| Primer | Forward primer (5'-3') | Reverse primer (5'-3') | Reference |
|-----------|-----------------------------------|----------------------------------|------------|
| PCR | | | |
| 411-BSH | CATGCCATGGGAATGTGTACTGGTTTAAGATTC | CGGCTCGAGCTAGTAAGTCACAAGACTGGTTG | This study |
| 338F/806R | ACTCCTACGGGA-GGCAGCAG | GGACTACHVGGGTWTCTAAT | |
| qPCR | | | |
| CYP7A1 | ACAGAAGCATAGACCCAA | TGCCAAACAGCGTTAGAT | 13 |
| CYP8B1 | AAGGCTGGCTTCCTGAGCTT | AACAGCTCATCGGCCTCATC | |
| CYP27A1 | TGCCTGGGTCGGAGGAT | GAGCCAGGGCAATCTCATACTT | 18 |
| CYP7B1 | TAGCCCTCTTTCCTCCACTCATA | GAACCGATCGAACCTAAATTCCT | |
| FXR | ACATCCCCATCTCTGCAC | TGTGAGGGCTGCAAAGGTTT | |
| SHP | CCTGGAGTCTTTCTGGAGCCTTGA | TGTTGCAGGTGTGCGATGTGG | |
| FGFR4 | GCATCTTTCAGGGGACACCA | TTGTACCAGTGACGACCACG | 19 |
| KLB | TCAGGCCTGCATAGGAAATG | CTGGATCGGGAGACTTAGGA | 20 |
| LRH-1 | TTGAGTGGGCCAGGAGTAGT | ACGCGACTTCTGTGTGTGAG | |
| FGF15 | ACGTCCTTGATGGCAATCG | GAGGACCAAAACGAACGAAATT | |
| IBABP | CAGGAGACGTGATTGAAAGGG | GCCCCCAGAGTAAGACTGGG | |
| ABCG5 | GCAGGGACCGAATTGTGATTG | AGGGATTGGAATGTTCAGGACAG | |
| ABCG8 | GGCTCAGGATCGGCTTTCAC | CCTTGACACAGGCATGAAGCA | 21 |
| HMGCR | GGACCAACCTTCTACCTC | CCATCACAGTGCCACATAC | |
| LDL-R | CAGTCCCAGGCAGCGTAT | TTGATCTTGGCGGGTGTT | |
| rpL32 | TCTGGTCCACAACGTCAAGG | GGATTGGTGACTCTGATGGC | |

1 Supplemental Table 1 PCR and quantitative PCR primers used in this study

3 Supplemental Table 2 Primer sequence for bsh qPCR

| Group Expected ²³ | Primer sequence (5'-3') | F/R | Reference <i>bsh</i> (Accession number)* | Product size (bp) |
|------------------------------|--------------------------|-----|---|----------------------|
| n | TGTGTGAGCAACGAGATGGC | F | AAR39435, BAF39637, AAF67801, AAN24611, | 105 |
| Z | ACACGAACGAAACGCGACGG | R | CDB23390, BAQ98062, BAP83517, AAX86039 | |
| 2 ۸ | AYTTTGGHMGWAAYYTHGAY | F | AAR39435, BAF39637, AAF67801, AAN24611, | 105 |
| 3A | RTTNARDCCDGCHATVCCWAVHCC | R | CDB23390, BAQ98062, BAP83517, AAX86039 | |
| 20 | GAGGATGGACTAGGAATGGC | F | ABE00605, CDK34338 | 104 |
| 30 | TGGAATAAACTCGAACGGCG | R | | |

4 *Accession number of the reference *bsh* is from EMBL database (http://www.ebi.ac.uk/)

5

7 Supplemental Figure legend:

8 Figure S1: Physiological and biochemical indexes (serum lipid: A; and liver lipid: B) were used to 9 evaluate the fat-reducing function of BSH strain in these five groups (ND: normal diet; HFD: high-fat 10 diet; HFD+NB5462: high-fat diet + empty plasmid control NB5462; HFD+YB334-H: high-fat diet + 11 recombinant YB334 with high BSH activity; and HFD+YB334-L: high-fat diet + recombinant YB334 12 with low BSH activity). TC: total cholesterol; TG: triacylglycerols; LDL-C: low-density lipoprotein cholesterol; and HDL-C: high-density lipoprotein cholesterol. Values were expressed as mean ± SD (n 13 14 = 8). * Represents a significant (p < 0.01) difference from HFD+NB5462 group. ** Represents a significant (p < 0.0001) difference from HFD+NB5462 group. # p < 0.01, ## p < 0.0001. 15 16 Figure S2: Fecal bile acid levels were used to evaluate the pathway of BSH involved in bile acid 17 18 metabolism in these five groups (ND: normal diet; HFD: high-fat diet; HFD+NB5462: high-fat diet + 19 empty plasmid control NB5462; HFD+YB334-H: high-fat diet + recombinant YB334 with high BSH 20 activity; and HFD+YB334-L: high-fat diet + recombinant YB334 with low BSH activity). UDCA: ursodeoxycholic acid; α -/ β -/ ω -MCA: α -/ β -/ ω -muricholic acid; CA: cholic acid; CDCA: 21 chenodeoxycholic acid; DCA: deoxycholic acid; LCA: lithocolic acid; TUDCA: tauroursodeoxycholic 22 acid; $T\alpha(\beta/\omega)MCA$: tauro- $\alpha(\beta/\omega)$ -muricholic acid; TCA: taurocholic acid; TCDCA: 23 24 taurochenodeoxycholic acid; TDCA: taurodeoxycholic acid; TLCA: taurolithocolic acid. Values were expressed as mean \pm SD (n = 8). * (p < 0.01) and ** (p < 0.0001) represent a significant difference 25 26 from HFD+NB5462 group, respectively. 27

