Supplementary methods

Animals

Animal experiments were conducted in accordance to the standards approved by the Faculty Animal Committee at the University of Santiago de Compostela, and the experiments were performed in agreement with the Rules of Laboratory Animal Care and International Law on Animal Experimentation. 8-12 weeks old wild-type (WT) mice (C57BL/6J), mice that carried floxed TAp63 alleles (with a C57BL/6J background), and conditional p63 liver KO mice (TAp63LKO) were housed in air-conditioned rooms (22-24 °C) under a 12:12h light/dark cycle. Animals were fed a standard diet and tap water *ad libitum* unless otherwise indicated. Mice that carried floxed TAp63 alleles (with a C57BL/6 background) were obtained from The Jackson Laboratory. TAp63LKO mice were generated in our laboratory crossing TAp63 floxed mice with Alfp-Cre mice, which express the Cre recombinase open reading frame (ORF) under the control of both the mouse albumin regulatory elements and the α-fetoprotein enhancers (Alfp-Cre transgene), a configuration that mimics the genomic organization of the mouse albumin gene [36]. Male and female mice with a C57BL/6 background were used indistinctly for p63 silencing or overexpression experiments. The number of animals used in each experiment is indicated in the corresponding figure legend. Animals were killed by decapitation, and tissues were removed rapidly and immediately frozen on dry ice. Tissues were kept at –80 °C until analysis. Homozygous wild-type (WT) and knockout (KO) mice originated from heterozygous mating; for each experiment, only littermate WT and KO animals were compared.

In Vivo Adenoviral Gene Transfer and *In Vivo* Virogenetic Procedures

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To achieve a specific effect on the liver, viruses were injected into the tail vein. Mice were held in a specific restrainer for intravenous injections, called Tailveiner (TV-150, Bioseb, France). Injections into the vein were carried out using a 27 G× 3/8" (0.40 mm × 10 mm) syringe. Mice were injected with 100 µl of adenoviral vectors diluted in saline. To down-regulate TAp63 specifically in liver of TAp63 floxed mice, associated adenoviruses serotype 8 (AAV8) was used, as it gives efficient and specific transgene expression in hepatocytes in vivo at low vector doses. AAV8-Cre and AAV8-GFP (1× 1010 VGml-1) (AAV8-GFP #SL100,833; AAV8-Cre-GFP #SL100,835 Signagen Laboratories, USA) were injected into Tap63 floxed mice (8–10 weeks), and one month later, experiments were performed. Overexpression of hepatic p63 was achieved by tail vein injection of adenoviral vector encoding p63 (#189SL100,865, SignaGen Laboratories, USA) or GFP (#SL100,833, SignaGen Laboratories, USA) (1×10^9 VG ml^{-1}). To recover the hepatic expression of p63 in the liver of KO mice (AlfCre p63 floxed mice), animals were injected into the tail vein with AAV8- P63, and controls with AAV8-GFP (1×10^9 VG ml^{-1}). To downregulate SIRT1 in liver, specific shRNA sequences for SIRT1 or luciferase (as a control) were designed using GPP Web Portal Tool (available at https://portals.broadinstitute.org/gpp/public/) following our optimized protocol [37]. In brief, oligonucleotides targeting the transcripts of interest were synthesized and subcloned into pLKO-Puro-IRES-eGFP (modified from pLKO.1 puro) vectors (Addgene). Briefly, HEK293T cells were maintained in high-glucose DMEM supplemented with 10% FBS (Gibco), 2 mM L-glutamine, and 1% penicillin/streptomycin, plated at a density of 8×10^6 cells per 150 mm dish, and transfected 24 hours later with PEI (Polyethylenimine; Sigma-Aldrich, 408727) and 20 µg of pLKO.shRNAs plasmids along with 10 µg of psPAX2 and pMD2.G packaging mix. After 24 hour, the medium was changed, and virus-containing supernatants were

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collected at 48 hours and 72 hours post-transfection. Lentiviral particles were concentrated using centrifugal filter units with 0.22 µm pore size (Amicon, UFC903024). The target sequences of the shRNAs used in this study were:

shSIRT1 #1: 5′-GCCATGTTTGATATTGAGTAT-3′

shSIRT1 #2: 5′- AGTGAGACCAGTAGCACTAAT-3′

shLuciferase: 5′-CCTAAGGTTAAGTCGCCCTCG-3′

Effect of Nutritional Status on Hepatic p63

WT animals were i) fed *ad libitu*m; ii) fasted for 6, 12, or 24 hours, or iii) refed *ad libitum* for 30 minutes, 1 hour, 2 hours or 24 hours after a 24-hour fasting.

Effect of Glucose on Hepatic p63 Protein Levels In Vivo

WT animals were i) fed *ad libitu*m; ii) fasted for 24 hours, or iii) fed only with sucrose *ad libitum* for 24 hours to maintain normal blood glucose levels and liver weight in the absence of other nutrients, as previously described.

Caloric Restriction

Five days before initiation of caloric restriction, mice were placed into individual cages and fed with chow diet *ad libitum.* During this time, food intake was monitored to determine the average amount of food consumed daily by each mouse. Thereafter, mice were randomly separated into two groups: one group continued to receive the chow diet *ad libitum*, while the other was subjected to 60% caloric restriction. Each mouse subjected to caloric restriction was fed at 6 p.m. every day with an amount of food equal to 40% of their daily food intake during the week of acclimation. Body weight and blood glucose

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were measured daily before feeding. Finally, mice were sacrificed at 5:30 p.m. (before feeding) on the fourth day of caloric restriction to collect blood and liver for analyses.

Glucose, Insulin, Pyruvate and Glutamine Tolerance Tests (GTT, ITT, PTT, and QTT respectively)

Basal blood glucose levels were measured after an overnight fast (12 hours) for GTT, PTT, and QTT, and after 6 hours for ITT, with a Glucocard Glucometer (ARKRAY, USA). GTT, ITT, QTT and PTT were done after an intraperitoneal injection of either 2 g kg^{-1} D-glucose (G8270, Sigma-Aldrich USA), 0.35 U kg^{-1} insulin (Actrapid, Novo Nordisk, Denmark), 1.5 g kg^{-1} L-glutamine (G3126, Sigma-Aldrich USA) or 1.25 g kg^{-1} sodium pyruvate (P2256, Sigma-Aldrich, USA), respectively, and area under curve values were determined.

Oral Glucose Tolerance Test (OGTT)

Oral glucose tolerance test (OGTT) was performed by oral gavage of glucose at a dose of 2 g kg^{-1} after overnight fasting. Blood glucose was measured before and 15, 30, 60, 90 and 120 min after D-glucose (G8270, Sigma-Aldrich USA) administration [38].

Postprandial Glucose Tolerance Test

Mice were fasted overnight and then refed with chow diet *ad libitum* for 4 hours. Blood glucose, food intake, and body weight were measured at 0, 30 minutes, 1 hour, 2 hours, and 4 hours after allowing the mice to eat *ad libitum.*

Hyperinsulinemic euglycemic clamp

Hyperinsulinemic-euglycemic clamps were performed in conscious unrestrained catheterized mice as previously described [39]. Briefly, catheters were surgically implanted 7 days prior to the experiment, in the right jugular vein and exteriorized above the neck using vascular access button (Instech Laboratories Inc, Plymouth Meeting, PA). Mice were fasted overnight, followed by a 2-hrs infusion of [3-3H] glucose (0.05 μCi/min) (Perkin Elmer, Walthman, MA). Continuous insulin infusion (2.5 mIU/kg body weight/min, Actrapid®, NovoNordisk A/S) was used for the induction of hyperinsulinemia. At reached steady state, in vivo insulin-stimulated glucose uptake in tissues was determined by a 10 μCi bolus injection of 2-[14C] deoxyglucose. After 30 min, mice were rapidly killed by cervical dislocation and tissues removed and stored at -80°C until use. Glucose concentration was measured using the glucose oxidase method (GLU, Roche Diagnostics, Rotkreuz, Switzerland) and insulin using an ELISA commercial kit (CrystalChem Inc., Downers Grove, IL). Measurements of 2-[14C] deoxyglucose-6-phosphate concentration in individual tissues allowed calculation of the glucose utilization index in tissues.

Hepatic Insulin Signalling In Vivo

To study the insulin signalling specifically in liver, mice were fasted for 6 hours and then anesthetized by an intraperitoneal injection of ketamine (100 mg kg^{-1} body weight)/xylazine (15 mg kg^{-1} body weight). Adequacy of the anesthesia was ensured by loss of pedal reflexes. The abdominal cavity of the mice was opened, and 125 μl of samples containing 5 units insulin (Actrapid, Novo Nordisk, Denmark) diluted in saline were injected into the inferior cava vein. Control injections were performed with 125 ml of saline. Samples of liver were harvested 2 minutes after injection.

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Patients

Liver samples were obtained from patients with morbid obesity undergoing bariatric surgery (n = 60) at the Clínica Universidad de Navarra. Obesity was defined as a BMI ≥ 30 kg/m^2 and body fat percentage as (BF) ≥ 35%. BMI was calculated as weight in kilograms divided by the square of height in meters, and BF was estimated by air-displacement plethysmography (Bod-Pod®, Life Measurements, Concord, CA, USA). Obese persons were sub-classified into two groups, of normoglycaemia (NG) or type 2 diabetes (T2D), following the criteria of the Expert Committee on the Diagnosis and Classification of Diabetes [40]. Inclusion criteria encompassed a complete diagnostic work-up including physical examination, laboratory investigation, ultrasound echography, and liver biopsy, consistent with the diagnosis of non-alcoholic fatty liver disease (NAFLD) according to the criteria of Kleiner and Brunt by an expert pathologist masked to all results of the assays [41]. Features of steatosis, lobular inflammation, and hepatocyte ballooning were combined to obtain a NAFLD activity score (NAS) (0-8) [41]. Exclusion criteria were: a) excess alcohol consumption (≥20 g for women and ≥30 g for men); b) the presence of hepatitis B virus surface antigen or hepatitis C virus antibodies in the absence of a history of vaccination; c) use of drugs linked to NAFLD, including amiodarone, valproate, tamoxifen, methotrexate, corticosteroids or anti-retrovirals; and d) evidence of other specific liver diseases, such as autoimmune liver disease, haemochromatosis, Wilson's disease, or α-1-antitrypsin deficiency. Patients with T2D were not on insulin therapy or medication likely to influence endogenous insulin levels. It has to be stressed that patients with T2D did not have a long diabetes history (less than 2–3 years, or even a *de novo* diagnosis, as evidenced from their anamnesis and biochemical determinations). All reported investigations were carried out in accordance with the principles of the Declaration of

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Helsinki, as revised in 2013, and approved by the Hospital's Ethical Committee responsible for research (protocol 2017.104). Written informed consent was obtained from all the participants.

