

### Supplemental Figure 1: Growth phenotypes of complex I mutants.

Previously published complex I mutants were collected. Seeds were plated on 0.5X MS medium with 1% sucrose and stratified for 2 days. The plates were incubated under long day conditions in a growth chamber for 3 weeks. Mutants used: *rug3-1* (Kühn et al., 2011), *ndufa1*, *ndufs4* (Meyer et al., 2009), *39kDa* (Meyer et al., 2011), *otp43* (Falcon de Longevialle et al., 2007), *css1* (Nakagawa and Sakurai, 2006), *ca2* (Perales et al., 2005).

### References

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0% sucrose

1% sucrose

*ndufs4*



*ndufv1-1*



*ndufv1-2*



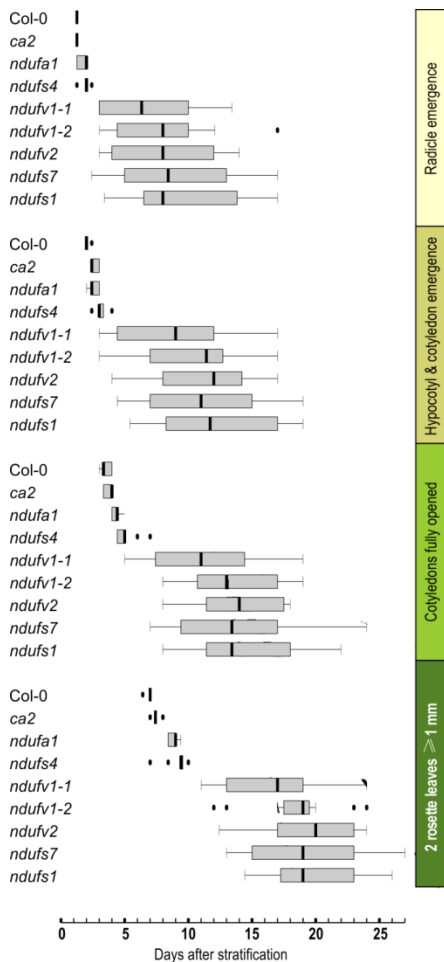
**Supplemental Figure 2: Growth phenotypes of complex I mutants in the absence or presence of sucrose.**

Seeds were surface sterilized and plated on 0.5X MS medium containing the sucrose concentrations indicated above the photos and stratified for 2 days. The plates were transferred to a growth chamber and incubated in a 12 h light/12 h dark regime. The photo was taken 3 weeks after sowing. Small yellow seedlings are aborted whereas green seedlings are established.

A

| mutant          | gene mutated     | accession    |
|-----------------|------------------|--------------|
| <i>ca2</i>      | <i>At1g47260</i> | SALK_010194  |
| <i>ndufa1</i>   | <i>At3g08610</i> | SAIL_150_H08 |
| <i>ndufs4</i>   | <i>At5g67590</i> | SAIL_596_E11 |
| <i>ndufv1-1</i> | <i>At5g08530</i> | SAIL_319_D07 |
| <i>ndufv1-2</i> | <i>At5g08530</i> | GK_062A01    |
| <i>ndufv2</i>   | <i>At4g02580</i> | SALK_150788  |
| <i>ndufs7</i>   | <i>At5g11770</i> | SALK_135972  |
| <i>ndufs1</i>   | <i>At5g37510</i> | emb1467      |

B



C

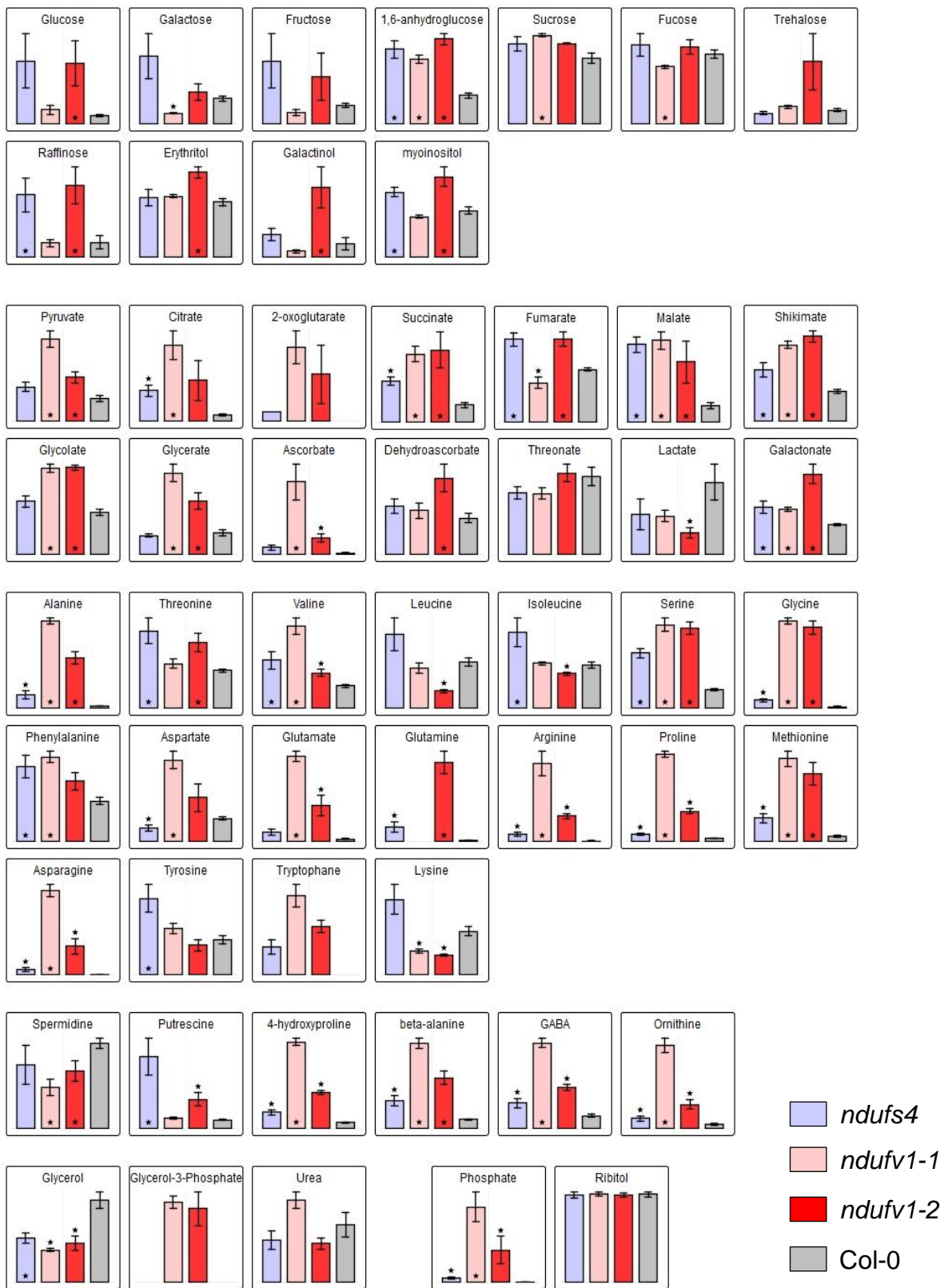


### Supplemental Figure 3: Analysis of the growth of severe complex I mutants.

A. Table of the mutants analyzed.

B. Box plot of the time required by several complex I mutants to reach the following growth stages: germination (radicle emergence), cotyledon emergence, cotyledons fully opened and seedling establishment (2 rosette leaves >1mm). Seeds were plated on 0.5X MS media containing 1% sucrose and stratified for 2 days.

