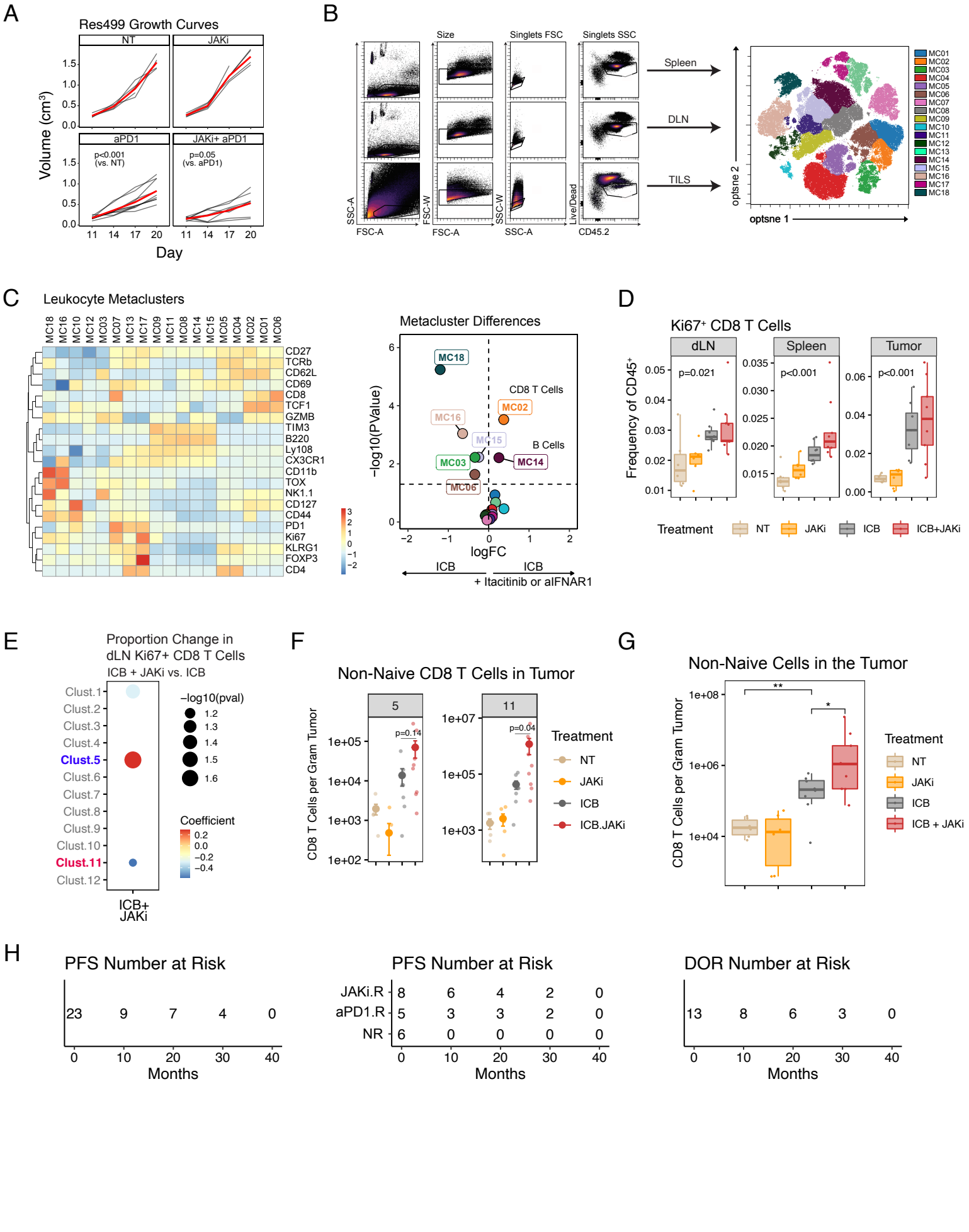
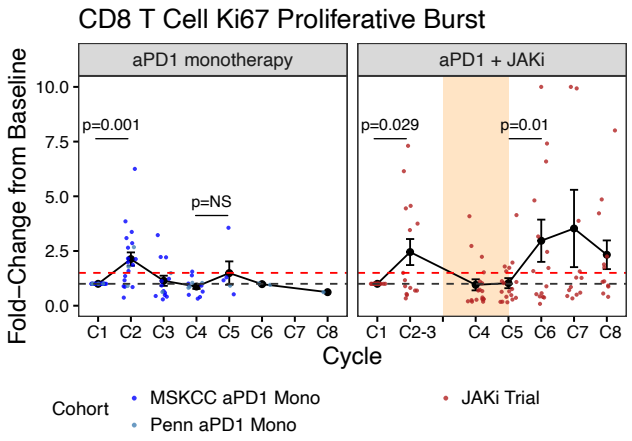
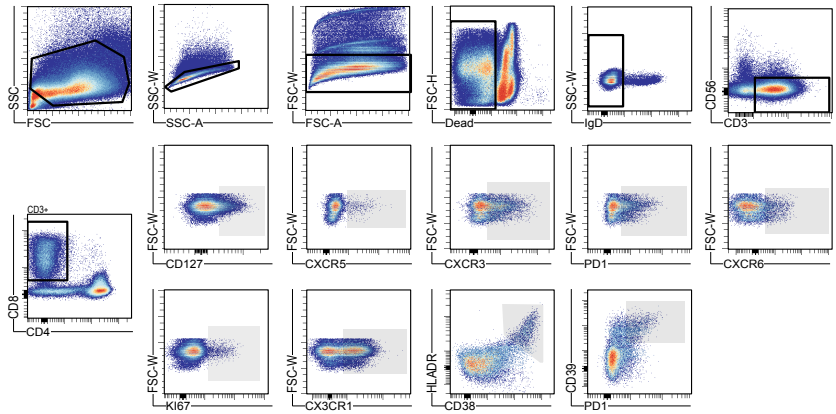

