Supplementary Figure Legends

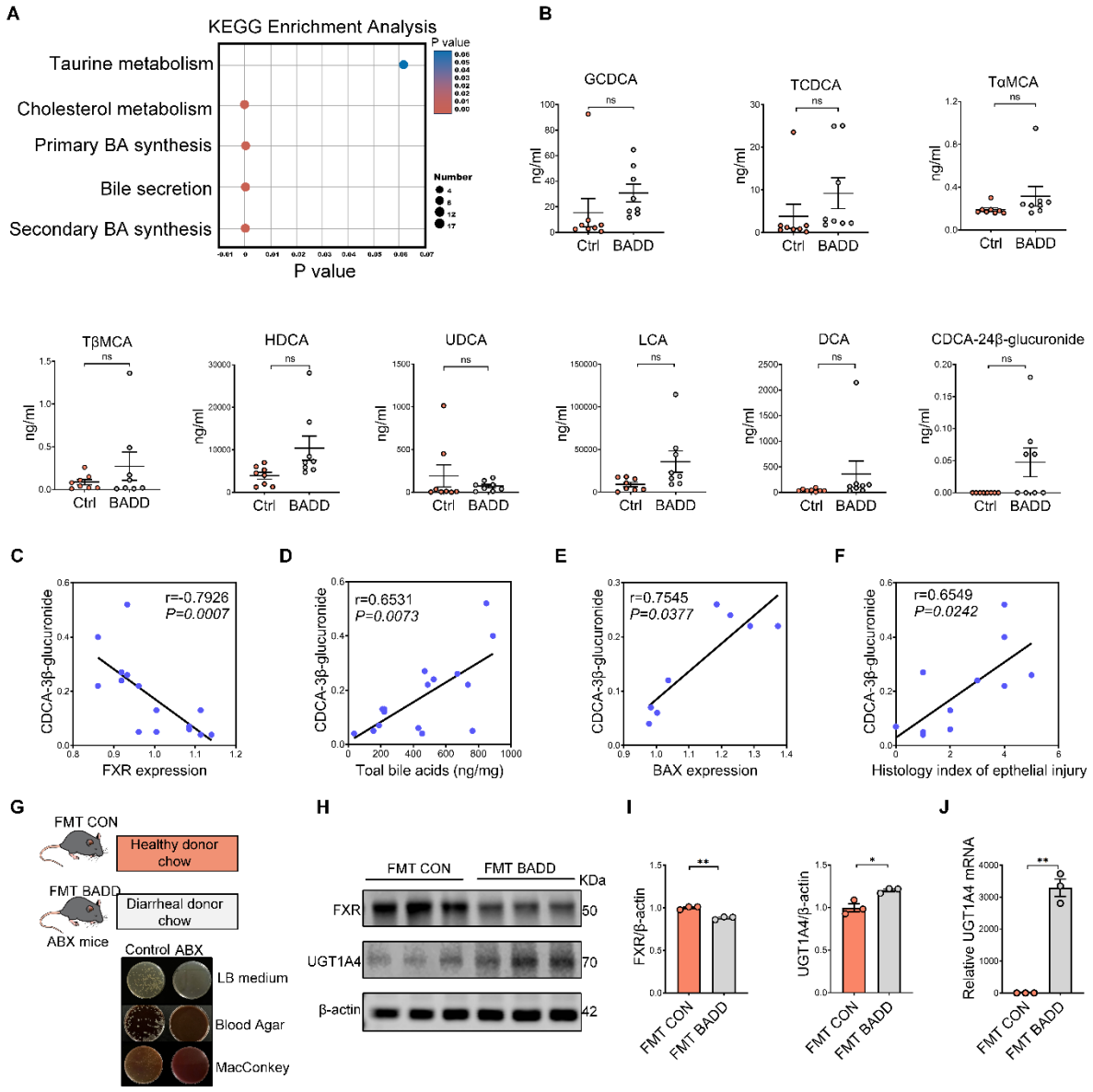

Figure. S1. UGT1A4 promotes CDCA-3β-glucuronide synthesis to cause BA disorder diarrhea, related to Figure 1.

(A) Changes of bile acid metabolism signals were enriched by PIRCUST between healthy pigs and BADD pigs (*n*=*8*). (B) Changes in the concentration of primary bile acids and secondary metabolites produced by CDCA metabolism (*n*=*8*). (C-F) Correlation between CDCA-3β-glucuronide and FXR level, total bile acids level, BAX level, and histology score. (G) Establishment of ABX mice and that were received microbiota transplantation from healthy donors or BADD donors. (H and I) Relative protein expressions of FXR and UGT1A4 in

mice(*n=3*). (J) Relative gene level of *UGT1A4* in the colon of mice (*n=3*). The differences between groups were compared using either student's t-test or two-way analysis of variance (ANOVA) followed by Tukey's test for multiple comparisons, and statistical significance was considered at $P < 0.05$. Data were represented as mean ± SEM, and exhibited $*P < 0.05$, $**P < 0.01$, $***P < 0.001$. Correlation played on spearman two-tailed test in 99% confidence interval.

