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Supplementary Materials for

Inhibition of microbial deconjugation of micellar bile acids protects against intestinal permeability and liver injury

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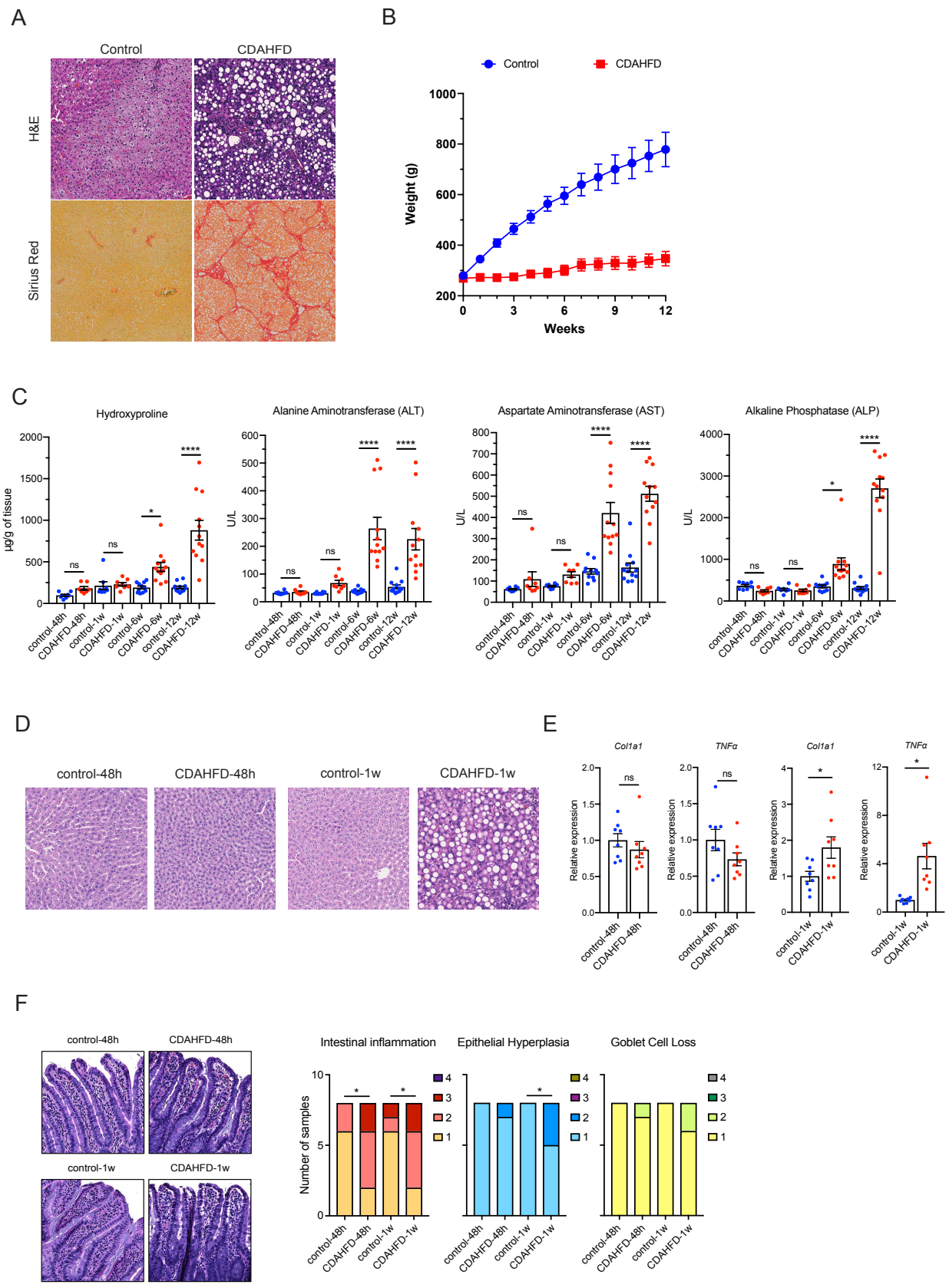
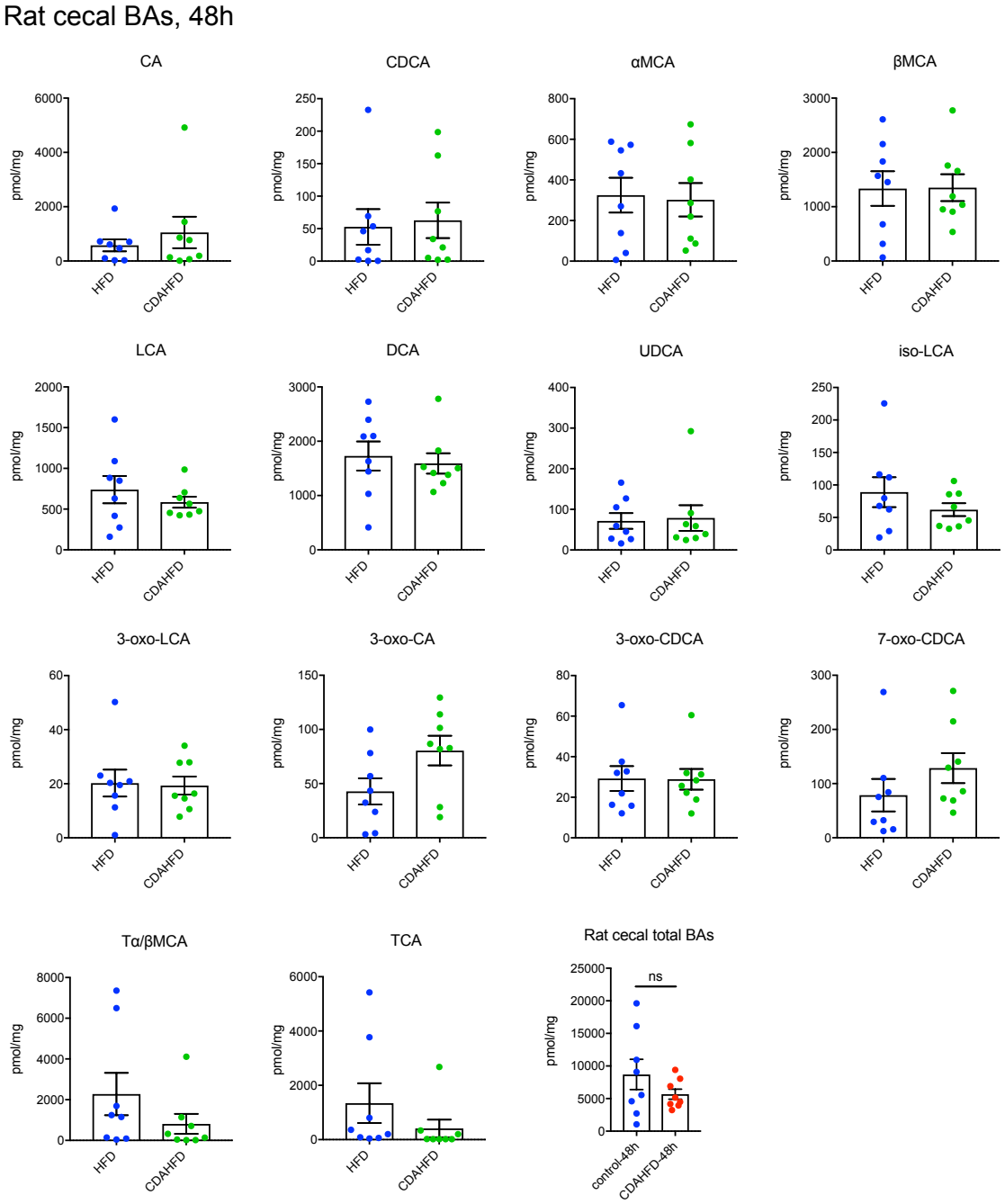
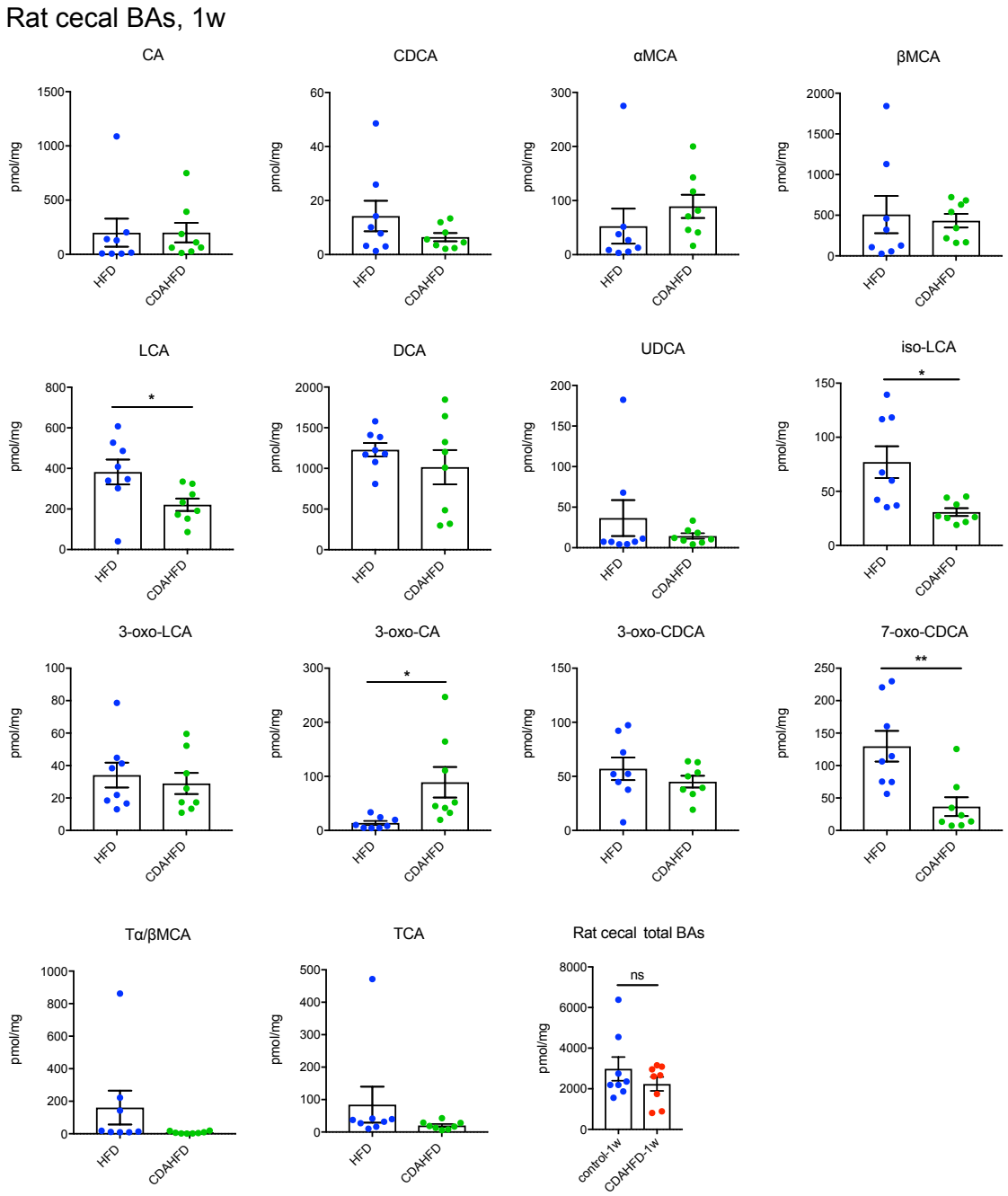

