

## Supplemental Data

# Bicarbonate Increases Tumor pH and Inhibits Spontaneous Metastases

Robey et al. CAN-07-5575R1

**Table S1. Blood analyses of control and bicarbonate-treated mice 60 days post-initiation of bicarbonate therapy.** Blood chemistries were measured from tail bleeds using an iSTAT® portable clinical analyzer with CG4+ cartridge (Heska, Fribourg, Switzerland) according to manufacturer instructions.

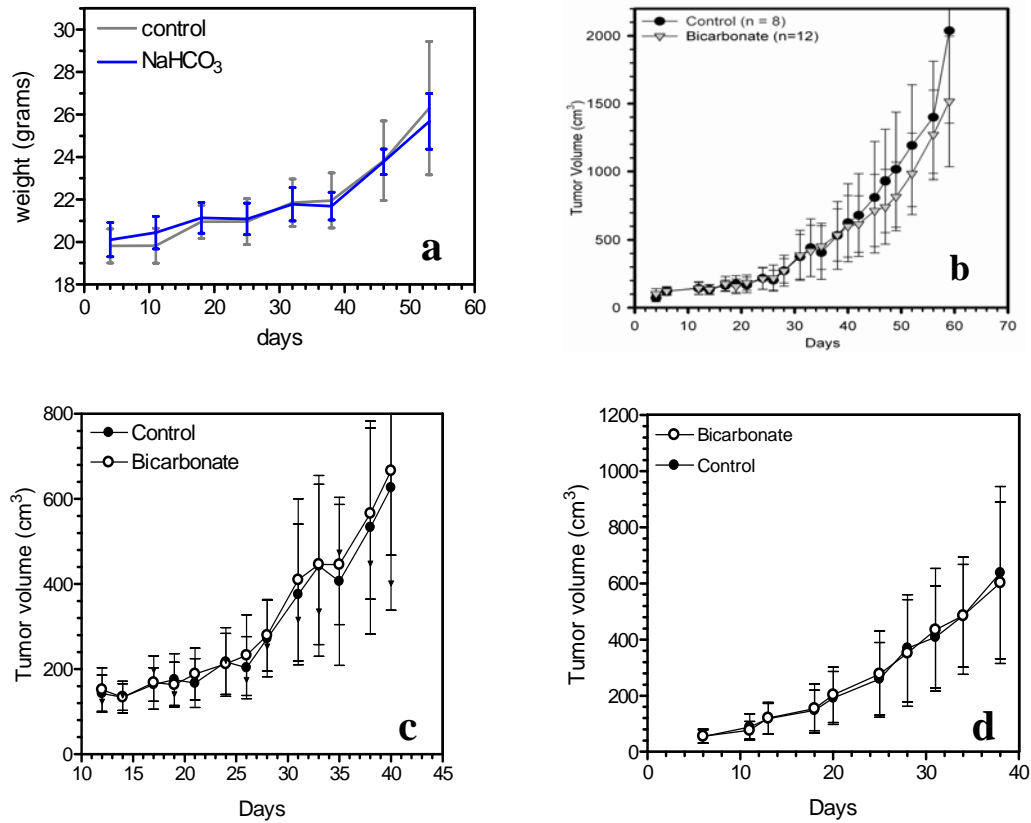
	<b>Bicarbonate (n=8)</b>		<b>Control (n=7)</b>	
	Mean	SD	Mean	SD
<b>pH</b>	7.06	0.10	7.07	0.11
<b>Na (meq/L)</b>	156.4	4.3	153.4	3.6
<b>K (meq/L)</b>	4.12	0.77	4.19	0.90
<b>Cl meq/L)</b>	114.2	2.3	116.3	1.7
<b>pCO<sub>2</sub> (mm Hg)</b>	57.9	13.8	57.2	11.6
<b>HCO<sub>3</sub><sup>-</sup> (mmol/L)</b>	15.3	1.51	16.1	1.21
<b>BUN (mmol/L)</b>	15.8	2.8	15.4	2.1
<b>Hematocrit (%)</b>	43.0	1.2	40.3	2.9

**Table S2. Dose Response data** showing fraction of animals (n=8 per group) with metastases following 30 days of primary tumor growth.

