

Supplementary figures

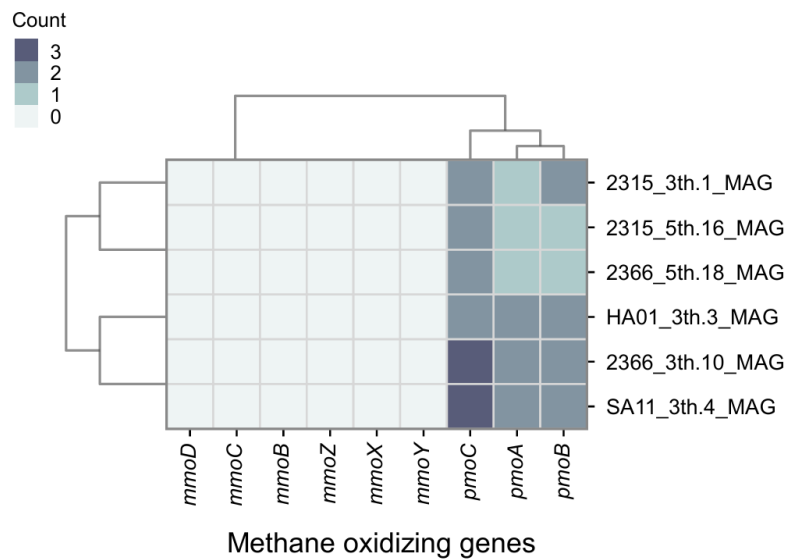


Fig. S1 The methane oxidizing genes (*pmoA*, *pmoB*, *pmoC*, *mmoB*, *mmoC*, *mmoD*, *mmoX*, *mmoY* and *mmoZ*) in metagenomic assembled genomes (MAGs).

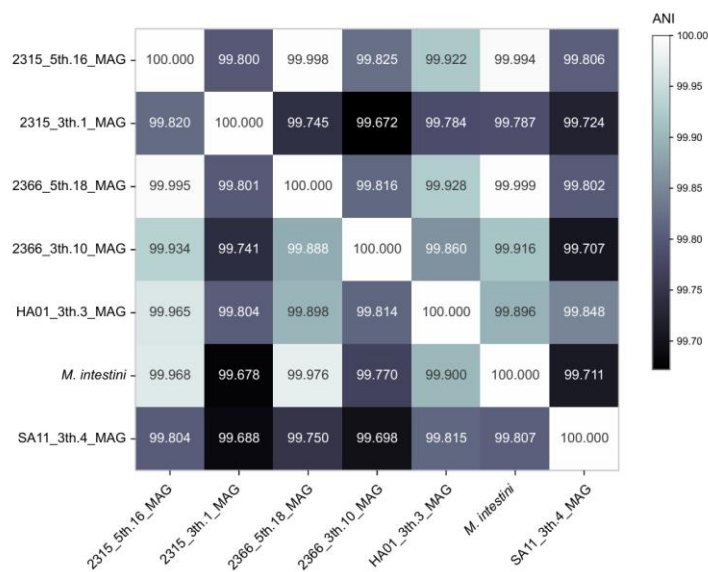


Fig. S2 The average nucleotide identity (ANI) between the *M. intestini* and MAGs with methane oxidizing genes.

