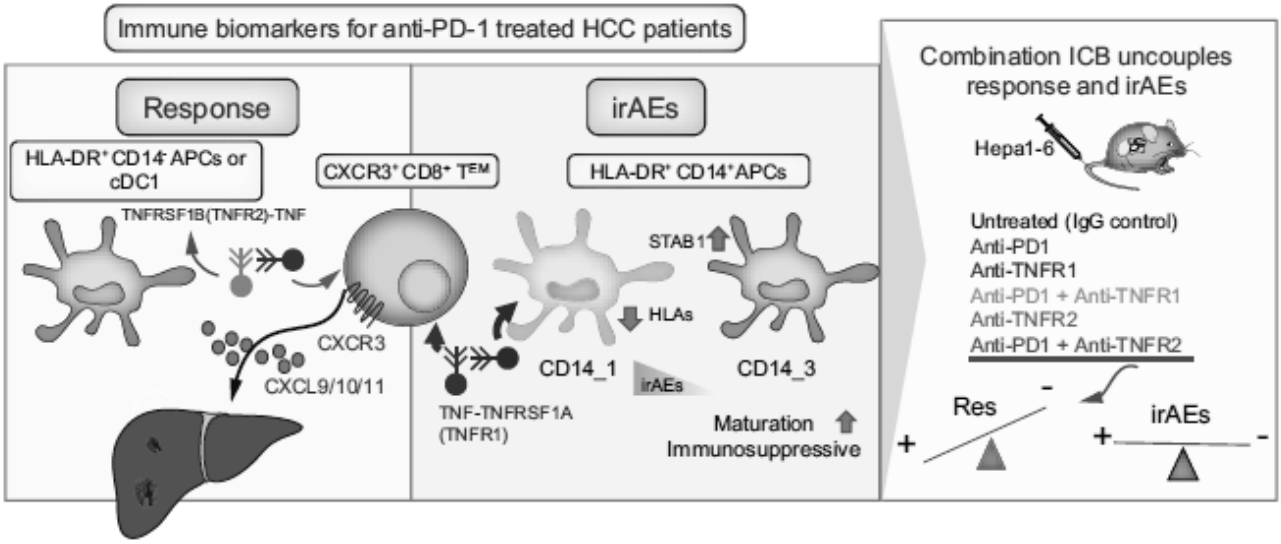

Immune biomarkers for anti-PD-1 treated HCC patients
Response
HLA-DR+ CD14- APCs or cDC1
TNFRSF1B(TNFR2)-TNF
CXCR3
CXCL9/10/11
CXCR3+ CD8+ TEM
irAEs
HLA-DR+ CD14+APCs
STAB1
HLAs
CD14_1
CD14_3
irAEs
TNF-TNFRSF1A (TNFR1)
Maturation
Immunosuppressive
Combination ICB uncouples response and irAEs
Hepa1-6
Untreated (IgG control)
Anti-PD1
Anti-TNFR1
Anti-PD1 + Anti-TNFR1
Anti-TNFR2
Anti-PD1 + Anti-TNFR2
Res
irAEs

**Figure 1**

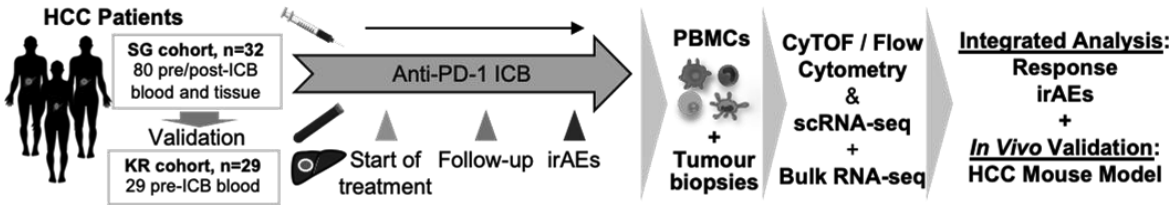



**Figure 2A**

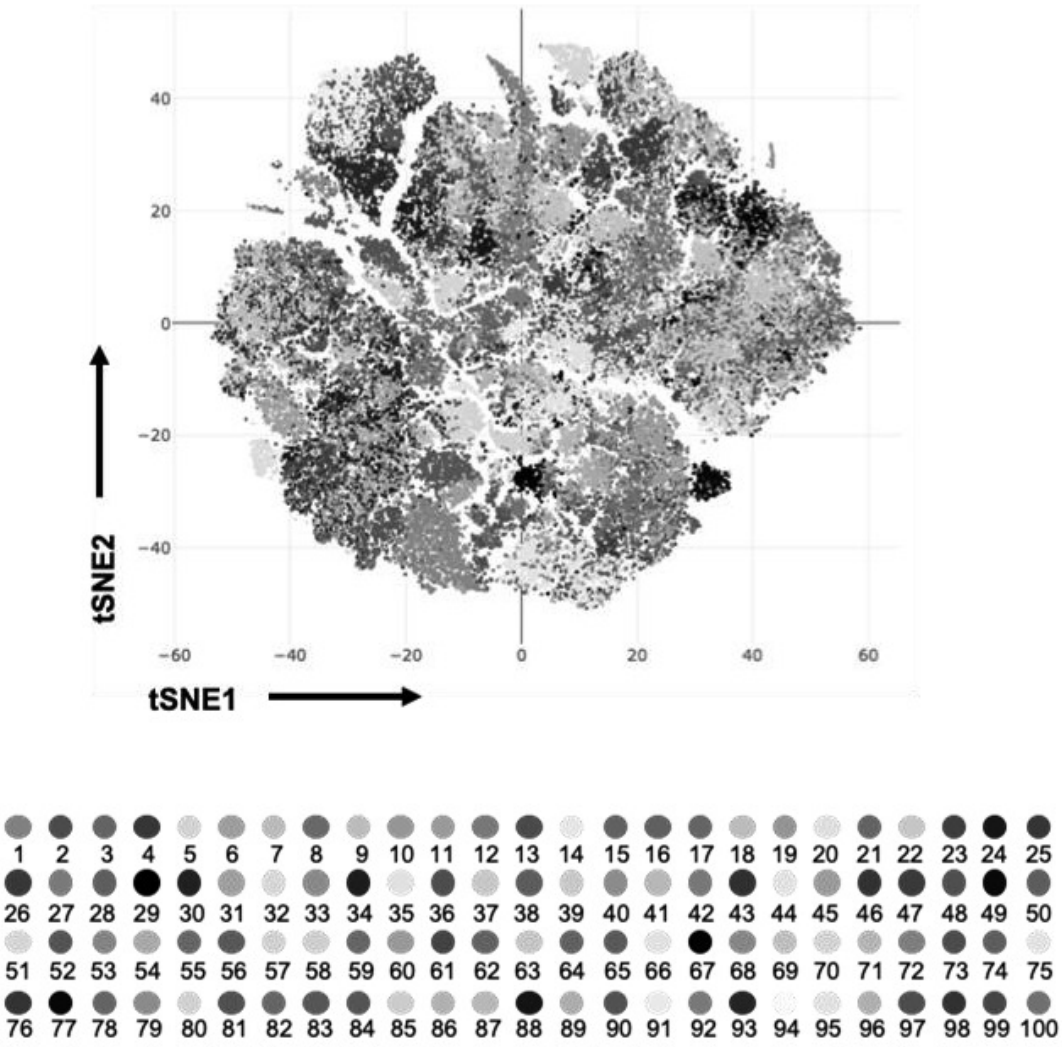



**Figure 2B**

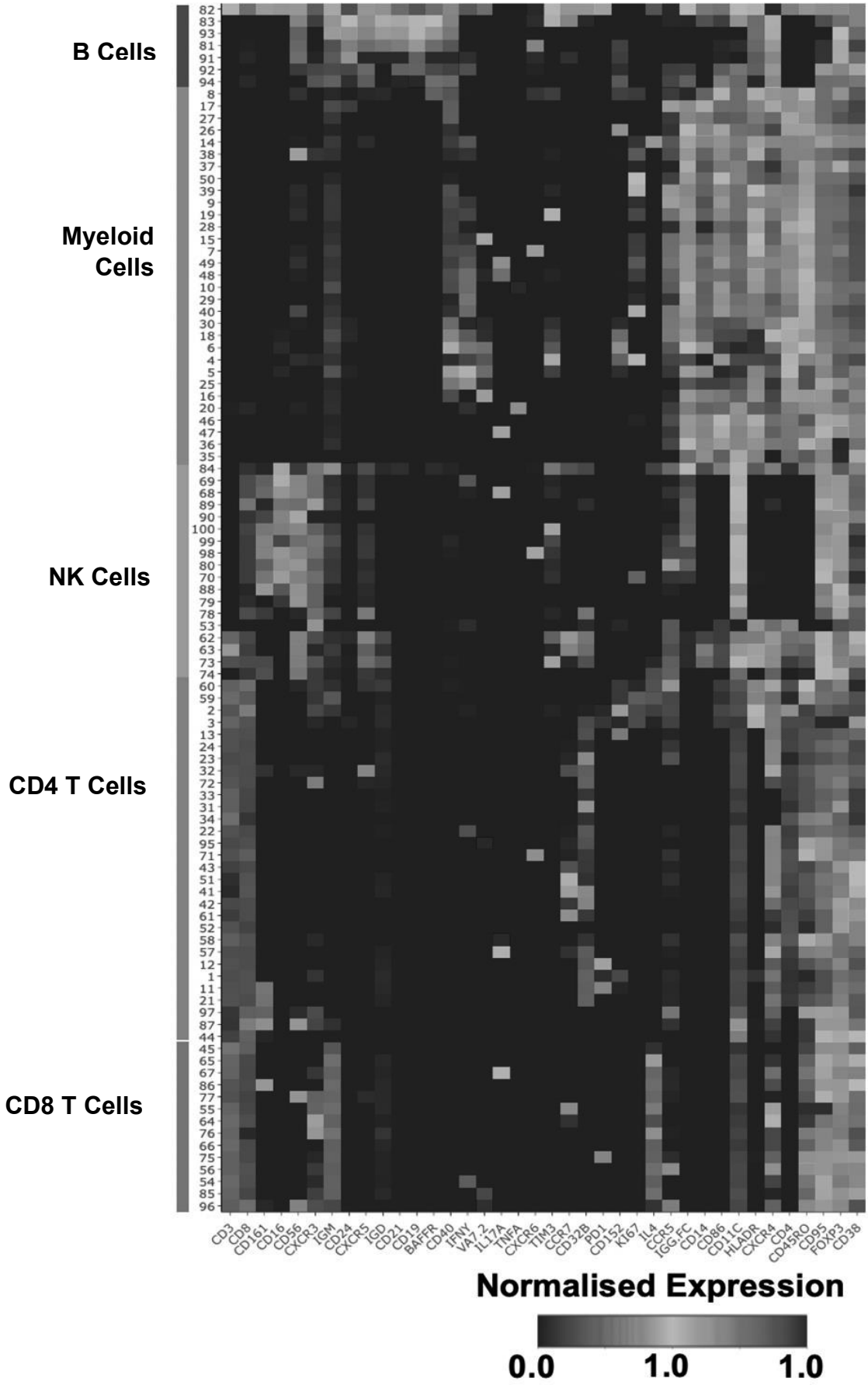

B Cells
Myeloid Cells
NK Cells
CD4 T Cells
CD8 T Cells
82 83 93 81 91 92 94 8 17 27 26 14 38 37 50 39 9 19 28 15 7 49 48 10 29 40 30 18 6 4 5 25 16 20 46 47 36 35 84 69 68 89 90 100 99 98 80 70 88 79 78 53 62 63 73 74 60 59 2 3 13 24 23 32 72 33 31 34 22 95 71 43 51 41 42 61 52 58 57 12 1 11 21 97 87 44 45 65 67 86 77 55 64 76 66 75 56 54 85 96
CD3 CD8 CD161 CD16 CD56 CXCR3 IGM CD24 CXCR5 IGD CD21 CD19 BAFFR CD40 IFNY VA7.2 IL17A TNFA CXCR6 TIM3 CCR7 CD32B PD1 CD152 KI67 IL4 CCR5 IGG.FC CD14 CD86 CD11C HLADR CXCR4 CD4 CD45RO CD95 FOXP3 CD38
Normalised Expression
0.0
1.0
1.0

