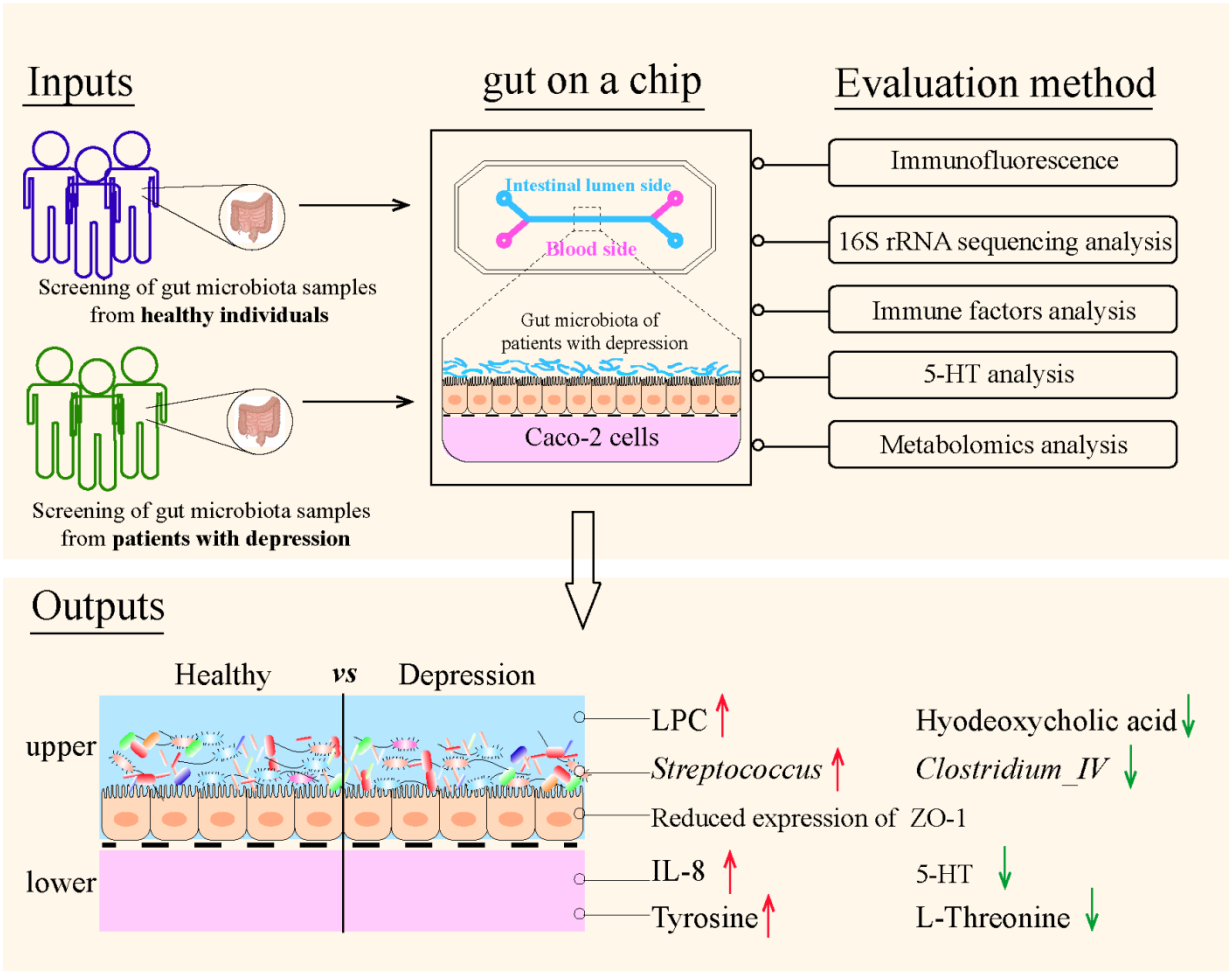
Electronic Supplementary Material (ESI) for Lab on a Chip.

