SUPPLEMENTARY DATA

Lactational delivery of Triclosan promotes non-alcoholic fatty liver disease in newborn mice

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Short Title: *Triclosan drives neonatal NAFLD*

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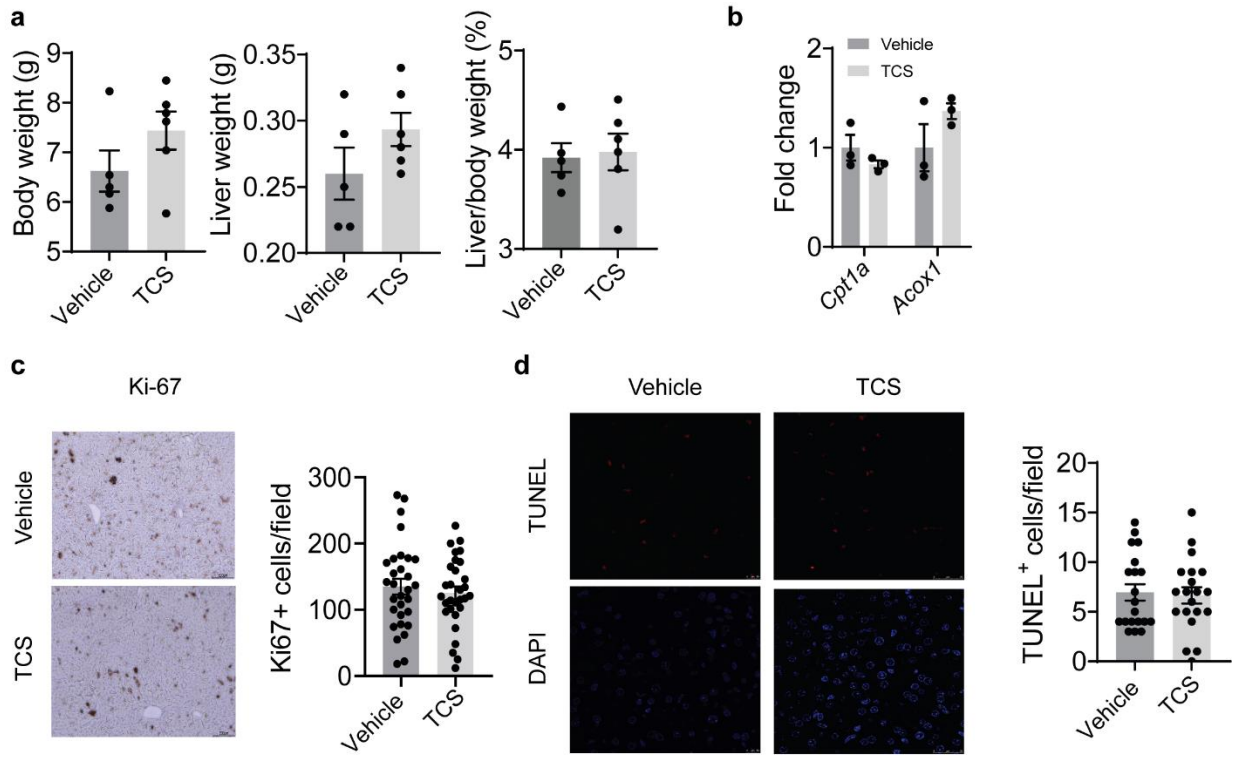

Figure S1. **TCS has no effect on body and liver weight and hepatic proliferation and cell death.** WT mice breastfed with vehicle or TCS for 21-days-old. **a**, Body weight, liver weight and Liver/Body Weight ratio (%) (n=5-6 mice per group). **b.** Expression of genes associated with fatty acid oxidation *Cpt1a* and *Acox1* (n=3 mice per group). **c**, Hepatocyte's proliferation was determined by Ki-67 immunostaining and quantitation of positive cells (n=30 images/5 mice per group). **d**, Liver cell apoptosis was determined by TUNEL staining and quantitation of positive cells (n=20 images/5 mice per group). Scale bars: **c**, 200µm and **d**, 20µm. **a-d** show mean ± S.E., determined by two-tailed Student`s test.

