

# Supporting Information

## **Metagenomic analysis of the human microbiome reveals the association between the abundance of gut bile salt hydrolases and host health**

Baolei Jia<sup>a,b\*</sup>, Dongbin Park<sup>b\*</sup>, Yoonsoo Hahn<sup>b</sup>, and Che Ok Jeon<sup>b</sup>

<sup>a</sup> State Key Laboratory of Biobased Material and Green Papermaking, School of Bioengineering, Qilu University of Technology (Shandong Academy of Sciences), Jinan, China; <sup>b</sup> Department of Life Science, Chung-Ang University, Seoul, Republic of Korea

CONTACT Che Ok Jeon [cojeon@cau.ac.kr](mailto:cojeon@cau.ac.kr). Department of Life Science, Chung-Ang University, Seoul 06974, Republic of Korea; [baoleijia@cau.ac.kr](mailto:baoleijia@cau.ac.kr), Department of Life Science, Chung-Ang University, Seoul 06974, Republic of Korea.

\*These authors contributed equally to this work.

**Supplementary Table S1. Characteristics of the experimentally characterized bile salt hydrolases.**

<b>Entry</b>	<b>Organism</b>	<b>Length</b>	<b>Cluster</b>	<b>References</b>
Q1WR93	<i>Lactobacillus salivarius</i>	316	Cluster2	(Fang et al., 2009)
C7AQX8	<i>Lactobacillus salivarius</i>	324	Cluster2	(Fang et al., 2009)
C7AQY2	<i>Lactobacillus salivarius</i>	324	Cluster2	(Bi et al., 2013)
C7AQX9	<i>Lactobacillus salivarius</i>	325	Cluster2	(Bi et al., 2013)
J7H3P9	<i>Lactobacillus salivarius</i>	324	Cluster2	(Wang et al., 2012)
A0A0R2G1E1	<i>Lactobacillus plantarum</i>	324	Cluster2	(Ren et al., 2011)
Q06115	<i>Lactobacillus plantarum</i>	324	Cluster2	(Christiaens et al., 1992)
M1R991	<i>Lactobacillus plantarum</i>	328	Cluster2	(Gu et al., 2014)
M1R367	<i>Lactobacillus plantarum</i>	317	Cluster6	(Gu et al., 2014)
B9V405	<i>Lactobacillus gasseri</i>	325	Cluster2	(Jiang et al., 2010)
A0A1Y0E209	<i>Lactobacillus gasseri</i>	326	Cluster2	(Rani et al., 2017)
Q5FKM3	<i>Lactobacillus acidophilus</i>	325	Cluster2	(McAuliffe et al., 2005)
Q5FK51	<i>Lactobacillus acidophilus</i>	325	Cluster2	(McAuliffe et al., 2005)
A5VJH0	<i>Lactobacillus reuteri</i>	325	Cluster2	(Bustos et al., 2016)
A0A1B3PS02	<i>Lactobacillus johnsonii</i>	326	Cluster2	(Chae et al., 2013)
A5HKP3	<i>Lactobacillus acidophilus</i>	316	Cluster2	(Chae et al., 2013)
A0A2M9NK78	<i>Lactobacillus johnsonii</i>	325	Cluster2	(Chae et al., 2013)
Q9F660	<i>Lactobacillus johnsonii</i>	326	Cluster2	(Elkins et al., 2001)
P97038	<i>Lactobacillus johnsonii</i>	316	Cluster2	(Elkins et al., 2001)
H6WSA2	<i>Lactobacillus fermentum</i>	325	Cluster5	(Kumar et al., 2013)
A0A1F0S498	<i>Lactobacillus sp.</i> HMSC056D05	338	Cluster7	(Kaya et al., 2017)
Q9KK62	<i>Bifidobacterium longum</i>	317	Cluster2	(Kumar et al., 2006)
A0A087BL81	<i>Bifidobacterium longum</i>	317	Cluster2	(Jarocki et al., 2014)
Q6R974	<i>Bifidobacterium bifidum</i>	316	Cluster2	(Kim et al., 2004)
G0YYC2	<i>Bifidobacterium animalis</i>	314	Cluster2	(Jarocki, 2011)
Q53CP8	<i>Bifidobacterium animalis</i>	314	Cluster2	(Kim and Lee, 2008)
A0A072MTF9	<i>Bifidobacterium pseudocatenulatum</i>	316	Cluster2	(Jarocki et al., 2014)
P54965	<i>Clostridium perfringens</i>	329	Cluster2	(Rossocha et al., 2005)
C7CXJ5	<i>Enterococcus faecalis</i> T2	324	Cluster2	(Chand et al., 2018)
E2YQS1	<i>Enterococcus faecalis</i> DAPTO 512	355	Cluster7	(Song et al., 2019)
R6GBZ3	<i>Eubacterium hallii</i> CAG:12	324	Cluster2	(Song et al., 2019)
A0A380KNN4	<i>Streptococcus infantarius</i>	325	Cluster2	(Song et al., 2019)
D6DDI0	<i>Bifidobacterium longum</i>	334	Cluster2	(Song et al., 2019)

