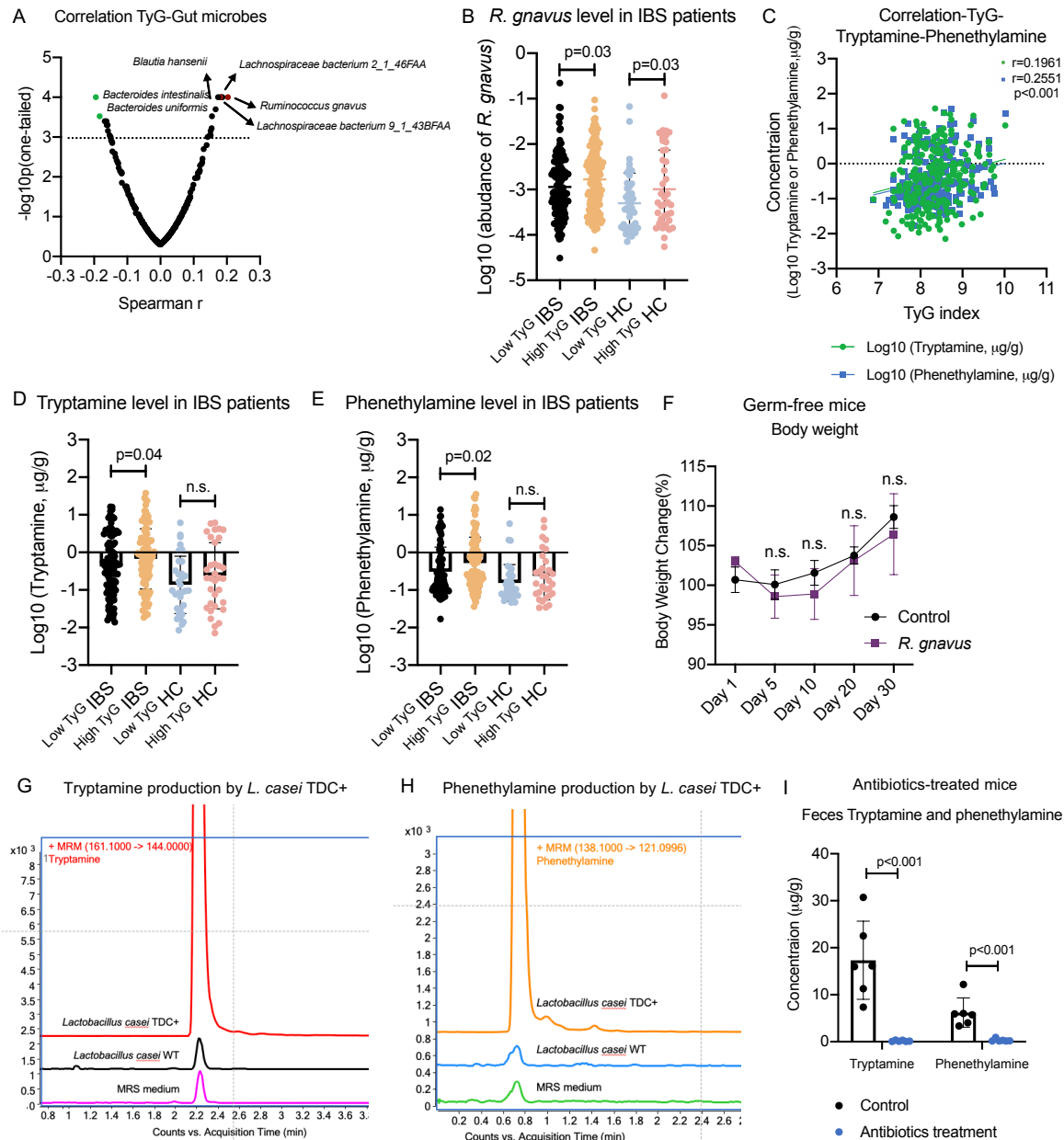


Supplemental Information

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

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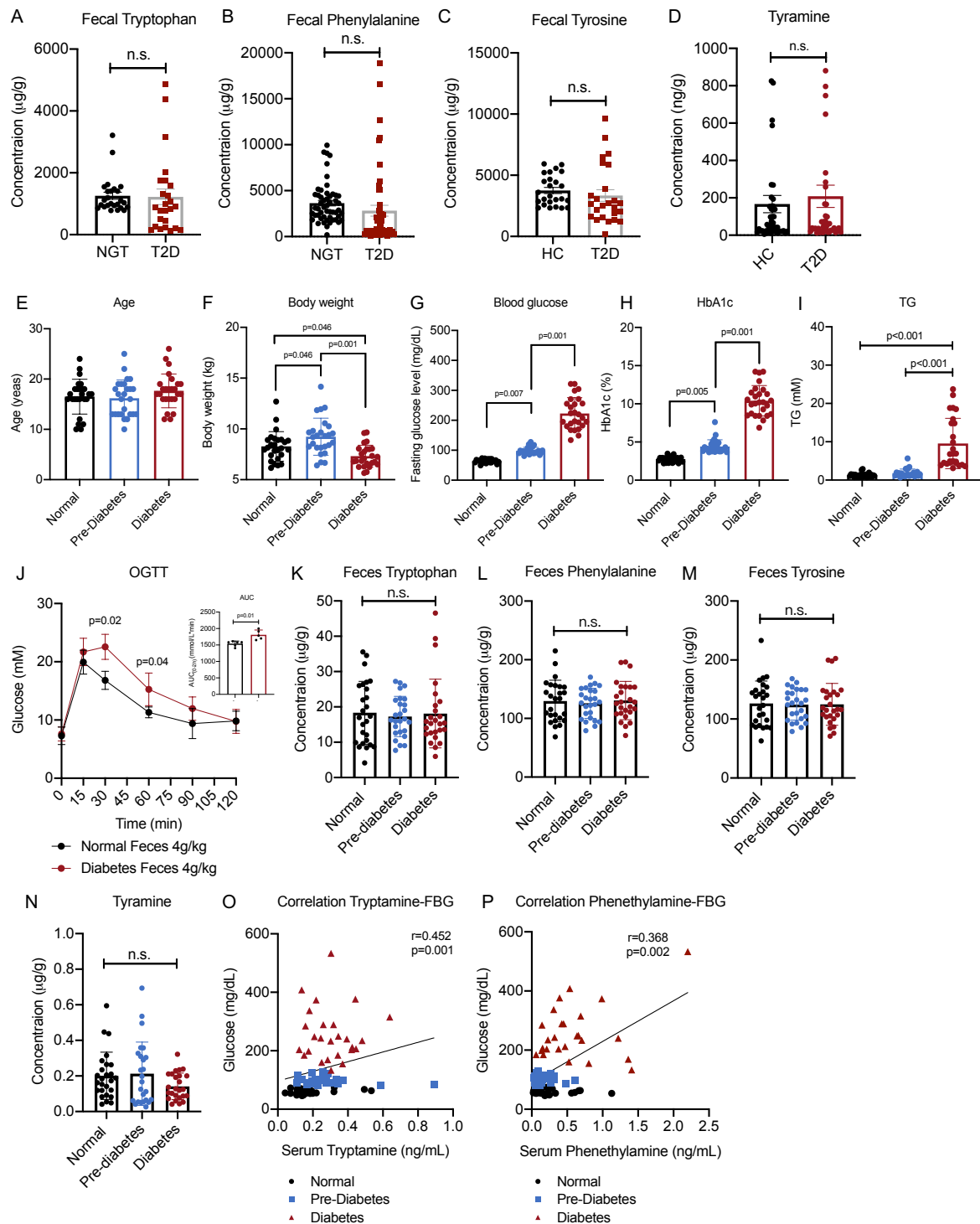


8

9 **Figure.S1 Positive association and causality between *R. gnnavus*-derived**
 10 **tryptamine/phenethylamine and insulin resistance in irritable bowel syndrome, Related**
 11 **to Figure.1**

12 (A) Spearman r and p-values (-log₁₀p) plot against gut bacteria species abundances and TyG
 13 level in human participants (n=412) (one-tailed test). (B) *R. gnnavus* abundances in IBS-D
 14 patients (n=290) and healthy control (HC, n=89) subjects with different TyG indexes using a
 15 50% cut-off value (two-tailed test). (C) Spearman's correlation between relative abundances

