Supplemental material

Figure S1. *Morc4* is a metastasis repressor in breast cancer

A. Collection diagram of primary tumor and metastatic lung tissue for each mouse strain (balb/c and nude) from three time points. (n=3 in each mice strain).

B. Typical GFP-positive metastatic nodules in the lungs of Nude mice at day 11, 18, and 25 after implantation of 4T1 parental cells and 4T1 cells expressing GeCKO library B into the mammary gland. (n=3).

C. Changes in tumor volume over time in nude mice as same shown in Figure S1B. Student's t-test, with $p < 0.05$ considered significant.

D. Typical GFP-positive metastatic nodules in the lungs of balb/c mice at days 18, 25, and 30. (n=3).

E. Changes in tumor volume over time in balb/c mice as same shown in Figure S1D. Student's t-test, with $p < 0.05$ considered significant.

F. Pearson's correlation statistic between sgRNA enrichment in plasmid DNA and 4T1 input cells（n=3）.

G. Average number of enriched sgRNAs targeting the 14 common candidate genes in all 36 tumors.(one duplicated gene)

H. Average number of enriched sgRNAs targeting the 14 common candidate genes in primary tumors from both mice strain.

I. Average number of enriched sgRNAs targeting the 14 common candidate genes in lung tumors from both mice strain.

Figure S2. Validation of *Morc4* as an immune-dependent metastasis suppressor.

A. RNA expression levels of the *Morc4* gene in the clover-tagged 4T1 cells with the expression of either sg1/2Morc4, sg2/3Morc4, or sg1/2/3Morc4 as determined by qPCR.

B. Sanger sequencing confirmation of the mutant status of the *Morc4* gene in 4T1 cell line.

C. Representative Images of GFP-positive metastatic nodules in the lungs of nude mice after implantation of sgMorc4 and parental 4T1-clover cells into the fat pad for 16 days. (n=9 mice).

D. The Quantification of nodule number in the metastasis lung tissue as same showed in Figure S2C. NS, not significant ($p > 0.05$).

E. The weight of primary tumor (g)(left) , spleen(g)(middle) , Lung(g)(right) of nude mice as same in Figure S2C.

F. Images of spleen and primary tumors after implantation of sgMorc4 and parental 4T1 cells into the fat pad of balb/c mice for 30 days. (n=12 mice in KO group, n=9 mice in control group).

G. The spleen weight (g) and lung weight (g) of balb/c mice shown in Figure S2F. Student's t-test, with $p < 0.05$ considered significant.

H. Measurement of primary tumor and spleen from balb/c mice implanted with 4T1-no labelled cells and 4T1 cells with expression of sgMorc4 into fat pad for 25days. (n=4)

I. Images of lung tissue from balb/c mice same as shown in Figure S2H.and Quantification of nodules on the surface of metastatic lungs. Student's t-test, with $p < 0.05$ considered significant.

J. Morc4 protein levels in the no labeled-EMT6 cells with the expressions of either sh1Morc4, sh2Morc4 by Western blots.

K. Image of the spleen in balb/c mice implanted with EMT6-no labelled cells and EMT6 cells with the expression of shMorc4 in mammary fat pads for 35 days (n=4 mice).

L. Images of lung from balb/c mice same as shown in Figure S2K.and Quantification of nodules on the surface of metastatic lungs(Lung tissues were fixed in Bouin's solution overnight).

M. Images of spleen and primary tumors after implantation of parental 4T1 cells and 4T1 cells with Morc4-cDNA into the fat pad of balb/c mice for 35 days. (n=6).

N. Tumor volume and primary tumor weight (g) of balb/c mice as same shown in Figure S2M, Tumor volume=1/2 * length * width^2.

Figure S3. *Morc4* Loss Impairs T Cell Differentiation leading to the Accumulation of Naïve T Cells

A. Dot plots of markers that describe the ten subpopulations in the primary tumor of balb/c mice with *Morc4*-KO, *Morc4*-OE, and control (Ctrl).

B. Dot plots of signature genes in the T cell population in Figure3C.

C. UMAP plot of major cell types in T cell population.

D. The t-SNE projection of naïve T cell markers (*Tcf7, Lef1, Sell*) along with CD8 and CD4 within T cell populations from primary tumors across the *Morc4*-KO, *Morc4*-OE, and control (Ctrl) groups in single-cell RNA sequencing data.

E. RNA expression of *Tcf7, Lef1, Sell* genes in naïve T cells population from primary tumors in *Morc4*-KO, *Morc4*-OE, and control (Ctrl) groups in single cell analysis data.

F. IHC staining for TCF7 (n=11) and SELL (n=15) and LEF1(n=8) in spleens derived from balb/c mice implanted with 4T1-*Morc4*-KO and control (Ctrl) cells.

