Supplemental Information

Reciprocal regulation of hepatic TGF-β1 and Foxo1 controls gluconeogenesis and energy expenditure

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Methods

Primary hepatocytes isolation and culturing

Primary mouse hepatocytes were isolated and cultured as previously described. Briefly, mice were infused with a calcium free HEPES-phosphate buffer I (Calcium-free HBSS containing 0.5 mM EGTA and 5.5 mM glucose, 1% Penicillin-Streptomycin (P/S), pH 7.4). After the color of the liver changed to a light brown color, collagenase containing buffer II (HBSS with 1.5 mM calcium, 0.5 mg/mL type II collagenase, 5.5 mM glucose, 1% P/S, pH7.4) was perfused into liver for digestion. After the appearance of cracking on the surface of liver, perfusion was stopped and the liver was excised into ice-cold serum-free DMEM medium. Cells from digested liver were teased out and suspended in serum-free DMEM medium, filtered through 70 μm cell strainer, and centrifuged at 1700 rpm for 2 min at 4° C. The pallet was washed with serum-free DMEM medium twice and mixed with Percoll (adjusted to physiological ionic strength with 10× PBS) to a final concentration of 36% and centrifuged at 1800 rpm for 6 min at 4° C. After removing the supernatant, hepatocyte pellet was washed once with serum-free DMEM medium and resuspended in DMEM medium supplemented with 10% fetal bovine serum (FBS) and 1% Penicillin-Streptomycin (P/S) for 3 h; after cell attachment, hepatocytes were cultured in serum-free DMEM medium overnight and then subjected to treatment for further analysis.

Adenovirus injection

Adenovirus expressing GFP (Ad-GFP) and TGF-β1 (Ad-TGF-β1) were generated with help of Vector Builder Inc. (Chicago, IL, USA) and delivered into the mouse liver by intravenous injection (i.v.) with a dose of 1×10^9 pfu in 200 ul saline (0.9% NaCl).

Nuclear and cytoplasmic protein extraction

Primary hepatocytes were washed twice with cold PBS and suspended in cold PBS. Nuclear and cytoplasmic proteins from primary hepatocytes were extracted with NE-PER nuclear and cytoplasmic extraction reagent (Thermo scientific) according to manufacturer's instructions. The cytoplasmic and nuclear extracts were all stored at -80 ℃ until use.

cAMP Assay

Mouse primary hepatocytes were isolated from WT mice and treated with 5 ng/ml TGF-β1 or different dose of TGF-β1, with or without 100 nM glucagon treatment for indicated time. Cellular cAMP was measured using cAMP ELISA kit (Cayman Chemicals).

Immunoprecipitation

Primary hepatocytes were isolated from WT mice and cultured in the 10 cm dish with DMEM medium supplemented with 10% FBS. Hepatocytes were collected by adding 1 ml TNE buffer (Sigma) and centrifuging for 15 min at 12,000 rpm at 4 ℃. The cell lysates were incubated with PKA-Ca antibody (Cell Signaling Technology) at room temperature for 30 min. The immune complexes were then precipitated by the IgG-coated magnetic beads (Invitrogen) at room temperature for 10 min. The complexes were then denatured by boiling at 95° C for 5 min in SDS sample buffer. The samples were then subjected to Western blot analysis.

Blood chemistry analysis

Insulin Elisa kit (Alpco) were used to measure serum insulin levels following the manufacturer's instruction. For serum FFA analysis, mice were fasted 16 h overnight, blood samples were collected for the measurement of overnight fasted FFA levels, then mice were injected intraperitoneally (i.p.) with insulin (1 u/kg body weight), blood samples were collected 30 and 60 min after insulin injection for the measurement of FFA levels. FFA Elisa kit (Cayman Chemical) were used to measure serum FFA levels, the change of serum FFA levels were presented in term of percentage of the overnight-fasted baseline.

Gene overexpression or knockdown in primary hepatocytes

For gene overexpression, primary hepatocytes were cultured in serum-free DMEM medium and infected with adenovirus (20 MOI) for 16 h, and then subjected to further analysis. For gen knockdown, primary hepatocytes were cultured in Opi-MEM medium for 6 h, and then subjected to Lipofectamine® 3000 (Life technologies) with siRNA according to manufacturer's instruction for 16 h, and then subjected to further analysis.

Glucose production assay

HGP assay was performed as previously described. Briefly, the primary mouse hepatocytes were isolated from 8-12-week-old mice and freshly isolated hepatocytes were resuspended in DMEM with 2% FBS for 3 h, then rinsed with PBS, and cultured in HGP buffer (118 mM NaCl, 2.5 mM CaCl2, 4.8 mM KCl, 25 mM NaHCO3, 1.1 mM KH2PO4, 1.2 mM MgSO4, 10 μM ZnSO4, 0.6% BSA, 10 mM HEPES, 10 mM sodium L-lactate, and 5 mM pyruvate, pH 7.4). Cell culture medium was collected at 3 h, and glucose in the medium measured according to the manufacturer's protocol, using Amplex® Red Glucose Assay (Invitrogen). For glycogenolysis assay, sodium L-lactate and pyruvate were removed from HGP buffer, and glucose released into the medium were measured after treatments. The difference between HGP and glycogenolysis was assumed to reflect the gluconeogenesis level.

