SUPPLEMENTAL DATA

SUPPLEMENTAL METHODS

1. Primers used for the generation of miR-122 targeting vectors and Southern blot probes:

miR122-5'armF: 5'GCCTAAAACAGCCAAATGGA

miR122-5'armR: 5'CACCCACTCTCACCTCGTCA

miR122-3'armF: 5'TTGACACTGCCTCTGTGGAG

miR122-3'armR: 5'TGGCTTCAACTTCCCATCAG

miR122-geneF: 5'cggatccTCTTCCTGGAATTCAAGCCTTT

miR122-geneR: 5'cggatccAGTGGGCCTAGTGCTGGAAA

mir122a-5'-ProbeF: 5'TAGAGAGGGGAAGGGGAAGG

mir122a-5'-ProbeR: 5'GGAAACCCATTTAGAAAGGGAGA

miR122 3' Probe F : 5'ACCCCCACTCCTTAGCATCATC

miR122 3' Probe R : 5'GCTCTTGGCTCCCTGTTTATGC

2. E2A-Cre genotyping primers

Cre1F: 5'CCTGTTTTGCACGTTCACCG

Cre1R: 5'ATG CTTCTGTCCGTTTGCCG

4. Alb-Cre genotyping primers

CreF: 5'GCGGTCTGGCAGTAAAAACTATC

CreR: 5'GTGAAACAGCATTGCTGTCACTT

5. qRT-PCR primers

Afp RTF: TCACATCCACGAGGAGTGTTGC

Afp RTR: CCTTCAGGTTTGACGCCATTC

Adam-10 RTF: GCTGGGAGGTCAGTATGGAAATC

Adam-10 RTR: TGGCACGCTGGTGTTTTTGG

Slc7a1 RTF: CACCACAGGGGAAGAAGTCAAG

Slc7a1 RTR: CGATGTCCAGGCAGAAGTAAGG

AldoA RTF: TCCATTGGCACCGAGAACAC

AldoA RTR: TTGATAACTTGGGGGAAGGGAC

Ccl2 RTF: TCTGTGCTGACCCCAAGAAGG

Ccl2 RTR: TGGTTGTGGAAAAGGTAGTGGAT

Ccl2 hnRNA-RTF: TCTCAACAGCATTGTCTCTATGGC

Ccl2 hnRNA-RTR: CATTCCTTCTTGGGGTCAGCAC

RhoA-RTF: TCTATGTGCCCACGGTGTTTG

RhoA-RTR: CATCGGTGTCTGGATAAGAGAGAGG

Epcam-RTF: GATGAAAAGGCACCCGAGTTC

Epcam-RTR: TAACCAGGACAACAATCCCCGC

Ucp2 RT-F: CAGCCAGCGCCCAGTACCG

Ucp2 RT-R: GCCAGGGCACCTGTGGTGCT

Srebf1-RT-F: TGGTGGGCACTGAAGCAAAG

Srebf1-RT-R: AAAGACAAGGGGCTACTCTGGGAG

Hmgcr-RT-F: TCTTGACGCTCTTGTGGAATGC

Hmgcr-RT-R: AAACTCTGGCAAAATGGCTGAG

Acly-RT-F: GGAGTCAAATCCTGGCTAAAACCTC

Acly-RT-R: TTTCGTCCACACCCACAAGCAG

Chrebp-RT-F: TGAACAACGCCATCTGGAGAG

Chrebp-RT-R: AATCCCCTTCCCTGCTGGACTTAC

Fatp1-RTF: ATCCGTCTGGTCAAGGTCAATG

Fatp1-RTR: TGGTGGCACTGTCACTAACATAACC

Mogat1 RT-F: CAGAGCAAGGAGGCAGAAGATG

Mogat1 RT-R: GAACAACGGGAAACAGAACCAG

Agpat3 RT-F: GTTATCCTCAACCACAACTTCG

Agpat3 RT-R: TTCCTCCCACTTCCGTTTGC

Agpat9 RT-F: TGGAGGATGAAGTGACCCAGAG

Agpat9 RT-R: AGAGGTAGCAGGAAGCAATAGCG

Dgat1 RT-F: GCCACAATCATCTGCTTCCCAG

Dgat1 RT-R: CCACTGACCTTCTTCCCTGTAGAG

Ppap2a RT-F: CCTTTGTCGGCAATCCCTACATAG

Ppap2a RT-R: CCTCCTTGACTTTCTCTTCATTCCC

Ppap2c RT-F: CTCATTGGGAGAAGCCTACC

Ppap2c RT-R: GCACATAGCCAGAACAGTTGAC

Agpat1-RT-F: TGCTTGGAATGATGGAGGTCC

Agpat1-RT-R: CCTTCAGGAAAAACCCAGACTCTC

Cidec-RT-F: GATGGCACAATCGTGGAGACAG

Cidec-RT-R: TCTTAGTTGGCTTCTGGGAAAGG

Ehhadh RT-F: ACAGCGATACCAGAAGCCAGTG

Ehhadh RT-R: AGCAACAGGAACTCCAACGACC

Acsl4 RT-F: GCCAGAAAACTTGAGCGTTCCTC

Acsl4 RT-R: GTAAGGGGTGAAGAGTATCCAATCC

Scd1 RT-F: ATTCACGACCCCACCTATCAGG

Scd1 RT-R: TAGTAGAAAATCCCGAAGAGGCAG

Mapkapk2 RT-F: CATCACCGACGACTACAAGGTCAC

Mapkapk2 RT-R: GACAATCAGCAGGCACTTCCTC

Gadd45b RT-F: GACATTGGGCACAACCGAAG

Gadd45b RT-R: TTTGGAGTGGGTCTCAGCGTTC

H19-RT-F: TGTATGCCCTAACCGCTCAGTC

H19 RT-R: ATTCATTCTGCCCCGCCTGC

H19-hnRNA RTF: TAAGTGTCTGTCCCGCTCGTG

H19-hnRNA RTR: GGAGTATGCTCACCAAGAAGGCTG

Igf2 RT-F: CCTGGAGACATACTGTGCCACC

Igf2 RT-R: TTGGAAGAACTTGCCCACGG

Igf2-hnRNA RT-F: GGTAGTTCCTTCTTCAGCCTTCCC

Igf2-hnRNA RT-R: ATTCCTTCAACCCCACCTCG

C-Jun RT-F: TGGGCACATCACCACTACACC

C-Jun RT-R: GGTTGAAGTTGCTGAGGTTGGC

Ctnnb1 RT-F: GCTGGTGAAAATGCTTGGGTC

Ctnnb1 RT-R: GCTTGCTCTCTTGATTGCCATAAG

Ccng1 RT-F: CTCAGTTCTTTGGCTTTGACACG

Ccng1 RT-R: ACATTCCTTTCCTCTTCAGTCGC

Ccnd1 RT-F: GAGCAGAAGTGCGAAGAGGAGG

Ccnd1 RT-R: TCACCAGAAGCAGTTCCATTTGC

Mapre1 RT-F: TGATTTGCCAGGAGAACG

Mapre1 RT-R: GCCCCCTTCATCAGGTATCA

c-Myc RT-F: AGGCTGGATTTCCTTTGGGC

c-Myc RT-R: TCGCTCTGCTGTTGCTGGTGATAG

Gapdh-RT-F: TCCTGCACCACCAACTGCTTAG

Gapdh-RT-R: TGCTTCACCACCTTCTTGATGTC

18S RT-F: TGACGGAAGGGCACCACCAG

18S RT-R: TCGCTCCACCAACTAAGAACGGC

6. Amplification and cloning of 3'-UTRs to psiCHECK2 (Promega) was performed as described (1). **The following primers were used.**

