



读书报告

汇报人：李帅

2017年8月20日

IF 29.886

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

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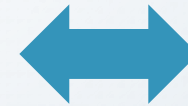
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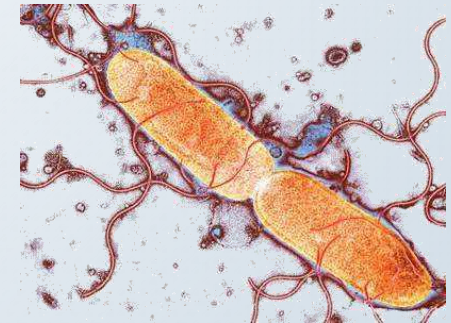
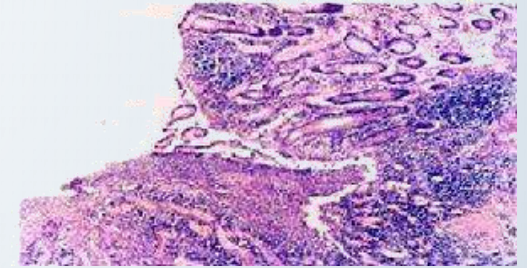
Obesity



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/ij.s.0.02873-0

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

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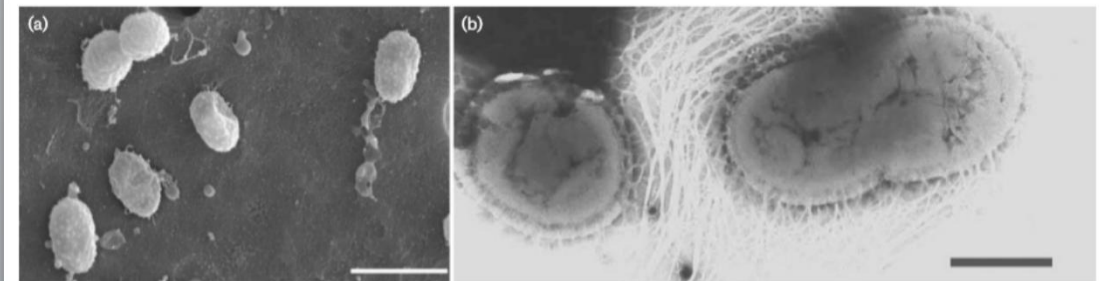


Fig. 3. EM images of strain Muc^T. (a) SEM image. Bar, 1 μ m. (b) TEM image of a negatively stained preparation. Note the thickened but extensive capsule fibres of the cells. Bar, 0.5 μ m.

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.

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

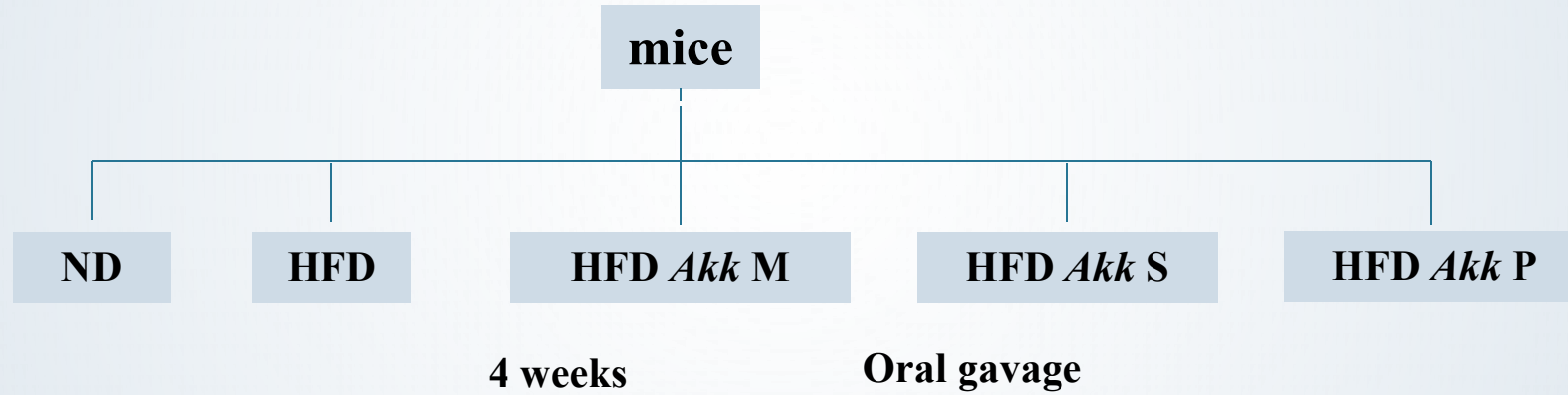
Normal chow diet (ND)

High-fat diet (HFD)

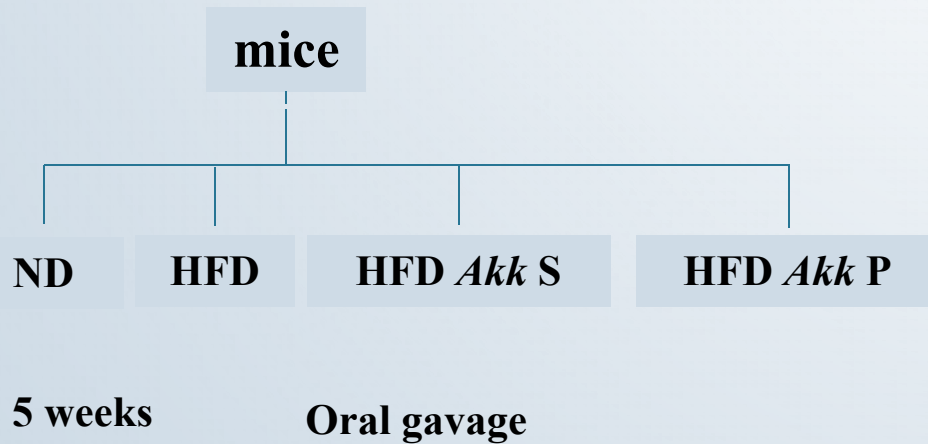
Body composition

7.5 MHz time domain nuclear magnetic resonance (TD-NMR)

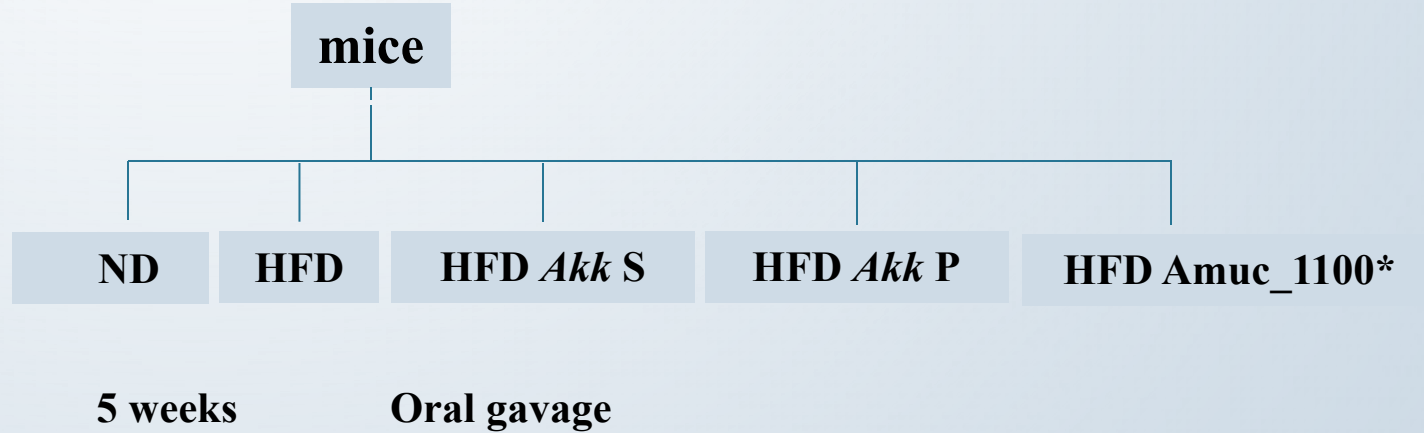
Experiment 1



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)

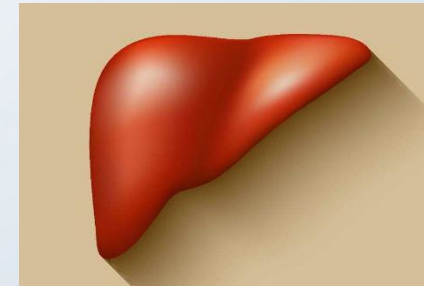
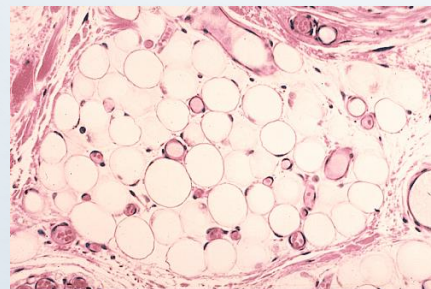
Insulin resistance index.