A
Control
CDAHFD
H&E
Sirius Red
B
Control
CDAHFD
Weight (g)
Weeks
C
Hydroxyproline
µg/g of tissue
Alanine Aminotransferase (ALT)
U/L
Aspartate Aminotransferase (AST)
U/L
Alkaline Phosphatase (ALP)
U/L
control-48h
CDAHFD-48h
control-1w
CDAHFD-1w
control-6w
CDAHFD-6w
control-12w
CDAHFD-12w
D
control-48h
CDAHFD-48h
control-1w
CDAHFD-1w
E
Col1a1
TNFα
Relative expression
F
control-48h
CDAHFD-48h
control-1w
CDAHFD-1w
Intestinal inflammation
Epithelial Hyperplasia
Goblet Cell Loss
Number of samples

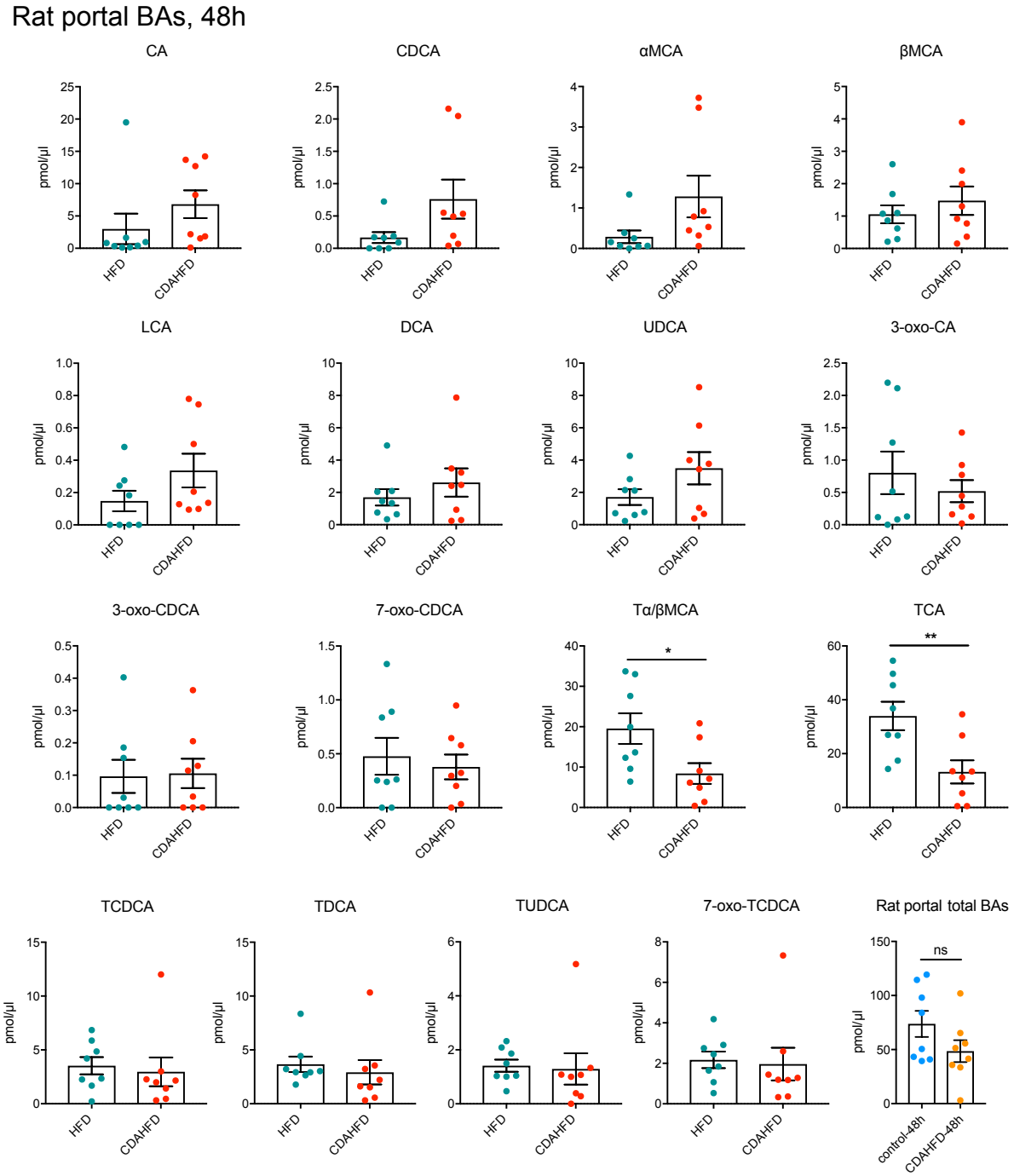
Supplementary Figure 1. Temporal development of liver damage in CDAHFD-fed rats. (A) CDAHFD-fed rats developed cirrhosis after 12 weeks of diet. Representative hematoxylin and eosin (H&E) and Sirius Red staining of livers from CDAHFD-fed and control rats after 12 weeks of diet. (B) Body weight of CDAHFD-fed and control rats, measured weekly. (C) CDAHFD-fed rats developed progressive hepatic inflammation and fibrosis as demonstrated by increased ALT, AST, alkaline phosphatase, and hepatic hydroxyproline from livers of control and CDAHFD-fed rats. n=8 per group for 48h and 1w timepoints (n=12 per group for 6w and 12w timepoints, one-way ANOVA followed by Tukey's multiple comparison test). (D) Histologic evidence of hepatic inflammation was evident after 1 week of CDAHFD but not after 48h. Representative H&E staining of liver tissue from control and CDAHFD-fed rats at indicated timepoints. (E) Expression of fibrosis and pro-inflammatory genes (hepatic *Col1a1* and *TNFα*, respectively) were increased after 1 week of CDAHFD but not after 48h as determined by qPCR (n=8 per group, two-tailed Welch's t test). (F) CDAHFD diet induced intestinal inflammation. Representative hematoxylin and eosin (H&E) staining of ileum from control and CDAHFD-fed rats with pathology scores (n=8 per group, Mann-Whitney test). ns = not significant, *p<0.05 **p<0.005, ***p<0.001, ****p<0.0001. Bars represent mean ± SEM.



