Cell Reports, Volume *21*

Supplemental Information

A Specific ChREBP and PPARα Cross-Talk Is Required for the Glucose-Mediated FGF21 Response

Alison Iroz, Alexandra Montagner, Fadila Benhamed, Françoise Levavasseur, Arnaud Polizzi, Elodie Anthony, Marion Régnier, Edwin Fouché, Céline Lukowicz, Michèle Cauzac, Emilie Tournier, Marcio Do-Cruzeiro, Martine Daujat-Chavanieu, Sabine Gerbal-Chalouin, Véronique Fauveau, Solenne Marmier, Anne-Françoise Burnol, Sandra Guilmeau, Yannick Lippi, Jean Girard, Walter Wahli, Renaud Dentin, Hervé Guillou, and Catherine Postic

Supplementary information

Generation of ChREBP knockout mice

Mice lacking exon 9 through 15 of the *Chrebp* gene were generated through homologous recombination (*Chrebp*$^{-/-}$ mice). Correspondence regarding *Chrebp*$^{-/-}$ mice should be addressed to renaud.dentin@inserm.fr and catherine.postic@inserm.fr. A vector containing the *Chrebp* sequence with the addition of two Lox-P sites flanking the coding exons 9-15 and a FRT-flanked hygromycin-resistant cassette was constructed (Figure S2). Clones were then selected after validation by 3 PCR sequences targeting i) 5' region homologue ii) 3' region homologue iii) floxed fragment. Homologous recombination of the target construct at the appropriate site was confirmed by Southern blot analysis followed by sequencing. Validated ES cells containing the target construct were injected in C57BL/6N derived blastocystes. Male agouti progeny were then bred with female C57BL/6J "flippase" mice to remove the hygromycin-resistant cassette. F1 progeny were crossed with *EllaCre* transgenic mice (Holzenberger et al. 2000) to ubiquitously remove the *loxP* floxed exons 9 to 15 from the *Chrebp* locus. This allowed generation of global *Chrebp*$^{-/-}$ mice (total excision of the *loxP*-flanked sequence) from a single germ-line mutation. Mice were genotyped using specific primers in Supplemental Table. S1. Loss of *Chrebp* expression was confirmed by qPCR and by western blot.

Nutritional challenges

All *in vivo* experiments were performed on adult (1à-12 week-old) male mice. All mice were sacrificed at ZT14 (14h after the start of light period in animal housing unit). Fasted mice were fasted starting at 11pm and sacrificed 24 hours later. Fasted and fed animals had free access to drinking water. Glucose treated mice were allowed ad libitum access to standard diet in addition to a 20% glucose solution (Sigma, G7021). To

measure sucrose intake, *Pparα*$^{hep+/+}$ and *Pparα*$^{hep-/-}$ mice (Montagner et al. 2016) were given free choice between a bottle containing a 10% sucrose solution or water. Consumption was measured daily during three days.

Culture conditions for primary mouse and human hepatocytes

For adenoviral infections, mouse hepatocytes from either female or male mice (as indicated in figure legens) were incubated under low glucose concentration (5 mM) with specific adenovirus (3 pfu/cell) for 24 h. For glucose stimulation experiments, hepatocytes were incubated with insulin (100 nM) in the presence of various glucose concentrations (5 to 30 mM) for 24 hours. For mannitol experiments, mouse hepatocytes were incubated with insulin (100 nM) in the presence of concentrations of mannitol (5 to 30 mM) for 24 hours. For HDAC inhibitor experiments, mouse hepatocytes were treated 10 μM of the HDAC inhibitor LBH589 (Panobinostat, a large spectrum pan-HDAC inhibitor (Selleckchem)) (Imai et al., 2016) for 24 h. DMSO was used as a control. Stimulation of human (100 μM) or mouse (10 μM) hepatocytes with Wy-14643 (DMSO used as a control) was done in combination with glucose stimulation (5 or 25 mM) 48 hours after seeding.

