读书报告

汇报人：李帅

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nature medicine

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A purified membrane protein from *Akkermansia muciniphila* or the pasteurized bacterium improves metabolism in obese and diabetic mice

Hubert Plovier[1], Amandine Everard[1,8], Céline Druart[1,8], Clara Depommier[1,8], Matthias Van Hul[1], Lucie Geurts[1], Julien Chilloux[2], Noora Ottman[3,7], Thibaut Duparc[4], Laeticia Lichtenstein[4], Antonis Myridakis[2], Nathalie M Delzenne[1], Judith Klievink[5], Arnab Bhattacharjee[5], Kees C H van der Ark[3], Steven Aalvink[3], Laurent O Martinez[4], Marc-Emmanuel Dumas[2], Dominique Maiter[6], Audrey Loumaye[6], Michel P Hermans[6], Jean-Paul Thissen[6], Clara Belzer[3], Willem M de Vos[3,5] & Patrice D Cani[1]

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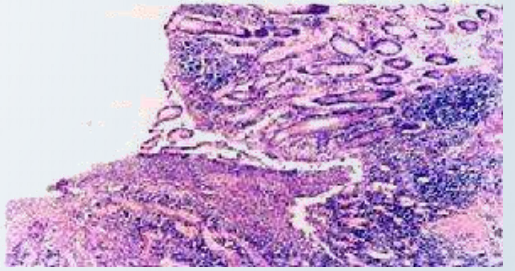
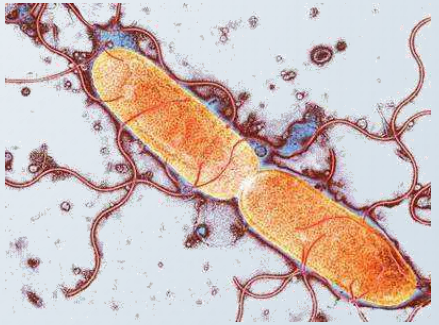
Obesity

Type 2 diabetes

Low-grade inflammation





Specific changes in gut microbiota composition

A. muciniphila **is a Gram-negative, strictly anaerobic, non-motile, non-spore-forming, oval-shaped bacterium. Its type strain is MucT (=ATCC BAA-835T =CIP 107961T).** ***A. muciniphila*** **is able to use mucin as its sole source of carbon and nitrogen, is culturable under anaerobic conditions on medium containing gastric mucin, and is able to colonize the gastrointestinal tracts of a number of animal species.** **1% ~ 5%**

Recently, ***A. muciniphila*** **strain Urmite became the first (evidently) unculturable bacterial strain to be sequenced in its entirety from a human stool sample.**

Akkermansia muciniphila	
Scientific classification	
Kingdom:	Bacteria
Phylum:	Verrucomicrobia
Class:	Verrucomicrobiae
Order:	Verrucomicrobiales
Family:	Verrucomicrobiaceae
Genus:	*Akkermansia*
Species:	***A. muciniphila***
Binomial name	
Akkermansia muciniphila	
Derrien et al 2004	

International Journal of Systematic and Evolutionary Microbiology (2004), **54**, 1469–1476 DOI 10.1099/ijs.0.02873-0

Akkermansia muciniphila gen. nov., sp. nov., a human intestinal mucin-degrading bacterium

Muriel Derrien, Elaine E. Vaughan, Caroline M. Plugge and Willem M. de Vos

Laboratory of Microbiology, Wageningen University, Hesselink van Suchtelenweg 4, 6703 CT Wageningen, The Netherlands

Correspondence
Muriel Derrien
muriel.derrien@wur.nl

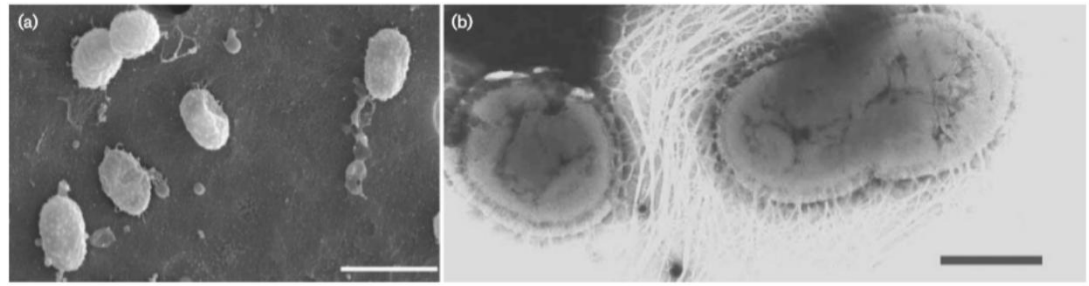


Fig. 3. EM images of strain Muc^T. (a) SEM image. Bar, 1 μm. (b) TEM image of a negatively stained preparation. Note the thickened but extensive capsule fibres of the cells. Bar, 0·5 μm.

Daily administration of live *A. muciniphila* grown on a mucusbased medium can counteract the development of high-fat diet (HFD)-induced obesity and gut barrier dysfunction.

Everard, A. *et al.* Proc. Natl. Acad. Sci. USA 110, 9066–9071 (2013)

The current growth requirements of *A. muciniphila* and its oxygen sensitivity render this bacterium unsuitable for human investigations and putative therapeutic opportunities.

Derrien, M., Vaughan, E.E., Plugge, C.M. & de Vos, W.M. *Int. J. Syst. Evol. Microbiol.* 54, 1469–1476 (2004)

如何解决？

Culture and pasteurization of *Akkermansia muciniphila*.

A. muciniphila MucT (ATTC BAA-835)

培养基替换 — mucin → 16 g/l soy-peptone, 4 g/l threonine, a mix of glucose and N-acetylglucosamine (25 mM each)

冲洗、收集 — Anaerobic PBS with 25% (vol/vol) glycerol

巴氏灭菌 — Pasteurization for 30 min at 70 °C.
Cultures were then immediately frozen and stored at −80 °C.

平板计数 — Plate counting using mucin media containing 1% agarose

Mice.

Cohorts of 10- to 11-week-old male C57BL/6J mice

12 h daylight cycle, lights off at 6 p.m.

Normal chow diet (ND)

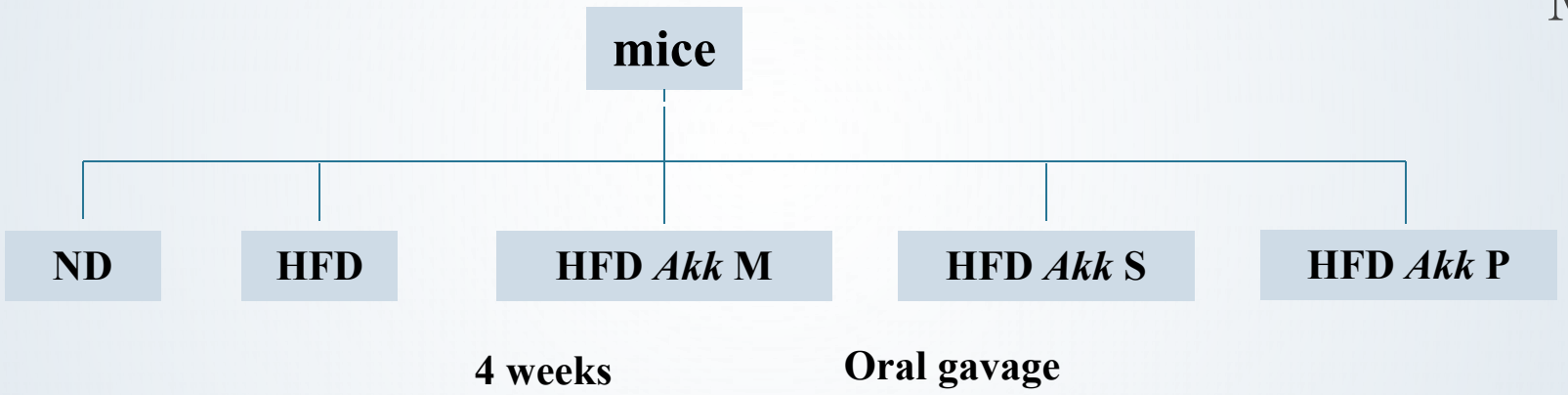
High-fat diet (HFD)

Body weight, food and water intake were recorded once weekly.