<b>NaHCO<sub>3</sub></b>				
0 mM	50mM	100mM	150mM	200mM
0.38	0.25	0.25	0.25	0.13

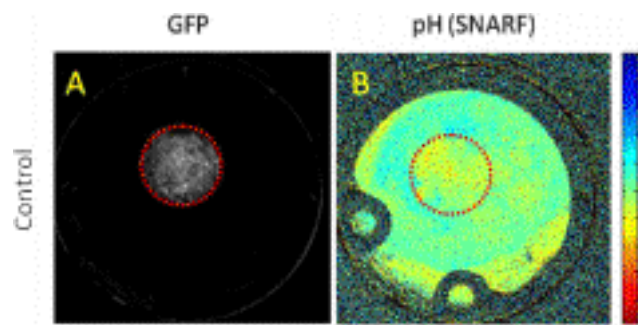
**Table S3. Lymph node (LN) and metastatic** infiltration by GFP-expressing MDA-MB-231 MFP tumors was quantified as measured in figure S3. Because the data are ordinal but not interval scaled, a Mann-Whitney-Wilcoxon rank order *U*-test was used to identify differences between the control and NaHCO<sub>3</sub> group (*p*=0.044, *U*=39 ).

Metastases	1 trace LN	≥2 trace LN	1 positive LN	≥2 positive LN	organ metastases	positive LN and organ metastases
<b>rank</b>	<b>1</b>	<b>2</b>	<b>3</b>	<b>4</b>	<b>5</b>	<b>6</b>
<b>Control (n=11)</b>	2	2	5	0	1	1
<b>NaHCO<sub>3</sub> (n=12)</b>	7	2	1	1	0	1

