**Supplementary Material: Quantification of metabolic rearrangements during neural stem cells differentiation into astrocytes by metabolic flux analysis**

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This document contains the following information in support of the article:

**Table S1 – Specific uptake and secretion rates of extracellular metabolites and their standard deviations (nmol/h/106cell) from replicate wells of NSCs and astrocytes.**

**Table S2 – Mass isotopomer distributions of intracellular metabolites for [1-13C] glucose experiments measured by GC-MS**.

**Table S3 – Metabolic fluxes and associated 95% confidence intervals (nmol/h/106cell) estimated after [1-13C] glucose administration to NSCs and astrocytes.**

**Table S1** – **Specific uptake and secretion rates of extracellular metabolites and their standard deviations (nmol/h/106cell) from replicate wells of NSCs and astrocytes.** The rates were calculated by linear regression of extracellular metabolite concentration profiles along 24 h after label administration. NaN represents undetermined transport rates.

|  |  |  |
| --- | --- | --- |
| **Metabolite** | **Neural Stem Cells** | **Astrocytes** |
| **Glucose** | -256.3 ± 1.3 | -175.7 ± 17.9 |
| **Lactate** | 237.8 ± 10.1 | 348.6 ± 9.6 |
| **Aspartate** | -2.6 ± 0.7 | -11.9 ± 5.8 |
| **Glutamate** | -1.8 ± 0.4 | -10.5 ± 3.4 |
| **Serine** | -2.8 ± 1.4 | -4.1 ± 1.7 |
| **Asparagine** | -0.6 ± 0.3 | -1.6 ± 0.3 |
| **Glycine** | 2.4 ± 1.1 | NaN |
| **Glutamine** | 26.1 ± 6.5 | 10.4 ± 2.2 |
| **Histidine** | NaN | -3.4 ± 1.0 |
| **Threonine** | NaN | 0.9 ± 0.6 |
| **Arginine** | NaN | 1.7 ± 0.3 |
| **Alanine** | 9.0 ± 1.4 | -6.6 ± 3.1 |
| **Proline** | 0.3 ± 0.1 | NaN |
| **Tyrosine** | -3.3 ± 1.9 | NaN |
| **Valine** | -3.0 ± 2.2 | NaN |
| **Methionine** | -0.8 ± 0.4 | -1.9 ± 0.3 |
| **Isoleucine** | -3.7 ± 2.2 | -1.5 ± 0.7 |
| **Leucine** | -3.9 ± 2.3 | NaN |
| **Phenylalanine** | NaN | 2.1 ± 0.8 |
| **Citrate** | NaN | 6.6 ± 0.9 |
| **Pyruvate** | -12.9 ± 5.1 | -48.0 ± 4.4 |

**Table S2 – Mass isotopomer distributions of intracellular metabolites for [1-13C] glucose experiments measured by GC-MS**. Data was corrected for natural isotope abundances.