Graphical Abstract



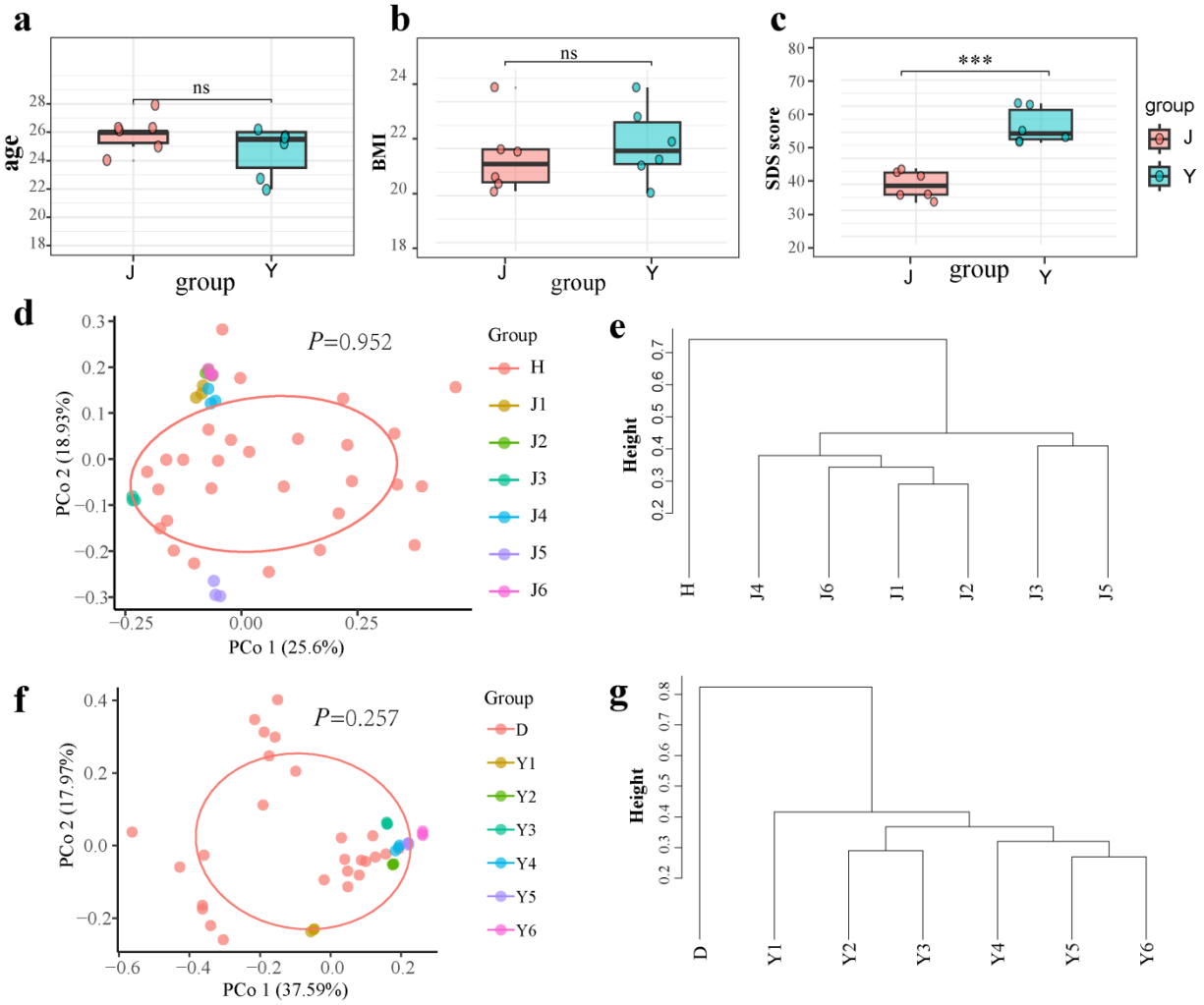


Fig.1 Recruitment of healthy volunteers and depression patients, along with the selection of their gut microbiota samples.

a-c, Age distribution, BMI statistics, and SDS score statistics for the healthy group and the depression group.（n=6，data are presented as mean ± s.d.; significance was calculated by one-way ANOVA; ***$P <$ 0.001; ns, not significant）."J" represents healthy volunteers, "Y" represents patients with depression.

d, Beta diversity analysis of gut microbiota between the healthy group samples (n=6) and the healthy individual database (n=28) was conducted. Significance of differences between the healthy group samples and the healthy individual database was evaluated using ANOSIM-based multivariate analysis of variance. J1-J6: 6 healthy volunteers;

e, Hierarchical clustering analysis of gut microbiota based on weighted UniFrac distances was performed for samples from the healthy group (n=6) and the healthy individual database (n=28). J1-J6: 6 healthy volunteers;

f, Beta diversity analysis of gut microbiota between samples from the depression group (n=6) and the depression patient database (n=26) was conducted. Significance of differences between samples from the depression group and the depression patient database was evaluated using ANOSIM-based multivariate analysis of variance.Y1-Y6: 6 depressed patients.

g, Hierarchical clustering analysis of gut microbiota based on weighted UniFrac distances was conducted for samples from the depression group (n=6) and the depression patient database (n=26).Y1-Y6: 6 depressed patients.