16 of *R. gnavus* with TyG level in human participants (n=412) (one-tailed test). **(D-E)** Tryptamine
17 and phenethylamine levels in IBS-D patients (n=290) and HC subjects (n=89) with different
18 TyG index using a 50% cut-off value (two-tailed test). **(F)** Body weight changes in germ-free
19 mice following colonization of *R. gnavus* ATCC 29149 (n=6 per group) (two-tailed test). **(G-**
20 **H)** LC-MS chromatogram of tryptamine and phenethylamine level in MRS culture medium of
21 *L. casei* TDC+ and *L. casei* vector control (WT). Differences in body weight changes were
22 determined by two-way ANOVA. Data are presented as mean $\pm$ S.D. **(I)** Tryptamine and
23 phenethylamine levels in fecal samples of normal mice from the control group (without
24 antibiotics treatment, n=6) and the group treated with antibiotics mixture (n=12) (two-tailed
25 test). Differences of phenethylamine and tryptamine levels in serum and fecal samples were
26 analyzed by one-tailed student t-tests. Data are presented as mean $\pm$ S.D.



27

28 **Figure.S2 Tryptamine and phenethylamine are positively correlated with glucose**
 29 **intolerance in patients with type 2 diabetes and monkeys with spontaneous diabetes,**
 30 **Related to Figure.2**

(A-D) Tryptophan and phenylalanine (substrates of tryptamine and phenethylamine) levels and tyrosine and tyramine in fecal samples from individuals with or without T2D (n=25 subjects with NGT, n=25 patients with T2D). (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-M) Tryptophan and phenylalanine (substrates of tryptamine and phenethylamine) levels and tyrosine and tyramine in fecal samples of monkeys without or with pre-diabetic or diabetes (n=26/per group). (O-P) Spearman's correlation between serum tryptamine and phenethylamine levels with FBG 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 two-tailed ordinary one-way ANOVA. Data are presented as mean $\pm$ S.D.

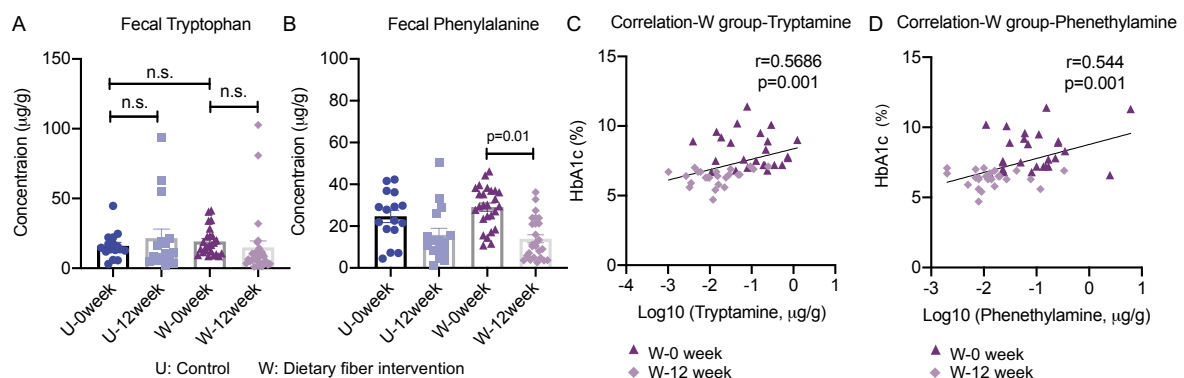


Figure.S3 Tryptamine and phenethylamine are negatively correlated with the improvement of insulin sensitivity in dietary fiber-treated patients with type 2 diabetes, Related to Figure.3

(A-B) Tryptophan and phenylalanine levels in fecal samples of individuals with or without type 2 diabetes (n=25 subjects with NGT, n=25 patients with T2D). (C-D) Spearman's

correlation analysis between fecal tryptamine and phenethylamine level and HbA1c index in T2D subjects consuming a high fiber diet (W group; n=27). Data are presented as mean±S.D. *P* values were determined by one-tailed test of ordinary one-way ANOVA or student's t-test.

