Science Advances
AAAS

Supplementary Materials for

Immunotherapy of glioblastoma explants induces interferon-γ responses and spatial immune cell rearrangements in tumor center, but not periphery

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The PDF file includes:

Other Supplementary Material for this manuscript includes the following:

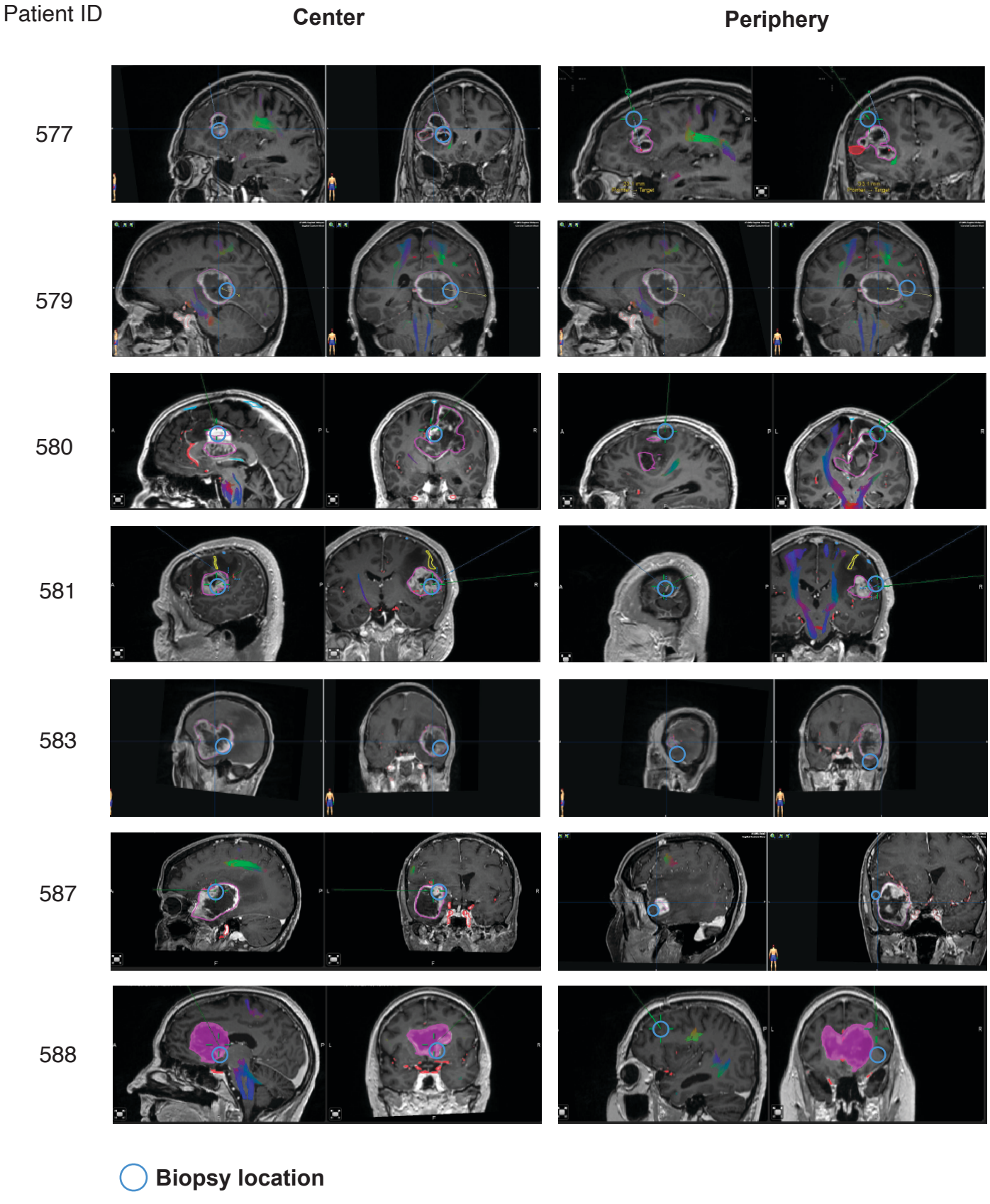
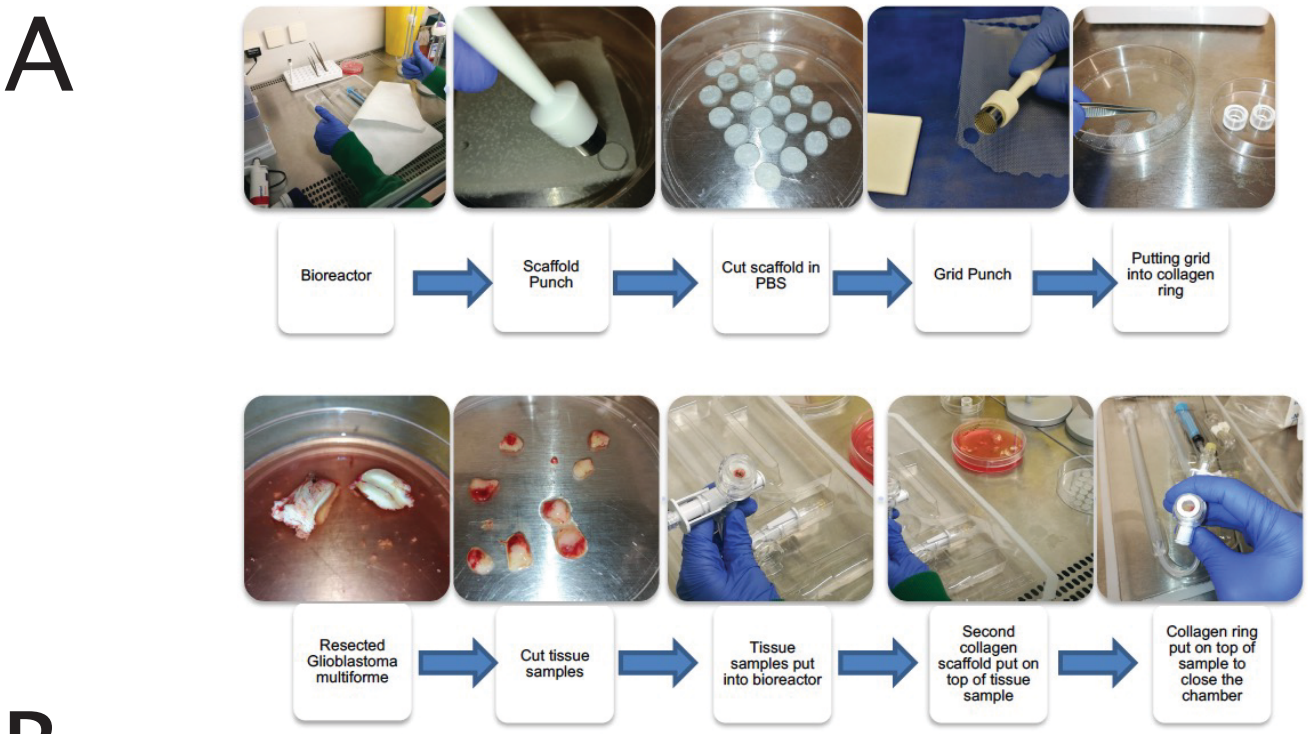
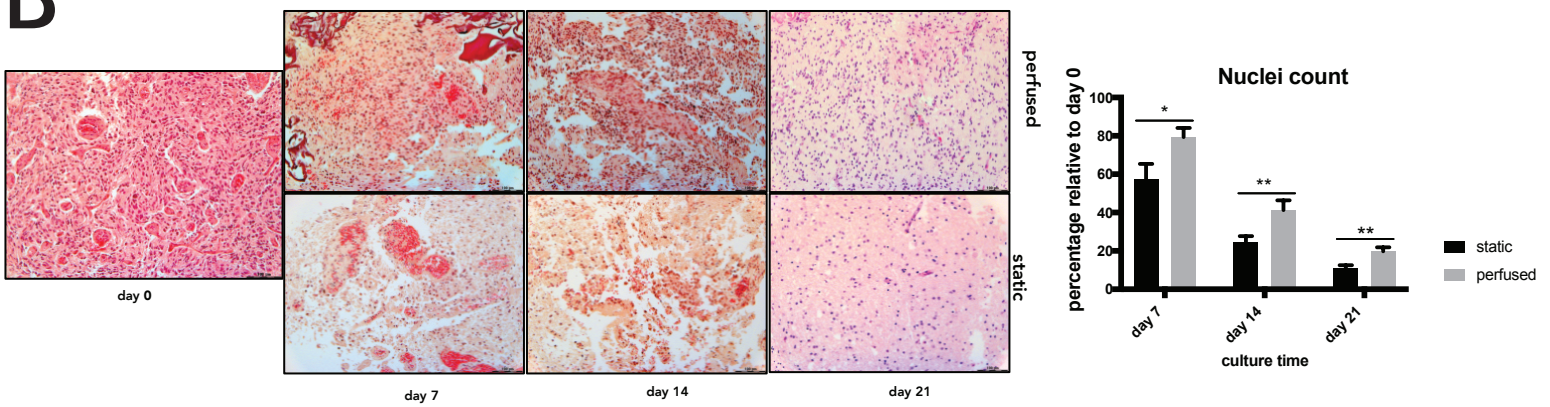

Patient ID
Center
Periphery
577
579
580
581
583
587
588
Biopsy location

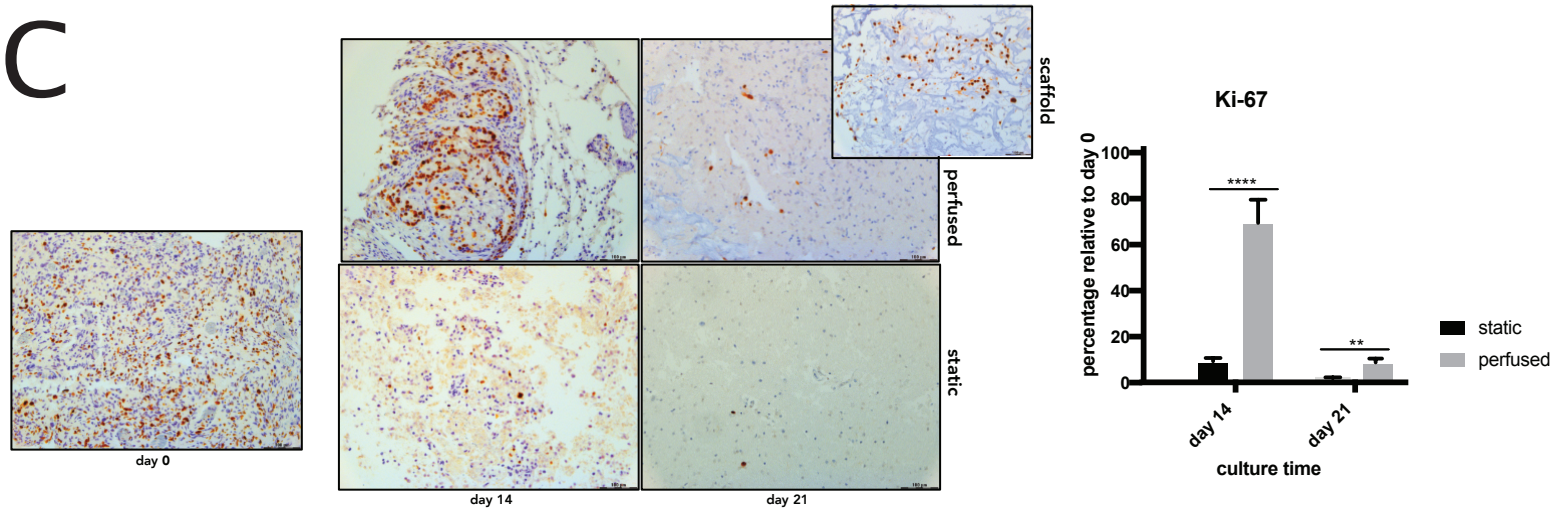
Figure S1

Fig. S1. Biopsy Locations.

Biopsies were acquired using the intraoperative navigation system Brainlab Automatic Image Registration (Brainlab). Preoperative planning of surgeries and labeling of important eloquent structures was performed in all cases, based on diffusion tensor imaging (DTI) or anatomical considerations. Intraoperative screen captures of both contrast enhancing center and peripheral invasion zone were acquired to document accuracy of the biopsy location. Intraoperative 5-ALA fluorescence was used as an additional stratifier for center-periphery discrimination (5-ALA fluorescence high for vital, contrast enhancing tumor vs. 5-ALA fluorescence low for infiltration zone).


A
Bioreactor
Scaffold Punch
Cut scaffold in PBS
Grid Punch
Putting grid into collagen ring
Resected Glioblastoma multiforme
Cut tissue samples
Tissue samples put into bioreactor
Second collagen scaffold put on top of tissue sample
Collagen ring put on top of sample to close the chamber


B
day 0
day 7
day 14
day 21
perfused
static
Nuclei count
percentage relative to day 0
culture time
static
perfused


C
day 0
day 14
day 21
scaffold
perfused
static
Ki-67
percentage relative to day 0
culture time
static
perfused

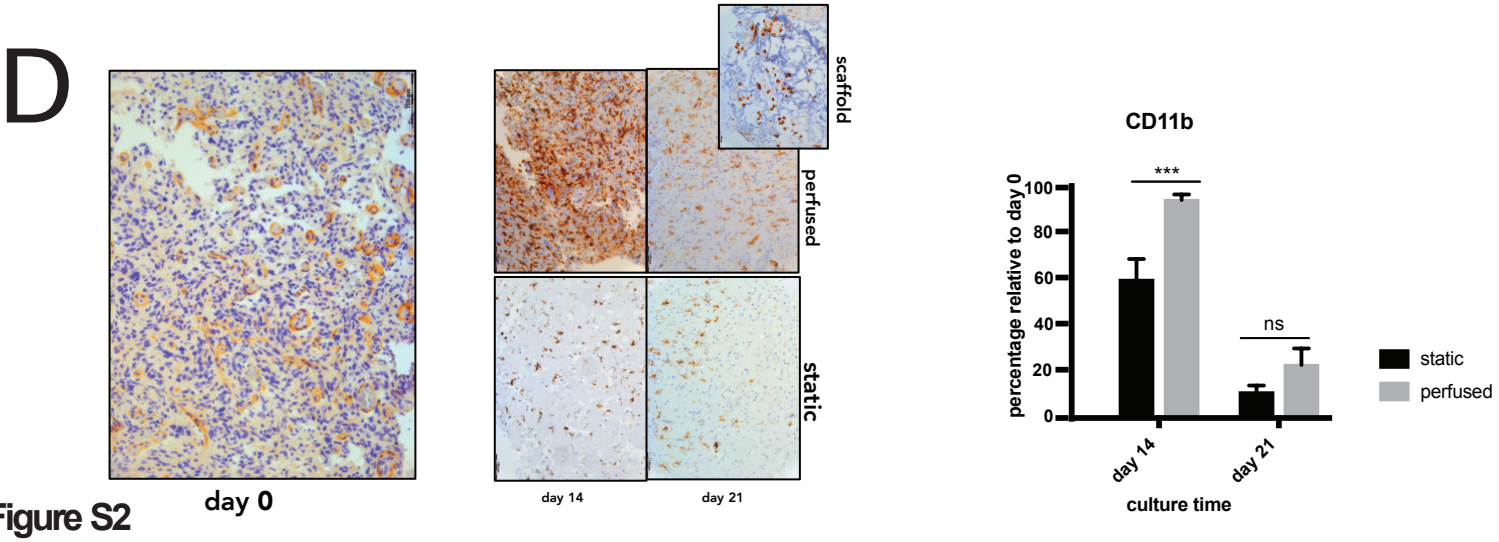

D
day 0
day 14
day 21
scaffold
perfused
static
CD11b
percentage relative to day 0
culture time
ns
static
perfused

Figure S2

Fig. S2. Bioreactor setup and validation.

(A) Workflow of assembling bioreactor 3D perfusion culture directly after tumor resection. **(B)** *Left:* Representative H&E stained microphotographs of individual tumor samples (center biopsy) at day 0, and after 7, 14 and 21 days of either perfusion culture or static culture condition (40x magnification). *Right:* Analysis of nuclear counts per 10 high power fields in 3 independent samples in relation to the D0 sample in perfused and static samples. **(C)** *Left:* Representative anti-Ki-67 immunohistochemical stained slides comparing static and perfused culture conditions at day 0, and 14 and days 21 after ex vivo culture. Insert: proliferating tumor cells invading the bioreactor scaffold after 21 days of *ex vivo* culture (40x magnification). *Right:* Relative quantification of Ki-67$^+$ proliferative cells after 14 and 21 days of *ex vivo* culture in static and perfused conditions, compared to freshly obtained GBM tissue (D0). **(D)** *Left:* Representative anti-CD11b immunohistochemical stained slides comparing static and perfused culture conditions at day 0, and 14 and days 21 after *ex vivo* culture. Insert: myeloid cells invading the bioreactor collagen scaffold after 21 days of *ex vivo* culture (40x magnification). *Right:* Relative quantification of CD11b$^+$ myeloid cells after 14 and 21 days of ex vivo culture in static and perfused conditions, compared to freshly obtained GBM tissue (D0). Data are obtained from 3 individual explants in each static and perfusion culture, and represented as mean +/- SEM. *Statistics:* *p<0.05, **p<0.01, ***p<0.005, student's t-tests.

Detailed clustering strategy

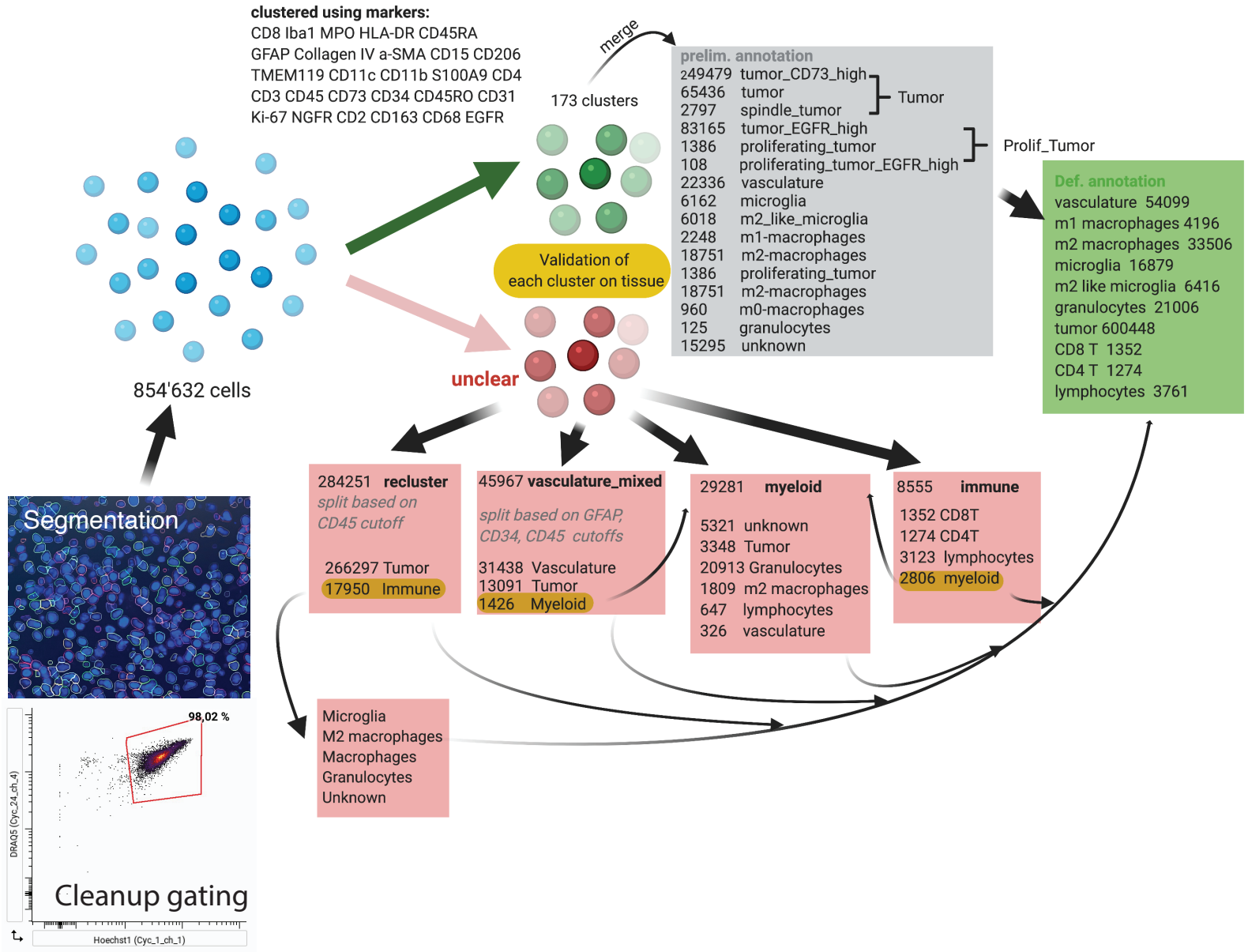


Figure S3

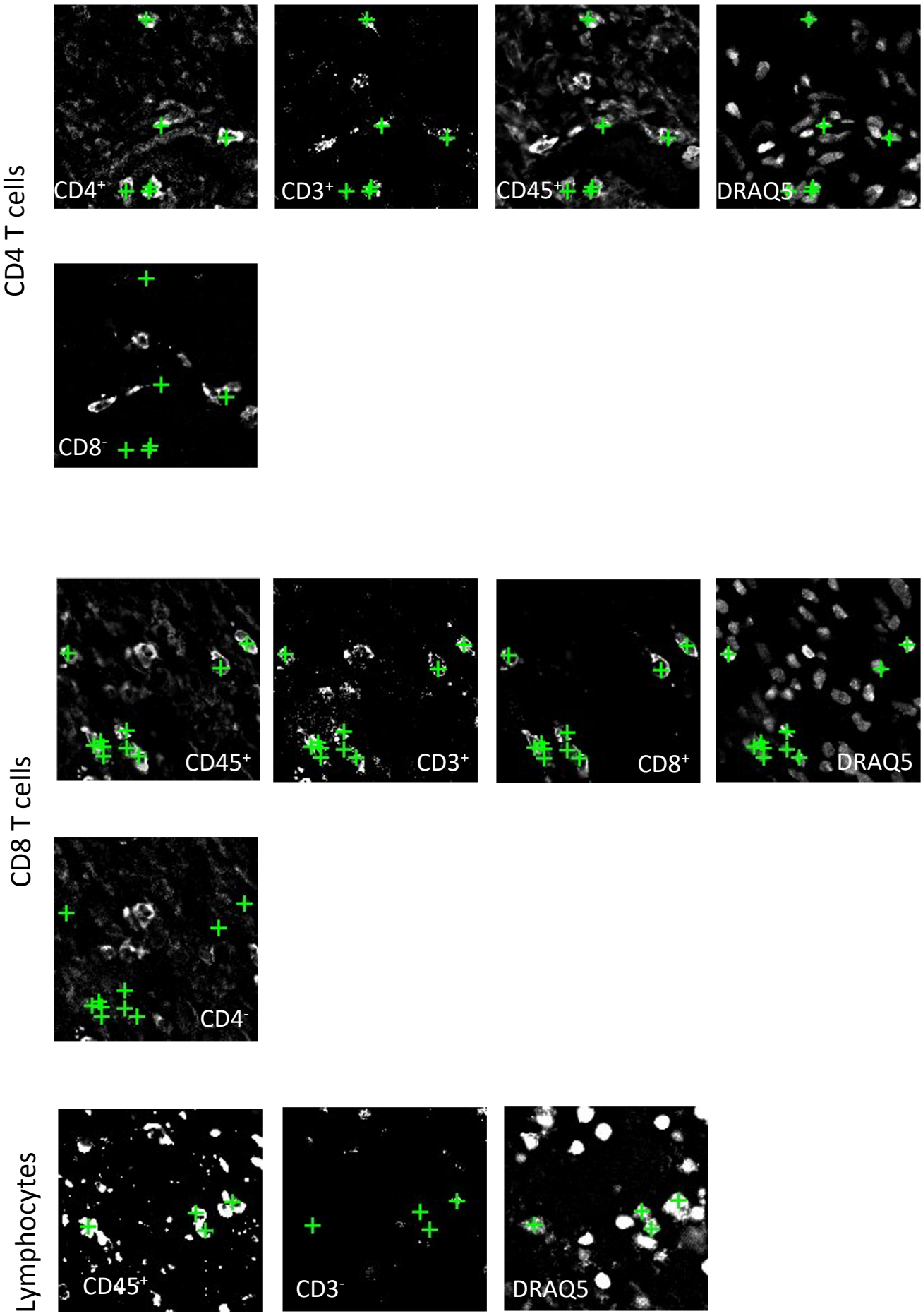

CD4 T cells
CD4+
CD3+
CD45+
DRAQ5
CD8-
CD8 T cells
CD45+
CD3+
CD8+
DRAQ5
CD4-
Lymphocytes
CD45+
CD3-
DRAQ5

Figure S3

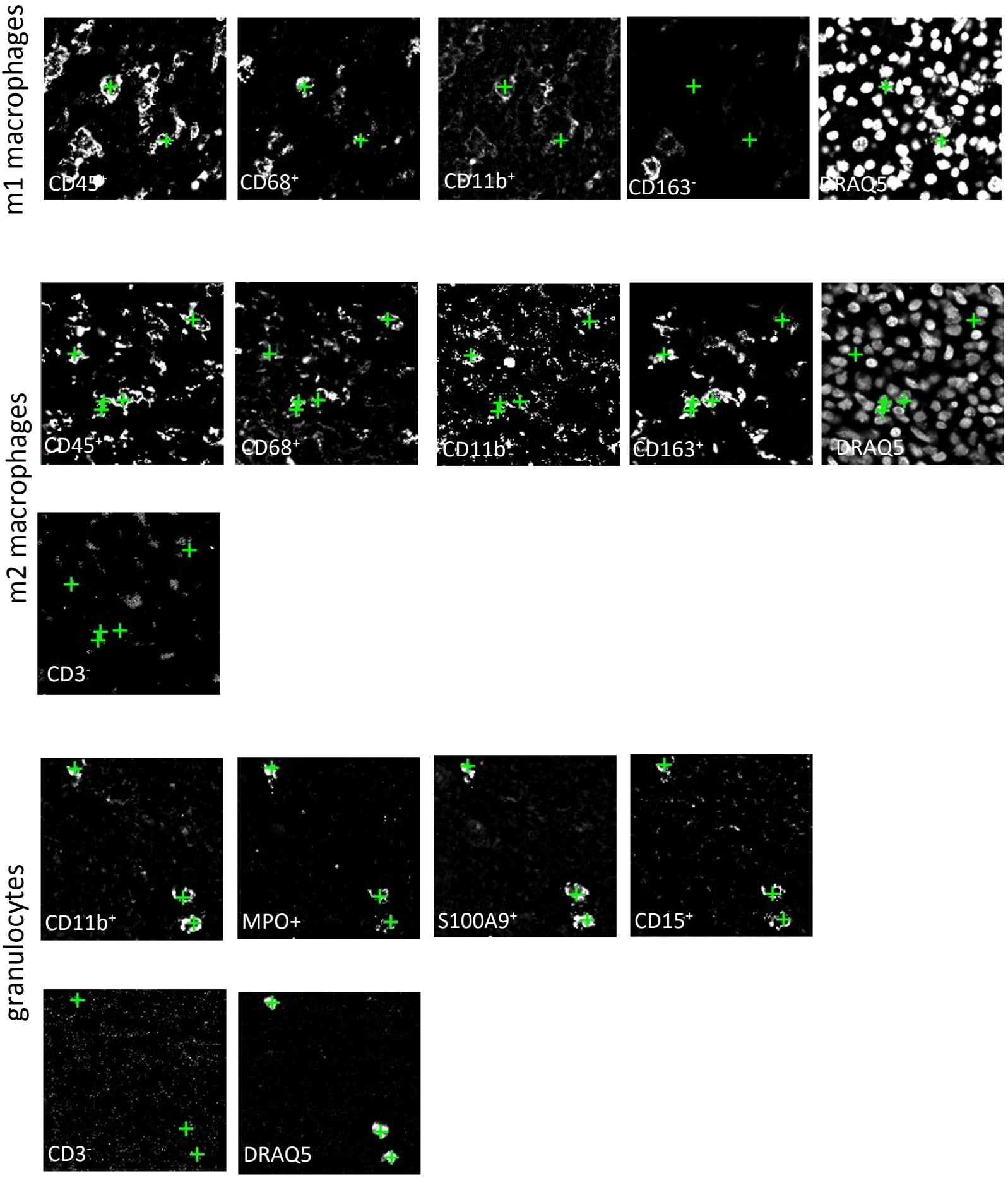

m1 macrophages
CD45+
CD68+
CD11b+
CD163-
DRAQ5
m2 macrophages
CD45+
CD68+
CD11b+
CD163+
DRAQ5
CD3-
granulocytes
CD11b+
MPO+
S100A9+
CD15+
CD3-
DRAQ5

