

Cell Reports, Volume 26

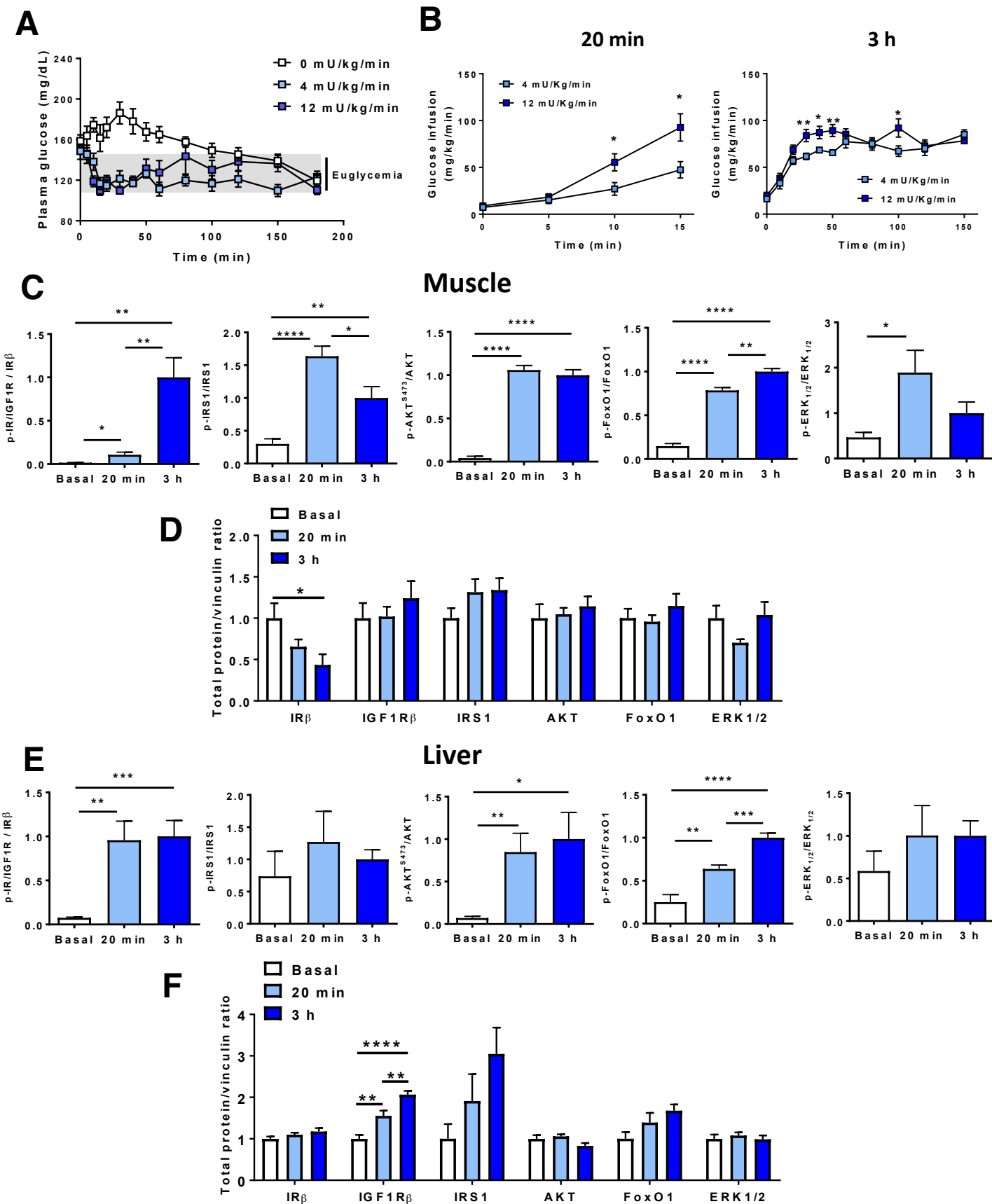
Supplemental Information

Multi-dimensional Transcriptional Remodeling

by Physiological Insulin *In Vivo*

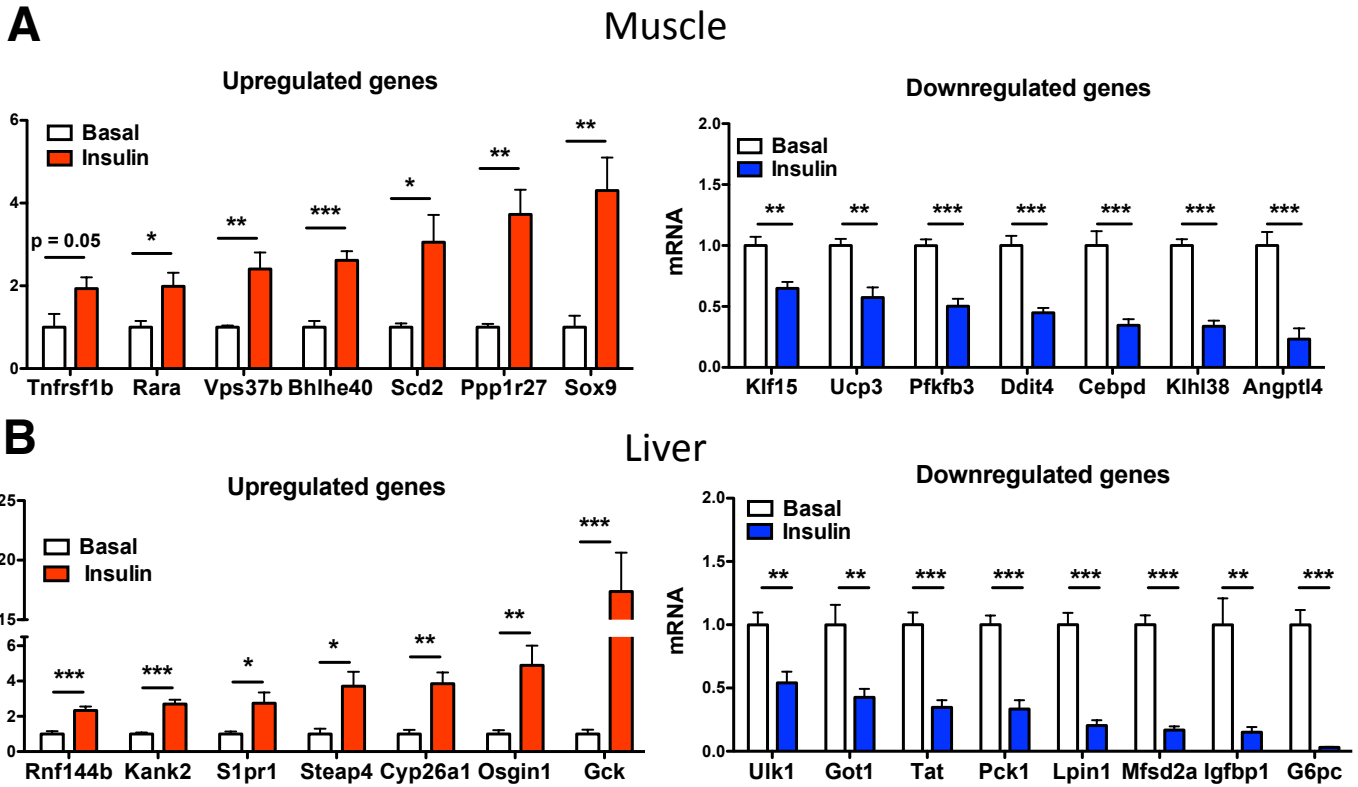
Thiago M. Batista, Ruben Garcia-Martin, Weikang Cai, Masahiro Konishi, Brian T. O'Neill, Masaji Sakaguchi, Jong Hun Kim, Dae Young Jung, Jason K. Kim, and C. Ronald Kahn

