

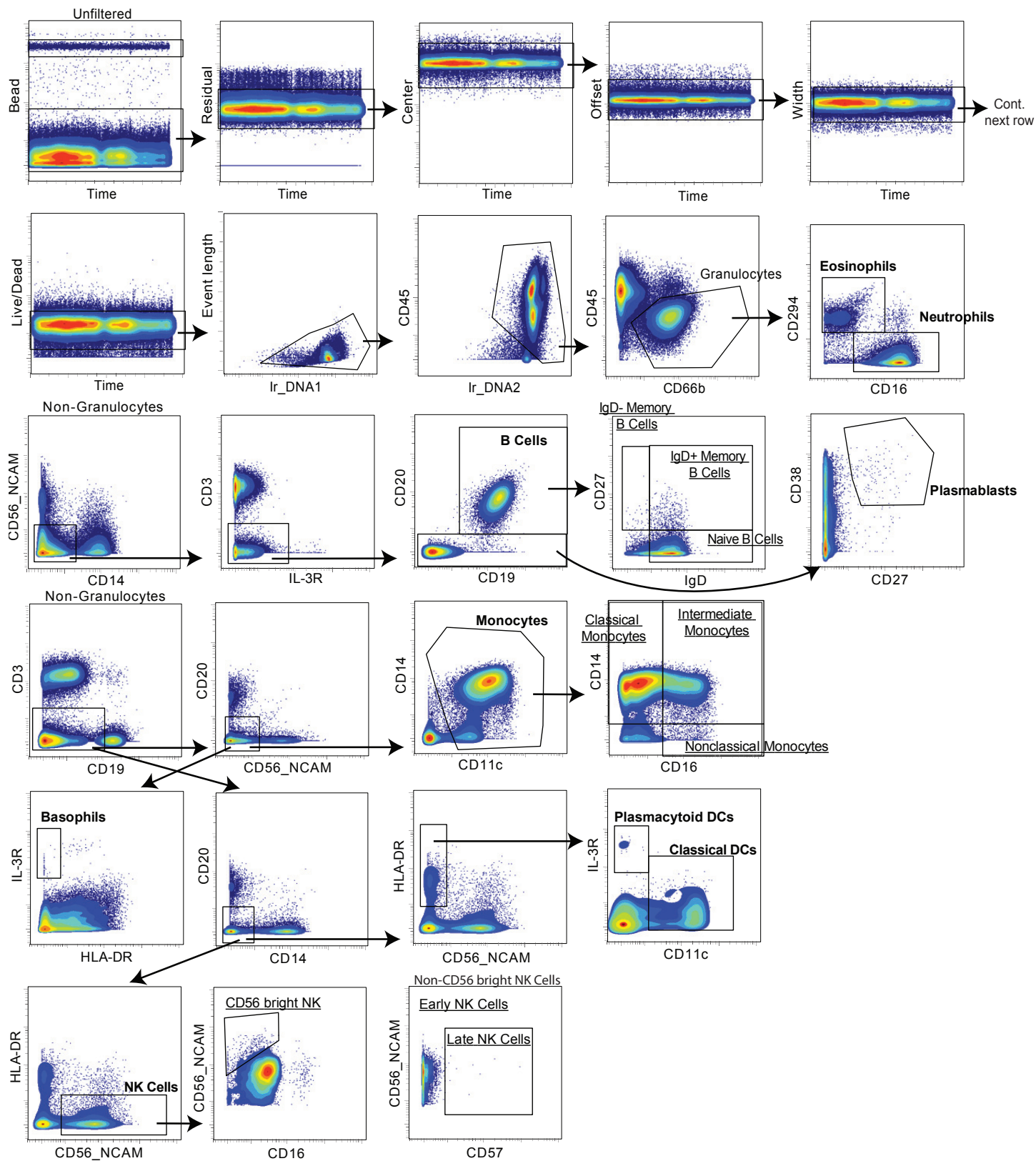
Supplemental Figure 1: Gating Strategy

Cells in bold were analyzed as a proportion of total CD45+ cells. Cells underlined were analyzed as a proportion of the parent cell in bold above it.

