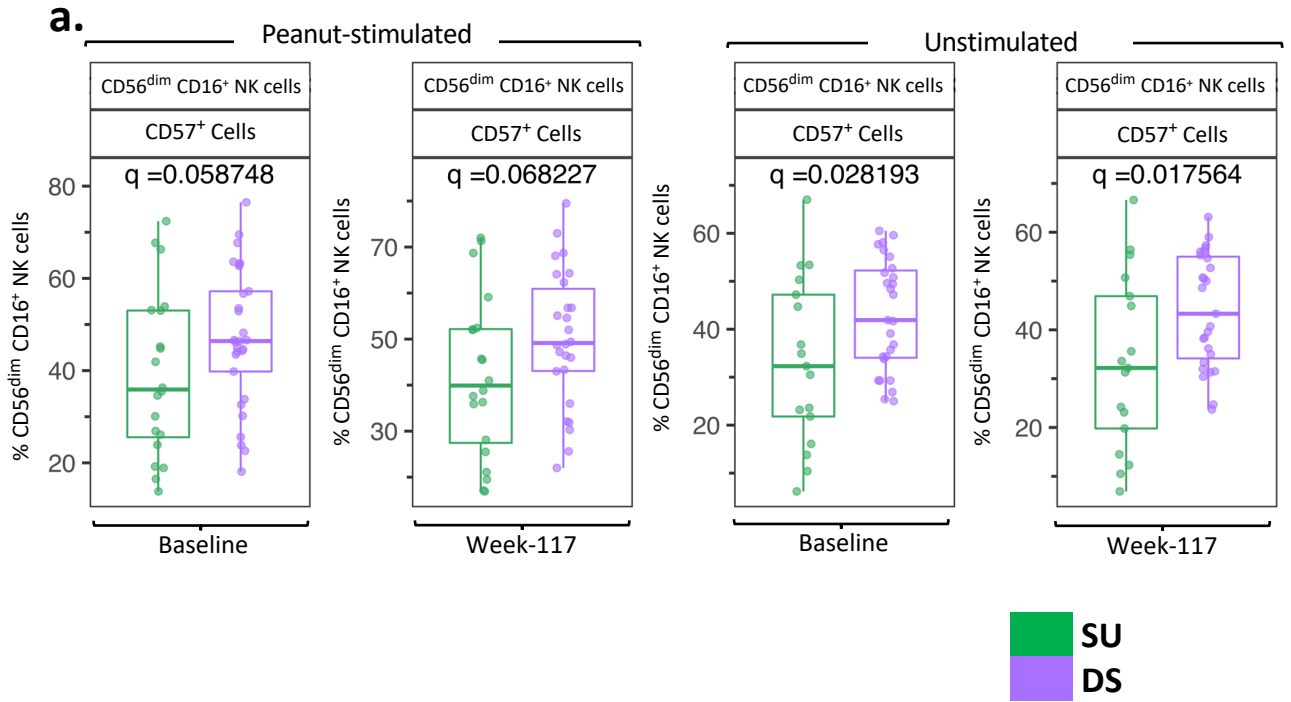
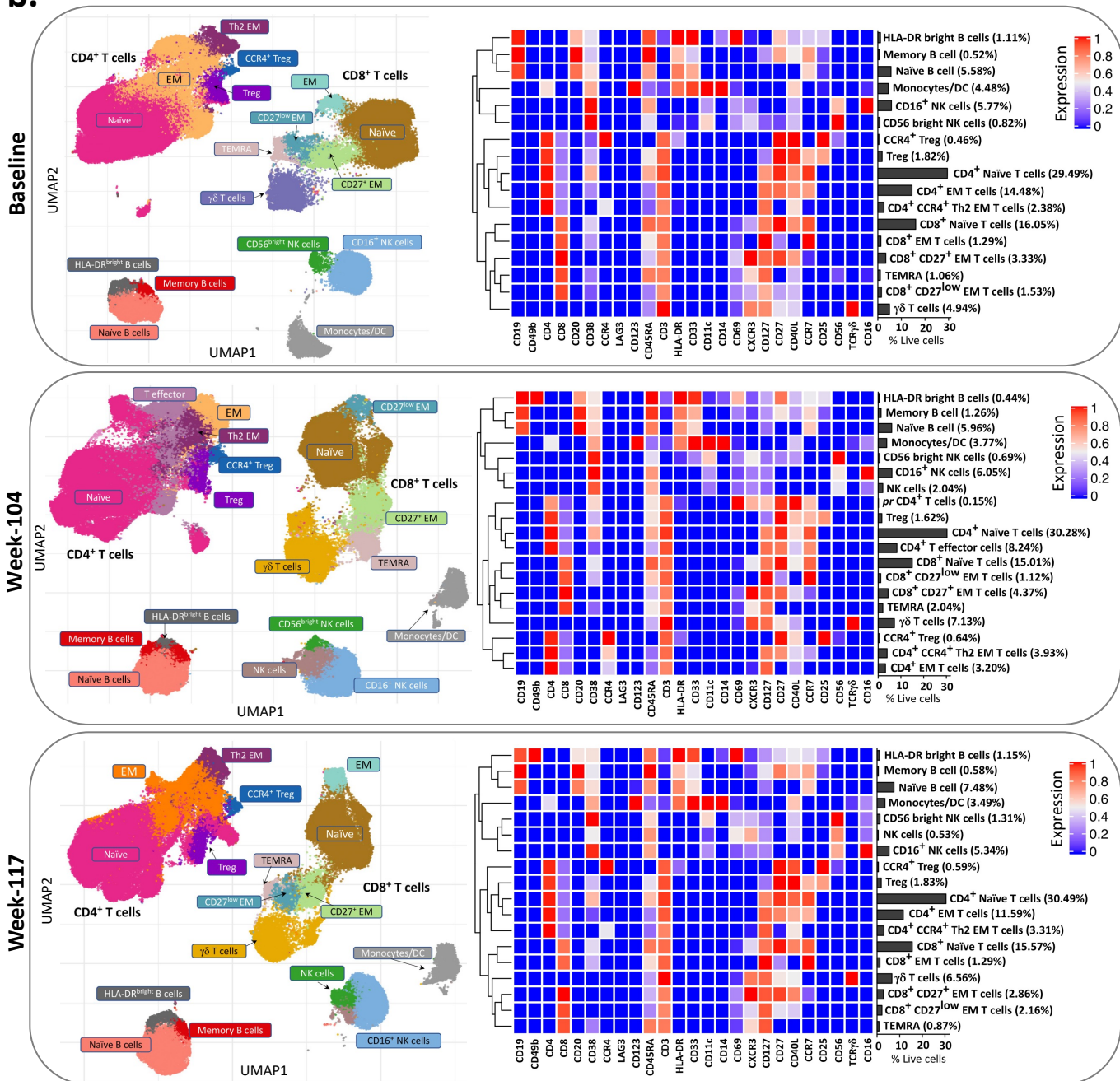


**Supplementary Fig. 1: Peanut OIT induces attenuation of the Th2 phenotype.** **a**, Uniform Manifold Approximation and Projection (UMAP) depicting distinct immune cell subsets identified using FlowSOM- based unsupervised clustering of peanut-stimulated BL, week 104, week 117 PBMCs. Clusters annotated as distinct immune cell subsets among peanut-stimulated week 104 PBMCs from participants on active peanut OIT (n = 80) were compared against those treated with placebo (n = 15). **b**, Significant changes in the expression of OX40 and CD27 in peanut-reactive CD4<sup>+</sup> T cell cluster cross-verified using manual gating are shown. [Week 104 (Peanut stim: Active, n = 80; Placebo, n = 15)]. **c,d**, Non-significant changes in marker expression and frequency within pr CD4<sup>+</sup> and pr CD8<sup>+</sup> T cells among peanut-stimulated [Week 104 (Peanut stim: Active, n = 80; Placebo, n = 15)] (**c**), and non-peanut reactive immune cell subsets among peanut-stimulated and unstimulated PBMCs (**d**). **e,f**, Significant changes in peanut-stimulated week 104 PBMC culture supernatant of active participants (n = 17), compared to those treated with placebo (n = 7) (**e**), and compared to BL (**f**). In the box-plots, medians are shown, and the ‘hinges’ represent the first and third quartiles. The whiskers are the smallest and largest values after exclusion of outliers (greater than the 75th percentile plus 1.5 times the interquartile range (IQR), or less than 25th percentile minus 1.5 times the IQR). Q values were computed using the  $\chi^2$ -test in mixed-effects models, adjusted for multiple hypothesis testing using the Benjamini and Hochberg method. Source data are provided as a Source Data file.