**Figure 2C**

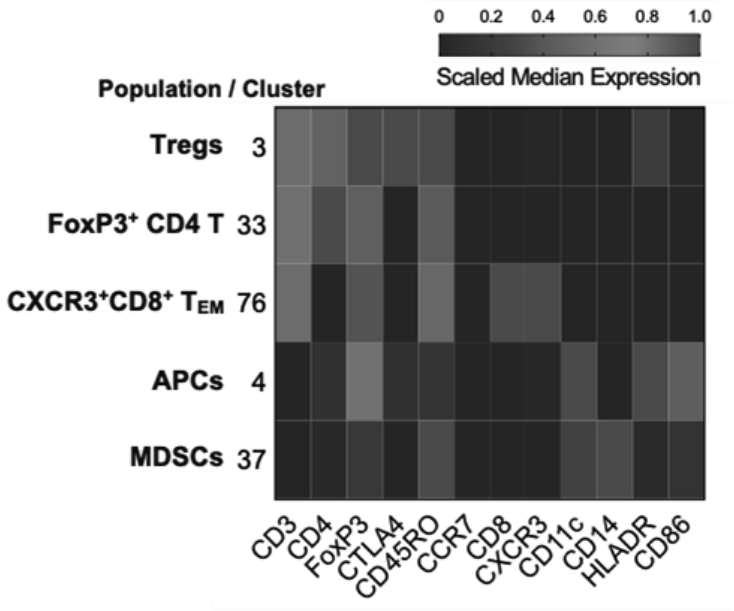



**Figure 2D**

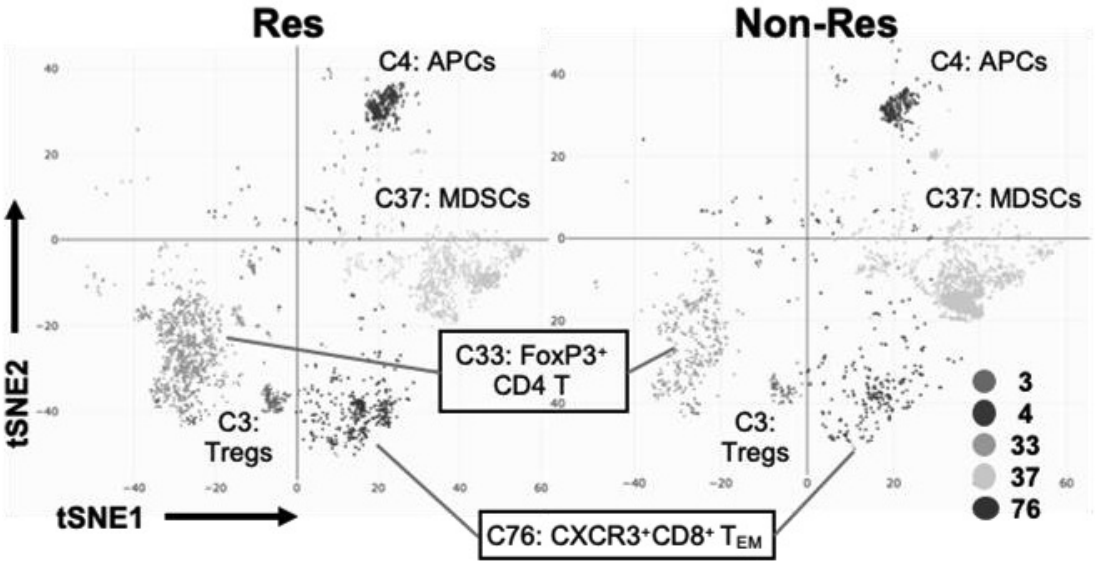



**Figure 2E**

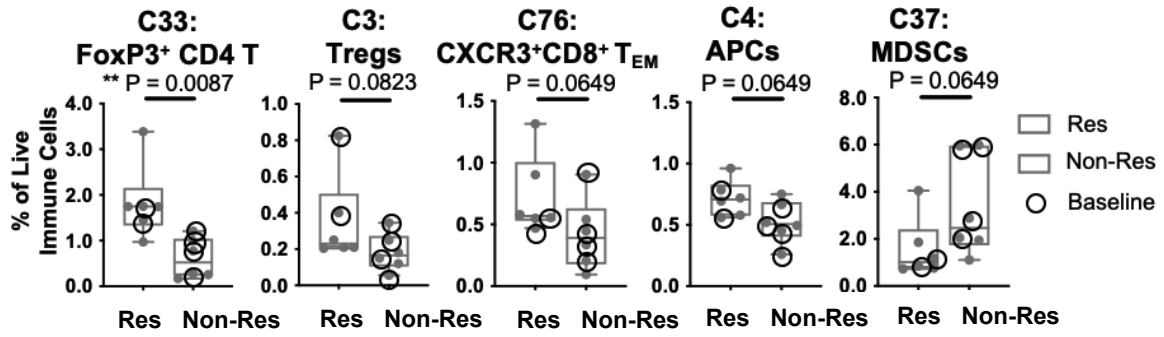



**Figure 2F**

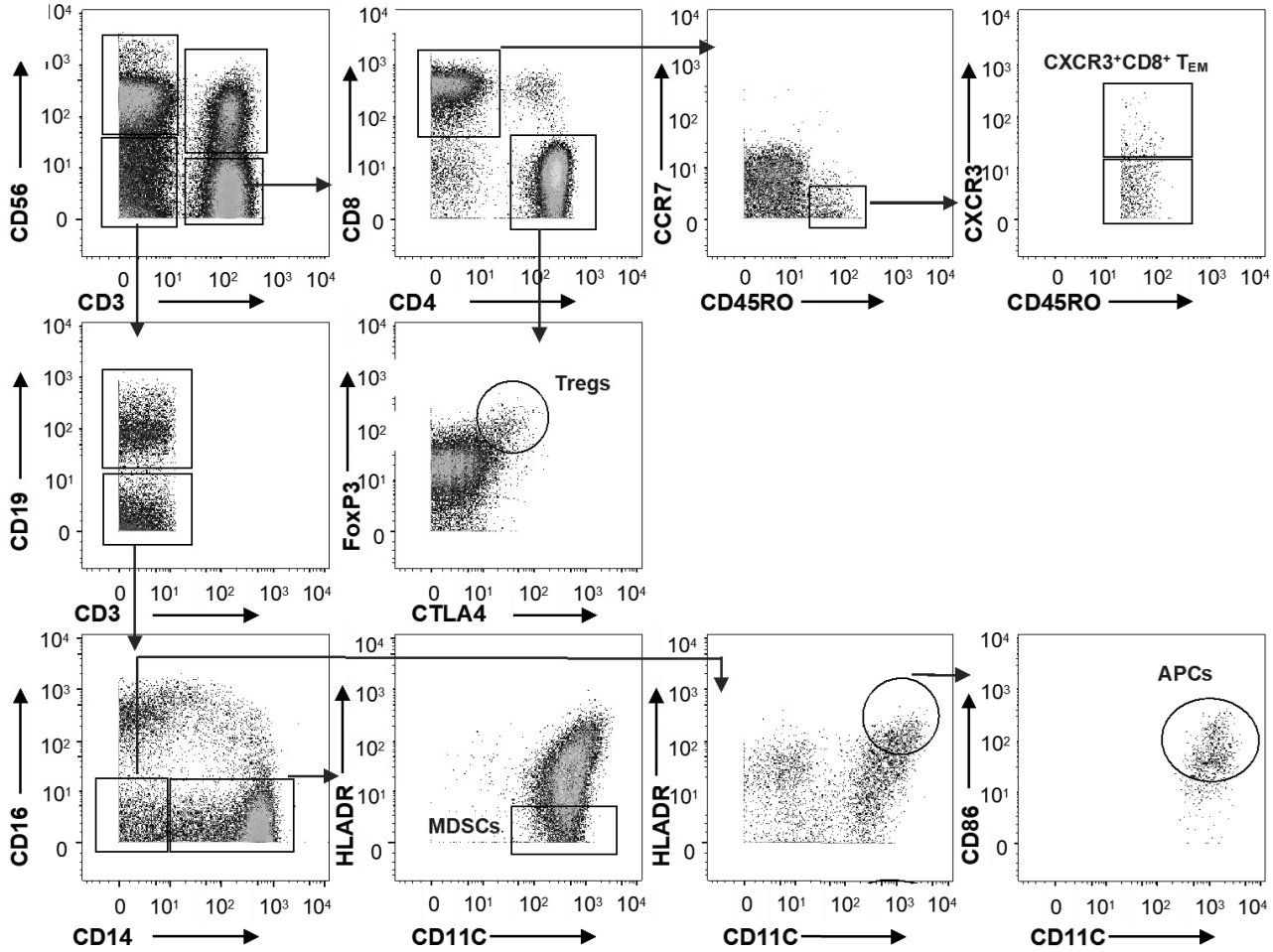



**Figure 2G**

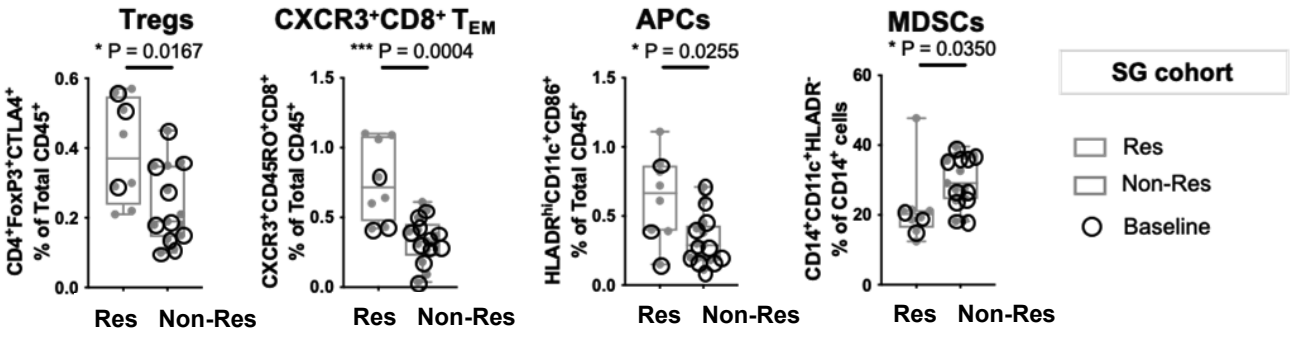



**Figure 2H**

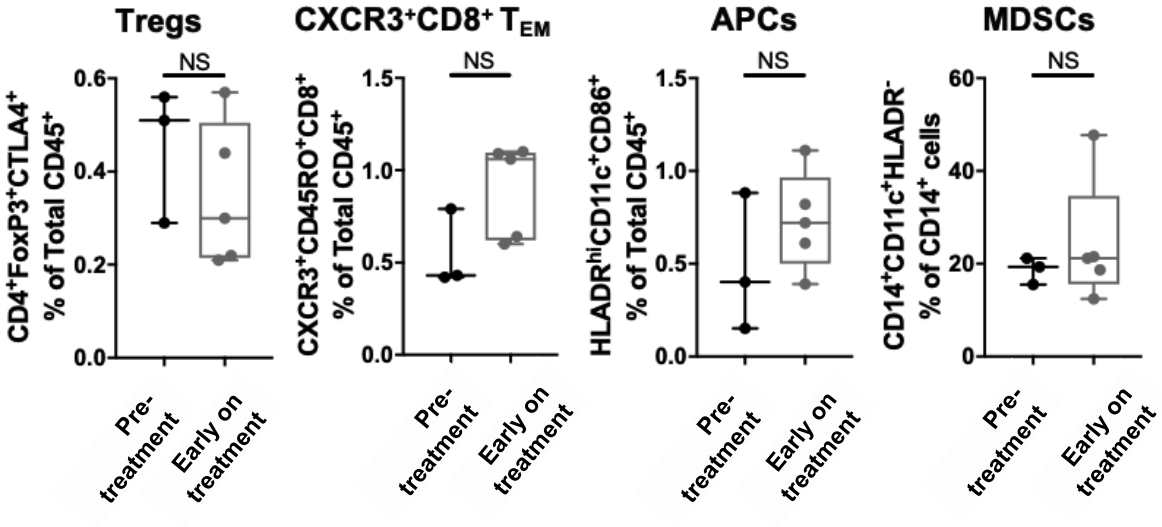



**Figure 2I**

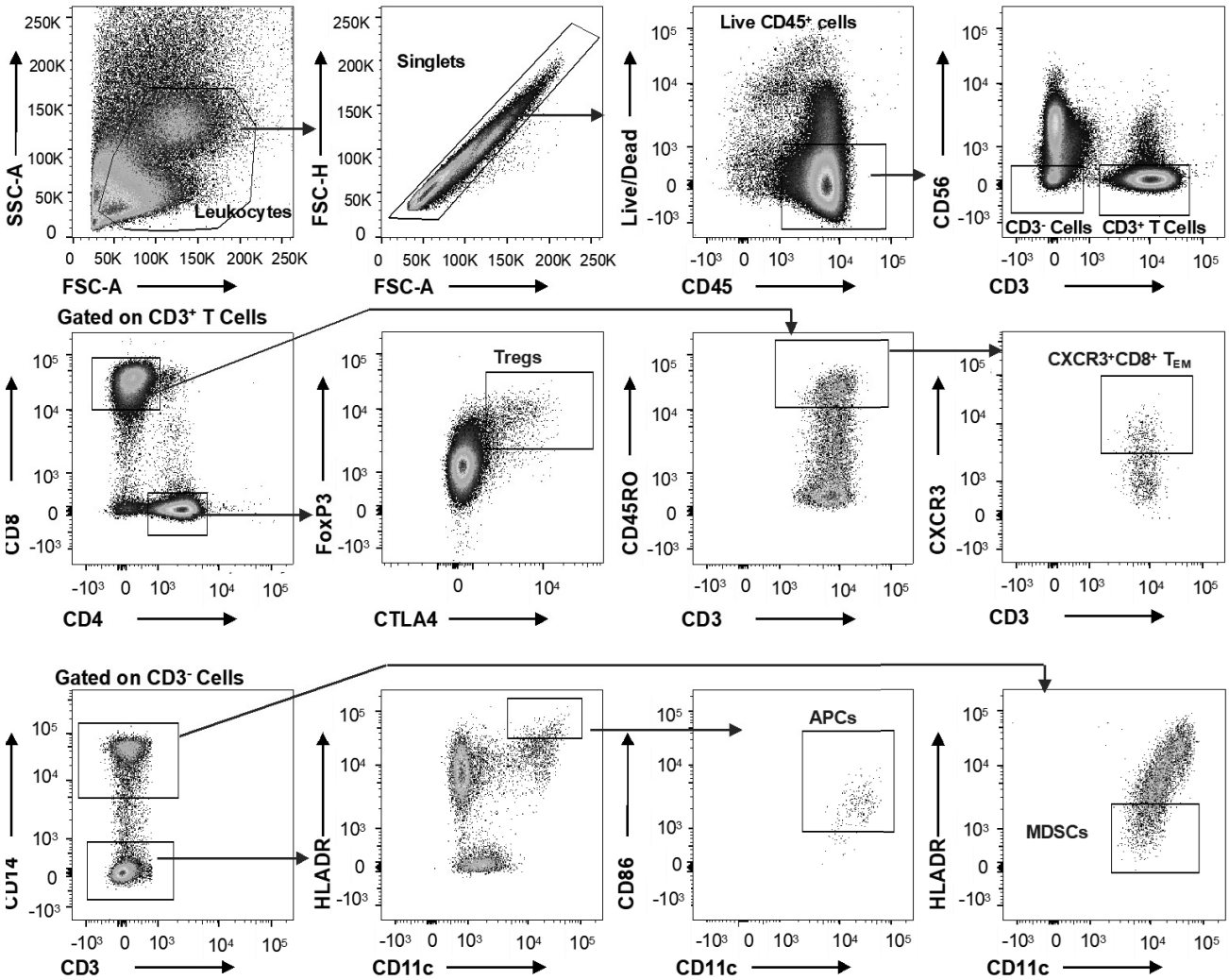



**Figure 2J**

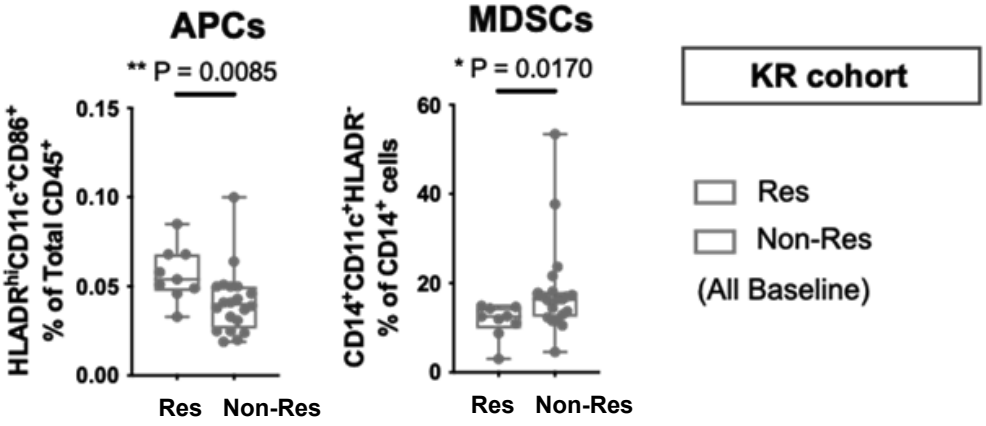



**Figure 2K**

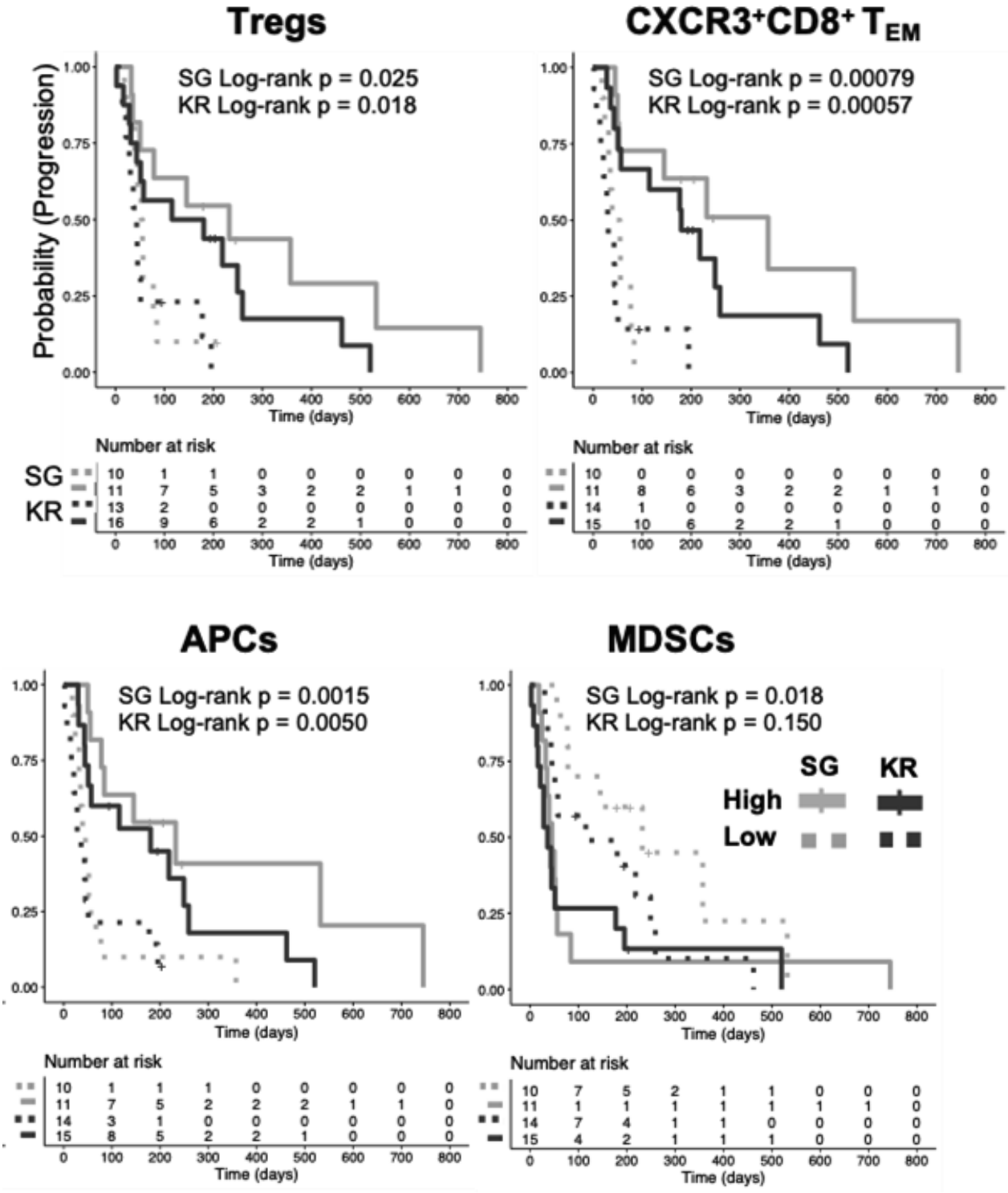

Tregs
SG Log-rank p = 0.025
KR Log-rank p = 0.018
Probability (Progression)
Time (days)
Number at risk
SG
KR
CXCR3+CD8+ TEM
SG Log-rank p = 0.00079
KR Log-rank p = 0.00057
APCs
SG Log-rank p = 0.0015
KR Log-rank p = 0.0050
MDSCs
SG Log-rank p = 0.018
KR Log-rank p = 0.150
SG
KR
High
Low