ChIP analysis

In vivo ChiP assays: Mouse liver tissue was submersed in PBS + 1% formaldehyde, cut into small pieces and incubated at room temperature for 15 minutes. Fixation was stopped by the addition of 0.125 M glycine (final). The tissue pieces were then treated with a TissueTearer and spun down and washed 2x in PBS. Chromatin was isolated by the addition of lysis buffer, followed by disruption with a Dounce homogenizer. Lysates were sonicated using the EpiShear™ Probe Sonicator (Active Motif, cat # 53051) with an

EpiShear™ Cooled Sonication Platform (Active Motif, cat # 53080) and the DNA sheared to an average length of 300-500 bp. Genomic DNA (Input) was prepared by treating aliquots of chromatin with RNase, proteinase K and heat for de-crosslinking (overnight at 65°C) followed by ethanol precipitation. Pellets were resuspended and the resulting DNA was quantified on a NanoDrop spectrophotometer. Extrapolation to the original chromatin volume allowed quantitation of the total chromatin yield. Aliquots of chromatin (30 μg) were pre-cleared with protein A or G agarose beads (Invitrogen). Genomic DNA regions of interest were isolated using antibodies against H3K9Ac (Active Motif, cat # 39917), RNA Pol II (Active Motif, cat# 39097), ChREBP (Novus, cat# NB400-135) and PPARα (Santa Cruz Biotechnologies, cat# sc-9000). Complexes were washed, eluted from the beads with SDS buffer, and subjected to RNase (20ug, 1 hour at 35°C) and proteinase K treatment (50μg, 3 hours at 35°C). Crosslinks were reversed by incubation overnight at 65°C, and ChIP DNA was purified by phenol-chloroform extraction and ethanol precipitation. QPCR reactions were carried out in triplicate using SYBR Green Supermix (Bio-Rad, Cat # 170-8882) on a CFX Connect™ Real Time PCR system. Positive and negative control sites were tested for each factor as well as the sites of interest. The resulting signals were normalized for primer efficiency by carrying out qPCR for each primer pair using input DNA (pooled unprecipitated genomic DNA from each sample). Specific enrichment was expressed as % of input.

In vitro ChiP assays from cultures hepatocytes were performed as previously described (Marmier et al. 2015). Proteins were cross-linked to DNA by addition of 1% formaldehyde for 10 minutes at room temperature. Crosslinking was stopped by a 5-minute incubation in 0.125 M glycine (final). Hepatocytes were scrapped and lysates were homogenized in low salt PIPES buffer and the nuclei fraction was enriched by a 10-

minute centrifugation. Sonication was performed in 10 seconds pulses, 15 times. Genomic DNA regions of interest were isolated using antibodies against ChREBP ChREBP (Novus, cat# NB400-135) and IgG (Cell Signaling). Immune complexes were captured with magnetic beads (Protein A-ChIP-Ademtech) and a saturated buffer. Positive and negative control sites were tested for each factor as well as the sites of interest. The resulting signals were normalized for primer efficiency by carrying out qPCR for each primer pair using input DNA (pooled unprecipitated genomic DNA from each sample). DNA fragments were quantified by qPCR, using primers described in Supplemental Table 1. Results are expressed in fold enrichment.

FAIRE qPCR

FAIRE qPCR was performed *Pparα*$^{hep+/+}$ and *Pparα*$^{hep-/-}$ hepatocytes using a protocol previously described (Simon et al., 2012). Cells were treated with 1% formaldehyde at room temperature for 5 minutes to form DNA-protein crosslinks and the crosslinking was stopped by addition of glycine to a final concentration of 125 mM. Cells were washed 3 times in 4°C PBS and lysed in cell lysis buffer (5mM PIPES, 85mM KCl, 0,5% NP40 and protease inhibitors) on ice for 10 minutes. Nuclei were pelleted and lysed in nuclei lysis buffer (50mM Tris, 10mM EDTA, 1% SDS and protease inhibitors) on ice for 10 minutes. Lysates were sonicated in a Bioruptor plus sonde (Diagenode). Cells debris was removed and DNA was extracted from supernatant by phenol/chloroform extraction. Under these conditions, DNA not crosslinked to proteins remains in the aqueous phase while the DNA cross-linked to proteins remains in the phenol phase. The FAIRE was analyzed by qPCR on genomic DNA using the ChoRE *Fgf21* primers (Figure 5C) and calculated by using relative enrichment for each amplicon using the comparative Ct method, such that a ratio is calculated for the signal from the FAIRE

sample relative to the signal from input control DNA. The results are expressed in fold enrichment.