A0A3A6KGT7	<i>Bacteroides sp.</i> AF35-22	361	Cluster3(S)	(Song et al., 2019)
A0A3A6KI09	<i>Bacteroides sp.</i> AF35-22	351	Cluster1(S)	(Song et al., 2019)
Q8A600	<i>Bacteroides thetaiotaomicron</i>	352	Cluster1(S)	(Yao et al., 2018)
A0A416F798	<i>Clostridium sp.</i> AF32-7AC	336	Cluster4	(Song et al., 2019)
A7LYY3	<i>Bacteroides ovatus</i>	359	Cluster3(S)	(Jones et al., 2008)
A7V5S3	<i>Bacteroides uniformis</i>	361	Cluster3(S)	(Jones et al., 2008)
A5ZA20	<i>Eubacterium ventriosum</i> ATCC 27560	329	Cluster2	(Jones et al., 2008)
A5ZWR0	<i>Blautia obeum</i> ATCC 29174	325	Cluster2	(Jones et al., 2008)
A5ZM11	<i>Blautia obeum</i> ATCC 29174	326	Cluster2	(Jones et al., 2008)
A4E835	<i>Collinsella aerofaciens</i> ATCC 25986	308	Cluster2	(Jones et al., 2008)
A7A5K1	<i>Bifidobacterium adolescentis</i> L2-32	347	Cluster2	(Jones et al., 2008)

(S): BSH with signal peptide.

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**Supplementary Table S2. Characteristics of the metagenomic datasets included in this study.**

<b>Accession Number</b>	<b>Health and Disease sample amounts</b>	<b>Countries</b>	<b>Sequencing Methods</b>	<b>References</b>
PRJEB5224	healthy (109) CD (39) UC (64)	Denmark, Spain	Illumina HiSeq 2000	(Li et al., 2014)
PRJNA389280	healthy (65) CD (151) UC (84)	USA	Illumina HiSeq 2000	(Schirmer et al., 2018)
PRJEB7774	healthy (63) CA (47) CRC (46)	Austria	Illumina HiSeq 2000	(Feng et al., 2015)
PRJEB6070	healthy (61) CA (42)	France, Germany	Illumina HiSeq 2000	(Zeller et al., 2014)
PRJEB12449	healthy (52) CRC (52)	USA	Illumina HiSeq 2000	(Vogtmann et al., 2016)
PRJEB10878	healthy (54) CRC (74)	China	Illumina HiSeq 2000	(Yu et al., 2017)
PRJNA389927	healthy (28) CA (28) CRC (28)	USA, Canada	Illumina HiSeq 4000	(Hannigan et al., 2018)
PRJNA447983	healthy (28) CRC (32)	Italy	Illumina HiSeq 2500	(Thomas et al., 2019)
PRJEB27928	healthy (60) CRC (22)	Germany	Illumina HiSeq 2000 Illumina HiSeq 4000	(Wirbel et al., 2019)
PRJEB4336	healthy (123) OB (169)	Denmark	Illumina Genome Analyzer II Illumina Genome Analyzer Iix Illumina HiSeq 2000	(Le Chatelier et al., 2013)
PRJNA422434	healthy (183) T2D (187)	China	Illumina Genome Analyzer II	(Qin et al., 2012)
PRJNA354235 PRJNA373901	healthy (308) m-NAFLD (74) a-NAFLD (12)	USA	Illumina HiSeq 2500	(Loomba et al., 2017; Abu-Ali et al., 2018)
PRJEB6337	healthy (114) LC (123)	China	Illumina HiSeq 2000	(Qin et al., 2014)

PRJEB21528	healthy (171) CVD (214)	China	Illumina HiSeq 2000	(Jie et al., 2017)
PRJEB17784	healthy (28) PD (31)	Germany	Illumina HiSeq 4000	(Bedarf et al., 2017)
PRJEB28847	healthy (22) epilepsy (24)	Sweden	Illumina MiSeq V3 chemistry	(Lindfeldt et al., 2019)
PRJNA453965	healthy (71) BC (62)	China	Illumina HiSeq X Ten	(Zhu et al., 2018)
<b>Validation cohorts</b>				
PRJEB1220	healthy (236) CD (13) UC (69)	Denmark, Spain	Illumina Genome Analyzer II, Illumina Genome Analyzer IIX, Illumina HiSeq 2000	(Nielsen et al., 2014)
PRJEB6070	healthy (61) CRC (91)	France, Germany	Illumina HiSeq 2000	(Zeller et al., 2014)

The sample numbers used in the analysis are shown after the cases. Abbreviations: UC: ulcerative colitis, CD: Crohn's disease, CA: colorectal adenomas, CRC: colorectal cancer, OB: obesity, T2D: Type 2 diabetes, NAFLD: non-alcoholic fatty liver disease, LC: liver cirrhosis, CVD: cardiovascular disease, PD: Parkinson's disease, BC: Breast cancer.