A
Res499 Growth Curves
NT
JAKi
aPD1
JAKi+ aPD1
p<0.001 (vs. NT)
p=0.05 (vs. aPD1)
Volume (cm3)
Day
B
Size
Singlets FSC
Singlets SSC
Spleen
DLN
TILS
SSC-A
FSC-A
FSC-W
SSC-W
Live/Dead
CD45.2
optsne 1
optsne 2
MC01
MC02
MC03
MC04
MC05
MC06
MC07
MC08
MC09
MC10
MC11
MC12
MC13
MC14
MC15
MC16
MC17
MC18
C
Leukocyte Metaclusters
CD27
TCRb
CD62L
CD69
CD8
TCF1
GZMB
TIM3
B220
Ly108
CX3CR1
CD11b
TOX
NK1.1
CD127
CD44
PD1
Ki67
KLRG1
FOXP3
CD4
Metacluster Differences
−log10(PValue)
logFC
CD8 T Cells
B Cells
ICB
ICB + Itacitinib or aIFNAR1
D
Ki67+ CD8 T Cells
dLN
Spleen
Tumor
Frequency of CD45+
p=0.021
p<0.001
p<0.001
Treatment
NT
JAKi
ICB
ICB+JAKi
E
Proportion Change in dLN Ki67+ CD8 T Cells
ICB + JAKi vs. ICB
−log10(pval)
Coefficient
ICB+ JAKi
F
Non-Naive CD8 T Cells in Tumor
CD8 T Cells per Gram Tumor
p=0.14
p=0.04
Treatment
NT
JAKi
ICB
ICB.JAKi
G
Non-Naive Cells in the Tumor
CD8 T Cells per Gram Tumor
Treatment
NT
JAKi
ICB
ICB + JAKi
H
PFS Number at Risk
Months
PFS Number at Risk
JAKi.R
aPD1.R
NR
Months
DOR Number at Risk
Months

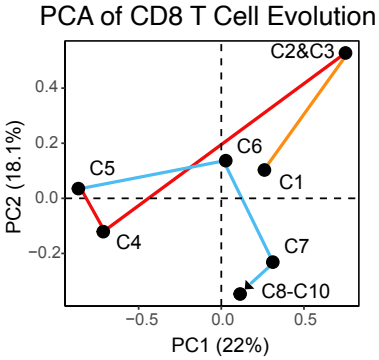
A


CD8 T Cell Ki67 Proliferative Burst
aPD1 monotherapy
aPD1 + JAKi
Fold−Change from Baseline
p=0.001
p=NS
p=0.029
p=0.01
Cycle
Cohort
MSKCC aPD1 Mono
Penn aPD1 Mono
JAKi Trial

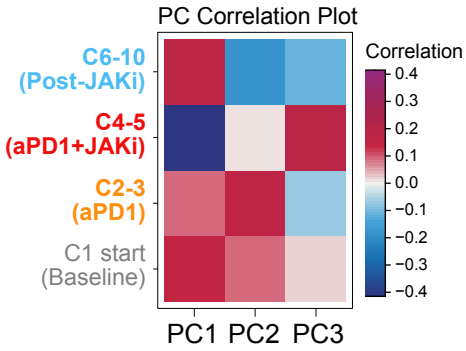
B


SSC
FSC
SSC-W
SSC-A
FSC-W
FSC-A
FSC-H
Dead
IgD
CD56
CD3
CD8
CD4
CD127
CXCR5
CXCR3
PD1
CXCR6
Ki67
CX3CR1
HLADR
CD38

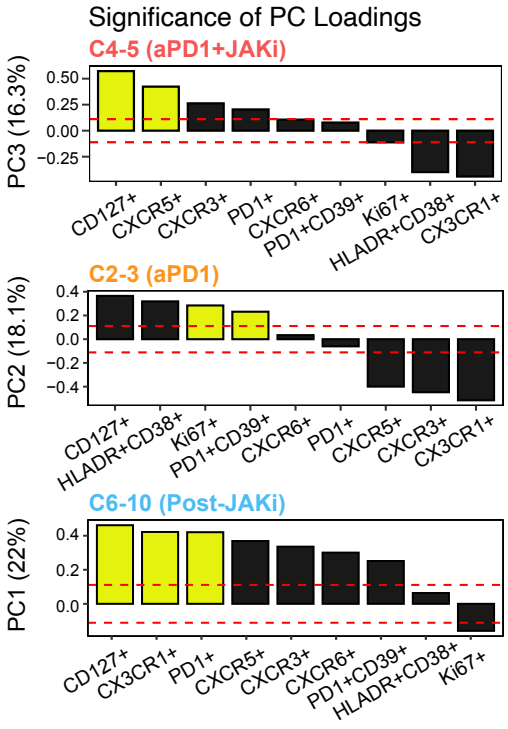
C


PCA of CD8 T Cell Evolution
C2&C3
C6
C5
C1
C4
C7
C8-C10
PC2 (18.1%)
PC1 (22%)

