Supplemental Information

MYC overrides HIF to regulate proliferating primary cell metabolism in hypoxia

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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 curve of lung fibroblast (LF) cell count *v.* total DNA by PicoGreen measurement used to interpolate cell numbers from DNA measurements. Data are mean ± SEM of three biological replicates. (**C**) Standard curve of PASMC cell count *v.* total DNA as in (B). (**D**) 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 statistically different. (**E**) Predicted well volumes were estimated from the change in culture plate mass over the experimental time course. Evaporation rates were different depending on the culture conditions and treatment. Although the mean evaporation rate is depicted, experiment-specific evaporation rates were used to calculate fluxes for each biological replicate (**F**) 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: **Effects of hypoxia on extracellular metabolite fluxes in lung fibroblasts.** (**A**) Lung fibroblasts (LFs) were cultured in 21% (*red*) or 0.2% (*dark blue*) oxygen beginning 24 h prior to time 0. Samples were collected every 24 h for 72 h. (**B**) Growth curves of LFs in each experimental condition (n = 4). (**C**) Growth rates from (B) were determined by robust linear modeling of log-transformed growth curves. (**D**) Representative immunoblot of LF protein lysates cultured as in (A). (**E**) Relative change in HIF-1α protein levels from (D) normalized to 21% oxygen at time 0 (n = 4). (**F**) Relative change in GLUT1 mRNA levels normalized to 21% oxygen treatment at time 0 (n = 4). (**G**) Relative change in LDHA mRNA levels as in (F). (**H**) Relative change in LDHA protein levels as in (E). (**I**) Extracellular fluxes of glucose (GLC) and lactate (LAC) (n = 4). (**J**) Extracellular fluxes of pyruvate (PYR) and amino acids. Data are mean ± SEM. Comparisons were made using linear mixed effects models with oxygen as the fixed effect and biological replicate as a random effect with p-values adjusted using the multivariate *t* distribution (\* p < 0.05).

Supplementary Figure 3: **Effects of hypoxia on extracellular metabolite fluxes in pulmonary artery smooth muscle cells.** (**A**) Pulmonary artery smooth muscle cells (PASMCs) were cultured in 21% (*red*) or 0.5% (*blue*) oxygen beginning 24 h prior to time 0. Samples were collected every 12 h for 48 h. (**B**) Growth curves of LFs in each experimental condition (n = 4). (**C**) Growth rates from (B) were determined by robust linear modeling of log-transformed growth curves. (**D**) Representative immunoblot of LF protein lysates cultured as in (A). (**E**) Relative change in HIF-1α protein levels from (D) normalized to 21% oxygen at time 0 (n = 4). (**F**) Relative change in GLUT1 mRNA levels normalized to 21% oxygen treatment at time 0 (n = 4). (**G**) Relative change in LDHA mRNA levels as in (F). (**H**) Relative change in LDHA protein levels as in (E). (**I**) Extracellular fluxes of glucose (GLC) and lactate (LAC) (n = 4). (**J**) Extracellular fluxes of pyruvate (PYR) and amino acids. Data are mean ± SEM. Comparisons were made using linear mixed effects models with oxygen as the fixed effect and biological replicate as a random effect with p-values adjusted using the multivariate *t* distribution (\* p < 0.05).

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 48 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 over the labeling time course.** LFs were cultured in 21% or 0.5% oxygen and labeled with the indicated tracers. Intracellular metabolites were analyzed by LC-MS (FBP, fructose-bisphosphate; PYR, pyruvate; CIT, citrate; MAL, malate). Mass isotopomer distributions were calculated and adjusted for natural abundance. Data show the total amount of metabolite labeling (*i.e.,* 1 - M0 fractional abundance). Data are the mean ± SEM of 4 biological replicates.

