Supplemental Information

Hypoxia uncouples HIF gene transcription and metabolic flux in proliferating primary cells

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# Tables

Table : Lung fibroblast fluxes in 21% and 0.5% oxygen

|  | | **21%a** | | | **0.5%b** | | |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **ID** | **Reaction** | **Flux** | **LB** | **UB** | **Flux** | **LB** | **UB** | **Ratio** |
| **NET** | | | | | | | | |
| **Transport** | | | | | | | | |
| GLUT | GLC.x -> GLC | 5.14e+02 | 5.11e+02 | 5.21e+02 | 4.41e+02 | 4.26e+02 | 4.58e+02 | 0.86 |
| PYRR | PYR.x -> PYR.c | 7.56e+01 | 7.31e+01 | 7.96e+01 | 6.21e+01 | 5.83e+01 | 6.60e+01 | 0.82 |
| MCT | LAC <-> LAC.x | 9.99e+02 | 9.98e+02 | 1.02e+03 | 8.91e+02 | 8.62e+02 | 9.25e+02 | 0.89 |
| ALAR | ALA -> ALA.x | 2.25e+00 | 1.95e+00 | 2.49e+00 | 5.84e-01 | 1.10e-03 | 1.16e+00 | 0.26 |
| GLNR | GLN.x -> GLN | 4.15e+01 | 4.06e+01 | 4.16e+01 | 1.43e+01 | 1.26e+01 | 1.94e+01 | 0.34 |
| GLUR | GLU <-> GLU.x | 1.62e+01 | 1.58e+01 | 1.68e+01 | 7.55e+00 | 6.88e+00 | 8.15e+00 | 0.47 |
| ASPR | ASP -> ASP.x | 2.57e+00 | 2.53e+00 | 2.68e+00 | 1.08e+00 | 4.17e-01 | 1.69e+00 | 0.42 |
| SERR | SER.x -> SER | 1.42e+01 | 1.35e+01 | 1.49e+01 | 5.49e+00 | 4.99e+00 | 6.06e+00 | 0.39 |
| CYSR | CYX.x -> CYS + CYS | 4.41e+00 | 4.23e+00 | 4.58e+00 | 1.65e+00 | 1.32e+00 | 2.08e+00 | 0.37 |
| GLYR | GLY -> GLY.x | 2.05e+00 | 1.90e+00 | 2.15e+00 | 2.60e-01 | 2.00e-02 | 4.92e-01 | 0.13 |
| **Glycolysis** | | | | | | | | |
| HK | GLC -> G6P | 5.14e+02 | 5.11e+02 | 5.21e+02 | 4.41e+02 | 4.26e+02 | 4.58e+02 | 0.86 |
| PGI | G6P <-> F6P | 5.11e+02 | 4.99e+02 | 5.24e+02 | 4.23e+02 | 4.04e+02 | 4.40e+02 | 0.83 |
| PFK | F6P -> FBP | 5.09e+02 | 5.00e+02 | 5.12e+02 | 4.32e+02 | 4.17e+02 | 4.49e+02 | 0.85 |
| ALDO | FBP <-> DHAP + GAP | 5.09e+02 | 5.00e+02 | 5.12e+02 | 4.32e+02 | 4.17e+02 | 4.49e+02 | 0.85 |
| TPI | DHAP <-> GAP | 5.08e+02 | 5.06e+02 | 5.08e+02 | 4.31e+02 | 4.15e+02 | 4.48e+02 | 0.85 |
| GAPDH | GAP <-> 3PG | 1.02e+03 | 9.96e+02 | 1.04e+03 | 8.69e+02 | 8.35e+02 | 9.03e+02 | 0.85 |
| ENO | 3PG -> PEP | 1.01e+03 | 9.99e+02 | 1.03e+03 | 8.68e+02 | 8.36e+02 | 9.00e+02 | 0.86 |
| PK | PEP -> PYR.c | 1.04e+03 | 9.95e+02 | 1.04e+03 | 8.78e+02 | 8.36e+02 | 9.21e+02 | 0.84 |
| LDH | PYR.c <-> LAC | 9.99e+02 | 9.98e+02 | 1.02e+03 | 8.91e+02 | 8.62e+02 | 9.25e+02 | 0.89 |
| GPT1 | PYR.c <-> ALA | 1.19e+01 | 9.12e+00 | 1.19e+01 | 5.55e+00 | -9.08e+02 | 6.13e+00 | 0.47 |
| GPT2 | PYR.m <-> ALA | -2.58e+00 | -4.56e+00 | 2.87e+00 | -2.40e-03 | -3.22e+01 | 9.11e+02 |  |
| **Pentose phosphate pathway** | | | | | | | | |
| G6PD | G6P -> P5P + CO2 | 1.26e-07 | 0.00e+00 | 3.91e-01 | 1.62e+01 | 4.41e+00 | 2.89e+01 | 128571428.57 |
| TK1 | P5P + P5P <-> S7P + GAP | -9.11e-01 | -9.29e-01 | -8.30e-01 | 4.76e+00 | -1.22e-01 | 9.62e+00 | -5.23 |
| TA | S7P + GAP <-> F6P + E4P | -9.11e-01 | -9.29e-01 | -8.30e-01 | 4.76e+00 | -1.22e-01 | 9.62e+00 | -5.23 |
| TK2 | P5P + E4P <-> F6P + GAP | -9.11e-01 | -9.29e-01 | -8.30e-01 | 4.76e+00 | -1.22e-01 | 9.62e+00 | -5.23 |
| **Anaplerosis** | | | | | | | | |
| PYRT | PYR.c -> PYR.m | 1.16e+02 | 1.16e+02 | 1.19e+02 | 4.42e+01 | 3.82e+01 | 9.58e+02 |  |
| PC | PYR.m + CO2 -> OAC | 1.88e+01 | 1.74e+01 | 1.91e+01 | 1.37e+01 | 9.82e+00 | 2.69e+01 |  |
| PEPCK | OAC -> PEP + CO2 | 2.56e+01 | 1.58e+01 | 2.57e+01 | 9.66e+00 | 0.00e+00 | 2.60e+01 |  |
| ME2 | MAL -> PYR.m + CO2 | 2.05e+00 | 9.51e-02 | 2.68e+00 | 1.00e-07 | 0.00e+00 | 2.25e+01 |  |
| ME1 | MAL -> PYR.c + CO2 | 2.78e-02 | 0.00e+00 | 2.63e+01 | 8.71e-05 | 0.00e+00 | 2.52e+01 |  |
| FAO | FAO -> AcCoA.m | 1.00e-07 | 0.00e+00 | 2.13e+00 | 6.58e-06 | 0.00e+00 | 7.73e-01 |  |
| GLDH | GLU <-> AKG | 1.71e+01 | 1.56e+01 | 1.84e+01 | 9.11e-01 | -6.16e-01 | 7.27e+00 | 0.05 |
| GLS | GLN <-> GLU | 3.78e+01 | 3.60e+01 | 3.86e+01 | 1.17e+01 | 1.01e+01 | 1.70e+01 | 0.31 |
| **Tricarboxylic acid cycle** | | | | | | | | |
| PDH | PYR.m -> AcCoA.m + CO2 | 1.02e+02 | 8.76e+01 | 1.15e+02 | 3.05e+01 | 2.86e+01 | 5.24e+01 | 0.30 |
| CS | AcCoA.m + OAC -> CIT | 1.02e+02 | 8.30e+01 | 1.11e+02 | 3.05e+01 | 2.88e+01 | 5.09e+01 | 0.30 |
| IDH | CIT <-> AKG + CO2 | 2.49e+01 | 2.42e+01 | 2.53e+01 | 1.01e+01 | 8.75e+00 | 1.41e+01 | 0.41 |