C. Growth phenotype of several complex I mutants. Top panel, 8 week old plants grown on soil. Bottom panel: 2 week old seedlings grown on 0.5X MS media containing 1% sucrose.



#### Supplemental Figure 4: Metabolite profiles of complex I mutants.

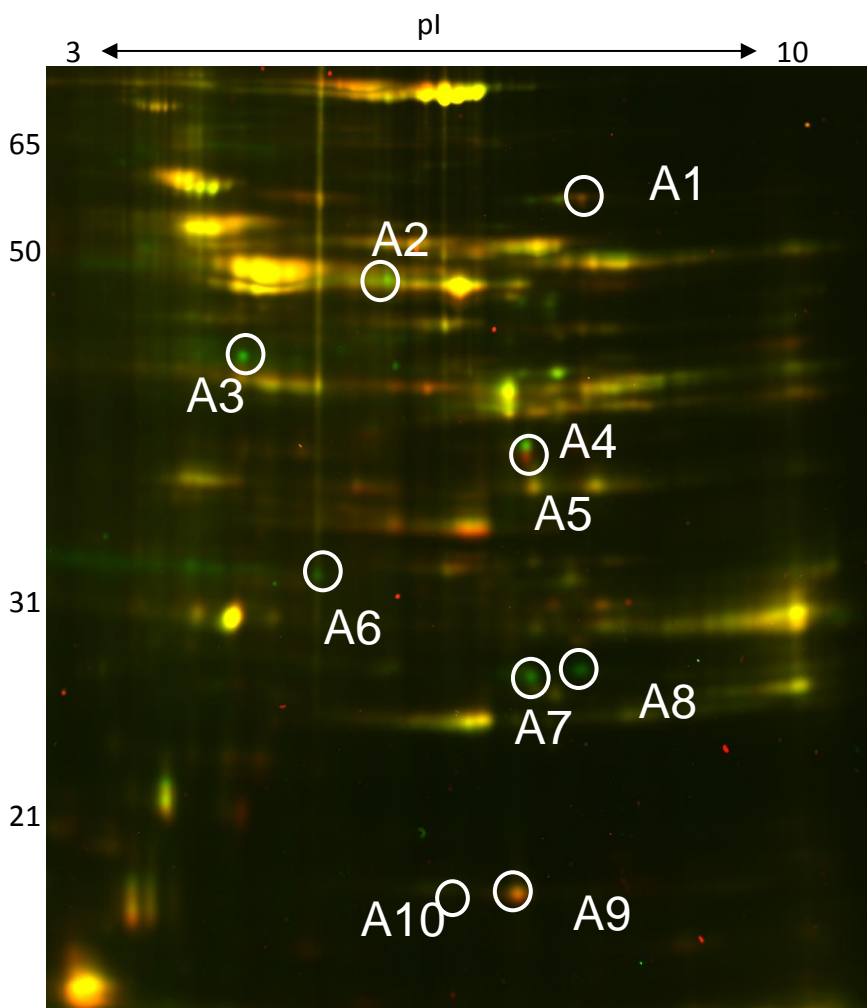
Rosette leaves of plants at the same physiological stage were harvested. Metabolites were extracted and analysed using GC-MS. For each metabolite, the bar of *Col-0* represents a normalised abundance of 1. Bars represent means  $\pm$  SE (n=6)

**Supplemental Figure 5: Comparison of the mitochondrial proteomes of *ndufv1-1* and *ndufs4* using DIGE.**

A. Representative gel with the proteins spots shown in false colors, *ndufv1-1* in red and *ndufs4* in green. Yellow indicates protein spots having the same intensity in both samples. The 10 spots that show a statistically significant differential abundance (more than 1.5 fold, n=3, p<0.05) are surrounded and labeled A1 to A10. The pI range is indicated on top of the gel and the molecular weight (in kD) marker in indicated on the left of the gel.

B. Table showing the results of the mass spectrometric analysis of the 10 spots, only identified proteins with a calculated pI and MW corresponding to the position of the spot are shown. Score : protein identification score given by MASCOT, pept : number of peptides identified, AGI : Arabidopsis Gene identifier. Proteins showing a higher abundance in *ndufv1-1* are highlighted in red, the other show a lower abundance in *ndufv1-1* compared to *ndufs4*.

