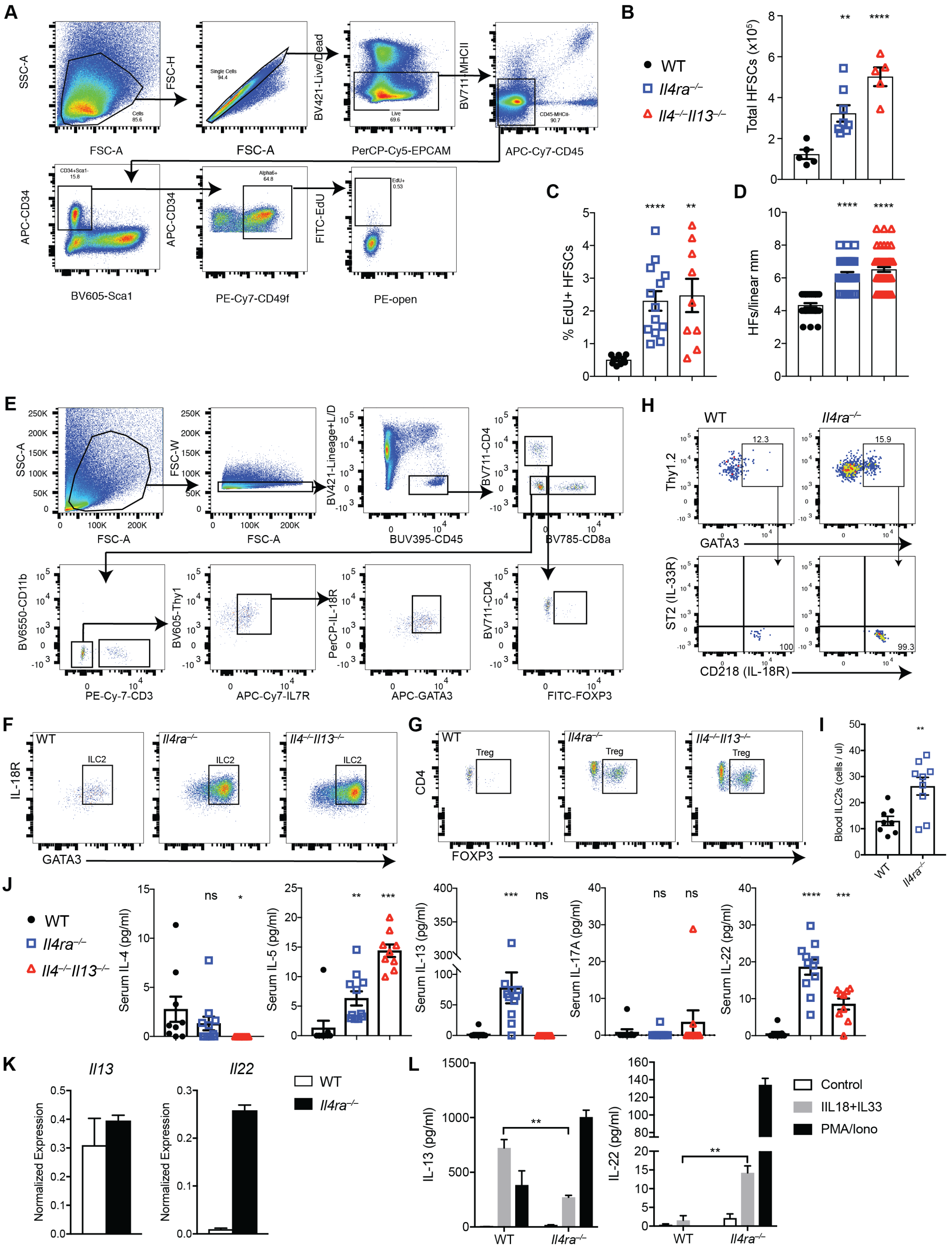
# Figure S1

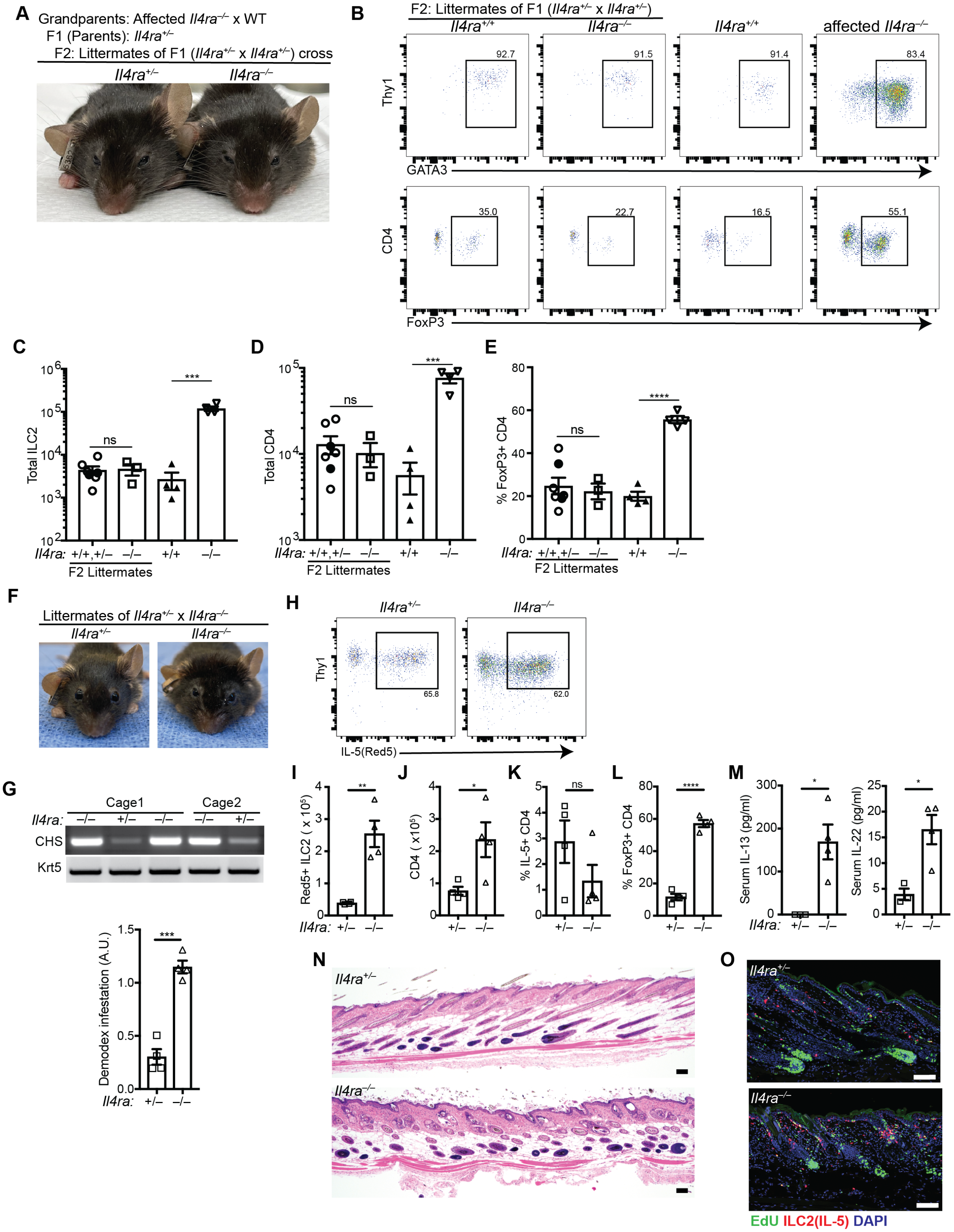


A
SSC-A
FSC-A
Cells 85.6
FSC-H
Single Cells 94.4
BV421-Live/Dead
Live 69.6
PerCP-Cy5-EPCAM
BV711-MHCII
CD45-MHCII- 90.7
APC-Cy7-CD45
APC-CD34
CD34+Sca1- 15.8
BV605-Sca1
Alpha6+ 64.8
PE-Cy7-CD49f
FITC-EdU
EdU+ 0.53
PE-open
B
● WT
□ Il4ra−/−
△ Il4−/−Il13−/−
Total HFSCs (x10^5)
**
****
C
% EdU+ HFSCs
****
**
D
HFs/linear mm
****
****
E
SSC-A
FSC-A
FSC-W
BV421-Lineage+L/D
BUV395-CD45
BV711-CD4
BV785-CD8a
BV6550-CD11b
PE-Cy-7-CD3
BV605-Thy1
APC-Cy7-IL7R
PerCP-IL-18R
APC-GATA3
BV711-CD4
FITC-FOXP3
H
WT
Il4ra−/−
Thy1.2
12.3
15.9
GATA3
ST2 (IL-33R)
100
99.3
CD218 (IL-18R)
F
WT
Il4ra−/−
Il4−/−Il13−/−
ILC2
IL-18R
GATA3
G
WT
Il4ra−/−
Il4−/−Il13−/−
Treg
CD4
FOXP3
I
Blood ILC2s (cells / ul)
**
WT
Il4ra−/−
J
● WT
□ Il4ra−/−
△ Il4−/−Il13−/−
Serum IL-4 (pg/ml)
ns
*
Serum IL-5 (pg/ml)
**
***
Serum IL-13 (pg/ml)
***
ns
Serum IL-17A (pg/ml)
ns
ns
Serum IL-22 (pg/ml)
****
***
K
Il13
Il22
Normalized Expression
□ WT
■ Il4ra−/−
L
IL-13 (pg/ml)
**
WT
Il4ra−/−
IL-22 (pg/ml)
**
□ Control
▨ IIL18+IL33
■ PMA/Iono

**Figure S1. Type 2 immune-deficient strains have an increased *Demodex* burden and expansion of skin ILC2s with extrusion into the circulation, related to Figure 1.**