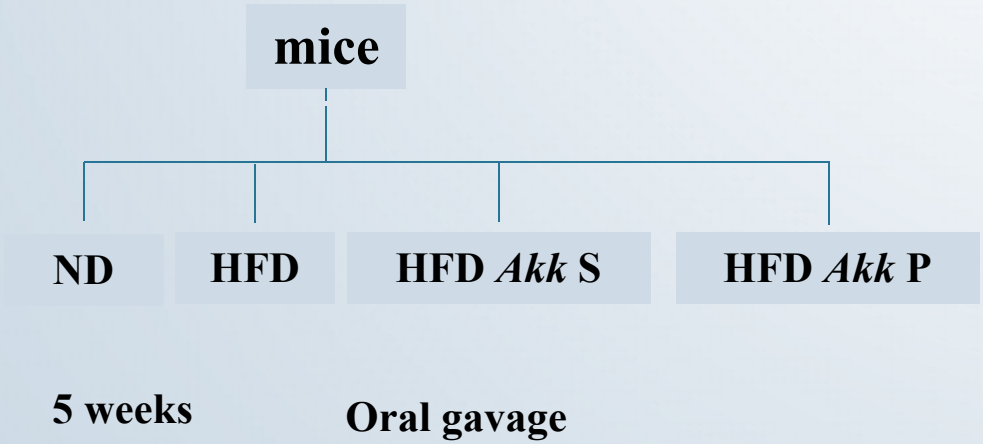
Body composition

7.5 MHz time domainnuclear magnetic resonance (TD-NMR)

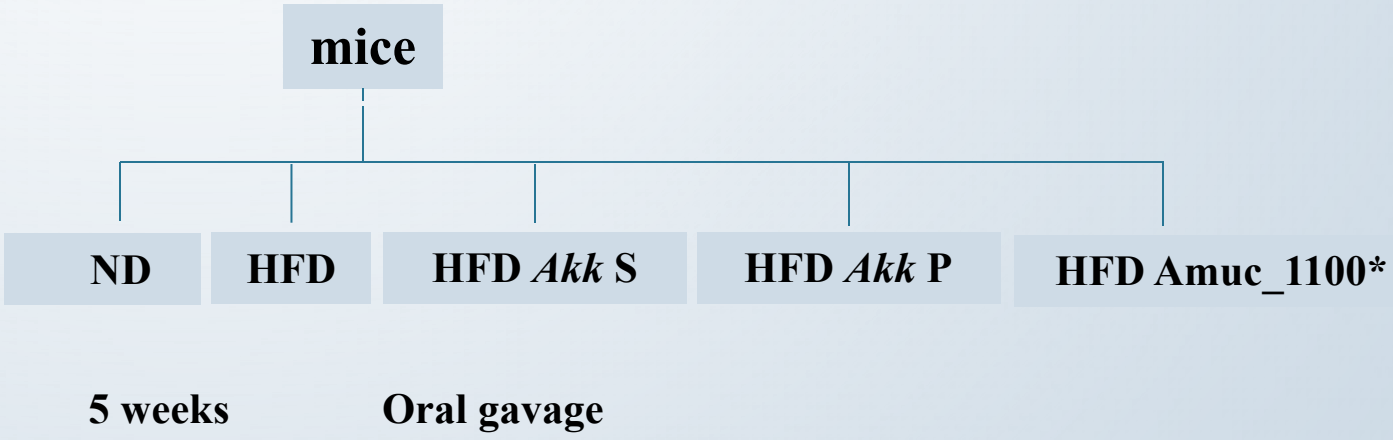
Experiment 1



Experiment 2



Experiment 3



also excluded. Finally, for all analyses and for each group, any exclusion decision was supported by the use of the Grubbs test for outlier detection. Moreover, during the second experiment, two mice from the same cage in the group HFD *Akk* S were excluded from analysis of the OGTT and insulin data displayed in **Supplementary Figure 1d–g**, because of aggressiveness and fighting throughout the OGTT leading to abnormal blood glucose and insulin values.

Oral glucose tolerance test.

- 6h-fasted mice
- Oral gavage
- 2 g glucose per kg body weight
- 0, 15, 30, 60, 90 and 120 min
- glucose meter (Accu Check, Roche, Switzerland)

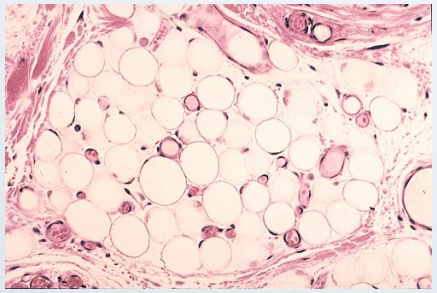
Insulin resistance index.

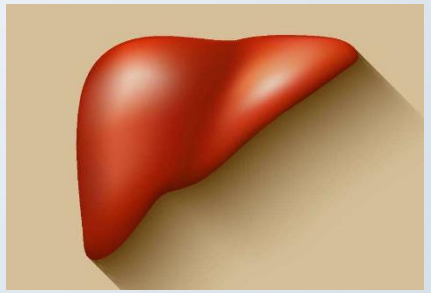
ELISA kit (Mercodia, Uppsala, Sweden)

Both blood glucose (−30 to 120 min)

Plasma insulin (−30 and 15 min)

Tissue sampling.



Histological analyses.

SAT → Ileal tissue

→ 4% paraformaldehyde — 24 h

→ ethanol 100% — 24 h

5 µm ileal paraffin sections

→ Periodic Acid Schiff (PAS) Staining

→ H.E. counter-Staining

→ Determine goblet cell density

Villus length ≥ 5 villi

Urinary metabolomics analyses.

光谱仪 **Spectrometer (Bruker)** **600.22 MHz 1H**

UPLC-MS/MS determination of plasma TMA and TMAO concentrations.

RNA preparation and real-time qPCR analysis.

Supplemental table 4: Primers used in this study.

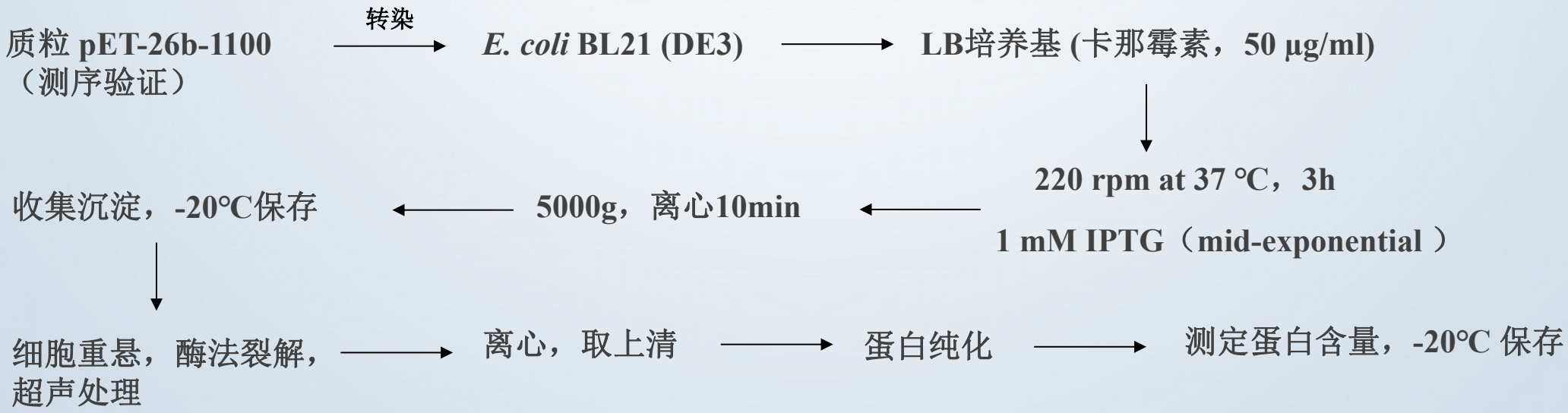
Gene	Forward primer sequence (5'-3')	Reverse primer sequence (5'-3')
Rpl19	GAAGGTCAAAGGGAATGTGTTCA	CCTTGTCTGCCTTCAGCTTGT
Fmo3	GGAACTTGCACTTTGCCTTC	TAGGAGATTGGGCTTTGCAC
Cnr1	CTGATGTTCTGGATCGGAGTC	TCTGAGGTGTGAATGATGATGC
Cldn3	TCATCGGCAGCAGCATCATCAC	ACGATGGTGATCTTGGCCTTGG
Ocln	ATGTCCGGCCGATGCTCTC	TTTGGCTGCTCTTGGGTCTGTAT
Napepld	TTCTTTGCTGGGGATACTGG	GCAAGGTCAAAAGGACCAAA
Naaa	ATTATGACCATTGGAAGCCTGCA	CGCTCATCACTGTAGTATAAATTGTGTAG
Lyz1	GCCAAGGTCTACAATCGTTGTGAGTTG	CAGTCAGCCAGCTTGACACCACG
DefA	GGTGATCATCAGACCCCAGCATCAGT	AAGAGACTAAAACTGAGGAGCAGC
Reg3g	TTCCTGTCCTCCATGATCAAA	CATCCACCTCTGTTGGGTTC
Pla2g2	AGGATTCCCCCAAGGATGCCAC	CAGCCGTTTCTGACAGGAGTTCTGG

Production of Amuc_1100* protein.

pET-26b *E. coli* XL1Blue (Novagen, Merck Millipore, MA, USA)

Primer sequences

5′-GGGTACCATATGATCGTCAATTCCAAACGC-3′ (Forward)
5′-CCTTGGCTCGAGATCTTCAGACGGTTCCTG-3′(Reverse).



