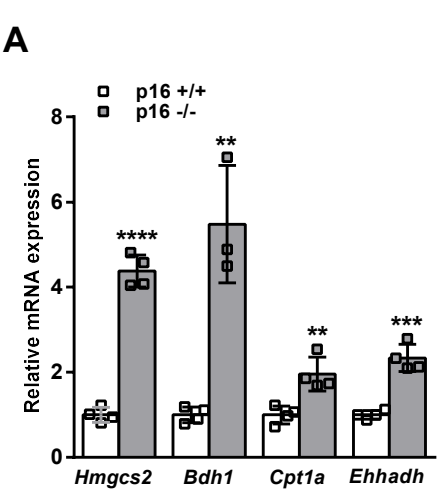

Figure S1. P16-deficiency increases fatty acid catabolism genes in primary hepatocytes. (**A**) *Hmgcs2*, *Bdh1*, *Cpt1α*, *Ehhadh* relative mRNA expression measured by QPCR in $p16^{+/+}$ and $p16^{-/-}$ primary hepatocytes.

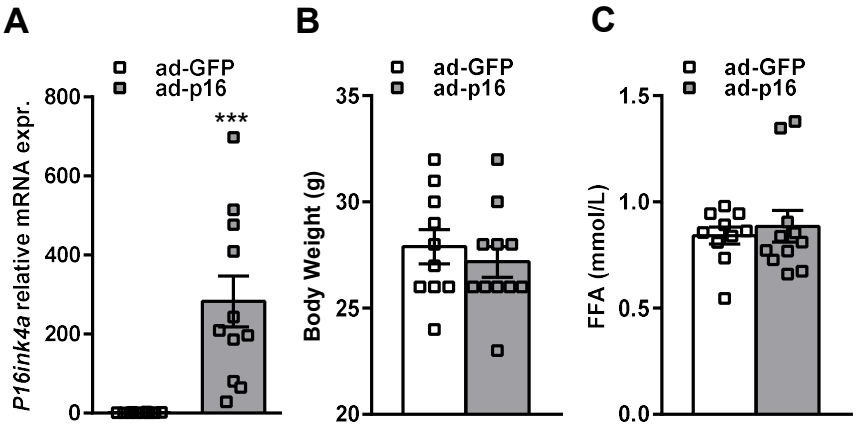

Figure S2. P16 overexpression has no impact on body weight and plasma FFA. (**A-C**) 12-week-old male mice were injected with adenovirus GFP (ad-GFP) (n=10) or adenovirus p16 (ad-*p16*) (n=11) (**A**) hepatic *P16ink4a* relative mRNA expression was measured by QPCR and (**B**) Body weight and (**C**) Circulating free fatty acids were measured after an overnight fasting. All values are expressed as means ± SEM. * compared to ad-GFP, t-test: *** $p<0.001$.

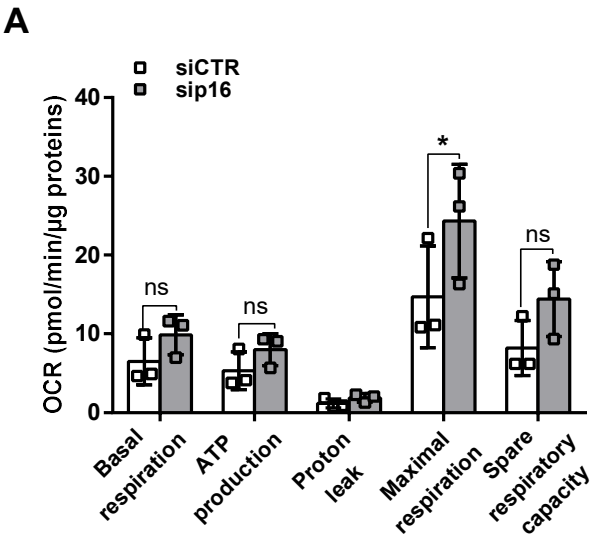

Figure S3. P16 silencing increases maximal respiration in IHH cells. (**A**) Bar graph of OCR measured by Seahorse apparatus in IHH cells transfected with siCTR or si*p16*. All values are expressed as means ± SD. * compared to siCTR; one-way ANOVA: * $p<0.05$.

