Supplemental Information

Gut microbiota-derived tryptamine and phenethylamine impair insulin sensitivity in metabolic syndrome and irritable bowel syndrome

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List of Supplementary Materials

Table.S1 Correlation between gut bacteria species with TyG in healthy controls and patients with IBS, Related to **Figure.1**

Table.S2 Phospho-peptides analysis of tryptamine effects on insulin-related pathways, Related to **Figure.4**

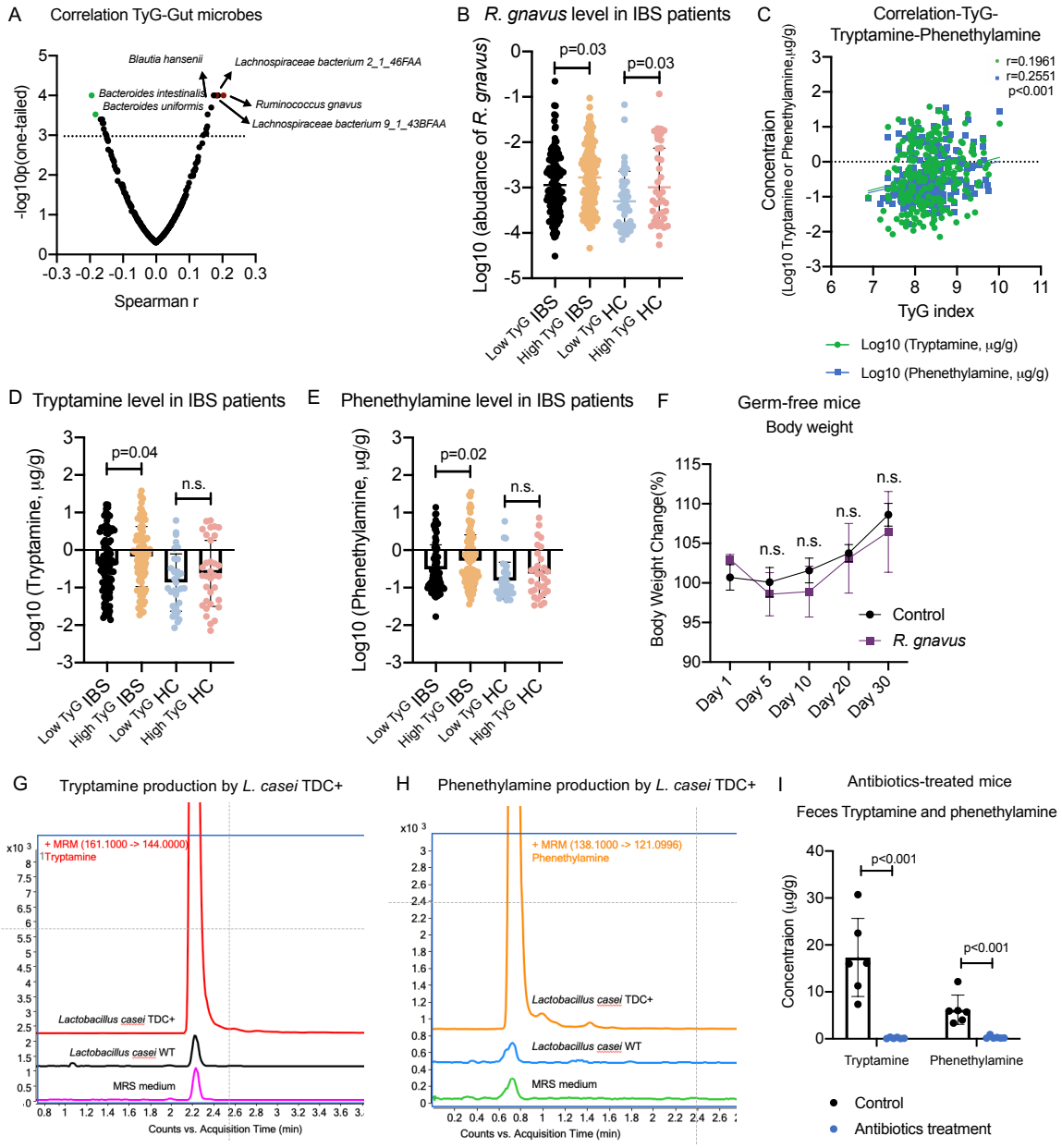
Table.S3 Tryptamine and phenethylamine levels in fecal samples from IBS donors for fecal microbiota transplantation, Related to **Figure.4**