Figure S3

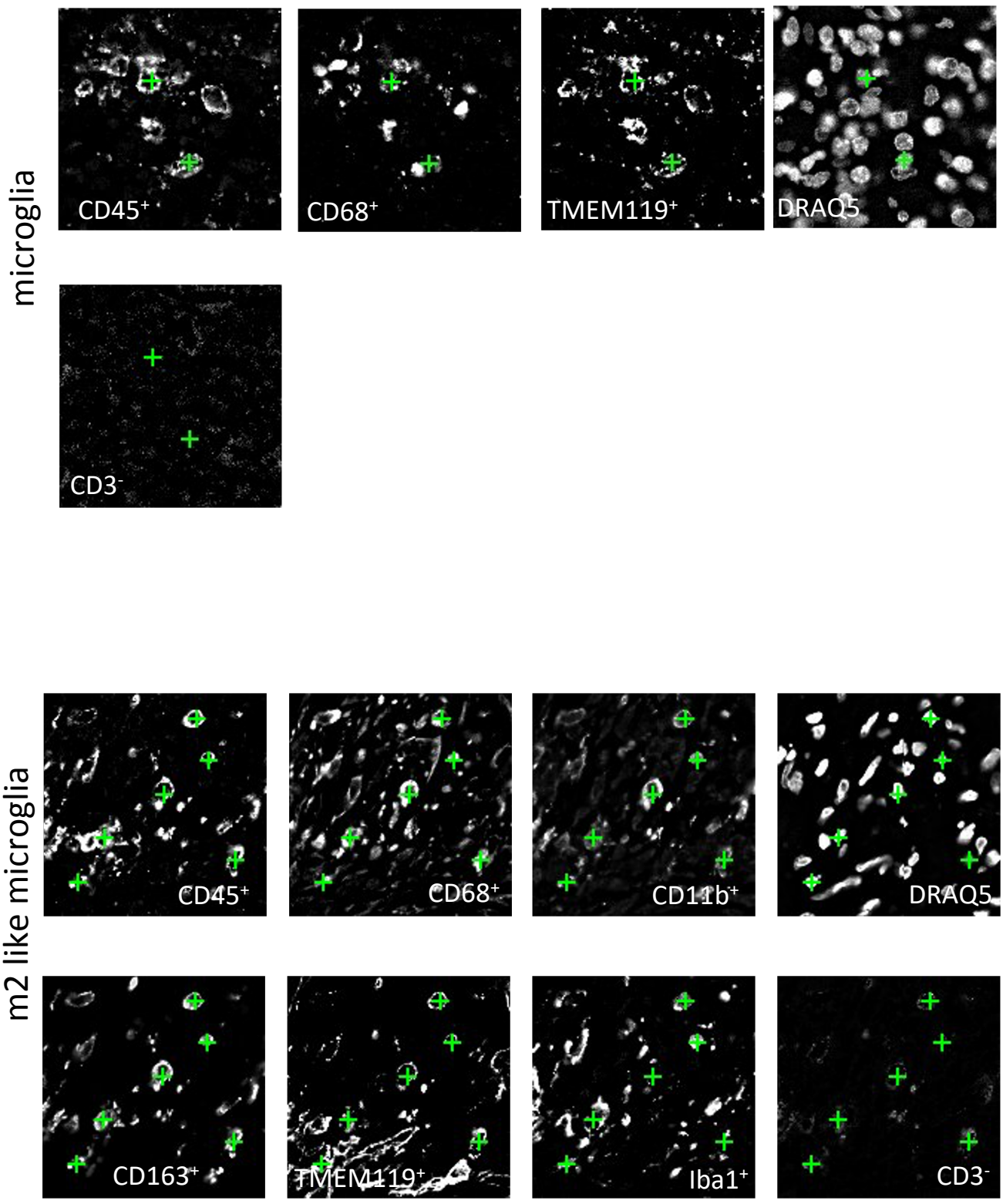

microglia
CD45+
CD68+
TMEM119+
DRAQ5
CD3-
m2 like microglia
CD45+
CD68+
CD11b+
DRAQ5
CD163+
TMEM119+
Iba1+
CD3-

Figure S3

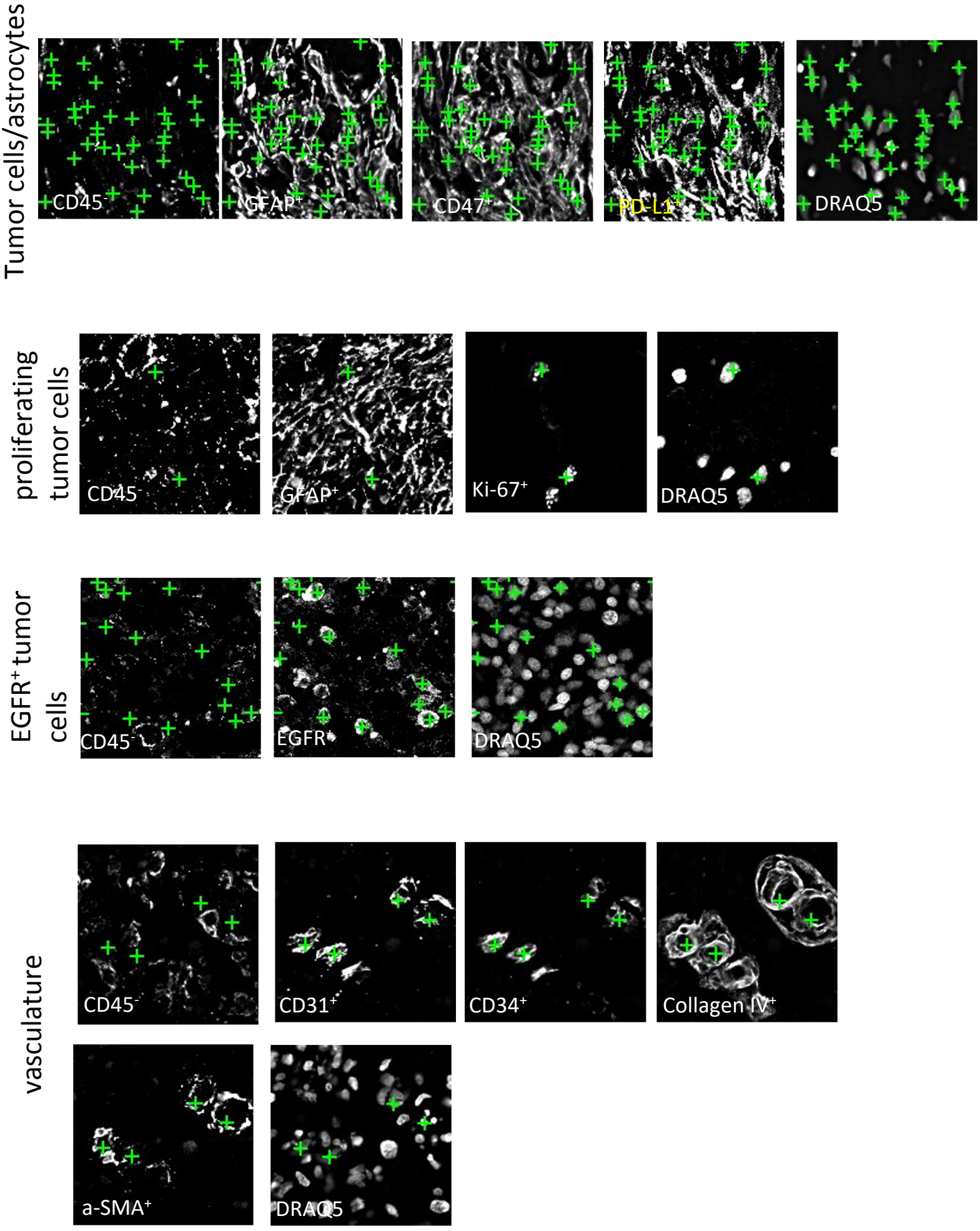

Tumor cells/astrocytes
CD45-
GFAP+
CD47+
PD-L1+
DRAQ5
proliferating tumor cells
CD45-
GFAP+
Ki-67+
DRAQ5
EGFR+ tumor cells
CD45-
EGFR+
DRAQ5
vasculature
CD45-
CD31+
CD34+
Collagen IV+
a-SMA+
DRAQ5

Figure S3

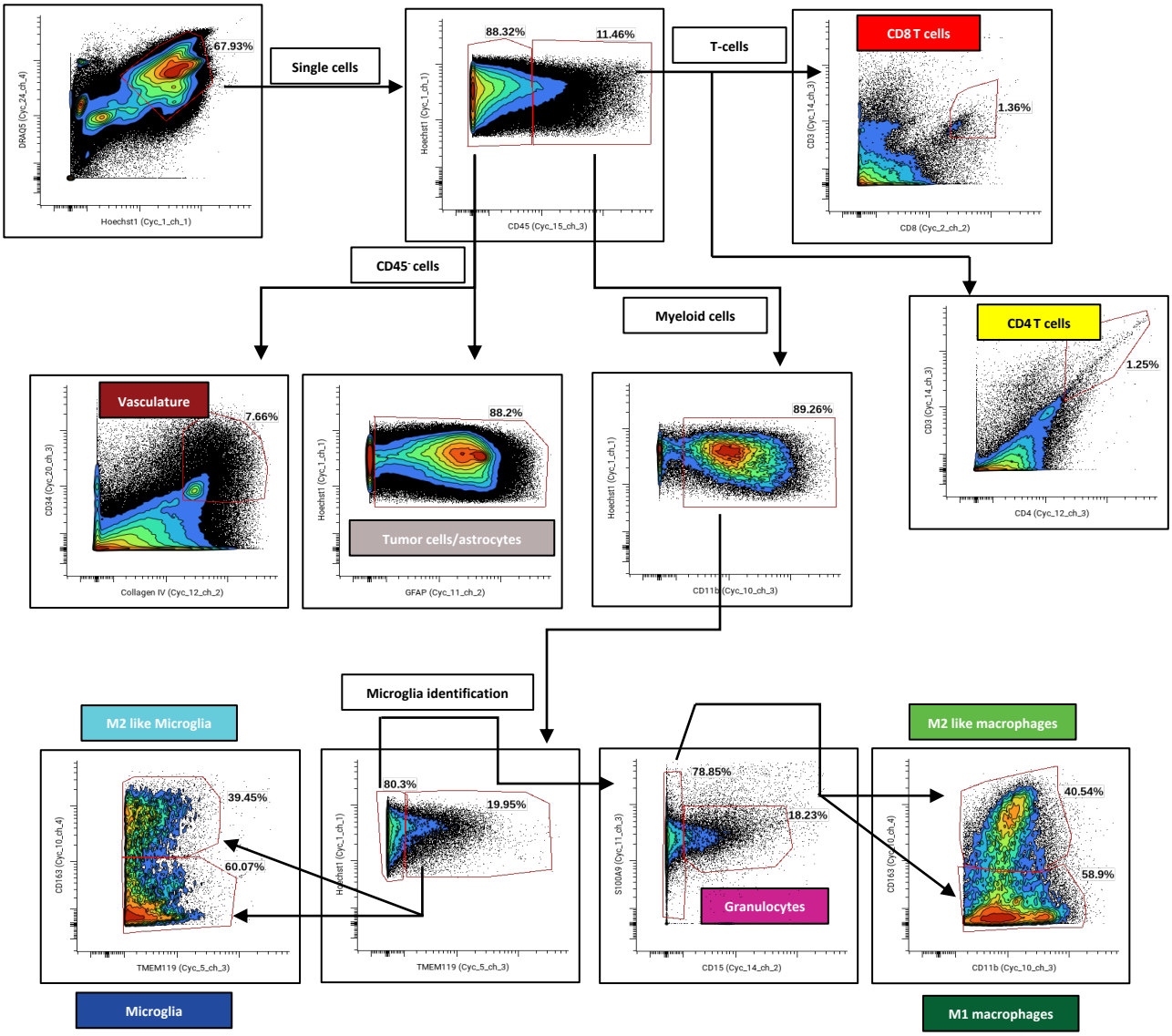

67.93%
DRAQ5 (Cyc_24_ch_4)
Hoechst1 (Cyc_1_ch_1)
Single cells
88.32%
11.46%
Hoechst1 (Cyc_1_ch_1)
CD45 (Cyc_15_ch_3)
T-cells
CD8 T cells
1.36%
CD3 (Cyc_14_ch_3)
CD8 (Cyc_2_ch_2)
CD45- cells
Myeloid cells
CD4 T cells
1.25%
CD3 (Cyc_14_ch_3)
CD4 (Cyc_12_ch_3)
Vasculature
7.66%
CD34 (Cyc_20_ch_3)
Collagen IV (Cyc_12_ch_2)
88.2%
Hoechst1 (Cyc_1_ch_1)
Tumor cells/astrocytes
GFAP (Cyc_11_ch_2)
89.26%
Hoechst1 (Cyc_1_ch_1)
CD11b (Cyc_10_ch_3)
Microglia identification
M2 like Microglia
39.45%
60.07%
CD163 (Cyc_10_ch_4)
TMEM119 (Cyc_5_ch_3)
80.3%
19.95%
Hoechst1 (Cyc_1_ch_1)
TMEM119 (Cyc_5_ch_3)
78.85%
18.23%
S100A9 (Cyc_11_ch_3)
CD15 (Cyc_14_ch_2)
Granulocytes
M2 like macrophages
40.54%
58.9%
CD163 (Cyc_10_ch_4)
CD11b (Cyc_10_ch_3)
Microglia
M1 macrophages

Figure S3

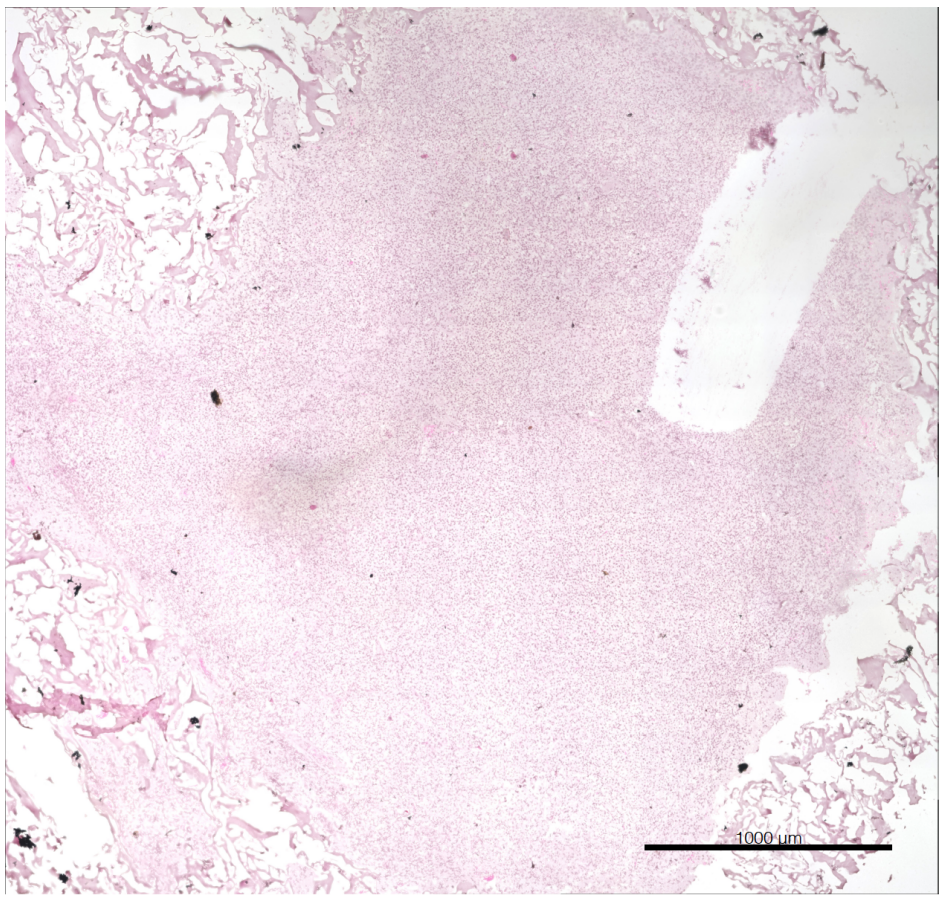
Figure S3: Detailed clustering strategy, cell passports, and FACS-like gating plots of segmented single cells.

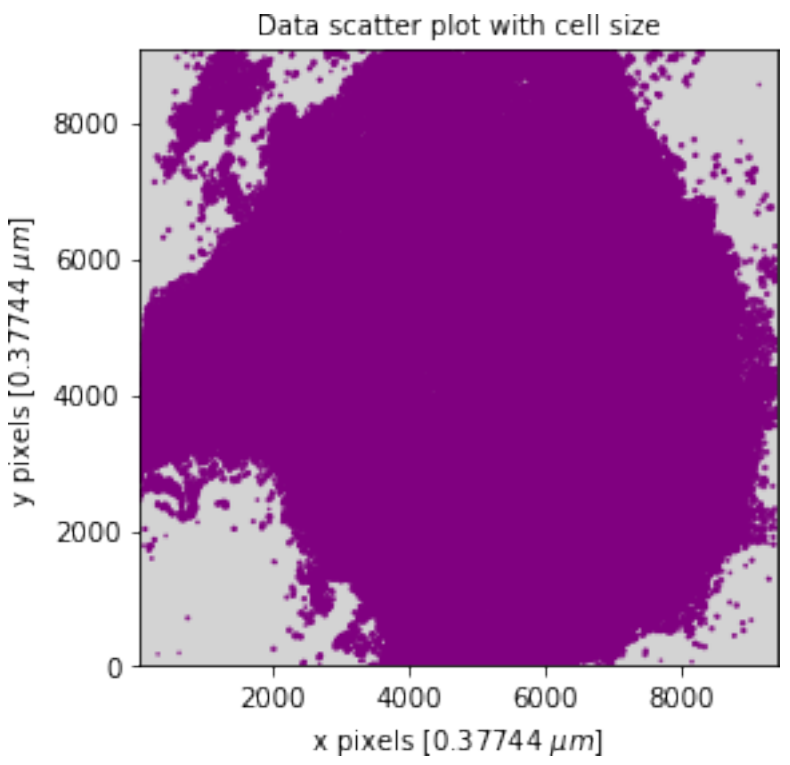
Page 1: Detailed clustering strategy as outlined in Figure 1C.

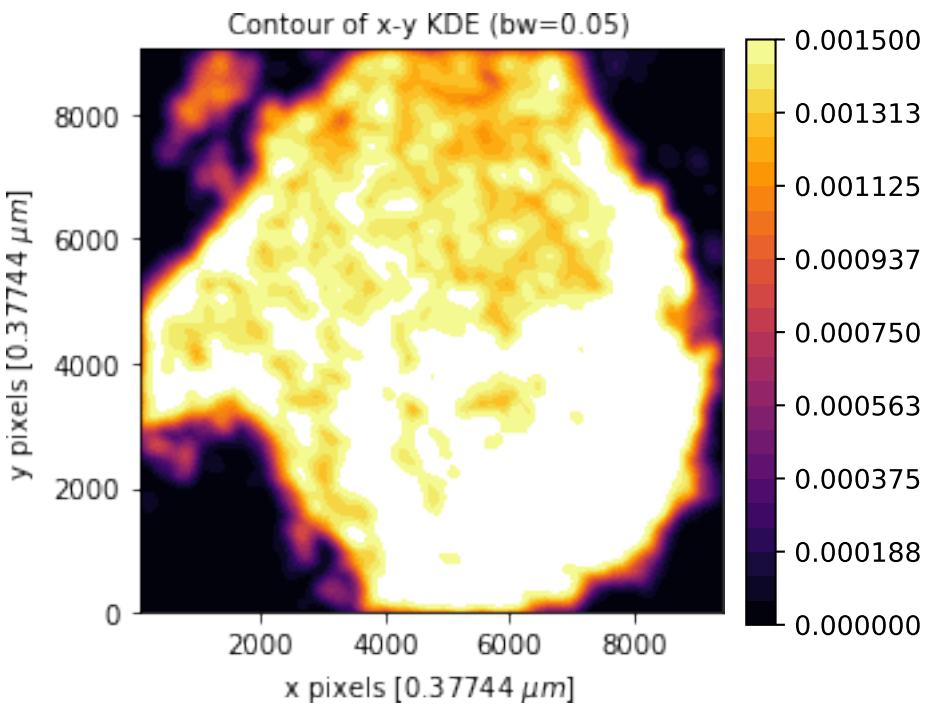
Page 2-5: After clustering, single cells from the 173 resulting initial clusters were overlaid on the raw data fluorescent images and on H&E stains of TMAs based on X/Y positions and visually verified based on marker expression profiles, morphology and localization within the tissue. Similar clusters were manually merged, resulting in 10 final clusters. Here, representative clusters are shown as green crosses based on X-Y coordinates of the cells contained in that cluster, overlaid on the montages of different samples. For each of these clusters, examples of markers important for the cluster identification (positive and negative) and DRAQ5 nuclear stain are shown.

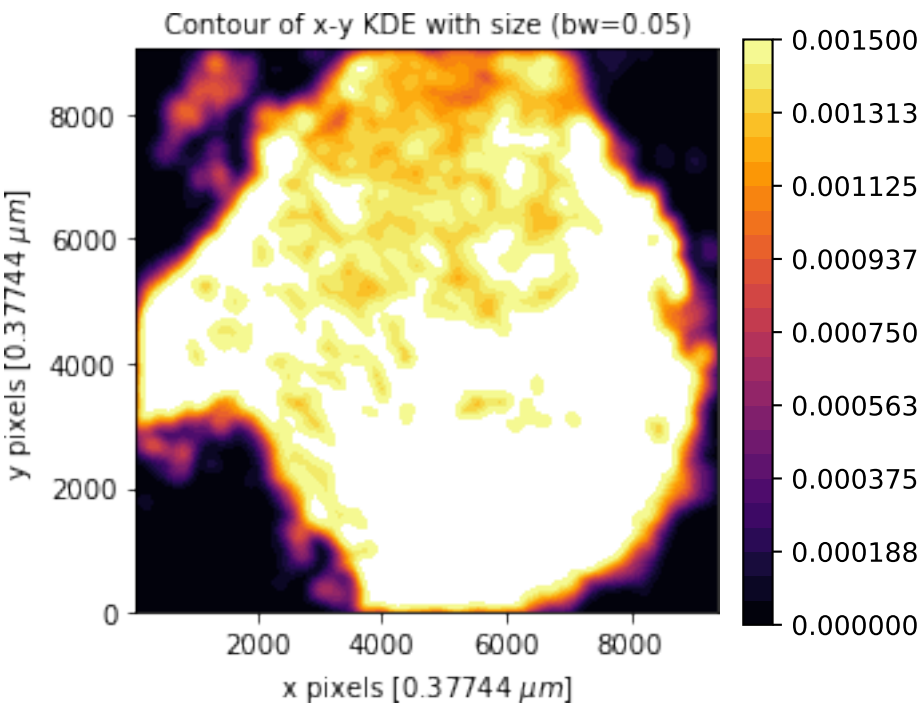
Page 6: Flow-cytometry-like plots gated on DRAQ5/Hoechst double-positive single cells were generated from concatenated FCS files of all included cells (CellEngine.com) to confirm the presence of phenotype defining markers on the cell populations of interest. As an example, CD4 T ($CD4^+$, $CD3^+$), CD8 T ($CD3^+$, $CD8^+$), microglia ($CD45^+$, $TMEM119^+$, $CD11b^+$, $CD163^-$), M2-like microglia ($CD45^+$, $TMEM119^+$, $CD11b^+$, $CD163^+$), M1 macrophages ($CD45^+$, $CD11b^+$, $CD163^-$), M2 macrophages ($CD45^+$, $CD11b^+$, $CD163^+$), granulocytes ($CD45^+$, $CD11b^+$, $CD15^+$, $S100A9^+$), tumor cells ($CD45^-$, $GFAP^+$, $CD31^-$) and vasculature ($CD45^-$, $CD31^+$, Collagen-IV^+) are shown.

