SUPPLEMENTARY METHODS

Antibodies and flow cytometry

For surface staining, cells were stained at 4 °C in the presence of Fc Block (2.4G2; BD Biosciences) in flow cytometry buffer (DPBS plus 2% FCS). After staining surface markers, cells were fixed and permeabilized for intracellular cytokine and transcription factor staining according to the manufacturer's instructions (BD Biosciences). Antibodies used for surface and intracellular staining were listed in the supplementary Table 2.

T cell lentiviral transduction

The lentiviral vector Gata3-shRNA and scramble-shRNA were purchased from Sigma. Lentivirus was produced in HEK293T cells. $CD4^+$ T cells were stimulated for 24 h with plate-bound anti-CD3 Ab (0.5 μg/ml) and then transduced with virus-containing medium supplemented with polybrene (8 μg/ml). 20 hours later, culture medium was replaced with complete medium with anti-CD3 Ab (0.5 μg/ml) for 2 days, rested for 2 days and then stimulated with PMA and Ionomycin for 4 hours for intracellular staining.

T cell stimulation

Mouse $CD4^+$ T cells were negatively purified and activated by plate-coated anti-CD3 (1 μg/ml) and anti-CD28 (1 μg/ml) Abs for 72 h in complete cell medium (RPMI1640) supplemented with 10% heat-inactivated FBS, 2 mM glutamine, penicillin-streptomycin, nonessential amino acids, sodium pyruvate, 10 mM HEPES and 50 μM 2-mercaptoethanol, then expanded for another 3 day in fresh medium containing 1 ng/ml recombinant mouse IL-2. In some experiments, cells cultured for 3 days and supernatants were collected for measuring cytokines by ELISA assay.

For isolation of infiltrating cells from lungs, lungs were minced and digested in 5 ml of RPMI1640 media plus 10% FBS with 250 μg/ml collagenase IV (Roche) and 30 U/ml DNase I (Sigma-Aldrich) for 60 min at 37 °C with shocking. Cells were passed through a 70-μm strainer and spin down. The cell pellet was resuspended in 40% Percoll, carefully layered onto 80% Percoll and centrifuged at 650 *g*, without brake, RT for 30 min, then collected the

layer in the interface between the two Percoll concentrations. Cells were washed with PBS before red blood cells were lysed with ACK lysis buffer. Cells were counted and 2×10^6 cells were activated with PMA and ionomycin for analysis of intracellular cytokines and surface markers by flow cytometry.

Tet-On System

Wild-type or mutant MCPIP1 (D141N, C306R, D225/226A) expressing CEM Tet-on cells were generated as described previously[1]. The cells were cultured for 24 hours with Dox (100 ng/ml) and then treated with PMA (10 ng/ml) and Ionomycin (0.5 µg/ml) for 4 hours. Total RNA was isolated and used to measure gene expression. For western blot, CEM Tet-On cells were treated with Dox (100 ng/ml) for 48 h and then lysed.

Luciferase assay

CEM-Tet-on cells were transfected with luciferase reporter plasmid containing the 3′ UTR of Gata3[2] using Nucleofector (Lonza). MCPIP1 expression was induced by Dox (100 ng/ml). Cells were lysed after 24 hours and luciferase activity in lysates was determined with Dual-Luciferase Reporter Assay system (Promega).

Quantitative RT-PCR

Quantitative PCR was performed with SYBR green (Invitrogen, Life Technologies) using an Applied Biosystems StepOne PCR system. Results were analyzed with ΔΔCT method with glyceraldehyde-3-phosphate dehydrogenase (GAPDH) as an endogenous reference control. Primer sequences are listed in supplementary table1.

ELISA

Cell culture supernatants were assayed by ELISA for mouse IL-4, IFN-γ (BD Biosciences), IL-5 and IL-13 (eBioscience), according to the manufacturers' instructions.