(**A**) Gating strategy for hair follicle stem cells (HFSCs). (**B**) Number of HFSCs by flow cytometry. (**C**) Percent EdU$^+$ HFSCs by flow cytometry. (**D**) Quantification of visualized hair follicles per 1mm of skin. (**E**) Gating strategy for skin cell populations using various mouse strains. (**F**) Flow cytometry of skin ILC2s (CD45$^+$Lin$^-$Thy1$^+$IL-7R$^+$GATA3$^+$) in 8-12 weeks old WT, *Il4ra*$^{-/-}$, and *Il4*$^{-/-}$,*Il13*$^{-/-}$ mice at homeostasis. (**G**) Flow cytometry of skin T regulatory (Treg) cells ((CD3$^+$CD4$^+$FoxP3$^+$) in 8-12-week-old WT, *Il4ra*$^{-/-}$, and *Il4*$^{-/-}$,*Il13*$^{-/-}$ mice at homeostasis. (**H**) Flow cytometry plot for blood ILC2s (gated CD45$^+$Lin$^-$Thy1$^+$GATA3$^+$) in WT or *Demodex*-infested *Il4ra*$^{-/-}$ mice. Expression of ST2 (IL-33R) and IL-18R in gated ILC2s as indicated. (**I**) Frequency of blood ILC2s in WT or Demodex-infested *Il4ra*$^{-/-}$ mice. (**J**) Serum IL-4, IL-5, IL-13, IL-17A, IL-22 in WT and Demodex-infested *Il4ra*$^{-/-}$ and *Il4*$^{-/-}$,*Il13*$^{-/-}$ mice. (**K**) QPCR analyses of *Il13* and *Il22* expression in sorted skin ILC2s from *Demode*-infested WT and *Il4ra*$^{-/-}$ mice. (**L**) IL-13 and IL-22 in supernatant of sorted skin ILC2s from *Demodex*-infested WT and *Il4ra*$^{-/-}$ mice cultured with IL-7+TSLP (Control); IL-7+TSLP+IL-18+IL-133 (IL-18+IL-33) or IL-7+TSLP+PMA/Ionomycin (PMA/Iono). Data presented as mean ± s.e.m. Data are from one representative experiment (B, K, L**)** of at least two independent experiments, or pooled from multiple independent experiments (C, D, I, J). * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$ by two-tailed Student's t test.

# Figure S2



A
Grandparents: Affected Il4ra−/− x WT
F1 (Parents): Il4ra+/−
F2: Littermates of F1 (Il4ra+/− x Il4ra+/−) cross
Il4ra+/−
Il4ra−/−
B
F2: Littermates of F1 (Il4ra+/− x Il4ra+/−)
Il4ra+/+
Il4ra−/−
Il4ra+/+
affected Il4ra−/−
Thy1
GATA3
92.7
91.5
91.4
83.4
CD4
FoxP3
35.0
22.7
16.5
55.1
C
Total ILC2
10^6
10^5
10^4
10^3
10^2
ns
***
Il4ra: +/+,+/− −/− +/+ −/−
F2 Littermates
D
Total CD4
10^5
10^4
10^3
ns
***
Il4ra: +/+,+/− −/− +/+ −/−
F2 Littermates
E
% FoxP3+ CD4
80
60
40
20
0
ns
****
Il4ra: +/+,+/− −/− +/+ −/−
F2 Littermates
F
Littermates of Il4ra+/− x Il4ra−/−
Il4ra+/−
Il4ra−/−
G
Cage1
Cage2
Il4ra: −/− +/− −/− −/− +/−
CHS
Krt5
Demodex infestation (A.U.)
1.5
1.0
0.5
0.0
***
Il4ra: +/− −/−
H
Il4ra+/−
Il4ra−/−
Thy1
IL-5(Red5)
65.8
62.0
I
Red5+ ILC2 ( x 10^5)
4
3
2
1
0
**
Il4ra: +/− −/−
J
CD4 ( x10^5)
4
3
2
1
0
*
+/− −/−
K
% IL-5+ CD4
5
4
3
2
1
0
ns
+/− −/−
L
% FoxP3+ CD4
80
60
40
20
0
****
+/− −/−
M
Serum IL-13 (pg/ml)
300
200
100
0
*
Il4ra: +/− −/−
Serum IL-22 (pg/ml)
25
20
15
10
5
0
*
+/− −/−
N
Il4ra+/−
Il4ra−/−
O
Il4ra+/−
Il4ra−/−
EdU ILC2(IL-5) DAPI

**Figure S2. Mice with intact type 2 immunity do not show the altered skin phenotype associated with *Demodex* infection, related to Figure 3.**

(**A**) Representative pictures of *Il4ra*$^{+/+}$ and *Il4ra*$^{-/-}$ F2 mice generated from F1 intercross of *Il4ra*$^{+/-}$ parent mice. The F1 *Il4ra*$^{+/-}$ mice were derived by crossing unaffected WT x affected *Il4ra*$^{-/-}$ grandparents. (**B**) Flow cytometry plots of ILC2s (top, gated CD45$^{+}$Lin$^{-}$CD3$^{-}$CD4$^{-}$Thy1$^{+}$IL7R$^{+}$) or CD4 (bottom, gated CD45$^{+}$CD3$^{+}$CD4$^{+}$) from F2 *Il4ra*$^{+/+}$, and *Il4ra*$^{-/-}$ mice generated from F1 *Il4ra*$^{+/-}$ intercross or age matched WT and affected *Il4ra*$^{-/-}$ experimental groups. (**C** to **E**) Quantification of ILC2 (C), CD4 (D), and frequency of Treg cells (E). In the *Il4ra*$^{+/+}$, and *Il4ra*$^{+/-}$ experimental group, closed circles represent WT mice and open circles represent heterozygous mice. (**F**) Representative pictures of *Il4ra*$^{+/-}$ *,Il5*$^{Red5}$ and *Il4ra*$^{-/-}$*,Il5*$^{Red5}$ littermates generated from visually unaffected *Il4ra*$^{+/-}$ x affected *Il4ra*$^{-/-}$ mice. The F1 *Il4ra*$^{+/-}$ mice were derived by crossing unaffected WT x affected *Il4ra*$^{-/-}$ parents. (**G**) PCR for *Demodex* chitinase synthase gene (CHS) or genomic DNA for the keratin 5 gene (Krt5) from back skin. Quantification of relative *Demodex* infestation for PCR is shown below. (**H**) Flow cytometry plots of Red5$^{+}$ ILC2s (pre-gated on Live CD45$^{+}$Lin$^{-}$Thy1$^{+}$) from littermate *Il4ra*$^{+/-}$ and *Il4ra*$^{-/-}$ mice. (**I** to **L**) Quantification of Red5$^{+}$ ILC2s (I), total CD4 (J), frequency of IL-5$^{+}$(Red5$^{+}$) CD4 T cells in skin (K) and frequency of Treg cells (CD3$^{+}$CD4$^{+}$FoxP3$^{+}$) in skin (L). (**M**) Serum IL-13 and IL-22 from littermate *Il4ra*$^{+/-}$ and *Il4ra*$^{-/-}$ mice. (**N**) Skin sections stained with H&E. Scale bar, 100 μm. (**O**) Back skin sections were stained with EdU (green), anti-tdTomato (red, highlighting IL-5$^{+}$ cells), and DAPI (blue). Scale bar, 100 μm. Data presented as mean ± s.e.m and representative of two independent experiments. Statistical significance shown by * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$ by one-way ANOVA (C-E) or two-tailed Student's t test (H-L). ns, not significant.

**Figure S3**

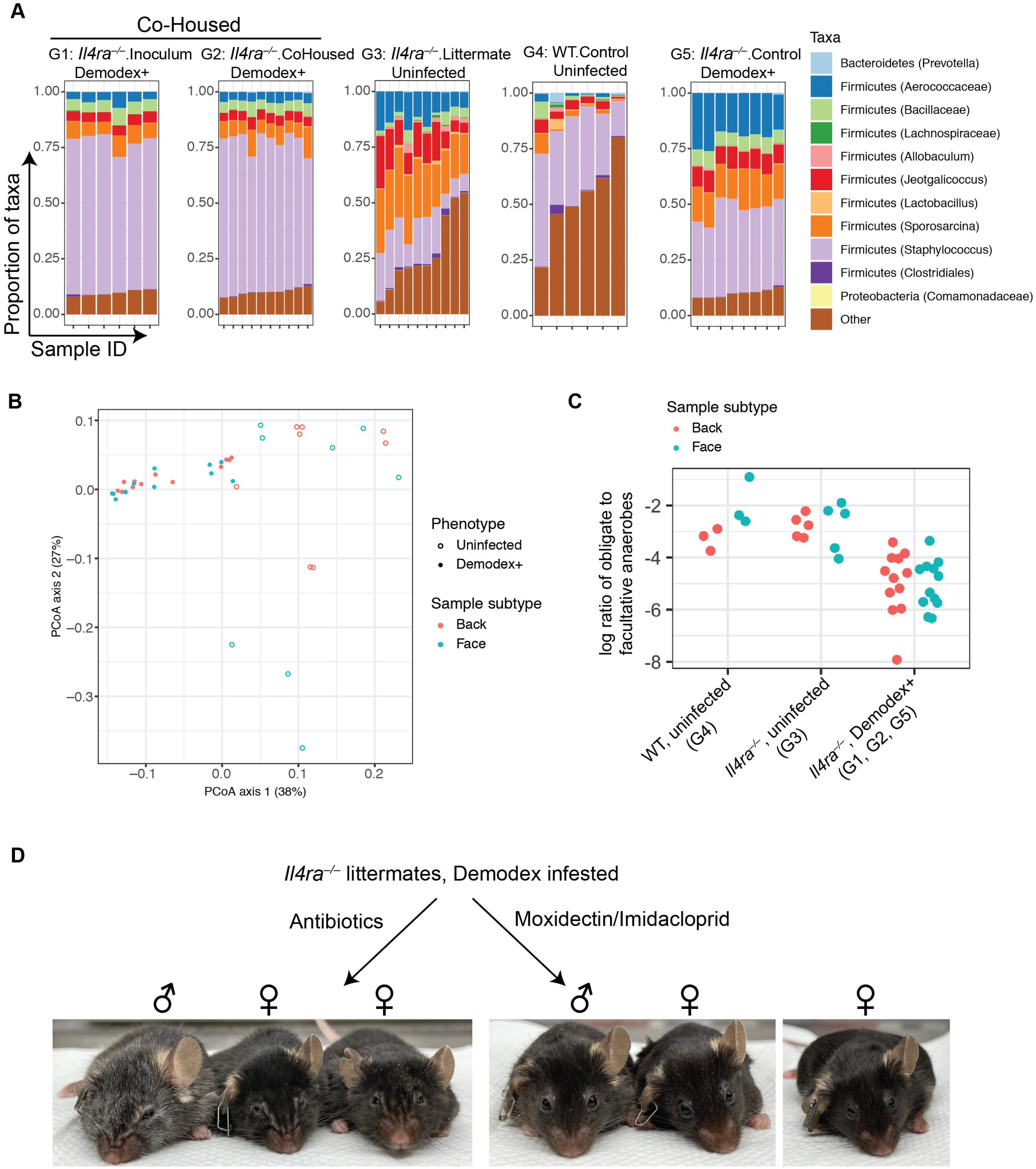


**Figure S3. 16S ribosomal RNA gene sequencing analysis of uninfected and *Demodex*-infected mice, related to Figure 3.**

(**A**) Compositional plots of the top 10 major taxa present in each sample of the co-housing experiment shown in Figure 3. Bacterial lineages are color coded and presented as stacked bar graphs. (**B**) PCoA plot based on the Bray-Curtis dissimilarity assessed using the microbiome data.