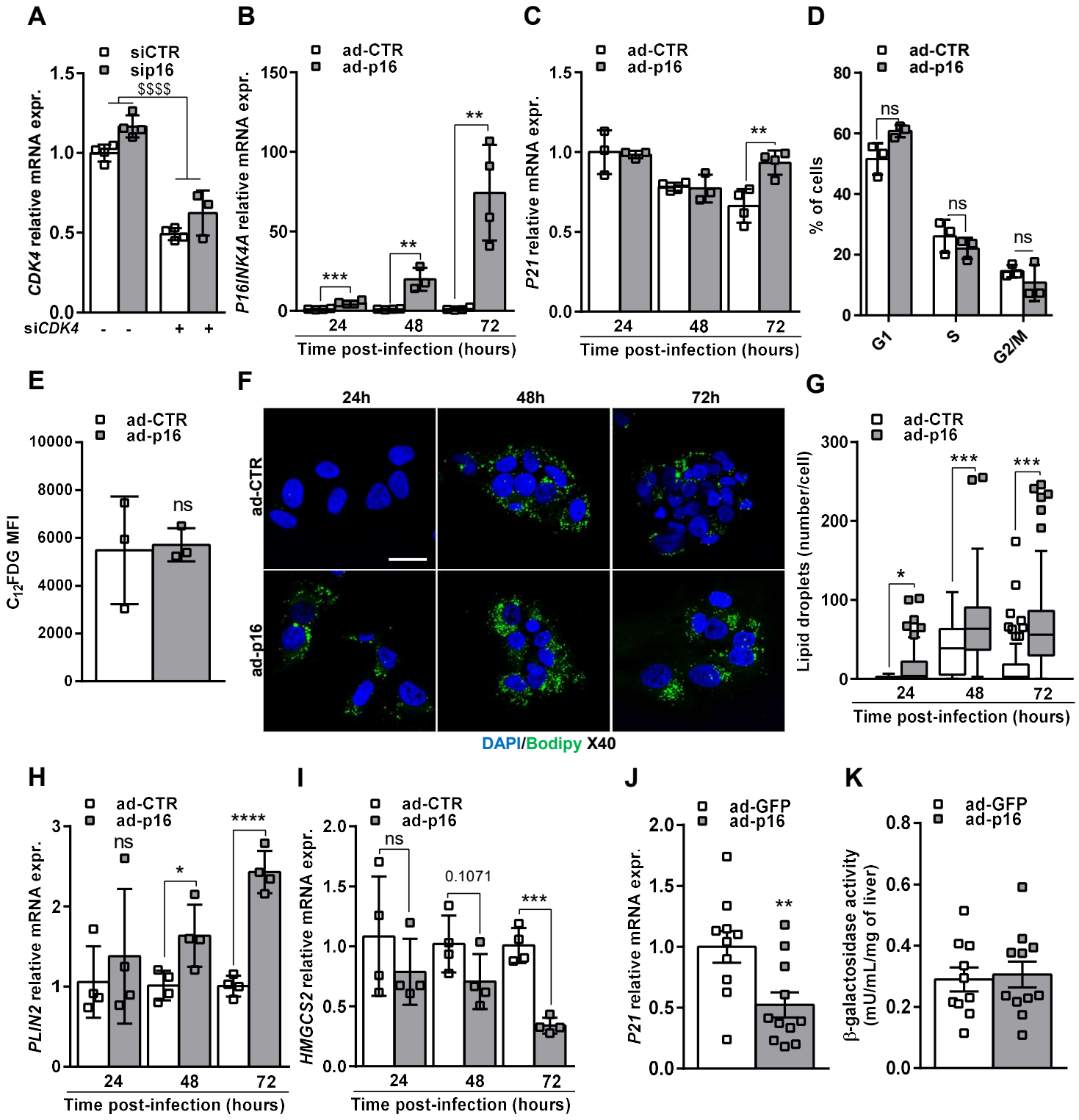

Figure S4. P16 overexpression does not modulate cell cycle and SA-β-Galactosidase activity in hepatocytes. (**A**) *CDK4* relative mRNA expression in IHH cells transfected with siCTR or si*p16* and cotransfected with si*CDK4*. (**B-I**) HepG2 cell line was infected with ad-CTR or ad-p16 for 24, 48 and 72h, (**B**) *P16* and (**C**) *P21* relative mRNA expression were measure by QPCR. (**D**) Percentage of cells in each phase of the cell cycle after propidium iodide staining were analyzed by FACS after 72h infection. (**E**) SA-β-Galactosidase activity was measured by C12FDG staining in HepG2 cells infected with ad-CTR or ad-p16 for 72 h. (**F**) Representative images of BODIPY 493/503 staining of neutral lipids; Scale bar = 20 µm. (**G**) Quantification of lipid droplets. (**H**) *PLIN2* and (**I**) *HMGCS2* relative mRNA expression were measured by QPCR. All values are expressed as means ± SD. * compared to siCTR of the same condition or ad-CTR, one-way ANOVA: **** p<0.0001; *** p<0.001, ** p<0.01 and * p<0.05. $ compared to siCTR or si*p16* in two different conditions (siCTR or si*CDK4*) $$$$ p<0.0001. (**J**) *P21* relative mRNA expression was measured by QPCR and (**K**) SA-β-Galactosidase activity was measured using Promokine beta-Galactosidase Activity Assay Kit (Promocell) in livers from ad-GFP (n=10) or ad-*p16* (n=11) infected mice. All values are expressed as means ± SEM. * compared to ad-GFP, unpaired t-test ** p<0.01.