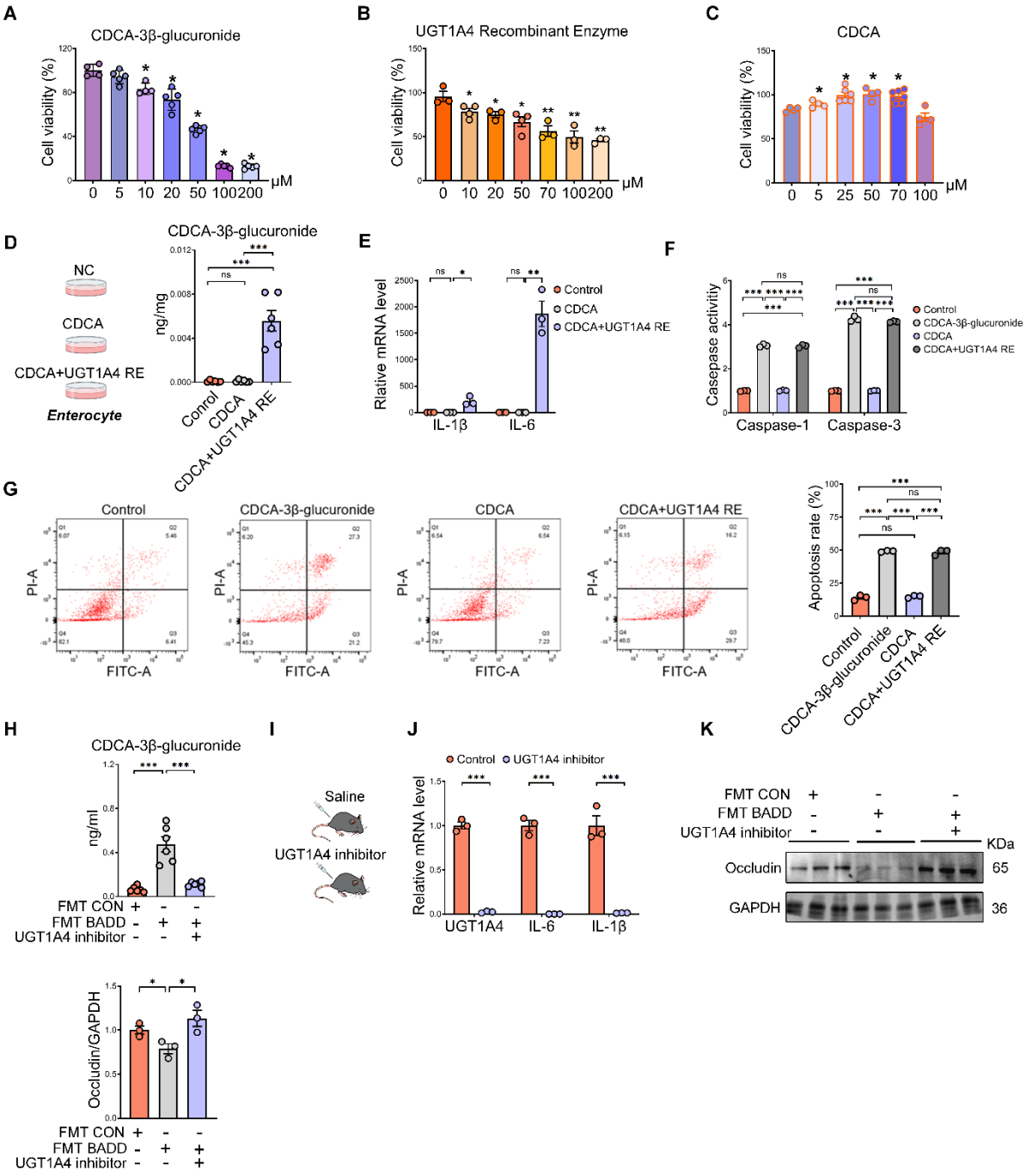

Figure. S2. Effects of UGT1A4 and CDCA-3β-glucuronide on inflammation, cell viability, and gut barrier function, related to Figure 2.

(A)Cell viability of gut epithelial cells after cells received treatment of CDCA-3β-glucuronide (*n=4-5*). (B) Cell viability of gut epithelial cells after cells received treatment of UGT1A4 recombinant enzyme (*n=3-4*). (C) Cell viability of gut epithelial cells after cells received treatment of CDCA (*n=4-6*). (D) Gut epithelial cells were treated by CDCA or CDCA coculture

with UGT1A4 recombinant enzyme, and measured the contents of CDCA-3β-glucuronide. (E) Relative gene levels of *IL1-β* and *IL-6* among control, CDCA, CDCA+UGT1A4 RE groups (*n=3*). (F) Casepase-1 activity and casepase-3 activity of gut epithelium cells were detected in control, CDCA-3β-glucuronide group, CDCA group, and co-treated CDCA and UGT1A4 RE group, respectively (*n=3*), results showed fold of control. (G) Apoptosis rates were measured by flow cytometry (*n=3*). FITC-A means the area of the pulse signal, which is an integral measurement of the fluorescence flux to reflect the sum of the cell fluorescence. (H) Concentration of CDCA-3β-glucuronide was measured in the colonic chyme (*n=3*) (I) A UGT1A4 depletion mouse model. (J) Relative gene levels of *UGT1A4, IL1-β*, and *IL-6* in the colon of mice (*n=3*). (K) Relative protein expression of Occludin (*n=3*). The differences between groups were compared using either student's t-test or two-way analysis of variance (ANOVA) followed by Tukey's test for multiple comparisons, and statistical significance was considered at $P < 0.05$. Data were represented as mean ± SEM, and exhibited $*P < 0.05$, $**P < 0.01$, $***P < 0.001$.

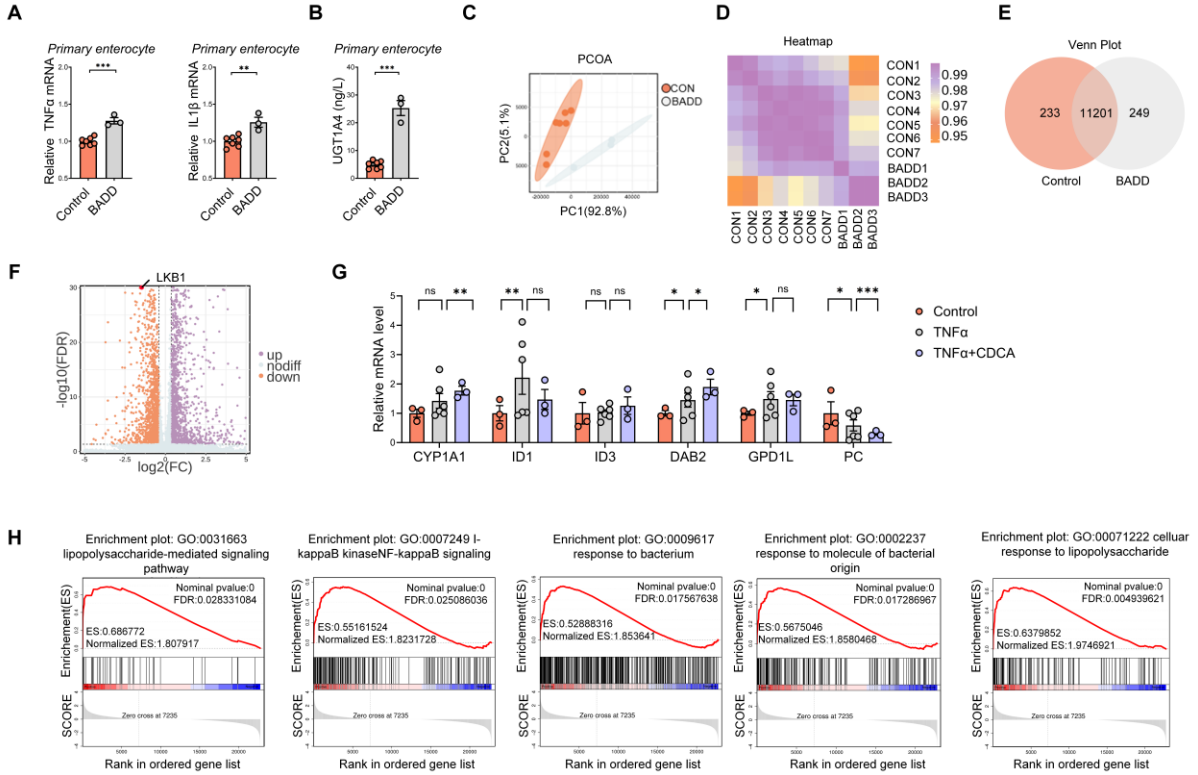

Figure. S3. Differential genes and GO signals are enriched in healthy and BA disorder diarrheal pigs, related to Figure 3.

