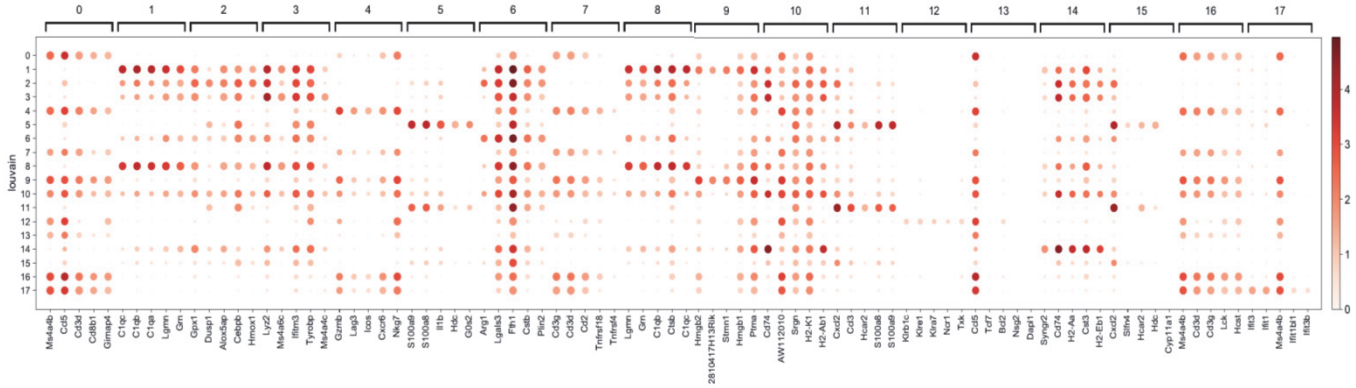
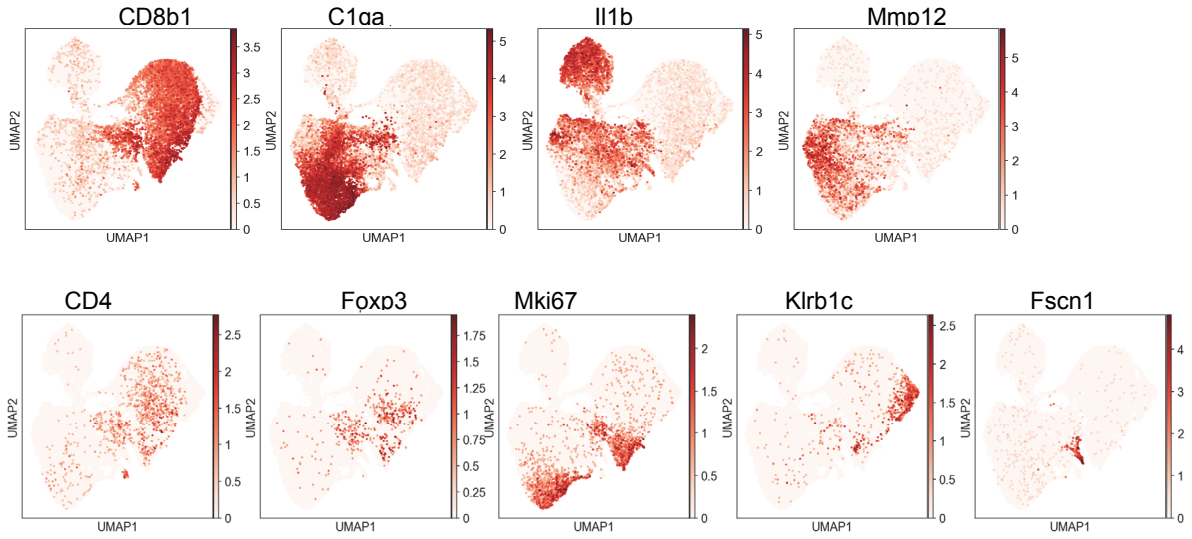
Figure S1