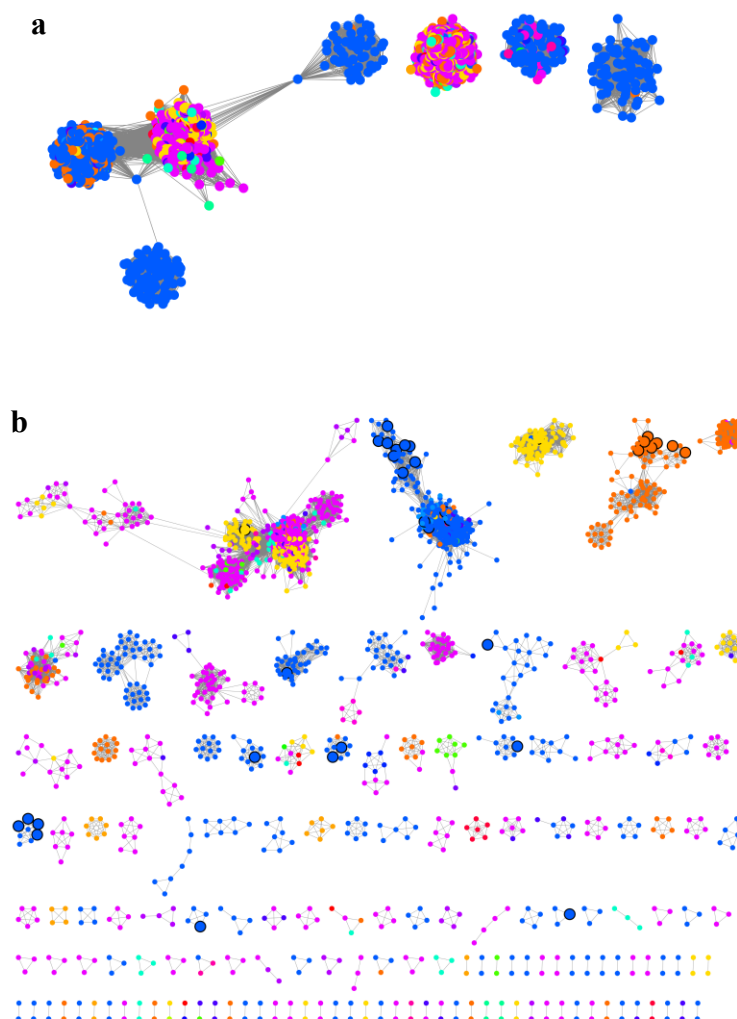
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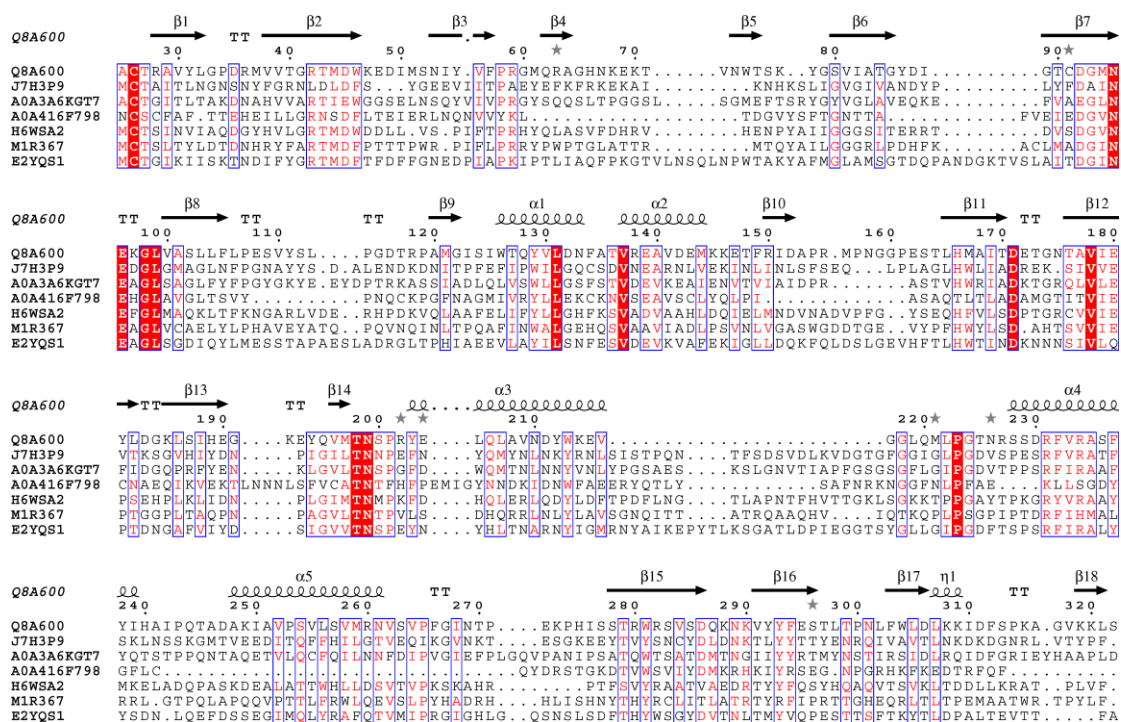