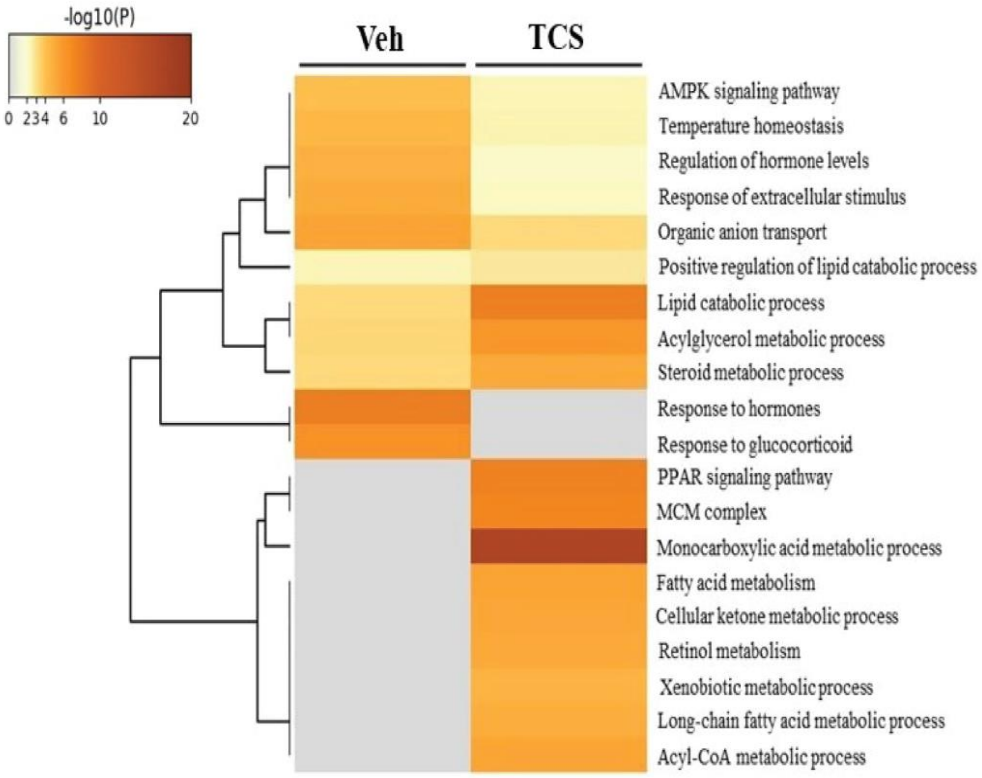

Figure S2. TCS-induced alterations in the liver transcriptome. WT mice breastfed with vehicle or TCS for 21-days-old. Heatmap depicting expression of HCC-related genes in liver tissue of neonatal mice treated with vehicle or TCS (n=3 mice per group). All genes showed in this figure had a *p*-adjusted value above 0.05, showing no differences between groups.

