Cell Reports, Volume *39*

Supplemental information

Single-cell RNA-seq of a soft-tissue sarcoma model reveals the critical role of tumor-expressed MIF in shaping macrophage heterogeneity

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Single-cell RNA-seq analysis of a soft-tissue sarcoma model reveals the critical role of tumor-expressed MIF in shaping macrophage heterogeneity.

Fernando H. G. Tessaro[1]*, Emily Y. Ko[1]*, Marco De Simone[1], Roberta Piras[1], Marina T. Broz[2], Helen S. Goodridge[3,4], Bonnie Balzer[5], Stephen L. Shiao[1,2,4,6], and Jlenia Guarnerio[1,2,3,6,#].

SUPPLEMENTARY FIGURES

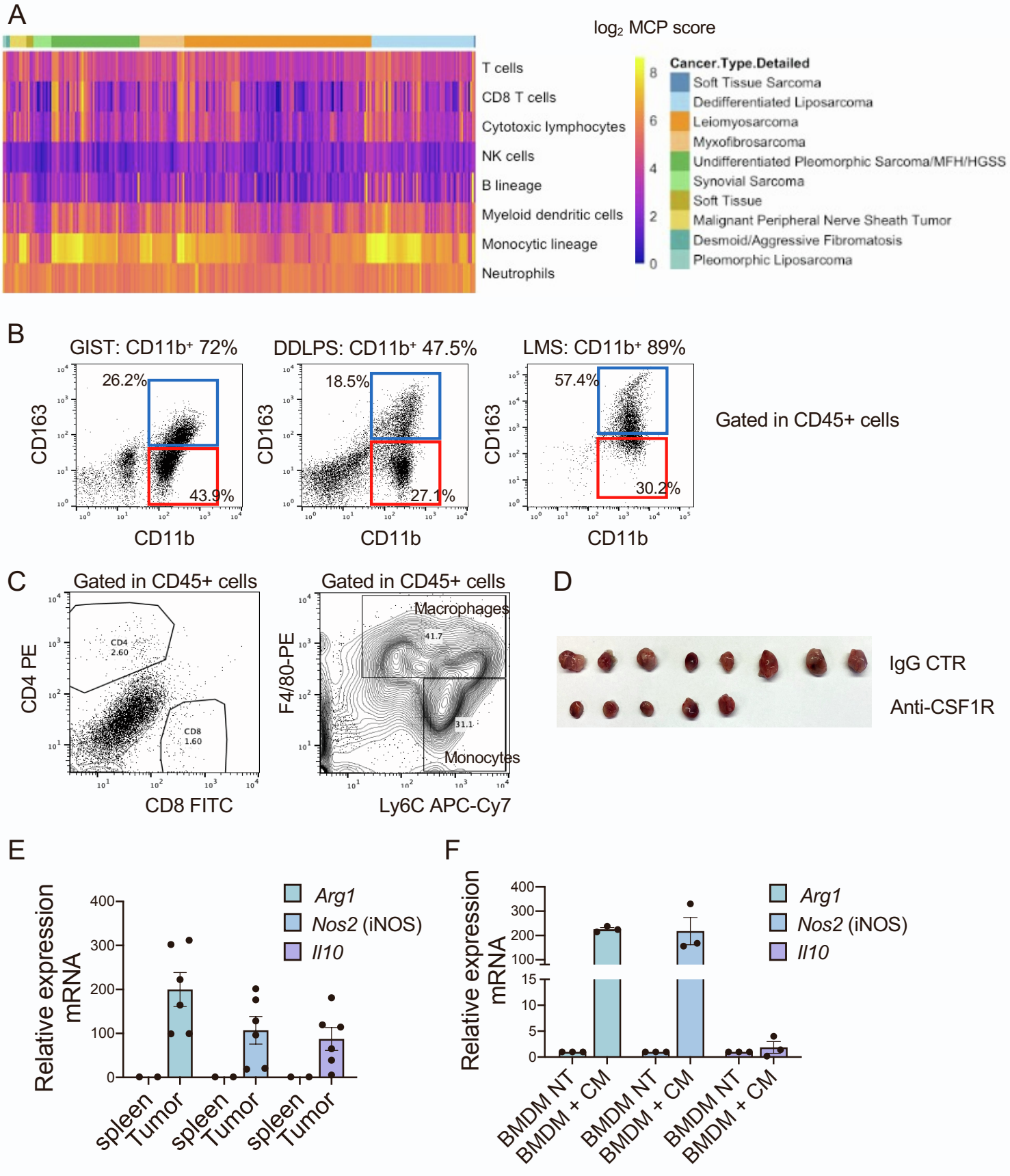

A
log2 MCP score
T cells
CD8 T cells
Cytotoxic lymphocytes
NK cells
B lineage
Myeloid dendritic cells
Monocytic lineage
Neutrophils
8
6
4
2
0
Cancer.Type.Detailed
Soft Tissue Sarcoma
Dedifferentiated Liposarcoma
Leiomyosarcoma
Myxofibrosarcoma
Undifferentiated Pleomorphic Sarcoma/MFH/HGSS
Synovial Sarcoma
Soft Tissue
Malignant Peripheral Nerve Sheath Tumor
Desmoid/Aggressive Fibromatosis
Pleomorphic Liposarcoma
B
GIST: CD11b+ 72%
DDLPS: CD11b+ 47.5%
LMS: CD11b+ 89%
26.2%
43.9%
18.5%
27.1%
57.4%
30.2%
CD163
CD11b
Gated in CD45+ cells
C
Gated in CD45+ cells
Gated in CD45+ cells
CD4 PE
CD8 FITC
CD4
2.60
CD8
1.60
F4/80-PE
Ly6C APC-Cy7
Macrophages
41.7
