Supplemental Figure Legends

Supplemental Figure 1: Effect of pyruvate vs. lactate on breast cancer cell proliferation, lactate dehydrogenase (LDH) activity and the NAD^+/NADH pool.
Media containing glucose (5.56 mM) and increasing concentrations of lactate (0.1-10 mM) were used to culture MCF7 cells for 72 h prior to assessment of total cell number **(A)**. MCF7 cells were cultured for 72 h in media containing glucose (5.56 mM) with pyruvate (1 mM) or lactate (1 mM). Cells were then harvested for spectrophotometric determination of LDH activity **(B)** or HPLC analysis of NAD^+/NADH **(C)**. LDH activity is expressed as μmol NADH consumed/min/mg protein. Data were normalized to total protein, and values represent means ± SEM, n=3-6. N.S. denotes no significant difference between groups.

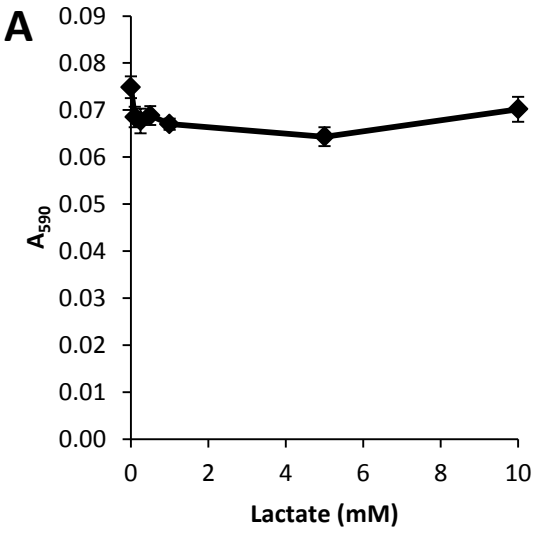
Supplemental Figure 2: Regulation of mitochondrial function by metabolic substrate supply.
MCF7 cells were seeded in specialized microplates and cultured for 24 h. Cells were then switched to unbuffered DMEM containing glucose only (5.5.6 mM), pyruvate only (1 mM), glucose and pyruvate (complete media), or complete media with CHC (500 μM) 1 h prior to measuring mitochondrial function assessed using sequential injection of oligomycin (O), FCCP (F), and Antimycin A (A). ATP-linked oxygen consumption rate (OCR), proton leak, and non-mitochondrial OCR are shown. Extracellular acidification rate (ECAR) was measured concomitantly. OCR and ECAR were normalized to total protein/well after completion of assay. Values represent means ± SEM, n=3-5. * $p < 0.05$ compared to complete media. N.S. denotes no significant difference between groups.

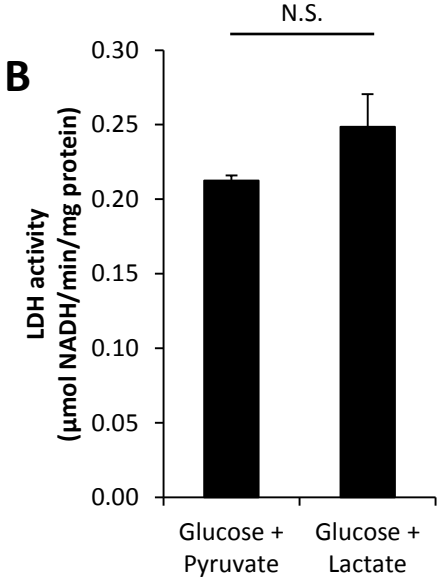
Supplemental Figure 3: Effect of pyruvate vs. lactate on mitochondrial function in breast cancer cells.
MCF7 cells were seeded in specialized microplates and cultured for 24 h. Cells were then switched to unbuffered DMEM media containing glucose (5.56 mM) supplemented with pyruvate (1 mM) or lactate (1 mM), and mitochondrial function was assessed using sequential injection of oligomycin (O), FCCP (F), and Antimycin A (A). Basal and maximal oxygen consumption rates (OCRs), ATP-linked OCR, proton leak, reserve capacity, and non-mitochondrial OCR are shown. Extracellular acidification rate (ECAR) was measured concomitantly. OCR and ECAR were normalized to total protein/well after completion of assay. Values represent means ± SEM, n=3-5. * $p < 0.05$ compared to pyruvate containing media. N.S. denotes no significant difference between groups.

