

## Supplement Data:

### Delta-tocotrienol modulates glutamine dependence by inhibiting ASCT2 and LAT1 Transporters in non-small cell lung cancer (NSCLC) cells: Metabolomic Approach

Lichchavi Dhananjaya Rajasinghe<sup>1</sup>, Melanie Hutchings<sup>1</sup>, Smiti Vaid Gupta<sup>1\*</sup>

<sup>1</sup> Department of Nutrition and Food Science, Wayne State University, Detroit, MI, U.S.A;

**Table S1: List of metabolite concentrations determined using Chenomx NMR Suite in A549 cells.** All <sup>1</sup>H-NMR spectra were processed and analyzed using the Chenomx NMR Suite Professional software (Chenomx Inc., Edmonton, AB), as previously described [1].

| A549 cells                     | Control 1          | Control 2 | Control 3 | Treatment 1 | Treatment 2 | Treatment 3 |
|--------------------------------|--------------------|-----------|-----------|-------------|-------------|-------------|
| Metabolite Name                | Concentration (μM) |           |           |             |             |             |
| 2-Oxoglutarate                 | 40.2               | 44        | 46.6      | 34.7        | 26.2        | 27          |
| Acetate                        | 25.4               | 29.7      | 28.6      | 23.2        | 17.7        | 26          |
| ADP                            | 42.9               | 57.3      | 43.1      | 40.9        | 36          | 45.5        |
| Alanine                        | 30.6               | 32.5      | 29.8      | 17.8        | 17.3        | 24.2        |
| AMP                            | 32.5               | 36.8      | 27        | 43.2        | 46.4        | 45.3        |
| Asparagine                     | 106.2              | 135.5     | 108.9     | 67.1        | 55.3        | 41.1        |
| Aspartate                      | 100.5              | 91.4      | 114.9     | 50.6        | 58          | 59.1        |
| ATP                            | 36                 | 45.2      | 45.4      | 53.9        | 31.4        | 43.3        |
| Citrate                        | 38.3               | 43        | 45.7      | 31.3        | 26.8        | 48.7        |
| Citrulline                     | 77.2               | 87.2      | 81.3      | 49          | 71.4        | 71.3        |
| Cystine                        | 84                 | 85.8      | 74.2      | 44.5        | 50.7        | 79.9        |
| Dimethylamine                  | 8                  | 6.2       | 9.8       | 403.6       | 210.8       | 66.9        |
| Formate                        | 318.9              | 216.9     | 347.1     | 303.7       | 321.3       | 313.4       |
| Fumarate                       | 21.6               | 27.9      | 25.4      | 28.3        | 22.4        | 32.3        |
| Glucose                        | 180.6              | 86.5      | 90        | 234.7       | 212.2       | 114.9       |
| Glutamate                      | 75                 | 89.7      | 77.5      | 44.9        | 47.2        | 53.9        |
| Glutamine                      | 103.7              | 92.2      | 103.8     | 62.7        | 52.5        | 78.7        |
| Glutaric acid monomethyl ester | 12.1               | 24.6      | 16.5      | 33.7        | 31.3        | 36.8        |
| Glutathione                    | 70.5               | 67.3      | 71        | 36.9        | 49.3        | 38.9        |
| Glycine                        | 31.8               | 33.4      | 34        | 22          | 15.6        | 23.4        |
| Histidine                      | 46.3               | 52.9      | 62.8      | 56.6        | 82.2        | 118.7       |
| Isoleucine                     | 22                 | 30.7      | 41.7      | 33.8        | 22.6        | 35.2        |
| Lactate                        | 132.3              | 143       | 140.2     | 103.5       | 99.8        | 96.4        |
| Leucine                        | 38.2               | 32.4      | 30.4      | 13.4        | 16.8        | 20.7        |
| Lysine                         | 66.3               | 36.9      | 21.6      | 32.9        | 41.5        | --          |
| Malate                         | 97.7               | 82.6      | --        | 49.9        | 58.2        | 37.8        |
| Methionine                     | 3.6                | 11.8      | 2         | 5.3         | 2.2         | 8.9         |
| N-Acetylglucosamine            | 31.4               | 13        | 21.1      | 8.2         | 11.8        | 18.4        |
| NAD+                           | 43.7               | 49.3      | 52.6      | 23.9        | --          | 53.7        |
| NADH                           | 36.4               | 42.4      | 36.3      | 32.1        | --          | 54.7        |
| NADP+                          | 71.8               | 29.9      | 22        | 6.6         | --          | 44.2        |