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# Figures

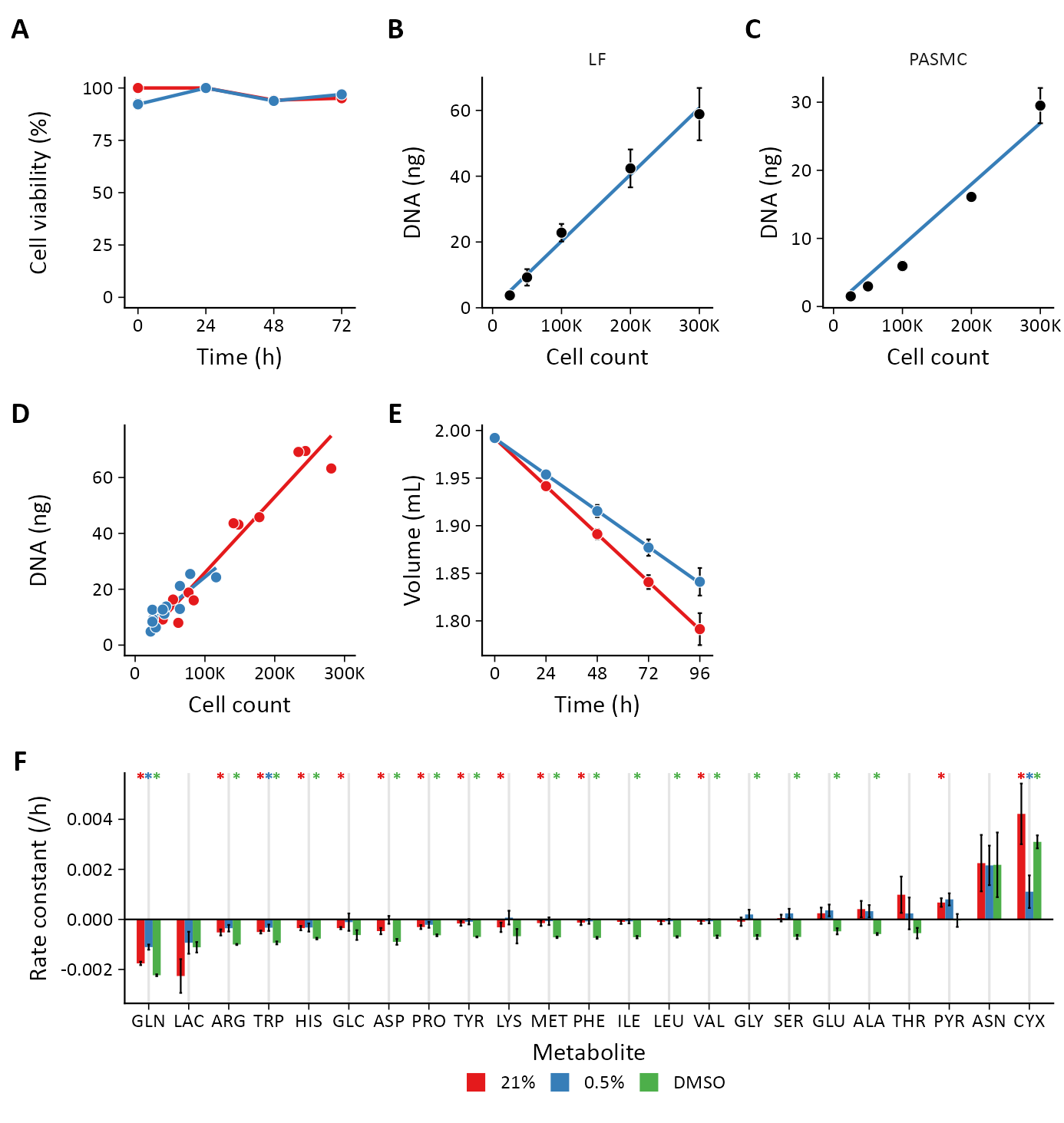


Figure 1: **Supporting data for extracellular flux calculations.** (**A**) Cell viability as assessed by live/dead cell staining with acridine orange plus propidium iodide staining did not differ between 21% and 0.5% oxygen culture conditions (n = 3 technical replicates). (**B**) Standard curve of lung fibroblast (LF) cell count *v.* total DNA by PicoGreen measurement used to interpolate cell numbers from DNA measurements. Data are mean ± SEM of three biological replicates. (**C**) Standard curve of PASMC cell count *v.* total DNA as in (B). (**D**) 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 statistically different. (**E**) Predicted well volumes were estimated from the change in culture plate mass over the experimental time course. Evaporation rates were different depending on the culture conditions and treatment. Although the mean evaporation rate is depicted, experiment-specific evaporation rates were used to calculate fluxes for each biological replicate (**F**) 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.