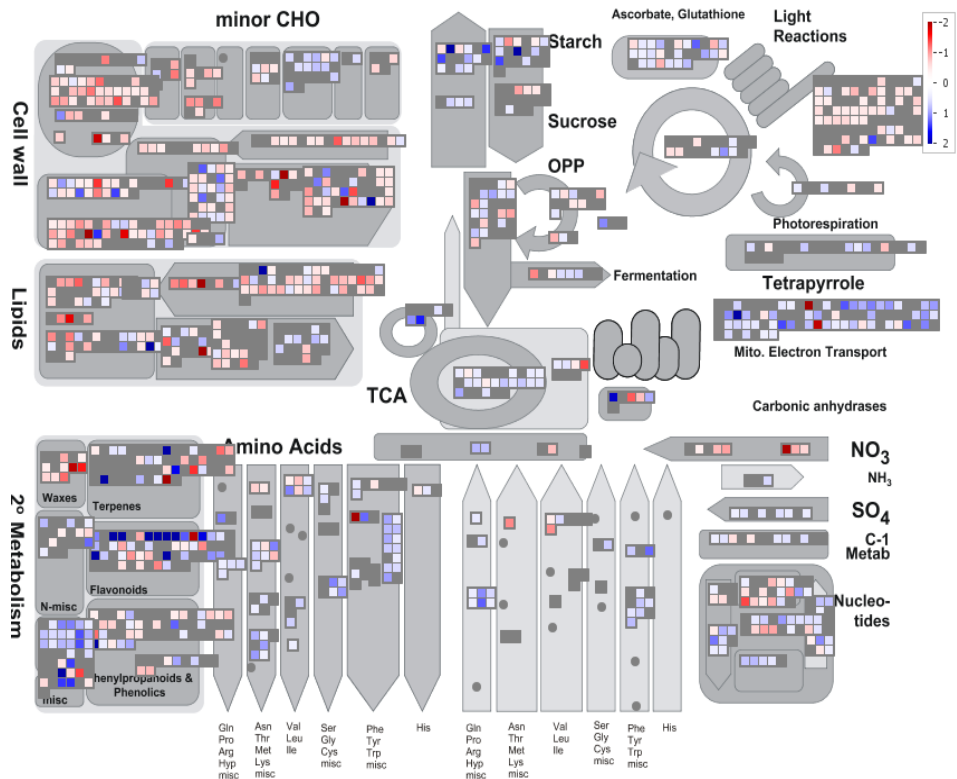
A



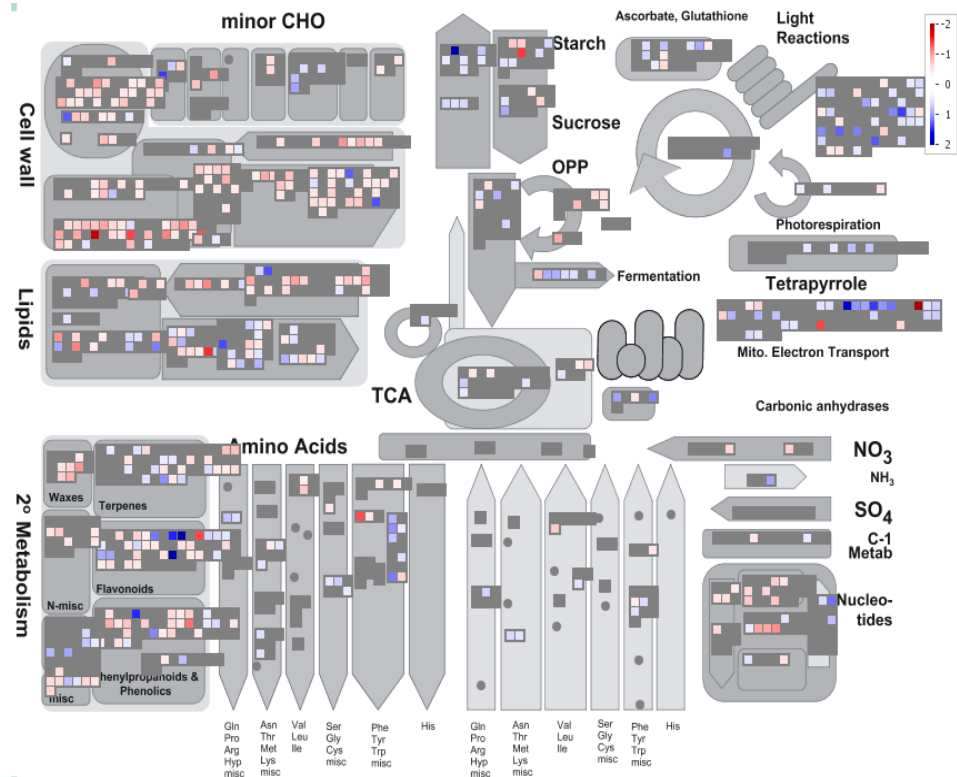
B

| spot | score | pept | AGI       | annotation                                   |
|------|-------|------|-----------|--|
| A1   | 137   | 10   | At3g47930 | L-galactono-1,4-lactone dehydrogenase (GLDH) |
| A2   | 2785  | 91   | At1g79440 | Aldehyde dehydrogenase                       |
|      | 901   | 45   | At3g48000 | Aldehyde dehydrogenase                       |
|      | 737   | 23   | At1g23310 | Glutamate:glyoxylate aminotransferase        |
|      | 661   | 19   | At1g70580 | Alanine-2-oxoglutarate aminotransferase      |
|      | A3    | 291  | 13        | At5g08270                                    |
| A4   | 2721  | 113  | At3g20000 | TOM40  |
| A5   | 701   | 31   | At1g13440 | Glyceraldehyde-3-phosphate dehydrogenase     |
| A6   | 162   | 6    | At1g18490 | Unknown protein                              |
| A7   | 1036  | 39   | At1g15390 | Peptide deformylase                          |
| A8   | 599   | 23   | At5g63510 | Carbonic anhydrase like                      |
| A9   | 1258  | 47   | At3g06050 | Peroxiredoxin IIF                            |
| A10  | 1077  | 43   | At3g06050 | Peroxiredoxin IIF                            |

A



B



### Supplemental Figure 6: Mapman metabolism maps

These maps represent the expression changes measured by our microarray analysis. Genes are grouped according to the Mapman categories. Only categories related to primary and secondary metabolism are shown. Each square represents one gene, only genes for which a significant expression value was measured (Holm-Bonferroni method,  $p\text{-val} < 0.05$ ) are represented. Genes down regulated are indicated in red and genes upregulated are indicated in blue, the darkest the color is, the strongest the change is, see scale on the top right of each panel. A. Map of *ndufv1-1*. B. Map of *ndufs4*.

Supplemental Table 1: Analysis of the gene categories showing differential response in *ndufv1-1* and *ndufs4*

|  | MapMan bin                           | direction | <i>ndufv1-1</i> |       | <i>ndufs4</i> |       |
|--|--------------------------------------|-----------|-----------------|-------|---------------|-------|
|  |                                      |           | proportion      | pval  | proportion    | pval  |
| PS   | lightreaction                        | down      | 0.50            | 0.033 | 0.24          | 0.739 |
| major CHO metabolism                         | raffinose family                     | down      | 0.55            | 0.007 | 0.45          | 0.849 |
| major CHO metabolism                         | myo-inositol                         | up        | 0.50            | 0.009 | 0.36          | 0.3   |
| OPP  | non-reductive PP                     | down      | 0.44            | 0.178 | 0.33          | 0.001 |
| mitochondrial electron transport / ATP synth | NADH-DH                              | up        | 0.60            | 0.001 | 0.29          | 0.544 |
| mitochondrial electron transport / ATP synth | cytochrome c reductase               | up        | 0.78            | 0.001 | 0.22          | 0.364 |
| cell wall                                    | pectin synthesis                     | down      | 0.57            | 0.001 | 0.29          | 0.839 |
| amino acid metabolism                        | amino acid synthesis                 | up        | 0.59            | 0.001 | 0.36          | 0.172 |
| metal handling                               | NA                                   | down      | 0.40            | 0.008 | 0.50          | 0.073 |
| metal handling                               | binding, chelation and storage       | down      | 0.38            | 0.263 | 0.45          | 0.008 |
| secondary metabolism                         | phenylpropanoids                     | down      | 0.43            | 0.055 | 0.51          | 0.003 |
| secondary metabolism                         | N misc                               | down      | 0.43            | 0.259 | 0.57          | 0.001 |
| secondary metabolism                         | sulfur-containing                    | up        | 0.56            | 0.001 | 0.35          | 0.239 |
| secondary metabolism                         | flavonoids                           | down      | 0.34            | 0.992 | 0.50          | 0.005 |
| hormone metabolism                           | abscisic acid                        | up        | 0.40            | 0.916 | 0.43          | 0.018 |
| hormone metabolism                           | cytokinin                            | down      | 0.42            | 0.884 | 0.47          | 0.001 |
| hormone metabolism                           | jasmonate                            | down      | 0.42            | 0.128 | 0.64          | 0.004 |
| stress                                       | biotic                               | up        | 0.36            | 0.591 | 0.43          | 0.006 |
| stress                                       | thioredoxin                          | down      | 0.45            | 0.006 | 0.34          | 0.055 |
| stress                                       | ascorbate and glutathione            | up        | 0.50            | 0.002 | 0.45          | 0.062 |
| nucleotide metabolism                        | synthesis                            | down      | 0.19            | 1     | 0.37          | 0.008 |
| nucleotide metabolism                        | synthesis                            | up        | 0.67            | 0.001 | 0.22          | 0.993 |
| nucleotide metabolism                        | phosphotransfer and pyrophosphatases | up        | 0.55            | 0.001 | 0.41          | 0.101 |
| misc   | cytochrome P450                      | down      | 0.41            | 0.399 | 0.45          | 0.036 |
| misc   | alcohol dehydrogenases               | up        | 0.33            | 0.272 | 0.56          | 0.007 |
| misc   | peroxidases                          | down      | 0.53            | 0.004 | 0.26          | 0.831 |
| misc   | acid and other phosphatases          | down      | 0.45            | 0.004 | 0.31          | 0.374 |
| misc   | myrosinases-lectin-jacalin           | down      | 0.46            | 0.194 | 0.35          | 0.014 |
| misc   | dynamain                             | up        | 0.33            | 0.543 | 0.50          | 0.026 |