RNA Immunoprecipitation

Th2-polarized cell lysates were prepared from WT mice after 6 d of *in vitro* polarization. Cell lysates were prepared in NT-2 buffer (50 mmol/L Tris-HCl, pH 7.4, 150 mmol/L NaCl,

1 mmol/L $MgCl_2$ and 0.05% NP-40) supplemented with 1 mmol/L dithiothreitol, 0.25 U/mL RNase out and 1× protease inhibitor and incubated on ice for 10 min. Cell lysates were obtained after high-speed centrifugation at 15,000 g for 15 min. Equal amounts of lysates were used for immunoprecipitation with MCPIP1 antibody and IgG1 control. Anti-MCPIP1 Ab or IgG1 were pre-coated onto protein A Sepharose beads and incubated with cell lysates. Protein A Sepharose beads were then washed with NT-2 buffer and incubated with 20 U RNase-free DNase I (15 min, 30°C) and further incubated in 100 μL NT-2 buffer containing 0.1% SDS and 0.5 mg/mL proteinase K (30 min, 55°C). The total RNAs were isolated by adding Trizol reagent (Invitrogen) to the beads and quantitative PCR (qPCR) was performed to measure GAPDH and GATA3.

Histological analysis

Peribronchiolar and perivascular inflammation were determined by a semiquantitatively graded scale as follows: 0, no inflammation; 1, mild inflammation; 2, moderate inflammation; 3, severe inflammation. Goblet-cell hyperplasia was assessed as follows: 0, no or rare PAS^+ cells; 1, <10% PAS^+ cells observed at a magnification of ×10; 2, 10–25% PAS^+ cells observed at a magnification of ×10; and 3, >25% PAS^+ cells observed at a magnification of ×4. A composite score was determined by multiplication of the graded score and the frequency of airways or vessels with a given pathological finding. All slides were examined in a random order and scored by a blinded pathologist.

References

1. Liu S, Qiu C, Miao R, et al. MCPIP1 restricts HIV infection and is rapidly degraded in activated CD4+ T cells. *Proc Natl Acad Sci U S A.* Nov 19 2013;110(47):19083-19088.

2. Yang Y, Ahn YH, Gibbons DL, et al. The Notch ligand Jagged2 promotes lung adenocarcinoma metastasis through a miR-200-dependent pathway in mice. *J Clin Invest.* Apr 2011;121(4):1373-1385.

FIGURE LEGENDS

Fig E1. Cell subsets in BAL of *zc3h12a*$^{-/-}$ mice. Total number of cells in the BAL of WT and *Zc3h12a*$^{-/-}$ mice were counted and summarized (3-4 mice per group). *: $p< 0.05$.

Fig E2. Effector CD4 T cells are increased in spleen of *zc3h12a*$^{-/-}$ mice. **(A)** The expression of CD62L and CD44 in CD4^{+} T cells of WT and *zc3h12a*$^{-/-}$ mice were measured by flow cytometry and summarized (n=3). **(B)** Percentages of CD8 and CD4 T cells in spleens of *zc3h12a*$^{-/-}$ mice and WT littermates were gated on CD3 (n=3). **(C)** The levels of IL-5 and IL-13 in culture supernatants of splenic CD4^{+} T cells were measured by ELISA after stimulation with anti-CD3/CD28 Abs (1 μg/ml) for different times (n=3). **(D)** Splenic cells were stimulated with P/I for 4 h and the percentages of IL-5^{+} and IFN-γ^{+} CD4 cells were measured by flow cytometry gated on CD44^{+} (n=4). *: $p< 0.05$, **: $p<0.01$, ***: $p< 0.001$.

Fig E3. Gene expression profiles of *zc3h12a*$^{-/-}$ CD4 T cells. The mRNA levels of Th2-related genes were measured by qRT-PCR in naïve WT littermate and *zc3h12a*$^{-/-}$ CD4 T cells stimulated with anti-CD3/CD28 Abs for 3 days (n=3). *: $p< 0.05$, **: $p<0.01$, ***: $p< 0.001$.

Fig E4. IL-5 and IL-13 expression depend on MCPIP1 in CD4 T cells. **(A-C)** WT littermate and *zc3h12a*$^{-/-}$ CD4^{+} T cells were pretreated with or without Malt1-specific inhibitor MI-2 (1 μM) for 30 min and then stimulated with anti-CD3/CD28 Abs for 4 hrs. The mRNA expression of cytokines was measured by qRT-PCR (n=3). *: $p< 0.05$, **: $p<0.01$.

