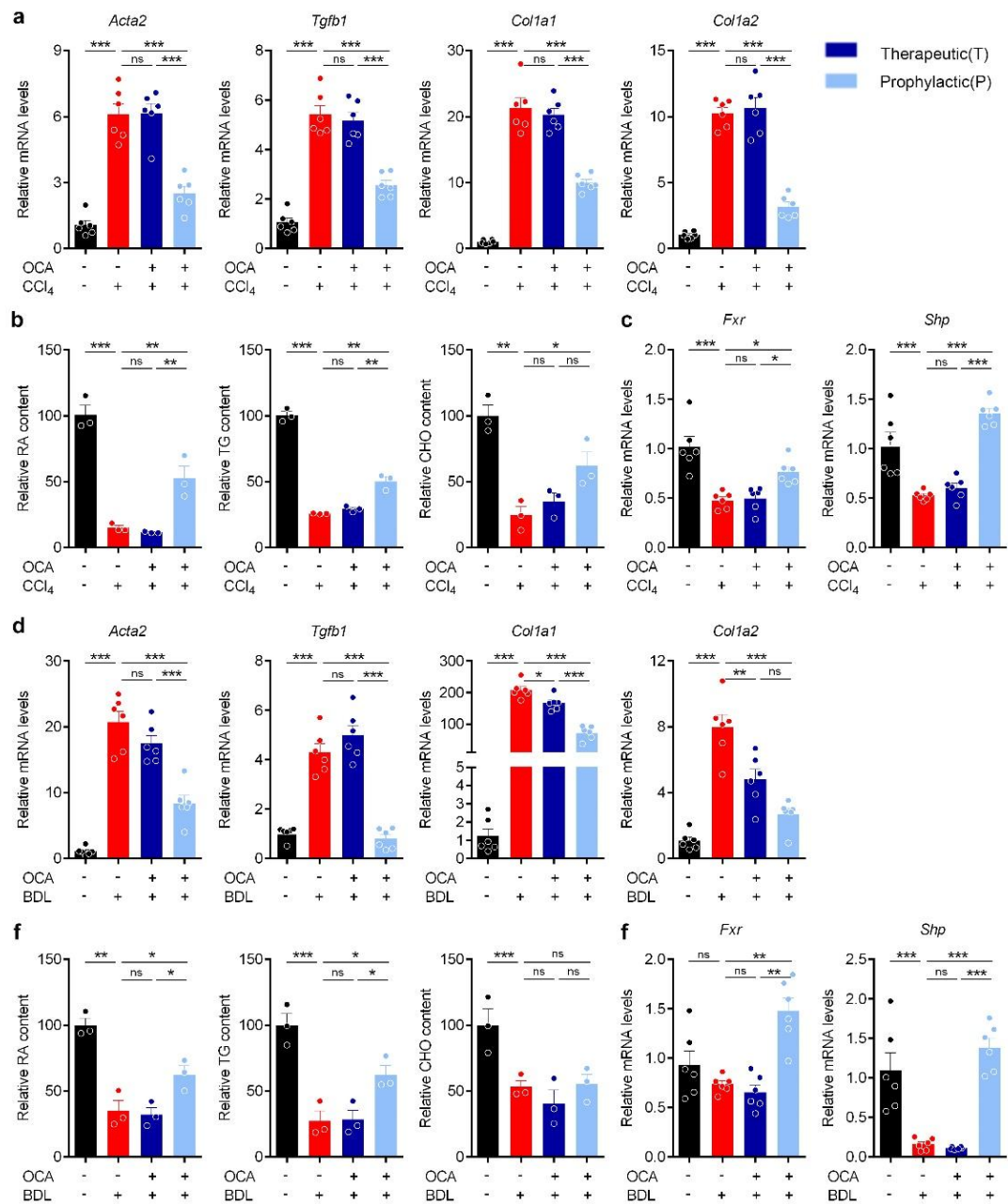


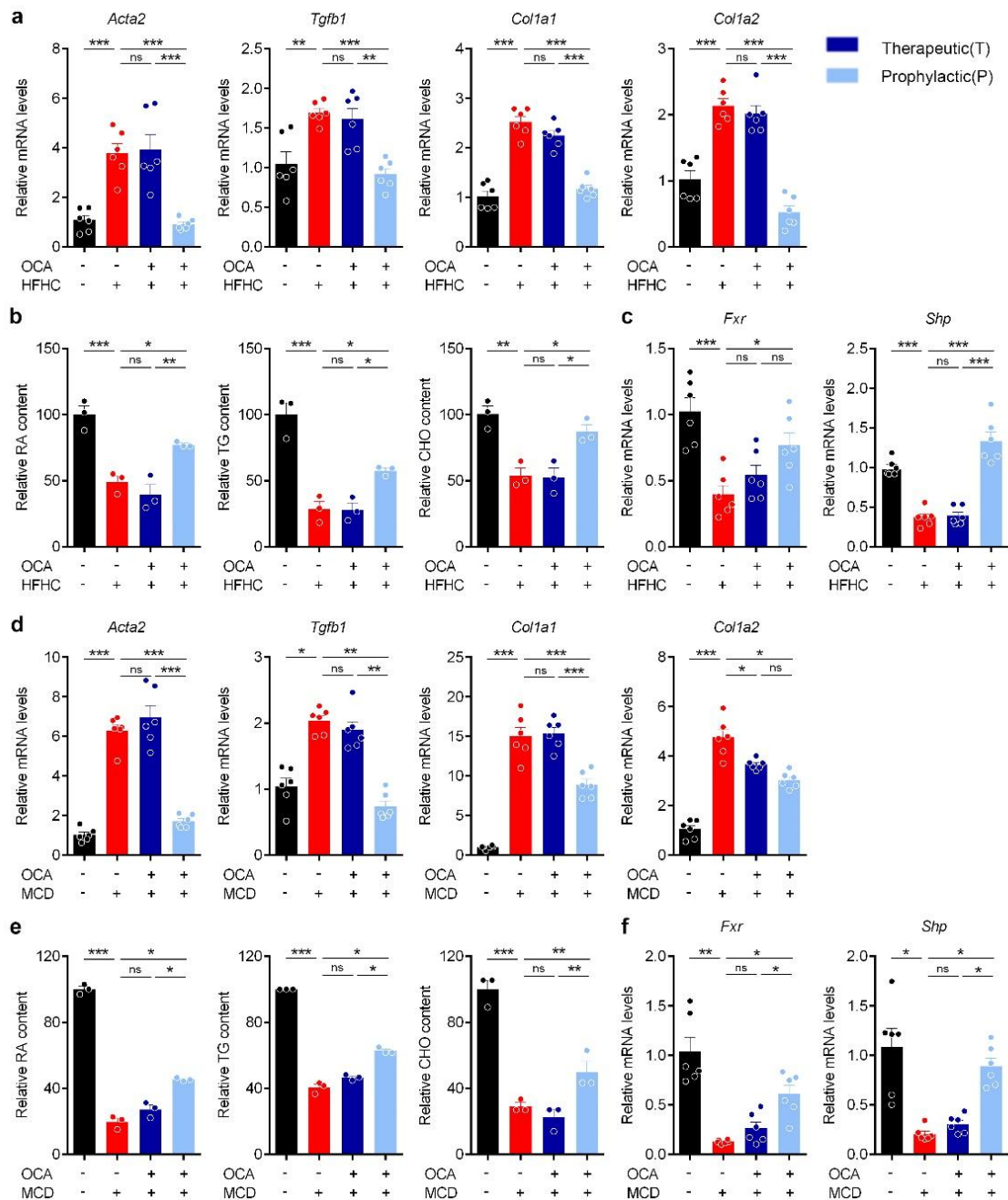
## **Supplementary Information**

### **SUMOylation inhibitors synergize with FXR agonists in combating liver fibrosis**

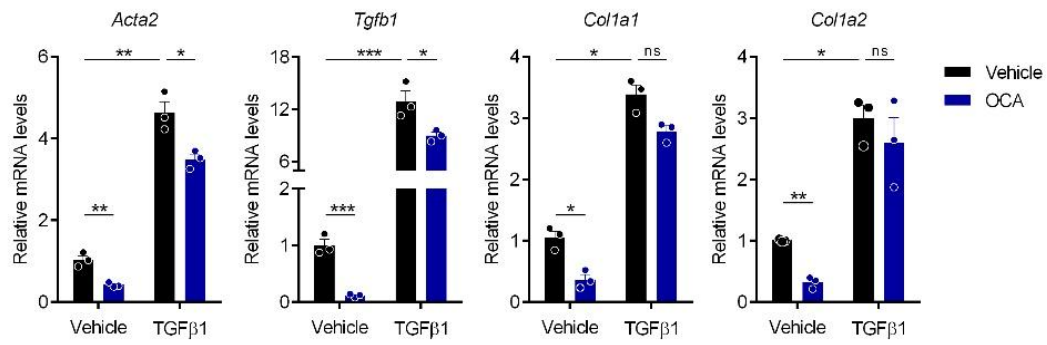
Zhou et al.



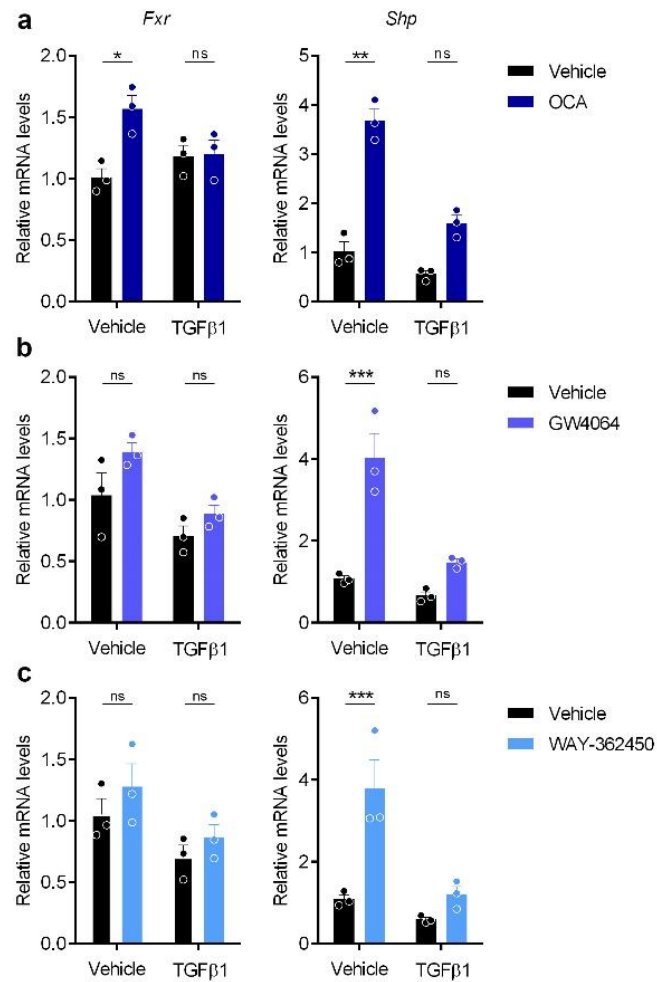
**Supplementary Fig. 1. Prophylactic but not therapeutic administration of OCA inhibits HSC activation and LD loss.** Anti-fibrotic effects of prophylactic and therapeutic administration of OCA were evaluated in freshly isolated HSCs from CCl<sub>4</sub>-induced (**a-c**) or BDL-induced (**d-f**) liver fibrosis models. **a** and **d**, Levels of *Acta2*, *Tgfb1*, *Col1a1* and *Col1a2* mRNAs in freshly isolated HSCs (n=6 biologically independent samples within these experiments). **b** and **e**, lipid quantitation analysis of isolated HSCs (n=3 biologically independent samples within these experiments). **c** and **f**, levels of *Fxr* and *Shp* mRNAs in freshly isolated HSCs (n=6 biologically independent samples within these experiments). Results are mean  $\pm$  SEM, \**P* < 0.05, \*\**P* < 0.01, \*\*\**P* < 0.001, and ns, statistically not significant, as assessed with ANOVA.



