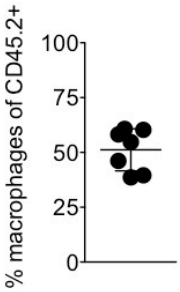
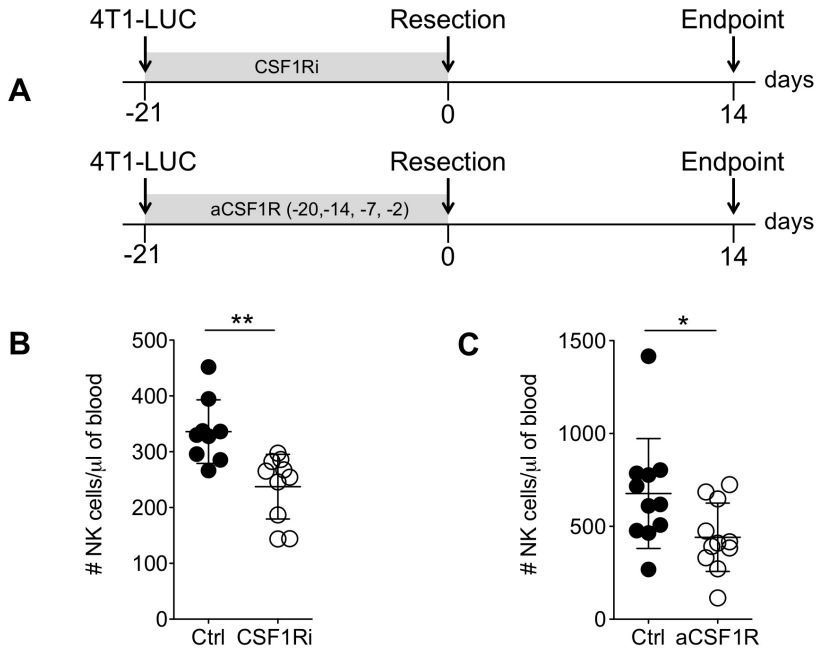


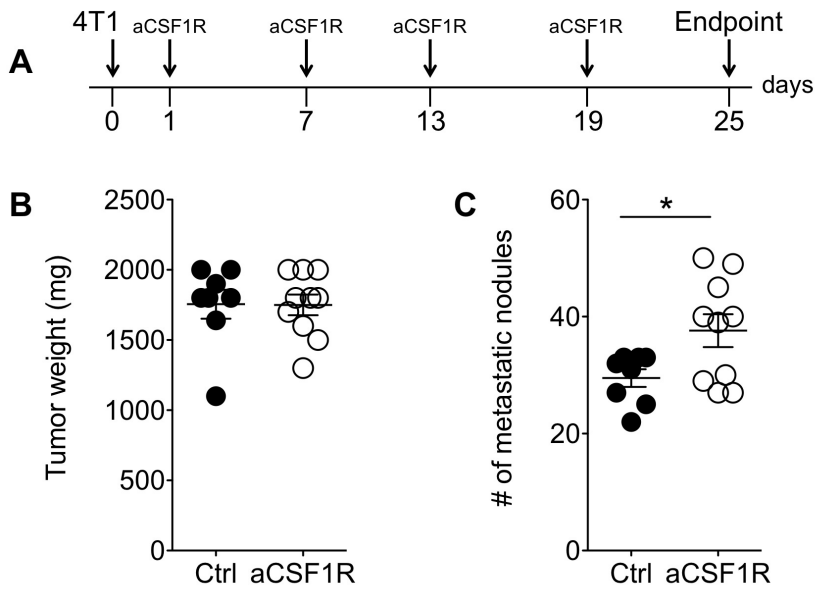
Supplementary Figure S1. A major proportion of tumor-infiltrating leukocytes consists of macrophages.

Percentage of macrophages (defined as $CD45.2^+$ $CD11b^+$ $Ly6G^-$ $F4/80^+$ $Ly6C^{int}$ cells) within live $CD45.2^+$ singlet cells associated with a primary LLC-LUC tumor at day 28 after tumor cell injection. Symbols represent individual mice (n=7). A representative experiment of 2 is shown.



