

Supplementary Materials for

Inception of early-life allergen-induced airway hyperresponsiveness is reliant on IL-13⁺CD4⁺ T cells

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Other Supplementary Material for this manuscript includes the following:

(available at immunology.sciencemag.org/cgi/content/full/3/27/eaan4128/DC1)

Table S2 (Microsoft Excel format). Raw data sets.

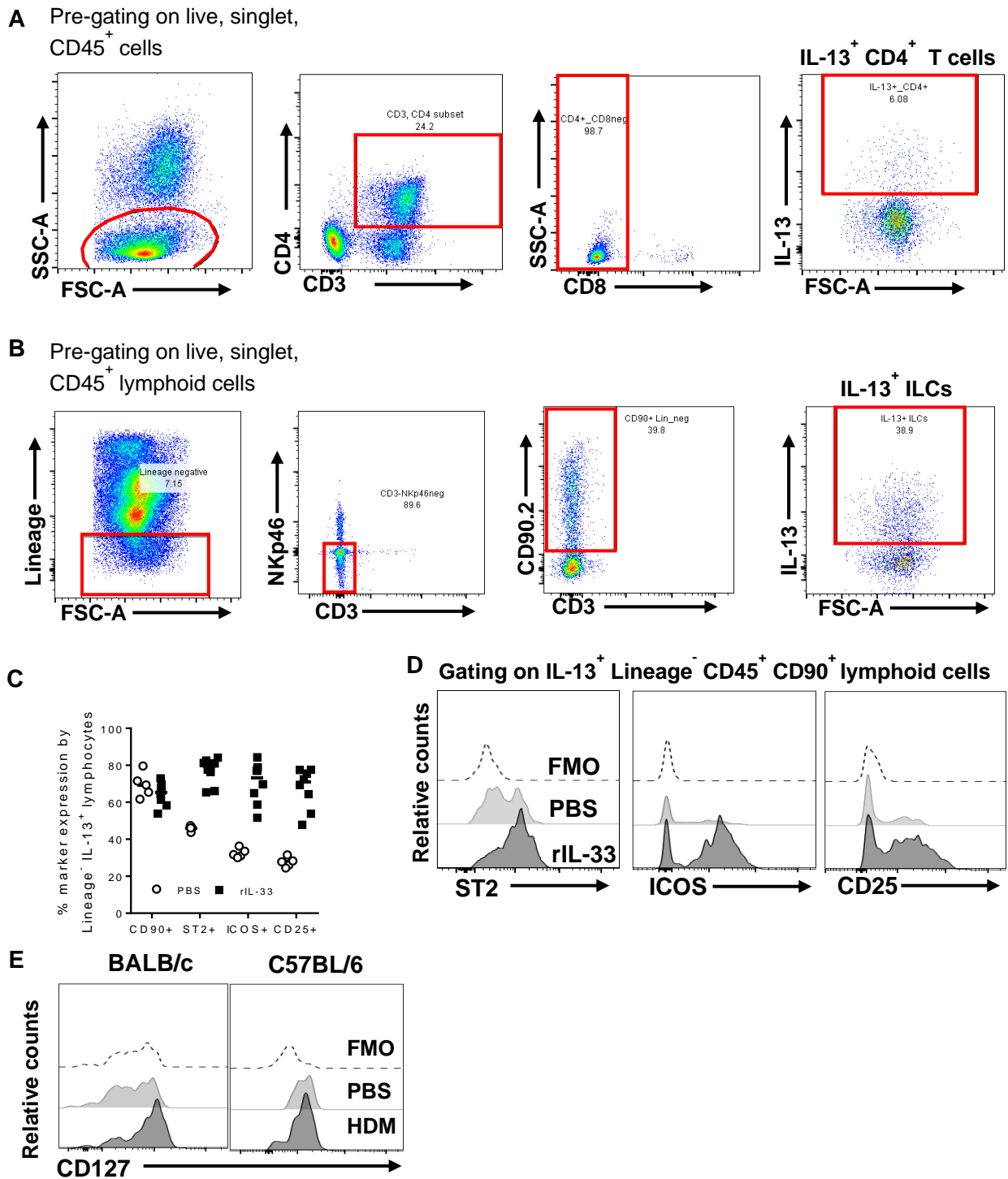


Fig. S1. Defining IL-13⁺ CD4⁺ T cells and ILCs in neonatal mice.