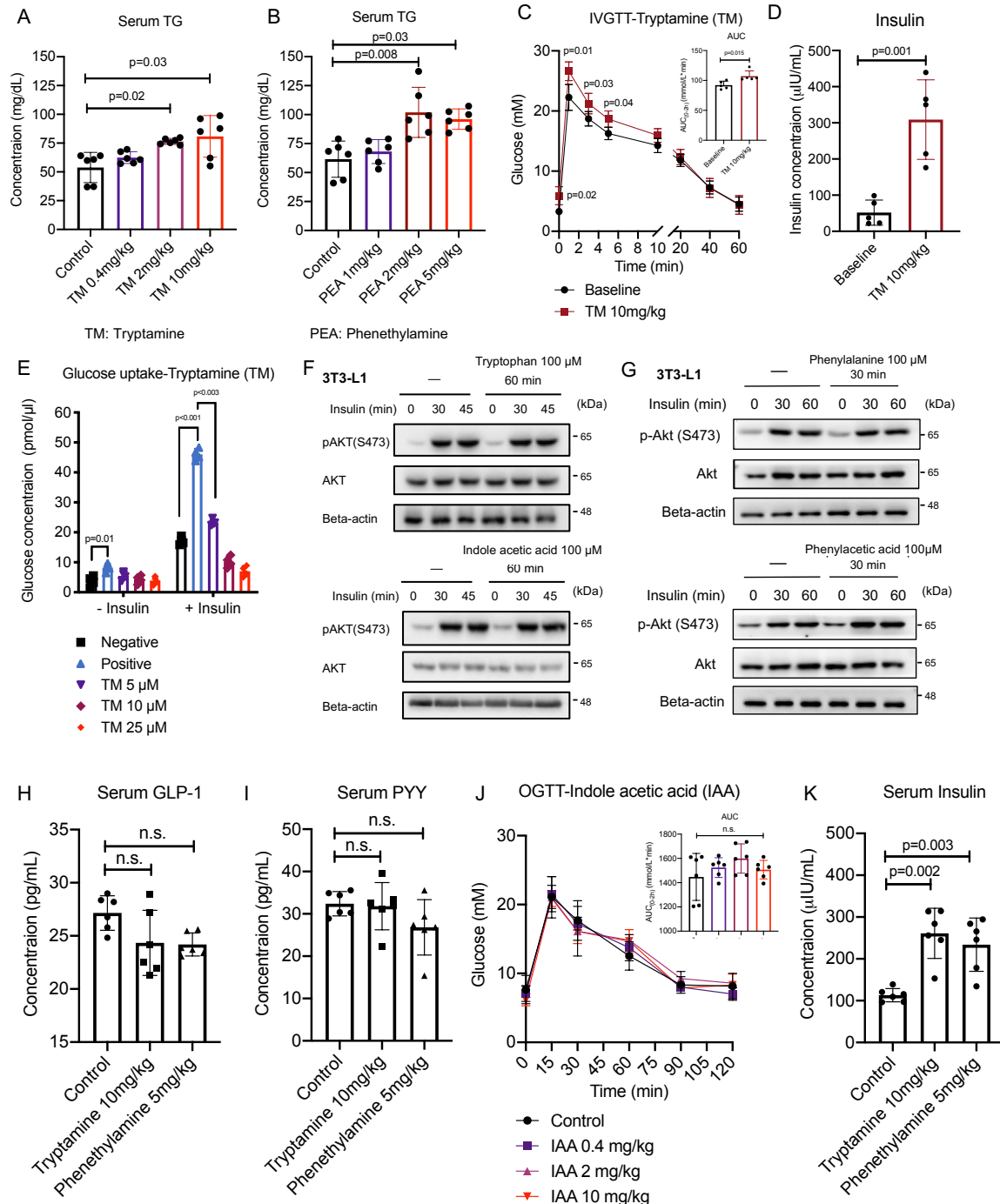