Figure S1, Blood glucose levels, glucose infusion during clamp experiment and western blot quantification, Related to Figure 1



(A) Blood glucose and (B) glucose infusion rates during euglycemic clamp experiments. For blood glucose, mice from 20 and 180 min time-points are shown together ($n = 6-12$). Western blot quantification in muscle showing (C) phospho/total ratios and (D) total/vinculin ratios. Western blot quantification in liver showing (E) phospho/total ratios and (F) total/vinculin ratios. Data are means \pm SEM, $n = 5-6$. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$, One-way ANOVA.

Figure S2, Validation of significantly regulated genes by high insulin at 3h, Related to Figure 2 and Figure 3

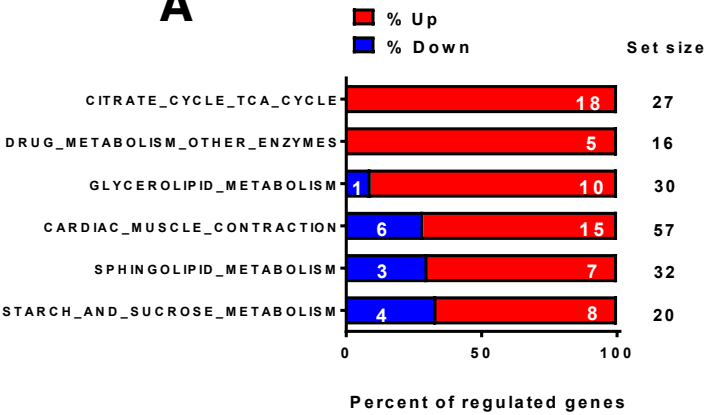


Semi-quantitative qPCR analysis in (A) muscle and (B) liver normalized to TBP. Data are means \pm SEM, n=6, * p < 0.05, ** p < 0.01, *** p < 0.001, Student's *t* test

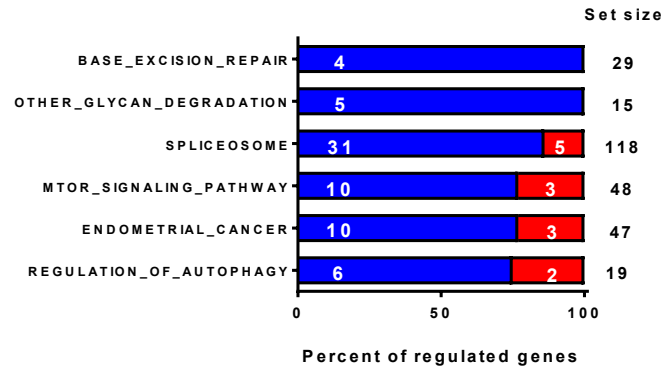
**Figure S3, KEGG pathways regulated by insulin in muscle and liver,
Related to Figure 2 and Figure 3**

