Reversal of Diet-induced Hepatic Steatosis by Peripheral CB1 Receptor Blockade in Mice is p53/miRNA-22/SIRT1/PPARα Dependent

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Inventory of Supplemental Material:

The following Supplemental Figures and Tables provide additional information supporting the role of hepatic CB_1R in the pathogenesis of obesity-induced hepatic steatosis:

Supplementary Figure 1. AM6545 neither binds to nor activates PPARα.

Supplementary Figure 2. Peripheral CB_1R blockade improves the HFD-induced metabolic abnormalities in both WT and PPARα$^{-/-}$ obese mice.

Supplementary Figure 3. Pharmacological blockade or genetic deletion of CB_1R in the liver affects the eCB levels.

Supplementary Figure 4. Peripheral CB_1R blockade improves the HFD-induced metabolic abnormalities in both WT and LSIRT1$^{-/-}$ obese mice.

Supplementary Figure 5. Analysis of miR-22 conservation across 113 species.

Supplementary Figure 6. miR-22 inhibitor and mimic-miR-22 alter miR-22 expression in HepG2 cells.

Supplementary Figure 7. miR-22 expression in hepatocytes is regulated by CB_1R, but not by CB_2R.

Supplementary Figure 8. Differential hepatic expression profiles of PPARα and SIRT1 in humans with NAFLD.

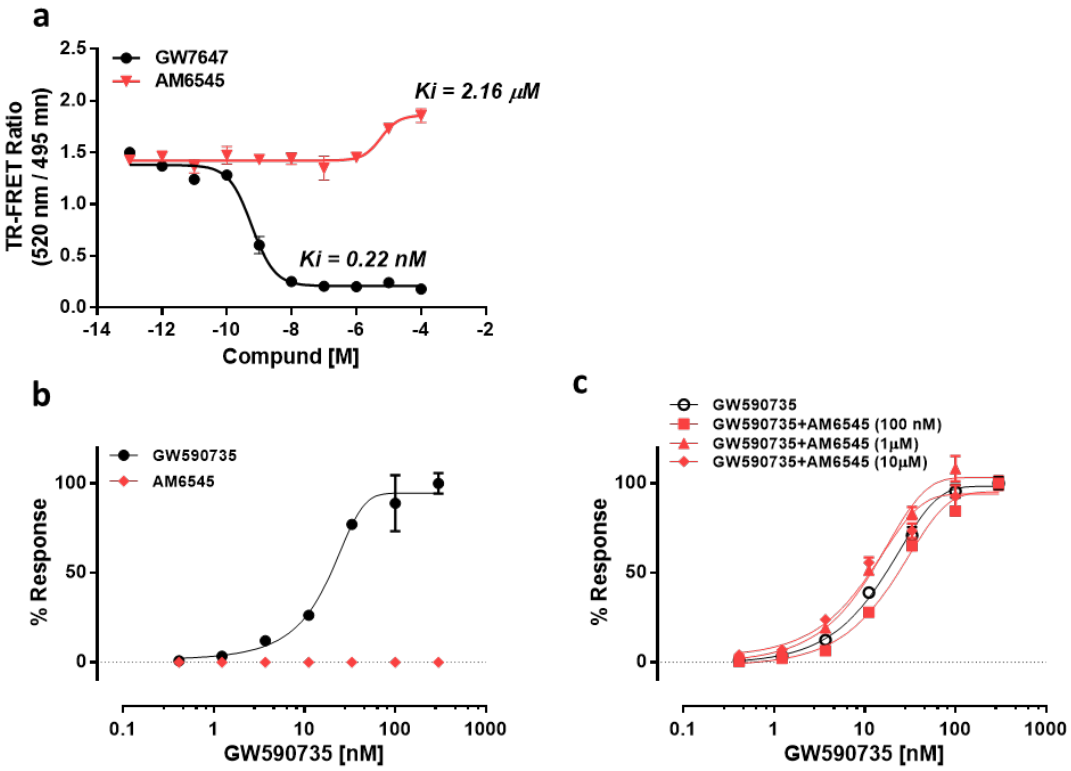
Supplementary Table 1. Unfiltered and filtered coevolved genes with *CNR1*.

Supplementary Table 2. Enrichment analysis of 100 coevolved genes with CNR1 employing GeneAnalytics tool.

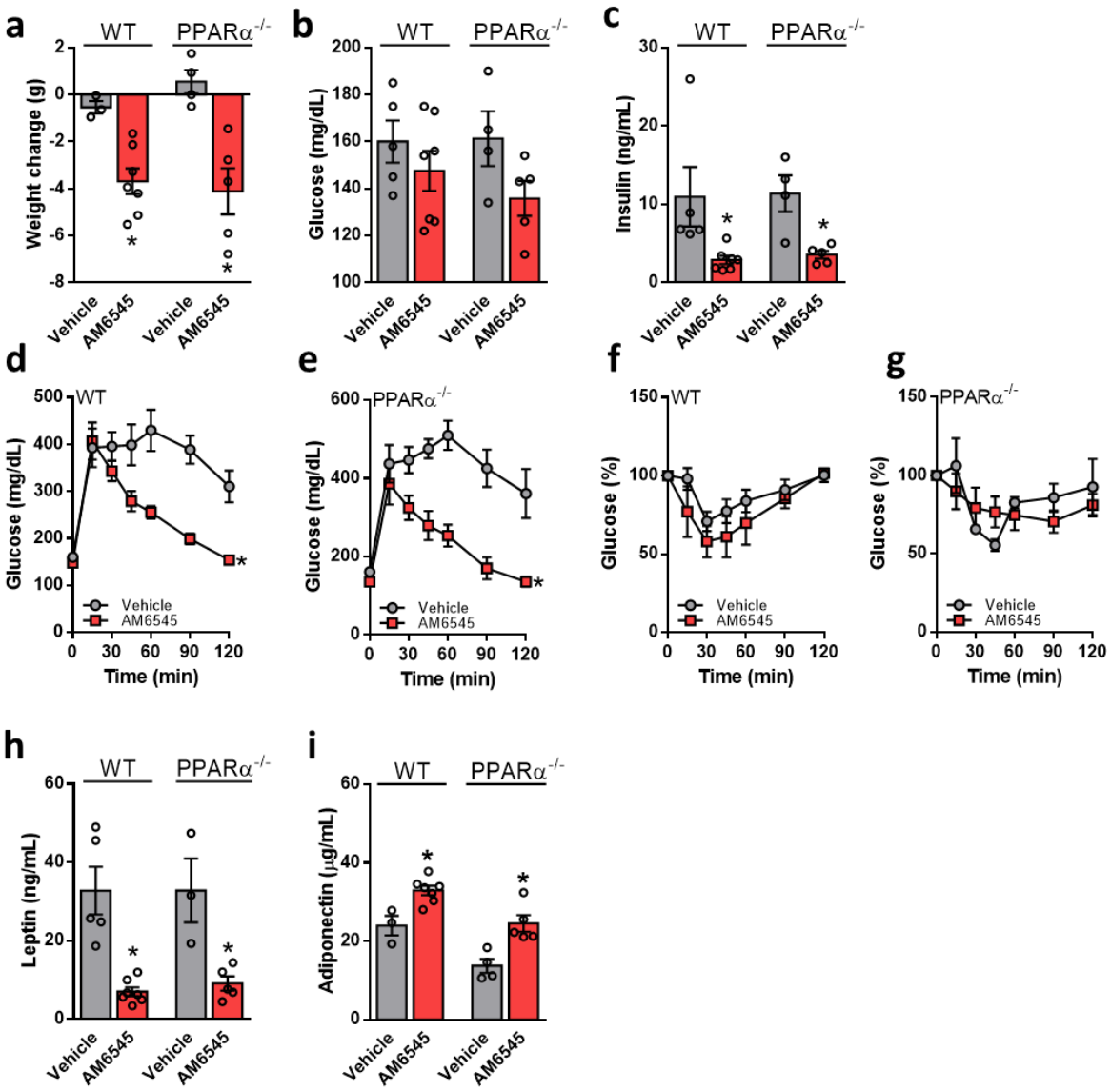
Supplementary Table 3. Elevated expression of hepatic microRNAs induced by AM6545 treatment.