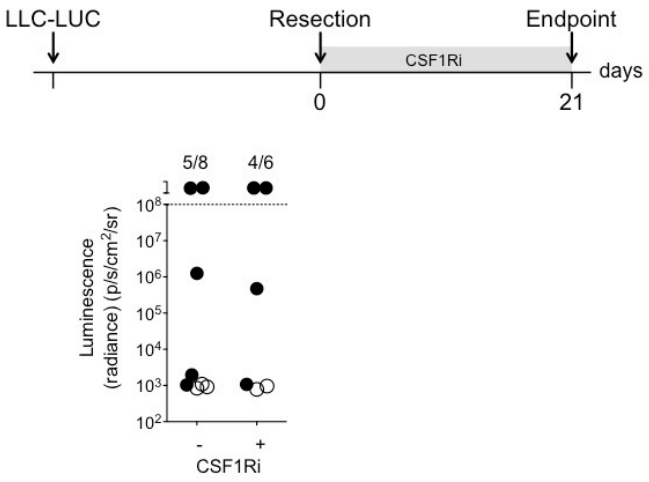
Supplementary Figure S2. Blocking CSF1R results in loss of circulating NK cells.

Spontaneous lung metastasis from autochthonous 4T1 tumors in BALB/c mice (**A**) Experimental timeline. Mice were treated daily with a small-molecule inhibitor of CSF1R (B) or with blocking anti-CSF1R antibody on the indicated days (C). **(B,C)** Number of circulating NK cells ($CD45^+CD3^-NK1.1^+$ after gating on live singlets) at resection. Mean ± SD. **p*<0.05 (two-tailed Student's t-test with Welch's correction). Each symbol represents an individual mouse. (B) Ctrl, n=9; aCSF1R, n=10. (C) Ctrl, n=11; aCSF1r, n=11. A representative experiment of 2 is shown.



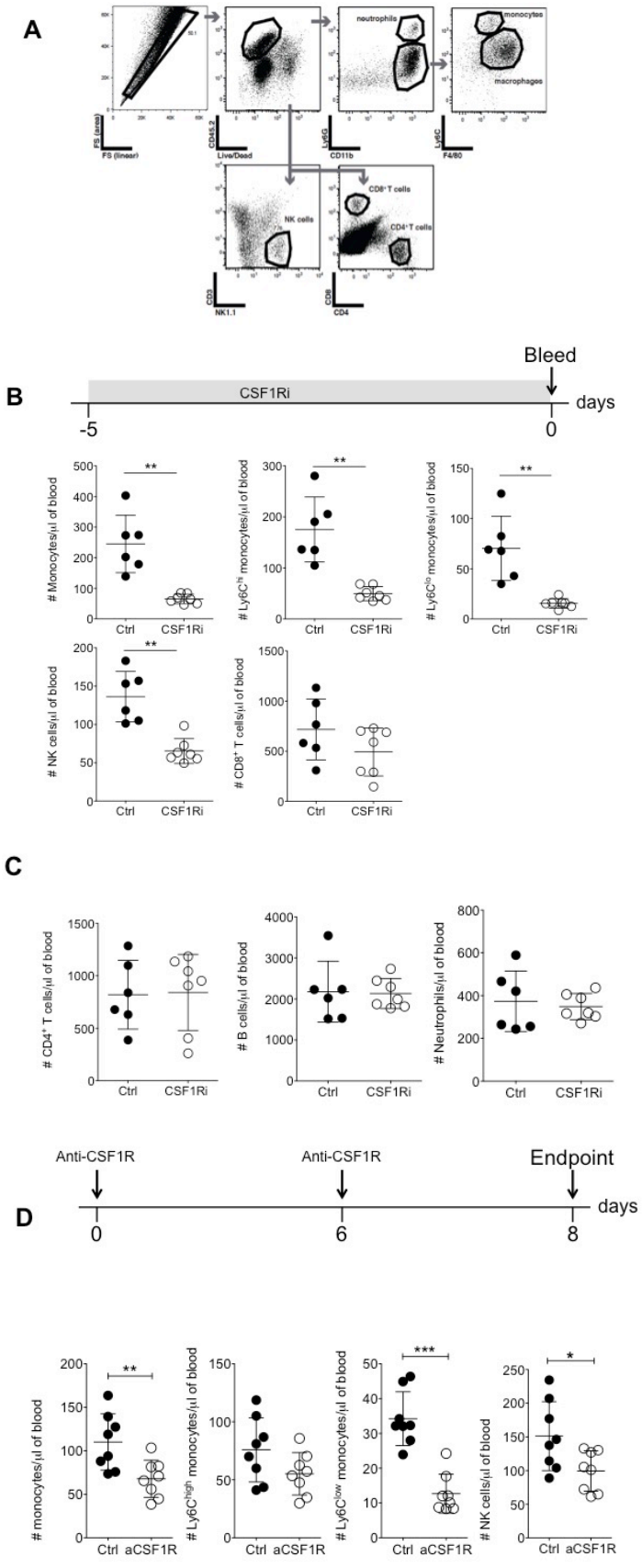
Supplementary Figure S3. Blocking CSF1R increases the risk of developing metastatic disease.

