

**Metabolomic and Mitochondrial Fingerprinting of the
Epithelial-to-Mesenchymal Transition (EMT) in
Non-Tumorigenic and Tumorigenic Human Breast Cells**

SUPPLEMENTAL INFORMATION

Table S1. Concentration of bioenergetic metabolites in HMLE/HMLE-EMT and HMLER/HMLER-EMT cells

| Metabolite | HMLE | HMLE-EMT | HMLER | HMLER-EMT |
|--------------------------------------|-------------|--------------|-------------|--------------|
| Pyruvate ^{c,d} | 3.8 (0.6) | 2.4 (0.6) | 2.7 (0.6) | 21.4 (2.0) |
| Lactate ^f | 78 (9) | 83 (5) | 86 (4) | 551 (57) |
| Alanine ^{c,f} | 48 (12) | 102 (12) | 45 (6) | 122 (22) |
| Valine ^{a,e} | 328 (17) | 574 (43) | 304 (17) | 361 (43) |
| Leucine ^{a,d} | 428 (39) | 676 (19) | 493 (41) | 419 (27) |
| Isoleucine ^{a,e} | 202 (28) | 366 (11) | 211 (28) | 280 (30) |
| Succinate ^c | 6.4 (0.6) | 5.9 (1.4) | 7.1 (0.8) | 3.7 (0.7) |
| Fumarate ^{c,f} | 0.7 (0.2) | 2.3 (0.4) | 0.9 (0.1) | 5.4 (0.5) |
| Serine ^{a,e} | 516 (30) | 959 (174) | 502 (41) | 632 (65) |
| Oxaloacetate ^f | 5.5 (1.3) | 9.6 (0.6) | 9.6 (1.9) | 21.6 (2.7) |
| Malate ^{a,e,f} | 1.6 (0.1) | 3.5 (0.9) | 1.9 (0.4) | 13.9 (0.8) |
| Aspartate ^{a,c,f} | 124 (8) | 182 (30) | 134 (12) | 215 (24) |
| Phosphoenolpyruvate ^{a,c,f} | 0.78 (0.25) | 10.40 (0.63) | 1.96 (0.24) | 84.67 (4.02) |
| Glutamate ^{a,f} | 183 (16) | 332 (29) | 204 (25) | 313 (21) |
| Glutamine | 184 (167) | 3490 (2215) | 621 (321) | 1512 (2742) |
| Glyceraldehyde 3-P | ULOD | ULOD | ULOD | 49 (8) |
| 3-phosphoglycerate ^{a,c,f} | 14.6 (0.8) | 62.2 (10.2) | 24.2 (5.8) | 209.0 (7.7) |
| Citrate ^d | 17.0 (3.4) | 25.7 (4.0) | 21.1 (2.4) | 7.5 (0.6) |
| Glucose ^{a,f} | 6.4 (1.5) | 22.7 (3.7) | 8.5 (1.5) | 24.2 (2.8) |
| Ribose 5-P ^d | 5.7 (1.4) | 2.9 (0.5) | 6.8 (0.6) | 11.0 (1.9) |
| Fructose 6-P ^{a,e,f} | 2.7 (0.8) | 34.5 (2.4) | 6.2 (1.3) | 12.6 (1.8) |
| Glucose 6-P ^a | 1.4 (0.1) | 42.3 (3.0) | 5.6 (1.2) | 5.2 (0.3) |
| Fructose 1,6-BisP ^{a,b,d} | 2.1 (0.4) | 34.9 (2.2) | 5.8 (1.2) | 4.5 (0.9) |
| 6-P-gluconate ^{a,d} | 20.6 (2.5) | 33.0 (4.1) | 24.3 (1.3) | 21.9 (1.3) |
| 3-hydroxybutyrate ^{a,e,f} | 55 (9) | 136 (7) | 70 (6) | 102 (15) |
| Succinyl-CoA ^c | 10.4 (1.8) | 10.5 (3.1) | 8.0 (1.1) | 16.2 (1.9) |
| Malonyl-CoA ^{a,e,f} | 12.6 (2.1) | 32.2 (4.5) | 15.8 (2.3) | 24.7 (2.9) |
| α -ketoglutarate | ULOD | ULOD | ULOD | 0.25 (0.03) |
| 2-hydroxyglutarate | ULOD | ULOD | ULOD | 21 (2) |

