Supplemental Figure Legends:

Supplemental Figure 1: ATP/ADP and NAD+/NADH ratios are not altered by changes in collagen extracellular matrix density.

A. ATP/ADP ratio calculated from total ion counts for ATP and ADP from U ^{13}C glutamine labeling experiment. Ratio was calculated for each replicate and then averaged (N=3, mean +/- SD). **B.** NAD+/NADH ratio calculated from total ion counts for NAD+ and NADH from U ^{13}C glutamine labeling experiment. Ratio was calculated for each replicate and then averaged (N=3, mean +/- SD).

Supplemental Figure 2: Oxygen consumption and extracellular acidification rates are altered by collagen matrix density in a normal mouse mammary gland cell line and a human breast carcinoma cell line.

A and B. Mean basal oxygen consumption and extracellular acidification rate for NMuMG normal mouse epithelial cells in LD and HD collagen matrices (N=12, SD, Significance via t-test $p<0.05$). **C and D.** Mean basal oxygen consumption and extracellular acidification rate for MDA-MB- 231 human carcinoma cells in LD and HD collagen matrices (N=7, SD, Significance via t-test $p<0.05$). Cells were analyzed in collagen spheroids using a Seahorse Flux analyzer, as described in Methods.

Supplemental Figure 3: Glucose flux through glycolysis and to lactate is not altered by changes in collagen extracellular matrix density.

A, B. Contribution of 1,2- ^{13}carbon glucose to the glycolytic intermediate fructose-1,6-bisphosphate and to lactate through lactate dehydrogenase. (N=3, mean +/- SD). **C**. Fraction of metabolite remaining unlabeled following glucose and glutamine labeling experiments (N=3 per labeling experiment, mean +/- SD).

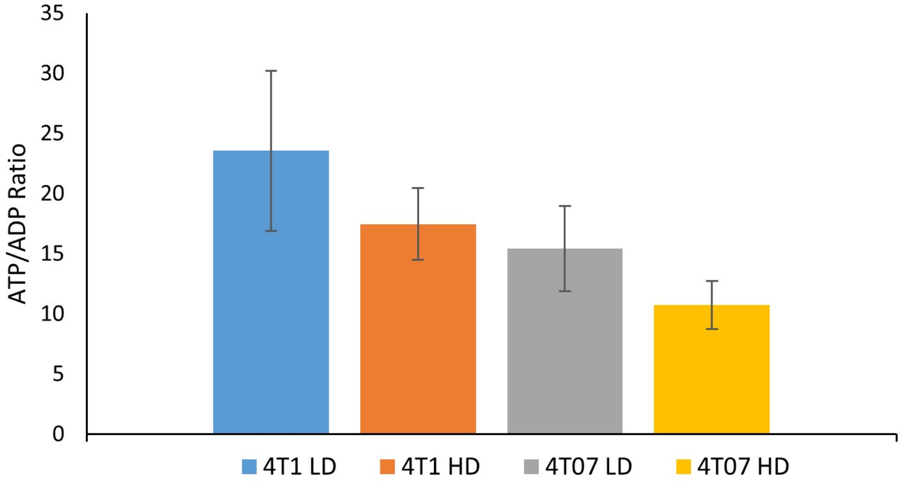
Supplemental Figure 4: Changes in oxygen consumption following addition of glutamine or glucose to minimal media lacking glucose or glutamine.

A. Percent change in oxygen consumption levels from baseline (first 3 measurements) after injection of glutamine (300 mg/L) to media lacking glucose and glutamine. Rotenone and Antimycin A were injected (1 μM final concentration for each) at the end of each experiment as a control for microgel displacement. (N=15, mean +/- SD). **B.** Percent change in oxygen consumption levels from baseline (first 3 measurements) after injection of glucose (2 g/L) to media lacking glucose and glutamine. Rotenone and Antimycin A were injected (1 μM final concentration for each) at the end of each experiment as a control for microgel displacement. (N=15, mean +/- SD). **C**. Percent change in extracellular acidification levels from baseline (first 3 measurements) after injection of glucose (2 g/L) to media lacking glucose and glutamine. Rotenone and Antimycin A were injected (1 μM final concentration for each) at the end of each experiment as a control for microgel displacement. (N=15, mean +/- SD).