**Figure 2L**

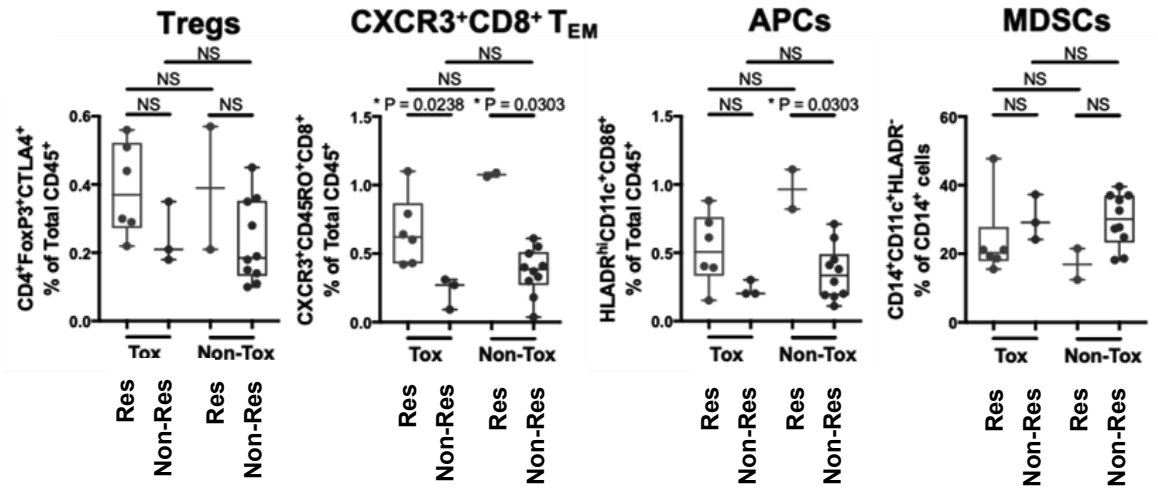



**Figure 2M**

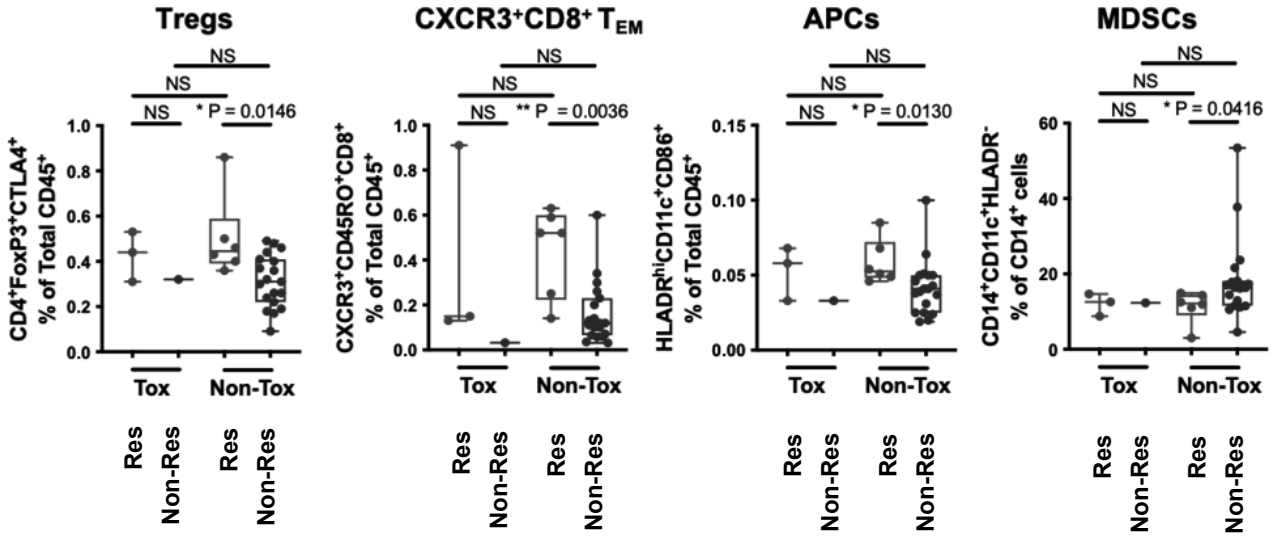



**Figure 2N**

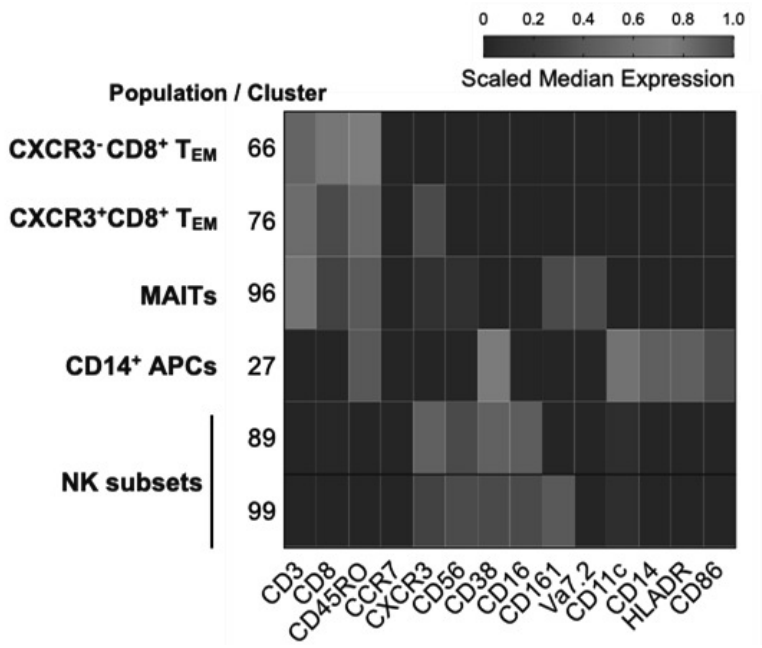



**Figure 3A**

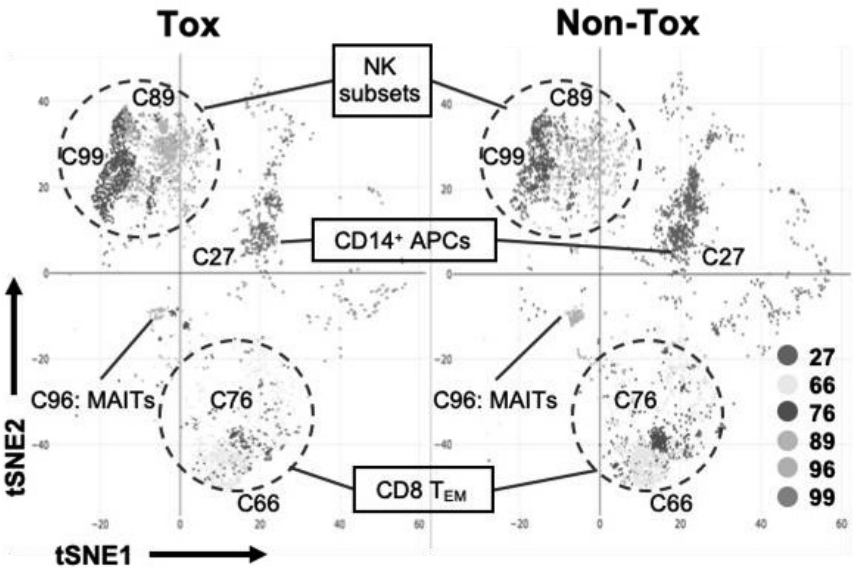



**Figure 3B**

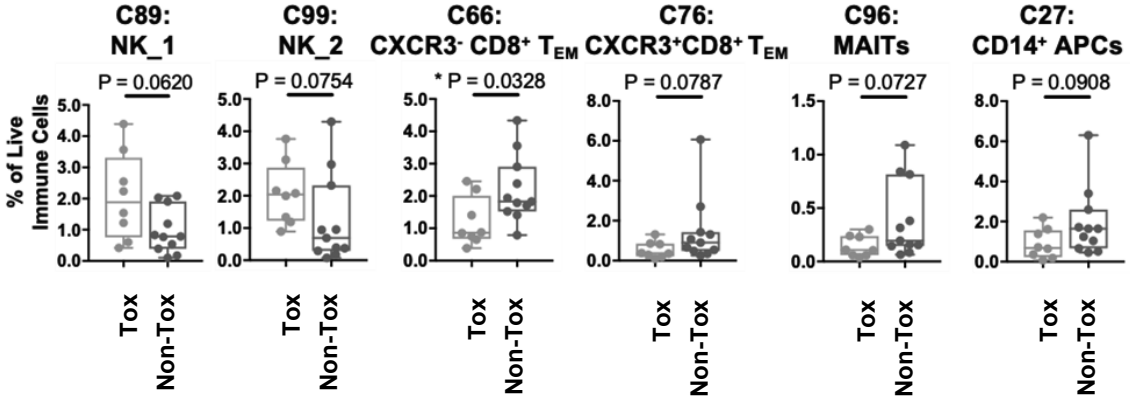



**Figure 3C**

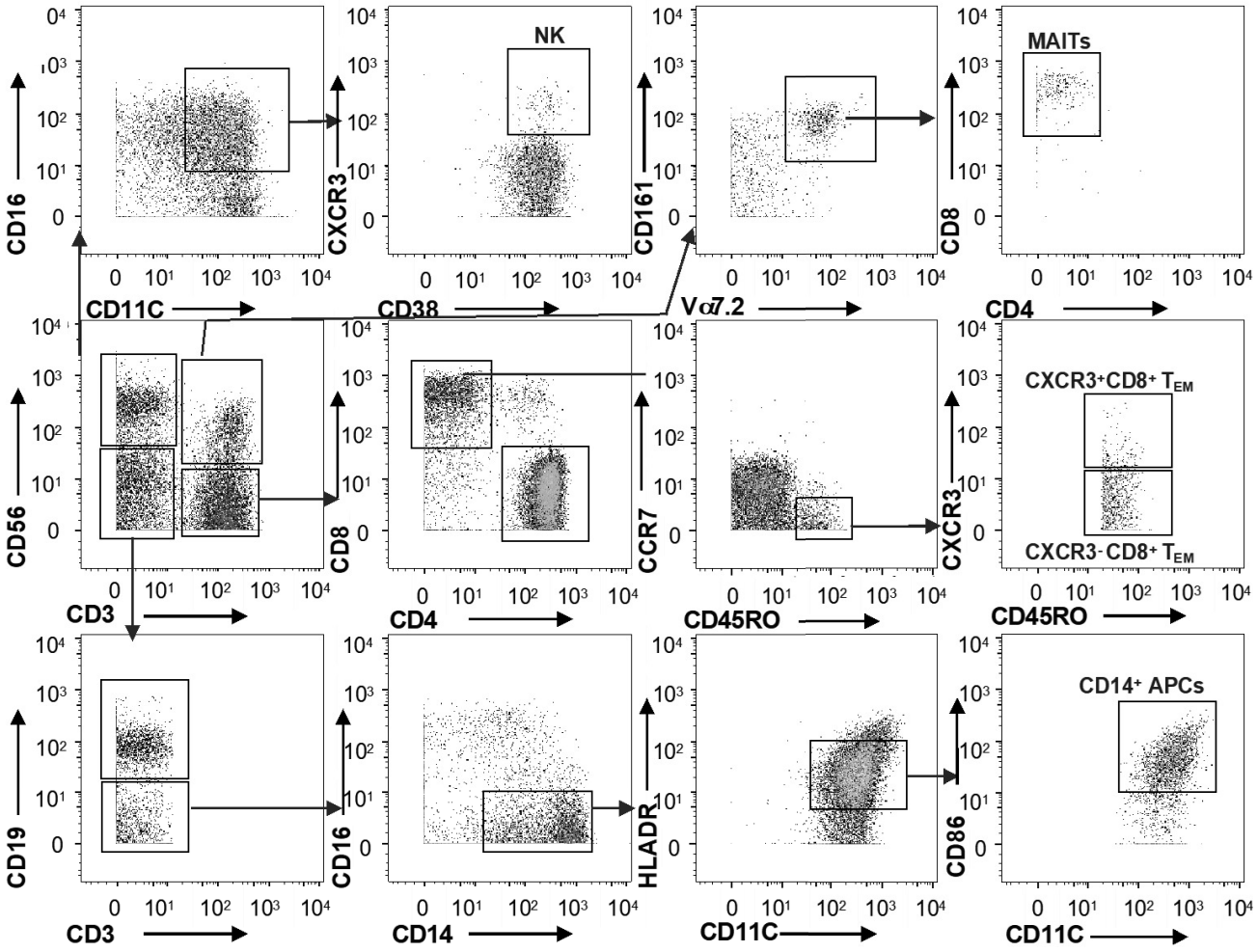



**Figure 3D**

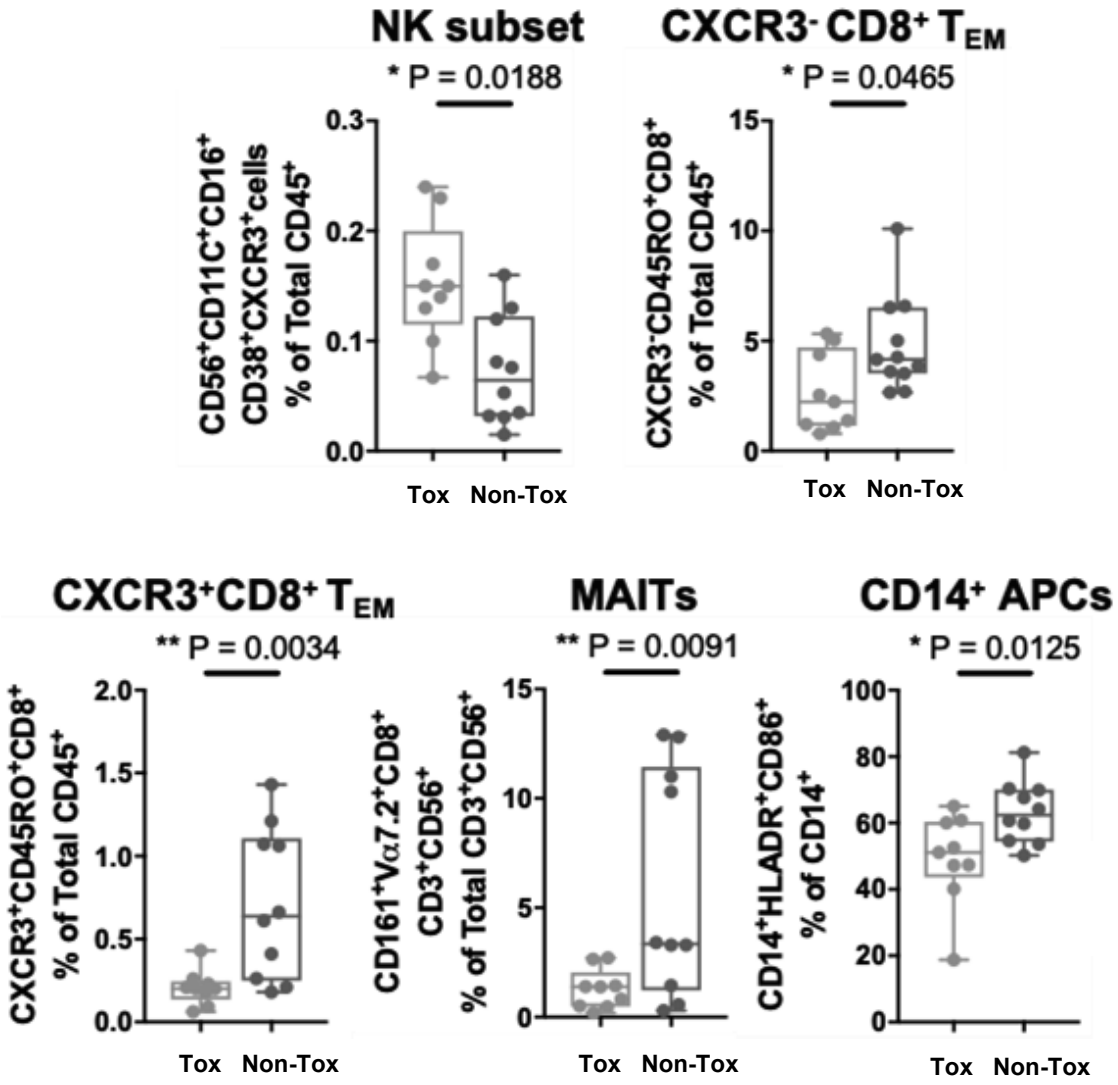

NK subset
* P = 0.0188
CD56+CD11C+CD16+ CD38+CXCR3+cells % of Total CD45+
0.3
0.2
0.1
0.0
Tox
Non-Tox
CXCR3- CD8+ TEM
* P = 0.0465
CXCR3-CD45RO+CD8+ % of Total CD45+
15
10
5
0
Tox
Non-Tox
CXCR3+CD8+ TEM
** P = 0.0034
CXCR3+CD45RO+CD8+ % of Total CD45+
2.0
1.5
1.0
0.5
0.0
Tox
Non-Tox
MAITs
** P = 0.0091
CD161+Vα7.2+CD8+ CD3+CD56+ % of Total CD3+CD56+
15
10
5
0
Tox
Non-Tox
CD14+ APCs
* P = 0.0125
CD14+HLADR+CD86+ % of CD14+
100
80
60
40
20
0
Tox
Non-Tox

**Figure 3E**

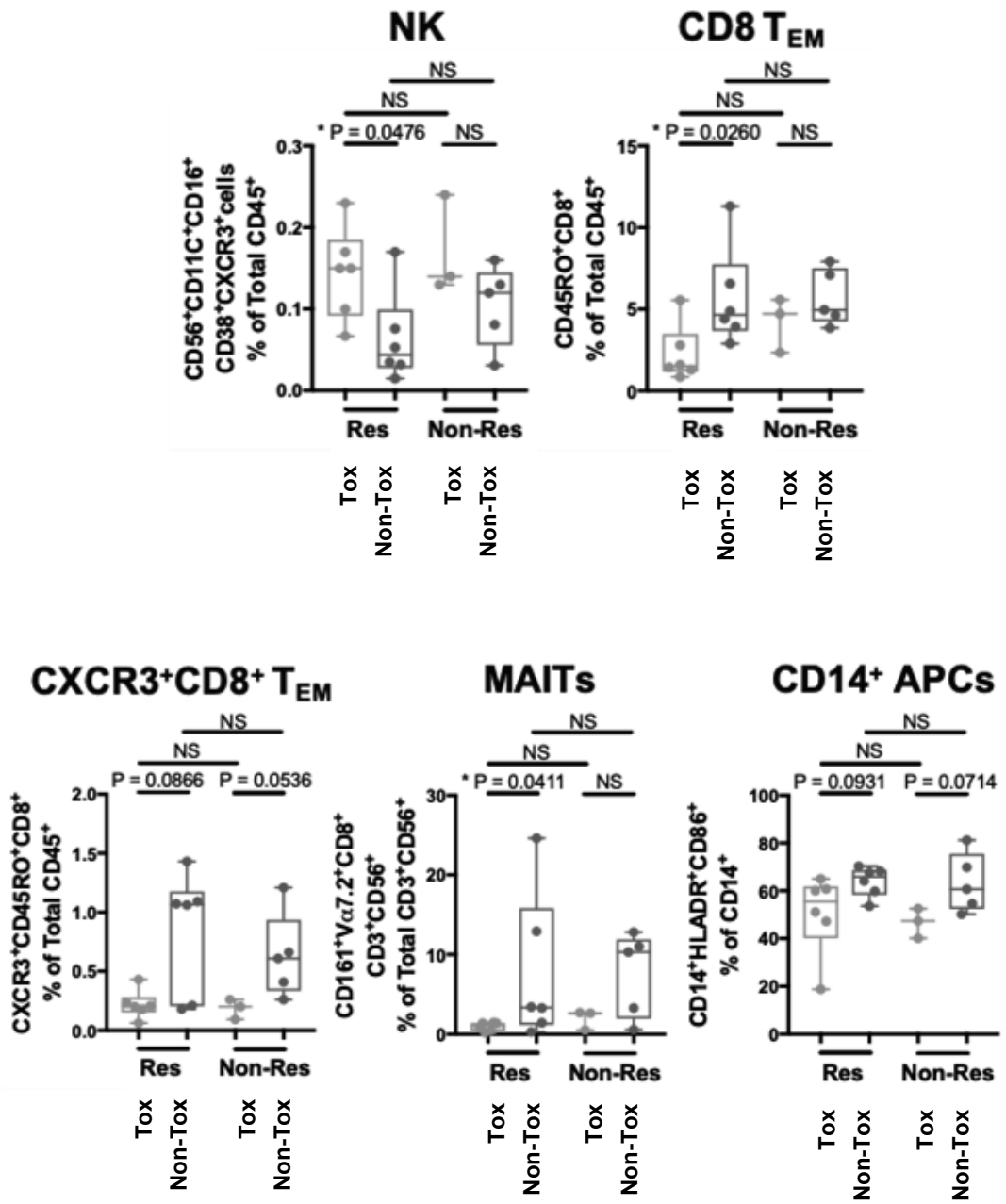

NK
CD8 T$_{EM}$
CXCR3$^+$CD8$^+$ T$_{EM}$
MAITs
CD14$^+$ APCs
CD56$^+$CD11C$^+$CD16$^+$ CD38$^+$CXCR3$^+$cells % of Total CD45$^+$
CD45RO$^+$CD8$^+$ % of Total CD45$^+$
CXCR3$^+$CD45RO$^+$CD8$^+$ % of Total CD45$^+$
CD161$^+$Vα7.2$^+$CD8$^+$ CD3$^+$CD56$^+$ % of Total CD3$^+$CD56$^+$
CD14$^+$HLADR$^+$CD86$^+$ % of CD14$^+$
NS
* P = 0.0476
* P = 0.0260
P = 0.0866
P = 0.0536
* P = 0.0411
P = 0.0931
P = 0.0714
Res
Non-Res
Tox
Non-Tox