Cell Culture

The human liver cell line THLE2 was purchased from ATCC (The Global Bioresource Center) and cultured in bronchial epithelial cell basal medium (BEBM) supplemented with additives (BEGM, BEGM Bullet Kit, CC3,170, Lonza/Clonetics Corporation, USA), 70 ng ml^{-1} phosphoethanolamine, 5 ng ml^{-1} epidermal growth factor (EGF), 10% fetal bovine serum (FBS) and 1% glutamine-penicillin-streptomycin solution (Sigma Aldrich, USA). THLE-2 cells should be grown on culture plates pre-coated with a mixture of 0.01 mg ml^{-1} fibronectin (33010018, Sigma Aldrich, USA), 0.01 mg ml^{-1} bovine serum albumin (A4503, Sigma Aldrich, USA) and 0.03 mg ml^{-1} collagen type I (#sc-136157, Santa Cruz, USA). AML12 cells, a murine liver cell line, was purchased from ATCC (The Global Bioresource Center) and was cultured in DMEM-F12, 10% FBS supplemented with 1× insulin-transferrin-selenium (ITS-G, 100×, 41400045, Thermofisher, USA) and 40 ng ml^{-1} dexamethasone (D4902, Sigma Aldrich, USA). Cell line was maintained at 37 °C in a humidified atmosphere containing 5% CO2 and was routinely tested for mycoplasma. 1.5×10^5 cells were seeded in a six-well plate for all experiments.

Cell Transfections

After 24 hours in culture, cells were transfected with specific small interference RNAs (si-RNAs) to knockdown the expression of p63 in THLE2 cells (C7-000110, Dharmacon,

USA). Non-targeting siRNAs were use as negative control (D-001810-10-05, Dharmacon, USA). Transfections were performed using Dharmafect 1 reagent (t-2001-03, Dharmacon, USA) following the protocol; briefly, 50 pmol of the sip63 diluted in 200 µl of optiMEM (#31985-070, Life Technologies, USA) was mixed with 6.5 µl of Dharmafect 1 diluted in 193.5 µl of optiMEM. This mixture was added into each well, resulting in a final volume of 1.5 ml with BEGM complete medium for 6 hours. After that, medium was replaced with fresh BEGM until the indicated treatments were performed. At 48 hours after the plasmid p63 transfection, cells were collected for protein extraction. THLE2 cells were transfected with a DNA plasmid containing the sequence necessary to increase the expression of TAp63 (plasmid, 27,008) (Addgene, USA) and control pcDNA (plasmid 16434) (Addgene, USA). Lipofectamine 2000 (#11668-019, Invitrogen, USA) was used to transfect cells with following protocol: 4 µl of Lipofectamine 2000 diluted on 150 µl of optiMEM mixed with 2.5 µg of DNA diluted on 150 µl of optiMEM. This mixture was incubated in a final volume of 1.5 ml of BEGM complete medium supplemented with FBS 5% for 6 hours. After that, medium was replaced with fresh complete BEGM medium until cells were subjected to the indicated treatments. p63 was upregulated for 24 hours before cells were collected for protein or mRNA extraction.

In Vitro Treatments

Cells were starved for 6 hours in Krebs–Henseleit–HEPES buffer (KHH; composition: 120 mmol l^{-1} NaCl, 4.7 mmol l^{-1} KCl, 2.5 mmol l^{-1} $CaCl_2$, 1.2 mmol l^{-1} MgSO4, 1.2 mmol l^{-1} KH2PO4, 25 mmol l^{-1} NaHCO3, 25 mmol l^{-1} HEPES pH 7.4), a fasting medium with neither nutrients nor hormones [30]. To study regulation of p63 protein expression by glucose in vitro, cell medium from untreated cells were changed to KHH, in the

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absence or presence of glucose (10 mM) (D-glucose, #G8270, Sigma-Aldrich, USA), and in the absence or presence of 2-Deoxy-D-glucose (10 mM) (2-D-D-glucose, #D8375, Sigma-Aldrich, USA). To study regulation by insulin in vitro, cells were starved and treated with insulin (10 nM) (Actrapid, Novo Nordisk, Denmark), and with LY-294,002 (10 nM) (LY-294,002, #L9908, Sigma-Aldrich, USA).

In vitro luciferase assay in Cells

For luciferase assays, AML12 cells were transfected in 24-well plates containing Lipofectamine 2000 or Dharmafect, 2,5 µl siTAp63 and non-targeting siRNA, 2.5 µg of TAp63 and control pcDNA overexpression vectors, 1 µg of each luciferase reporter plasmids pTa luc, pTa-202-sirt1luc, and 50 ng of pRL-TK-Renilla (as transfection control) for 48 hours. Luciferase was measured in a Mithras LB 940 apparatus (Berthold Technologies, Bad Wildbad, Germany).

Glucose Measurement in Cell Culture Medium

Cells were incubated in glucogenic medium (KHH medium supplemented with lactate 20 mM and pyruvate 2 mM) for the indicated times. Glucose released to the media was quantified using the High Sensitivity Glucose Assay Kit (MAK181-1KT, Sigma-Aldrich, USA). Results were normalized by the amount of protein.

SIRT1 Deacetylase Activity

SIRT1 activity was measured using a deacetylase fluorometric assay kit (SIRT1 Activity Assay Kit, Abcam, ab156065) [42]. First, subcellular fractions from cells were isolated using the Proteoextract Subcellular Proteome Extraction kit (539790, Calbiochem) (without protease inhibitor cocktail) [43]. Next, 25 µg of crude nuclear extract was used

to evaluate the SIRT1 deacetylase activity. The fluorescence intensity at 440 nm (exc. 340 nm) was measured every minute for a total of 60 min immediately after the addition of fluorosubstrate peptide.

Chromatin immunoprecipitation (ChIP) assays

ChIP assays were performed using QuilkChip Kit (Novus Biologicals, Englewood, USA). Soluble chromatin fractions were immunoprecipitated with 1 µg of monoclonal anti-FLAG antibody (Abcam, Cambridge, UK), or no target human immunoglobulin G (IgG) (Sigma-Aldrich, St. Louis, USA) as control. The histone-DNA crosslinks were reversed by overnight incubation at 65°C. PCR was used to analyze the DNA fragment from ChIP assays. The PCR was run for 1 min at 95, 56, and 72ºC within each cycle, for 36 cycles in total.

The oligonucleotides used for SIRT1 promoter amplification were: forward, -182/-161 bp, 5´-acgtgacccggcgtgttgt-3´, and reverse, -20/-1 bp, 5´-tcttccaactgcctctctgg-3´ (PCR product 182 bp). PCR products were quantified using the ImageJ software and represented as mean ± SEM.

***In vitro* immunofluorescence studies**

Immunofluorescence studies were performed using standard procedures in cells grown on precoated and fixed with formalin 10% wt/vol. Fixed cells were incubated with permeabilizing- blocking solution (5% Donkey Serum, 0,3%TritónX-100) for 1 hour and then incubated ON with FOXO-1 antibody (#52857, Abcam, United Kingdom) in a dilution buffer (1%BSA, 0,3% TritónX-100) at 1:250. Secondary antibody Donkey anti-rabbit alexa-488 (#711-545-152, Jackson ImmunoResearch, UK) was incubated in the same dilution buffer at 1:1000 together with Hoescht 33342 (#62249, Thermo Scientific,

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USA) (1:100) for 1 hour. All intermediate washing steps were performed using PBS. Coverslips were mounted in slides with Fluoro-Gel (#17982-10, Electron Microscopy Sciences, UK). Images were acquired using Thunder DM4 B microscope (Leica Microsystems, Germany) and nuclear-to-cytoplasmic ratio was determined using the Leica Las X Version 3.7.2.22383 and ImageJ software.

Western Blot Analysis

Total protein lysates from liver (20 µg µl^{-1}) and THLE2 cells (2 µg µl^{-1}) were subjected to SDS-PAGE, electrotransferred onto polyvinylidene difluoride membranes (Millipore), and probed with the indicated antibodies. The conditions for each antibody are described in Supplementary Table S4. For the immunoprecipitation assay, extracts were incubated overnight at 4°C with control IgG (m-IgG2a. sc-542731, Santa Cruz Biotechnology) or specific primary antibody (PGC1α Antibody (D-5). sc-518025, Santa Cruz Biotechnology). Antibodies were precipitated with Protein G Agarose beads (Protein G Sepharose 4 Fast Flow. 17-0618-01, GE Healthcare). The captured proteins were centrifuged, the supernatants discarded, and the beads washed in lysis buffer. Beads were boiled for 5 minutes at 95 °C in 20 µl sample buffer. Immunoprecipitated were analysed by Western blot. The antibodies employed were Acetylated-Lysine Antibody (9441, Cell Signalling) and PGC1α (ST1202, Calbiochem). For protein detection, horseradish peroxidase-conjugated secondary antibodies and chemiluminescence were used (Amersham Biosciences, Little Chalfont, UK). Protein levels were normalized to GAPDH for each sample.

Quantitative Reverse-Transcriptase PCR (qRT-PCR) Analysis

RNA was extracted using TRIzol reagent (ThermoFisher, USA) according to the manufacturer's instructions. Total RNA of 100 ng were used for each RT reaction, and cDNA synthesis was performed using the SuperScript First-Strand Synthesis System (ThermoFisher, USA) and random primers. Negative control reactions, containing all reagents except the sample, were used to ensure specificity of the PCR amplification. For analysis of gene expression, real-time reverse-transcription polymerase chain reaction (RT-PCR) assays were used with a fluorescent temperature cycler (TaqMan; Applied Biosystems, USA) following the manufacturer's instructions and TaqMan and SYBR green reagent (Agilent Technologies, USA). The PCR cycling conditions included an initial denaturation at 50 ºC for 10 min followed by 40 cycles at 95 ºC for 15 s and 60 ºC for 1 min. The oligonucleotide specific primers are shown in Supplementary Tables S5-S6. For data analysis, the input value of gene expression was standardized to the HPRT value for the sample group and expressed as a comparison with the average value for the control group. All samples were run in duplicate, and the average values were calculated.