Extraction of *A. muciniphila* LPS.

Hot phenol-water extraction method

Zhang, L. & Skurnik, M. J. Bacteriol. 176, 1756–1760 (1994).

Dynamic light scattering analysis.

Heat induced aggregation of Amuc_1100* was measured by light scattering on a Carry Eclipse Fluorescence spectrophotometer (Agilent Biosciences, Santa Clara, CA, USA) equipped with Cary temperature controller and thermophobes. Amuc_1100* (at the concentration of 15 μM) was heated in presence of PBS (pH 7.4) at a constant rate of 1 ° C/min from 30 ° C to 100 ° C. The light scattering at 350 nm was measured with excitation and emission slits at 2.5 nm.

Fast protein liquid chromatography.

Plasma total cholesterol and triglycerides (TG) **lipoproteins**

Western blotting.

p-IRβ (1:1,000; sc-25103, Santa Cruz, CA, USA),
p-Akt^{Thr308} (1:1,000; #2965L, Cell Signaling, Danvers, MA, USA)
p-Akt^{Ser473} (1:1,000; #4060L, Cell Signaling).

β-actin (1:10,000; ab6276).

Plasma LPS analysis.

Endosafe-Multi-Cartridge System (Charles River Laboratories, MA, USA)

Everard, A. et al. Acad. Sci. USA 110, 9066–9071 (2013).

Safety assessment of live and pasteurized *A. muciniphila*.

Sterile PBS containing glycerol

10^{10} CFU live *A. muciniphila* (Akk S - 10^{10})

10^{9} CFU live *A. muciniphila* (Akk S - 10^{9})

10^{10} CFU pasteurized *A. muciniphila* (Akk P - 10^{10})

Statistical analysis.

mean ± s.e.m.

one-way ANOVA — Tukey *post-hoc* test; Kruskal-Wallis test; Dunnett *post-hoc* test

two-way ANOVA — Bonferonni *post-hoc* test; ……

***, P < 0.05; **, P < 0.01 ; ***, P < 0.001**

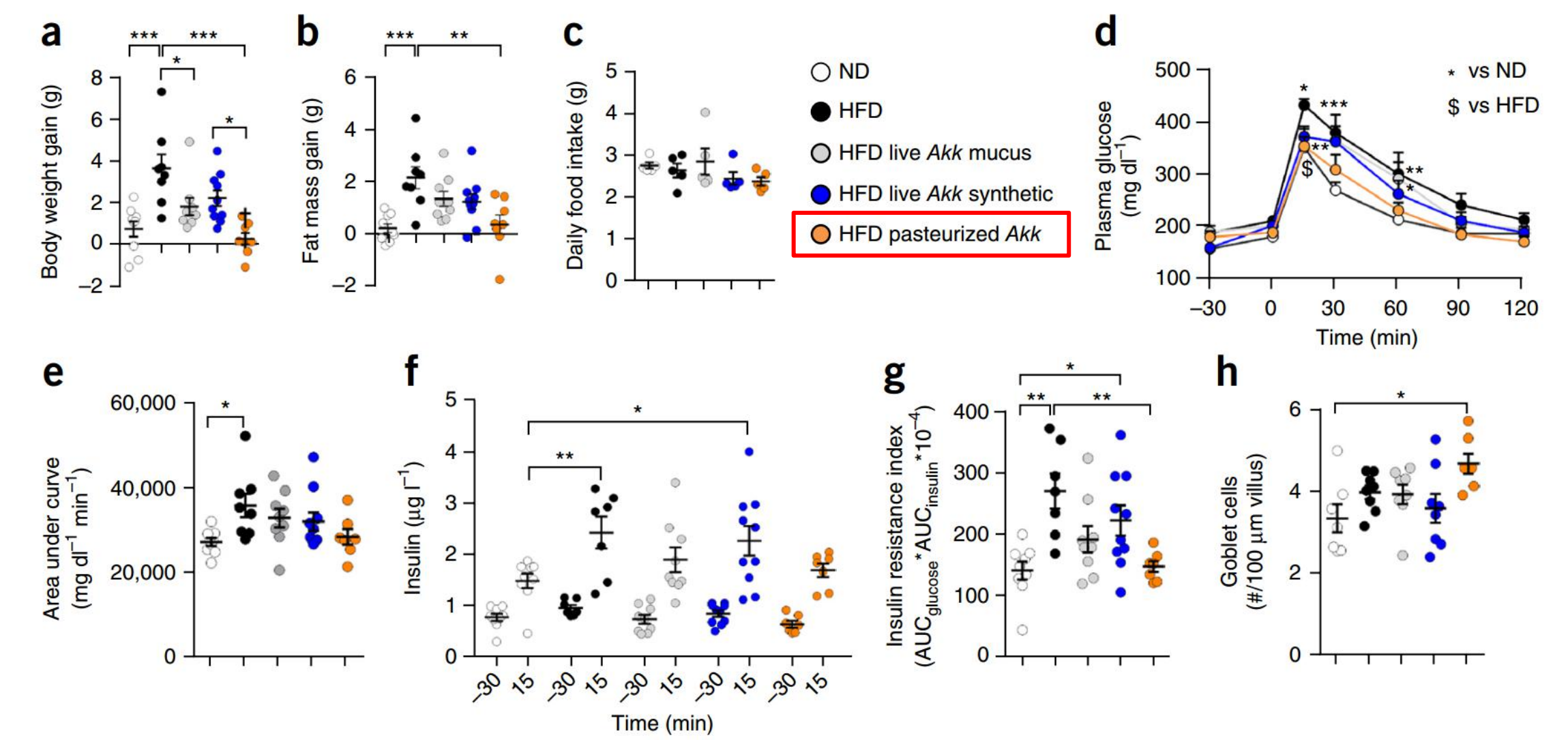
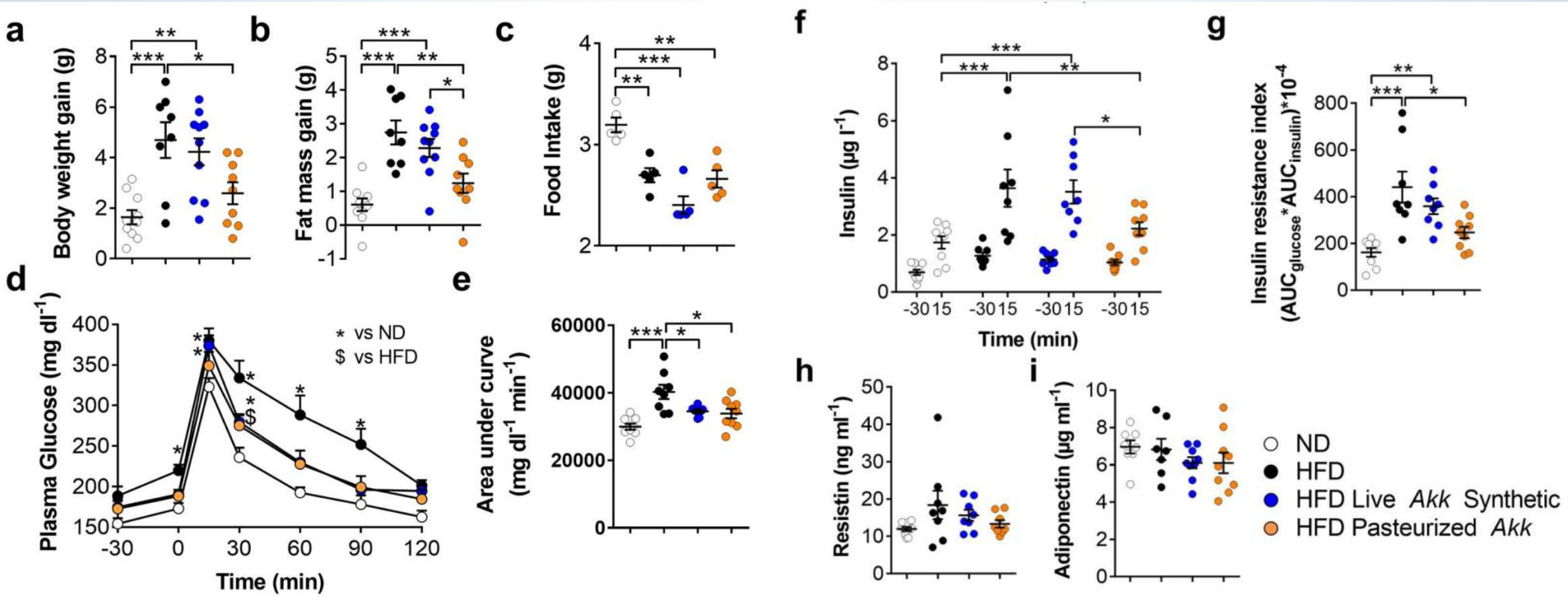
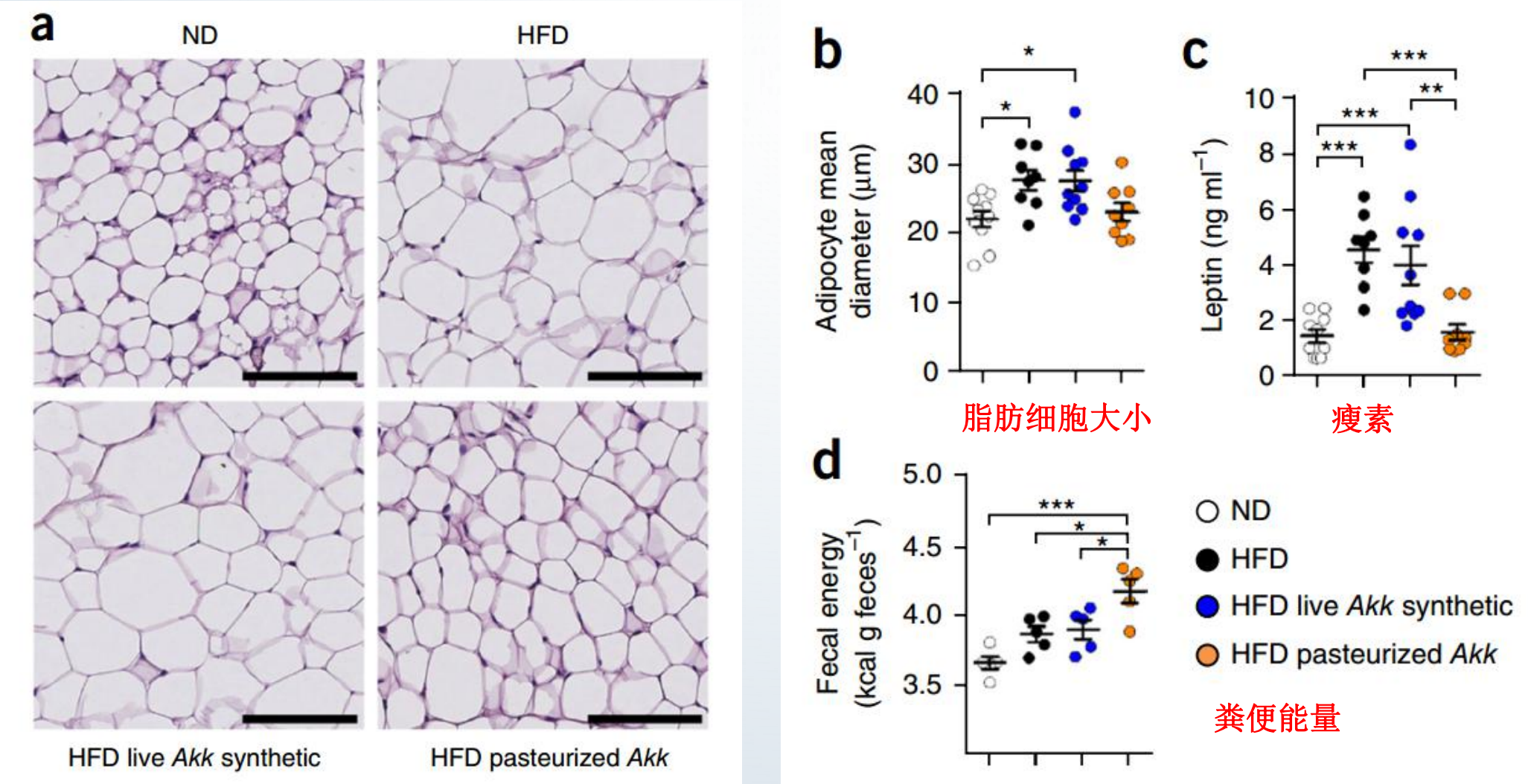


