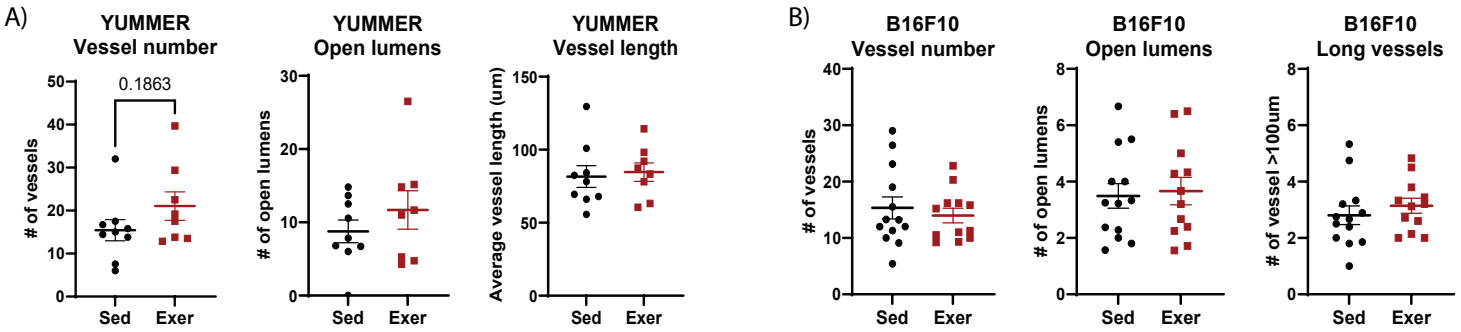
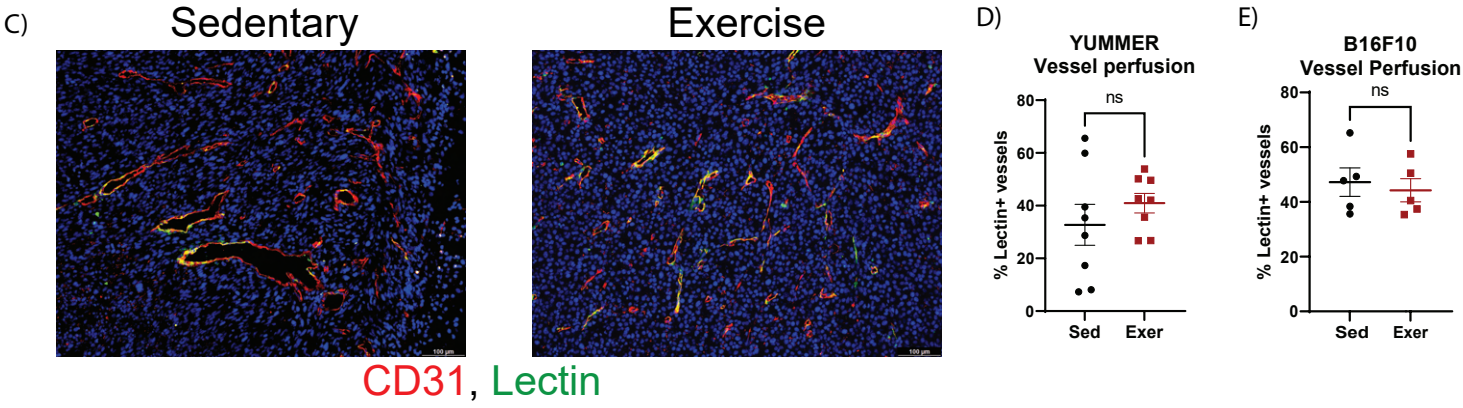
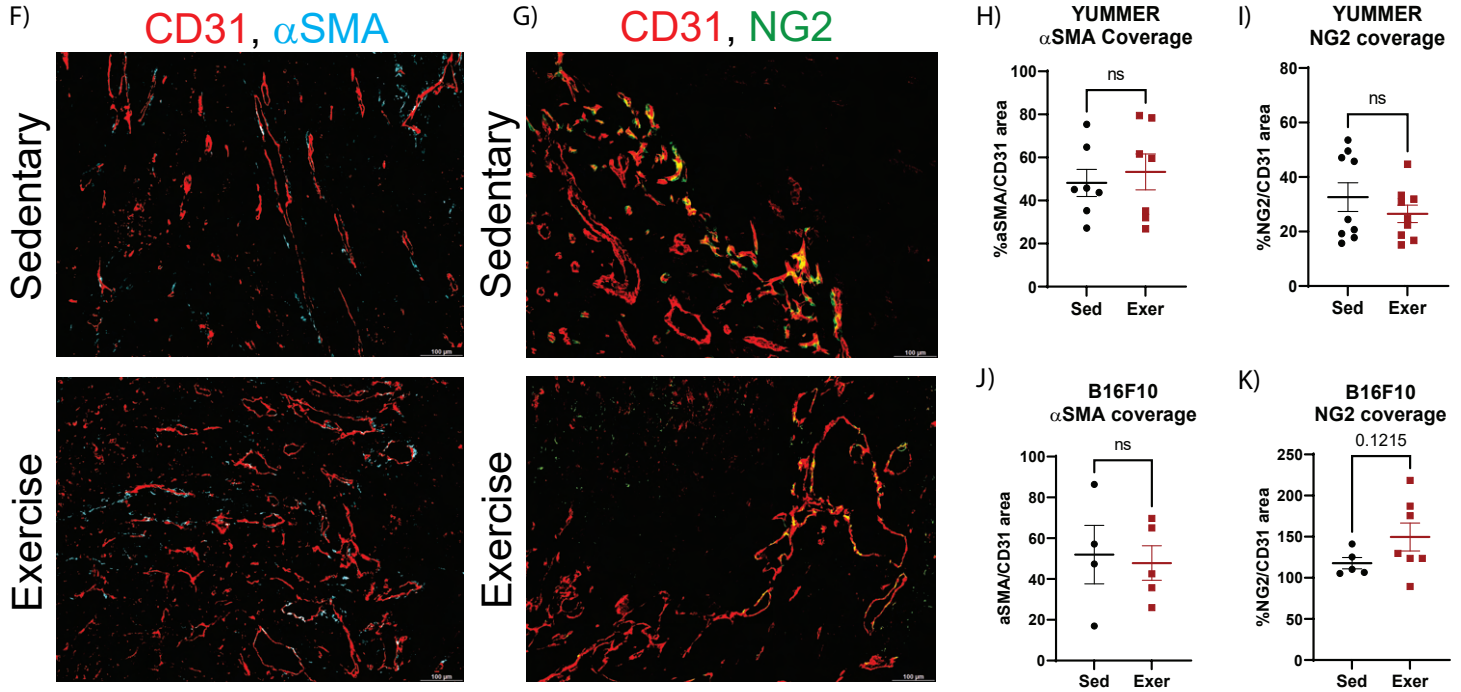
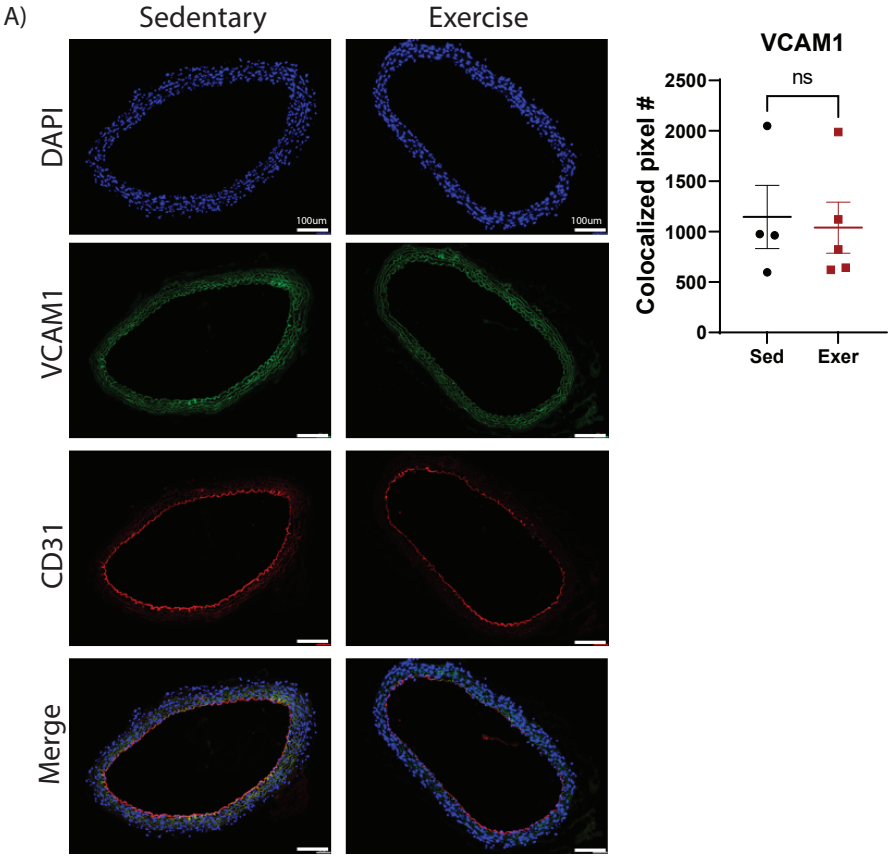