Spontaneous lung metastasis from autochthonous 4T1 tumors in BALB/c mice. (**B**) Primary tumor weight at end point. **(C)** Number of metastatic nodules in lung at the end point. (B,C) Ctrl, n=8; aCSF1R, n=10. Mean ± SD. *p<0.05 (two-tailed Student's t-test with Welch's correction). Each symbol represents an individual mouse. A representative experiment of 2 is shown.



Supplementary Figure S4. Adjuvant treatment with CSF1Ri has no impact on the metastatic load.

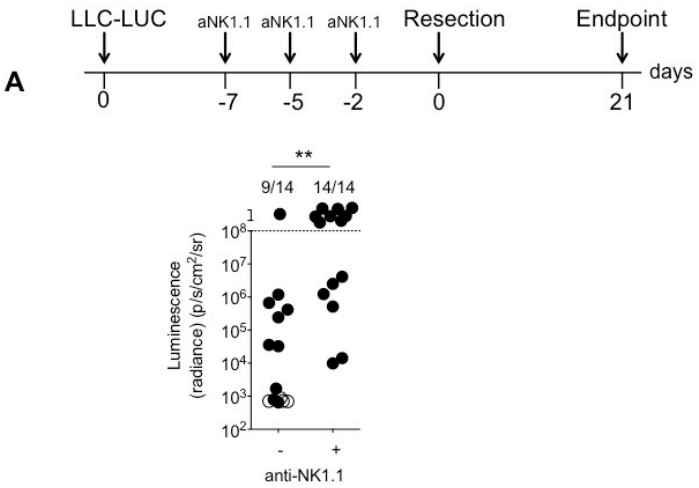
Metastatic burden in mice treated according to the experimental timeline. Each symbol represents a sum of luminescent signal from lung, liver and two tumor-draining lymph nodes. Filled circles represent mice with detectable metastasis, open circles represent metastasis-free mice. Mice above the dotted line had to be sacrifice before the endpoint due to metastatic burden. Numbers above the graph show number of mice with metastasis in total cohort of mice (Ctrl, n=8; CSF1Ri, n=6). Both groups are not statistically significantly different (Chi-square test).


A
neutrophils
monocytes
macrophages
FS (area)
FS (linear)
CD45.2
Live/Dead
Ly6G
CD11b
Ly6C
F4/80
NK cells
CD8+ T cells
CD4+ T cells
CD3
NK1.1
CD8
CD4
B
Bleed
CSF1Ri
days
-5
0
Monocytes/µl of blood
Ly6C^hi monocytes/µl of blood
Ly6C^lo monocytes/µl of blood
NK cells/µl of blood
CD8+ T cells/µl of blood
Ctrl
CSF1Ri
**
C
CD4+ T cells/µl of blood
B cells/µl of blood
Neutrophils/µl of blood
D
Anti-CSF1R
Anti-CSF1R
Endpoint
days
0
6
8
monocytes/µl of blood
Ly6C^high monocytes/µl of blood
Ly6C^low monocytes/µl of blood
NK cells/µl of blood
Ctrl
aCSF1R
***
*

Supplementary Figure S5. Blocking CSF1R results in loss of circulating NK, $CD8^+$ and myeloid cells in non-tumor-bearing mice.