ELISA kit (Merckodia, Uppsala, Sweden)

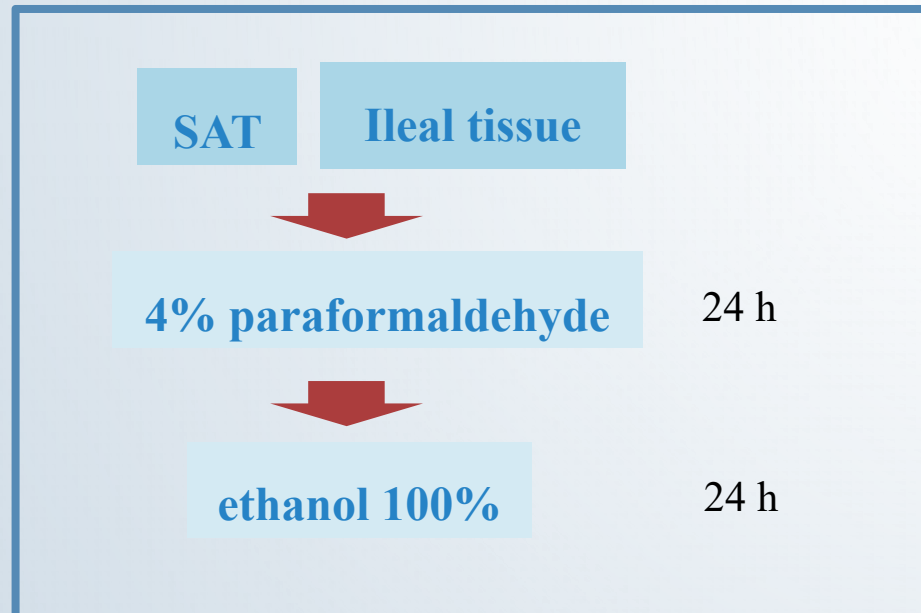
Both blood glucose (−30 to 120 min)

Plasma insulin (−30 and 15 min)

Tissue sampling.



Histological analyses.



5 μ m 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')
<i>Rpl19</i>	GAAGGTCAAAGGGAATGTGTCA	CCTTGTCTGCCTTCAGCTTGT
<i>Fmo3</i>	GGAACTTGCACTTTGCCTTC	TAGGAGATTGGGCTTTGCAC
<i>Cnr1</i>	CTGATGTTCTGGATCGGAGTC	TCTGAGGTGTGAATGATGATGC
<i>Cldn3</i>	TCATCGGCAGCAGCATCATCAC	ACGATGGTGATCTTGGCCTTGG
<i>Ocln</i>	ATGTCCGGCCGATGCTCTC	TTTGGCTGCTCTGGGTCTGTAT
<i>Napepld</i>	TTCTTTGCTGGGGATACTGG	GCAAGGTCAAAGGACCAAA
<i>Naaa</i>	ATTATGACCATTGGAAGCCTGCA	CGCTCATCACTGTAGTATAAATTGTGTAG
<i>Lyz1</i>	GCCAAGGTCTACAATCGTTGTGAGTTG	CAGTCAGCCAGCTTGACACCACG
<i>DefA</i>	GGTGATCATCAGACCCAGCATCAGT	AAGAGACTAAAAGTGGAGCAGC
<i>Reg3g</i>	TTCTGTCTCCATGATCAAA	CATCCACCTCTGTTGGGTTT
<i>Pla2g2</i>	AGGATTCCCCCAAGGATGCCAC	CAGCCGTTTCTGACAGGAGTTCTGG

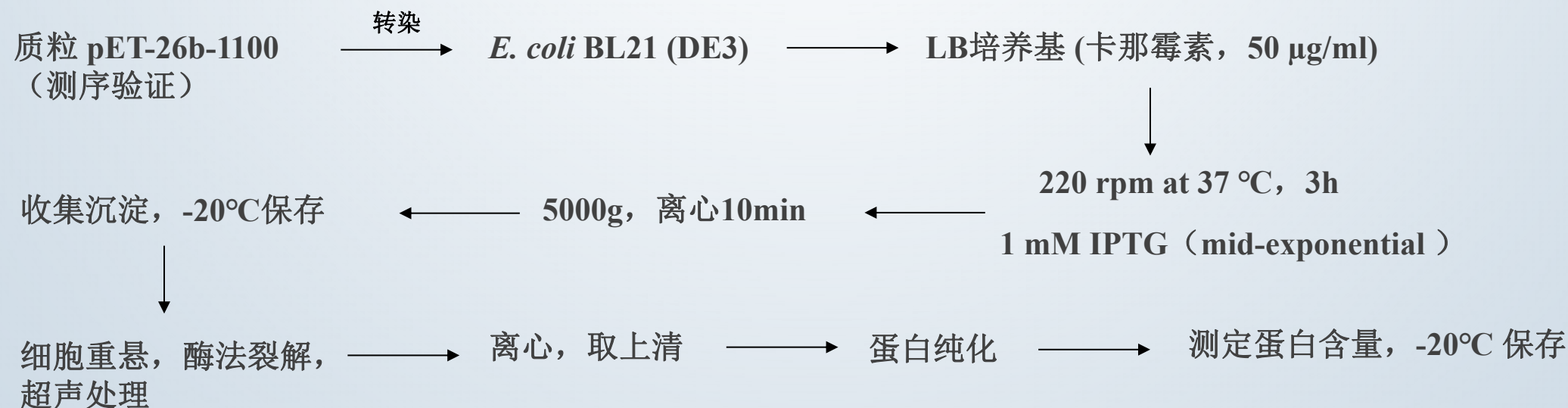
Production of Amuc_1100* protein.

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 μ M) 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-IR β (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

10¹⁰ CFU live *A. muciniphila* (Akk S - 10¹⁰)

10⁹ CFU live *A. muciniphila* (Akk S - 10⁹)

10¹⁰ CFU pasteurized *A. muciniphila* (Akk P - 10¹⁰)

Statistical analysis.

mean ± s.e.m.

one-way ANOVA

Tukey *post-hoc* test

two-way ANOVA

Bonferonni *post-hoc* test

Kruskal-Wallis test

.....

