Circulating Metabolites in Progression to Islet Autoimmunity and Type 1 Diabetes

Electronic supplementary material
ESM Methods

Two step derivatization:

In the first step 25 μl of methoxyamine hydrochloride (TS-45950; Thermo Scientific; USA) was added to the sample. While mixing, the solution was incubated for one hour at 45 °C. In the second step, 25μl of N-methyl-N-trimethylsilyl trifluoroacetamide (Sigma-Aldrich; Steinheim, Germany) was added. Incubation was again performed for one hour at 45 °C. Before injection 50 μl of hexane was added to increase the volatility of the solvent. Additional standards here added during derivatization. n-alkanes (c = 8 mg/l in MSTFA) were used for calculation of retention indexes and 4,4′-dibromoocctafluorobiphenyl (c = 9.8 mg/l in hexane) were used as syringe standard to control the quality of injection. 1 μl of derivatized sample was injected after derivatization program was completed.
ESM Figure 1. PCA score plot of the second principal component obtained from 405 plasma samples.
ESM Figure 2. ASCA PC1 loading plot for Fig 1. The loading explains the pattern seen in the score plot that provides the means to interpret the class specific metabolic alteration related to age. x-axis is the variable order and y-axis represents the metabolic pattern corresponding to the score plot. Here, colour of the loadings indicates the loading scores in PC1.
ESM Figure 3. PCA score plots and loadings based on ANOVA-simultaneous component analysis (ASCA). (a) PC1 score plot obtained based on the factor study cases score in ASCA analysis at age of 3 months. Here, each sample is represented by a point and coloured according to the case (red diamond: subjects who remained islet autoantibody negative during the follow-up age (CTR), green square: subjects who tested positive for at least one antibody in a minimum of two consecutive samples but did not progress to clinical T1D during the follow-up (P1Ab), blue triangle up: progressors to type-1-diabetes (PT1D). Samples with similar score are clustered together. (b) The corresponding PC1 loading plot. The loadings explain the pattern seen in the score plot which provides the means to interpret the metabolic alteration related to case. X-axis is the variable order and y-axis represents the metabolic pattern corresponding to the score plot. Here, colour of the loadings indicates the loading scores in PC1.
**ESM Figure 4.** Concentrations of 3-Indole acetic acid at 6 months of age.
**ESM Table 1.** Anthropometric characteristics of study population.

|                          | PT1D       | PIAb      | CTR       |
|--------------------------|------------|-----------|-----------|
| Sex (female, male)       | (14, 26)   | (14, 26)  | (14, 26)  |
| Age at time of diagnosis | 4.75 ± 2.94| NA        | NA        |
| Age at time of first seroconversion | 1.34 ± 0.58| 3.05 ± 2.50 | NA        |
| HLA information          |            |           |           |
| High risk (DR3 - DQA1*05 - DQB1*02) /DRB1*0401/2/4/5 - DQA1*03 - DQB1*0302) | 9         | 4         | 7         |
| Moderately increased risk* | 21        | 22        | 14        |
| Slightly increased risk* | 10         | 9         | 7         |
| Neutral*                 | 3          | 4         |           |
| Decreased risk and/or not possible to form haplotype* | 2         | 8         |

*other than DR3 - DQA1*05 - DQB1*02) /DRB1*0401/2/4/5 - DQA1*03 - DQB1*0302. This table has been adapted from [10]
**ESM Table 2.** Coefficient of variation across the analysis (415 samples).

