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The gut microbiota mediates the protective effects of anserine supplementation on hyperuricaemia and associated renal inflammation

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Table Legends

Table S1. Sequences of the primers used for qRT-PCR analysis.

Gene	Forward primer	Reverse primer
β-actin	CAGGCATTGCTGACAGGATG	TGCTGATCCACATCTGCTGG
XOD	TATGCATCCAAGGCTGTCGG	GATCCACACAAGCGTTTCGG
ADA	CATGCGGTTGTTCGCTTCAA	CTGCGTTGATGTTCAGTCGC
PNP	AGGCCCCAACTTTGAGACTG	ACAGTGCCTTGCGACGATAA
ABCG2	TTCTGGTGGAGAGAAACGCC	TCTGGGGCCACAACTGTAGA
MRP4	TCAGCAACTCGTCTTCCCAC	GGCCAGTGCAGATACATGGT
GLUT9	GTCGGCATGGGTTTCCAGTA	GGAAGGCAGCTGAGATCTGG
URAT1	ACACAGCCAGTCTCTTGATGGAGTG	CCGTGATGAGCCAGCGTGCC
UMOD	TAGCAGAACATGCATTGAGCCT	TGTGCTGGGCATTACATGGT
NLRP3	CAGACCTCCAAGACCACGACTG	CATCCGCAGCCAATGAACAGAG
Caspase-1	TCCAATAATGCAAGTCAAGCC	GCTGTACCCCAGATTTTGTAGCA
ASC	GTGCTTAGAGACATGGGCTTAC	GTTCTGGCTGTACTCTGAGCA
TLR4	GGACTATGTGATGTGACCATTGAT	TTATAGATACACCTGCCAGAGACA
MyD88	CTACAGAGCAAGGAATGTGACT	ACCTGATGCCATTTGCTGTCC
TRAF6	ATCACTTGGCACGACACTTG	TAGGCGACTCTCCAACTGTT
NF-κB	GTCCTCCGTGAGCTGCTG	GGGATGGCCTCAAGAAGGAG
ZO-1	AGTGAACTACGAGACGCTGG	AGACGATCAACCGCATTTGG
Occludin	GCTGTGATGTGTGTGAGCTG	GACGG TCTACCT GGAGGAAC
Claudin-1	TCAGGTCTGGCGACATTAGT	GACAGGAGCAGGAAAGTAGGA

Table S2. RDP classifications of the sequence reads at the class and genus levels in hyperuricaemic mice. The data are expressed as the mean ± SD, n=8. In a, b and c, significant differences (P<0.05) were determined by ANOVA.

Class	control	model	ans	Genus	control	model	ans
Bacteroidia	45.35±4.29%a	43.92±2.05%a	42.25±2.13%a	*Lactobacillus*	35.70±1.04%b	24.4±1.89%c	45.89±3.87%a
Bacilli	26.06±1.89%b	37.35±4.04%a	40.05±2.29%a	*Porphyromonas*	17.65±3.9%b	28.01±2.34%a	15.25±3.1%b
Clostridia	20.78±3.57%a	10.04±2.62%b	14.06±1.62%a	*Lachnospira*	19.35±3.6%a	18.74±3.44%a	16.06±2.03%b
Gammaproteobacteria	2.28±0.44%a	1.60±0.82%a	0.62±0.23%b	*Pseudomonas*	2.16±0.42%a	2.66±1.09%a	0.40±0.28%b
Actinobacteria	2.74±0.85%a	2.41±1.22%a	0.92±0.22%b	*Coriobacteriaceae*	1.96±0.65%a	1.18±0.11%b	0.60±0.12%c
Alphaproteobacteria	0.11±0.04%a	0.57±0.96%a	0.13±0.08%a	*Brevundimonas*	0.04±0.01%b	0.09±0.06%a	0.06±0.03%a
Erysipelotrichia	0.34±0.12%a	0.34±0.13%a	0.16±0.08%b	*Bacteroides*	2.78±1.31%a	2.16±1.19%a	0.99±0.49%b
Saccharibacteria	0.63±0.42%b	2.18±0.23%a	1.18±0.61%b	*Clostridium*	10.24±0.92%a	3.24±0.66%b	4.00±3.13%b
others	1.75±0.52%	1.61±0.64%	0.78±0.70%	*Barnesiella*	3.70±1.38%c	5.49±0.38%b	6.99±1.10%a
				Saccharibacteria	0.48±0.13%c	2.10±0.35%a	1.01±0.57%b
				Alloprevotella	0.64±0.28%b	2.50±0.55%a	3.25±1.01%a
				others	5.31±1.03%	9.44±1.34%	5.5±0.89%

