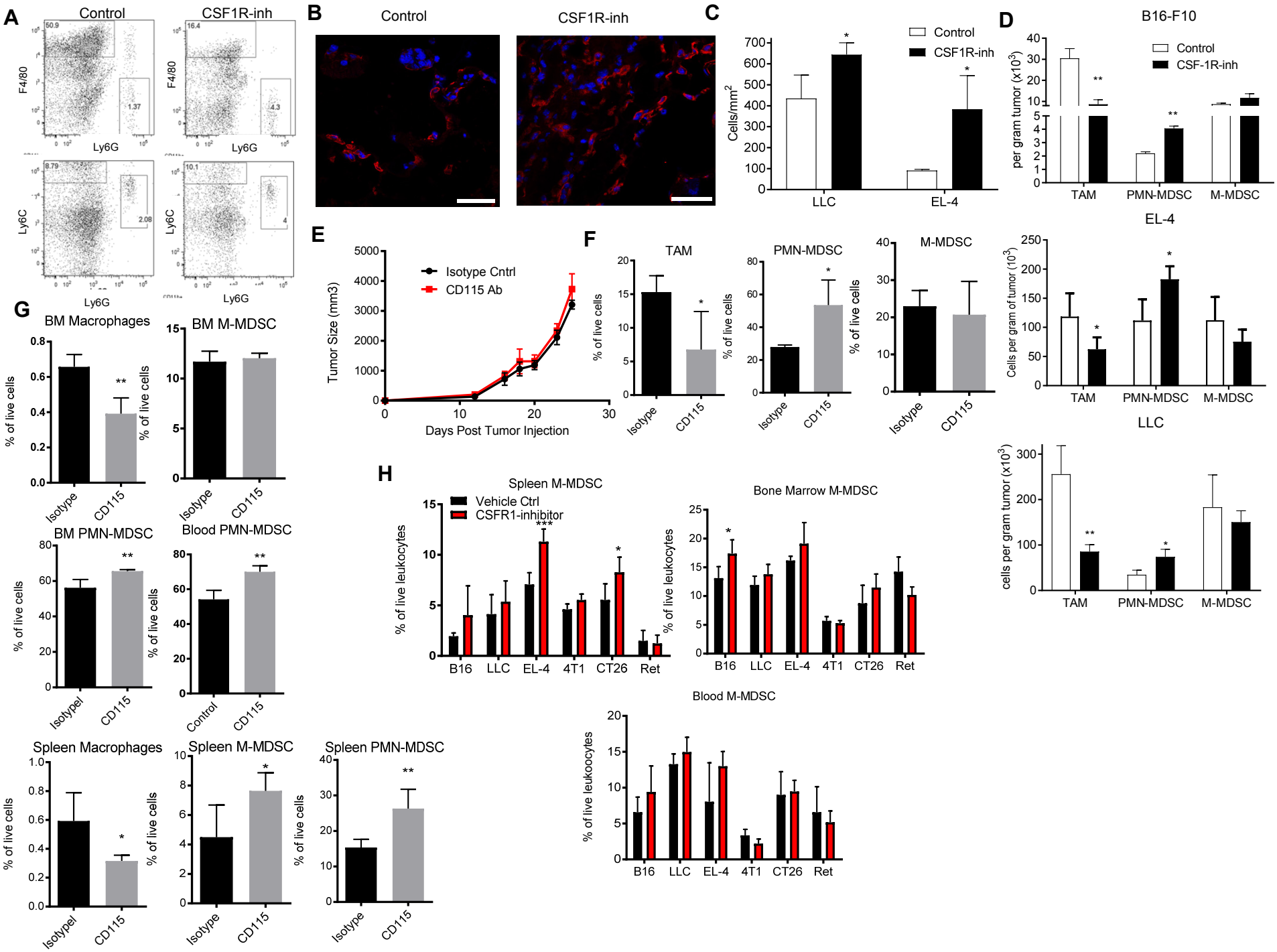

A
Control
CSF1R-inh
F4/80
Ly6G
Ly6C
50.9
1.37
16.4
4.3
8.79
2.08
10.1
4
B
Control
CSF1R-inh
C
Control
CSF1R-inh
Cells/mm2
LLC
EL-4
D
B16-F10
Control
CSF-1R-inh
per gram tumor (x10^3)
TAM
PMN-MDSC
M-MDSC
EL-4
Cells per gram of tumor (10^3)
LLC
cells per gram tumor (x10^3)
E
Isotype Cntrl
CD115 Ab
Tumor Size (mm3)
Days Post Tumor Injection
F
TAM
PMN-MDSC
M-MDSC
% of live cells
Isotype
CD115
G
BM Macrophages
BM M-MDSC
BM PMN-MDSC
Blood PMN-MDSC
Spleen Macrophages
Spleen M-MDSC
Spleen PMN-MDSC
% of live cells
Isotype
CD115
Isotypel
Control
H
Spleen M-MDSC
Vehicle Ctrl
CSFR1-inhibitor
Bone Marrow M-MDSC
Blood M-MDSC
% of live leukocytes
B16
LLC
EL-4
4T1
CT26
Ret

Figure S1. Related to Figure 1: PMN-MDSC in tumor tissues of mice treated with CSF-1R inhibitor. **A**. A Typical example of staining of tumor tissues of TRAMP mice treated with vehicle (control) or JNJ-40346527 (CSF-1R-inh). **B**. A Typical example of staining of tumor tissues with Ly6G antibody. Scale bar = 50 μm. **C**. The number of Ly6G+ cells per mm2 in mice treated with JNJ-40346527. Each group included 4 mice. * - $p<0.05$ between groups. **D**. The number of myeloid cells in tumor tissues of mice treated with JNJ-40346527 (CSF1R inh) or vehicle (control) 10 days after tumor inoculation. Tumor model is indicated on the graph. Each group included 4 mice. * - $p<0.05$; ** - $p<0.01$ between groups. **E**. Effect of 3-week treatment of CD115 ab (250 μg/mouse, twice a week) on tumor growth in LLC tumor model (n=5). **F**. Proportion of myeloid cells in tumors. **G**. Proportion of myeloid cells in BM and spleens of the mice. Mean and SD from 4 mice are shown. * - $p<0.05$; ** - $p<0.01$ between groups. **H**. Proportion of M-MDSC in different tissues of mice treated with CSF-1R inhibitor (n=4). In all experiments Mean and SD are shown. p values were calculated using Student's t-test.

