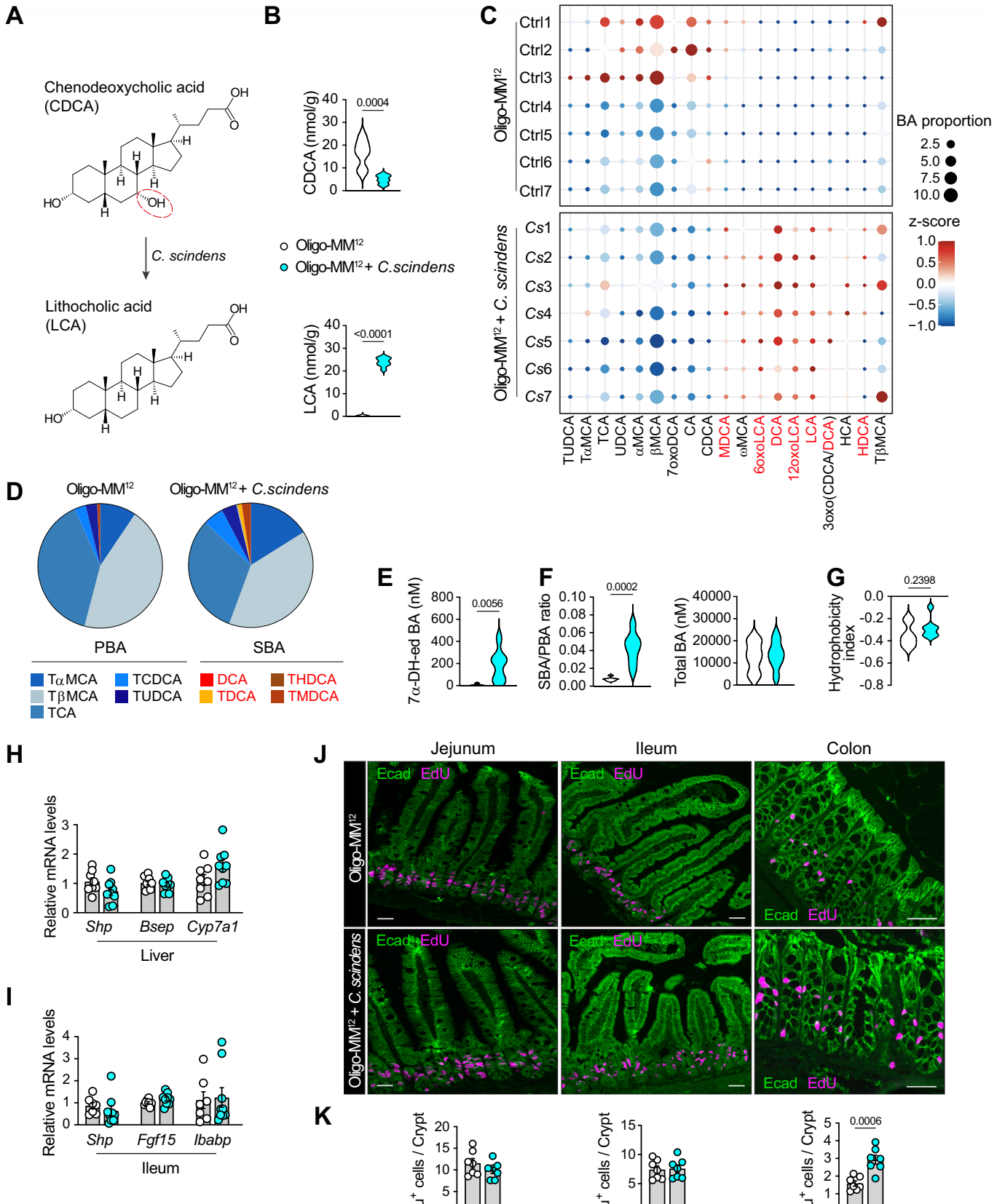
Expanded View Figures

Figure EV1. Stable colonization of Oligo-MM^{12} mice with *C. scindens* modulates the BA composition and promotes proliferation of colonic stem cells under basal conditions.