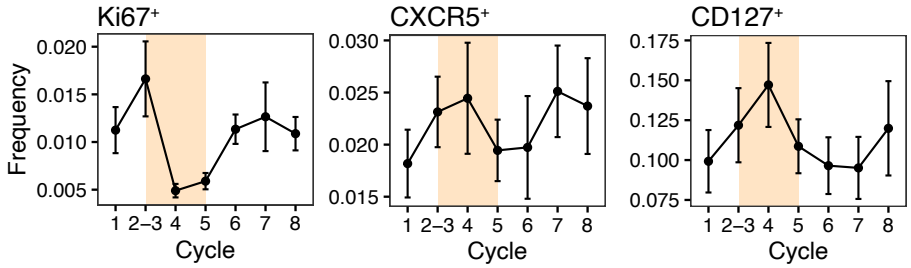
D

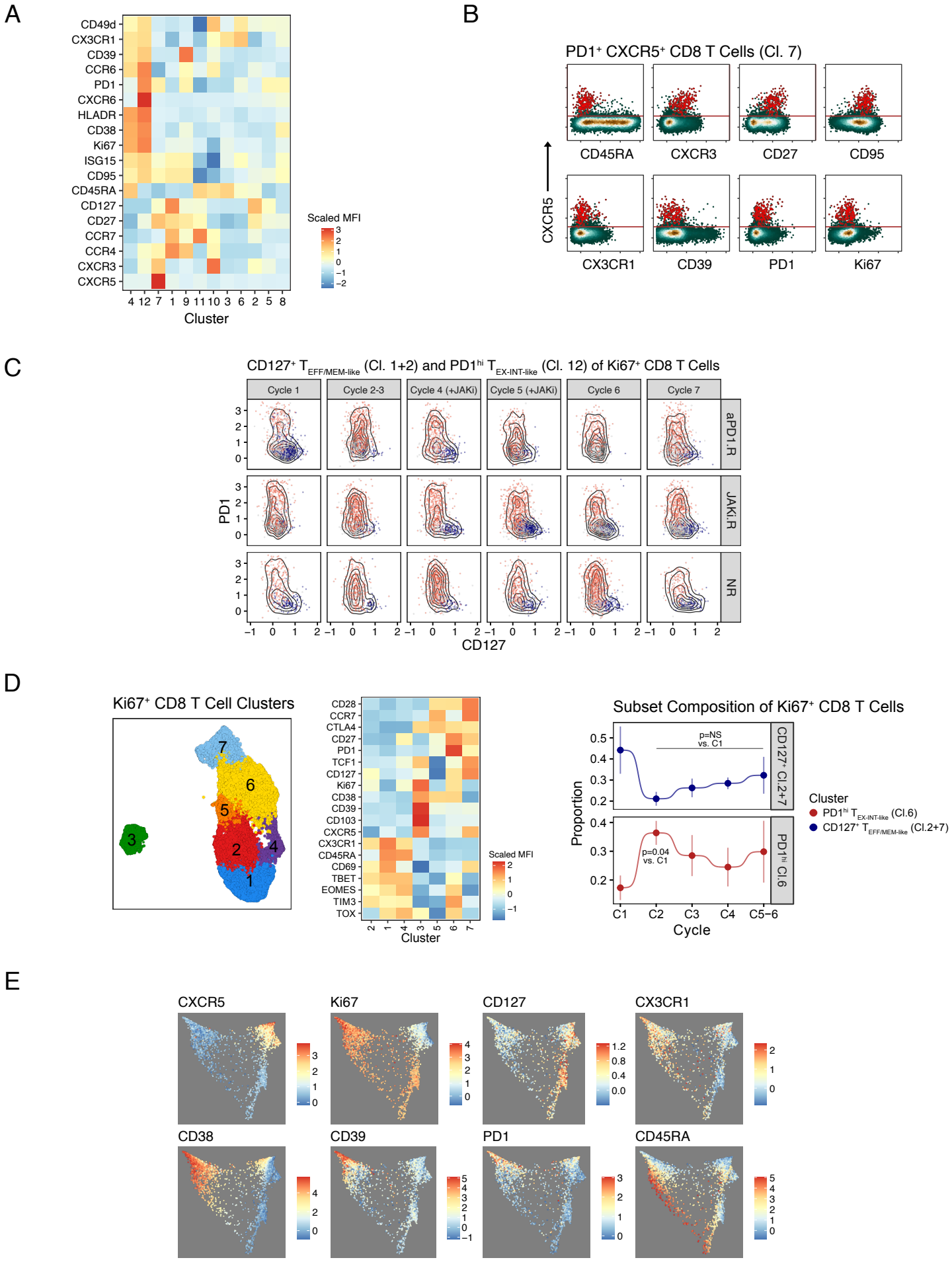

PC Correlation Plot
C6-10
(Post-JAKi)
C4-5
(aPD1+JAKi)
C2-3
(aPD1)
C1 start
(Baseline)
Correlation
PC1 PC2 PC3