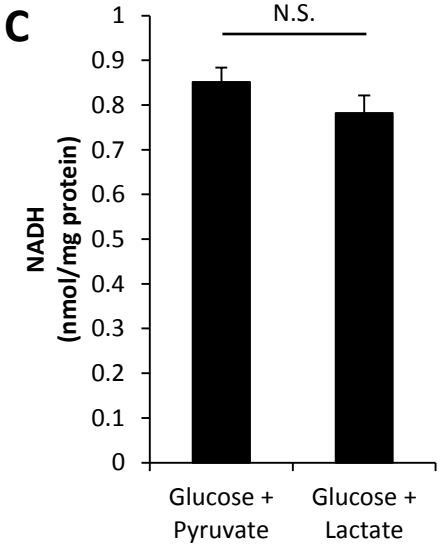
Supplemental Figure 4: Effect of metabolic substrates on adenine nucleotide pools.
MCF7 cells were cultured for 72 h in media containing glucose only (5.56 mM), pyruvate only (1 mM), lactate only (1 mM), glucose and lactate, glucose and pyruvate (complete media), or complete media containing CHC (500 μM). Cells were then harvested for HPLC analysis of ATP **(A)** and ADP **(B).** Data were normalized to total protein, and values represent means ± SEM, n=3. N.S. denotes no significant difference between groups.

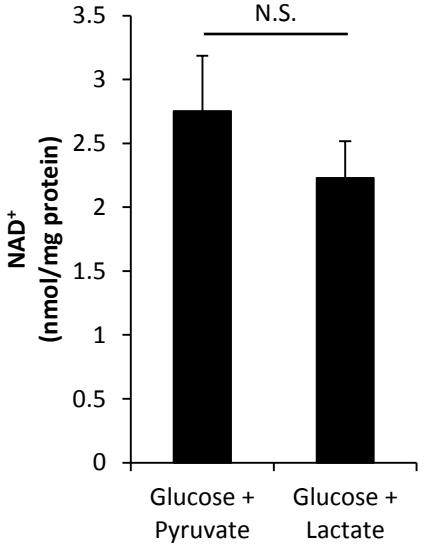
Supplemental Figure 5: Effect of CHC on mitochondrial function in the presence of different metabolic substrates.
MCF7 cells were seeded in specialized microplates and cultured for 24 h. 1 h prior to assessment of mitochondrial function, cells were switched to unbuffered DMEM containing glucose only (5.56 mM), pyruvate only (1 mM), or glucose and pyruvate (complete media) in the absence (closed bars) or presence (open bars) of CHC (500 μM), and mitochondrial function was assessed using sequential injection of oligomycin (O), FCCP (F), and Antimycin A (A). ATP-linked oxygen consumption rate (OCR), proton leak, and non-mitochondrial OCR are shown. Extracellular acidification rate (ECAR) was measured concomitantly. OCR and ECAR were normalized to total protein/well after completion of assay. Values represent means ± SEM, n=3-4. * $p < 0.05$ compared to media condition without CHC. N.S. denotes no significant difference between groups.

