Supplementary materials for

Microbiome-metabolomics analysis reveals the potential effect of verbascoside in alleviating cognitive impairment in db/db mice

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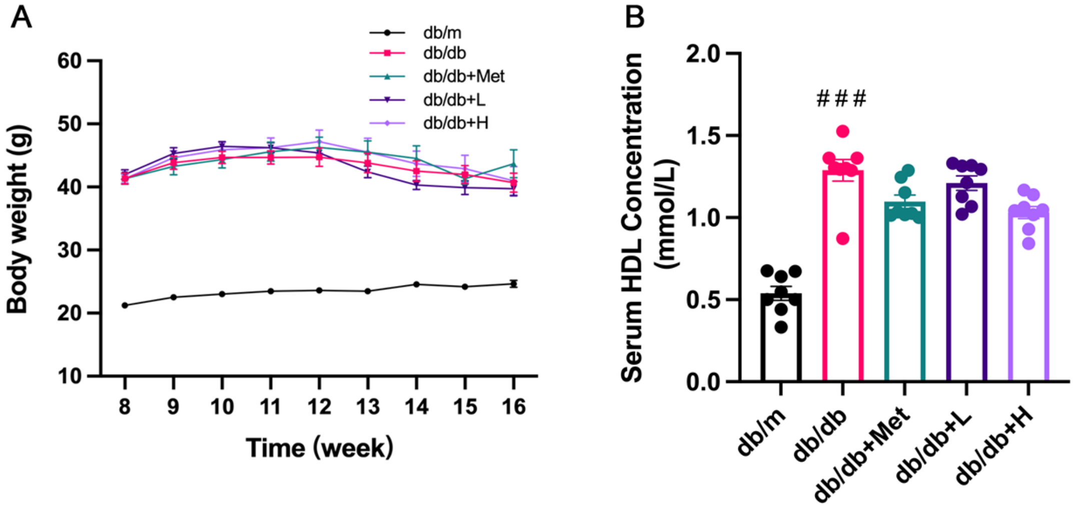
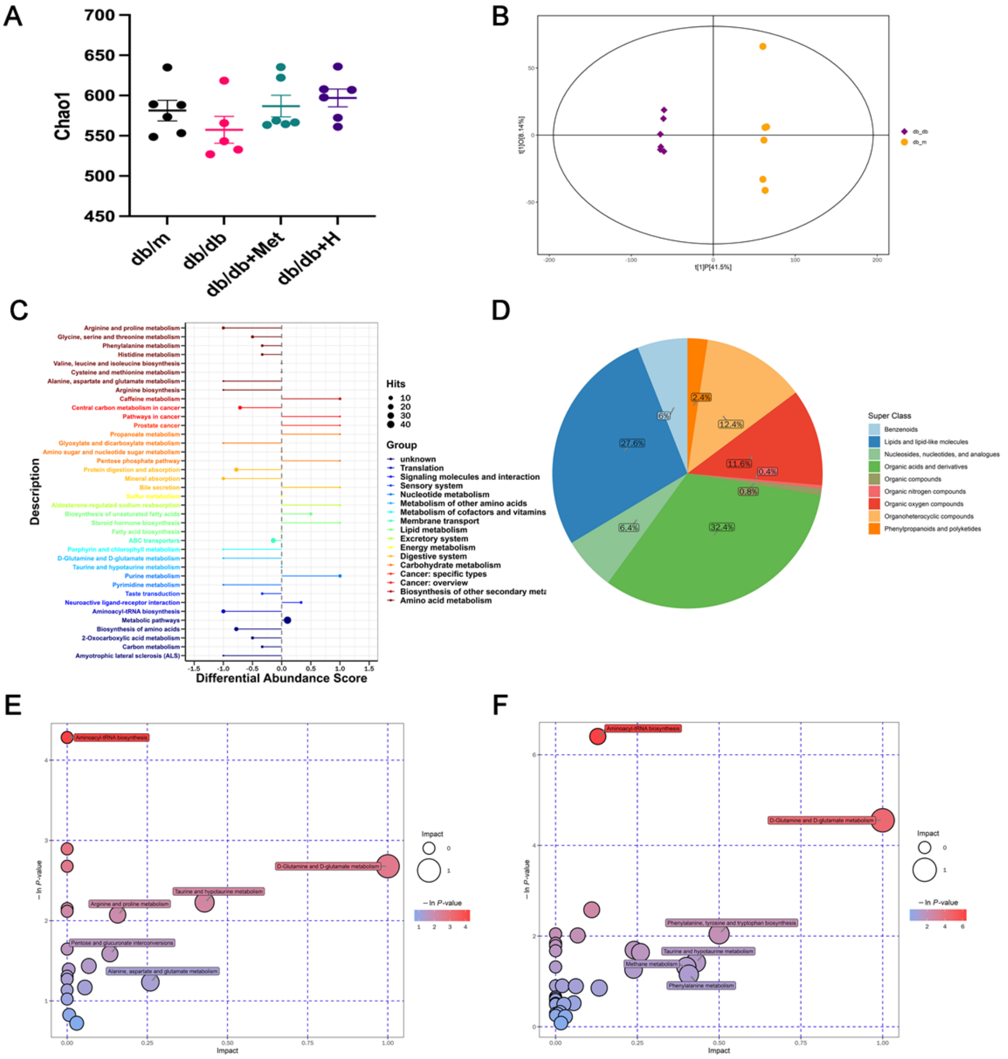


