

Supplementary Table 3. Tests for detecting horizontal and directional pleiotropy in forward MR analysis.

| Exposure | MR-PRESSO global test | | MR-Egger intercept pEgger | | | Cochran's Q test | | |
|----------------|---------------------------|---------|---------------------------|-------------------|---------|------------------|-----------------|-------|
| | MR-PRESSO RSSobs | P value | Egger- intercept | Standard Error | P value | IVW (P) | MR-Egger (P) | |
| Gut microbiota | <i>Collinsella</i> | 11.512 | 0.409 | -0.044 | 0.035 | 0.252 | 0.341 | 0.391 |
| | <i>Oscillibacter</i> | 7.355 | 0.905 | -0.025 | 0.027 | 0.372 | 0.903 | 0.910 |
| | <i>Coprobacter</i> | 3.576 | 0.960 | -0.025 | 0.031 | 0.445 | 0.969 | 0.973 |
| | <i>Olsenella</i> | 13.639 | 0.315 | 0.003 | 0.029 | 0.921 | 0.261 | 0.190 |
| | <i>Lachnoclostridium</i> | 20.241 | 0.167 | -0.005 | 0.031 | 0.855 | 0.138 | 0.101 |
| | <i>Prevotella9</i> | 6.633 | 0.977 | -0.015 | 0.021 | 0.467 | 0.973 | 0.972 |
| | <i>Methanobrevibacter</i> | 6.006 | 0.568 | 0.074 | 0.043 | 0.162 | 0.950 | 0.901 |
| | <i>Ruminococcus2</i> | 17.376 | 0.363 | 0.009 | 0.017 | 0.597 | 0.363 | 0.314 |
| | Serine | 27.181 | 0.651 | 0.011 | 0.012 | 0.362 | 0.589 | 0.583 |
| | Pyruvate | 9.313 | 0.802 | 0.027 | 0.021 | 0.218 | 0.808 | 0.874 |
| Gut metabolite | Pipecolate | 7.775 | 0.802 | -0.001 | 0.023 | 0.962 | 0.807 | 0.731 |
| | Indoleacetate | 25.269 | 0.175 | -0.011 | 0.011 | 0.314 | 0.128 | 0.137 |
| | Glycodeoxycholate | 3.880 | 0.751 | 0.013 | 0.048 | 0.803 | 0.758 | 0.636 |
| | Adrenate | 3.852 | 0.957 | -0.001 | 0.021 | 0.963 | 0.954 | 0.919 |
| | Carnitine | 168.085 | 0.185 | -0.004 | 0.005 | 0.483 | 0.174 | 0.167 |
| | Phenylacetate | 5.637 | 0.803 | -0.005 | 0.017 | 0.776 | 0.869 | 0.798 |

Supplementary Table 4. Reverse MR analyses of GDM on gut microbiota by different methods.

| Outcome | Inverse variance weighted | | Maximum likelihood | | Weighted median | | MR.RAPS | | MR Egger | |
|---------------------------|------------------------------|-------|--------------------------|-------|--------------------------|-------|--------------------------|-------|--------------------------|-------|
| | β (95%CI) | P | β (95%CI) | P | β (95%CI) | P | β (95%CI) | P | β (95%CI) | P |
| <i>Collinsella</i> | -0.022 (-0.103,0.058) | 0.592 | -0.023 (-0.102,0.057) | 0.581 | 0.004 (-0.100,0.107) | 0.941 | -0.035 (-0.089,0.019) | 0.203 | -0.38 (-0.431,0.355) | 0.872 |
| <i>Oscillibacter</i> | -0.004 (-0.116,0.111) | 0.951 | -0.04 (-0.118,0.111) | 0.950 | -0.014 (-0.157,0.128) | 0.842 | 0.049 (-0.029,0.127) | 0.221 | 0.033 (-0.466,0.533) | 0.913 |
| <i>Coprobacter</i> | -0.037 (-0.107,0.033) | 0.302 | -0.038 (-0.109,0.033) | 0.290 | -0.023 (-0.109,0.063) | 0.600 | -0.009 (-0.058,0.039) | 0.700 | 0.153 (0.126,0.433) | 0.390 |
| <i>Olsenella</i> | 0.068 (-0.090,0.225) | 0.401 | 0.068 (-0.09,0.226) | 0.400 | 0.005 (-0.183,0.193) | 0.962 | 0.055 (-0.053,0.162) | 0.320 | 0.048 (-0.579,0.676) | 0.890 |
| <i>Lachnoclostridium</i> | 0.097 (-0.058,0.252) | 0.222 | 0.096 (-0.058,0.253) | 0.221 | 0.077 (-0.107,0.261) | 0.410 | 0.008 (-0.098,0.114) | 0.881 | 0.126 (-0.490,0.742) | 0.731 |
| <i>Prevotella9</i> | 0.032 (-0.076,0.139) | 0.564 | 0.032 (-0.069,0.133) | 0.532 | 0.016 (-0.104,0.135) | 0.798 | 0.002 (-0.066,0.070) | 0.964 | 0.260 (-0.150,0.670) | 0.340 |
| <i>Methanobrevibacter</i> | 0.006 (-0.119,0.131) | 0.920 | 0.007 (-0.087,0.100) | 0.893 | 0.020 (-0.102,0.142) | 0.753 | 0.027 (-0.036,0.090) | 0.403 | -0.086 (-0.684,0.511) | 0.800 |
| <i>Ruminococcus2</i> | 0.018 (-0.078,0.113) | 0.710 | 0.018 (-0.058,0.046) | 0.642 | -0.021 (-0.119,0.077) | 0.680 | -0.006 (-0.058,0.046) | 0.826 | 0.111 (-0.337,0.558) | 0.676 |