Supplementary Table 4. Reduced expression of hepatic microRNAs induced by AM6545 treatment.

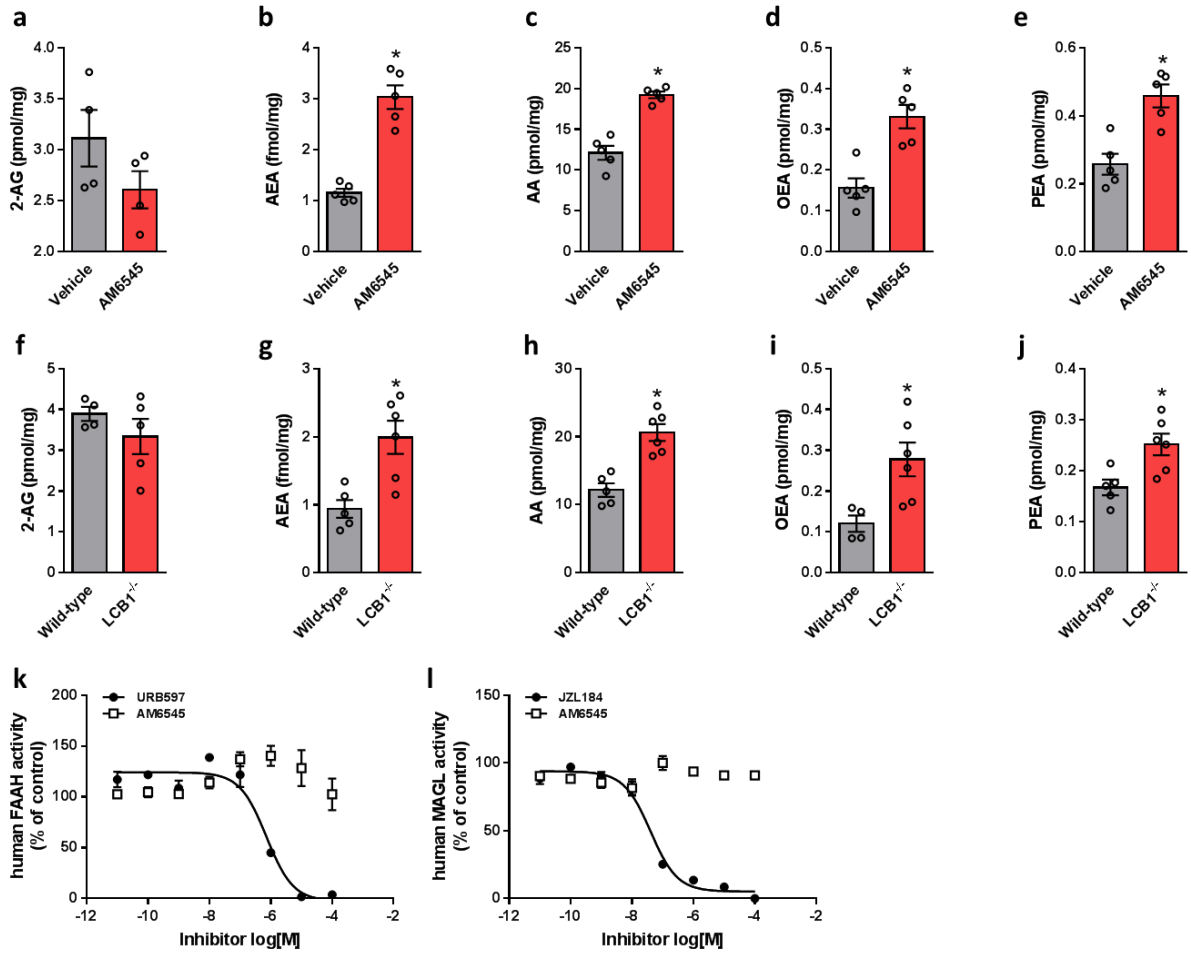
Supplementary Table 5. Primers list.