Supplemental Figure 5: Basal oxygen consumption and extracellular acidification rate is not significantly altered in 4T1 cells in LD collagen matrix by pretreatment of Sodium Dichloroacetate

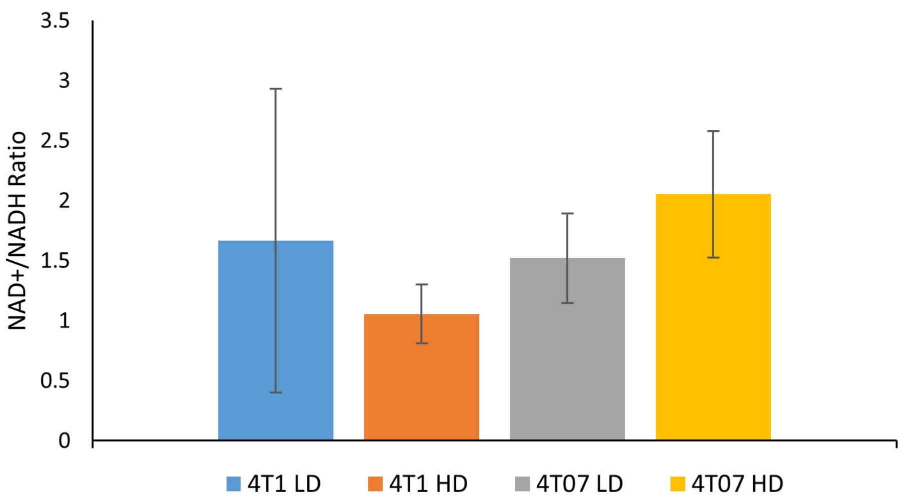
A and B. Mean basal oxygen consumption and extracellular acidification rate for 4T1 cells in LD collagen matrices following a 16 hour pretreatment of 0, 10 or 25 mM dichloroacetate (DCA, N=12, +/- SD). **C**. Representative western blot of collagen microgel used for basal OCR and ECAR SeaHorse experiment. Phosphorylation of pyruvate dehydrogenase was decreased by addition of 10mM and 25 mM DCA.

a.