31.1
Monocytes
D
IgG CTR
Anti-CSF1R
E
Relative expression mRNA
400
300
200
100
0
Arg1
Nos2 (iNOS)
Il10
spleen
Tumor
F
Relative expression mRNA
400
300
200
100
15
10
5
0
Arg1
Nos2 (iNOS)
Il10
BMDM NT
BMDM + CM

Figure S1. Macrophages promote sarcoma growth, related to Figure 1.

(A) Proportions of major infiltrating immune cell populations inferred by MCP-counter for all adult Soft-Tissue Sarcoma samples, including Undifferentiated Pleiomorphic Sarcomas, collected in the TCGA bulk RNA-seq database. The categories *Soft Tissue Sarcoma* (blue) and *Soft Tissue* (tan) correspond to STS cases for which a sarcoma subtype was not determined.

(B) Flow cytometry analysis of monocytes ($CD11b^{+}CD163^{-}$) and macrophages ($CD11b^{+}CD163^{+}$) infiltrating 3 human sarcoma samples.

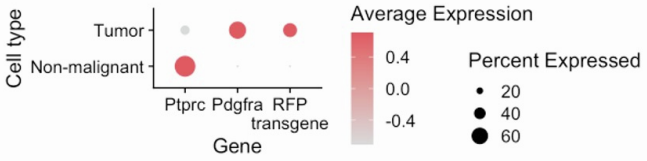
(C) Gating strategy to define T cells and myeloid cells from the mouse sarcoma samples.

(D) Representative pictures of relative sarcoma size after treatment with anti-CSF1R antibodies.

(E) mRNA expression of *Arg1* and *Nos2* (iNOS) in tumor-infiltrating myeloid cells (CD11b+) compared to tumor-naïve myeloid cells isolated from healthy mouse spleen (left panel). Expression of *Arg1* and *Nos2* in BMDM, cultured in normal medium or in medium conditioned by sarcoma cells (right panel).

