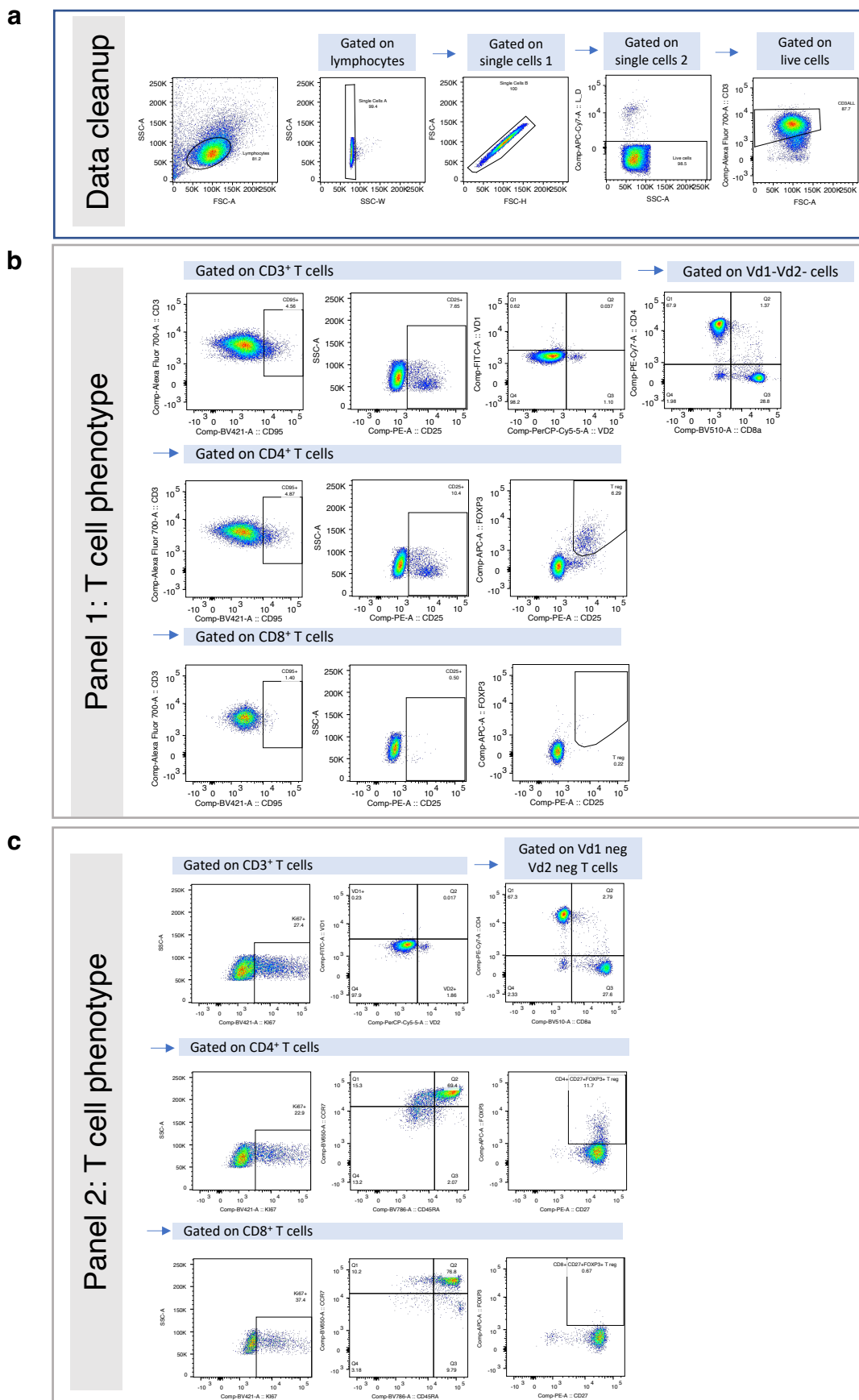
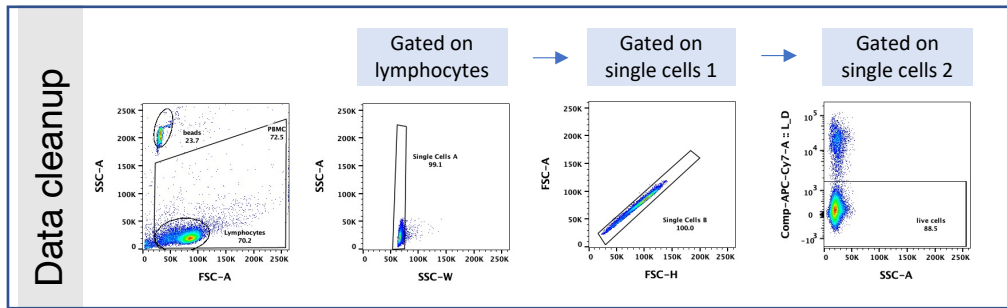
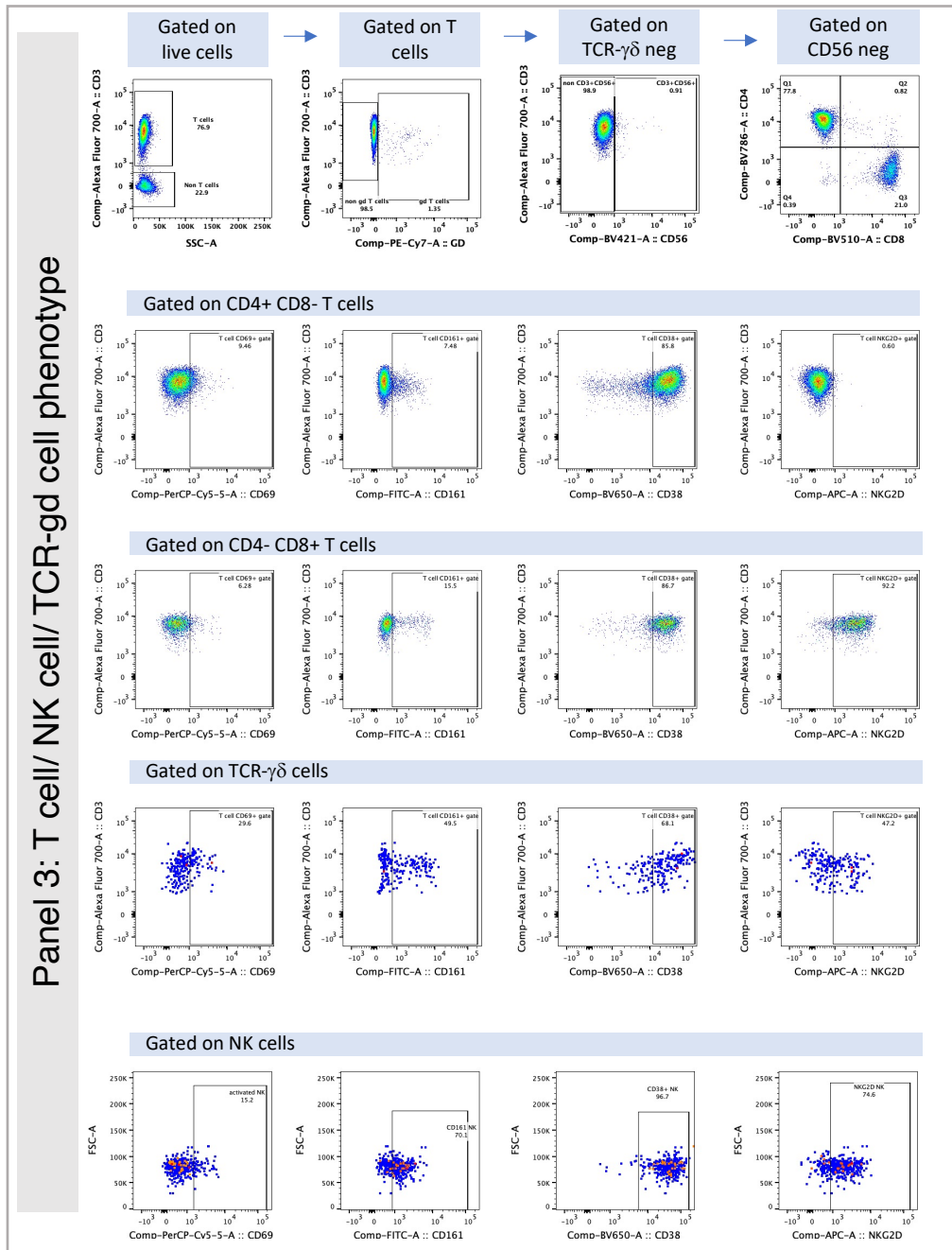




