

## SUPPLEMENTAL FIGURE LEGENDS

### Supplemental Figure 1: CCR2 expression in spontaneous MMTV-PyMT tumors and lungs

- a. Purity of cancer cells is greater than 90% after cell isolation, as determined by flow cytometry for EpCAM (black, a marker expressed on epithelial-derived MMTV-PyMT cells) compared to background (gray). Representative of three separate isolations.
- b. mRNA was collected from MMTV-PyMT;*Ccr2*<sup>+/+</sup>, MMTV-PyMT;*Ccr2*<sup>+/-</sup>, and MMTV-PyMT;*Ccr2*<sup>-/-</sup> tumor cells, and qPCR was performed for *Ccr2* and normalized to  $\beta$ -actin expression (mean +/- SEM,  $p < 0.05$ , one-way ANOVA;  $n = 3$ ).
- c. Tumors from *Ccr2*<sup>+/-</sup> cancer cells phenocopy those from *Ccr2*<sup>-/-</sup> cancer cells, regardless of whether the host is *Ccr2*<sup>+/+</sup> or *Ccr2*<sup>-/-</sup>. Tumor burden was determined by weekly caliper measurement (mean +/- SEM, two-way ANOVA;  $n = 8$  for all conditions).
- d. Metastatic burden is unchanged in MMTV-PyMT;*Ccr2*<sup>+/+</sup>, MMTV-PyMT;*Ccr2*<sup>+/-</sup>, and MMTV-PyMT;*Ccr2*<sup>-/-</sup> mice, as determined by quantification from H&E-stained lung sections, indicated as percentage of the area of the lung tissues (mean +/- SEM, ANOVA;  $n = 24$  MMTV-PyMT;*Ccr2*<sup>+/+</sup>, 33 MMTV-PyMT;*Ccr2*<sup>+/-</sup>, and 27 MMTV-PyMT;*Ccr2*<sup>-/-</sup> mice).
- e. The number of metastatic foci is unchanged in MMTV-PyMT;*Ccr2*<sup>+/+</sup>, MMTV-PyMT;*Ccr2*<sup>+/-</sup>, and MMTV-PyMT;*Ccr2*<sup>-/-</sup> mice. Mice were classified as having either  $\leq$  or  $>$  than 0.1 foci/mm<sup>2</sup> lung area (Chi-square test;  $n = 24$  MMTV-PyMT;*Ccr2*<sup>+/+</sup>, 33 MMTV-PyMT;*Ccr2*<sup>+/-</sup>, and 27 MMTV-PyMT;*Ccr2*<sup>-/-</sup> mice).
- f. The average size of the metastatic foci is decreased in MMTV-PyMT;*Ccr2*<sup>+/-</sup> and MMTV-PyMT;*Ccr2*<sup>-/-</sup> mice compared to foci in MMTV-PyMT;*Ccr2*<sup>+/+</sup> mice. The number of mice with an average of large vs. small metastatic foci is indicated (Chi-square test;  $n = 24$  MMTV-PyMT;*Ccr2*<sup>+/+</sup>, 33 MMTV-PyMT;*Ccr2*<sup>+/-</sup>, and 27 MMTV-PyMT;*Ccr2*<sup>-/-</sup> mice).

### **Supplemental Figure 2: Cytokine analysis in tumor lysate and conditioned medium**

- a.** Cytokine analysis from MMTV-PyMT;*Ccr2*<sup>+/+</sup> and MMTV-PyMT;*Ccr2*<sup>-/-</sup> tumors using the Proteome Profiler Mouse Cytokine Array Kit, Panel A (R&D Systems; mean +/- SEM, multiple Student's t-tests; n=4).
- b.** Cytokine analysis from supernatant of MMTV-PyMT;*Ccr2*<sup>+/+</sup> and MMTV-PyMT;*Ccr2*<sup>-/-</sup> tumor cells using the Proteome Profiler Mouse Cytokine Array Kit, Panel A after serum starvation for 24 h (R&D Systems; mean +/- SEM, multiple Student's t-tests; n=4).