a



b



c

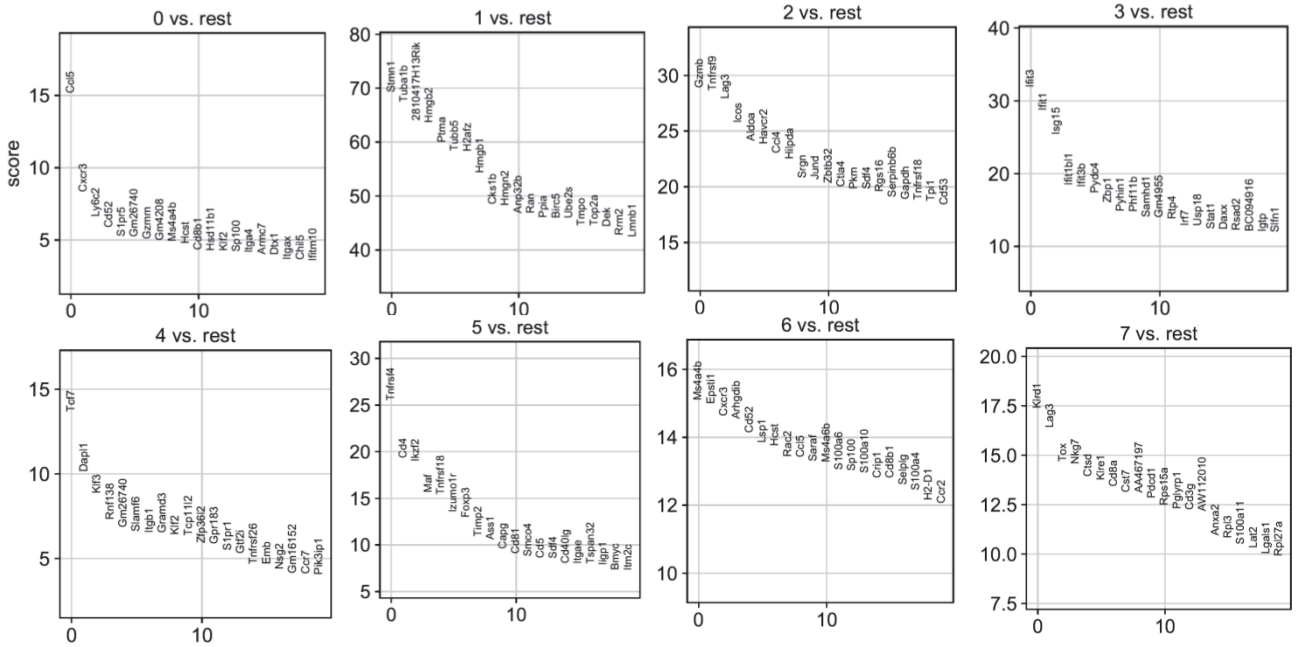


Figure S2

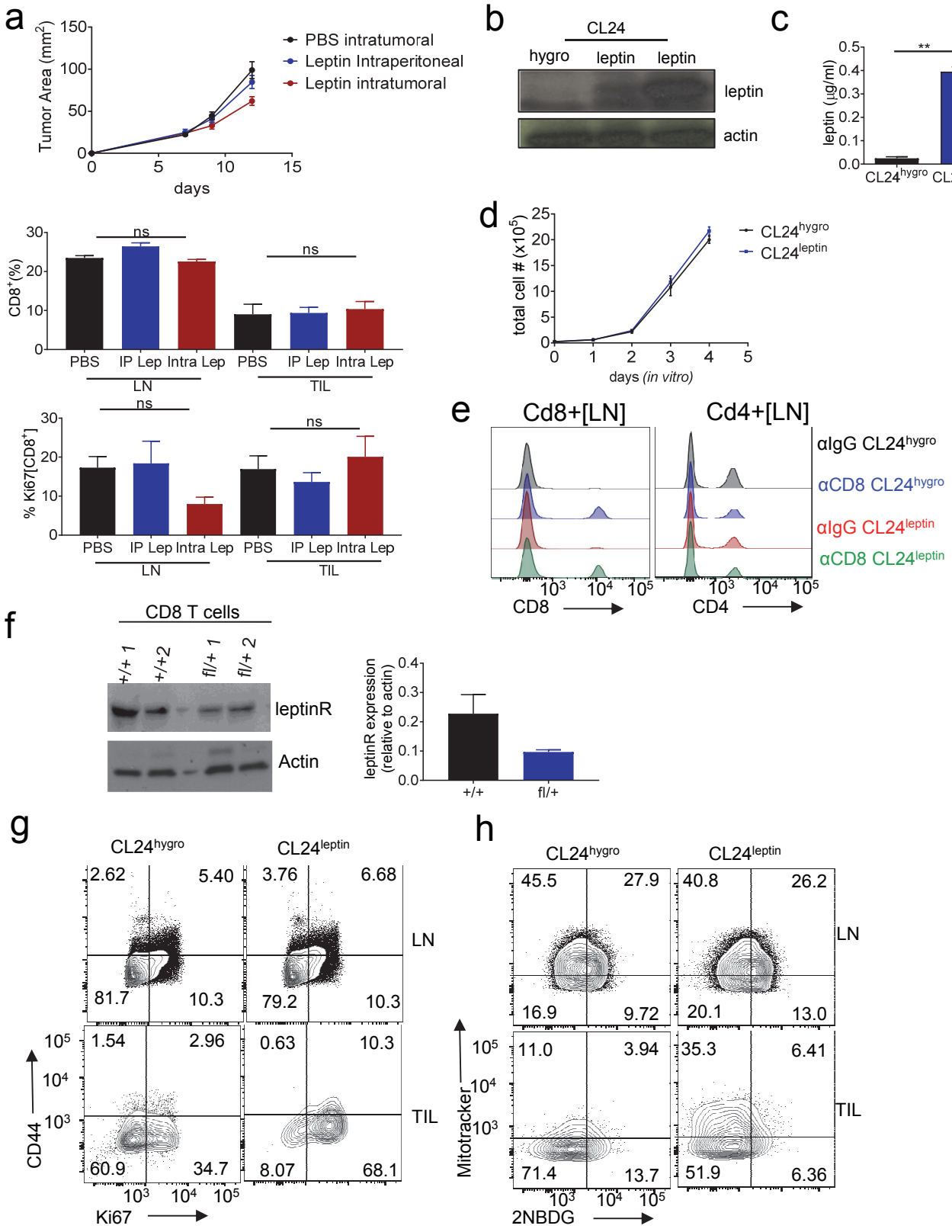

a
Tumor Area (mm2)
days
PBS intratumoral
Leptin Intraperitoneal
Leptin intratumoral
b
CL24
hygro
leptin
leptin
leptin
actin
c
leptin (µg/ml)
CL24hygro
CL24leptin
d
total cell # (x10^5)
days (in vitro)
CL24hygro
CL24leptin
CD8+(%)
% Ki67[CD8+]
PBS
IP Lep
Intra Lep
LN
TIL
ns
e
Cd8+[LN]
Cd4+[LN]
αIgG CL24hygro
αCD8 CL24hygro
αIgG CL24leptin
αCD8 CL24leptin
CD8
CD4
f
CD8 T cells
+/+ 1
+/+2
fl/+ 1
fl/+ 2
leptinR
Actin
leptinR expression (relative to actin)
+/+
fl/+
g
CL24hygro
CL24leptin
LN
TIL
CD44
Ki67
h
CL24hygro
CL24leptin
LN
TIL
Mitotracker
2NBDG

