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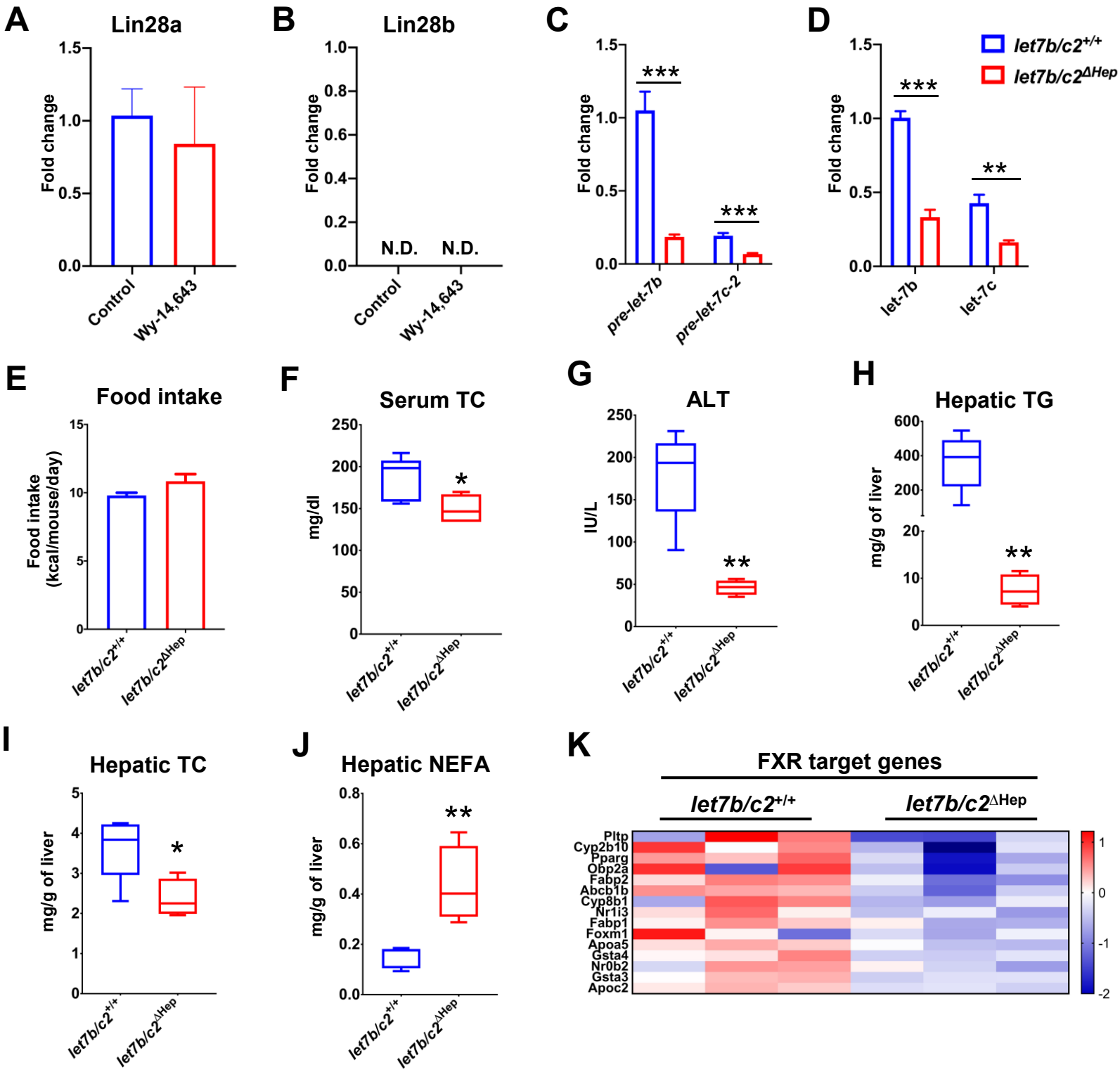
Supplemental information

Feedback repression of PPARα signaling by *Let-7* microRNA

Tomoki Yagai, Tingting Yan, Yuhong Luo, Shogo Takahashi, Daisuke Aibara, Donghwan Kim, Chad N. Brocker, Moshe Levi, Hozumi Motohashi, and Frank J. Gonzalez



Supplemental Figure 1. Lin28 expressions in liver and phenotypic analyses of *let7b/c2*$^{+/+}$ and *let7b/c2*$^{\Delta Hep}$ mice. Related to Figure 1 and 2.

