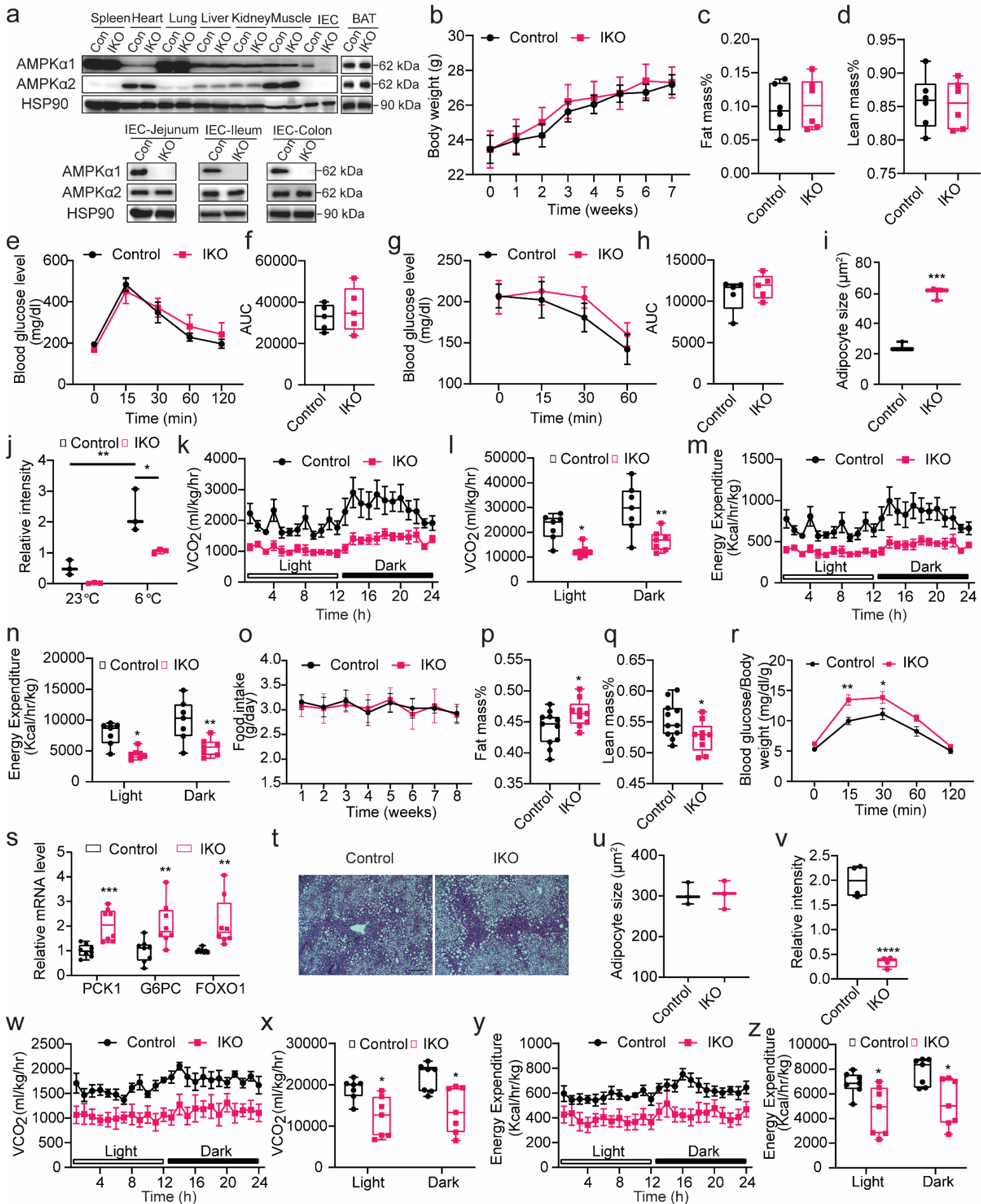


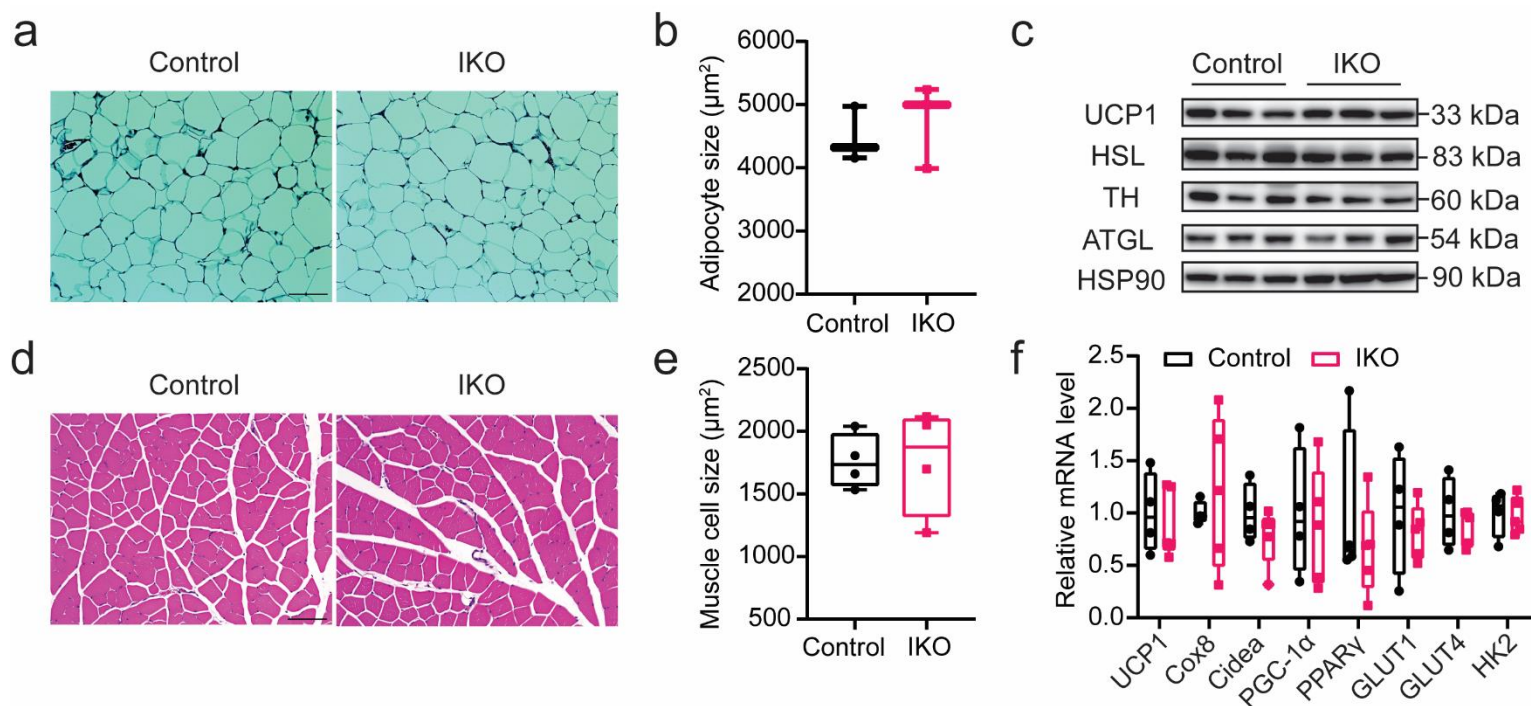
## **Supplementary Information**

**Intestinal AMPK modulation of microbiota mediates crosstalk with brown fat to control thermogenesis**

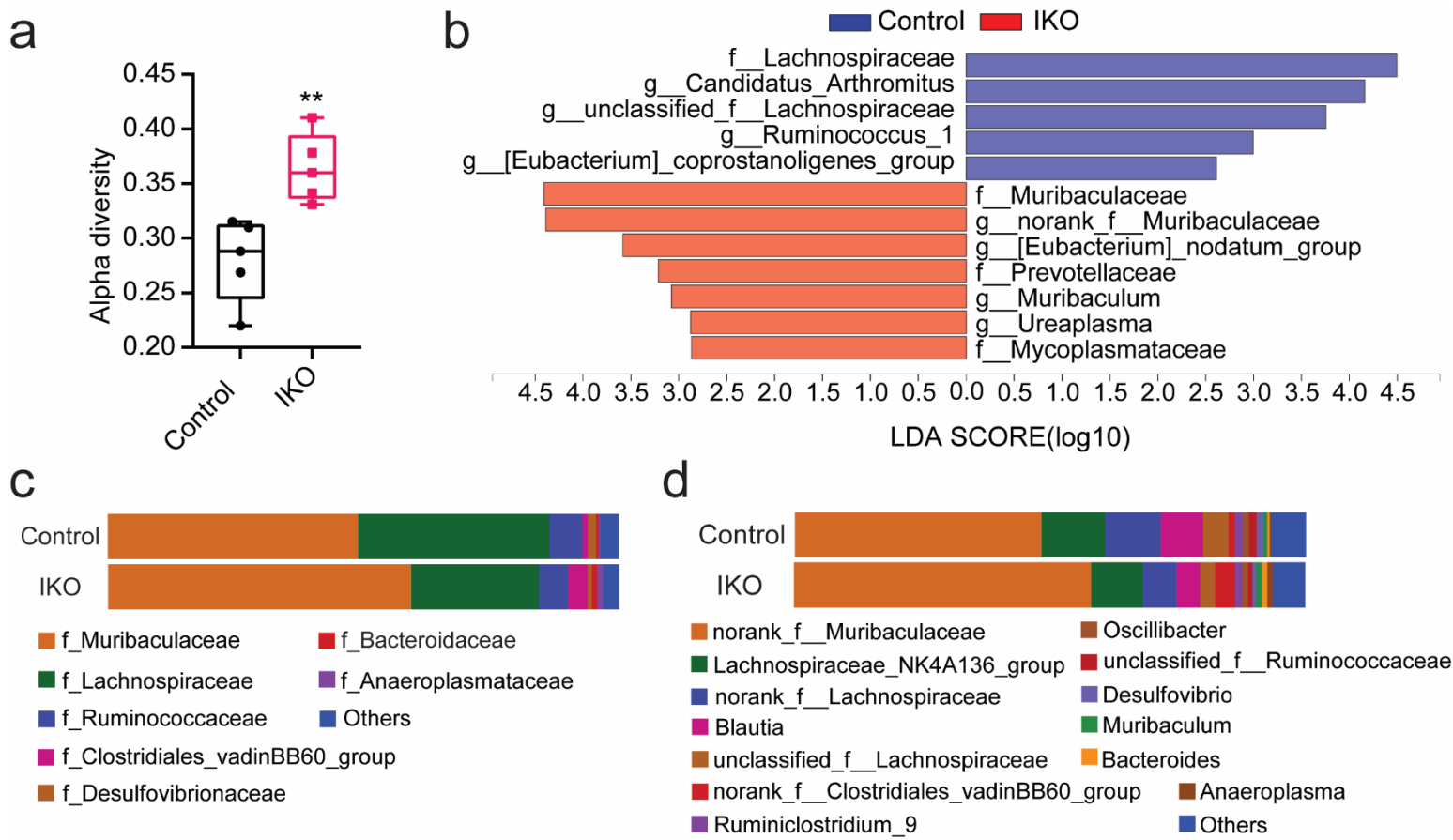
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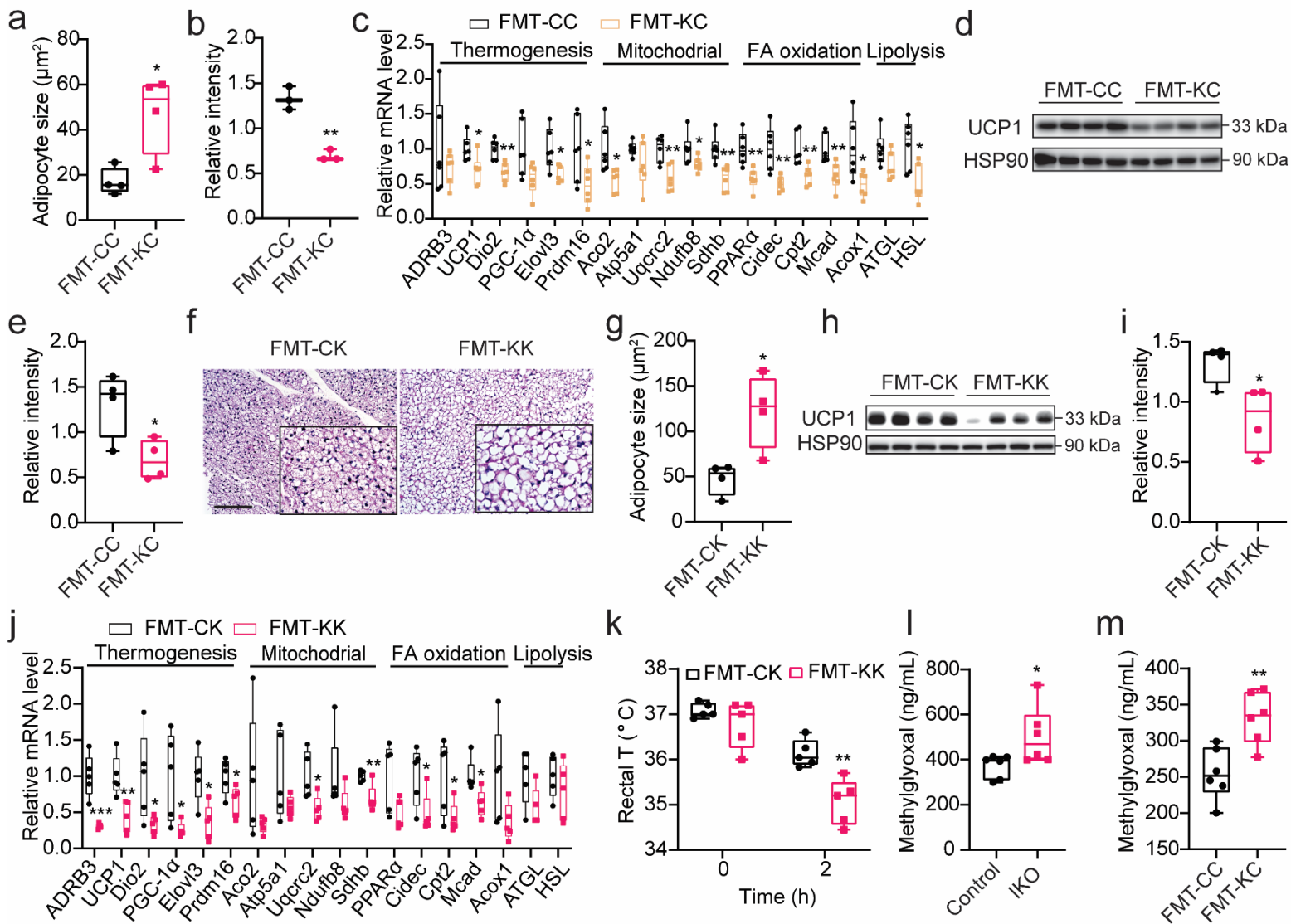
Supplementary Fig. 1 Metabolic phenotypes of AMPK $\alpha$ 1-IKO mice. For a-n, mice were fed chow diet. (a) Western blot analysis of AMPK $\alpha$ 1 protein in various tissues of AMPK $\alpha$ 1<sup>fl/fl</sup> (Con) and AMPK $\alpha$ 1-IKO mice. (b) Body weight of mice from 6-week-old (n=7 biologically independent samples for control group and n=6 biologically independent samples for IKO group). (c-d) The ratio of fat mass (c) and lean mass (d) to the body weights of mice (n=6 biologically independent samples). (e-h) Glucose tolerance test (e, f) and insulin tolerance test (g, h) results, including blood glucose levels and the area under the curve (AUC) for 12-week-old mice (n=5 biologically independent samples). (i) Adipocyte size analysis in Figure 1a (n=3 biologically independent samples), *P* value: 0.0003. (j) The quantified densities of the Western blot bands in Figure 1c (n=3 biologically independent samples), *P* value: 0.0023, 0.0210. (k-n) VCO<sub>2</sub> (k-l) and energy expenditure (m-n) of mice over 24 h (n=7 biologically independent samples), *P* value for l: 0.0009, 0.0081. *P* value for n: 0.0010, 0.0085. For o-z, mice were fed HFD. (o) Daily food intake (n=5 biologically independent samples). (p-q) The