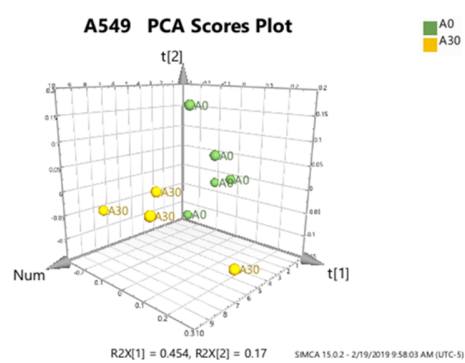
|                         |      |       |       |       |      |       |
|-------------------------|------|-------|-------|-------|------|-------|
| NADPH                   | 40.9 | 53.3  | 46.7  | 50.6  | 39.1 | 64    |
| Proline                 | 42   | 8.2   | 105.3 | 38.7  | 62.2 | 90    |
| Succinate               | 8.1  | 7.5   | 12.1  | 2.3   | 5.5  | 7.7   |
| Taurine                 | 98.4 | 104.8 | 67.6  | 104.2 | 79.6 | 50.8  |
| Tryptophan              | 64   | 88.8  | 91    | 54.9  | 57.3 | 105.7 |
| Tyramine                | 7.7  | 22.7  | 53.9  | --    | 34.1 | 78.3  |
| UDP-N-Acetylglucosamine | 7.2  | 8.8   | 4.7   | 0     | 5.6  | 6     |
| Uracil                  | 86.4 | 113.6 | 94    | 80.7  | 65.8 | 33.8  |
| Valine                  | 22.4 | 25    | 24    | 24    | 14.5 | 26.2  |

**Table S2: List of metabolite concentrations determined using Chenomx NMR Suite in H1299 cells.** All <sup>1</sup>H-NMR spectra were processed and analyzed using the Chenomx NMR Suite Professional software (Chenomx Inc., Edmonton, AB), as previously described [1].

| H1299 cells                    | Control 1          | Control 2 | Control 3 | Treatment 1 | Treatment 2 | Treatment 3 |
|--------------------------------|--------------------|-----------|-----------|-------------|-------------|-------------|
| Metabolite Name                | Concentration (μM) |           |           |             |             |             |
| 2-Oxoglutarate                 | 23.8               | 30.8      | 43        | 15.1        | 18.2        | 18.2        |
| ADP                            | 37.7               | 26.4      | 36.6      | 10          | 8.9         | 25.7        |
| Alanine                        | 30                 | 30.7      | 25.8      | 20.4        | 19.2        | 15.1        |
| AMP                            | 27.2               | 27.7      | 27.6      | 15.9        | 11          | 14.3        |
| Arginine                       | 44.9               | 46.4      | 40        | 37.6        | 24.9        | 22.7        |
| Asparagine                     | 128.8              | 108.4     | 77.8      | 70.5        | 116.8       | 64.7        |
| Aspartate                      | 100.6              | 107.2     | 108.8     | 77          | 72.4        | 82.8        |
| ATP                            | 43.9               | 52.8      | 33.7      | 14.6        | 24.6        | 27.3        |
| Citrate                        | 34.5               | 34.7      | 36.3      | 31.1        | 25.1        | 20.6        |
| Citrulline                     | 65.6               | 90.5      | 40.1      | 36.8        | 89.3        | 34.1        |
| Cystine                        | 55.5               | 56.2      | 71.2      | 12.2        | --          | 40.3        |
| Fumarate                       |                    |           |           |             |             |             |
| Formate                        | 479.3              | 319.2     | 265.1     | 356.4       | 391.4       | 292.3       |
| Glucose                        | 228.5              | 235.1     | 125.6     | 133.9       | 174.6       | 132.9       |
| Glutamate                      | 88.2               | 75.3      | 76.9      | 56.5        | 50.1        | 41.4        |
| Glutamine                      | 82.4               | 72.2      | 71.2      | 56.5        | 62.2        | 42.3        |
| Glutaric acid monomethyl ester | --                 | --        | 27.4      | 26.5        | 10.1        | 25.1        |
| Glutathione                    | 41.9               | 48        | 37        | 21.1        | 36.8        | 26.2        |
| Glycine                        | --                 | 32.8      | 23.5      | 21          | 19.6        | 13.7        |
| Histidine                      |                    |           |           |             |             |             |
| Isoleucine                     | 41.1               | 40.2      | 30.3      | 23.4        | 27.3        | 20.6        |
| Lactate                        | 124                | 117.9     | 135.4     | 128.7       | 136.6       | 100.8       |
| Leucine                        | 31.1               | 30.7      | 33.6      | 19          | 18.8        | 17.2        |
| Lysine                         | 50.6               | 42.3      | 23.6      | 27.3        | 21          | 10          |
| Malate                         | 132.8              | 120.5     | 139.4     | 84.1        | 73.9        | 96          |