Fig E5. Notch signal is required for the initiation of IL-5-producing Th2 cells mediated by MCPIP1. **(A)** The percentages of Gata3^{+}*zc3h12a*$^{-/-}$ CD4^{+}T cells were analyzed after pretreatment with GSI and then with anti-CD3/CD28 Abs (n=3). **(B)** Total CD4^{+} T cells of WT and *zc3h12a*$^{-/-}$ were pretreated with GSI and then cultured with anti-CD3/CD28 Abs. Cytokine-producing cells were gated on CD4 and summarized (n=3). *: $p< 0.05$, **: $p<0.01$, ***: $p< 0.001$.

Fig E6. MCPIP1 inhibits the expression of Th2-associated transcription factors. **(A)** The

percentages of *Gata3*$^{+}$*Zc3h12a*$^{-/-}$ CD4^{+} as in Fig. 6C were summarized from three independent experiments. **(B)** The expression of GFP/MCPIP1 fusion protein in CEM-Tet-On cells after adding Dox. **(C)** CEM-Tet-On cells were treated with Dox and P/I, followed by measuring Gata3 and IL-13 mRNA expression by qRT-PCR (n=3). **(D)** CD4^{+} T cells from WT and *zc3h12a*$^{-/-}$ mice were stimulated with anti-CD3/CD28 Abs and ActD/DRB. Then total RNAs were harvested for measuring mRNA levels by qRT-PCR. **(E)** The levels of Gata3 and MCPIP1 mRNA in WT CD4^{+} Th2 cells were measured by qRT-PCR. **(F)** Cytoplasmic extracts were collected from the Th2 cells and immunoprecipitated with anti-MCPIP1 or isotype matched Ab, followed by RNA extraction. Gata3 mRNA was detected by PCR (n=3). **(G)** A schematic diagram for putative functional conserved domains of MCPIP1. **(H)** The expression of Gata3 mRNA in CEM Tet-On cells after adding Dox and stimulating with P/I for 3 h (n=3). **(I)** The MFI of Gata3 in CEM-Tet-On cells after adding Dox and then stimulating with P/I. **(J)** Stable CEM-Tet-On cells were treated with Dox and P/I, and then treated with ActD and DRB for different times. The remaining Gata3 mRNA was measured by qRT-PCR (n=3). *: $p< 0.05$, **: $p<0.01$, ***: $p< 0.001$.

Fig E7. MCPIP1 deficiency enhances Th2 cell-mediated disease in an allergen-induced asthma model. WT and *zc3h12a*$^{-/-}$ mice were sensitized and challenged with OVA as in Fig. 7A. H&E and PAS staining of lung tissue sections were examined on day 26 **(A)** and inflammation was scored **(B)** (n=3). **(C)** The percentages of CD44^{high} cells were gated on CD4^{+} T cells in spleens of the mice as in (A) and summarized (n=3). **(D)** Splenocytes from the mice in (A) were stimulated with P/I and the percentages of IL-5^{+} and IL-4^{+} CD4^{+} T cells were measured and analyzed (n=4). **(E)** Splenocytes from the mice in (A) were re-stimulated with OVA (200 μg/ml) and IL-2 (10 ng/ml) for 3 days, and stimulated with P/I. The percentages of cytokine-producing CD4^{+} T cells were measured by FACS. **(F)** Cell subtypes in BAL of the CD45.1 recipient mice as in Fig. 7C were counted and summarized (n=4). **(G)** Splenocytes from the CD45.1^{+} recipient mice as in (D) were used to detect donor cells (CD45.2^{+}) and host cells (CD45.1^{+}) gated on CD3^{+}CD4^{+} and CD3^{+}CD4^{-} cells. *: $p< 0.05$, **: $p<0.01$, ***: $p< 0.001$.

Table E1.

