

Electronic Supplementary Material Table 1. Baseline Characteristics in the Analytic Study Population and the Overall ARIC Study

|                                    | Analytic Study Population<br>(N=2,939) | ARIC Study Population<br>(N=15,792) |
|------------------------------------|--|-------------------------------------|
| Age, years                         | 53.3 (5.7)                             | 54.2 (5.8)                          |
| Female sex                         | 1,755 (59.7)                           | 8,710 (55.2)                        |
| African American                   | 1,665 (56.7)                           | 4,266 (27.0)                        |
| Study center                       |  |                                     |
| Forsyth County, North Carolina     | 485 (16.5)                             | 4,035 (25.6)                        |
| Jackson, Mississippi               | 1,566 (53.3)                           | 3,728 (23.6)                        |
| Minneapolis, Minnesota             | 455 (15.5)                             | 4,009 (25.4)                        |
| Washington County, Maryland        | 433 (14.7)                             | 4,020 (25.5)                        |
| Batch                              |  |                                     |
| Batch 1                            | 1,275 (43.4)                           | N/A                                 |
| Batch 2                            | 1,664 (56.6)                           | N/A                                 |
| Education, years                   |  |                                     |
| ≤11                                | 835 (28.4)                             | 3,767 (23.9)                        |
| 12-16                              | 1,029 (35.0)                           | 6,412 (40.6)                        |
| 17-21                              | 1,075 (36.6)                           | 5,586 (35.4)                        |
| Systolic blood pressure, mmHg      | 122.8 (19.8)                           | 121.4 (19.0)                        |
| Diastolic blood pressure, mmHg     | 76.3 (12.3)                            | 73.7 (11.3)                         |
| Body mass index, kg/m <sup>2</sup> | 28.2 (5.6)                             | 27.7 (5.4)                          |
| HDL cholesterol, mg/dL             | 54.4 (17.4)                            | 51.6 (17.1)                         |
| LDL cholesterol, mg/dL             | 136.8 (39.7)                           | 137.6 (39.4)                        |
| Physical activity index            | 2.3 (0.8)                              | 2.4 (0.8)                           |
| eGFR, mL/min/1.73 m <sup>2</sup>   | 106.4 (17.4)                           | 102.5 (15.8)                        |
| Current smoking                    | 779 (26.5)                             | 4,132 (26.2)                        |
| History of CVD                     | 269 (9.2)                              | 1,365 (8.6)                         |
| Fasting glucose, mmol/L            | 5.5 (0.6)                              | 6.0 (2.3)                           |

CVD, cardiovascular disease; eGFR, estimated glomerular filtration rate; HDL, high-density lipoprotein; LDL, low-density lipoprotein; N/A, not applicable

Data are expressed as means (SD) and *n* (%).

Electronic Supplementary Material Table 2. Heat Map of Hazard Ratios<sup>a</sup> for Incident Diabetes Per One Standard Deviation Increase in Log-Transformed Serum Metabolites

