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SUPPLEMENTARY INFORMATION

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The fecal metabolome as a functional readout of the gut microbiome

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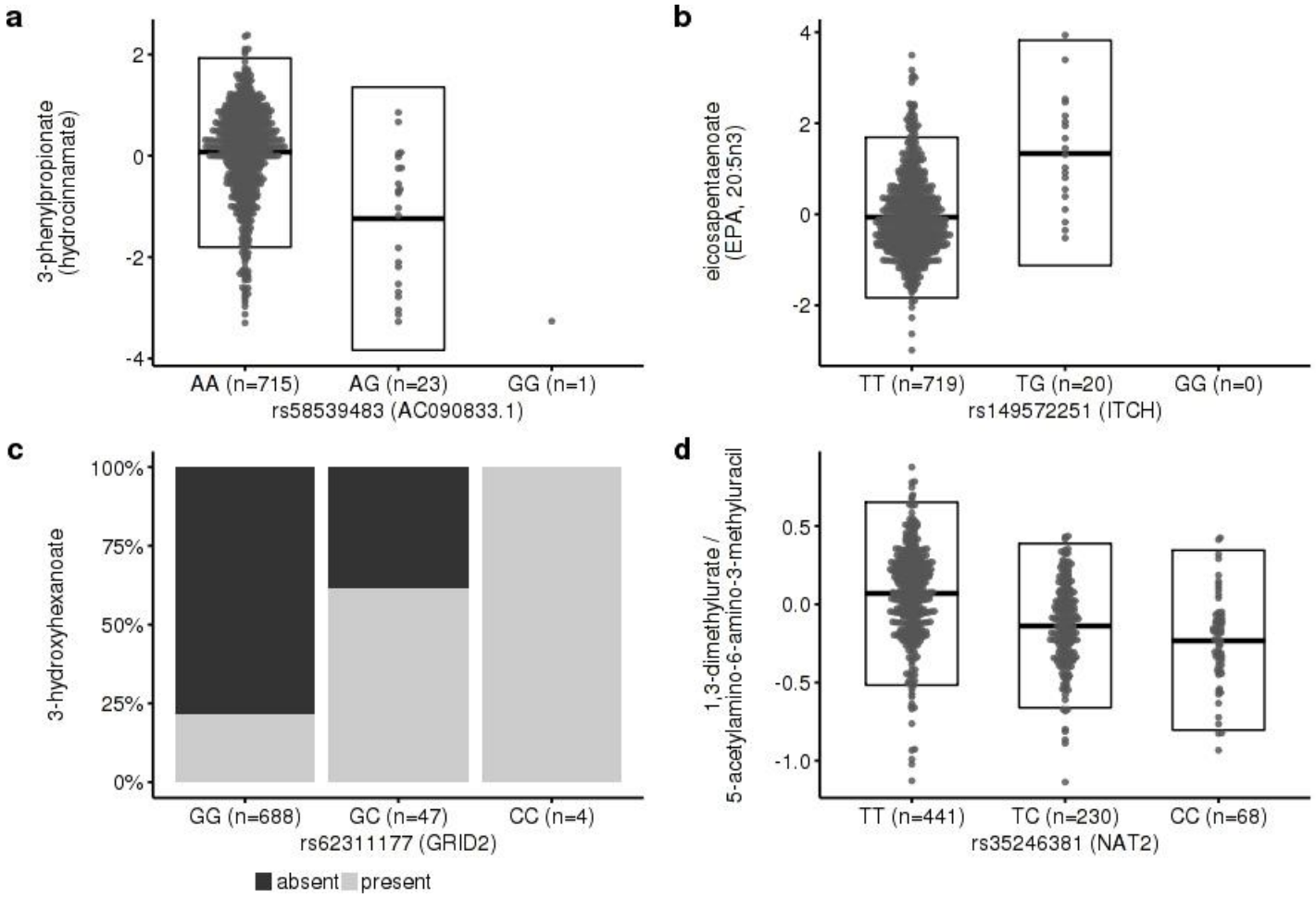
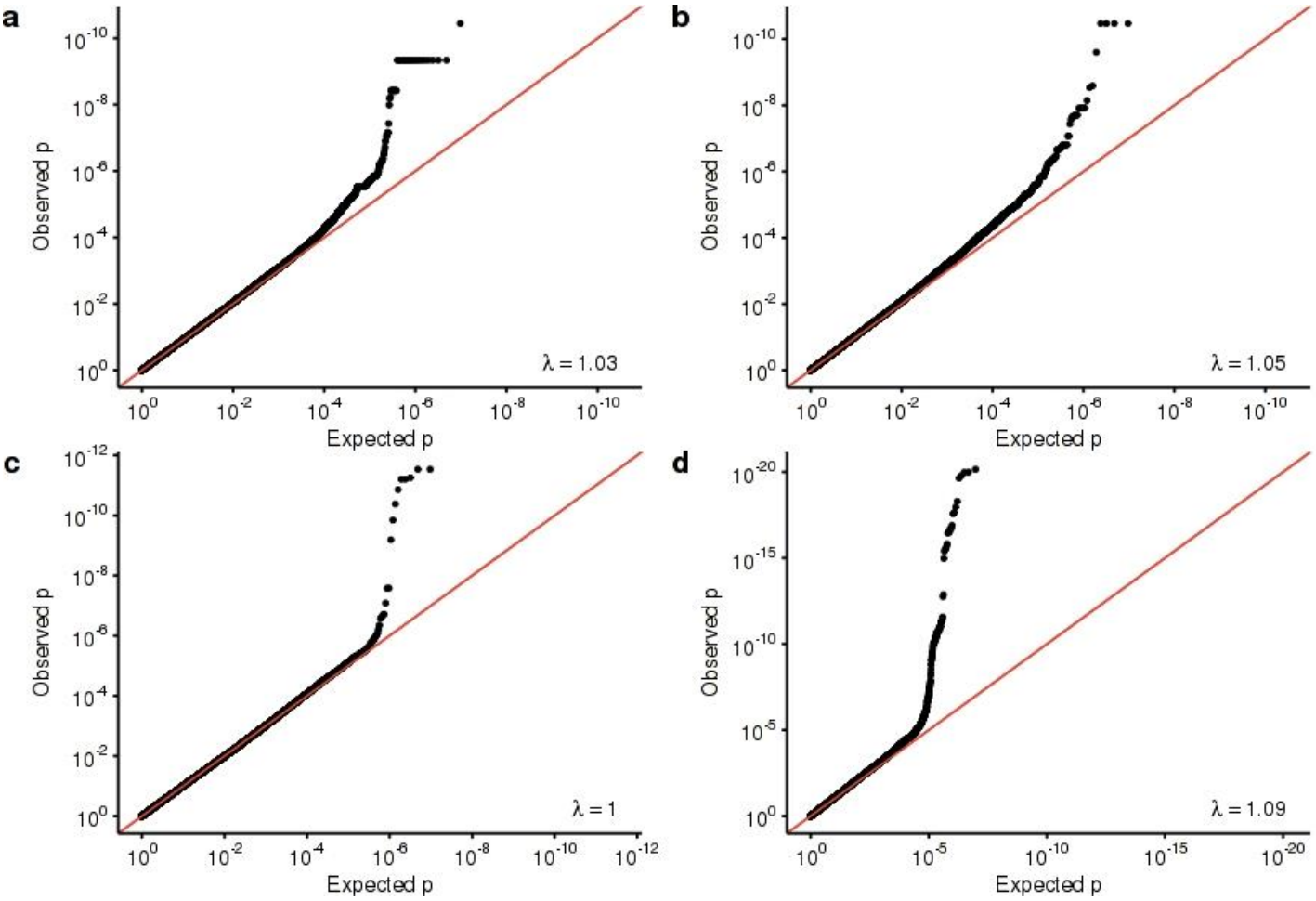


