Fig S5

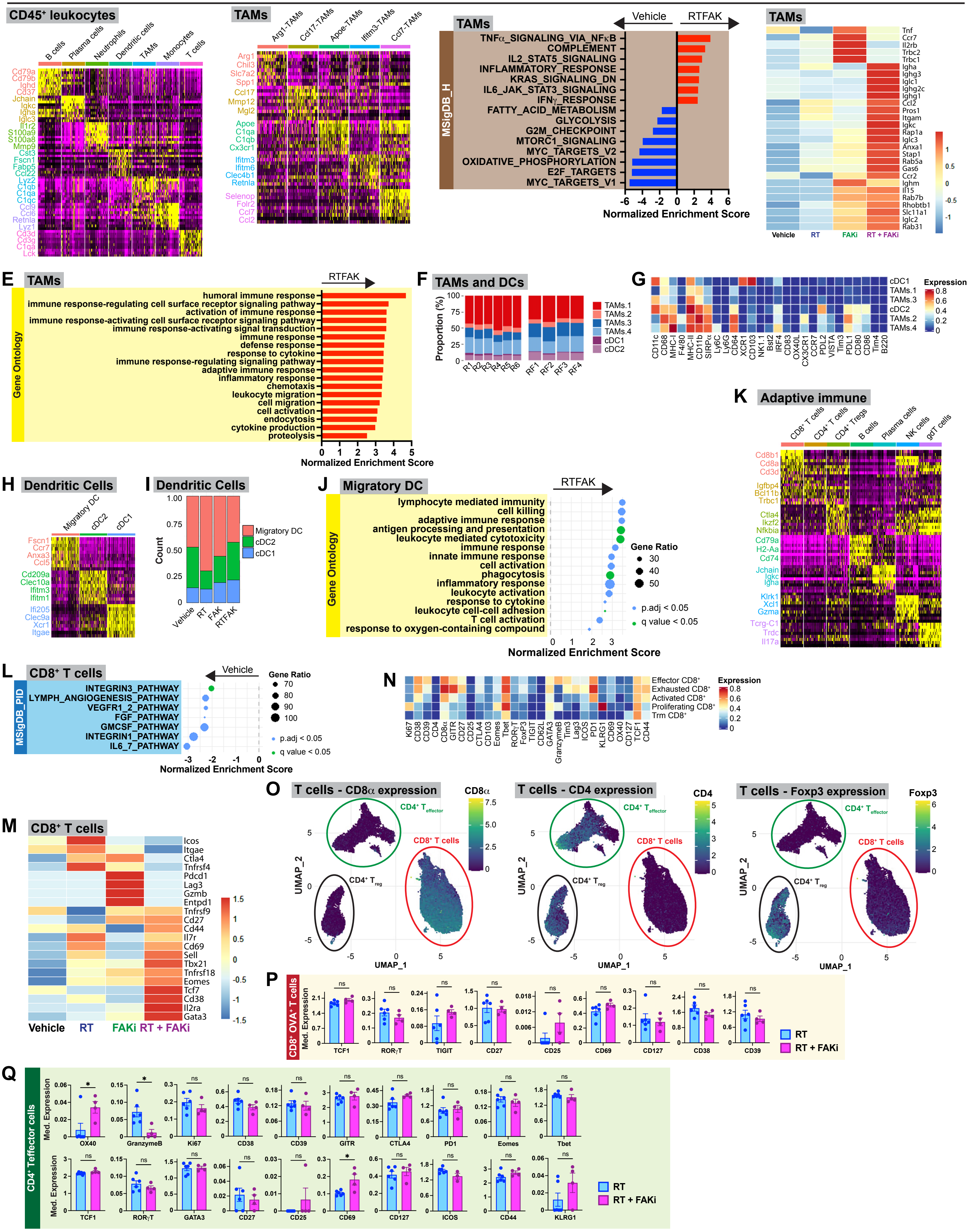
A
CD45⁺ leukocytes
B cells
Plasma cells
Neutrophils
Dendritic cells
TAMs
Monocytes
T cells
Cd79a
Cd79b
Ighd
Cd37
Jchain
Igkc
Igha
Iglc3
Il1r2
S100a9
S100a8
Mmp9
Cst3
Fscn1
Fabp5
Ccl22
Lyz2
C1qb
C1qa
C1qc
Ccl9
Ccl6
Retnla
Lyz1
Cd3d
Cd3g
C1qa
Lck
B
TAMs
Arg1-TAMs
Ccl17-TAMs
Apoe-TAMs
Ifitm3-TAMs
Ccl7-TAMs
Arg1
Chil3
Slc7a2
Spp1
Ccl17
Mmp12
Mgl2
Apoe
C1qa
C1qb
Cx3cr1
Ifitm3
Ifitm6
Clec4b1
Retnla
Selenop
Folr2
Ccl7
Ccl2
C
TAMs
Vehicle
RTFAK
MSigDB_H
TNFα_SIGNALING_VIA_NFκB
COMPLEMENT
IL2_STAT5_SIGNALING
INFLAMMATORY_RESPONSE
KRAS_SIGNALING_DN
IL6_JAK_STAT3_SIGNALING
IFNγ_RESPONSE
FATTY_ACID_METABOLISM
GLYCOLYSIS
G2M_CHECKPOINT
MTORC1_SIGNALING
MYC_TARGETS_V2
OXIDATIVE_PHOSPHORYLATION
E2F_TARGETS
MYC_TARGETS_V1
-6 -4 -2 0 2 4 6
Normalized Enrichment Score
D
TAMs
Tnf
Ccr7
Il2rb
Trbc2
Trbc1
Igha
Ighg3
Iglc1
Ighg2c
Ighg1
Ccl2
Pros1
Itgam
Igkc
Rap1a
Iglc3
Anxa1
Stap1
Rab5a
Gas6
Ccr2
Ighm
Il15
Rab7b
Rhobtb1
Slc11a1
Iglc2
Rab31
1
0.5
0
-0.5
-1
Vehicle
RT
FAKi
RT + FAKi
E
TAMs
RTFAK
Gene Ontology
humoral immune response
immune response-regulating cell surface receptor signaling pathway
activation of immune response
immune response-activating cell surface receptor signaling pathway
immune response-activating signal transduction
immune response
defense response
response to cytokine
immune response-regulating signaling pathway
adaptive immune response
inflammatory response
chemotaxis
leukocyte migration
cell migration
cell activation
endocytosis
cytokine production
proteolysis
0 1 2 3 4 5
Normalized Enrichment Score
F
TAMs and DCs
Proportion (%)
100
75
50
25
0
R1
R2
R3
R4
R5
R6
RF1
RF2
RF3
RF4
TAMs.1
TAMs.2
TAMs.3
TAMs.4
cDC1
cDC2
G
cDC1
TAMs.1
TAMs.3
cDC2
TAMs.2
TAMs.4
Expression
0.8
0.6
0.4
0.2
0
CD11c
CD68
MHC-I
F4/80
MHC-II
CD11b
SIRPα
Ly6C
Ly6G
CD64
XCR1
CD103
NK1.1
Bst2
IRF4
CD83
OX40L
CX3CR1
CCR7
PDL2
VISTA
Tim3
PDL1
CD80
CD86
Tim4
B220
H
Dendritic Cells
Migratory DC
cDC2
cDC1
Fscn1
Ccr7
Anxa3
Ccl5
Cd209a
Clec10a
Ifitm3
Ifitm1
Ifi205
Clec9a
Xcr1
Itgae
I
Dendritic Cells
Count
1.00
0.75
0.50
0.25
0
Vehicle
RT
FAK
RTFAK
Migratory DC
cDC2
cDC1
J
Migratory DC
RTFAK
Gene Ontology
lymphocyte mediated immunity
cell killing
adaptive immune response