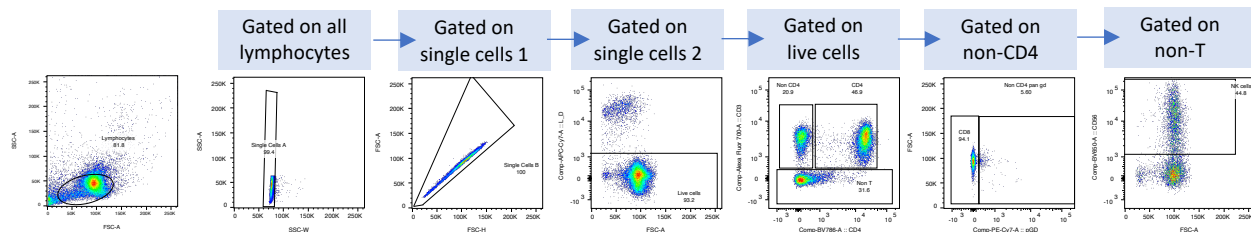
Supplementary Figure 1: Flow cytometry gating strategies for panels 1 and 2. **a**, Data clean-up and a shared initial gating workflow is shown. **b**, Panel 1: Gating strategy used to examine frequencies of Vδ1, Vδ2, CD4⁺ and CD8⁺ T cells, CD4⁺ T regs, CD8⁺ FOXP3⁺CD25⁺ T cells and frequencies of T cells expressing CD25 or CD95/FAS. **c**, Panel 2: Gating strategy to examine frequencies of proliferating (Ki67⁺) cells, naïve and memory cells as well as CD27⁺FOXP3⁺ cells within CD4⁺ and CD8⁺ T cells.