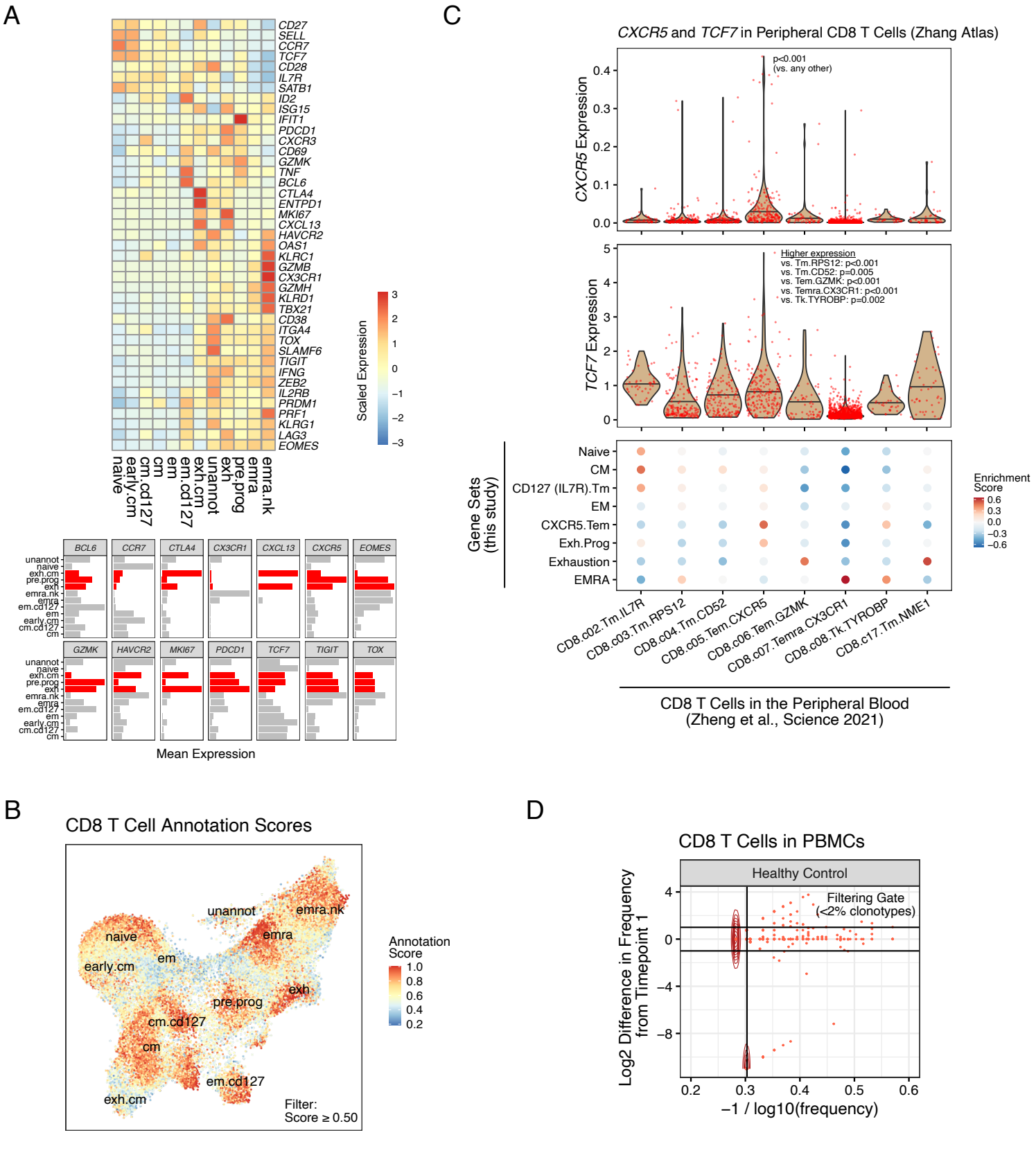
E

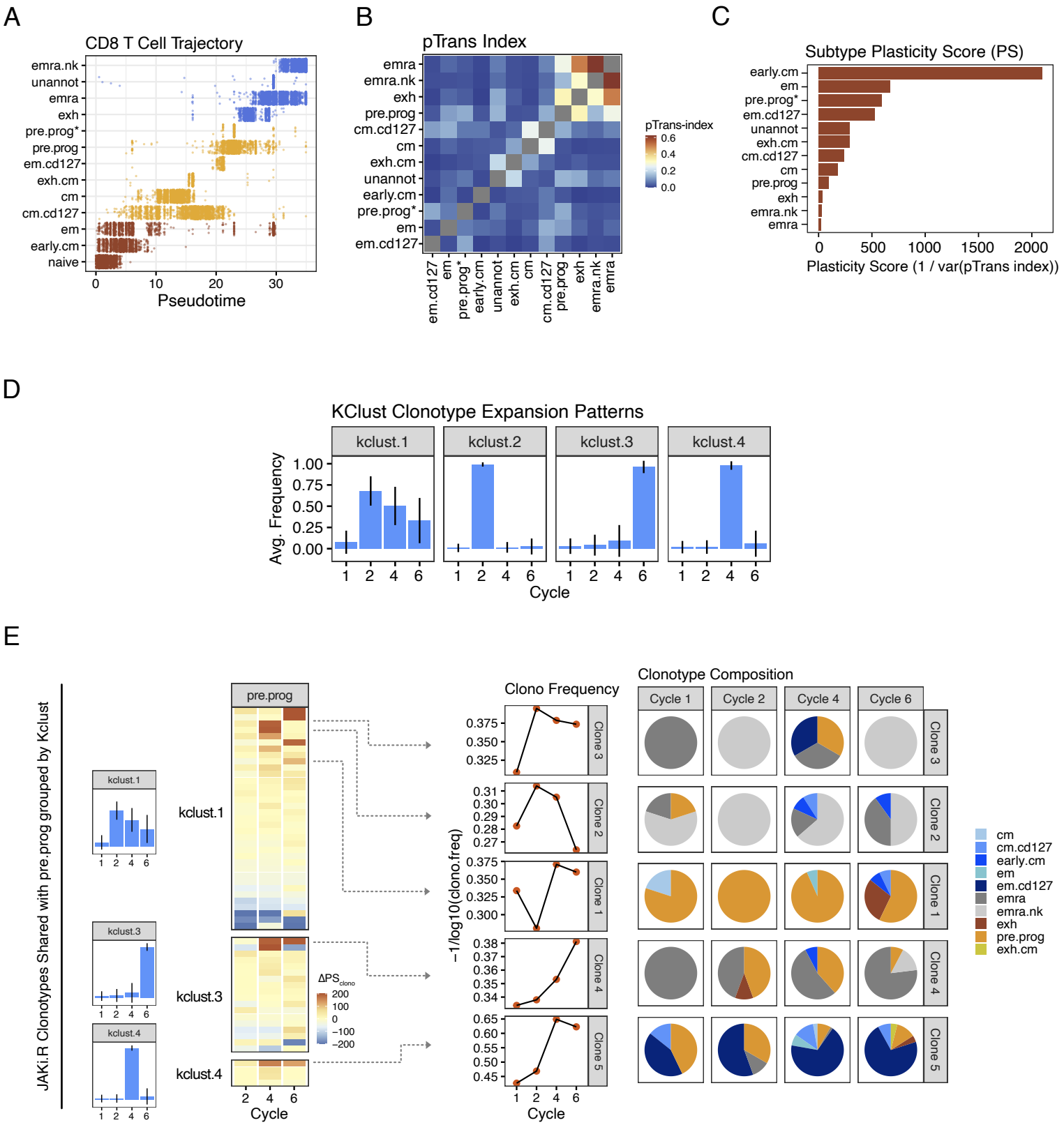

Significance of PC Loadings
C4-5 (aPD1+JAKi)
PC3 (16.3%)
CD127+
CXCR5+
CXCR3+
PD1+
CXCR6+
PD1+CD39+
Ki67+
HLADR+CD38+
CX3CR1+
C2-3 (aPD1)
PC2 (18.1%)
C6-10 (Post-JAKi)
PC1 (22%)