(A)Relative mRNA of TNFα and IL-1β in primary gut epithelium cells isolated from healthy piglets and BADD piglets (*n=3-7*) (B) Content of UGT1A4 in primary gut epithelium cells isolated from healthy piglets and BADD piglets (*n=3-7*) (C) PCOA plot of genes on bray-curtis dissimilarity matrices in pigs (*n=3-7*). (D) Heatmap plot of genes (*n=3-7*). (E) Venn plot of genes (*n=3-7*). (F) Volcano plot of genes, compared to control group, purple represented up-regulated genes, orange down-regulated genes, and gray represented undifferentiated genes (*n=3-7*). (G) Expect from LKB1, rest differential gene were verified after FXR activation in the inflammation (*n=3-6*). (H) Changes of LPS mediated signaling, NF-κB signaling, signals of response to bacterium, LPS, and molecule of bacterial origin were showed by GO plots (n=3-7). The differences between groups were compared using either student's t-test or two-way analysis of variance (ANOVA) followed by Tukey's test for multiple comparisons, and statistical significance was considered at $P < 0.05$. Data were represented as mean ± SEM, and exhibited $*P < 0.05$, $**P < 0.01$, $***P < 0.001$.

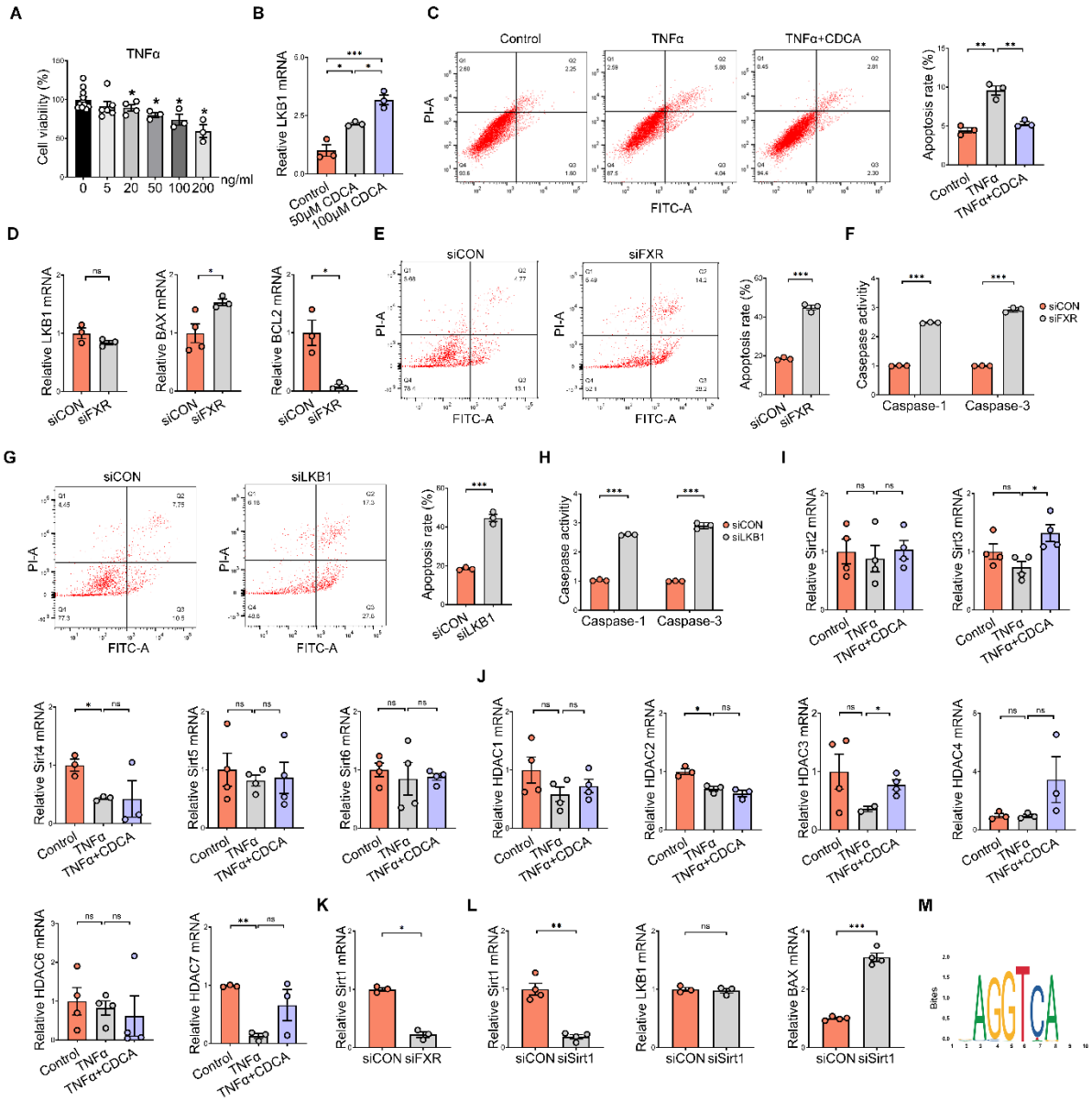

Figure. S4. Effects of FXR activation on changes of Sirtuins, HDACs, LKB1, and apoptosis gene expression, related to Figure 4.

(A) Cell viability of gut epithelial cells after cells received treatment of TNF-α (*n=3-11*). (B) Relative gene level of LKB1 in different concentrations of CDCA (*n=3*). (C) Apoptosis level was detected by flow cytometry (*n=3*), FITC-A means an integral measurement of the fluorescence flux to reflect the sum of the cell fluorescence. (D) Relative gene levels of *LKB1, BAX*, and *BCL2* in treatment of FXR siRNA (*n=3*). (E) Apoptosis level was detected by flow cytometry in the siCON group and siFXR group (*n=3*). (F) Activities of caspase-1 and caspase-

3 in the siCON group and siFXR group (*n=3*), results showed fold of control. (G) Apoptosis level was detected by flow cytometry in the siCON group and siLKB1 group (*n=3*). (H) Activities of caspase-1 and caspase-3 in the siCON group and siLKB1 group (*n=3*), results showed fold of control. (I and J) Relative gene levels of *Sirtuins* and *HDACs* families associated with bile acids (*n=3-4*). (K) Relative gene level of *SIRT1* in treatment of FXR siRNA (*n=3*). (L) Relative gene levels of *SIRT1, LKB1*, and *BAX* in treatment of SIRT1 siRNA (*n=3*). (M) FXR and SIRT1 DNA promoter binding region was predicted by JASPER. The differences between groups were compared using either student's t-test or two-way analysis of variance (ANOVA) followed by Tukey's test for multiple comparisons, and statistical significance was considered at $P < 0.05$. Data were represented as mean ± SEM, and exhibited $*P < 0.05$, $**P < 0.01$, $***P < 0.001$.

