Supplementary Information

Table S1. Baseline characteristics of the study population.

Characteristic, mean (STD) or counts %	Male n=4,081 (46.1%)	Female n=4,778 (53.9%)	All n=8,859
Age	51.2 (7.8)	52.3 (7.8)	51.8 (7.8)
Body Composition			
BMI (kg/m^2)	26.5 (3.8)	25.6 (4.4)	26.0 (4.1)
Total fat mass (g)	22,958.3 (7,992.0)	25,744.5 (8,479.0)	24,436.9 (8,369.8)
Scanned VAT mass (g)	1,180.5 (729.6)	637.6 (452.4)	892.5 (657.2)
Android tissue fat (%)	0.3 (0.1)	0.4 (0.1)	0.4 (0.1)
Liver Ultrasound			
Liver sound speed (m/s)	1,548.5 (27.8)	1,540.7 (27.5)	1,544.4 (27.9)
Liver viscosity (Pa.s)	1.7 (0.2)	1.7 (0.3)	1.7 (0.3)
Liver elasticity (kPa)	5.4 (1.0)	4.6 (0.9)	5.0 (1.0)
Liver attenuation (dB/cm/MHz)	0.4 (0.1)	0.4 (0.1)	0.4 (0.1)
CGM-derived measures			
% above 180	0.3 (2.5)	0.2 (1.1)	0.2 (1.9)
% below 70	4.8 (9.4)	6.0 (10.1)	5.5 (9.8)
% in 70-180	94.9 (9.6)	93.8 (10.0)	94.3 (9.9)
ADRR	10.2 (4.5)	11.1 (4.6)	10.7 (4.6)
AUC	97.0 (12.2)	94.2 (10.9)	95.5 (11.6)
COGI	0.9 (0.2)	0.9 (0.2)	0.9 (0.2)
CONGA	18.2 (5.9)	17.6 (5.1)	17.9 (5.5)
CV	15.8 (4.0)	16.1 (3.8)	16.0 (3.9)
eA1C	5.0 (0.4)	4.9 (0.4)	5.0 (0.4)
GMI	5.6 (0.3)	5.6 (0.3)	5.6 (0.3)
GVP	1.1 (0.0)	1.1 (0.0)	1.1 (0.0)
GRADE	1.3 (1.0)	1.2 (0.8)	1.3 (0.9)
HBGI	0.2 (0.7)	0.1 (0.3)	0.2 (0.5)
Hyper index	0.0 (0.2)	0.0 (0.1)	0.0 (0.1)
Hypo index	0.7 (1.5)	0.8 (1.4)	0.8 (1.5)
IGC	0.7 (1.5)	0.8 (1.4)	0.8 (1.4)
IQR	17.6 (5.5)	16.9 (4.8)	17.2 (5.1)
J_index	12.8 (3.9)	12.1 (3.1)	12.4 (3.5)
LBGI	2.2 (1.9)	2.5 (1.9)	2.4 (1.9)

MAD	12.3 (3.7)	11.8 (3.2)	12.1 (3.4)
MAG	10.7 (2.8)	10.6 (2.7)	10.6 (2.7)
MAGE	37.8 (11.9)	37.6 (10.8)	37.7 (11.3)
MODD	13.3 (4.1)	12.7 (3.5)	13.0 (3.8)
SD	15.3 (4.5)	15.1 (4.1)	15.2 (4.3)
SD.Roc	0.4 (0.1)	0.4 (0.1)	0.4 (0.1)
SDb	12.6 (3.9)	12.1 (3.4)	12.4 (3.6)
SDbdm	11.5 (3.4)	11.1 (3.0)	11.3 (3.2)
SDdm	5.1 (3.3)	4.7 (2.6)	4.9 (3.0)
SDw	13.7 (3.8)	13.7 (3.6)	13.7 (3.7)
SDwsh	5.2 (1.2)	5.1 (1.1)	5.2 (1.2)

BMI: Body Mass Index; VAT: Visceral Adipose Tissue; For CGM-derived measures abbreviations see Table S5.

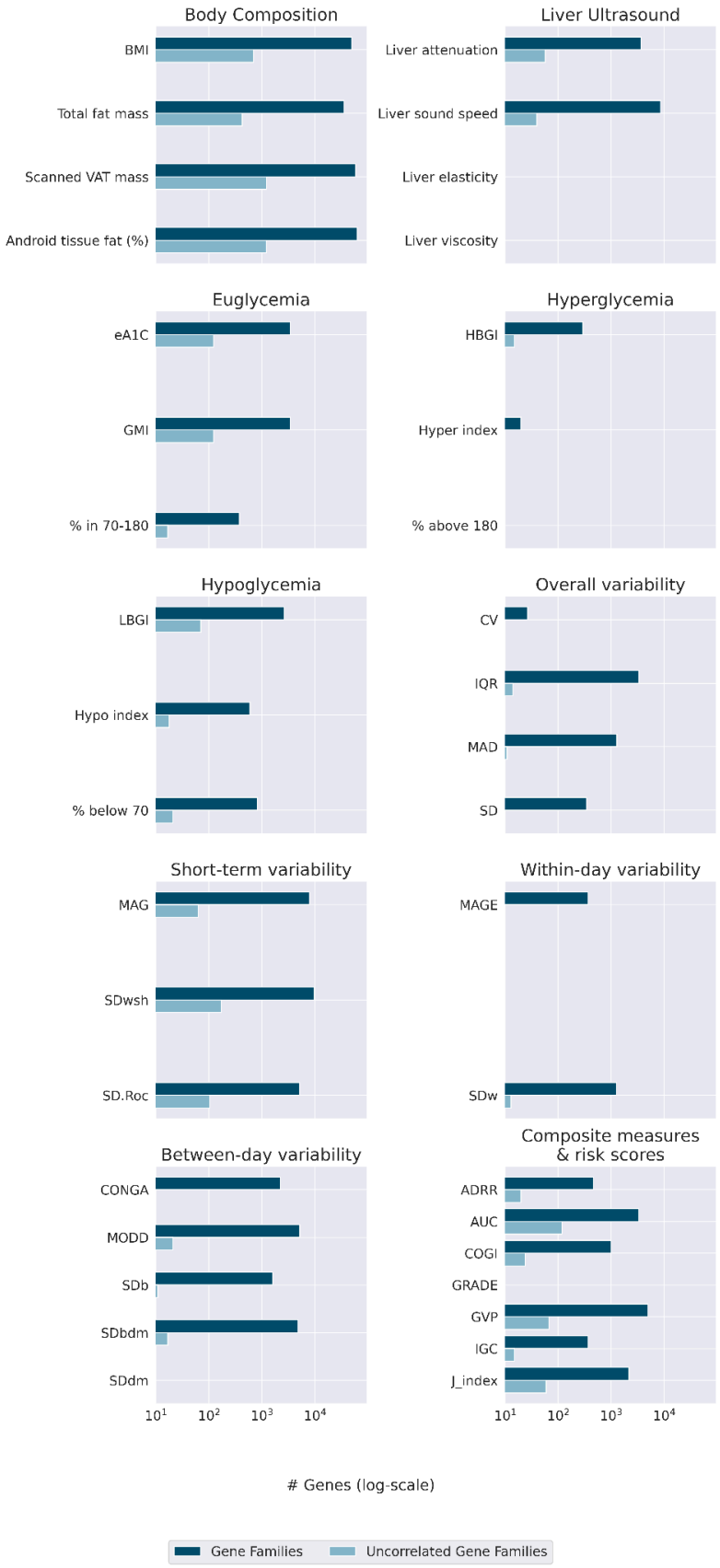

Figure S1: Number of Significantly Associated Bacterial Gene Families and Clumped Independent Bacterial Gene Families with metabolic health measures. Sub-plots present metabolic health measures in each category; Body composition, Liver US, Euglycemia, Hyperglycemia, Hypoglycemia, Overall variability, Short-term variability, Within-day variability, Between-day variability, Composite measures & risk scores. Bars show the number of significantly correlated bacterial gene families (dark blue) and clumped independent bacterial gene families (light blue) for each measure, on a log-scale.