References

Hozelberger, M, Lenzner, C., Leneuve, P., Zaoui, R.,Hammard, G., Vaulont, S., and Le Bouc, Y., (2000). Cre-mediated germline mosaicism: a method allowing rapid generation of several alleles on target genes. Nucleaic Acid Research. 28, e92.

Imai, Y. , Ohta, E.,Takeda, S., Sunamura, S., Ishibashi, M., Tamura, H., Wang, Y., Deguchi, A., Tanaka, J., Maru, Y., and Motoji, T. (2016). Histone deacetylase inhibitor panobinostat induces calcineurin degradation in multiple myeloma. JCI Insight;1(5):e85061.

Marmier, S., Dentin, R., Daujat-Chavanieu, M., Guillou, H., Bertrand-Michel, J., Gerbal-Chaloin, S., Girard, J., Lotersztajn S., Postic,C. (2015). Novel role for carbohydrate responsive element binding protein in the control of ethanol metabolism and susceptibility to binge drinking. Hepatology. 62(4):1086-100.

Montagner, A., Polizzi, A., Fouché, E., Ducheix, S., Lippi, Y., Lasserre, F., Barquissau, V., Régnier, M., Lukowicz, C., Benhamed, F., Iroz, A., Bertrand-Michel, J., Saati, T.A., Cano, P., Mselli-Lakhal, L., Mithieux, G., Rajas, F., Lagarrigue, S., Pineau, T., Loiseau, N., Postic, C., Langin, D., Wahli, W., and Guillou, H. (2016). Liver PPARα is crucial for whole-body fatty acid homeostasis and is protective against NAFLD. Gut 65(7):1202-1214.

Simon, J.M., Giresi, P.G., Davis, I.J., Lieb, J.D. (2012). Using formaldehyde-assisted isolation of regulatory elements (FAIRE) to isolate active regulatory DNA. Nat Protoc.;7(2):256-67.