- > CD45+ Cells (CD45+, DNA+)
- >> Granulocytes (CD66b+, CD45 lo-mid)
- >>> **Neutrophils** (CD294-, CD16hi)
- >>> **Eosinophils** (CD294+, CD16lo)
- >> Mononuclear Cells (non-Granulocytes)
- >>> Non-monocyte, non-NK cell (CD14-, CD56-)
- >>>> Non-T (CD3-, IL3R-)
- >>>>> **B cells** (CD19+, CD20+)
- >>>>>> Naïve B cell (IgD+, CD27-)
- >>>>>> IgD negative Memory B cell (CD27+, IgD-)
- >>>>>> IgD positive Memory B cell (CD27+, IgD+)
- >>>>> CD20 negative (CD20-)
- >>>>>> **Plasmablast (CD38+, CD27+)**
- >>> Non-T cell, non-B cell (CD3-, CD19-)
- >>>> Non-NK cell (CD56-, CD20-)
- >>>>> **Monocytes (CD11c+)**
- >>>>>> Classical Monocytes (CD14+, CD16-)
- >>>>>> Intermediate Monocytes (CD14+, CD16+)
- >>>>>> Non-Classical Monocytes (CD14-, CD16+)
- >>>>>> **Basophils (IL-3R+, HLA-DR-)**
- >>>> Non-Monocyte (CD14-, CD20-)
- >>>>> Dendritic Cells (HLA-DR+, CD56-)
- >>>>>> **Plasmacytoid Dendritic Cells (CD11c-, IL-3R+)**
- >>>>>> **Classic Dendritic Cells (CD11c+, IL3R-)**
- >>>>>> **NK Cells (CD56+, HLA-DR-)**
- >>>>>>> CD56 bright NK Cells (CD56 hi, CD16-)
- >>>>>>> CD56 dim NK cells (CD56 lo-mid)
- >>>>>>>> Early NK Cells (CD57-)
- >>>>>>>> Late NK Cells (CD57+)
- >>> T cells (CD3+, CD19-)
- >>>> **NK T cells (CD56+)**
- >>>> $\gamma\delta$ T cells (TCR $\gamma\delta$ +
- >>>> ab T -cells (TCR $\gamma\delta$ -)
- >>>>> **CD8 T cells (CD8+, CD4-)**
- >>>>> **CD4 T cells (CD8-, CD4+)**
- >>>>> **Double positive T cells (CD8+, CD4+)**
- >>>>> **Double negative T cells (CD8-, CD4-)**
- The following applies to CD8+ T cells, CD4+ T cells, and $\gamma\delta$ T cells each--
- >>>>>> CD161+ T cells (CD161+)
- >>>>>> CD57+ T cells (CD57+)
- >>>>>> CD27+ T cells (CD27+)
- >>>>>>> Effector Memory 1 T cells (CCR7-, CD45RA-)
- >>>>>>> CCR7+ T cells (CCR7+)
- >>>>>>>> Central Memory T cells (CD45RO+, CD45RA-)
- >>>>>>>> Naïve T cells (Non-Central Memory T cells)
- >>>>>>>> CD27- CD45RA- T cells (CD27-, CD45RA-)
- >>>>>>>>> Effector Memory 2 T cells (CCR7+)
- >>>>>>>>> Effector Memory 3 T cells (CCR7-)
- >>>>>>>>>> T-effector memory re-expressing CD45RA cells (CD27-, CD45RA+)

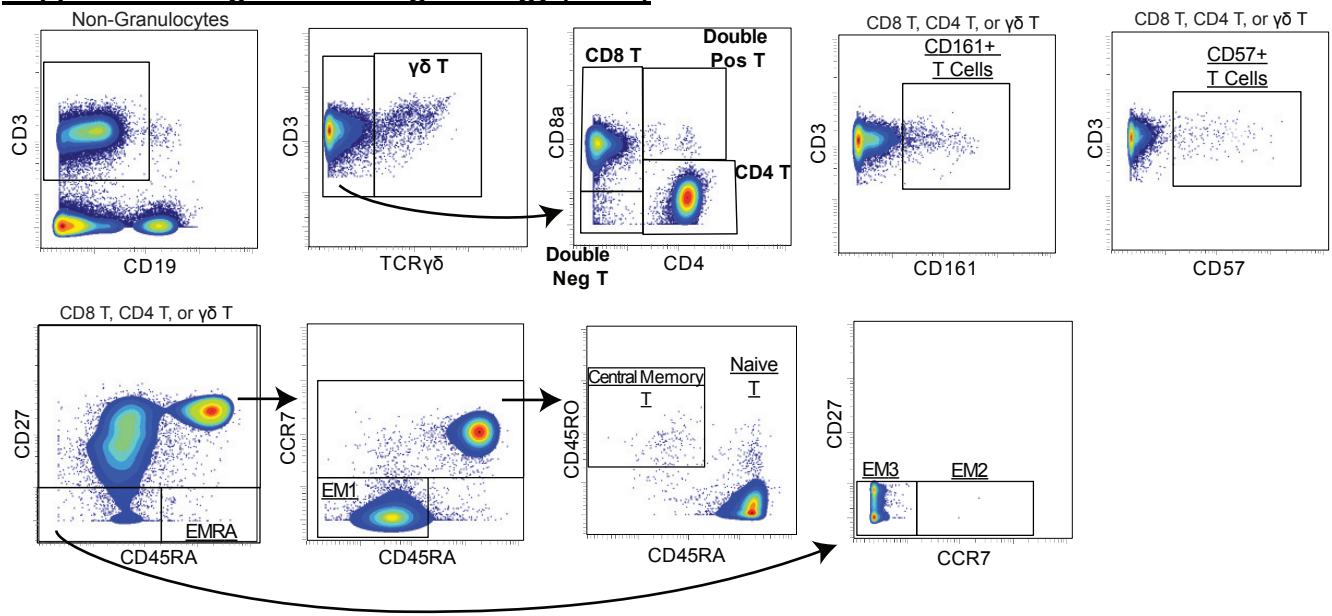
Supplemental Figure 1: Gating Strategy (cont.)