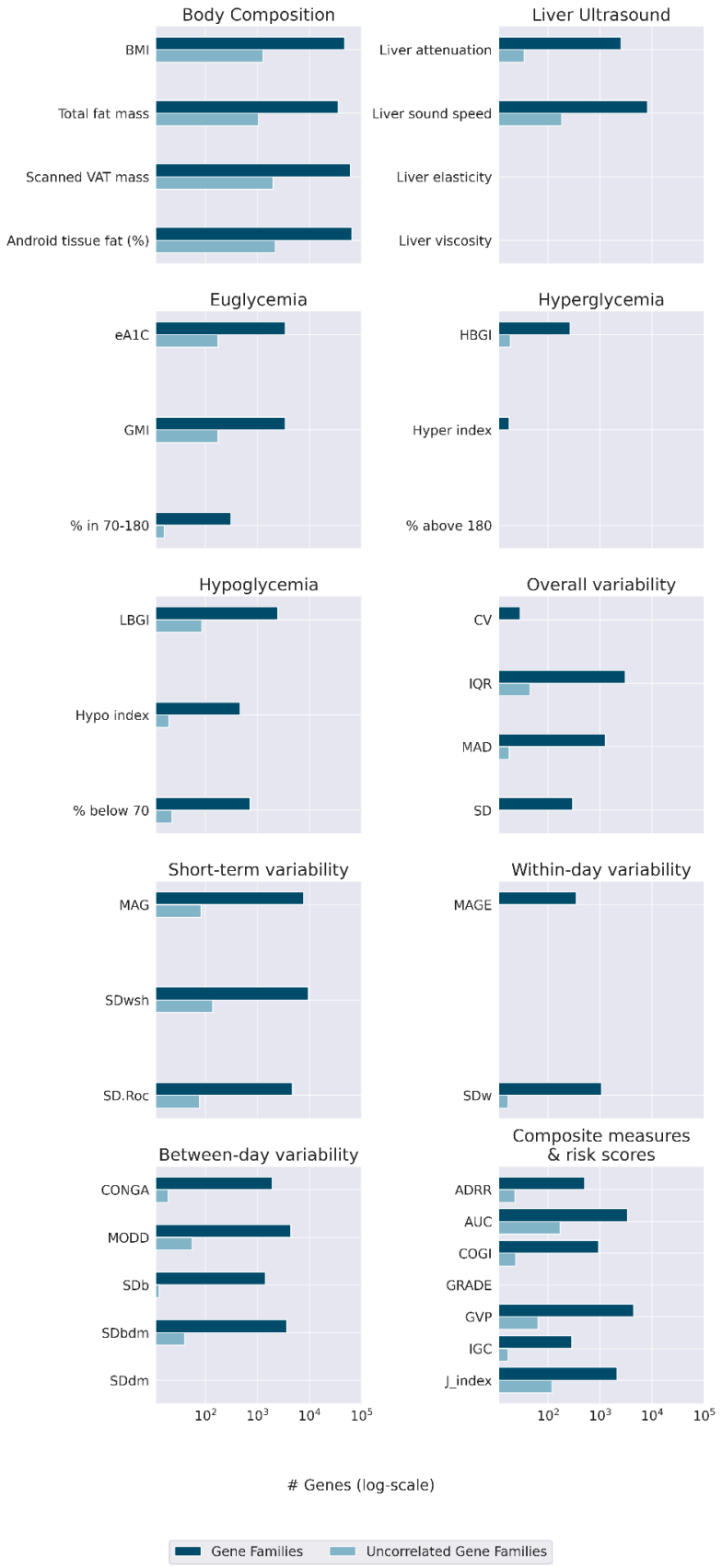

Figure S2: Number of Significantly Associated Bacterial Gene Families and Pre-Filtered Independent Bacterial Gene Families with metabolic health measures. Sub-plots present metabolic health measures in each category; Body composition, Liver US, Euglycemia, Hyperglycemia, Hypoglycemia, Overall variability, Short-term variability, Within-day variability, Between-day variability, Composite measures & risk scores. Bars show the number of significantly correlated bacterial gene families (dark blue) and pre-filtered independent bacterial gene families (light blue) for each measure, on a log-scale.

Table S2. “Key” Bacterial Pathways - Metabolic health group selection and Hierarchical clustering

Pathway	Body Composition	Liver US	CGM-derived Measures	Cluster
PWY-7977: L-methionine biosynthesis IV			X	Cluster I
ASPASN-PWY: superpathway of L-aspartate and L-asparagine biosynthesis			X	
PWY0-1586: peptidoglycan maturation (meso-diaminopimelate containing)	X			
PWY0-1296: purine ribonucleosides degradation	X	X		Cluster II
PWY-7761: NAD salvage pathway II (PNC IV cycle)			X	
PWY-7383: anaerobic energy metabolism (invertebrates, cytosol)	X	X	X	
PWY66-399: gluconeogenesis III			X	
PWY-7953: UDP-N-acetylmuramoyl-pentapeptide biosynthesis III (meso-diaminopimelate containing)		X		Cluster III
PWY-6609: adenine and adenosine salvage III	X			
PWY-3841: folate transformations II (plants)		X		
TRNA-CHARGING-PWY: tRNA charging	X	X		

Table S3. “Key” Bacterial Gene Families (Clumped) - Metabolic health group selection and Hierarchical clustering

Gene Family	Body composition	Liver US	CGM-derived Measures	Cluster
Uncharacterized protein		X		I
Uncharacterized protein		X		
Uncharacterized protein | Faecalibacterium prausnitzii			X	II
Phosphate/phosphite/phosphonate ABC transporters, periplasmic binding protein			X	
Uncharacterized protein			X	
Site-specific recombinase, phage integrase family			X	
PG1 protein	X			
DNA-directed RNA polymerase subunit beta	X			
Hydroxylamine reductase	X			
Translation initiation factor IF-2	X			
L(+)-tartrate dehydratase subunit beta	X			
ATPase BadF/BadG/BcrA/BcrD type domain-containing protein		X		
AAA+ ATPase domain-containing protein		X		
Uncharacterized protein | uncultured Blautia sp		X		
FAD-dependent oxidoreductase			X	III

Table S4. “Key” Bacterial Gene Families (Pre-Filtered) - Metabolic health group selection and Hierarchical clustering

Gene Family	Body composition	Liver US	CGM-derived Measures	Cluster
Uncharacterized protein | Faecalibacterium prausnitzii			X	I
Uncharacterized protein | Faecalibacterium prausnitzii M21/2			X	
DUF3847 domain-containing protein			X	II
Stage V sporulation protein S (SpoVS)	X			
Pyridoxamine 5'-phosphate oxidase family protein	X			
Mutator family transposase	X	X		
Aminotransferase		X		
Molecular chaperone		X		
Uncharacterized protein | Faecalibacterium duncaniae		X		
CBM-cenC domain-containing protein	X	X		
Uncharacterized protein | Faecalibacterium prausnitzii M21/2			X	
Uncharacterized protein | Faecalibacterium prausnitzii			X	
Uncharacterized protein	X			III