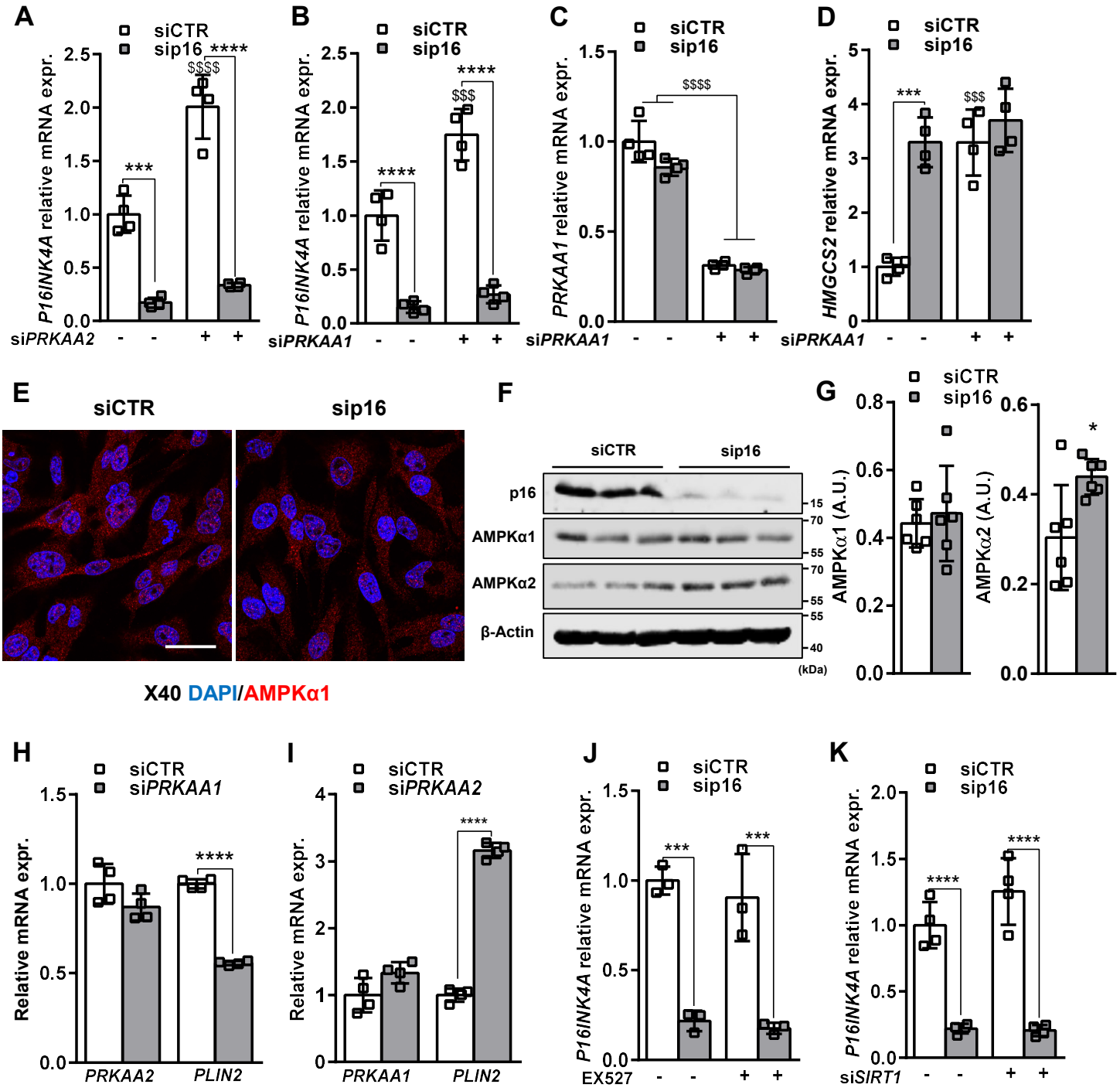

Figure S5. P16 modulates HMGCS2 expression via activation of AMPKα2 but not AMPKα1 in IHH cells. (**A**) Relative mRNA expression of *P16* measured by QPCR in IHH cells transfected with siCTR or si*p16* and cotransfected with si*PRKAA2*. (**B**) *P16* (**C**) *PRKAA1* and (**D**) *HMGCS2* relative mRNA expression measured by QPCR in IHH cells transfected with siCTR or si*p16* and cotransfected with si*PRKAA1*. (**E**) Representative images of AMPKα1 immunofluorescent staining in IHH cells transfected with siCTR or si*p16*. The threshold for red fluorescence was enhanced compared to AMPKα2 immunofluorescent staining to improve the visibility of AMPKα1; Scale bar = 20 µm. (**F**) Western blot assay showing the protein levels of p16, AMPKα1, AMPKα2 and β-actin and (**G**) quantification of AMPKα1 and AMPKα2 protein levels using β-actin as a loading control (pool of 2 separate experiments) in IHH cells transfected with siCTR or sip16. Relative mRNA expression of (**H**) *PRKAA2* and *PLIN2* and (**I**) *PRKAA1* and *PLIN2* in IHH cells transfected with si*PRKAA1* or si*PRKAA2*. (**J**) *P16* and (**K**) *SIRT1* relative mRNA expression in IHH cells transfected with siCTR or si*p16,* cotreated with EX527 or cotransfected with si*SIRT1* respectively. All values are expressed as mean ± SD. * compared to siCTR of the same condition (siCTR, si*PRKAA1*, si*PRKAA2*, si*SIRT1*, DMSO or EX527); one-way ANOVA (**A, B, C, D, H, I, J, K**) or unpaired t-test (**G**) : **** p<0.0001; *** p<0.001, ** p<0.01 and * p<0.05; $ compared to siCTR or sip16 in two different conditions (siCTR vs si*PRKAA1*, siCTR vs si*PRKAA2*, siCTR vs si*SIRT1* or DMSO vs EX527) $$$$ p<0.0001, $$$ p<0.001.