Table.S4 Correlation between fecal tryptamine with gut bacteria abundances in T2D subjects, Related to **Figure.5**

Table.S5 BLASTP alignments with E-value < 1e-5 and identity > 30% to identify tryptamine and phenethylamine producers in T2D subjects, Related to **Figure.5**

Table.S6 Reagents and resources used in this study, Related to **Materials and Method**

Table.S7 MRM transition and parameters used in targeted metabolomics, Related to **Materials and Method**



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35 **Figure.S1 Colonization of *R. gnavus* impairs insulin sensitivity in germ-free mice, Related**

36 **to Figure.1**

37 (**A**) Spearman r and p-values (-log10p) plot against gut bacteria species abundances and TyG

38 level in human participants (n=412). (**B**) *R. gnavus* abundances in in IBS-D patients (n=290)

39 and healthy control (HC, n=89) subjects with different TyG index using a 50% cut-off value.

40 (**C**) Spearman's correlation between relative abundances of *R. gnavus* with TyG level in human

participants (n=412). (**D-E**) Tryptamine and phenethylamine levels in IBS-D patients and HC subjects with different TyG index using a 50% cut-off value. (**F**) Body weight changes in germ-free mice following colonization of *R. gnavus* ATCC 29149 (n=6 per group). **(G-H)** LC-MS chromatogram of tryptamine and phenethylamine level in MRS culture medium of *L. casei* TDC+ and *L. casei* vector control (WT). Differences in body weight changes were determined by two-way ANOVA. Data are presented as mean±S.D. **(I)** Tryptamine and phenethylamine levels in fecal samples of normal mice from the control group (without antibiotics treatment, n=6) and the group treated with antibiotics mixture (n=12). Differences of phenethylamine and tryptamine levels in serum and fecal samples were analyzed by one-tailed student t-tests. Data are presented as mean ± S.D.

