Supplementary Methods

Western Blot. PyMT-WT and EO771 cell lysates were generated and protein expression analysed by Western blotting as previously described (9). Antibody details listed in antibody section.

Conditioned media assays. Conditioned media was generated by filtering (0.22 μm filter, Millipore) serum-free, phenol red-free low glucose Dulbecco's Modified Eagles Medium (Invitrogen) cultured on confluent EO771, PyMT-WT or PyMT-Siah2$^{-/-}$ cells plated in 6-well plates for 10 hrs under normoxic (20% O_2) or hypoxic conditions (2% O_2) as previously described (6). To generate the pre-metastatic niche in the lung *in vivo* and for studies of BMDC lung infiltration, conditioned media (300 μl) was injected daily for 7 days (day 0-6) into the peritoneum of immune competent WT or eGFP bone marrow-chimeric mice, and lungs harvested the next day (day 7) for flow cytometry analysis or embedding in OCT (Tissue-Tek).

Experimental metastasis models. Mice were injected with 2 x 10^5 EO771/PyMT-WT Cherry or B16F10 cells intravenously via the tail vein on day 7 after treatment with conditioned medium for 7 days. Lungs were harvested 2-4 weeks later and analyzed by flow cytometry for Cherry positive tumor cells in EO771 and PyMT-WT models. In B16F10 models, macrometastatic foci in whole lungs was counted using a dissecting microscope, and micrometastatic foci counted in a similar manner on H&E stained-lung sections.

MCP-1/CCL2 neutralization. Conditioned medium was incubated with anti-MCP-1 antibody at 40 ng/ml for 90 min at room temperature on a rotating wheel. The antibody was then removed from the conditioned medium by incubation with 50 μl Protein A/G PLUS-Agarose beads (Santa Cruz Biotechnologies) per 20 ml conditioned medium on rotating wheel for 1 hour at room temperature. Samples were centrifuged at 1400 rpm for 4 min to pellet beads. Conditioned medium neutralized with an MCP-1 antibody was then injected into immune competent WT mice in place of normal conditioned medium as above.

NK cell depletion. Immune competent WT mice were treated as above with additional intraperitoneal injections of 100 μg asialoGM1 (rabbit; Wako Pure Chemical Industries), or isotype Ig antibody on days -1, 0, 7, 14, 21 and 28. Eyebleeds were done on day 0 and 7 to confirm NK cell depletion via flow cytometry analysis, and 2 x 10^5 Cherry-positive tumor cells were injected intravenously on day 7. Mice were harvested on day 35 and whole lung analyzed by flow cytometry.

Antibodies. Antibodies used in Western blot, neutralization and flow cytometry analysis are listed below.

Antibody	Details	Source
Hif-1α	Rabbit anti-mouse pAb	Novus Biologicals
Hif-2α	Rabbit anti-mouse pAb	Novus Biologicals
α-Tubulin	Mouse anti-mouse mAb	Sigma Aldrich
MCP-1	Anti-mouse CCL2/JE/MCP-1 pAb	R&D Systems
GM1	Rabbit anti-asialo GM1	Wako Pure Chemical Industries, Ltd.
CD45.2	Clone 104	eBioscience
CD11b	Clone M1/70	eBioscience
Gr-1	Clone RB6-8C5	eBioscience
Ly6G	Clone 1A8	BD Bioscience
Ly6C	Clone AL-21	BD Bioscience
CD3e	Clone 145-2C11	eBioscience
NK1.1	Clone PK136	eBioscience
CD4	Clone RM4-5	eBioscience
CD8α	Clone 53-6.7	eBioscience
F4/80	Clone BM8	eBioscience
MCH Class II	Clone M5/114.15.2	eBioscience
CD11c	Clone N418	eBioscience
CD69	Clone H1.2F3	eBioscience
CD27	Clone LG.7F9	eBioscience