Agpat1-3'UTR- XhoI-F: ccgctcgagACAGAAGGGCTGACACCAGATGAC

Agpat1-3'UTR-NotI-R: aagaatgcggccgcAACGCCACTGGGAGGAAATG

Agpat1-3'UTRmut1-F: CTTCTGAAGTGAATGTCAGAGGATGTTCTTTCCCTGCCCC

Agpat1-3'UTRmut1-R: GGGGCAGGGAAAGAACATCCTCTGACATTCACTTCAGAAG

Agpat1-3'UTRmut2-F: GTGGGTGCAGTCTCCAGAGGAAGCCCCACCTGCCATC

Agpat1-3'UTRmut2-R: GATGGCAGGTGGGGCTTCCTCTGGAGACTGCACCCAC

Agpat1-3'UTRmut2-F: GAAGCTGCACCTGACAGAGGTCACAGGCTCACCTG

Agpat1-3'UTRmut2-R: CAGGTGAGCCTGTGACCTCTGTCAGGTGCAGCTTC

Cidec-3'UTR-XhoI-F: ccgctcgagTACATGCAGCAGTTCCTGGATGCC

Cidec-3'UTR-NotI-R: aagaatgcggccgcCAGAATCATCTGCATTCCCTAAGG

Cidec-3'UTRmut1-F: CGTCTTCCGTGCCTGAGGCTCTCTGGTGCAC

Cidec-3'UTRmut1-R: GTGCACCAGAGAGCCTCAGGCACGGAAGACG

Cidec-3'UTRmut2-F: GGTCCTGGCTGCCCAGTGAGATCATGCAGTAGAC

Cidec-3'UTRmut2-R: GTCTACTGCATGATCTCACTGGGCAGCCAGGACC

Mapre1-3'UTR-XhoI-F: ccgctcgagAGCAGAGCAACATCCGAAG

Mapre1-3'UTR-NotI-R: aagaatgcggccgcTGACAATGGGGAGCAGTGAC

Mapre1-3'UTR-del-XhoI-F: ccgctcgagCAGAAGTCTCACCTTTTCCG

Ccl2-3'UTR-XhoI-F: ccgctcgagTGTGACTCGGACTGTGATGC

Ccl2-3'UTR-NotI-R: aagaatgcggccgcGGAATCTCAAACACAAAGTTTACCC
Ccl2-3'UTRmut-F: CTGTGAATCCAGATTCAAGTGAATCAATGTATGAGAGATG
Ccl2-3'UTRmut-R: CATCTCTCATACATTGATTCACTTGAATCTGGATTCACAG

7. Primers used for cloning miR-122 into scAAV.EF1α.eGFP

miR-122-FseI-F: ATCGATGGCCGGCCTGACAAGGTTCCCCTATTATCA
miR-122-FseI-R: ATCGATGGCCGGCCGTAAGTACACAAGATTGAGAAGACTGA

8. Sequence of short hairpin RNA targeting luciferase in a miR-30 backbone used for construction of scAAV.shLuc.eGFP

GGCCGGCCCGTTGCCTGCACATCTTGGAAACACTTGCTGGGATTACTTCTTCAGGTT
AACCCAACAGAAGGCTCGAGAAGGTATATTGCTGTTGACAGTGAGCGCC**CGCCTGAA**
GTCTCTGATTAATAGTGAAGCCACAGATGTATTAATCAGAGACTTCAGGCGGTTGC
CTACTGCCTCGGAATTCAAGGGGCTACTTTAGGAGCAATTATCTTGTTTACTAAAACT
GAATACCTTGCTATCTCTTTGATACATTGGCCGGCC
(shRNA sequence in bold)

Plasmid construction: Coding regions and/or 3´ UTRs were PCR amplified from 129/SvJ genomic DNA and subsequently cloned into the multiple cloning sites of psiCHECK2 (Promega), a reporter vector expressing both renilla and firefly luciferase.

Antibodies used in western blot analysis:

Agpat1 (Santa Cruz, sc-68592), Agpat3 (Santa Cruz, sc-83190), Agpat9 (Santa Cruz, sc68372), Ccnd1 (Santa Cruz, sc-7865), Afp (Santa Cruz, sc-8108), Adam10 (Santa Cruz, sc-28358), Dgat1 (Santa Cruz, sc-32861), Ppap-2a (Santa Cruz, sc-51390), EpCAM (Santa Cruz, sc-66020), c-Jun (Santa Cruz, sc-44), Mapre1 (Santa Cruz, sc-15347), Iqgap1 (Santa Cruz, sc-10792), RhoA (67B9) (Cell Signaling, 2117), Cidec (GenWay, GWB-269046), Igf2 (Abcam, ab9574), c-Myc (Santa Cruz, sc-40), Smarcd1/Baf60a (Santa Cruz, sc-135843), β-catenin (Abcam, ab6302), Mogat1 (Mgat1) (Santa Cruz, sc-32387), Cyp2e1 (Abcam, ab28146), Srf (sc-335), Igf1ra (sc-712), Histone H3 (Abcam, ab1791), HNF6 (Santa Cruz, sc-13050) and Gapdh (Chemicon, MAB374).

MicroRNA mimics and inhibitors

miRIDIAN mimic mmu-miR-122 (cat # C-310464) and negative control RNA (cat # CN-001000) (Figs. 5F-I, S8 and S10), miRIDIAN Hairpin Inhibitor mmu-miR-122 (IH-310464) and control inhibitor (cat # IN-001005) (Figs. S8 and S10) were obtained from Thermo scientific. LNA-modified anti-miR-122 (cat # 199002) and control (cat # 410039) (Figures 5H, I) were obtained from Exiqon.

Generation of liver specific (LKO) and germ-line miR-122 knockout (KO) mice. The targeting vector was constructed by amplifying homology arms from 129SvJ genomic DNA and cloning them into pBlueScript SK (pBSK) (Strategene). *mmu-miR-122* gene with the flanking region (569bp) was PCR amplified and cloned into pFlox-Frt-Neo (2). Floxed *mmu-miR-122*-neo was subcloned into pBSK flanked by 5'- and 3'-arms (**Figure S1**). Electroporation of mouse R1ES cells, provided by Dr. Andras Nagy (3), and subsequent generation of chimeric mice from targeted clones were performed at the University of Michigan Knockout Mouse Core Facility. Two of the mutant clones were transmitted through the mouse germ line, which was confirmed by analysis of tail DNA by PCR. *miR-122*$^{loxP/loxP}$ littermates served as controls in all studies. Animals were housed in a helicobacter-free facility and were handled and euthanized following institutional guidelines.

Measurement of hepatic triglyceride synthesis. The *in vivo* triglyceride synthesis rate was determined by measuring $^{3}H_{1}$-glycerol incorporation into hepatic triglycerides following a published protocol (4, 5). Briefly, 8-10 week-old mice were trained to feed during 3h periods from 9 A.M. to 12 noon every day for two weeks. On the day of the experiment,

after a 3 hour feeding and subsequent 1h fasting, $^{3}H_{1}$ glycerol (50μCi/150μl) was injected IP. Twenty minutes later, mice were sacrificed and livers were harvested. Total lipid was extracted from 0.3g of liver tissue and separated on a TLC plate. Triglyceride spots were scraped from the plate, suspended in the scintillation cocktail and counted in a scintillation counter. The tritium incorporated into hepatic triglycerides was normalized to serum $^{3}H_{1}$ level in each mouse.

Determination of Triglyceride synthesis in primary mouse hepatocytes transfected with siRNA. Primary hepatocytes were isolated from LKO or KO mice with collagenase-based method as previously described (6). The cell viability over 85% was determined by trypan blue staining before seeded onto 12 well plates at $5x10^{5}$ cells per well in culture medium (Williams' Medium E with 10% FBS, 10mM HEPES and 10 nm insulin plus penicillin and streptomycin) and cultured overnight. The hepatocytes were transfected with a mixture of 50nmol/L gene-specific siRNA or scrambled si-RNA (Smartpool si-Genome from Dharmacon) and 2ul/ml lipofectamine 2000 (Invitrogen) in culture medium without antibiotics. After 6 hours of incubation, the mixture was replaced with culture medium. After 48h the culture medium was replaced with 10uCi/ml [$^{3}H_{1}$]-glycerol in FBS free culture medium containing 5% fatty acid free BSA and 0.3mM oleic acid. TG synthesis in transfected mouse hepatocytes was determined by extracting lipids and separating TG on TLC plates followed by counting $^{3}H_{1}$ incorporation in TG in a scintillation counter.