(**A**) Schematic illustrating the 7α-dehydroxylation reaction at carbon 7 (highlighted in red) of CDCA to form LCA. (**B**) Violin plots showing the amount of the indicated BA species in the feces of 8-week-old male Oligo-MM^{12} mice gavaged with live *C. scindens* (10^7 CFU – Oligo-MM^{12} + *C. scindens*) or PBS (control – Oligo-MM^{12}) under basal conditions ($n = 7$/group) (For LCA (nmol/g): Oligo-MM^{12} vs Oligo-MM^{12} + *C. scindens* $P < 0.0001$). (**C**) Dot plots representing the BA proportion and the z score in stools of mice in (**B**). The dot color represents the z-score for each BA species, whereas the dot size represents the proportion of each BA species over the total BA amount per mouse. 7α-dehydroxylated BAs are highlighted in red. Ctrl = Oligo-MM^{12} and *Cs* = Oligo-MM^{12} + *C. scindens*. (**D**) Plasma BA composition of mice in (**B**). BA data are normalized to the total amount of BAs. 7α-dehydroxylated BAs are in red. (**E**, **F**) Concentrations (nM) of the indicated BAs and secondary (SBA)-to-primary (PBA) ratio in plasma of mice in (**B**). 7α-DH-ed: 7α-dehydroxylated BAs. (**G**) BA hydrophobicity index in plasma of mice in (**B**). (**H**, **I**) Relative mRNA levels of the indicated genes in liver (**H**) or ileum (**I**) of mice in (**B**). (**J**, **K**) Representative images (**J**) and quantification of EdU$^+$ cells per crypt (**K**) in the jejunum, ileum, and colon of mice in (**B**). Graphs represent mean ± SEM. *n* refers to biological replicates. *P* values (exact values) were calculated using 2-tailed Student's t test (**B**, **E**, **F**, **G**, **K**).


A
Chenodeoxycholic acid (CDCA)
C. scindens
Lithocholic acid (LCA)
B
CDCA (nmol/g)
0.0004
Oligo-MM12
Oligo-MM12+ C.scindens
LCA (nmol/g)
<0.0001
C
Oligo-MM12
Ctrl1
Ctrl2
Ctrl3
Ctrl4
Ctrl5
Ctrl6
Ctrl7
Oligo-MM12 + C. scindens
Cs1
Cs2
Cs3
Cs4
Cs5
Cs6
Cs7
TUDCA
TαMCA
TCA
UDCA
αMCA
βMCA
7oxoDCA
CA
CDCA
MDCA
ωMCA
6oxoLCA
DCA
12oxoLCA
LCA
3oxo(CDCA/DCA)
HCA
HDCA
TβMCA
BA proportion
2.5
5.0
7.5
10.0
z-score
1.0
0.5
0.0
−0.5
−1.0
D
Oligo-MM12
Oligo-MM12+ C.scindens
PBA
SBA
TαMCA
TβMCA
TCA
TCDCA
TUDCA
DCA
TDCA
THDCA
TMDCA
E
7α-DH-ed BA (nM)
0.0056
F
SBA/PBA ratio
0.0002
Total BA (nM)
G
Hydrophobicity index
0.2398
H
Relative mRNA levels
Shp
Bsep
Cyp7a1
Liver
I
Relative mRNA levels
Shp
Fgf15
Ibabp
Ileum
J
Jejunum
Ileum
Colon
Oligo-MM12
Oligo-MM12 + C. scindens
Ecad EdU
K
Edu+ cells / Crypt
0.0006