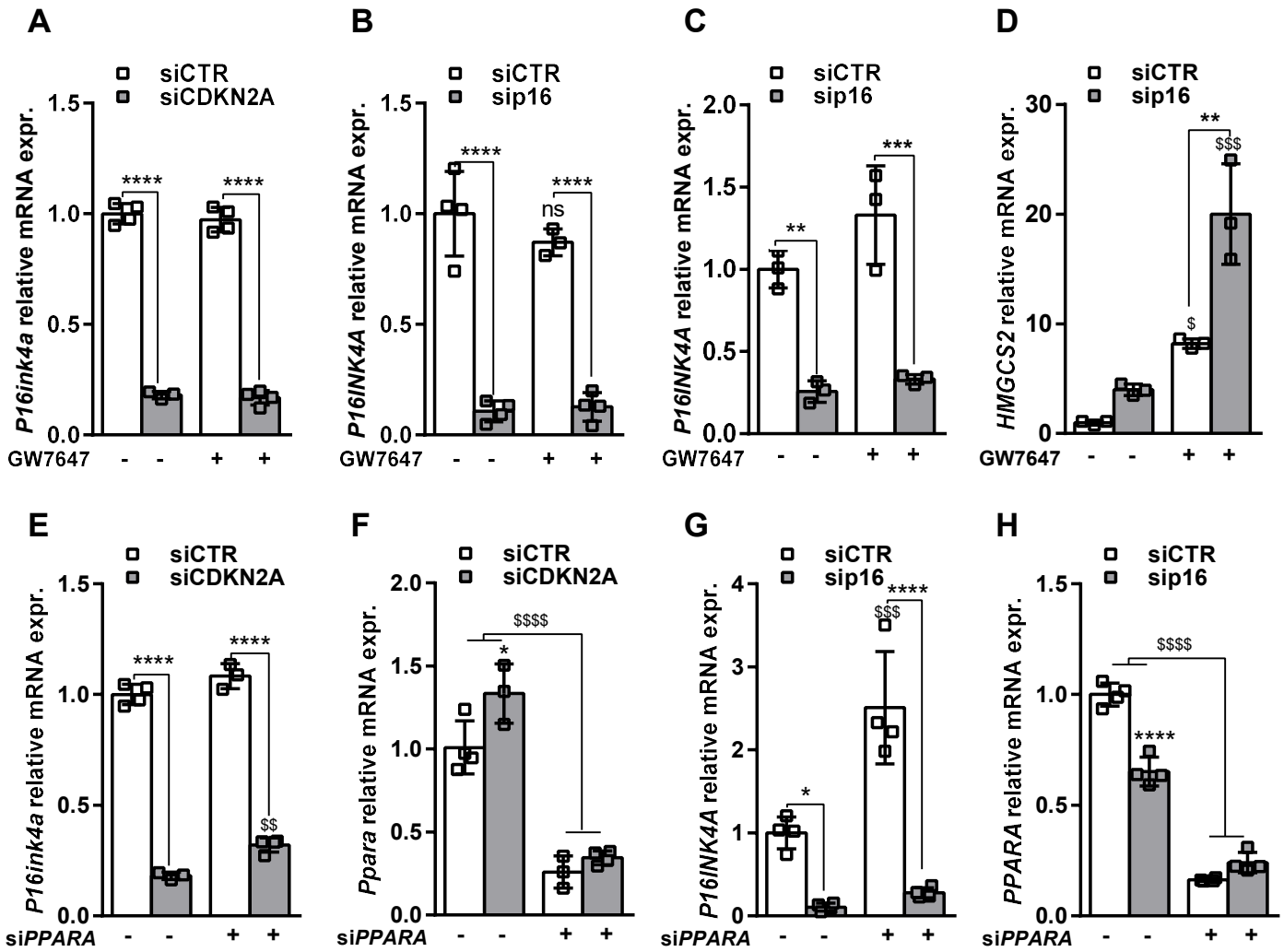

Figure S6. P16-deficiency increases fatty acid catabolism gene expression via activation of PPARα in hepatocytes. (A-B) *P16* relative mRNA expression measured by QPCR in (**A**) murine AML12 cell line transfected with siCTR or si*CDKN2A* and (**B**) in human IHH cell line transfected with siCTR or si*p16* and treated with 600 nM GW7647 or DMSO (control) for 8h. (**C**) *P16* and (**D**) *HMGCS2* relative mRNA expression measured by QPCR in human HepG2 cell line transfected with siCTR or si*p16* and treated with 600 nM GW7647 or DMSO. (**E**) *P16* and (**F**) *Ppara* relative mRNA expression measured by QPCR in the murine AML12 cell line transfected with siCTR or si*CDKN2A* and cotransfected with si*PPARA*. (**G**) *P16* and (**H**) *PPARA* relative mRNA expression measured by QPCR in the human IHH cell line transfected with siCTR or si*p16* and cotransfected with si*PPARA*. All values are expressed as means ± SD. * compared to siCTR of the same condition (DMSO, GW7647, siCTR or si*PPARA*); one-way ANOVA: **** p<0.0001; *** p<0.001, ** p<0.01 and * p<0.05; $ compared between two different conditions (DMSO vs GW7647 or siCTR vs si*PPARA*) $$$$ p<0.0001; $$$ p<0.001, $$ p<0.01 and $ p<0.05.

n = 910 patients	**median [IQR]**
Age (years)	43.0 [33.0, 51.0]
Sex (% men)	27.6%
Weight (kg)	129.0 [114.0, 146.0]
BMI (kg/m²)	45.8 [41.3, 51.3]
HbA_1c	5.9 [5.5, 6.6]
HOMA2	1.36 [0.47, 2.35]
Fasting Glucose (mmol/L)	5.6 [5.1, 6.8]
Plasma Triglycerides (mmol/L)	1.4 [1.1, 1.9]