Figure 1 Pasteurization enhances *A. muciniphila*–mediated effects on high-fat diet-induced obesity.



Supplemental figure 1: Increased protection against HFD-induced obesity in mice receiving pasteurized *A. muciniphila* for 5 weeks.



Leptin：能够参与糖、脂肪及能量代谢的调节，促使机体减少摄食，增加能量释放，抑制脂肪细胞的合成，进而使体重减轻。

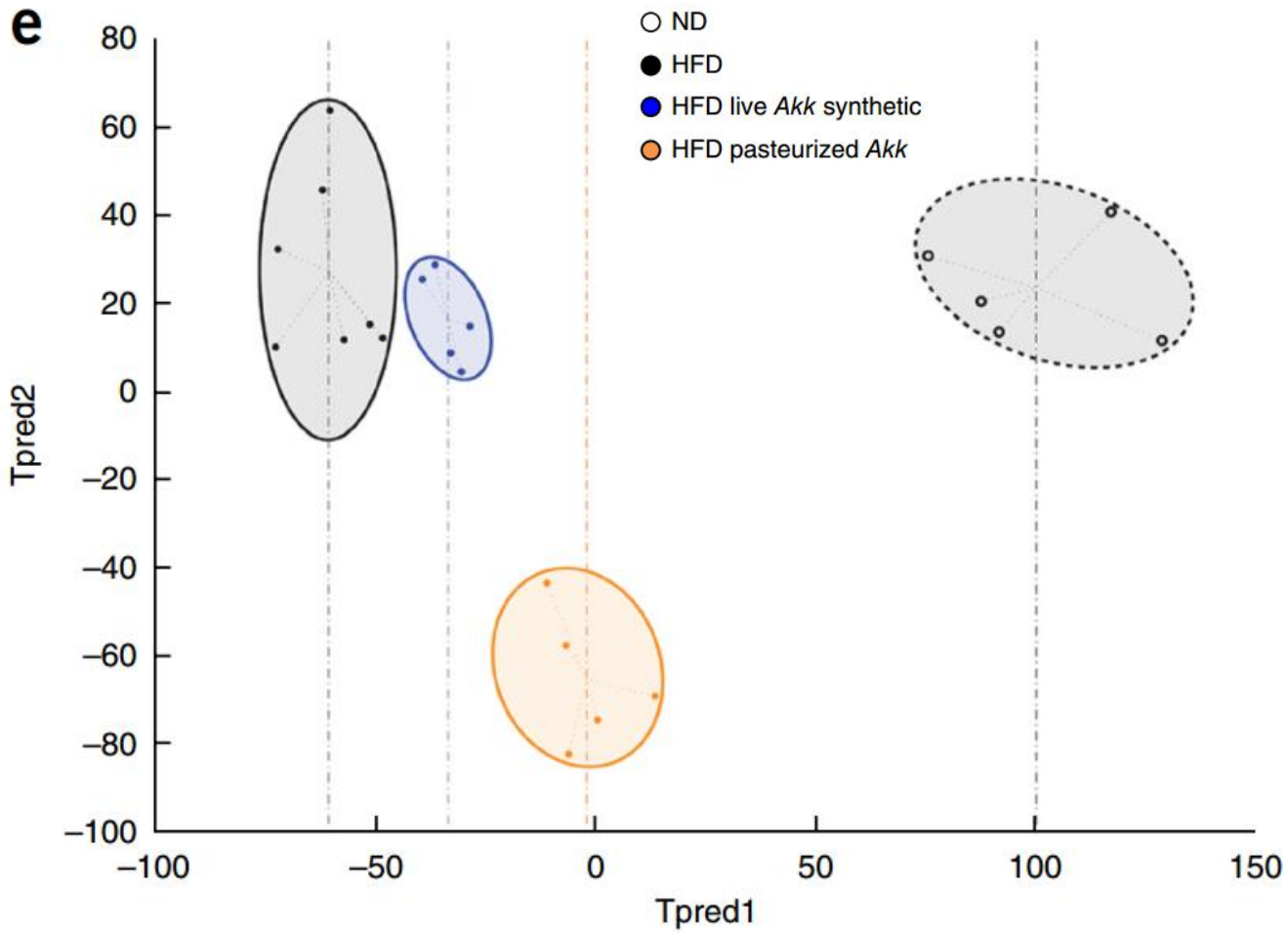
Figure 2 Pasteurized *A. muciniphila* modulates adipose tissue physiology, intestinal energy absorption and urinary metabolome.

