

Supplemental Information

Gut microbiota analysis

Genomic DNA purification and 16S rRNA gene amplification.

Genomic DNA was isolated from approximately 60 mg of caecal content using the repeated bead beating method previously described (1). The V4 region of the bacterial 16S rRNA gene was amplified using primers as reported by Kozich et al (2). PCR was carried out under the following conditions: initial denaturation for 3 min at 94°C, followed by 25 cycles of denaturation for 45 s at 94°C, annealing for 60 s at 50°C and elongation for 90 s at 72°C, and a final elongation step for 10 min at 72°C and further treated as reported previously (3).

Sequence analysis

Sequencing was done at the Genomic Core Facility of Gothenburg University. The forward and reverse reads from the pair-end sequencing were joined by exploiting the long overlap between both reads using in-house codes. Identical bases in the overlap sequence increase the assurance accuracy of the sequencing and therefore we assigned the highest possible quality score for those matching bases. The FASTX-Toolkit was used to filter out low-quality reads and reads with a quality Phread score over 20 in at least 98% of their sequences passed the filter.

The sequencing data were then analyzed using the software package Quantitative Insights Into Microbial Ecology (QIIME), version 1.9.0. Sequences were clustered into operational taxonomic units (OTUs) at a 97% identity threshold using a closed-reference OTU picking approach with UCLUST (4) against the Greengenes reference database (5). Representative sequences for the OTUs were Greengenes reference sequences or cluster seeds, and were taxonomically assigned using the Greengenes taxonomy and the Ribosomal Database Project Classifier (6). Representative OTUs were aligned using PyNAST (7) and used to build a

phylogenetic tree with FastTree (8), which was used to estimate the β -diversity of samples using phylogenetic diversity (9) and weighted unifrac (10). Three-dimensional principal coordinates analysis plots were visualized using Emperor (11). With this approach, a total of 4 364 287 sequences grouped in 709 OTUs were obtained for the 43 samples sequenced, with a median of 71 524 sequences assigned to each sample. To correct for differences in sequencing depth, 48 400 sequences were randomly sub-sampled for each sample and used for diversity analyses. Sequences with very low abundance (relative abundance < 0.005%) were excluded from the analysis.

LDA Effect Size algorithm (12) was used to identify taxa that discriminated caecal microbiota profiles according to the colonization origin.

Bile acid analysis

BAs from 50 μ l serum (from portal and caval veins) were extracted using protein precipitation with 10 volumes of methanol containing deuterium-labelled internal standards (2.5 μ M and 50 nM of each BA standard for portal and caval vein respectively) (13). The samples were vortexed for 10 min and then centrifuged at 20000g for 10 min. For serum from portal vein, the supernatant was diluted 10 times in methanol:water (1:1) and from caval vein, the supernatant was evaporated and reconstituted in 200 μ l of methanol:water (1:1). BAs from liver and caecum were extracted after homogenizing the tissue in 2 ml propylene tubes with 6 zirconium oxide beads (3 mm) (Retsch GmbH, Haan, Germany). Approximately 50 mg of tissue was placed in the tube and 500 μ l methanol containing internal standards were added (2.5 μ M of each BA standard). The tissue was homogenised using a TissueLyser II instrument (Qiagen, Hilden, Germany) at 25 Hz for 10 min. The homogenate was then centrifuged at 20 000g for 10 min and the supernatant was diluted 50 times with water: methanol [1:1] before

analysis. BAs from gallbladder were extracted as above using the whole gallbladder and 500 μ l of methanol. After homogenisation and centrifugation, the supernatant was diluted 1000 times with water:methanol [1:1] before analysis. Because of the high BA concentration in gallbladder, the internal standards (50 nM of each) were added after the dilution.

BAs were analysed using ultra-high performance liquid chromatography tandem mass spectrometry (UPLC-MS/MS). The analytical setup consisted of a Rheos Allegro quaternary ultra-performance pump (Flux Instruments, Basel, Switzerland) and a QTRAP 5500 mass spectrometer (ABSciex, Concord, Canada). The BAs (5 μ l injection) were separated on a Kinetex™ C18 column (2.1 x 100 mm 1.7 μ m particles) (Phenomenex, Torrance, CA, USA) kept at 60°C. The mobile phases were 7.5 mM ammonium formate in water adjusted to pH 4.5 with formic acid (A-phase) and 0.1% formic acid in acetonitrile (B-phase). The UPLC gradient started with a 1 min isocratic elution with 25% B. From 1 to 5 min, B was increased to 35%; from 5 to 14.5 min, B was increased to 95%; the gradient was then kept at this level for 0.5 min and then, from 15 to 15.5 min, returned to 25% B. The method ended with 2.5 min equilibration to give a total runtime of 18 min per sample. The flow rate was 400 μ l/min and detection of BAs was made using scheduled MRM in negative mode. MRM detection window was 90 s and target scan time was 0.3 s. Quantification was made against calibration curves generated from non-labelled standards.

Reagents

Methanol and formic acid were from Merck (Darmstadt, Germany). Ammonium formate was bought from Sigma-Aldrich (Steinheim, Germany) and acetonitrile was from Rathburn Chemicals Ltd (Walkburn, UK). The BAs taurocholic acid (TCA), tauroursodeoxycholic acid (TUDCA), taurohyodeoxycholic acid (THDCA), taurochenodeoxycholic acid (TCDCA), taurodeoxycholic acid (TDCA), cholic acid (CA), ursodeoxycholic acid (UDCA), hyodeoxycholic acid (HDCA), chenodeoxycholic acid (CDCA), deoxycholic acid (DCA) and