Plasma Total Cholesterol (mmol/L)	4.9 [4.3, 5.5]
Plasma HDL-C (mmol/L)	1.1 [1.0, 1.3]

Table S1: Clinical parameters of obese patients used for liver transcriptomic analysis

SUPPLEMENTARY METHODS

SiRNA

Name	Sequence/reference	supplier
MOUSE		
Control-F	D-001810-10 Dharmacon ON-TARGETplus Non-targeting Pool	Horizon
Control-R		
CDKN2A-F	L-043107-00 Dharmacon SMARTpool ON-TARGETplus	Horizon
CDKN2A-R		
PPARα-F	L-040740-01 Dharmacon SMARTpool: ON-TARGETplus	Horizon
PPARα-R		
HUMAN		
Control-F	AGGUAGUGUAAUCGCCUUG	EUROFINS GENOMICS
Control-R	CAAGGCGAUUACACUACCU	
P16-F	CGCACCGAAUAGUUACGGU	EUROFINS GENOMICS
P16-R	ACCGUAACUAUUCGGUGCG	
PPARα-F	CUUCUUUCGGCGAACGAUU	EUROFINS GENOMICS
PPARα-R	AAUCGUUCGCCGAAAGAAG	
CDK4-F	M-003238-02 Dharmacon siGENOME	Horizon
CDK4-R		
PRKAA1-F	M-005027 Dharmacon SMARTPOOL siGENOME	Horizon
PRKAA1-R		
PRKAA2-F	M-005361 Dharmacon SMARTpool siGENOME	Horizon
PRKAA2-R		
SIRT1-F	M-003540 Dharmacon SMARTpool siGENOME	Horizon
SIRT1-R		

QPCR primers

Name	Sequence
HOUSEKEEPING GENE	
Cyclophilin-F	GCATACGGGTCCTGGCATCTTGTCC
Cyclophilin-R	ATGGTGATCTTCTTGCTGGTCTTGC
MOUSE	
P16-F	TTGAGCAGAAGAGCTGCTACGT
P16-R	CGTACCCCGATTCAGGTGAT
PPARα-F	ATCGCGTACGGCAATGGCTTTA
PPARα-R	CAGGCCGATCTCCACAGCAAATTA
HMGCS2-F	CAGCCTCCCTCTTCAATGCT
HMGCS2-R	ACCACTCGGGTAGACTGCAA
BDH1-F	GCGCACCACAAAATCCTTCC
BDH1-R	TGGTGATGCAGTATGGCGAG
CPT1α-F	CATCATGACTATGCGCTACTC
CPT1α-R	CAGTGCTGTCATGCGTTGG
EHHADH-F	TTGCCAATGCAAAGGCTCGT
EHHADH-R	GCAACAGGAACTCCAACGAC
PLIN2-F	GATTGAATTCGCCAGGAAGA
PLIN2-R	TGGCATGTAGTCTGGAGCTG

