Figure S1. *Identification of immune cells in lung tissues.* The general schemata for flow cytometry used to identify immune cells is shown. The cell surface marker used were as follows: eosinophils, SiglecF$^+$ CCR3$^+$ (A); Th1 lymphocytes, CD45$^+$CD3$^+$IFNγ$^+$ (B); Th2 lymphocytes, CD45$^+$CD3$^+$IL4$^+$ (B); Th17 lymphocytes, CD45$^+$CD3$^+$IL4$^+$ (B); ILCs, Lin$^-$IL7Rα$^+$Flt3$^-$ST2$^+$, IL-17RB$^+$ or ICOS$^+$ (C); myeloid dendritic cells (mDCs), CD45$^+$ CD11b$^+$ CD11c$^+$ B220$^-$ (D); for mDCs we used CD45$^+$ CD11b$^+$ CD11c$^+$ B220$^-$ CD103$^+$ (D); plasmacytoid dendritic cells (pDCs), CD45$^+$ CD11b$^-$ CD11c$^+$ B220$^+$ (D). Activated mDCs and pDCs were identified using the surface expression of CD86 and I-A/E on these cells (D).

Figure S2. *Expression of FOXA3 causes goblet cell differentiation.* Immunofluorescence confocal microscopy is shown for Muc5ac (white) Muc5B (red), Scgb1a1 (green) (upper panels) and acetylated tubulin (white) (lower panels) from airway sections for *Scgb1a1.rtTA/Foxa3* transgenic mice and control single transgenic littermates treated with doxycycline from E16 to PN15 at the time of sacrifice. In controls, Scgb1a1 staining was present at high levels in Club cells. Expression of FOXA3 induced goblet cell metaplasia and Muc5AC and Muc5B staining in goblet cells in which Scgb1a1 staining, while present, was decreased (A). Neither Muc5b nor Scgb1a1 were present in ciliated cells identified by acetylated tubulin staining (white) (B). Scale bar: 50 μm.

Figure S3. *Spdef$^{+/+}$and Spdef$^{-/-}$ induces similar levels of serum IgE and Il4 mRNA in the splenocytes and peripheral lymph nodes.* The adult *Spdef$^{+/+}$and Spdef$^{-/-}$ mice* were injected intraperitoneally with 200µl of donkey anti-mouse IgD serum. On day 7, serum, spleen and LNs (inguinal and axillary) were collected. Serum total IgE and IgG1 in *Spdef$^{+/+}$* (white bars) and *Spdef$^{-/-}$* mice (black bars) was assessed by ELISA (A,B). *Il4* mRNA in spleen and lymph nodes was assessed by qRT-PCR (C,D). Graphs represent mean ± S.E., significance was calculated using Student's t-test (2-tailed, unpaired), n=3-4 in each group. Serum total IgE and HDM specific IgE and IgG1 were assessed by ELISA in adult (E-G) and PN15 (H-J) *Spdef$^{+/+}$*and *Spdef$^{-/-}$* mice. Graphs represent mean ± S.E., significance was analyzed using Student's t-test (2-tailed, unpaired), n=4-6 for each group.

Figure S4. *SPDEF mediates FOXA3 induced Th2 responses.* Control littermate, *Scgb1ab-rtTA/Foxa3/Spdef$^{+/+}$* (red line), *Scgb1ab-rtTA/Foxa3/Spdef$^{+/-}$* (blue line) and *Scgb1ab-rtTA/Foxa3/Spdef$^{-/-}$* (green line) were placed on doxycycline from E16.5 – PN15 and euthanized after testing in Penh (A). Graphs represent mean± S.E, *** = $p<0.001$ and # = $p<0.05$, analyzed using two-way Anova with Bon-Ferroni post test, n=6 mice in each group. Lung tissue was obtained from the pups at PN15 for analysis. *Scgb1ab-rtTA/Foxa3/Spdef$^{-/-}$* pups did not develop spontaneous eosinophilic inflammation or goblet cell metaplasia as assessed by H&E and compared with *Scgb1ab-rtTA/Foxa3/Spdef$^{+/-}$* mice (C). Lung RNAs were measured by qRT-PCR in control littermates (white bars), *Scgb1ab-rtTA/Foxa3/Spdef$^{+/+}$* (black bars), *Scgb1ab-rtTA/Foxa3/Spdef$^{+/-}$* (light gray bars) and *Scgb1ab-rtTA/Foxa3/Spdef$^{-/-}$* (dark gray bars) (D). Graphs represent mean ± S.E., * $p<0.05$ analyzed using two-way Anova with Bon-Ferroni post test, n=6 mice for each group. *Scgb1ab-rtTA/Foxa3/Spdef$^{+/+}$* (red line) and *Scgb1ab-*

rtTA/Foxa3/Spdef$^{+/-}$ (green line) were placed on doxycycline from E16.5 – PN15 and exposed to a single treatment of intranasal HDM and AHR tested by Penh (B) 2 days later, demonstrating inhibition of AHR on the *Spdef*$^{/+}$ background. Graphs represent mean± S.E, * = $p<0.001$, analyzed using two-way Anova with Bon-Ferroni post test, n=6 mice in each group.

