

Supplemental Material

GDF10 blocks hepatic PPAR γ activation to protect against diet-induced liver injury

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Table 1: List of antibodies used for IHC and immunoblotting.

Antibody	Manufacturer and Product Number	Application	Dilution
C/EBP	sc-7273, Santa Cruz Biotechnology	IHC	1:50, HIER
CD36	NB400-144, Novus Biologicals	IHC	1:100, no retrieval
GRP78	sc-1050, Santa Cruz Biotechnology	IHC	1:40, no retrieval
GRP94	ADI-SPA-850, Enzo Life Sciences	IHC	1:100, HIER
Perilipin	ab3526, Abcam	IHC	1:100, HIER
PPAR γ	sc-7273, Santa Cruz Biotechnology	IHC	1:50, HIER
pPERK	ab192591, Abcam	IHC	1:50, HIER
GAPDH	2118, Cell Signaling Technology	IB	1:1000
GDF10	16630, Novus Biologicals	IHC, IB	1:50, 1:1000
Histone H3	9715, Cell Signaling Technology	IB	1:1000
C/EBP α	sc-150, Santa Cruz Biotechnology	IF, IB	1:50, 1:200
PPAR γ	sc-7273, Santa Cruz Biotechnology	IF, IB	1:50, 1:200
Smad2/3	3102, Cell Signaling Technology	IB	1:500

Phospho-Smad2/3	8828, Cell Signaling Technology	IB	1:500
Smad1/5/9	12656, Cell Signaling Technology	IB	1:500
Phospho-Smad1/5/9	13820, Cell Signaling Technology	IB	1:500

IB, immunoblot; IHC, immunohistochemical staining; IF, immunofluorescence; HIER, heat-induced epitope retrieval

Table 2: List of primers used for quantitative real time PCR

Gene	Species	Forward	Reverse
ANGPTL4	Mouse	CATCCTGGGACGAGATGAACT	TGACAAGCGTTACCACAGGC
ACOX1	Mouse	GGGAGTGCTACGGGTACATG	CCGATATCCCCAACAGTGATG
ACOT2	Mouse	GTTGTGCCAACAGGATTGGAA	GCTCAGCGTCGCATTTGTC
Acadm	Mouse	AGGGTTTAGTTTTGAGTTGACGG	CCCCGCTTTTGTTCATATCCG
ATF4	Mouse	ATGGCCGGCTATGGATGAT	CGAAGTCAAACCTCTTTCAGATCCATT
BBC3	Mouse	TGTGGAGGAGGAGGAGTGG	TGCTGCTCTTCTTGCTCCG
CEBPa	Mouse	CAAGAACAGCAACGAGTACCG	GTCACTGGTCAACTCCAGCAC
CD36	Mouse	GATGACGTGGCAAAGAACAG	TCCTCGGGGTCTGAGTTAT
CHOP	Mouse	CTGCCTTTCACCTTGAGAC	CGTTTCTGGGGATGAGATA
Casp3	Mouse	CCTCAGAGAGACATTCATGG	GCAGTAGTCGCCTCTGAAGA
Casp7	Mouse	GGACCGAGGGCCCACTTATC	TCGCTTTGTCTGAAGTTCTTGTT
CRCT2	Mouse	ATGAACCCTAACCCCAAGAC	CGTTCTCCTCAATAGCAGGGA
FOXO1	Mouse	CCCAGGCCGGAGTTTAACC	GTTGCTCATAAAGTCGGTGCT
FGF21	Mouse	AGATCAGGGAGGATGGAACA	TCAAAGTGAGGCGATCCATA
FN1	Mouse	CGAGGTGACAGAGACCACCA	CTGGGAGTCAAGCCAGACACA
FSP27	Mouse	ATGGACTACGCCATGAAGTCT	CGGTGCTAACACGACAGGG
FATP5	Mouse	GAATCGGGAGGCAGAGAACT	AGCGGGTCATACAAGTGAGC
GRP78	Mouse	GTCCTGCATCATCAGCAAAG	GGTAGCCACATACTGAACACC
GRP94	Mouse	GATGGTCTGGCAACATGGAG	CGCCTTGGTGTCTGGTAGAA
GDF10	Mouse	AATGATCGACAAAAGCCTGT	CTTGCAGAATACCTCACGAGC