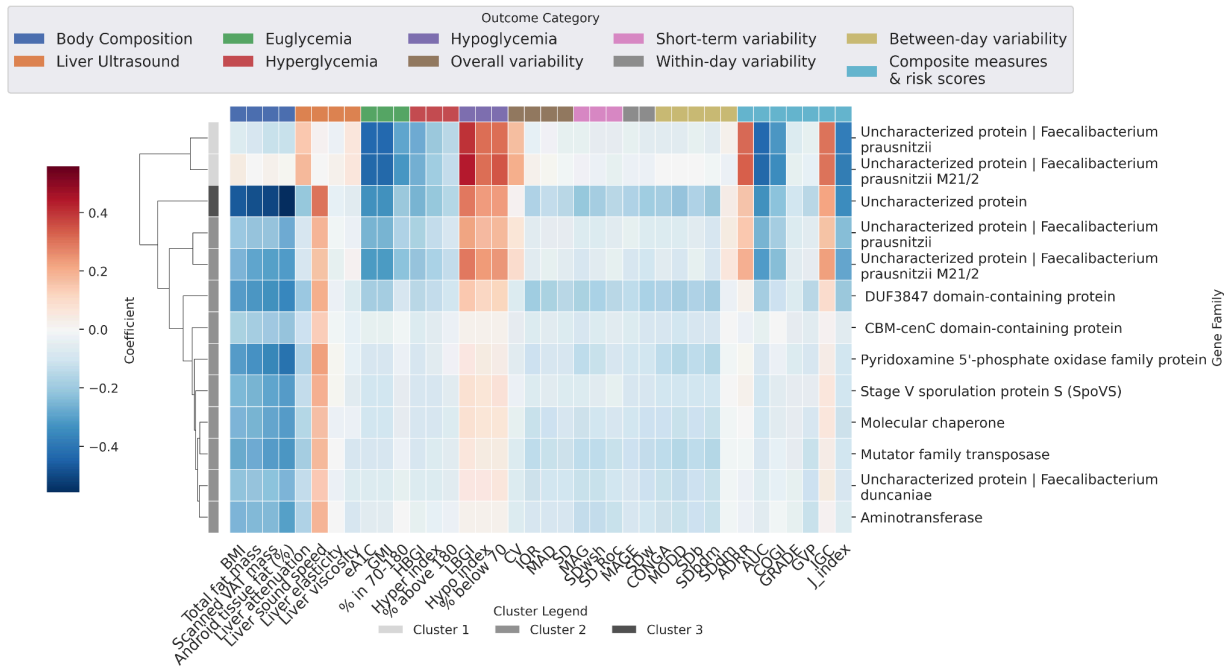

Figure S3: Heatmap of "Key" Pre-filtered Bacterial Gene Families. Heatmaps showing the correlation coefficient of the 13 "Key" Pre-filtered Bacterial Gene Families with the highest number of significantly associated metabolic health measures and lowest p-value ranking across groups (see Selecting "Key" associated Bacterial Gene Families and Pathways in Methods).

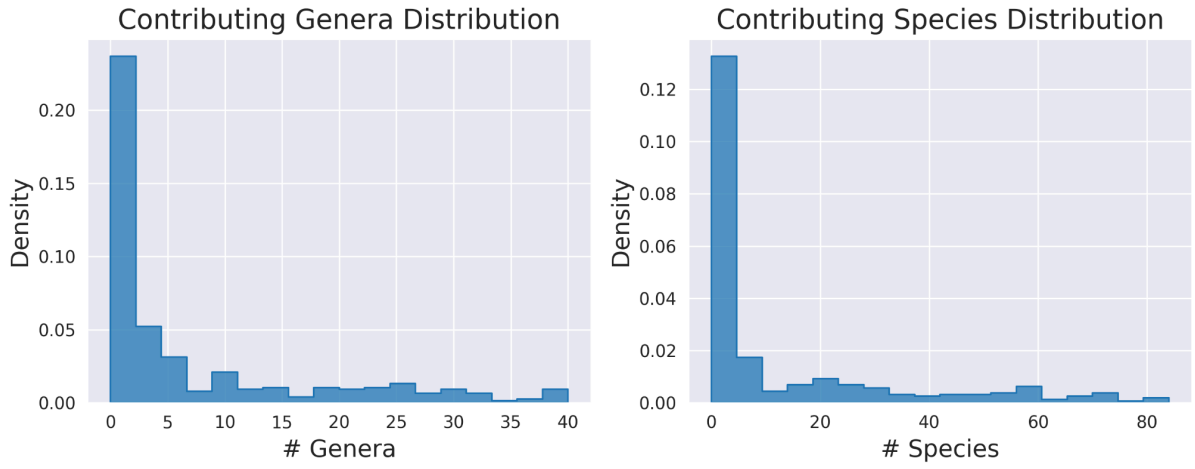

Figure S4: Number of Contributing Genera and Species to the abundance of bacterial pathways. Distribution plots show the density of the number of contributing **Genera (Left)** and **Species (Right)** to the abundances of the analyzed bacterial pathways. Pathways for which all abundances were from unclassified genera were excluded from the distribution.

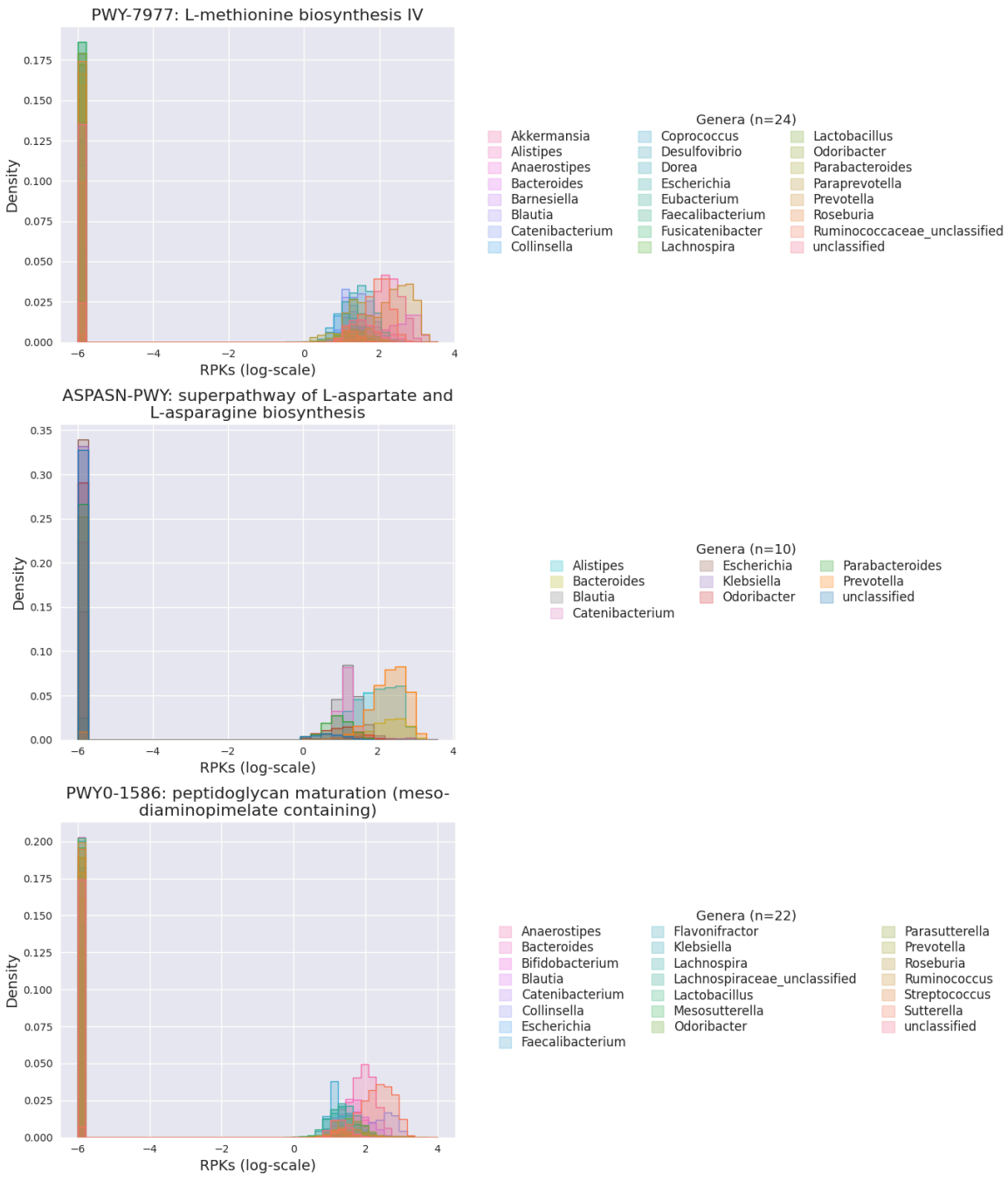

Figure S5: Genera contributing to the abundance of "Key" Pathways from Cluster I.
Histogram of RPKs (log-scale) contributed by each genera to the 3 "Key" pathways from cluster I (top to bottom): **PWY-7977, ASPASN-PWY, PWY0-1586 .** Legend beside each figure shows the number of genera that contributed to the pathway, along with its corresponding color.