A

TYPE (STRAIN)
GENOME SERVER

JOB SUMMARY

Query	Subject	d_0	C.I. d_0	d_4	C.I. d_4	d_6	C.I. d_6	Diff. G+C Percent
'MOB.fa'	<i>Methylocystis parva</i> OBBP	42.6	[39.2 - 46.0]	27.7	[25.3 - 30.2]	37.9	[35.0 - 41.0]	1.91
'MOB.fa'	<i>Methylocystis iwaonis</i> JCM 34278T	28.8	[25.5 - 32.5]	25.7	[23.3 - 28.1]	27.0	[24.1 - 30.1]	2.11
'MOB.fa'	<i>Methylocystis echinoides</i> LMG 27198	25.0	[21.7 - 28.6]	24.5	[22.2 - 27.0]	23.8	[20.9 - 26.9]	1.38
'MOB.fa'	<i>Methylocystis hirsuta</i> DSM 18500	17.8	[14.8 - 21.4]	22.7	[20.4 - 25.1]	17.7	[15.0 - 20.7]	2.84
'MOB.fa'	<i>Methylocystis suflittae</i> NLS-7	17.8	[14.7 - 21.4]	22.5	[20.2 - 25.0]	17.6	[15.0 - 20.6]	2.76
'MOB.fa'	<i>Methylosinus trichosporium</i> OB3b	16.7	[13.7 - 20.2]	22.5	[20.2 - 25.0]	16.7	[14.1 - 19.6]	0.68
'MOB.fa'	<i>Methylocystis rosea</i> SV97	17.8	[14.7 - 21.3]	22.5	[20.3 - 25.0]	17.6	[15.0 - 20.6]	2.77
'MOB.fa'	<i>Methylocystis silviterrae</i> FST	18.1	[15.0 - 21.6]	22.3	[20.0 - 24.7]	17.8	[15.2 - 20.8]	2.66
'MOB.fa'	<i>Methylocystis bryophila</i> DSM21852	14.7	[11.9 - 18.1]	21.6	[19.4 - 24.0]	14.9	[12.4 - 17.8]	2.07
'MOB.fa'	<i>Methylosinus sporium</i> DSM 17706	17.4	[14.4 - 21.0]	21.5	[19.2 - 23.9]	17.2	[14.6 - 20.2]	0.02

Strain	Conclusion	Identification result
'MOB'	potential new species	

MOB: *M. intestini* in this study

B

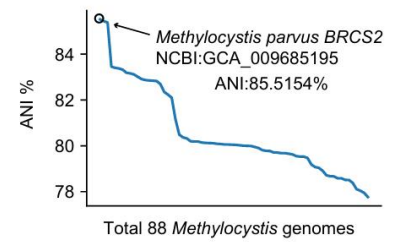


Fig. S3 (A) Identification of *M. intestini* in this study using TYGS database (<https://tygs.dsmz.de>). This table showed pairwise comparisons of *M. intestini* vs. type-strain genomes.

- formula d_0 (a.k.a. GGDC formula 1): length of all HSPs divided by total genome length
- formula d_4 (a.k.a. GGDC formula 2): sum of all identities found in HSPs divided by overall HSP length
- formula d_6 (a.k.a. GGDC formula 3): sum of all identities found in HSPs divided by total genome length

(B) The Average Nucleotide Identity (ANI) of *M. intestini* and other all 88 *Methylocystis* genomes collected in NCBI database (<https://www.ncbi.nlm.nih.gov>).

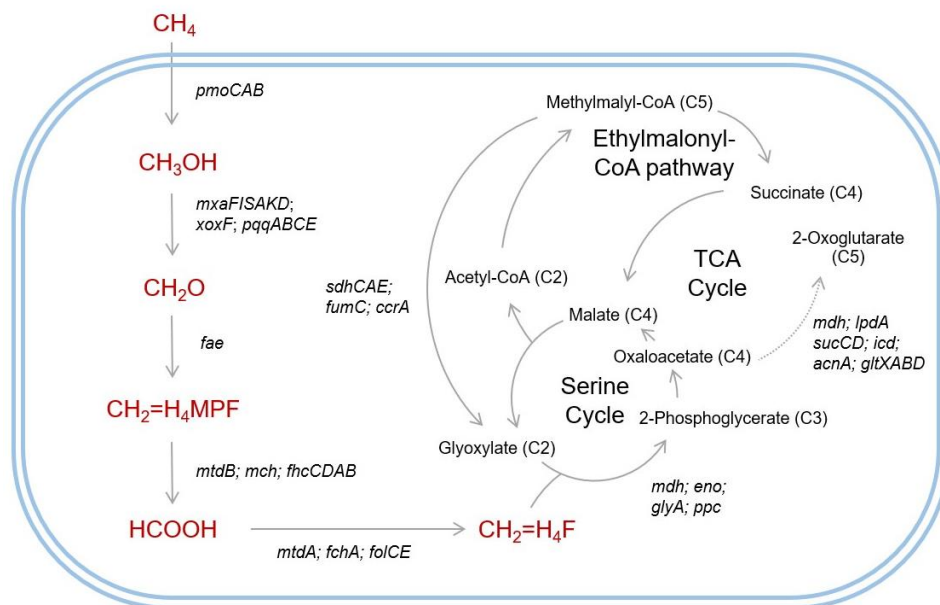


Fig. S4 Methylotrophy pathway of *M. intestini* as deduced from the genome sequence.

