Figure S1

Gating strategy for H3.3K27M-reactive CD8+ T-cells. A representative gating strategy to identify CD8+ T-cells from patient-derived PBMCs that exhibit reactivity for K27M on CyTOF above background levels. Cells staining double-positive with iridium (Ir) intercalator in channels 191 and 193 were identified as intact cells (*left*). Cells with low cisplatin staining were identified as live cells (*second to left*). Cells staining positive for CD45 and CD3 were selected as T-cells (*middle*). Cells staining positive for CD8+ were identified as CD8+ T-cells (*second to right*). Cells staining above background levels with H3.3K27M dextramer were identified as H3.3K27M-reactive CD8+ T-cells (*right*). Healthy donor (**A**) and H3.3K27M-specific TCR-transduced CD8+ T-cells (**B**) were used to validate the specificity and sensitivity of the H3.3K27M dextramer respectively. CD8+ T-cells derived from a representative patient (**C**) exhibiting reactivity for H3.3K27M above background were classified as H3.3K27M-reactive CD8+ T-cells.

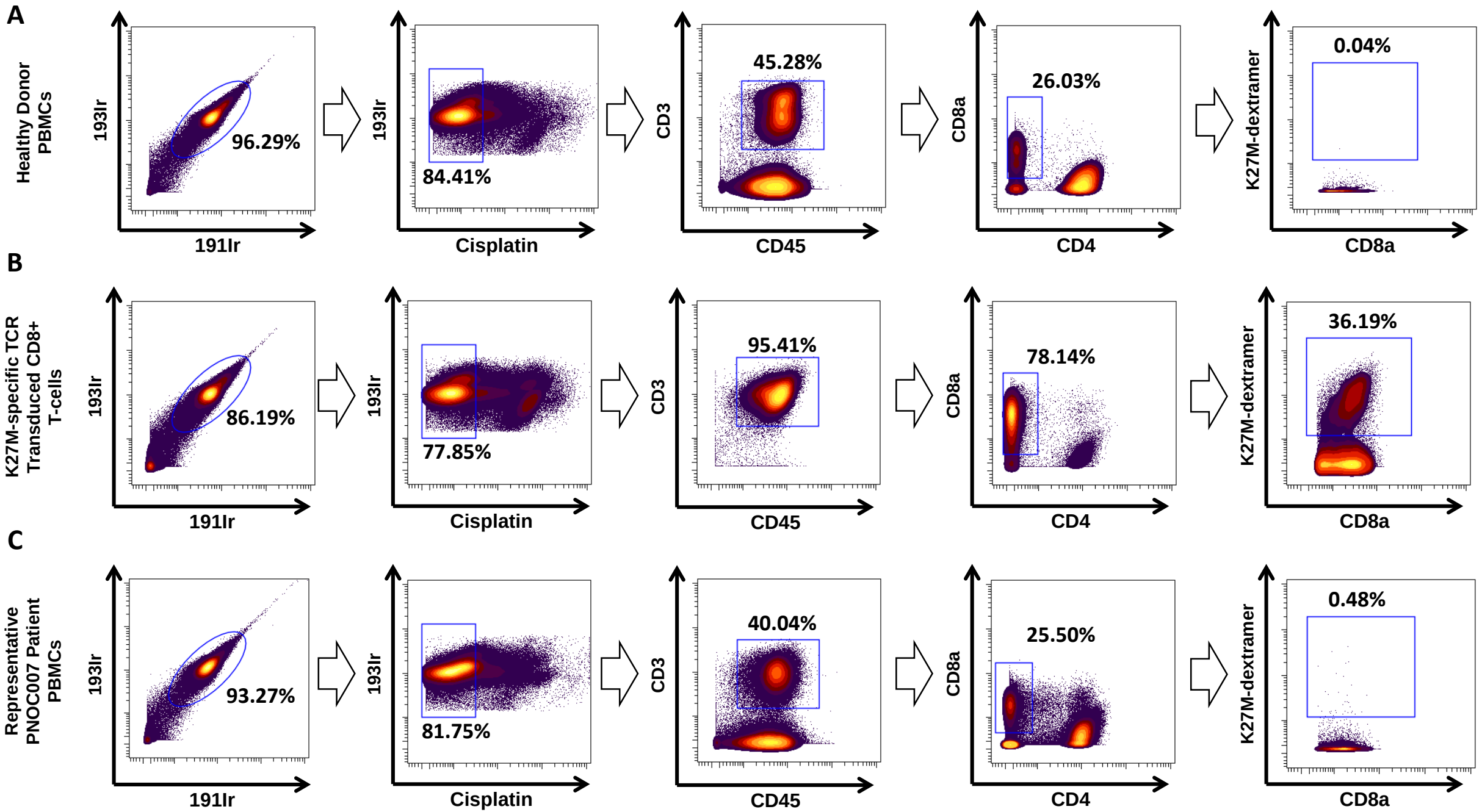

A
Healthy Donor PBMCs
193Ir
191Ir
96.29%
193Ir
Cisplatin
84.41%
CD3
CD45
45.28%
CD8a
CD4
26.03%
K27M-dextramer
CD8a
0.04%
B
K27M-specific TCR Transduced CD8+ T-cells
193Ir
191Ir
86.19%
193Ir
Cisplatin
77.85%
CD3
CD45
95.41%
CD8a
CD4
78.14%
K27M-dextramer
CD8a
36.19%
C
Representative PNOC007 Patient PBMCs
193Ir
191Ir
93.27%
193Ir
Cisplatin
81.75%
CD3
CD45
40.04%
CD8a
CD4
25.50%
K27M-dextramer
CD8a
0.48%

Figure S2

Mass cytometry-based detection of H3.3K27M-reactive CD8+ T-cell is as sensitive as conventional flow cytometry using H3.3K27M dextramer. CD8+ T-cells were detected from the same patient on both flow cytometry (*above*) and mass cytometry (*below*), exhibiting comparable percentages of overall CD8+ T-cells that were identified as H3.3K27M-reactive.

PNOC007-12 CD8+ T-cells

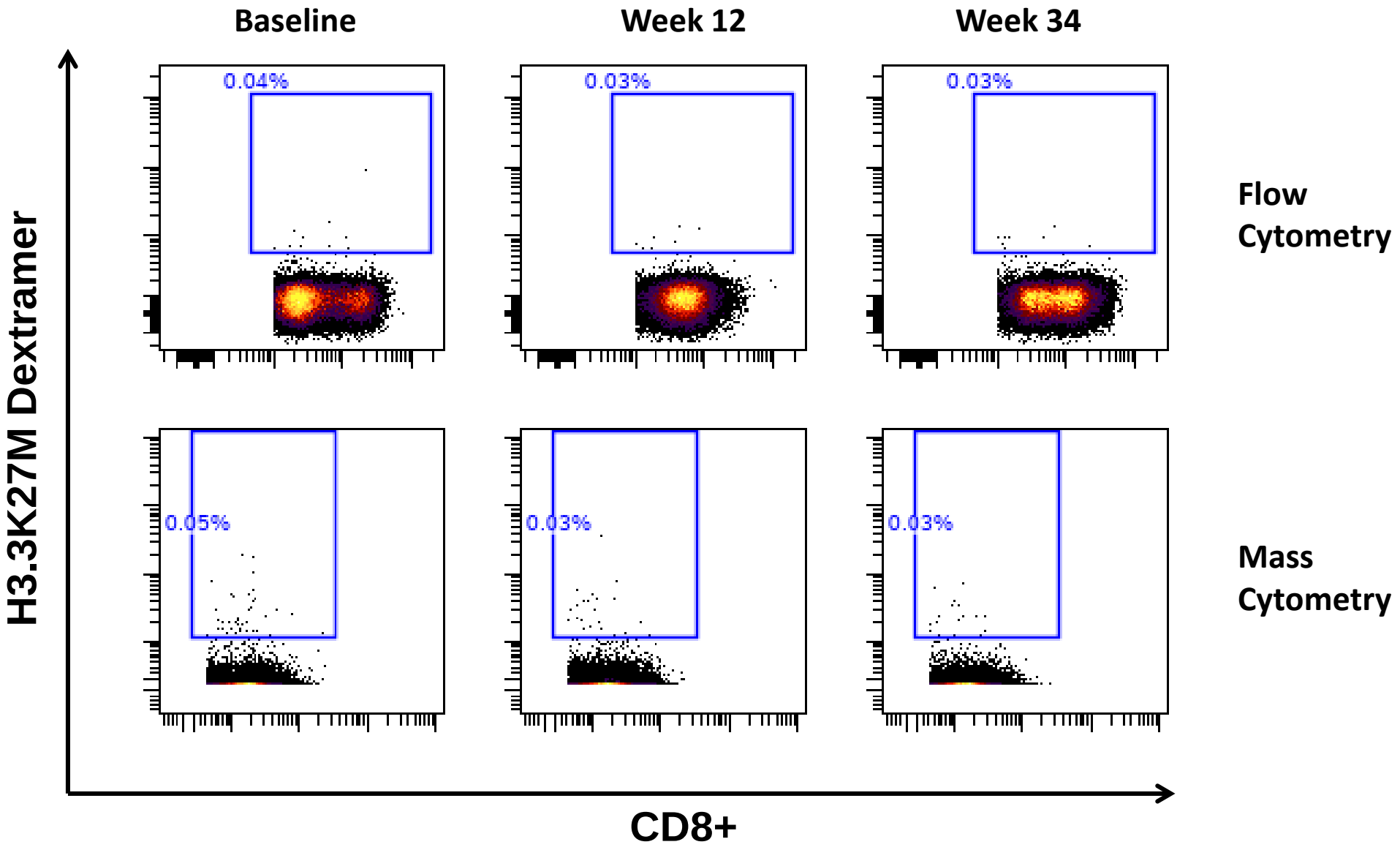
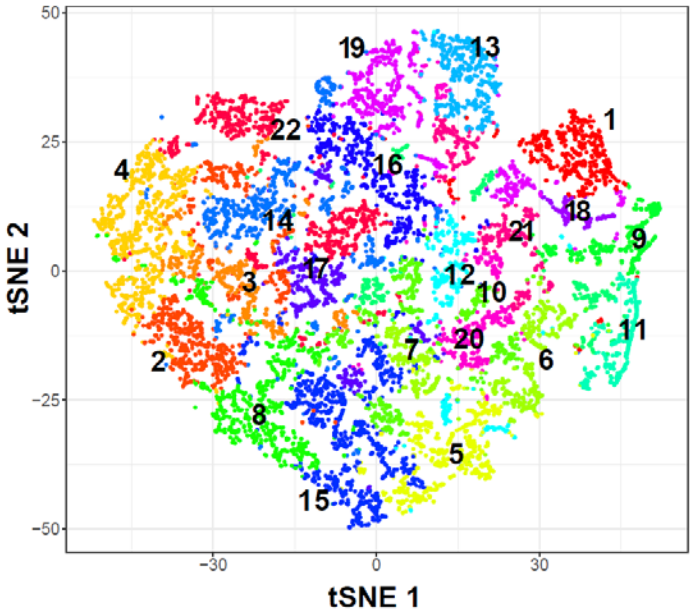
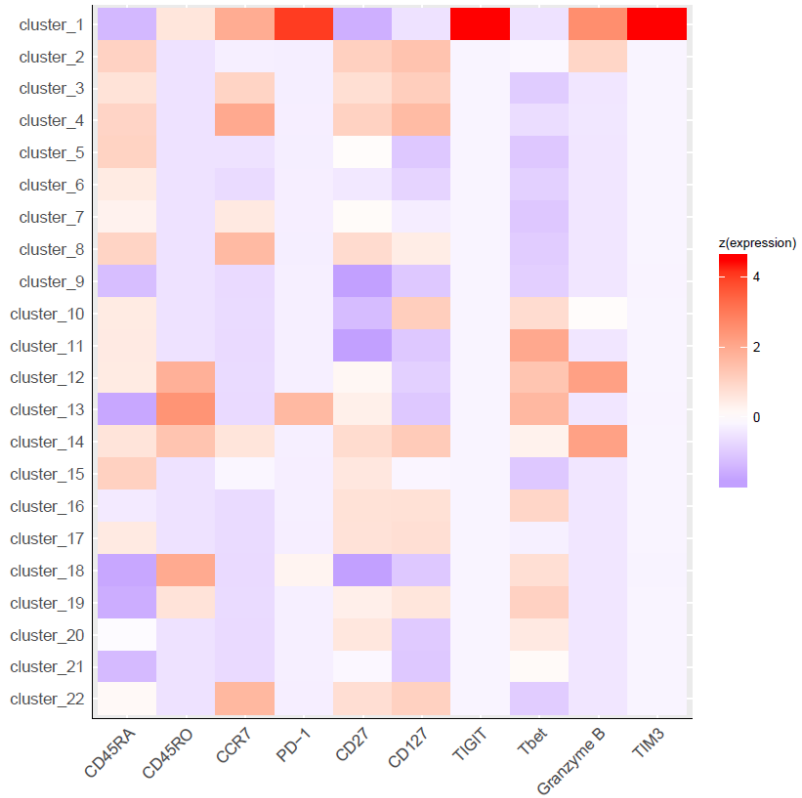


Figure S3

tSNE stratification and expression profiles of discrete H3.3K27M-reactive CD8+ T-cell subpopulations. *Top-left*, H3.3K27M-reactive CD8+ T-cells were stratified on a tSNE plot using the CD8+ T-cell canonical markers: CD45RA, CD45RO, CCR7, CD27, and CD127. *Bottom-left*, heat map visualizing the z-score normalized expression of resultant H3.3K27M-reactive CD8+ T-cell clusters. *Right,* CyTOF-based marker expression profiles and phenotypic characterizations of H3.3K27M-reactive CD8+ T-cell clusters identified via tSNE stratification.





Cluster Number	Phenotype
1	CD45RO^{+} CD27^{-} CD127^{-} TIM3^{+} PD-1^{+} Tbet^{-}
2	CD45RA^{+} CCR7^{lo} CD27^{+} CD127^{+} CD29^{-} Tbet^{-}
3	CD45RA^{+} CCR7^{+} CD27^{+} CD127^{+} CD29^{-} Tbet^{-}
4	CD45RA^{+} CCR7^{+} CD27^{+} CD127^{+} CD29^{-} Tbet^{-}
5	CD45RA^{+} CCR7^{-} CD27^{mid} CD127^{-} CD29^{-} Tbet^{-}
6	CD45RA^{+} CCR7^{-} CD27^{-} CD127^{-} CD29^{-} Tbet^{-}
7	CD45RA^{+} CCR7^{hi} CD27^{mid} CD127^{-} CD29 Tbet^{-}
8	CD45RA^{+} CCR7^{++} CD27^{+} CD127^{+} CD29^{-} Tbet^{-}
9	All markers low
10	CD45RA^{+} CCR7^{-} CD27^{-} CD127^{+} CD29^{hi} Tbet^{+} CD49d^{+}
11	CD45RA^{+} CCR7^{-} CD27^{-} CD127^{-} CD29^{hi} Tbet^{+} CD49d^{-}
12	CD45RA^{+} CD45RO^{+} CCR7^{mid} CD27^{hi} CD127^{+} CD29^{+} Tbet^{+} CD49d^{mid}
13	CD45RO^{+} CCR7^{-} CD27^{hi} CD127^{-} CD29^{+} Tbet^{+}
14	CD45RA^{+} CD45RO^{+} CCR7^{+} CD27^{+} CD127^{+} CD29^{+} Tbet^{hi} CD49d^{+}
15	CD45RA^{+} CCR7^{mid} CD27^{+} CD127^{-} CD29^{-} Tbet^{-}
16	CD45RA^{+} CCR7^{-} CD27^{+} CD127^{+} CD29^{hi} Tbet^{+}
17	CD45RA^{+} CCR7^{-} CD27^{+} CD127^{+} CD29^{-} Tbet^{-}
18	CD45RO^{+} CCR7^{-} CD27^{-} CD127^{-} CD29^{+} Tbet^{+}
19	CD45RO^{+} CCR7^{-} CD27^{hi} CD127^{+} CD29^{+} Tbet^{+} CD49d^{mid}
20	CD45RA^{+} CCR7^{-} CD27^{+} CD127^{-} CD29^{mid} Tbet^{hi}
21	Almost all markers low
22	CD45RA^{+} CCR7^{+} CD27^{+} CD127^{+} CD29^{-} Tbet^{-}

Color Key
Early Activated
Stem Cell Memory
Effector
Effector Memory
Exhausted

Figure S4

Spider plot depicting longitudinal patient-specific H3.3K27M-reactive CD8+ T-cell frequencies of both Strata A+B patients and Stratum A patients alone

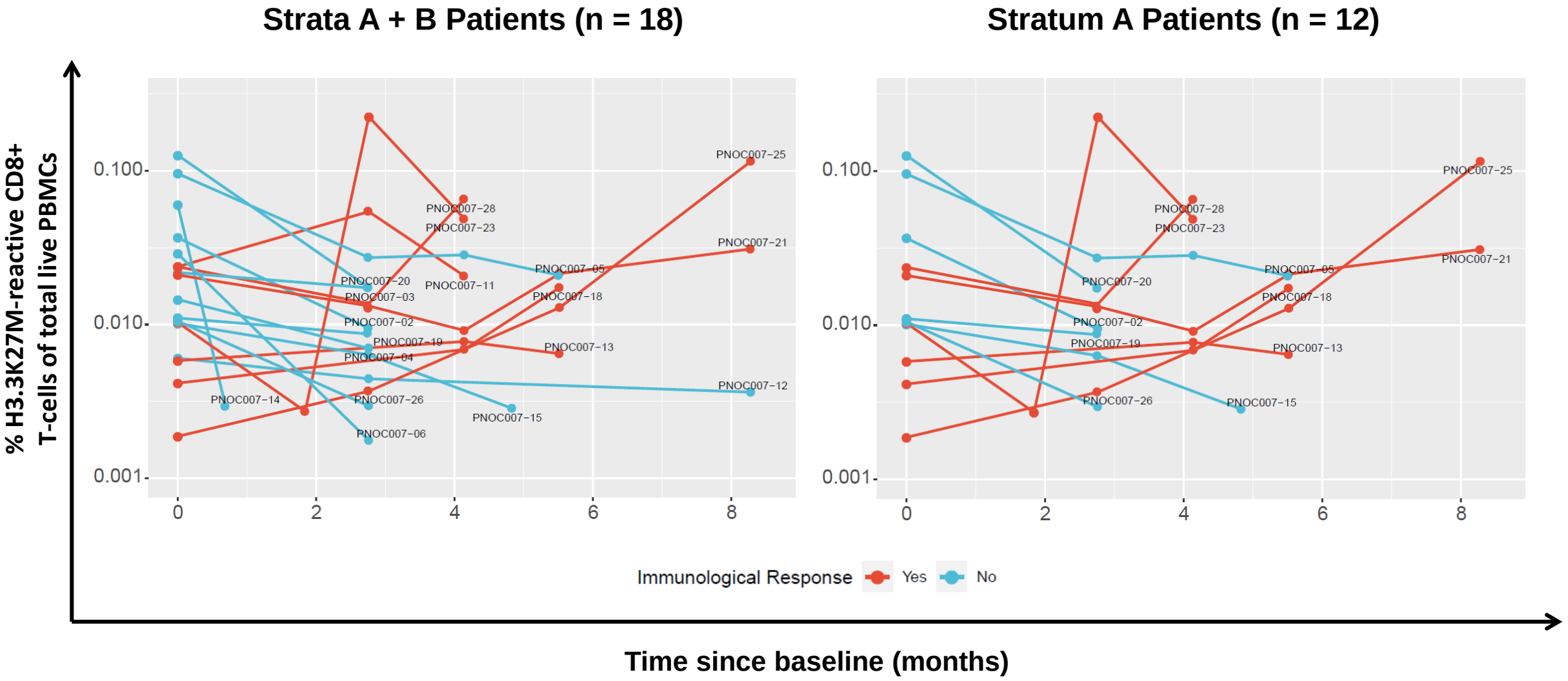

Strata A + B Patients (n = 18)
Stratum A Patients (n = 12)
% H3.3K27M-reactive CD8+
T-cells of total live PBMCs
0.100
0.010
0.001
0
2
4
6
8
PNOC007-25
PNOC007-28
PNOC007-23
PNOC007-21
PNOC007-05
PNOC007-20
PNOC007-11
PNOC007-03
PNOC007-18
PNOC007-02
PNOC007-19
PNOC007-13
PNOC007-04
PNOC007-12
PNOC007-14
PNOC007-26
PNOC007-15
PNOC007-06
Immunological Response
Yes
No
Time since baseline (months)

Table S1

Table depicting multivariate cox proportional hazards analyses using overall survival for both Strata A+B and Stratum A patients alone for non-confounding immune profiles

Strata A+B Patients

Table S1: Multivariate Analysis of Immune Profiles

Parameter	Condition	Hazard Ratio* (95% CI)	P-value	Confounding (P-value via Fisher's Exact Test)			
				Immunological Response	Bulk CD8+ T-cell Expansion	Baseline MDSC Abundance	H3.3K27M-reactive T_{EX} Expansion
Immunological Response	No (n=11)	Reference					
	Yes (n=7)	0.04 (0.004 – 0.045)	p < 0.01**		p = 0.33	p = 0.34	p = 0.32
Bulk CD8+ T-cell Expansion	No (n=13)	Reference					
	Yes (n=5)	4.00 (0.67 – 23.88)	p = 0.13			p = 1.00	p = 0.61
Baseline MDSC Abundance	High (n=14)	Reference					
	Low (n=14)	0.34 (0.08 – 1.51)	p = 0.15				p = 0.62
H3.3K27M-reactive T_{EX} Expansion	No (n=6)	Reference					
	Yes (n=12)	3.75 (0.85 – 16.54)	p = 0.08				

Stratum A Patients

Parameter	Condition	Hazard Ratio (95% CI)	P-value	Confounding (P-value via Fisher's Exact Test)			
				Immunological Response	Bulk CD8+ T-cell Expansion	Baseline MDSC Abundance	H3.3K27M-reactive T_{EX} Expansion
Immunological Response	No (n=6)	Reference					
	Yes (n=6)	0.03 (0.001 – 1.960)	p = 0.10		p = 0.55	p = 1.00	p = 0.55
Bulk CD8+ T-cell Expansion	No (n=8)	Reference					
	Yes (n=4)	15.21 (0.33 – 668.70)	p = 0.16			p = 0.58	p = 1.00
Baseline MDSC Abundance	High (n=10)	Reference					
	Low (n=9)	0.09 (0.01 – 0.92)	p = 0.04*				p = 0.58
H3.3K27M-reactive T_{EX} Expansion	No (n=4)	Reference					
	Yes (n=8)	2.12 (0.30 – 14.92)	p = 0.45				

*Hazard ratios derived from overall survival

Figure S5

Kaplan-Meier survival curves visualizing OS among strata A + B patients based on the expansion of H3.3K27M-reactive CD8+ T-cell subpopulations.