| OGDH | AKG -> SUC + CO2 | 4.19e+01 | 4.01e+01 | 4.25e+01 | 1.10e+01 | 7.87e+00 | 2.02e+01 | 0.26 |
| SDH | SUC <-> FUM | 4.19e+01 | 4.01e+01 | 4.25e+01 | 1.10e+01 | 7.87e+00 | 2.02e+01 | 0.26 |
| FH | FUM <-> MAL | 4.19e+01 | 4.01e+01 | 4.25e+01 | 1.10e+01 | 7.87e+00 | 2.02e+01 | 0.26 |
| MDH | MAL <-> OAC | 1.17e+02 | 1.08e+02 | 1.24e+02 | 3.14e+01 | 2.62e+01 | 5.70e+01 | 0.27 |
| GOT | OAC <-> ASP | 8.11e+00 | 8.06e+00 | 8.23e+00 | 4.98e+00 | 4.32e+00 | 5.64e+00 | 0.61 |
| **Amino acid metabolism** | | | | | | | | |
| PST | 3PG -> SER | 1.95e+00 | 1.63e+00 | 2.00e+00 | 2.42e-01 | 1.34e-01 | 3.57e+01 |  |
| SHT | SER <-> GLY + MEETHF | 6.38e+00 | 6.22e+00 | 6.43e+00 | 3.91e+00 | 3.71e+00 | 4.10e+00 | 0.61 |
| CYST | SER <-> CYS | -7.12e+00 | -7.19e+00 | -6.81e+00 | -2.10e+00 | -2.97e+00 | -1.44e+00 | 0.30 |
| SD | SER -> PYR.c | 1.17e+01 | 1.04e+01 | 1.20e+01 | 2.82e-01 | 0.00e+00 | 1.47e+00 | 0.02 |
| GLYS | CO2 + MEETHF -> GLY | 3.39e+00 | 3.35e+00 | 3.49e+00 | 1.80e+00 | 1.66e+00 | 1.93e+00 | 0.53 |
| **Biomass** | | | | | | | | |
| BIOMASS | 1216\*AcCoA.c + 295.6\*ALA + 232.4\*ASP + 114.7\*CO2 + 71.43\*CYS + 57.14\*DHAP + 142.4\*G6P + 158.6\*GLN + 190.1\*GLU + 324.2\*GLY + 125.6\*MEETHF + 114.7\*P5P + 217.2\*SER -> biomass | 2.38e-02 | 2.34e-02 | 2.39e-02 | 1.68e-02 | 1.61e-02 | 1.75e-02 | 0.71 |
| ACL | CIT -> AcCoA.c + MAL | 7.74e+01 | 6.29e+01 | 1.04e+02 | 2.04e+01 | 1.95e+01 | 3.71e+01 | 0.26 |
| LIPS | AcCoA.c -> lipid | 4.84e+01 | 4.55e+01 | 4.84e+01 | 1.00e-07 | 0.00e+00 | 1.68e+01 | 0.00 |
| **Mixing** | | | | | | | | |
| cPYR | 0\*PYR.c -> PYR.ms | 1.00e+00 | 8.47e-01 | 1.00e+00 | 1.42e-01 | 0.00e+00 | 1.00e+00 |  |
| mPYR | 0\*PYR.m -> PYR.ms | 1.00e-07 | 0.00e+00 | 1.53e-01 | 8.58e-01 | 0.00e+00 | 1.00e+00 |  |
| sPYR | PYR.ms -> PYR.fix | 1.00e+00 | 1.00e+00 | 1.00e+00 | 1.00e+00 | 1.00e+00 | 1.00e+00 |  |
| **EXCH** | | | | | | | | |
| **Transport** | | | | | | | | |
| MCT | LAC <-> LAC.x | 1.00e-07 | 0.00e+00 | 1.05e-01 | 1.52e+03 | 1.35e+03 | 2.41e+03 | 15200000000.00 |
| GLUR | GLU <-> GLU.x | 5.10e+00 | 4.77e+00 | 5.23e+00 | 1.54e+00 | 1.11e+00 | 2.54e+00 | 0.30 |
| **Glycolysis** | | | | | | | | |
| PGI | G6P <-> F6P | 2.78e+05 | 1.77e+05 | Inf | 2.46e+05 | 0.00e+00 | Inf |  |
| ALDO | FBP <-> DHAP + GAP | 1.43e+02 | 1.43e+02 | 1.43e+02 | 3.20e+02 | 2.79e+02 | 3.60e+02 | 2.24 |
| TPI | DHAP <-> GAP | 4.33e+03 | 4.33e+03 | 1.09e+04 | 1.70e+03 | 1.06e+03 | 3.06e+03 | 0.39 |
| GAPDH | GAP <-> 3PG | 4.42e+02 | 4.72e+00 | 4.50e+02 | 1.00e-07 | 0.00e+00 | 2.39e+02 |  |
| LDH | PYR.c <-> LAC | 1.63e+03 | 1.62e+03 | 1.80e+03 | 4.80e+00 | 0.00e+00 | 3.51e+02 | 0.00 |
| GPT1 | PYR.c <-> ALA | 1.00e-07 | 0.00e+00 | 2.61e-01 | 8.32e+02 | 0.00e+00 | 9.06e+02 |  |
| GPT2 | PYR.m <-> ALA | 4.21e-04 | 0.00e+00 | 2.92e+00 | 1.28e-04 | 0.00e+00 |  |  |
| **Pentose phosphate pathway** | | | | | | | | |
| TK1 | P5P + P5P <-> S7P + GAP | 9.97e+04 | 6.27e+03 | Inf | 1.47e+02 | 6.67e+01 | 2.60e+02 | 0.00 |
| TA | S7P + GAP <-> F6P + E4P | 5.93e+00 | 5.79e+00 | 6.97e+00 | 2.35e-04 | 0.00e+00 | 7.54e+00 |  |
| TK2 | P5P + E4P <-> F6P + GAP | 1.00e+07 | -Inf | Inf | 9.05e+00 | 4.10e+00 | 1.43e+01 |  |
| **Anaplerosis** | | | | | | | | |
| GLDH | GLU <-> AKG | 1.52e+03 | 1.52e+03 | 7.13e+03 | 3.78e+02 | 1.93e+02 | 1.94e+03 |  |
| GLS | GLN <-> GLU | 3.99e-01 | 0.00e+00 | 8.04e-01 | 1.00e-07 | 0.00e+00 | 3.84e-01 |  |
| **Tricarboxylic acid cycle** | | | | | | | | |
| IDH | CIT <-> AKG + CO2 | 4.55e+00 | 4.03e+00 | 5.19e+00 | 2.52e+00 | 1.80e+00 | 4.50e+00 |  |
| SDH | SUC <-> FUM | 1.22e+03 |  | Inf | 7.60e+01 | 2.57e+01 | Inf |  |
| FH | FUM <-> MAL | 3.66e+05 | 1.95e+05 | Inf | 5.05e+05 | 3.06e+02 | Inf |  |
| MDH | MAL <-> OAC | 1.11e+03 | 7.88e+02 | 2.38e+03 | 1.33e+02 | 7.22e+01 | 3.25e+02 | 0.12 |
| GOT | OAC <-> ASP | 1.00e+07 | -Inf | Inf | 4.42e+01 | 0.00e+00 | Inf |  |
| **Amino acid metabolism** | | | | | | | | |
| SHT | SER <-> GLY + MEETHF | 5.10e+00 | 8.92e-01 | 5.25e+00 | 6.07e-07 | 0.00e+00 | 3.32e+02 |  |
| CYST | SER <-> CYS | 1.52e-05 | 0.00e+00 | 2.55e-04 | 1.46e-02 | 0.00e+00 | Inf |  |
| a SSR 391.7 [311.2-416.6] (95% CI, 362 DOF) | | | | | | | | |
| b SSR 334.3 [311.2-416.6] (95% CI, 362 DOF) | | | | | | | | |