### **Supplemental Figure 3. Gating strategy for immune cells**

Gating strategy for immune cells. For flow cytometry analysis, cells from tumors were gated on live CD45<sup>+</sup> cells and were further analyzed using the indicated markers to characterize infiltration of tumor-associated macrophages (TAMs), DCs, T cells, granulocytes, monocytes, and MDSCs. SSC=side scatter.

### **Supplemental Figure 4: T cell infiltration in tumors derived from *Ccr2*<sup>+/+</sup> or *Ccr2*<sup>-/-</sup> cancer cells**

- a.** There is no difference between *Ccr2*<sup>+/+</sup> and *Ccr2*<sup>-/-</sup> tumors in the percentage of CD3<sup>+</sup> T cells among CD45<sup>+</sup> leukocytes, as determined by flow cytometry for CD45<sup>+</sup>CD3<sup>+</sup> cells (mean +/- SEM, Student's t-test; n=5).
- b.** FoxP3<sup>+</sup>CD4<sup>+</sup> regulatory T cell infiltration is increased in *Ccr2*<sup>+/+</sup> tumors compared to *Ccr2*<sup>-/-</sup> tumors, as determined by flow cytometry gated on CD45<sup>+</sup>CD3<sup>+</sup>CD4<sup>+</sup> cells (mean +/- SEM, Student's t-test; n=5).

- c. Immune suppression induced by cancer cell CCR2 signaling is confined to the local tumor microenvironment. *Ccr2*<sup>+/+</sup> cancer cells transplanted into one mammary gland did not alter the growth of tumors from *Ccr2*<sup>-/-</sup> cancer cells transplanted to the contralateral gland. Tumor burden was determined by weekly caliper measurement (mean +/- SEM, two-way ANOVA; n=10 for all conditions).

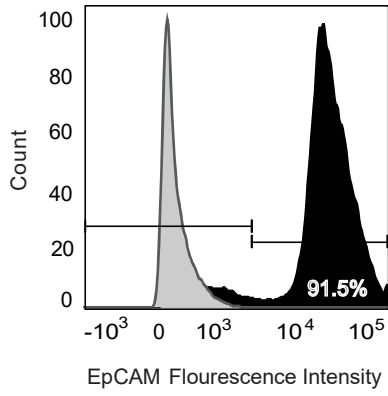
**Supplemental Figure 5: Myeloid cell infiltration in tumors derived from *Ccr2*<sup>+/+</sup> or *Ccr2*<sup>-/-</sup> cancer cells**

- a. IFN- $\gamma$  expression is unchanged on tumor lysates from *Ccr2*<sup>+/+</sup> and *Ccr2*<sup>-/-</sup> transplants, as determined by qPCR normalized to GAPDH (mean +/- SEM, Student's t-test; n=3).
- b. IFN- $\gamma$  expression on infiltrating CD8<sup>+</sup> cells is unchanged between *Ccr2*<sup>+/+</sup> and *Ccr2*<sup>-/-</sup> tumors, as determined by flow cytometry gated on CD45<sup>+</sup>CD8<sup>+</sup> cells (mean +/- SEM, Student's t-test; n=5).
- c. CCR2 and PD-L1 gene expression, represented as RPKM value, correlates in luminal A, luminal B, and basal-like human breast cancer (Pearson correlation coefficient=0.59, p=3.25\*10<sup>-23</sup>, n=231 patients; Pearson correlation coefficient=0.64, p=4.6\*10<sup>-16</sup>, n=127 patients; and Pearson correlation coefficient=0.64, p=2.17\*10<sup>-19</sup>, n=98 patients, respectively).
- d. Macrophage infiltration is unchanged between *Ccr2*<sup>+/+</sup> and *Ccr2*<sup>-/-</sup> tumors, as determined by flow cytometry gated on CD45<sup>+</sup>CD11b<sup>+</sup>MHC class II<sup>+</sup> F4/80<sup>+</sup> cells (mean +/- SEM, Student's t-test; n=5 and 4 for *Ccr2*<sup>+/+</sup> and *Ccr2*<sup>-/-</sup> tumors, respectively).
- e. Percentage of CD11b<sup>+</sup>MHC class II<sup>-</sup> cells is unchanged between *Ccr2*<sup>+/+</sup> and *Ccr2*<sup>-/-</sup> tumors, as determined by flow cytometry gated on CD45<sup>+</sup> cells (mean +/- SEM, Student's t-test; n=5 and 4 for *Ccr2*<sup>+/+</sup> and *Ccr2*<sup>-/-</sup> tumors, respectively).