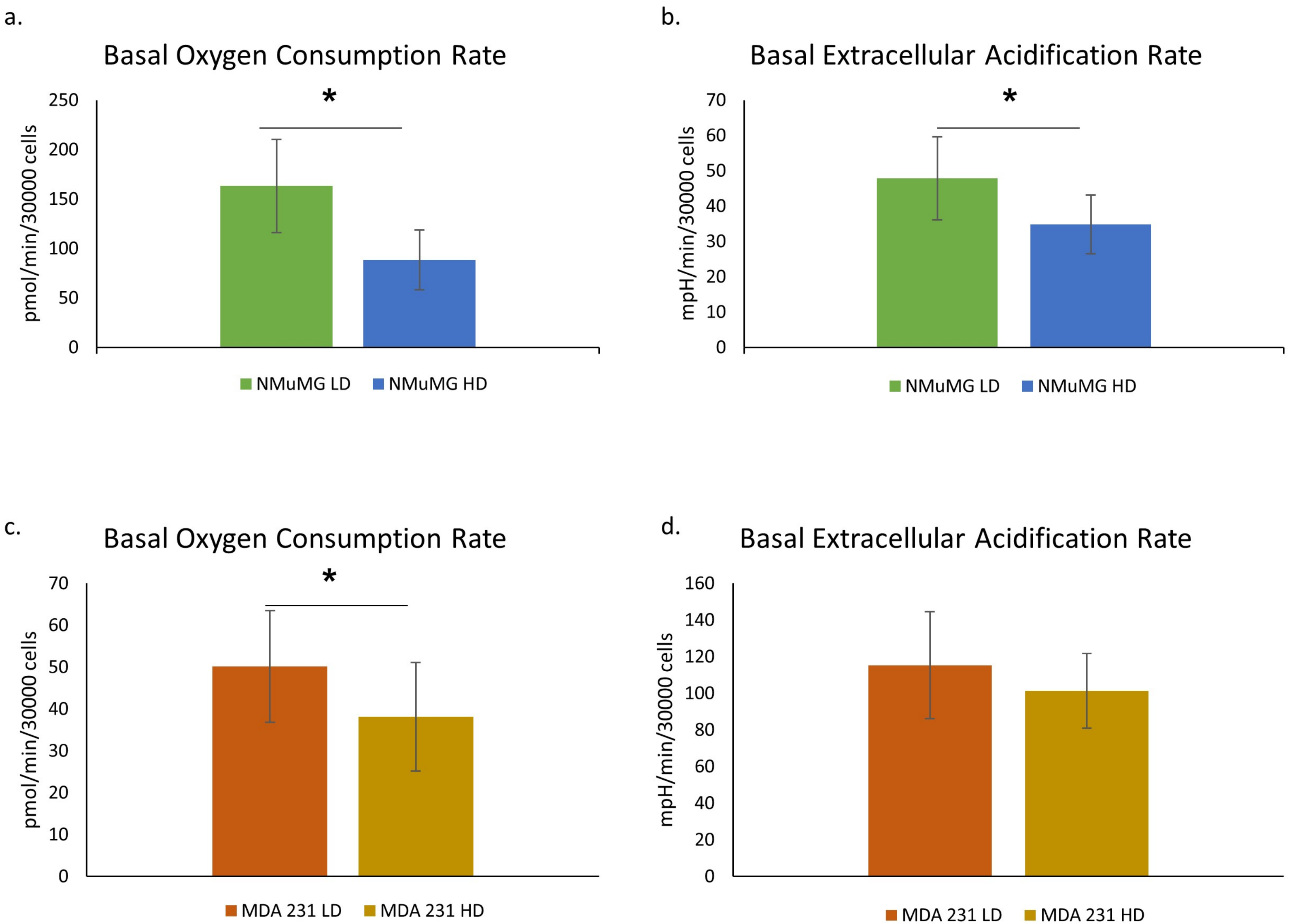


35
30
25
20
15
10
5
0
ATP/ADP Ratio
4T1 LD
4T1 HD
4T07 LD
4T07 HD

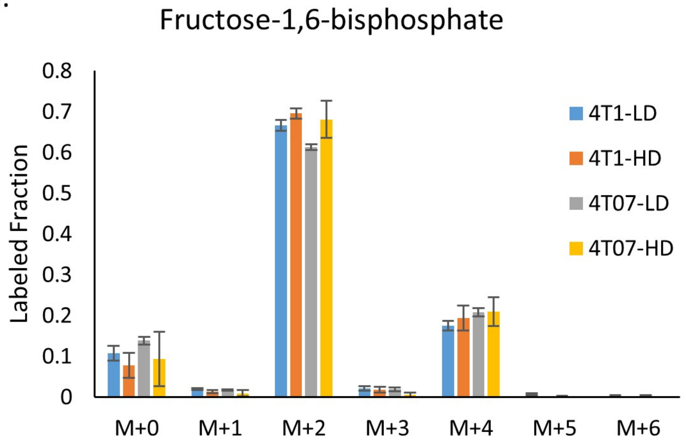
b.