Dunnett *post-hoc* test

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

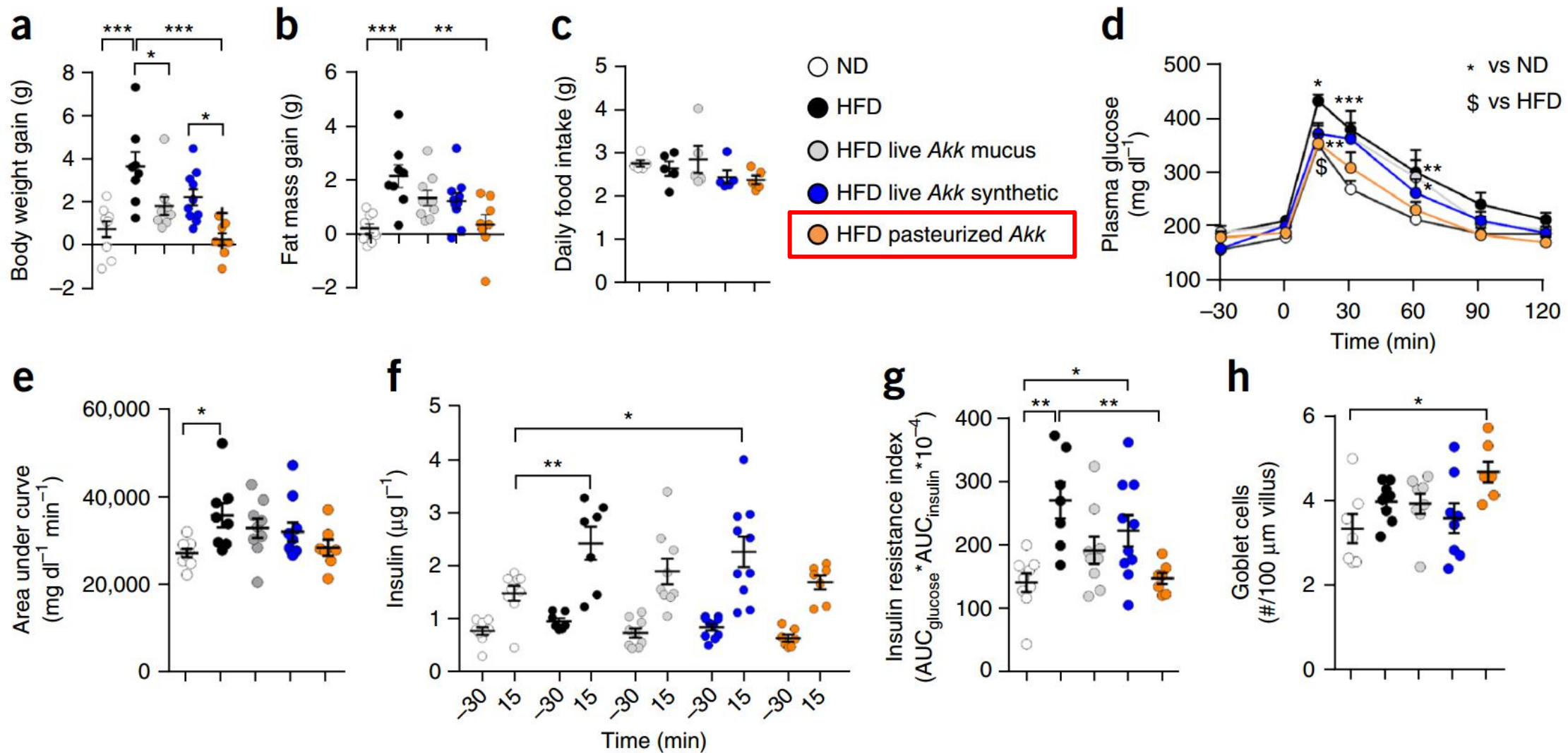
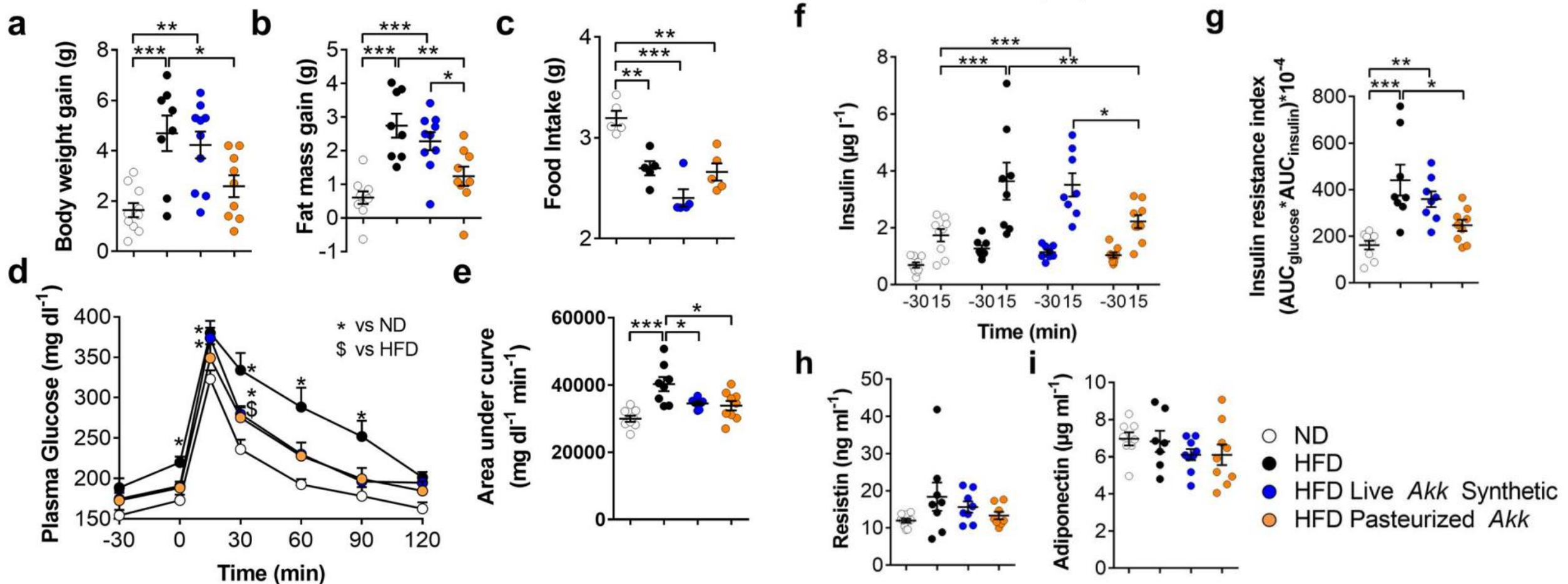
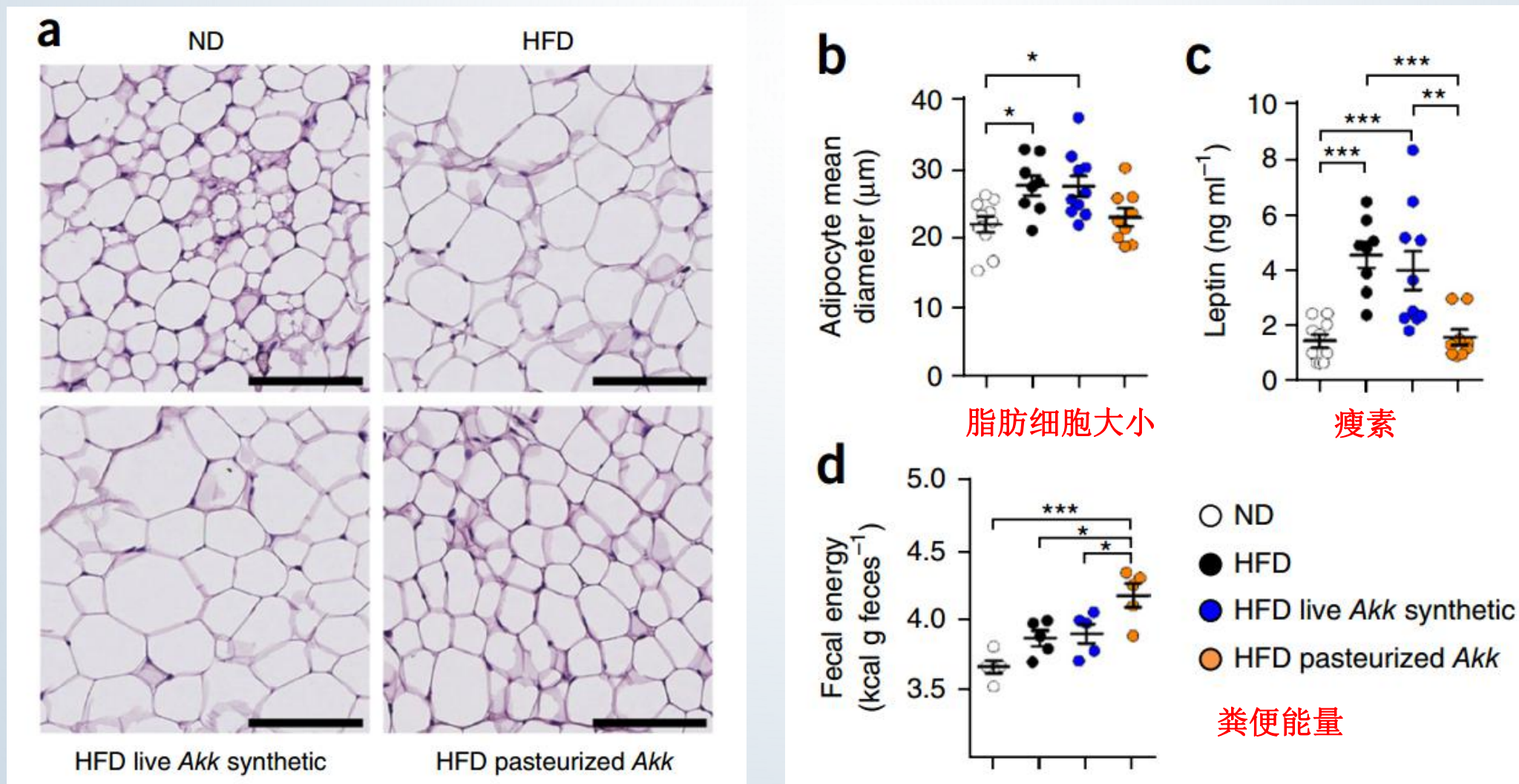


Figure 1 Pasteurization enhances *A. muciniphila*-mediated effects on high-fat diet-induced obesity.