| Metabolite                                | % Coefficient of variation (CV) |
|-------------------------------------------|---------------------------------|
| Nonadecanoic acid                         | 32.61                           |
| Diethylene glycol                         | 32.7                            |
| Myo-Inositolphosphate                     | 34.86                           |
| Fumaric acid                              | 37.55                           |
| 2-Phenylisopropanol                       | 39.87                           |
| Valine                                    | 41.32                           |
| p-Hydroxydiisopropylbenzene               | 41.49                           |
| L-5-Oxoproline                            | 43.44                           |
| α-D-(+)-Mannopyranose                     | 44.42                           |
| Cholesterol                               | 44.52                           |
| Serine                                    | 44.64                           |
| Aminomalonic acid                         | 45.53                           |
| Lactic acid                               | 45.69                           |
| Alanine                                   | 46.6                            |
| 2-Palmitoylglycerol                       | 46.89                           |
| Methylmaleic acid                         | 47.02                           |
| 3,4-Dihydroxybutanoic acid               | 47.68                           |
| Glycerol-2-phosphate                      | 47.95                           |
| Itaconic acid                             | 48.22                           |
| α-D-Glucopyranose                         | 48.31                           |
| Octanoic acid                             | 49.4                            |
| Threonine                                 | 49.93                           |
| Malic acid                                | 50.06                           |
| Stearic acid                              | 50.63                           |
| L-Threonic acid                           | 50.88                           |
| Palmitic acid                             | 51.35                           |
| Isoleucine                                | 52.58                           |
| 1-Dodecanol                               | 53.48                           |
| 4-Hydroxyphenyllactic acid                | 53.51                           |
| Linoleic acid                             | 53.82                           |
| 2-Hydroxybutyric acid                     | 53.89                           |
| Leucine                                   | 54.34                           |
| Arachidic acid                            | 54.44                           |
| Arachidonic acid                          | 54.51                           |
| L-Threonine                               | 54.52                           |
| 4-Hydroxybutanoic acid                    | 55.59                           |
| Pentadecanoic acid                        | 56.62                           |
| Stigmasterol                              | 57.5                            |
| Doconexent                                | 58.25                           |
| Creatinine                                | 59.25                           |
| 1-Monopalmitin                            | 59.73                           |
| Succinic acid                             | 59.97                           |
| Compound                                         | MW  |
|--------------------------------------------------|-----|
| Phenylalanine                                    | 60.71 |
| Proline                                          | 61.17 |
| 9-Hexadecenoic acid                             | 61.55 |
| Oleic acid                                       | 61.65 |
| (R<sup>*</sup>, R<sup>*</sup>)-2,3-Dihydroxybutanoic acid | 61.81 |
| 9-Tetradecenoic acid                            | 61.84 |
| Aconitic acid, (Z)-                              | 63.7 |
| Pentanoic acid, 2-oxy                            | 64.1 |
| L-Threonic acid                                  | 64.54 |
| L-Valine                                         | 64.73 |
| Decanoic acid                                    | 64.79 |
| D-Allofuranose                                   | 64.9 |
| Glycine                                          | 65.03 |
| Triethylene glycol                               | 65.28 |
| Pyroglutamic acid                                | 66.76 |
| 1-Octadecanol                                    | 67.35 |
| Heptanoic acid                                   | 69.93 |
| 2-Ketoisocaproic acid                            | 71.48 |
| D(-)-Lyxofuranose                                | 72.28 |
| Glycerol monostearate                            | 72.45 |
| 11-Eicosenoic acid, (E)-                         | 72.57 |
| L-Hydroxyproline, (E)-                           | 73.85 |
| L-Hydroxyproline, (E)-                           | 74.66 |
| 3-Indoleacetic acid                              | 74.9 |
| Tyrosine                                         | 75.16 |
| 2,4-Dihydroxybutanoic acid                      | 76.21 |
| 3,4,5-Trihydroxypentanoic acid                  | 77.85 |
| 2-Oleoylglycerol                                 | 80.69 |
| Glyceric acid                                    | 81.65 |
| alpha-Tocopherol                                 | 83.87 |
| L-(+)-Threose                                    | 84.3 |
| D-Arabinose                                      | 84.91 |
| 1,5-Anhydrohexitol                               | 85.65 |
| L-Tryptophan                                     | 89.45 |
| Dodecanoic acid                                  | 91.4 |
| Tryptophan                                       | 92.05 |
| 3-Hydroxyisovaleric acid                        | 94.33 |
| Glutamic acid                                    | 100.48 |
| 3-Hydroxybutyric acid                           | 114.25 |
| Pyruvic acid                                     | 114.32 |
| Methionine                                       | 116.18 |
| ?-Alanine                                        | 117.19 |
| D(-)-Tagatose                                    | 120.25 |
| Aspartic acid                                    | 124.85 |
| Azelaic acid                                     | 127.08 |
| Compound                        | MW    |
|--------------------------------|-------|
| Bisphenol A                    | 131.44|
| Levoglucosan                   | 133.58|
| Ribonic acid                   | 148.97|
| 1,5-Anhydrohexitol             | 171.48|
| Ethanolamine                   | 194.86|
| Glycolic acid                  | 225.34|
| 2-Hydroxy-3-methylbutyric acid| 245.9  |