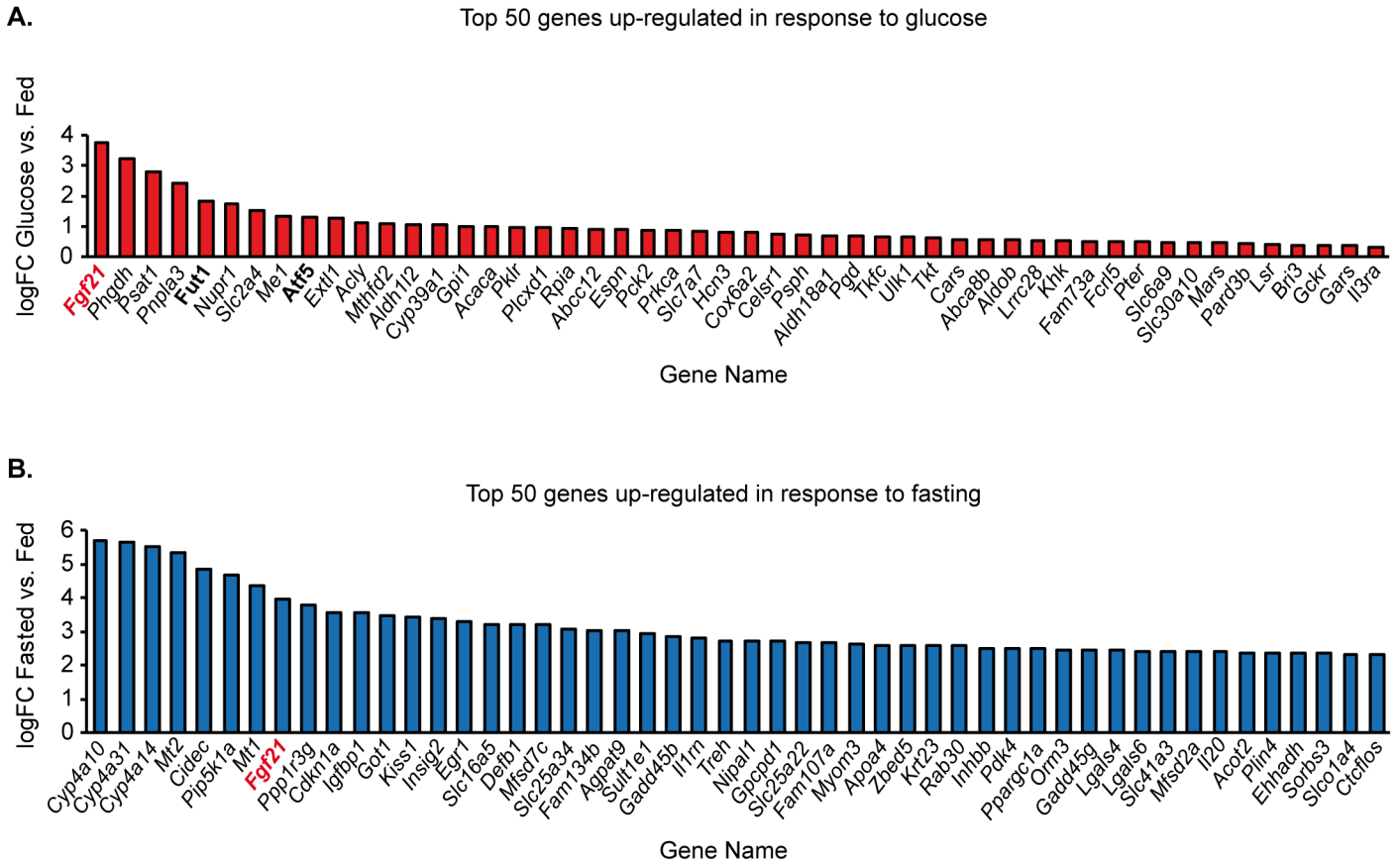


Figure S1. Related to Figure 1. Most significant induced genes in response to glucose and to fasting. (A) Microarray was performed and revealed that the top 50 genes showing up-regulation of mRNA levels in response to 24 hours glucose challenge (Glucose) when compared to fed mice (Fed). These genes were selected on the basis of significantly different mean of expression level (Glucose vs Fed, n=6 per group) with an adjusted p value <0.05. **(B)** Microarray was performed and revealed that the top 50 genes showing up-regulation of mRNA levels in response to 24 hours fasting (Fasted) when compared to fed mice (Fed). These genes were selected on the basis of significantly different mean of expression level (fasted vs Fed, n=6 per group) with an adjusted p value <0.05.

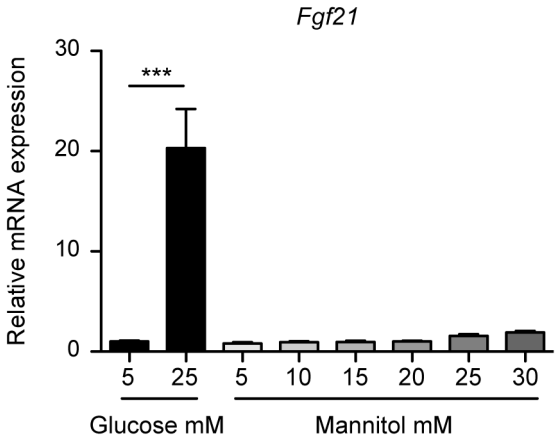
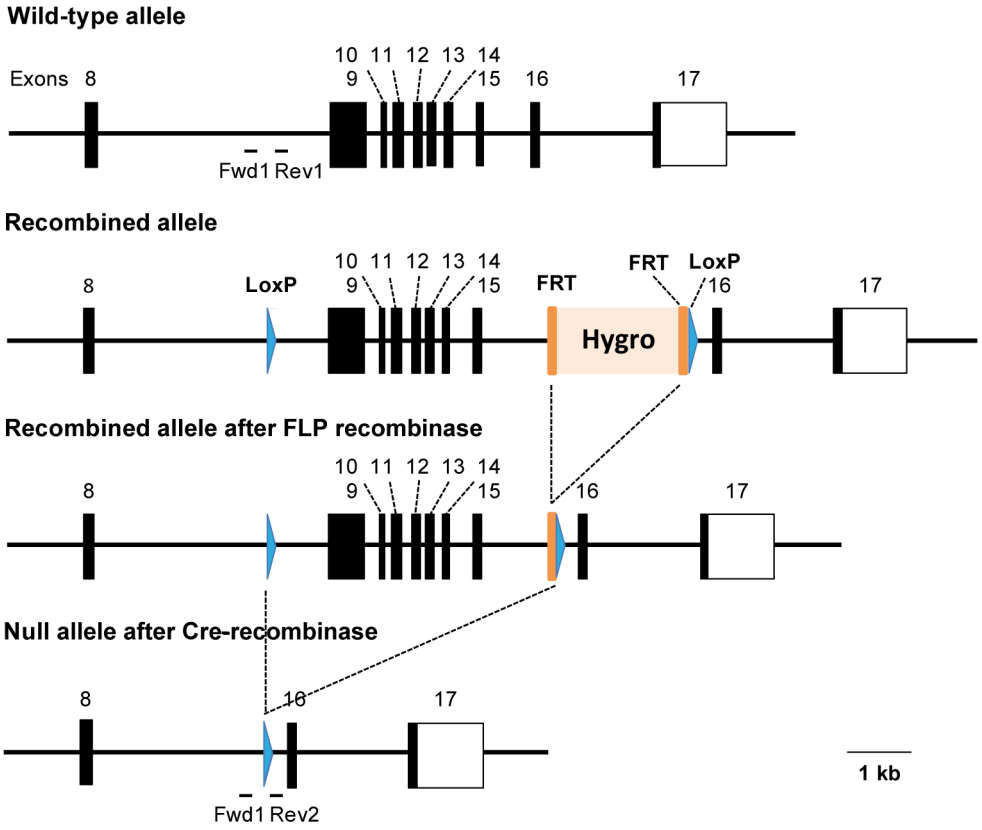