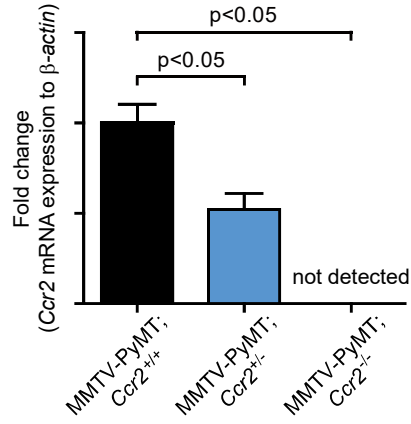
- f.** Granulocytes and granulocytic MDSCs are decreased on *Ccr2*<sup>-/-</sup> tumors, as determined by flow cytometry gated on CD45+CD11b+CD11c-Ly6G+Ly6C+ cells (mean +/- SEM, Student's t-test; n=5).
- g.** Inflammatory monocytes and monocytic MDSCs are increased on *Ccr2*<sup>-/-</sup> tumors, as determined by flow cytometry gated on CD45+CD11b+CD11c-Ly6G-Ly6C+ cells (mean +/- SEM, Student's t-test; n=5).
- h.** CD103+ DCs are increased in MMTV-PyMT;*Ccr2*<sup>-/-</sup> tumors compared to MMTV-PyMT;*Ccr2*<sup>+/+</sup> tumors, as determined by flow cytometry gated on CD11c+MHCII+ cells within the CD45+ population (mean +/- SEM, Student's t-test; n=4 and 5 for MMTV-PyMT;*Ccr2*<sup>+/+</sup> and MMTV-PyMT;*Ccr2*<sup>-/-</sup> tumors, respectively).