ratio of fat mass (p) and lean mass (q) to the body weights of mice. (n=11 biologically independent samples for control group and n=9 biologically independent samples for IKO group), *P* value for p:0.0448. *P* value for q:0.0439. (r) Glucose tolerance test relative to the body weight at 10 weeks of HFD feeding (n=7 biologically independent samples for control group and n=5 biologically independent samples for IKO group), *P* value:0.0025, 0.0271. (s) Gene expression of PCK1, G6PC and FOXO1 in the liver tissue (n=8 biologically independent samples), *P* value:0.0008, 0.0074, 0.0066. (t-u) Representative images (t) and lipid droplet size analysis (u, n=3 biologically independent samples) of H&E-stained liver sections. Scale bar =100  $\mu$ m. (v) The quantified densities of the Western blot bands in Figure 1m (n=7 biologically independent samples). *P* value: <0.0001. (w-z) VCO<sub>2</sub> (w-x) and energy expenditure (y-z) of mice (n=7 biologically independent samples). *P* value for x:0.0071, 0.0078. *P* value for z:0.0189, 0.0105. Values are means  $\pm$  s.e.m. for b, e, g, k, m, o, r, w, y. The boxplot elements (for c-d, f, h-j, l, n, p-q, s, u-v, x, z) are defined as following: center line, median; box limits, upper and lower quartiles; whiskers, 1.5  $\times$  interquartile range. \**P*<0.05, \*\**P*<0.01, \*\*\**P*<0.001 and \*\*\*\**P*<0.0001 cf. Control mice by two-tailed Student's t-tests (for c-d, f, h-i, l, n, p-q, s, u-v, x, z), and two-way ANOVA with Tukey's post-hoc tests (for b, e, g, j, o, r). Source data are provided as a Source Data file.



