iScience, Volume *25*

Supplemental information

Targeted deletion of Interleukin-3

results in asthma exacerbations

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Supplementary Figures

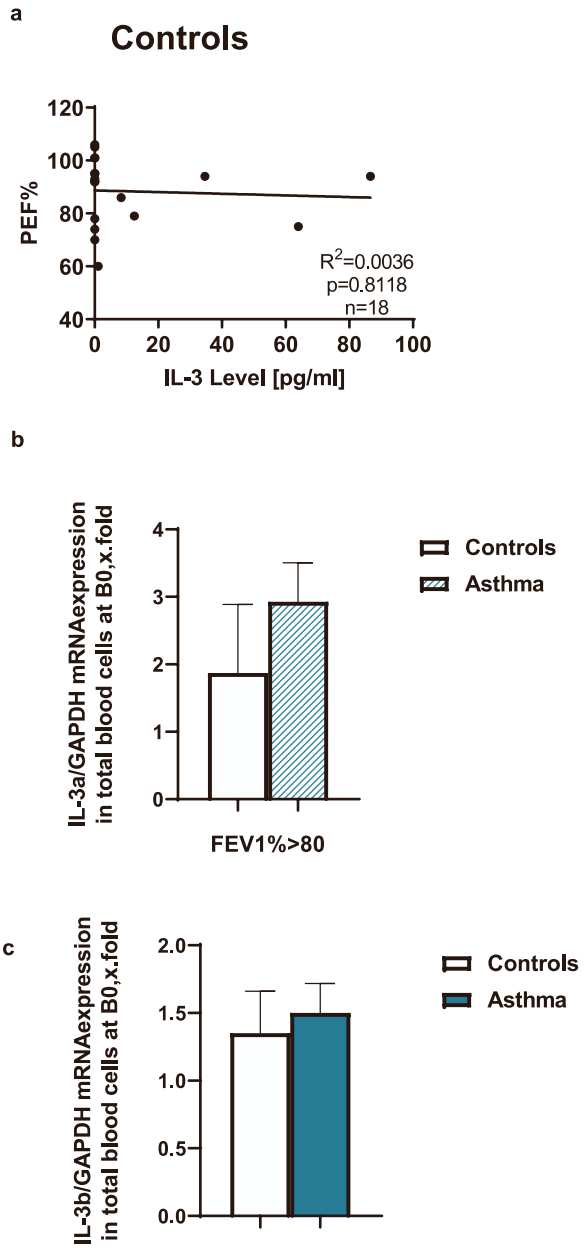
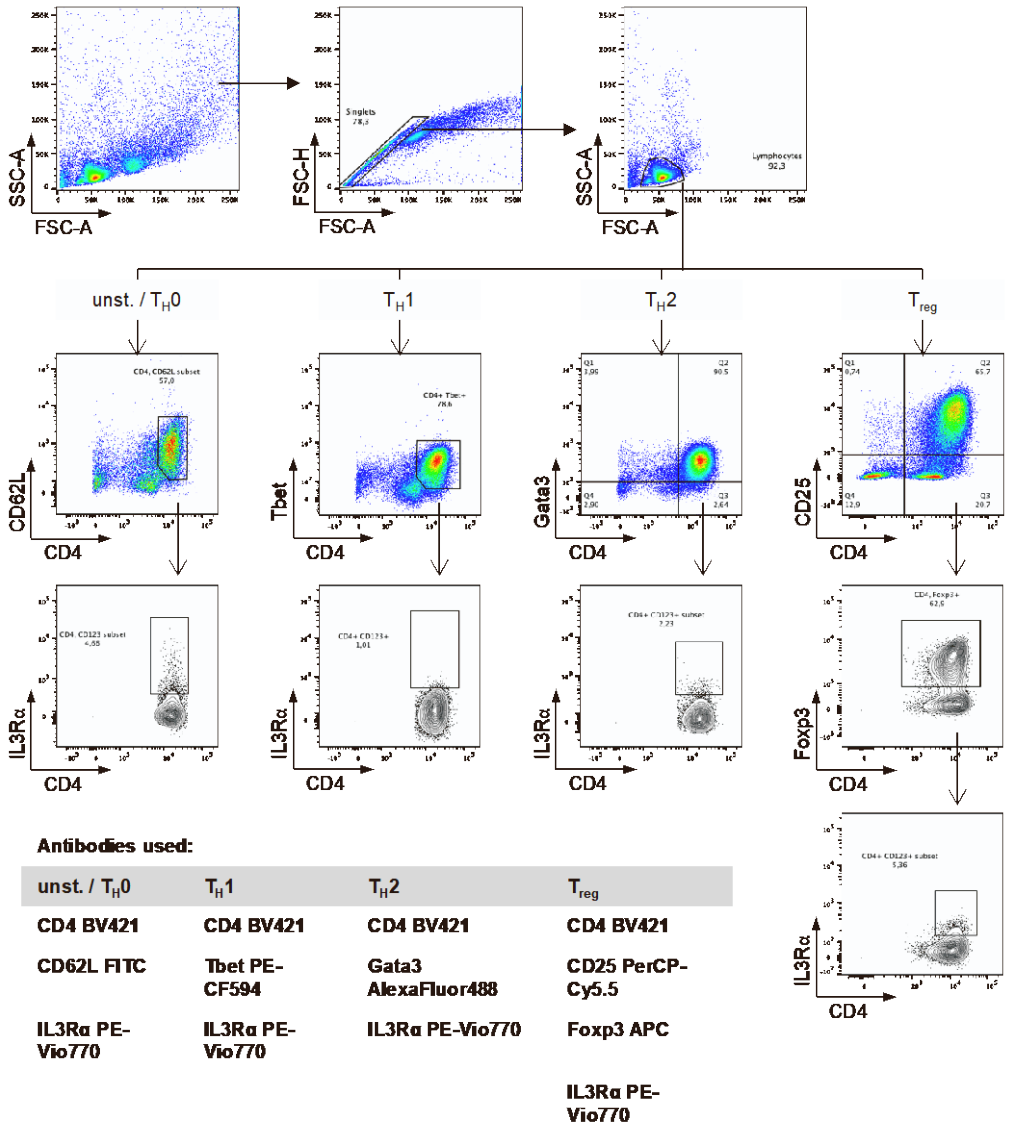