Figure S3

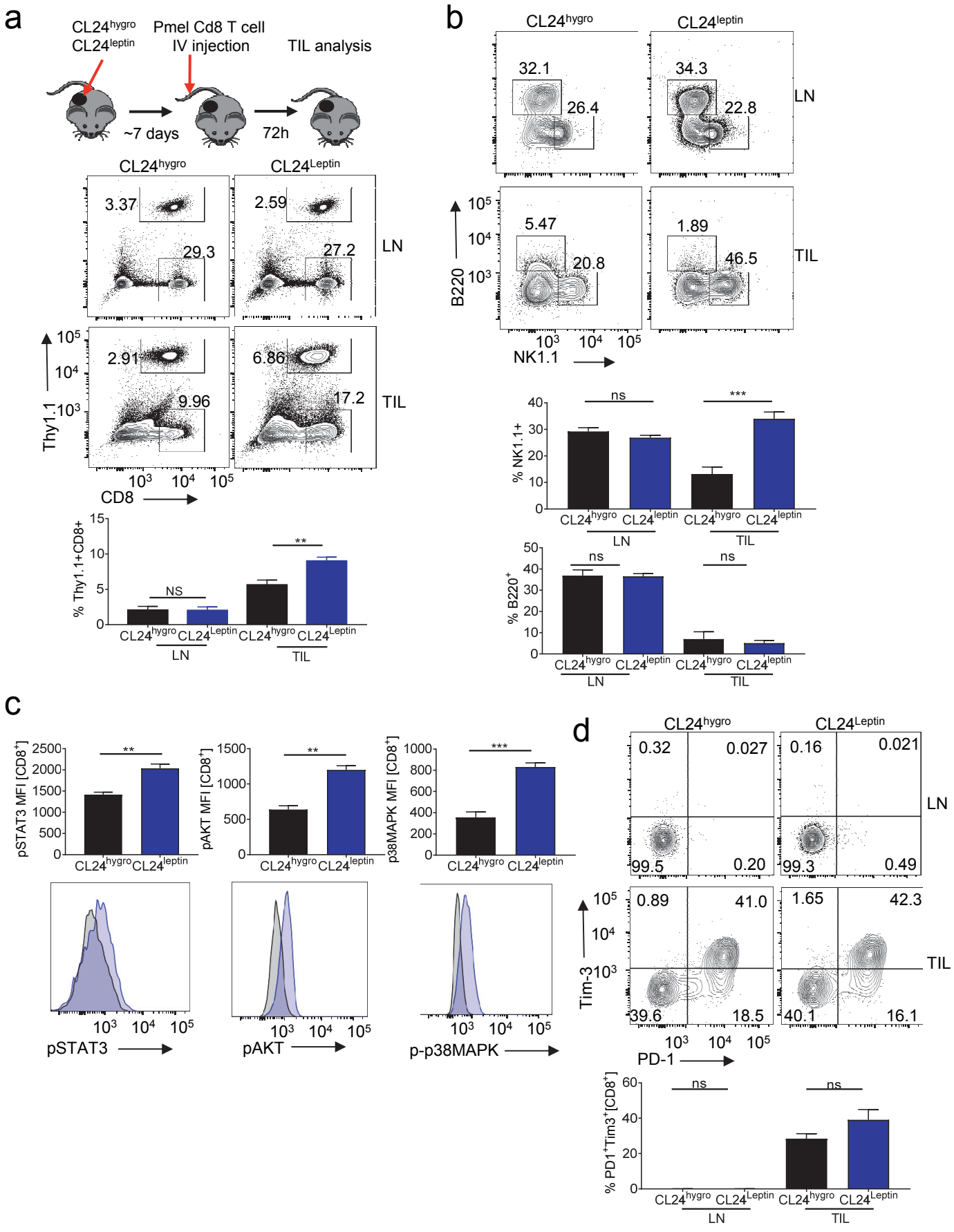

a
CL24hygro
CL24leptin
Pmel Cd8 T cell
IV injection
TIL analysis
~7 days
72h
CL24hygro
CL24Leptin
3.37
29.3
2.59
27.2
LN
Thy1.1
2.91
9.96
6.86
17.2
TIL
CD8
% Thy1.1+CD8+
NS
**
b
CL24hygro
CL24leptin
32.1
26.4
34.3
22.8
LN
B220
5.47
20.8
1.89
46.5
TIL
NK1.1
% NK1.1+
ns
***
% B220+
c
pSTAT3 MFI [CD8+]
pAKT MFI [CD8+]
p38MAPK MFI [CD8+]
pSTAT3
pAKT
p-p38MAPK
d
CL24hygro
CL24Leptin
0.32
0.027
0.16
0.021
LN
99.5
0.20
99.3
0.49
Tim-3
0.89
41.0
1.65
42.3
TIL
39.6
18.5
40.1
16.1
PD-1
% PD1+Tim3+[CD8+]