Supplemental material

KEY RESOURCES TABLE

REAGENT OR RESOURCE	SOURCE	IDENTIFIER
Antibodies		
Anti-Phospho-CREB Ser133	*Cell signaling technology*	Cat# 9198 (1:1000 dilution); RRID: AB_ 2561044
Anti-CREB	*Cell signaling technology*	Cat# 9197 (1:1000 dilution); RRID: AB_331277
Anti-Foxo1	*Cell signaling technology*	Cat# 2880 (1:1000 dilution); RRID: AB_2106495
Anti-GAPDH	*Cell signaling technology*	Cat# 2118s (1:1000 dilution); RRID: AB_ 561053
Anti-Phospho-Foxo1 Ser273	*Covance*	Cat# N/A (customized antibody) (1:500 dilution)
Anti-Phospho-Smad3 Ser423/425	*Cell signaling technology*	Cat# 9520 (1:1000 dilution); RRID: AB_2193207
Anti-Smad3	*Cell signaling technology*	Cat# 9523 (1:1000 dilution); RRID: AB_2193182
Anti-Phospho-AKT Ser473	*Cell signaling technology*	Cat# 4060 (1:1000 dilution); RRID: AB_2315049
Anti-AKT	*Cell signaling technology*	Cat# 9272 (1:1000 dilution); RRID: AB_329827
Anti-TGF-β	*Cell signaling technology*	Cat# 3711 (1:1000 dilution); RRID: AB_2063354
Anti-rabbit IgG, HRP-linked Antibody	*Cell signaling technology*	Cat# 7074 (1:5000 dilution); RRID: AB_2099233
TGF-beta1 Monoclonal Antibody (9016)	ThermoFisher Scientific	Cat# MAB5-23702 (0.5 µg/ml)
Mouse IgG1 Isotype Control	ThermoFisher Scientific	Cat# 02-6100 (0.5 µg/ml); RRID AB_2532935
Chemicals, Peptides, and Recombinant Proteins		
Collagenase II	ThermoFisher Scientific	Cat# 17101015
Percoll	GE Healthcare Life Sciences	Cat# 17-0891-01
Lipofectamine™ 3000 transfection reagent	ThermoFisher Scientific	Cat# L3000001
Opi-MEM	ThermoFisher Scientific	Cat# 51985034
TRIzol RNA isolation reagent	ThermoFisher Scientific	Cat# 15596026
iScript™ Reverse Transcription Supermix	Bio-rad	Cat# 1708840
Ssoadvanced Universal SYBR® Green Supermix	Bio-rad	Cat# 1725274
RIPA buffer (10×)	Cell signaling technology	Cat# 9806
NE-PER™ Nuclear and Cytoplasmic Extraction Reagents	ThermoFisher Scientific	Cat# 78833
Mouse TGF-β1	Cell signaling technology	Cat# 5231LC
Sodium L-lactate	Sigma-Aldrich	Cat# L7022
BSA	Sigma-Aldrich	Cat# A3294
H89	TOCRIS	Cat# 2910
LY2157299	AChemBlock	*Cat# 10348*
DMSO	*Sigma-Aldrich*	*Cat#* D8418
High fat diet (42%)	Envigo	Cat# TD.88137
Low fat control diet (13%)	Envigo	Cat# TD.08485
Dextrose	Sigma-Aldrich	Cat# G8270-1KG
Human insulin used in ITTs	Novo-Nordisk	Cat# NDC 0169-1833-11
Glucagon	Sigma-Aldrich	Cat# G2044
Sodium pyruvate	*Sigma-Aldrich*	Cat# P5280
MitoTracker™ Green FM	ThermoFisher Scientific	Cat# M7514
TruSeq Stranded mRNA Library Prep Kit	*Illumina*	Cat# 20020594
Protein A Agarose	Thermo fisher	Cat# AM20333

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REAGENT OR RESOURCE	SOURCE	IDENTIFIER
Critical Commercial Assays		
Amplex™ Red Glucose/Glucose Oxidase assay Kit	ThermoFisher Scientific	Cat# A22189
Insulin ELISA Kit	ALPCO	Cat# 80-INSHU-E01.1
Triglyceride Assay Kit- Quantification	Abcam	Cat# ab65336
Free Fatty Acid Fluorometric Assay Kit	Cayman Chemical	Cat# 700310
LEGEND MAX™ Free Active TGF-β1 ELISA Kit	BioLegend	Cat# 437707
Dual-Glo Luciferase Assay System	Promega	Cat# E2920
Oligonucleotides		
Scramble siRNA	ThermoFisher Scientific	Cat# AM4636
PKAc siRNA	ThermoFisher Scientific	Cat# AM16708 (63544)
Cyclophilin forward	*Integrated DNA Technologies*	ACTGAATGGCTGGATGGCAAG
Cyclophilin reverse	*Integrated DNA Technologies*	TGCCCGCAAGTCAAAAGAAAT
G6pc forward	*Integrated DNA Technologies*	CATTGTGGCTTCCTTGGTCC
G6pc reverse	*Integrated DNA Technologies*	GGCAGTATGGGATAAGACTG
Pck1 forward	*Integrated DNA Technologies*	CCATCGGCTACATCCCTAAG
Pck1 reverse	*Integrated DNA Technologies*	GACCTGGTCCTCCAGATA
Gck forward	*Integrated DNA Technologies*	CAACTGGACCAAGGGCTTCAA
GCK reverse	*Integrated DNA Technologies*	TGTGGCCACCGTGTCATTC
Foxo1 forward	*Integrated DNA Technologies*	AGATGAGTGCCCTGGGCAGC
Foxo1 reverse	*Integrated DNA Technologies*	GATGGACTCCATGTCACAGT
Tgfb1 forward	*Integrated DNA Technologies*	ATCCTGTCCAAACTAAGGCTCG
Tgfb1 reverse	*Integrated DNA Technologies*	ACCTCTTTAGCATAGTAGTCCGC
Irs1 forward	*Integrated DNA Technologies*	CCCGTTCGGTGCCAAATAGC
Irs1 reverse	*Integrated DNA Technologies*	GCCACTGGTGAGGTATCCACATAGC
Irs2 forward	*Integrated DNA Technologies*	ACTTCCCAGGGTCCCACTGCTG
Irs2 reverse	*Integrated DNA Technologies*	GGCTTTGGAGGTGCCACGATAG
Fasn forward	*Integrated DNA Technologies*	ATGGCGAGGACTTGGGTGCT
Fasn reverse	*Integrated DNA Technologies*	GGAGCTATGGATGATGTTGA
Scd1 forward	*Integrated DNA Technologies*	CTGTACGGGATCATACTGGTTC
Scd1 reverse	*Integrated DNA Technologies*	GCCGTGCCTTGTAAGTTCTG
Srebp1c forward	*Integrated DNA Technologies*	GGAGCCATGGATTGCACATT
Srebp1c reverse	*Integrated DNA Technologies*	GGCCCGGGAAGTCACTGT
Acc1 forward	*Integrated DNA Technologies*	CCTCCGTCAGCTCAGATACA
Acc1 reverse	*Integrated DNA Technologies*	TTTACTAGGTGCAAGCCAGACA
Cpt1 forward	*Integrated DNA Technologies*	GCTGGAGGTGGCTTTGGT
Cpt1 reverse	*Integrated DNA Technologies*	GCTTGGCGGATGTGGTTC
Acox1 forward	*Integrated DNA Technologies*	GCCAAGGCGACCTGAGTGAGC
Acox1 reverse	*Integrated DNA Technologies*	ACCGCAAGCCATCCGACATTC
Ucp1 forward	*Integrated DNA Technologies*	ACTGCCACACCTCCAGTCATT
Ucp1 reverse	*Integrated DNA Technologies*	CTTTGCCTCACTCAGGATTGG
Pgc1a forward	*Integrated DNA Technologies*	TGTGGAACTCTCTGGAACTGC
Pgc1a reverse	*Integrated DNA Technologies*	GCCTTGAAAGGGTTATCTTGG
Fgf21 forward	*Integrated DNA Technologies*	AGATGGAGCTCTCTATGGATCG
Fgf21 reverse	*Integrated DNA Technologies*	GGGCTTCAGACTGGTACACAT