Supplemental Figure 1

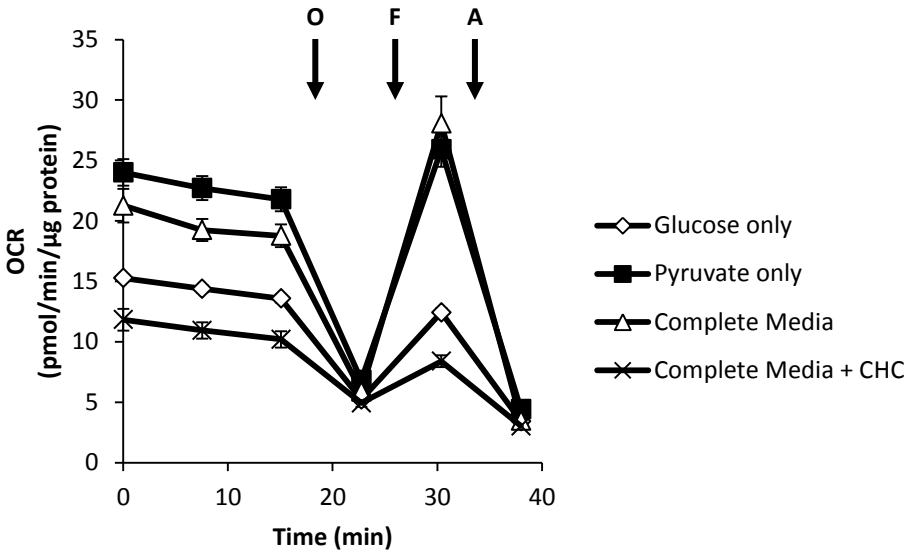


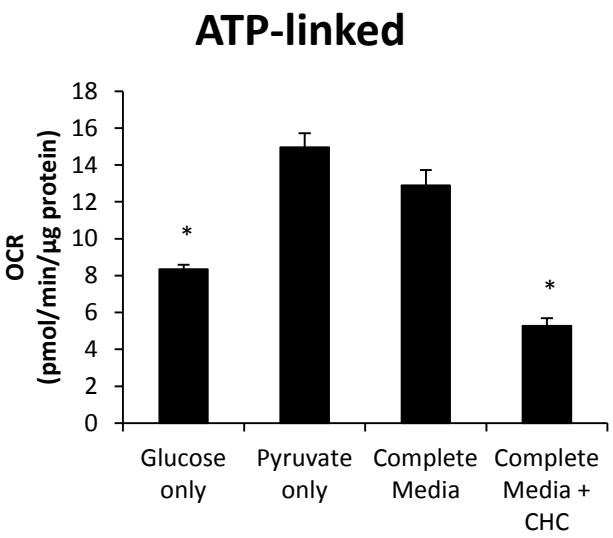


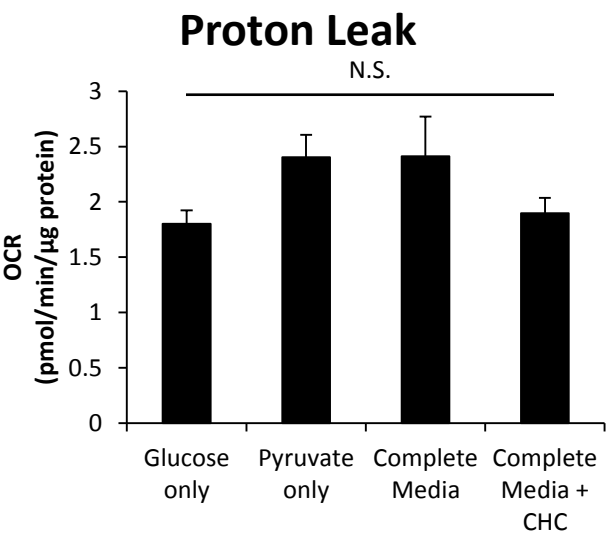


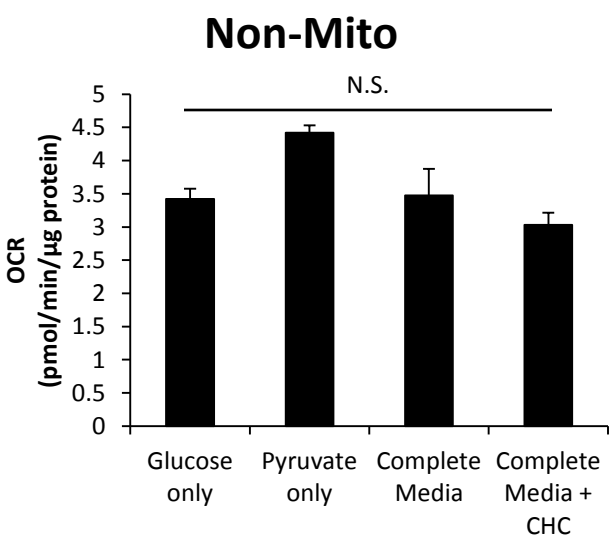