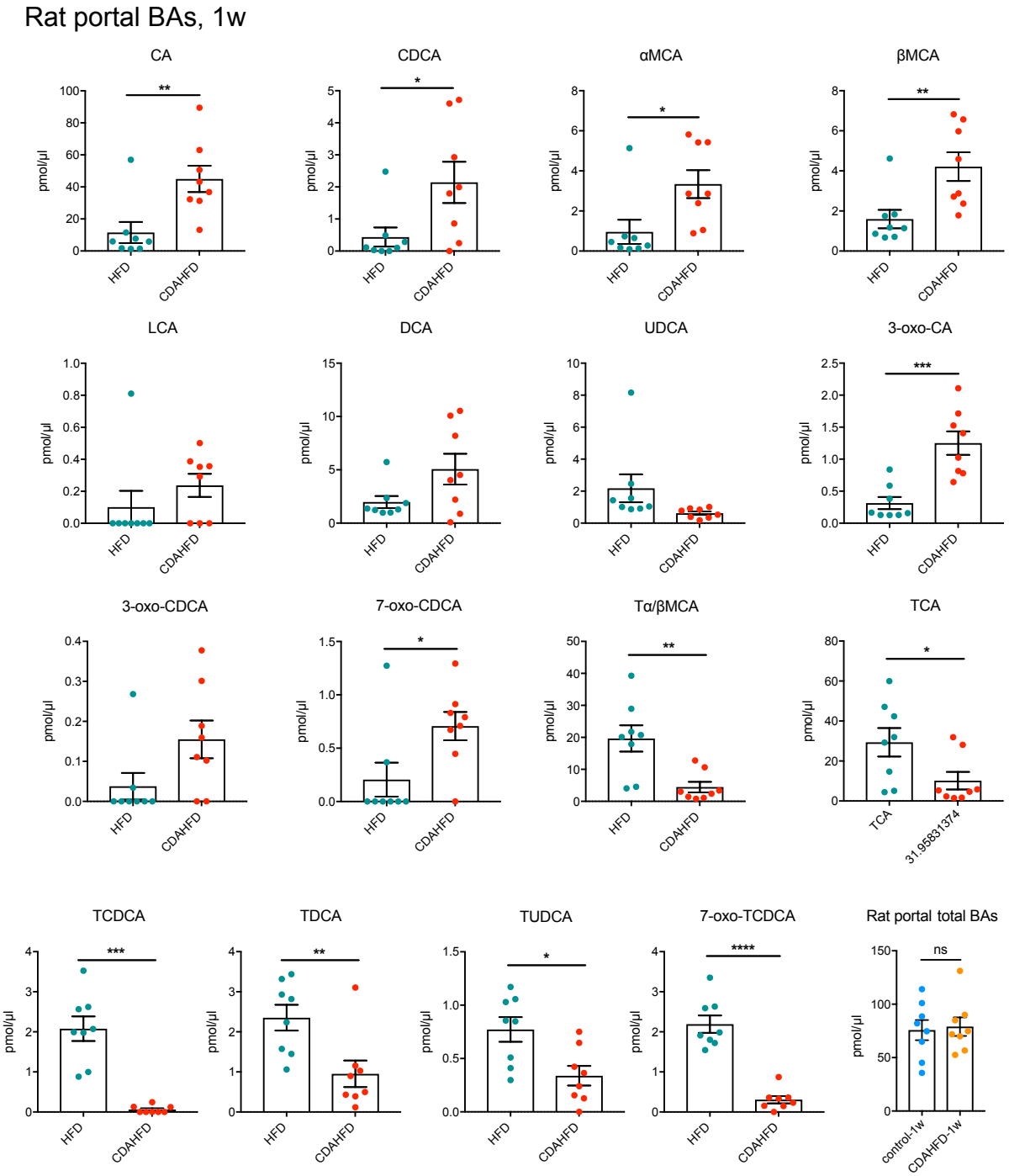
Supplementary Figure 2. **Bile acid concentrations in cecal contents of rats 48 hours post HFD control or CDAHFD diet intervention**. Bile acids were quantified using UPLC-MS. All bile acids with measurable concentrations above the limit of detection are shown (n=8 per group, two-tailed Welch's t test, data not marked with asterisk(s) were not significant, ns = not significant, bars represent mean ± SEM).



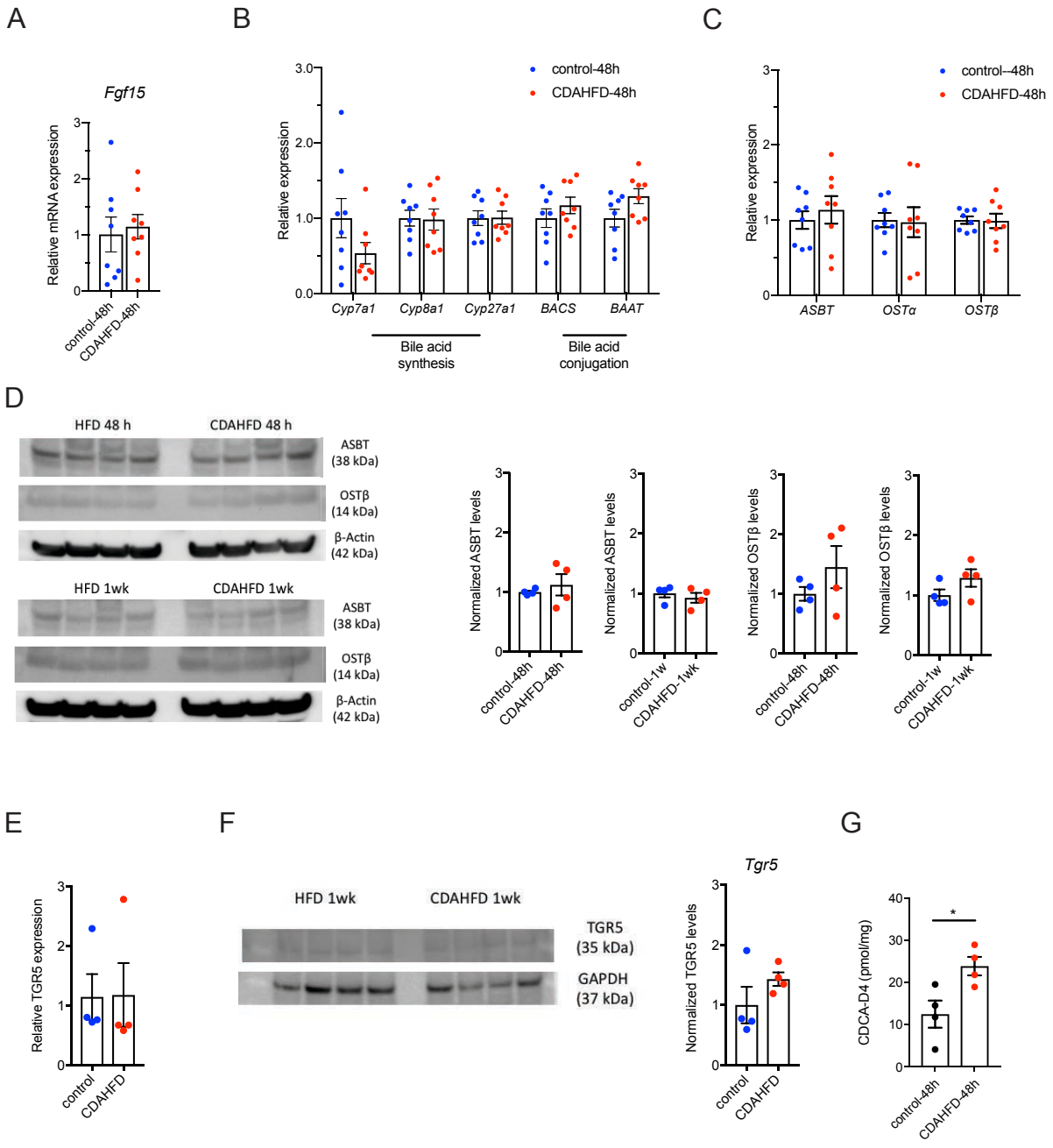
Supplementary Figure 3. **Bile acid concentrations in cecal contents of rats 1 week post HFD control or CDAHFD diet intervention**. Bile acids were quantified using UPLC-MS. All bile acids with measurable concentrations above the limit of detection are shown (n=8 per group, two-tailed Welch's t test, data not marked with asterisk(s) were not significant, ns = not significant, *p<0.05, **p<0.005. Bars represent mean ± SEM).



Supplementary Figure 4. **Bile acid concentrations in portal veins of rats 48 hours post HFD control or CDAHFD diet intervention**. Bile acids were quantified using UPLC-MS. All bile acids with measurable concentrations above the limit of detection are shown (n=8 per group, two-tailed Welch's t test, data not marked with asterisk(s) were not significant, ns = not significant, *p<0.05, **p<0.005. Bars represent mean ± SEM).



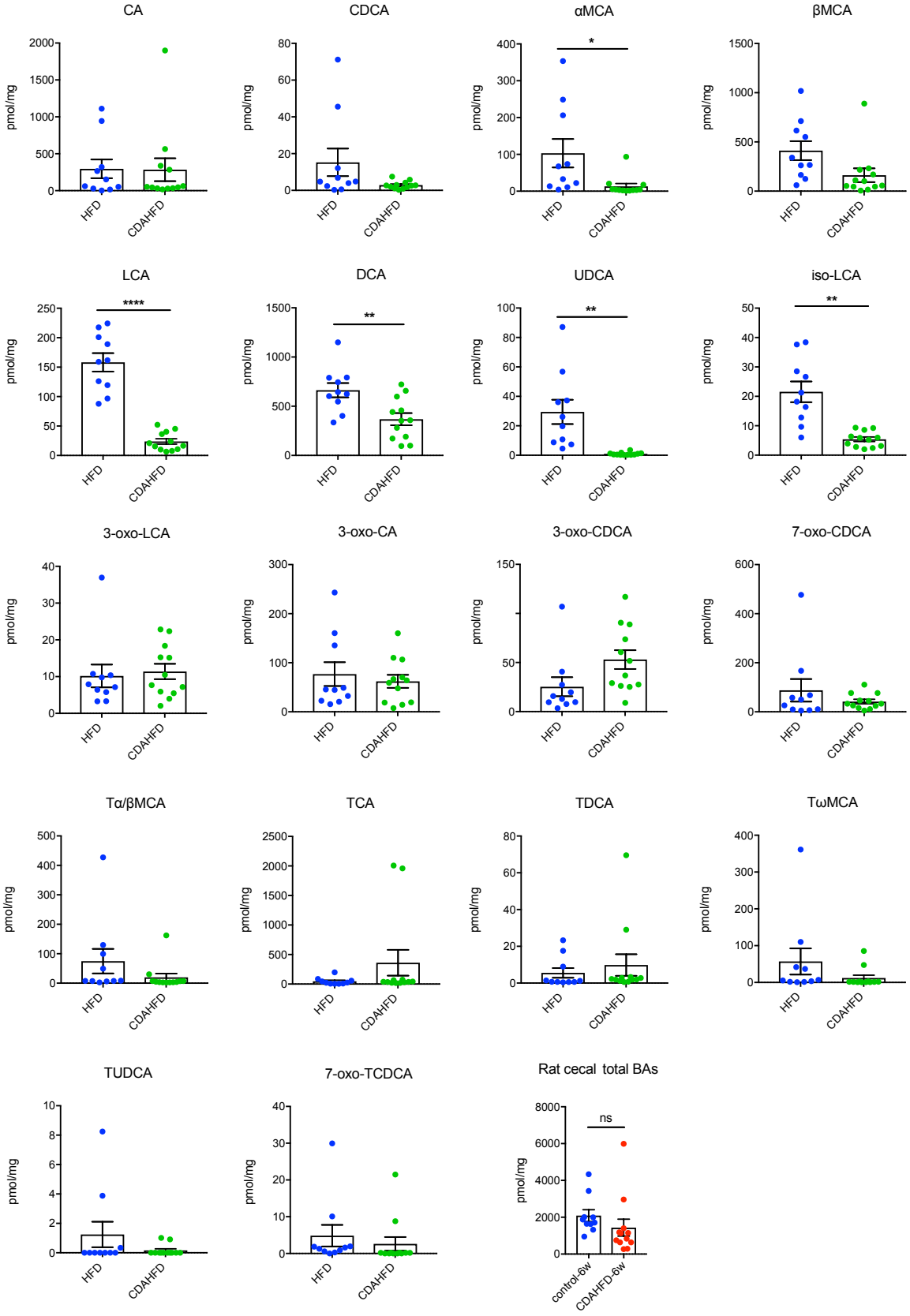
Supplementary Figure 5. **Bile acid concentrations in portal veins of rats 1 week post HFD control or CDAHFD diet intervention**. Bile acids were quantified using UPLC-MS. All bile acids with measurable concentrations above the limit of detection are shown (n=8 per group, two-tailed Welch's t test, data not marked with asterisk(s) were not significant, ns = not significant, *p<0.05, **p<0.005, ***p<0.001, ****p<0.0001. Bars represent mean ± SEM).