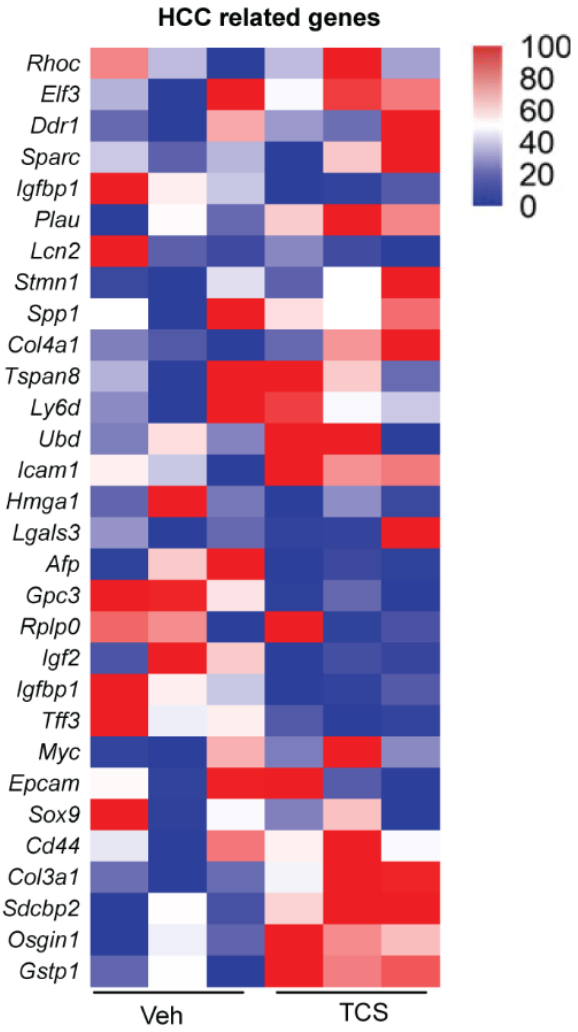

Figure S3. TCS has little effect on HCC-related genes expression. WT mice breastfed with vehicle or TCS for 21-days-old. Heatmap depicting expression of HCC-related genes in liver tissue of neonatal mice treated with vehicle or TCS (n=3 mice per group). All genes showed in this figure had a *p*-adjusted value above 0.05, showing no differences between groups.

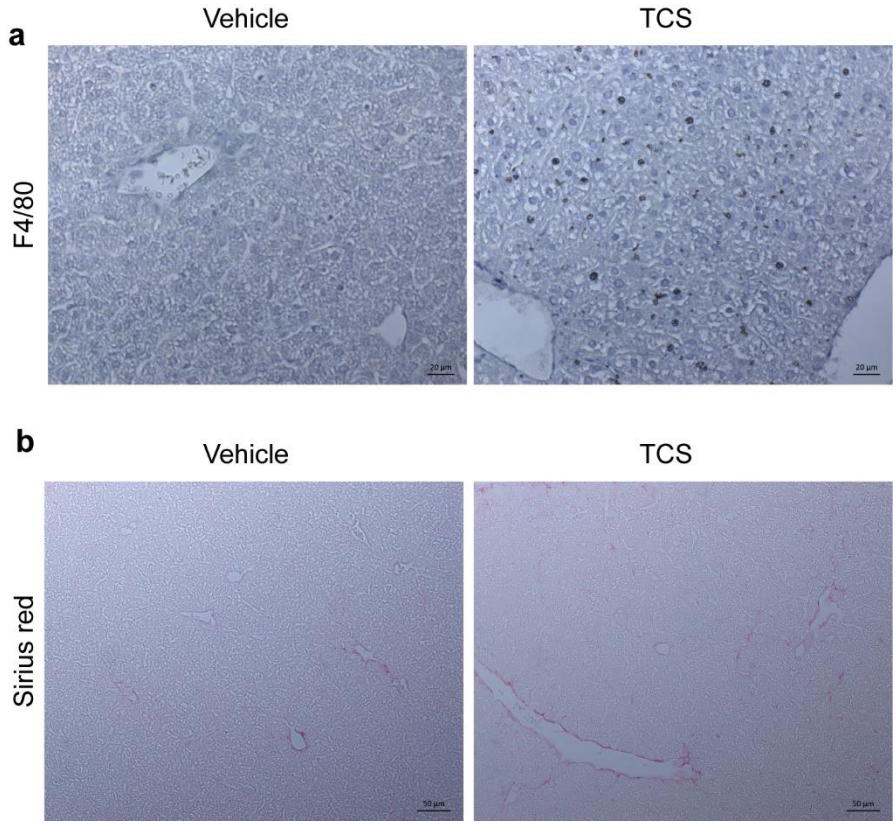

Figure S4. TCS increases immune infiltration and fibrosis. WT mice breastfed with vehicle or TCS milk for 21-days-old. **a**, IHC of formalin-fixed paraffin-embedded liver sections from vehicle and TCS group F4/80 immunostaining show macrophage infiltration (n=4 mice per group). **b**, Frozen sections were stained by Sirius Red to visualize collagen deposition (n=4 mice per group). Scale bars: **a**, 20µm and **b**, 50µm.

