Supplemental figure Captions

Figure S1: Boxplot of best overall radiologic response (%) per histological subtype

Figure S2: Swimmer plot

Figure S3: PD-L1 and radiologic response
A. PD-L1 by histology (Fisher's exact test)
B. Spider plot
C. Best overall response by PD-L1 expression in EAC and EAC and ESCC combined (categorical BOR)
D. Best overall response by PD-L1 expression in EAC and EAC and ESCC combined (boxplot, numerical BOR)

Figure S4: PD-L1 and survival
This includes PD-L1 CPS from archival tissue.
A. EAC and ESCC, CPS < 1 vs. ≥ 1
B. EAC and ESCC, CPS < 1 vs. 1-10 vs. ≥ 10

Figure S5: On-treatment flow cytometry data on tumor biopsy
A. Spider plot of the patients with on treatment biopsies analyzed by flow cytometry
B. Overview of changes in major cell populations relative to CD45 during treatment
C. Bar graph of major cell populations as a percentage of CD45+ cells, showing baseline and on treatment / end of treatment sample

Figure S6: PBMC flow by response
TCM = central memory T cells, TEM = effector memory T cells, TEMRA = effector memory T cells re-expressing CD45RA

Figure S7: PBMC flow by the number of prior lines of therapy
TCM = central memory T cells, TEM = effector memory T cells, TEMRA = effector memory T cells re-expressing CD45RA

Figure S8: PBMC flow by prior radiotherapy
TCM = central memory T cells, TEM = effector memory T cells, TEMRA = effector memory T cells re-expressing CD45RA

Figure S9: Kaplan Meier plots comparing progression-free and overall survival of patients with neutrophil-to-lymphocyte ratios below vs. above or equal to median at baseline.

Figure S10: NanoString, baseline
A. Heatmap of gene expression at baseline
B. Volcano plot of differential gene expression analysis, comparing patients with progressive disease with patients with stable disease or a partial response as best overall radiologic response
C. Gene set enrichment analysis (GSEA)

Figure S11: NanoString, on-treatment and archival
A. Heatmap showing unsupervised time series clustering of gene expression in on-treatment compared to baseline biopsies
B. Heatmap showing unsupervised time series clustering of gene expression in baseline compared to archival biopsies

Figure S1

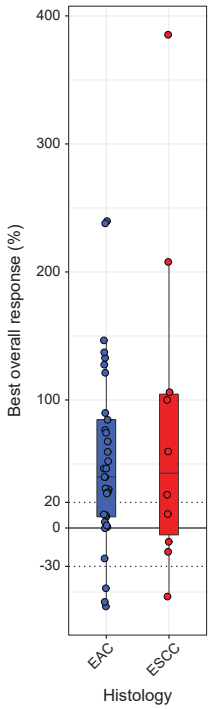

400
300
200
100
20
0
-30
Best overall response (%)
EAC
ESCC
Histology

Figure S2

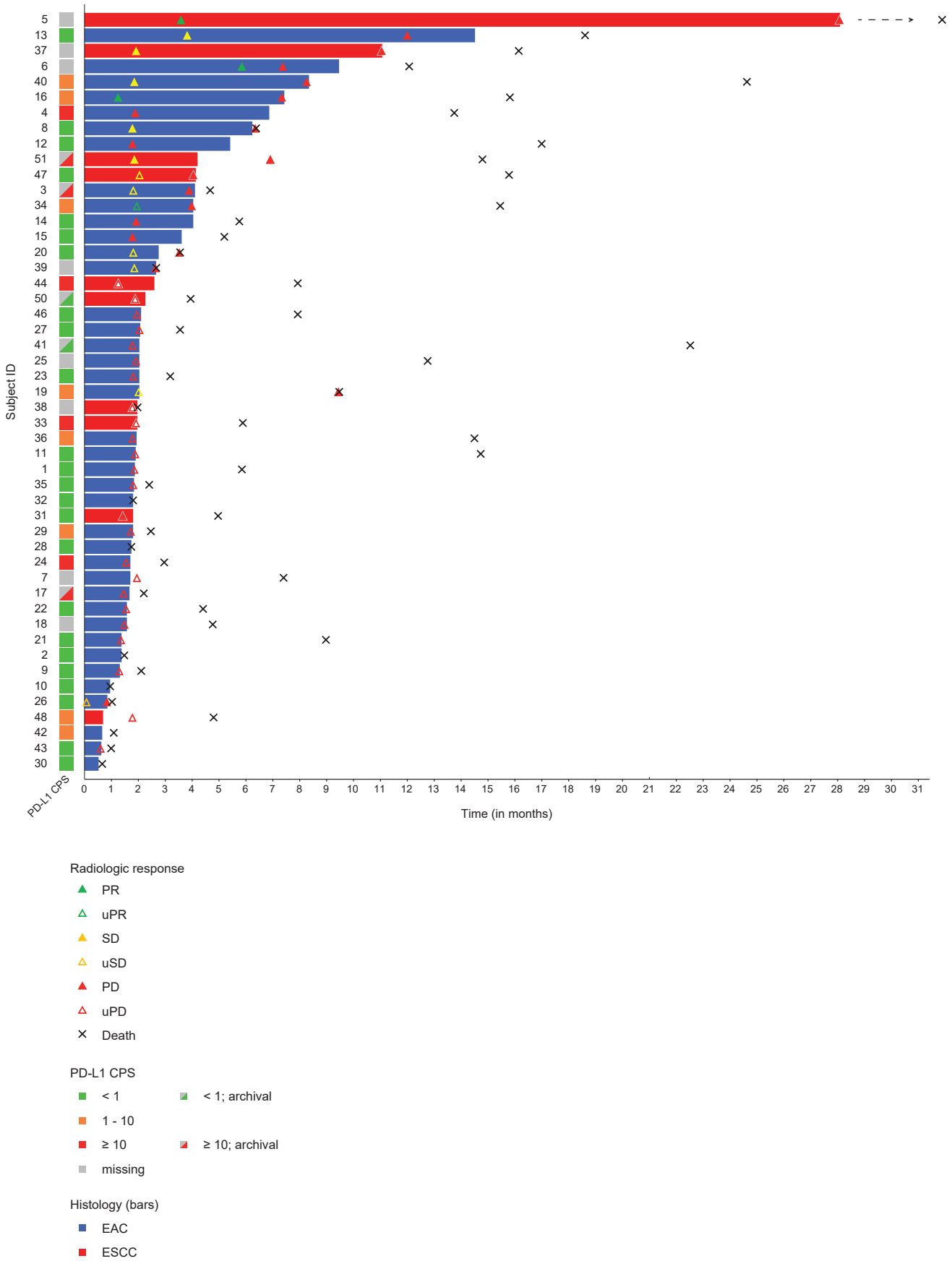

Subject ID
5
13
37
6
40
16
4
8
12
51
47
3
34
14
15
20
39
44
50
46
27
41
25
23
19
38
33
36
11
1
35
32
31
29
28
24
7
17
22
18
21
2
9
10
26
48
42
43
30
PD-L1 CPS
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31
Time (in months)
Radiologic response
PR
uPR
SD
uSD
PD
uPD
Death
PD-L1 CPS
< 1
< 1; archival
1 - 10
≥ 10
≥ 10; archival
missing
Histology (bars)
EAC
ESCC