Microarray analysis. Total RNA from the livers of male mice fasted overnight was isolated using Trizol (Invitrogen), purified using mini RNeasy columns (Qiagen), and the

integrity and quantity of the RNA was assessed using an Agilent Bioanalyzer and Nanodrop RNA 6000, respectively. Total RNA was labeled using the Affymetrix Whole Transcript Sense Labeling kit and hybridized to the Affymetrix Mouse Exon 1.0 ST array following the manufacturer's protocol at the Microarray Shared Resource Facility, The Ohio State University Comprehensive Cancer Center.

Affymetrix GeneChip Mouse Exon 1.0 ST Array with 23,332 probe-sets was used for gene expression profiling of 5 control (miR-122$^{loxP/loxP}$) and 5 LKO mice. Signal intensities were quantified by Affymetrix software. Background correction and normalization was performed and gene expression level was summarized over probes using the RMA method (7). A filtering method based on the percentage of samples with expression values below the noise level was applied to filter out probe-sets with little or no expression, resulting in 11,670 detectable probe-sets. Generalized linear models were used to detect differentially expressed genes between the control (floxed) and LKO mice. In order to improve the estimates of variability and statistical tests for differential expression, a variance smoothing method was employed (8). The significance level was determined by controlling the average number of false positives (9). A p-value of 0.0001 was used as the significance cutoff, allowing an average number of false positives of 1.2.

Microarray analysis of the tumor RNA. The Agilent 4X44 platform was used to assess gene expression in liver tumors from 4 LKO and 4 KO mice and in normal liver from age-matched control mice using manufacturer's protocol. Briefly, highly purified total RNA from each group was hybridized to microarray slides overnight, washed, and then scanned with

an Agilent G2505C Microarray Scanner. The raw signal intensity for each probe was extracted from the image data using Agilent Feature Extraction 10.5 (FE) and analyzed by the mathematical software package “R”. The log2 intensity ratio of red to green was normalized to the sum of log2 intensities of red and green. This normalization adjusts the red and green intensities relative to one another so that the red/green ratios are an unbiased representation of true ratios.

Ingenuity Pathway Analysis: The IPA application (http://www.ingenuity.com/products/IPA/Free-Trial-Software.html) was used to identify gene networks that were overrepresented among the genes that exhibited ≥1.5 fold up- or down-regulation with a *P*-value ≤0.0001 in LKO livers. A significance score of ≥3 indicates that there is a less than 1 in 1000 chance that the highlighted genes were assembled into a network due to a random chance.

SUPPLEMENTAL TABLES

Table S1. Serum profiles of 5 week old control and miR-122 KO mice

	ALP (U/L)	ALT (U/L)	Chl (mg/dL)	HDL-Chl (mg/dL)	TG (mg/dL)
Control (n=7) Median ± SE	262.9 ± 51.5	42.5 ± 15.3	130.2 ± 70.7	47.4 ±27.7	194 ± 101.5
KO (n=8) Median ± SE	615.1 ± 69.2	44.2 ± 13.7	61.8 ± 25.0	21.6 ± 10.0	156.2 ± 74.7
***P*-value**	**5.82E-07***	0.812	**0.033***	**0.039***	0.421

Serum was collected from mice by cardiac puncture after overnight fasting. Control mice included 4 male and 3 female. KO mice included 4 male and 4 female. Statistical significance was determined by student's 2-tailed t test. *P*-value<0.05 indicated by asterisks.

Table S2. Networks identified by Ingenuity Pathway Analysis among dysregulated genes in livers of miR-122 LKO mice.

#	IPA Score	Network functions
1	33	Organismal Survival, Cellular Movement, Cellular Growth and Proliferation
2	18	Lipid Metabolism, Small Molecule Biochemistry, Nucleic Acid Metabolism
3	16	Lipid Metabolism, Small Molecule Biochemistry, Molecular Transport
4	15	Amino Acid Metabolism, Molecular Transport, Small Molecule Biochemistry
5	15	Organismal Development, Hematological Disease, Immunological Disease
6	13	Lipid Metabolism, Molecular Transport, Small Molecule Biochemistry
7	13	Cell Death, Gene Expression, Cancer

The IPA application (http://www.ingenuity.com/products/IPA/Free-Trial-Software.html) was used to identify gene networks that were overrepresented among the genes that exhibited ≥1.5 fold up- or down-regulation with a *P*-value ≤0.0001 in LKO livers. A significance score of ≥3 indicates that there is a less than 1 in 1000 chance that the highlighted genes were assembled into a network due to a random chance.

Table S3. Genes involved in lipid metabolism that are dysregulated in the livers of miR-122 LKO mice.

Pathway	Gene	qPCR (LKO/control)	*P*-value	Microarray (LKO/control)	*P*-value
Triglyceride synthesis	Agpat1	10.8	2.5E-07	4.72	3.15E-12
	Agpat3	2.2	0.015	1.25	0.002
	Agpat9	2.0	0.007	1.22	0.037
	Mogat1	14.5	0.000	3.23	0.000
	Dgat1	2.1	0.036	1.2	0.004
	Ppap2a	2.0	0.022	1.3	0.001
	Ppap2c	1.6	0.011	1.3	0.034
Fatty acid synthesis	Acly	0.62	0.031	0.65	1.93E-05
	Chrebp	0.59	0.000	0.58	2.86E-07
	Srebp1c	0.56	0.010	0.58	7.05E-05
	Scd1	0.18	0.050	0.28	0.0002
	Acs14	2.34	0.010	1.91	2.65E-05
Fatty acid oxidation	Ehhadh	2.10	0.002	2.32	1.38E-05
	Ucp2	4.92	0.000	4.39	1.00E-09
Cholesterol synthesis	Hmgcr	0.69	0.050	0.68	0.000
Lipid transport	Stard4	0.59	0.014	0.69	3.24E-06
	(Stard2)	3.38	0.05	1.78	6.00E-05
	Slc27a1 (FATP1)	3.52	0.008	2.19	0.000
Lipid storage	Cidec (Fsp27)	10.42	0.000	6.29	1.99E-07

qPCR analyses were performed in triplicate. The primer sequences are provided in the supplemental methods. Relative expression was calculated using the $\Delta\Delta C_T$ method (1). Statistical analysis of the qPCR data was performed using the student's t 2-tailed test.

Table S4. Genes related to hepatocarcinogenesis are significantly upregulated in miR-122 LKO livers.

Pathway	Gene	qPCR (LKO/control)	*P*-value	Microarray (LKO/control)	*P*-value
Cancer	H19	493	0.000	118.45	0.000
	Afp	8.33	0.001	1.72	0.001
	Igf2	185	0.050	4.41	0.000
	MapKapk2	1.73	0.020	1.72	0.000
	Ctnnb1	1.27	0.000	1.71	0.000
	c-Jun	1.9	0.000	1.93	0.001
	Epcam	2.58	0.003	1.59	0.002
	Ccng1	3.12	0.010	2.27	0.000
	Ccnd1	7.13	0.030	3.54	0.000
	Gadd45B	5.66	0.012	2.30	0.000
	c-Myc	1.60	0.035	1.21	0.097
	RhoA	1.60	0.010	1.26	0.000

qRT-PCR analyses were performed in triplicate. The primer sequences are provided in the supplemental methods. Relative expression was calculated using the $\Delta\Delta C_T$ method (1). Statistical analysis of the qPCR data was performed using the student's t 2-tailed test.