**Figure 3F**

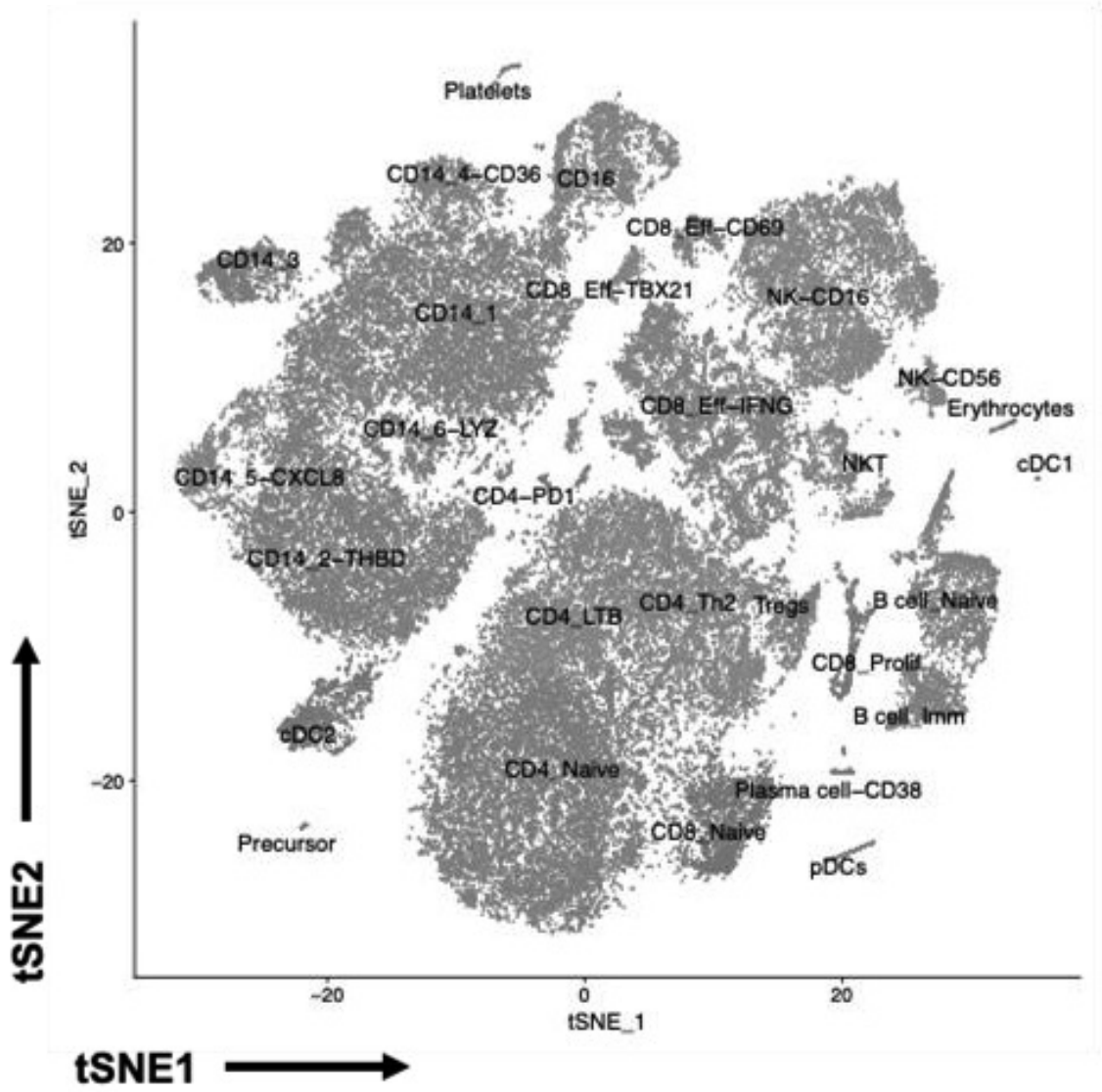

Platelets
CD14_4-CD36
CD16
CD8_Eff-CD69
CD14_3
CD8_Eff-TBX21
NK-CD16
CD14_1
NK-CD56
Erythrocytes
CD8_Eff-IFNG
CD14_6-LYZ
NKT
cDC1
CD14_5-CXCL8
CD4-PD1
CD14_2-THBD
CD4_LTB
CD4_Th2_Tregs
B cell_Naive
CD8_Prolif
B cell_Imm
cDC2
CD4_Naive
Plasma cell-CD38
Precursor
CD8_Naive
pDCs
20
0
-20
tSNE_2
-20
0
20
tSNE_1
tSNE2
tSNE1

**Figure 4A**

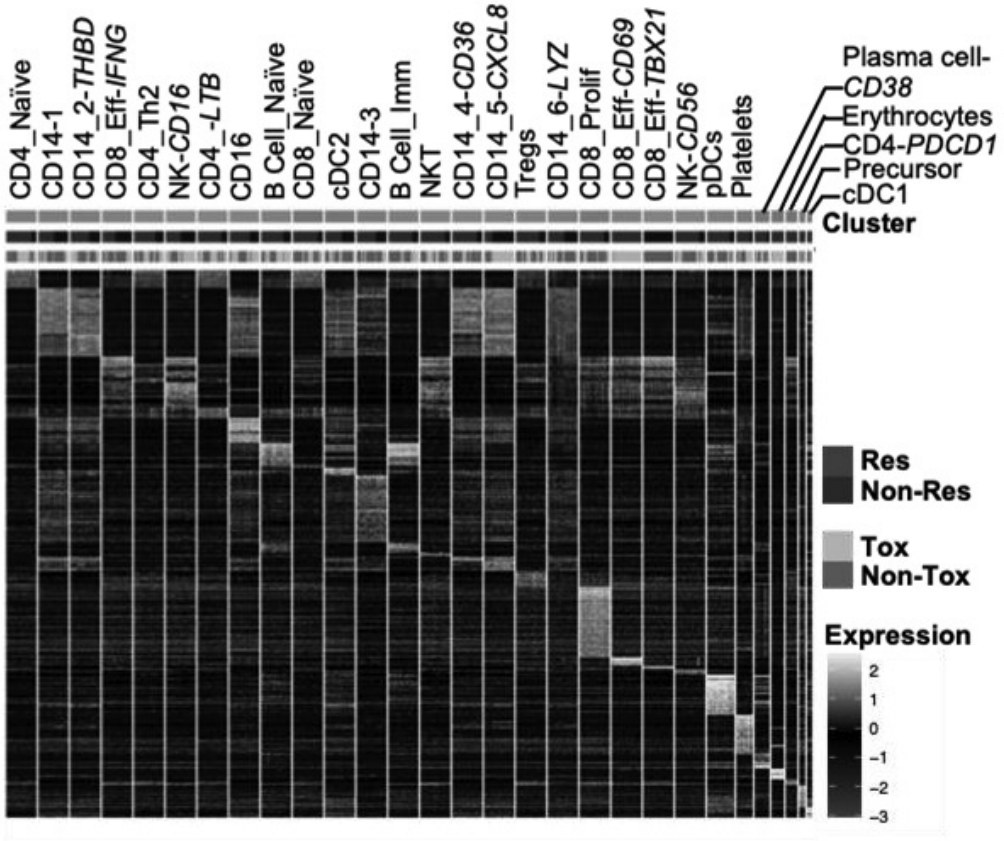

CD4_Naïve
CD14-1
CD14_2-THBD
CD8_Eff-IFNG
CD4_Th2
NK-CD16
CD4_-LTB
CD16
B Cell_Naïve
CD8_Naïve
cDC2
CD14-3
B Cell_Imm
NKT
CD14_4-CD36
CD14_5-CXCL8
Tregs
CD14_6-LYZ
CD8_Prolif
CD8_Eff-CD69
CD8_Eff-TBX21
NK-CD56
pDCs
Platelets
Plasma cell-CD38
Erythrocytes
CD4-PDCD1
Precursor
cDC1
Cluster
Res
Non-Res
Tox
Non-Tox
Expression
2
1
0
−1
−2
−3

**Figure 4B**

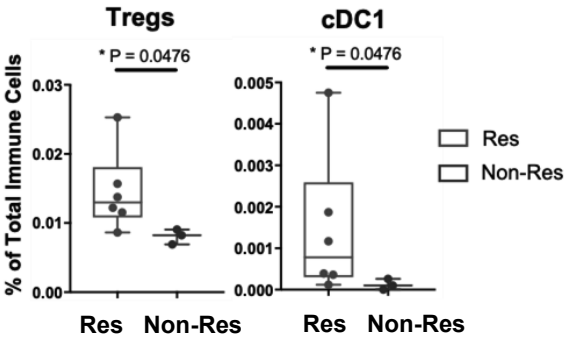



Figure 4C

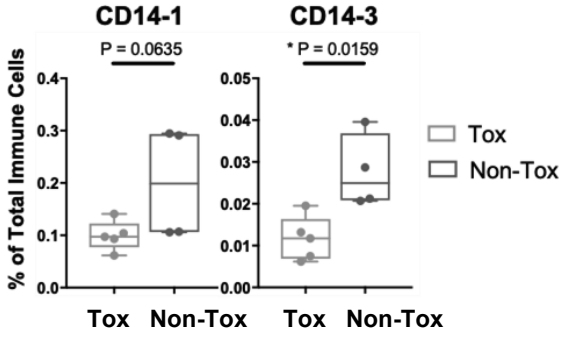



Figure 4D

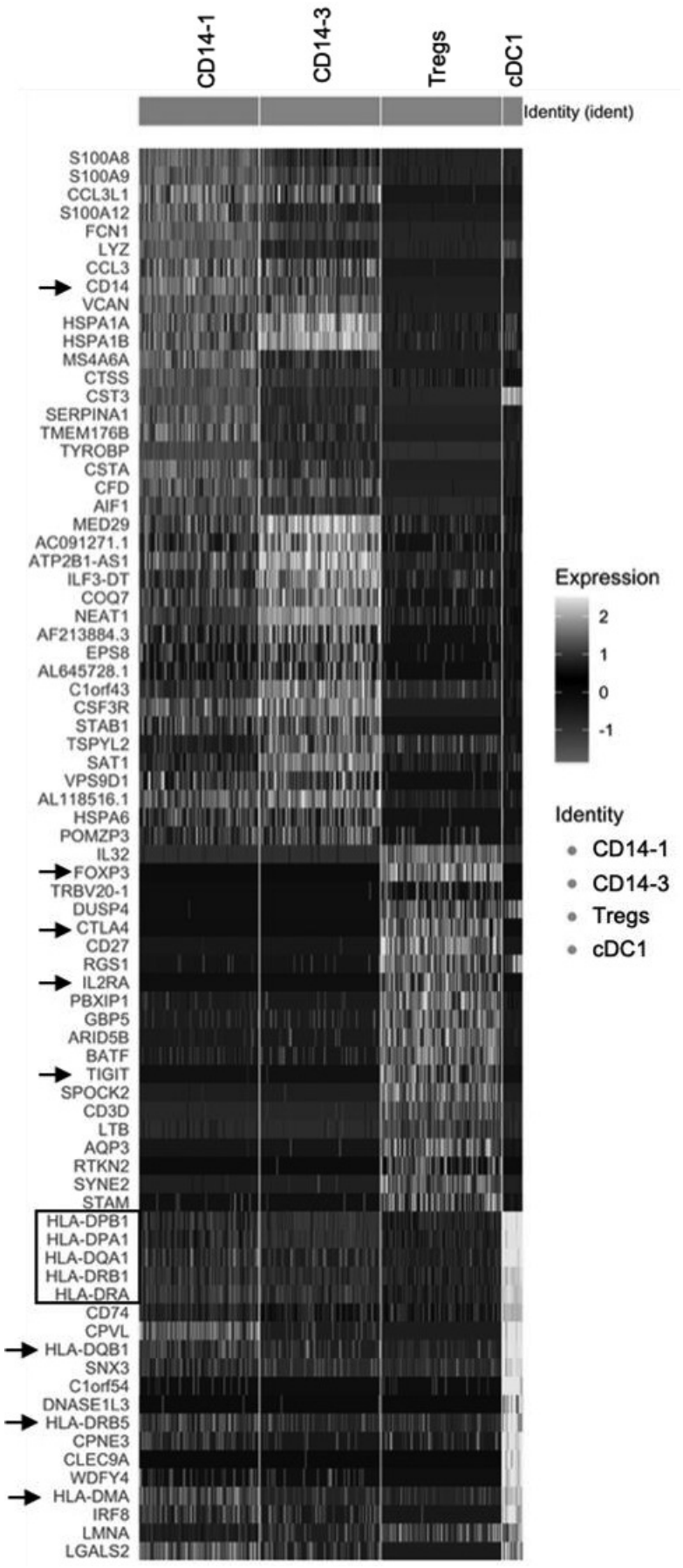

CD14-1
CD14-3
Tregs
cDC1
Identity (ident)
S100A8
S100A9
CCL3L1
S100A12
FCN1
LYZ
CCL3
CD14
VCAN
HSPA1A
HSPA1B
MS4A6A
CTSS
CST3
SERPINA1
TMEM176B
TYROBP
CSTA
CFD
AIF1
MED29
AC091271.1
ATP2B1-AS1
ILF3-DT
COQ7
NEAT1
AF213884.3
EPS8
AL645728.1
C1orf43
CSF3R
STAB1
TSPYL2
SAT1
VPS9D1
AL118516.1
HSPA6
POMZP3
IL32
FOXP3
TRBV20-1
DUSP4
CTLA4
CD27
RGS1
IL2RA
PBXIP1
GBP5
ARID5B
BATF
TIGIT
SPOCK2
CD3D
LTB
AQP3
RTKN2
SYNE2
STAM
HLA-DPB1
HLA-DPA1
HLA-DQA1
HLA-DRB1
HLA-DRA
CD74
CPVL
HLA-DQB1
SNX3
C1orf54
DNASE1L3
HLA-DRB5
CPNE3
CLEC9A
WDFY4
HLA-DMA
IRF8
LMNA
LGALS2
Expression
2
1
0
-1
Identity
CD14-1
CD14-3
Tregs
cDC1