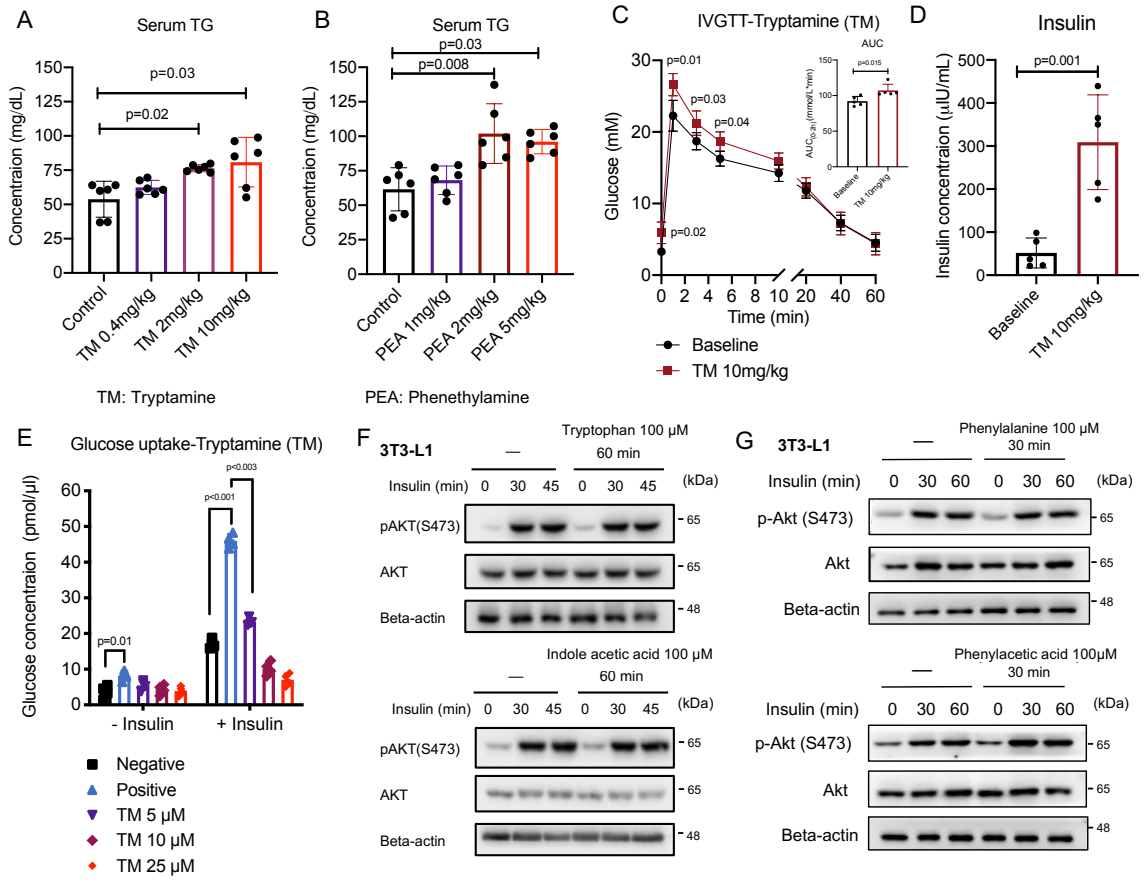


Figure.S2 Tryptamine and phenethylamine impair insulin sensitivity in mice, monkeys and *in vitro* models, Related to Figure.2

(A-B) Serum TG level in mice (n=6/group) after treatment with tryptamine or phenethylamine at indicated dosages or control (1% DMSO in saline) by i.p. **(C-D)** IVGTT index and serum insulin levels in monkeys after treatment with tryptamine (10mg/kg) or control (1% CMC-Na in water) (n=5/group). **(E)** Effect of tryptamine (5μM, 10μM and 25μM) on glucose uptake stimulated by insulin (20nM) in 3T3-L1 cells (n=3/group). **(F)** Western blot (and quantification) of tryptophan (100μM) and indole acetic acid (100μM) treatment (precursor and metabolite of tryptamine) on insulin signaling stimulated by insulin (20nM) in 3T3-L1 cells (n=3/group). **(G)** Western blot (and quantification) of phenylalanine (100μM) and phenylacetic acid (100μM) treatment (precursor and metabolite of phenethylamine) on insulin signaling stimulated by insulin (20nM) in 3T3-L1 cells (n=3/group). Data are presented as mean±S.D. *P* values were determined by ordinary one-way ANOVA or Student's t-test.

