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Supplemental Information

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Supplementary Information

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Transparent Methods

Materials

The human hepatocellular carcinoma cell line Huh7.5 was a kind gift from Dr. Charles M. Rice (Rockefeller University, New York, NY). All *mir*Vana mimics and inhibitors, along with controls, were purchased from Ambion (Austin, TX).

Cell culture, transfections, and viral infection

Huh7.5 cells were maintained in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10 % fetal bovine serum (FBS) and 100 nM non-essential amino acids. For transfections of uninfected cells, cells were seeded in 6-well plates. The following day, the transfection mixture containing RNAiMax (Thermo Fisher Scientific) and the miRNA control mimic/inhibitor or miR-124 mimic/inhibitor at a final concentration of 100 nM in Opti-MEM was added to the cells. For RNA analysis, after 48 h, cells were harvested in RLT Plus lysis buffer (RNeasy Plus kit, Qiagen, Mississauga, ON). For western blot analysis, cells were lysed 48 h post-transfection in 1X SDS lysis buffer (50 mM Tris-HCl [pH 6.8], 2 % SDS, and 10 % glycerol).

Serum starvation experiments were performed by seeding Huh7.5 cells in 6-well plates and growing cells in serum free media for 48 h. Cells were then lysed using ML Buffer (NucleoSpin miRNA Isolation Kit lysis buffer; Macherey-Nagel, Düren, Germany).

HCV infections of Huh7.5 cells were performed with the HCV $JFH1_T$ strain. This strain is derived from the cell culture-adapted JFH-AM1 strain and contains three amino acid substitutions (Russell et al., 2008). Briefly, cells were infected at an MOI of 0.1 then virus was removed following a 5 h infection. Forty-eight hours post infection, supernatant was collected for virus titration and cells were lysed in ML Buffer (Macherey-Nagel) for RNA extraction. For examining miRNA's antiviral effects, Huh7.5 cells were transfected with 100 nM of miRNA

mimic (control or miR-124). Twenty-four hours post-transfection, cells were infected with HCV $JFH1_T$. At 48 h post-infection, cell supernatants were removed and used for infectious titer determination, and cells were lysed with RLT Plus lysis buffer (Qiagen) for RNA isolation.

Quantitative real-time PCR

RNA was quantified using a NanoDrop (Thermo Fisher Scientific). Reverse transcription of 250 ng of RNA was performed with random hexamers using a SuperScript II Reverse Transcriptase kit (Life Technologies, Carlsbad, CA) according to the manufacturer's protocol. qPCR was subsequently performed on a CFX Connect Real-Time PCR Detection System (Bio-Rad, Hercules, CA) using SsoAdvanced Universal SYBR Green Supermix (Bio-Rad) according to the manufacturer's instructions. Primers were added to a final concentration of 500 nM in a total reaction volume of 10 µL. Primers used for qPCR are listed in Table S3. All primers were validated prior to use with melt curves and standard curves. For determination of miRNA expression levels, 5 ng total RNA was reverse transcribed using the TaqMan MicroRNA Reverse Transcription Kit (Life Technologies), and qPCR reactions were performed using TaqMan Universal PCR Mastermix (No AmpErase UNG; Life Technologies) with primers specific for miR-124 or RNU6B. For both mRNA and miRNA quantification, the $2^{-\Delta\Delta Ct}$ method was used to calculate fold changes in expression relative to control samples, with 18S rRNA or RNU6B levels being used for normalization (Schmittgen, 2001).

Immunoblotting

Lysates were thawed and passed through a 21G needle (BD Biosciences) before determining their protein concentration by DC assay (Bio-Rad). SDS-PAGE was performed using 10 to 20 ug of protein lysate loaded on a 12 % TGX gel (Bio-Rad) and the migrated proteins were visualized