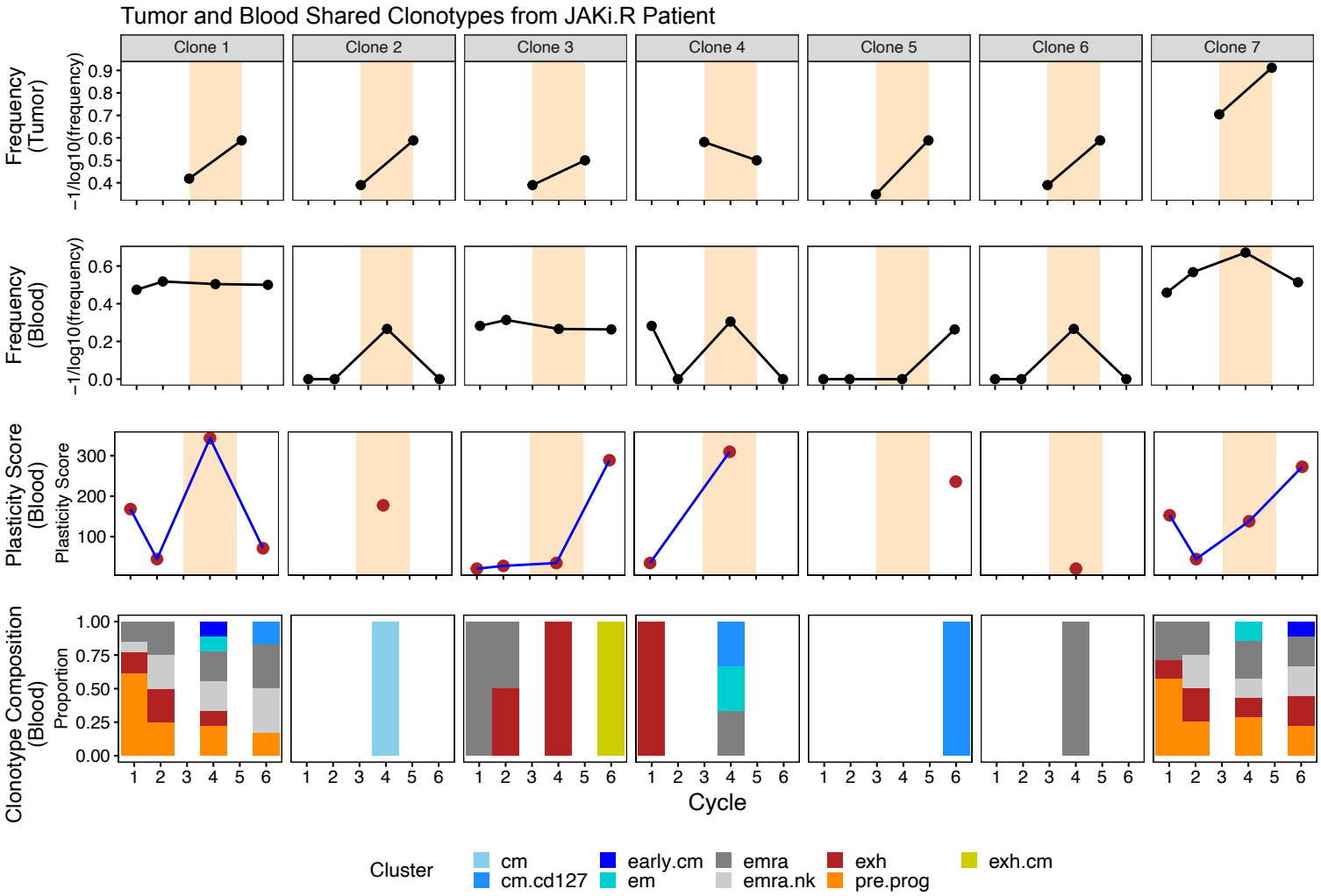
F


Ki67⁺
CXCR5⁺
CD127⁺
Frequency
Cycle

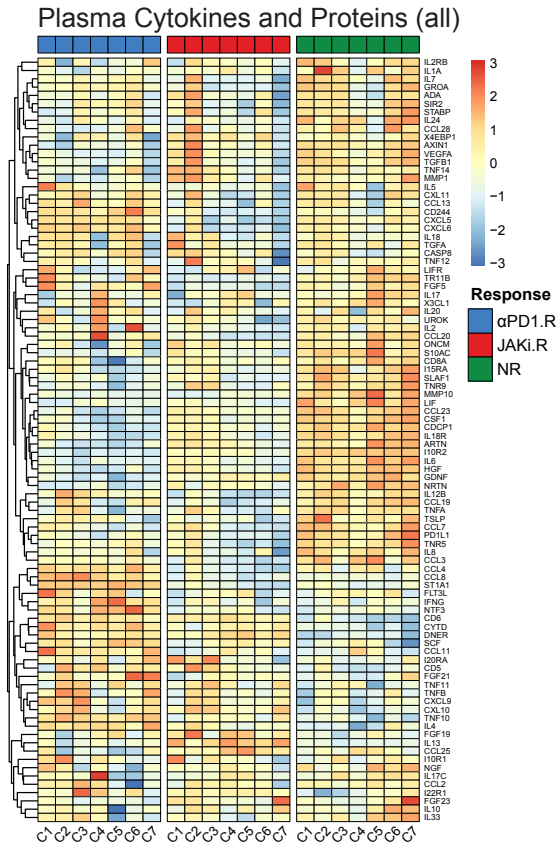

A
CD49d
CX3CR1
CD39
CCR6
PD1
CXCR6
HLADR
CD38
Ki67
ISG15
CD95
CD45RA
CD127
CD27
CCR7
CCR4
CXCR3
CXCR5
4 12 7 1 9 11 10 3 6 2 5 8
Cluster
Scaled MFI
B
PD1+ CXCR5+ CD8 T Cells (Cl. 7)
CD45RA
CXCR3
CD27
CD95
CXCR5
CX3CR1
CD39
PD1
Ki67
C
CD127+ TEFF/MEM-like (Cl. 1+2) and PD1hi TEX-INT-like (Cl. 12) of Ki67+ CD8 T Cells
Cycle 1
Cycle 2-3
Cycle 4 (+JAKi)
Cycle 5 (+JAKi)
Cycle 6
Cycle 7
aPD1.R
JAKi.R
NR
PD1
CD127
D
Ki67+ CD8 T Cell Clusters
CD28
CCR7
CTLA4
CD27
PD1
TCF1
CD127
Ki67
CD38
CD39
CD103
CXCR5
CX3CR1
CD45RA
CD69
TBET
EOMES
TIM3
TOX
Cluster
Scaled MFI
Subset Composition of Ki67+ CD8 T Cells
p=NS
vs. C1
p=0.04
vs. C1
CD127+ Cl.2+7
PD1hi Cl.6
Proportion
C1
C2
C3
C4
C5-6
Cycle
Cluster
PD1hi TEX-INT-like (Cl.6)
CD127+ TEFF/MEM-like (Cl.2+7)
E
CXCR5
Ki67
CD127
CX3CR1
CD38
CD39
PD1
CD45RA