P21-F	GTGGCCTTGTCGCTGTCT
P21-R	GACCAATCTGCGCTTGGAGTG
HUMAN	
P16-F	GAGCAGCATGGAGCCTTC
P16-R	GGCCTCCGACCGTAACTATT
PPARα-F	CCAGCGTCTTCTCAGCCATACACAG
PPARα-R	TTTCTGTCGGGATGTCACACAACG
HMGCS2-F	AGGCTGGAAGTAGGCACTGA
HMGCS2-R	GTGGGACGAGCATTACCACT
PLIN2-F	GGGCTAGACAGGATTGAGGA
PLIN2-R	GCCCCAGTCACAGTAGTCGT
CPT1α-F	TCTGGCTGGACATGCAGTTGGC
CPT1α-R	TATCGTGGTGGTGGGCGTGATG
EHHADH-F	GGTCAACGCGATCAGTACGAC
EHHADH-R	CTGAAGCCACGAATATCAGCAC
PRKAA1-F	GGCTCACCCAACTATGCTG
PRKAA1-R	GAGAATAACCCCACTGCTCCA
PRKAA2-F	GCAGCACCTGAAGTCATCTC
PRKAA2-R	TCATCAAATGGGAGGGTGCC
CDK4-F	GTGGACATGTGGAGTGTTGG
CDK4-R	CGGCTTCAGAGTTTCCACAG
SIRT1-F	GCAGATTAGTAGGCGGCTTG
SIRT1-R	TCTGGCATGTCCCACTATCA

Oxygen consumption profile.

Basal oxygen consumption rate (OCR) was normalized by subtracting the minimum rate after rotenone and antimycin A addition. The remaining oxygen consumption referred to non-mitochondrial OCR. ATP production-linked oxygen consumption was calculated as the difference between basal oxygen consumption and OCR, measured after the addition of oligomycin. Proton leak was calculated as the difference between OCR obtained after the addition of oligomycin and non-mitochondrial OCR. Maximal OCR was determined by subtraction the non-mitochondrial OCR from the maximal OCR reading after the addition of FCCP. The spare respiratory capacity was calculated by subtraction basal OCR from maximum OCR.

β-Galactosidase activity analysis

Beta-galactosidase activity assays were performed using the Promokine Assay Kit (Promocell) according to the manufacturer's instructions. Briefly, five milligrams of liver from each mouse were weighed and lysed in 100 µL of beta-Gal Assay Buffer, followed by centrifugation at 10,000g for 5 min at 4°C. The supernatants of each sample were loaded in duplicate in a 96-well plate and diluted 1:2.5 in the beta-Gal Assay Buffer. The fluorescein standard and the beta-Gal Positive Control were prepared as described in the protocol and loaded in duplicate. Immediately after addition of the Reaction Mix to the wells, the plate was loaded in a SpectraMax® i3 microplate reader (Molecular Devices). Fluorescence was measured (Ex/Em = 480/520 nm) every 5 min for 30 or 60 min at 37°C. The measures from T20min and T30min

were used for the calculation of beta-Galactosidase activity. Data are presented as mU/mL/mg of liver.

Senescence-Associated (SA)-β-Galactosidase staining

C12FDG (Invitrogen) and SA-β-Galactosidase (Thermo fisher) staining were done according to Debacq-Chainiaux et al. (45). C12FDG fluorescence was analyzed with blue laser 488 nm and 530 nm emission filter by flow cytometry with BD LSR Fortessa X-20 cell analyzer (BD Biosciences). SA-βGal activity related to C12FDG staining was estimated using the mean fluorescence intensity (MFI) of the live single-cell population after removing autofluorescence. SA-β-Galactosidase positive cells were counted in 15 fields with phase-contrast microscopy.