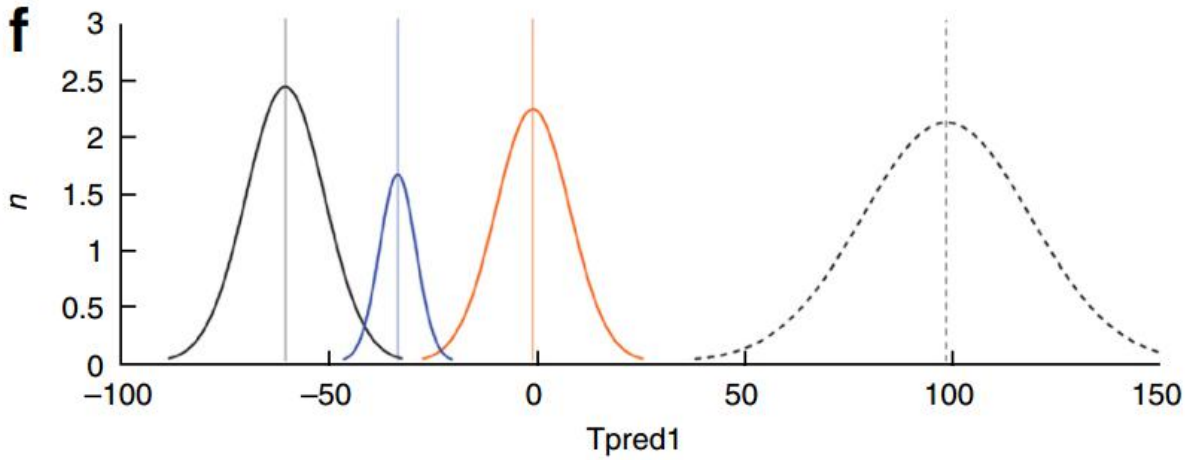
尿液代谢分析

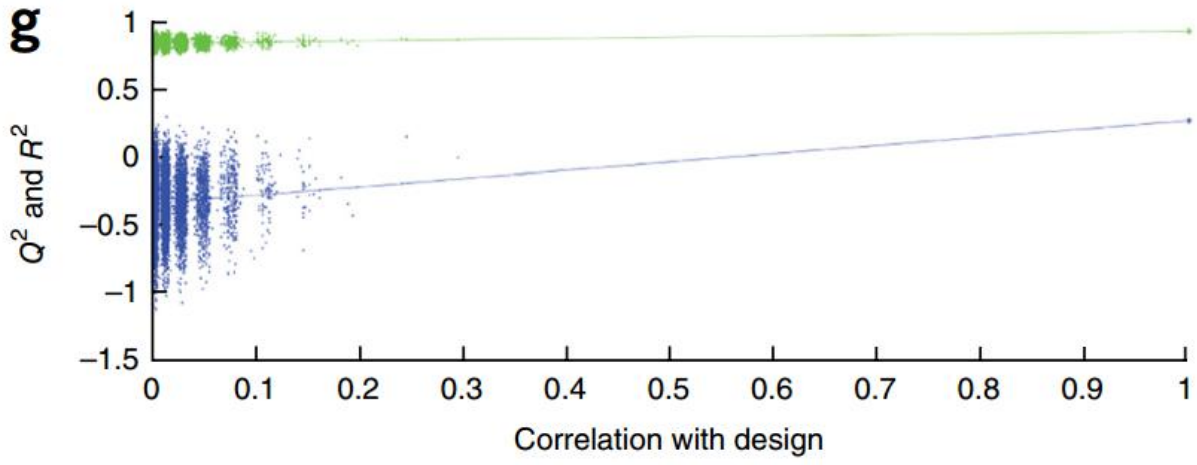
(e) Orthogonal partial least-squares discriminant analysis (OPLS-DA) predictive score plot for urine metabolic profiles representing predictive component 1 (Tpred1) versus Tpred2.

(f) Projection of all treatment groups on the first predictive score of the OPLS-DA model.

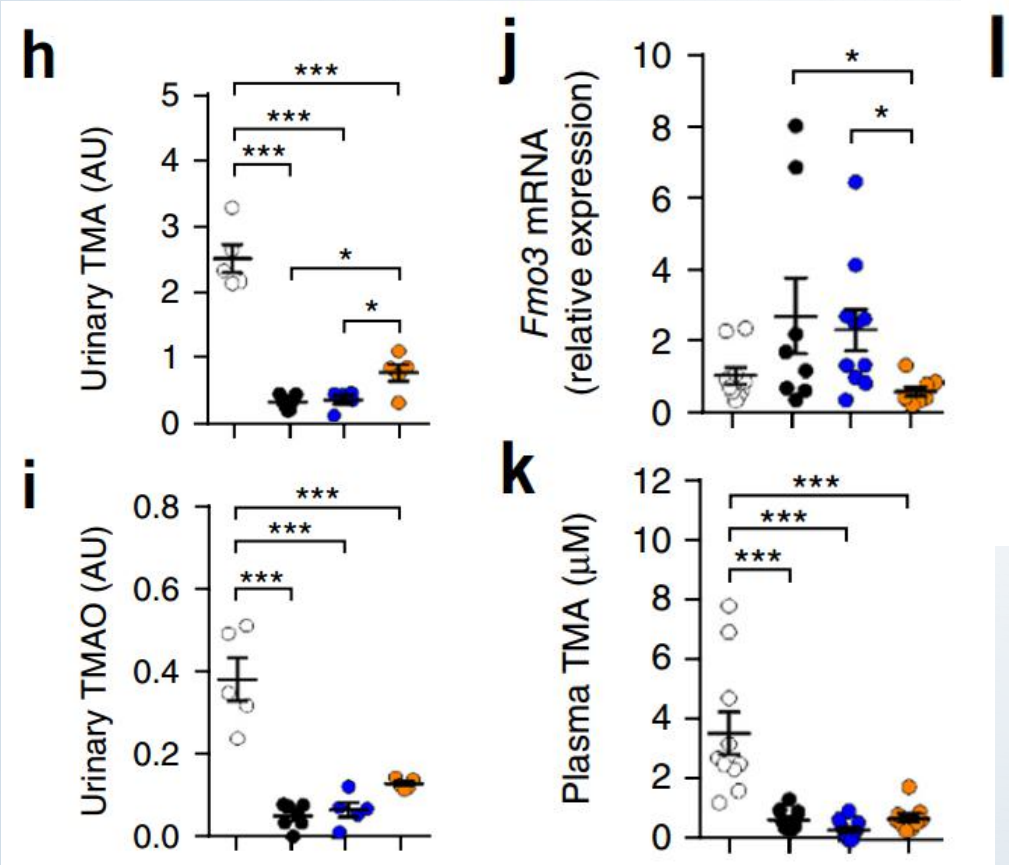
(g) Empirical assessment of the significance of O-PLS goodness-of-fit parameters.