on a ChemiDoc MP (Bio-Rad) using the Stain Free activation protocol for 5 min. The migrated proteins were then transferred onto a PVDF membrane using a Trans-Blot Turbo (Bio-Rad) Membranes were then blocked with 5 % BSA or 5 % milk in TBS-T prior to incubation with primary antibodies. The membrane was then probed with either mouse anti-AADAC (1:1000 dilution; Santa Cruz Biotechnology, sc-390591), mouse anti-HADHA (1:1000 dilution, Santa Cruz Biotech., sc-374497), or mouse anti-PECI (1:1000 dilution, Santa Cruz Biotech., sc-136374) followed by goat anti-mouse antibody conjugated to horseradish peroxidase (1:20000; Jackson ImmunoResearch Laboratories, Inc., 115-035-062). Blots were visualized with Clarity ECL Western blotting reagents (Bio-Rad) on a ChemiDoc MP (Bio-Rad). Stain-free detection of total protein loading was used for the control. Blot images were cropped and adjusted for contrast using Image Lab (Bio-Rad).

Triglyceride assays

Triglyceride levels were quantified by spectrophotometric analyses, using the TG quantification kit (BioVision) as per manufacturer's protocols, with a few minor modifications. Cells were lysed from a 6-well plate in 5 % NP-40 substitute (BioShop, Canada) in water. Samples were diluted 1 in 10 in TG assay buffer; then 50 uL was added to a 96-well plate in duplicate, with and without lipase. The remainder of the protocol mirrored the manufacturer's protocol.

Oil red O lipid staining and Microscopy

Huh7.5 were seeded in 4-well chamber slides. The next day cells were transfected with 100 nM of miRNA mimic (control or miR-124). 48h post-transfection, cells were washed with 1X with PBS and then fixed with 10% formaldehyde for 10 mins at RT. Following the incubation, 10% formaldehyde was replaced with fresh 10% formaldehyde and incubated at RT for an hour. Fixed cells were then washed 2X with water and incubated for 5 minutes with 60%

isopropanol. Cells were completely dried before incubation with 60% isopropanol containing oil red O for 10 minutes. Oil Red O (sigma) stock solution was prepared at 0.35% final concentration in isopropanol and filtered through 0.2μm filter. Cells were immediately washed 5X with water and chamber slide was then mounted with ProLong™ Gold Antifade Mountant with DAPI (Thermofisher). Images were then acquired with Axiophot fluorescence microscope (Zeiss) attached to a DP-70 Colour CCD camera (Olympus) with a 50W mercury fluorescence excitation light source. Images were taken using ImagePro 6 software suite (mediaCybernetics) and analyzed using ImageJ (NIH).

Infectivity assay

Supernatants of HCV infected cells were filtered through a 45 μm PES filter (Ultident, Saint-Laurent, Canada) before being serially diluted 10-fold in medium. For HCV infectivity assays, 100 μL of each dilution was then used to infect Huh7.5 cells seeded (at 5×10^4 per well) onto 8-well Lab-Tek II chamber slides (NUNC) for 4 h. Following incubation, the infectious medium was removed and replaced with fresh medium. Forty-eight hours post infection, cells were fixed and stained with HCV core monoclonal antibody (1:100; ThermoFisher Scientific; MA1080), followed by a secondary antibody, Alexa Fluor 488–conjugated goat anti-mouse (1:250; ThermoFisher Scientific; A-11029). Viral titers are expressed as the number of focus-forming units (FFU) per mL of supernatant.

mRNA microarray

RNA isolation from Huh7.5 cells was performed with the RNeasy kit (Qiagen). Gene expression profiling was performed using Affymetrix Human Gene ST.2.0 arrays. Data was normalized and analyzed using the Applied Biosystems Transcriptome Analysis Console (v4.0), according to the

manufacturer's protocols. Pathway enrichment analysis was performed on genes repressed by miR-124 more than 1.5-fold and predicted by TargetScan (Agarwal et al., 2015), irrespective of conservation, to be a miR-124 target. This analysis was performed using the ToppGene Suite (Chen et al., 2009). For ToppGene pathway enrichment analysis, *P*-values were adjusted with Bonferroni correction.