Figure S1: IL-3 in pediatric asthma, related to Figure 1c (a) and Figure 2 (b,c), respectively. **(a)** Correlation between the IL-3 level quantified by ELISA in the supernatant from PHA stimulated PBMCs isolated from healthy control children and the PEF% predicted (PEF% predicted healthy controls n=18) at the baseline visit; related to Figure 1c. **(b, c)** *IL3Ra/GAPDH* mRNA (b) and *IL3Rb/GAPDH* mRNA (c) expression in total blood cells from healthy control children (n=8) and asthmatic children (n=5) at baseline visit; related to Figure 2. Data are presented as means ± SEMs. Two-tailed student *t* test was used to calculate statistical significance. * $p \leq 0.05$; ** $p \leq 0.01$, *** $p \leq 0.001$, **** $p \leq 0.0001$.



Antibodies used:

unst. / T_H0	T_H1	T_H2	T_{reg}
CD4 BV421	CD4 BV421	CD4 BV421	CD4 BV421
CD62L FITC	Tbet PE-CF594	Gata3 AlexaFluor488	CD25 PerCP-Cy5.5
IL3Rα PE-Vio770	IL3Rα PE-Vio770	IL3Rα PE-Vio770	Foxp3 APC
			IL3Rα PE-Vio770

Figure S2: Spleen T cell differentiation of WT and IL-3 deficient mice, related to figure 3. Gating strategy of differentiated spleen T cells of WT and IL-3 deficient mice including the analysis of the IL3Rα expression on the differentiated T cell subtypes related to Figure 3. The respective used flow cytometry antibodies are listed below. Representative dot- and contourplots, respectively, are shown.