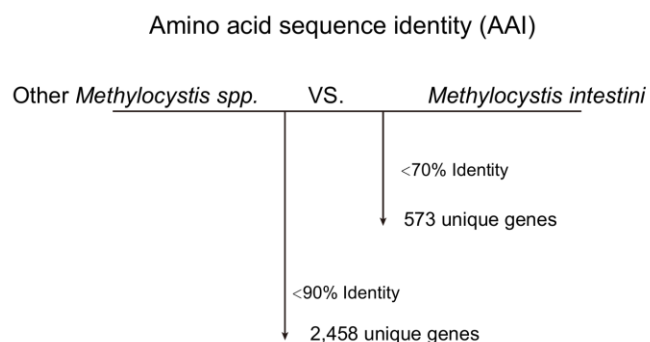


Fig. S5 Comparison of genomic genes between other *Methylocystis* spp. and *M. intestini*. 2,458 and 573 unique genes (at 90% and 70% amino acid sequence identity) are detected.

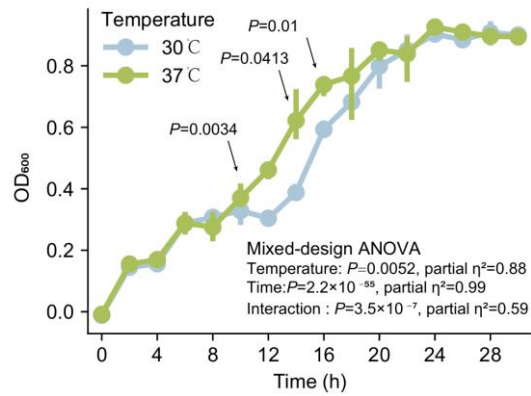


Fig. S6 The growth curve of *M. intestini* at 30 and 37 degrees Celsius. The mixed-design ANOVA test and two-tailed unpaired t-test with Benjamini/Hochberg correction are used (n=6). Data are presented as means $\pm$ standard of mean.

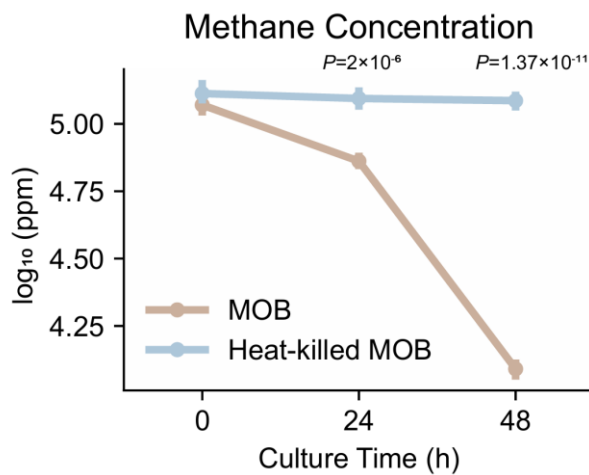


Fig. S7 Methane concentration in headspace gas of the cultured bottle with NMS1 medium. MOB: *M. intestini*. Two-tailed unpaired t-test is used (n=5). Data are presented as means $\pm$ standard of mean.

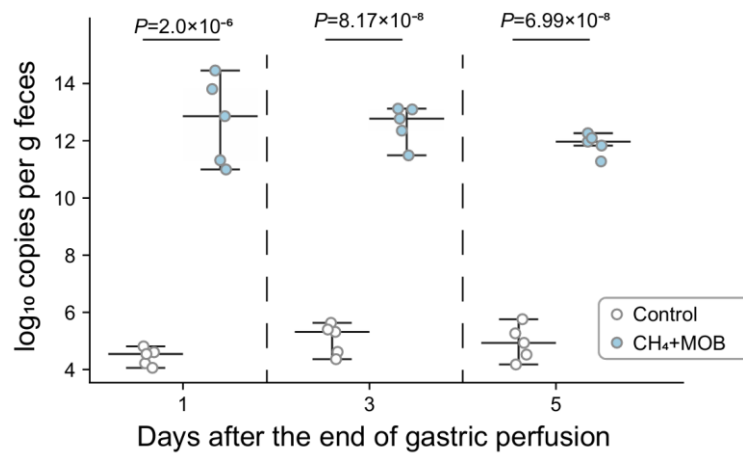


Fig. S8 qPCR of the abundance of *M. intestini* in feces of mice from different groups.

