# (Estrella et al. Acid-mediated Invasion)

## Supplemental Figure Legends

**Figure S1: Photomicrographs of cells in this study.** HMEC, HCT116/GFP and MDAmb231 cells were grown to 70-80% confluency. Samples were then viewed with an automated Zeiss Observer Z.1 inverted microscope through a 10x /0.3NA or 20x/0.4NA objective and a phase contrast ring. Images were produced using the AxioCam MRm CCD camera and Axiovision version 4.6 software suite. (Carl Zeiss Inc., Germany).

**Figure S2: Comparison of normal vs tumor cells by size.** Using these phase contrast images, ROI were chosen and area of cells was determined by pixel number using Image-Pro Plus v6.2. Once area was measured, data analysis revealed statistical significance ($p<0.0001$) between cell size.

**Figure S3: Growth of MDAmb231 tumor within the dorsal window chamber.** Mean growth of MDAmb23/GFP tumors within the dorsal window chamber. Cells were embedded in a 0.8 mg/mL collagen type 1 matrix followed by inoculation into the window chamber. Tumor area was measured at days 2, 3, and 16 by counting the number of pixels. Tumor area measured by fluorescence pixel number obtained using Image-Pro Plus 6.2.

**Figure S4: Quantification of tumor growth.** Images are shown for mouse tumor #23 on day 4 (green) and day 14 (red). Images were co-registered using the ring clip of the DWC as a fiducial. The centroid of the tumor on day 4 was calculated and radial lines were drawn every $22.5^{o}$ of arc, beginning at the ring. Growth was calculated as the

number of pixels that the day 14 tumor extended beyond the edge of the day 4 tumor using Definiens Developer XD®.

**Figure S5: Relation between tumor growth and pH for all 5 mice**. Data for all 5 mice were parsed as in figure 2D wherein the growth (in pixels) and pH were calculated every 22.5$^{o}$ of arc. These data show that growth increased with decreasing pH for all animals, and that there was a threshold of pH above which no growth occurred. Lines represent the trend below threshold for each mouse.

**Figure S6: *Ex vivo* comparison of vessel density in stroma vs tumor tissue.** Using the Image Scope v7.1 regions of viable tumor were manually selected. 200μm micrometers were placed around the circumference of the tumor region perpendicular to the tumor edge. A second manually selected region was drawn around the perimeter of the micrometers. This effectively segments the entire tissue section into three regions of interest; the tumor, the edge within 200μm of the tumor and the distant stromal region. The Aperio (Vista, CA, USA) microvessel algorithm v1.0 was used with the following parameters (smoothing 2; dark threshold160; light threshold 210; regioning 6; completion 7) to identify the vascular density which was defined as the vessel area divided by total area (μm2).

**Figure S7: Segmentation and analysis of vasculature *In vivo*.** In vivo vessels and tumors were independently segmented and quantified within four ordinal quadrants emanating from the tumor centroid as described in Methods.

**Figure S8: *In vivo* relation between vessel density and tumor growth**. The data from Figure S5 were used to compare tumor growth (change in number of pixels from day 4 to day 13) to vessel density (on day 4) in each of the 4 quadrants.

**Figure S9: *In vitro* degradation of collagen I and IV by HCT116 cells.** A) Images show 2-day cultures of HCT116 cells grown in 2D collagen I and 3D rBM overlay cultures at pHe 7.4 and 6.8, respectively. Images represent degradation products of DQ-collagen I and DQ-collagen IV in the entire 2D and 3D volume of cells. B) Using the Definiens imaging software, proteolytic activity was measured, correlated to fluorescence intensity and normalized to cell area. Proteolytic activity at acidic pHe was statistically significant ($p<0.0051$ for DQ 1 and $p<0.03$ for DQ IV).

**Figure S10: Inhibition of tumor growth as a result of $NaHCO_3$ treatment.** Tumor area was measured at days 4 and 13 by fluorescent pixel number using Image-Pro Plus v6.2. The mean growth illustrated a significant increase in size of the control tumor over 2 weeks ($p<0.033$). In contrast, following bicarbonate treatment, the size of the tumor significantly decreased ($p<0.003$). Comparing control vs. bicarbonate mice on day 13 showed a significant difference ($p<0.028$) using three different statistical approaches.