Supporting Information

Title: “Flow-encoded oxygen control to track the time-dependence of molecular changes induced by static or cycling hypoxia:

Authors: Ming Yao,[†] Zahid N. Rabbani,[‡] Tyler Sattler,[‡] Khue G. Nguyen,[‡] David A. Zaharoff,[‡] Glenn Walker,[‡] Michael P. Gamcsik[‡]

[†]Department of Mechanical and Aerospace Engineering, NC State University, Raleigh, NC 27695

[‡] UNC/NCSU Joint Department of Biomedical Engineering, Raleigh, NC 27695

Corresponding Author: Michael P. Gamcsik, mgamcsi@ncsu.edu

Table of Contents - Supplementary Figures

Figure S1 Legend: COMSOL modeling

Heat maps of pO_2 content in flow-encoding manifold at different flow-rates of Input 1 Gas 95% N2 /5% CO2 and Input 2 Gas 95% Air/5% CO_2 at an initial time point (a) and arbitrary time points t_1-t_7 (b)-(h). The flow rates of the two gases are given above the blue and red arrows in each figure. The heat maps of content show sequential switching of output gas channels of each row A-C from air/CO_2 to N_2/CO_2 at each time point. (h) Show theredicted oxygen gradient along one row of the plate when, there are 50K mcf7 cells in each well, consumption rate is 3.5e-17 mol O_2·cells^{-1}·s^{-1} at a gas flow rate of air/5% CO_2 of 3 mL/min. COMSOL predicts a gradient of <0.04 mmHg. Note the difference in scale for the heat map.