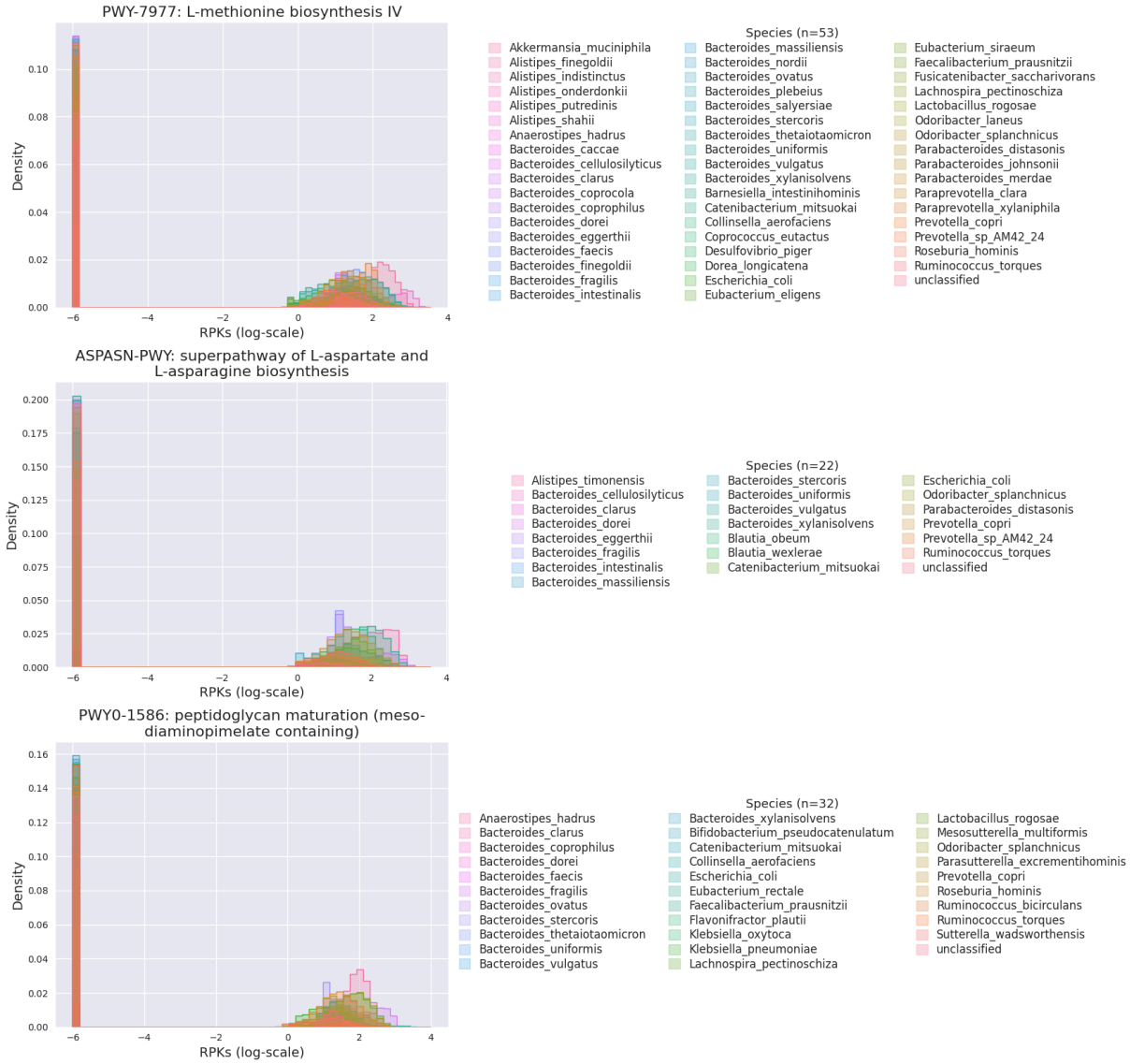

Figure S6: Species contributing to the abundance of "Key" Pathways from Cluster I.
Histogram of RPKs (log-scale) contributed by each species to the 3 "Key" pathways from cluster I (top to bottom): **PWY-7977, ASPASN-PWY, PWY0-1586 .** Legend beside each figure shows the number of species that contributed to the pathway, along with its corresponding color.

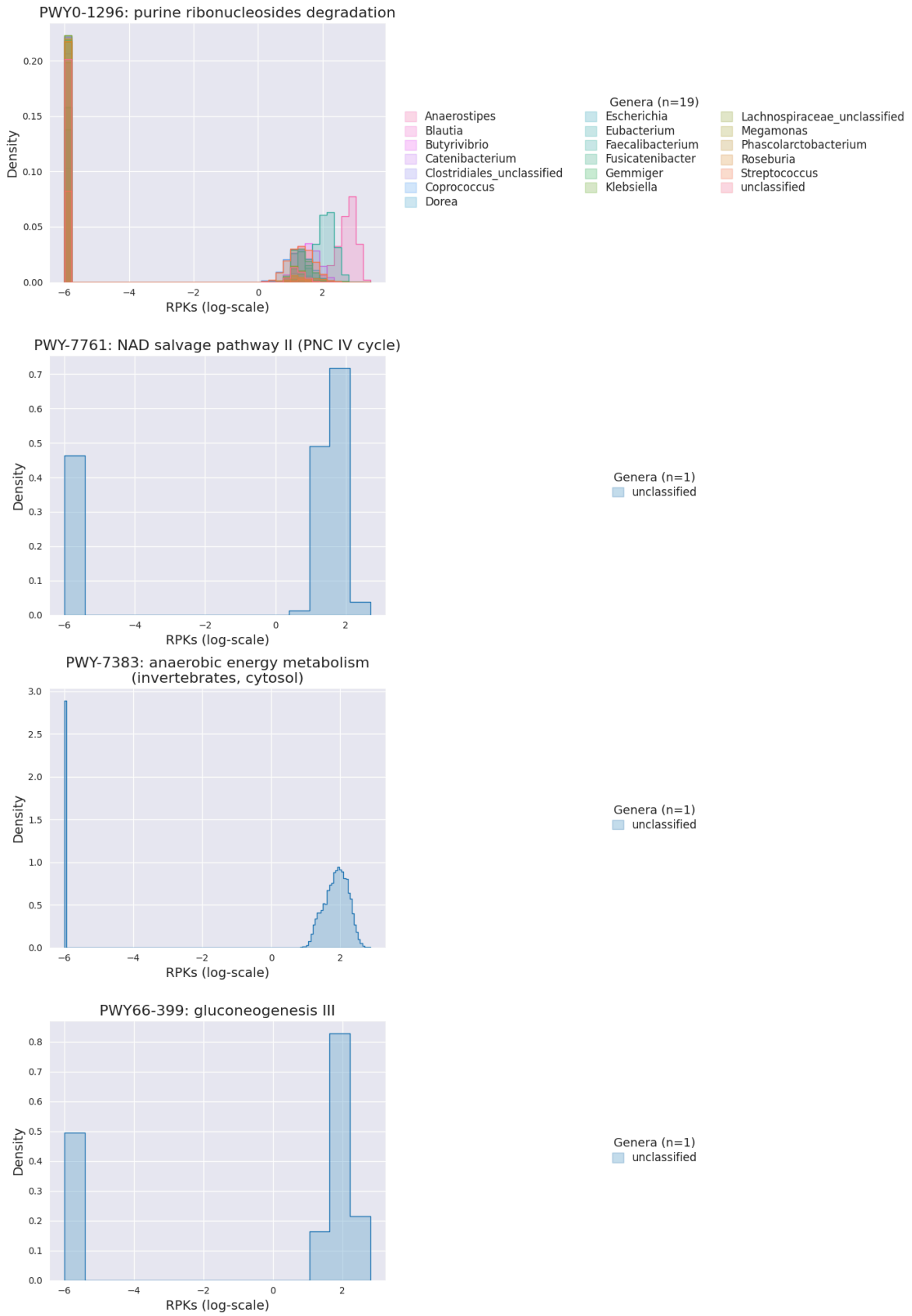

Figure S7: Genera contributing to the abundance of "Key" Pathways from Cluster II.
Histogram of RPKs (log-scale) contributed by each genera to the 3 "Key" pathways from cluster I (top to bottom): **PWY0-1296, PWY-7761, PWY-7383, PWY66-399 .** Legend beside each figure shows the number of genera that contributed to the pathway, along with its corresponding color.

