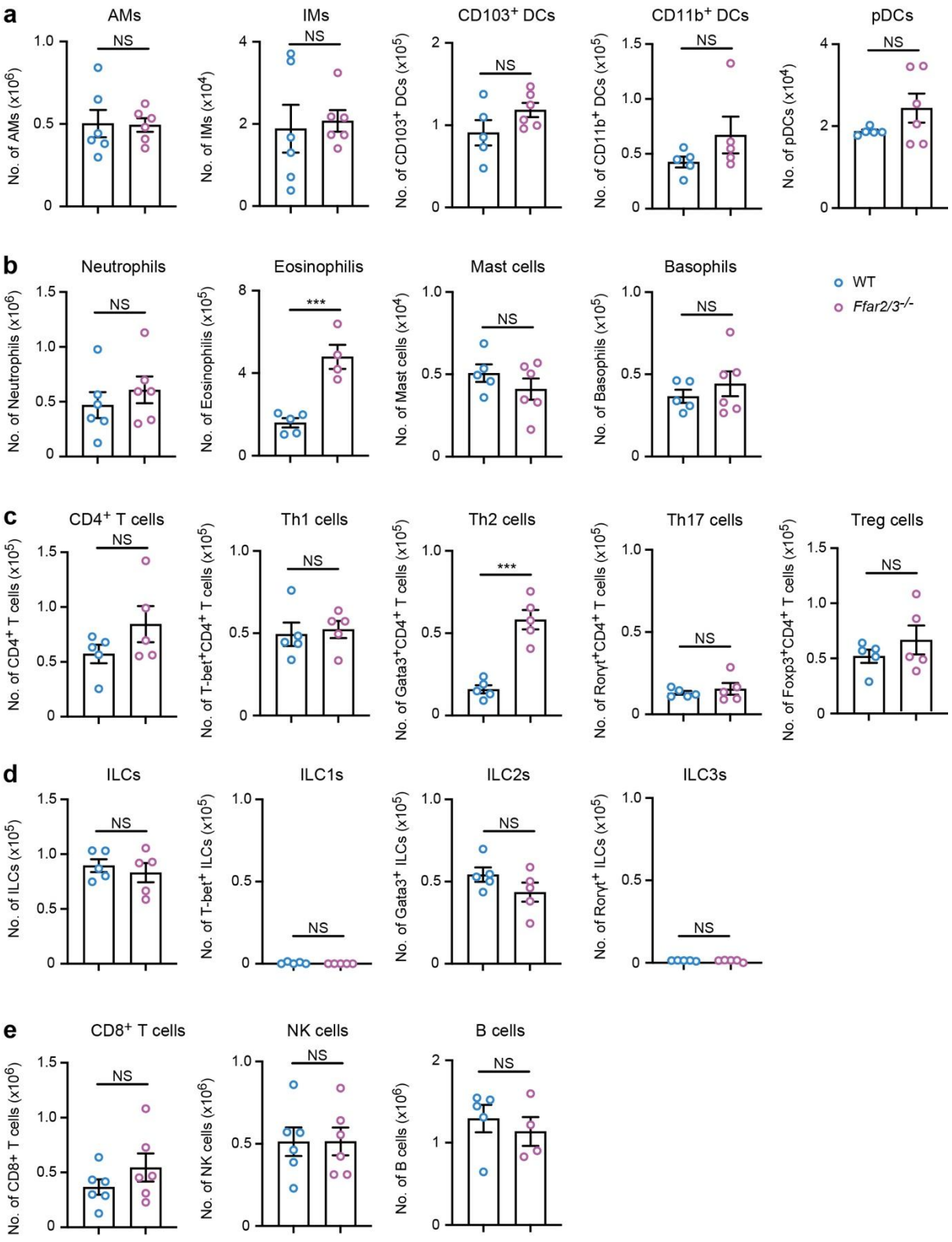


Supplementary Fig. 3. Gating strategy for flow cytometry. Flow cytometry analysis of lung ILC2s and $ST2^{+}CD4^{+}$ T cells in wild-type and *Ffar2/Ffar3*-deficient mice treated with PBS-treated or IL-33-treated condition.