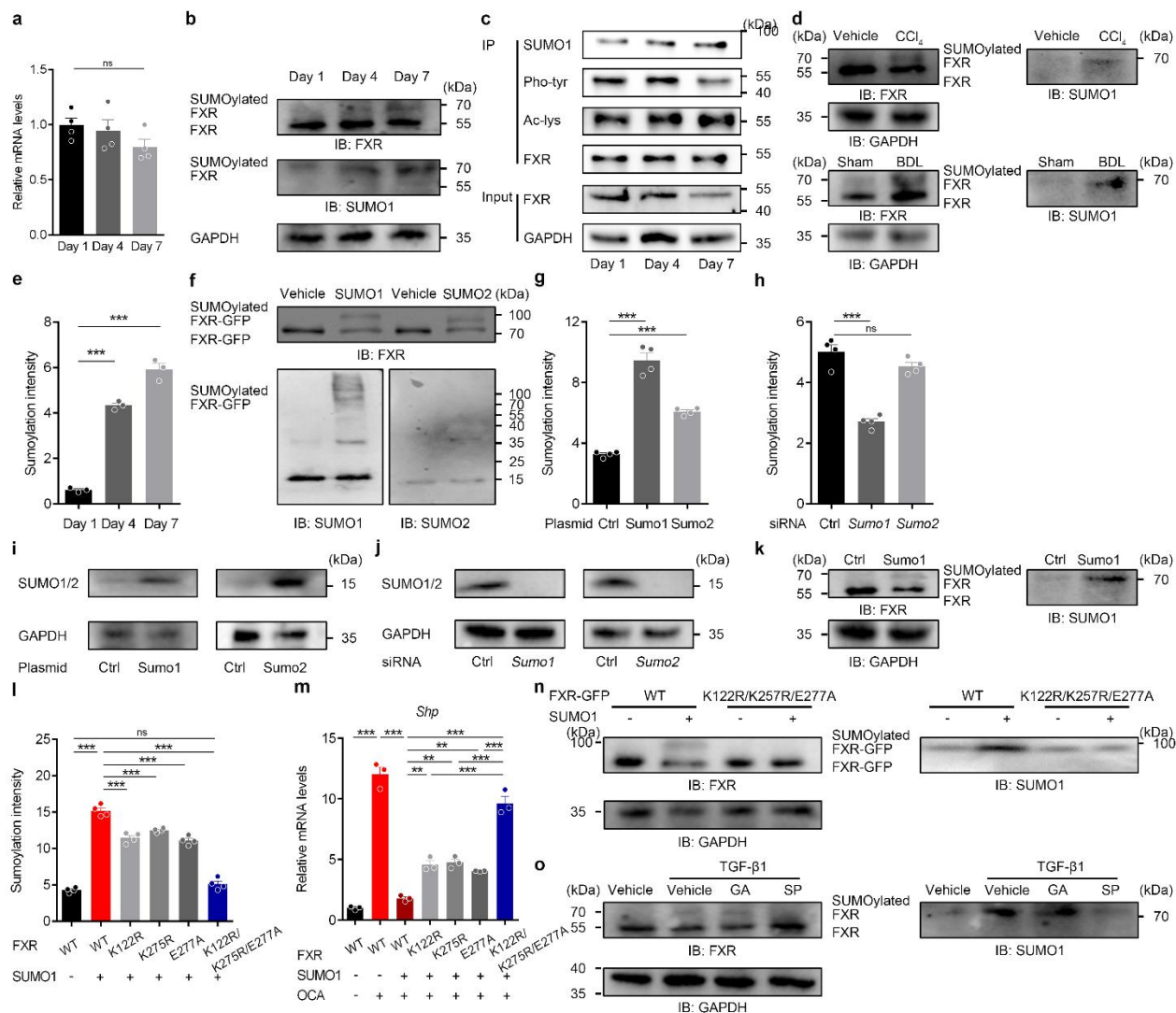
**Supplementary Fig. 2. Prophylactic but not therapeutic administration of OCA inhibits HSC activation and LD loss in NASH models.** Anti-fibrotic effects of prophylactic and therapeutic administration of OCA were evaluated in freshly isolated HSCs from mice fed with HFHC (a-c) or MCD diet (d-f). **a** and **d**, Levels of *Acta2*, *Tgfb1*, *Col1a1* and *Col1a2* mRNAs in freshly isolated HSCs (n=6 biologically independent samples within these experiments). **b** and **e**, lipid quantitation analysis of isolated HSCs (n=3 biologically independent samples within these experiments). **c** and **f**, levels of *Fxr* and *Shp* mRNAs in freshly isolated HSCs (n=6 biologically independent samples within these experiments). Results are mean  $\pm$  SEM, \* $P$  < 0.05, \*\* $P$  < 0.01, \*\*\* $P$  < 0.001, and ns, statistically not significant, as assessed with ANOVA.