representative center sample









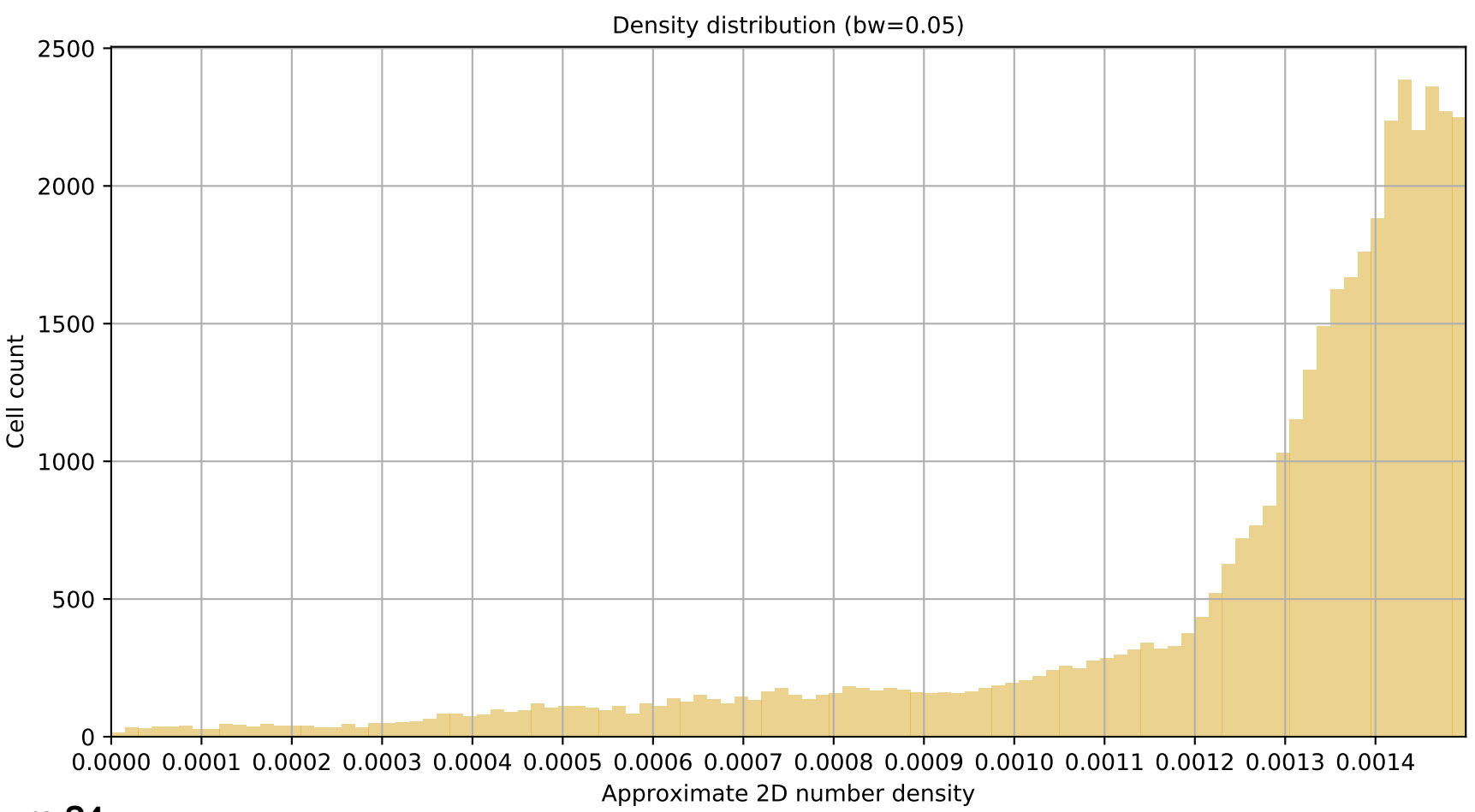
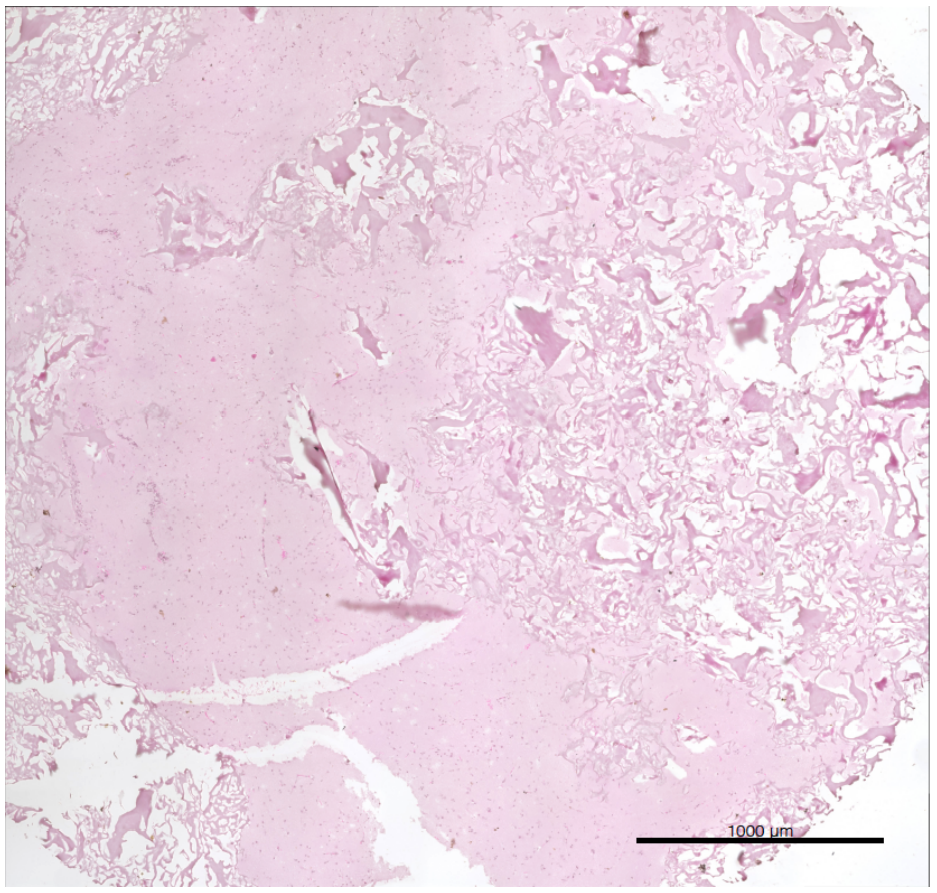
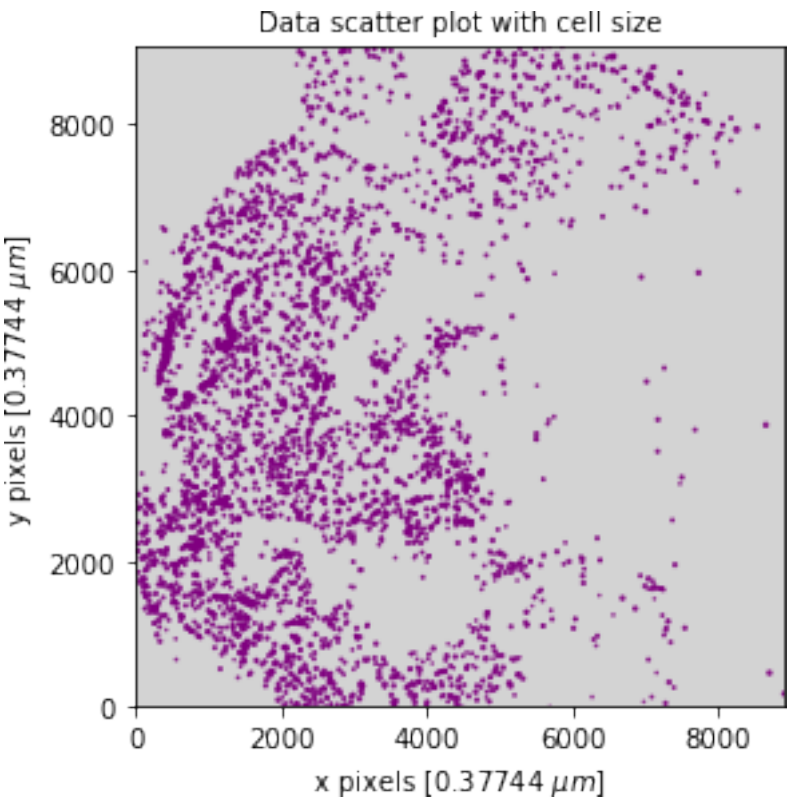
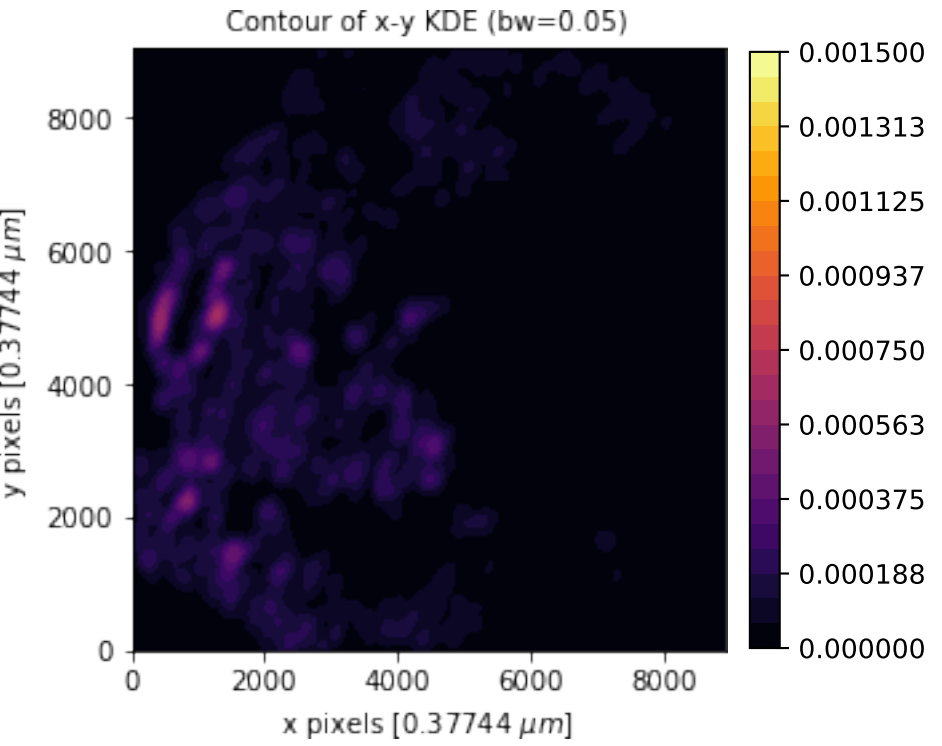


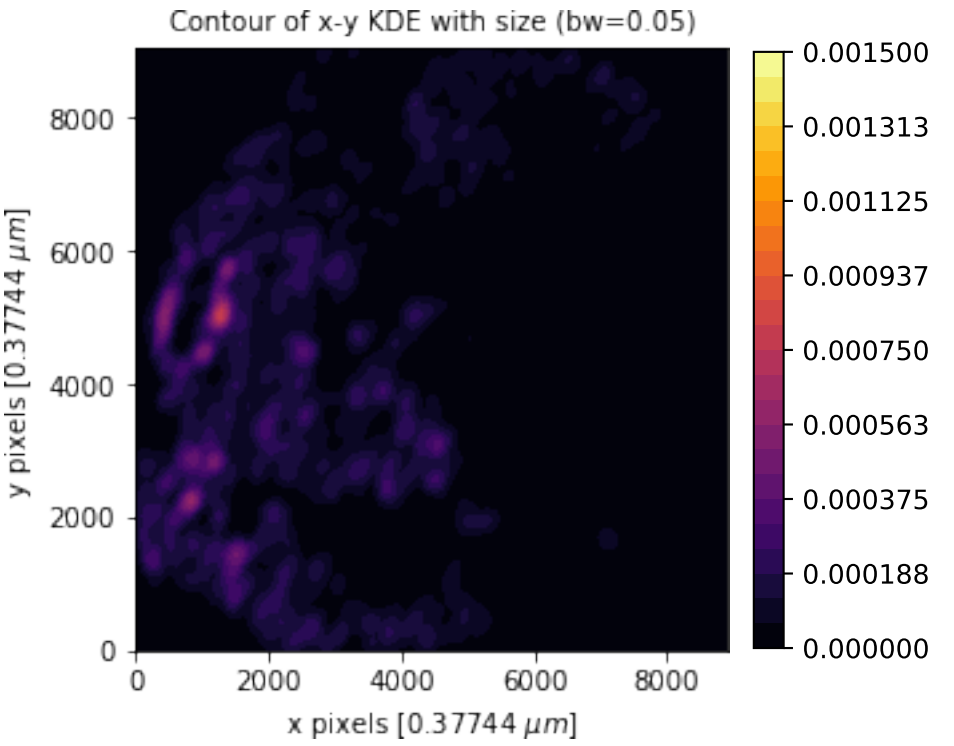
Figure S4

representative periphery sample









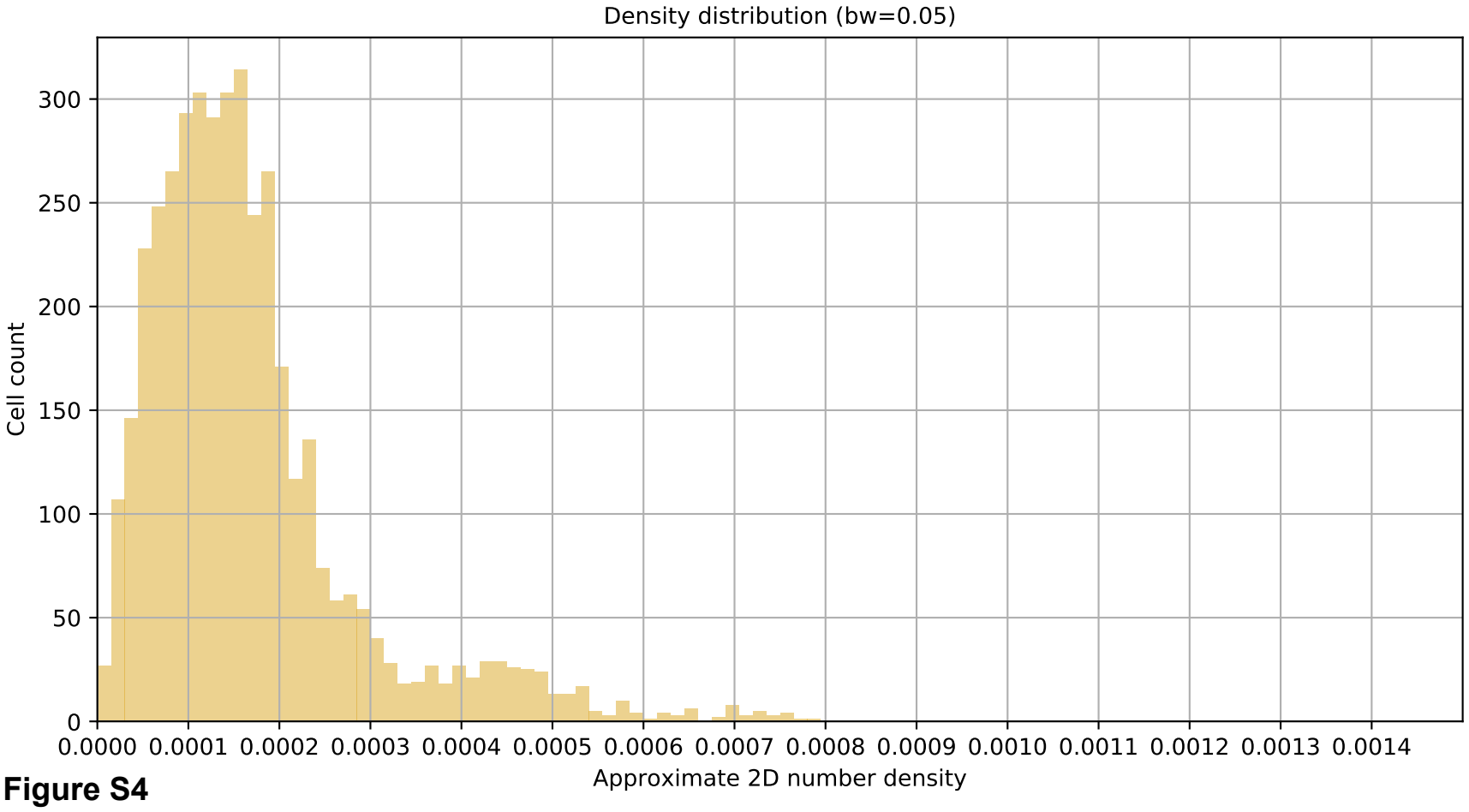


Figure S4

Figure S4

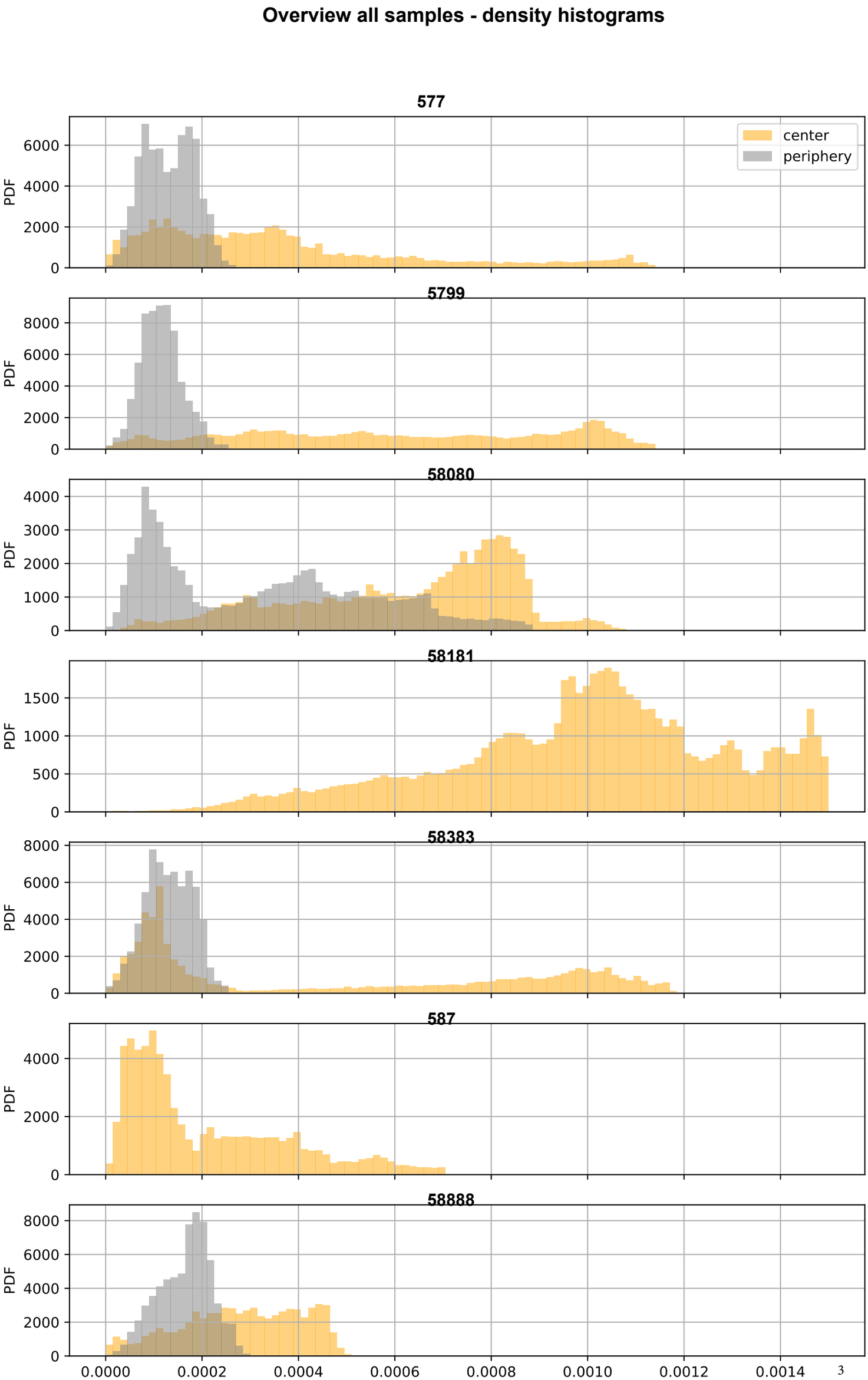

Overview all samples - density histograms
577
5799
58080
58181
58383
587
58888
center
periphery
PDF
0.0000
0.0002
0.0004
0.0006
0.0008
0.0010
0.0012
0.0014

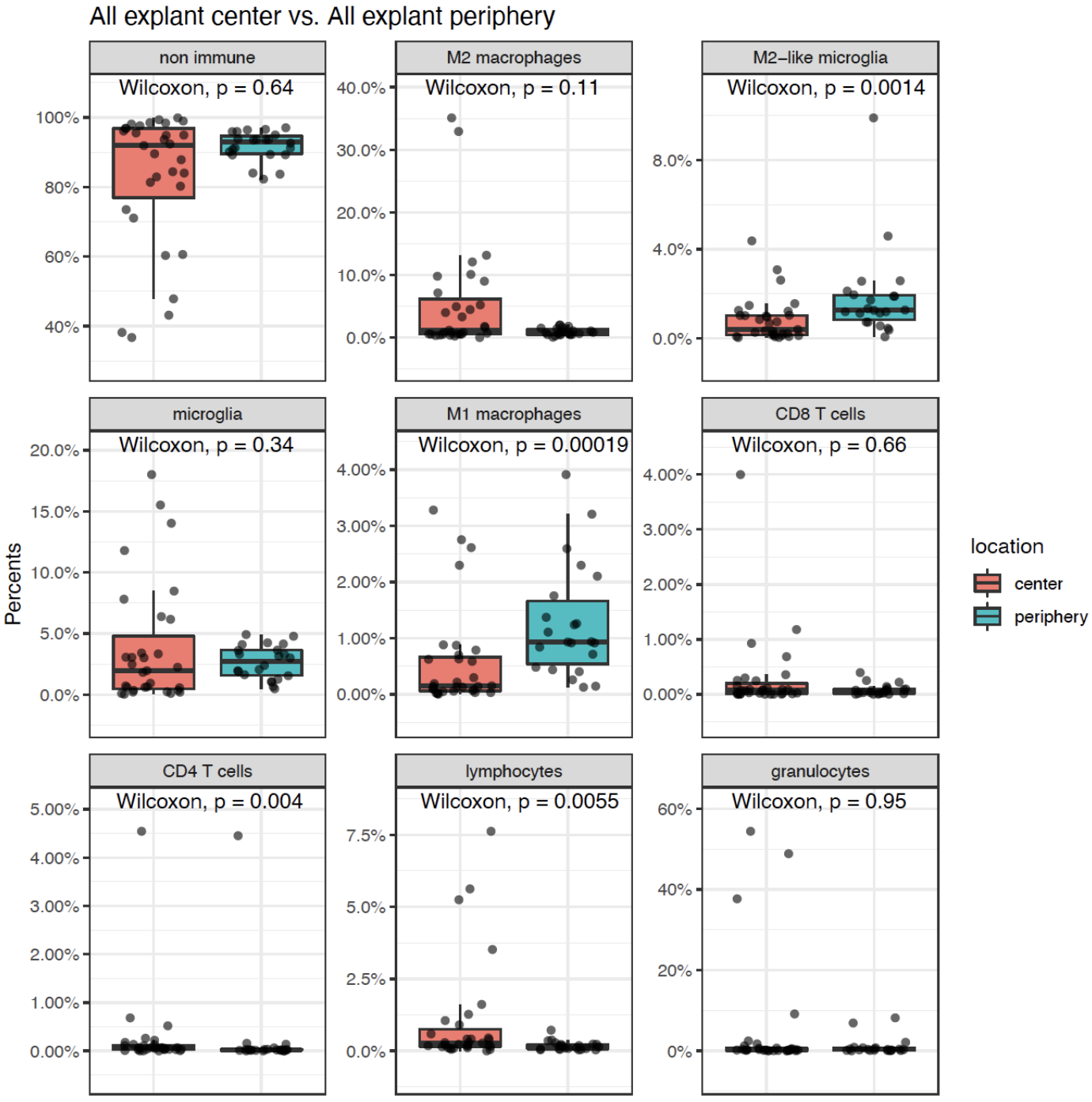
Fig. S4: Cell density calculations across center and periphery explants

Page 1: exemplary center explant. Top: H&E overview (left), scatter plot of cells with size representation (right). Scale bar: 1000 µm. Middle: KDE (Kernel Density Estimation) of cells according to location based on x-y coordinates (left), and in consideration of cell size (right). Bottom: density distribution histogram and cell number.

Page 2: exemplary periphery explant: Top: H&E overview (left), scatter plot of cells with size representation (right). Scale bar: 1000 µm. Middle: KDE (Kernel Density Estimation) of cells according to location based on x-y coordinates (left), and in consideration of cell size (right).. Bw = bandwith, which determines how smooth the KDE becomes. Bottom: density distribution histogram and cell number.

Page 3: smoothened cell density histograms across center and periphery explants. PDF=probability density function (y-axis), approximate 2D number density (x-axis).

A


All explant center vs. All explant periphery
non immune
Wilcoxon, p = 0.64
M2 macrophages
Wilcoxon, p = 0.11
M2–like microglia
Wilcoxon, p = 0.0014
microglia
Wilcoxon, p = 0.34
M1 macrophages
Wilcoxon, p = 0.00019
CD8 T cells
Wilcoxon, p = 0.66
CD4 T cells
Wilcoxon, p = 0.004
lymphocytes
Wilcoxon, p = 0.0055
granulocytes
Wilcoxon, p = 0.95
Percents
location
center
periphery

B

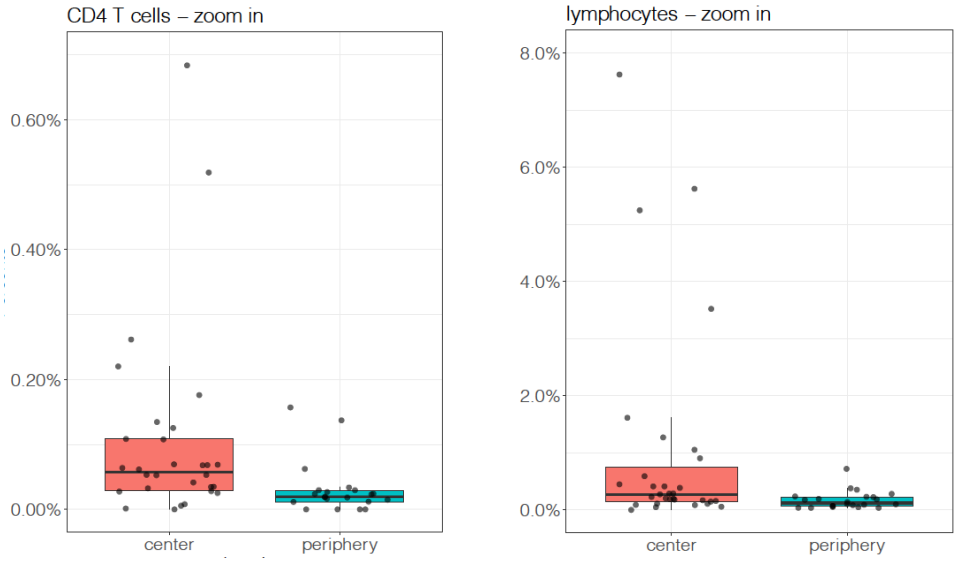
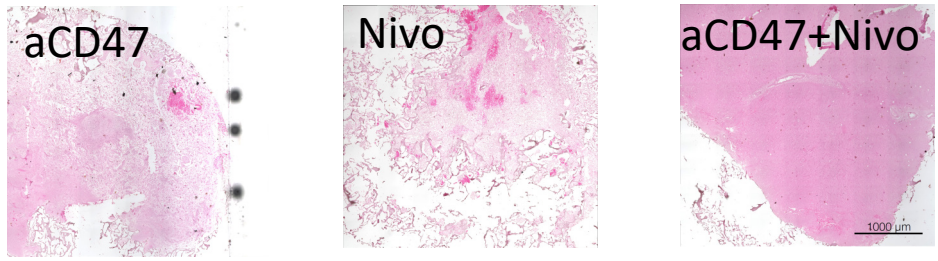

CD4 T cells – zoom in
lymphocytes – zoom in
center
periphery

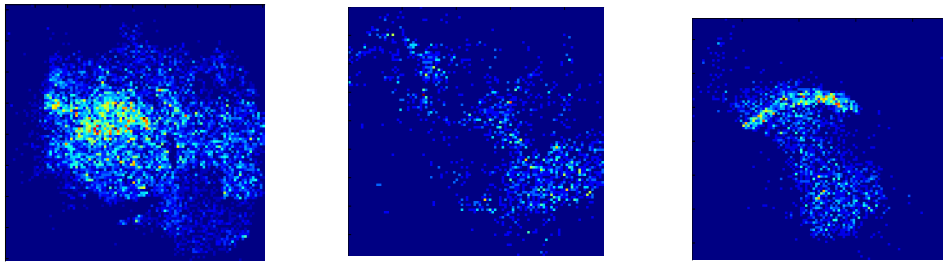
Figure S5

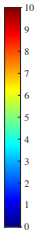
Fig. S5. Frequencies of cellular phenotypes (center vs. periphery) in explants.

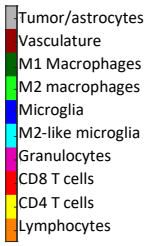
Proportions of cellular phenotypes per biopsy location in explants: The proportions of annotated cells per explant are plotted against biopsy location (center vs. periphery). Each dot in the bar graphs corresponds to one bioreactor sample. N=30 center samples and n=23 periphery samples. Statistics: Wilcoxon tests.