Continued		
REAGENT OR RESOURCE	SOURCE	IDENTIFIER
Oligonucleotides		
Cpt1 forward	*Integrated DNA Technologies*	GCTGGAGGTGGCTTTGGT
Cpt1 reverse	*Integrated DNA Technologies*	GCTTGGCGGATGTGGTTC
Acox1 forward	*Integrated DNA Technologies*	GCCAAGGCGACCTGAGTGAGC
Acox1 reverse	*Integrated DNA Technologies*	ACCGCAAGCCATCCGACATTC
Ucp1 forward	*Integrated DNA Technologies*	ACTGCCACACCTCCAGTCATT
Ucp1 reverse	*Integrated DNA Technologies*	CTTTGCCTCACTCAGGATTGG
Pgc1a forward	*Integrated DNA Technologies*	TGTGGAACTCTCTGGAACTGC
Pgc1a reverse	*Integrated DNA Technologies*	GCCTTGAAAGGGTTATCTTGG
Fgf21 forward	*Integrated DNA Technologies*	AGATGGAGCTCTCTATGGATCG
Fgf21 reverse	*Integrated DNA Technologies*	GGGCTTCAGACTGGTACACAT
Prdm16 forward	*Integrated DNA Technologies*	TGACCATACCCGGAGGCATATGC
Prdm16 reverse	*Integrated DNA Technologies*	TGGGGTTAAAGGCTCCGGACTC
Tfam forward	*Integrated DNA Technologies*	CAAAGGATGATTCGGCTCAG
Tfam reverse	*Integrated DNA Technologies*	AAGCTGAATATATGCCTGCTTTTC
Bmp8 forward	*Integrated DNA Technologies*	ATGTGGAAACCGAGGATGG
Bmp8 reverse	*Integrated DNA Technologies*	CCTGAAGAAACCAACCATGAA
Acta2 forward	*Integrated DNA Technologies*	ATGAAGCCCAGAGCAAGAGA
Acta2 reverse	*Integrated DNA Technologies*	ATGTCGTCCAGTTGGTGAT
Col1a forward	*Integrated DNA Technologies*	GCGAGTGCTGTGCTTTCTG
Col1a reverse	*Integrated DNA Technologies*	GGTCCCTCGACTCCTACATCT
Col3a forward	*Integrated DNA Technologies*	GTTCTAGAGGATGGCTGTACTAAACACA
Col3a reverse	*Integrated DNA Technologies*	TTGCCTTGCGTGTTTGATATTC
Elastin forward	*Integrated DNA Technologies*	TGGTGACATGATCCCTCTCTCTT
Elastin reverse	*Integrated DNA Technologies*	CCAGGGTGTCCCAGATGTG
Timp1 forward	*Integrated DNA Technologies*	GGCATCCTCTTGTTGCTATCACTG
Timp1 reverse	*Integrated DNA Technologies*	GTCATCTTGATCTCATAACGCTGG
Mcp1 forward	*Integrated DNA Technologies*	CAGGTGTCCCAAAGAAGCTGTAG
Mcp1 reverse	*Integrated DNA Technologies*	GGGTCAGCACAGACCTCTCTCT
Tnfα forward	*Integrated DNA Technologies*	GAGAAAGTCAACCTCCTCTCTG
Tnfα reverse	*Integrated DNA Technologies*	GAAGACTCCTCCCAGGTATATG
Il1b forward	*Integrated DNA Technologies*	TGTTCTTTGAAGTTGACGGACCC
Il1b reverse	*Integrated DNA Technologies*	TCATCTCGGAGCCTGTAGTGC
Trl4 forward	*Integrated DNA Technologies*	CGAGGCTTTTCCATCCAATA
Trl4 reverse	*Integrated DNA Technologies*	AGGCAGCAGGTGGAATTGTAT
Virus Strains		
Adenovirus-GFP	*Vector Builder*	N/A
Adenovirus-shTGF-β1	*Vector Builder*	N/A
Adenovirus-TGF-β1	*Vector Builder*	N/A
Adenovirus-Foxo1	*Vector Builder*	N/A
Experimental Models: Cell Lines		
HepG2 cell line	*ATCC*	HB-8065™
Experimental Models: Organisms/Strains		
Mouse: TGF- β1 L/L (also as Tgfb1$^{flox\ ex6}$)	*Jackson Laboratories*	JAX: 033001

Mouse: TGFβ1-tg (also as $\beta1^{glo}$)	*Jackson Laboratories*	JAX: 018393
Mouse: TβRII L/L (also as $Tgfbr2^{tm1Karl}$)	*Jackson Laboratories*	JAX: 012603
Mouse: Albumin-Cre	*Jackson Laboratories*	JAX: 003574
Mouse: WT C57BL6/J	*Jackson Laboratories*	JAX: 000664
Mouse: db/db (also as BSK db)	*Jackson Laboratories*	JAX: 000642
Software and Algorithms		
GraphPad Prism version 6 for windows	GraphPad software	http://graphpad.com
ImageJ 1.51K	National Institutes of Health	http://imagej.nih.gov/ij
Others		
CONTOUR® NEXT ONE blood glucose meter	*Ascensia Diabetes Care*	N/A
CONTOUR NEXT test strips, 100ct	*Ascensia Diabetes Care*	Cat# 7312
TH-8 Thermalert Clinical Monitoring Thermometer	*Physitemp Instruments*	N/A
TSE PhenoMaster	*TSE Systems*	N/A
EchoMRI™-100H	*Echo Medical Systems*	N/A