Supplementary Figure 1

Genetic associations of fecal metabolites

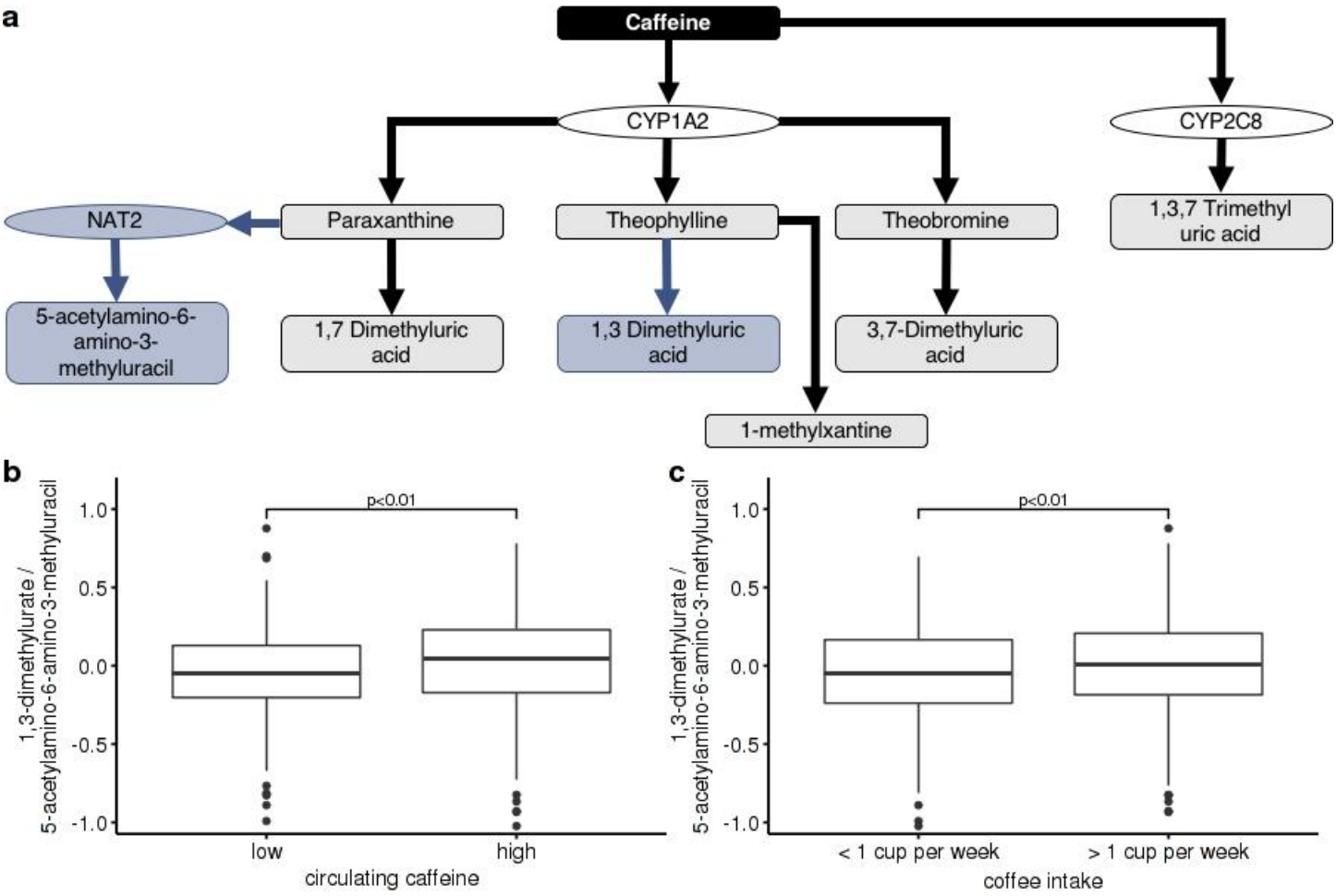
We found three fecal metabolites and one metabolite ratio significantly associated with genetic loci. Each panel shows one of these associations with the respective lead SNP. 3-hydroxyhexanoate was found in less than 80% of all samples and was, thus, analyzed as dichotomous trait. The other metabolites are observed in at least 80% of the samples and were analyzed as continuous traits.