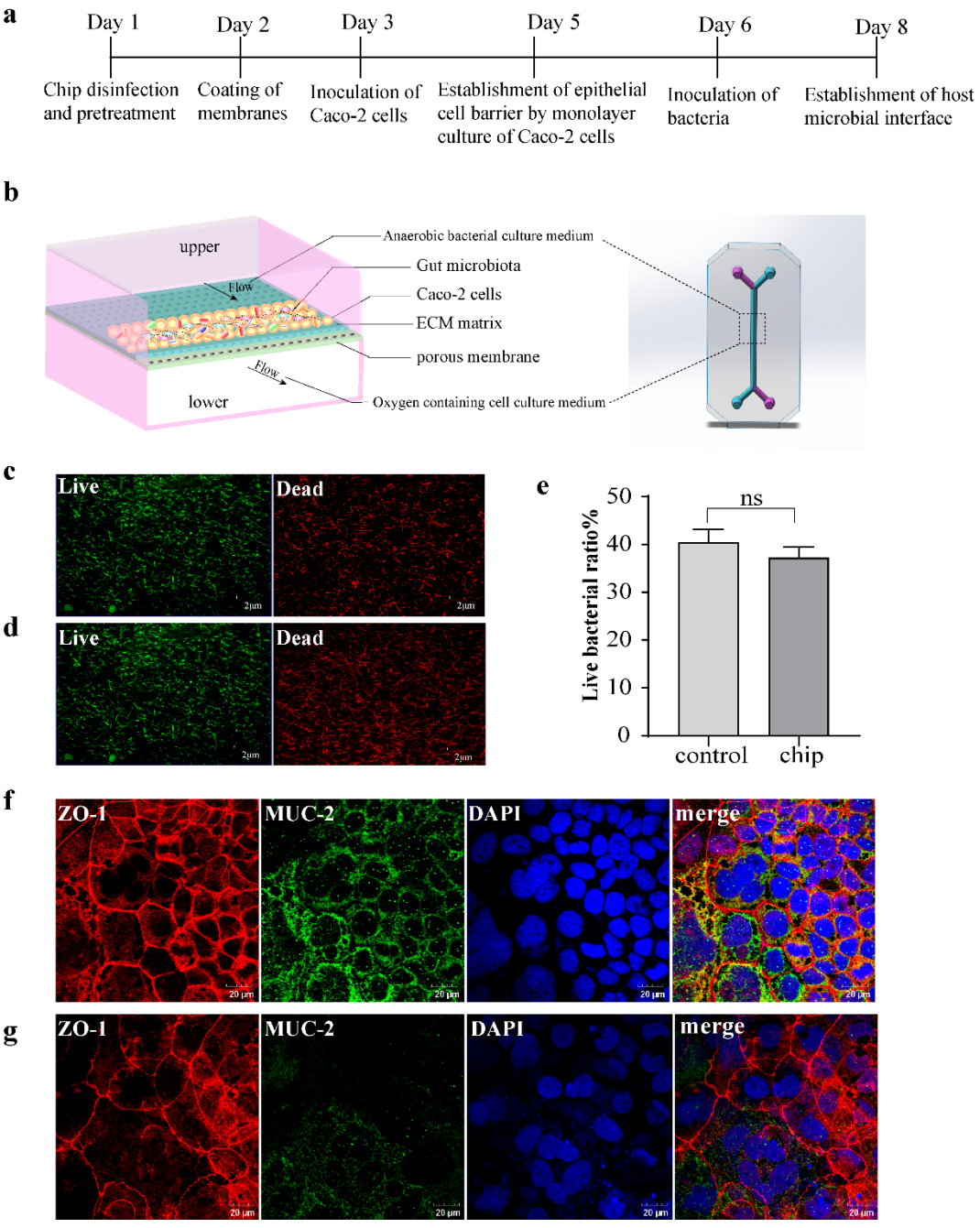


Fig.2 Analysis of co cultivation of Caco-2 cells on chip with specific anaerobic bacteria *Faecalibacterium praussnitzii (F. prausnitzii)*.

a, Timeline overview of experimental design.

b, A schematic diagram of the chip channel design and a photograph of the Intestine-Chip.

c,Staining diagram of live and dead bacteria cultured under conventional anaerobic conditions for *Faecalibacterium praussnitzii (F. prausnitzii).* Scale bar: 2μm.

d, Staining map of *F.P* co-cultured with caco-2 cells on the chip.Scale bar: 2μm.

e, The proportion of viable bacteria cultured on the chip and under conventional anaerobic conditions for

Faecalibacterium praussnitzii (F. prausnitzii) (n=5).

f, Caco-2 cells form a tight monolayer with tight junctions (ZO-1) on the chip surface, and secrete a mucus layer (Muc-2). DAPI is used to stain the cell nuclei (blue). Scale bar: 20μm.

g, After co-culturing caco-2 cells on the chip with *Faecalibacterium praussnitzii (F.P)*, immunofluorescence staining of caco-2 cells was performed.Scale bar: 20μm.

