Supplemental Information

MYC uncouples HIF target gene expression from glycolytic flux in hypoxic proliferating primary cells

Courtney A. Copeland1, Benjamin A. Olenchock1, David R. Ziehr1,2, Sarah McGarrity1,3, Kevin Leahy1, Jamey D. Young4, Joseph Loscalzo1, and William M. Oldham1,‡

1 Department of Medicine, Brigham and Women’s Hospital and Harvard Medical School, Boston, MA  
2 Department of Medicine, Massachusetts General Hospital and Harvard Medical School, Boston, MA  
3 Center for Systems Biology, School of Health Sciences, University of Iceland, Reykjavik, Iceland  
4 Departments of Chemical & Biomolecular Engineering and Molecular Physiology & Biophysics, Vanderbilt University, Nashville, TN

‡ Correspondence: [William M. Oldham <[woldham@bwh.harvard.edu](mailto:woldham@bwh.harvard.edu)>](mailto:woldham@bwh.harvard.edu)

# Tables

Table 1: 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 2: 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 3: 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.

Supplementary Figure 7: **Isotopically non-stationary metabolic flux analysis.** (**A**) Metabolic flux model of LF metabolism in 21% oxygen. (**B**) Metabolic flux model of PASMC metabolism in 21% oxygen. (**C**) LF fluxes were normalized to cell growth rate. Graph depicts the ratio of normalized metabolic fluxes in LFs cultured in 0.5% oxygen compared to 21% oxygen control. Fluxes with non-overlapping confidence intervals are highlighted to indicate significant changes. (**D**) Ratio of metabolic fluxes in 0.5% oxygen compared to 21% oxygen in PASMCs.

Supplementary Figure 8: **Metabolomic profiling of hypoxia and BAY treated lung fibroblasts.** (**A**, **B**) Volcano plots of differentially regulated metabolites with 0.5% oxygen in DMSO-treated cells (A) or BAY treatment in 21% oxygen-cultured cells (B). Significantly increased metabolites with 0.5% oxygen (*blue*) or with BAY treatment (*purple*) are indicated. The top 10 up- and down-regulated metabolites are labeled. (**C**) Venn diagram illustrating the number of differentially regulated metabolites following hypoxia (*blue*) or BAY treatment (*purple*). (**D**, **E**) Results of a metabolite set enrichment analysis of KEGG pathways based on the data from (A) and (B). Significantly enriched pathways with p < 0.05 are indicated.

Supplementary Figure 9: **Transcriptomic profiling of hypoxia and BAY treated lung fibroblasts.** (**A**, **B**) Volcano plots of differentially regulated transcripts with 0.5% oxygen in DMSO-treated cells (A) or BAY treatment in 21% oxygen-cultured cells (B). The top 10 up- and down-regulated metabolites are highlighted. (**C**) Venn diagram illustrating the number of differentially regulated genes following hypoxia (*blue*) or BAY treatment (*purple*). (**D**) Venn diagram illustrating the number of differentially enriched Hallmark gene sets with hypoxia (*blue*) or BAY treatment (*purple*). (**E**, **F**) Normalized enrichment scores from gene set enrichment analysis of Hallmark gene sets affected by 0.5% oxygen (E) or BAY treatment (F).

# 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.

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.

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.

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.

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.

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.

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.

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.

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.

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.

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.

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.

Figure 7: Isotopically non-stationary metabolic flux analysis. (A) Metabolic flux model of LF metabolism in 21% oxygen. (B) Metabolic flux model of PASMC metabolism in 21% oxygen. (C) LF fluxes were normalized to cell growth rate. Graph depicts the ratio of normalized metabolic fluxes in LFs cultured in 0.5% oxygen compared to 21% oxygen control. Fluxes with non-overlapping confidence intervals are highlighted to indicate significant changes. (D) Ratio of metabolic fluxes in 0.5% oxygen compared to 21% oxygen in PASMCs.