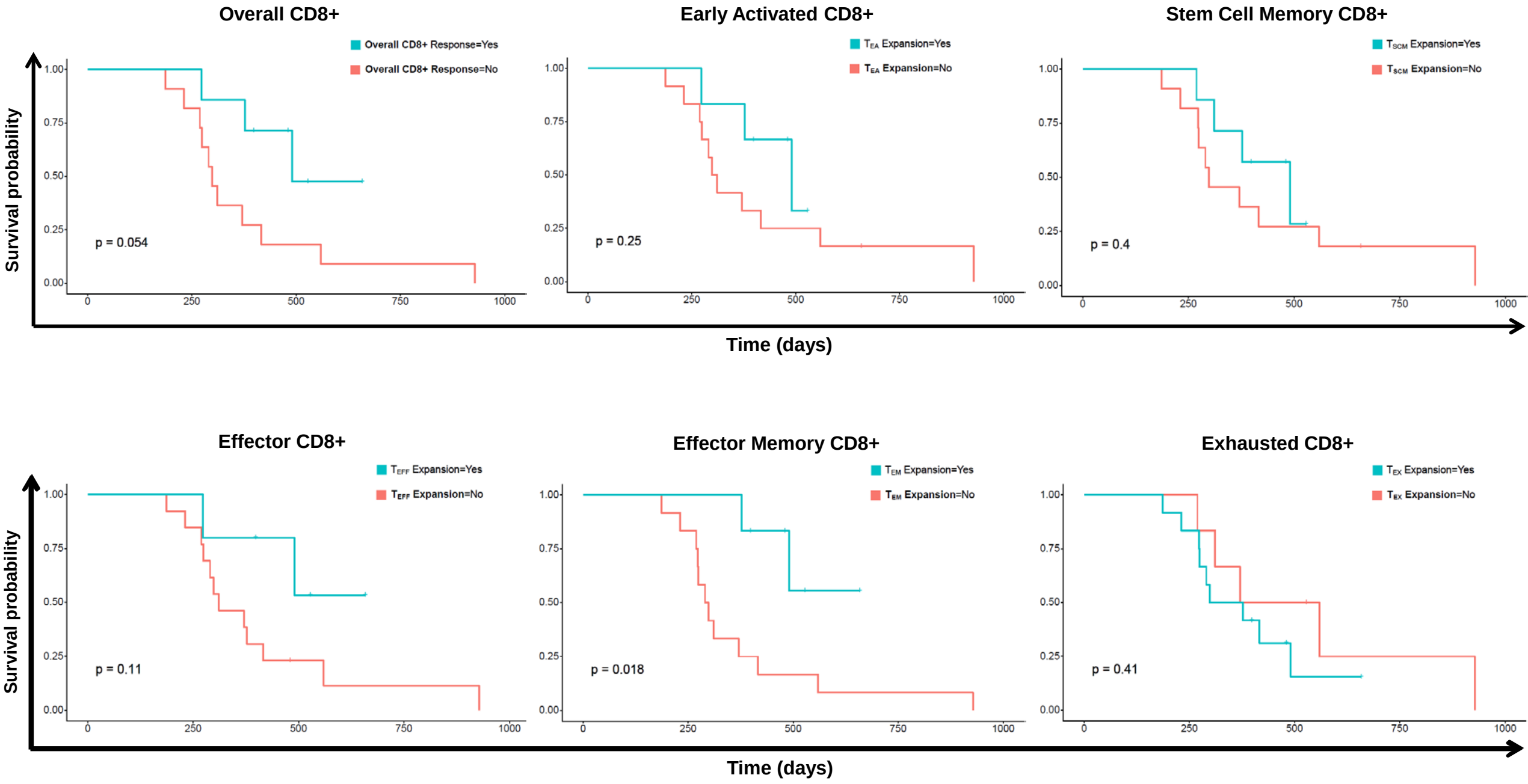

Overall CD8+
Overall CD8+ Response=Yes
Overall CD8+ Response=No
p = 0.054
Early Activated CD8+
TEA Expansion=Yes
TEA Expansion=No
p = 0.25
Stem Cell Memory CD8+
TSCM Expansion=Yes
TSCM Expansion=No
p = 0.4
Effector CD8+
TEFF Expansion=Yes
TEFF Expansion=No
p = 0.11
Effector Memory CD8+
TEM Expansion=Yes
TEM Expansion=No
p = 0.018
Exhausted CD8+
TEX Expansion=Yes
TEX Expansion=No
p = 0.41
Survival probability
1.00
0.75
0.50
0.25
0.00
0
250
500
750
1000
Time (days)

Figure S6

Kaplan-Meier survival curves visualizing PFS among strata A + B patients based on the expansion of H3.3K27M-reactive CD8+ T-cell subpopulations.

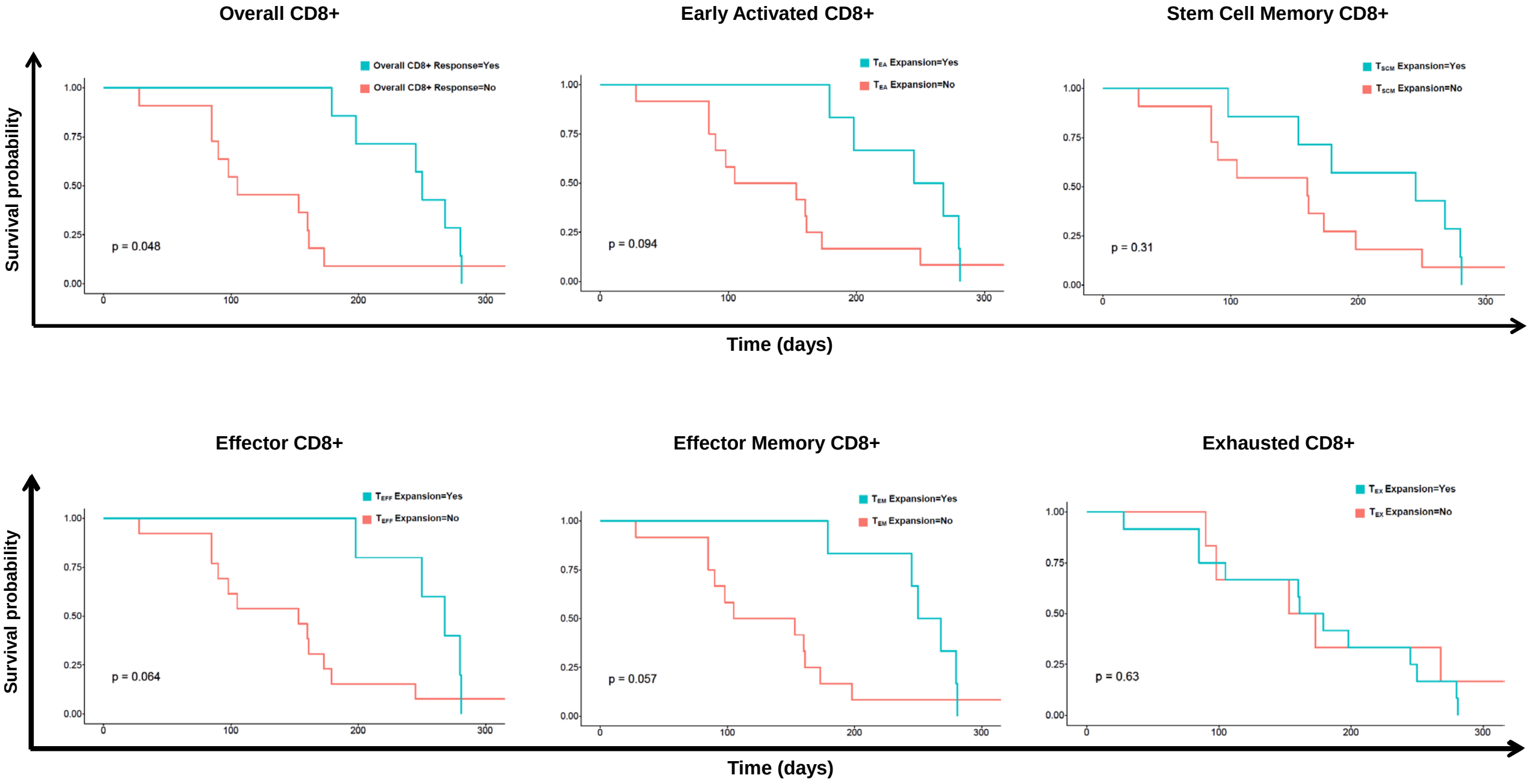

Overall CD8+
Overall CD8+ Response=Yes
Overall CD8+ Response=No
p = 0.048
Early Activated CD8+
TEA Expansion=Yes
TEA Expansion=No
p = 0.094
Stem Cell Memory CD8+
TSCM Expansion=Yes
TSCM Expansion=No
p = 0.31
Survival probability
Time (days)
Effector CD8+
TEFF Expansion=Yes
TEFF Expansion=No
p = 0.064
Effector Memory CD8+
TEM Expansion=Yes
TEM Expansion=No
p = 0.057
Exhausted CD8+
TEX Expansion=Yes
TEX Expansion=No
p = 0.63
Survival probability
Time (days)

Figure S7

Kaplan-Meier survival curves visualizing OS among stratum A patients based on the expansion of H3.3K27M-reactive CD8+ T-cell subpopulations.

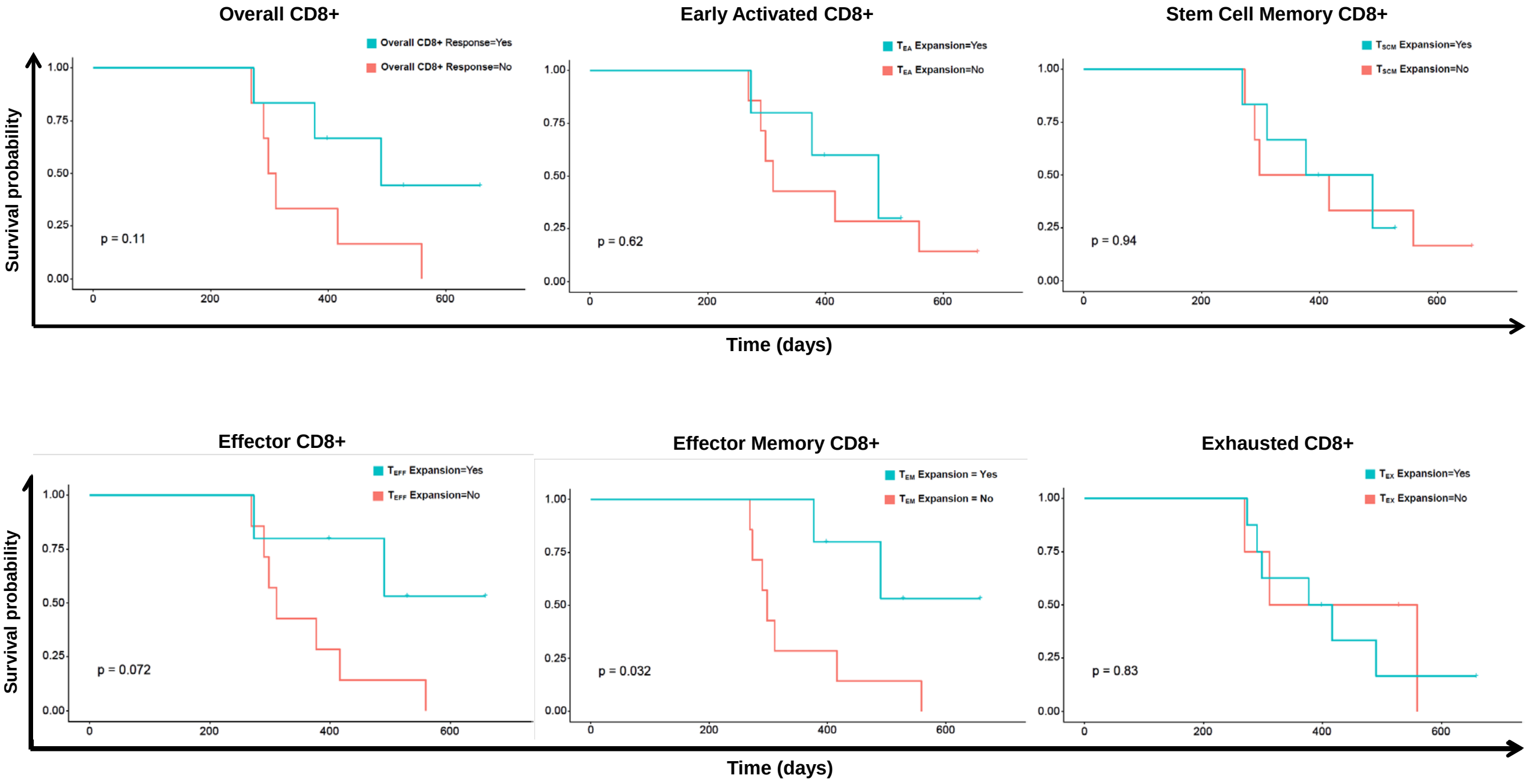

Overall CD8+
Overall CD8+ Response=Yes
Overall CD8+ Response=No
p = 0.11
Early Activated CD8+
T_{EA} Expansion=Yes
T_{EA} Expansion=No
p = 0.62
Stem Cell Memory CD8+
T_{SCM} Expansion=Yes
T_{SCM} Expansion=No
p = 0.94
Effector CD8+
T_{EFF} Expansion=Yes
T_{EFF} Expansion=No
p = 0.072
Effector Memory CD8+
T_{EM} Expansion = Yes
T_{EM} Expansion = No
p = 0.032
Exhausted CD8+
T_{EX} Expansion=Yes
T_{EX} Expansion=No
p = 0.83
Survival probability
1.00
0.75
0.50
0.25
0.00
0
200
400
600
Time (days)

Figure S8

Kaplan-Meier survival curves visualizing PFS among stratum A patients based on the expansion of H3.3K27M-reactive CD8+ T-cell subpopulations.

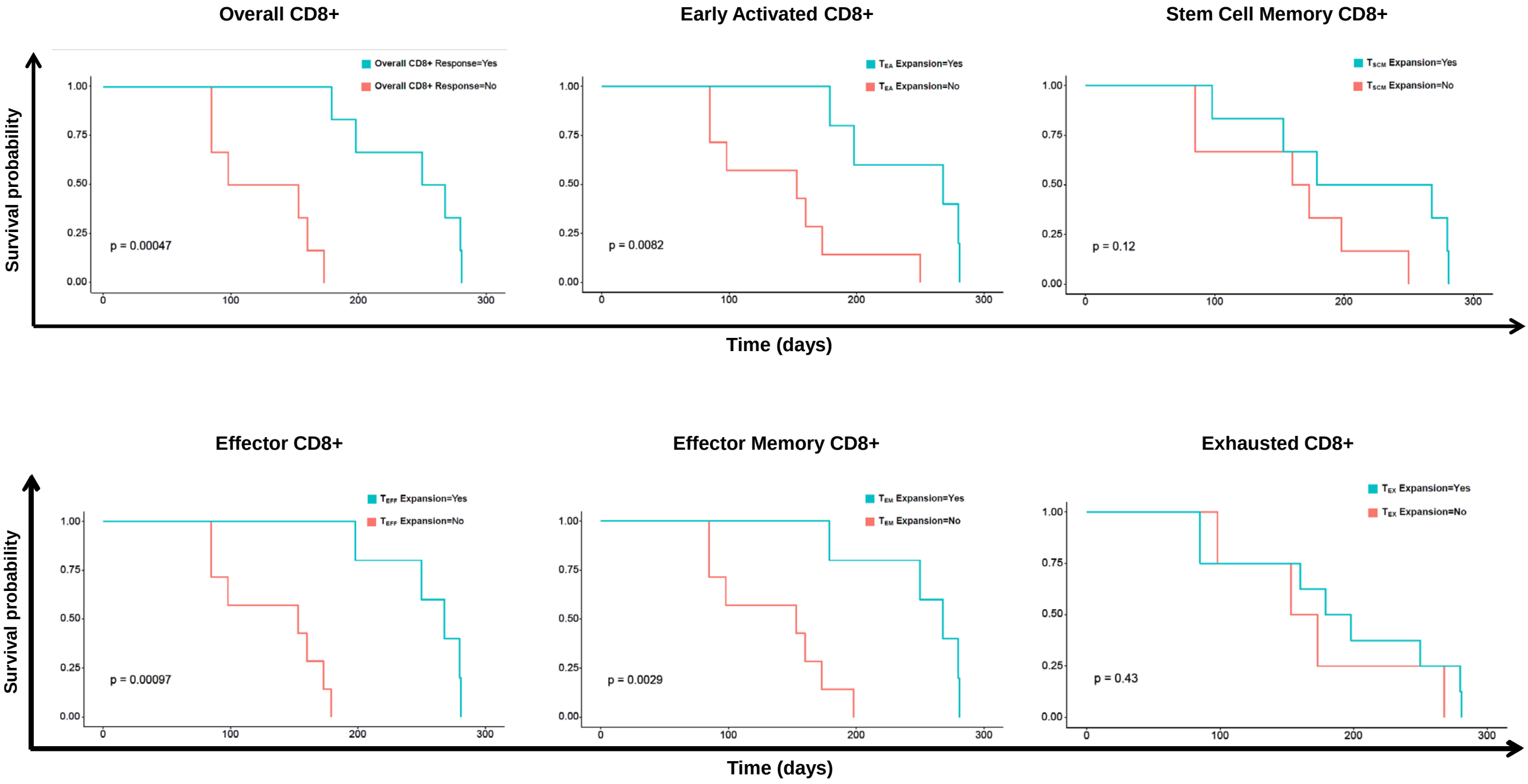
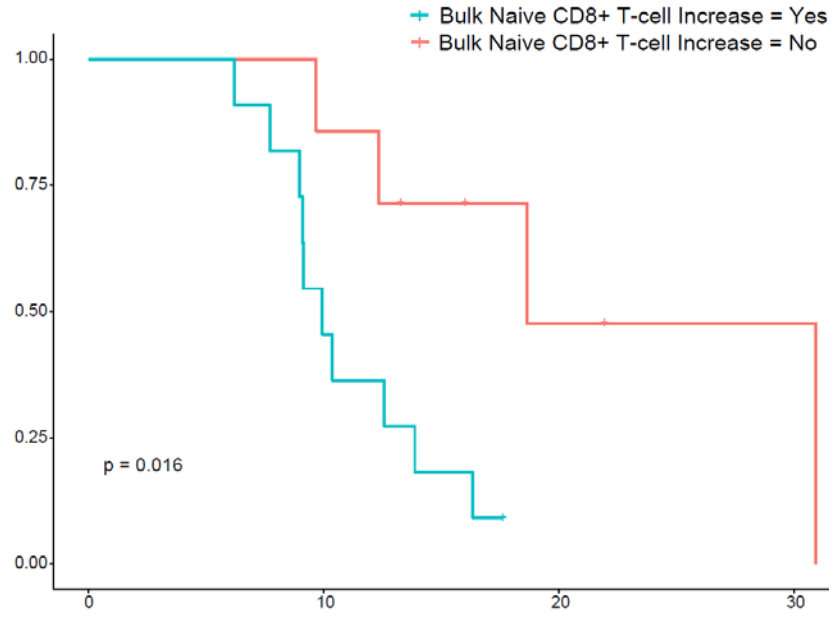

Overall CD8+
Overall CD8+ Response=Yes
Overall CD8+ Response=No
p = 0.00047
Early Activated CD8+
TEA Expansion=Yes
TEA Expansion=No
p = 0.0082
Stem Cell Memory CD8+
TSCM Expansion=Yes
TSCM Expansion=No
p = 0.12
Effector CD8+
TEFF Expansion=Yes
TEFF Expansion=No
p = 0.00097
Effector Memory CD8+
TEM Expansion=Yes
TEM Expansion=No
p = 0.0029
Exhausted CD8+
TEX Expansion=Yes
TEX Expansion=No
p = 0.43
Survival probability
1.00
0.75
0.50
0.25
0.00
0
100
200
300
Time (days)

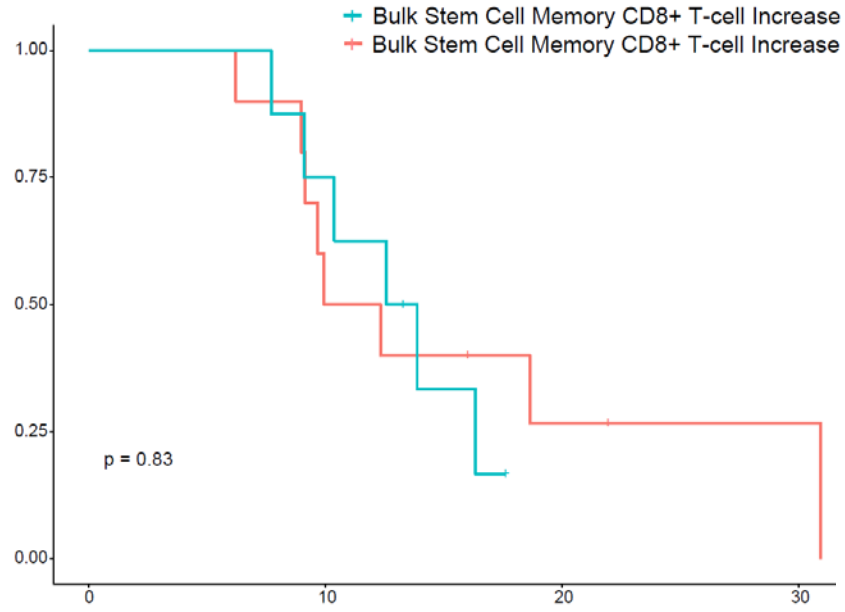
Figure S9

Kaplan-Meier survival curves visualizing OS among strata A + B patients based on the expansion of bulk CD8+ T-cell subpopulations.