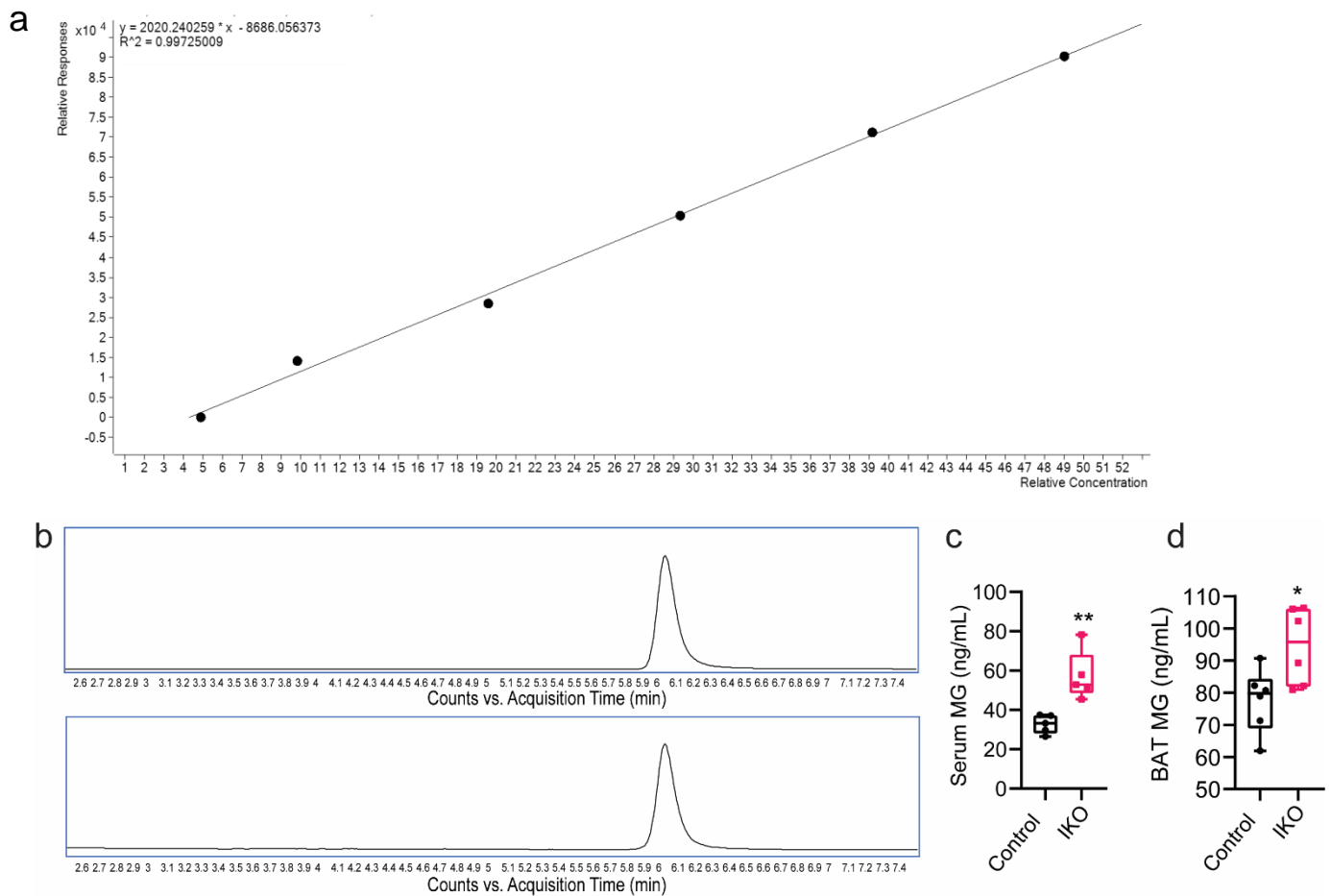
Supplementary Fig. 2. Phenotype of inguinal WAT (iWAT) and muscle in the HFD-fed AMPK $\alpha$ 1<sup>fl/fl</sup> and IKO mice. (a-b) Representative images (a) and adipocyte size analysis (b, n=3 biologically independent samples) of H&E-stained iWAT sections. Scale bar =100  $\mu\text{m}$ . (c) Representative Western blot analysis of UCP1 protein levels in the iWAT. (d-e) Representative images (e) and muscle cell size analysis (f, n=4 biologically independent samples) of H&E-stained muscle sections. Scale bar =100  $\mu\text{m}$ . (f) Relative mRNA levels of genes expressed in the muscle (n=4 biologically independent samples for control group and n=5 biologically independent samples for IKO group). The boxplot elements (for b-c, e-f) are defined as following: center line, median; box limits, upper and lower quartiles; whiskers, 1.5  $\times$  interquartile range. Source data are provided as a Source Data file.