All samples were used to make a common plot. Samples were further classified by gross skin phenotype (uninfected, open circles; *Demodex*-infected (Demodex+), closed circles). The color of the symbol indicates the sampling site. (**C**) Ratio of obligate to facultative anaerobes abundance in uninfected and Demodex+ mice. (**D**) 8 week old, *Demodex*-infested $Il4ra^{-/-}$ littermates were separated and treated with broad spectrum antibiotics for 1 month in the drinking water or topically with Moxidectin and Imidacloprid weekly for 8 weeks.

# Figure S4

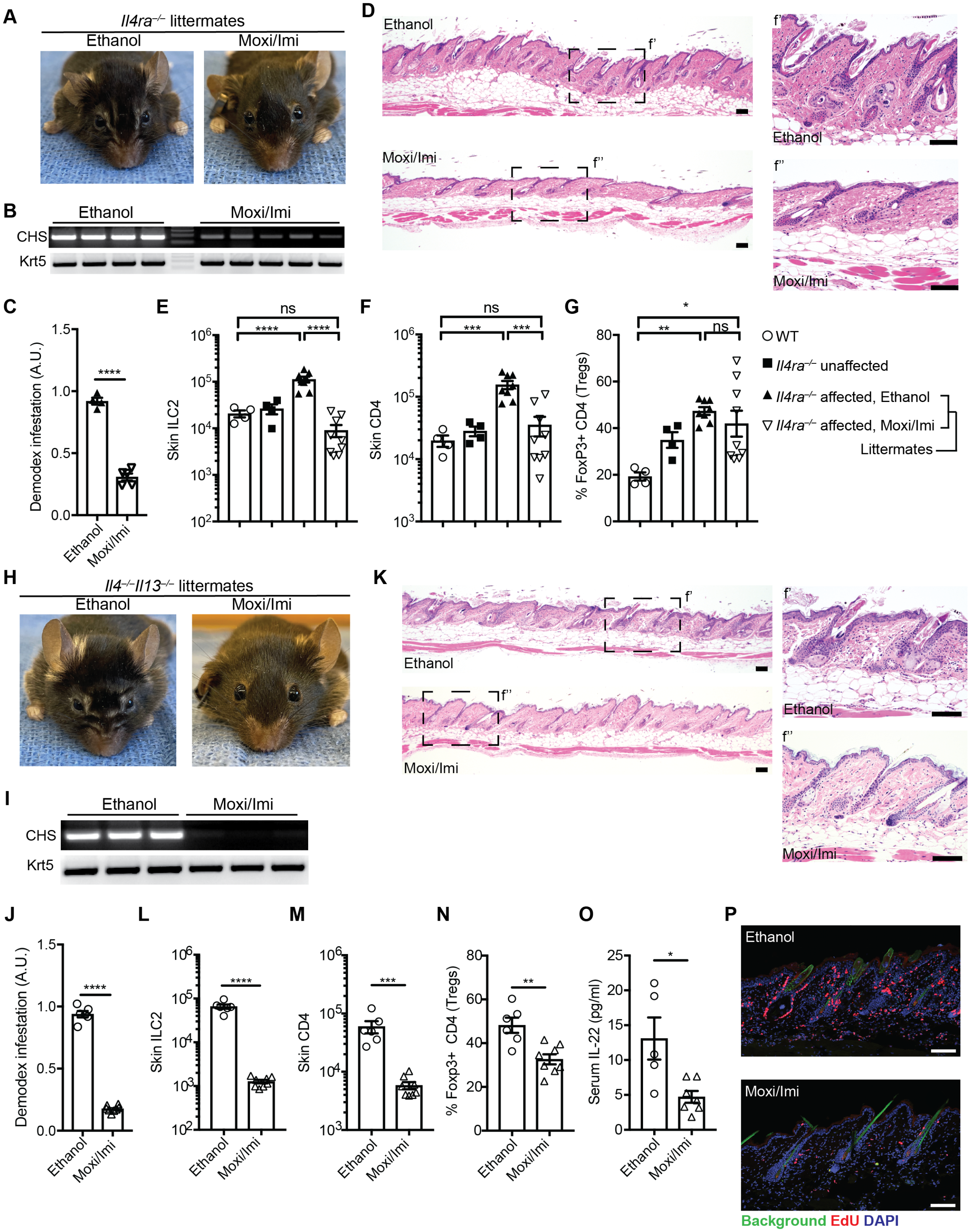

A
Il4ra−/− littermates
Ethanol
Moxi/Imi
B
Ethanol
Moxi/Imi
CHS
Krt5
C
Demodex infestation (A.U.)
1.5
1.0
0.5
0.0
****
Ethanol
Moxi/Imi
D
Ethanol
f'
Moxi/Imi
f''
E
Skin ILC2
$10^6$
$10^5$
$10^4$
$10^3$
$10^2$
ns
****
****
F
Skin CD4
$10^6$
$10^5$
$10^4$
$10^3$
ns
***
***
G
% FoxP3+ CD4 (Tregs)
80
60
40
20
0
*
**
ns
○ WT
■ Il4ra−/− unaffected
▲ Il4ra−/− affected, Ethanol
▽ Il4ra−/− affected, Moxi/Imi
Littermates
H
Il4−/−Il13−/− littermates
Ethanol
Moxi/Imi
I
Ethanol
Moxi/Imi
CHS
Krt5
K
Ethanol
f'
Moxi/Imi
f''
J
Demodex infestation (A.U.)
1.5
1.0
0.5
0.0
****
Ethanol
Moxi/Imi
L
Skin ILC2
$10^6$
$10^5$
$10^4$
$10^3$
$10^2$
****
Ethanol
Moxi/Imi
M
Skin CD4
$10^6$
$10^5$
$10^4$
$10^3$
***
Ethanol
Moxi/Imi
N
% Foxp3+ CD4 (Tregs)
80
60
40
20
0
**
Ethanol
Moxi/Imi
O
Serum IL-22 (pg/ml)
25
20
15
10
5
0
*
Ethanol
Moxi/Imi
P
Ethanol
Moxi/Imi
Background EdU DAPI

**Figure S4. Targeted therapy prevents the phenotype in *Demodex*-infested type 2 immunodeficient mice, related to Figure 3.**

(**A**) *Demodex*-infested *Il4ra*$^{-/-}$ littermates that were separated and treated with Ethanol (vehicle control) or Moxidectin and Imidacloprid mixture (Moxi/Imi) once a week for 8 weeks. (**B**) PCR for *Demodex* chitin synthase gene (CHS, top) or genomic DNA for the Keratin gene (Krt5, bottom) in Ethanol- or Moxi/Imi-treated mice. Each lane represents an individual mouse. (**C**) Quantification of relative band intensity (CHS/Krt5) for the PCR in (B). (**D**) Sections from back skin of Ethanol- or Moxi/Imi-treated mice were stained with H&E. Scale bar, 100 μm. (**E–G**) Quantification of skin ILC2 (D), CD4 (E), and frequencies of Treg cells in skin (F) from control WT, or unaffected *Il4ra*$^{-/-}$ or from *Demodex*-infested *Il4ra*$^{-/-}$ littermates that were treated with Ethanol or Moxi/Imi. (**H**) Representative pictures of *Il4*$^{-/-}$*,Il13*$^{-/-}$ mice. Littermates of known *Demodex* infested *Il4*$^{-/-}$*,Il13*$^{-/-}$ were separated at 3-6 weeks of age and treated with topical Ethanol or Moxi/Imi once a week for 8 weeks. (**I**) PCR for *Demodex* chitinase synthase gene (CHS) or genomic DNA for the keratin 5 gene (Krt5) from back skin. (**J**) Quantification of relative band intensity (CHS/Krt5) for the PCR in (I). (**K**) Sections from back skin of *Il4*$^{-/-}$*,Il13*$^{-/-}$ Ethanol- or Moxi/Imi-treated mice were stained with H&E. Scale bar, 100 μm. (**L–N**) Quantification of ILC2s (J), CD4s (K), and frequency of Treg cells (J) in *Il4*$^{-/-}$*,Il13*$^{-/-}$ mice treated with Ethanol or Moxi/Imi for 8 weeks. (**O**) Serum IL-22. (**P**) Sections from back skin of *Il4*$^{-/-}$*,Il13*$^{-/-}$ Ethanol- or Moxi/Imi-treated mice were stained for EdU (red) and DAPI (blue). Scale bar, 100 μm. Data presented as mean ± s.e.m and pooled from two independent experiments. Statistical significance shown by * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$ by one-way ANOVA (C, E-G) or two-tailed Student's t test (J-O). ns, not significant.