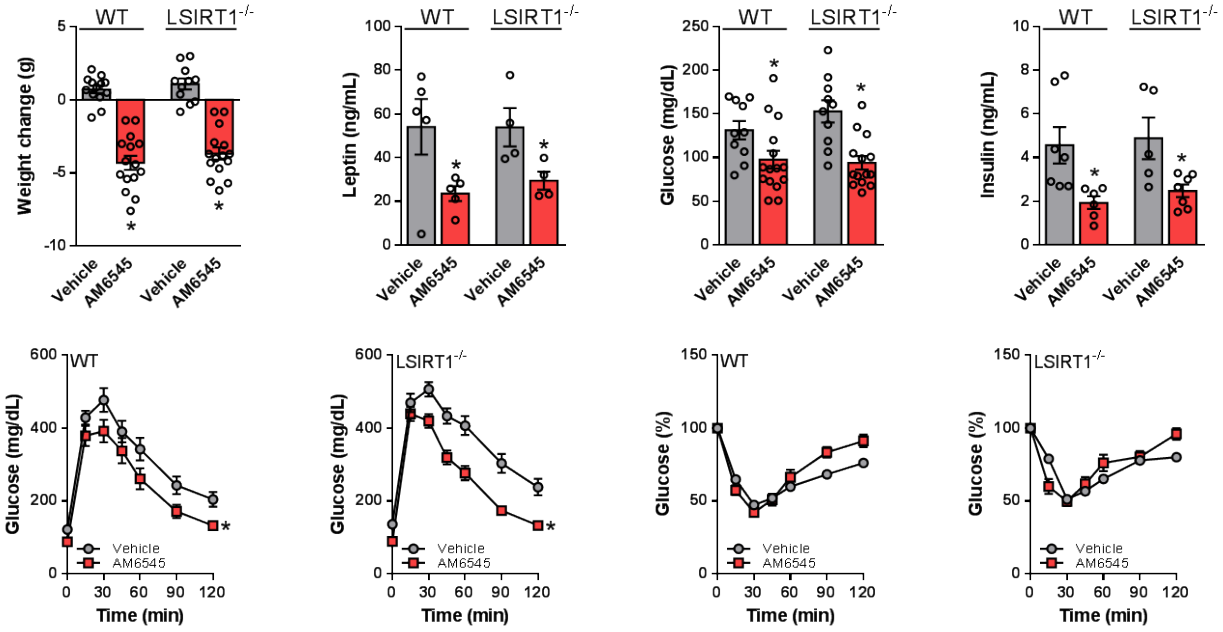
Supplementary Figure 1. AM6545 neither binds to nor activates PPARα. (**a**) Competitive *in vitro* ligand binding assay for PPARα with AM6545 using a PPARα LanthaScreen TR-FRET assay, demonstrating that AM6545 did not affect the binding of the Fluormone™ Pan-PPAR Green tracer to recombinant PPARα-LBD. For comparison, GW7645, an agonist for PPARα, bound the PPARα-LBD and displaced the tracer at a nanomolar range. (**b**) Effect of various doses of AM6545 on PPARα activation. GW590735, a selective PPARα agonist, but not AM6545, dose-dependently increased the activity of PPARα. (**c**) AM6545 was not able to modulate the agonist-dependent increased activity of PPARα. Data represent the mean ± SEM from three independent experiments.



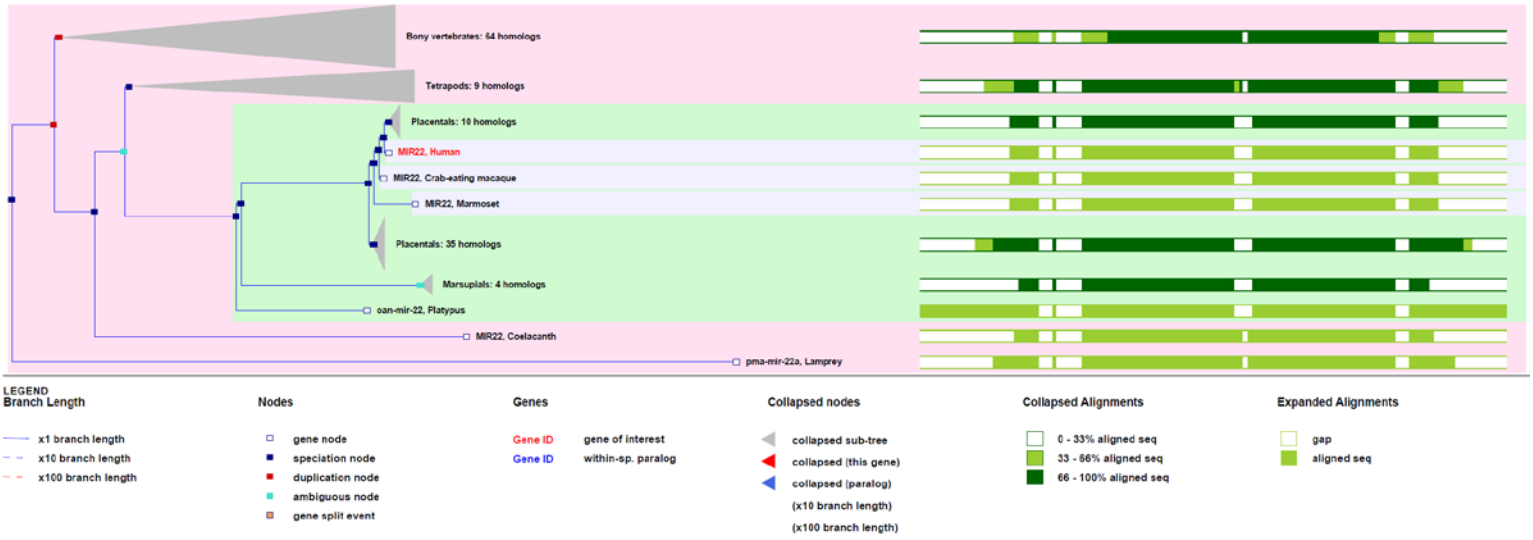
Supplementary Figure 2. Peripheral CB_1R blockade improves the HFD-induced metabolic abnormalities in both WT and PPARα$^{-/-}$ obese mice. Male six-week-old PPARα$^{-/-}$ and their littermate control mice were fed a HFD for 14 weeks and then treated with AM6545 (10 mg/kg, ip) for 7 days. AM6545 reduced the body weight (**a**) without affecting fasting glucose levels (**b**), but significantly reduced serum insulin levels (**c**) and improved glucose tolerance (**d, e**) without affecting insulin sensitivity (**f, g**) in both mouse strains. AM6545 was equally effective in reducing the HFD-induced hyperleptinemia (**h**) and in increasing the HFD-induced hypoadiponectinemia (**i**) in WT and PPARα$^{-/-}$ obese mice. Data represent the mean ± SEM from 5-7 mice per group. *P<0.05 relative to the Veh-treated group from the same strain.