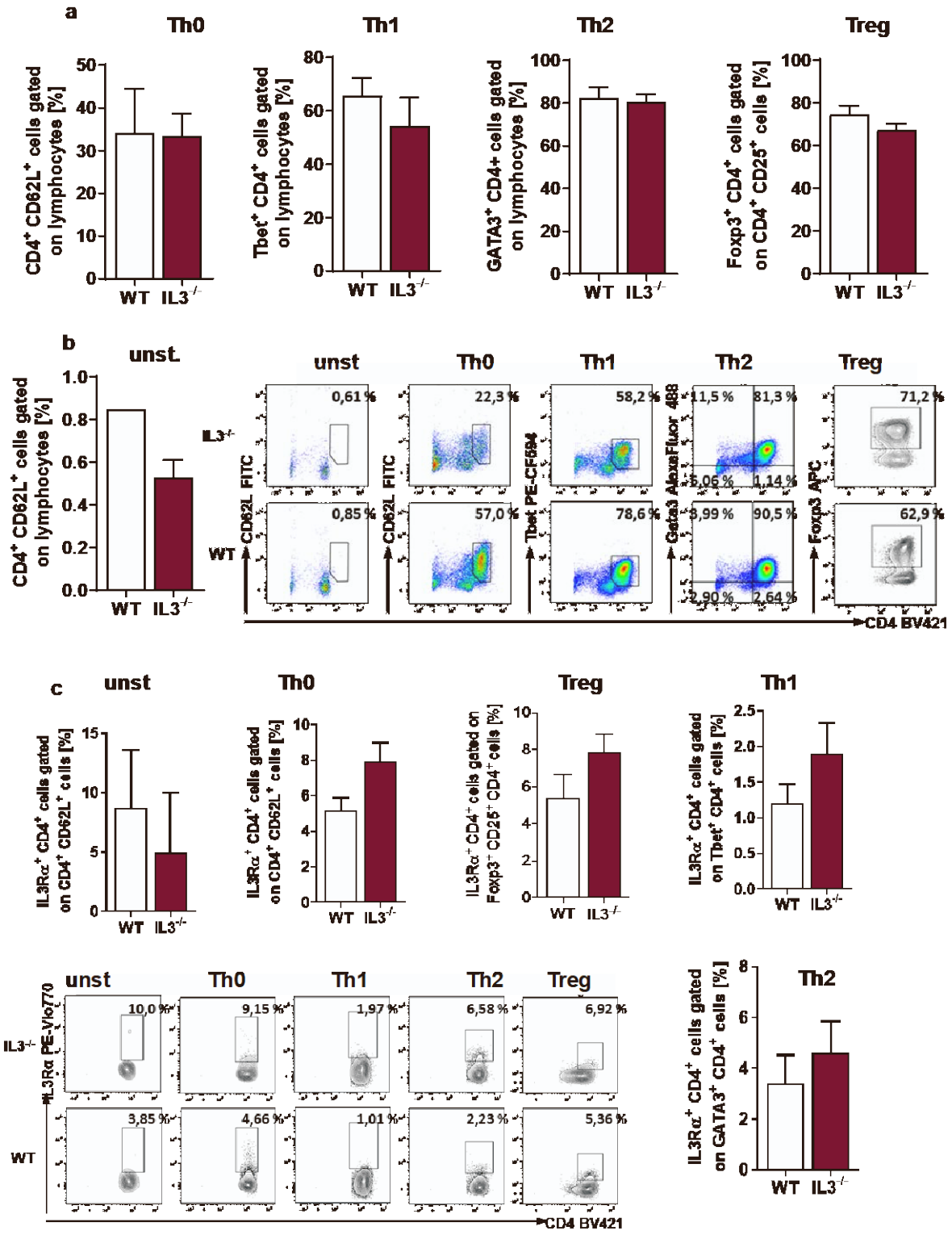


Figure S3: IL-3 and IL-3Rα characterization in the spleen T cells of WT and IL-3 deficient mice, related to Figure 3. (**a, b)** Flow cytometry analysis of the different T cell subtypes based on the indicated, specific cell markers (n=2/2 (unstimulated), n= 4/4 (T_h0, T_{reg}), n=4/3 (T_h1), n=3/4 (T_h2). **(c)** Flow cytometry analysis of the IL3Rα expression on the differentiated T cell subtypes (n=2/2 (unstimulated), n= 4/4 (T_h0, T_{reg}), n=4/3 (T_h1), n=3/4 (T_h2)). Representative dot- and contourplots, respectively, are shown. Data are presented as means ± SEMs. Two-tailed student *t* test, Two-way ANOVA or ordinary One-way ANOVA was used to calculate statistical significance. * $p \leq 0.05$; ** $p \leq 0.01$, *** $p \leq 0.001$, **** $p \leq 0.0001$.