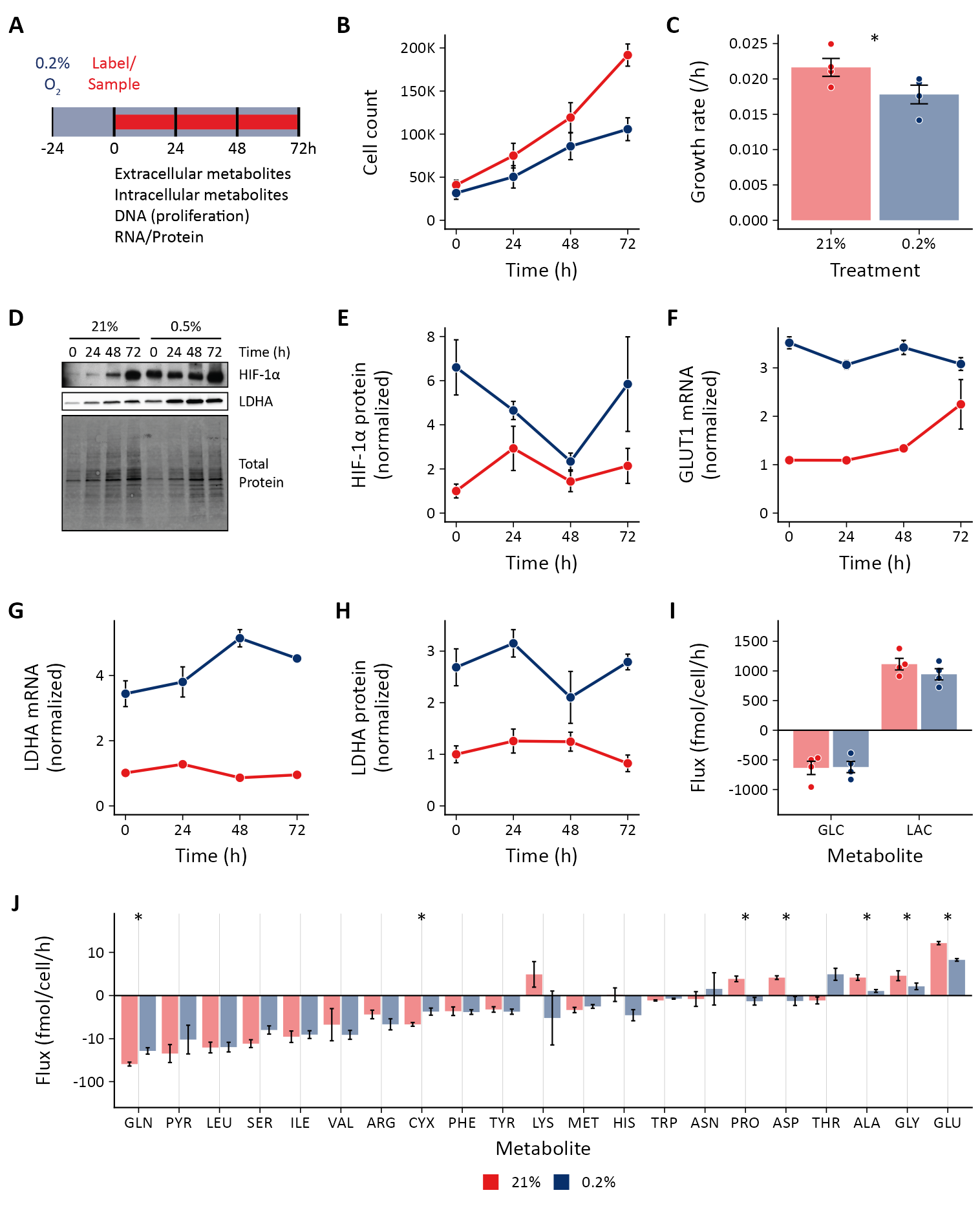


Figure 2: **Effects of hypoxia on extracellular metabolite fluxes in lung fibroblasts.** (**A**) Lung fibroblasts (LFs) were cultured in 21% (*red*) or 0.2% (*dark blue*) oxygen beginning 24 h prior to time 0. Samples were collected every 24 h for 72 h. (**B**) Growth curves of LFs in each experimental condition (n = 4). (**C**) Growth rates from (B) were determined by robust linear modeling of log-transformed growth curves. (**D**) Representative immunoblot of LF protein lysates cultured as in (A). (**E**) Relative change in HIF-1α protein levels from (D) normalized to 21% oxygen at time 0 (n = 4). (**F**) Relative change in GLUT1 mRNA levels normalized to 21% oxygen treatment at time 0 (n = 4). (**G**) Relative change in LDHA mRNA levels as in (F). (**H**) Relative change in LDHA protein levels as in (E). (**I**) Extracellular fluxes of glucose (GLC) and lactate (LAC) (n = 4). (**J**) Extracellular fluxes of pyruvate (PYR) and amino acids. Data are mean ± SEM. Comparisons were made using linear mixed effects models with oxygen as the fixed effect and biological replicate as a random effect with p-values adjusted using the multivariate *t* distribution (\* p < 0.05).