Figure S3

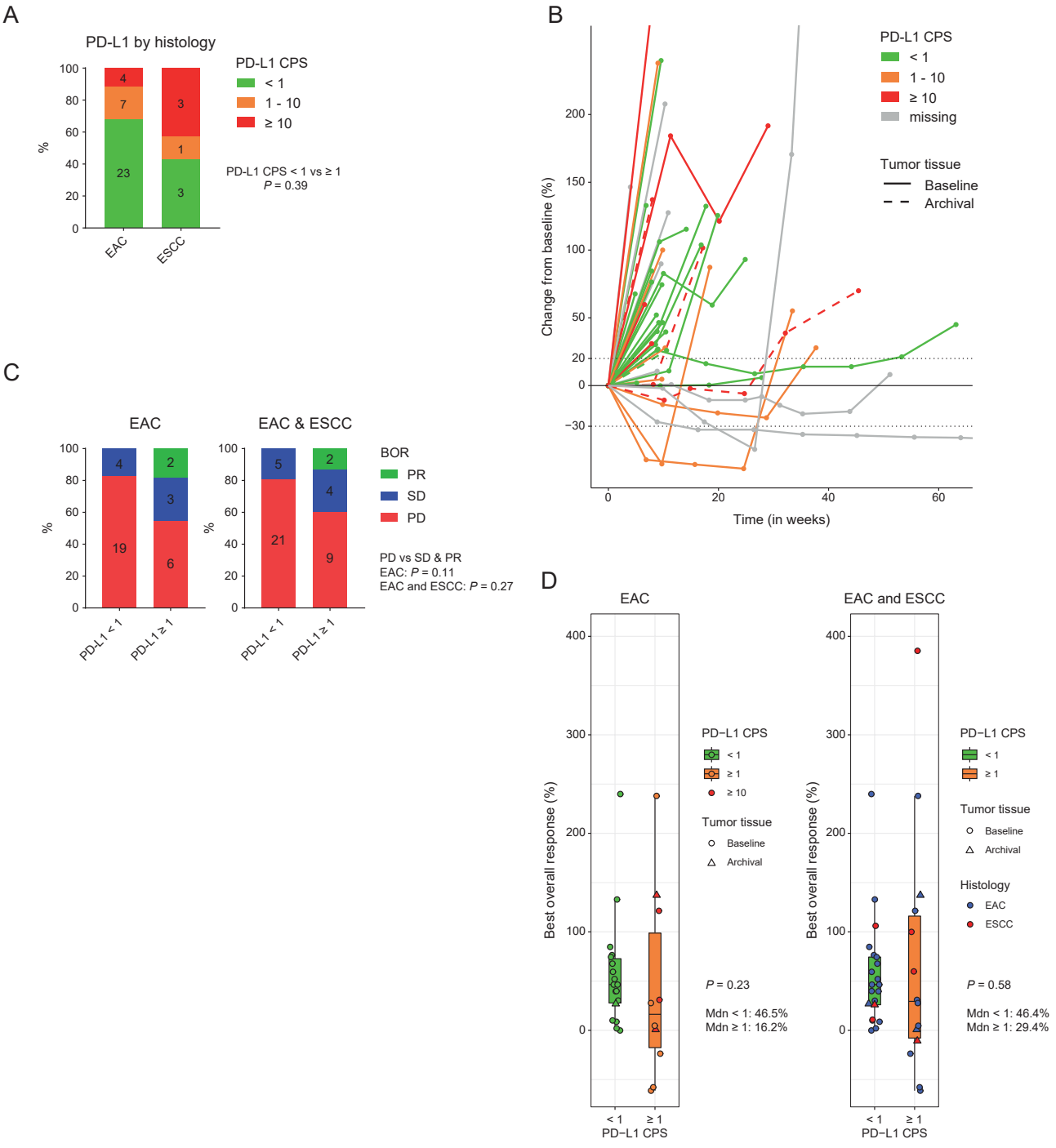
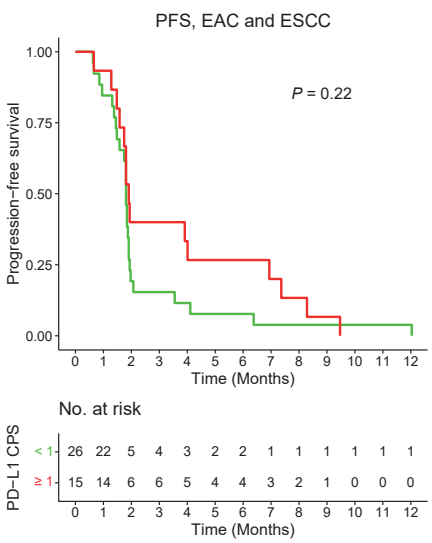
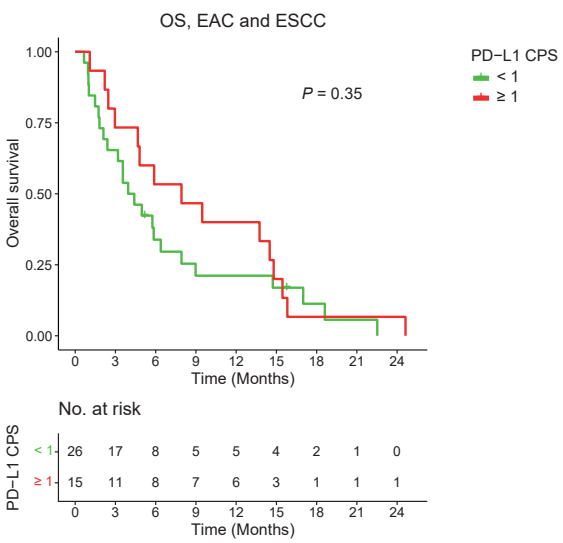

A
PD-L1 by histology
PD-L1 CPS
< 1
1 - 10
≥ 10
%
EAC
ESCC
PD-L1 CPS < 1 vs ≥ 1
P = 0.39
B
PD-L1 CPS
< 1
1 - 10
≥ 10
missing
Tumor tissue
Baseline
Archival
Change from baseline (%)
Time (in weeks)
C
EAC
EAC & ESCC
BOR
PR
SD
PD
PD vs SD & PR
EAC: P = 0.11
EAC and ESCC: P = 0.27
PD-L1 < 1
PD-L1 ≥ 1
D
EAC
EAC and ESCC
Best overall response (%)
PD−L1 CPS
Tumor tissue
Baseline
Archival
Histology
EAC
ESCC
P = 0.23
Mdn < 1: 46.5%
Mdn ≥ 1: 16.2%
P = 0.58
Mdn < 1: 46.4%
Mdn ≥ 1: 29.4%

Figure S4

A

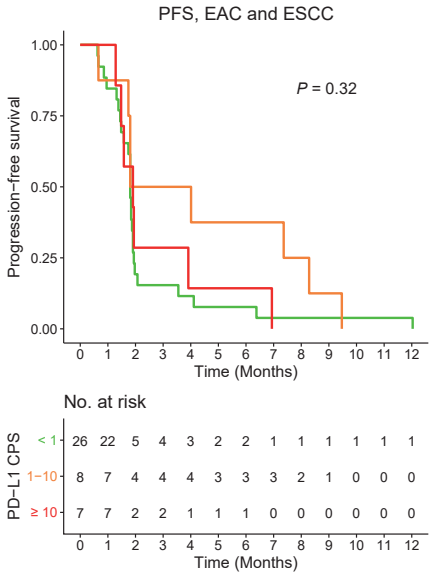


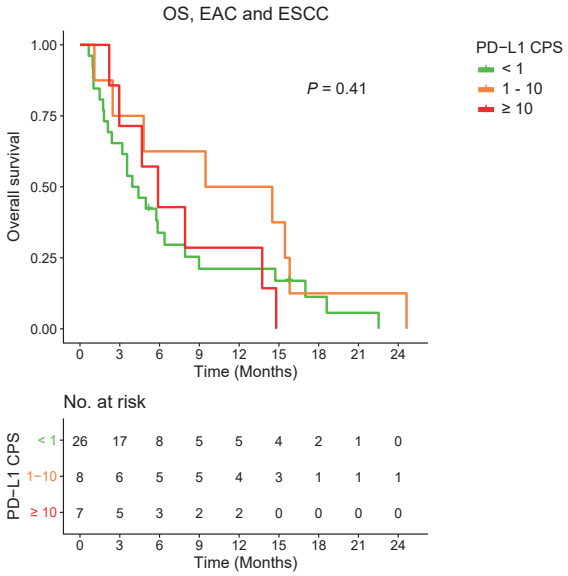


Population (n)	Median PFS (months) (95% CI)	6-month PFS rate (%, 95% CI)	12-month PFS rate (%, 95% CI)
Overall (49)	1.84 (1.81-1.97)	18.4 (10.2-33.1)	4.1 (1.1-15.9)
PD-L1 < 1 (26)	1.81 (1.58-1.94)	7.7 (2.0-29.1)	3.9 (0.6-26.3)
PD-L1 ≥ 1 (15)	1.91 (1.74-7.36)	26.7 (11.5-61.7)	0 (NA)