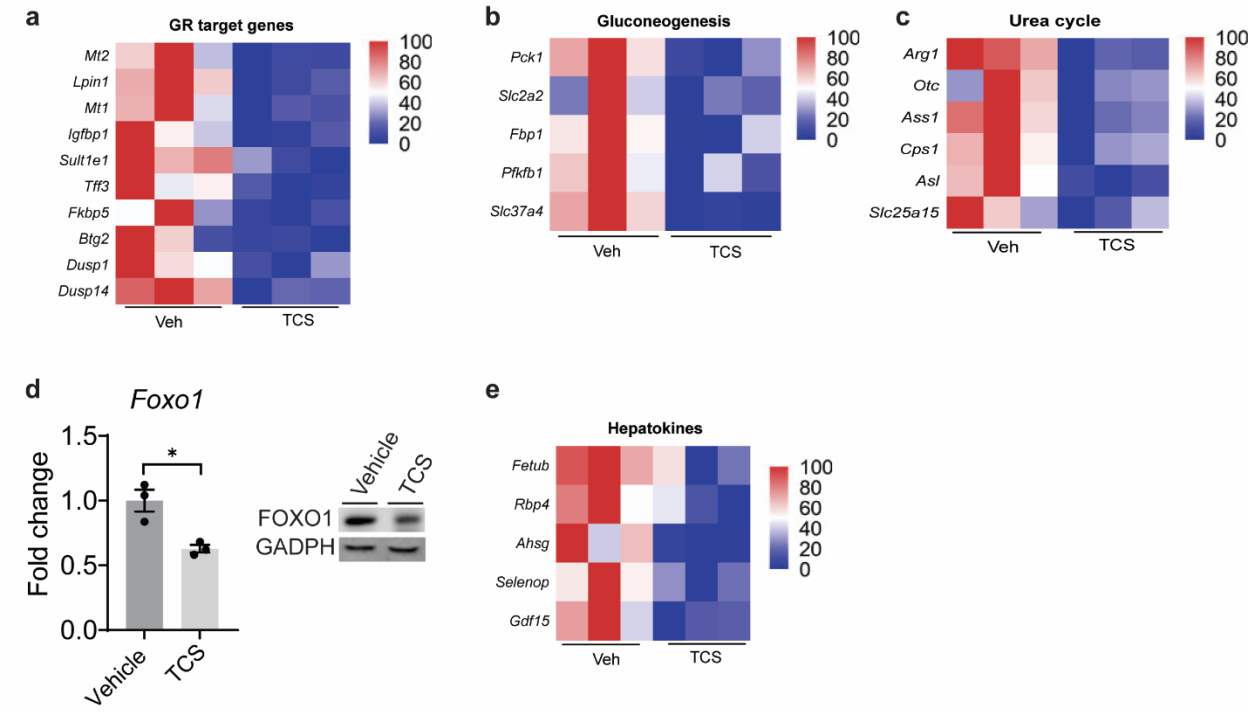

Figure S5. TCS downregulates glucocorticoid receptor and downstream pathways. WT mice breastfed with vehicle or TCS for 21-days-old. **a-c** Heatmaps showing differential expression of genes associated with glucocorticoid receptor (GR) signaling (**a**), and genes associated with downstream GR pathways, gluconeogenesis (**b**) and urea cycle (**c**) (n=3 per group). **d**, gene expression and representative IB image of FOXO1 in liver. **e**, Heatmap showing differential expression of hepatokines in liver (n=3 per group). **d** show mean ± S.E., determined by two-tailed Student`s test; **P*<0.05. All genes used are significant and p adjusted value below the cut-off level of 0.05.