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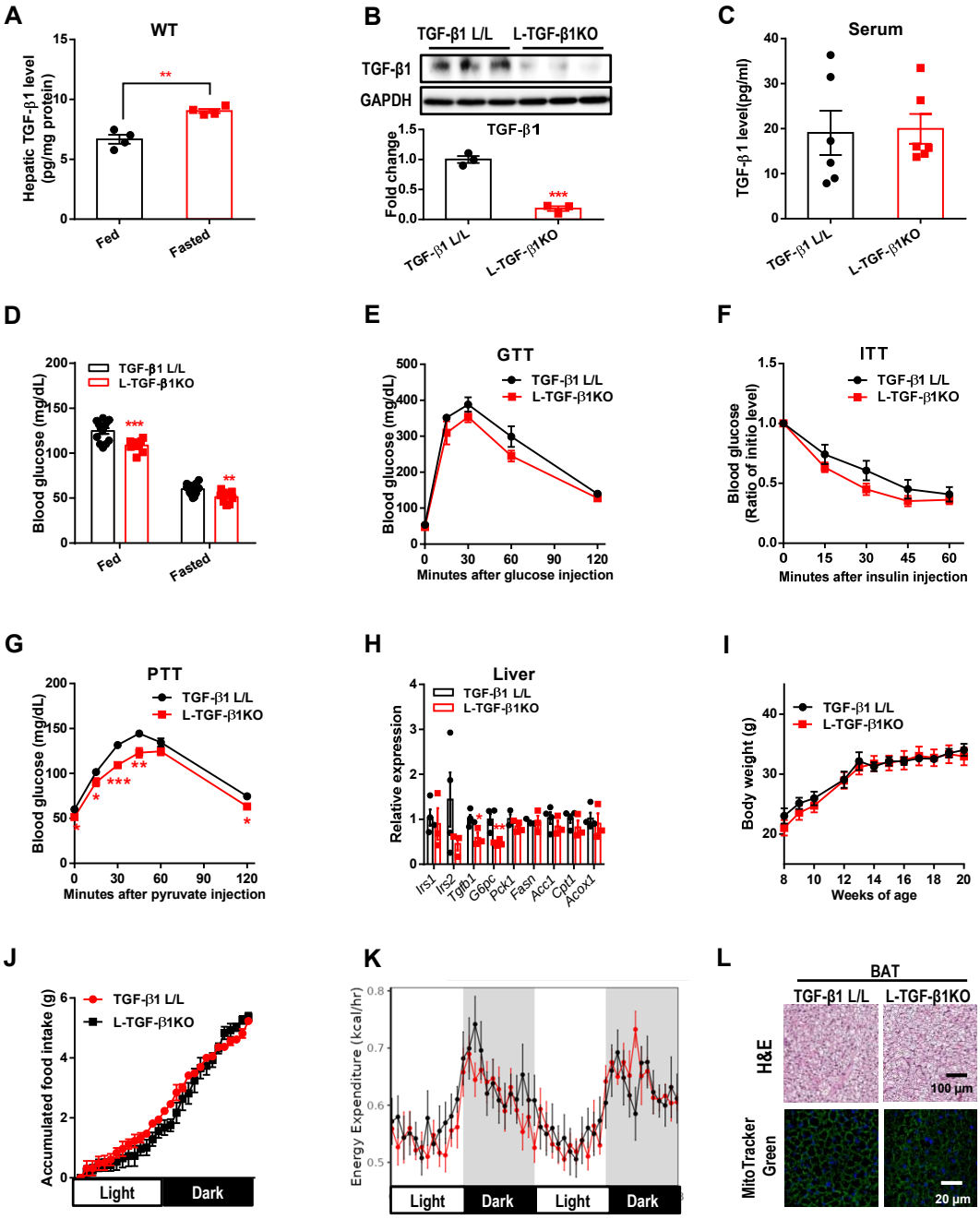
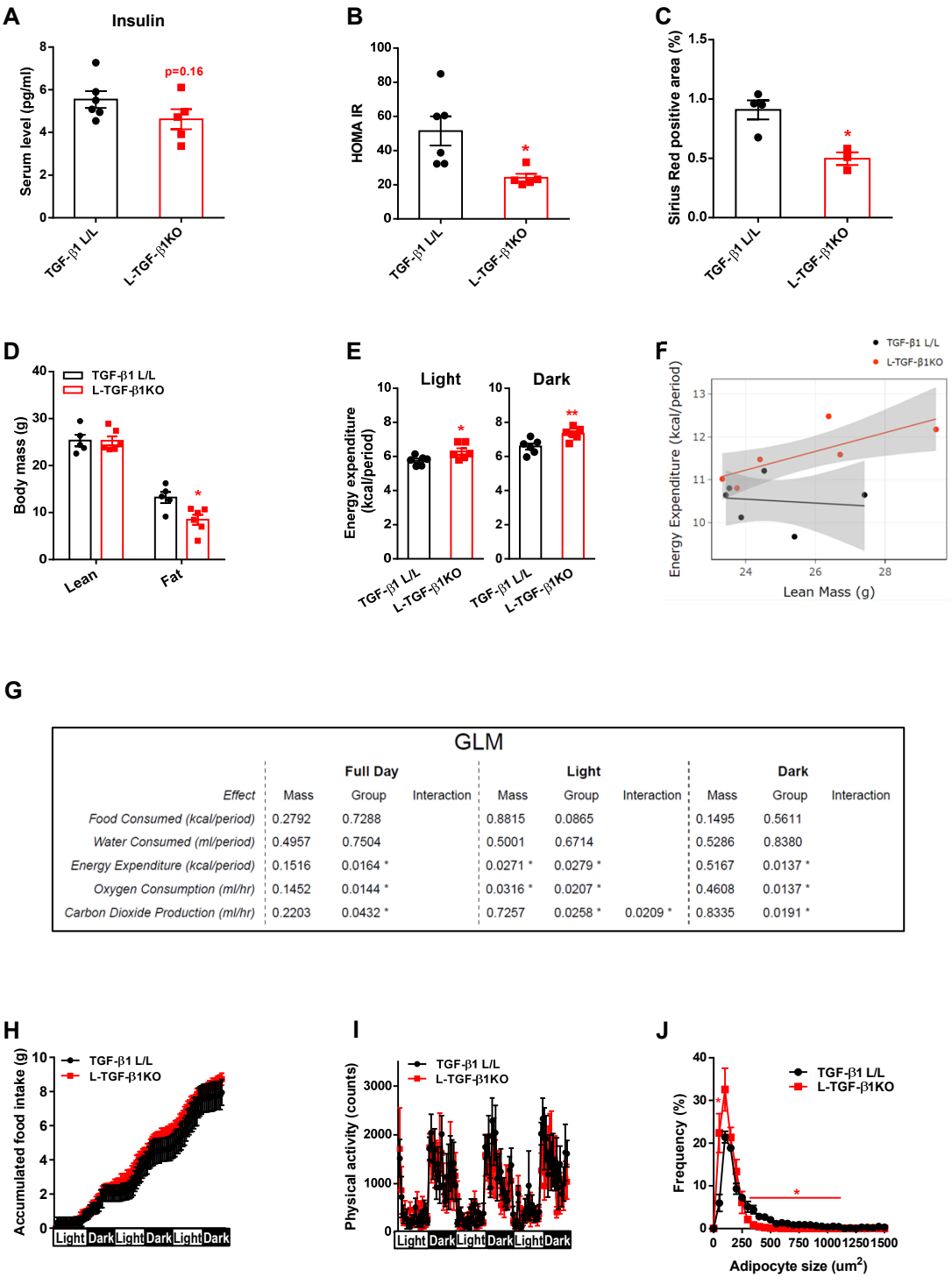