(A) Hierarchical (left to right) gating strategy for IL-13⁺ CD4⁺ T cells, defined as live, singlet, CD45⁺, FSC/SSC low (lymphoid), CD4⁺, CD3⁺, CD8⁻ cells with positive intracellular IL-13 staining were then selected. (B) Hierarchical (left to right) gating strategy for IL-13⁺

ILCs, defined as live, singlet, CD45⁺ lymphoid, lineage⁻ (TCR β , TCR $\gamma\delta$, CD3e, CD5, CD19, CD11b, CD11c, FC ϵ R1, GR-1, F4/80, NKp46 and TER-119) ⁻negative, CD90.2⁺ cells with positive intracellular IL-13 staining. Plots in **(A)** and **(B)** are from representative lung samples from IL-33-treated neonatal mice. **(C)** Percentages of IL-13⁺ Lineage⁻ CD45⁺ lymphoid cells in lung tissue expressing typical ILC surface markers. **(D)** Representative histograms showing surface expression of ST2, ICOS and CD25 on pulmonary IL-13⁺ CD90.2⁺ CD45⁺ Lineage⁻ ILCs, relative to FMO controls. **(E)** Representative histograms showing CD127 surface staining on IL-13⁺ CD90.2⁺ Lineage⁻ ILC2 from lungs of neonatal BALB/c (left) and C57BL/6 mice treated with HDM or PBS for 2 weeks, relative to FMO controls. Data are representative of 4-8 replicate animals per group.

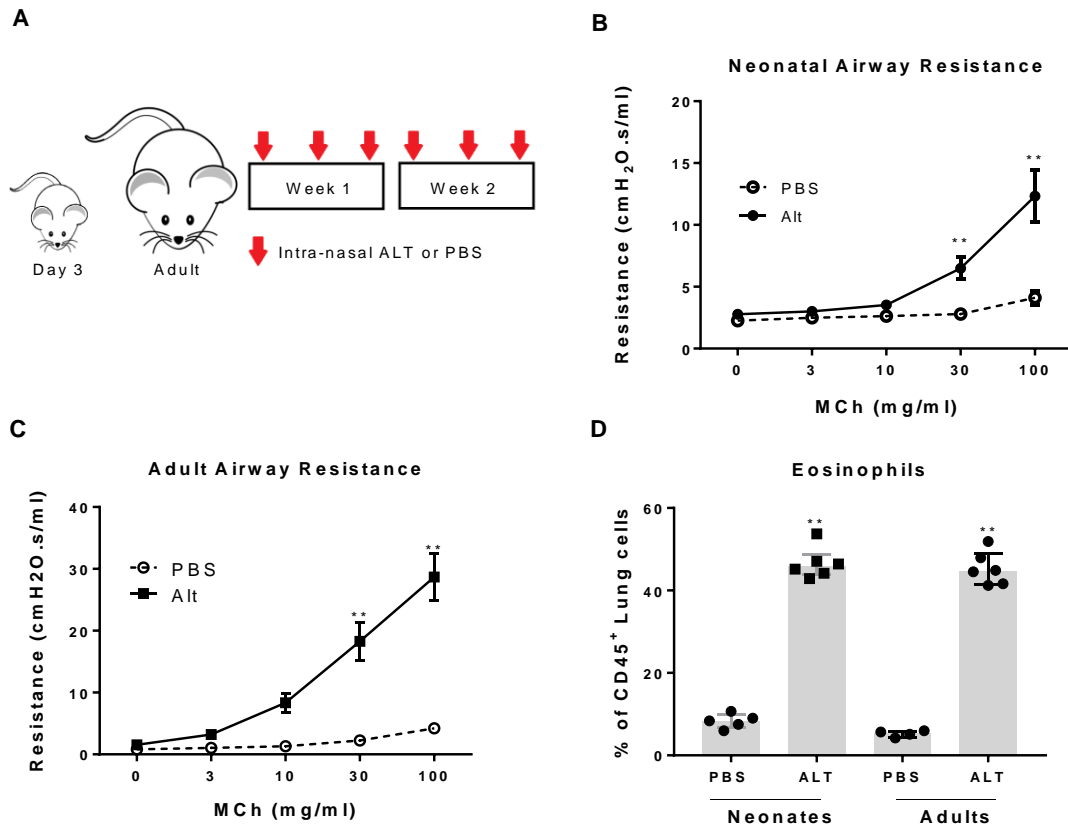


Fig. S2. *A. alternata* induces AHR in neonatal mice.