A)
YUMMER
Vessel number
0.1863
# of vessels
Sed
Exer
YUMMER
Open lumens
# of open lumens
YUMMER
Vessel length
Average vessel length (um)
B)
B16F10
Vessel number
B16F10
Open lumens
B16F10
Long vessels
# of vessel >100um


C)
Sedentary
Exercise
CD31, Lectin
D)
YUMMER
Vessel perfusion
ns
% Lectin+ vessels
E)
B16F10
Vessel Perfusion

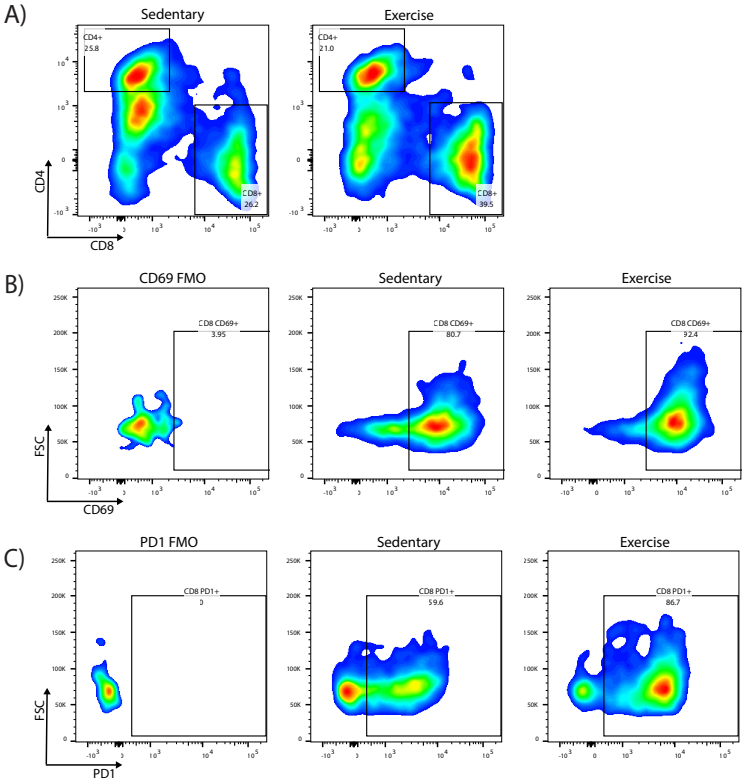

F)
CD31, αSMA
Sedentary
Exercise
G)
CD31, NG2
H)
YUMMER
αSMA Coverage
%aSMA/CD31 area
I)
YUMMER
NG2 coverage
%NG2/CD31 area
J)
B16F10
αSMA coverage
aSMA/CD31 area
K)
B16F10
NG2 coverage
0.1215

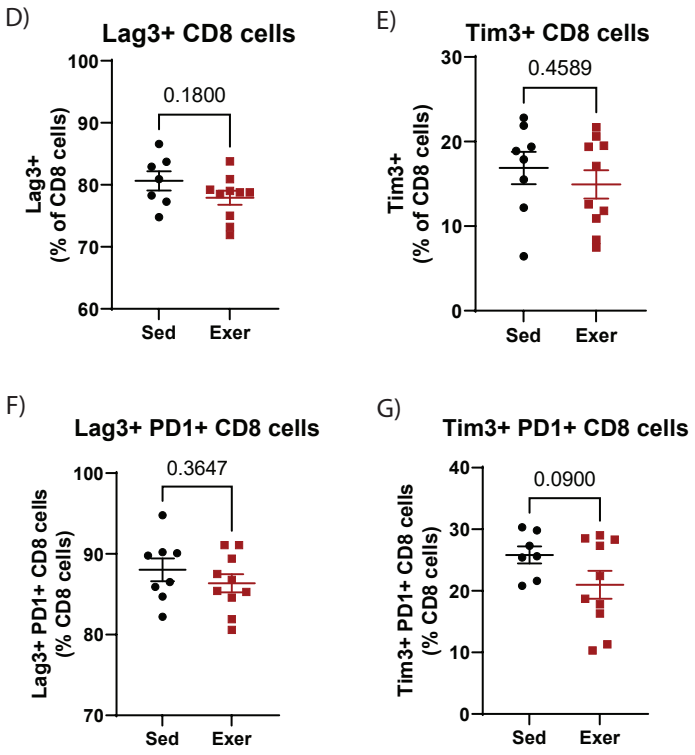
Supplementary Figure S1. Exercise does not change perfusion or mural cell coverage in melanoma tumor vasculature. A) YUMMER or B) B16F10 vessel structure was evaluated using CD31 immunofluorescent staining. A,B) Vessel number, number of open lumens, and vessel length were unchanged in A) YUMMER tumors and B) B16F10 tumors. C) Representative immunofluorescent images of CD31 (red), Texas-red conjugated lectin (green), and DAPI (blue) in sedentary or exercise YUMMER tumor samples. D,E) Quantification of percentage of lectin$^+$ vessels in D) YUMMER and E) B16F10 tumor. F,G) Representative immunofluorescent images of F) CD31 (red) and αSMA (cyan), or G) CD31 (red) and NG2 (green) in sedentary or exercise YUMMER tumor samples. H,I) Quantification of H) αSMA area: CD31 area ratio, or I) NG2 area: CD31 area ratio in YUMMER tumors. J,K) Quantification of J) αSMA area: CD31 area ratio, or K) Quantification of NG2 area: CD31 area ratio in B16F10 tumors. Graphs are displayed with mean +/- SEM. T-test results are represented on graphs according to the following: ns= $p > 0.05$, *$p \leq 0.05$, **$p \leq 0.01$, ***$p \leq 0.001$, ****$p \leq 0.0001$. Scale bar = 100μm.