Figure S1. Hepatic TGF-β1 deficiency lowers blood glucose and hepatic gluconeogenesis in chow diet fed mice. **(A)** Hepatic TGF-β1 levels in WT mice under fed and 16 h fasted conditions. **(B-L)** TGF-β1 L/L and L-TGF-β1KO mice at the age of 8-10 weeks were fed with normal chow diet. **(B)** Western blots analysis of TGF-β1 and GAPDH protein levels in the primary hepatocytes from these mice. **(C)** Serum TGF-β1 levels of these mice (n=6). **(D)** Blood glucose levels of these mice under fed condition and 16 h fasted condition (n=9-14). **(E)** GTT, **(F)** ITT, and **(G)** PTT in these mice (n=6). **(H)** mRNA expression of *Irs1*, *Irs2*, *Tgfb1*, *G6pc*, *Pck1*, *Fasn*, *Acc1*, *Cpt1* and *Acox1* in the liver of these mice under 16 h fasted condition (n=4). **(I)** Body weight of these mice at the indicated weeks of age. **(J)** Accumulated food intake and **(K)** energy expenditure in these mice during light and dark phases (n=4). **(L)** H&E staining and MitoTracker Green staining of the BAT of these mice. Data are presented as the means ± SEM. * p< 0.05, ** p< 0.01, *** p< 0.001 vs TGF-β1 L/L or between assigned groups using two-way ANOVA or t-test.

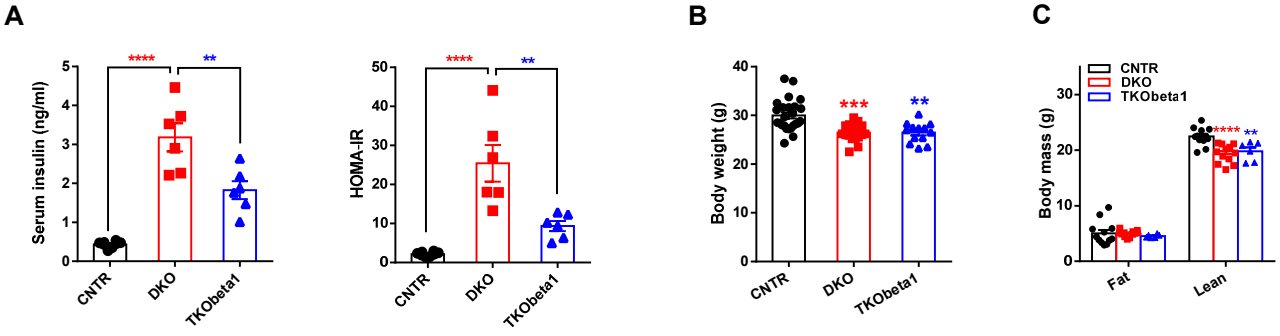


G

GLM

	Full Day			Light			Dark		
Effect	Mass	Group	Interaction	Mass	Group	Interaction	Mass	Group	Interaction
Food Consumed (kcal/period)	0.2792	0.7288		0.8815	0.0865		0.1495	0.5611	
Water Consumed (ml/period)	0.4957	0.7504		0.5001	0.6714		0.5286	0.8380	
Energy Expenditure (kcal/period)	0.1516	0.0164 *		0.0271 *	0.0279 *		0.5167	0.0137 *	
Oxygen Consumption (ml/hr)	0.1452	0.0144 *		0.0316 *	0.0207 *		0.4608	0.0137 *	
Carbon Dioxide Production (ml/hr)	0.2203	0.0432 *		0.7257	0.0258 *	0.0209 *	0.8335	0.0191 *	

Figure S2. Characteristics of TGF-β1 L/L and L-TGF-β1KO mice fed with HFD, related to Figure 2. TGF-β1 L/L and L-TGF-β1KO mice at the age of 3-month were fed with HFD for 3 months. **(A)** Serum insulin levels of these mice under overnight fasting conditions. **(B)** HOMA IR of these mice. **(C)** Sirius Red positive area in the liver of these mice. **(D)** Body composition. **(E)** Averaged energy expenditure during light and dark phases. **(F)** The plot of energy expenditure vs lean body mass. **(G)** ANCOVA analysis table of the regression plot of energy expenditure vs lean body mass. **(H and I)** Accumulated food intake **(H)** and physical activity **(I)** of these mice. **(J)** The distribution of the size of adipocytes in BAT of these mice. Data are presented as the means ± SEM. * p< 0.01, ** p< 0.01 vs TGF-β1 L/L or between assigned groups using one-way ANOVA or t-test.



D

GLM (DKO vs CNTR)

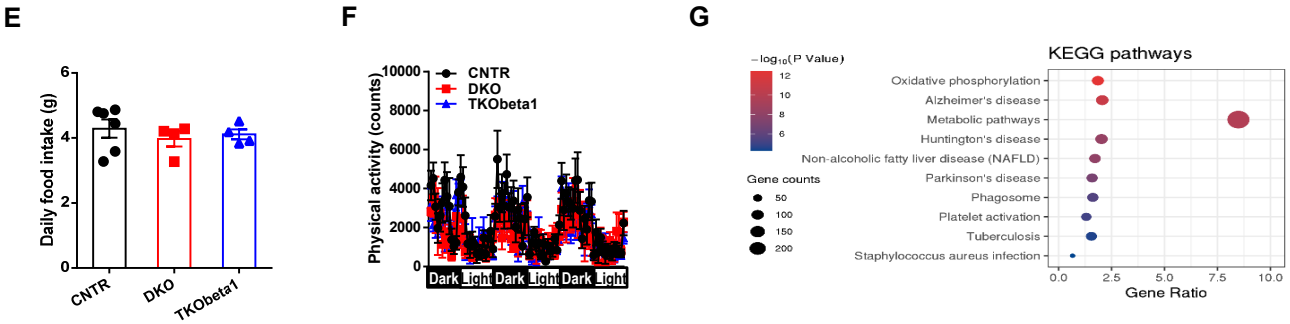
	Full Day			Light			Dark		
Effect	Mass	Group	Interaction	Mass	Group	Interaction	Mass	Group	Interaction
Food Consumed (kcal/hr)	0.0442 *	0.3162		0.2206	0.6758		0.2277	0.4949	
Water Consumed (ml/hr)	0.6870	0.2744		0.3891	0.7197		0.7501	0.0704	
Energy Expenditure (kcal/hr)	0.0155 *	0.0116 *		0.0029 **	0.0077 **		0.0542	0.0196 *	
Oxygen Consumption (ml/hr)	0.0171 *	0.0115 *		0.0040 **	0.0085 **		0.0524	0.0179 *	
Carbon Dioxide Production (ml/hr)	0.0116 *	0.0141 *		<0.001 ***	0.0073 **		0.0652	0.0295 *	

