Supplementary Information

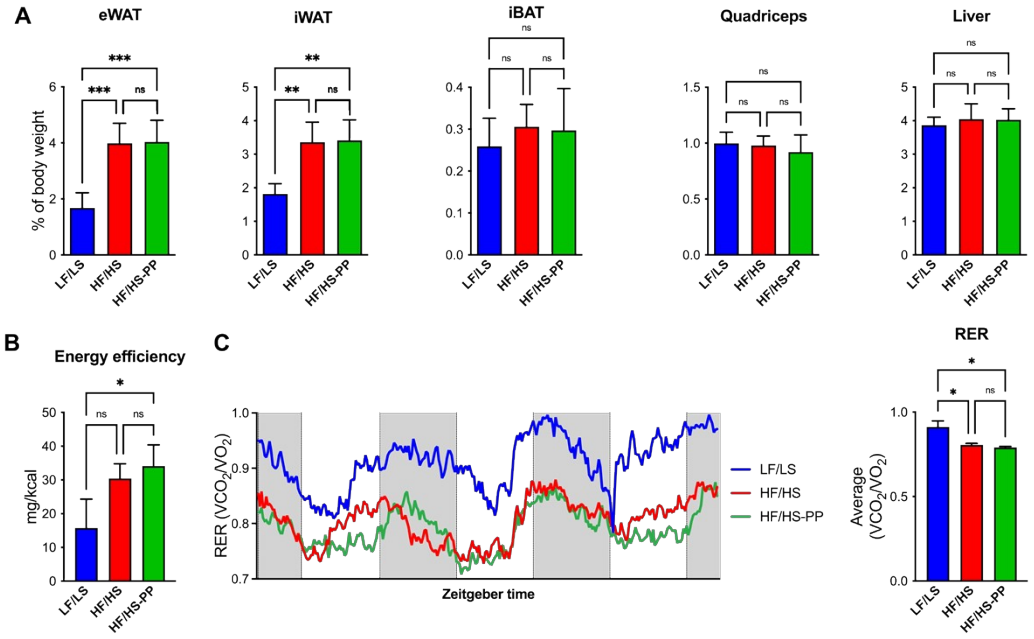

Figure S1. Dietary pot-pollen does not improve body composition, energy efficiency or RER in HF/HS-fed C57BL/6J mice. (A) From left, the relative weight of epididymal white adipose tissue (eWAT), inguinal white adipose tissue (iWAT), interscapular brown adipose tissue (iBAT), quadriceps muscle and liver compared to body weight. (B) Energy efficiency calculated as weight gain over accumulated energy intake during 12 weeks. (C) Continuous (left) and average (right) respiratory exchange ratio (RER) over a 72h period. Non-shaded area, lights on. Data are shown as mean ± SD. Differences between the groups were analyzed using two-way ANOVA followed by Tukey-Kramer test. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, ns: no significant difference.

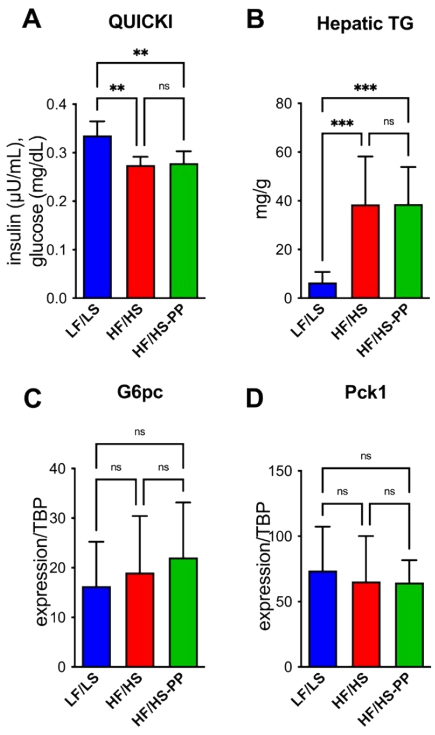

Figure S2. Supplementation of an HF/HS diet with pot-pollen does not improve insulin resistance, change liver triglyceride level or expression of genes for gluconeogenesis. (A) Quantitative insulin sensitivity check index (QUICKI). (B) Hepatic triglyceride (TG) levels. (C) Glucose-6-phosphatases (G6pc) hepatic expression. (D) Phosphoenolpyruvate carboxykinase-1 (Pck1) hepatic expression. Data are shown as means ± SD. Differences between the groups were analyzed using two-way ANOVA followed by Tukey-Kramer test. $^{**}P < 0.01$, $^{***}P < 0.001$, ns: no significant difference.