Fmo3：TMA → TMAO



TMAO的用途

6.1 饲料添加剂

氧化三甲胺作为一种天然、安全的饲料添加剂，在畜牧业中具有广泛的发展前景。

主要功能有：

1、促进肌肉细胞的增殖来促进肌肉组织的生长

2、增加胆汁体积，减少脂肪沉积

3、参与水生动物渗透压调节

4、稳定蛋白质结构

5、提高饲料转化率

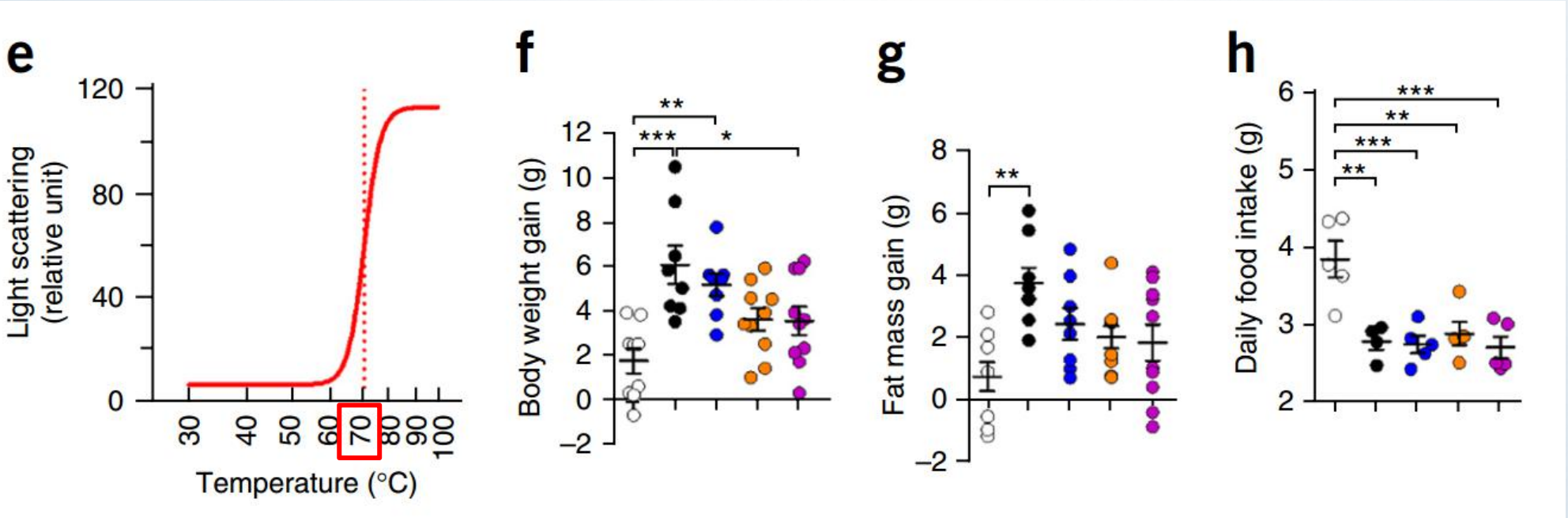
6、提高瘦肉率（通过降低酮体脂肪含量）

7、特殊的鲜味和爽口的甜味，有诱食作用

用法过量：

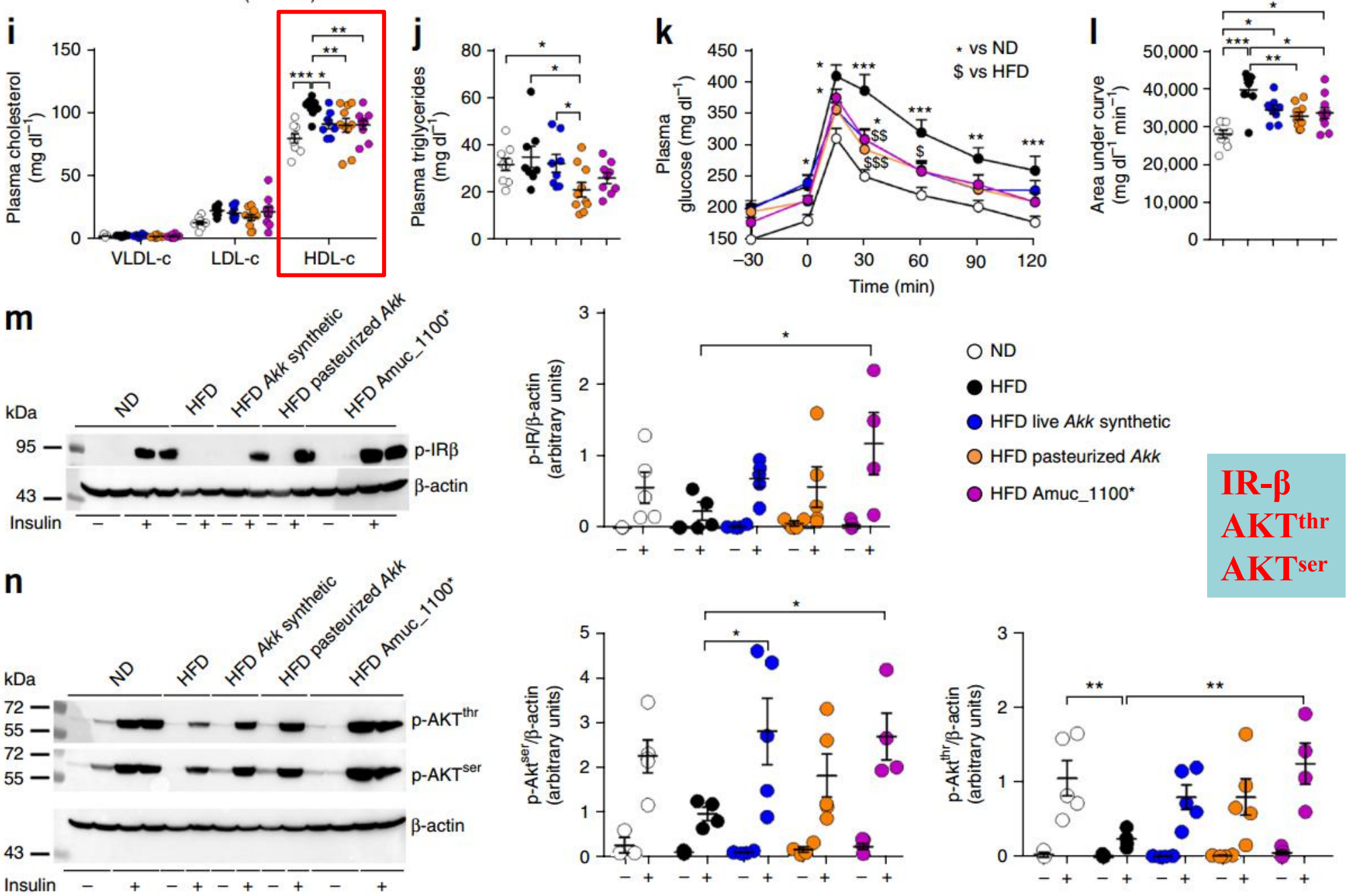
对虾、海水鱼、鳗鱼、甲鱼，添加1~2kg/吨全价配合饲料

青虾、淡水鱼：1~1.5kg/吨全价配合饲料

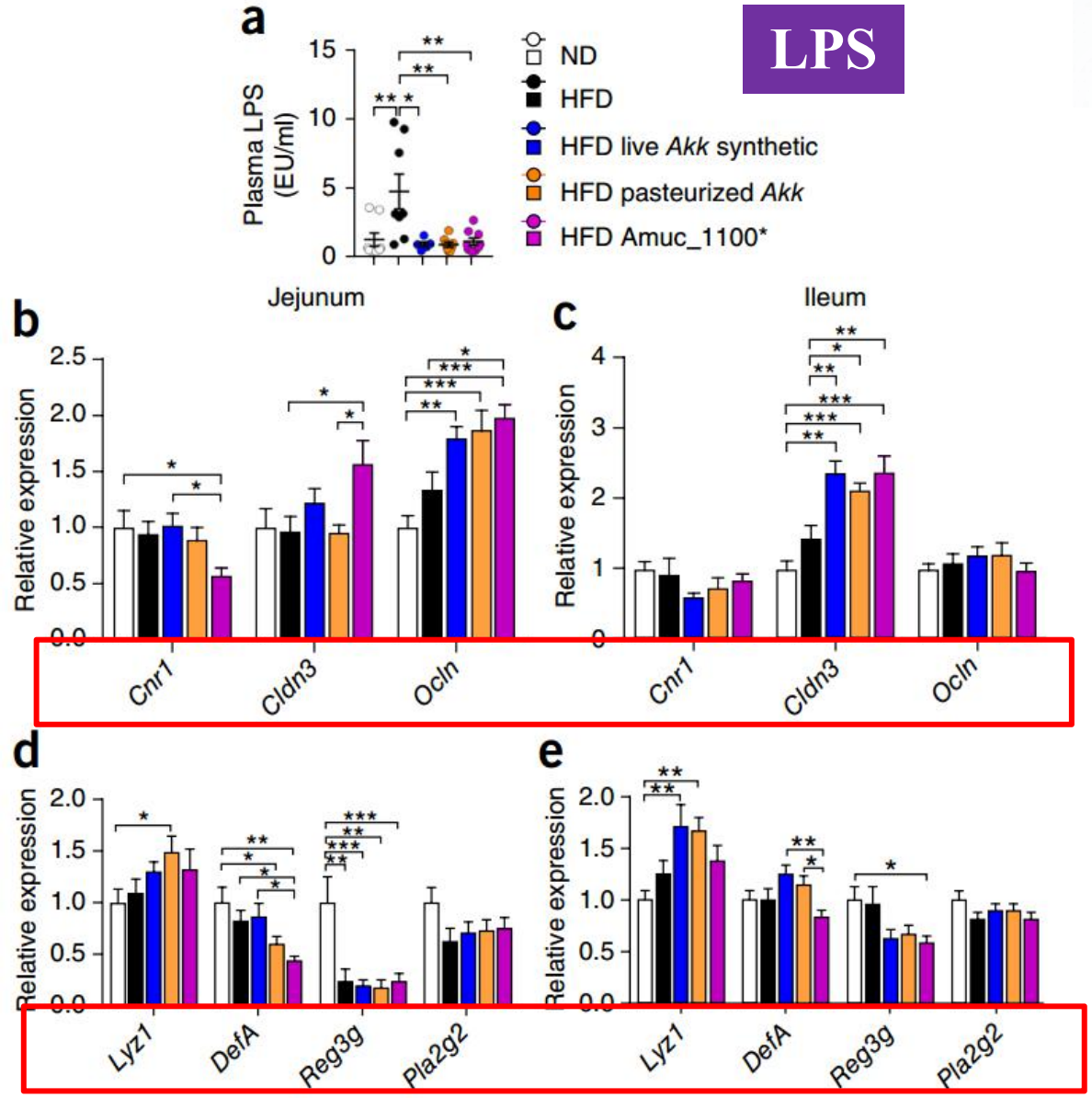


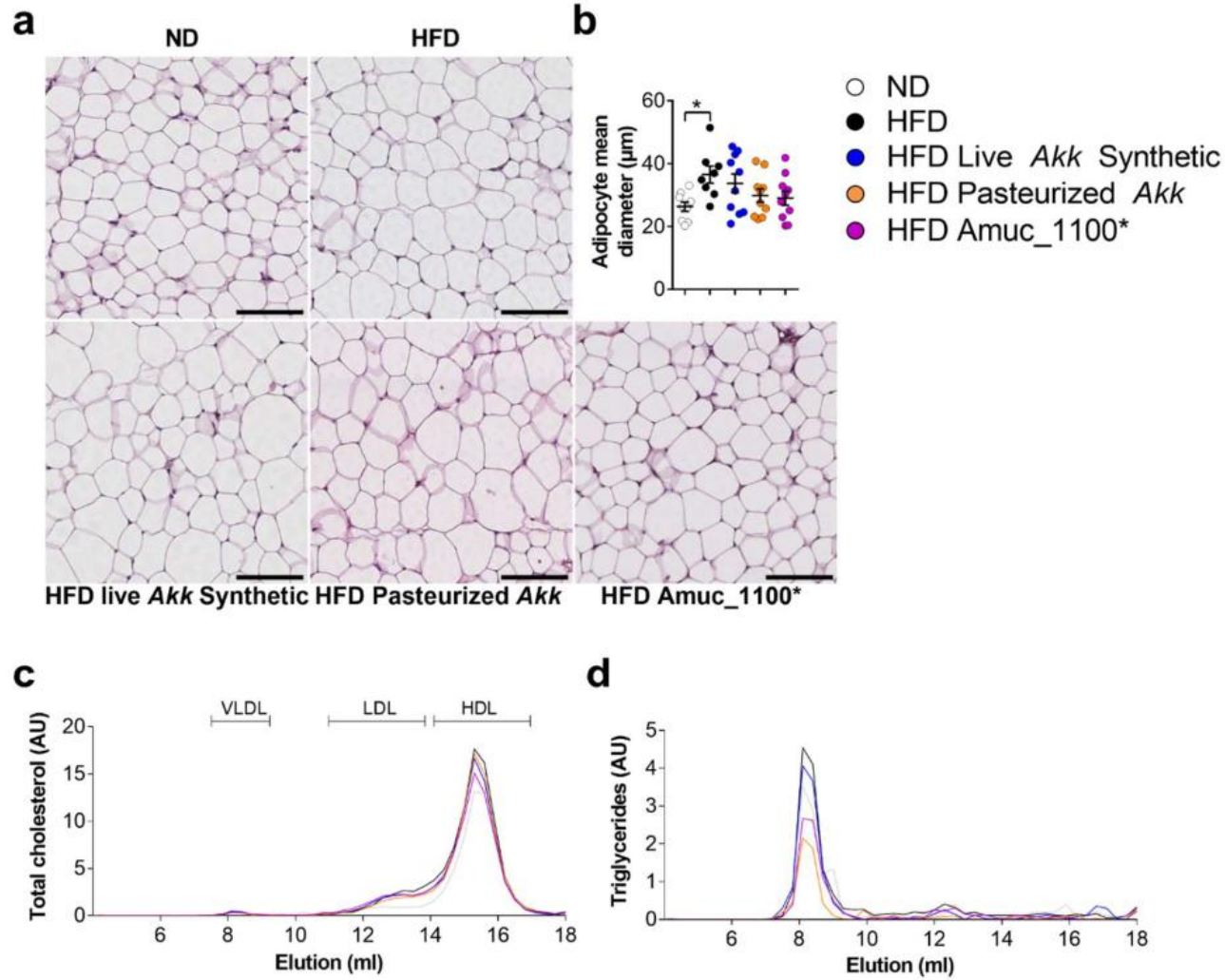
Amuc_1100*热稳定性

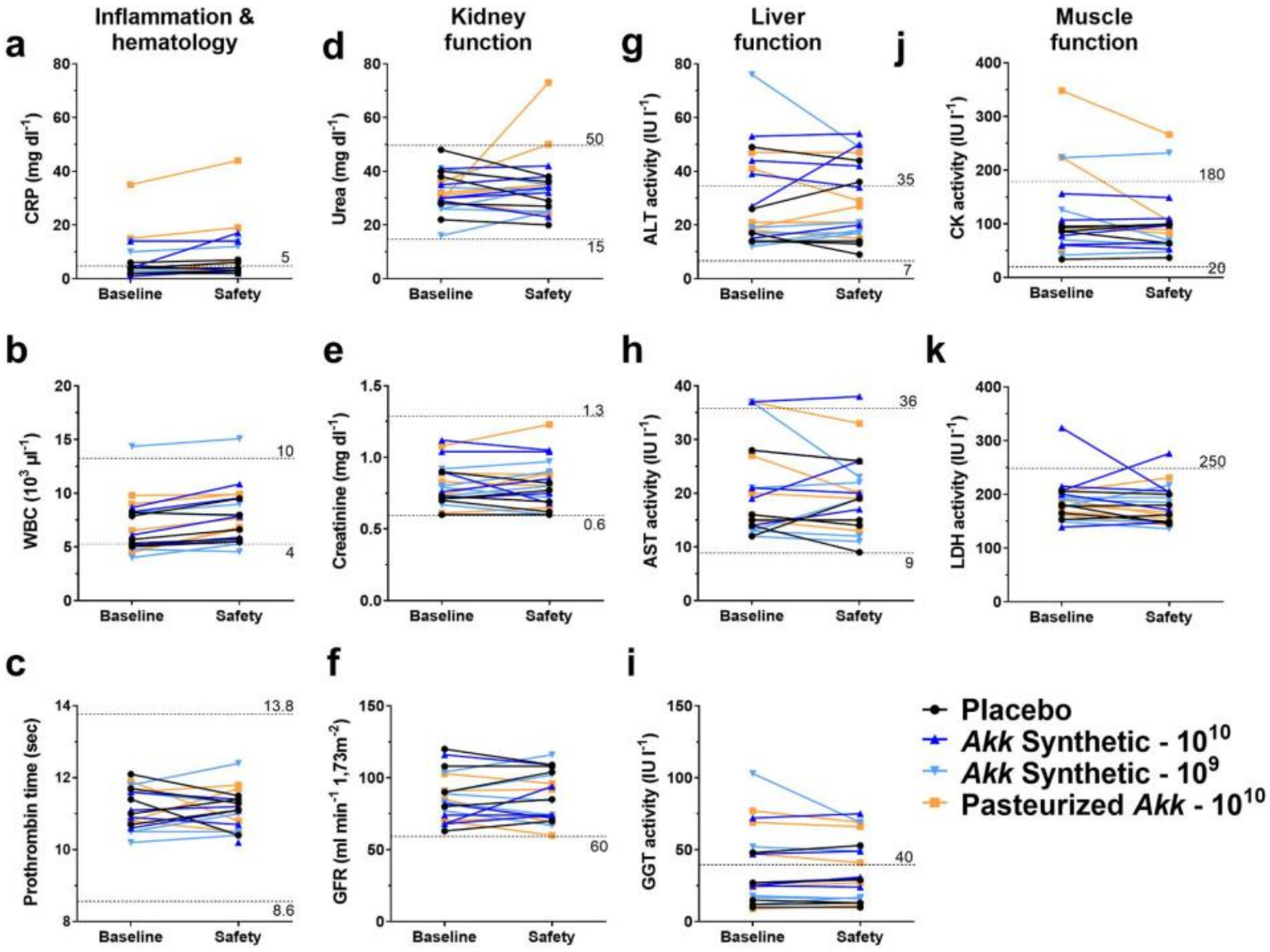
RESULTS



IR-β
AKT^{thr}
AKT^{ser}

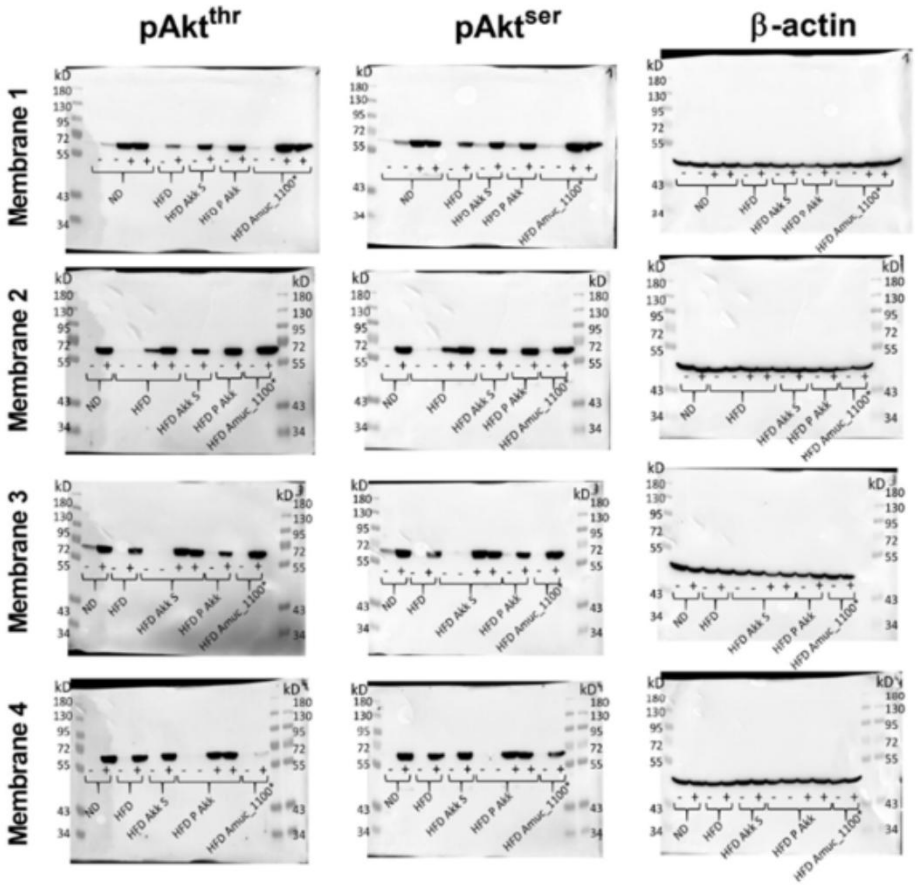

LPS
a
Plasma LPS (EU/ml)
ND
HFD
HFD live Akk synthetic
HFD pasteurized Akk
HFD Amuc_1100*
Jejunum
Ileum
b
c
Relative expression
Cnr1
Cldn3
Ocln
d
e
Lyz1
DefA
Reg3g
Pla2g2


a
ND
HFD
b
Adipocyte mean diameter (µm)
ND
HFD
HFD Live Akk Synthetic
HFD Pasteurized Akk
HFD Amuc_1100*
HFD live Akk Synthetic
HFD Pasteurized Akk
HFD Amuc_1100*
c
Total cholesterol (AU)
VLDL
LDL
HDL
Elution (ml)
d
Triglycerides (AU)
Elution (ml)

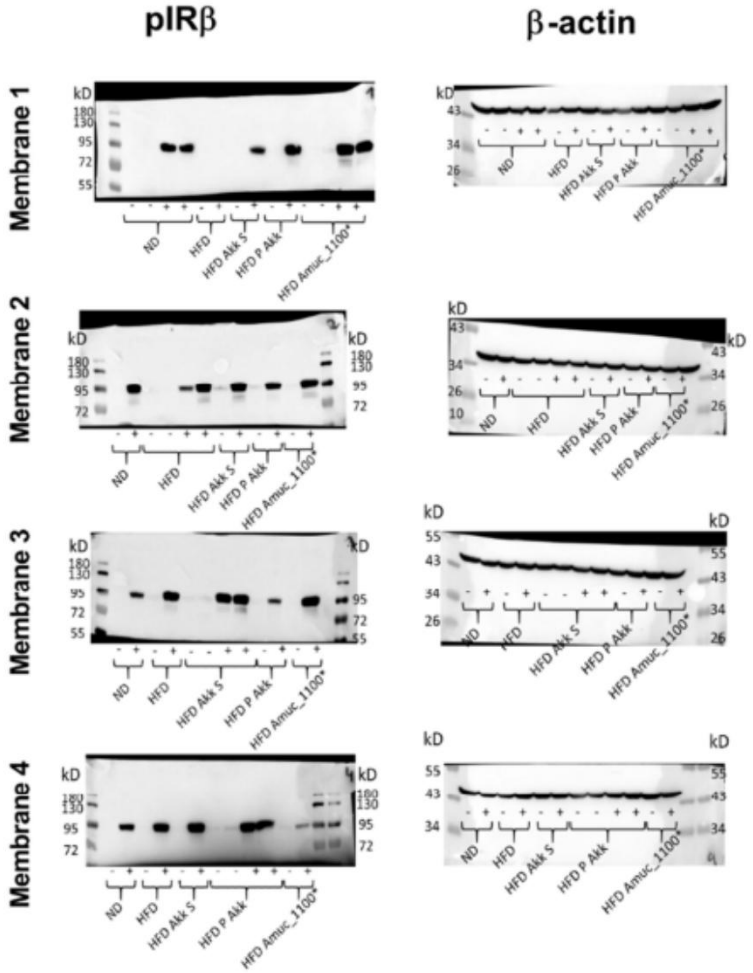


Supplemental figure 5: Safety assessment of daily *A. muciniphila* oral administration in individuals with excess body weight after two weeks of treatment.

