

Supplemental Information for

A SUMO-dependent LRH-1/OSBP pathway promoting nonalcoholic fatty liver disease

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Figure S1

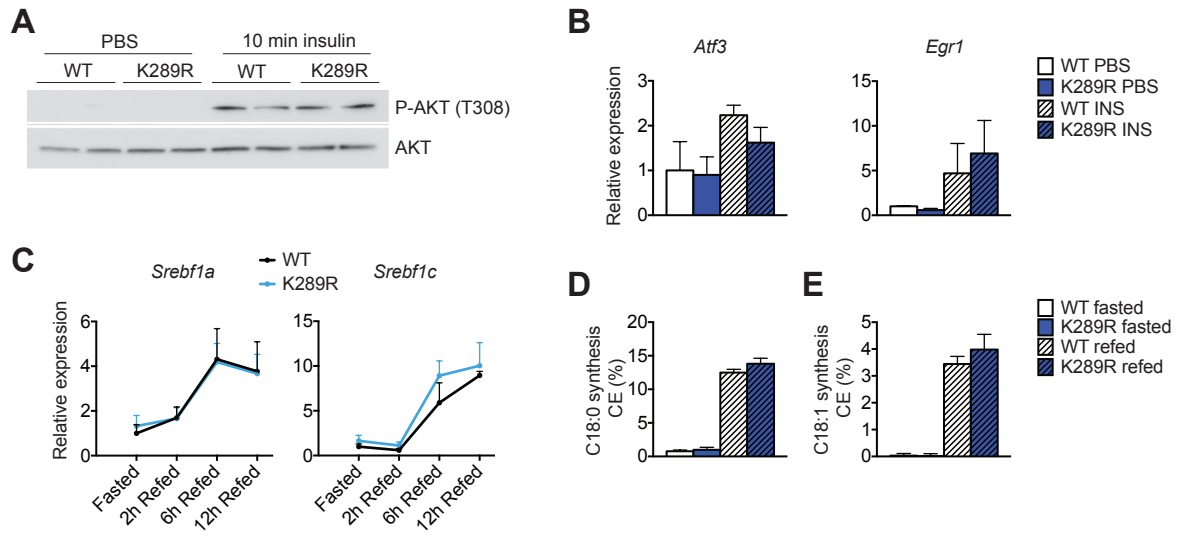


Figure S1. *Lrh-1* K289R mice do not show changes in the early insulin response. (A, B) Phospho and total AKT immunoblots (A), and mRNA expression of early response genes (B) in livers from fasted WT or K289R mice that were injected with PBS or insulin 10 min prior to sacrifice. $n = 3$ for PBS groups, 4 for insulin groups. (C) Hepatic expression of the two *Srebf1* isoforms, *Srebf1a* and *Srebf1c*, in fasted and refeed WT and K289R mice. $n = 4$ per genotype. (D, E) Fractional chain elongation (CE) of pre-existing palmitate to hepatic stearate (D) and oleate (E). $n = 6$ per genotype. Error bars represent means \pm S.E.M. WT, *Lrh-1* WT; K289R, *Lrh-1* K289R mice.

Figure S2

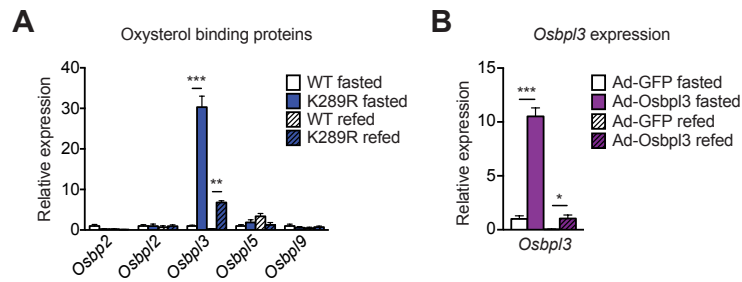


Figure S2. *Osbp13* is a direct transcriptional target of LRH-1. (A) Hepatic mRNA expression of oxysterol binding protein family members in fasted or 6-hour-refed K289R and WT mice. $n = 10$ per genotype. **(B)** *Osbp13* expression in hepatic lysates of fasted or refed mice infected with Ad-GFP or Ad-OSBPL3. $n = 2$ per fasted group, $n = 5$ for refed group. Error bars represent means \pm S.E.M. * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$ relative to WT, as determined by two-way ANOVA with Bonferroni post-hoc test (A, B). WT, *Lrh-1* WT; K289R, *Lrh-1* K289R mice.