| Pathway and Sub-Pathway                              | Metabolites                   | Model 1      | Model 2      | Model 3     | Color Scale |
|--|-------------------------------|--------------|--------------|-------------|-------------|
| <b>Amino Acid</b>                                    |                               |              |              |             |             |
| Alanine and Aspartate Metabolism                     | N-acetylalanine               | <b>2.24</b>  | 1.44         | 1.59        | 0           |
| Alanine and Aspartate Metabolism                     | asparagine                    | <b>0.67</b>  | <b>0.75</b>  | <b>0.78</b> | 1           |
| Alanine and Aspartate Metabolism                     | alanine                       | <b>2.57</b>  | <b>1.84</b>  | 1.26        | 2           |
| Glutamate Metabolism                                 | glutamate                     | <b>2.25</b>  | <b>2.12</b>  | 1.86        | 3           |
| Glutathione Metabolism                               | 5-oxoproline                  | <b>0.63</b>  | <b>0.67</b>  | 0.76        | 4           |
| Glycine, Serine and Threonine Metabolism             | glycine                       | <b>0.44</b>  | <b>0.60</b>  | 0.73        | 5           |
| Glycine, Serine and Threonine Metabolism             | N-acetylthreonine             | <b>1.50</b>  | 1.19         | 1.10        | 6           |
| Leucine, Isoleucine and Valine Metabolism            | alpha-hydroxyisovalerate      | <b>1.28</b>  | <b>1.30</b>  | 1.17        | 7           |
| Leucine, Isoleucine and Valine Metabolism            | 2-methylbutyrylcarnitine (C5) | <b>1.49</b>  | 1.25         | 1.14        | 8           |
| Leucine, Isoleucine and Valine Metabolism            | leucine                       | <b>4.58</b>  | <b>3.12</b>  | <b>2.37</b> | 9           |
| Leucine, Isoleucine and Valine Metabolism            | isoleucine                    | <b>7.57</b>  | <b>4.42</b>  | <b>2.96</b> | 10          |
| Leucine, Isoleucine and Valine Metabolism            | 3-hydroxyisobutyrate          | <b>1.65</b>  | <b>1.53</b>  | 1.29        | 11          |
| Leucine, Isoleucine and Valine Metabolism            | valine                        | <b>6.49</b>  | <b>3.59</b>  | <b>2.41</b> | 12          |
| Leucine, Isoleucine and Valine Metabolism            | isovalerylcarnitine           | <b>1.50</b>  | 1.26         | 1.21        | 13          |
| Leucine, Isoleucine and Valine Metabolism            | isovalerate                   | <b>2.07</b>  | <b>2.05</b>  | 1.41        | 14          |
| Lysine Metabolism                                    | lysine                        | <b>2.20</b>  | 1.62         | 1.30        |             |
| Lysine Metabolism                                    | N6-acetyllysine               | <b>1.63</b>  | 1.21         | 1.23        |             |
| Methionine, Cysteine, SAM and Taurine Metabolism     | 2-hydroxybutyrate (AHB)       | <b>1.61</b>  | <b>1.57</b>  | 1.30        |             |
| Phenylalanine and Tyrosine Metabolism                | N-acetylphenylalanine         | <b>1.36</b>  | 1.20         | 1.04        |             |
| Phenylalanine and Tyrosine Metabolism                | phenyllactate (PLA)           | <b>1.57</b>  | <b>1.36</b>  | 1.23        |             |
| Phenylalanine and Tyrosine Metabolism                | phenylalanine                 | <b>3.71</b>  | <b>2.17</b>  | 1.69        |             |
| Phenylalanine and Tyrosine Metabolism                | tyrosine                      | <b>4.86</b>  | <b>2.65</b>  | 1.73        |             |
| Phenylalanine and Tyrosine Metabolism                | 3-(4-hydroxyphenyl)lactate    | <b>2.57</b>  | <b>2.00</b>  | <b>1.55</b> |             |
| Tryptophan Metabolism                                | tryptophan                    | <b>2.33</b>  | 1.98         | 1.40        |             |
| Tryptophan Metabolism                                | kynurenine                    | <b>2.09</b>  | 1.25         | 1.14        |             |
| Urea cycle; Arginine and Proline Metabolism          | ornithine                     | <b>1.43</b>  | 1.28         | 1.25        |             |
| Urea cycle; Arginine and Proline Metabolism          | pro-hydroxy-pro               | <b>0.66</b>  | 0.74         | 0.77        |             |
| Urea cycle; Arginine and Proline Metabolism          | proline                       | <b>2.10</b>  | 1.45         | 1.10        |             |
| <b>Carbohydrate</b>                                  |                               |              |              |             |             |
| Disaccharides and Oligosaccharides                   | trehalose                     | <b>1.31</b>  | <b>1.26</b>  | <b>1.16</b> |             |
| Fructose, Mannose and Galactose Metabolism           | mannose                       | <b>4.13</b>  | <b>2.53</b>  | 1.28        |             |
| Glycolysis, Gluconeogenesis, and Pyruvate Metabolism | glucose                       | <b>14.14</b> | <b>11.21</b> | 2.24        |             |
| Glycolysis, Gluconeogenesis, and Pyruvate Metabolism | lactate                       | <b>2.94</b>  | <b>2.23</b>  | 1.51        |             |
| <b>Cofactors and Vitamins</b>                        |                               |              |              |             |             |