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.

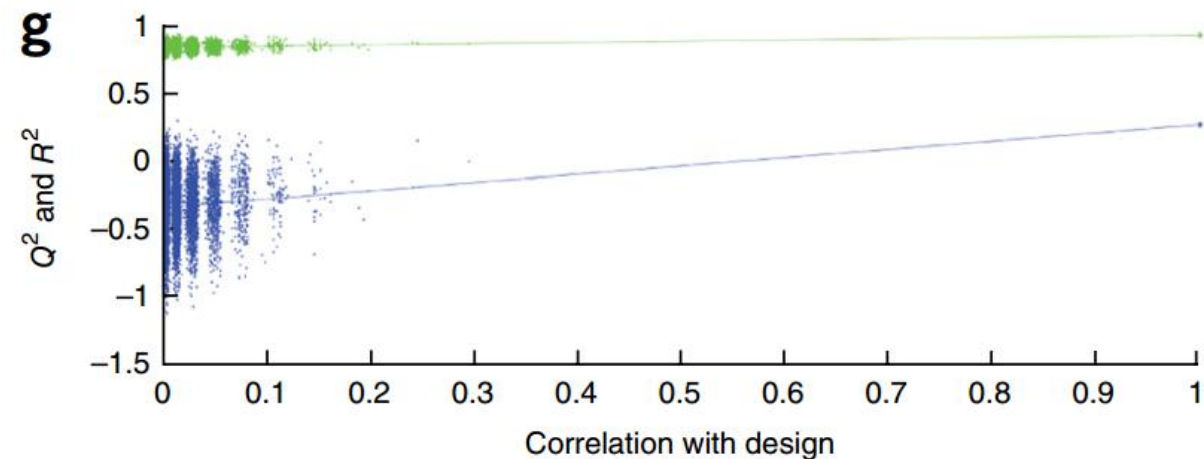
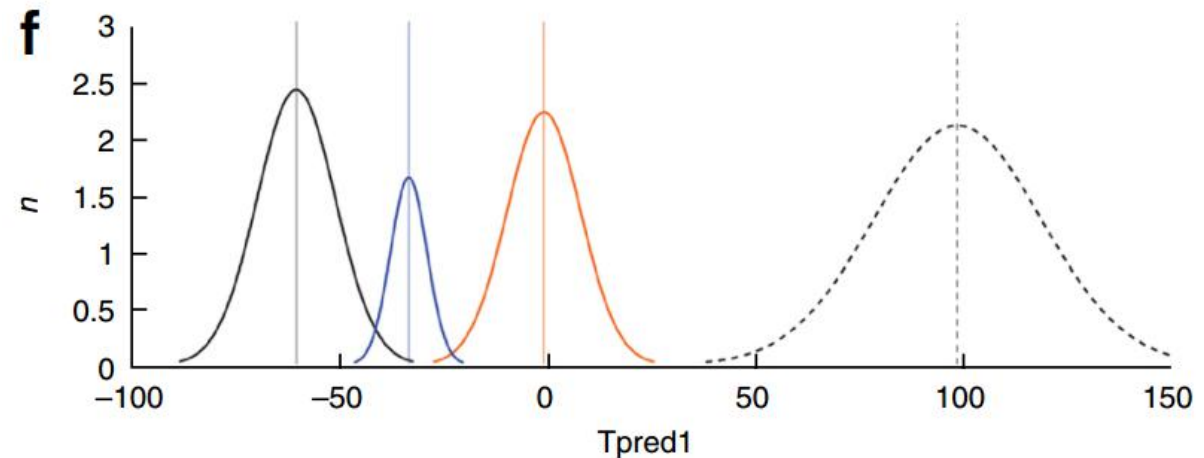
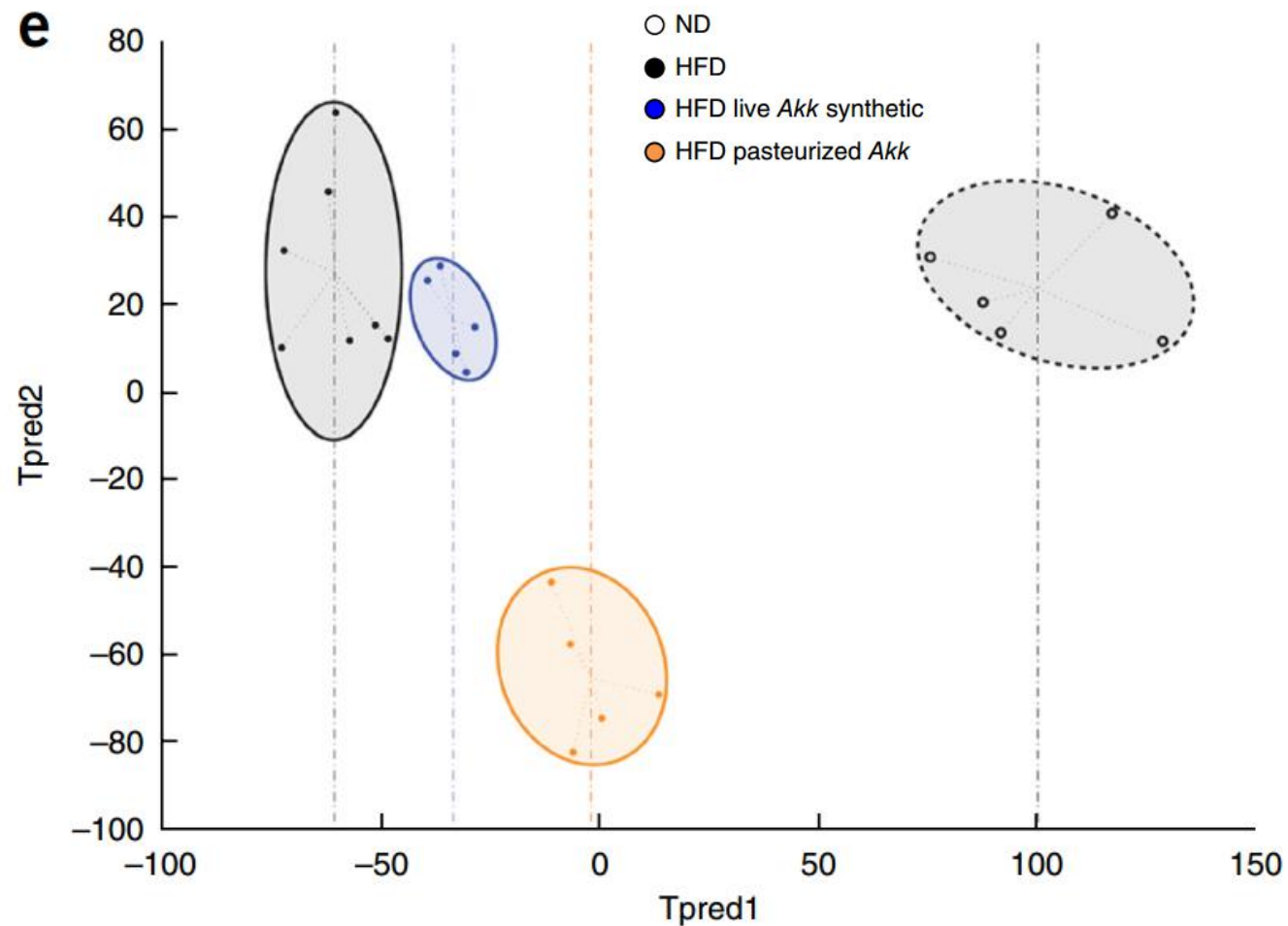
尿液代谢分析