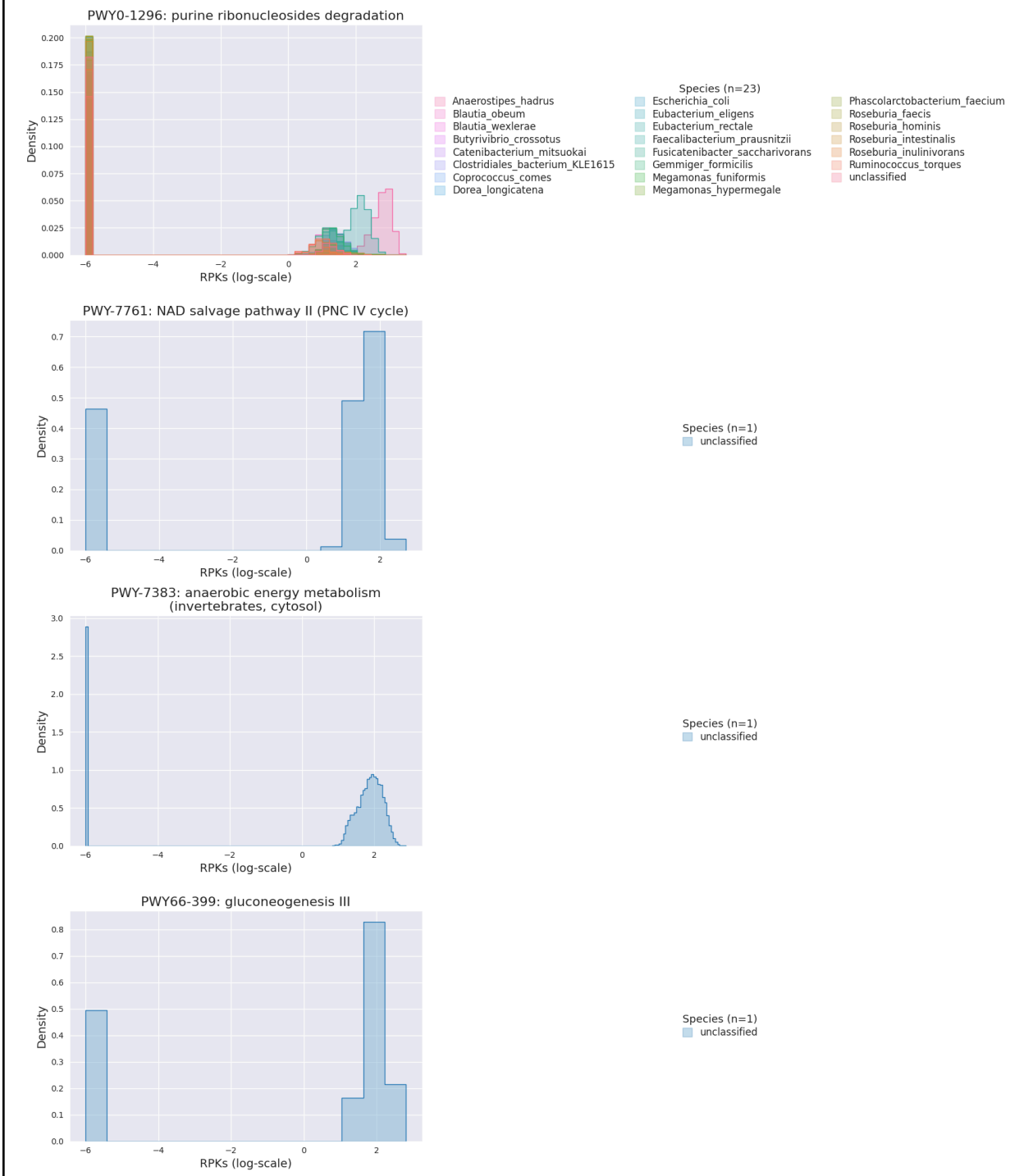

Figure S8: Species contributing to the abundance of "Key" Pathways from Cluster II.
Histogram of RPKs (log-scale) contributed by each species to the 3 "Key" pathways from cluster I (top to bottom): **PWY0-1296, PWY-7761, PWY-7383, PWY66-399 .** Legend beside each figure shows the number of species that contributed to the pathway, along with its corresponding color.