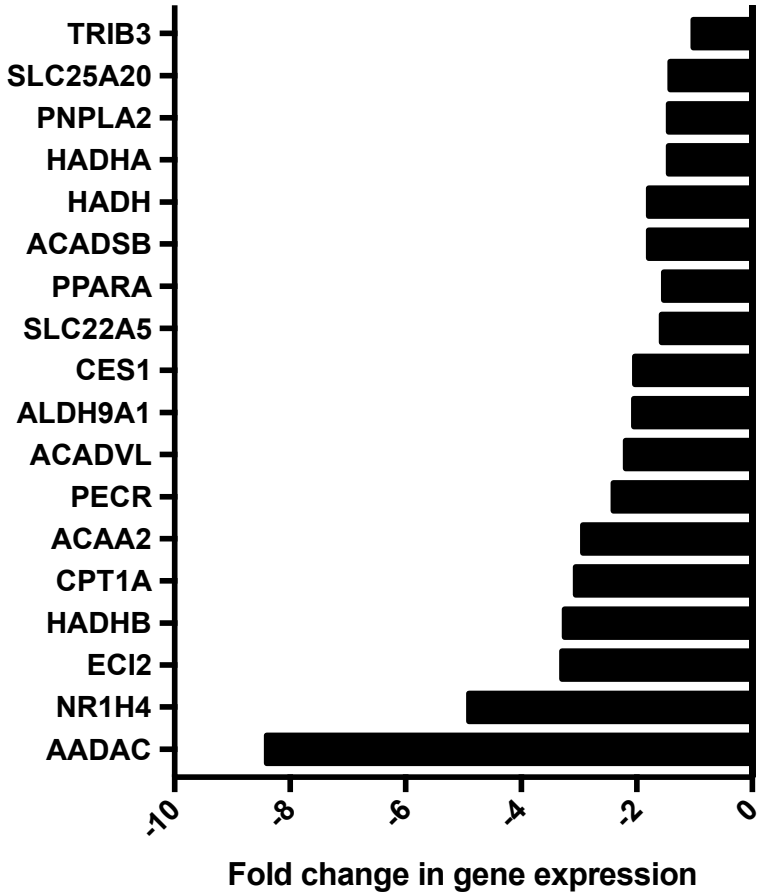
Supplemental References

Agarwal, V., Bell, G.W., Nam, J.W., and Bartel, D.P. (2015). Predicting effective microRNA target sites in mammalian mRNAs. eLife *4*, e05005.

Chen, J., Bardes, E.E., Aronow, B.J., and Jegga, A.G. (2009). ToppGene Suite for gene list enrichment analysis and candidate gene prioritization. Nucleic Acids Res. *37*, W305-W311.

Russell, R.S., Meunier, J.C., Takikawa, S., Faulk, K., Engle, R.E., Bukh, J., Purcell, R.H., and Emerson, S.U. (2008). Advantages of a single-cycle production assay to study cell culture-adaptive mutations of hepatitis C virus. Proc. Natl. Acad. Sci. USA *105*, 4370-4375.

Schmittgen, T.D. (2001). Real-time quantitative PCR. Methods *25*, 383-385.



Supplementary Figure S1. Microarray gene expression analysis in miR-124 transfected mimic Huh7.5 cells. Related to Figure 2. Fold changes in gene expression of select lipid-metabolism-related genes in Huh7.5 cells transfected with 100 nM of miR-124 mimic are shown. Fold changes were calculated relative to gene expression in 100 nM control mimic-transfected Huh7.5 cells.

a

miR-124-3p U**AAGGCA**CGCGGUGAAUGCC

b

CPT1A (ENST00000265641.5)
3 miR-124 binding sites
3'UTR position 641-648

...AGUC**UUCAC**AACCCA**GUGCCUUA**...

3'UTR position 1240-1246

...GUCC**UUCA**GAUAGGA**GUGCCUU**C...