Supplementary Fig. 3 Alteration of gut microbiota profile in AMPK $\alpha$ 1-IKO mice. (a) Alpha diversity analysis of the gut microbiota in AMPK $\alpha$ 1<sup>fl/fl</sup> (Control) and AMPK $\alpha$ 1-IKO mice fed chow diet (n=5 biologically independent samples), *P* value: 0.0055. (b) Linear discriminant analysis (LDA) effect size (LEfSe) score was calculated to evaluate bacterial families or genera overrepresented in mice fed chow diet (n=5 biologically independent samples). (c, d) Bacterial taxon-based analysis at the family level (c) and genus level (d) in mice fed chow diet (n=5 biologically independent samples). The boxplot elements (for a) are defined as following: center line, median; box limits, upper and lower quartiles; whiskers, 1.5  $\times$  interquartile range. \*\**P* < 0.01 by two-tailed Student's *t*-test. Source data are provided as a Source Data file.

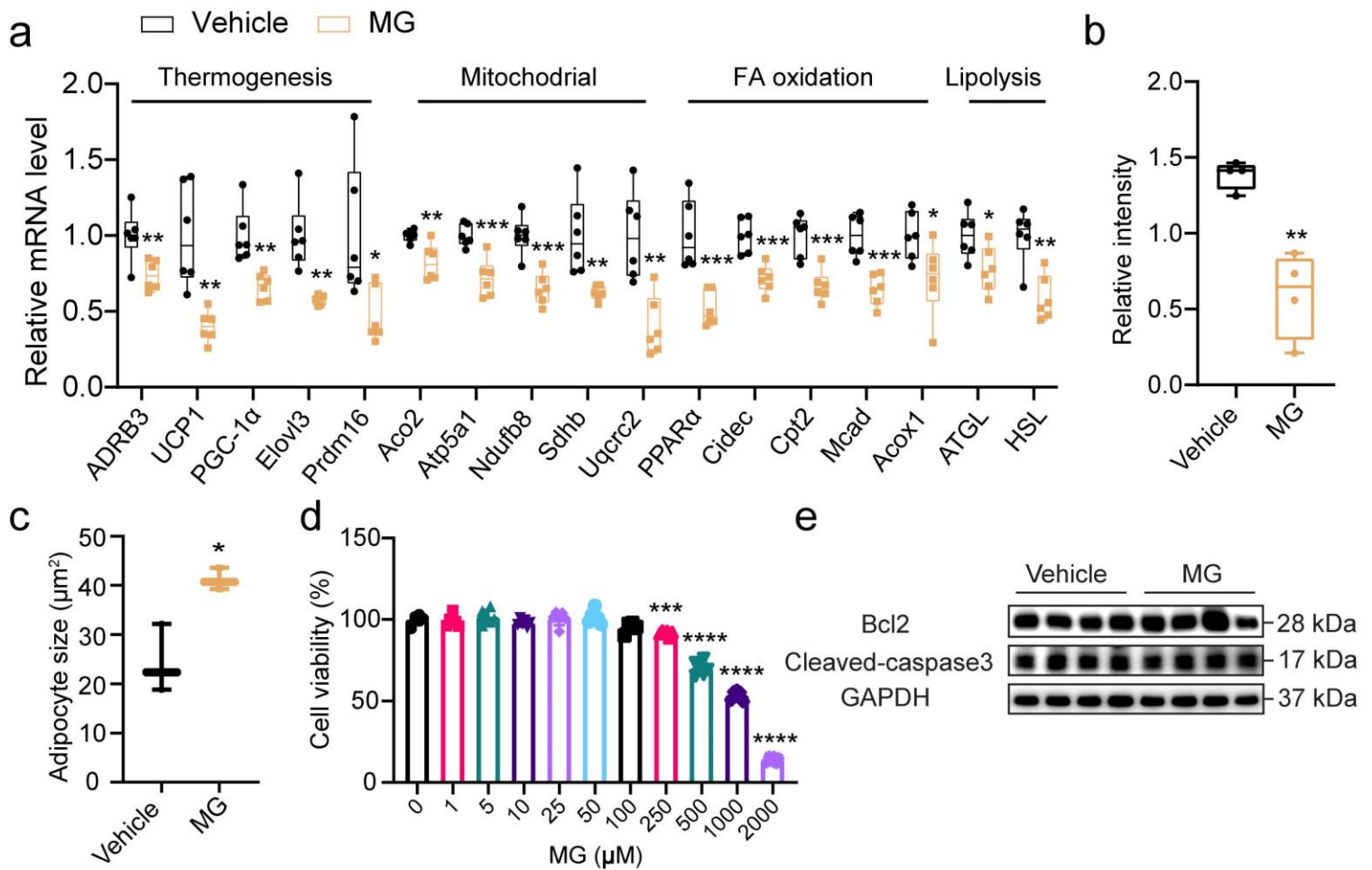


Supplementary Fig. 4 Gut microbiota alteration in AMPK $\alpha$ 1-IKO mice are sufficient to impair BAT thermogenesis. Fresh feces from AMPK $\alpha$ 1<sup>fl/fl</sup> and AMPK $\alpha$ 1-IKO mice were transplanted to WT or AMPK $\alpha$ 1-IKO recipient mice. FMT-CC, FMT from AMPK $\alpha$ 1<sup>fl/fl</sup> mice to WT mice; FMT-KC, FMT from AMPK $\alpha$ 1-IKO mice to WT mice. FMT-CK, FMT from AMPK $\alpha$ 1<sup>fl/fl</sup> mice to AMPK $\alpha$ 1-IKO mice. FMT-KK, FMT from AMPK $\alpha$ 1-IKO mice to AMPK $\alpha$ 1-IKO mice. (a) Adipocyte size analysis in Figure 2c (n=4 biologically independent samples), *P* value: 0.0162. (b) The quantified densities of the Western blot bands in Figure 2e (n=3 biologically independent samples), *P* value: 0.0015. (c) Relative mRNA levels of genes expressed in the BAT of recipient mice exposed to cold for 6 h (n=6 biologically independent samples), *P* value: 0.0209, 0.0016, 0.0129, 0.0442, 0.0159, 0.0027, 0.0471, 0.0026, 0.0031, 0.0039, 0.0067, 0.0069, 0.0216, 0.0100. (d-e) Western blot analysis of UCP1 protein levels in the BAT of recipient mice exposed to cold for 6 h (n=4 biologically independent samples), *P* value: 