# Supplemental Figure 1

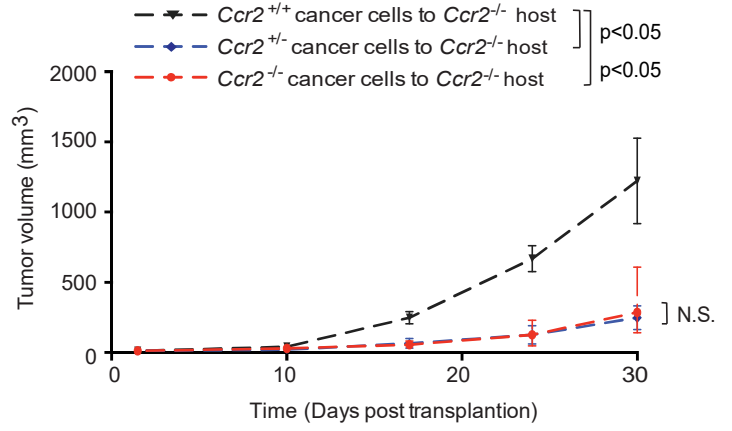
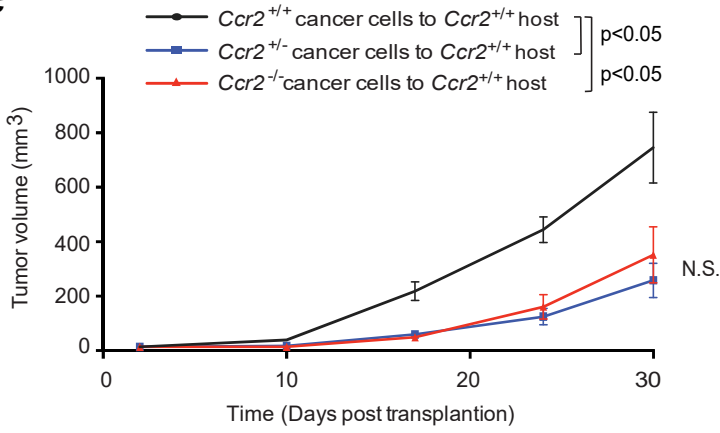
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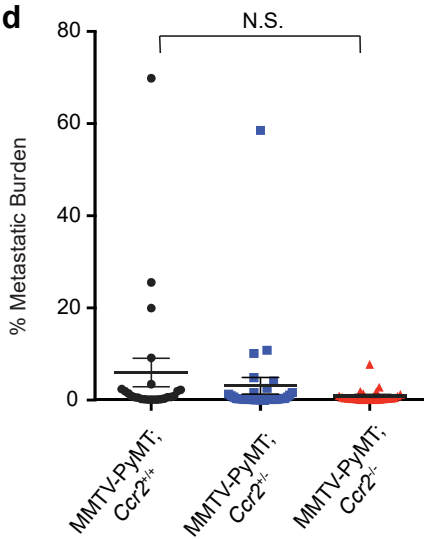
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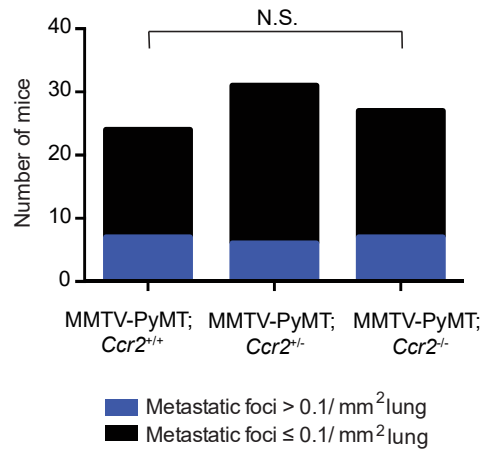
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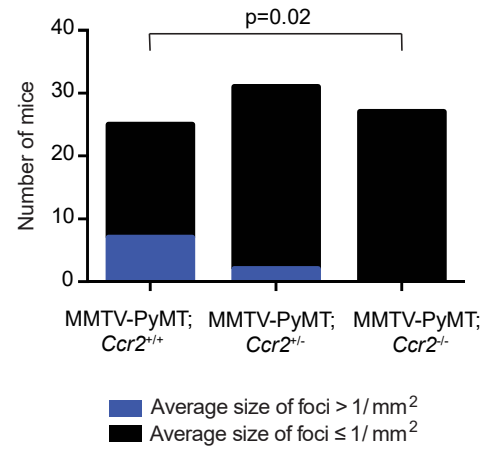
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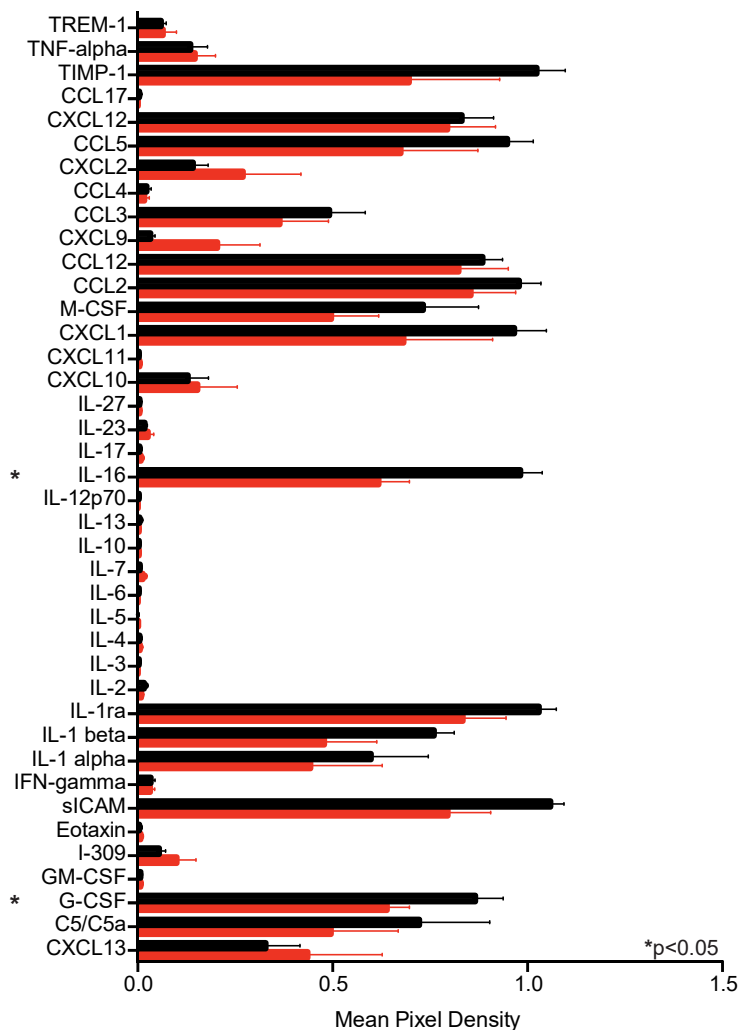