A)
Sedentary
Exercise
DAPI
VCAM1
CD31
Merge
100um
100um
VCAM1
ns
Colocalized pixel #
2500
2000
1500
1000
500
0
Sed
Exer

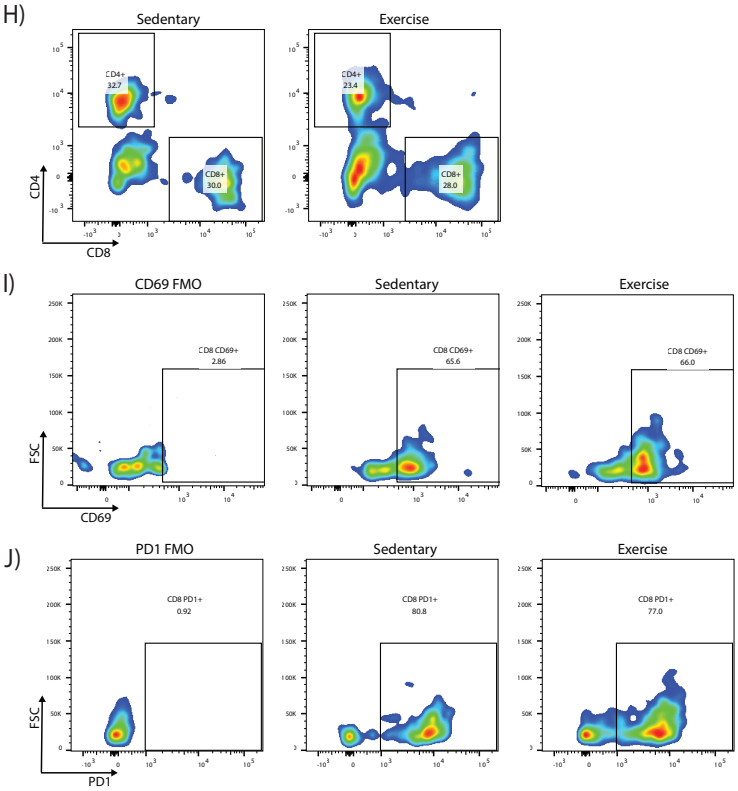
Supplementary Figure S2. Exercise does not alter VCAM1 expression in mouse aortas. A) Representative immunofluorescent images of aortas from sedentary or exercised C57Bl/6 mice stained for VCAM1 (green), CD31 (red), or DAPI (blue). VCAM1 colocalization with CD31 was quantified, scale bar: 100μm. Graphs are displayed with mean +/- SEM. T-test results are represented on graphs according to the following: ns= $p > 0.05$, *$p \leq 0.05$, **$p \leq 0.01$, ***$p \leq 0.001$, ****$p \leq 0.0001$.