Table : Lung fibroblast fluxes following DMSO and BAY treatment

|  | | **DMSOa** | | | **BAYb** | | |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **ID** | **Reaction** | **Flux** | **LB** | **UB** | **Flux** | **LB** | **UB** | **Ratio** |
| **NET** | | | | | | | | |
| **Transport** | | | | | | | | |
| GLUT | GLC.x -> GLC | 6.12e+02 | 6.12e+02 | 6.12e+02 | 8.80e+02 | 8.80e+02 | 8.80e+02 | 1.44 |
| PYRR | PYR.x -> PYR.c | 9.98e+01 | 9.95e+01 | 1.01e+02 | 6.06e+01 | 6.06e+01 | 6.06e+01 | 0.61 |
| MCT | LAC <-> LAC.x | 8.19e+02 | 8.17e+02 | 8.20e+02 | 1.33e+03 | 1.33e+03 | 1.33e+03 | 1.62 |
| ALAR | ALA -> ALA.x | 2.67e+00 | 2.36e+00 | 3.29e+00 | 5.98e+00 | 5.88e+00 | 6.24e+00 | 2.24 |
| GLNR | GLN.x -> GLN | 3.78e+01 | 3.77e+01 | 3.79e+01 | 2.06e+01 | 2.06e+01 | 2.06e+01 | 0.54 |
| GLUR | GLU <-> GLU.x | 1.61e+01 | 1.56e+01 | 1.62e+01 | 1.68e+01 | 1.68e+01 | 1.68e+01 | 1.05 |
| ASPR | ASP -> ASP.x | 2.36e+00 | 2.32e+00 | 2.49e+00 | 1.80e+00 | 1.80e+00 | 1.81e+00 | 0.76 |
| SERR | SER.x -> SER | 1.03e+01 | 1.03e+01 | 1.06e+01 | 2.50e+00 | 2.50e+00 | 2.50e+00 | 0.24 |
| CYSR | CYX.x -> CYS + CYS | 2.79e+00 | 2.79e+00 | 2.95e+00 | 3.07e-01 | 3.06e-01 | 3.07e-01 | 0.11 |
| GLYR | GLY -> GLY.x | 2.52e+00 | 2.30e+00 | 2.73e+00 | 5.52e-01 | 4.30e-01 | 7.45e-01 | 0.22 |
| **Glycolysis** | | | | | | | | |
| HK | GLC -> G6P | 6.12e+02 | 6.12e+02 | 6.12e+02 | 8.80e+02 | 8.80e+02 | 8.80e+02 | 1.44 |
| PGI | G6P <-> F6P | 6.09e+02 | 6.08e+02 | 6.09e+02 | 8.42e+02 | 8.42e+02 | 8.42e+02 | 1.38 |
| PFK | F6P -> FBP | 6.07e+02 | 6.07e+02 | 6.07e+02 | 8.65e+02 | 8.65e+02 | 8.65e+02 | 1.43 |
| ALDO | FBP <-> DHAP + GAP | 6.07e+02 | 6.07e+02 | 6.07e+02 | 8.65e+02 | 8.65e+02 | 8.65e+02 | 1.43 |
| TPI | DHAP <-> GAP | 6.06e+02 | 6.06e+02 | 6.06e+02 | 8.65e+02 | 8.65e+02 | 8.65e+02 | 1.43 |
| GAPDH | GAP <-> 3PG | 1.21e+03 | 1.21e+03 | 1.21e+03 | 1.74e+03 | 1.74e+03 | 1.74e+03 | 1.44 |
| ENO | 3PG -> PEP | 1.21e+03 | 1.21e+03 | 1.21e+03 | 1.57e+03 | 1.57e+03 | 1.57e+03 | 1.30 |
| PK | PEP -> PYR.c | 1.23e+03 | 1.19e+03 | 1.23e+03 | 1.65e+03 | 1.65e+03 | 1.65e+03 | 1.34 |
| LDH | PYR.c <-> LAC | 8.19e+02 | 8.17e+02 | 8.20e+02 | 1.33e+03 | 1.33e+03 | 1.33e+03 | 1.62 |
| GPT1 | PYR.c <-> ALA | 9.62e+00 | 9.44e+00 | 9.62e+00 | 9.36e+00 | 9.32e+00 | 9.42e+00 | 0.97 |
| GPT2 | PYR.m <-> ALA | 1.14e-01 |  |  | 2.28e-07 | -1.22e-05 | 6.41e-04 |  |
| **Pentose phosphate pathway** | | | | | | | | |
| G6PD | G6P -> P5P + CO2 | 2.02e-02 | 0.00e+00 | 1.08e+00 | 3.64e+01 | 3.64e+01 | 3.64e+01 | 1801.98 |
| TK1 | P5P + P5P <-> S7P + GAP | -9.06e-01 | -9.28e-01 | -9.06e-01 | 1.17e+01 | 1.17e+01 | 1.17e+01 | -12.89 |
| TA | S7P + GAP <-> F6P + E4P | -9.06e-01 | -9.28e-01 | -9.06e-01 | 1.17e+01 | 1.17e+01 | 1.17e+01 | -12.89 |
| TK2 | P5P + E4P <-> F6P + GAP | -9.06e-01 | -9.28e-01 | -9.06e-01 | 1.17e+01 | 1.17e+01 | 1.17e+01 | -12.89 |
| **Anaplerosis** | | | | | | | | |
| PYRT | PYR.c -> PYR.m | 4.99e+02 | 4.97e+02 | 4.99e+02 | 5.50e+02 | 5.50e+02 | 5.50e+02 | 1.10 |
| PC | PYR.m + CO2 -> OAC | 2.11e+01 | 2.07e+01 | 2.17e+01 | 9.05e+01 | 9.05e+01 | 9.05e+01 | 4.28 |
| PEPCK | OAC -> PEP + CO2 | 1.36e+01 | 1.36e+01 | 1.37e+01 | 8.58e+01 | 8.58e+01 | 8.58e+01 | 6.31 |
| ME2 | MAL -> PYR.m + CO2 | 1.30e+01 | 1.28e+01 | 1.37e+01 | 1.00e-07 | 0.00e+00 | 9.49e-06 | 0.00 |
| ME1 | MAL -> PYR.c + CO2 | 3.20e-03 | 0.00e+00 | 1.73e+00 | 1.00e-07 | 0.00e+00 | 2.15e-05 |  |
| FAO | FAO -> AcCoA.m | 1.00e-07 | 0.00e+00 | 3.48e+00 | 1.09e-04 | 8.34e-06 | 4.14e-02 |  |
| GLDH | GLU <-> AKG | 1.33e+01 | 1.31e+01 | 1.35e+01 | -2.46e-01 | -2.47e-01 | -2.46e-01 | -0.02 |
| GLS | GLN <-> GLU | 3.40e+01 | 3.35e+01 | 3.42e+01 | 1.88e+01 | 1.88e+01 | 1.88e+01 | 0.55 |
| **Tricarboxylic acid cycle** | | | | | | | | |
| PDH | PYR.m -> AcCoA.m + CO2 | 4.90e+02 | 4.90e+02 | 4.92e+02 | 4.60e+02 | 4.60e+02 | 4.60e+02 | 0.94 |
| CS | AcCoA.m + OAC -> CIT | 4.90e+02 | 4.84e+02 | 4.91e+02 | 4.60e+02 | 4.60e+02 | 4.60e+02 | 0.94 |
| IDH | CIT <-> AKG + CO2 | 2.70e+01 | 2.70e+01 | 2.76e+01 | 1.45e+01 | 1.45e+01 | 1.45e+01 | 0.54 |
| OGDH | AKG -> SUC + CO2 | 4.03e+01 | 3.99e+01 | 4.04e+01 | 1.43e+01 | 1.43e+01 | 1.43e+01 | 0.35 |