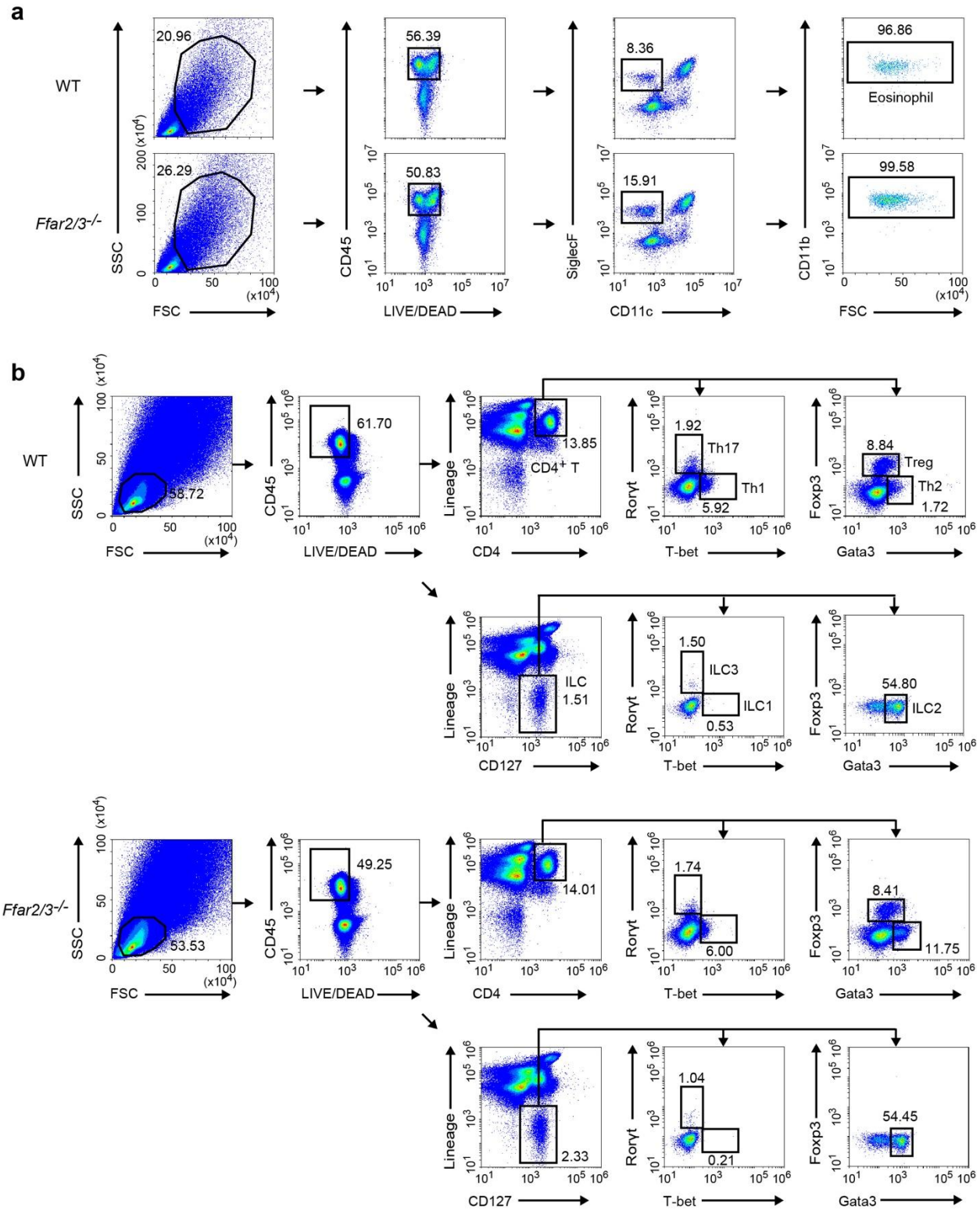


Supplementary Fig. 4. Comparison of gene expression in ILC2s and ST2$^+$CD4$^+$ T cells isolated from lung tissue of IL-33-treated mice and cytokine production in CD4$^+$ T cells derived from spleen under IL-33 stimulation. The mRNA expression levels of *Gata3* **(a, d)**, *Il1rl1* **(b, e)**, type 2 cytokines (*Il4*, *Il5*, and *Il13*) **(c, f)** in ILC2 **(a–c)** and ST2$^+$CD4$^+$ T cells **(d–f)**

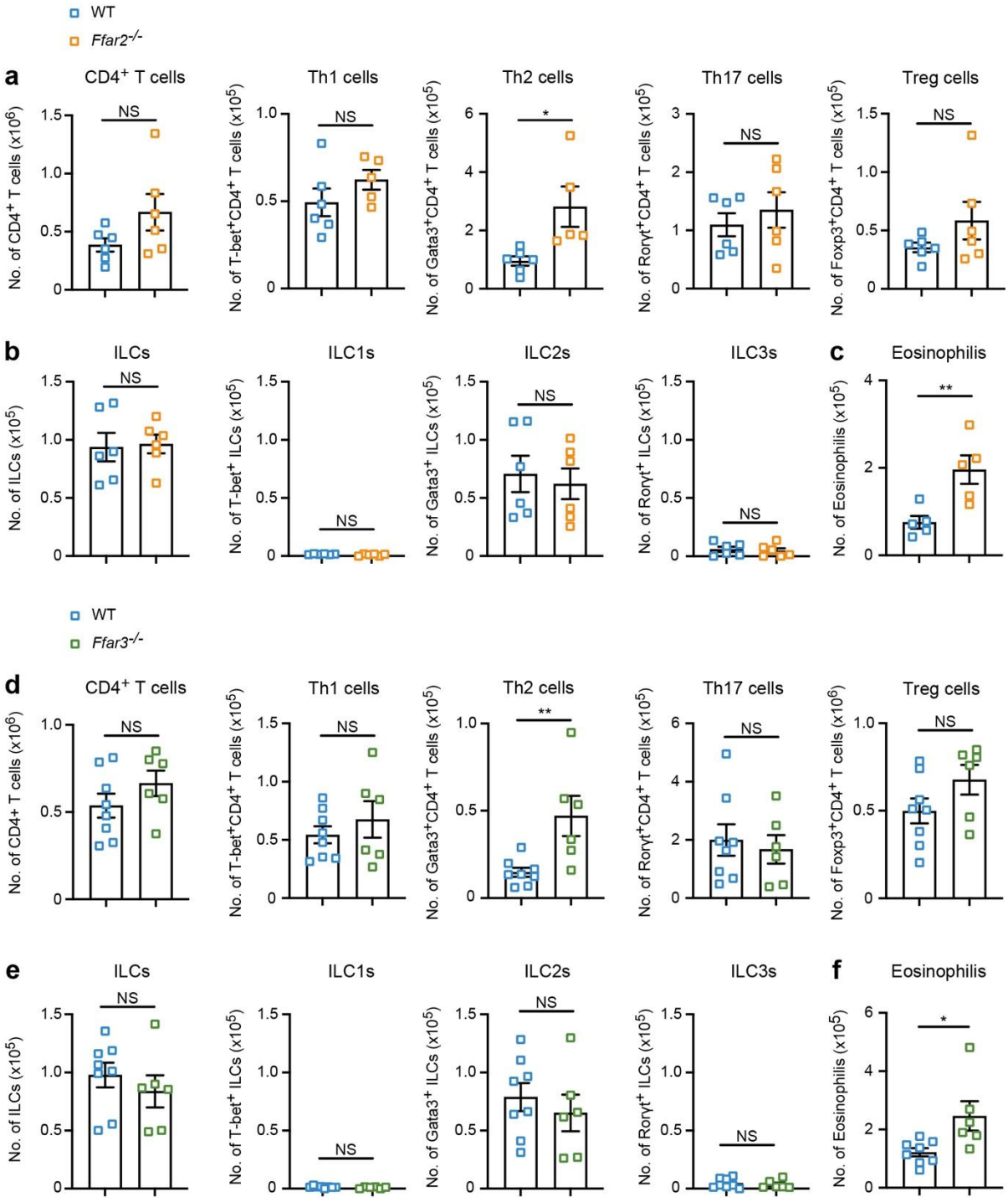
isolated from the lung tissue of wild-type and *Ffar2/Ffar3*-deficient mice in the IL-33–induced eosinophilic airway inflammation model. **g** $CD4^+$ T cells (2.0×10^5 cells/well) isolated from the spleens of wild-type and *Ffar2/Ffar3*-deficient mice were stimulated with IL-2 (10 ng/ml) and IL-33 (10 ng/ml) for 72 h. IL-4, IL-5, and IL-13 concentrations in the culture supernatants. Mean ± s.e.m., n = 5-6 mice per group. **P* < 0.05, ***P* < 0.01, *****P* < 0.0001 (Student's *t*-test [**a**–**f**], one-way analysis of variance (ANOVA) and Tukey's post hoc test [**g**]). NS: not significant.