Here, the median % CV (50th centile % CV) is 61.82 and the 90th centile % CV is 120.71.
ESM Table 3. Plasma metabolites different between CTR and PT1D at age 3 months.

| S:N | Metabolite               | Nominal p-values | Adjusted p-values | Log2F |
|-----|--------------------------|------------------|-------------------|-------|
| 1   | 11-Eicosenoic acid, (E)- | 0.0013           | 0.057             | -0.341|
| 2   | L-5-Oxoproline           | 0.0013           | 0.057             | -0.143|
| 3   | Stearic acid             | 0.0021           | 0.057             | -0.102|
| 4   | Palmitic acid            | 0.0024           | 0.057             | -0.084|
| 5   | Oleic acid               | 0.0032           | 0.0616            | -0.097|
| 6   | Arachidonic acid         | 0.0043           | 0.067             | -0.132|
| 7   | 1-Monopalmitin           | 0.0057           | 0.067             | -0.186|
| 8   | Linoleic acid            | 0.0057           | 0.067             | -0.095|
| 9   | Glutamic acid            | 0.0065           | 0.068             | -0.312|
| 10  | 3-Hydroxybutyric acid    | 0.023            | 0.22              | -0.171|
| 11  | Doconexent               | 0.033            | 0.26              | -0.19 |
| 12  | D-Arabinose              | 0.037            | 0.26              | -0.216|
| 13  | Ribonic acid             | 0.041            | 0.26              | -0.755|
| 14  | L-Threonic acid          | 0.041            | 0.26              | -0.162|
| 15  | Glyceric acid            | 0.041            | 0.26              | -0.438|
## ESM Table 4. Plasma metabolites different between CTR and PT1D at age 6 months.

| S:N | Metabolite                              | Nominal P-values | Adjusted-pvalues | Log2F |
|-----|-----------------------------------------|------------------|------------------|-------|
| 1   | Ribonic acid                            | 7.4E-05          | 0.0070           | -0.919|
| 2   | D-Arabinose                             | 0.00063          | 0.020            | -0.23 |
| 3   | L-Threonic acid                         | 0.00063          | 0.020            | -0.198|
| 4   | Methionine                              | 0.0015           | 0.036            | 2.049 |
| 5   | Pentadecanoic acid                      | 0.0019           | 0.036            | -0.177|
| 6   | Glyceric acid                           | 0.0025           | 0.036            | -0.402|
| 7   | L-5-Oxoproline                          | 0.0027           | 0.036            | -0.093|
| 8   | Succinic acid                           | 0.0040           | 0.045            | -0.187|
| 9   | 1,5-Anhydrohexitol                      | 0.00431          | 0.045            | -0.125|
| 10  | Glutamic acid                           | 0.0056           | 0.052            | -0.291|
| 11  | L-Hydroxyproline, (E)-                  | 0.0077           | 0.0655           | -0.221|
| 12  | 4-Hydroxyphenyllactic acid              | 0.0098           | 0.075            | -0.334|
| 13  | Aspartic acid                           | 0.0100           | 0.075            | -0.732|
| 14  | D-(-)-Lyxofuranose                      | 0.014            | 0.093            | -0.482|
| 15  | Tryptophan                              | 0.015            | 0.093            | -0.295|
| 16  | 3-Indoleacetic acid                     | 0.026            | 0.15             | -0.166|
| 17  | alpha-Tocopherol                        | 0.027            | 0.15             | 0.405 |
| 18  | Malic acid                              | 0.046            | 0.22             | -0.085|
| 19  | 9-Tetradecenoic acid                    | 0.048            | 0.21             | -0.223|
| 20  | Creatinine                              | 0.048            | 0.26             | 0.153 |
**ESM Table 5.** The detailed results of potential metabolic pathways that altered between CTRL and PT1D at age 3 months