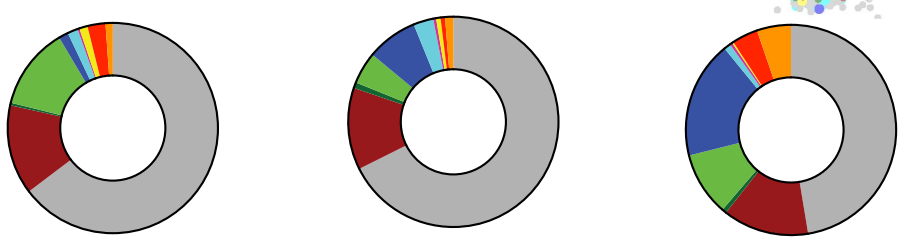


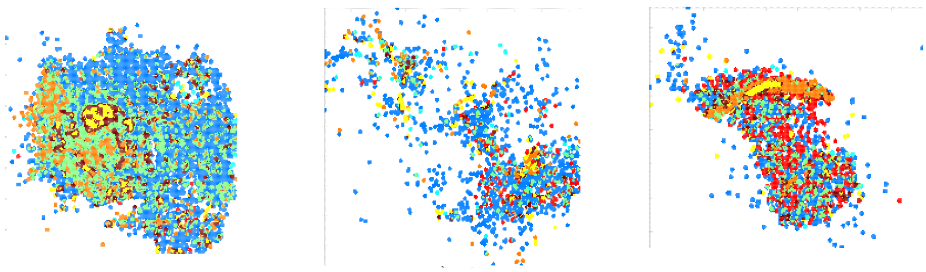
Age: 73
Gender: m
Subtype: MES
MGMT -
OS: 500d

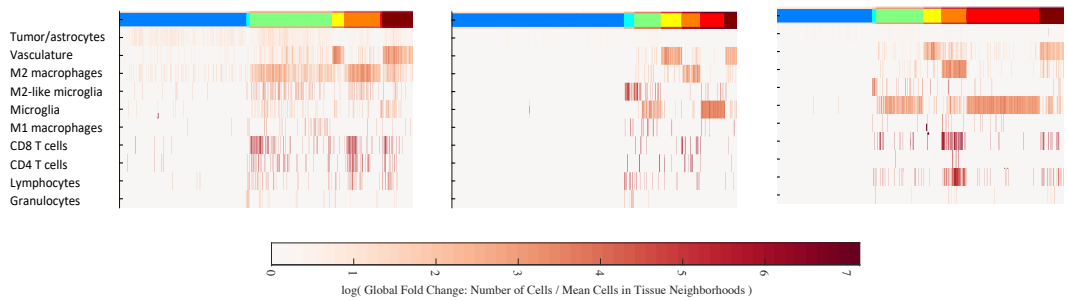


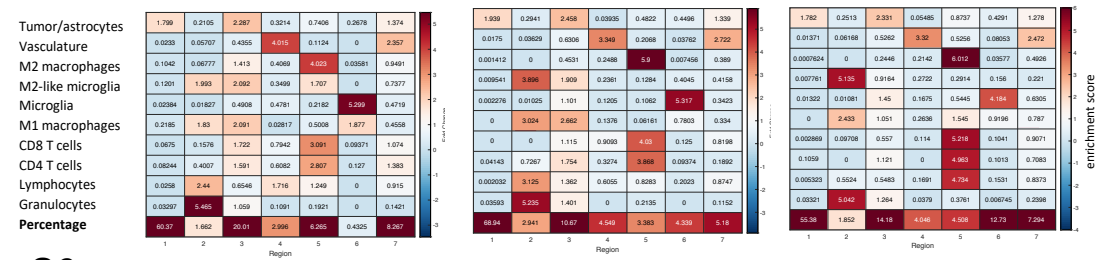












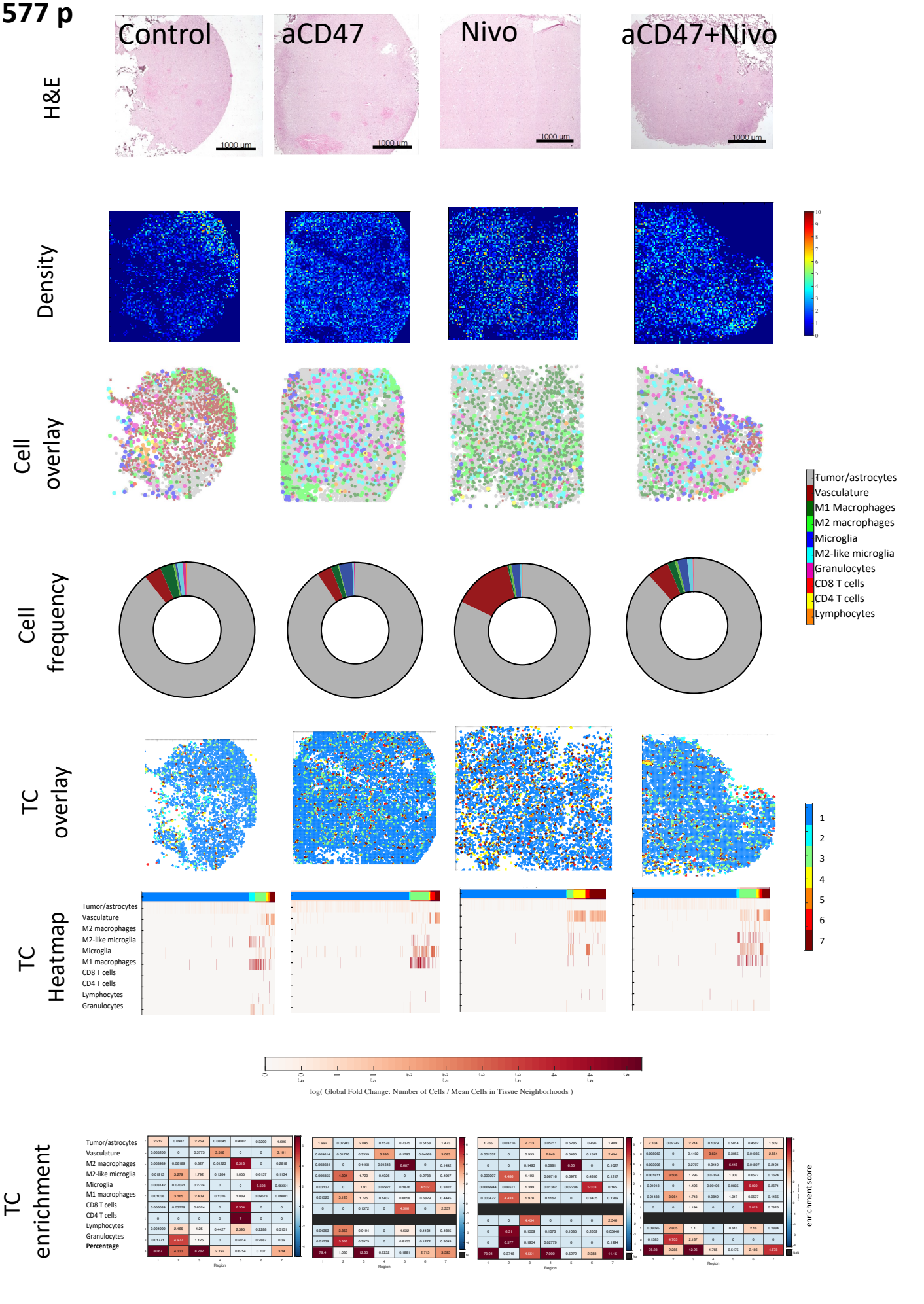

H&E
Control
aCD47
Nivo
aCD47+Nivo
1000 um
Density
Cell overlay
Tumor/astrocytes
Vasculature
M1 Macrophages
M2 macrophages
Microglia
M2-like microglia
Granulocytes
CD8 T cells
CD4 T cells
Lymphocytes
Cell frequency
TC overlay
TC Heatmap
log(Global Fold Change: Number of Cells / Mean Cells in Tissue Neighborhoods)
TC enrichment
Region
enrichment score

Figure S6

579c

Age: 58
Gender: f
Subtype: MES
MGMT -
OS: 505d

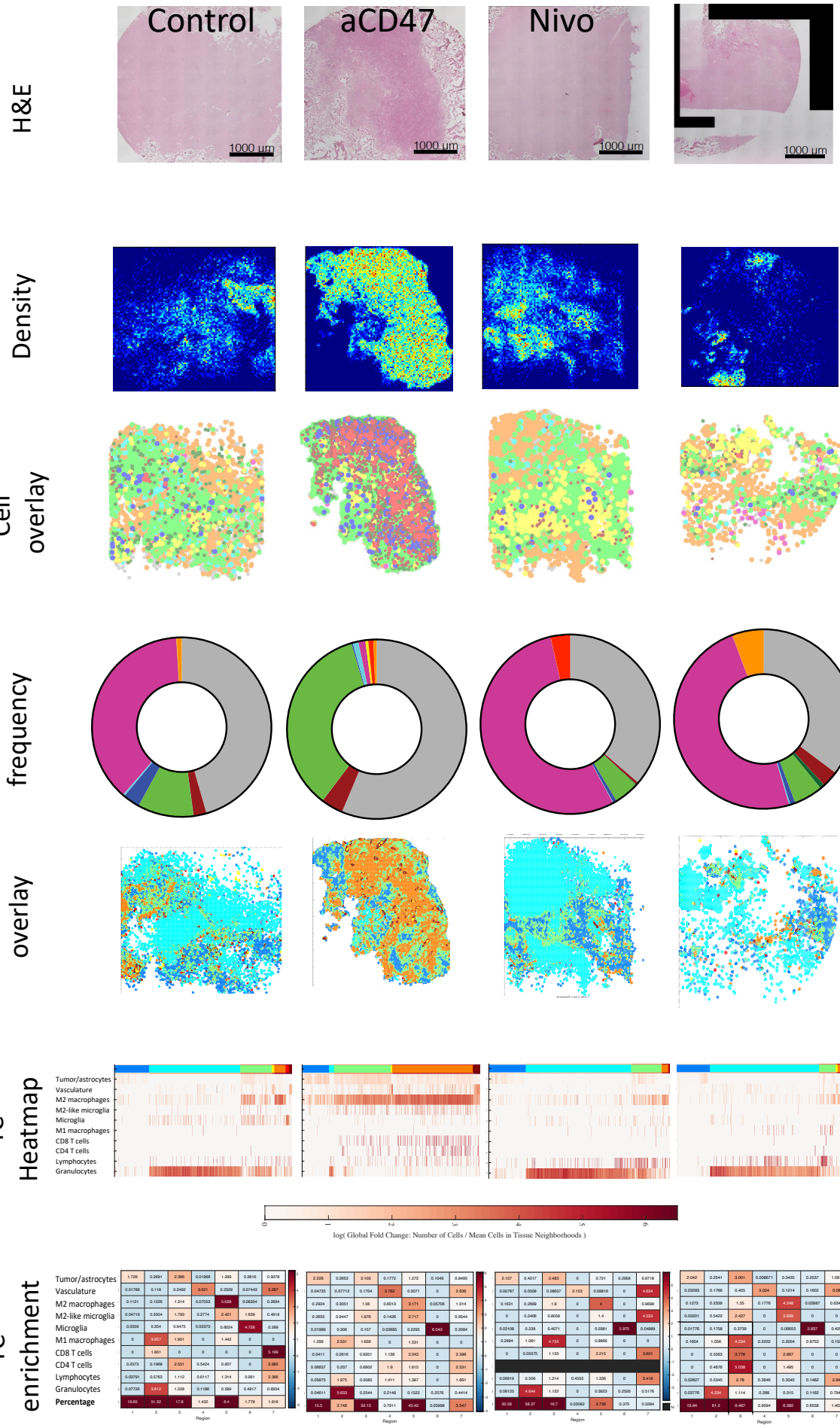


Figure S6

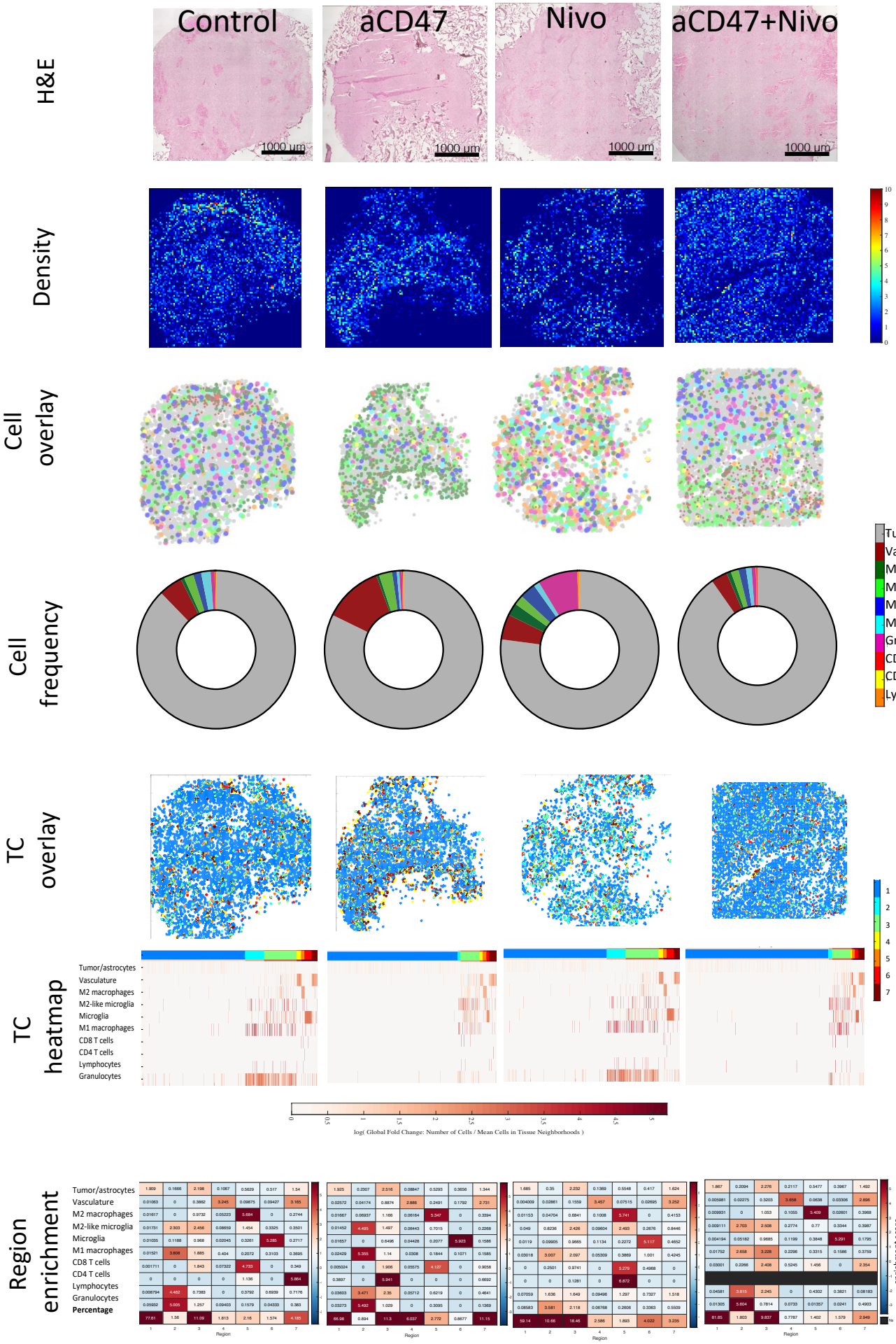

H&E
Control
aCD47
Nivo
aCD47+Nivo
1000 um
Density
Cell overlay
Cell frequency
Tumor/astrocytes
Vasculature
M1 Macrophages
M2 macrophages
Microglia
M2-like microglia
Granulocytes
CD8 T cells
CD4 T cells
Lymphocytes
TC overlay
TC heatmap
log(Global Fold Change: Number of Cells / Mean Cells in Tissue Neighborhoods)
Region enrichment
Percentage
Region
enrichment score

Figure S6

580 c

Age: 39
Gender:m
Subtype: CL
MGMT +
OS: 651

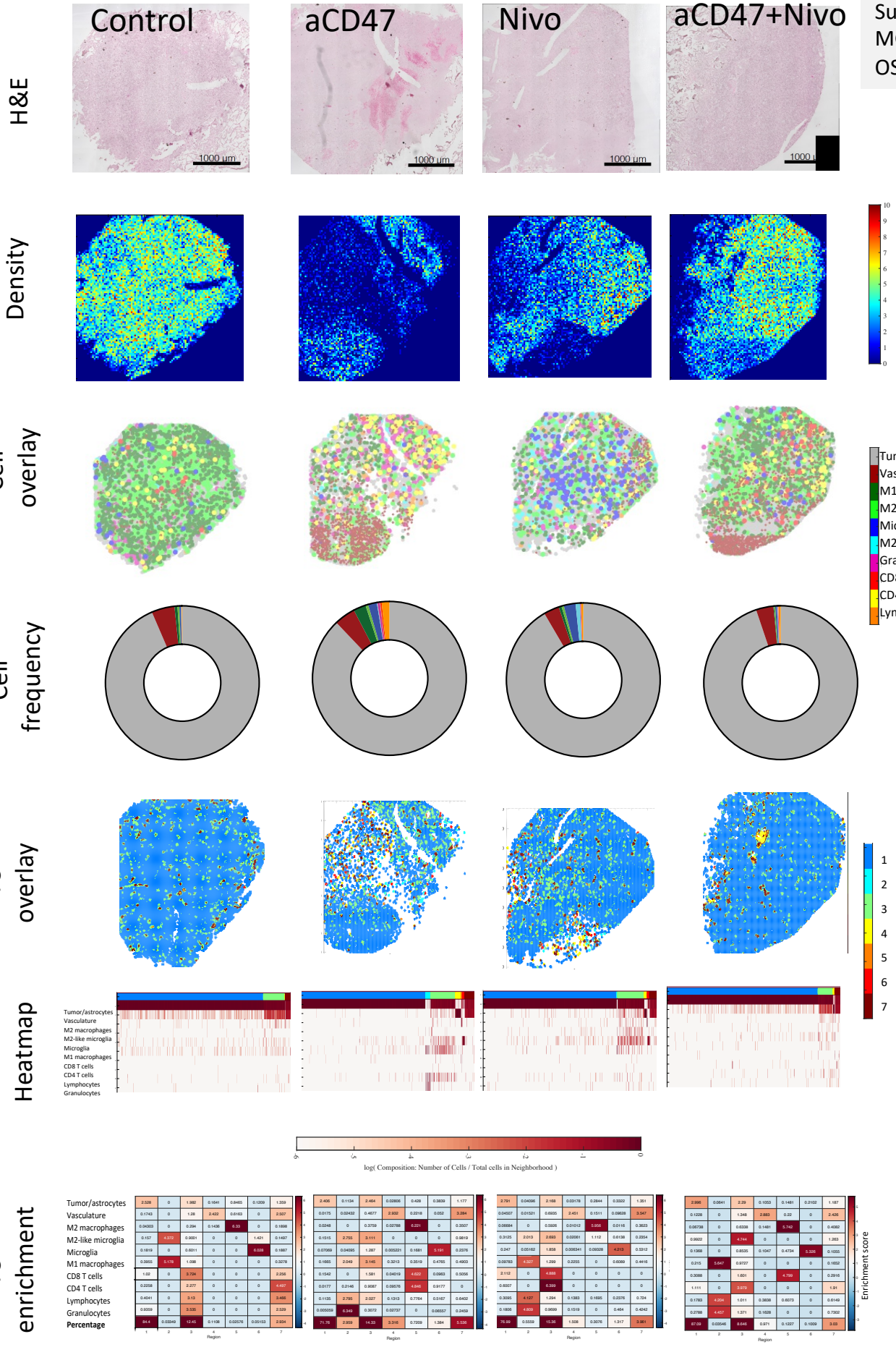


Figure S6

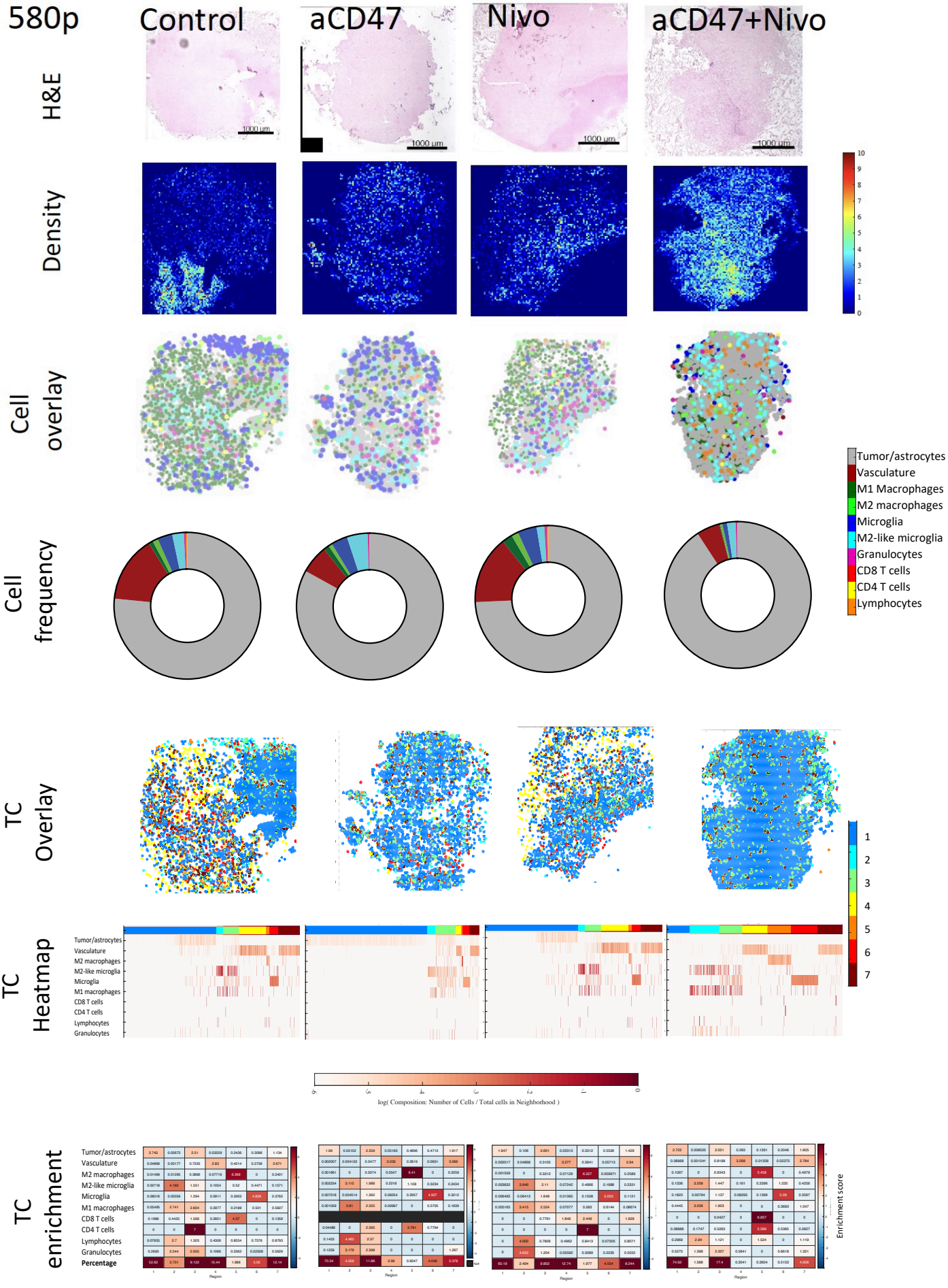

580p
Control
aCD47
Nivo
aCD47+Nivo
H&E
1000 µm
Density
Cell overlay
Tumor/astrocytes
Vasculature
M1 Macrophages
M2 macrophages
Microglia
M2-like microglia
Granulocytes
CD8 T cells
CD4 T cells
Lymphocytes
Cell frequency
TC Overlay
TC Heatmap
log(Composition: Number of Cells / Total cells in Neighborhood)
TC enrichment
Percentage
Region
Enrichment score