## Supplementary Figure S1.



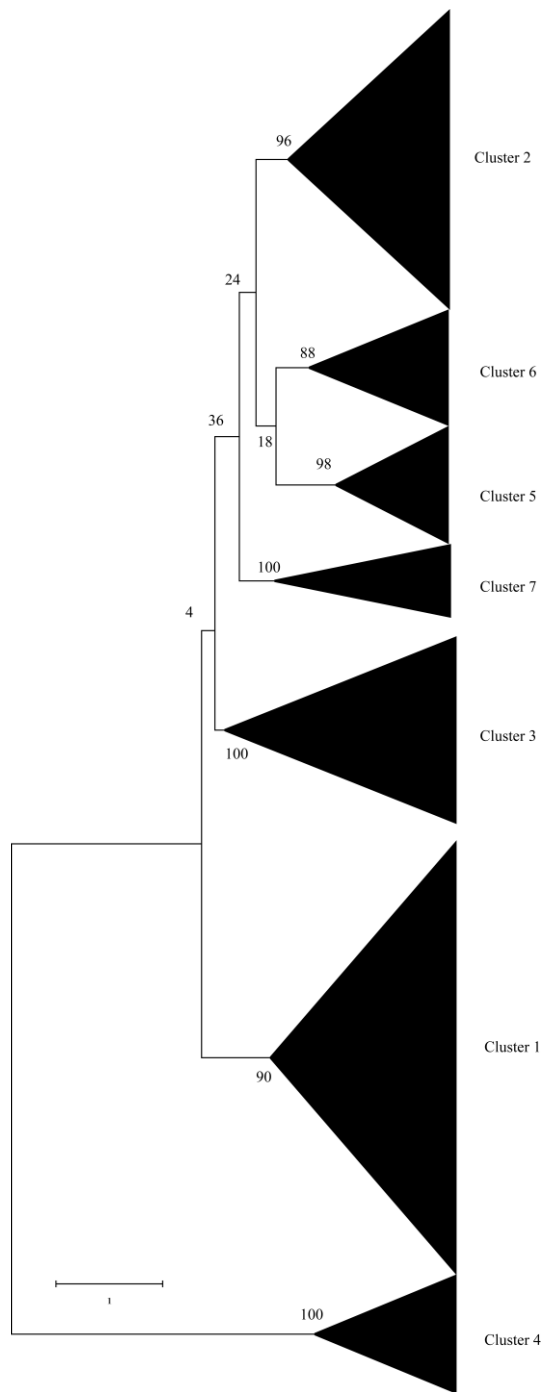
**Supplementary Figure S1. Protein sequence similarity network (SSN) of bile salt hydrolases (BSHs).** The proteins listed in Table S1 were used as queries to BLAST against the UniProt database with the parameters described in the “Methods.” The queries and BLAST results (5,790 proteins in total) were used to generate the network using an e-value threshold of  $10^{-40}$  (>30% sequence identity, (a)) or an e-value threshold of  $10^{-75}$  (>50% sequence identity, (b)). The network displayed 2,396 nodes representing 5,790 proteins filtered at 90% sequence identity. Each cluster was sequentially ranked and labeled based on node number. In B, the enzymes listed Supplementary Table 1 is enlarged. Nodes from the same phylum in the network have the same color, which is shown in figure 1.