| Metabolic pathway                               | Total Cpd | Hits | Nominal p | -log(p) | Holm adjust | FDR | Impact |
|------------------------------------------------|-----------|------|-----------|---------|-------------|-----|--------|
| Alanine, aspartate and glutamate metabolism    | 24        | 2    | 0.0007    | 7.1429  | 0.0166      | 0.002 | 0.441  |
| Aminoacyl-tRNA biosynthesis                     | 75        | 2    | 0.0007    | 7.14    | 0.016       | 0.002 | 0.112  |
| D-Glutamine and D-glutamate metabolism          | 11        | 1    | 0.001     | 6.44    | 0.025       | 0.002 | 0.112  |
| Tryptophan metabolism                           | 79        | 1    | 0.052     | 2.93    | 0.106       | 0.055 | 0.047  |
| Arginine and proline metabolism                 | 77        | 2    | 0.0007    | 7.14    | 0.016       | 0.002 | 0.035  |
| Glyoxylate and dicarboxylate metabolism         | 50        | 1    | 0.017     | 4.02    | 0.106       | 0.020 | 0.032  |
| Pentose phosphate pathway                       | 32        | 1    | 0.017     | 4.02    | 0.106       | 0.020 | 0.021  |
| Glycerolipid metabolism                         | 32        | 1    | 0.017     | 4.02    | 0.106       | 0.020 | 0.020  |
| Glutathione metabolism                          | 38        | 1    | 0.001     | 6.44    | 0.025       | 0.002 | 0.010  |
| Butanoate metabolism                            | 40        | 2    | 0.0004    | 7.67    | 0.0101      | 0.002 | 0.004  |
| Ubiquinone and other terpenoid-quinone biosynthesis | 36      | 1    | 0.065     | 2.73    | 0.106       | 0.065 | 0.0006 |
| Nitrogen metabolism                             | 39        | 2    | 0.0007    | 7.14    | 0.016       | 0.002 | 0.006  |
| Histidine metabolism                            | 44        | 2    | 0.0007    | 7.14    | 0.016       | 0.002 | 0.0005 |
| Glycine, serine and threonine metabolism        | 48        | 2    | 0.003     | 5.72    | 0.025       | 0.004 | 0.0004 |
| Porphyrin and chlorophyll metabolism            | 104       | 1    | 0.0015    | 6.44    | 0.025       | 0.002 | 0      |
| Cysteine and methionine metabolism              | 56        | 1    | 0.001     | 6.39    | 0.025       | 0.002 | 0      |
| Lysine biosynthesis                             | 32        | 1    | 0.001     | 6.39    | 0.025       | 0.002 | 0      |
| beta-Alanine metabolism                         | 28        | 1    | 0.001     | 6.39    | 0.025       | 0.002 | 0      |
| Cyanooamino acid metabolism                     | 16        | 1    | 0.001     | 6.39    | 0.025       | 0.002 | 0      |
| Nicotinate and nicotinamide metabolism          | 44        | 1    | 0.001     | 6.39    | 0.025       | 0.002 | 0      |
| Pantotenate and CoA biosynthesis                | 27        | 1    | 0.001     | 6.39    | 0.025       | 0.002 | 0      |
| Synthesis and degradation of ketone bodies      | 6         | 1    | 0.030     | 3.48    | 0.106       | 0.033 | 0      |
ESM Table 6. The detailed results of potential metabolic pathways that altered between CTRL and PT1D at age 6 months

