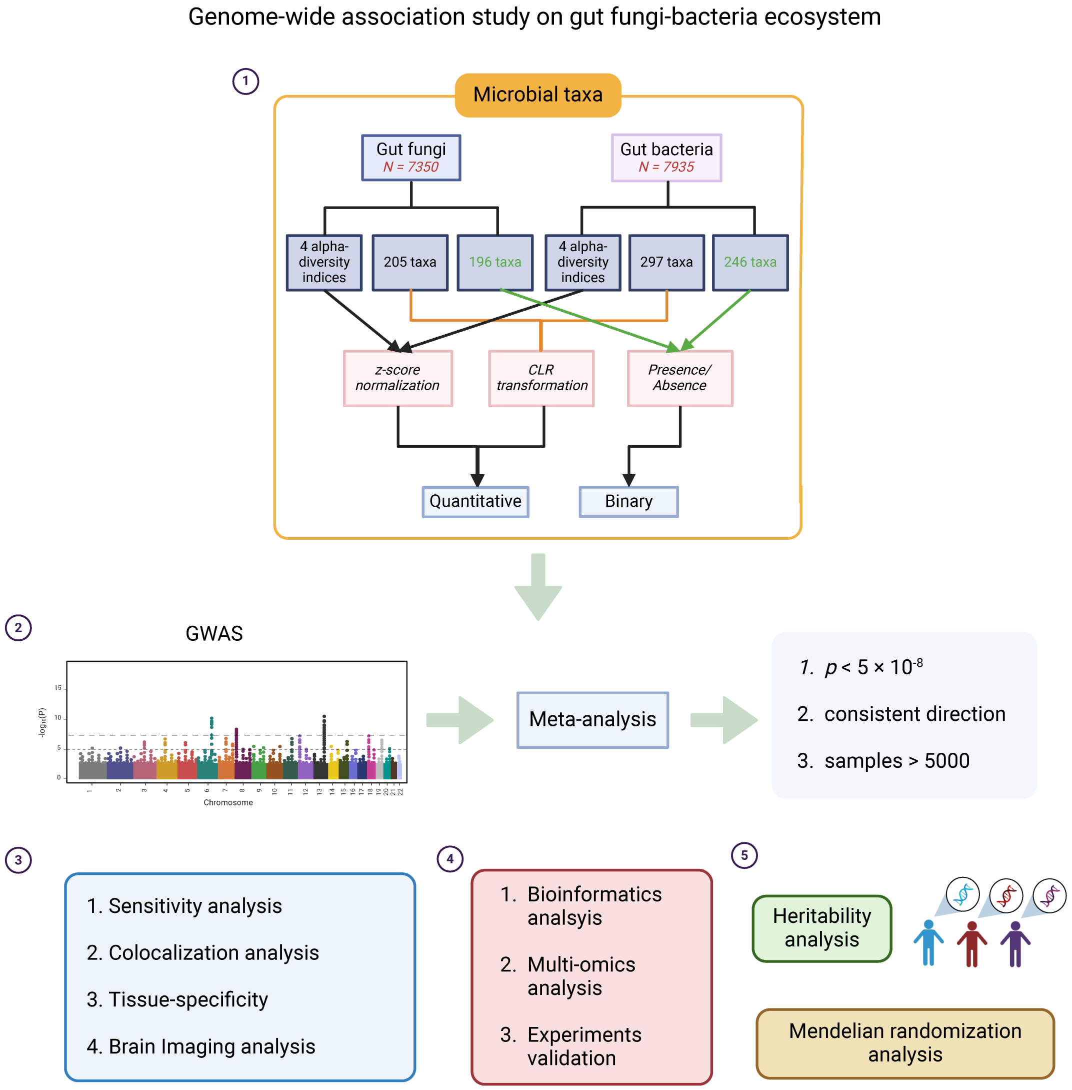

Fig. S1 Flowchart of the study design. GWAS, genome-wide association study; CLR, centered log ratio.