Tessaro et al, Figure S2

A



B

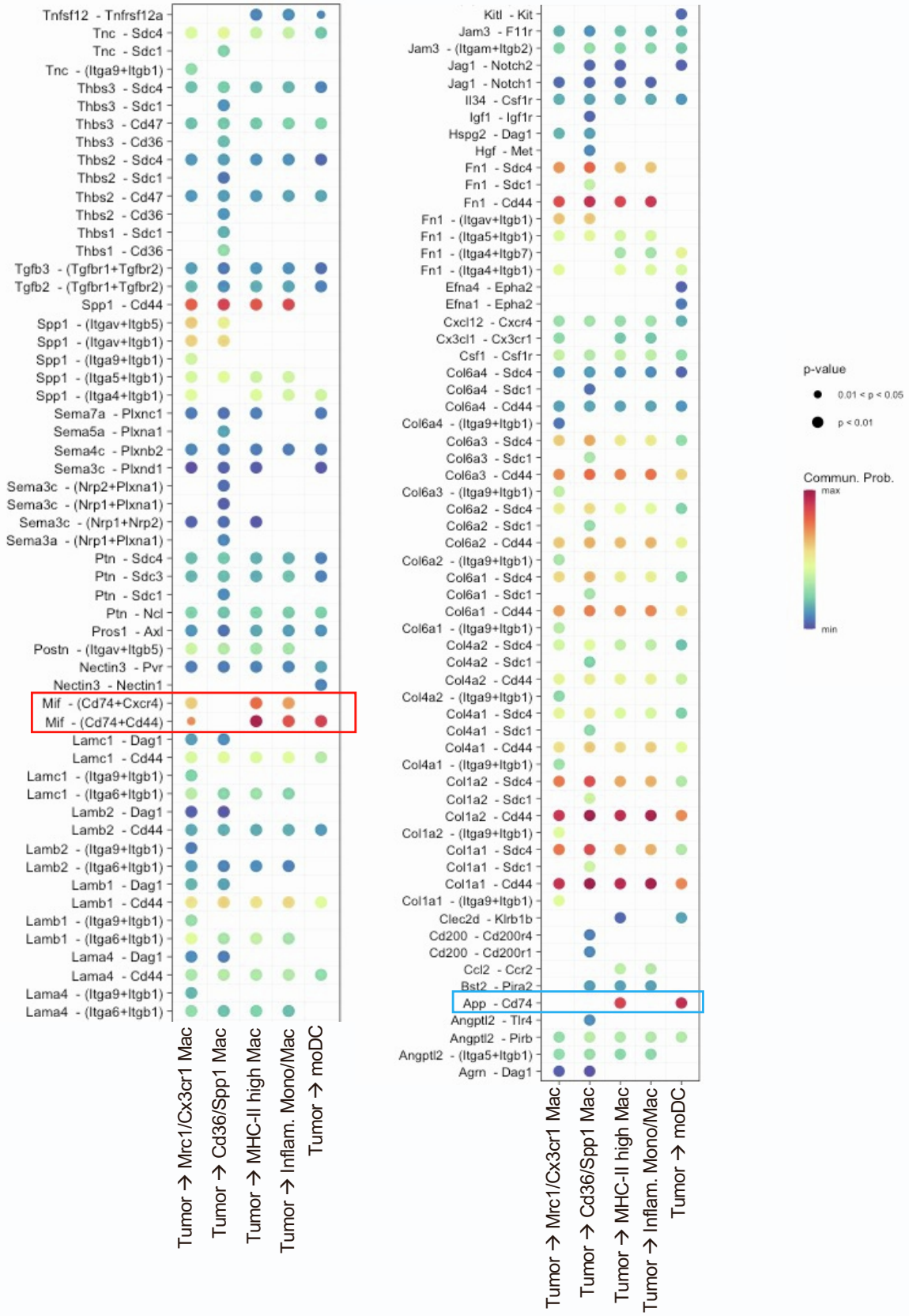
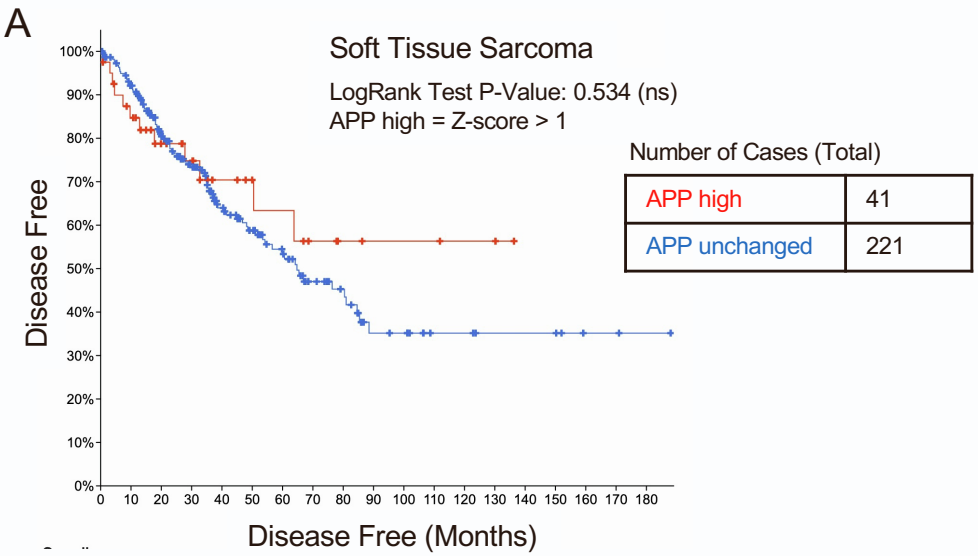
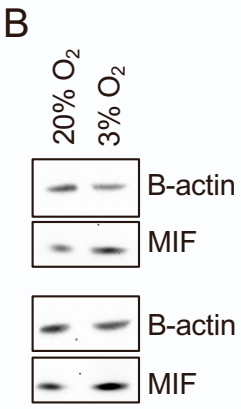


