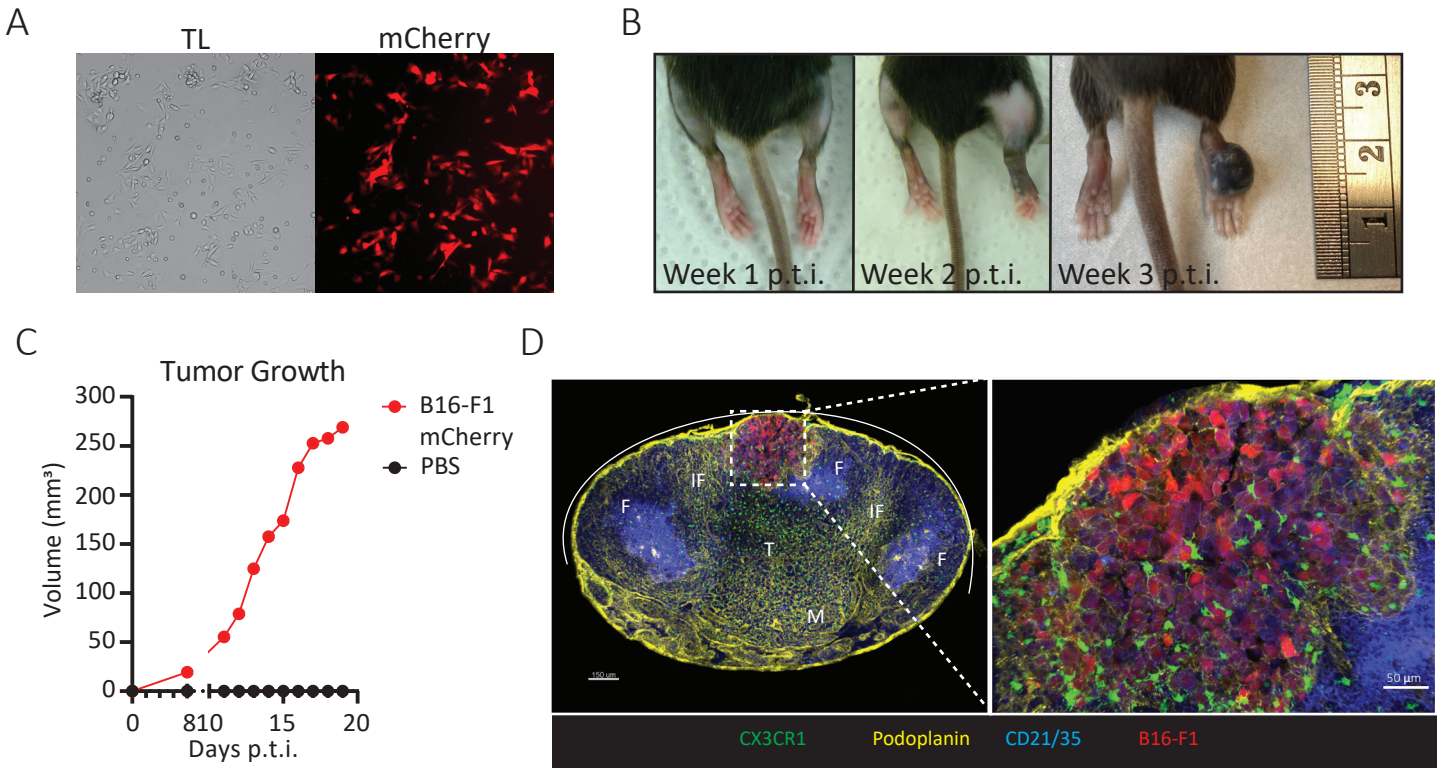
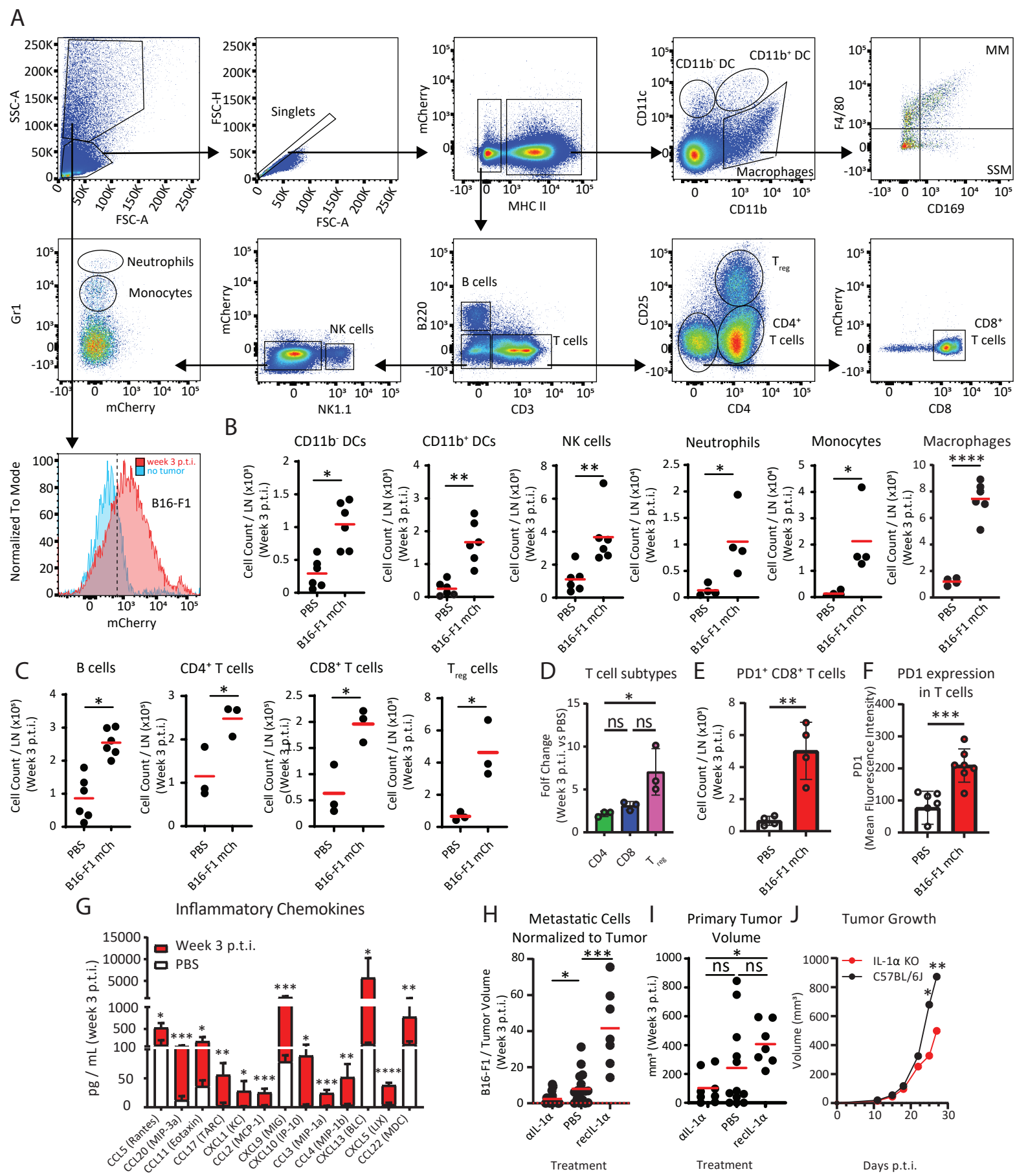
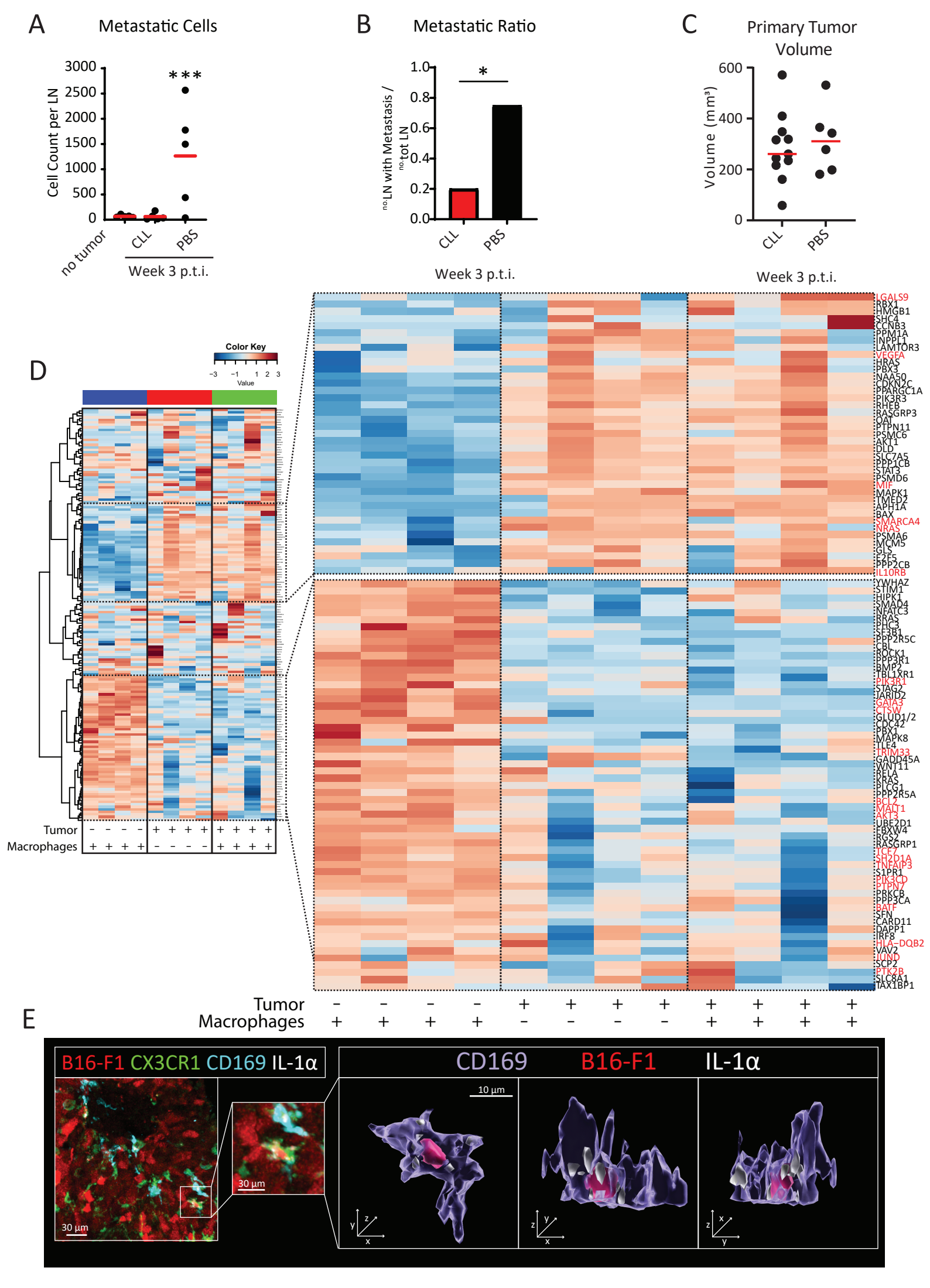


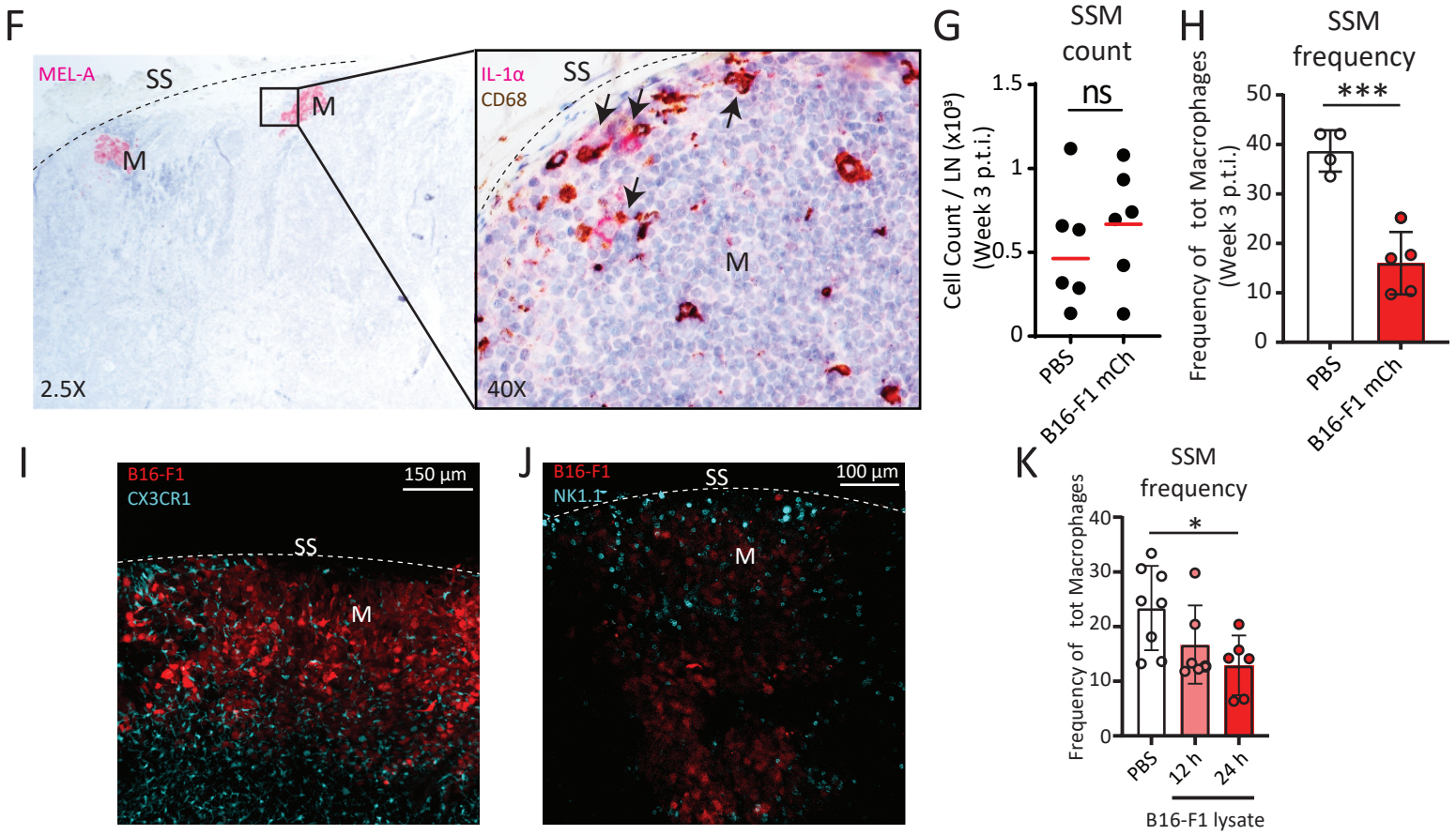
Supplementary Figure 1 - Mouse melanoma metastases growth in the sentinel LN
(A, left) Transmitted light and (A, right) mCherry microscope pictures of transduced B16-F1 mCherry. (B) Representative pictures of primary tumor growth at week one, two and three p.t.i.. (C) Timecourse indicating tumor volume in the first three weeks p.t.i.. (D) Confocal micrograph showing sLN (left) at week three p.t.i. and (right) magnification of the metastasis invading the SS and IF areas.