Supplementary Figure 2

Q–Q plots for host genetic associations with fecal metabolites

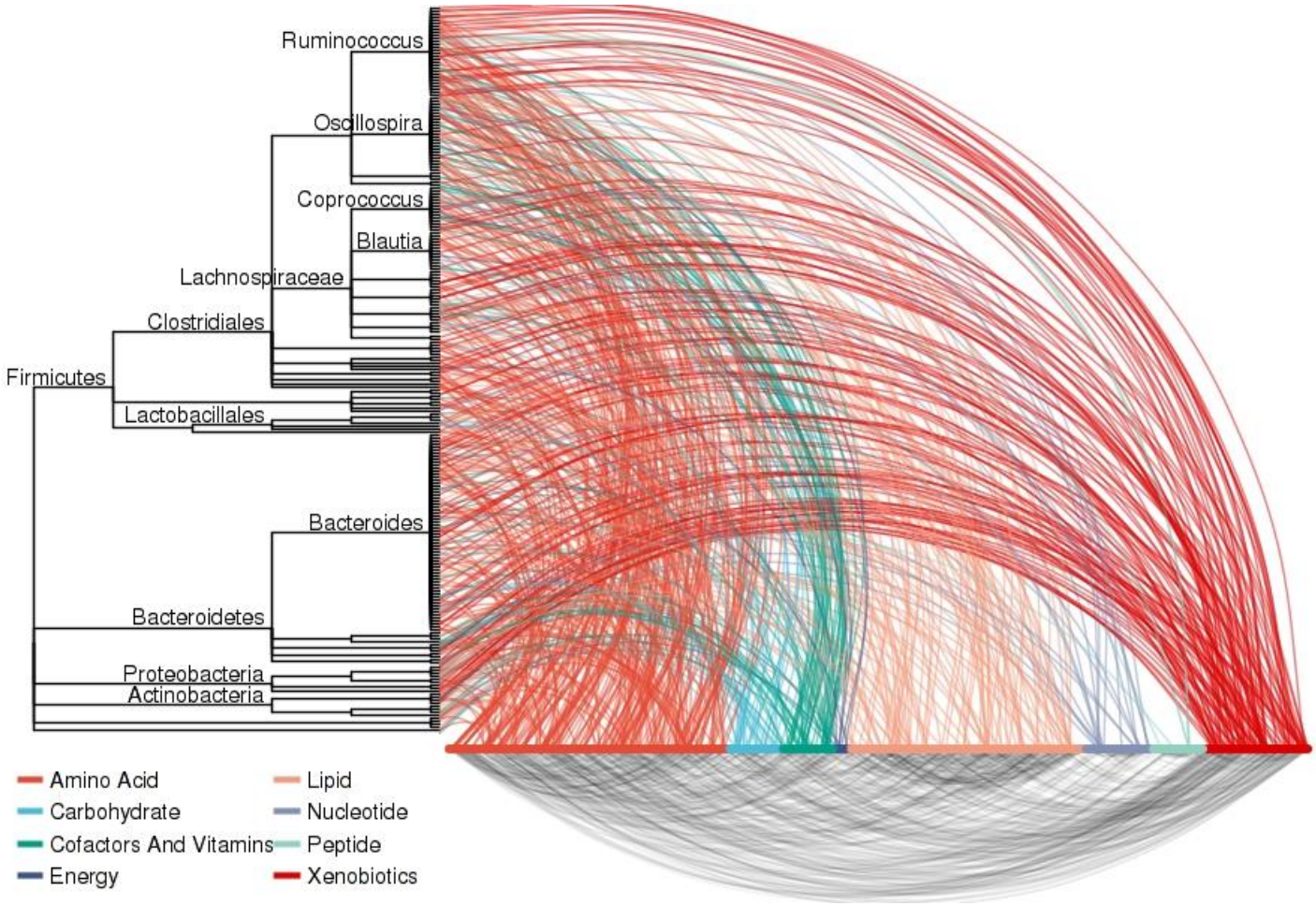
Each panel shows the qq-plot for one of the (a-c) three metabolites and (d) metabolite ratio, which have a genome-wide significant association with a genetic locus in the discovery cohort (n=739). P-values were calculated using the score test implemented in GEMMA.