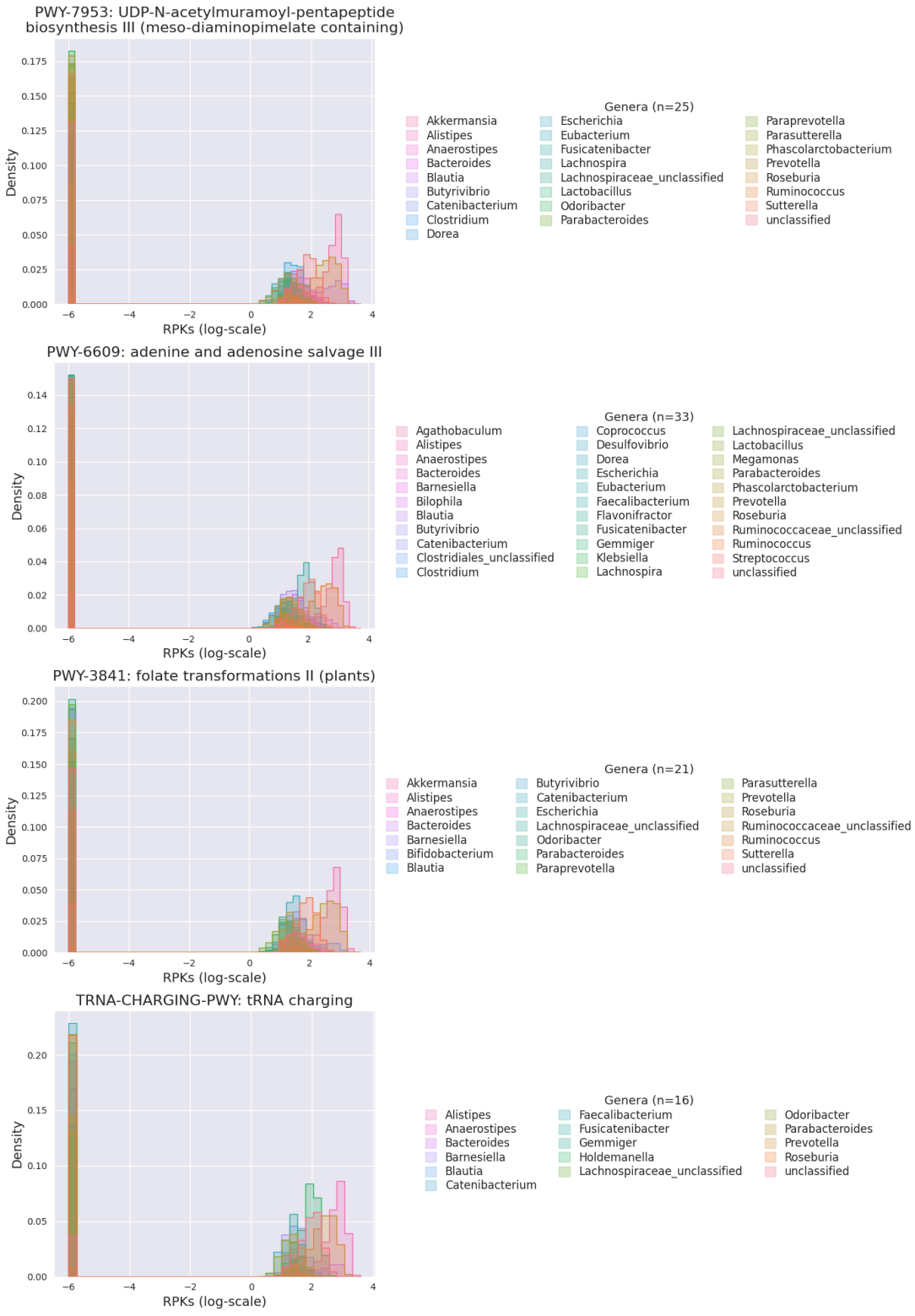

Figure S9: Genera contributing to the abundance of "Key" Pathways from Cluster III.
Histogram of RPKs (log-scale) contributed by each genera to the 3 "Key" pathways from cluster I (top to bottom): **PWY-7953, PWY-6609, PWY-3841, TRNA-CHARGING-PWY.** Legend beside each figure shows the number of genera that contributed to the pathway, along with its corresponding color.

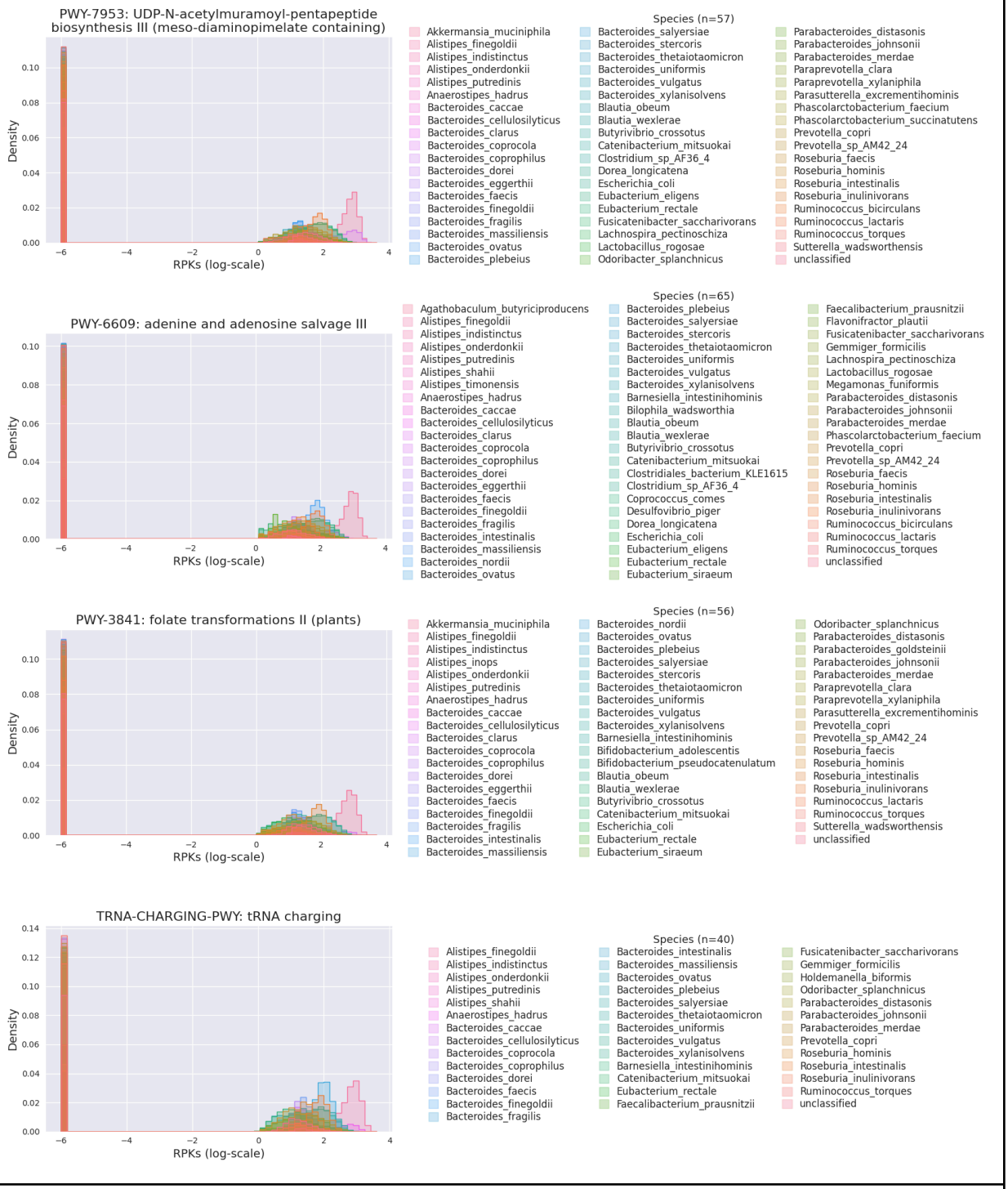

Figure S10: Species contributing to the abundance of "Key" Pathways from Cluster III.
Histogram of RPKs (log-scale) contributed by each species to the 3 "Key" pathways from cluster I (top to bottom): **PWY-7953, PWY-6609, PWY-3841, TRNA-CHARGING-PWY .** Legend beside each figure shows the number of species that contributed to the pathway, along with its corresponding color.