Figure S3.

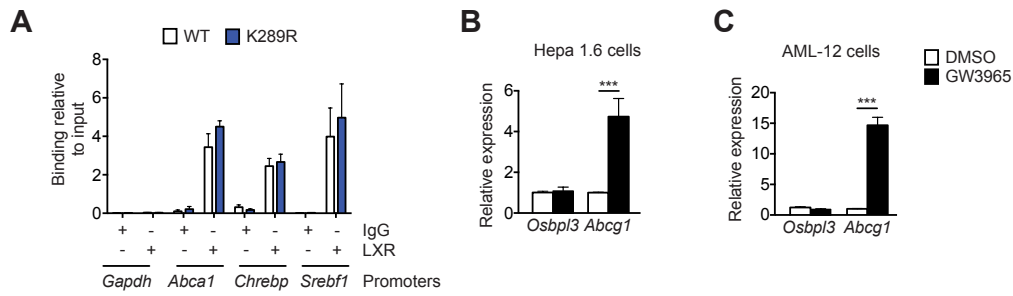


Figure S3. Crosstalk between LXR and LRH-1 pathways. (A) LXR chromatin immunoprecipitation on hepatic lysates from WT and K289R mice. n = 6 per genotype. (B, C) Expression of *Osbp13* and *Abcg1* in Hepa 1.6 (B) or AML-12 (C) cells upon treatment with the LXR agonist GW3965. n = 3 per treatment. Error bars represent means \pm S.E.M. *** $p < 0.001$ relative to WT, as determined by unpaired Student's *t*-test (B, C). WT, *Lrh-1* WT; K289R, *Lrh-1* K289R mice.

Figure S4

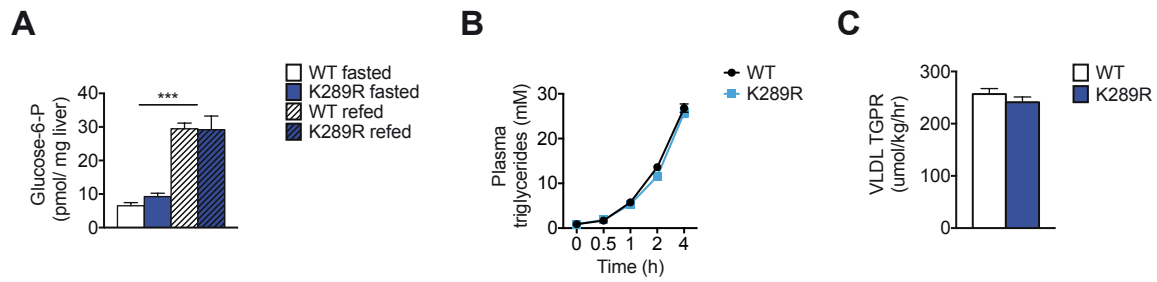


Figure S4. No change in glucose-6-phosphate production and VLDL secretion in *Lrh-1* K289R mice. (A) Quantification of glucose-6-phosphate (glucose-6-P) in hepatic lysates of fasted or refed WT and K289R mice. $n = 4$ WT fasted, 7 K289R fasted, 7 WT refed, and 6 K289R refed. (B, C) Plasma triglyceride synthesis over time (B), and triglyceride production rate (TGPR) in WT and K289R mice (C). $n = 7$ per genotype. Error bars represent means \pm S.E.M. *** $p < 0.001$ refed mice relative to fasted mice, as determined by two-way ANOVA with Bonferroni post-hoc test (A). WT, *Lrh-1* WT; K289R, *Lrh-1* K289R mice.