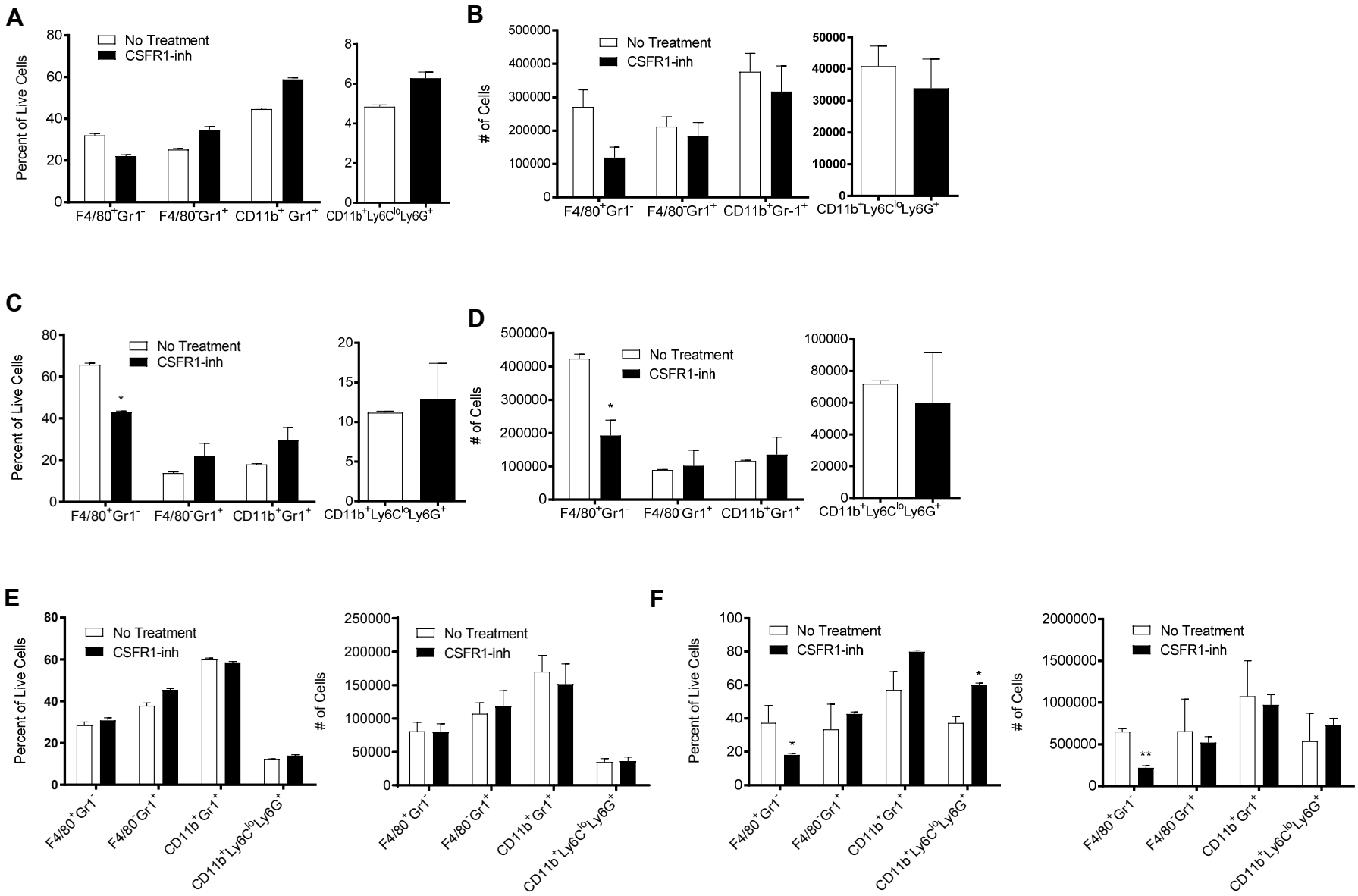

A
B
C
D
E
F
No Treatment
CSFR1-inh
Percent of Live Cells
# of Cells
F4/80+Gr1-
F4/80-Gr1+
CD11b+ Gr1+
CD11b+Gr-1+
CD11b+Ly6CloLy6G+
*
**

Figure S2. Related to Figure 2: Effect of JNJ-40346527 on myeloid cell differentiation. A-D. Percentage and absolute number of cells generated from enriched BM HPC during 3-day (**A,B**) or 6-day (**C,D**) culture with GM-CSF and JNJ-40346527. **E, F** – Percentage and absolute number of cells generated from enriched BM HPC without treatment with TES. 3 days (E) and 6 days (F). Each group included 3 replicates. * - $p<0.05$; ** - $p<0.01$ from no JNJ-40346527 group.