Figure S6

581c

Age: 59
Gender: m
Subtype: CL
MGMT +
OS: >700

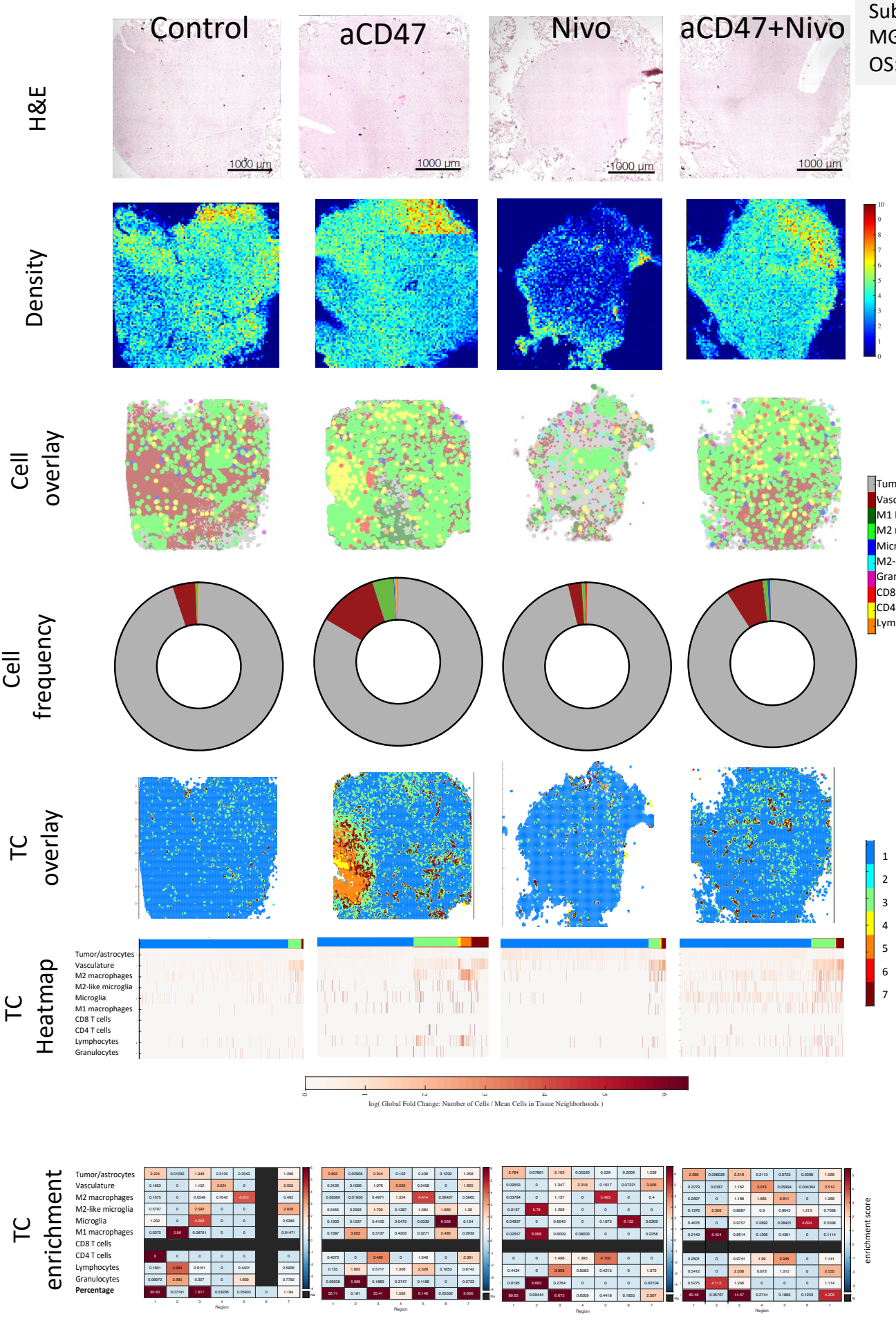


Figure S6

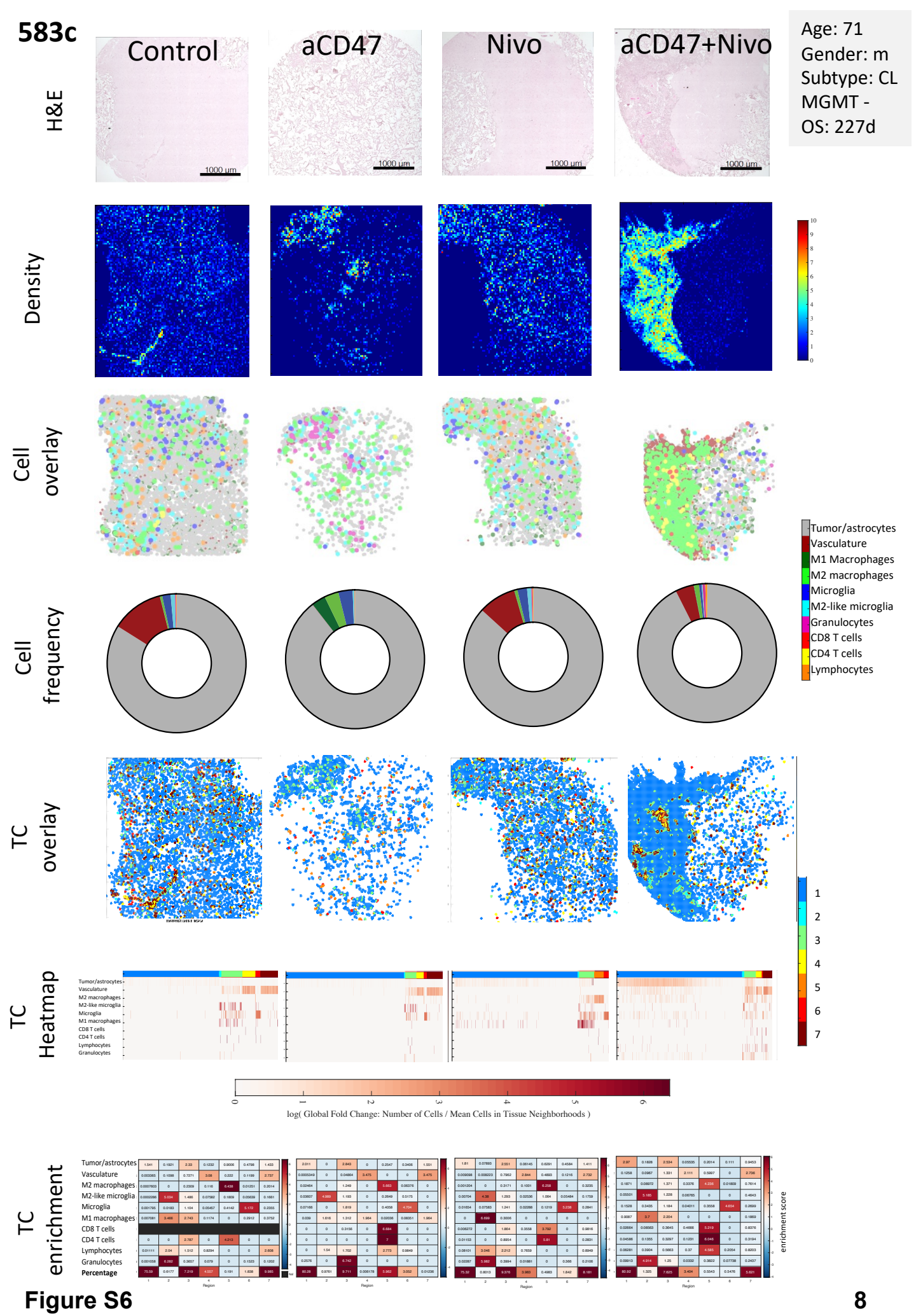

583c
Age: 71
Gender: m
Subtype: CL
MGMT -
OS: 227d
H&E
Control
aCD47
Nivo
aCD47+Nivo
1000 μm
Density
Cell overlay
Tumor/astrocytes
Vasculature
M1 Macrophages
M2 macrophages
Microglia
M2-like microglia
Granulocytes
CD8 T cells
CD4 T cells
Lymphocytes
Cell frequency
TC overlay
TC Heatmap
log(Global Fold Change: Number of Cells / Mean Cells in Tissue Neighborhoods)
TC enrichment
Percentage
enrichment score
Region

Figure S6

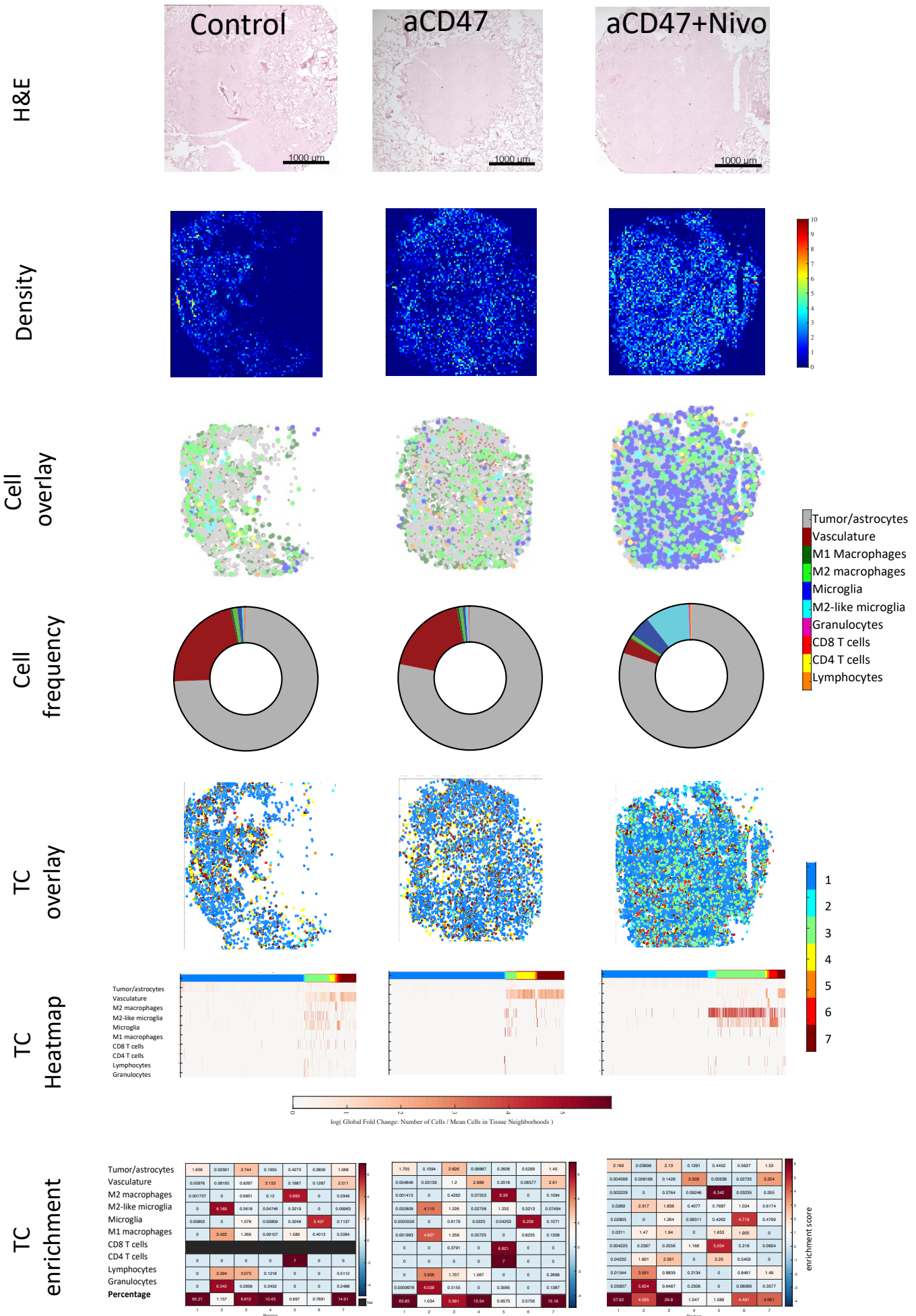

H&E
Control
aCD47
aCD47+Nivo
1000 um
Density
Cell overlay
Tumor/astrocytes
Vasculature
M1 Macrophages
M2 macrophages
Microglia
M2-like microglia
Granulocytes
CD8 T cells
CD4 T cells
Lymphocytes
Cell frequency
TC overlay
TC Heatmap
log(Global Fold Change: Number of Cells / Mean Cells in Tissue Neighborhoods)
TC enrichment
Percentage
Region
enrichment score

Figure S6

587c

Age: 69
Gender: f
Subtype: MES
MGMT -
OS: 470d

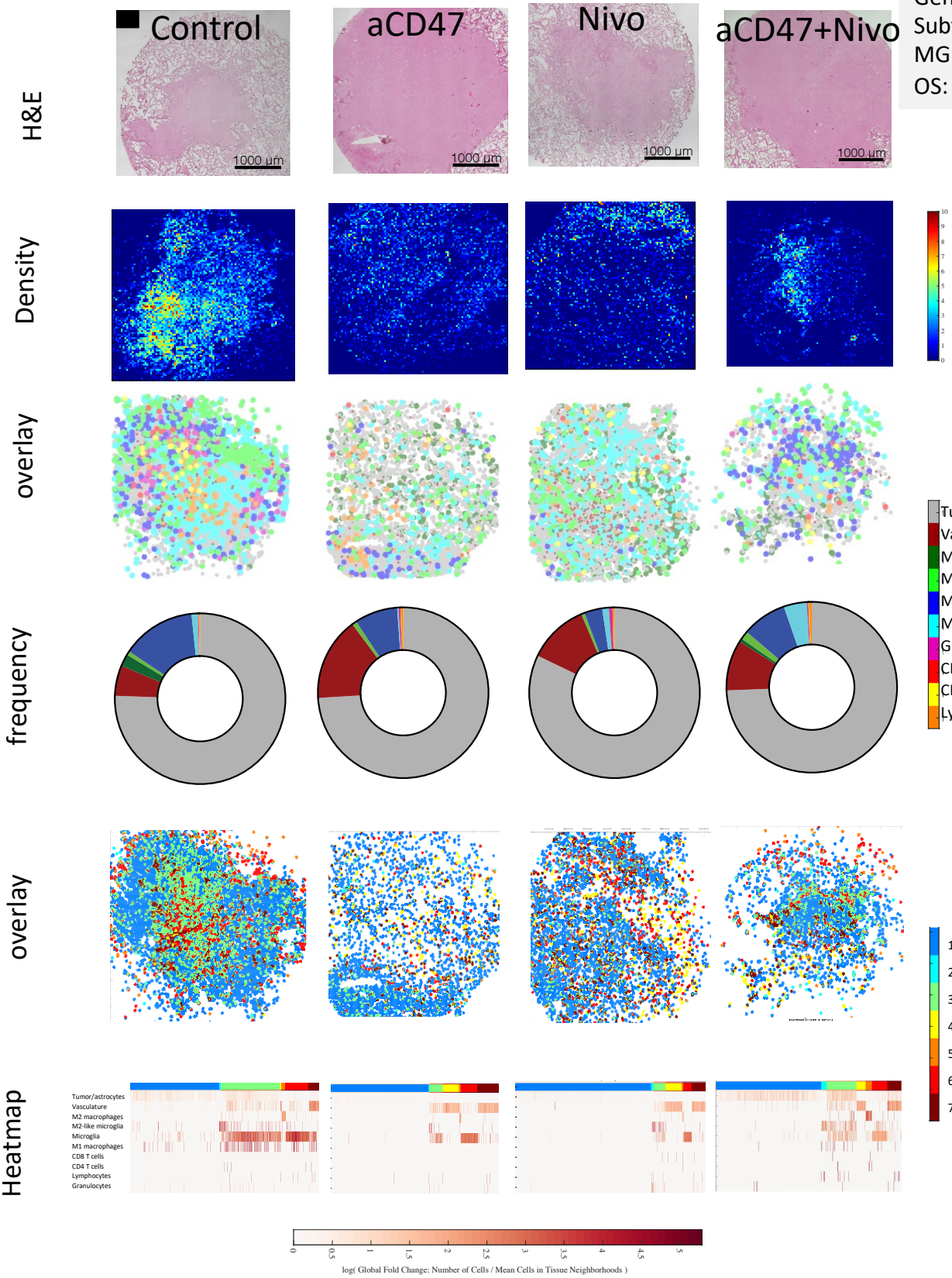


Figure S6

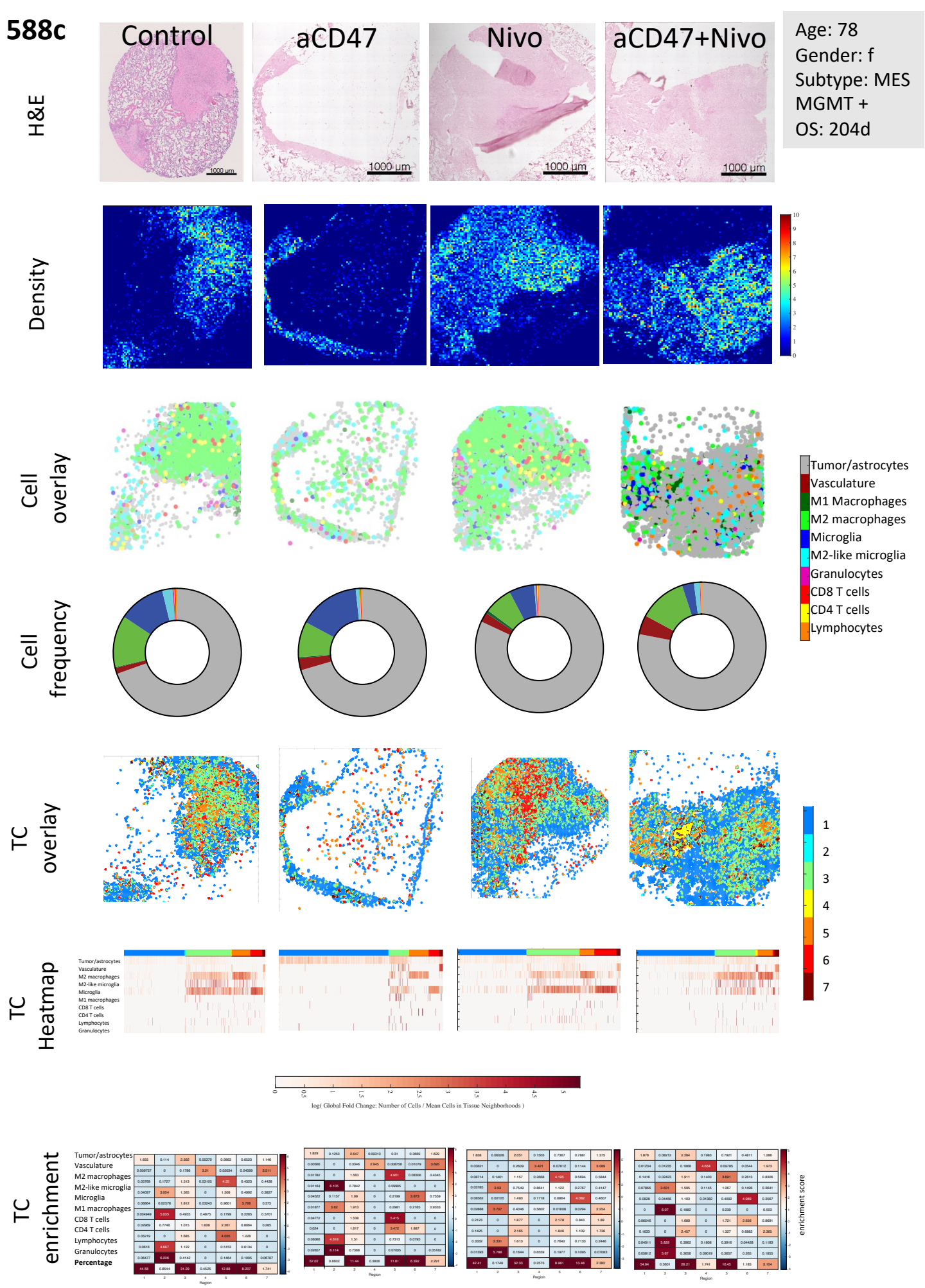

588c
Control
aCD47
Nivo
aCD47+Nivo
Age: 78
Gender: f
Subtype: MES
MGMT +
OS: 204d
H&E
1000 µm
Density
Cell overlay
Tumor/astrocytes
Vasculature
M1 Macrophages
M2 macrophages
Microglia
M2-like microglia
Granulocytes
CD8 T cells
CD4 T cells
Lymphocytes
Cell frequency
TC overlay
TC Heatmap
log(Global Fold Change: Number of Cells / Mean Cells in Tissue Neighborhoods)
TC enrichment
Percentage
Region
enrichment score

Figure S6

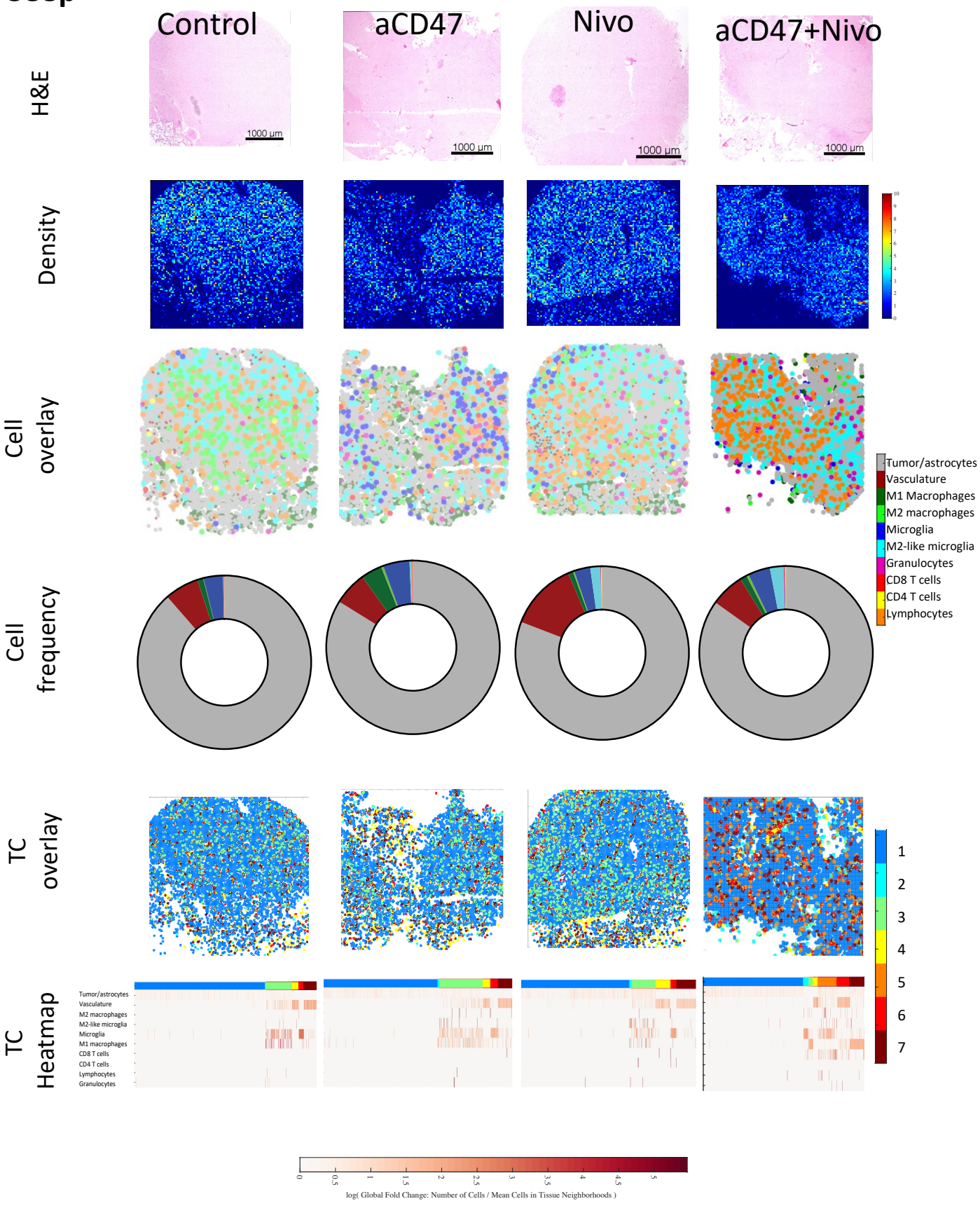

Control
aCD47
Nivo
aCD47+Nivo
H&E
1000 µm
Density
Cell overlay
Tumor/astrocytes
Vasculature
M1 Macrophages
M2 macrophages
Microglia
M2-like microglia
Granulocytes
CD8 T cells
CD4 T cells
Lymphocytes
Cell frequency
TC overlay
TC Heatmap
TC enrichment
Percentage
Region
log(Global Fold Change: Number of Cells / Mean Cells in Tissue Neighborhoods)
enrichment score

Figure S6

Fig. S6. Individualized treatment outcomes per patient and per region.

For each patient (center and periphery), a set of personalized calculations were generated. *1st row*: H&E (stitched together after CODEX run) stains of explants control (GBM) and treatment conditions, scale bar: 1000µm. *2nd row:* point density plot (x-y plot is divided into a 100x100 grid, in which every rectangle is colored corresponding to the number of elements within this square). *3rd row:* Cell overlay, each dot represents an individual, annotated cell type according to the final cell type annotation. Color code of cell types displayed on the right; 4th row: pie chart of cell type proportions within each individual explant. *5th row*: tissue compartment (TC) overlay, according to 7 TCs outlined in Figure 3 (cf. Figure 3 and text for TC naming and numbering). *6th row:* TC region heatmap depicting the TC frequency (top bar, similar color code as in TC overlay), and the global fold change (number of cells per mean cells in TC) of specific cell types per TC. *7th row*: region enrichment heatmaps depicting the fold change enrichment of cell type composition per TC, and the overall percentage of the TC within an individual sample.

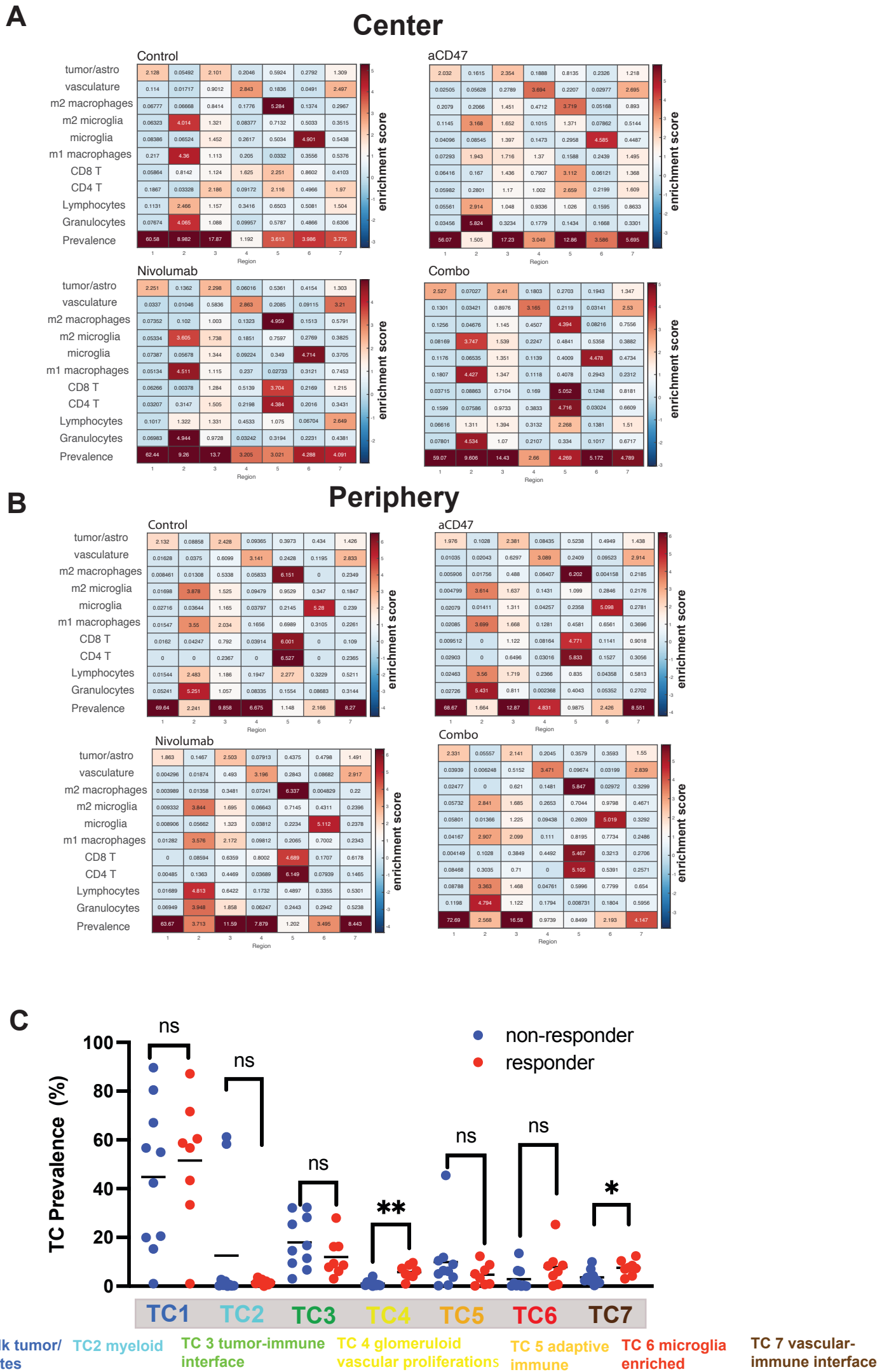

A
Center
Control
aCD47
Nivolumab
Combo
tumor/astro
vasculature
m2 macrophages
m2 microglia
microglia
m1 macrophages
CD8 T
CD4 T
Lymphocytes
Granulocytes
Prevalence
Region
enrichment score
B
Periphery
Control
aCD47
Nivolumab
Combo
C
TC Prevalence (%)
non-responder
responder
ns
**
*
TC1
TC2
TC3
TC4
TC5
TC6
TC7
TC1 bulk tumor/ astrocytes
TC2 myeloid
TC 3 tumor-immune interface
TC 4 glomeruloid vascular proliferations
TC 5 adaptive immune
TC 6 microglia enriched
TC 7 vascular-immune interface

Figure S7

Fig. S7. TC enrichment scores per therapeutic condition and location; TC prevalence changes in respondig vs. non responding explants.