Figure S5

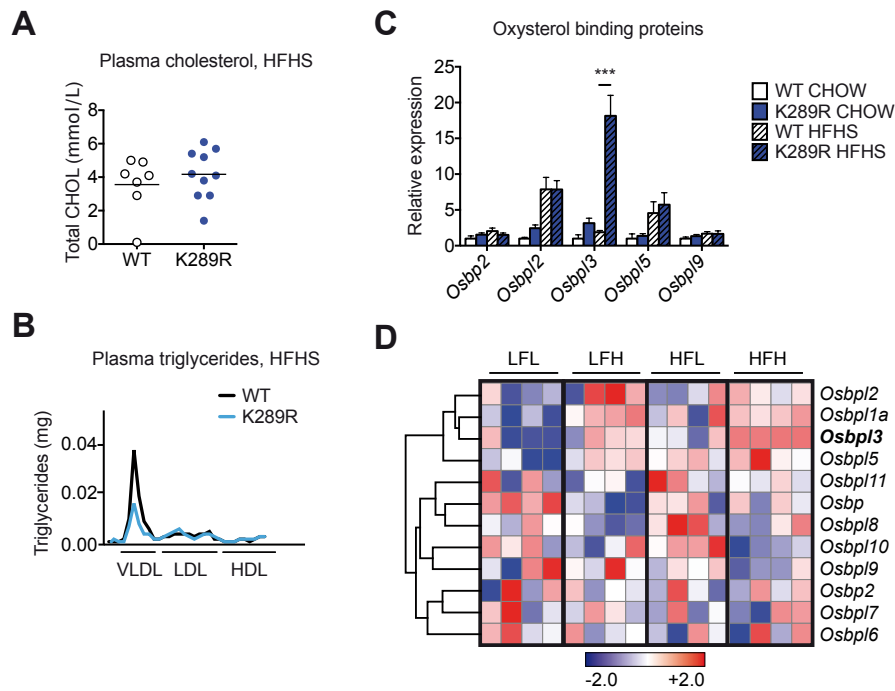


Figure S5. *Lrh-1* K289R mice develop NAFLD upon HFHS diet feeding. (A) Plasma cholesterol levels in WT and K289R mice fed a HFHS diet. WT, n = 7; K289R, n = 10 per genotype. (B) Triglyceride (TG) content in lipoprotein subfractions. VLDL, very-low density lipoprotein; LDL, low-density lipoprotein; HDL, high-density lipoprotein. n = 1 pooled sample from 7 mice per genotype. (C) Hepatic mRNA expression of oxysterol binding protein family members in K289R and WT mice upon chow or HFHS feeding. n = 9 per genotype. (D) Heatmap displaying the expression of oxysterol binding protein family members as well as markers of matrix degradation, fibrosis, and inflammation in mice that were classified as low-fat low (LFL) responders, low-fat high (LFH) responders, high-fat low (HFL) responders, and high fat high (HFH) responders according to the development of NAFLD/NASH upon chow or high-fat diet feeding (1). Normalized expression values are in Log₂ scale. Error bars represent means ± S.E.M. ***p<0.001 relative to WT, as determined by two-way ANOVA with Bonferroni post-hoc test (C). WT, *Lrh-1* WT; K289R, *Lrh-1* K289R mice.

