Supplementary Information

Defective FXR-SHP regulation in obesity aberrantly increases *miR-802* expression, promoting insulin resistance and fatty liver

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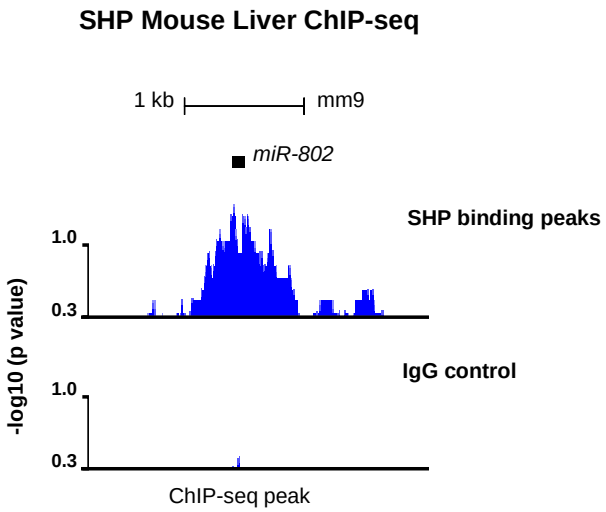
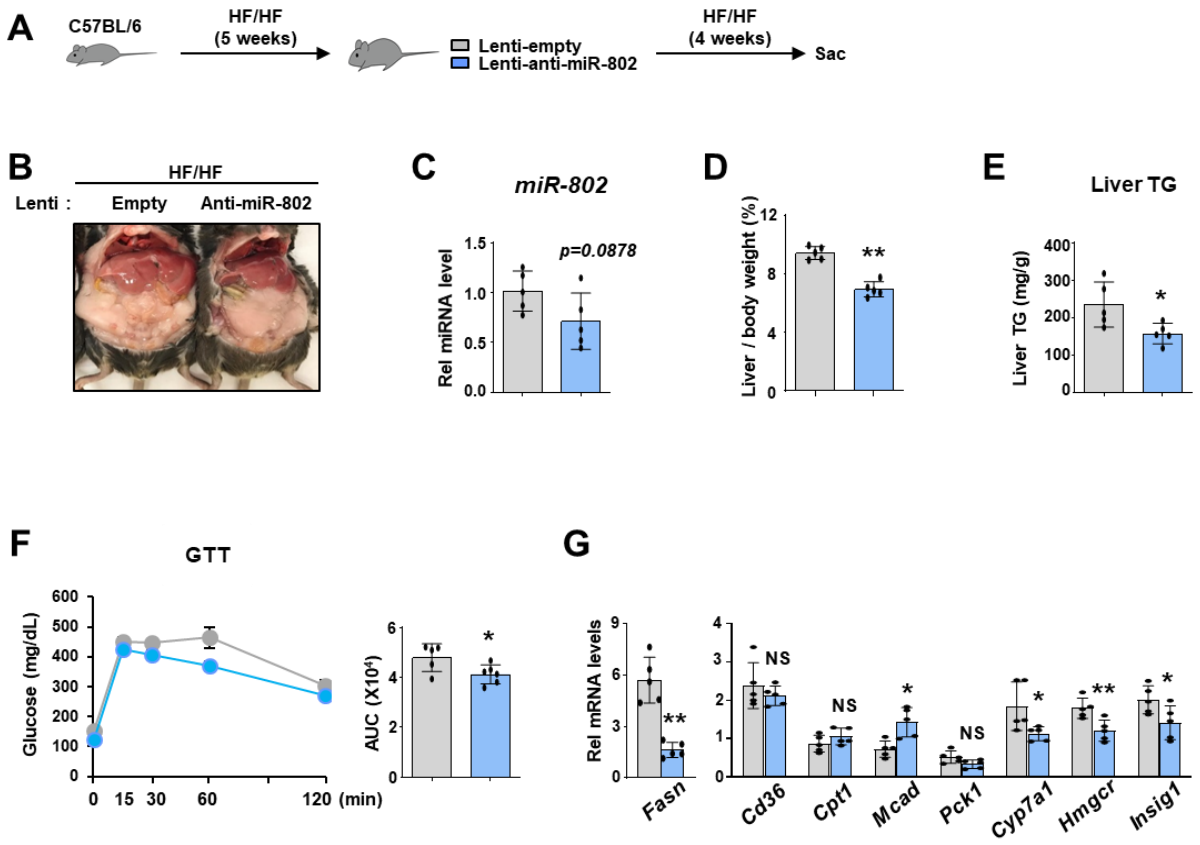
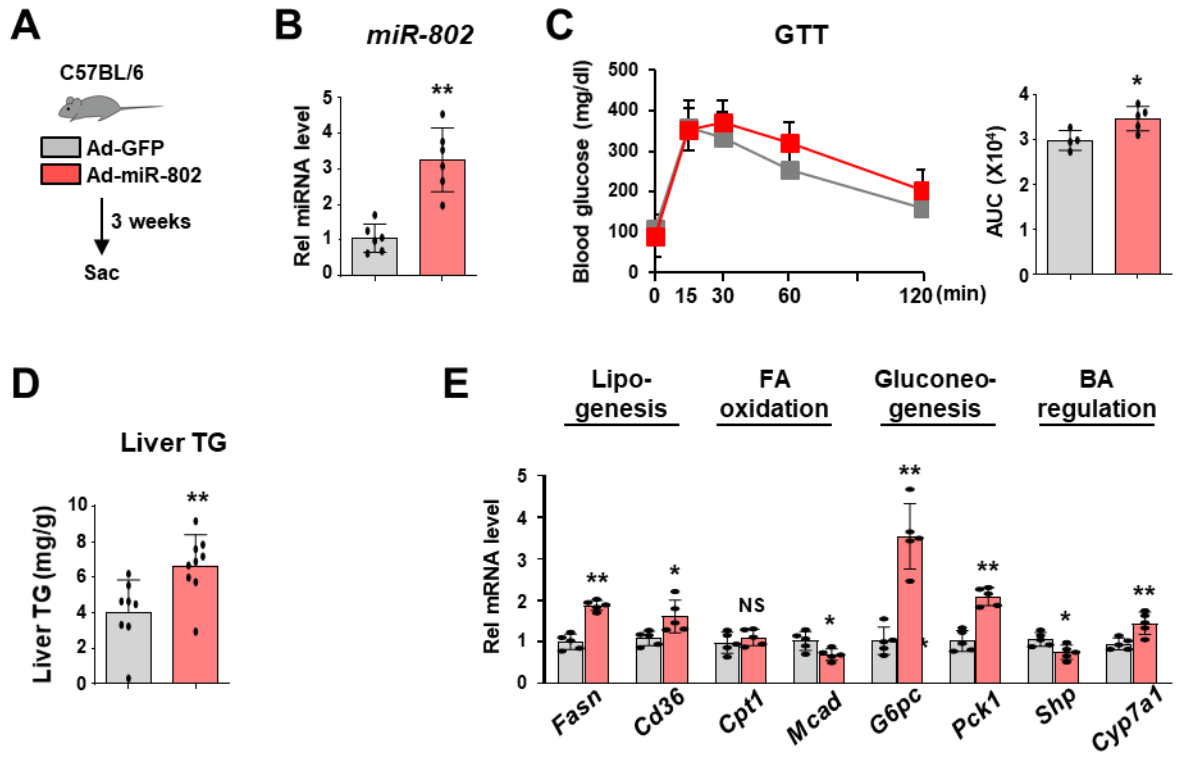
Supplementary Figures