IRE1a	Mouse	TGAAACACCCCTTCTTCTGG	CCTCCTTTTCTATTCCGGTCACTT
Il1b	Mouse	GCACTACAGGCTCCGAGATGAAC	TTGTGCTTGCTTGGTTCTCCTTGT
LPL	Mouse	GGGCTCTGCCTGAGTTGTAG	AGAAATTTTGAAGGCCTGGT
PPARa	Mouse	GAAGGGCACACGCGTGCGAGTTTT CAG	GAAGGGCACACGCGTGCGAGTTTT CAG
Perilipin	Mouse	CTGTGTGCAATGCCTATGAG A	CTGGAGGGTATTGAAGAGCC G
PGC1b	Mouse	TCCTGTAAAAGCCCGGAGTA T	GCTCTGGTAGGGGCAGTGA
PDK4	Mouse	AGGGAGGTCGAGCTGTTCTC	GGAGTGTTCACTAAGCGGTCA
PARP1	Mouse	GGAAAGGGATCTACTTTGCCG	TCGGGTCTCCCTGAGATGTG
PCK1	Mouse	CTGCATAACGGTCTGGACTTC	CAGCAACTGCCGTACTCC
sXBP1	Mouse	GAGTCCGCAGCAGGTG	GTGTCAGAGTCCATGGGA
STAT3	Mouse	CACCTTGGATTGAGAGTCAAGAC	AGGAATCGGCTATATTGCTGGT
TNFa	Mouse	CATGAGCACAGAAAGCATGATCCG	AAGCAGGAATGAGAAGAGGCTGAG
TGF beta	Mouse	CAACAATTCCTGGCGTTACCTTGG	GAAAGCCCTGTATTCCGTCTCCTT

Figure legends

Fig. S1 GDF10 treatment reduces cytotoxicity in response to TG and TM. (A) ORO staining and isopropanol extract quantification of HepG2 cells treated with TG (100 nM), PA (200 μ M) and rhGDF10 (250 ng/mL) for 24 hours. (B) LDH release assay in the medium from Huh7 cells treated with TG (100 nM) and TM (2 μ g/mL). Scale bar, 50 μ m. All data are shown as the mean \pm S.D. *, $p < 0.05$ by unpaired two-tailed Student's *t*-test or one-way ANOVA.

Fig. S2 GDF10^{-/-} exhibit a reduction in plasma Leptin. Plasma content of circulating Leptin in wild type and GDF10^{-/-} mice fed NCD (n=5). All data are shown as the mean \pm S.D. *, $p < 0.05$ by unpaired two-tailed Student's *t*-test.

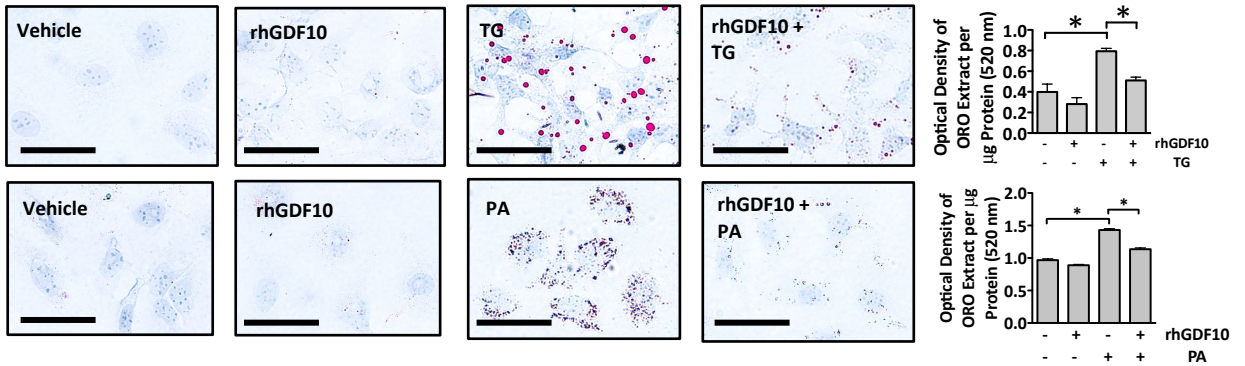
Fig. S3 GDF10 modulates PPAR γ and C/EBP α nuclear abundance. Immunofluorescent staining of PPAR γ and C/EBP α in (A) HepG2 cells and (B) primary human hepatocytes treated with rhGDF10 or CGTZ. Scale bars, 50 μ m.