0.0262. (f-g) Representative images (f) and adipocyte size analysis (g, n=4 biologically independent samples) of H&E-stained BAT sections from FMT recipient mice. Scale bar =100  $\mu$ m. *P* value: 0.0152. (h-i) Western blot analysis of UCP1 protein levels in the BAT of recipient mice (n=4 biologically independent samples), *P* value: 0.0272. (j) Relative mRNA levels of genes expressed in the BAT of recipient mice. (n=6 biologically independent samples), *P* value: 0.0007, 0.0069, 0.0388, 0.0273, 0.0105, 0.0380, 0.0235, 0.0084, 0.0445, 0.0480, 0.0200. (k) Rectal temperatures of recipient mice exposed to 6 °C for 2 h (n=5 biologically independent samples), *P* value: 0.0013. (l) Methylglyoxal levels in the fecal samples in the chow diet-fed AMPK mice measured by LC-MS/MS methods (n=6 biologically independent samples), *P* value: 0.0405. (m) Methylglyoxal levels in the fecal samples in FMT recipient mice measured by LC-MS/MS methods (n=6 biologically independent samples), *P* value: 0.0041. The boxplot elements (for a-c, e, g, i-m ) are defined as following: center line, median; box limits, upper and lower quartiles; whiskers, 1.5  $\times$  interquartile range. \**P* < 0.05, \*\**P* < 0.01 and \*\*\**P* < 0.01 by two-tailed Student's t-tests (for a-c, e-g, i-j, l ), and two-way ANOVA with Tukey's post-hoc tests for m. Source data are provided as a Source Data file.

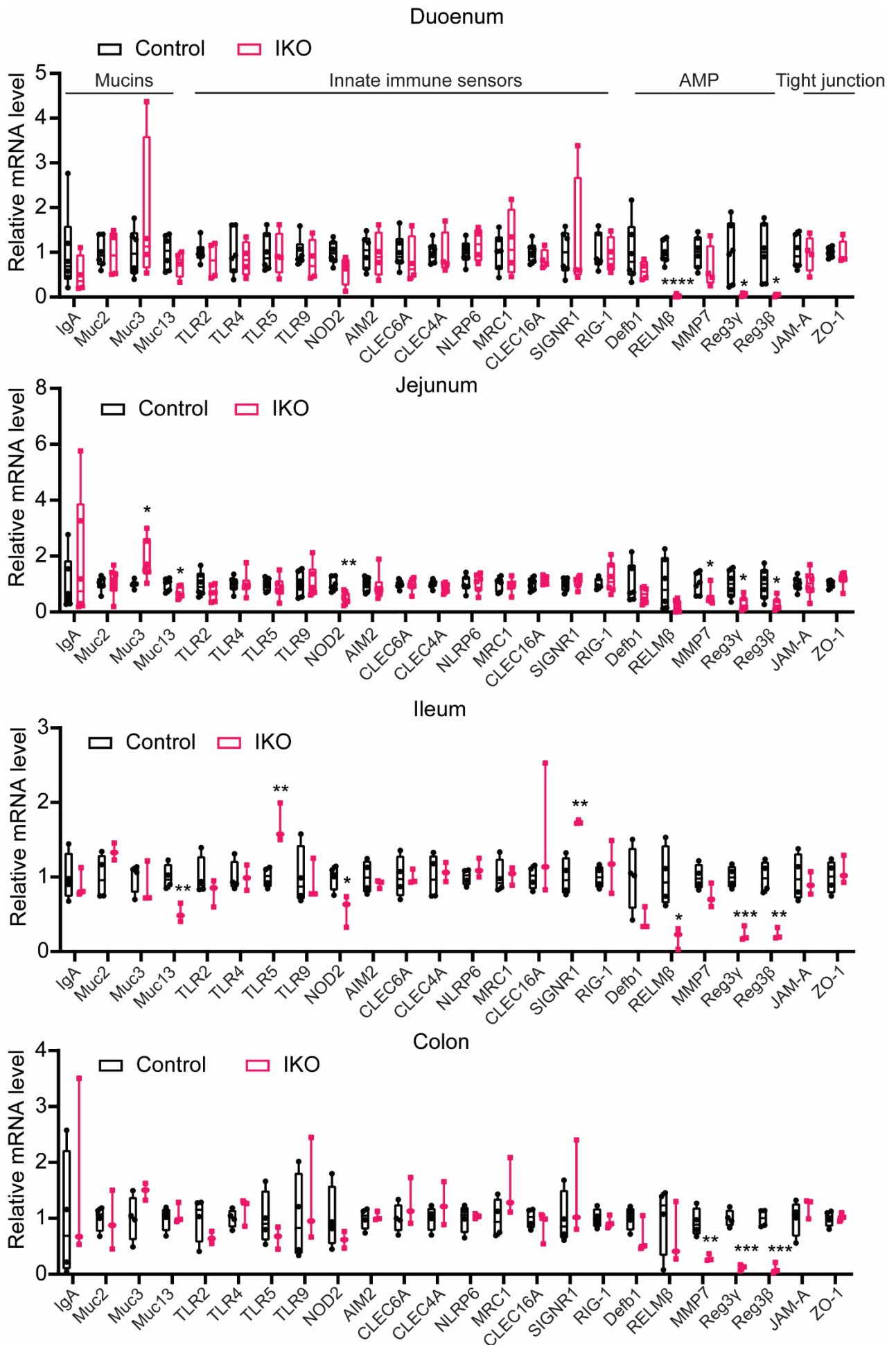


Supplementary Fig. 5 The analysis of methylglyoxal using by LC-MS/MS. (a) The standard curve. (b) the sample curve (below) matched with the standard curve (upper). (c) MG levels in the serum of DIO mice ( $n=5$  biologically independent samples),  $P$  value: 0.0036. (d) MG levels in the BAT tissues of chow diet-fed mice ( $n=6$  biologically independent samples),  $P$  value: 0.0230. The boxplot elements (for c-d) are defined as following: center line, median; box limits, upper and lower quartiles; whiskers,  $1.5 \times$  interquartile range. \* $P < 0.05$ , \*\* $P < 0.01$  by two-tailed Student's  $t$ -tests. Source data are provided as a Source Data file.