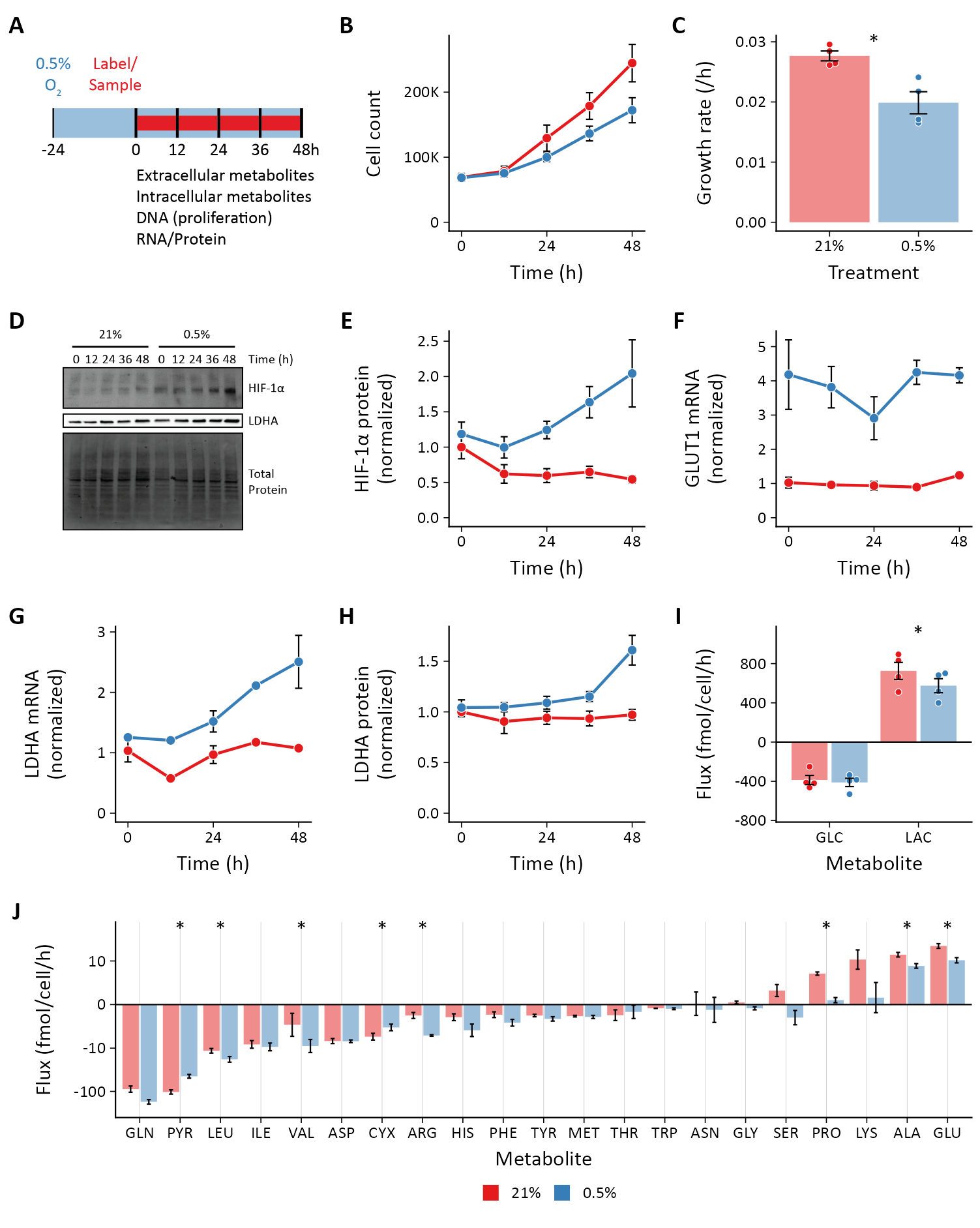


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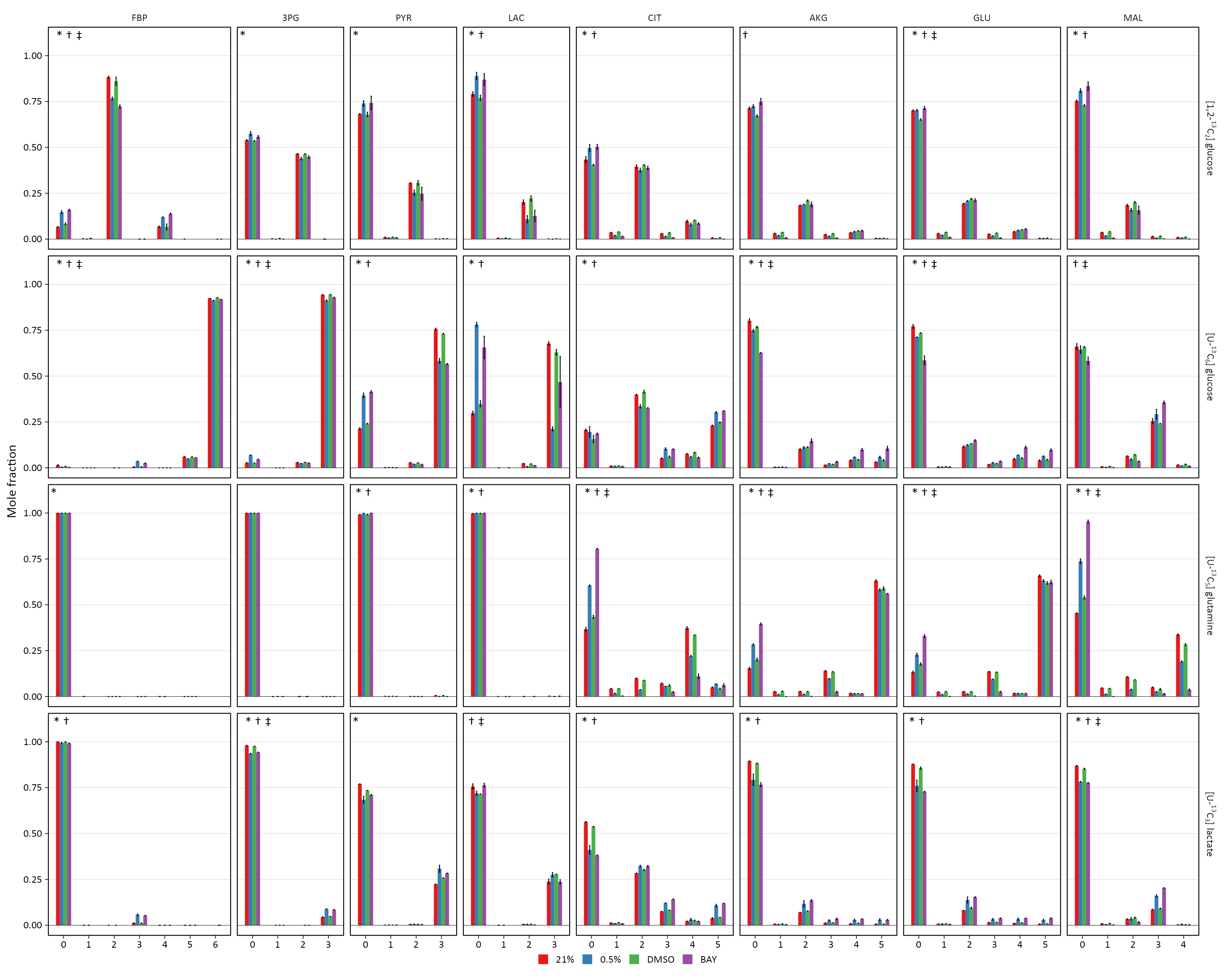