Supplementary figure 2: Pro-tumoral release of IL-1α in the metastatic LN

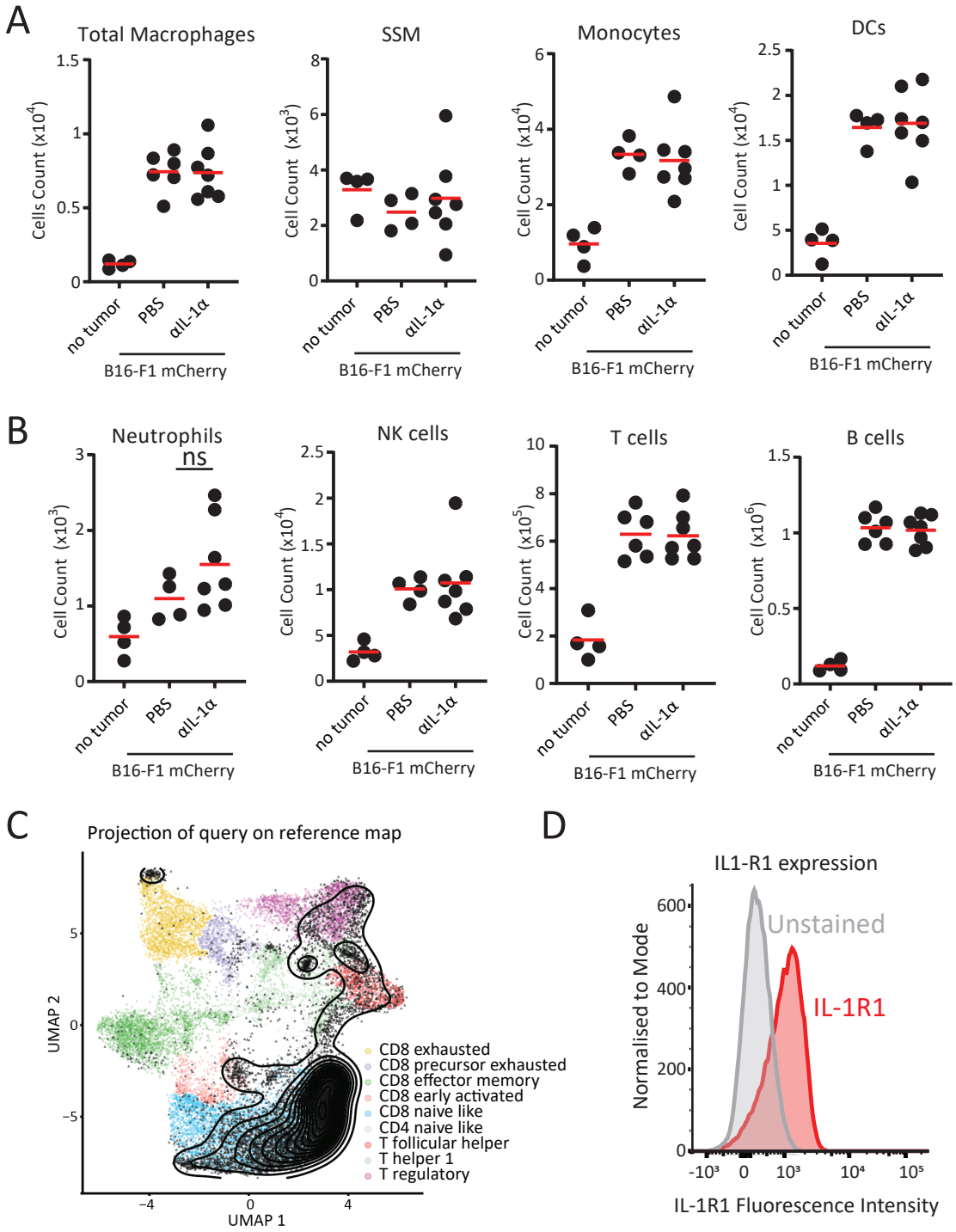
(A) FACS gating strategy to detect immune cells. In addition to B16 melanoma (mCherry$^+$), different cells are detectable from single cells, including CD11b$^-$ DCs (MHC II$^+$, CD11c$^+$, CD11b$^-$), CD11b$^+$ DCs (MHC II$^+$, CD11c$^+$, CD11b$^+$), subcapsular sinus macrophages (SSM, MHC II$^+$, CD11c^{intlow}, CD11b$^+$, CD169$^+$, F4/80$^-$), medullary macrophages (MM, MHC