Supplementary Fig.1 Effect of verbascoside on body weight and HDL in db/db mice. (A) Changes in body weight of mice; (B) Serum HDL concentration. All statistical data are expressed as mean±SEM, n=8/group. compared to db/m group: $^{\#\#\#}p<0.001$.



Supplementary Fig.2 Effect of verbascoside on intestinal flora and serum metabolites in db/db mice. (A) Chao1 index of intestinal flora in each group; (B) The scatter plot obtained by the orthogonal partial least squares-discriminant analysis (OPLS-DA) model of serum untargeted metabolomics data in negative ion mode; (C) Differential abundance analysis of differential metabolites KEGG pathway; (D) The percentage of serum metabolite classification; (E) Bubble plots of db/db+H VS db/db metabolic pathway enrichment analysis; (F) Bubble plots of db/db VS db/m metabolic pathway enrichment analysis.

Supplementary Table 1

Significance analysis of β diversity between groups (Unweighted unifrac significance, anosim)

Group 1	Group 2	Sample size	Permutations	R	p-value	q-value
db.8w	dbm.8w	12	200	0.014814815	0.437810945	0.454026165
Met.8w	db.8w	12	200	0.057407407	0.243781095	0.325041459
H.8w	db.8w	12	200	0.259259259	0.014925373	0.052238806

The value of R ranges from 0 to 1. The closer it is to 1, the greater the difference between groups.

Supplementary Table 2

Metabolite pathway changes in serum (db/db+H VS db/db, NEG mod)

Pathway	Total	Hits	Raw p	-ln(p)	Holm adjust	FDR	Impact
Aminoacyl-tRNA biosynthesis	69	4	0.013812	4.2822	1	1	0
Lysine biosynthesis	4	1	0.055331	2.8944	1	1	0
Biotin metabolism	5	1	0.068702	2.678	1	1	0
D-Glutamine and D-glutamate metabolism	5	1	0.068702	2.678	1	1	1
Taurine and hypotaurine metabolism	8	1	0.10774	2.228	1	1	0.42857
Biosynthesis of unsaturated fatty acids	42	2	0.11644	2.1503	1	1	0
Nitrogen metabolism	9	1	0.12041	2.1169	1	1	0
Arginine and proline metabolism	44	2	0.12583	2.0728	1	1	0.15747
Histidine metabolism	15	1	0.19289	1.6457	1	1	0
Pentose and glucuronate interconversions	16	1	0.2044	1.5877	1	1	0.13333
Pentose phosphate pathway	19	1	0.23801	1.4354	1	1	0.06757
Purine metabolism	68	2	0.24876	1.3913	1	1	0.0048
Butanoate metabolism	22	1	0.27027	1.3083	1	1	0
Lysine degradation	23	1	0.28074	1.2703	1	1	0
Alanine, aspartate and glutamate metabolism	24	1	0.29105	1.2342	1	1	0.25949
Glutathione metabolism	26	1	0.31127	1.1671	1	1	0.05534
Porphyrin and chlorophyll metabolism	27	1	0.32118	1.1358	1	1	0
Glycine, serine and threonine metabolism	31	1	0.35945	1.0232	1	1	0
Tryptophan metabolism	40	1	0.43821	0.82506	1	1	0.00642
Primary bile acid biosynthesis	46	1	0.48551	0.72256	1	1	0.02976

Hits represent the matched number of metabolites in the pathway. Raw p represents the original p-value calculated from the enrichment analysis. Holm p represents the p-value further adjusted using the Holm–Bonferroni method. FDR (false discovery rate) represents the p-value adjusted using the false discovery rate.