Primer sequences

Gene symbol	Forward primer (5’---3’)	Reverse primer (5’—3’)
mIrf4	5’-GCAGCCTTCAGGGCTCGTCG	5’-GCAGCCTTCAGGGCTCGTCG
mIl4	5’-GGTCTCAACCCCCAGCTAGT	5’-GCCGATGATCTCTCTCAAGTGAT
mIl5	5’-CTCTGTTGACAAGCAATGAGACG	5’-TCTTCAGTATGTCTAGCCCCT
mIl13	5’-CCTGGCTCTTGCTTGCCT	5’-GGTCTTGTGTGATGTTGCTCA
mIfng	5’-ATGAACGCTACACACTGCATC	5’-CCATCCTTTTGCCAGTTCCTC
mIl17a	5’- CTCCAGAAGGCCCTCAGACTAC	5’-GGGTCT TCATTGCGGTGG
mTgf-b	5’-TAAGAGGTCACCCGCGTGCT	5’- AAAGACAGCCACTCAGGCGTA
mCcl24	5’-ATTCTGTGACCATCCCCTCAT	5’- TGTATGTGCCTCTGAACCCAC
mMuc5ac	5’-CTCCTCTCGGTGACAGAGTCT	5’-GTGGTTTGACACTGACTTCCC
mGob5	5’-CGTGGTCATTGGCGATGACG	5’-CTGTCTTCCTCTTGATCCTCCA
mSocs3	5’-ATGGTCACCCACAGCAAGTTT	5’-TCCAGTAGAATCCGCTCTCCT
mGfi	5’-GATGAGCTTTGCACACTGGA	5’-AGGAACGCAGCTTTGACTGT
mRorc	5’-AAGATCTGCAGCTTTTCCACA	5’-TTTGGAACTGGCTTTCCATC
mGata3	5’-CTCGGCCATTCGTACATGGAA	5’-GGATACCTCTGCACCGTAGC
mStat6	5’-CTCTGTGGGGCCTAATTTCCA	5’-CATCTGAACCGACCAGGAACT
mTbx21,	5’-TTTCCAAGAGACCCAGTTCATTG	5’-ATGCGTACATGGACTCAAAGTT
mFoxp3	5’-CCCATCCCCAGGAGTCTTG	5’-ACCATGACTAGGGGCACTGTA
mIcosl	5’-TAAAGTGTCCCTGTTTTGTGTCC	5’-ATTGCACCGACTTCAGTCTCT
mCd40	5’-TGTCATCTGTGAAAAGGTGGTC	5’-ACTGGAGCAGCGGTGTTATG
mOx40l	5’-GGGATGCTTCTGTGCTTCATCT	5’-TGTTCTGCACCTCCATAGTTTGA
mCcr3	5’-TCAACTTGGCAATTTCTGACCT	5’-CAGCATGGACGATAGCCAGG
mCxcr4	5’-AGCTAAGCAGCATGAACAACT	5’-AACGCTGCTGTAGAGGTTGACACT
mCcl5	5’-TCGTGCCCACGTCAAGGAGTA	5’-TAGAGCAAGCAATGACAGGGA
mJag1	5’-CCTCGGGTCAGTTTGAGCTG	5’-CCTTGAGGCACACTTTGAAGTA
mJag2	5’-CAATGACACCACTCCAGATGAG	5’-GGCCAAAGA AGTCGTTGCG
mDll1	5’-CAGGACCTTCTTTCGCGTATG	5’-AAGGGGAATCGGATGGGGTT
mDll3	5’- CTGGTGTCTTCGAGCTACAAAT	5’-TGCTCCGTATAGACCGGGAC

mDll4	5’-TTCCAGGCAACCTTCTCCGA	5’- ACTGCCGCTATTCTTGTCCC
mGapdh	5’- AACTTTGGCATTGTGGAAGG	5’- ACACATTGGGGGTAGGAACA
hGata3	5’-GCCCCTCATTAAGCCCAAG	5’-TTGTGGTGGTCTGACAGTTCG
hZc3h12a	5’-GGCAGTGGTTTCTTACGAAGGA	5’-CCCATCACAGACCAGCACAT
hIl13	5’-CCTCATGGCGCTTTTGTTGAC	5’-TCTGGTTCTGGGTGATGTTGA
pLA-up	5’-ATGCCTTCCTGATCCTATTGG	
pLA-low		5’-CTTTCATGAGCAATGGTCGCA
pLaz-low		5’-GTGCGGGCCTCTTCGCTATTAC

152

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Table E2.