a**b**

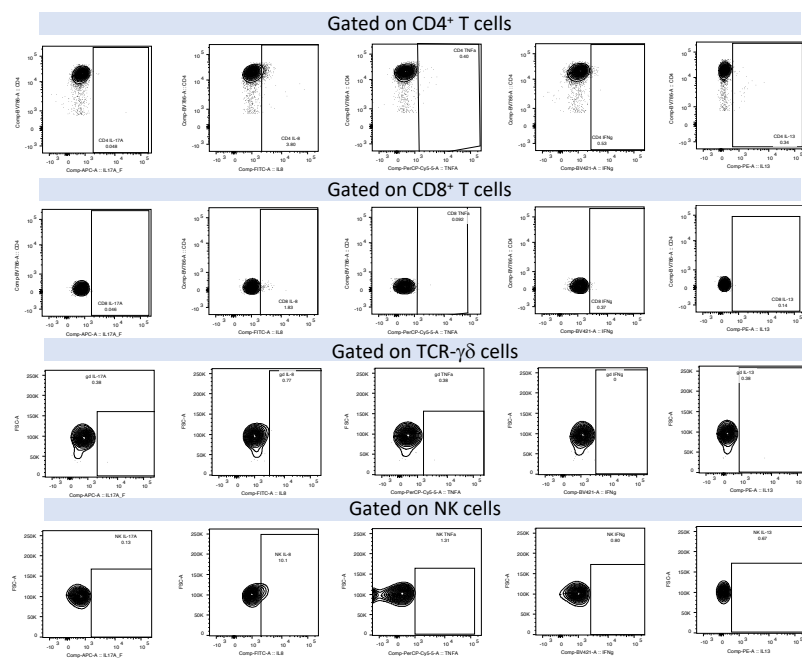
Supplementary Figure 2: Flow cytometry gating strategy for panel 3. a, Data clean-up and a shared initial gating workflow is shown. **b**, Gating strategy to examine expression of CD161, CD38, NKG2D and CD69 on T cells, NK and TCR- $\gamma\delta$ cells.