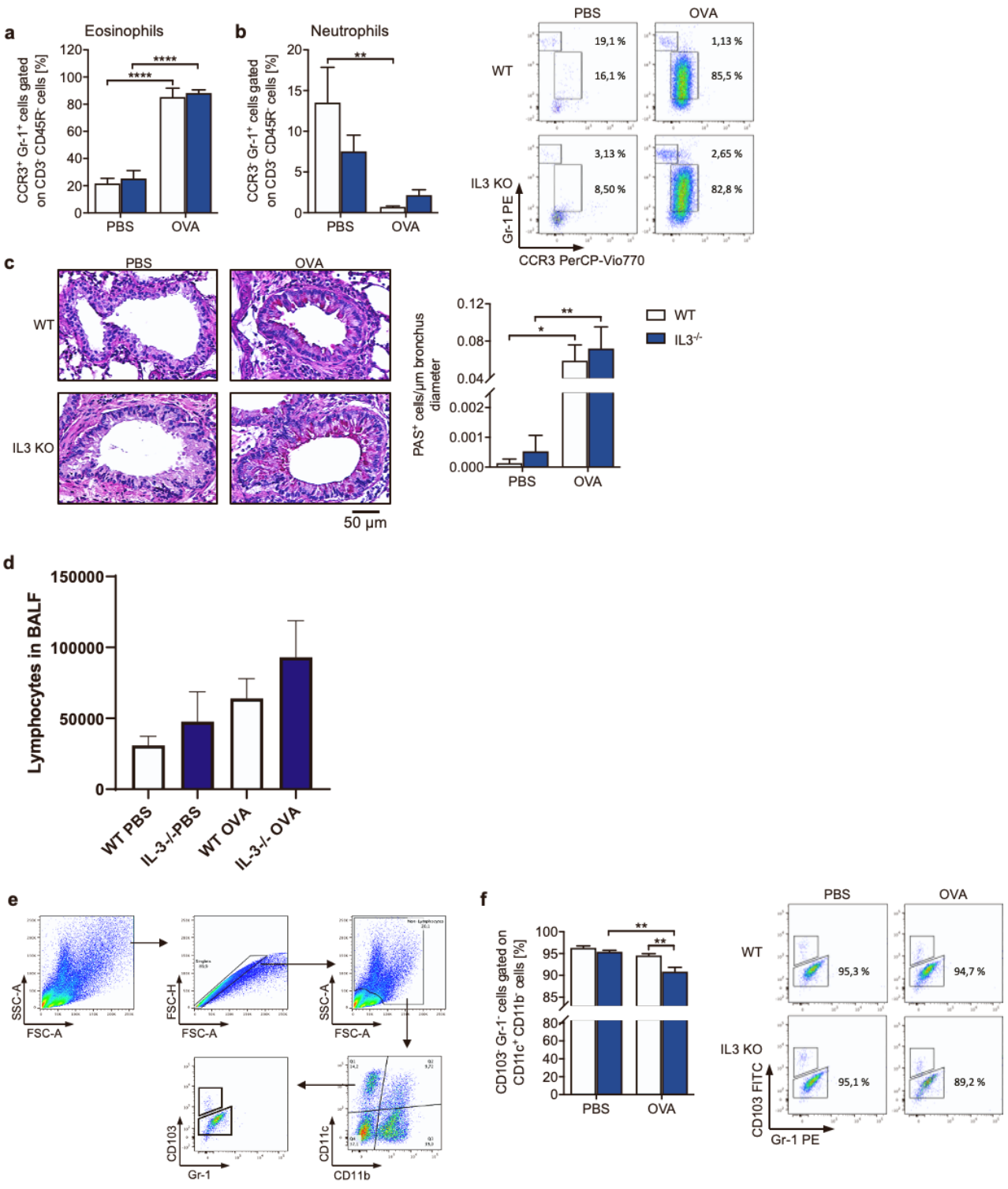


Figure S4: Characterization of IL-3$^{-/-}$ mice in an OVA-induced asthma model, **Figure S4a-d related to Figure 4 and Figure S4e, f related to Figure 5b. (a, b)** Flow cytometry analysis of CCR3^{+} Gr-1^{+} eosinophils and CCR3^{-} Gr-1+ neutrophils in the BALF from Balb/c WT and IL3$^{-/-}$ mice with and without asthma (n=10/10/9/10). **(c)** Analysis of PAS^{+} cells per µm bronchus diameter (n=15/15/13/14) in the lungs of WT and IL3$^{-/-}$ mice with and without asthma and corresponding pictures of Periodic acid-Schiff (PAS) staining. **(d)** Analysis of Lymphocytes in the BALF of WT and IL3$^{-/-}$ mice with and without asthma (n=5,2,4,5). **(e, f)** Flow cytometry analysis of CD103^{-} Gr-1^{-} cells in total lung cells isolated from Balb/c WT and IL3$^{-/-}$ mice with and without asthma (n=4/4/5/5). A representative dot plot is shown for each group. Data are presented as means ± SEMs. Two-way

ANOVA was used to calculate statistical significance. * p ≤ 0.05; ** p ≤ 0.01, *** p ≤ 0.001, **** p ≤ 0.0001.