Figure S5. *Expression of FOXA3 in airway epithelial cells enhanced HDM induced goblet cell differentiation, inflammation and AHR.* Dams were placed on doxycycline from E16.5-PN17. On PN15 *Scgb1a1-rtTA/Foxa3* (black bars) and control (white bars) pups were exposed to a single treatment with intranasal HDM and euthanized 2 days later. AHR was assessed by Penh and compared to saline treated littermate controls (A). Graphs represent mean± S.E *** = $p<0.001$ and # = $p<0.05$, using two-way Anova with Bon-Ferroni post test, n = 6 mice in each group. Lung histology and IHC staining for of FOXA3 and SPDEF are shown (B). mRNAs associated with goblet cell differentiation, dendritic cell and Th2 inflammatory responses were assessed in whole lung by qRT-PCR (B and C). Graphs represent mean± S.E., *= $p<0.05$ using Student's t-test (2-tailed, unpaired), n=6 in each group.

Figure S6. *Chromatin immunoprecipitation sequencing (ChIP-Seq) identifies TSLP and CCL17 as potential transcriptional targets of FOXA3.* ChIP-Seq was performed on BEAS2B cells after infection with lenti-Foxa3 as described by Chen et al., 2014 (24). Peaks were visualized as custom tracks in the UCSC genome browser with HG19 as reference genome. UCSC Genome browser tracks depict FOXA3 ChIP-Seq peaks located in the promoter and 3″ UTL of TSLP gene, in the first intronic and 3′ intergenic region (near 3′-UTR of the CCL17 gene, respectively. R0 = peak calling with no redundant sequences.

Figure S7. *Anti-TSLP inhibits FOXA3 induced Th2 lung inflammation.* Dams were placed on doxycycline from E16.5–PN8. *Scgb1a1-rtTA/Foxa3* (black bar) and littermate single transgenic control pups (white) were treated with 50μg of anti-TSLP antibody on PND3 and lung tissues obtained on PND8. Anti-TSLP decreased eosinophilic infiltration in H and E (A), and decreased MUC5B immunohistochemistry staining (A). *Foxa3*, *Spdef*, *Muc5ac*, *Csf2*, *Tslp*, *Il4*, *Il13, Ccl17, Il5*, *Ear11*, *Ccl11, and Ccl24* mRNAs were assessed by qRT-PCR (B). The graphs represent mean ± S.E.,*, $p<0.05$ analyzed using two-way Anova with Bon-Ferroni post test, n= 6 for each group.

Mouse Line		Primer sequence
Scgb1a1-rtTA (line 2)	Forward	ACT GCC CAT TGC CCA AAC AC
	Reverse	AAA ATC TTG CCA GCT TTC CCC
Otet7-TRE2-Spdef	Forward	TTC CAG GAG CTG GGC GGT AA
	Reverse	GGT CCA TGG TGA TAC AAG GGA CAT
Otet7-Foxa3-IRES-EGFP	Forward	AGC AAA GAC CCC AAC GAG AAG C
	Reverse	CAA ACA ACA GAT GGC TGG CAA C
Spdef$^{/-}$	Primer 1	CCC ACC TCC TAT GTC AGC CAT GGC
	Primer 2	CAA TCC TGT ACC ATA TCT GGC ATG G
	Primer 3	CAG ATT AGA GAT GCA CAA CCT GCC
	Primer 4	GCA TCG CAT TGT CTG AGT AGG TGT CA

Supplemental Table 1

Genes	TaqMan Probes	Source
EUK 18S rRNA	4352930	Applied Biosystems
Foxa3	Mm00484714_m1	Applied Biosystems
Spdef	Mm00600221_m1	Applied Biosystems
Muc5ac	Mm01276725_g1	Applied Biosystems
Muc5b	Mm00466391_m1	Applied Biosystems
Tslp	Mm01157588_m1	Applied Biosystems
Il33	Mm01195786_m1	Applied Biosystems
Csf2	Mm01290062_m1	Applied Biosystems
Ccl20	Mm01268753_g1	Applied Biosystems
Il25	Mm00499822_m1	Applied Biosystems
Il4	Mm00445259_m1	Applied Biosystems
Il13	Mm00434204_m1	Applied Biosystems
Ccl17	Mm01244826_g1	Applied Biosystems
Il17a	Mm00439618_m1	Applied Biosystems
Ifng	Mm01168134_m1	Applied Biosystems
Ear11	Mm00519056_s1	Applied Biosystems
Ccl11	Mm00441238_m1	Applied Biosystems
Ccl24	Mm00444701_m1	Applied Biosystems
Il5	Mm00439646_m1	Applied Biosystems
Acta2	Mm00725412_s1	Applied Biosystems

Supplemental Table 2

Flow Antibodies	Clone	Source
CD3e FITC	145-2C11	BioLegend
CCR3 FITC	J073E5	BioLegend
IL7Rα FITC	A7R34	BioLegend
CD86 FITC	PO3	BioLegend
I-A/I-E PE	M5/114.15.2	BioLegend
IL13 PE	eBio13A	eBioscience
SiglecF PE	E50-2440	BD Pharmingen
IL33Rα PE	DIH9	BioLegend
CD11b Pacific Blue	M1/70	BioLegend
ICOS Pacific Blue	C398.4A	BioLegend
CD4 Pacific Blue	RM4-4	BioLegend
CD45 Alexa Fluor 700	30-F11	BioLegend
CD3/Ly-6G(Ly-6C)/CD11b/CD45R/Ter-119 Alexa Fluor 700	Cat# 79923	BioLegend
CD103 PerCP/Cy5.5	2 E 7	BioLegend
IL17A PerCP/Cy5.5	TC11-18H10.1	BioLegend
CD11c APC	N418	BioLegend
Flt3 APC	A2F10	BioLegend
IFNγ APC	XMG1.2	BioLegend
B220 PE/Cy7	RA3-6B2	BioLegend
IL4 PE/Cy7	11B11	BioLegend
Goat anti-rat IgG PE/Cy7	Poly4054	BioLegend
IL17RB Purified Rat Monoclonal IgG1	752101	R&D