Population (n)	Median OS (months) (95% CI)	6-month OS rate (%, 95% CI)	12-month OS rate (%, 95% CI)
Overall (49)	5.75 (3.95-9.47)	44.6 (32.6-61.1)	31.9 (21.1-48.2)
PD-L1 < 1 (26)	4.18 (2.40-7.92)	33.9 (19.6-58.4)	21.2 (9.8-45.5)
PD-L1 ≥ 1 (15)	7.92 (4.67-15.45)	55.3 (33.2-85.6)	40.0 (21.5-74.3)

B

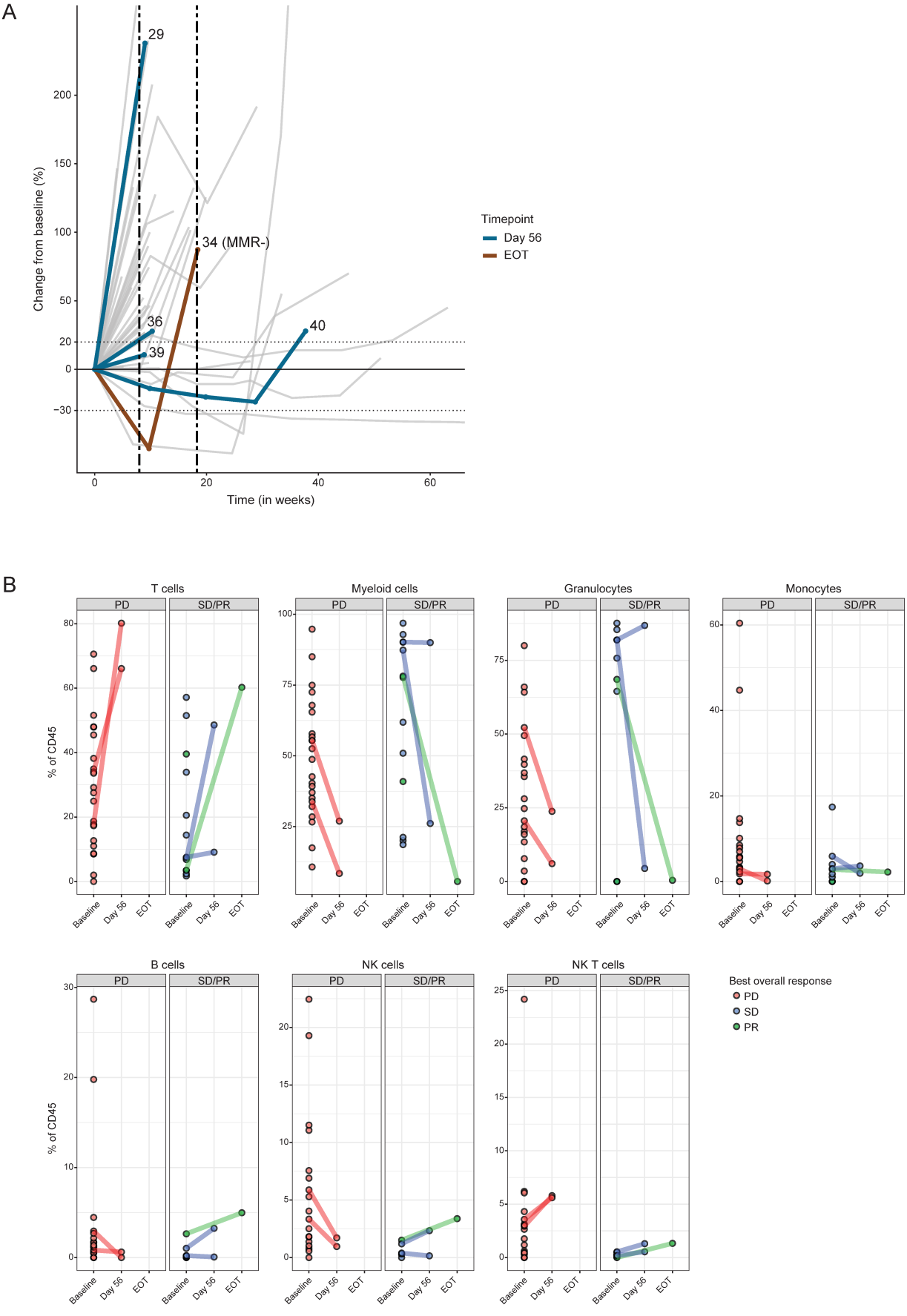




Population (n)	Median PFS (months) (95% CI)	6-month PFS rate (%, 95% CI)	12-month PFS rate (%, 95% CI)
Overall (49)	1.84 (1.81-1.97)	18.4 (10.2-33.1)	4.1 (1.1-15.9)
PD-L1 < 1 (26)	1.81 (1.58-1.94)	7.7 (2.0-29.1)	3.9 (0.6-26.3)
PD-L1 1-10 (8)	2.91 (1.81-NA)	37.5 (15.3-91.7)	0 (NA)
PD-L1 ≥ 10 (7)	1.91 (1.48-NA)	14.3 (2.3-87.7)	0 (NA)

Population (n)	Median OS (months) (95% CI)	6-month OS rate (%, 95% CI)	12-month OS rate (%, 95% CI)
Overall (49)	5.75 (3.95-9.47)	44.6 (32.6-61.1)	31.9 (21.1-48.2)
PD-L1 < 1 (26)	4.18 (2.40-7.92)	33.9 (19.6-58.4)	21.2 (9.8-45.5)
PD-L1 1 - 10 (8)	11.98 (4.80-NA)	62.5 (36.5-100)	50.0 (25.0-100)
PD-L1 ≥ 10 (7)	5.88 (2.96-NA)	42.9 (18.2-100)	28.6 (8.9-92.2)

Figure S5


A
29
34 (MMR-)
36
39
40
Change from baseline (%)
Time (in weeks)
Timepoint
Day 56
EOT
B
T cells
Myeloid cells
Granulocytes
Monocytes
B cells
NK cells
NK T cells
PD
SD/PR
% of CD45
Baseline
Day 56
EOT
Best overall response
PD
SD
PR