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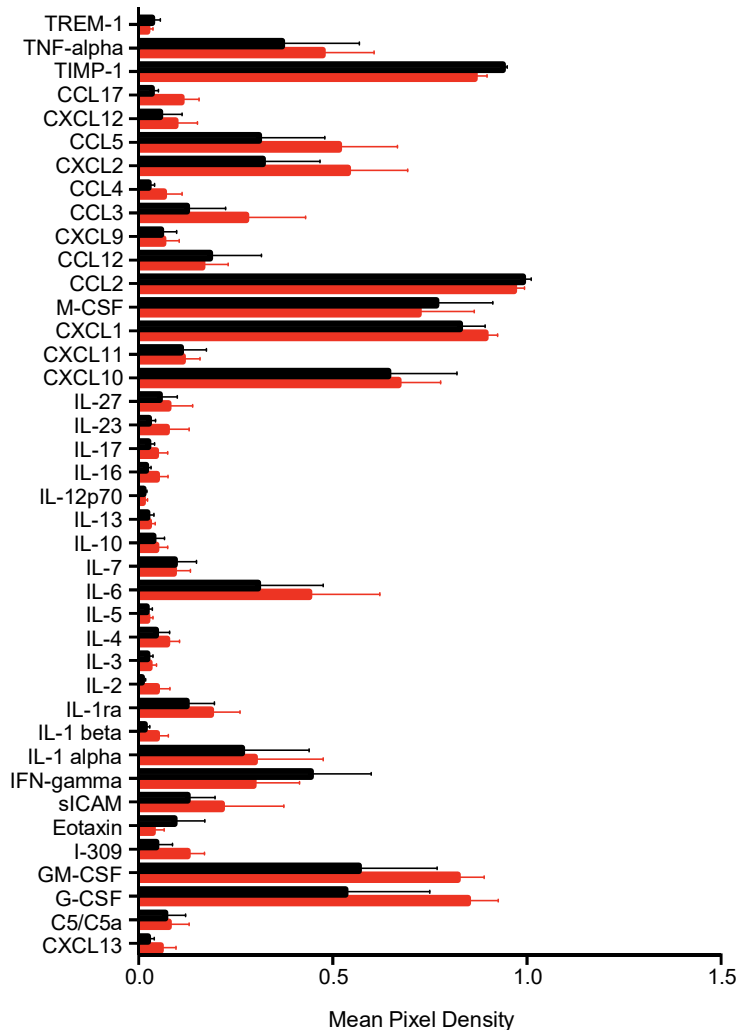
# Supplemental Figure 2

**a**



■ MMTV-PyMT; *Ccr2*<sup>+/+</sup> tumor lysate  
 ■ MMTV-PyMT; *Ccr2*<sup>-/-</sup> tumor lysate

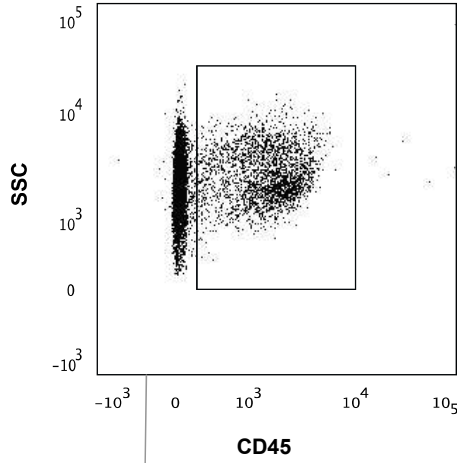
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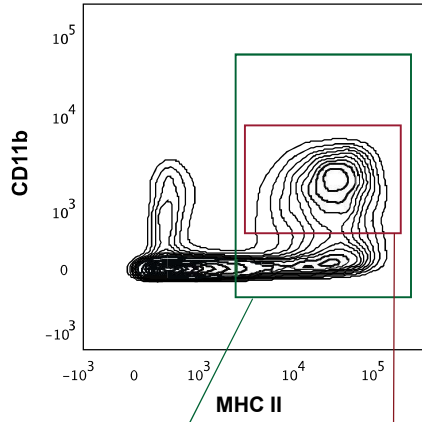
■ MMTV-PyMT; *Ccr2*<sup>+/+</sup> conditioned media  
 ■ MMTV-PyMT; *Ccr2*<sup>-/-</sup> conditioned media