Antibody list

Antibody	clone	Company
anti-CD3-APC	145-2C11	BD Biosciences
anti-CD4-PE	H129.19	BD Biosciences
anti-CD8-PE	53-6.7	BD Biosciences
anti-CD4-PerCP	RM4-5	BD Biosciences
anti-CD62L-PE	MEL-14	BD Biosciences
anti-CD44-APC-cy7	IM7	BD Biosciences
anti-CD44-FITC	IM7	BD Biosciences
anti-CD11b-FITC	M1/70	BD Biosciences
anti-CD45.1-AF700	A20	BD Biosciences
Anti-CD45-pecy7	104	BD Biosciences
anti-Ly6G-APC	1A8	BD Biosciences
anti-Siglec F-PE	E50-2440	BD Biosciences
Siglec F-PE	E50-2440	BD Biosciences
anti-IFN-γ-APC	XMG1.2	BD Biosciences
anti-IL-17A-PE	TC11-18H10.1	BD Biosciences
anti-IL-17A-AF488	TC11-18H10.1	BD Biosciences
anti-IL4-AF488	11B11	BD Biosciences
anti-IFN-γ-AF700	XMG1.2	BD Biosciences
anti-IL-5-APC	TRFK5	BD Biosciences
anti-Gata3-PE	C50-823	BD Biosciences
anti-IL-13-PercPeF710	eBio13A	eBioscience
anti-CD11b-FITC	M1/70	Biolegend
anti-CD45-PE-Cy7	N418	Biolegend

Figure E1.

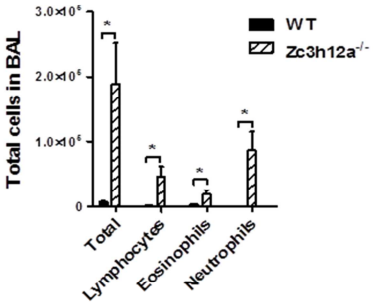

Total cells in BAL
3.0×10⁶
2.0×10⁶
1.0×10⁶
0
WT
Zc3h12a-/-
*
*
*
*
Total
Lymphocytes
Eosinophils
Neutrophils

Figure E2.