C

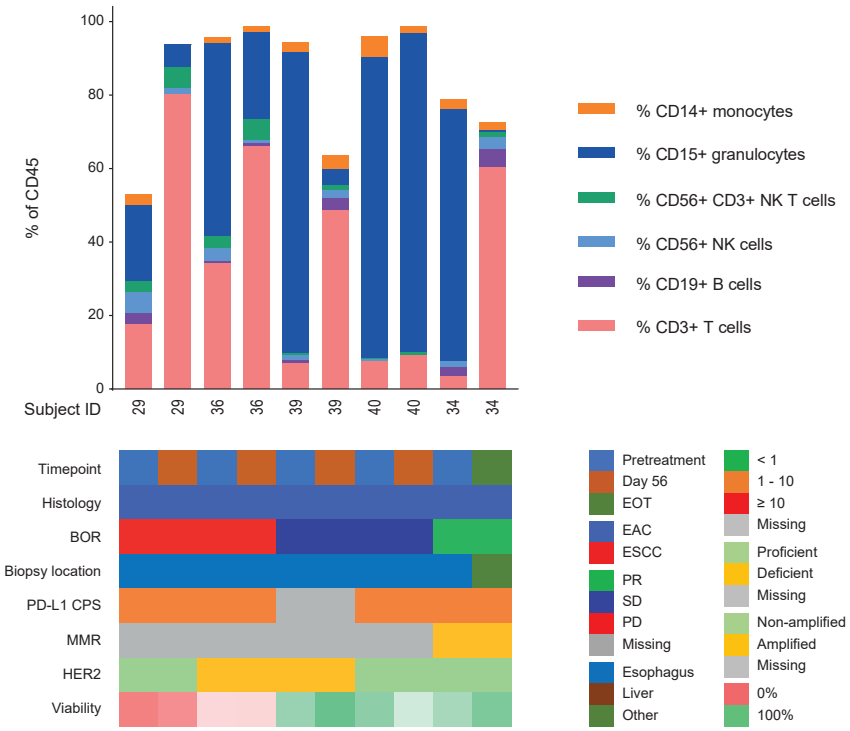
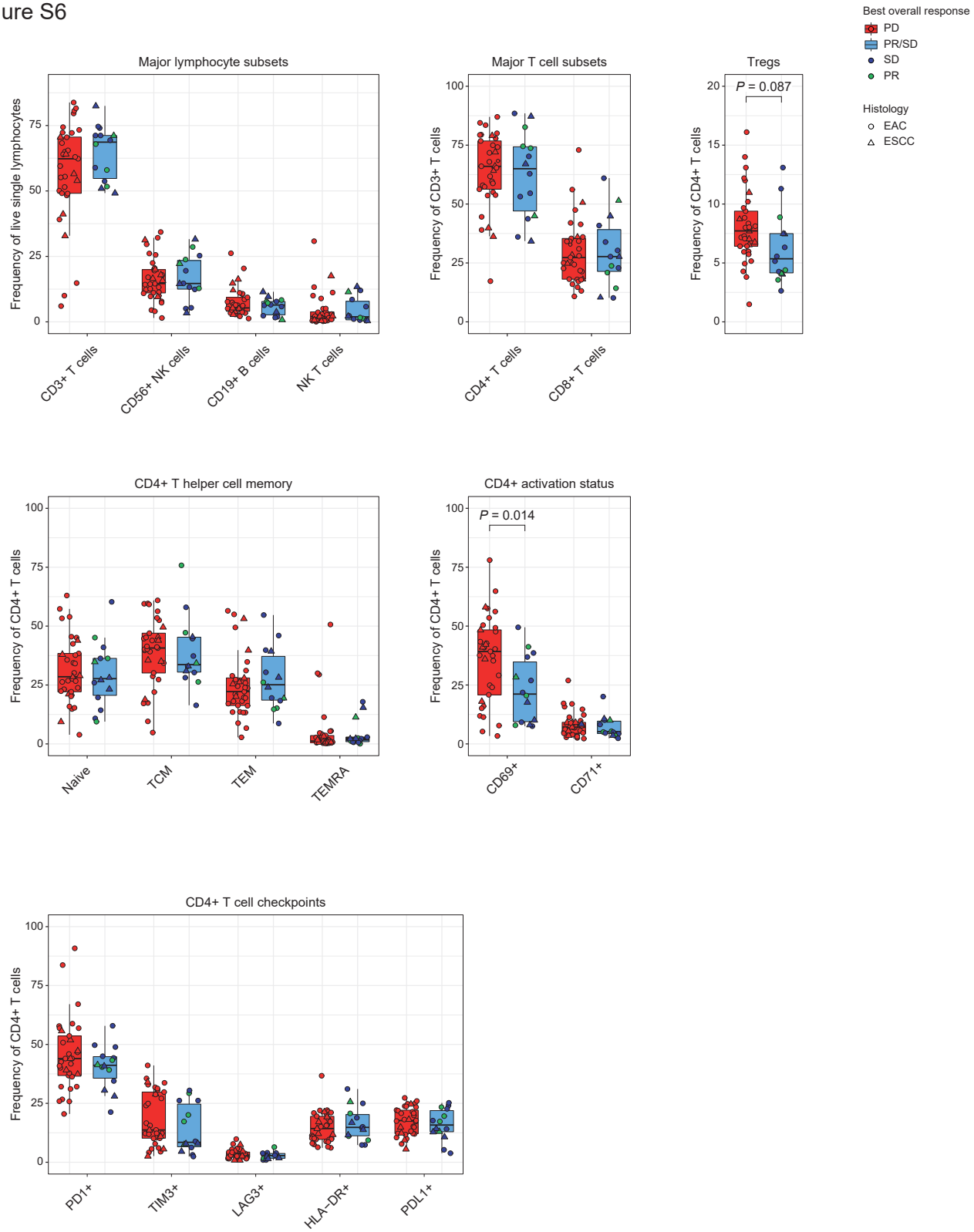
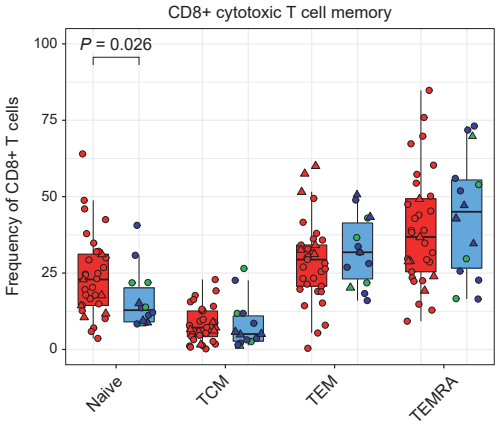
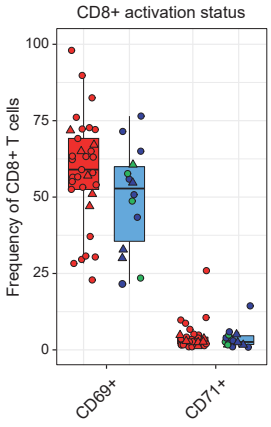
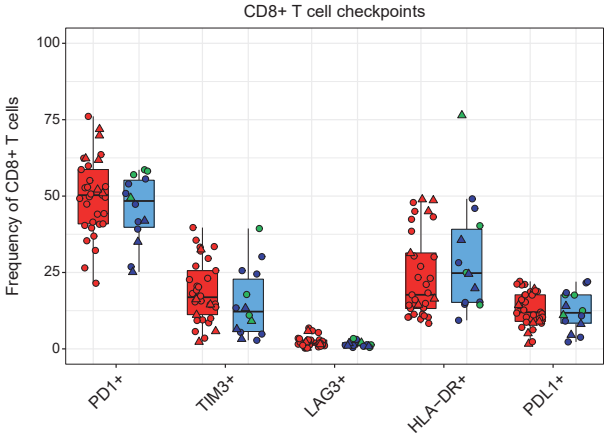

100
80
60
40
20
0
% of CD45
Subject ID
29
29
36
36
39
39
40
40
34
34
% CD14+ monocytes
% CD15+ granulocytes
% CD56+ CD3+ NK T cells
% CD56+ NK cells
% CD19+ B cells
% CD3+ T cells
Timepoint
Histology
BOR
Biopsy location
PD-L1 CPS
MMR
HER2
Viability
Pretreatment
Day 56
EOT
EAC
ESCC
PR
SD
PD
Missing
Esophagus
Liver
Other
< 1
1 - 10
≥ 10
Missing
Proficient
Deficient
Missing
Non-amplified
Amplified
Missing
0%
100%

Figure S6


Best overall response
PD
PR/SD
SD
PR
Histology
EAC
ESCC
Major lymphocyte subsets
Frequency of live single lymphocytes
CD3+ T cells
CD56+ NK cells
CD19+ B cells
NK T cells
Major T cell subsets
Frequency of CD3+ T cells
CD4+ T cells
CD8+ T cells
Tregs
P = 0.087
Frequency of CD4+ T cells
CD4+ T helper cell memory
Naive
TCM
TEM
TEMRA
CD4+ activation status
P = 0.014
CD69+
CD71+
CD4+ T cell checkpoints
PD1+
TIM3+
LAG3+
HLA−DR+
PDL1+







PFS

	P-value	HR	95% CI
Tregs, % of CD4 T cells	0.011	1.133	1.03-1.25
CD71+ CD8 T cells, % of CD8 T cells	0.0062	1.129	1.04-1.23
CD71+ CD4 T cells, % of CD4 T cells	0.0045	1.104	1.03-1.18
CD3, % of live single lymphocytes	0.053	0.982	0.96-1.00