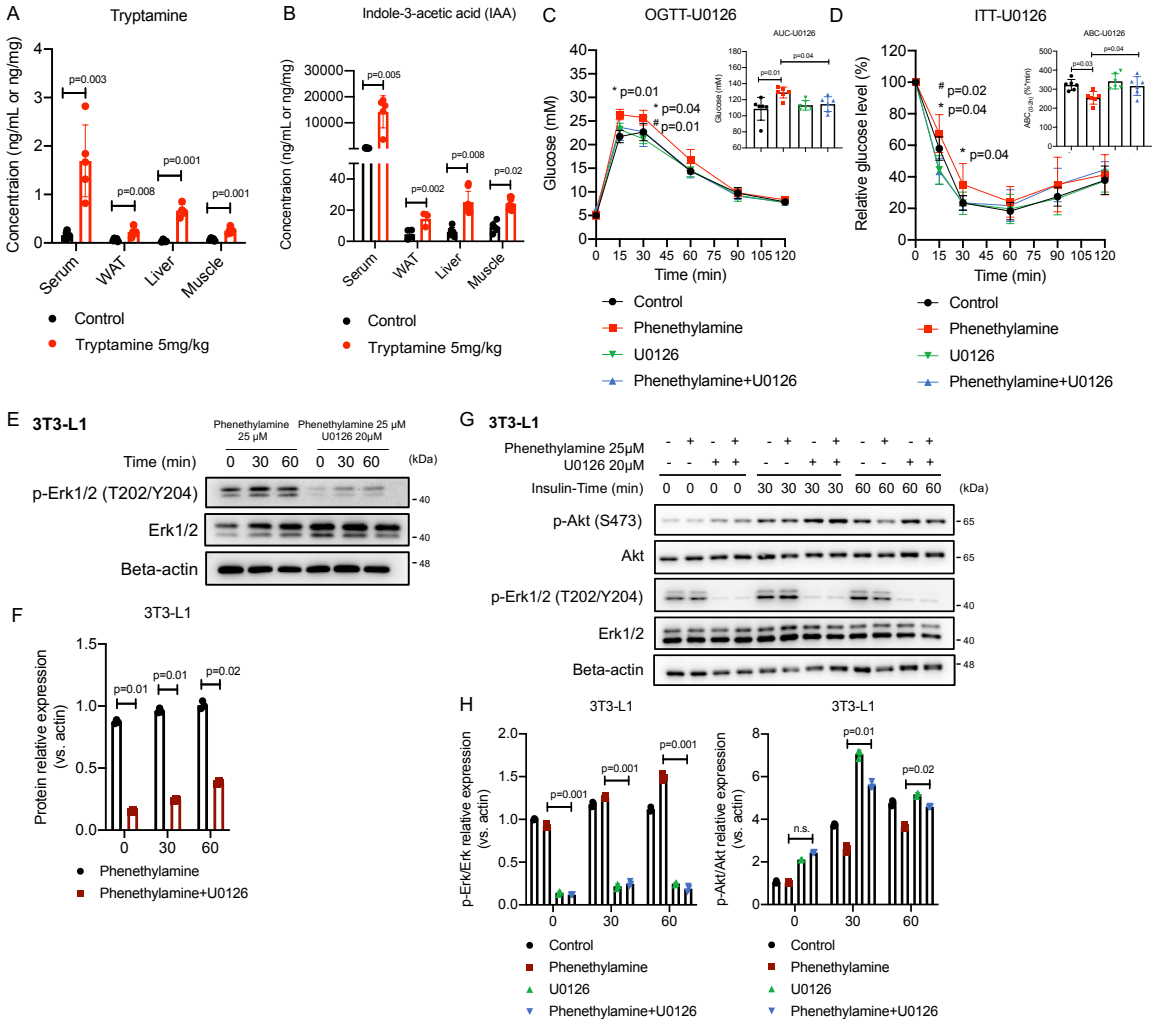


Figure.S3 Tryptamine and phenethylamine impair insulin signaling via ERK activation

(A-B) Tryptamine and indole acetic acid levels in serum, WAT, liver, and skeletal muscle after administration of tryptamine (5mg/kg) at indicated times (n=6). **(C-D)** OGTT and ITT indexes in mice after treatment of tryptamine (10mg/kg), ERK inhibitor PD98509 (10mg/kg) or control (1% DMSO in saline) (n=6/group). * Comparisons between control group and tryptamine group (10 mg/kg). #Comparisons between tryptamine group and tryptamine+ERK inhibitor (PD98059) group. **(E-F)** Western blot (and quantification) of the effect of phenethylamine (25μM) and ERK inhibitor U0126 on ERK activation in 3T3-L1 cells (n=3/group). **(G-H)** Western blot (and quantification) of the effects of tryptamine (25μM) and ERK inhibitor

U0126 treatment (20μM) on ERK activation and insulin (20nM)-stimulated AKT activation in 3T3-L1 cells (n=3/group). Data are presented as mean±S.D. *P* values were determined by ordinary one-way ANOVA or Student's t-test.