Figure S4

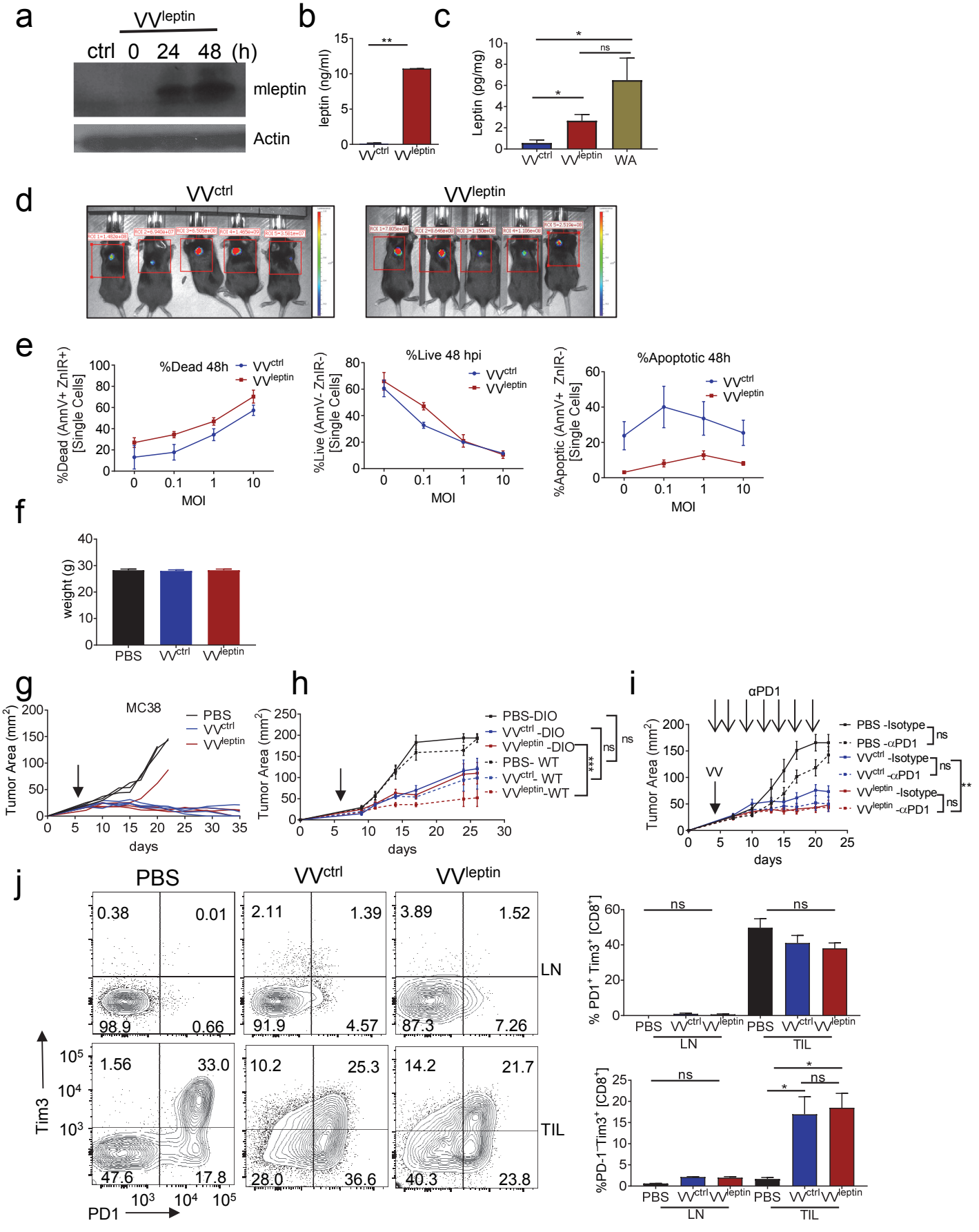

a
VVleptin
ctrl 0 24 48 (h)
mleptin
Actin
b
leptin (ng/ml)
VVctrl VVleptin
c
Leptin (pg/mg)
VVctrl VVleptin WA
d
VVctrl
VVleptin
e
%Dead 48h
%Dead (AnnV+ ZnIR+) [Single Cells]
MOI
%Live 48 hpi
%Live (AnnV- ZnIR-) [Single Cells]
%Apoptotic 48h
%Apoptic (AnnV+ ZnIR-) [Single Cells]
f
weight (g)
PBS VVctrl VVleptin
g
MC38
PBS
Tumor Area (mm2)
days
h
PBS-DIO
VVctrl -DIO
VVleptin -DIO
PBS- WT
VVctrl- WT
VVleptin-WT
i
αPD1
VV
PBS -Isotype
PBS -αPD1
VVctrl -Isotype
VVctrl -αPD1
VVleptin -Isotype
VVleptin -αPD1
j
PBS
VVctrl
VVleptin
LN
TIL
Tim3
PD1
% PD1+ Tim3+ [CD8+]
%PD-1+Tim3+ [CD8+]