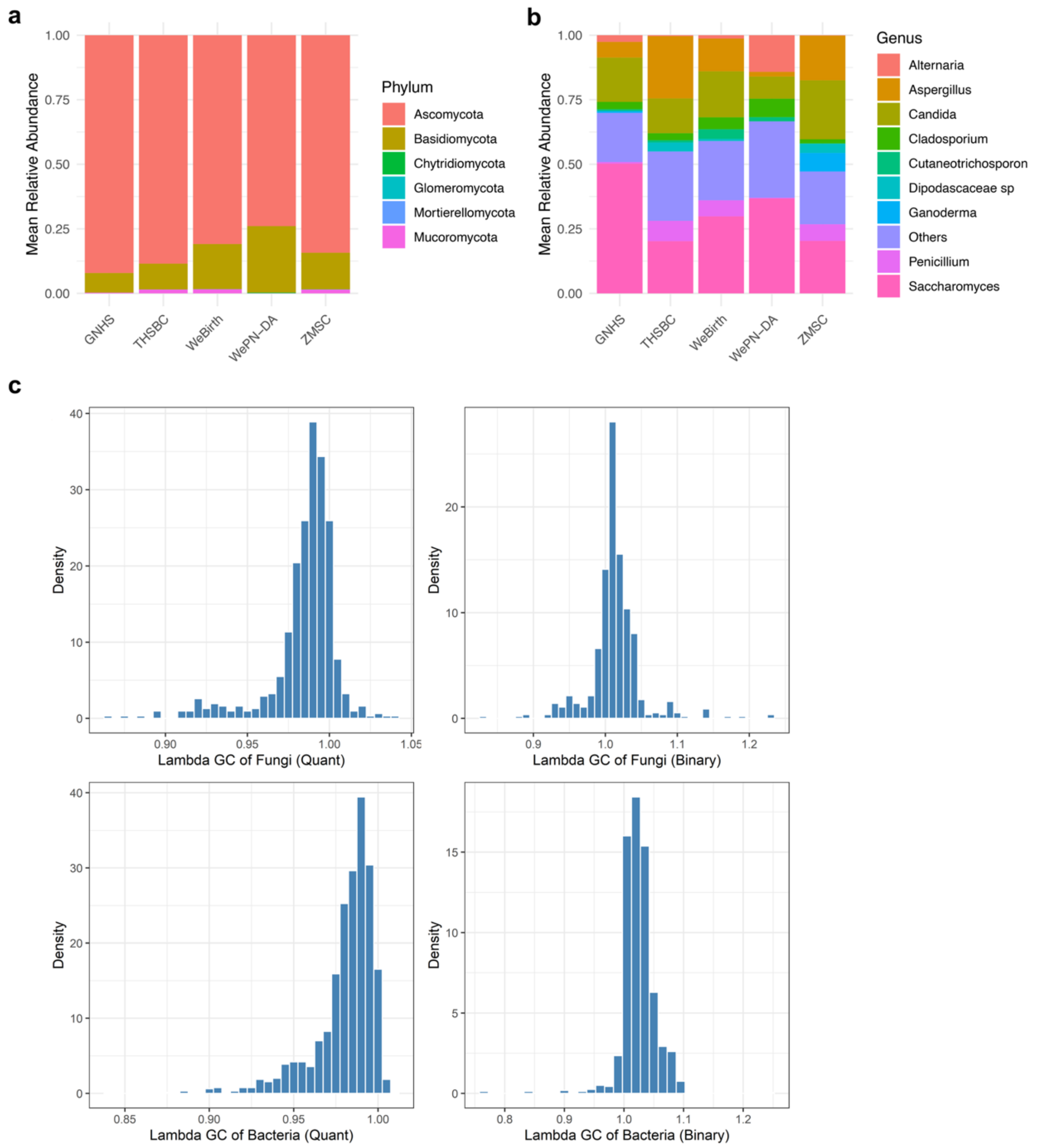

Fig. S2 Gut mycobiome composition across all cohorts. a-b, Bar plots showed the mean relative abundance of gut fungal phylum and genus among five cohorts. **c,** The distribution of genomic inflation factor (λGC).