Muscle

A

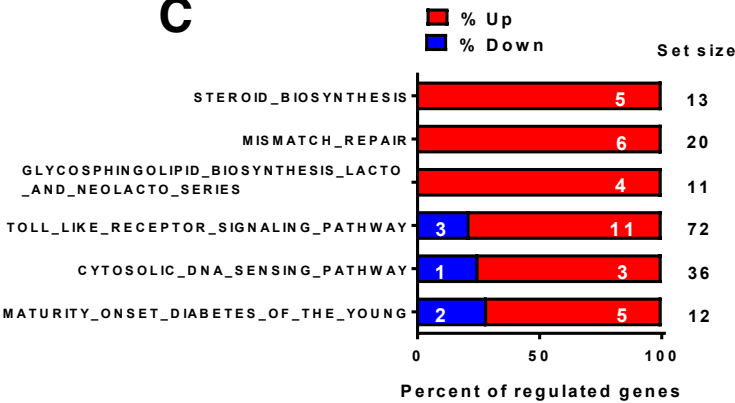


B

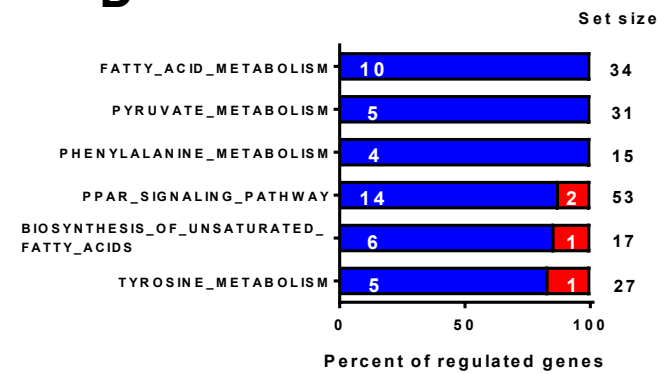


Liver

C

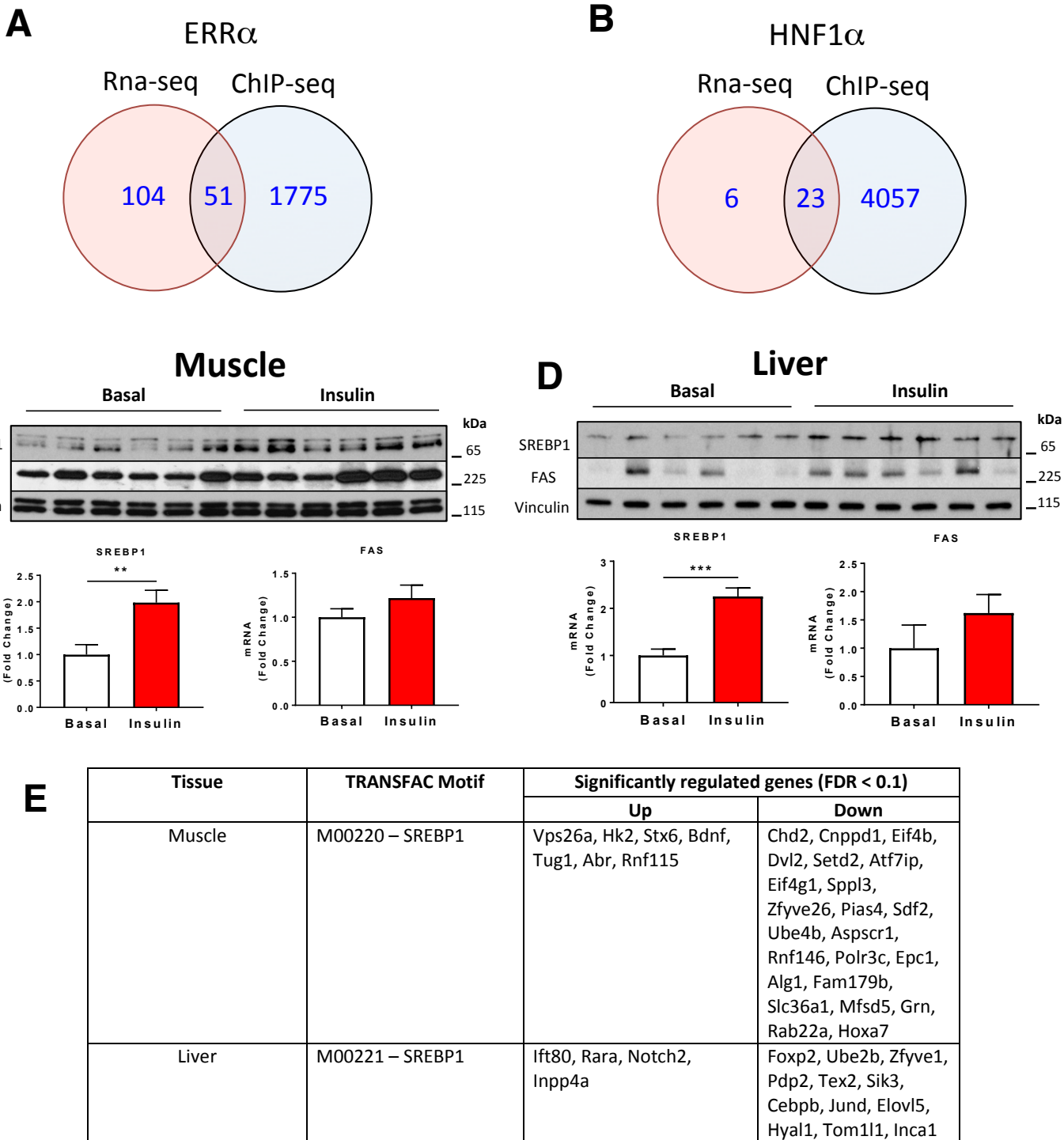


D



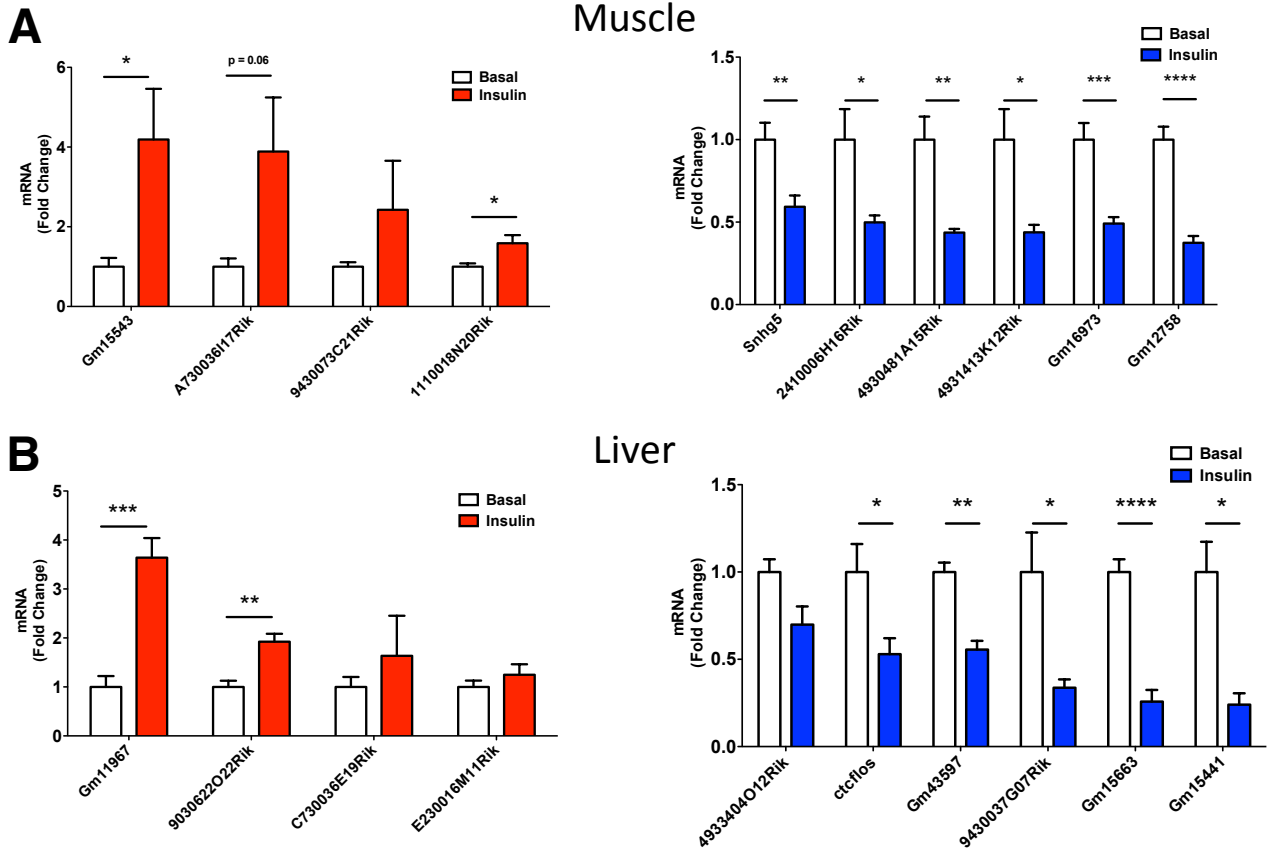
Significant (FDR < 0.05) insulin-regulated pathways in (A, B) skeletal muscle and (C, D) liver at 3 h assessed by mroast analysis. Plots are percentage of up and down-regulated genes over total regulated genes (FDR < 0.1) within each pathway. Numbers of regulated genes are indicated inside bars. Set size represents the total number of genes annotated in each pathway from KEGG database. Pathway analysis was performed using a set in which data from low and high insulin samples was combined (n = 12).