Figure S2. *Fgf21* is not induced by Mannitol in primary hepatocytes. Related to Figure 2. Primary hepatocytes derived from adult male C57BL/6J mice. Hepatocytes were stimulated one day after platting for 24 hours with medium containing 5, or 25 mM glucose; or increasing concentrations of mannitol (from 5 to 30 mM). Relative *Fgf21* gene expression was determined by qPCR. Data are presented as means ± SEM from 3 independent cultures. Each culture was completed in triplicate. Significance is based on 2-way ANOVA followed by Bonferroni post test, $p \leq 0.001$ (***).

A.



B.

Primer	*Sequence*	*Position*
Fwd1	CCACCCCTATGGAATGGTTC	Arm 5'
Rev1	GCACCCATTTACCAACTTAGTC	Floxable zone
Rev2	TCCCACATCTCTAGGCTCAG	Arm 3'

C.

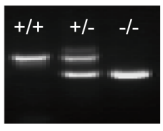


Figure S3. Generation of global ChREBP knockout mice. Related to Experimental procedures generation of knockout mice. Related to Figure 2. (A) Wild type and recombined alleles are indicated. Exons 9-15 were floxed to generate *Chrebp* conditional mutant allele. **(B)** PCR analysis was used to identify offspring that carry Cre-mediated recombination between *loxP1* and *loxP2* using primers Fwd1 and Rev1 for wild type allele: 319 bp and for the allele including *LoxP* site: 375 pb. Primer Fwd1 and Rev2: 477 bp were used for mutant allele. **(C)** The first lanes is a DNA sample from a wild type mice (+/+) detecting the 319 bp band, while the second lane is a DNA sample from a heterozygous mouse (+/-) detected the 375 bp band and the third lane is a *Chrebp* knockout mouse (-/-) detected at 477 bp band.