Table S3. RDP classifications of the sequence reads at the class and genus levels in microbiota transplantation mice. The data are

expressed as the mean ± SD, n=8. In a, b and c, significant differences (P<0.05) were determined by ANOVA among the MT-control, MT-model and MT-ans groups. In A, B and C, significant differences (P<0.05) were determined by ANOVA among the A-MT-control, A-MT-model and A-MT-ans groups.

Class	MT-control	MT-model	MT-ans	A-MT-control	A-MT-model	A-MT-ans
Bacilli	42.46±3.08%b	65.01±2.65%a	41.36±0.87%b	46.67±7.67%B	68.59±2.81%A	38.05±2.59%B
Bacteroidia	32.23±3.58%a	10.57±1.72%b	31.33±2.00%a	25.41±3.30%B	16.72±2.28%C	34.31±1.30%A
Clostridia	15.22±3.30%a	6.51±1.74%b	20.21±3.16%a	20.12±4.86%A	6.57±1.86%B	20.76±2.77%A
Actinobacteria	2.28±0.34%c	6.65±2.08%a	3.45±1.46%b	2.08±0.41%A	2.38±0.86%A	2.15±1.28%A
Gammaproteobacteria	1.46±0.62%b	5.66±0.73%a	0.51±0.23%c	0.80±0.27%B	0.85±0.15%B	1.38±0.26%A
others	6.33±1.84%	5.58±1.58%	3.15±1.94%	4.93±1.41%	4.89±2.60%	3.34±2.10%
Genus	MT-control	MT-model	MT-ans	A-MT-control	A-MT-model	A-MT-ans
Lactobacillus	66.37±3.12%a	42.86±3.42%b	61.70±1.65%a	63.88±2.39%A	48.40±2.09%B	66.24±3.73%A
Porphyromonas	1.87±1.18%b	17.69±1.86%a	5.85±1.57%b	3.62±1.35%B	14.08±2.33%A	3.63±1.77%B
Lachnospira	5.58±1.60%a	3.66±0.33%b	5.55±0.50%a	8.32±0.94%C	12.67±1.42%B	14.46±1.42%A
Bacteroides	12.88±1.07%a	2.89±0.55%b	2.92±0.37%b	6.82±0.56%A	3.33±0.77%B	1.76±0.52%B
Alistipes	1.08±0.48%c	13.45±1.30%a	2.49±0.21%b	5.89±1.57%A	2.68±0.26%B	0.89±0.49%C
Barnesiella	1.75±0.98%b	2.34±0.62%b	3.90±0.84%a	1.43±0.26%B	4.94±1.04%A	0.50±0.18%C
Parabacteroides	2.05±0.59%a	1.68±0.55%a	1.41±0.42%b	1.43±0.23%A	1.42±0.26%A	0.59±0.20%B
Coriobacteriaceae	1.58±0.18%c	3.53±0.61%b	4.26±0.31%a	1.50±0.18%B	3.24±0.65%A	1.56±0.60%B
Alloprevotella	1.02±0.42%b	0.83±0.29%c	5.98±1.05%a	0.56±0.17%B	0.38±0.09%C	3.08±1.66%A
Odoribacter	0.64±0.13%c	2.84±0.63%a	2.01±0.38%b	2.41±0.32%A	1.81±0.23%B	1.04±0.42%C
others	5.15±3.90%	7.23±2.73%	3.91±2.44%	4.14±1.95%	7.06±2.20%	6.24±1.98%

Figure legends

Fig. S1. The molecular formula of anserine drawn with ChemDraw Pro 18.0.

Fig. S2. Animal experimental design. (A) Analysis of anserine treatment in hyperuricaemic mice. (B) The microbiota transplantation experiment. (C) The antibiotic treatment experiment in mice.

Fig. S3. Effect of anserine treatment on the transcription of genes related to uric acid metabolism pathways. (A) Expression of uric acid excretion-related genes, UMOD; (B) Expression of uric acid excretion-related genes, MRP4. The results are shown as the mean ± SD, n=8. In a, b and c, significant differences (P<0.05) were determined by ANOVA.