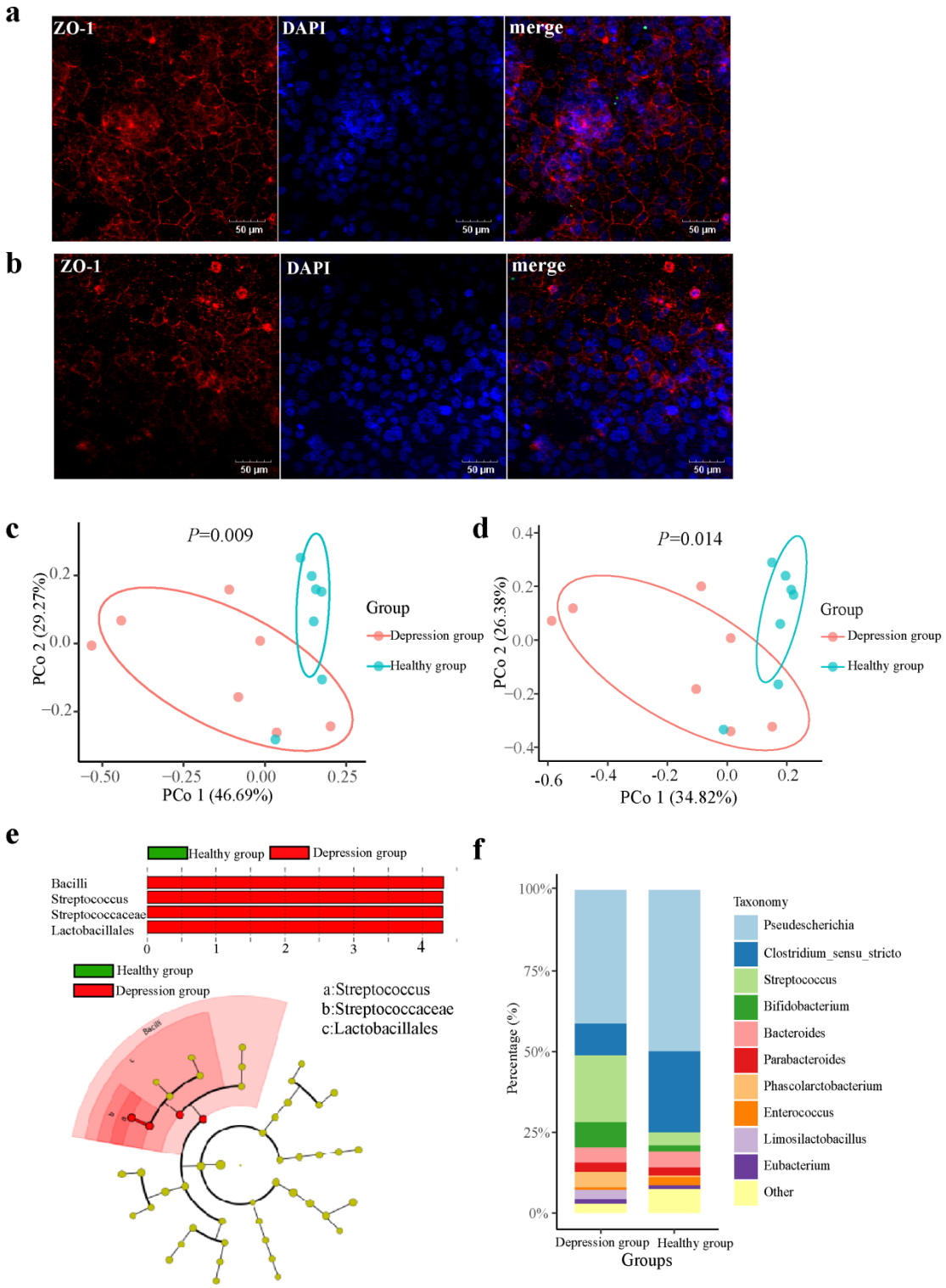


Fig.3 Construction of a depression-on-gut--chip(DoGC) and 16s DNA analysis.

a, Co-cultivation of healthy volunteers (J1) gut microbiota and caco-2 cells on the chip. Caco-2 cells form a tight monolayer with tight junctions (ZO-1) on the chip surface, DAPI is used to stain the cell nuclei (blue). Scale bar: 50μm.

b, Co-cultivation of depression patients(Y1) gut microbiota and caco-2 cells on the chip.Caco-2 cells form a tight monolayer with tight junctions (ZO-1) on the chip surface, DAPI is used to stain the cell nuclei (blue). Scale bar: 50μm.

c, Compared to the healthy group, PCoA analysis based on Bray-Curtis distance of gut microbiotawas performed in the DoGC. ANOSIM analysis was used to test the inter-group differences and compare the community structures between the two groups.

d, PCoA analysis based on unifrac distance was conducted, and ANOSIM analysis was used to test inter-group differences and compare community structures between the two groups.

e, Compared to the healthy group, LefSe analysis of gut microbiota in the DoGC effluents.

f, Compared to the healthy group, the taxonomic abundance of gut microbiota at the genus level in DoGC.