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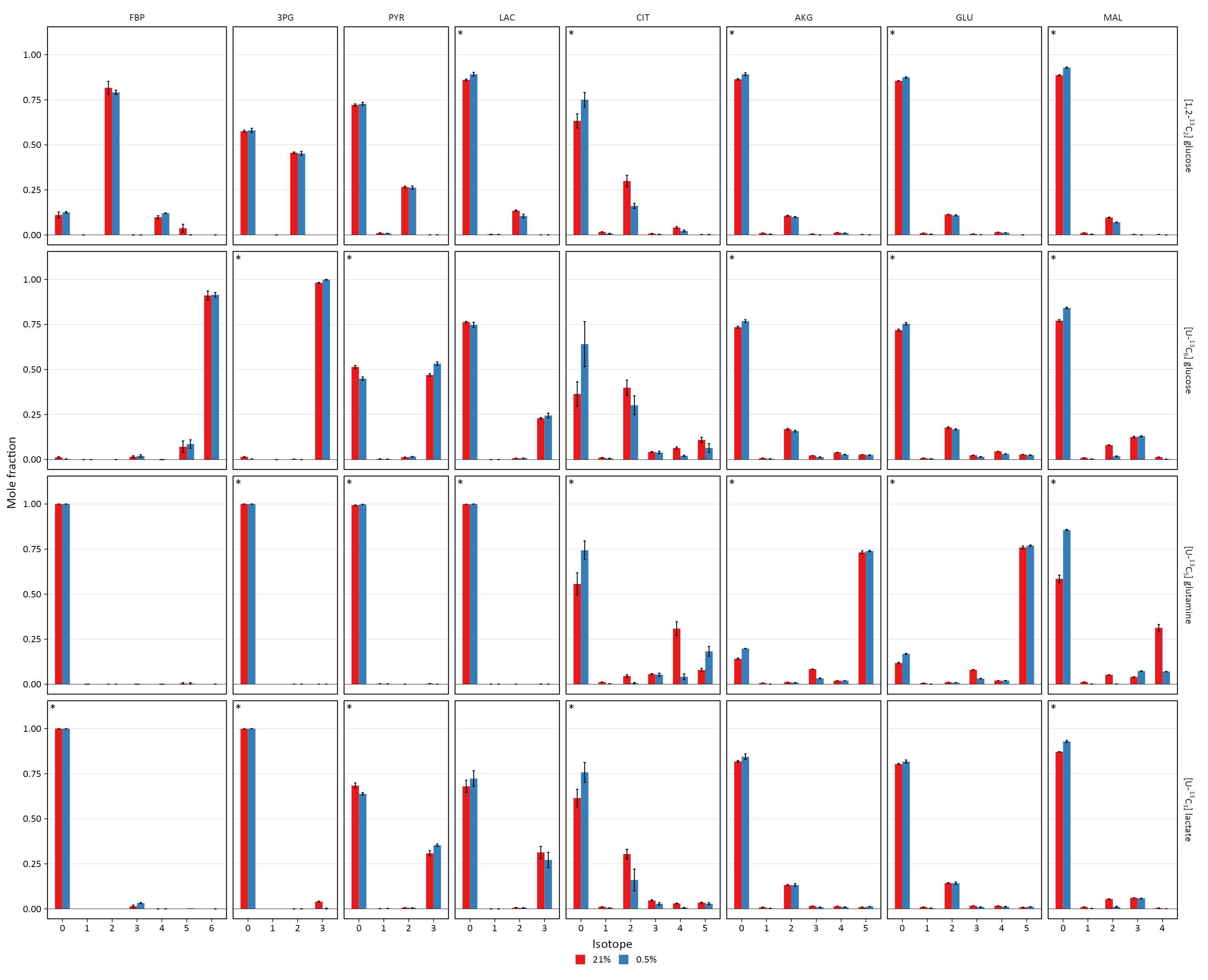