28 Figure S3: Alpha diversity (A), Bacteria composition (phylum level, B), and Heatmap clustering

29 analysis of biodiversity (C) of mouse gut microbiota in these four groups (HFD+NB5462: high-fat diet

30 + empty plasmid control NB5462; HFD: high-fat diet; HFD+YB334-H: high-fat diet + recombinant

31 YB334 with high BSH activity; ND: normal diet).

32

33 Figure S4: Cecum short-chain fatty acids (SCFAs) were detected to verify the relationship between

34 intestinal flora and fat metabolism. HFD+NB5462: high-fat diet + empty plasmid control NB5462;

35 HFD: high-fat diet; HFD+YB334-H: high-fat diet + recombinant YB334 with high BSH activity; ND:

36 normal diet. Values were expressed as mean \pm SD (n = 8). ** Represents a significant (p < 0.0001)

37 difference from HFD+NB5462 group.

38

A. Serum lipid



Fig. S1: Physiological and biochemical indexes (serum lipid: A; and liver lipid: B) were used to evaluate 41 the fat-reducing function of BSH strain in these five groups (ND: normal diet; HFD: high-fat diet; 42 HFD+NB5462: high-fat diet + empty plasmid control NB5462; HFD+YB334-H: high-fat diet + 43 recombinant YB334 with high BSH activity; and HFD+YB334-L: high-fat diet + recombinant YB334 with 44 45 low BSH activity). TC: total cholesterol; TG: triacylglycerols; LDL-C: low-density lipoprotein cholesterol; and HDL-C: high-density lipoprotein cholesterol. Values were expressed as mean \pm SD (n = 8). * 46 Represents a significant (p < 0.01) difference from HFD+NB5462 group. ** Represents a significant (p 47 < 0.0001) difference from HFD+NB5462 group. # *p* < 0.01, ## *p* < 0.0001. 48

49





Fig. S2: Fecal bile acid levels were used to evaluate the pathway of BSH involved in bile acid metabolism 52 in these five groups (ND: normal diet; HFD: high-fat diet; HFD+NB5462: high-fat diet + empty plasmid 53 control NB5462; HFD+YB334-H: high-fat diet + recombinant YB334 with high BSH activity; and 54 HFD+YB334-L: high-fat diet + recombinant YB334 with low BSH activity). UDCA: ursodeoxycholic acid; 55 α -/ β -/ ω -MCA: α -/ β -/ ω -muricholic acid; CA: cholic acid; CDCA: chenodeoxycholic acid; DCA: 56 deoxycholic acid; LCA: lithocolic acid; TUDCA: tauroursodeoxycholic acid; $T\alpha(\beta/\omega)MCA$: tauro- $\alpha(\beta/\omega)$ -57 muricholic acid; TCA: taurocholic acid; TCDCA: taurochenodeoxycholic acid; TDCA: taurodeoxycholic 58 acid; TLCA: taurolithocolic acid. Values were expressed as mean \pm SD (n = 8). * (p < 0.01) and ** (p < 59 0.0001) represent a significant difference from HFD+NB5462 group, respectively. 60

- 61
- 62



Fig. S3: Alpha diversity (A), Bacteria composition (phylum level, B), and Heatmap clustering analysis of
biodiversity (C) of mouse gut microbiota in these four groups (HFD+NB5462: high-fat diet + empty
plasmid control NB5462; HFD: high-fat diet; HFD+YB334-H: high-fat diet + recombinant YB334 with
high BSH activity; ND: normal diet).



Fig. S4: Cecum short-chain fatty acids (SCFAs) were detected to verify the relationship between intestinal flora and fat metabolism. HFD+NB5462: high-fat diet + empty plasmid control NB5462; HFD: high-fat diet; HFD+YB334-H: high-fat diet + recombinant YB334 with high BSH activity; ND: normal diet. Values were expressed as mean \pm SD (n = 8). ** Represents a significant (*p* < 0.0001) difference from HFD+NB5462 group.