a
AMs
IMs
CD103+ DCs
CD11b+ DCs
pDCs
No. of AMs (x10^6)
No. of IMs (x10^4)
No. of CD103+ DCs (x10^5)
No. of CD11b+ DCs (x10^5)
No. of pDCs (x10^4)
NS
b
Neutrophils
Eosinophilis
Mast cells
Basophils
No. of Neutrophils (x10^6)
No. of Eosinophilis (x10^5)
No. of Mast cells (x10^4)
No. of Basophils (x10^5)
***
WT
Ffar2/3-/-
c
CD4+ T cells
Th1 cells
Th2 cells
Th17 cells
Treg cells
No. of CD4+ T cells (x10^5)
No. of T-bet+CD4+ T cells (x10^5)
No. of Gata3+CD4+ T cells (x10^5)
No. of Rorγt+CD4+ T cells (x10^5)
No. of Foxp3+CD4+ T cells (x10^5)
d
ILCs
ILC1s
ILC2s
ILC3s
No. of ILCs (x10^5)
No. of T-bet+ ILCs (x10^5)
No. of Gata3+ ILCs (x10^5)
No. of Rorγt+ ILCs (x10^5)
e
CD8+ T cells
NK cells
B cells
No. of CD8+ T cells (x10^6)
No. of NK cells (x10^6)
No. of B cells (x10^6)

Supplementary Fig. 5. Comprehensive analysis of lung immune cell populations in a healthy state. Numbers of macrophage and dendritic cell subsets **(a)**, granulocyte subsets **(b)**, $CD4^+$ T cell subsets **(c)**, ILC subsets **(d)**, and other lymphoid cells **(e)** isolated from the lung tissue of wild-type and *Ffar2/Ffar3*-deficient mice. Mean ± s.e.m., n = 4–6 mice per group. ****P* < 0.001, (Student's *t*-test). NS: not significant. AMs: alveolar macrophages. IMs: Interstitial macrophages. DCs; dendritic cells. NK cells, natural killer cells.