Supplementary Table 5. Tests for detecting horizontal and directional pleiotropy in reverse MR analysis.

| Exposure | MR-PRESSO global test | | MR-Egger intercept pEgger | | | Cochran's Q test | | |
|----------------|---------------------------|---------|---------------------------|-------------------|---------|------------------|--------------|-------|
| | MR-PRESSO RSSobs | P value | Egger-intercept | Standard error | P value | IVW (P) | MR-Egger (P) | |
| Gut microbiota | <i>Collinsella</i> | 7.280 | 0.377 | 0.003 | 0.037 | 0.943 | 0.366 | 0.206 |
| | <i>Oscillibacter</i> | 5.403 | 0.432 | -0.043 | 0.038 | 0.377 | 0.324 | 0.348 |
| | <i>Coprobacter</i> | 5.260 | 0.512 | -0.007 | 0.047 | 0.895 | 0.485 | 0.298 |
| | <i>Olsenella</i> | 0.794 | 0.935 | -0.005 | 0.058 | 0.934 | 0.932 | 0.807 |
| | <i>Lachnoclostridium</i> | 4.161 | 0.564 | -0.036 | 0.026 | 0.301 | 0.503 | 0.802 |
| | <i>Prevotella9</i> | 12.033 | 0.235 | 0.017 | 0.056 | 0.783 | 0.136 | 0.071 |
| | <i>Methanobrevibacter</i> | 2.462 | 0.730 | 0.003 | 0.059 | 0.956 | 0.736 | 0.531 |
| | <i>Ruminococcus2</i> | 9.898 | 0.266 | -0.017 | 0.042 | 0.715 | 0.189 | 0.112 |
| | Serine | 306.675 | 0.049 | 0.009 | 0.006 | 0.217 | 0.002 | 0.026 |
| Gut metabolite | Pyruvate | -29.025 | 0.001 | -0.022 | 0.005 | 0.028 | 0.001 | 0.714 |
| | Pipecolate | 1.836 | 0.882 | 0.004 | 0.006 | 0.548 | 0.873 | 0.856 |
| | Indoleacetate | 1.764 | 0.887 | 0.003 | 0.005 | 0.635 | 0.852 | 0.783 |
| | Glycodeoxycholate | 6.721 | 0.446 | -0.024 | 0.023 | 0.357 | 0.279 | 0.302 |
| | Adrenate | 15.901 | 0.172 | -0.001 | 0.009 | 0.973 | 0.034 | 0.015 |
| | Carnitine | 37.744 | 0.076 | -0.005 | 0.002 | 0.115 | 0.061 | 0.073 |
| | Phenylacetate | 11.931 | 0.257 | 0.011 | 0.007 | 0.208 | 0.137 | 0.287 |

Supplementary Table 6. Reverse MR analyses of GDM on gut metabolites by different methods.

| Outcome | Inverse variance weighted | | Maximum likelihood | | Weighted median | | MR.RAPS | | MR Egger | |
|-------------------|---------------------------|-------|---------------------------|-------|---------------------------|-------|----------------------------|-------|--------------------------|-------|
| | β (95%CI) | P | β (95%CI) | P | β (95%CI) | P | β (95%CI) | P | β (95%CI) | P |
| Serine | 0.004 (-0.019,0.027) | 0.711 | 0.005 (-0.007,0.016) | 0.438 | 0.001 (-0.012,0.013) | 0.934 | 0.005 (-0.007,0.016) | 0.430 | -0.034 (-0.085,0.018) | 0.290 |
| Pyruvate | -0.016 (-0.058,0.027) | 0.470 | -0.017 (-0.037,0.004) | 0.120 | -0.002 (-0.026,0.022) | 0.880 | -0.017 (-0.036,0.003) | 0.107 | 0.080 (0.029,0.132) | 0.054 |
| Pipecolate | 0.003 (-0.017,0.023) | 0.790 | 0.003 (-0.017,0.023) | 0.790 | -0.001 (-0.023,0.022) | 0.960 | 0.003 (-0.017,0.023) | 0.790 | -0.014 (-0.066,0.038) | 0.640 |
| Indoleacetate | -0.002 (-0.020,0.016) | 0.810 | -0.002 (-0.020,0.016) | 0.810 | -0.001 (-0.021,0.019) | 0.930 | -0.002 (-0.020,0.016) | 0.820 | -0.014 (-0.061,0.033) | 0.610 |
| Glycodeoxycholate | -0.058 (-0.138,0.023) | 0.160 | -0.058 (-0.130,0.014) | 0.113 | -0.052 (-0.132,0.027) | 0.200 | -0.058 (-0.131,0.015) | 0.117 | 0.042 (-0.155,0.240) | 0.703 |
| Adrenate | -0.019 (-0.048,0.009) | 0.177 | -0.020 (-0.038,-0.002) | 0.029 | -0.023 (-0.044,-0.003) | 0.026 | -0.020 (-0.042,0.001) | 0.057 | -0.018 (-0.104,0.068) | 0.708 |
| Carnitine | -0.006 (-0.018,0.007) | 0.390 | -0.006 (-0.012,0.000) | 0.059 | -0.003 (-0.011,0.005) | 0.495 | -0.006 (-60.766,90.754) | 0.053 | 0.016 (-0.005,0.037) | 0.236 |
| Phenylacetate | -0.015 (-0.043,0.013) | 0.300 | -0.015 (-0.036,0.006) | 0.170 | -0.020 (-0.048,0.007) | 0.150 | -0.015 (-0.037,0.007) | 0.170 | -0.063 (-0.126,0.001) | 0.150 |