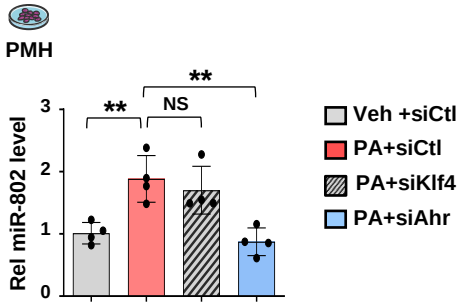
Supplementary Figure 1. UCSD genome browser display of the SHP binding peaks at the *miR-802* gene generated from published SHP mouse liver ChIP-seq data (Kim et al., Liver ChIP-seq analysis in FGF19-treated mice reveals SHP as a global transcriptional partner of SREBP-2, Genome Biology, 2015. 16:268).



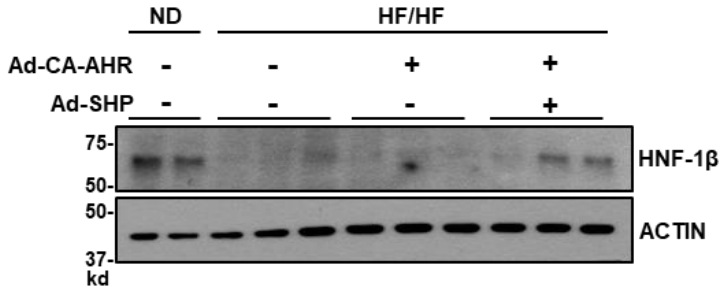
Supplementary Figure 2. Downregulation of miR-802 in dietary obese mice: Downregulation of miR-802 in obese mice resulted in decreased liver TG levels and improved glucose tolerance and altered expression of genes involved in hepatic lipid, glucose, bile acid, and cholesterol metabolism. (A) Experimental outline: C57BL/6 mice were fed high fat/high fructose (HF/HF) chow for 5 weeks and injected via the tail vein with lentivirus expressing miR-802 sponge for miR-802 downregulation for 4 weeks before sacrifice. (B) Representative pictures of obese mice infected with indicated viruses. (C) Hepatic miR-802 levels (n=5). (D) The ratio of liver/body weight, an indicator of fatty liver (n=5). (E) Liver TG levels (n=5). (F) Glucose tolerance test (GTT) and calculated area under the curve (AUC) (right) (n=6). (G) Levels of the mRNAs for genes involved in hepatic lipid, glucose, bile acid and cholesterol metabolism determined by RT-qPCR (n=5). Statistical significance was determined by the Student's t-test (n=5-6), * $p < 0.05$, ** $p < 0.01$, NS, not significant.



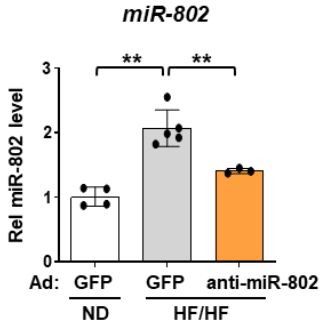
Supplementary Figure 3. Overexpression of miR-802 in normal chow fed mice: Overexpression of miR-802 in lean mice resulted in increased glucose intolerance and liver TG levels and altered expression of genes involved in hepatic lipid, glucose, and bile acid metabolism. (A) Experimental outline: C57BL/6 mice were fed normal chow and were injected via the tail vein with Ad virus expressing miR-802 for 3 weeks before sacrifice. (B) Hepatic miR-802 levels (n=6). (C) Glucose tolerance test (GTT) and calculated area under the curve (AUC) (right) (n=5). (D) Liver TG levels (n=9). (E) Levels of the mRNAs for genes involved in hepatic lipid, glucose, and bile acid metabolism determined by RT-qPCR (n=5). The mean and SD are plotted. Statistical significance was determined by the Student's t-test (n=5-9), * $p < 0.05$, ** $p < 0.01$, NS, not significant.



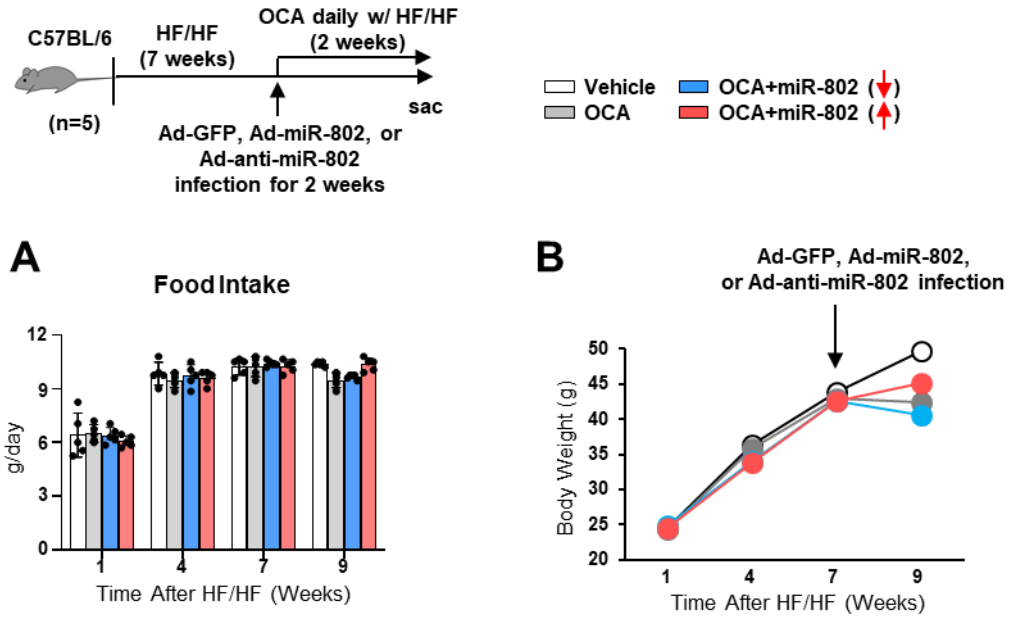
Supplementary Figure 4. Downregulation of AHR, but not KLF4, decreased miR-802 levels in primary mouse hepatocytes (PMHs) treated with palmitic acid to mimic obesity. PMHs were transfected with siRNA for KLF4 or AhR and 24 h later, treated with palmitic acid (PA) (300 μM) for 24 h. Levels of miR-802 determined by RT-qPCR. The mean and standard deviation are plotted. Statistical significance was determined by the one-way ANOVA (n=4), ** $p < 0.01$, NS, not significant.