|  |   |             |             |      |
|--|---|-------------|-------------|------|
| Nicotinate and Nicotinamide Metabolism               | N1-Methyl-2-pyridone-5-carboxamide          | <b>1.27</b> | 1.10        | 1.14 |
| Energy   |   |             |             |      |
| TCA Cycle  | succinate                                   | <b>2.73</b> | <b>2.11</b> | 1.46 |
| Lipid  |   |             |             |      |
| Carnitine Metabolism                                 | carnitine                                   | <b>2.63</b> | 1.63        | 1.15 |
| Fatty Acid Metabolism(Acyl Carnitine)                | hydroxybutyrylcarnitine                     | <b>1.21</b> | 1.13        | 1.09 |
| Long Chain Fatty Acid                                | myristate (14:0)                            | <b>1.46</b> | <b>1.50</b> | 1.36 |
| Long Chain Fatty Acid                                | palmitate (16:0)                            | <b>2.10</b> | <b>1.99</b> | 1.71 |
| Long Chain Fatty Acid                                | 10-heptadecenoate (17:1n7)                  | <b>1.46</b> | 1.36        | 1.24 |
| Long Chain Fatty Acid                                | palmitoleate (16:1n7)                       | <b>1.41</b> | 1.32        | 1.26 |
| Long Chain Fatty Acid                                | oleate (18:1n9)                             | <b>1.49</b> | <b>1.47</b> | 1.41 |
| Long Chain Fatty Acid                                | stearate (18:0)                             | <b>1.76</b> | <b>2.01</b> | 1.72 |
| Long Chain Fatty Acid                                | 10-nonadecenoate (19:1n9)                   | <b>1.38</b> | <b>1.36</b> | 1.26 |
| Lysolipid  | 1-arachidonoylglycerophosphoinositol        | <b>1.53</b> | <b>1.65</b> | 1.48 |
| Lysolipid  | 1-oleoylglycerophosphocholine (18:1)        | <b>0.64</b> | 0.78        | 0.81 |
| Monoacylglycerol                                     | 1-oleoylglycerol (1-monoolein)              | <b>1.60</b> | <b>1.29</b> | 1.21 |
| Phospholipid Metabolism                              | glycerophosphorylcholine (GPC)              | <b>0.69</b> | 0.87        | 0.93 |
| Phospholipid Metabolism                              | choline                                     | <b>0.43</b> | 0.58        | 0.62 |
| Polyunsaturated Fatty Acid (n3 and n6)               | dihomo-linolenate (20:3n3 or n6)            | <b>2.12</b> | <b>1.80</b> | 1.57 |
| Polyunsaturated Fatty Acid (n3 and n6)               | dihomo-linoleate (20:2n6)                   | <b>1.40</b> | <b>1.44</b> | 1.32 |
| Polyunsaturated Fatty Acid (n3 and n6)               | stearidonate (18:4n3)                       | <b>1.28</b> | <b>1.32</b> | 1.18 |
| Polyunsaturated Fatty Acid (n3 and n6)               | arachidonate (20:4n6)                       | <b>1.63</b> | <b>1.76</b> | 1.47 |
| Polyunsaturated Fatty Acid (n3 and n6)               | adrenate (22:4n6)                           | <b>1.59</b> | <b>1.51</b> | 1.33 |
| Polyunsaturated Fatty Acid (n3 and n6)               | eicosapentaenoate (EPA; 20:5n3)             | <b>1.31</b> | <b>1.36</b> | 1.17 |
| Secondary Bile Acid Metabolism                       | glycocholate sulfate                        | <b>1.46</b> | <b>1.30</b> | 1.22 |
| Steroid  | 4-androsten-3beta,17beta-diol disulfate (1) | <b>1.24</b> | <b>1.23</b> | 1.09 |
| Steroid  | 4-androsten-3beta,17beta-diol disulfate (2) | <b>1.31</b> | 1.20        | 1.05 |
| Nucleotide   |   |             |             |      |
| Purine Metabolism, (Hypo)Xanthine/Inosine containing | urate                                       | <b>2.79</b> | <b>1.78</b> | 1.42 |
| Pyrimidine Metabolism, Uracil containing             | N-acetyl-beta-alanine                       | <b>1.50</b> | 1.33        | 1.32 |
| Peptide  |   |             |             |      |
| Dipeptide  | phenylalanylleucine                         | <b>1.36</b> | 1.22        | 1.17 |
| Dipeptide  | phenylalanylphenylalanine                   | <b>1.43</b> | <b>1.34</b> | 1.19 |
| Dipeptide  | pyroglutamylglycine                         | <b>1.23</b> | <b>1.17</b> | 1.09 |
| Dipeptide  | leucylleucine                               | <b>1.28</b> | 1.18        | 1.06 |
| Dipeptide Derivative                                 | N-acetylcarnosine                           | <b>1.38</b> | 1.20        | 1.12 |
| Gamma-glutamyl Amino Acid                            | gamma-glutamylglutamate                     | <b>0.82</b> | <b>0.85</b> | 0.90 |
| Gamma-glutamyl Amino Acid                            | gamma-glutamylthreonine                     | <b>0.71</b> | <b>0.76</b> | 0.84 |
| Polypeptide  | HWESASLLR                                   | <b>1.12</b> | 1.08        | 1.07 |
| Xenobiotics  |   |             |             |      |