a

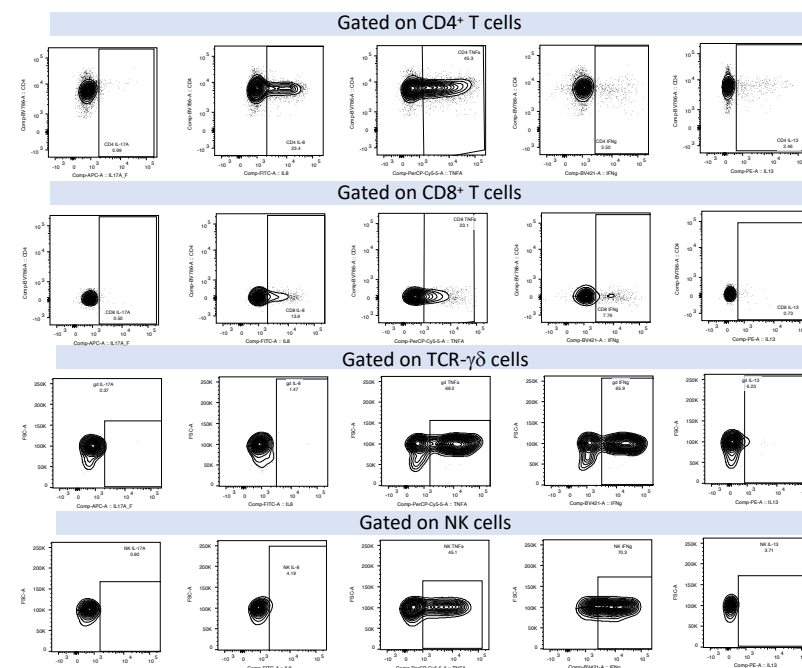
Data cleanup

**b**

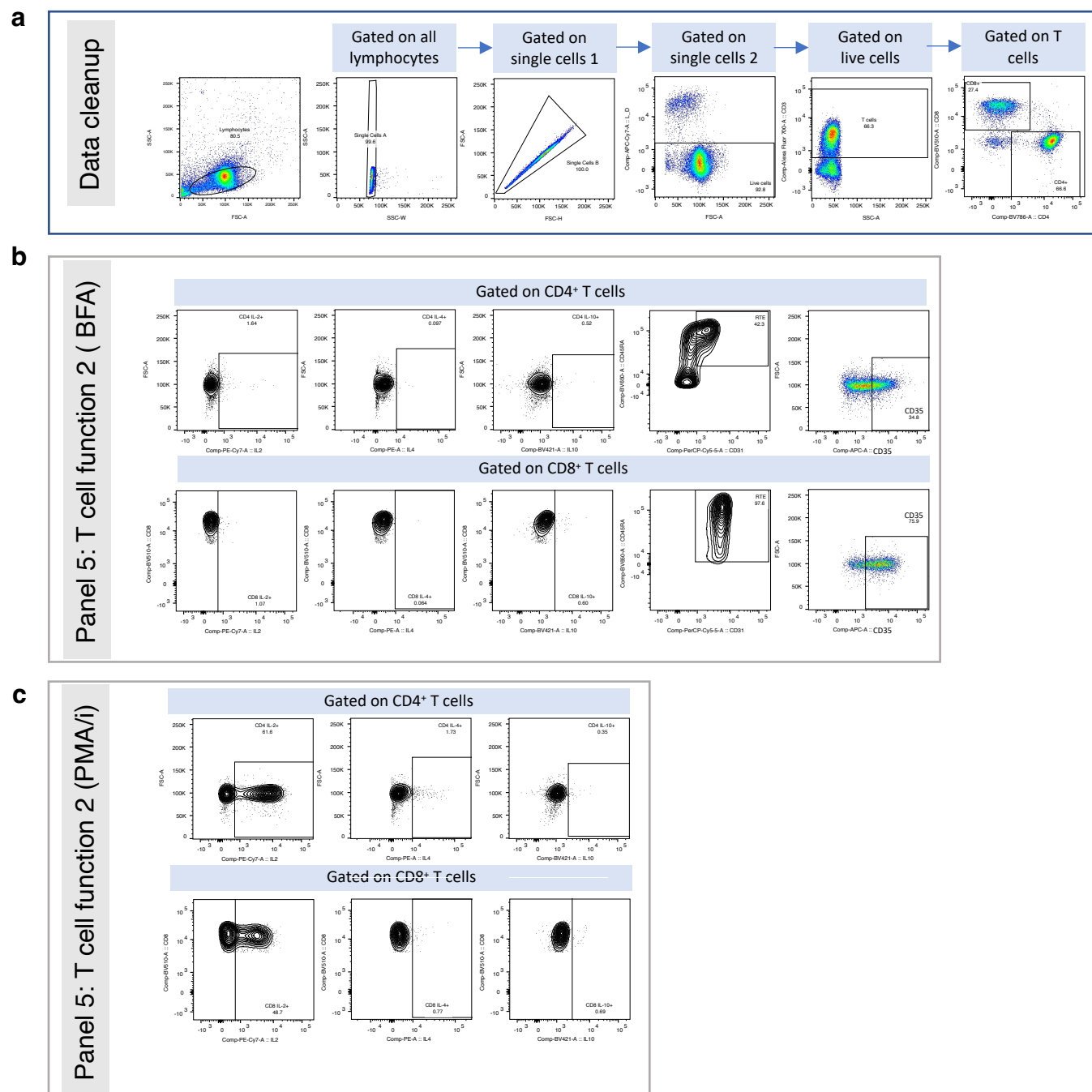
Panel 4: T /NK /TCR- $\gamma\delta$ cell function (BFA)