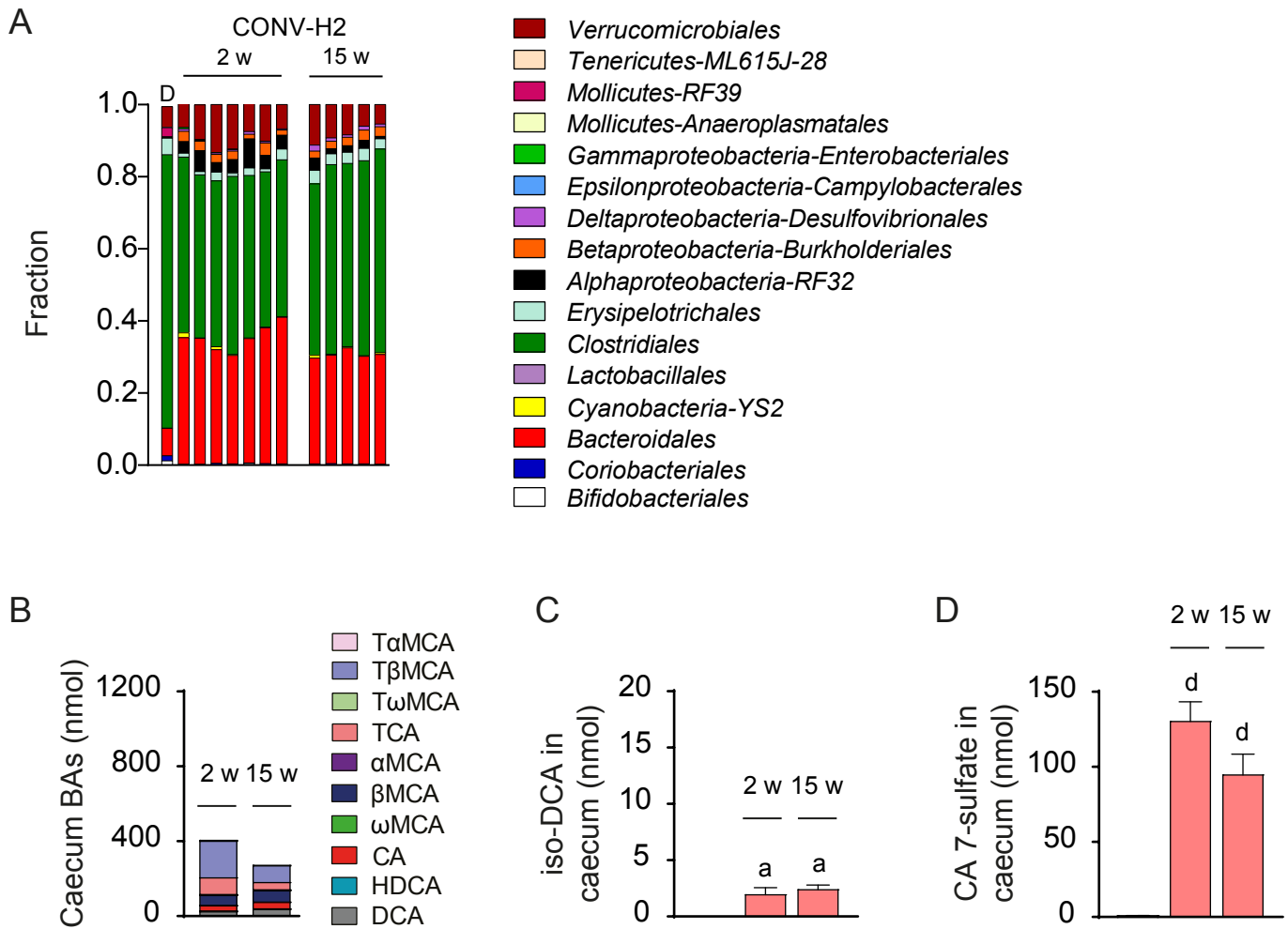
lithocholic acid (LCA) were purchased from Sigma Aldrich (Stockholm, Sweden). Muricholic acids (α -, β -, and ω -MCA, taurine-conjugated and unconjugated) and murideoxycholic acid (MDCA) were purchased from Steraloids (Newport, RI, USA). Deuterated bile acids d_4 -TCA, d_4 -UDCA, d_4 -CDCA and d_4 -LCA were purchased from CDN isotopes (Quebec, Canada). Iso-DCA was a kind gift from Jan Sjövall and CA-7 sulfate was a kind gift from AstraZeneca in Mölndal, Sweden.

In vitro assay of BA metabolism by a human microbiota

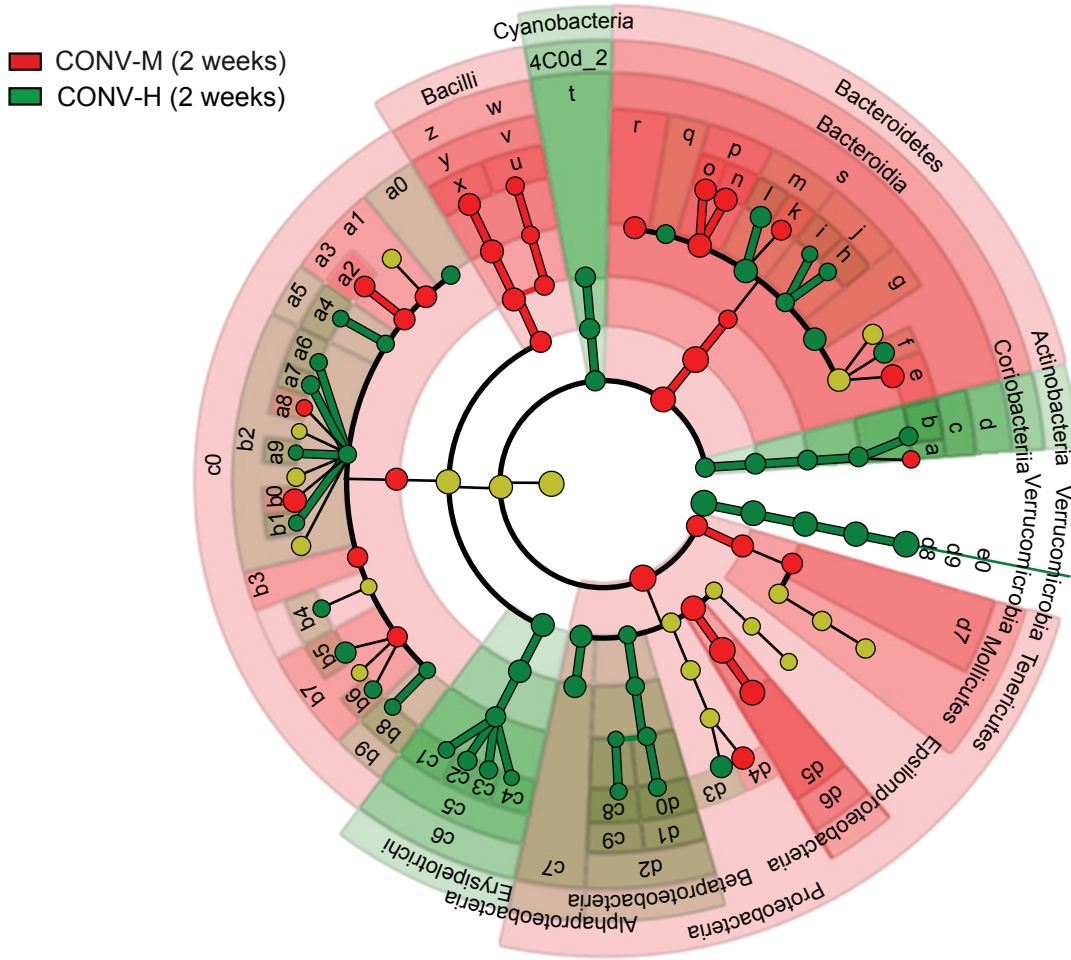
Faecal samples from the first human donor were inoculated (1% w/v) in 5 ml sulfate-rich anaerobic BHI medium (SRANB-BHI containing cellobiose (1 g/L), maltose (1 g/L), cysteine (0.5 g/L), yeast extract (2.5 g/L), ammonium sulfate (3.31 g/L), hemin (10 mg/L) and BHI (37 g/L)) supplemented with 0.2% TCA or CA. All chemicals were obtained from Sigma Aldrich except BHI which was purchased from OXOID, UK. The faecal samples were incubated as batch cultures under strict anaerobic conditions maintained in COY chamber for 24 hours at 37°C. The anaerobic atmosphere of the COY chamber was composed of 5% hydrogen, 10% carbon dioxide and 85% nitrogen. Samples (50 μ l) were taken for BA analysis at start and after 24 hours' incubation and were kept in -20 °C until the time of analysis.