(A) Day 3 old and adult BALB/c mice were exposed to intermittent doses of *Alternaria alternata* (ALT) or PBS for 2 weeks (↓). Analysis was carried out 24 hours post final dose. Airway resistance to methacholine (Mch) in (B) neonatal and (C) adult mice. (D) Numbers of eosinophils (SiglecF⁺CD11c^{low/neg}) in lung enumerated by flow cytometry. N=6-8 ALT, n=4-6 PBS. Data representative of 2 experiments. *p<0.05, **<0.01, ***p<0.001.

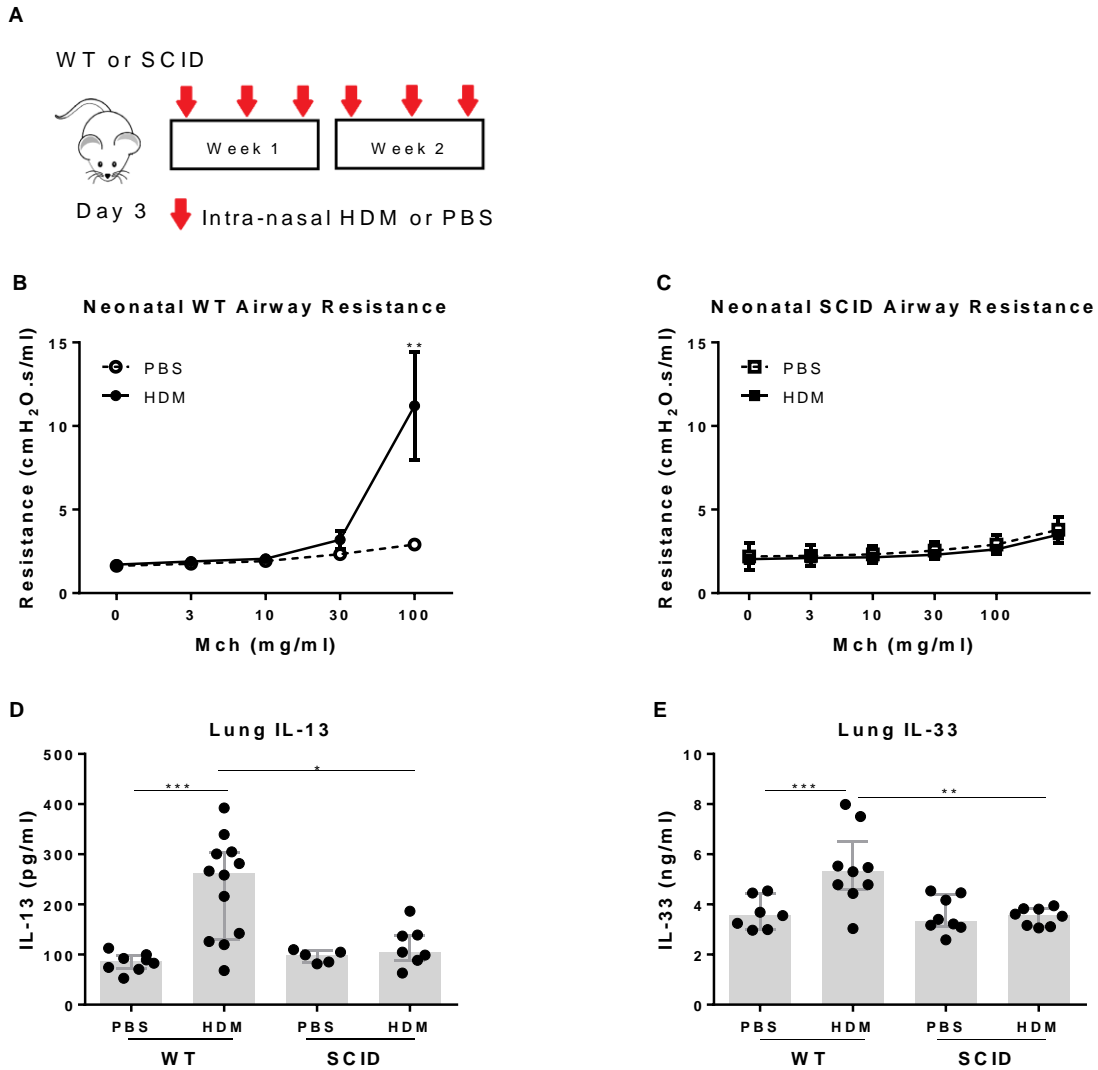


Fig. S3. Allergen exposure in neonatal SCID mice does not result in AHR.