**c**

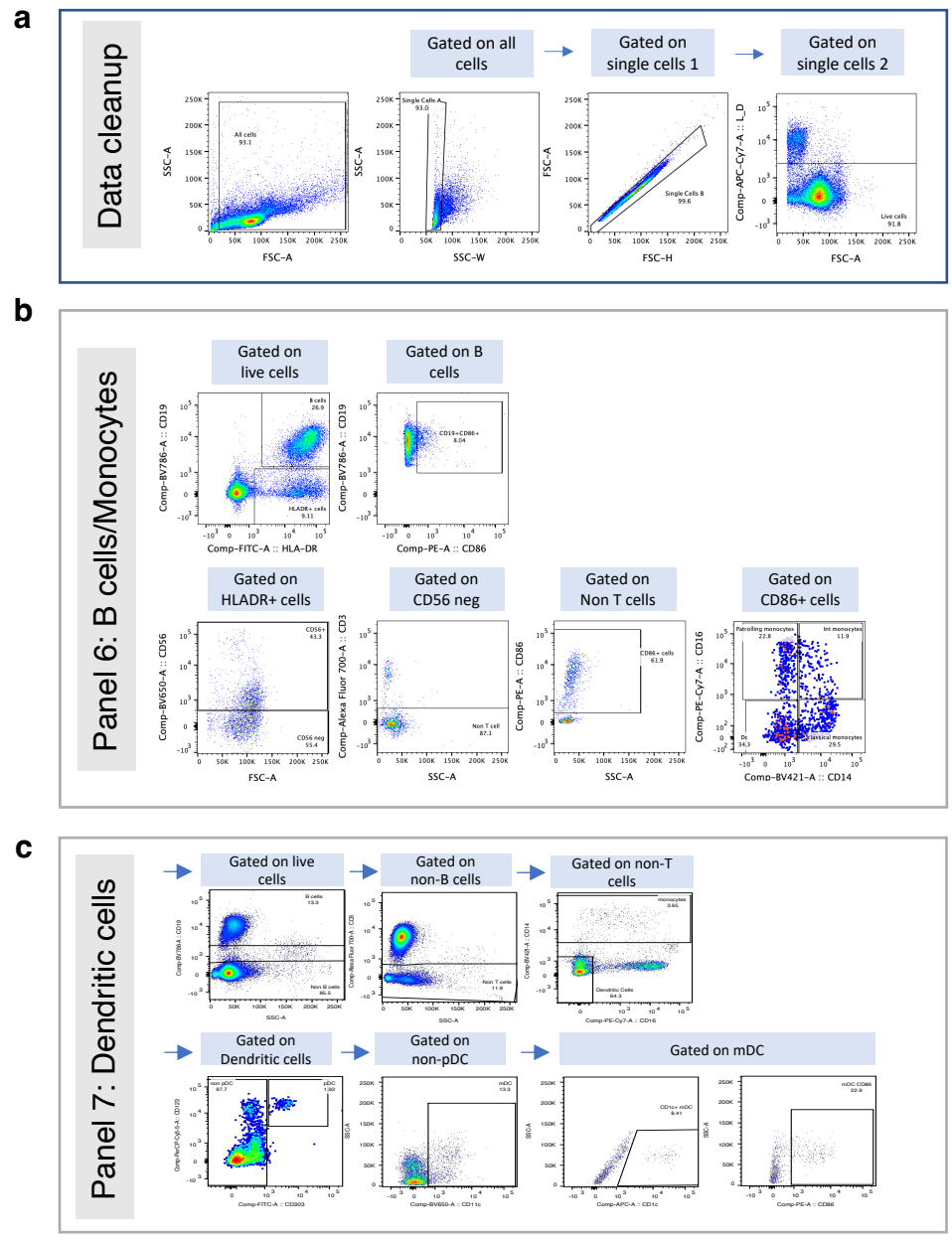
Panel 4: T /NK /TCR- $\gamma\delta$ cell function (PMA/i)



