

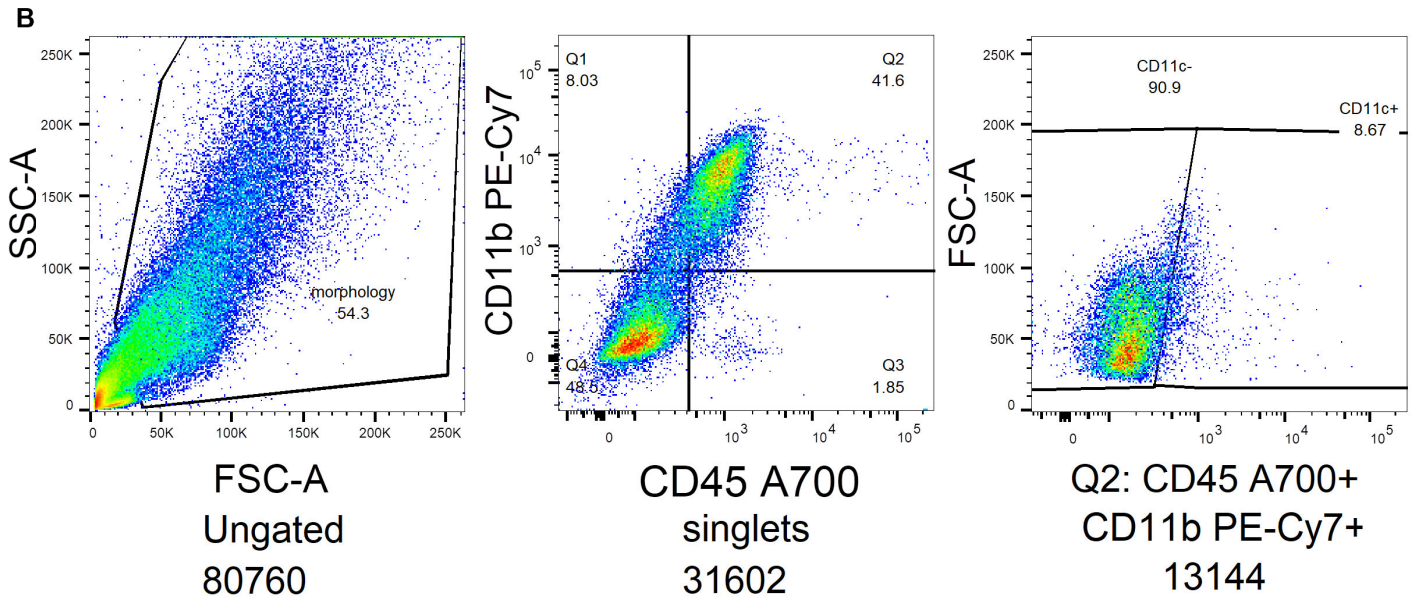
Supplemental Figure S1

A Panel 1

Marker	CD45	CD11b	CD11c	Ly6C	Ly6G	F4/80	MHCII
Antibody #	BioLegend #103128	BioLegend #101216	BioLegend #117328	Thermo Fisher Scientific #48-5932-82	BioLegend #127614	BioLegend #123110	BioLegend #107636
Macrophages	+	+	+	-	-	+	+
M-MDSC	+	+	-	+	-	Med	-
G-MDSC	+	+	-	-	+	-	-
Classical Dendritic Cell 1	+	-	+	-	-	-	+
Classical Dendritic Cell 2	+	+	+	-	-	-	+
Inflammatory Dendritic Cell	+	+	+	+	-	+	+
Eosinophils	+	+	-	-	-	Med	-

Panel 2

Marker	CD45	CD3	CD8	CD4	FoxP3	NK1.1	CD19
Antibody #	BioLegend #103128	BioLegend #100228	BioLegend #100751	BioLegend #100434	R&D Systems #C8214A-100	BioLegend #108708	BioLegend #115520
CD8 T cell	+	+	+	-	-	-	-
CD4 T cell	+	+	-	+	-	-	-
T reg	+	+	-	+	+	-	-
Double-negative T cell	+	+	-	-	-	-	-
NK cell	+	-	-	-	-	+	-
B Cell	+	-	-	-	-	-	+



1 **Supplemental Figure S1. Flow Cytometry Panel and Gating Strategy. A.** A complete list of
2 the flow cytometry antibodies used and the markers for each cell type. **B.** Representative flow
3 cytometry initial gating strategy for eosinophil-containing populations, prior to Ly6C and
4 Ly6G gating.