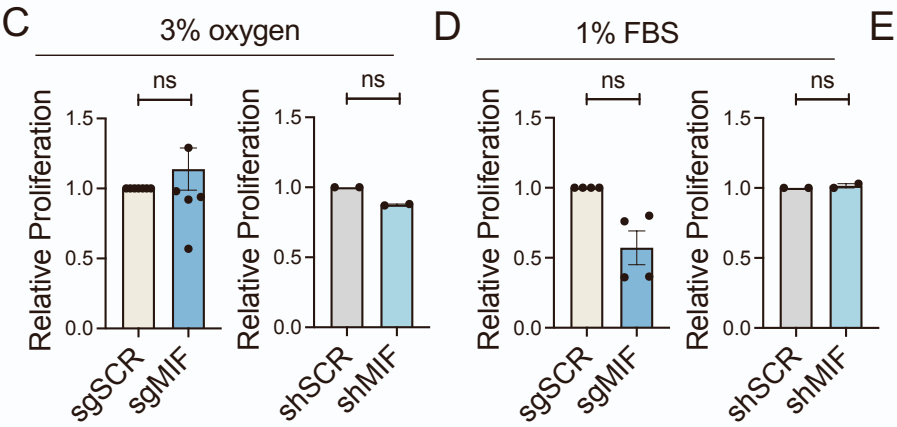
Figure S2. Ligand-receptor pairs mediate interactions between tumor and myeloid cells, related to Figure 2.

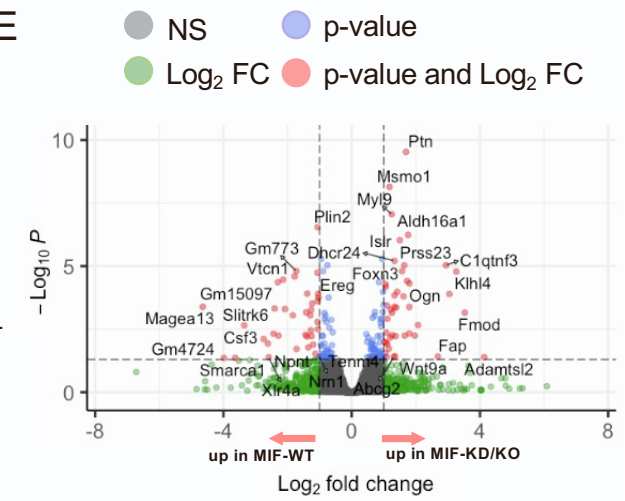
(**A**) Specific expression of the red fluorescent transgene (RFP) in the scRNAseq cell cluster annotated as Tumor. The sequence of this transgene was appended to the reference mouse genome prior to alignment of FASTQ files.

(**B**) All ligand-receptor signaling pairs from tumor to myeloid cells which were identified in CellChatDB to be statistically significant in at least one celltype-celltype pairing, based on the differentially expressed signaling genes of each cell type. High-probability interactions from tumor-expressed *Mif* or tumor-expressed *App* to myeloid-expressed *Cd74* are highlighted (red box and blue boxes, respectively).