Supplementary Figure 6. **CDAHFD-fed rats displayed increased microbial BA deconjugation with no difference in FXR signaling, BA synthesis, conjugation, or transport.** (A) *Fgf15* expression as measured by RT-qPCR was unchanged 48 hours after dietary intervention (n=8 per group, two-tailed Welch's t-test). (B-D) Liver mRNA expression of bile acid synthesis and conjugation genes (B), and intestinal BA transporters measured by RT-qPCR (C) and Western blot analysis (D) were similar between control and CDAHFD-fed rats after 48h post-diet intervention (n=8 per group for RT-qPCR, n=4 for Western blot, two-tailed Welch's t test). (E-F) TGR5 mRNA (E) and protein levels (F) were similar between control

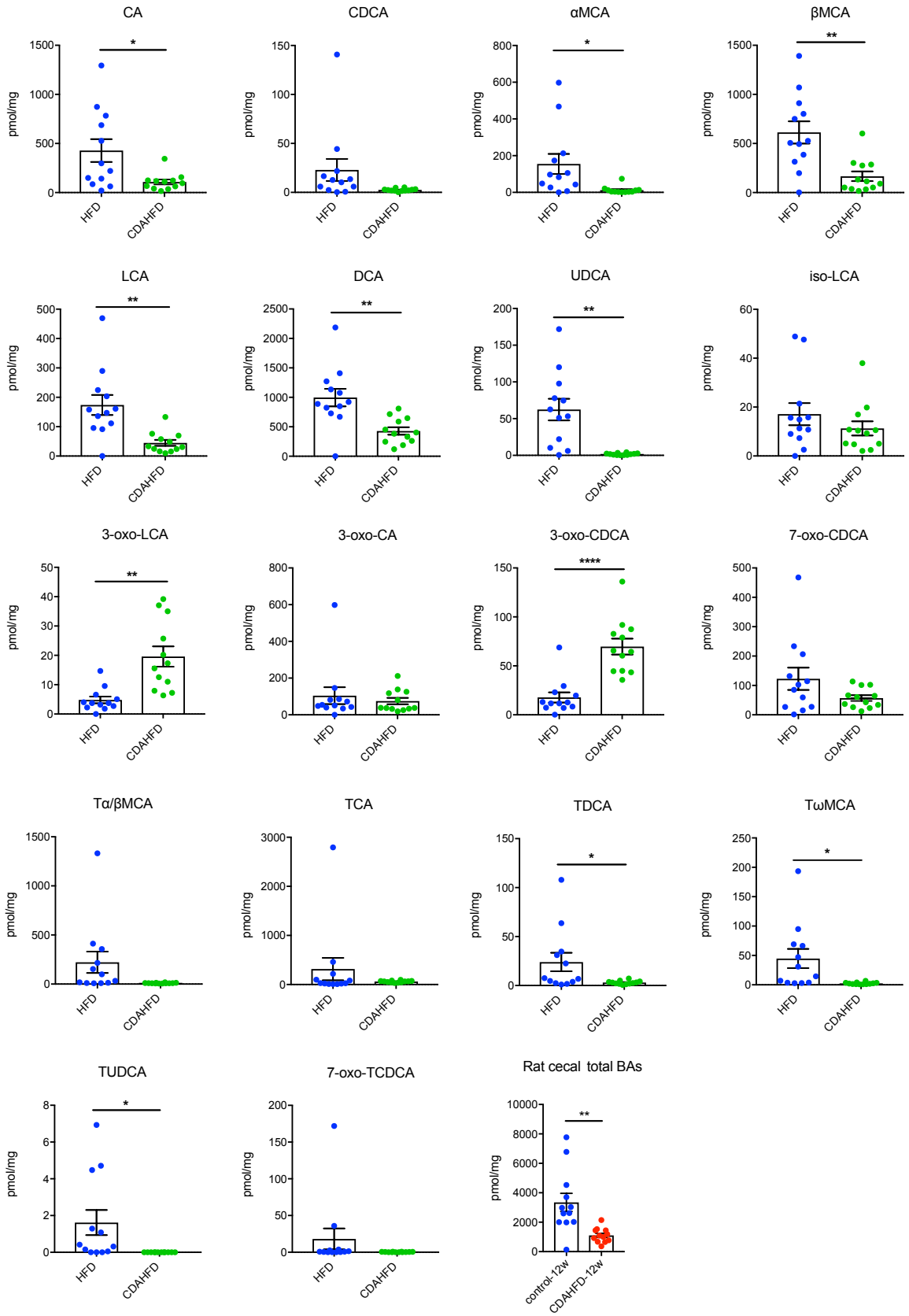
and CDAHFD-fed rats (n=4 per group, two-tailed Welch's t test). (G) Cecal BSH activity was increased in CDAHFD-fed rats compared to control rats at 48h of diet as measured by conversion of deuterated glyco-CDCA (GCDCA-D4) to deuterated CDCA (CDCA-D4) (n=4 per group, two-tailed Welch's t test). Data not marked with asterisk(s) were not significant, $*p<0.05$, bars represent mean ± SEM.

Rat cecal BAs, 6w



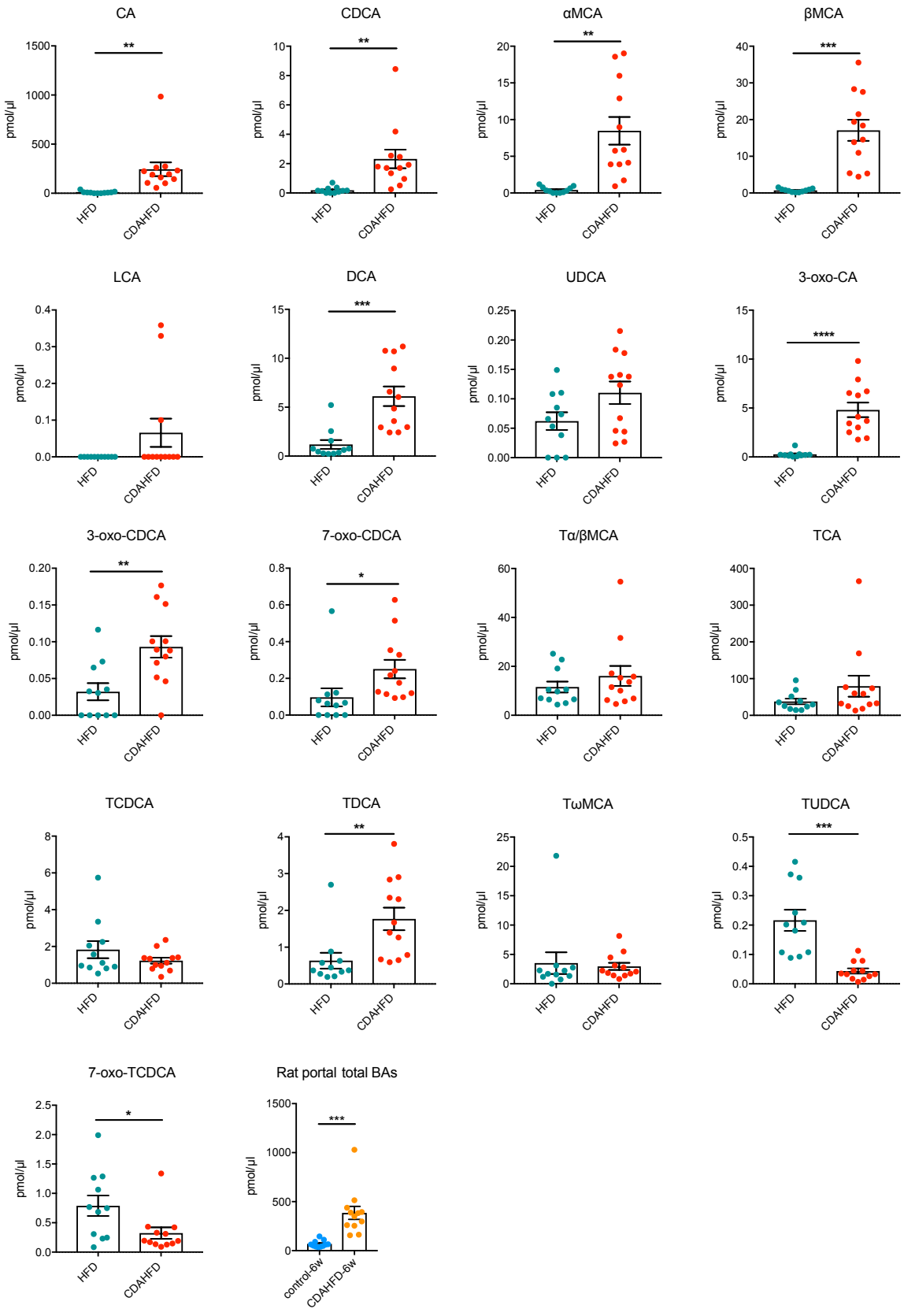
Supplementary Figure 7. **Bile acid concentrations in cecal contents of rats 6 weeks post HFD control or CDAHFD diet intervention**. Bile acids were quantified using UPLC-MS. All bile acids with measurable concentrations above the limit of detection are shown (HFD n=10, CDAFD n=12, two-tailed Welch's t test, data not marked with asterisk(s) were not significant, ns = not significant, *p<0.05, **p<0.005, ****p<0.0001, bars represent mean ± SEM).

