PGC-1α isoforms coordinate to balance hepatic metabolism and apoptosis in inflammatory environments

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Short title: PGC1A4 inhibits hepatocyte apoptosis

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ABSTRACT

Liver is exposed to changing metabolic and inflammatory environments. It must sense and adapt to metabolic need while balancing resources required to protect itself from insult. PGC-1α is a transcriptional coactivator that both coordinates metabolic adaptation to diverse stimuli and protects against inflammation in several tissues. However, it is not known how PGC-1α integrates extracellular signals to balance metabolic and anti-inflammatory outcomes. PGC-1α exists as multiple, alternatively spliced variants expressed from different promoters. We show in human liver, NALFD/NASH preferentially activated the alternative *PPARGC1A* promoter. Gene expression analysis in primary mouse hepatocytes identified shared and isoform-specific roles for PGC-1α variants in response to TNFα. PGC-1α1 primarily impacted gene programs of nutrient and mitochondrial metabolism, while TNFα signaling revealed that PGC-1α4 influenced several pathways related to innate immunity and cell death. Gain- and loss-of-function models showed that PGC-1α4 specifically enhanced expression of anti-apoptotic gene programs and attenuated hepatocyte apoptosis in response to TNFα or LPS. This was in contrast to PGC-1α1, which reduced expression of a wide inflammatory gene network, but did not prevent liver cell death. We conclude that PGC-1α variants have distinct, yet complimentary roles in hepatic responses to inflammation and identify PGC-1α4 as an important mitigator of apoptosis.

Keywords: PGC-1 isoforms, PPARGC1A, cell death, inflammation, NASH, NAFLD, apoptosis, liver, hepatic metabolism

INTRODUCTION

The liver is a vital organ with various physiological roles in metabolism, detoxification, and innate immunity. Before entering the systemic circulation, blood enters the liver via the portal vein and is filtered through the sinusoids. Thus, the liver is one of the first organs to encounter absorbed nutrients as well as microbial products from the gastrointestinal tract, some of which are toxins, antigens, and other metabolites that can cause inflammation. The unique anatomical architecture of the liver allows it to perform a broad range of metabolic functions, but at the same time it must exert powerful immunocompetence, surveilling portal blood and acting as a protective barrier (Bogdanos, Gao, & Gershwin, 2013). The liver must adapt quickly to various metabolic and inflammatory signals from the digestive tract or systemic circulation, concurrently responding to changing glucose and lipid homeostasis, while integrating inflammatory signals, and if needed, initiating an immune response. Importantly, hepatic metabolism can be reprogramed by an inflammatory response (Ganeshan et al., 2019), allowing a trade-off between energy destined for nutrient metabolism versus tolerance to infection. However, mechanisms helping to balance metabolic demand with inflammatory response are not clear.

The peroxisome proliferator activated receptor gamma coactivator-1 alpha (PGC-1α) regulates many transcriptional programs related to nutrient metabolism, energy homeostasis and mitochondrial respiration (Wu et al., 1999) by binding to nuclear receptors and other transcription factors to enhance their activity (Kelly & Scarpulla, 2004). PGC-1α is important for mitochondrial homeostasis in several tissues, but also activates expression of gene programs within a broader set of biological functions. For example, PGC-1α is induced in skeletal muscle during exercise and stimulates expression of genes involved in fiber type switching, angiogenesis and regulation of the neuromuscular junction (Arany et al., 2008; Baar et al., 2002; Handschin et

al., 2007; Lin et al., 2002). In liver, PGC-1α is induced during fasting and to increase gluconeogenesis, heme biosynthesis, modulate insulin response and enhance fatty acid oxidation (Besse-Patin et al., 2019; Estall, Kahn, et al., 2009; Estall, Ruas, et al., 2009; Yoon et al., 2001).

Evidence suggests that PGC-1α is also an essential component of the inflammatory response, but mechanisms for this are unclear. Over-expression in muscle protects mice from disease, exercise, and age-related inflammatory damage (Chan et al., 2014; Dinulovic et al., 2016; Eisele, Furrer, Beer, & Handschin, 2015; Eisele, Salatino, Sobek, Hottiger, & Handschin, 2013) and preservation of PGC-1α activity blunts lipopolysaccharide (LPS)-induced inflammatory damage to heart and kidney (Schilling et al., 2011; Tran et al., 2011). On the other hand, reduced PGC-1α increases pro-inflammatory cytokine expression and increases inflammation damage to muscle and liver tissue in response to stresses (Besse-Patin et al., 2017; Eisele et al., 2015; Sczelecki et al., 2014). Over-expression of PGC-1α decreases expression of pro-inflammatory cytokines, while simultaneously inducing expression of secreted anti-inflammatory factors (Buler et al., 2012; Eisele et al., 2015) that can feed back to dampen inflammatory signaling. How PGC-1α regulates the inflammatory response within cells is not yet understood.

