

## **Supplementary Information for**

### **Phosphorylation of RXR $\alpha$ mediates the effect of JNK to suppress hepatic FGF21 expression and promote metabolic syndrome**

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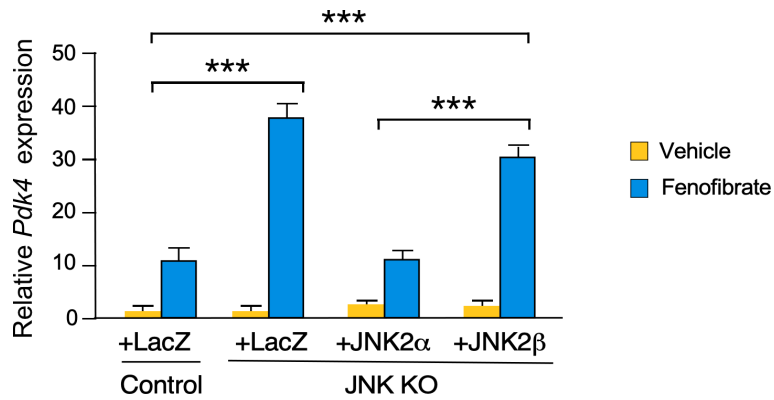
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#### **This PDF file includes:**

Figures S1 to S4  
Table S1  
SI References

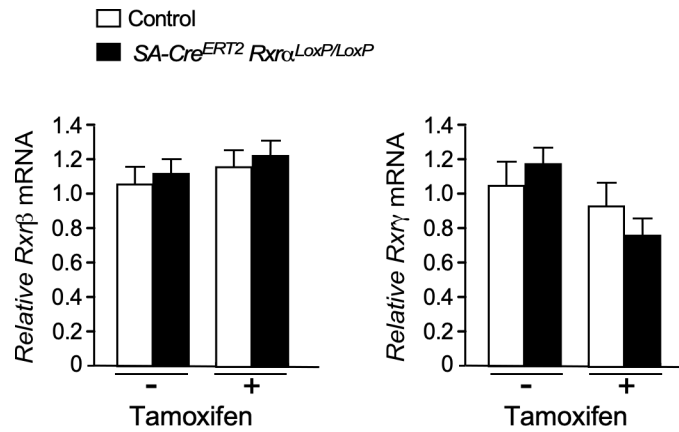
#### **Other supplementary materials for this manuscript include the following:**

Dataset S1



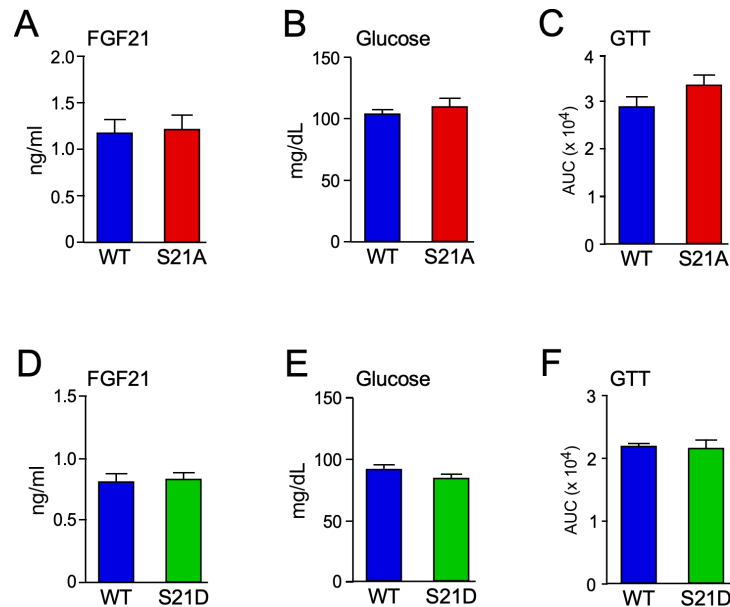
**Fig. S1. Effect of hepatic JNK2 $\alpha$  and JNK2 $\beta$  on the expression of the PPAR $\alpha$  target gene *Pdk4*.**