3.5
3
2.5
2
1.5
1
0.5
0
NAD+/NADH Ratio
4T1 LD
4T1 HD
4T07 LD
4T07 HD

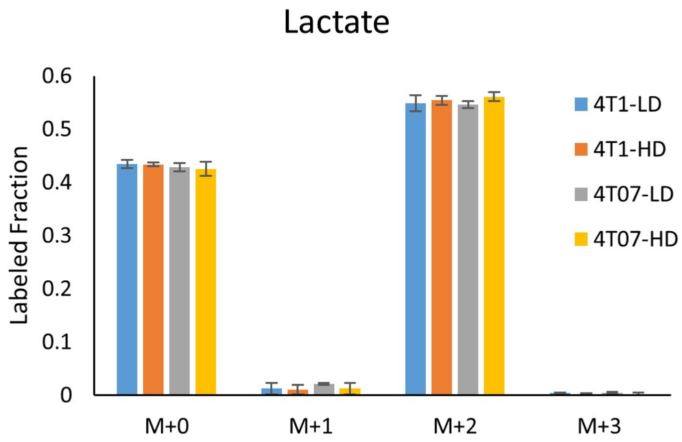

a.
Basal Oxygen Consumption Rate
*
pmol/min/30000 cells
250
200
150
100
50
0
NMuMG LD
NMuMG HD
b.
Basal Extracellular Acidification Rate
*
mpH/min/30000 cells
70
60
50
40
30
20
10
0
NMuMG LD
NMuMG HD
c.
Basal Oxygen Consumption Rate
*
pmol/min/30000 cells
70
60
50
40
30
20
10
0
MDA 231 LD
MDA 231 HD
d.
Basal Extracellular Acidification Rate
mpH/min/30000 cells
160
140
120
100
80
60
40
20
0
MDA 231 LD
MDA 231 HD

a.



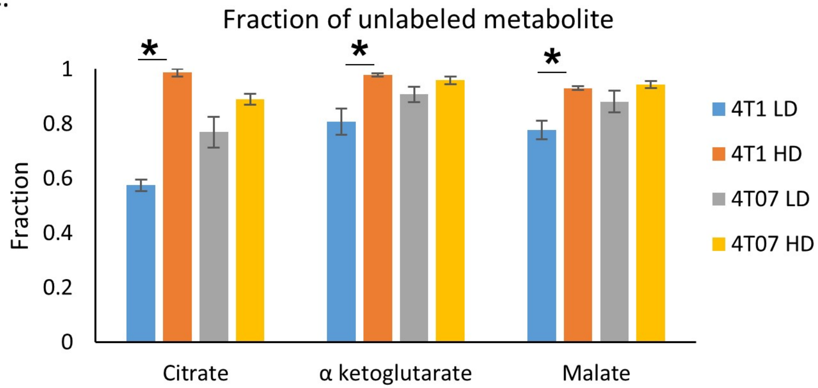
Fructose-1,6-bisphosphate
Labeled Fraction
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
M+0
M+1
M+2
M+3
M+4
M+5
M+6
4T1-LD
4T1-HD
4T07-LD
4T07-HD

b.