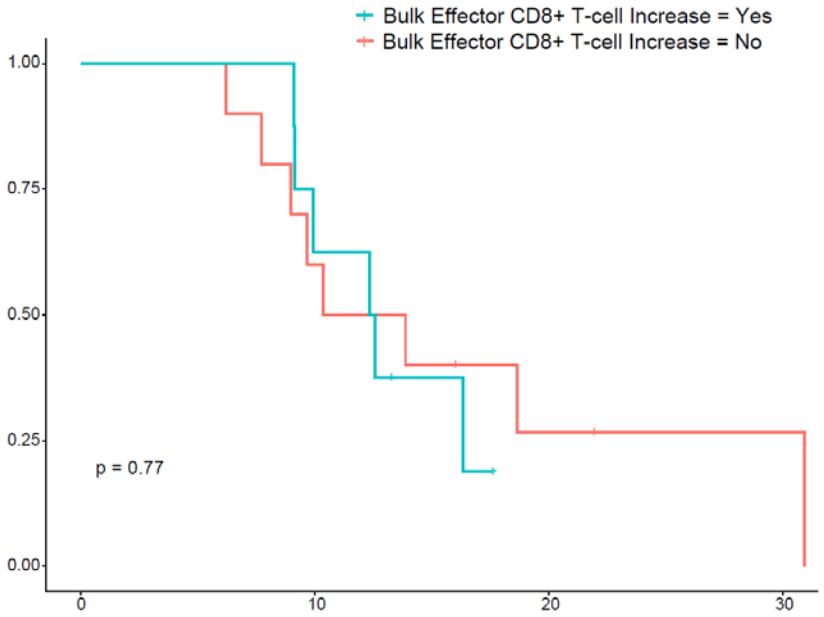
Early Activated CD8+



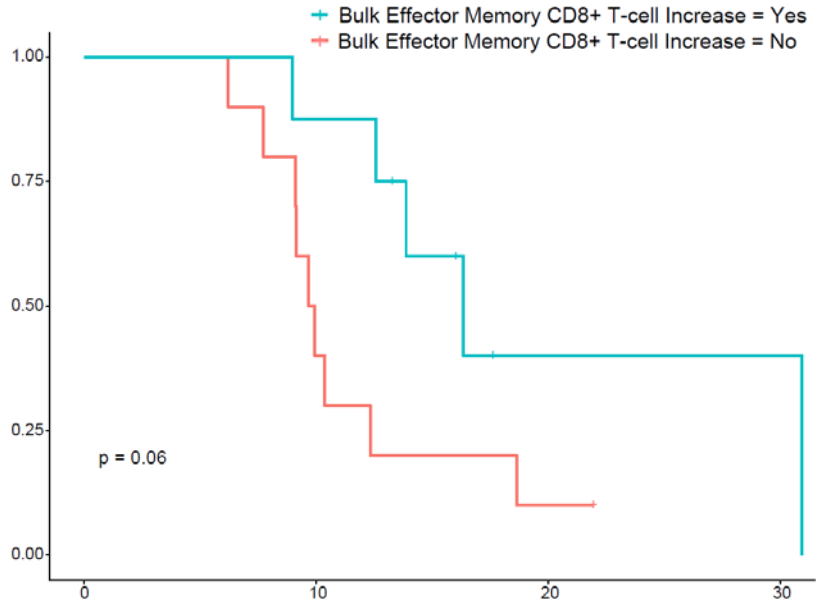
Stem Cell Memory CD8+



Effector CD8+



Effector Memory CD8+



Exhausted CD8+

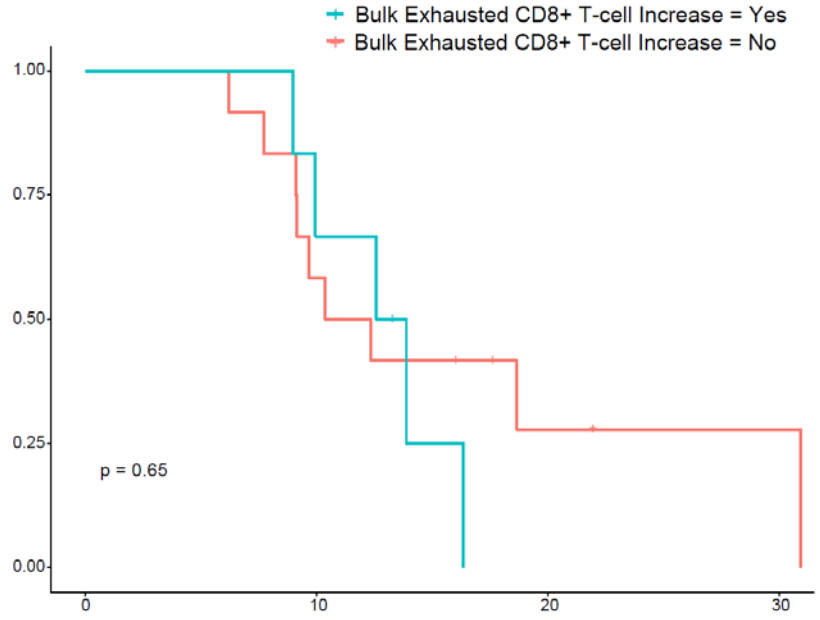
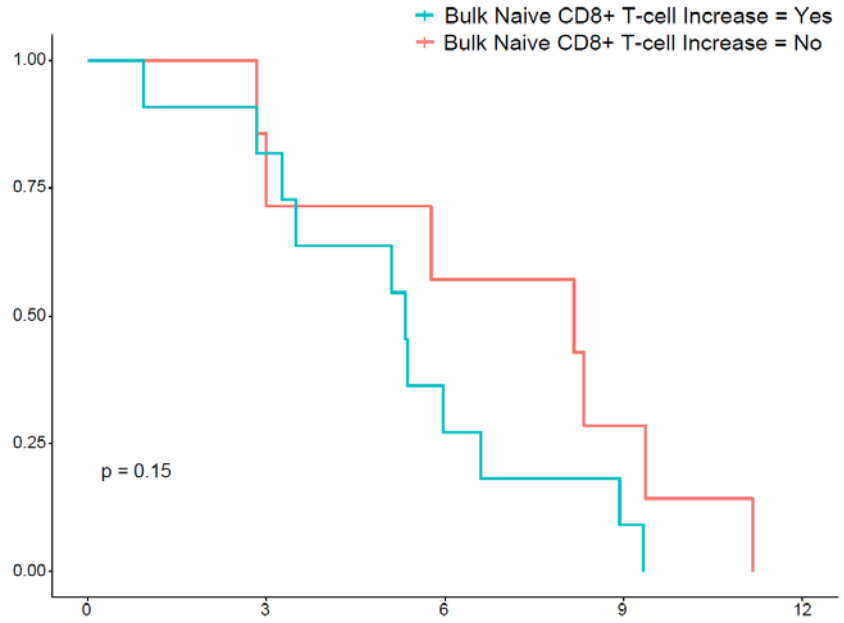


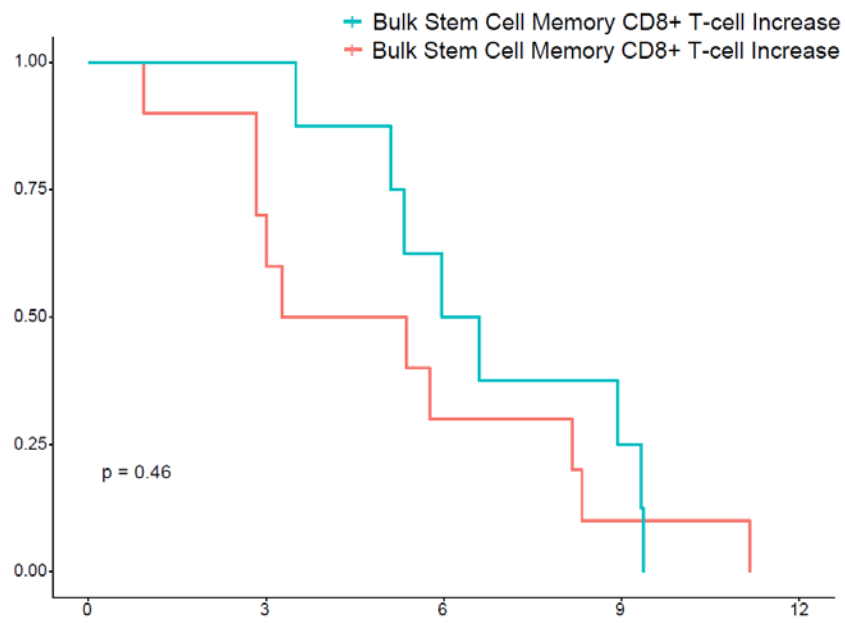
Figure S10

Kaplan-Meier survival curves visualizing PFS among strata A + B patients based on the expansion of bulk CD8+ T-cell subpopulations.

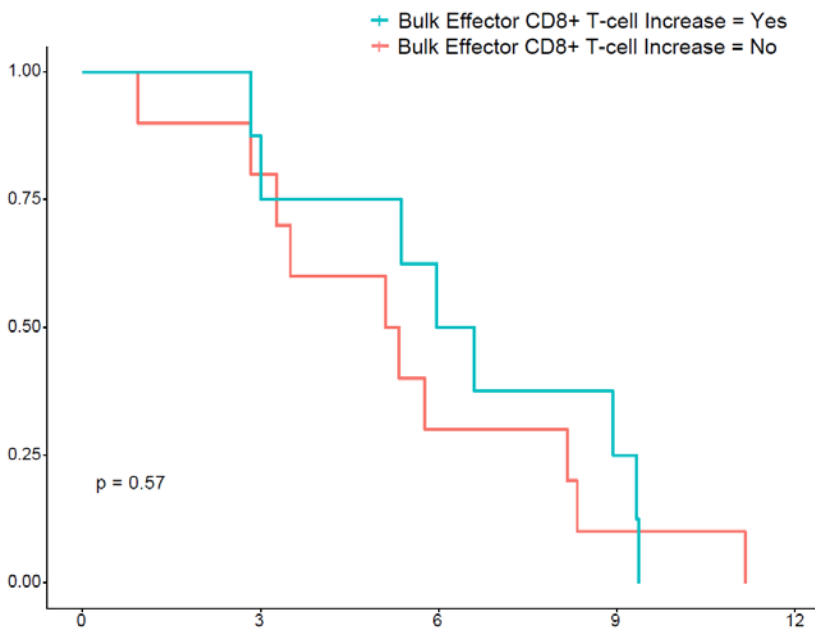
Early Activated CD8+



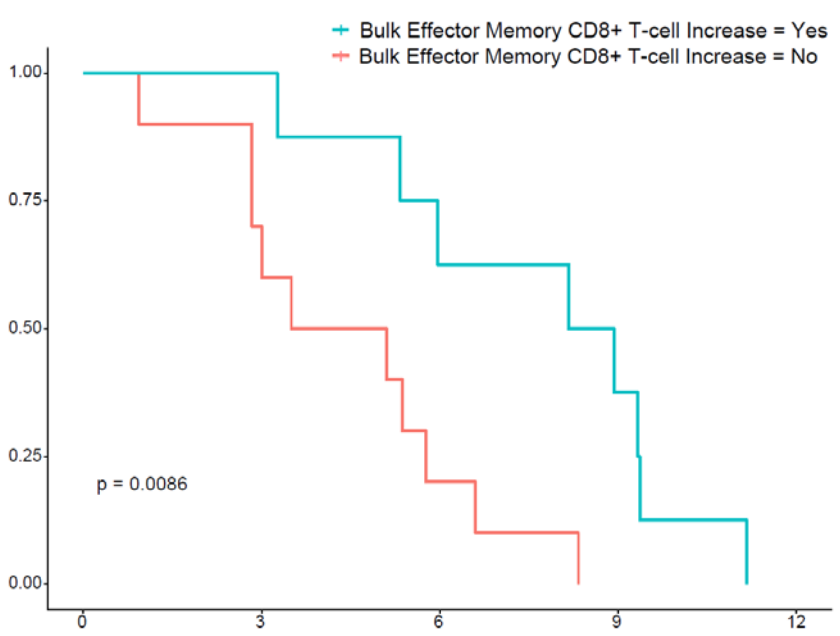
Stem Cell Memory CD8+



Effector CD8+



Effector Memory CD8+



Exhausted CD8+

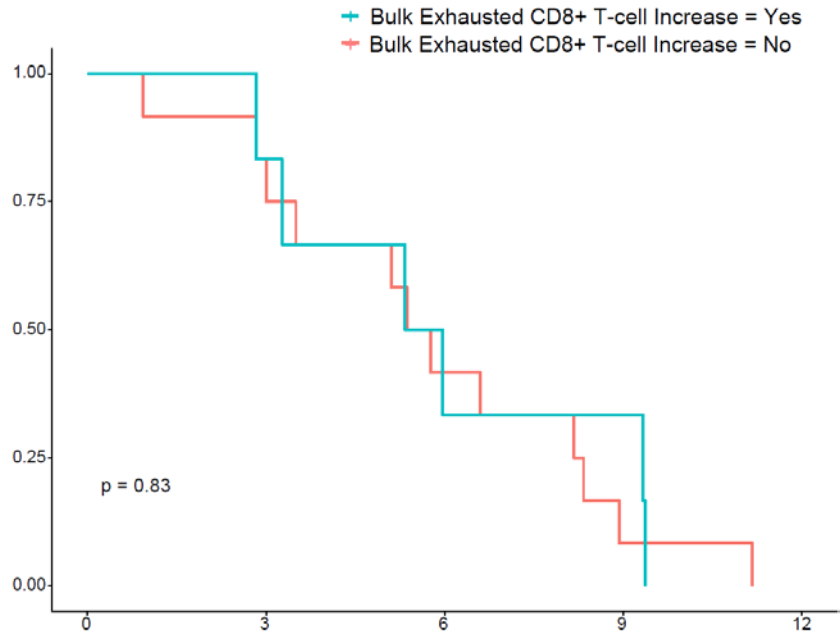
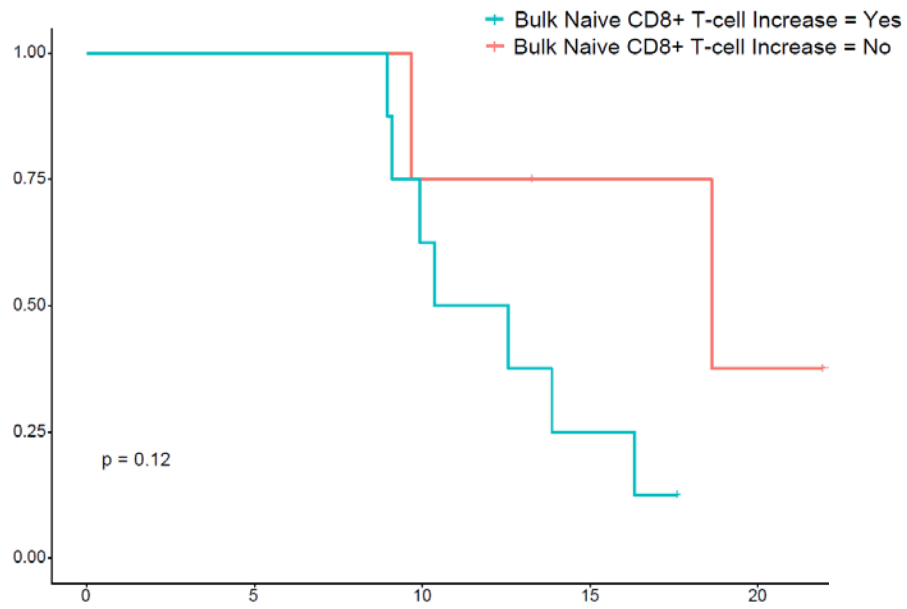


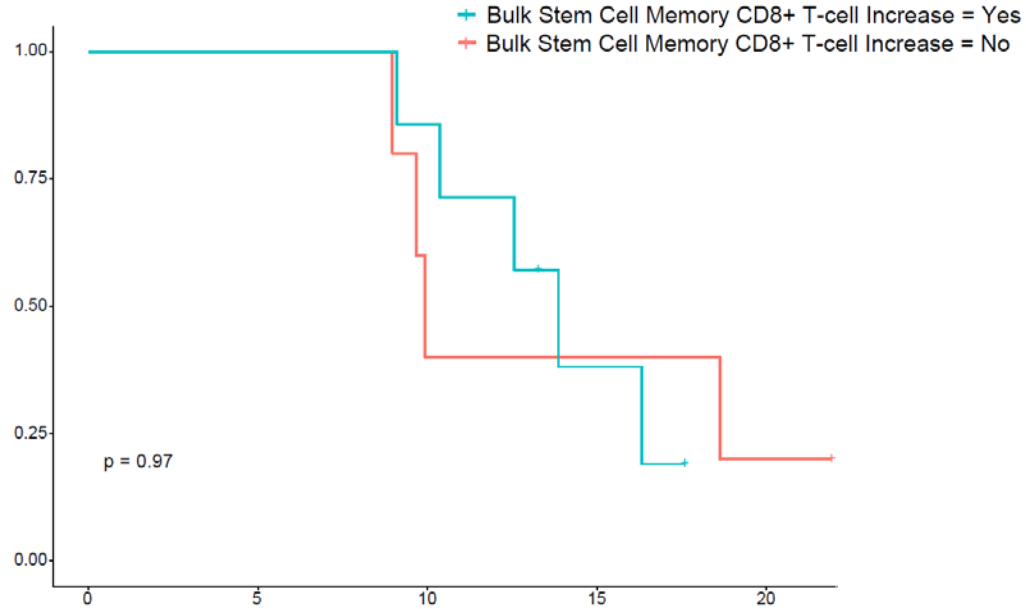
Figure S11

Kaplan-Meier survival curves visualizing OS among stratum A patients based on the expansion of bulk CD8+ T-cell subpopulations.