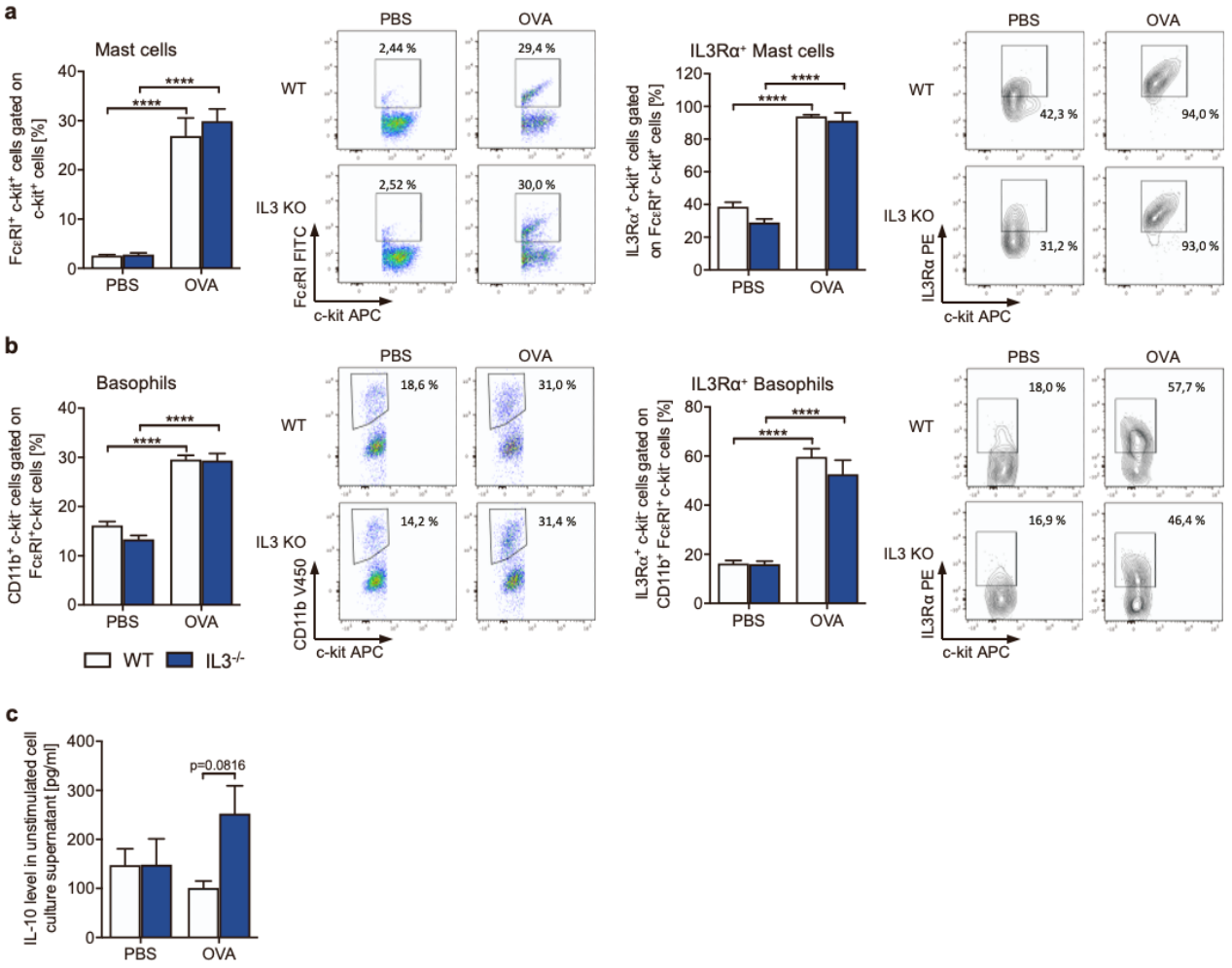


Figure S5: Mast cells and basophils in OVA-treated IL-3$^{-/-}$ mice, Figure S5a, b related to Figure 5 and Figure S5c related to Figure 6.(a) Flow cytometry analysis of mast cells (left) and IL3Rα^{+} mucosal mast cells (right) in total lung cells isolated from Balb/c WT and IL3$^{-/-}$ mice with and without asthma (n=4/4/5/5). **(b)** Flow cytometry analysis of basophils (left) and IL3Rα^{+} basophils (right) in total lung cells isolated from Balb/c WT and IL3$^{-/-}$ mice with and without asthma (n=5/5/5/5). **(c)** ELISA analysis of the IL-10 level in cell culture supernatants obtained from total lung cells and cultured *in vitro* for 24h of Balb/c WT and IL-3$^{-/-}$ mice with and without asthma (n=9/9/10/10). A representative dot plot is shown for each group. Data are presented as means ± SEMs. Two-way ANOVA was used to calculate statistical significance. * p ≤ 0.05; ** p ≤ 0.01, *** p ≤ 0.001, **** p ≤ 0.0001

Supplementary Tables

Table S1: Demographic and clinical data of the healthy PreDicta cohort WP1-UK-ER analyzed at the baseline visit. Related to STAR Methods (section ‘Human Study’).

Patient	Age	Gender	Skin Prick Test*	Atopic dermatitis
C1	6	male	n.d.	no
C2	6	female	n.d.	no
C3	5	male	n.d.	no
C4	4	male	n.d.	No
C5	4	female	n.d.	No
C6	5	female	n.d.	No
C7	5	female	negative	No
C8	3	male	n.d.	No
C9	6	male	n.d.	Yes
C10	4	female	n.d.	No
C11	6	male	n.d.	No
C12	4	male	negative	No
C13	5	female	n.d.	No
C14	5	female	al	No
C15	4	male	ca, f	No
C16	5	male	n.d.	No
C17	4	male	negative	No
C18	4	female	negative	No
C19	5	male	negative	No
C20	4	male	negative	No
C21	5	male	negative	Yes
Average	4.71 ± 0.18	m. = 61.9 % f. = 38.1 %	pos. = 9.5 % neg. = 33.3 %	yes = 9.5 % no = 90.5 %

*al, *Alternaria* species; ca, cat; f, *Dermatophagoides* mix; n.d., not done.