Rat cecal BAs, 12w



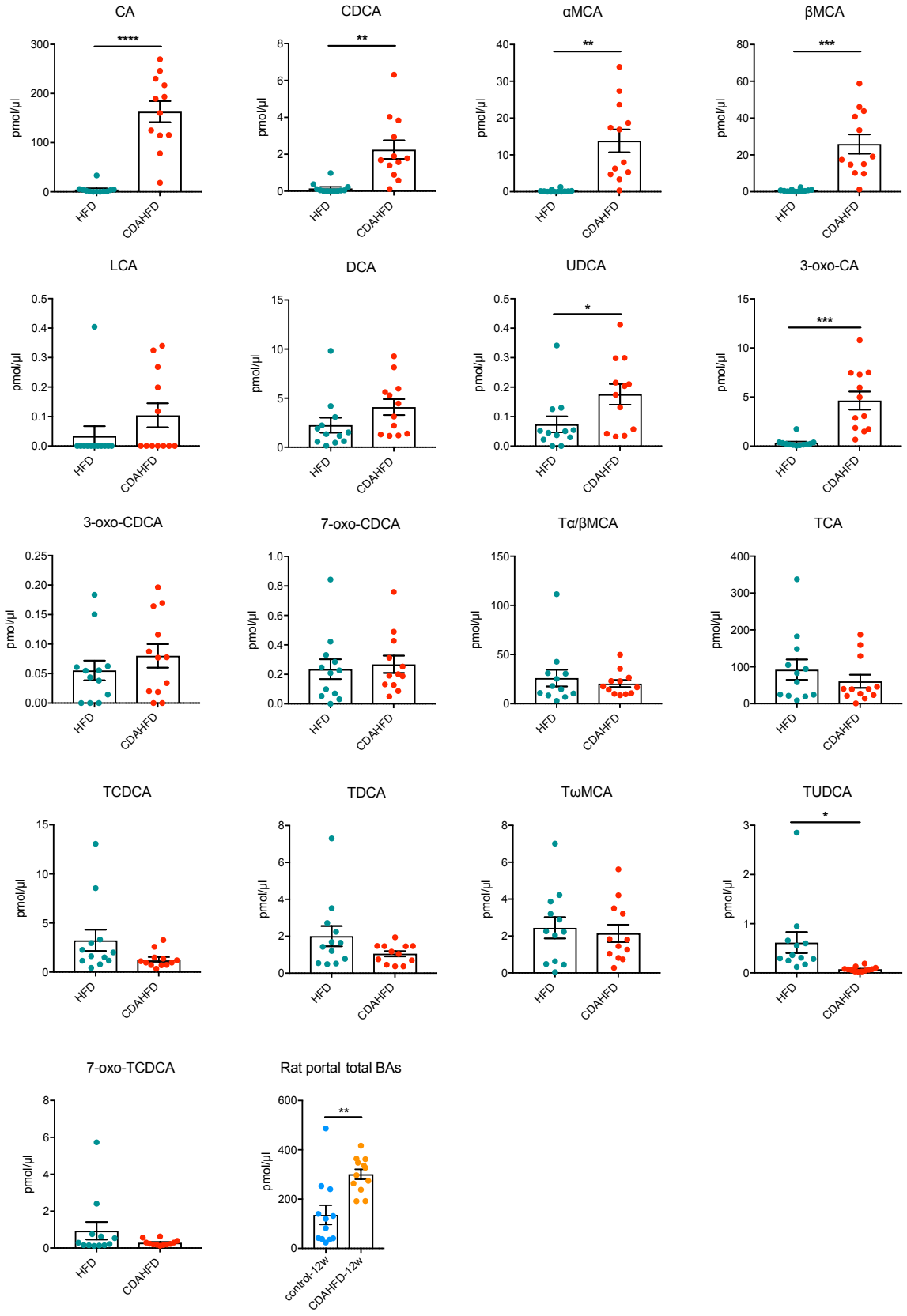
Supplementary Figure 8. **Bile acid concentrations in cecal contents of rats 12 weeks post HFD control or CDAHFD diet intervention**. Bile acids were quantified using UPLC-MS. All bile acids with measurable concentrations above the limit of detection are shown (n=12 per group, two-tailed Welch's t test, data not marked with asterisk(s) were not significant, *p<0.05, **p<0.005, ****p<0.0001, bars represent mean ± SEM).

Rat portal BAs, 6w

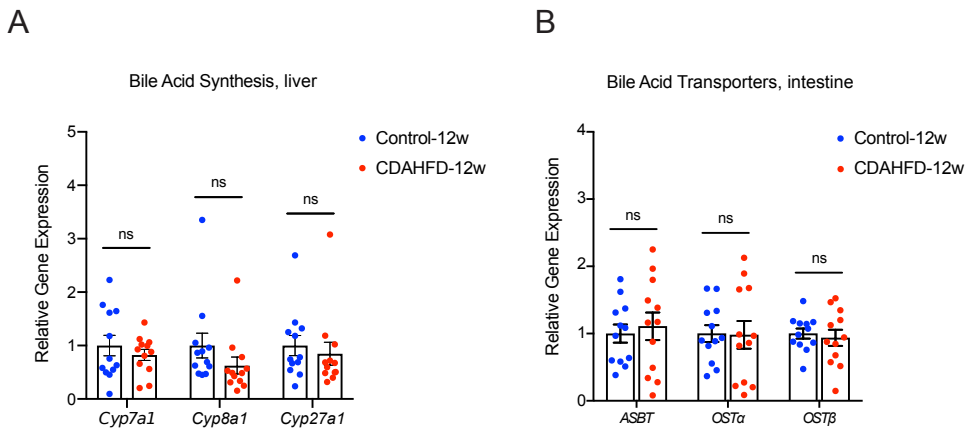


Supplementary Figure 9. **Bile acid concentrations in portal veins of rats 6 weeks post HFD control or CDAHFD diet intervention**. Bile acids were quantified using UPLC-MS. All bile acids with measurable concentrations above the limit of detection are shown (n=12 per group, two-tailed Welch's t test, data not marked with asterisk(s) were not significant, *p<0.05, **p<0.005, ***p<0.001, ****p<0.0001, bars represent mean ± SEM).