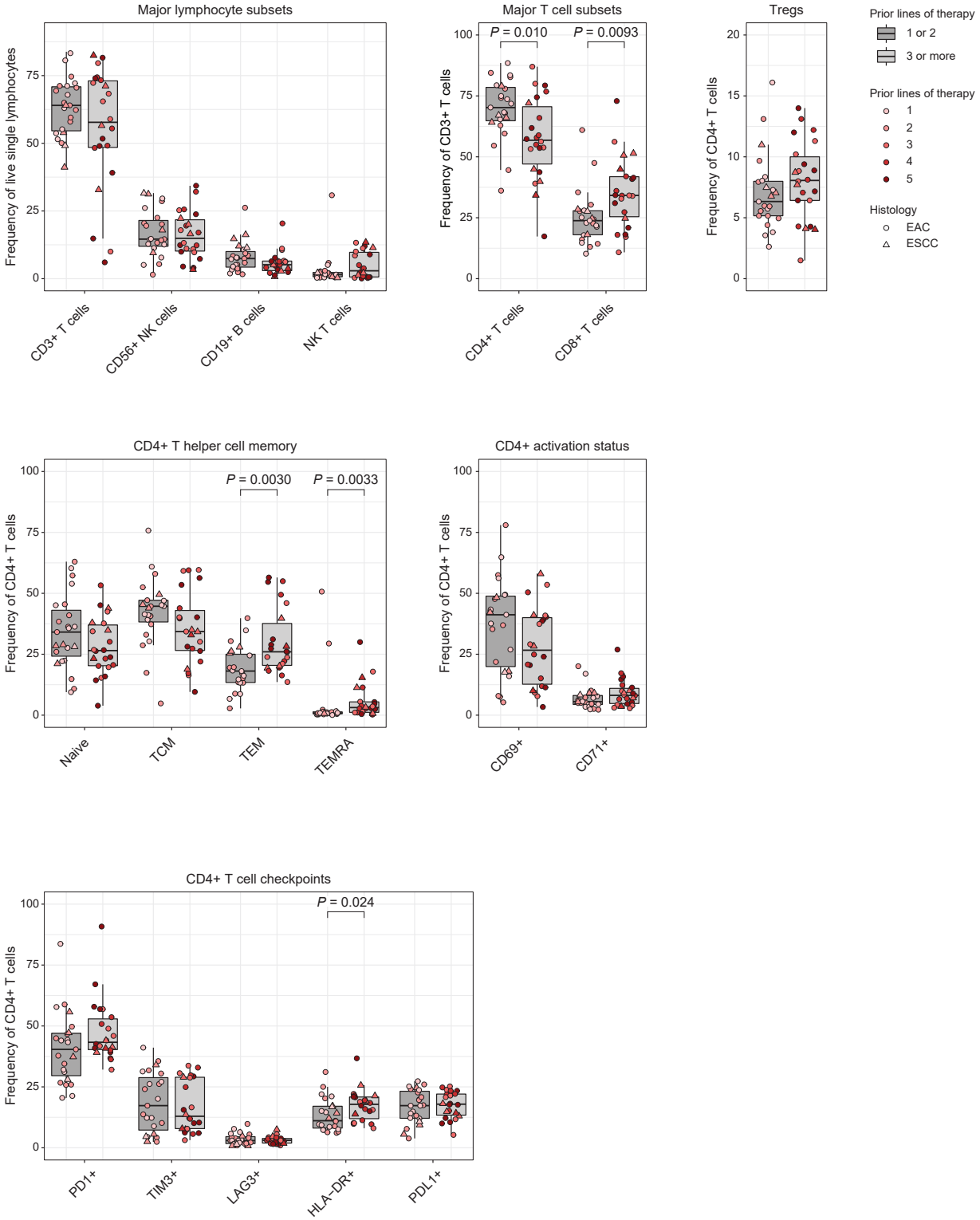
0.9 1.0 1.1 1.2 1.3 1.4 1.5 1.6

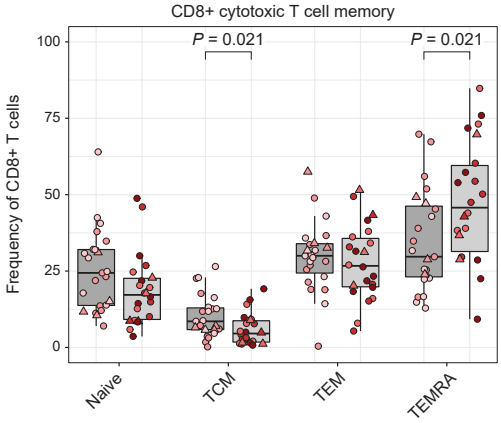
OS

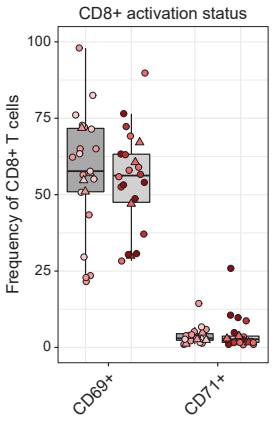
	P-value	HR	95% CI
Tregs, % of live lymphocytes	0.018	1.299	1.05-1.61
Tregs, % of CD4 T cells	0.00092	1.195	1.08-1.33
CD71+ CD4 T cells, % of CD8 T cells	0.017	1.129	1.02-1.25
CD71+ CD4 T cells, % of CD4 T cells	0.016	1.095	1.02-1.18
TIM3+ CD4 T cells, % of CD4 T cells	0.084	1.029	1.00-1.06
CD3+ T cells, % of live single lymphocytes	0.039	0.980	0.96-1.00
CD8+ NK T cells, % of NK T cells	0.0036	0.973	0.95-0.99

0.9 1.0 1.1 1.2 1.3 1.4 1.5 1.6

Figure S7


Major lymphocyte subsets
Frequency of live single lymphocytes
CD3+ T cells
CD56+ NK cells
CD19+ B cells
NK T cells
Major T cell subsets
Frequency of CD3+ T cells
P = 0.010
P = 0.0093
CD4+ T cells
CD8+ T cells
Tregs
Frequency of CD4+ T cells
Prior lines of therapy
1 or 2
3 or more
Prior lines of therapy
1
2
3
4
5
Histology
EAC
ESCC
CD4+ T helper cell memory
Frequency of CD4+ T cells
P = 0.0030
P = 0.0033
Naive
TCM
TEM
TEMRA
CD4+ activation status
CD69+
CD71+
CD4+ T cell checkpoints
P = 0.024
PD1+
TIM3+
LAG3+
HLA-DR+
PDL1+


CD8+ cytotoxic T cell memory
P = 0.021
P = 0.021
Frequency of CD8+ T cells
100
75
50
25
0
Naive
TCM
TEM
TEMRA