## Supplementary Figure S2.



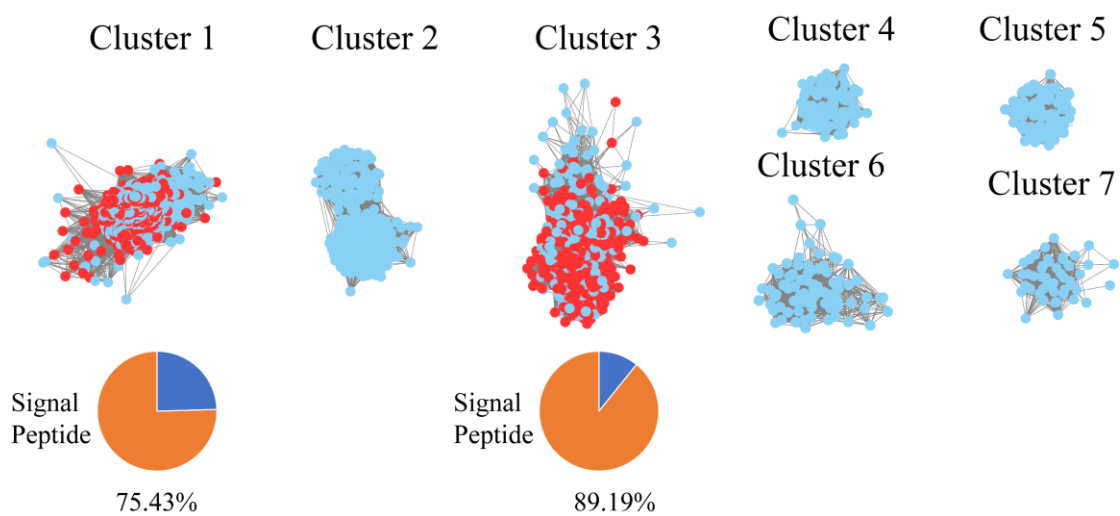
**Supplementary Figure S2.** Sequence alignment of representative sequences from Clusters 1–7. The UniProt IDs of the 7 enzymes from Clusters 1–7 are listed on the left. The secondary structure was predicted based on the enzyme from *Bacteroides thetaiotaomicron* VPI (UniProt ID: Q8A600; PDB ID: 3HBC). Identical and similar amino acids are shown in red boxes.

**Supplementary Figure S3.**



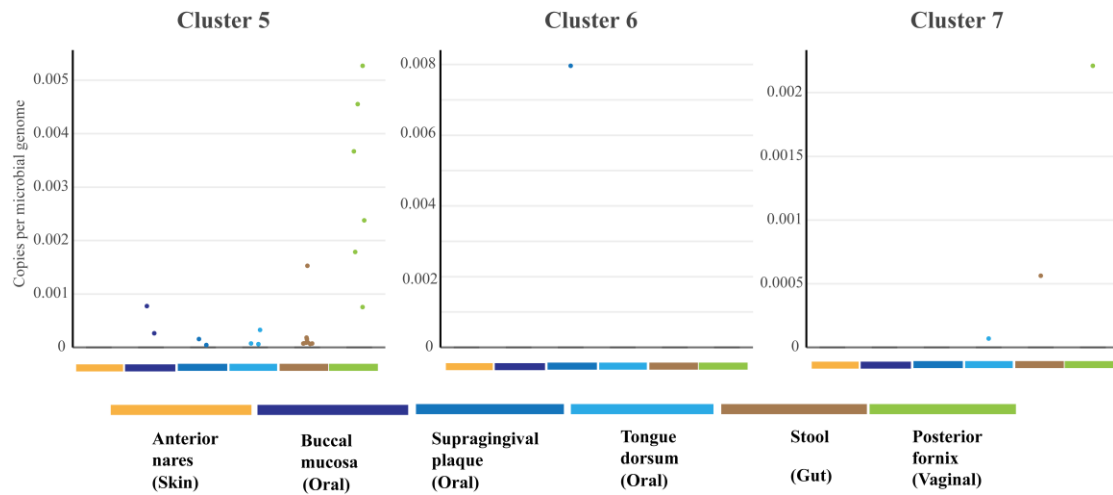
**Supplementary Figure S3.** Evolutionary relationships of bile salt hydrolases (BSHs) in the 7 clusters. Maximum-likelihood phylogenetic tree for the BSHs listed in the Supplementary dataset were generated using MEGA X. The percentage of replicate trees in the bootstrap test (500 replicates) is shown next to the branches.

**Supplementary Figure S4.**



**Supplementary Figure S4.** Protein sequence similarity network (SSN) of bile salt hydrolases (BSHs) annotated by signal peptides. The network was built as shown in Figure 1. The proteins with signal peptides are shown in red. The protein percentages with signal peptides in the clusters are listed at the bottom.