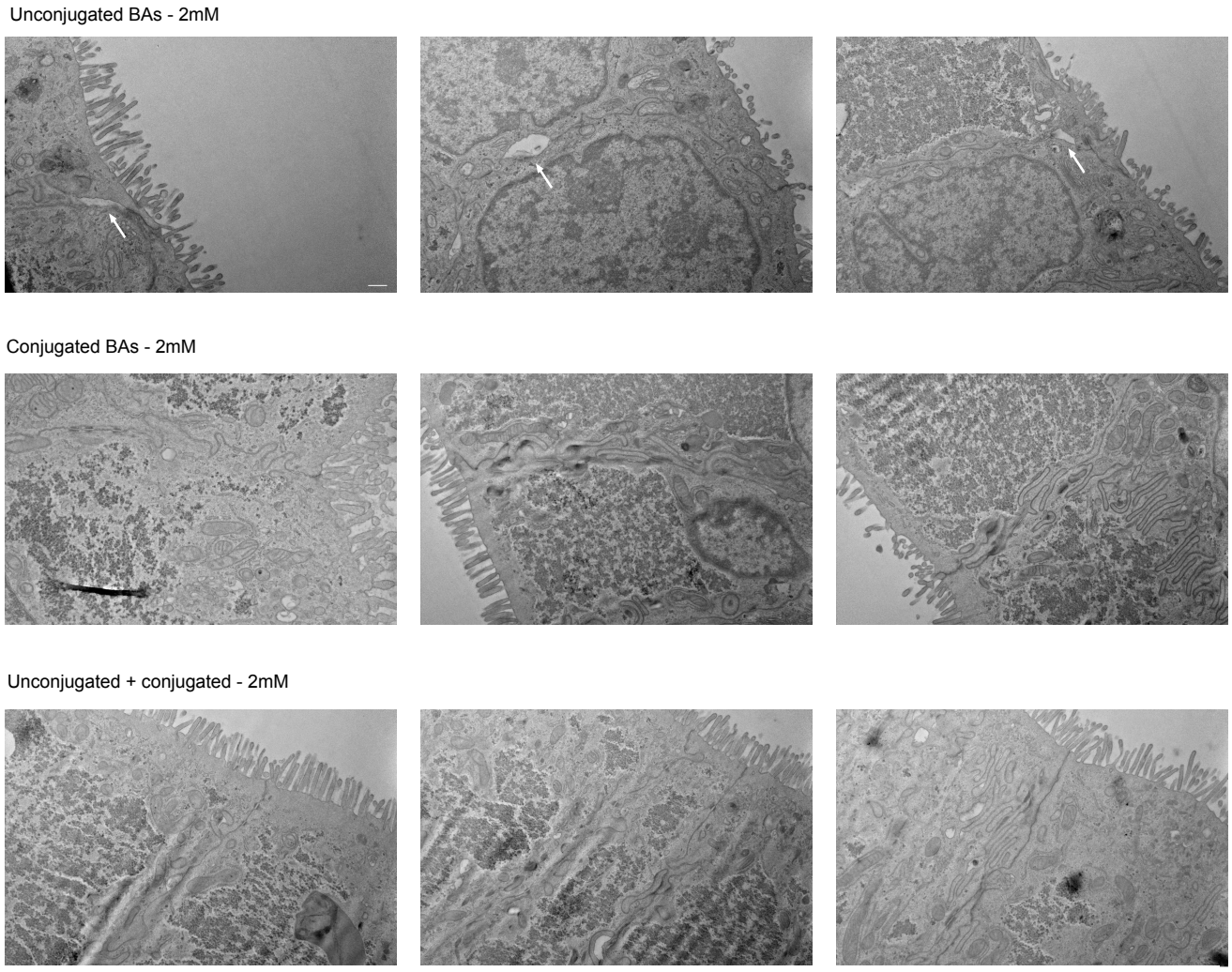
Rat portal BAs, 12w



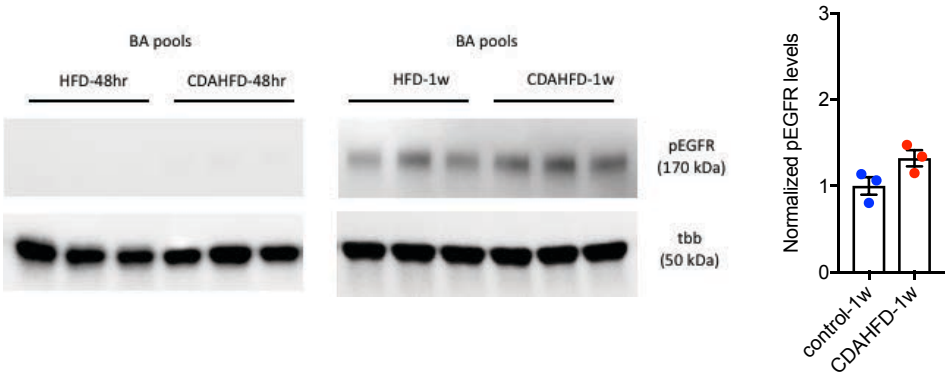
Supplementary Figure 10. **Bile acid concentrations in portal veins of rats 12 weeks post HFD control or CDAHFD diet intervention**. Bile acids were quantified using UPLC-MS. All bile acids with measurable concentrations above the limit of detection are shown (n=12 per group, two-tailed Welch's t test, data not marked with asterisk(s) were not significant, *p<0.05, **p<0.005, ***p<0.001, ****p<0.0001, bars represent mean ± SEM).



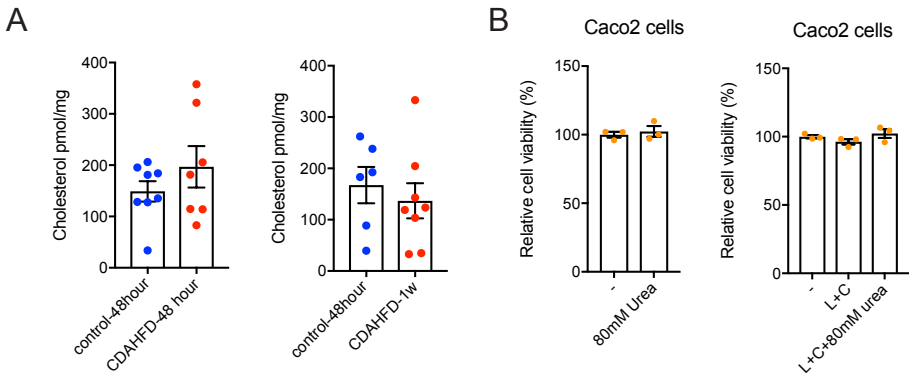
Supplementary Figure 11. **CDAHFD-fed rats at 12 weeks showed no difference in BA synthesis or transport.** (A, B) Liver mRNA expression of bile acid synthesis (A) and intestinal BA transporters (B) measured by RT-qPCR was similar between control and CDAHFD-fed rats after 12w post-diet intervention (n=12 per group, two-tailed Welch's t test). ns = not significant, bars represent mean ± SEM.



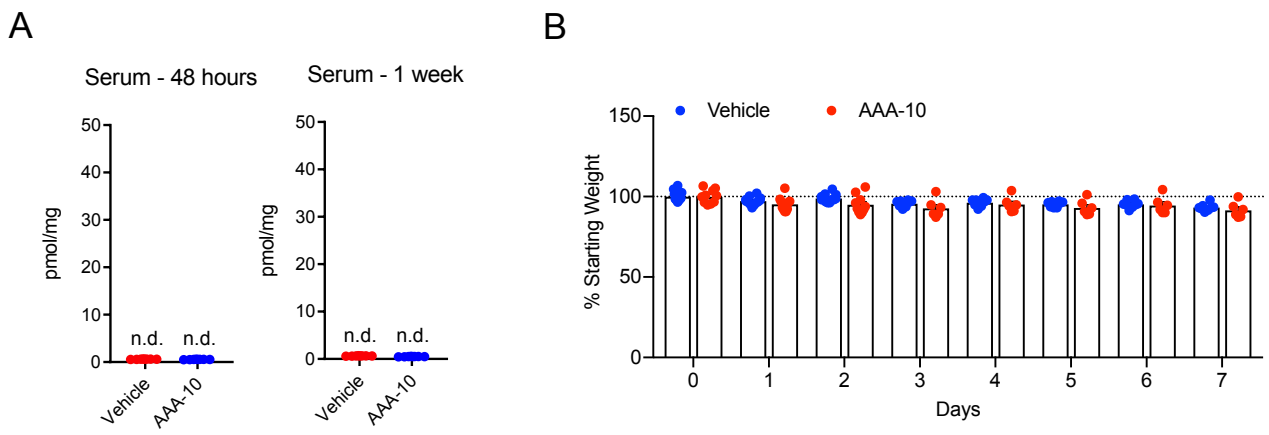
Supplementary Figure 12. Conjugated BAs prevented the development of unconjugated BA-induced tight junction dilatation. Additional representative EM images of Caco2 cells from transwells after exposure to conjugated, unconjugated, and combined BA pools at indicated concentrations. The white arrows point to examples of tight junction dilatation. Scale bar=500 nm.



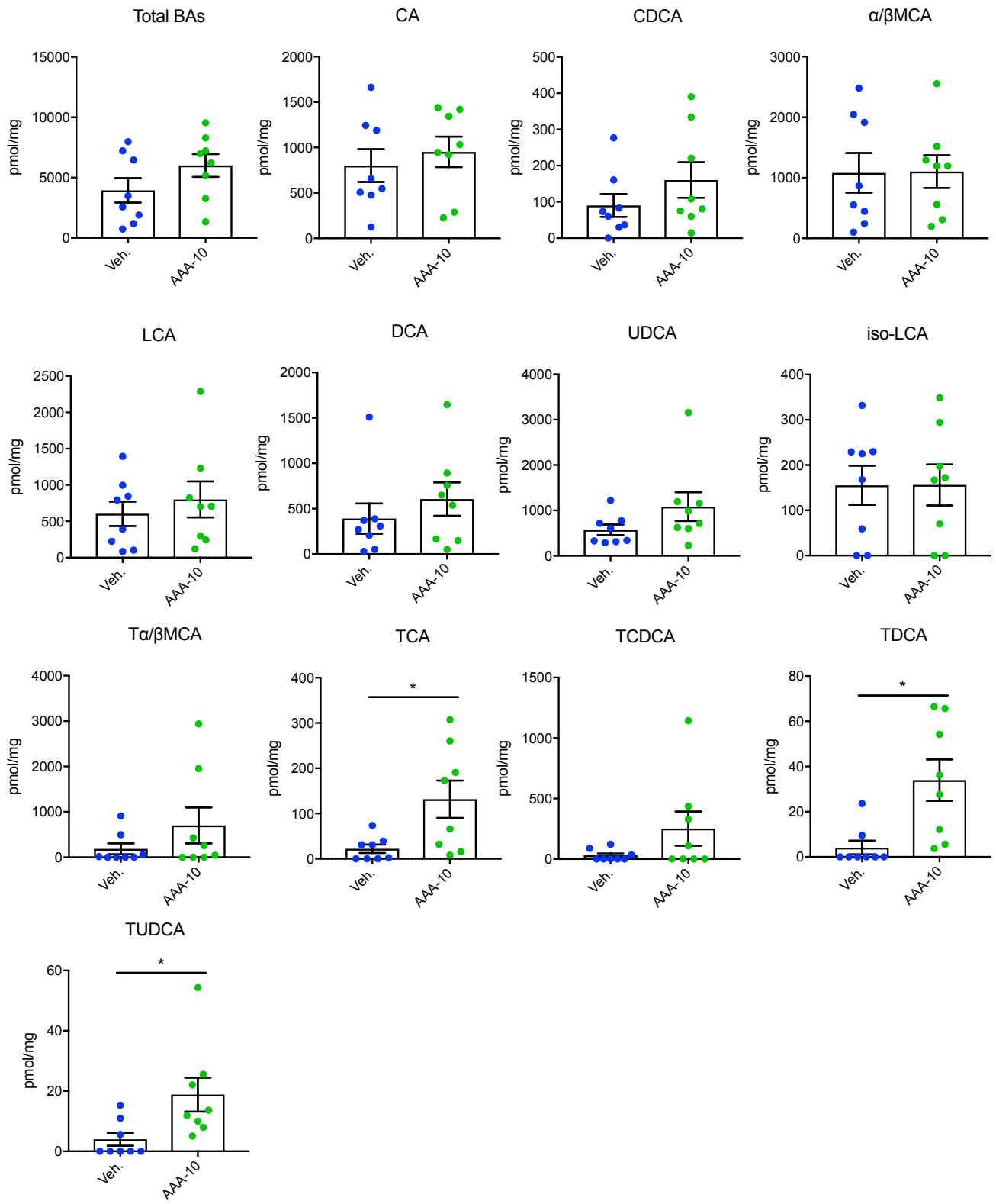
Supplemental Figure 13. CDAHFD-fed rat cecal BA pools did not differentially induce phosphorylation of EGFR compared to control BA pools on Caco-2 cells. Phosphorylated EGFR (pEGFR) and tubulin (tbb) protein levels measured by Western blot analysis in Caco-2 cells that were treated for 20 min with indicated pools of BAs (n=3 per group, not significant, two-tailed Welch's t-test).