Individual enrichment scores of cell types in respective TCs stratified per treatment and biopsy locations in pooled explants- (**A**) center explants: n=6 for control, n=7 for anti-CD47, n=7 for Nivolumab and n=7 for anti-CD47+Nivolumab; (**B**) periphery explants: n=5 for control, n=5 for anti-CD47, n=4 for Nivolumab and n=5 for anti-CD47+Nivolumab. (**C**) TC prevalence changes in non-responding vs. responding center explants, cf. Fig. 6A, lower panels. Statistics: *$p<0.05$, **$p<0.01$, Welch's t-test,

ADA
p = 0.092
ADGRG1
p = 0.0013
ANGPT1
p = 7.3e−05
ANGPT2
p = 0.11
ARG1
p = 0.0062
CAIX
p = 1.4e−05
CASP−8
p = 7.3e−05
CCL17
p = 0.22
CCL19
p = 0.062
CCL20
p = 0.45
CCL23
p = 0.62
CCL3
p = 0.062
CCL4
p = 0.26
CD244
p = 0.036
CD27
p = 0.036
CD28
p = 0.00059
CD4
p = 0.028
CD40
p = 0.0086
CD40−L
p = 0.29
CD5
p = 5.6e−05
CD70
p = 0.038
CD83
p = 0.9
CD8A
p = 0.00031
CRTAM
p = 0.013
CSF−1
p = 0.097
CX3CL1
p = 0.029
CXCL1
p = 0.96
CXCL10
p = 0.2
CXCL11
p = 0.53
CXCL12
p = 0.078
CXCL13
p = 1.9e−05
CXCL5
p = 0.52
CXCL9
p = 0.012
DCN
p = 0.0037
EGF
p = 0.19
FASLG
p = 0.015
FGF2
p = 0.13
Gal−1
p = 0.0031
Gal−9
p = 0.0086
GZMA
p = 0.001
GZMB
p = 0.84
GZMH
p = 0.00022
HGF
p = 0.2
HO−1
p = 4.3e−05
ICOSLG
p = 9.4e−05
IFN−gamma
p = 0.00066
IL−1 alpha
p = 0.36
IL10
p = 0.53
IL12
p = 0.15
IL12RB1
p = 0.049
IL13
p = 0.011
IL15
p = 0.0024
IL18
p = 0.022
IL2
p = 0.58
IL33
p = 0.066
IL4
p = 0.0046
IL5
p = 0.008
IL6
p = 0.21
IL7
p = 0.002
IL8
p = 0.0028
KIR3DL1
p = 0.84
KLRD1
p = 0.097
LAG3
p = 0.0016
LAMP3
p = 0.041
LAP TGF−beta−1
p = 5.7e−06
MCP−1
p = 6.4e−05
MCP−2
p = 0.24
MCP−3
p = 0.13
MCP−4
p = 0.0086
MIC−A/B
p = 0.049
MMP12
p = 0.88
MMP7
p = 0.014
MUC−16
p = 0.0057
NCR1
p = 0.016
NOS3
p = 0.0018
PD−L1
p = 0.01
PD−L2
p = 1.2e−05
PDCD1
p = 0.33
PDGF subunit B
p = 0.34
PGF
p = 0.55
PTN
p = 2.6e−06
TIE2
p = 0.049
TNF
p = 0.96
TNFRSF12A
p = 0.21
TNFRSF21
p = 0.36
TNFRSF4
p = 0.0034
TNFRSF9
p = 0.33
TNFSF14
p = 6.4e−05
TRAIL
p = 0.074
TWEAK
p = 0.73
VEGFA
p = 0.00048
VEGFR−2
p = 0.013
NPX
Group
c
p

Figure S8

Fig. S8. Differential expression of cytokines in treated center and periphery explants.

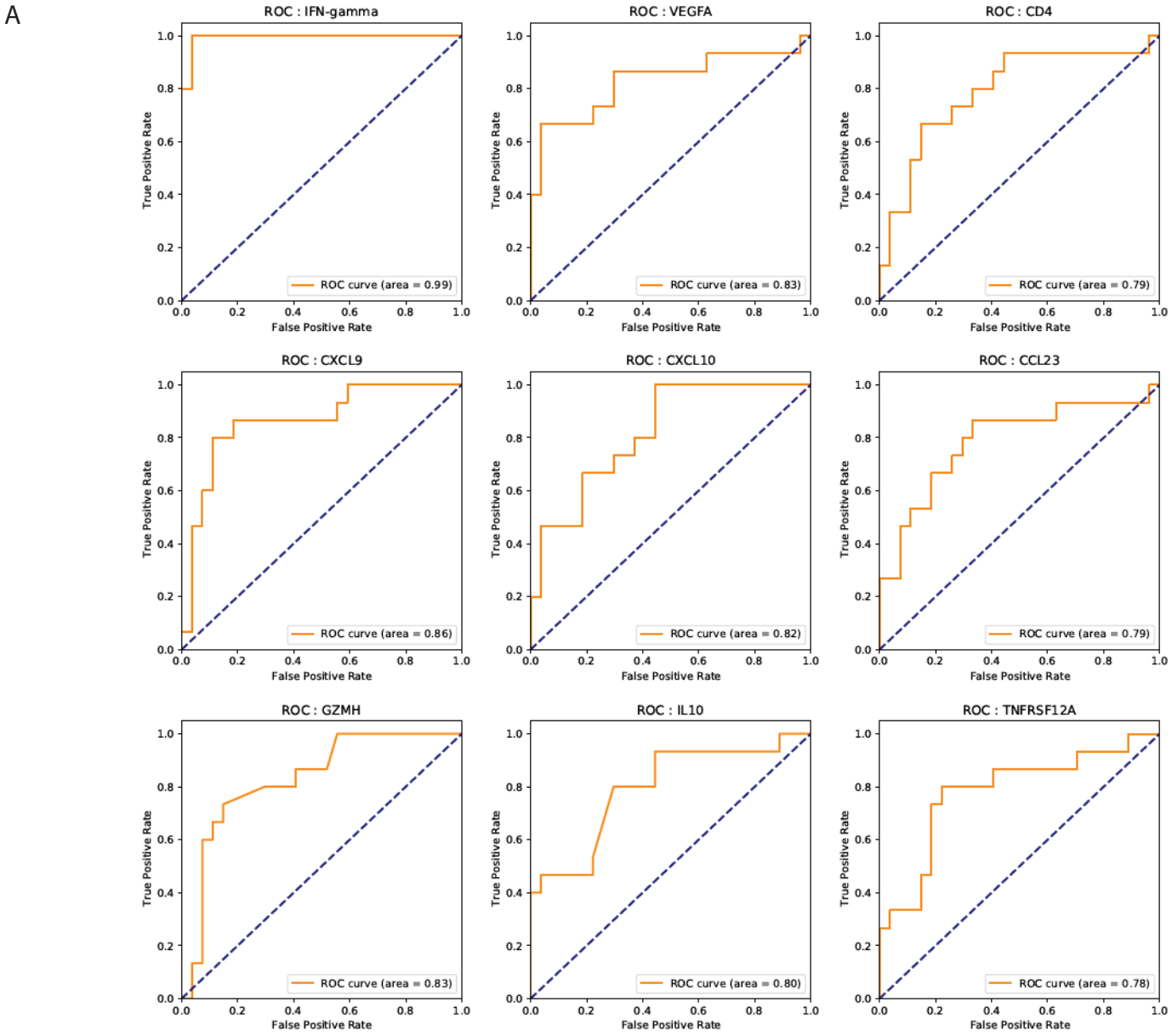
Results from multiplexed bead based proximity extension assay of 92 analytes in the bioreactor culture supernatant. Comparison of pooled treated (all conditions) center (n=21) and periphery (n=21) explants independent of response. Only significant results are displayed. Values are displayed as mean +/- SEM. NPX: Log2 scaled **normalized protein expression**. Statistics: ANOVA with multiple comparison adjustments. Welch's t-test between the groups.


PCA 0 - 36.2%
PCA-0 weights
0.1600
0.1575
0.1550
0.1525
0.1500
CASP-8
LAP TGF-beta-1
CSF-1
CD40
ANGPT2
TNF
MMP7
Gal-9
TNFRSF12A
PCA 1 - 14.0%
PCA-1 weights
−0.18
−0.20
−0.22
−0.24
IL8
MCP-1
MCP-3
CCL4
MCP-2
CCL3
IL6
CCL20
CXCL1
PCA 2 - 9.8%
PCA-2 weights
0.2
0.1
0.0
−0.1
−0.2
TIE2
TRAIL
CD244
PDGF subunit B
IL2
ARG1
CD27
FASLG
KLRD1
PCA 3 - 6.2%
PCA-3 weights
0.2
0.0
−0.2
KIR3DL1
IFN-gamma
CXCL9
CCL19
ANGPT1
NOS3
CXCL12
TNFRSF21
IL-1 alpha
PCA 4 - 4.7%
PCA-4 weights
0.2
0.1
0.0
−0.1
−0.2
−0.3
EGF
IL5
CD28
CD40-L
IL7
CRTAM
CD4
KIR3DL1
TNFSF14

Figure S9

Fig. S9. PCA Loadings of unbiased cytokine analysis in center and periphery.

PCA loadings of the principal component analysis in **Figure 5D**.



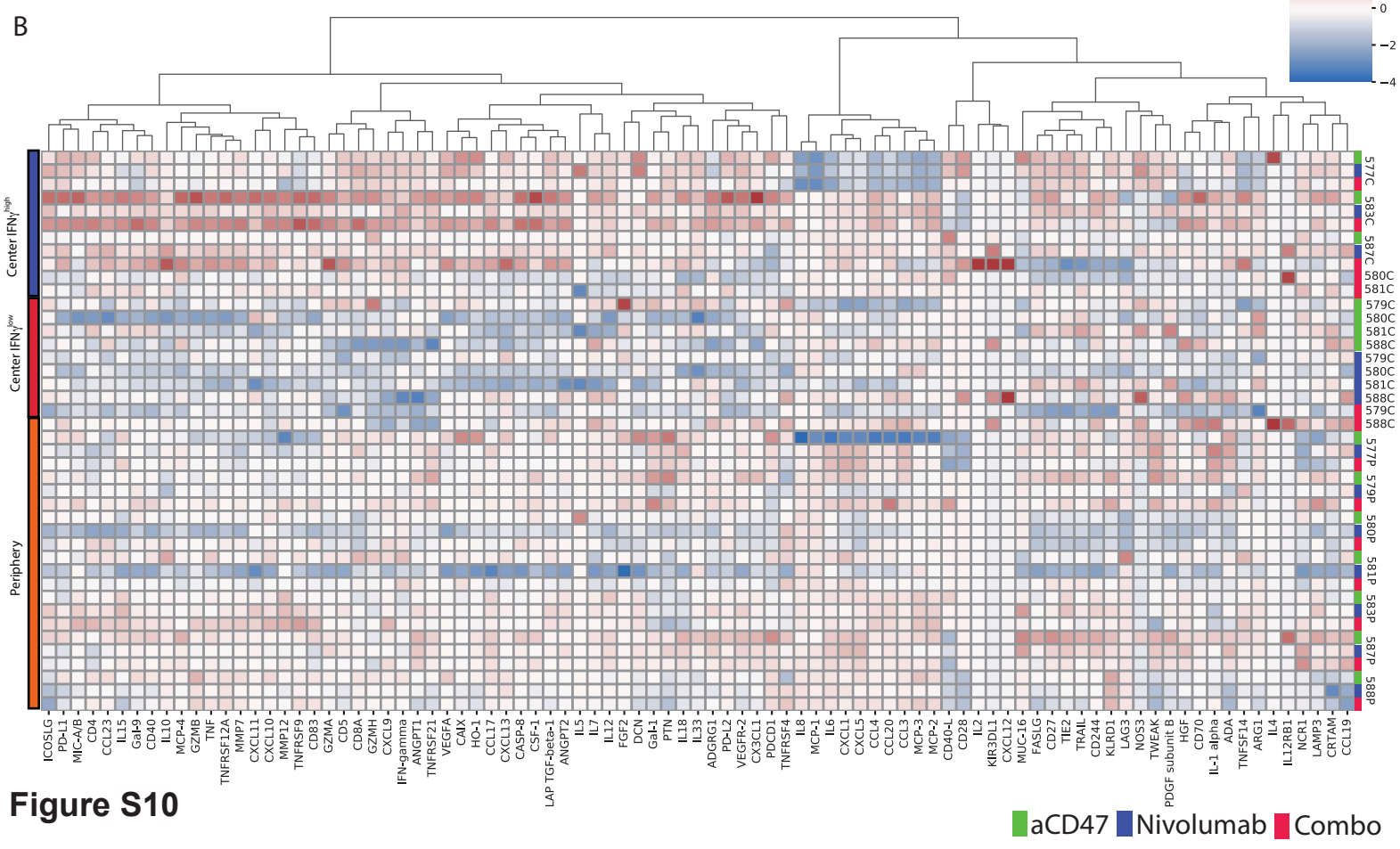
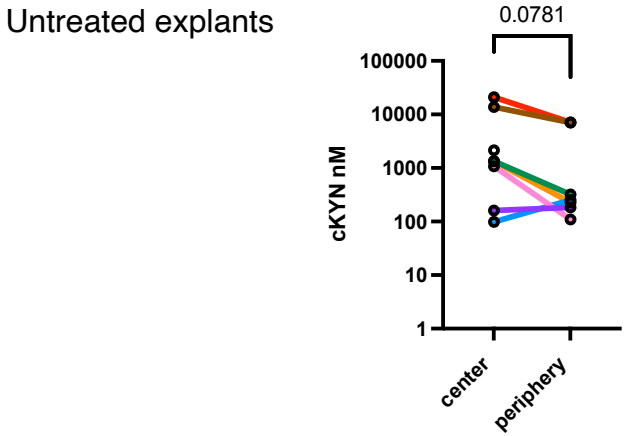


Figure S10

aCD47 Nivolumab Combo

Fig. S10. AUC-ROC analysis of cytokines associated with IFNγ, heatmap clustering of cytokines stratified per IFNγ status and location.

(A) Based on the IFNγ stratification criterion, we performed an AUC-ROC analysis of other associated cytokines in center explants. Cytokines with a AUC > 0.78 were determined to have a high association with IFNγ and are listed. **(B)** Normalized heatmap depicting the overall explant cytokine response per treatment in IFNγ^{high} and IFNγ^{low} center explants, and periphery explants. Samples were normalized to internal control condition.


Untreated explants
0.0781
cKYN nM
100000
10000
1000
100
10
1
center
periphery

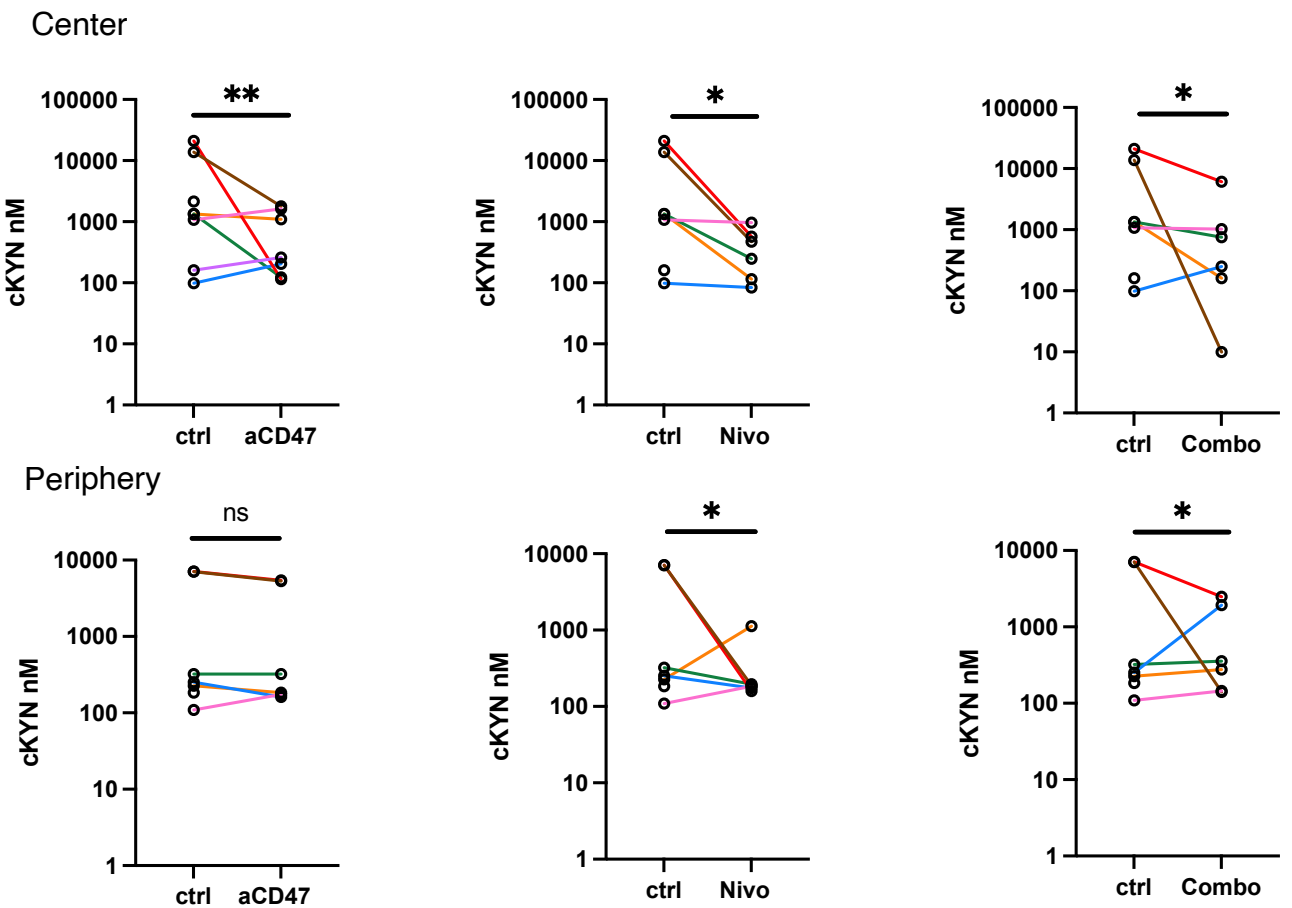
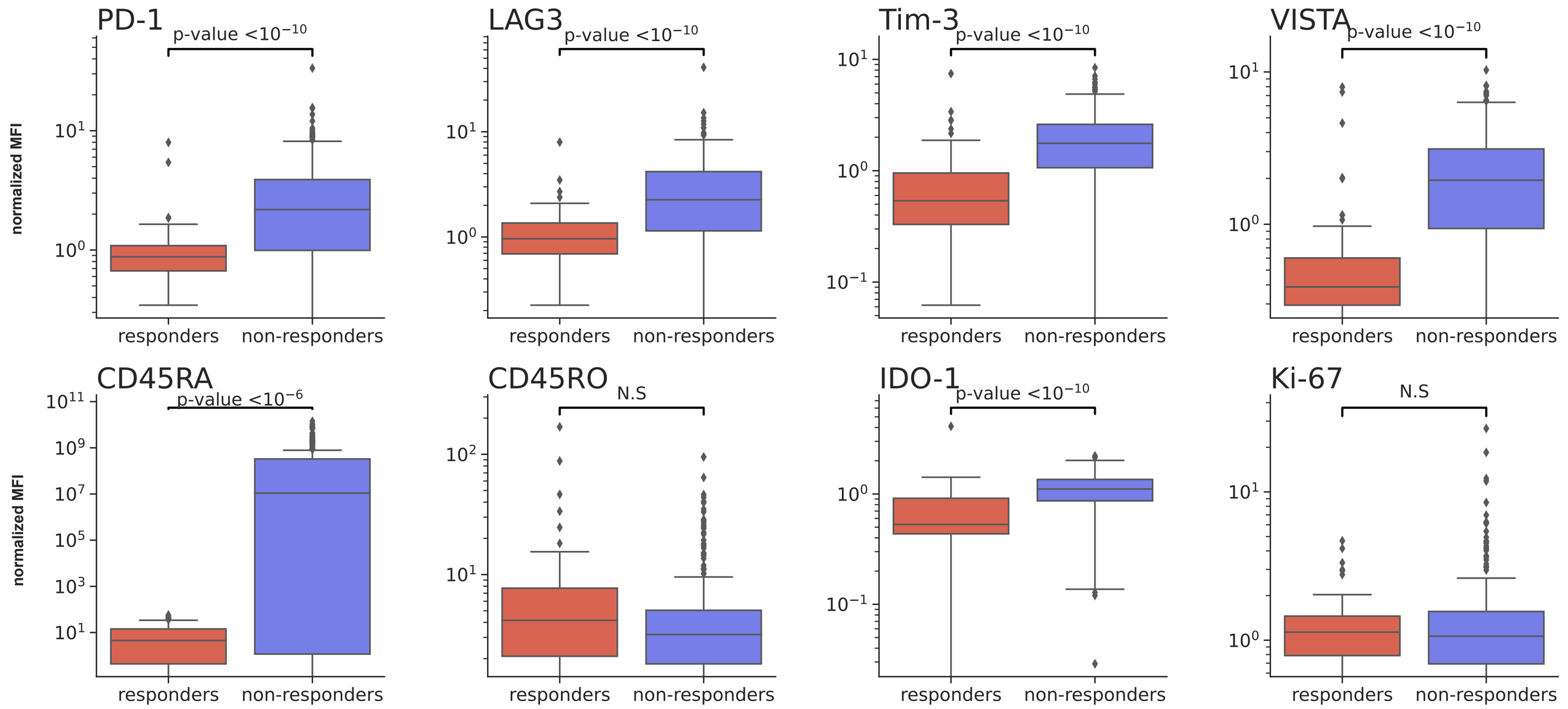

Center
**
*
*
cKYN nM
ctrl
aCD47
Nivo
Combo
Periphery
ns
*
*
577
580
583
588
579
581
587

Figure S11

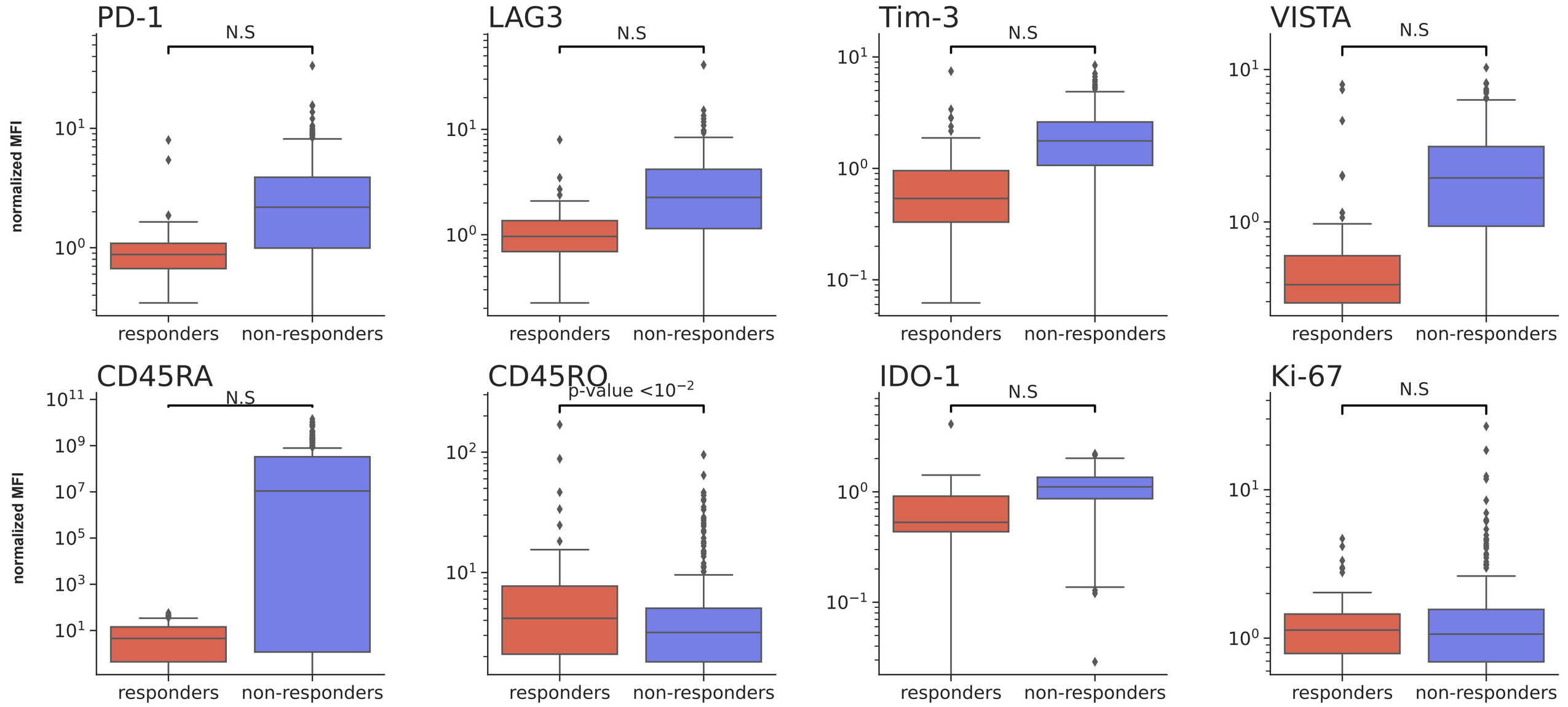
Fig. S11. KYN modulation in individual patient explants after immunotherapy, and based on IFNγ status.

Assessment of KYN concentrations (nM) in explant culture supernatant by LC-MS in control and immunotherapy treated center and periphery explants. Each dot represents an individual LC-MS measurement. The coloured connecting lines are pairs of individual patients. *Statistics:* *p<0.05, **p<0.01, ***p<0.005, Wilcoxon test.