A
CD27
SELL
CCR7
TCF7
CD28
IL7R
SATB1
ID2
ISG15
IFIT1
PDCD1
CXCR3
CD69
GZMK
TNF
BCL6
CTLA4
ENTPD1
MKI67
CXCL13
HAVCR2
OAS1
KLRC1
GZMB
CX3CR1
GZMH
KLRD1
TBX21
CD38
ITGA4
TOX
SLAMF6
TIGIT
IFNG
ZEB2
IL2RB
PRDM1
PRF1
KLRG1
LAG3
EOMES
naive
early.cm
cm.cd127
cm
em
em.cd127
exh.cm
unannot
exh
pre.prog
emra
emra.nk
Scaled Expression
3
2
1
0
−1
−2
−3
BCL6
CCR7
CTLA4
CX3CR1
CXCL13
CXCR5
EOMES
GZMK
HAVCR2
MKI67
PDCD1
TCF7
TIGIT
TOX
Mean Expression
C
CXCR5 and TCF7 in Peripheral CD8 T Cells (Zhang Atlas)
CXCR5 Expression
p<0.001
(vs. any other)
TCF7 Expression
Higher expression
vs. Tm.RPS12: p<0.001
vs. Tm.CD52: p=0.005
vs. Tem.GZMK: p<0.001
vs. Temra.CX3CR1: p<0.001
vs. Tk.TYROBP: p=0.002
Gene Sets
(this study)
Naive
CM
CD127 (IL7R).Tm
EM
CXCR5.Tem
Exh.Prog
Exhaustion
EMRA
Enrichment
Score
CD8.c02.Tm.IL7R
CD8.c03.Tm.RPS12
CD8.c04.Tm.CD52
CD8.c05.Tem.CXCR5
CD8.c06.Tem.GZMK
CD8.c07.Temra.CX3CR1
CD8.c08.Tk.TYROBP
CD8.c17.Tm.NME1
CD8 T Cells in the Peripheral Blood
(Zheng et al., Science 2021)
B
CD8 T Cell Annotation Scores
unannot
emra.nk
naive
emra
em
early.cm
exh
pre.prog
cm.cd127
cm
em.cd127
exh.cm
Filter:
Score ≥ 0.50
Annotation
Score
1.0
0.8
0.6
0.4
0.2
D
CD8 T Cells in PBMCs
Healthy Control
Filtering Gate
(<2% clonotypes)
Log2 Difference in Frequency
from Timepoint 1
−1 / log10(frequency)

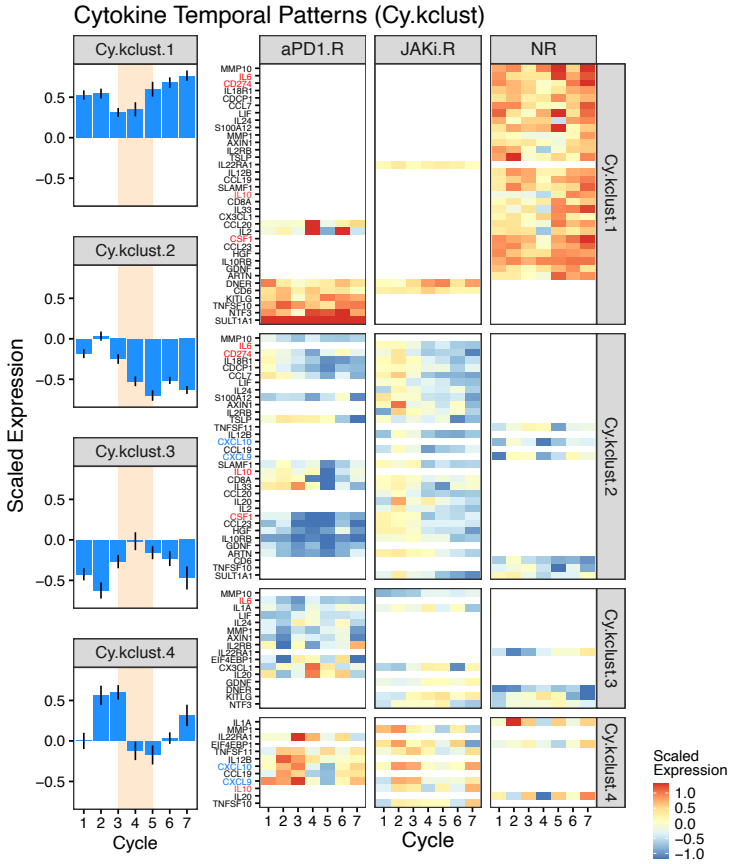

A
CD8 T Cell Trajectory
emra.nk
unannot
emra
exh
pre.prog*
pre.prog
em.cd127
exh.cm
cm
cm.cd127
em
early.cm
naive
0
10
20
30
Pseudotime
B
pTrans Index
emra
emra.nk
exh
pre.prog
cm.cd127
cm
exh.cm
unannot
early.cm
pre.prog*
em
em.cd127
pTrans-index
0.6
0.4
0.2
0.0
C
Subtype Plasticity Score (PS)
early.cm
em
pre.prog*
em.cd127
unannot
exh.cm
cm.cd127
cm
pre.prog
exh
emra.nk
emra
0
500
1000
1500
2000
Plasticity Score (1 / var(pTrans index))
D
KClust Clonotype Expansion Patterns
kclust.1
kclust.2
kclust.3
kclust.4
Avg. Frequency
1.00
0.75
0.50
0.25
0.00
1 2 4 6
Cycle
E
JAKi.R Clonotypes Shared with pre.prog grouped by Kclust
kclust.1
kclust.3
kclust.4
1 2 4 6
pre.prog
kclust.1
kclust.3
kclust.4
ΔPS clono
200
100
0
−100
−200
2 4 6
Cycle
Clono Frequency
−1/log10(clono.freq)
Clone 3
Clone 2
Clone 1
Clone 4
Clone 5
1 2 4 6
Cycle
Clonotype Composition
Cycle 1
Cycle 2
Cycle 4
Cycle 6
Clone 3
Clone 2
Clone 1
Clone 4
Clone 5
cm
cm.cd127
early.cm
em
em.cd127
emra
emra.nk
exh
pre.prog
exh.cm