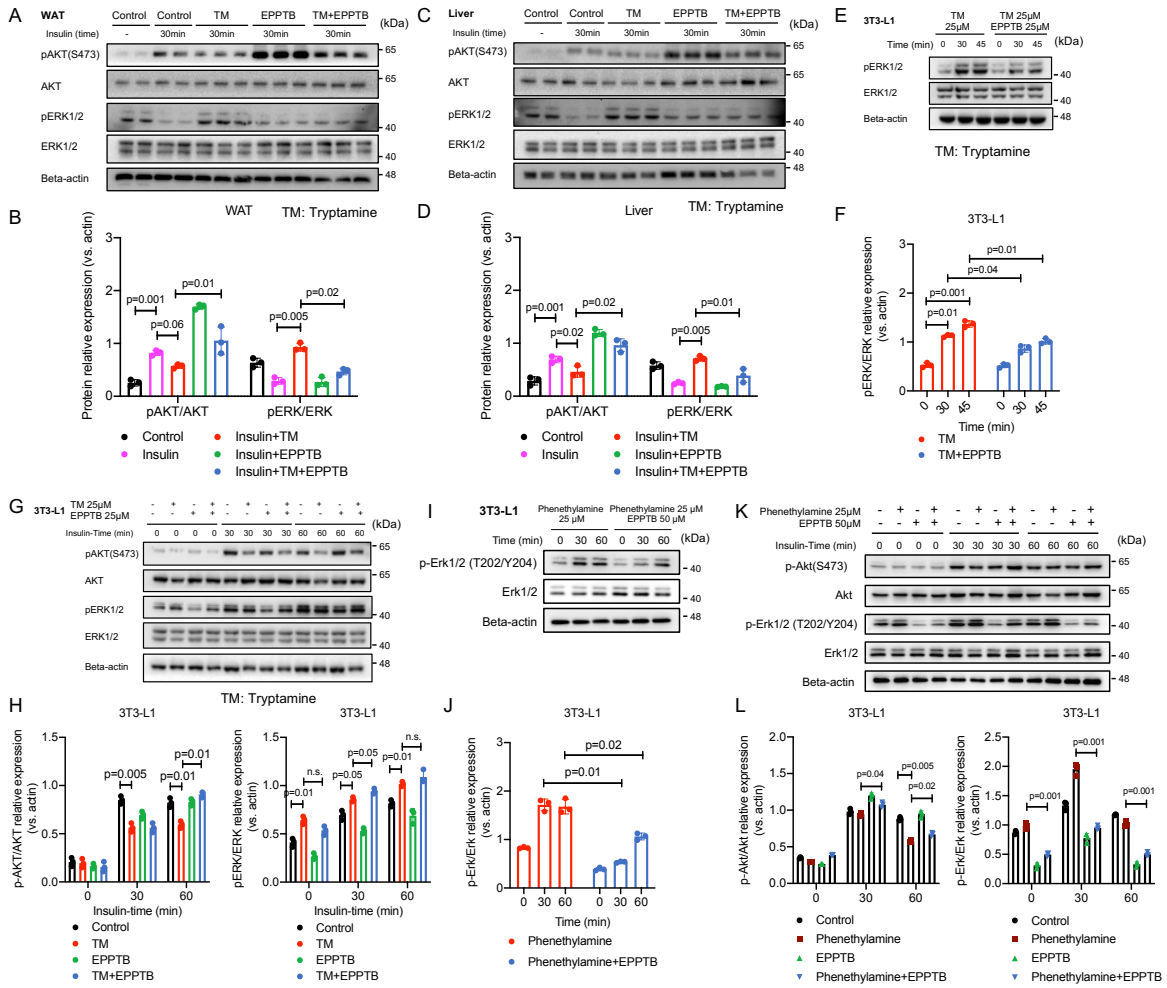