Hematoxylin/Eosin Staining and Immunohistochemistry

Fresh liver samples were fixed in 4% formaldehyde for 24 h, dehydrated using alcohol 70%, followed to alcohol 80% and 90%, and then embedded in paraffin. Sections of 4 μ m were cut with a microtome and stained using a standard Hematoxylin and Eosin alcoholic procedure according to the manufacturer´s instructions (BioOptica). After successive rinse with distilled water, sections were dried at 37 ^{o}C for 30 min and mounted with permanent (non-alcohol, non-xylene based) mounting media. For Oil Red O staining, frozen liver samples were cut in 8 µm sections with a cryostat and stained in filtered Oil Red O for 10 min. After being washed in distilled water, sections were counterstained with Mayer ´s hematoxylin for 3 min and mounted in aqueous mounting

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(glycerine jelly). For Ki67 and CC3 immunohistochemistry staining, samples fixated in paraffin were dewaxed, hydrated, pre-treated in PTLink TE buffer pH 9 and blocked with 3% peroxidase for 10 minutes. Next, sections were incubated with the primary antibody at a concentration of 1:500 overnight and at 4ºC, followed by an incubation with the secondary antibody (EnVision, DAKO) for 30 minutes at room temperature. After that, DAB developer was used for 1 minute and sections were counterstained with Mayer´s haematoxylin for 10 min, dehydrated and mounted. In all the histological staining, we used up to 3-5 representative microphotographs of each animal were taken at 20X or 40X with a BX51 microscope equipped with a DP70 digital camera (Olympus). Lipids in Oil Red O-stained sections, were quantified using ImageJ software (National Institutes of Health and the Laboratory for Optical and Computational Instrumentation, LOCI, University of Wisconsin).

Data Analysis and Statistics

Results are given as mean ± standard error mean (SEM). Animals were excluded if an objective experimental failure was observed (reference: A call for transparent reporting to optimize the predictive value of preclinical research). In the molecular analysis, values detected by the two-fold of standard deviation observed were considered a failure in the technique and were discarded. For that, all experimental groups were made with set of animals of the same age and similar body weight. Studies were not blinded to investigators. All experiments were performed once if not otherwise indicated in the figure legends. To test if the populations follow a Gaussian distribution, a normality test was performed (Kolgomorov–Smirnof test for n between 5–7; Shapiro-Wilk test for $n \geq 7$). For normal distributions, parametric test was used; for two population comparisons, an unpaired t-tests (two-tailed for treatment and phenotyping experiment, otherwise one-

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tailed) were used as indicated in figure legends; for multiple comparison test, a one-way ANOVA followed by Bonferroni *post-hoc* multiple comparison test was performed. For non-Gaussian distributions, non-parametric test was used; Mann-Whitney *U* test were used for two comparison test, and Kruskal–Wallis followed by Dunn *post-hoc* test for multiple comparison. Pearson's correlation coefficients (r) and stepwise multiple linear regression analysis were used to determine the association between variables. $P<0.05$ was considered significant for all the analysis. Test were performed with GraphPad Prism Software Version 6.0 (GraphPad, San Diego, CA).

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Supplementary table 1. Anthropometric, biochemical and clinical characteristics of obese patients with normoglycemia (NG) and type 2 diabetes (T2D).

	NG	T2D
n	30	30
Age (years, mean ± SD)	44 ± 10	50 ± 9*
Gender (F / M)	15 / 15	15 / 15
Weight (kg ± SD)	116.9 ± 25.5	117.6 ± 23.8
BMI	41.2 ± 6.6	41.9 ± 7.1
LDL cholesterol (mg/dl ± SD)	118 ± 29	113 ± 38
HDL cholesterol (mg/dl ± SD)	53 ± 17	45 ± 14
Triglycerides (mg/dl ± SD)	105 ± 53.6	144 ± 70*
Cholesterol (mg/dl ± SD)	191 ± 34	190 ± 43
AST (U/L ± SD)	19 ± 6	20 ± 7
ALT (U/L ± SD)	25 ± 12	26 ± 11
Glucose (mg/dl ± SD)	92 ± 6	144 ± 55***
OGTT (mg/dl ± SD)	110 ± 24	212 ± 54***
HOMA	4.32 ± 2.96	7.75 ± 5.41 **
Insulin (mU/ml)	18.6 ± 12.1	32 ± 57.1
A1c (%)	5.7 ± 0.6	8.4 ± 1.8 **

BMI, body mass index; LDL, low-density lipoprotein; HDL, high-density lipoprotein; AST, aspartate transaminase; ALT, alanine transaminase.

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Supplementary Table 2. Univariate analysis of the correlations between hepatic TAp63 protein levels and variables related to glucose metabolism.

	TAp63 protein	
	r	*P*
Glucose	-0.52	**0.001**
Glucose 2 h OGTT	-0.50	**0.013**
Insulin	-0.03	0.855
Insulin 2 h OGTT	-0.18	0.392
Glycated haemoglobin	-0.86	**<0.0001**
HOMA	-0.33	**0.036**
QUICKI	0.21	0.174

Values are Pearson's correlation coefficients and associated *P* values after age-, sex- and BMI-adjustment. OGTT, oral glucose tolerance test; HOMA, homeostasis model assessment; QUICKI, quantitative insulin sensitivity check index.

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Supplementary Table 3. Multiple linear regression analyses with hepatic TAp63 protein as dependent variable for all subjects in the cross-sectional study.

	TAp63 protein	
Model I	*β*	*P*
Age	-1.254	**0.023**
Sex	-19.972	0.087
BMI	-2.166	**0.014**
AST	2.624	0.165
ALT	-1.517	0.145
Adjusted R^2	0.264	**0.024**
Model II	*β*	*P*
Age	-1.004	**0.049**
Sex	-19.688	0.085
BMI	-2.474	**0.004**
HOMA	-3.563	**0.007**
Adjusted R^2	0.351	**0.001**

BMI, body mass index; HOMA, homeostasis model assessment. β is the regression coefficient, which allows evaluating the relative significance of each independent variable in multiple linear regression analyses. Adjusted R^2 expresses the percentage of the variance explained by the independent variables in the different models (i.e. 0.351 is 35.1%). Statistical significant values are in bold.

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Supplementary Table 4. Antibodies used for western blot.

Protein target	Manufacter (catalog number)	Species reactivity	Dilution
Phosphoenolpyruvate Carboxykinase 1 (PCK1)	Abcam (ab70358)	Rabbit polyclonal	1:1000
Glyceraldehyde 3-phosphate Dehydrogenase (GAPDH)	Merck (CB1001)	Mouse monoclonal	1:5000
Phospho-AKT (pAKT) (Ser 473)	Cell Signaling (9271)	Rabbit polyclonal	1:1000
Phospho-Pyruvate Dehydrogenase E1-alpha subunit antibody (pPDH)	Abcam (ab177461)	Rabbit monoclonal	1:1000
Glucose-6-phosphatase	Abcam (ab83690)	Rabbit polyclonal	1:1000
SIRT1	Cell Signaling (8469S)	Mouse monoclonal	1:1000
DNp63	Biolegend (619002)	Rabbit polyclonal	1:1000
TAp63	Biolegend (938102)	Mouse monoclonal	1:1000

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Supplementary Table 5. Primers used for gene amplification by Syber green.

Gene	Sequence FW (5'->3')	Sequence RV (3'->5')
DNp63 (*Mus musculus*)	GCAGCCTTGACCAGTCTCACTGC	TCCATGCTGTTCAGGAGCCCCA
SIRT1 (*Mus musculus*)	AGTTCCAGCCGTCTCTGTGT	CTCCACGAACAGCTTCACAA
HPRT (*Mus musculus*)	TGCTGACCTGCTGGATTACATT	CCCCGTTGACTGATCATTACAGTA
PCK1	ACCGACCTCCCTTACGAAAT	CCCTAGCCTGTTCTCTGTGC
G6pase	AGGAAGGATGCAGGAAGGAA	TGGAACCAGATGGGAAAGAG

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Supplementary Table 6. Primers used for gene amplification by Taqman.

Gene	**Reference**
TAp63 (*Mus musculus*)	Mm00495793_m1
TAp63 (*Homo sapiens*)	Hs00978343_m1
HPRT (*Mus musculus*)	Mm01545399_m1
HPRT (*Homo sapiens*)	Hs02800695_m1
SIRT1 (*Mus musculus*)	Mm01168521_m1

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Supplementary figure legends

Supplementary Figure 1. TAp63 protein levels are decreased during fasting. A) p63 mRNA levels in WT and glucagon receptor-deficient mice (GCNR KO) injected with saline or glucagon. B) p63 mRNA levels in WT and GCNR KO mice during fasting. C) FGF21 mRNA levels and D) p63 mRNA levels in WT mice injected with sh-luciferase or sh-FGF21. E) TAp63 protein levels and F) DNp63 protein levels in WT mice fed *ad libitum* or fasted for 24 h. G) DNp63 mRNA levels in WT mice fed *ad libitum*, fasted for 24h or fasted for 24h and then refed for 24h. H) p63 mRNA levels in extrahepatic tissues (WAT, BAT, muscle, kidney, and intestine) from WT mice fed *ad libitum*, fasted for 24h or fasted for 24h and then refed for 24h. PCK1 and G6Pase mRNA levels in jejum (I) and kidney (J) from WT mice fed *ad libitum*, fasted for 24h or fasted for 24h and then refed for 24h. Expression of GAPDH (western blot) or HPRT (qRT-PCR) served as loading control, and control values were normalized to 100%. Data are presented as mean ± standard error mean (SEM). ∗p <0.05, ∗∗p <0.01, ***p<0,001, using a Student's t test (A), (B), (C), (D), (E) and (G), or one-way ANOVA followed by a Bonferroni Multiple Comparison Test (G), (H), (I) and (J).

Supplementary Figure 2. Food intake, cumulative body weight gain, and blood glucose levels in mice lacking hepatic TAp63. A) p63 and SIRT1 mRNA levels in extrahepatic tissues (WAT, BAT, muscle, kidney, and intestine) from floxTAp63 mice injected with AAV8-GFP (control) or AAV8-Cre. B,C) Cumulative food intake, cumulative body weight gain, glucose tolerance test (GTT), insulin tolerance test (ITT) and glutamine tolerance test (QTT) in B) floxTAp63 mice injected with AAV8-GFP (control) or AAV8-Cre to decrease Tap63 levels in the liver; and C) control mice and AlfpCre floxTAp63 (Tap63 LKO) mice. D) Oral glucose tolerance test in control mice

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and Tap63 LKO mice. Area under the curve (AUC) is also shown. Data are presented as mean ± standard error mean (SEM). ∗∗p <0.01 using a Student's t test (D).

Supplementary Figure 3. Blood glucose, serum FGF21, serum glucagon and AST/ALT levels in mice lacking hepatic TAp63 under fasting conditions.