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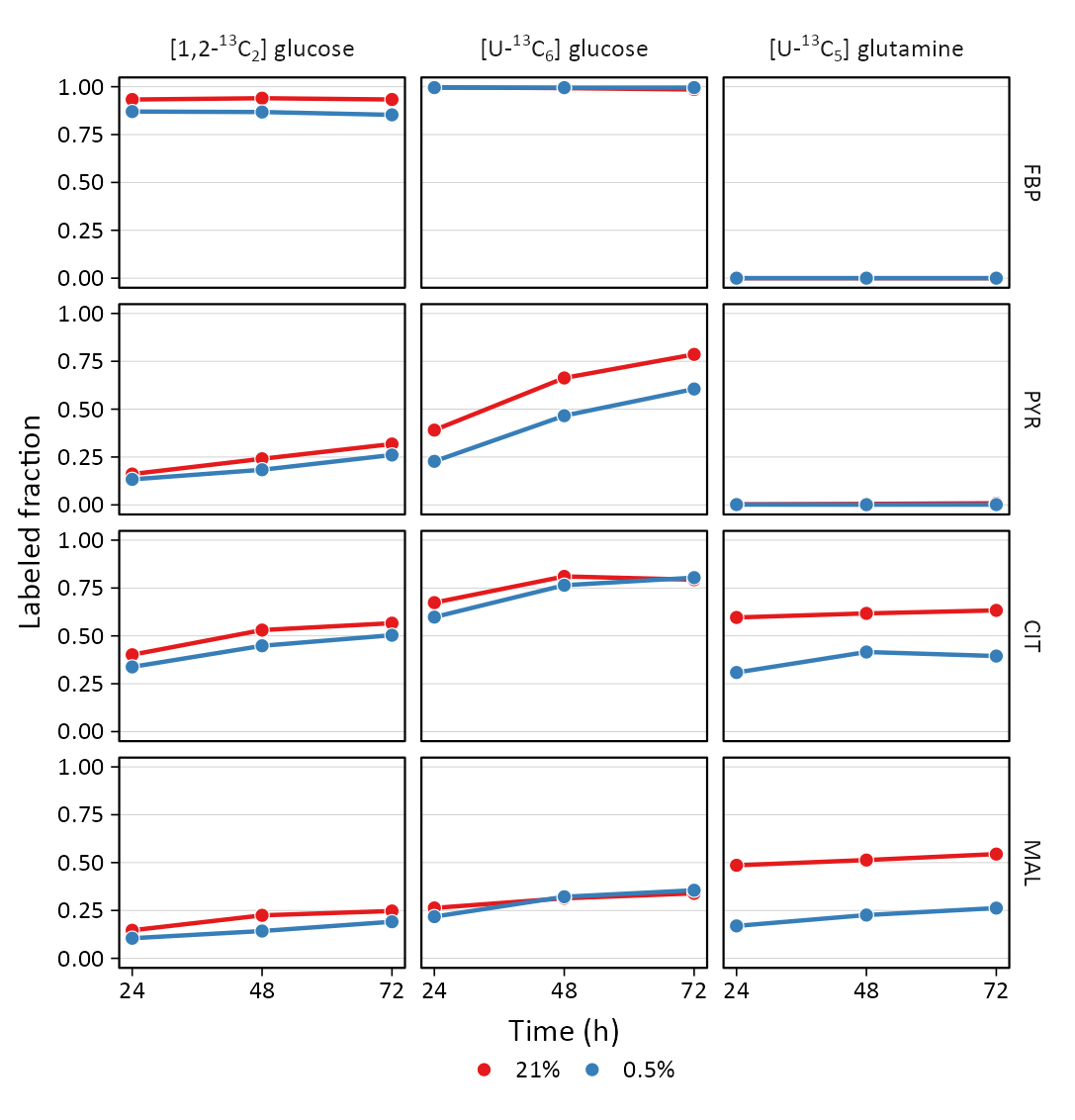