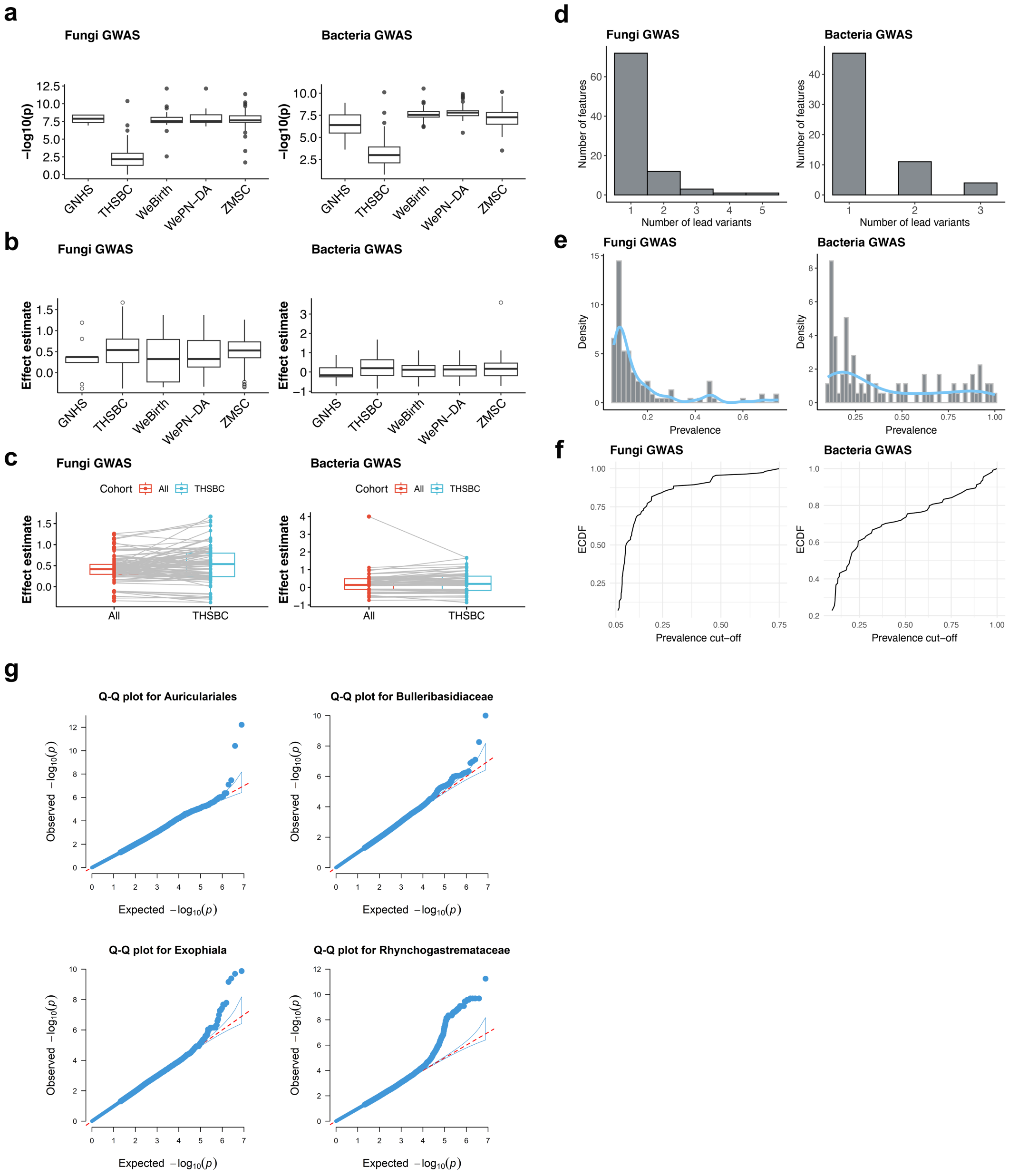

Fig. S3 Sensitivity analyses and summary of the associations identified in the present GWAS. a-c, Results of the leave-one-out analysis. Box plots showed the statistical significance (**a**) and effect estimate (**b**) of the associations identified in the present study after excluding each cohort. (**c**) Box plots showed the change in effect estimate of each association identified in the present study after excluding the THSBC participants. Dots connected by the grey line indicate identical associations. **d,** The number of lead variants per feature. **e,** Distribution of the GWAS association numbers by feature prevalence. **f**, The empirical cumulative distribution plot showed the proportion of GWAS association numbers under various feature prevalence cut-offs. **g,** Quantile-quantile (Q-Q) plot for the GWAS of gut fungi.