Supplemental Figure 14. Cholesterol levels were similar in CDAHFD-fed rat and control rat ceca and urea, cholesterol, and lecithin did not affect Caco-2 viability. (A) Cholesterol levels in CDAHFD and HFD-fed rats at 48 hour and 1 week time points as measured in rat cecum (n=8 control, n=7 CDAHFD in 48 hour group; n=6 control, n=8 CDAHFD in 1 week group, not significant, two-tailed Welch's t-test). Average amount of cholesterol found in the cecum is 150 pmol/mg, which is approximately 150 μM. (B) MTT assay determined that 80 mM urea with or without lecithin (1 mM) and cholesterol (150 μM) does not affect Caco-2 cell viability (n=3 per group, not significant, two-tailed Welch's t-test). Bars represent mean ± SEM.



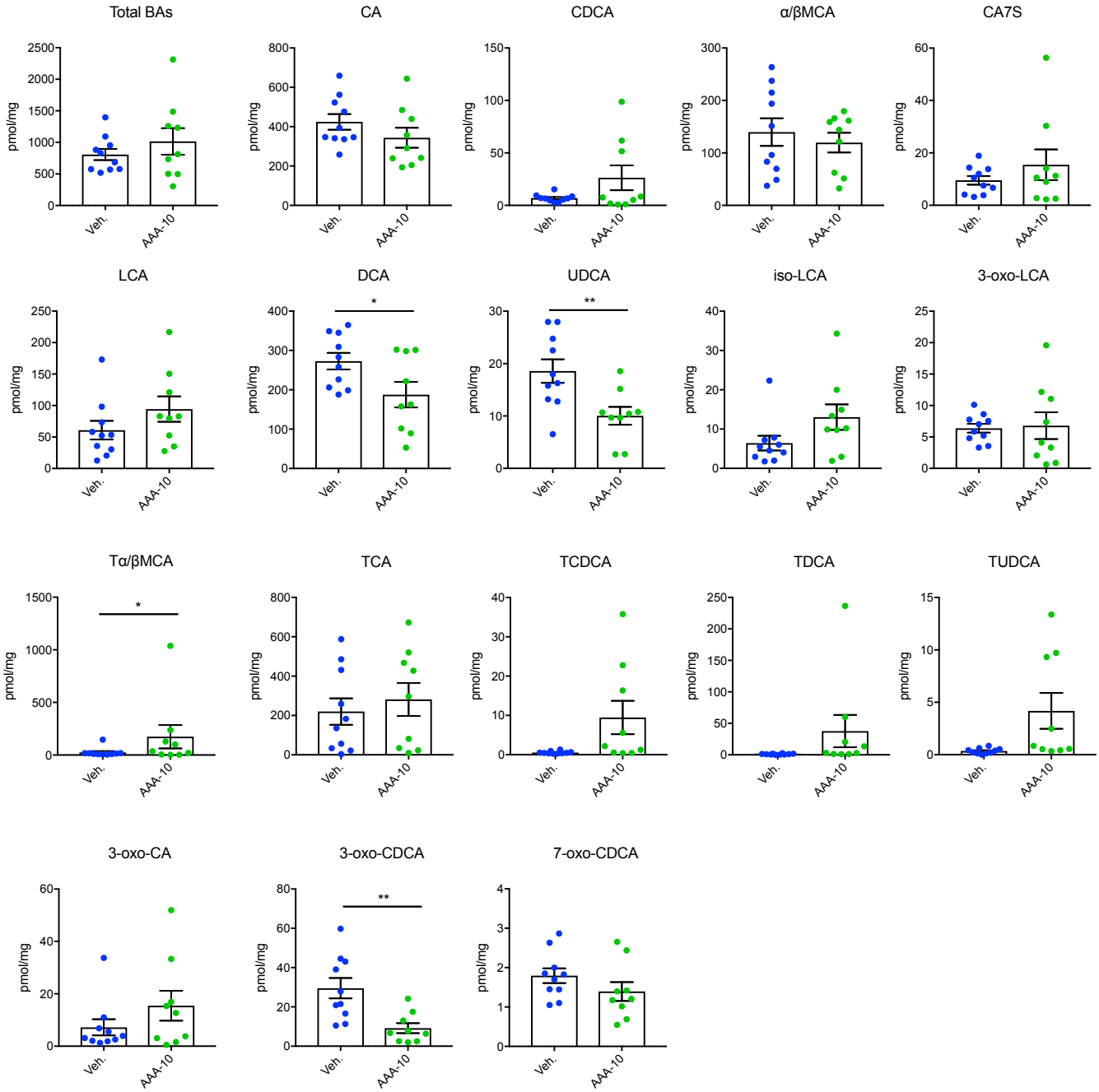
Supplemental Figure 15. Metrics for AAA-10 treatment of CDAHFD rats after 48 hours and 1 week. (A) UPLC-MS analysis of serum demonstrated no detection of AAA-10 in treated animals (n=8 per group, two-tailed Welch's t test). (B) Weight of vehicle and AAA-10 treated CDAHFD-fed rats over 7 days of treatment normalized to starting weight (n=8 per group, two-tailed Welch's t test). Data not marked with asterisk(s) were not significant, ns = not significant, bars represent mean ± SEM.



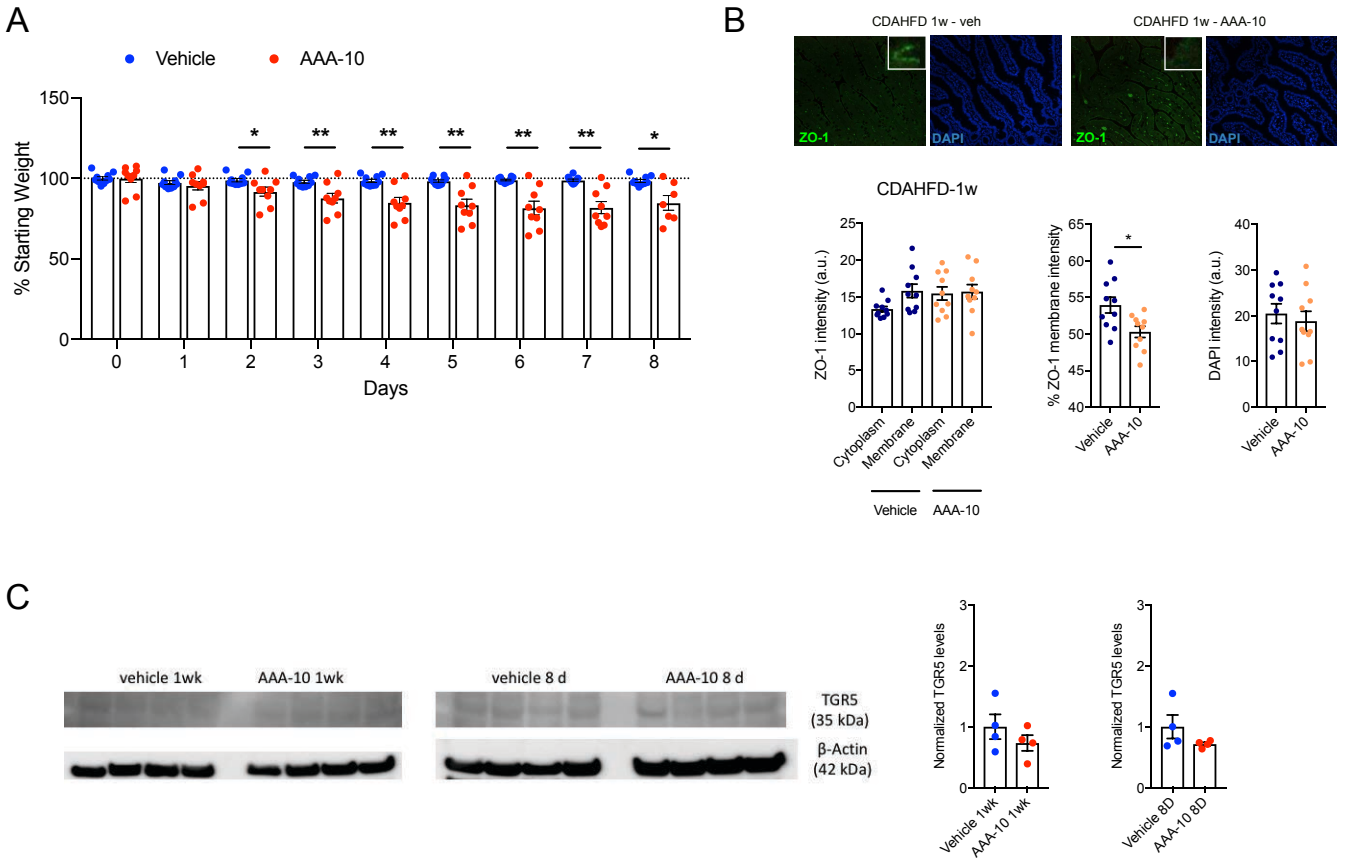
Supplementary Figure 16. **Bile acid concentrations in cecal contents of rats 1 week post CDAHFD diet intervention and AAA-10 treatment**. Bile acids were quantified using UPLC-MS. All bile acids with

measurable concentrations above the limit of detection are shown (n=8 per group, two-tailed Welch's t test, data not marked with asterisk(s) were not significant, *p<0.05, bars represent mean ± SEM).