| SDH | SUC <-> FUM | 4.03e+01 | 3.99e+01 | 4.04e+01 | 1.43e+01 | 1.43e+01 | 1.43e+01 | 0.35 |
| FH | FUM <-> MAL | 4.03e+01 | 3.99e+01 | 4.04e+01 | 1.43e+01 | 1.43e+01 | 1.43e+01 | 0.35 |
| MDH | MAL <-> OAC | 4.91e+02 | 4.91e+02 | 4.92e+02 | 4.60e+02 | 4.60e+02 | 4.60e+02 | 0.94 |
| GOT | OAC <-> ASP | 7.91e+00 | 7.76e+00 | 7.98e+00 | 4.46e+00 | 4.46e+00 | 4.46e+00 | 0.56 |
| **Amino acid metabolism** | | | | | | | | |
| PST | 3PG -> SER | 4.03e-01 | 3.74e-01 | 5.04e-01 | 1.73e+02 | 1.73e+02 | 1.73e+02 | 429.83 |
| SHT | SER <-> GLY + MEETHF | 6.63e+00 | 6.59e+00 | 6.65e+00 | 2.85e+00 | 2.79e+00 | 2.93e+00 | 0.43 |
| CYST | SER <-> CYS | -3.88e+00 | -3.91e+00 | -3.87e+00 | 2.03e-01 | 2.02e-01 | 2.03e-01 | -0.05 |
| SD | SER -> PYR.c | 2.80e+00 | 2.80e+00 | 2.80e+00 | 1.70e+02 | 1.70e+02 | 1.70e+02 | 60.81 |
| GLYS | CO2 + MEETHF -> GLY | 3.63e+00 | 3.50e+00 | 3.65e+00 | 1.41e+00 | 1.30e+00 | 1.46e+00 | 0.39 |
| **Biomass** | | | | | | | | |
| BIOMASS | 1216\*AcCoA.c + 295.6\*ALA + 232.4\*ASP + 114.7\*CO2 + 71.43\*CYS + 57.14\*DHAP + 142.4\*G6P + 158.6\*GLN + 190.1\*GLU + 324.2\*GLY + 125.6\*MEETHF + 114.7\*P5P + 217.2\*SER -> biomass | 2.39e-02 | 2.39e-02 | 2.50e-02 | 1.14e-02 | 1.14e-02 | 1.14e-02 | 0.48 |
| ACL | CIT -> AcCoA.c + MAL | 4.63e+02 | 4.63e+02 | 4.66e+02 | 4.45e+02 | 4.45e+02 | 4.45e+02 | 0.96 |
| LIPS | AcCoA.c -> lipid | 4.34e+02 | 4.29e+02 | 4.34e+02 | 4.32e+02 | 4.32e+02 | 4.32e+02 |  |
| **Mixing** | | | | | | | | |
| cPYR | 0\*PYR.c -> PYR.ms | 1.00e+00 | 9.99e-01 | 1.00e+00 | 1.00e-07 | 0.00e+00 | 1.00e+00 |  |
| mPYR | 0\*PYR.m -> PYR.ms | 1.00e-07 | 0.00e+00 | 9.83e-04 | 1.00e+00 | 0.00e+00 | 1.00e+00 |  |
| sPYR | PYR.ms -> PYR.fix | 1.00e+00 | 1.00e+00 | 1.00e+00 | 1.00e+00 | 1.00e+00 | 1.00e+00 |  |
| **EXCH** | | | | | | | | |
| **Transport** | | | | | | | | |
| MCT | LAC <-> LAC.x | 6.24e-04 | 0.00e+00 | 3.56e+00 | 7.11e+02 | 7.11e+02 | 7.11e+02 | 1139423.08 |
| GLUR | GLU <-> GLU.x | 5.06e+00 | 4.82e+00 | 5.75e+00 | 3.48e+00 | 3.48e+00 | 3.48e+00 | 0.69 |
| **Glycolysis** | | | | | | | | |
| PGI | G6P <-> F6P | 1.40e+06 | 1.39e+06 | Inf | 4.31e+06 | 4.31e+06 | 4.31e+06 |  |
| ALDO | FBP <-> DHAP + GAP | 2.38e+02 | 2.38e+02 | 2.38e+02 | 1.02e+03 | 1.02e+03 | 1.02e+03 | 4.28 |
| TPI | DHAP <-> GAP | 9.99e+06 |  | Inf | 7.57e+03 | 7.57e+03 | 7.57e+03 |  |
| GAPDH | GAP <-> 3PG | 5.81e+02 | 5.81e+02 | 7.25e+02 | 1.09e+02 | 1.07e+02 | 1.09e+02 | 0.19 |
| LDH | PYR.c <-> LAC | 2.65e+03 | 2.58e+03 | 2.65e+03 | 4.92e+01 | 4.91e+01 | 4.94e+01 | 0.02 |
| GPT1 | PYR.c <-> ALA | 1.00e-07 | 0.00e+00 | 5.60e-02 | 2.45e+03 | 2.45e+03 | 2.45e+03 | 24500000000.00 |
| GPT2 | PYR.m <-> ALA | 1.00e-07 | 0.00e+00 | 5.65e-02 | 1.00e-07 | 0.00e+00 | 1.20e-05 |  |
| **Pentose phosphate pathway** | | | | | | | | |
| TK1 | P5P + P5P <-> S7P + GAP | 1.28e+06 | 9.01e+03 | Inf | 1.00e+07 | -Inf | Inf |  |
| TA | S7P + GAP <-> F6P + E4P | 8.89e+00 | 8.88e+00 | 9.53e+00 | 5.10e+01 | 5.10e+01 | 5.10e+01 | 5.74 |
| TK2 | P5P + E4P <-> F6P + GAP | 6.93e+00 | 5.12e+00 | 6.98e+00 | 1.00e-07 | 0.00e+00 | 1.56e-04 | 0.00 |
| **Anaplerosis** | | | | | | | | |
| GLDH | GLU <-> AKG | 5.63e+03 | 4.43e+03 | 5.66e+03 | 1.42e+03 | 1.42e+03 | 1.42e+03 | 0.25 |
| GLS | GLN <-> GLU | 1.27e+00 | 1.20e+00 | 1.50e+00 | 5.52e-01 | 5.51e-01 | 5.55e-01 | 0.43 |
| **Tricarboxylic acid cycle** | | | | | | | | |
| IDH | CIT <-> AKG + CO2 | 3.36e+00 | 3.24e+00 | 3.92e+00 | 4.66e+00 | 4.66e+00 | 4.66e+00 | 1.39 |
| SDH | SUC <-> FUM | 4.30e+02 | 4.30e+02 | 1.46e+06 | 1.04e+04 | 1.04e+04 | 1.04e+04 |  |
| FH | FUM <-> MAL | 7.29e+06 | -Inf | Inf | 4.56e+06 | 4.56e+06 | 4.56e+06 |  |
| MDH | MAL <-> OAC | 5.49e+02 | 5.47e+02 | 5.49e+02 | 1.00e-07 | 0.00e+00 | 6.30e-03 | 0.00 |
| GOT | OAC <-> ASP | 1.04e+02 | 1.04e+02 | 1.04e+02 | 4.76e+05 | 4.76e+05 | 4.76e+05 | 4576.92 |
| **Amino acid metabolism** | | | | | | | | |
| SHT | SER <-> GLY + MEETHF | 1.39e+00 | 1.37e+00 | 1.41e+00 | 1.86e+03 | 1.86e+03 | 1.86e+03 | 1338.13 |
| CYST | SER <-> CYS | 1.25e-07 | 0.00e+00 | 4.22e-02 | 1.33e-01 | 1.33e-01 | 1.33e-01 | 1064000.00 |
| a SSR 393.5 [311.2-416.6] (95% CI, 362 DOF) | | | | | | | | |
| b SSR 392.4 [308.4-413.4] (95% CI, 359 DOF) | | | | | | | | |