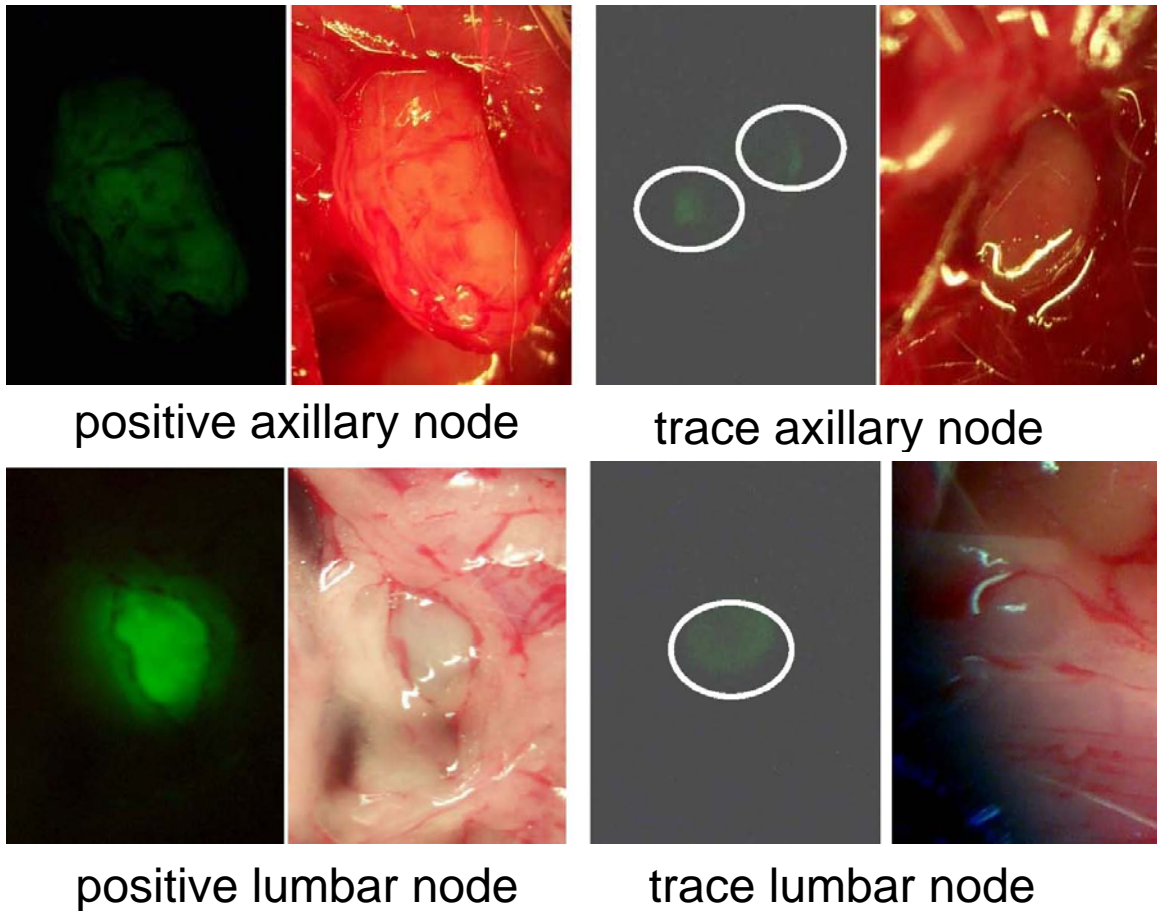


**Figure S1 | The effect of NaHCO<sub>3</sub> on tumor and animal growth.** Tumor bearing animals were randomized onto bicarbonate (200 mM ad lib) or control water six days following tumor inoculation. Animals were weighed weekly. **Panel (a)** shows animal weights in control and bicarbonate groups from a typical experiment indicating that animals did not fail to thrive or become dehydrated. Volumes of primary tumors in mammary fat pads were measured twice weekly and calculated from orthogonal measurements of external dimensions as  $(\text{width})^2 \times (\text{length}) / 2$ . **Panels (b-d)** show growth rates of primary tumors (in cm<sup>3</sup>) are shown for three different experiments, showing that

bicarbonate therapy had no effect on the growth of primary tumors. For these data two-tailed, unpaired t test; between these groups yielded a  $p=0.98$ .

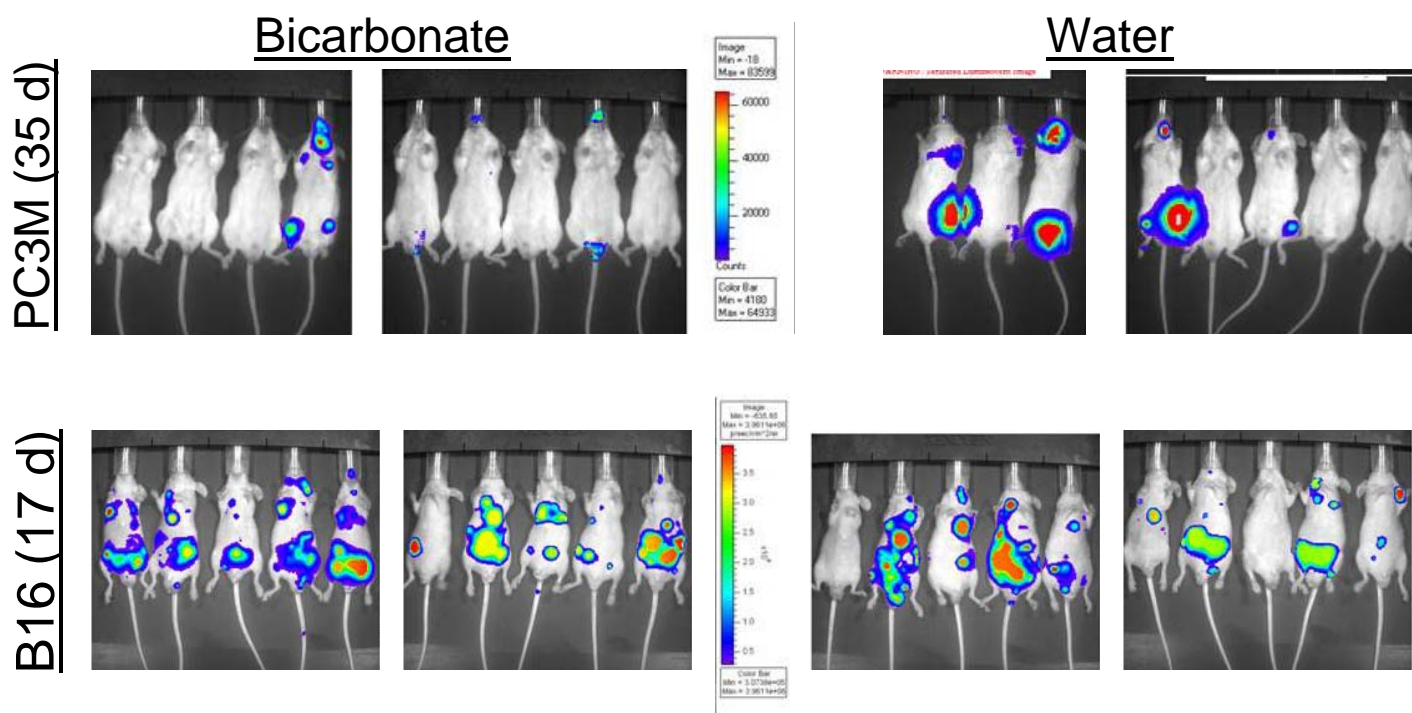


**Figure S2 | (a)** Extent and density of MDA-MB-231/GFP tumors measured by Argon laser (488 nm) excitation and emissions with a 515/30 nm bandpass filter. 10X FOV = 12.5 mm **(b)** Extracellular pH was measured following injection of SNARF-1 free acid by excitation with a He/Ne laser at 543 nm and emissions were collected in channel 1 with a 595/50 nm bandpass and in channel 2 with a 640 nm long pass filter. The pH was calculated from ratio of emission in channel 2 to channel 1 and thus the measurement is independent of the dye concentration. 10X FOV = 12.5 mm