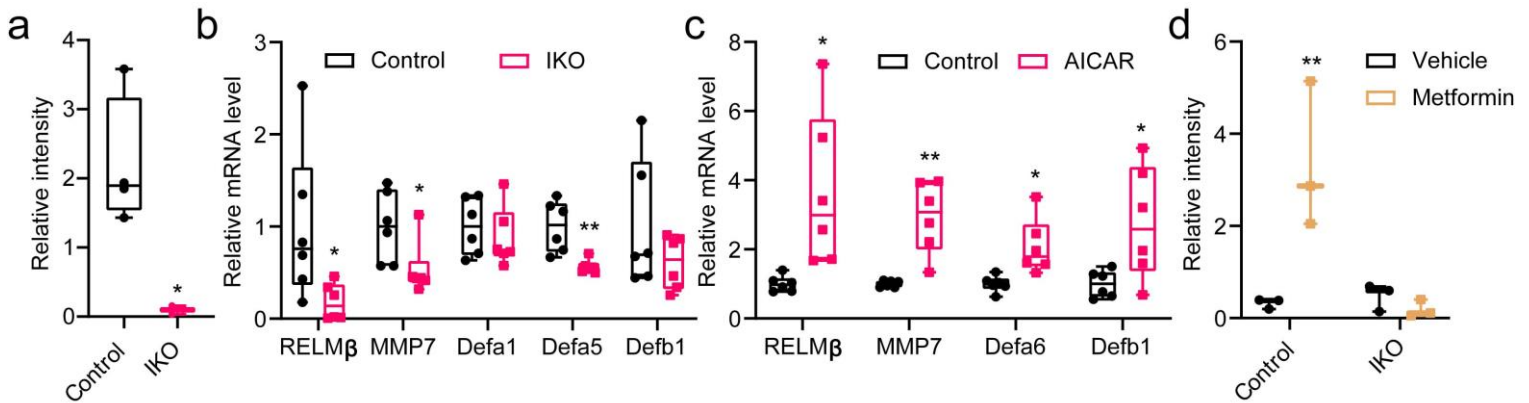




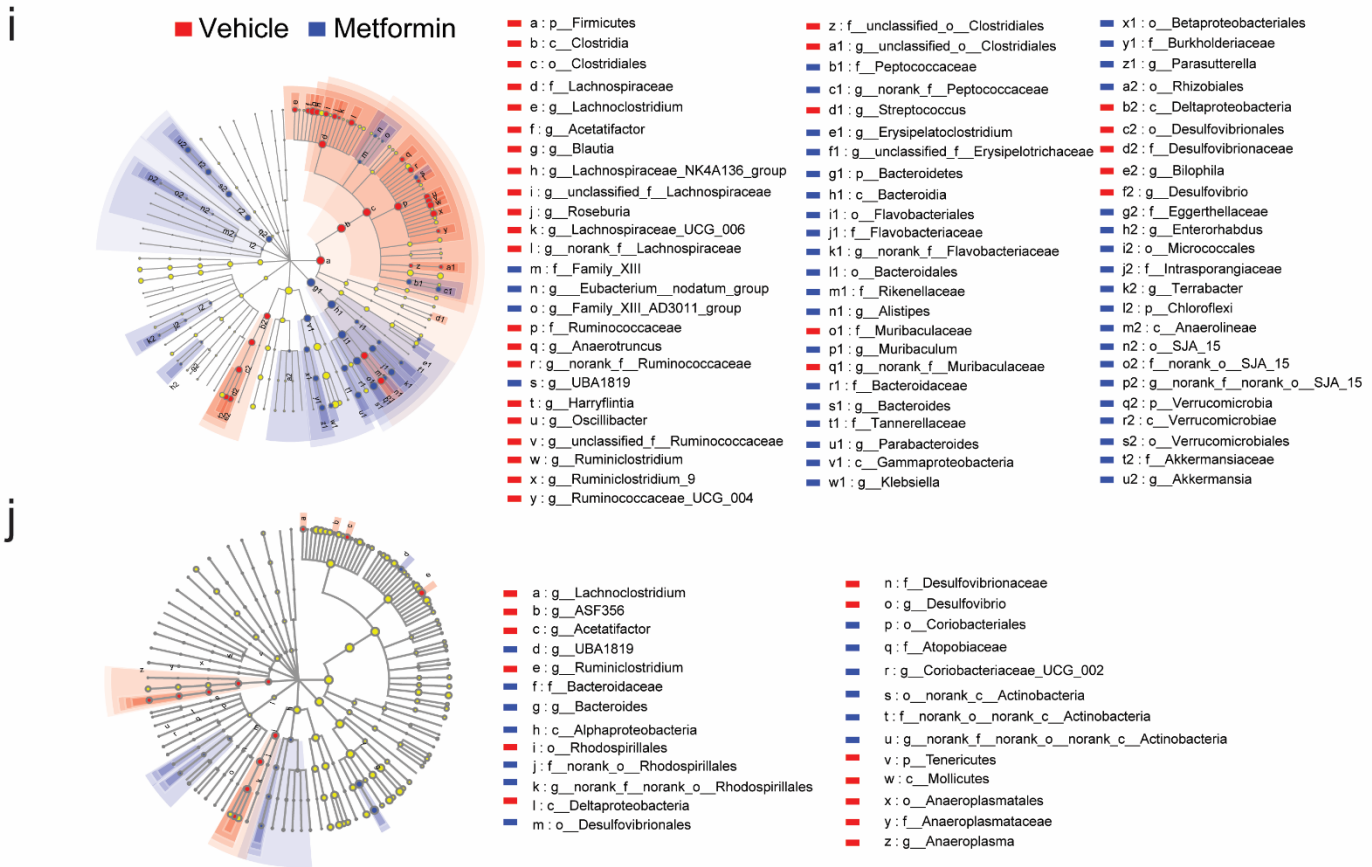
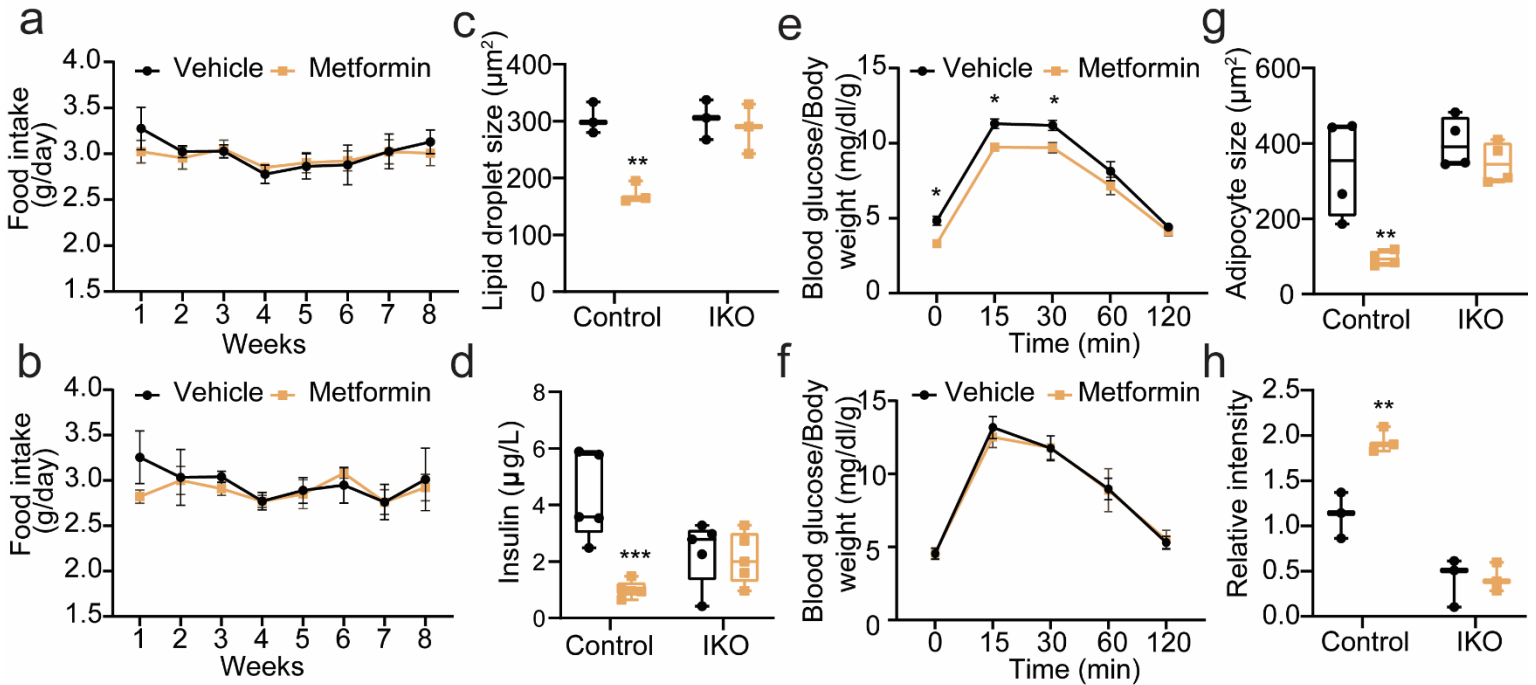
Supplementary Fig. 6 Methylglyoxal treatment reduces the expression of functional genes in brown adipocyte. (a) Gene expression in mature HIB1B cell line treated with 50  $\mu\text{M}$  methylglyoxal ( $n=6$  biologically independent samples),  $P$  value: 0.0086, 0.0019, 0.0021, 0.0010, 0.0246, 0.0063, 0.0008, 0.0004, 0.0060, 0.0018, 0.0008, 0.0006, 0.0007, 0.0005, 0.0348, 0.0268, 0.0014. (b) The quantified densities of the Western blot bands in Figure 2k ( $n=4$  biologically independent samples),  $P$  value: 0.0018. (c) Adipocyte size analysis in Figure 2i ( $n=3$  biologically independent samples),  $P$  value: 0.0163. (d) Cell viability test in the HIB1B cells treated with gradient concentrations of Methylglyoxal (MG) ( $n=6$  biologically independent samples),  $P$  value: 0.0006,  $<0.0001$ ,  $<0.0001$ ,  $<0.0001$ . (e) Bcl2 and Cleaved-caspase3 protein expression in the BAT of WT mice after i.p. injections of methylglyoxal (50 mg/kg) once daily for 2 weeks. The boxplot elements (for a-d) are defined as following: center line, median; box limits, upper and lower quartiles; whiskers,  $1.5 \times$  interquartile range. \* $P<0.05$ , \*\* $P<0.01$ , \*\*\* $P<0.001$  and \*\*\*\* $P<0.0001$  by two-tailed Student's t-tests or one-way ANOVA with Tukey's post-hoc tests. Source data are provided as a Source Data file.



Supplementary Fig. 7 Epithelium-associated factors gene expression in duodenum (n=6 biologically independent samples for control group and n=4 biologically independent samples for IKO group, *P* value: <0.0001, 0.0255, 0.0184), jejunum (n=6 biologically independent samples, *P* value: 0.0180, 0.0323, 0.0035, 0.0405, 0.0111, 0.0157), ileum (n=4 biologically