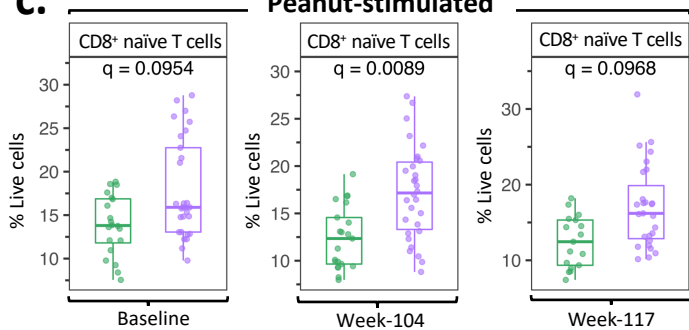
b.

## Unsupervised clustering (FlowSOM)



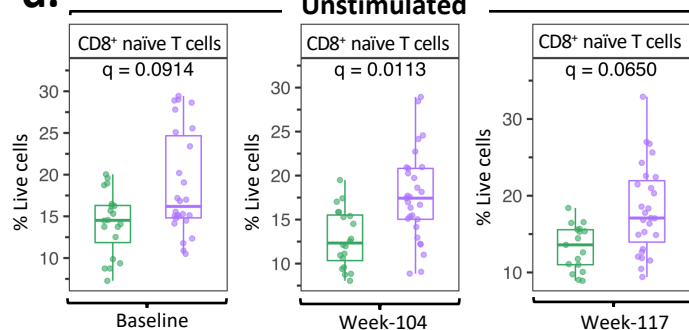
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## Peanut-stimulated

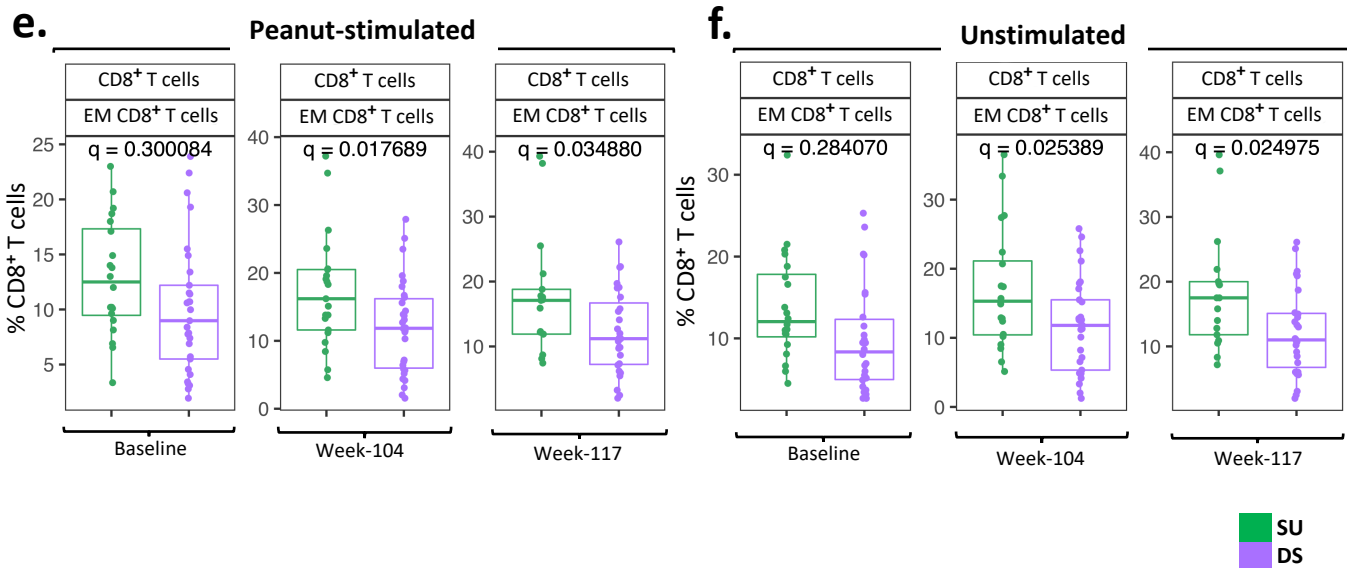


d.