Blood glucose (A), serum FGF21 (B), serum glucagon (C) and ALT/AST (D) levels in 24 hours fasting floxTAp63 mice injected with AAV8-GFP (control) or AAV8-Cre. Blood glucose (E), serum FGF21 (F), serum glucagon (G) and ALT/AST (H) levels in 24 hours fasting control mice and Tap63 LKO mice. PCK1 and G6Pase mRNA levels in the intestine (I) and kidneys (J) in 24 hours fasted floxTAp63 mice injected with AAV8-GFP (control) or AAV8-Cre. PCK1 and G6Pase mRNA levels in the intestine (K) and kidneys (L) in 24 hours fasted control mice and Tap63 LKO mice. Expression of GAPDH (western blot) or HPRT (qRT-PCR) served as loading control, and control values were normalized to 100%. Data are presented as mean ± standard error mean (SEM). ∗p <0.05 using a Student's t test (A) (E).

Supplementary Figure 4. Serum FGF21 and serum glucagon levels in mice lacking hepatic TAp63 under refeeding conditions. Serum glucagon (A) and FGF21 (B) levels after refeeding in floxTAp63 mice injected with AAV8-GFP (control) or AAV8-Cre. Serum glucagon (C) and FGF21 (D) levels after refeeding in control and TAp63 LKO mice. Data are presented as mean ± standard error mean (SEM).

Supplementary Figure 5. Inhibition of hepatic TAp63 does not alter cell cycle or cell death. Hematoxylin and Eosin (upper panels), cleaved caspase 3 (CC3) (central panels) and Ki67 (lower panels) staining of liver sections.

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Supplementary Figure 6. Recover of TAp63 expression in TAp63 LKO mice. Glucose tolerance test (GTT) and insulin tolerance test (ITT) in control mice and Tap63 LKO mice injected with a AAV8-TAp63 virus. Area under the curve (AUC) is also shown. Data are presented as mean ± standard error mean (SEM).

Supplementary Figure 7. Putative binding sites for TP63 in the SIRT1 gene promoter. A) Predicted putative binding sites for TP63 in the *Mus musculus* and *Homo sapiens* SIRT1 gene promoter with a p<0.01. B) Predicted putative binding sites for TP63 in the *Mus musculus* and *Homo sapiens* SIRT1 gene promoter with a p<0.001. C) SIRT1 promoter activity in THLE-2 cells transfected with pTA (control), pTA-202-Sirt1, p0 or ppTAp63 as indicated. Data are presented as mean ± standard error mean (SEM). ∗∗p <0.01, ***p<0.001, using one-way ANOVA followed by a Bonferroni Multiple Comparison Test (C).

Supplementary Figure 8. Inhibition of hepatic TAp63 and SIRT1 does not cause significant alterations in lipid storage, inflammation or cholesterol metabolism.

A) Hematoxylin Eosin and Oil Red Staining of liver sections in control or TAp63 LKO mice after being injected with shLuciferase or shSIRT1. B) Protein levels of enzymes implicated in *de novo* lipogenesis (FAS, pACC/ACC), lipid oxidation (CPT1) and lipid uptake (LPL) in control and TAp63 LKO mice after being injected with sh-Luciferase or sh-SIRT1. mRNA levels of genes implicated in inflammation (C) (Tnfα, Il1β, Il6, Tgfβ, F4/80) and lipid transport/cholesterol metabolism (D) (Cyp7a1, Apoc2, Apoa1, Hmgcr) in control or TAp63 LKO mice after being injected with sh-Luciferase or sh-SIRT1. E) Protein levels of total and phosphorylated CREB in control or TAp63 LKO mice after

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being injected with sh-Luciferase or sh-SIRT1. Expression of GAPDH (western blot) or HPRT (qRT-PCR) served as loading control, and control values were normalized to 100%. Data are presented as mean ± standard error mean (SEM).

Supplementary Figure 9. Hepatic TAp63 over-expression does not cause alterations in cell cycle progression or cell death. A) Hematoxylin and Eosin (upper panels), cleaved caspase 3 (CC3) (central panels) and ki67 (lower panels) staining of liver sections. B) pCREB and CREB protein levels in fasted WT mice injected with a AAV8 expressing either GFP or TAp63. Expression of GAPDH (western blot) served as loading control, and control values were normalized to 100%. Data are presented as mean ± standard error mean (SEM).

Supplementary Figure 10. SIRT1 regulation in fasting.

A) Blood glucose, p63 and SIRT1 mRNA levels in WT mice *fed ad libitum* or fasted for 6, 12 and 24 hours. B) Correlation between SIRT1 and TAp63 mRNA levels, between SIRT1 and blood glucose levels, and between blood glucose and TAp63 mRNA levels. C) SIRT1 mRNA levels in WT mice fed *ad libitum*, fasted for 24h or fasted for 24h and then refed for 24h. D) SIRT1 mRNA levels in THLE-2 cells maintained in complete medium (CM) or starved in KHH with or without glucose (10 mM). E) SIRT1 mRNA levels in different tissues (WAT, BAT, muscle, kidney, and intestine) after 24 hours of fasting and further refeeding for 24h. F) SIRT1 mRNA levels in the WAT, BAT, muscle, kidney and intestine of TAp63 flox mice injected with AAV8-GFP (control) or AAV8-Cre. Expression of HPRT served as loading control, and control values were normalized to 100%. Data are presented as mean ± standard error mean (SEM). $*p < 0.05$, $***p < 0.001$ using a one-way ANOVA followed by a Bonferroni Multiple Comparison Test (D).

Supplementary Figure 11. PGC1a levels in Tap63 KO mice and FOXO1 localitation with the Tap63 inhibition/overexpression.

A) Liver protein levels of acetylated-PGC1a in control and TAp63 LKO mice. B) Intracellular localization of Foxo1 in THLE-2 with or without p63 expression, kept in KHH medium in presence or absence of insulin 10 nM. Expression of GAPDH served as loading control, and control values were normalized to 100%. Data are presented as mean ± standard error mean (SEM). ∗p <0.05 using a Student's t test (A) or one-way ANOVA followed by a Bonferroni Multiple Comparison Test (B).

Supplementary Figure 12. Uncropped western blots.

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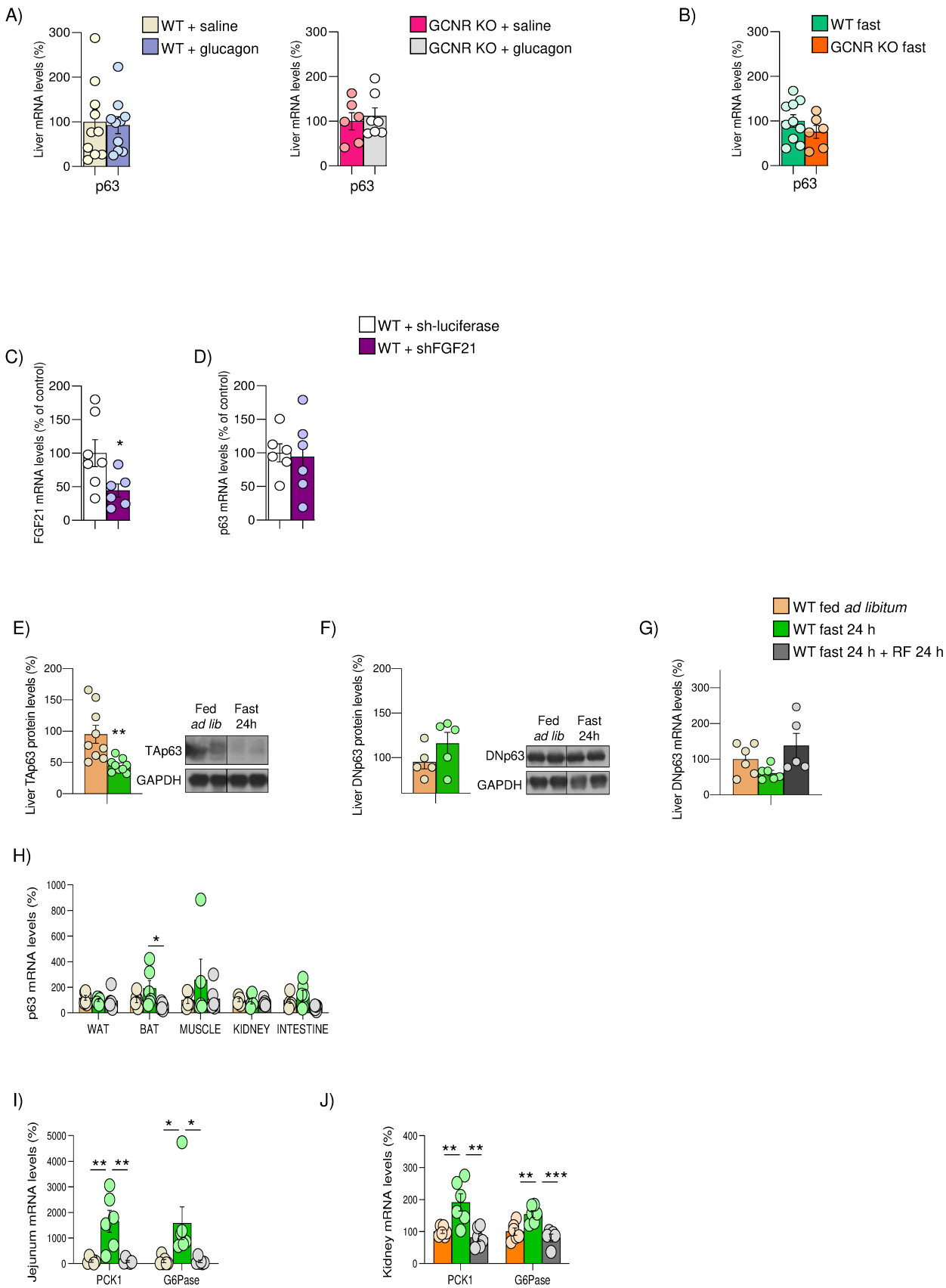
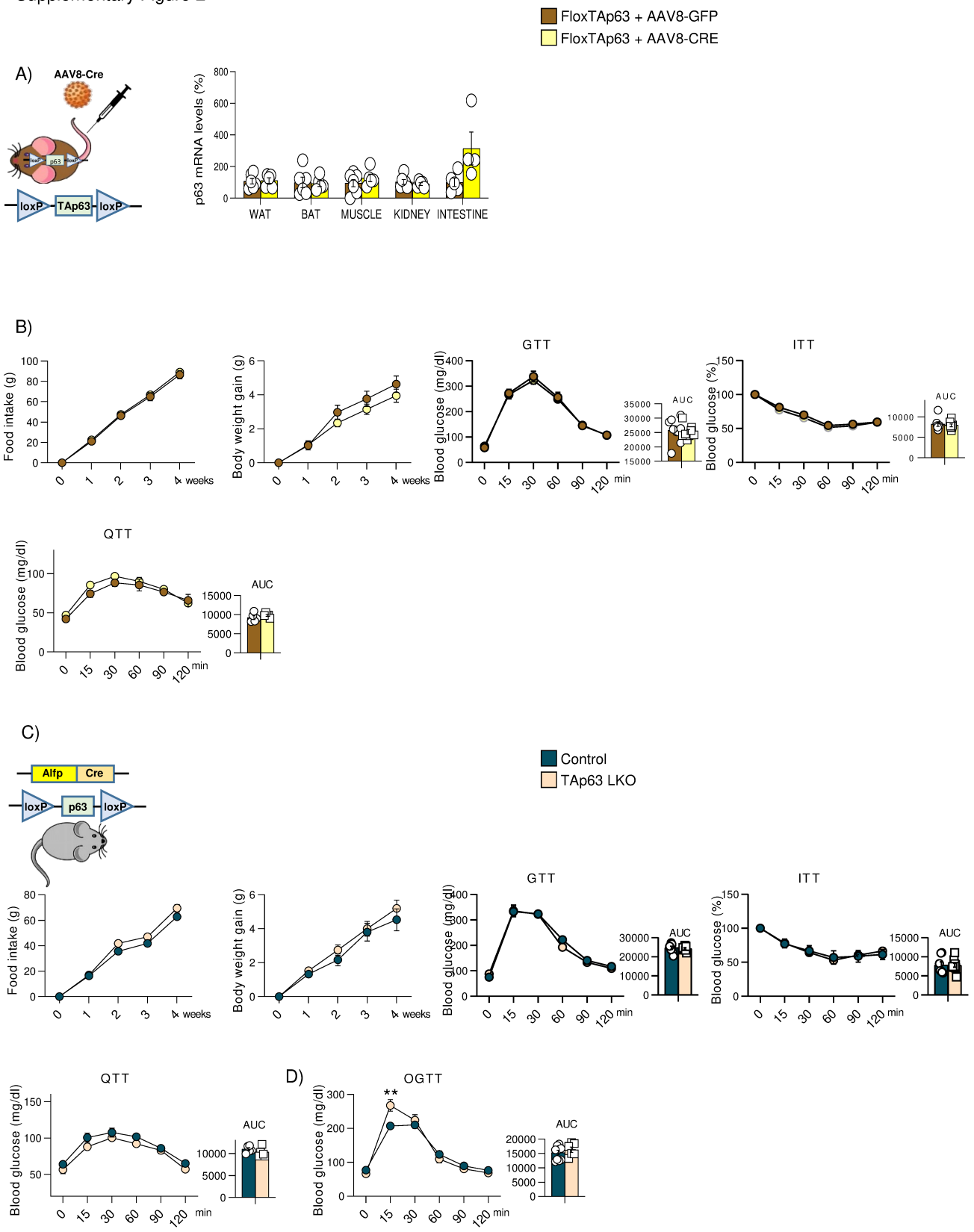
Supplementary Figure 1



