**Cell Metabolism, Volume *29***

## Supplemental Information

# Profound Perturbation of the Metabolome in Obesity Is Associated with Health Risk

**Elizabeth T. Cirulli, Lining Guo, Christine Leon Swisher, Naisha Shah, Lei Huang, Lori A. Napier, Ewen F. Kirkness, Tim D. Spector, C. Thomas Caskey, Bernard Thorens, J. Craig Venter, and Amalio Telenti**

# Profound perturbation of the metabolome in obesity associates with health risk

Elizabeth T. Cirulli[1*], Lining Guo[2], Christine Leon Swisher[1], Naisha Shah[1], Lei Huang[1], Lori A. Napier[1], Ewen F. Kirkness[1], Tim D. Spector[3], C. Thomas Caskey[4], Bernard Thorens[5], J. Craig Venter[6], and Amalio Telenti[7*]

## Supplementary Figures

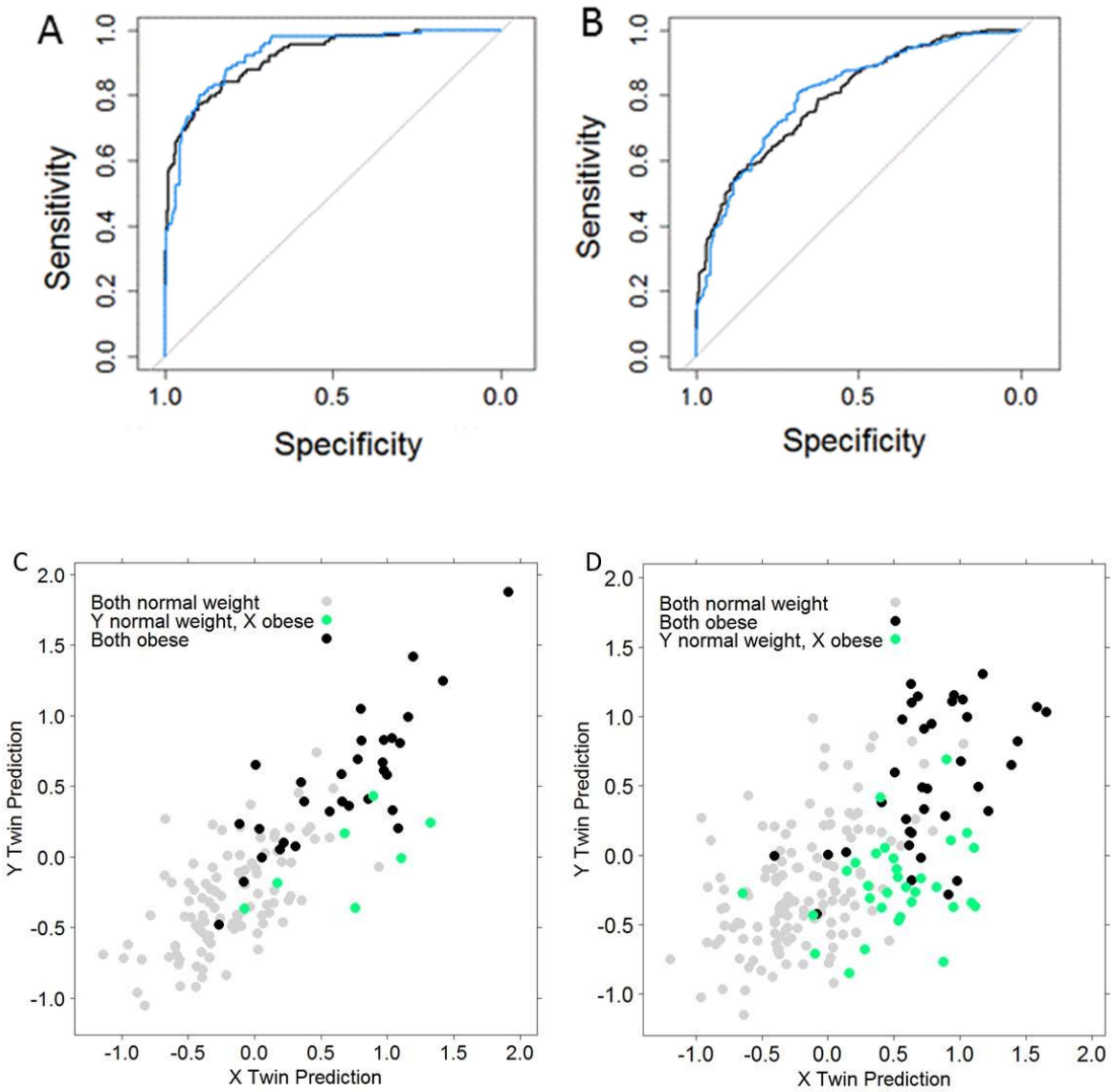


**Figure S1. Obesity prediction, Related to Figure 2.** Shown is the receiver operating characteristic (ROC) curve for the BMI prediction model to distinguish A) obese (BMI>=30) from normal weight (BMI 18.5-25) and B) overweight or obese (BMI>=25) from normal weight (BMI 18.5-25). The train (black) AUC were 0.918 (A) and 0.795 (B), and the test (blue) AUC were 0.926 (A) and 0.804 (B). The test specificities were 89.7% (A) and 68.7% (B), with 80.2% (A) and 80.7% (B) sensitivity. C) and D) show the obesity prediction and actual obesity status for 350 sets of twins. Shown is the BMI model prediction for each individual plotted against their twin's prediction. The heavier twin is always on the X axis, and twins are color-coded to indicate their actual BMI status. C) shows the 144 monozygotic twins ($r^2$=0.64), and D) shows the 206 dizygotic twins ($r^2$=0.33). When both twins were obese, they both generally had high BMI model predictions, and when both twins were normal weight, they both generally had low BMI predictions. When only one twin was obese (green, X axis) and the other was normal weight (green, Y axis), the obese twin almost always had the higher BMI

prediction.