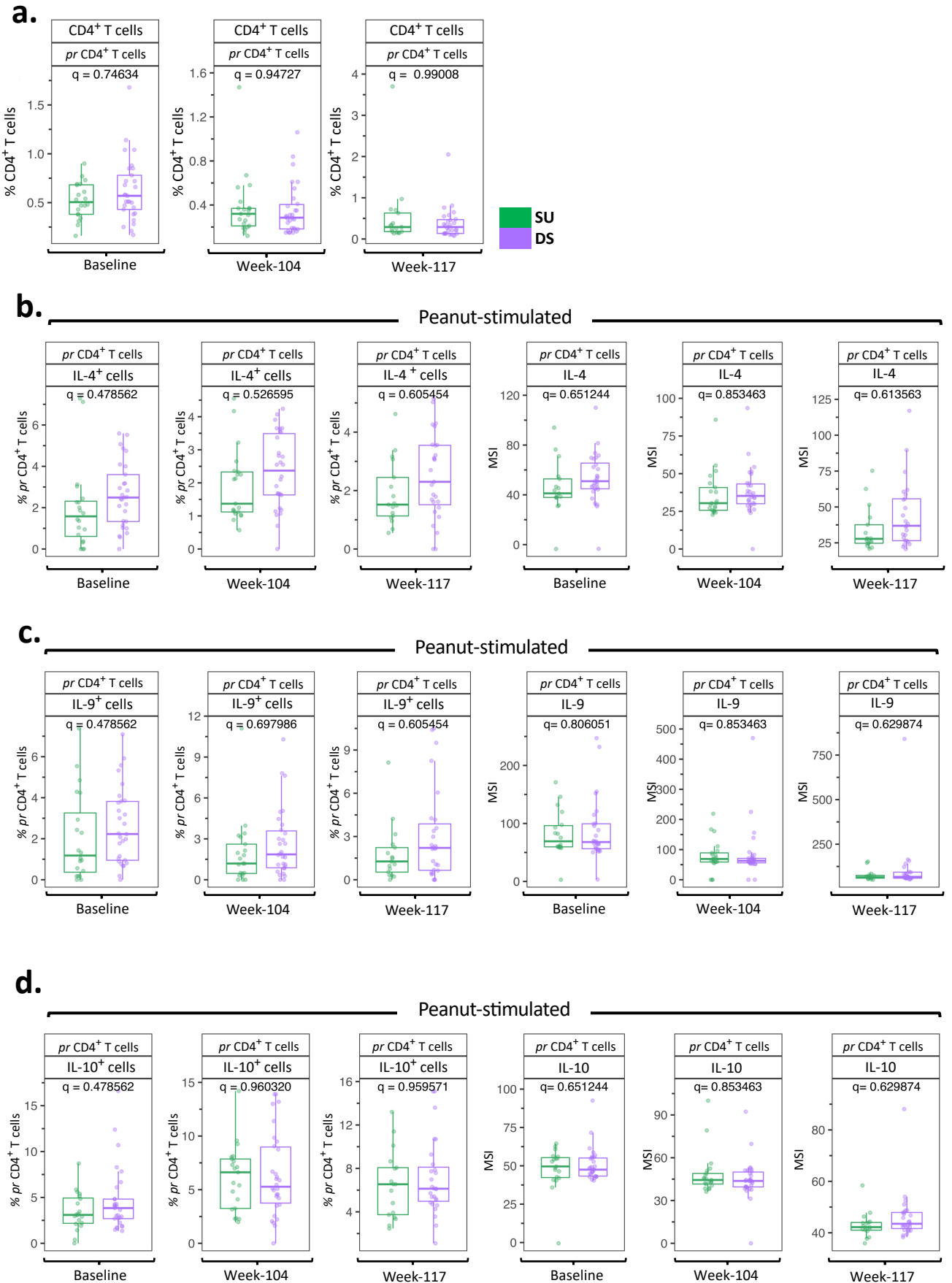
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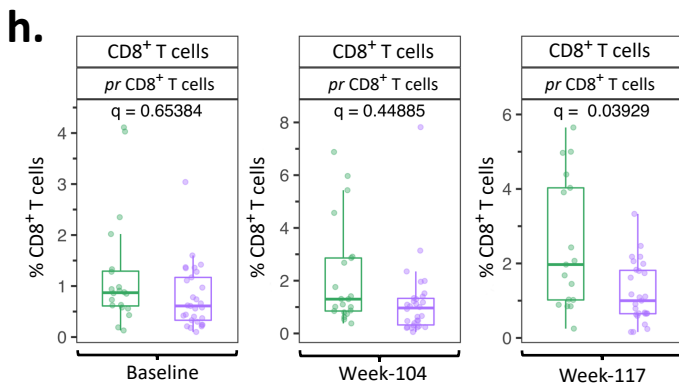
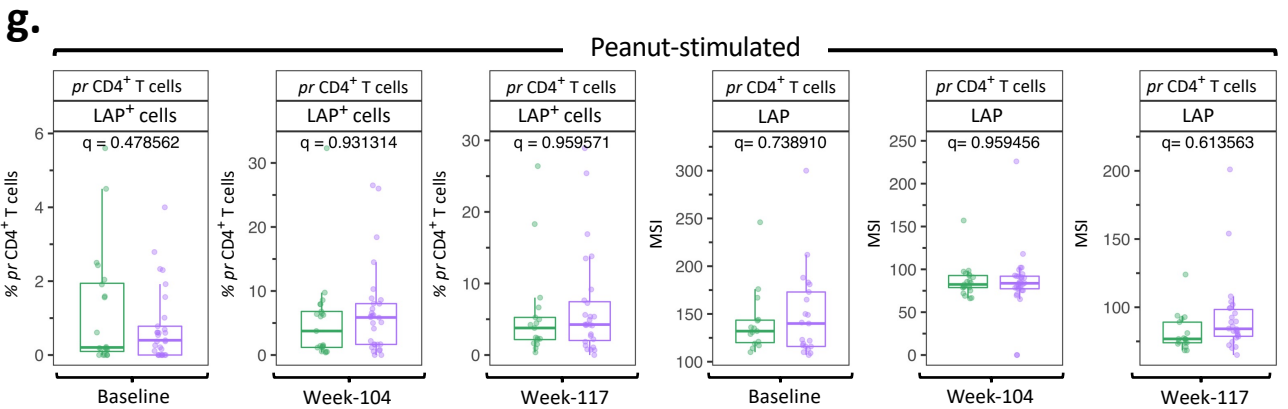
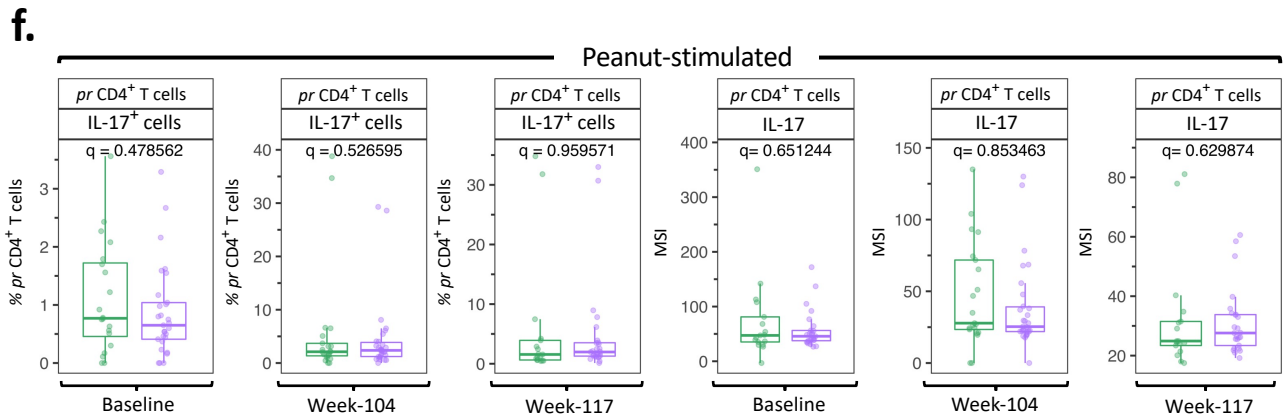
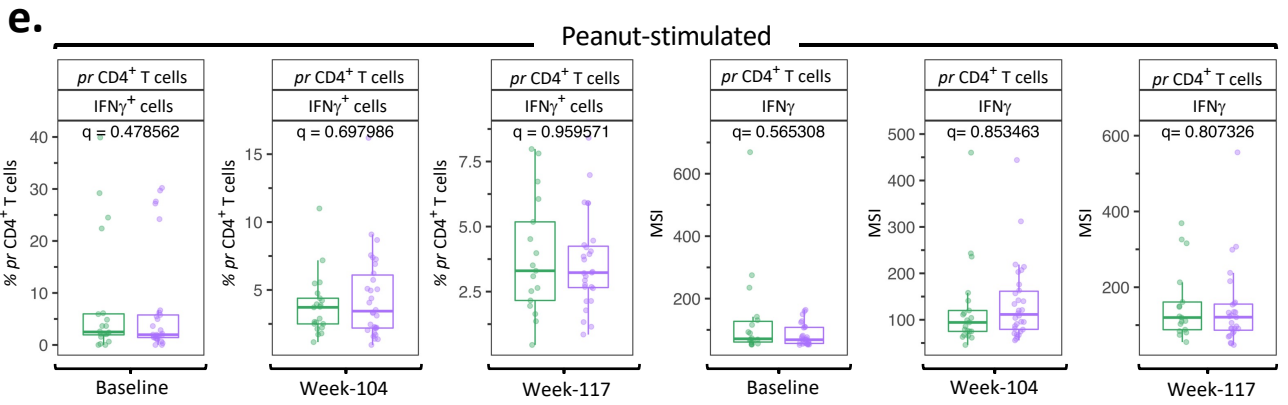