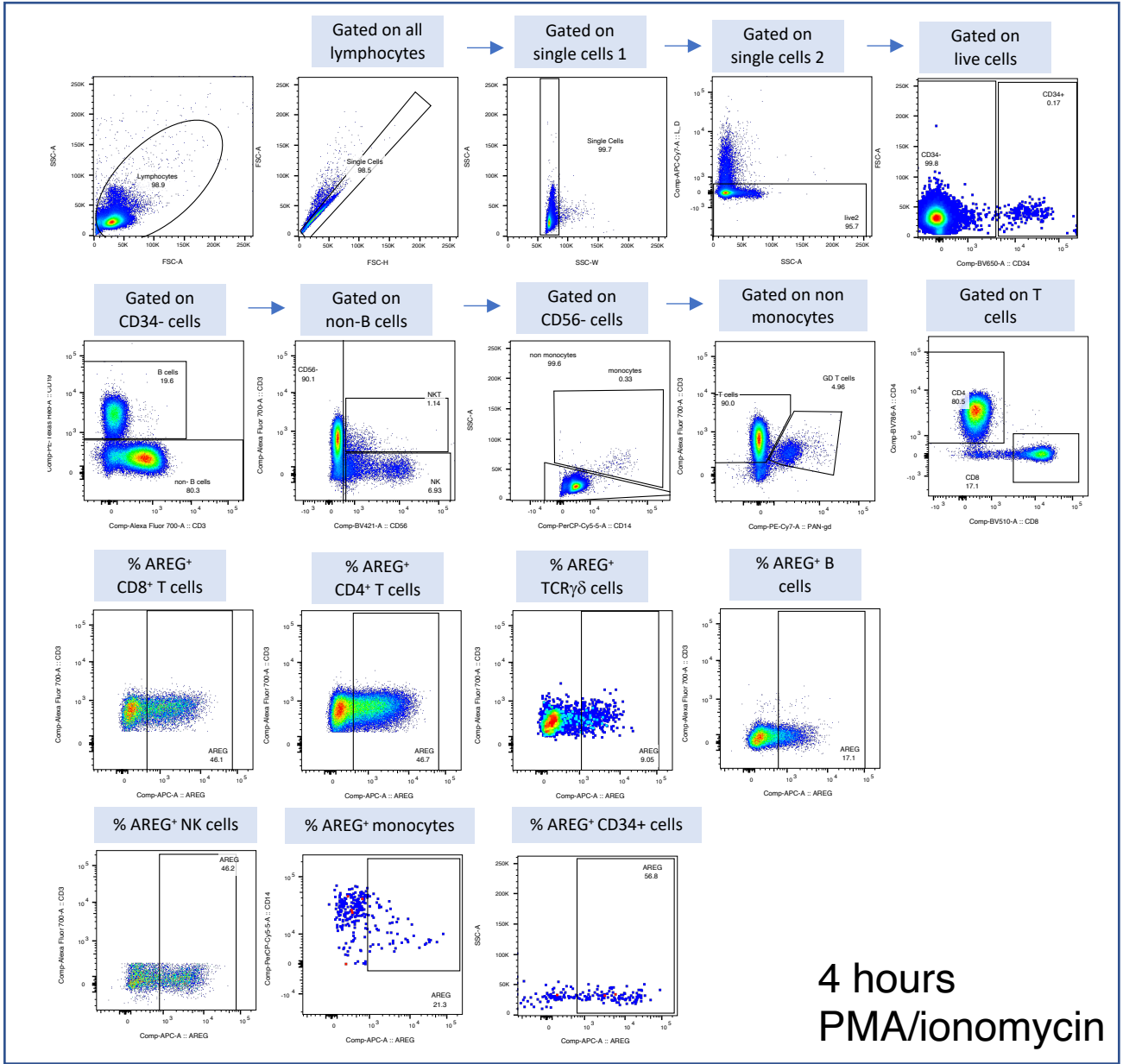
Supplementary Figure 3: Flow cytometry gating strategy for panel 4. **a**, Data clean-up and a shared initial gating workflow is shown. Gating strategy to examine production of CXCL8, TNF α , IL-17A/F, IFN γ and IL-13 within T cells, NK and TCR- $\gamma\delta$ cells by intracellular cytokine staining. Cells were incubated with **b**, Brefeldin A (BFA) only to demonstrate spontaneous cytokine production, or **c**, BFA in addition to PMA and ionomycin to determine cytokine production after stimulation.