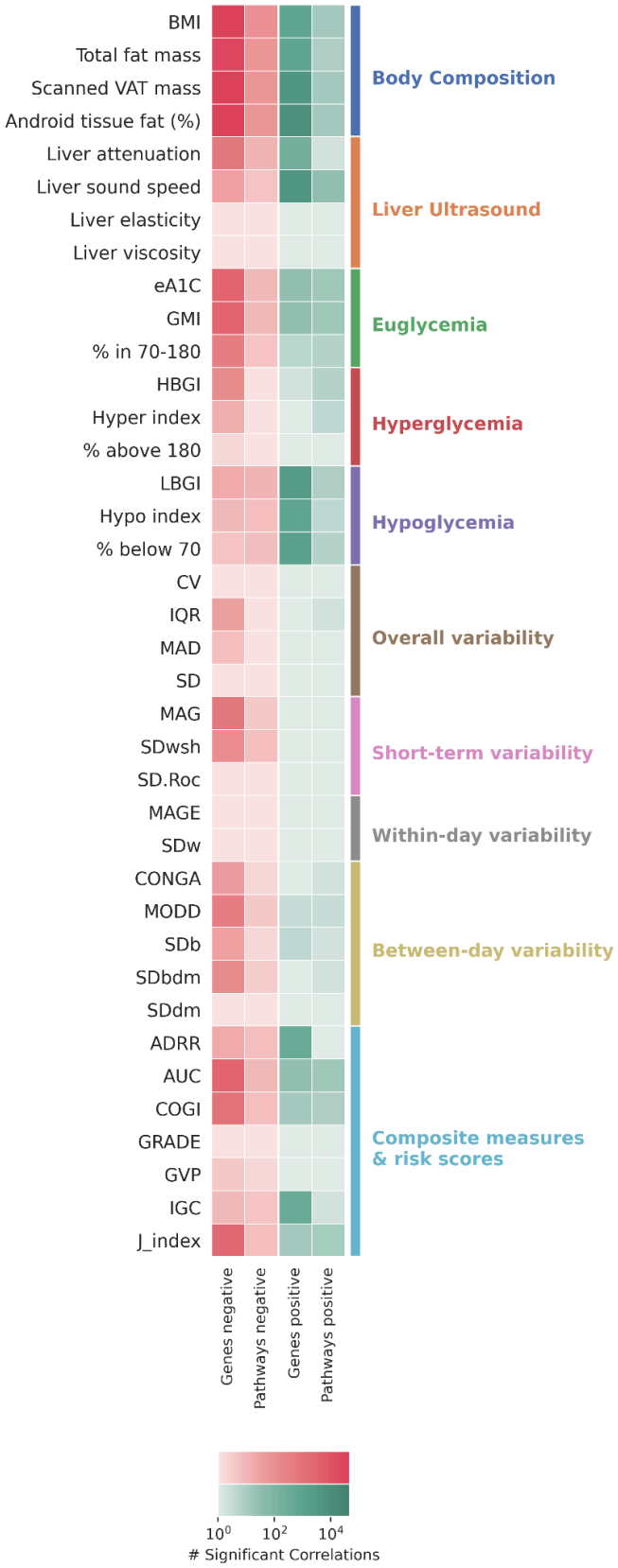

Figure S11: Associations adjusted for nutritional habits summaries. Number of Significantly Associated Bacterial Gene Families and Pathways with metabolic health measures.
Heatmap showing the number of positive and negative significant associations between each metabolic health measure and bacterial gene families and pathways. Associations were obtained using two-sided linear regression adjusted for age, sex and nutritional summaries (see Nutritional summaries as sensitivity analysis in Methods), and corrected for multiple comparisons using the Bonferroni method. Associations with Bonferroni-corrected p-value < 0.05 were considered significant. Colored bar on the right shows the category assigned for each metabolic health measure.

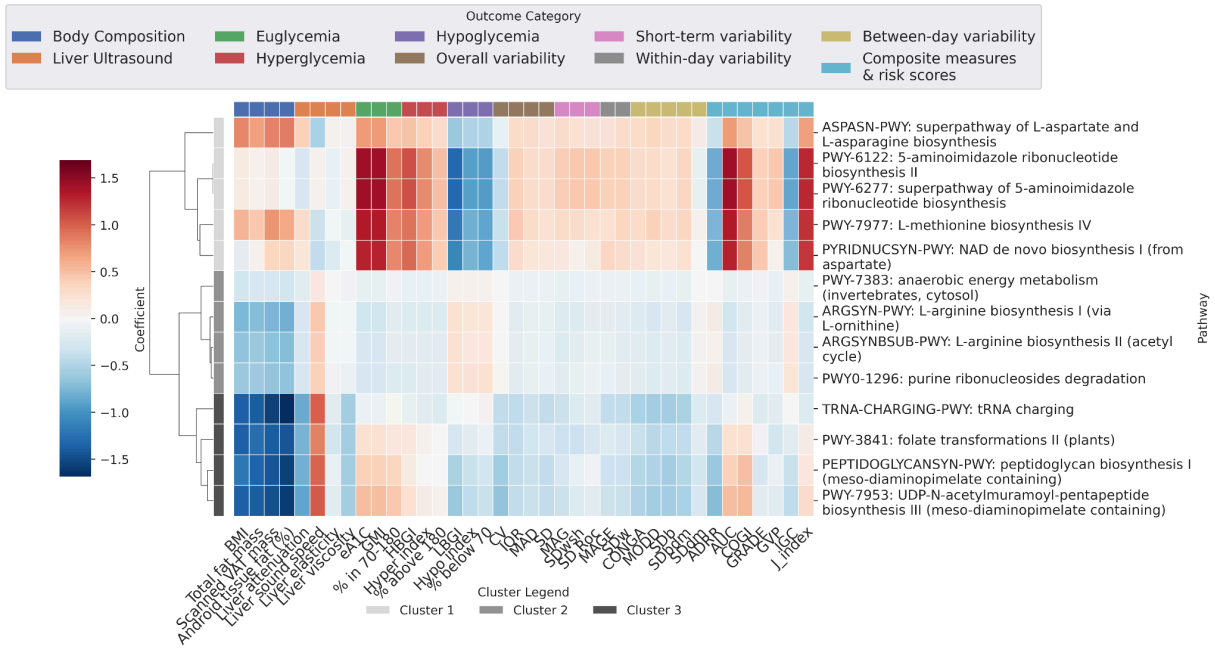

Figure S12: Heatmap of "Key" Bacterial Pathways after adjusting for nutritional habits summaries. Heatmaps showing the correlation coefficient of the 13 "Key" Bacterial Pathways with the highest number of significantly associated metabolic health measures and lowest p-value ranking across groups (see Selecting "Key" associated Bacterial Gene Families and Pathways in Methods).

Table S5. "Key" Bacterial Pathways - Metabolic health group selection and Hierarchical clustering, adjusted for nutritional summaries

Pathway	Body composition	Liver US	CGM-derived Measures	Cluster
PWY-7977: L-methionine biosynthesis IV			X	Cluster I
ASPASN-PWY: superpathway of L-aspartate and L-asparagine biosynthesis			X	
PYRIDNUCSYN-PWY: NAD de novo biosynthesis I (from aspartate)			X	
PWY-6277: superpathway of 5-aminoimidazole ribonucleotide biosynthesis			X	
PWY-6122: 5-aminoimidazole ribonucleotide biosynthesis II			X	
PWY0-1296: purine ribonucleosides degradation	X			Cluster II
ARGSYN-PWY: L-arginine biosynthesis I (via L-ornithine)	X			
ARGSYNBSUB-PWY: L-arginine biosynthesis II (acetyl cycle)	X			
PWY-7383: anaerobic energy metabolism (invertebrates, cytosol)	X	X		
PWY-7953: UDP-N-acetylmuramoyl-pentapeptide biosynthesis III (meso-diaminopimelate containing)		X		Cluster III
PEPTIDOGLYCANSYN-PWY: peptidoglycan biosynthesis I (meso-diaminopimelate containing)		X		
PWY-3841: folate transformations II (plants)		X		
TRNA-CHARGING-PWY: tRNA charging	X	X		

Table S6. Baseline characteristics of the replication population.

Characteristic, mean (STD) or counts %	Male n=3,451 (42.1%)	Female n=4,754 (57.9%)	All n=8,205
Age	49.2 (15.9)	47.9 (13.9)	48.4 (14.8)
Anthropometric			
BMI (kg/m^2)	25.6 (3.9)	25.5 (4.7)	25.6 (4.4)