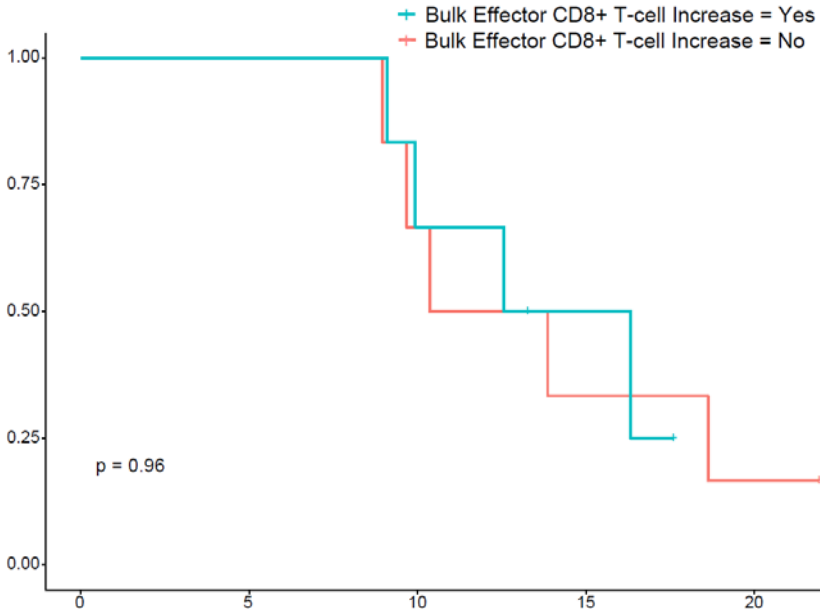
Early Activated CD8+



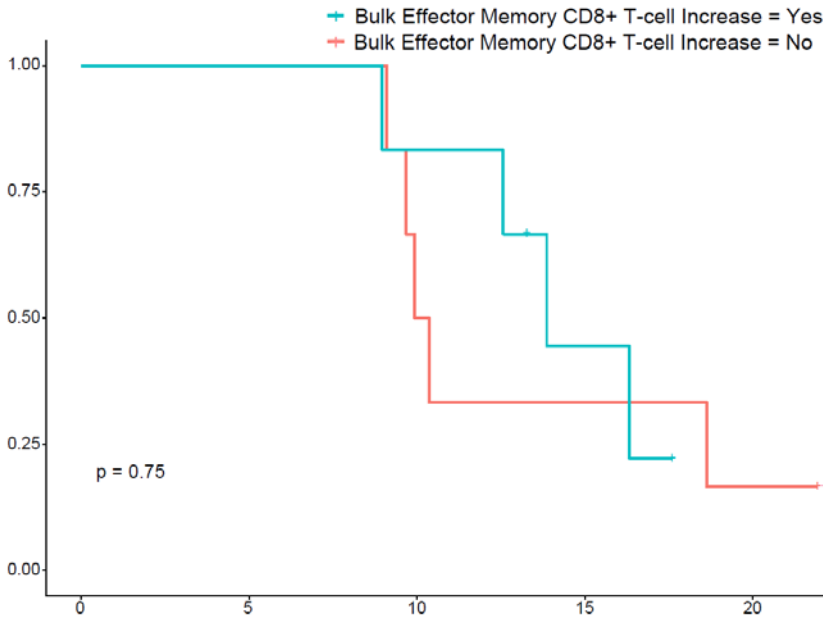
Stem Cell Memory CD8+



Effector CD8+



Effector Memory CD8+



Exhausted CD8+

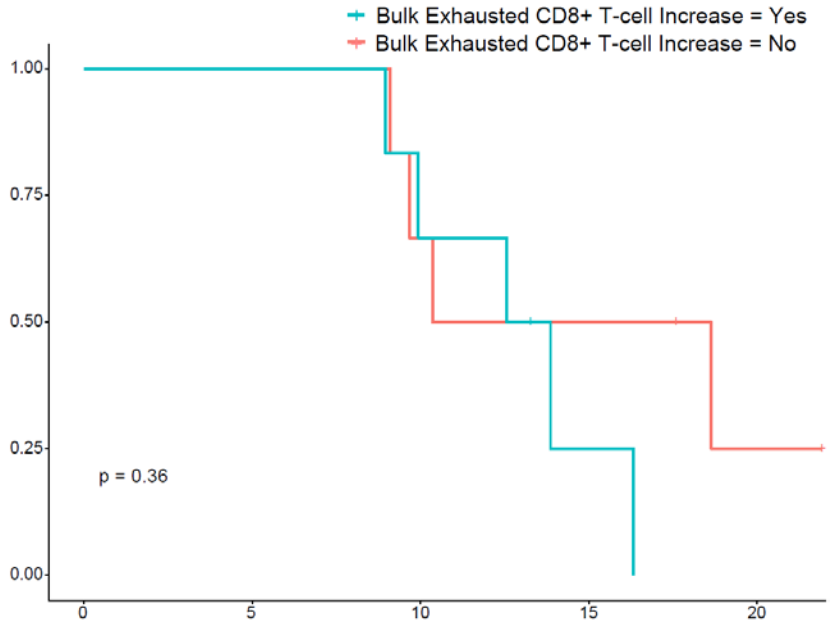
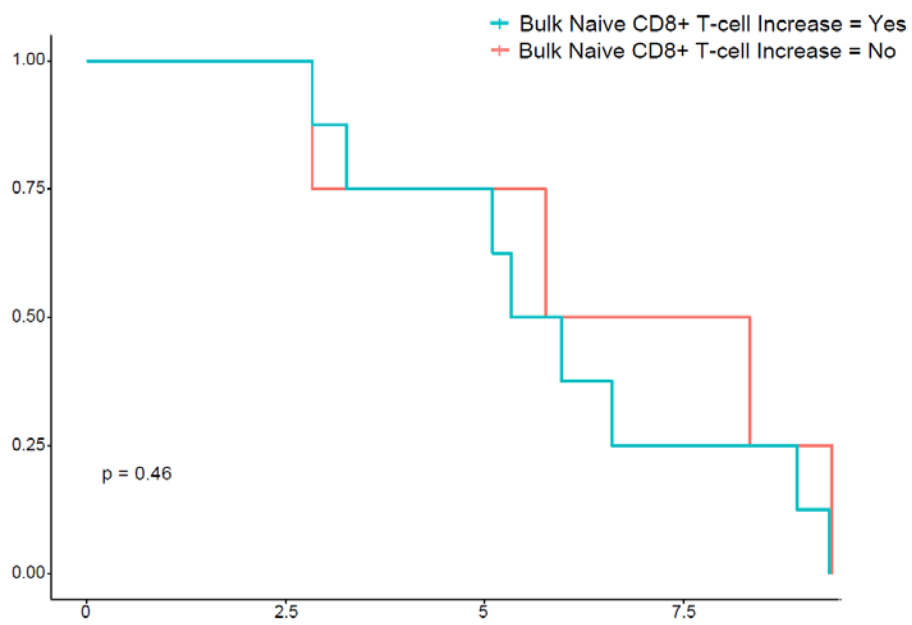


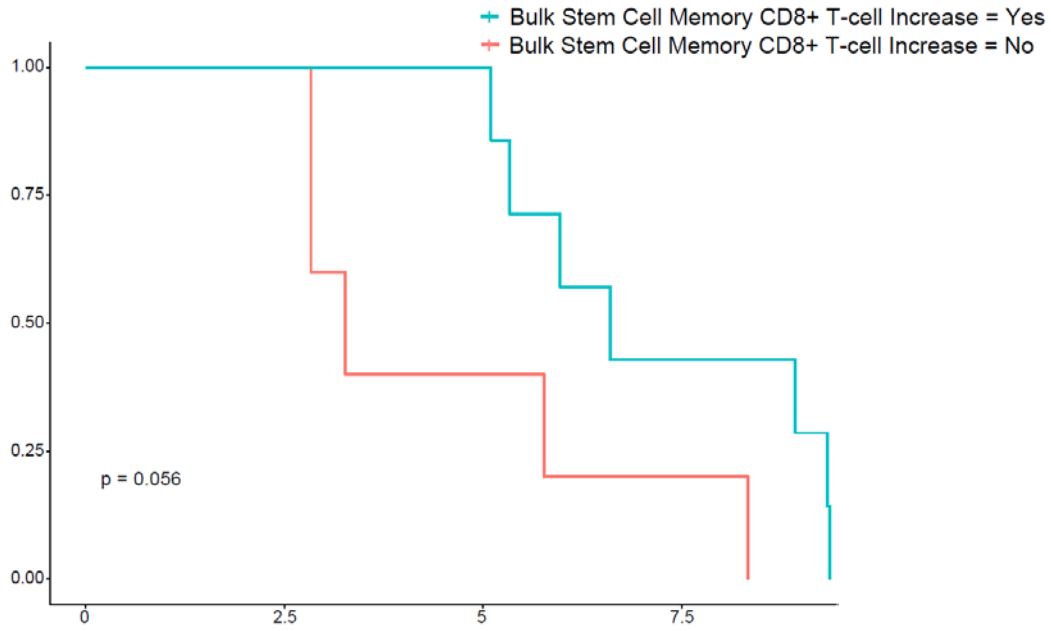
Figure S12

Kaplan-Meier survival curves visualizing PFS among stratum A patients based on the expansion of bulk CD8+ T-cell subpopulations.

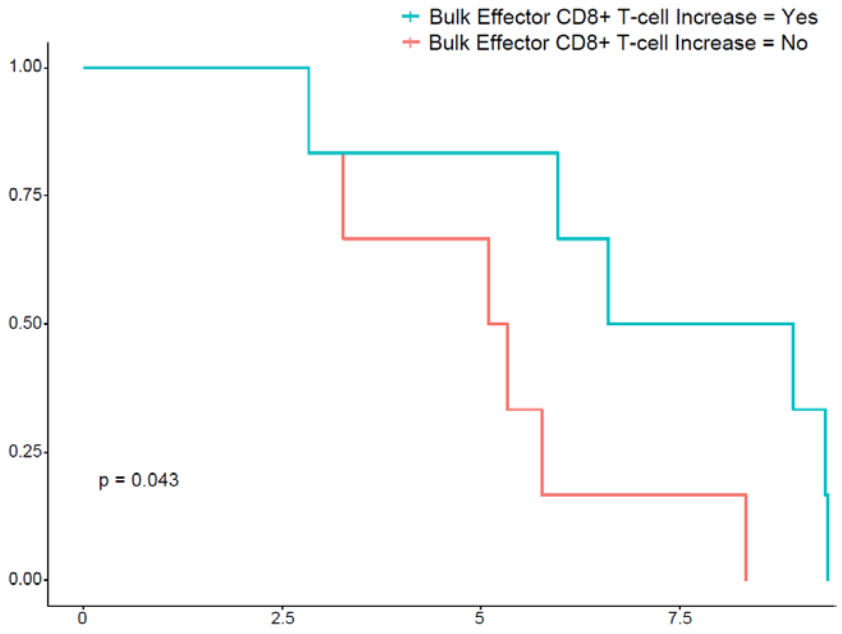
Early Activated CD8+



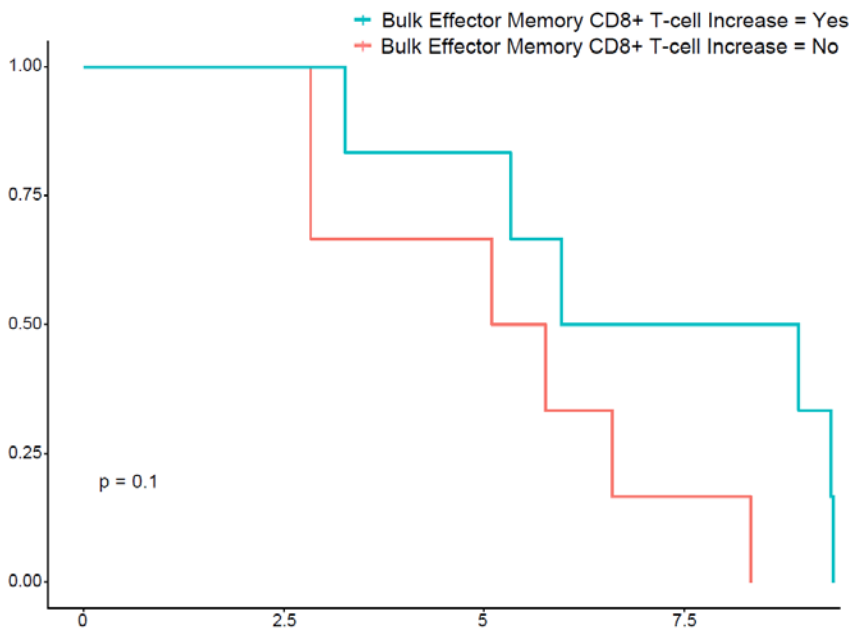
Stem Cell Memory CD8+



Effector CD8+



Effector Memory CD8+



Exhausted CD8+

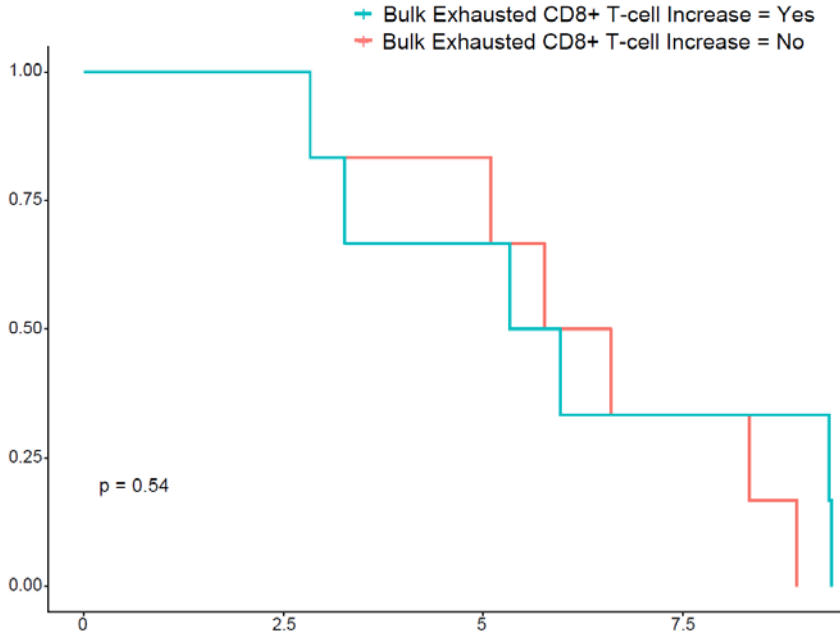


Figure S13

Linear model correlating the relationship between number of vaccines administered and relative percent change of H3.3K27M-reactive CD8+ T-cells utilizing Spearman correlation coefficient. Models both with and without outlier patient exhibiting greatest H3.3K27M-reactive CD8+ T-cell expansion (PNOC007-25) reveal significant correlation.

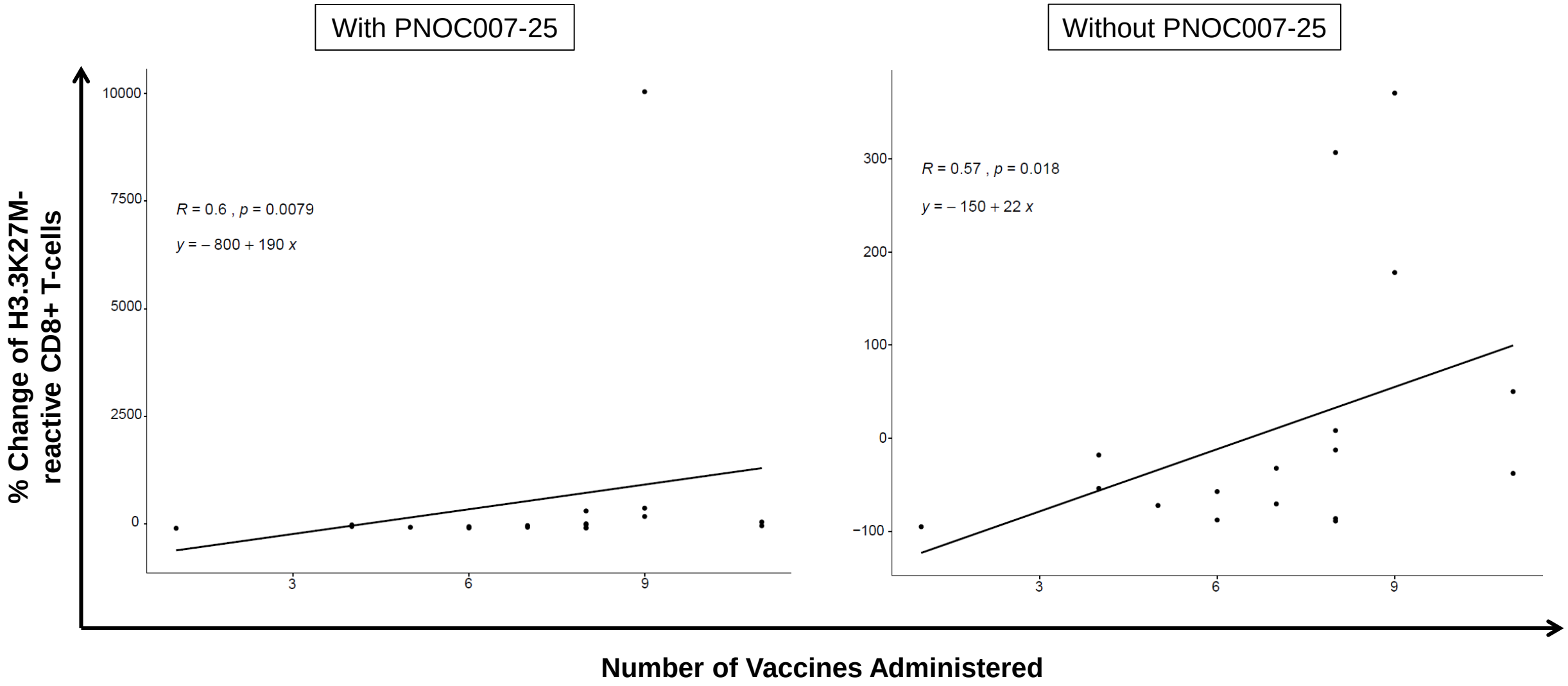
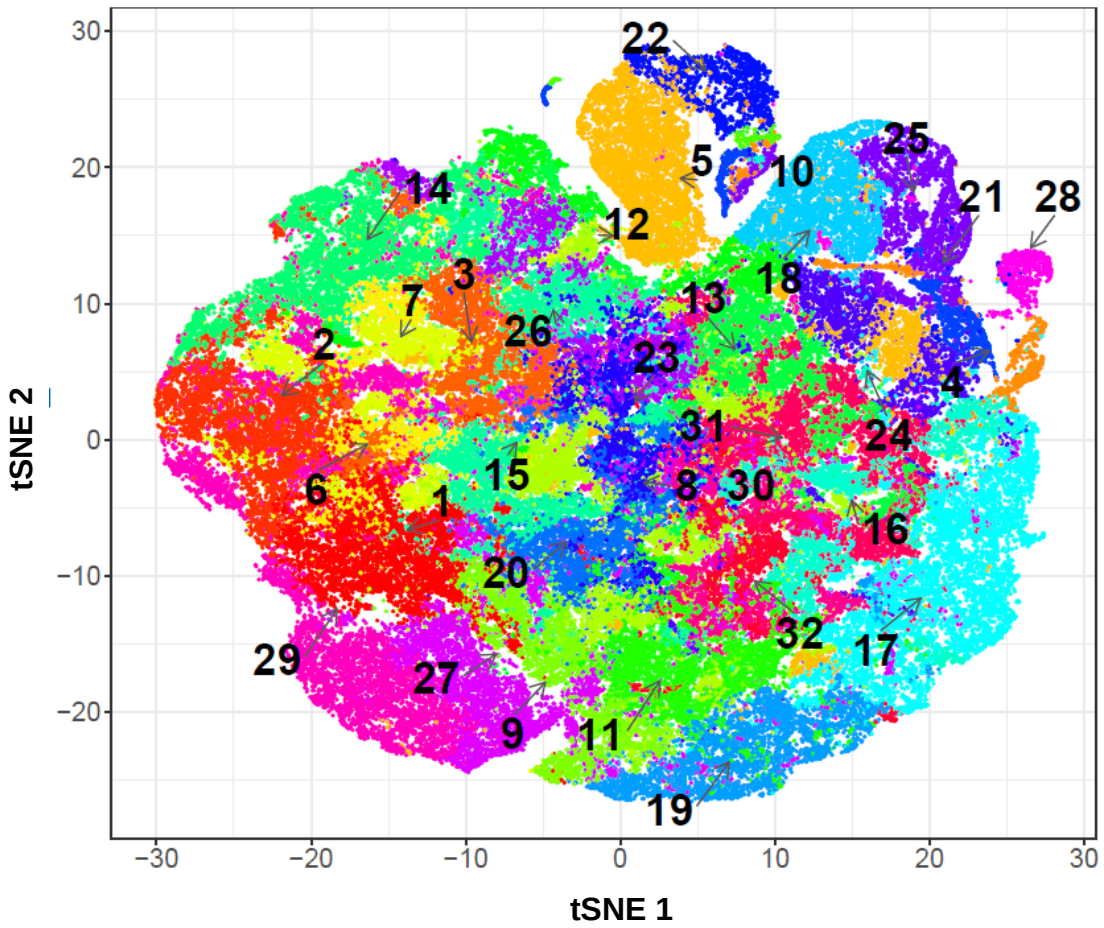

With PNOC007-25
Without PNOC007-25
% Change of H3.3K27M-reactive CD8+ T-cells
10000
7500
5000
2500
0
R = 0.6 , p = 0.0079
y = – 800 + 190 x
3
6
9
300
200
100
0
−100
R = 0.57 , p = 0.018
y = – 150 + 22 x
3
6
9
Number of Vaccines Administered

Figure S14

tSNE stratification and expression profiles of discrete myeloid subpopulations. *Left*, patient-derived myeloid cells were stratified on a tSNE plot using the myeloid canonical markers: CD33, CD11b, CD11c, CD14, CD15 and CD16. *Right*, heat map visualizing the z-score normalized expression of resultant myeloid cell clusters.


tSNE 2
tSNE 1
30
20
10
0
−10
−20
−30
22
5
10
25
21
28
14
12
3
7
18
13
26
2
23
4
31
24
15
6
1
8
30
16
20
32
17
29
27
9
11
19