GLM (TKObeta1 vs CNTR)

	Full Day			Light			Dark		
Effect	Mass	Group	Interaction	Mass	Group	Interaction	Mass	Group	Interaction
Food Consumed (kcal/hr)	0.0442 *	0.8897		0.2206	0.8509		0.2277	0.9997	
Water Consumed (ml/hr)	0.6870	0.8225		0.3891	0.7677		0.7501	0.3790	
Energy Expenditure (kcal/hr)	0.0155 *	0.8800		0.0029 **	0.8633		0.0542	0.7441	
Oxygen Consumption (ml/hr)	0.0171 *	0.8483		0.0040 **	0.9132		0.0524	0.7182	
Carbon Dioxide Production (ml/hr)	0.0116 *	0.9811		<0.001 ***	0.6538		0.0652	0.8545	

ANOVA

	Full Day		Light		Dark	
Effect	DKO vs CNTR	TKObeta1 vs CNTR	DKO vs CNTR	TKObeta1 vs CNTR	DKO vs CNTR	TKObeta1 vs CNTR
Respiratory Exchange Ratio	0.0684	0.9044	0.0744	0.9000	0.1828	0.7106
Locomotor Activity (beam breaks/hr)	0.0274 *	0.1042	0.0350 *	0.1659	0.0356 *	0.1094
Ambulatory Activity (beam breaks/hr)	0.0106 *	0.0316 *	0.0166 *	0.0486 *	0.0155 *	0.0414 *



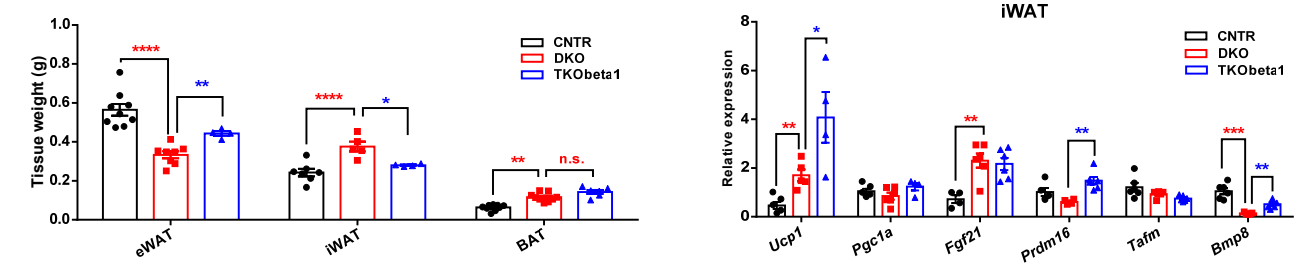


Figure S3. Characteristics of CNTR, DKO, TKObeta1 mice, related Figure 3. **(A)** Serum insulin levels of these mice under overnight fasting conditions and HOMA IR of these mice. **(B and C)** Body weight **(B)** and body compositions **(C)** of CNTR, DKO and TKObeta1 mice (n=13-25). **(D)** The ANCOVA analysis of the regression plots of energy expenditure vs lean body mass. **(E and F)** Averaged daily food intake **(E)** and physical activity **(F)** of CNTR, DKO and TKObeta1 mice during light and dark phases (n=4-6). **(G)** KEGG pathways enrichment analysis of DEGs in the BAT of CNTR and DKO mice. **(H)** Adipose tissue (eWAT, iWAT, and BAT) weight of WT, DKO and TKObeta1 mice (n=6-8). **(I)** mRNA expression of Ucp1, Pgc1a, Fgf21, Prdm16, Tfam, and Bmp8 in the BAT of WT, DKO and TKObeta1 mice (n=4-6). * p< 0.05, ** p< 0.01, *** p< 0.001, **** p< 0.0001 vs CNTR or between assigned groups ANOVA or t-test.