Supplemental Table 3

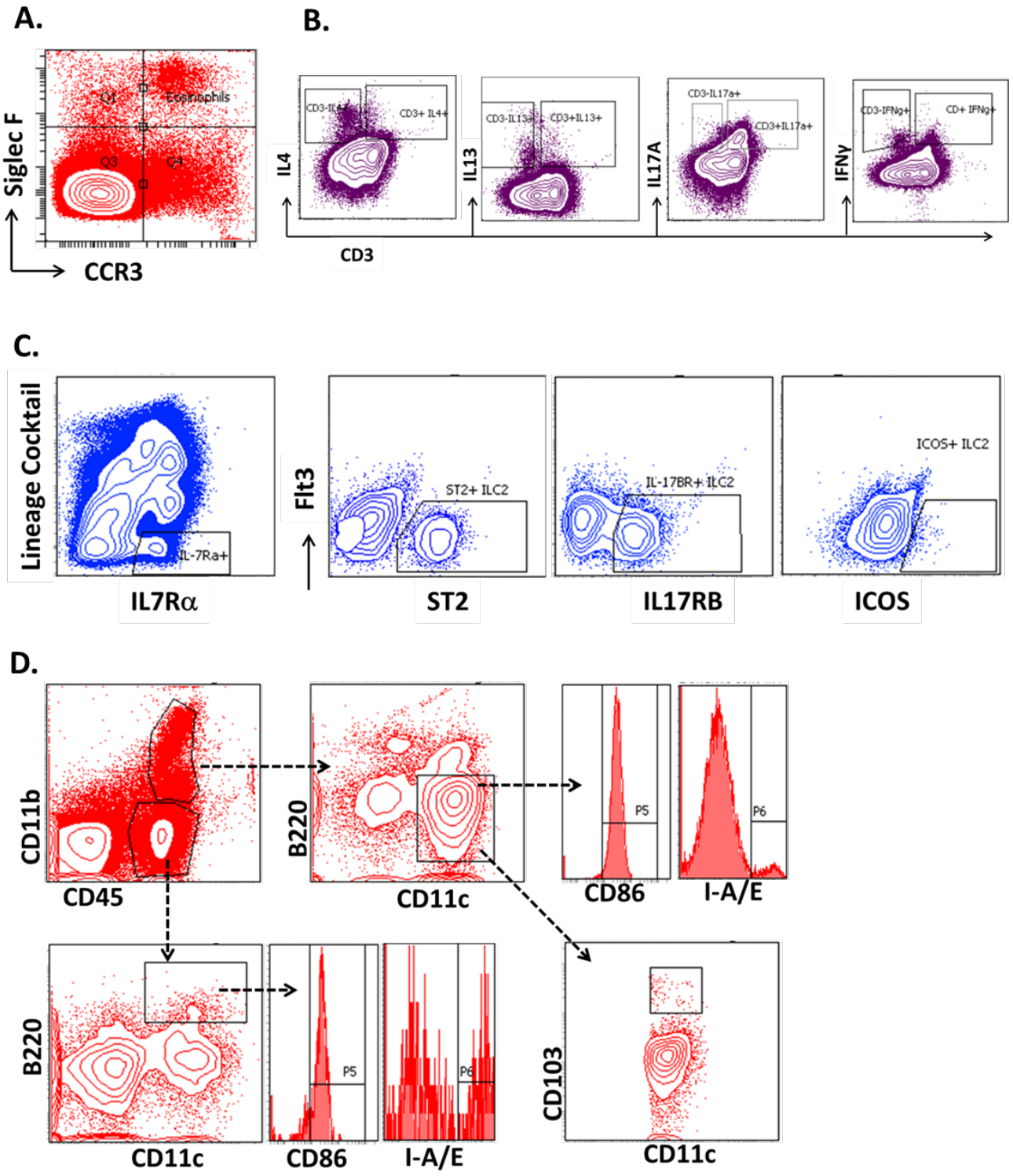

A.
B.
C.
D.
Siglec F
CCR3
Q1
Eosinophils
Q3
Q4
IL4
IL13
IL17A
IFNγ
CD3
CD3-IL4+
CD3+ IL4+
CD3-IL13+
CD3+IL13+
CD3-IL17a+
CD3+IL17a+
CD3-IFNg+
CD+ IFNg+
Lineage Cocktail
IL-7Ra+
IL7Rα
Flt3
ST2+ ILC2
ST2
IL-17BR+ ILC2
IL17RB
ICOS+ ILC2
ICOS
CD11b
CD45
B220
CD11c
P5
P6
CD86
I-A/E
CD103

