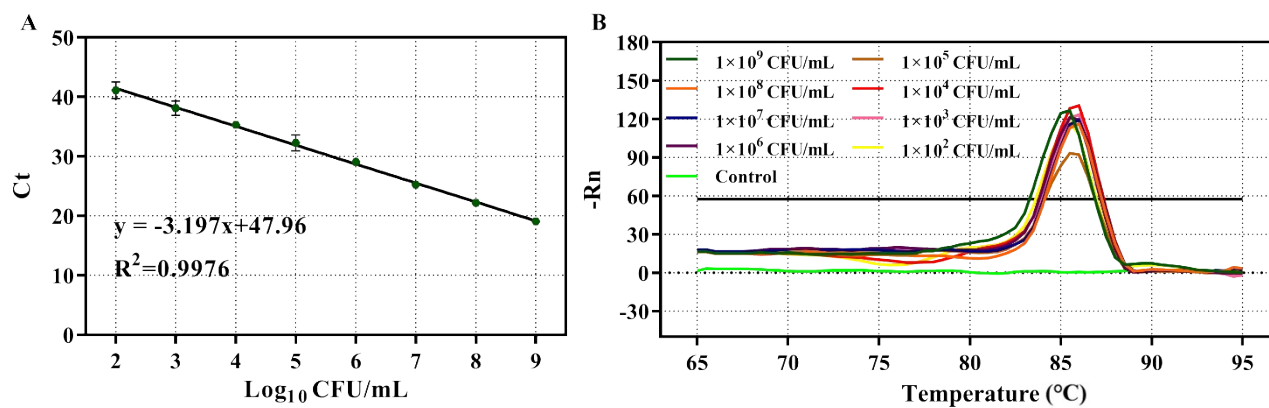
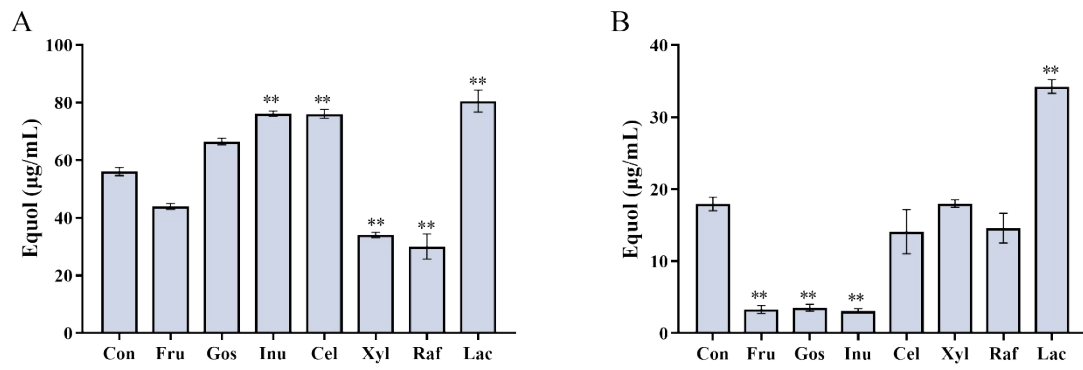


### Supplementary figure captions



**Fig. S1.** Detection of PMAxx-qPCR for *Lactobacillus rhamnosus* ATCC 7469. Plotted values represent the mean and standard deviation obtained from three replicate tests. (A) Standard curve; (B) Melting curve analysis of qPCR products.