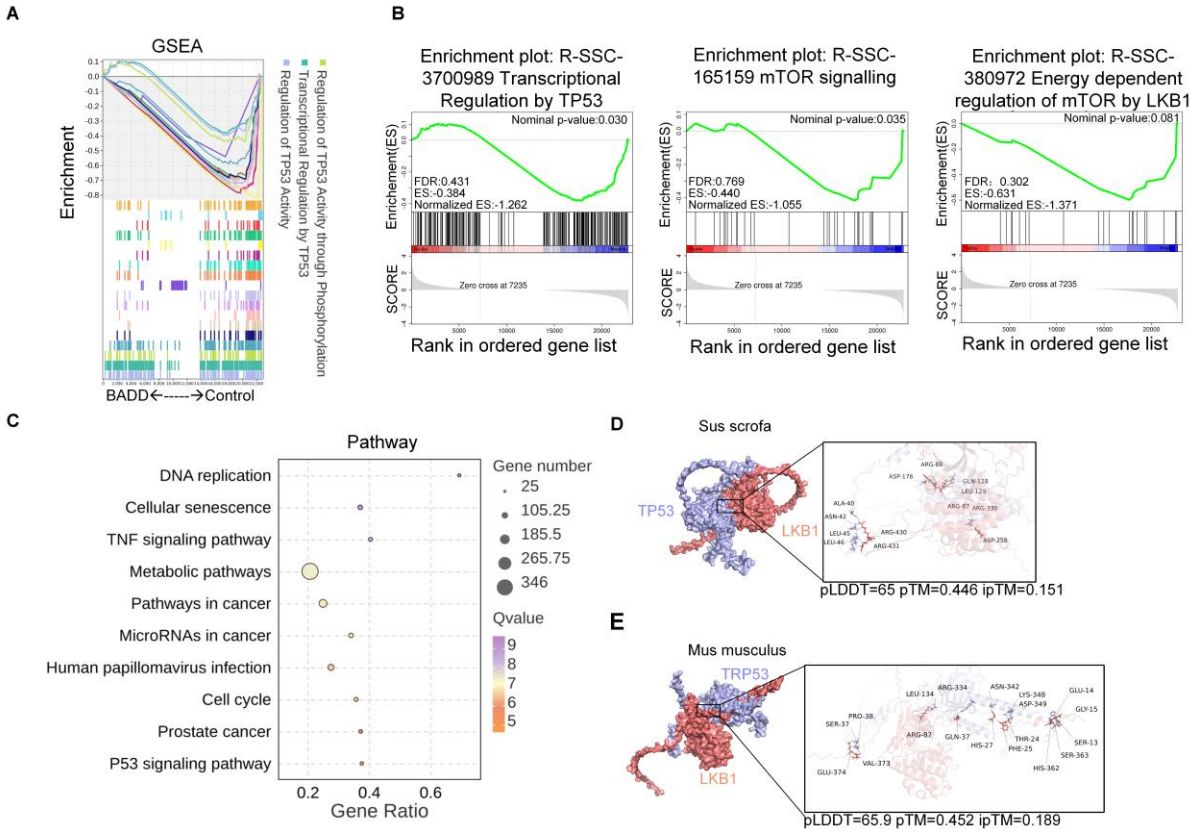

Figure. S5. Interactions between proteins of LKB1 and P53 are predicted, related to Figure5.

(A and B) GSEA enriched signals of LKB1 (*n=3-7*). (C) Enrichment of GO signal pathways (*n=3-7*). (D) Docking of LKB1 and TP53 was predicted in pigs. (E) Docking of LKB1 and TPR53 was predicted in mice. Data were analyzed by transcriptome sequencing (A to C).

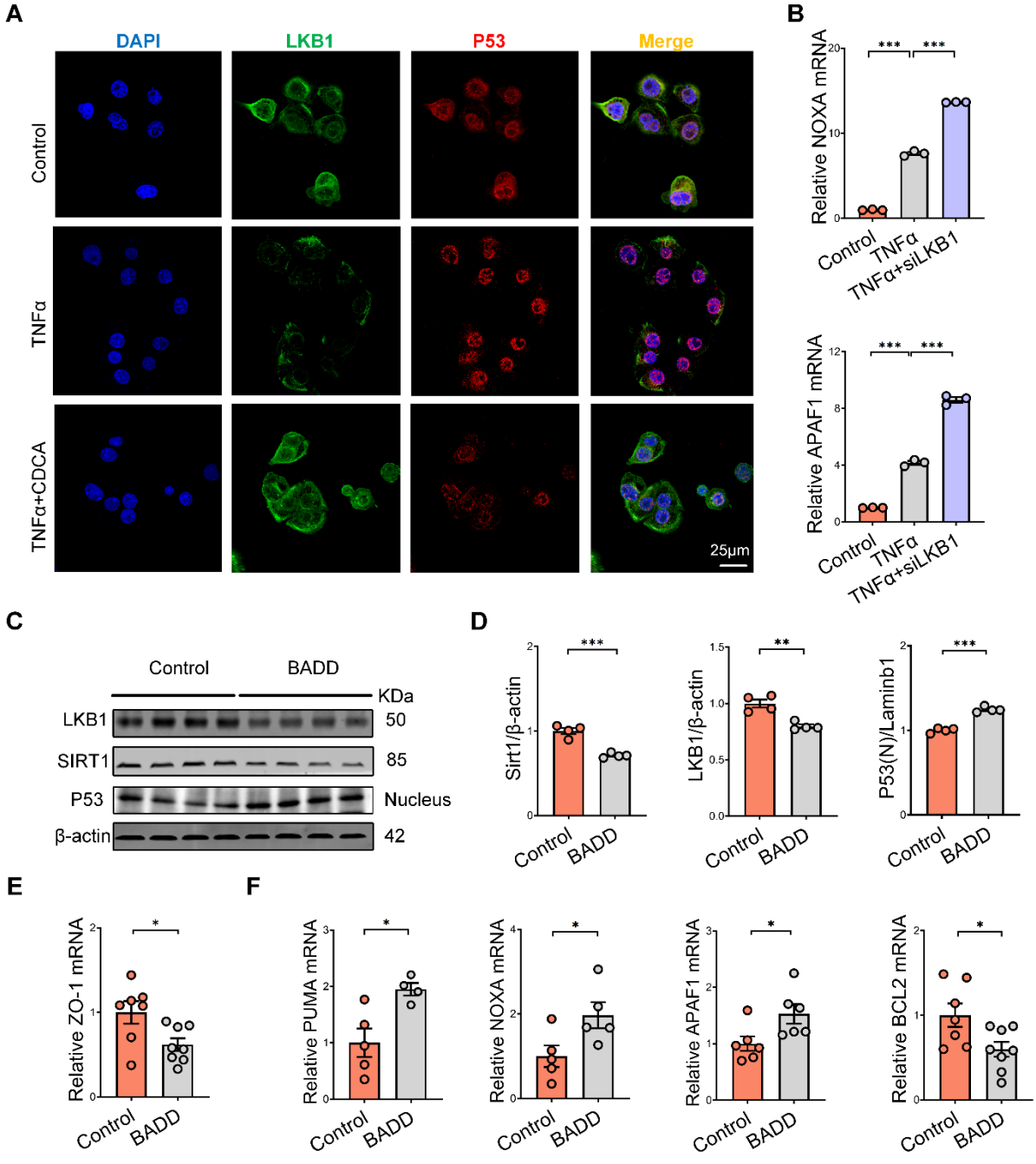

Figure. S6. Apoptosis associated genes regulated by P53 are measured, related to Figure5. (A) Co-immunofluorescence stain of LKB1 and P53 showed LKB1 and P53 both location in the cells. Scale bars, 25 μm. Green fluorescence is LKB1. Red fluorescence is P53. (B) Relative

gene levels of *NOXA* and *APAF-1* (*n=3*). (C and D) Relative protein expressions of SIRT1, LKB1, P53 of nucleus in the colon (*n=4*). (E) Relative gene levels of *ZO-1* (*n=3*). (F) Relative gene levels of *PUMA, NOXA, APAF-1,* and *BCL2* in the colon (*n=4-8*). The differences between groups were compared using either student's t-test or two-way analysis of variance (ANOVA) followed by Tukey's test for multiple comparisons, and statistical significance was considered at $P < 0.05$. Data were represented as mean ± SEM, and exhibited $*P < 0.05$, $**P < 0.01$, $***P < 0.001$.