Figure S1

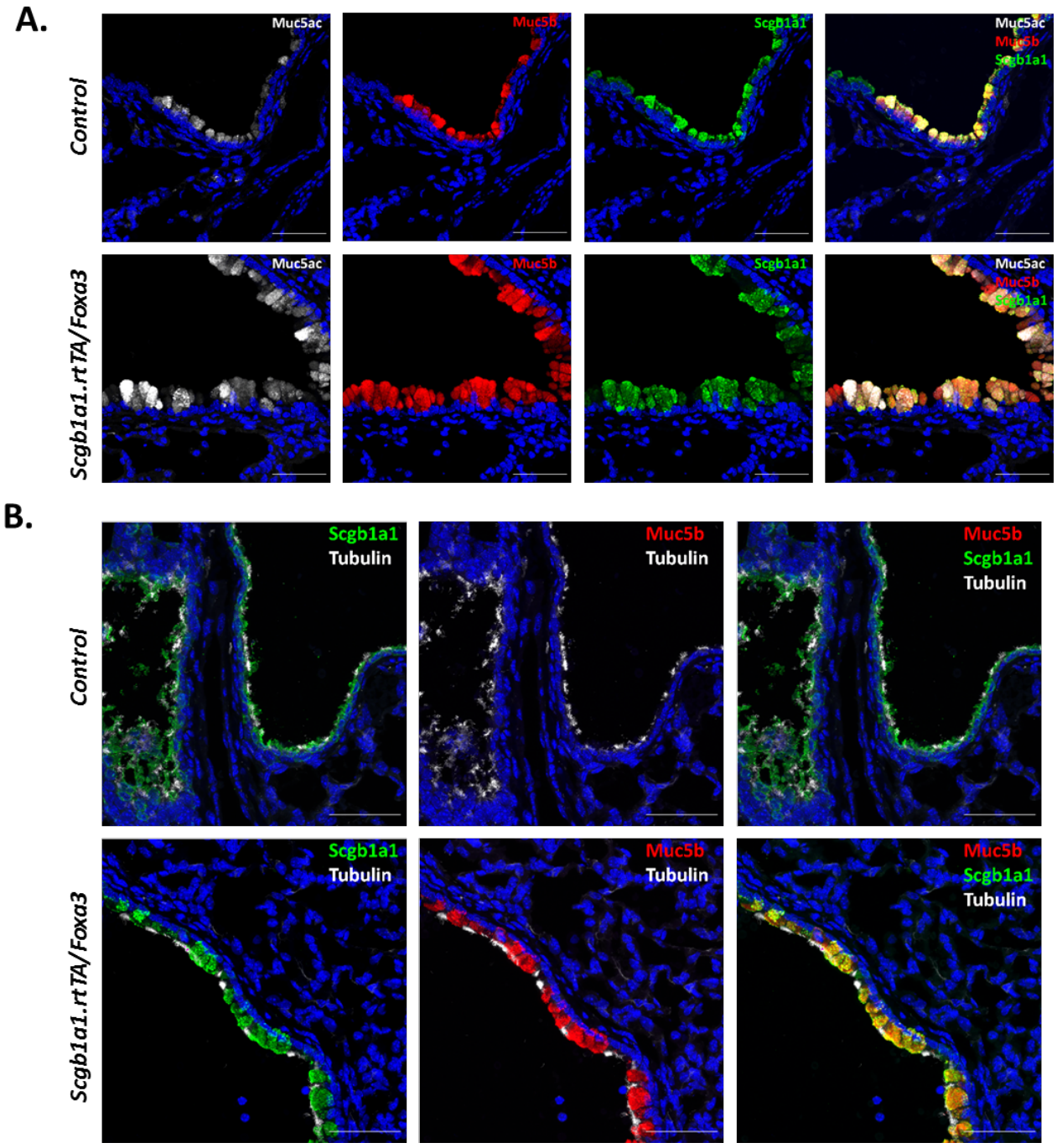

A.
Control
Scgb1a1.rtTA/Foxa3
Muc5ac
Muc5b
Scgb1a1
B.
Control
Scgb1a1.rtTA/Foxa3
Scgb1a1
Tubulin
Muc5b
Tubulin
Muc5b
Scgb1a1
Tubulin

