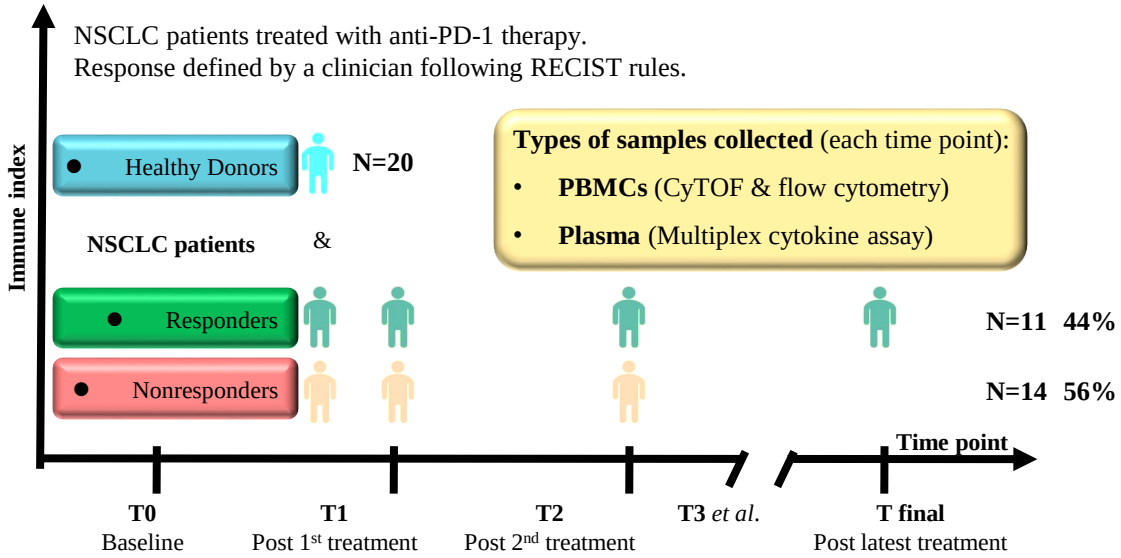
Supplementary data

Supplementary data

a



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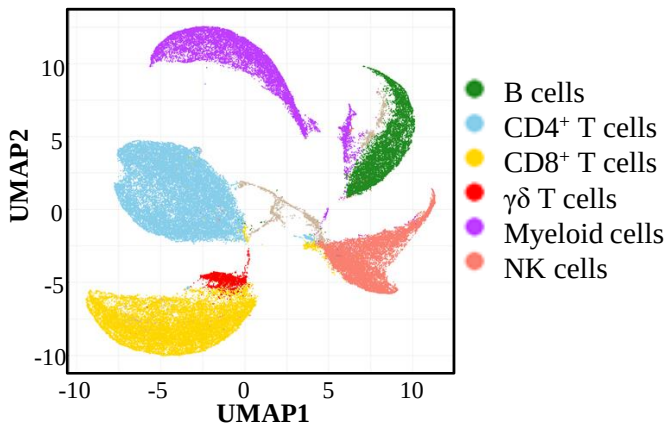


c

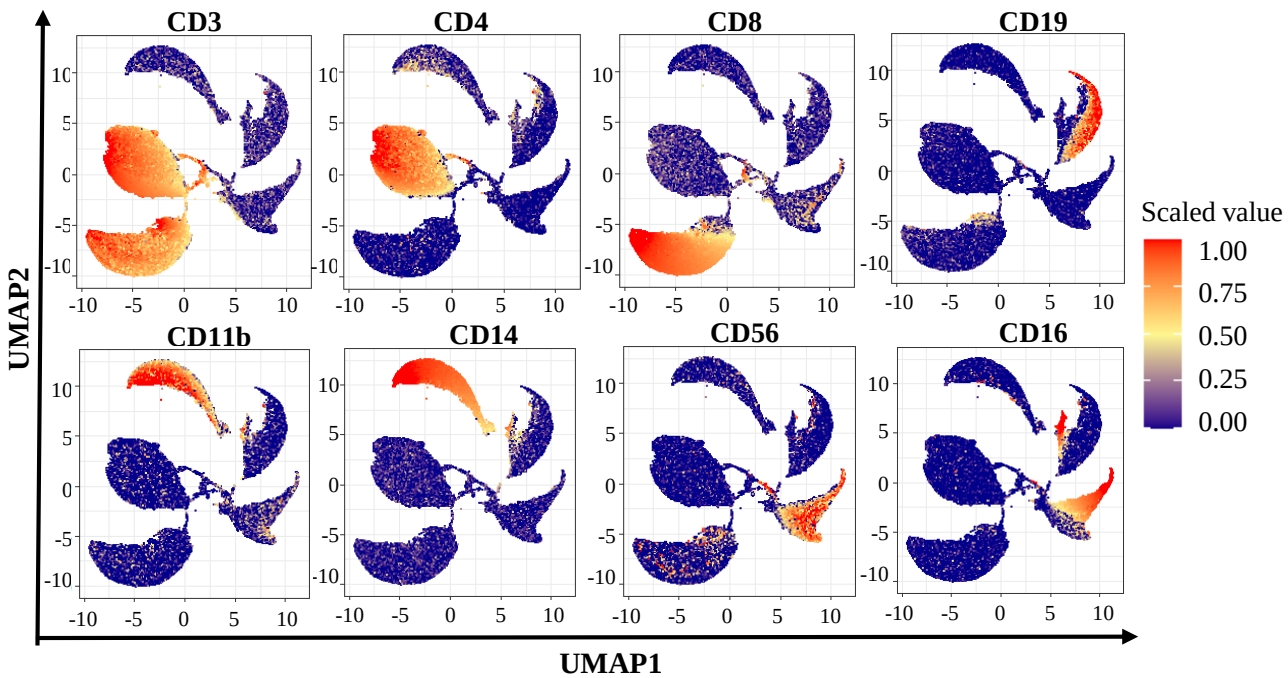
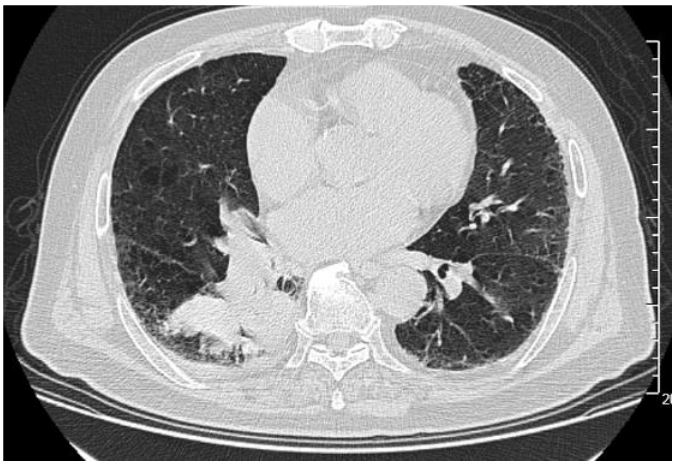


Figure S1. **High-dimensional single-cell analysis of PBMCs from NSCLC patients identifies immunophenotypes.** a. Illustration of the study design. The analysis was performed on PBMCs from healthy donors (n=20) and NSCLC patients (total patient number=25, responder number=11, nonresponder number=14). b. Manual clustering for major immune cell types. Cells were colored according to the cluster they were assigned to using the FlowSOM algorithm and manual annotation. c. Bioinformatic analysis of phenotypic profiling by UMAP dimension reduction and clustering algorithms (colored by sample features).

Supplementary data

CT scan

Before immunotherapy



After immunotherapy

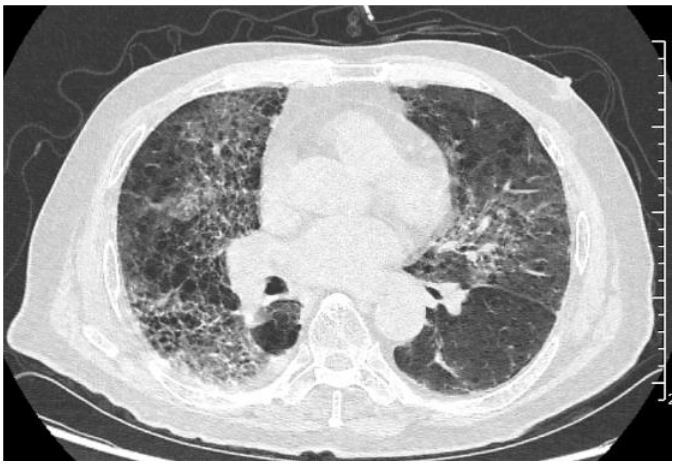


Figure S2. Above, representative patient CT scans. CT scan comparison for an 83-year-old patient with lung cancer before and after immunotherapy. Immune-related pneumonitis was also identified. Below, representative IHC images.

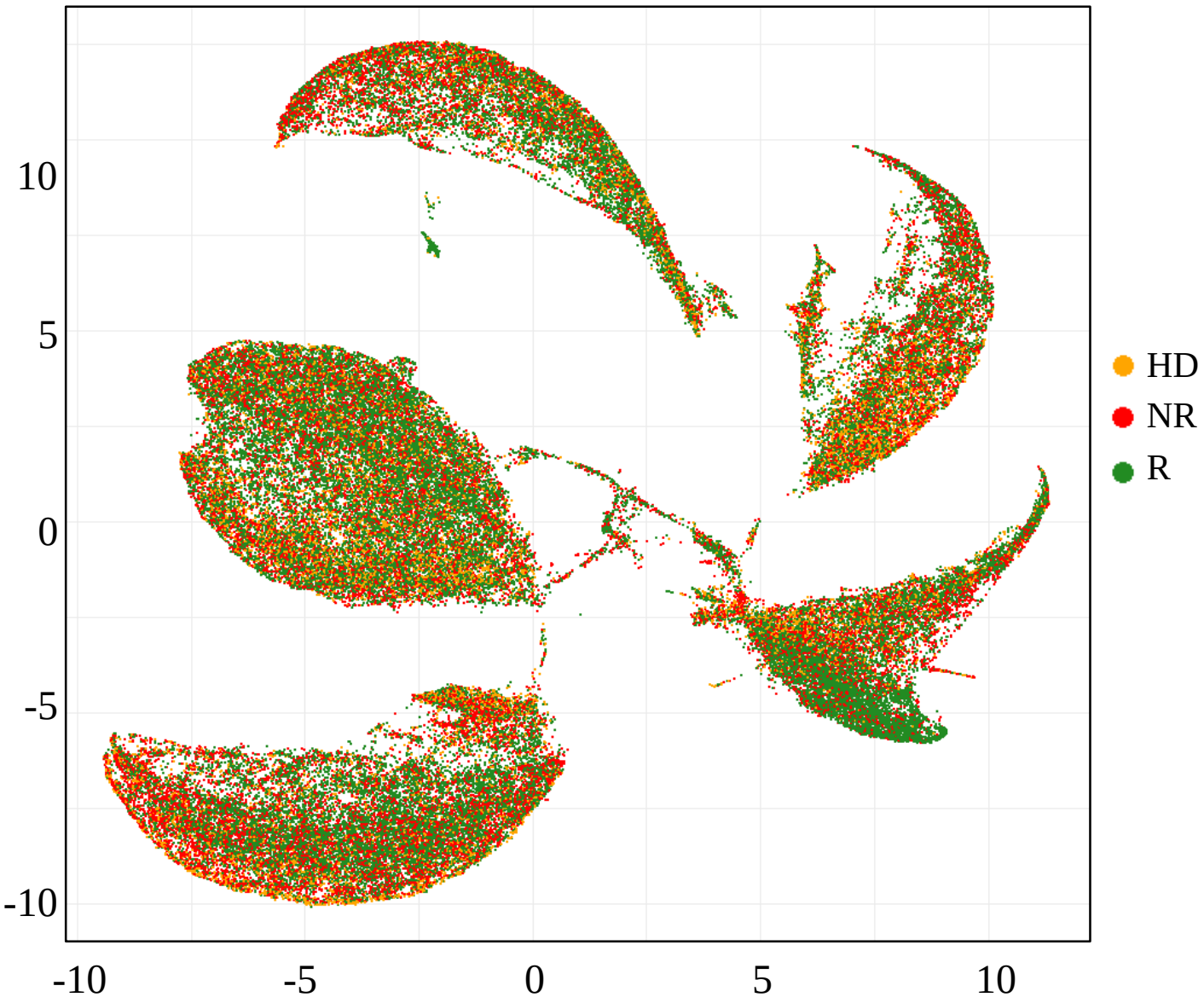


Figure S3. Exemplified UMAP visualization for healthy donors, responders and nonresponders. HD, healthy donors. NR, nonresponders. R, responders.

Supplementary data

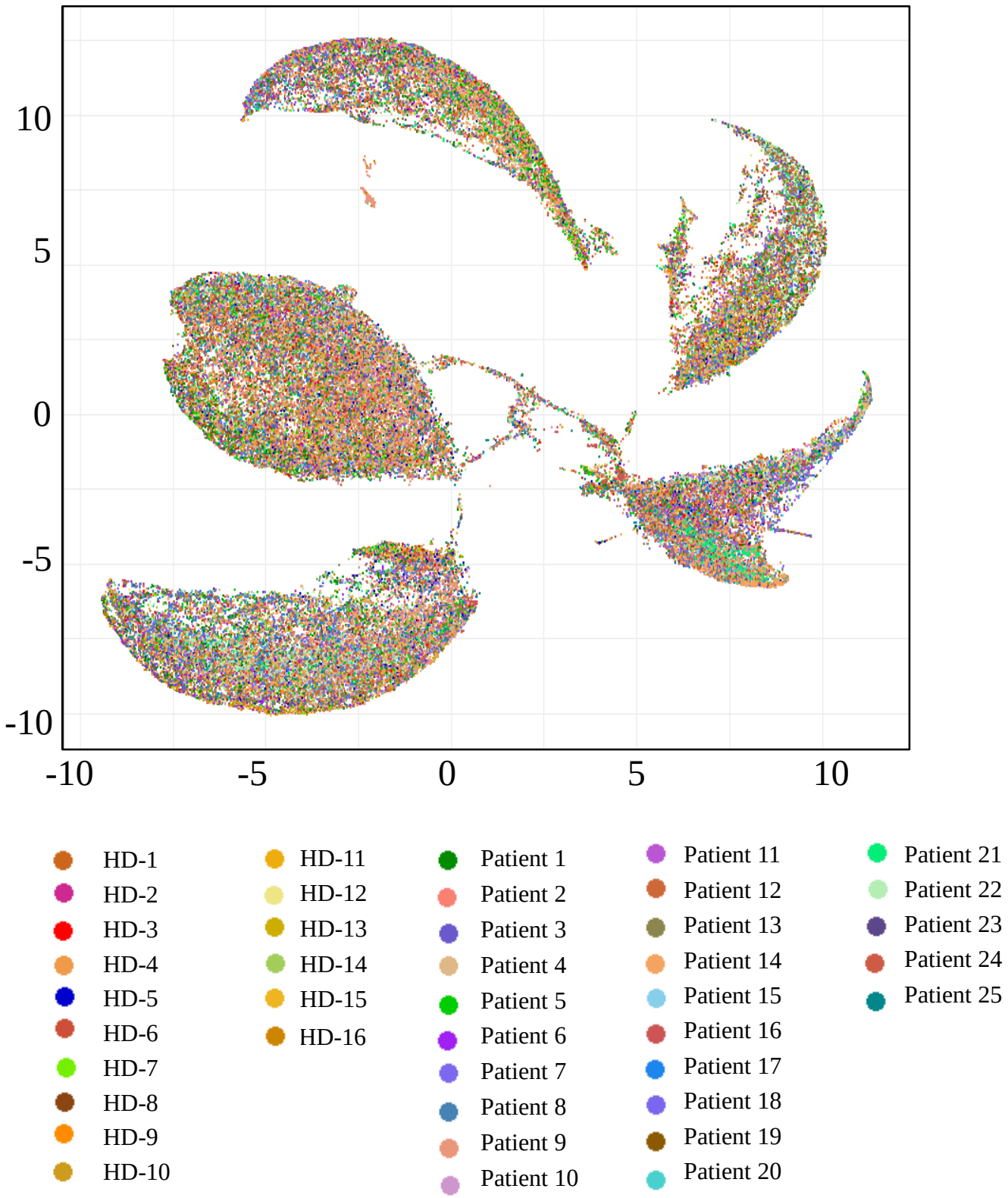


Figure S4. Exemplified UMAP visualization for different patients.

Supplementary data

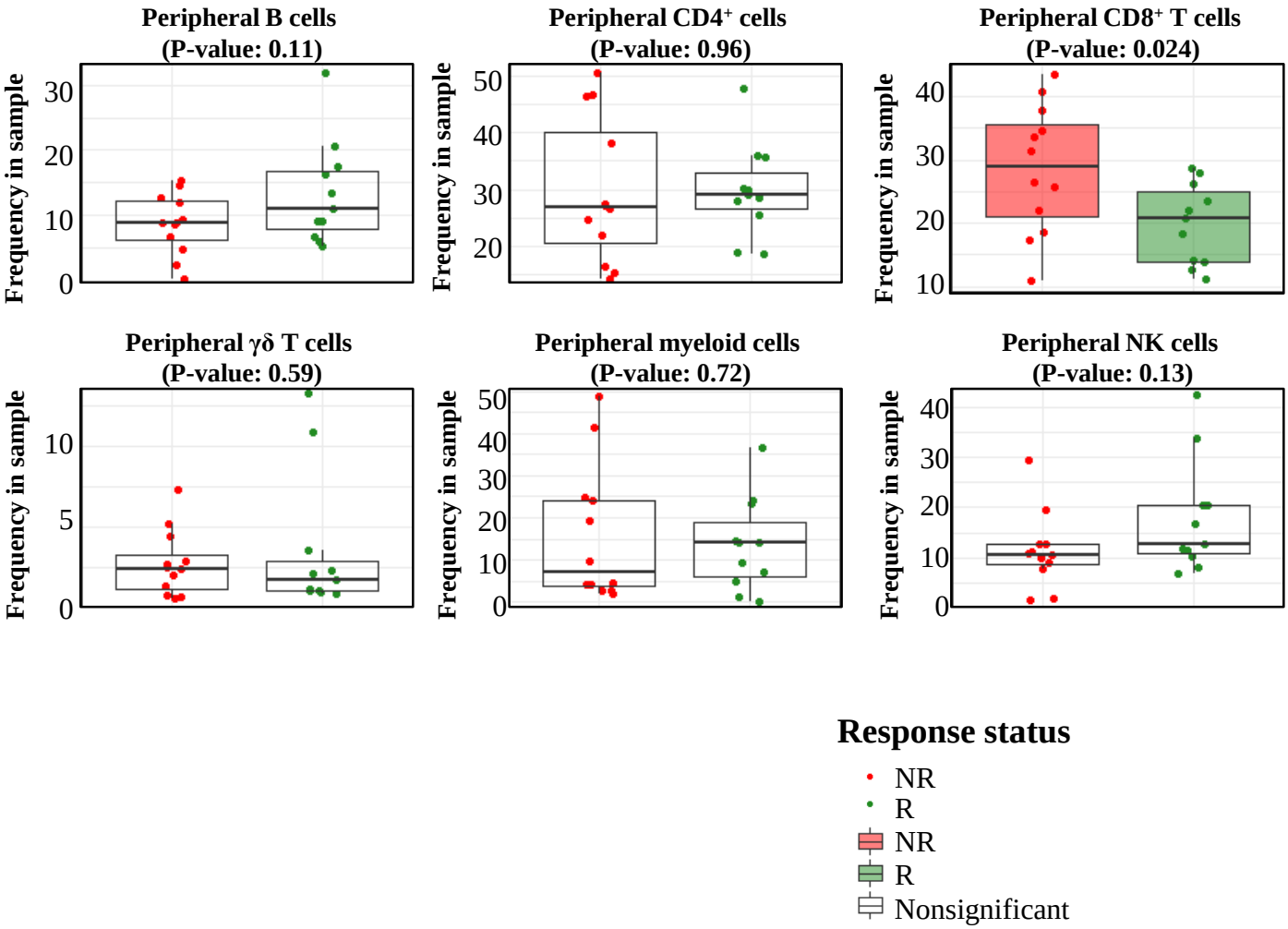


Figure S5. Overview of the main types of immune cells in responders (n=11) and nonresponders (n=14) evaluated by a physician after receipt of anti-PD-1 immunotherapy. The box plots represent the interquartile range (IQR), with the horizontal line indicating the median. The whiskers extend to the farthest data point within a maximum of 1.5 × IQR. All p-values were calculated using two-sided t-tests and were corrected for multiple comparisons using the Benjamini-Hochberg adjustment.