YUMMER tumor

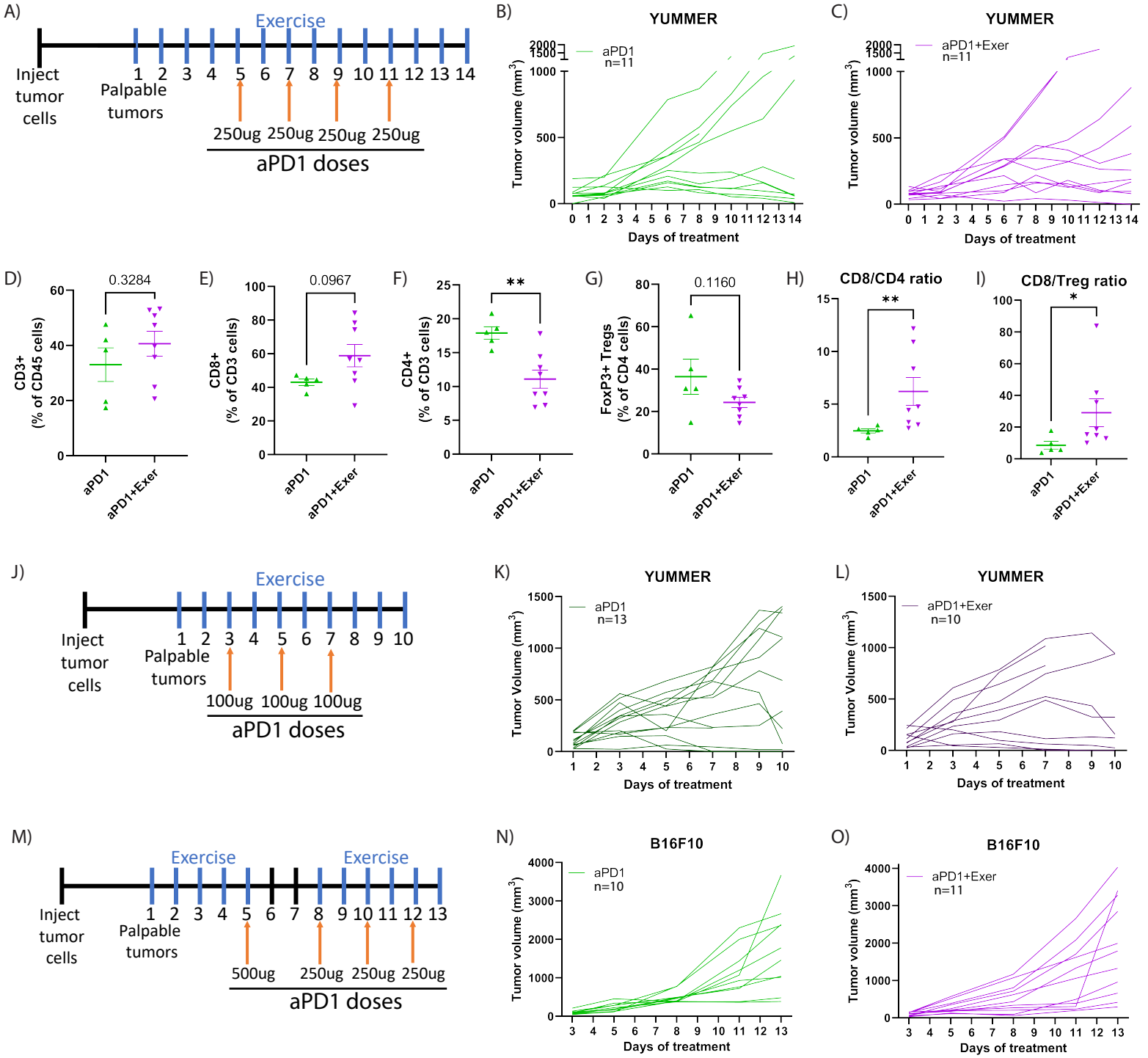




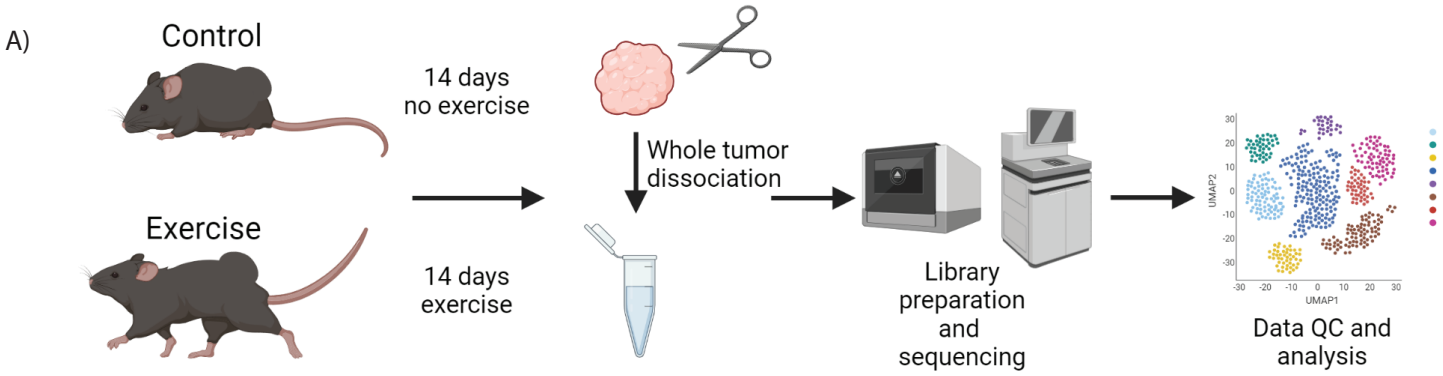
B16F10 tumor

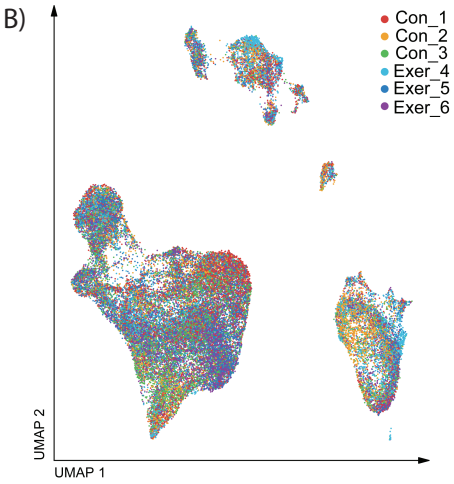


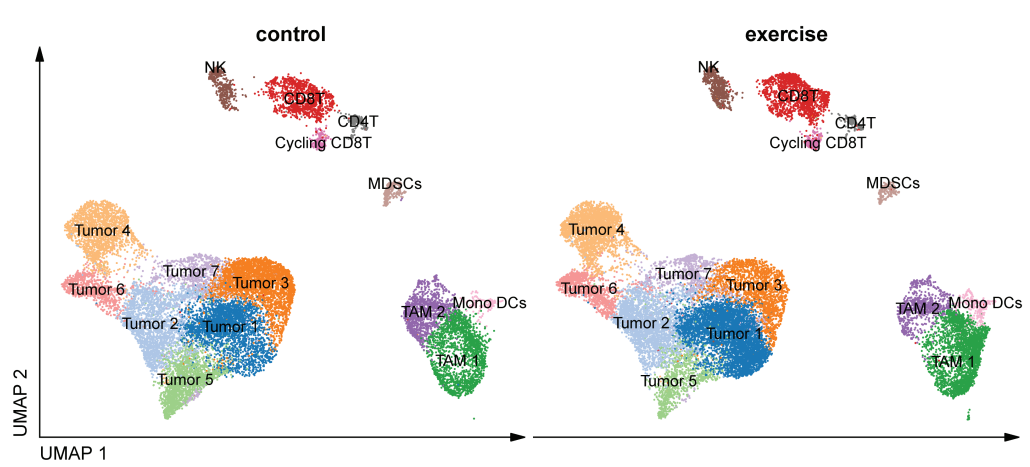
Supplementary Figure S3. T cell flow cytometry in melanoma tumors. Flow cytometry gating in YUMMER tumors of: A) CD8 versus CD4, B) forward scatter (FSC) versus CD69, C) FSC versus PD1. Flow cytometry quantification of percent of D) $Lag3^+$ $CD8^+$ cells relative to $CD8^+$ T cells, E) $Tim3^+$ $CD8^+$ cells relative to $CD8^+$ T cells, F) $Lag3^+$ $PD1^+$ $CD8^+$ cells relative to $CD8^+$ T cells, G) $Tim3^+$ $PD1^+$ $CD8^+$ cells relative to $CD8^+$ T cells. Graphs are displayed with mean +/- SEM. T-test results are reported on graphs. Flow cytometry gating in B16F10 tumors of: H) CD8 versus CD4, I) FSC versus CD69, J) FSC versus PD1.