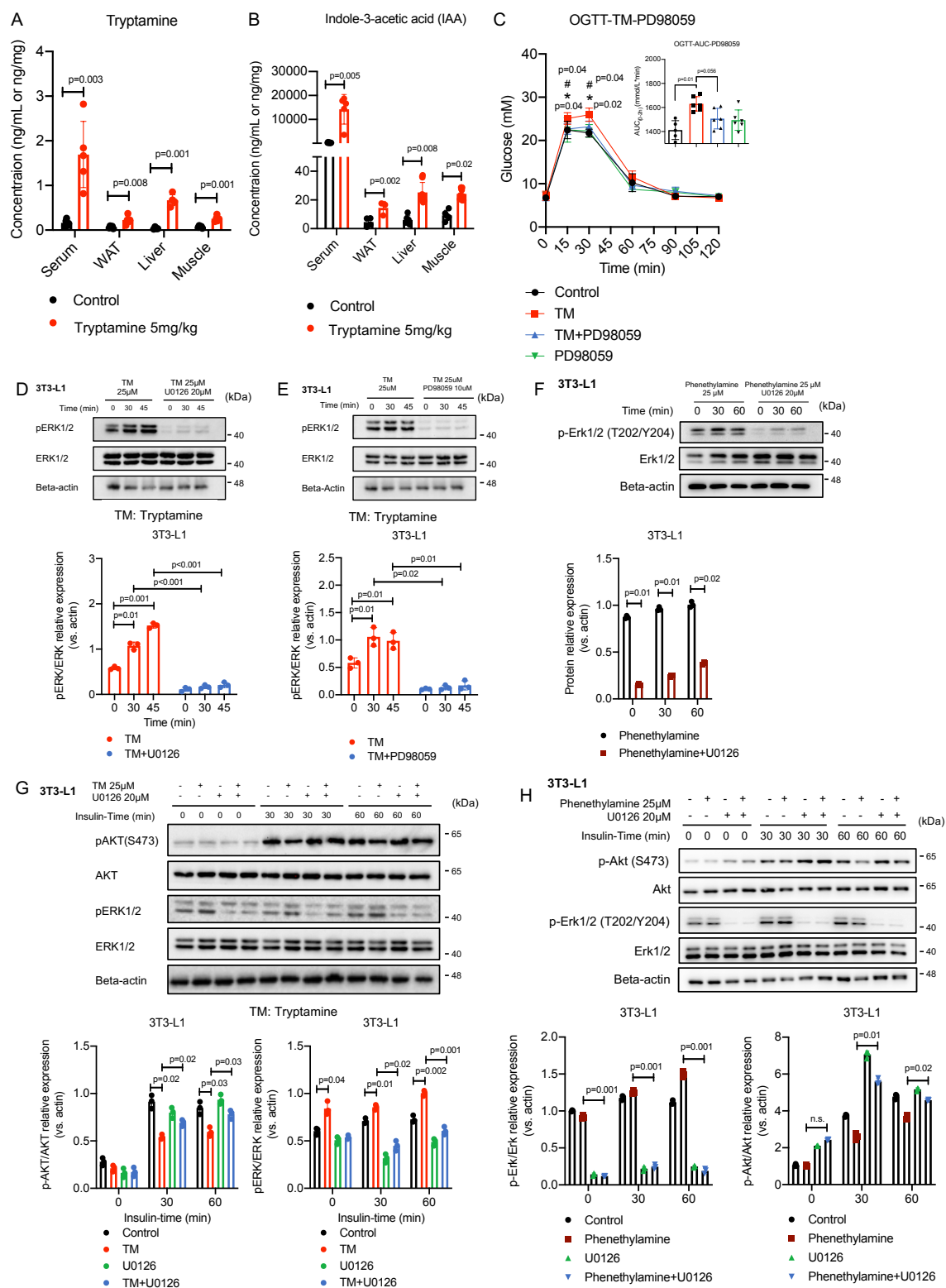