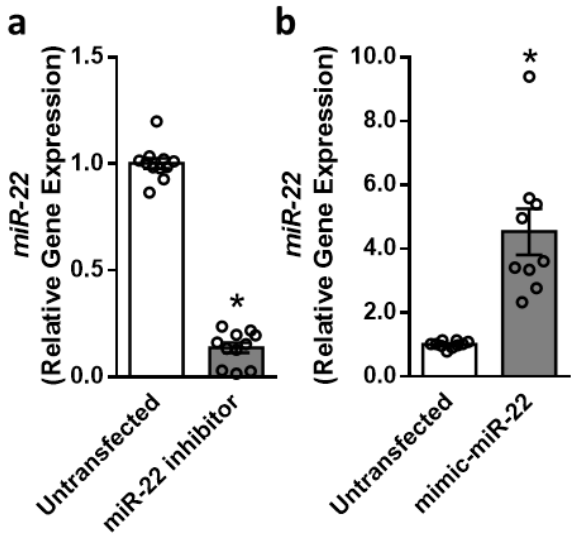
Supplementary Figure 3. Pharmacological blockade or genetic deletion of CB_1R in the liver affects the eCB levels. Male six-week-old C57Bl/6 and $LCB1^{-/-}$ mice were fed a HFD for 14 weeks. Then, C57Bl/6 mice were treated with AM6545 (10 mg/kg, i.p.) for 7 days. AM6545 did not affect the hepatic levels of 2-AG (**a**); however, it significantly increased the AEA (**b**), AA (**c**), OEA (**d**), and PEA (**e**) levels. Similarly, genetic deletion of hepatic CB_1R did not alter the hepatic 2-AG levels (**f**); however, significant elevations in the hepatic levels of AEA (**g**), AA (**h**), OEA (**i**), and PEA (**j**) were found in $LCB1^{-/-}$ mice fed a HFD vs. a STD. Note that AM6545 is not an inhibitor of both FAAH (**k**) or MAGL (**l**), compared to their potent inhibitors, URB597 and JZL184, respectively. Data represent the mean ± SEM from 4-6 mice per group. *P<0.05 relative to the Veh-treated or the wild-type group.



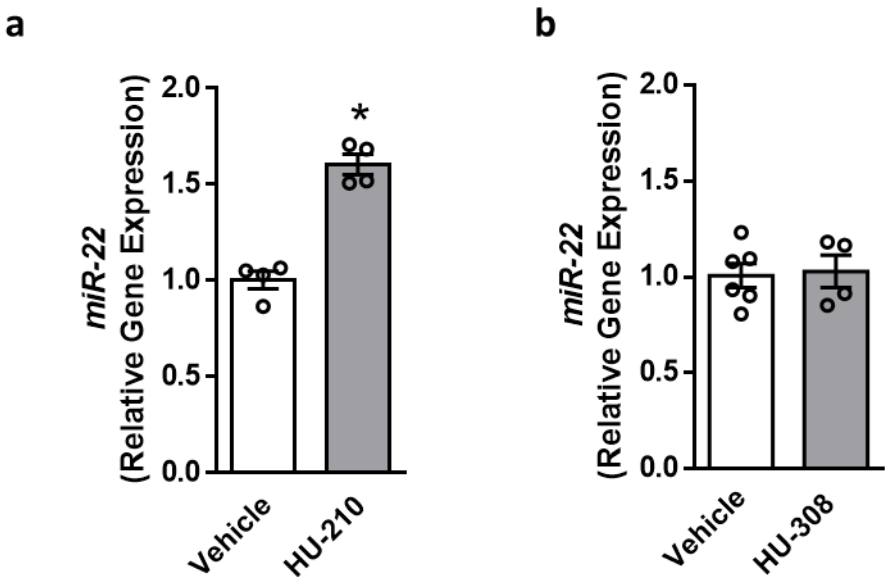
Supplementary Figure 4. Peripheral CB_1R blockade improves the HFD-induced metabolic abnormalities in both WT and LSIRT1$^{-/-}$ obese mice. Male six-week-old liver-specific SIRT1 knockout (LSIRT1$^{-/-}$) mice and their littermate control mice were fed a HFD for 14 weeks and then treated with AM6545 (10 mg/kg, ip) for 7 days. AM6545 reduced the body weight (**a**), hyperleptinemia (**b**), fasting glucose (**c**), and serum insulin levels (**d**), and improved glucose tolerance (**e, f**) without affecting insulin sensitivity (**g, h**) in both mouse strains. Data represent the mean ± SEM from 4-15 mice per group. *P<0.05 relative to the Veh-treated group from the same strain.



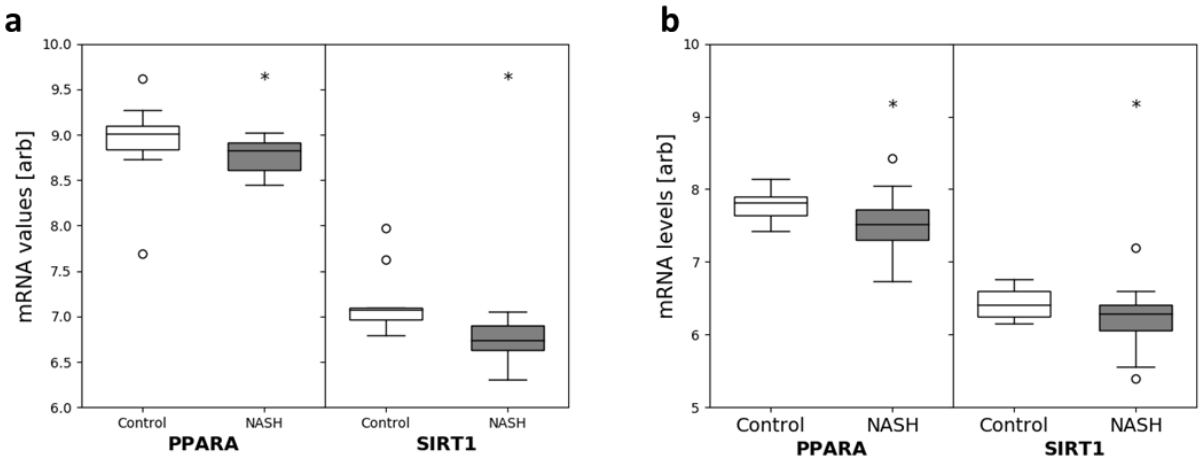
Supplementary Figure 5. Analysis of miR-22 conservation across 113 species from Ensembl (doi.org/10.1093/nar/gkz966). miR-22 is highly and medium conserved across most vertebrates.