Figure S5

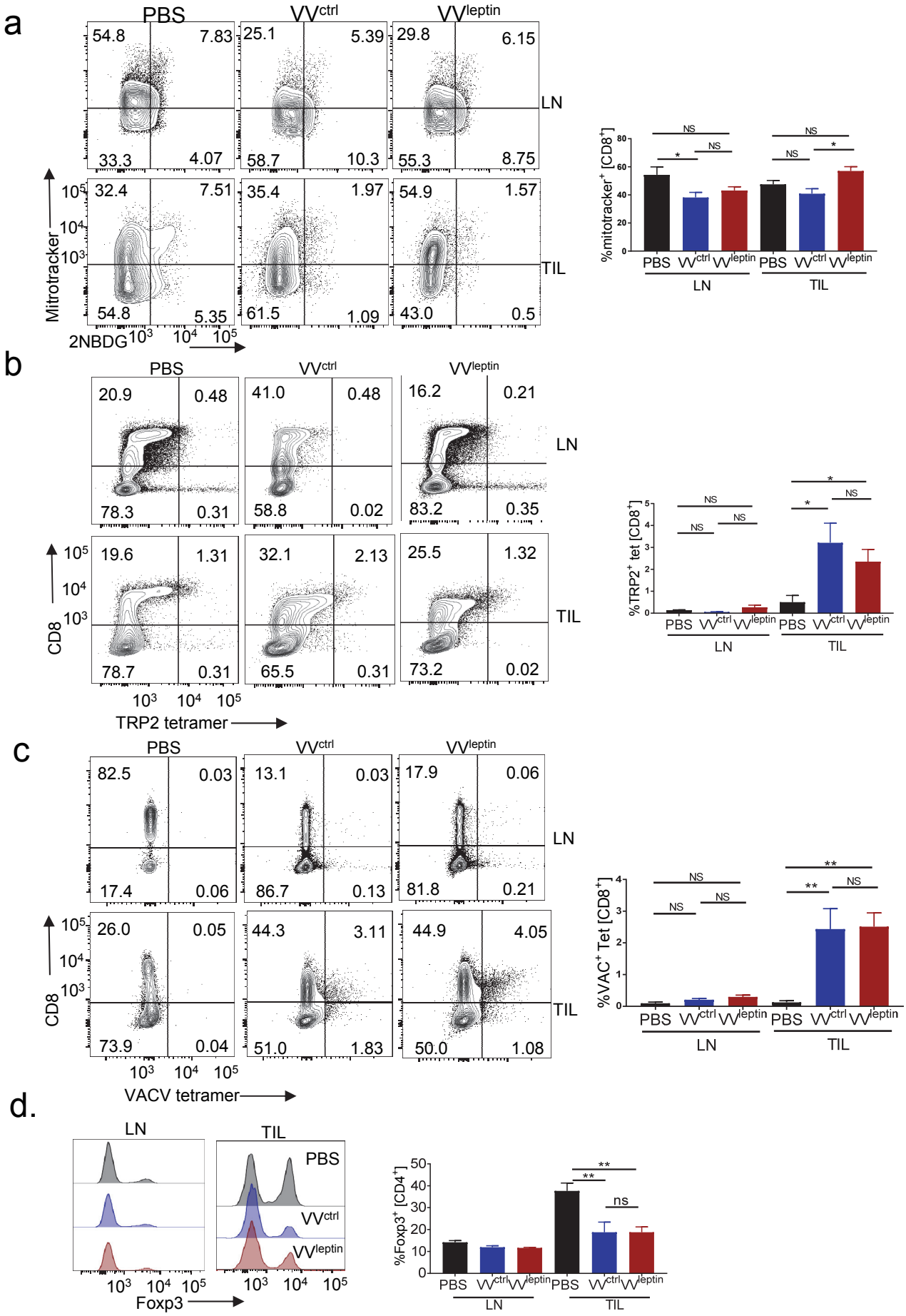