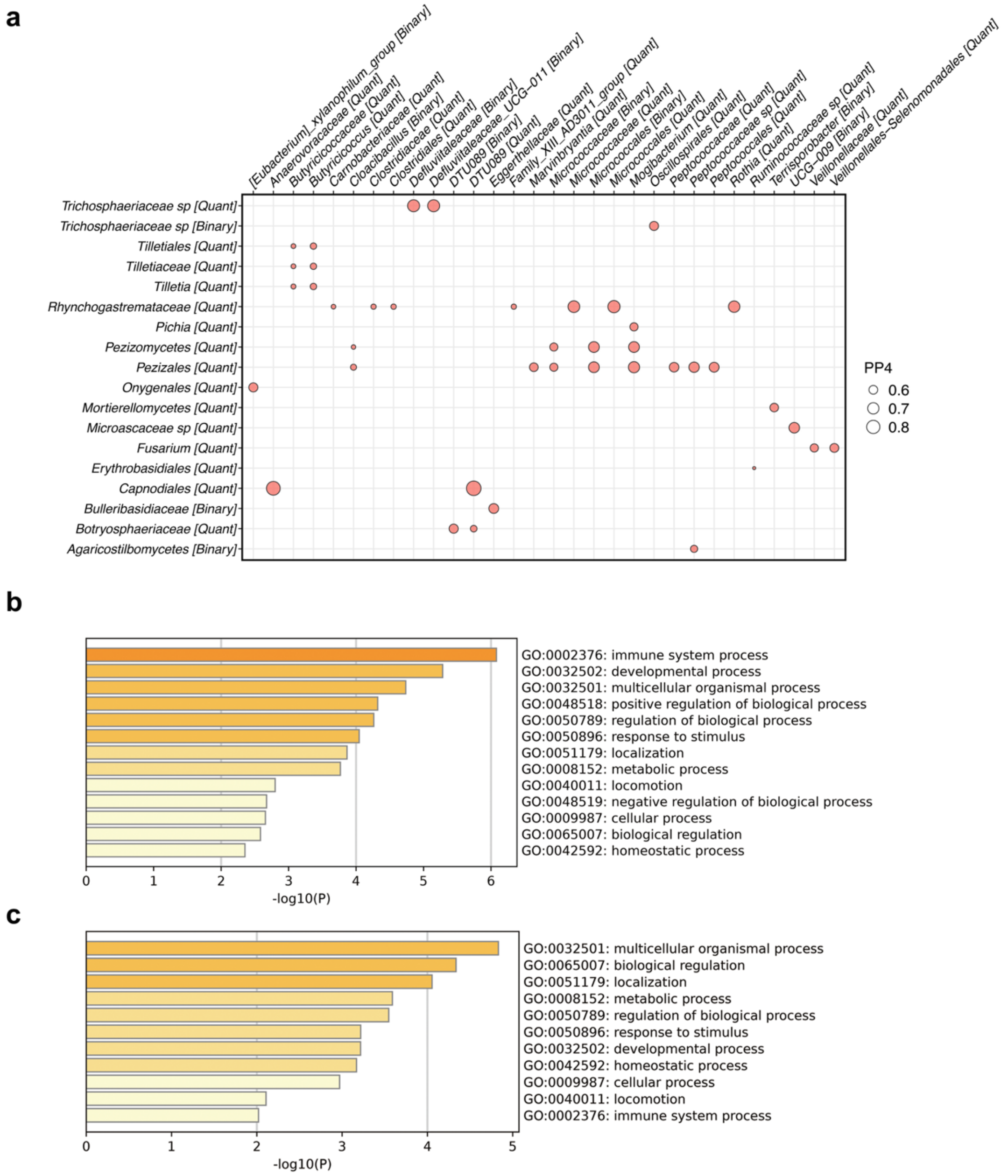

Fig. S4 Colocalization between the gut fungi and bacteria and Functional annotation based on the Metascape. a, The bubble plot showed the colocalization evidence of gut fungi and bacteria (PP4 > 0.5). The size of circles indicates the posterior probability of colocalization (PP4). **b-c,** Bar plots showed the enriched parent Gene Ontology biological processes, which was obtained based on the genes identified by the fungi (**b**) and bacteria GWAS (**c**). The bars were colored by the *p* values.