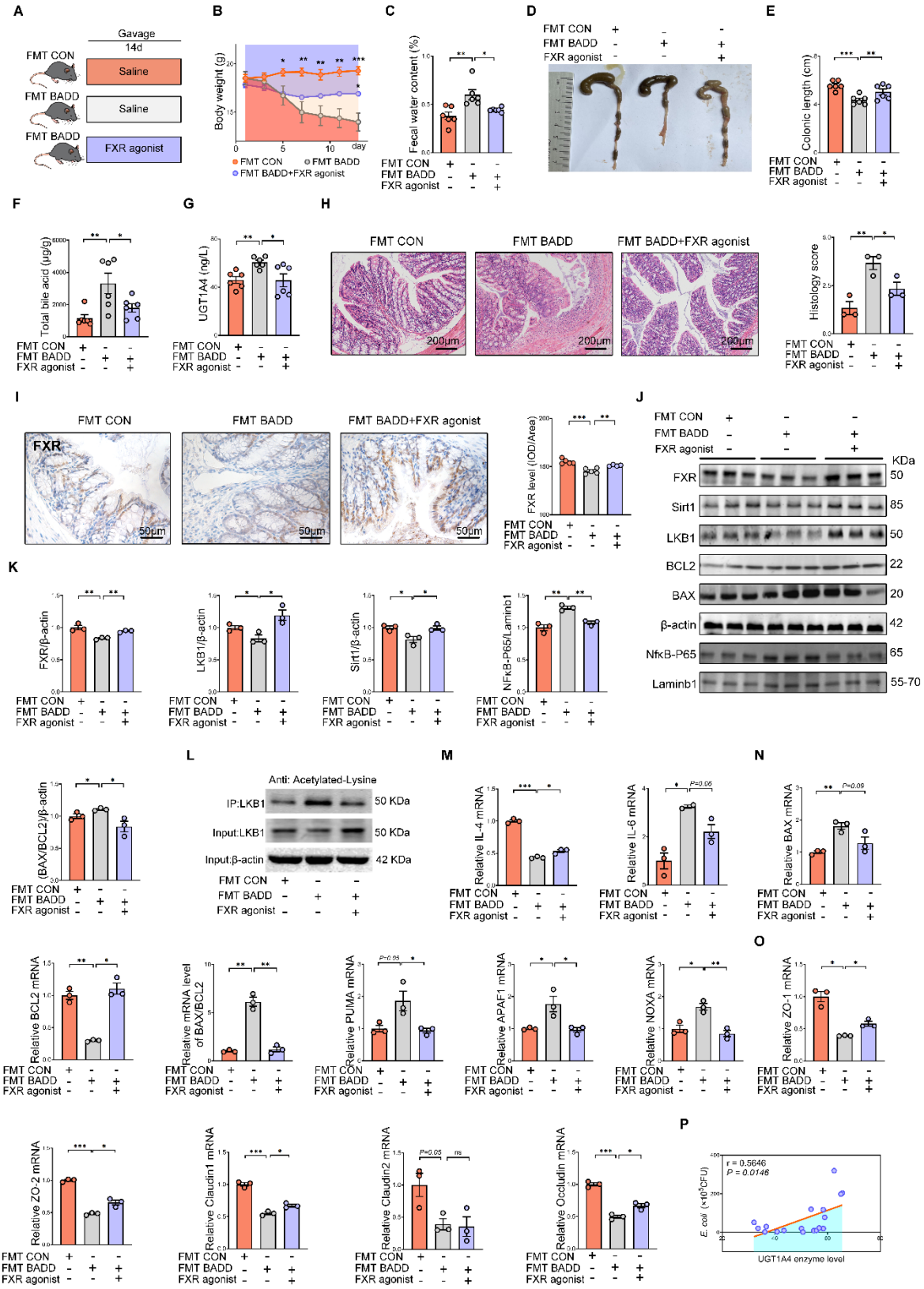

Figure. S7. Microbiota transplantation induced BA metabolism disorders are ameliorated by FXR activation, related to Figure 6.

(A) ABX mice after microbiota transplantation from healthy and BADD donors, were received oral gavage by FXR agonist. (B) Changes of body weight of mice in 14 days (*n=6*). (C) Faecal water index (*n=6*). (D and E) Colonic lengthen statistics (*n=6*). (F) Total bile acid contents in the faeces (*n=6*). (G) UGT1A4 enzyme content in the colon (*n=6*). (H) Histological staining and score (*n=3*). Scale bars, 100 μm. (I) Location and expression of FXR were measured by immunohistochemistry (*n=3*). Scale bars, 50 μm. (J and K) Relative protein expressions of FXR, SIRT1, LKB1, BAX/BCL2, and NFκB-P65 in the colon (*n=3*). (L) Acetylation-IP was used to measure acetylated LKB1 protein in colonic tissue of mice. (M) Relative gene levels of *interleukin 4*(*IL-4*) and *IL-6* (*n=3)*. (N) Relative gene levels of *BAX*, *BCL2*, *BAX/BCL2*, *PUMA*, *APAF-1*, *NOXA* (*n=3*). (O) Relative gene levels of *ZO-1*, *ZO-2*, *Claudin-1*, *Clauin-2*, *Occludin* (*n=3*). (P) Correlation between counts of faecal *E. coli* and UGT1A4 level (*n=6*). The differences between groups were compared using either student's t-test or two-way analysis of variance (ANOVA) followed by Tukey's test for multiple comparisons, and statistical significance was considered at $P < 0.05$. Data were represented as mean ± SEM, and exhibited $*P < 0.05$, $**P < 0.01$, $***P < 0.001$. Correlation played on spearman two-tailed test in 99% confidence interval.