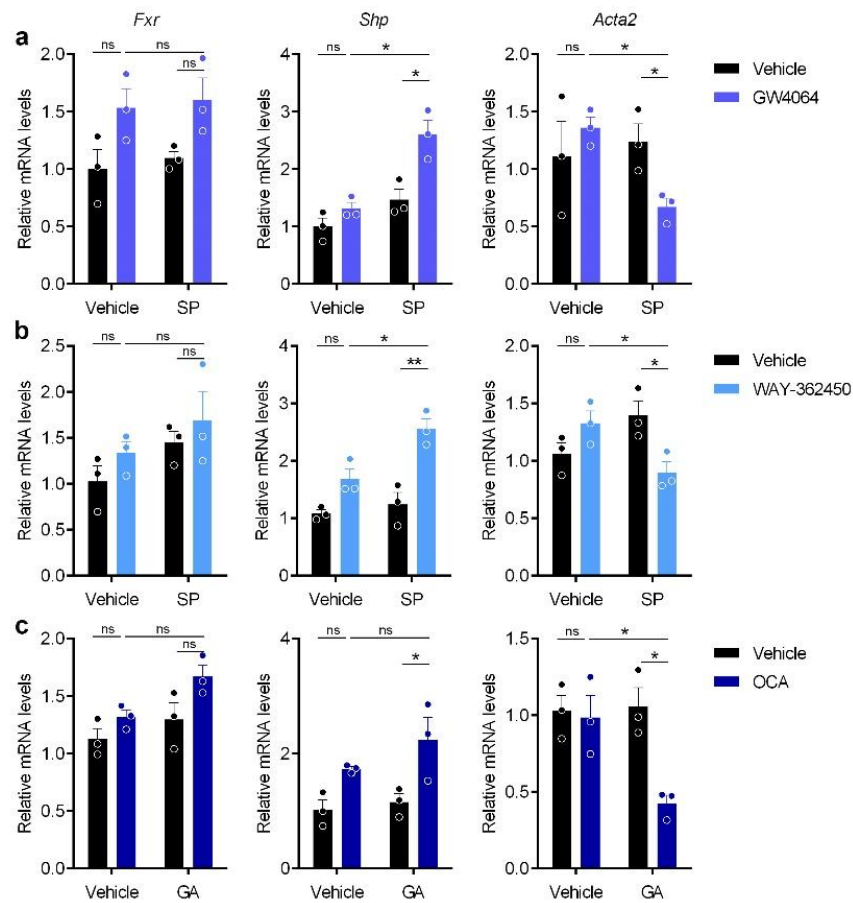
**Supplementary Fig. 3. OCA is ineffective against already activated HSC-T6 cells.** Cultured HSC-T6 cells, exposed to vehicle or TGFβ1, were then treated with OCA. Levels *Acta2*, *Col1a1*, *Col1a2* and *Tgfb1* mRNAs. n=3 biologically independent samples. Results are mean ± SEM, \* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$ , and ns, statistically not significant, as assessed with ANOVA.



**Supplementary Fig. 4. Activated HSCs show limited functional response to FXR ligands.** Cultured HSC-T6 cells, exposed to vehicle or TGFβ1, were then treated with FXR ligands, including OCA (a), GW4064 (b), and WAY-362450 (c). Levels of *Fxr* and *Shp* mRNAs.  $n=3$  biologically independent samples within these experiments. Results are mean  $\pm$  SEM, \*\* $P < 0.01$ , \*\*\* $P < 0.001$ , and ns, statistically not significant, as assessed with ANOVA.