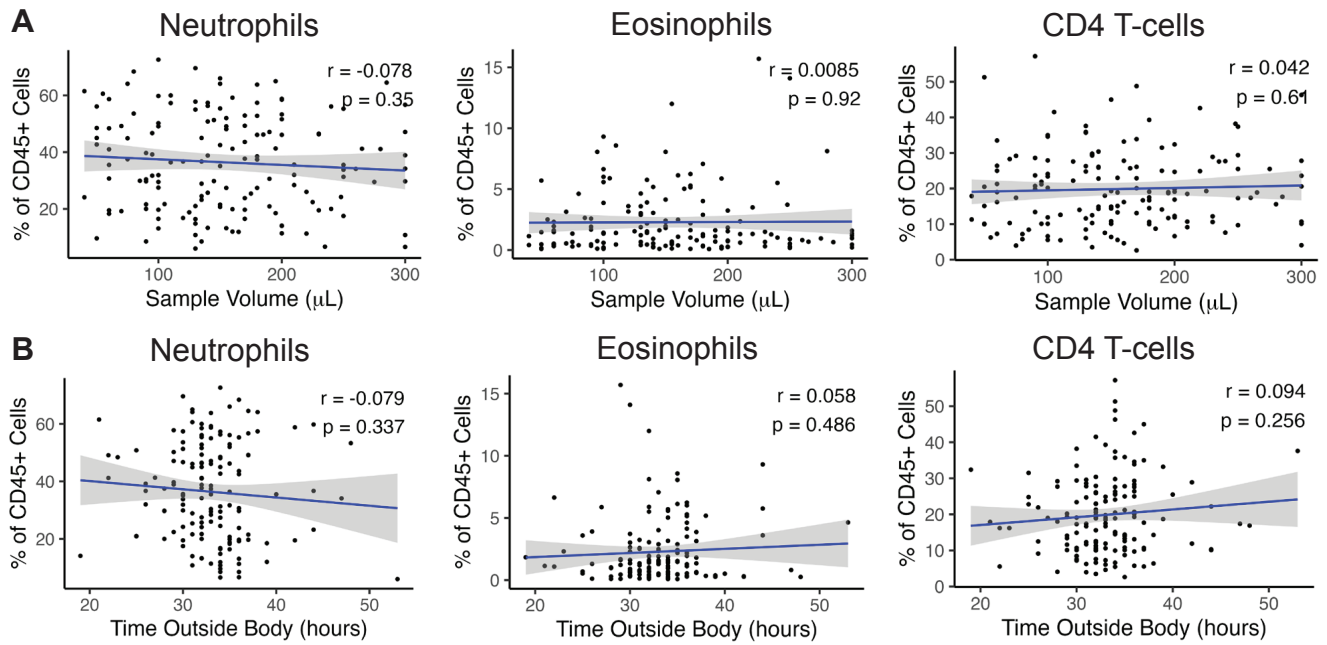
Representative gating strategy from a single infant P14 sample.