Tumor and Blood Shared Clonotypes from JAKi.R Patient
Clone 1
Clone 2
Clone 3
Clone 4
Clone 5
Clone 6
Clone 7
Frequency (Tumor)
−1/log10(frequency)
Frequency (Blood)
Plasticity Score (Blood)
Plasticity Score
Clonotype Composition (Blood)
Proportion
Cycle
Cluster
cm
cm.cd127
early.cm
em
emra
emra.nk
exh
pre.prog
exh.cm

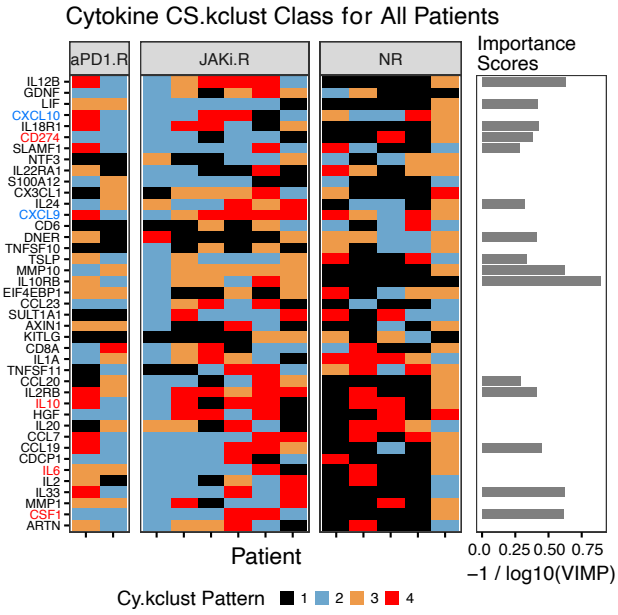
A


Plasma Cytokines and Proteins (all)
Response
αPD1.R
JAKi.R
NR
C1 C2 C3 C4 C5 C6 C7

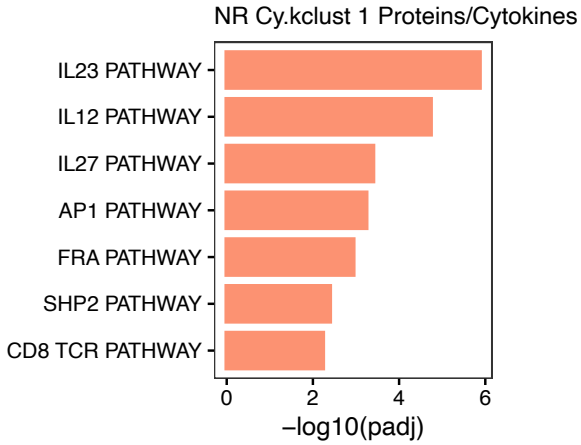
B


Cytokine Temporal Patterns (Cy.kclust)
Cy.kclust.1
Cy.kclust.2
Cy.kclust.3
Cy.kclust.4
aPD1.R
JAKi.R
NR
Scaled Expression
Cycle
Scaled Expression

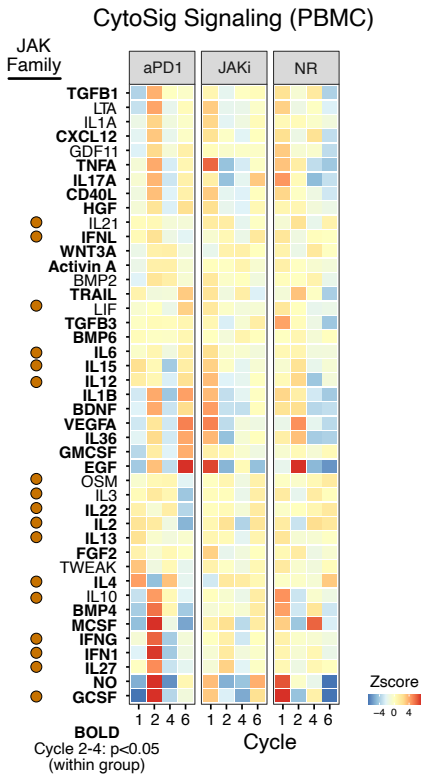
C


Cytokine CS.kclust Class for All Patients
aPD1.R
JAKi.R
NR
Importance Scores
Patient
−1 / log10(VIMP)
Cy.kclust Pattern 1 2 3 4