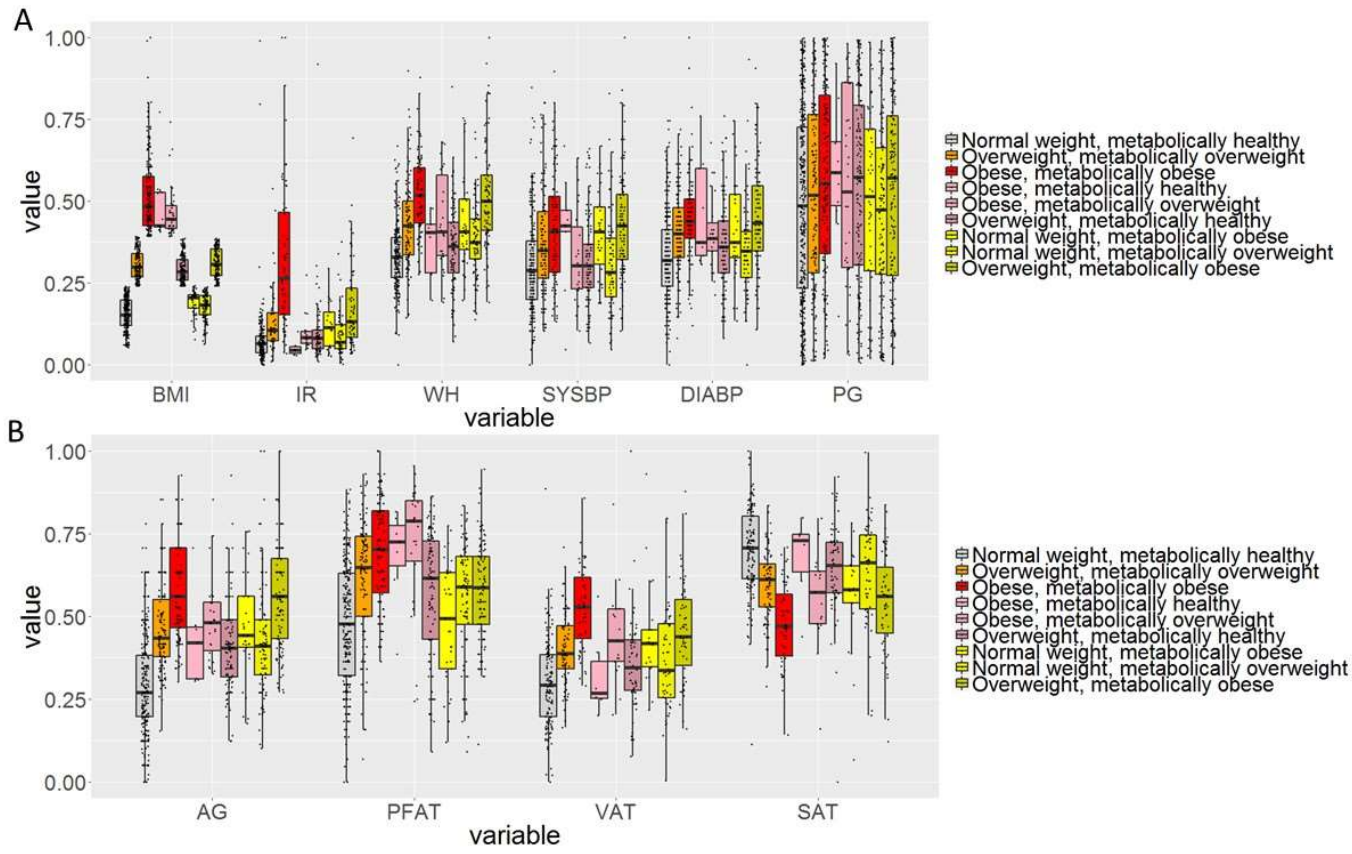


**Figure S2. Factors associated with having a metabolic BMI different from actual BMI, Related to Figure 2.** A) Participants were split into 9 groups: normal weight, metabolically healthy (gray; BMI 18.5-25, BMI prediction below overweight cutoff from Figure S2 B); overweight, metabolically overweight (orange; BMI 25-30, BMI prediction above overweight cutoff but below obese cutoff from Figure S2 A); obese, metabolically obese (red; BMI >=30, BMI prediction above obese cutoff from Figure S2 A); obese, metabolically healthy (pink 1; BMI >=30, BMI prediction below overweight cutoff); obese, metabolically overweight (pink 2; BMI >=30, BMI prediction below obese cutoff); overweight, metabolically healthy (pink 3; BMI 25-30, BMI prediction below overweight cutoff); normal, metabolically obese (yellow 1; BMI 18.5-25, BMI prediction above obese cutoff); normal, metabolically overweight (yellow 