Table : PASMC fluxes in 21% and 0.5% oxygen

|  | | **21%a** | | | **0.5%b** | | |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **ID** | **Reaction** | **Flux** | **LB** | **UB** | **Flux** | **LB** | **UB** | **Ratio** |
| **NET** | | | | | | | | |
| **Transport** | | | | | | | | |
| GLUT | GLC.x -> GLC | 4.28e+02 | 4.28e+02 | 4.28e+02 | 3.65e+02 | 3.65e+02 | 3.65e+02 | 0.85 |
| PYRR | PYR.x -> PYR.c | 1.04e+02 | 1.02e+02 | 1.09e+02 | 4.53e+01 | 4.31e+01 | 4.57e+01 | 0.44 |
| MCT | LAC <-> LAC.x | 8.01e+02 | 8.01e+02 | 8.04e+02 | 6.49e+02 | 6.49e+02 | 6.49e+02 | 0.81 |
| ALAR | ALA -> ALA.x | 1.43e+01 | 1.43e+01 | 1.46e+01 | 7.83e+00 | 7.83e+00 | 8.24e+00 | 0.55 |
| GLNR | GLN.x -> GLN | 7.73e+01 | 7.53e+01 | 7.73e+01 | 1.77e+02 | 1.77e+02 | 1.77e+02 | 2.29 |
| GLUR | GLU <-> GLU.x | 2.53e+01 | 2.52e+01 | 2.54e+01 | 1.19e+01 | 1.19e+01 | 1.22e+01 | 0.47 |
| ASPR | ASP -> ASP.x | 7.01e+00 | 6.99e+00 | 7.02e+00 | 6.92e+00 | 6.84e+00 | 7.00e+00 |  |
| SERR | SER.x -> SER | 2.54e+00 | 2.48e+00 | 2.55e+00 | 2.57e+00 | 2.55e+00 | 2.57e+00 | 1.01 |
| CYSR | CYX.x -> CYS + CYS | 6.39e+00 | 6.34e+00 | 6.45e+00 | 3.75e+00 | 3.75e+00 | 3.75e+00 | 0.59 |
| GLYR | GLY -> GLY.x | 3.66e-01 | 3.03e-01 | 4.19e-01 | 4.06e-01 | 3.86e-01 | 4.25e-01 |  |
| **Glycolysis** | | | | | | | | |
| HK | GLC -> G6P | 4.28e+02 | 4.28e+02 | 4.28e+02 | 3.65e+02 | 3.65e+02 | 3.65e+02 | 0.85 |
| PGI | G6P <-> F6P | 4.06e+02 | 4.06e+02 | 4.07e+02 | 3.62e+02 | 3.62e+02 | 3.63e+02 | 0.89 |
| PFK | F6P -> FBP | 4.17e+02 | 4.17e+02 | 4.18e+02 | 3.61e+02 | 3.60e+02 | 3.61e+02 | 0.87 |
| ALDO | FBP <-> DHAP + GAP | 4.17e+02 | 4.17e+02 | 4.18e+02 | 3.61e+02 | 3.60e+02 | 3.61e+02 | 0.87 |
| TPI | DHAP <-> GAP | 4.16e+02 | 4.16e+02 | 4.16e+02 | 3.60e+02 | 3.60e+02 | 3.60e+02 | 0.87 |
| GAPDH | GAP <-> 3PG | 8.39e+02 | 8.39e+02 | 8.41e+02 | 7.21e+02 | 7.21e+02 | 7.21e+02 | 0.86 |
| ENO | 3PG -> PEP | 8.36e+02 | 8.35e+02 | 8.53e+02 | 7.20e+02 | 7.20e+02 | 7.20e+02 | 0.86 |
| PK | PEP -> PYR.c | 9.31e+02 | 9.30e+02 | 9.31e+02 | 9.24e+02 | 9.24e+02 | 9.24e+02 | 0.99 |
| LDH | PYR.c <-> LAC | 8.01e+02 | 8.01e+02 | 8.04e+02 | 6.49e+02 | 6.49e+02 | 6.49e+02 | 0.81 |
| GPT1 | PYR.c <-> ALA | 1.64e+02 | 1.62e+02 | 1.92e+02 | -1.36e+01 | -1.39e+01 | -1.35e+01 | -0.08 |
| GPT2 | PYR.m <-> ALA | -1.43e+02 | -1.43e+02 | -1.42e+02 | 2.62e+01 | 2.51e+01 | 2.65e+01 | -0.18 |
| **Pentose phosphate pathway** | | | | | | | | |
| G6PD | G6P -> P5P + CO2 | 1.89e+01 | 1.57e+01 | 1.93e+01 | 1.16e-07 | 0.00e+00 | 1.10e-03 | 0.00 |
| TK1 | P5P + P5P <-> S7P + GAP | 5.46e+00 | 4.44e+00 | 5.96e+00 | -6.15e-01 | -6.15e-01 | -5.77e-01 | -0.11 |
| TA | S7P + GAP <-> F6P + E4P | 5.46e+00 | 4.44e+00 | 5.96e+00 | -6.15e-01 | -6.15e-01 | -5.77e-01 | -0.11 |