Concentrations are shown in $\mu\text{M}/\text{mg}$ of protein \pm SEM (n=5, in triplicate)

Decimals were set according to the first significant digit of the measured SEM

^a Changes between HMLE and HMLE-EMT are statistically significant (p-value < 0.05)

^b Changes between HMLE and HMLER are statistically significant (p-value < 0.05)

^c Changes between HMLER and HMLER-EMT are statistically significant (p-value < 0.05)

^d Changes between HMLE-EMT and HMLER-EMT are statistically significant (p-value < 0.05)

^e Changes between HMLER and HMLE-EMT are statistically significant (p-value < 0.05)

^f Changes between HMLE and HMLER-EMT are statistically significant (p-value < 0.05)

ULOD: Upper Limit of Detection

Table S2. Concentration of homocysteine-methionine 1C metabolites in HMLE/HMLE-EMT and HMLER/HMLER-EMT cells

| Metabolite | HMLE | HMLE-EMT | HMLER | HMLER-EMT |
|--------------------------------|-----------------|-----------------|-----------------|------------------|
| Cystathionine ^{a,c,d} | 0.0034 (0.0008) | 0.0012 (0.0004) | 0.0021 (0.0005) | 0.0087 (0.0013) |
| Cysteine ^d | 4.2 (0.6) | 1.9 (0.4) | ULOQ | 9.6 (1.3) |
| Betaine ^{a,e} | 0.044 (0.004) | 0.028 (0.001) | 0.042 (0.008) | 0.041 (0.008) |
| Choline | 2.4 (0.5) | 2.3 (0.3) | 2.3 (0.3) | 2.3 (0.4) |
| Dimethylglycine | 2.3 (0.2) | 2.3 (0.3) | 2.2 (0.3) | 2.4 (0.5) |
| Homocysteine ^a | 0.19 (0.03) | 0.72 (0.06) | 0.29 (0.01) | 0.28 (0.07) |
| Met ^{a,e,f} | 0.61 (0.10) | 2.10 (0.36) | 0.89 (0.10) | 1.24 (0.20) |
| SAM ^{a,c,d} | 0.031 (0.006) | 0.008 (0.002) | 0.014 (0.002) | 0.099 (0.023) |
| AMP | 0.036 (0.012) | 0.031 (0.002) | 0.034 (0.007) | 0.034 (0.005) |
| NADH ^{a,c,d} | 0.46 (0.03) | 0.32 (0.04) | 0.40 (0.03) | 2.82 (0.56) |
| SAH ^{b,c,d} | 0.014 (0.004) | 0.006 (0.002) | 0.004 (0.001) | 0.039 (0.004) |
| Riboflavin ^{a,e} | 0.015 (0.003) | 0.033 (0.006) | 0.017 (0.001) | 0.020 (0.002) |
| Gly ^a | 98 (15) | 217 (9) | 138 (6) | 143 (7) |
| Ser ^{a,d} | 501 (67) | 943 (98) | 537 (53) | 528 (42) |

Concentrations are shown in $\mu\text{M}/\text{mg}$ of protein \pm SEM (n=5, in triplicate)

Decimals were set according to the first significant digit of the measured SEM

^a Changes between HMLE and HMLE-EMT are statistically significant (p-value < 0.05)

^b Changes between HMLE and HMLER are statistically significant (p-value < 0.05)

^c Changes between HMLER and HMLER-EMT are statistically significant (p-value < 0.05)

^d Changes between HMLE-EMT and HMLER-EMT are statistically significant (p-value < 0.05)

^e Changes between HMLER and HMLE-EMT are statistically significant (p-value < 0.05)

^f Changes between HMLE and HMLER-EMT are statistically significant (p-value < 0.05)

Met: methionine; SAM:5-adenosyl-methionine; AMP:3' 5' adenosine monophosphate; NADH; nicotinamide adenine dinucleotide phosphate, reduced; SAH: 5-adenosyl-homocysteine; Gly: glycine; Ser: serine; ULOD: Upper Limit of Detection