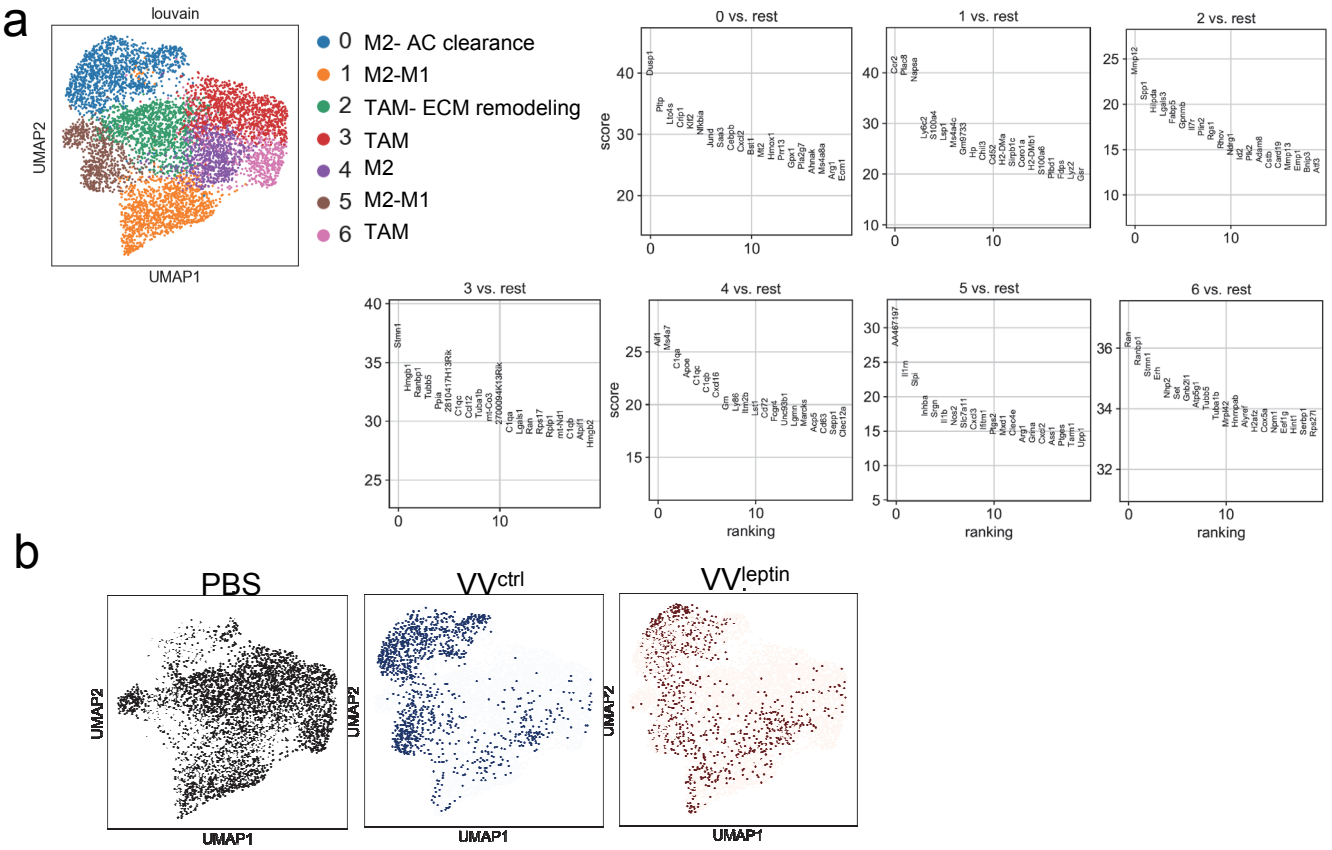