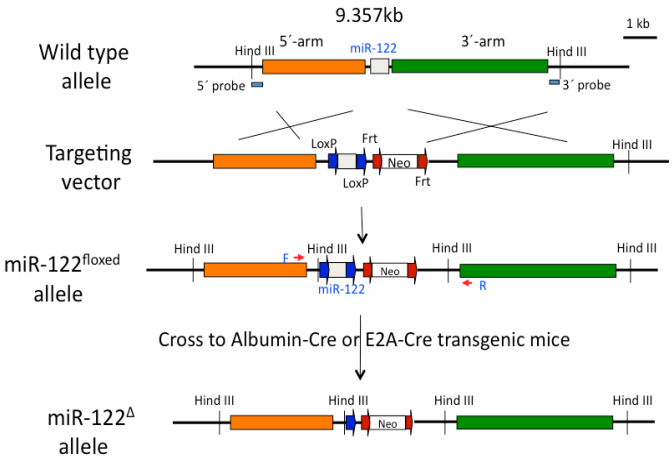
Table S5. Serum profiles of 6 month old control and miR-122 KO mice

	ALP (U/L)	ALT (U/L)	GGT (U/L)
Control (n=7) Median ± SE	81.0 ± 42.64	54.6 ± 18.2	0.67 ±0.82
KO (n=5) Median ± SE	252.3 ± 63.0	54.6 ± 18.2	4.2 ± 2.4
***P*-value**	**0.0002***	0.10	**0.007***

Serum was collected from mice by cardiac puncture after overnight fasting. Control mice included 7 males and KO mice included 5 males. Statistical significance was determined by student's 2-tailed t test. *P*-value<0.05 indicated by asterisks.

SUPPLEMENTAL FIGURE 1

A



B

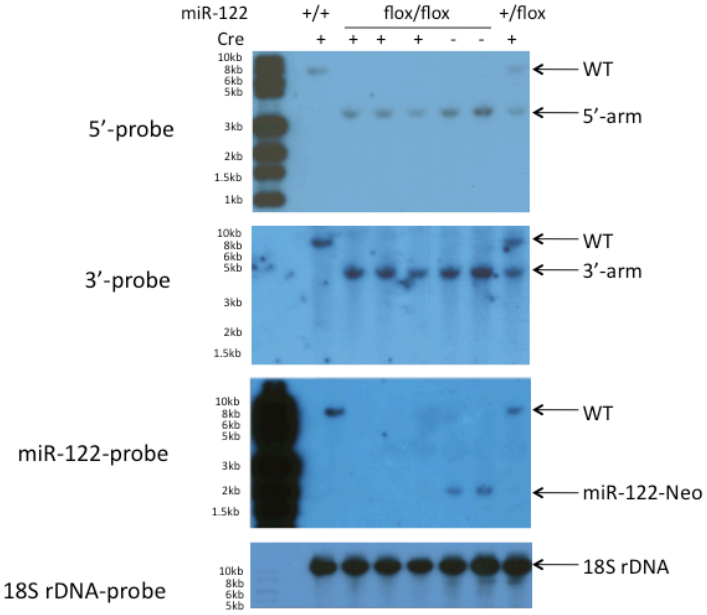


Figure S1. (A) Schematic representation of the generation of conditional and germ-line miR-122 knockout mice. The targeting vector was generated by amplifying homology arms from 129SvJ genomic DNA, which were cloned into pBSK. Targeted mouse ES cell clones and chimeric mice were generated at the University of Michigan Knockout Mouse Core Facility. Two independent targeted clones were transmitted through the mouse germ line. **(B) Southern blot analysis of liver DNA from 10 week-old mice of the indicated genotypes.** Hind III-digested liver DNA was subjected to Southern blot analysis with probes specific for 5´- and 3´- arms as well as the *miR-122* locus to confirm correct targeting and Cre-mediated recombination. The 5´ probe recognizes a ~9.5 kb fragment from the WT allele and a ~3.3 kb fragment from miR-122^{loxP} allele. The 3´ probe recognizes a ~9.5 kb WT fragment and a ~4.6 kb miR-122^{loxP} fragment. Cre-mediated deletion of *miR-122* was confirmed by loss of a ~1.8 kb fragment detected with the miR-122 probe.

SUPPLEMENTAL FIGURE 2

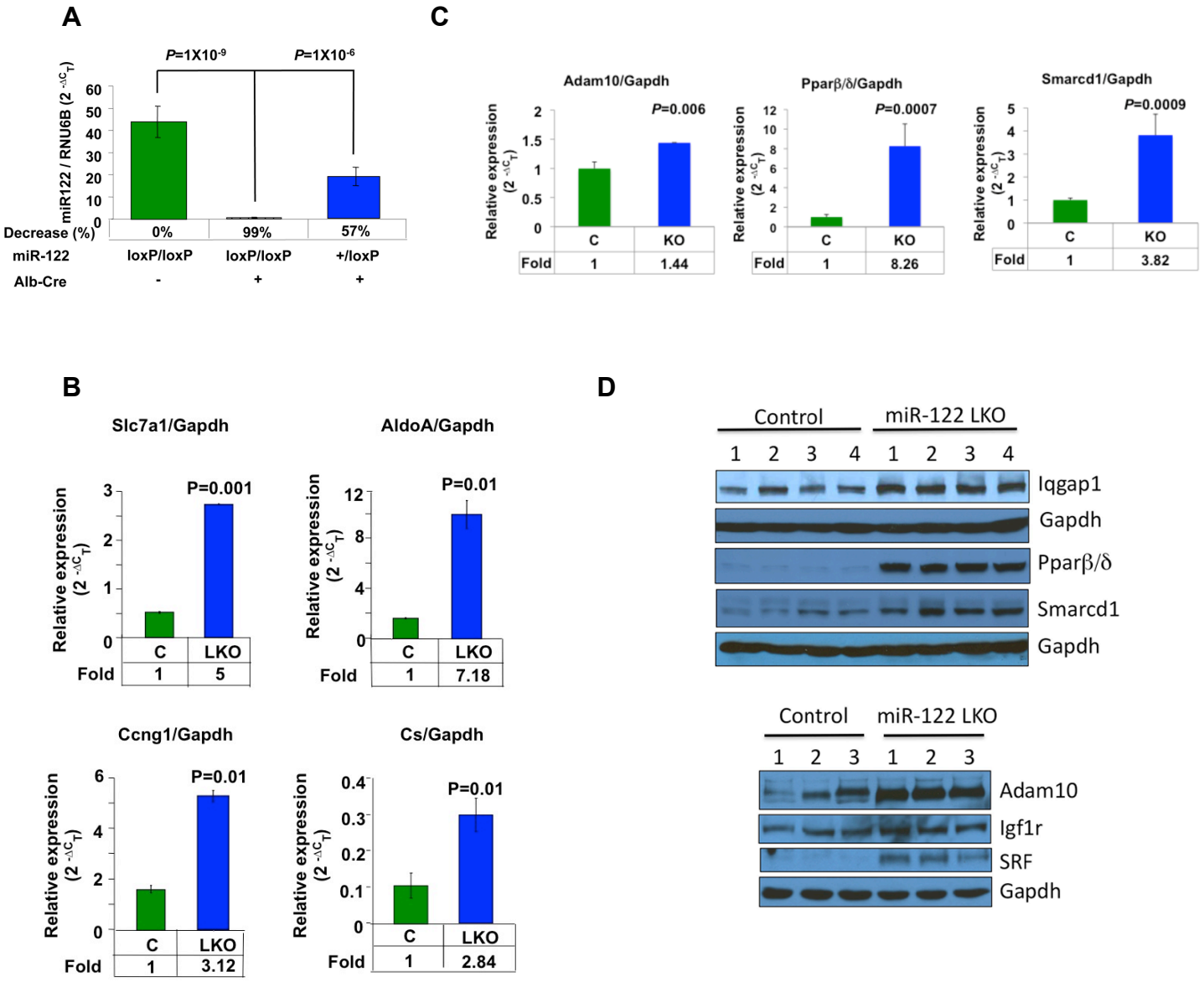


Figure S2. (A) Quantitative PCR (qPCR) analysis of liver RNA from 10 week-old mice confirmed reduced miR-122 expression in heterozygous and homozygous LKO mice. miR-122 and RNU6B were measured in DNase-treated total RNA using respective Taqman assay kits (Invitrogen). Each sample was analyzed in triplicate (n=4). **(B,C) qPCR analysis confirmed upregulation of validated miR-122 targets in LKO (B) and KO livers (C).** DNase-treated total RNA was subjected to qPCR using the SYBR Green method (n=4 mice per condition). Data was normalized to Gapdh. C, LKO, and KO denote control (floxed), liver-specific (LKO), and germline (KO) miR-122 knockout mice, respectively. **(D) Protein levels of validated miR-122 targets are increased in livers of 10 week-old miR-122 LKO mice.** Whole liver extracts prepared as described (1) were subjected to western blot analysis with specific antibodies.