药物安全性测试



Supplemental figure 6: Uncropped western blots for Figure 3m.



Supplemental figure 7: Uncropped western blots for Figure 3n.

Supplemental table 1: Descriptive characteristics at the beginning of treatment for all subjects included in the clinical study.

Sex (M/W)
Age (Years)
Body weight (kg)
Body mass index
Waist circumfere
Fasting glycaemi

Supplemental table 2: Clinical parameters measured in all groups during the clinical study (mean ± SD).

	Placebo		Live *Akk* Synthetic - 10^{10}		Live *Akk* Synthetic - 10^{9}		Pasteurized *Akk* - 10^{10}	
Inflammation & Hematology	**Baseline**	**Safety**	**Baseline**	**Safety**	**Baseline**	**Safety**	**Baseline**	**Safety**
C-reactive protein (mg dl^{-1})	3,60 ± 1,67	4,40 ± 2,07	5,20 ± 5,17	7,80 ± 7,12	6,60 ± 5,18	6,40 ± 6,07	11,40 ± 14,33	15,20 ± 17,38
White blood cells (10^3 μl^{-1})	6,43 ± 1,49	7,07 ± 1,68	6,67 ± 1,69	7,94 ± 2,29	7,91 ± 4,08	8,36 ± 4,17	6,89 ± 2,44	8,20 ± 1,61
Prothrombin time (sec)	11,38 ± 0,55	11,1						
Liver enzymes	**Baseline**	**Sa**						
Alanine aminotransferase activity (IU l^{-1})	24,00 ± 14,82	23,2(						