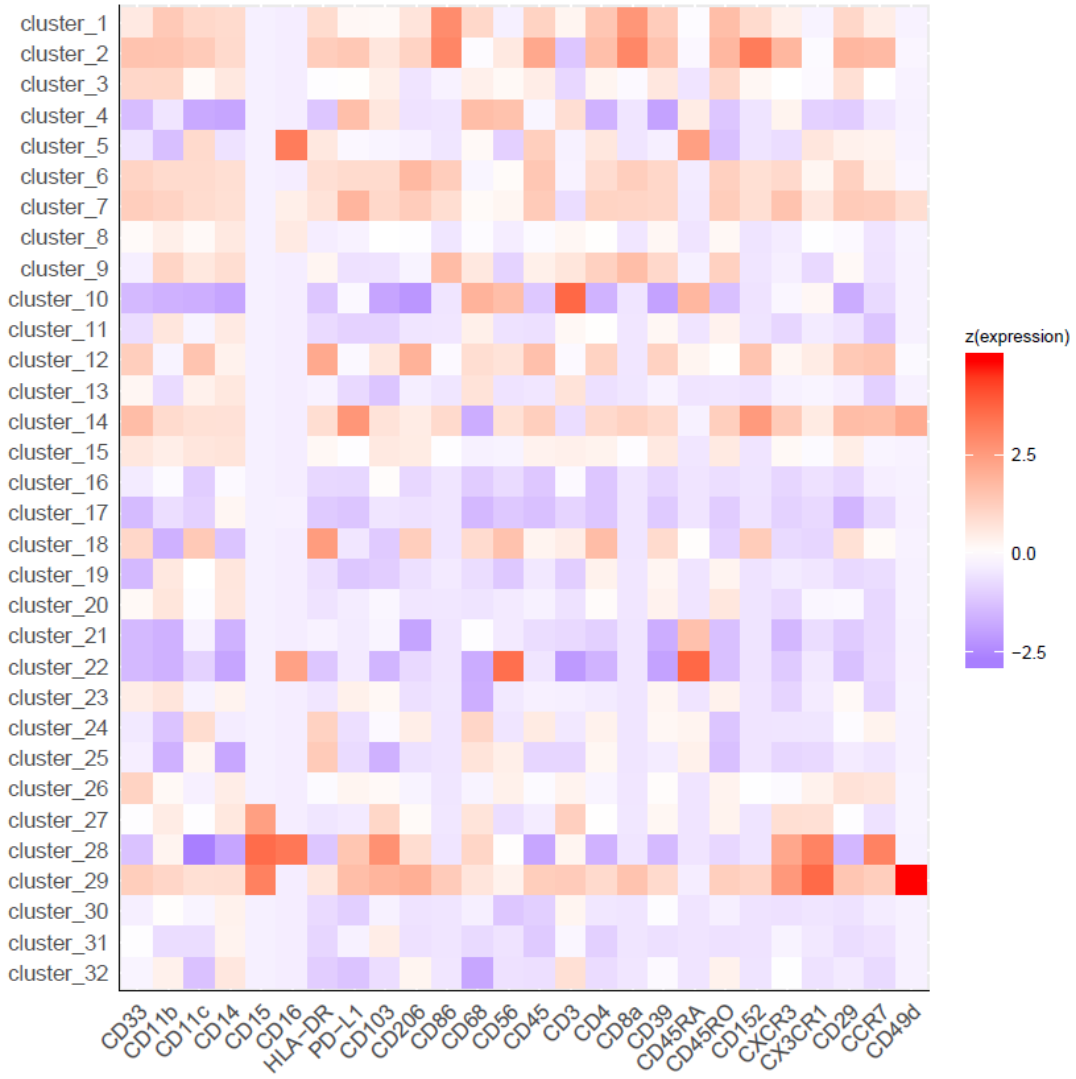

cluster_1
cluster_2
cluster_3
cluster_4
cluster_5
cluster_6
cluster_7
cluster_8
cluster_9
cluster_10
cluster_11
cluster_12
cluster_13
cluster_14
cluster_15
cluster_16
cluster_17
cluster_18
cluster_19
cluster_20
cluster_21
cluster_22
cluster_23
cluster_24
cluster_25
cluster_26
cluster_27
cluster_28
cluster_29
cluster_30
cluster_31
cluster_32
CD33
CD11b
CD11c
CD14
CD15
CD16
HLA−DR
PD−L1
CD103
CD206
CD86
CD68
CD56
CD45
CD3
CD4
CD8a
CD39
CD45RA
CD45RO
CD152
CXCR3
CX3CR1
CD29
CCR7
CD49d
z(expression)
2.5
0.0
−2.5

Table S2

CyTOF-based marker expression profiles and MDSC phenotypic characterizations of myeloid cell clusters identified via tSNE stratification.

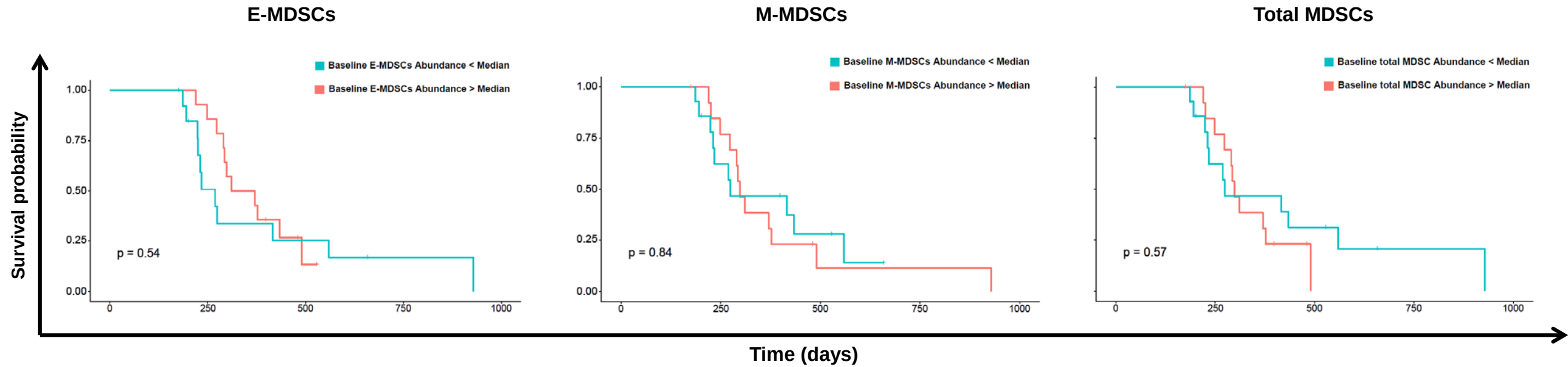
Cluster Number	Phenotype
1	CD33+ CD11b+ CD14+ CD86+ CD68^{hi}
2	CD33+ CD11b+ CD14+ CD86+ CD68^{mid}
3	CD33+ CD11b+ CD11c^{mid} CD14+ CD206^{lo} CD68^{mid}
4	Almost all markers low
5	CD11c+ CD16+ HLA-DR+
6	CD33+ CD11b+ CD11c+ CD14+ CD206+ HLA-DR^{hi}
7	CD33+ CD11b+ CD11c+ CD14+ CD16^{hi} HLA-DR^{hi}
8	CD33^{mid} CD11b+ CD11c+ CD14^{hi} CD16^{hi} HLA-DR^{lo}
9	CD33^{mid} CD11b+ CD11c+ CD14+ HLA-DR^{mid} CD206-
10	Almost all markers low
11	CD33^{lo} CD11b+ CD11c^{mid} CD14^{hi} CD206- CD68+
12	CD33+ CD11c+ HLA-DR+ CD103^{hi} CD206+
13	CD33^{hi} CD11b^{lo} CD11c^{hi} CD14+ HLA-DR^{lo} CD68^{hi}
14	CD33+ CD11b+ CD11c+ CD14+ HLA-DR^{hi}
15	CD33+ CD11b+ CD11c+ CD14+ HLA-DR^{mid-hi} CD103^{hi} CD206^{hi}
16	CD33^{mid} CD11b^{mid} CD14^{lo} HLA-DR^{lo}
17	CD11b^{lo} CD14^{hi} HLA-DR-
18	CD33+ CD11c+ CD206+ HLA-DR+
19	CD11b+ CD11c^{mid} CD14+
20	CD33+ CD11b+ CD11c^{mid} CD14^{hi} HLA-DR^{lo}
21	CD11c^{mid} HLA-DR^{mid} CD103^{mid} CD68^{mid}
22	CD11c^{lo} CD16+ HLA-DR-
23	CD33+ CD11b+ CD14+ HLA-DR-
24	CD11c+ HLA-DR+ CD206^{hi}
25	CD11c+ HLA-DR+ CD68^{hi}
26	CD33+ CD11b+ CD14+ HLA-DR^{mid-lo}
27	CD33+ CD11b+ CD11c^{mid} CD15+ HLA-DR^{lo}
28	CD11b+ CD15+ CD16+ HLA-DR^{lo}
29	CD33+ CD11b+ CD11c^{hi} CD15+ HLA-DR^{hi} CD103+
30	CD33^{lo} CD11b^{mid} CD14^{hi} HLA-DR^{lo}
31	CD33^{mid} CD11b^{lo} CD14^{hi} HLA-DR^{lo}
32	CD33^{lo} CD11b^{hi} CD14^{hi} HLA-DR^{lo}

Color Key
E-MDSC
M-MDSC

Figure S15

Kaplan-Meier survival curves visualizing OS and PFS among strata A + B patients based on the abundance of circulatory MDSC subtypes at baseline.

OS:


E-MDSCs
Baseline E-MDSCs Abundance < Median
Baseline E-MDSCs Abundance > Median
p = 0.54
M-MDSCs
Baseline M-MDSCs Abundance < Median
Baseline M-MDSCs Abundance > Median
p = 0.84
Total MDSCs
Baseline total MDSC Abundance < Median
Baseline total MDSC Abundance > Median
p = 0.57
Survival probability
1.00
0.75
0.50
0.25
0.00
0
250
500
750
1000
Time (days)

PFS:

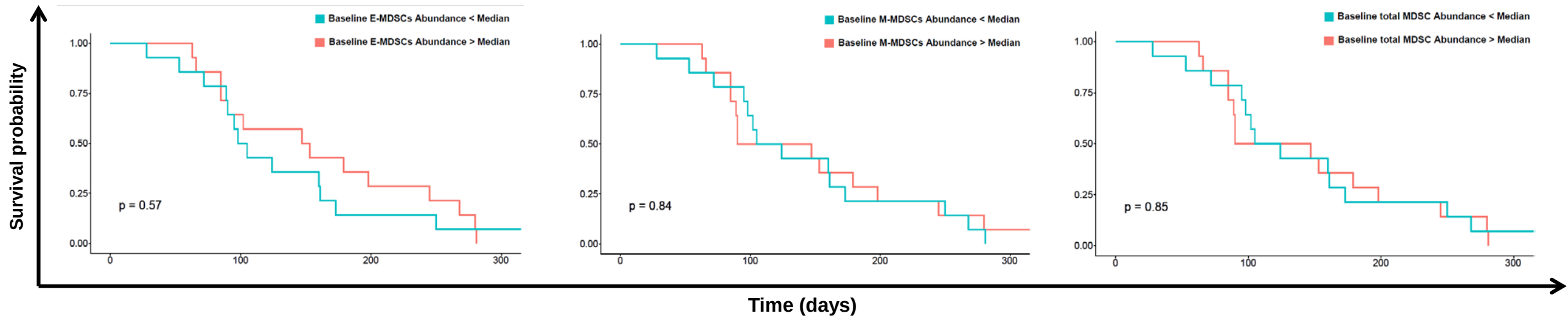
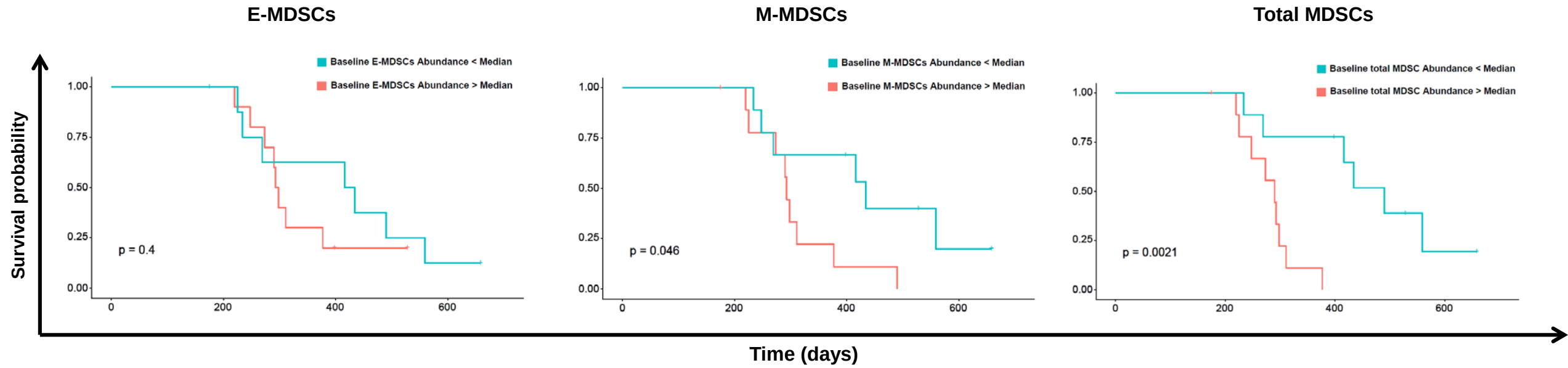

E-MDSCs
Baseline E-MDSCs Abundance < Median
Baseline E-MDSCs Abundance > Median
p = 0.57
M-MDSCs
Baseline M-MDSCs Abundance < Median
Baseline M-MDSCs Abundance > Median
p = 0.84
Total MDSCs
Baseline total MDSC Abundance < Median
Baseline total MDSC Abundance > Median
p = 0.85
Survival probability
1.00
0.75
0.50
0.25
0.00
0
100
200
300
Time (days)

Figure S16

Kaplan-Meier survival curves visualizing OS and PFS among stratum A patients based on the abundance of circulatory MDSC subtypes at baseline.

OS:


E-MDSCs
M-MDSCs
Total MDSCs
Baseline E-MDSCs Abundance < Median
Baseline E-MDSCs Abundance > Median
Baseline M-MDSCs Abundance < Median
Baseline M-MDSCs Abundance > Median
Baseline total MDSC Abundance < Median
Baseline total MDSC Abundance > Median
p = 0.4
p = 0.046
p = 0.0021
Survival probability
Time (days)

PFS:

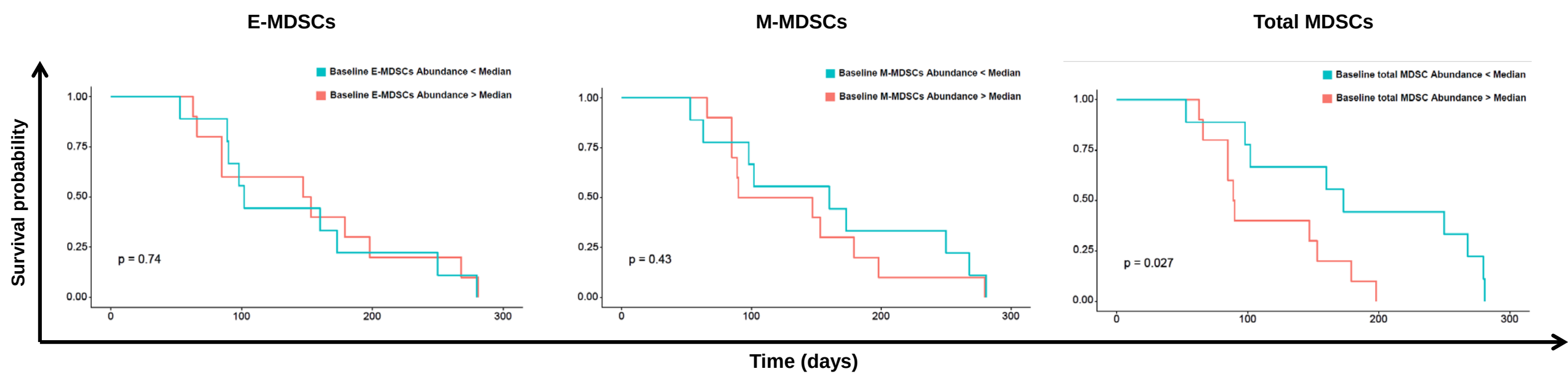

E-MDSCs
M-MDSCs
Total MDSCs
Baseline E-MDSCs Abundance < Median
Baseline E-MDSCs Abundance > Median
Baseline M-MDSCs Abundance < Median
Baseline M-MDSCs Abundance > Median
Baseline total MDSC Abundance < Median
Baseline total MDSC Abundance > Median
p = 0.74
p = 0.43
p = 0.027
Survival probability
Time (days)

Figure S17

Kaplan-Meier survival curves visualizing OS of strata A+B and stratum A patients with MDSC abundances in upper and lower quartile of their respective cohorts

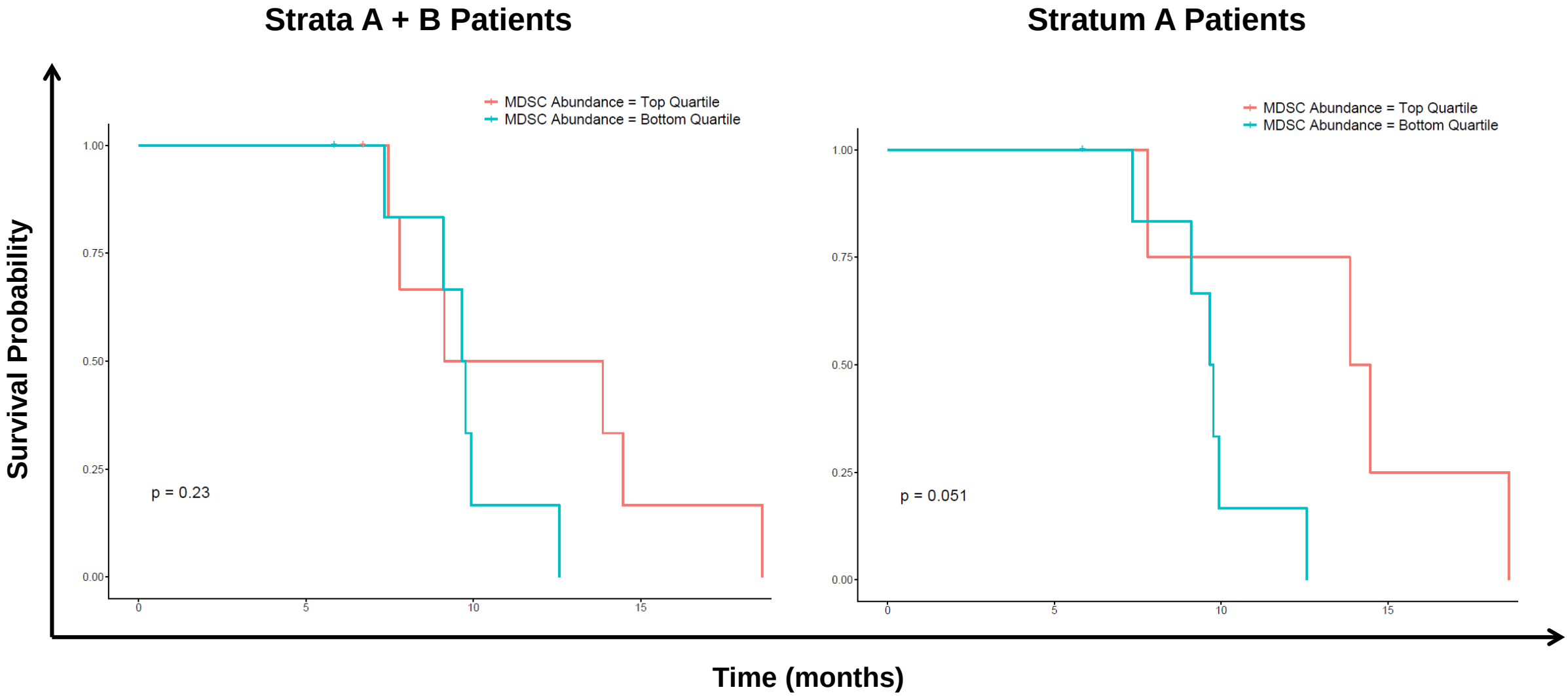
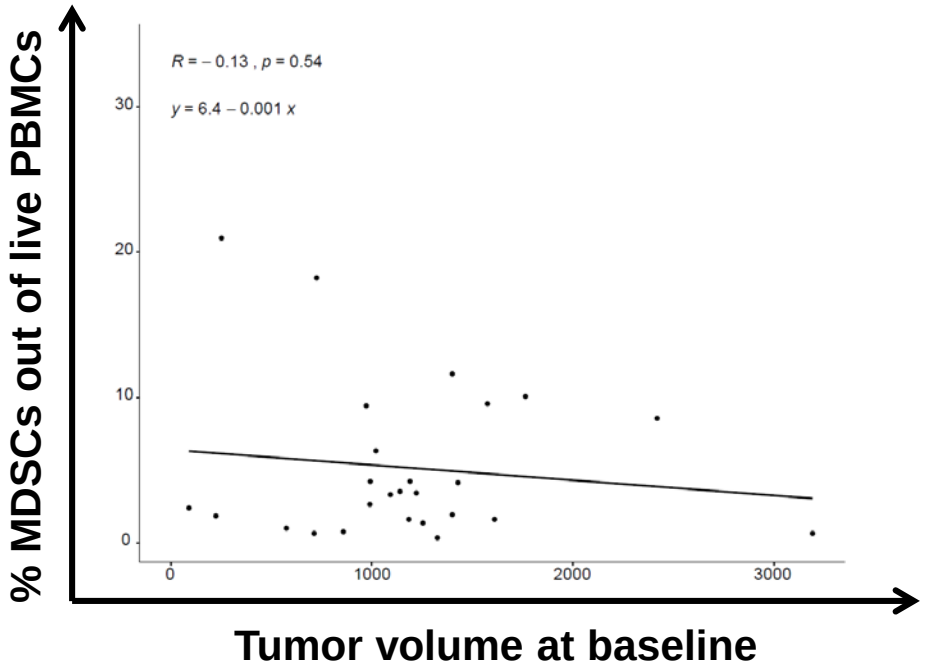