A
Soft Tissue Sarcoma
LogRank Test P-Value: 0.534 (ns)
APP high = Z-score > 1
Number of Cases (Total)
APP high 41
APP unchanged 221
Disease Free
Disease Free (Months)


B
20% O2
3% O2
B-actin
MIF
B-actin
MIF


C
3% oxygen
D
1% FBS
Relative Proliferation
ns
sgSCR
sgMIF
shSCR
shMIF


E
NS
p-value
Log2 FC
p-value and Log2 FC
−Log10 P
up in MIF-WT
up in MIF-KD/KO
Log2 fold change

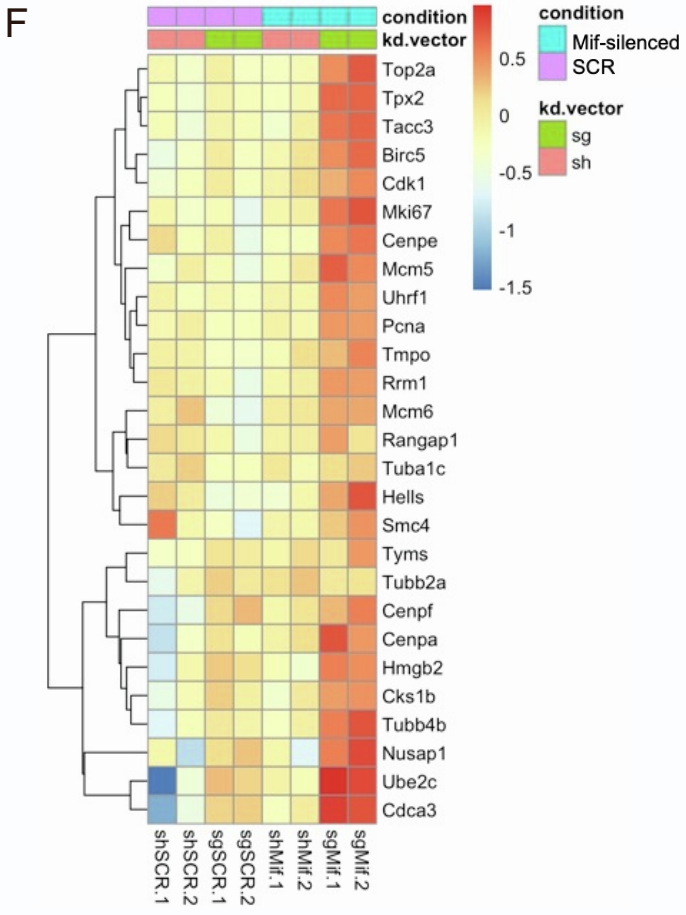

F
condition
kd.vector
Mif-silenced
SCR
sg
sh
shSCR.1
shSCR.2
sgSCR.1
sgSCR.2
shMif.1
shMif.2
sgMif.1
sgMif.2

Figure S3. MIF silencing in the tumor cells reduces sarcoma growth through non-cell autonomous mechanisms, related to Figure 3.

(A) Survival analysis of all soft-tissue sarcoma patients in the TCGA, stratified by APP mRNA expression level.

(B) Expression levels of the MIF protein in sarcoma cells grown in normal oxygen concentration (20%) or in hypoxic conditions (3%).

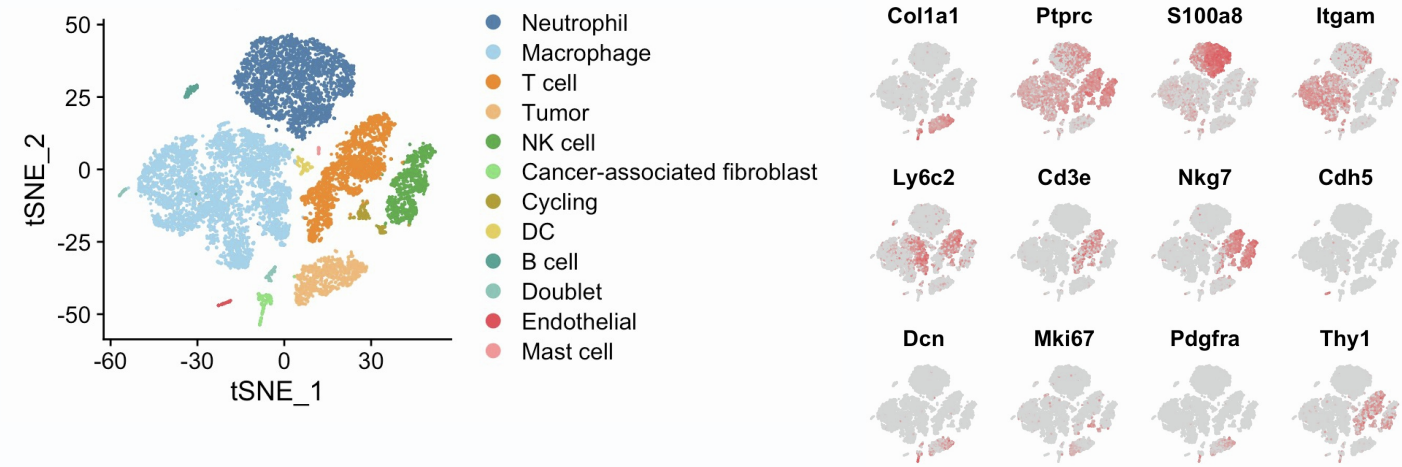
(C) Relative proliferation *in vitro* of MIF-KO (sgMif, left panel) or MIF-KD (shMif, right panel) sarcoma cells compared to MIF-WT cells, when maintained in hypoxic conditions (3% O_2).