independent samples for control group and n=3 biologically independent samples for IKO group, *P* value: 0.0086, 0.0062, 0.0301, 0.0048, 0.0239, 0.0006, 0.0022) and colon (n=4 biologically independent samples for control group and n=3 biologically independent samples for IKO group, *P* value: 0.0093, 0.0003, 0.0003) tissues in the AMPK $\alpha$ 1<sup>fl/fl</sup> (Control) and AMPK $\alpha$ 1-IKO mice fed chow diet. The boxplot elements are defined as following: center line, median; box limits, upper and lower quartiles; whiskers, 1.5  $\times$  interquartile range. \**P*<0.05, \*\**P*<0.01, \*\*\**P*<0.001 and \*\*\*\**P*<0.0001 by two-tailed Student's t-tests. Source data are provided as a Source Data file.



Supplementary Fig. 8 Intestinal AMPK regulates the expression of AMPs. (a) The quantified densities of the Western blot bands in Figure 3b (n=4 biologically independent samples for control group and n=3 biologically independent samples for IKO group), *P* value: 0.0132. (b) Relative mRNA levels of AMPs in the jejunum of AMPK $\alpha$ 1<sup>fl/fl</sup> (Control) and AMPK $\alpha$ 1-IKO mice fed chow diet (n=6 biologically independent samples), *P* value: 0.0438, 0.0405, 0.0040. (c) Relative mRNA levels of AMPs in HT-29 cells treated with 500  $\mu$ M of AICAR (n=6 biologically independent samples), *P* value: 0.0161, 0.0011, 0.0108, 0.0275. (d) The quantified densities of the Western blot bands in Figure 3h (n=3 biologically independent samples), *P* value: 0.0040. The boxplot elements are defined as following: center line, median; box limits, upper and lower quartiles; whiskers,  $1.5 \times$  interquartile range. \**P* < 0.05, \*\**P* < 0.01 by two-tailed Student's *t*-tests (for a-c) or two-way ANOVA with Tukey's post-hoc tests for d. Source data are provided as a Source Data file.