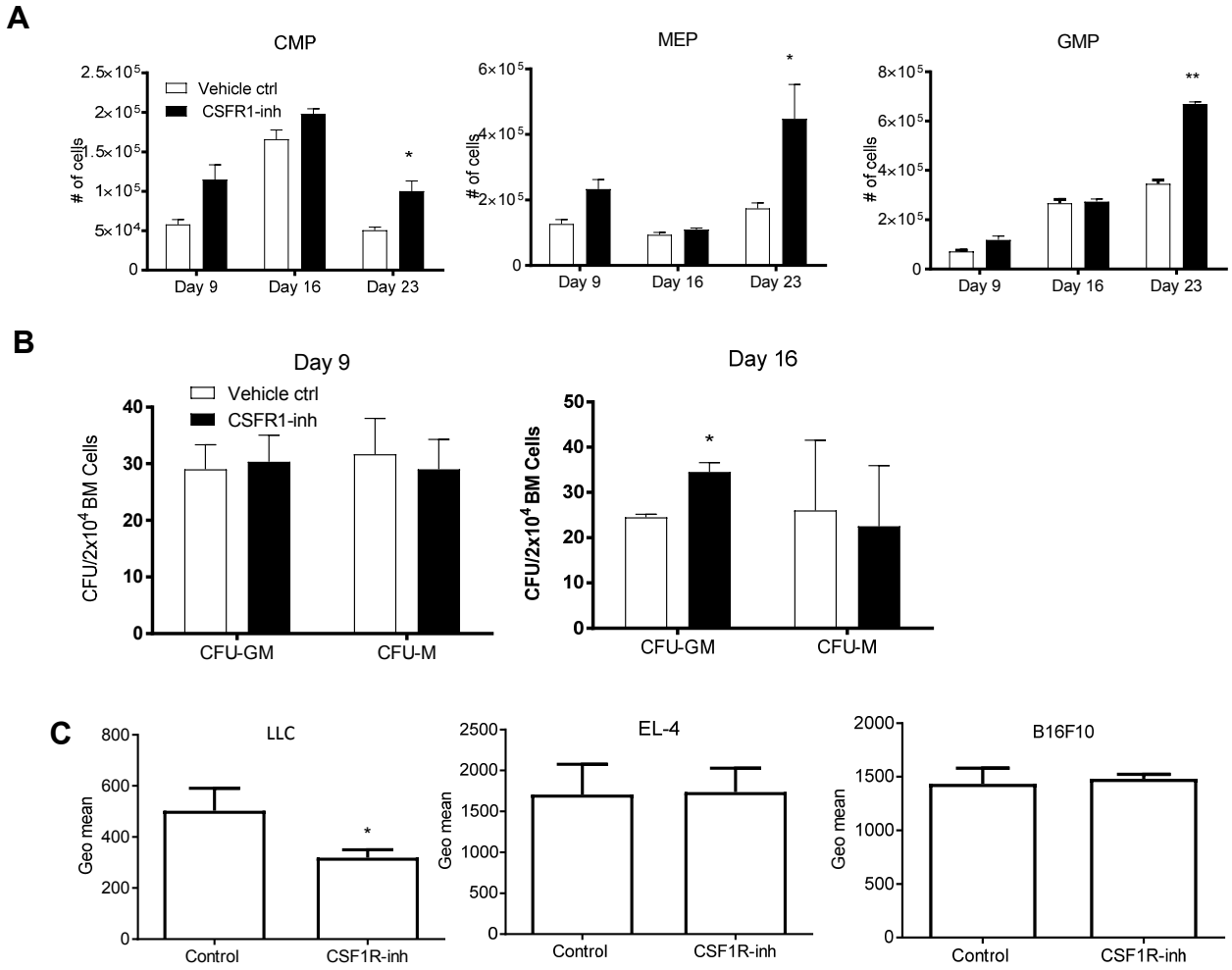

A
CMP
MEP
GMP
Vehicle ctrl
CSFR1-inh
# of cells
Day 9
Day 16
Day 23
B
Day 9
Day 16
CFU/2x10^4 BM Cells
CFU-GM
CFU-M
C
LLC
EL-4
B16F10
Geo mean
Control
CSF1R-inh

Figure S3. Related to Figure 2: Effect of JNJ-40346527 on myeloid progenitors. A. Effect of JNJ-40346527 on CMP and GMP in BM at different time during the treatment. **B**. Effect of JNJ-40346527 treatment on BM cell colony formation (n=3). Treatment was performed as described in Figure 2. **C**. Expression of CXCR2 on BM PMN-MDSC of mice treated with CSF1R inhibitor for 3 weeks. Each experiment included 3 mice. * - $p<0.05$; between groups.