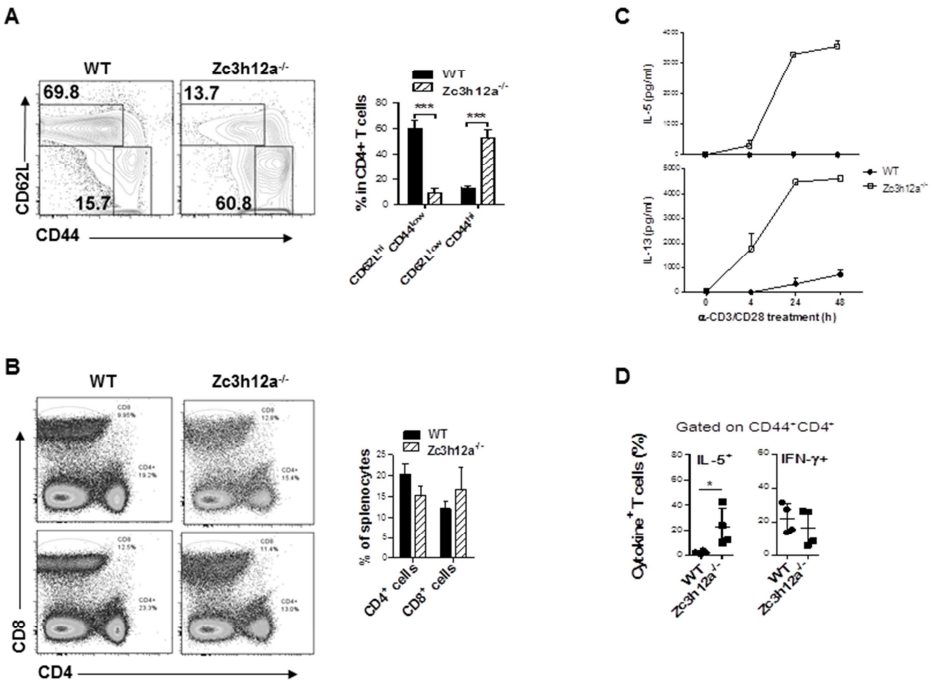

A
WT
Zc3h12a-/-
69.8
13.7
15.7
60.8
CD62L
CD44
WT
Zc3h12a-/-
% in CD4+ T cells
***
***
CD62L hi CD44low
CD62L low CD44hi
C
IL-5 (pg/ml)
IL-13 (pg/ml)
WT
Zc3h12a-/-
0
4
24
48
α-CD3/CD28 treatment (h)
B
WT
Zc3h12a-/-
CD8
CD4
WT
Zc3h12a-/-
% of splenocytes
CD4+ cells
CD8+ cells
D
Gated on CD44+CD4+
Cytokine+ T cells (%)
IL-5+
IFN-γ+
*
WT
Zc3h12a-/-
WT
Zc3h12a-/-

Figure E3.

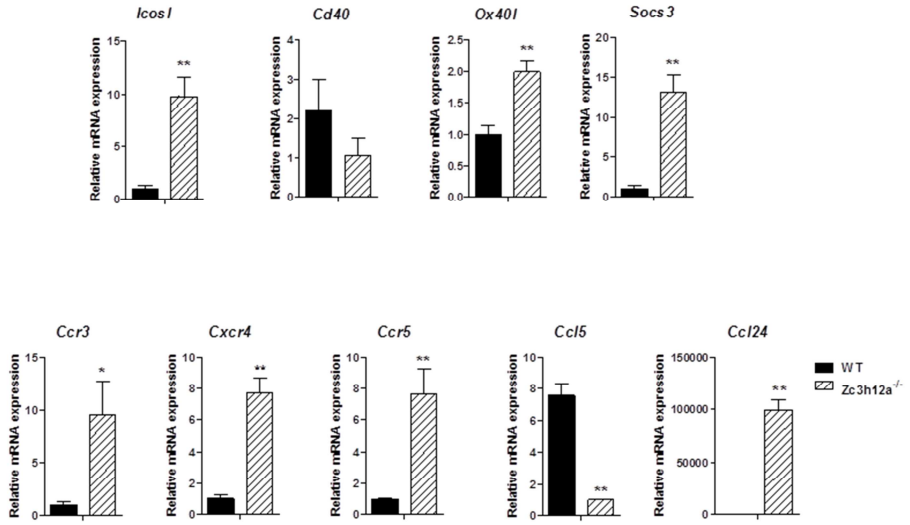

Icos1
Cd40
Ox40l
Socs3
Ccr3
Cxcr4
Ccr5
Ccl5
Ccl24
Relative mRNA expression
WT
Zc3h12a-/-

Figure E4.

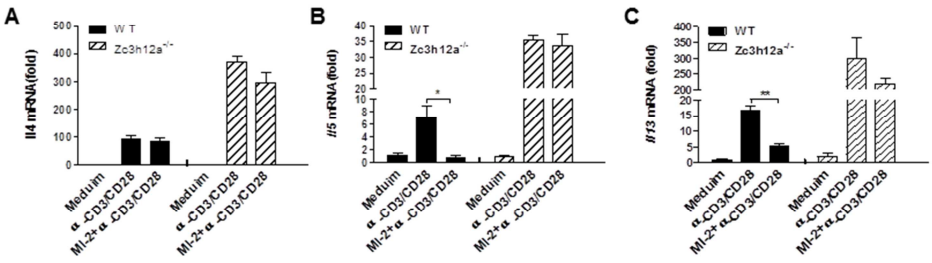

A
B
C
WT
Zc3h12a-/-
Il4 mRNA(fold)
Il5 mRNA (fold)
Il13 mRNA (fold)
Medium
α-CD3/CD28
MI-2+α-CD3/CD28
*
**

Figure E5.