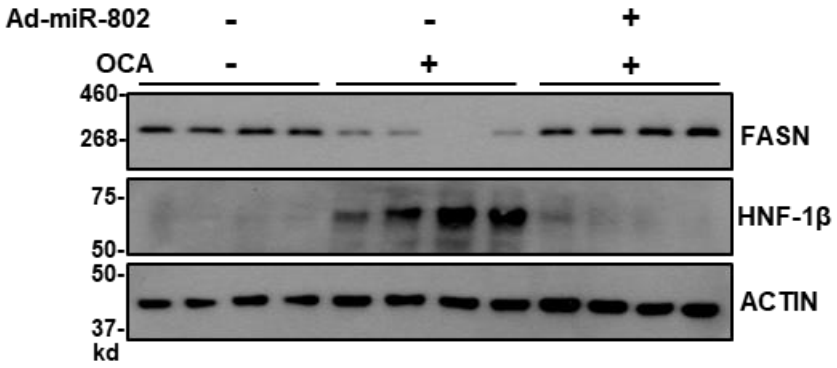
Supplementary Figure 5. Hepatic protein levels of HNF-1β are decreased in dietary obese mice, and overexpression of SHP partially restores the levels. Mice were fed a HF/HF diet for 8 weeks and then, injected via the tail vein with Ad viruses expressing AHR or SHP as indicated 3 weeks before sacrifice. Protein levels of HNF-1β and a loading control Actin in liver extracts determined by IB (n=2 for ND mice, n=3 for HF/HF mice).



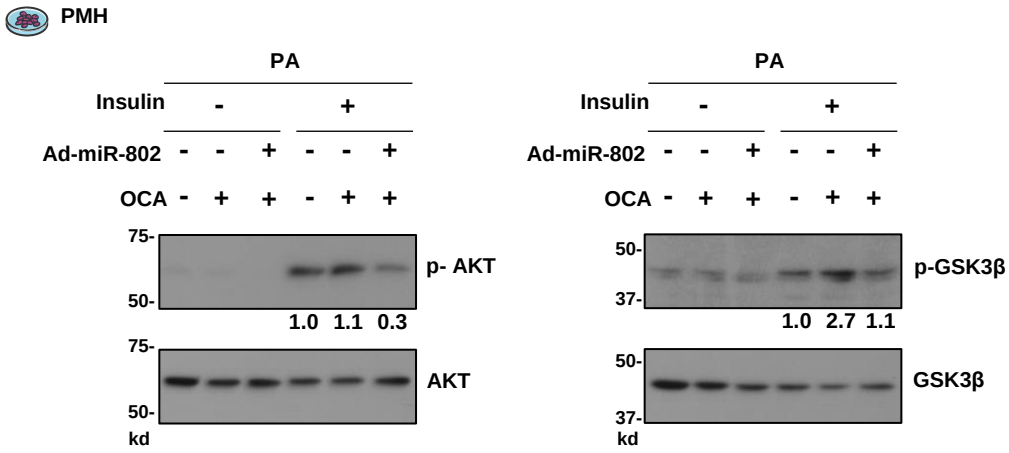
Supplementary Figure 6. Adenoviral expression of antisense RNA for miR-802 in dietary obese mice resulted in significant reduction in hepatic miR-802 levels. Mice were fed a HF/HF diet or normal chow diet (ND) for 7 weeks and infected with the indicated adenovirus 2 weeks before sacrifice. Hepatic miR-802 levels measured by RT-qPCR. The mean and standard deviation are plotted. Statistical significance was determined by one-way ANOVA (n=3-5), ** $p < 0.01$.