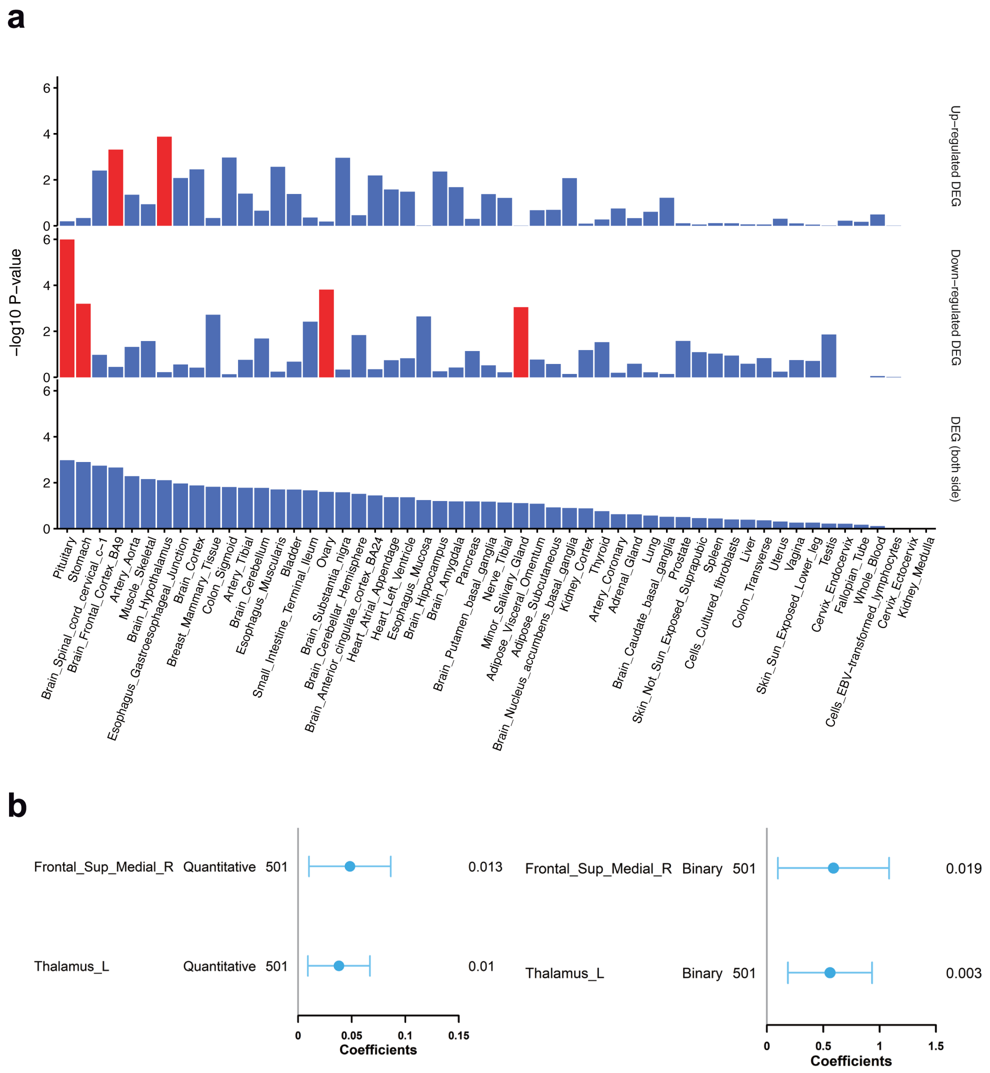

Fig. S5 Summary of the tissue-specificity. a, The bar plots showed the results of tissue specificity analysis targeting 54 tissue types using the GENE2FUNC module of the FUMA website. The input gene lists were obtained from the bacteria GWAS. Significant enrichment at Benjamini-Hochberg corrected *P* value ≤ 0.05 are colored in red. **b,** Forest plot for the associations between the abundance of *Eremothecium* and brain MRI structural volumes. DEG, Differentially Expressed Gene; Frontal_Sup_Medial_R, right superior frontal gyrus (medial); Thalamus_L, left thalamus.