Figure S6

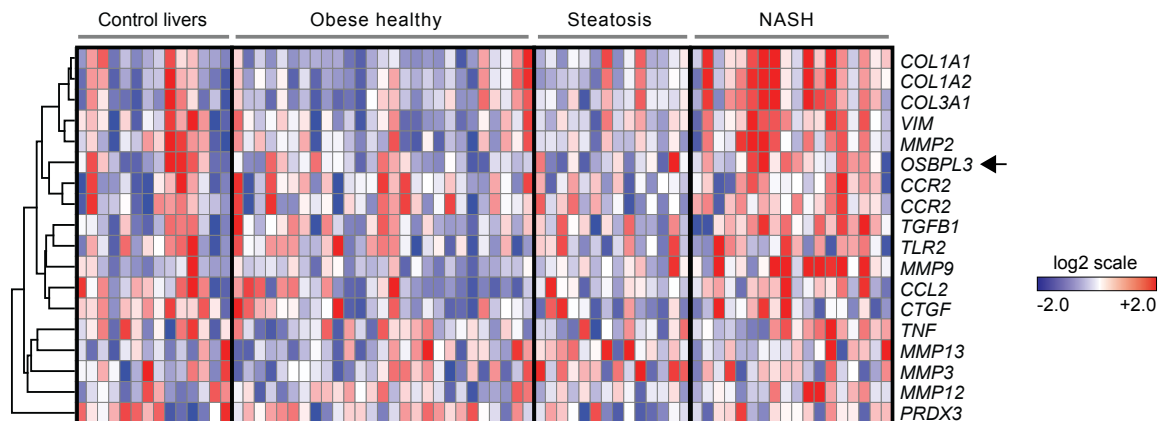


Figure S6. Expression of *OSBPL3* in NASH patients. Expression of *OSBPL3* and markers of matrix degradation, fibrosis, and inflammation in transcriptomic data from human subjects that had livers ranging from healthy controls to steatosis and further to NASH (2). Arrow indicates *OSBPL3*. Normalized expression values are in Log_2 scale.

Figure S7

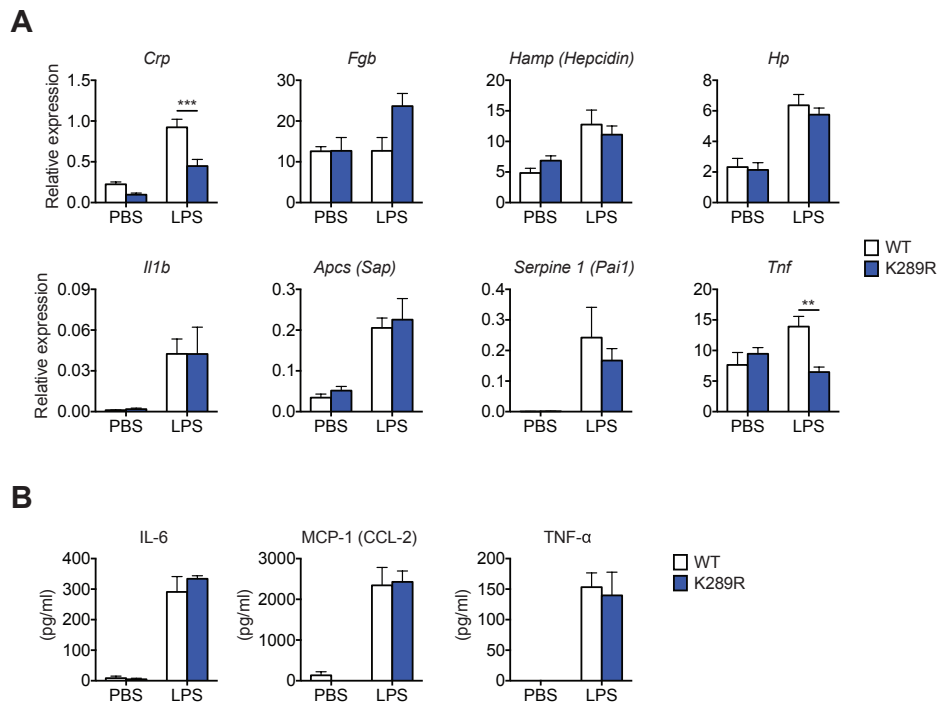


Figure S7. Hepatic acute phase response in *Lrh-1* WT and *K289R* mice. (A) Hepatic expression of the indicated acute phase response genes in WT and K289R mice that were challenged with either PBS or lipopolysaccharide (LPS) for 2.5 hours. $n = 5$ PBS-treated groups, $n = 6$ LPS-treated groups. (B) ELISA assays to determine the plasma content of IL-6, MCP-1 or TNF- α . $n = 5$ PBS-treated groups, $n = 6$ LPS-treated groups. ** <0.01 and *** $p < 0.001$ relative to WT, as determined by two-way ANOVA with Bonferroni post-hoc test (A). WT, *Lrh-1* WT; K289R, *Lrh-1* K289R mice.

Table S1. Q-PCR primer table.