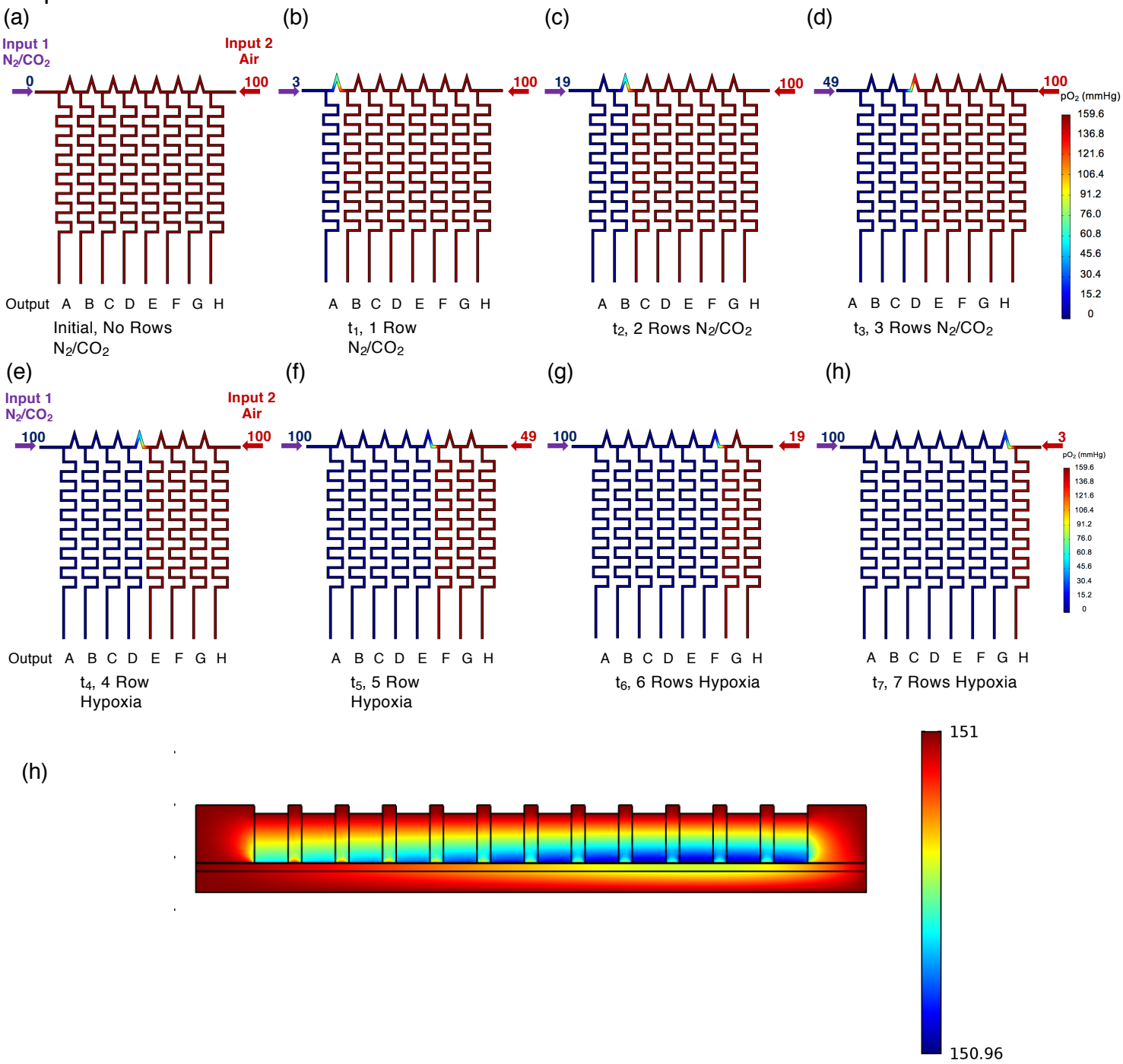

Figure S2: Relative cell counts and cell viability (ATP content) for cells under normoxia (0 h) or 1 – 24 h of static hypoxia. In all cases, Cells were initially seeded at approximately 1.5 x 10^4 cells/well. They were allowed to attach for 24 h under air/CO_2 and then exposed to hypoxia for periods for up to 24h. Data shown is the average +/- the standard deviation from 3 wells at each time point. There was no statistically significant difference in cell numbers or viability when comparing the 0 and 24 h timepoints.

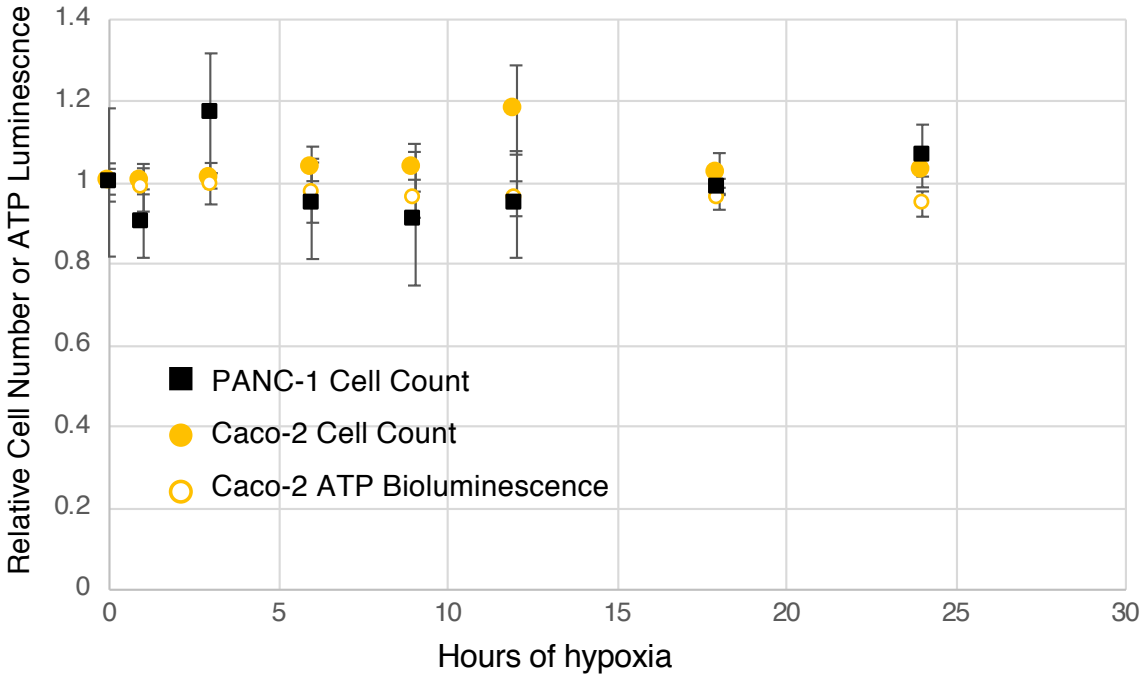

Figure S3: Histochemistry of Caco-2 cells exposed to static (1 mmHg) or cycled hypoxia consisting of 30 min cycles of 134 and 1 mmHg for periods between 0 and 24 h. The top row in each series shows HIF-1α staining. DAPI staining shows cell nuclei. The plate used for cycling hypoxia includes one row of cells is exposed to static hypoxia for 24 h as a comparison to cells exposed to cycled hypoxia for 24 h on the same plate.