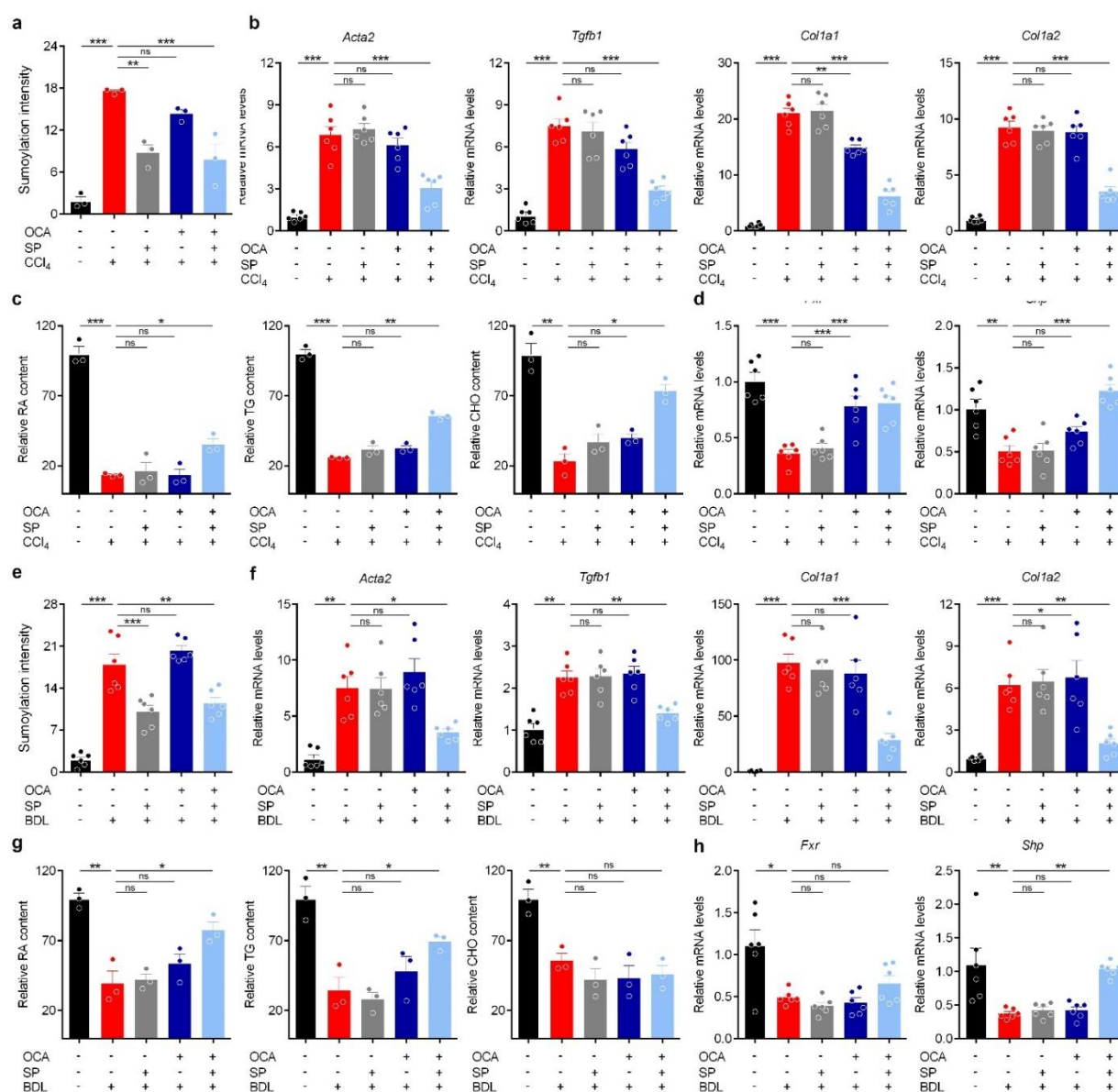


**Supplementary Fig. 5. Expression and PTMs of FXR during the activation of HSCs.** **a,b**, mRNA (n=4 biologically independent samples) (**a**) and protein (**b**) levels of FXR in primary HSCs cultured for 1, 4, and 7 days. **c**, PTMs of FXR in primary HSCs cultured for 1, 4, and 7 days as analyzed by Co-IP. **d, e**, SUMOylation of FXR in primary HSCs cultured for 1, 4, and 7 days as analyzed by western blot (**d**) and Protein SUMOylation Assay Ultra Kit (**e**) (n=3 biologically independent samples). **f**, SUMOylation analysis of recombinant FXR-GFP protein. **g, h**, SUMOylation analysis of FXR in HSCs transfected with Sumo1/2 plasmids (**g**) or siRNA (**h**). **i, j**, Protein expression of SUMO1/2 in HSCs transfected with Sumo1/2 plasmids (**i**) or siRNA (**j**). **k**, SUMOylation of FXR in HSCs transfected with Sumo1 plasmid. **l, m**, FXR SUMOylation (**l**) and Shp levels (**m**) in cells transfected with WT or SUMO-site mutant FXR plasmids. **n**, FXR SUMOylation by western blot analysis in cells transfected with WT or triple mutant FXR plasmid. **o**, FXR SUMOylation by western blot analysis in cells treated with GA or SP in the presence of TGF- $\beta$ 1. Results are mean  $\pm$  SEM, \* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$ , and ns, statistically not significant, as assessed with ANOVA.