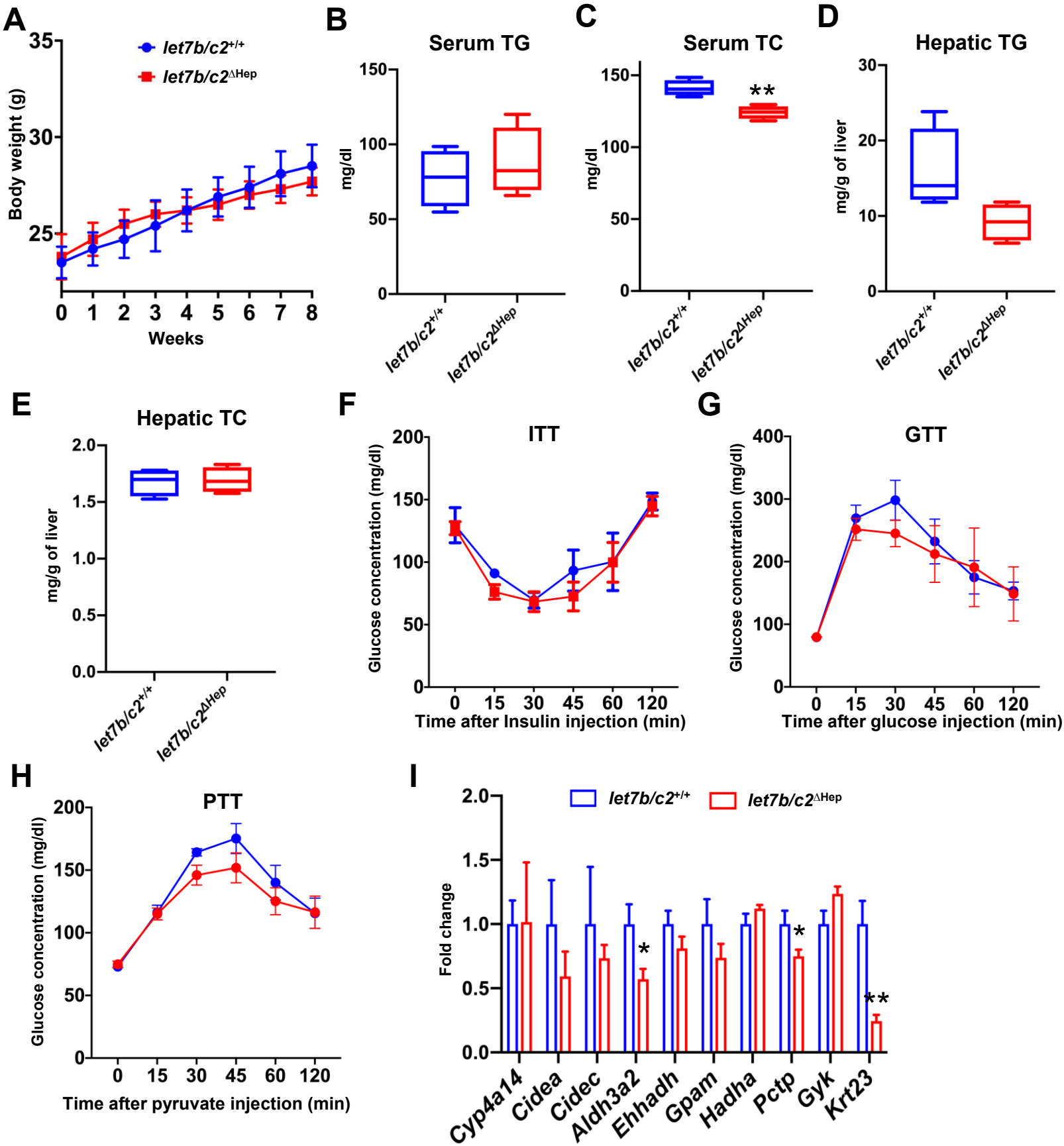
(A and B) Fold change of Lin28a (A) and Lin28b (B) mRNA abundance in Wy-14,643 treated liver. N.D.:Non-Detected.

(C and D) Fold change of *pre-let-7b* and *pre-let-7c-2* (C) and mature *let-7b* and *let-7c* (D) abundance in *let7b/c2*$^{+/+}$ and *let7b/c2*$^{\Delta Hep}$ livers.

(E) Average daily food intake (kcal/mouse/day) of *let7b/c2*$^{+/+}$ and *let7b/c2*$^{\Delta Hep}$ when HFD-feeding.

(F-J) Biochemical analyses for serum total cholesterol (TC) (F); alanine aminotransferase activity (ALT) (G); hepatic triglycerides (TG) (H); hepatic TC (I); and hepatic non-esterified fatty acid (NEFA) (J) in *let7b/c2*$^{+/+}$ and *let7b/c2*$^{\Delta Hep}$ mice after HFD-feeding.

(K) Heat map of FXR target genes identified by differential gene expression analysis of RNA-seq data from *let7b/c2*$^{+/+}$ and *let7b/c2*$^{\Delta Hep}$ livers after HFD-feeding. n = 5 mice/ group. Data are presented as mean ± SEM.