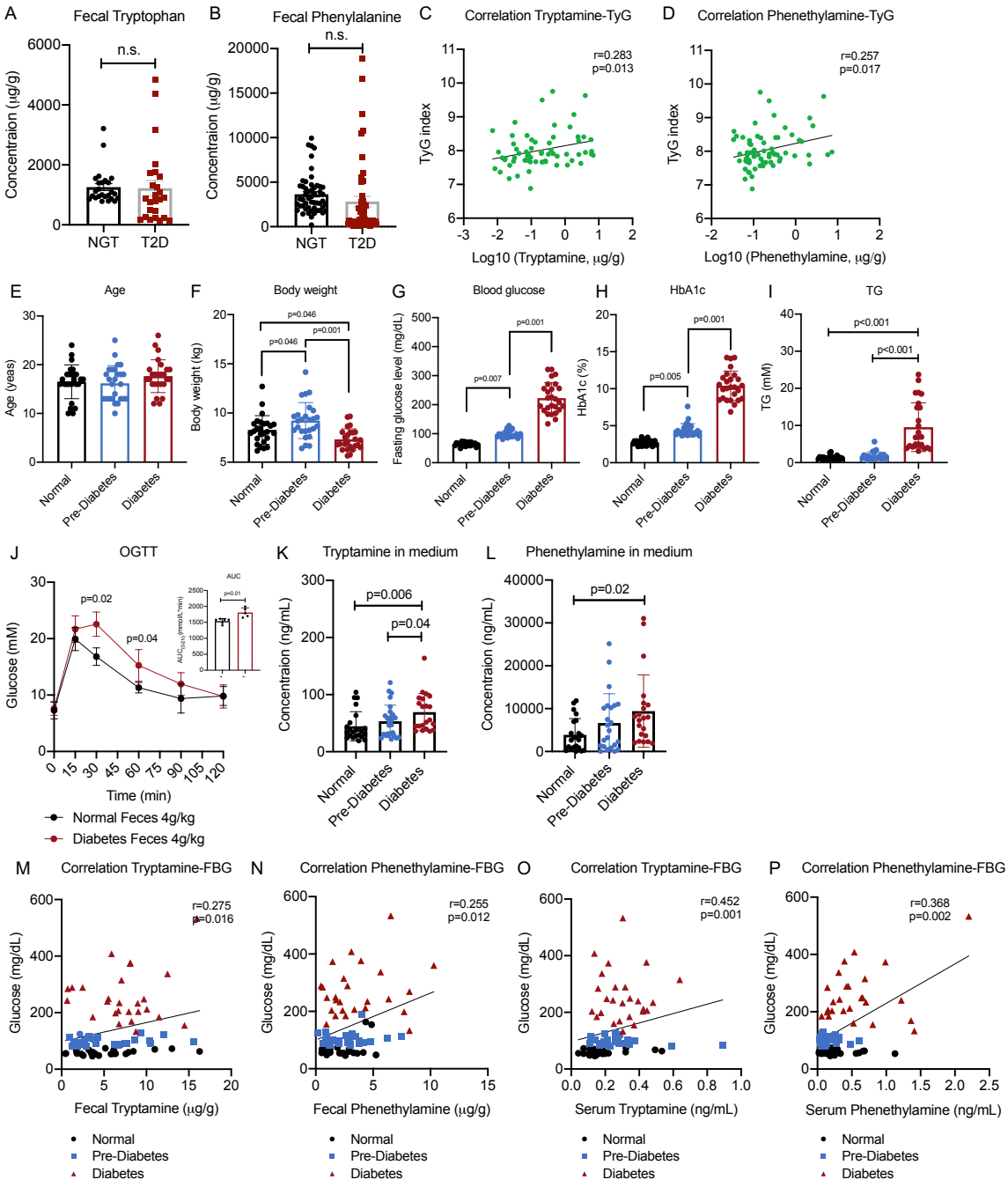