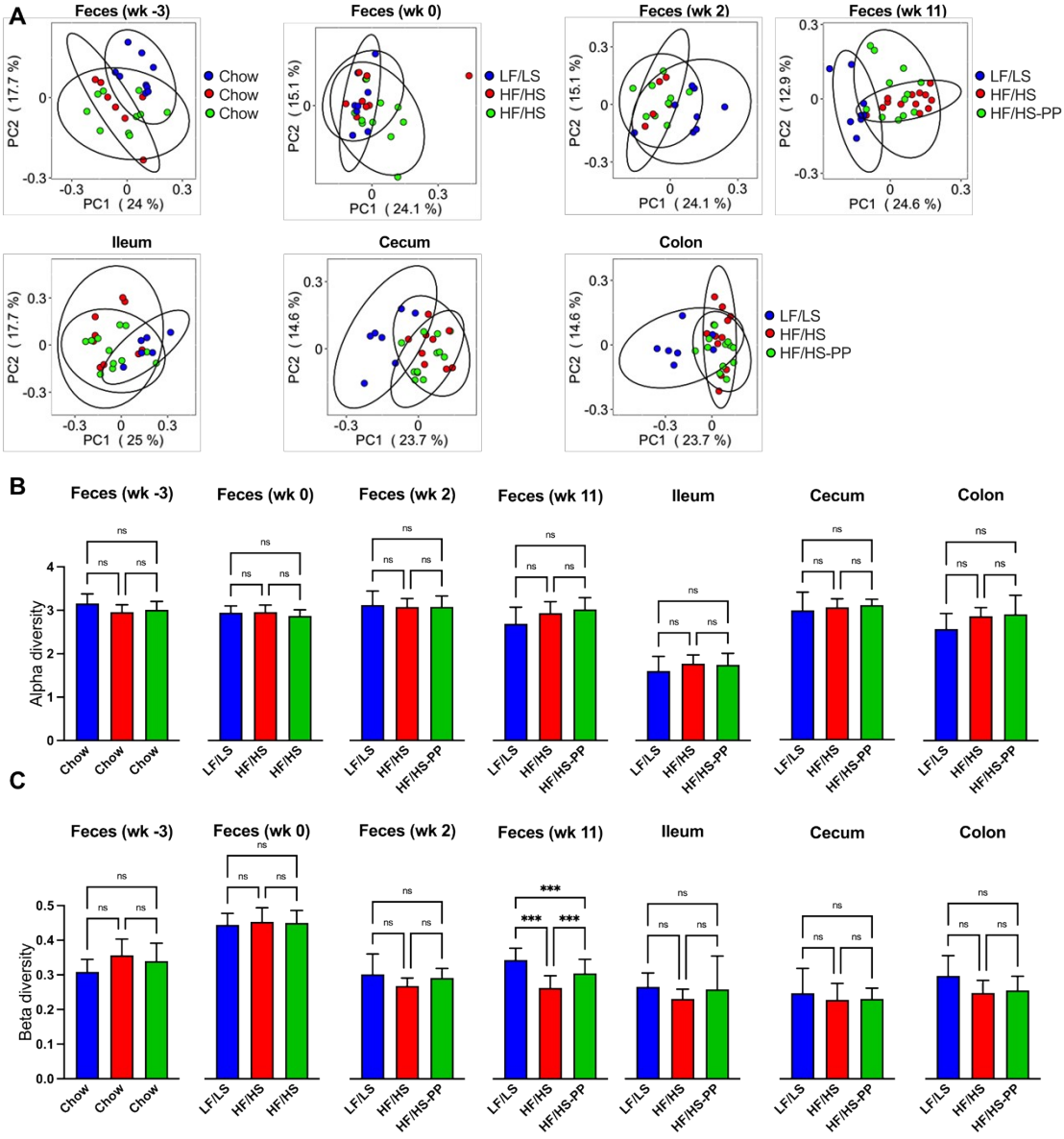

Figure S3. Supplementation with pot pollen does not change composition of gut microbiota but increase diversity after 11 weeks. (A) Principal coordinates analysis (PCoA) of operational-taxonomic units (OTUs) of gut microbiota at genus level in feces (1 week after HF/HS feeding and, week 0, 2 and 11 of supplementation), ileum, cecum, and colon fecal content. (B) Shannon and (C) beta diversity using unweighted UniFrac distances in the feces, ileum, cecum and colon content. Data are shown as means ± SD. Differences between the groups were analyzed using two-way ANOVA followed by Tukey-Kramer. *$P < 0.05$, **$P < 0.001$, ns: no significant difference.