II$^+$, CD11c^{intlow}, CD11b$^+$, CD169$^+$, F4/80$^+$), B cells (MHC II$^-$, CD3$^-$, B220$^+$), CD4$^+$ T cells (MHC II$^-$, B220$^-$, CD3$^+$, CD4$^+$, CD25$^-$), T$_{reg}$ (MHC II$^-$, B220$^-$, CD3$^+$, CD4$^+$, CD25$^+$), CD8$^+$ T cells (MHC II$^-$, B220$^-$, CD3$^+$, CD4$^-$, CD25$^-$, CD8$^+$), NK cells (MHC II$^-$, B220$^-$, CD3$^-$, NK1.1$^+$), neutrophils (MHC II$^-$, B220$^-$, CD3$^-$, NK1.1$^-$, Gr1^{high}) and monocytes (MHC II$^-$, B220$^-$, CD3$^-$, NK1.1$^-$, Gr1^{int}) and B16 melanoma (mCherry$^+$). Quantification of the total number of (B) innate and (C) adaptive immune cells in metastatic sLN at week three p.t.i.. (D) Fold change increase of CD4$^+$, CD8$^+$ and regulatory T cells in tumor in comparison to control. (E) Quantification of the number of PD1$^+$ cells and (F) PD1 mean expression in CD8$^+$ T cells at week three p.t.i. in comparison to controls. (G) Inflammatory chemokines in metastatic sLN (red) in comparison to mice without tumor (white). (H) Number of metastatic melanoma cells in the sLN in mice treated with anti IL-1α or recombinant IL-1α, normalized to the primary tumor volume. The red dashed line indicates the background, calculated on the number of events in non-tumor-bearing mice. (I) Primary tumor volume in mice treated with anti IL-1α blocking antibody or with recombinant IL-1α in comparison to untreated. (J) Primary tumor growth in IL-1α wild type and IL-1α KO mice.


A
Metastatic Cells
Cell Count per LN
3000
2500
2000
1500
1000
500
0
***
no tumor
CLL
PBS
Week 3 p.t.i.
B
Metastatic Ratio
no LN with Metastasis / no tot LN
1.0
0.8
0.6
0.4
0.2
0.0
*
CLL
PBS
Week 3 p.t.i.
C
Primary Tumor Volume
Volume (mm³)
600
400
200
0
CLL
PBS
Week 3 p.t.i.