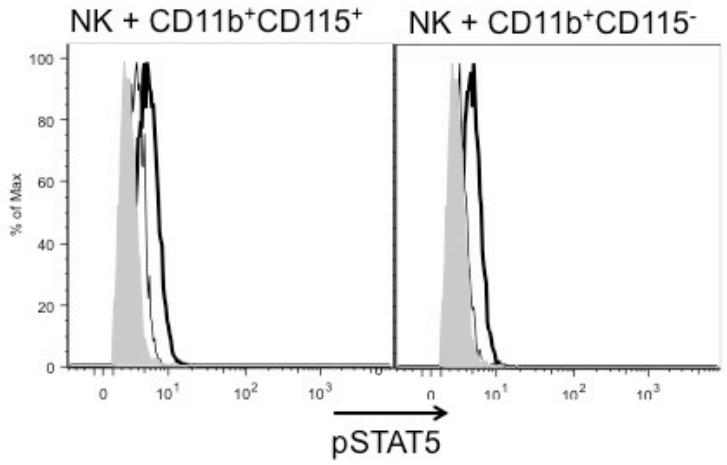
(A) Gating strategy for tumor infiltrating immune cells in LLC-LUC tumors. **(B,C)** Administration of CSF1Ri during 5 d and subsequent quantification by flow cytometry of **(B)** circulating monocytes, NK and $CD8^+$ T cells, and **(C)** circulating $CD4^+$ T cells, B cells and neutrophils. **(D)** Administration of anti-CSF1R results in loss of circulating monocytes, patrolling monocytes and NK cells. Each symbol represents an individual mouse. (B,C) Ctrl, n=6; CSF1Ri, n=7. (D) Ctrl and CSF1Ri, n=8.

Mean ± SD. *p<0.05, **p<0.01, ***p<0.005 (two-tailed Student's t-test with Welch's correction). A representative experiment of 2 is shown.

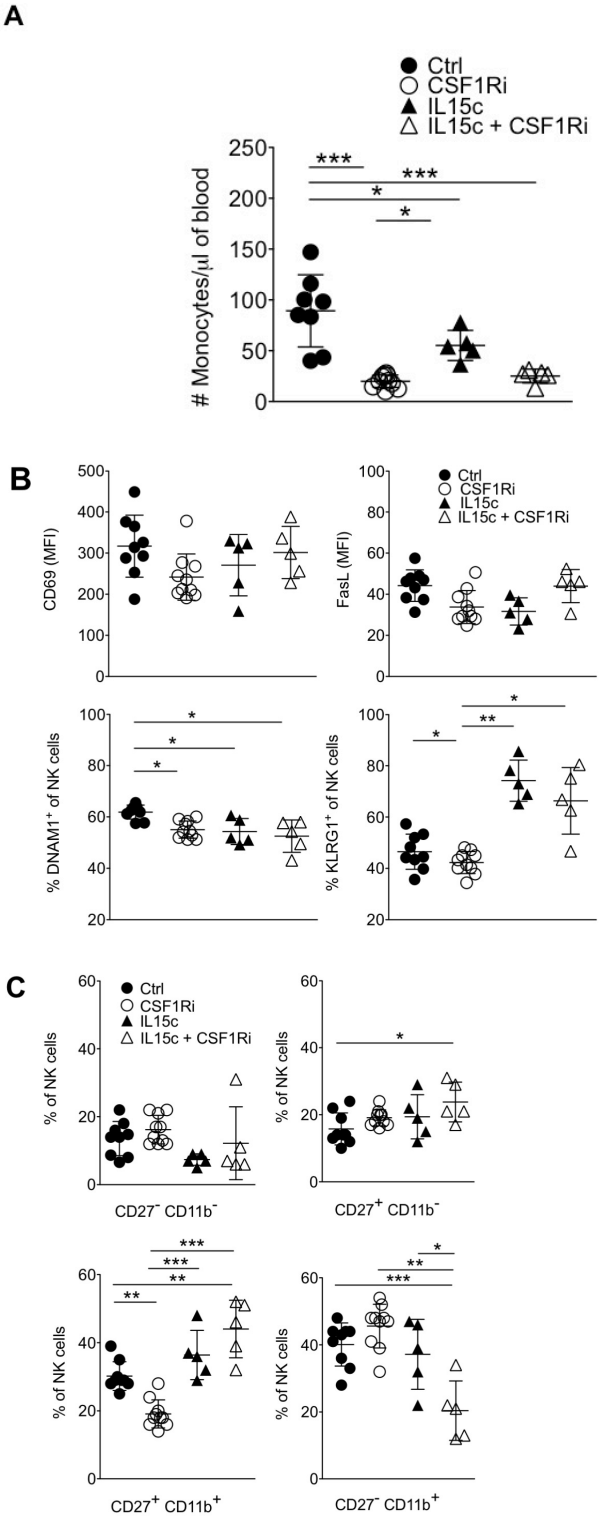


Supplementary Figure S6. NK cells control metastatic seeding of LLC-LUC cells to the lungs.