SUPPLEMENTAL FIGURE 3

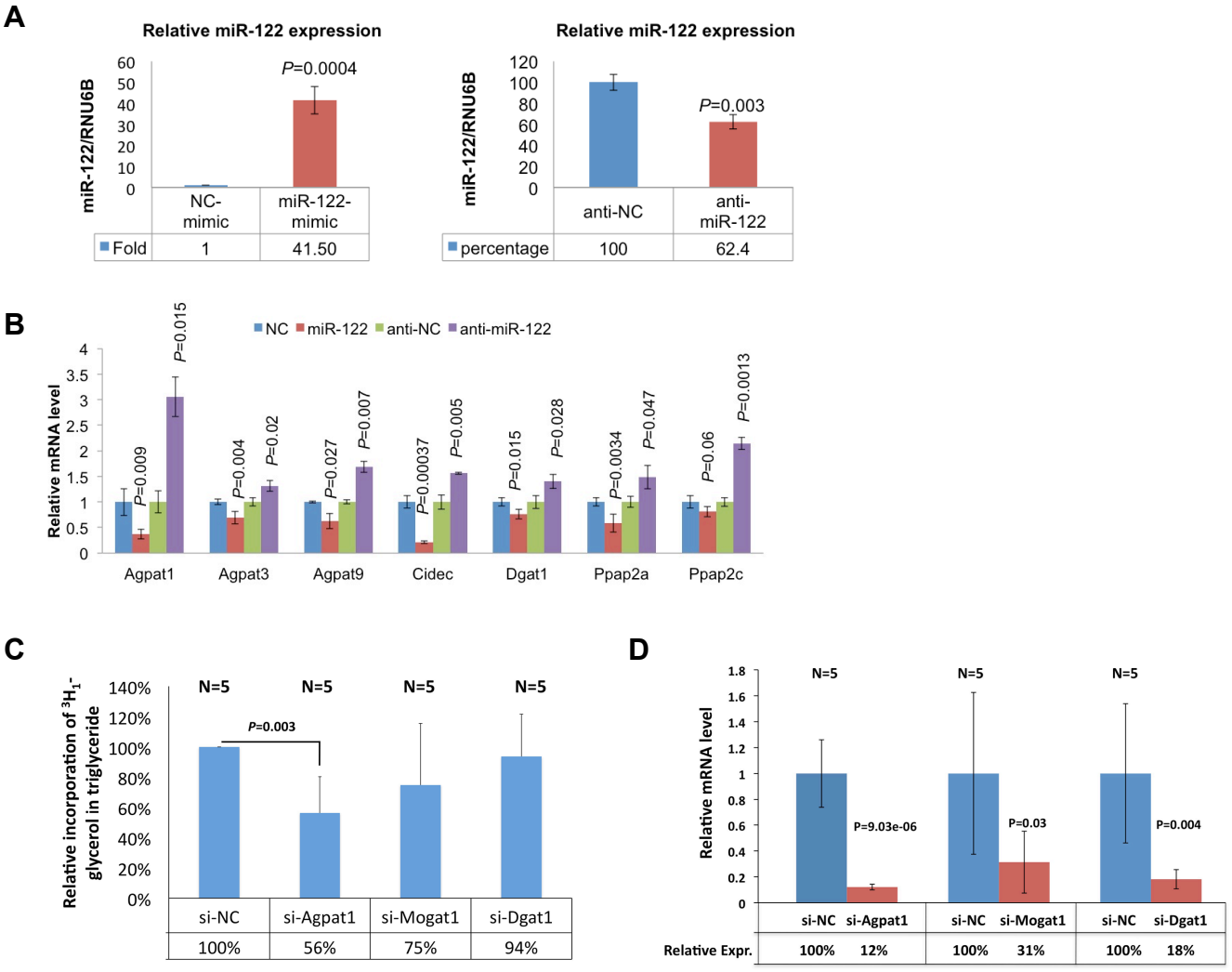
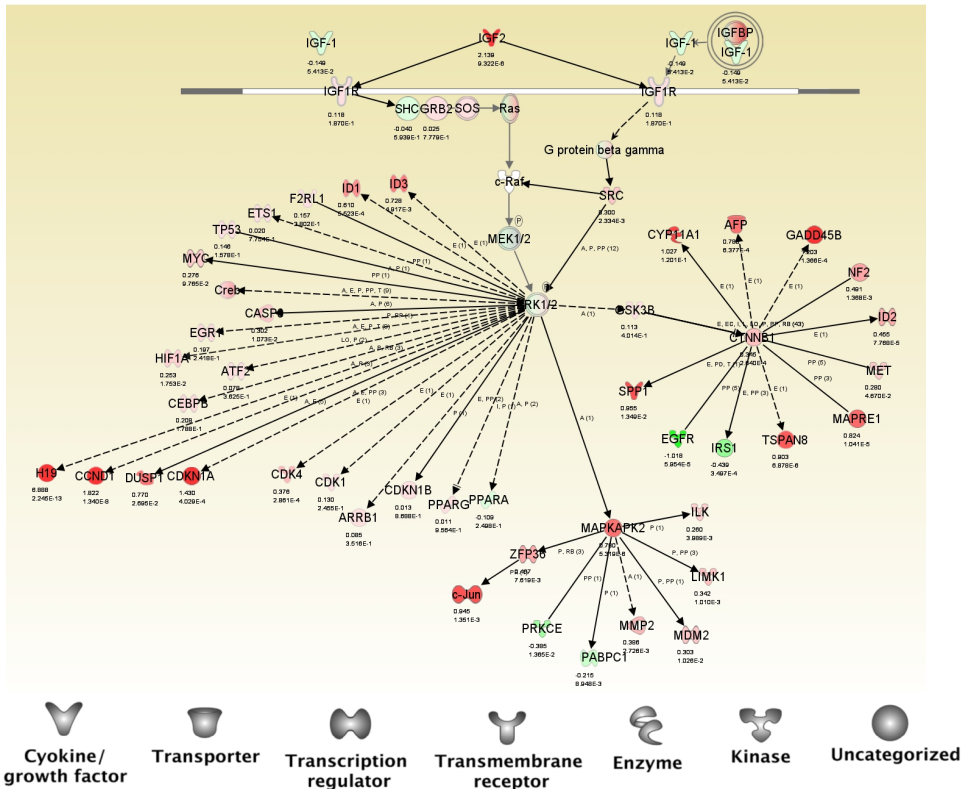


Figure S3. miR-122 negatively regulates the expression of genes involved in triglyceride biosynthesis. (A) miR-122 expression in mouse Hepa cells after transient transfection of miR-122 mimic or inhibitor. NC-mimic and anti-NC represent negative controls. **(B)** Relative expression of genes in the triglyceride biosynthesis pathway normalized to Gapdh expression 40-48 hours after transfection with miR-122 mimic or inhibitor. Expression in negative control-transfected cells was assigned a value of 1. The data represent the mean of 2 independent experiments ± standard deviations (each sample analyzed in triplicate). Statistical analyses were performed using the 2-tailed t test. **(C)** Hepatocytes isolated from 3 LKO and 2 KO mice were transfected with 50nM gene-specific or scrambled siRNA for 6h. After 48h, cells were incubated with $^{3}H_{1}$-glycerol for 15 minutes and $^{3}H_{1}$–incorporation in purified TG was measured in $5x10^{5}$ cells. **(D)** qRT-PCR analysis demonstrating depletion of specific RNAs in hepatocytes transfected with gene-specific siRNAs. The data in panels (C) and (D) represent the mean ± standard deviations (each sample analyzed in triplicate). Statistical analyses were performed using the 2-tailed t test.