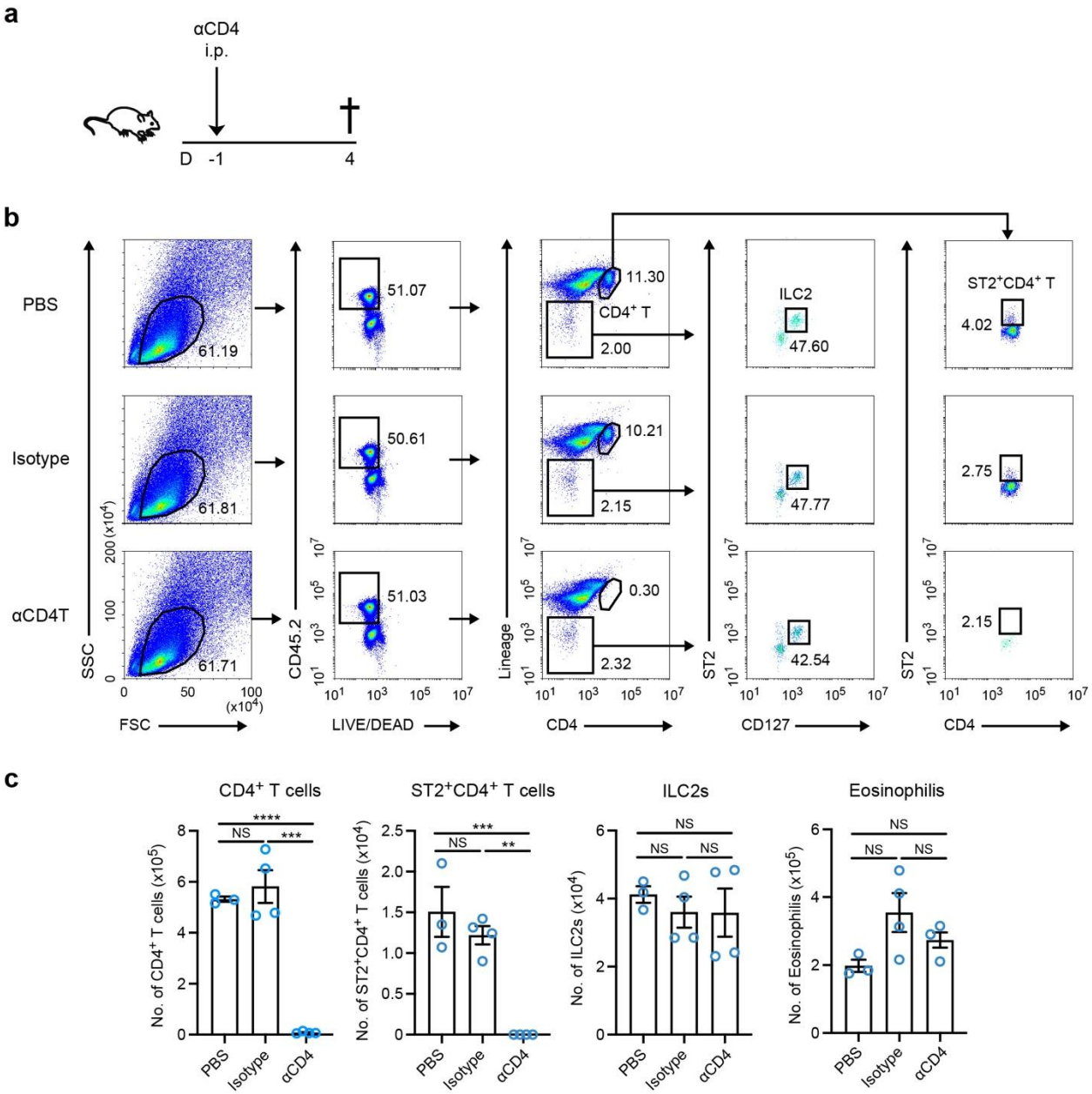

a
WT
Ffar2/3-/-
20.96
26.29
SSC
FSC
56.39
50.83
CD45
LIVE/DEAD
8.36
15.91
SiglecF
CD11c
96.86
Eosinophil
99.58
CD11b
FSC
b
WT
58.72
61.70
13.85
CD4+ T
1.92
Th17
Th1
5.92
8.84
Treg
Th2
1.72
Lineage
CD4
Roryt
T-bet
Foxp3
Gata3
ILC
1.51
1.50
ILC3
ILC1
0.53
54.80
ILC2
CD127
Ffar2/3-/-
53.53
49.25
14.01
1.74
6.00
8.41
11.75
1.04
0.21
2.33
54.45