Supplemental References

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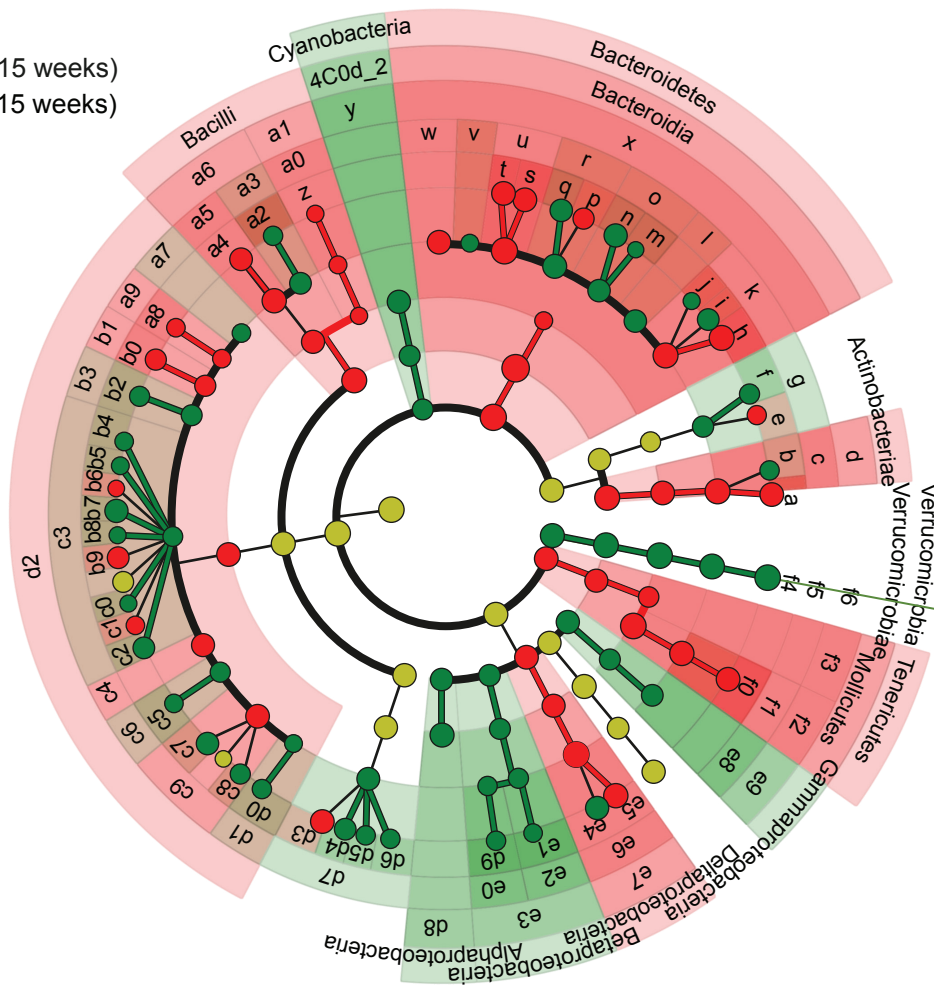
Supplemental Figure S1. Gut microbiota and BA composition in caecum of mice colonized with a second human donor. (A) Relative abundance of orders in caecal bacteria from CONV-H2 mice colonized for 2 weeks (2w) or 15 weeks (15w). (B-D) Whole organ amounts of BAs (B) iso-DCA (C) and CA-7 sulfate (D) in caecum. $n=5-7$ mice/group; D, donor sample; MCA, muricholic acid; CA, cholic acid; HDCA, hyodeoxycholic acid; DCA, deoxycholic acid; T, taurine-conjugated species.



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|---|--|---|
| ■ a: <i>Adlercreutzia</i> | ■ a0: <i>Christensenellaceae</i> | ■ c6: <i>Erysipelotrichales</i> |
| ■ b: <i>Collinsella_aerofaciens</i> | ■ a1: <i>Clostridiaceae</i> | ■ c7: <i>RF32</i> |
| ■ c: <i>Coriobacteriaceae</i> | ■ a2: <i>Dehalobacterium</i> | ■ c8: <i>Sutrella</i> |
| ■ d: <i>Coriobacteriales</i> | ■ a3: <i>Dehalobacteriaceae</i> | ■ c9: <i>Alcaligenaceae</i> |
| ■ e: <i>Bacteroides</i> | ■ a4: <i>Anaerofustis</i> | ■ d0: <i>Oxalobacter_formigenes</i> |
| ■ f: <i>Bacteroides_ovatus</i> | ■ a5: <i>Eubacteriaceae</i> | ■ d1: <i>Oxalobacteraceae</i> |
| ■ g: <i>Barnesiellaceae</i> | ■ a6: <i>Blautia</i> | ■ d2: <i>Burkholderiales</i> |
| ■ h: <i>Butyricimonas</i> | ■ a7: <i>Blautia_obeum</i> | ■ d3: <i>Bilophila</i> |
| ■ i: <i>Odoribacter</i> | ■ a8: <i>Coprococcus</i> | ■ d4: <i>Desulfovibrio</i> |
| ■ j: <i>Odoribacteraceae</i> | ■ a9: <i>Dorea</i> | ■ d5: <i>Helicobacteraceae</i> |
| ■ k: <i>Parabacteroides</i> | ■ b0: <i>Roseburia</i> | ■ d6: <i>Campylobacteriales</i> |
| ■ l: <i>Parabacteroides_distasonis</i> | ■ b1: <i>Ruminococcus</i> | ■ d7: <i>RF39</i> |
| ■ m: <i>Porphyromonadaceae</i> | ■ b2: <i>Lachnospiracheae</i> | ■ d8: <i>Akkermansia_muciniphila</i> |
| ■ n: <i>Prevotella</i> | ■ b3: <i>Mogibacteriaceae</i> | ■ d9: <i>Verrucomicrobiaceae</i> |
| ■ o: <i>Prevotella_copri</i> | ■ b4: <i>rc4_4</i> | ■ e0: <i>Verrucomicrobiales</i> |
| ■ p: <i>Prevotellaceae</i> | ■ b5: <i>Feacalibacterium_prausnitzii</i> | |
| ■ q: <i>Rikenellaceae</i> | ■ b6: <i>Ruminococcus</i> | |
| ■ r: <i>S24_7</i> | ■ b7: <i>Ruminococcaceae</i> | |
| ■ s: <i>Bacteroidales</i> | ■ b8: <i>Phascolarctobacterium</i> | |
| ■ t: <i>YS2</i> | ■ b9: <i>Vellionellaceae</i> | |
| ■ u: <i>Staphylococcus</i> | ■ c0: <i>Clostridiales</i> | |
| ■ v: <i>Staphylococcaceae</i> | ■ c1: <i>Allobaculum</i> | |
| ■ w: <i>Bacillales</i> | ■ c2: <i>Coprobacillus</i> | |
| ■ x: <i>Lactobacillus</i> | ■ c3: <i>Eubacterium_dolichum</i> | |
| ■ y: <i>Lactobacillaceae</i> | ■ c4: <i>cc115</i> | |
| ■ z: <i>Lactobacillales</i> | ■ c5: <i>Erysipelotrichaceae</i> | |