# Figure S5

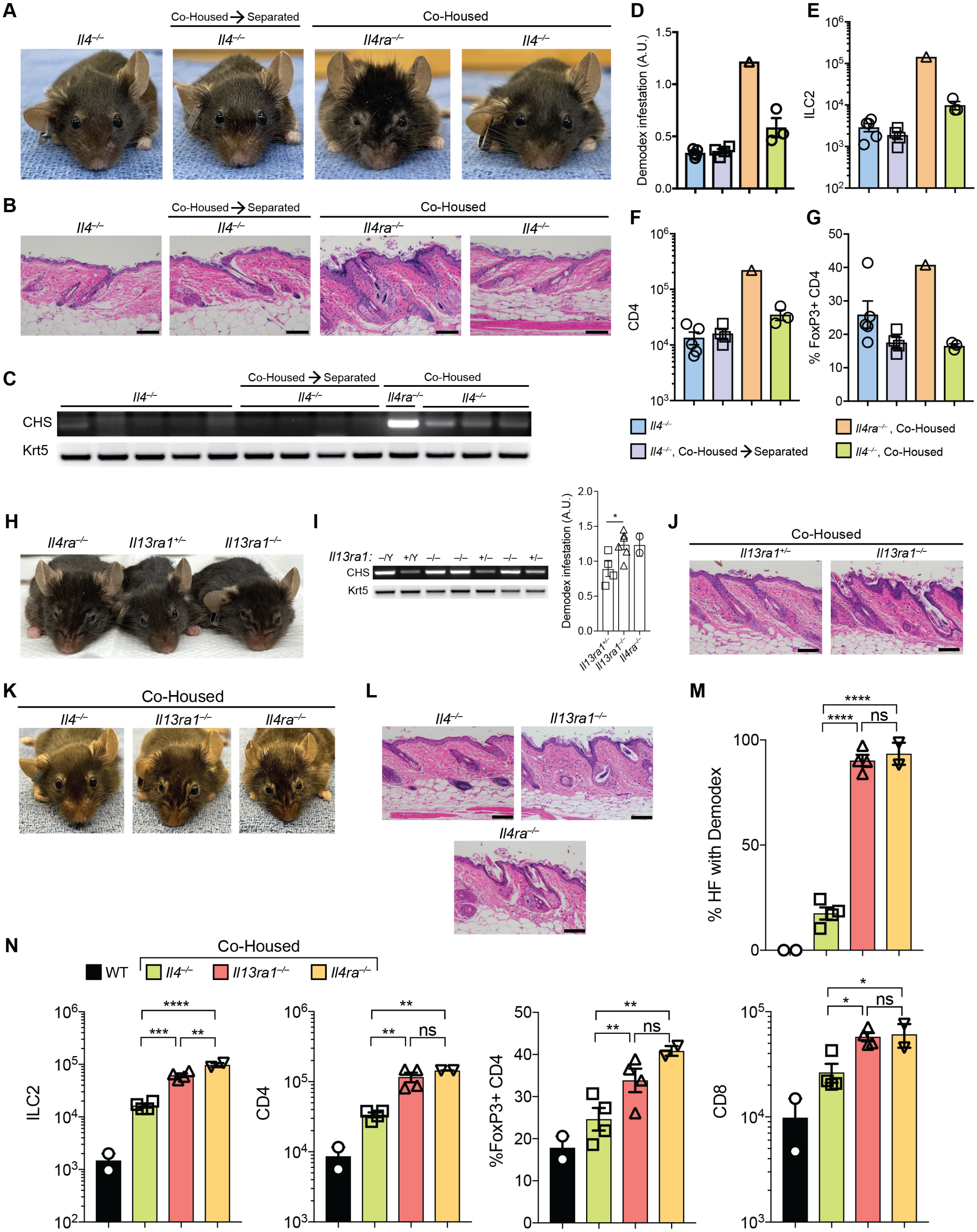

A
Il4–/–
Co-Housed → Separated
Il4–/–
Co-Housed
Il4ra–/–
Il4–/–
B
Il4–/–
Co-Housed → Separated
Il4–/–
Co-Housed
Il4ra–/–
Il4–/–
C
Co-Housed → Separated
Co-Housed
Il4–/–
Il4–/–
Il4ra–/–
Il4–/–
CHS
Krt5
D
Demodex infestation (A.U.)
0.0
0.5
1.0
1.5
E
ILC2
10^2
10^3
10^4
10^5
10^6
F
CD4
10^3
10^4
10^5
10^6
G
% FoxP3+ CD4
0
10
20
30
40
50
Il4–/–
Il4–/–, Co-Housed → Separated
Il4ra–/–, Co-Housed
Il4–/–, Co-Housed
H
Il4ra–/–
Il13ra1+/–
Il13ra1–/–
I
Il13ra1: –/Y +/Y –/– –/– +/– –/– +/–
CHS
Krt5
Demodex infestation (A.U.)
0.0
0.5
1.0
1.5
2.0
*
Il13ra1+/–
Il13ra1–/–
Il4ra–/–
J
Co-Housed
Il13ra1+/–
Il13ra1–/–
K
Co-Housed
Il4–/–
Il13ra1–/–
Il4ra–/–
L
Il4–/–
Il13ra1–/–
Il4ra–/–
M
% HF with Demodex
0
50
100
****
****
ns
N
Co-Housed
WT
Il4–/–
Il13ra1–/–
Il4ra–/–
ILC2
10^2
10^3
10^4
10^5
10^6
****
***
**
CD4
10^3
10^4
10^5
10^6
**
**
ns
%FoxP3+ CD4
0
10
20
30
40
50
**
**
ns
CD8
10^3
10^4
10^5
*
*
ns

**Figure S5. Susceptibility to *Demodex* infection is dependent on IL-13Ra1 but not IL-4 deficiency, related to Figure 4.**

(**A**) Representative uninfected *Il4*$^{-/-}$, *Il4*$^{-/-}$ co-housed with *Demodex*-infested *Il4ra*$^{-/-}$ mice for 8 weeks then separated for 8 weeks, or co-housed *Il4*$^{-/-}$ mice with *Demodex*-infested *Il4ra*$^{-/-}$ for 12 weeks. (**B**) Skin sections, as in (A) stained for H&E. Scale bar, 100 μm.
(**C**) PCR for *Demodex* chitinase synthase gene (CHS, top) or genomic DNA for the keratin 5 gene (Krt5, bottom) from back skin. (**D**) Quantification of relative *Demodex* infestation by PCR in (C). (**E** to **G**) Number of skin ILC2s (E), CD4 (F), and frequency of Treg cells (G) (as percentage of total CD4) in back skin. (**H**) Representative co-housed *Demodex*-infested *Il4ra*$^{-/-}$, *Il13ra1*$^{+/-}$ and *Il13ra1*$^{-/-}$ mice. (**I**) PCR for *Demodex* chitinase synthase gene (CHS, top) or genomic DNA for the keratin 5 gene (Krt5, bottom) from back skin and quantification of relative *Demodex* infestation (right). (**J**) Skin sections stained for H&E from *Demodex*-infested *Il13ra1*$^{+/-}$ and *Il13ra1*$^{-/-}$ mice. Scale bar, 100 μm. (**K**) Representative co-housed *Il4*$^{-/-}$, *Il13ra1*$^{-/-}$ and *Il4ra*$^{-/-}$ mice. (**L**) Skin sections stained for H&E from mice as in (K). Scale bar, 100 μm. (**M**) Quantification of proportion of HF visually infested by *Demodex* mites. (**N**) Number of skin ILC2s, CD4, frequency of Treg cells (as percentage of total CD4), and CD8 in back skin. Data presented as mean ± s.e.m and representative of two independent experiments. Statistical significance shown by * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$ by one-way ANOVA. ns, not significant.

# Figure S6

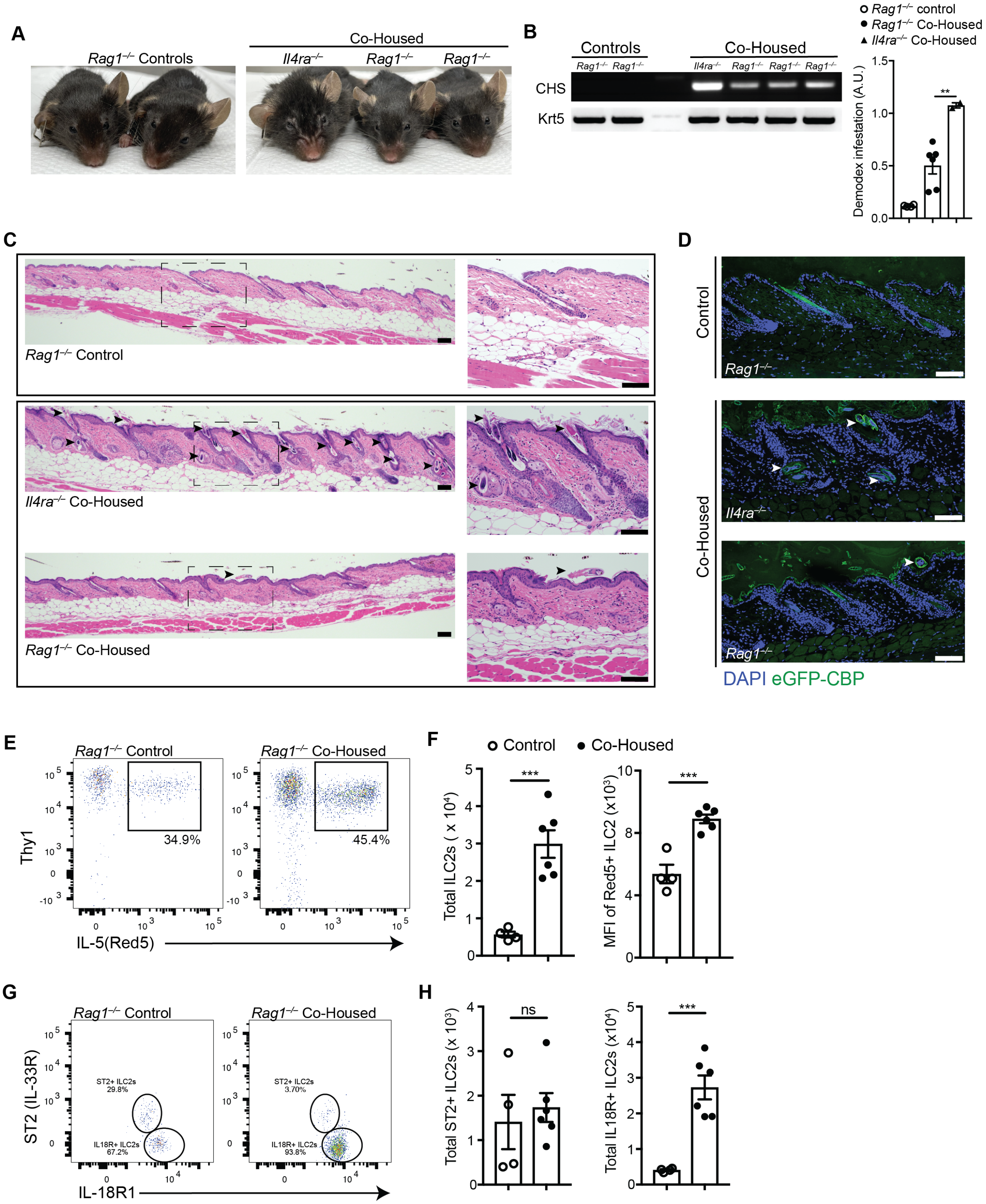
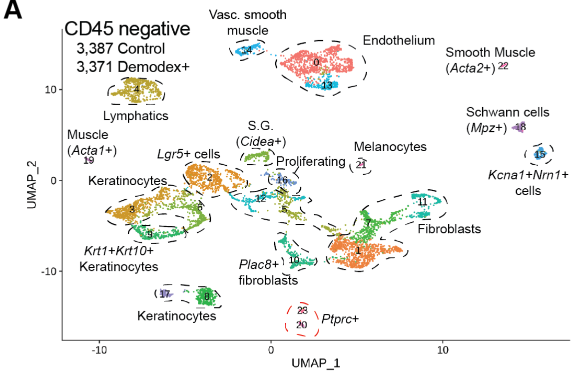