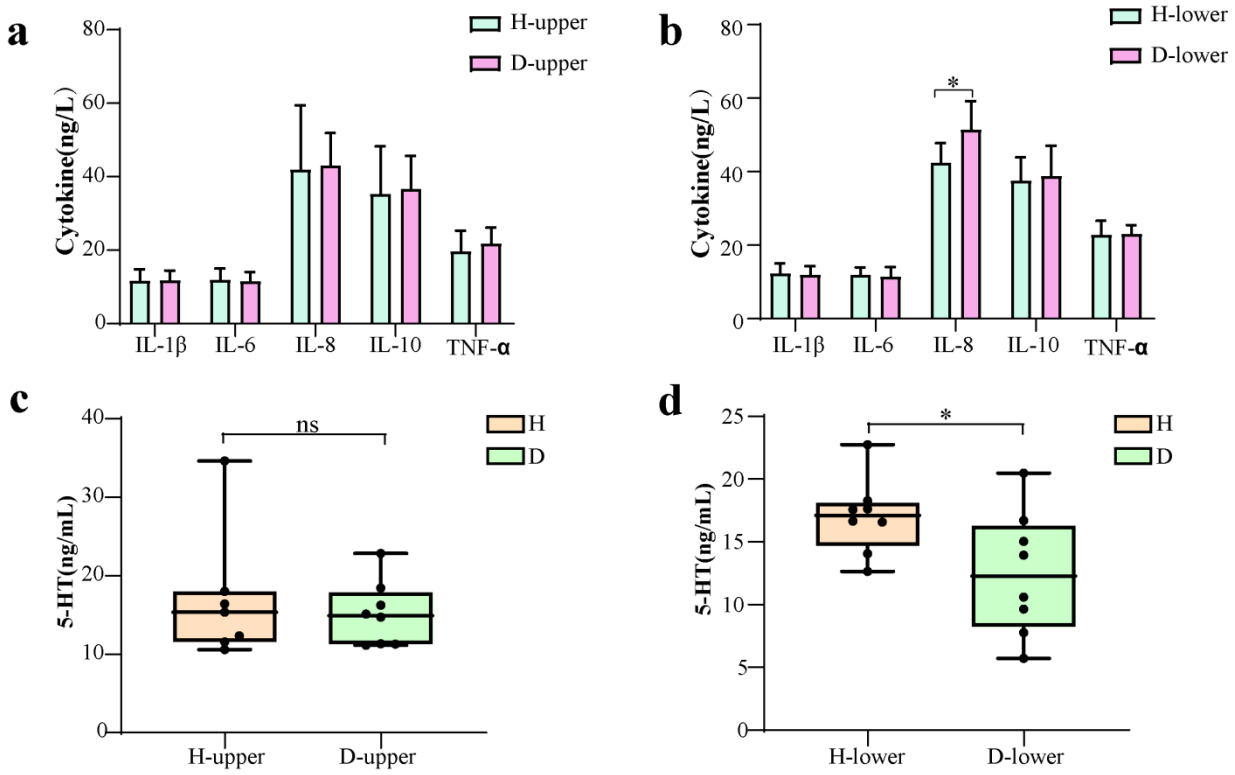


Fig.4 Analysis of cytokine levels and 5-HT levels in the effluent of depression intestinal chip.

a, Compared to the healthy group, Changes in cytokine levels in the upper effluents from the DoGC (n=8, '*' indicates $P < 0.05$, 'ns' indicates $P > 0.05$).

b, Compared to the healthy group, Changes in cytokine levels in the lower effluents from the DoGC. (n=8, '*' indicates $P < 0.05$, 'ns' indicates $P > 0.05$).

c, Compared to the healthy group, Changes in neurotransmitter 5-HT levels in the upper effluents from the DoGC. (n=8, "*" indicates $P < 0.05$, "ns" indicates $P > 0.05$).

d, Compared to the healthy group, Changes in neurotransmitter 5-HT levels in the lower effluents from the DoGC.(n=8, '*' indicates $P < 0.05$, 'ns' indicates $P > 0.05$).

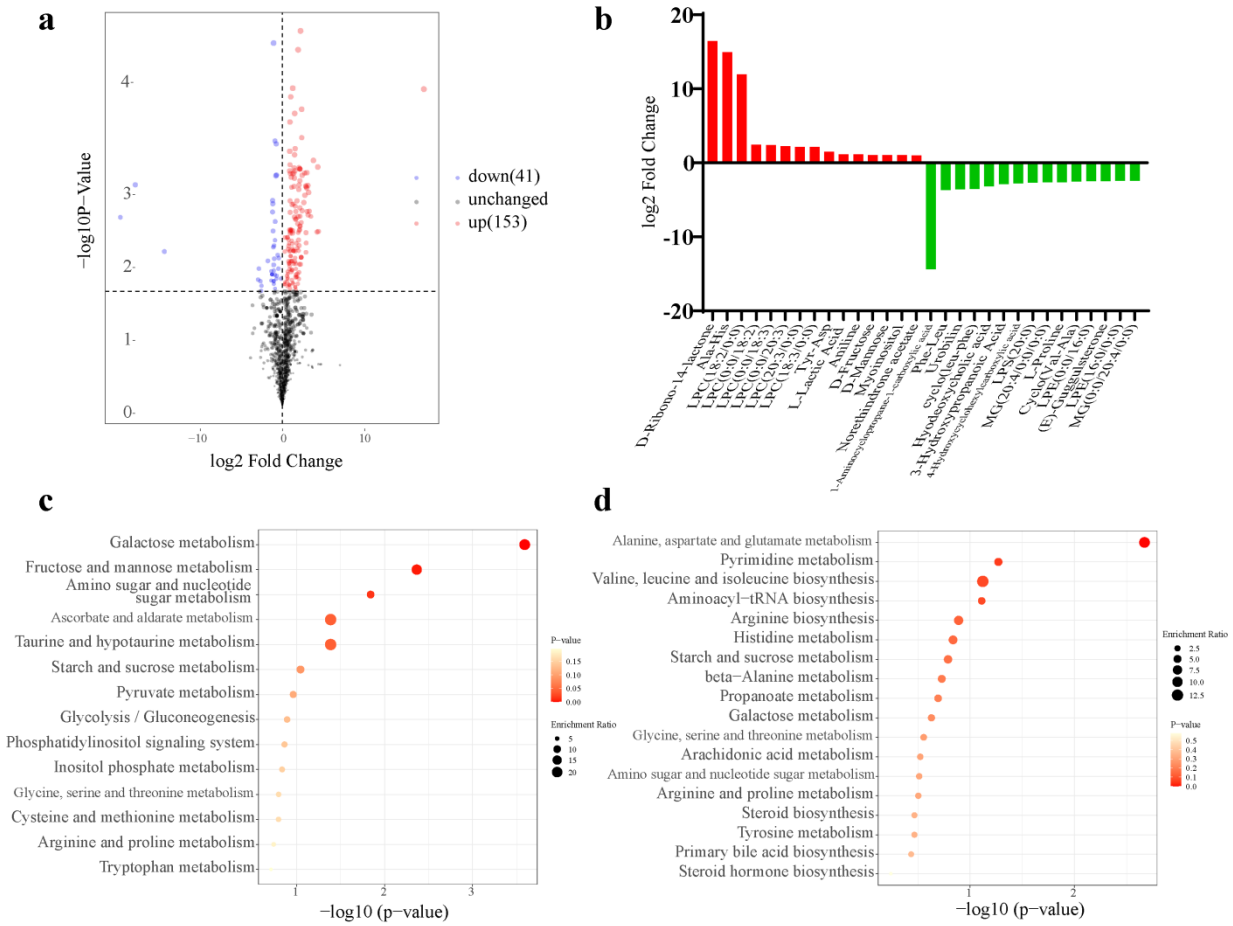


Fig.5 Metabolism characteristics of upper effluent in DoGC and its related KEGG enrichment pathways.