Supplementary Figure 6. miR-22 inhibitor and mimic-miR-22 alter miR-22 expression in HepG2 cells. (**a**) Transient-transfected hepatocytes with a miR-22 inhibitor reduced the endogenous expression of miR-22. (**b**) Transient-transfected hepatocytes with a mimic-miR-22 increased the endogenous expression of miR-22. Data represent the mean ± SEM from three independent experiments. *P<0.05 relative to the untransfected cells.



Supplementary Figure 7. miR-22 expression in hepatocytes is regulated by CB_1R, but not by CB_2R. HepG2 cells incubated with the CB_1R agonist, HU-210 (100 nM) (**a**), and with the CB_2R agonist, HU-308 (100 nM) (**b**) for 24 hours. HU-210 increased miR-22 expression, whereas HU-308 did not alter its expression in these cells. Data represent the mean ± SEM. *P<0.05 relative to Vehicle-treated group.



Supplementary Figure 8. Differential hepatic expression profiles of SIRT1, and PPARα in humans with NAFLD. PPARα and SIRT1 mRNA expression levels of control and NASH samples in Ahrens et al. 2013 (**a**), and Haas et al. 2019 (**b**). In Ahrens et al. 2013, control samples were obtained from exclusion of liver malignancy during oncological surgery. From Haas et al. 2019, control samples were collected from patients without NASH, w/wo T2D. Mann Whitney U test was applied for Gene level comparison of SIRT1 and PPAR between control and NASH samples. *P<0.05 (Mann Whitney U test).

Supplementary Table 1. Unfiltered and filtered coevolved genes with *CNR1*

Unfiltered 100 coevolved genes with *CNR1*	**Filtered 53 coevolved genes with *CNR1***
MC2R	*MC2R*
MC5R	*CNR2*
CNR2	*PTAFR*
MC3R	*TGFB3*
MC4R	*GPR1*
LPAR1	*FN1*
PTAFR	*ACKR4*
TGFB3	*GPR85*
TGFB2	*LGI2*
GPR12	*RXFP3*
OXGR1	*CDH2*
GPR6	*MDGA2*
GPR1	*LRRN3*
FN1	*OLFM1*
ACKR4	*MTNR1A*
LPAR3	*NETO1*
GPR183	*LRP12*
GPR85	*CDH6*
LGI2	*NR3C1*
S1PR5	*FRS2*
P2RY1	*P2RY14*
RXFP3	*ETV6*
CDH2	*NRP1*
MDGA2	*EDNRA*
LRRN3	*LCORL*
CDH4	*ITGA11*
LGI1	*BOC*
OLFM1	*OR51E1*
MTNR1A	*RHO*
NETO1	*MYBPC1*
MDGA1	*CADM2*
OLFM3	*SEMA3C*
GPR3	*ADGRB3*
LRP12	*IL1RAPL1*
CDH6	*OIT3*
NETO2	*GPR63*
NR3C1	*KRT8*
LPAR2	*DSP*
FRS2	*CHST1*
CDH11	*LRRC3B*
CDH10	*MID1*
P2RY14	*PDGFRA*
ETV6	*KRT17*
CDH12	*ADCYAP1R1*

LRRN1	*GPR139*
NRP1	*PALLD*
EDNRA	*PPARA*
INHBA	*OR52B2*
LCORL	*SRPX*
OLFM2	*GRB10*
ITGA11	*PROKR2*
CDH9	*MC1R*
BOC	*CCDC88A*
LPAR4	
OR51E1	
CDH8	
CXCR4	
RHO	
MYBPC1	
EDNRB	
P2RY10	
P2RY4	
CADM2	
LGI3	
SEMA3C	
ADGRB3	
IL1RAPL1	
NRP2	
LRFN5	
OIT3	
GPR63	
KRT8	
S1PR1	
DSP	
GPR37	
CDH20	
CHST1	
LRRC3B	
MID1	
PDGFRA	
CDH18	
CDH1	
KRT17	
KRT19	
TGFB1	
SEMA3D	
CDH7	
ADCYAP1R1	
NCAM1	
GPR139	
P2RY6	
PALLD	

DCBLD2
PPARA
OR52B2
SRPX
GRB10
PROKR2
MC1R
CCDC88A

Supplementary Table 2. Enrichment analysis of 100 coevolved genes with *CNR1* employing GeneAnalytics tool

	Name	GeneAnalytics Score*	P-value (calculated with hypergeometric test)	Matched genes
Diseases	Breast Cancer	14.31	$6.9x10^{-7}$	*BOC, CDH1, CDH11, CDH2, CXCR4, EDNRA, ETV6, FN1, INHBA, KRT17, KRT19, KRT8, MDGA1, MID1, MYBPC1, NR3C1, NRP1, NRP2, PDGFRA, TGFB1, TGFB2, TGFB3*
	Body Mass Index Quantitative Trait Locus 11 (Termed as Obesity & Energy Metabolism)	10.41	$1.2x10^{-5}$	*CNR2, EDNRA, FN1, GPR183, MC1R, MC2R, MC3R, MC4R, MC5R, MID1, NR3C1, PALLD, PPARA, SEMA3C, TGFB1*
	Heart Disease (including Dilated Cardiomyopathy, Heart Disease, Arrhythmogenic Right Ventricular Cardiomyopathy)	8.36	$1.4x10^{-7}$	*CDH2, DSP, EDNRA, EDNRB, FN1, MYBPC1, PPARA, TGFB1, TGFB2, TGFB3, CXCR4, ITGA11*
	Liver Disease	3.64	$1.7x10^{-3}$	*KRT19, KRT8, PPARA, TGFB1*
Pathways	Peptide Ligand-binding Receptors	94.24	$6.2x10^{-29}$	*ACKR4, GPR1, ADCYAP1R1, CNR2, GPR12, MC4R, EDNRB, GPR85, GPR183, GPR3, GPR6, GPR37, CXCR4, EDNRA, LPAR2, GPR63, MC1R, LPAR4, MTNR1A, LPAR3, LPAR1, MC3R, MC5R, P2RY6, MC2R, P2RY10, NR3C1, P2RY4, OXGR1, P2RY1, PTAFR, S1PR5, S1PR1, PROKR2, RHO, P2RY14, RXFP3*
	Signaling By GPCR	49.68	$3.0x10^{-15}$	*BOC, ACKR4, ADCYAP1R1, CNR2, INHBA, GRB10, CDH1, MC4R, EDNRB, GPR183, GPR37, CXCR4, EDNRA, LPAR2, FRS2, FN1, MC1R, OR52B2, LPAR4, MTNR1A, NCAM1, LPAR3, LRP12, LPAR1, MC3R, MC5R, P2RY6, MC2R, P2RY10, NR3C1, NRP1, P2RY4, PDGFRA, OXGR1, P2RY1, NRP2, OR51E1, PTAFR, S1PR5, S1PR1, PROKR2, RHO, P2RY14, RXFP3, TGFB1*
	Developmental Biology	20.1	$1.1x10^{-6}$	*BOC, CDH4, KRT17, LGI2, GRB10, KRT19, LGI3, DSP, KRT8, LGI1, FRS2, FN1, NCAM1, CDH2, NRP1, PDGFRA, NRP2, PPARA, TGFB1*