Strata A + B Patients
Stratum A Patients
MDSC Abundance = Top Quartile
MDSC Abundance = Bottom Quartile
Survival Probability
1.00
0.75
0.50
0.25
0.00
0
5
10
15
p = 0.23
p = 0.051
Time (months)

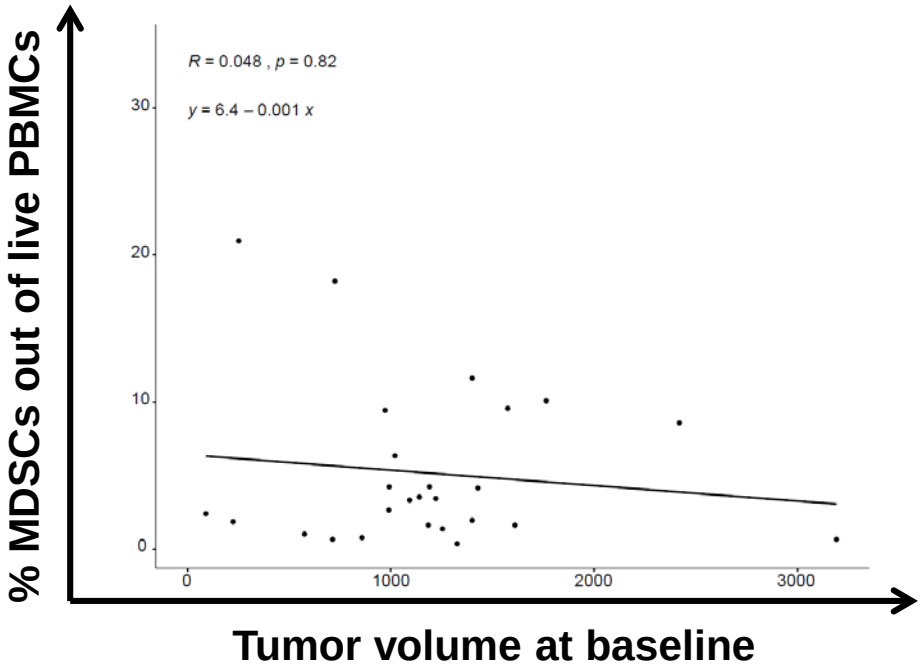
Figure S18

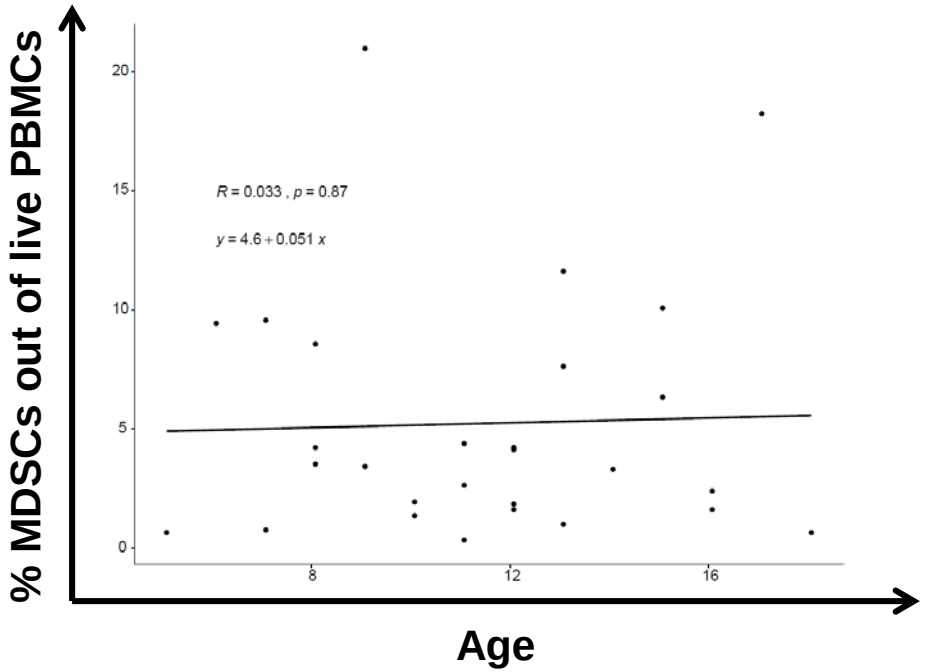
Linear models correlating baseline tumor volume and age with baseline MDSC abundance utilizing Spearman and Pearson correlation methods

Pearson product moment correlation



Spearman rank-order correlation





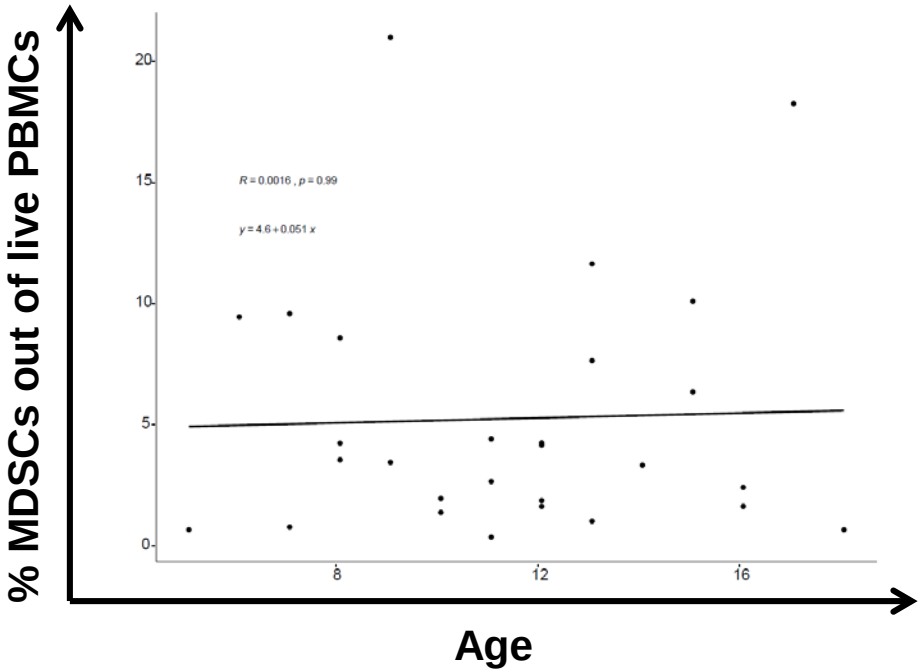
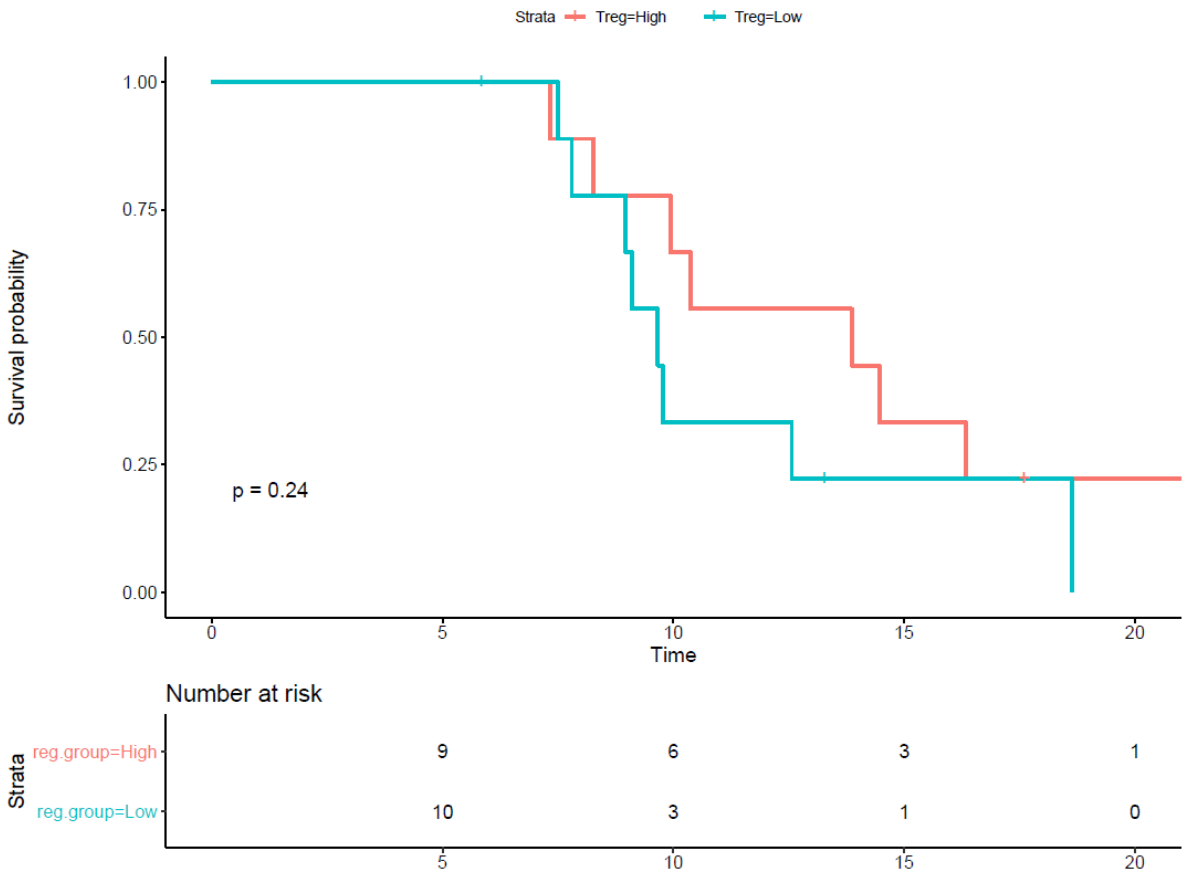


Figure S19

Kaplan-Meier survival curves visualizing OS and PFS of stratum A patients stratified based on Treg abundances above and below median

Overall Survival



Progression-free Survival

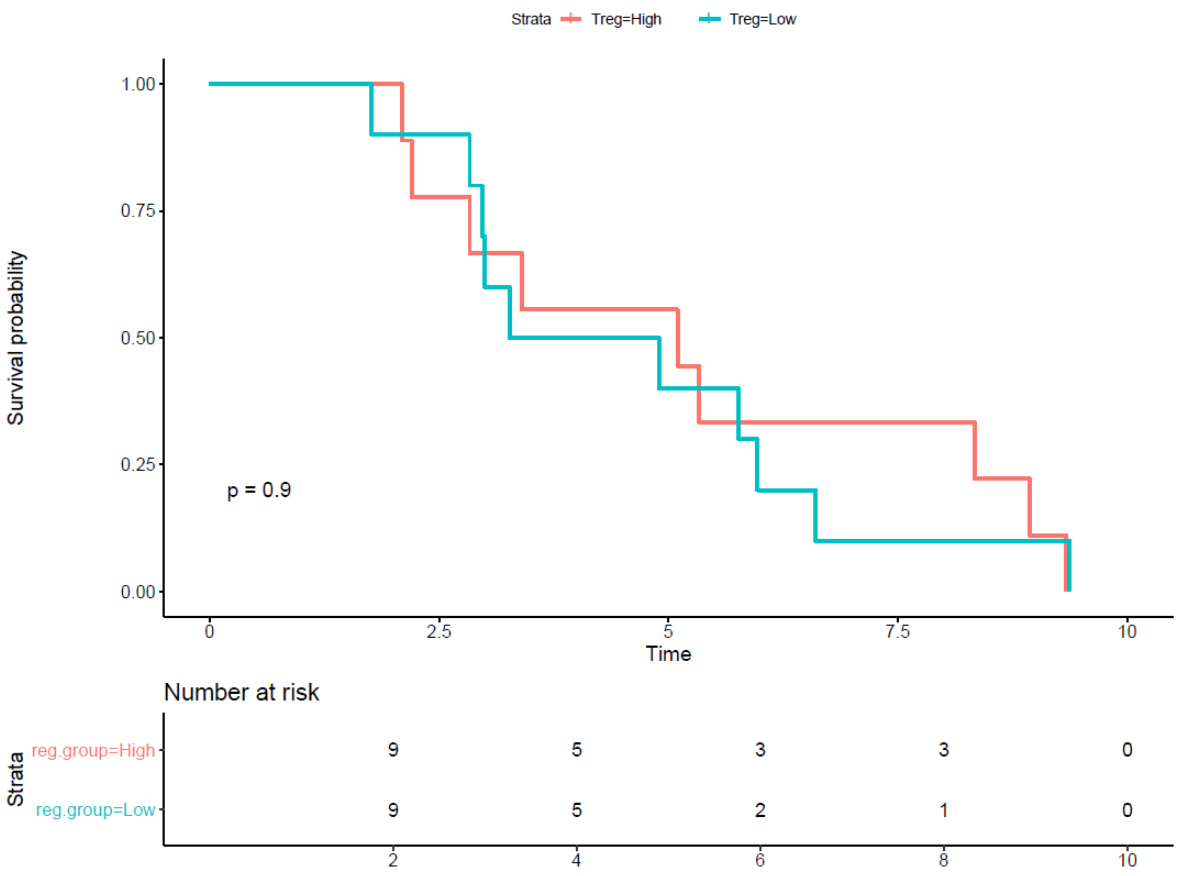


Figure S20

Spider plot depicting longitudinal patient-specific H3.3K27M-reactive CD8+ T-cell frequencies of patients based on dexamethasone administration during vaccine schedule

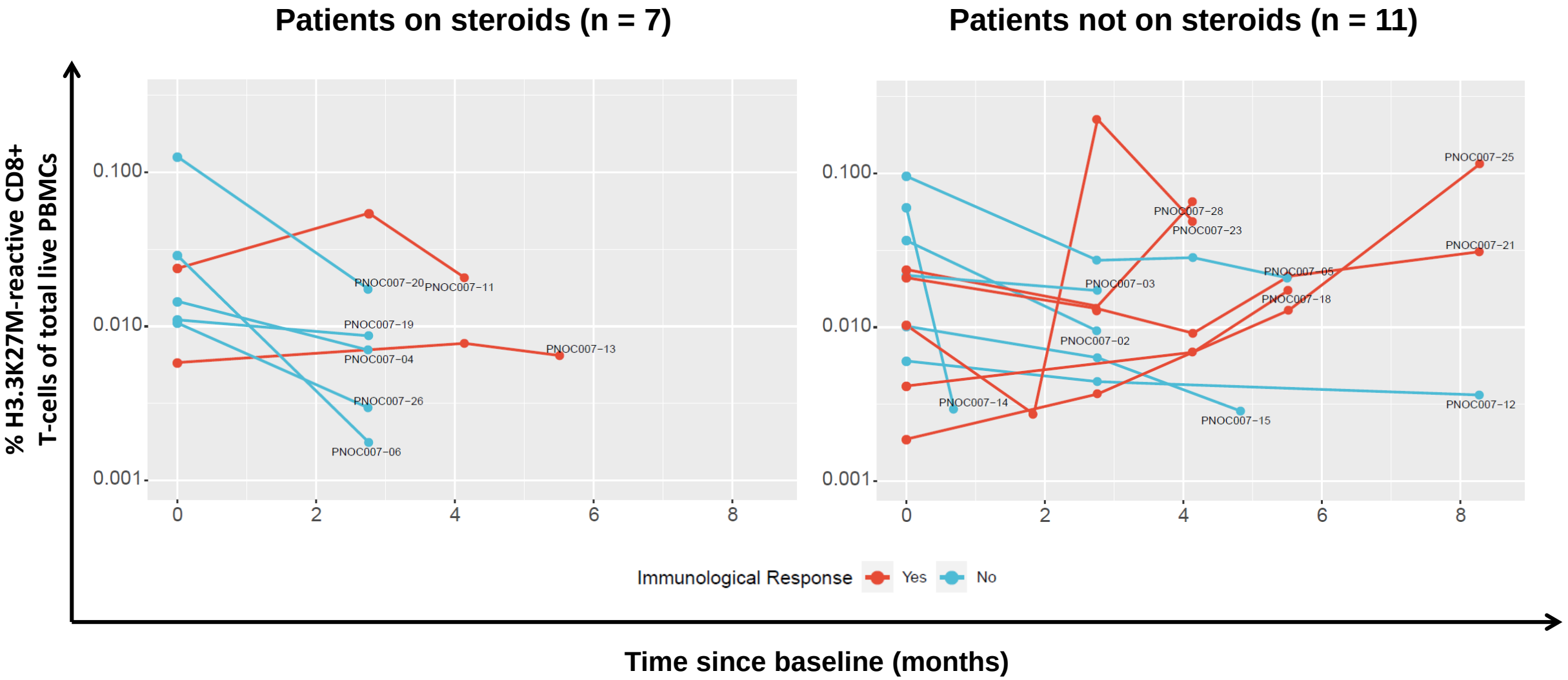
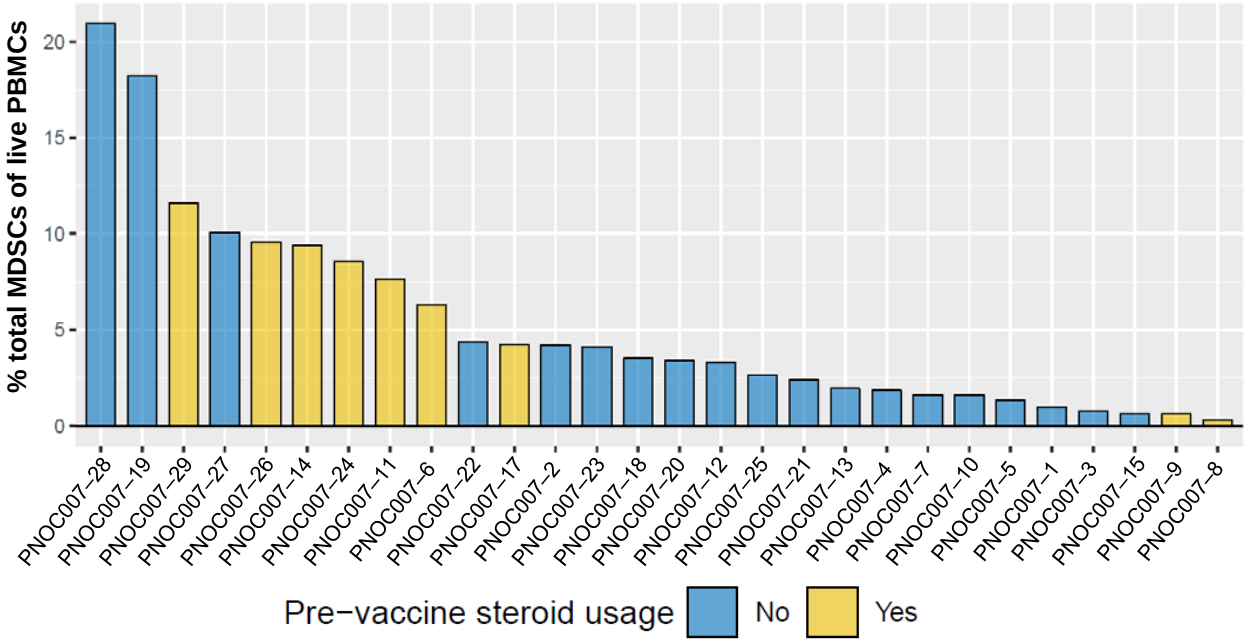
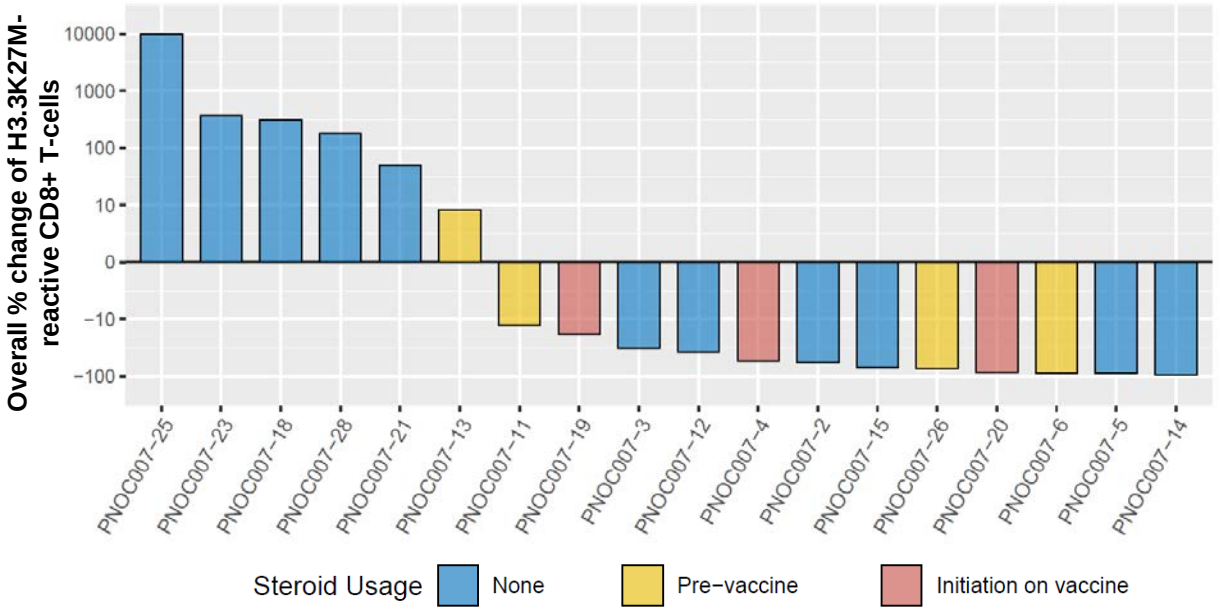
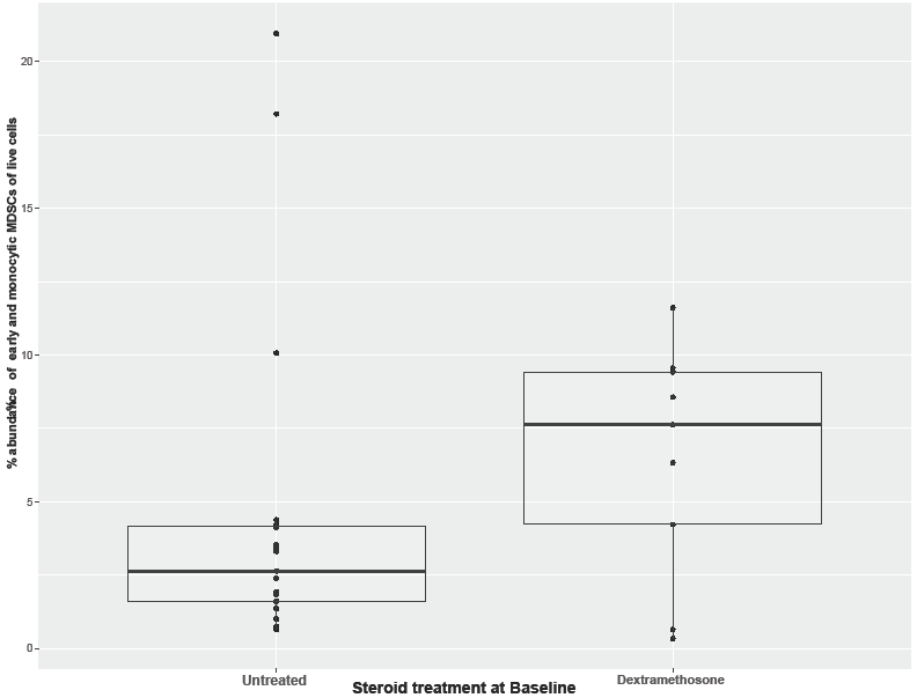