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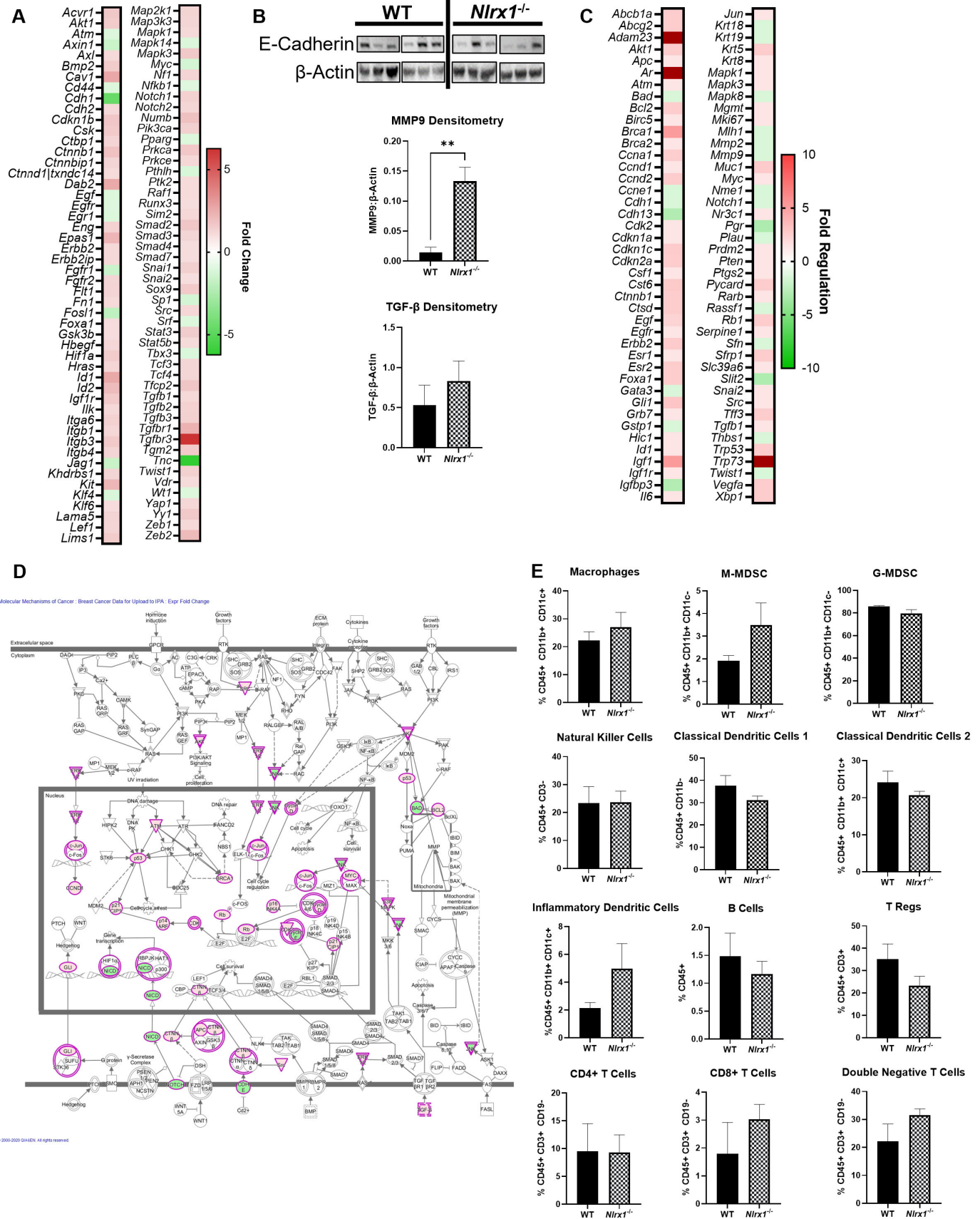