Two-tailed unpaired t-test with Benjamini/Hochberg correction is used ($n=5$). Data are presented as means $\pm$ standard of mean.

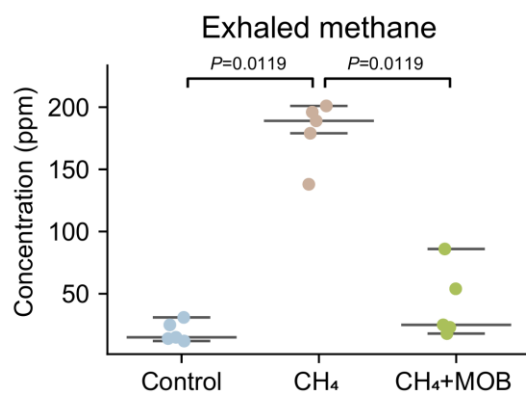


Fig. S9 Exhaled methane concentrations of mice in different groups. Two-tailed Mann-

Whitney-Wilcoxon test with Benjamini/Hochberg correction is used ($n=5$). Data are presented as means $\pm$ standard of mean.

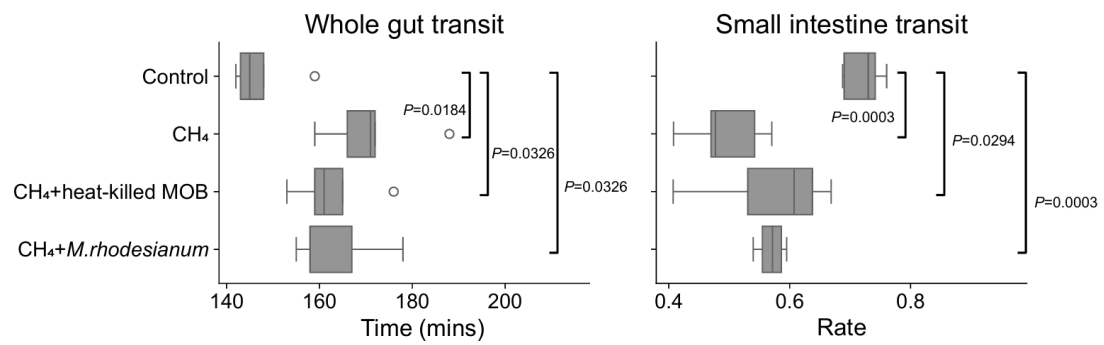


Fig. S10 Left: The whole gut transit time. Right: Small intestine transit rate. MOB: *M. intestini*. *M. rhodesianum*: *Methylobacterium rhodesianum*. Two-tailed unpaired t-test with Benjamini/Hochberg correction is used (n=5). (center line, median; box limits, 25th and 75th percentile; whiskers, Tukey; points, outliers)