SU  
DS

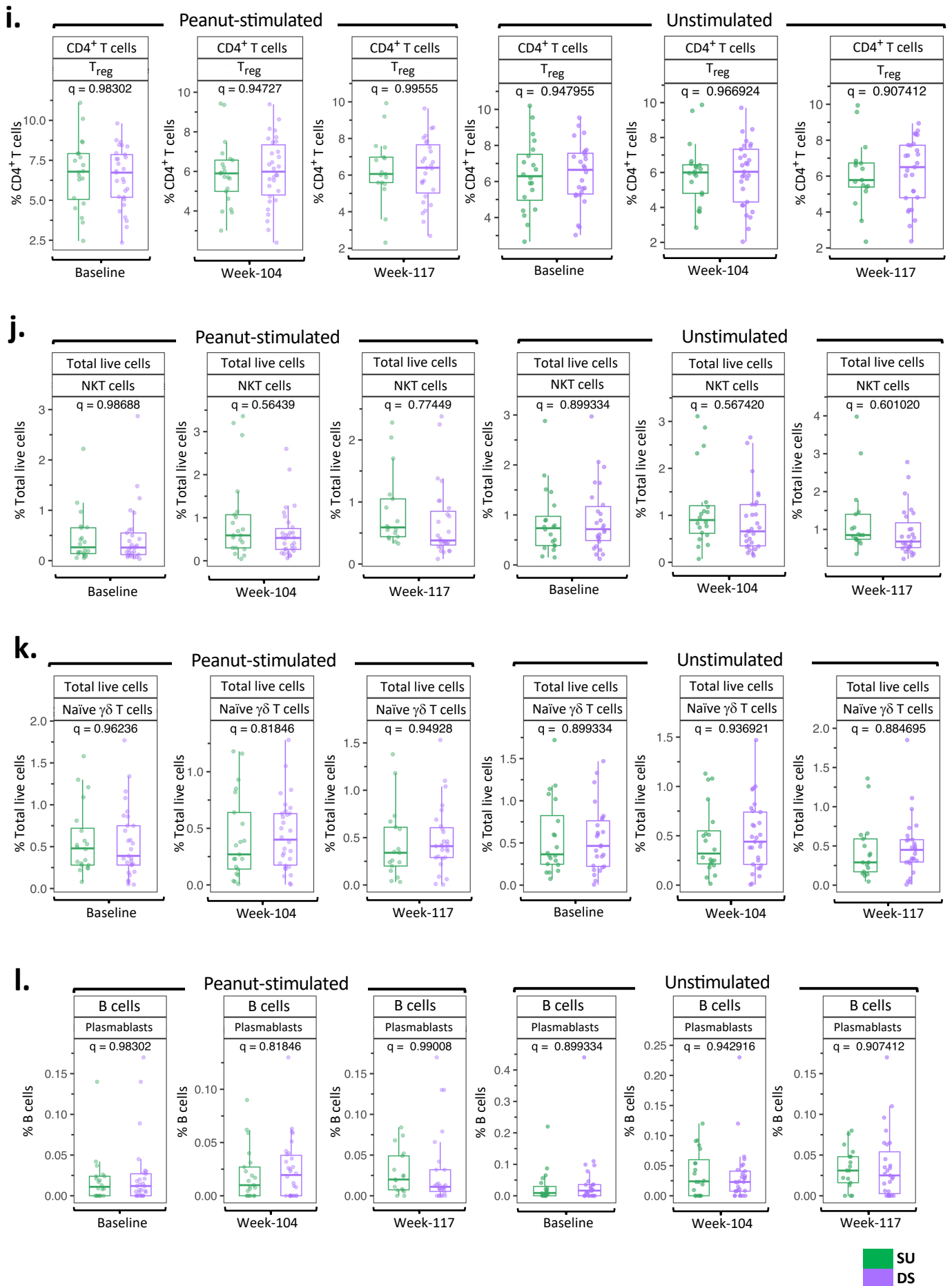


**Supplementary Fig. 2: Higher CD57<sup>+</sup> CD56<sup>dim</sup> CD16<sup>+</sup> NK cell and higher naïve CD8<sup>+</sup> vis-à-vis lower EM CD8<sup>+</sup> T cell frequencies discern DS participants from SU.** **a**, Higher frequencies of CD57<sup>+</sup>CD56<sup>dim</sup>CD16<sup>+</sup> NK cell in DS participants compared to SU at BL and week 117 in peanut-stimulated and unstimulated PBMCs. [BL (pea stim: SU, n = 20, DS, n = 29; unstim: SU, n = 20, DS, n = 26), week 117 (peanut stimulated: SU, n = 17, DS, n = 27; unstim: SU, n = 17, DS, n = 27)]. **b**, UMAP depicting distinct immune cell subsets identified using FlowSOM-based unsupervised clustering of unstimulated BL, week 104, week 117 PBMCs. Frequency and marker expression on immune cell clusters among unstimulated PBMCs from SU participants were respectively compared with those from DS participants. **c,d**, Significant change in the abundance of naïve CD8<sup>+</sup> T cell cluster per time point cross-verified using manual gating of peanut- stimulated (**c**) and unstimulated (**d**) PBMCs at BL, week 104 (pea stim: SU, n = 21, DS, n = 30; unstim: SU, n = 20, DS, n = 29), and week 117 are shown. **e,f**, Frequency of EM CD8<sup>+</sup> T cells among total CD8<sup>+</sup> T cells at BL, week 104, and week 117 in peanut-stimulated (**e**) and unstimulated (**f**) PBMCs estimated by manual gating. In the box-plots, medians are shown, and the ‘hinges’ represent the first and third quartiles. The whiskers are the smallest and largest values after exclusion of outliers (greater than the 75th percentile plus 1.5 times the interquartile range (IQR), or less than 25th percentile minus 1.5 times the IQR. Q values were computed using the  $\chi^2$ -test in mixed-effects models, adjusted for multiple hypothesis testing using the Benjamini and Hochberg method. Source data are provided as a Source Data file.



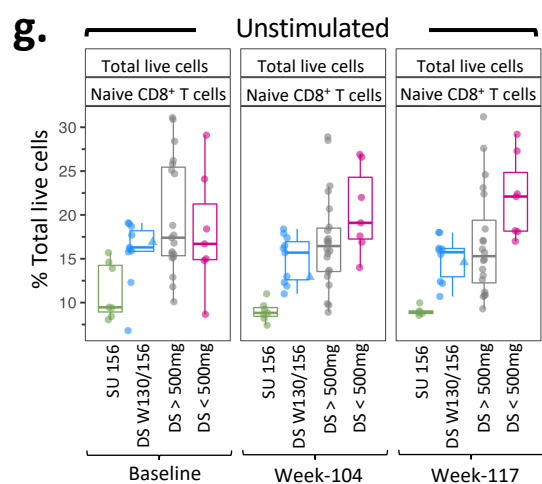
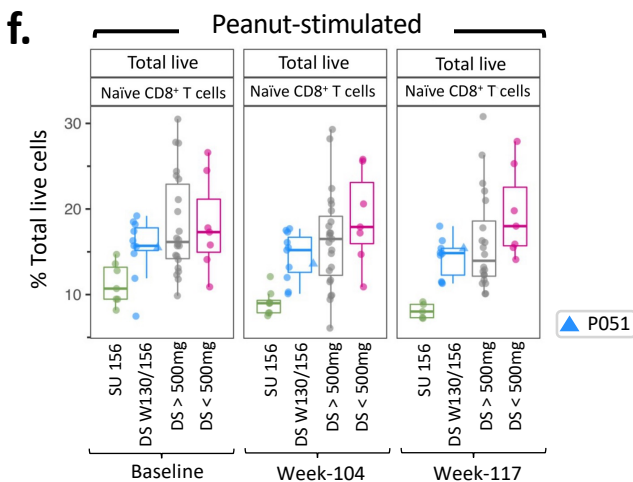
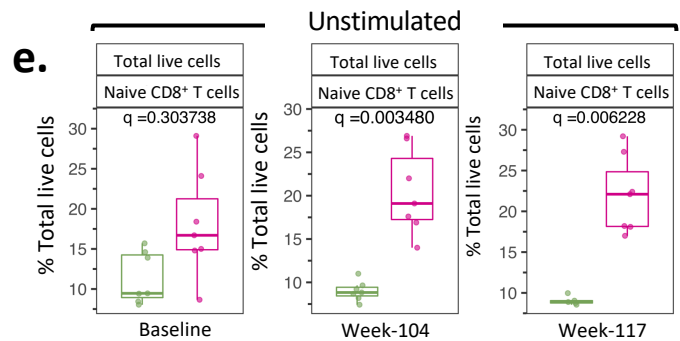
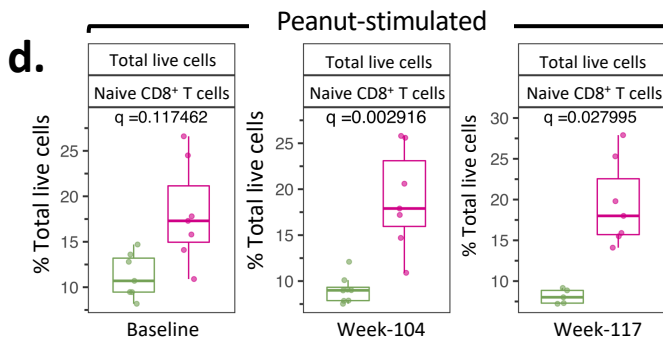
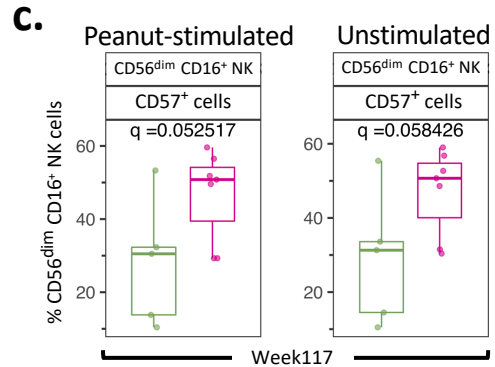
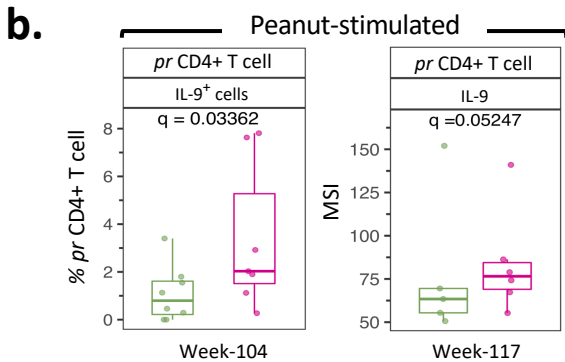
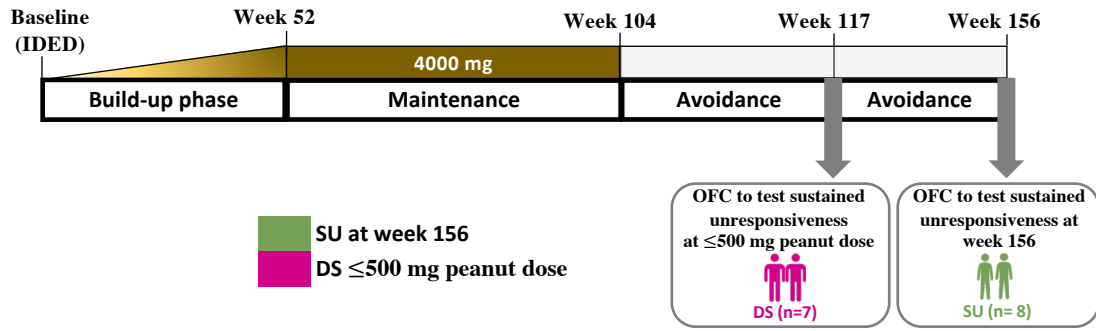