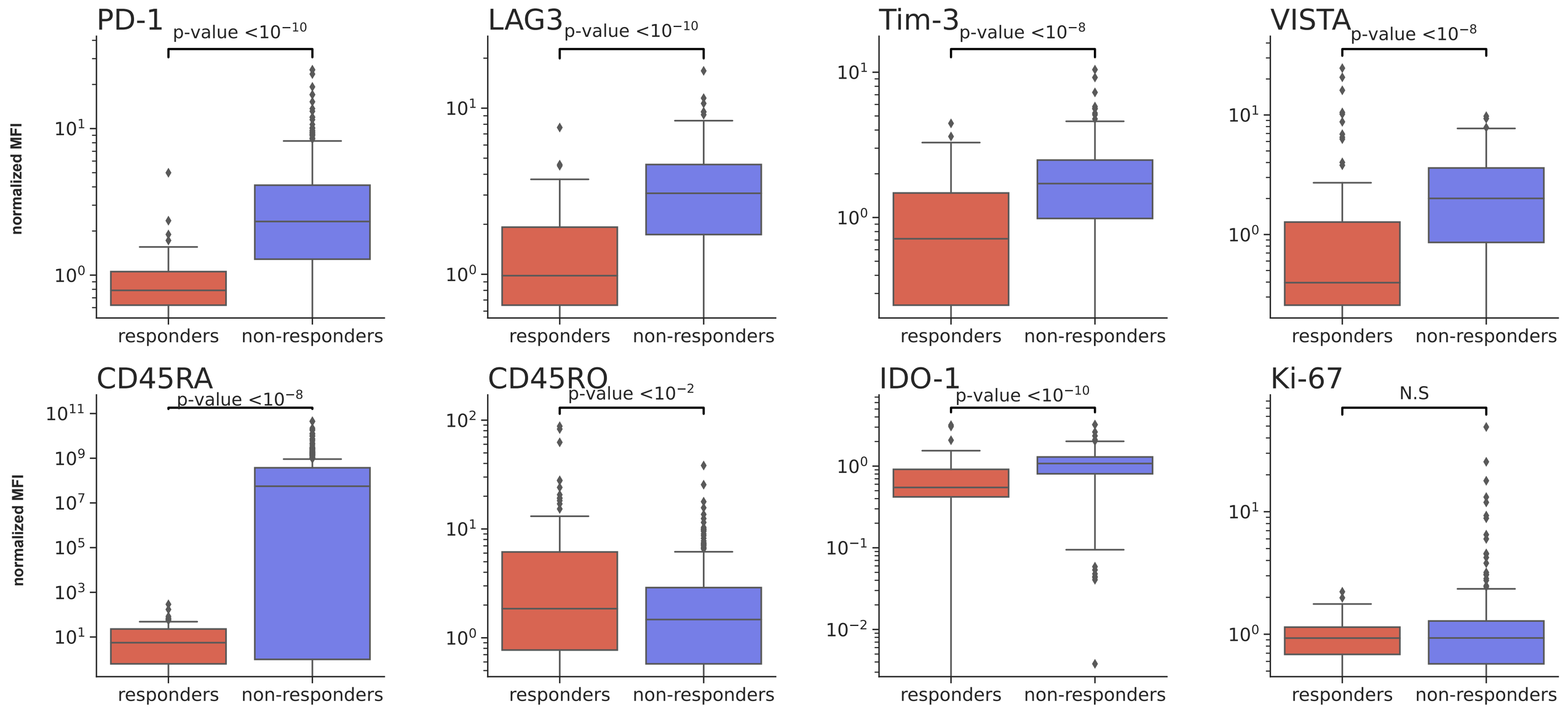
Center, CD4 T-cells



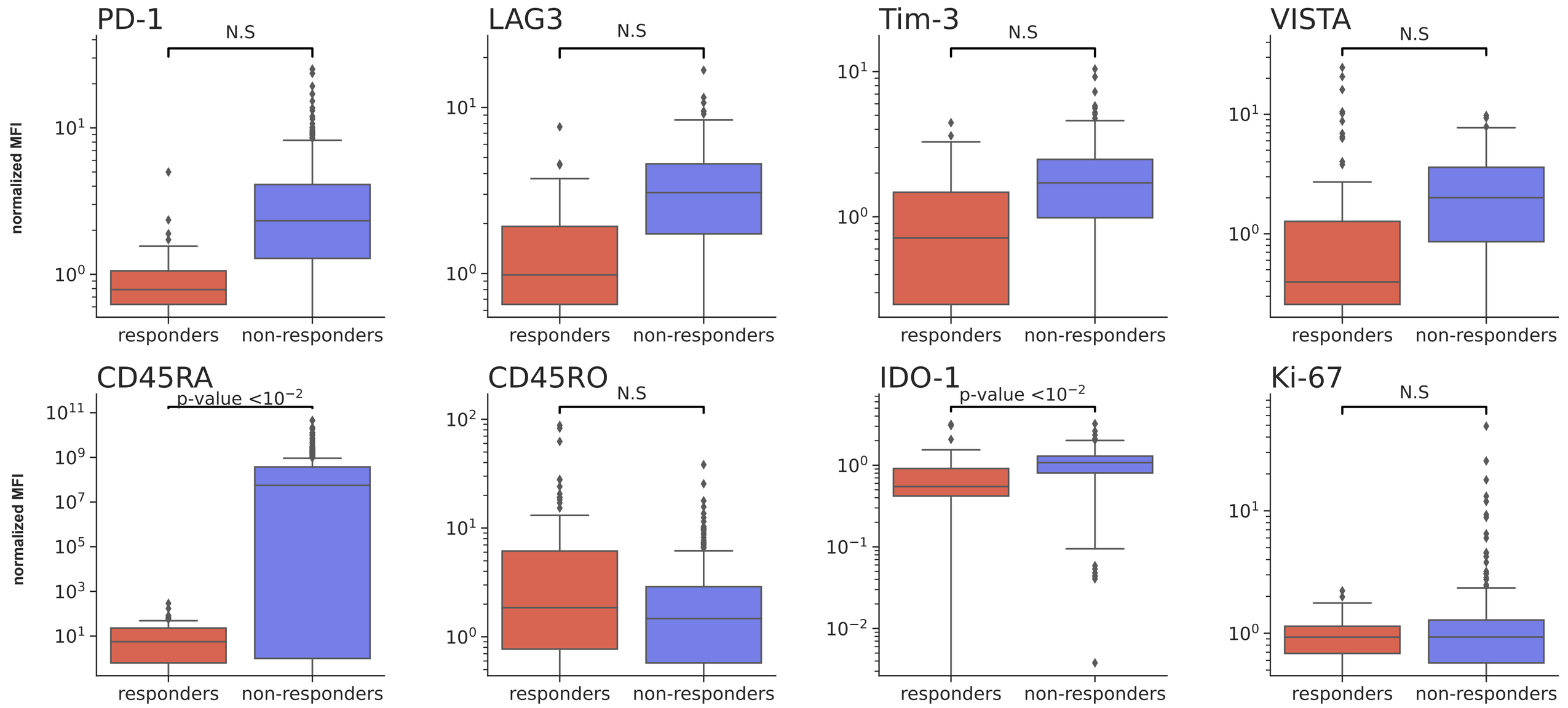
Periphery, CD4 T-cells



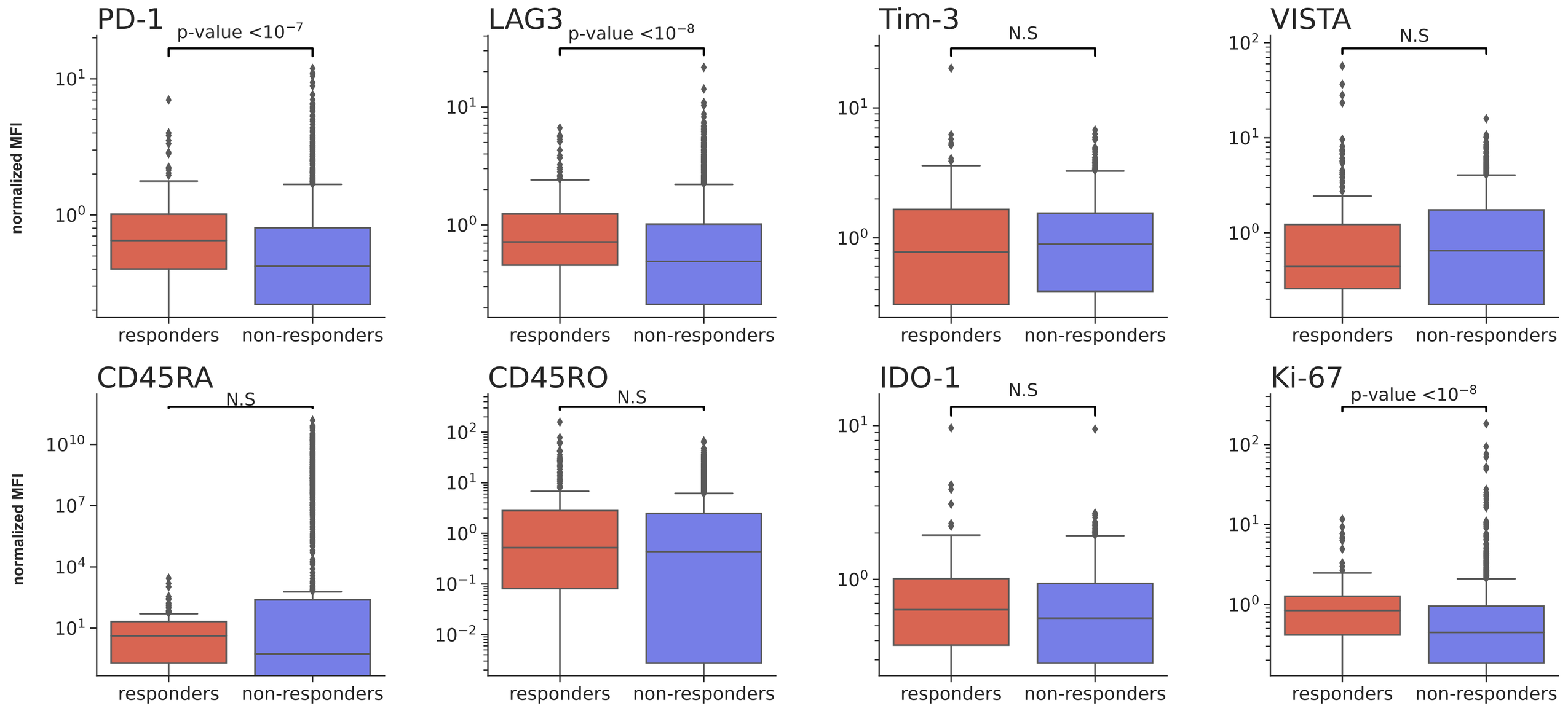
Center, CD8 T-cells



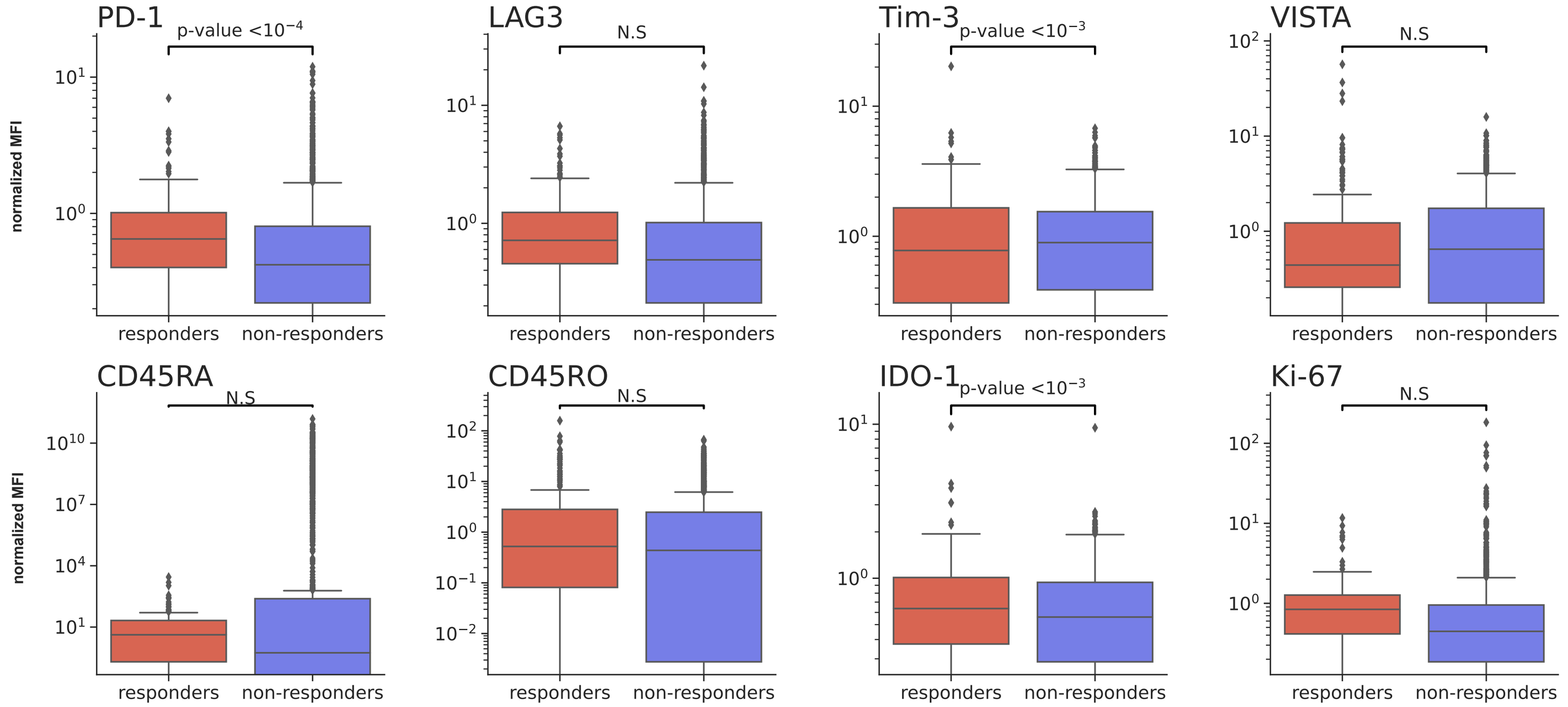
Periphery, CD8 T-cells



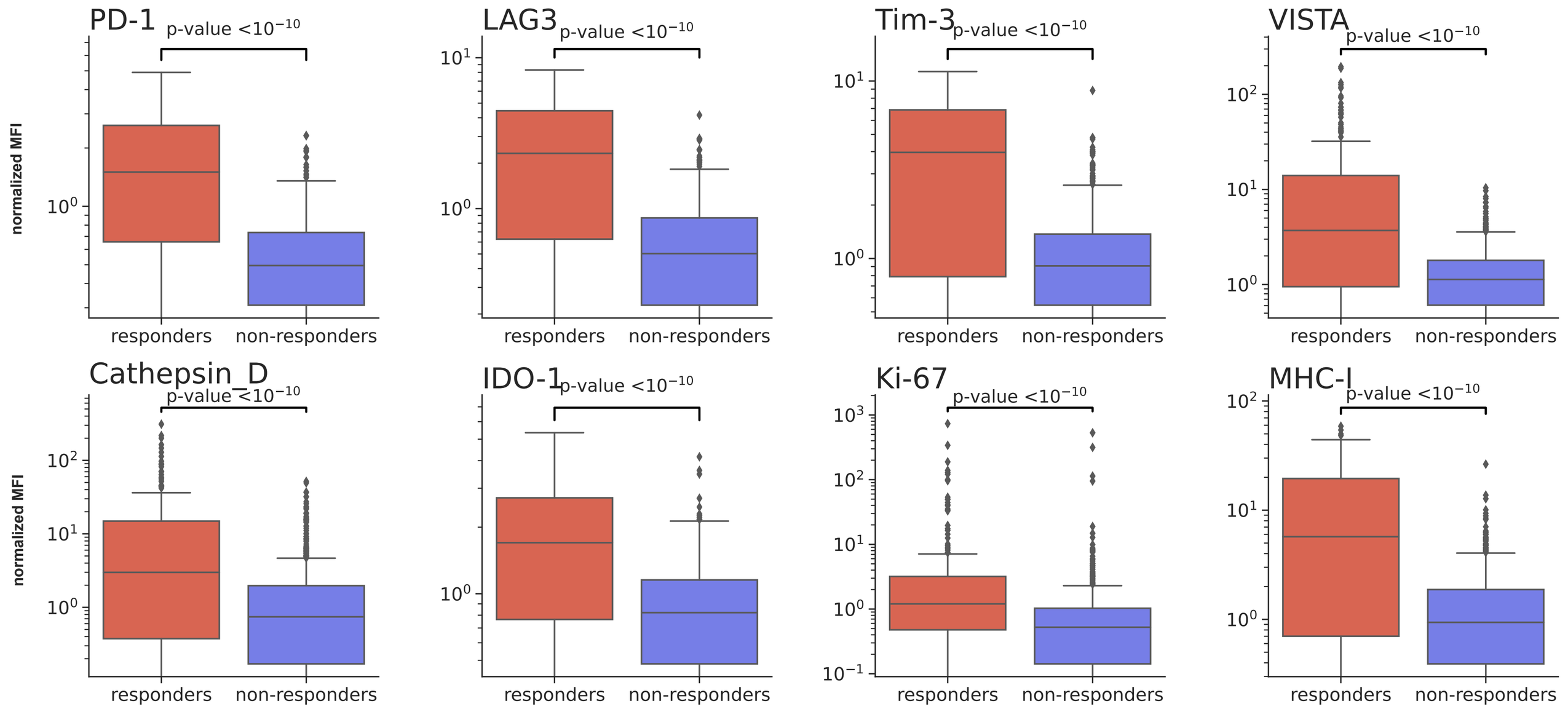
Center, Lymphocytes



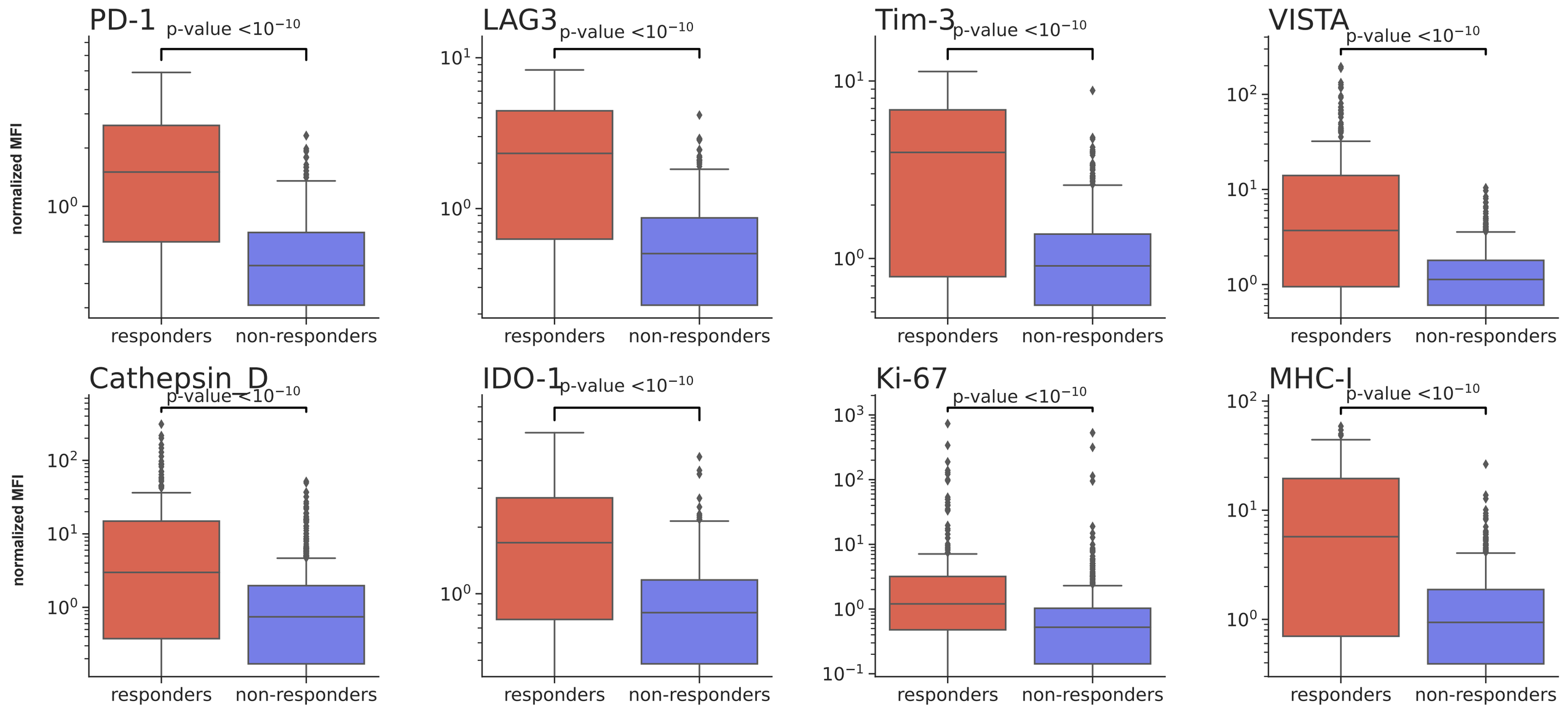
Periphery, Lymphocytes



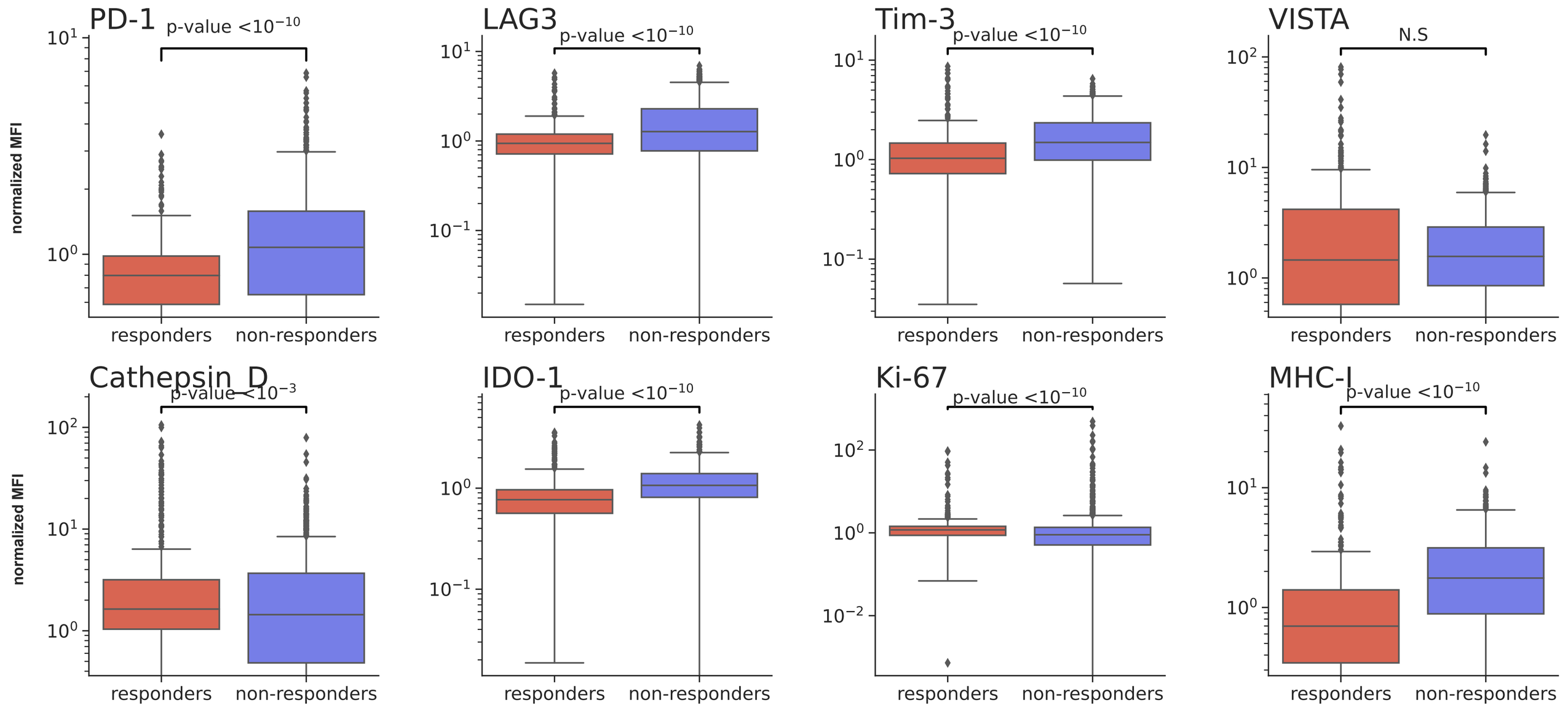
Center, M1 macrophages



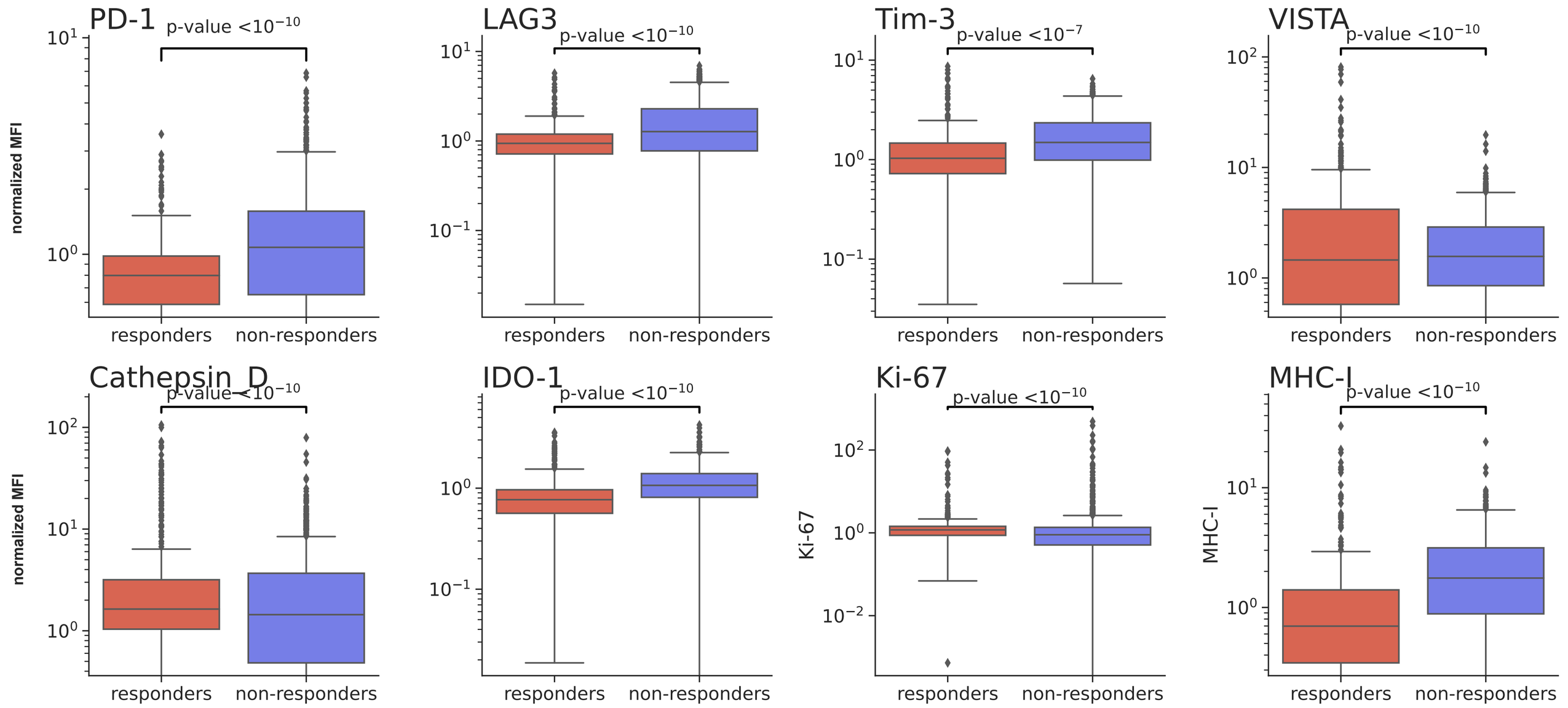
Periphery, M1 macrophages



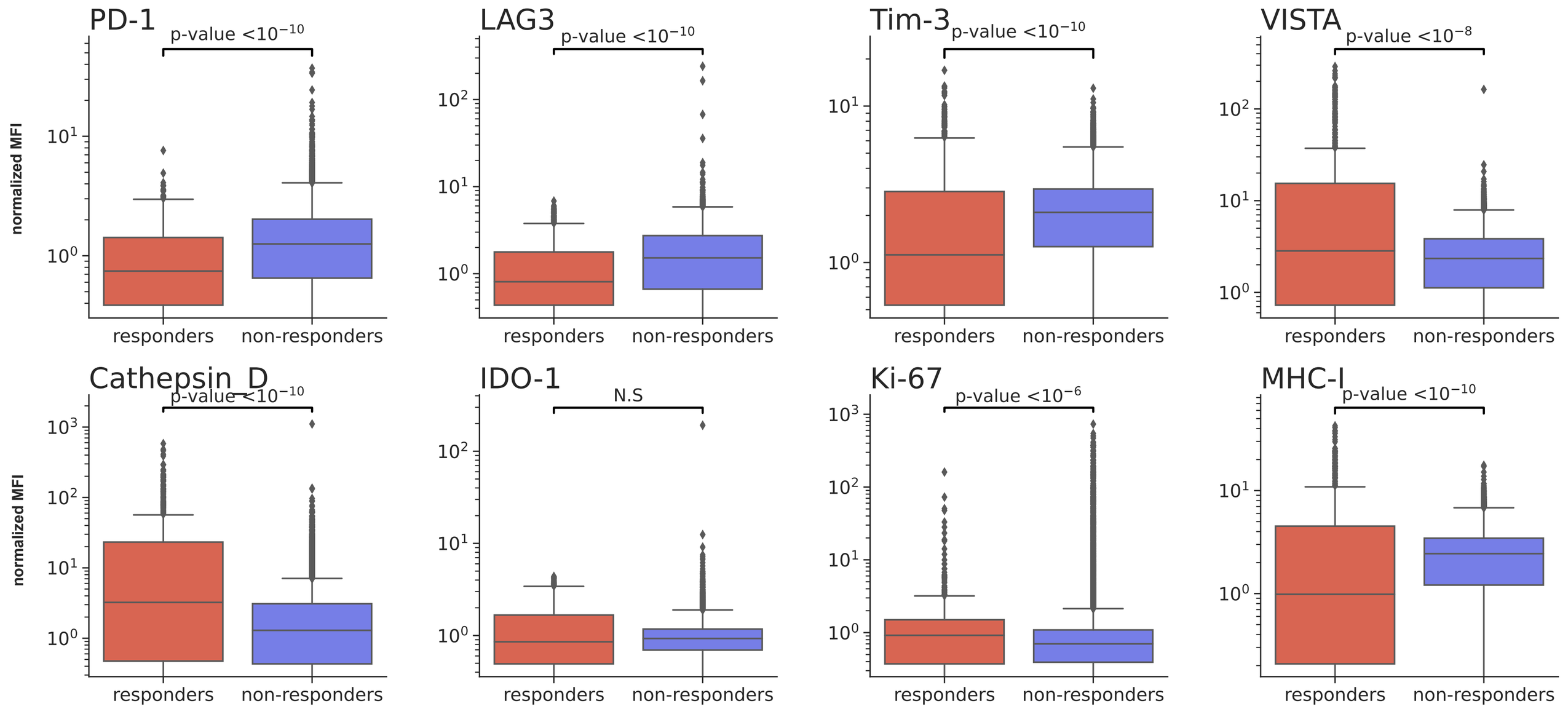
Center, M2-like microglia



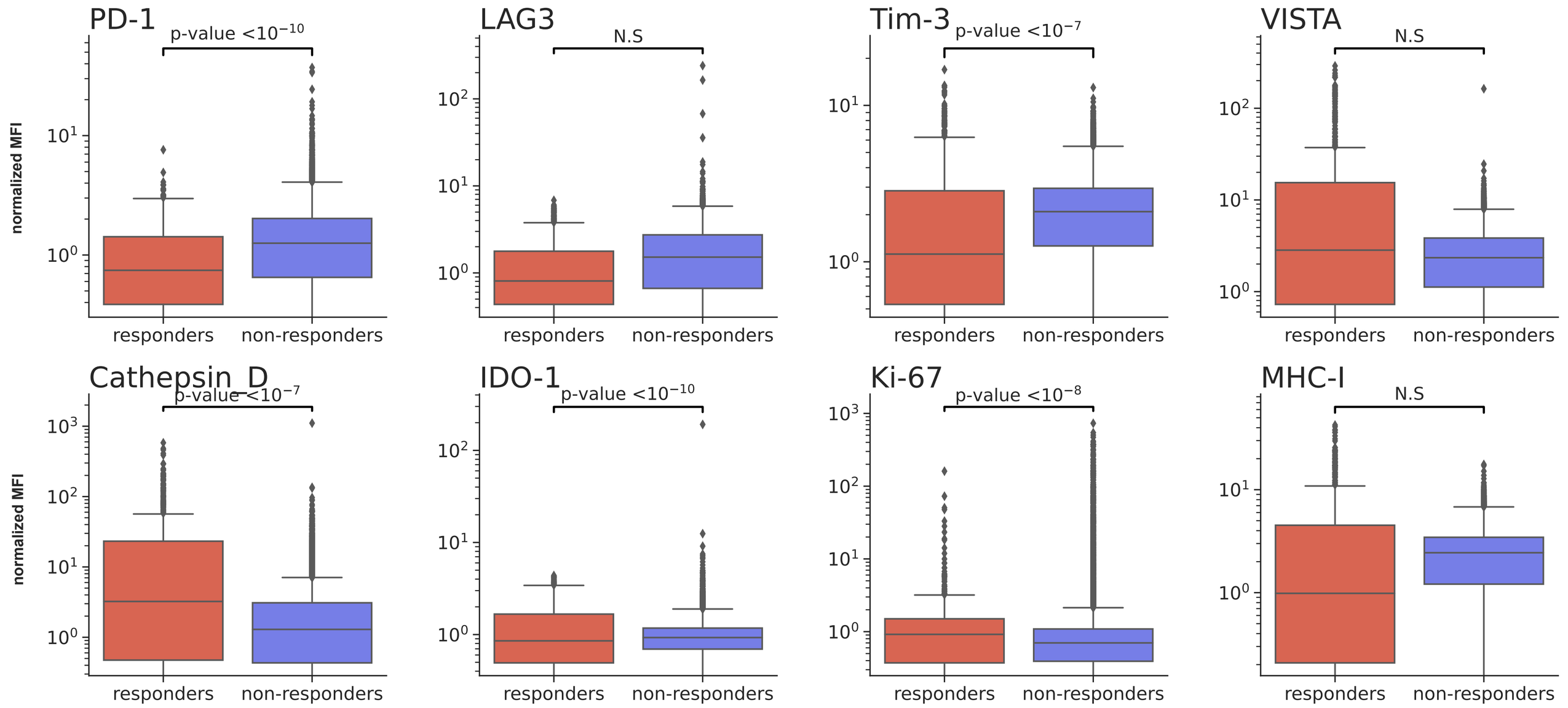
Periphery, M2-like microglia



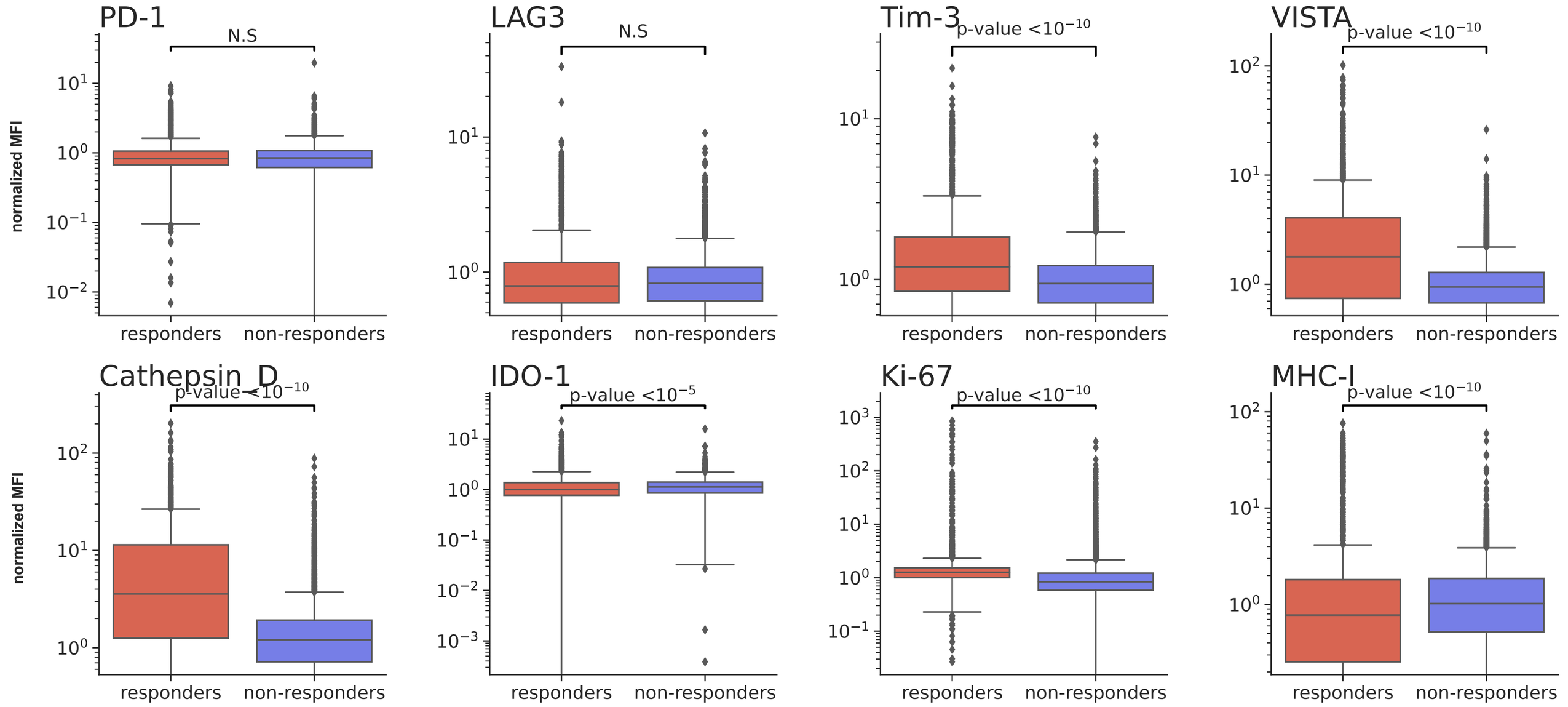
Center, M2 macrophages



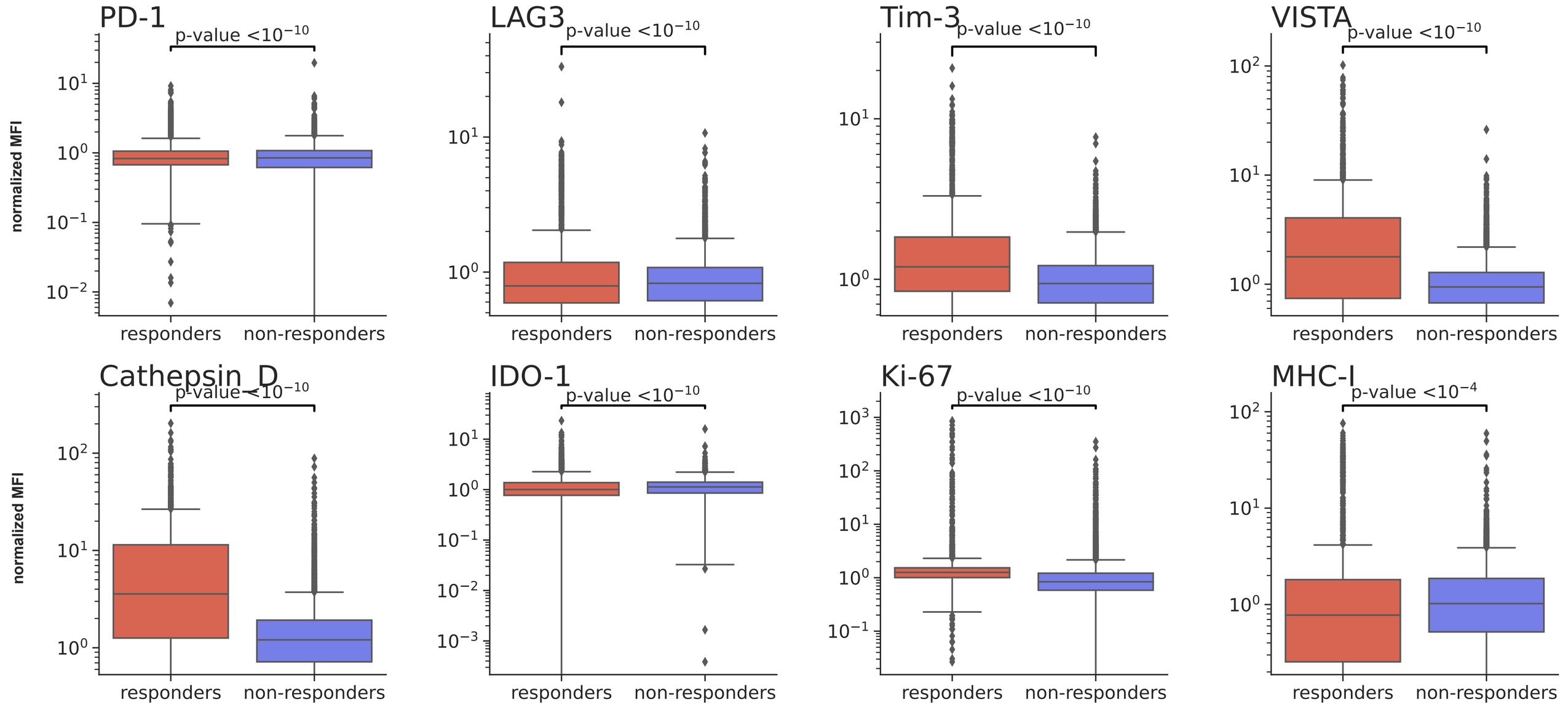
Periphery, M2 macrophages



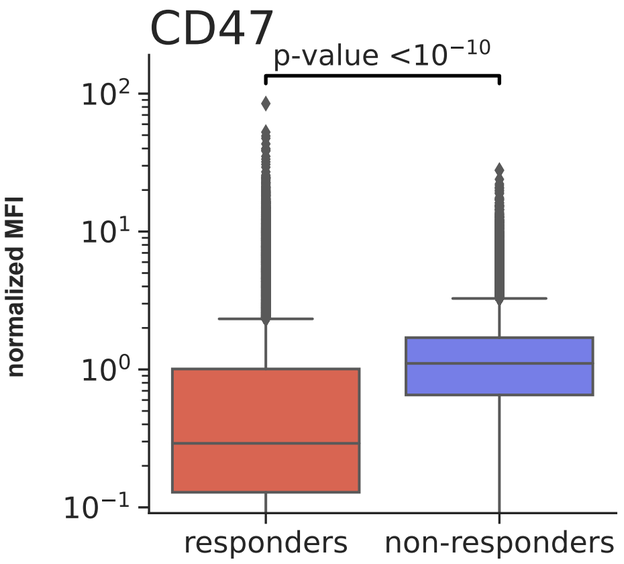
Center, Microglial cells

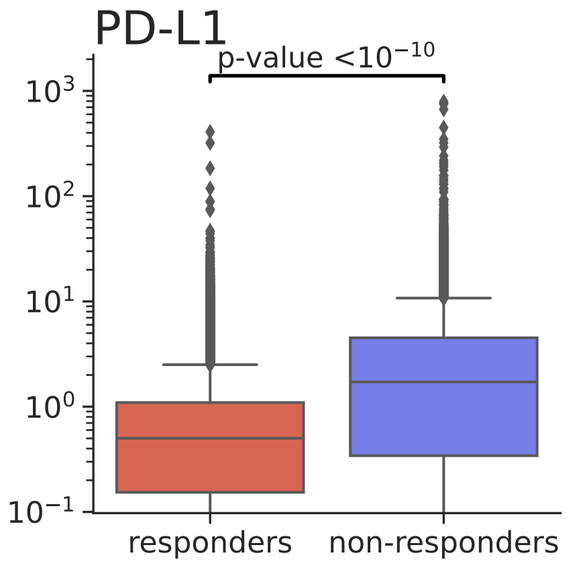


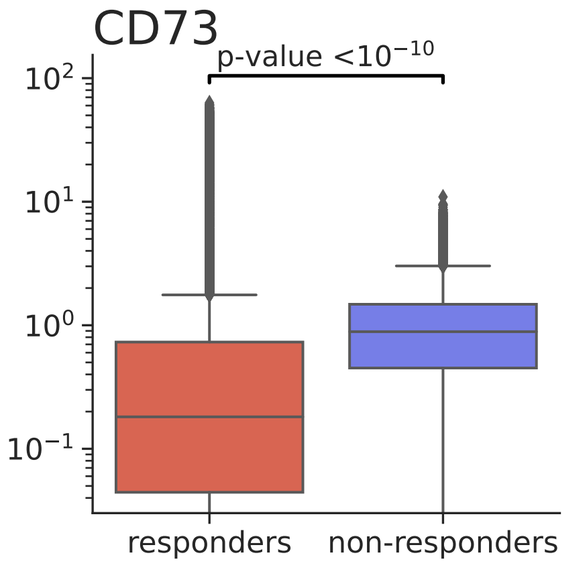
Periphery, Microglial cells

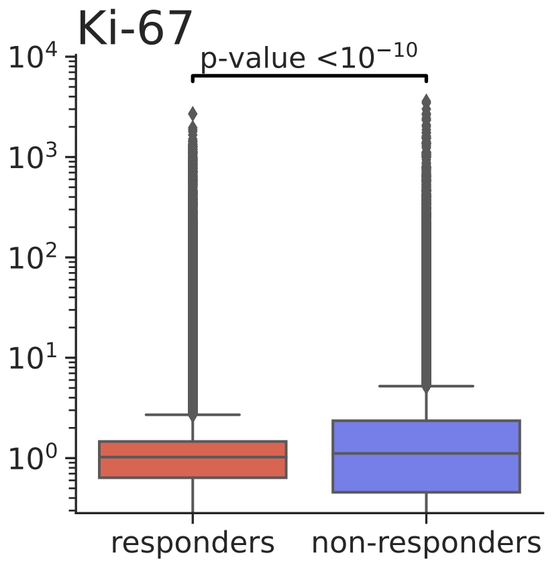


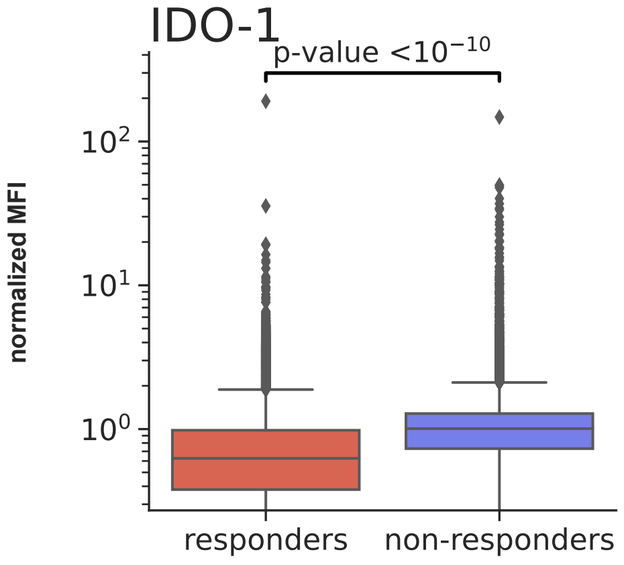
Center, tumor cells/astrocytes

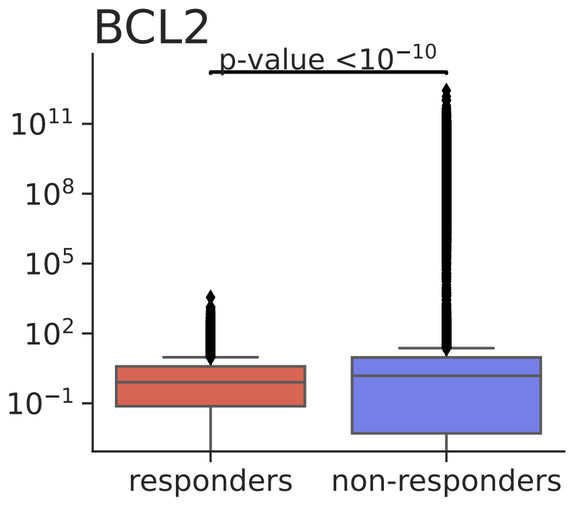




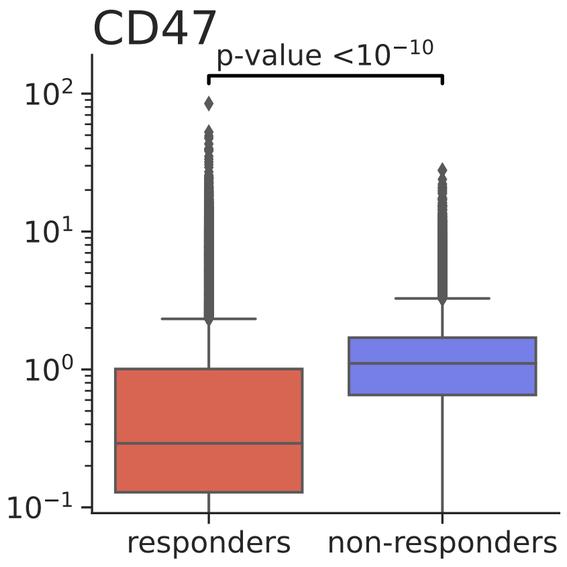


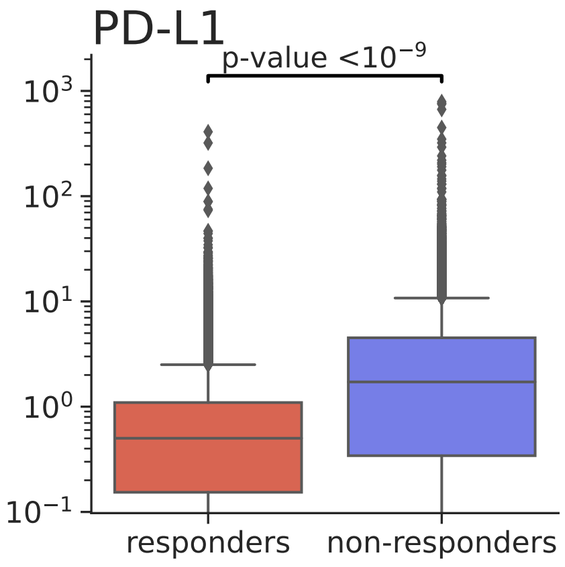


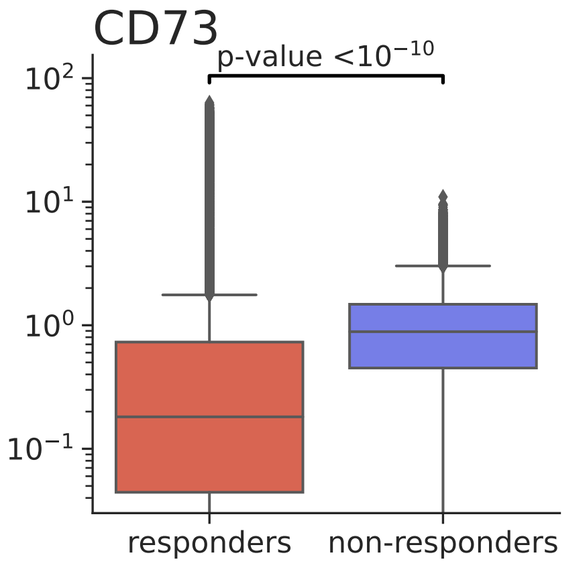


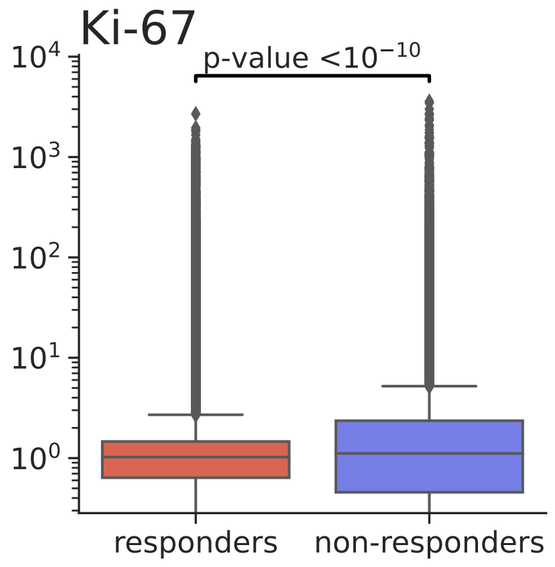


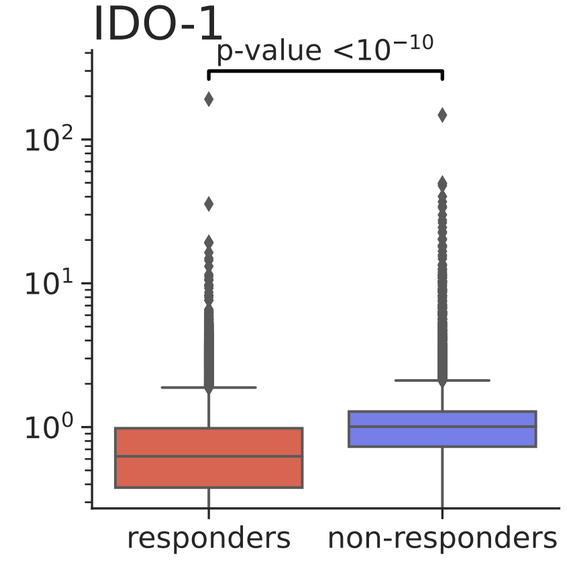
Periphery, tumor cells/astrocytes











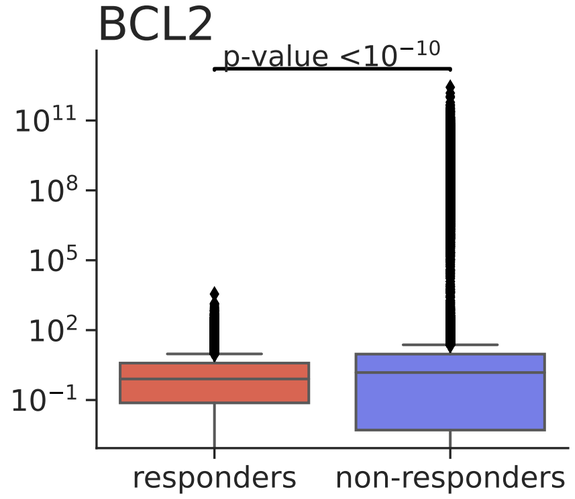


Fig. S12. Assessment of immune checkpoint, proliferation and activation markers on cellular subsets, stratified per IFNγ^{high} and IFNγ^{low} responses, and biopsy location.

Patient samples were stratified according to IFNγ response after immunomodulatory treatment (see Figure 5C and G), and pooled. Log of normalized mean fluorescent intensity (MFI) values of the respective markers in individual cellular phenotypes per region (center vs. periphery) and per IFNγ response (low vs. high) were plotted.