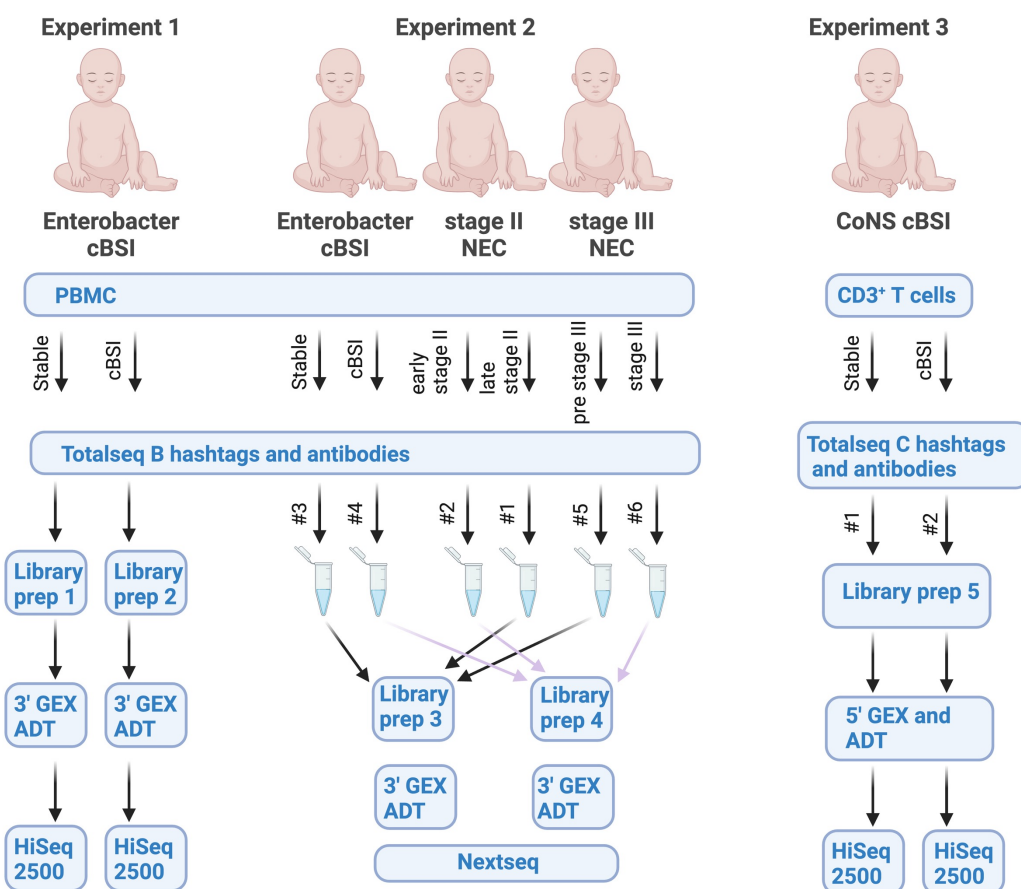
Supplementary Figure 4: Flow cytometry gating strategy for panel 5. **a**, Data clean-up and an initial gating workflow is shown. IL2, IL4 and IL-10 production by intracellular cytokine staining in CD4⁺ and CD8⁺ T cells. Cells were incubated with **b**, Brefeldin A (BFA) only to demonstrate spontaneous cytokine production, or **c**, BFA in addition to PMA and ionomycin to determine cytokine production after stimulation. Additionally for all BFA treated samples, frequencies of recent thymic emigrant (RTE) and CD35⁺ CD4⁺ and CD8⁺ T cells were assessed.