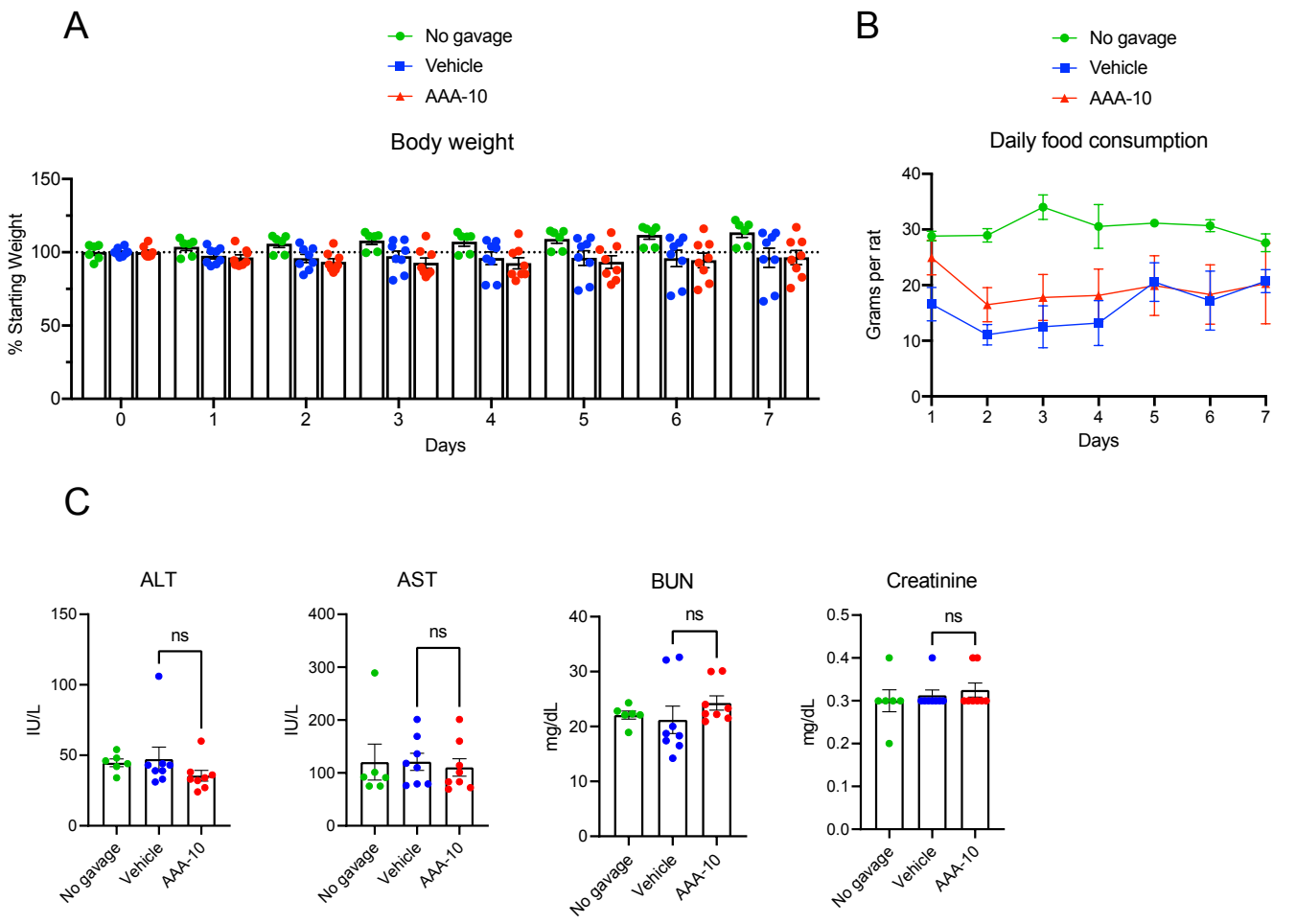
8 day AAA-10 treatment, cecal BAs



Supplementary Figure 17. **Bile acid concentrations in cecal contents of rats 8 days post CDAHFD diet intervention and AAA-10 treatment**. Bile acids were quantified using UPLC-MS. All bile acids with measurable concentrations above the limit of detection are shown (n=8 per group, two-tailed Welch's t test, data not marked with asterisk(s) were not significant, *p<0.05, **p<0.005, bars represent mean ± SEM).



Supplemental Figure 18. Metrics and TGR5 analysis for AAA-10 treatment of CDAHFD rats for 8 days. (A) Weight of vehicle and AAA-10 treated CDAHFD-fed rats over 8 days of treatment normalized to starting weight (n=10 in vehicle group, n=9 in AAA-10 group except day 8, where n=7 in AAA-10 group, two-tailed Welch's t test). (B) AAA-10 treatment prevented aberrant ZO-1 subcellular localization in absence of weight loss. ZO-1 immunofluorescence and DAPI counterstaining of rat ileum with quantification from vehicle and AAA-10 treated CDAHFD-fed rats at indicated timepoints. Intestines from 4 animals per group were stained (n=10 intestinal cell images per group were analyzed. For ZO-1 intensity, one-way ANOVA followed by Tukey's multiple comparison test, for %ZO-1 membrane intensity and DAPI intensity, two-tailed Welch's t test). (C) TGR5 protein levels were similar between vehicle and AAA-10 treated rats (n=4 per group, two-tailed Welch's t test). Data not marked with asterisk(s) were not significant). *p<0.05, **p<0.005. Bars represent mean ± SEM.



Supplemental Figure 19. Toxicity assessment of AAA-10 in chow-fed rats. (A) No difference in body weight was observed between AAA-10-treated and vehicle-treated rats. Daily body weights measured and normalized to starting weight on day 0 (n=8 per group for both vehicle- and AAA-10 treated, n=6 in no gavage control, not significant, one-way ANOVA followed by Tukey's multiple comparison test). (B) AAA-10 did not induce differences in food consumption between vehicle and AAA-10 treated rats. Daily food consumption was monitored (n=8 per group for both vehicle- and AAA-10 treated, n=6 in no gavage control, not significant one-way ANOVA followed by Tukey's multiple comparison test). (C) Serum measurement of alanine aminotransferase (ALT), aspartate aminotransferase (AST), blood urea nitrogen (BUN), and creatinine (n=8 per group for both vehicle- and AAA-10 treated, n=6 in no gavage control, one-way ANOVA followed by Tukey's multiple comparison test). Data not marked with asterisk(s) were not significant, ns = non-significant, bars represent mean ± SEM.

Supplementary Table 1. Primer sequences used in this manuscript for quantitative PCR analysis.

Gene	Forward Sequence (5' to 3')	Reverse Sequence (5' to 3')
CYP7A1	GGGCAGGCTTGGGAATTTTG	GGGCAGGCTTGGGAATTTTG
CYP8B1	CAGGTTGGAAGCCGAGACAT	CAGGTTGGAAGCCGAGACAT
CYP27A1	TGGACAACCACCTTTGGGAC	TGGACAACCACCTTTGGGAC
BACS	TTCAGGGACCACTGGACTTCCAAA	ACCACATCATCAGCTGTTCTCCCA
BAAT	GGTTGGCATCCTTTCTGTGTGCAT	ATTCTTCACTGCAGGGTGTAGGCT
ASBT	GGTTGCGCTTGTTATTCCTGT	GGTTCAATGATCCAGGCACTT
OSTα	GGCCCTTTCCAGTATGCCTT	CAGGTGCAACTTGGCTTGAC
OSTβ	GAAGCAGCCACAAGACAACG	TCTCTTAGGATGCCCAGGCT
Col1a1	TCTGACTGGAAGAGCGGAGA	GGGTTTGGGCTGATGTACCA
Acta2	GGAGATGGCGTGACTCACAA	CGCTCAGCAGTAGTCACGAA
IL-1β	CACCTCTCAAGCAGAGCACA	ACGGGTTCCATGGTGAAGTC
Tnfα	ATGGGCTCCCTCTCATCAGT	TTTGCTACGACGTGGGCTAC
IFNγ	CGAGGTGAACAACCCACAGA	TTTGCTACGACGTGGGCTAC
Cxcl2	ACCATCAGGGTACAGGGGTT	CAACCCTTGGTAGGGTCGTC
Cxcl9	GTTTGCCCCAAGCCCTAACT	GCTGAATCTGGGTCTAGGCA
Cxcl16	TTTGGACCCTTGGCCCTTAC	AGTAGCAACTTCCAGCGACA
Ccl2	AGTTAATGCCCCACTCACCTG	GTAGTTCTCCAGCCGACTCA
TGR5	CTGGGCTACTCACAGGGTTG	CAGATTGGCAAGCAGGGAGA
GAPDH	ATGACTCTACCCACGGCAAG	CTGGAAGATGGTGATGGGTT