Fig. S4 TGF β R1 inhibition reduces CGTZ-mediated lipid droplet accumulation in HepG2 cells. ORO staining in HepG2 cells treated with CGTZ (10 μ M), rhGDF10 (250 ng/mL) and IN1130 (100 nM). Scale bar, 50 μ m.

Fig. S5 GDF10^{-/-} mice exhibit increased hepatic fibrosis, apoptosis and gluconeogenesis. (A) PSR staining in the livers of mice fed NCD or HFD. Real time PCR analysis of hepatic mRNA abundance of indicated genes involved in **(B)** apoptosis and **(C)** gluconeogenesis (n=5). Scale bars, 200 μ m. All data are shown as the mean \pm S.D. *, $p < 0.05$ by one-way ANOVA.

Fig. S6. ER stress-inducing agent TM induces the expression of GDF10 in 3T3-L1 adipocytes. Real time PCR analysis of GDF10 mRNA transcript levels in 3T3-L1 adipocytes treated with TM (2 μ g/ml) for 24 hours. All data are shown as the mean \pm S.D. *, $p < 0.05$ by unpaired two-tailed Student's t -test.

A



B

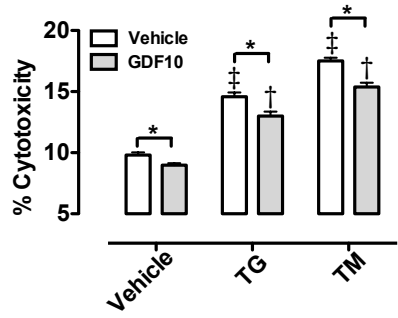


Figure S2.

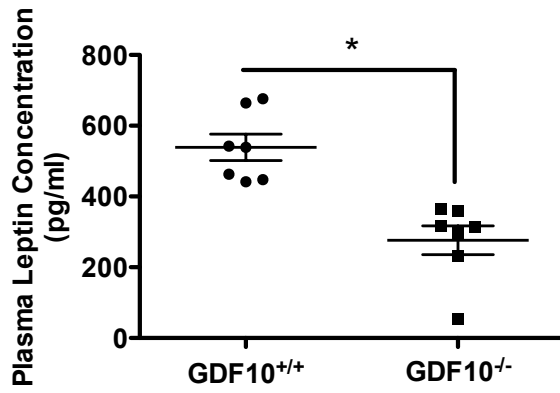


Figure S3.