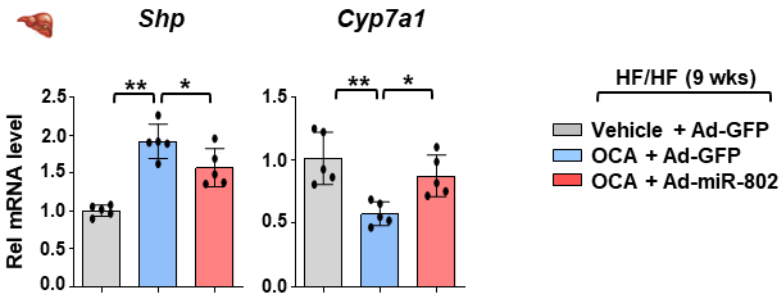
Supplementary Figure 7. The effects of HF/HF, OCA treatment, and overexpression of miR-802 on body weight and food intake in mice. Mice were fed a HF/HF diet for 7 weeks and then, injected via the tail vein Ad-miR-802, Ad-anti-miR-802, or control virus and treated daily with OCA for an additional 2 weeks as indicated in Fig. 5. (A) food intake. (B) body weight.



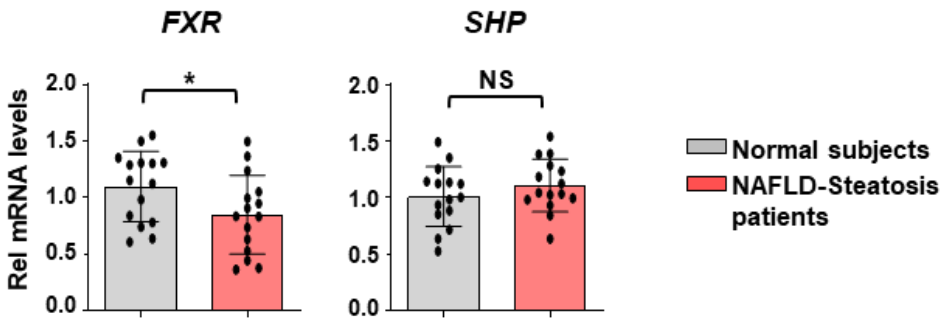
Supplementary Figure 8. OCA-mediated effects on protein levels of FASN and HNF-1β are largely reversed by overexpression of miR-802. Mice were fed a HF/HF diet for 7 weeks and then, injected via the tail vein with Ad-miR-802 or control virus and treated daily with OCA for 2 weeks before sacrifice as indicated in Fig. 5. Protein levels of FASN and HNF-1β in liver extracts determined by IB (n=4 mice)



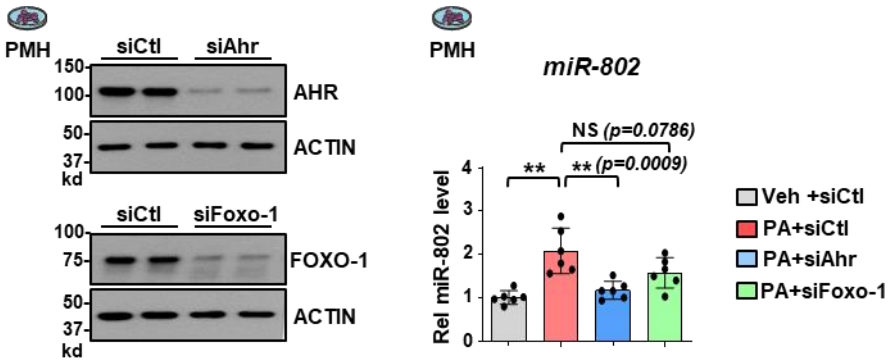
Supplementary Figure 9. Overexpression of miR-802 decreased insulin signaling in OCA-treated hepatocytes. PMHs were infected with Ad-miR802 and treated with OCA (10 μM) and PA (300 μM) for 1 day to mimic obesity, and then, treated with insulin (100 nM) for 10 min. Levels of the indicated proteins measured by IB.