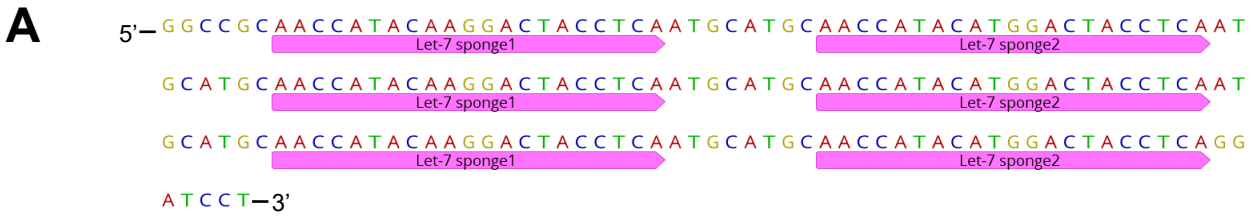
Supplemental Figure 2. Biochemical assays for normal chow diet-fed *let7b/c2*$^{+/+}$ and *let7b/c2*$^{\Delta Hep}$ mice. Related to Figure 2.

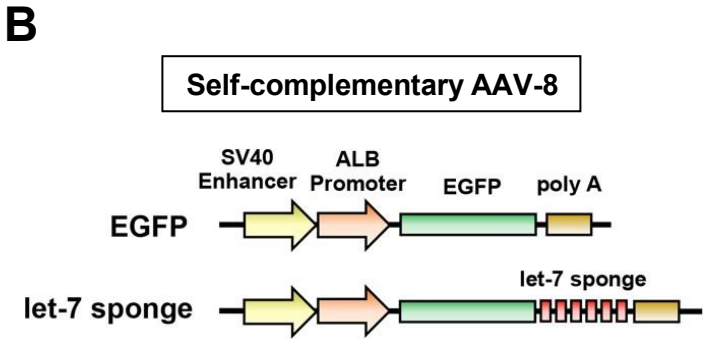
Body weight alterations of *let7b/c2*$^{+/+}$ and *let7b/c2*$^{\Delta Hep}$ mice during normal chow diet-feeding. Average daily food intake (kcal/mouse/day). N = 5 mice/ group. Data are presented as mean ± SEM.

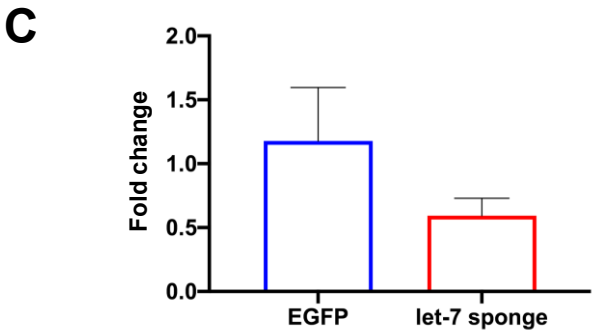
(B-E) Biochemical analyses for serum TG (B); serum TC (C); Hepatic TG (D); and Hepatic TC (E) in *let7b/c2*$^{+/+}$ and *let7b/c2*$^{\Delta Hep}$ mice after normal chow diet-feeding.

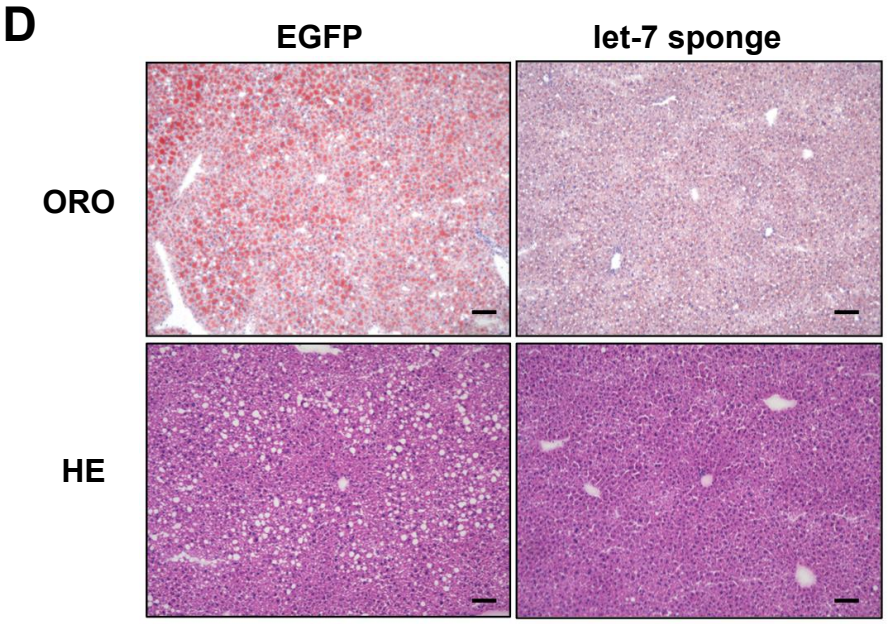
(F-H) ITT (F); GTT (G); and PTT (H) for normal chow diet-fed *let7b/c2*$^{+/+}$ and *let7b/c2*$^{\Delta Hep}$ mice.