A
Rag1–/– Controls
Co-Housed
Il4ra–/–
Rag1–/–
Rag1–/–
B
Controls
Rag1–/–
Rag1–/–
Co-Housed
Il4ra–/–
Rag1–/–
Rag1–/–
Rag1–/–
CHS
Krt5
○ Rag1–/– control
● Rag1–/– Co-Housed
▲ Il4ra–/– Co-Housed
Demodex infestation (A.U.)
1.5
1.0
0.5
0.0
**
C
Rag1–/– Control
Il4ra–/– Co-Housed
Rag1–/– Co-Housed
D
Control
Rag1–/–
Co-Housed
Il4ra–/–
Rag1–/–
DAPI eGFP-CBP
E
Rag1–/– Control
Rag1–/– Co-Housed
Thy1
$10^5$
$10^4$
$10^3$
0
$-10^3$
34.9%
45.4%
0
$10^3$
$10^5$
IL-5(Red5)
F
○ Control
● Co-Housed
Total ILC2s ( x $10^4$)
5
4
3
2
1
0
***
MFI of Red5+ ILC2 (x$10^3$)
10
8
4
0
***
G
Rag1–/– Control
Rag1–/– Co-Housed
ST2 (IL-33R)
ST2+ ILC2s
29.8%
IL18R+ ILC2s
67.2%
ST2+ ILC2s
3.70%
IL18R+ ILC2s
93.8%
$10^5$
$10^4$
$10^3$
0
$10^4$
IL-18R1
H
Total ST2+ ILC2s (x $10^3$)
4
3
2
1
0
ns
Total IL18R+ ILC2s (x$10^4$)
5
4
3
2
1
0
***

**Figure S6. ILC-sufficient mice are protected from high-burden *Demodex* infection, related to Figure 5.**

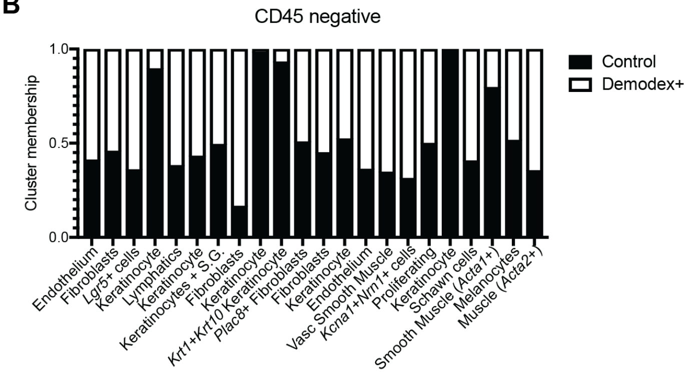
(**A**) Representative images of *Rag1*$^{-/-}$,*Il5*$^{Red5/Red5}$ littermates that were maintained by themselves (Control) or Co-Housed with *Demodex*-infested *Il4ra*$^{-/-}$ mice (Co-Housed) for 8 weeks. (**B**) PCR for *Demodex* chitin synthase gene (CHS, top) or genomic DNA for the keratin 5 gene (Krt5, bottom) from back skin and quantification of relative *Demodex* infestation (right). (**C**) Skin sections from *Rag1*$^{-/-}$,*Il5*$^{Red5/Red5}$ control mice and *Demodex*-infected *Il4ra*$^{-/-}$ mice co-housed with *Rag1*$^{-/-}$,*Il5*$^{Red5/Red5}$ mice were stained with H&E. (**D**) Skin sections as in (C), stained for chitin (green, eGFP-CBP) and DAPI (blue). Arrowheads highlight *Demodex* mites. All scale bars, 100 μm. (**E**) Flow cytometry plots of Red5$^{+}$ ILC2s (pre-gated on Live CD45$^{+}$Lin$^{-}$Thy1$^{+}$) from *Rag1*$^{-/-}$,*Il5*$^{Red5/Red5}$ (control) or *Rag1*$^{-/-}$,*Il5*$^{Red5/Red5}$ that were co-housed with *Demodex*-infested *Il4ra*$^{-/-}$ mice (Co-Housed). (**F**) Quantification of the number of Red5$^{+}$ ILC2s (left), and mean fluorescence intensity (MFI) of Red5$^{+}$ ILC2s (right) in skin. (**G**) Flow cytometry plots showing ST2 (IL-33R) and IL-18R expression by skin ILC2s (pre-gated on Live CD45$^{+}$Lin$^{-}$Thy1$^{+}$Red5$^{+}$) from *Rag1*$^{-/-}$,*Il5*$^{Red5/Red5}$ (control) or *Rag1*$^{-/-}$,*Il5*$^{Red5/Red5}$ that were co-housed with *Il4ra*$^{-/-}$ mice (Co-Housed). (**H**) Quantification of the total of ST2$^{+}$ (left) or IL-18R$^{+}$ (right) Red5$^{+}$ ILC2s in full thickness back skin. Data presented as mean ± s.e.m and pooled from 2 independent cohorts. Statistical significance shown by *** $P < 0.001$ by two-tailed Student's t test. ns, not significant.

# Figure S7

**A**



**B**

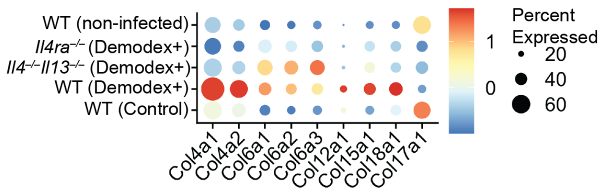


**C**

### Upregulated in WT Demodex+ vs. WT Control

| GO term | Description | P-value | FDR q-value |
|---|---|---|---|
| GO:0030020 | extracellular matrix structural constituent conferring tensile strength | 3.98E-06 | 4.32E-03 |
| GO:0005201 | extracellular matrix structural constituent | 4.14E-05 | 2.25E-02 |
| GO:0061134 | peptidase regulator activity | 5.98E-05 | 2.17E-02 |
| GO:0005198 | structural molecule activity | 8.33E-05 | 2.26E-02 |
| GO:0005125 | cytokine activity | 8.75E-05 | 1.90E-02 |
| GO:0008009 | chemokine activity | 2.10E-04 | 3.80E-02 |
| GO:0030414 | peptidase inhibitor activity | 8.43E-04 | 1.31E-01 |

**D**



**E**

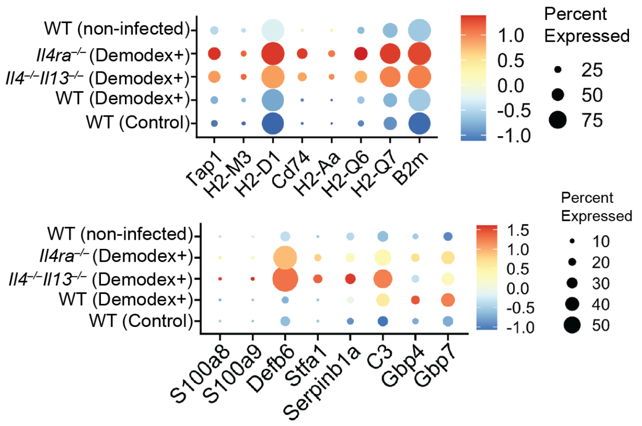
### Upregulated in *Il4ra*$^{-/-}$ Demodex+ vs. WT (uninfected)

| GO term | Description | P-value | FDR q-value |
|---|---|---|---|
| GO:0043207 | response to external biotic stimulus | 2.55E-14 | 2.31E-10 |
| GO:0002376 | immune system process | 1.50E-13 | 4.52E-10 |
| GO:0006952 | defense response | 3.18E-13 | 5.76E-10 |
| GO:0019882 | antigen processing and presentation | 4.39E-10 | 3.98E-07 |
| GO:0009617 | response to bacterium | 2.24E-09 | 1.45E-06 |
| GO:0019730 | antimicrobial humoral response | 2.65E-08 | 1.41E-05 |
| GO:0098542 | defense response to other organism | 3.06E-08 | 1.54E-05 |
| GO:0034341 | response to interferon-gamma | 3.65E-08 | 1.74E-05 |