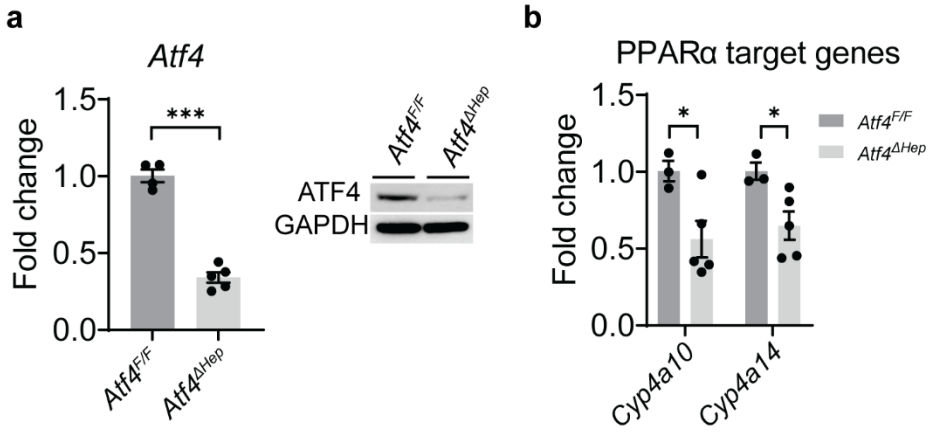

Figure S6. Downstream **PPARα target genes are downregulated in *Atf4*$^{\Delta Hep}$ mice treated with TCS.** *Atf4*$^{F/F}$ and *Atf4*$^{\Delta Hep}$ mice breastfed with TCS milk 21-days-old. **a**, Hepatic expression of ATF4 gene in both *Atf4*$^{F/F}$ and *Atf4*$^{\blacktriangle Hep}$ livers and IB of ATF4 (n=4-5 mice per group). **b**, Hepatic expression of downstream PPARα target genes in *Atf4*$^{F/F}$ and *Atf4*$^{\blacktriangle Hep}$ (n=3-5 mice per group). **a, b** show mean ± S.E., determined by two-tailed Student`s test; *P<0.05, ***P<0.001.

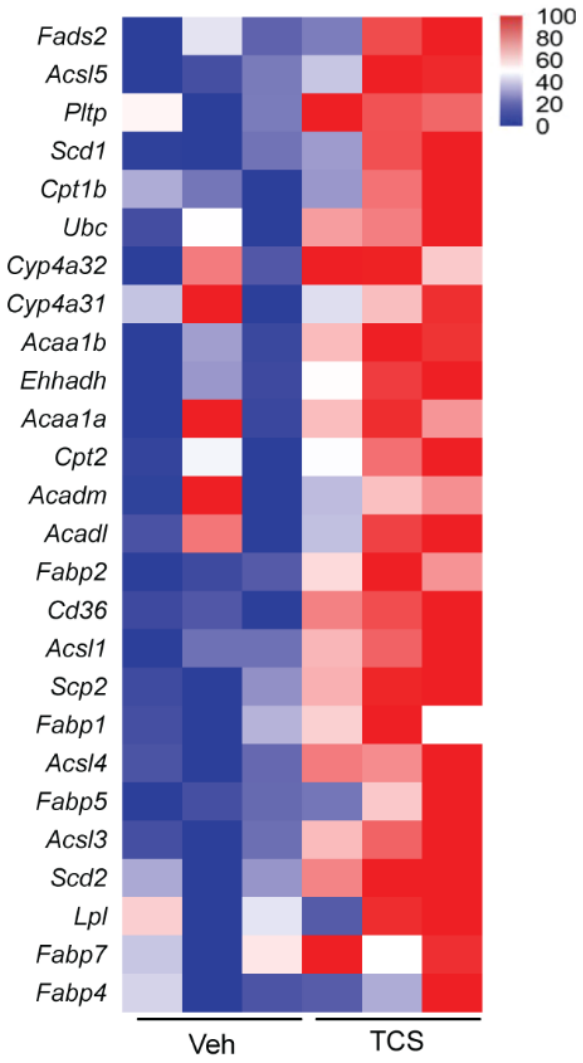

Figure S7. TCS has strong effect on PPARα target genes. WT mice breastfed with vehicle or TCS for 21-days-old. Heatmap depicting expression of downstream PPARα target genes in liver tissue of neonatal mice treated with vehicle or TCS (n=3 mice per group). All genes showed in this figure had a p-adjusted value above 0.05, showing no differences between groups.