Figure 7: **Isotopically non-stationary metabolic flux analysis.** (**A**) Metabolic flux model of LF metabolism in 21% oxygen. (**B**) Metabolic flux model of PASMC metabolism in 21% oxygen. (**C**) LF fluxes were normalized to cell growth rate. Graph depicts the ratio of normalized metabolic fluxes in LFs cultured in 0.5% oxygen compared to 21% oxygen control. Fluxes with non-overlapping confidence intervals are highlighted to indicate significant changes. (**D**) Ratio of metabolic fluxes in 0.5% oxygen compared to 21% oxygen in PASMCs.

Figure 8: Metabolomic profiling of hypoxia and BAY treated lung fibroblasts. (A, B) Volcano plots of differentially regulated metabolites with 0.5% oxygen in DMSO-treated cells (A) or BAY treatment in 21% oxygen-cultured cells (B). Significantly increased metabolites with 0.5% oxygen (blue) or with BAY treatment (purple) are indicated. The top 10 up- and down-regulated metabolites are labeled. (C) Venn diagram illustrating the number of differentially regulated metabolites following hypoxia (blue) or BAY treatment (purple). (D, E) Results of a metabolite set enrichment analysis of KEGG pathways based on the data from (A) and (B). Significantly enriched pathways with p < 0.05 are indicated.

Figure 8: **Metabolomic profiling of hypoxia and BAY treated lung fibroblasts.** (**A**, **B**) Volcano plots of differentially regulated metabolites with 0.5% oxygen in DMSO-treated cells (A) or BAY treatment in 21% oxygen-cultured cells (B). Significantly increased metabolites with 0.5% oxygen (*blue*) or with BAY treatment (*purple*) are indicated. The top 10 up- and down-regulated metabolites are labeled. (**C**) Venn diagram illustrating the number of differentially regulated metabolites following hypoxia (*blue*) or BAY treatment (*purple*). (**D**, **E**) Results of a metabolite set enrichment analysis of KEGG pathways based on the data from (A) and (B). Significantly enriched pathways with p < 0.05 are indicated.

Figure 9: Transcriptomic profiling of hypoxia and BAY treated lung fibroblasts. (A, B) Volcano plots of differentially regulated transcripts with 0.5% oxygen in DMSO-treated cells (A) or BAY treatment in 21% oxygen-cultured cells (B). The top 10 up- and down-regulated metabolites are highlighted. (C) Venn diagram illustrating the number of differentially regulated genes following hypoxia (blue) or BAY treatment (purple). (D) Venn diagram illustrating the number of differentially enriched Hallmark gene sets with hypoxia (blue) or BAY treatment (purple). (E, F) Normalized enrichment scores from gene set enrichment analysis of Hallmark gene sets affected by 0.5% oxygen (E) or BAY treatment (F).

Figure 9: **Transcriptomic profiling of hypoxia and BAY treated lung fibroblasts.** (**A**, **B**) Volcano plots of differentially regulated transcripts with 0.5% oxygen in DMSO-treated cells (A) or BAY treatment in 21% oxygen-cultured cells (B). The top 10 up- and down-regulated metabolites are highlighted. (**C**) Venn diagram illustrating the number of differentially regulated genes following hypoxia (*blue*) or BAY treatment (*purple*). (**D**) Venn diagram illustrating the number of differentially enriched Hallmark gene sets with hypoxia (*blue*) or BAY treatment (*purple*). (**E**, **F**) Normalized enrichment scores from gene set enrichment analysis of Hallmark gene sets affected by 0.5% oxygen (E) or BAY treatment (F).

# Supplemental References

1. R Core Team. [*R: A language and environment for statistical computing*](https://www.R-project.org/). (R Foundation for Statistical Computing, 2020).

2. Xie, Y. [*Bookdown: Authoring books and technical documents with r markdown*](https://CRAN.R-project.org/package=bookdown). (2020).

3. Robinson, D. & Hayes, A. [*Broom: Convert statistical analysis objects into tidy tibbles*](https://CRAN.R-project.org/package=broom). (2020).

4. Bryan, J. [*Cellranger: Translate spreadsheet cell ranges to rows and columns*](https://CRAN.R-project.org/package=cellranger). (2016).

5. Wilke, C. O. [*Cowplot: Streamlined plot theme and plot annotations for ’ggplot2’*](https://CRAN.R-project.org/package=cowplot). (2019).