D
Color Key
−3 −1 1 2 3
Value
Tumor
Macrophages
LGALS9
RBX1
HMGB1
SHC4
CCNB3
PPM1A
INPPL1
LAMTOR3
VEGFA
HRAS
PBX3
NAA50
CDKN2C
PPARGC1A
PIK3R3
RHEB
RASGRP3
OAT
PTPN11
PSMC6
AKT1
DLD
SLC7A5
PPP1CB
STAT3
PSMD6
MIF
MAPK1
TMED2
APH1A
BAX
SMARCA4
NRAS
PSMA6
MCM5
GLS
EIF5
PPP2CB
IL10RB
YWHAZ
STIM1
HIPK1
SMAD4
NFATC3
RRAS
PHC3
SF3B1
PPP2R5C
CBL
ROCK1
PPP3R1
BMP2
TBL1XR1
PIK3R1
STAG2
ARID2
GAIA3
CTSW
GLUD1/2
CDC42
PBX1
MAPK8
TLR4
TRIM33
GADD45A
WNT11
RELA
KRAS
PLCG1
PPP2R5A
BCL2
MALT1
AKT3
UBE2D1
FBXW4
RGS2
RASGRP1
FCF7
SH2D1A
TNFAIP3
S1PR1
PIK3CD
PTPN7
PRKCB
PPP3CA
BATF
SFN
CARD11
DAPP1
IRF8
HLA_DQB2
VAV2
IUND
SCP2
PTK2B
SLC8A1
TAX1BP1
Tumor
Macrophages
E
B16-F1 CX3CR1 CD169 IL-1α
30 µm
30 µm
CD169
B16-F1
IL-1α
10 µm

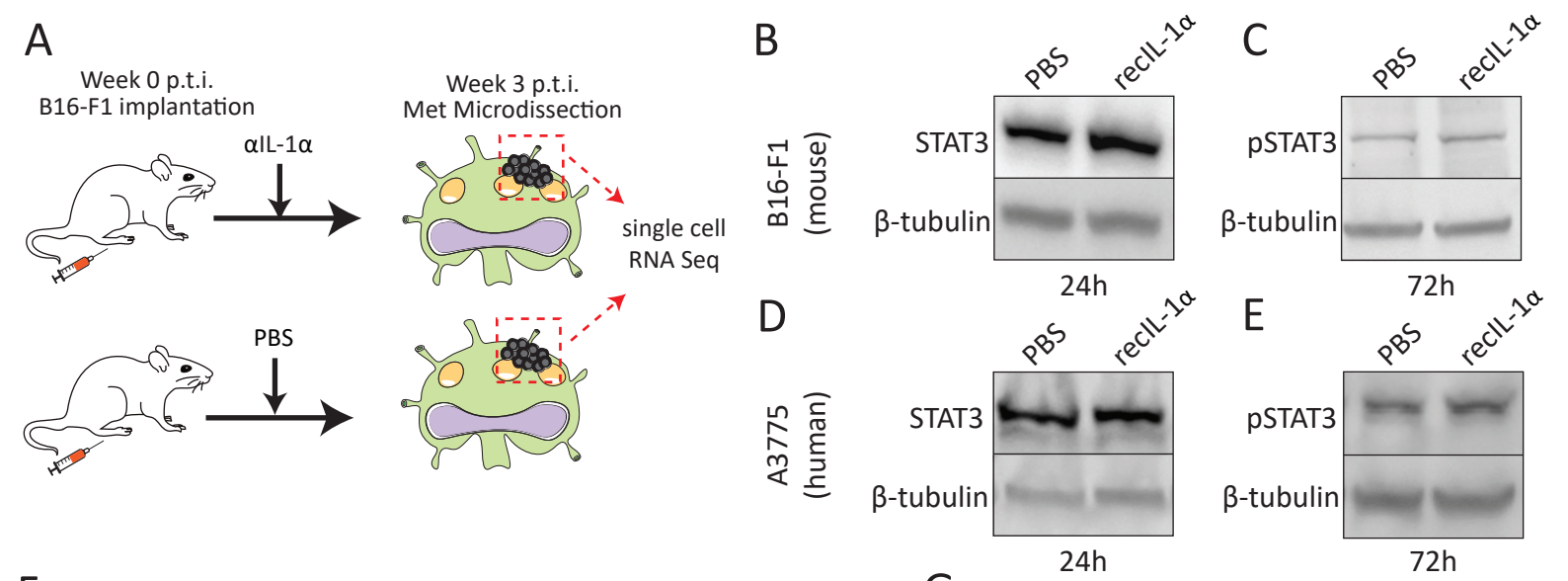


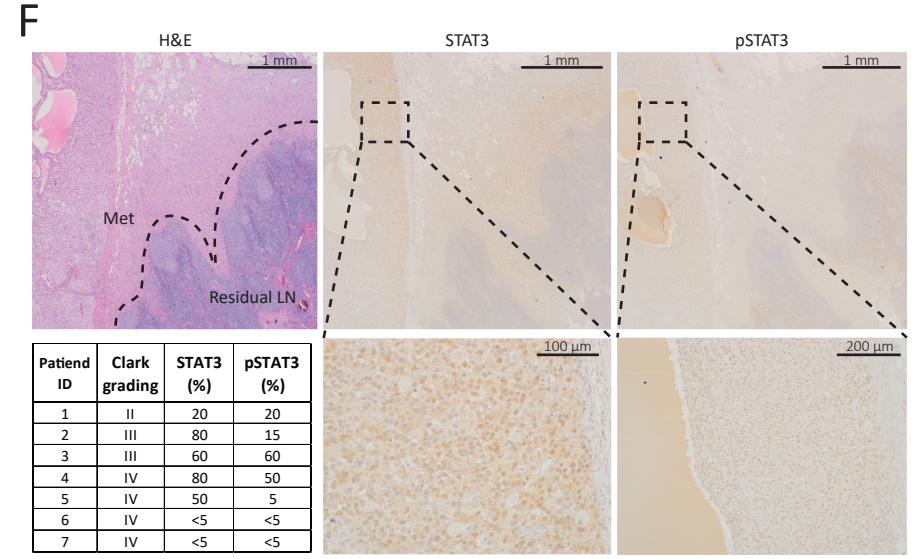
Supplementary figure 3: SSM are the main source of IL-1α

(A) Total number of metastatic cells in sLN of mice treated with clodronate liposomes (CLL) in comparison to negative controls. (B) Metastatic ratio and (C) Primary tumor volume in mice treated with CLL in comparison to untreated mice. (D, left) Heatmap representing average expression of genes related to immunosuppression and immune exhaustion in metastatic LN three weeks p.t.i. from mice depleted for macrophages (green) in comparison to untreated mice (red) and mice without tumor (blue). (D, right) Magnifications of two regions of the previous heatmap showing, highlighted in red, some relevant genes associated with immune suppression (above) and immune activation (below).