(D) Relative proliferation *in vitro* of MIF-KO (sgMif, left panel) or MIF-KD (shMif, right panel) sarcoma cells compared to MIF-WT cells, when maintained in starvation conditions (1% FBS).

(E) Volcano plot (top) and heat map (bottom) showing the most differentially expressed genes in bulk RNA-sequencing of FACS-sorted tumor cells *ex vivo* (MIF-KO/KD vs MIF-WT sarcoma cells). Dotted lines are placed at a significance threshold equivalent to 5% FDR, and absolute log2 fold-change greater than 1. Red points indicate significant genes.

(F) Heat map of the top differentially expressed genes related to cell cycle and proliferation. Data are derived from bulk RNA-sequencing of FACS-sorted tumor *cells ex vivo*.

A


tSNE_2
tSNE_1
50
25
0
-25
-50
-60
-30
0
30
Neutrophil
Macrophage
T cell
Tumor
NK cell
Cancer-associated fibroblast
Cycling
DC
B cell
Doublet
Endothelial
Mast cell
Col1a1
Ptprc
S100a8
Itgam
Ly6c2
Cd3e
Nkg7
Cdh5
Dcn
Mki67
Pdgfra
Thy1

B

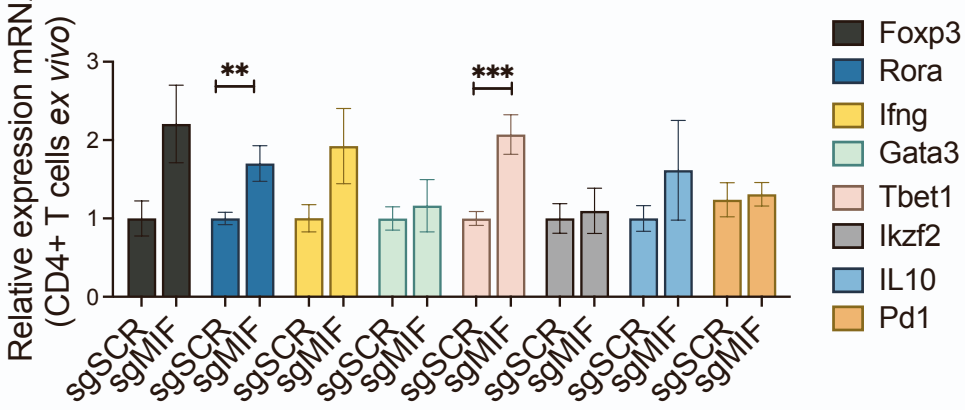

Relative expression mRNA
(CD4+ T cells ex vivo)
3
2
1
0
**
***
sgSCR
sgMIF
Foxp3
Rora
Ifng
Gata3
Tbet1
Ikzf2
IL10
Pd1

Figure S4. MIF silencing in the tumor cells re-shape the tumor microenvironment, related to Figure 4.

(A) tSNE plot of major cell populations defined by single-cell RNA-sequencing of MIF-WT (sgSCR) and MIF-KO (sgMIF) tumors. Top marker genes are overlaid on the right.

(B) Differential expression of genes related to the activation state of CD4+ T cells in FACS-sorted from the MIF-WT (sgSCR) and MIF-KO (sgMIF) tumors.