Supplementary Figure 3

Associations of fecal metabolites with caffeine metabolism

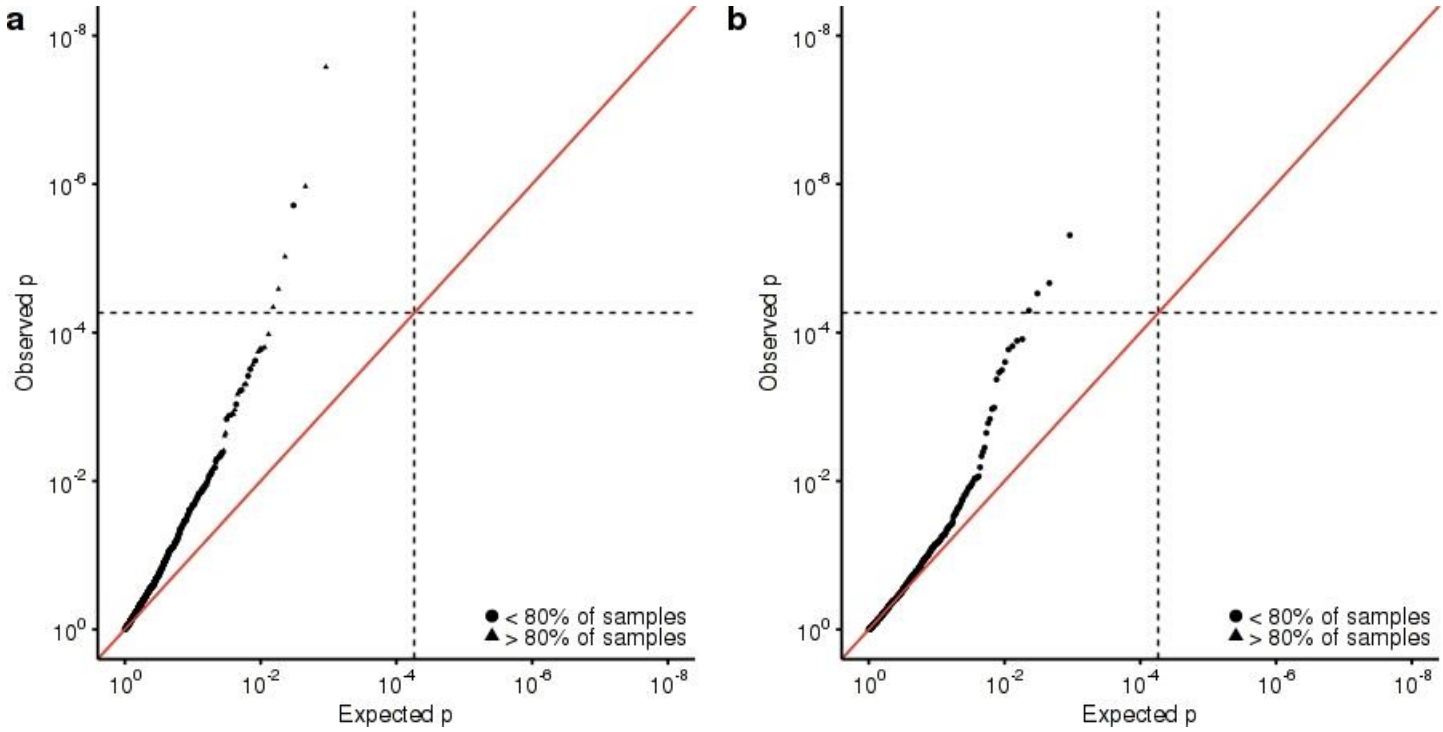
(a) Caffeine metabolism pathway showing the generation of the two metabolites that form the fecal metabolite ratio associated with *NAT2* genetic variants. (b) Box plot showing the relationship between circulating caffeine (bottom versus top tertile) and the metabolite ratio 1,3-dimethylurate / 5-acetylamino-6-amino-3-methyluracil (n=444). (c) Box plot showing the relationship between coffee intake (from food frequency questionnaires) and 1,3-dimethylurate / 5-acetylamino-6-amino-3-methyluracil (n=676). P-values were calculated from linear mixed models.



Supplementary Figure 4

Multivariate dependencies of fecal metabolites and the gut microbiome

We used a Gaussian graphical model to illustrate multivariate dependencies of fecal metabolite levels and gut microbes (n=644). Microbes on the y-axis are ordered by taxonomy, metabolites on the x-axis by pathway and hierarchical clustering of the partial correlation matrix. Connections indicate significant (FDR < 5%) shrinkage partial correlations of fecal metabolites and microbial OTUs, given all other metabolites and microbes in the model. Edges are colored by the metabolic pathway.



Supplementary Figure 5

Effect of storage time on fecal metabolite levels

To assess the effect of storage (a) in the participants' fridge before being stored in the TwinsUK Biobank and (b) in the freezer at -80°C before being analyzed, we calculated linear regression models of fecal metabolites against both measures (n=786). Here we present qq-plots where the dashed lines indicate Bonferroni-cutoff.

Supplemental Table 1. Study population characteristics.

	Discovery	Replication
n	786	230
Zygosity (MZ/DZ/Singleton)	296/310/180	0/110/120
Age	65.2 ± 7.6	66.9 ±8.6
Gender (female)	734 (93.4%)	226 (98.3%)
BMI	26.1 ± 4.7	27.2 ± 5.2