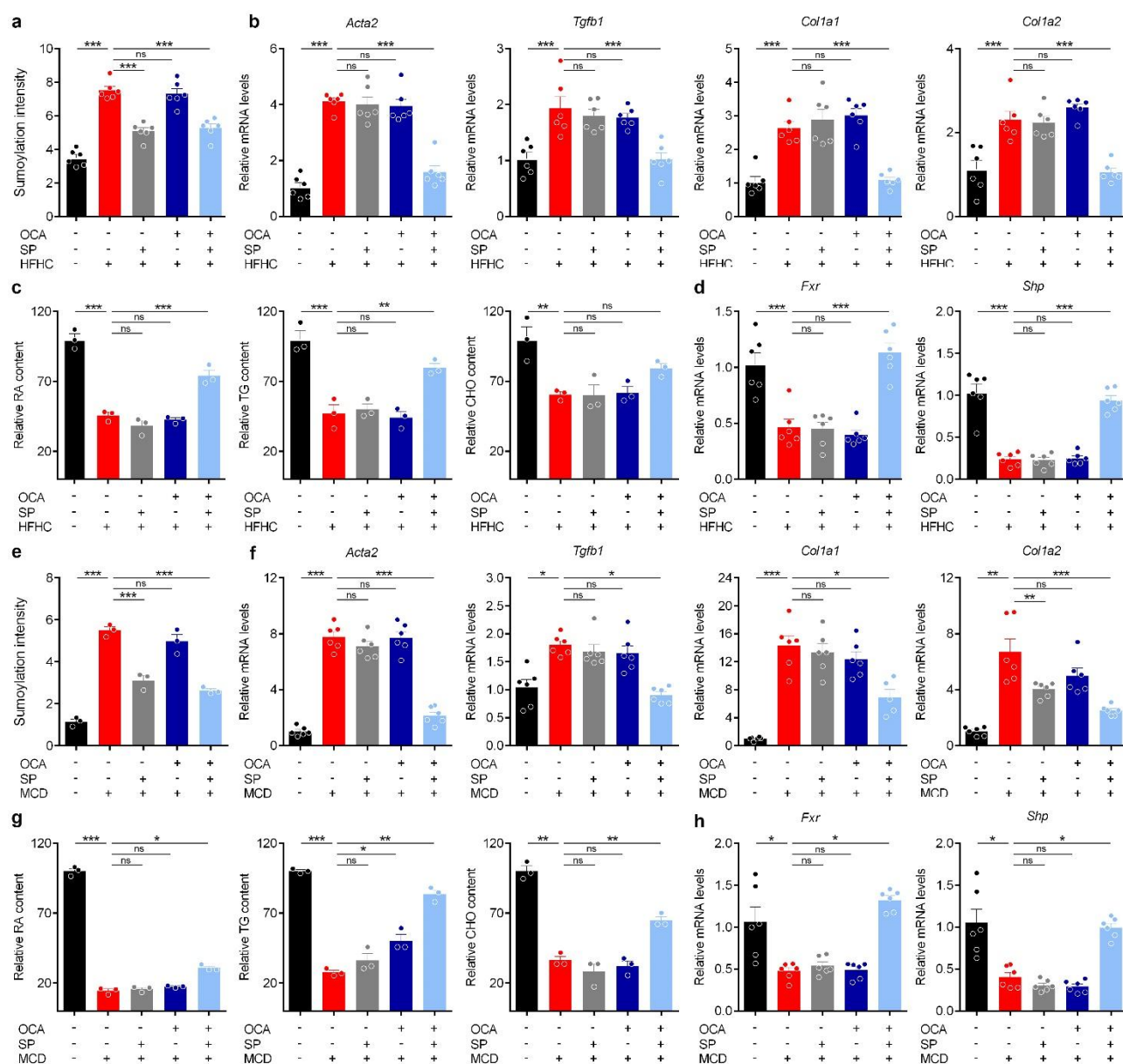


**Supplementary Fig. 6. SUMOylation inhibition restores the activity of FXR in activated HSCs.**

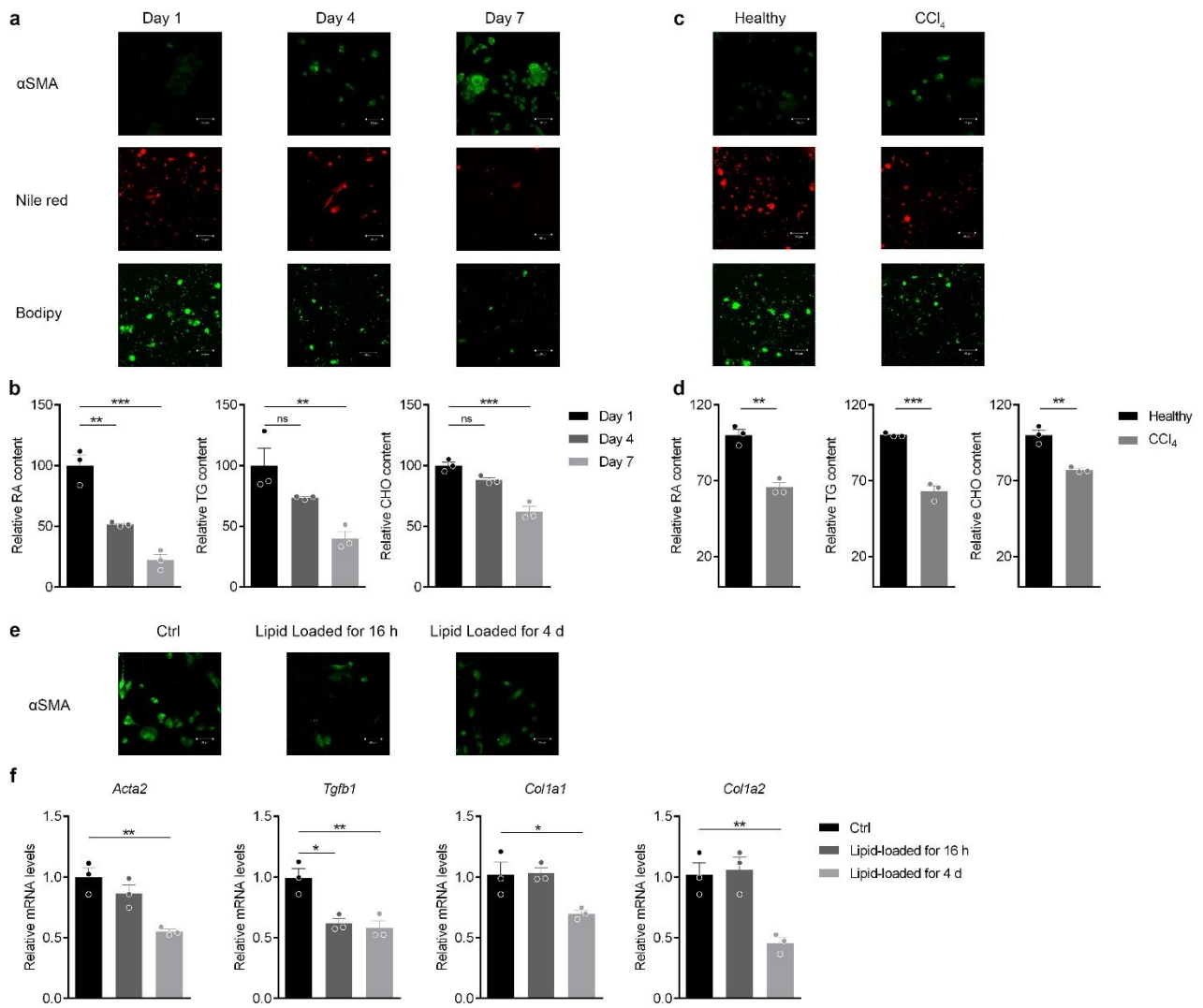
Primary HSCs were isolated and cultured for 4 days. These cells, exposed to vehicle or SUMOylation inhibitors, including SP (**a**, **b**) and GA (**c**), were then treated with various FXR ligands, including GW4064 (**a**), WAY-362450 (**b**), and OCA (**c**). Levels of *Fxr*, *Shp* and *Acta2* mRNAs. n=3 biologically independent samples within these experiments. Results are mean  $\pm$  SEM, \* $P$ < 0.05, and ns, statistically not significant, as assessed with ANOVA.