SUPPLEMENTAL FIGURE 4

A



B

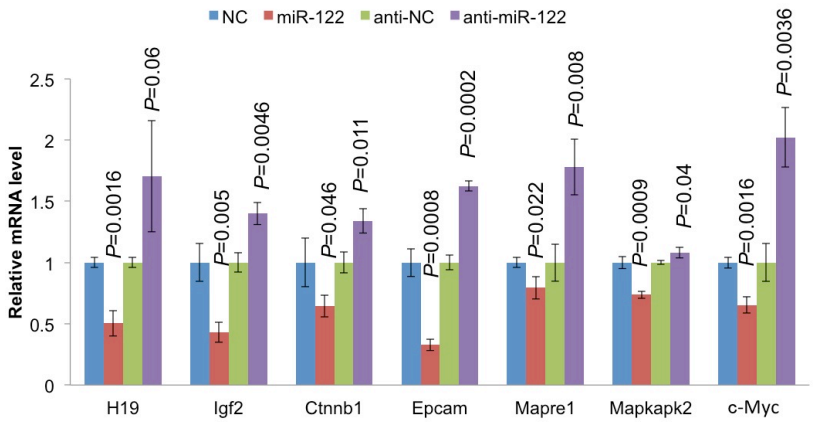
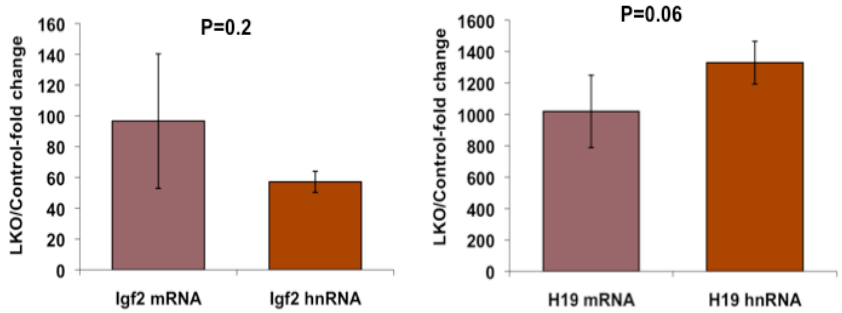


Figure S4. (A) The IPA network of Igf2 signaling and downstream effectors including Ras and β-catenin (CTNNB1) is dysregulated in LKO livers. The first number below each gene represents the fold change in expression (log_2) in LKO livers compared to controls while the second number represents the *P* value associated with the expression change. The shapes represent the functional class of each gene. **(B) miR-122 negatively regulates the expression of genes involved in hepatocarcinogenesis.** The experiment was performed as described in Figure S3B.

SUPPLEMENTAL FIGURE 5

A



B

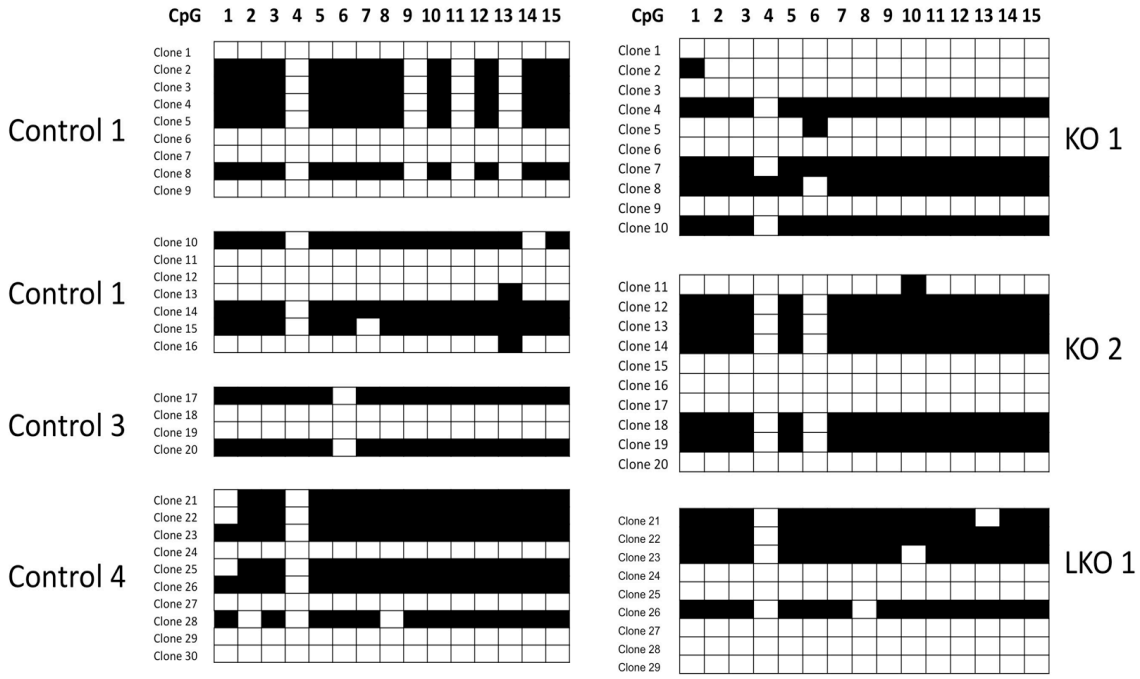


Figure S5. (A) Ig2 and H19 are transcriptionally upregulated in miR-122 LKO livers. a, qPCR analysis demonstrated comparable upregulation of Igf2 and H19 unspliced hnRNA and fully spliced mRNA in LKO livers. n=4. **(B) Methylation profile of DMR (Differentially Methylated Region) upstream of H19 gene is not significantly altered.** Genomic DNA from LKO/KO and control livers was subjected to bisulfite sequencing with DMR-specific primers (10). Black and white boxes represent methylated and unmethylated CpG respectively.