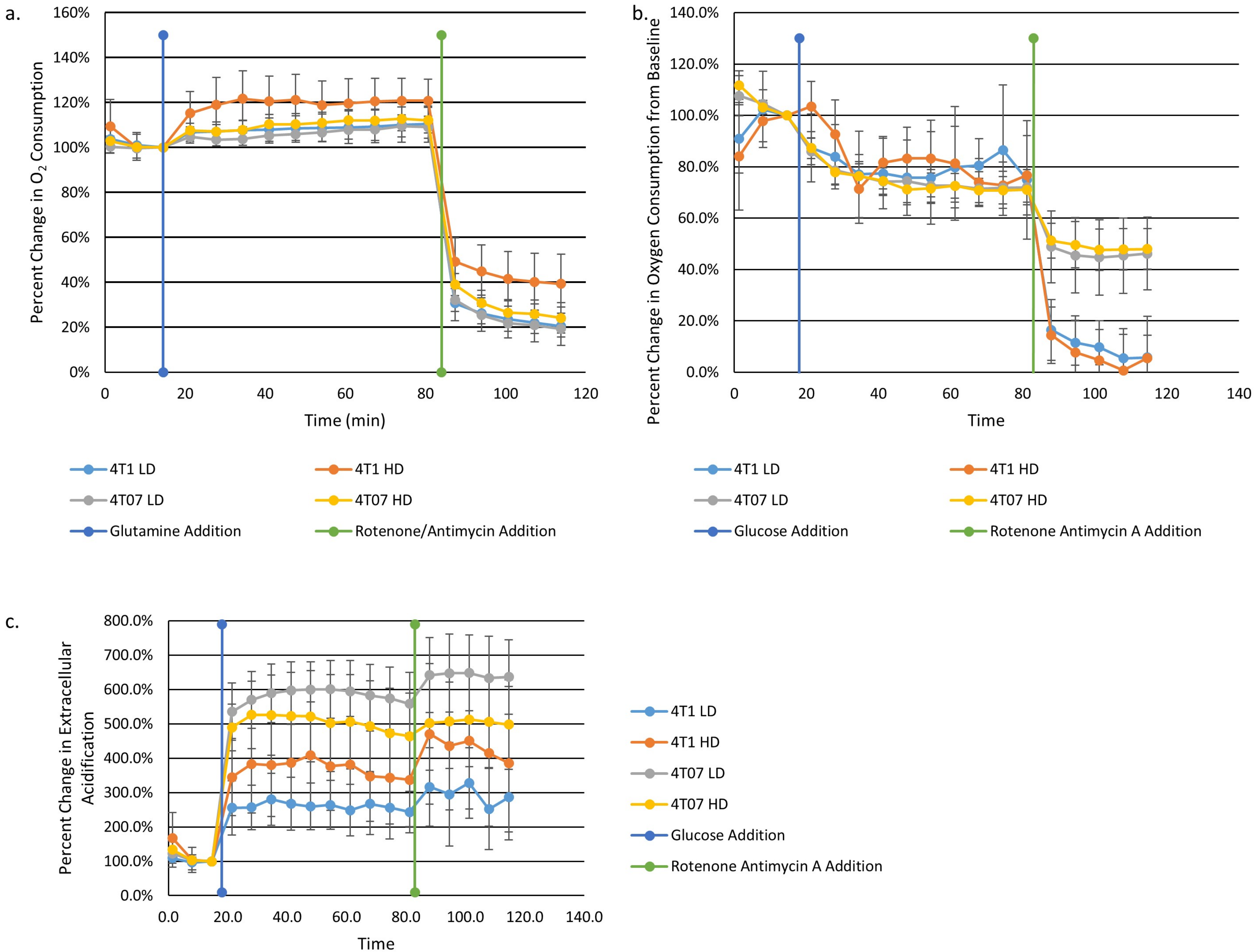


Lactate
Labeled Fraction
0.6
0.5
0.4
0.3
0.2
0.1
0
M+0
M+1
M+2
M+3
4T1-LD
4T1-HD
4T07-LD
4T07-HD

c.

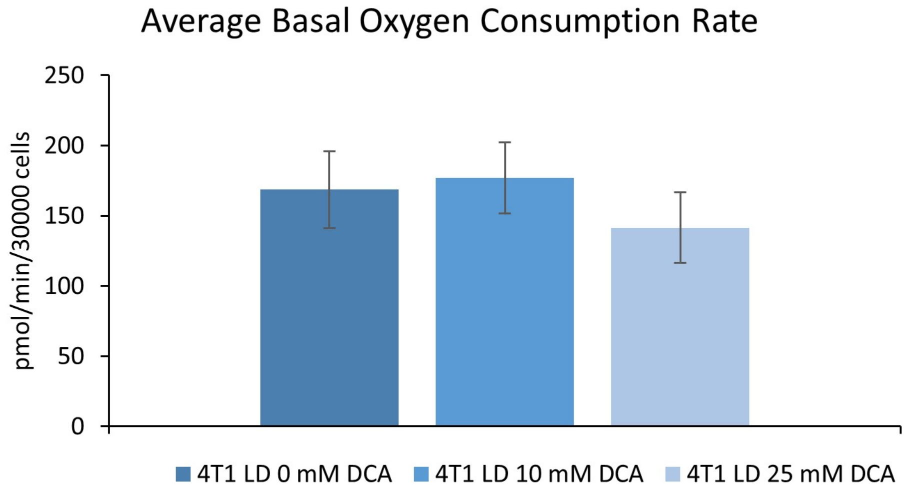


Fraction of unlabeled metabolite
Fraction
1
0.8
0.6
0.4
0.2
0
*
*
*
Citrate
α ketoglutarate
Malate
4T1 LD
4T1 HD
4T07 LD
4T07 HD



