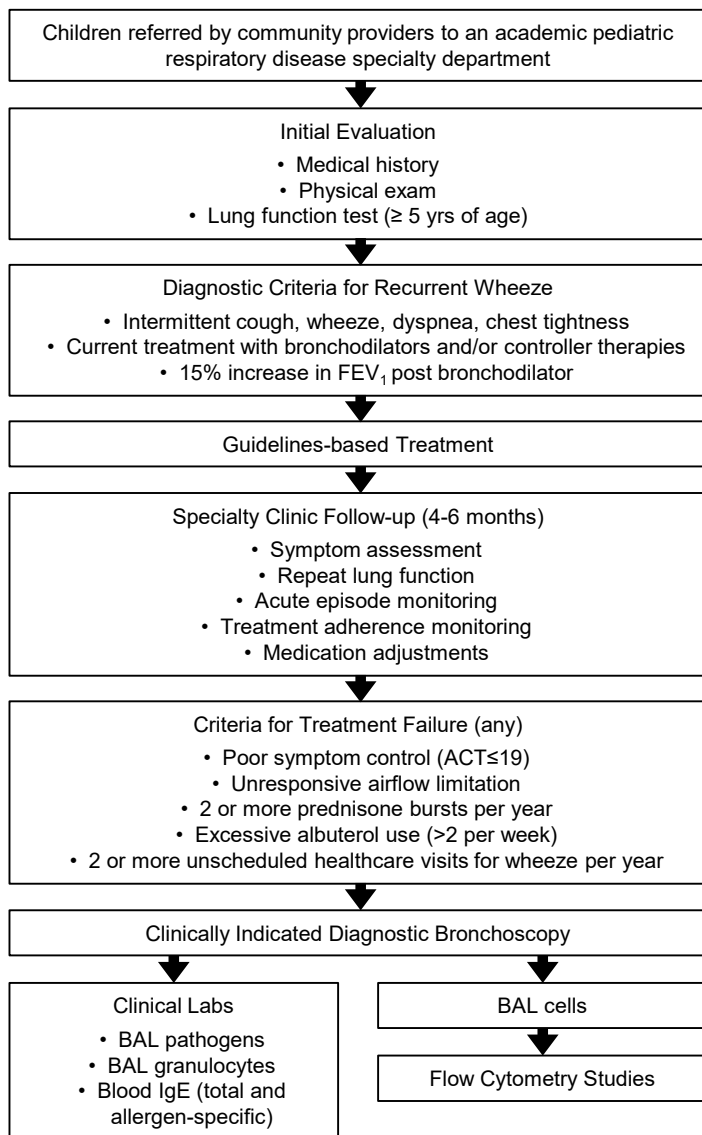
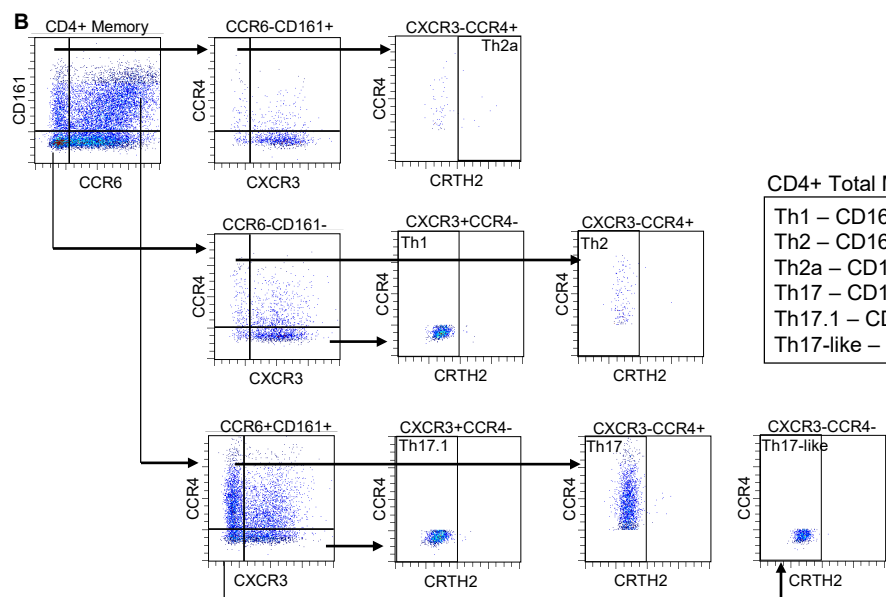
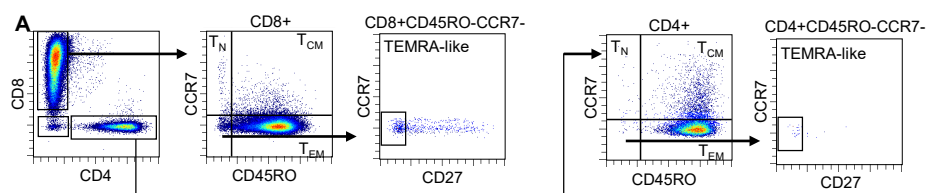




Supplemental Figure 1. Flow chart showing the clinical assessment, treatment, and indications for a clinically indicated bronchoscopy in children with treatment-refractory recurrent wheeze.

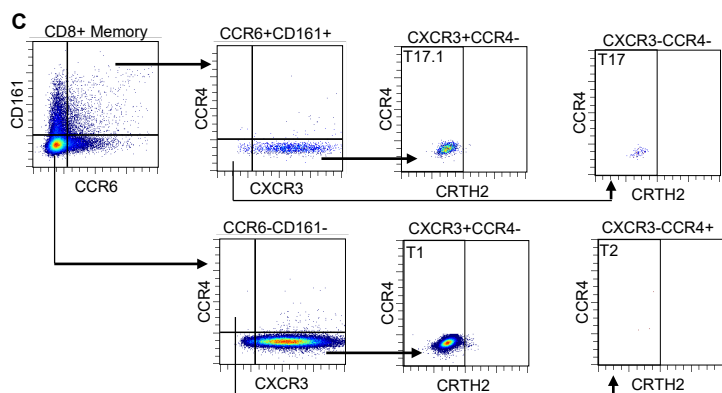
Supplemental Table 1. Spectral flow cytometry panel for T-cell phenotyping.

Marker	Color	Clone	Vendor	Catalog number	Dilution
Viability	Live/Dead Blue		Invitrogen	L23105	1:4000
CD3	BUV395	SK7	BD	564001	1:50
CD4	BUV496	OKT4	BD	750980	1:50
CD45RO	BUV563	UCHL1	BD	748369	1:100
PD-1	BUV615	EH12.1	BD	612991	1:50
CXCR3	BUV661	1C6/CXCR3	BD	741649	1:50
CCR5	BUV737	2D7/CCR5	BD	612808	1:25
CD69	BUV805	FN50	BD	748763	1:100
IL-4R α	BV421	G077F6	Biolegend	355014	1:50
CRTH2	BV480	BM16	BD	746388	1:200
CCR4	BV605	L291H4	Biolegend	359418	1:25
CCR7	BV650	G043H7	Biolegend	353234	1:20
CCR6	BV711	G034E3	Biolegend	353436	1:50
CXCR5	BV750	J252D4	Biolegend	356942	1:50
CD161	BV785	HP-3G10	Biolegend	339930	1:20
ICOS	BB515	C398.4A	BD	565881	1:100
CD14	FITC	M5E2	Biolegend	301804	1:20
CD19	FITC	H1B19	Biolegend	302206	1:20
CD8	PerCP	SK1	Biolegend	344708	1:200
CD95	BB700	DX2	BD	566543	1:100
CD103	Per-CP eFluor710	Ber-ACT8	Invitrogen	46103742	1:50
ST2	PE	Polyclonal	R&D Systems	FAB5231P100	1:10
CD25	PE-FIRE700	M-A251	Biolegend	356146	1:50
T-bet	PE-CY7	4B10	Biolegend	644823	1:800
Ki-67	APC	20Raj1	Invitrogen	17-5699-41	1:200
TCF-1	AF647	C63D9	Cell Signaling Technology	6709S	1:200
CD127	APC-R700	HIL-7R-M21	BD	565185	1:200
CD27	APC-CY7	O323	Biolegend	302816	1:50
CD38	APC-FIRE810	HIT2	Biolegend	303550	1:50