2; BMI 18.5-25, BMI prediction above overweight cutoff); and overweight, metabolically obese (yellow 3; BMI 25-30, BMI prediction above obese cutoff). All y-axis values are scaled to a range from 0-1 to allow comparison across groups. The same process is used in B) to show imaging (DEXA or MRI) values associated with metabolic BMI outliers. BMI = body mass index; IR = insulin resistance; WH = waist/hip ratio; SYSBP = systolic blood pressure; DIABP = diastolic blood pressure; PG = polygenic risk score; AG = android/gynoid ratio; PFAT = percent fat; VAT = visceral fat; SAT = subcutaneous fat.

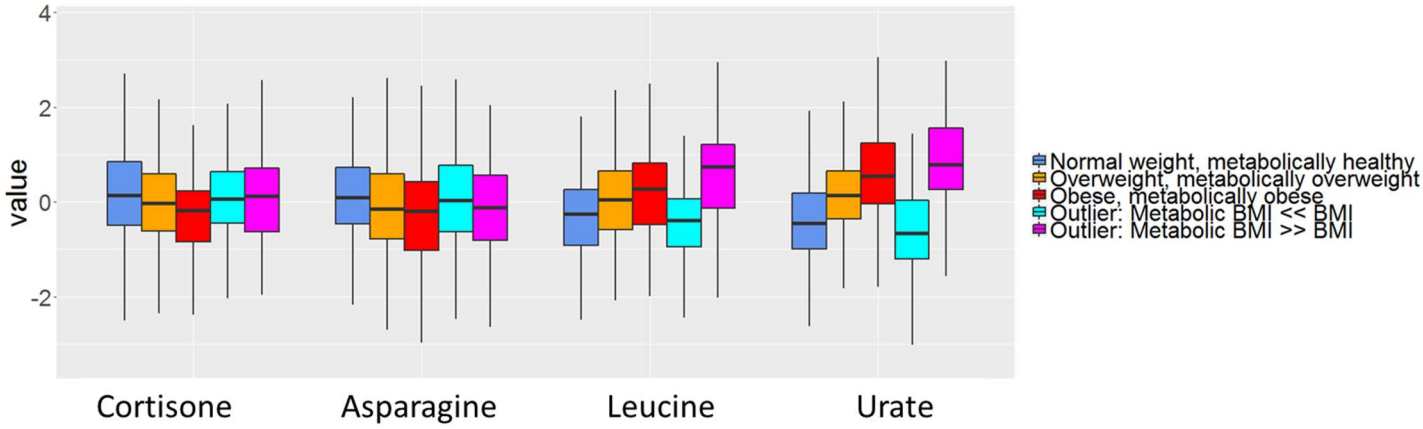


**Figure S3. Metabolite levels and their relationship to mBMI/BMI category, Related to Table 1.** Cortisone and asparagine show no statistically significant difference between the mBMI>>BMI and mBMI<<BMI groups, while leucine and urate (representative metabolites) do show significant differences.