|                      |              |             |             |             |
|----------------------|--------------|-------------|-------------|-------------|
| Food Component/Plant | erythritol   | <b>2.41</b> | <b>1.93</b> | <b>1.66</b> |
| Food Component/Plant | gluconate    | <b>1.35</b> | <b>1.30</b> | 1.16        |
| Food Component/Plant | piperine     | <b>1.11</b> | 1.07        | 1.02        |
| Xanthine Metabolism  | caffeine     | <b>1.11</b> | 1.08        | 1.06        |
| Xanthine Metabolism  | theobromine  | <b>1.13</b> | <b>1.11</b> | 1.09        |
| Xanthine Metabolism  | theophylline | <b>1.11</b> | 1.09        | 1.06        |

<sup>a</sup> Hazard ratios are bolded if the association is statistically significant at the Bonferroni-corrected level ( $0.05/245=2.04 \times 10^{-4}$ ).

Model 1: Adjusted for age, sex, race-center, and batch

Model 2: Adjusted for covariates in Model 1 as well as education level, systolic blood pressure, diastolic blood pressure, body mass index, high density lipoprotein cholesterol, low density lipoprotein cholesterol, smoking status, physical activity level, history of cardiovascular disease, and estimated glomerular filtration rate

Model 3: Adjusted for covariates in Model 2 as well as fasting glucose

Electronic Supplementary Material Table 3. Serum Metabolites Significantly Associated with Incident Diabetes According to Metabolic Pathway and Stratified by Race