JNK knockout (JNK KO) mice with compound deficiency of JNK1 plus JNK2 in hepatocytes (*Alb-Cre<sup>+/-</sup> Mapk8<sup>loxP/loxP</sup> Mapk9<sup>loxP/loxP</sup>*) were transduced with AAV8 vectors expressing LacZ, JNK2 $\alpha$ , or JNK2 $\beta$ . Control (*Alb-Cre<sup>+/-</sup> Mapk8<sup>loxP/loxP</sup> Mapk9<sup>loxP/loxP</sup>*) mice were transduced with an AAV8 vector expressing LacZ. The mice were euthanized at age 24 wks and primary hepatocytes were prepared. The cells were cultured 24 h and then treated without or with 100  $\mu$ M fenofibrate (16 h). The expression of *Pdk4* mRNA was measured by RT-qPCR assays (mean  $\pm$  SEM; \*\*\*,  $p < 0.001$ ;  $n = 6$ ).



**Fig. S2. Effect of *Rxrα* gene ablation on the expression on hepatic *Rxrβ* and *Rxrγ* mRNA expression.**

Control SA-Cre<sup>ERT2</sup> mice and SA-Cre<sup>ERT2</sup> *Rxrα*<sup>loxP/loxP</sup> mice were treated without or with Tamoxifen at age 8 wks, fed a HFD starting at age 10 wks, and euthanized at age 18 wks. The expression of *Rxrβ*, and *Rxrγ* mRNA in the liver was measured by RT-qPCR assays (mean ± SEM; n = 5-9).



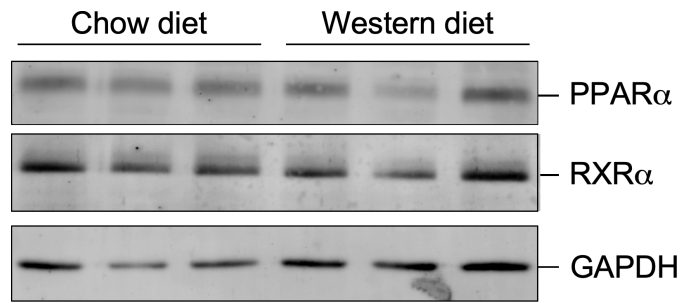
**Fig. S3. Hepatic hXRRA phosphorylation on Ser<sup>21</sup> causes no change in FGF21 signaling.**

(A,B) Control *SA-Cre<sup>ERT2</sup>* mice and *SA-Cre<sup>ERT2</sup> Rxra<sup>loxP/loxP</sup>* mice (age 7 wks) were transduced with AAV8 viruses expressing hXRRA or Ser21Ala-hXRRA. The mice were treated without or with Tamoxifen at age 8 wks, fed a HFD starting at age 10 wks, and euthanized at age 18 wks. Fasting blood FGF21 and glucose concentration in HFD-fed mice expressing hXRRA or Ser21Ala hXRRA in hepatocytes was measured (mean +/- SEM; n = 9 ~ 10).

(C) Glucose tolerance tests (GTT) on mice expressing hXRRA or Ser21Ala hXRRA in hepatocytes were performed (mean +/- SEM; n = 9 ~ 10).

(D,E) Fasting blood FGF21 and glucose concentration in CD-fed mice expressing hXRRA or Ser21Asp hXRRA in hepatocytes was measured (mean +/- SEM; \*, p<0.05; n = 11 ~ 12).

(F) GTT on mice expressing hXRRA or Ser21Asp hXRRA in hepatocytes were performed (mean +/- SEM; n = 11 ~ 12).



**Fig. S4. Effect of nutritional stress on the hepatic expression of PPAR $\alpha$  and RXR $\alpha$ .**

Wild-type mice (aged 10 wks.) were fed a chow diet (CD) or a Western Diet (WD) and euthanized at age 18 wks. Liver extracts prepared from 3 mice per group were examined by immunoblot analysis by probing with antibodies to PPAR $\alpha$ , RXR $\alpha$ , and GAPDH.