|             |   |      |      |       |      |       |
|-------------|---|------|------|-------|------|-------|
| misc        | short chain dehydrogenase/reductase (SDR) | up   | 0.50 | 0.001 | 0.38 | 0.459 |
| misc        | GCN5-related N-acetyltransferase          | down | 0.38 | 0.579 | 0.44 | 0.008 |
| RNA         | regulation of transcription               | up   | 0.41 | 0.105 | 0.41 | 0.037 |
| DNA         | synthesis/chromatin structure             | down | 0.38 | 0.901 | 0.36 | 0.026 |
| protein     | synthesis                                 | up   | 0.62 | 0.001 | 0.27 | 0.857 |
| protein     | postranslational modification             | down | 0.39 | 0.033 | 0.32 | 0.998 |
| protein     | postranslational modification             | up   | 0.37 | 0.968 | 0.45 | 0.003 |
| protein     | degradation                               | down | 0.44 | 0.001 | 0.36 | 0.132 |
| signalling  | in sugar and nutrient physiology          | up   | 0.46 | 0.093 | 0.46 | 0.001 |
| signalling  | receptor kinases                          | down | 0.49 | 0.002 | 0.38 | 0.45  |
| signalling  | phosphoinositides                         | down | 0.43 | 0.018 | 0.24 | 0.985 |
| signalling  | phosphoinositides                         | up   | 0.24 | 0.983 | 0.40 | 0.016 |
| signalling  | MAP kinases                               | down | 0.46 | 0.003 | 0.40 | 0.445 |
| signalling  | light                                     | up   | 0.39 | 0.129 | 0.56 | 0.001 |
| development | late embryogenesis abundant               | up   | 0.59 | 0.006 | 0.36 | 0.537 |
| development | squamosa promoter binding like (SPL)      | up   | 0.38 | 0.824 | 0.54 | 0.033 |
| transport   | potassium                                 | down | 0.62 | 0.002 | 0.35 | 0.185 |
| transport   | unspecified anions                        | down | 0.55 | 0.022 | 0.36 | 0.246 |



**Supplemental Table 2.** Overview of the metabolite reporting list.

**GC-TOF-MS metabolites**

|    | A  | B             | C              | D              | E  | F                                      | G                | H  | I                          |
|----|--|---------------|----------------|----------------|--|--|------------------|--|----------------------------|
| 1  |  |               |                |                |  |  |                  |  |                            |
| 2  | <b>Experiment title:</b> Metabolite profiling of Arabidopsis complex I mutants                       |               |                |                |  |  |                  |  |                            |
| 3  | <b>Organism/Plant specie</b> <i>Arabidopsis thaliana</i>   |               |                |                |  |  |                  |  |                            |
| 4  | <b>Organ/tissue:</b> Leaf  |               |                |                |  |  |                  |  |                            |
| 5  | <b>Analytical tool:</b> GC-TOF-MS  |               |                |                |  |  |                  |  |                            |
| 6  |  |               |                |                |  |  |                  |  |                            |
| 7  | Peak/compound no.- number referenced back to the main text   |               |                |                |  |  |                  |  |                            |
| 8  | Ret. Time- Time expected, Tag Time Index and Time deviation  |               |                |                |  |  |                  |  |                            |
| 9  | Putative Name- putative identification of the metabolite/derivative                                  |               |                |                |  |  |                  |  |                            |
| 10 | Corresponding metabolite name in literature  |               |                |                |  |  |                  |  |                            |
| 11 | Mol. Formula- molecular formula of the metabolite or its FA adduct;                                  |               |                |                |  |  |                  |  |                            |
| 12 | Mass to charge ratio (m/z)   |               |                |                |  |  |                  |  |                            |
| 13 | (S)- identification confirmed by a standard compound   |               |                |                |  |  |                  |  |                            |
| 14 | I, II, III- different isomers  |               |                |                |  |  |                  |  |                            |
| 15 | Identification level (A; B; C; D)- (A) standard or NMR; (B) MS/MS; (C) MS <sup>E</sup> ; (D) MS only |               |                |                |  |  |                  |  |                            |
| 16 |  |               |                |                |  |  |                  |  |                            |
| 17 | Peak/Compound no.  | Time Expected | Tag Time Index | Time Deviation | Putative metabolite name (Derivative)                            | Corresponding Metabolite in Literature | Metabolite Class | Mol formula                                    | Mass to charge ratio (m/z) |
| 18 | 1  | 190250        | 190298         | -0.08          | M000100_A105001-101_CONT-METB_190250_TOF_Lactic acid, DL- (2TMS) | Lactate                                | Acid (Hydroxy)   | C <sub>3</sub> H <sub>6</sub> O <sub>3</sub>   | 117                        |
| 19 | 2  | 206867        | 206910         | 0.01           | M000517_A106002-101_CONT-MST_206867_TOF_Glycolic acid (2TMS)     | Glycolate                              | Acid (Hydroxy)   | C <sub>2</sub> H <sub>4</sub> O <sub>3</sub>   | 177                        |
| 20 | 3  | 208565        | 208986         | 0.29           | M000026_A110001-101_METB_208565_TOF_Alanine, DL- (2TMS)          | Alanine                                | Amino acids      | C <sub>3</sub> H <sub>7</sub> NO <sub>2</sub>  | 116                        |
| 21 | 4  | 222650        | 222654         | -0.01          | M000071_A104002-101_METB_222650_TOF_Pyruvic acid (1MEOX) (1TMS)  | Pyruvate                               | Acid (Oxo)       | C <sub>3</sub> H <sub>4</sub> O <sub>3</sub>   | 174                        |
| 22 | 5  | 271580        | 271581         | 0              | M000030_A122001-101_METB_271580_TOF_Valine, DL- (2TMS)           | Valine                                 | Amino acids      | C <sub>5</sub> H <sub>11</sub> NO <sub>2</sub> | 144                        |
| 23 | 6  | 291783        | 291650         | -0.05          | M000053_A129003-101_METB_291783_TOF_Glycerol (3TMS)              | Glycerol                               | Polyol (Triol)   | C <sub>3</sub> H <sub>8</sub> O <sub>3</sub>   | 103                        |
| 24 | 7  | 305020        | 304900         | -0.01          | M000025_A129002-101_METB_301020_TOF_Leucine, DL- (2TMS)          | Leucine                                | Acid (Amino)     | C <sub>6</sub> H <sub>13</sub> NO <sub>2</sub> | 158                        |
| 25 | 8  | 319193        | 318906         | -0.05          | M000017_A132002-101_METB_319193_TOF_Isoleucine, L- (2TMS)        | Isoleucine                             | Amino acids      | C <sub>6</sub> H <sub>13</sub> NO <sub>2</sub> | 158                        |
| 26 | 9  | 325180        | 325031         | 0.02           | M000031_A133001-101_METB_325180_TOF_Glycine (3TMS)               | Glycine                                | Acid (Amino)     | C <sub>2</sub> H <sub>5</sub> NO <sub>2</sub>  | 174                        |
| 27 | 10   | 327380        | 326918         | -0.12          | M000015_A128001-101_METB_327380_TOF_Serine, DL- (2TMS)           | Serine                                 | Acid (Amino)     | C <sub>3</sub> H <sub>7</sub> NO <sub>3</sub>  | 116                        |
| 28 | 11   | 333520        | 332458         | -0.29          | M000075_A129001-101_METB_333520_TOF_Phosphoric acid (3TMS)       | Phosphate                              | Inorganic acid   | H <sub>3</sub> O <sub>4</sub> P                | 299                        |
| 29 | 12   | 338693        | 338470         | -0.01          | M000029_A132003-101_METB_338693_TOF_Proline, L- (2TMS)           | Proline                                | Amino acids      | C <sub>5</sub> H <sub>9</sub> NO <sub>2</sub>  | 142                        |
| 30 | 13   | 340357        | 339685         | -0.21          | M000364_A127002-101_METB_340357_TOF_Urea (2TMS)                  | Urea                                   | Amide            | CH <sub>4</sub> N <sub>2</sub> O               | 189                        |
| 31 | 14   | 345050        | 344408         | -0.18          | M000073_A135003-101_METB_345050_TOF_Glyceric acid, DL- (3TMS)    | Glycerate                              | Acid (Hydroxy)   | C <sub>3</sub> H <sub>6</sub> O <sub>4</sub>   | 189                        |
| 32 | 15   | 365427        | 365088         | -0.09          | M000074_A134001-101_METB_365427_TOF_Succinic acid (2TMS)         | Succinate                              | Organic acids    | C <sub>4</sub> H <sub>6</sub> O <sub>4</sub>   | 247                        |