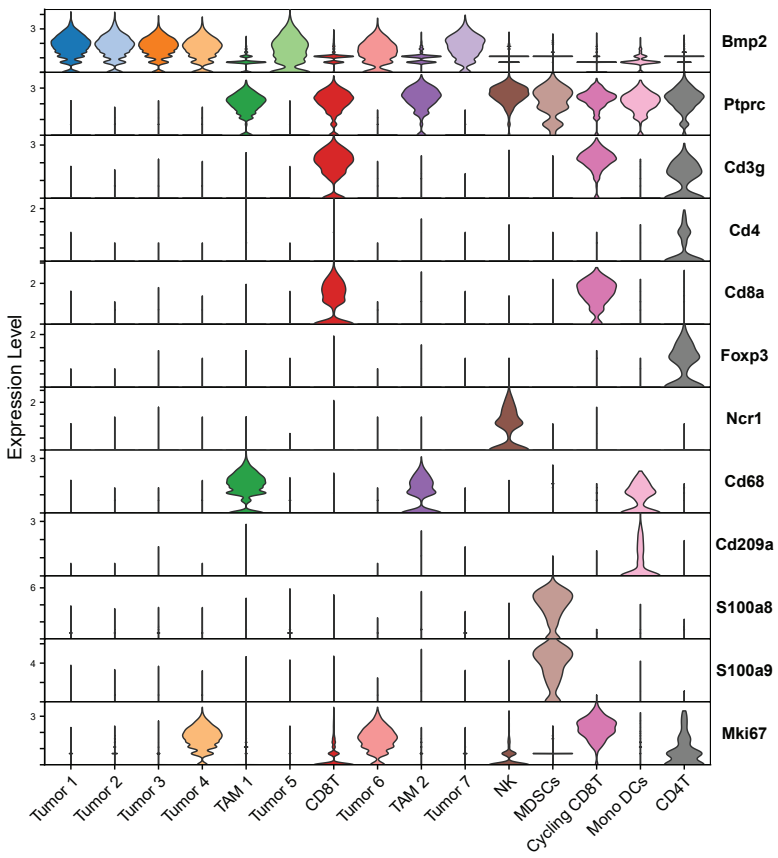

A)
Exercise
Inject tumor cells
1 2 3 4 5 6 7 8 9 10 11 12 13 14
Palpable tumors
250ug 250ug 250ug 250ug
aPD1 doses
B)
YUMMER
aPD1 n=11
Tumor volume (mm3)
Days of treatment
C)
YUMMER
aPD1+Exer n=11
Tumor volume (mm3)
Days of treatment
D)
0.3284
CD3+ (% of CD45 cells)
aPD1
aPD1+Exer
E)
0.0967
CD8+ (% of CD3 cells)
F)
**
CD4+ (% of CD3 cells)
G)
0.1160
FoxP3+ Tregs (% of CD4 cells)
H)
CD8/CD4 ratio
**
I)
CD8/Treg ratio
*
J)
Exercise
Inject tumor cells
1 2 3 4 5 6 7 8 9 10
Palpable tumors
100ug 100ug 100ug
aPD1 doses
K)
YUMMER
aPD1 n=13
Tumor Volume (mm3)
Days of treatment
L)
YUMMER
aPD1+Exer n=10
Tumor Volume (mm3)
Days of treatment
M)
Exercise
Exercise
Inject tumor cells
1 2 3 4 5 6 7 8 9 10 11 12 13
Palpable tumors
500ug 250ug 250ug 250ug
aPD1 doses
N)
B16F10
aPD1 n=10
Tumor volume (mm3)
Days of treatment
O)
B16F10
aPD1+Exer n=11
Tumor volume (mm3)
Days of treatment

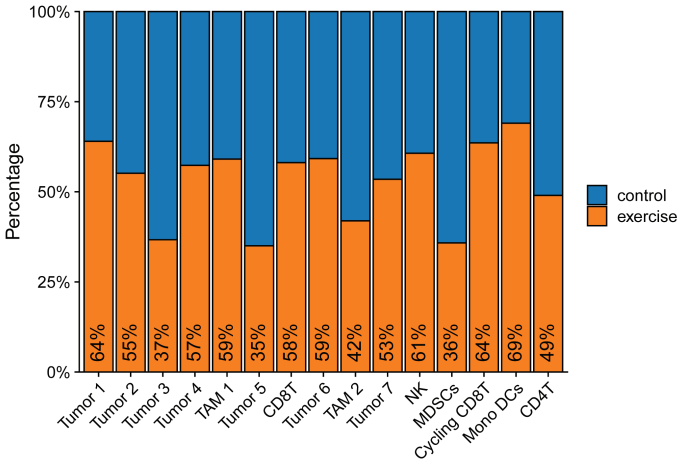
Supplementary Figure S4. Anti-PD1 treatment with aerobic exercise in melanoma models. A) Experimental timeline of anti-PD1 and exercise treatment in mice injected with YUMMER tumors. Tumor growth was monitored over time with calipers in B) anti-PD1 treated mice (green) and C) anti-PD1 and exercise (aPD1+Exer, purple) treated mice. Each line represents one mouse. Lines ending prior to experiment completion are mice that were euthanized due to tumor ulceration. Flow cytometry quantifications of YUMMER tumors to identify the percent of D) $CD3^+$ T cells relative to $CD45^+$ cells, E) $CD8^+$ T cells relative to $CD3^+$ cells, F) $CD4^+$ T cells relative to $CD3^+$ cells, G) $FoxP3^+$ T regs relative to $CD4^+$ cells, H) ratio of CD8/CD4 T cells, and I) ratio of CD8/Treg cells. Graphs are displayed with mean +/- SEM. T-test results are represented on graphs according to the following: ns= $p > 0.05$, *$p \leq 0.05$, **$p \leq 0.01$, ***$p \leq 0.001$, ****$p \leq 0.0001$. J) Second experimental timeline of anti-PD1 and exercise treatment in mice injected with YUMMER tumors. Tumor growth was monitored over time with calipers in K) anti-PD1 treated mice (dark green) and L) anti-PD1 and exercise (aPD1+Exer, dark purple) treated mice. M) Experimental timeline of anti-PD1 and exercise treatment in mice injected with B16F10 tumors. Tumor growth was monitored over time with calipers in N) anti-PD1 treated mice (green) and O) anti-PD1 and exercise (aPD1+Exer, purple) treated mice.


A)
Control
14 days
no exercise
Exercise
14 days
exercise
Whole tumor
dissociation
Library
preparation
and
sequencing
Data QC and
analysis