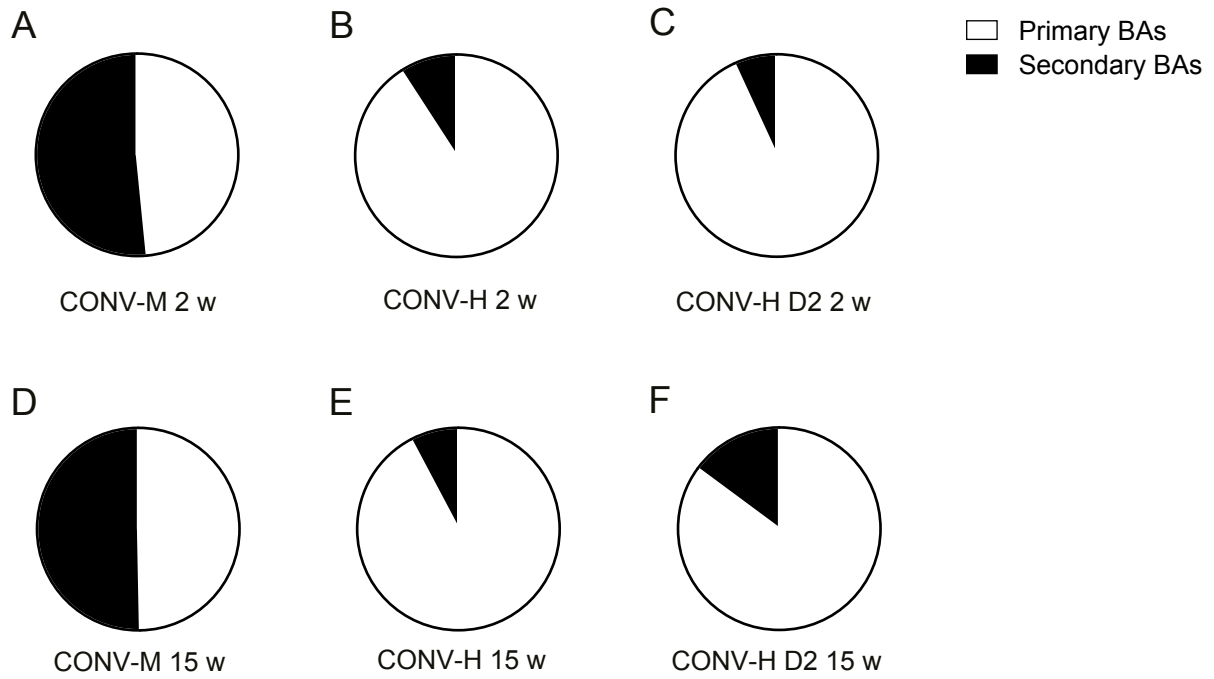
Supplemental Figure S2. Cladogram representing OTUs of statistical and biological difference between caecum microbiota of CONV-M and CONV-H mice after 2 weeks of colonization. Each circle diameter is proportional to OTU's abundance. n=6 samples/group.

■ CONV-M (15 weeks)
■ CONV-H (15 weeks)