Supplementary Figure 5: Flow cytometry gating strategy for panels 6 and 7 a, Data clean-up and a shared initial gating workflow is shown. Gating strategies to examine **b**, frequencies of B cells, monocytes and **c**, dendritic cells.



Supplementary Figure 6: Flow cytometry gating strategy for panel 8. Assessment of AREG production within PBMC subsets after activation with PMA and ionomycin in the presence of Brefeldin A (4 hours).



Baby	Clinical event	Feature barcoding antibody	Clone	Barcode sequence	Cat no
A11	stable	TotalSeq™-B0253 anti-human Hashtag 3	LNH-94, 2M2	TTCCGCCTCTCTTTG	394635
A11	Enterobacter cBSI	TotalSeq™-B0254 anti-human Hashtag 4	LNH-94, 2M2	AGTAAGTTCAGCGTA	394637
A5	stage II NEC (pre-antibiotics)	TotalSeq™-B0252 anti-human Hashtag 2	LNH-94, 2M2	TGATGGCCTATTGGG	394633
A5	late NEC (on antibiotics)	TotalSeq™-B0251 anti-human Hashtag 1	LNH-94, 2M2	GTCAACTCTTTAGCG	394631
B6	pre stage III NEC	TotalSeq™-B0255 anti-human Hashtag 5	LNH-94, 2M2	AAGTATCGTTTCGCA	394639
B6	stage III NEC	TotalSeq™-B0256 anti-human Hashtag 6	LNH-94, 2M2	GGTTGCCAGATGTCA	394641
A13	stable	TotalSeq™-C0251 anti-human Hashtag 1	LNH-94, 2M2	GTCAACTCTTTAGCG	394661
A13	CoNS cBSI	TotalSeq™-C0252 anti-human Hashtag 2	LNH-94, 2M2	TGATGGCCTATTGGG	394663
A5, A11, B6, A12		TotalSeq™-B0139 anti-human TCR γ/δ	B1	CTTCCGATTCATTCA	331233
		TotalSeq™-B0072 anti-human CD4	RPA-T4	TGTTCCCGCTCAACT	300565
		TotalSeq™-B0080 anti-human CD8a	RPA-T8	GCTGCGCTTTCCATT	301069
A13		TotalSeq™-C0072 anti-human CD4	RPA-T4	TGTTCCCGCTCAACT	300567
		TotalSeq™-C0080 anti-human CD8a	RPA-T8	GCTGCGCTTTCCATT	301071
		TotalSeq™-C0154 anti-human CD27	O323	GCACTCCTGCATGTA	302853

Supplementary Figure 7. Diagram of the scRNAseq workflow. GEX = gene expression library. ADT = Antibody Derived Tag library. HiSeq 2500= Illumina HiSeq 2500 sequencing system. Nextseq = Illumina Nextseq 500 platform. A table of all Totalseq hashtags and antibodies is shown below.