CD8+ activation status
Frequency of CD8+ T cells
100
75
50
25
0
CD69+
CD71+

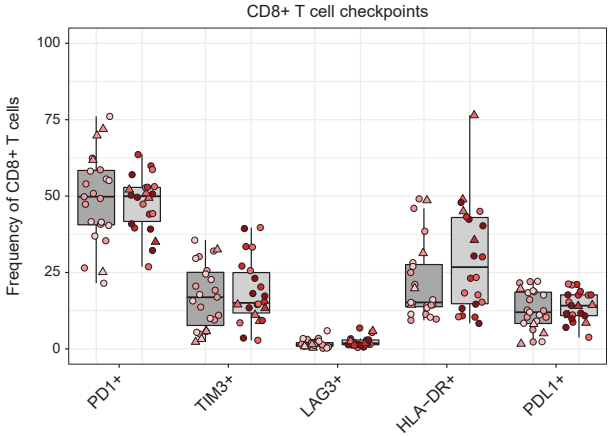
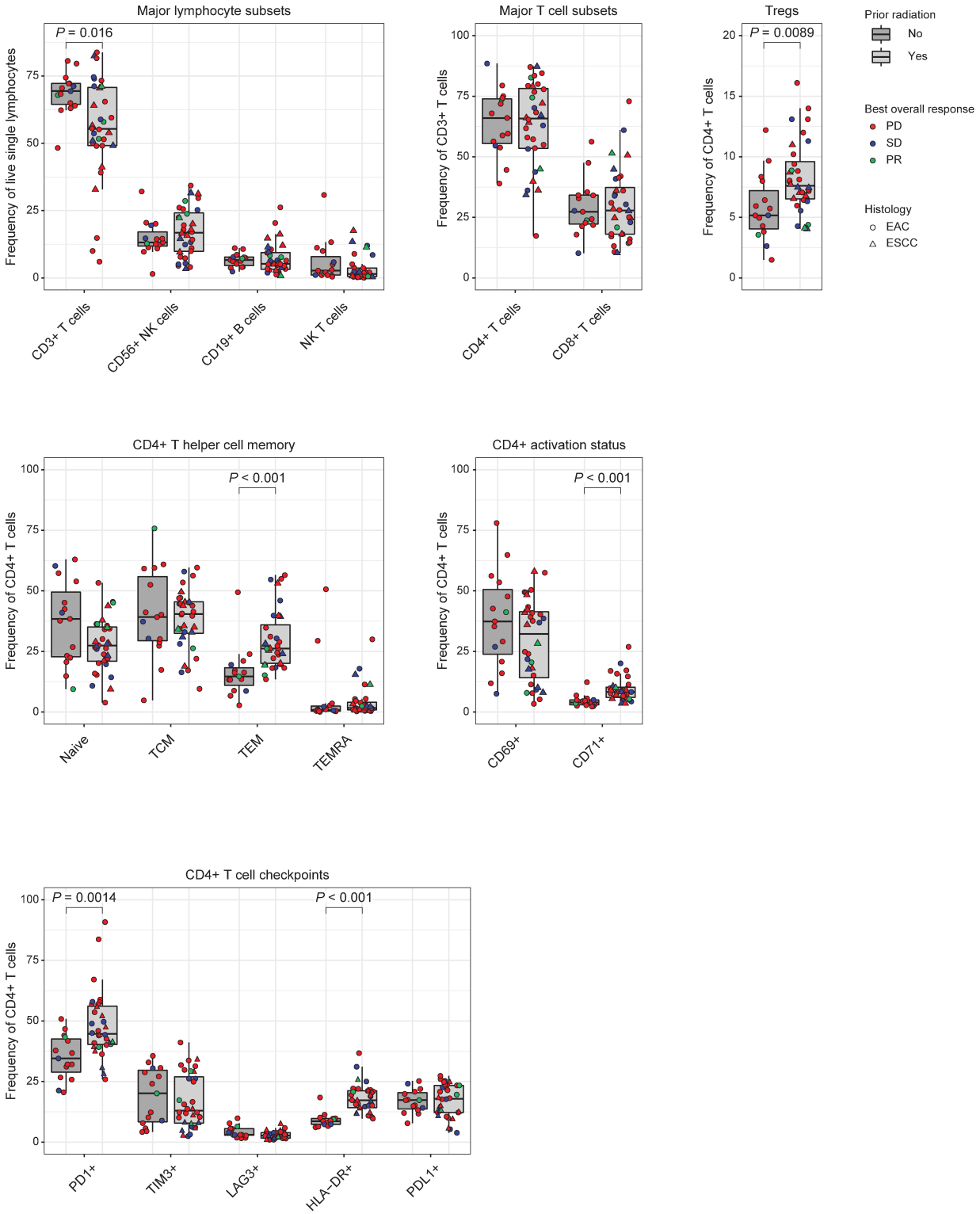
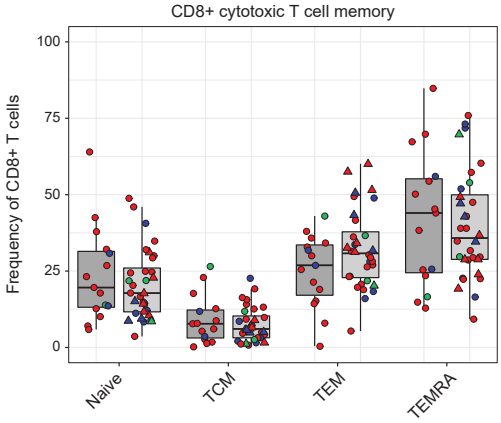
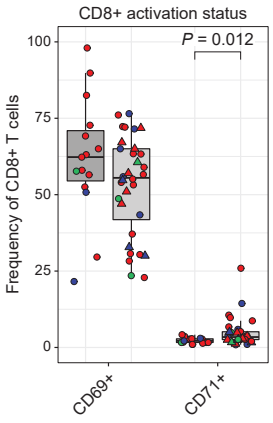

CD8+ T cell checkpoints
Frequency of CD8+ T cells
100
75
50
25
0
PD1+
TIM3+
LAG3+
HLA-DR+
PDL1+

Figure S8


Major lymphocyte subsets
P = 0.016
Frequency of live single lymphocytes
CD3+ T cells
CD56+ NK cells
CD19+ B cells
NK T cells
Major T cell subsets
Frequency of CD3+ T cells
CD4+ T cells
CD8+ T cells
Tregs
P = 0.0089
Frequency of CD4+ T cells
Prior radiation
No
Yes
Best overall response
PD
SD
PR
Histology
EAC
ESCC
CD4+ T helper cell memory
P < 0.001
Frequency of CD4+ T cells
Naive
TCM
TEM
TEMRA
CD4+ activation status
P < 0.001
Frequency of CD4+ T cells
CD69+
CD71+
CD4+ T cell checkpoints
P = 0.0014
P < 0.001
Frequency of CD4+ T cells
PD1+
TIM3+
LAG3+
HLA-DR+
PDL1+


CD8+ cytotoxic T cell memory
Frequency of CD8+ T cells
100
75
50
25
0
Naive
TCM
TEM
TEMRA


CD8+ activation status
P = 0.012
Frequency of CD8+ T cells
100
75
50
25
0
CD69+
CD71+

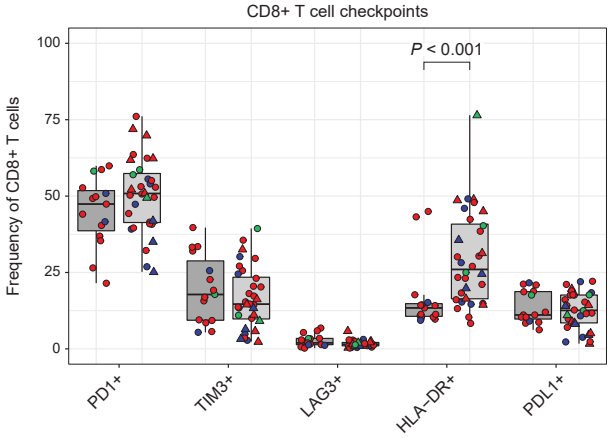
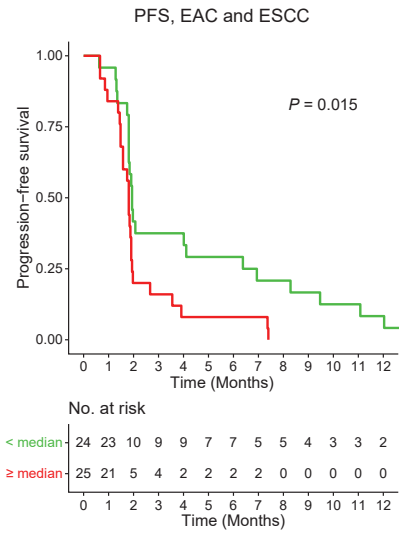
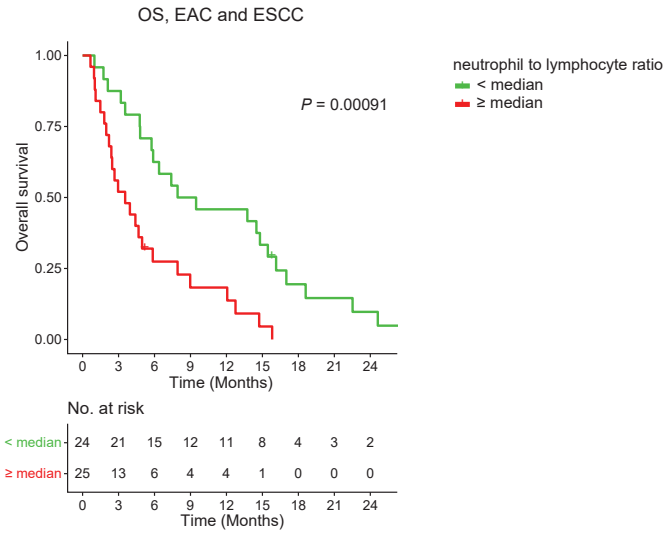