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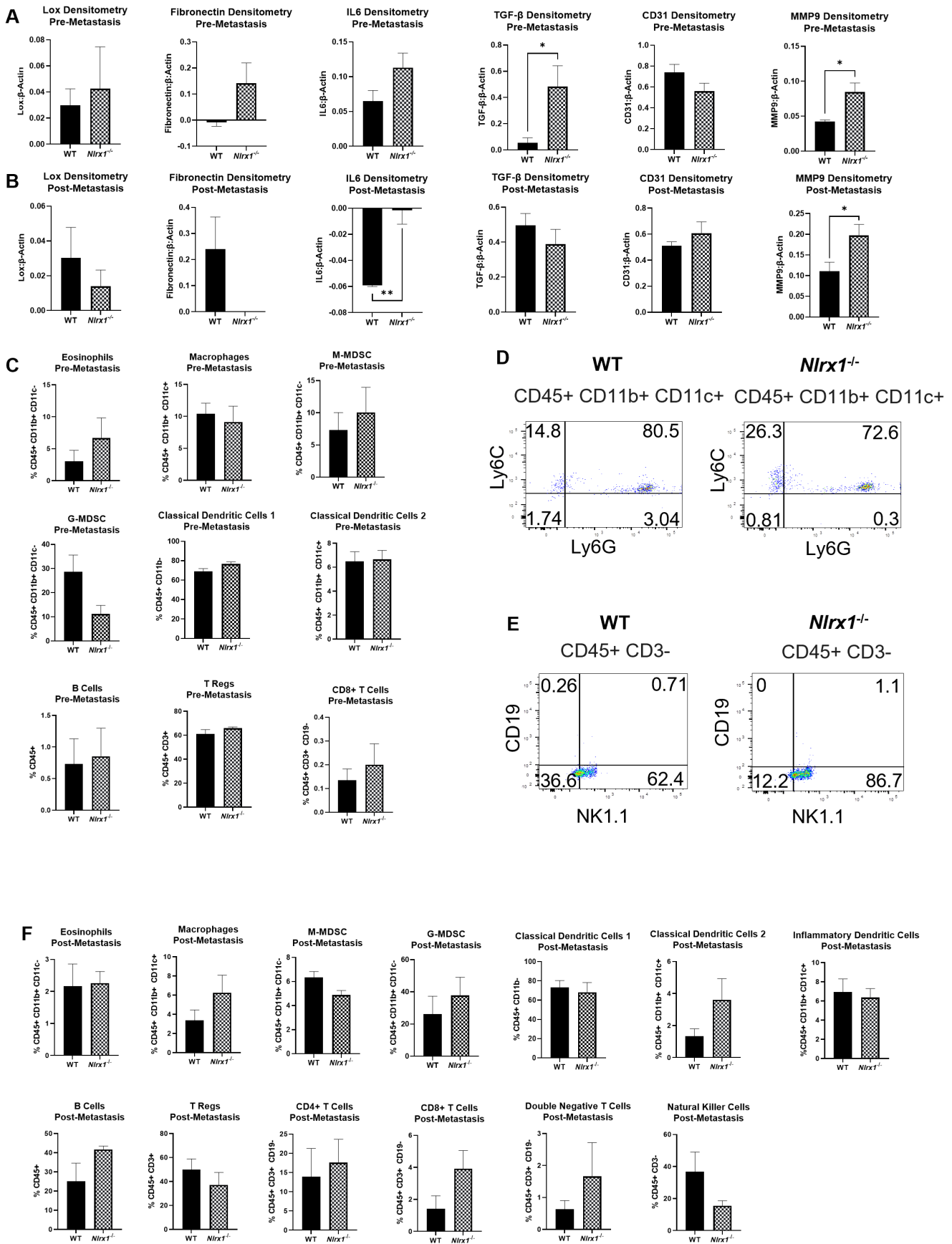
Supplemental Figure S2