Gene	Forward (5' to 3' sequence)	Reverse (5' to 3' sequence)
<i>36B4</i>	AGATTCGGGATATGCTGTTGG	AAAGCCTGGAAGAAGGAGGTC
<i>Acaca</i>	CCATCCAAACAGAGGGAACATC	CTACATGAGTCATGCCATAGTGGTT
<i>Acly</i>	GCCAGCGGGAGCACATC	CTTTGCAGGTGCCACTTCATC
<i>Acta2</i>	CCCAGACATCAGGGAGTAATGG	TCTATCGGATACTTCAGCGTCA
<i>Apcs</i>	GGACCAAGCATGGACAAGCTA	TGTCTGACAAAAGGCTTCTGAAAG
<i>Atf3</i>	GGTCGCACTGACTTCTGAGG	CTCTGGCCGTTCTCTGGA
<i>B2m</i>	TTCTGGTGCTTGTCTCACTG	TATGTTCCGGCTTCCCATTCT
<i>Col1a1</i>	TGTTTCAGCTTTGTGGACCTC	TCAAGCATACTCGGGTTTC
<i>Col1a1</i>	AACCCCTGCCCCGCACATG	CAGACGGCTGAGTAGGGAACA
<i>Crp</i>	CCCTCTTCAGATCCTTTCCT	GCCCTCTGATAGATTATCC
<i>Des</i>	CTCGGAAGTTGAGAGCAGAGA	GTGAAGATGGCCTTGGATGT
<i>Egr1</i>	TGGGATAACTCGTCTCCACC	GAGCGAACAACCCATAGAGC
<i>Elovl6</i>	AACTTGGCTCGCTTGTTCAT	CCAATGGATGCAGGAAAAC
<i>Fasn</i>	AGCTTCGGCTGCTGTTGGAAGT	TCGGATGCCTCTGAACCACTCACA
<i>Fgb</i>	GTATCTCATCCAGCCTGACA	CATCCTGACGGTCTGTATG
<i>Gfap</i>	CCTTCTGACACGGATTTGGT	ACATCGAGATCGCCACCTAC
<i>Gpam</i>	GCTATCATGTCCACCCACATTTG	ACTTCTCTCTCATCACAAAGAAGTC
<i>Hamp</i>	GCTGCCTGTCTCCTGCTTCT	AGCTCTGTAGTCTGTCTCATCTGTTG
<i>Hp</i>	GAAGCAATGGGTGAACACAG	TGCCTTTGGCATCCATAGAG
<i>Il1b</i>	CAACCAACAAGTGATATTCTCCATG	GATCCACACTCTCCAGCTGCA
<i>Mcp1/Ccl2</i>	AGGTCCCTGTCATGCTTCTG	GCTGCTGGTGATCCTCTTGT
<i>Mip1a/Ccl3</i>	GTGGAATCTTCCGGCTGTAG	ACCATGACACTCTGCAACCA
<i>Mmp13</i>	CTTCTTCTTGTGAGCTGGACTC	CTGTGGAGGTCACGTGACT
<i>Mmp2</i>	CAAGTTCCTCCGGCGATGTC	TTCTGGTCAAGGTCACCTGTC
<i>Mmp3</i>	ACATGGAGACTTTGTCCCTTTTG	TTGGCTGAGTGGTAGAGTCCC
<i>Mmp9</i>	CTGGACAGCCAGACACTAAAG	CTCGCGCAAGTCTTCAGAG
<i>Osbp2</i>	TGTGGTGGAGTTCACGTGTTG	CAAGGCTATCCGTGTGATGA
<i>Osbp12</i>	TCTATATTTATGTTGAAGTTGTGTGGA	CTTGGGTGTCAGAGGGTTG
<i>Osbp13</i>	TCAATCCTTCCACGACTTCC	CGGTGTGTCTCAAAAGTTGGT
<i>Osbp15</i>	AGAAAGGCCTCCTCCTTCAT	GGCCCTGAGCATCTTGTCT
<i>Osbp19</i>	TCCAAGGGACTAGGCTGGTA	CAACAAATAGCATGGTAGAATCAA
<i>Ppib</i>	CAGGGGAGATGGCACAGGAG	CGGCTGTCTGTCTTGGTGTCTCC
<i>Reln</i>	ACATGAGAGGCCACCACACT	CTTCTCAGAGCATTTGGAGGC
<i>Scd1</i>	CCGGAGACCCCTTAGATCGA	TAGCCTGTAAAAGATTTCTGCAAACC
<i>Scd1</i>	CTGTACGGGATCATACTGGTTCCC	CAGCCGAGCCTTGTAAGTTCTGTG
<i>Serpine 1</i>	TGGCTCAGAGCAACAAGTTCAA	TCAAAGGTTGCAGCGATGAACA
<i>Srebf1a</i>	GCCGGCGCCATGGACGAGCTGGCC	CAGGAAGGCTTCCAGAGAGGAGGC
<i>Srebf1c</i>	GGAGCCATGGATTGCACATT	GGCCCGGGAAGTCACTGT
<i>Tgfb1</i>	TGACGTCACCTGGAGTTGTACGG	GGTTCATGTTCATGGATGGTGC
<i>Tnf</i>	GTAGCCACGTCGTAGCAAAC	AGTTGGTTGTCTTTGAGATCCATG
<i>Vim</i>	GGATTCCACTTTCCGTTCAA	GAAATTCGAGGAGGAGATGC