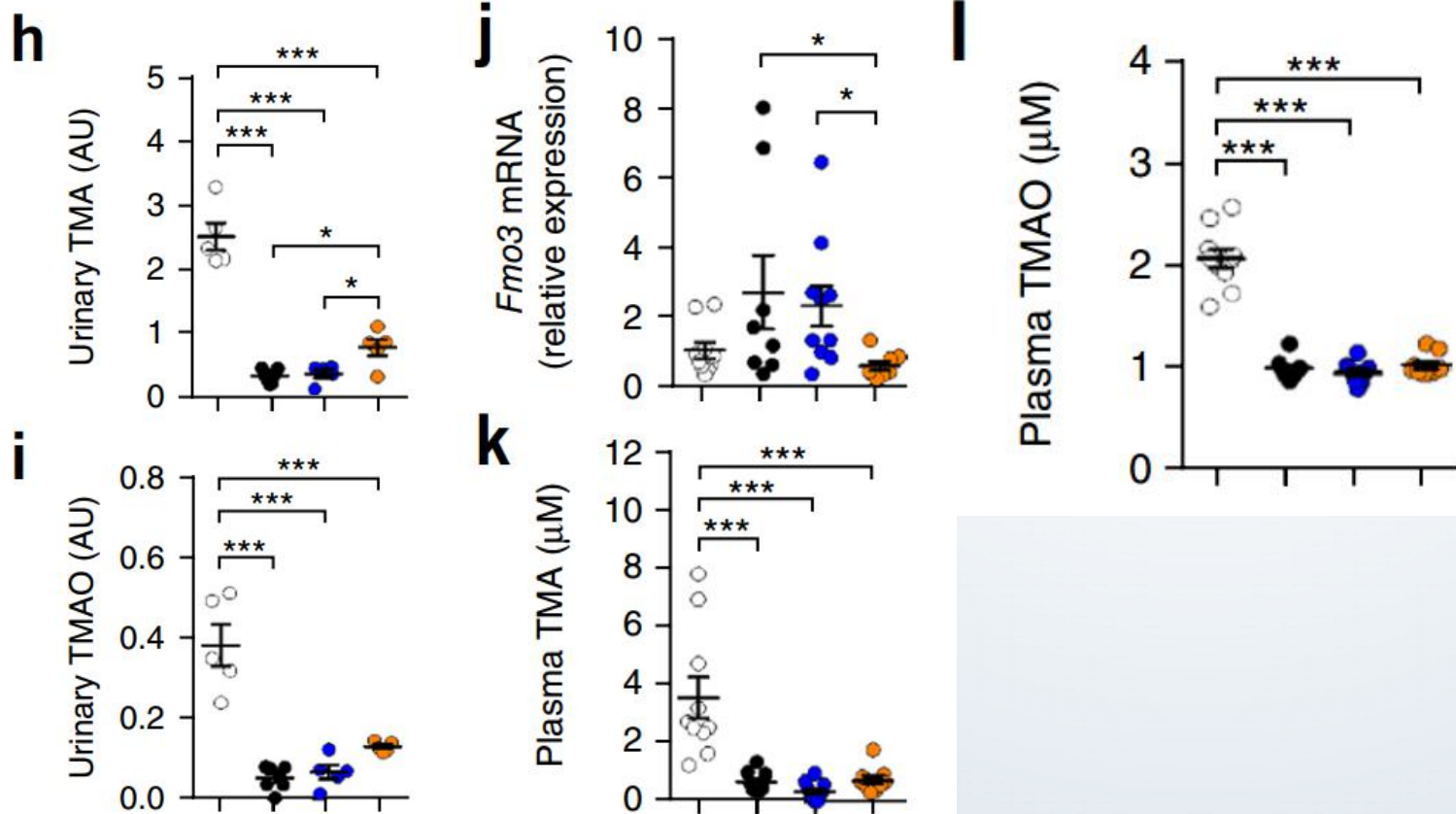
RESULTS

(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。

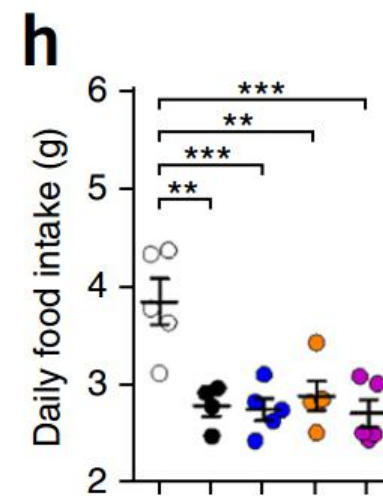
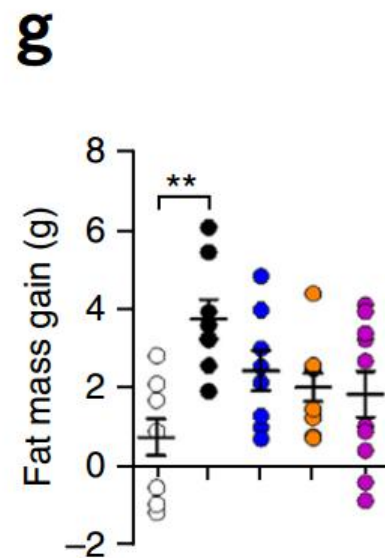
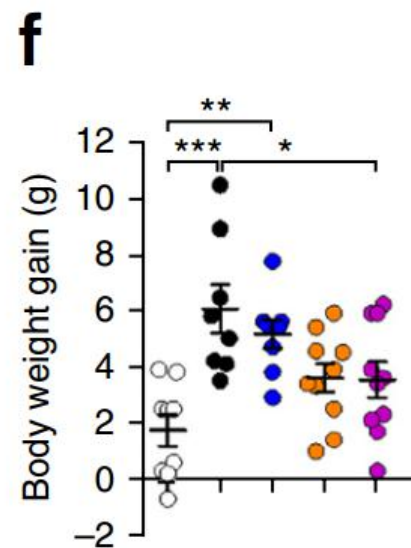
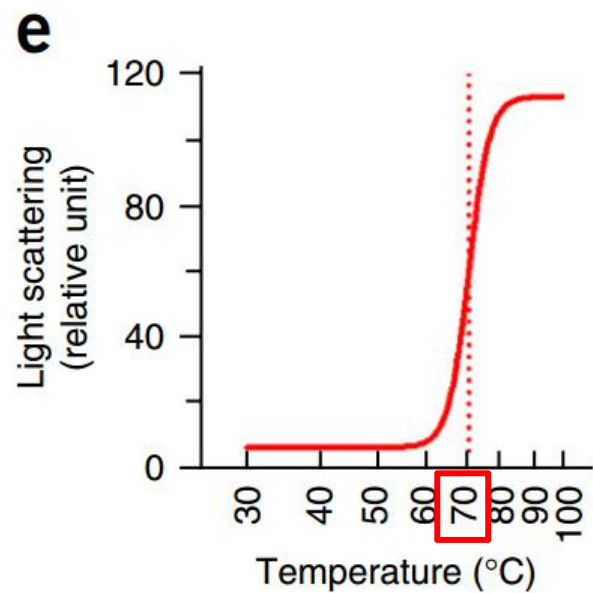
主要功能有：