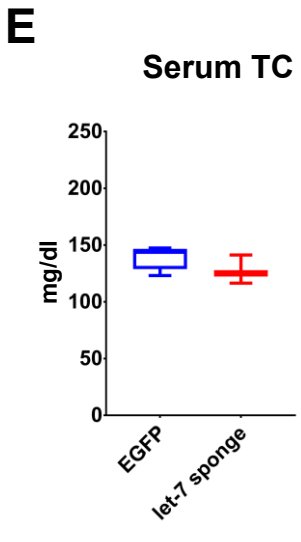
(I) mRNA analysis by qRT-PCR of PPARα target genes in *let7b/c2*$^{+/+}$ and *let7b/c2*$^{\Delta Hep}$ livers after 1-week HFD-feeding. n = 5 mice/ group. Data are presented as mean ± SEM.

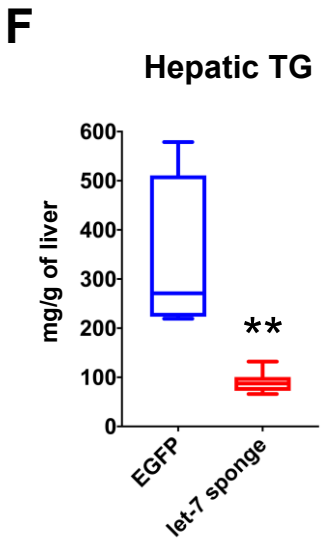

A
5'— GGCCGCAACCATACAAGGACTACCTCAATGCATGCAACCATACATGGACTACCTCAAT
Let-7 sponge1
Let-7 sponge2
GCATGCAACCATACAAGGACTACCTCAATGCATGCAACCATACATGGACTACCTCAAT
Let-7 sponge1
Let-7 sponge2
GCATGCAACCATACAAGGACTACCTCAATGCATGCAACCATACATGGACTACCTCAGG
Let-7 sponge1
Let-7 sponge2
ATCCT—3'

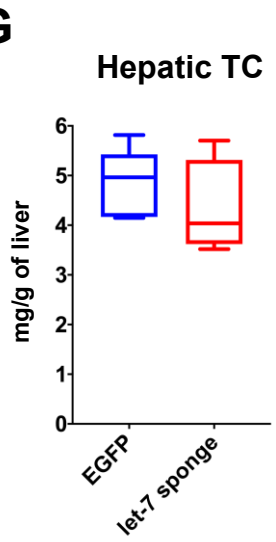

B
Self-complementary AAV-8
SV40 Enhancer
ALB Promoter
EGFP
poly A
EGFP
let-7 sponge
let-7 sponge