Supplementary data

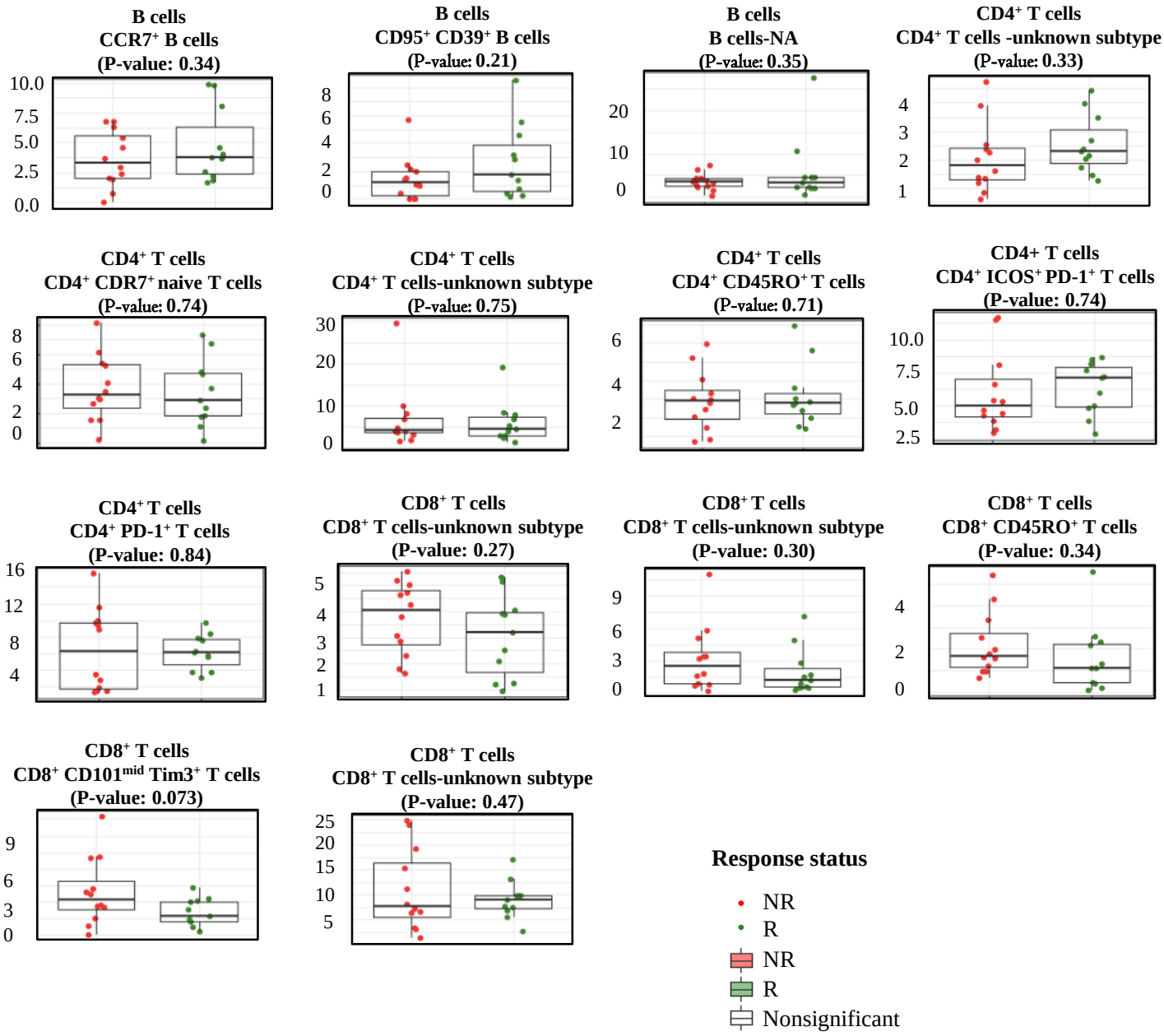


Figure S6-1. Overview of subsets of immune cells in responders and nonresponders. All p-values were calculated using two-sided t-tests comparing the R to the NR and were corrected for multiple comparisons using the Benjamini-Hochberg adjustment.

Supplementary data

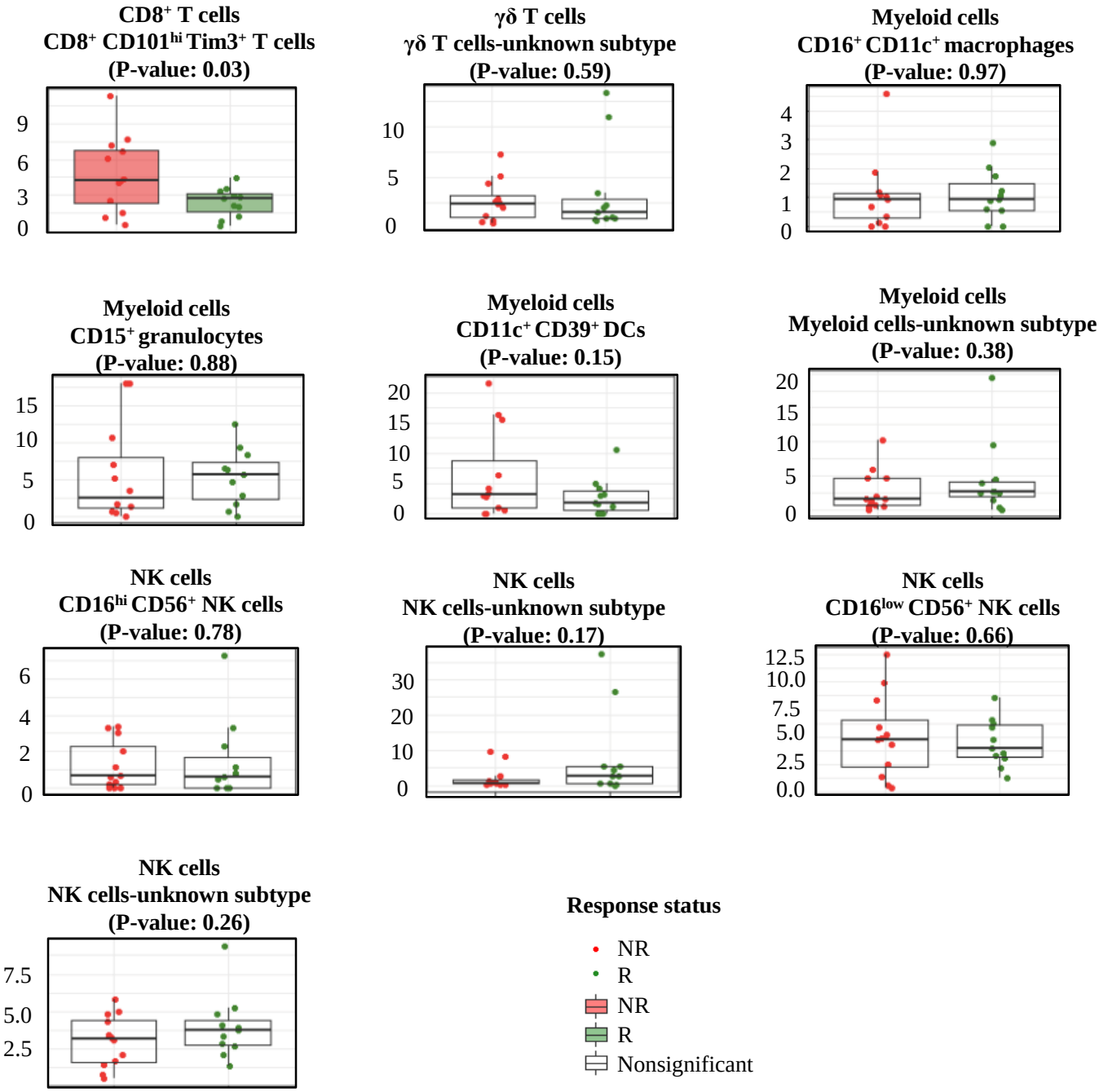


Figure S6-2. Overview of subsets of immune cells in responders and nonresponders. All p-values were calculated using two-sided t-tests comparing the R to the NR and were corrected for multiple comparisons using the Benjamini-Hochberg adjustment.

Supplementary data

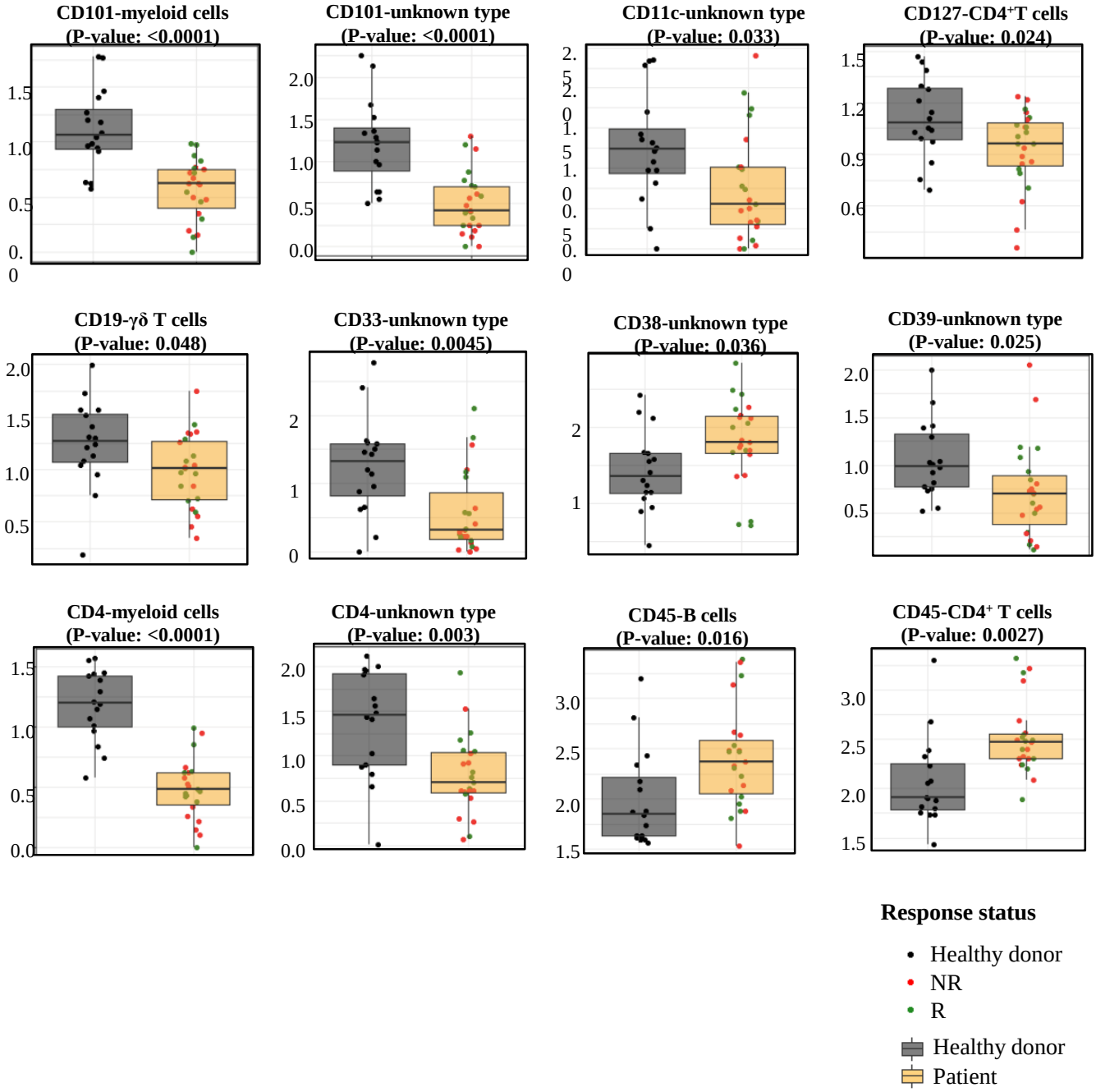


Figure S7-1. Subsets of immune cells with a p-value < 0.05 for the comparison of patients and healthy donors (at baseline). All p-values were calculated using two-sided t-tests comparing the patients to the HDs and were corrected for multiple comparisons using the Benjamini-Hochberg adjustment.

Supplementary data

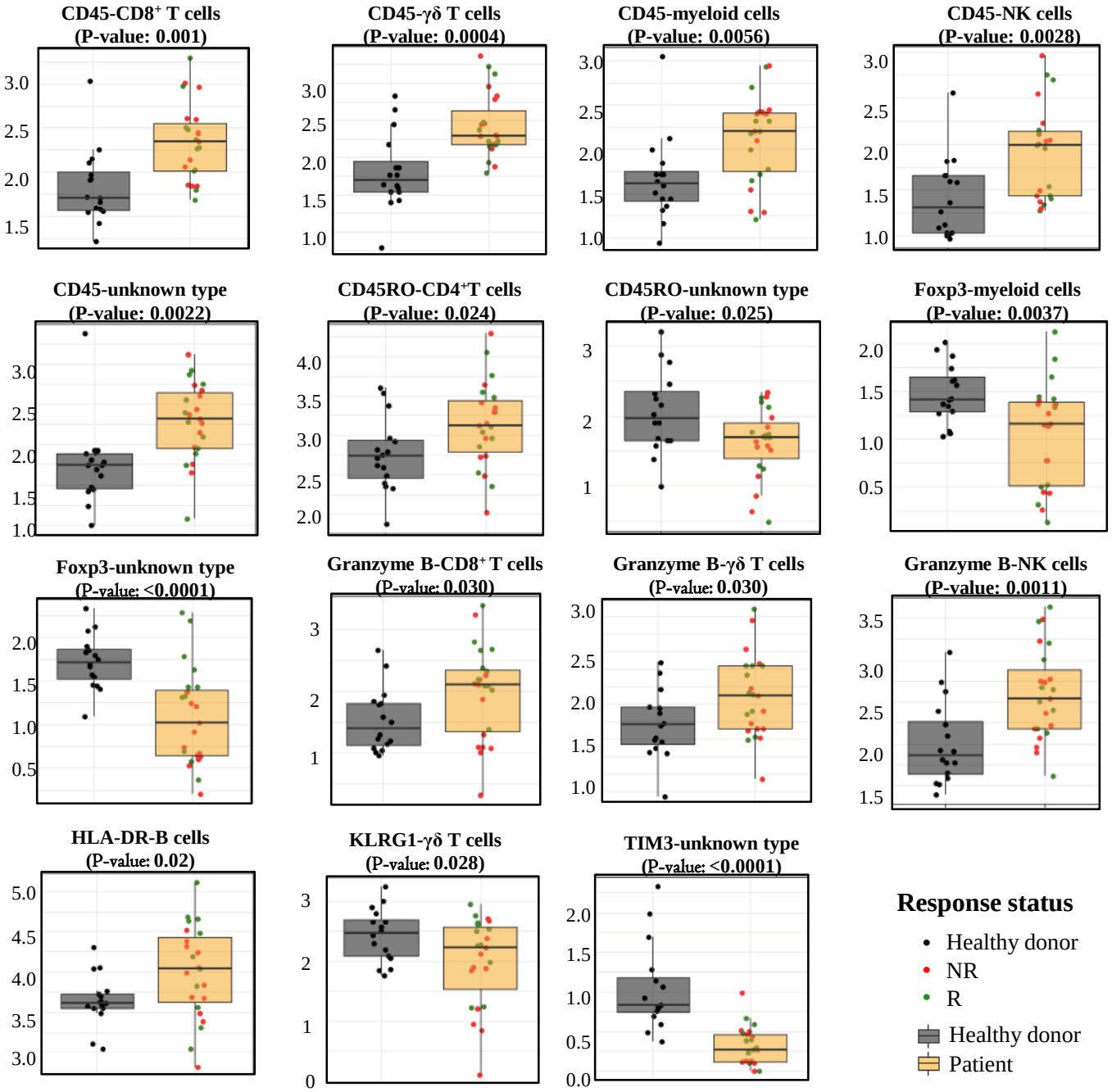


Figure S7-2. Subsets of immune cells with a p-value < 0.05 for the comparison of patients and healthy donors (at baseline). All p-values were calculated using two-sided t-tests comparing the patients to the HDs and were corrected for multiple comparisons using the Benjamini-Hochberg adjustment.

Supplementary data

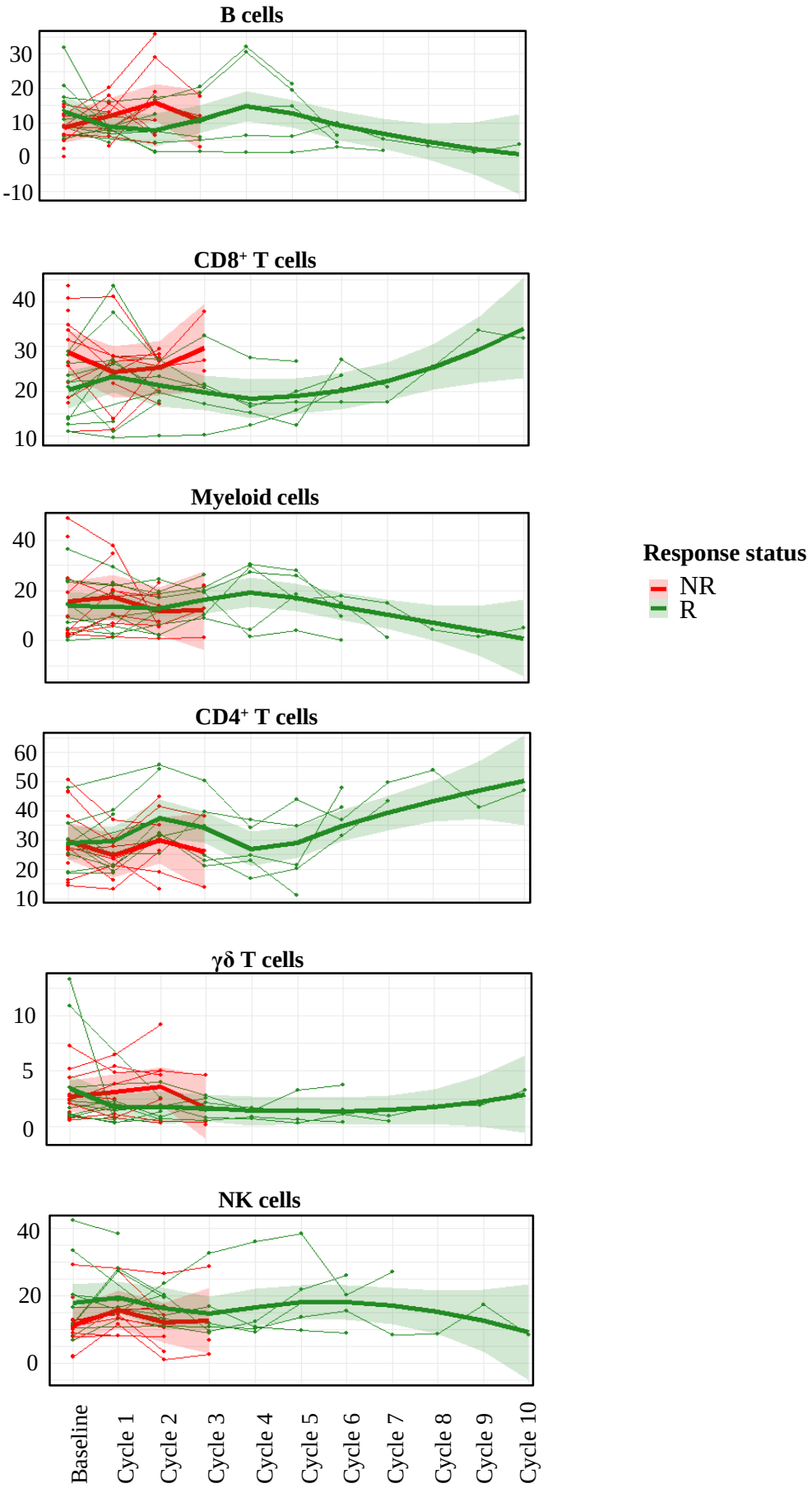


Figure S8. Longitudinal behavior of antigens in major types of immune cells compared between responders and nonresponders.