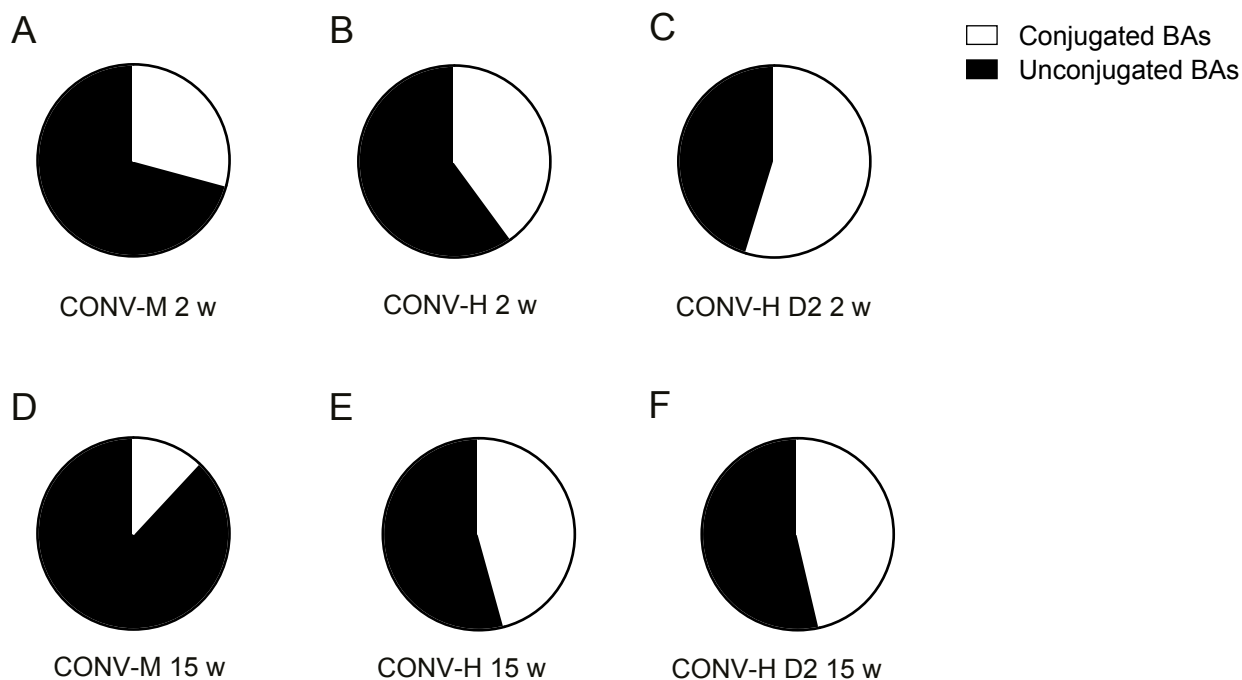


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|---|--|---|
| ■ a: <i>Bifidobacterium</i> | ■ a7: <i>Christensenellaceae</i> | ■ e0: <i>Alcaligenaceae</i> |
| ■ b: <i>Bifidobacterium_longum</i> | ■ a8: <i>Candidatus_arthromitus</i> | ■ e1: <i>Oxalobacter_formigenes</i> |
| ■ c: <i>Bifidobacteriaceae</i> | ■ a9: <i>Clostridiaceae</i> | ■ e2: <i>Oxalobacteraceae</i> |
| ■ d: <i>Bifidobacteriales</i> | ■ b0: <i>Dehalobacterium</i> | ■ e3: <i>Burkholderiales</i> |
| ■ e: <i>Adlercreutzia</i> | ■ b1: <i>Dehalobacteriaceae</i> | ■ e4: <i>Bilophia</i> |
| ■ f: <i>Collinsella_aerofaciens</i> | ■ b2: <i>Anaerofustis</i> | ■ e5: <i>Desulfovibrio</i> |
| ■ g: <i>Coriobacteriaceae</i> | ■ b3: <i>Eubacteriaceae</i> | ■ e6: <i>Desulfovibrionaceae</i> |
| ■ h: <i>Bacteroides</i> | ■ b4: <i>Blautia</i> | ■ e7: <i>Desulfovibrionales</i> |
| ■ i: <i>Bacteroides_ovatus</i> | ■ b5: <i>Blautia_obeum</i> | ■ e8: <i>Enterobacteriaceae</i> |
| ■ j: <i>Bacteroides_uniformis</i> | ■ b6: <i>Coprococcus</i> | ■ e9: <i>Enterobacteriales</i> |
| ■ k: <i>Bacteroidaceae</i> | ■ b7: <i>Coprococcus_eutactus</i> | ■ f0: <i>Anaeroplasmataceae</i> |
| ■ l: <i>Barnesiellaceae</i> | ■ b8: <i>Dorea</i> | ■ f1: <i>Anaeroplasmatales</i> |
| ■ m: <i>Butyrivomona</i> | ■ b9: <i>Dorea_formicigenerans</i> | ■ f2: <i>Anaeroplasmatales</i> |
| ■ n: <i>Odoribacter</i> | ■ c0: <i>Ruminococcus</i> | ■ f3: <i>RF39</i> |
| ■ o: <i>Odoribacteriaceae</i> | ■ c1: <i>Ruminococcus_gnavus</i> | ■ f4: <i>Akkermansia_muciniphila</i> |
| ■ p: <i>Parabacteroides</i> | ■ c1: <i>Ruminococcus_torques</i> | ■ f5: <i>Verrucomicrobiaceae</i> |
| ■ q: <i>Parabacteroides_distasonis</i> | ■ c3: <i>Lachnospirachaeae</i> | ■ f6: <i>Verrucomicrobiales</i> |
| ■ r: <i>Porphyromonadaceae</i> | ■ c4: <i>Mogibacteriaceae</i> | |
| ■ s: <i>Prevotella</i> | ■ c5: <i>rc4_4</i> | |
| ■ t: <i>Prevotella_copri</i> | ■ c6: <i>Peptococcaceae</i> | |
| ■ u: <i>Prevotellaceae</i> | ■ c7: <i>Feacalibacterium_prausnitzii</i> | |
| ■ v: <i>Rikenellaceae</i> | ■ c8: <i>Ruminococcus</i> | |
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| ■ z: <i>Staphylococcus</i> | ■ d2: <i>Clostridiales</i> | |
| ■ a0: <i>Staphylococcaceae</i> | ■ d3: <i>Allobaculum</i> | |
| ■ a1: <i>Bacillales</i> | ■ d4: <i>Coprobaillus</i> | |
| ■ a2: <i>Enterococcus</i> | ■ d5: <i>Eubacterium_dolichum</i> | |
| ■ a3: <i>Enterococcaceae</i> | ■ d6: <i>cc115</i> | |
| ■ a4: <i>Lactobacillus</i> | ■ d7: <i>Erysipelotrichaceae</i> | |
| ■ a5: <i>Lactobacillaceae</i> | ■ d8: <i>RF32</i> | |
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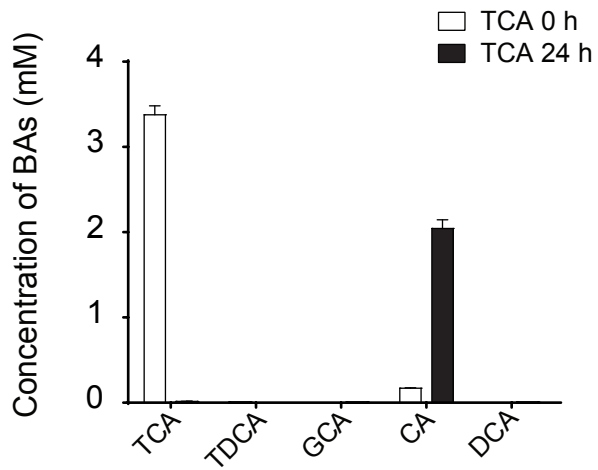
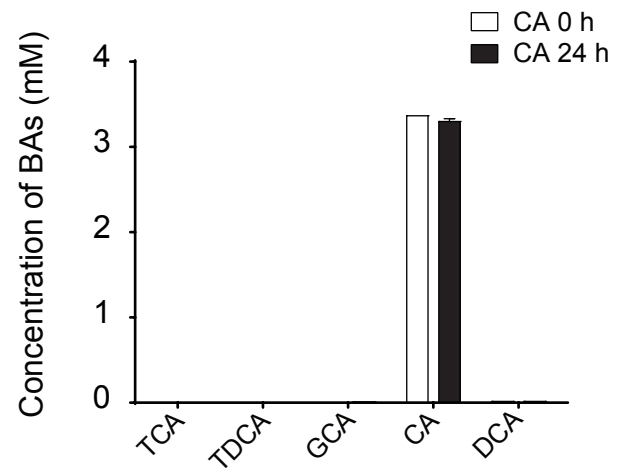
Supplemental Figure S3. Cladogram representing OTUs of statistical and biological difference between caecum microbiota of CONV-M and CONV-H mice after 15 weeks of colonization. Each circle diameter is proportional to OTU's abundance. n=6-10 samples/group.