a, Volcano plots depicting differentially identified metabolites in DoGC. Red dots, upregulated metabolites($P < 0.05$). Blue dots, downregulated metabolites($P < 0.05$). Grey dots, nondifferentially expressed metabolites($P > 0.05$).

b, Compared to the healthy group, the top 10 differential metabolites in DOGC. Red: up-regulation, Green:down-regulation.

c, Compared to the healthy group, the upregulated differential metabolites in DoGC are enriched in KEGG.

d, Compared to the healthy group, the downregulated differential metabolites in DoGC are enriched in KEGG.

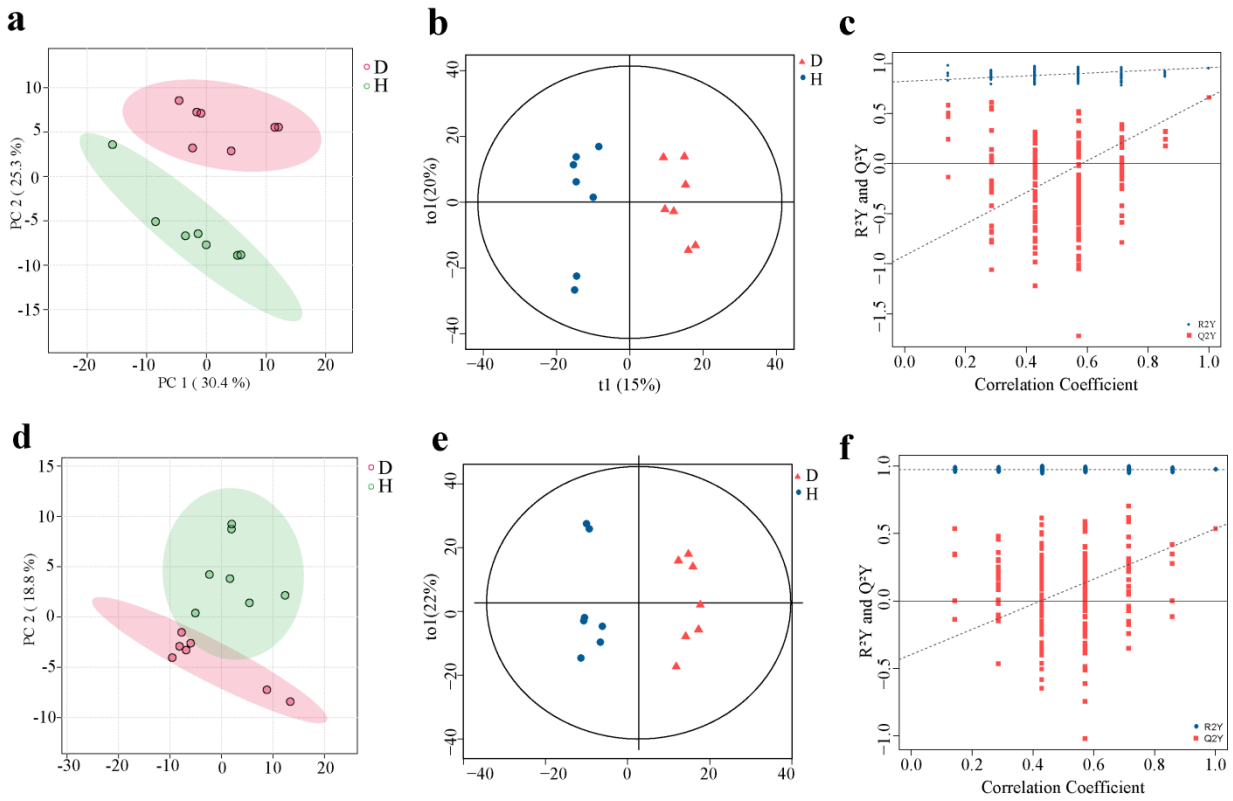


Fig.S1 Construction and analysis of metabolomic models for chip effluents from healthy and depressed groups.

a, Compared to the healthy group,the PCA analysis results of upper effluents from the DoGC.

b, Compared to the healthy group,the OPLS-DA score plots of upper effluents from the DoGC.

c, Compared to the healthy group,the OPLS-DA model permutation test plots for upper effluents from the DoGC.

d, Compared to the healthy group,the PCA analysis results of lower effluents from the DoGC.

e, Compared to the healthy group,the OPLS-DA score plots of lower effluents from the DoGC.

f, Compared to the healthy group,the OPLS-DA model permutation test plots for lower effluents from the DoGC.