* The score for diseases and pathways is calculated in two different ways, and can be review at: http://geneanalytics.genecards.org/user-guide/#1986

Supplementary Table 3. Elevated expression of hepatic microRNAs induced by AM6545 treatment.

					Pparα						Sirt1					
miRBase 14	**miRBase 22**	**Accession**	**log2FC**	**pval**	**diana_microt**	**elmmo**	**miranda**	**pictar**	**pita**	**targetscan**	**diana_microt**	**elmmo**	**miranda**	**pictar**	**pita**	**targetscan**
mmu-miR-223	mmu-miR-223-3p	MIMAT0000665	3.44	0.00		0.12			-3.56	-0.03			-0.18			
mmu-miR-363	mmu-miR-363-3p	MIMAT0000708	7.89	0.00											-1.48	
mmu-miR-106b*	mmu-miR-106b-3p	MIMAT0004582	2.8	0.00												
mmu-miR-410	mmu-miR-410-3p	MIMAT0001091	7.4	0.00					1.28		0.75	0.42	-0.11		2.44	
mmu-miR-15b	mmu-miR-15b-5p	MIMAT0000124	2.09	0.00	0.46				-3.21	-0.07					-2.63	
mmu-miR-369-3p	mmu-miR-369-3p	MIMAT0003186	6.89	0.00		0.05			-2.09	-0.02	0.80	0.29	-0.89	15.16	-2.88	-0.09
mmu-miR-326	mmu-miR-326-3p	MIMAT0000559	2.52	0.00					-2.50	-0.01	0.48	0.23			-4.63	
mmu-miR-376c	mmu-miR-376c-3p	MIMAT0003183	4.44	0.00					-0.13	-0.03	0.54				-2.53	
mmu-miR-409-3p	mmu-miR-409-3p	MIMAT0001090	3.51	0.00					-1.81		0.74				-1.96	
mmu-miR-654-3p	mmu-miR-654-3p	MIMAT0004898	3.36	0.00					-1.73						1.49	
mmu-miR-487b	mmu-miR-487b-3p	MIMAT0003184	4.36	0.00					-5.54						-6.00	
mmu-miR-495	mmu-miR-495-3p	MIMAT0003456	6.32	0.00					6.67		0.81	0.12	-0.35		-0.44	-0.01
mmu-miR-451	mmu-miR-451a	MIMAT0001632	1.71	0.00					-1.97							
mmu-miR-486	mmu-miR-486a-5p	MIMAT0003130	1.96	0.00						-0.01						

mmu-miR-744	mmu-miR-744-5p	MIMAT0004187	2.19	0.00					-15.65							
mmu-miR-197	NA	NA	3.13	0.00												
mmu-miR-93*	mmu-miR-93-3p	MIMAT0004636	3.38	0.00		0.08				-0.05						
mmu-miR-598	mmu-miR-598-3p	MIMAT0004942	3.7	0.00					-1.34		0.49				-0.65	
mmu-miR-431	mmu-miR-431-5p	MIMAT0001418	3.21	0.00					-5.52	-0.01						
mmu-miR-376a	mmu-miR-376a-3p	MIMAT0000740	5.64	0.00												
mmu-miR-323-3p	mmu-miR-323-3p	MIMAT0000551	5.56	0.00							0.83	0.23	-0.38	8.55	-2.38	-0.03
mmu-miR-10b	mmu-miR-10b-5p	MIMAT0000208	2.64	0.00		0.14		1.68	-0.20	-0.15						
mmu-miR-18a	mmu-miR-18a-5p	MIMAT0000528	1.64	0.00	0.56	0.07	-0.20		-6.02	-0.08						
mmu-miR-505	mmu-miR-505-3p	MIMAT0003513	2.66	0.00					-3.67						-2.97	
mmu-miR-23a	mmu-miR-23a-3p	MIMAT0000532	1.4	0.00					0.24		0.55	0.43	-0.22	7.02	-7.00	-0.04
mmu-miR-652	mmu-miR-652-3p	MIMAT0003711	1.54	0.00	0.60				-9.25							
mmu-miR-150	mmu-miR-150-5p	MIMAT0000160	1.68	0.00												
mmu-miR-151-5p	mmu-miR-151-5p	MIMAT0004536	1.47	0.01					-1.82							
mmu-miR-339-5p	mmu-miR-339-5p	MIMAT0000584	1.57	0.01					-3.34							
mmu-miR-9	mmu-miR-9-5p	MIMAT0000142	5.48	0.01		0.85		12.48	-2.17	-0.25	0.96	0.91	-1.18	8.38	-4.71	-0.36
mmu-miR-28*	mmu-miR-28a-3p	MIMAT0004661	1.73	0.01												