SUPPLEMENTAL FIGURE 6

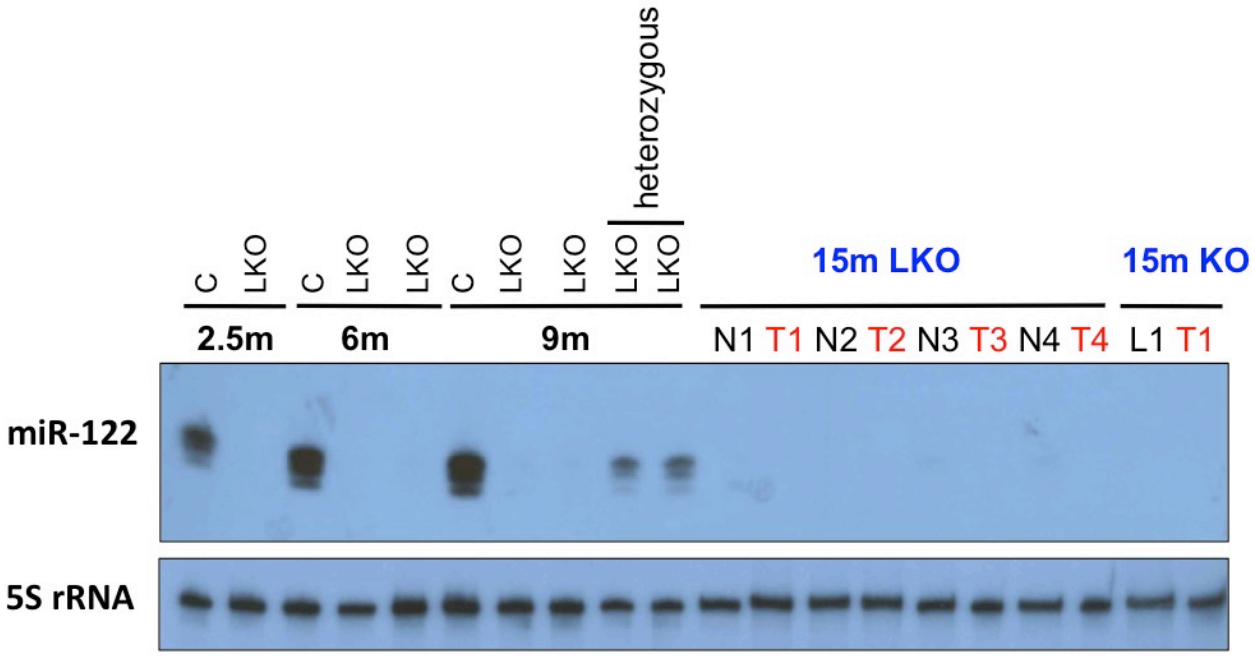


Figure S6. miR-122 LKO livers do not undergo repopulation with non-recombined miR-122-expressing hepatocytes. Northern blot analysis confirmed that expression of miR-122 is negligible in tumors (T) and matching benign liver tissues (N or L) in aged LKO mice. C, control mice.

SUPPLEMENTAL FIGURE 7

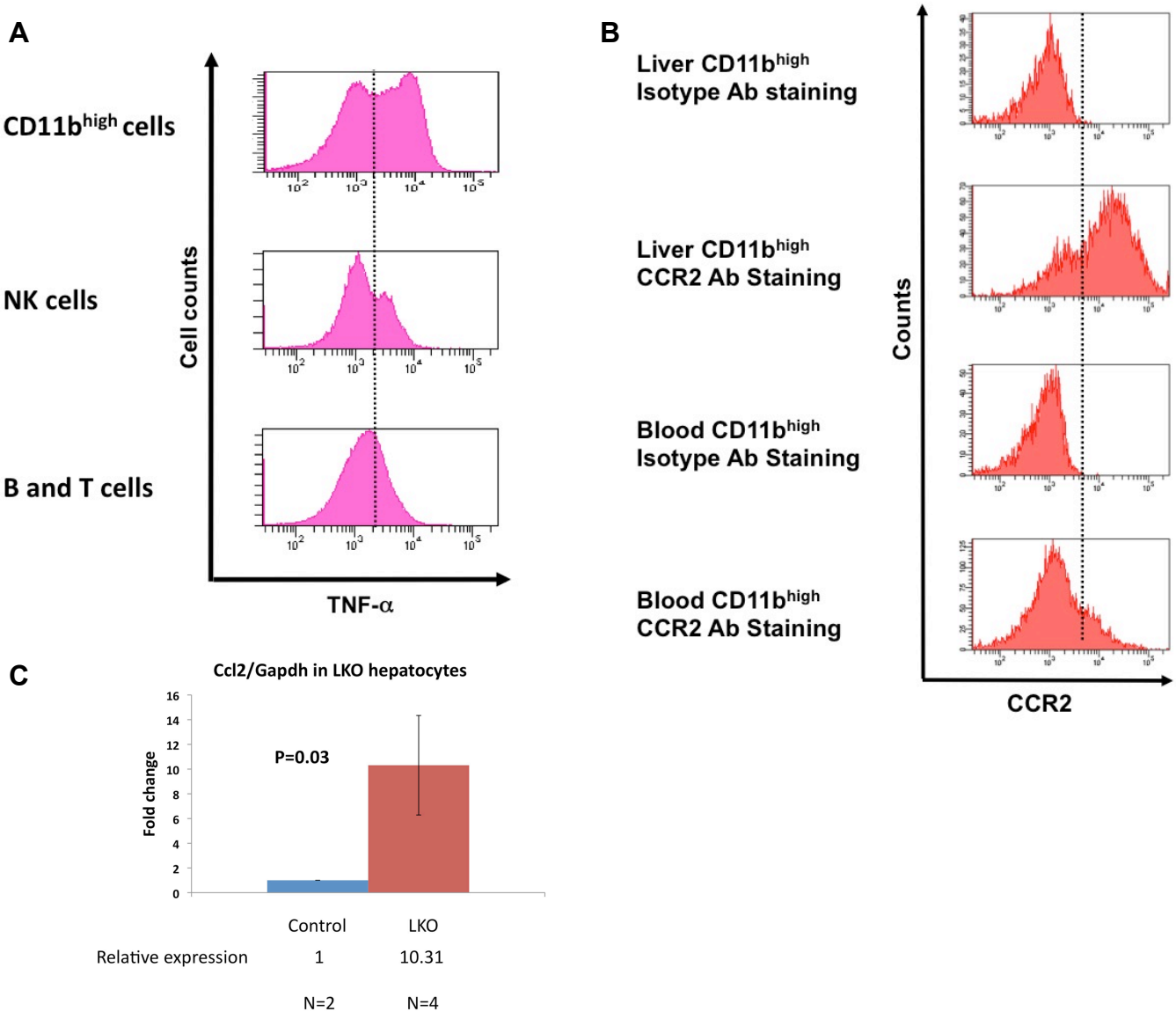


Figure S7. (A) Liver CD11b^{high}Gr1^{+} cells produce TNF-α. Liver immune cells were enriched by Percoll density gradient centrifugation and subjected to surface staining with Gr1, CD11b, CD3, CD19, and NK1.1 antibodies, followed by intracellular staining for TNF-α. **(B) The fraction of intrahepatic CD11b^{high}Gr1^{+} cells expressing Ccr2, the Ccl2 receptor, was much greater than the fraction of CD11b^{high}Gr1^{+} cells expressing Ccr2 in peripheral blood.** Liver immune cells were enriched by Percoll density gradient centrifugation and peripheral blood cells were collected after clearance by red blood cell lysis buffer. Both liver and blood immune cells were subjected to surface staining with Gr1, CD11b, CCR2, CD3, CD19, and NK1.1 antibodies. **(C) *Ccl2* expression is increased in LKO hepatocytes.** Hepatocytes isolated from LKO and control (floxed) male mice were cultured overnight before RNA isolation. *Ccl2* mRNA abundance was measured by qPCR and the data was normalized to Gapdh.