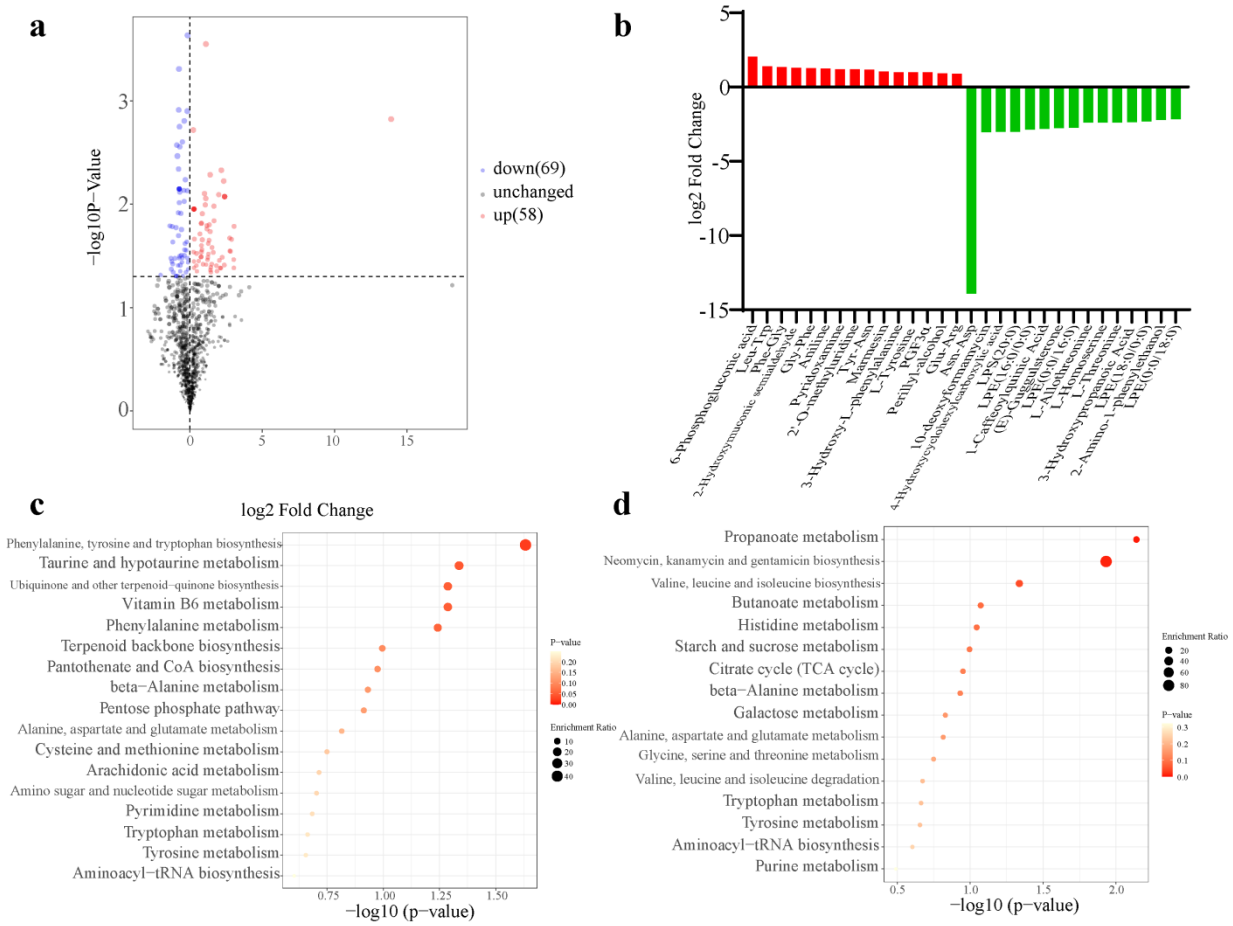


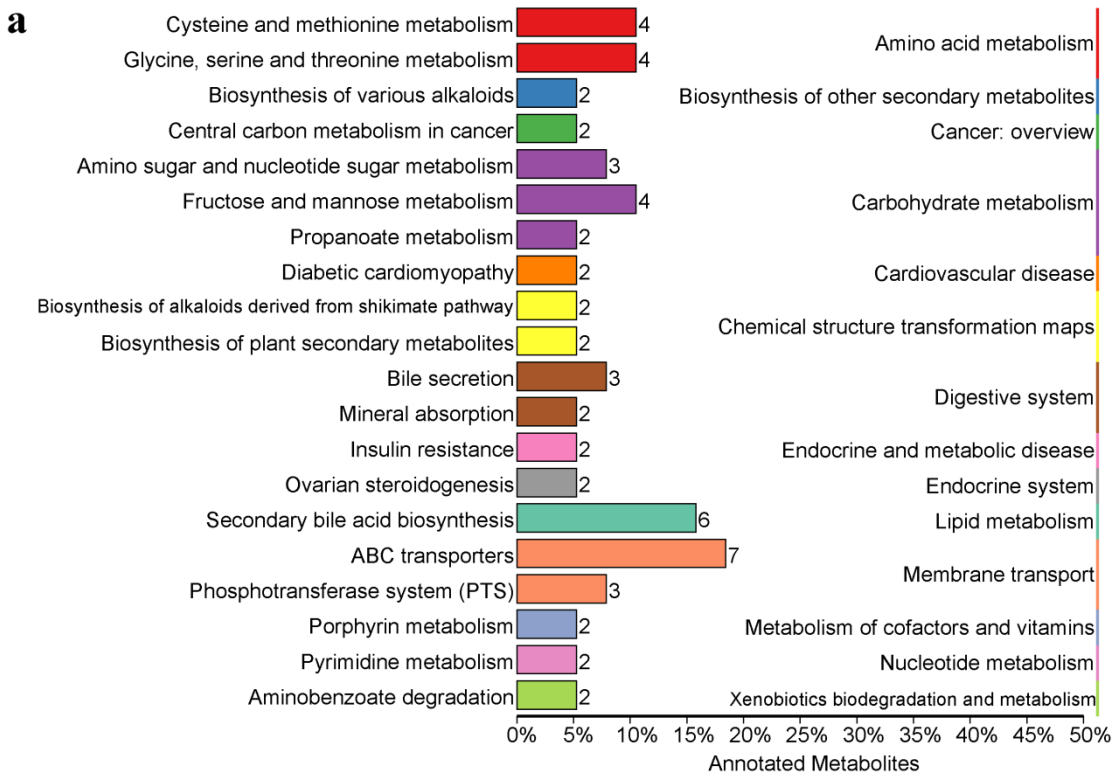
Fig.S2 Metabolism characteristics of lower effluent in DoGC and its related KEGG enrichment pathways.