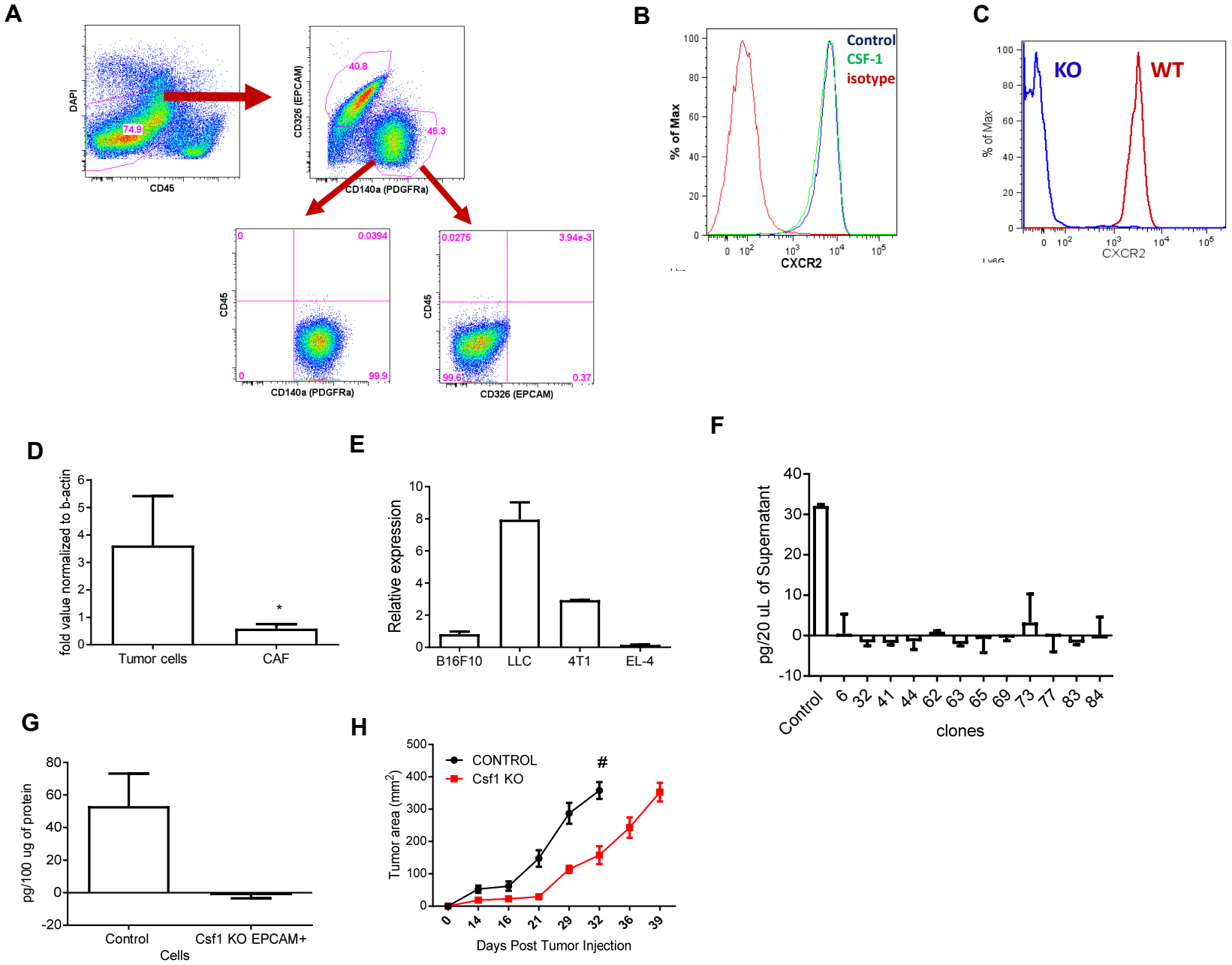

A
DAPI
CD45
74.9
CD326 (EPCAM)
CD140a (PDGFRa)
40.8
45.3
CD45
CD140a (PDGFRa)
0
0.0394
0
99.9
CD45
CD326 (EPCAM)
0.0275
3.94e-3
99.63
0.37
B
% of Max
CXCR2
Control
CSF-1
isotype
C
KO
WT
% of Max
CXCR2
D
fold value normalized to b-actin
Tumor cells
CAF
*
E
Relative expression
B16F10
LLC
4T1
EL-4
F
pg/20 uL of Supernatant
Control
6
32
41
44
62
63
65
69
73
77
83
84
clones
G
pg/100 ug of protein
Control
Csf1 KO EPCAM+
Cells
H
Tumor area (mm2)
CONTROL
Csf1 KO
#
0
14
16
21
29
32
36
39
Days Post Tumor Injection

Figure S4. Related to Figure 4. Deletion of CSF1 in tumor cells. A. Typical gating and sorting strategy for isolation of fibroblasts. **B**. Expression of CXCR2 on neutrophils cultured for 24 hr with CSF1. **C**. Expression of CXCR2 on neutrophils in peripheral blood of mice 5 weeks after reconstitution with wild-type (WT) and CXCR2-KO bone marrow. **D**. Expression of csf1 mRNA (qPCR) in CAF and tumor cells in LLC-TB mice (n=3). **E**. Expression of csf1 mRNA (qPCR) in different tumor cell lines. **F**. csf1 expression in different clones of LLC tumor cells after CRISPR-CAS9 deletion of csf1. **G**. Clone of LLC tumor cells lacking Csf1 production after 3 weeks of tumor growth in vivo. **H**. Tumor growth of WT and M-CSF deleted LLC tumor cells (n=5).