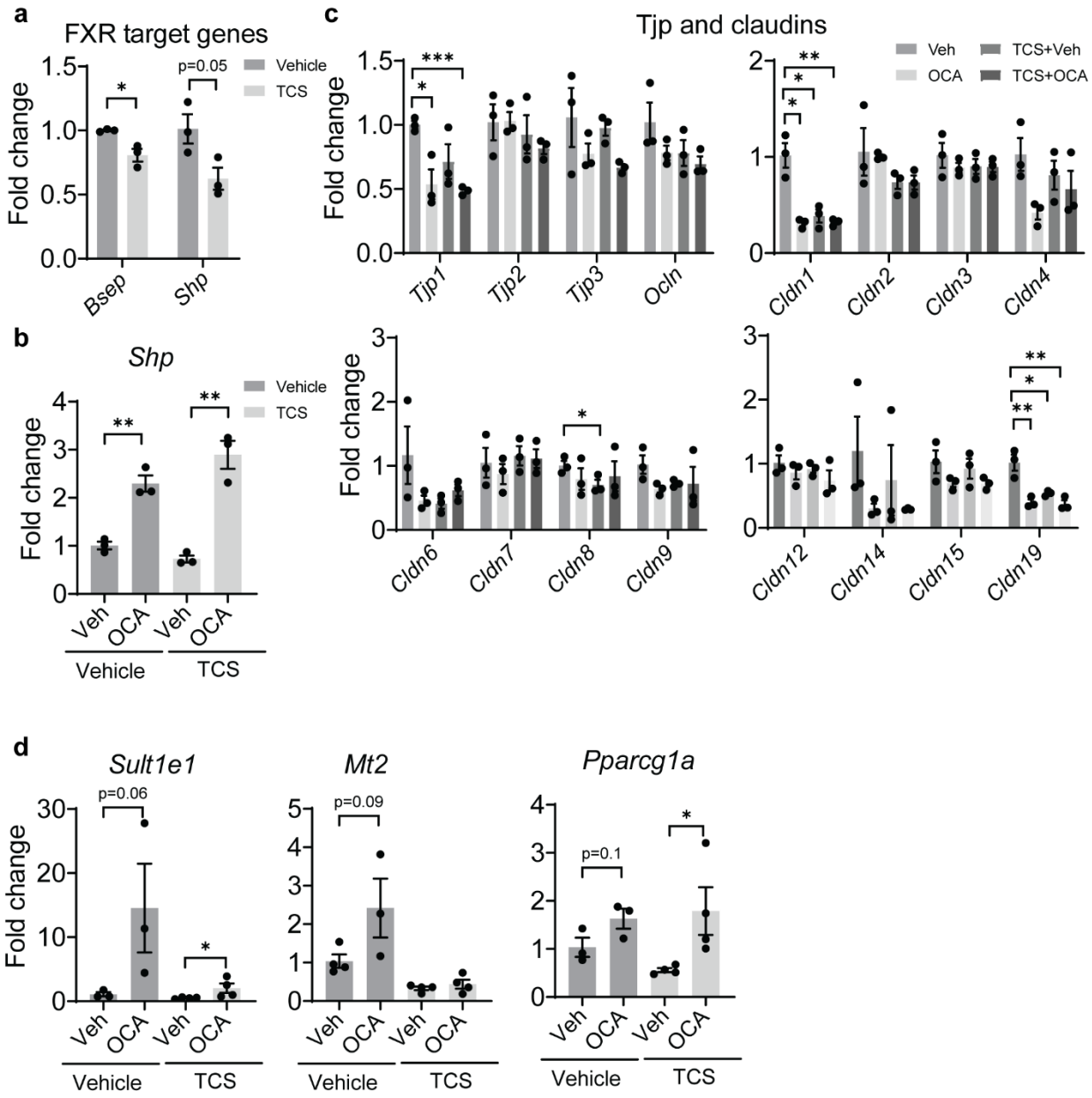

Figure S8. OCA has no effect in intestinal barrier genes but increases GR response in liver in the absence of TCS. WT neonatal mice breastfed with vehicle or TCS milk for 21 days and treated with vehicle or OCA. **a**, Hepatic expression of genes associated with FXR (n=3 mice per group). **b**, Intestinal expression of *Shp* (n=3 mice per group). **c**, Intestinal expression of TJPs, occludin and claudins (n=3 mice per group). **d**, Hepatic expression GR related genes (n=3-4 mice per group). **a-d** show mean ± S.E., determined by two-tailed Student`s test; *P<0.05, **P<0.01, ***P<0.001.