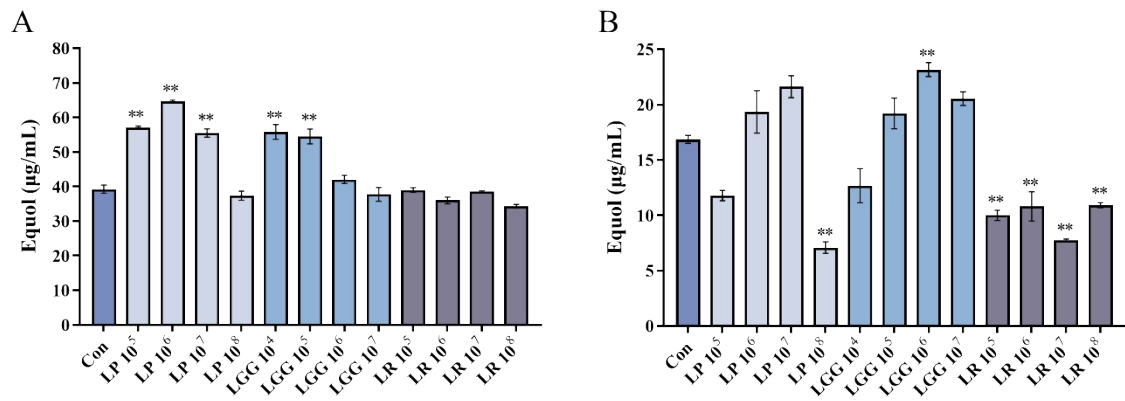


**Fig. S2.** Effects of different prebiotics on the biotransformation ability of high- and low-producing bacteria.

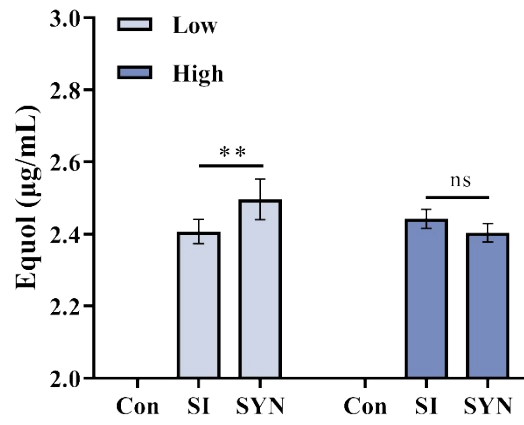
(A) High-producing bacteria; (B) Low-producing bacteria. Con: control group; Fru: fructooligosaccharide;

Gos: galactooligosaccharides; Inu: inulin; Cel: cellobiose; Xyl: xylitol; Raf: raffinose; Lac: lactulose. \*\*p

< 0.01 compared with the control group.