Supplementary Fig. 6. Gating strategy for flow cytometry. Flow cytometric analysis of lung eosinophils **(a)**, $CD4^+$ T cell subsets, and ILC subsets **(b)** in wild-type and *Ffar2/Ffar3*-deficient mice in a healthy state.



Supplementary Fig. 7. Comparison of CD4$^+$ T cell subset, ILC subsets, and eosinophils of lung tissue of *Ffar2*-deficient and *Ffar3*-deficient mice in a healthy state. CD4$^+$ T cell subsets **(a, d)**, ILC subsets (**b**, **e**), and eosinophils (**c**, **f**) in wild-type, *Ffar2*-deficient **(a–c)**, and

Ffar3-deficient **(d–f)** mice in a healthy state. Mean ± s.e.m., n = 5–8 mice per group. **P* < 0.05, ***P* < 0.01 (Student's *t*-test). NS: not significant.



Supplementary Fig. 8. CD4$^+$ T cell depletion. a The experimental schemes of CD4$^+$ T cell depletion. Wild-type mice were intraperitoneally administered PBS or Isotype control (100 μg/mouse) or anti-CD4 antibodies (100 μg/mouse) once (day -1) and evaluated on day 4. **b** Flow cytometric analysis of lung ILC2s and ST2$^+$CD4$^+$ T cells. **c** The number of CD4$^+$ T cells, ST2$^+$CD4$^+$ T cells, ILC2s, and Eosinophils. Mean ± s.e.m., n = 3–4 mice per group. $^{**}P < 0.01$,

***$P < 0.001$, ****$P < 0.0001$ (one-way analysis of variance (ANOVA) and Tukey's post hoc test). NS; not significant.

Supplementary Table 1. Antibodies and reagents

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Antibodies		
Biotin-conjugated anti-CD3 (17A2)	BioLegend	Cat# 100244
Biotin-conjugated anti-CD19 (6D5)	BioLegend	Cat# 115503
Biotin-conjugated anti-B220 (RA3-6B2)	BioLegend	Cat# 103204
Biotin-conjugated anti-CD11c (N418)	BioLegend	Cat# 117304
Biotin-conjugated anti-CD11b (M1/70)	BioLegend	Cat# 101204
Biotin-conjugated anti-Gr-1 (RB6-8C5)	BioLegend	Cat# 108404
Biotin-conjugated anti-CD5 (53-7.3)	eBioscience	Cat# 13-0051-85
Biotin-conjugated anti-CD8a (53-6.7)	eBioscience	Cat# 553029
Biotin-conjugated anti-NK1.1 (PK136)	eBioscience	Cat# 553163
Biotin-conjugated anti-FcεRI (MAR-1)	eBioscience	Cat# 13-5898-82
Biotin-conjugated anti-TER119	eBioscience	Cat# 13-5921-85
FITC-conjugated anti-CD45.2 (104)	BioLegend	Cat# 109805

