Supplementary Figure 1 Growth and metastatic timeline of E0771 BCA and M3-9-M ERMS.

A-B. Primary tumor growth was measured over time for (A) E0771 ffluc-eGFP BCA injected into the mammary fat pad or (B) M3-9-M ffluc-eGFP ERMS injected into the gastrocnemius muscle. Numbers of mice per time point are indicated above marker.

C-D. Freshly dissected lungs from E0771 ffluc-eGFP (C) or M3-9-M ffluc-eGFP (D) tumor-bearing mice were assayed for tumor-derived luminescent signal by IVIS. (n>5 mice per time point)

E. Representative IVIS images of lungs from E0771 ffluc-eGFP or M3-9-M ffluc-eGFP tumor-bearing mice. Luminescence scale bar is shown at right.

D. Immunofluorescence for GFP from lungs of mice bearing E0771 ffluc-eGFP primary tumors for 12 days (top) or 21 days (bottom). Images acquired with 63x magnification. Scale bar is 10μm.

Supplementary Figure 2 Analysis of the bone marrow microenvironment and CXCR4 expression on E0771 BCA Tumor cells.

A. qRT-PCR expression of *Cxcl12* relative to beta actin from total bones of HBSS control or E0771 tumor-bearing mice. (*n*=4 for HBSS; *n*=5 for E0771; data are presented as fold change +/- SEM)

B. Quantification of CXCR4-expressing LSK cells per milliliter of total blood in control or E0771 BCA tumor-bearing mice. (*n*=5 mice per group)

C. Flow cytometry quantification of CXCR4 geometric mean fluorescence signal intensity on LSK cells in peripheral blood. (*n*=5 mice per group)

D. Flow cytometry analysis of the percent of LSK cells in peripheral blood that express CXCR4. (*n*=5 mice per group)

E. Flow cytometry evaluation of CXCR4 expression on E0771 tumor cells prior to tail-vein injection. **P*<0.05; ***P*<0.005; ****P*<0.005.

Supplementary Figure 3 Myeloid-derived suppressor cells localize with spontaneous metastases.

A. Representative immunofluorescence of lungs with the indicated antibodies (CD11b, Gr-1, GFP) and DAPI and imaged under 20x magnification. Scale bar is 50μm.

B. 63x magnification of the boxed region in (A). Scale bar is 10μm.

C. Representative immunofluorescence of lungs with the indicated antibodies (CD11b, and Gr-1) and DAPI and imaged under 20x magnification. "T" indicates tumor. Tumor borders are indicated with a dotted white line. "BV" indicates a blood vessel. Blood vessel borders are indicated with a dotted green line. Scale bar is 50μm.

Supplementary Figure 4 Phenotypic and functionally immunosuppressive MDSCs in the primary tumor and spleen of tumor-bearing mice.

A. Representative flow cytometry gating strategy of E0771-GFP primary tumor at 12 days following tumor implantation.

B. Quantification of myeloid cells within the primary tumor 12 days following tumor implantation. (n=4 primary tumors)

C. Suppression of T cell division by MDSCs isolated from either control mice spleen or E0771 primary tumor (n=3 replicates per group). *P<0.05; **P<0.005; ***P<0.005.

D. Representative flow cytometry gating strategy of control or E0771-GFP tumor-bearing mice 12 days following HBSS injection or tumor implantation.

E. Quantification of myeloid cells within the spleen of control or E0771-GFP tumor-bearing mice 12 days following HBSS injection or tumor implantation (n=4 mice per group).

F. Suppression of T cell division by MDSCs isolated from spleen of control mice or spleen of tumor-bearing mice implanted with E0771 for 12 days (n=3 replicates per group). *P<0.05; **P<0.005; ***P<0.005.

Supplementary Figure 5 Myeloid subsets in pre/early metastatic lung.

A. C57Bl/6 mice that had received a bone marrow transplant from GFP-transgenic mice were given no tumor or implanted with E0771 ffluc-mCherry for the indicated time. Percentage of GFP^+ cells expressing the indicated lineage markers in lung are presented. (*n*=3 mice per group)

B-C. Quantification of $CD11b^+Ly6g^+$ cells per lung of control or E0771 BCA tumor-bearing mice (B) or M3-9-M ERMS tumor-bearing mice (C) at the indicated times following tumor implantation. (*n*=5 for no tumor mice; *n*=5-6 for each tumor-bearing mouse group)

D-E. Quantification of $CD11b^+Ly6c^{high}$ cells per lung of control or E0771 BCA tumor-bearing mice (D) or M3-9-M ERMS tumor-bearing mice (E) at the indicated times following tumor implantation. (*n*=5 for no tumor mice; *n*=5-6 for each tumor-bearing mouse group)

F-G. Quantification of $CD11c^+$ cells (F) or $CD11b^+Ly6c^{high}F4/80^+CD115^+$ cells (G) per lung of control or M3-9-M ERMS tumor-bearing mice at the indicated times following tumor implantation. (*n*=5 for no tumor mice; *n*=5-6 for each tumor-bearing mouse group)

H-I. Quantification of $CD11b^+Ly6c^{high}CD206^+CD115^+$ cells (F) or $CD11b^+Ly6c^{high}CD80^+$ cells per lung of control or E0771 BCA tumor-bearing mice at the indicated times following tumor implantation. (*n*=3 for no tumor mice; *n*=3 for each tumor-bearing mouse group)

Supplementary Figure 5 *Ex vivo* tumor-mediated bone marrow expansion.

A-D. Lineage-depleted bone marrow was incubated with StemSpan, E0771 tumor-conditioned StemSpan, or M3-9-M tumor-conditioned StemSpan that was heated to the indicated temperature for 30 minutes. The indicated cell types were quantified by flow cytometry after 7 days of *ex vivo* culture. (*n*=2 wells per condition)

E. Lineage-depleted bone marrow was incubated with StemSpan, E0771 tumor-conditioned StemSpan, or M3-9-M tumor-conditioned StemSpan. Cultures were supplemented with FLT3 ligand at 5, 10, 25, or 50ng/mL. LSK cells were quantified by flow cytometry after 7 days of *ex vivo* culture. (*n*=2 wells per condition)