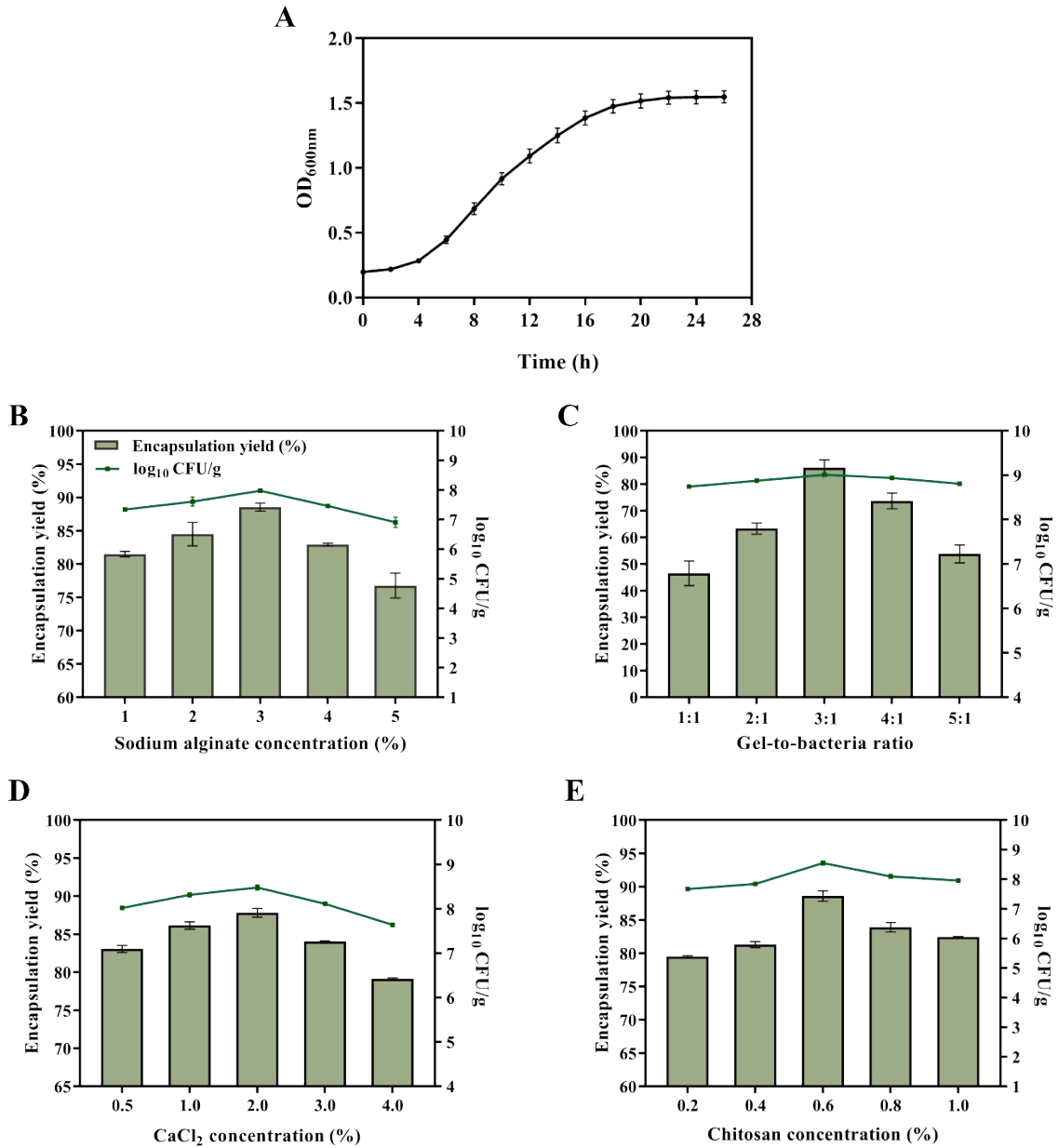


**Fig. S3.** Effects of different concentrations of three probiotics on the biotransformation ability of high- and low-producing bacteria. (A) High-producing bacteria; (B) Low-producing bacteria. LP: *Lactobacillus plantarum* ATCC 8014; LGG: *Lactobacillus rhamnosus* ATCC 7469; LR: *Lactobacillus reuteri* ATCC 23272. \*\* $p < 0.01$  compared with the control group.

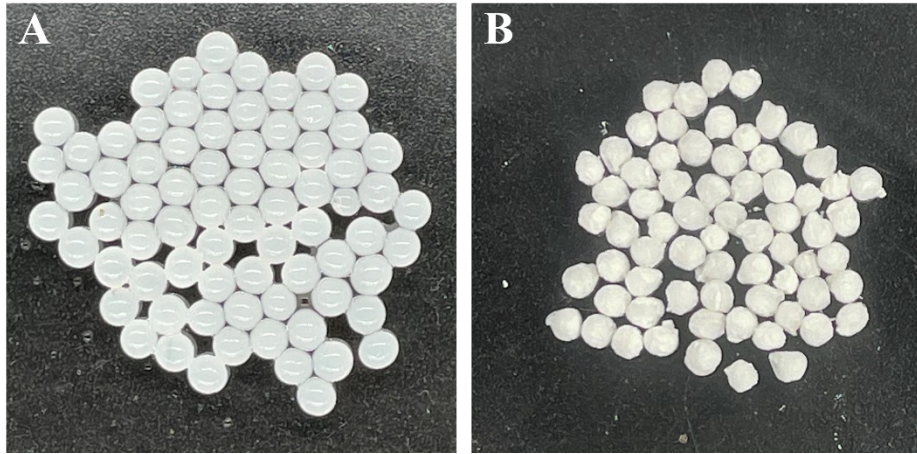


**Fig. S4.** Plasma equol concentration of HFA mice after four weeks of synbiotic preparation intervention.

Con: control group; SI: soy isoflavones group; SYN: synbiotic preparation group. \*\*p < 0.01; ns: no significance.



**Fig. S5.** Optimization of the microcapsule preparation process. (A) Growth curve of *Lactobacillus rhamnosus* ATCC 7469; (B) Effect of different sodium alginate concentrations on the encapsulation yield of microcapsules and the number of viable probiotics; (C) Effect of different gel-to-bacteria ratios on the encapsulation yield of microcapsules and the number of viable probiotics; (D) Effect of different CaCl<sub>2</sub> concentrations on the encapsulation yield of microcapsules and the number of viable probiotics; (E) Effect of different chitosan concentrations on the encapsulation yield of microcapsules and the number of viable probiotics.



**Fig. S6.** Chitosan-coated sodium alginate microcapsules. (A) Fresh microcapsules; (B) Freeze-dried microcapsules.

## Supplementary table captions

**Table S1.** Factors and levels used in the orthogonal tests.

Level	Variable		
	A	B	C
	Daidzein (%)	Lactulose (%)	Probiotic microcapsules (g)
1	0.015	1	0.5
2	0.025	1.5	1
3	0.035	2	1.5

