## SUPPORTING INFORMATION

## A Paper-Based Invasion Assay: Assessing Chemotaxis of Cancer Cells in Gradients of Oxygen

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## **Supplemental Figures:**



**Supplemental Figure 1.** A-B) A549-HGF-M cellsed in layer +4 after 24 hours within the invasion stack. All cells expressed GFP. (A) Single confocal scan of layer +4. (B) Maximum intensity projection of total thickness of layer +4. Green is GFP, Blue is DAPI.



**Supplemental Figure 2.** A-C) Image of a mouse lung, 23 days post-implantation with A549 (A), A549-HGF (B), and A549-HGF-M (C) cells implanted subcutaneously on the hind flank of the mouse. All three cell lines expressed GFP. D) Image of mouse lung when the A549 xenograft tumor reached 1,000 mm<sup>3</sup>. E) Plot of number of lung metastases 23 days post-implantation of A549, A549-HGF, and A549-HGF-M cells. F) Plot of number of metastases to the lung when the primary tumor reached at least 1000 mm<sup>3</sup> for A549, A549-HGF, and A549-HGF-M cells.



**Supplemental Figure 3.** Schematic of the invasion stack with the seeded layer shown in green (positioned at the top, middle, or bottom of the stack), layers of matrigel shown in pink, layer of PDMS shown in blue (permeable to oxygen but not nutrients), and acrylic holder shown in black (impermeable to oxygen and nutrients). A) Cells were positioned at the top of the stack, nutrients were available only from the top of the stack, and oxygen was available from both the top and bottom of the stack. B) Cells were positioned at the bottom of the stack, nutrients were available only from the top of the stack, and oxygen was available from both the top and bottom of the stack. B) Cells were positioned at the stack nutrients were available only from the top of the stack, and oxygen was available from both the top and bottom of the stack. Error bars represent the standard deviation for 20 replicate zones.