3'UTR position 1506-1512

... CUUGCCGGAGAACAC**GUGCCUU**U...

c

ACADVL (ENST00000356839.5)
1 miR-124 binding site
3'UTR position 31-37

...GCCUGUCCCAGUUAU**GUGCCUU**C...

d

ACADSB (ENST00000358776.4)
1 miR-124 binding site
3'UTR position 75-81

...UCUUGUUGGGAGUAA**GUGCCUU**G...

e

ECI2 (ENST00000380118.3)
1 miR-124 binding site
3'UTR position 87-93

...UAAAUAAGC**UUCA**UU**GUGCCUU**U...

f

HADHA (ENST00000380649.3)
1 miR-124 binding site
3'UTR position 136-142

...UGCUCCCUGAUUAAA**GUGCCUU**C...

g

SLC25A20 (ENST00000430379.1)
1 miR-124 binding site
3'UTR position 141-151

...ACUUGGUGAGACUGU**UGCCUU**AA...

h

PNPLA2 (ENST00000336615.4)
1 miR-124 binding site
3'UTR position 189-196

... AGGCCCCGCG**CACC**U**GUGCCUUA**...

i

ACAA2 (ENST00000285093.10)
2 miR-124 binding sites
3'UTR position 291-297

... AGUAAC**CACC**ACUUC**UGCCUUAG**...

3'UTR position 331-337

... AAAUCAAAUAAAUGU**UGCCUUAA**...

j

HADH (ENST00000403312.1)
1 miR-124 binding site
3'UTR position 251-257

... AGCUUUA**CACC**CUUG**GUGCCUU**G...

k

PECR (ENST00000265322.7)
2 miR-124 binding sites
3'UTR position 32-38

... GUCCUCCAUCCCCCA**GUGCCUU**C...

3'UTR position 792-798

... CUUUAGGAGCAAUAA**UGCCUUA**U...

l

ALDH9A1 (ENST00000354775.4)
1 miR-124 binding site
3'UTR position 181-187

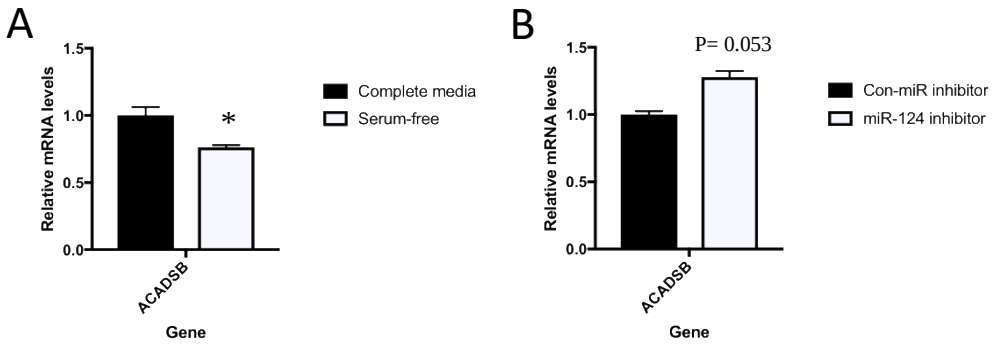
... GAAAAUGUGCAUUAA**GUGCCUU**G...

m

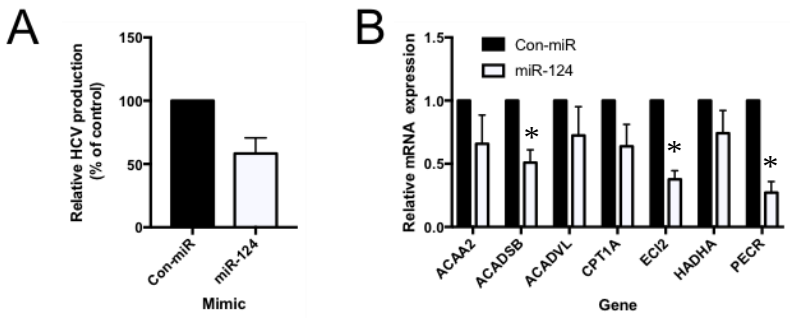
SLC22A5 (ENST00000245407.3)
1 miR-124 binding site
3'UTR position 1290-1296

... GUGAAAGCUACUGAA**GUGCCUU**G...