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | **Neural Stem Cells** | | | | **Astrocytes** | | | |
|  | 0.33 h | 3 h | 12 h | 24 h | 0.33 h | 3 h | 12 h | 24 h |
| 3PG (M0) | 0.639 | 0.787 | 0.604 | 0.564 | 0.586 | 0.568 | 0.667 | 0.582 |
| 3PG (M1) | 0.361 | 0.213 | 0.396 | 0.436 | 0.414 | 0.432 | 0.333 | 0.418 |
| PEP (M0) | 0.695 | 0.771 | 0.618 | 0.578 | 0.608 | 0.639 | 0.754 | 0.674 |
| PEP (M1) | 0.305 | 0.229 | 0.382 | 0.422 | 0.392 | 0.361 | 0.246 | 0.326 |
| Lac (M0) | 0.906 | 0.882 | 0.891 | 0.844 | 0.992 | 0.986 | 0.968 | 0.824 |
| Lac (M1) | 0.094 | 0.118 | 0.109 | 0.156 | 0.008 | 0.014 | 0.032 | 0.176 |
| Lac (M2) | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Ala (M0) | 0.782 | 0.726 | 0.667 | 0.646 | 0.861 | 0.828 | 0.791 | 0.710 |
| Ala (M1) | 0.218 | 0.274 | 0.333 | 0.354 | 0.139 | 0.172 | 0.209 | 0.290 |
| Ala (M2) | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Cit (M0) | 0.812 | 0.713 | 0.677 | 0.656 | 0.894 | 0.880 | 0.775 | 0.726 |
| Cit (M1) | 0.186 | 0.261 | 0.270 | 0.282 | 0.106 | 0.119 | 0.189 | 0.227 |
| Cit (M2) | 0.002 | 0.025 | 0.047 | 0.056 | 0 | 0.002 | 0.034 | 0.046 |
| Cit (M3) | 0 | 0 | 0.006 | 0.006 | 0 | 0 | 0.002 | 0.001 |
| Suc (M0) | 0.974 | 0.945 | 0.934 | 0.923 | 0.993 | 0.993 | 0.983 | 0.959 |
| Suc (M1) | 0.026 | 0.054 | 0.063 | 0.070 | 0.007 | 0.007 | 0.017 | 0.036 |
| Suc (M2) | 0 | 0.001 | 0.003 | 0.007 | 0 | 0 | 0.001 | 0.004 |
| Suc (M3) | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Fum (M0) | 0.965 | 0.898 | 0.854 | 0.820 | 0.991 | 0.955 | 0.881 | 0.872 |
| Fum (M1) | 0.031 | 0.093 | 0.126 | 0.153 | 0.008 | 0.043 | 0.103 | 0.116 |
| Fum (M2) | 0.004 | 0.009 | 0.020 | 0.026 | 0.001 | 0.002 | 0.016 | 0.011 |
| Fum (M3) | 0 | 0 | 0.001 | 0.002 | 0 | 0 | 0 | 0.001 |
| Mal (M0) | 0.964 | 0.894 | 0.825 | 0.809 | 0.994 | 0.952 | 0.810 | 0.767 |
| Mal (M1) | 0.035 | 0.101 | 0.156 | 0.167 | 0.006 | 0.048 | 0.174 | 0.213 |
| Mal (M2) | 0 | 0.003 | 0.017 | 0.022 | 0 | 0 | 0.016 | 0.020 |
| Mal (M3) | 0 | 0.001 | 0.001 | 0.002 | 0 | 0 | 0 | 0 |
| Asp (M0) | 0.976 | 0.910 | 0.826 | 0.817 | 1 | 0.957 | 0.877 | 0.869 |
| Asp (M1) | 0.024 | 0.089 | 0.158 | 0.163 | 0 | 0.043 | 0.116 | 0.124 |
| Asp (M2) | 0 | 0.001 | 0.015 | 0.018 | 0 | 0 | 0.006 | 0.005 |
| Asp (M3) | 0 | 0 | 0.001 | 0.001 | 0 | 0 | 0.001 | 0.002 |
| Glu (M0) | 0.941 | 0.867 | 0.796 | 0.798 | 0.980 | 0.922 | 0.664 | 0.616 |
| Glu (M1) | 0.057 | 0.121 | 0.173 | 0.167 | 0.020 | 0.077 | 0.285 | 0.321 |
| Glu (M2) | 0.001 | 0.011 | 0.029 | 0.032 | 0 | 0.001 | 0.050 | 0.062 |
| Glu (M3) | 0 | 0 | 0.002 | 0.003 | 0 | 0 | 0.001 | 0.001 |
| Gln (M0) | 1 | 0.995 | 0.993 | 0.994 | 1 | 0.964 | 0.789 | 0.716 |
| Gln (M1) | 0 | 0.005 | 0.007 | 0.006 | 0 | 0.036 | 0.184 | 0.240 |
| Gln (M2) | 0 | 0 | 0 | 0 | 0 | 0 | 0.026 | 0.043 |
| Gln (M3) | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

**Table S3** – **Metabolic fluxes and associated 95% confidence intervals (nmol/h/106cell) estimated after [1-13C]glucose administration to NSCs and astrocytes.** For flux estimation, experimentally determined errors were associated with the extracellular rates and an error of 1.5 mol% was attributed for the MS measurements. Flux estimation solutions with statistically accepted fits were obtained for both cell populations. For NSC the sum of square residuals (SSR) was 36.0 (the system has 86 degrees of freedom; assuming the minimized SSR follows a *χ*2-distribution, the expected 95% confidence region of SSR is [62.2, 113.5]. For astrocytes the SSR was 89.1 (the expected SSR interval was [51.3, 98.5], with 73 degrees of freedom).