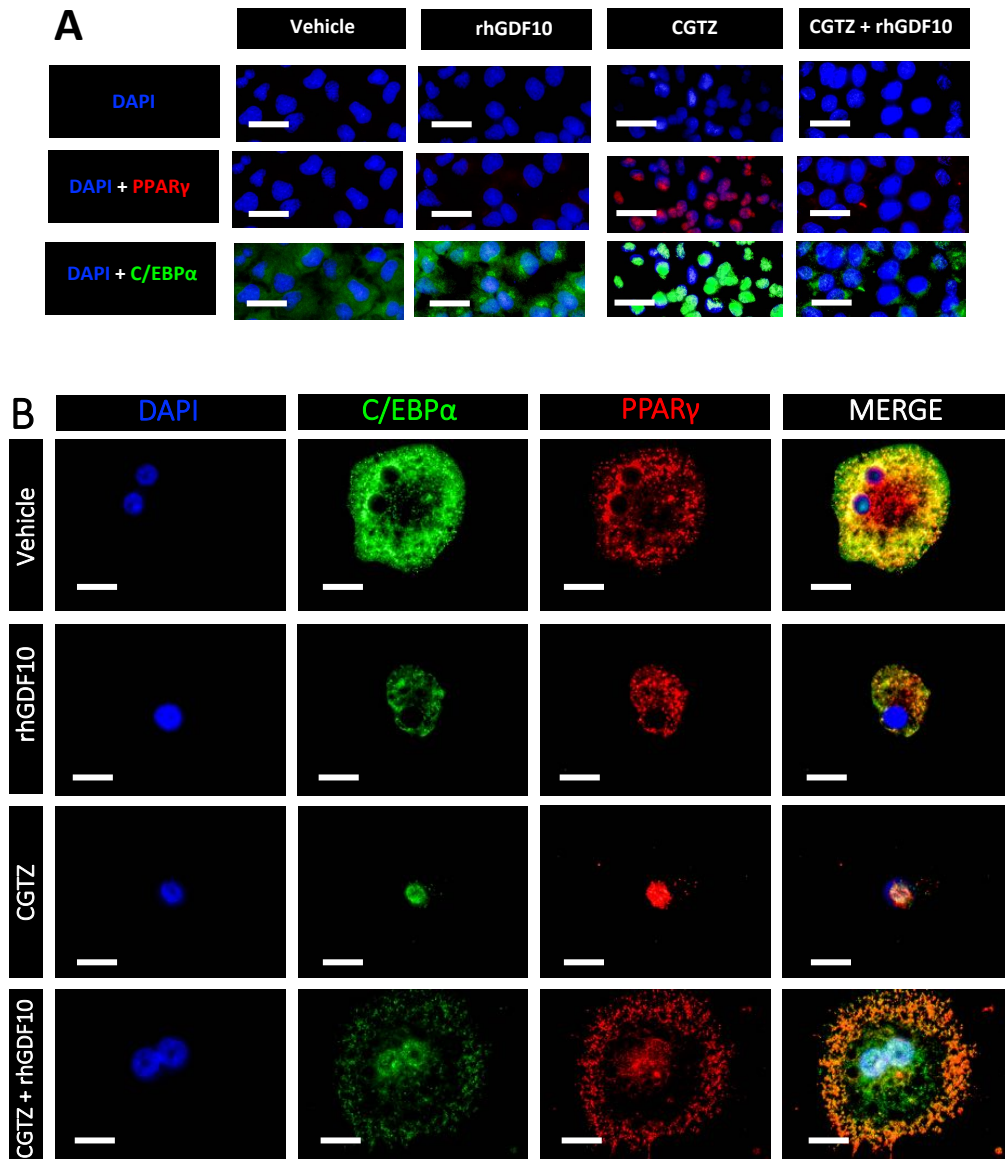


Figure S4.