Table S2: Demographic and clinical data of the asthmatic PreDicta cohort WP1-UK-ER analyzed at the baseline visit. Related to STAR Methods (section 'Human Study').

Patient	Age	Gender	Asthma Severity *	Phenotype **	Skin Prick Test***	Atopic dermatitis
A1	6	male	I	v	al, ca, g	yes
A2	6	male	II	u	al, b, g	yes
A3	5	female	II	u	ca	no
A4	6	male	II	a	al, am, ca, f, g	yes
A5	5	male	I	u	ca	no
A6	5	female	I	u	al	no
A7	5	male	I	v	g	yes
A8	4	female	II	v,a	g	yes
A9	6	female	I	v	b, g	yes
A10	5	male	II	e,v	negative	no
A11	4	male	III	e	negative	no
A12	5	female	III	a,v	ca, f, g	no
A13	6	female	I	a,e,v	b, ca, f, g	yes
A14	5	male	I	v	ca, f, g	yes
A15	4	female	I	v	negative	no
A16	4	male	I	v	negative	no
A17	5	male	I	v	ca, f, g	no
A18	4	male	I	v	al, b, ca, f, g	yes
A19	5	male	I	v	al, am, b, ca, f, g	yes
A20	4	male	I	v	b	no
A21	4	male	I	v	negative	no
A22	5	female	I	e	n.d.	no
A23	5	male	II	a,e,v	al, b, ca, f, g	no
A24	5	female	II	v	negative	no
Average	4.92 ± 0.15	m. = 62.5 % f. = 37.5 %	I = 62.5 % II = 29.2 % III = 8.3 %	u = 16.7 % v = 70.8 % a =4.2 % e = 8.3 %	pos. = 73.9 % neg. = 26.1 %	yes = 41.7 % no = 58.3 %

* I=Intermittent: FEV1 > 80 %, MEF > 65 %, symptom-free interval > 2 months; II= Mild Persistent: FEV1 > 80 %, MEF > 65 %, symptom-free interval < 2 months; III= Moderate persistent: FEV1 < 80 %, MEF < 65 %, symptoms several days a week; IV= Severe persistent: FEV1 < 60 %, Symptoms during the day and night. ** v, virus-induced; a, allergen-induced; e, exercise-induced; u, unresolved. ***al, *Alternaria* species; am, ambrosia; b, birch; ca, cat; f, *Dermatophagoides* mix; g, grass pollen mix; n.d., not done. FEV1: forced expiratory volume in 1 second / forced vital capacity; MEF: mean expiratory flow.

Table S3: Spirometry (pre-bronchodilation) data of the healthy and asthmatic PreDicta cohort WP1-UK-ER analyzed at the baseline visit. Related to STAR Methods (section 'Spirometry').

Patient	PEF % predicted	FVC % predicted	FEV1 % predicted
C1	75	73	77
C2	94	117	121
C3	94	100	110
C4	78	104	118
C5	92	99	111
C6	n.e.	n.e.	n.e.
C7	60	101	84
C8	n.e.	n.e.	n.e.
C9	86	113	105
C10	93	99	109
C11	95	86	87
C12	70	89	100
C13	105	102	112
C14	95	109	119
C15	75	103	113
C16	101	95	111
C17	101	97	109
C18	74	83	92
C19	79	113	123
C20	106	106	121
C21	92	97	109
A1	132	119	126
A2	n.e.	n.e.	n.e.
A3	80	89	95
A4	127	115	128
A5	86	97	102
A6	119	129	129
A7	117	125	143
A8	88	106	115
A9	77	100	98
A10	84	91	96
A11	106	102	115
A12	75	114	92
A13	104	106	111
A14	90	85	99
A15	107	122	135
A16	82	90	99
A17	65	92	88
A18	65	92	87
A19	86	97	101
A20	60	64	71
A21	54	76	77
A22	92	103	98
A23	99	91	81

A24	53	92	69

FEV1: forced expiratory volume in 1 second / forced vital capacity; FVC: forced vital capacity; n.e.: not evaluable; n.m.: not measured; PEF: peak expiratory flow.