Patients on steroids (n = 7)
Patients not on steroids (n = 11)
% H3.3K27M-reactive CD8+
T-cells of total live PBMCs
0.100
0.010
0.001
0
2
4
6
8
PNOC007-20
PNOC007-11
PNOC007-19
PNOC007-04
PNOC007-13
PNOC007-26
PNOC007-06
PNOC007-28
PNOC007-23
PNOC007-25
PNOC007-21
PNOC007-05
PNOC007-03
PNOC007-18
PNOC007-02
PNOC007-14
PNOC007-15
PNOC007-12
Immunological Response
Yes
No
Time since baseline (months)

Figure S21

Dexamethasone administration is associated with circulatory MDSC levels and negatively associated with rates of CD8+ T-cell responses and survival in strata A and B patients. *Top left,* waterfall plot depicting the overall levels of circulatory MDSCs at baseline among strata A and B patients. *Bottom left,* boxplot depicting overall levels of circulatory MDSCs at baseline among strata A and B patients. *Top right,* waterfall plot depicting the overall longitudinal percent change of H3.3K27M-reactive CD8+ T-cells at the final time point analyzed relative to baseline for patients treated with dexamethasone pre-treatment (*yellow*), patients receiving dexamethasone after commencement of vaccination (*red*), and patients not receiving dexamethasone on study. (*blue*). *Bottom right,* Kaplan-Meier survival curves visualizing the OS of strata A and B patients based on pre-treatment dexamethasone administration. Cohorts were divided by patients not receiving dexamethasone at baseline (*blue*), patients receiving 1mg doses of dexamethasone (*red*), and patients receiving 2mg doses of dexamethasone (*green*).


% total MDSCs of live PBMCs
20
15
10
5
0
PNOC007−28
PNOC007−19
PNOC007−29
PNOC007−27
PNOC007−26
PNOC007−14
PNOC007−24
PNOC007−11
PNOC007−6
PNOC007−22
PNOC007−17
PNOC007−2
PNOC007−23
PNOC007−18
PNOC007−20
PNOC007−12
PNOC007−25
PNOC007−21
PNOC007−13
PNOC007−4
PNOC007−7
PNOC007−10
PNOC007−5
PNOC007−1
PNOC007−3
PNOC007−15
PNOC007−9
PNOC007−8
Pre−vaccine steroid usage
No
Yes


Overall % change of H3.3K27M-reactive CD8+ T-cells
10000
1000
100
10
0
−10
−100
PNOC007−25
PNOC007−23
PNOC007−18
PNOC007−28
PNOC007−21
PNOC007−13
PNOC007−11
PNOC007−19
PNOC007−3
PNOC007−12
PNOC007−4
PNOC007−2
PNOC007−15
PNOC007−26
PNOC007−20
PNOC007−6
PNOC007−5
PNOC007−14
Steroid Usage
None
Pre−vaccine
Initiation on vaccine


% abundance of early and monocytic MDSCs of live cells
20
15
10
5
0
Untreated
Dextramethosone
Steroid treatment at Baseline

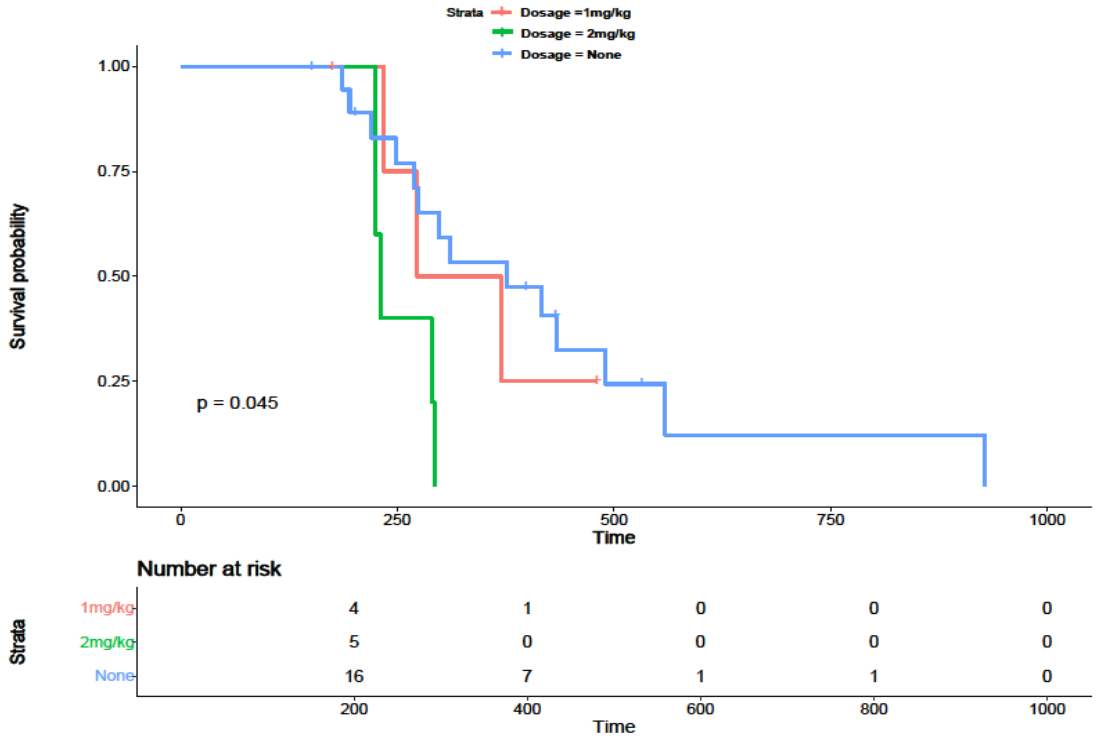
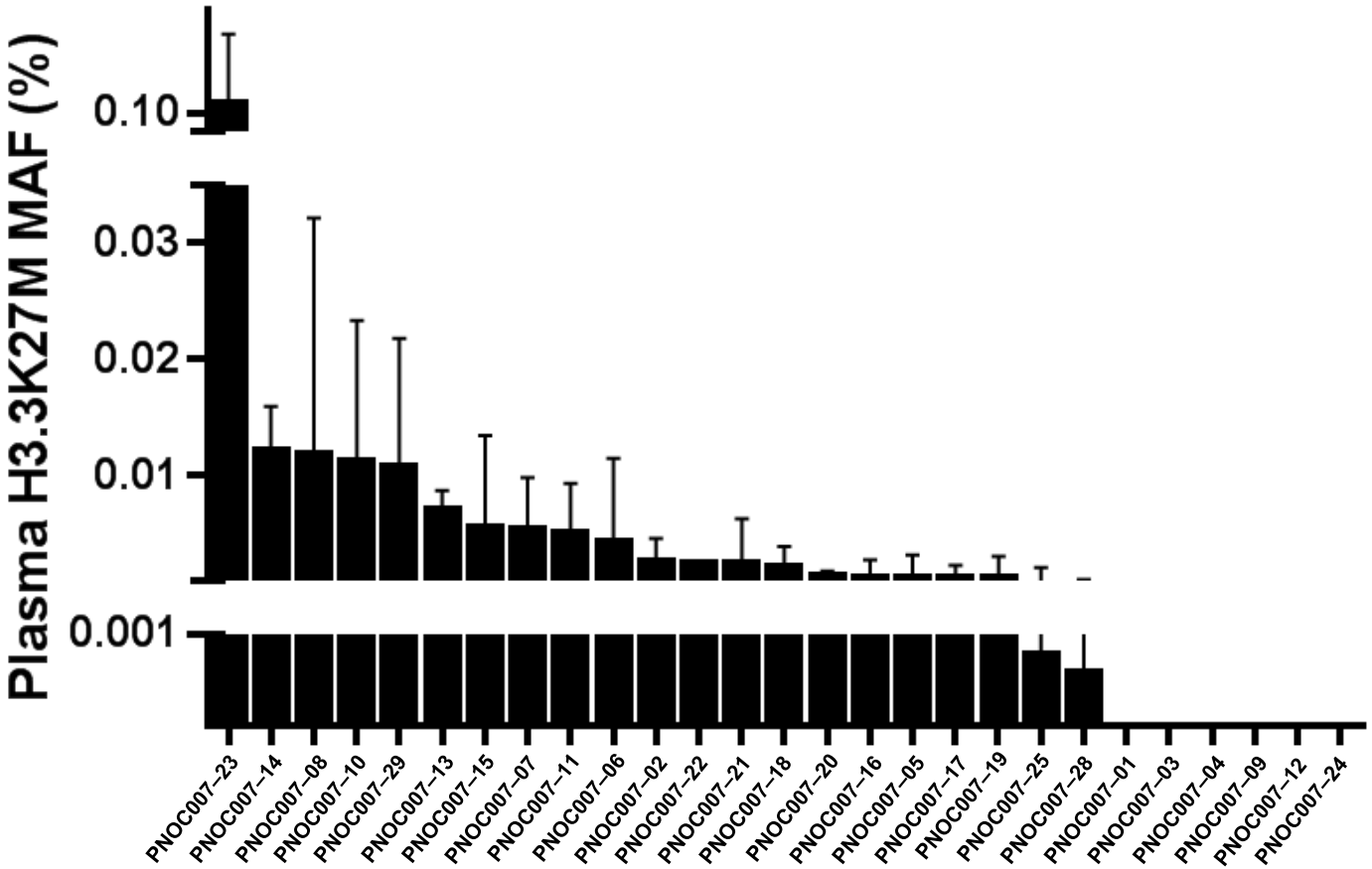
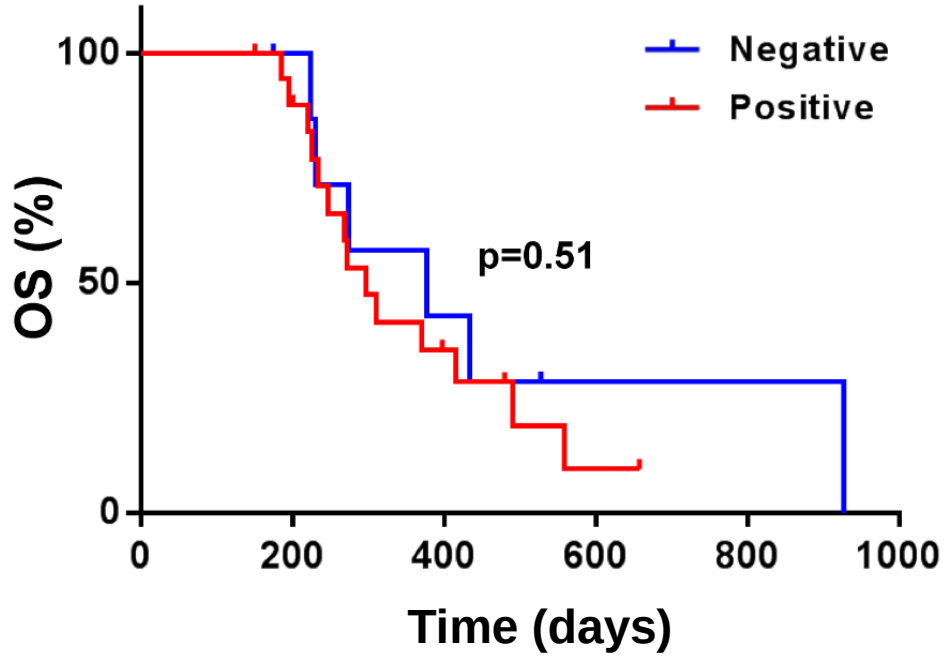

Strata
Dosage =1mg/kg
Dosage = 2mg/kg
Dosage = None
Survival probability
1.00
0.75
0.50
0.25
0.00
p = 0.045
0
250
500
750
1000
Time
Number at risk
Strata
1mg/kg
2mg/kg
None
4 1 0 0 0
5 0 0 0 0
16 7 1 1 0
200
400
600
800
1000
Time

Figure S22

Post-radiation therapy mutation allelic frequency as assessed by liquid biopsy is not predictive of improved patient outcomes. *Left*, plasma ctDNA, defined by H3.3K27M mutation allele frequency (MAF) >=0.001%, was detected at baseline (Week 0), in 19 out of 27 patients (70.4%; n=13 Stratum A, n=6 Stratum B). *Right,* Kaplan-Meier survival curves visualizing the OS (top) and PFS (bottom) for patients from which plasma ctDNA was detected at baseline (*red*) compared to patients who did not exhibit plasma ctDNA (*blue*).


Plasma H3.3K27M MAF (%)
0.10
0.03
0.02
0.01
0.001
PNOC007–23
PNOC007–14
PNOC007–08
PNOC007–10
PNOC007–29
PNOC007–13
PNOC007–15
PNOC007–07
PNOC007–11
PNOC007–06
PNOC007–02
PNOC007–22
PNOC007–21
PNOC007–18
PNOC007–20
PNOC007–16
PNOC007–05
PNOC007–17
PNOC007–19
PNOC007–25
PNOC007–28
PNOC007–01
PNOC007–03
PNOC007–04
PNOC007–09
PNOC007–12
PNOC007–24


Negative
Positive
OS (%)
100
50
0
p=0.51
0
200
400
600
800
1000
Time (days)

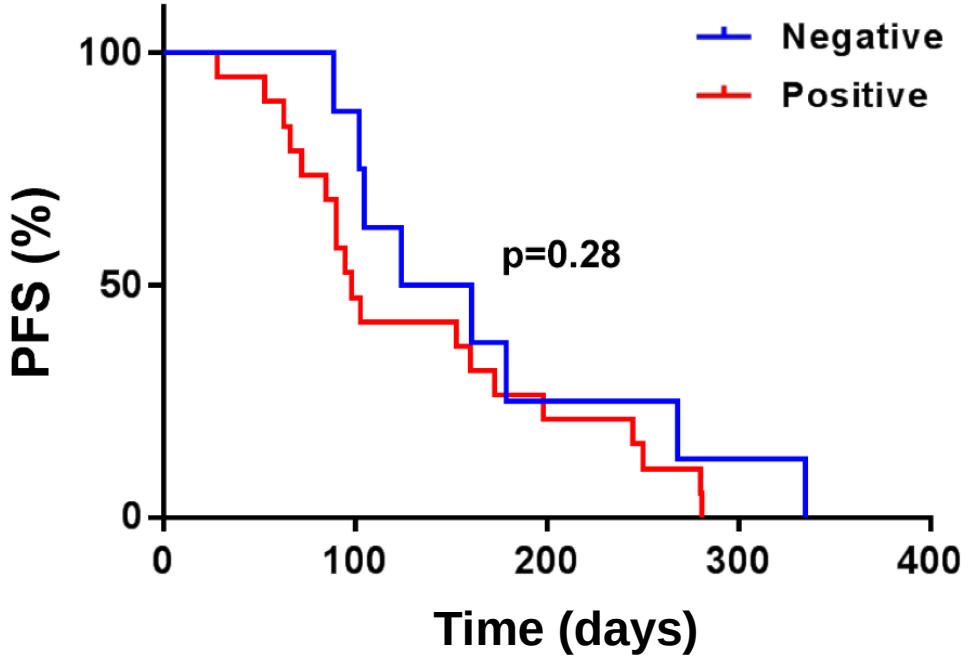
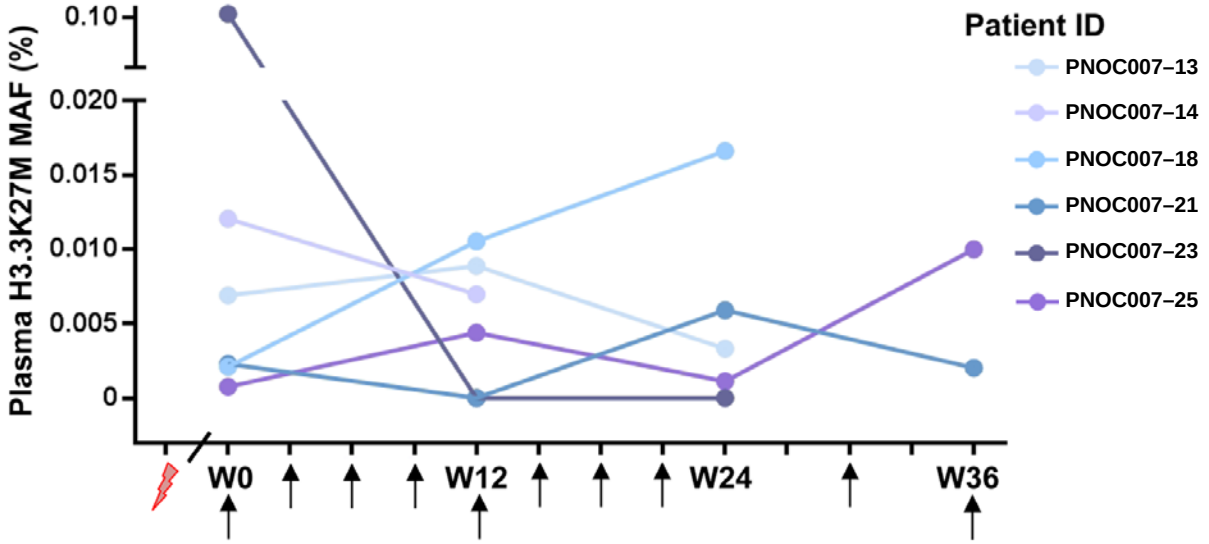

Negative
Positive
PFS (%)
100
50
0
p=0.28
0
100
200
300
400
Time (days)

Figure S23

Longitudinal fluctuations in plasma ctDNA do not correlate with patient outcomes. *Left,* longitudinal monitoring of plasma ctDNA indicates changes in ctDNA abundance during the course of therapy, as exemplified by serial ctDNA analysis from six patients (PNOC007-13, -14, -18, -21, -23 and -25). *Right*, Kaplan-Meier curve visualizing the OS of patients exhibiting an increase in plasma ctDNA between baseline and week 12 (*red*) compared to patients who showed a decrease during this time (*blue*).