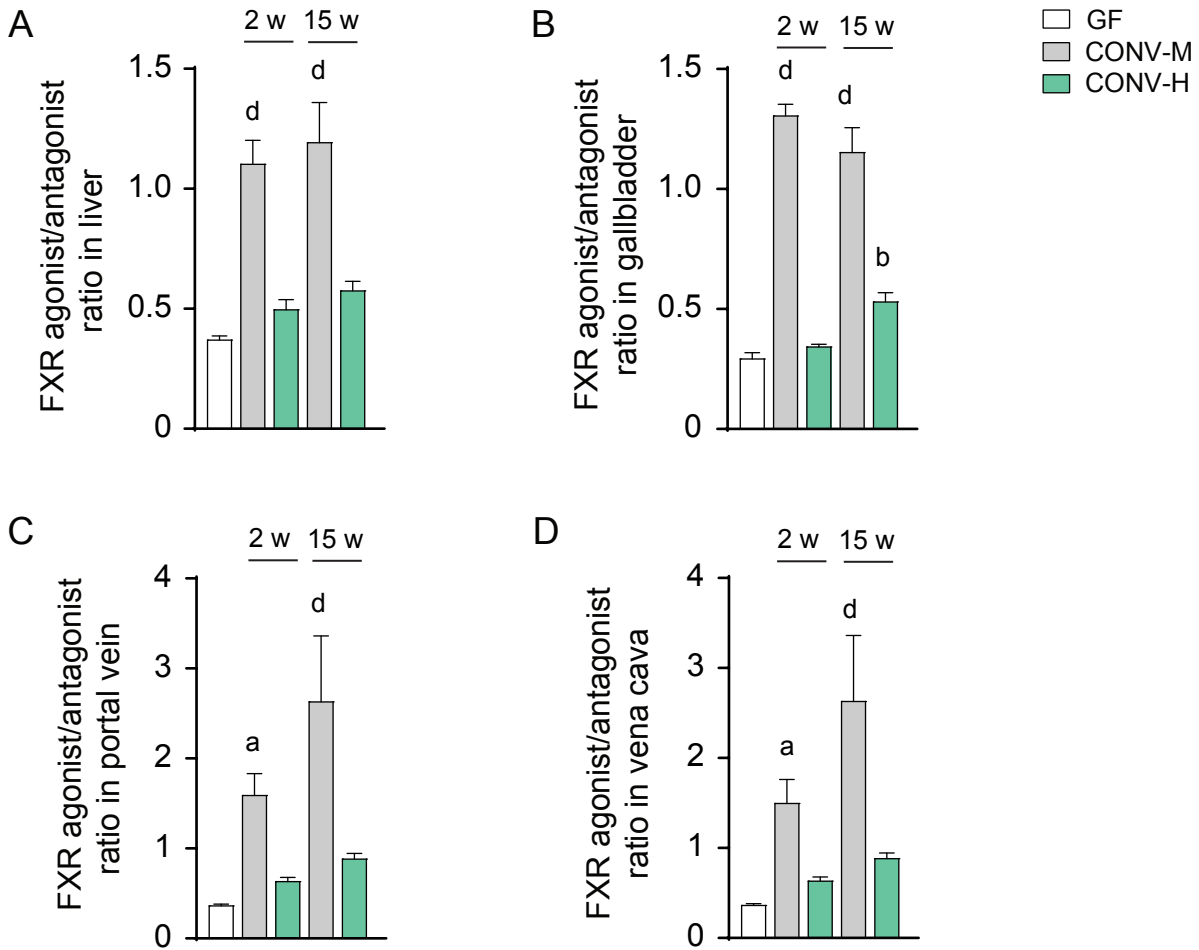
Supplemental Figure S4. Relative amounts of primary and secondary BAs in caecum of mice colonized with mouse or human microbiota. (A-F) Primary and secondary BAs in caecum after 2 weeks' (A-C) or 15 weeks' (D-F) colonization with mouse microbiota (A and D), human microbiota from the first human donor (CONV-H) (B and E) or the second human donor (CONV-H D2) (C and F). Primary bile acids, TCA, CA, TCDCA, CDCA, T α MCA, α MCA, T β MCA, β MCA, TUDCA, UDCA); secondary BAs, DCA, iso-DCA, HCA, HDCA, LCA, MDCA, ω MCA,



Supplemental Figure S5. Relative amounts of conjugated and unconjugated BAs in caecum of mice colonized with mouse or human microbiota. (A-F) Conjugated and unconjugated BAs in caecum after 2 weeks' (A-C) or 15 weeks' (D-F) colonization with mouse microbiota (CONV-M) (A and D), human microbiota from the first human donor (CONV-H) (B and E) or the second human donor (CONV-H D2) (C and F). Conjugated BAs, TCA, TCDCA, TDCA, THDCA, T α MCA, T β MCA, T ω MCA, TUDCA; unconjugated BAs, CA, CDCA, DCA, iso-DCA, HCA, HDCA, LCA, α MCA, β MCA, ω MCA, MDCA, UDCA.

A**B****Supplemental Figure S6. Metabolism of TCA and CA by human microbiota in vitro.**

(A) Concentration of BAs in suspensions of human faecal samples incubated in BHI medium with TCA (A) or CA (B) for 24 hours. Mean values \pm SEM are plotted; n=2 samples for each time point.



Supplemental Figure S7. Changes in the ratio between FXR agonists and antagonists after colonization. (A-D) Ratio between FXR agonists and antagonists in liver (A), gallbladder (B), portal vein (C) and caval vein (D). Mean values \pm SEM are plotted. $n = 4-9$ samples/group; ^a $P < 0.05$, ^b $P < 0.01$, ^c $P < 0.001$, ^d $P < 0.0001$ indicate differences vs GF with ANOVA and Dunnett's multiple comparisons test. FXR agonists, TCA, TCDCA, TDCA, TLCA, CA, CDCA, DCA, LCA; FXR antagonists, T α MCA, T β MCA.