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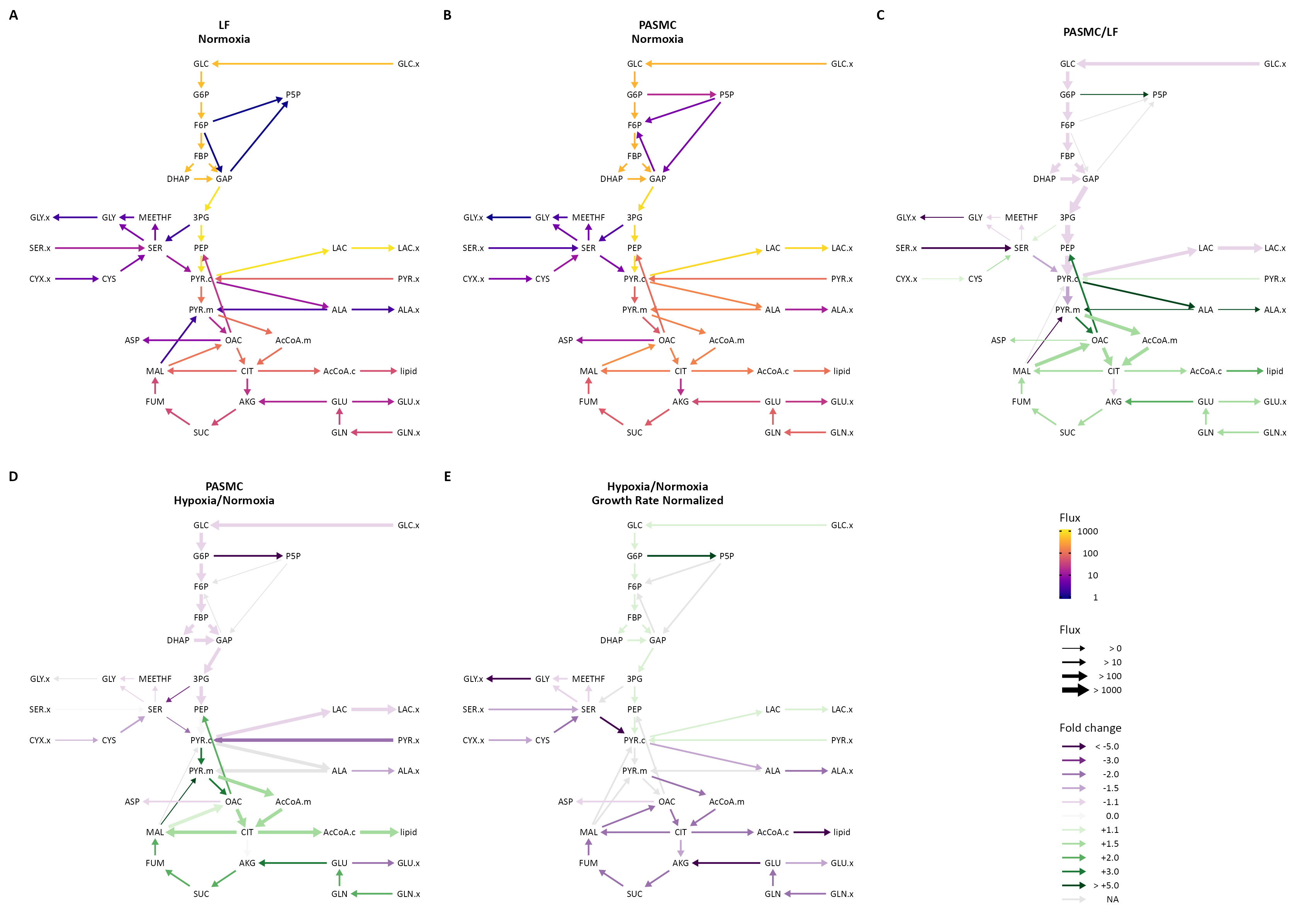


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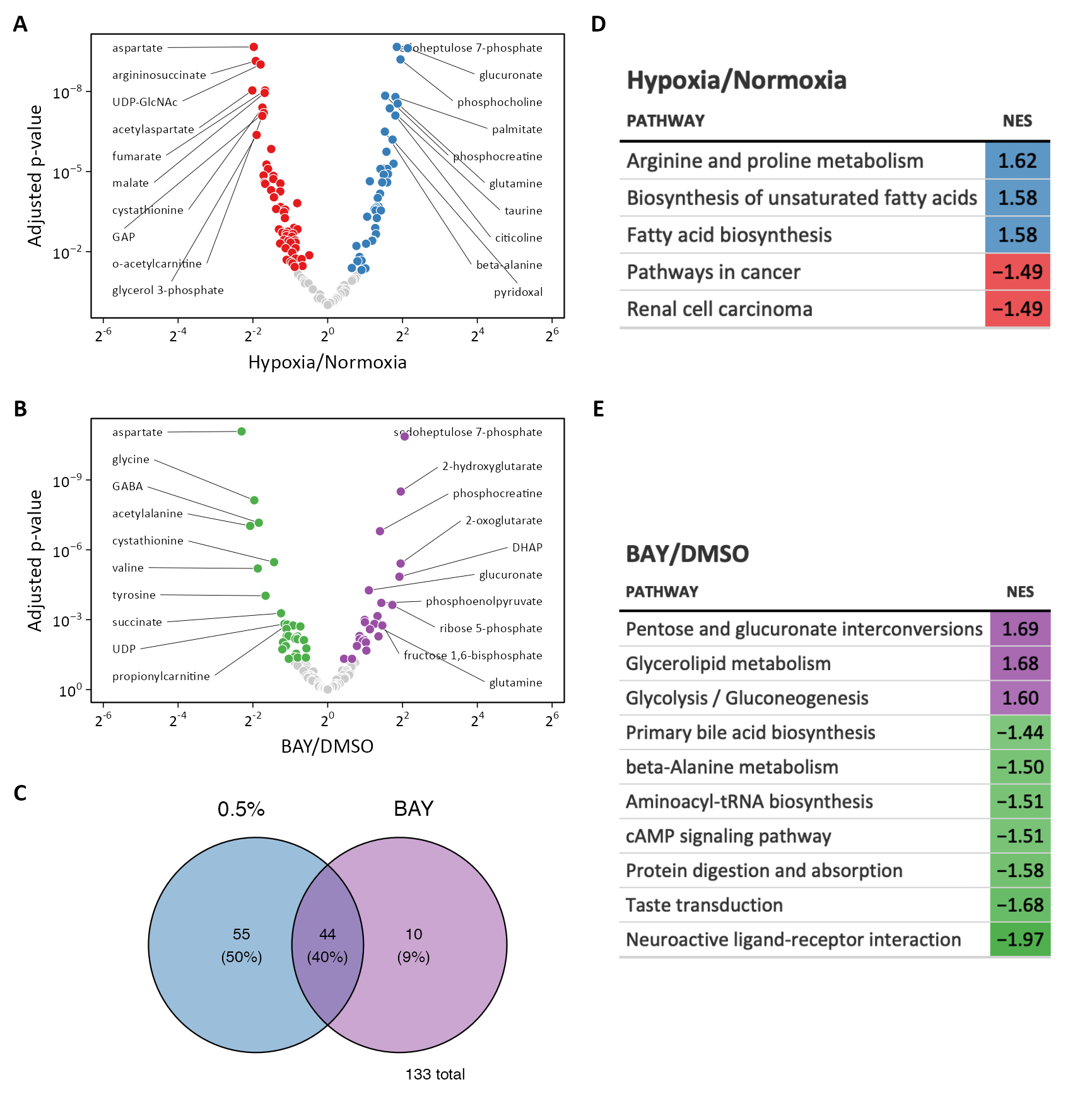