CD4+ Total Memory

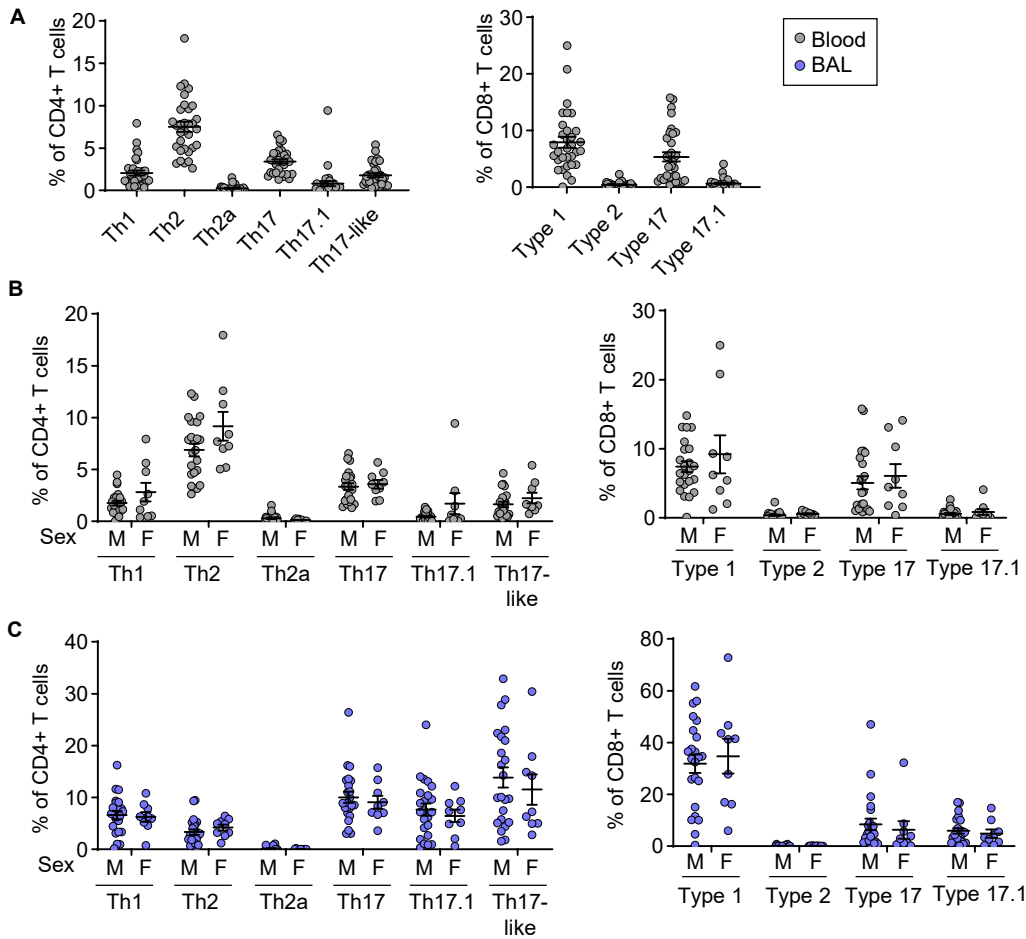
Th1 – CD161-CCR6-CXCR3+CCR4-CRTH2-
 Th2 – CD161-CCR6-CXCR3-CCR4+CRTH2-
 Th2a – CD161+CCR6-CXCR3-CCR4+CRTH2+
 Th17 – CD161+CCR6+CXCR3-CCR4+CRTH2-
 Th17.1 – CD161+CCR6+CXCR3+CCR4-CRTH2-
 Th17-like – CD161+CCR6+CXCR3-CCR4-CRTH2-



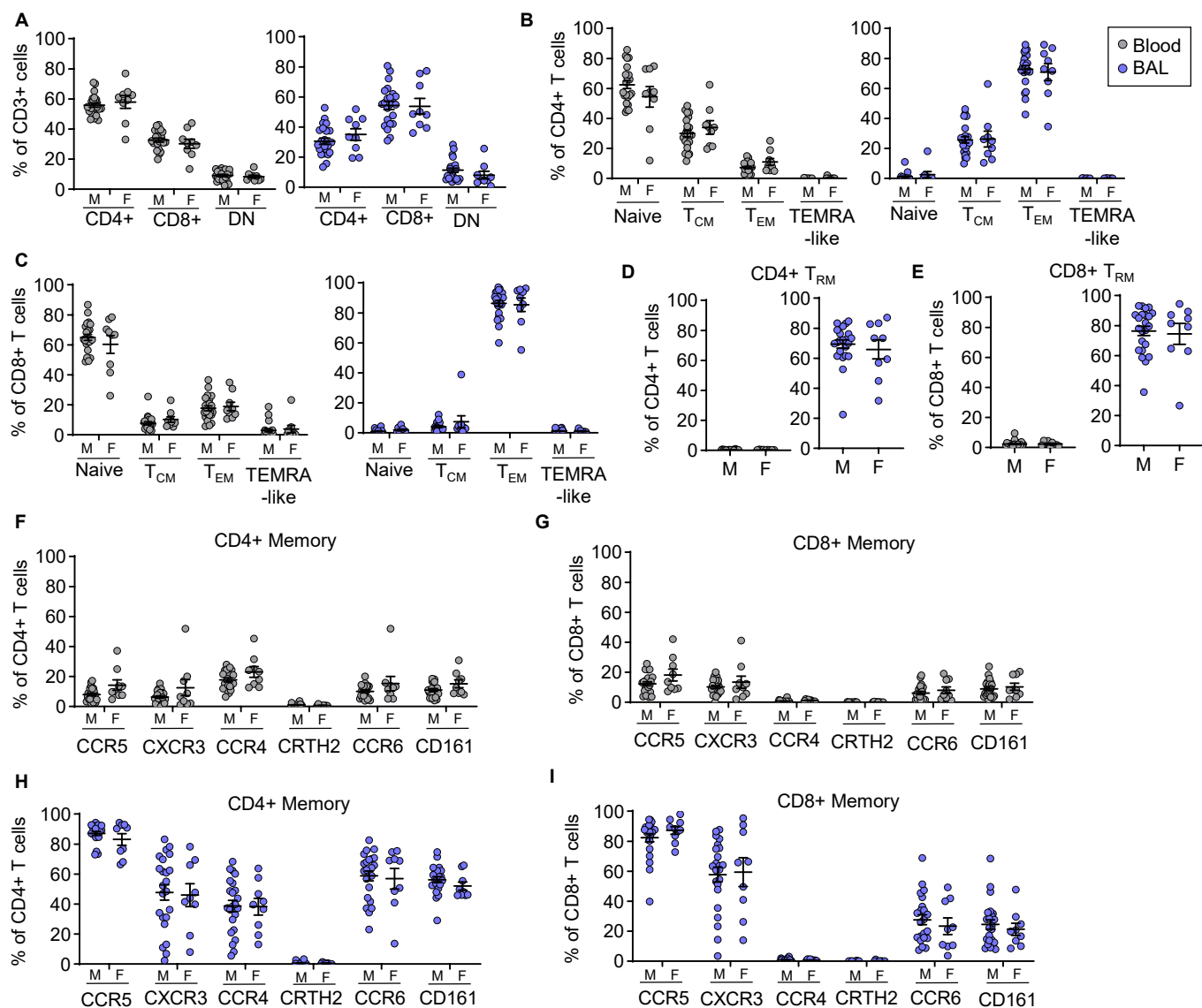
CD8+ Total Memory

T1 – CD161-CCR6-CXCR3+CCR4-CRTH2-
 T2 – CD161-CCR6-CXCR3-CCR4+CRTH2-
 T17 – CD161+CCR6+CXCR3-CCR4-CRTH2-
 T17.1 – CD161+CCR6+CXCR3+CCR4-CRTH2-