Figure S4, Motif enrichment and ChIP-Seq comparisons, SREBP1 protein expression and target genes, Related to Figure 4



Overlap between transcription factor motif enrichment analysis (RNA-Seq) and ChIP-Seq analysis of (A) ERR α and (B) HNF1 α . Protein expression of mature SREBP1 isoform and FAS in (C) muscle and (D) liver in response to high insulin at 3h. Data are means \pm SEM, n=6, ** p < 0.01, *** p < 0.001, Student's *t* test. (E) Significantly regulated genes showing enrichment of SREBP1 motifs within promoter regions.

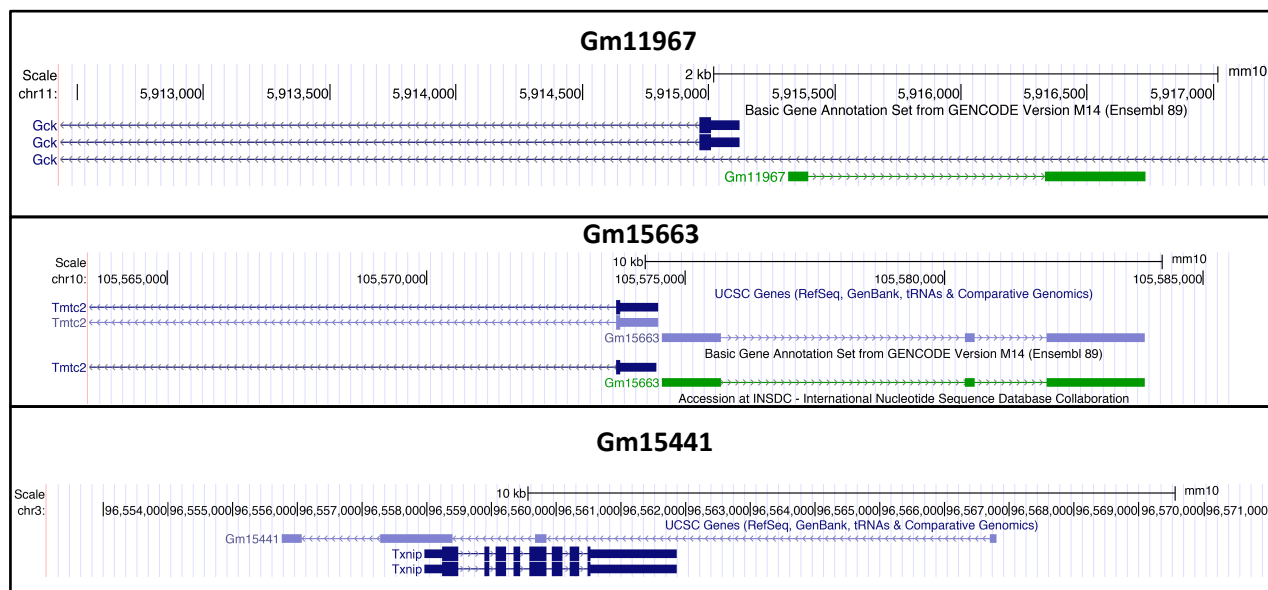
Figure S5, Validation of significantly regulated ncRNA genes by high insulin at 3 h, Related to Figure 6



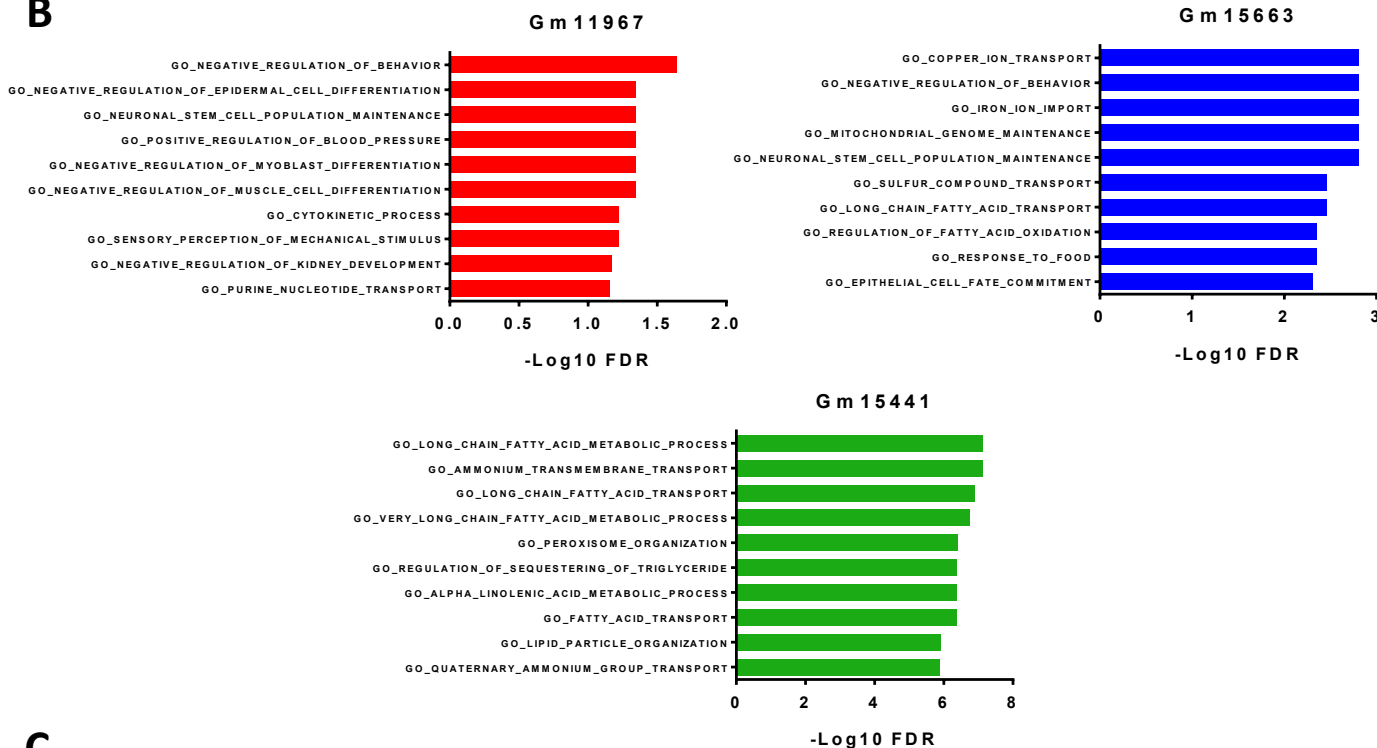
Semi-quantitative qPCR analysis in (A) muscle and (B) liver normalized to TBP. Data are means \pm SEM, n=6, * p < 0.05, ** p < 0.01, *** p < 0.001, **** p < 0.0001, Student's *t* test.