**Table S2.** Experimental design and results for response surface analysis.

Run	A Sodium alginate concentration	B Gel-to-bacteria ratio	C CaCl <sub>2</sub> concentration	D Chitosan concentration	Encapsulation yield (%)
1	0	-1	0	1	72.24 ± 0.03
2	0	0	0	0	93.50 ± 0.12
3	1	0	0	1	90.83 ± 0.01
4	0	0	0	0	93.10 ± 0.25
5	0	1	1	0	92.30 ± 0.40
6	-1	0	-1	0	90.50 ± 0.41
7	0	-1	-1	0	90.92 ± 0.01
8	0	1	0	1	75.21 ± 0.02
9	0	-1	1	0	72.00 ± 0.12
10	1	0	0	-1	72.42 ± 0.57
11	0	-1	0	-1	74.69 ± 0.21
12	0	0	0	0	92.90 ± 0.14
13	0	0	1	-1	85.28 ± 0.39
14	0	1	0	-1	68.47 ± 0.05
15	-1	-1	0	0	76.09 ± 0.07
16	0	0	0	0	91.45 ± 0.09
17	-1	0	0	1	75.35 ± 0.06
18	0	0	-1	1	90.28 ± 0.17
19	1	0	1	0	92.81 ± 0.04
20	0	1	-1	0	69.17 ± 0.06
21	1	1	0	0	85.22 ± 0.02
22	-1	0	0	-1	89.94 ± 0.09
23	0	0	-1	-1	74.37 ± 0.63
24	0	0	0	0	90.30 ± 0.10
25	-1	0	1	0	88.31 ± 0.83
26	0	0	1	1	77.99 ± 0.08
27	1	0	-1	0	91.16 ± 0.02
28	1	-1	0	0	83.49 ± 0.01
29	-1	1	0	0	86.43 ± 0.07



**Table S3.** Quadratic regression model analysis of variance.

Source	Sum of squares	Df	Mean square	F-value	P-value > F	
Model	2026.02	14	144.72	32.00	< 0.0001	**
A-sodium alginate concentration	7.22	1	7.22	1.60	0.2269	
B-gel-to-bacteria ratio	4.53	1	4.53	1.00	0.3341	
C-calcium chloride concentration	0.4370	1	0.4370	0.0966	0.7605	
D-chitosan concentration	23.32	1	23.32	5.16	0.0395	*
AB	18.53	1	18.53	4.10	0.0424	*
AC	3.69	1	3.69	0.8152	0.3819	
AD	272.25	1	272.25	60.20	< 0.0001	**
BC	442.05	1	442.05	97.75	< 0.0001	**
BD	21.11	1	21.11	4.67	0.0485	*
CD	134.56	1	134.56	29.76	< 0.0001	**
A <sup>2</sup>	0.2616	1	0.2616	0.0579	0.8134	
B <sup>2</sup>	615.46	1	615.46	136.10	< 0.0001	**
C <sup>2</sup>	8.33	1	8.33	1.84	0.1962	
D <sup>2</sup>	602.27	1	602.27	133.18	< 0.0001	**
Residual	63.31	14	4.52			
Lack of Fit	56.16	10	5.62	3.14	0.1406	Non Significant
Pure Error	7.15	4	1.79			
Cor Total	2089.33	28				

[\*. Significant difference (P < 0.05); \*\*. The difference is highly significant (P < 0.01).]

**Table S4.** Model credibility analysis

parameters	SD	Mean	CV%	PRESS	R <sup>2</sup>	Adj R <sup>2</sup>	Pred R <sup>2</sup>	Adeq Precision
Value	2.13	83.68	2.54	334.66	0.9697	0.9394	0.8398	15.0691

**Table S5.** Design and results of orthogonal experiments.

Number	Variable			Growth ratio
	A Daidzein	B Lactulose	C Probiotic microcapsules	
1	1	1	1	1.092
2	2	2	1	1.099
3	3	3	1	1.350
4	3	2	2	1.412
5	2	1	2	1.402
6	1	3	2	1.380
7	1	2	3	1.074
8	2	3	3	1.062
9	3	1	3	1.023
K <sub>1</sub>	3.545	3.518	3.541	
K <sub>2</sub>	3.563	3.586	4.194	
K <sub>3</sub>	3.786	3.791	3.159	
k <sub>1</sub>	1.182	1.173	1.180	
k <sub>2</sub>	1.188	1.195	1.398	
k <sub>3</sub>	1.262	1.264	1.053	
R	0.080	0.091	0.345	

**Table S6.** Contents of daidzein and equol in the fecal fermentation broth.

Fecal flora	Daidzein concentration ( $\mu\text{g/mL}$ )	Equol concentration ( $\mu\text{g/mL}$ )	Equol/Daidzein	$\log_{10}$ (equol/daidzein)
low-equol- producing fecal flora	59.72	20.96	0.35	-0.45
high-equol- producing fecal flora	4.17	59.39	14.24	1.15