(E, left) Confocal micrographs showing a tumor infiltrating SSM and (E, right) corresponding 3D reconstruction indicating intracellular accumulation of tumor (red) and IL-1α (white). (F, left) Representative section of human sLN showing presence of melanoma metastases (pink), indicated with the letter M. (F, right) Magnification of metastatic area highlighting infiltration of $CD68^+$ macrophages (brown) and expression of IL-1α (pink). Representative macrophages positive to IL-1α are indicated by black arrows. (G) Total number of SSM and (H) frequency on total LN macrophages at three weeks p.t.i.. Confocal micrographs of metastasis infiltrated by (I) myeloid $CX3CR1^+$ cells and (J) NK cells. (K) Frequency of SSM at 12 and 24 hours after injection of tumor cell lysate.

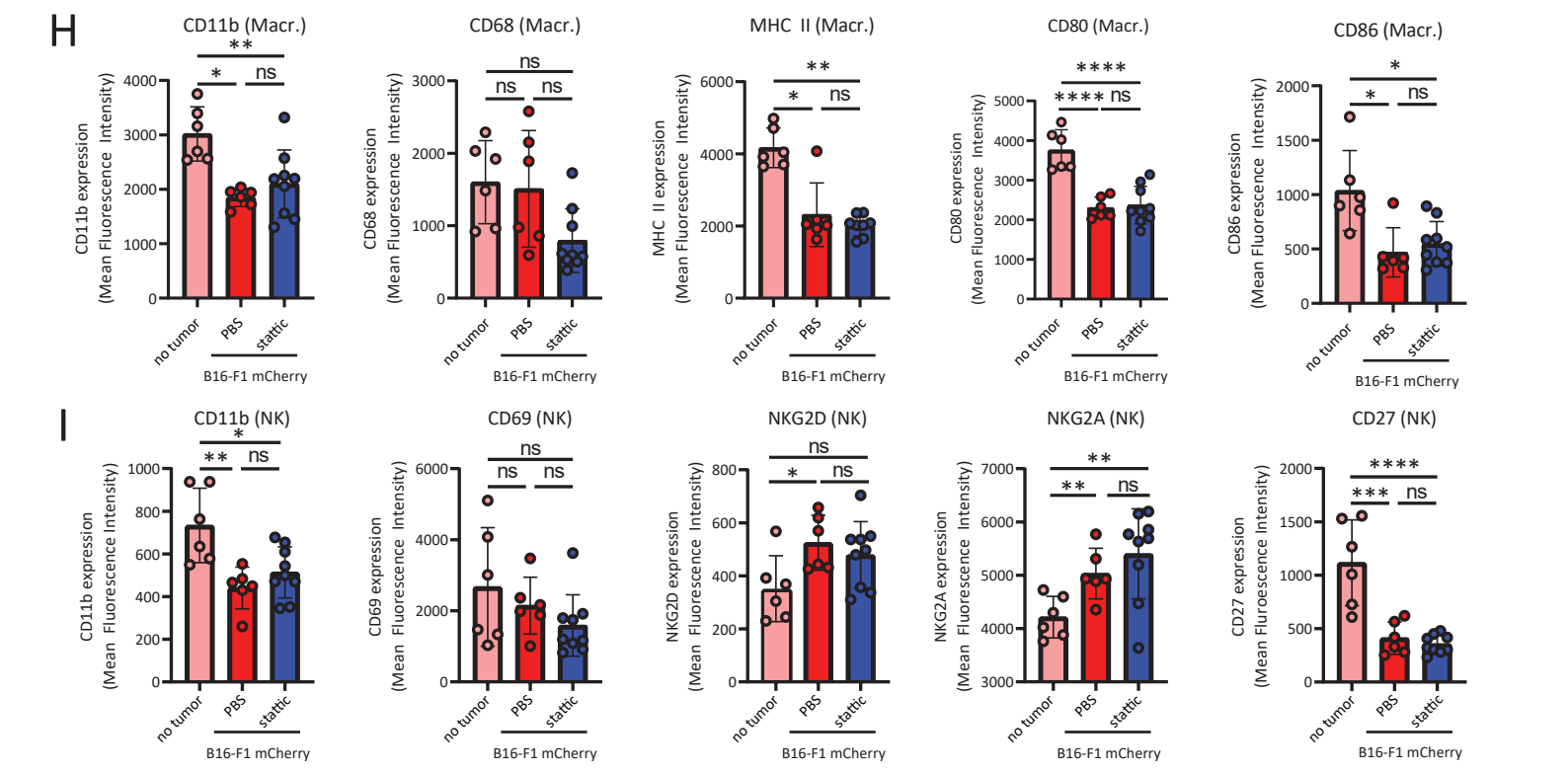


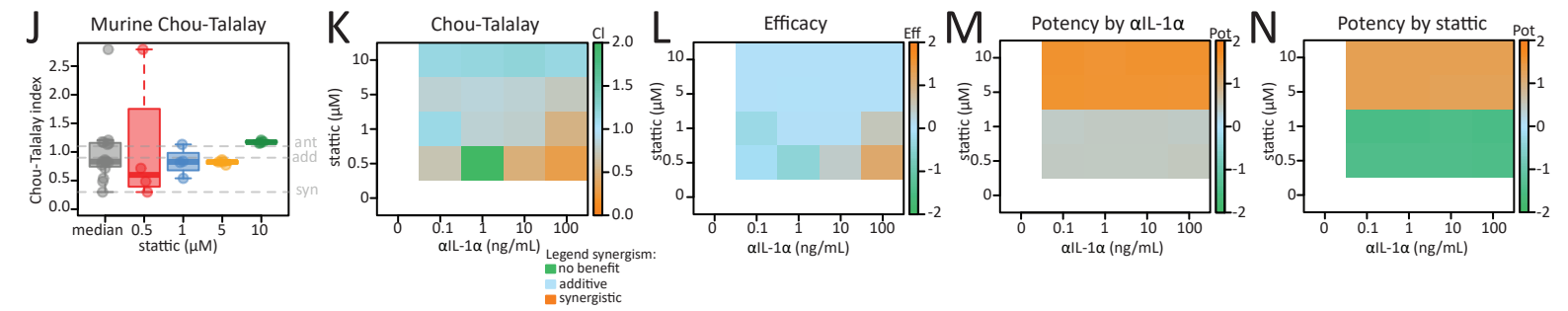
Supplementary figure 4: SSM derived IL-1α directly supports tumor proliferation
Total number of (A) phagocytic and (B) non phagocytic immune cells in sLN of mice treated with anti IL-1α antibody in comparison to untreated at three weeks p.t.i.. (C) Projection of the T cell subsets identity on the scSeq UMAP map. (D) Expression of IL-1R1 in B16-F1 *in vitro*.





Patiend ID	Clark grading	STAT3 (%)	pSTAT3 (%)
1	II	20	20
2	III	80	15
3	III	60	60
4	IV	80	50
5	IV	50	5
6	IV	<5	<5
7	IV	<5	<5