Figure S2

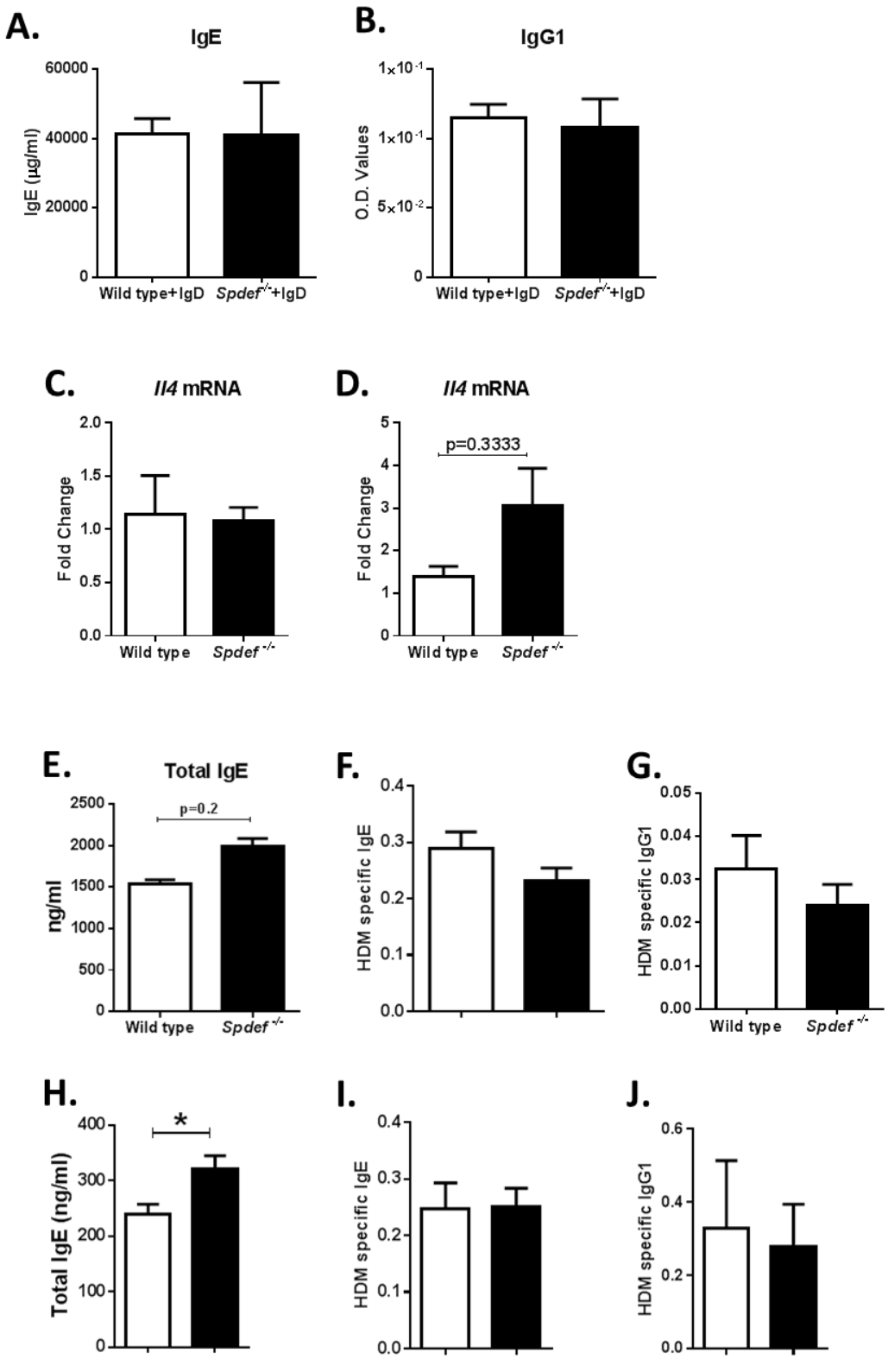
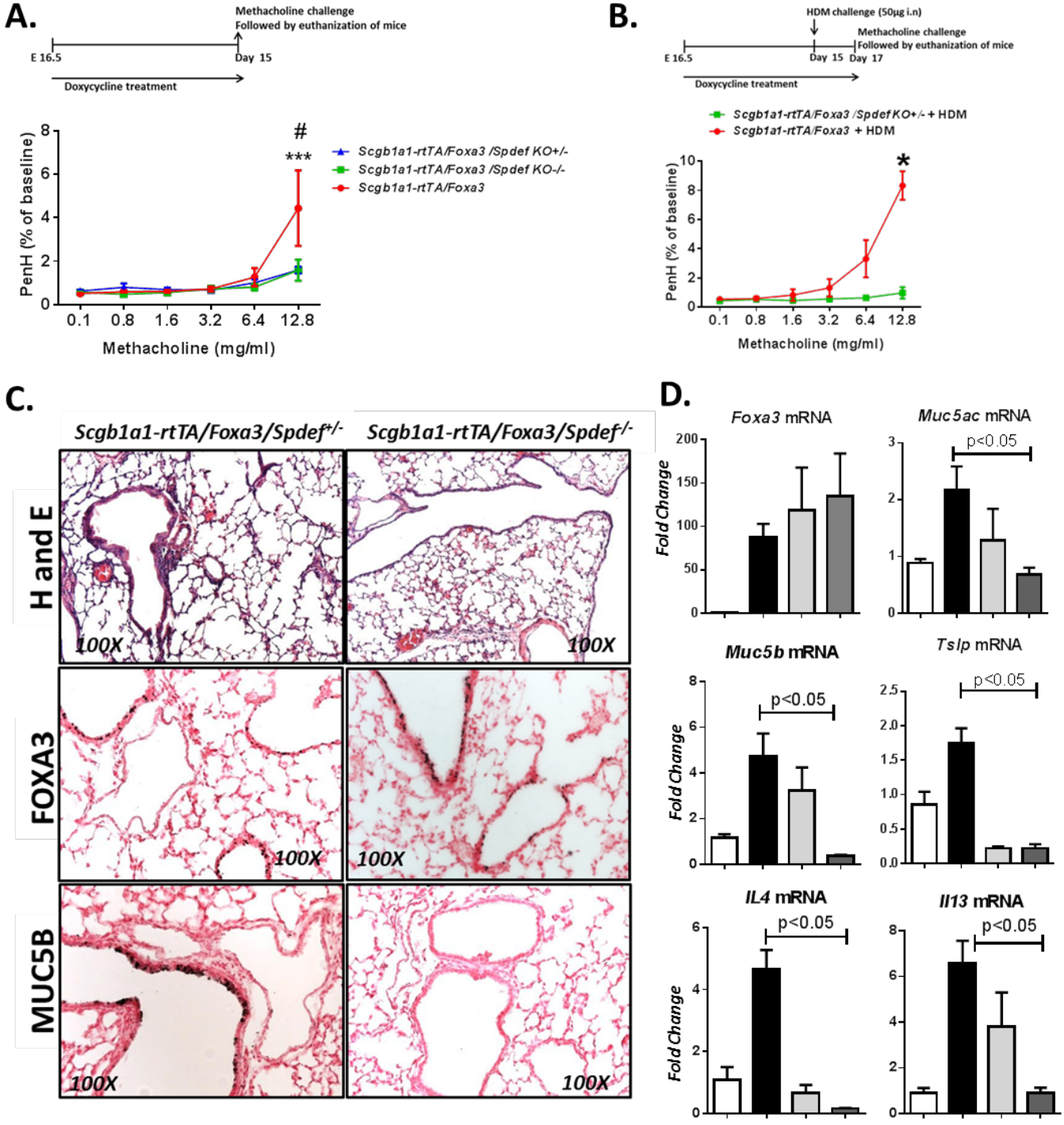