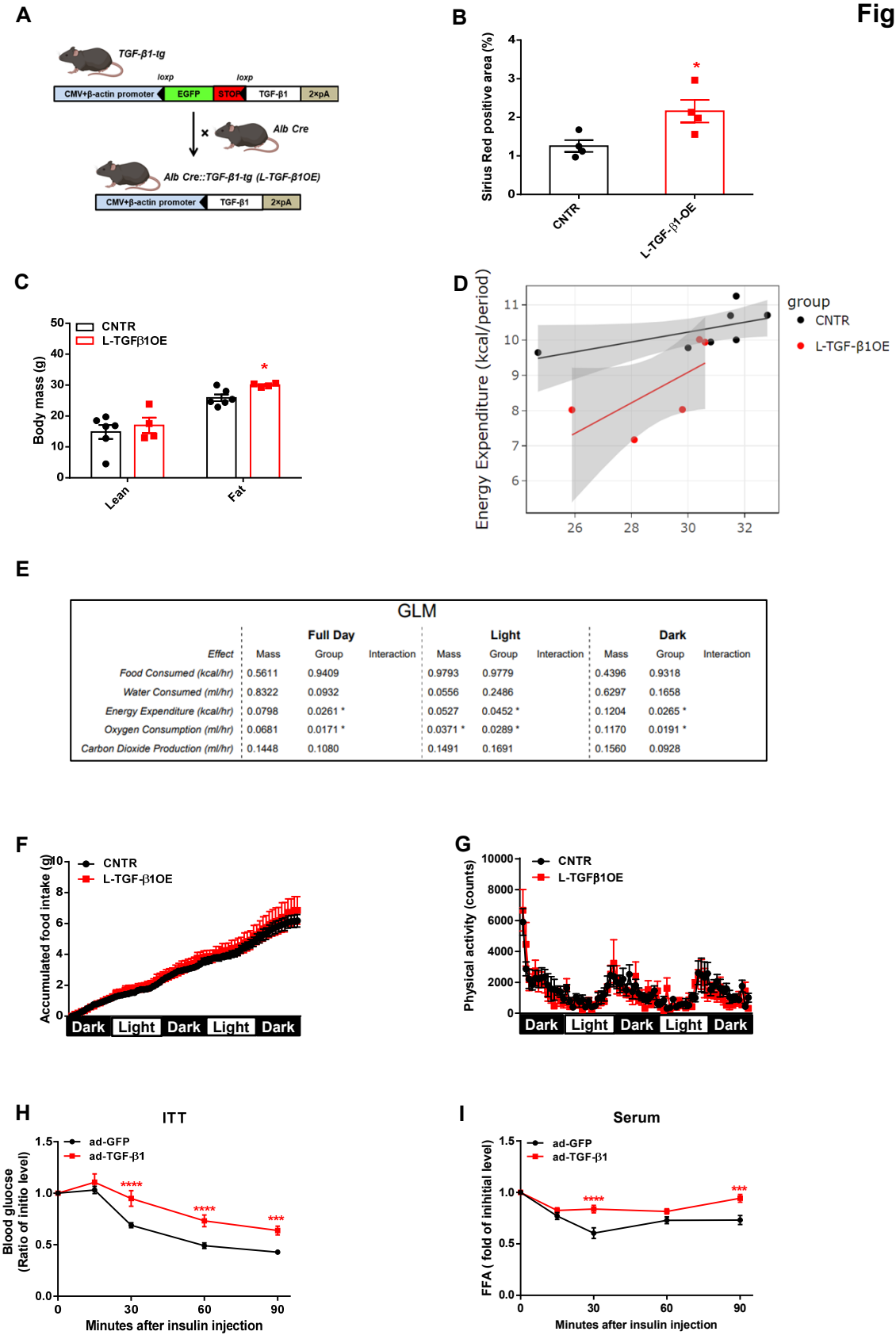


Figure S4. Characteristics of liver TGF-β1 overexpressing mice (L-TGF-β1OE and ad-TGF-β1) fed with HFD, related to Figure 4. **(A)** Schematic diagram of the breeding strategy for generating L-TGF-β1OE mice. **(B-G)** CNTR and L-TGF-β1-OE mice at the age of 3-month were fed with HFD for 3 months. **(B)**Sirius Red positive area in the liver of these mice. **(C)** Body composition, **(D)** The plot of energy expenditure vs lean body mass, **(E)** ANCOVA analysis table of the regression plot of energy expenditure vs lean body mass, **(F)** accumulated food intake, and **(G)** physical activity of these mice (n=6-9). **(H and I)** WT mice at the age of 2-months-old were fed with HFD for 3 months and then injected (*i.v.*) with adenovirus expression GFP (ad-GFP) and TGF-β1 (ad-TGF-β1) for 2 weeks. **(H)** ITT in mice injected with ad-GFP and ad- TGF-β1 under 4 h fasted condition (n=6-8 mice/group). **(I)** Fold change of serum FFA levels in mice injected with ad-GFP and ad-TGF-β1 30 min or 60 min after insulin injection (n=6). Data are presented as the means ± SEM. * p< 0.05, *** p< 0.001, **** p< 0.001 vs CNTR or ad-GFP using two-way ANOVA or t-test.