**Figure 4E**

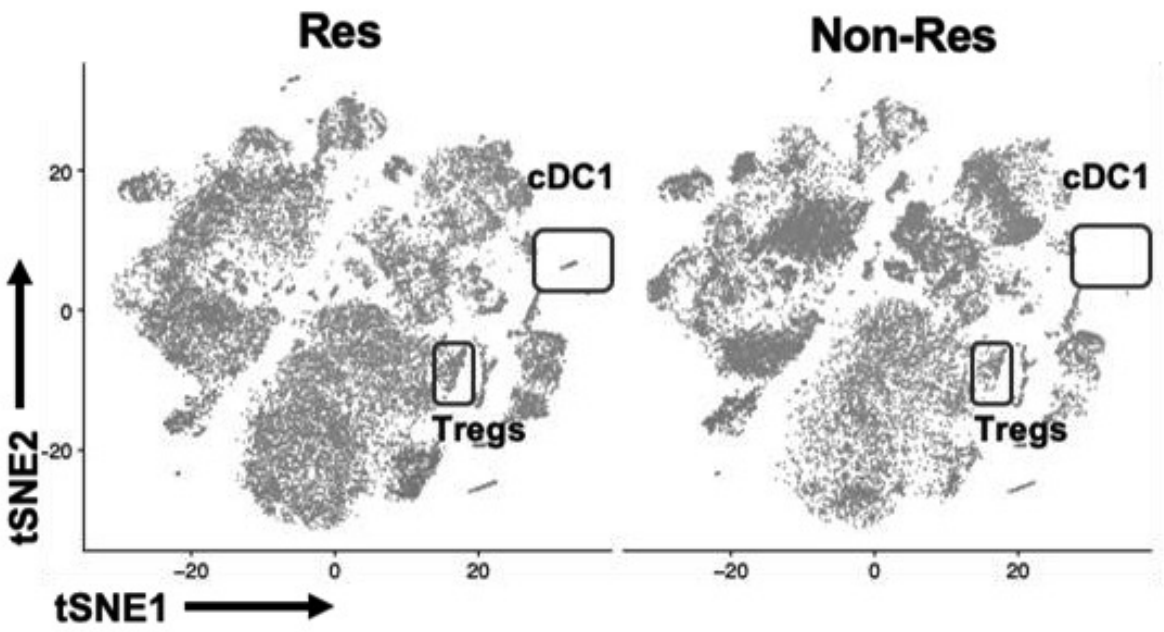



**Figure 4F**

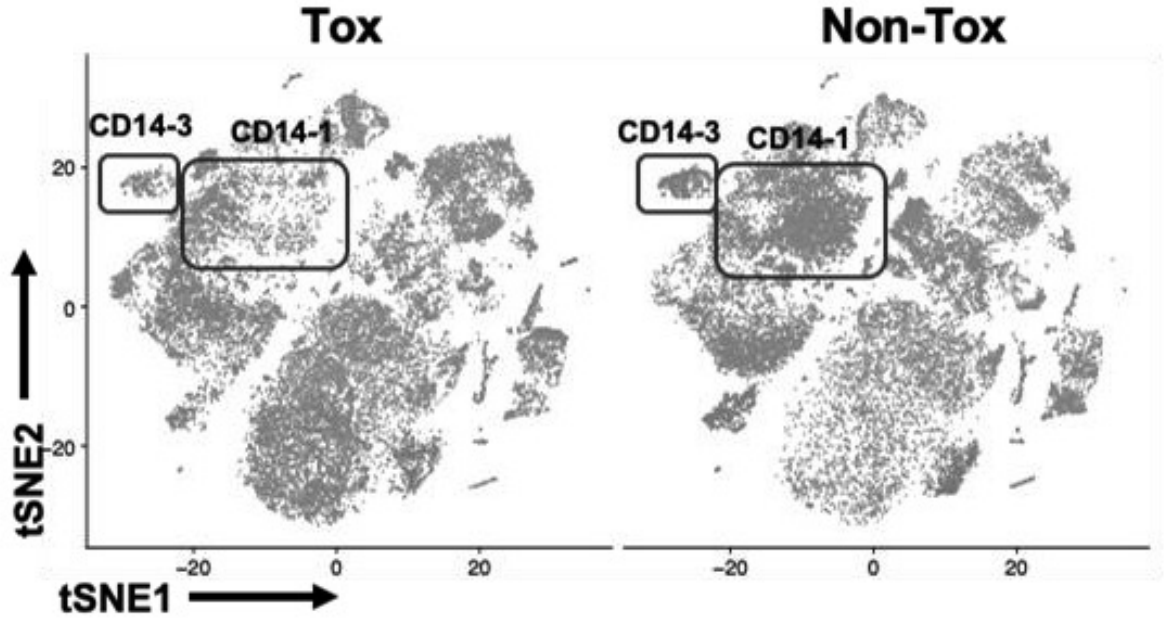



**Figure 4G**

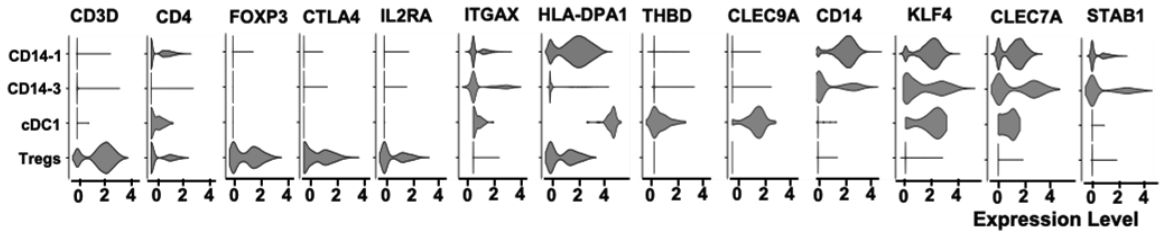



**Figure 4H**

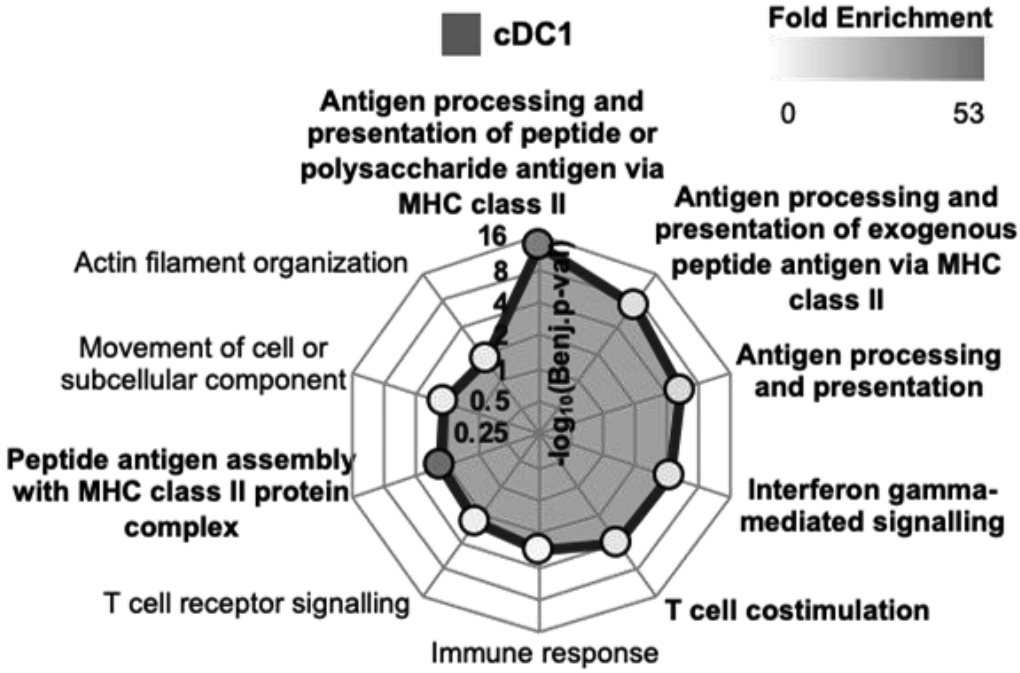



**Figure 4I**

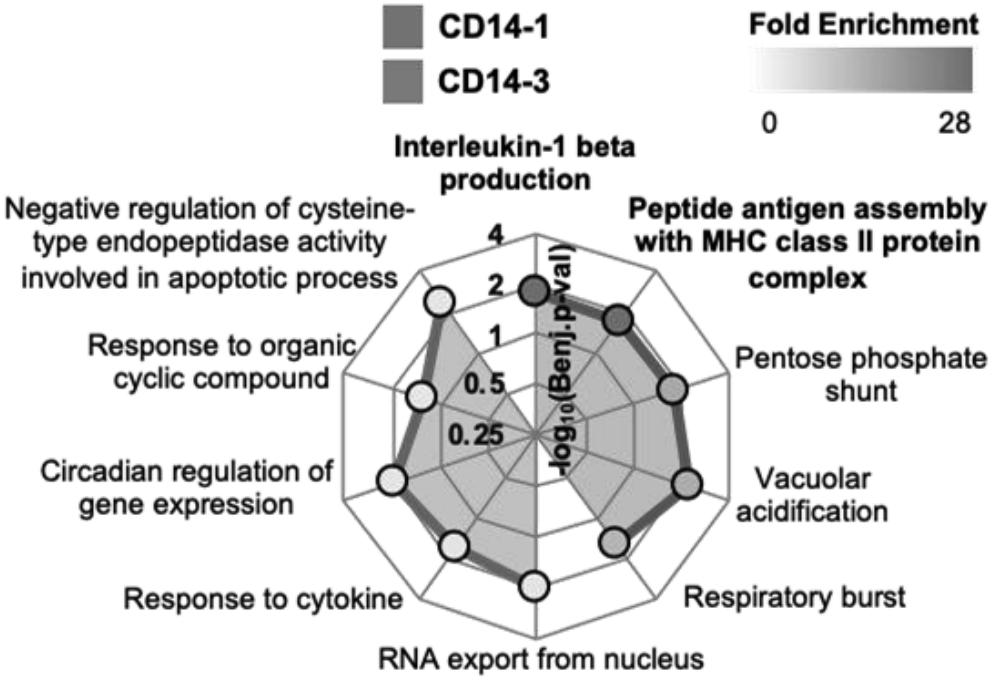

CD14-1
CD14-3
Fold Enrichment
0
28
Interleukin-1 beta production
Peptide antigen assembly with MHC class II protein complex
Pentose phosphate shunt
Vacuolar acidification
Respiratory burst
RNA export from nucleus
Response to cytokine
Circadian regulation of gene expression
Response to organic cyclic compound
Negative regulation of cysteine-type endopeptidase activity involved in apoptotic process
4
2
1
0.5
0.25
-log10(Benj.p-val)

**Figure 4J**

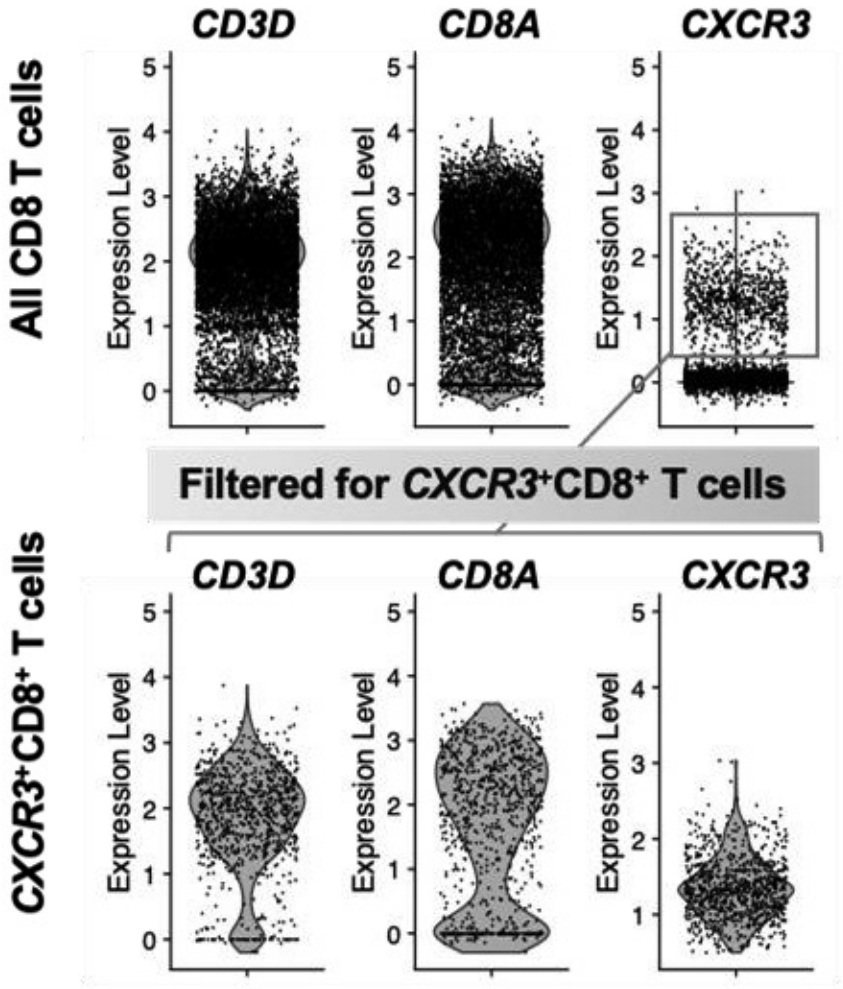

All CD8 T cells
CD3D
CD8A
CXCR3
Expression Level
Filtered for CXCR3+CD8+ T cells
CXCR3+CD8+ T cells
CD3D
CD8A
CXCR3
Expression Level

**Figure 5A**

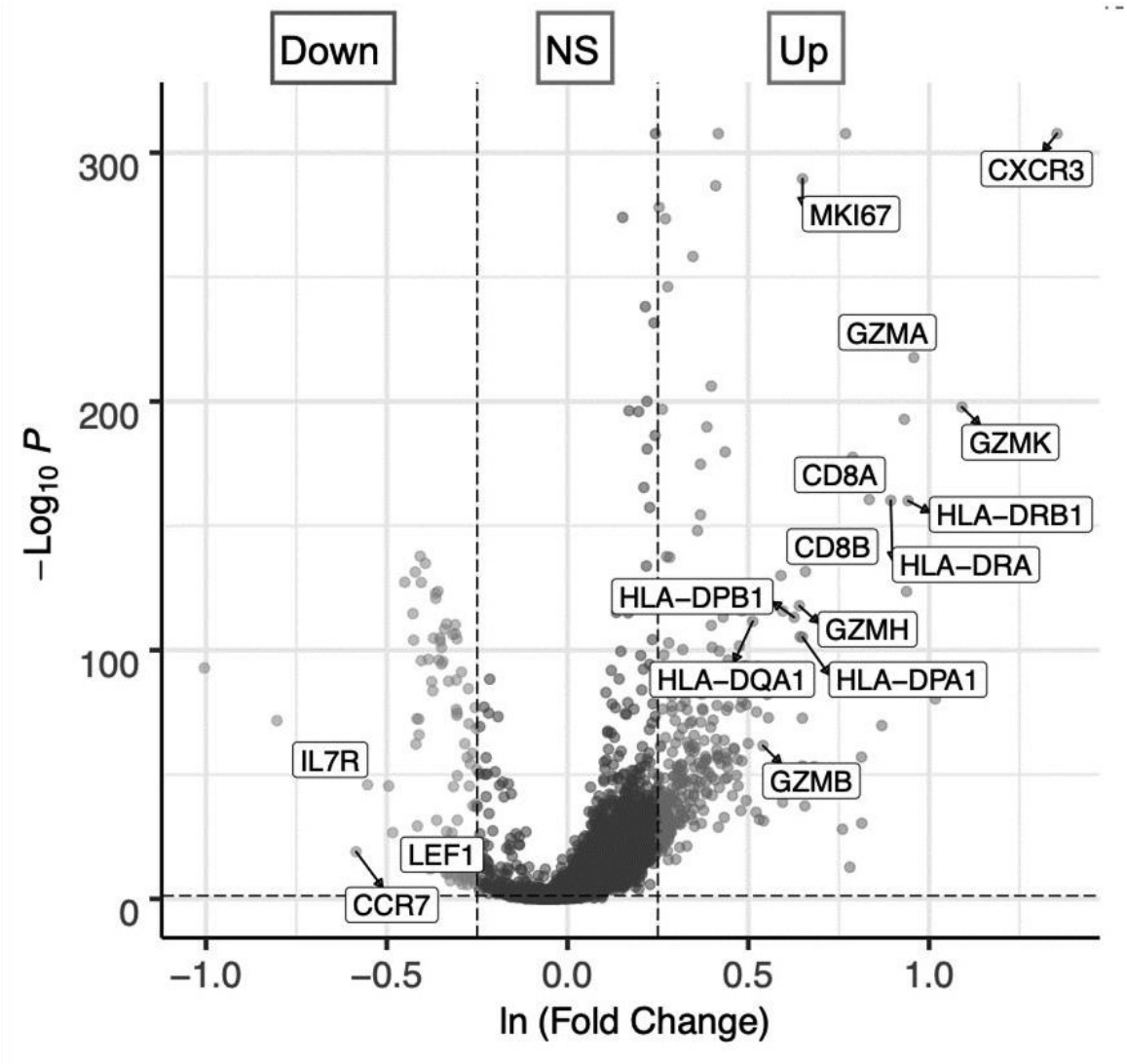

Down
NS
Up
300
200
100
0
−Log10 P
−1.0
−0.5
0.0
0.5
1.0
ln (Fold Change)
CXCR3
MKI67
GZMA
GZMK
CD8A
HLA−DRB1
CD8B
HLA−DRA
HLA−DPB1
GZMH
HLA−DQA1
HLA−DPA1
IL7R
GZMB
LEF1
CCR7