G. The number of TCF7 positive cells was quantified in Figure S3F using Image J.

H. The number of LEF1 positive cells was quantified in Figure S3F.

I. The number of SELL positive cells was quantified in Figure S3F.

J. Dot plots of signature genes in the single cell analysis of primary tumor in human breast cancer patients.

K. UMAP plots of each cluster across MORC4 high and MORC4 low in human breast cancer patients from Figure3M.

L. Mean cell composition of Naïve T cell populations across MORC4 high and MORC4 low in human breast cancer patients.

Figure S4. *Morc4* loss alters chromatin modifications and upregulates *Tslp* expression in breast cancer models.

A. Western blot analysis of chromatin-associated proteins (H3K27me3, H3K9me3, H3K27ac) in primary tumor and lung tissues of balb/c mice with either 4T1-*Morc4*-KO or control.

B. Immunohistochemical (IHC) staining of H3K27ac in the tumor tissues of balb/c mice post-implantation with either 4T1-*Morc4*-KO(n=18) or control (n=13) cells for 30 days. And Quantification of H3K27ac positive cells were counted per view. Student's t-test, with $p < 0.05$ considered statistically significant.

C. Immunohistochemical (IHC) staining was performed to detect H3K9me3 in the lung tissues of balb/c mice same as shown in Figure S4B, and the Quantification of H3K9me3 -positive cells was counted. (n=15)

D. IHC staining of H3K27me3 in the primary tumor from balb/c mice as shown in Figure S4B.(n=12)

E. IHC staining of H3K27me3 in metastatic lung from balb/c mice as shown in Figure S4B.(n=32)

F. RNA expression levels of *Tslp* mRNA in 4T1 parental and 4T1 cells with *Morc4*-shRNA, *Morc4*-sh/Tslp-KO cells.

G. Western blot analysis of MORC4, TSLP protein in the 4T1 cell line, comparing cells with *Morc4* gene knockdown (*Morc4*-sh1, *Morc4*-sh2).

H. Protein levels of TSLP in Control (Ctrl) and EMT6-shRNA (shRNA1 and shRNA2) cells.

I. Western blot analysis of TSLP and MORC4 protein expression in the 231 parental and 231-cell line with 3 clones of MORC4 gene overexpression.

J. Immunohistochemical (IHC) staining using the EpCAM antibody in spleen from balb/c mice implanted with parental 4T1 cells and 4T1-Morc4-KO cells and the Quantification of EpCAM positive cells was counted. (n=5).

K. Immunohistochemical (IHC) staining using the EpCAM antibody in lymph Node from balb/c mice same in FigureS4J.

Figure S5. Morc4 loss promotes naive T cell expansion and tumor invasion via TSLP-IL7Ra signaling.

A. Protein level of Morc4 in 4T1 parental cells and 4T1 cells transfected with 3 different clones of *Morc4*-shRNA.

B. Quantitative PCR (qPCR) analysis of *Tcf7* and *Lef1* RNA expression levels in inactivated Naive T cells co-cultured with 4T1 control (4T1-ctrl), 4T1 cells transfected with Morc4-shRNA, and Morc4-sh/Tslp-ko.

C. Protein level of LEF1 and SELL in the Naïve T cells incubated with TSLP protein. (28F12,1ug/ml).

D. RNA expression level of *Lef1* and *Sell* in Naïve T cell incubated with TSLP protein. Student's t-test, with $p < 0.05$ considered statistically significant.

E. CyTOF analysis of T cell subset distributions in primary tumors from BALB/c mice across Morc4-KO, control, and Morc4-KO/Tslp-KO groups.

F. RNA expression levels of *Il7ra* in inactivated Naive T cells co-cultured with 4T1 control (4T1-ctrl), 4T1 cells transfected with *Morc4*-KO and Morc4-sh/Tslp-KO.

G. Expression of EMT markers (Twist, Zeb1, Fn1) in 4T1 cells co-cultured with naïve T cells from Morc4-KO vs. control BALB/c mice, with or without anti-SELL(Biocell-Mel-14)/anti-CCL5(MCE-R6G9) blockade. Student's t-test, with $p < 0.05$ considered statistically significant.

H. Trans well migration of 4T1 cells co-cultured with naïve T cells from Morc4-KO vs. control of BALB/c mice, with or without anti-SELL/anti-CCL5 neutralizing antibodies blockade. Scale bar = 500 μm.

I. Quantification of migrated 4T1 cells in Trans well assays. Statistical significance was determined by unpaired Student's t-test; (n = 5 independent experiments).

Figure S6. Disruption of TSLP-IL-7Rα signaling or depletion of naïve T cells effectively attenuates metastatic progression.

A. The image of Primary tumor and spleen across treatment groups in the balb/c mice on day 29 after tumor cell implantation (n=5).

B. Image of metastasis lung tissue from *Morc4*-KO tumor-bearing balb/c mice were treated with either Tslp inhibitor or anti-CD62L(clone:Mel-14) neutralizing antibody, or combination therapy. (100ug/100ulPBS)

C. CyTOF dot plots of T cell within $CD3^+$ cell population subsets in primary tumors across treatment groups of balb/c mice on day 29.