Figure S6, lncRNA loci, GO analysis and knockdown efficiency in primary hepatocytes, Related to Figure 7

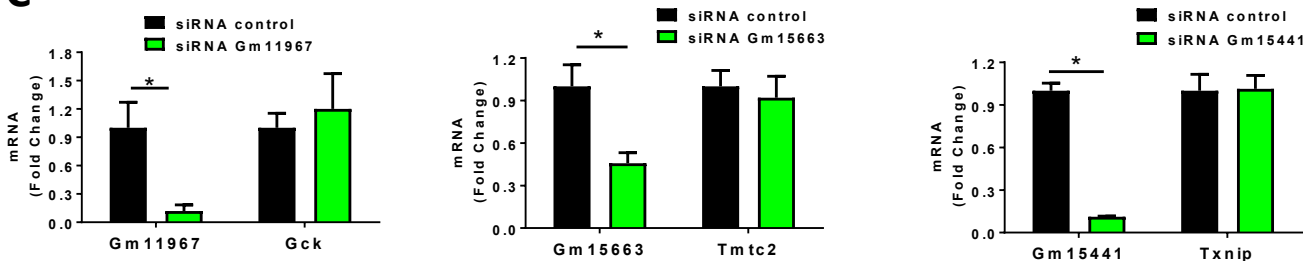
A



B



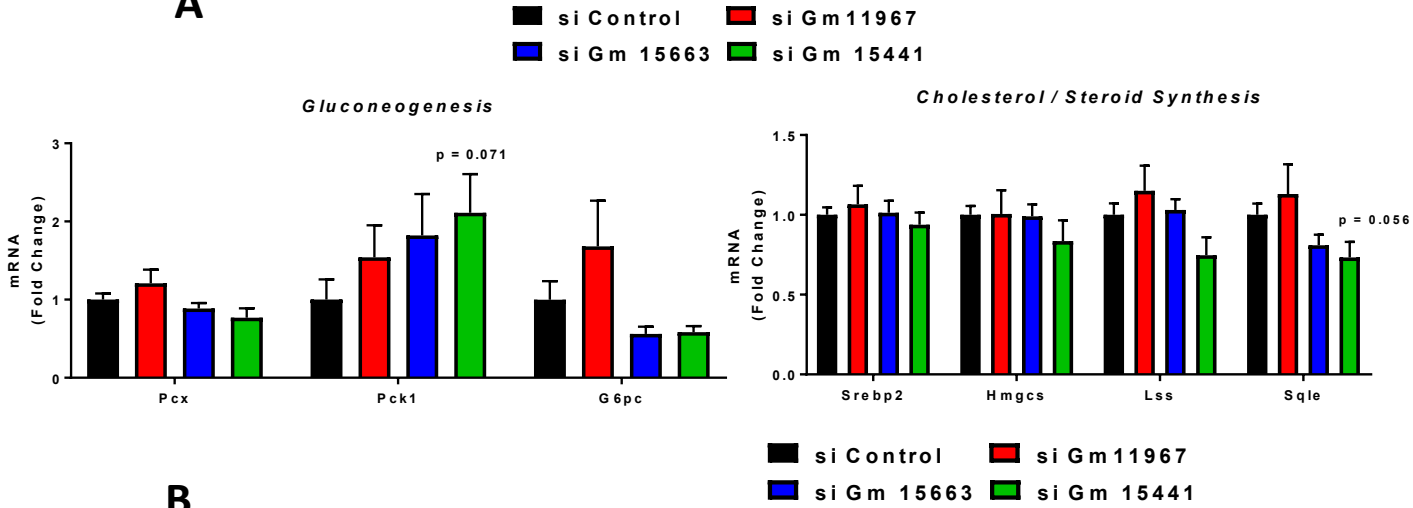
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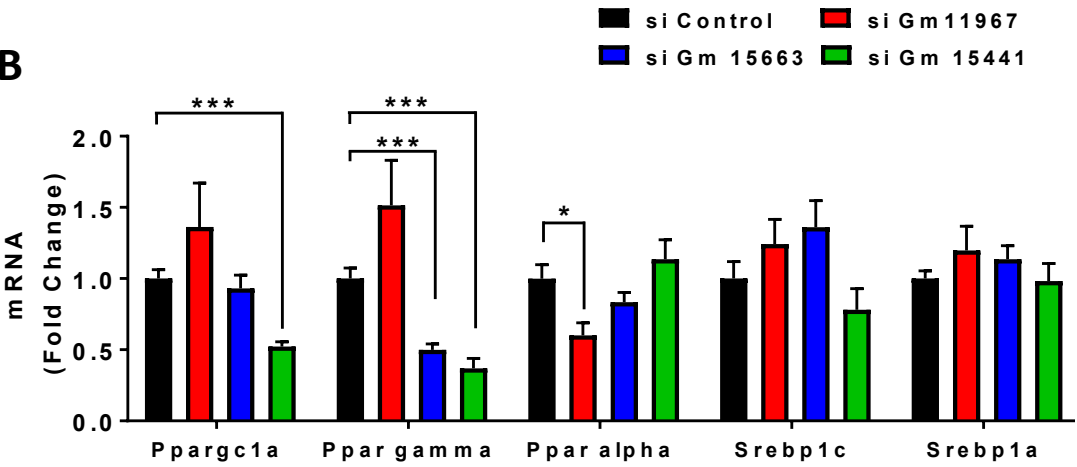
(A) Genome browser screen shots from UCSC (GRCm38/mm10) indicating the position of the indicated lncRNAs and neighboring genes. (B) Top 10 significantly (FDR < 0.1) enriched GO Biological Process terms from mRNA-lncRNA correlation analysis (C) Semi-quantitative qPCR analysis, normalized to TBP, showing knockdown efficiency of lncRNAs in primary hepatocytes and no effects on neighboring genes. Data are means \pm SEM, n= 4-5, * p < 0.05, Student's *t* test.