Supplementary data

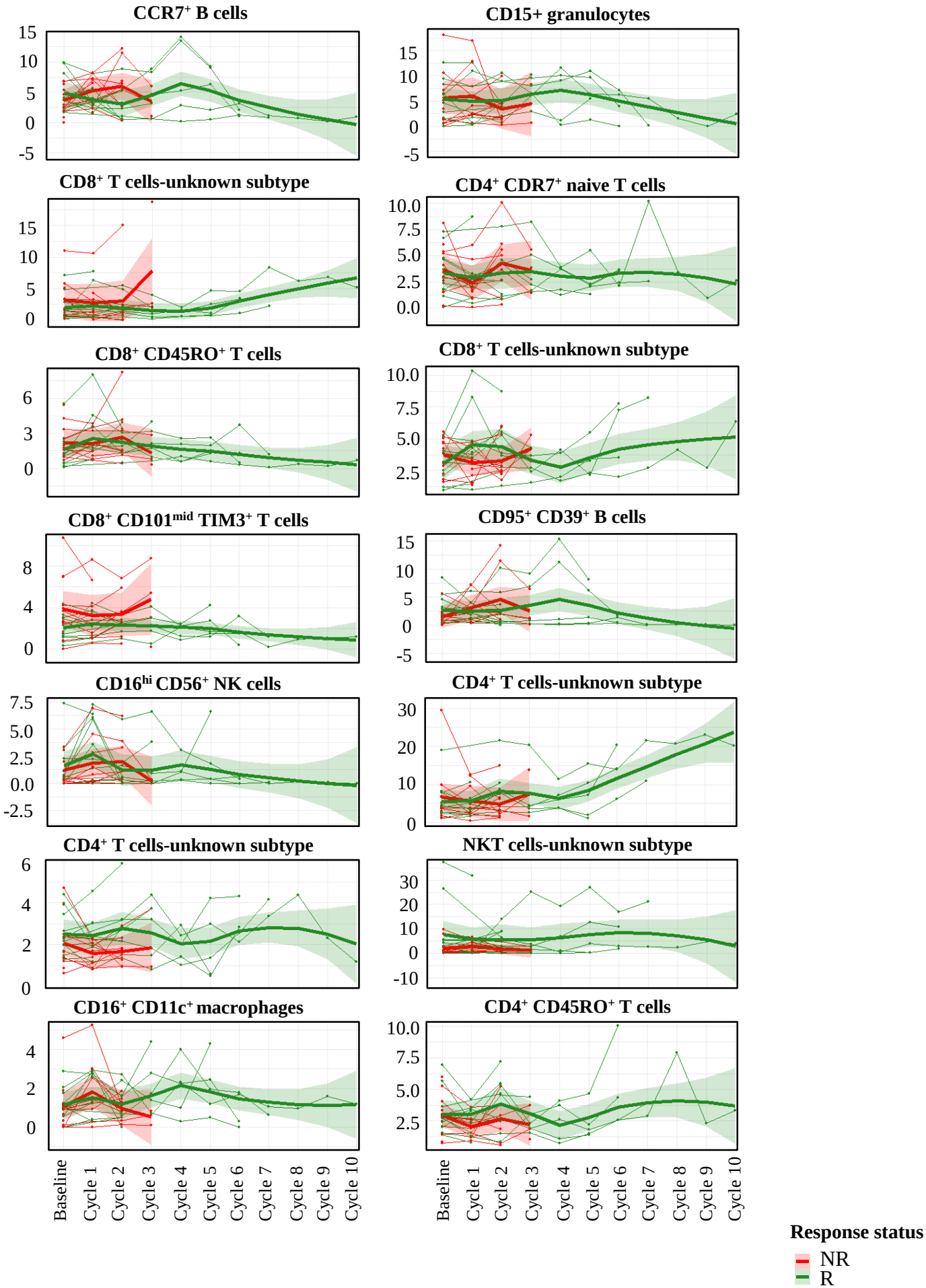


Figure S9-1. Longitudinal behavior of subtypes of immune cells compared between responders and nonresponders.

Supplementary data

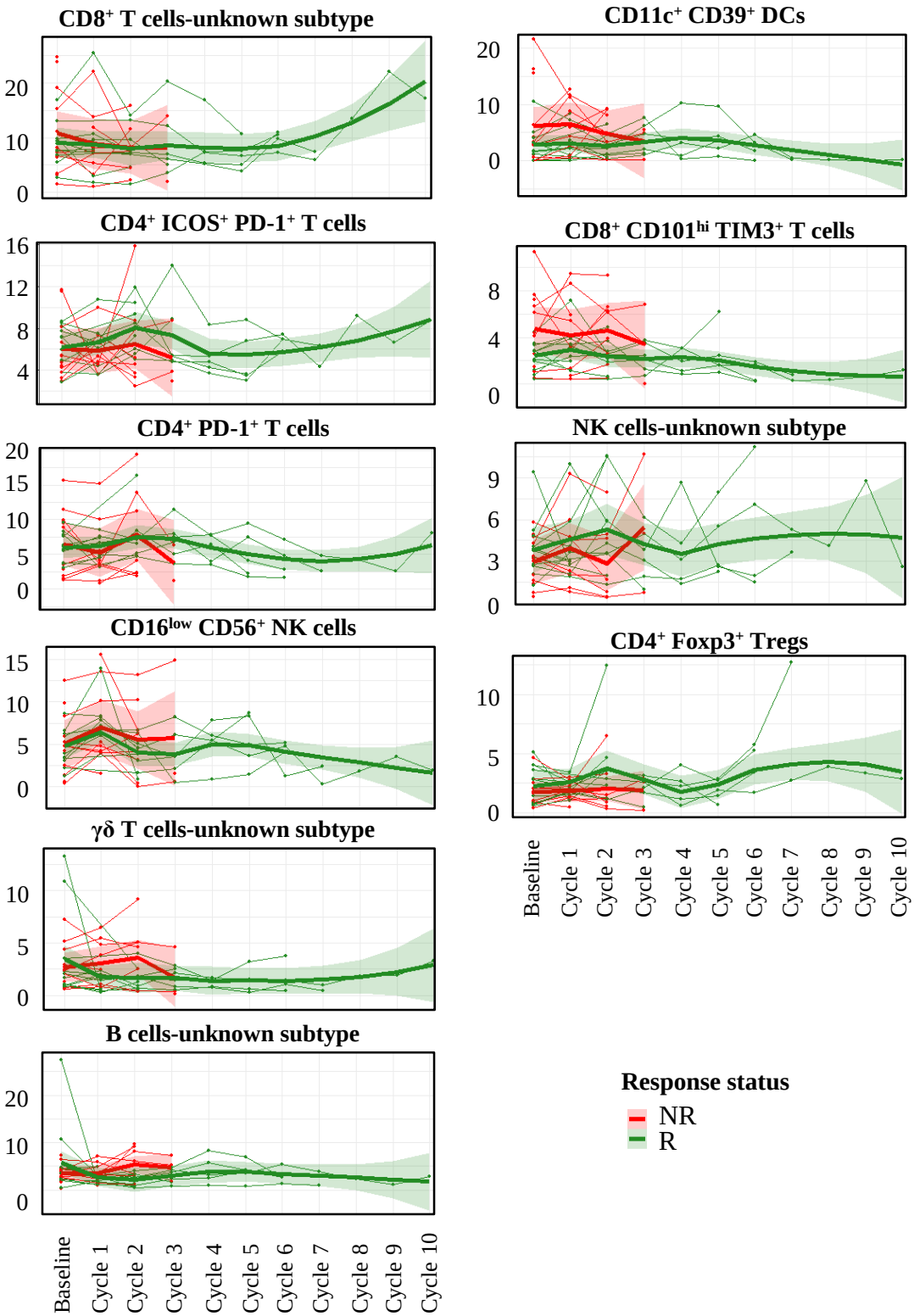


Figure S9-2. Longitudinal behavior of subtypes of immune cells compared between responders and nonresponders.

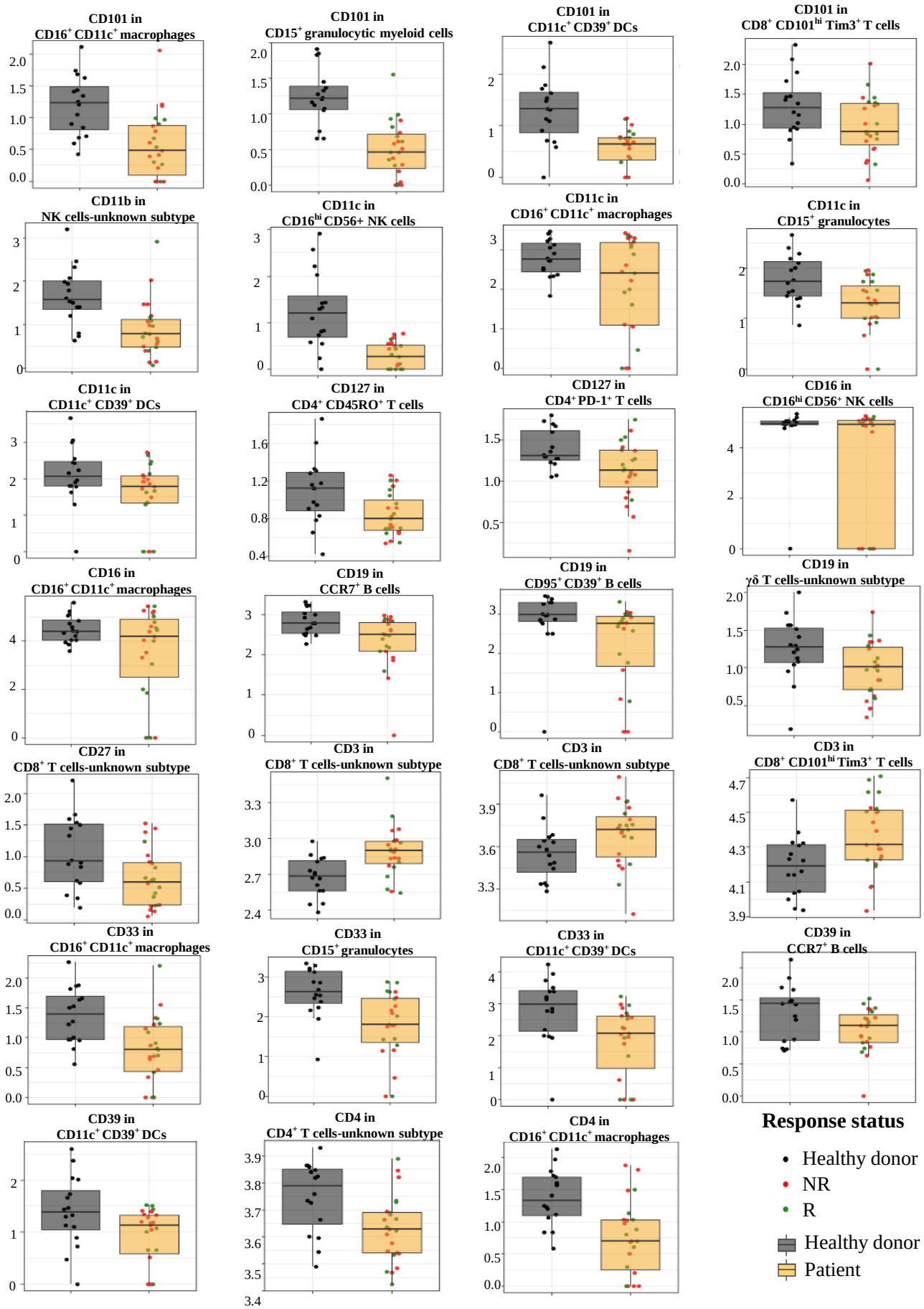


Figure S10-1. Antigens of immune cells with a p-value < 0.05 when comparing patients and healthy donors (at baseline). All p-values were calculated using two-sided t-tests comparing the patients to the HDs and were corrected for multiple comparisons using the Benjamini-Hochberg adjustment.

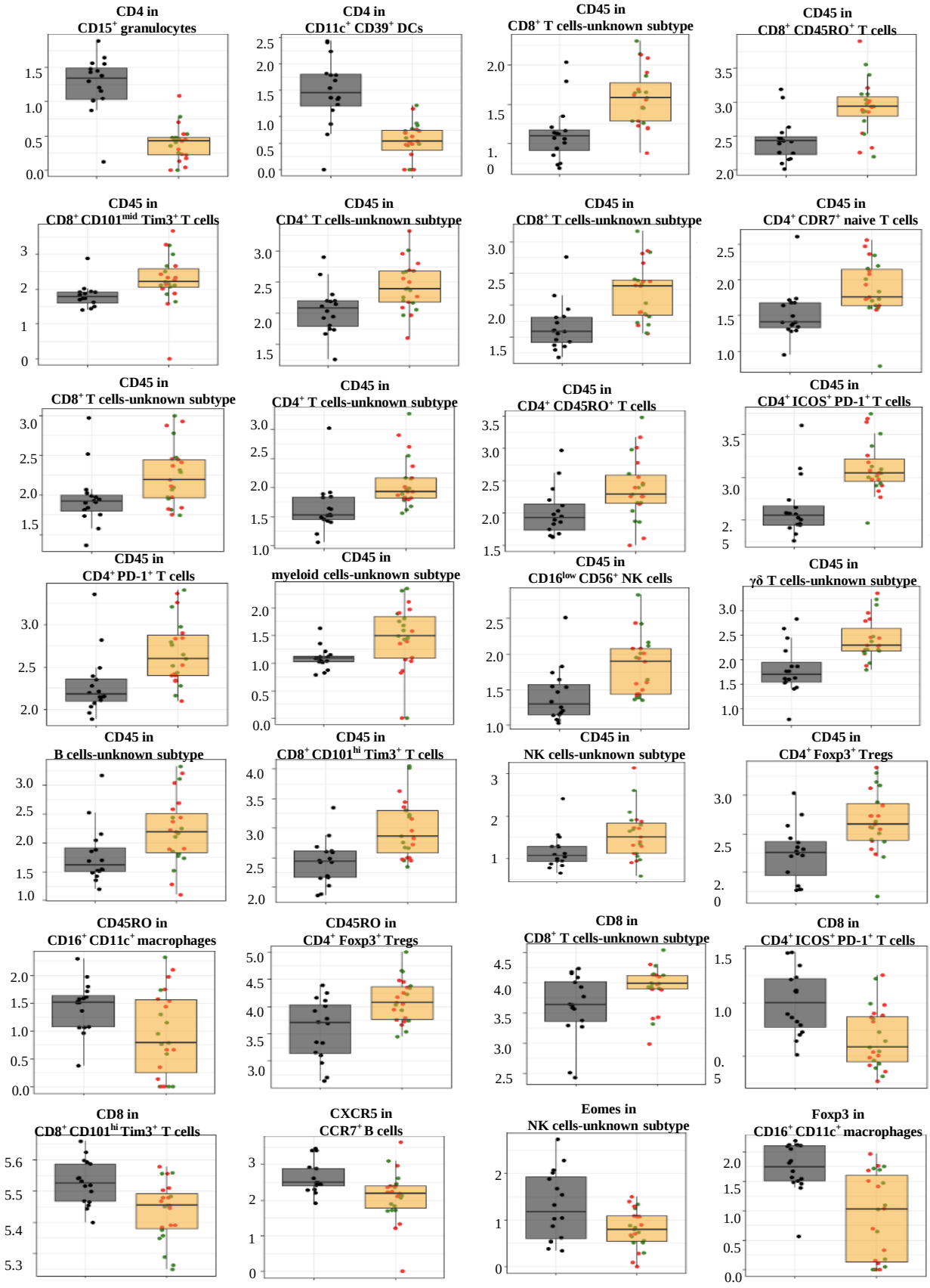


Figure S10-2. Antigens of immune cells with a p-value < 0.05 when comparing patients and healthy donors (at baseline). All p-values were calculated using two-sided t-tests comparing the patients to the HDs and were corrected for multiple comparisons using the Benjamini-Hochberg adjustment.

Supplementary data

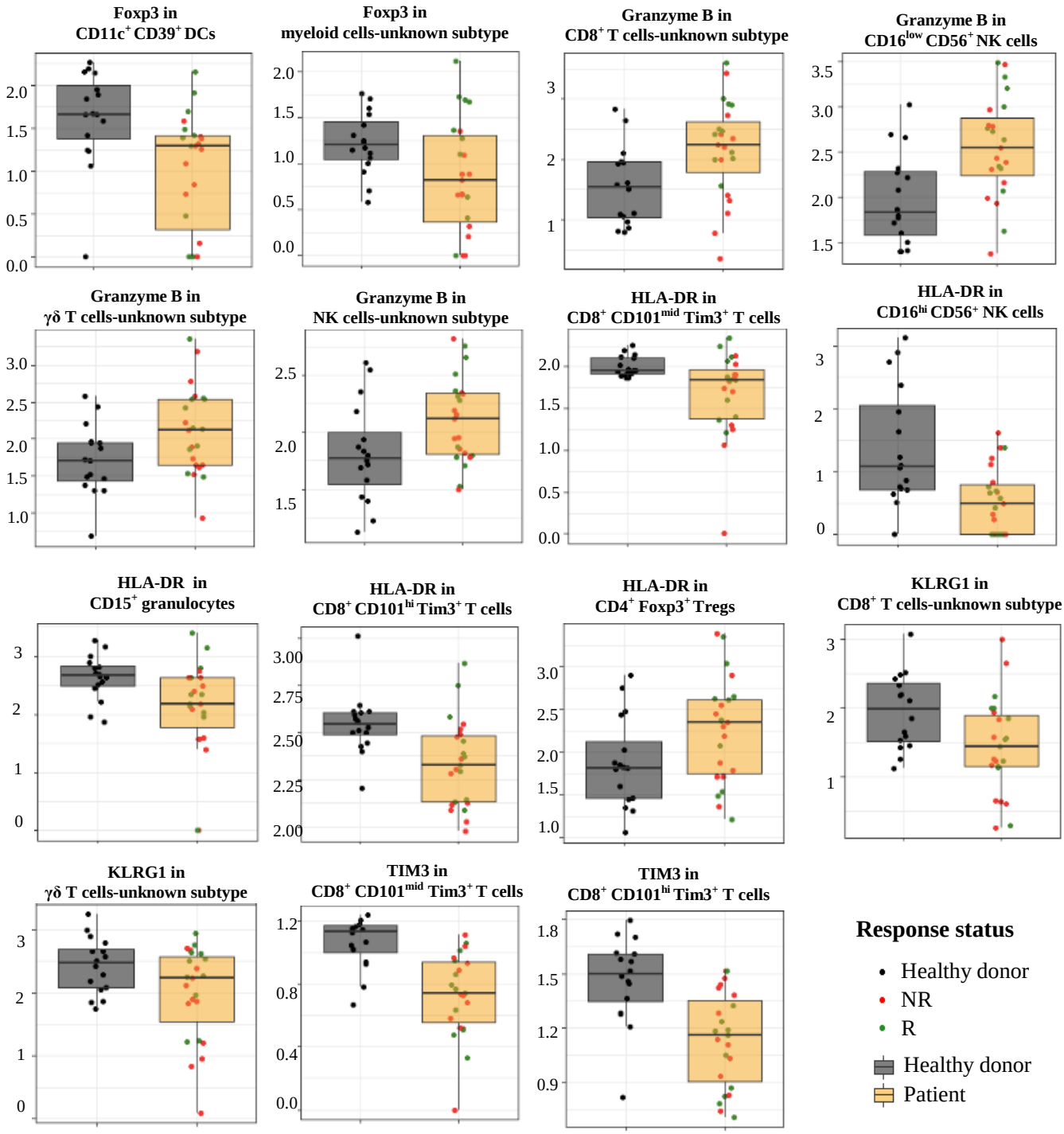


Figure S10-3. Antigens of immune cells with a p-value < 0.05 when comparing patients and healthy donors (at baseline). All p-values were calculated using two-sided t-tests comparing the patients to the HD and were corrected for multiple comparisons using the Benjamini-Hochberg adjustment.