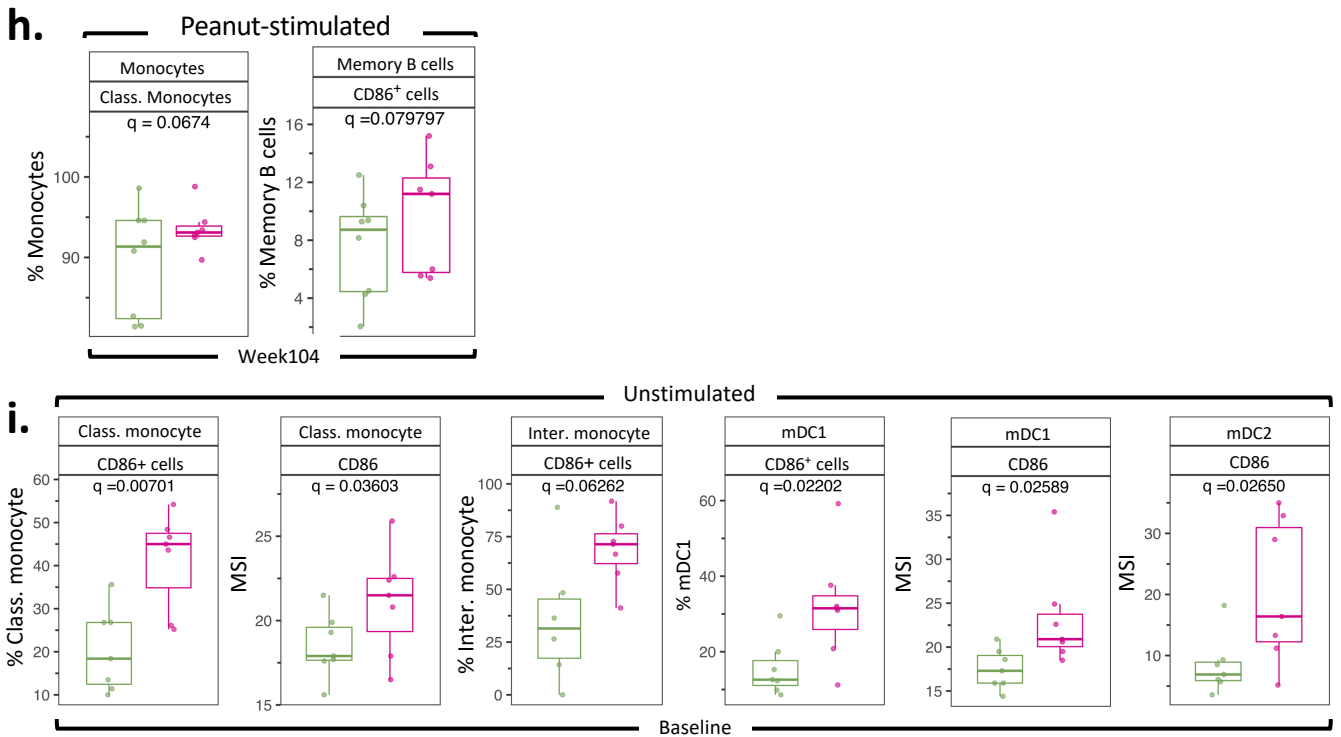




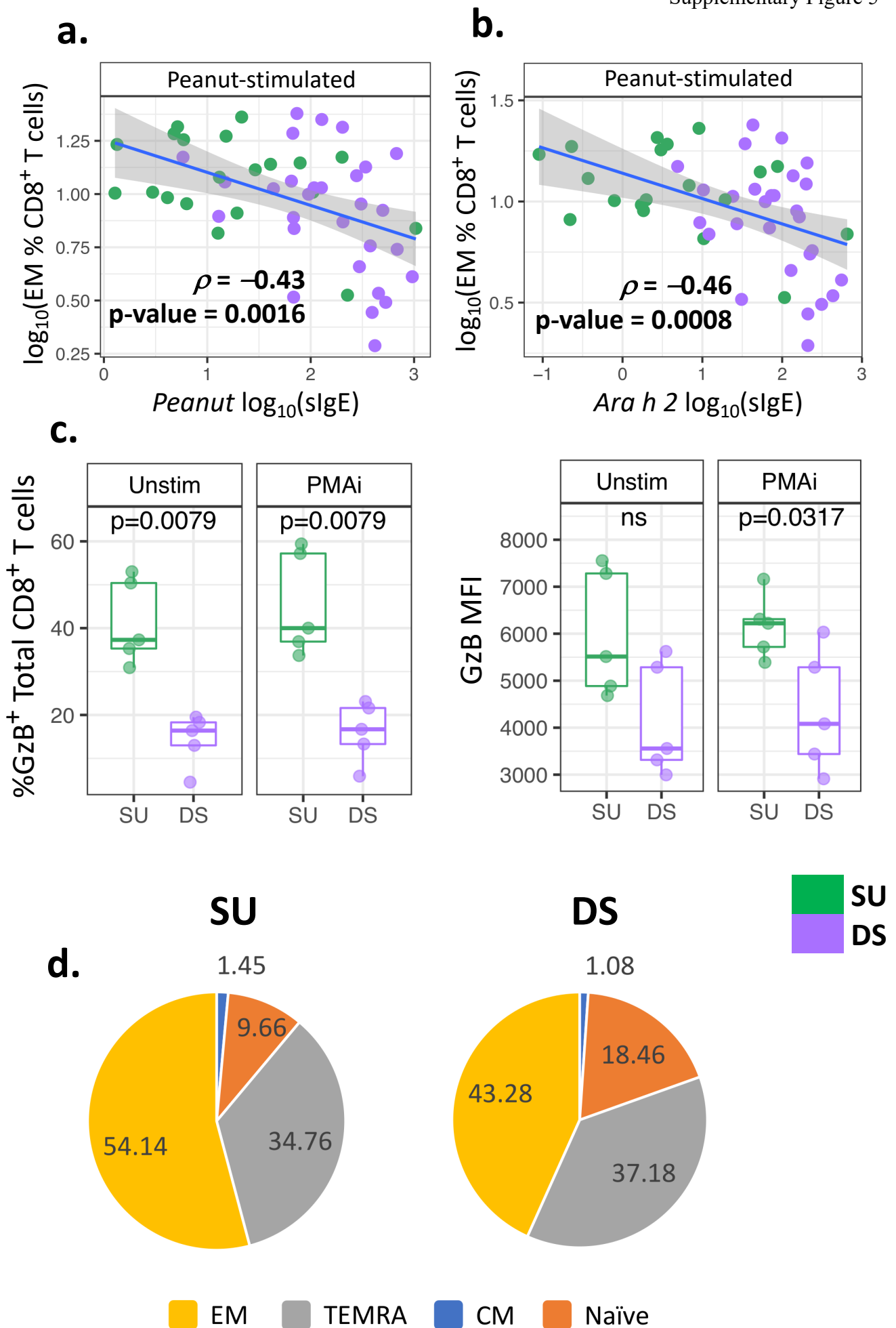
**Supplementary Fig. 3: Frequencies and marker expression of immune cell subsets other than CD8<sup>+</sup> T cells remain comparable between SU and DS participants.** Immune cell subsets among peanut- stimulated and unstimulated PBMCs from SU participants were compared with DS participants at BL, week 104, and week 117. [Baseline (Peanut stim: SU, n = 20, DS, n = 29; unstim: SU, n = 20, DS, n = 26); Week 104 (Peanut stim: SU, n = 21, DS, n = 30; unstim: SU, n = 20, DS, n = 29); Week 117 (Peanut stim and unstim: SU, n = 17, DS, n = 27)]. Non-significant changes in marker expression and frequency within pr CD4<sup>+</sup> and pr CD8<sup>+</sup> T cells among peanut-stimulated (**a-h**), and non-peanut reactive immune cell subsets among peanut- stimulated and unstimulated PBMCs (**i-l**). In the box-plots, medians are shown, and the ‘hinges’ represent the first and third quartiles. The whiskers are the smallest and largest values after exclusion of outliers (greater than the 75th percentile plus 1.5 times the interquartile range (IQR), or less than 25th percentile minus 1.5 times the IQR. Q values were computed using the  $\chi^2$ -test in mixed-effects models, adjusted for multiple hypothesis testing using the Benjamini and Hochberg method. Source data are provided as a Source Data file.