|    |    |         |         |       |   |                      |                     |   |     |
|----|----|---------|---------|-------|---|----------------------|---------------------|---|-----|
| 33 | 16 | 368365  | 367370  | -0.27 | M000016_A140001-101_METB_368365_TOF_Threonine, DL- (3TMS)                       | Threonine            | Amino acids         | C <sub>4</sub> H <sub>9</sub> NO <sub>3</sub>                 | 291 |
| 34 | 17 | 371255  | 371391  | 0.04  | M000067_A137001-101_METB_371255_TOF_Fumaric acid (2TMS)                         | Fumarate             | Organic acids       | C <sub>4</sub> H <sub>4</sub> O <sub>4</sub>                  | 245 |
| 35 | 18 | 394250  | 393926  | -0.08 | M000027_A144001-101_METB_394250_TOF_Alanine, beta- (3TMS)                       | beta-alanine         | Amino acids         | C <sub>3</sub> H <sub>7</sub> NO <sub>2</sub>                 | 174 |
| 36 | 19 | 408455  | 408460  | 0.01  | M000054_A150002-101_METB_408455_TOF_Erythritol (4TMS)                           | Erythritol           | Polyol (Tetraol)    | C <sub>4</sub> H <sub>10</sub> O <sub>4</sub>                 | 217 |
| 37 | 20 | 440995  | 440812  | -0.03 | M000065_A149001-101_METB_440995_TOF_Malic acid, DL- (3TMS)                      | Malate               | Organic acids       | C <sub>4</sub> H <sub>6</sub> O <sub>5</sub>                  | 245 |
| 38 | 21 | 448453  | 448236  | -0.02 | M000034_A153001-101_METB_448453_TOF_Proline, 4-hydroxy-, DL-, trans- (3TMS)     | 4-hydroxyproline     | Acid (Amino)        | C <sub>5</sub> H <sub>9</sub> NO <sub>3</sub>                 | 140 |
| 39 | 22 | 452200  | 452146  | 0.02  | M000114_A153003-101_METB_452200_TOF_Butyric acid, 4-amino- (3TMS)               | GABA                 | Amino acids         | C <sub>4</sub> H <sub>9</sub> NO <sub>2</sub>                 | 174 |
| 40 | 23 | 457283  | 457044  | -0.05 | M000033_A152002-101_METB_457283_TOF_Aspartic acid, L- (3TMS)                    | Aspartate            | Amino acids         | C <sub>4</sub> H <sub>7</sub> NO <sub>4</sub>                 | 232 |
| 41 | 24 | 458330  | 457912  | -0.13 | M000078_A156001-101_METB_458330_TOF_Threonic acid (4TMS)                        | Threonate            | Acid (Hydroxy)      | C <sub>4</sub> H <sub>8</sub> O <sub>5</sub>                  | 292 |
| 42 | 25 | 474325  | 474088  | -0.06 | M000018_A152001-101_METB_474325_TOF_Methionine, DL- (2TMS)                      | Methionine           | Acid (Amino)        | C <sub>5</sub> H <sub>11</sub> NO <sub>2</sub> S              | 128 |
| 43 | 26 | 507780  | 507893  | 0.01  | M000036_A163001-101_METB_507780_TOF_Glutamic acid, DL- (3TMS)                   | Glutamate            | Amino acids         | C <sub>9</sub> H <sub>9</sub> NO <sub>5</sub>                 | 348 |
| 44 | 27 | 517180  | 517401  | 0.05  | M000186_A175002-101_METB-METB_517180_TOF_Putrescine (4TMS)                      | Putrescine           | Polyamine           | C <sub>4</sub> H <sub>12</sub> N <sub>2</sub>                 | 214 |
| 45 | 28 | 522140  | 521998  | 0.03  | M000591_A173002-101_METB_522140_TOF_Fucose, DL- (1MEOX) (4TMS)                  | Fucose               | Sugar (Hexose, de   | C <sub>6</sub> H <sub>12</sub> O <sub>5</sub>                 | 117 |
| 46 | 29 | 523847  | 523650  | 0.03  | M000571_A158004-101_METB_523847_TOF_Glutaric acid, 2-oxo- (1MEOX) (2TMS)        | 2-oxoglutarate       | Acid (Oxo)          | C <sub>5</sub> H <sub>6</sub> O <sub>5</sub>                  | 198 |
| 47 | 30 | 531145  | 530862  | -0.13 | M000011_A164001-101_METB_531145_TOF_Phenylalanine, DL- (2TMS)                   | Phenylalanine        | Amino acids         | C <sub>9</sub> H <sub>11</sub> NO <sub>2</sub>                | 192 |
| 48 | 31 | 550100  | 549922  | -0.01 | M000013_A168001-101_METB_550100_TOF_Asparagine, DL- (3TMS)                      | Asparagine           | Amino acids         | C <sub>4</sub> H <sub>8</sub> N <sub>2</sub> O <sub>3</sub>   | 116 |
| 49 | 32 | 557320  | 556396  | -0.13 | M000248_A172001-101_METB_557320_TOF_Glucose, 1,6-anhydro, beta-D- (3TMS)        | 1,6-anhydroglucose   | Sugar (Hexose, al   | C <sub>6</sub> H <sub>10</sub> O <sub>5</sub>                 | 204 |
| 50 | 33 | 570427  | 570358  | -0.01 | M000028_A182002-101_METB-METB_570427_TOF_Ornithine, DL- (4TMS)                  | Ornithine            | Acid (Amino)        | C <sub>5</sub> H <sub>12</sub> N <sub>2</sub> O <sub>2</sub>  | 142 |
| 51 | 34 | 574230  | 573794  | -0.08 | M000328_A177002-101_METB_574230_TOF_Glycerol-3-phosphate, DL- (4TMS)            | Glycerol-3-Phosphate | Triol phosphate     | C <sub>3</sub> H <sub>9</sub> O <sub>6</sub> P                | 357 |