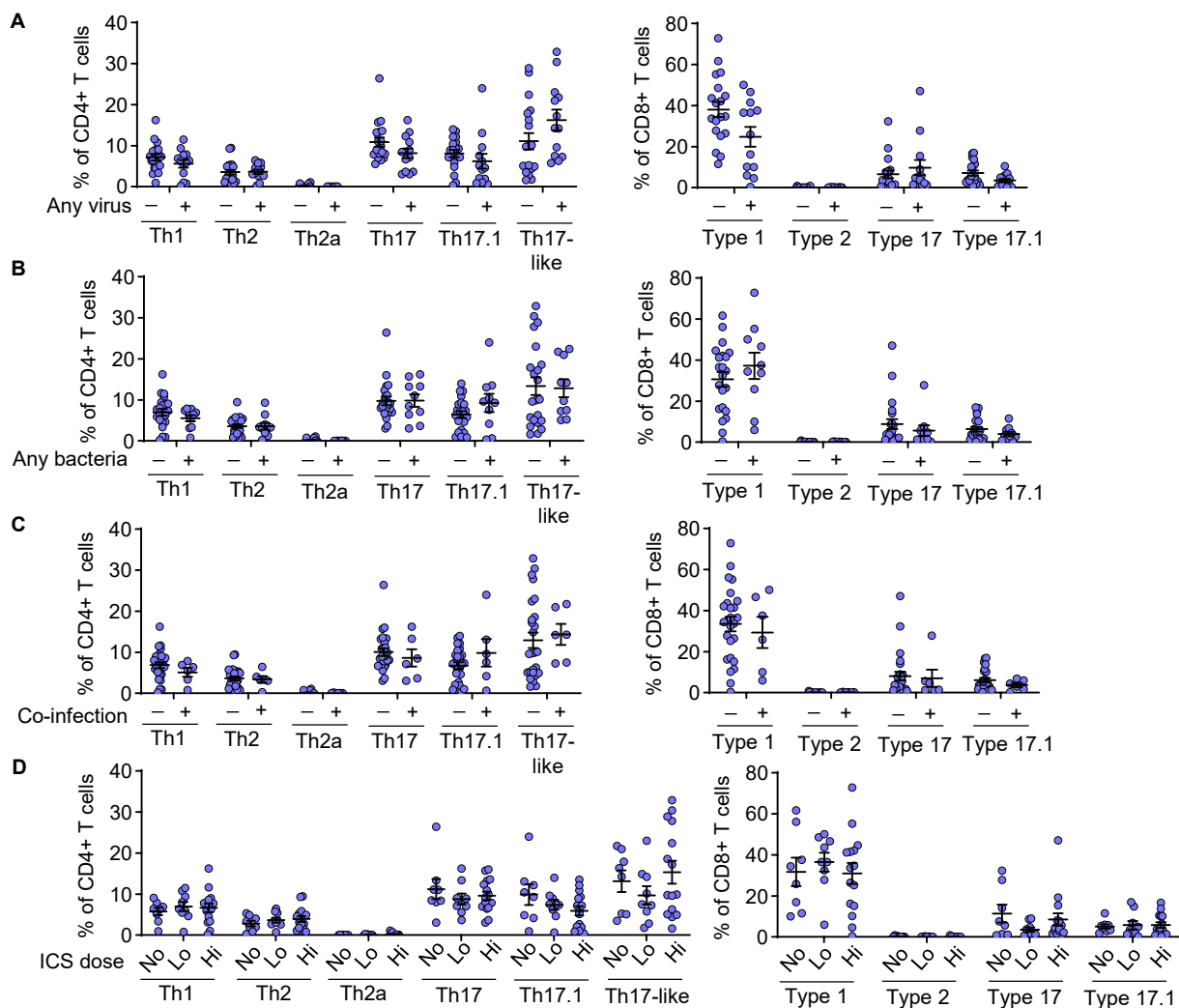
Supplemental Figure 2. Representative scatter plots showing the gating strategy for (A) memory T cells and (B and C) type 1, type 2, and type 17 subsets.



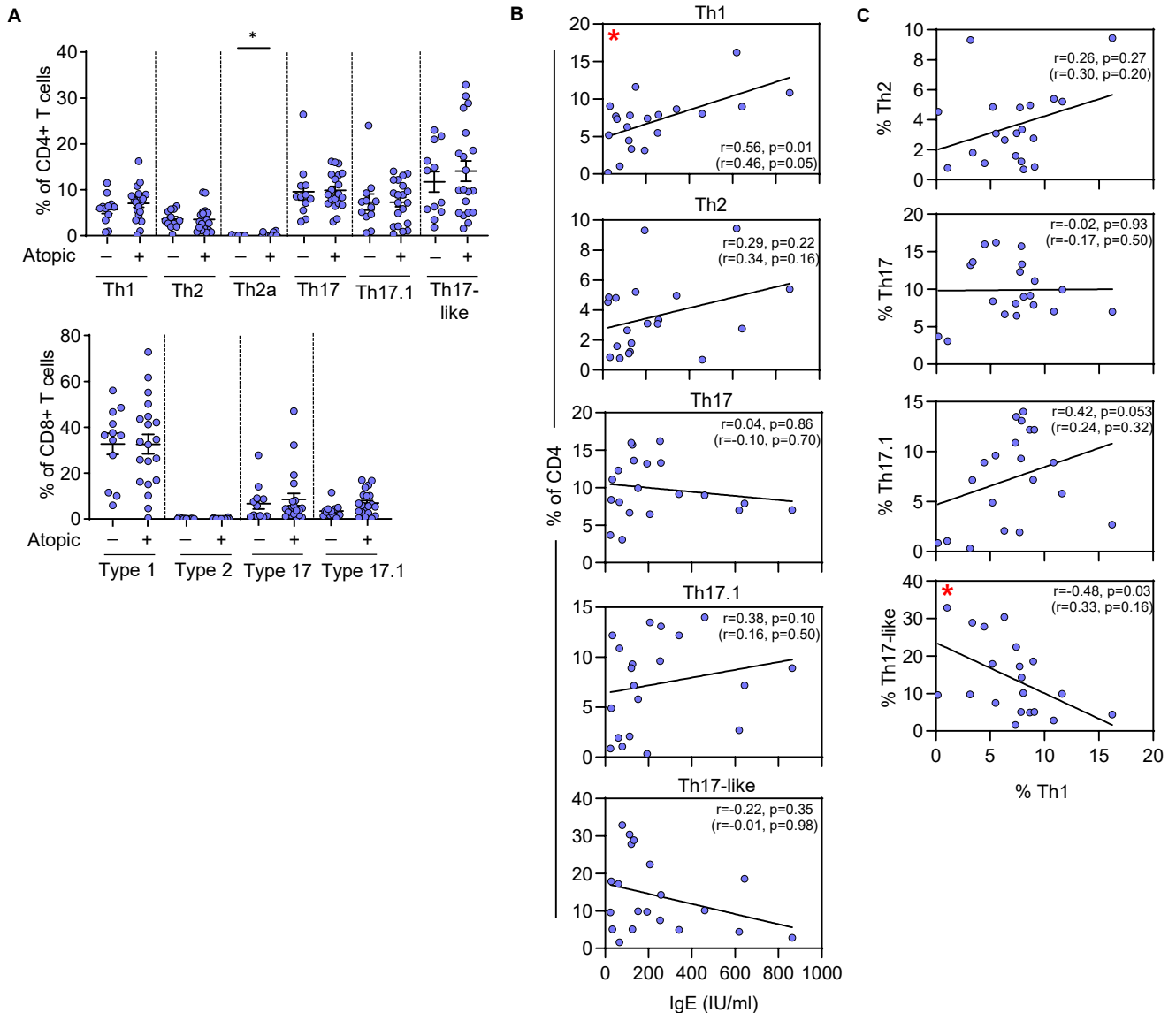
Supplemental Figure 3. Frequencies of type 1, type 2, and type 17 signatures by sex. (A) Frequencies of T-cell subsets in the blood (n=32). (B and C) Frequencies of T-cell subsets in the blood (B) and BAL (C) of males (n=23; M) and females (n=9; F). Bars denote mean $\pm$ SEM. Multiple Mann-Whitney tests with Holm-Sidak correction (B and C).