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| *#* | *Reaction* |  | *NSC* | | *Astrocytes* | | | |
|  | *Flux* | *95% Confidence Interval* | *Flux* | *95% Confidence Interval* | | |
| *R1* | G6P ⭤ F6P | net | 246.7 | [239.7, 257.8] | 147.3 | | [129.9, 163.2] |
|  | G6P ⭤ F6P | exch | 7734.4 | [0, Inf] | 2646.0 | [0, Inf] | | |
| *R2* | F6P ⭢ FBP |  | 253.1 | [249.6, 257.7] | 152.8 | [135.8, 170.7] | | |
| *R3* | FBP ⭤ DHAP + GAP | net | 253.1 | [249.6, 257.7] | 152.8 | [135.8, 170.7] | | |
|  | FBP ⭤ DHAP + GAP | exch | 1E-7 | [0, Inf] | 1E-7 | [0, Inf] | | |
| *R4* | DHAP ⭤ GAP | net | 253.1 | [249.6, 257.7] | 152.8 | [135.8, 170.7] | | |
|  | DHAP ⭤ GAP | exch | 120.9 | [0, Inf] | 119.0 | [0, Inf] | | |
| *R5* | GAP ⭤ 3PG | net | 509.3 | [503.5, 516.6] | 308.4 | [274.3, 342.8] | | |
|  | GAP ⭤ 3PG | exch | 0.6 | [0, Inf] | 1E-7 | [0, Inf] | | |
| *R6* | 3PG ⭤ PEP | net | 509.3 | [503.5, 516.6] | 308.4 | [274.3, 342.8] | | |
|  | 3PG ⭤ PEP | exch | 1E-7 | [0, Inf] | 0.2 | [0, Inf] | | |
| *R7* | PEP ⭢ Pyr.c |  | 509.3 | [503.5, 516.6] | 308.4 | [274.3, 342.8] | | |
| *R8* | G6P ⭢ P5P + CO2 |  | 9.5 | [NaN, 16.1] | 8.3 | [4.2, 13.9] | | |
| *R9* | P5P + P5P ⭤ GAP + S7P | net | 3.2 | [NaN, 5.3] | 2.8 | [1.4, 4.6] | | |
|  | P5P + P5P ⭤ GAP + S7P | exch | 277.5 | [172.5, 404.7] | 1E-7 | [0, Inf] | | |
| *R10* | S7P + GAP ⭤ E4P + F6P | net | 3.2 | [NaN, 5.3] | 2.8 | [1.4, 4.6] | | |
|  | S7P + GAP ⭤ E4P + F6P | exch | 1E-7 | [0, 15.6] | 1E-7 | [0, Inf] | | |
| *R11* | E4P + P5P ⭤ GAP + F6P | net | 3.2 | [NaN, 5.3] | 2.8 | [1.4, 4.6] | | |
|  | E4P + P5P ⭤ GAP + F6P | exch | 1E-7 | [0, 4.1] | 1E-7 | [0, Inf] | | |
| *R12* | Pyr.c ⭤ Lac | net | 239.3 | [217.9, 262.1] | 354.1 | [323.8, 384.1] | | |
|  | Pyr.c ⭤ Lac | exch | 139.2 | [0, 204.7] | 77.8 | [0, 236.7] | | |
| *R13* | Pyr.c ⭤ Ala | net | 9.0 | [6.1, 12.3] | -6.5 | [-12.7, -0.3] | | |
|  | Pyr.c ⭤ Ala | exch | 73.1 | [0, Inf] | 10329 | [8.3, Inf] | | |