Supplementary Table 7. Metabolic pathway associated with GDM.

| Metabolic pathway | Involved metabolites | <i>P</i> value | Database |
|---|-----------------------------|-----------------------|------------------|
| Phenylalanine metabolism | Phenylacetic acid | 0.026 | KEGG SMP |
| Citrate cycle (TCA cycle) | Pyruvate | 0.041 | KEGG SMP |
| Pyruvate metabolism | Pyruvate | 0.056 | KEGG SMP |
| Lysine degradation | L-Pipecolate | 0.063 | KEGG SMP |
| Glycolysis / Gluconeogenesis | Pyruvate | 0.065 | KEGG SMP SMP |
| Alanine, aspartate and glutamate metabolism | Pyruvate | 0.070 | KEGG SMP SMP SMP |
| Glyoxylate and dicarboxylate metabolism | Pyruvate | 0.080 | KEGG |
| Glycine, serine and threonine metabolism | Pyruvate | 0.083 | KEGG SMP |
| Cysteine and methionine metabolism | Pyruvate | 0.083 | KEGG SMP SMP |
| Arginine and proline metabolism | Pyruvate | 0.095 | KEGG SMP |
| Tryptophan metabolism | Indole-3-acetate | 0.102 | KEGG SMP |
| Tyrosine metabolism | Pyruvate | 0.104 | KEGG SMP SMP |

Supplementary Table 8. Detailed information for genome-wide association studies involved in the present Mendelian randomization study.

| Variable | Consortium or study | Sample size | Journal | Year | Cohort | Nation of cohort | Number of samples |
|-------------------------|---------------------|-------------|------------|------|---------------|------------------|--------------------------------|
| | | | | | BSPSPC | Germany | 721 |
| | | | | | CARDIAw | USA | 257 |
| | | | | | COPSAC | Denmark | 380 |
| | | | | | DanFunD16 | Denmark | 2,396 |
| | | | | | FGFP | Belgian | 2,259 |
| | | | | | FOCUS | Germany | 960 |
| | | | | | GEM_HCE_v12 | Canada | 378 |
| | | | | | GEM_HCE_v24 | Canada | 203 |
| | | | | | GEM_ICHIP_HCE | Canada | 662 |
| | | | | | GenR | The Netherlands | 1,328 |
| | | | | | HCHS/SOL | USA | 1,097 |
| Gut microbiota | MiBioGen | 18,340 | Nat Genet. | 2021 | KSCS | South Korea | 811 |
| | | | | | LLD | The Netherlands | 875 |
| | | | | | METSIM | Finland | 522 |
| | | | | | MIBS | The Netherlands | 80 |
| | | | | | NGRC | USA | 77 |
| | | | | | NTR | The Netherlands | 279 |
| | | | | | PNP | Israel | 481 |
| | | | | | POPCOL | Sweden | 134 |
| | | | | | RS3 | The Netherlands | 1,220 |
| | | | | | SHIP | Germany | 996 |
| | | | | | SHIP-TREND | Germany | 905 |
| | | | | | TwinsUK | UK | 1,205 |
| Gut metabolites | TwinsUK, KORA F4 | 7,824 | Nat Genet. | 2017 | KORA F4 | Germany | 1,768 |
| | | | | | TwinsUK | UK | 6,056 |
| BMI | UK Biobank | 461,460 | | 2018 | UK Biobank | European | 461,460 |
| alcohol drinking | UK Biobank | 462,346 | | 2018 | UK Biobank | European | 462,346 |
| smoking | GSCAN | 607,291 | | 2019 | GSCAN | European | 311,629 cases/321,173 controls |
| hypertension | UK Biobank | 462,346 | | 2018 | UK Biobank | European | 462,346 |
| GDM | FinnGen | 123,579 | | 2021 | FinnGen | European | 5,687 cases/117,892 controls |