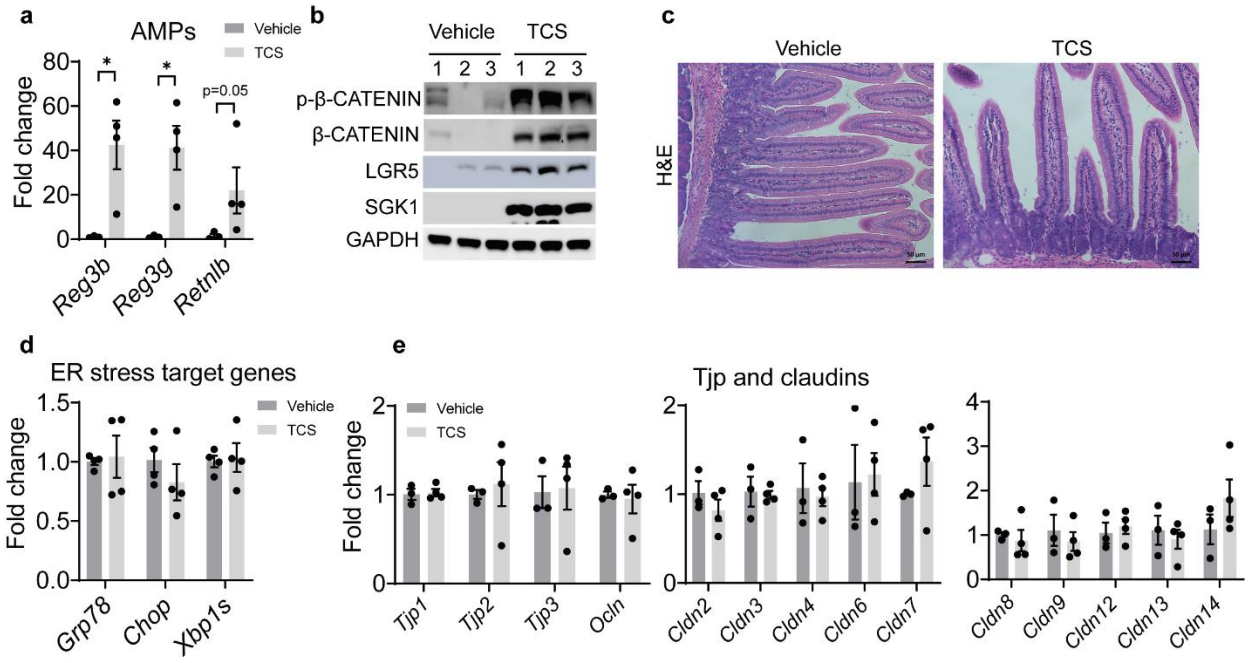

Figure S9. TCS treatment upregulates AMPs and β-catenin pathway in intestines. WT neonatal mice breastfed with vehicle or TCS milk for 21 days. **a**, Intestinal expression of *Reg3b*, *Reg3g* and *Retnlb* (n=3-4 mice per group). **b**, Intestinal IB of total and phosphorylated β-catenin and LGR5 and SGK1 (n=3 mice per group). **c**, Jejunum histological sections were stained with H&E (n=3 per group), Scale bars=50μm. **d,** Intestinal expression of genes associated with ER stress (n=4 mice per group). **e**. Intestinal TJP and claudins genes (n=3 mice per group). **a**, **d** and **e** show mean ± S.E., determined by two-tailed Student`s test; *P<0.05.

Table

Table S1. NAFLD semi-quantitative score in neonatal mice breastfed with TCS, according to Brunt (Brunt, E.M. Nonalcoholic steatohepatitis: pathologic features and differential diagnosis. *Semin Diagn Pathol* **22**, 330-338, 2005)

Histopathology	Vehicle	TCS
Steatosis	0	1
Inflammation	0	1
Fibrosis	0	1
Ballooning	0	0
Necrosis	-	-
Total score	**0**	**3**

Steatosis: hepatocytes exhibiting macro/microvacuolization, 0 (<5%); 1 (5-33%); 2 (33-66%); 4 (>66%). **Inflammation**: foci per 200x field, 0 (no foci); 1 (<2 foci per field); 2 (2-4 foci per field); 3 (>4 foci per field). **Fibrosis**: 0 (none); 1 (mild); 2 (moderate); 3 (severe); 4 (cirrhosis). **Ballooning**: 0 (none); 1 (few balloon cells); 2 (many balloon cells). **Necrosis**: not reported in NAFLD.

Table S2. Primer sequences used for Reverse Transcription Quantitative-PCR (RT-qPCR).