|                         |      |      |       |      |       |      |
|-------------------------|------|------|-------|------|-------|------|
| Methionine              | 7.9  | 9.8  | 8.3   | 5.4  | 9.4   | 5.2  |
| N-Acetylglucosamine     |      |      |       |      |       |      |
| NADH                    | 80.4 | 51.8 | 63.7  | 11.4 | 84.9  | 34.8 |
| NADPH                   | 37.3 | 44.7 | 63.7  | 11.5 | 68.6  | 34.2 |
| Proline                 | 89.1 | 42.6 | 138.8 | 32.8 | 118.1 | 71.9 |
| Succinate               | 14.5 | 15.6 | 11.5  | 0    | 8.3   | 7.6  |
| Taurine                 |      |      |       |      |       |      |
| Tryptophan              | 39   | 37.3 | 34.2  | 33.4 | 13.5  | 6.6  |
| UDP-N-Acetylglucosamine | 12.3 | --   | 13.4  | --   | 58.6  | 76.9 |
| Uracil                  | 94.7 | 99   | 71.9  | 21.2 | 61.1  | 38.4 |
| Valine                  | 33.6 | --   | 23.9  | 20.9 | 28.9  | 15.2 |

A



B

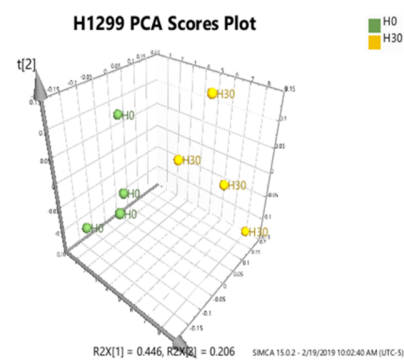


Figure S1: **Effects of  $\delta T$  on A549 (A ) and H1299 (B) on the metabolome of lung cancer cell lines.** 3 Dimensional Principal component analysis (PCA) scores plot( A and B), PC1 (t[1]) versus PC2 (t[2]) and numbers showing the unsupervised separation of metabolites profiles among the  $\delta T$  treatments 0(Green) and30 (yellow) after 72 hours incubation. Each symbol represents metabolites from the one Petri dish described at the method section; The ellipses shown in A and B represents the Hotelling’s T2 95% confidence interval for the multivariate data. Data is parito scaled.

## Reference

1. Wishart DS, Lewis MJ, Morrissey JA, Flegel MD, Jeroncic K, et al. (2008) The human cerebrospinal fluid metabolome. *J Chromatogr B* 871: 164-173.