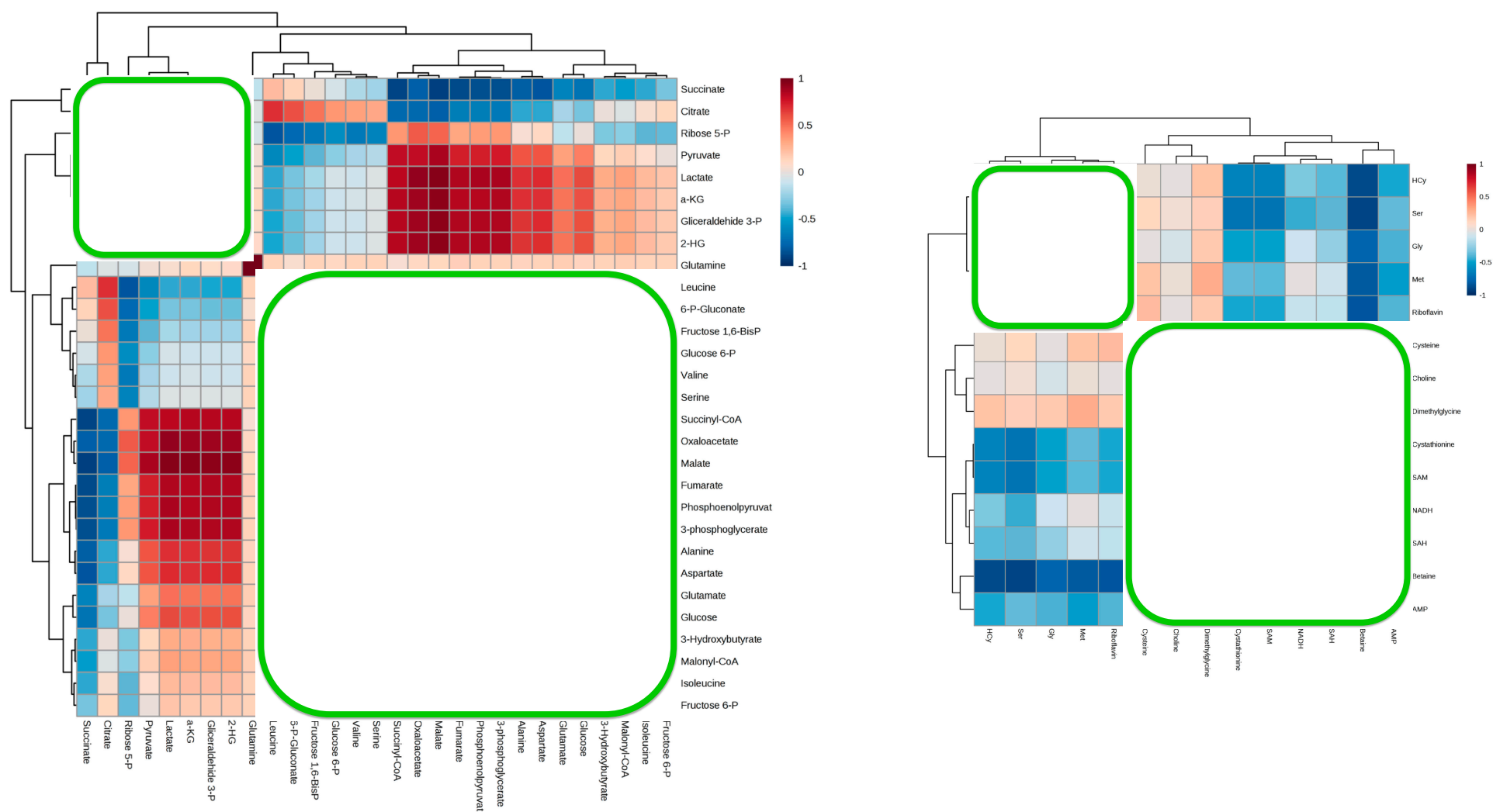


Figure S1. Correlation of the metabolomic data. Correlation matrix between all bioenergetic (*left*) and homocysteine-methionine 1C (*right*) metabolites collected from HMLE/HMLE-EMT and HMLER/HMLER-EMT cells based on the game's metrics. The color correlates to the intensity of metabolite concentration, where red indicates high intensity and blue indicates low intensity.