23 **Supplemental Figure S2. Expansion on NLRX1 Expressed in Healthy Host Cells Decreases**
24 **Metastasis by Limiting Epithelial-Mesenchymal Transition (EMT) Characteristics and**
25 **Immune Cell Recruitment. A.** Heatmap of the complete list of 100 EMT-related genes (*Nlr1^{-/-}*
26 tumors vs WT tumors). **B.** Western blot of E-Cadherin and densitometry of MMP9 and TGF- β
27 western blots. n=5-6 per genotype. Data were analyzed with two-way unpaired t tests and are
28 shown as mean \pm SE. **C.** Heatmap of the complete list of genes in the murine breast cancer RT²
29 Profiler PCR Array (*Nlr1^{-/-}* tumors vs WT tumors). **D.** Ingenuity Pathway Analysis output. **E.**
30 Quantification of immune cell populations in the tumor that were not statistically significant
31 between WT (n=5) and *Nlr1^{-/-}* (n=5) mice. Data were analyzed with two-way unpaired t tests and
32 are shown as mean \pm SE. *p \leq 0.05, **p \leq 0.01, ***p \leq 0.001, **** p \leq 0.0001.

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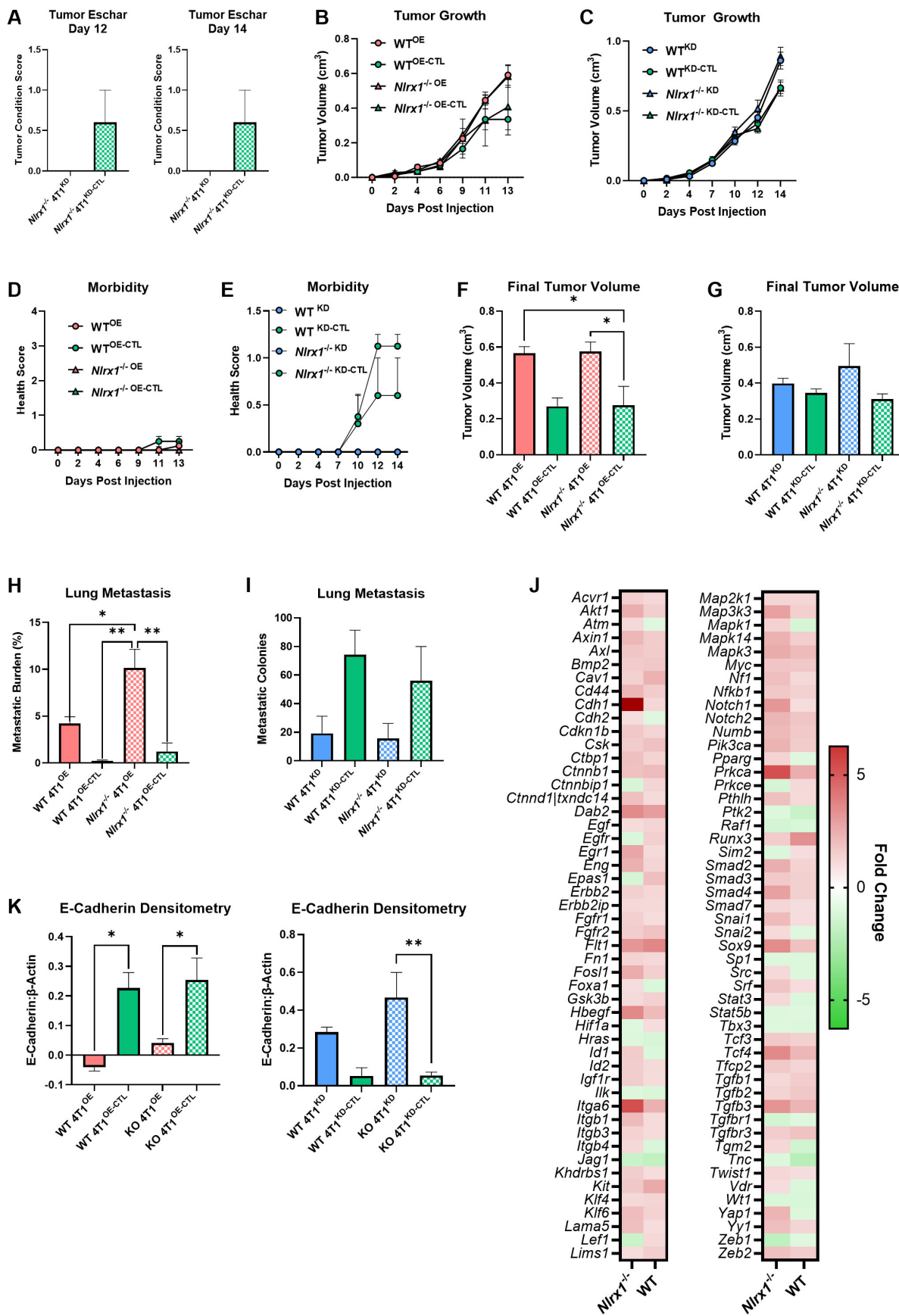
Supplemental Figure S3