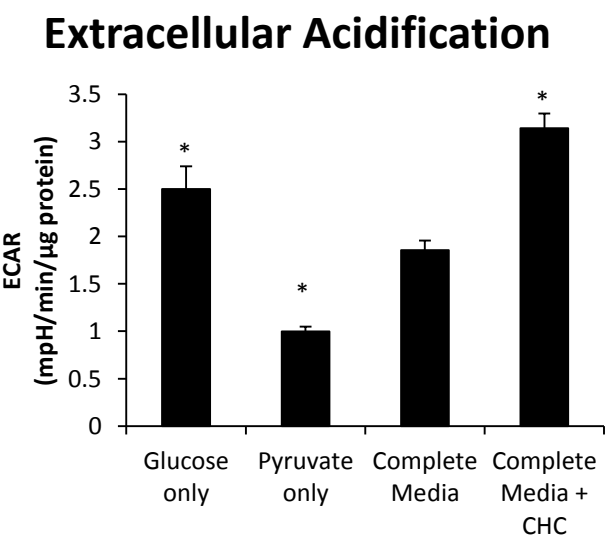
Supplemental Figure 2



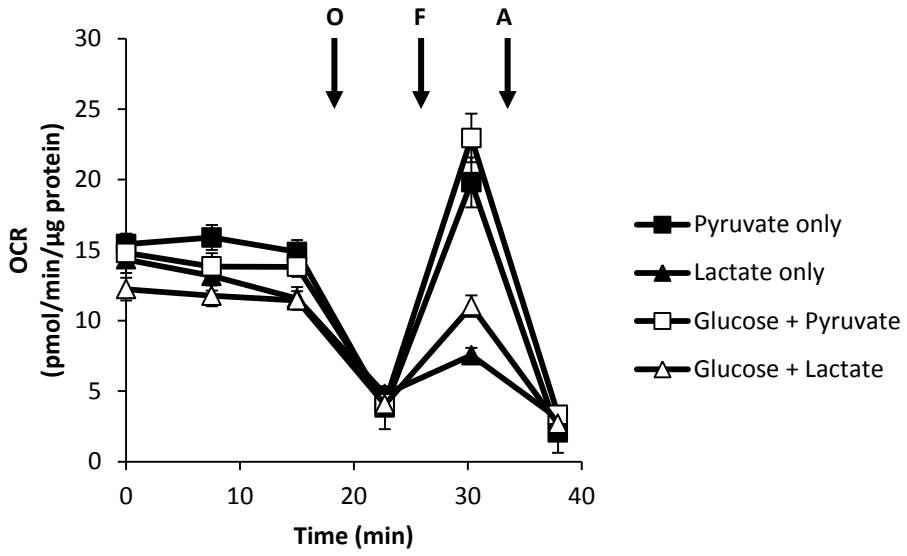


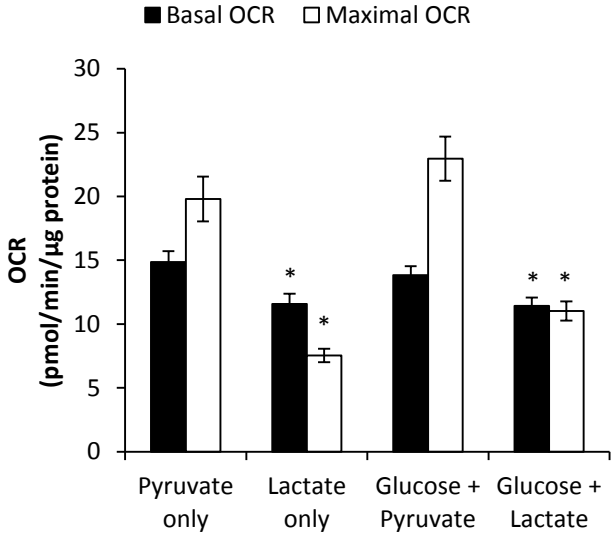