Figure.S4 Tryptamine and phenethylamine impair insulin sensitivity in mice, monkeys and *in vitro* models, Related to Figure.4

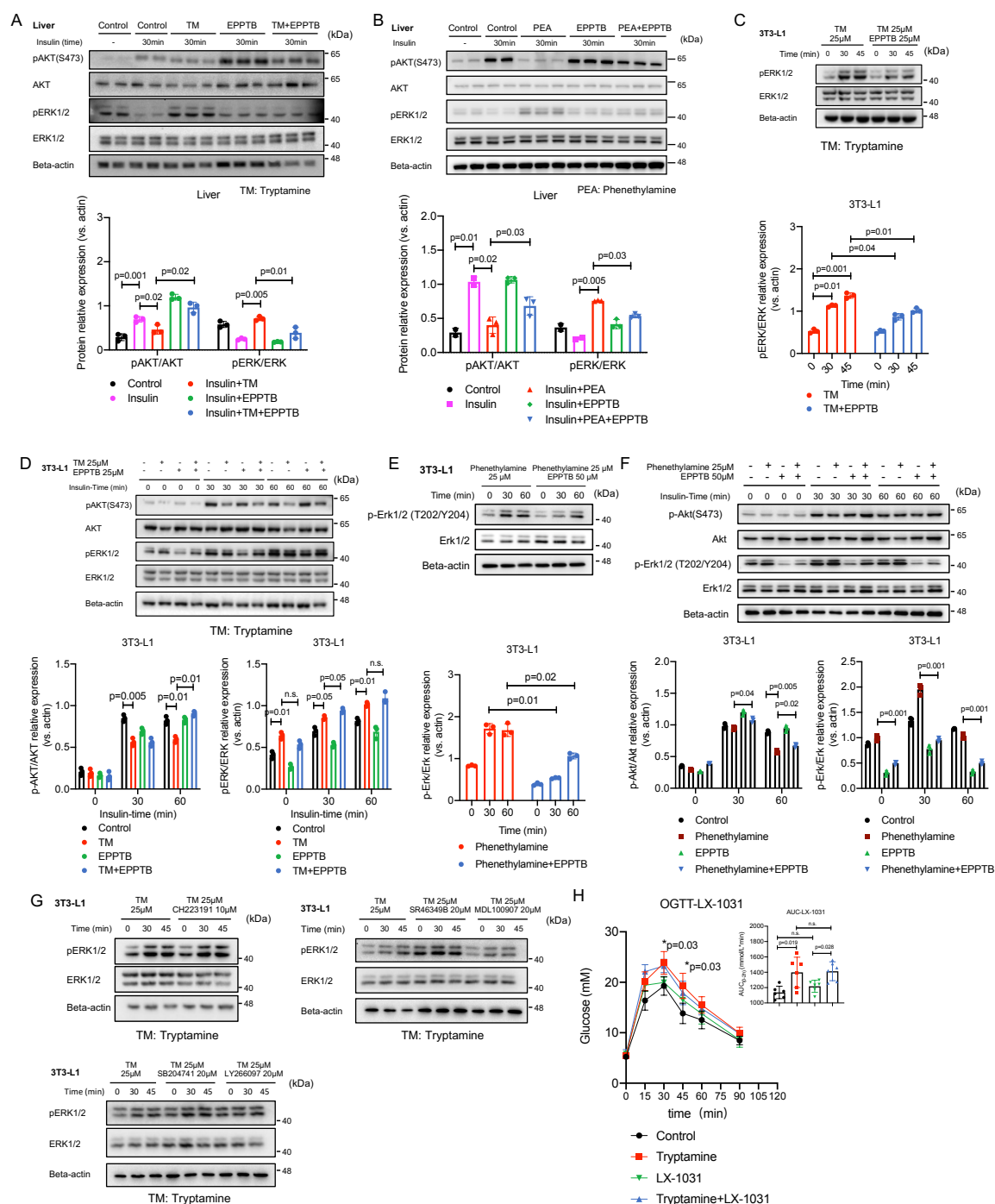
(A-B) Serum TG level in mice (n=6/group) after treatment with tryptamine or phenethylamine at indicated dosages or control (1% DMSO in saline) by i.p. **(C-D)** IVGTT index and serum insulin levels in monkeys after treatment with tryptamine (10mg/kg) or control (1% CMC-Na in water) (n=5/group). **(E)**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



72

73 **Figure.S5 Tryptamine and phenethylamine impair insulin signaling via ERK activation**