45 **Supplemental Figure S3. Expansion on NLRX1 Expressed in Healthy Host Cells Attenuates**
46 **Lung Metastasis Through Limiting the Formation of the Metastatic Niche. A-B.**
47 Densitometry of Lox, Fibronectin, IL6, TGF- β , CD31, and MMP9 western blots at the (A) pre-
48 metastasis timepoint (n=4 per genotype) and the (B) post-metastasis timepoint (n=5 per genotype).
49 **C-E.** In the pre-metastatic lungs (n=4 per genotype), (C) quantification of immune cell populations
50 that were not statistically significant between WT and *Nlr1^{-/-}* mice, and (D-E) the representative
51 flow cytometry scatter plots of immune cell populations that were statistically significant,
52 including (D) inflammatory DCs, and (E) NK cells. **F.** In the post-metastatic lung (n=4 per
53 genotype), quantification of immune cell populations that were not statistically significant between
54 WT and *Nlr1^{-/-}* mice. Data were analyzed with two-way unpaired t tests and are shown as mean
55 \pm SE. * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$, **** $p \leq 0.0001$.

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Supplemental Figure S4



67 **Supplemental Figure S4. Expansion on NLRX1 Expressed in 4T1 Cells Promotes Tumor**
68 **Growth and Metastasis *In Vivo* and Expansion on NLRX1 Expressed in 4T1 Cells Augments**
69 **Lung Metastasis Through Promoting Epithelial-Mesenchymal Transition. A.** Comparison of
70 tumor eschar at Days 12 and 14 between 4T1^{KD} and 4T1^{KD-CTL} tumors in *Nlrp1*^{-/-} mice using a two-
71 way unpaired T test. **B-I.** Ordinary one-way ANOVAs with Tukey's multiple comparisons test
72 were performed to compare tumor growth in the **(B)** overexpression and **(C)** knock-down studies,
73 morbidity in the **(D)** overexpression and **(E)** knock-down studies, final tumor volume in the **(F)**
74 overexpression studies and **(G)** knock-down studies, and lung metastasis in the **(H)** overexpression
75 studies and **(I)** knock-down studies. n = 3-5 for each group per study. **J.** Heatmap of the complete
76 list of 100 EMT-related genes (4T1OE vs 4T1OE-CTL tumors). **K.** Densitometry of E-Cadherin
77 western blots from 4T1^{OE}, 4T1^{OE-CTL}, 4T1^{KD}, and 4T1^{KD-CTL} tumors in WT and *Nlrp1*^{-/-} mice. n=3-
78 5 per group. All data are shown as mean ± SE. *p ≤ 0.05, **p ≤ 0.01, ***p ≤ 0.001, **** p ≤
79 0.0001.

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