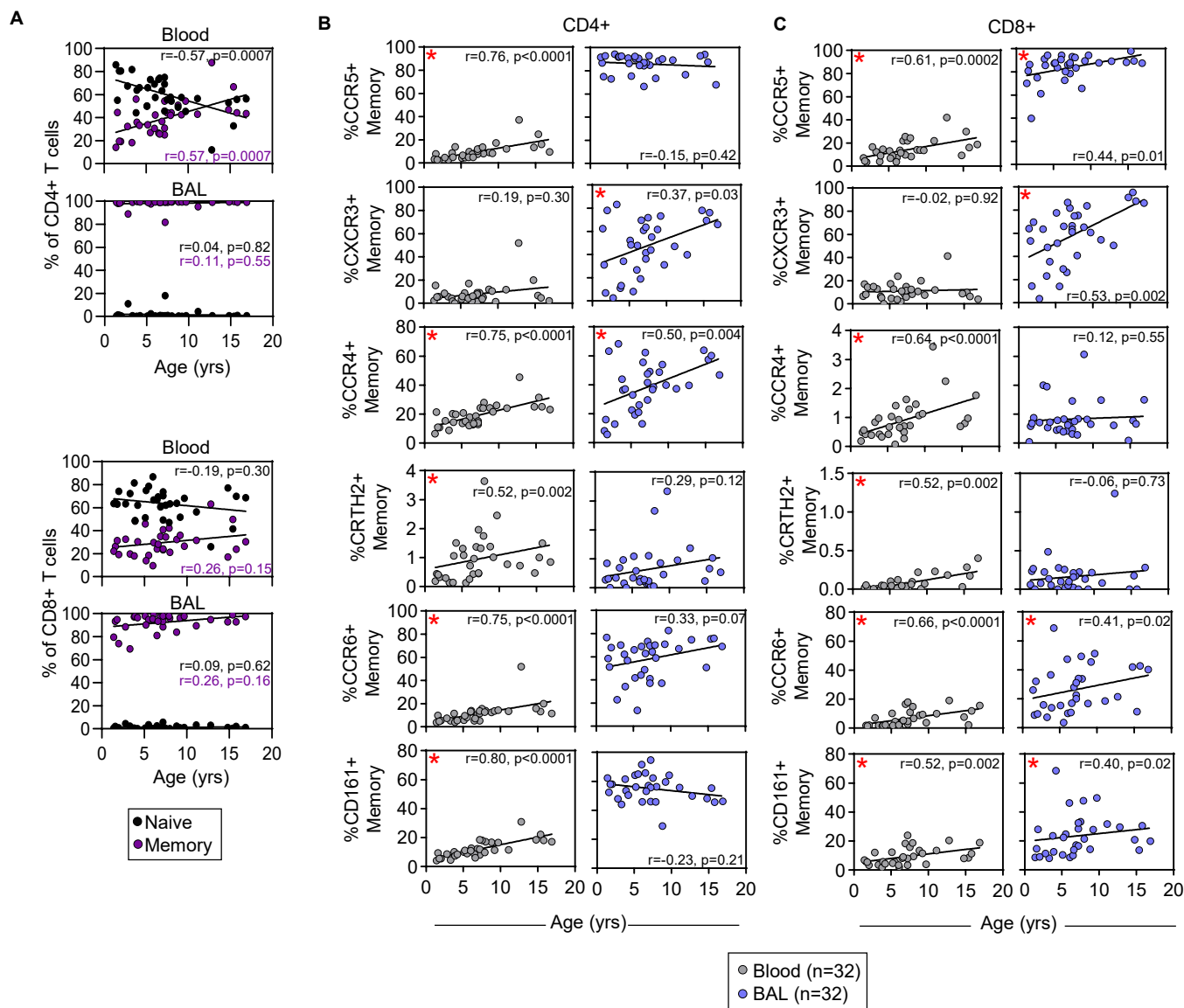
Supplemental Figure 4. Frequencies of broad T-cell subsets by sex. (A) Frequencies of CD4+, CD8+, and DN T cells in blood and BAL, as a percentage of CD3+ cells. (B and C) Frequencies of naïve, T_{CM}, T_{EM}, and TEMRA-like T cells in blood and BAL, as a percentage of CD4+ and CD8+ T cells. (D and E) Frequencies of CD4+ and CD8+ T_{RM} in blood and BAL. (F-I) Frequencies of marker-positive memory (T_{CM}, T_{EM}, and TEMRA-like) CD4+ and CD8+ T cells in blood and BAL. Mean $\pm$ SEM. Multiple Mann-Whitney tests with Holm-Sidak correction (A, B, C, F, G, H, and I) and Mann-Whitney test (D and E). M, Male, n=23; F, Female, n=9.



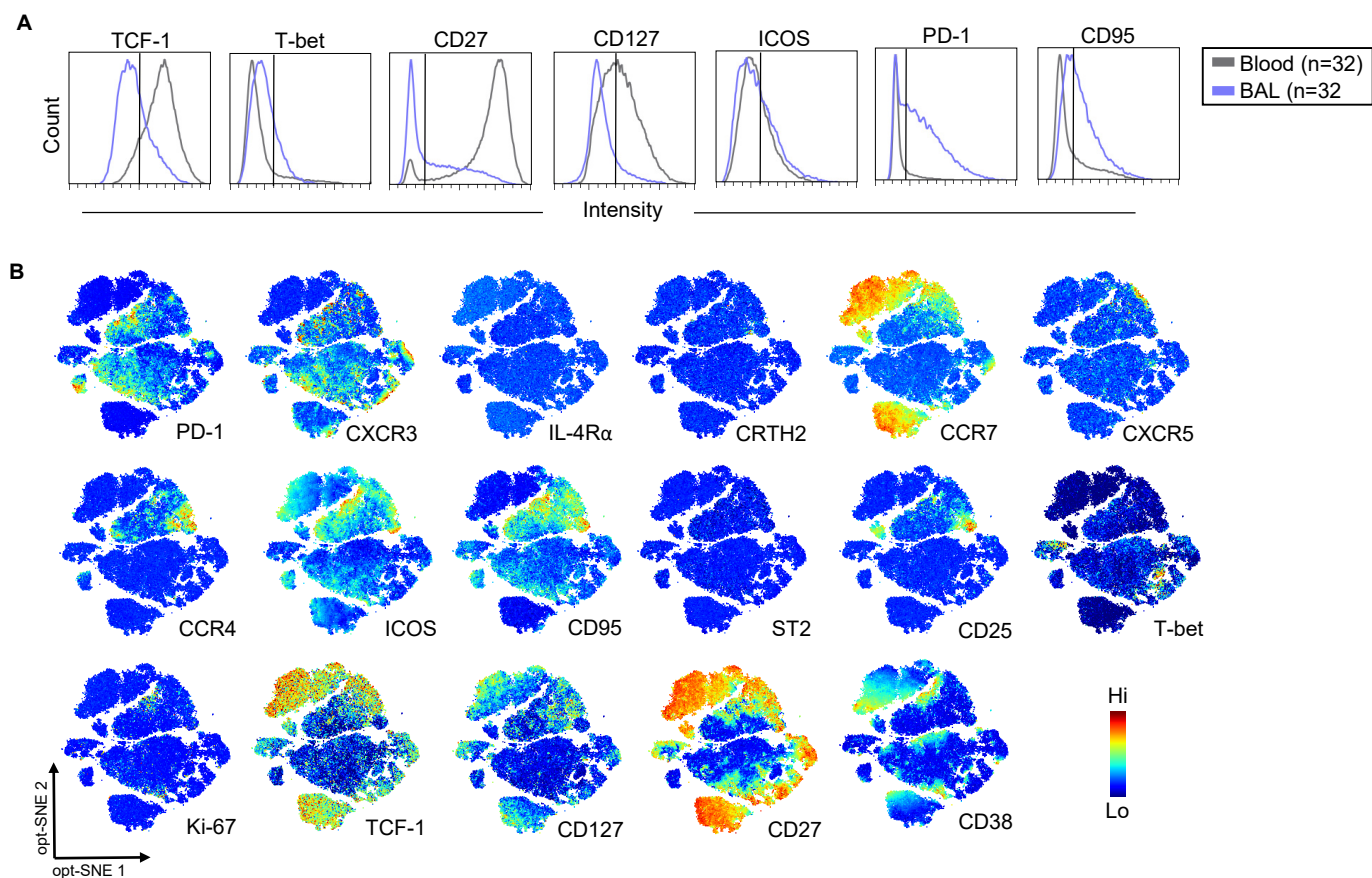
Supplemental Figure 5. Frequencies of type 1, type 2, and type 17 signatures in the airways by infection status and medication use. (A-D) Frequencies of T-cell subsets in the BAL in relation to (A) any virus (- n=19, + n=13), (B) any bacteria (- n=22, + n=10), and (C) co-infection (- n=26, + n=6). (D) Frequencies of T-cell subsets in the BAL of patients on no ICS (n=8), low dose ICS (n=9; Lo), and high dose ICS (n=15; Hi). Mean $\pm$ SEM. Multiple Mann-Whitney tests with Holm-Sidak correction (A-C) and Kruskal-Wallis test with Dunn's multiple comparisons test (D).