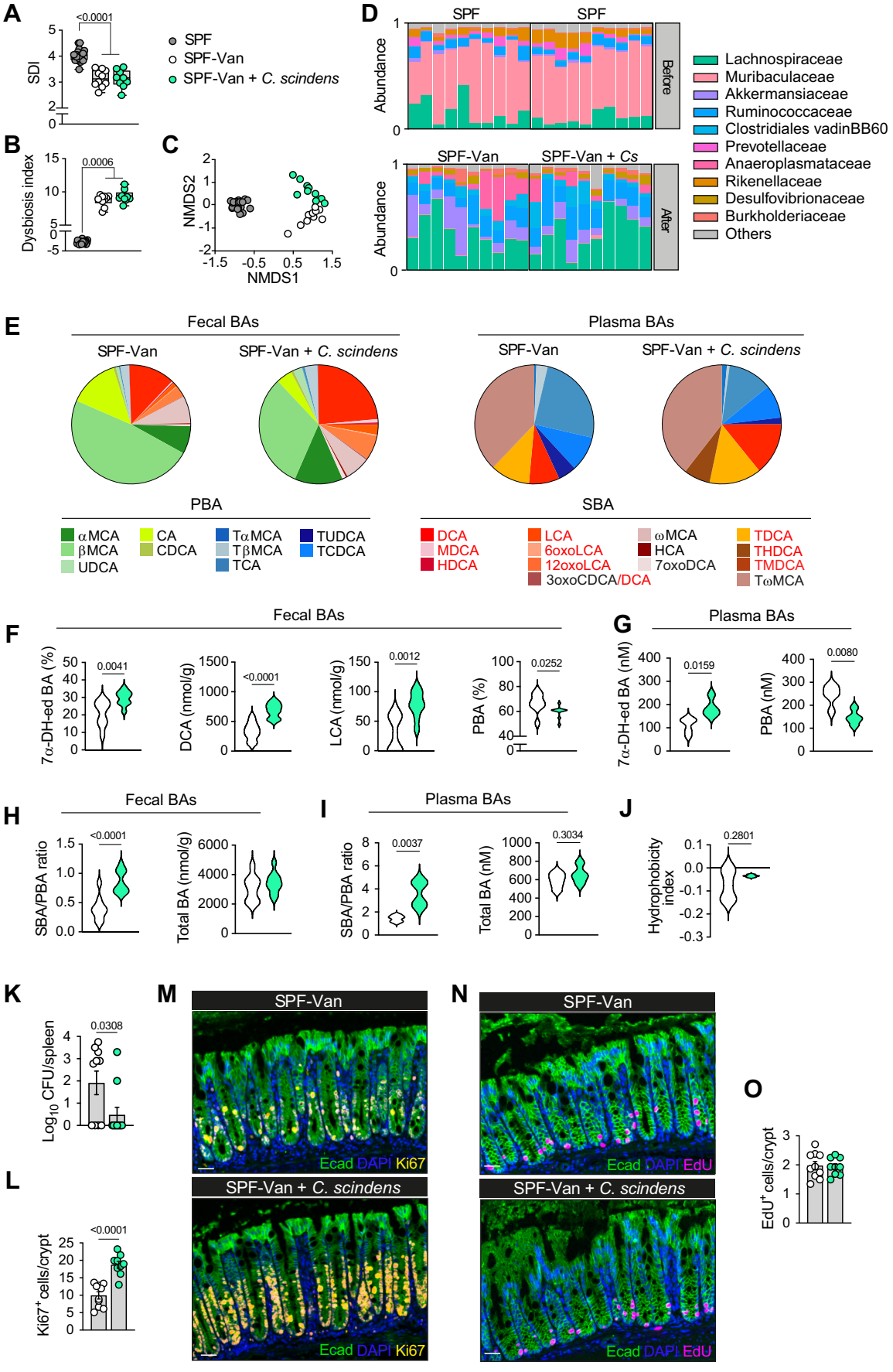

A
SDI
<0.0001
SPF
SPF-Van
SPF-Van + C. scindens
B
Dysbiosis index
0.0006
C
NMDS2
NMDS1
D
SPF
SPF
Before
SPF-Van
SPF-Van + Cs
After
Abundance
Lachnospiraceae
Muribaculaceae
Akkermansiaceae
Ruminococcaceae
Clostridiales vadinBB60
Prevotellaceae
Anaeroplasmataceae
Rikenellaceae
Desulfovibrionaceae
Burkholderiaceae
Others
E
Fecal BAs
Plasma BAs
SPF-Van
SPF-Van + C. scindens
PBA
αMCA
βMCA
UDCA
CA
CDCA
TαMCA
TβMCA
TCA
TUDCA
TCDCA
SBA
DCA
MDCA
HDCA
LCA
6oxoLCA
12oxoLCA
3oxoCDCA/DCA
ωMCA
HCA
7oxoDCA
TDCA
THDCA
TMDCA
TωMCA
F
Fecal BAs
7α-DH-ed BA (%)
0.0041
DCA (nmol/g)
<0.0001
LCA (nmol/g)
0.0012
PBA (%)
0.0252
G
Plasma BAs
7α-DH-ed BA (nM)
0.0159
PBA (nM)
0.0080
H
Fecal BAs
SBA/PBA ratio
<0.0001
Total BA (nmol/g)
I
Plasma BAs
SBA/PBA ratio
0.0037
Total BA (nM)
0.3034
J
Hydrophobicity index
0.2801
K
Log10 CFU/spleen
0.0308
L
Ki67+cells/crypt
<0.0001
M
SPF-Van
Ecad DAPI Ki67
SPF-Van + C. scindens
Ecad DAPI Ki67
N
SPF-Van
Ecad DAPI EdU
SPF-Van + C. scindens
Ecad DAPI EdU
O
EdU+cells/crypt

Figure EV2. Characterization of *C. scindens* colonization in SPF-Van mice.