**a.** Participant subset analysis

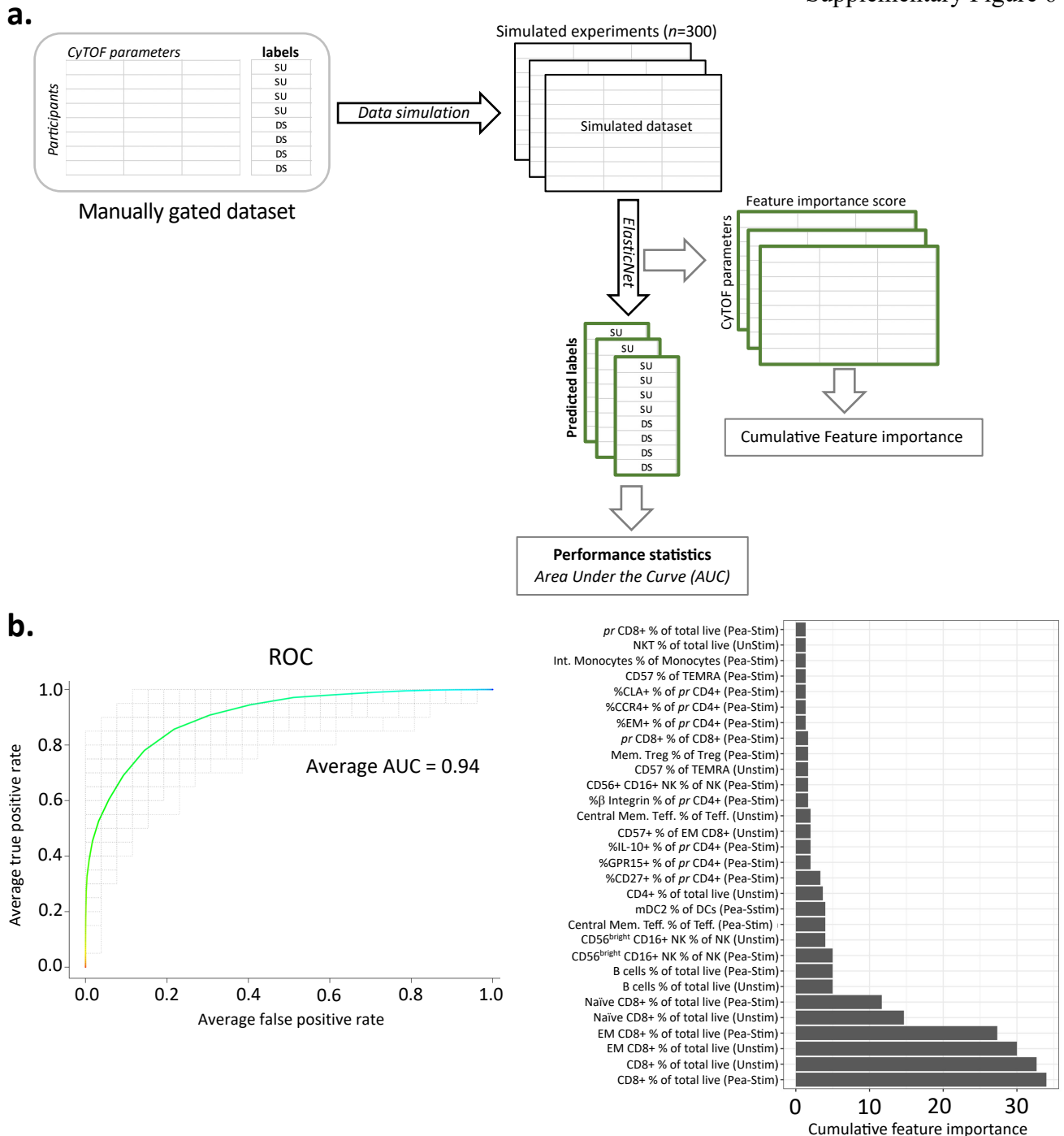




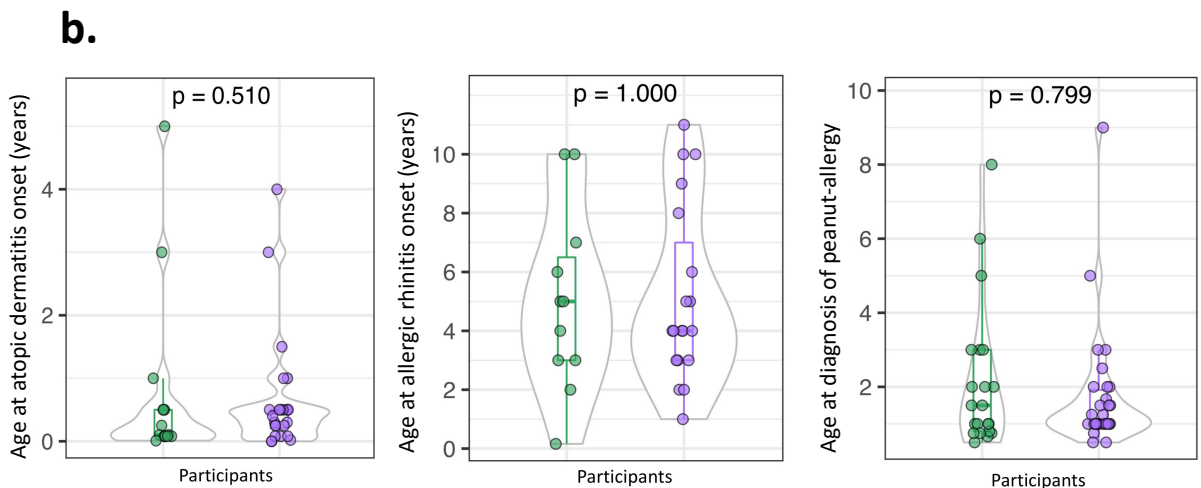
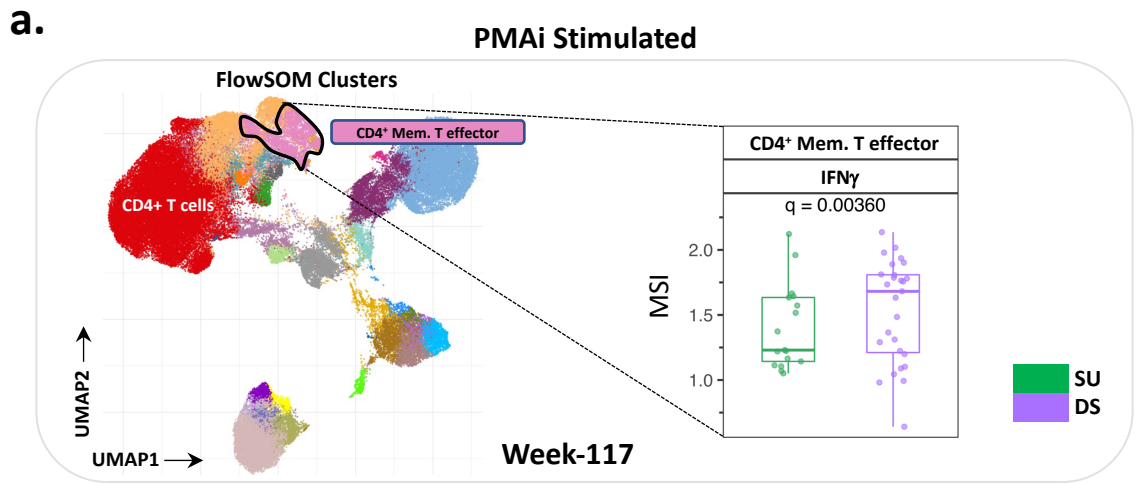
**Supplementary Fig. 4: Participants exhibiting long-term SU (SU wk 156) compared with DS  $\leq 500$  mg showed identical immune distinctions as observed in SU vs. DS comparison. a,** Schematic representation of POISED study, showing DBPCFC at week 156 and depiction of subset of SU (SU wk 156) and DS (DS  $\leq 500$  mg) participants. **b-i,** Frequency and expression changes among immune cell subsets in peanut-stimulated or unstimulated PBMCs at BL, week 104, and week 117 from SU wk 156 participants (n=8) were compared with those from DS  $\leq 500$  mg participants, who failed a DBPCFC at a dose  $\leq 500$  mg peanut at week 117 (n=7). Significant differences in frequency or expression of IL-9 in *pr* CD4<sup>+</sup> T cells (**b**), CD57 in CD56<sup>dim</sup>CD16<sup>+</sup> NK cells (**c**), naïve CD8<sup>+</sup> T cell (**d, e**), and CD86 in APC subsets (**h, i**) are shown. Frequency of naïve CD8<sup>+</sup> T cells in peanut-stimulated (**f**) and unstimulated (**g**) PBMCs from all per-protocol avoidance group participants classified by time point and dose of peanut OFC failure. In the box-plots, medians are shown, and the ‘hinges’ represent the first and third quartiles. The whiskers are the smallest and largest values after exclusion of outliers (greater than the 75th percentile plus 1.5 times the interquartile range (IQR), or less than 25th percentile minus 1.5 times the IQR). Q values were computed using the  $\chi^2$ -test in mixed-effects models, adjusted for multiple hypothesis testing using the Benjamini and Hochberg method. Source data are provided as a Source Data file.