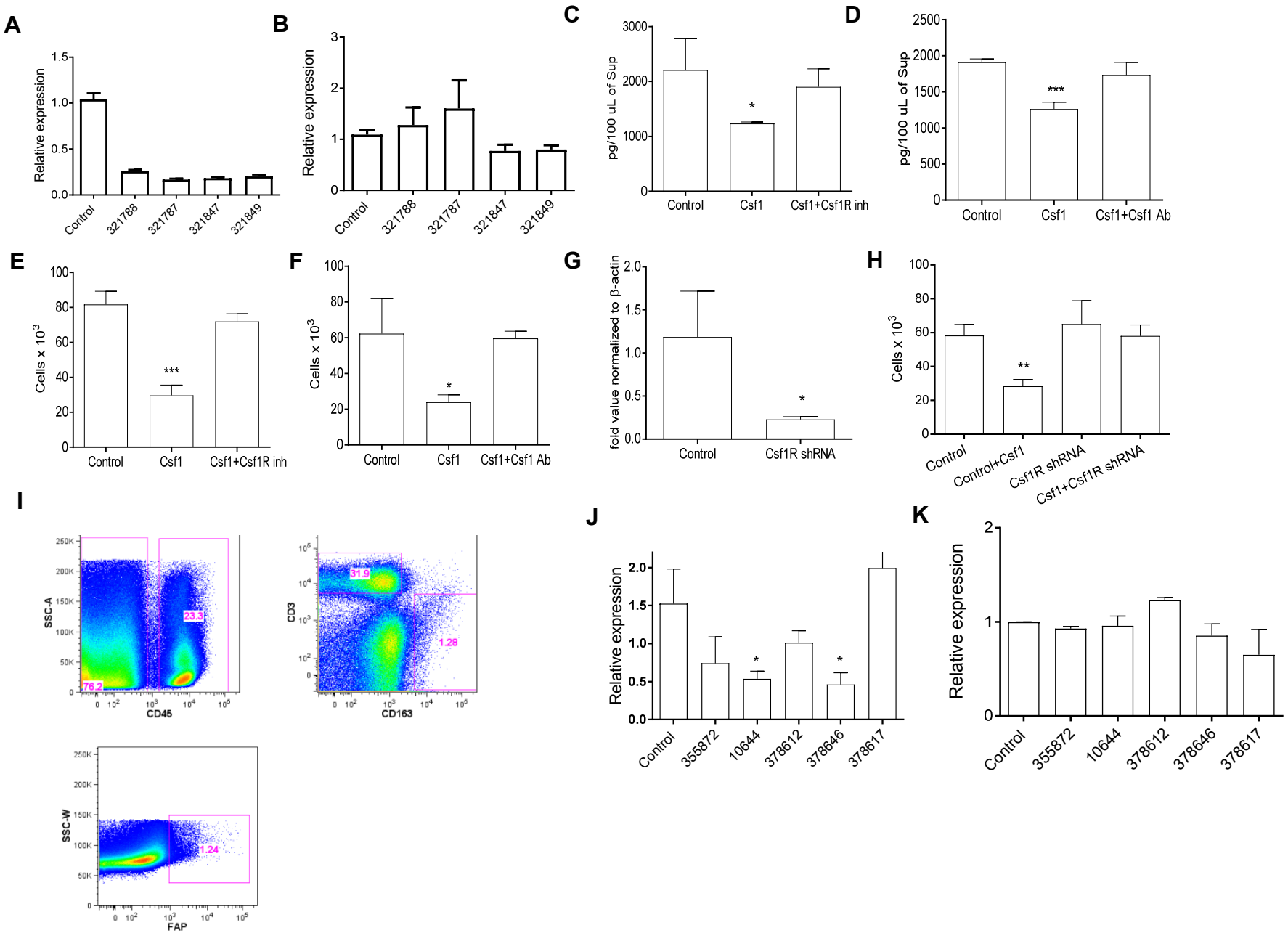
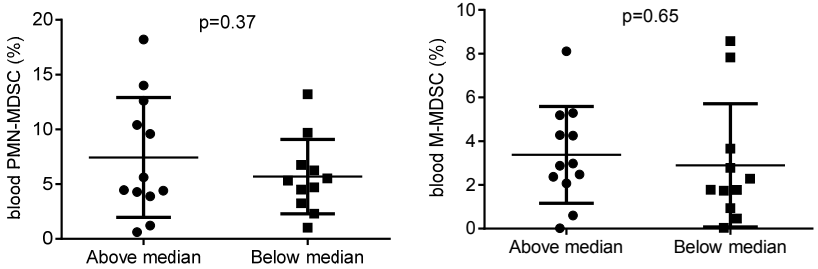
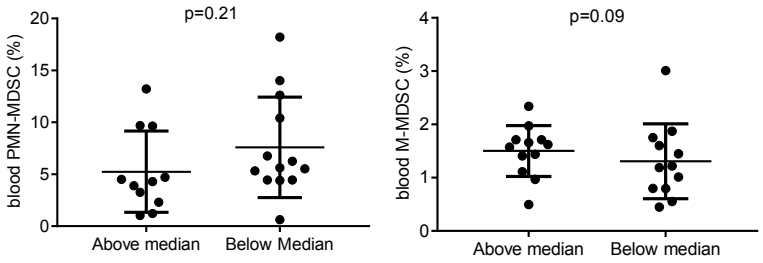