Figure S7, Effects of IncRNA knockdown in primary and AML-12 hepatocytes, Related to Figure 7

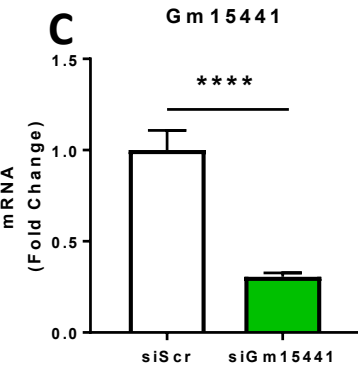
A



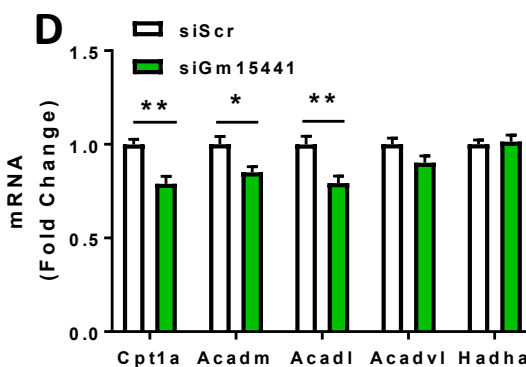
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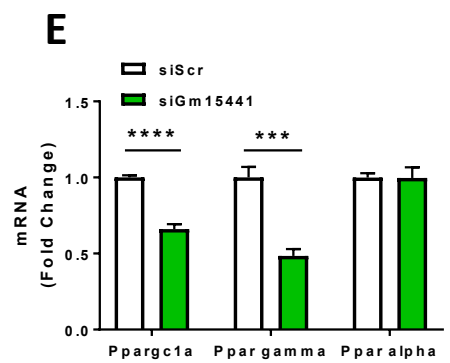
C



D



E



Semi-quantitative qPCR analysis of (A) genes involved in gluconeogenesis and cholesterol/steroid synthesis and (B) gene expression of major transcriptional regulators of lipid metabolism in primary hepatocytes transfected with siRNAs against *Gm11967*, *Gm15663*, *Gm15441* or scramble controls, normalized to 18S. Data are means \pm SEM. For each condition, hepatocyte cultures from 4-5 mice were used, * *p* < 0.05, *** *p* < 0.001, Student's *t* test. (C) Knockdown efficiency and expression of (D) fatty acid oxidation genes and (E) transcriptional regulators of lipid metabolism in AML-12 hepatocytes after transfection with siRNAs against *Gm15441* or scramble controls, normalized to TBP. Data are means \pm SEM. *n* = 5-6 biological replicates, * *p* < 0.05, ** *p* < 0.01, *** *p* < 0.001, **** *p* < 0.0001, Student's *t* test.

Table S3 – Regulation of Mitochondrial DNA-encoded genes in muscle by high insulin at 3 h, Related to Figure 2

Ensembl accession n°	Gene symbol	Fold change (Ins/Basal)	FDR
16 rRNA			
ENSMUSG00000064339	mt-Rnr2	1.87	0.0933
Electron transport chain			
ENSMUSG00000064351	mt-Co1	1.59	0.0542
ENSMUSG00000064341	mt-Nd1	1.74	0.063
ENSMUSG00000064345	mt-Nd2	1.68	0.0771
ENSMUSG00000064360	mt-Nd3	2.14	0.0765
ENSMUSG00000064363	mt-Nd4	1.68	0.0755
ENSMUSG00000064367	mt-Nd5	1.62	0.0392
ENSMUSG00000064368	mt-Nd6	1.52	0.0545
tRNA			
ENSMUSG00000064347	mt-Ta	2.53	0.042
ENSMUSG00000064361	mt-Tr	3.5	0.0465
ENSMUSG00000064348	mt-Tn	2.7	0.0578
ENSMUSG00000064349	mt-Tc	2.32	0.0493
ENSMUSG00000064343	mt-Tq	2.92	0.0311
ENSMUSG00000064359	mt-Tg	3.01	0.025
ENSMUSG00000064364	mt-Th	2.75	0.0175
ENSMUSG00000064342	mt-Ti	4.43	0.02
ENSMUSG00000064340	mt-Tl1	2.72	0.00348
ENSMUSG00000064372	mt-Tp	6.49	0.0345
ENSMUSG00000064346	mt-Tw	3.42	0.00306

Table S4, Genes inversely regulated by insulin and diabetic models (STZ and HFD), Related to Figure 5