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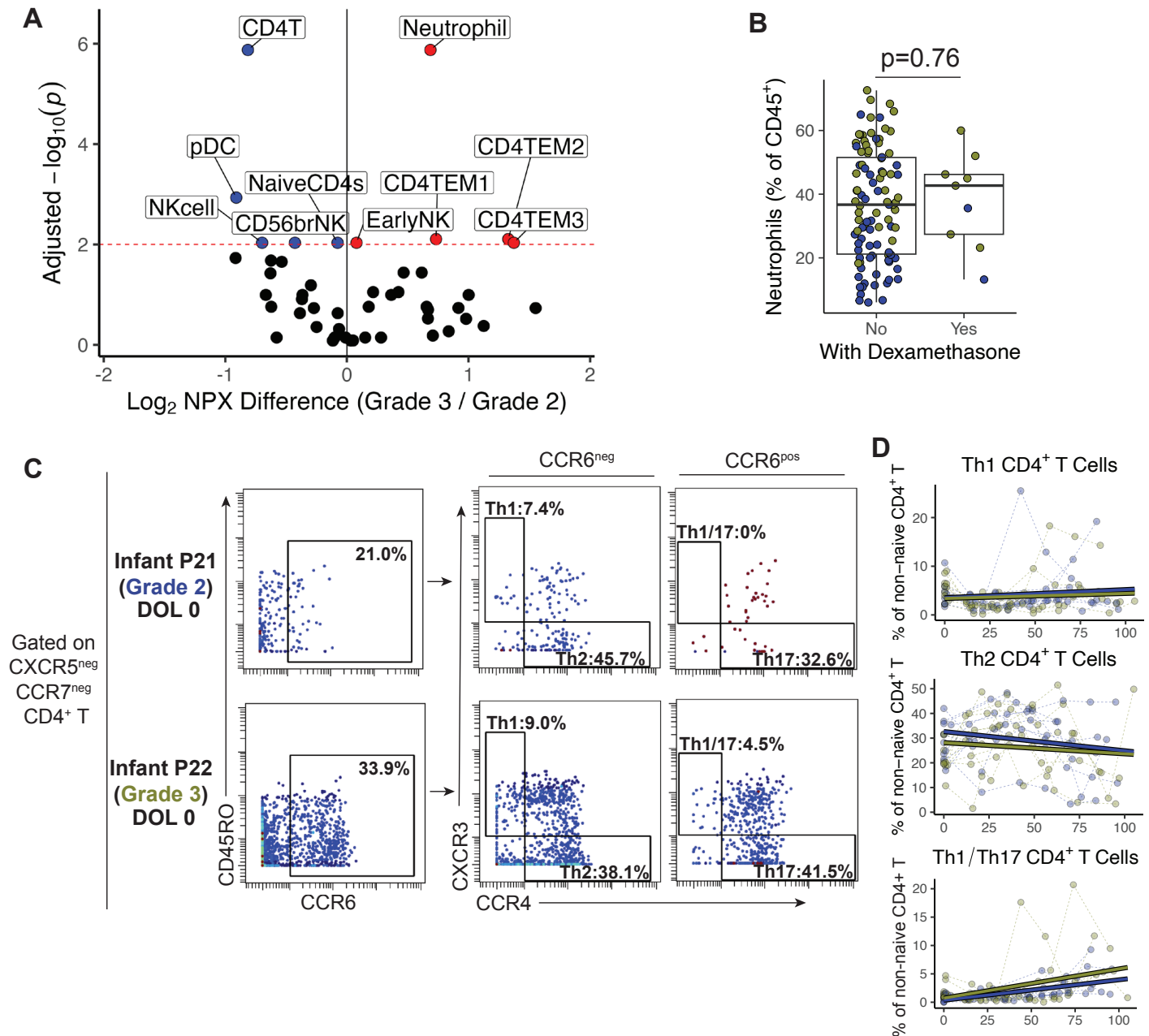
Supplemental Figure 1: Gating Strategy (cont.)





Supplemental Figure 2: Sample volume and time outside of body does not impact overall cell composition

A) Neutrophils, eosinophils, and CD4⁺ T cells proportions in all samples over a range of residual sample volumes.
 B) Cells in all samples over the time each sample rested in the clinical lab before processing for this study.



Supplemental Figure 3: Infants with severe BPD have altered neutrophil and CD4⁺ T cell profiles

A) Volcano plot demonstrating cell populations that differ between infants with Grade 3 and Grade 2 BPD. B) Proportional neutrophil levels in each sample by whether the sample was collected during active dexamethasone administration. Infants with severe (green) and moderate (blue) BPD shown. P-value derived from Wilcoxon test. C) Representative CyTOF plots showing the distribution of Th subpopulations within non-naïve CXCR5⁺, CCR7⁺ T cells from two infants, one ultimately developing moderate BPD (P21) and one severe BPD (P22), at one timepoint on DOL 0. D) Mixed linear model of Th1, Th2, and Th1/Th17 cells as a proportion of non-naïve CD4⁺ T cells over time from severe BPD (green) and moderate BPD (blue).