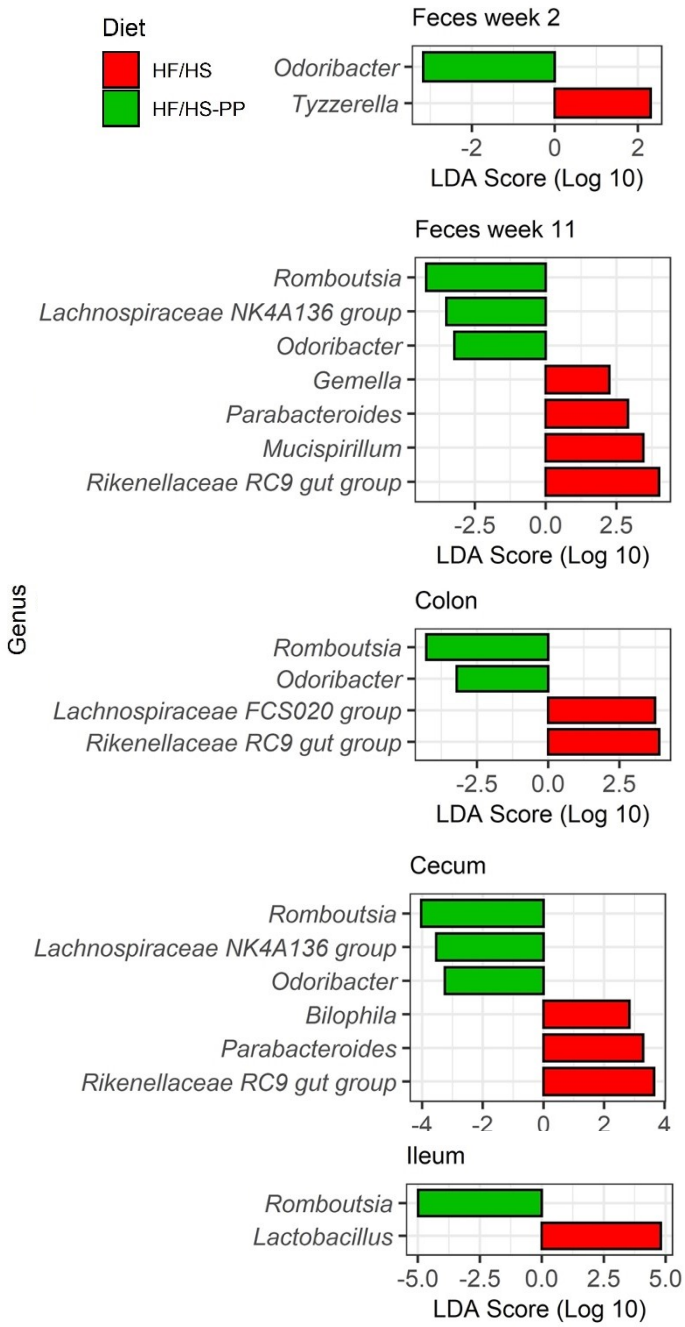

Figure S4. LEfSe identifies increased abundance of Lachnospiraceae NK4A136 group in feces and the colon in HF/HS-PP fed mice. Linear discriminant analysis (LDA) score in the feces (n = 10-12), colon (n = 10-12), cecum (n = 10-12) and the ileum (n = 10-12).