| *R14* | Pyr.m ⭢ AcCoA.m + CO2 |  | 318.0 | [290.2, 353.5] | 9.1 | [3.2, 20.0] | | |
| *R15* | Pyr.m + CO2 ⭢ OAC |  | 57.7 | [22.8, 323.0] | 39.2 | [18.8, 106.4] | | |
| *R16* | OAC + AcCoA.m ⭢ Cit |  | 347.1 | [306.6, 391.9] | 17.9 | [13.2, 30.2] | | |
| *R17* | Cit ⭤ AKG + CO2 | net | -27.7 | [-42.1, 56.1] | 11.2 | [6.8, 17.3] | | |
|  | Cit ⭤ AKG + CO2 | exch | 54545.1 | [19.5, Inf] |  |  | | |
| *R18* | AKG ⭢ SucCoA + CO2 |  | 0.08 | [0, 83.0] | 1E-7 | [-2.8E-15, 5.5] | | |
| *R19* | SucCoA ⭤ Suc | net | 0.08 | [0, 83.0] | 1E-7 | [0, 5.5] | | |
|  | SucCoA ⭤ Suc | exch | 1E-7 | [0, Inf] | 50.3 | [0, Inf] | | |
| *R20* | Suc ⭤ Fum | net | 6.7 | [0.4, 87.6] | 3.4 | [2.0, 21.0] | | |
|  | Suc ⭤ Fum | exch | 4.0 | [0, 10.9] | 1E-7 | [0, 36.4] | | |
| *R21* | Fum ⭤ Mal | net | 10.2 | [2.6, 94.1] | 5.2 | [2.2, 11.7] | | |
|  | Fum ⭤ Mal | exch | 64.8 | [14.2, 815.3] | 5.8 | [1.7, 43.6] | | |
| *R22* | OAC ⭤ Mal | net | 88.7 | [43.7, 345.9] | 32.4 | [6.5, 98.4] | | |
|  | OAC ⭤ Mal | exch | 1E-7 | [0, Inf] | 1E-7 | [0, 48.9] | | |
| *R23* | Mal ⭢ Pyr.m + CO2 |  | 98.9 | [52.2, 358.9] | 37.6 | [10.5, 103.9] | | |
| *R24* | Cit ⭢ OAC + AcCoA.c |  | 374.8 | [331.4, 425.5] | 1E-7 | [0, NaN] | | |
| *R25* | Gln ⭤ Glu | net | 25.9 | [13.1, 38.6] | -9.5 | [-14.0, -5.5] | | |
|  | Gln ⭤ Glu | exch | 1.1 | [0, 185.9] | 1E-7 | [0, 2.4] | | |
| *R26* | Glu ⭤ AKG | net | 27.8 | [14.5, 43.0] | -11.2 | [-16.5, -6.9] | | |
|  | Glu ⭤ AKG | exch | 53.5 | [15.8, 387.6] | 404.5 | [0, Inf] | | |
| *R27* | Asn ⭤ Asp | net | 0.6 | [0.08, 1.2] | 1.6 | [1.0, 2.1] | | |
|  | Asn ⭤ Asp | exch | 260.2 | [0, Inf] | 2600.3 | [0, Inf] | | |
| *R28* | Asp ⭤ OAC | net | 3.3 | [1.8, 5.0] | 11.0 | [1.3, 20.5] | | |
|  | Asp ⭤ OAC | exch | 103.5 | [14.3, Inf] | 11.2 | [2.6, 69.5] | | |
| *R29* | Ser ⭢ Pyr.c |  | 2.9 | [0.05, 5.8] | 4.1 | [0, 11.4] | | |
| *R30* | Ser ⭤ Gly + C1 | net | -0.8 | [-2.1, 0] | -1.9 | [-8.5, 3.8] | | |