Supplemental Table S1. Bile acid (BA) content in liver. Amounts of BAs in the whole organ for each group of mice. BAs >0.1% abundance are included. BAs (nmol/organ), relative liver weight (mg/g body weight) and body weight (g) are presented as mean \pm SEM; n = 5-9 samples/group; ^a P<0.05, ^b P <0.01, ^c P <0.001, ^d P <0.0001 indicate differences vs GF mice with ANOVA and Tukey's multiple comparisons test.

Liver BAs	GF	CONV-M (2 w)	CONV-H (2w)	CONV-M (15 w)	CONV-H (15w)
T α MCA	0.019 \pm 0.003	0.021 \pm 0.004	0.019 \pm 0.004	0.008 \pm 0.002	0.007 \pm 0.001
T β MCA	0.770 \pm 0.134	0.288 \pm 0.068	0.646 \pm 0.136	0.108 \pm 0.021 ^b	0.279 \pm 0.044 ^a
T ω MCA		0.077 \pm 0.018 ^d		0.031 \pm 0.007	0.001 \pm 0.000
TCA	0.291 \pm 0.059	0.308 \pm 0.061	0.310 \pm 0.063	0.129 \pm 0.028	0.168 \pm 0.032
TUDCA	0.004 \pm 0.001	0.003 \pm 0.001	0.005 \pm 0.001	0.002 \pm 0.000	0.002 \pm 0.000
THDCA		0.003 \pm 0.001 ^d	traces		
TCDCA	0.008 \pm 0.001	0.005 \pm 0.001	0.005 \pm 0.001	0.002 \pm 0.000 ^b	0.002 \pm 0.000 ^c
TDCA		0.009 \pm 0.002 ^d	0.002 \pm 0.001	0.004 \pm 0.001 ^a	0.001 \pm 0.000
β MCA	0.003 \pm 0.001	traces	0.004 \pm 0.002	traces	0.003 \pm 0.001
CA				traces	
Total BA	1.095 \pm 0.197	0.713 \pm 0.147	0.992 \pm 0.205	0.285 \pm 0.056 ^b	0.463 \pm 0.077 ^b
Relative liver weight	44.00 \pm 1.94	46.00 \pm 0.95	44.47 \pm 1.41	42.02 \pm 1.98	42.23 \pm 0.71
Body weight	29.2 \pm 0.9	31.4 \pm 1.6	28.2 \pm 0.8	37.1 \pm 2.1 ^c	29.3 \pm 0.8

Supplemental Table S2. Bile acid (BA) content in gallbladder. Amounts of BAs in the whole organ for each group of mice. BAs >0.1% abundance are included. BAs (nmol/organ) and relative weights (mg/g body weight) are presented as mean \pm SEM; n = 5-9 samples/group; ^a P<0.05, ^b P <0.01, ^c P <0.001, ^d P <0.0001 indicate differences vs GF mice with ANOVA and Tukey's multiple comparisons test.

Gallbladder BAs	GF	CONV-M (2 w)	CONV-H (2 w)	CONV-M (15 w)	CONV-H (15 w)
T α MCA	215.10 \pm 21.95	137.73 \pm 32.00	54.31 \pm 9.34 ^b	288.85 \pm 56.57	85.57 \pm 9.90
T β MCA	15663.31 \pm 2839.06	2268.06 \pm 459.61 ^b	3636.47 \pm 674.71 ^b	3645.80 \pm 394.42	4127.95 \pm 337.93
T ω MCA		499.58 \pm 118.65 ^c		1057.73 \pm 105.28 ^d	13.37 \pm 3.56
TCA	3950.31 \pm 433.79	3040.48 \pm 576.06	1230.32 \pm 214.20 ^b	4302.08 \pm 483.50	2180.74 \pm 75.13
TUDCA	61.54 \pm 5.12	33.97 \pm 6.60 ^a	15.63 \pm 3.53 ^c	66.35 \pm 1.05	31.00 \pm 1.01
THDCA		23.39 \pm 5.06		36.86 \pm 5.77 ^b	14.26 \pm 1.26
TCDCA	39.63 \pm 4.52	14.29 \pm 2.48 ^b	12.23 \pm 1.40 ^b	23.77 \pm 6.06	traces
TDCA		40.05 \pm 6.16 ^d		94.05 \pm 9.44 ^d	traces
β MCA				24.30 \pm 14.41 ^a	traces
ω MCA				20.98 \pm 10.55 ^b	
CA		traces		78.11 \pm 42.31 ^b	
Total BA	19929.89 \pm 3202.28	6057.56 \pm 1190.50 ^c	4948.96 \pm 900.31 ^c	9638.87 \pm 1190.50 ^a	6452.89 \pm 434.66 ^b
Relative gallbladder weight	2.85 \pm 0.10	1.05 \pm 0.08 ^d	0.67 \pm 0.08 ^d	1.52 \pm 0.11 ^d	1.32 \pm 0.15 ^d

Supplemental Table S3. Bile acid (BA) content in caecum. Amounts of BAs in the whole organ for each group of mice.

BAs >0.1% abundance are included. BAs (nmol/organ) and relative weights (mg/g body weight) are presented as mean ± SEM; n = 5-9 samples/group; ^a P<0.05, ^b P <0.01, ^c P <0.001 ^d P <0.0001 indicate differences vs GF mice with ANOVA and Tukey's multiple comparisons test.

Caecum BAs	GF	CONV-M (2 w)	CONV-H (2 w)	CONV-M (15 w)	CONV-H (15 w)
T α MCA	11.74 ± 2.04	5.92 ± 3.09	1.79 ± 0.42	4.41 ± 2.39	2.71 ± 5.50
T β MCA	821.30 ± 146.30	109.64 ± 53.39 ^c	96.86 ± 18.52 ^c	54.13 ± 31.00 ^c	148.72 ± 38.95 ^c
T ω MCA		27.02 ± 13.52		14.13 ± 7.57	traces
TCA	219.71 ± 30.85	80.83 ± 42.92	37.04 ± 6.27 ^b	31.76 ± 14.83 ^b	64.76 ± 16.05 ^a
TUDCA	3.13 ± 0.35	traces	0.68 ± 0.09 ^c		traces
THDCA		1.65 ± 0.73	traces	traces	
TCDCA	2.33 ± 0.37		traces		
TDCA		3.05 ± 1.39	traces	1.67 ± 0.30	
α MCA		3.50 ± 0.58	4.36 ± 1.27	12.01 ± 2.80 ^d	2.68 ± 0.47
β MCA		23.10 ± 5.83	112.22 ± 26.26	109.88 ± 25.90	146.80 ± 27.34 ^b
ω MCA		141.44 ± 26.59 ^d	1.57 ± 0.40	205.61 ± 25.47 ^d	3.17 ± 0.89
CA		29.26 ± 10.70	59.20 ± 11.23	159.35 ± 55.66 ^b	52.94 ± 12.58
HCA				traces	
UDCA		0.98 ± 0.25	1.97 ± 0.58 ^a	2.65 ± 0.38 ^c	1.33 ± 0.22
MDCA			traces	traces	traces
HDCA		13.20 ± 3.52 ^c	2.58 ± 0.60	12.48 ± 1.91 ^c	5.00 ± 0.74
CDCA			0.88 ± 1.19 ^a	traces	traces
DCA		111.11 ± 13.74 ^c	22.90 ± 7.58	155.60 ± 23.66 ^d	42.23 ± 11.10
LCA		5.63 ± 0.92 ^c	4.24 ± 0.93 ^a	4.48 ± 0.82 ^b	1.81 ± 0.53
Total BA	1058.22 ± 179.15	556.33 ± 142.30	345.61 ± 69.04 ^a	768.16 ± 138.28	472.13 ± 68.91
Relative caecum weight	73.15 ± 5.58	15.20 ± 1.08 ^d	21.65 ± 0.93 ^d	13.31 ± 1.02 ^d	20.05 ± 1,80 ^d