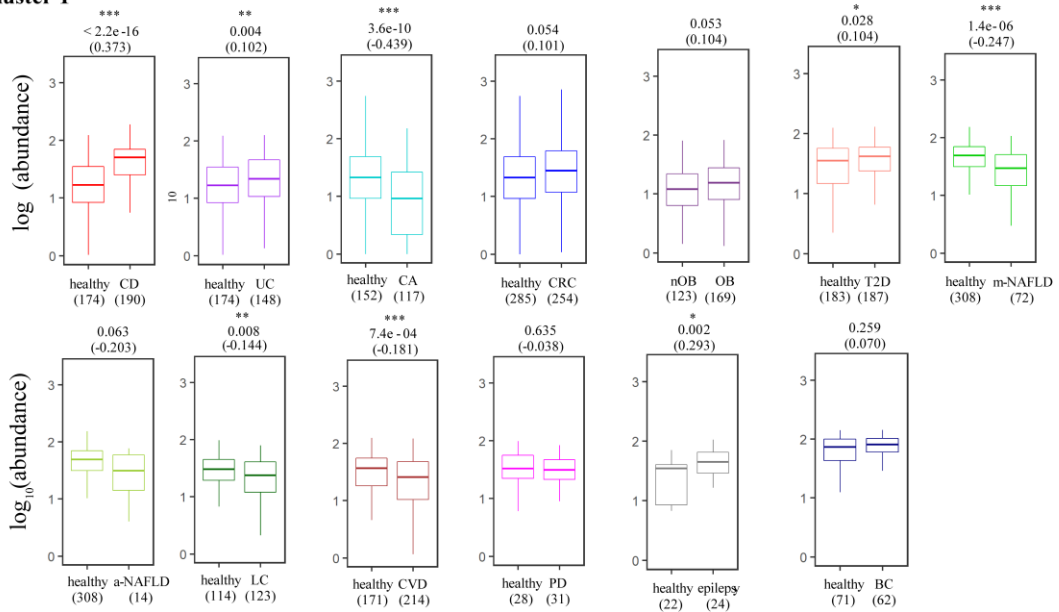
### Supplementary Figure S5.



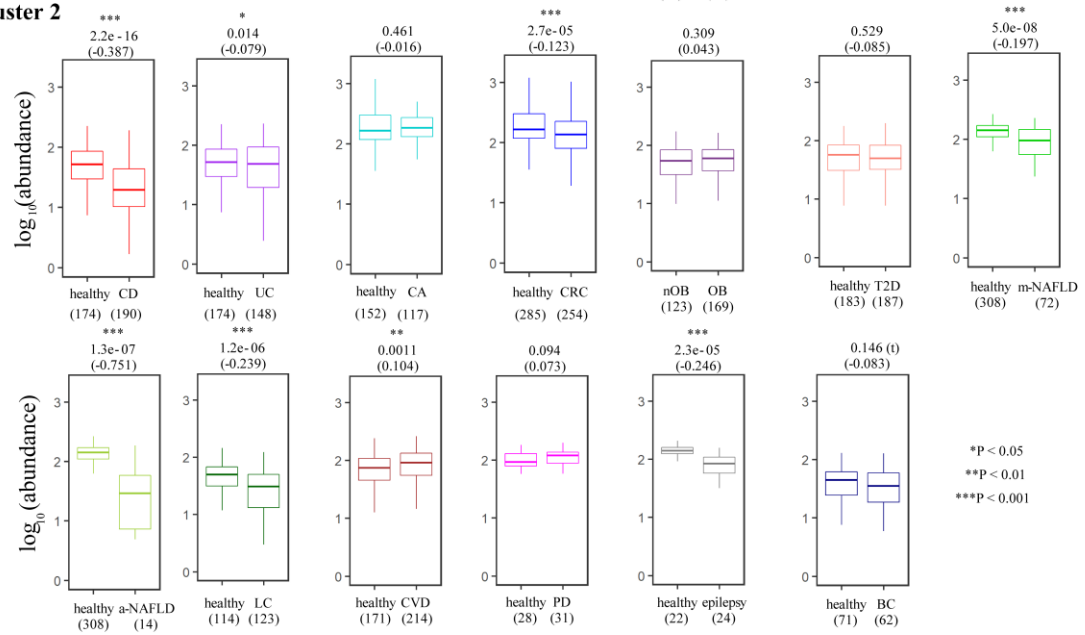
**Supplementary Figure S5.** Boxplots of the abundances of Clusters 1–4 across six body sites. Tukey boxplots in the main text show the median, first quartile ( $Q_1$ ), and third quartile ( $Q_3$ ). Whiskers are extended to include data points between  $Q_1-1.5$  ( $Q_3-Q_1$ ) and  $Q_1$  and between  $Q_3$  and  $Q_1+1.5$  ( $Q_3-Q_1$ ) (the lower and upper inner fences, respectively). Values outside of this range are individually marked with dots.