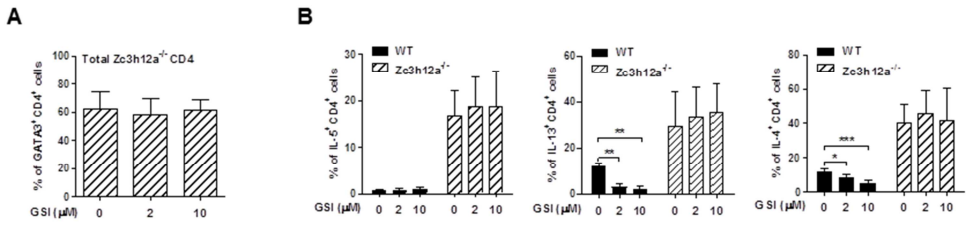

A
Total Zc3h12a-/- CD4
% of GATA3+ CD4+ cells
GSI (μM) 0 2 10
B
WT
Zc3h12a-/-
% of IL-5+ CD4+ cells
% of IL-13+ CD4+ cells
% of IL-4+ CD4+ cells
GSI (μM) 0 2 10 0 2 10
**
**
*
***

Figure E6.

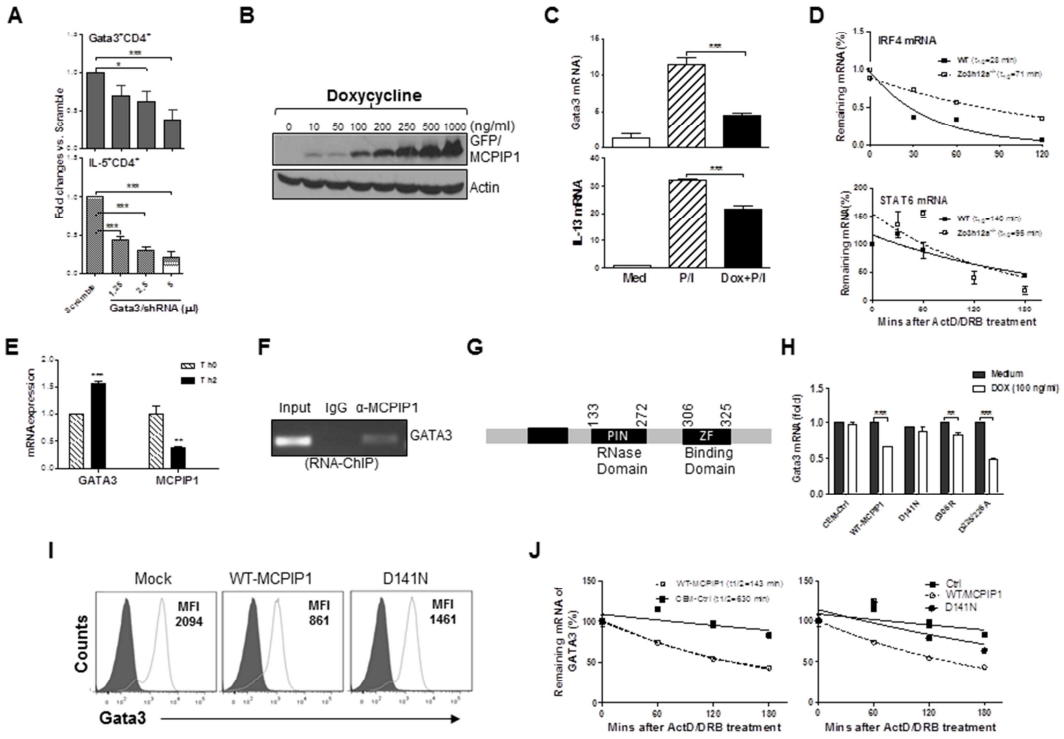
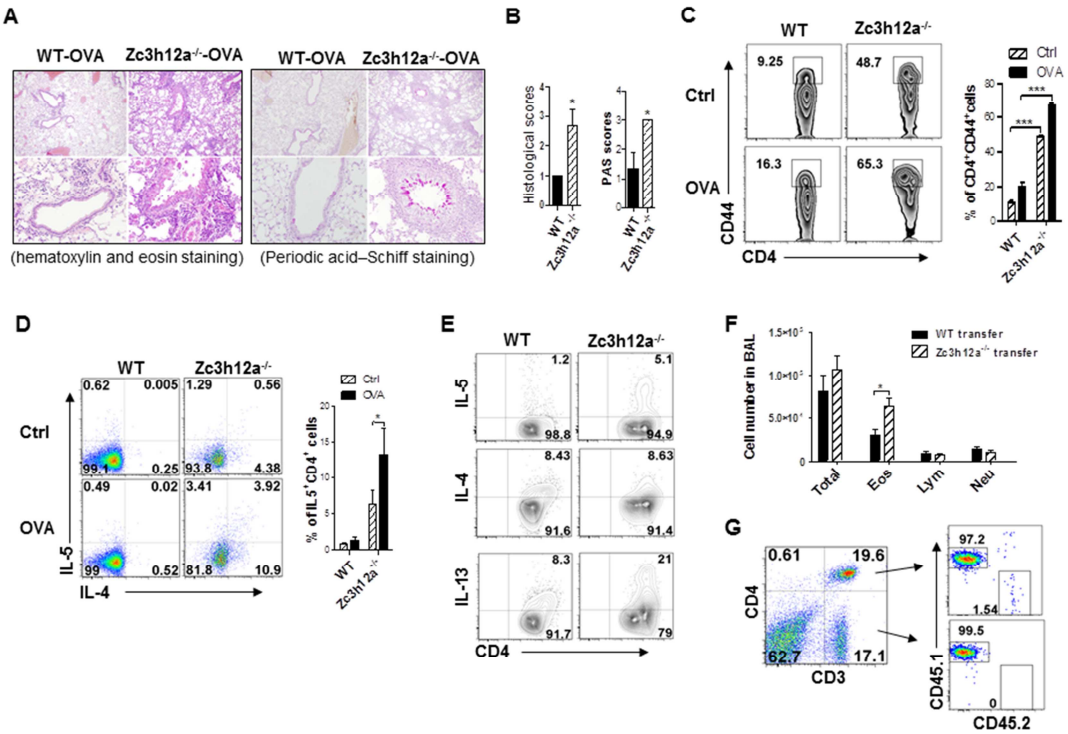