| TK2 | P5P + E4P <-> F6P + GAP | 5.46e+00 | 4.44e+00 | 5.96e+00 | -6.15e-01 | -6.15e-01 | -5.77e-01 | -0.11 |
| **Anaplerosis** | | | | | | | | |
| PYRT | PYR.c -> PYR.m | 7.60e+01 | 7.59e+01 | 7.66e+01 | 3.36e+02 | 3.36e+02 | 3.36e+02 | 4.42 |
| PC | PYR.m + CO2 -> OAC | 6.30e+01 | 6.29e+01 | 6.59e+01 | 2.37e+02 | 2.36e+02 | 2.37e+02 | 3.76 |
| PEPCK | OAC -> PEP + CO2 | 9.51e+01 | 9.51e+01 | 9.53e+01 | 2.03e+02 | 2.03e+02 | 2.04e+02 | 2.14 |
| ME2 | MAL -> PYR.m + CO2 | 1.20e-03 | 0.00e+00 | 5.20e-03 | 1.82e+02 | 1.81e+02 | 1.82e+02 | 151517.08 |
| ME1 | MAL -> PYR.c + CO2 | 3.29e-05 | 0.00e+00 | 1.15e+00 | 5.91e-05 | 0.00e+00 | 8.06e-02 |  |
| FAO | FAO -> AcCoA.m | 1.00e-07 | 0.00e+00 | 1.32e-02 | 1.15e-04 | 0.00e+00 | 1.56e-01 |  |
| GLDH | GLU <-> AKG | 4.43e+01 | 4.42e+01 | 4.45e+01 | 1.59e+02 | 1.59e+02 | 1.59e+02 | 3.60 |
| GLS | GLN <-> GLU | 7.38e+01 | 7.36e+01 | 7.38e+01 | 1.74e+02 | 1.74e+02 | 1.74e+02 | 2.36 |
| **Tricarboxylic acid cycle** | | | | | | | | |
| PDH | PYR.m -> AcCoA.m + CO2 | 1.56e+02 | 1.48e+02 | 1.66e+02 | 2.55e+02 | 2.55e+02 | 2.55e+02 | 1.63 |
| CS | AcCoA.m + OAC -> CIT | 1.56e+02 | 1.56e+02 | 1.58e+02 | 2.55e+02 | 2.55e+02 | 2.55e+02 | 1.63 |
| IDH | CIT <-> AKG + CO2 | 2.11e+01 | 2.10e+01 | 2.11e+01 | 2.16e+01 | 2.16e+01 | 2.16e+01 | 1.03 |
| OGDH | AKG -> SUC + CO2 | 6.54e+01 | 6.51e+01 | 6.59e+01 | 1.81e+02 | 1.80e+02 | 1.81e+02 | 2.77 |
| SDH | SUC <-> FUM | 6.54e+01 | 6.51e+01 | 6.59e+01 | 1.81e+02 | 1.80e+02 | 1.81e+02 | 2.77 |
| FH | FUM <-> MAL | 6.54e+01 | 6.51e+01 | 6.59e+01 | 1.81e+02 | 1.80e+02 | 1.81e+02 | 2.77 |
| MDH | MAL <-> OAC | 2.01e+02 | 2.01e+02 | 2.01e+02 | 2.32e+02 | 2.32e+02 | 2.33e+02 | 1.16 |
| GOT | OAC <-> ASP | 1.22e+01 | 1.17e+01 | 1.24e+01 | 1.07e+01 | 1.06e+01 | 1.07e+01 | 0.87 |
| **Amino acid metabolism** | | | | | | | | |
| PST | 3PG -> SER | 2.69e+00 | 2.57e+00 | 2.80e+00 | 7.12e-01 | 7.01e-01 | 7.21e-01 | 0.26 |
| SHT | SER <-> GLY + MEETHF | 5.19e+00 | 5.15e+00 | 5.20e+00 | 3.82e+00 | 3.81e+00 | 3.86e+00 | 0.74 |
| CYST | SER <-> CYS | -1.12e+01 | -1.17e+01 | -1.11e+01 | -6.35e+00 | -6.35e+00 | -6.35e+00 | 0.57 |
| SD | SER -> PYR.c | 6.39e+00 | 6.23e+00 | 6.44e+00 | 2.33e+00 | 2.33e+00 | 2.33e+00 | 0.36 |
| GLYS | CO2 + MEETHF -> GLY | 2.39e+00 | 2.36e+00 | 2.42e+00 | 1.80e+00 | 1.79e+00 | 1.81e+00 | 0.75 |
| **Biomass** | | | | | | | | |
| BIOMASS | 978\*AcCoA.c + 237.8\*ALA + 187\*ASP + 92.3\*CO2 + 57.46\*CYS + 45.97\*DHAP + 114.5\*G6P + 127.6\*GLN + 153\*GLU + 260.8\*GLY + 101.1\*MEETHF + 92.3\*P5P + 174.8\*SER -> biomass | 2.77e-02 | 2.70e-02 | 2.79e-02 | 2.00e-02 | 2.00e-02 | 2.00e-02 | 0.72 |
| ACL | CIT -> AcCoA.c + MAL | 1.35e+02 | 1.34e+02 | 1.38e+02 | 2.33e+02 | 2.33e+02 | 2.33e+02 | 1.72 |
| LIPS | AcCoA.c -> lipid | 1.08e+02 | 9.99e+01 | 1.08e+02 | 2.14e+02 | 2.14e+02 | 2.14e+02 | 1.98 |