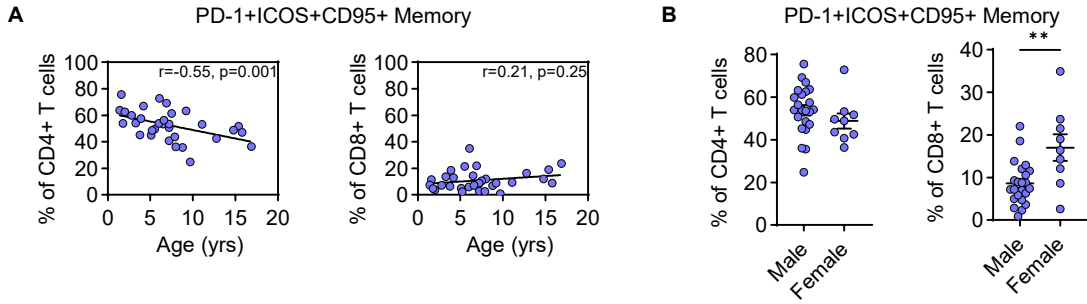
Supplemental Figure 6. Th1 cells are linked to total IgE in atopic children. (A) Frequencies of type 1, type 2, and type 17 subsets in the BAL of patients with (n=20) and without (n=12) atopy as defined by detection of specific IgE to at least one allergen. Mean $\pm$ SEM. (B) Spearman correlations between levels of total IgE and frequencies of CD4+ T-cell subsets in atopic patients. Lines denote linear regression and red asterisks denote significant correlations. Multiple Mann-Whitney tests with Holm-Sidak correction (A). Values in parentheses corrected for age (B and C). *p<0.05



Supplemental Figure 7. Preferential accumulation of type 1 T cells in the airways with age. (A) Spearman correlations between age and frequencies of naïve and memory (T_{CM} , T_{EM} , TEMRA-like) CD4+ and CD8+ T cells in blood and BAL from 32 children. (B and C) Spearman correlations between age and frequencies of marker-positive memory CD4+ T cells (B) and CD8+ T cells (C) in blood and BAL. Lines denote linear regression and red asterisks denote significant correlations.



Supplemental Figure 8. Expression profile of T cells in the blood and BAL of children with recurrent wheeze. (A) Histograms showing the expression of select markers on blood and BAL CD3⁺ cells. (B) Expression of select phenotypic markers across all samples (n=64).

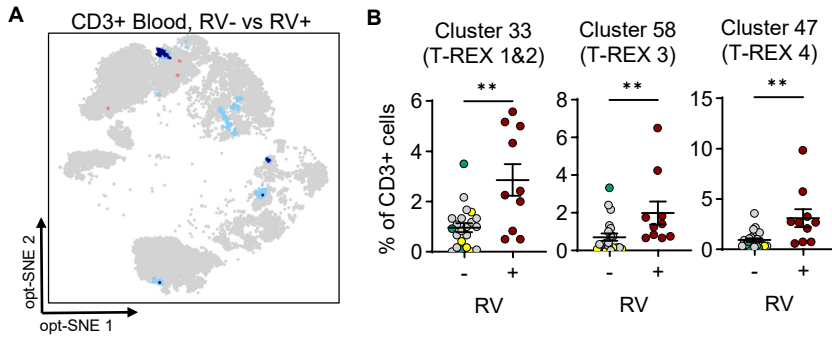


Supplemental Figure 9. Activated T cells in the lower airways by age and sex. (A) Spearman correlations between age and frequencies of activated CD4+ and CD8+ memory T cells in the BAL (n=32). Lines denote linear regression. (B) Frequencies of activated CD4+ and CD8+ memory T cells in the BAL of males (n=23) and females (n=9). Mean $\pm$ SEM. Mann-Whitney test. ** $p \leq 0.01$

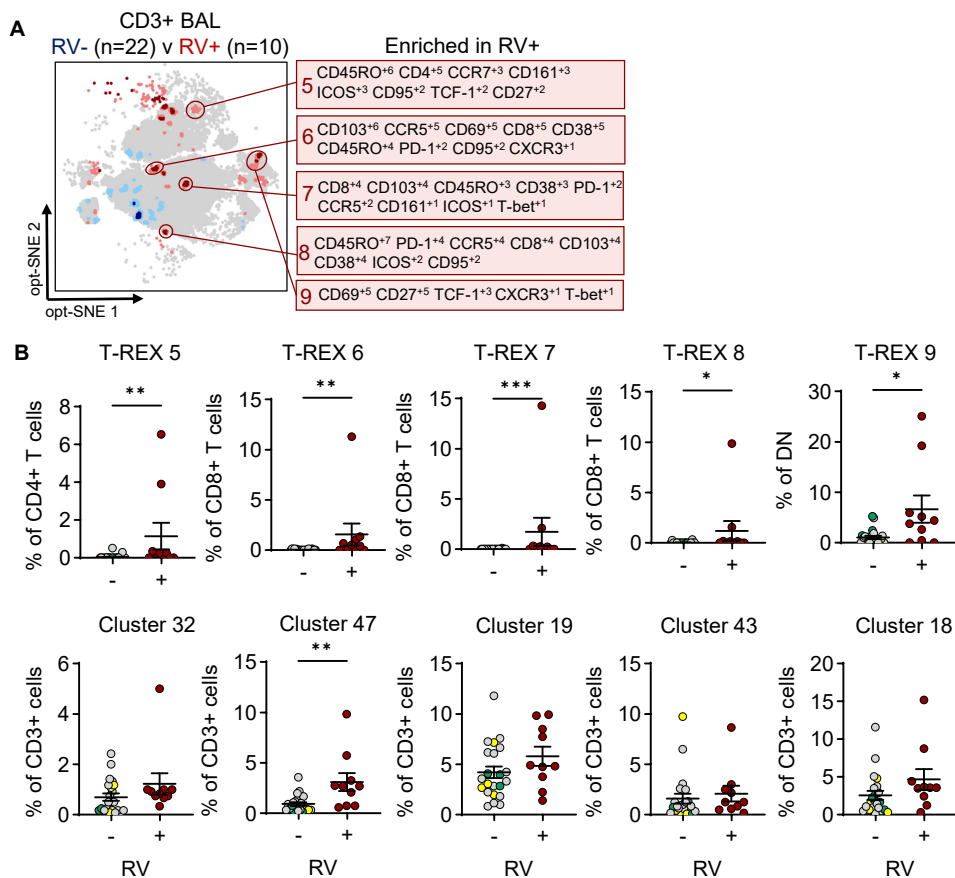
Supplemental Table 2. Demographic and clinical data of RV- and RV+ patients