- 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

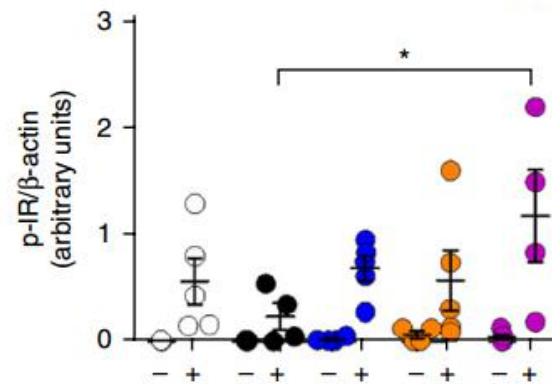
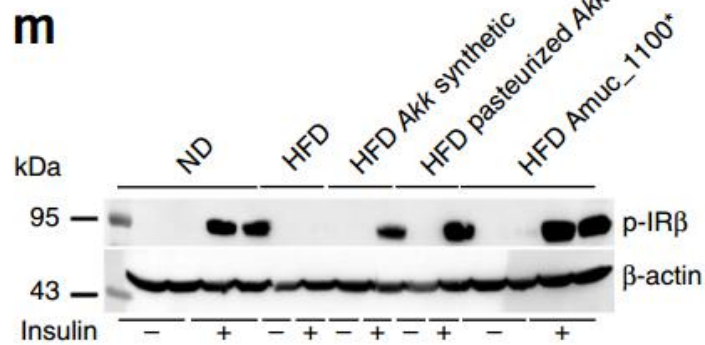
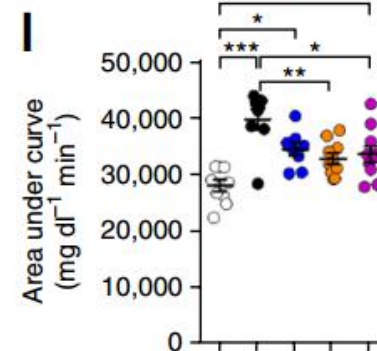
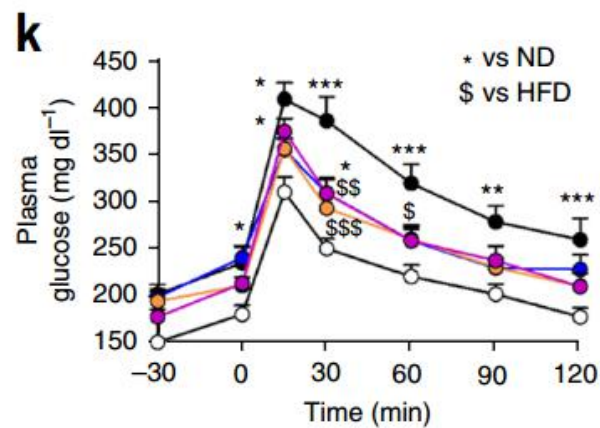
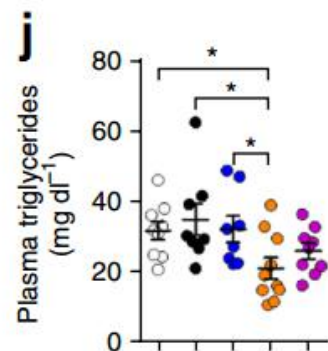
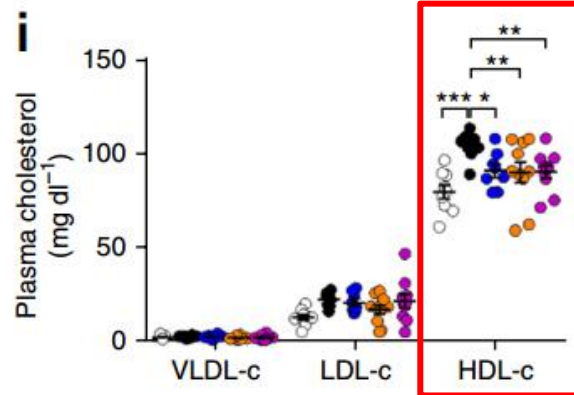
青虾、淡水鱼：1~1.5kg/吨全价配合饲料



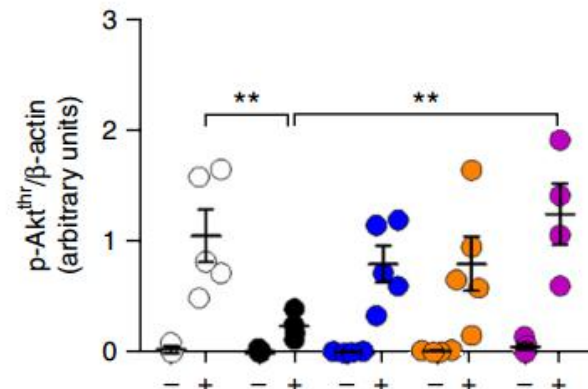
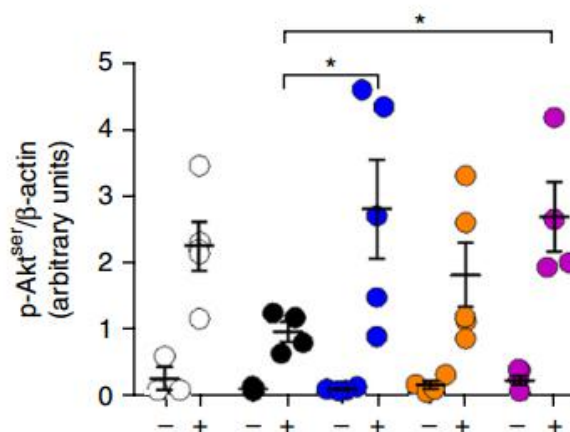
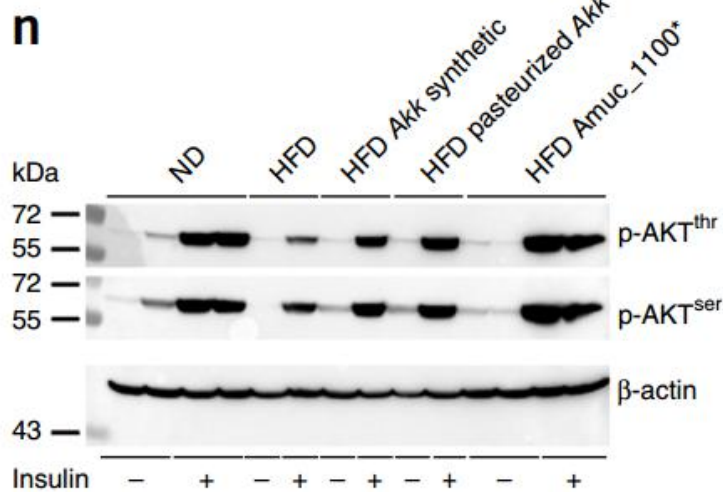
- ND
- HFD
- HFD live *Akk* synthetic
- HFD pasteurized *Akk*
- HFD Amuc_1100*