A.
IgE
IgE (µg/ml)
60000
40000
20000
0
Wild type+IgD
Spdef-/-+IgD
B.
IgG1
O.D. Values
1x10-1
1x10-1
5x10-2
0
Wild type+IgD
Spdef-/-+IgD
C.
Il4 mRNA
Fold Change
2.0
1.5
1.0
0.5
0.0
Wild type
Spdef -/-
D.
Il4 mRNA
p=0.3333
Fold Change
5
4
3
2
1
0
Wild type
Spdef -/-
E.
Total IgE
p=0.2
ng/ml
2500
2000
1500
1000
500
0
Wild type
Spdef -/-
F.
HDM specific IgE
0.4
0.3
0.2
0.1
0.0
G.
HDM specific IgG1
0.05
0.04
0.03
0.02
0.01
0.00
Wild type
Spdef -/-
H.
*
Total IgE (ng/ml)
400
300
200
100
0
I.
HDM specific IgE
0.4
0.3
0.2
0.1
0.0
J.
HDM specific IgG1
0.6
0.4
0.2
0.0

Figure S3


A.
Methacholine challenge
Followed by euthanization of mice
E 16.5
Day 15
Doxycycline treatment
PenH (% of baseline)
Methacholine (mg/ml)
0.1 0.8 1.6 3.2 6.4 12.8
#
***
Scgb1a1-rtTA/Foxa3 /Spdef KO+/-
Scgb1a1-rtTA/Foxa3 /Spdef KO-/-
Scgb1a1-rtTA/Foxa3
B.
HDM challenge (50µg i.n)
Methacholine challenge
Followed by euthanization of mice
E 16.5
Day 15
Day 17
Doxycycline treatment
Scgb1a1-rtTA/Foxa3 /Spdef KO+/- + HDM
Scgb1a1-rtTA/Foxa3 + HDM
*
C.
Scgb1a1-rtTA/Foxa3/Spdef+/-
Scgb1a1-rtTA/Foxa3/Spdef-/-
H and E
FOXA3
MUC5B
100X
D.
Foxa3 mRNA
Muc5ac mRNA
Muc5b mRNA
Tslp mRNA
IL4 mRNA
Il13 mRNA
Fold Change
p<0.05