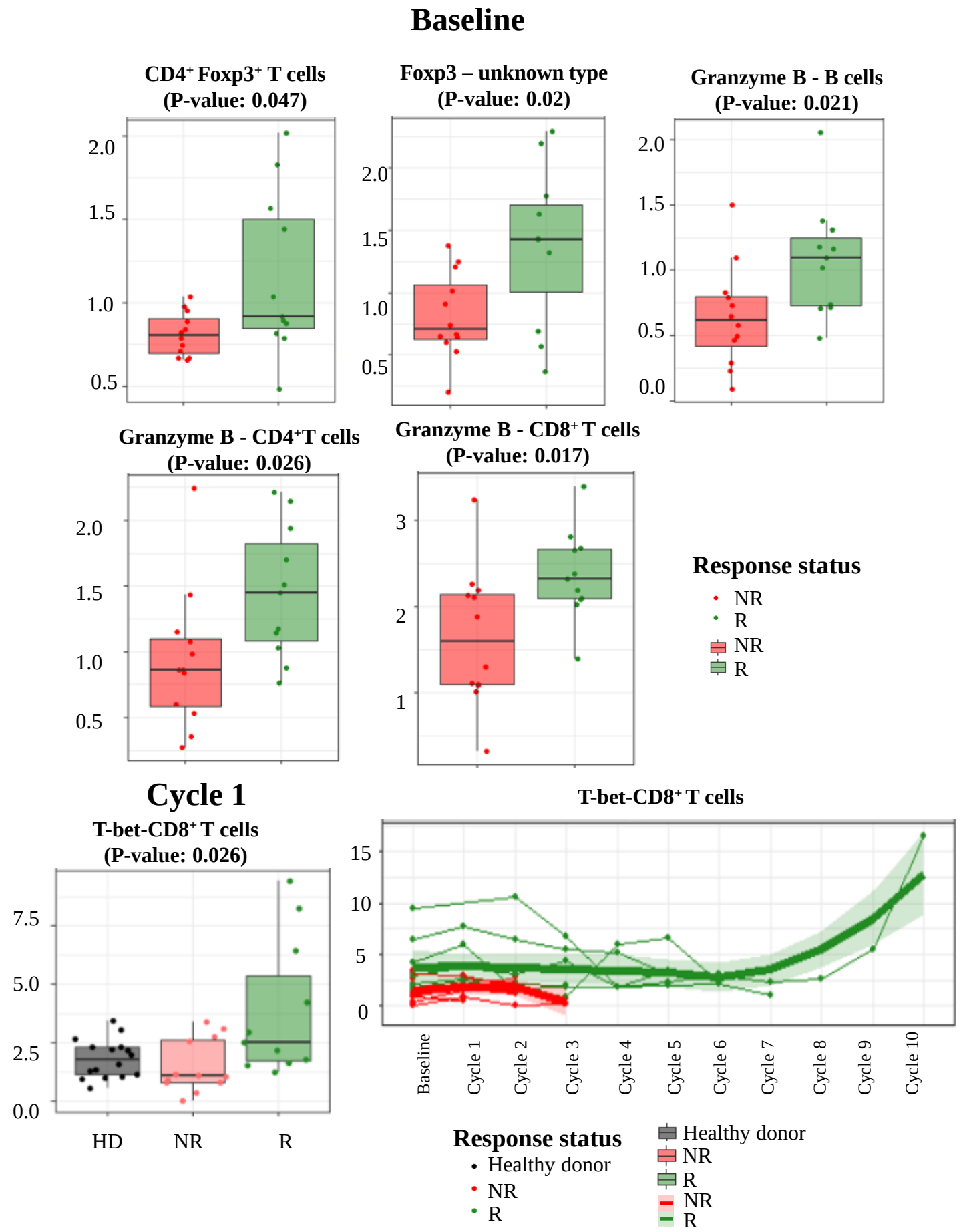


Figure S11. Antigens on major types of immune cells with a p-value < 0.05 when comparing R and NR (at baseline and after one cycle of anti-PD1 treatment) as well as the longitudinal behavior in patients with NSCLC.

Supplementary data

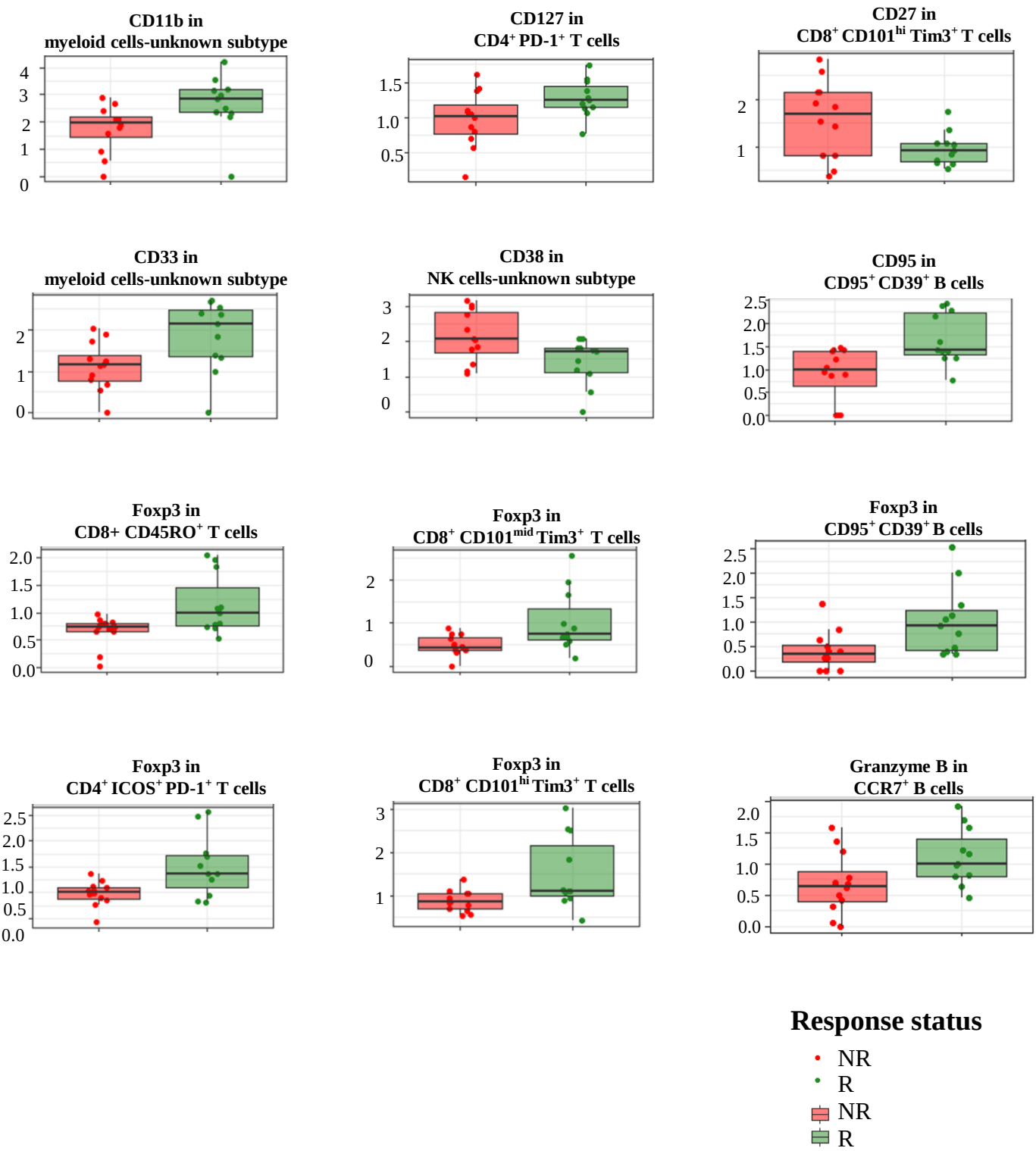


Figure S12-1. Antigens on subsets of immune cells with a p-value < 0.05 when comparing R and NR (at baseline).

Supplementary data

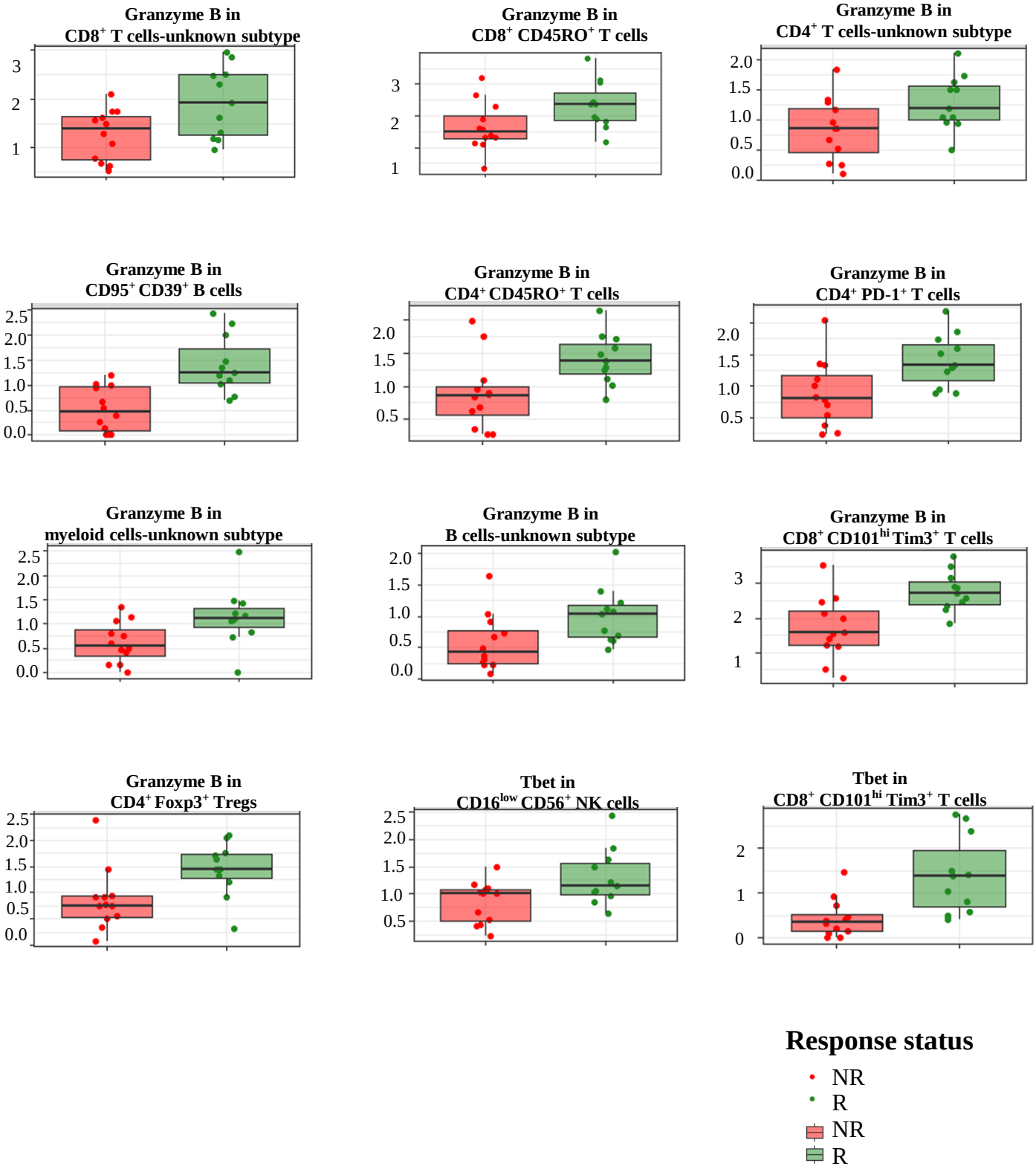


Figure S12-2. Antigens on subsets of immune cell with a p-value < 0.05 when comparing R and NR (at baseline).

Supplementary data

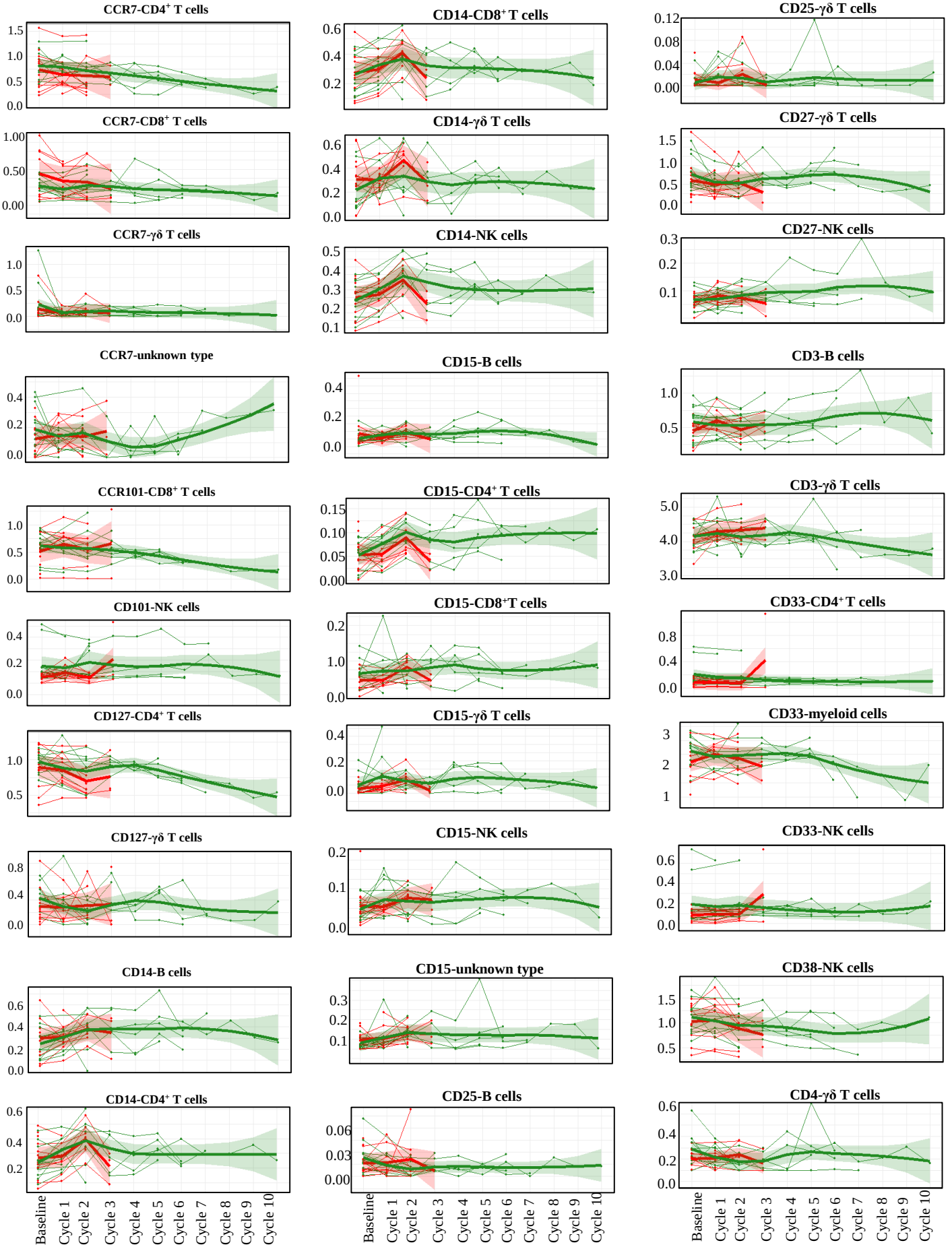


Figure S13-1. Longitudinal behavior of antigens in major types of immune cells compared between responders and nonresponders.

Supplementary data

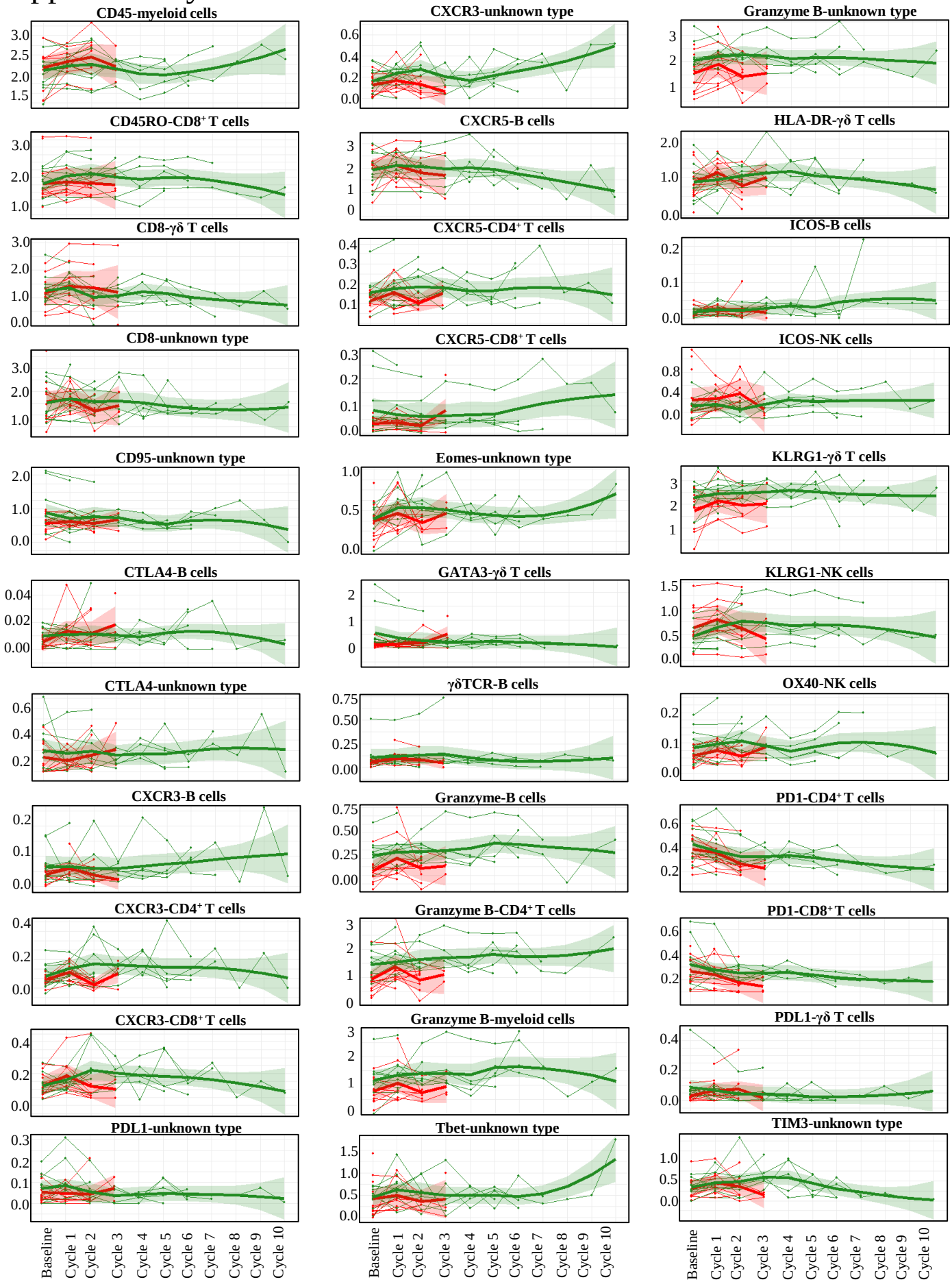


Figure S13-2. Longitudinal behavior of antigens in major types of immune cells compared between responders and nonresponders.