FITC-conjugated anti-MHCII (M5/114.15.2)	BioLegend	Cat# 107605
FITC-conjugated anti-CD11c (N418)	BioLegend	Cat# 117305
FITC-conjugated anti-CD49b (DX5)	BioLegend	Cat# 108906
FITC-conjugated anti-CD3 (17A2)	BioLegend	Cat# 100203
FITC-conjugated anti-Foxp3 (FJK-16s)	eBioscience	Cat# 11-5773-82
PE-conjugated anti-FcεRIa (MAR-1)	BioLegend	Cat# 134307
PE-conjugated anti-NK1.1 (PK136)	BioLegend	Cat# 108708
PE-conjugated anti-Rorγt (AFKJS-9)	eBioscience	Cat# 12-6988-80
PE-conjugated anti-SiglecF (1RNM44N)	eBioscience	Cat# 12-1702-82
PE-conjugated anti-ST2 (U29-93)	BD Pharmingen	Cat# 566311
PerCP Cy5.5-conjugated anti-CD45 (30-F11)	BioLegend	Cat# 103132
PerCP Cy5.5-conjugated anti-CD4 (30-GK1.5)	BioLegend	Cat# 100434
PECy7-conjugated anti-Gr-1 (RB6-8C5)	BioLegend	Cat# 108415

PECy7-conjugated anti-CD103 (2E7)	BioLegend	Cat# 121425
PECy7-conjugated anti-CD64 (X54-5/7.1)	BioLegend	Cat# 139313
PECy7-conjugated anti-CD127 (A7R34)	BioLegend	Cat# 135013
PECy7-conjugated anti-streptavidin	BD Pharmingen	Cat# 557598
APC-conjugated anti-CD317 (927)	BioLegend	Cat# 127015
APC-conjugated anti-CD127 (SB/199)	BioLegend	Cat# 121121
APC-conjugated anti-CD8a (53-6.7)	BioLegend	Cat# 100712
APC-conjugated anti-SiglecF (S17007L)	BioLegend	Cat# 155507
APC-conjugated anti-CD24 (M1/69)	BioLegend	Cat# 101813
Alexa Fluor 647-conjugated anti-Gata3 (L50-823)	BD Pharmingen	Cat# 560078
Alexa Fluor 700-conjugated anti-CD45 (30-F11)	BioLegend	Cat# 103127
APC Cy7-conjugated anti-CD4 (GK1.5)	BioLegend	Cat# 100413
APC Cy7-conjugated anti-CD11b (M1/70)	BioLegend	Cat# 101226
APC Cy7-conjugated anti-CD19 (6D5)	BioLegend	Cat# 115530

APC Cy7-conjugated anti-streptavidin	BioLegend	Cat# 405208
BV421-conjugated anti-CD11b (M1/70)	BioLegend	Cat# 101235
BV421-conjugated anti-CD11c (N418)	BioLegend	Cat# 117329
BV421-conjugated anti-T-bet (O4-46)	BD Pharmingen	Cat# 644815
CD16/32 (2.4G2) (93)	BioLegend	Cat# 101302
LIVE/DEAD Fixable Aqua Dead Cell Stain Kit for 405 nm excitation	Thermo Fisher Scientific	Cat# L34965
Chemicals, peptides, and recombinant proteins		
Papain, Carica papaya	Roche Diagnostics GmbH	Cat# 10108014001
Alternaria. Alternata (Alternaria tenuis), Lyophilized Form	Greer Laboratories	Cat#
Recombinant mouse IL-2	R&D Systems	Cat#
Recombinant mouse IL-33	R&D Systems	Cat#
Critical commercial assays		
RNeasy Mini Kit	Qiagen	Cat# 74106
SYBR Premix Ex Taq II	TAKARA	Cat# RR820B

Foxp3 / Transcription Factor Staining Buffer Set	eBioscience	Cat# 00-5523-00
Sodium propionate	Fujifilm Wako	Cat# 198-03015
InVivoMAb anti-mouse CD4 (clone YTS 191)	Bio X Cell	Cat# BE0119
InVivoMAb rat IgG2b isotype control, anti-keyhole limpet hemocyanin	Bio X Cell	Cat# BE0090
Quantikine ELISA Kits Mouse IL-4 Immunoassay	R&D Systems	Cat# M4000B-1
Quantikine ELISA Kits Mouse IL-5 Immunoassay	R&D Systems	Cat# M5000
Quantikine ELISA Kits Mouse IL-13 Immunoassay	R&D Systems	Cat# M1300CB
Naive CD4+ T cell isolation Kit, mouse	Miltenyi Biotec	Cat# 130-104-453