Supplementary Figure S2. miRNA recognition elements in direct targets of miR-124, related to Figures 2 & 4. (a) Sequence of mature miR-124 with seed sequence highlighted in yellow. Sequence of miR-124 binding sites in the 3'UTRs of (b) *CPT1A*, (c) *ACADVL*, (d) *ACADSB*, (e) *ECI2* (f) HADHA, (g) *SLC25A20*, (h) *PNPLA2*, (i) *ACAA2*, (j) *HADH*, (k) *PECR*, (l) *ALDH9A1* and (m) *SLC22A5*. Sequences were taken from TargetScan. Seed sequences are highlighted in yellow, and nucleotides predicted to be involved in supplementary interactions with the 3' end of miR-124 are highlighted in green.



Supplementary Figure S3. Expression of ACADSB during serum depletion and miR-124 inhibition, related to Figure 2. (A) Huh7.5 cells were cultured in complete or serum-free media for 48 h. qRT-PCR was performed to measure relative ACADSB expression (n = 4). (B) Huh7.5 cells were transfected with control or miR-124 inhibitor for 6 hours and were then cultured in complete or serum-free media for 48 hours. qRT-PCR was performed to measure relative ACADSB expression (n = 2). Data are represented as the mean ± SEM (*$P < 0.05$).



Supplementary Figure S4. miR-124 regulates HCV infection, related to Figures 2 & 4. (A) Relative HCV production and (B) miR-124 target expression in control (Con-miR) and miR-124 mimic transfected Huh7.5 cells infected with HCV. Viral production was assessed by a focus-forming assay while target expression was measured via qRT-PCR analysis. Data are represented as the mean ± SEM.

NM_000183.2

TAGATCCAGAAGAAGTGACCTGAAGTTTCTGTGCAACACTCACACTAGGCAATGCC
ATTTCAATGCATTACTAAATGACATTTGTAGTTCCTAGCTCCTCTTAGGAAAACAGTT
CTTG**TGGCCTT**CTATTAAATAGTTTGCACTTAAGCCTTGCCAGTGTTCTGAGCTTTT
CAATAATCAGTTTACTGCTCTTTCAGGGATTTCTAAGCCACCAGAATCTCACATGAG
ATGTGTGGGTGGTTGTTTTTGGTCTCTGTTGTCACTAAAGACTAAATGAGGGTTTG
CAGTTGGGAAAGAGGTCAACTGAGATTTGGAAATCATCTTTGTAATATTTGCAAATT
ATACTTGTTCTTATCTGTGTCCTAAAGATGTGTTCTCTATAAAATACAAACCAACGTG
CCTAATTAATTATGGAAAAATAATTCAGAATCTAAACACCACTGAAAACTTATAAAAA
ATGTTTAGATACATAAATATGGTGGTCAGCGTTAATAAAGTGGAGAAATATTGGAAA
AAAAAA

NM_001281512.1

TAGATCCAGAAGAAGTGACCTGAAGTTTCTGTGCAACACTCACACTAGGCAATGCC
ATTTCAATGCATTACTAAATGACATTTGTAGTTCCTAGCTCCTCTTAGGAAAACAGTT
CTTG**TGGCCTT**CTATTAAATAGTTTGCACTTAAGCCTTGCCAGTGTTCTGAGCTTTT
CAATAATCAGTTTACTGCTCTTTCAGGGATTTCTAAGCCACCAGAATCTCACATGAG
ATGTGTGGGTGGTTGTTTTTGGTCTCTGTTGTCACTAAAGACTAAATGAGGGTTTG
CAGTTGGGAAAGAGGTCAACTGAGATTTGGAAATCATCTTTGTAATATTTGCAAATT
ATACTTGTTCTTATCTGTGTCCTAAAGATGTGTTCTCTATAAAATACAAACCAACGTG
CCTAATTAATTATGGAAAAATAATTCAGAATCTAAACACCACTGAAAACTTATAAAAA
ATGTTTAGATACATAAATATGGTGGTCAGCGTTAATAAAGTGGAGAAATATTGGAAA
AAAAAA