A
Gata3+CD4+
IL-5+CD4+
Fold changes vs. Scramble
Scramble
1.25
2.5
5
Gata3/shRNA (μl)
B
Doxycycline
0 10 50 100 200 250 500 1000 (ng/ml)
GFP/
MCPIP1
Actin
C
Gata3 mRNA
IL-13 mRNA
Med
P/I
Dox+P/I
D
IRF4 mRNA
Remaining mRNA (%)
STAT6 mRNA
Mins after ActD/DRB treatment
E
mRNA expression
T h0
T h2
GATA3
MCPIP1
F
Input IgG α-MCPIP1
GATA3
(RNA-ChIP)
G
133
272
306
325
PIN
ZF
RNase
Domain
Binding
Domain
H
Medium
DOX (100 ng/ml)
Gata3 mRNA (fold)
CBM-Ctrl
WT-MCPIP1
D141N
CBM-N
D252/256A
I
Mock
WT-MCPIP1
D141N
MFI
2094
MFI
861
MFI
1461
Counts
Gata3
J
WT-MCPIP1 (t1/2=143 min)
CBM-Ctrl (t1/2=630 min)
Remaining mRNA of GATA3 (%)
Mins after ActD/DRB treatment
Ctrl
WT-MCPIP1
D141N
Mins after ActD/DRB treatment

Figure E7.


A
WT-OVA
Zc3h12a-/--OVA
WT-OVA
Zc3h12a-/--OVA
(hematoxylin and eosin staining)
(Periodic acid–Schiff staining)
B
Histological scores
PAS scores
WT
Zc3h12a-/-
C
WT
Zc3h12a-/-
Ctrl
OVA
9.25
48.7
16.3
65.3
CD44
CD4
% of CD4+CD44+cells
D
WT
Zc3h12a-/-
Ctrl
OVA
0.62
0.005
1.29
0.56
99.1
0.25
93.8
4.38
0.49
0.02
3.41
3.92
99
0.52
81.8
10.9
IL-5
IL-4
% of IL5+CD4+ cells
E
WT
Zc3h12a-/-
IL-5
IL-4
IL-13
1.2
5.1
98.8
94.9
8.43
8.63
91.6
91.4
8.3
21
91.7
79
CD4
F
WT transfer
Zc3h12a-/- transfer
Cell number in BAL
Total
Eos
Lym
Neu
G
0.61
19.6
62.7
17.1
CD4
CD3
97.2
1.54
99.5
0
CD45.1
CD45.2