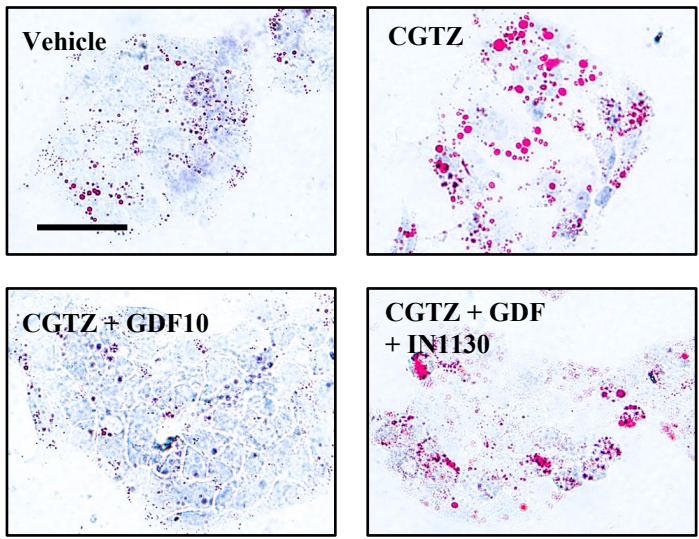


Figure S5.

GDF10^{+/+} NCD GDF10^{-/-} NCD GDF10^{+/+} HFD GDF10^{-/-} HFD

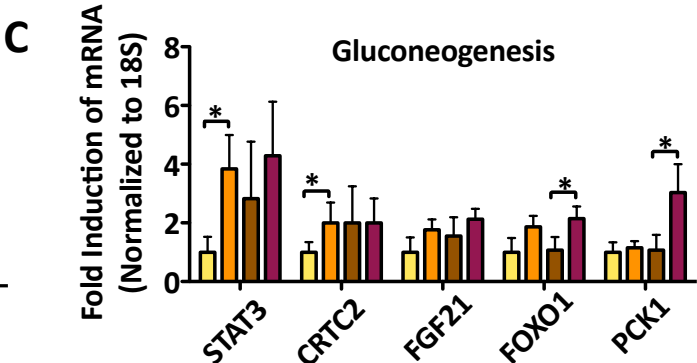
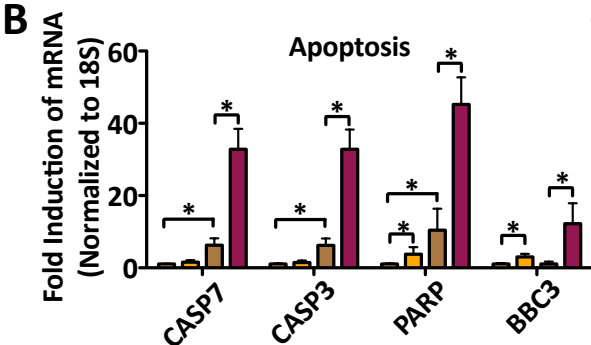
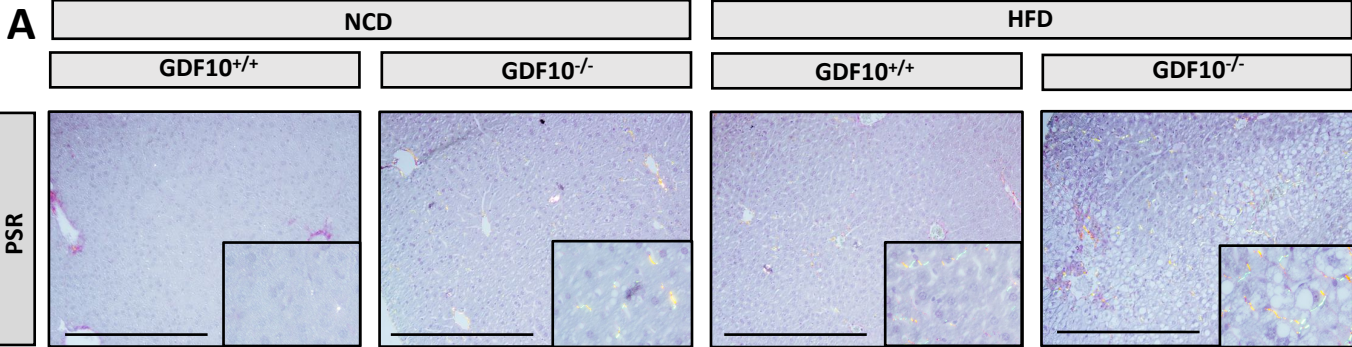


Figure S6.

A