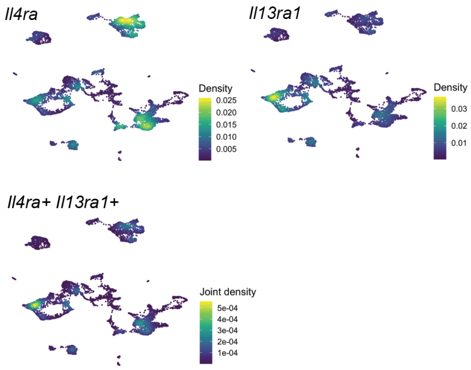
### Upregulated in *Il4*$^{-/-}$*Il13*$^{-/-}$ Demodex+ vs. WT (uninfected)

| GO term | Description | P-value | FDR q-value |
|---|---|---|---|
| GO:0006952 | defense response | 1.41E-16 | 1.33E-12 |
| GO:0002682 | regulation of immune system process | 7.22E-14 | 3.40E-10 |
| GO:0043207 | response to external biotic stimulus | 1.07E-12 | 2.03E-09 |
| GO:0006954 | inflammatory response | 1.25E-10 | 1.18E-07 |
| GO:0070098 | chemokine-mediated signaling pathway | 1.47E-10 | 1.26E-07 |
| GO:0009617 | response to bacterium | 2.33E-09 | 1.57E-06 |
| GO:0071621 | granulocyte chemotaxis | 7.98E-09 | 4.19E-06 |
| GO:0019882 | antigen processing and presentation | 1.49E-08 | 6.71E-06 |
| GO:0034341 | response to interferon-gamma | 5.30E-07 | 1.35E-04 |

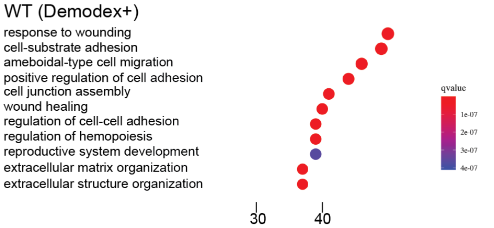
**F**



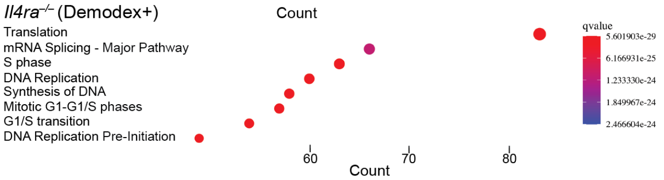
**G**



**H**



**I**



**J**

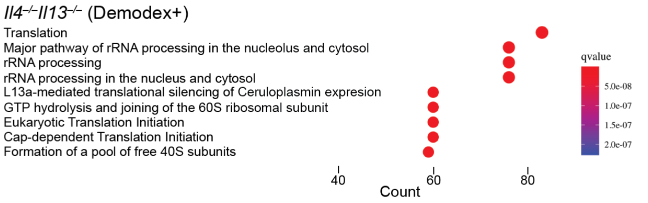
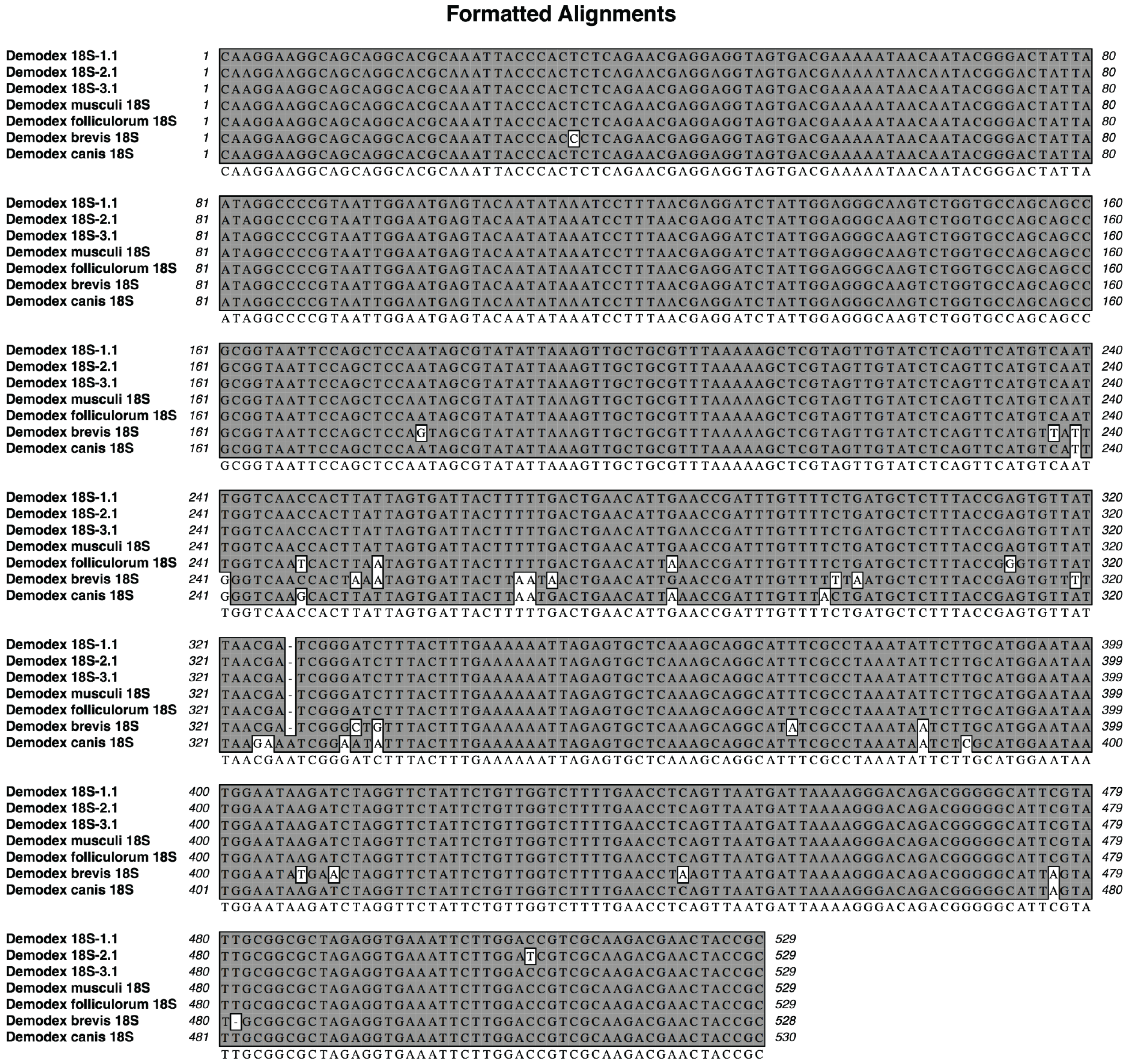


**Figure S7. scRNA-seq of *Demodex*-infected mice reveal a divergent tissue response in WT and type 2 immunodeficient mice, related to Figure 6.**

(**A**) UMAP projection of the CD45$^{-}$ cells from skin of WT control and *Demodex*-infected mice. (**B**) Bar plot showing the percentage of cells in (A) for each cluster by experimental condition. S.G., sebaceous glands. (**C**) Gene Ontology (GO) analyses for biological processes upregulated across all CD45$^{-}$ cells in *Demodex*-infected WT mice vs. WT control. (**D**) Dot plot of representative genes associated with GO term related to tensile strength. (**E**) Gene Ontology (GO) analyses for biological processes upregulated across all CD45$^{-}$ cells in *Demodex*-infected *Il4ra*$^{-/-}$ (top) and *Il4*$^{-/-}$,*Il13*$^{-/-}$ (bottom) *Demodex*-infected mice vs. WT (non-infected) control. (**F**) Dot plot of representative genes associated with antigen presentation and anti-microbial response. (**G**) Density plot showing expression of *Il4ra*, *Il13ra1* or joint *Il4ra* and *Il13ra1* by CD45$^{-}$ cells (UMAP as in A). (**H–J**) GO biological processes upregulated in stem cells in WT (H), IL-4Ra$^{-/-}$ (I), or *Il4*$^{-/-}$,*Il13*$^{-/-}$ (J) *Demodex*-infested skin (I) vs. WT (non-infected) control.