Ensembl accession n°	Gene Symbol	Log2 Fold Change			FDR		
		Ins 3h High vs Basal	HFD vs Chow	STZ vs Vehicle	Ins 3h High vs Basal	HFD vs Chow	STZ vs Vehicle
<i>Muscle</i>							
ENSMUSG00000018893	Mb	1.85	-3.25	-1.28	0.0216	5.09E-06	0.0204
ENSMUSG00000021622	Ckmt2	1.28	-2.12	-1.68	0.00514	0.000934	0.00613
ENSMUSG00000002416	Ndufb2	0.91	-2.86	-0.805	0.0289	1.23E-05	0.0602
ENSMUSG00000022551	Cyc1	0.869	-1.53	-0.659	0.00441	0.0106	0.0568
ENSMUSG00000046668	Cxc5	0.82	-1.59	-0.618	0.0976	1.30E-05	0.082
ENSMUSG00000038375	Trp53inp2	0.733	-2.52	-0.761	0.0183	0.000196	0.0357
ENSMUSG00000026664	Phyh	0.699	-0.589	-1.16	0.0169	0.0461	0.00882
ENSMUSG00000022186	Oxct1	0.637	-1.96	-1.23	0.0319	0.000287	0.0204
ENSMUSG00000025968	Ndufs1	0.578	-1.56	-0.856	0.0214	0.0145	0.041
ENSMUSG00000032279	ldh3a	0.542	-1.08	-0.88	0.0281	0.00455	0.0486
ENSMUSG00000027332	lvd	0.536	-0.551	-1.1	0.0477	0.00245	0.0555
ENSMUSG00000024181	Mrpl28	0.502	-0.657	-0.65	0.0833	0.000478	0.0556
ENSMUSG00000026895	Ndufa8	0.491	-1.5	-0.671	0.0931	0.000937	0.0414
ENSMUSG00000019179	Mdh2	0.441	-1.26	-0.673	0.0827	0.00589	0.0435
ENSMUSG00000032216	Nedd4	-0.354	0.792	0.657	0.0822	0.000489	0.0878
ENSMUSG00000027651	Rprd1b	-0.376	0.434	0.699	0.0416	0.00463	0.0602
ENSMUSG00000026473	Glul	-0.515	0.564	1.31	0.0436	0.00683	0.0142
ENSMUSG00000028329	Xpa	-0.67	0.359	0.982	0.0757	0.0632	0.0238
ENSMUSG00000074340	Ovgp1	-0.864	0.57	0.816	0.0248	0.00544	0.0841
ENSMUSG00000054640	Slc8a1	-0.874	0.41	0.808	0.0464	0.044	0.0718
ENSMUSG00000048249	Crebrf	-0.897	0.874	1.07	0.0156	2.00E-04	0.075
ENSMUSG00000031939	Taf1d	-0.99	0.939	1.4	0.00744	0.00435	0.028
<i>Liver</i>							
ENSMUSG00000052562	Slc22a30	0.903	-0.606	-1.02	0.0104	0.0139	0.0106
ENSMUSG00000022010	Tsc22d1	0.814	-0.58	-0.707	0.00849	0.00818	0.0768
ENSMUSG00000022508	Bcl6	0.765	-1.21	-1.34	0.0857	0.000451	0.0364
ENSMUSG00000067071	Hes6	0.741	-0.752	-1.3	0.0859	0.000302	2.32E-06
ENSMUSG00000020484	Xbp1	0.737	-0.408	-0.245	0.0254	0.0681	0.0368
ENSMUSG00000073155	1810058124Rik	0.681	-0.379	-0.419	0.00455	0.00194	0.0256
ENSMUSG00000038267	Slc22a23	0.41	-0.51	-0.249	0.0175	0.00676	0.0144
ENSMUSG00000037242	Clic4	-0.412	0.277	0.157	0.0303	0.0152	0.0835
ENSMUSG00000029178	Klf3	-0.469	1.4	0.284	0.0598	2.55E-06	0.0925
ENSMUSG00000030934	Oat	-0.528	0.202	0.53	0.0535	0.0959	0.000591
ENSMUSG00000071076	Jund	-0.601	0.541	0.241	0.0346	0.00027	0.0972
ENSMUSG00000037573	Tob1	-0.752	0.405	0.31	0.00422	0.0944	0.0186
ENSMUSG00000031596	Slc7a2	-0.967	0.263	0.484	6.08E-05	0.0541	0.00555
ENSMUSG00000022091	Sorbs3	-1.14	0.374	0.926	7.02E-06	0.0013	7.71E-05
ENSMUSG00000034634	Ly6d	-1.22	0.885	2.8	0.0537	2.16E-07	0.000418
ENSMUSG00000020423	Btg2	-1.26	0.612	1.17	0.0138	6.75E-05	0.000229
ENSMUSG00000032715	Trib3	-1.35	0.23	0.426	0.0296	0.063	0.00764
ENSMUSG00000041773	Enc1	-1.67	0.198	0.483	0.000285	0.079	0.00575
ENSMUSG00000037887	Dusp8	-1.82	0.272	0.238	0.0176	0.0436	0.0717
ENSMUSG00000028654	Mycl	-2.11	0.261	0.206	1.07E-06	0.0394	0.0343