CD8+ T cell checkpoints
P < 0.001
Frequency of CD8+ T cells
100
75
50
25
0
PD1+
TIM3+
LAG3+
HLA-DR+
PDL1+

Figure S9

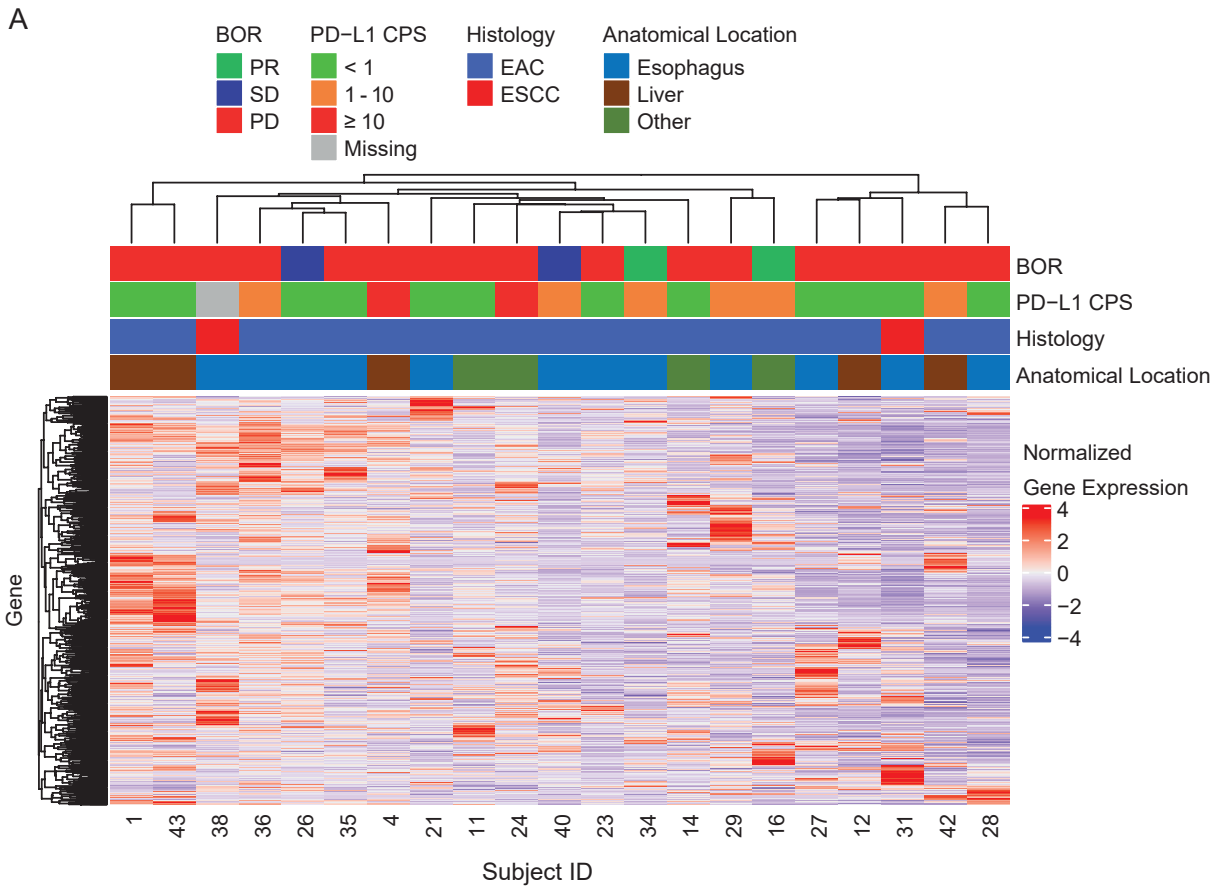


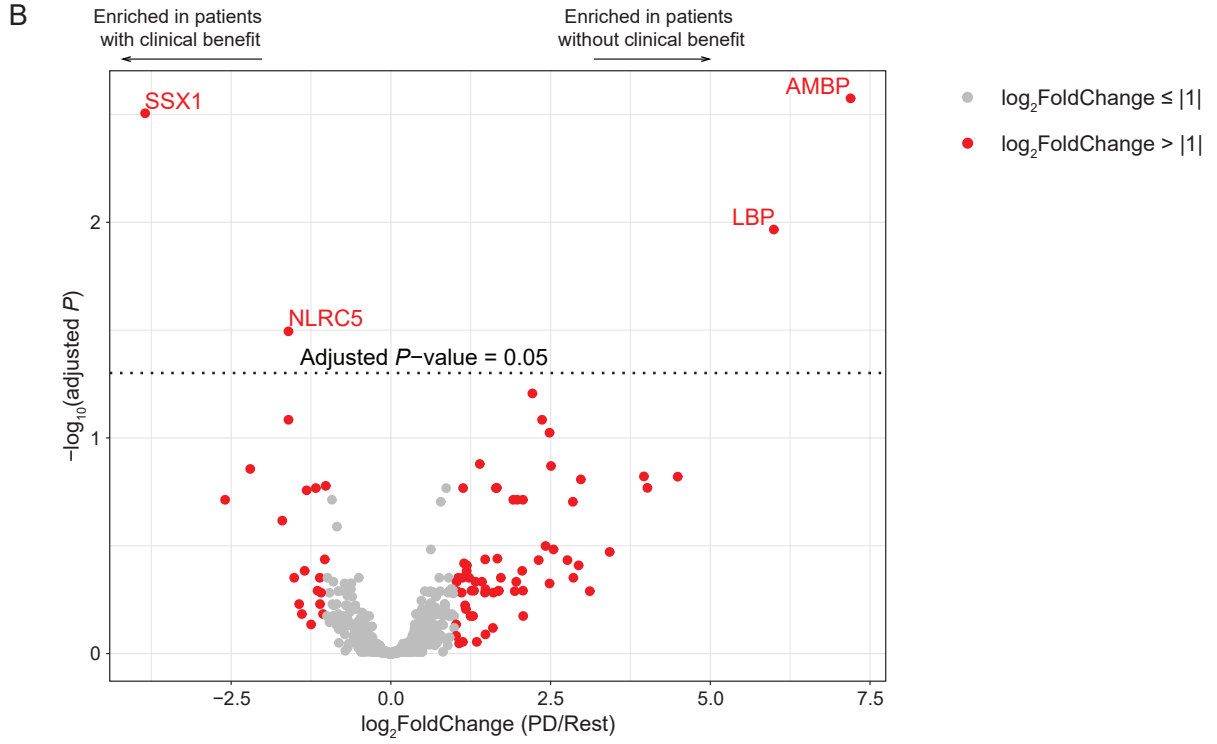


Population (n)	Median PFS (months) (95% CI)	6-month PFS rate (%, 95% CI)	12-month PFS rate (%, 95% CI)
Overall (49)	1.84 (1.81-1.97)	18.4 (10.2-33.1)	4.1 (1.1-15.9)
NLR < median	1.94 (1.81-6.38)	29.2 (15.6-54.4)	8.3 (2.2-31.4)
NLR ≥ median	1.81(1.58-1.94)	8.0 (2.1-30.2)	NA