Supplementary figure 5: IL-1α induces STAT3 expression and phosphorylation in tumor
(A) Scheme representing the design of scSeq experiment. Representative immunoblot of (B) STAT3 and (C) pSTAT3 in murine melanoma and (D) STAT3 and (E) pSTAT3 in human melanoma. (F) Histological sections of a representative metastatic lesion in the sLN of a human patient, stained with haematoxylin and eosin (H&E, left) in comparison to IHC staining of STAT3 (center) and pSTAT3 (right), with corresponding magnifications (below). (F, below left) Table reporting Clark grading and percentage of STAT3 and pSTAT3 positive cells in 7 human metastatic sLN. (G) Survival probability of NF1 mutated melanoma patients according to high (red) or low (blue) expression of pSTAT3. Flow cytometric quantification of activation markers of (H) macrophages (CD11b, CD68, MHC II, CD80 and CD86) and (I) NK cells (CD11b, CD69, NKG2D, NKG2A and CD27), expressed as mean fluorescence intensity, in metastatic lesions of untreated mice in comparison to animals treated with the STAT3i stattic and mice without tumor. (J) Samples distribution of the Chou-Talalay Index of the combination therapy, indicating the thresholds for synergistic, additive and antagonist effect in grey. (K) Chou-Talalay Index, (L) efficacy, (M) potency by anti IL-1α, and (N) potency by stattic of the combination therapy, all indicated as average expression of samples.