Supplementary Figure 2



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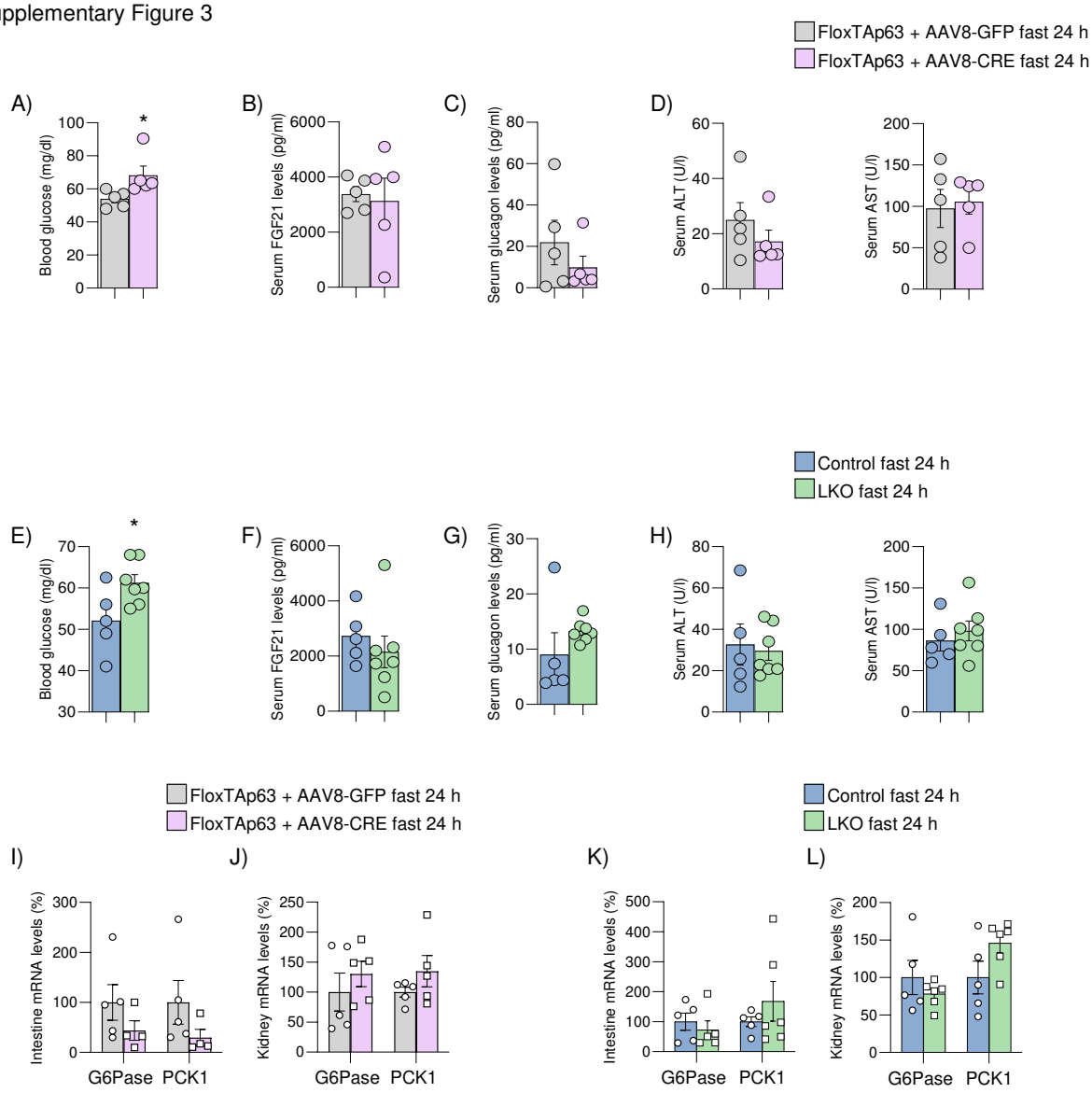

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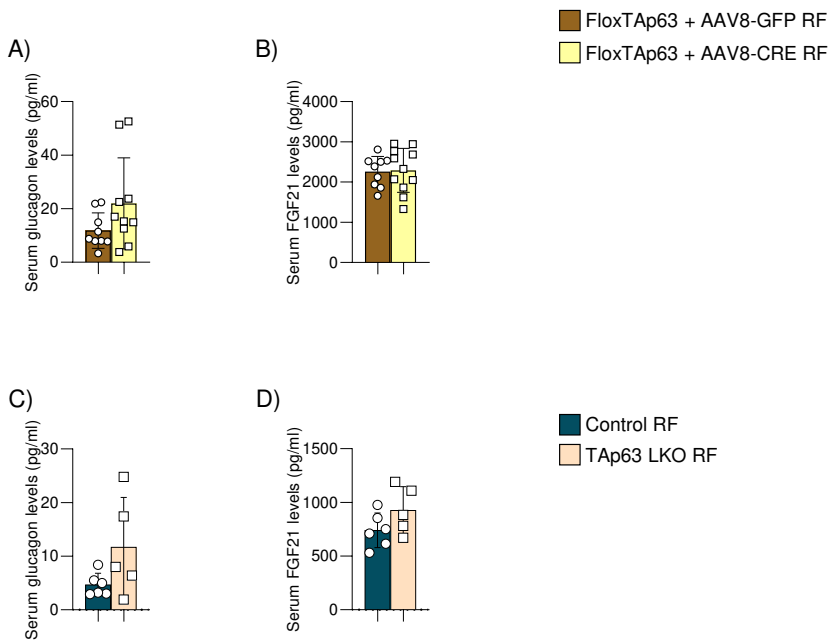
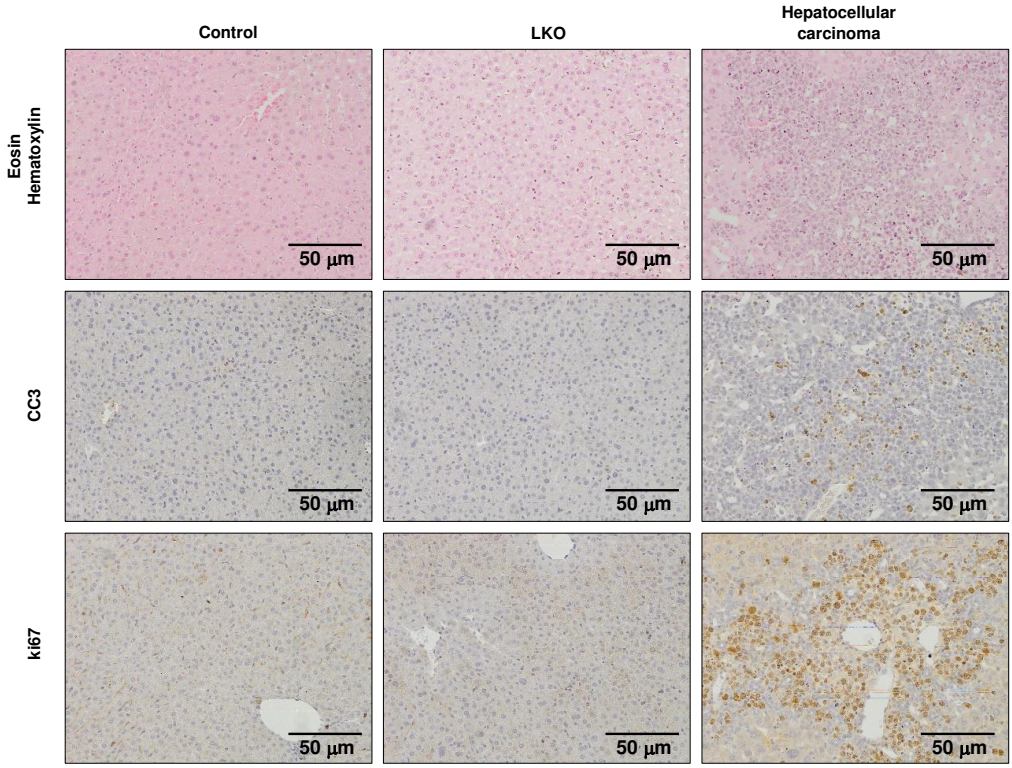
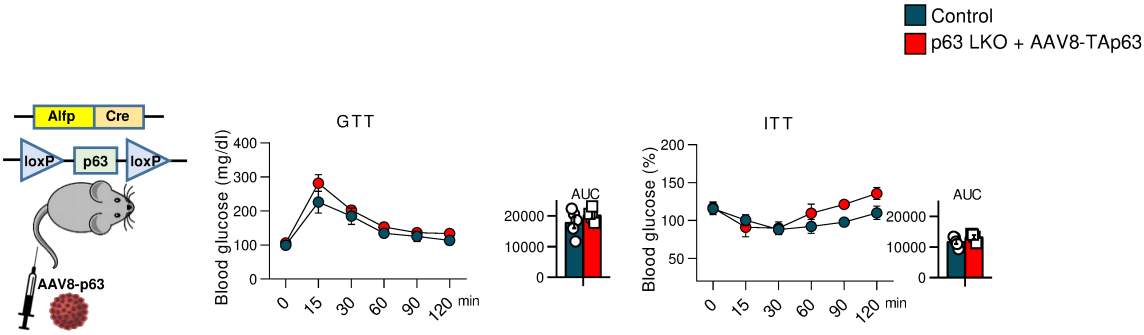