Aspartate aminotransferase activity (IU l^{-1})	17,00 ± 6,33	16,6						
γ-Glutamyltransferase activity (IU l^{-1})	22,40 ± 15,76	23,6(						
Kidney function	**Baseline**	**Sa**						
Urea (mg dl^{-1})	35,20 ± 10,26	30,0						
Creatinine (mg dl^{-1})	0,73 ± 0,11	0,7						
Glomerular filtration rate (ml min^{-1} $1,73m^{-2}$)	92,20 ± 22,52	95,2(						
Muscle enzymes	**Baseline**	**Sa**						
Creatinine kinase activity (IU l^{-1})	78,80 ± 25,37	79,4(						
Lactate dehydrogenase activity (IU l^{-1})	176,60 ± 19,86	167,2						

Supplemental table 3: Proportion of subjects experiencing self-reported adverse effects.

	Placebo	Live *Akk* Synthetic - 10^{10}	Live *Akk* Synthetic - 10^{9}	Pasteurized *Akk* -10^{10}
Nausea	1/5	0	2/5	1/5
Flatulence	0	1/5	3/5	1/5
Bloating	1/5	1/5	0	0
Cramps	1/5	1/5	0	1/5
Borborygmi	0	3/5	3/5	0
Gastric reflux	1/5	0	1/5	0

1. *A. muciniphila* ——治疗肥胖及相关代谢紊乱疾病的希望；
2. 新一代益生菌的研究领域——粘液层；
3. 应用障碍——大多肠道菌对氧气高度敏感；
4. 巴氏灭菌的*A. muciniphila* 对治疗更加有效；
5. *A. muciniphila* 外膜蛋白amuc_1100 对治疗起着关键作用；
6. 通过巴氏灭菌法对有益效果的改善是否适用于除*A.muciniphila*的其它细菌，仍需进一步测试；
7. 巴氏杀菌是一种利用厌氧菌作为治疗工具的创新方法；
8. 分离和鉴定这些特殊细菌（益生菌）的产物对未来针对疾病的治疗会很有帮助；
9. 在合成培养基上培养的和巴氏灭菌的*A. muciniphila* 对人类来说是安全可用的。

谢 谢 ！