# Supplemental Figure 3

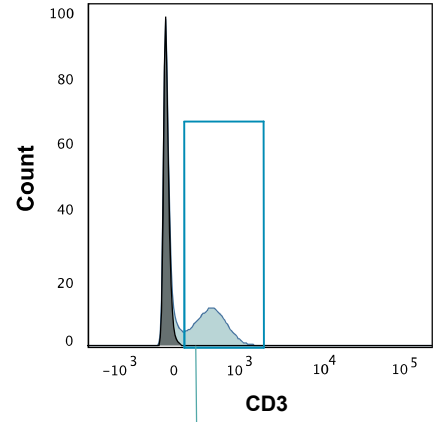
## Tumor-infiltrating immune cells



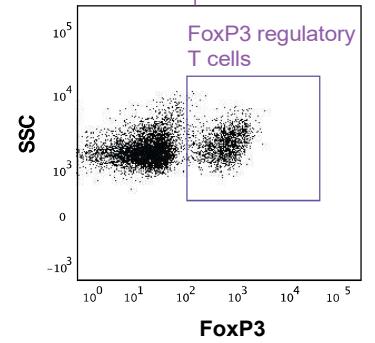
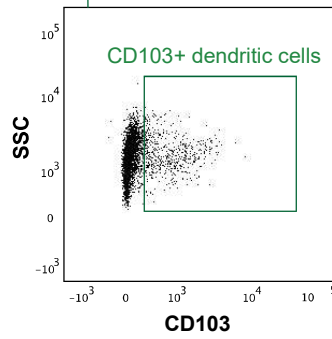
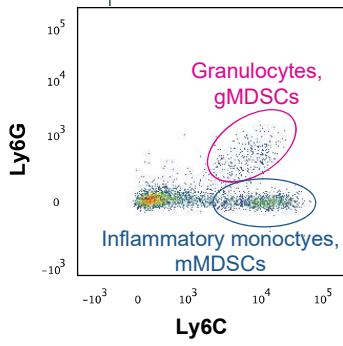
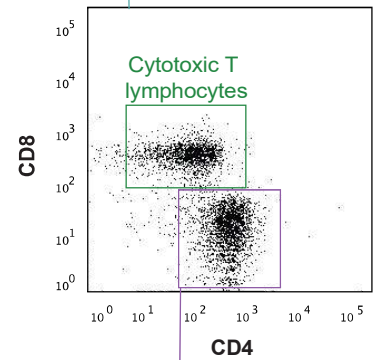
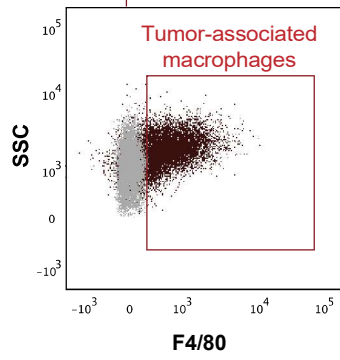
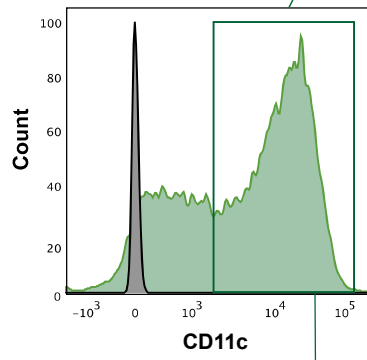
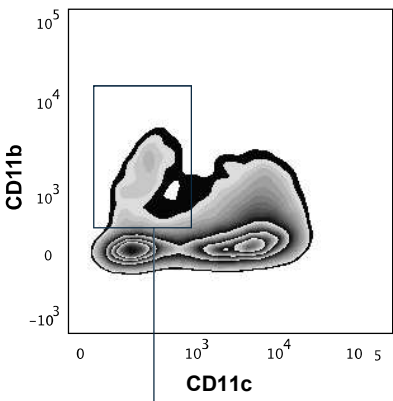
## TAMs, DCs