(**A**) Shannon diversity index (SDI) showing the fecal bacterial community diversity of C57BL/6J mice (SPF) ($n = 19$) or preconditioned with vancomycin and gavaged daily for 5 days with live *C. scindens* (10^8 CFU – SPF-Van + *C. scindens*) ($n = 9$) or PBS vehicle (SPF-Van) ($n = 10$) (SPF vs all other groups $P < 0.0001$). (**B**) Gut dysbiosis index of mice in (**A**). (**C**) Non-metric multidimensional scaling (NMDS) of amplicon sequence variant (ASV) counts based on the Bray-Curtis dissimilarity of mice in (**A**). (**D**) Relative abundance of bacterial families representing more than 10% of total abundance of mice in (**A**). (**E**) BA composition in feces ($n = 9$ for SPF-Van and $n = 10$ for SPF-Van + *C. scindens*) and plasma ($n = 5$ for SPF-Van and $n = 4$ for SPF-Van + *C. scindens*), respectively. BA data are normalized to the total amount of BAs. 7α-dehydroxylated BAs are in red. (**F**) Violin plots showing BA amount (nmol/g of feces) or proportion of the indicated BAs to total BA fecal amount of mice in (**E**) (For DCA (nmol/g): SPF-Van vs SPF-Van + *C. scindens* $P < 0.0001$). (**G**) Violin plots showing the BA concentration (nM) of the indicated BAs in the plasma of mice in (**E**). (**H**) Secondary (SBA)-to-primary (PBA) BA ratio and total BA amount in feces of mice in (**E**) (For SBA/PBA ratio: SPF-Van vs SPF-Van + *C. scindens* $P < 0.0001$). (**I**) Secondary (SBA)-to-primary (PBA) BA ratio and total BA concentration in plasma of mice in (**E**). (**J**) BA hydrophobicity index in plasma of mice in (**E**). (**K**) Bacterial colony-forming units (CFU) in the spleen of 10-week-old male SPF-Van mice gavaged daily for 5 days with live *C. scindens* (10^8 CFU – SPF-Van + *C. scindens*) ($n = 11$) or PBS (control – SPF-Van) ($n = 10$) and subjected to a 7-day treatment with DSS (2.5% in drinking water) (Fig. 2F). (**L**, **M**) Quantification (**L**) and representative images (**M**) of $Ki67^+$ cells per crypt in the colon of 10-week-old male SPF-Van mice gavaged daily for 5 days with live *C. scindens* (10^8 CFU – SPF-Van + *C. scindens*) ($n = 9$) or vehicle (PBS – SPF-Van) ($n = 10$) (SPF-Van vs SPF-Van + *C. scindens* $P < 0.0001$). 2 days after the colonization, experimental colitis was induced by a 7-day treatment with DSS (2.5% in drinking water) followed by 3 days of drinking water (recovery period) (Fig. 2M). (**N**, **O**) Representative images (**N**) and quantification of EdU^+ cells per crypt (**O**) in the colon of unchallenged mice in (**A**) at the time of sacrifice. Scale bar = 50 µm (**M**, **N**). Graphs represent mean ± SEM. *n* refers to biological replicates. *P* values (exact values) were calculated using one-way ANOVA followed by Bonferroni's post hoc correction (**A**, **B**) or 2-tailed Student's t test (**F**, **G**, **H**, **I**, **K**, **L**).