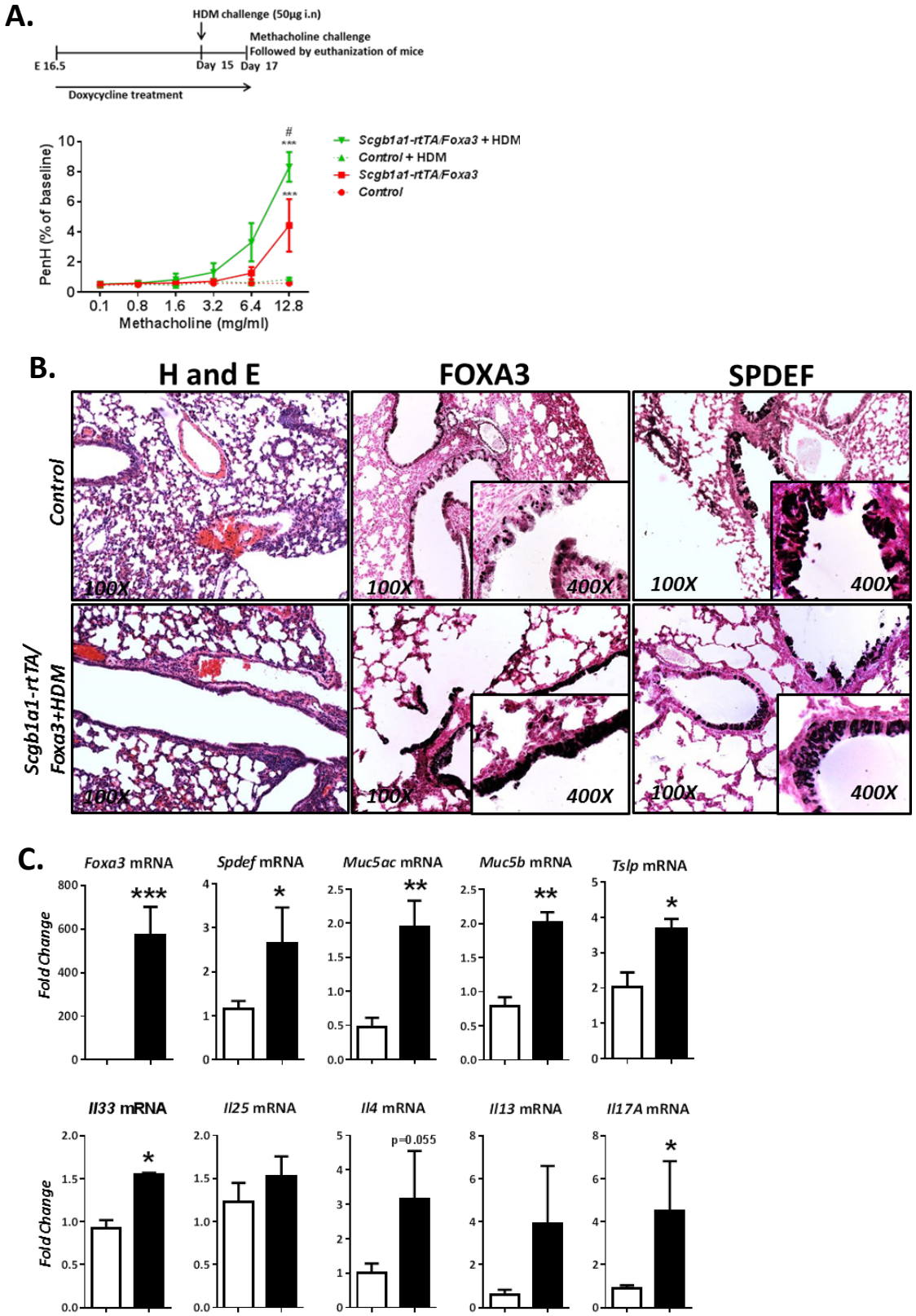
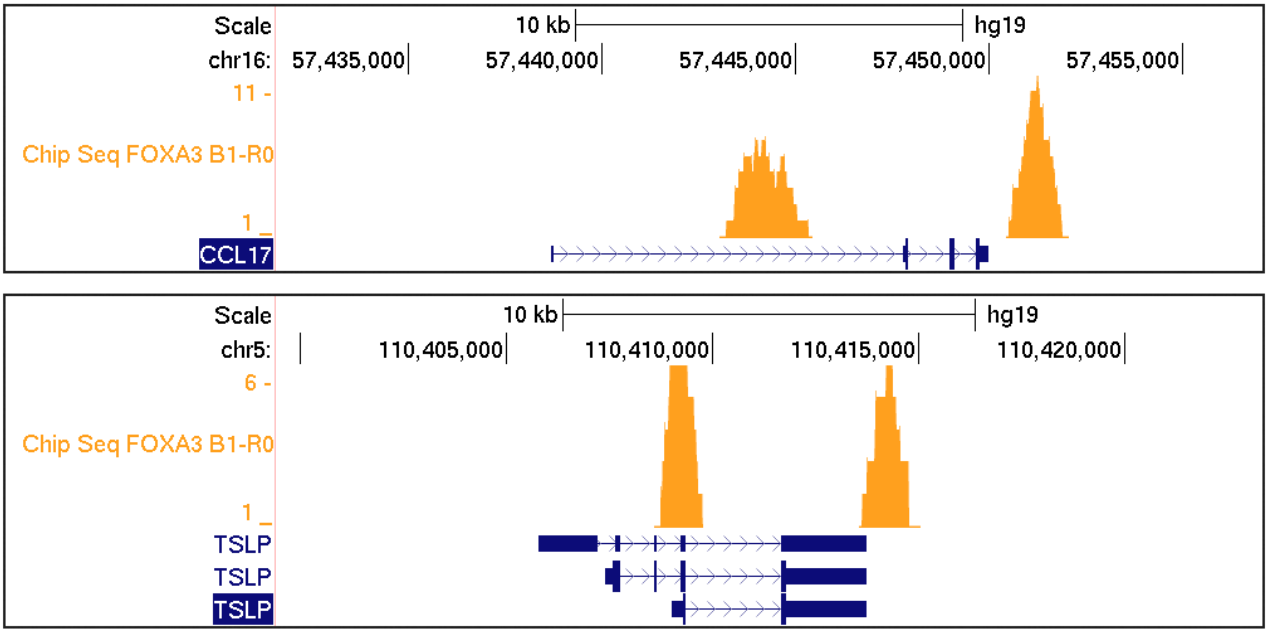

A.
HDM challenge (50µg i.n)
Methacholine challenge
Followed by euthanization of mice
E 16.5
Day 15
Day 17
Doxycycline treatment
PenH (% of baseline)
Methacholine (mg/ml)
0.1
0.8
1.6
3.2
6.4
12.8
Scgb1a1-rtTA/Foxa3 + HDM
Control + HDM
Scgb1a1-rtTA/Foxa3
Control
B.
H and E
FOXA3
SPDEF
Control
Scgb1a1-rtTA/ Foxa3+HDM
100X
400X
C.
Foxa3 mRNA
Spdef mRNA
Muc5ac mRNA
Muc5b mRNA
Tslp mRNA
Fold Change
Il33 mRNA
Il25 mRNA
Il4 mRNA
Il13 mRNA
Il17A mRNA
p=0.055