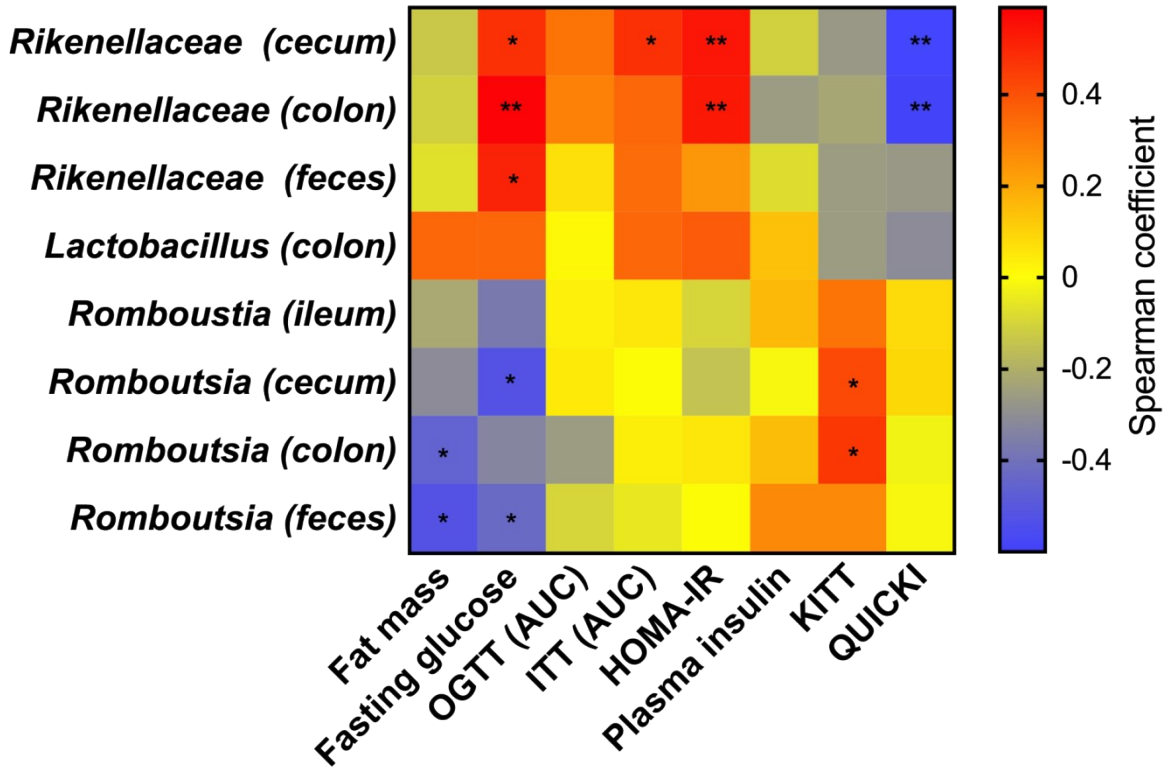

Figure S5. Heatmap showing Spearman correlation coefficients between gut microbiota and host characteristics. OGTT, oral glucose tolerance test; ITT, insulin tolerance test; HOMA-IR, homeostasis model assessment of insulin resistance; Plasma insulin 15 min after glucose stimulation; KITT, the rate constant for insulin tolerance test; QUICKI, quantitative insulin sensitivity check index. *$P < 0.05$, **$P < 0.01$.