Supplementary Fig. 9 Intestinal AMPK is required for the metabolic benefits of metformin. DIO AMPK $\alpha$ 1<sup>fl/fl</sup> and AMPK $\alpha$ 1-IKO mice were orally gavage with metformin (100 mg/kg) once daily for 8 weeks. (a-b) Daily food intake of AMPK $\alpha$ 1<sup>fl/fl</sup> mice (a) and AMPK $\alpha$ 1-IKO mice (b) (n=5 biologically independent samples). (c) Lipid droplet size analysis in Figure 4c and 4h (n=3 biologically independent samples), *P* value: 0.0023. (d) Fasting serum levels of insulin in AMPK $\alpha$ 1<sup>fl/fl</sup> mice and AMPK $\alpha$ 1-IKO mice (n=5 biologically independent samples), *P* value: 0.0004. (e-f) Glucose tolerance test relative to the body weight at 8 weeks of metformin administration in the AMPK $\alpha$ 1<sup>fl/fl</sup> (e) and IKO (f) mice (n=7 biologically independent samples), *P* value for e: 0.0248, 0.0203, 0.0301. (g) Adipocyte size analysis in Figure 4k (n=4 biologically independent samples), *P* value: 0.0020. (h) The quantified densities of the Western blot bands in Figure 4l (n=3 biologically independent samples), *P* value: 0.0031. (i-j) Cladogram analysis from the phylum to genus level in AMPK $\alpha$ 1<sup>fl/fl</sup> mice (i) and AMPK $\alpha$ 1-IKO mice (j) (n=5 biologically independent samples). The different color nodes represent the microbial groups with significant enrichment in the corresponding groups. Circles indicate phylogenetic levels from domain to genus. The diameter of each circle is proportional to the abundance of the group. The yellow nodes represent the microbial groups that have no significant difference in different groups. Values are means  $\pm$  s.e.m. for a-b, e-f. The boxplot elements (for c-d, g-h) are defined as following: center line, median; box limits, upper and lower quartiles; whiskers, 1.5  $\times$  interquartile range. \**P*<0.05, \*\**P*<0.01 and \*\*\**P*<0.001 by two-way ANOVA with Tukey's post-hoc tests. Source data are provided as a Source Data file.

Supplementary Table 1 Clinical characteristics.

Characters	Patients without obesity and diabetes	Patients with obesity and diabetes	P value
Age	51.2±2.5	51.0±3.9	0.9332
Gender, M/F	3/2	3/2	
BMI	23.62±2.44	36.42±2.35	<0.0001
HbA1c (%)	5.44±0.27	8.58±1.59	0.0046

Table 1. Clinical characteristics. Data was presented as mean value ± standard deviation. T2D, type II diabetes; BMI, Body mass index. Two tailed-Student's t-tests were used to determine the statistical significance of differences between two groups.

Supplementary Table 2. Sequences of primers for qPCR.

Name	Forward primer (5'-3')	Reverse primer (5'-3')
m_Ucp1	GTACACCAAGGAAGGACCGA	TTTATTCGTGGTCTCCCAGC
m_ADRB3	GGCCCTCTCTAGTTCCCAG	TAGCCATCAAACCTGTTGAGC
m_IRF4	TCCGACAGTGGTTGATCGAC	CCTCACGATTGTAGTCCTGCTT
m_Dio2	AATTATGCCTCGGAGAAGACCG	GGCAGTTGCCTAGTGAAAGGT
m_PGC-1 $\alpha$	TATGGAGTGACATAGAGTGTGCT	CCACTTCAATCCACCCAGAAAG
m_Elov13	TTCTCACGCGGGTAAAAATGG	GAGCAACAGATAGACGACCAC
m_Cox8	TGTGGGGATCTCAGCCATAGT	AGTGGGCTAAGACCCATCCTG
m_Prmd16	CCAAGGCAAGGGCGAAGAA	AGTCTGGTGGGATTGGAATGT
m_Aco2	ATCGAGCGGGGAAAGACATAC	TGATGGTACAGCCACCTTAGG
m_Atp5a1	TCTCCATGCCTCTAACACTCG	CCAGGTCAACAGACGTGTCAG
m_Ndufb8	TGTTGCCGGGGTCATATCCTA	AGCATCGGGTAGTCGCCATA
m_Sdhd	AATTTGCCATTTACCGATGGGA	AGCATCCAACACCATAGGTCC
m_Uqcrc2	AAAGTTGCCCGAAGGTTAAA	GAGCATAGTTTTCCAGAGAAGCA
m_Ppara	AGAGCCCCATCTGTCCTCTC	ACTGGTAGTCTGCAAAACCAAA
m_Cidec	ATGGACTACGCCATGAAGTCT	CGGTGCTAACACGACAGGG
m_Cpt2	CAGCACAGCATCGTACCCA	TCCCAATGCCGTTCTCAAAAT
m_Acox1	TAACCTCCTCACTCGAAGCCA	AGTTCCATGACCCATCTCTGTC
m_Mcad	AGGGTTTAGTTTTGAGTTGACGG	CCCCGCTTTTGTATATTCCG
m_ATGL	GGATGGCGGCATTTACAGACA	CAAAGGGTTGGGTTGGTTCAG
m_Scd1	TTCTTGCGATACTCTGGTGC	CGGGATTGAATGTTCTTGTGCT
m_36B4	AGATTCCGGATATGCTGTTGGC	TCGGGTCCTAGACCAGTGTTT
m_Reg3 $\gamma$	ATGCTTCCCCGTATAACCATCA	GGCCATATCTGCATCATAACCAG
m_Reg3 $\beta$	ACTCCCTGAAGAATATACCCTCC	CGCTATTGAGCACAGATACGAG
m_RELM $\beta$	AAGCCTACACTGTGTTTCCTTTT	GCTTCCTTGATCCTTTGATCCAC
m_Defb1	AGGTGTTGGCATTCTCACAAG	GCTTATCTGGTTTACAGGTTCCC
m_MMP7	CTGCCACTGTCCCAGGAAG	GGGAGAGTTTTCCAGTCATGG
m_Defa1	TCAAGAGGCTGCAAAGGAAGAGAAC	TGGTCTCCATGTTTACGCGACAGC
m_Defa5	TCAAAAAAGCTGATATGCTATTG	AGCTGCAGCAGAATACGAAAG
h_Reg3a	AGCTACTCATACTGCTGGATTGG	CACCTCAGAAATGCTGTGCTT
h_RELM $\beta$	CCGTCTCTTGCCTCCTTC	CTTTTGACACTAGCACACGAGA
h_Defb1	ATGAGAACTTCCTACCTTCTGCT	TCTGTAACAGGTGCCTTGAATTT
h_Defa6	CTGAGCCACTCCAAGCTGAG	GTTGAGCCCAAAGCTCTAAGAC
h_MMP7	GAGTGAGCTACAGTGGGAACA	CTATGACGCGGGAGTTTAACAT
h_36B4	AACATGCTCAACATCTCCCC	CCGACTCCTCCGACTCTTC