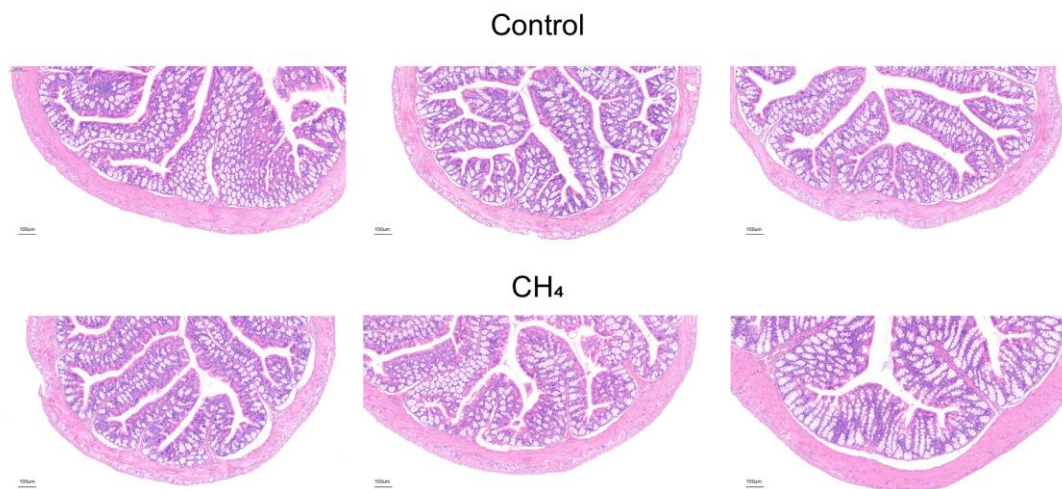


Fig. S11 The Hematoxylin-Eosin (H&E) staining of mice colon in Control and CH₄ groups.

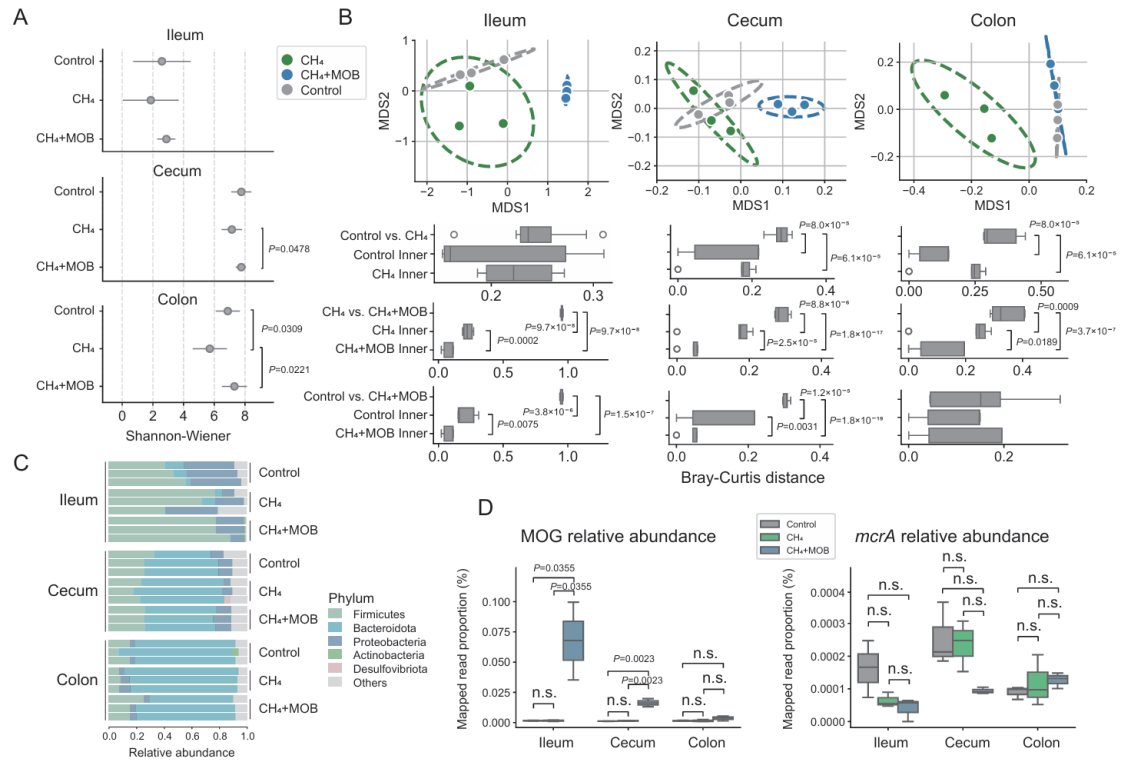


Fig. S12 The gut microbiome in different groups. **(A)** The alpha-diversity, 95% Confidence interval are shown (n=3). **(B)** The non-metric multidimensional scaling analysis. The box plot below shows the Bray-Curtis distance between different groups and within groups (n=3). **(C)** The proportion of gut microbial composition at phylum level. Only the top three phyla are displayed in each sample, and the rest are classed as 'Others' (n=3). **(D)** The mapped read proportion of methane oxidation gene (MOG) and *mcrA* gene in different groups (n=3). Two-tailed unpaired t-test with Benjamini/Hochberg correction is used. Boxplots (center line, median; box limits, 25th and 75th percentile; whiskers, Tukey; points, outliers). Data in **A** are presented as means $\pm$ standard of mean. n.s. represents $P < 0.05$.