**Figure 5B**

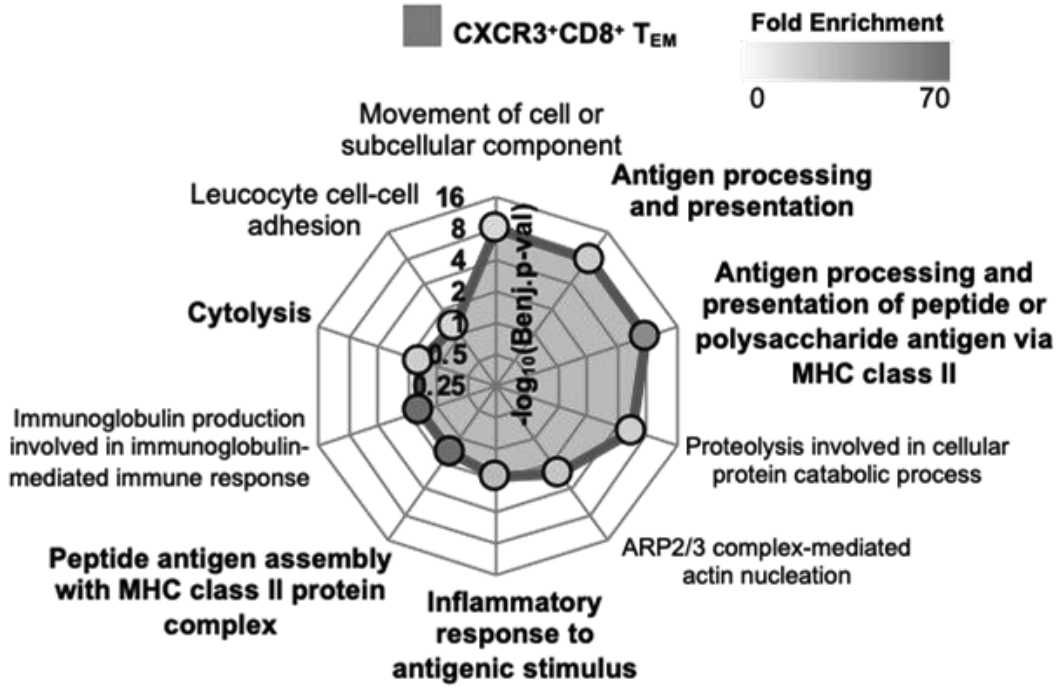



**Figure 5C**

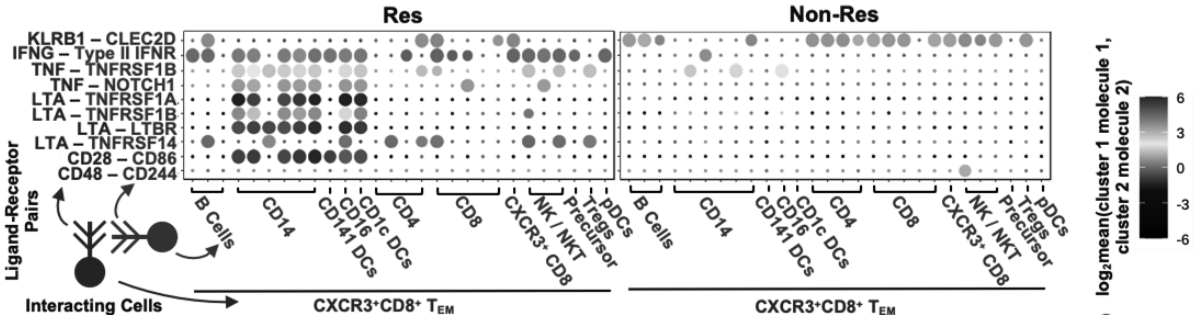



**Figure 5D**

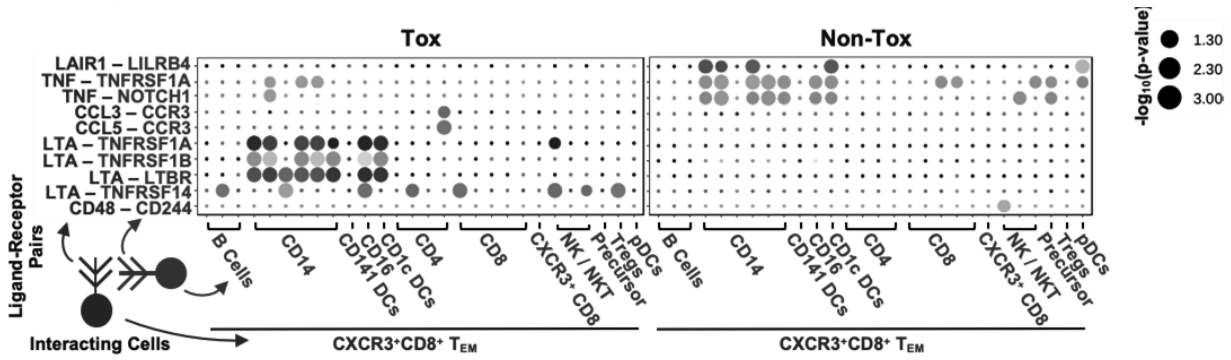



**Figure 5E**

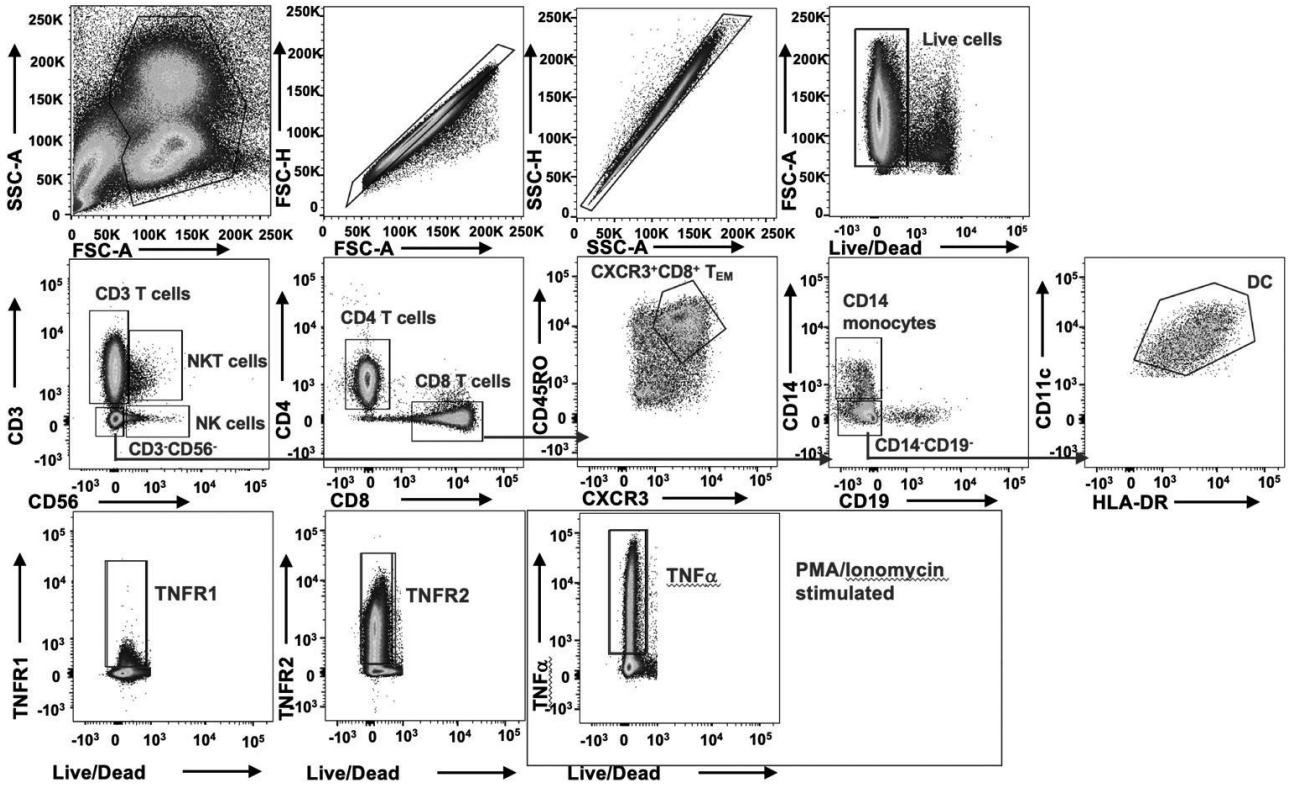



**Figure 5F**

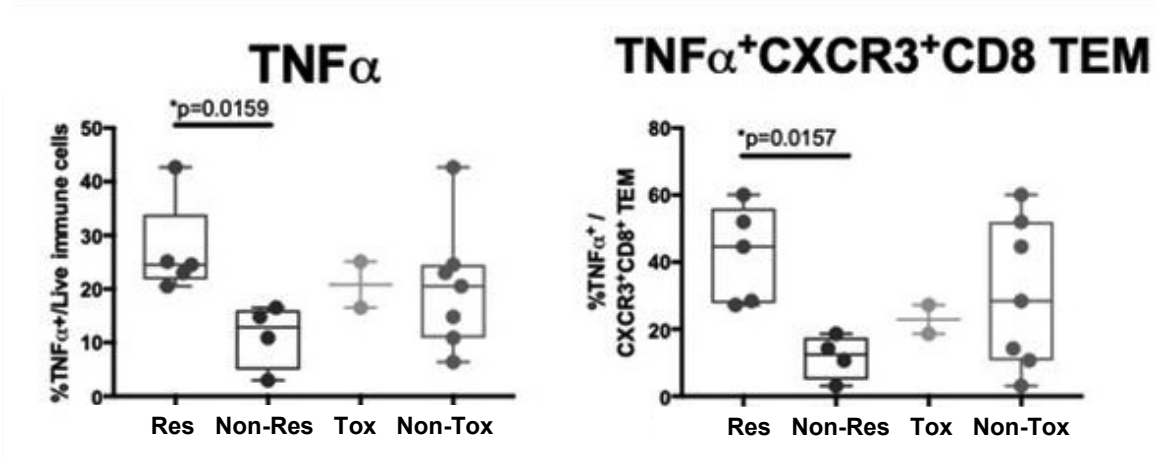



**Figure 5G**

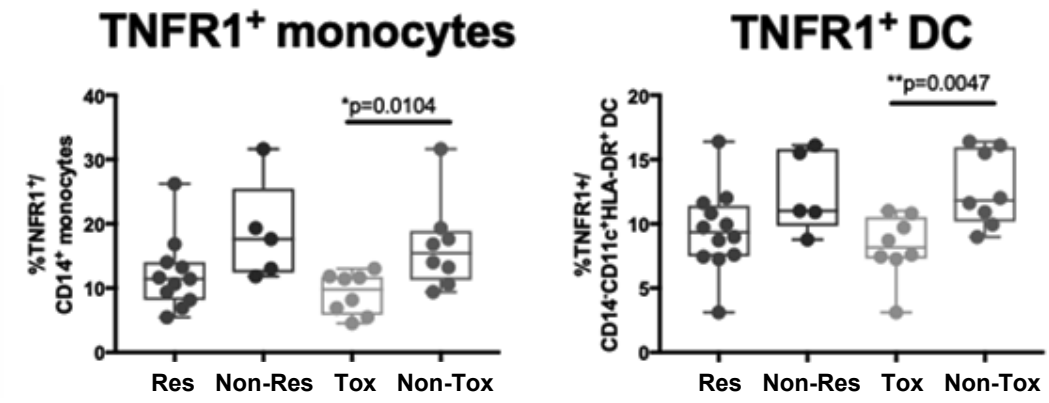



**Figure 5H**

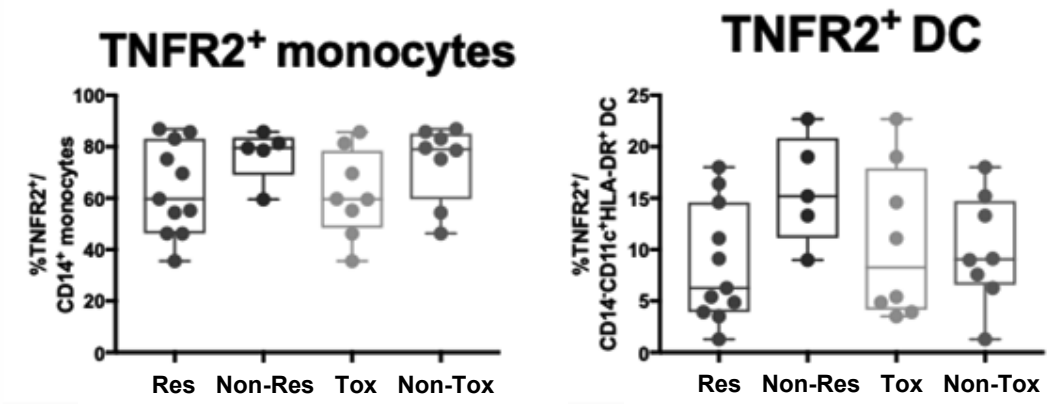



**Figure 5I**

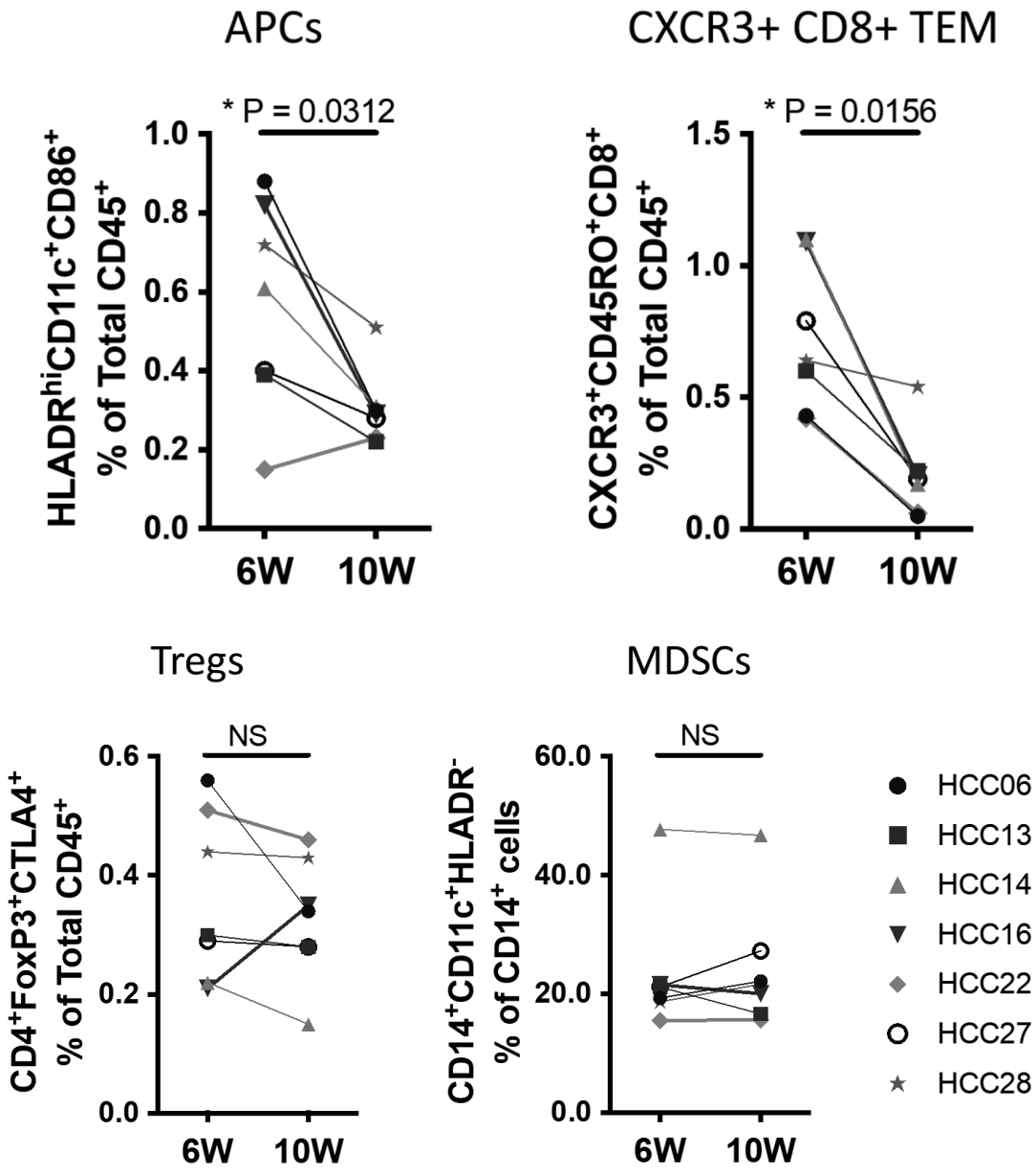

APCs
* P = 0.0312
HLADR^hiCD11c^+CD86^+ % of Total CD45^+
1.0
0.8
0.6
0.4
0.2
0.0
6W
10W
CXCR3+ CD8+ TEM
* P = 0.0156
CXCR3^+CD45RO^+CD8^+ % of Total CD45^+
1.5
1.0
0.5
0.0
6W
10W
Tregs
NS
CD4^+FoxP3^+CTLA4^+ % of Total CD45^+
0.6
0.4
0.2
0.0
6W
10W
MDSCs
NS
CD14^+CD11c^+HLADR^- % of CD14^+ cells
60.0
40.0
20.0
0.0
6W
10W
HCC06
HCC13
HCC14
HCC16
HCC22
HCC27
HCC28

**Figure 6A**

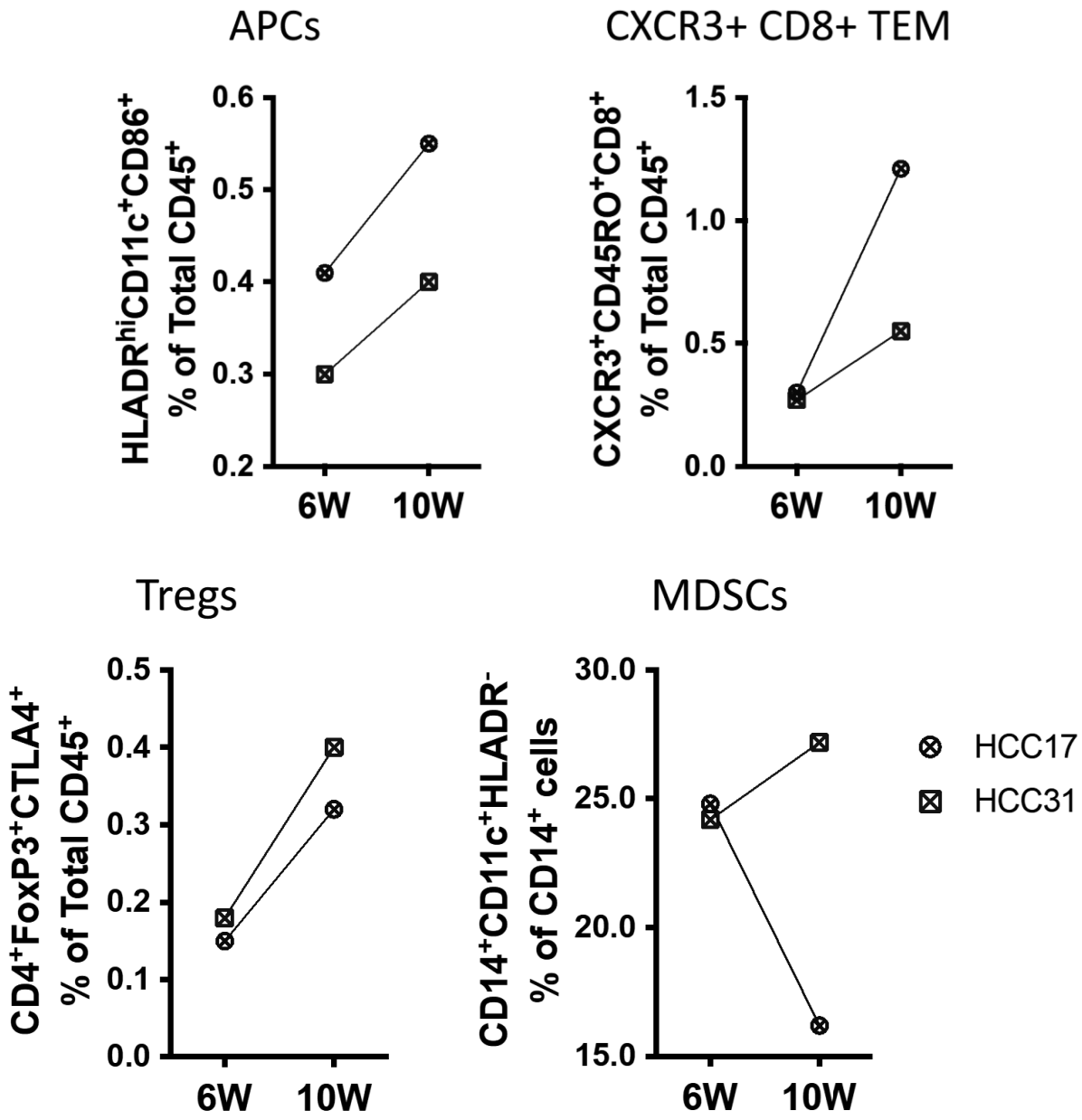

APCs
HLADR^hiCD11c^+CD86^+ % of Total CD45^+
0.6
0.5
0.4
0.3
0.2
6W
10W
CXCR3+ CD8+ TEM
CXCR3^+CD45RO^+CD8^+ % of Total CD45^+
1.5
1.0
0.5
0.0
6W
10W
Tregs
CD4^+FoxP3^+CTLA4^+ % of Total CD45^+
0.5
0.4
0.3
0.2
0.1
0.0
6W
10W
MDSCs
CD14^+CD11c^+HLADR^- % of CD14^+ cells
30.0
25.0
20.0
15.0
6W
10W
HCC17
HCC31