Population (n)	Median OS (months) (95% CI)	6-month OS rate (%, 95% CI)	12-month OS rate (%, 95% CI)
Overall (49)	5.75 (3.95-9.47)	44.6 (32.6-61.1)	31.9 (21.1-48.2)
NLR < median	8.70 (5.88-16.14)	62.5 (45.8-85.2)	45.8 (29.7-70.8)
NLR ≥ median	3.55 (2.40-7.92)	27.4 (14.4-52.4)	18.3 (7.7-43.2)

Figure S10


A
BOR
PR
SD
PD
PD−L1 CPS
< 1
1 - 10
≥ 10
Missing
Histology
EAC
ESCC
Anatomical Location
Esophagus
Liver
Other
BOR
PD−L1 CPS
Histology
Anatomical Location
Normalized
Gene Expression
4
2
0
−2
−4
Gene
1 43 38 36 26 35 4 21 11 24 40 23 34 14 29 16 27 12 31 42 28
Subject ID


B
Enriched in patients with clinical benefit
Enriched in patients without clinical benefit
SSX1
AMBP
LBP
NLRC5
Adjusted P−value = 0.05
log2FoldChange ≤ |1|
log2FoldChange > |1|
−log10(adjusted P)
2
1
0
−2.5
0.0
2.5
5.0
7.5
log2FoldChange (PD/Rest)

C

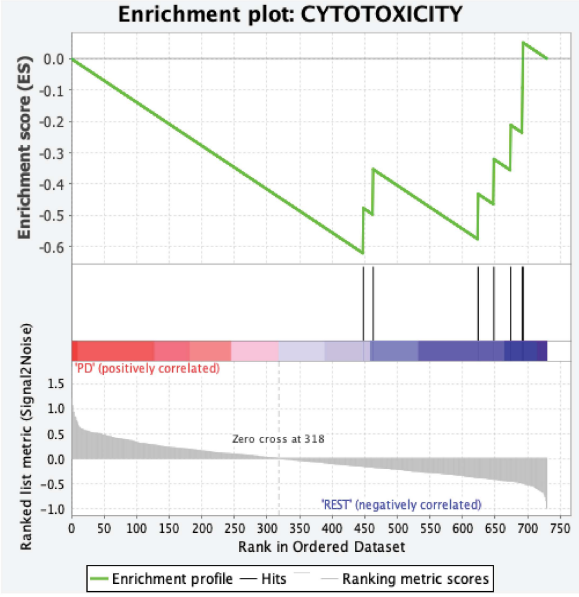
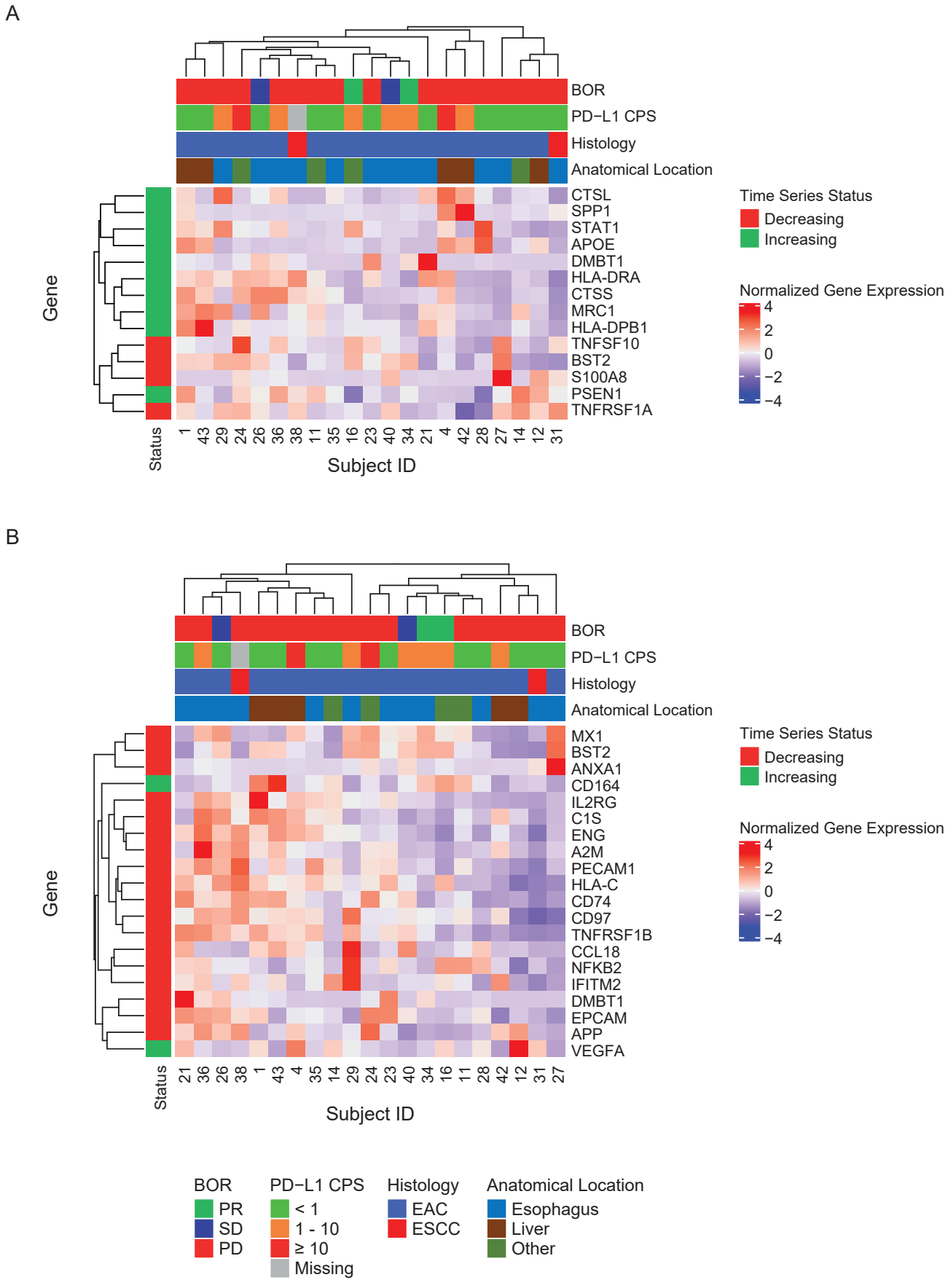

Enrichment plot: CYTOTOXICITY
Enrichment score (ES)
0.0
-0.1
-0.2
-0.3
-0.4
-0.5
-0.6
'PD' (positively correlated)
Ranked list metric (Signal2Noise)
1.5
1.0
0.5
0.0
-0.5
-1.0
Zero cross at 318
'REST' (negatively correlated)
0 50 100 150 200 250 300 350 400 450 500 550 600 650 700 750
Rank in Ordered Dataset
Enrichment profile
Hits
Ranking metric scores

Figure S11


A
BOR
PD−L1 CPS
Histology
Anatomical Location
CTSL
SPP1
STAT1
APOE
DMBT1
HLA-DRA
CTSS
MRC1
HLA-DPB1
TNFSF10
BST2
S100A8
PSEN1
TNFRSF1A
Gene
Status
1 43 29 24 26 36 38 11 35 16 23 40 34 21 4 42 28 27 14 12 31
Subject ID
Time Series Status
Decreasing
Increasing
Normalized Gene Expression
4
2
0
−2
−4
B
BOR
PD−L1 CPS
Histology
Anatomical Location
MX1
BST2
ANXA1
CD164
IL2RG
C1S
ENG
A2M
PECAM1
HLA-C
CD74
CD97
TNFRSF1B
CCL18
NFKB2
IFITM2
DMBT1
EPCAM
APP
VEGFA
Gene
Status
21 36 26 38 1 43 4 35 14 29 24 23 40 34 16 11 28 42 12 31 27
Subject ID
Time Series Status
Decreasing
Increasing
Normalized Gene Expression
4
2
0
−2
−4
BOR
PR
SD
PD
PD−L1 CPS
< 1
1 - 10
≥ 10
Missing
Histology
EAC
ESCC
Anatomical Location
Esophagus
Liver
Other