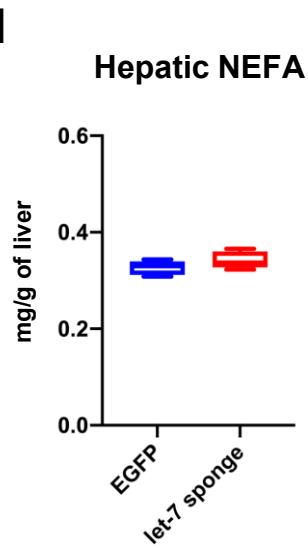

C
Fold change
2.0
1.5
1.0
0.5
0.0
EGFP
let-7 sponge

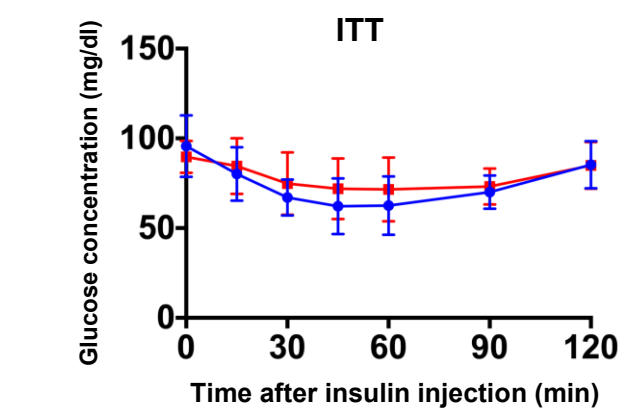

D
EGFP
let-7 sponge
ORO
HE

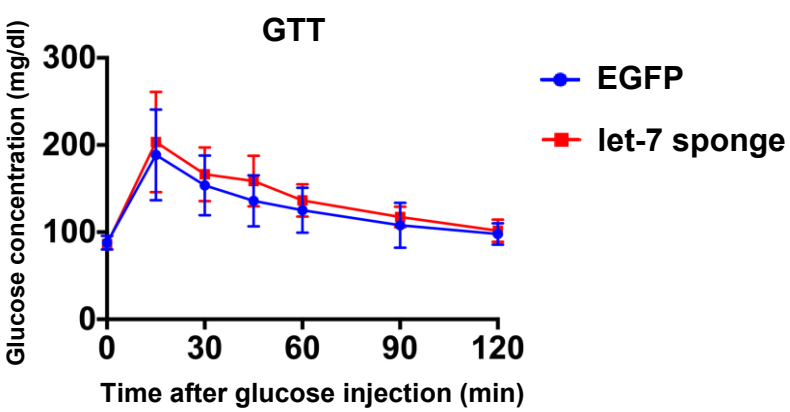

E
Serum TC
mg/dl
250
200
150
100
50
0
EGFP
let-7 sponge


F
Hepatic TG
mg/g of liver
600
500
400
300
200
100
0
**
EGFP
let-7 sponge


G
Hepatic TC
mg/g of liver
6
5
4
3
2
1
0
EGFP
let-7 sponge


H
Hepatic NEFA
mg/g of liver
0.6
0.4
0.2
0.0
EGFP
let-7 sponge


I
ITT
Glucose concentration (mg/dl)
150
100
50
0
0
30
60
90
120
Time after insulin injection (min)


J
GTT
Glucose concentration (mg/dl)
300
200
100
0
0
30
60
90
120
Time after glucose injection (min)
EGFP
let-7 sponge

Supplemental Figure 3. let-7 sponge AAV construction and phenotypes of let-7 sponge AAV-transduced mice related to Figure 2.

(A) Sequence of let-7 sponge inserted in let-7 sponge AAV vector.

(B) Scheme of let-7 sponge expression AAV vector.

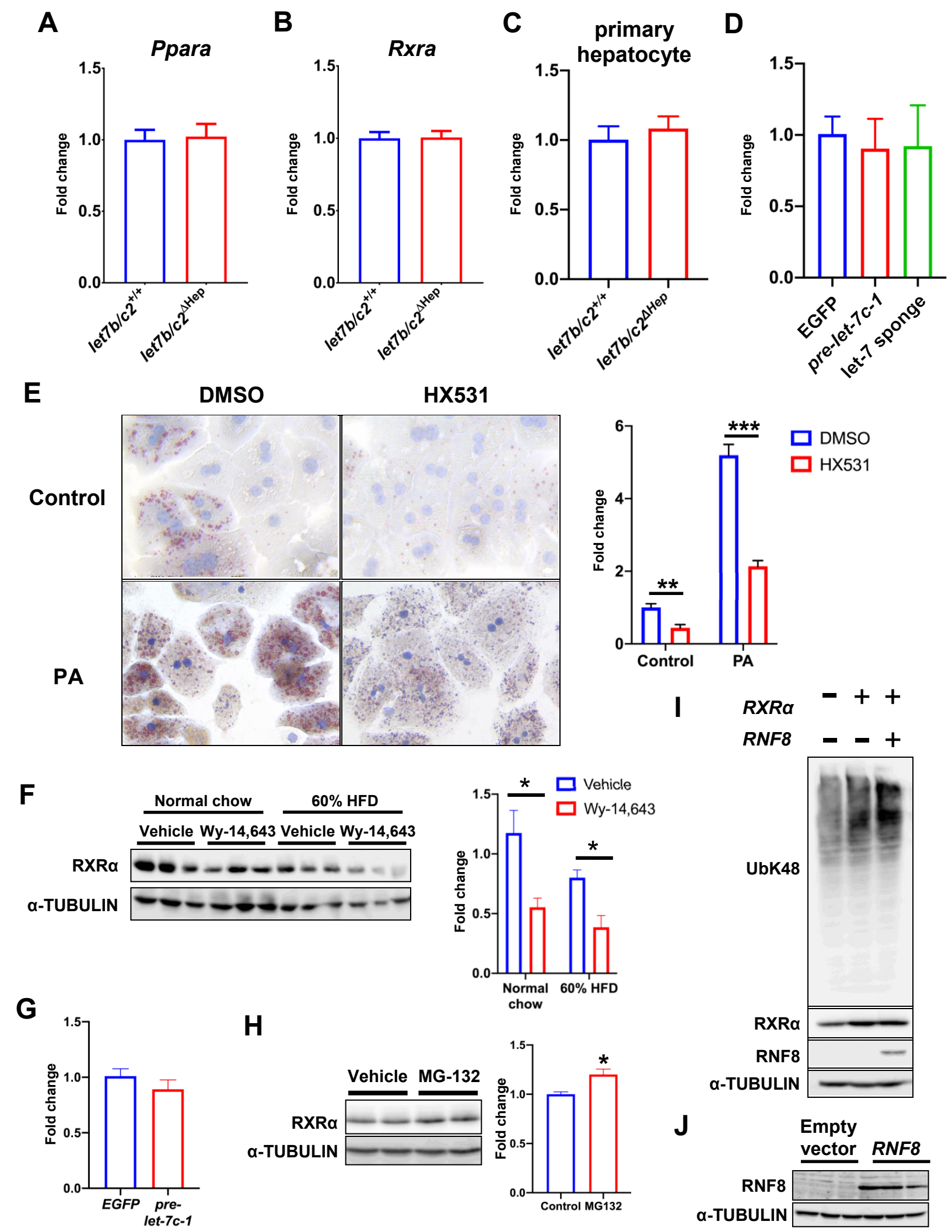
(C) qPCR analysis of GFP DNA in primary hepatocytes transduced by EGFP and let-7 sponge expressing AAV.