	RV-	RV+	P value
n	22	10	N/A
Age (yrs) ^A	6.7 (5.0-8.9)	4.8 (3.0-7.7)	0.19 ^D
Male sex, n (%)	16 (72.7)	7 (70.0)	>0.99 ^E
White, n (%)	16 (72.7)	8 (80.0)	>0.99 ^E
Age Category			0.35 ^E
Pre-school (1-5yrs)	8 (36.4)	4 (40.0)	
School-age (6-11yrs)	9 (40.9)	6 (60.0)	
Teen-age (12-17yrs)	5 (22.7)	0 (0.0)	
Total IgE (IU/ml) ^A	72.7 (33.9-156.0)	43.5 (14.8-128.1)	0.35 ^D
Atopic, n (%)	15 (68.2)	5 (50.0)	0.44 ^E
Medication, n (%)			
Inhaled corticosteroid (ICS)	16 (72.7)	8 (80.0)	>0.99 ^E
Long-acting β -agonist	13 (59.1)	1 (10.0)	0.02 ^E
Antileukotriene	7 (31.9)	2 (20.0)	0.68 ^E
Oral prednisone	2 (9.1)	1 (10.0)	>0.99 ^E
Dupilumab	2 (9.1)	0 (0.0)	>0.99 ^E
Mepolizumab	1 (4.5)	0 (0.0)	>0.99 ^E
Omalizumab	1 (4.5)	0 (0.0)	>0.99 ^E
BAL composition % ^B		(n=9)	
Eosinophils	0 (0-4)	0 (0-14)	0.43 ^D
Neutrophils	2.5 (0-68)	29 (0-90)	0.03 ^D
Macrophage	75.5 (0-95)	47 (0-88)	0.05 ^D
Lymphocytes	4 (0-11)	3 (0-9)	0.60 ^D
Infection status, n (%)			
Rhinovirus	0 (0.0)	10 (100.0)	<0.0001 ^E
Other virus ^C	3 (13.6)	0 (0.0)	0.53 ^E
Any bacteria	6 (27.3)	4 (40.0)	0.68 ^E
Co-infection	2 (9.1)	4 (40.0)	0.06 ^E

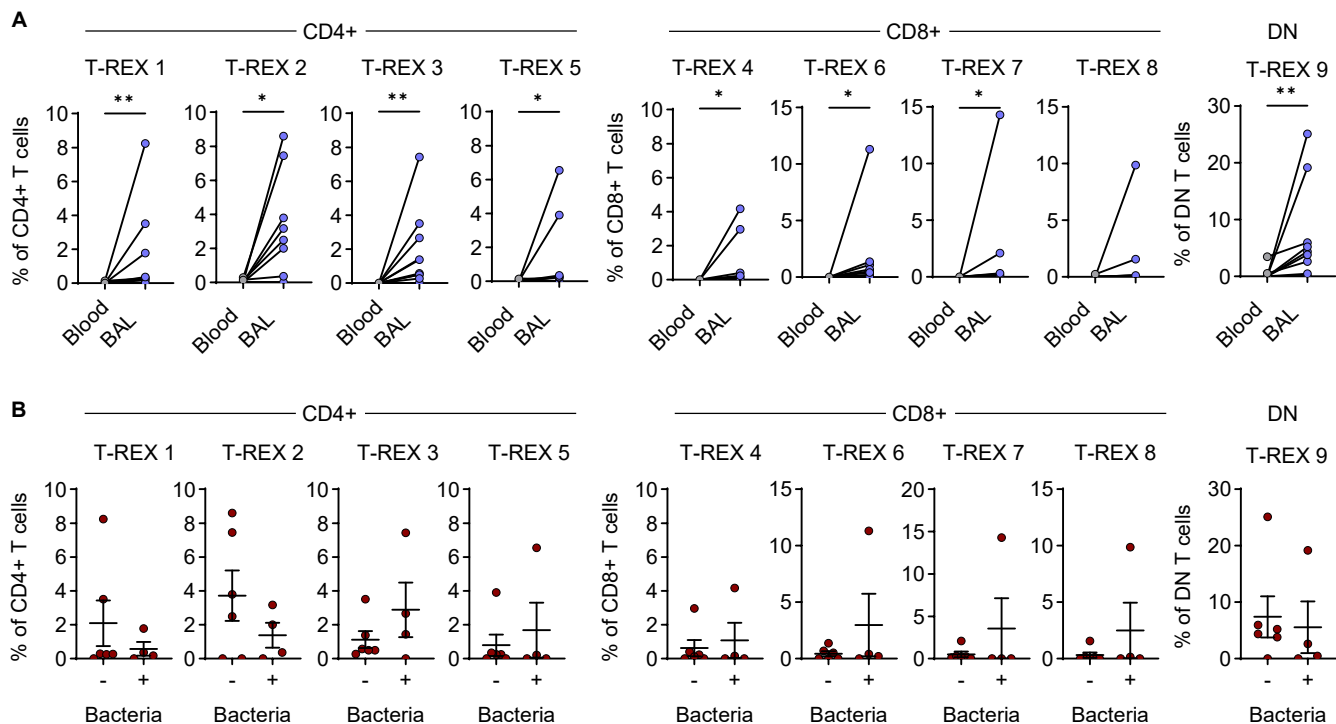
^A Geometric mean (95% CI); ^B Median (range); ^C Adenovirus and Metapneumovirus; ^D Mann-Whitney test; ^E Fisher's exact test.



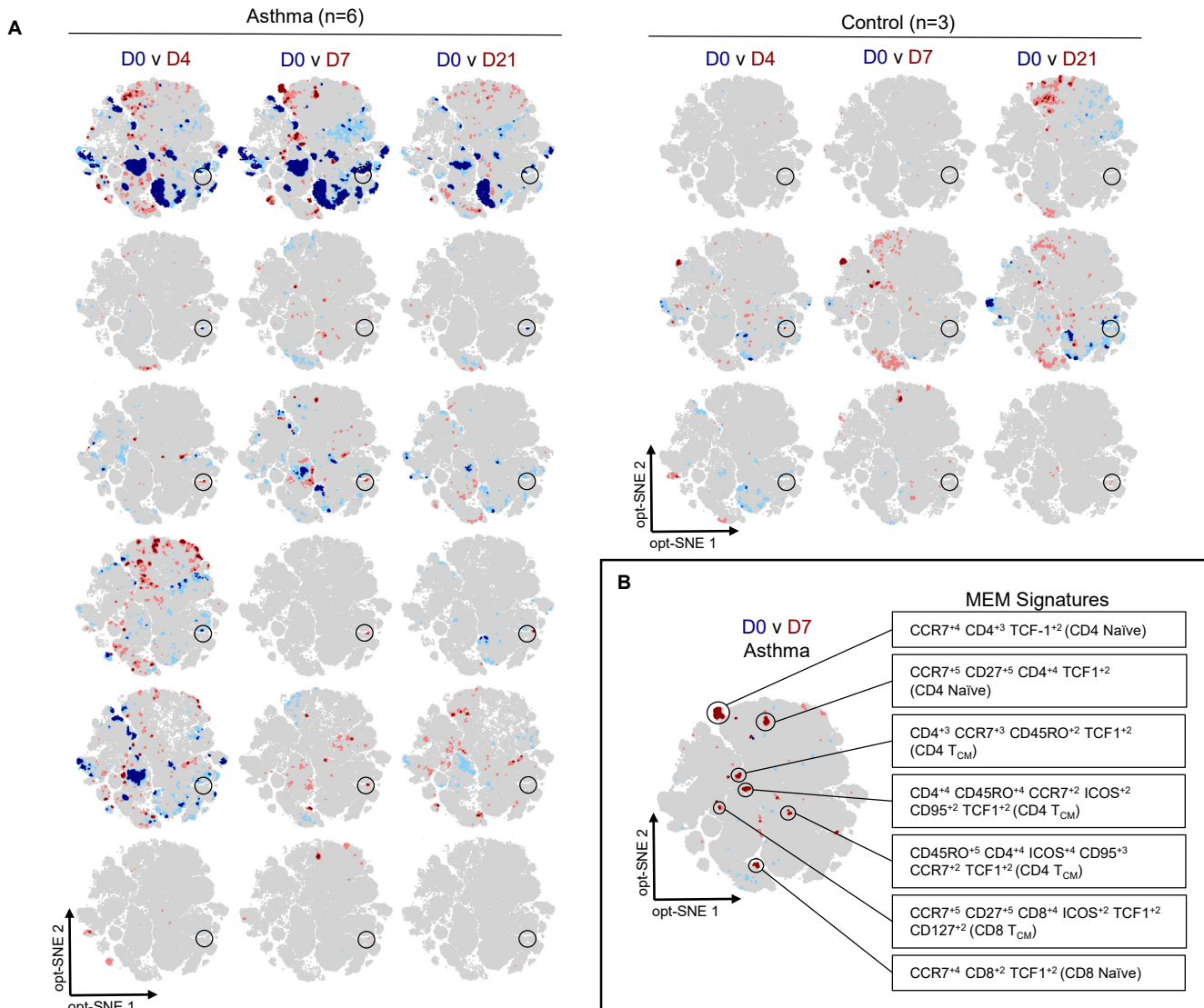
Supplemental Figure 10. T-REX analysis of blood, and frequencies of PhenoGraph clusters containing T-REX populations in RV+ and RV- children. (A) T-REX analysis of T cells comparing RV- (n=22) and RV+ (n=10) groups. **(B)** Frequencies of PhenoGraph clusters containing T-REX populations in RV- and RV+ groups. Colored symbols denote patients positive for other viruses (green, n=3), RV+ patients (red, n=10), patients negative for any virus (grey, n=15) and patients who received a biologic (yellow, n=4). Mean $\pm$ SEM. Mann-Whitney test.