## T cells

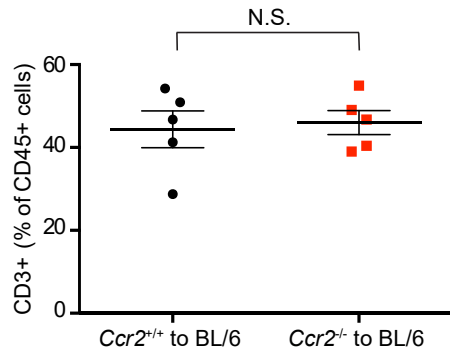


## Granulocytes Monocytes MDSCs

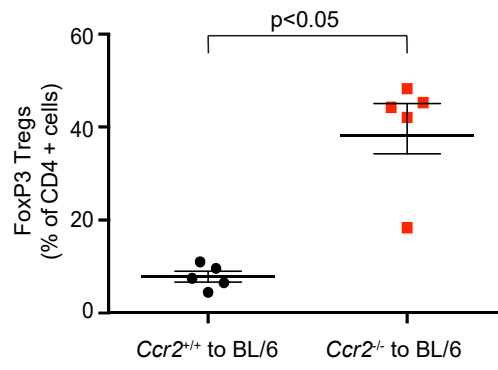


# Supplemental Figure 4

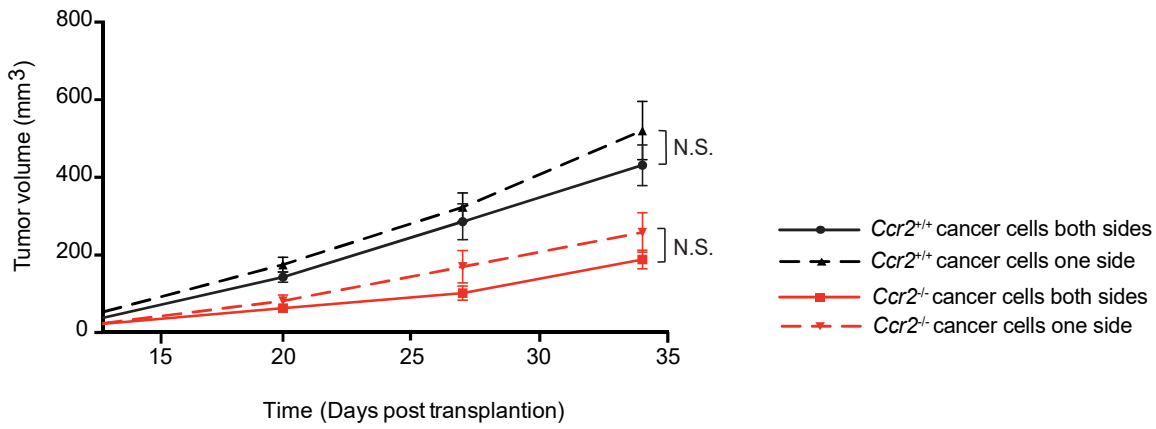
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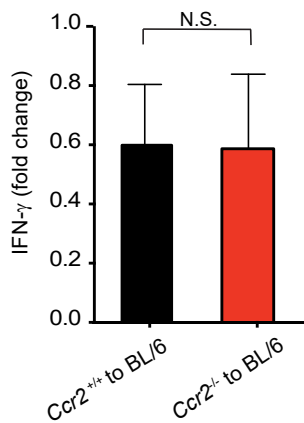
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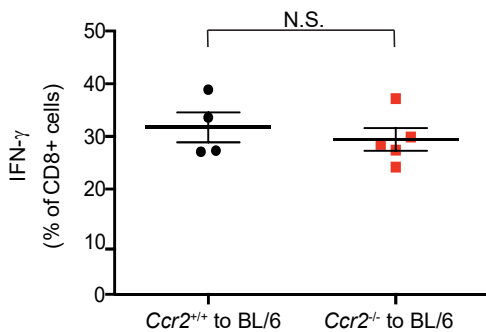