antigen processing and presentation
leukocyte mediated cytotoxicity
immune response
innate immune response
cell activation
phagocytosis
inflammatory response
leukocyte activation
response to cytokine
leukocyte cell-cell adhesion
T cell activation
response to oxygen-containing compound
Gene Ratio
30
40
50
p.adj < 0.05
q value < 0.05
0 1 2 3
Normalized Enrichment Score
K
Adaptive immune
CD8⁺ T cells
CD4⁺ T cells
CD4⁺ Tregs
B cells
Plasma cells
NK cells
gdT cells
Cd8b1
Cd8a
Cd3d
Igfbp4
Bcl11b
Trbc1
Ctla4
Ikzf2
Nfkbia
Cd79a
H2-Aa
Cd74
Jchain
Igkc
Igha
Klrk1
Xcl1
Gzma
Tcrg-C1
Trdc
Il17a
L
CD8⁺ T cells
Vehicle
MSigDB_PID
INTEGRIN3_PATHWAY
LYMPH_ANGIOGENESIS_PATHWAY
VEGFR1_2_PATHWAY
FGF_PATHWAY
GMCSF_PATHWAY
INTEGRIN1_PATHWAY
IL6_7_PATHWAY
Gene Ratio
70
80
90
100
p.adj < 0.05
q value < 0.05
-3 -2 -1 0
Normalized Enrichment Score
M
CD8⁺ T cells
Icos
Itgae
Ctla4
Tnfrsf4
Pdcd1
Lag3
Gzmb
Entpd1
Tnfrsf9
Cd27
Cd44
Il7r
Cd69
Sell
Tbx21
Tnfrsf18
Eomes
Tcf7
Cd38
Il2ra
Gata3
1.5
1
0.5
0
-0.5
-1
-1.5
Vehicle
RT
FAKi
RT + FAKi
N
Effector CD8⁺
Exhausted CD8⁺
Activated CD8⁺
Proliferating CD8⁺
Trm CD8⁺
Expression
0.8
0.6
0.4
0.2
0
Ki67
CD38
CD4
CD8α
GITR
CD27
CD25
CTLA4
CD103
Eomes
Tbet
RORγT
FoxP3
TIGIT
CD62L
GATA3
GranzymeB
Tim3
Lag3
ICOS
PD1
KLRG1
CD69
OX40
CD127
TCF1
CD44
O
T cells - CD8α expression
CD8α
7.5
5.0
2.5
0
CD4⁺ $T_{effector}$
CD8⁺ T cells
CD4⁺ T_{reg}
UMAP_2
UMAP_1
5
0
-5
-5 0 5
T cells - CD4 expression
CD4
5
4
3
2
1
0
CD4⁺ $T_{effector}$
CD8⁺ T cells
CD4⁺ T_{reg}
UMAP_2
UMAP_1
T cells - Foxp3 expression
Foxp3
6
4
2
0
CD4⁺ $T_{effector}$
CD8⁺ T cells
CD4⁺ T_{reg}
UMAP_2
UMAP_1
P
CD8⁺ OVA⁺ T cells
Med. Expression
ns
TCF1
RORγT
TIGIT
CD27
CD25
CD69
CD127
CD38
CD39
RT
RT + FAKi
Q
CD4⁺ Teffector cells
Med. Expression
*
ns
OX40
GranzymeB
Ki67
CD38
CD39
GITR
CTLA4
PD1
Eomes
Tbet
TCF1
RORγT
GATA3
CD27
CD25
CD69
CD127
ICOS
CD44
KLRG1
RT
RT + FAKi

Figure S5: Tumor-infiltrating immune cells from RT+FAKi treated mice have better anti-tumor signatures