|  | Ser ⭤ Gly + C1 | exch | 26.4 | [0, Inf] | 0.02 | [0, Inf] | | |
| *R31* | Glu ⭤ Pro | net | 0.3 | [0.08, 0.5] | 1E-7 | [-0.5, 1.3] | | |
|  | Glu ⭤ Pro | exch | 0.3 | [0, Inf] | 1E-7 | [0, Inf] | | |
| *R32* | Val + CO2 ⭢ Suc + CO2 + CO2 |  | 2.4 | [0, 8.2] | 1E-7 | [0, 1.8] | | |
| *R33* | Ile + CO2 ⭢ Suc + AcCoA.m + CO2 |  | 3.5 | [0, 7.8] | 1.6 | [0.2, 2.9] | | |
| *R34* | Leu + CO2 ⭢ AcCoA.m + AcCoA.m + AcCoA.m + CO2 |  | 3.9 | [0, 9.0] | 0.5 | [0, 2.0] | | |
| *R35* | Thr ⭢ AcCoA.m + Gly |  | 2.3 | [0, 4.5] | 0.09 | [0, 1.3] | | |
| *R36* | Phe ⭢ Tyr |  | 0.7 | [0, 3.5] | 0.2 | [0, 1.7] | | |
| *R37* | Tyr ⭢ Fum + AcCoA.m + AcCoA.m + CO2 |  | 3.5 | [0, 8.7] | 1.8 | [0., 5.2] | | |
| *R38* | Met + Ser + CO2 ⭢ Suc + Cys.snk + CO2 + C1 |  | 0.7 | [0, 1.6] | 1.9 | [1.3, 2.5] | | |
| *R39* | Lys ⭢ CO2 + CO2 + AcCoA.m + AcCoA.m |  | 2.2 | [0, 7.9] | 0.9 | [0, 3.3] | | |
| *R40* | His ⭢ Glu + C1 |  | 0.1 | [0, 1.1] | 1E-7 | [0, 1.1] | | |
| *R41* | Arg ⭢ Glu + Urea.snk |  | 72.3 | [0, 1325.4] | 1E-7 | [0, 42.7] | | |
| *R42* | Glu + CO2 ⭢ Arg |  | 72.1 | [0, 1338.0] | 1.7 | [1.0, 73.3] | | |
| *R43* | Pyr.c ⭤ Pyr.m | net | 276.8 | [251.6, 303.7] | 10.8 | [-1.2, 25.0] | | |
|  | Pyr.c ⭤ Pyr.m | exch | 1E-7 | [0, Inf] | 516.1 | [239.1, 791.6] | | |
| *R44* | CO2 ⭤ CO2.ext | net | 279.4 | [-890.7, 398.2] | 29.0 | [-44.2, 50.9] | | |
|  | CO2 ⭤ CO2.ext | exch | 1E-7 | [0, Inf] | 1E-7 | [0, 43.6] | | |
| *R45* | Glc.ext ⭢ G6P |  | 256.2 | [253.8, 258.7] | 155.7 | [138.5, 172.9] | | |
| *R46* | Lac ⭤ Lac.ext | net | 239.3 | [215.5, 262.1] | 354.1 | [323.8, 384.1] | | |
|  | Lac ⭤ Lac.ext | exch | 542.8 | [344.5, 721.8] | 1E-7 | [0, 135.6] | | |
| *R47* | Ala ⭤ Ala.ext | net | 9.0 | [6.1, 12.3] | -6.5 | [-12.7, -0.3] | | |
|  | Ala ⭤ Ala.ext | exch | 1E-7 | [0, 113.9] | 1E-7 | [0, 88.3] | | |
| *R48* | Pyr.ext ⭤ Pyr.c | net | 12.8 | [5.0, 21.9] | 45.9 | [35.4, 56.5] | | |