| 52 | 35 | 579380  | 578894  | -0.08 | M000606_A187002-101_METB_579380_TOF_Fructose, D- (1MEOX) (5TMS)                 | Fructose             | Sugar (Hexose)      | C <sub>6</sub> H <sub>12</sub> O <sub>6</sub>                 | 307 |
| 53 | 36 | 585405  | 585031  | -0.09 | M000607_A181002-101_METB_585405_TOF_Shikimic acid (4TMS)                        | Shikimate            | Acid (Hydroxy)      | C <sub>7</sub> H <sub>10</sub> O <sub>5</sub>                 | 204 |
| 54 | 37 | 587270  | 586972  | -0.05 | M000043_A191002-101_METB-METB_587270_TOF_Galactose, D- (1MEOX) (5TMS)           | Galactose            | Sugar (Hexose, al   | C <sub>6</sub> H <sub>12</sub> O <sub>6</sub>                 | 160 |
| 55 | 38 | 592883  | 592536  | -0.06 | M000069_A182004-101_METB-METB_592883_TOF_Citric acid (4TMS)                     | Citrate              | Organic acid        | C <sub>6</sub> H <sub>8</sub> O <sub>7</sub>                  | 273 |
| 56 | 39 | 598300  | 598120  | 0.05  | M000032_A178001-101_METB_598300_TOF_Glutamine, DL- (3TMS)                       | Glutamine            | Acid (Amino)        | C <sub>5</sub> H <sub>10</sub> N <sub>2</sub> O <sub>3</sub>  | 245 |
| 57 | 40 | 598880  | 598513  | -0.04 | M000040_A191001-101_METB-METB_598880_TOF_Glucose, D- (1MEOX) (5TMS)             | Glucose              | Sugar (Hexose, al   | C <sub>6</sub> H <sub>12</sub> O <sub>6</sub>                 | 160 |
| 58 | 41 | 605940  | 605763  | -0.02 | M000038_A183001-101_METB-METB_605940_TOF_Arginine, DL-, -NH <sub>3</sub> (3TMS) | Arginine             | Acid (Amino)        | C <sub>6</sub> H <sub>11</sub> N <sub>3</sub> O <sub>2</sub>  | 157 |
| 59 | 42 | 615467  | 615058  | -0.08 | M000014_A192003-101_METB_615467_TOF_Lysine, L- (4TMS)                           | Lysine               | Amino acids         | C <sub>6</sub> H <sub>14</sub> N <sub>2</sub> O <sub>2</sub>  | 174 |
| 60 | 43 | 624990  | 624668  | 0     | M000082_A185002-101_METB-METB_624990_TOF_Dehydroascorbic acid dimer             | Dehydroascorbate     | Hydroxy acid        | C <sub>6</sub> H <sub>6</sub> O <sub>6</sub>                  | 173 |
| 61 | 44 | 626890  | 626336  | -0.1  | M000596_A199002-101_METB_626890_TOF_Galactonic acid (6TMS)                      | Galactonate          | Acid (Hexonic)      | C <sub>6</sub> H <sub>12</sub> O <sub>7</sub>                 | 292 |
| 62 | 45 | 651990  | 650829  | -0.17 | M000001_A195002-101_METB_651990_TOF_Ascorbic acid, L(+)- (4TMS)                 | Ascorbate            | Acid (Hydroxy)      | C <sub>6</sub> H <sub>8</sub> O <sub>6</sub>                  | 117 |
| 63 | 46 | 653910  | 654002  | 0.02  | M000060_A209002-101_METB_653910_TOF_Inositol, myo- (6TMS)                       | myoinositol          | Polyol              | C <sub>6</sub> H <sub>14</sub> O <sub>6</sub>                 | 191 |
| 64 | 47 | 658337  | 658004  | -0.06 | M000035_A194002-101_METB_658337_TOF_Tyrosine, DL- (3TMS)                        | Tyrosine             | Amino acids         | C <sub>9</sub> H <sub>11</sub> NO <sub>3</sub>                | 218 |
| 65 | 48 | 723585  | 723554  | -0.03 | M000106_A226002-101_METB_723585_TOF_Spermidine (5TMS)                           | Spermidine           | Amine (Poly)        | C <sub>7</sub> H <sub>19</sub> N <sub>3</sub>                 | 144 |
| 66 | 49 | 790560  | 789999  | -0.08 | M000012_A223001-101_METB_790560_TOF_Tryptophan, L- (3TMS)                       | Tryptophane          | Amino acids         | C <sub>11</sub> H <sub>12</sub> N <sub>2</sub> O <sub>2</sub> | 202 |
| 67 | 50 | 840783  | 841026  | 0.04  | M000044_A264001-101_METB_840783_TOF_Sucrose, D- (8TMS)                          | Sucrose              | Sugar (disaccharid  | C <sub>12</sub> H <sub>22</sub> O <sub>11</sub>               | 451 |
| 68 | 51 | 876240  | 875263  | -0.11 | M000671_A274002-101_METB_876240_TOF_Trehalose, alpha, alpha'-, D- (8TMS)        | Trehalose            | Sugar (disaccharid  | C <sub>12</sub> H <sub>22</sub> O <sub>11</sub>               | 191 |
| 69 | 52 | 940280  | 938950  | -0.14 | M000673_A299002-101_METB_940280_EI/ROE_Galactinol (9TMS)                        | Galactinol           | Conjugate (Hexos    | C <sub>12</sub> H <sub>22</sub> O <sub>11</sub>               | 204 |
| 70 | 53 | 1033367 | 1032568 | -0.08 | M000049_A337002-101_METB_1033367_TOF_Raffinose (11TMS)                          | Raffinose            | Sugar (trisaccharid | C <sub>18</sub> H <sub>32</sub> O <sub>16</sub>               | 204 |