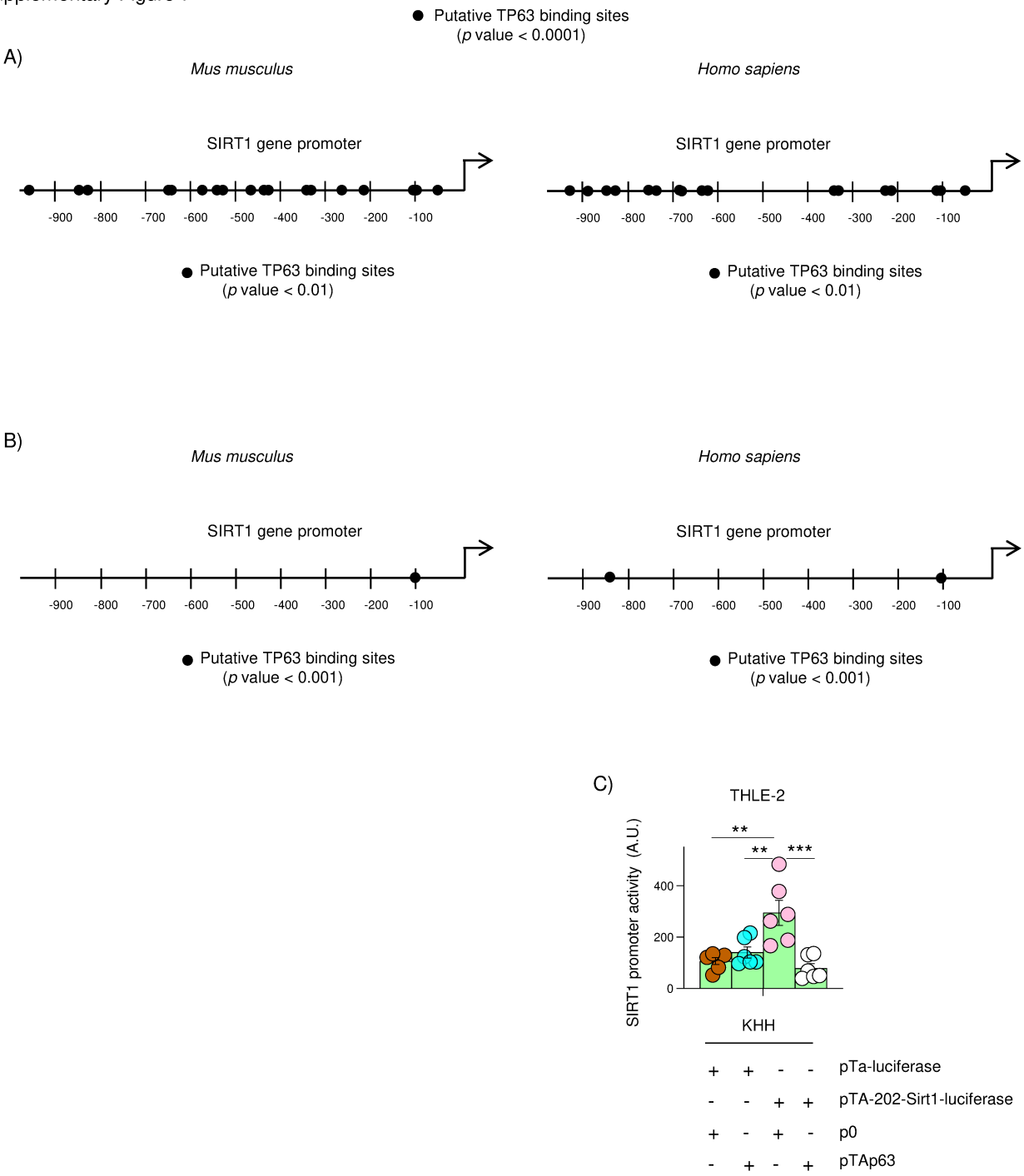
Supplementary Figure 5



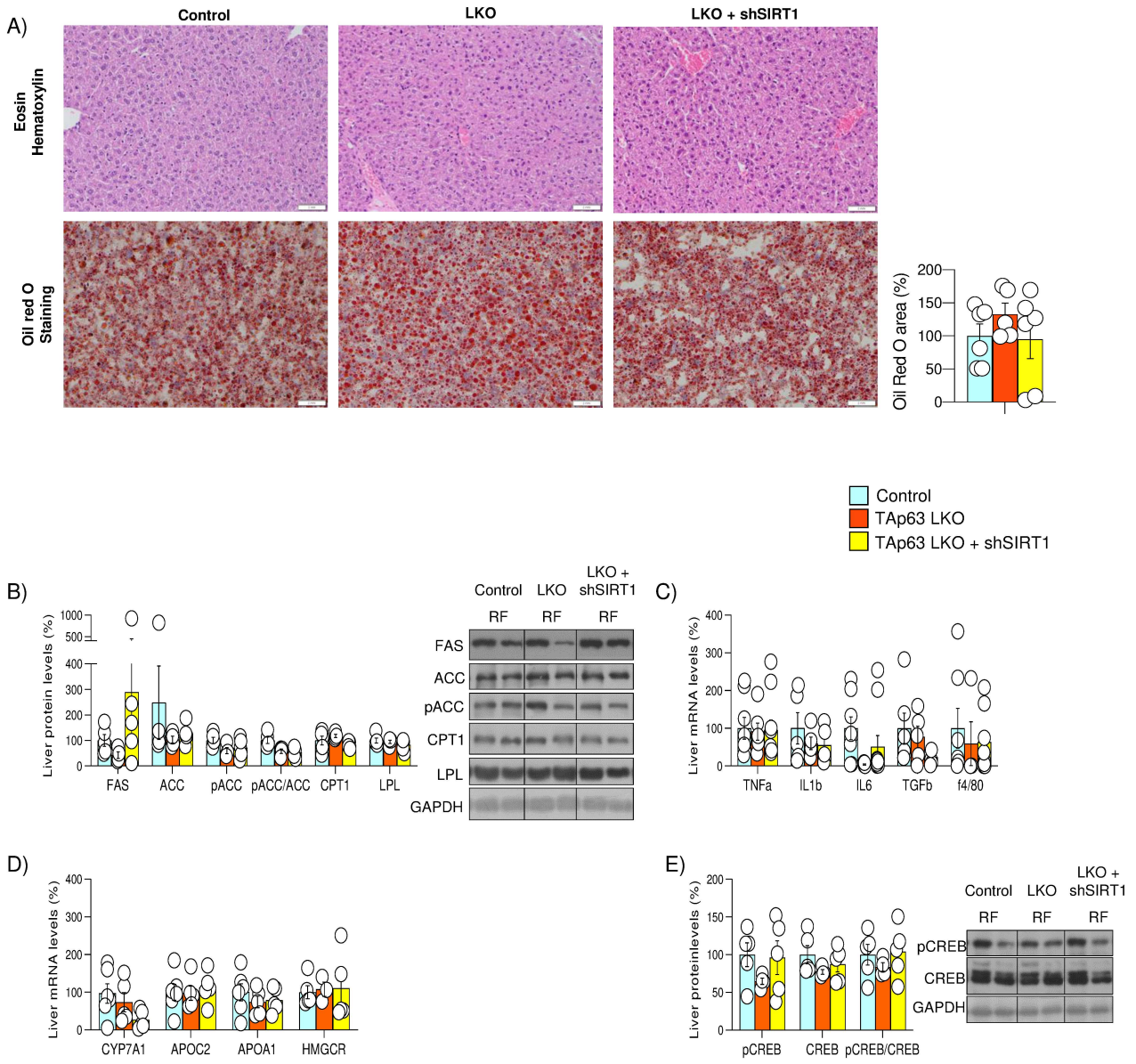
Supplementary Figure 6



Supplementary Figure 7

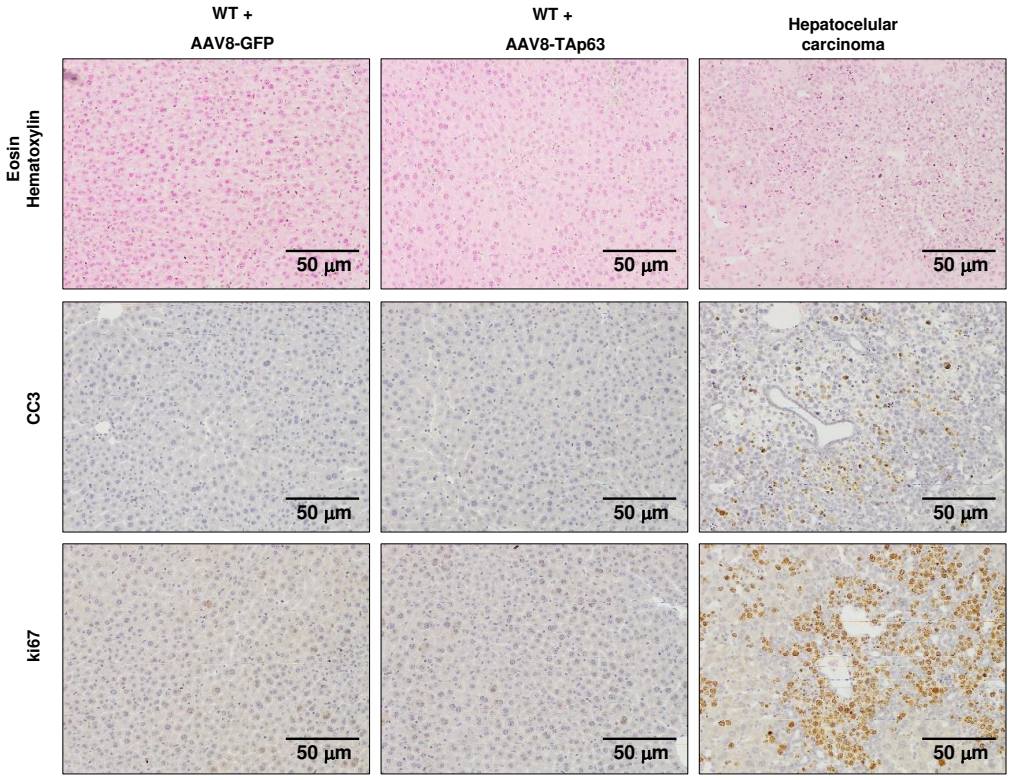


Supplementary Figure 8



Supplementary Figure 9

A)