Fig. S4. Effect of microbiota transplantation on the body weight and the kidney and liver indexes in hyperuricaemic mice. (A) The body weight gain of hyperuricaemic mice with microbiota transplantation treatment; (B) Changes in the kidney index of hyperuricaemic mice that received microbiota transplantation treatment; (C) Changes in the liver index of hyperuricaemic mice that received microbiota transplantation treatment. The results are shown as the mean ± SD, n=8. In a, b and c, significant differences (P<0.05) were determined by ANOVA among the MT-control, MT-model and MT-ans groups. In A, B and C, significant differences (P<0.05) were determined by ANOVA among the A-MT-control, A-MT-model and A-MT-ans groups.

Fig. S5. Effect of microbiota transplantation on uric acid metabolism pathways. (A) Expression of uric acid excretion-related genes, UMOD; (B) Expression of uric acid excretion-related genes, MRP4. The results are shown

as the mean ± SD, n=8. In a, b and c, significant differences (P<0.05) were determined by ANOVA among the MT-control, MT-model and MT-ans groups. In A, B and C, significant differences (P<0.05) were determined by ANOVA among the A-MT-control, A-MT-model and A-MT-ans groups.

Fig. S6. Analysis of the binding of anserine and uric acid-related enzymes by DS simulation. (A) The interactions of anserine with XOD. (B) The interactions of the amino acid residues of anserine with XOD. (C) The interactions of anserine with ADA. (D) The interactions of the amino acid residues of anserine with ADA. (E) The interactions of anserine with PNP. (F) The interactions of the amino acid residues of anserine with PNP. (G) The interactions of anserine with URAT1. (H) The interactions of the amino acid residues of anserine with URAT1.

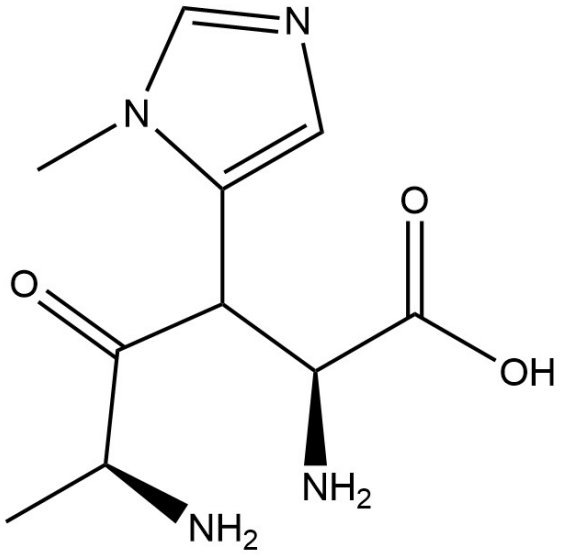
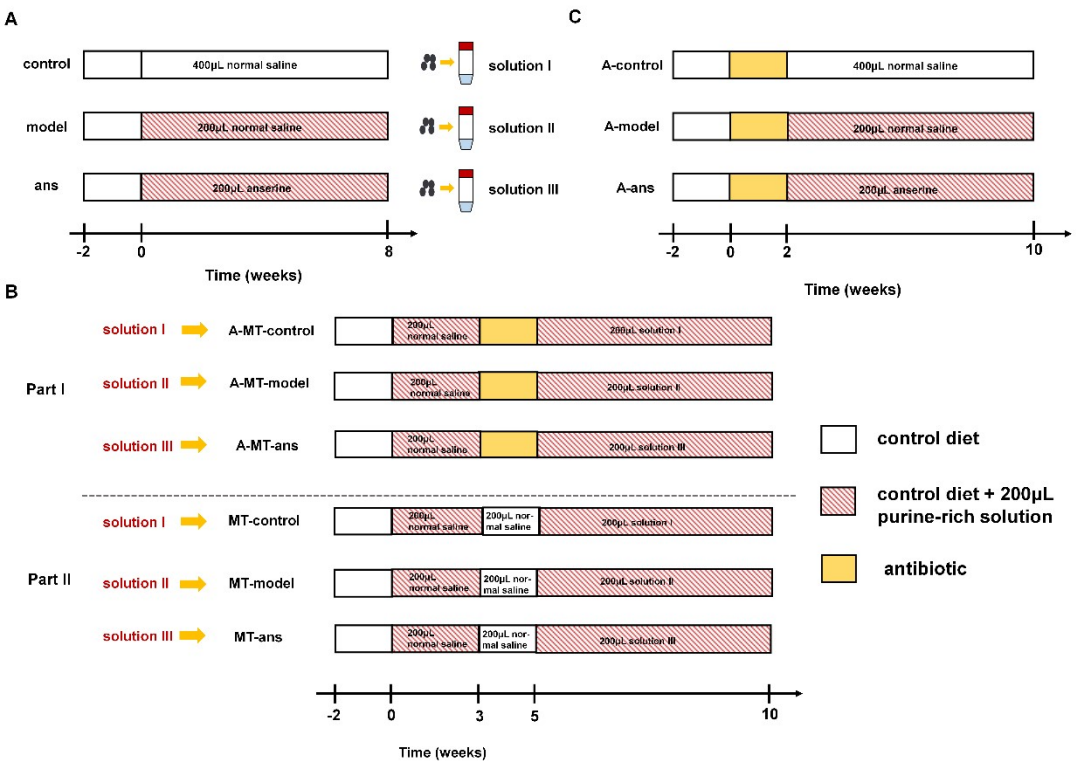