| Pathway and Sub-Pathway                   | Metabolites                | White                    |                       | Black                    |                       | <i>p</i> value for interaction |
|---|----------------------------|--------------------------|-----------------------|--------------------------|-----------------------|--------------------------------|
|   |                            | HR <sup>a</sup> (95% CI) | <i>p</i> value        | HR <sup>a</sup> (95% CI) | <i>p</i> value        |                                |
| <b>Amino Acid</b>                         |                            |                          |                       |                          |                       |                                |
| Leucine, Isoleucine and Valine Metabolism | isoleucine                 | 2.73 (1.48, 5.02)        | 1.26×10 <sup>-3</sup> | 2.93 (1.78, 4.83)        | 2.48×10 <sup>-5</sup> | 0.94                           |
| Alanine and Aspartate Metabolism          | asparagine                 | 0.69 (0.60, 0.80)        | 3.70×10 <sup>-7</sup> | 0.83 (0.73, 0.93)        | 1.26×10 <sup>-3</sup> | 0.05                           |
| Leucine, Isoleucine and Valine Metabolism | leucine                    | 2.81 (1.51, 5.22)        | 1.06×10 <sup>-3</sup> | 2.06 (1.28, 3.33)        | 3.06×10 <sup>-3</sup> | 0.49                           |
| Phenylalanine and Tyrosine Metabolism     | 3-(4-hydroxyphenyl)lactate | 1.69 (1.18, 2.41)        | 4.05×10 <sup>-3</sup> | 1.48 (1.13, 1.92)        | 4.08×10 <sup>-3</sup> | 0.90                           |
| Leucine, Isoleucine and Valine Metabolism | valine                     | 2.61 (1.30, 5.23)        | 7.03×10 <sup>-3</sup> | 2.12 (1.21, 3.72)        | 8.48×10 <sup>-3</sup> | 0.43                           |
| <b>Carbohydrate</b>                       |                            |                          |                       |                          |                       |                                |
| Disaccharides and Oligosaccharides        | trehalose                  | 1.13 (1.02, 1.25)        | 2.08×10 <sup>-2</sup> | 1.20 (1.10, 1.32)        | 1.23×10 <sup>-4</sup> | 0.24                           |
| <b>Xenobiotic</b>                         |                            |                          |                       |                          |                       |                                |
| Food Component/Plant                      | erythritol                 | 2.91 (1.72, 4.92)        | 6.89×10 <sup>-5</sup> | 1.42 (1.05, 1.93)        | 2.50×10 <sup>-2</sup> | 0.12                           |

CI, confidence interval; HR, hazard ratio

<sup>a</sup> Hazard ratio per one standard deviation increase in the log-transformed metabolite in multivariable regression models adjusted for age, sex, center, batch, education level, systolic blood pressure, diastolic blood pressure, body mass index, high density lipoprotein cholesterol, low density lipoprotein cholesterol, smoking status, physical activity level, history of cardiovascular disease, estimated glomerular filtration rate, and fasting glucose.

Electronic Supplementary Material Table 4. Pearson's Correlation Coefficients for Serum Metabolites Significantly Associated with Incident Diabetes

|                             | Isoleucine               | Asparagine               | Leucine                 | Trehalose               | 3-(4-hydroxyphenyl) lactate | Valine                  | Erythritol |
|-----------------------------|--------------------------|--------------------------|-------------------------|-------------------------|-----------------------------|-------------------------|------------|
| Isoleucine                  | 1                        |                          |                         |                         |                             |                         |            |
| Asparagine                  | -0.07<br><i>p</i> <0.001 | 1                        |                         |                         |                             |                         |            |
| Leucine                     | 0.86<br><i>p</i> <0.001  | 0.009<br><i>p</i> =0.64  | 1                       |                         |                             |                         |            |
| Trehalose                   | 0.11<br><i>p</i> <0.001  | -0.11<br><i>p</i> <0.001 | 0.11<br><i>p</i> <0.001 | 1                       |                             |                         |            |
| 3-(4-hydroxyphenyl) lactate | 0.50<br><i>p</i> <0.001  | -0.10<br><i>p</i> <0.001 | 0.45<br><i>p</i> <0.001 | 0.10<br><i>p</i> <0.001 | 1                           |                         |            |
| Valine                      | 0.83<br><i>p</i> <0.001  | -0.06<br><i>p</i> <0.001 | 0.86<br><i>p</i> <0.001 | 0.11<br><i>p</i> <0.001 | 0.42<br><i>p</i> <0.001     | 1                       |            |
| Erythritol                  | 0.28<br><i>p</i> <0.001  | -0.009<br><i>p</i> =0.64 | 0.24<br><i>p</i> <0.001 | 0.03<br><i>p</i> =0.06  | 0.31<br><i>p</i> <0.001     | 0.23<br><i>p</i> <0.001 | 1          |

| Color rules for abs(corr) | 0 | 0.2 | 0.4 | 0.6 | 0.8 | 1 |
|---------------------------|---|-----|-----|-----|-----|---|
|                           |   |     |     |     |     |   |