(D) Oil Red O staining of liver sections from wild-type mice infected with EGFP or let-7 sponge expressing AAV after being on HFD for eight weeks. Bars = 100μm

(E-H) Biochemical analyses for serum TC (D); hepatic TG (E); hepatic TC (F); and hepatic NEFA (G) concentration in EGFP and let-7 sponge AAV-infected mice after HFD-feeding.

(I and J) ITT (H); and GTT (I) for normal chow diet-fed EGFP and let-7 sponge AAV-infected mice.

Data are presented as mean ± SEM. (n = 4-5 mice per group; *$p < 0.05$)


A
Ppara
Fold change
1.5
1.0
0.5
0.0
let7b/c2+/+
let7b/c2ΔHep
B
Rxra
Fold change
1.5
1.0
0.5
0.0
let7b/c2+/+
let7b/c2ΔHep
C
primary hepatocyte
Fold change
1.5
1.0
0.5
0.0
let7b/c2+/+
let7b/c2ΔHep
D
Fold change
1.5
1.0
0.5
0.0
EGFP
pre-let-7c-1
let-7 sponge
E
DMSO
HX531
Control
PA
Fold change
6
4
2
0
***
**
DMSO
HX531
Control
PA
F
Normal chow
60% HFD
Vehicle
Wy-14,643
Vehicle
Wy-14,643
RXRα
α-TUBULIN
Fold change
1.5
1.0
0.5
0.0
*
*
Vehicle
Wy-14,643
Normal chow
60% HFD
G
Fold change
1.5
1.0
0.5
0.0
EGFP
pre-let-7c-1
H
Vehicle
MG-132
RXRα
α-TUBULIN
Fold change
1.5
1.0
0.5
0.0
*
Control
MG132
I
RXRα
RNF8
UbK48
RXRα
RNF8
α-TUBULIN
J
Empty vector
RNF8
RNF8
α-TUBULIN

Supplemental Figure 4. RXRα expressions, functions and protein ubiquitination by let-7 miRNA related to Figure 3 and 4.

(A-B) Fold change of PPARα (A); and RXRα (B) mRNA in *let7b/c2*$^{+/+}$ and *let7b/c2*$^{\Delta Hep}$ livers fed HFD.

(C) Fold change of RXRα mRNA in *let7b/c2*$^{+/+}$ and *let7b/c2*$^{\Delta Hep}$ primary hepatocytes.

(D) Fold change of RXRα mRNA in EGFP, pre-let-7c-1 and let-7 sponge AAV-transduced liver after HFD-feeding.

(E) Oil red O staining and the morphometric analysis for lipid accumulation in mouse primary hepatocytes treated with Palmitic acid (800 μM) and/or HX-531 (5 μM).

(F) Western blot analysis and the densitometric quantification of RXRα in 60% HFD and/or Wy-14,643-treated mouse livers.

(G) Fold change of Rnf8 mRNA in EGFP and pre-let-7c-1 AAV-transduced liver.

(H) Western blot analysis and the densitometric quantification of RXRα in *Rxra* transfected Hepa-1 cells treated with the proteasome inhibitor MG-132.

(I) Western blot analysis of K48 polyubiquitin chain in *Rxra* and *Rnf8* transfected Hepa-1 cells.

(J) Western blot analysis of RNF8 protein in *Rnf8* transfected Hepa-1 cells.