NM_001281513.1

TAGATCCAGAAGAAGTGACCTGAAGTTTCTGTGCAACACTCACACTAGGCAATGCC
ATTTCAATGCATTACTAAATGACATTTGTAGTTCCTAGCTCCTCTTAGGAAAACAGTT
CTTG**TGGCCTT**CTATTAAATAGTTTGCACTTAAGCCTTGCCAGTGTTCTGAGCTTTT
CAATAATCAGTTTACTGCTCTTTCAGGGATTTCTAAGCCACCAGAATCTCACATGAG
ATGTGTGGGTGGTTGTTTTTGGTCTCTGTTGTCACTAAAGACTAAATGAGGGTTTG
CAGTTGGGAAAGAGGTCAACTGAGATTTGGAAATCATCTTTGTAATATTTGCAAATT
ATACTTGTTCTTATCTGTGTCCTAAAGATGTGTTCTCTATAAAATACAAACCAACGTG
CCTAATTAATTATGGAAAAATAATTCAGAATCTAAACACCACTGAAAACTTATAAAAA
ATGTTTAGATACATAAATATGGTGGTCAGCGTTAATAAAGTGGAGAAATATTGGAAA
AAAAAA

Supplementary Figure S5. miR-124 G-bulge binding site in HADHB 3'UTR, Related to Figure 2. miR-124 G-bulge recognition sites are highlighted in red and bold for all three HADHB transcripts isoforms (NCBI reference sequences shown).

ATGGGAAGAAAATCGCTGTACCTTCTGATTGTGGGGATCCTCATAGCATATTATATT
TATACGCCTCTCCCAGATAACGTTGAGGAGCCATGGAGAATGATGTGGATAAACG
CACATCTGAAAACTATACAAAATTTGGCTACATTTGTGGAGCTCCTGGGACTTCAC
CATTTTATGGATTCCTTTAAGGTTGTCGGGAGCTTTGATGAAGTCCCACCAACCTCA
GATGAAAATGTCACTGTGACTGAGACAAAATTCAACAACATTCTTGTTCGGGTATAT
GTGCCAAAGAGAAAGTCTGAAGCACTAAGAAGGGGGTTGTTTTACATCCATGGTG
GAGGCTGGTGCGTGGGAAGTGCTGCTCTAAGTGGTTATGACTTGCTGTCAAGATG
GACAGCAGACAGACTTGATGCTGTCGTCGTATCAACCAACTACAGATTAGCACCTA
AGTATCATTTCCCAATTCAATTTGAAGATGTATATAATGCCTTAAGGTGGTTCTTAC
GTAAAAAAGTTCTTGCAAAATATGGTGTGAACCCTGAGAGAATCGGTATTTCTGGA
GATAGTGCAGGAGGGAATTTAGCTGCAGCAGTGACTCAACAGCTCCTTGATGACC
CAGATGTCAAGATCAAACTCAAGATCCAGTCTTTAATTTATCCTGCCCTTCAGCCTC
TTGATGTAGATTTACCGTCATATCAAGAAAATTCAAATTTTCTATTTCTATCCAAATC
ACTCATGGTCAGATTCTGGAGTGAATATTTTACCACTGATAGATCACTTGAAAAAGC
CATGCTTTCCAGACAACATGTACCTGTGGAATCAAGTCATCTCTTCAAATTTGTTAA
TTGGAGTTCCCTGCTCCCTGAGAGGTTTATAAAAGGACATGTTTATAACAATCCAAA
TTATGGCAGTTCTGAGCTGGCTAAAAAATATCCAGGGTTCCTAGATGTGAGGGCAG
CCCCTTTGTTGGCTGATGACAACAAATTACGTGGCTTACCCCTGACCTATGTCATC
ACCTGTCAATATGATCTCTTAAGAGATGATGGACTCATGTATGTCACCCGACTTCG
CAACACTGGGGTTCAGGTGACTCATAACCATGTTGAGGATGGATTCCATGGAGCAT
TTTCATTTCTGGGACTTAAAATTAGTCACAGACTTATAAATCAGTATATTGAGTGGCT
AAAGGAAAATCTATAG

Supplementary Figure S6. miR-124 binding site in AADAC ORF, related to Figure 4. Sequence for AADAC ORF is shown (NCBI: NM_001086.2). The miR-124 binding site is shown in red letters, while the three rare codons are underlined and highlighted in yellow.

Table S1. Summary of top up- and down-regulated genes from microarray analysis of miR-124 mimic transfected Huh7.5 cells, Related to Figure 2.