# Supplemental Figure 5

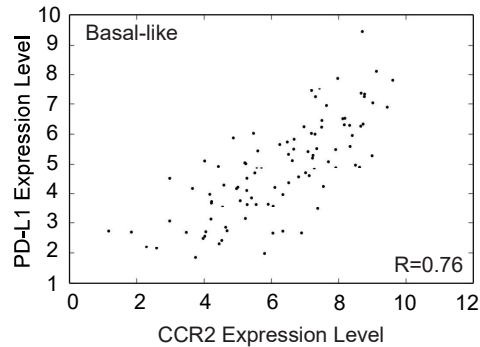
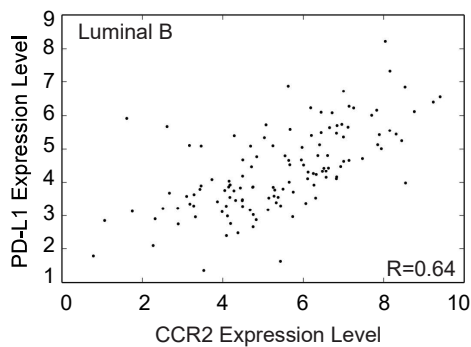
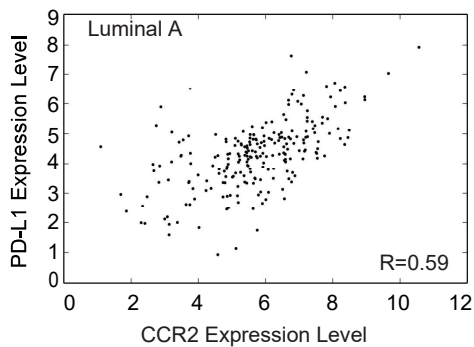
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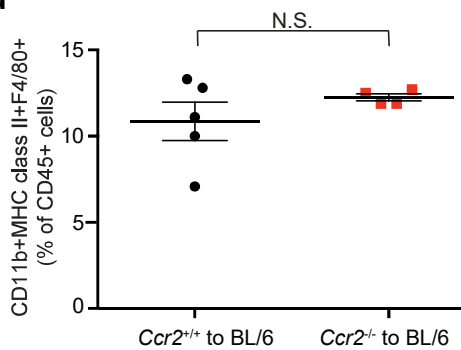
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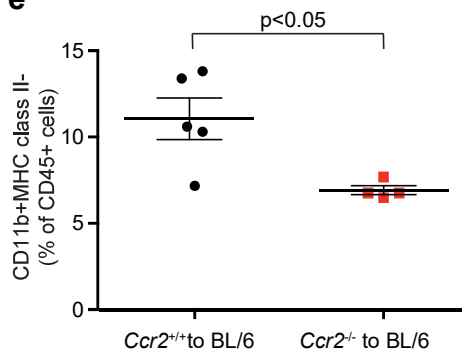
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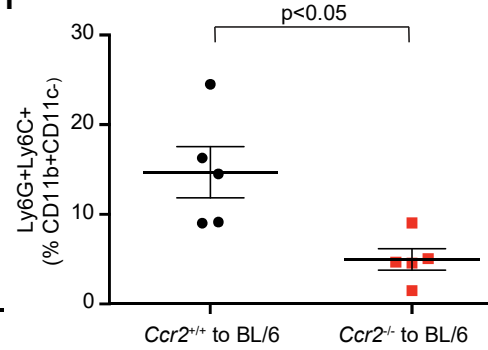
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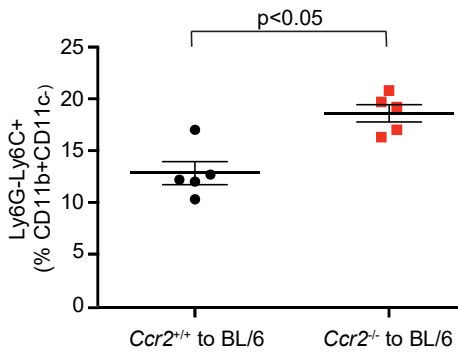
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**f**



**g**



**h**