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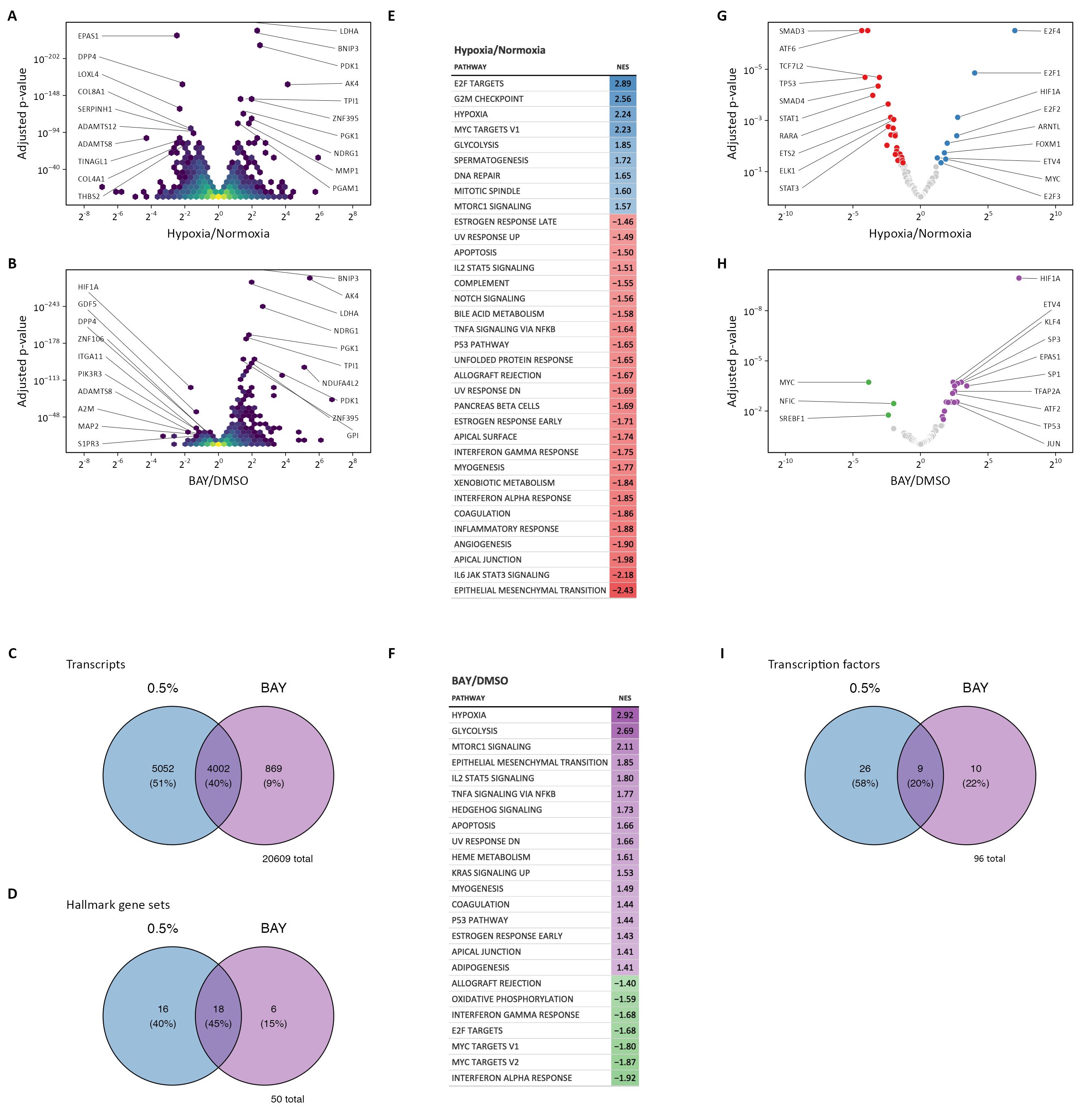


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