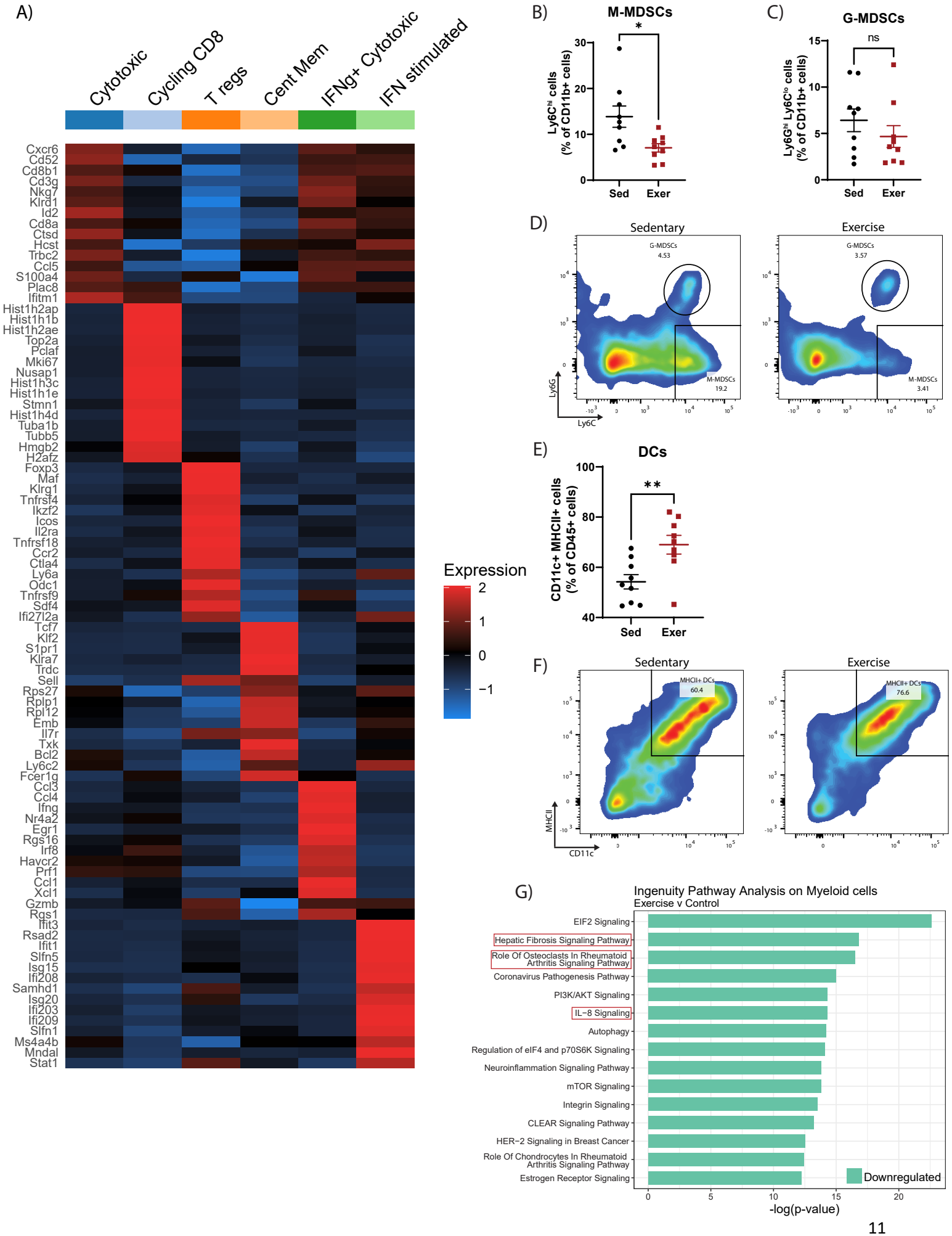

B)
Con_1
Con_2
Con_3
Exer_4
Exer_5
Exer_6
UMAP 2
UMAP 1


C)
control
exercise
NK
CD8T
CD4T
Cycling CD8T
MDSCs
Tumor 4
Tumor 7
Tumor 6
Tumor 3
Tumor 2
Tumor 1
Tumor 5
TAM 2
Mono DCs
TAM 1
UMAP 2
UMAP 1

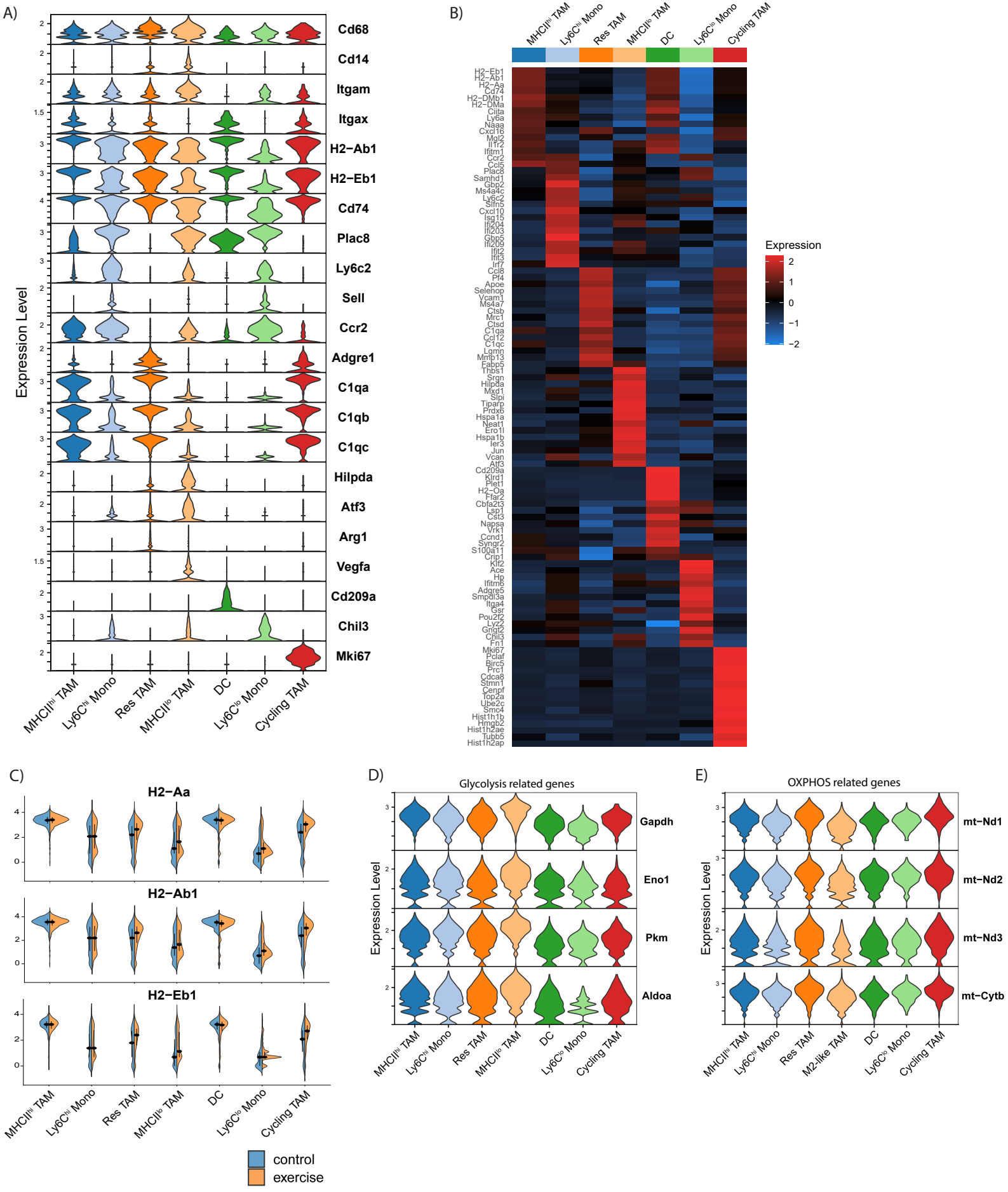

D)
Expression Level
Bmp2
Ptprc
Cd3g
Cd4
Cd8a
Foxp3
Ncr1
Cd68
Cd209a
S100a8
S100a9
Mki67
Tumor 1
Tumor 2
Tumor 3
Tumor 4
TAM 1
Tumor 5
CD8T
Tumor 6
TAM 2
Tumor 7
NK
MDSCs
Cycling CD8T
Mono DCs
CD4T