Gene name	Forward (5' - 3')	Reverse (5' - 3')
Acaca	TTCCACGTAGGAGGAGCTTCC	CCTCCGGTGCCTTCTCATTAC
Acly	CTGTGCCACCATGTTCTCCTC	AGGCCTGGTTCTTGGCTACTG
Atf4	TTGTCCGTTACAGCAACACTG	GCAGCAGCACCAGGCTCT
Bsep	AAGGACAGCCACACCAACTC	CCAGAACATGACAAACGGAA
Ccne1	TTGCAAGACCCAGATGAAGA	TCCACGCATGCTGAATTATC
Cldn1	ATCACCTTCGGGAGCTCAGGT	TGATGGGGGTCAAGGGGTCAT
Cldn2	ATGCCTTCTTGAGCCTGCTT	CAGTGTCTCTGGCAAGCTGA
Cldn3	CGTACCGTCACCACTACCAG	CAGCCTAGCAAGCAGACTGT
Cldn4	CCATGGAACCCTTCCGTTGA	ACCCGTCCATCCACTCTACA
Cldn6	CCCTTGGTGGCTGATGCTCAA	AGGTGGAGCTTGGACTCAGGT
Cldn7	TGTACCTACCTGGTCCTGGG	TCTCAGAAAAGACGGGACGC
Cldn8	GGAATGCCAATCCATCACGC	CTCTTTTATCCCCAGGCCCC
Cldn9	AAGTGGTATGGGAGGGGCTGT	CGCAGGTGGAAGCTTCTGGAA
Cldn12	TGTGTGCAGATGTGCTCCTGT	GCAGGAGGGCTTGAGCTGTAT
Cldn14	CTAACCAGAGGGCATGTGTGC	AGTCCCATCCACCTTGATGCT
Cldn15	GGTGGCTATCTCGTGGTACG	GCACTCCAGCCCAAGTAGAG
Cldn19	GGAATTCTTCAACCCCAGCAC	ATAGGGCTGTGGGATGCTGTT
Chop	CCACCACACCTGAAAGCAGAA	AGGTGAAAGGCAGGGACTCA
Ctgf	CAGACTGGAGAAGCAGAGCC	GCTTGGCGATTTTAGGTGTC
Cyclophiline	CAGACGCCACTGTCGCTT	TGTCTTTGGAACTTTGTCTGC
Cyp4a14	ACCCCTCTAGATTTGCACCA	AGCAAACTGTTTCCCAATGC
Cyp4a10	CACACCCTGATCACCAACAG	TCCTTGATGCACATTGTGGT
Edem1	CTACCTGCGAAGAGGCCG	GTTCATGAGCTGCCCACTGA
Ehhadh	CTATGATCCGCCTCTGCAA	TGGCTCTAACCGTATGGTCC
Fabp1	TGCAGAGCCAGGAGAACTTT	GATTTCTGACACCCCCTTGA
Fgf21	CTCCAGCAGTTCTCTGA	CCTGGGTGTCAAAGCCTCTA
Fasn	AGAAGAGCCATGGAGGAGGTG	ATGTCCACACCACCAATGAGG
Grp78	TTCAGCCAATTATCAGCAAACTCT	TTTTCTGATGTATCCTCTTCACCAGT
Mlxipl	CGGATACGGACTTGGAGGATC	GAAGTGTCCGCTGTGGATGAC
Mt2	TTGCGCTCGACCCAATACTC	CATTGTTTGCATTTGCAGGCG
Ocln	TCCGGATCCTGTCTATGCTCA	ATAGCCACCTCCGTAGCCAAA
Perk	TCATCCAGCCTTAGCAAACC	ATGCTTTCACGGTCTTGGTC

Pparcg1a	AAGTGGTGTAGCGACCAATCG	AATGAGGGCAATCCGTCTTCA
Reg3b	CCCAAGGGCTCCCAGGCTTA	GAGGTGTCCTCCAGGCCTCT
Reg3g	TGGCGCTGAAGCTTCCTTCC	TCATAGCCCAGTGTCGGGTCA
Retnlb	CCATTTCCTGAGCTTTCTGG	AGCACATCCAGTGACAACCA
Scd1	GCTCTACACCTGCCTCTTCG	CAGCCGAGCCTTGTAAGTTC
Shp	CACGATCCTCTTCAACCCAG	AGACTTCACACAGTGCCCA
Srebf1	GGCTCTGGAACAGACACTGG	TGGTTGTTGATGAGCTGGAG
Sult1e1	AAACTCACCTGCCACCCAAG	TTGGCGTTCCGGCAAAGATA
Xbp1s	CTGAGTCCGAATCAGGTGCAG	GTCCATGGGAAGATGTTCTGG
Tjp1	GAAACTCTGCTGAGCCCCCTA	GTTTTAGGGTCACCCGACGAG
Tjp2	CGAAGCAGTCTGGGTCTCTGA	CCGGCTCCTCTAGCTCATTGT
Tjp3	ATGGTATGCCATTTCGGAACC	CCGGGTACAACGTGTCCACTA