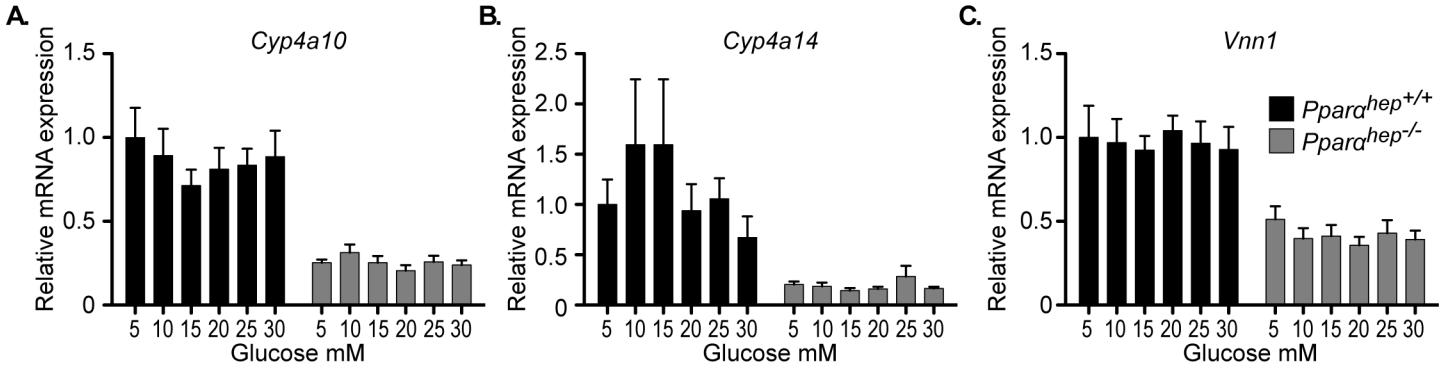


Figure S4. The expression of *Cyp4a10*, *Cyp4a14* and *Vnn1* is not induced by glucose. Related to Figure 4. Primary cultures of hepatocytes derived from adult male *Pparα*$^{hep+/+}$ or *Pparα*$^{hep-/-}$ mice. Hepatocytes were stimulated one day after platting for 24 hours with medium containing 5, 10, 15, 20, 25, or 30 mM glucose. Relative gene expression was determined by qPCR. Primers used were described in Montagner *et al.* 2016.