(A) Heatmap displaying top 10 genes of different immune cells in **Fig. 5A**. **(B)** Heatmap displaying top 10 genes of TAMs in **Fig. 5B**. **(C)** Bar graphs displaying GSEA results from MSigDB_Hallmark database comparing TAMs in **Fig. 5B**. All graphs display comparisons of vehicle to RT+FAKi-treated mice. All pathways were filtered with p.adj value < 0.05. **(D)** Heatmap displaying relative expression level of genes related to antigen processing and presentation taken from MSigDB_Hallmark database in TAMs from **Fig. 5B**. **(E)** Bar graphs displaying GSEA results from GO database comparing TAMs in **Fig. 5B**. All graphs display comparisons of vehicle to RT+FAKi-treated mice. All pathways were filtered with p.adj value < 0.05. **(F)** Count ratio from CyTOF analysis of the 4 TAM clusters and 2 cDC clusters from **Fig. 5F**. R1-R6 denotes RT-treated samples. RF1-RF4 denotes RT+FAKi-treated samples. **(G)** Heatmap displaying expression level of markers used in CyTOF analysis for cell clustering in **Fig. 5F**. **(H)** Heatmap displaying top 10 genes of different cDCs in **Fig. 5H**. **(I)** Count ratio from scRNA-seq analysis of the 3 cDC clusters from **Fig. 5H**. **(J)** Dot plot displaying GSEA results from GO database comparing migratory DCs clusters. All graphs display comparisons of vehicle to RT+FAKi-treated mice. All pathways were filtered with p.adj value < 0.05 or q value < 0.05. **(K)** Heatmap displaying top 10 genes of different adaptive immune cells in **Fig. 5I**. **(L)** Dot plot displaying GSEA analysis from MSigDB_PID database taken from DEGs of $CD8^+$ T cells in **Fig. 5I**. All graphs display comparisons of vehicle to RT+FAKi-treated mice. All pathways were filtered with p.adj value < 0.05 or q value < 0.05. **(M)** Heatmap displaying relative expression level of genes related to effector/activation markers from $CD8^+$ T cells in **Fig. 5I**. **(N)** Heatmap displaying expression level of markers used in CyTOF analysis for cell clustering in **Fig. 5O**. **(O)** CD8, CD4, and Foxp3 expression levels projected onto UMAP plots in **Fig. 5N**. **(P-Q)** Analysis of median expression level of various markers on $CD8^+$ OVA-$Dextramer^+$ T cells (P) and $CD4^+$ T effector cells (Q) in **Fig. 5N**. n = at least 4 groups of pooled mice/group. All graphs depict mean +/- SEM. "*" denotes $p < 0.05$ by two-tailed t-test or one-way ANOVA as appropriate. "ns" denotes not significant.