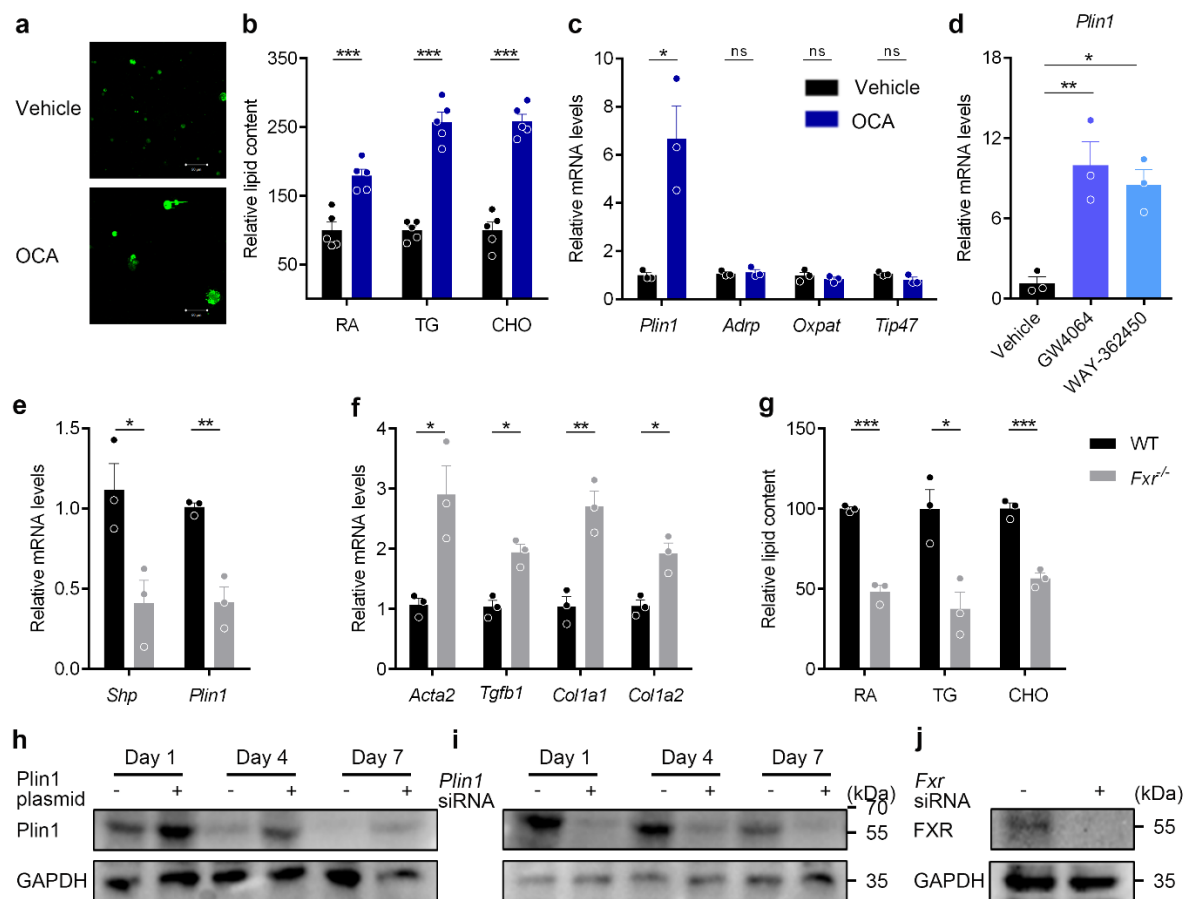
**Supplementary Fig. 7. SUMOylation inhibition rescues the effects of FXR agonists in inhibiting HSC activation and LD loss caused by toxin or cholestasis.** Fibrotic mice, caused by CCl<sub>4</sub> treatment (**a-d**) or BDL operation (**e-h**), were treated with OCA in the presence or absence of SP. HSCs were then isolated and analyzed. **a** and **d**, SUMOylation of FXR as analyzed by Protein SUMOylation Assay Ultra Kit. **b** and **e**, Levels of *Acta2*, *Col1a1*, *Col1a2* and *Tgfb1* mRNAs in freshly isolated HSCs (n=6 biologically independent samples within these experiments). **c** and **f**, lipid quantitation analysis of isolated HSCs (n=3 biologically independent samples within these experiments). **d** and **h**, Levels of *Fxr* and *Shp* of mRNAs in freshly isolated HSCs (n=6 biologically independent samples within these experiments). Results are mean ± SEM, \**P*< 0.05, \*\**P*< 0.01, \*\*\**P*< 0.001, and ns, statistically not significant, as assessed with ANOVA.



**Supplementary Fig. 8. SUMOylation inhibition rescues the effects of FXR agonists in inhibiting HSC activation and LD loss caused by NASH.** Fibrotic mice, caused by HFHC (**a-d**) or MCD diet (**e-h**), were treated with OCA in the presence or absence of SP. HSCs were then isolated and analyzed. **a** and **d**, SUMOylation of FXR as analyzed by Protein SUMOylation Assay Ultra Kit. **b** and **e**, Levels of *Acta2*, *Colla1*, *Colla2* and *Tgfb1* mRNAs in freshly isolated HSCs (n=6 biologically independent samples within these experiments). **c** and **f**, lipid quantitation analysis of isolated HSCs (n=3 biologically independent samples within these experiments). **d** and **h**, Levels of *Fxr* and *Shp* of mRNAs in freshly isolated HSCs (n=6 biologically independent samples within these experiments). Results are mean  $\pm$  SEM, \* $P$ < 0.05, \*\* $P$ < 0.01, \*\*\* $P$ < 0.001, and ns, statistically not significant, as assessed with ANOVA.



**Supplementary Fig. 9. LD loss controls the activation of HSCs.** **a-d**, Activation of HSCs paralleled with LD loss,  $n=3$  biologically independent samples within these experiments. **a**,  $\alpha$ SMA, Nile red and Bodipy staining of isolated primary HSCs cultured for variant days (representative of  $n=3$ . Scale bar, 50  $\mu$ m.). **b**, Lipid quantitation analysis of isolated primary HSCs cultured for variant days. **c**,  $\alpha$ SMA, Nile red and Bodipy staining of isolated primary HSCs from healthy or CCl<sub>4</sub>-treated mice (representative of  $n=3$ . Scale bar, 50  $\mu$ m.). **d**, Lipid quantitation analysis of isolated primary HSCs from healthy or CCl<sub>4</sub>-treated mice. **e,f**, Lipid loaded HSCs showed repressed activation phenotype,  $n=3$  biologically independent samples within these experiments. **e**,  $\alpha$ SMA staining (representative of  $n=3$ . Scale bar, 50  $\mu$ m.). **f**, Levels of pro-fibrotic gene mRNAs. Results are mean  $\pm$  SEM, \* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$ , as assessed with Student's t-test or ANOVA.