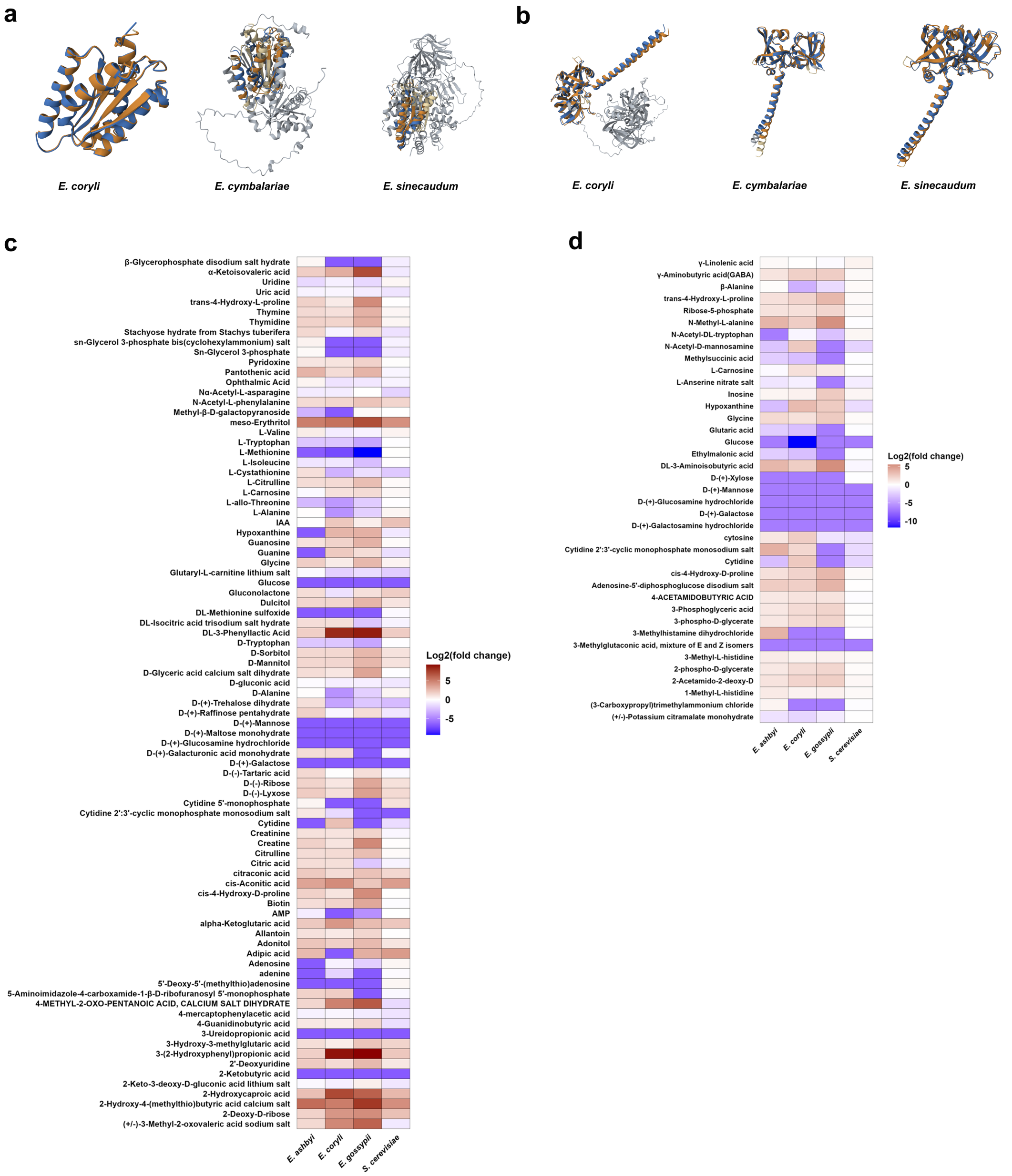

Fig. S6 Fungal strain analysis. a-b, Protein structural alignment between *Eremothecium* species and *Saccharomyces cerevisiae*. The plot shows the alignment of predicted protein structure with the best gene sequence similarity with RIB4 enzyme (**a**) and RIB5 enzyme (**b**). **c-d,** Metabolic profiles of the four gut fungal strains. The heatmap shows the fold change of metabolites compared with the control medium based on UPLC-MS/MS methods with negative ion mode (**c**) and positive ion mode (**d**).

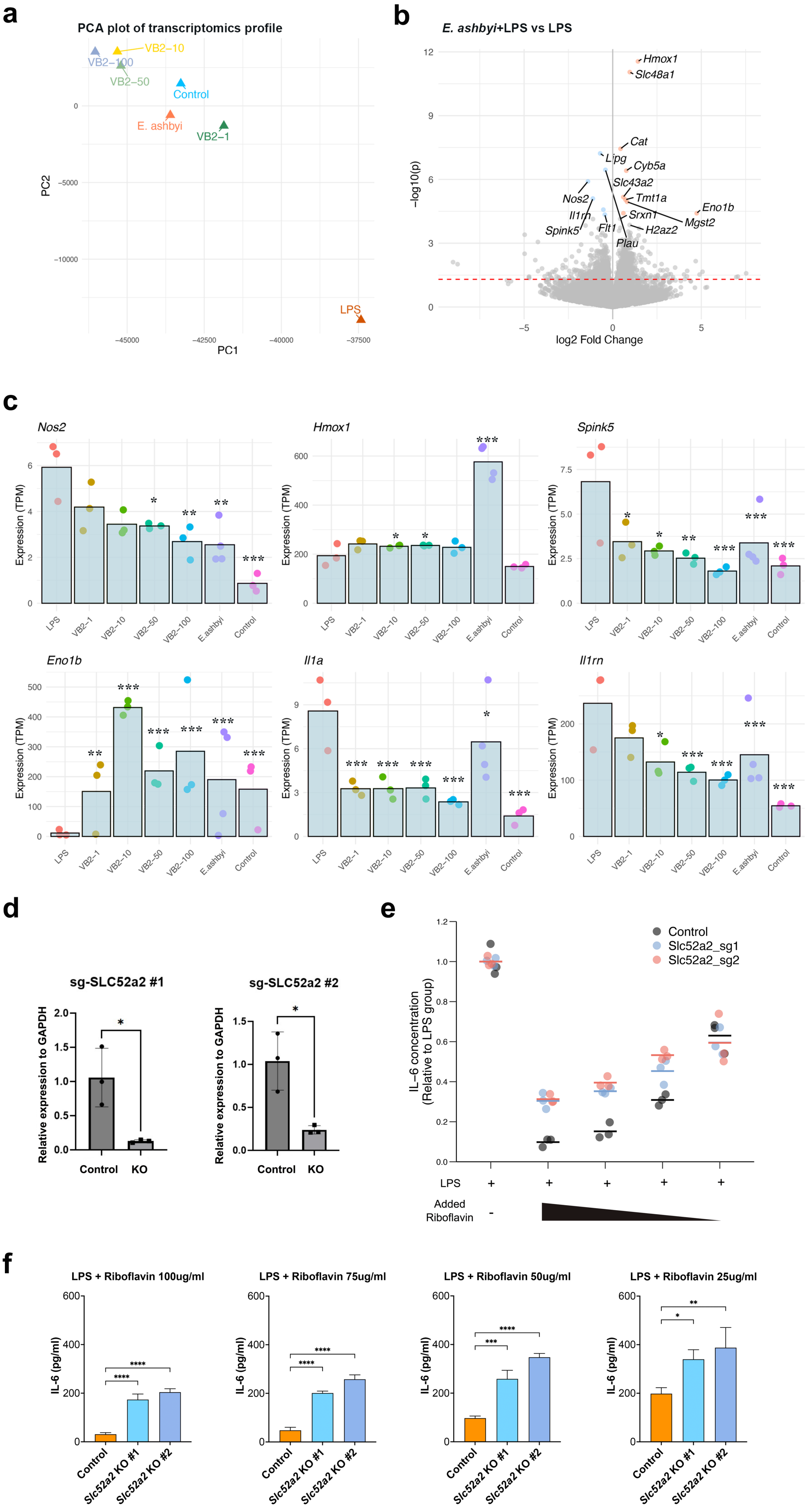
a
PCA plot of transcriptomics profile
VB2−10
VB2−100
VB2−50
Control
E. ashbyi
VB2−1
LPS
PC2
PC1
b
E. ashbyi+LPS vs LPS
Hmox1
Slc48a1
Cat
Lipg
Cyb5a
Slc43a2
Nos2
Tmt1a
Il1rn
Srxn1
Eno1b
Mgst2
Spink5
Flt1
H2az2
Plau
−log10(p)
log2 Fold Change
c