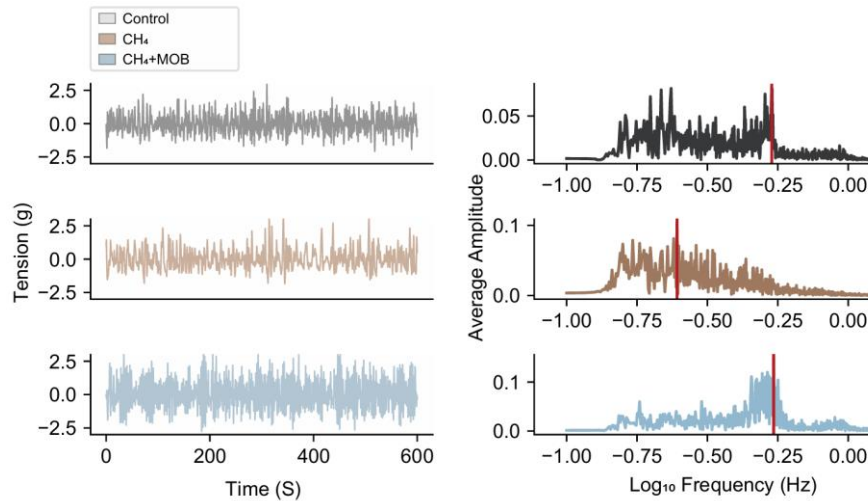


Fig. S13 Spontaneous contractile activity of rat intestinal segment from different groups, and the frequency spectrums of them. The frequency with highest average amplitude is marked with a red line.

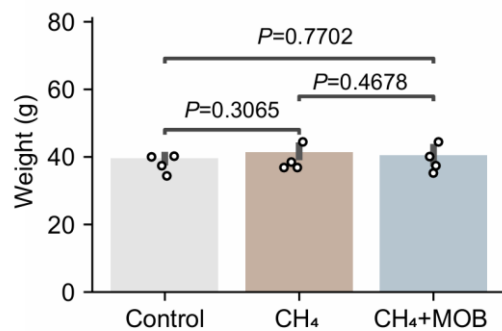


Fig. S14 Weight of mice after four weeks of intervention. Two-tailed Mann-Whitney-Wilcoxon test is used ($n=4$). Data are presented as means $\pm$ standard of mean.

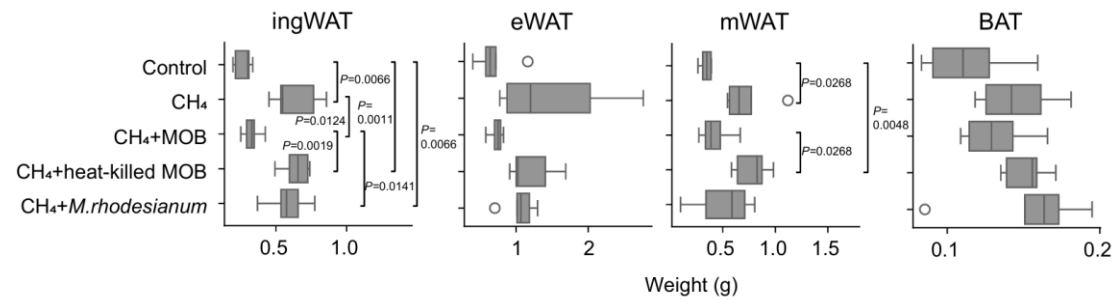


Fig. S15 Comparisons among different groups in adipose tissue weight from different depots in KM mice. (WAT: white adipose tissue; BAT, brown adipose tissue; eWAT: epididymal WAT; ingWAT: inguinal WAT; mWAT: mesenteric WAT.) Two-tailed unpaired t-test with Benjamini/Hochberg correction is used (n=5). (center line, median; box limits, 25th and 75th percentile; whiskers, Tukey; points, outliers)