# Table S1. Identification of *Demodex musculi*, related to Figure 1.



```
Demodex 18S-1.1             1  CAAGGAAGGCAGCAGGCACGCAAATTACCCACTCTCAGAACGAGGAGGTAGTGACGAAAAATAACAATACGGGACTATTA  80
Demodex 18S-2.1             1  CAAGGAAGGCAGCAGGCACGCAAATTACCCACTCTCAGAACGAGGAGGTAGTGACGAAAAATAACAATACGGGACTATTA  80
Demodex 18S-3.1             1  CAAGGAAGGCAGCAGGCACGCAAATTACCCACTCTCAGAACGAGGAGGTAGTGACGAAAAATAACAATACGGGACTATTA  80
Demodex musculi 18S         1  CAAGGAAGGCAGCAGGCACGCAAATTACCCACTCTCAGAACGAGGAGGTAGTGACGAAAAATAACAATACGGGACTATTA  80
Demodex folliculorum 18S    1  CAAGGAAGGCAGCAGGCACGCAAATTACCCACTCTCAGAACGAGGAGGTAGTGACGAAAAATAACAATACGGGACTATTA  80
Demodex brevis 18S          1  CAAGGAAGGCAGCAGGCACGCAAATTACCCACCCTCAGAACGAGGAGGTAGTGACGAAAAATAACAATACGGGACTATTA  80
Demodex canis 18S           1  CAAGGAAGGCAGCAGGCACGCAAATTACCCACTCTCAGAACGAGGAGGTAGTGACGAAAAATAACAATACGGGACTATTA  80
                               CAAGGAAGGCAGCAGGCACGCAAATTACCCACTCTCAGAACGAGGAGGTAGTGACGAAAAATAACAATACGGGACTATTA

Demodex 18S-1.1            81  ATAGGCCCCGTAATTGGAATGAGTACAATATAAATCCTTTAACGAGGATCTATTGGAGGGCAAGTCTGGTGCCAGCAGCC  160
Demodex 18S-2.1            81  ATAGGCCCCGTAATTGGAATGAGTACAATATAAATCCTTTAACGAGGATCTATTGGAGGGCAAGTCTGGTGCCAGCAGCC  160
Demodex 18S-3.1            81  ATAGGCCCCGTAATTGGAATGAGTACAATATAAATCCTTTAACGAGGATCTATTGGAGGGCAAGTCTGGTGCCAGCAGCC  160
Demodex musculi 18S        81  ATAGGCCCCGTAATTGGAATGAGTACAATATAAATCCTTTAACGAGGATCTATTGGAGGGCAAGTCTGGTGCCAGCAGCC  160
Demodex folliculorum 18S   81  ATAGGCCCCGTAATTGGAATGAGTACAATATAAATCCTTTAACGAGGATCTATTGGAGGGCAAGTCTGGTGCCAGCAGCC  160
Demodex brevis 18S         81  ATAGGCCCCGTAATTGGAATGAGTACAATATAAATCCTTTAACGAGGATCTATTGGAGGGCAAGTCTGGTGCCAGCAGCC  160
Demodex canis 18S          81  ATAGGCCCCGTAATTGGAATGAGTACAATATAAATCCTTTAACGAGGATCTATTGGAGGGCAAGTCTGGTGCCAGCAGCC  160
                               ATAGGCCCCGTAATTGGAATGAGTACAATATAAATCCTTTAACGAGGATCTATTGGAGGGCAAGTCTGGTGCCAGCAGCC

Demodex 18S-1.1           161  GCGGTAATTCCAGCTCCAATAGCGTATATTAAAGTTGCTGCGTTTAAAAAGCTCGTAGTTGTATCTCAGTTCATGTCAAT  240
Demodex 18S-2.1           161  GCGGTAATTCCAGCTCCAATAGCGTATATTAAAGTTGCTGCGTTTAAAAAGCTCGTAGTTGTATCTCAGTTCATGTCAAT  240
Demodex 18S-3.1           161  GCGGTAATTCCAGCTCCAATAGCGTATATTAAAGTTGCTGCGTTTAAAAAGCTCGTAGTTGTATCTCAGTTCATGTCAAT  240
Demodex musculi 18S       161  GCGGTAATTCCAGCTCCAATAGCGTATATTAAAGTTGCTGCGTTTAAAAAGCTCGTAGTTGTATCTCAGTTCATGTCAAT  240
Demodex folliculorum 18S  161  GCGGTAATTCCAGCTCCAATAGCGTATATTAAAGTTGCTGCGTTTAAAAAGCTCGTAGTTGTATCTCAGTTCATGTCAAT  240
Demodex brevis 18S        161  GCGGTAATTCCAGCTCCAGTAGCGTATATTAAAGTTGCTGCGTTTAAAAAGCTCGTAGTTGTATCTCAGTTCATGTTATT  240
Demodex canis 18S         161  GCGGTAATTCCAGCTCCAATAGCGTATATTAAAGTTGCTGCGTTTAAAAAGCTCGTAGTTGTATCTCAGTTCATGTCATT  240
                               GCGGTAATTCCAGCTCCAATAGCGTATATTAAAGTTGCTGCGTTTAAAAAGCTCGTAGTTGTATCTCAGTTCATGTCAAT

Demodex 18S-1.1           241  TGGTCAACCACTTATTAGTGATTACTTTTTGACTGAACATTGAACCGATTTGTTTTCTGATGCTCTTTACCGAGTGTTAT  320
Demodex 18S-2.1           241  TGGTCAACCACTTATTAGTGATTACTTTTTGACTGAACATTGAACCGATTTGTTTTCTGATGCTCTTTACCGAGTGTTAT  320
Demodex 18S-3.1           241  TGGTCAACCACTTATTAGTGATTACTTTTTGACTGAACATTGAACCGATTTGTTTTCTGATGCTCTTTACCGAGTGTTAT  320
Demodex musculi 18S       241  TGGTCAACCACTTATTAGTGATTACTTTTTGACTGAACATTGAACCGATTTGTTTTCTGATGCTCTTTACCGAGTGTTAT  320
Demodex folliculorum 18S  241  TGGTCAATCACTTAATAGTGATTACTTTTTGACTGAACATTAAACCGATTTGTTTTCTGATGCTCTTTACCGGGTGTTAT  320
Demodex brevis 18S        241  GGGTCAACCACTAAATAGTGATTACTTAATAACTGAACATTGAACCGATTTGTTTTTTAATGCTCTTTACCGAGTGTTTT  320
Demodex canis 18S         241  GGGTCAAGCACTTATTAGTGATTACTTAATGACTGAACATTAAACCGATTTGTTTACTGATGCTCTTTACCGAGTGTTAT  320
                               TGGTCAACCACTTATTAGTGATTACTTTTTGACTGAACATTGAACCGATTTGTTTTCTGATGCTCTTTACCGAGTGTTAT

Demodex 18S-1.1           321  TAACGA-TCGGGATCTTTACTTTGAAAAAATTAGAGTGCTCAAAGCAGGCATTTCGCCTAAATATTCTTGCATGGAATAA  399
Demodex 18S-2.1           321  TAACGA-TCGGGATCTTTACTTTGAAAAAATTAGAGTGCTCAAAGCAGGCATTTCGCCTAAATATTCTTGCATGGAATAA  399
Demodex 18S-3.1           321  TAACGA-TCGGGATCTTTACTTTGAAAAAATTAGAGTGCTCAAAGCAGGCATTTCGCCTAAATATTCTTGCATGGAATAA  399
Demodex musculi 18S       321  TAACGA-TCGGGATCTTTACTTTGAAAAAATTAGAGTGCTCAAAGCAGGCATTTCGCCTAAATATTCTTGCATGGAATAA  399
Demodex folliculorum 18S  321  TAACGA-TCGGGATCTTTACTTTGAAAAAATTAGAGTGCTCAAAGCAGGCATTTCGCCTAAATATTCTTGCATGGAATAA  399
Demodex brevis 18S        321  TAACGA-TCGGGCTGTTTACTTTGAAAAAATTAGAGTGCTCAAAGCAGGCATATCGCCTAAATAATCTTGCATGGAATAA  399
Demodex canis 18S         321  TAAGAAATCGGAATATTTACTTTGAAAAAATTAGAGTGCTCAAAGCAGGCATTTCGCCTAAATAATCTCGCATGGAATAA  400
                               TAACGAATCGGGATCTTTACTTTGAAAAAATTAGAGTGCTCAAAGCAGGCATTTCGCCTAAATATTCTTGCATGGAATAA

Demodex 18S-1.1           400  TGGAATAAGATCTAGGTTCTATTCTGTTGGTCTTTTGAACCTCAGTTAATGATTAAAAGGGACAGACGGGGGCATTCGTA  479
Demodex 18S-2.1           400  TGGAATAAGATCTAGGTTCTATTCTGTTGGTCTTTTGAACCTCAGTTAATGATTAAAAGGGACAGACGGGGGCATTCGTA  479
Demodex 18S-3.1           400  TGGAATAAGATCTAGGTTCTATTCTGTTGGTCTTTTGAACCTCAGTTAATGATTAAAAGGGACAGACGGGGGCATTCGTA  479
Demodex musculi 18S       400  TGGAATAAGATCTAGGTTCTATTCTGTTGGTCTTTTGAACCTCAGTTAATGATTAAAAGGGACAGACGGGGGCATTCGTA  479
Demodex folliculorum 18S  400  TGGAATAAGATCTAGGTTCTATTCTGTTGGTCTTTTGAACCTCAGTTAATGATTAAAAGGGACAGACGGGGGCATTCGTA  479
Demodex brevis 18S        400  TGGAATATGAACTAGGTTCTATTCTGTTGGTCTTTTGAACCTAAGTTAATGATTAAAAGGGACAGACGGGGGCATTAGTA  479
Demodex canis 18S         401  TGGAATAAGATCTAGGTTCTATTCTGTTGGTCTTTTGAACCTCAGTTAATGATTAAAAGGGACAGACGGGGGCATTAGTA  480
                               TGGAATAAGATCTAGGTTCTATTCTGTTGGTCTTTTGAACCTCAGTTAATGATTAAAAGGGACAGACGGGGGCATTCGTA

Demodex 18S-1.1           480  TTGCGGCGCTAGAGGTGAAATTCTTGGACCGTCGCAAGACGAACTACCGC  529
Demodex 18S-2.1           480  TTGCGGCGCTAGAGGTGAAATTCTTGGATCGTCGCAAGACGAACTACCGC  529
Demodex 18S-3.1           480  TTGCGGCGCTAGAGGTGAAATTCTTGGACCGTCGCAAGACGAACTACCGC  529
Demodex musculi 18S       480  TTGCGGCGCTAGAGGTGAAATTCTTGGACCGTCGCAAGACGAACTACCGC  529
Demodex folliculorum 18S  480  TTGCGGCGCTAGAGGTGAAATTCTTGGACCGTCGCAAGACGAACTACCGC  529
Demodex brevis 18S        480  T-GCGGCGCTAGAGGTGAAATTCTTGGACCGTCGCAAGACGAACTACCGC  528
Demodex canis 18S         481  TTGCGGCGCTAGAGGTGAAATTCTTGGACCGTCGCAAGACGAACTACCGC  530
                               TTGCGGCGCTAGAGGTGAAATTCTTGGACCGTCGCAAGACGAACTACCGC
```

Alignments of putative *Demodex spp.* 18S rRNA gene amplicon sequences obtained from 3 affected mice (one representative for each sample) with the corresponding sequences of *Demodex musculi* (GenBank #JF834894), *Demodex folliculorum* (GenBank #KY922187), *Demodex brevis* (GenBank JN885466.1), and *Demodex canis* (GenBank JN885468.1). Single nucleotide sequence which differs from the *Demodex musculi* reference is highlighted and the consensus sequence is also shown.