E)
Percentage
100%
75%
50%
25%
0%
64%
55%
37%
57%
59%
35%
58%
59%
42%
53%
61%
36%
64%
69%
49%
Tumor 1
Tumor 2
Tumor 3
Tumor 4
TAM 1
Tumor 5
CD8T
Tumor 6
TAM 2
Tumor 7
NK
MDSCs
Cycling CD8T
Mono DCs
CD4T
control
exercise

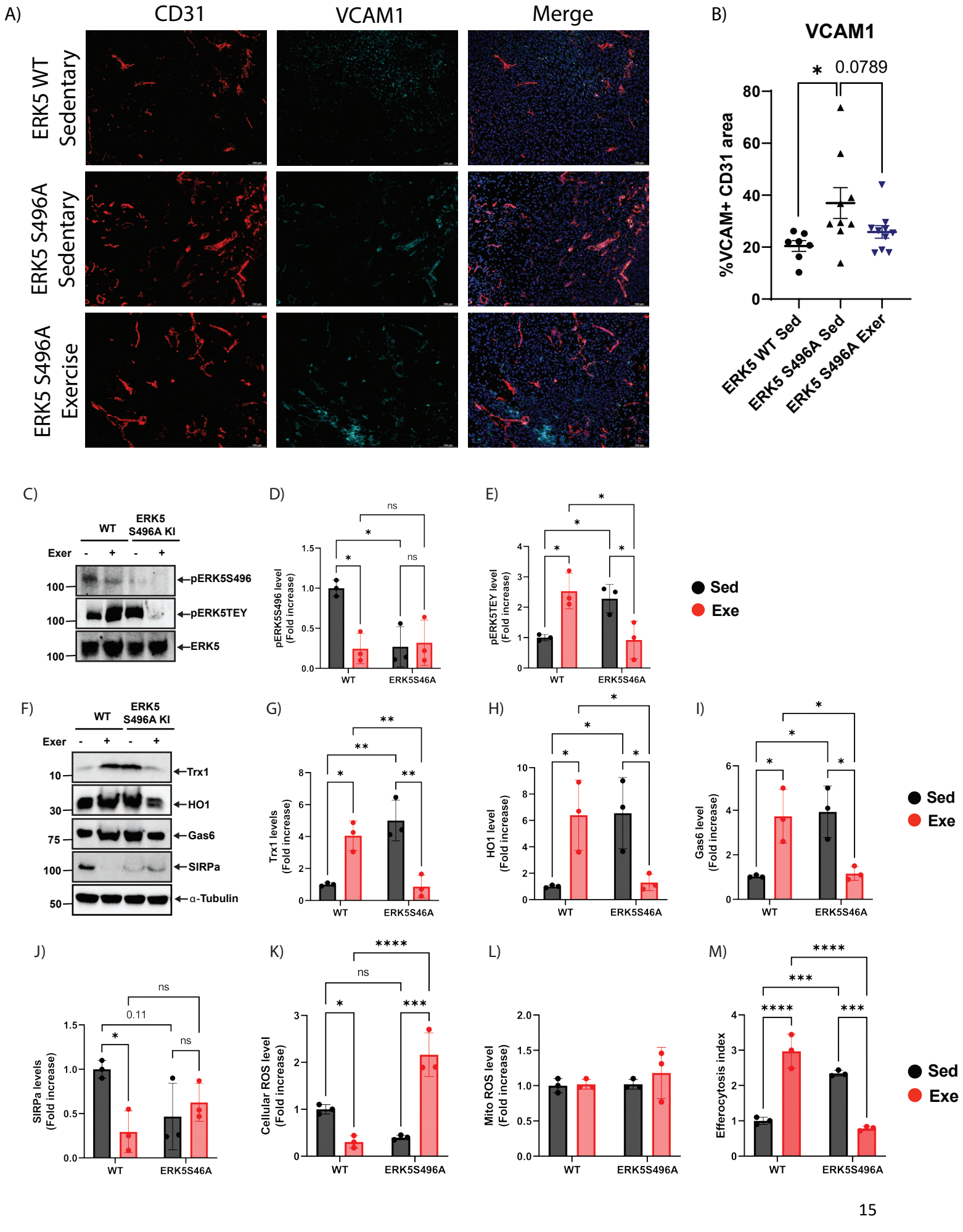
Supplementary Figure S5. Single cell RNA sequencing on YUMMER tumors from sedentary and exercised mice. A) Schematic of scRNA-seq workflow. B) UMAP plot of three control and three exercise tumor samples colored by sample name. C) UMAP plot of all cells from control (left) or exercise (right) YUMMER tumors. D) Stacked violin plot of cell marker expression across clusters. E) Proportions of each cluster from control (blue) samples versus exercised (orange) samples.


A)
Cytotoxic
Cycling CD8
T regs
Cent Mem
IFNg+ Cytotoxic
IFN stimulated
Cxcr6
Cd52
Cd8b1
Cd3g
Nkg7
Klrd1
Id2
Cd8a
Ctsd
Hcst
Trbc2
Ccl5
S100a4
Plac8
Ifitm1
Hist1h2ap
Hist1h1b
Hist1h2ae
Top2a
Pclaf
Mki67
Nusap1
Hist1h3c
Hist1h1e
Stmn1
Hist1h4d
Tuba1b
Tubb5
Hmgb2
H2afz
Foxp3
Maf
Klrg1
Tnfrsf4
Ikzf2
Icos
Il2ra
Tnfrsf18
Ccr2
Ctla4
Ly6a
Odc1
Tnfrsf9
Sdf4
Ifi27l2a
Tcf7
Klf2
S1pr1
Klra7
Trdc
Sell
Rps27
Rplp1
Rpl12
Emb
Il7r
Txk
Bcl2
Ly6c2
Fcer1g
Ccl3
Ccl4
Ifng
Nr4a2
Egr1
Rgs16
Irf8
Havcr2
Prf1
Ccl1
Xcl1
Gzmb
Rgs1
Ifit3
Rsad2
Ifit1
Slfn5
Isg15
Ifi208
Samhd1
Isg20
Ifi203
Ifi209
Slfn1
Ms4a4b
Mndal
Stat1
Expression
2
1
0
−1
B)
M-MDSCs
*
Ly6C^hi cells (% of CD11b+ cells)
30
20
10
0
Sed
Exer
C)
G-MDSCs
ns
Ly6G^hi Ly6C^lo cells (% of CD11b+ cells)
15
10
5
0
Sed
Exer
D)
Sedentary
Exercise
G-MDSCs
4.53
M-MDSCs
19.2
G-MDSCs
3.57
M-MDSCs
3.41
Ly6G
Ly6C
E)
DCs
**
CD11c+ MHCII+ cells (% of CD45+ cells)
100
80
60
40
Sed
Exer
F)
Sedentary
Exercise
MHCII+ DCs
60.4
MHCII+ DCs
76.6
MHCII
CD11c
G)
Ingenuity Pathway Analysis on Myeloid cells
Exercise v Control
EIF2 Signaling
Hepatic Fibrosis Signaling Pathway
Role Of Osteoclasts In Rheumatoid Arthritis Signaling Pathway
Coronavirus Pathogenesis Pathway
PI3K/AKT Signaling
IL−8 Signaling
Autophagy
Regulation of eIF4 and p70S6K Signaling
Neuroinflammation Signaling Pathway
mTOR Signaling
Integrin Signaling
CLEAR Signaling Pathway
HER−2 Signaling in Breast Cancer
Role Of Chondrocytes In Rheumatoid Arthritis Signaling Pathway
Estrogen Receptor Signaling
Downregulated
0
5
10
15
20
-log(p-value)