**Table S1. Source of Research Materials**

REAGENT or RESOURCE	SOURCE	IDENTIFIER
<b>Antibodies</b>		
Mouse monoclonal antibody to JUN	Cell Signaling Technology	Cat# 2315; RRID:AB_490780)
Rabbit polyclonal antibody to pSer <sup>63</sup> -JUN	Cell Signaling Technology	Cat# 9261; RRID:AB_213016292
Rabbit monoclonal antibody to RXR $\alpha$	Abcam	Cat# ab125001, RRID:AB_10975632
Rabbit polyclonal antibody to pSer <sup>22</sup> RXR $\alpha$	This study	N/A
Mouse polyclonal antibody to PPAR $\alpha$ (Immunofluorescence)	Millipore Sigma	Cat# SAB1406277, RRID:AB_10741281
Mouse monoclonal antibody to PPAR $\alpha$ (Immunoblot)	Millipore Sigma	Cat# MAB3890; RRID:AB_2165744
Mouse monoclonal antibody to JNK1/2	BD Biosciences	Cat# 554285, RRID:AB_395344
Rabbit polyclonal antibody to GAPDH	Santa Cruz Biotechnology	Cat# sc-25778, RRID:AB_10167668
Mouse monoclonal antibody to $\alpha$ Tubulin	Millipore Sigma	Cat# T5168, RRID:AB_477579
IRDye 680RD conjugated-goat anti-mouse IgG antibody	LI-COR Biosciences	Cat# 926-68070, RRID:AB_10956588
IRDye 800CW conjugated-goat anti-rabbit IgG antibody	LI-COR Biosciences	Cat# 926-32211, RRID:AB_621843
Alexa-Fluor 488 conjugated-goat anti-mouse IgG (H + L) antibody	ThermoFisher Scientific	Cat# A21052, RRID:AB_2535719
Alexa-Fluor 633 conjugated-goat anti-rabbit IgG (H + L) antibody	ThermoFisher Scientific	Cat# A-11034, RRID:AB_2576217
<b>Bacterial and Virus Strains</b>		
N/A		
Biological Samples		
N/A		
<b>Chemicals, Peptides, and Recombinant Proteins</b>		
Pyruvate	Millipore Sigma	Cat# P5280
Glucose	J.T. Baker	Cat#1 916-01
Fenofibrate	Millipore Sigma	Cat# 6020
Liver Perfusion Media	ThermoFisher Scientific	Cat# 17701038
Liver Digest Buffer	ThermoFisher Scientific	Cat# 17703034
Rat tail collagen I	ThermoFisher Scientific	Cat# A1048301
DMEM	ThermoFisher Scientific	Cat# 11960051
Bovine Growth Serum	ThermoFisher Scientific	Cat# SH3054103
L-Glutamine (100X)	ThermoFisher Scientific	Cat# 250300081
Penicillin/Streptomycin (100X)	ThermoFisher Scientific	Cat# 15140122

Lipofectamine 2000	Thermo Fisher Scientific	Cat# 11668500
DAPI	ThermoFisher Scientific	Cat#D3571, RRID:AB_2307445
O.C.T. compound	ThermoFisher Scientific	Cat# 23-730-571
Alexa-Fluor 546-conjugated phalloidin	ThermoFisher Scientific	Cat# A22283
Chow diet	Purina	Cat# Iso Pro 3000
High Fat Diet	Bio-Serv	Cat# S3282
Western Diet	Research Diets	Cat# D17063001Bi
<b>Critical Commercial Assays</b>		
RNAeasy kit	Qiagen	Cat# 74106
Taqman Fast Universal PCR Master Mix	ThermoFisher Scientific	Cat# 4352042
High Capacity cDNA Reverse Transcription Kit	ThermoFisher Scientific	Cat# 4368813
Taqman Probe for mouse <i>Pdk4</i>	ThermoFisher Scientific	Mm01166878_m1
Taqman Probe for human <i>Rxra</i>	ThermoFisher Scientific	Hs01067640_m1
Universal ProbeLibrary Probe #63	Roche	Cat# 04688619001
FGF21 ELISA	Millipore Sigma	Cat# EZRMFGF21-26K
<b>Deposited Data</b>		
Mass Spec Data	ProteomeXchange Consortium via the PRIDE partner repository	Accession Number: PXD034183. <a href="http://www.ebi.ac.uk/pride/archive/projects/PXD034183">http://www.ebi.ac.uk/pride/archive/projects/PXD034183</a>
<b>Experimental Models: Cell Lines</b>		
N/A		
<b>Experimental Models: Organisms/Strains</b>		
C57BL/6J mice	The Jackson Laboratory	RRID:IMSR_JAX:000664
B6.Cg-Speer6-ps1 <sup>Tg(alb-cre)21Mgn</sup> /J mice	The Jackson Laboratory	RRID:IMSR_JAX:003574
<i>Rxra</i> <sup>tm1Krc</sup> /J mice	The Jackson Laboratory	<i>Rxra</i> <sup>tm1Krc</sup> /J mice (RRID:IMSR_JAX:013086)
<i>Alb</i> <sup>tm1(cre/ERT2)Mtz</sup> mice	Metzger colony	(1)
<i>Mapk8</i> <sup>LoxP/LoxP</sup> mice	Davis colony	(2)
<i>Mapk9</i> <sup>LoxP/LoxP</sup> mice	Davis colony	(3).
<b>Oligonucleotides</b>		
Oligonucleotides	Eurofins Genomics LLC.	N/A
<b>Recombinant DNA</b>		
pAAV-CB6-PI	N/A	(4)
pAAV-CB6-PI-LacZ	N/A	Rashnonejad et al., 2016)