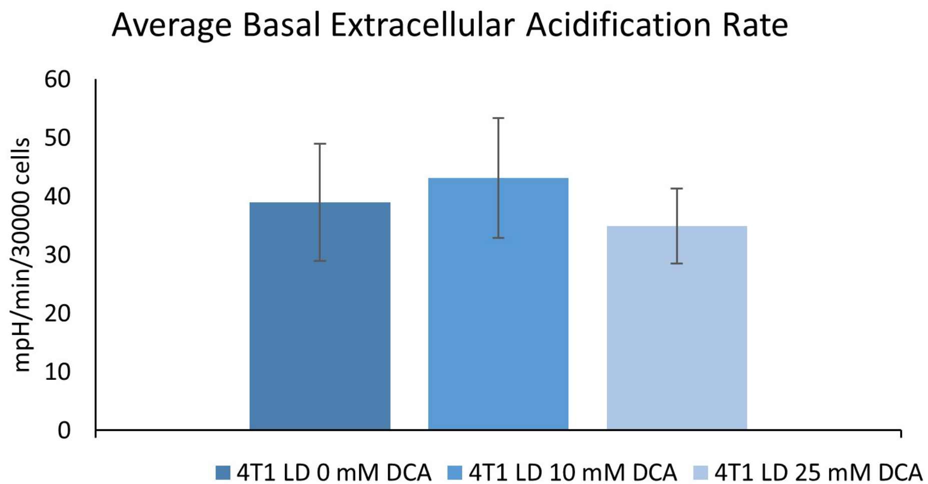
a.
Percent Change in O_2 Consumption
160%
140%
120%
100%
80%
60%
40%
20%
0%
0
20
40
60
80
100
120
Time (min)
4T1 LD
4T1 HD
4T07 LD
4T07 HD
Glutamine Addition
Rotenone/Antimycin Addition
b.
Percent Change in Oxygen Consumption from Baseline
140.0%
120.0%
100.0%
80.0%
60.0%
40.0%
20.0%
0.0%
0
20
40
60
80
100
120
140
Time
4T1 LD
4T1 HD
4T07 LD
4T07 HD
Glucose Addition
Rotenone Antimycin A Addition
c.
Percent Change in Extracellular Acidification
800.0%
700.0%
600.0%
500.0%
400.0%
300.0%
200.0%
100.0%
0.0%
0.0
20.0
40.0
60.0
80.0
100.0
120.0
140.0
Time
4T1 LD
4T1 HD
4T07 LD
4T07 HD
Glucose Addition
Rotenone Antimycin A Addition

a.



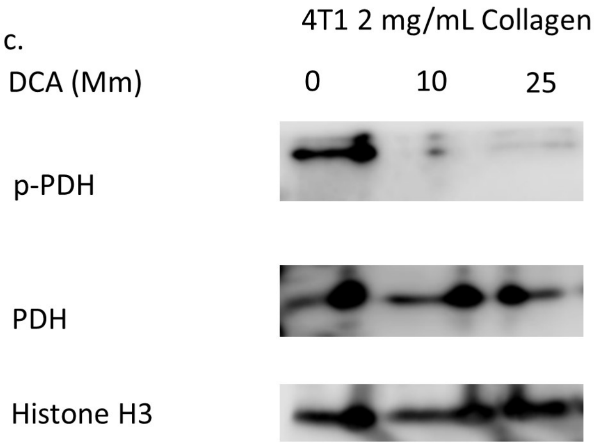
Average Basal Oxygen Consumption Rate
pmol/min/30000 cells
250
200
150
100
50
0
4T1 LD 0 mM DCA
4T1 LD 10 mM DCA
4T1 LD 25 mM DCA

b.



Average Basal Extracellular Acidification Rate
mpH/min/30000 cells
60
50
40
30
20
10
0
4T1 LD 0 mM DCA
4T1 LD 10 mM DCA
4T1 LD 25 mM DCA

c.



4T1 2 mg/mL Collagen
DCA (Mm)
0
10
25
p-PDH
PDH
Histone H3