(A) Day 3 old WT BALB/c and beige SCID mice were exposed to intermittent doses of house dust mite (HDM) or PBS for 2 weeks (↓). Analysis was carried out 24 hours post final dose. Airway resistance to methacholine (Mch) in (B) WT mice and (C) SCID mice. Levels of (D) IL-13 and (E) IL-33 from lung homogenates of WT mice compared to SCID mice. N=7-12 HDM, n=6-8 PBS. Data representative of 2 experiments. *p<0.05, **p<0.01, ***p<0.001.

Table S1. Antibodies used for flow cytometry.

Antigen	Clone	Fluorochrome	Target species	Manufacturer
CD103	2E7	FITC	Mouse	Thermo Fisher/ eBiosciences
CD11b	M1/70	APC	Mouse	Biolegend
CD11c	N418	APC or APC Cy7	Mouse	Biolegend
CD127	A7R34	BV711	Mouse	Biolegend
CD19	ebio 1D3	APC	Mouse	Biolegend
CD25	PC61	BV510	Mouse	Biolegend
CD3e	145-2C11	APC cy7 or PE Cy7	Mouse	Biolegend
CD4	RM4-5	BV421 or APC Cy7	Mouse	Biolegend
CD44	IM7	PERCP Cy5.5	Mouse	Biolegend
CD45	30-F11	PerCP Cy5.5 or BV711 or PE/Dazzle™ 594	Mouse	Biolegend
CD5	53-7.3	APC	Mouse	Biolegend
CD62L	MEL-14	BV605	Mouse	Biolegend
CD64	X54-5/7.1	BV421	Mouse	Biolegend
CD8a	53-6.7	BV605	Mouse	Biolegend
CD90.2	53-2.1	BV605	Mouse	Biolegend
F4/80	BM8	APC	Mouse	Biolegend
FCεR1	MAR1	APC	Mouse	Biolegend
GR-1	RB6-8C5	APC	Mouse	Biolegend

IA/IE	M5/114.15.2	PECRP cy5.5	Mouse	Biolegend
ICOS	7E.17G9	PE cy7	Mouse	Biolegend
IFN γ	XMG1.2	FITC	Mouse	Biolegend
IL-10	JES5-16E3	APC	Mouse	Biolegend
IL-13	ebio 13A	PE	Mouse	Thermo Fisher/ eBiosciences
IL-17A	TC11-18H10	Alexa Fluor 700 or PERCP Cy5.5	Mouse	Biolegend
IL-5	TRFK5	BV421	Mouse	Biolegend
Ly6G	1A8	BV510	Mouse	Biolegend
Nkp46	29A1.4	PE/Dazzle™ 594 or BV605	Mouse	Biolegend
Siglec F	E50-2440	PE	Mouse	BD
ST2	DJ8	FITC	Mouse	MD Biosciences
TCR β	H57-597	APC	Mouse	Biolegend
TCR $\gamma\delta$	ebio GL3	APC	Mouse	Biolegend
TER-119	TER-119	APC	Mouse	Biolegend
CD11c	3.9	FITC	Human	Biolegend
CD123	6H6	FITC	Human	Biolegend
CD127	A019D5	PERCP Cy5.5	Human	Biolegend
CD14	63D3	FITC	Human	Biolegend
CD16	B73.1	FITC	Human	Biolegend
CD161	HP-3G10	PE Cy7	Human	Biolegend
CD19	HIB19	FITC	Human	Biolegend

CD1a	HI149	FITC	Human	Biolegend
CD20	2H7	FITC	Human	Biolegend
CD3	OKT3	BV510	Human	Biolegend
CD34	581	FITC	Human	Biolegend
CD4	OKT4	Alexa Fluor 700	Human	Biolegend
CD45	HI30	BV605	Human	Biolegend
CD5	L17F12	FITC	Human	Biolegend
CD56	HCD56	PE Texas Red	Human	Biolegend
CD8	SK1	FITC	Human	Biolegend
CRTH2	BM16	APC Cy7	Human	Biolegend
FCεR1	AER-37	FITC	Human	Biolegend
IL-13	JES10-5A2	PE	Human	Biolegend

Table S2. Raw data sets.