Supplementary Tables & Figures:

Table S1: Tissue Identification

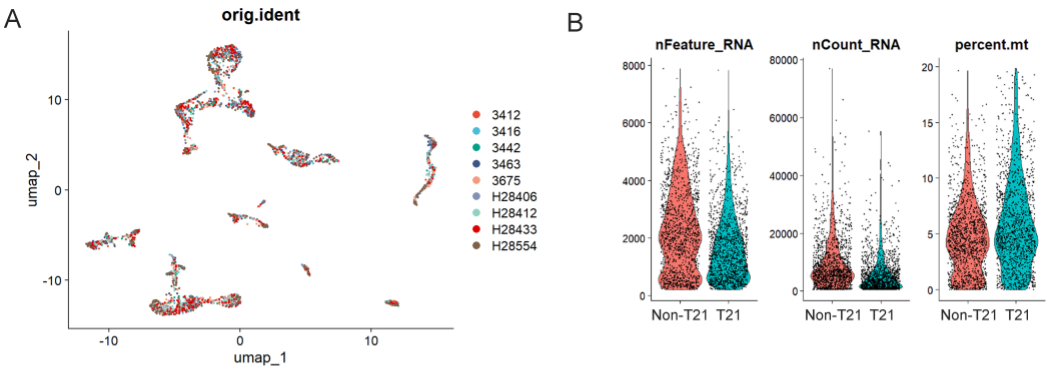
Sample Type	Identification	Gestational Age (Wks)	Sex	Application
Non-T21	H29488	20.1	F	Immune cell isolation, IF, FISH
T21	H28811	19.6	F	Immune cell isolation, IF, FISH
Non-T21	H29008	20.1	F	Immune cell isolation IF, FISH
T21	H29493	19.3	F	Immune cell isolation IF, FISH
Non-T21	H29411	14.5	M	Immune cell isolation
T21	H29746	14.5	M	Immune cell isolation
Non-T21	H29345	19.1	M	Immune cell isolation IF, FISH
T21	H29370	19.1	M	Immune cell isolation IF, FISH
Non-T21	H29427	16	F	Immune cell isolation IF, FISH
T21	H29424	15.5	F	Immune cell isolation IF, FISH
Non-T21	H29205	16.5	M	Immune cell isolation
T21	H29910	17.3	M	Immune cell isolation
Non-T21	H28758	17.4	M	Immune cell isolation IF, FISH
T21	H28790	18.1	M	Immune cell isolation IF, FISH
Non-T21	359	19.5	F	Immune cell isolation IF, FISH
T21	H28433	19.4	F	Immune cell isolation IF, FISH

Table S2: TaqMan probes

Target gene	Probe ID
GAPDH	HS02786624
PTPRC	Hs04189704
CD19	Hs01047413
CD38	Hs01120071
CD22	Hs00998488
RAG1	Hs01920694
SPN	Hs01872322
CR2	Hs00153398

Table S3: Antibody list

Antibody	Company	RRID	Host species	Dilution
CDH1 E-Cadherin (24E10)	Cell signaling technology, Danvers, MA, USA	RRID:AB_2291471	Rabbit	1/200
CD20	Proteintech, Rosemont, IL, USA	RRID, AB_2881391	Mouse	1/100
CD38	Proteintech	RRID, AB_716994	Mouse	1/100



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Cluster	Non-T21	T21	Total
0	171	260	431
1	207	198	405
2	183	112	295
3	113	159	272
4	121	131	252
5	99	110	209
6	79	118	197
7	68	99	167
8	129	37	166
9	75	49	124
10	42	44	86
11	41	37	78
12	45	31	76
13	35	25	60
Total	1408	1410	2818

Figure S1. Prenatal T21 immune cell quality control A) Distribution of epithelial cells by sample in UMAP shows no sample-level bias. B) Quality control panels for genes expressed, UMI counts, and mitochondrial gene proportions within each immune sub-cluster. C) Table shows cell distribution by condition (Non-T21 vs T21) for each sub-cluster.