A
Relative expression
Control
321788
321787
321847
321849
B
Relative expression
C
pg/100 uL of Sup
Control
Csf1
Csf1+Csf1R inh
D
pg/100 uL of Sup
Control
Csf1
Csf1+Csf1 Ab
E
Cells x 10^3
Control
Csf1
Csf1+Csf1R inh
F
Cells x 10^3
Control
Csf1
Csf1+Csf1 Ab
G
fold value normalized to β-actin
Control
Csf1R shRNA
H
Cells x 10^3
Control
Control+Csf1
Csf1R shRNA
Csf1+Csf1R shRNA
I
SSC-A
CD45
76.2
23.3
CD3
CD163
31.9
1.28
SSC-W
FAP
1.24
J
Relative expression
Control
355872
10644
378612
378646
378617
K
Relative expression
Control
355872
10644
378612
378646
378617

Figure S5. Related to Figure 5: CSF1 shRNA in fibroblasts. **A, B**. Mouse lung fibroblasts transduced with different csf1r constructs. Expression of csf1r (A) and pdgfra (B) are shown. Constructs 321847 and 321849 were used in the studies. Control - lentivirus with empty vector. **C, D**. CAF isolated from tumors of LLC TB mice were cultured with Csf1 (20 ng/ml) in the presence or absence of Csf1R inh (**C**) or Csf1 neutralizing Ab (5ug/ml) (**D**) for 4 days. Cxcl1 protein in supernatants was measured by ELISA. Cumulative results of three experiments (Mean and SD) are shown. **E,F**. CAF isolated from tumors of LLC TB mice were treated with CSF-1 alone or with either CSF-1R inh (**E**) or CSF-1 neutralizing antibody (**F**). Supernatants collected from culture were used for BM neutrophils chemotaxis. Mean and SD, n=3 are shown. * $p<0.05$; ***$p<0.001$. **G**. CAF were transduced either with control vector or Csf1r shRNA construct. Expression of csf-1r was analyzed by qPCR. β-actin was used as a loading control. **H**. CAF transduced either with lentiviral control vector or Csf1R shRNA construct were treated with Csf1 for 4 days. Supernatants obtained from culture were used for BM PMN-MDSC chemotaxis. Mean and SD, n=3 are shown. ** $p<0.01$. **I**. Gating strategy for analysis of cells in human tumors; **J, K**. Human fibroblasts from NSCLC patient transduced with different CSF1R shRNA constructs. Expression of CSF1R (**J**) and PDGFAR (**K**) are shown. Constructs 10644 and 378646 were used in the studies. Construct 378617 was used as additional control. Control - lentivirus with empty vector. * - significant ($p<0.05$) differences from control.