mmu-miR-331-3p	mmu-miR-331-3p	MIMAT0000571	2.33	0.01					2.34							
mmu-miR-423-3p	mmu-miR-423-3p	MIMAT0003454	1.43	0.01												
mmu-miR-130b*	mmu-miR-130b-5p	MIMAT0004583	2.96	0.01		0.00	-0.73			-0.02			-0.12			
mmu-miR-485*	mmu-miR-485-3p	MIMAT0003129	2.99	0.01						-0.02	0.84					-0.11
mmu-miR-199a-3p	mmu-miR-199a-3p	MIMAT0000230	1.33	0.01					-8.33	-0.05					-6.70	
mmu-miR-425*	mmu-miR-425-3p	MIMAT0001342	2.22	0.01												
mmu-miR-484	mmu-miR-484	MIMAT0003127	1.27	0.01		0.08			-5.50	-0.07	0.55		-0.80		-3.12	-0.14
mmu-miR-142-5p	mmu-miR-142a-5p	MIMAT0000154	1.29	0.01												
mmu-miR-199b	mmu-miR-199b-3p	MIMAT0004667	1.32	0.01						-0.05						
mmu-miR-133a	mmu-miR-133a-3p	MIMAT0000145	4.59	0.01		0.33			-2.73		0.72	0.83	-0.25	5.78	-2.29	
mmu-miR-130b	mmu-miR-130b-3p	MIMAT0000387	3.4	0.02		0.11	-0.38		-5.60						-4.89	
mmu-miR-324-3p	mmu-miR-324-3p	MIMAT0000556	1.55	0.02					-9.22		0.53					
mmu-miR-144	mmu-miR-144-3p	MIMAT0000156	1.31	0.02		0.52		17.36	-0.67	-0.09			-0.57		-2.54	
mmu-miR-27a	mmu-miR-27a-3p	MIMAT0000537	1.11	0.02		0.62		16.13	-7.82	-0.17	0.51				-3.44	
mmu-miR-128	mmu-miR-128-3p	MIMAT0000140	1.6	0.02		0.53		13.93	-5.48	-0.09	0.77	0.53	-0.73	4.45	-5.98	-0.20
mmu-miR-411	mmu-miR-411-5p	MIMAT0004747	3.14	0.02											-1.68	
mmu-miR-181a-2*	mmu-miR-	MIMAT0005443	2.61	0.03												

	181a-2-3p															
mmu-miR-328	mmu-miR-328-3p	MIMAT0000565	1.35	0.03					-2.65	-0.02						
mmu-miR-196a	mmu-miR-196a-5p	MIMAT0000518	2.31	0.03	0.47		-0.20		-1.37	-0.12	0.54	0.02	-0.19		-4.32	-0.12
mmu-miR-92a	mmu-miR-92a-3p	MIMAT0000539	1.02	0.03											-6.78	
mmu-miR-301b	mmu-miR-301b-3p	MIMAT0004186	4.19	0.04		0.11	-0.16		-4.44						-1.23	
mmu-miR-379	mmu-miR-379-5p	MIMAT0000743	2.47	0.04					-2.20							
mmu-miR-25	mmu-miR-25-3p	MIMAT0000652	0.95	0.04											-4.89	
mmu-miR-374	mmu-miR-374b-5p	MIMAT0003727	1.06	0.05							0.60		-0.17		0.58	
mmu-miR-127	mmu-miR-127-3p	MIMAT0000139	1.72	0.05												
mmu-miR-99b*	mmu-miR-99b-3p	MIMAT0004525	2.99	0.05												
mmu-miR-320	mmu-miR-320-3p	MIMAT0000666	1.51	0.05					-3.39		0.48					

Supplementary Table 4. Reduced expression of hepatic microRNAs induced by AM6545 treatment.

					Pparα						Sirt1					
miRBase 14	**miRBase 22**	**Accession**	**log2FC**	**pval**	**diana_microt**	**elmmo**	**miranda**	**pictar**	**pita**	**targetscan**	**diana_microt**	**elmmo**	**miranda**	**pictar**	**pita**	**targetscan**
mmu-miR-205	mmu-miR-205-5p	MIMAT0000238	-6.17	0.00					-3.07							
mmu-miR-802	mmu-miR-802-5p	MIMAT0004188	-1.79	0.00					-3.40	-0.01	0.70				1.17	
mmu-miR-203	mmu-miR-203-3p	MIMAT0000236	-1.87	0.00		0.17			-0.88			0.17			-1.39	
mmu-miR-497	mmu-miR-497a-5p	MIMAT0003453	-1.8	0.00						-0.05						
mmu-miR-29b	mmu-miR-29b-3p	MIMAT0000127	-1.47	0.00		0.33			-3.91	-0.07	0.55	0.69	-0.20		3.17	-0.10
mmu-miR-195	mmu-miR-195a-5p	MIMAT0000225	-1.79	0.00	0.50				-0.08	-0.06					-3.83	
mmu-miR-214*	mmu-miR-214-5p	MIMAT0004664	-4.19	0.00						-0.12						
mmu-miR-99a	mmu-miR-99a-5p	MIMAT0000131	-1.39	0.00					-3.94	-0.34						
mmu-miR-148a*	mmu-miR-148a-5p	MIMAT0004617	-3.11	0.00		0.21					0.82	0.24				
mmu-miR-143	mmu-miR-143-3p	MIMAT0000247	-1.24	0.01					-6.40						-4.03	
mmu-miR-378	mmu-miR-378a-3p	MIMAT0003151	-1.13	0.01						-0.05						
mmu-let-7c	mmu-let-7c-5p	MIMAT0000523	-1.11	0.01		0.87	-0.12	14.68	-5.87	-0.18			-0.13			
mmu-miR-149	mmu-miR-149-5p	MIMAT0000159	-1.44	0.01					-5.73	-0.02	0.55				-4.16	
mmu-miR-193	mmu-miR-193a-3p	MIMAT0000223	-1.27	0.01					-0.79	-0.03						