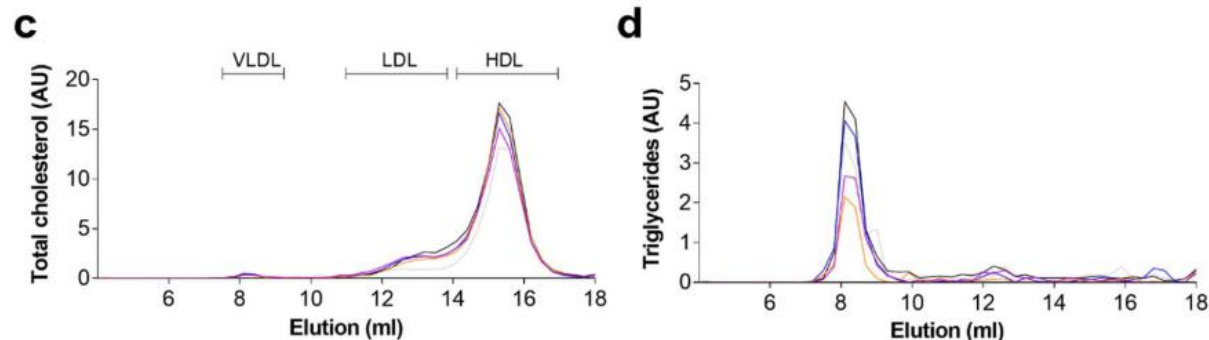
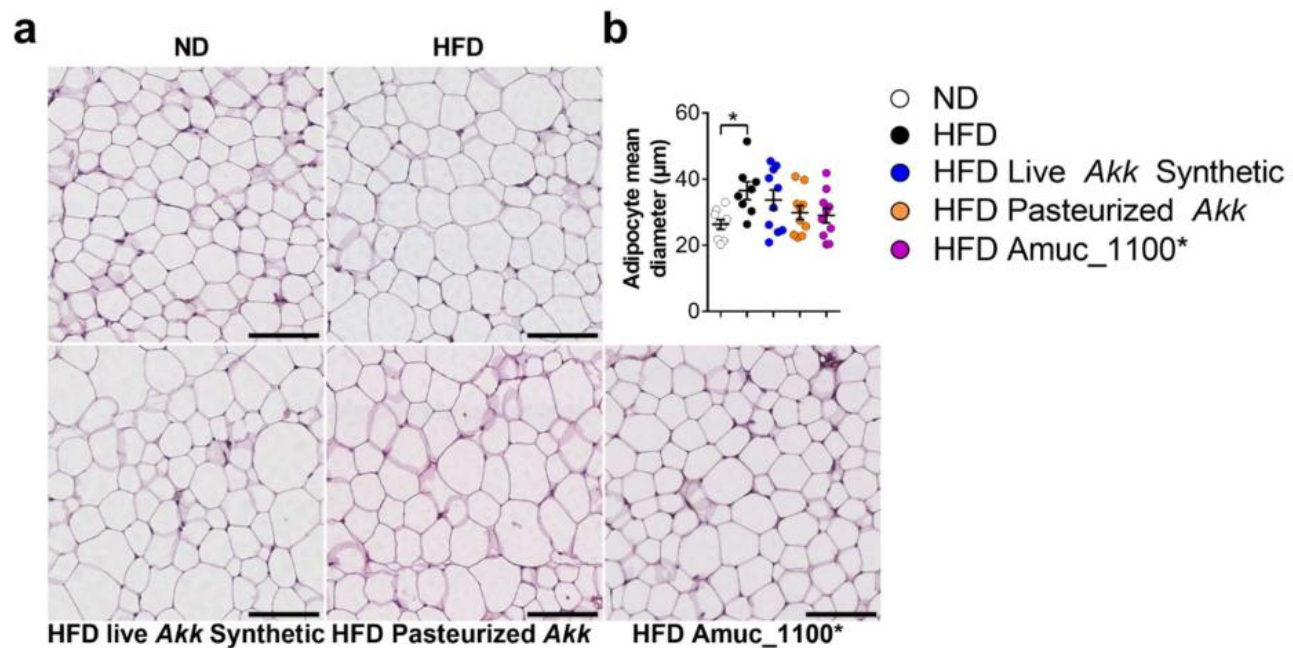
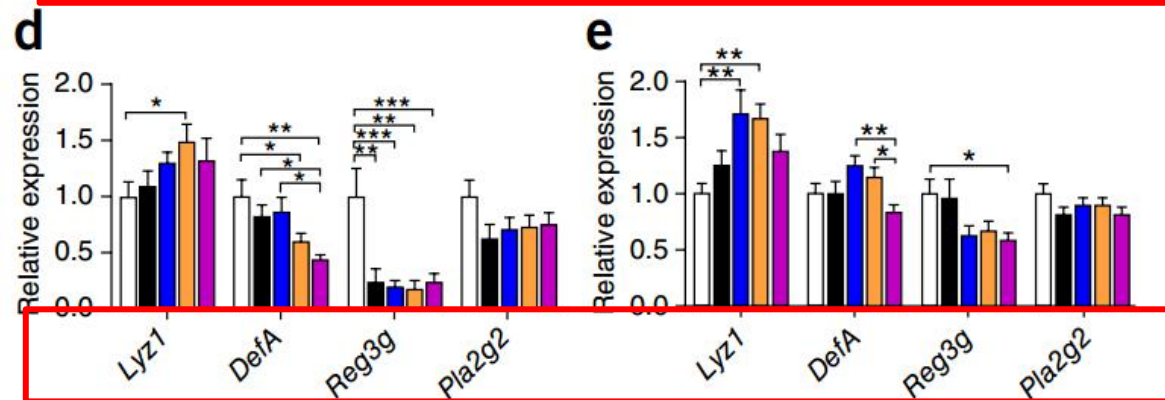
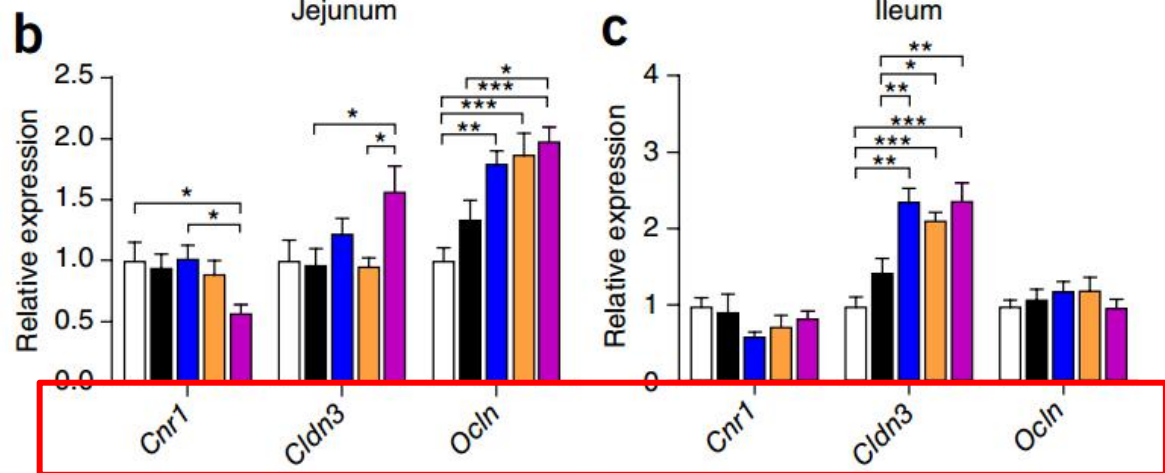
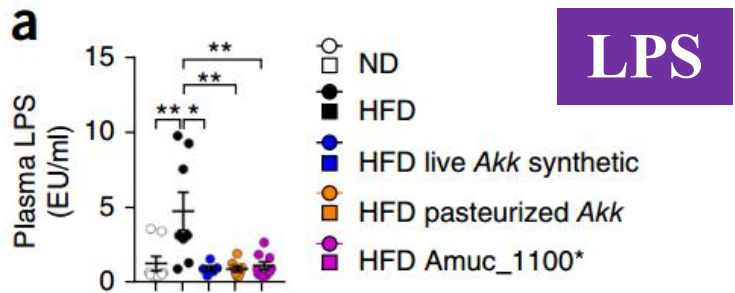
Amuc_1100*热稳定性