**Figure 6B**

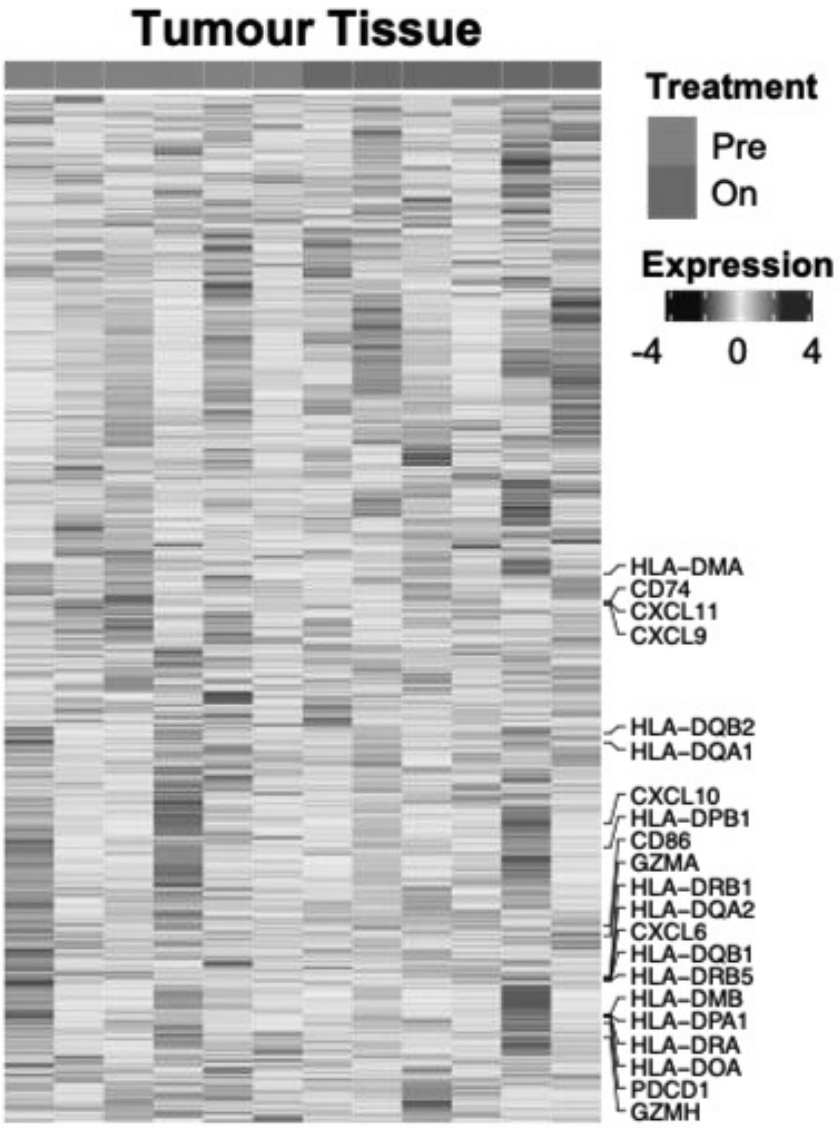

Tumour Tissue
Treatment
Pre
On
Expression
-4
0
4
HLA-DMA
CD74
CXCL11
CXCL9
HLA-DQB2
HLA-DQA1
CXCL10
HLA-DPB1
CD86
GZMA
HLA-DRB1
HLA-DQA2
CXCL6
HLA-DQB1
HLA-DRB5
HLA-DMB
HLA-DPA1
HLA-DRA
HLA-DOA
PDCD1
GZMH

**Figure 6C**

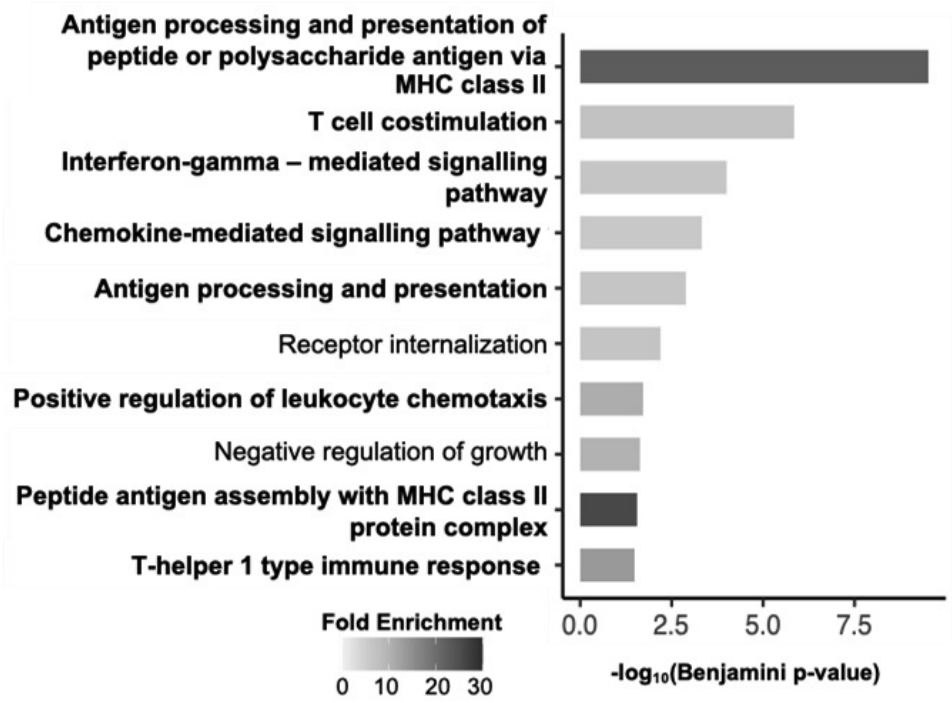

Antigen processing and presentation of peptide or polysaccharide antigen via MHC class II
T cell costimulation
Interferon-gamma – mediated signalling pathway
Chemokine-mediated signalling pathway
Antigen processing and presentation
Receptor internalization
Positive regulation of leukocyte chemotaxis
Negative regulation of growth
Peptide antigen assembly with MHC class II protein complex
T-helper 1 type immune response
Fold Enrichment
0 10 20 30
0.0 2.5 5.0 7.5
-log10(Benjamini p-value)

**Figure 6D**

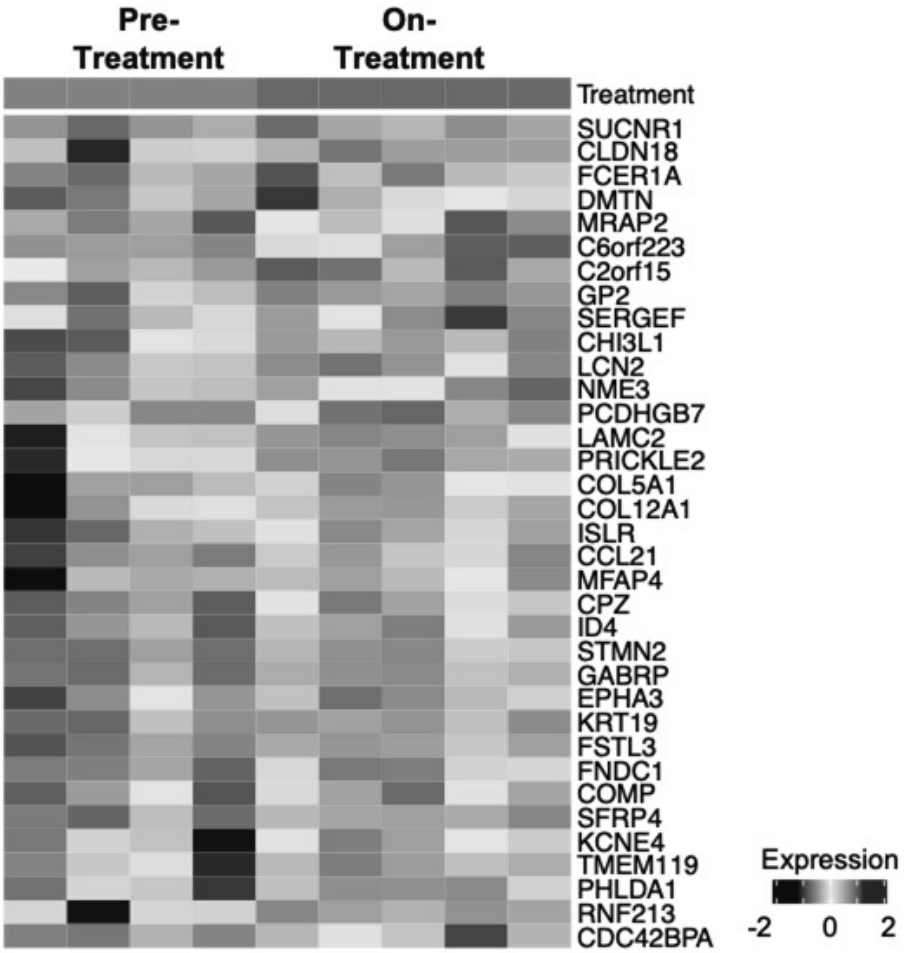

Pre-
Treatment
On-
Treatment
Treatment
SUCNR1
CLDN18
FCER1A
DMTN
MRAP2
C6orf223
C2orf15
GP2
SERGEF
CHI3L1
LCN2
NME3
PCDHGB7
LAMC2
PRICKLE2
COL5A1
COL12A1
ISLR
CCL21
MFAP4
CPZ
ID4
STMN2
GABRP
EPHA3
KRT19
FSTL3
FNDC1
COMP
SFRP4
KCNE4
TMEM119
PHLDA1
RNF213
CDC42BPA
Expression
-2
0
2

**Figure 6E**

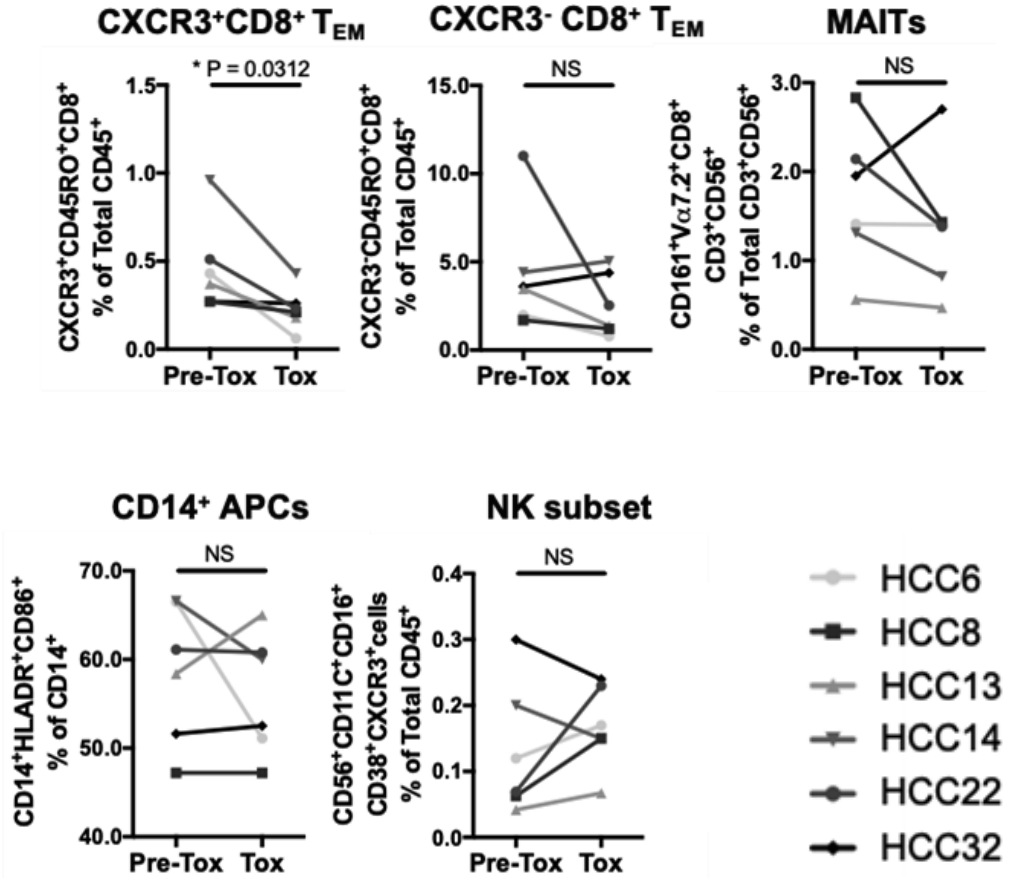

CXCR3+CD8+ TEM
CXCR3- CD8+ TEM
MAITs
* P = 0.0312
NS
NS
CXCR3+CD45RO+CD8+ % of Total CD45+
CXCR3-CD45RO+CD8+ % of Total CD45+
CD161+Vα7.2+CD8+ CD3+CD56+ % of Total CD3+CD56+
1.5
1.0
0.5
0.0
15.0
10.0
5.0
0.0
3.0
2.0
1.0
0.0
Pre-Tox Tox
Pre-Tox Tox
Pre-Tox Tox
CD14+ APCs
NK subset
NS
NS
CD14+HLADR+CD86+ % of CD14+
CD56+CD11C+CD16+ CD38+CXCR3+cells % of Total CD45+
70.0
60.0
50.0
40.0
0.4
0.3
0.2
0.1
0.0
Pre-Tox Tox
Pre-Tox Tox
HCC6
HCC8
HCC13
HCC14
HCC22
HCC32