a
PBS
VVctrl
VVleptin
54.8
7.83
25.1
5.39
29.8
6.15
LN
33.3
4.07
58.7
10.3
55.3
8.75
32.4
7.51
35.4
1.97
54.9
1.57
TIL
54.8
5.35
61.5
1.09
43.0
0.5
Mitrotracker
2NBDG
%mitotracker+ [CD8+]
NS
*
PBS VVctrl VVleptin PBS VVctrl VVleptin
LN
TIL
b
20.9
0.48
41.0
0.48
16.2
0.21
78.3
0.31
58.8
0.02
83.2
0.35
19.6
1.31
32.1
2.13
25.5
1.32
78.7
0.31
65.5
0.31
73.2
0.02
CD8
TRP2 tetramer
%TRP2+ tet [CD8+]
c
82.5
0.03
13.1
0.03
17.9
0.06
17.4
0.06
86.7
0.13
81.8
0.21
26.0
0.05
44.3
3.11
44.9
4.05
73.9
0.04
51.0
1.83
50.0
1.08
VACV tetramer
%VAC+ Tet [CD8+]
**
d.
LN
TIL
PBS
VVctrl
VVleptin
Foxp3
%Foxp3+ [CD4+]
ns

Figure S6


a
louvain
UMAP2
UMAP1
0 M2- AC clearance
1 M2-M1
2 TAM- ECM remodeling
3 TAM
4 M2
5 M2-M1
6 TAM
0 vs. rest
1 vs. rest
2 vs. rest
3 vs. rest
4 vs. rest
5 vs. rest
6 vs. rest
score
ranking

b
PBS
VVctrl
VVleptin
UMAP2
UMAP1

SUPPLEMENTARY FIGURE LEGENDS

Figure S1 related to Figure 1. Single cell RNA sequencing analysis from TIL treated with oncolytic vaccinia virus. (A) Dot plot of top 5 differentially expressed genes defining each cluster of cells. Intensity of red color indicates the normalized level of gene expression and the size of the dot represents the percentage of cells expressing that gene. (B) UMAP plots with canonical marker genes colored which were used to assign identities to cell clusters. (C) Top 20 differentially expressed genes in each cluster as compared to all the other clusters.

Figure S2 related to Figure 4. Systemic delivery of recombinant leptin, characterization of leptin overexpression in tumor cells and leptin receptor knock out transgenics. (A) C57BL/6J mice were inject subdermally with CL24 cells. 5-7 days after injection tumors were treated either systemically by IP with recombinant leptin 3 consecutive or intratumorally (1ug/g) and tumor growth monitored. Representative flow histogram and tabulated flow cytometric data for CD8+ T cells from LN and TIL from mice and ki67 expression. (B) Immunoblot analysis of mouse leptin protein expression of CL24 cell line stably transduced with control plasmid ($CL24^{hygro}$) and mouse leptin gene plasmid ($CL24^{leptin}$). (C) ELISA analysis of leptin in the media of cells transduced with control plasmid and leptin gene. (D) In vitro growth analysis between $CL24^{hygro}$ and $CL24^{leptin}$ cell lines. (E) C57BL/6J mice were treated every other day with anti-CD8 (200ug). At day 6 mice were injected with either $CL24^{hygro}$ or $CL24^{leptin}$ and tumor growth was monitored. CD8 and CD4 expression analysis in lymph node (LN). (F) CD8 T cells isolated from ObR fl/+ CD4 CRE mice and analyzed by immunoblot for Leptin receptor (LeptinR), actin was used as a loading control. C57BL/6J mice were injected with either $CL24^{hygro}$ or $CL24^{leptin}$. (G) Representative flow cytogram of LN and TIL for metabolic markers Mitotracker FM staining and 2NBDG uptake. Error bars indicate s.e.m. Data represents at least 3 independent experiments *p <0.05, **p <0.01, ***p <0.001 by two-way ANOVA. Error bars indicate s.e.m.