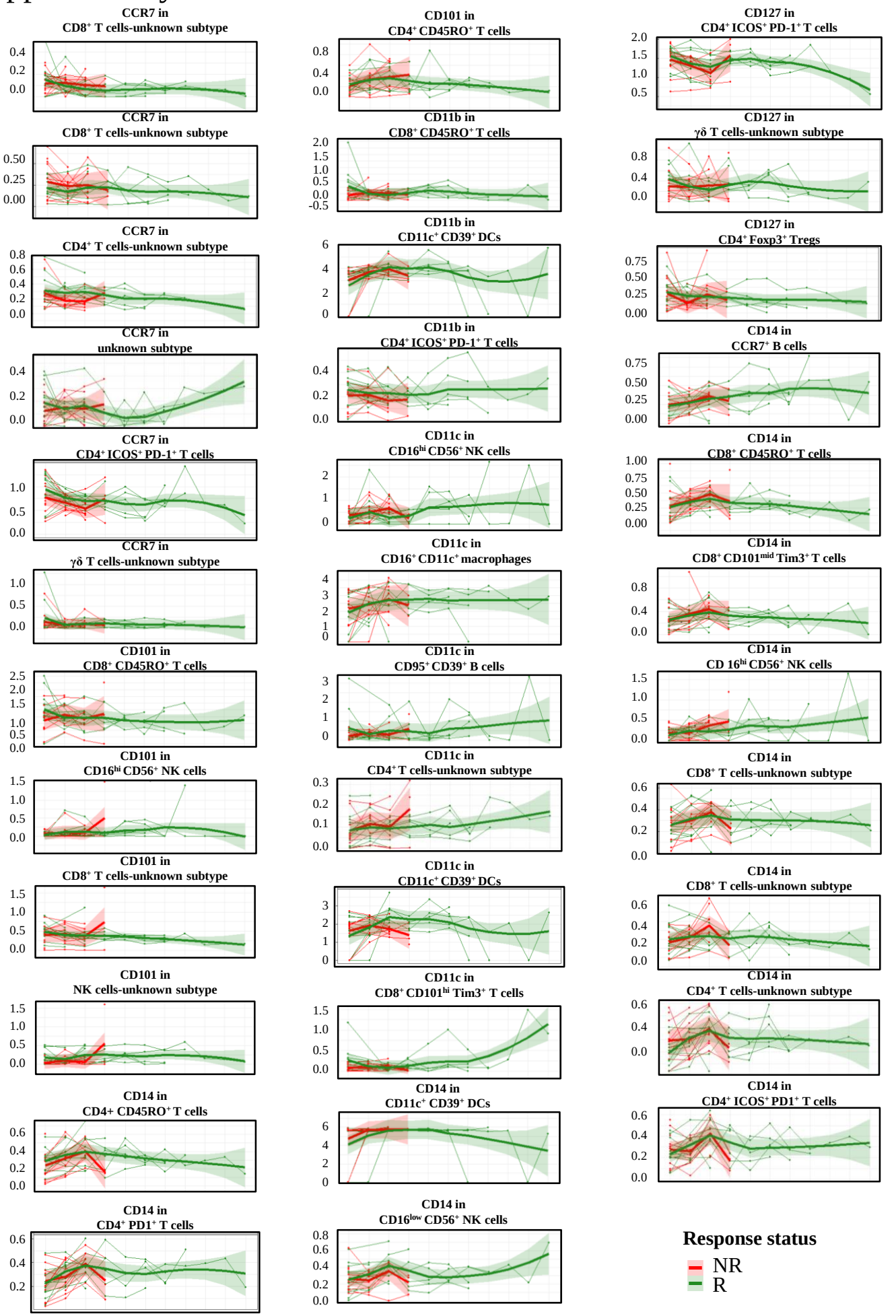


Figure S14-1. Longitudinal behavior of antigens in different subsets of immune cells compared between responders and nonresponders.

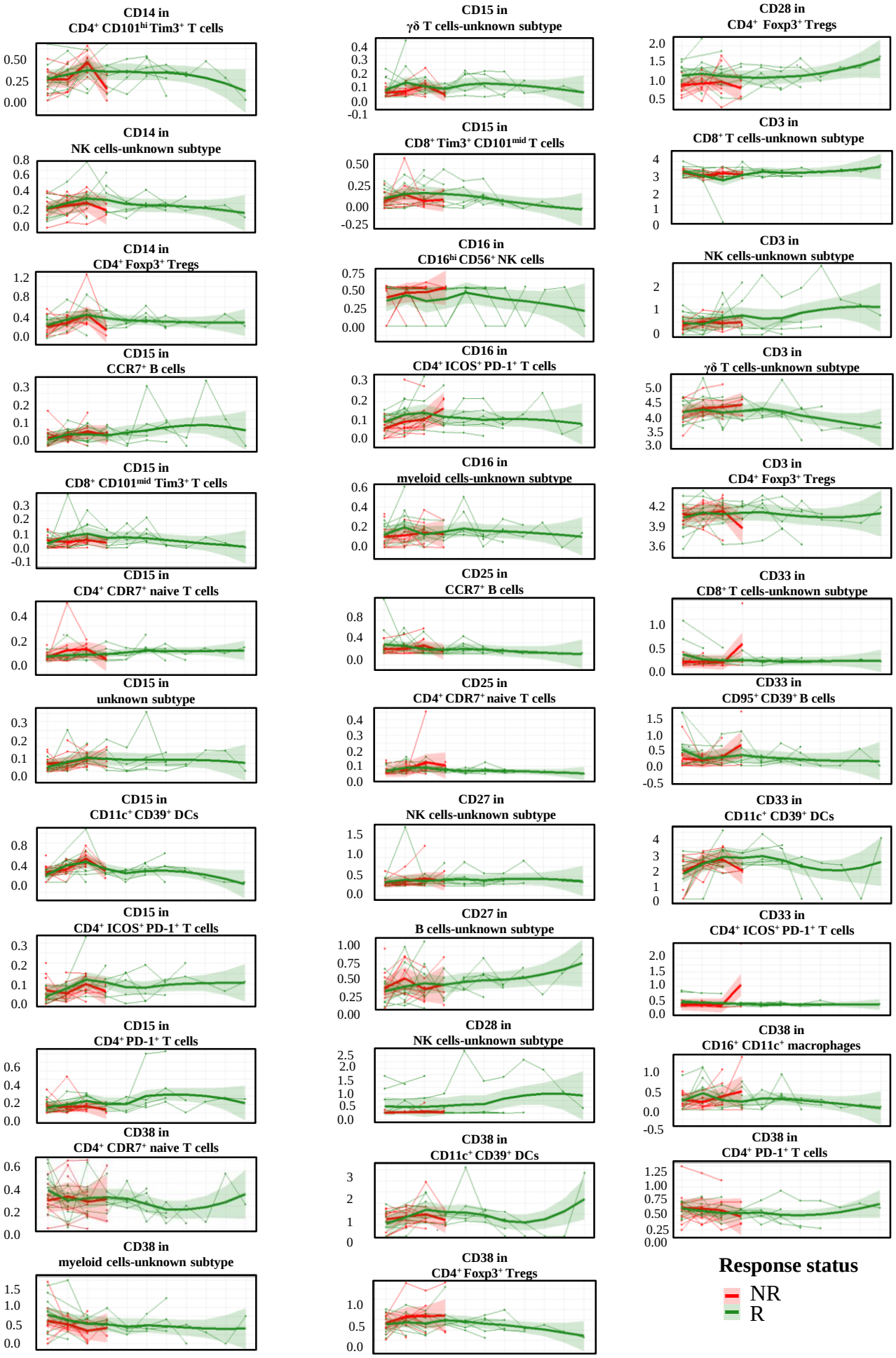


Figure S14-2. Longitudinal behavior of antigens in different subsets of immune cells compared between responders and nonresponders.

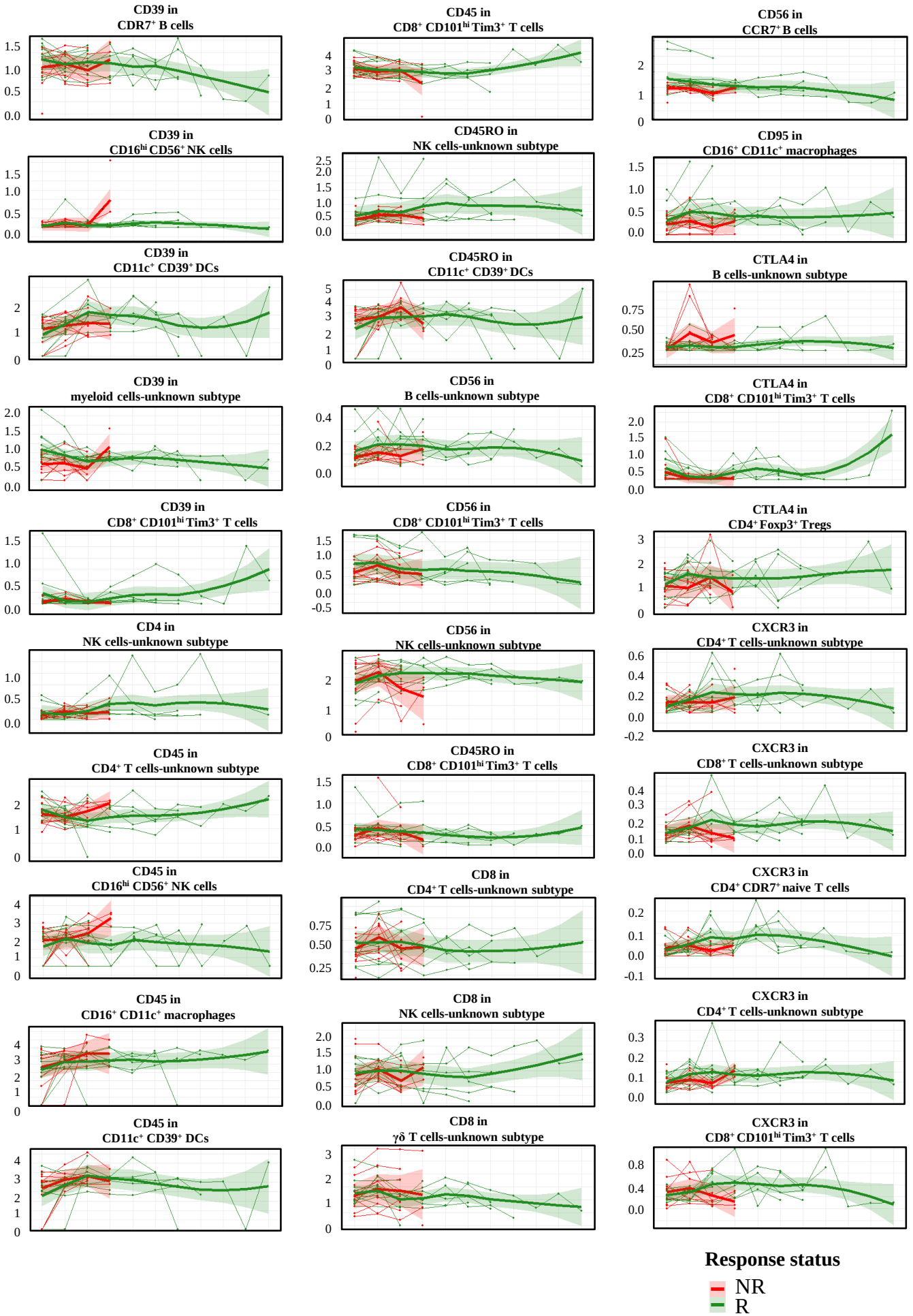


Figure S14-3. Longitudinal behavior of antigens in different subsets of immune cells compared between responders and nonresponders.

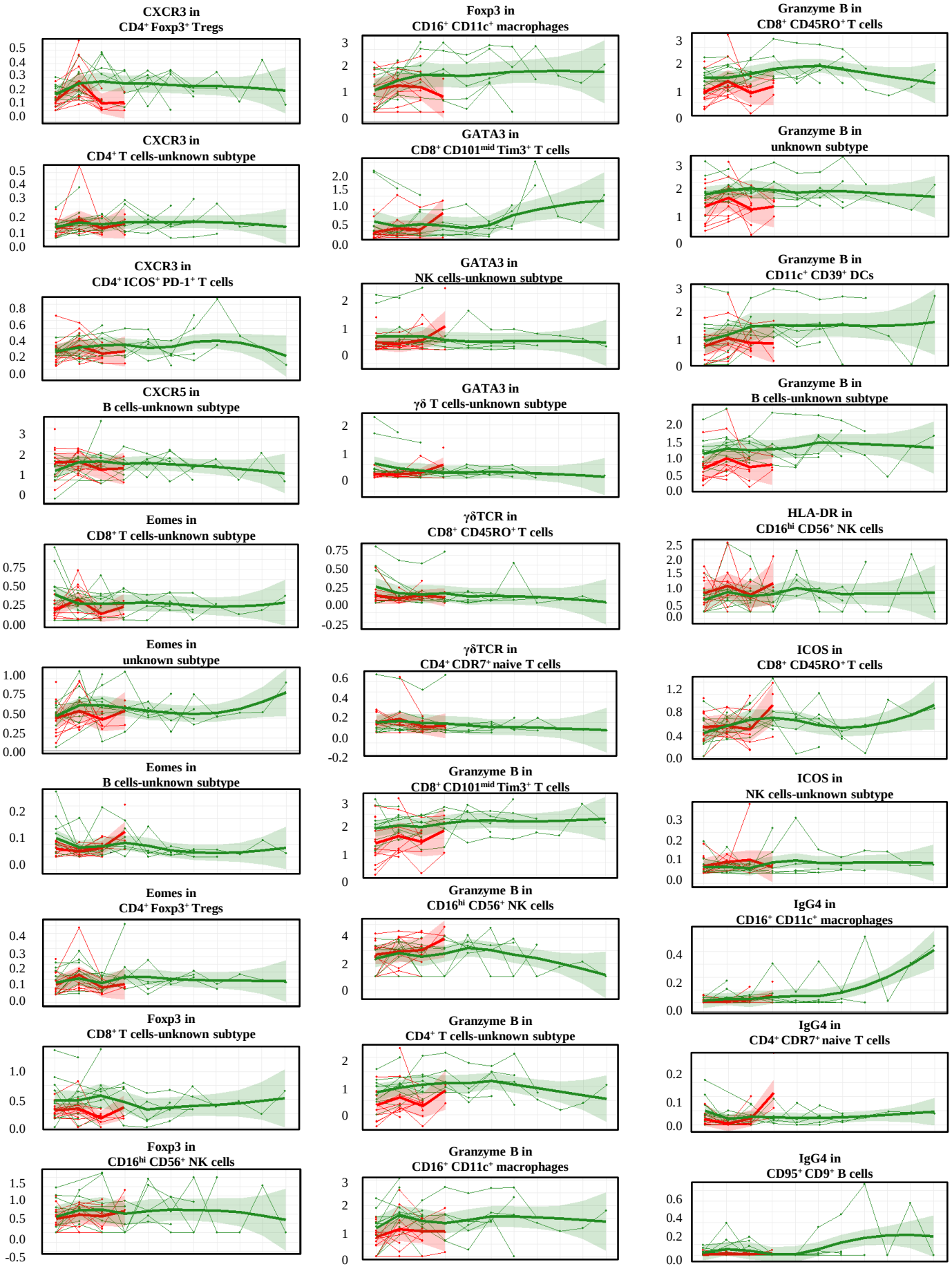


Figure S14-4. Longitudinal behavior of antigens in different subsets of immune cells compared between responders and nonresponders.

Supplementary data

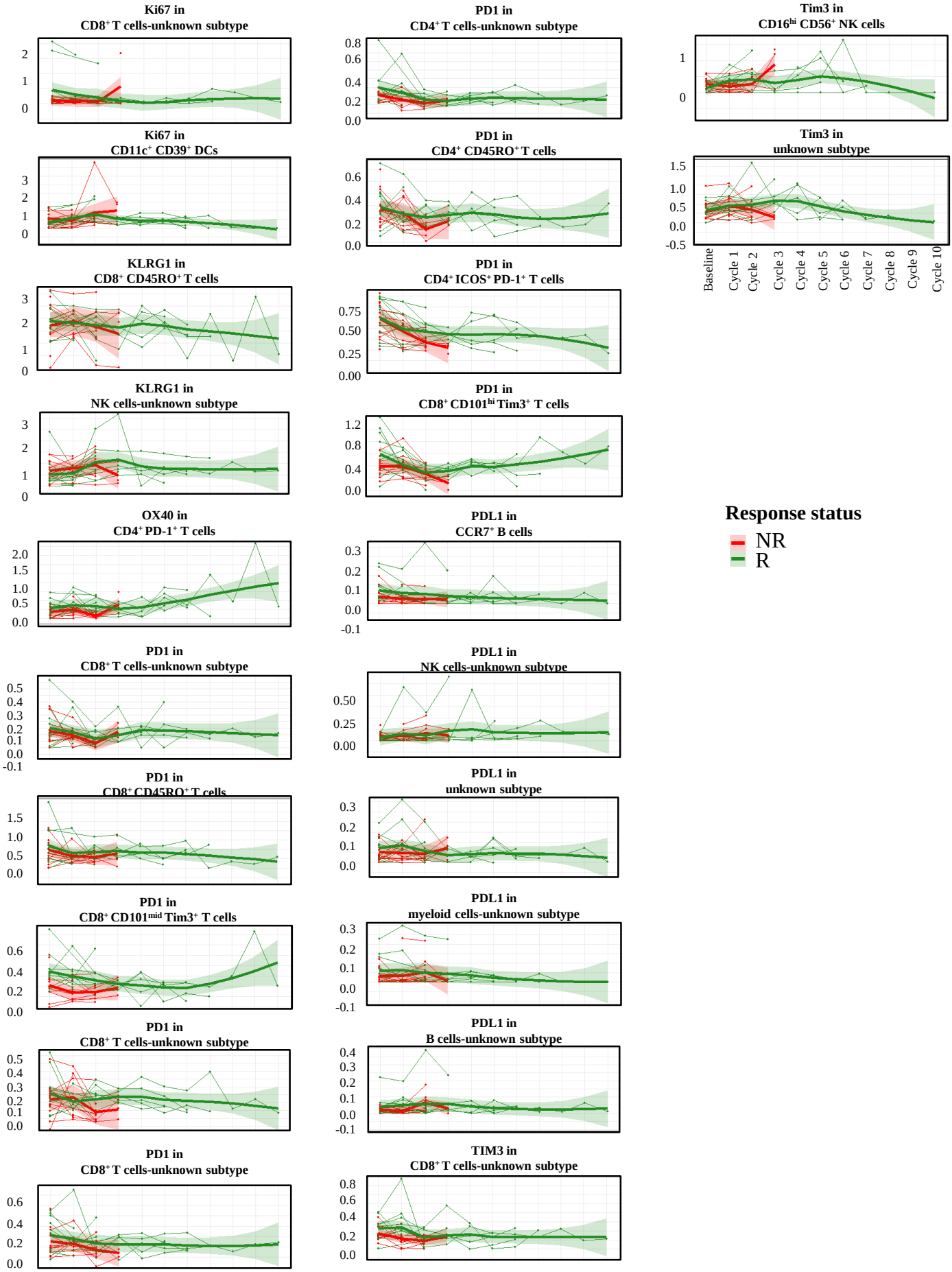


Figure S14-5. Longitudinal behavior of antigens in different subsets of immune cells compared between responders and nonresponders.