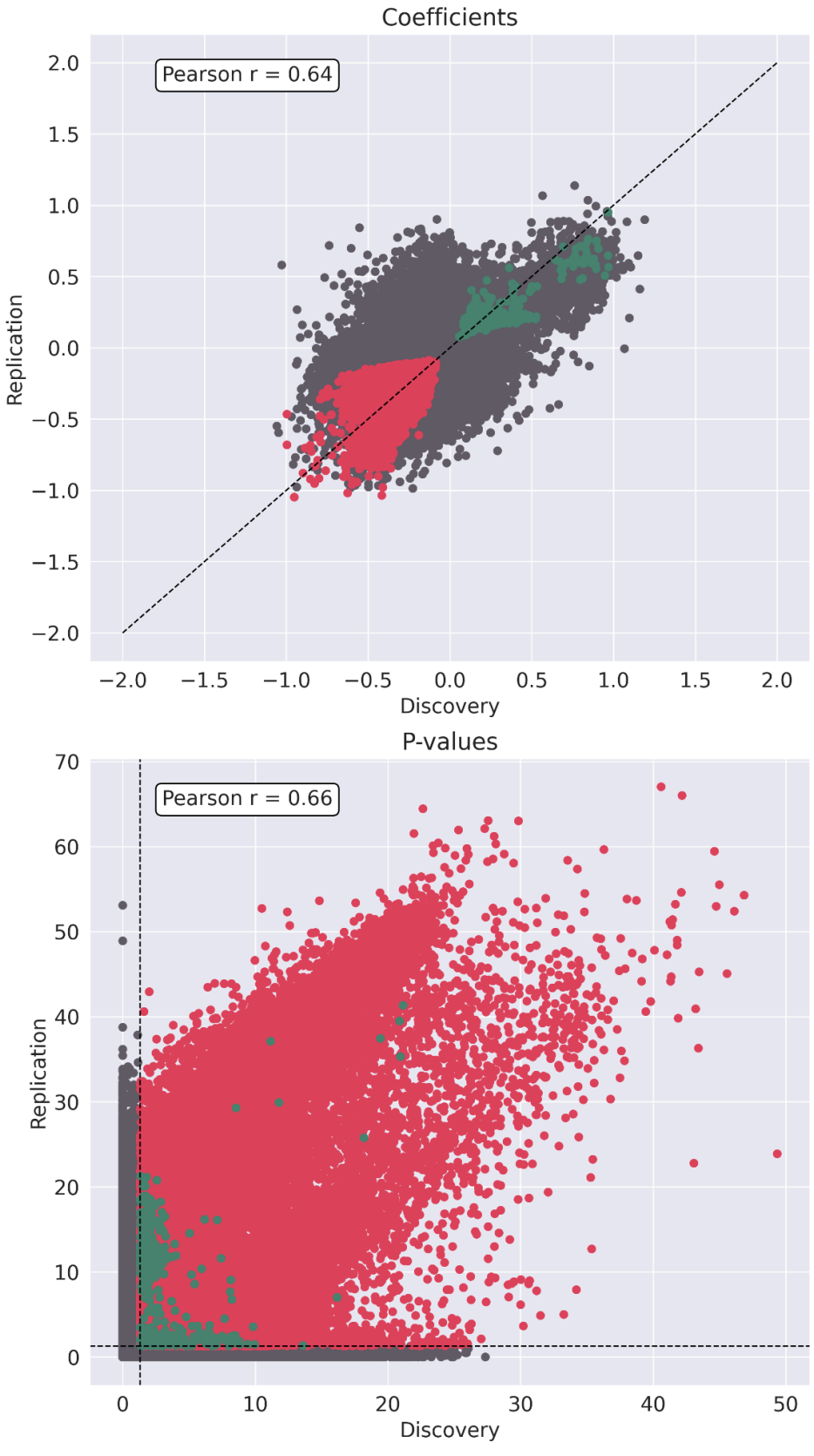

Figure S13: Bacterial Gene Families correlate with BMI replicate in an independent cohort.
Coefficients (top) and **P-values (-log scale) (bottom)** of the associations between bacterial gene families and BMI, in the discovery vs. the replication cohort. Associations were performed using two-sided linear regression adjusted for age and sex. Multiple comparisons were corrected using the Bonferroni method, and associations with Bonferroni-corrected p-value < 0.05 were considered significant. Colored dots present bacterial gene families with significant associations in both cohorts, and with either a negative (red) or positive (green) correlation coefficient in both cohorts.

Table S7. Summary of CGM-derived measures

Measure	Short description	Reference
% above value	Percent of glucose measures that were larger than a specified value.	
% below value	Percent of glucose measures that were lower than a specified value.	
% in range	Percent of glucose measures that were in a specified range of glucose values.	
ADRR	Average daily risk range (ADRR) is a variability measure that was designed to be sensitive to both hyperglycemia and hypoglycemia. The ADRR is composed of the HBGI and LGBI.	[1]
AUC	Hourly average Area Under the Curve (AUC). This measure integrates, to some extent, the severity of a high or low glucose along with the duration of the abnormality. It's calculated for each hour in the CGM, then averaged for each day over a 24-hour period, then once again averaged between all days to receive a single AUC measure.	[2]
COGI	Continuous Glucose Monitoring Index (COGI). COGI uses three measures of the CGM - time in range, time below range and glucose variability (GV) and averages them using a weighted mean, usually with weights of 50%, 35% and 15% accordingly.	[3]
CONGA	Continuous Overall Net Glycemic Action (CONGA). This measure assesses intra-day glycemic variability, by calculating the standard deviation of differences in glucose measures taken n hours apart.	[4]
CV	Coefficient of variation of all glucose values.	[5]
eA1C	A linear transformation of the mean glucose value, meant to estimate the HbA1C blood test. Calculated by the following formula: $(46.7 + mean(Glucose))/28.7$.	[6]
GMI	A linear transformation of the mean glucose value, meant to improve the eA1C measure. Calculated by the following formula: $3.31 + 0.02329 * mean(Glucose)$.	[7]
GRADE	Glycaemic Risk Assessment Diabetes Equation (GRADE). This clinical risk score aims to evaluate the degree of risk presented by a glucose profile. This score was constructed by 50 diabetes healthcare professionals, which scored glucose values with a risk range of 0-50. Then, each glucose value was assigned as a single risk score as the average of the 50, and a function was found to fit these risk scores.	[8]
GVP	Glucose Variability Percentage (GVP), which is designed to capture both the amplitude and frequency of glucose oscillations.	[9]
HBGI	High Blood Glucose Index (HBGI) and Low Blood Glucose Index (LBGI) - measures of the frequency and extent of high BG and low BG readings. These are nonnegative numbers, where larger LBGI or HBGI indicates more frequent and/or extensive hypoglycemia or hyperglycemia (Kovatchec et al. 2002). Prior to their calculation, the blood glucose levels are transformed using a nonlinear transformation to create a symmetric distribution around the "clinical center", and create equal-sized intervals for hyperglycemic and hypoglycemic ranges (without this transformation the range of hyperglycemia is much larger than the range for hypoglycemia).	[10]
Hyper index	This is a weighted average of hyperglycemic values, with larger penalties for more extreme values.	[5]
Hypo index	This is a weighted average of hypoglycemic values, with progressively larger penalties for more extreme values	[5]
IGC	Index of Glycemic Control (IGC), a sum of the hypoglycemia and hyperglycemia indexes. This index was shown to be highly correlated with percentage of time in target range.	[5]

IQR	Interquartile range (IQR), calculated as the distance between the 25th percentile and the 75th percentile of the glucose values.	
J_index	This index was designed to stress the importance of the two major glycaemia components: the mean level and the variability of glycaemia. Calculated by the following formula: $0.001 * [mean(Glucose) + SD(Glucose)]2$.	[11]
LBGI	See HBGI.	[10]
MAD	Median Absolute Deviation (MAD). This is a measure of glycemic variability, which is calculated by taking the median of the differences between glucose values and their median.	
MAG	Mean Absolute Glucose (MAG). This is a measure of glycemic variability that's meant to be less dependent on the mean glucose value and to take into account all variability over time.	[12]
MAGE	Mean Amplitude of Glycemic Excursions (MAGE), an index for glycemic variability. This index is focused on the amplitude of blood glucose changes, and as such it takes into account only changes in the blood glucose (either upwards or downwards) that are large enough to be considered as significant responses.	[13]
MODD	Mean difference between glucose values obtained at the same time of day (MODD). This is a measure of glycemic variability that is calculated by taking the mean of the absolute differences between glucose values measured at the same time, a day apart.	[13]
SD	Standard deviation of all glucose values.	
SD.Roc	Standard deviation of all the rate of change (ROC) values of an individual. ROC is calculated between every two consecutive glucose measures.	[14]
SDb	SD between days, within time points. Mean value of the standard deviations calculated for values taken at each time point across all days.	[15]
SDbdm	SD between days, within time points, corrected for changes in daily means. Calculated by subtracting the daily mean from each glucose value, then calculating the SD of the corrected glucose values across days for each time point, and finally taking the mean of those standard deviations.	[15]
SDdm	Horizontal SD. SD of the mean glucose values, taken for each day separately.	[15]
SDw	Vertical SD within days. Average value of the SD values for each day separately.	[15]
SDwsh	SD within series. Taking hour-long intervals that start at each point of the glucose measures, the SD of each hour-long window is computed, and then the mean of all those SDs is taken.	[15]

Supplementary appendix references

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