**Figure 6F**

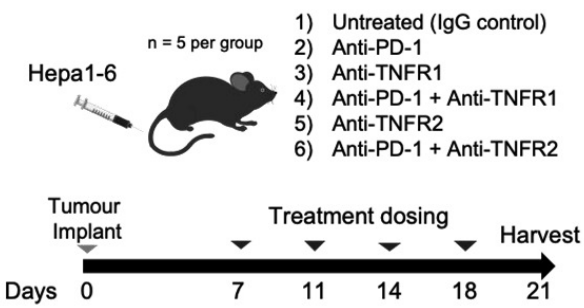



Figure 7A

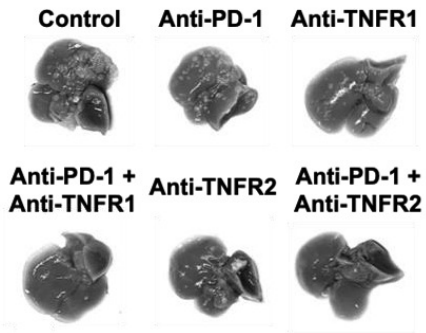



Figure 7B

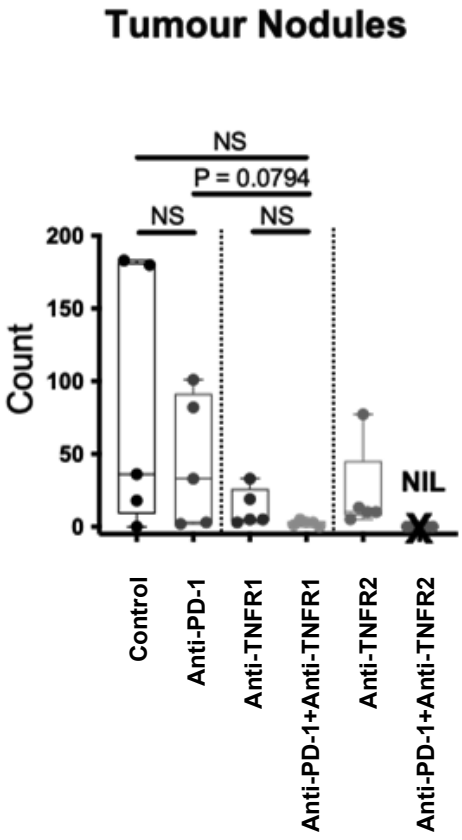



Figure 7C

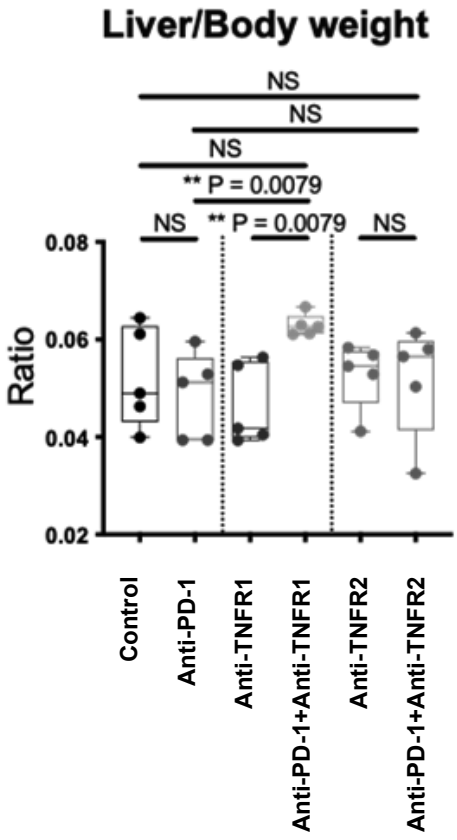



Figure 7D

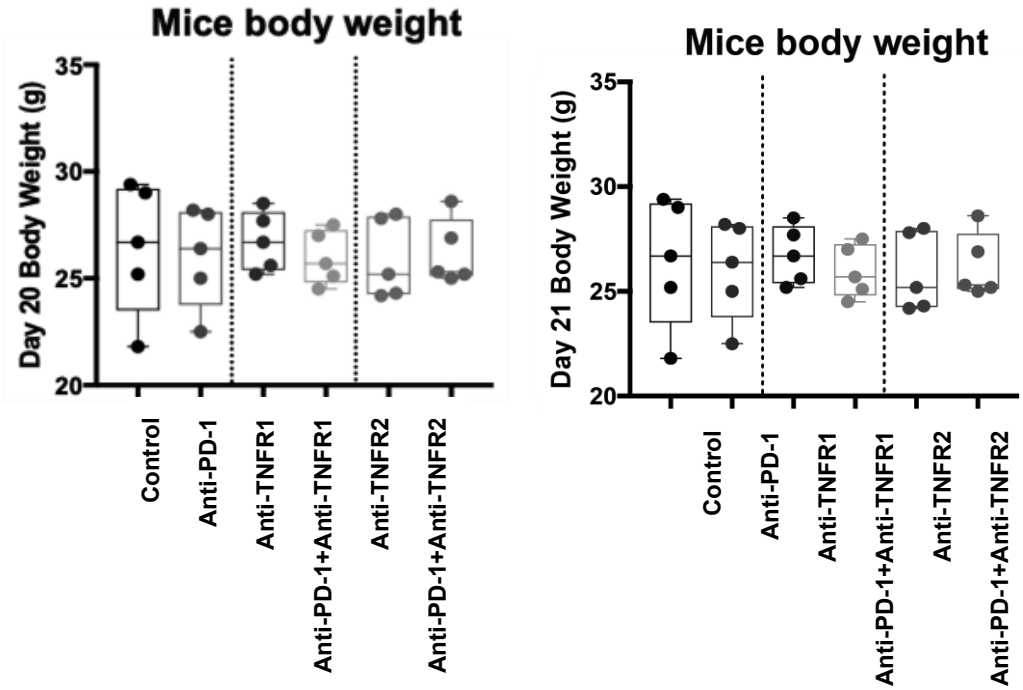



**Figure 7E**

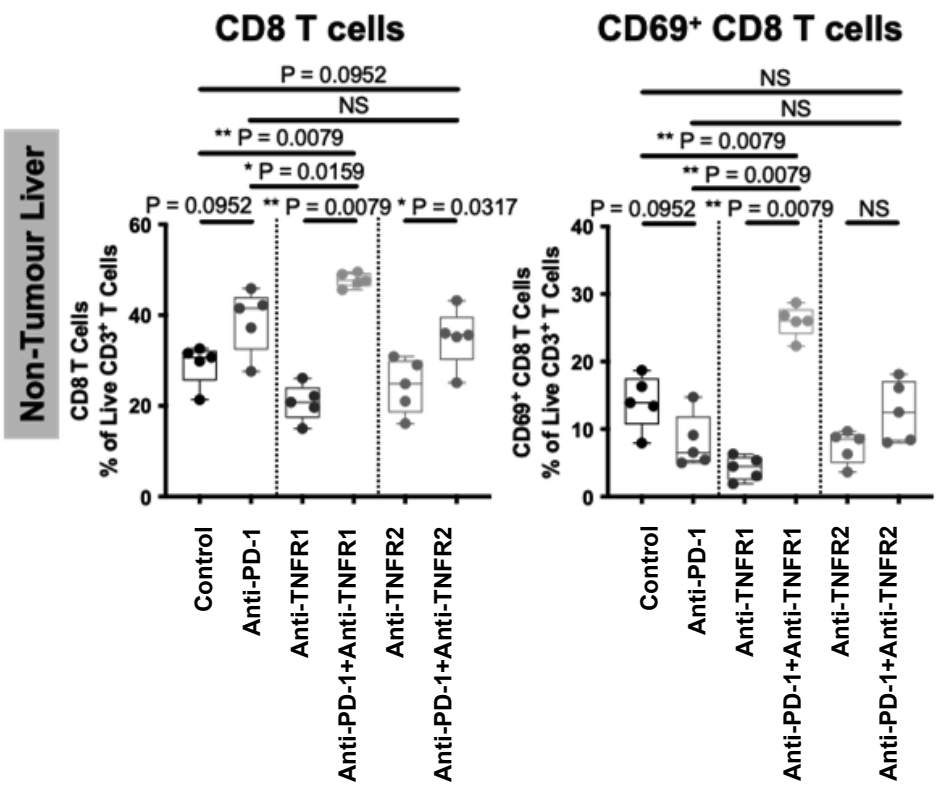



**Figure 7F**

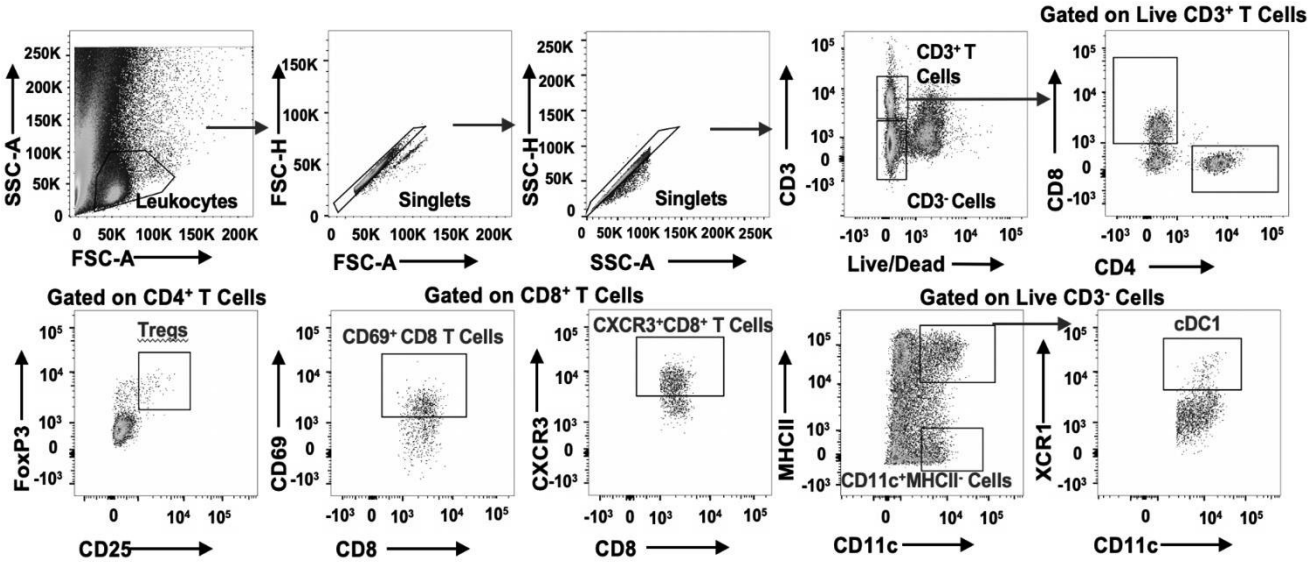



**Figure 7G**

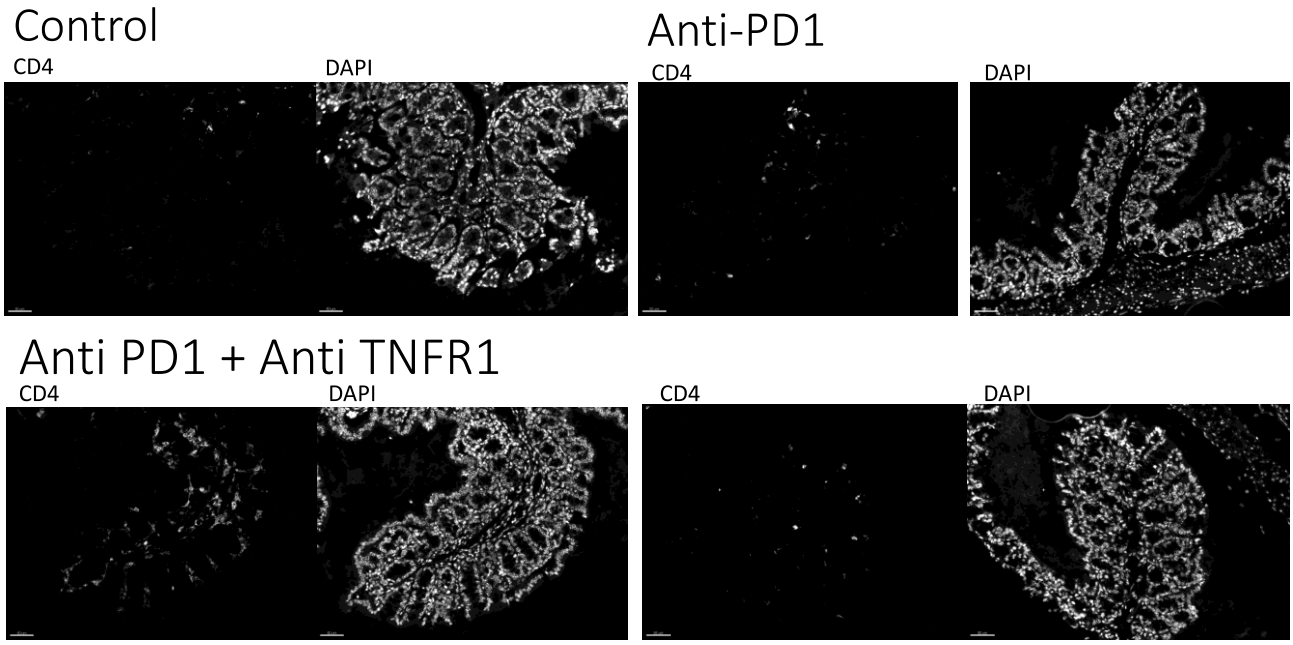



**Figure 7H**

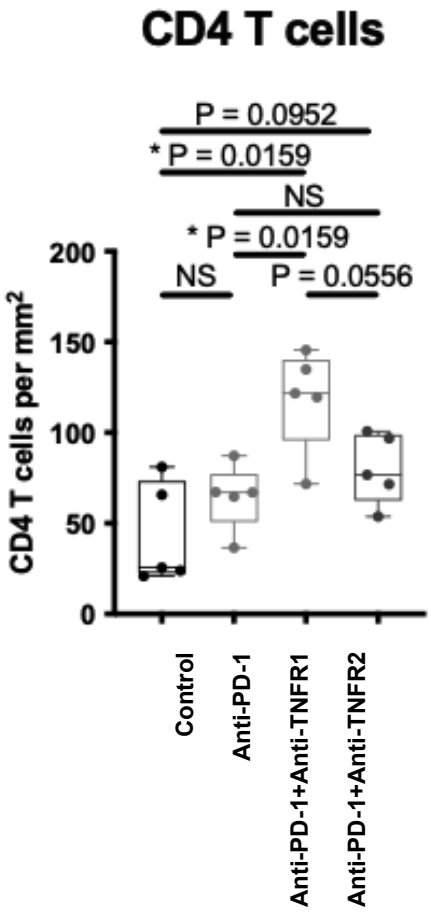

CD4 T cells
P = 0.0952
* P = 0.0159
NS
* P = 0.0159
NS
P = 0.0556
CD4 T cells per mm2
200
150
100
50
0
Control
Anti-PD-1
Anti-PD-1+Anti-TNFR1
Anti-PD-1+Anti-TNFR2

**Figure 7I**

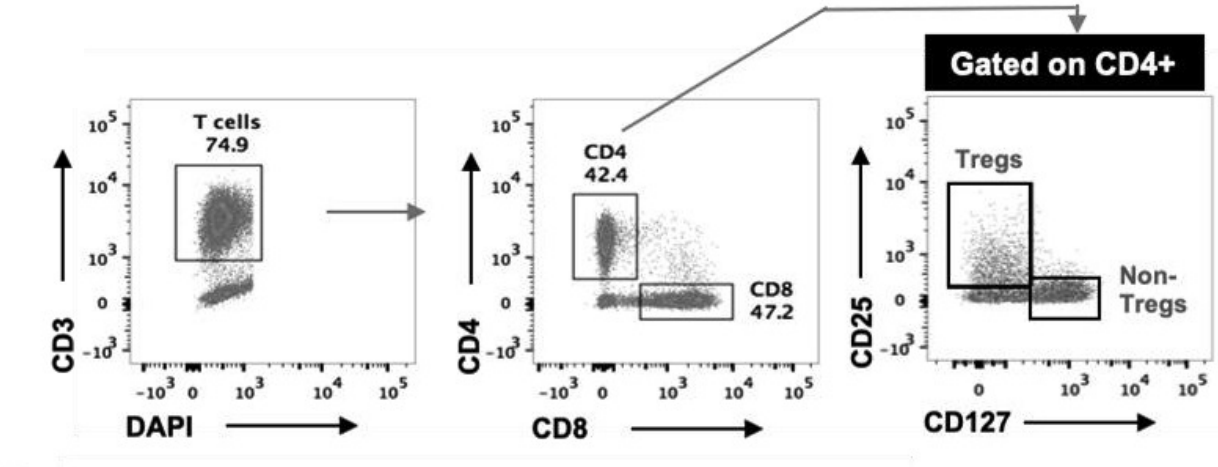



**Figure 7J**

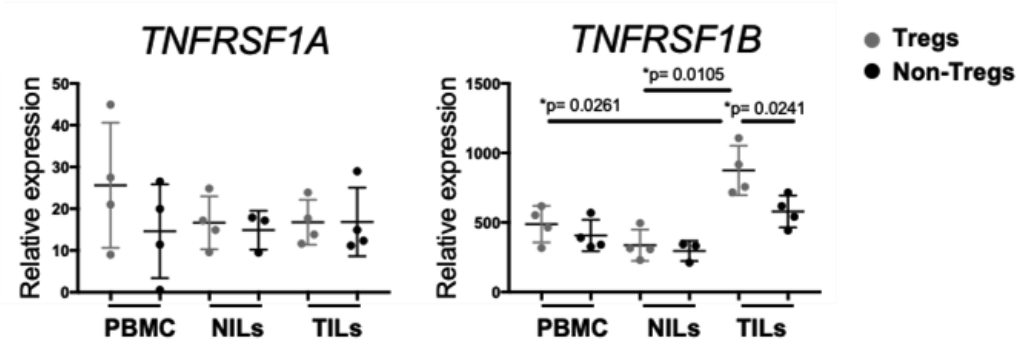



**Figure 7K**

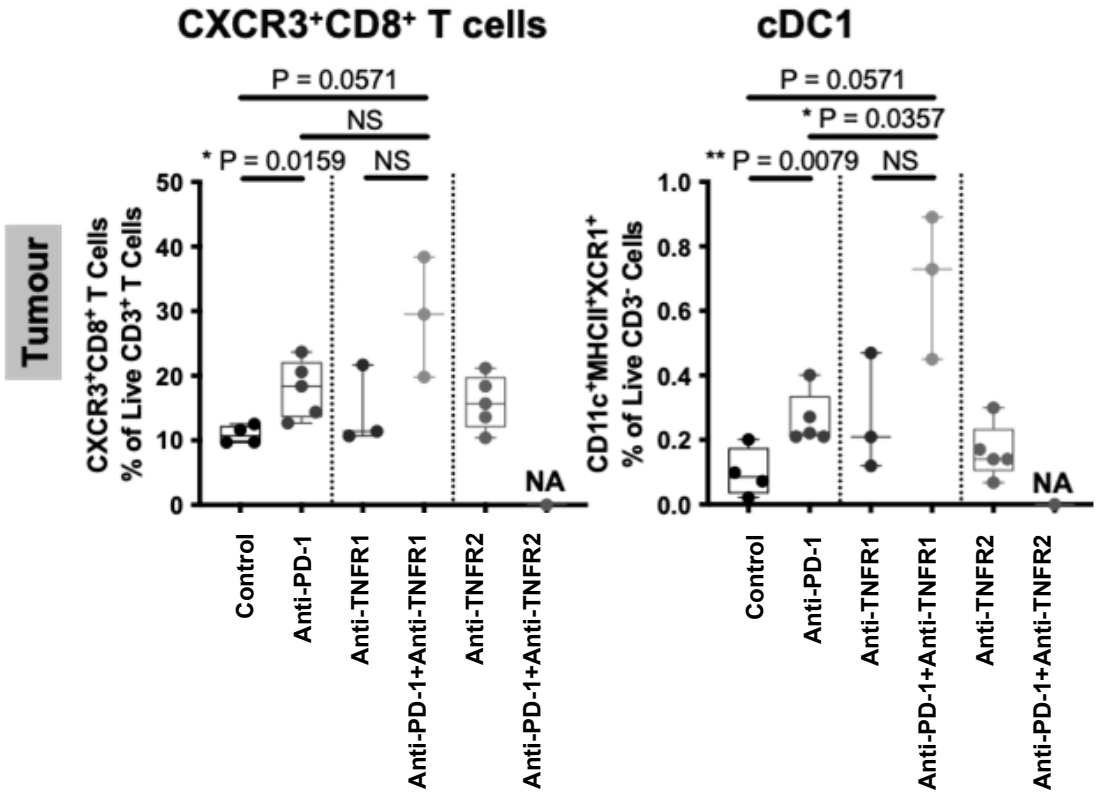



**Figure 7L**

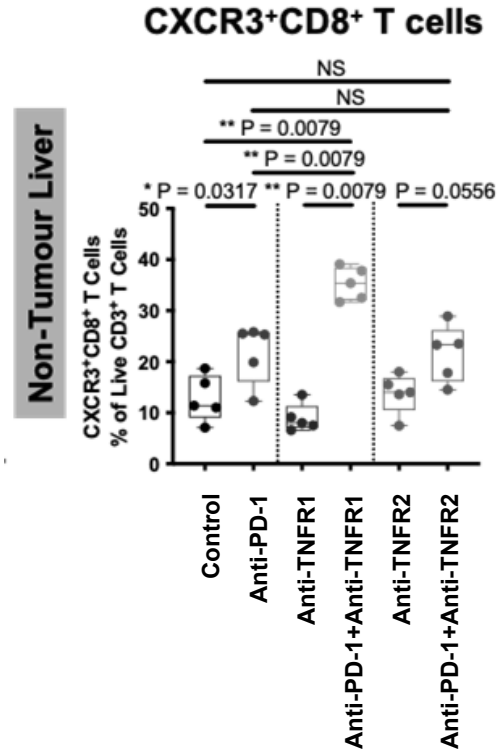



**Figure 7M**