## Table S2. List of antibodies used for flow cytometry and immunofluorescence, related to STAR Methods.

| Company | Catalog Number | Antibody Name | Clone | Dilution |
|---|---|---|---|---|
| BioLegend | 115549 | Brilliant Violet 421™ anti-mouse CD19 Antibody, 50 µg | 6D5 | 1:300 |
| BioLegend | 116234 | Brilliant Violet 421™ anti-mouse TER-119/Erythroid Cells Antibody, 50 µg | TER-119 | 1:300 |
| Biolegend | 108445 | Brilliant Violet 421™ anti-mouse Ly-6G/Ly-6C (Gr-1) Antibody, 50 µg | RB6-8C5 | 1:300 |
| Biolegend | 101236 | Brilliant Violet 421™ anti-mouse/human CD11b Antibody, 500ul | M1/70 | 1:300 |
| Biolegend | 101259 | Brilliant Violet 650™ anti-mouse/human CD11b Antibody | M1/70 | 1:300 |
| Biolegend | 117322 | Pacific Blue™ anti-mouse CD11c Antibody, 100 µg | N418 | 1:300 |
| BioLegend | 108918 | Pacific Blue™ anti-mouse CD49b (pan-NK cells) Antibody, 100 µg | DX5 | 1:300 |
| BioLegend | 137612 | Brilliant Violet 421™ anti-mouse CD335 (NKp46) Antibody, 50 µg | 29A1.4 | 1:300 |
| BioLegend | 108741 | Brilliant Violet 421™ anti-mouse NK-1.1 Antibody, 50 µg | PK136 | 1:300 |
| BioLegend | 118120 | Brilliant Violet 421™ anti-mouse TCR γ/δ Antibody, 50 µg | GL3 | 1:500 |
| Biolegend | 134314 | Pacific Blue™ anti-mouse FcεRIα Antibody, 100 µg | MAR-1 | 1:300 |
| BioLegend | 100220 | PE/Cy7 anti-mouse CD3 Antibody, 100 µg | 17A2 | 1:200 |
| BioLegend | 123124 | Pacific Blue™ anti-mouse F4/80 Antibody, 100 µg | BM8 | 1:300 |
| Biolegend | 103147 | Brilliant Violet 711™ anti-mouse CD45 Antibody, 50 µg | 30-F11 | 1:300 |
| BD Biosciences | 564279 | BUV395 Rat Anti-Mouse CD45, 50 µg | 30-F11 | 1:300 |
| BioLegend | 103116 | APC/Cyanine7 anti-mouse CD45 Antibody, 100 µg | 30-F11 | 1:400 |
| BioLegend | 105331 | Brilliant Violet 785™ anti-mouse CD90.2 Antibody, 50 µg | 30-H12 | 1:1000 |
| Biolegend | 105343 | Brilliant Violet 605™ anti-mouse CD90.2 Antibody | 30-H12 | 1:1000 |
| BioLegend | 100550 | Brilliant Violet 711™ anti-mouse CD4 Antibody, 500 µl | RM4-5 | 1:200 |
| BioLegend | 100725 | Pacific Blue™ anti-mouse CD8a Antibody, 100 µg | 53-6.7 | 1:200 |
| BioLegend | 100750 | Brilliant Violet 785™ anti-mouse CD8a Antibody | 53-6.7 | 1:200 |
| BioLegend | 100642 | Pacific Blue™ anti-mouse CD5 Antibody | 53-7.3 | 1:300 |
| BioLegend | 108133 | Brilliant Violet 605™ anti-mouse Ly-6A/E (Sca-1) Antibody, 125 µL | D7 | 1:300 |
| BioLegend | 107643 | Brilliant Violet 711™ anti-mouse I-A/I-E Antibody | M5/114.15.2 | 1:400 |
| BioLegend | 118220 | PerCP/Cyanine5.5 anti-mouse CD326 (Ep-CAM) Antibody | G8.8 | 1:300 |
| BioLegend | 313606 | FITC anti-human/mouse CD49f Antibody | GoH3 | 1:300 |
| BioLegend | 313622 | PE/Cyanine7 anti-human/mouse CD49f Antibody | GoH3 | 1:300 |
| BD Biosciences | 560230 | Alexa Fluor® 647 Rat anti-Mouse CD34 | RAM34 | 1:200 |
| Invitrogen | 47-1271-82 | CD127 (IL-7R) Monoclonal Antibody (A7R34), APC-eFluor 780 | A7R34 | 1:100 |
| Invitrogen | 50-9966-42 | Gata-3 Monoclonal Antibody (TWAJ), eFluor 660, eBioscience™, 100 tests | TWAJ | 1:25 |
| Biolegend | 300514 | APC anti-human CD4 Antibody | RPA-T4 | 1:20 |
| Invitrogen | 11-5773-82 | FOXP3 Monoclonal Antibody (FJK-16s), eBioscience™ | FJK-16s | 1:100 |
| BD Biosciences | 745257 | BV605 Rat Anti-Mouse IL-33R (ST2) , 50 µg | U29-93 | 1:200 |
| Invitrogen | 46-5183-82 | CD218a (IL-18Ra) Monoclonal Antibody (P3TUNYA), PerCP-eFluor 710, eBioscience™, 100 µg | P3TUNYA | 1:300 |
| TakaraBio | 632496 | Living Colors® DsRed Polyclonal Antibody (100 µl) | Polyclonal, Lot 1904182 | 1:500 |
| Invitrogen | 11-5698-82 | Ki-67 Monoclonal Antibody (SolA15), FITC, eBioscience™ | SolA15 | 1:100 |
| BD Biosciences | 612131 | FITC Mouse Anti- E-Cadherin | Clone 36 | 1:400 |
| Invitrogen | A-21428 | Goat anti-Rabbit IgG (H+L) Cross-Adsorbed Secondary Antibody, Alexa Fluor 555 | Polyclonal, Lot 2011559 | 1:1000 |

**Table S3. List of antibodies used for the CyTOF experiment, related to STAR Methods.**

| Channel | Metal | Antigen | Conc. Stock (µg/ml) | Clone | Vendor |
|---|---|---|---|---|---|
| 113 | In | Ter119 | 0.4 | TER119 | BioLegend |
| 115 | In | CD45 | 0.5 | 30-F11 | BioLegend |
| 139 | La | pSTAT3 | 3 | 49 | BD Biosciences |
| 140 | Ce | CD326 | 0.5 | G8.8 | BioLegend |
| 141 | Pr | CD11b | 0.5 | M1/70 | BioLegend |
| 142 | Nd | pH-H3 | 0.5 | HTA28 | Abcam |
| 143 | Nd | CD11c | 0.2 | HL3 | BD Biosciences |
| 144 | Nd | pMAPKAPK2 | 1.5 | 27B7 | Cell Signaling Technology |
| 145 | Nd | pCREB | 4 | 87G3 | Cell Signaling Technology |
| 146 | Nd | pPLCg2 | 1 | K86-689.37 | BD Biosciences |
| 147 | Sm | pSTAT1 | 2.5 | 4a | BD Biosciences |
| 148 | Nd | Myc | 0.2 | 9E10 | Fluidigm |
| 149 | Sm | pSTAT5 | 3 | 47 | BD Biosciences |
| 150 | Nd | pS6 | 7 | 2F9 | Cell Signaling Technology |
| 151 | Eu | Ly6C | 0.5 | HK1.4 | BioLegend |
| 152 | Sm | pErk1/2 | 10 | D13.14.4E | Cell Signaling Technology |
| 153 | Eu | CyclinB1-153 | 0.2 | GNS-1 | Fluidigm |
| 154 | Sm | p-p38 | 3 | 36/p38 | BD Biosciences |
| 155 | Gd | CD8 | 0.2 | 53-6.7 | BioLegend |
| 156 | Gd | CD4 | 0.5 | RM4-5 | Biolegend |
| 157 | Gd | CD3 | 0.5 | 17A2 | BD Biosciences |
| 158 | Gd | Ly6G | 0.5 | 1A8 | BioLegend |
| 159 | Tb | p4EBP1 | 1 | 236B4 | Cell Signaling Technology |
| 160 | Gd | pSyk/ZAP70 | 1.25 | 17a | BD Biosciences |
| 161 | Dy | pTBK1 | 6 | D52C2 | Cell Signaling Technology |
| 162 | Dy | TCRgd | 0.853 | GL3 | BioLegend |
| 163 | Dy | IkBa | 3 | L35A5 | Cell Signaling Technology |
| 164 | Dy | AFP-1 | 0.2 | SPM334 | Novus |
| 165 | Ho | pSTAT6 | 3 | 18 | BD Biosciences |
| 166 | Er | pRB | 0.2 | J112906 | Fluidigm |
| 167 | Er | Foxp3 | 5 | NRRF-30 | eBioscience |
| 168 | Er | NK1.1 | 0.5 | PK136 | BioLegend |
| 169 | Tm | Ki67 | 2 | SolA15 | eBioscience |
| 170 | Er | pSTAT4 | 3 | 38 | BD Biosciences |
| 171 | Yb | CD62L | 5 | MEL-14 | BioLegend |
| 172 | Yb | SiglecF | 0.5 | E50-2440 | BD Biosciences |
| 173 | Yb | CD19 | 0.5 | 6D5 | BioLegend |
| 174 | Yb | CD34 | 0.2 | RAM34 | BD Biosciences |
| 175 | Lu | CD44 | 0.5 | IM7 | BD Biosciences |
| 209 | Bi | MHC II | 0.5 | M5/114.15.2 | BioLegend |

**Table S4. Patient sample characteristics, related to STAR Methods.**

| Sample | Diagnosis | Age | Sex | Location | Reason for tissue |
|---|---|---|---|---|---|
| 1 | Rhinophyma | 70 | M | nose | Rhinophyma excision |
| 2 | Rhinophyma | 47 | M | nose | Rhinophyma excision |
| 3 | Rhinophyma | 68 | M | nose | Rhinophyma excision |
| 4 | Rhinophyma | 62 | M | nose | Rhinophyma excision |
| 5 | Rhinophyma | 62 | M | nose | Rhinophyma excision |
| 6 | Rhinophyma | 63 | M | nose | Rhinophyma excision |
| 7 | Rhinophyma | 63 | M | nose | Rhinophyma excision |
| 8 | Rhinophyma | 59 | M | nose | Rhinophyma excision |
| 9 | Normal | 78 | M | nose | BCC excision |
| 10 | Normal | 65 | F | nose | BCC excision |
| 11 | Normal | 66 | F | nose | BCC excision |
| 12 | Normal | 65 | M | nose | MIS excision |
| 13 | Normal | 59 | F | nose | MIS excision |
| 14 | Normal | 65 | F | nose | MIS excision |
| 15 | Normal | 57 | F | nose | Nevus excision |
| 16 | Normal | 75 | M | nose | BCC excision |

Note: Normal skin was obtained from the tips of elliptical excisions. Cases were selected where normal skin in the tips, well away from the reason for the excision, was available.