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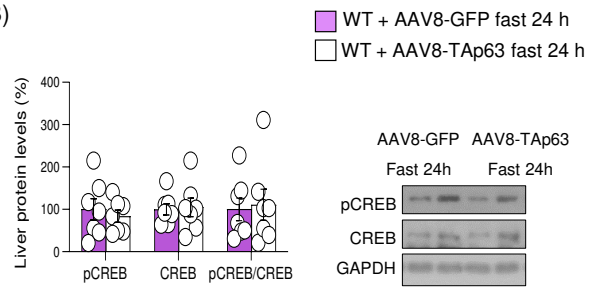

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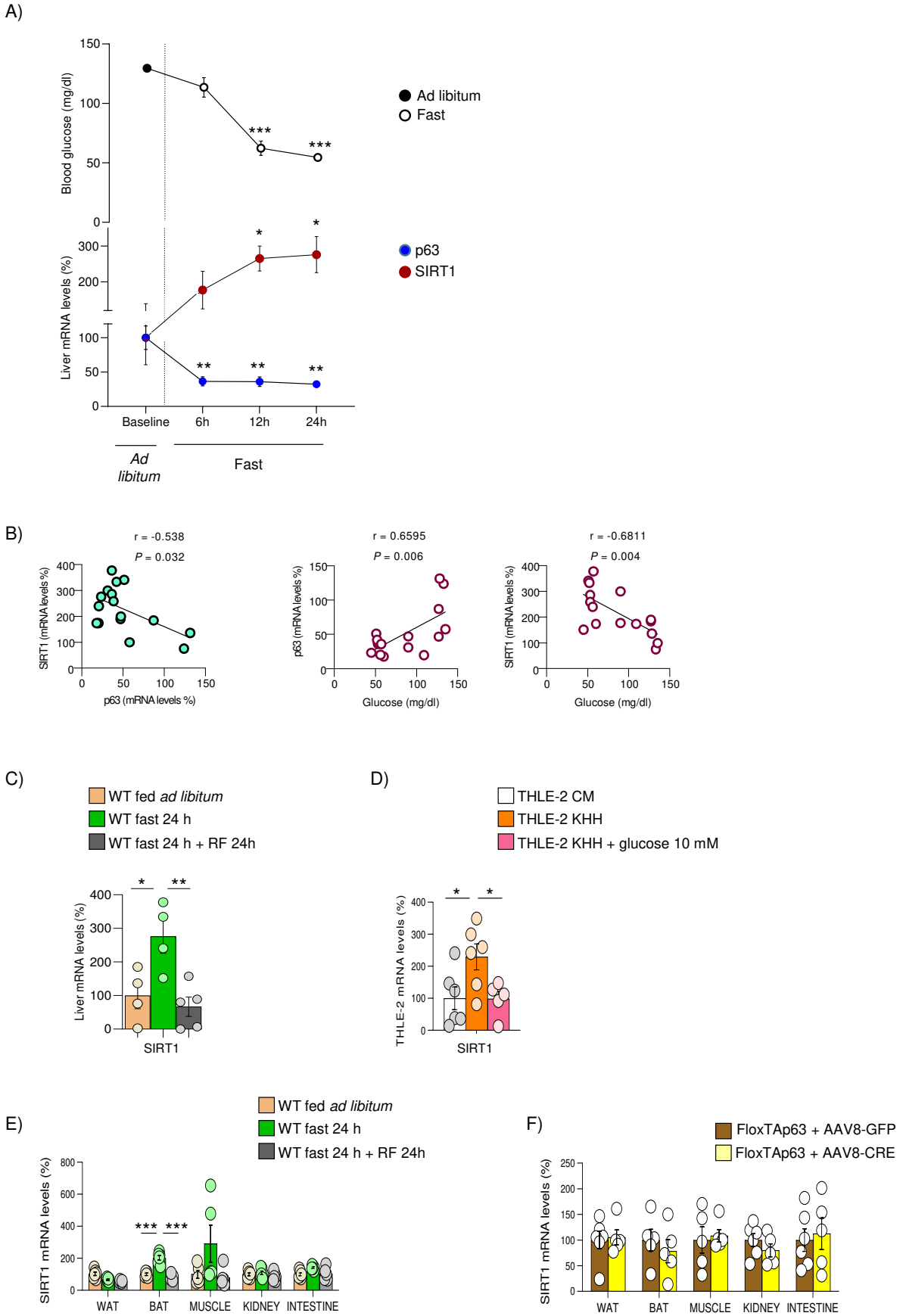

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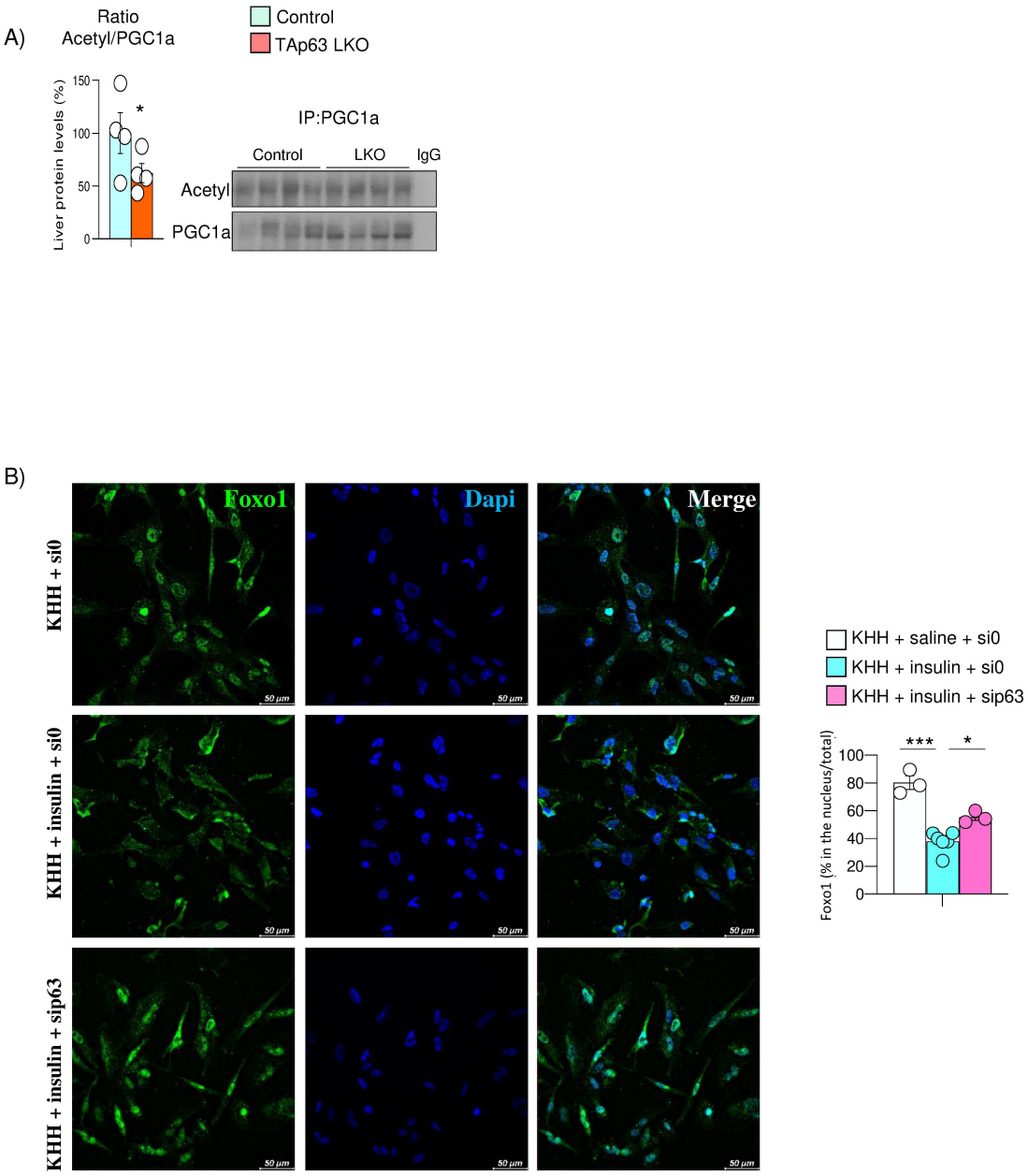


Supplementary Figure 12

Fig 2E

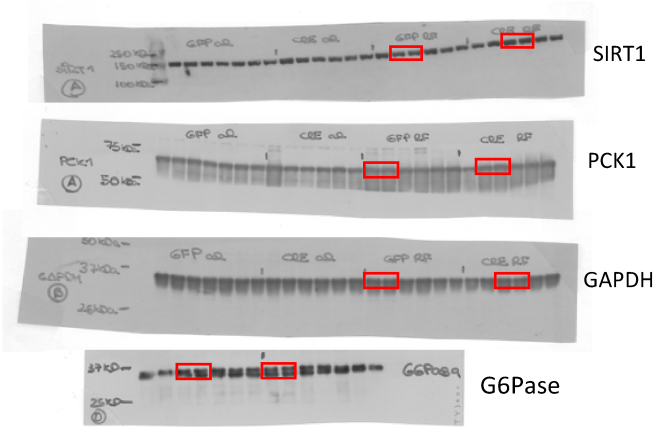
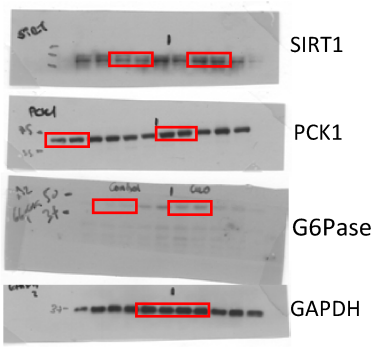