| Species detected before                             | Identification level (A-D) |
|---|----------------------------|
| Arabidopsis   | A                          |
| Arabidopsis   | A                          |
| Tomato, Arabidopsis, potato, <i>Lotus japonicus</i> | A                          |
| Tomato, Arabidopsis, potato, <i>Lotus japonicus</i> | A                          |
| Tomato, Arabidopsis, potato, <i>Lotus japonicus</i> | A                          |
| Tomato, Arabidopsis, potato, <i>Lotus japonicus</i> | A                          |
| Tomato, Arabidopsis, potato, <i>Lotus japonicus</i> | A                          |
| Tomato, Arabidopsis, potato, <i>Lotus japonicus</i> | A                          |
| Tomato, Arabidopsis, potato, <i>Lotus japonicus</i> | A                          |
| Tomato, Arabidopsis, potato, <i>Lotus japonicus</i> | A                          |
| Tomato, Arabidopsis, potato, <i>Lotus japonicus</i> | A                          |
| Tomato, Arabidopsis, potato, <i>Lotus japonicus</i> | A                          |
| Tomato, Arabidopsis                                 | A                          |
| Tomato, Arabidopsis, potato, <i>Lotus japonicus</i> | A                          |

| Time Expected | Tag_Time _Index | Time Deviation | Name Analyte          | Tag_Mass |
|---------------|-----------------|----------------|-----------------------|----------|
| 190250        | 190298          | -0.08          | M000100_ / Lactate    | 117      |
| 206867        | 206910          | 0.01           | M000517_ / Glycolate  | 177      |
| 208565        | 208986          | 0.29           | M000026_ / Alanine    | 116      |
| 222650        | 222654          | -0.01          | M000071_ / Pyruvate   | 174      |
| 271580        | 271581          | 0              | M000030_ / Valine     | 144      |
| 291783        | 291650          | -0.05          | M000053_ / Glycerol   | 103      |
| 305020        | 304900          | -0.01          | M000025_ / Leucine    | 158      |
| 319193        | 318906          | -0.05          | M000017_ / Isoleucine | 158      |
| 325180        | 325031          | 0.02           | M000031_ / Glycine    | 174      |
| 327380        | 326918          | -0.12          | M000015_ / Serine     | 116      |
| 333520        | 332458          | -0.29          | M000075_ / Phosphate  | 299      |
| 338693        | 338470          | -0.01          | M000029_ / Proline    | 142      |
| 340357        | 339685          | -0.21          | M000364_ / Urea       | 189      |
| 345050        | 344408          | -0.18          | M000073_ / Glycerate  | 189      |
| 365427        | 365088          | -0.09          | M000074_ / Succinate  | 247      |

|   |   |         |         |       |                               |     |
|---|---|---------|---------|-------|-------------------------------|-----|
| Tomato, Arabidopsis, potato                         | A | 368365  | 367370  | -0.27 | M000016_ / Threonine          | 291 |
| Tomato, Arabidopsis, potato, <i>Lotus japonicus</i> | A | 371255  | 371391  | 0.04  | M000067_ / Fumarate           | 245 |
| Tomato, Arabidopsis, potato, <i>Lotus japonicus</i> | A | 394250  | 393926  | -0.08 | M000027_ / beta-alanine       | 174 |
| Tomato, Arabidopsis, potato                         | A | 408455  | 408460  | 0.01  | M000054_ / Erythritol         | 217 |
| Tomato, Arabidopsis, potato, <i>Lotus japonicus</i> | A | 440995  | 440812  | -0.03 | M000065_ / Malate             | 245 |
| Tomato, Arabidopsis, potato                         | A | 448453  | 448236  | -0.02 | M000034_ / 4-hydroxyproline   | 140 |
| Tomato, Arabidopsis, potato, <i>Lotus japonicus</i> | A | 452200  | 452146  | 0.02  | M000114_ / GABA               | 174 |
| Tomato, Arabidopsis, potato                         | A | 457283  | 457044  | -0.05 | M000033_ / Aspartate          | 232 |
| Tomato, Arabidopsis, potato                         | A | 458330  | 457912  | -0.13 | M000078_ / Threonate          | 292 |
| Tomato, Arabidopsis, potato, <i>Lotus japonicus</i> | A | 474325  | 474088  | -0.06 | M000018_ / Methionine         | 128 |
| Tomato, Arabidopsis, potato, <i>Lotus japonicus</i> | A | 507780  | 507893  | 0.01  | M000036_ / Glutamate          | 348 |
| Tomato, Arabidopsis, potato, <i>Lotus japonicus</i> | A | 517180  | 517401  | 0.05  | M000186_ / Putrescine         | 214 |
| Tomato, Arabidopsis, potato                         | A | 522140  | 521998  | 0.03  | M000591_ / Fucose             | 117 |
| Tomato, Arabidopsis, potato                         | A | 523847  | 523650  | 0.03  | M000571_ / 2-oxoglutarate     | 198 |
| Tomato, Arabidopsis, potato, <i>Lotus japonicus</i> | A | 531145  | 530862  | -0.13 | M000011_ / Phenylalanine      | 192 |
| Tomato, Arabidopsis, potato                         | A | 550100  | 549922  | -0.01 | M000013_ / Asparagine         | 116 |
| Tomato, Arabidopsis, potato                         | A | 557320  | 556396  | -0.13 | M000248_ / 1,6-anhydroglucose | 204 |
| Arabidopsis   | A | 570427  | 570358  | -0.01 | M000028_ / Ornithine          | 142 |
| Tomato, Arabidopsis, potato                         | A | 574230  | 573794  | -0.08 | M000328_ / Glycerol-3-Phospha | 357 |
| Tomato, Arabidopsis, potato, <i>Lotus japonicus</i> | A | 579380  | 578894  | -0.08 | M000606_ / Fructose           | 307 |
| Tomato, Arabidopsis                                 | A | 585405  | 585031  | -0.09 | M000607_ / Shikimate          | 204 |
| Tomato, Arabidopsis, potato, <i>Lotus japonicus</i> | A | 587270  | 586972  | -0.05 | M000043_ / Galactose          | 160 |
| Tomato, Arabidopsis, potato, <i>Lotus japonicus</i> | A | 592883  | 592536  | -0.06 | M000069_ / Citrate            | 273 |
| Tomato, Arabidopsis, potato, <i>Lotus japonicus</i> | A | 598300  | 598120  | 0.05  | M000032_ / Glutamine          | 245 |
| Tomato, Arabidopsis, potato, <i>Lotus japonicus</i> | A | 598880  | 598513  | -0.04 | M000040_ / Glucose            | 160 |
| Tomato, Arabidopsis, potato                         | A | 605940  | 605763  | -0.02 | M000038_ / Arginine           | 157 |
| Tomato, Arabidopsis, potato                         | A | 615467  | 615058  | -0.08 | M000014_ / Lysine             | 174 |
| Tomato, Arabidopsis, potato                         | A | 624990  | 624668  | 0     | M000082_ / Dehydroascorbate   | 173 |
| Tomato, Arabidopsis, potato                         | A | 626890  | 626336  | -0.1  | M000596_ / Galactonate        | 292 |
| Tomato, Arabidopsis, potato                         | A | 651990  | 650829  | -0.17 | M000001_ / Ascorbate          | 117 |
| Tomato, Arabidopsis, potato                         | A | 653910  | 654002  | 0.02  | M000060_ / myoinositol        | 191 |
| Tomato, Arabidopsis, potato, <i>Lotus japonicus</i> | A | 658337  | 658004  | -0.06 | M000035_ / Tyrosine           | 218 |
| Arabidopsis   | A | 723585  | 723554  | -0.03 | M000106_ / Spermidine         | 144 |
| Tomato, Arabidopsis, potato                         | A | 790560  | 789999  | -0.08 | M000012_ / Tryptophane        | 202 |
| Tomato, Arabidopsis, potato, <i>Lotus japonicus</i> | A | 840783  | 841026  | 0.04  | M000044_ / Sucrose            | 451 |
| Tomato, Arabidopsis, potato                         | A | 876240  | 875263  | -0.11 | M000671_ / Trehalose          | 191 |
| Tomato, Arabidopsis, potato, <i>Lotus japonicus</i> | A | 940280  | 938950  | -0.14 | M000673_ / Galactinol         | 204 |
| Tomato, Arabidopsis, potato, <i>Lotus japonicus</i> | A | 1033367 | 1032568 | -0.08 | M000049_ / Raffinose          | 204 |