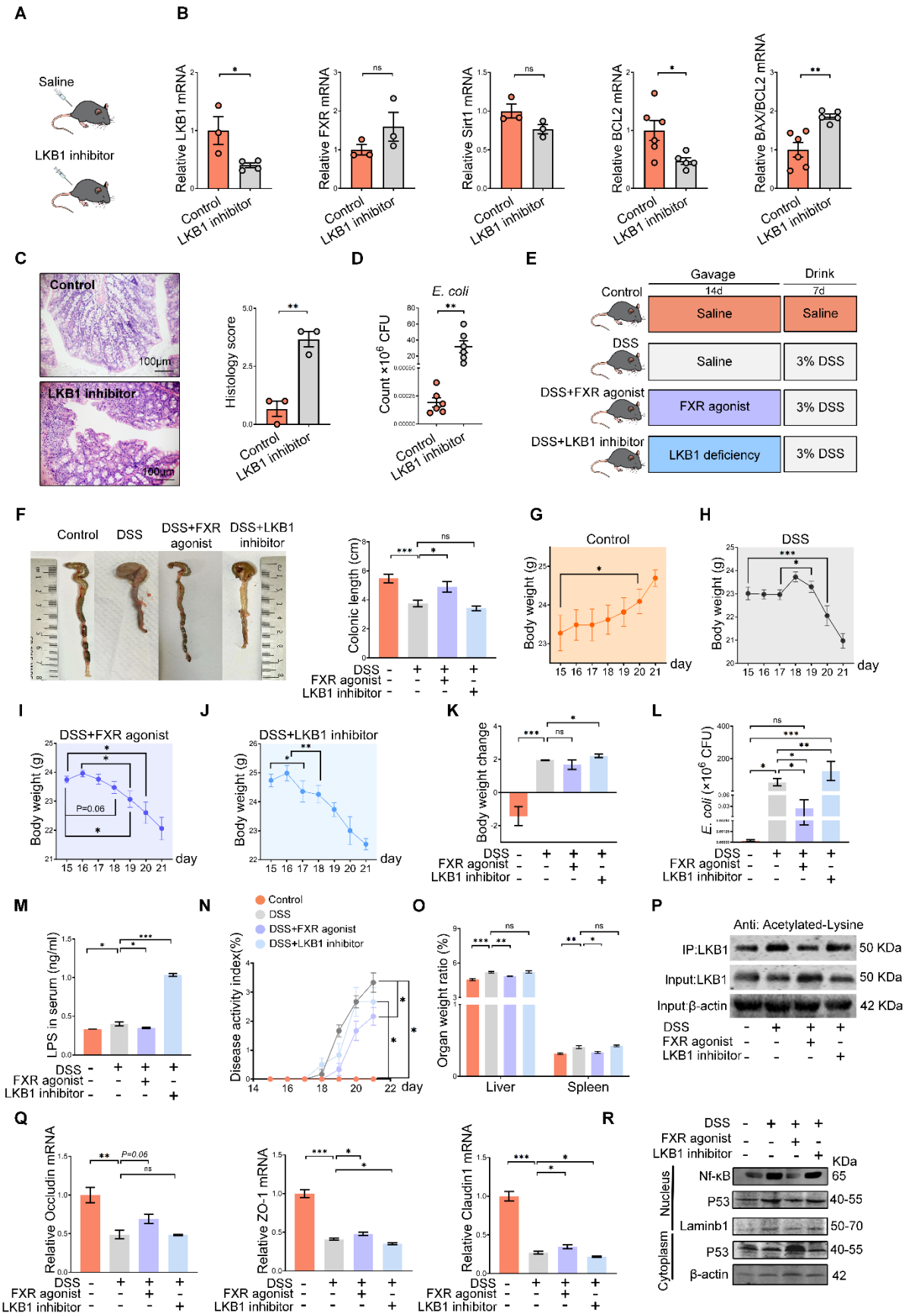
A
Saline
LKB1 inhibitor
B
Relative LKB1 mRNA
Relative FXR mRNA
Relative Sirt1 mRNA
Relative BCL2 mRNA
Relative BAX/BCL2 mRNA
Control
LKB1 inhibitor
C
Control
LKB1 inhibitor
100μm
Histology score
D
E. coli
Count ×10^6 CFU
E
Gavage
Drink
14d
7d
Control
Saline
Saline
DSS
Saline
3% DSS
DSS+FXR agonist
FXR agonist
3% DSS
DSS+LKB1 inhibitor
LKB1 deficiency
3% DSS
F
Control
DSS
DSS+FXR agonist
DSS+LKB1 inhibitor
Colonic length (cm)
DSS
FXR agonist
LKB1 inhibitor
G
Control
Body weight (g)
day
H
DSS
I
DSS+FXR agonist
P=0.06
J
DSS+LKB1 inhibitor
K
Body weight change
L
E. coli (×10^6 CFU)
M
LPS in serum (ng/ml)
N
Control
DSS
DSS+FXR agonist
DSS+LKB1 inhibitor
Disease activity index(%)
O
Organ weight ratio (%)
Liver
Spleen
P
Anti: Acetylated-Lysine
IP:LKB1
Input:LKB1
Input:β-actin
50 KDa
50 KDa
42 KDa
Q
Relative Occludin mRNA
Relative ZO-1 mRNA
Relative Claudin1 mRNA
R
KDa
Nucleus
Nf-κB
65
P53
40-55
Laminb1
50-70
Cytoplasm
P53
40-55
β-actin
42

Figure. S8. DSS induced UGT1A4-BA disorders is alleviated by FXR-SIRT1-LKB1 axis, related to Figure 6.

(A)LKB1 depletion mouse model. (B)Relative gene levels of *LKB1*, *FXR*, *SIRT1*, *BCL2*, and *BAX/BCL2* (*n=3*). (C) Histological staining and score (*n=3*). (D) Abundance of *E. coli* in the faeces. (E) DSS mouse model in treatment of FXR agonist and LKB1 inhibitor. (F) Colonic lengthen count (*n=6*). (G-J) Changes of body weight after DSS challenge (*n=6*). (K) Body weight change from 15d to 21d (*n=6*). (L) Abundance of *E. coli* in the faeces. (M) LPS concentration in the serum (*n=6*). (N) Disease activity index (*n=6*). DAI of DSS-treated mice and DSS+LKB1 inhibitor mice occurred significant differences compared to control group from 18d-21d. DAI of DSS+FXR agonist mice occurred significant decrease compared to DSS group from 19d-21d. (O) Organ weight ratio of liver and spleen (*n=6*). (P) Acetylation level of LKB1 protein *in vivo*. (Q) P53 protein expression *in vivo*. (R) Relative gene levels of *Occludin*, *ZO-1*, *Claudin-1* in the colon (*n=3*). The differences between groups were compared using either student's t-test or two-way analysis of variance (ANOVA) followed by Tukey's test for multiple comparisons, and statistical significance was considered at $P < 0.05$. Data were represented as mean ± SEM, and exhibited $*P < 0.05$, $**P < 0.01$, $***P < 0.001$.