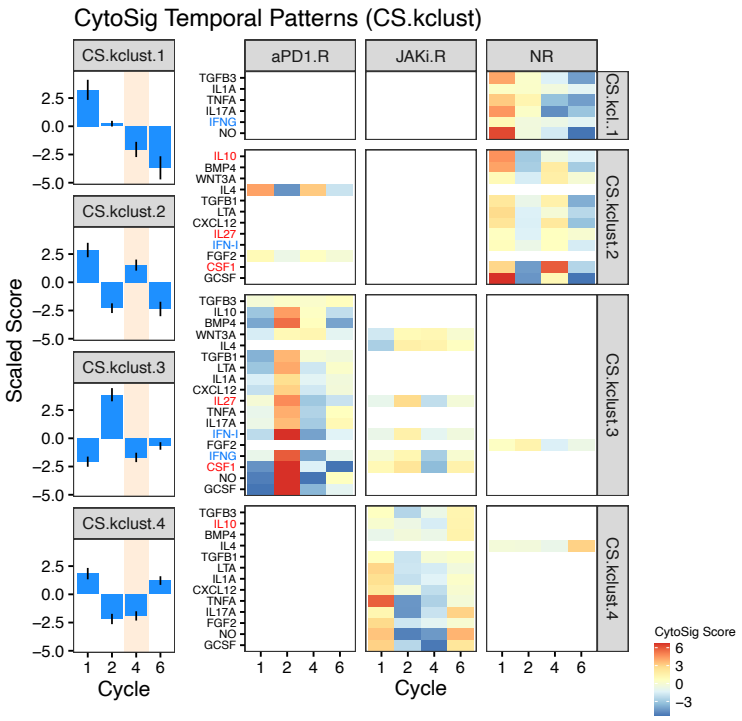
D


NR Cy.kclust 1 Proteins/Cytokines
IL23 PATHWAY
IL12 PATHWAY
IL27 PATHWAY
AP1 PATHWAY
FRA PATHWAY
SHP2 PATHWAY
CD8 TCR PATHWAY
−log10(padj)

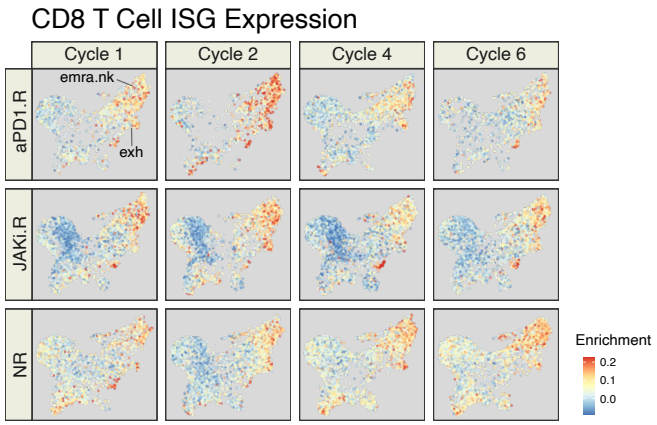
A


CytoSig Signaling (PBMC)
JAK Family
aPD1
JAKi
NR
TGFB1
LTA
IL1A
CXCL12
GDF11
TNFA
IL17A
CD40L
HGF
IL21
IFNL
WNT3A
Activin A
BMP2
TRAIL
LIF
TGFB3
BMP6
IL6
IL15
IL12
IL1B
BDNF
VEGFA
IL36
GMCSF
EGF
OSM
IL3
IL22
IL2
IL13
FGF2
TWEAK
IL4
IL10
BMP4
MCSF
IFNG
IFN1
IL27
NO
GCSF
1 2 4 6
Cycle
Zscore
−4 0 4
BOLD
Cycle 2-4: p<0.05
(within group)

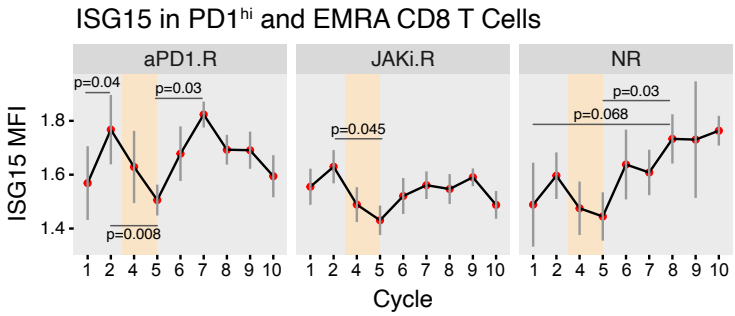
B


CytoSig Temporal Patterns (CS.kclust)
CS.kclust.1
CS.kclust.2
CS.kclust.3
CS.kclust.4
Scaled Score
2.5
0.0
−2.5
−5.0
aPD1.R
JAKi.R
NR
CS.kcl..1
CS.kclust.2
CS.kclust.3
CS.kclust.4
TGFB3
IL1A
TNFA
IL17A
IFNG
NO
IL10
BMP4
WNT3A
IL4
TGFB1
LTA
CXCL12
IL27
IFN-I
FGF2
CSF1
GCSF
1 2 4 6
Cycle
CytoSig Score
6
3
0
−3

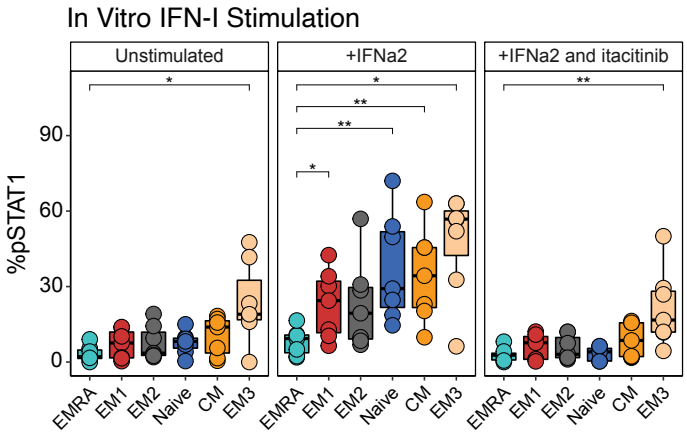
A


CD8 T Cell ISG Expression
Cycle 1
Cycle 2
Cycle 4
Cycle 6
aPD1.R
JAKi.R
NR
emra.nk
exh
Enrichment
0.2
0.1
0.0

B


ISG15 in PD1hi and EMRA CD8 T Cells
aPD1.R
JAKi.R
NR
ISG15 MFI
1.8
1.6
1.4
p=0.04
p=0.03
p=0.008
p=0.045
p=0.03
p=0.068
1 2 4 5 6 7 8 9 10
Cycle

C


In Vitro IFN-I Stimulation
Unstimulated
+IFNa2
+IFNa2 and itacitinib
%pSTAT1
90
60
30
0
EMRA
EM1
EM2
Naive
CM
EM3