Gene Symbol	Description	Fold Change
	Top up-regulated genes	
SLC2A2	solute carrier family 2 (facilitated glucose transporter), member 2	16.97
BEX1	brain expressed X-linked 1	12.08
RANBP3L	RAN binding protein 3-like	11.48
LOC101927482	uncharacterized LOC101927482	11.07
CYP3A5	cytochrome P450, family 3, subfamily A, polypeptide 5	8.65
LOC105375361	uncharacterized LOC105375361	6.84
B3GALT1	UDP-Gal:betaGlcNAc beta 1,3-galactosyltransferase 1	6.68
KCNJ3	potassium channel, inwardly rectifying subfamily J, member 3	6.34
KIF5C	kinesin family member 5C	6.22
DIO1	deiodinase, iodothyronine, type I	6.21
	Top down-regulated genes	
IQGAP1	IQ motif containing GTPase activating protein 1	-5.75
CP	ceruloplasmin (ferroxidase)	-5.76
LGALS3	lectin, galactoside-binding, soluble, 3	-5.92
AIM1	absent in melanoma 1	-6.01
TNFSF4	tumor necrosis factor (ligand) superfamily, member 4	-6.02
HEPACAM2	HEPACAM family member 2	-6.50
CD38	CD38 molecule	-6.99
AADAC	arylacetamide deacetylase	-8.40
VAMP3	vesicle associated membrane protein 3	-9.11
HP	haptoglobin	-15.69

Table S2. Gene ontology analysis classifying genes repressed by >1.5 fold in miR-124 mimic transfected Huh7.5 cells by biological process, Related to Figure 2.

Name	Source	*P*-value[†]
Fatty acid metabolism	SMPDB	1.63E-2
Fatty acid degradation	BioSystems: KEGG	1.65E-2

*****Only biological processes with $P < 0.05$ are listed.

[†]Adjusted with Bonferroni correction.

Table S3. List of oligonucleotides used in this study, related to Figures 2 & 4.

Oligonucleotide	Sequence
qPCR primers	
18S rRNA – FWD	GAGCTCCTGGGACTTCACCAT
18S rRNA – REV	ATCTGGGTCATCAAGGAGCTGT
AADAC – FWD	CTGCTCCGAGGTGTGTTTGTA
AADAC – REV	GGCAGCAAATTCAGACAAGTCA
ACADSB – FWD	GATGGCAAATGTAGACCCTACC
ACADSB – REV	AAGGCCCGGAGTATCACGA
ACADVL – FWD	TAGGAGAGGCAGGCAAACAGCT
ACADVL – REV	CACAGTGGCAAACTGCTCCAGA
CPT1A – FWD	TCCAGTTGGCTTATCGTGGTG
CPT1A – REV	TCCAGAGTCCGATTGATTTTTGC
ECI2 – FWD	TTCAACCGGCCCAAAAAGAAA
ECI2 – REV	ATTCCCTCAGTAAAACGGCATT
HADHA – FWD	AAATTGACAGCGTATGCCATGA
HADHA – REV	GCTTTCGCACTTTTTCTTCCACT
JFH1 HCV– FWD	GTCTGCGGAACCGGTGAGTA
JFH1 HCV – REV	GCCCAAATGGCCGGGATA
PECR – FWD	CGGAAAAGCCATCGTGAAGGAG
PECR – REV	ATGACTCGTGCCTGCTTTGTGG
PPARA – FWD	CTATCATTTGCTGTGGAGATCG
PPARA – REV	AAGATATCGTCCGGGTGGTT
PPARGC1A – FWD	GCTTTCTGGGTGGACTCAAGT
PPARGC1A – REV	GAGGGCAATCCGTCTTCATCC
SLC25A20 - FWD	CCGAGAACTGACAGACGGAG
SLC25A20 - REV	CCAAAGAAGCACACGGCAAA
CES1 - FWD	AACTGTCGCCCTTCCACGAT
CES1 - REV	CATCCCCTGTGCTGAAGAATCC
ALDH9A1 - FWD	GCAACCGGCCGAGTGATAG
ALDH9A1 - REV	ACCACATTGAAGAGCCCAGG