Supplemental Table S4. Bile acid (BA) content in portal vein. BA concentrations (nmol/mL) in portal vein serum for each group of mice. BAs >0.1% abundance are included and presented as mean \pm SEM; n = 5-9 samples/group; ^a P<0.05, ^b P <0.01, ^c P <0.001, ^d P <0.0001 indicate differences vs GF mice with ANOVA and Tukey's multiple comparisons test.

Portal vein BAs	GF	CONV-M (2 w)	CONV-H (2 w)	CONV-M (15 w)	CONV-H (15 w)
T α MCA	6.95 \pm 1.36	2.66 \pm 0.84	2.61 \pm 0.63	3.97 \pm 1.13	1.98 \pm 0.44 ^a
T β MCA	398.11 \pm 79.58	37.29 \pm 11.14 ^c	81.46 \pm 15.00 ^b	38.30 \pm 12.56 ^c	67.21 \pm 11.86 ^c
T ω MCA		10.04 \pm 3.43 ^b		10.68 \pm 3.28 ^b	traces
TCA	147.89 \pm 35.28	38.35 \pm 9.27	47.89 \pm 8.68	46.82 \pm 11.03	49.66 \pm 7.66 ^a
TUDCA	2.01 \pm 0.36	0.36 \pm 0.11 ^b	0.69 \pm 0.11 ^a	0.35 \pm 0.10 ^b	0.56 \pm 0.10 ^c
THDCA		0.32 \pm 0.08	traces	0.46 \pm 0.17	0.31 \pm 0.06
TCDCa	1.47 \pm 0.29	0.28 \pm 0.05 ^b	0.53 \pm 0.09 ^a	traces	0.34 \pm 0.06 ^c
TDCA		0.76 \pm 0.18 ^a	traces	0.85 \pm 0.20 ^b	0.24 \pm 0.04
α MCA		0.24 \pm 0.05		0.52 \pm 0.16	traces
β MCA		1.80 \pm 0.50	3.66 \pm 1.73	8.02 \pm 2.58	8.39 \pm 1.82 ^a
ω MCA		1.37 \pm 0.37		4.01 \pm 0.86	
CA		7.06 \pm 2.22	2.42 \pm 1.30	23.55 \pm 5.65 ^b	6.57 \pm 1.43
UDCA		traces		0.55 \pm 0.10	0.26 \pm 0.05
HDCA		0.13 \pm 0.04		0.27 \pm 0.03	
CDCA				traces	
DCA		1.27 \pm 0.22	0.39 \pm 0.07	2.19 \pm 0.24	0.34 \pm 0.05
Total BA	556.43 \pm 115.82	101.93 \pm 22.88 ^b	139.67 \pm 25.16 ^a	140.53 \pm 31.47 ^b	135.88 \pm 19.56 ^b

Supplemental Table S5. Bile acid (BA) content in caval vein. BA concentrations (nmol/mL) in caval vein serum for each group of mice. BAs >0.1% abundance are included and presented as mean \pm SEM; n = 5-9 samples/group;

^a P<0.05, ^b P <0.01, ^c P <0.001, ^d P <0.0001 indicate differences vs GF mice with ANOVA and Tukey's multiple comparisons test.

Caval vein BAs	GF	CONV-M (2 w)	CONV-H (2 w)	CONV-M (15 w)	CONV-H (15 w)
T α MCA	0.37 \pm 0.13	0.02 \pm 0.01	0.09 \pm 0.04	0.04 \pm 0.01	0.05 \pm 0.02
T β MCA	14.39 \pm 5.4	0.19 \pm 0.07	1.86 \pm 0.67	0.30 \pm 0.06	1.14 \pm 0.49
T ω MCA		0.08 \pm 0.02		0.14 \pm 0.04 ^a	
TCA	7.05 \pm 2.59	0.41 \pm 0.13	1.48 \pm 0.46	0.75 \pm 0.23	1.14 \pm 0.52
TUDCA	0.06 \pm 0.02	traces	0.01 \pm 0.00	traces	0.01 \pm 0.00
THDCA	traces	0.01 \pm 0.00	0.01 \pm 0.00	0.01 \pm 0.00	0.01 \pm 0.00
TCDCA	0.07 \pm 0.02	0.01 \pm 0.00	0.02 \pm 0.00		0.01 \pm 0.00
TDCA		0.03 \pm 0.01	0.01 \pm 0.01	0.03 \pm 0.01	0.01 \pm 0.00
α MCA		0.02 \pm 0.01		0.02 \pm 0.01	0.01 \pm 0.00
β MCA	0.09 \pm 0.03	0.08 \pm 0.02	0.33 \pm 0.19	0.53 \pm 0.22	0.80 \pm 0.23
ω MCA		0.11 \pm 0.04		0.44 \pm 0.12	0.01 \pm 0.00
CA		0.19 \pm 0.05	0.12 \pm 0.08	0.99 \pm 0.27 ^b	0.28 \pm 0.08
UDCA		traces	0.01 \pm 0.01	0.03 \pm 0.01	0.02 \pm 0.01
MDCA				traces	
HDCA		0.01 \pm 0.00	traces	0.01 \pm 0.00	0.01 \pm 0.00
CDCA		traces		traces	
DCA		0.07 \pm 0.02	0.02 \pm 0.01	0.13 \pm 0.01	0.02 \pm 0.00
Total BA	22.02 \pm 8.17	1.20 \pm 0.19 ^a	3.97 \pm 1.28 ^a	3.42 \pm 0.67 ^a	3.50 \pm 1.25 ^a