Supplementary Figure 10. The effect of OCA treatment on expression of *Shp and Cyp7a1* in obese mice is blunted by overexpression of miR-802. Mice were fed a HF/HF diet for 7 weeks and then, injected via the tail vein with Ad-miR-802 or control virus and treated daily with OCA for 2 weeks as indicated in Fig. 5 before sacrifice. Levels of the mRNAs for *Shp and Cyp7a1* determined by RT-qPCR. The mean and SD is plotted. Statistical significance was determined by one-way ANOVA (n=5 mice), * $p < 0.05$, ** $p < 0.01$.



Supplementary Figure 11. Hepatic mRNA levels of *FXR* are modestly reduced, whereas those of *SHP* are not changed, in NAFLD patients compared to normal individuals. Liver samples from 15 normal individuals or 15 NAFLD-steatosis patients were analyzed. Hepatic levels of mRNAs for *FXR* and *SHP* determined by RT-qPCR. The mean and SD are plotted. Statistical significance was determined by the Student's t-test (n=15 individuals), * $p < 0.05$, NS, not significant.



Supplementary Figure 12. Downregulation of AHR, but not FOXO-1, significantly decreased miR-802 levels in PMHs treated with palmitic acid. PMHs were transfected with siRNA for AhR or Foxo-1 and 24 h later, treated with palmitic acid (PA) (300 μM) for 24 h. Protein levels of AHR and FOXO-1 determined by IB analysis (left). Levels of miR-802 determined by RT-qPCR (right). The mean and standard deviation are plotted. Statistical significance was determined by the one-way ANOVA (n=6), ** $p < 0.01$, NS, not significant.

Supplementary Tables

Supplementary Table 1: Primer sequences used for RT-qPCR

A. List of primer sequences for mouse RT-qPCR

No.	Gene	Forward (5'-3')	Reverse (5'-3')
1	Hnf-1b	AGGTCCGTGTCTACAACTGG	GTGACCTCATTGTTTCCCGG
2	Hnf4	AGCCACTGTCCCAATCAGTC	TGCAACATAGCATCCTCAGC
3	Foxo1	AACCAGTCCAACTCGACCAC	TGCTCATAAAGTCGGTGCTG
4	Creb1	AGTGCTGTTCTCCCGTCAAG	TGTACCCACCTTCCTTCTGC
5	Crtc2	CCCTGCTATTGAGGAGAACG	GCTGGTCAGGAGATGGAAAG
6	Pck1	CTTCTCTGCCAAGGTCATCC	TTTTGGGGATGGGCAC
7	G6pc	GCTGAAACTTTCAGCCACATCC	TCCAAGCGGGAAACCAAAC
8	Fasn	CCTGGATAGCATTCCGAACCT	AGCACATCTCGAAGGCTACACA
9	Srebp1	GCTGTTGGCATCCTGCTATC	ATGCTGGAAGTGACGGTGGT
10	CD36	GCCAAGCTATTGCGACATGA	AAGGCATTGGCTGGAAGAAC
11	Cpt1	TCGAAACATCTACCATGCAGCA	CAGCATTCTTCGTGACGTTGG
12	Mcad	GATCGCAATGGGTGCTTTTGATAGAA	AGCTGATTGGCAATGTCTCCAGCAAA
13	36B4	CGACTCACAGAGCAGGC	CACCGAGGCAACAGTTGG

B. List of primer sequences for human RT-qPCR

No.	Gene	Forward (5'-3')	Reverse (5'-3')
1	HNF-1b	CAGGAAGGAGGAGGCATTC	CAGCTTGTTTGGAGGAGAGG
2	PCK1	ATCAAGTCAATGCCGACCTC	TATGGATGGGAAAGGGAATG
3	G6PC	TGAGGATGGAGGAAGGAATG	TCACGGACACCAAGATGAAC
4	36B4	AAGGCTGTGGTGCTGATG	GGTCCTCCTTGGTGAACA

Supplementary Table 2: Primer sequences used for ChIP-qPCR

A. List of primer sequences for mouse ChIP-qPCR

Gene	Forward (5'-3')	Reverse (5'-3')
miR-802	TAGGGAAGGAATGTGGCAAG	GGGCAGGCAGTATCCTCTTC
miR-802(No SHP peak)	GACCTCCAGAATCCTCCTT	AGAGTTGCTTTGGGCTGTG
Cyp7a1	ACCTTCGGCTTATCGACTATTGC	TATCTGGCCTTGAACTAAGTCCATCT

B. List of primer sequences for human ChIP-qPCR

Gene	Forward (5'-3')	Reverse (5'-3')
miR-802	AGGAAGGTGGGAGAAGGAAG	GCAGCAAAGATCCGCAAAG