Page 1: center CD4 T cells, page 2: periphery CD4 T cells; page 3: center CD8 T cells, page 4: periphery CD8 T cells; page 5: center lymphocytes; page 6: periphery lymphocytes; page 7: center M1 macrophages, page 8: periphery M1 macrophages; page 9: center M2 like microglia, page 10: periphery M2 like microglia; page 11: center M2 macrophages, page 12: periphery M2 macrophages; page 13: center microglia, page 14: periphery microglia; page 15: center tumor cells/astrocytes; page 16: periphery tumor cells/astrocytes.

Statistics: non-parametric Kruskal-Wallis H-test [21], using Benjamini-Hochberg procedure to control the false discovery rate.

Baseline Parameters					
ID	**Sex**	**Age**	**DG**	**WHO**	**Survival**
577	M	73	GBM	IV	500
579	F	58	GBM	IV	505
580	M	39	GBM	IV	651
581	M	59	GBM	IV	>700*
583	M	71	GBM	IV	227
587	F	69	GBM	IV	470
588	F	78	GBM	IV	204
					* still alive

Genetical characterization					
ID	**Subclass**	***IDH***	**MGMT**	***TERT***	***EGFR***
577	MES	WT	UNM	WT	2n
579	MES	WT	UNM	WT	2n
580	RTK II CL	WT	MET	G228A	2n
581	RTK II CL	WT	MET	G228A	>2n
583	RTK II CL	WT	UNM	G228A	2n
587	MES	WT	UNM	WT	2n
588	MES	WT	MET	G228A	2n

Tissue availability				
	Explant (D7) tissue		***Cytokines***	
ID	Center	Periphery	Center	Periphery
577	y	y	y	y
579	y	y	y	y
580	y	y	y	y
581	y	NA	y	y
583	y	y	y	y
587	y	NA	y	y
588	y	y	y	y

Steroid dosage (Dexamethasone)			
ID			
	Pre-operative	***Intra-operative***	***Post-operative***
577	8mg/day (104mg total)	none	28mg
579	12mg/day (108mg total)	8mg	na
580	12mg/day (60mg total)	12mg	12mg/d
581	12mg/day (84mg total)	none	12mg
583	different doses/day (80mg total)	none	na
587	different doses/day (32mg total)	12mg	na
588	24mg/day (24mg total)	4mg	20mg

Table S1. Clinical data and pathological-molecular tumor characteristics.

Abbreviations: MES mesenchymal; RTK II CL classical;WT wildtype; UNM unmethylated; MET methylated; y yes; NA not available

Data S1. (separate .xlsx file)

List of purified antibodies (sheet 1), CODEX multicycle panel (sheet 2) and CODEX oligonucleotides (sheet 3) used in this study.