| Metabolic pathway                                           | Total Cpd | Hits | Nominal p    | -log(p) | Holm adjust | FDR       | Impact  |
|-------------------------------------------------------------|-----------|------|--------------|---------|-------------|-----------|---------|
| Alanine, aspartate and glutamate metabolism                | 24        | 3    | 0.00086      | 7.06    | 0.019       | 0.00256   | 0.4416  |
| Tryptophan metabolism                                      | 79        | 2    | 0.0084       | 4.78    | 0.046       | 0.00871   | 0.15633 |
| Arginine and proline metabolism                            | 77        | 4    | 0.00016      | 8.74    | 0.004       | 0.001581  | 0.1325  |
| Aminoacyl-tRNA biosynthesis                                | 75        | 4    | 9.7E-05      | 9.24    | 0.002       | 0.001581  | 0.1168  |
| D-Glutamine and D-glutamate metabolism                    | 11        | 1    | 0.0011       | 6.85    | 0.021       | 0.00256   | 0.1123  |
| Cysteine and methionine metabolism                         | 56        | 2    | 0.0024       | 8.33    | 0.006256    | 0.001581  | 0.03806 |
| Ubiquinone and other terpenoid-quinone biosynthesis        | 36        | 2    | 0.0044       | 5.43    | 0.035311    | 0.005776  | 0.03749 |
| Glyoxylate and dicarboxylate metabolism                    | 50        | 2    | 0.0018       | 6.30    | 0.031202    | 0.00321   | 0.03291 |
| Pentose phosphate pathway                                  | 32        | 1    | 0.0022       | 6.12    | 0.035311    | 0.00321   | 0.02181 |
| Glycerolipid metabolism                                    | 32        | 1    | 0.0022       | 6.12    | 0.035311    | 0.00321   | 0.0206  |
| Butanoate metabolism                                       | 40        | 2    | 0.0010       | 6.87    | 0.021825    | 0.00256   | 0.01774 |
| Citrate cycle (TCA cycle)                                  | 20        | 1    | 0.0071       | 4.95    | 0.045828    | 0.00762   | 0.01446 |
| Glutathione metabolism                                     | 38        | 2    | 0.00382      | 7.87    | 0.00971     | 0.001581  | 0.01285 |
| Ascorbate and aldarate metabolism                          | 45        | 2    | 0.00018      | 8.61    | 0.004902    | 0.001581  | 0.0080  |
| Propanoate metabolism                                      | 35        | 1    | 0.0071       | 4.95    | 0.045828    | 0.00762   | 0.00334 |
| Nitrogen metabolism                                        | 39        | 3    | 0.00027      | 8.21    | 0.006815    | 0.001581  | 0.0006  |
| Histidine metabolism                                       | 44        | 2    | 0.0010       | 6.86    | 0.021825    | 0.00256   | 0.00051 |
| Glycine, serine and threonine metabolism                   | 48        | 3    | 0.00038      | 7.87    | 0.00971     | 0.001581  | 0.00047 |
| Amino sugar and nucleotide sugar metabolism                | 88        | 1    | 0.0065       | 5.03    | 0.045828    | 0.00762   | 7.0E-05 |
| Porphyrin and chlorophyll metabolism                       | 104       | 1    | 0.0011       | 6.85    | 0.021825    | 0.00256   | 0        |
| Lysine biosynthesis                                        | 32        | 1    | 0.0022       | 6.11    | 0.035311    | 0.00321   | 0        |
| beta-Alanine metabolism                                    | 28        | 1    | 0.0022       | 6.11    | 0.035311    | 0.00321   | 0        |
| Cyanoamino acid metabolism                                 | 16        | 1    | 0.0022       | 6.11    | 0.035311    | 0.00321   | 0        |
| Nicotinate and nicotinamide metabolism                     | 44        | 1    | 0.0022       | 6.11    | 0.035311    | 0.00321   | 0        |
| Pantothenate and CoA biosynthesis                          | 27        | 1    | 0.0022       | 6.11    | 0.035311    | 0.00321   | 0        |
| Tyrosine metabolism                                       | 76        | 2    | 0.0028       | 5.87    | 0.035311    | 0.00389   | 0        |
| Pentose and glucuronate interconversions                   | 53        | 1    | 0.0065       | 5.03    | 0.045828    | 0.00762   | 0        |
| Phenylalanine metabolism                                  | 45        | 1    | 0.0071       | 4.95    | 0.045828    | 0.00762   | 0        |
| Phenylalanine, tyrosine and tryptophan biosynthesis        | 27        | 1    | 0.011        | 4.47    | 0.045828    | 0.01493   | 0        |
**ESM Table 7.** Plasma metabolites different between B-P1Ab and A-P1Ab.

