

Supplementary Figure 1.

A) Western blot of Hif-1 α /Hif-2 α protein expression in PyMT-WT and EO771 cell lysates after exposure to normoxic (20% O₂) or hypoxic conditions (2% O₂) for 2 or 10 hours. **B)** Schematic of experimental metastasis procedure. Cell-free NCM and HCM from PyMT-WT and EO771 mammary tumor cells was injected intraperitoneally into mice daily for 7 days, followed by intravenous injection of Cherry-labelled PyMT-WT or EO771 mammary or B16F10 melanoma tumor cells on day 7. Lungs were harvested at 2 or 4 weeks post tumor cell injection.

Supplementary Figure 2.

A) Representative H&E images of micro- and macrometastases in lungs 2 weeks post injection of B16F10 tumor cells in mice pre-treated with PyMT-WT NCM or HCM. B16F10 metastases (indicated by black arrow heads) counted by dissecting microscope and summarised in graph below (n=10/group). Mean \pm SEM. **B)** Cytokines/growth factors increased or decreased in PyMT-WT and EO771 HCM. Previously published factors in pre-metastatic niche formation are highlighted in green, and references for corresponding target positions on arrays in Figure 2B & C shown in brackets.

Supplementary Figure 3.

A/B) Flow cytometry analysis of CD11b⁺/Ly6C^{med} (**A**) and CD11b⁺/Ly6C^{high} (**B**) myeloid cell lung infiltrate in mice treated with PyMT-WT NCM/HCM alone (n=5-8) or NCM/HCM neutralized with MCP-1 antibody (anti-MCP-1 + NCM/HCM; n=10). Mean percentage \pm SEM.

Supplementary Figure 4.

A) Flow cytometry analysis of eGFP⁺ BMDC lung infiltrate for CD3⁺/CD4⁺ T cells, CD3⁺/CD8⁺ T cells, CD11b⁺/MHC Class II⁺/F4/80⁺ macrophages and CD11c⁺/MHC Class II⁺ dendritic cell subpopulations (n=5/group). **B)** Flow cytometry analysis of mean intensity CD69 expression on CD3⁻/NK1.1⁺ cells compared to isotype control (n=5/group). **C)** Flow cytometry analysis of % CD3⁻/NK1.1⁺ NK cells in peripheral blood from mice after treatment with isotype (n=9) or asialoGM1 antibody (n=18) but before NCM/HCM injection. Mean percentage \pm SEM.