pAAV-CB6-PI-mJNK2 $\alpha$ 2	This study	N/A
pAAV-CB6-PI-mJNK2 $\beta$ 2	This study	N/A
pCV-Sport-hR $\alpha$	Addgene #8882	RRID:Addgene_8882
pAAV-CB6-PI-hR $\alpha$	This study	N/A
pAAV-CB6-PI-hR $\alpha$ (Ser260Ala)	This study	N/A
pAAV-CB6-PI-hR $\alpha$ (Ser260Asp)	This study	N/A
pAAV-CB6-PI-hR $\alpha$ (Ser21Ala)	This study	N/A
pAAV-CB6-PI-hR $\alpha$ (Ser21Asp)	This study	N/A
<b>Software and Algorithms</b>		
ImageJ2 (Version 2.3.0/1.53q, Build d544a3f481)	Open Source	<a href="https://imagej.nih.gov/ij">https://imagej.nih.gov/ij</a>
Photoshop (Version CC 2017)	Adobe	<a href="http://www.adobe.com/Photoshop">www.adobe.com/Photoshop</a>
Image Studio (Version 3.1)	LI-COR	<a href="https://www.licor.com/bio/products/software/image_studio_lite">https://www.licor.com/bio/products/software/image_studio_lite</a>
QuantStudio 12K Flex Software (version 1.3)	ThermoFisher Scientific	<a href="https://www.thermofisher.com/us/en/home/technical-resources/software-downloads/quantstudio-12k-software.html">https://www.thermofisher.com/us/en/home/technical-resources/software-downloads/quantstudio-12k-software.html</a>
Excel (Version 16.38)	Microsoft	Office 365
Canvas X DRAW 7 (Version 7.0.3)	Canvas GFX	<a href="https://www.canvasgfx.com/en/products/canvas-x/">https://www.canvasgfx.com/en/products/canvas-x/</a>
Complex heatmap package (version 1.10.2)	N/A	(5)
Leica Application Suite X (version 3.5.18963.2)	Leica	<a href="https://www.leica-microsystems.com/products/microscope-software/p/leica-las-x-ls/">https://www.leica-microsystems.com/products/microscope-software/p/leica-las-x-ls/</a>
GraphPad Prism 7 (Version 7.0)	GraphPad Software	<a href="https://www.graphpad.com/scientific-software/prism">https://www.graphpad.com/scientific-software/prism</a>



**Dataset S1 (separate file).** Quantitative Phosphoproteomic Analysis.

### **SI References**

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3. M. S. Han *et al.*, JNK expression by macrophages promotes obesity-induced insulin resistance and inflammation. *Science* **339**, 218-222 (2013).
4. A. Rashnonejad, G. A. Chermahini, S. Li, F. Ozkinay, G. Gao, Large-Scale Production of Adeno-Associated Viral Vector Serotype-9 Carrying the Human Survival Motor Neuron Gene. *Mol Biotechnol* **58**, 30-36 (2016).
5. Z. Gu, R. Eils, M. Schlesner, Complex heatmaps reveal patterns and correlations in multidimensional genomic data. *Bioinformatics* **32**, 2847-2849 (2016).