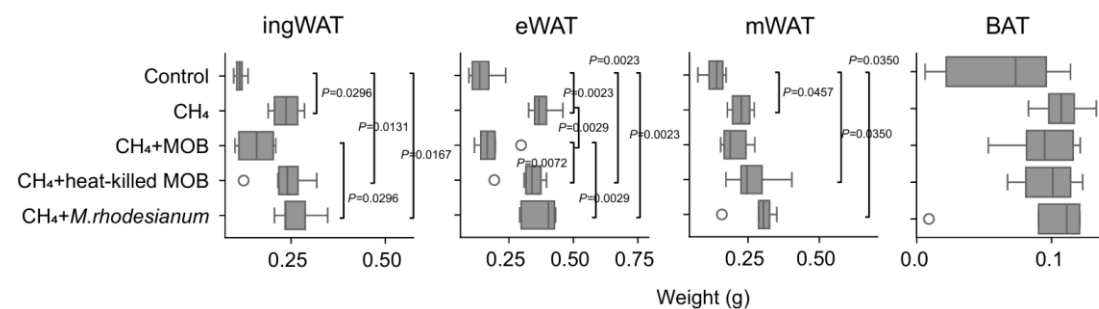


Fig. S16 Comparisons among different groups in adipose tissue weight from different depots in C57BL/6 mice. (WAT: white adipose tissue; BAT, brown adipose tissue; eWAT: epididymal WAT; ingWAT: inguinal WAT; mWAT: mesenteric WAT.) Two-tailed unpaired t-test with Benjamini/Hochberg correction is used (n=5). (center line, median; box limits, 25th and 75th percentile; whiskers, Tukey; points, outliers)

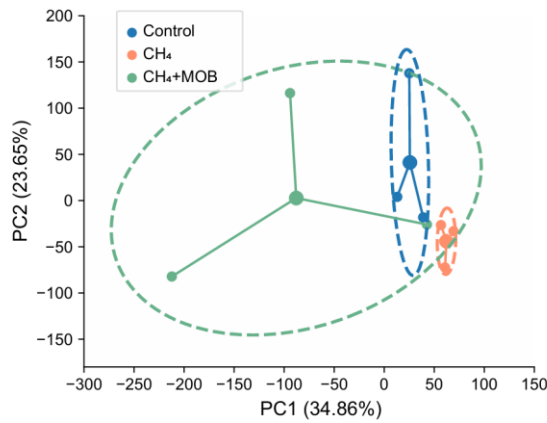


Fig. S17 Principal Component Analysis (PCA) of three groups of RNA sequencing data of the ileum collected after one week of intervention. The large dots in the figure represent the centroids obtained for each group, and the circle formed by the dashed line represents the 65% confidence interval.

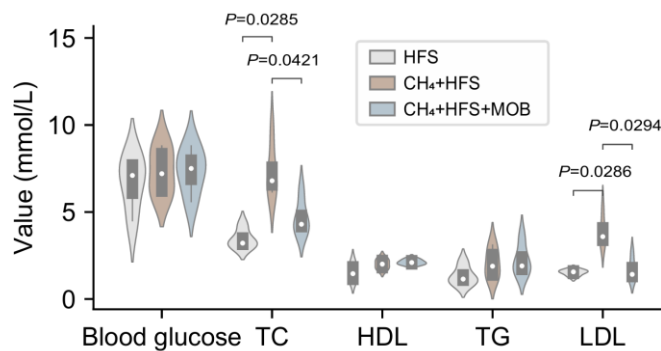


Fig. S18 Blood indexes of mice in different groups. TC: total cholesterol. HDL: high-density lipoprotein. TG: triglyceride. LDL: low-density lipoprotein. Violin plot showing distribution of blood indexes. Horizontal bars within boxes denote medians. Tops and bottoms of boxes represent 25th and 75th percentiles, and lines extend to the 1.5 $\times$ interquartile range. The two-tailed Mann-Whitney-Wilcoxon test was performed (n=4).

Supplementary Tables

Table S1. Composition of NMS1 medium

	Weight (g)
MgSO ₄ ·7H ₂ O	0.2
CaCl ₂ ·2H ₂ O	0.14
KNO ₃	1
KH ₂ PO ₄	0.26
Na ₂ HPO ₄	0.51

Dissolve the above substances in 1L of water.