A


blood PMN-MDSC (%)
20
15
10
5
0
p=0.37
Above median
Below median
blood M-MDSC (%)
10
8
6
4
2
0
p=0.65
Above median
Below median

B


blood PMN-MDSC (%)
20
15
10
5
0
p=0.21
Above median
Below Median
blood M-MDSC (%)
4
3
2
1
0
p=0.09
Above median
Below median

C

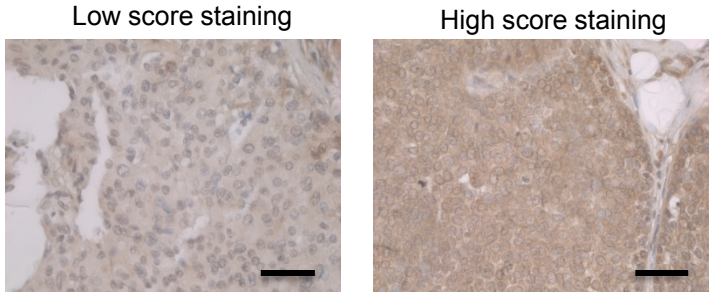

Low score staining
High score staining

Figure S6. Related to Figure 6: MDSC in peripheral blood of cancer patients. A. Proportion of PMN-MDSC (left) and M-MDSC (right) in peripheral blood of samples split based on CXCL8 levels in tumors. **B**. Proportion of PMN-MDSC (left) and M-MDSC (right) in peripheral blood of samples split based on CSF-1 levels in tumors. **C**. Examples of staining of tissues for CSF-1. Scale bar – 50 μm.

Table S1, related to STAR Methods Sequence of primers used for qPCR

Murine

Mif **-** F-CAT CGC AGT GCA CGT GGT CC; R- GGC ACC ACC GAT CTT GCC GA
cxcl-1 **-** F- CGA GGC TTG CCT TGA CCC TGA A; R- GGG TCA TAT GCC AGT CCC ACC
cxcl-2 **-** F-TTT GCC TTG ACC CTG AAG CCC C; R- TTC CGT TGA GGG ACA GCA GCC
cxcl-5 **-** F- GCA TCC CCA GCG GTT CCA TCT; R- GCT CCG TTG CGG CTA TGA CTG A
cxcl-7**-**F- CTC AGA CCT ACA TCG TCC TGC ACC; R- CTG TAA GGG GAG CCA GCG CA
cxcl-12**-**F-GAG GAA GGC TGA CAT CCG TGG G; R-ATG ACC CCA GTC AGT GCT GTC C
Csf-1**-** F-CATCCAGGCAGAGACTGACA; R-CTTGCTGATCCTCCTTCCAG
ccl-2**-**F-CCCAATGAGTAGGCTGGAGA; R-AAAATGGATCCACACCTTGC
ccl-3**-**F-CCA AGT CTT CTC AGC GCC ATA-3' ; R-GAT GAA TTG GCG TGG AAT CTT C-3'
ccl-4**-**F-TGC TCG TGG CTG CCT TCT-3' ; R-CTG CCG GGA GGT GTA AGA GA-3'
ccl-5**-**F-TGC CCA CGT CAA GGA GTA TT-3’; R-CAG GAC CGG AGT GGG AGT A-3’
ccl-6**-**F- 5’ ATGAGAAACTCCAAGACTGCC- 3’; R- 5’ TTATTGGAGGGTTATAGCGACG -3’
ccl-7**-**F- CCA AGT GTG GGC CCA ACC AGA T; R- GCT TCC CAG GGA CAC CGA CT
pdgfra-F- AGAAAATCCGATACCCGGAG; R- AGAGGAGGAGCTTGAGGGAG
csf-1r-F- TTGCCTTCGTATCTCTCGATG; R- CTCTGCTGGTGCTACTGCTG
β-actin-F-ACCGCTCGTTGCCAATAGTGATGA-3’; R -TGAGAGGGAAATCGTGCGTGACAT-3’

Human

Cxcl-8**-**F- GACCACACTGCGCCAACAC; R- CTTCTCCACAACCCTCTGCAC
Pdgfra- F- GCTCAGCCCTGTGAGAAGAC; R- ATTGCGGAATAACATCGGAG
Csf-1r-F- TTGGGCTGATCCTCTTCTTC; R- AAAGCGTGAGAGCACGAAGT
Gapdh**-**F- GCATCTTCTTTTGCGTCG; R- GACCAAATCCGTTGACTC