**Supplementary Fig. 5: Negative correlation of EM CD8<sup>+</sup> with peanut- and Ara h 2-specific IgE at BL and distinction in GzB expression among SU vs. DS participants.** **a,b**, Spearman correlations of frequency of EM CD8<sup>+</sup> T cells among total CD8<sup>+</sup> cells in peanut-stimulated BL PBMCs with peanut-specific (a), and Ara h 2-specific (b) IgE at BL. (pea stim: SU, n = 20, DS, n = 29). Plots display the data points and a linear regression line with a 95% confidence interval shading. P-values in the figure were determined by an unpaired two-tailed Student's t test. **c**, Frequency (left panels) and expression (right panels) of GzB in total CD8<sup>+</sup> T cells among unstimulated and PMAi-stimulated week 52 PBMCs from 5 SU and 5 DS participants evaluated using flow cytometry. **d**, Pie charts depicting relative mean proportion of unstimulated CD8<sup>+</sup> T cell subsets expressing GzB in SU vs. DS participants. The whiskers are the smallest and largest values after exclusion of outliers (greater than the 75th percentile plus 1.5 times the interquartile range (IQR), or less than 25th percentile minus 1.5 times the IQR). Plots display the correlation coefficient ( $\rho$ ) and linear regression line with a 95% confidence interval shading. P value was computed using unpaired, two-sided, non-parametric Mann-Whitney test. Data are presented as the mean  $\pm$  s.e. Source data are provided as a Source Data file.



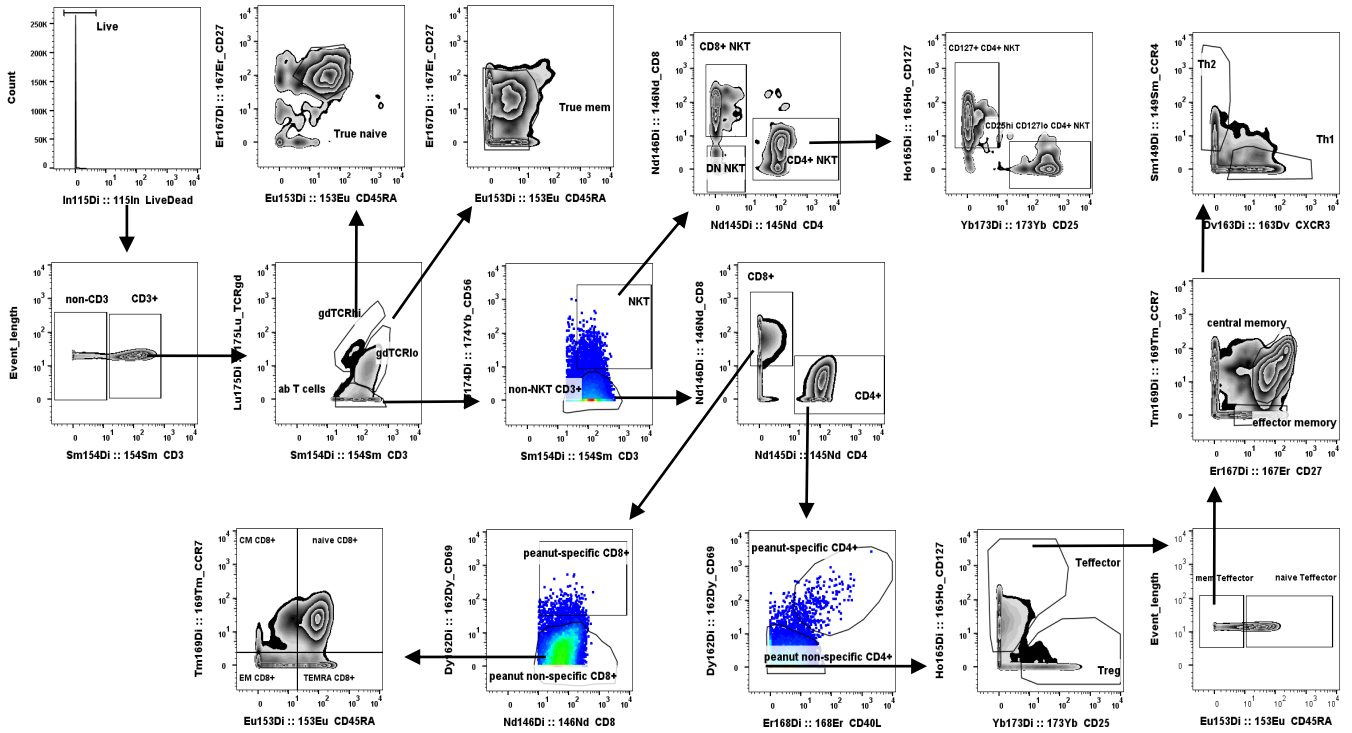
**Supplementary Fig. 6: Machine learning analysis underlines CD8<sup>+</sup> T cell differentiation status as a major predictive feature of SU vs. DS.** **a**, Machine learning analysis workflow. The BL dataset with CyTOF parameters, (i.e., cell type frequencies and marker expression in peanut-simulated and unstimulated PBMCs) was subjected to elastic net modeling for classification of SU vs. DS participants. The performance of elastic net models was evaluated using average AUC and ROC analysis across 300 iterations. The cumulative feature importance score was enumerated for the CyTOF parameters that had feature importance score greater than 80 in at least one elastic net model. **b**, The average AUC and ROC predicted for the given simulated BL datasets. The cumulated feature importance score highlights the most common and important CyTOF parameters used by the models to distinguish SU from DS participants. Source data are provided as a Source Data file.