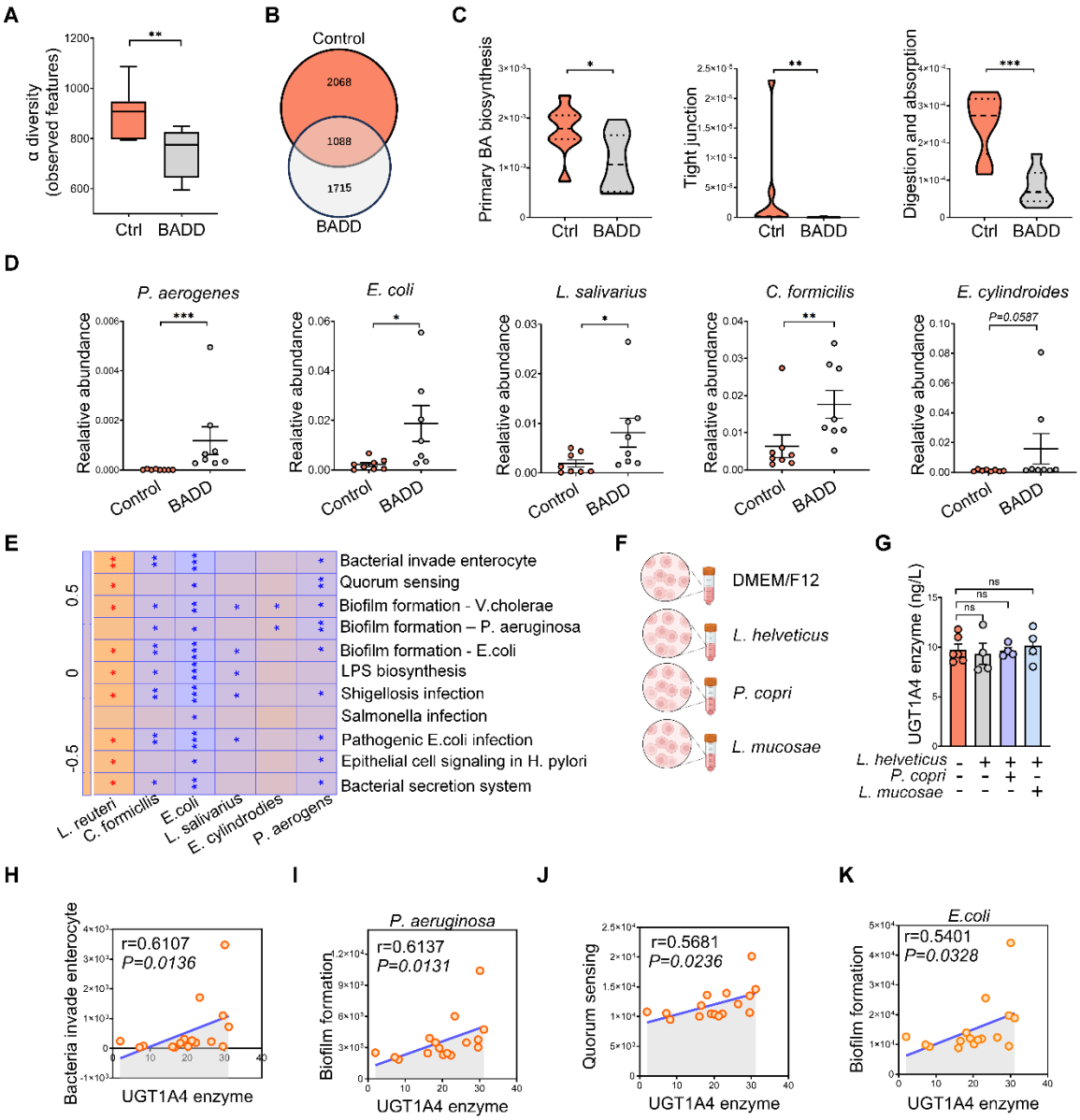

Figure. S9. Relationship between microbiota and UGT1A4 content in BA disorder induced diarrhea, related to Figure 7.

(A) Observed feature index of α diversity (*n=8*). (B) Venn plot (n=8). (C) Enrichment of metabolic signaling pathway (*n=8*). (D) Relative abundance of *P. aerogenes*, *E. coli*, *L. salivarius*, *C. formicilis*, and *E. cylindroides* (*n=8*). (E) Heatmap of correlation, red * represents negative relation, blue* represents positive relation (*n=8*). (F and G) *L. helveticus*, *P. copri*, and *L. mucosae* in treatment of cell to measure UGT1A4 level, respectively (*n=4*). (H) Correlation between bacterial signaling pathways and level of UGT1A4 enzyme (*n=8*). The

differences between groups were compared using either student's t-test or two-way analysis of variance (ANOVA) followed by Tukey's test for multiple comparisons, and statistical significance was considered at $P < 0.05$. Data were represented as mean ± SEM, and exhibited $*P < 0.05$, $**P < 0.01$, $***P < 0.001$. Correlation played on spearman two-tailed test in 90 % confidence interval.

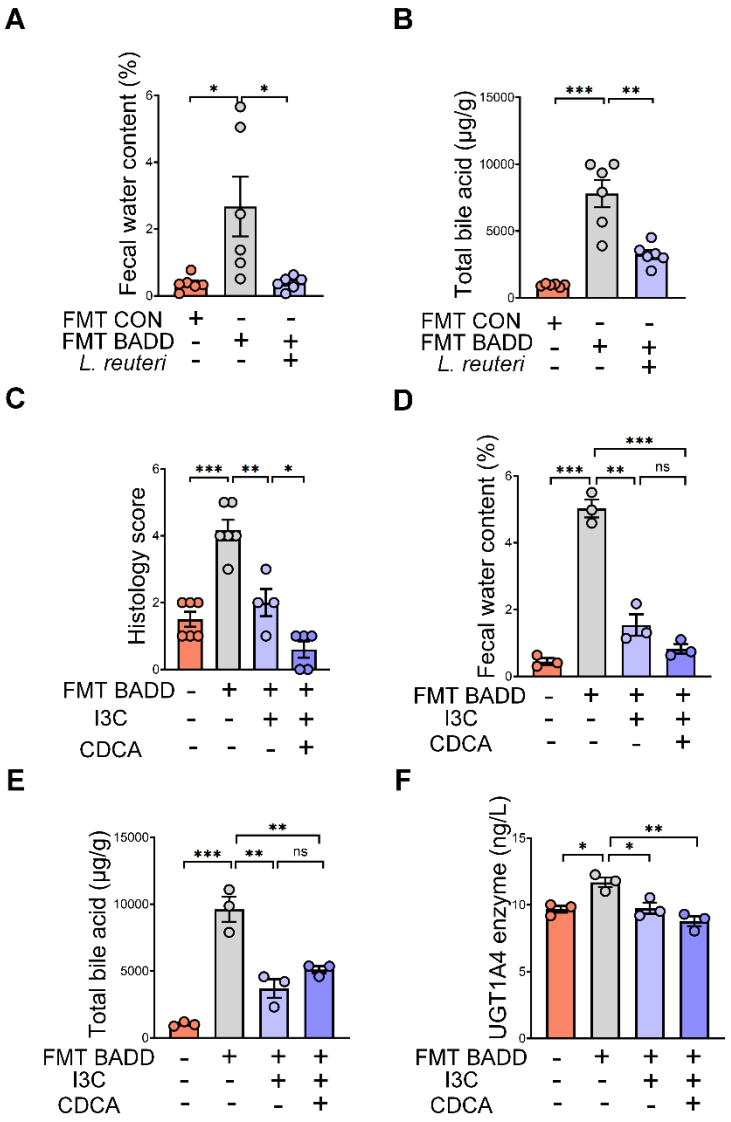

Figure. S10. *L. reuteri* and it derived I3C prevent CDCA glucuronidation induced diarrhea, related to Figure 8.

(A) Faecal water content in FMT mice received *L. reuteri* orally gavage ($n=6$). (B) Total bile acid content in FMT mice received *L. reuteri* orally gavage ($n=6$). (C) Histological score of

colonic tissues (*n=4-6*). (D) Faecal water content in FMT mice received I3C and CDCA orally gavage (*n=3*). (E) Total bile acid content in FMT mice received I3C and CDCA orally gavage (*n=3*). (F) Level of UGT1A4 enzyme in FMT mice received I3C and CDCA orally gavage (*n=3*). The differences between groups were compared using either student's t-test or two-way analysis of variance (ANOVA) followed by Tukey's test for multiple comparisons, and statistical significance was considered at $P < 0.05$. Data were represented as mean ± SEM, and exhibited $*P < 0.05$, $**P < 0.01$, $***P < 0.001$.