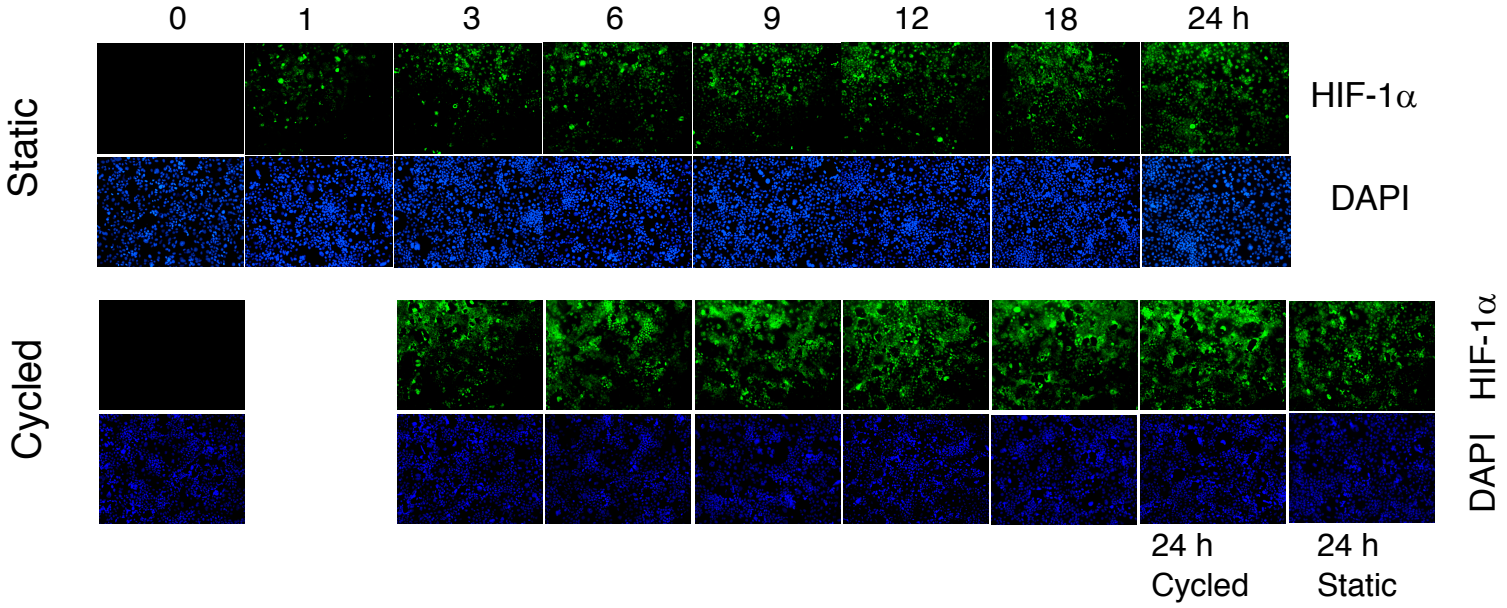

Figure S4 Uptake of the fluorescent 2-deoxyglucose analog 2-NBDG in (A) PANC-1 and (B) Caco-2 cells exposed to static (1 mmHg) or cycled hypoxia consisting of 30 min cycles of 134 and 1 mmHg for periods between 0 and 24 h. the plate used for cycling hypoxia includes one row of cells is exposed to static hypoxia for 24 h as a comparison to cells exposed to cycled hypoxia for 24 h on the same plate.