Supplementary data

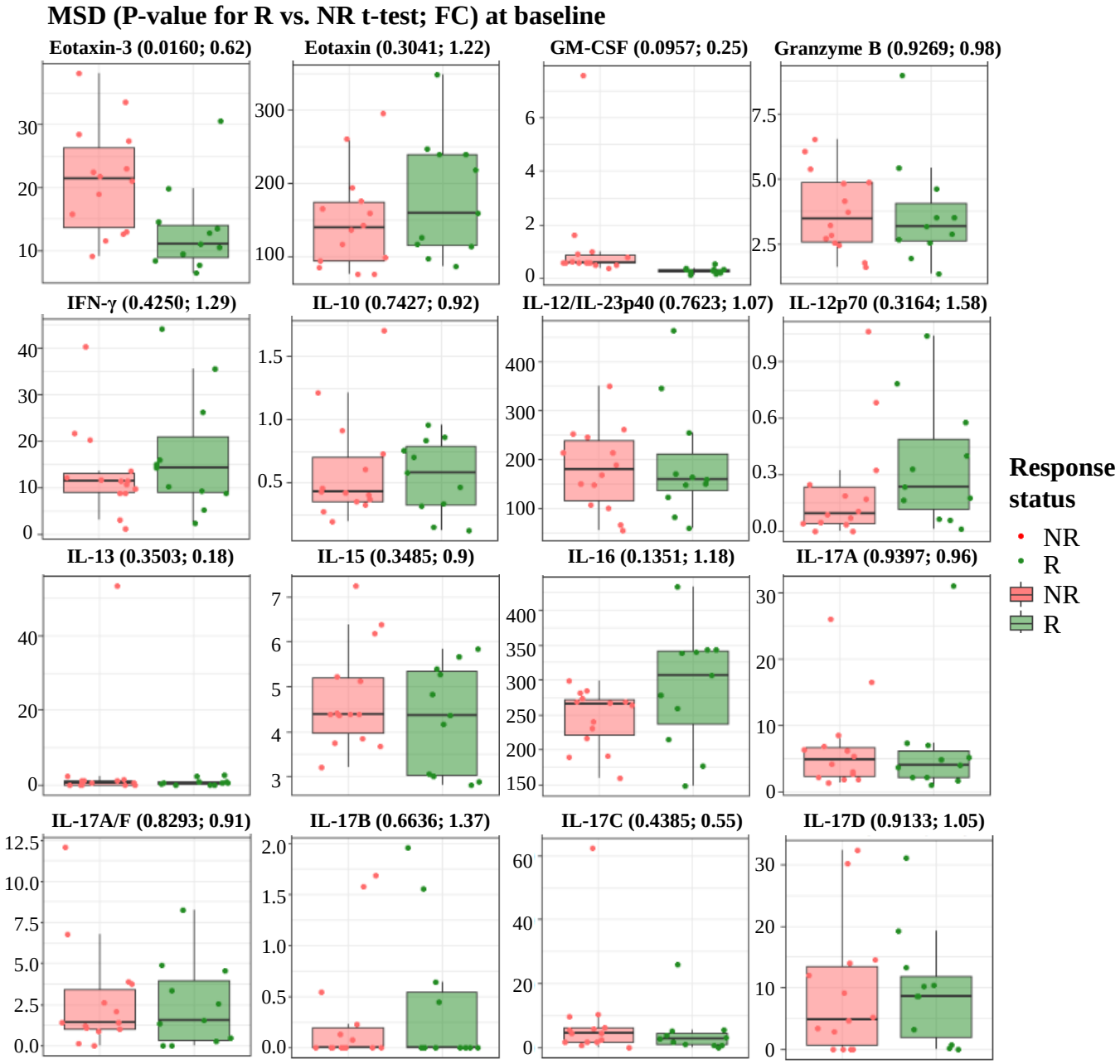


Figure S15-1. Cytokine levels tested by MSD at baseline and compared between responders and nonresponders in a cohort of NSCLC patients receiving anti-PD-1 treatment. All p-values were calculated using two-sided t-tests comparing the R to the NR and were corrected for multiple comparisons using the Benjamini-Hochberg adjustment.

Supplementary data

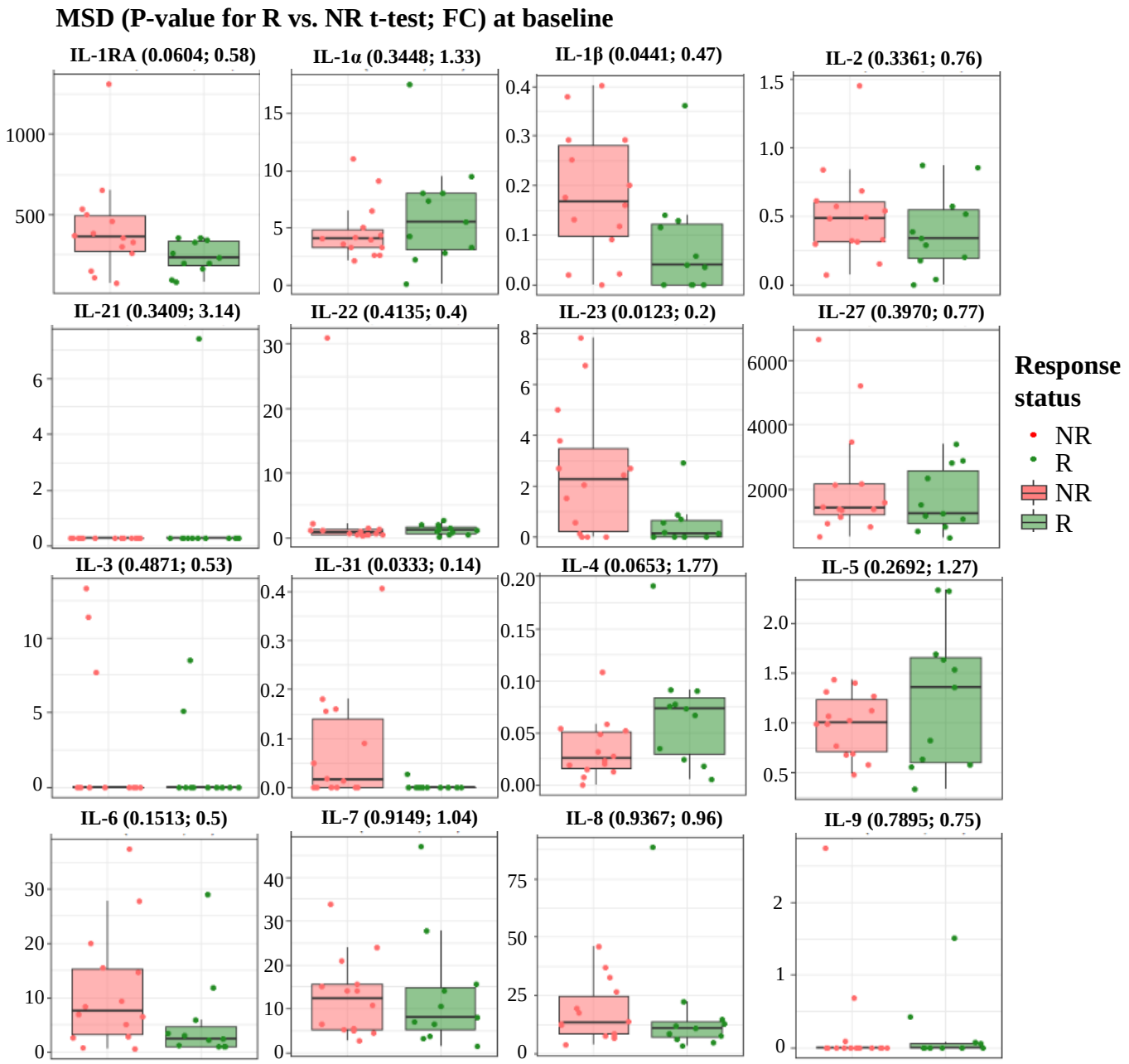


Figure S15-2. Cytokine levels tested by MSD at baseline and compared between responders and nonresponders in a cohort of NSCLC patients receiving anti-PD-1 treatment. All p-values were calculated using two-sided t-tests comparing the R to the NR and were corrected for multiple comparisons using the Benjamini-Hochberg adjustment.

Supplementary data

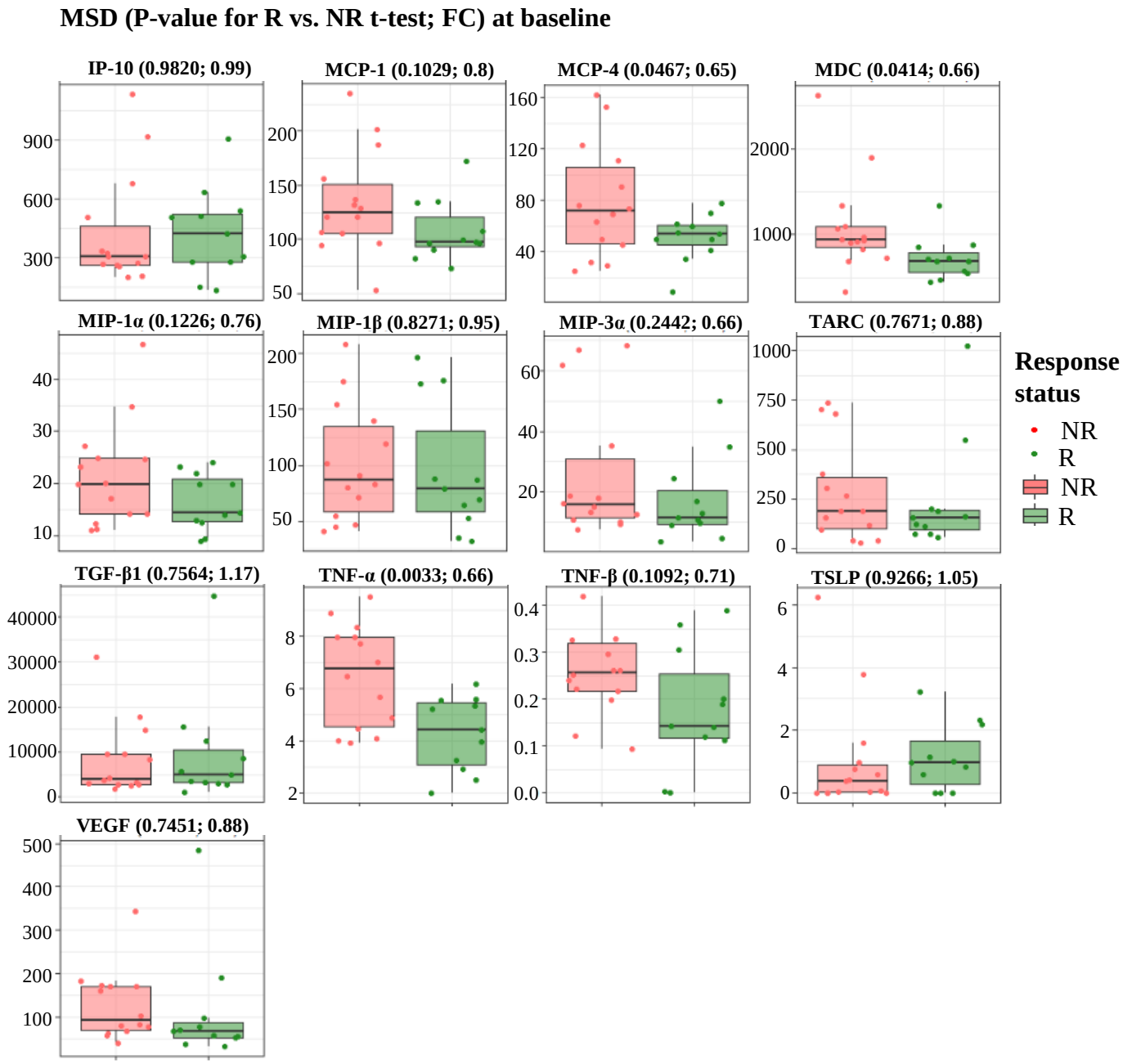


Figure S15-3. Cytokine levels tested by MSD at baseline and compared between responders and nonresponders in a cohort of NSCLC patients receiving anti-PD-1 treatment. All p-values were calculated using two-sided t-tests comparing the R to the NR and were corrected for multiple comparisons using the Benjamini-Hochberg adjustment.

Supplementary data

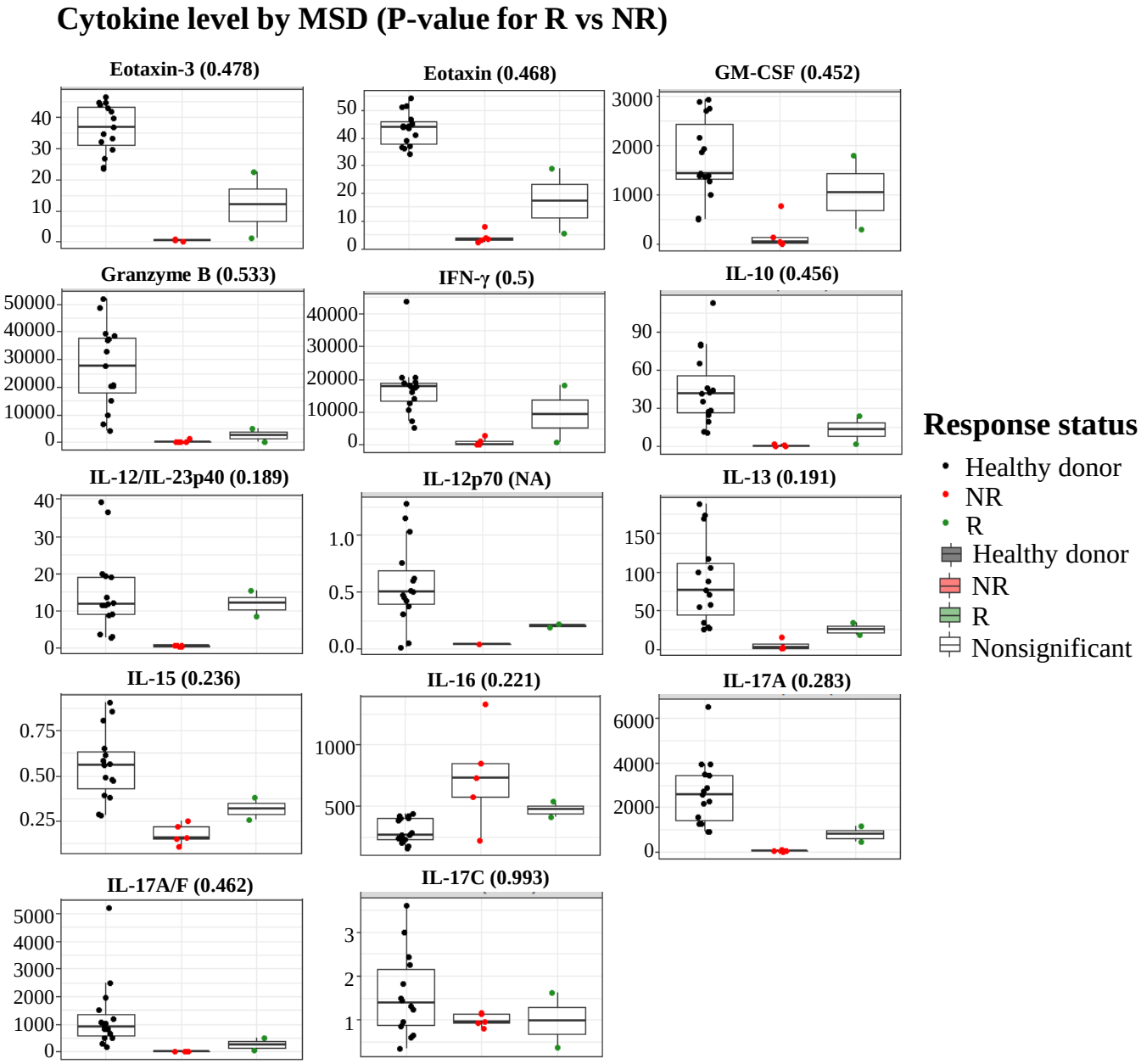


Figure S16-1. Cytokine levels tested by MSD after T cell stimulation and compared among healthy donors and responders and nonresponders in a cohort of NSCLC patients receiving anti-PD-1 treatment. All p-values were calculated using two-sided t-tests comparing patients to the HDs and were corrected for multiple comparisons using the Benjamini-Hochberg adjustment.

Supplementary data

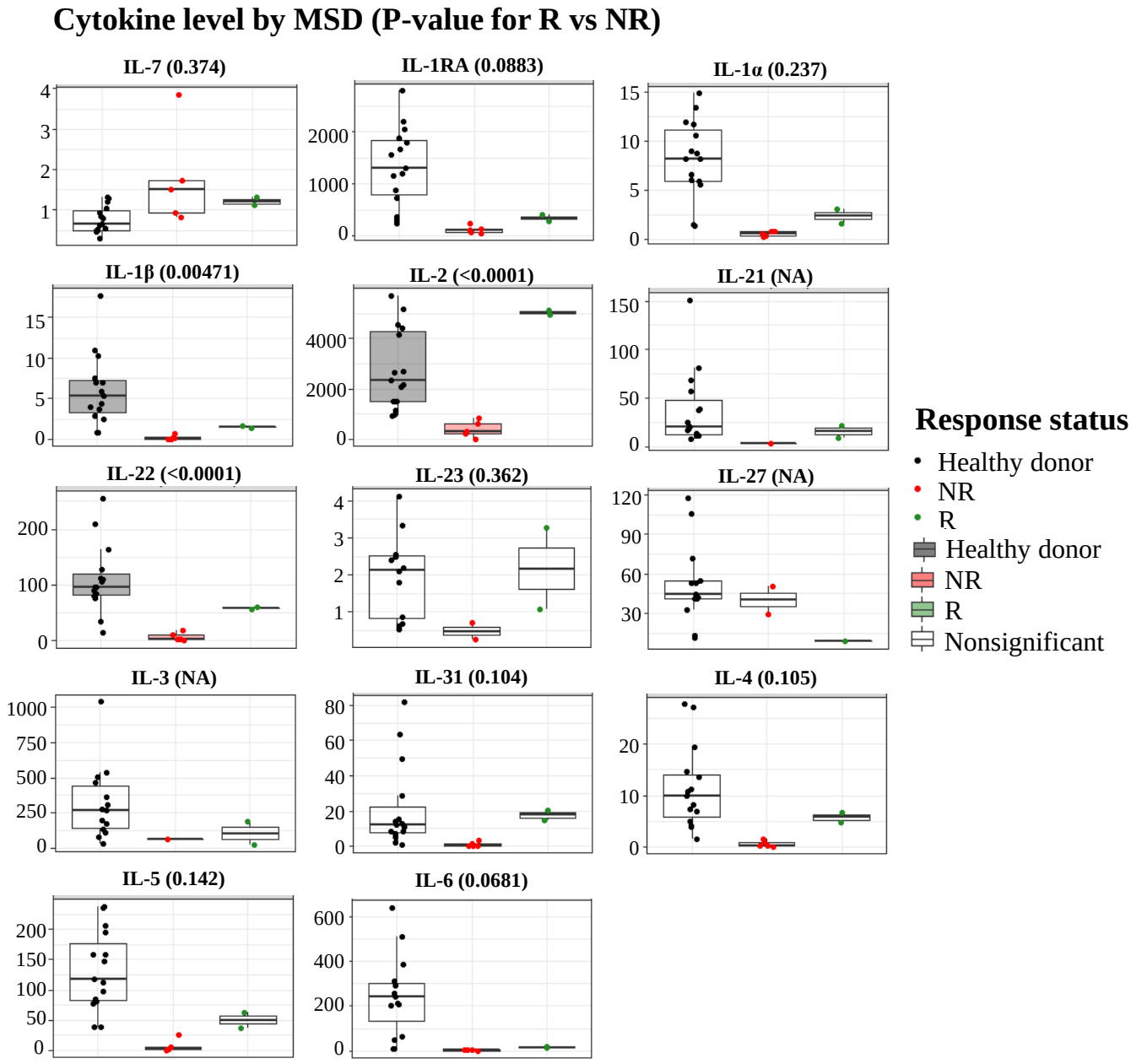


Figure S16-2. Cytokine levels tested by MSD after T cell stimulation and compared among healthy donors and responders and nonresponders in a cohort of NSCLC patients receiving anti-PD-1 treatment. All p-values were calculated using two-sided t-tests comparing patients to the HDs and were corrected for multiple comparisons using the Benjamini-Hochberg adjustment.