| S:N | Metabolite                                      | Nominal p-values | Adjusted-p-values |
|-----|------------------------------------------------|------------------|-------------------|
| 1   | Glutamic acid                                  | 0.00017          | 0.016             |
| 2   | Aspartic acid                                  | 0.0015           | 0.047             |
| 3   | Malic acid                                     | 0.0015           | 0.047             |
| 4   | 3,4-Dihydroxybutanoic acid                    | 0.0022           | 0.052             |
| 5   | Glyceric acid                                  | 0.010            | 0.19              |
| 6   | 3-Hydroxybutyric acid                          | 0.016            | 0.26              |
| 7   | (R*,R*)-2,3-Dihydroxybutanoic acid            | 0.024            | 0.29              |
| 8   | Ribonic acid                                   | 0.025            | 0.29              |
| 9   | D-Arabinose                                    | 0.040            | 0.38              |
| 10  | 3-Indoleacetic acid                            | 0.048            | 0.38              |
| 11  | alpha-Tocopherol                               | 0.049            | 0.38              |

Abbreviations: Before seroconversion in P1Ab (B-P1Ab), after seroconversion in P1Ab (A-P1Ab).
ESM Table 8. Plasma metabolites different between B-PT1D and A-PT1D.

| S:N | Metabolite                      | Nominal p-values | Adjusted p-values |
|-----|--------------------------------|------------------|-------------------|
| 1   | Malic acid                     | 0.0017           | 0.16              |
| 2   | Tyrosine                       | 0.0037           | 0.17              |
| 3   | Glycolic acid                  | 0.0074           | 0.19              |
| 4   | L-Threonine                    | 0.0080           | 0.19              |
| 5   | 3,4,5-Trihydroxypentanoic acid | 0.023            | 0.40              |
| 6   | Glutamic acid                  | 0.026            | 0.40              |
| 7   | 2-Phenylisopropanol            | 0.044            | 0.60              |

Abbreviations: Before seroconversion in progressors (B-PT1D), after seroconversion in progressors (A-PT1D).
**ESM Table 9.** The detailed results of potential metabolic pathways that altered between B-P1Ab and A-P1Ab.

| Metabolic pathway                                  | Total Cpd | Hits | Nominal p   | -log(p)  | Holm adjust | FDR   | Impact |
|---------------------------------------------------|-----------|------|--------------|----------|-------------|-------|--------|
| Alanine, aspartate and glutamate metabolism       | 24        | 2    | 0.00079      | 7.1429   | 0.0166      | 0.002455 | 0.4416 |
| Aminoacyl-tRNA biosynthesis                       | 75        | 2    | 0.00079      | 7.1429   | 0.0166      | 0.002455 | 0.11268|
| D-Glutamine and D-glutamate metabolism            | 11        | 1    | 0.001595     | 6.4409   | 0.025519    | 0.002455 | 0.1123 |
| Tryptophan metabolism                             | 79        | 1    | 0.052983     | 2.9378   | 0.10694     | 0.055506 | 0.0478 |
| Arginine and proline metabolism                   | 77        | 2    | 0.00079      | 7.1429   | 0.0166      | 0.002455 | 0.03582|
| Glyoxylate and dicarboxylate metabolism           | 50        | 1    | 0.017823     | 4.0273   | 0.10694     | 0.020637 | 0.03291|
| Pentose phosphate pathway                         | 32        | 1    | 0.017823     | 4.0273   | 0.10694     | 0.020637 | 0.02181|
| Glycerolipid metabolism                           | 32        | 1    | 0.017823     | 4.0273   | 0.10694     | 0.020637 | 0.0206 |
| Glutathione metabolism                            | 38        | 1    | 0.001595     | 6.4409   | 0.025519    | 0.002455 | 0.01095|
| Butanoate metabolism                              | 40        | 2    | 0.000462     | 7.6796   | 0.010168    | 0.002455 | 0.0048 |
| Ubiquinone and other terpenoid-quinone biosynthesis| 36        | 1    | 0.065137     | 2.7313   | 0.10694     | 0.065137 | 0.00069|
| Nitrogen metabolism                               | 39        | 2    | 0.00079      | 7.1429   | 0.0166      | 0.002455 | 0.00067|
| Histidine metabolism                              | 44        | 2    | 0.00079      | 7.1429   | 0.0166      | 0.002455 | 0.00051|
| Glycine, serine and threonine metabolism          | 48        | 2    | 0.003253     | 5.7281   | 0.025519    | 0.00447  | 0.00047|
| Porphyrin and chlorophyll metabolism              | 104       | 1    | 0.001595     | 6.4409   | 0.025519    | 0.002455 | 0       |
| Cysteine and methionine metabolism                | 56        | 1    | 0.001674     | 6.3928   | 0.025519    | 0.002455 | 0       |
| Lysine biosynthesis                               | 32        | 1    | 0.001674     | 6.3928   | 0.025519    | 0.002455 | 0       |
| beta-Alanine metabolism                           | 28        | 1    | 0.001674     | 6.3928   | 0.025519    | 0.002455 | 0       |
| Cyanoamino acid metabolism                        | 16        | 1    | 0.001674     | 6.3928   | 0.025519    | 0.002455 | 0       |
| Nicotinate and nicotinamide metabolism            | 44        | 1    | 0.001674     | 6.3928   | 0.025519    | 0.002455 | 0       |
| Pantothenate and CoA biosynthesis                 | 27        | 1    | 0.001674     | 6.3928   | 0.025519    | 0.002455 | 0       |
| Synthesis and degradation of ketone bodies        | 6         | 1    | 0.030629     | 3.4858   | 0.10694     | 0.033692 | 0       |
**ESM Table 10.** The detailed results of potential metabolic pathways that altered between B-PT1D and A-PT1D.