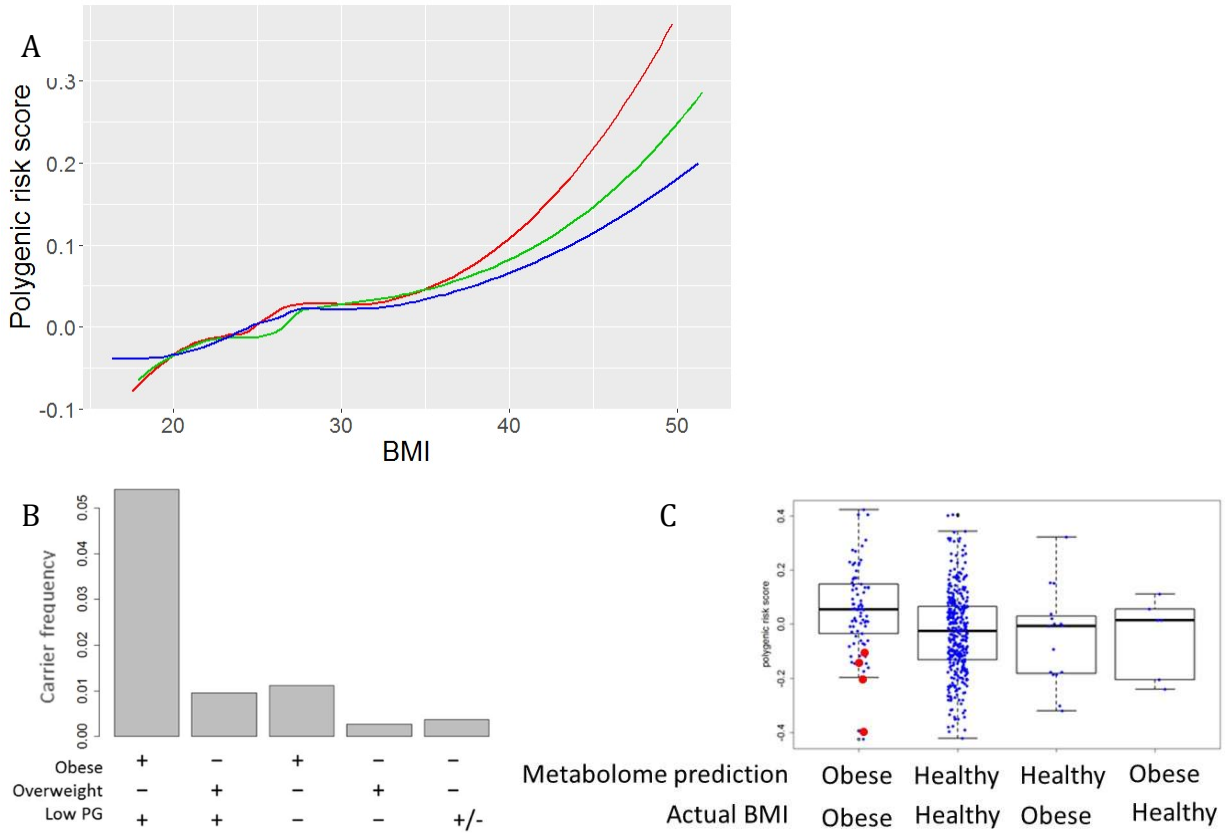


**Figure S4. Genetics of BMI, Related to Table 2.** A) Mean polygenic risk score at each BMI as a function of age for timepoints 1, 2 and 3 in TwinsUK in red, green and blue, respectively. Note that the association of higher polygenic risk scores with higher BMIs appears stronger at earlier visits, i.e., younger ages. B) Shown is the carrier frequency of individuals with rare (MAF<0.001%) coding variants in *MC4R* broken down by obesity status and having a low (first quartile) polygenic risk score (PG). We identified 8 such carriers in the subset of unrelated participants, with an enrichment in participants who were obese despite a low polygenic risk score. Out of 37 participants who were obese with polygenic risk scores in the lowest quartile, 5.4% were *MC4R* variant carriers, while the carrier frequency was just 0.4% in those of normal weight. C) Of four obese *MC4R* variant carriers, two had a dizygotic twin who was also a carrier of the variant. In both cases, twins were obese despite having polygenic risk scores in the bottom quartile. Both sets of twins were predicted to be obese from their metabolome. Three of the four unrelated obese carriers of *MC4R* variants were also predicted to be obese from their metabolomes. Here, the polygenic risk scores of the twin pairs in the TwinsUK cohort are shown, broken down by whether both twins were obese (BMI>30) or normal weight (BMI 18.5-25) and predicted by the metabolome to be obese or normal weight. Obese twin pairs that carry *MC4R* variants are shown in red.