Figure S3 related to Figure 4. Characterization of tumor infiltrating lymphocytes of leptin overexpression in tumor cells. (A) C57BL/6J mice were inject subdermally with $CL24^{hygro}$ and $CL24^{leptin}$ cells. 5-7 days after injection mice were given an adoptive transfer of $10x10^6$ previously activated PMEL CD8 T cells. 3 days after transfer tumors were analyzed for infiltrated

T cells. (B) NK1.1 and B220 analysis for natural killer cells and B cells respectively on LN and TIL from mice injected with $CL24^{hygro}$ and $CL24^{leptin}$. Representative flow histogram for NK1.1 and B220 staining in LN and TIL and tabulated flow cytometric data are shown. (C) Representative flow histogram and tabulated flow cytometric data for CD8+ T cells from LN and TIL from mice injected with $CL24^{hygro}$ and $CL24^{leptin}$ analyzed for pSTAT3, pAKT and p-p38MAPK expression. (D) Representative flow cytograms and tabulated flow cytometric data for Tim3 and PD-1 expression leptin from tumors treated as in (B). Error bars indicate s.e.m. Data represents at least 3 independent experiments *p <0.05, **p <0.01, ***p <0.001 by two-way ANOVA. Error bars indicate s.e.m.

Figure S4 related to Figure 5. Additional tumor models and therapeutic regiments with leptin-engineered oncolytic *Vaccinia* virus and tumor infiltrating lymphocyte analysis
(A) Immunoblot analysis of mouse leptin protein expression of CL24 cell line treated with VV^{leptin} at $2.5x10^6$ PFU in vitro 24h and 48h. (B) ELISA analysis of leptin in the media of CL24 cells treated with VV^{leptin}. (C) ELISA analysis of leptin in interstitial fluid of tumors treated with VV^{ctrl} or VV^{leptin}. Interstitial fluid from white adipose tissue (WA) used as control. Data represents at least 3 independent experiments *p <0.05 by two-way ANOVA. Error bars indicate s.e.m. (D) C57BL/6J mice were injected subdermally with CL24 cells. 5-7 days after tumor cell injection tumors were treated intratumorally with PBS, VV^{ctrl}, or VV^{leptin} 24h later mice were injected with luciferin (30mg/ml) IP for 10min and conducted In Vivo Bioluminescence Imaging. (E) CL24 cells were infected in vitro with VV^{ctrl}, or VV^{leptin} at MOI 01, 1, 10. Representative flow cytogram represents Zombie staining (live-dead) and AnnexinV (apoptosis). (F) C57BL/6J mice were injected subdermally with CL24 cells. 5-7 days after tumor cell injection tumors were treated intratumorally with PBS, VV^{ctrl}, or VV^{leptin}. Mice were monitored for weight at the final time point of tumor growth analysis. (G) C57BL/6J mice were injected subdermally with MC38 cells. 5-7 days after tumor cell injection tumors were treated intratumorally with PBS, VV^{ctrl}, or VV^{leptin}. Mice were monitored for tumor growth. (H) C57BL/6J DIO mice were injected subdermally with CL24 cells. 5-7 days after tumor cell injection tumors were treated intratumorally with PBS, VV^{ctrl}, or VV^{leptin}. Mice were monitored for tumor growth. (I) C57BL/6J mice we treated as in (H) in addition to anti-PD1 treatment every other day. Mice were monitored for tumor growth. C57BL/6J mice were injected subdermally with CL24 cells. 5-7 days after tumor cell injection tumors were treated intratumorally with PBS, VV^{ctrl}, or VV^{leptin}. Representative flow cytogram and tabulated flow cytometric data represents Tim3 and PD1 expressions (J). Error bars indicate

s.e.m. Data represents at least 3 independent experiments *p <0.05, **p <0.01, ***p <0.001 by two-way ANOVA. Error bars indicate s.e.m.

Figure S5 related to Figure 5 and Figure 6. Tumor infiltrating lymphocyte analysis for after leptin-engineered oncolytic *Vaccinia* virus vaccinia treatment. C57BL/6J mice were injected subdermally with CL24 cells. 5-7 days after tumor cell injection tumors were treated intratumorally with PBS, VV^{ctrl}, or VV^{leptin}. Mice were monitored for tumor growth. (A) Representative flow cytogram and tabulated flow cytometric data represents mitotracker-2NBDG expression (A). C57BL/6J mice we treated as in (A) Representative flow cytogram and tabulated flow cytometric data represents CD8- TRP2 tetramer expression (B) and CD8 and Vaccinia tetramer expression (C). C57BL/6J mice we treated as in (A) Representative flow cytograms and tabulated flow cytometric data for $CD4^+$ $Foxp3^+$T cells (T regulatory cells) from LN and TIL (D). Error bars indicate s.e.m. Error bars indicate s.e.m. Data represents at least 3 independent experiments *p <0.05, **p <0.01, ***p <0.001 by two-way ANOVA. Error bars indicate s.e.m.

Figure S6 related to Figure 6. Single cell RNA sequencing analysis from TIL treated with oncolytic vaccinia virus for macrophage subpopulation. (A) Unsupervised clustering of macrophage subpopulation. (B) Top 20 differentially expressed genes for each cluster seen in (A). (C) UMAPs colored by treatments depicting the cell distribution through the macrophage subpopulation clustering.