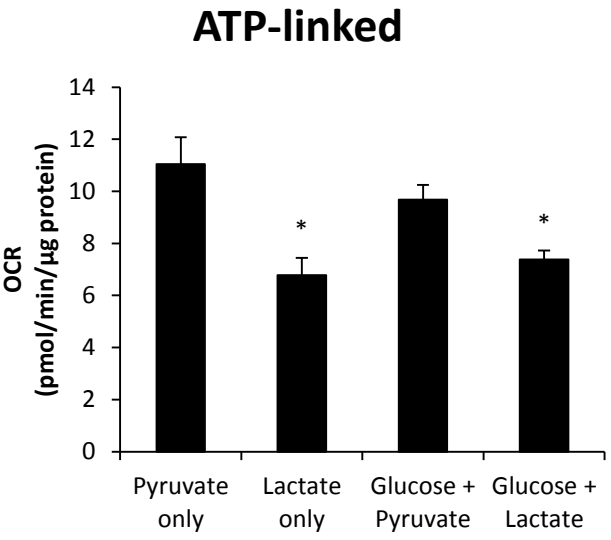


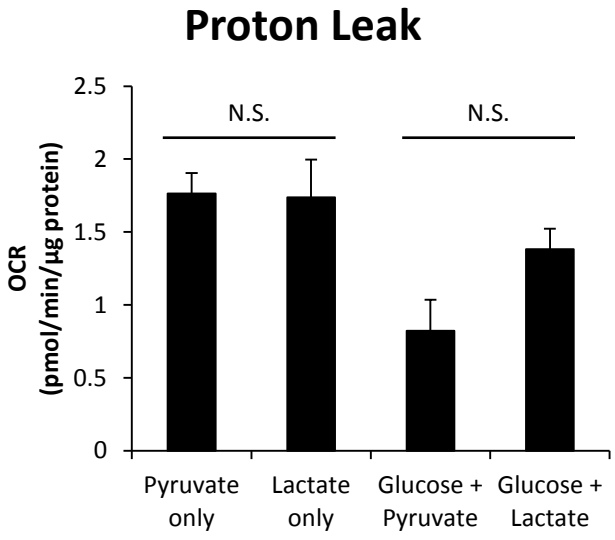


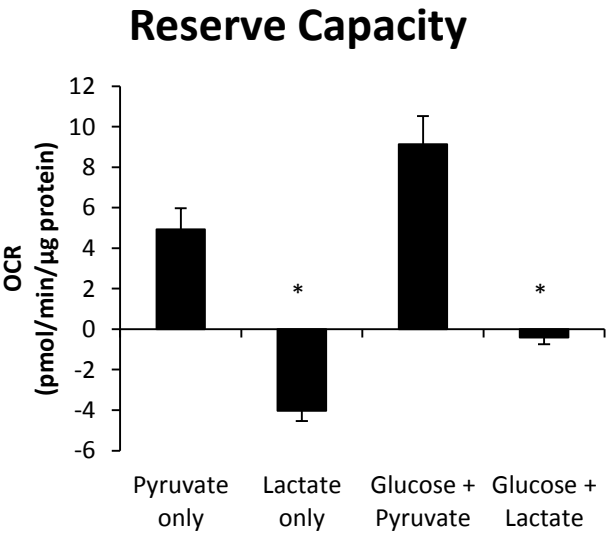
Supplemental Figure 3











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