Table S7, Primer sequences used in the study, Related to STAR Methods

Primer	Sequence		Primer	Sequence		Primer	Sequence	
	Coding Genes				Non-Coding Genes			
Tbp	F	ACCCTTCACCAATGACTCCTATG	Mfsd2a	F	AGAAGCAGCAACTGTCCATTT	Gm15543	F	GAGAGGCACAGGAAGGACTG
	R	TGACTGCAGCAAAATCGCTTGG		R	CTCGGCCACAAAAAGGATAAT		R	CAGCCAGACAGCAGAGACTG
Tnfrsf1b	F	GACTGTGAGGCAAGCATGTAT	lgfbp1	F	CTGCCAAACTGCAACAAGAATG	A73003617Rik	F	CAGGGGTGGAGGAAGTACAA
	R	GGTCTCTGCTTTAGTGCAGG		R	GGTCCCCTCTAGTCTCCAGA		R	CTCTCTCCGCACACTGAA
Rara	F	TTCTTTCCCCTATGCTGGGT	G6pc	F	CTGTTTGACAAACGCCGTAT	9430073C21Rik	F	TAGCTGCTTTGCTCCCTGAG
	R	GGGAGGGCTGGGTACTATCTC		R	AGGTGACAGGGAGACTGTTA		R	AGTCCCAGGTCAACAAGCTG
Vps37b	F	ACAACGCTTCTTGGAGACTC	Gck var1	F	CTAGGGTACTTCTGCTTTGG	1110018N20Rik	F	CTGAGCGTCAACCAGGAG
	R	CGCTTGTCTGGTAGACATCA		R	CGTAGGTGGGCAACATCTTT		R	AATGGCTGCTCTCTGTTT
Bhlhe40	F	ACGGAGACCTGTACAGGGATG	Gck var2	F	TCGGGAGTCAGGAACATCTC	Snhg5	F	GCACGTTACAAGGACAATG
	R	GGCAGTTTGAAGTTTCTTTC		R	CGTAGGTGGGCAACATCTTT		R	ACTGGCATCTCTCGCAAGTT
Scd2	F	GCAITTTGGGACGTTGTACG	Txnip	F	TATGGTACAGGATGACTTCC	2410006H16Rik	F	ATCATGGCTGTGCTTCTC
	R	AGCCGTGCCTTGTATGTTCTG		R	GTTCCCCGCTGTAGAGACTG		R	TCATGGTGGGATGATACTGC
Ppp1r27	F	TGCTAGTCAAATACGGGGCTG	Tmtc2	F	GCAAGGACCTATGAGGAAG	4930481A15Rik	F	CTCCATCTGCTCCCTCAG
	R	CCGGGTCAATAAAGTCCGGAG		R	GGTGAGATGAGCAGGGATGT		R	CAGCCAGTGATTGAGCAAAA
Sox9	F	CGGAACAGCCACATCTCTCC	Cd36	F	TCAATGGAAAGGATAACAAAGCA	4931413K12Rik	F	CTGCCCTCTCAGCACTTC
	R	GCTTGACAGCTCGGTTTTGG		R	TTCAACAAAAGGTGAAAAGGA		R	CTGTTCTAAGGGACGGCTTG
Klf15	F	GAGACCTTCTCGTACCAGAAA	Fatp1	F	TTCTCGTGGGCGAGATCAAC	Gm16973	F	AAGTCTGGGCTCAGACTGCT
	R	GCTGGAGACATCGTGTGAT		R	AGCACGTACCTGAGAGGTA		R	GACAGGTGGGAAGGACAGAA
Ucp3	F	CTGCACCGCCAGCTTGTAGTT	Cpt1a	F	AGTGGCCTCACAGACTCCAG	Gm12758	F	TACCATTATGCCTGGCTTCC
	R	ATCATGGCTTGAATCGGACC		R	GCCCATGTTGTACAGCTTCC		R	GAACTGTGCAGGAGCTGGAT
Pfkfb3	F	CGATGCTGGTGTGTGTGAG	Hadha	F	CTGGTCAGCAGAGCAGAAGA	2810425M01Rik	F	CCTCTGACGAAGGGAAGTCTG
	R	TCTCTGGGCTCTAATTC		R	ATTGGCAGTCTCAGTCTGCTT		R	CCACTCTGGTTAGGGTCTCTG
Ddit4	F	GTGCTGGCTCTGGACTCTC	Acadm	F	AGCAGGAGAAATGAACTCC	2310015A10Rik	F	GCATGAGCGGTTTCTCTTTC
	R	CCGGTACTTAGCGTCAGGG		R	AGCTCTAGACGAAGCCACGA		R	AGCAGGTGACCATACCCAAG
Cebpd	F	CGACTTCAGCGCTACATTGA	Acadl	F	TCITGCGATCAGCTTITCA	Gm26908	F	CCTCCTCGTCCAGTCTTCTG
	R	CTAGCGACAGACCCACAC		R	GGTACATGTGGGAGTACCCG		R	CGCCGACCCCTACATTA
Klhl38	F	TGAGGAGTTACCAGATGGGGT	Acadvl	F	CTGATGAGCTCCAGGGTAA	Gm9828	F	TTGTCCAGTAGGCTGCAAGT
	R	CAGTCAGGATCTTGCTTTGCTT		R	TTGGGCTCTCTAATACCCA		R	CTGGAAGCATTCCGAACAT
Angptl4	F	CATCTGGGACGAGATGAACT	Sreb1c	F	GAGCCATGGATTGCACATTT	2410006H16Rik	F	ATCATGGGATCGTGCTTCTC
	R	TGACAAGCGTTTACCACAGGC		R	CTCAGGAGAGTTGGCACCTG		R	TCATGGTGGGATGATACTGC
Rnf144b	F	CATGACTCGGAAAACCAC	Sreb1a	F	GAAGTGGACACAGCGGTTTT	Gm15622	F	CGTTGACTTCTGCCCTAA
	R	CTGGCACTCTGGAGCATGG		R	GGCCAGAGAAGCAGAAGAGA		R	CGTTGTGTCTCAGTCTCG
Kank2	F	GGAGGAAATTCGATGGATCTG	Pparg	F	TCAGTCTGTGGACCTCTCC	Gm16559	F	ATGGGATTTCTGCCCTCTCT
	R	ACTTTCAGTCTCGCTCTGTGA		R	ACCCTTGCATCCTTCAAAAG		R	TGTAGCTTCTGTCGGAATGA
S1pr1	F	ATGGTGTCCACTAGCATCCC	Ppara	F	AACATCGAGTGTCAATATGTGG	Gm42940	F	CCCAGTTTGCATCTCAAT
	R	CGATGTTCAACTTCCCTGTGTAG		R	CCGAATAGTTCGCCGAAAAGAA		R	AGGTGTTTTCCCAATCACA
Steap4	F	GGGAAGTCACTGGGATTGAAAA	Pcx	F	GGGATGCCACCAGTCACT	B430119L08Rik	F	ATCCTGTGCTGCCCTACAGT
	R	CCGAATAGCTCAGGACCTCTG		R	CATAGGGGCAATCTTTTTGA		R	AATGCCACTGCTGCTTCTCT
Cyp26a1	F	AAGCTCTGGGACCTGTACTGT	Sreb2	F	GCGTTCTGGAGACCATGGA	Malat1	F	CTTTGCGGGTGTGTAGGTT
	R	CTCCGCTGAAGCACCATCT		R	ACAAAGTTGCTCTGAAAACAATCA		R	CAAAAGTGGAGGTTGTGCT
Osgin1	F	CCTCCGGTATCTGCCTGTC	Hmgcs	F	TGCTGGAAAGTATACCATCGGCCT	Gm11967	F	TGATGATCGGATGCTTTGAA
	R	GGAAAAGTACTTAGGTCTGGG		R	GCCGCCAATGCAATCATAGGAAA		R	CAGAGTTGCTCTGGGTGCTT
Gck	F	TGAGCCGGATGCAGAAGGA	Lss	F	TGTTCTGGTTCAGTGGATG	9030622O22Rik	F	GCTGAAGCCCTTTGATCTTG
	R	GCAACATCTTACACTGGCCT		R	GATAGCAAAATGAAGTGTCCAG		R	CATCAGTCCCAATGTTGTGC
Ulk1	F	AAGTTCGAGTCTCTCGCAAG	Sqle	F	TTGTTGCGGATGGACTCTTCTCA	C730036E19Rik	F	GCCAGTAAATCCGAAGACCA
	R	CGATGTTTTCTGCTTTAGTTC		R	GTTGACCAGAACAAGCTCCGCAA		R	GGCATGTCCATTCTGACTT
Got1	F	GCGCTCCATCAGTCTTTG	Ppargc1a	F	TGATGTGAATGACTTGGATACAGACA	E230016M11Rik	F	AATCTCTCCAGCCGACTCT
	R	ATTATCTGTGCGGTACGCTC		R	GCTCATTGTTGACTGGTTGGATATG		R	TGTGATGACTTCAGCTTGG
Tat	F	TGCTGGATGTTCCGCTCAATA	18S	F	GTTCCGACCATAAACGATGCC	4933404O12Rik	F	TGTGATCTTGAACCCTGAG
	R	CGGCTTCACTTCATGTTGTC		R	TGGTGGTGCCTTCCGTCAT		R	AAGGGGAATGAACACTGTC
Pck1	F	TGACAGACTCGCCTATGTG	Lpin1	F	CATGCTTCCGAAAGTCTTCA	Ctcflos	F	CCGAGAACAAGAAGAGCTGG
	R	CCCAGTTGTTGACCAAGGC		R	GGTATTCTTTGGCGTCAACCT		R	GCTAGTTGCGGGAAGACTTG
						Gm43597	F	ACCAACAGCCTCGCTCTAAA
							R	TCTACAGCCCAGCAGTTTCT
						9430037G07Rik	F	CGTTCCTTAGTCCCAGTTTGG
							R	CTGGTCATGCAGTTCTGCTG
						Gm15663	F	ATTTTCTGGCACTGGGTTG
							R	AGCAAGCAGAGCAGAACTCC
						Gm15441	F	TTCTGGTGAATGCTCTGGTG
							R	GAGATGGCTTGGTGGTTGAT