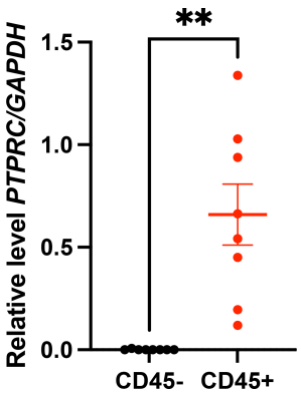

Figure S2. PTPRC Expression Confirms Purity of CD45⁺ Immune Cell Fraction RT-qPCR analysis of PTPRC expression in CD45⁺ immune cells and CD45⁻ cells isolated from T21 and non-T21 prenatal lungs. PTPRC transcripts were detected only in the CD45⁺ fraction, confirming the specificity and efficiency of the CD45⁺ isolation. Results are shown as individual data points and mean ± SEM; **p < 0.01.

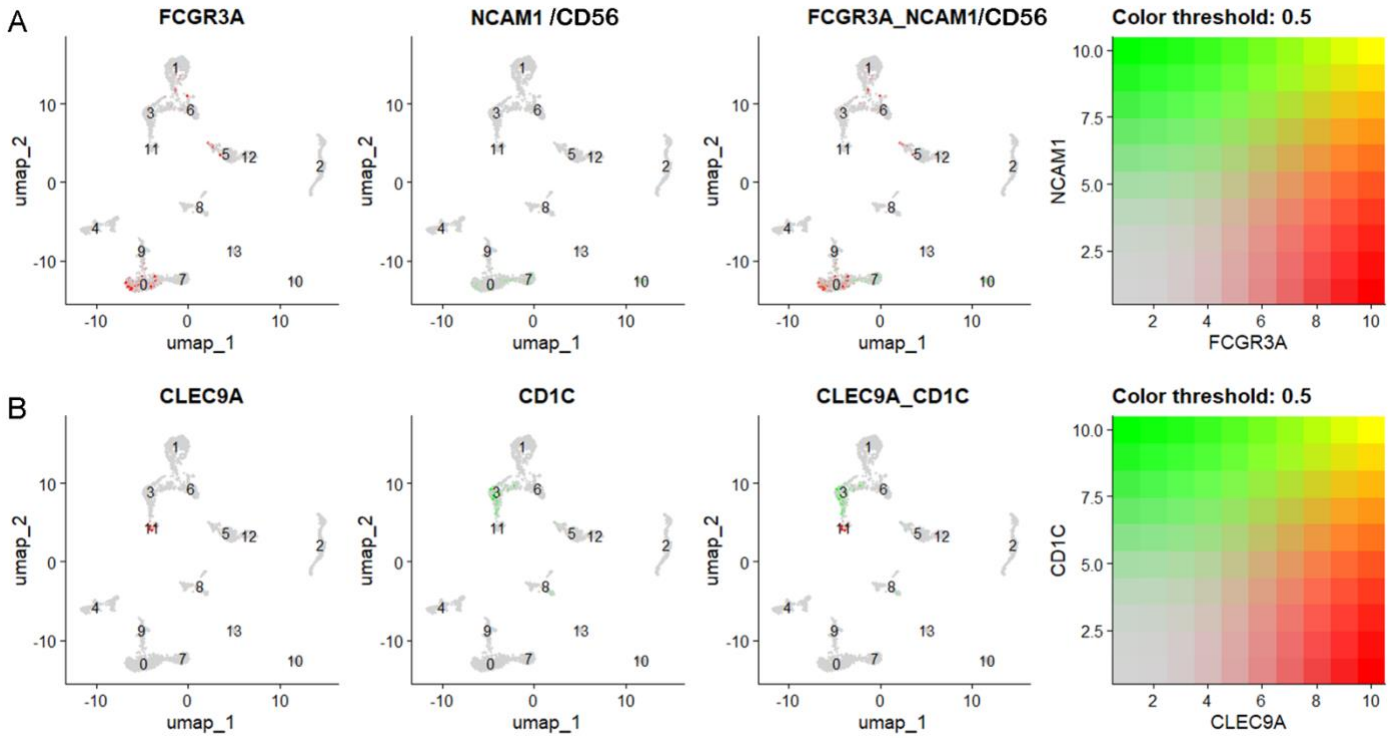

Figure S3. Gene co-expression to validate cell type identification. (A) Immune marker co-expression of FCGR3A (Red) and NCAM1/CD56 (Green) represented by UMAP. Each dot represents a single cell. Cells demonstrating FCGR3A/NCAM1 co-expression would be represented in yellow. However, we did not observe any cells co-expressing both markers. (B) Immune marker co-expression of CLEC9A (Red) and CD1C (Green) represented by UMAP. Each dot represents a single cell. Cells demonstrating CLEC9A/CD1C co-expression would be represented in yellow. However, we did not observe any cells co-expressing both markers.