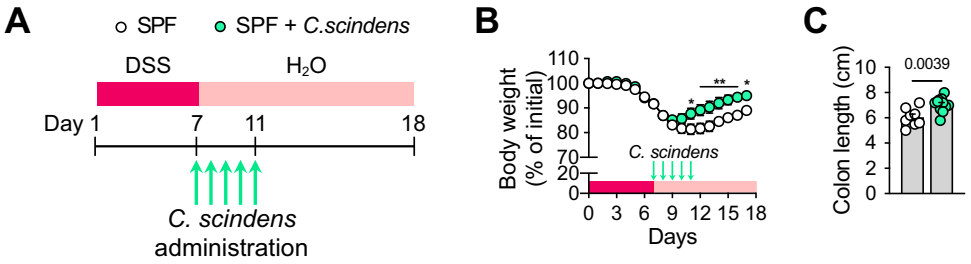


Figure EV3. Therapeutic effect of *C. scindens* administration in SPF mice.

(**A**) Workflow of the therapeutic DSS-induced acute experimental colitis experiment. 10-week-old male C57BL/6J mice were treated with DSS (2.5% in drinking water) for 7 days followed by a recovery phase. From the first day of the recovery period, mice were gavaged daily for 5 days with live *C. scindens* (10^8 CFU – SPF + *C. scindens* ($n = 11$)) or vehicle (PBS – SPF ($n = 9$)). Mouse body weight was monitored daily until one of the four experimental groups reached the initial body weight. (**B**) Percentage of body weight loss of mice in (**A**) during exposure to DSS and the recovery phase (SPF-Van vs SPF-Van + *C. scindens* day 11: $^*P = 0.02$; day 12: $^{**}P = 0.003$; day 13: $^{**}P = 0.002$; day 14: $^{**}P = 0.003$; day 15: $^{**}P = 0.004$; day 16: $^{**}P = 0.004$; day 17: $^*P = 0.04$). (**C**) Colon length of mice in (**A**) at the end of the experiment. Graphs represent mean ± SEM. *n* refers to biological replicates. *P* values (exact values) were calculated using two-way ANOVA followed by Bonferroni's post hoc correction (**B**) or 2-tailed Student's t-test (**C**).

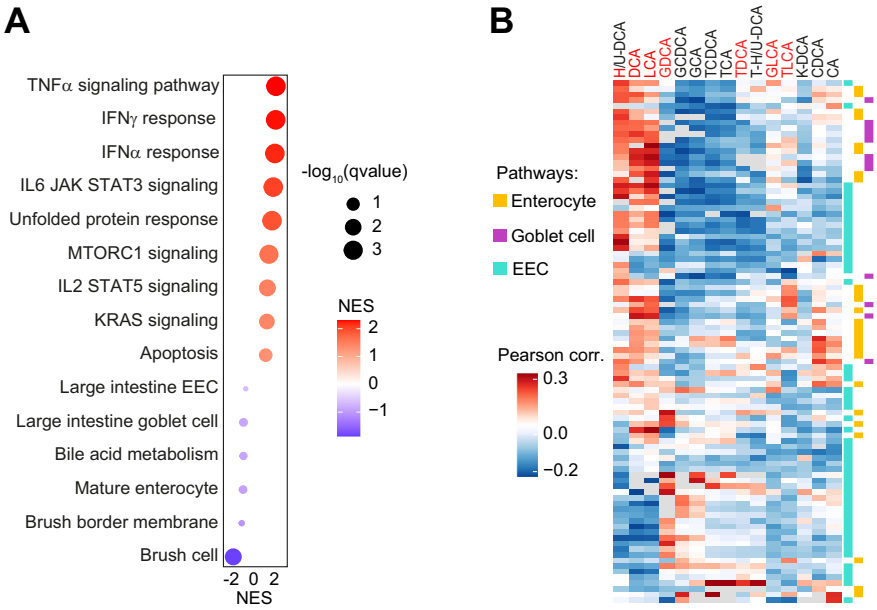


Figure EV4. 7α-dehydroxylated BAs are associated with intestinal cell differentiation in the colon of UC patients.

(**A**) Gene set enrichment analysis (GSEA) representing a selection of the most modulated biological processes in the colon of UC patients compared to non-IBD controls (publicly available dataset from (Lloyd-Price et al, 2019)), ordered by normalized enrichment score (NES). (**B**) Heatmap representing the correlation (Pearson correlation) between the abundance of different fecal BA species and the genes most significantly involved in biological processes of enterocytes, goblet cells and enteroendocrine cells (EEC) of colon in UC patients and non-IBD controls in (**A**). 7α-dehydroxylated BAs are highlighted in red.