SUPPLEMENTAL FIGURE 8

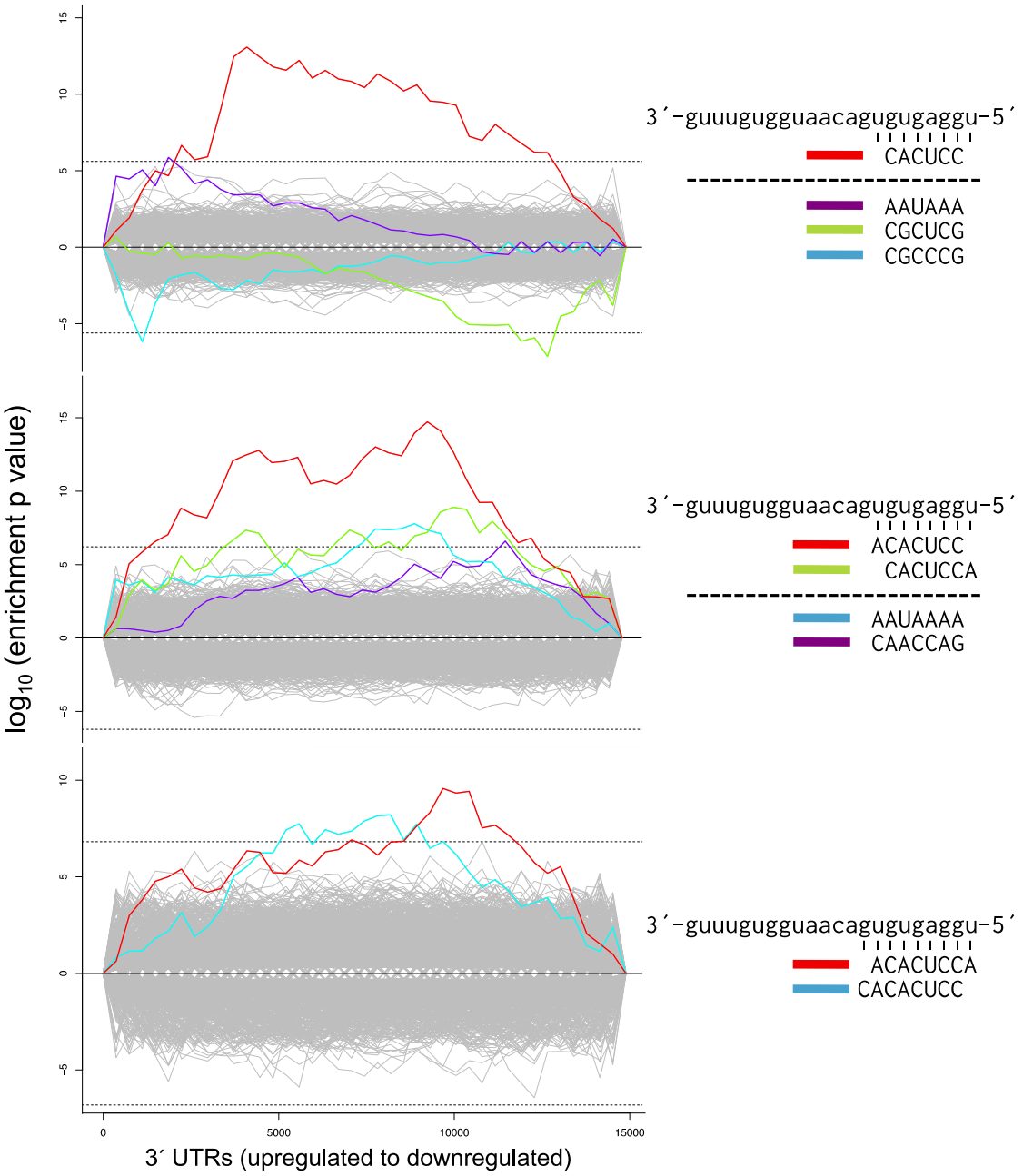


Figure S8. Enrichment of miR-122 target sites in genes upregulated in tumors from KO/LKO mice. Sylamer plots showing the enriched and depleted hexamers (upper), heptamers (middle), and octamers (lower) in transcripts that are upregulated in KO/LKO tumors. All motifs that reached statistical significance are highlighted in color on the plots. On the right, motifs that are complementary to the miR-122 seed sequence are shown above the dotted lines while those that are unrelated to miR-122 are shown below the dotted lines.

SUPPLEMENTAL FIGURE 9

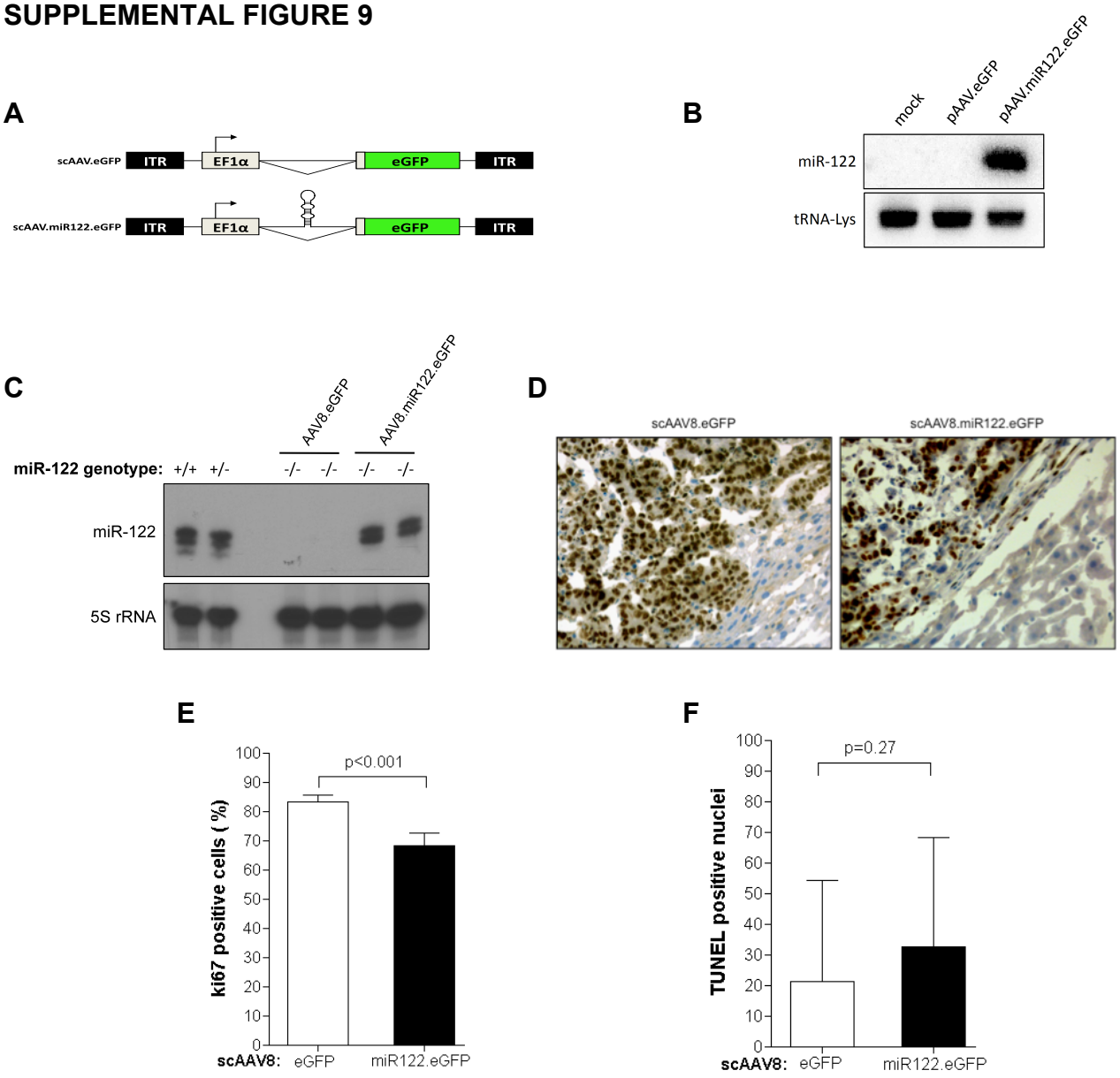


Figure S9. Delivery of miR-122 suppresses tumorigenesis in tet-o-*MYC*; LAP-tTA mice. **(A)** Schematic representation of scAAV.miR-122.eGFP vector illustrating locations of inverted terminal repeats (ITRs), elongation factor 1 α promoter (EF1α), miRNA (shown as a hairpin), and enhanced green fluorescent protein (eGFP) open reading frame. **(B)** Northern blot showing expression of miR-122 in HeLa cells after transfection of the indicated vectors. **(C)** Northern blot showing restitution of miR-122 expression in the liver after administration of scAAV8.miR122.eGFP to KO mice. **(D)** Representative ki67-stained sections from mice after administration of control (left) or miR-122-encoding (right) AAV vectors. The dashed lines show the boundary between normal liver (lower right) and tumor tissue (upper left). **(E)** Quantification of ki67 staining (n=6 mice per condition, 3-4 randomly chosen fields quantified per animal). **(F)** Quantification of TUNEL staining reveals no measureable difference in apoptosis in control vs. miR-122-treated tumors.

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