Supplementary data

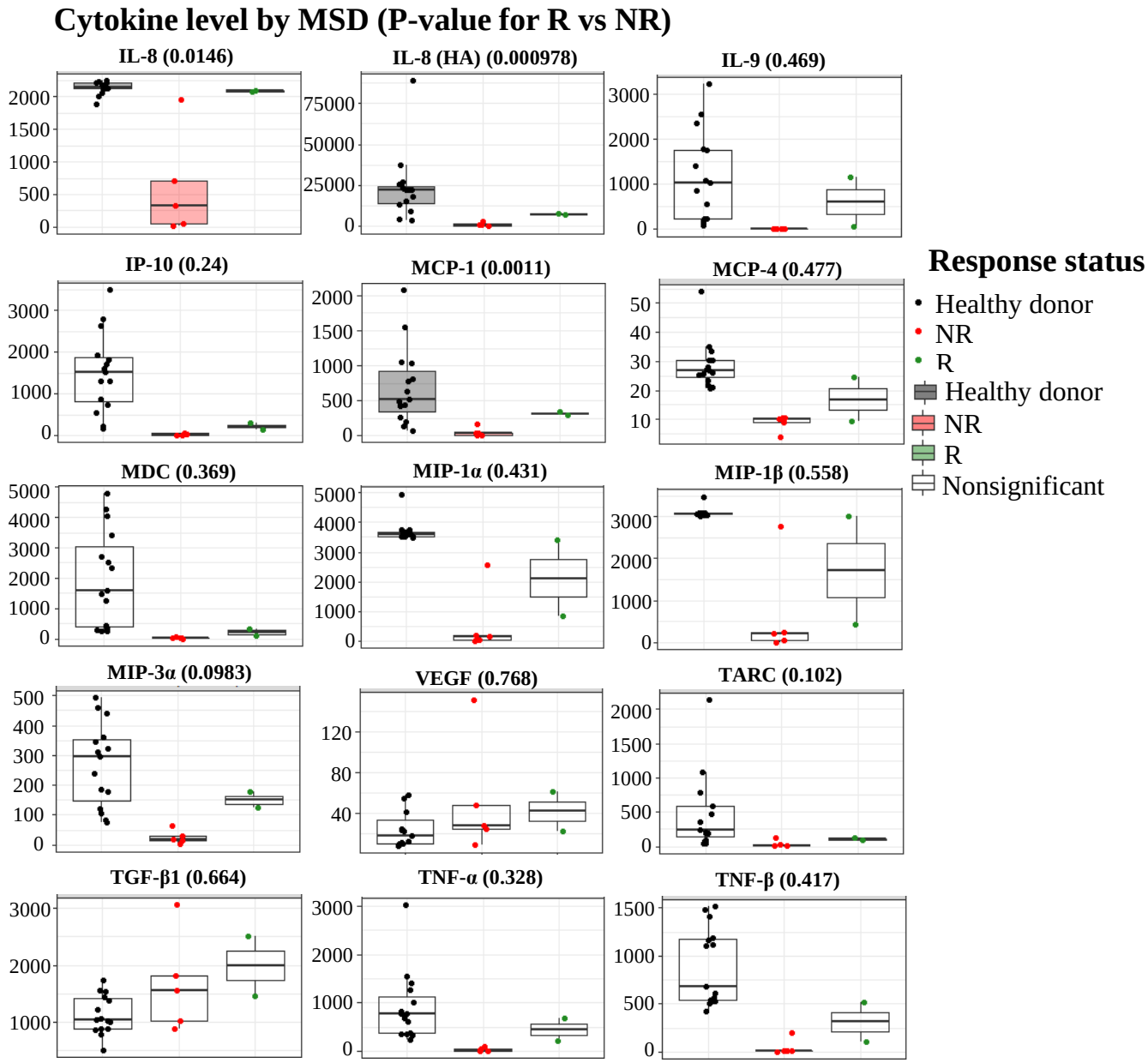


Figure S16-3. Cytokine levels tested by MSD after T cell stimulation and compared among healthy donors and responders and nonresponders in a cohort of NSCLC patients receiving anti-PD-1 treatment. All p-values were calculated using two-sided t-tests comparing patients to the HDs and were corrected for multiple comparisons using the Benjamini-Hochberg adjustment.

Supplementary data-Correlations between cytokines and immune cell types

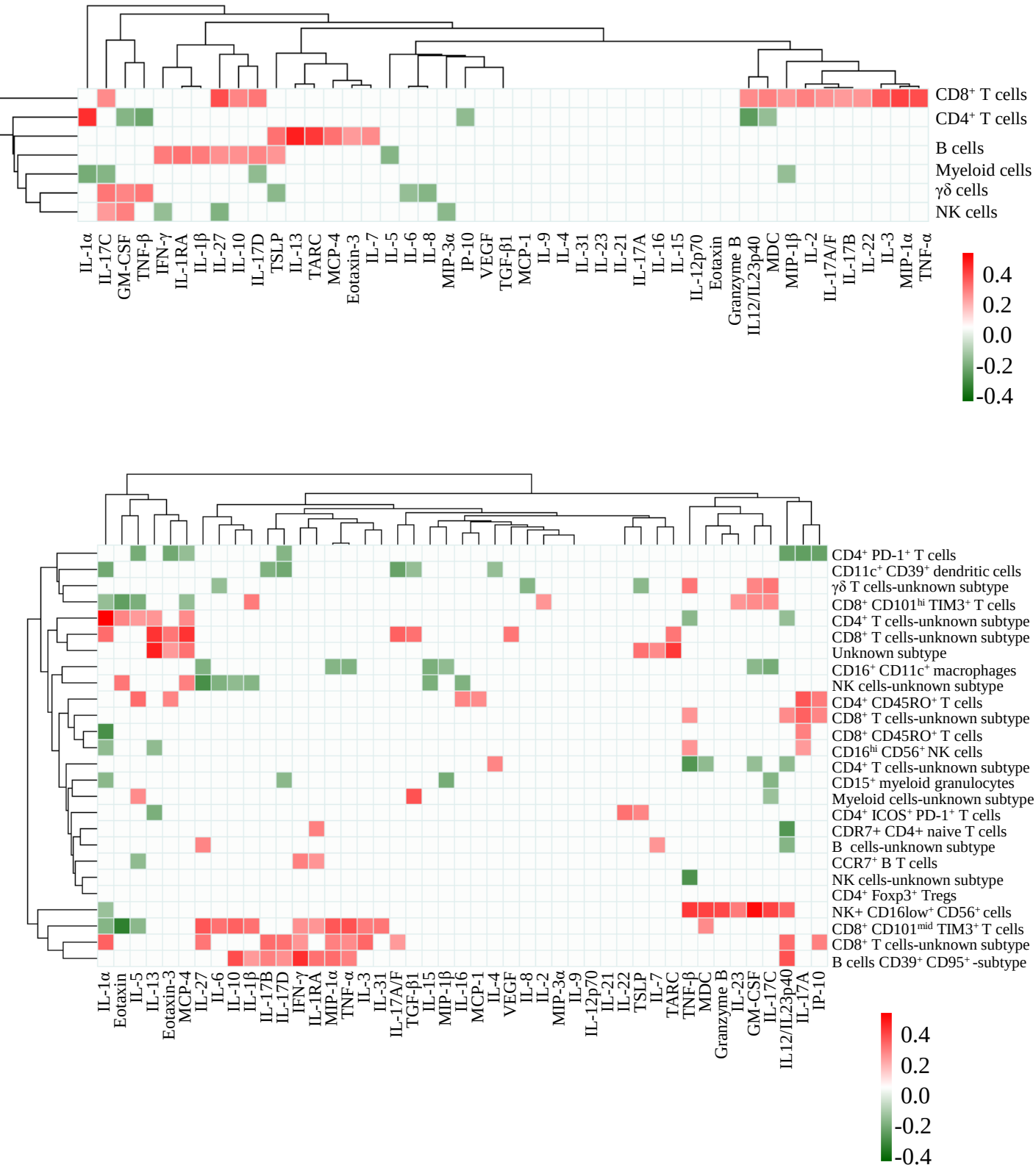


Figure S17. Heatmap of Pearson correlation coefficient (PCC) values, indicating the correlations between different immune cell types and levels of cytokines in patients. A PCC value larger than 0 indicates a positive relationship. A value less than 0 indicates a negative relationship. If the value equals 0, the correlation between the two objects was not statistically significant.

Supplementary data

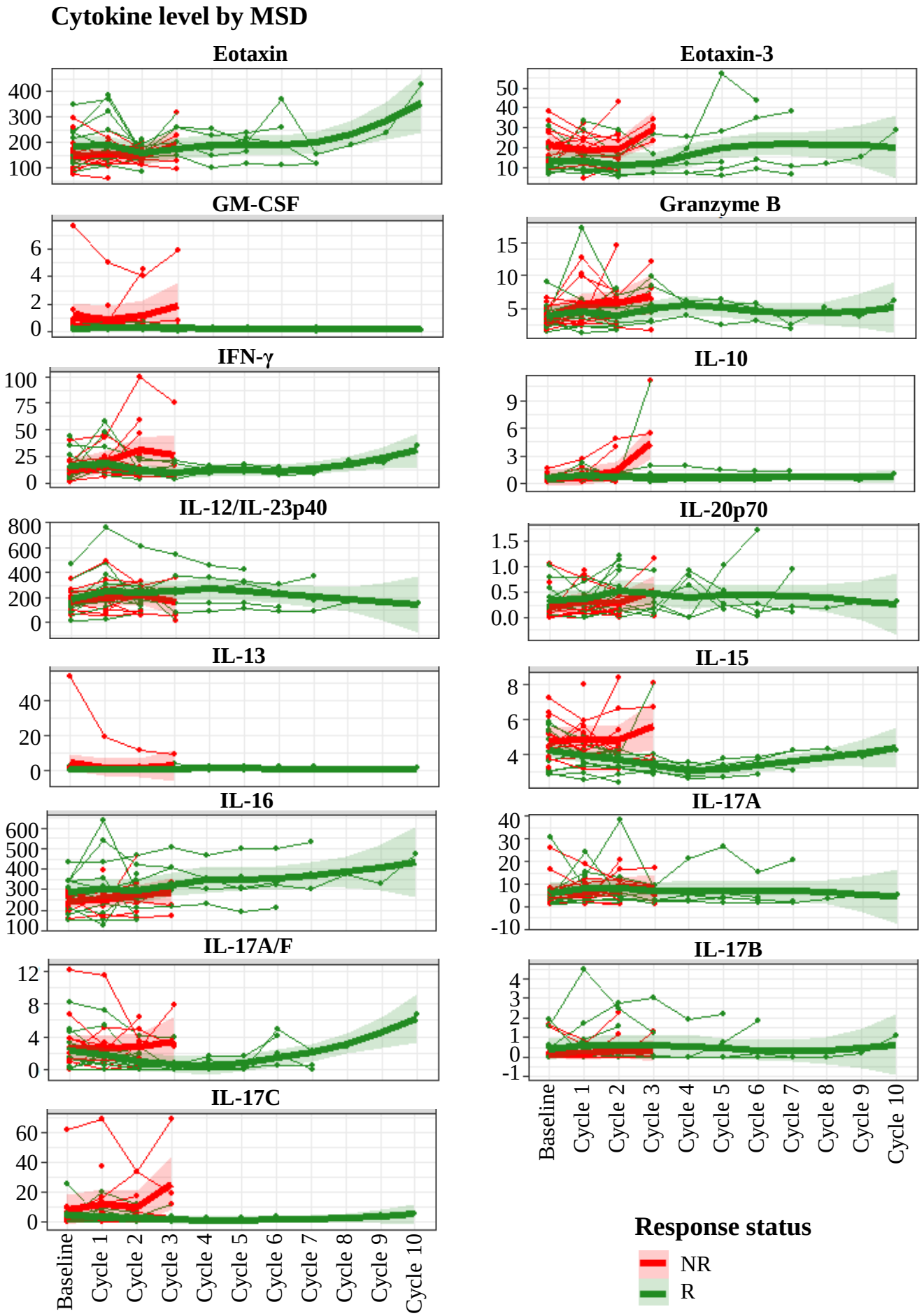


Figure S18-1. Examples of the longitudinal behavior of cytokines under unstimulated conditions compared between responders and nonresponders.

Supplementary data

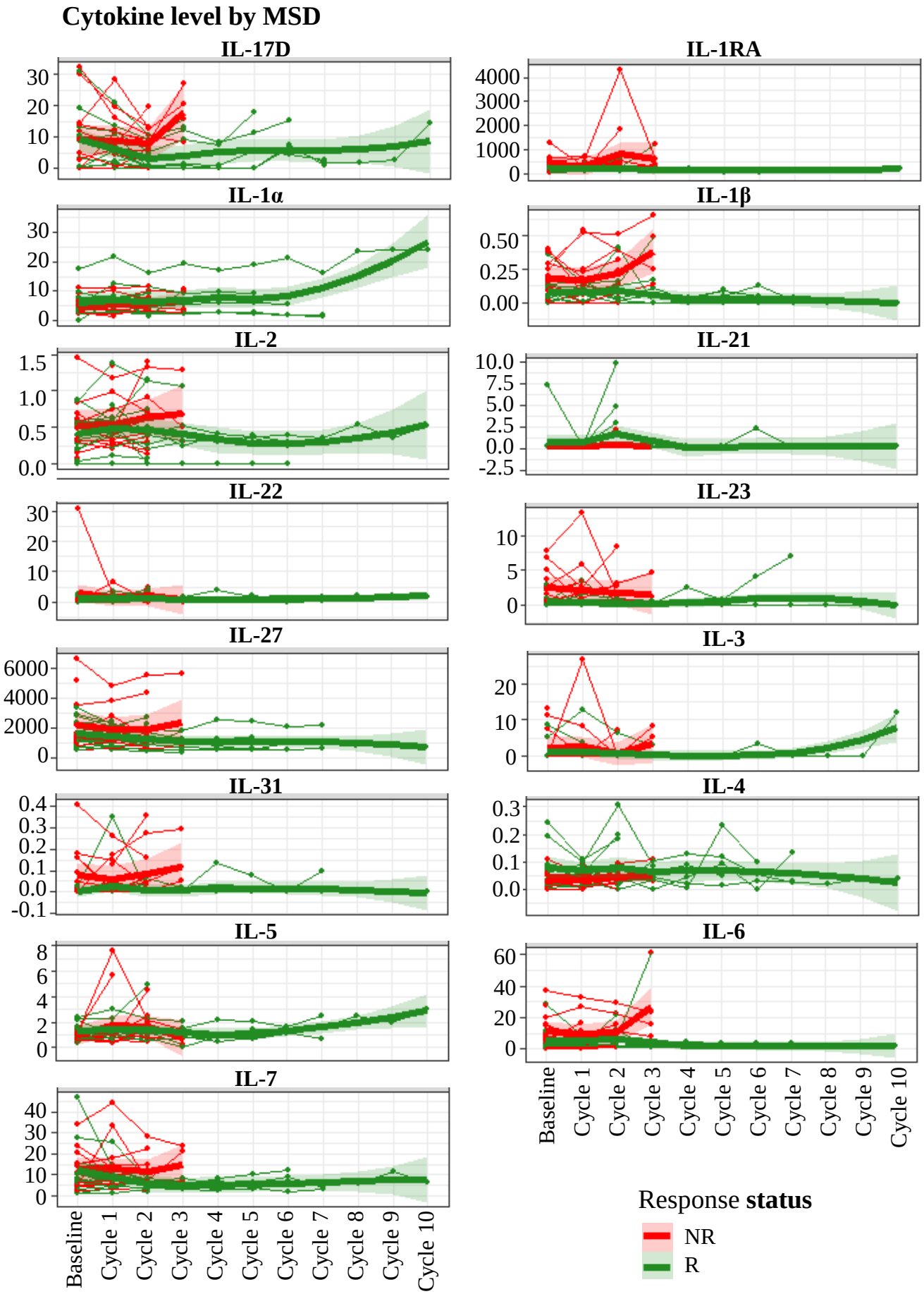


Figure S18-2. Examples of the longitudinal behavior of cytokines under unstimulated conditions compared between responders and nonresponders.