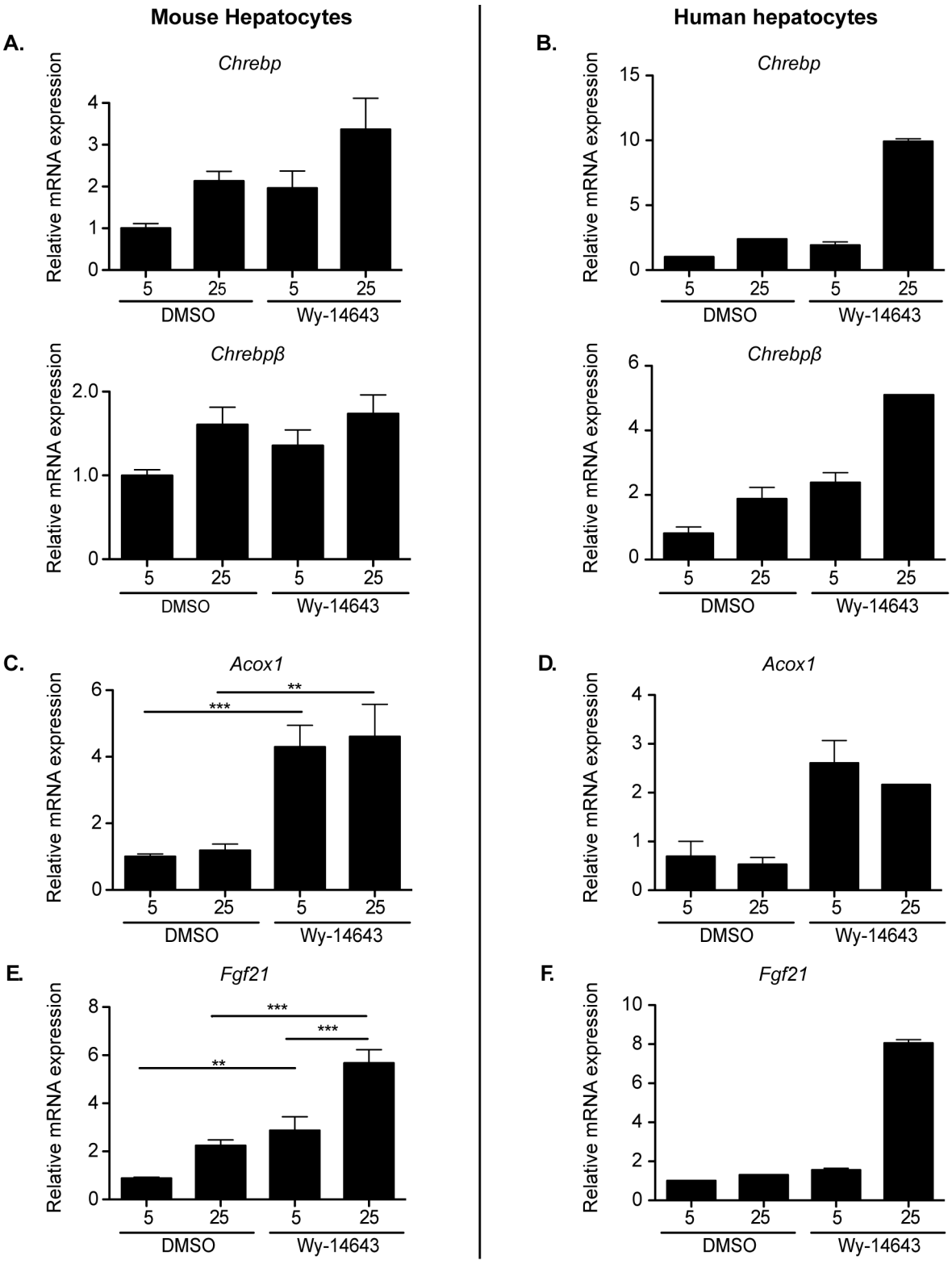


Figure S5. FGF21 synergistically responds to glucose and a pharmacological PPARα activator in both mouse and human hepatocytes. Primary mouse **(A-E)** or human hepatocytes **(B-F)** stimulated 24 hours with medium containing 5 or 25 mM glucose and of 100 µM Wy-14643, 10 µl of DMSO was used as a control. Relative gene expression was determined by qPCR. Mouse data are presented as means ± SEM from 4 independent cultures. Each culture was completed in triplicate. Significance is based on 2-way ANOVA followed by Bonferroni post test $p \leq 0.01$ (**), $p \leq 0.001$ (***). Human hepatocyte data are presented as means ± SEM from 1 culture in triplicate assays.

Table S1. Primers used for qPCR ChIP analysis. Related to Figure 2

Mouse Genes		Sequences for ChIP	T fusion °C
ChoRE *Fgf21*	forward	TAGCCCTTTTCATTCAGCCCCT	60
	reverse	CTCTGTGTTGAACTCCCAGCTGA	
ChoRE *L-pk*	forward	GTCCCACACTTTGGAAGCAT	60
	reverse	CCCAACACTGATTCTACCC	

Table S2. Primers used for qPCR analysis of mouse mRNA. Related to Figures 1 to 6.

Mouse Genes		Sequences for qPCR	T fusion °C
ChREBP	forward	AATGGGATGGTGTCTACCGC	58
	reverse	GGCGAAGGGAATTCAGGACA	
ChREBP β	forward	TCTGCAGATCGCGTGGAG	58
	reverse	CT GTCCCGGCATAGCAAC	
L-pk	forward	CTTGCTCTACCGTGAGCCTC	58
	reverse	ACCACAATCACCAGATCACC	
Scd1	forward	CCGGAGACCCCTTAGATCGA	58
	reverse	TAGCCTGTAAAAGATTTCTGCAAA	
Fgf21	forward	TACAATGTGTACCAGTCTGAAG	58
	reverse	ACAGCCCTAGATTCAGGA	
Acox1	forward	CCGCCTATGCCTTCCACTTTC	60
	reverse	CAAGCCATCCGACATTCTTCG	
TBP	forward	CCCCACAACTCTTCCATTCT	58
	reverse	GCAGGAGTGATAGGGGTCAT	

Table S3. Primers used for qPCR analysis of human mRNA. Related to Figure S5.

Human Genes		Sequences for qPCR	T fusion °C
ChREBP	forward	CCA GCC TCA AGG TGA GCA AA	58
	reverse	CAC GCT CCT GCT GTA GCA	
ChREBP β	forward	AGCGGATTCCAGGTGAGG	58
	reverse	TTGTTCAGGCGGATCTTGTC	
L-pk	forward	TGGGCCTCATGCCTCTGACA	58
	reverse	TCCTGGGTCAGTTGGGCCAC	
Fgf21	forward	GGATTCGGACTGGTAAACAT	58
	reverse	GGGAGTCAAGACATCCAGGT	
Acox1	forward	GTGGGCTTGGAAAGACTTCA	58
	reverse	CCGATGTCACCAACGGTAAT	
18S	forward	CCATCCAATCGGTAGTAGCG	58
	reverse	GTAACCCGTTGAACCCCATT	