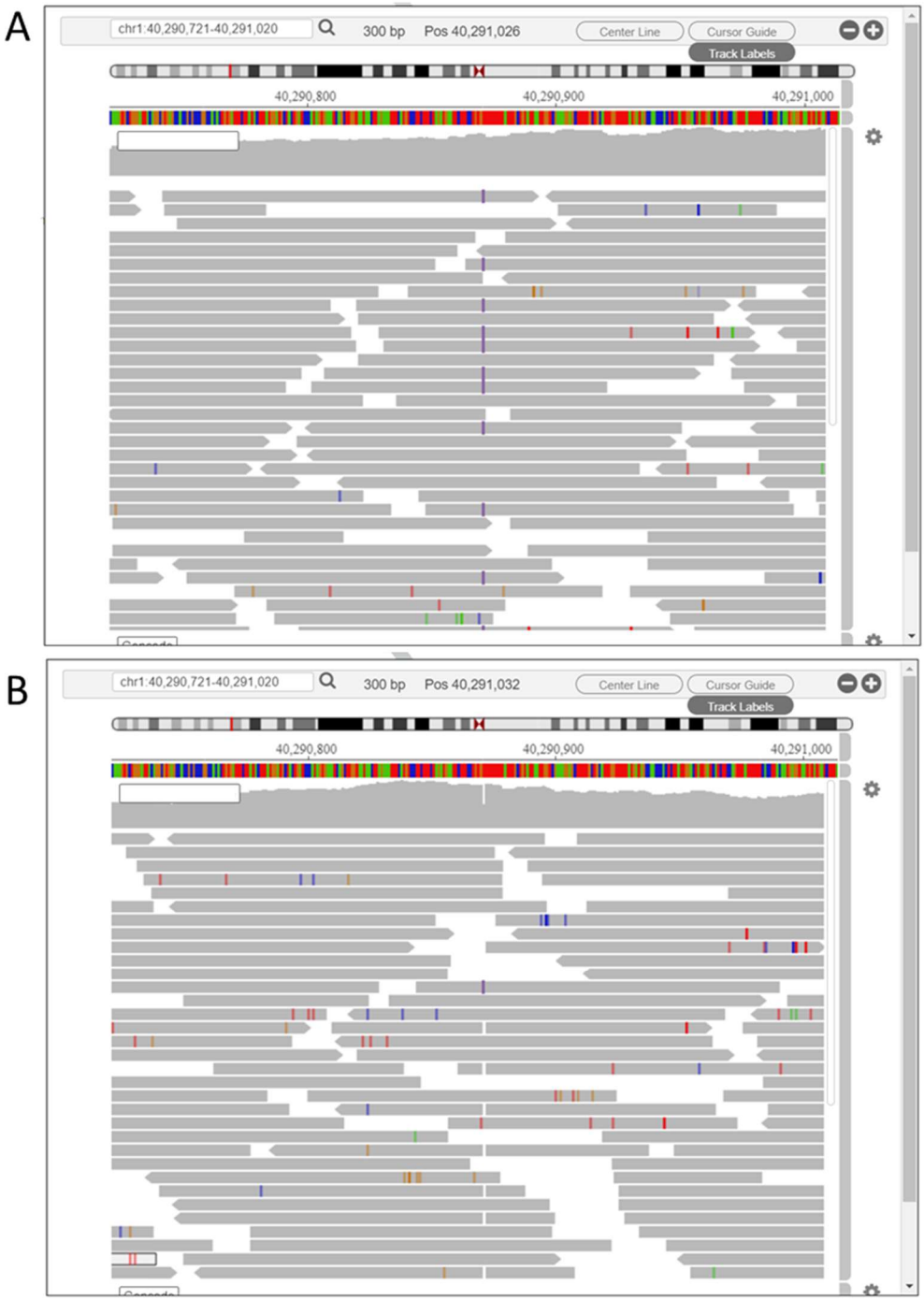


**Figure S5. Alignments at ZMPSTE24 p.Leu362fs, Related to Table 2.** A) Alignments of the T insertion in a carrier; B) alignments of the T deletion. The insertion is annotated as causal for recessive mandibuloacral dysplasia with type B lipodystrophy, and the deletion has not been previously annotated.