Nos2
Hmox1
Spink5
Eno1b
Il1a
Il1rn
Expression (TPM)
LPS
VB2−1
VB2−10
VB2−50
VB2−100
E.ashbyi
Control
d
sg-SLC52a2 #1
sg-SLC52a2 #2
Relative expression to GAPDH
Control
KO
e
Control
Slc52a2_sg1
Slc52a2_sg2
IL−6 concentration (Relative to LPS group)
LPS
Added Riboflavin
f
LPS + Riboflavin 100ug/ml
LPS + Riboflavin 75ug/ml
LPS + Riboflavin 50ug/ml
LPS + Riboflavin 25ug/ml
IL-6 (pg/ml)
Control
Slc52a2 KO #1
Slc52a2 KO #2

Fig. S7 Transcriptome changes in BV2 microglia cells and *Slc52a2* knockout experiments. a, PCA plot of the whole transcriptomics profile under each condition (VB2-1, 1 μg/mL riboflavin; VB2-10, 10 μg/mL; VB2-50, 50 μg/mL; VB2-100, 100 μg/mL). The triangles indicate the centroids of each group. **b,** Volcano plot of the differential gene expression between *E. ashbyi*+LPS and LPS group. Only significantly changed genes (FDR < 0.05) were colored according to direction. The dashed line indicates the threshold of $p = 0.05$. **c,** Bar plot showed the gene expressions after the LPS intervention of each group. The asterisks indicate the statistical significance of each group in DESeq2 results compared to the LPS group. **d,** The bar plot showed the relative expression of Slc52a2 in BV2 microglia, which indicates the knockout efficiency. **e,** The dot plot showed the IL-6 concentration relative to the LPS group. **f,** The bar plot showed the levels of IL-6 in each group. One-way analysis of variance (ANOVA) was used followed by Dunnett post hoc test. **** $p < 0.0001$, *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$. PCA, principal component analysis. VB2, vitamin B2. FDR, false-discovery rate. LPS, lipopolysaccharide; KO, knockout.