Fig. S1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model
200μL normal saline
solution II
ans
200μL anserine
solution III
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0
8
Time (weeks)
C
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400μL normal saline
A-model
200μL normal saline
A-ans
200μL anserine
-2
0
2
10
Time (weeks)
B
Part I
solution I
A-MT-control
solution II
A-MT-model
solution III
A-MT-ans
Part II
solution I
MT-control
solution II
MT-model
solution III
MT-ans
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200μL solution II
200μL solution III
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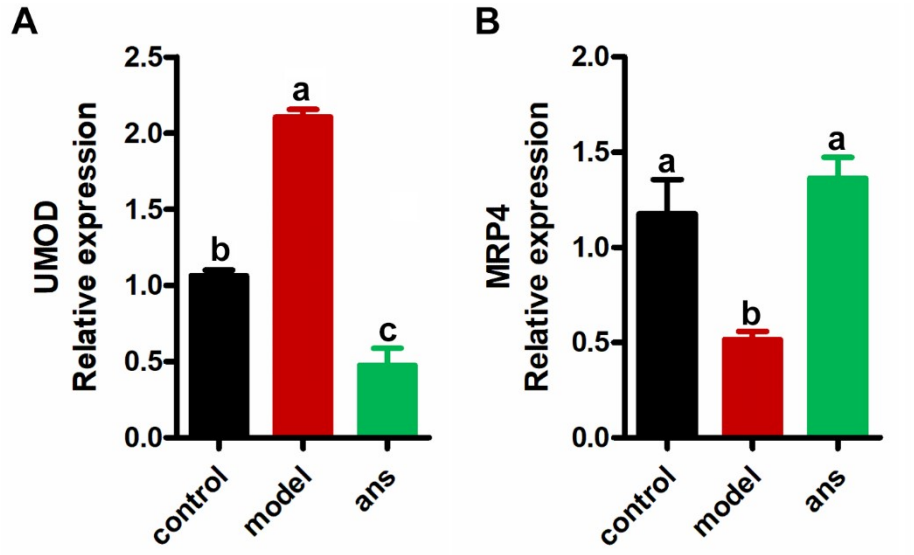
-2
0
3
5
10
Time (weeks)
control diet
control diet + 200μL purine-rich solution
antibiotic

Fig. S2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Relative expression
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2.0
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b
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control
model
ans
B
MRP4
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1.0
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0.0
a
b
a
control
model
ans