(A) Mice bearing subcutaneous LLC-LUC tumors were depleted from NK cells before resection. Spontaneous metastasis to the lungs is presented as sum of luminescent signals from lung, liver and both tumor-draining lymph nodes for each mouse. Filled circles represent mice with detectable metastasis, open circles represent metastasis-free mice. Mice above the dotted line had to be sacrificed before the endpoint due to metastatic burden. Numbers above the graph show number of mice with metastasis in total cohort of animals (n=14). $^{**}p<0.01$ (Chi-square test).



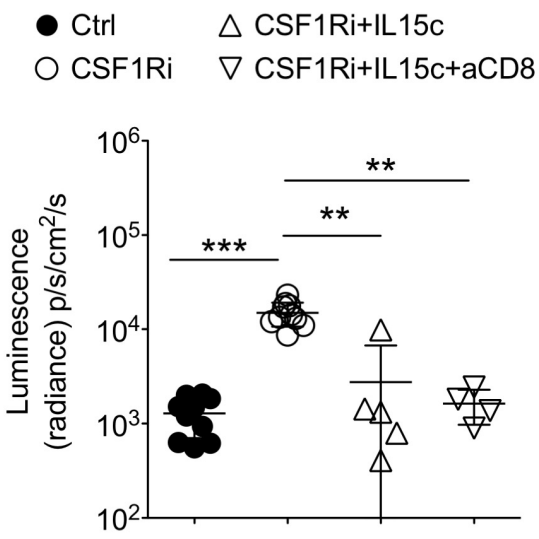
Supplementary Figure S7. Both CD115$^+$CD11b$^+$ and CD115$^-$CD11b$^+$ cells trans-present IL-15 to NK cells. Sorted C57BL/6 NK cells were incubated with CD11b$^+$CD115$^+$ or CD11b$^+$CD115$^-$ cells sorted from C57BL/6 or *Il15ra*$^{-/-}$ mice. Cells were subsequently stained for pSTAT5. The figure shows pSTAT5 staining after gating on sorted NK cells. Left panel: C57BL/6 NK cells were incubated with medium (filled grey), C57BL/6 CD11b$^+$CD115$^+$ (bold line) or *Il15ra*$^{-/-}$ CD11b$^+$CD115$^+$ cells (thin line). Right panel: C57BL/6 NK cells were incubated with medium (filled grey), C57BL/6 CD11b$^+$CD115$^-$ (bold line) or *Il15ra*$^{-/-}$ CD11b$^+$CD115$^-$ cells (thin line). One representative FACS plot of three is shown.


A
Ctrl
CSF1Ri
IL15c
IL15c + CSF1Ri
Monocytes/µl of blood
B
CD69 (MFI)
FasL (MFI)
% DNAM1+ of NK cells
% KLRG1+ of NK cells
C
% of NK cells
CD27- CD11b-
CD27+ CD11b-
CD27+ CD11b+
CD27- CD11b+

Supplementary Figure S8. The effect of CSF1Ri and/or exogenous IL-15c circulating monocytes and NK phenotype.

Mice were treated as described in Figure 4A. **(A)** Circulating monocyte counts determined by flow cytometry. **(B,C)** Circulating NK cells as shown in Figure 4C were further characterized by flow cytometry.

Each symbol represents an individual mouse. Ctrl, n=9; CSF1Ri, n=10; IL15c, n=5; CSF1Ri+IL15c, n=5. Mean ± SD. *p<0.05, **p<0.01, *** p<0.005 (one-way ANOVA with Bonferroni's correction). One-way ANOVA with Bonferroni's correction. A representative experiment of 2 is shown.



Supplementary Figure S9. Exogenous IL-15 prevents CSF1Ri-induced metastasis independently of CD8$^+$ T cells.

Mice were treated essentially as described in the legends to Figure 4: Daily CSF1Ri administration was started on day -5, IL-15c was given on day -3, 10^6 sorted NK cells (CD45.2$^+$CD3$^-$NK1.1$^+$ live singlets) were adoptively transferred on day 0, LLC-LUC cells were injected on day 0. The 4 different treatment groups are displayed in the figure. Quantification of tumor burden in resected lungs by bioluminescence. Each symbol represents an individual mouse. Ctrl, n=10; CSF1Ri, n=10; CSF1Ri+IL15c, n=5; CSF1Ri+IL15c+aCD8, n=4. Mean ± SD. **p<0.01, ***p<0.005 (one-way ANOVA with Bonferroni's correction). A representative experiment of 2 is shown.