Supplementary data

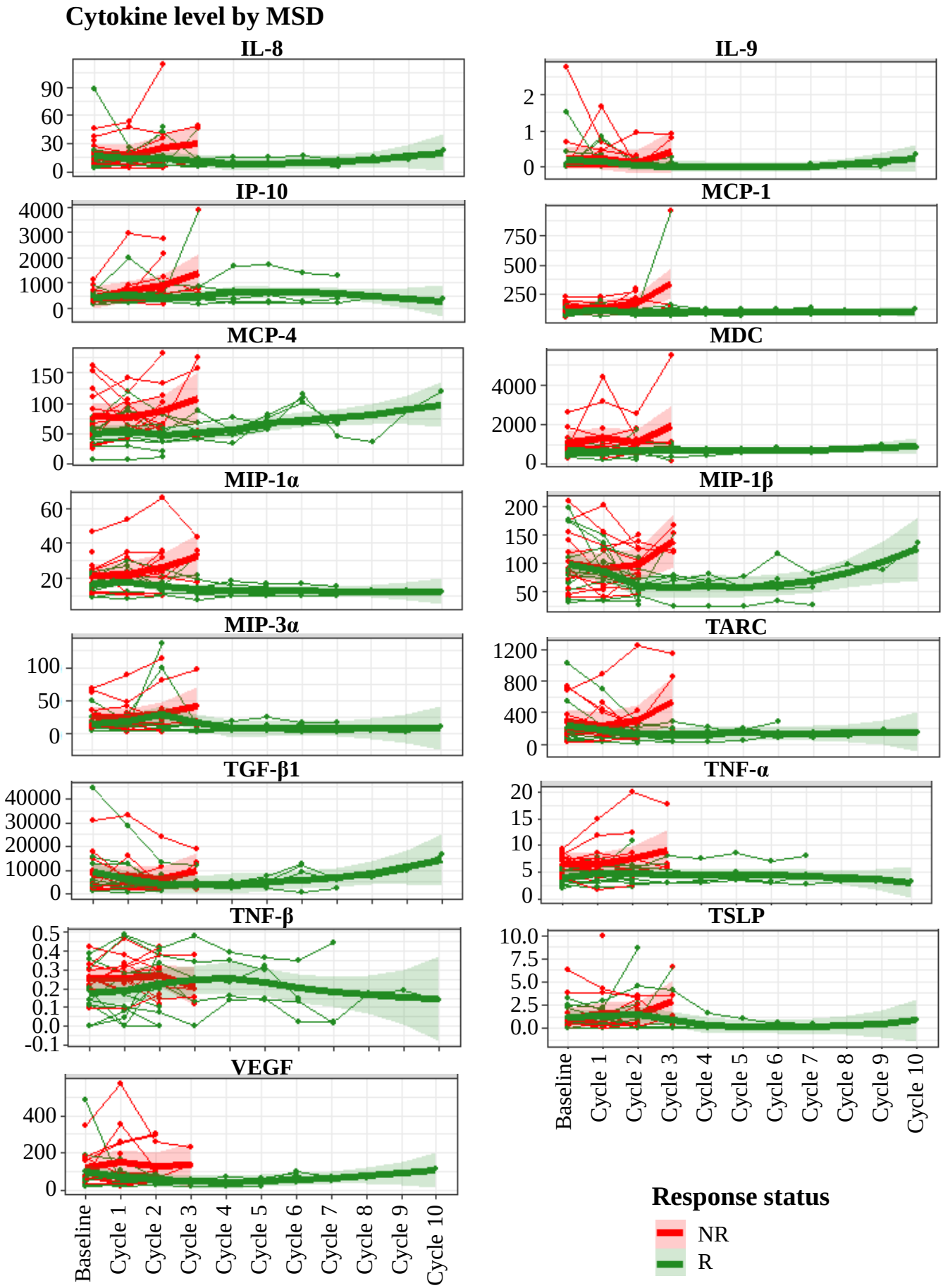


Figure S18-3. Examples of the longitudinal behavior of cytokines under unstimulated conditions compared between responders and nonresponders.

Supplementary data

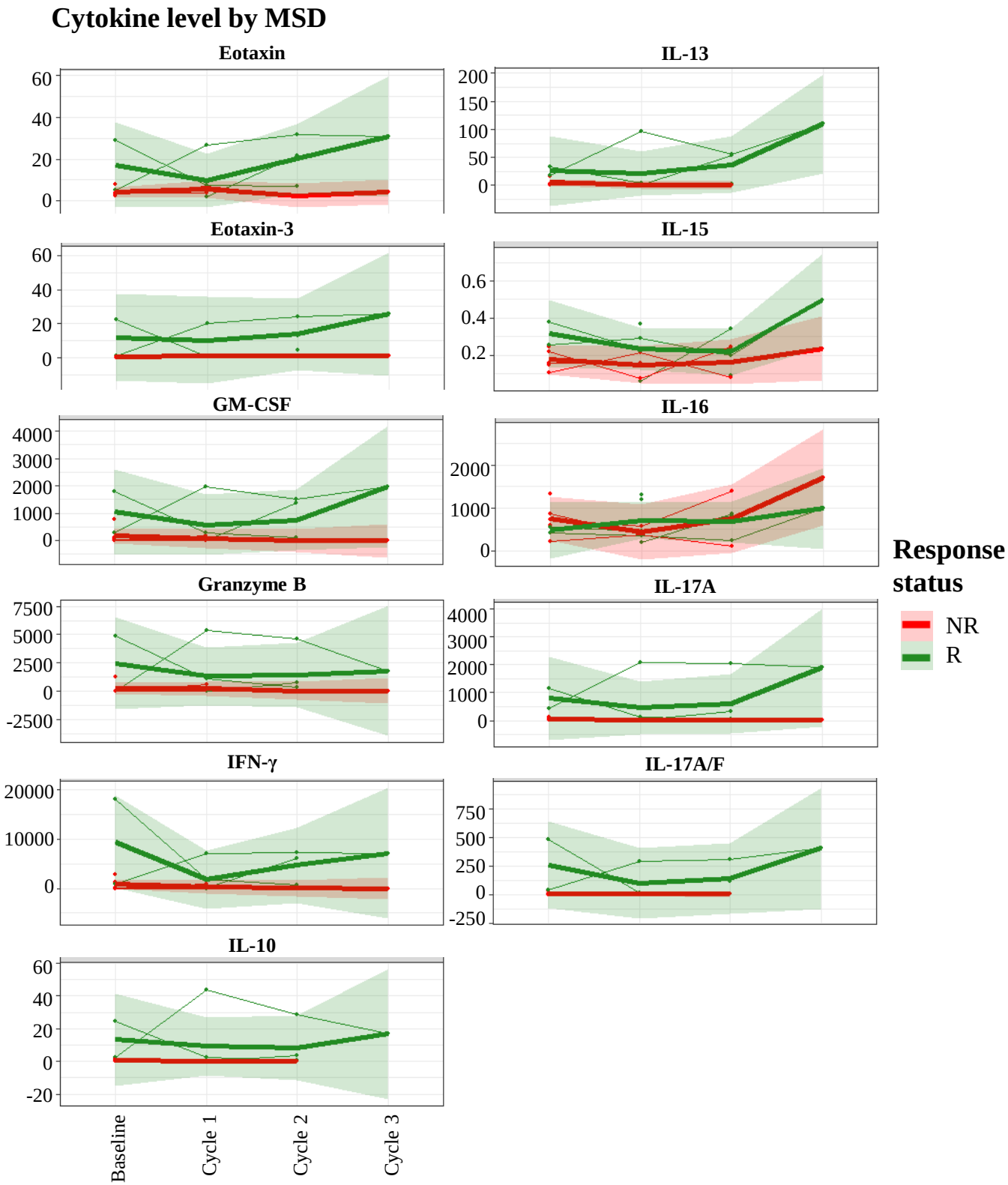


Figure S19-1. Comparison of the longitudinal behavior of cytokines after stimulation of T cells within the PBMC population of responders or nonresponders.

Supplementary data

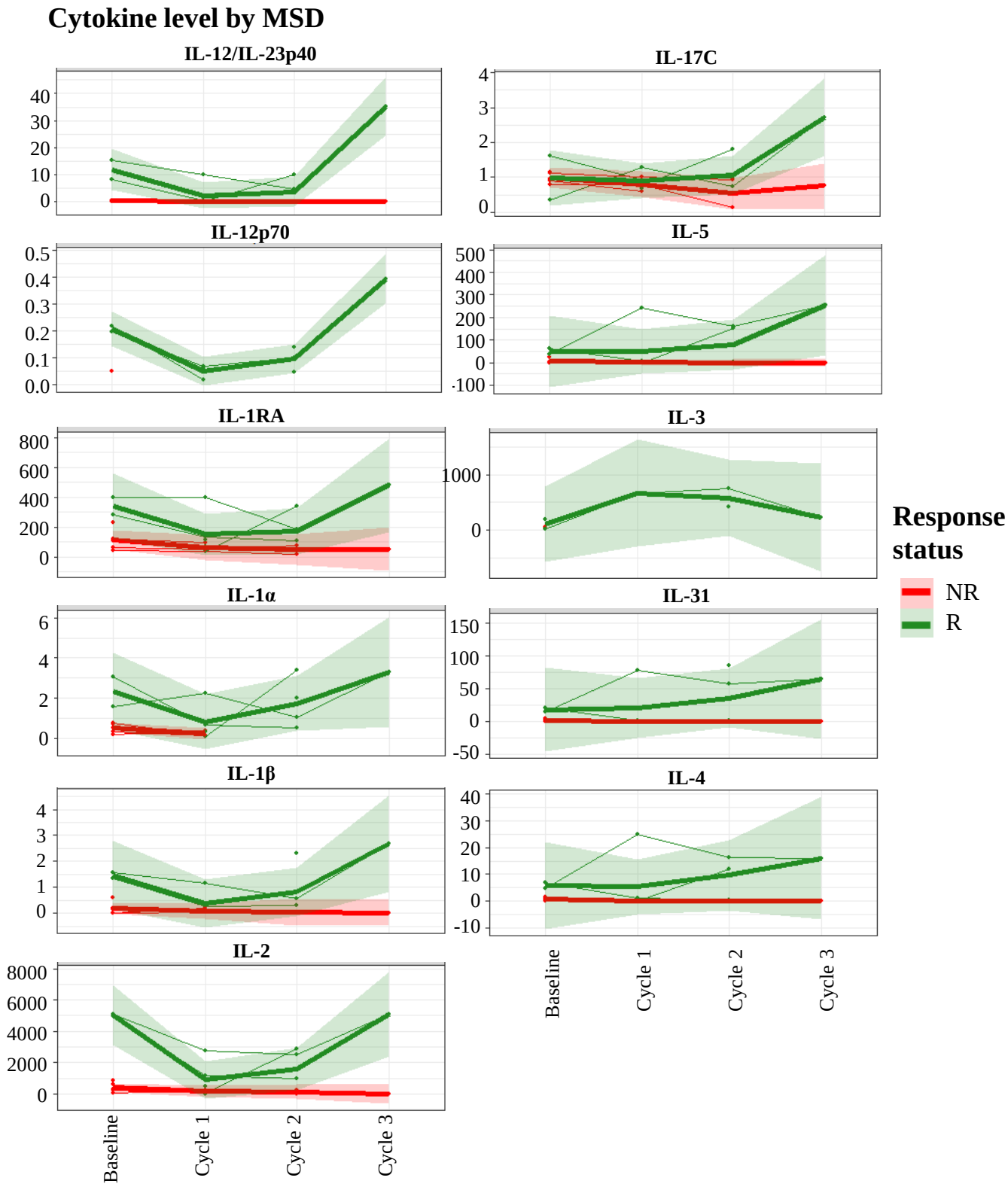


Figure S19-2. Comparison of the longitudinal behavior of cytokines after stimulation of T cells within the PBMC population of responders or nonresponders.

Supplementary data

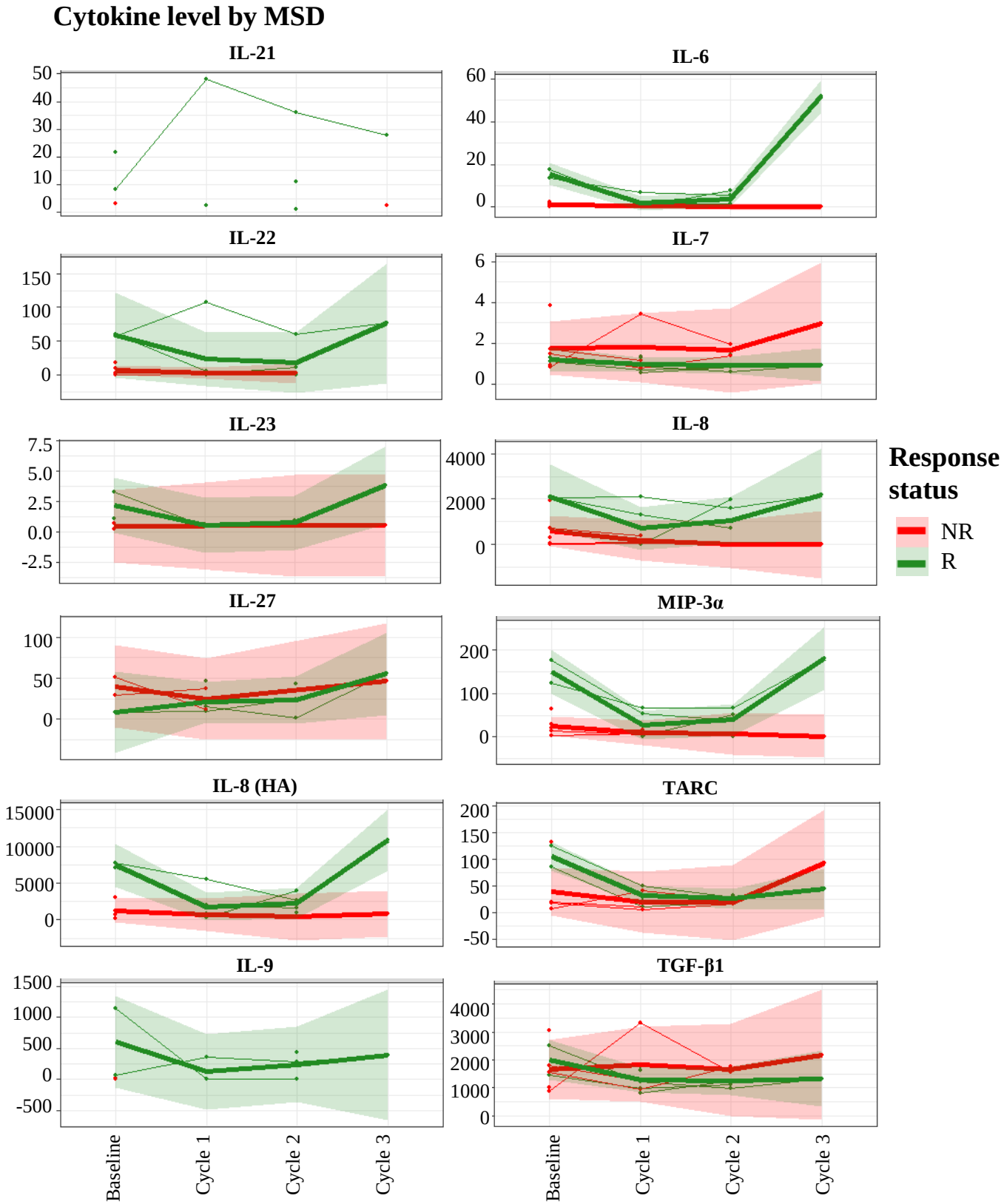


Figure S19-3. Comparison of the longitudinal behavior of cytokines after stimulation of T cells within the PBMC population of responders or nonresponders.

Supplementary data

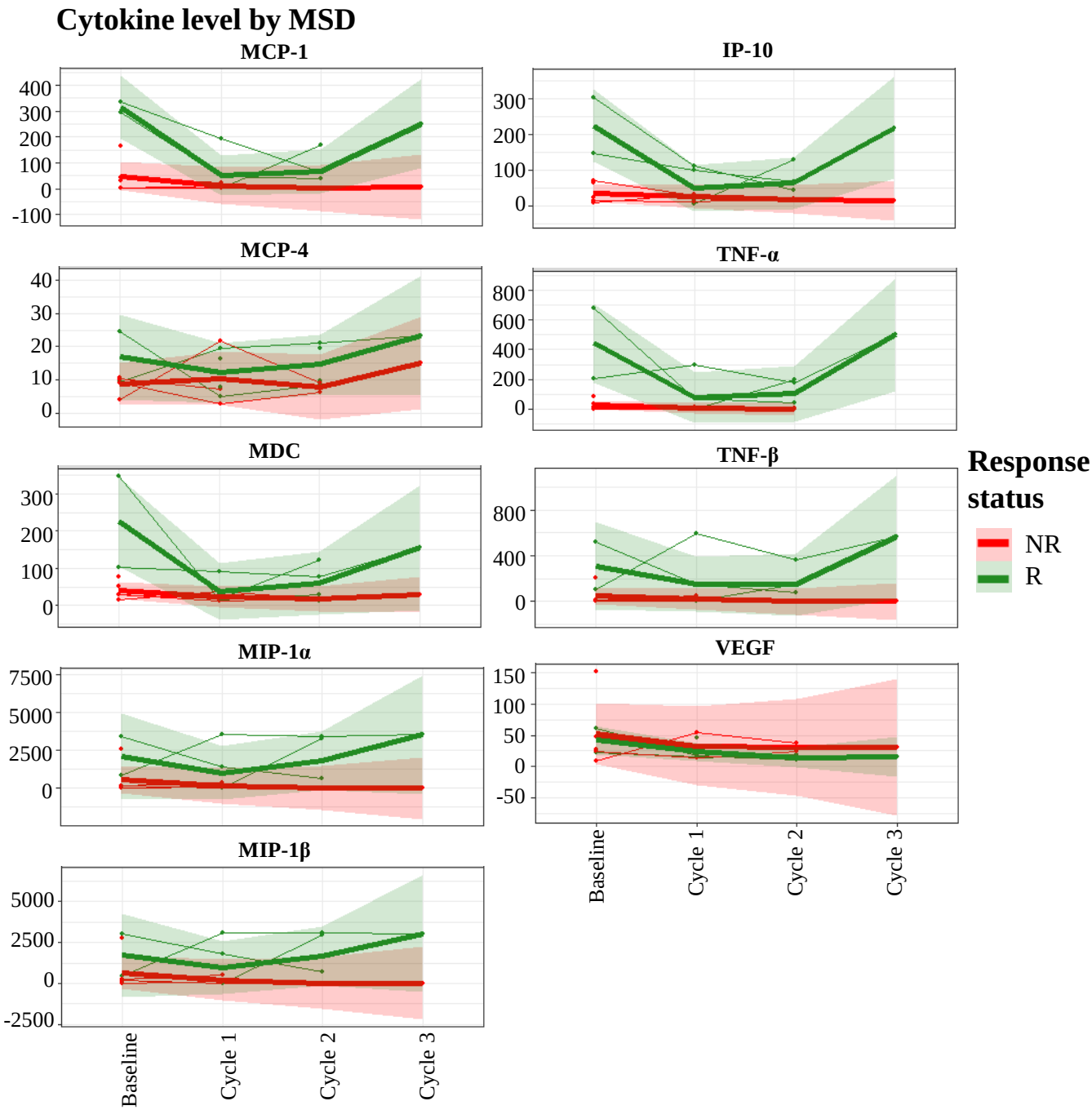


Figure S19-4. Comparison of the longitudinal behavior of cytokines after stimulation of T cells within the PBMC population of responders or nonresponders.

Supplementary data

Characteristic	Patient (N=25)	Responder (N=11)	Non-responder (N=14)
Sex - no. (%)			
Male	19 (76)	10 (91)	9 (64)
Female	6 (24)	1 (9)	5 (36)
Age - mean±sd (min, median, max)	63.5±12.2 (38, 63, 89)	68.4±11.4 (54, 65, 89)	59.6±11.8 (38, 62, 76)
Histology - no. (%)			
Adenocarcinoma	21 (84)	8 (73)	13 (93)
Squamous carcinoma	4 (16)	3 (27)	1 (7)
Tumor Stage – no. (%)			
IIIB	1 (4)	1 (9)	0 (0)
IV	24 (96)	10 (91)	14 (100)
PDL1 IHC level – no. (%)			
>49%	13 (52)	6 (55)	7 (50)
1%~49%	3 (12)	2 (18)	1 (7)
<1%	8 (32)	3 (27)	5 (36)
N/A	1 (4)	0 (0)	1 (7)
Duration of response (in month) - mean±sd (min, median, max)	6.2±4.5 (0, 5, 15)	9.4±3.5 (4, 10, 15)	3.6±3.6 (0, 3, 15)

Table S1. Summary of the clinical evaluation results for all the patients (n=25) after receipt of anti-PD1 treatment.

Supplementary data

Parameters	Immune cell panel	Parameters	Immune cell panel
1	CD3	21	CD56
2	CD4	22	CCR7
3	TCRγδ	23	CD69
4	CD8a	24	T-bet
5	CD11b	25	CD95
6	CD11c	26	CD101
7	CD14	27	CD127
8	CD15	28	XCR5
9	CD16	29	CTLA-4
10	CD19	30	Eomes
11	CXCR3	31	Foxp3
12	KLRG-1	32	Gata-3
13	CD27	33	HLA-DR
14	CD25	34	ICOS
15	CD28	35	Ki-67
16	CD33	36	LAG3
17	CD38	37	PD-1
18	CD39	38	PD-L1
19	CD45	39	Granzyme B
20	CD45RO	40	TIM-3

Table S2. Composition of the 40-target panel for CyTOF tests.