Supplemental Figure 11. “Highly variable” RV-related T-cell signatures. (A) MEM signatures of “highly variable” populations enriched in the RV⁺ group. (B) Frequencies of “highly variable” T-REX populations enriched in the RV⁺ group and their corresponding PhenoGraph clusters. Colored symbols denote patients positive for other viruses (green, n=3), RV⁺ patients (red, n=10), patients negative for any virus (grey, n=15) and patients who received a biologic (yellow, n=4). Mean ± SEM. Mann-Whitney test. *p<0.05, **p≤0.01, ***p≤0.001.



Supplemental Figure 12. Analysis of frequencies of RV-related T-Rex populations 1-9. (A) Frequencies of T-Rex populations in matched blood and BAL of RV+ patients (n=10). (B) Frequencies of T-Rex populations in RV+ children who tested negative (n=6) or positive (n=4) for bacteria. Mean $\pm$ SEM. Wilcoxon matched-pairs signed rank test (A) and Mann-Whitney test (B). *p<0.05, **p<0.01.



Supplemental Figure 13. Variability in the T-cell response to RV challenge. (A) T-REX plots for individual subjects (asthma, n=6 and healthy controls, n=4) comparing changes on days 4, 7, and 21 with day 0 of RV challenge. Black circles denote T-REX A. (B) “Highly variable” T-REX populations induced by RV in asthma (day 7 versus day 0).

Supplemental Table 3. Characteristics of RV-challenge study participants.

	Asthma	Control	P value
n	6	3	N/A
Age (yrs) ^A	21.5 (18.6-24.8)	21.3 (18.6-24.4)	0.76 ^B
Male sex, n (%)	2 (33.3)	1 (33.3)	>0.99 ^C
White, n (%)	4 (66.7)	3 (100.0)	0.50 ^C
Total IgE (IU/ml) ^A	1139 (473.7-2737)	9.7 (1-96.4)	0.02 ^B

^A Geometric mean (95% CI); ^B Mann-Whitney test; ^C Fisher's exact test.

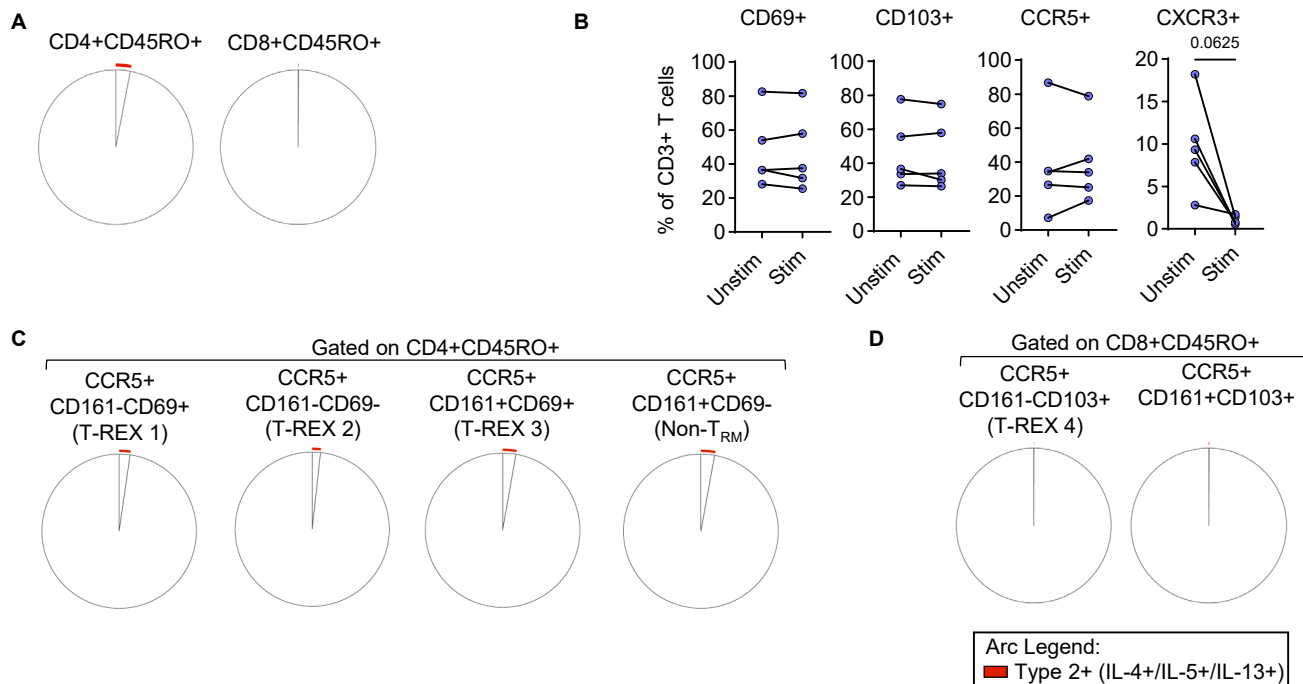
Supplemental Table 4. Demographics and clinical data for patients included in the intracellular cytokine analysis.

	Cytokine profiling	T-cell phenotyping	P value
n	6	32	N/A
Age (yrs) ^A	8.6 (6.3-11.5)	6.0 (4.7-7.6)	0.17 ^C
Male sex, n (%)	5 (83.3)	23 (71.9)	>0.99 ^D
White, n (%)	3 (50.0)	24 (75.0)	0.64 ^D
Total IgE (IU/ml) ^A	78.2 (10.9-559.0)	61.9 (34.1-112.3)	0.82 ^C
Atopic, n (%)	4 (66.7)	20 (62.5)	>0.99 ^D
Medication, n (%)			
Inhaled corticosteroid (ICS)	5 (83.3)	24 (75.0)	>0.99 ^D
Long-acting β -agonist	5 (83.3)	14 (43.8)	0.08 ^D
Antileukotriene	2 (33.3)	9 (28.1)	>0.99 ^D
Oral prednisone	0 (0.0)	3 (9.4)	>0.99 ^D
Dupilumab	1 (16.7)	2 (6.3)	0.41 ^D
Mepolizumab	1 (16.7)	1 (3.1)	0.29 ^D
Omalizumab	0 (0.0)	1 (3.1)	>0.99 ^D
BAL phenotype, n (%)		(n=31)	0.75 ^D
Isolated eosinophilia	0 (0.0)	2 (6.5)	
Isolated neutrophilia	1 (16.7)	9 (29.0)	
Mixed granulocytes	1 (16.7)	2 (6.5)	
Pauci-granulocytic	4 (66.7)	18 (58.1)	
Infection status, n (%)			
Rhinovirus	0 (0.0)	10 (31.3)	0.17 ^D
Other virus ^B	0 (0.0)	3 (9.4)	>0.99 ^D
Any bacteria	0 (0.0)	10 (31.3)	0.17 ^D
Co-infection	0 (0.0)	6 (18.8)	0.56 ^D