Fig 2J



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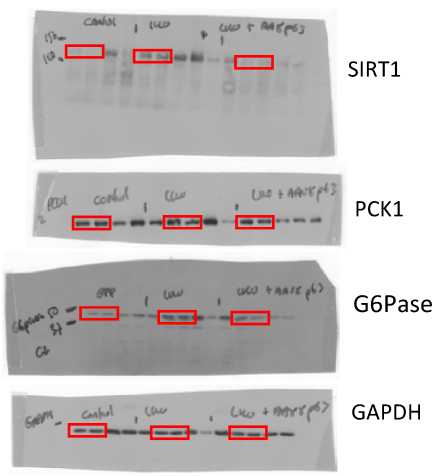


Supplementary Figure 12

Fig 4C

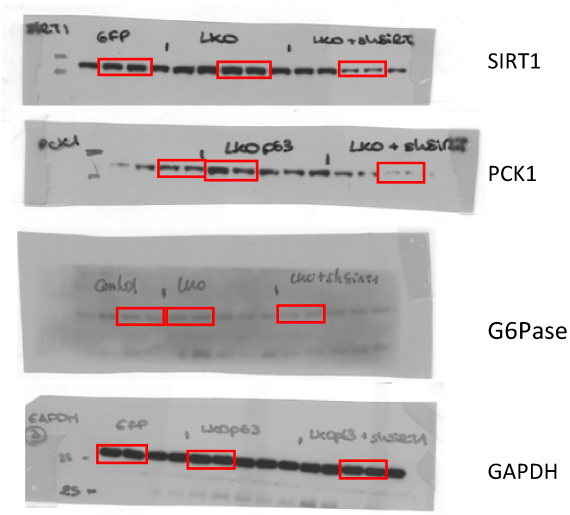


Fig 5G

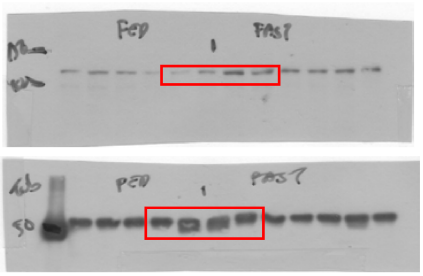
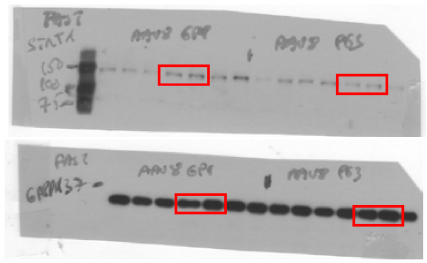


Fig 5I



Supplementary Figure 12

Fig 6D

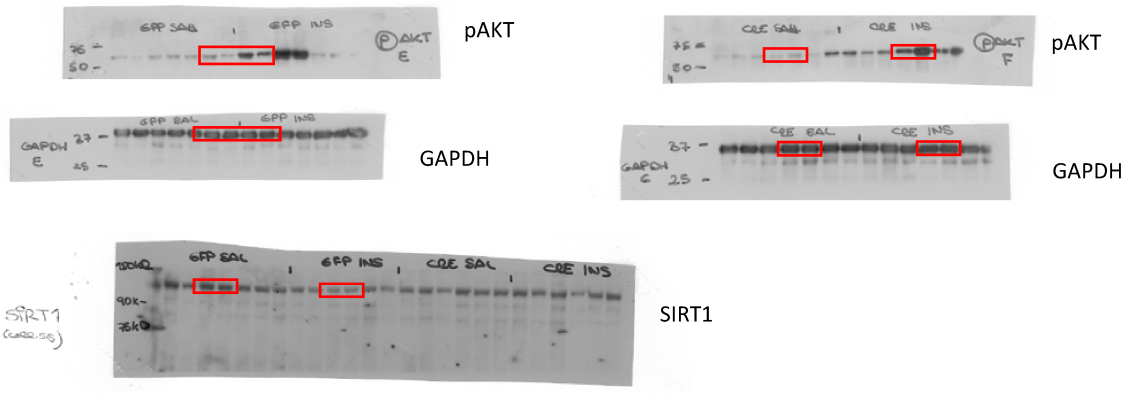


Fig 6E

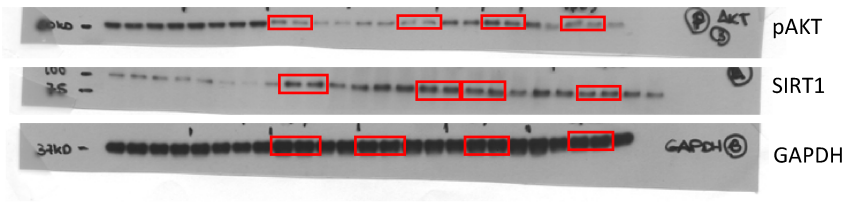
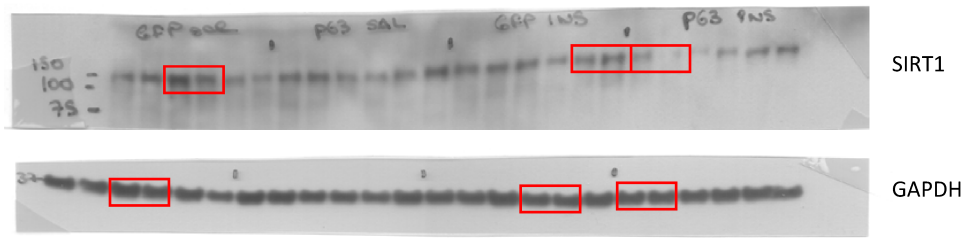
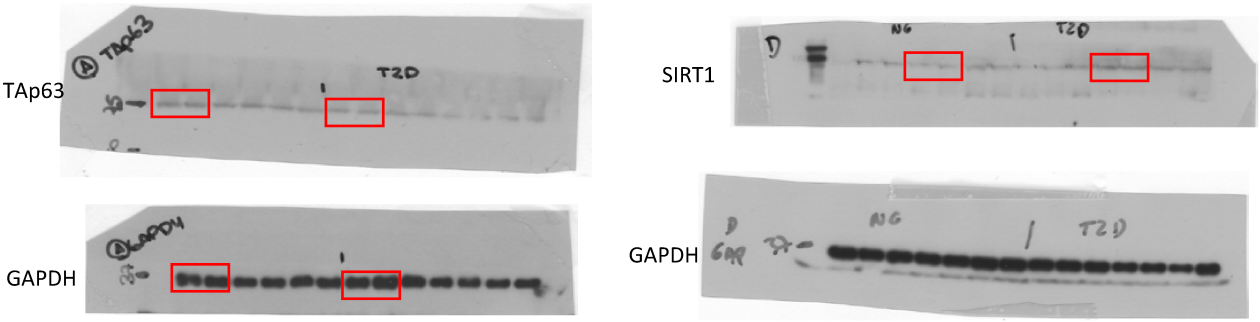


Fig 6G



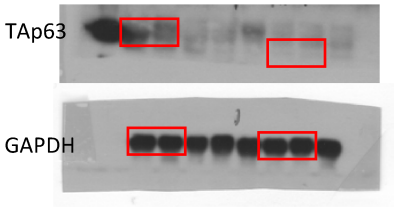
Supplementary Figure 12

Fig 7A



Supplementary Figure 12

Supplementary Fig 1E



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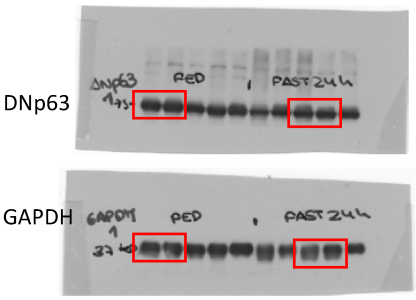
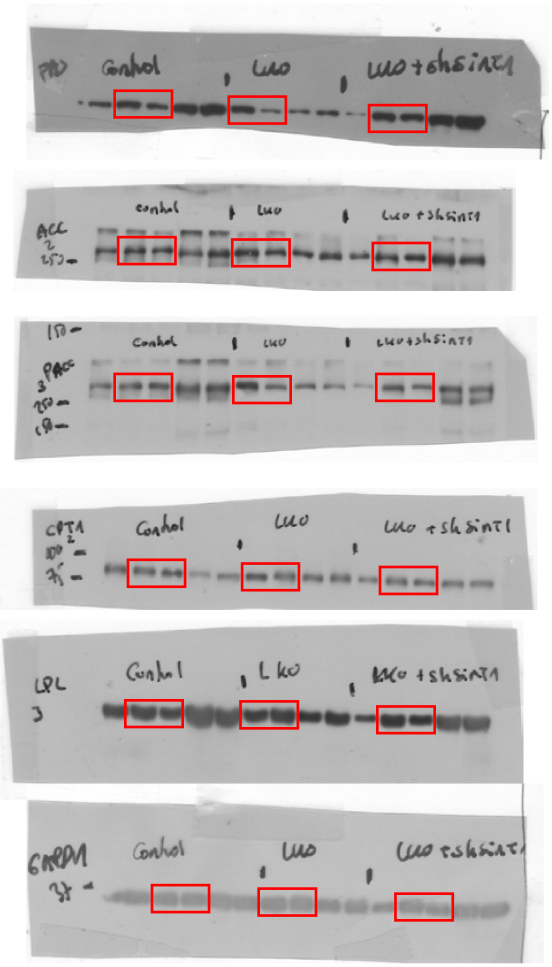


Supplementary Figure 12

Supplementary Fig 8B



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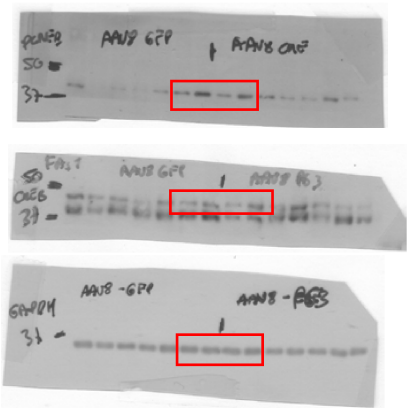

Supplementary Fig 8E