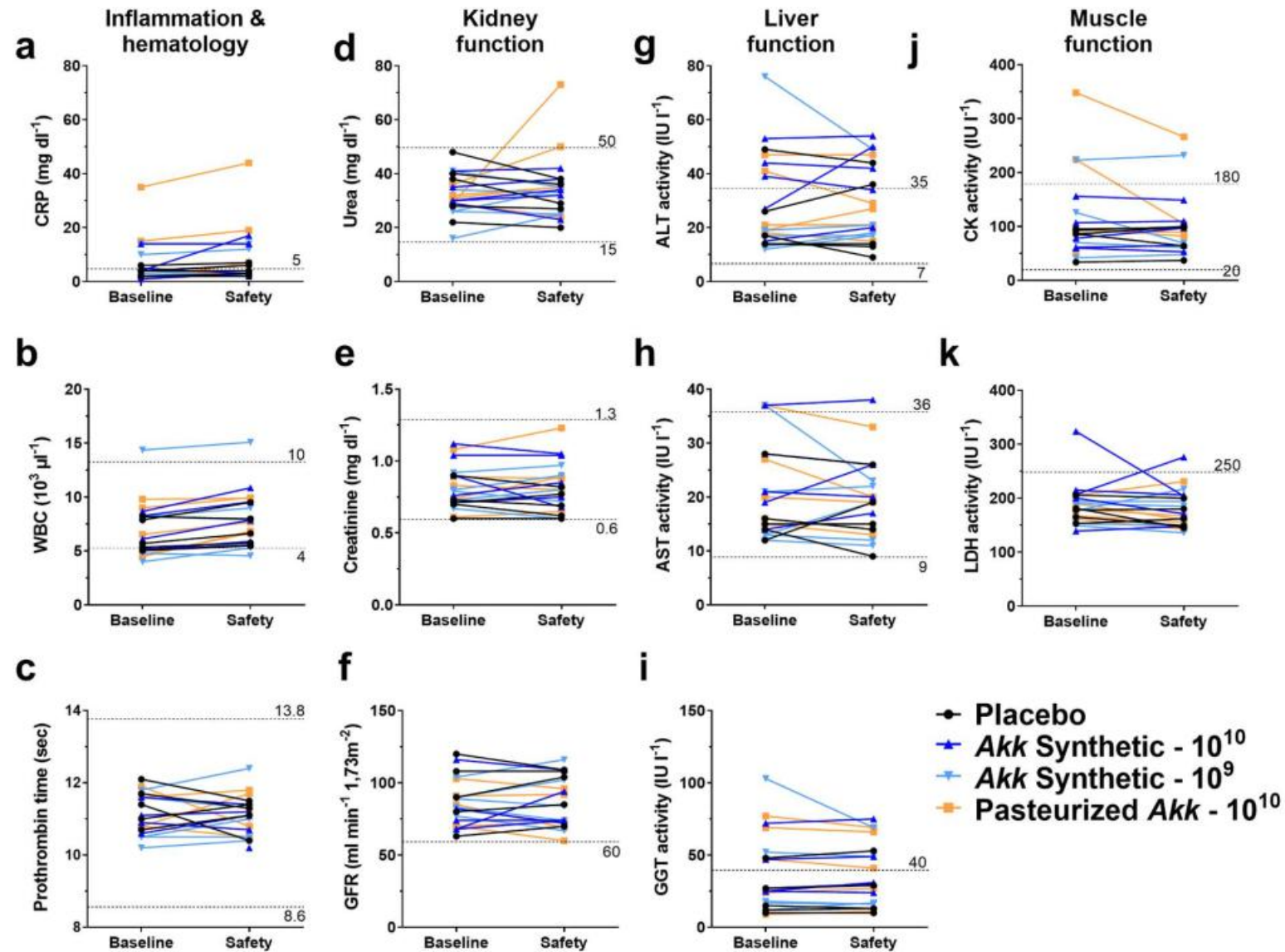
RESULTS



IR-β
AKT^{thr}
AKT^{ser}

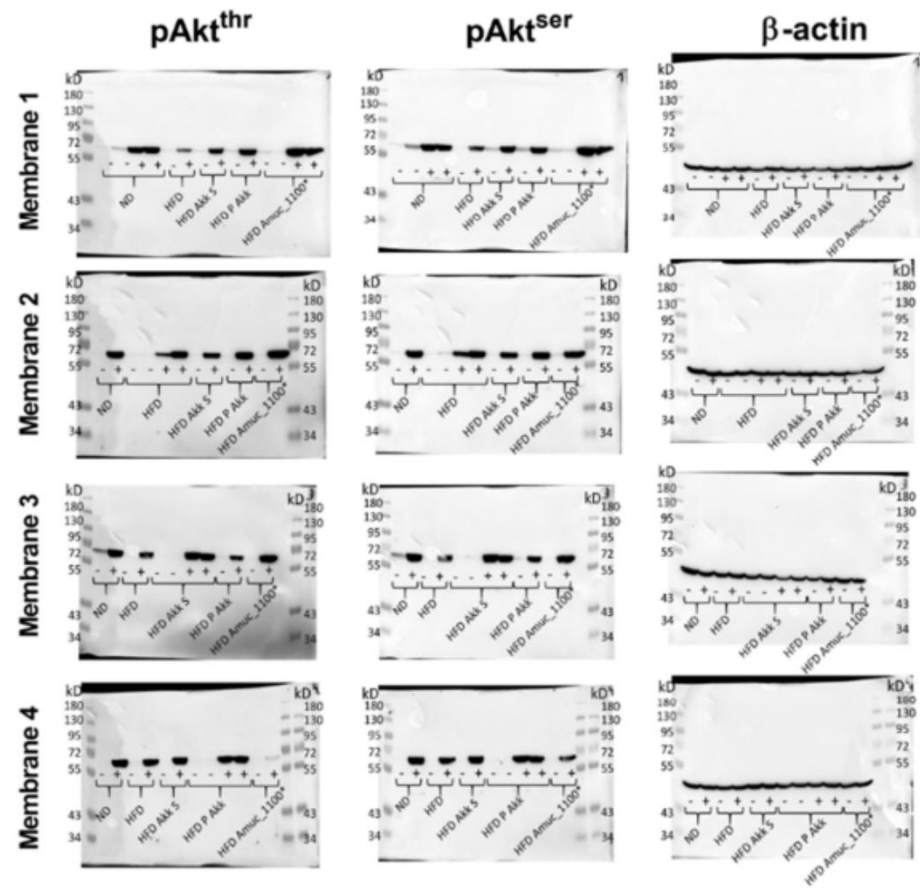




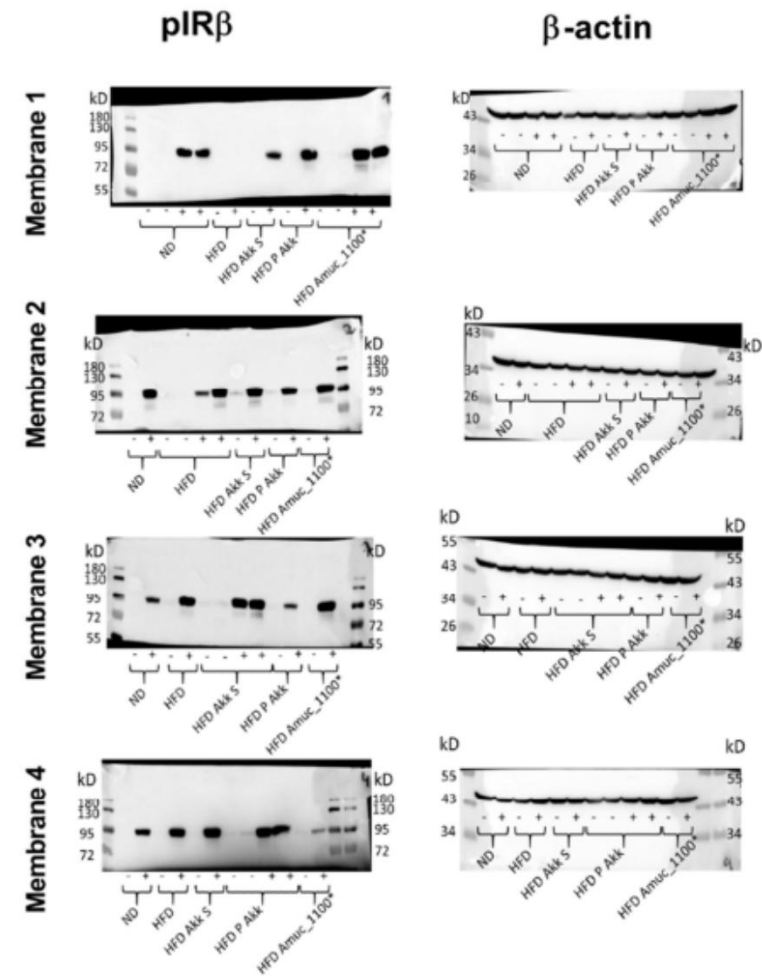


Supplemental figure 5: Safety assessment of daily *A. muciniphila* oral administration in individuals with excess body weight after two weeks of treatment.

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 circumference
Fasting glycaemia

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

Inflammation & Hematology	Placebo		Live Akk Synthetic - 10 ¹⁰		Live Akk Synthetic - 10 ⁹		Pasteurized Akk - 10 ¹⁰	
	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	23,20						
Aspartate aminotransferase activity (IU l ⁻¹)	17,00 ± 6,33	16,6						
γ-Glutamyltransferase activity (IU l ⁻¹)	22,40 ± 15,76	23,60						
Kidney function	Baseline	Sa						
Urea (mg dl ⁻¹)	35,20 ± 10,26	30,0						
Creatinine (mg dl ⁻¹)	0,73 ± 0,11	0,71						
Glomerular filtration rate (ml min ⁻¹ 1,73m ⁻²)	92,20 ± 22,52	95,20						
Muscle enzymes	Baseline	Sa						
Creatinine kinase activity (IU l ⁻¹)	78,80 ± 25,37	79,40						
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！