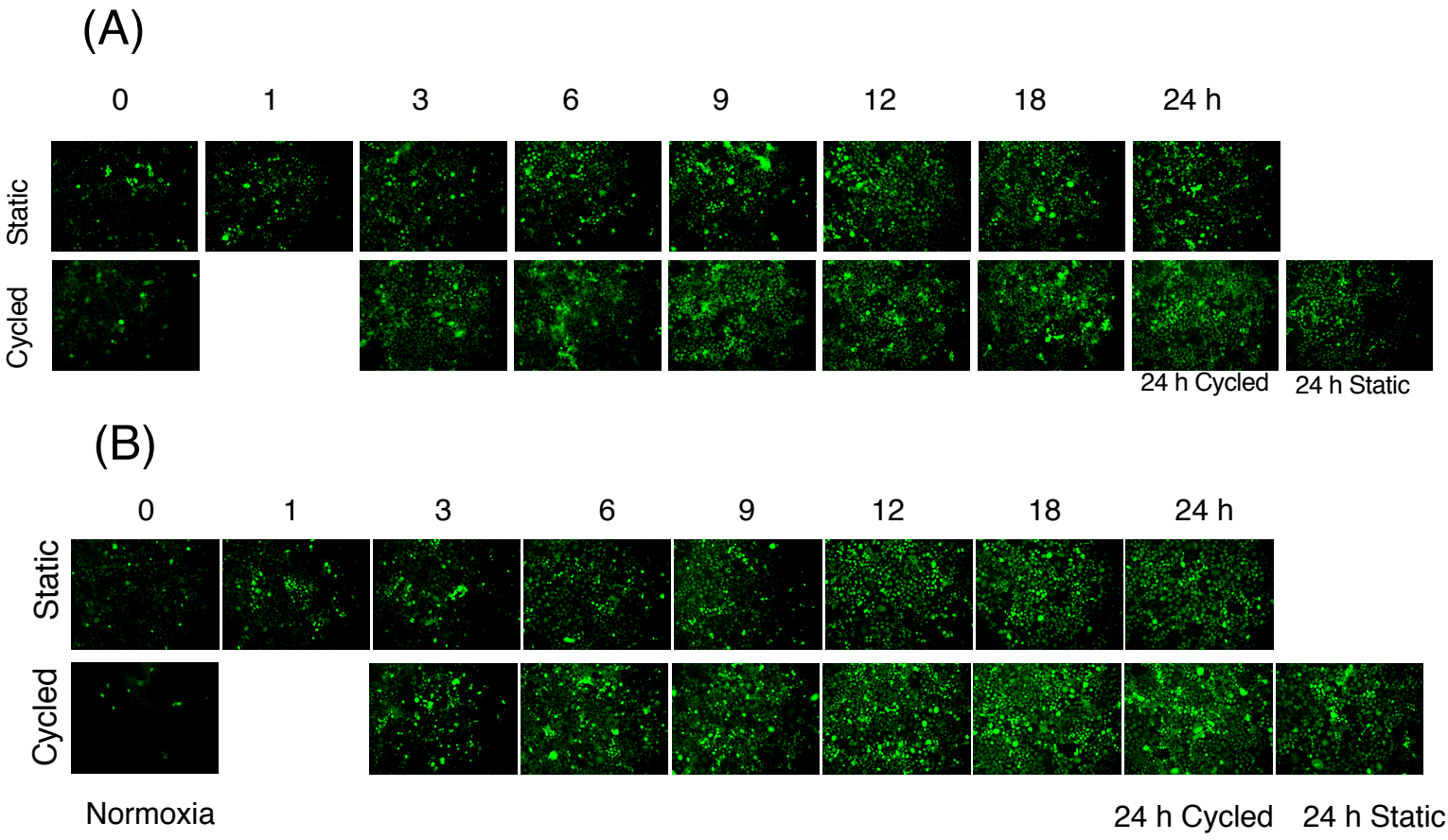

Figure S5: Fluorescent micrographs of ALDH activity in PANC-1 cells as measured by the uptake and metabolism of BAAA reagent. The plate used for cycling hypoxia includes one row of cells is exposed to static hypoxia for 24 h as a control for comparison to cells exposed to cycled hypoxia for 24 h on the same plate.

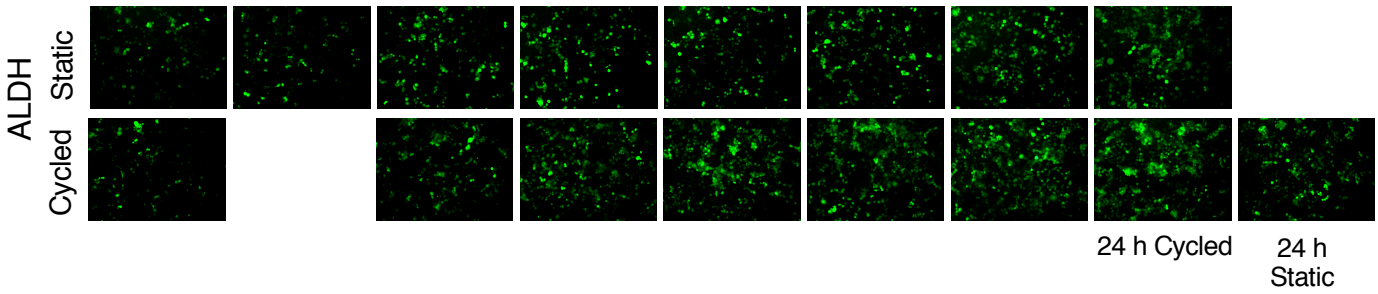

Figure S6: Flow cytometry data for PANC-1 cells (A) DEAB treated control under static hypoxia and (B) DEAB treated control under cycling hypoxia (C) Test group under static hypoxia for 0 – 24