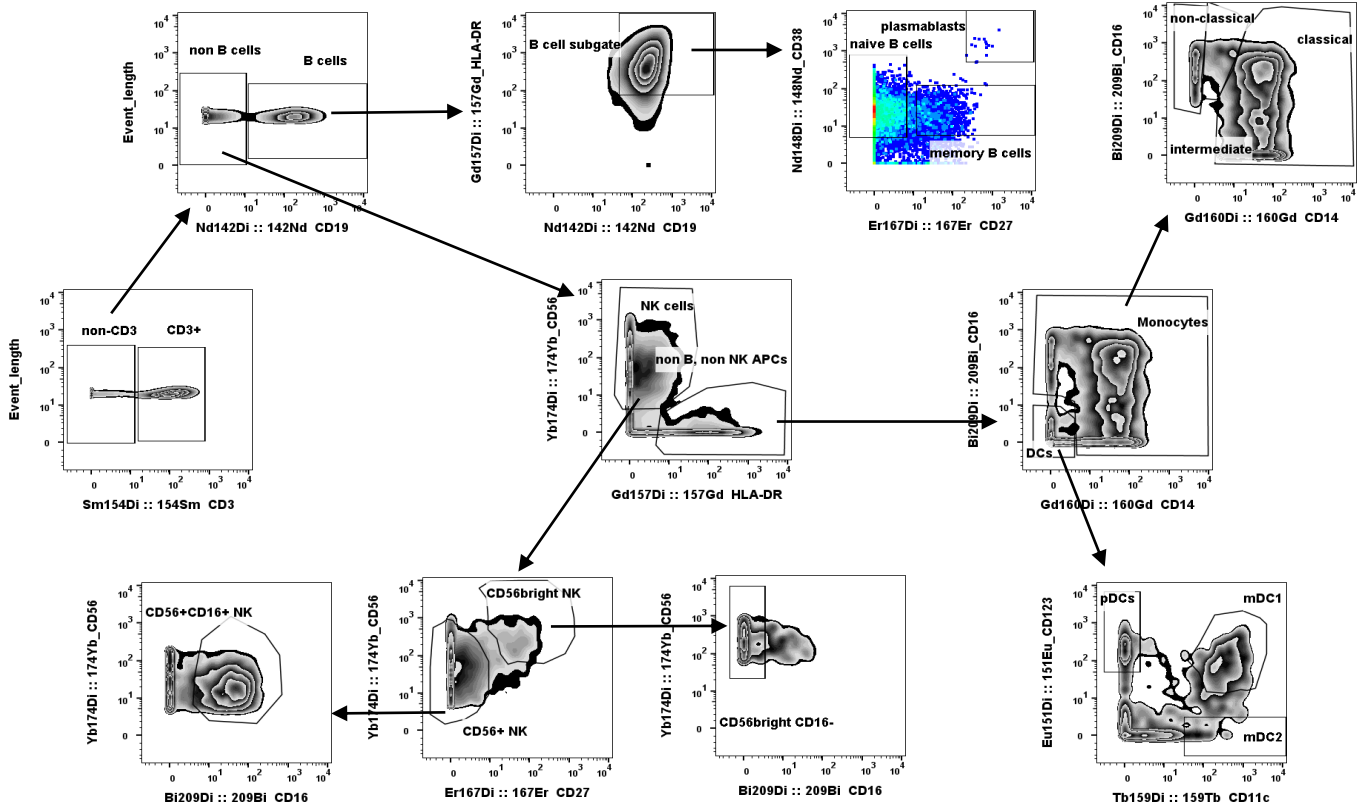
**Supplementary Fig. 7: Lower expression of IFN $\gamma$  in memory CD4<sup>+</sup> T effector cluster in SU participants.** **a**, UMAP depicting distinct immune cell subsets identified using FlowSOM-based unsupervised clustering of PMA/Ionomycin-stimulated week 117 PBMCs. Expression of IFN $\gamma$  in memory CD4<sup>+</sup> T effector cluster in SU (n = 17) vs. DS (n = 27) participants. P-values by  $\chi^2$ -tests in mixed-effect model adjusted with Benjamini and Hochberg method. **b**, Comparison of age at onset of atopic dermatitis [SU (n = 15) vs. DS (n = 24)], allergic rhinitis [SU (n = 17) vs. DS (n = 27)], and age at diagnosis of peanut allergy [SU (n = 21) vs. DS (n = 30)]. The whiskers are the smallest and largest values after exclusion of outliers (greater than the 75th percentile plus 1.5 times the interquartile range (IQR), or less than 25th percentile minus 1.5 times the IQR). P value was computed using unpaired, two-sided, non-parametric Mann-Whitney test. Source data are provided as a Source Data file.



**a.**



**b.**



**Supplementary Fig. 8: Gating scheme for identification of CD3<sup>+</sup> and CD3<sup>-</sup> immune cell subsets among total live PBMCs in CyTOF dataset.** Gating scheme for identification of CD3<sup>+</sup> and CD3<sup>-</sup> immune cell subsets among total live PBMCs in CyTOF dataset. PBMCs stimulated with 200 µg/ml peanut solution for 24 hours with the addition of brefeldin A for the last 4 hours were gated using FlowJo v10. **a**, Representative gating for  $\gamma\delta$  cell subsets, NKT cells, peanut-reactive CD4<sup>+</sup> T cells, non-peanut reactive CD4<sup>+</sup> T effector subsets and regulatory T cells, peanut-reactive CD8<sup>+</sup> T cells, and non-peanut-reactive CD8<sup>+</sup> T cell subsets is depicted. **b**, Representative gating for B cells, NK cells, monocytes, and DC subsets among CD3<sup>-</sup> parent population is depicted.

**Supplementary Table 1. Participant demographics**

	<b>CyTOF</b>				
	<b>Active (n=95)</b>	<b>Placebo (n=25)</b>	<b>SU (n=21)</b>	<b>DS (n=30)</b>	<b>Luminex (n=27)</b>
Age in years, median (IQR, range)	10 (5, 7-47)	11 (5, 7-53)	9 (2, 7-43)	11 (4, 7-29)	9 (4, 7-27)
Female sex, n (%)	24 (30.00%)	6 (26.09%)	7 (33.33%)	9 (30.00%)	8 (29.62%)
Non-Hispanic or Latino ethnicity, n (%)	80 (100.00%)	21 (91.30%)	21 (100.00%)	30 (100.00%)	27 (100%)
History of asthma, n (%)	45 (56.25%)	20 (86.96%)	14 (66.67%)	17 (56.67%)	18 (66.67%)
History of atopic dermatitis, n (%)	56 (70.00%)	16 (69.57%)	15 (71.43%)	24 (80.00%)	19 (70.37%)
History of allergic rhinitis, n (%)	57 (71.25%)	19 (82.61%)	14 (66.67%)	22 (73.33%)	16 (59.25%)
Median CTD of peanut in mg, median (IQR, range)	25 (95, 0-500)	25 (70, 0-375)	75 (150, 5-375)	25 (70, 0-375)	25 (70, 0-375)
Total IgE in kUL/L, median (IQR, range)	552 (977.5, 18-4803)	383 (805.5, 17-4734)	470 (812, 18-2819)	958 (1368.25, 61-4803)	550 (716.5, 35-3225)
Peanut-reactive IgE in kUL/L, median (IQR, range)	76.55 (213.8, 0.62-2186)	74.6 (206.34, 0.41-964)	13 (26.22, 0.76-2186)	139 (360.4, 8.57-854)	89.5 (401.95, 0.76-2186)
Median SPT wheel diameter of peanut in mm, median (IQR, range)	12 (7.625, 4-29.5)	12 (5, 6-33)	12 (10, 4-23.5)	12.25 (7.375, 5-29.5)	11 (6.5, 4-22)

**Supplementary Table 2. Sample count**

		CyTOF			Luminex		
		PBMC			Supernatant		Plasma
Time	Stimulation	<b>Peanut stim</b>	<b>Unstim</b>	<b>PMAi stim</b>	<b>Peanut stim</b>	<b>Unstim</b>	-
	<b>Baseline</b> [#SU; #DS]	110 [20; 29]	107 [20; 26]	109 [20; 27]	19 [07;07]	19 [07;07]	24 [08;10]
	<b>Week-104</b> [#Active; #Placebo] [#SU; #DS]	95 [80; 15] [21;30]	91 [76; 15] [20;29]	93 [78; 15] [21;29]	24 [16; 07] [10;07]	23 [16; 07] [07;09]	28 [21; 07] [08;13]
	<b>Week-117</b> [#SU; #DS]	75 [17;27]	72 [17;27]	74 [17;27]	17 [07;09]	16 [07;09]	21 [08;13]

**Supplementary Table 3.** Lineage and functional markers used in FlowSOM unsupervised clustering.

Stimulation	Lineage Markers	Functional markers
Peanut-stimulated and Unstimulated samples	CD19, CD49b, CD4, CD8, CD20, CD38, CCR4, LAG3, CD123, CD45RA, CD3, HLA-DR, CD33, CD11c, CD14, CD69, CXCR3, CD27, CD40L, CCR7, CD25, CD56, TCR $\gamma\delta$ , CD16	CD86, OX40, CD28, GPR15, PD1, integrin, CLA, CD57, IL-4, IL-9, IFN $\gamma$ , IL-17, LAP, IL-10
PMA/ionomycin-stimulated samples	CD19, CD4, CD8, CD20, CD38, CD45RA, CD3, HLA-DR, CD11c, CD14, CD27, CCR7, CD25, CD56, TCR $\gamma\delta$	IL-4, IL-9, IFN $\gamma$ , IL-17, LAP, IL-10