a, Volcano plots depicting differentially identified metabolites in DoGC. Red dots, upregulated metabolites($P < 0.05$). Blue dots, downregulated metabolites($P < 0.05$). Grey dots, nondifferentially expressed metabolites($P > 0.05$).

b, Compared to the healthy group, the top 10 differential metabolites in DOGC. Red: up-regulation, Green:down-regulation.

c, Compared to the healthy group, the upregulated differential metabolites in DoGC are enriched in KEGG.

d, Compared to the healthy group, the downregulated differential metabolites in DoGC are enriched in KEGG.



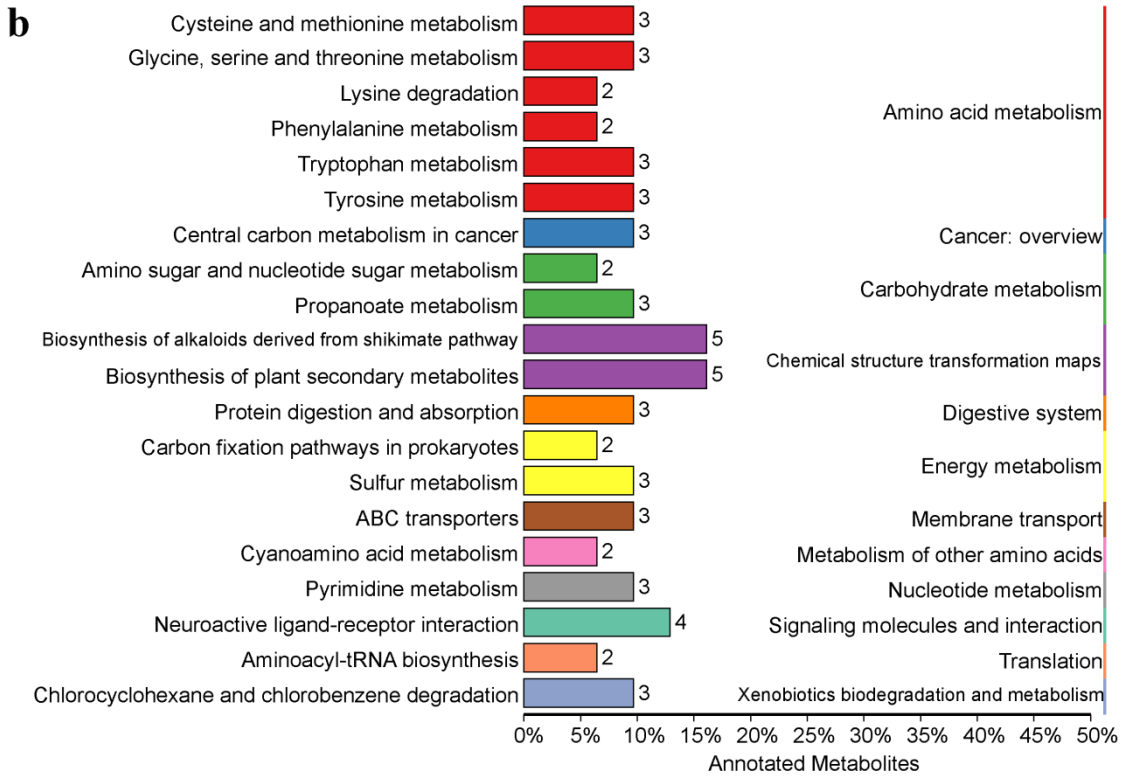


Fig.S3 Pathway classification of differentially identified metabolites in intestinal chip effluents.

a, Compared to the healthy group, differential metabolite pathway classification chart in upper effluent of DoGC.

b, Compared to the healthy group, differential metabolite pathway classification chart in lower effluent of DoGC.

Table S1. The SDS scores and depression severity levels for both the healthy volunteer group and the depression patient group are presented in the table.

Number	Sex	SDS scores	Depression level
J1	female	36	-
J2	female	45	-
J3	male	43	-
J4	male	46	-
J5	male	33	-
J6	female	36	-
Y1	female	71	severe
Y2	female	61	moderate
Y3	male	58	mild
Y4	female	57	mild
Y5	male	56	mild
Y6	male	71	major

J1-J6: 6 healthy volunteers; Y1-Y6: 6 depressed patients.