Supplemental table 3: list of primers used in this study

genotyping of *ndufv1-1*

LP        ACGGCCATCAGAACTTTAGG  
RP        GTTGGATCGTCTTACGGGAAC  
LB        TAGCATCTGAATTCATAACCAATCTCGATACAC

genotyping of *ndufv1-2*

LP        CGATCTGTTTGGTTACCTCCTG  
RP        CATGTGGTTCTGGTTATGATTTTG  
LB        ATATTGACCATCATACTCATTGC

genotyping of *ndufv2*

LP        TGTGATTCATTAAATCATTCTGCC  
RP        GAGACATCGAATCAGCTTTGC  
LB        TGGTTCACGTAGTGGGCCATCG

genotyping of *ndufS7*

LP        TCAACTGGTAAACAACGGGAG  
RP        TTTGATTGTGGTTGTATGACTCG  
LB        TGGTTCACGTAGTGGGCCATCG

genotyping of *ndufS1*

LP        TGAAAACCCAAGTTAAAGCTCTC  
RP        GCAAACACCTCGGGACTG  
LB        TAGCATCTGAATTCATAACCAATCTCGATACAC

## Supplemental Experimental procedures

### Metabolite profiling

GC-TOF-MS based metabolite profiling was performed basically as described by Lisec et al. (Lisec et al., 2006). Polar metabolites were extracted from 50 mg of frozen leaf material and 150  $\mu$ l of each extract was used for the analysis. TagFinder (Luedemann et al., 2012) was used for peak annotation and quantification with Golm Metabolome Database (<http://gmd.mpimp-golm.mpg.de>; (Kopka et al., 2005) as a reference library. The parameters used for the peak annotation are listed in Supplemental Table 2 according to (Fernie et al., 2011). The intensity of each fragment was normalized by that of ribitol which was added into the extraction solution as an internal standard.

### Differential Gel Electrophoresis (DIGE)

Mitochondrial proteins from *ndufv1-1* and *ndufs4* plants were precipitated overnight at -20°C in 100% (v/v) acetone. After centrifugation at 20,000g and 4°C for 20 min, pellets were resuspended in 10 mL of DIGE lysis buffer (8 M urea, 4% (w/v) CHAPS, and 40 mM Tris, pH 8.5) and centrifuged again at 12,000g and 4°C for 10 min in order to remove insoluble material. Fifty micrograms of proteins from each sample was labeled with a different Cy dye (Cy-3 or Cy-5) by addition of 400 pmol of dye (freshly prepared in dimethylformamide according to the manufacturer's instructions) on ice in the dark for 30 min. The reaction was stopped by addition of 10 mM lysine for 10 min on ice in the dark. An equal volume of DIGE lysis buffer with 22 mM DTT was added to each sample. Each of the labeled protein samples were mixed, and rehydration buffer (8 M urea, 4% (w/v), CHAPS, 0.5% (v/v), 3–10 nonlinear immobilized pH gradient buffer, 18 mM DTT, and 0.001% (w/v) bromophenol blue) was added to give a final volume of 450  $\mu$ L. The mix was loaded onto a 24-cm-long strip with immobilized nonlinear pH gradient of 3 to 10 (Immobiline DryStrip; GE Healthcare). Rehydration of the strips and the first IEF dimension electrophoresis were performed on an IPGphor unit (GE Healthcare) using the following settings: 12 h at 30 V (rehydration step), 1 h at 500 V, 1 h gradient from 500 V to 1,000 V, 1 h gradient from 1,000 V to 3,000 V, 2 h gradient from 3,000 V to 8,000 V, and 5 h at 8,000 V. After IEF, strips were incubated for 15 min in an equilibration buffer (6 M urea, 2% (w/v) SDS, 26% (v/v) glycerol, 65 mM DTT, 0.001% (w/v) bromophenol blue, and 50 mM Tris-HCl, pH 8.8) and then for 15 min in an equilibration buffer containing iodoacetamide (6 M urea, 2% (w/v) SDS, 26% (v/v) glycerol, 135 mM DTT, 0.001% (w/v) bromophenol blue, and 50mM Tris-HCl, pH8.8). The equilibrated strips were then loaded on top of a 12% (w/v) acrylamide gel. Following separation, gels were scanned using the Typhoon Trio Variable Mode Imager at a resolution of 100 (pixel size) with the photomultiplier tube set to 500 V. Proteins were processed (quantification) using the DeCyder 2-D Differential Analysis software version 6.5 (GE Healthcare). In order to get statistical significance from these experiments, three sets of proteins from three independent experiments were labeled and submitted to electrophoresis. Standard gels were also performed, and precipitation, IEF, and SDS-PAGE were run in parallel with labeled samples. These standard gels were loaded with a mix of 150 mg of each protein sample. After electrophoresis, proteins were visualized by colloidal Coomassie Brilliant Blue (G250) staining. The aim of the standard gel is to allow identification of proteins by excision gel spots followed by mass spectrometry.

## Identification of Proteins by Liquid Chromatography-Tandem Mass Spectrometry

Protein spots were excised from the gel and subjected to tryptic digestion following the standard protocol from (Shevchenko et al., 1996). Prior LC MS/MS analysis the samples were desalted with C<sub>18</sub> ZipTips (Millipore, Bedford, MA) following the manufacturer's instructions. The desalted samples were resuspended in 10 µl 2.5% acetonitrile and 0.5% TFA. The reversed phase separation of the peptides was performed on a nanoflow HPLC (Proxeon Biosystems, Odense, Denmark) using a Chromolith® CapRod® RP-18e 150-0.2 (Millipore, Darmstadt, Germany) column. A binary solvent system (solution A: 0.5% acetic acid, 5% isopropanol, solution B: 0.5% acetic acid, 5% isopropanol, 80% acetonitrile) was used for separation of the peptides in a 30 min linear gradient (95–40% A, followed by a final peptide elution step for 5 min at 20% A). The HPLC was coupled via a nano ESI ion source to a high resolution Orbitrap hybrid mass spectrometer (LTQ-Orbitrap, Thermo Fisher Scientific, San Jose, CA). The spectral acquisition for the full scan MS spectra was performed at a full-width half-maximum resolution of 30,000 in the Orbitrap section of the MS, while the data dependent MS/MS, with up to five spectra per preceding full scan, were obtained in the linear ion trap of the LTQ. Database search was performed by Mascot 2.4 (Matrix Science Ltd., London, UK) with the criteria as follows: *Arabidopsis* protein database (TAIR10 protein DB, version September 2012); proteolytic enzyme: trypsin allowing up to one missed cleavage; maximal precursor and fragment ion errors: 10 ppm and 0.8 Da. As modifications: fixed: Carbamidomethylation of cysteine; variable: oxidation of methionine. We set the threshold for identification to three different peptides and a score for the protein above 100.

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