Supplementary Table 2. Primers used for qRT-PCR

Gene	Forward 5'- -3'	Reverse 5'- -3'
mGapdh	CGTCCCGTAGACAAAATGGT	TTGATGGCAACAATCTCCAC
mIl4	GGTCTCAACCCCCAGCTAGT	GCCGATGATCTCTCTCAAGTGAT
mIl5	GCAATGAGACGATGAGGCTT	CCCACGGACAGTTTGATTCT
mIl13	TGTGTCTCTCCCTCTGACCC	CACACTCCATACCATGCTGC
mIl1rl1	TGTATTTGACAGTTACGGAGGGC	ACTTCAGACGATCTCTTGAGACA
mGata3	CTCGGCCATTCGTACATGGAA	GGATACCTCTGCACCGTAGC
mTnfrs4	TACCTACCCCAGTGGTCACAA	ACGGATGACATAGAGTATCCCTG
mMaf	CTGCCGCTTCAAGAGGGTGCAGC	GATCTCCTGCTTGAGGTGGTC
mCcr3	TCGAGCCCGAACTGTGACT	CCTCTGGATAGCGAGGACTG

Supplementary Table 3. Gating strategies for flowcytometry

Cell strain	Flowcytometry gating
Alveolar macrophages (AMs)	$CD45^{+}CD11b^{int}CD11c^{+}SiglecF^{+}CD64^{+}$
Interstitial macrophages (IMs)	$CD45^{+}CD11b^{+}CD11c^{-}SiglecF^{-}CD64^{+}$
$CD103^{+}$ dendritic cells ($CD103^{+}$DCs)	$CD45^{+}CD11c^{+}MHCII^{+}$ $CD24^{+}CD103^{+}$
$CD11b^{+}$ dendritic cells ($CD11b^{+}$DCs)	$CD45^{+}$ $CD11c^{+}MHCII^{+}$ $CD24^{+}$ $CD11b^{+}$
Plasmacytoid dendritic cells (pDCs)	$CD45^{+}CD11b^{-}MHCII^{+}CD317^{+}$
Neutrophils	$CD45^{+}CD11b^{+}Gr\text{-}1^{+}$
Eosinophils	$CD45^{+}CD11c^{-}SiglecF^{+}CD11b^{+}$
Basophils	$CD45^{+}Fc\varepsilon RI^{+}CD49b^{+}$
Mast cells	$CD45^{+}Fc\varepsilon RI^{+}c\text{-}kit^{+}$
$CD4^{+}$ T cells	$CD45^{+}CD4^{+}$

Th1 cells	$CD45^+CD4^+T\text{-}bet^+$
Th2 cells	$CD45^+CD4^+Gata3^+$
$ST2^+CD4^+$ T cells	$CD45^+CD4^+ST2^+$
Th17 cells	$CD45^+CD4^+Ror\gamma^+$
Regulatory T cells (Treg cells)	$CD45^+CD4^+Foxp3^+$
ILCs	$CD45^+Lineage^-CD4^-CD127^+$
ILC1s	$CD45^+Lineage^-CD4^-CD127^+T\text{-}bet^+$
ILC2s	$CD45^+Lineage^-CD4^-CD127^+Gata3^+$ or $CD45^+Lineage^-CD4^-$ $CD127^+ST2^+$
ILC3s	$CD45^+Lineage^-CD4^-CD127^+Ror\gamma^+$
$CD8^+$ T cells	$CD45^+CD3^+CD8^+$
NK cells	$CD45^+NK1.1^+$

B cells	$CD45^{+}CD19^{+}$