Table. S1. Fecal total bile acid content and *E. coli* number of pigs.

Sample	Total bile acid (ng/mg)	*E. coli* abundance (CFU/g)
Control1	2.10×10^{4}	7.04×10^{6}
Control2	0.36×10^{4}	3.20×10^{6}
Control3	1.55×10^{4}	1.24×10^{6}
Control4	4.31×10^{4}	0.56×10^{6}
Control5	4.54×10^{4}	0.88×10^{6}
Control6	2.21×10^{4}	3.20×10^{6}
Control7	2.21×10^{4}	2.48×10^{6}
Control8	1.89×10^{4}	0.72×10^{6}
Diarrhea1	4.88×10^{4}	4.80×10^{10}
Diarrhea2	6.70×10^{4}	5.60×10^{10}
Diarrhea3	7.35×10^{4}	1.04×10^{10}
Diarrhea4	5.25×10^{4}	4.88×10^{10}
Diarrhea5	8.99×10^{4}	0.96×10^{10}
Diarrhea6	8.48×10^{4}	3.84×10^{10}
Diarrhea7	7.63×10^{4}	2.00×10^{10}
Diarrhea8	4.7×10^{4}	3.04×10^{10}

159 **Table. S2. Primers for qRT-PCR.**

	Forward Primer (5'-3')	Reverse Primer (5'-3')
M-FXR	GGCACTCCCATTTACAGGCT	CAACACACAGCTCATCCCCT
M-SIRT1	CGGCTACCGAGGTCCATATAC	AACATGGCTTGAGGGTCTGG
M-LKB1	ACACCTTCATCCACCGCAT	ACCTCCTTCACCTTGCCGTA
M-TNFα	CCACGCTCTTCTGTCTACTG	ACTTGGTGGTTTGCTACGA
M-IL6	GAGCCCACCAAGAACGATA	TTGTCACCAGCATCAGTCC
M-IL1β	CCTTCCAGGATGAGGACATGA	TGAGTCACAGAGGATGGGCTC
M-BAX	GGTTGCCCTCTTCTACTTTGC	AGCCACCCTGGTCTTGGAT
M-BCL2	TCATTTCCCATCCCGCTGT	GGTCCTTGTCTCATTATCCCA
M-PUMA	CATCGACACCGACCCTCAC	CGGATTTCTCCATTGTCATCTCCA
M-APAF1	GATCCACACAGGCCATCACA	GGAGCCATCGGGAGAAAACA
M-NOXA	GTTCGCAGCTCAACTCAGGA	ACCACAGTTATGTCCGGTGC
M-CHIP-SIRT1	CCTGCACTTATGCTTTTCTGGA	CAGGATCTTCTAGCTCCAGGC
M-UGT1A4(5)	CCCTACAAGGATTAGTGGGGC	CGAGTACAAAGTCCCCTCGG
M-ZO1	CTCAAGTTCCTGAAGCCCGT	CGACGAGGAGTCGGATGATT
M-ZO2	CCCATCCAGAGACCCGCTAT	CTCCCTTCTTGAACCTCACCA
M-Claudin1	AGCTGTGCATGGCCTCTTGT	CCAATGTCAATGGCAACACCCT
M-Claudin2	AACAAGTTCTTATGTCGGTGC	TGGTAGGCATCGTAGTAGTTG
M-Occludin	TGGCAAAGTGAATGGCAAGC	TCATAGTGGTCAGGGTCCGT
P-FXR	GATGAGCATGAAGCCTGCCA	AAACCTTTGCACCCCTCACA
P-SIRT1	GCTCCGCAAGAAACCGC	TTGTTCGAGGATCTGTGCCAA
P-LKB1	CGCTCTACAACATCACAACAG	GCATTCCTCTCAGCAGGTCT
P-SIRT2	CGGACACTGAGGAAGGAGCA	TTGTTGATGAGCAGGCGTG
P-SIRT3	CGTTCATCTGTTGCTGCCCT	GTTCTGGGTGTAGAGCCGCA
P-SIRT4	TAGTGATGACTGGAGCAGGAA	GCCAAGTTATCCGACCGT
P-SIRT5	GCCGATTACTTTCCCAGC	GTTCTCTCCAAACCACACGAC
P-SIRT6	GTCTGGACAATGGAGGAGC	GGCTGAAGGTTGACAATGA
P-HDAC1	TGACGAGTCCTATGAGGCCA	CAAACTCCACACACTTGGCG
P-HDAC2	GCTTTCAACTGGTGGCTC	TTCTGGAGTGTTCTGGTTTG
P-HDAC3	GCTTGGCCTTGACCCATAGT	GGGCACATTGAGGCAGTAGT
P-HDAC4	CTCCACGAGCACATCAAA	TTCTGAGCGGAAAGTCATC
P-HDAC6	TAGCGGATGTAAAGAAGAAAGGCA	TCATAGCGGTGGATGGAGAAA
P-HDAC7	AACGGTTTTGCTGTGGTTCG	ACATTTGGCGGAGACATGGT
P-IL6	GGAGACCTGCTTGATGAGA	GTACTAATCTGCACAGCCTC
P-IL1β	GGAGACCTGCTTGATGAGA	GTACTAATCTGCACAGCCTC
P-BAX	AGAAGTTGAGCGAGTGTCTC	GCCGTCAGCAAACATTTCG
P-BCL2	GGGAGGATTGTGGCCTTCTT	CTGGGCCCATACAGCTCCAC
P-NOXA	ACACTCAGACGAACCCTACG	AGGCGGAAGAGTTTGGCTAT
P-PUMA	GGAGACAAGAGGAGCAGCAG	CTGGGTAAGGGCAGAAGTCC
P-APAF1	CCATCACAGCACCATCCAGT	CACTTGGGCTTCCGTCAGAT
P-CYP1A1	CCTCCTTCGTTCCCTTCACC	CAGTGCCTTGTTGATGGTGC
P-ID1	ACCCTCAACGGCGAAATCAG	TCAGCGACACAAGATGCGA

P-ID3	CACTGCTACTCGCGTCTCC	ATGTAGTCGATGACGCGCTG
P-DAB2	TCTGTCCAGTCCTCACCACA	AGCAGGGATAACTGGCACAC
P-PC	ATGGAGCGCGTGTTTGACTA	TTTGATGTGCAGCGTCTTGC
P-GDP1L	GTTTGTCATCCCCCACCAGT	TGGTCTCACAGAACTTGCCG
P-ZO1	GCCATCCACTCCTGCCTAT	CGGGACCTGCTCATAACTTC

160 M, mouse; P, pig.

161

Table. S3. Criteria of disease activity index (DAI) [56].

Score	Body weight loss (%)	Fecal viscosity	Degree of faecal blood
0	0%	Normal	None
1	1-5%	/	/
2	5-10%	Sticky	Occult blood positive
3	10-20%	/	/
4	>20%	Diarrheal	Revealed hemorrhage