Figure S5


Scale
10 kb
hg19
chr16:
57,435,000
57,440,000
57,445,000
57,450,000
57,455,000
11 -
Chip Seq FOXA3 B1-R0
1 _
CCL17
Scale
10 kb
hg19
chr5:
110,405,000
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110,420,000
6 -
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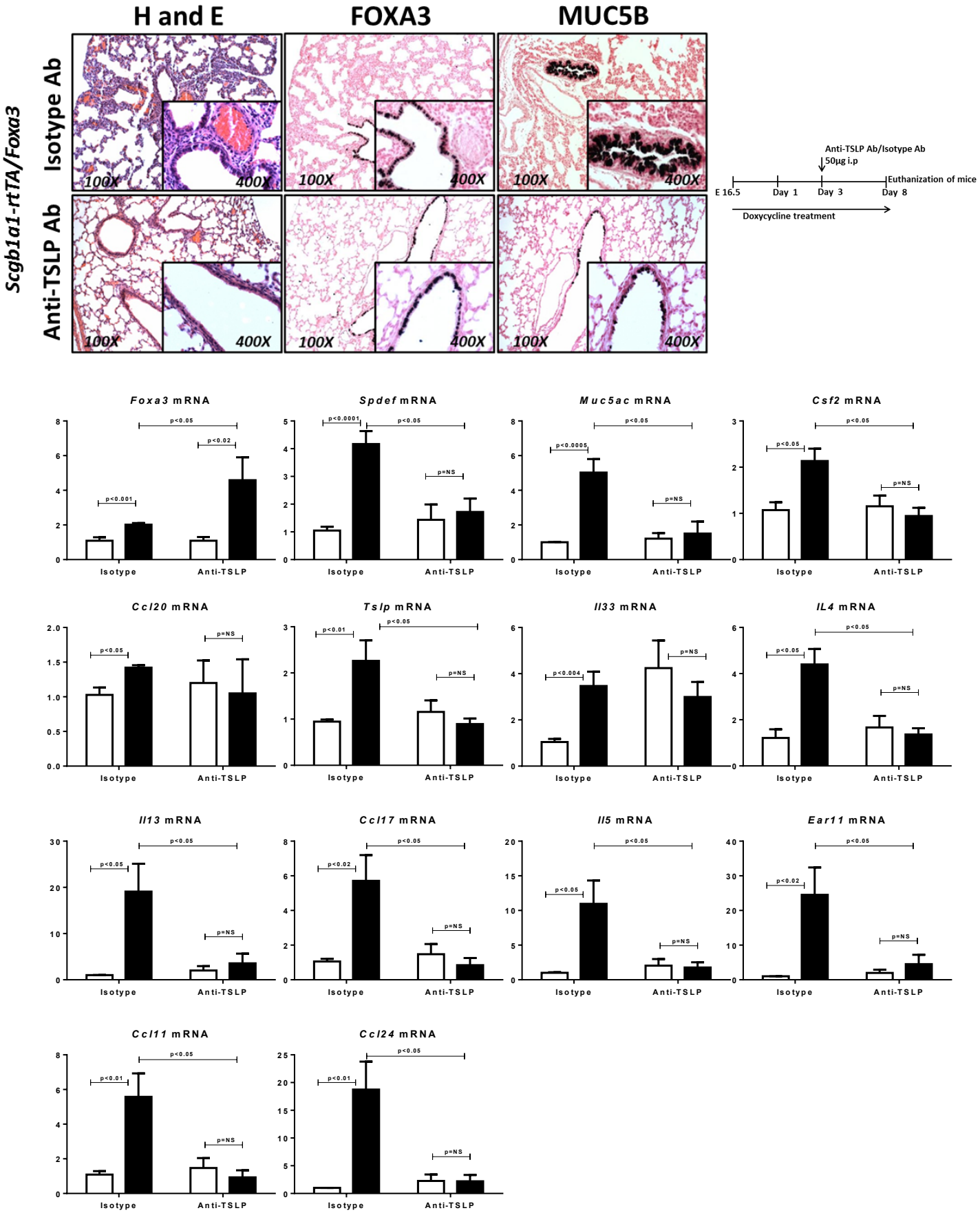
1 _
TSLP
TSLP
TSLP

Figure S6


H and E
FOXA3
MUC5B
Scgb1a1-rtTA/Foxa3
Isotype Ab
Anti-TSLP Ab
100X
400X
Anti-TSLP Ab/Isotype Ab
50µg i.p
E 16.5
Day 1
Day 3
Day 8
Euthanization of mice
Doxycycline treatment
Foxa3 mRNA
Spdef mRNA
Muc5ac mRNA
Csf2 mRNA
Ccl20 mRNA
Tslp mRNA
Il33 mRNA
IL4 mRNA
Il13 mRNA
Ccl17 mRNA
Il5 mRNA
Ear11 mRNA
Ccl11 mRNA
Ccl24 mRNA
Isotype
Anti-TSLP
p<0.05
p<0.02
p<0.001
p<0.0001
p=NS
p<0.0005
p<0.01
p<0.004