6. Wickham, H., Hester, J. & Chang, W. *Devtools: Tools to make developing r packages easier*. (2020).

7. Wickham, H., François, R., Henry, L. & Müller, K. *Dplyr: A grammar of data manipulation*. (2020).

8. Lenth, R. [*Emmeans: Estimated marginal means, aka least-squares means*](https://CRAN.R-project.org/package=emmeans). (2020).

9. Wickham, H. [*Forcats: Tools for working with categorical variables (factors)*](https://CRAN.R-project.org/package=forcats). (2020).

10. Clarke, E. & Sherrill-Mix, S. [*Ggbeeswarm: Categorical scatter (violin point) plots*](https://CRAN.R-project.org/package=ggbeeswarm). (2017).

11. Wickham, H. *et al.* [*ggplot2: Create elegant data visualisations using the grammar of graphics*](https://CRAN.R-project.org/package=ggplot2). (2020).

12. Pedersen, T. L. *Ggraph: An implementation of grammar of graphics for graphs and networks*. (2020).

13. Zhu, H. [*kableExtra: Construct complex table with ’kable’ and pipe syntax*](https://CRAN.R-project.org/package=kableExtra). (2019).

14. Xie, Y. [*Knitr: A general-purpose package for dynamic report generation in r*](https://CRAN.R-project.org/package=knitr). (2020).

15. Bates, D., Maechler, M., Bolker, B. & Walker, S. [*lme4: Linear mixed-effects models using ’eigen’ and S4*](https://CRAN.R-project.org/package=lme4). (2020).

16. Kuznetsova, A., Bruun Brockhoff, P. & Haubo Bojesen Christensen, R. [*lmerTest: Tests in linear mixed effects models*](https://CRAN.R-project.org/package=lmerTest). (2020).

17. Spinu, V., Grolemund, G. & Wickham, H. [*Lubridate: Make dealing with dates a little easier*](https://CRAN.R-project.org/package=lubridate). (2020).

18. Ooms, J. [*Magick: Advanced graphics and image-processing in r*](https://CRAN.R-project.org/package=magick). (2020).

19. Bache, S. M. & Wickham, H. [*Magrittr: A forward-pipe operator for r*](https://CRAN.R-project.org/package=magrittr). (2014).

20. Ripley, B. [*MASS: Support functions and datasets for venables and ripley’s MASS*](https://CRAN.R-project.org/package=MASS). (2020).

21. Oldham, W. [*Mzrtools: Make molecular formulas useful for mass spectrometry*](https://github.com/wmoldham/mzrtools). (2020).

22. Pedersen, T. L. *Patchwork: The composer of plots*. (2020).

23. <sorenh@math.aau.dk>, U. H. S. H. [*Pbkrtest: Parametric bootstrap and kenward roger based methods for mixed model comparison*](https://CRAN.R-project.org/package=pbkrtest). (2020).

24. Henry, L. & Wickham, H. [*Purrr: Functional programming tools*](https://CRAN.R-project.org/package=purrr). (2020).

25. Neuwirth, E. [*RColorBrewer: ColorBrewer palettes*](https://CRAN.R-project.org/package=RColorBrewer). (2014).

26. Wickham, H., Hester, J. & Francois, R. [*Readr: Read rectangular text data*](https://CRAN.R-project.org/package=readr). (2018).

27. Wickham, H. & Bryan, J. [*Readxl: Read excel files*](https://CRAN.R-project.org/package=readxl). (2019).

28. Ushey, K. [*Renv: Project environments*](https://CRAN.R-project.org/package=renv). (2020).

29. Henry, L. & Wickham, H. [*Rlang: Functions for base types and core r and ’tidyverse’ features*](https://CRAN.R-project.org/package=rlang). (2020).

30. Allaire, J. *et al.* [*Rmarkdown: Dynamic documents for r*](https://CRAN.R-project.org/package=rmarkdown). (2020).

31. Wickham, H., Danenberg, P., Csárdi, G. & Eugster, M. [*roxygen2: In-line documentation for r*](https://CRAN.R-project.org/package=roxygen2). (2020).

32. Wickham, H. [*Stringr: Simple, consistent wrappers for common string operations*](https://CRAN.R-project.org/package=stringr). (2019).