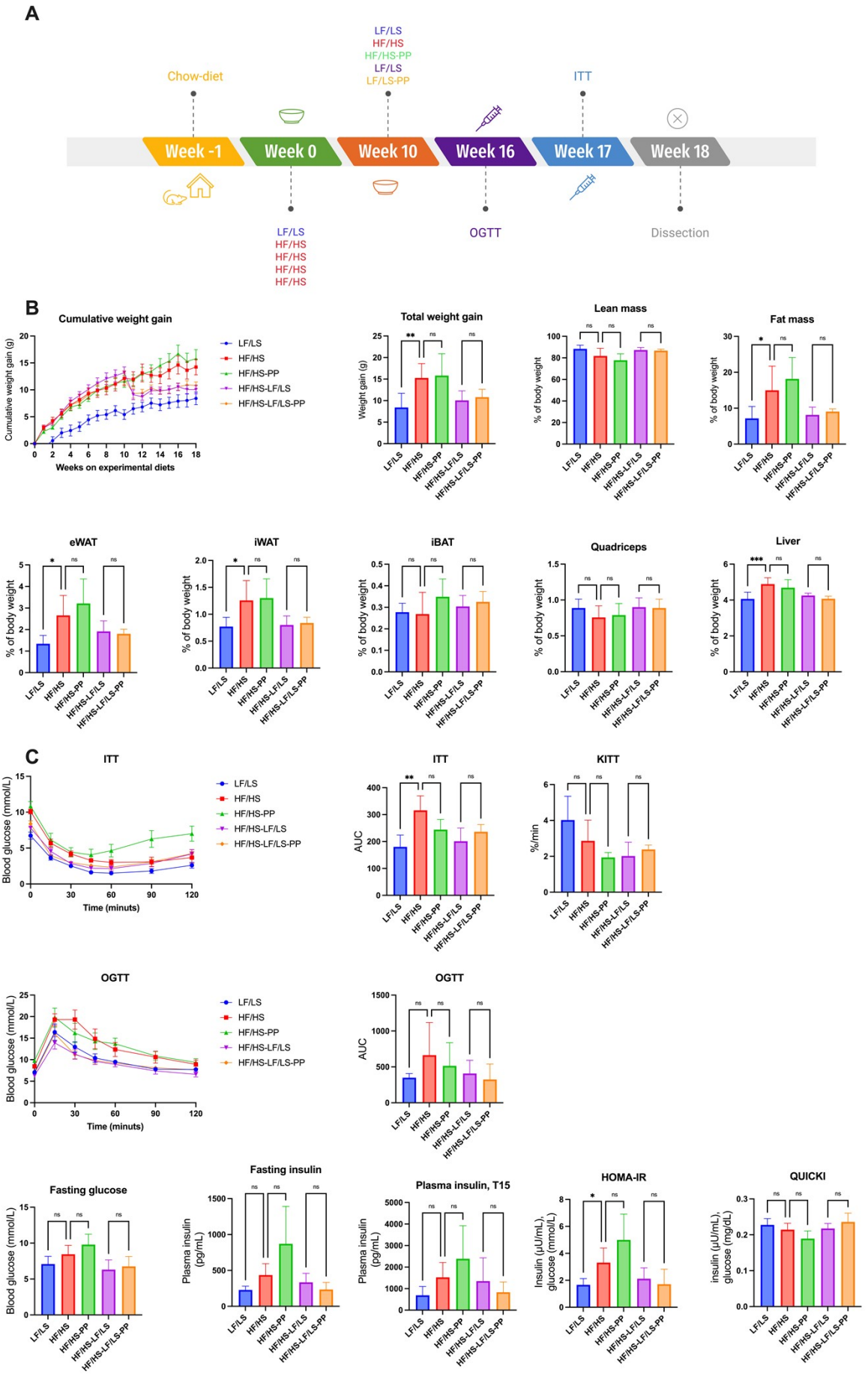
A
Chow-diet
LF/LS
HF/HS
HF/HS-PP
LF/LS
LF/LS-PP
ITT
Week -1
Week 0
Week 10
Week 16
Week 17
Week 18
LF/LS
HF/HS
HF/HS
HF/HS
HF/HS
OGTT
Dissection
B
Cumulative weight gain
Cumulative weight gain (g)
Weeks on experimental diets
LF/LS
HF/HS
HF/HS-PP
HF/HS-LF/LS
HF/HS-LF/LS-PP
Total weight gain
Weight gain (g)
Lean mass
% of body weight
Fat mass
% of body weight
eWAT
% of body weight
iWAT
% of body weight
iBAT
% of body weight
Quadriceps
% of body weight
Liver
% of body weight
C
ITT
Blood glucose (mmol/L)
Time (minuts)
LF/LS
HF/HS
HF/HS-PP
HF/HS-LF/LS
HF/HS-LF/LS-PP
ITT
AUC
KITT
%/min
OGTT
Blood glucose (mmol/L)
Time (minuts)
OGTT
AUC
Fasting glucose
Blood glucose (mmol/L)
Fasting insulin
Plasma insulin (pg/mL)
Plasma insulin, T15
Plasma insulin (pg/mL)
HOMA-IR
Insulin (µU/mL), glucose (mmol/L)
QUICKI
insulin (µU/mL), glucose (mg/dL)

Figure S6. Experimental design and results from an experiment including the group LF/LS-PP. (A) Mice were acclimatized with free access to water and chow-diet for 1 week (week -1). Then, one group was fed a low-fat/low-sucrose diet (LF/LS) and the other four groups were fed a high-fat/high-sucrose diet (HF/HS). After 10 weeks, one group of LF/LS fed mice and one HF/HS group were kept on the same diet, whereas the other three HF/HS groups were fed with either HF/HS diet containing 0.1% pot-pollen (HF/HS-PP), LF/LS diet, or LF/LS diet containing 0.1% pot-pollen (LF/LS-PP) for 8 weeks. (B) From left, cumulative weight gain; total weight gain; percent lean and fat body mass; the relative weight of epididymal white adipose tissue (eWAT); inguinal white adipose tissue (iWAT); interscapular brown adipose tissue (iBAT); quadriceps muscle and liver compared to body weight. (C) From left, insulin tolerance test (ITT) at week 17; area under the curve (AUC) of ITT for each group normalized to initial blood glucose level; glucose disappearance rate (KITT), derived from the ITT; oral glucose tolerance test (OGTT) at week 16; AUC of OGTT for each group normalized to initial blood glucose level; blood glucose after 5h of fasting; fasting plasma insulin; plasma insulin, 15 min after glucose stimulation, homeostasis model assessment of insulin resistance (HOMA-IR) calculated from fasting levels of plasma insulin and blood glucose; and quantitative insulin sensitivity check index (QUICKI). Data are shown as mean ± SD (n = 6-9). Differences between groups were analyzed using two-way ANOVA followed by Tukey-Kramer post-hoc test. *P < 0.05, **P < 0.01, ***P < 0.001, ns: no significant difference.