|  | Pyr.ext ⭤ Pyr.c | exch | 0.2 | [0, 35.5] | 1E-7 | [0, 93.0] | | |
| *R49* | Cit ⭢ Cit.ext |  |  |  | 6.7 | [4.1, 9.4] | | |
| *R50* | Gln ⭤ Gln.ext | net | -25.9 | [-38.6, 13.1] | 9.5 | [5.5, 14.0] | | |
|  | Gln ⭤ Gln.ext | exch |  |  | 2.1 | [0, 5.2] | | |
| *R51* | Glu ⭤ Glu.ext | net | -1.8 | [-2.7, -1.0] | -1E-7 | [-0.5, 1.7] | | |
|  | Glu ⭤ Glu.ext | exch | 14.5 | [0, 157.5] | 1E-7 | [0, 1.8] | | |
| *R52* | Asp ⭤ Asp.ext | net | -2.6 | [-4.0, -1.2] | -9.4 | [-19.3, 0.3] | | |
|  | Asp ⭤ Asp.ext | exch | 5.4 | [0, 121.7] | 1E-7 | [0, 50.1] | | |
| *R53* | Asn ⭤ Asn.ext | net | -0.6 | [-1.2, -0.08] | -1.6 | [-2.1, -1.0] | | |
|  | Asn ⭤ Asn.ext | exch | 0.7 | [0, 158.7] | 1E-7 | [0, Inf] | | |
| *R54* | Ser.ext ⭤ Ser | net | 2.8 | [-0.4, 5.6] | 4.1 | [0.7, 7.6] | | |
|  | Ser.ext ⭤ Ser | exch | 1E-7 | [0, Inf] | 0.03 | [0, Inf] | | |
| *R55* | Gly ⭤ Gly.ext | net | 1.5 | [-0.5, 3.8] | -1.9 | [-8.4, 3.9] | | |
|  | Gly ⭤ Gly.ext | exch | 0.005 | [0, Inf] | 1E-7 | [0, Inf] | | |
| *R56* | Pro.ext ⭤ Pro | net | -0.3 | [-0.5, -0.08] | -1E-7 | [-1.3, 0.5] | | |
|  | Pro.ext ⭤ Pro | exch | 0.9 | [0, Inf] | 1.0 | [0, Inf] | | |
| *R57* | Val.ext ⭢ Val |  | 2.4 | [0, 8.2] | 1E-7 | [0, 1.8] | | |
| *R58* | Ile.ext ⭢ Ile |  | 3.5 | [0, 7.8] | 1.6 | [0.2, 2.9] | | |
| *R59* | Leu.ext ⭢ Leu |  | 3.9 | [0, 9.0] | 0.5 | [0, 2.0] | | |
| *R60* | Thr.ext ⭢ Thr |  | 2.3 | [0, 4.5] | 0.09 | [0, 1.3] | | |
| *R61* | Phe.ext ⭢ Phe |  | 0.7 | [0, 3.5] | 0.2 | [0, 1.7] | | |
| *R62* | Tyr.ext ⭢ Tyr |  | 2.8 | [0, 7.1] | 1.6 | [0, 4.8] | | |
| *R63* | Met.ext ⭢ Met |  | 0.7 | [0, 1.6] | 1.9 | [1.3, 2.5] | | |
| *R64* | Lys.ext ⭢ Lys |  | 2.2 | [0, 7.9] | 0.9 | [0, 3.3] | | |
| *R65* | His.ext ⭢ His |  | 0.1 | [0, 1.1] | 1E-7 | [0, 1.1] | | |
| *R66* | Arg.ext ⭤ Arg | net | 0.2 | [-2.6, 3.3] | -1.7 | [-2.4, -1.0] | | |
|  | Arg.ext ⭤ Arg | exch | 1E-7 | [0, 305.4] | 1E-7 | [0, Inf] | | |