## Supplementary Figure S6.

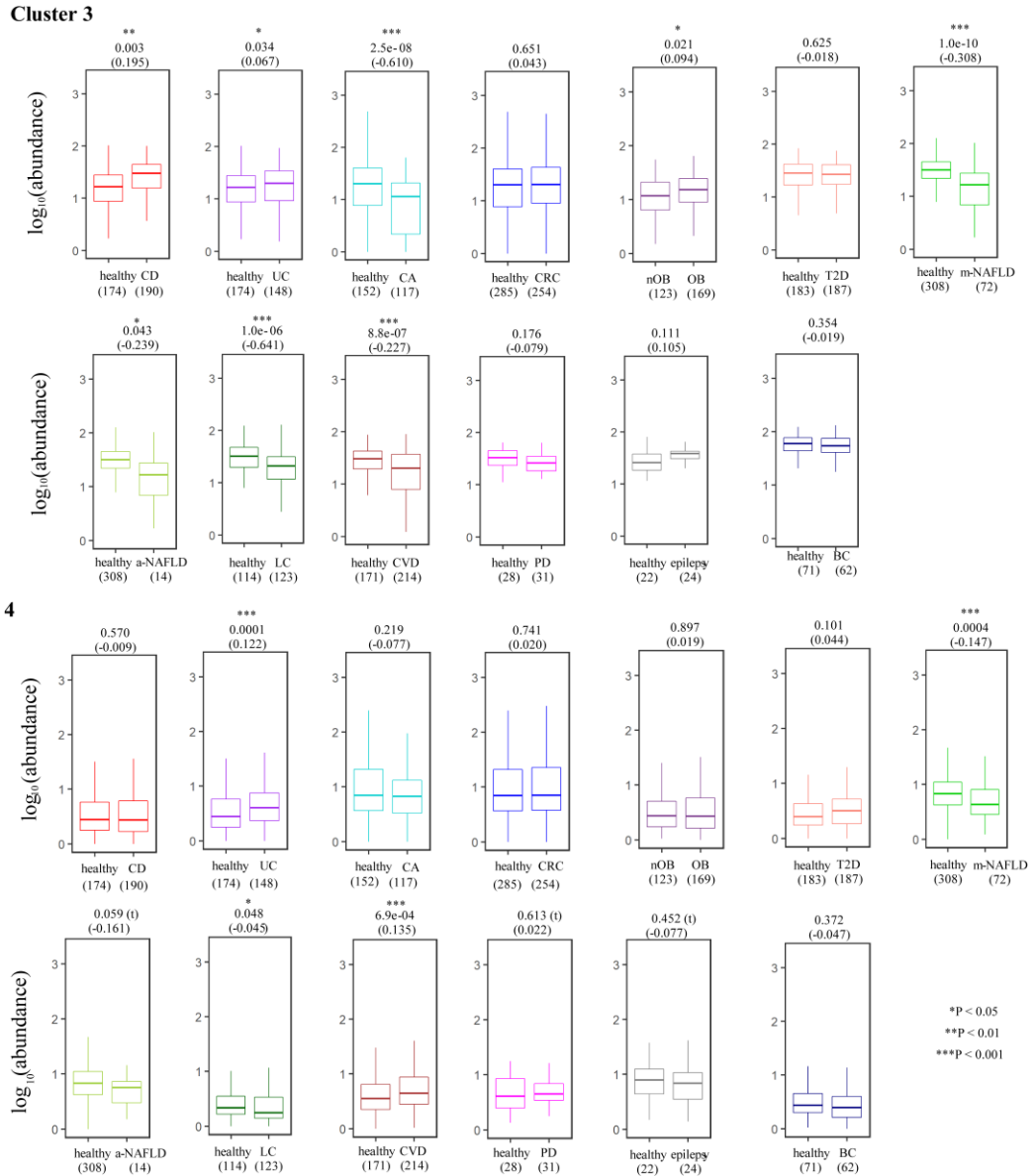
### Cluster 1



### Cluster 2

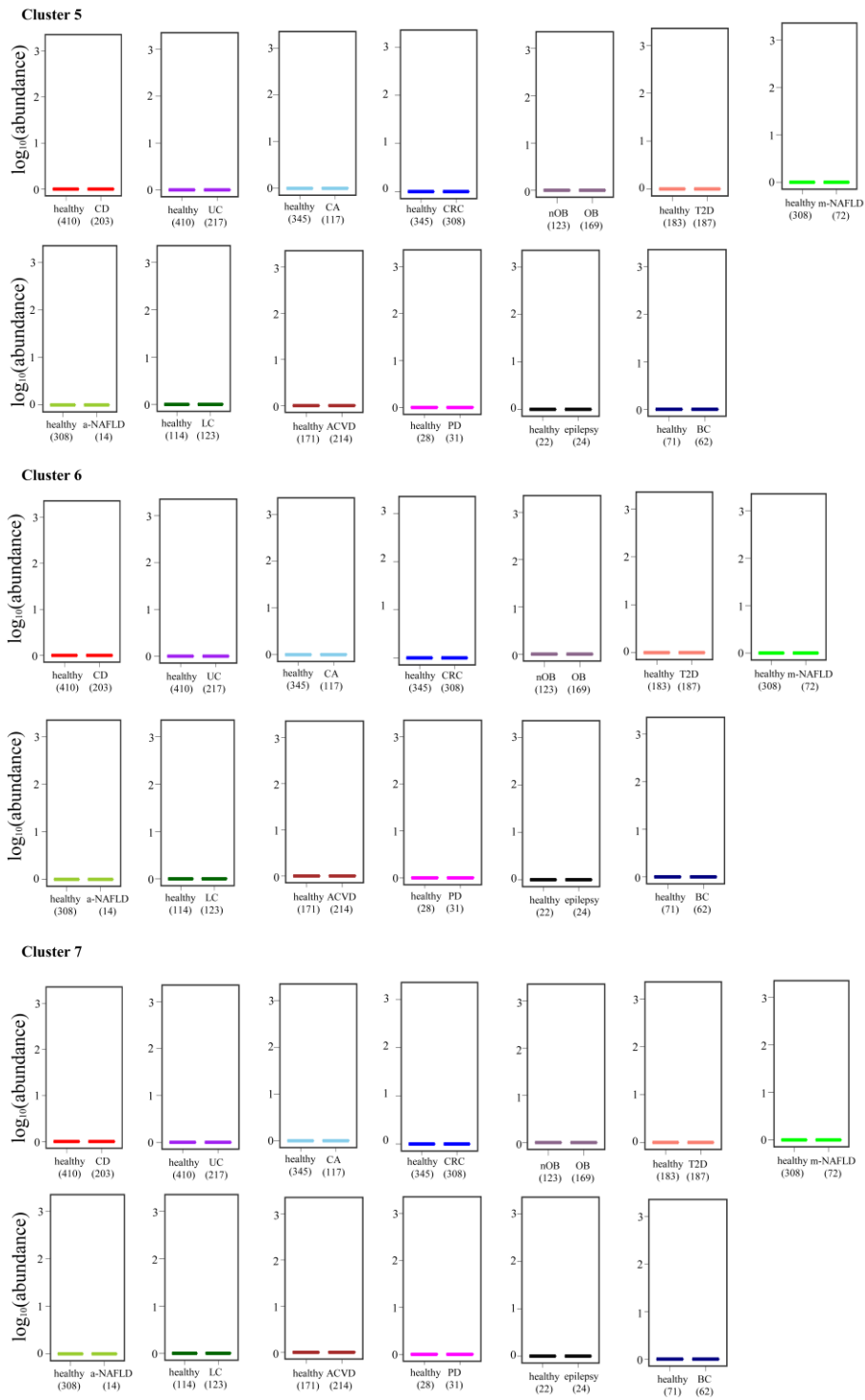


\*P < 0.05  
 \*\*P < 0.01  
 \*\*\*P < 0.001



**Supplementary Figure S6.** Abundance of BSH genes in metagenomic data from controls and individuals with diseases. The sample number of controls and diseases are shown below the box plot. The abbreviations of the diseases are: UC: ulcerative colitis, CD: Crohn's disease, CA: colorectal adenomas, CRC: colorectal cancer, NAFLD: non-alcoholic fatty liver disease, LC: liver cirrhosis, CVD: cardiovascular disease, PD: Parkinson's disease, T2D: Type 2 diabetes, OB: obesity, BC: Breast cancer. The number above the plot shows the generalized fold change. The number above the generalized fold change showed the significant value, which is determined using which is determined using two-sample Wilcoxon rank sum test or two-sample Student's t-test (shown as t after the significant value) as described in Methods.  $*p < 0.05$ ,  $**p < 0.01$ ,  $***p < 0.001$  compared with control.

## Supplementary Figure S7



**Supplementary Figure S7.** Abundance of BSH genes in Cluster 2-7 in metagenomic data from controls and individuals with diseases. The sample number of controls and diseases are shown below the box plot. The abbreviations of the diseases are: UC: ulcerative colitis, CD: Crohn's disease, CA: colorectal adenomas, CRC: colorectal cancer, NAFLD: non-alcoholic fatty liver disease, LC: liver cirrhosis, CVD: cardiovascular disease, PD: Parkinson's disease, T2D: Type 2 diabetes, OB: obesity, BC: Breast cancer. The number above the plot shows the generalized fold change.



## Supplementary Dataset S1: Uniprot ID of protein sequences used to generate sequence similarity network.

Uniprot ID in Cluster 1:

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Uniprot ID in Cluster 5:

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Uniprot ID in Cluster 7:

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## Supplementary Dataset S2: NCBI accession number of metagenomic data used for ShortBRED.

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