| Metabolic pathway                                      | Total Cpd | Hits | Nominal p  | -\(\log(p)\) | Holm adjust | FDR | Impact   |
|--------------------------------------------------------|-----------|------|------------|---------------|-------------|------|----------|
| Alanine, aspartate and glutamate metabolism            | 24        | 1    | 0.077339   | 2.5596        | 0.46403     | 0.077339 | 0.17664  |
| D-Glutamine and D-glutamate metabolism                 | 11        | 1    | 0.077339   | 2.5596        | 0.46403     | 0.077339 | 0.1123   |
| Glycine, serine and threonine metabolism               | 48        | 1    | 0.037713   | 3.2778        | 0.307       | 0.058283 | 0.09661  |
| Aminoacyl-tRNA biosynthesis                            | 75        | 3    | 0.00216    | 6.1376        | 0.026103    | 0.005246 | 0.05634  |
| Tyrosine metabolism                                   | 76        | 1    | 0.001631   | 6.4183        | 0.026103    | 0.004622 | 0.04724  |
| Arginine and proline metabolism                       | 77        | 1    | 0.077339   | 2.5596        | 0.46403     | 0.077339 | 0.03582  |
| Glutathione metabolism                                | 38        | 1    | 0.077339   | 2.5596        | 0.46403     | 0.077339 | 0.01095  |
| Phenylalanine, tyrosine and tryptophphan biosynthesis  | 27        | 1    | 0.001631   | 6.4183        | 0.026103    | 0.004622 | 0.00738  |
| Glyoxylate and dicarboxylate metabolism               | 50        | 1    | 0.01235    | 4.4887        | 0.1123      | 0.023874 | 0.00686  |
| Histidine metabolism                                  | 44        | 1    | 0.077339   | 2.5596        | 0.46403     | 0.077339 | 0.00051  |
| Nitrogen metabolism                                   | 39        | 2    | 0.001151   | 6.7676        | 0.09558     | 0.004622 | 0        |
| Ubiquinone and other terpenoid-quinone biosynthesis    | 36        | 1    | 0.001631   | 6.4183        | 0.026103    | 0.004622 | 0        |
| Phenylalanine metabolism                              | 45        | 1    | 0.001631   | 6.4183        | 0.026103    | 0.004622 | 0        |
| Thiamine metabolism                                   | 24        | 1    | 0.001631   | 6.4183        | 0.026103    | 0.004622 | 0        |
| Porphyrin and chlorophyll metabolism                  | 104       | 2    | 0.026077   | 3.6467        | 0.23469     | 0.049257 | 0        |
| Valine, leucine and isoleucine biosynthesis            | 27        | 1    | 0.037713   | 3.2778        | 0.307       | 0.058283 | 0        |
| Butanoate metabolism                                  | 40        | 1    | 0.077339   | 2.5596        | 0.46403     | 0.077339 | 0        |