Fig. S3

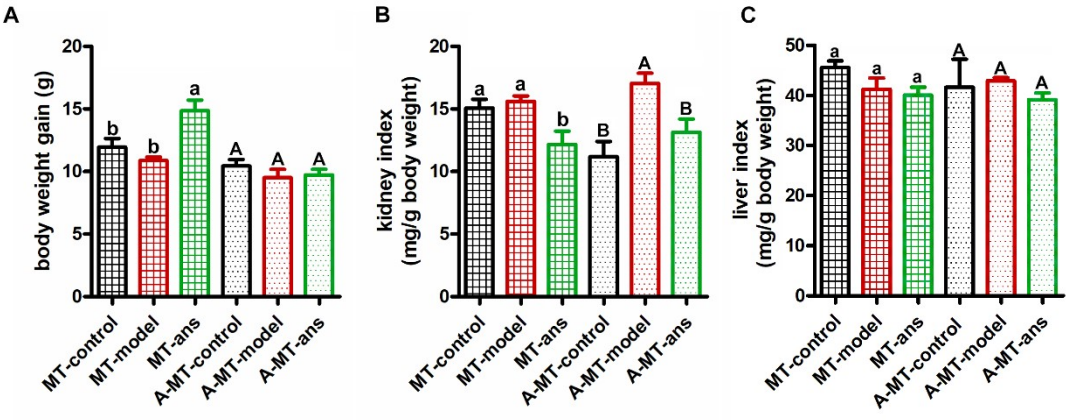

Fig. S4

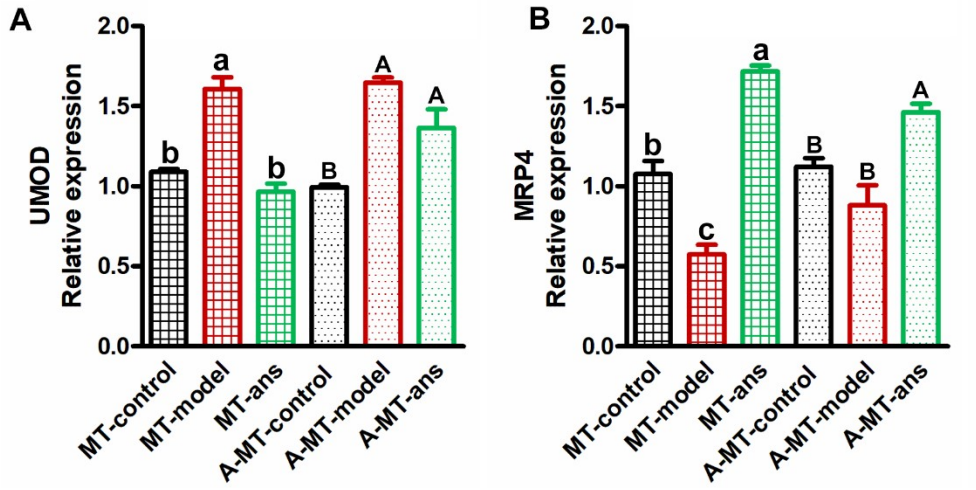

Fig. S5

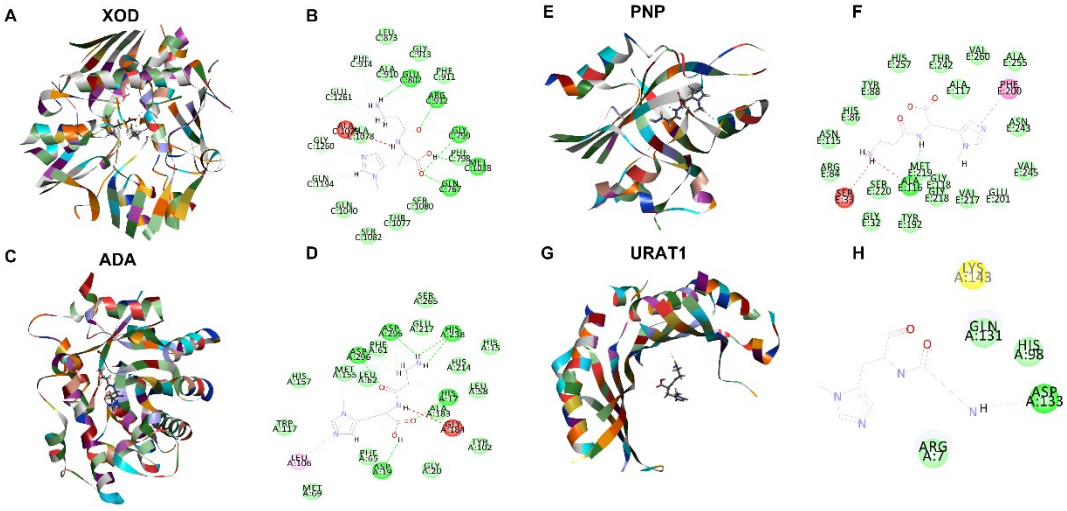

Fig. S6