74 **(A-B)** Tryptamine and indole acetic acid levels in serum, WAT, liver, and skeletal muscle after
75 administration of tryptamine (5mg/kg) at indicated times (n=6). **(C)** OGTT index in mice after
76 treatment of tryptamine (10mg/kg), ERK inhibitor PD98509 (10mg/kg) or control (1% DMSO
77 in saline) (n=6/group). * Comparisons between control group and tryptamine group (10 mg/kg).
78 [#]Comparisons between tryptamine group and tryptamine+ERK inhibitor (PD98059) group. **(D-**
79 **F)** Western blot (and quantification) of the effect of tryptamine (25μM), phenethylamine
80 (25μM) and ERK inhibitor U0126 on ERK activation in 3T3-L1 cells (n=3/group). **(G-H)**
81 Western blot (and quantification) of the effects of tryptamine (25μM) and ERK inhibitor
82 U0126 treatment (20μM) on ERK activation and insulin (20nM)-stimulated AKT activation in
83 3T3-L1 cells (n=3/group). Data are presented as mean±S.D. *P* values were determined by two-
84 tailed test of ordinary one-way ANOVA or Student's t-test.



85

86 **Figure.S6 Tryptamine and phenethylamine weaken insulin signaling via TAAR1-ERK**
 87 **signaling axis, Related to Figure.6**

88 (A-B) Western blot (and quantification) of the effects of tryptamine (10mg/kg) and TAAR1
 89 antagonist EPPTB (10mg/kg) treatment on ERK activation and insulin (1U/kg)-stimulated

90 AKT activation in liver lysates from mice (n=2 in control and insulin group, n=3 in other
91 groups). **(C)** Western blot (and quantification) of the effects of tryptamine (25μM) and TAAR1
92 antagonist EPPTB treatment (20μM) on ERK activation in 3T3-L1 cells (n=3/group). **(D)**
93 Western blot (and quantification) of the effects of tryptamine (25μM) and TAAR1 antagonist
94 EPPTB treatment (20μM) on ERK activation and insulin (20nM)-stimulated AKT activation
95 in 3T3-L1 cells (n=3/group). **(E)** Western blot (and quantification) of the effects of
96 phenethylamine (25μM) and TAAR1 antagonist EPPTB treatment (20μM) on ERK activation
97 in 3T3-L1 cells (n=3/group). **(F)** Western blot (and quantification) of the effects of
98 phenethylamine (25μM) and TAAR1 antagonist EPPTB treatment (20μM) on ERK activation
99 and insulin (20nM)-stimulated AKT activation in 3T3-L1 cells (n=3/group). **(G)** Western blot
100 of tryptamine (25μM), AhR antagonist CH223191 (10μM), 5-HT2A receptor antagonist
101 SR46349B (25μM) and MDL100907 (20μM) as well as 5-HT2B receptor antagonist
102 SB204741 (20μM) and LY266097 (20μM) in 3T3-L1 cells (n=3/group). **(H)** OGTT index in
103 mice after treatment of tryptamine (10mg/kg), TPH1 inhibitor LX-1031 (10mg/kg) or control
104 (1% DMSO in saline) (n=6/group). * Comparisons between control group and tryptamine
105 group (10 mg/kg). Data are presented as mean±S.D. *P* values were determined by two-tailed
106 test of ordinary one-way ANOVA or Student's t-test.