Figure.S4 Tryptamine and phenethylamine weaken insulin signaling via TAAR1-ERK signaling axis

(**A-D**) Western blot (and quantification) of the effects of tryptamine (10mg/kg) and TAAR1 antagonist EPPTB (10mg/kg) treatment on ERK activation and insulin (1U/kg)-stimulated AKT activation in WAT lysates and liver lysates from mice (n=2-3/group). **(E-F)** Western blot (and quantification) of the effects of tryptamine (25μM) and TAAR1 antagonist EPPTB

85 treatment (20μM) on ERK activation in 3T3-L1 cells (n=3/group). **(G-H)** Western blot (and
86 quantification) of the effects of tryptamine (25μM) and TAAR1 antagonist EPPTB treatment
87 (20μM) on ERK activation and insulin (20nM)-stimulated AKT activation in 3T3-L1 cells
88 (n=3/group). **(I-J)** Western blot (and quantification) of the effects of phenethylamine (25μM)
89 and TAAR1 antagonist EPPTB treatment (20μM) on ERK activation in 3T3-L1 cells
90 (n=3/group). **(K-L)** Western blot (and quantification) of the effects of phenethylamine (25μM)
91 and TAAR1 antagonist EPPTB treatment (20μM) on ERK activation and insulin (20nM)-
92 stimulated AKT activation in 3T3-L1 cells (n=3/group). Data are presented as mean±S.D. *P*
93 values were determined by ordinary one-way ANOVA or Student's t-test.



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95 **Figure.S5 Tryptamine and phenethylamine are positively correlated with glucose**
96 **intolerance in patients with type 2 diabetes and monkeys with spontaneous diabetes**

97 **(A-B)** Tryptophan and phenylalanine (substrates of tryptamine and phenethylamine) levels in
98 fecal samples from individuals with or without T2D (n=25 subjects with NGT, n=25 patients
99 with T2D). **(C-D)** Spearman's correlation between fecal tryptamine/phenethylamine levels

with TyG level in healthy volunteers (n=89). **(E-I)** Age, body weight, FBG, HbA1c and TG levels in monkeys without or with pre-diabetic or diabetes (n=26/per group). **(J)** OGTT index in HFD-fed mice after treatment with fecal suspension from normal monkeys and diabetes monkeys once per day for 5 days (n=6/group). (**K-L**) Tryptamine and phenethylamine production in batch culture experiments using feces from monkeys with or without pre-diabetes and diabetes (n=26/group). **(M-P)** Spearman's correlation between fecal/serum tryptamine and phenethylamine levels with FBG and HbA1c level in monkeys with or without pre-diabetes and diabetes (n=26/group). Differences of phenethylamine and tryptamine levels in fecal and serum samples were analyzed by one-tailed student t-tests. Differences of age, body weight, FBG, HbA1c, TG and OGTT indexes in monkeys were analyzed by ordinary one-way ANOVA. Data are presented as mean $\pm$ S.D.