Supplementary Figure S6. Immune cell populations in sedentary or exercise treated YUMMER tumors. A) Heatmap of top 15 differentially expressed genes in each T cell cluster. Flow cytometry quantification of percent of B) $Ly6C^{hi}$ M-MDSCs relative to $CD11b^{+}$ cells, and C) $Ly6G^{hi}Ly6C^{lo}$ G-MDSCs relative to $CD11b^{+}$ cells. D) Flow cytometry gating of MDSC populations in sedentary and exercised samples. E) Percent of $CD11c^{+}$ $MHCII^{+}$ DCs relative to $CD45^{+}$ cells. F) Flow cytometry gating of DCs in sedentary and exercised samples. Graphs are displayed with mean +/- SEM. T-test results are represented on graphs according to the following: ns= $p > 0.05$, *$p \leq 0.05$, **$p \leq 0.01$, ***$p \leq 0.001$, ****$p \leq 0.0001$. G) Top 15 pathways regulated by exercise in myeloid cells performed using Ingenuity Pathway Analysis (IPA). Pathways highlighted are associated with inflammation.


A)
Expression Level
Cd68
Cd14
Itgam
Itgax
H2−Ab1
H2−Eb1
Cd74
Plac8
Ly6c2
Sell
Ccr2
Adgre1
C1qa
C1qb
C1qc
Hilpda
Atf3
Arg1
Vegfa
Cd209a
Chil3
Mki67
MHCIIhi TAM
Ly6Chi Mono
Res TAM
MHCIIlo TAM
DC
Ly6Clo Mono
Cycling TAM
B)
Expression
C)
H2−Aa
H2−Ab1
H2−Eb1
control
exercise
D)
Glycolysis related genes
Gapdh
Eno1
Pkm
Aldoa
E)
OXPHOS related genes
mt−Nd1
mt−Nd2
mt−Nd3
mt−Cytb
M2−like TAM

Supplementary Figure S7. scRNA-seq Monocyte/ Macrophage/ DC populations in YUMMER tumors. A) Stacked violin plot of myeloid cell gene expression across clusters colored by cluster. B) Heatmap of top 15 differentially expressed genes in each Monocyte/ Macrophage/ DC cluster. Violin plots of C) glycolysis and D) OXPHOS-related gene expression across clusters colored by cluster. C) Violin plots of MHCII genes across clusters in control (blue) and exercise (orange) samples. Black point represents median and whiskers represent interquartile range (IQR). Violin plots of D) glycolysis and E) OXPHOS-related genes across clusters in control (blue) and exercise (orange) samples. Black point represents median and whiskers represent IQR.


A)
CD31
VCAM1
Merge
ERK5 WT Sedentary
ERK5 S496A Sedentary
ERK5 S496A Exercise
B)
VCAM1
* 0.0789
%VCAM+ CD31 area
ERK5 WT Sed
ERK5 S496A Sed
ERK5 S496A Exer
C)
WT
ERK5 S496A KI
Exer
pERK5S496
pERK5TEY
ERK5
D)
pERK5S496 level (Fold increase)
WT
ERK5S46A
E)
pERK5TEY level (Fold increase)
Sed
Exe
F)
Trx1
HO1
Gas6
SIRPa
α-Tubulin
G)
Trx1 levels (Fold increase)
H)
HO1 level (Fold increase)
I)
Gas6 level (Fold increase)
J)
SIRPa levels (Fold increase)
0.11
K)
Cellular ROS level (Fold increase)
L)
Mito ROS level (Fold increase)
ERK5S496A
M)
Efferocytosis index

Supplementary Figure S8. The effect of exercise on tumor vessel adhesion molecule expression and BMDM function in WT and ERK5 S496 KI mice.

A) Representative immunofluorescent images of CD31 (red), VCAM1 (cyan), and DAPI (blue) in YUMMER tumors; scale bar: 100μm. B) Percent of $VCAM1^+$ $CD31^+$ tumor vessel area quantified in WT ERK5 sedentary, ERK5 S496A KI sedentary and ERK5 S496A KI exercised mice. Graphs are displayed with mean +/- standard error of the mean (SEM); each point represents one tumor value obtained by the average of > 5 10x microscopic fields. T-test results are represented on graphs according to the following: ns= $p > 0.05$, *$p \leq 0.05$, **$p \leq 0.01$, ***$p \leq 0.001$, ****$p \leq 0.0001$. C) Representative western blot on isolated BMDM from WT sedentary, WT exercised, ERK5 S496A sedentary, and ERK5 S496A exercised mice for pERK5 TEY, pERK5 S496, and ERK5 protein levels. D) Western blot quantification of pERK5 TEY: total ERK5 ratio. E) Western blot quantification of pERK5 S496: total ERK5 ratio. F) Representative western blot on isolated BMDM from WT sedentary, WT exercised, ERK5 S496A sedentary, and ERK5 S496A exercised mice for Trx1, HO1, Gas6, SIRPα, and α-Tubulin protein levels. G) Western blot quantification of Trx1: α-Tubulin ratio. H) Western blot quantification of HO1: α-Tubulin ratio. I) Western blot quantification of Gas6: α-Tubulin ratio. J) Western blot quantification of SIRPα: α-Tubulin ratio. K) Cellular ROS level quantification in BMDMs. L) Mitochondrial ROS level quantification in BMDMs. M) Efferocytosis index quantification in BMDMs. Graphs are displayed with mean +/- SD. Two-way ANOVA post-hoc comparisons are represented on graphs according to the following: ns= $p > 0.05$, *$p \leq 0.05$, **$p \leq 0.01$, ***$p \leq 0.001$, ****$p \leq 0.0001$.