**Supplementary Fig. 10. Activation of FXR-Plin1 pathway prevents HSC activation and LD loss.**

**a, b**, OCA treatment prevents LD loss. **a**, Bodipy staining of HSCs (representative of  $n=3$  biologically independent samples. Scale bar, 50  $\mu$ m.). **b**, Lipid quantitation analysis ( $n=5$  biologically independent samples). **c**, Effect of OCA treatment on the expression of LD associated proteins ( $n=3$  biologically independent samples within these experiments). **d**, Effect of FXR agonist on the mRNA expression of *Plin1* ( $n=3$  biologically independent samples within these experiments). **e-g**, HSCs of *Fxr*<sup>-/-</sup> mouse shows suppressed expression of *Plin1* (**e**), enhanced expression of pro-fibrotic genes (**f**), as well as decreased lipid content (**g**),  $n=3$  biologically independent samples within these experiments. **h,i**, Plin1 protein expression in primary HSCs transfected with Plin1 plasmid (**h**) or siRNA (**i**) and cultured for 1, 4, or 7 days. **j**, FXR protein expression in primary HSCs transfected with *Fxr* siRNA. Results are mean  $\pm$  SEM, \* $P < 0.05$ , \*\* $P < 0.01$ , and ns, statistically not significant, as assessed with Student's t-test or ANOVA.

**Supplementary table 1. Primer sequences for RT-PCR.**

| Genes               | Sequences (5'-3') |                           |
|---------------------|-------------------|---------------------------|
| Human <i>FXR</i>    | Forward           | GACTTTGGACCATGAAGACCAG    |
|                     | Reverse           | GCCCAGACGGAAGTTTCTTATT    |
| Human <i>SHP</i>    | Forward           | AGGAATATGCCTGCCTGAAAGGG   |
|                     | Reverse           | CTGGTCGGAATGGACTTGAGGGT   |
| Human <i>PLIN1</i>  | Forward           | ACATCAGTCTTGACAACCTTGAGGA |
|                     | Reverse           | GCACGGTGTATCGAGAGAGG      |
| Human <i>GAPDH</i>  | Forward           | AATGAAGGGGTCATTGATGG      |
|                     | Reverse           | AAGGTGAAGGTCGGAGTCAA      |
| Mouse <i>Fxr</i>    | Forward           | GCACGCTGATCAGACAGCTA      |
|                     | Reverse           | CAGGAGGGTCTGTTGGTCTG      |
| Mouse <i>Shp</i>    | Forward           | GTACCTGAAGGGCACGATCC      |
|                     | Reverse           | GTGAAGTCTTGGAGCCCTGGT     |
| Mouse <i>Acta2</i>  | Forward           | GCACCCAGCATGAAGATCAAG     |
|                     | Reverse           | TCTGCTGGAAGGTAGACAGCGAAG  |
| Mouse <i>Col1a1</i> | Forward           | TAGGCCATTGTGTATGCAGC      |
|                     | Reverse           | ACATGTTTCAGCTTTGTGGACC    |
| Mouse <i>Col1a2</i> | Forward           | GCAGGGTTCCAACGATGTTG      |
|                     | Reverse           | GCAGCCATCGACTAGGACAGA     |
| Mouse <i>Tgfb1</i>  | Forward           | AGGAGACGGAATACAGGGCT      |
|                     | Reverse           | CCACGTAGTAGACGATGGGC      |
| Mouse <i>Plin1</i>  | Forward           | GGAGTGGCTGCAAGTGTTTC      |
|                     | Reverse           | ATCCCAGATTGCAGCAGACC      |
| Mouse <i>Gapdh</i>  | Forward           | TTGATGGCAACAATCTCCAC      |
|                     | Reverse           | CGTCCCGTAGACAAAATGGT      |
| Rat <i>Fxr</i>      | Forward           | TGGA CTCATACAGCAAACAGAGA  |
|                     | Reverse           | GTCTGAAACCCTGGAAGTCTTTT   |

|                   |         |                        |
|-------------------|---------|------------------------|
| Rat <i>Shp</i>    | Forward | CCTGGAGCAGCCCTCGT      |
|                   | Reverse | AACACTGTATGCAAACCGAGGA |
| Rat <i>Acta2</i>  | Forward | GCTCCATCCTGGCTTCTCTA   |
|                   | Reverse | TAGAAGCATTTGCGGTGGAC   |
| Rat <i>Col1a1</i> | Forward | AACCCCAAGGAGAAGAAGCA   |
|                   | Reverse | AGCGTGCTGTAGGTGAATCG   |
| Rat <i>Col1a2</i> | Forward | TCGCTCACAGCCTTCACTCAG  |
|                   | Reverse | TGCGGGCAGGGTTCTTTCTA   |
| Rat <i>Plin1</i>  | Forward | GTGGCTCTCAGCTGCATGT    |
|                   | Reverse | CTGGAAGCACTCACAGGTCC   |
| Rat <i>Adrp</i>   | Forward | AACCTGCCCTTGGTGAGCTC   |
|                   | Reverse | GCCATTGACCACAGACTTGG   |
| Rat <i>Tip47</i>  | Forward | GTGGCTCACCTGGAATCAGAA  |
|                   | Reverse | CTGAACACACTGAGTGCCTGG  |
| Rat <i>Oxpat</i>  | Forward | GGATGTCCGGTGATCAGAC    |
|                   | Reverse | GTGCACGTGGCCCTGACCAG   |
| Rat <i>Tgfb1</i>  | Forward | TGCTTCAGCTCCACAGAGAA   |
|                   | Reverse | TGGTTGTAGAGGGCAAGGAC   |
| Rat <i>Gapdh</i>  | Forward | AACGGCACAGTCAAGGCTGA   |
|                   | Reverse | ACGCCAGTAGACTCCACGACAT |