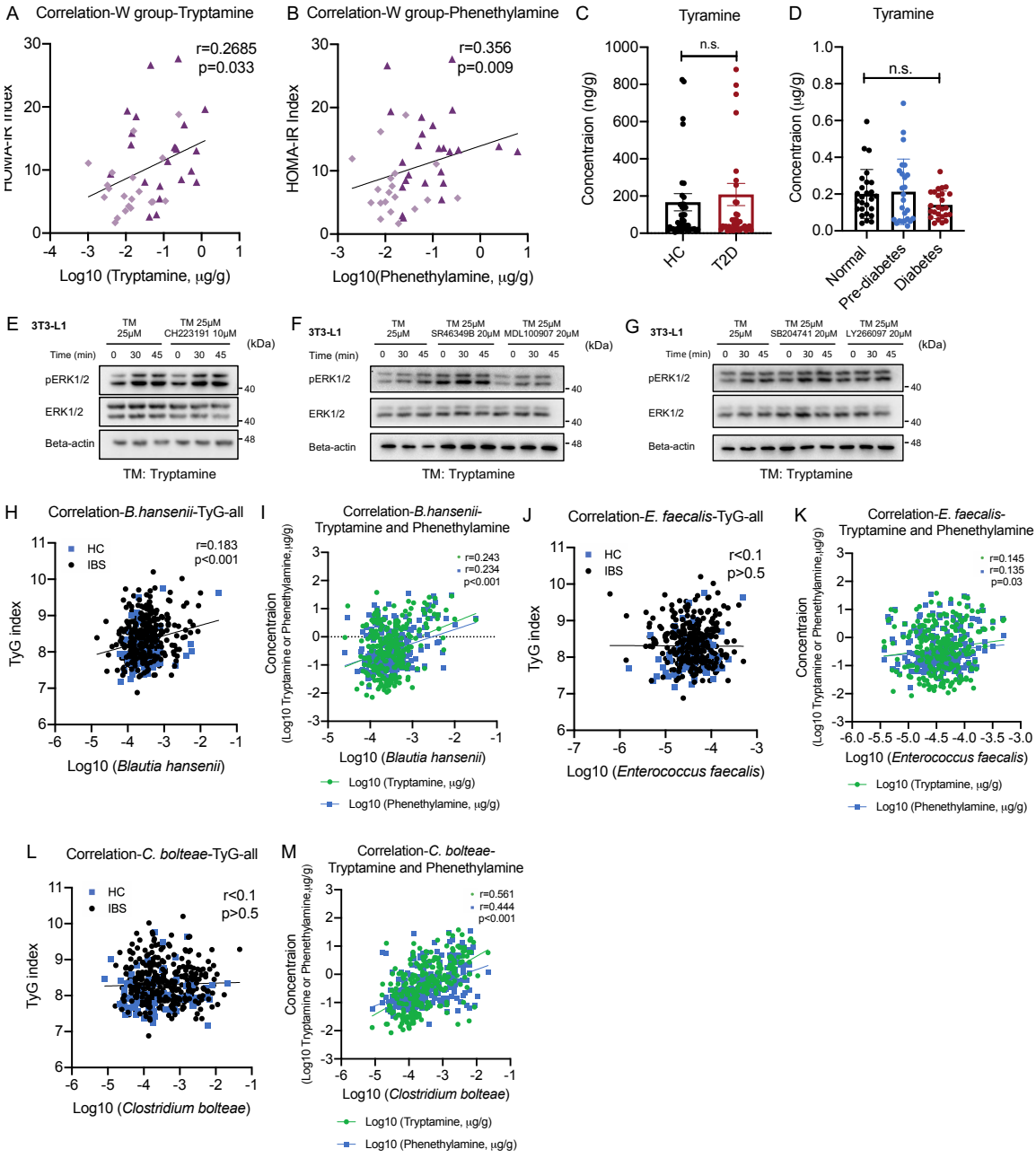

Figure.S6 Tryptamine and phenethylamine are negatively correlated with the improvement of insulin sensitivity in dietary fiber-treated patients with type 2 diabetes

(**A-B**) Spearman's correlation analysis between fecal tryptamine and phenethylamine level and HOMA-IR index in T2D subjects consuming a high fiber diet (W group; n=27). **(C)** Tyramine levels in fecal samples of individuals with or without type 2 diabetes (n=25 subjects with NGT, n=25 patients with T2D). **(D)** Tyramine levels in fecal samples of age-matched monkeys

without or with pre-diabetes or diabetes (n=26/group). **(E-G)** Western blot of tryptamine (25μM), AhR antagonist CH223191 (10μM), 5-HT2A receptor antagonist SR46349B (25μM) and MDL100907 (20μM) as well as 5-HT2B receptor antagonist SB204741 (20μM) and LY266097 (20μM) in 3T3-L1 cells (n=3/group). Data are presented as mean±S.D. *P* values were determined by ordinary one-way ANOVA or Student's t-test. **(H-I)** Spearman's correlation analysis between relative abundances of *Blautia hansenii* with TyG level and fecal tryptamine/phenethylamine level in human participants (n=412). **(J-K)** Spearman's correlation analysis between relative abundances of *Enterococcus faecalis* with TyG level and fecal tryptamine/phenethylamine level in human participants (n=412). **(L-M)** Spearman's correlation analysis between relative abundances of *Clostridium boltae* with TyG level and fecal tryptamine/phenethylamine level in human participants (n=412). Data are presented as mean±S.D. *P* values were determined by ordinary one-way ANOVA or student's t-test.