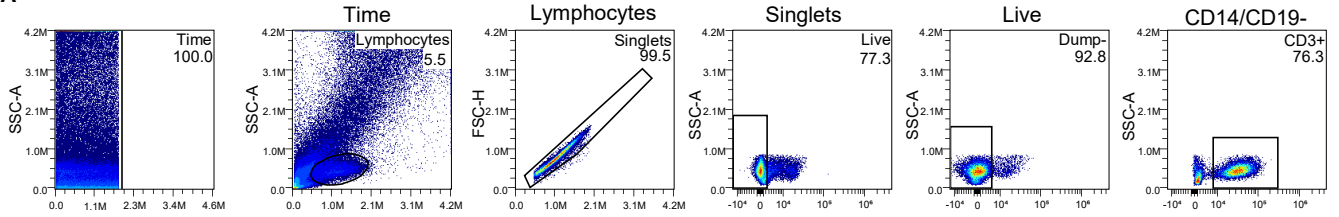
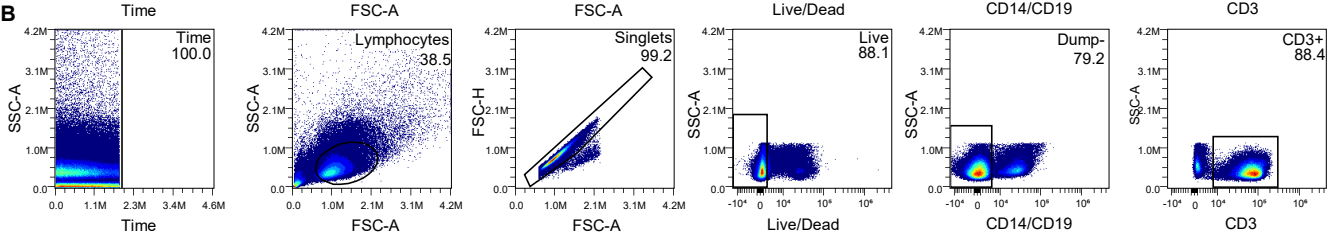
^A Geometric mean (95% CI). ^B Adenovirus and Metapneumovirus. ^C Mann-Whitney test. ^D Fisher's exact test.

Supplemental Table 5. Spectral flow cytometry panel for intracellular cytokine analysis.

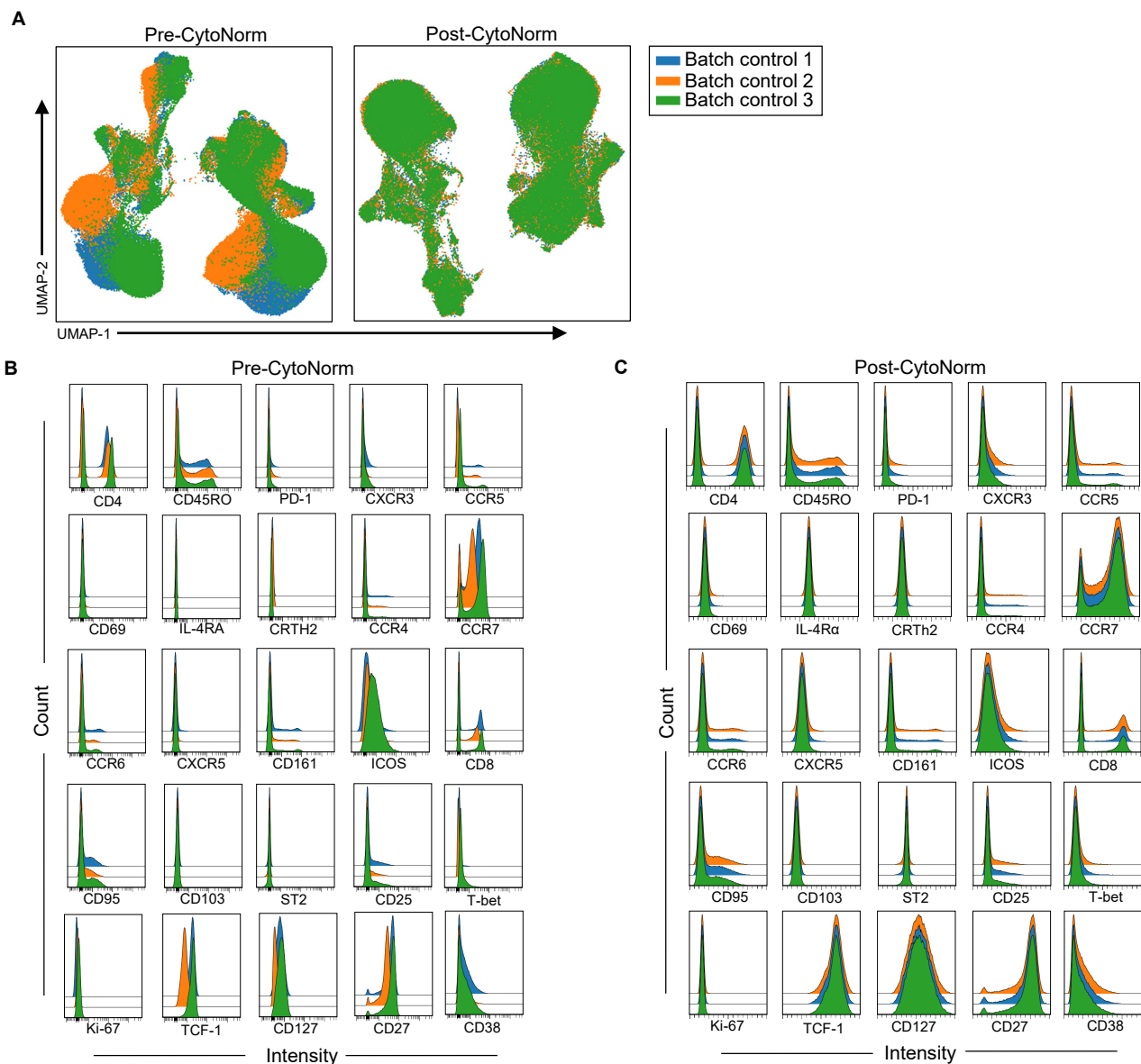
Marker	Color	Clone	Vendor	Catalog number	Dilution
Viability	Live/Dead Blue		Invitrogen	L23105	1:4000
CD3	BUV395	SK7	BD	564001	1:50
CD4	BUV496	OKT4	BD	750980	1:50
CD45RO	BUV563	UCHL1	BD	748369	1:100
CXCR3	BUV661	1C6/CXCR3	BD	741649	1:50
CCR5	BUV737	2D7/CCR5	BD	612808	1:25
CD69	BUV805	FN50	BD	748763	1:100
CD14	BV510	M5E2	Biolegend	301842	1:20
CD19	BV510	HIB19	Biolegend	302242	1:20
IL-4	BV605	MP4-25D2	Biolegend	500828	1:40
CD103	BV711	Ber-ACT8	Biolegend	350221	1:50
TNF- α	BV750	MAb11	BD	566359	1:200
IL-17A	BV785	BL168	Biolegend	512338	1:100
IL-13	FITC	B-P6	Invitrogen	BMS133FI	1:50
CD8	PerCP	SK1	Biolegend	344708	1:200
Granzyme B	RB705	GB11	BD	570275	1:100
IL-21	PE	3A3-N2.1	BD	562042	1:50
CD161	PE-Dazzle 594	HP-3G10	Biolegend	339940	1:20
IL-2	PE-Cy7	MQ1-17H12	BD	560707	1:50
IL-5	APC	JES1-39D10	Miltenyi Biotec	130-127-364	1:20
IFN- γ	Alexa Fluor 700	4S.B3	Biolegend	502520	1:1000
IL-22	APC-Fire 750	2G12A41	Biolegend	366713	1:50



Supplemental Figure 14. Type 2 cytokine production is minimal in the lower airways of children with recurrent wheeze. (A) SPICE plots showing average type 2 cytokine signatures in CD4+ and CD8+ memory T cells in the BAL (n=6). **(B)** Change in marker expression after stimulation of BAL cells (n=5). Mean $\pm$ SEM. **(C and D)** Average type 2 cytokine signatures in candidate CD4+ and CD8+ T-REX populations (n=6). Wilcoxon matched-pairs signed rank test **(B)**.

A**B**

Supplemental Figure 15. Representative scatter plots showing manual gating strategy for pre-processing of (A) blood and (B) BAL samples.



Supplemental Figure 16. Results of CytoNorm data normalization. (A) UMAP of batch control samples, pre- and post-normalization using Cytonorm. Histograms showing the distribution of each marker within each batch control, (B) pre- and (C) post-normalization.