Plasma H3.3K27M MAF (%)
0.10
0.020
0.015
0.010
0.005
0
W0
W12
W24
W36
Patient ID
PNOC007–13
PNOC007–14
PNOC007–18
PNOC007–21
PNOC007–23
PNOC007–25

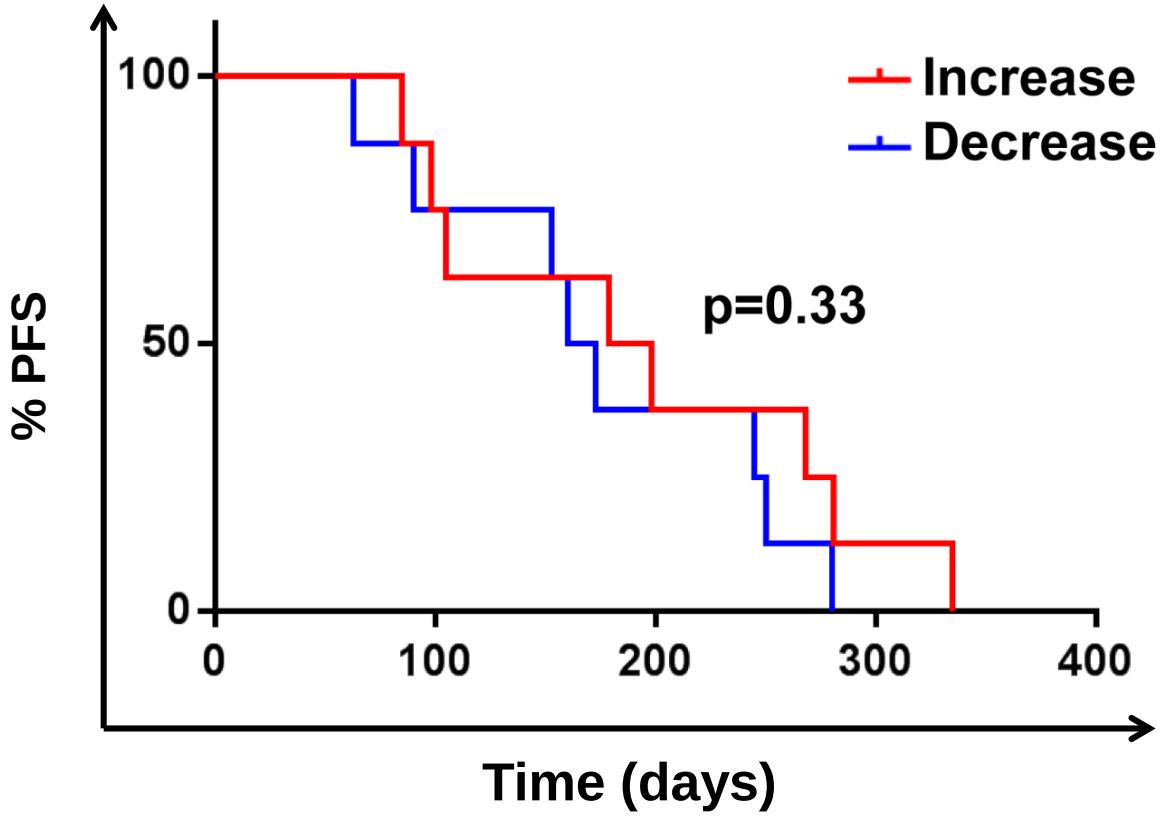

% PFS
100
50
0
0
100
200
300
400
Time (days)
Increase
Decrease
p=0.33

Figure S24

Kaplan-Meier survival curve visualizing OS of patients stratified based on a median threshold of tumor volume at baseline

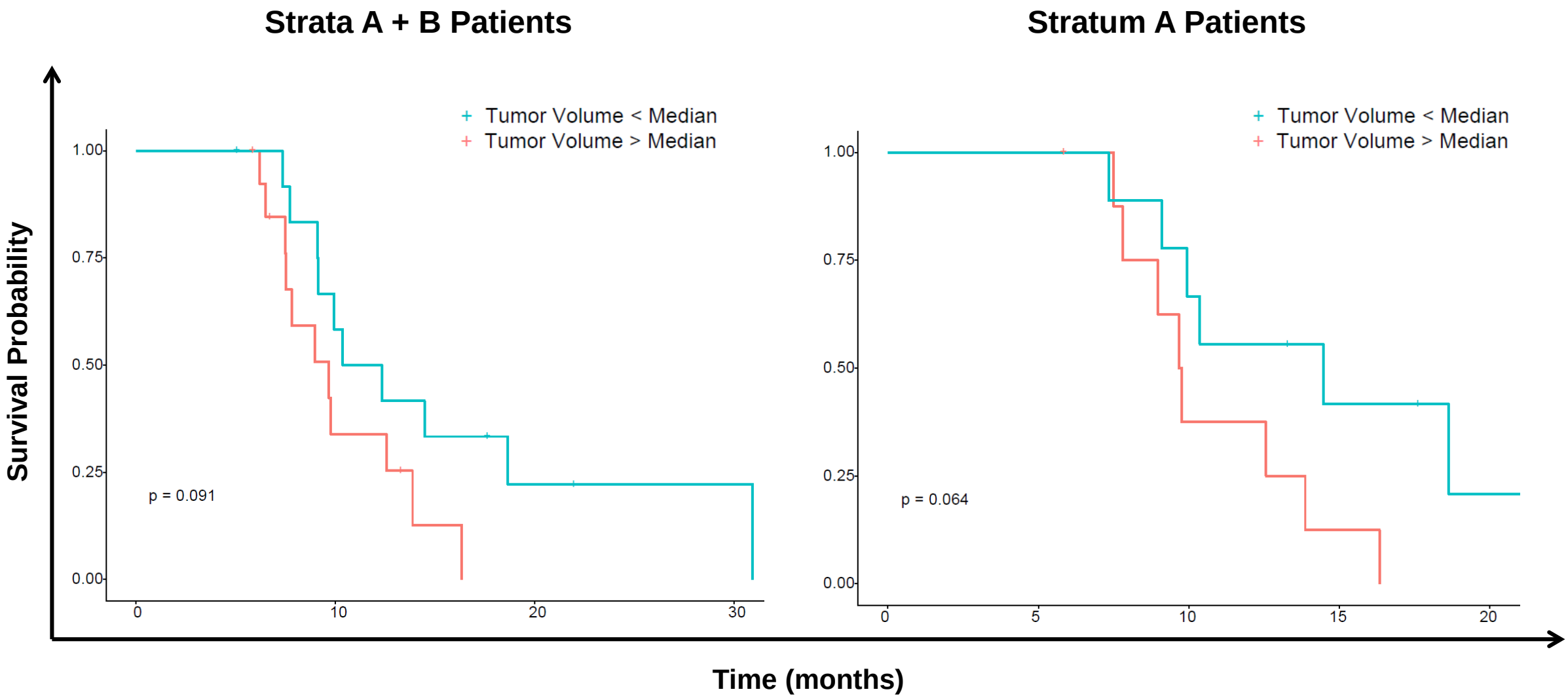

Strata A + B Patients
Stratum A Patients
Tumor Volume < Median
Tumor Volume > Median
Survival Probability
1.00
0.75
0.50
0.25
0.00
p = 0.091
p = 0.064
0
10
20
30
5
15
Time (months)

Figure S25

Kaplan-Meier survival curve visualizing OS of patients stratified based on upper and lower quartiles of tumor volume at baseline

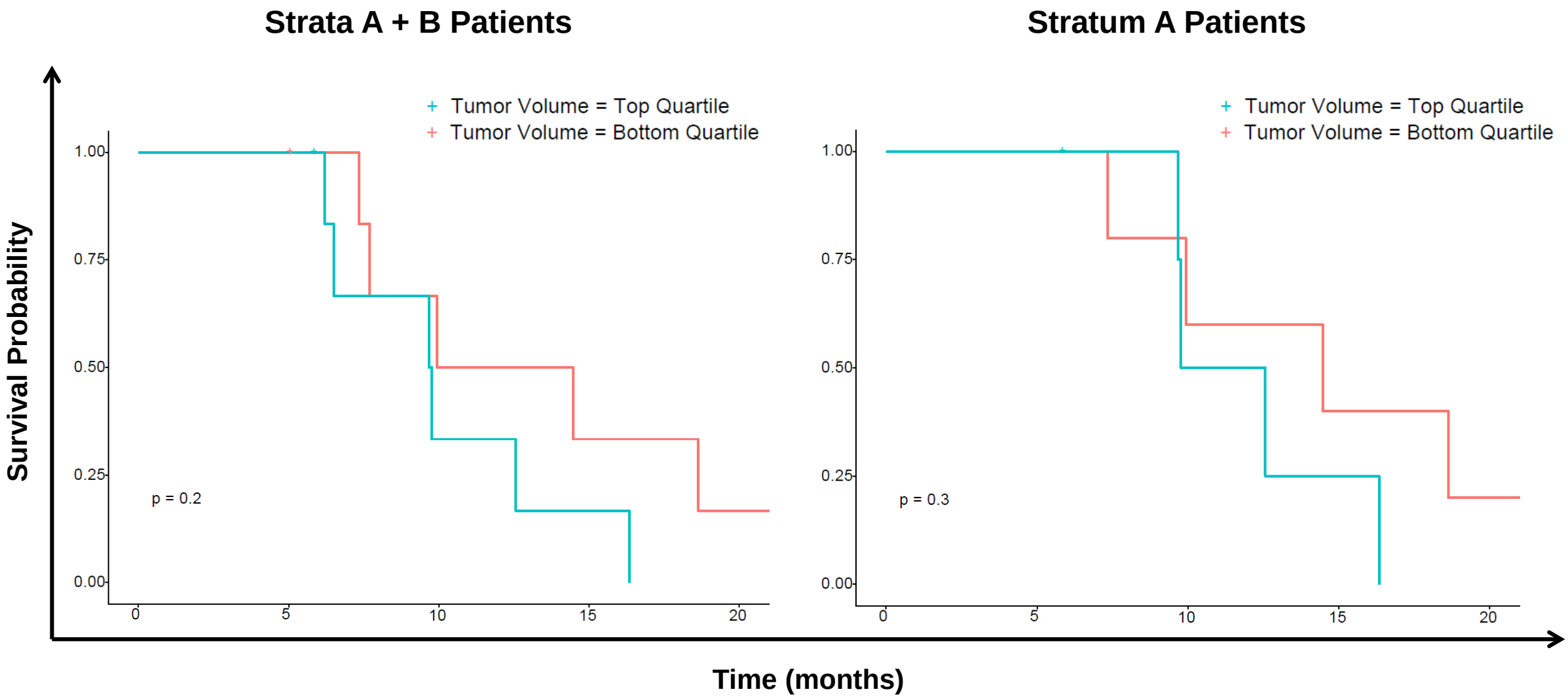
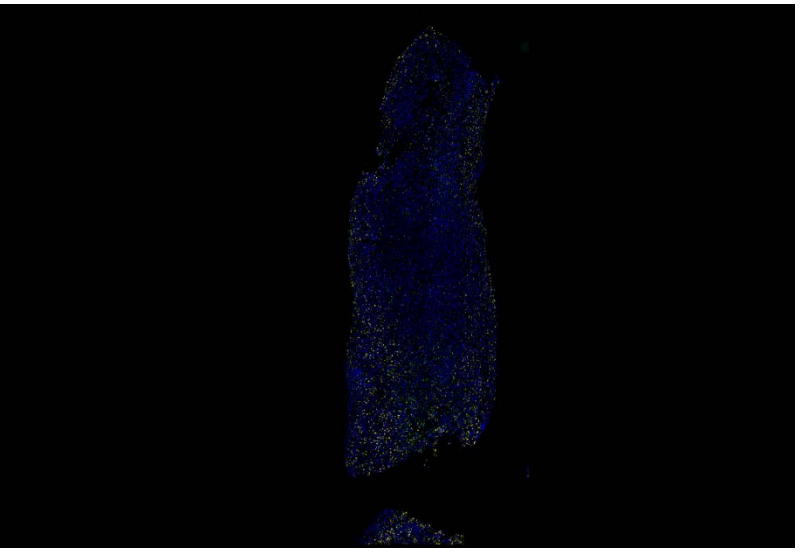

Strata A + B Patients
Tumor Volume = Top Quartile
Tumor Volume = Bottom Quartile
1.00
0.75
0.50
0.25
0.00
p = 0.2
0
5
10
15
20
Stratum A Patients
Tumor Volume = Top Quartile
Tumor Volume = Bottom Quartile
1.00
0.75
0.50
0.25
0.00
p = 0.3
0
5
10
15
20
Survival Probability
Time (months)

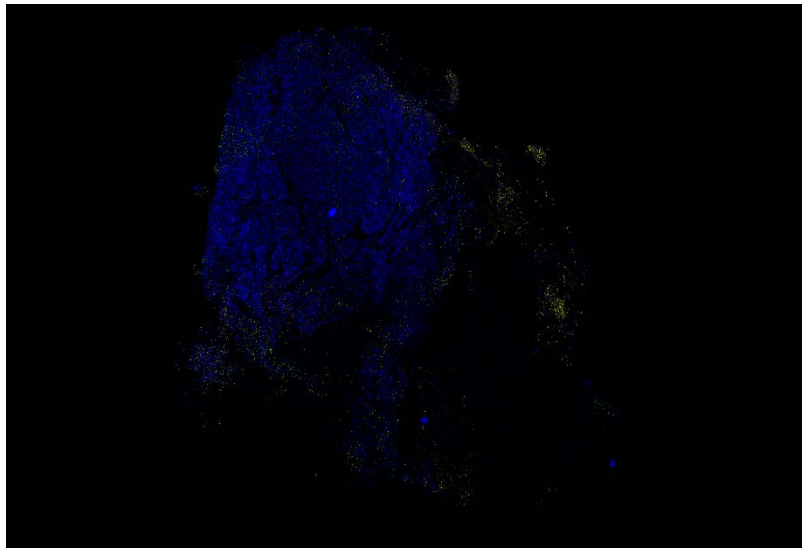
Figure S26

Representative immunofluorescence images quantifying MDSC abundance in tumor microenvironment. Live cells were identified using DAPI nuclear stain. MDSCs were identified using FITC-conjugated Iba1, Cy5-conjugated CD33, and rhodamine-conjugated HLA-DR antibodies. MDSCs were classified as live cells that were $Iba1^{+}$ $CD33^{+}$ $HLA\text{-}DR^{lo/-}$.

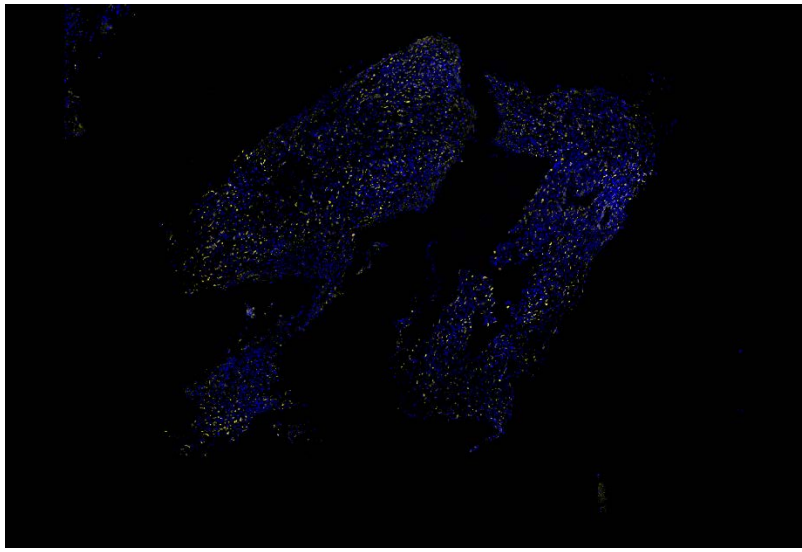
PNOC007-05



PNOC007-17



PNOC007-22



Patient ID	Number of cells analyzed:	% MDSCs of live cells in TME	% MDSCs of live cells in PBMCs
PNOC007-05	4.25e4	0.019%	1.36%
PNOC007-17	6.44e6	0.153%	4.22%
PNOC007-22	4.22e5	0.0047%	4.38%

Figure S27

Corroboratory plot between circulatory MDSCs analyzed on CyTOF and MDSCs identified through immunohistochemistry.

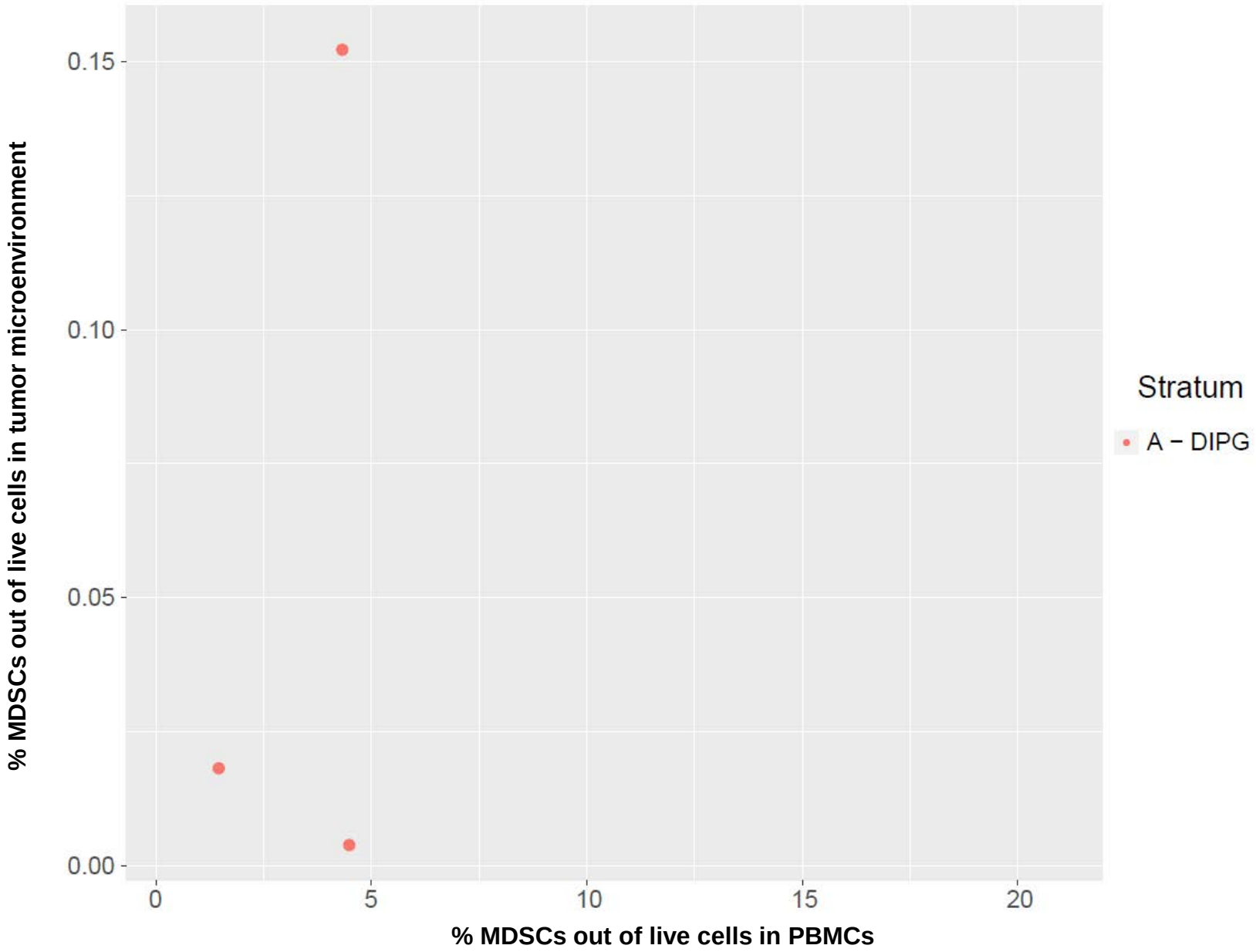

0.15
0.10
0.05
0.00
% MDSCs out of live cells in tumor microenvironment
0
5
10
15
20
% MDSCs out of live cells in PBMCs
Stratum
A − DIPG

Figure S28

PNOC007 clinical trial therapy schema

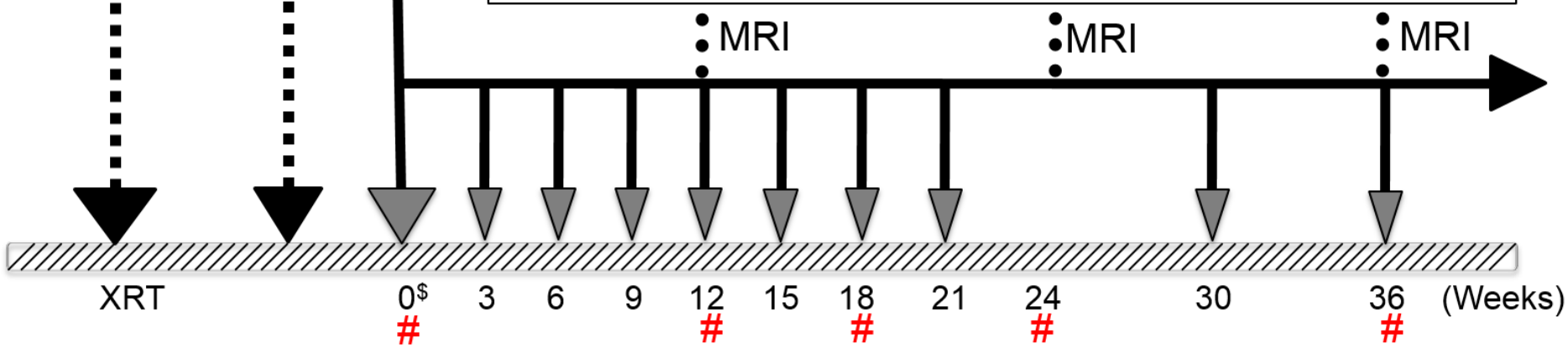
Therapy Schema

XRT following diagnosis of H3.3K27M and HLA-A2 positive DIPG (n=19) and other H3.3K27M and HLA-A2 positive gliomas including spinal cord gliomas (n=10)

Baseline MRI/Lab/Clinic evaluation must be obtained within 2 weeks of enrollment; therapy should start 2-8 weeks after completion of XRT

H3.3K27M specific peptide-vaccines every 3 weeks starting 2-8 weeks following the completion of XRT. Up to 8 doses of the vaccine/poly-ICLC combination will be given in the first 21 weeks. Children who tolerate therapy well and have stable or improved disease evaluations can continue with vaccine (after first 8 doses, vaccine will be given every 6 weeks starting at Week 30; total 96 weeks)

MRIs every 12 weeks at Week 12, 24, 36, 48, etc.
Immuno-monitoring (#) Week 0,12,18, 24 and then every 12 weeks thereafter (Week 36, 48, 60, etc.)



$If evidence of radiation induced pseudo-progression on baseline MRI, poly-ICLC administration can be held for up to 12 weeks