| **Mixing** | | | | | | | | |
| cPYR | 0\*PYR.c -> PYR.ms | 5.77e-01 | 5.64e-01 | 5.92e-01 | 1.00e+00 | 9.96e-01 | 1.00e+00 | 1.73 |
| mPYR | 0\*PYR.m -> PYR.ms | 4.23e-01 | 4.08e-01 | 4.36e-01 | 1.00e-07 | 0.00e+00 | 4.40e-03 | 0.00 |
| sPYR | PYR.ms -> PYR.fix | 1.00e+00 | 1.00e+00 | 1.00e+00 | 1.00e+00 | 1.00e+00 | 1.00e+00 |  |
| **EXCH** | | | | | | | | |
| **Transport** | | | | | | | | |
| MCT | LAC <-> LAC.x | 1.00e-07 | 0.00e+00 | 1.36e+02 | 1.64e+03 | 1.63e+03 | 1.65e+03 | 16400000000.00 |
| GLUR | GLU <-> GLU.x | 1.00e-07 | 0.00e+00 | 2.27e-02 | 5.69e-05 | 0.00e+00 | 1.71e-02 |  |
| **Glycolysis** | | | | | | | | |
| PGI | G6P <-> F6P | 4.88e+06 | 4.88e+06 | Inf | 9.92e+06 | 9.85e+04 | Inf |  |
| ALDO | FBP <-> DHAP + GAP | 2.89e+02 | 2.80e+02 | 2.89e+02 | 2.57e+02 | 2.56e+02 | 2.57e+02 | 0.89 |
| TPI | DHAP <-> GAP | 9.86e+06 | -Inf | Inf | 1.65e+03 | 1.63e+03 | 1.68e+03 |  |
| GAPDH | GAP <-> 3PG | 1.12e+03 | 0.00e+00 | 5.88e+05 | 1.00e-07 | 0.00e+00 | 2.27e-01 |  |
| LDH | PYR.c <-> LAC | 1.47e+03 | 1.39e+03 | 1.47e+03 | 4.49e+02 | 4.49e+02 | 4.49e+02 | 0.31 |
| GPT1 | PYR.c <-> ALA | 2.74e+02 | 2.73e+02 | 2.77e+02 | 1.00e-07 | 0.00e+00 | 4.28e-02 | 0.00 |
| GPT2 | PYR.m <-> ALA | 1.38e+02 | 1.38e+02 | 1.49e+02 | 9.64e+01 | 0.00e+00 | 1.01e+02 | 0.70 |
| **Pentose phosphate pathway** | | | | | | | | |
| TK1 | P5P + P5P <-> S7P + GAP | 7.99e+02 | 7.97e+02 | 8.08e+02 | 3.54e+01 | 3.54e+01 | 3.55e+01 | 0.04 |
| TA | S7P + GAP <-> F6P + E4P | 1.53e-01 | 0.00e+00 | 5.82e-01 | 2.55e+00 | 2.54e+00 | 2.57e+00 | 16.67 |
| TK2 | P5P + E4P <-> F6P + GAP | 3.33e+00 | 2.62e+00 | 3.35e+00 | 1.29e+01 | 1.29e+01 | 1.29e+01 | 3.88 |
| **Anaplerosis** | | | | | | | | |
| GLDH | GLU <-> AKG | 5.36e+02 | 5.34e+02 | 8.37e+02 | 1.23e+03 | 1.23e+03 | 1.23e+03 | 2.29 |
| GLS | GLN <-> GLU | 3.20e-01 | 0.00e+00 | 2.74e+00 | 1.12e+00 | 1.07e+00 | 1.74e+00 |  |
| **Tricarboxylic acid cycle** | | | | | | | | |
| IDH | CIT <-> AKG + CO2 | 1.04e+01 | 1.02e+01 | 1.04e+01 | 6.30e+01 | 6.30e+01 | 6.31e+01 | 6.09 |
| SDH | SUC <-> FUM | 2.78e-01 | 0.00e+00 | Inf | 3.34e+06 | 3.34e+06 | 3.34e+06 |  |
| FH | FUM <-> MAL | 1.03e-04 | 0.00e+00 | 1.58e+01 | 2.18e+02 | 2.18e+02 | 2.18e+02 | 2114238.83 |
| MDH | MAL <-> OAC | 1.01e+03 | 8.27e+02 | 1.01e+03 | 3.67e+03 | 3.67e+03 | 3.69e+03 | 3.63 |
| GOT | OAC <-> ASP | 2.27e+02 | 2.27e+02 | 2.47e+02 | 1.54e+01 | 1.54e+01 | 1.55e+01 | 0.07 |
| **Amino acid metabolism** | | | | | | | | |
| SHT | SER <-> GLY + MEETHF | 3.55e+00 | 3.52e+00 | 3.59e+00 | 1.60e-01 | 1.36e-01 | 1.70e-01 | 0.05 |
| CYST | SER <-> CYS | 1.04e+03 | 1.03e+03 | 1.04e+03 | 2.00e-03 | 0.00e+00 | 2.00e-03 | 0.00 |
| a SSR 575.6 [499.1-630.6] (95% CI, 563 DOF) | | | | | | | | |
| b SSR 521.3 [482.2-611.6] (95% CI, 545 DOF) | | | | | | | | |