mmu-miR-30e	mmu-miR-30e-5p	MIMAT0000248	-1.05	0.02					0.46		0.58	0.93	-0.91	1.09	-6.66	-0.25
mmu-miR-872	mmu-miR-872-5p	MIMAT0004934	-2.17	0.02	0.67	0.05	-0.22		-6.44	-0.12	0.46				-4.24	
mmu-miR-192	mmu-miR-192-5p	MIMAT0000517	-1.02	0.02					-1.93							
mmu-miR-30c-2*	mmu-miR-30c-2-3p	MIMAT0005438	-1.83	0.02						-0.06						
mmu-miR-455*	mmu-miR-455-5p	MIMAT0003485	-1.44	0.03					-0.08	-0.18		0.07			-3.41	
mmu-miR-22	mmu-miR-22-3p	MIMAT0000531	-0.93	0.03	0.66	0.28	-0.33	11.96	-7.80	-0.22	0.82	0.44	-1.20	4.99	-10.24	-0.55
mmu-miR-29c	mmu-miR-29c-3p	MIMAT0000536	-1.01	0.03		0.33			-2.31	-0.07	0.54	0.69	-0.20		4.11	-0.10
mmu-miR-187	mmu-miR-187-3p	MIMAT0000216	-1.76	0.03					-7.30							
mmu-miR-148a	mmu-miR-148a-3p	MIMAT0000516	-0.91	0.04		0.04			-2.82	-0.14						
mmu-miR-30a	mmu-miR-30a-5p	MIMAT0000128	-0.9	0.04					1.86		0.55	0.93	-0.92	1.09	-5.56	-0.22
mmu-miR-7a	mmu-miR-7a-5p	MIMAT0000677	-1.27	0.04					-6.17	-0.11	0.50		-0.15		-6.30	
mmu-miR-101a*	mmu-miR-101a-5p	MIMAT0004526	-1.65	0.04						-0.01	0.73		-1.25			-0.18
mmu-miR-345-3p	mmu-miR-345-3p	MIMAT0004656	-2.33	0.04		0.06			-5.74	-0.08						-0.07
mmu-miR-31	mmu-miR-31-5p	MIMAT0000538	-1.02	0.04			-0.40		-7.60		0.95	0.24			-9.60	
mmu-miR-22*	mmu-miR-22-5p	MIMAT0004629	-1.07	0.04	0.49						0.69					
mmu-miR-107	mmu-miR-107-3p	MIMAT0000647	-0.98	0.04					-7.69	-0.01					-7.43	-0.14

mmu-miR-122	mmu-miR-122-5p	MIMAT0000246	-0.87	0.05					-9.54							
mmu-miR-378*	mmu-miR-378a-5p	MIMAT0000742	-1.25	0.05					-6.00	-0.02						-0.08

Supplementary Table 5. Primers list.

Gene	Forward primer
	Reverse primer
hSIRT1	5' GCGGGAATCCAAAGGATAAT 3'
	5' CTGTTGCAAAGGAACCATGA 3'
hβ-ACTIN	5' TCCCTGGAGAAGAGCTACGA 3'
	5' AGCACTGTGTTGGCGTACAG 3'
hGAPDH	5' AATCCCATCACCATCTTCCA 3'
	5' TGGACTCCACGACGTACTCA 3'
hPPARα	5' CATTACGGAGTCCACGCGT 3'
	5' ACCAGCTTGAGTCGAATCGTT 3'
hACAA2	5' CTGCTCCGAGGTGTGTTTGTA 3'
	5' GGCAGCAAATTCAGACAAGTCA 3'
hDECR1	5' CTATGCTGAGACTGGTTCAGGT 3'
	5' CCAGACGGCTAAAGGCACC 3'
hDECR2	5' TCCTTCAACGCCTTCAAGACC 3'
	5' GGTGGCAGTGATGTTCACGAT 3'
hMLYCD	5' ACGTCCGGGAAATGAATGGG 3'
	5' GTAACCCGTTCTAGGTTCAGGA 3'
hHADH_B	5' CTGTCCAGACCAAAACGAAGAA 3'
	5' CGATGCAACAAACCCGTAAGC 3'
hCORT	5' GCCTCCTGACTTTCCTCGC 3'
	5' GGGCTTCCTCTCCTATGAGGG 3'

hCPT2	5' CATACAAGCTACATTTCGGGACC 3'
	5' AGCCCGGAGTGTCTTCAGAA 3'
hPri-MIR-22	5' GCTGAGCCGCAGTAGTTCTT 3'
	5' GGCAGAGGGCAACAGTTCTT 3'
hp21	5' TGAGCCGCGACTGTGATG 3'
	5' GTCTCGGTGACAAAGTCGAGGTT 3'
hBAX	5' GCTGTTGGGCTGGATCCAAG 3'
	5' TCAGCCCATCTTCTTCCAGA 3'
hPUMA	5' GGAGACAAGAGGAGCAG 3'
	5' CTGGGTAAGGGCAGGAGT 3'
hPPARα_SG	Qiagen QuantiTect Primer Assay-QT00017451
mSirt1	5' ATGACGCTGTGGCAGATTGTT 3'
	5' CCGCAAGGCGAGCATAGAT 3'
mβ-Actin	5'GGCTGTATTCCCCTCCATCG 3'
	5' CCAGTTGGTAACAATGCCATGT 3'
mGapdh	5' AGGTCGGTGTGAACGGATTTG 3'
	5' TGTAGACCATGTAGTTGAGGTCA 3'
mPparα	5' TACTGCCGTTTTCACAAGTGC 3'
	5' AGGTCGTGTTCACAGGTAAGA 3'
mAcaa2	5' ATGTGCGCTTCGGAACCAAA 3'
	5' CAAGGCGTATCTGTCACAGTC 3'
mDecr1	5' GATCCGGGTCCTCAGAGGTTT 3'
	5' ATCAGGTGGTAGCATAGGCTT 3'

mDecr2	5' CACGGCTGCTAAGAAGTTGGT 3'
	5' AGCTGCACAGTTAATGAGGATG 3'
mMlycD	5' GCACGTCCGGGAAATGAAC 3'
	5' GCCTCACACTCGCTGATCTT 3'
mHadh_B	5' ACTACATCAAAATGGGCTCTCAG 3'
	5' AGCAGAAATGGAATGCGGACC 3'
mCort	5' GAGCGGCCTTCTGACTTTCC 3'
	5' GGGCTTTTTATCCAGGTGTGG 3'
mCpt2	5' CAGCACAGCATCGTACCCA 3'
	5' TCCCAATGCCGTTCTCAAAAT 3'
mPri-miR-22	5' GCTGAGCCGCAGTAGTTCTTC 3'
	5' GCAGAGGGCAACAGTTCTTCAA 3'
mPparα_SG	Qiagen QuantiTect Primer Assay-QT00137984