Data are presented as mean ± SEM. (n = 4-5 blots or fields per group; *p < 0.05, **p < 0.01, ***p < 0.001)

Supplemental Table 1. *let-7* microRNA family in mice related to Figure 1

Mature *let-7* microRNA	Precursor	Primary transcript	Clustered *let-7* precursor	Chromosome	miRBase ID
let-7a	*pre-let-7a-1*	let-7adf cluster	*pre-let-7d, pre-let-7f-1*	Chr.13	MI0000556
	pre-let-7a-2	3110039I08Rik		Chr.9	MI0000557
let-7b	*pre-let-7b*	Lincppara	*pre-let-7c-1*	Chr.15	MI0000558
let-7c	*pre-let-7c-1*	2810055G20Rik		Chr.16	MI0000559
	pre-let-7c-2	Lincppara	*pre-let-7b*	Chr.15	MI0000560
let-7d	*pre-let-7d*	let-7adf cluster	*pre-let-7a-1, pre-let-7f-1*	Chr.13	MI0000405
let-7e	*pre-let-7e*	Spaca6		Chr.17	MI0000561
let-7f	*pre-let-7f-1*	let-7adf cluster	*pre-let-7a-1, pre-let-7d*	Chr.13	MI0000562
	pre-let-7f-2	Huwe1	*pre-miR-98*	Chr.X	MI0000563
let-7g	*pre-let-7g*	Wdr82		Chr.9	MI0000137
let-7i	*pre-let-7i*	mmu-let-7i		Chr.10	MI0000138
miR-98	*pre-miR-98*	Huwe1	*pre-let-7f-2*	Chr.X	MI0000586

Summary of mature *let-7* miRNA, the precursors and the primary transcripts. Mature *let-7a*, *c* and *f* are processed from two precursors. *Pre-let-7a-1*, *pre-let-7d* and *pre-let-7f-1* are processed from an identical primary transcript. *Pre-let-7b* and *pre-let-7c-1* are also processed from an identical primary transcript

Supplemental Table 2. Downregulated pathways in *let7b/c2*$^{\Delta Hep}$ mouse liver compared with *let7b/c2*$^{+/+}$ related to Figure 3

Upstream Regulator	Expr Log Ratio	Molecule Type	Predicted Activation State	Activation z-score
PPARA	0.032	ligand-dependent nuclear receptor	Inhibited	-6.751
INSIG1	0.13	other	Inhibited	-3.564
Alpha catenin		group	Inhibited	-3.389
HOXA3		transcription regulator	Inhibited	-3.357
CFTR		ion channel	Inhibited	-3.194
PPARG	-1.333	ligand-dependent nuclear receptor	Inhibited	-3.171
NR1H4	0.035	ligand-dependent nuclear receptor	Inhibited	-3.134
PPARD	0.348	ligand-dependent nuclear receptor	Inhibited	-3.119
KLF15	0.058	transcription regulator	Inhibited	-2.721
LPL	1.748	enzyme	Inhibited	-2.619
TP73		transcription regulator	Inhibited	-2.601
PNPLA2	0.618	enzyme	Inhibited	-2.498
LCAT	-0.135	enzyme	Inhibited	-2.449
ABCG1	0.414	transporter	Inhibited	-2.433
INSIG2	-0.922	other	Inhibited	-2.415
let-7a-5p (and other miRNAs w/seed GAGGUAG)		mature microRNA	Inhibited	-2.414
ZFP36	-0.182	transcription regulator	Inhibited	-2.412
let-7		microRNA	Inhibited	-2.401
ZBTB20	0.568	transcription regulator	Inhibited	-2.4

Pathway analysis for RNA-seq data from 60% HFD-fed *let7b/c2*$^{+/+}$ and *let7b/c2*$^{\Delta Hep}$ mouse livers. PPAR and FXR pathways (blue labeled) were significantly repressed by *let7b/c2* deletion in liver.

Supplemental Table 3. Primers and Oligos used in this paper related to STAR Methods