# Figure legends

Supplementary Figure 1: **Supporting data for extracellular flux calculations.** (**A**) Cell viability as assessed by live/dead cell staining with acridine orange plus propidium iodide staining did not differ between 21% and 0.5% oxygen culture conditions (n = 3 technical replicates). (**B**) Standard curves were generated to interpolate cell counts from total DNA by seeding lung fibroblasts (LF) and pulmonary artery smooth muscle cells (PASMC) at the indicated densities in basal medium. Data are mean ± SEM of three biological replicates. (**C**) Total DNA measurements were compared to direct cell counts over the experimental time course. Cell counts and total DNA were obtained from the same sample wells. The slopes of the best-fit lines for 21% (*red*) and 0.5% (*blue*) samples were not different. (**D**) Predicted well volumes were estimated from the change in culture plate mass over the time course of the experiment. Evaporation rates were different depending on the treatment. Although the mean evaporation rate is depicted, experiment-specific evaporation rates were used to calculate fluxes for each experiment. (**E**) Metabolite accumulation (positive values) and degradation (negative values) rates. Data are mean ± SEM of 3-8 biological replicates. Rates significantly different from 0 (\*) based on a probability value < 0.05 using Student’s one-sample *t*-test were incorporated into flux calculations.

Supplementary Figure 2: **Extracellular flux measurements in 0.2% oxygen.** Lung fibroblasts (LFs) were cultured with 21% oxygen (*red*) or 0.2% oxygen (*dark blue*) beginning 24 h prior to time 0. (**A**) Growth curves of LFs in each experimental condition (n = 4). (**B**) Growth rates from (A) were determined by robust linear modeling of log-transformed growth curves. (**C**) Representative immunoblot of LF protein lysates cultured as in (A). (**D**, **E**) Relative change in HIF-1α (D) and LDHA (E) protein levels normalized to 21% oxygen time 0 (n = 4). (**F**, **G**) Relative changes in GLUT1 (G) and LDHA (H) mRNA levels normalized to 21% oxygen time 0 (n = 4). (**H**, **I**) Extracellular fluxes of the indicated metabolites (n = 4). Data are mean ± SEM. Comparisons were made using linear mixed effects models with treatment group as a fixed effect and biological replicate as a random effect. Tukey’s *post hoc* test was applied to determine differences between 21% and 0.2% oxygen (\*) with p-values < 0.05 considered significant.

Supplementary Figure 3: **Extracellular flux measurements in pulmonary artery smooth muscle cells in 0.5% oxygen.** Pulmonary artery smooth muscle cells (PASMCs) were cultured with 21% oxygen (*red*) or 0.5% oxygen (*blue*) beginning 24 h prior to time 0. (**A**) Growth curves of PASMCs under in each experimental condition (n = 8). (**B**) Growth rates from (A) were determined by robust linear modeling of log-transformed growth curves. (**C**) Representative immunoblot of PASMC protein lysates cultured as in (A). (**D**, **E**) Relative change in HIF-1α (D) and LDHA (E) protein levels normalized to 21% oxygen time 0 (n = 4). (**F**, **G**) Relative changes in GLUT1 (G) and LDHA (H) mRNA levels normalized to 21% oxygen time 0 (n = 4). (**H**, **I**) Extracellular fluxes of the indicated metabolites (n = 8). Data are mean ± SEM. Comparisons were made using linear mixed effects models with treatment group as a fixed effect and biological replicate as a random effect. Tukey’s *post hoc* test was applied to determine differences between 21% and 0.5% oxygen (\*) with p-values < 0.05 considered significant.

Supplementary Figure 4: **Mass isotopomer distributions after 72 h of labeling in lung fibroblasts.** Lung fibroblasts (LFs) were labeled with the indicated tracers and intracellular metabolites were analyzed by LC-MS after 72 h. Mass isotopomer distributions were adjusted for natural abundance. Data are the mean ± SEM of 4 biological replicates. Significant differences in labeling patterns between 21% and 0.5% oxygen (\*), DMSO and BAY treatment (†), and 0.5% oxygen and BAY treatment (‡) for each combination of metabolite and tracer are highlighted.

Supplementary Figure 5: **Mass isotopomer distributions after 72 h of labeling in pulmonary artery smooth muscle cells.** Pulmonary artery smooth muscle cells (PASMCs) were labeled with the indicated tracers and intracellular metabolites were analyzed by LC-MS after 36 h. Mass isotopomer distributions were adjusted for natural abundance. Data are the mean ± SEM of 4 biological replicates. Significant differences in labeling patterns between 21% and 0.5% oxygen (\*) for each combination of metabolite and tracer are highlighted.

Supplementary Figure 6: **Isotope incorporation in key metabolites over the experimental time course.** (**A**, **B**) LFs were cultured in 21% (A) or 0.5% (B) oxygen and labeled with the indicated tracers and intracellular metabolites were analyzed by LC-MS (PYR, pyruvate; CIT, citrate; MAL, malate). Mass isotopomer distributions were adjusted for natural abundance. Data are the mean ± SEM of 4 biological replicates.

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Supplementary Figure 9: **Transcriptomic profiling of hypoxia and BAY treated lung fibroblasts.** (**A**)

# Figures

![Figure 1: Supporting data for extracellular flux calculations. (A) Cell viability as assessed by live/dead cell staining with acridine orange plus propidium iodide staining did not differ between 21% and 0.5% oxygen culture conditions (n = 3 technical replicates). (B) Standard curves were generated to interpolate cell counts from total DNA by seeding lung fibroblasts (LF) and pulmonary artery smooth muscle cells (PASMC) at the indicated densities in basal medium. Data are mean ± SEM of three biological replicates. (C) Total DNA measurements were compared to direct cell counts over the experimental time course. Cell counts and total DNA were obtained from the same sample wells. The slopes of the best-fit lines for 21% (red) and 0.5% (blue) samples were not different. (D) Predicted well volumes were estimated from the change in culture plate mass over the time course of the experiment. Evaporation rates were different depending on the treatment. Although the mean evaporation rate is depicted, experiment-specific evaporation rates were used to calculate fluxes for each experiment. (E) Metabolite accumulation (positive values) and degradation (negative values) rates. Data are mean ± SEM of 3-8 biological replicates. Rates significantly different from 0 (*) based on a probability value < 0.05 using Student’s one-sample t-test were incorporated into flux calculations.](data:application/pdf;base64,)

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![Figure 2: Extracellular flux measurements in 0.2% oxygen. Lung fibroblasts (LFs) were cultured with 21% oxygen (red) or 0.2% oxygen (dark blue) beginning 24 h prior to time 0. (A) Growth curves of LFs in each experimental condition (n = 4). (B) Growth rates from (A) were determined by robust linear modeling of log-transformed growth curves. (C) Representative immunoblot of LF protein lysates cultured as in (A). (D, E) Relative change in HIF-1α (D) and LDHA (E) protein levels normalized to 21% oxygen time 0 (n = 4). (F, G) Relative changes in GLUT1 (G) and LDHA (H) mRNA levels normalized to 21% oxygen time 0 (n = 4). (H, I) Extracellular fluxes of the indicated metabolites (n = 4). Data are mean ± SEM. Comparisons were made using linear mixed effects models with treatment group as a fixed effect and biological replicate as a random effect. Tukey’s post hoc test was applied to determine differences between 21% and 0.2% oxygen (*) with p-values < 0.05 considered significant.](data:application/pdf;base64,)

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