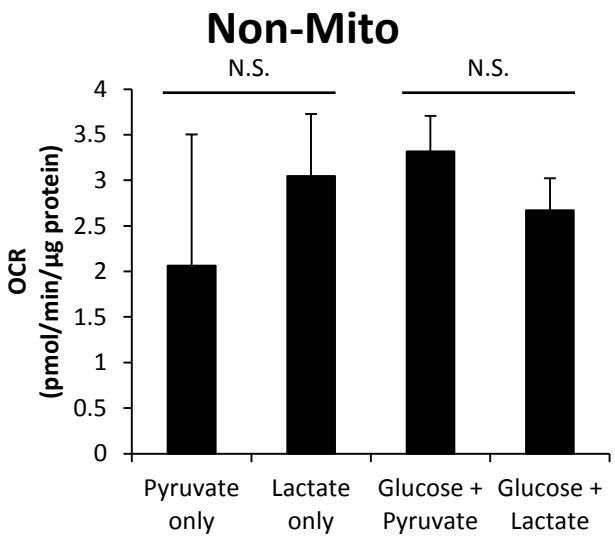
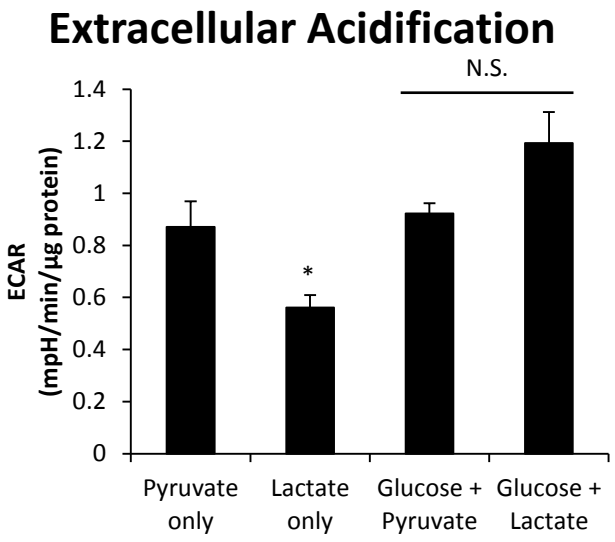
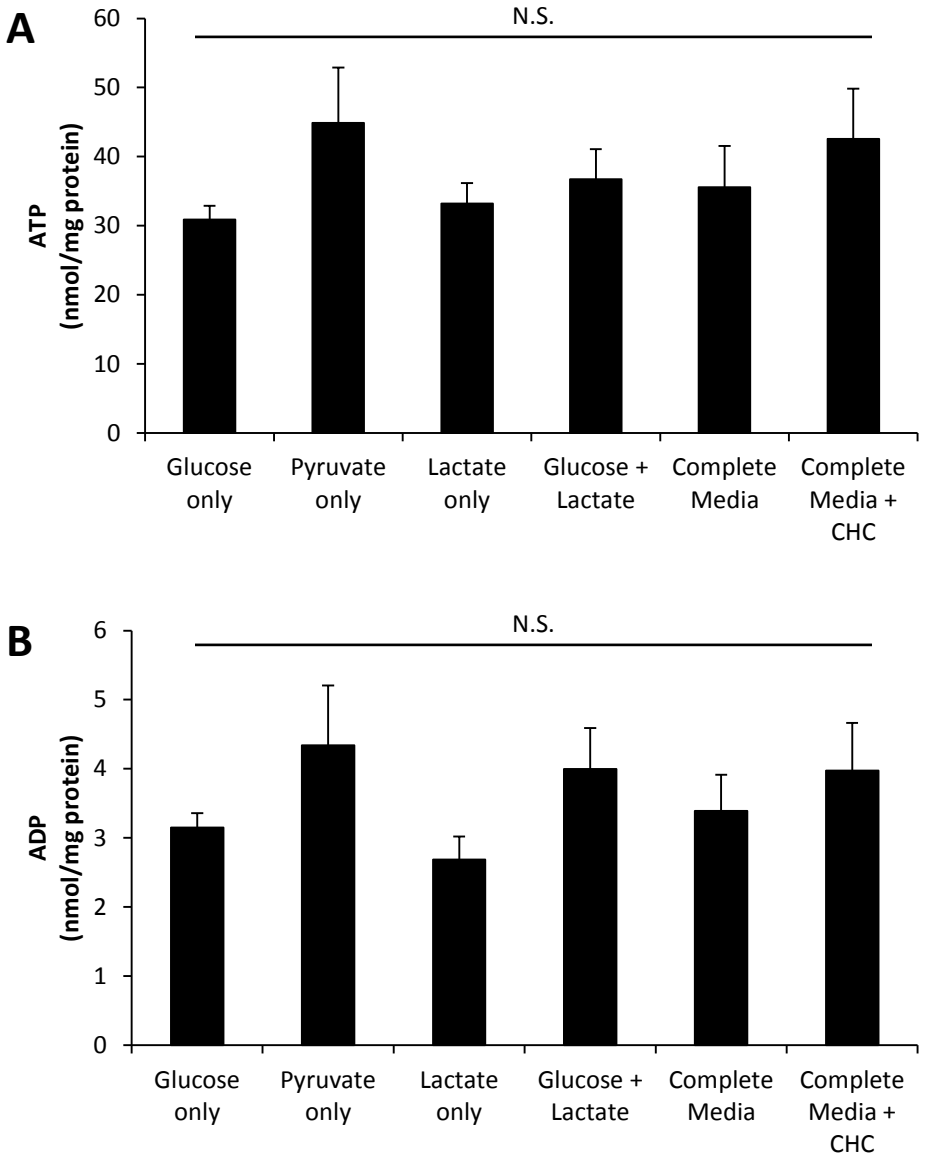




Supplemental Figure 4



Supplemental Figure 5