Primers for mouse Quantitative RT-PCR		
	Forward (5' to 3')	Reverse (5’ to 3’)
pre-Let-7a-1	TTAGGGTCACACCCACCAC	ATCACCTTAGGAAAGACAGTAGATTG
pre-Let-7a-2	TAGAGTTACATCAAGGGAGATAACTG	GTGCAAGTCCCAAGGAAAG
pre-Let-7b	TCAGGGCAGTGATGTTGCC	TCAGGGAAGGCAGTAGGTTG
pre-Let-7c-1	GAGTTACACCCTGGGAGTTAACTG	AGTGTGCTCCAAGGAAAGCTAG
pre-Let-7c-2	TTTTGGGCTCTGCCCCGC	GCGGCCACTTCAGGAAAGACAG
pre-Let-7d	TTAGGGCAGAGATTTTGCCC	GCCCTAAGAAAGGCAGCAGG
pre-Let-7e	TGAGGAAGACACCCGAGGAG	GCAGCCTGGGGAAAGCTAG
pre-Let-7f-1	GTGGGGTAGTGATTTTACCCTG	CTCAGGGAAGGCAATAGATTGTATAG
pre-Let-7f-2	TTAGGGTCATACCCCATCTTGG	CGTGGGAAAGACAGTAGACTGTATAG
pre-Let-7g	TGAGGGTCTATGATACCACCCG	GCAAGGCAGTGGCCTGTAC
pre-Let-7i	GGTCGGGTTGTGACATTGC	CTAGCAAGGCAGTAGCTTGCG
pre-miR98	GTGGGGTAGGGATTTTAGGCC	ATGCCACACACCAAGGAAAGTAG
Cyp4a14	CCTGACTTTCTTTCGCCTGC	TGATCACTCCATCTGTGTGCT
Ehhadh	CCGGTCAATGCCATCAGT	CCGGTCAATGCCATCAGT
Hadha	AGGATCCTACGCTCCAGAGG	AAGCAGGATCCACTGATGGC
Krt23	CTGATTCAGCTCCGCCAAGA	TCCATCGTCCCTTCAGTGTC
Aldh3a2	GGTCAAACCTGCATTGCTCC	TCCCCATAAAAGTCCTTCACCG
Cidea	GACAGAAATGGACACCGGGTA	TGACATTGAGACAGCCGAGG
Cidec	TGGCACAATCGTGGAGACAG	CTCTCTTCTTGCGCTGTTCTG
Gpam	TGTGTACCCGAAGGTCTCCA	TGTTTACATCGGCCAAGGCT
Pctp	GGCTCTCCTCGCAGATGTTT	TGAGCCAGGACGGAATTTGG
Gyk	TGGCCTAATGAAAGCTGGGG	TGCACTGGGCTCCCAATAAG
Gapdh	AGGTCGGTGTGAACGGATTTG	TGTAGACCATGTAGTTGAGGTCA
Ppara	CACGCATGTGAAGGCTGTAA	CACGCATGTGAAGGCTGTAA
Rxra	CTCAATGGCGTCCTCAAGGT	ACCCCATAGTGTTTGCCTGAG
Rnf8	GACTTTCCAGGAGGAGGCAC	CCAGGGAGTTGGTTCTGGAC
Egfp	AAGCTGACCCTGAAGTTCATCTGC	CTTGTAGTTGCCGTCGTCCTTGAA
Oligos for Let-7 sponge AAV construction (5' to 3')		
Let-7 sponge	GGCCGCAACCATACAAGGACTACCTCAATGCATGCAACCATACATGGACTACCTCAATGCATGCAACCATACAAGG ACTACCTCAATGCATGCAACCATACATGGACTACCTCAATGCATGCAACCATACAAGGACTACCTCAATGCATGCAA CCATACATGGACTACCTCAGGATCCT	
Let-7 sponge complement	CTAGAGGATCCTGAGGTAGTCCATGTATGGTTGCATGCATTGAGGTAGTCCTTGTATGGTTGCATGCATTGAGGTAG TCCATGTATGGTTGCATGCATTGAGGTAGTCCTTGTATGGTTGCATGCATTGAGGTAGTCCATGTATGGTTGCATGC ATTGAGGTAGTCCTTGTATGGTTGC	
Primers for *pre-Let-7c-1* AAV construction (5' to 3')		
Let-7c-1 NotI EcoRV	AAAGCGGCCGCTAATGGAAGTGTGGTCAAGG	AAAGATATCGCGTTGTGTCATTGCTTACAAG
Oligos for *Rnf8* 3'UTR luciferase reporter vectors		
Rnf8 3'UTR Wild-type	AAAGCTAGCGACTGAGTTAGGAAGTCTGTGCCACTGGCCTTCCGTCATGCAGGCCCTGCTGCTACCACCCCCCAA CGTGTGCTGGGACCCTGCTCGGACTGCCTGACTGTGCATTAAATGAGTTGAGTTCTGTGTTTCTTAATTTGTTGATT GTTTTTGATACCTCATGCACCCTCTTGACATCATTGTGGACAGCTGGGTGTGAGTCCTGTGGCTGTGTGGAAGGT GGCCTGCATTTTGAGAGCCTGGTTTACCTTTTTATGAAAACATGATGTCATTTCTTGGGAATAAAATGTATAAAACTC TCAGTTGCTCAGCTGGCTCGAGAAA	
Rnf8 3'UTR Mutant	AAAGCTAGCGACTGAGTTAGGAAGTCTGTGCCACTGGCCTTCCGTCATGCAGGCCCTGCTGCTACCACCCCCCAA CGTGTGCTGGGACCCTGCTCGGACTGCCTGACTGTGCATTAAATGAGTTGAGTTCTGTGTTTCTTAATTTGTTGATT GTTTTTGAGCGGCCGCTGCACCCTCTTGACATCATTGTGGACAGCTGGGTGTGAGTCCTGTGGCTGTGTGGAAG GTGGCCTGCATTTTGAGAGCCTGGTTTACCTTTTTATGAAAACATGATGTCATTTCTTGGGAATAAAATGTATAAAAC TCTCAGTTGCTCAGCTGGCTCGAGAAA	

Primers and oligos used for qRT-PCR. AAV constructs and *Rnf8* 3'UTR reporter vectors.