Table S2. CHIP primer table.

Gene	Forward (5' to 3' sequence)	Reverse (5' to 3' sequence)	Reference
<i>Abca1</i>	GCTTTCTGCTGAGTGACTGAACTAC	GAATTACTGCTTTTTGCCGCG	(3)
<i>Actin</i>	GCGGCCAACGCCAAAACCTCTCC	GGCCCCGCGCCGCTCACTCAC	
<i>Chrebp</i>	TCTGTGGATCGTGAACCCTATTT	TTCGTCTCGGGTGGCAACGGGGGACA	(4)
<i>Gapdh</i>	AGTGCCAGCCTCGTCCCGTAGACAAAATG	AAGTGGGCCCCGGCCTTCTCCAT	
<i>Osbpl3-site1</i>	ATTTGCCAGGCACTACCAAC	TCCCCGAAAGGTAAGAGTT	
<i>Osbpl3-site2</i>	TCCTTACCCACACTTTGAG	CTTCCCCTCTCCCATGCTCC	
<i>Osbpl3-site3</i>	TTGGCATCCAAAACACACTG	ACATTTCCCCGACTTCATCA	
<i>Osbpl3-site4</i>	TCATGTGTGGCAGGTTTGT	ATAAAAGCCACCCCTTCCAT	
<i>Osbpl3-site5</i>	CCCAGCTTCTCAGCATCTTC	CTCAATCCTCTTGCCCTCTGC	
<i>Osbpl3-site6</i>	CCTTCTCCCTTTTCTCTGC	ACGGATCTTGACTGGAGCAC	
<i>Srebf1</i>	GAACCAGCGGTGGGAACACAGAGC	GACGGCGGCAGCTCGGGTTTCTC	(3)

Table S3. Cloning primer table.

Gene	Forward (5' to 3' sequence)	Reverse (5' to 3' sequence)
<i>Osbpl3 Topo</i>	CACCATGAGTGACGAGAAGAATCTCG	TCACCATAAGACGGGATGGT

Table S4. siRNA table (HPLC purified).

Gene	Sense sequence (5' to 3' sequence)	Overhangs
<i>Osbpl3 siRNA</i>	AAG UUG GUU UCA CCU UCA ATT	dTdT
<i>Scrambled siRNA</i>	ACA GAC GGA GAC GCA CAC CTT	dTdT

Supplemental references

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specific roles for liver X receptor/corepressor complexes in the regulation of ABCA1 and SREBP1 gene expression. *Mol Cell Biol* 23:5780-5789.

4. Cha, J.Y., and Repa, J.J. 2007. The liver X receptor (LXR) and hepatic lipogenesis. The carbohydrate-response element-binding protein is a target gene of LXR. *J Biol Chem* 282:743-751.