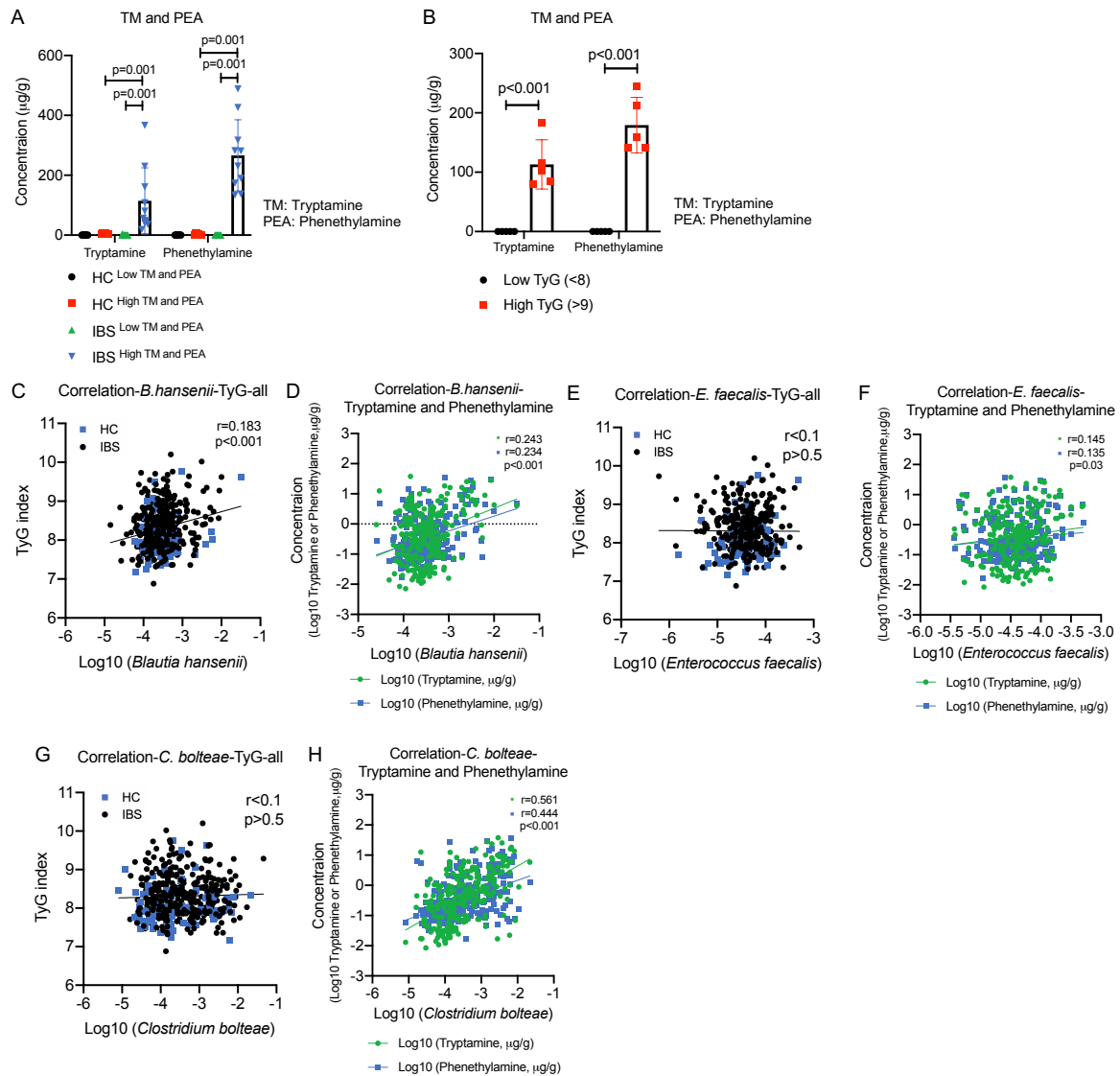


Figure.S7 TAAR1 inhibition alleviates insulin resistance induced by tryptamine and phenethylamine-producing bacteria

(A) Tryptamine and phenethylamine levels in fecal samples of HC and IBS subjects with low and high tryptamine and phenethylamine index (n=10/group). (B) Tryptamine and phenethylamine levels in fecal samples of HC and IBS subjects with low and high TyG index (n=5/group). (C-D) Spearman's correlation analysis between relative abundances of *Blautia hansenii* with TyG level and fecal tryptamine/phenethylamine level in human participants (n=412). (E-F) Spearman's correlation analysis between relative abundances of *Enterococcus*

116 *faecalis* with TyG level and fecal tryptamine/phenethylamine level in human participants
117 (n=412). **(G-H)** Spearman's correlation analysis between relative abundances of *Clostridium*
118 *boltae* with TyG level and fecal tryptamine/phenethylamine level in human participants
119 (n=412). Data are presented as mean $\pm$ S.D. *P* values were determined by one-tailed ordinary
120 one-way ANOVA or student's t-test.