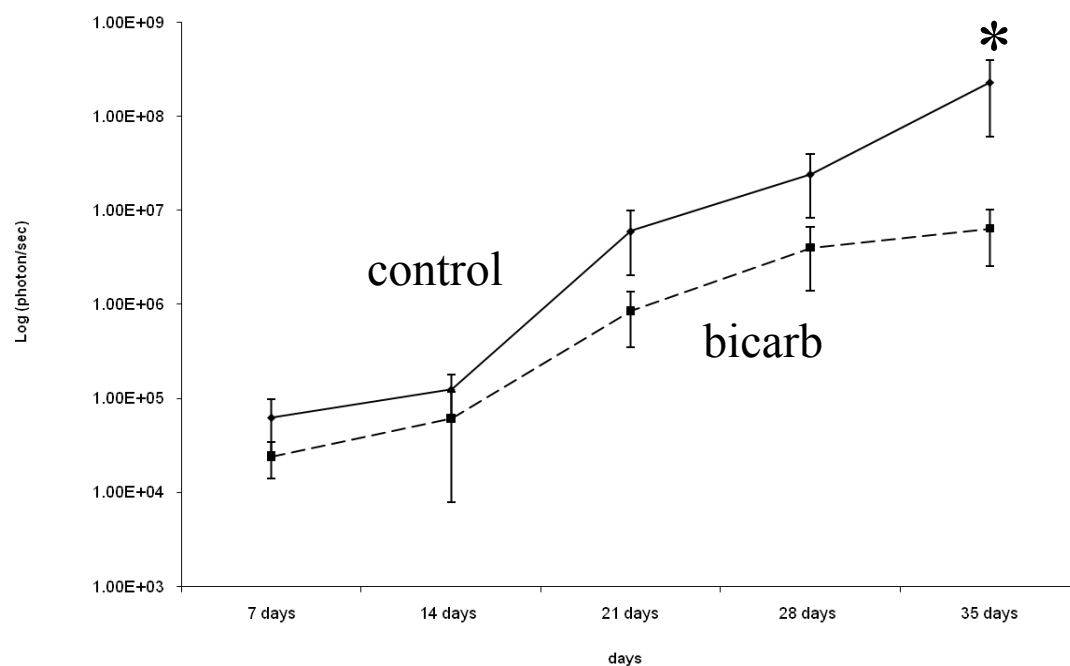


**Figure S3 | Representative GFP and white light images of lymph node infiltrates.**

GFP-expressing MDA-MB-231 tumors were grown for 30 d in mammary fat pads of mice that were given tap water or 200mM NaHCO<sub>3</sub> to drink. At time of necropsy, animals were examined by fluorescence in a dissecting scope for evidence of metastases. Images were captured at the same focal plane in the presence of 480 nm excitation and > 490 nm filtered emission with an exposure time of 4 sec for GFP images, and white light illumination with an exposure time of 1/10 sec. GFP images from ‘trace’ infiltrates have been enhanced by increasing intensity 32-fold.



**Figure S4 | Representative in vivo bioluminescence images.** PC3M-luc-C6 and B16-F10-luc-G5 were obtained from Caliper Life Sciences (Hopkinton, MA) and maintained in MEM/EBSS media supplemented with 10%FBS.  $5 \times 10^5$  B16-F10-luc cells or  $5 \times 10^6$  PC3M-luc cells were injected intravenously through the tail vein of male Nu/Nu mice or male SCID beige mice (n=20 for each cell line). For B16 cells, mice were imaged biweekly from dorsal and ventral views, from day 0 to day 17; they were sacrificed on day 17, and *ex-vivo* images of excised lungs, livers and rib cages were taken immediately after sacrifice. PC3M cells were treated identically, except mice were imaged weekly for 7 weeks. For each experiment, 10 mice were started on drinking water (*ad libitum*) supplemented with 200mM NaHCO<sub>3</sub> six (6) days prior to injections. Water consumption and mice weights were recorded biweekly. Images were obtained using Xenogen IVIS 200 imaging system and analyzed using Xenogen Living Image® software. These data we collected at 17 and 35 days post-injection for B16 and PC3M, respectively.



**Figure S5 | Log bioluminescence (photons/sec) from mice injected with PC3M cells.**

Mice were tail vein injected with f-luc expressing PC3M cells and monitored weekly during tumor growth using a Caliper IVIS-200 imaging system. Data are expressed as average of 10 mice per group  $\pm$  SEM. \*  $p < 0.05$ .