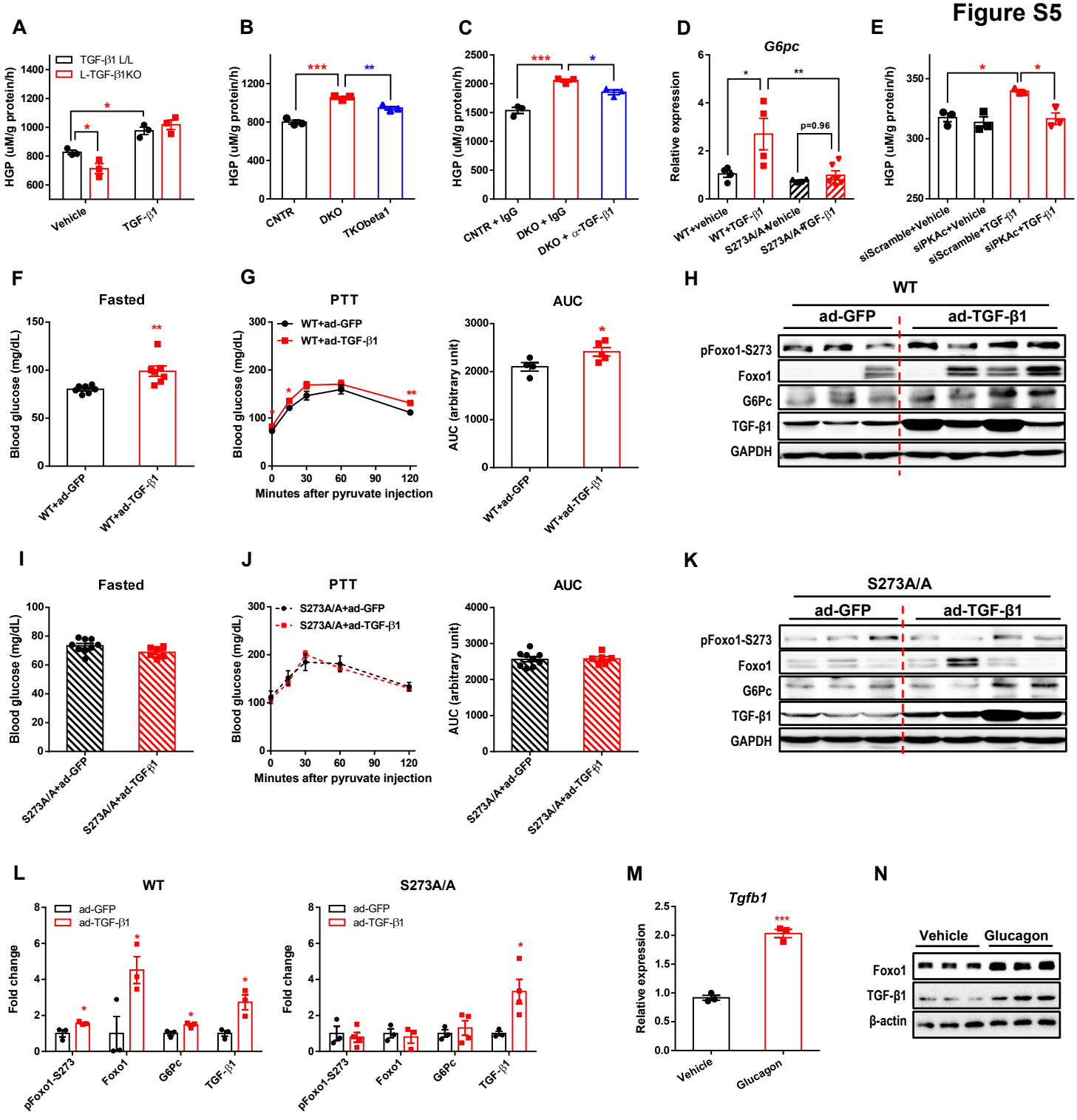


Figure S5. The effect of TGF-β1 on HGP and Foxo1 expression in primary hepatocytes and mice, related to Figure 5. For TGF-β1 treatment, the dose of TGF-β1 was 2.5 ng/ml. **(A)** HGP in TGF-β1 L/L and L-TGF-β1KO primary hepatocytes treated with TGF-β1 for 3 h. **(B)** HGP in CNTR, DKO, and TKObeta1 primary hepatocytes. **(C)** HGP in CNTR and DKO hepatocytes with or without neutralization of TGF-β1 by α-TGF-β1 antibody. **(D)** *G6pc* mRNA levels in WT and S273A/A primary hepatocytes. **(E)** Primary hepatocytes were isolated from WT mice and transfected with siScramble or siPKAc for 16 h in Opi-MEM medium, then treated with TGF-β1 (5 ng/ml) for 3 h in HGP buffer and determined the HGP levels. **(F-L)** WT and S273A/A mice at the age of 3-months-old were injected (*i.v.*) with adenovirus expression GFP (ad-GFP) and TGF-β1 (ad-TGF-β1) for 3 weeks. Fasted blood glucose **(F)**, PTT **(G)**, western blot analysis **(H)** and corresponding quantification **(L)** of hepatic protein levels of pFoxo1-S273, Foxo1, G6pc, TGF-β1 and GAPDH in WT mice. Fasted blood glucose **(I)**, PTT **(J)**, western blot analysis **(K)** and corresponding quantification **(L)** of hepatic protein levels of pFoxo1-S273, Foxo1, G6pc, TGF-β1 and GAPDH in S273A/A mice. **(M and N)** WT primary hepatocytes were treated with 100nM glucagon for 12 h. *Tgfb1* mRNA **(M)** and protein levels **(N)** in these cells were determined by qPCR and western blot. * p< 0.05, ** p< 0.01, *** p< 0.001 between assigned groups or vs vehicle group using one-way ANOVA or t-test.

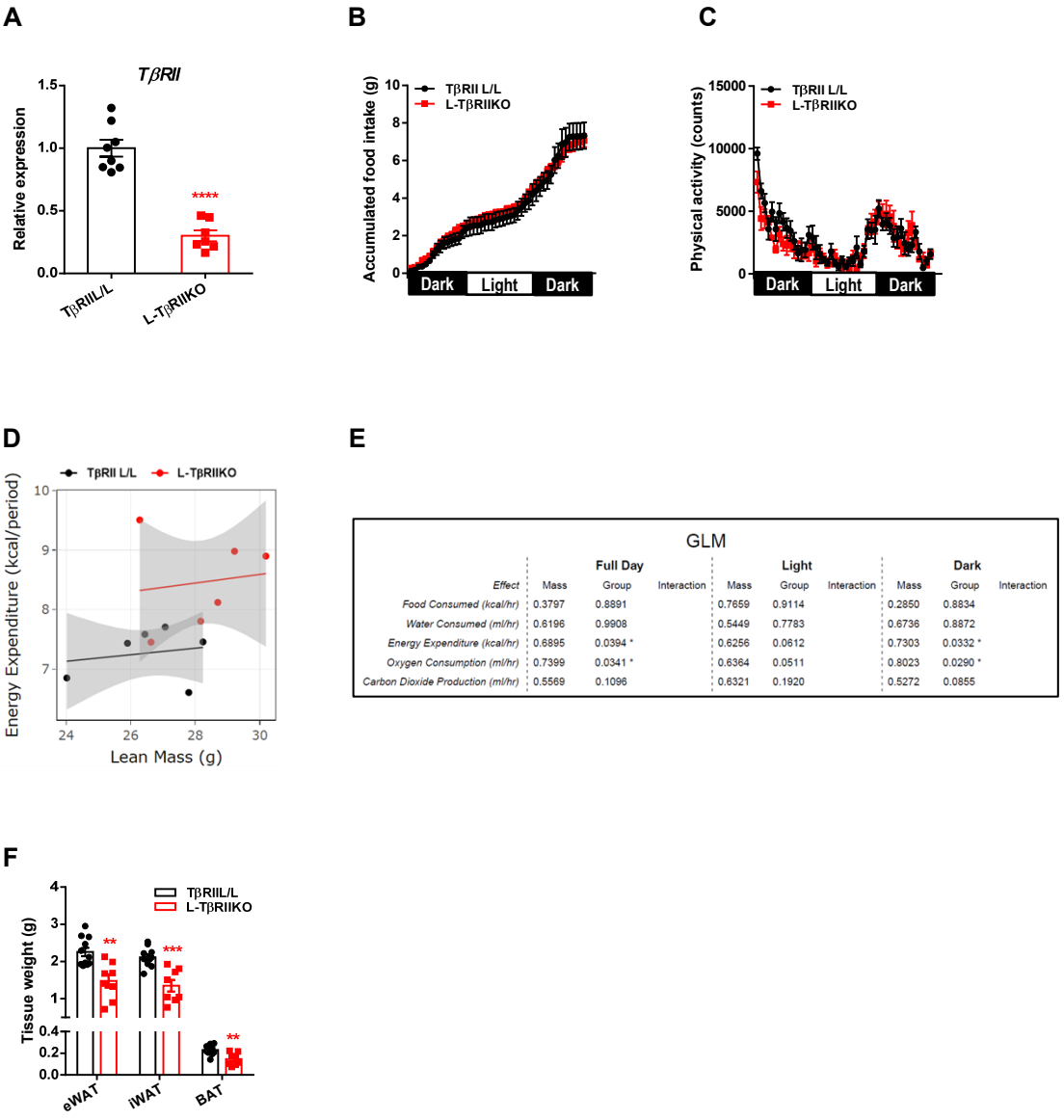


Figure S6. Characteristics of TβRII L/L and L-TβRIIKO mice fed with HFD, related to Figure 6. TβRII L/L and L-TβRIIKO mice at the age of 3-month were fed with HFD for 3 months. **(A)** TβRII mRNA expression in liver of these mice (n=7-8). **(B)** Accumulated food intake and **(C)** physical activities in these mice (n=6). **(D)** The plot of energy expenditure vs lean body mass, **(E)** ANCOVA analysis table of the regression plot of energy expenditure vs lean body mass. **(F)** Adipose tissue (eWAT, iWAT, and BAT) weight of TβRII L/L and L-TβRIIKO mice (n=11-13). Data are presented as the means ± SEM. * p< 0.05, ** p< 0.01, *** p< 0.001, **** p< 0.001 vs TβRII L/L using two-way ANOVA or t-test.