33. Müller, K. & Wickham, H. [*Tibble: Simple data frames*](https://CRAN.R-project.org/package=tibble). (2020).

34. Pedersen, T. L. *Tidygraph: A tidy API for graph manipulation*. (2020).

35. Wickham, H. & Henry, L. [*Tidyr: Tidy messy data*](https://CRAN.R-project.org/package=tidyr). (2020).

36. Wickham, H. *Tidyverse: Easily install and load the ’tidyverse’*. (2020).

37. Xie, Y. [*Tinytex: Helper functions to install and maintain TeX live, and compile LaTeX documents*](https://CRAN.R-project.org/package=tinytex). (2020).

38. Wickham, H. & Bryan, J. [*Usethis: Automate package and project setup*](https://CRAN.R-project.org/package=usethis). (2020).

39. Garnier, S. [*Viridis: Default color maps from ’matplotlib’*](https://CRAN.R-project.org/package=viridis). (2018).

40. Oldham, W. [*Wmo: Personal utility functions*](https://github.com/wmoldham/wmo). (2020).

41. Xie, Y. [*Bookdown: Authoring books and technical documents with R markdown*](https://github.com/rstudio/bookdown). (Chapman; Hall/CRC, 2016).

42. Wickham, H. [*ggplot2: Elegant graphics for data analysis*](https://ggplot2.tidyverse.org). (Springer-Verlag New York, 2016).

43. Xie, Y. [*Dynamic documents with R and knitr*](https://yihui.org/knitr/). (Chapman; Hall/CRC, 2015).

44. Bates, D., Mächler, M., Bolker, B. & Walker, S. [Fitting linear mixed-effects models using lme4](https://doi.org/10.18637/jss.v067.i01). *Journal of Statistical Software* **67**, 1–48 (2015).

45. Kuznetsova, A., Brockhoff, P. B. & Christensen, R. H. B. [lmerTest package: Tests in linear mixed effects models](https://doi.org/10.18637/jss.v082.i13). *Journal of Statistical Software* **82**, 1–26 (2017).

46. Grolemund, G. & Wickham, H. [Dates and times made easy with lubridate](http://www.jstatsoft.org/v40/i03/). *Journal of Statistical Software* **40**, 1–25 (2011).

47. Venables, W. N. & Ripley, B. D. [*Modern applied statistics with s*](http://www.stats.ox.ac.uk/pub/MASS4). (Springer, 2002).

48. Halekoh, U. & Højsgaard, S. [A kenward-roger approximation and parametric bootstrap methods for tests in linear mixed models – the R package pbkrtest](http://www.jstatsoft.org/v59/i09/). *Journal of Statistical Software* **59**, 1–30 (2014).

49. Xie, Y., Allaire, J. J. & Grolemund, G. [*R markdown: The definitive guide*](https://bookdown.org/yihui/rmarkdown). (Chapman; Hall/CRC, 2018).

50. Wickham, H. *et al.* [Welcome to the tidyverse](https://doi.org/10.21105/joss.01686). *Journal of Open Source Software* **4**, 1686 (2019).

51. Korotkevich, G., Sukhov, V. & Sergushichev, A. Fast gene set enrichment analysis. *bioRxiv* (2019) doi:[10.1101/060012](https://doi.org/10.1101/060012).

52. Puente-Santamaria, L., Wasserman, W. & del Peso, L. TFEA.ChIP: A tool kit for transcription factor binding site enrichment analysis capitalizing on ChIP-seq datasets. *Bioinformatics* (2019) doi:[10.1093/bioinformatics/btz573](https://doi.org/10.1093/bioinformatics/btz573).

53. Sebastian, C. & Hackermüller, J. [multiGSEA: A GSEA-based pathway enrichment analysis for multi-omics data](https://doi.org/10.1186/s12859-020-03910-x). *BMC Bioinformatics* **21**, (2020).

54. Ritchie, M. E. *et al.* [limma powers differential expression analyses for RNA-sequencing and microarray studies](https://doi.org/10.1093/nar/gkv007). *Nucleic Acids Research* **43**, e47 (2015).