Although PGC-1α is a coactivator, data suggest that PGC-1α may indirectly represses NF-κB target gene transcription though coactivation of anti-inflammatory transcriptional networks linked to PPARs (Eisele et al., 2013). It may also bind to the p65 subunit of nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) (Alvarez-Guardia et al., 2010), but it is unclear whether this leads to direct repression of its activity. Thus, mechanistic understanding of links between inflammatory signaling and PGC-1α activity remain limited and likely goes beyond regulation of NF-κB. Data support PGC-1α as an important regulator of both mitochondria metabolism and inflammation, yet it is not known how PGC-1α integrates multiple extra-cellular signals to coordinate and balance each cellular response. In this study, we show that differentially

97 spliced variants of the PGC-1α protein have unique functions in regulating hepatocyte responses
98 to concurrently integrate metabolic and inflammatory signals.
99
100 **MATERIALS AND METHODS:**
101 *Mice*
102 Mice with a floxed *Ppargc1a* allele (B6.129-*Ppargc1a*$^{tm2.1Brsp}$/J) were crossed with mice
103 expressing Cre-recombinase under control of the albumin promoter Tg(Alb-cre)$^{21Mgn/J}$. Interbred
104 mice created hepatocyte-specific PGC-1α knockout mice (LKO: *Ppargc1a*$^{fl/fl,\ Alb\text{-}cre}$) and littermate
105 controls (WT:*Ppargc1a*$^{fl/fl}$ and Alb-Cre+). Age-matched, male mice on a C57BL/6J background
106 were used. For tissue-specific PGC-1α4 over-expression (^{LSL}PGC-1α4), tdTomato was replaced
107 with murine PGC-1α4 cDNA in the Ai9 vector downstream of the Lox-stop-Lox signal
108 (Supplemental Fig. S1). Recombination at the *ROSA26* locus was confirmed in neomycin-resistant
109 C57BL/6 embryonic stem cells clones and founder mice backcrossed 10 generations onto a
110 C57BL/6N background. Genotyping primers are listed in Supplemental Table S1.
111 The *Ppargc1a* Alternative Promoter Knock-out mouse line (AltPromKO) was generated by
112 InGenious Targeting Laboratory (Ronkonkoma, New York). Briefly, a targeting construct was
113 used to insert LoxP sites flanking exon 1b and 1b' of the alternative *Ppargc1a* promoter
114 (Supplemental Fig. S1). Recombination was confirmed in C57BL/6 embryonic stem cells and
115 founder mice backcrossed three times with C57BL/6N mice. Experiments were performed in
116 accordance with IRCM institutional animal care and use committee regulations.
117
118 *Mouse housing, diets, and lipopolysaccharide treatment*
119 Mice were maintained on *ad libitum* chow (Tekland #2918) at 22°C (12h light/dark cycle). For *in*
120 *vivo* model of steatohepatitis, mice were fed a methionine-choline deficient (MCD) diet

(A02082002B, Research Diets) or matched control diet (A02082003B) starting at 8 weeks of age for up to 42 days. Serum alanine aminotransferase (ALT) was measured by Liquid ALT (SGPT) kit (Pointe Scientific). For LPS treatment, livers of 10-week-old male or female mice were harvested 6 hours after tail-vein injection of LPS (2 mg/kg, Invivogen) or vehicle (PBS).

Primary hepatocyte isolation and treatment

Primary mouse hepatocytes from 12-week-old mice were isolated by two-step liberase perfusion (Liberase TL, Roche) and 50% Percoll gradient purification (Besse-Patin et al., 2017). Cells were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 0.2% BSA (Fatty acid free, Fisher Scientific), 25 mM glucose, 2 mM sodium pyruvate, 0.1 μM dexamethasone, 1% Penicillin/Streptomycin and 1 nM insulin. One day after isolation, hepatocytes were infected with adenovirus (5 MOI) overnight and starved of insulin and dexamethasone for 24 hours prior to treatment with TNFα (Fitzgerald) at 2 ng/mL for 2 hours for signaling/gene expression, or 20 ng/mL for 8 hours for apoptosis. Apoptosis was measured by Cell Death Detection ELISA (Roche). For reporter assays, cells were transfected (Lipofectamine) with a construct expressing firefly luciferase downstream of 3x NF-κB response elements. Activity was normalized to total protein following quantification using the Dual Luciferase Reporter Assay System (Promega).

Protein isolation, immunoprecipitation and immunoblotting

Proteins were homogenized/solubilized in radioimmunoprecipitation assay buffer containing protease and phosphatase inhibitors. Hepatic PGC-1α was immunoprecipitated from liver using anti-PGC-1α (Millipore, ST1202) in 1% Triton/TBS. Elutes and total proteins were resolved by SDS-PAGE, blotted, and probed with antibodies (Supplemental Table S2).

Immunofluorescence

H2.35 cells cultured in DMEM, supplemented with 10% Fetal Bovine Serum (FBS, Wisent), 1% penicillin/streptomycin, 0.2 μM dexamethasone were incubated on poly-L-lysine coated coverslips and transfected with V5-tagged PGC-1α variants for 24 hours (Lipofectamine). Cells were starved overnight of dexamethasone prior to TNFα treatment (50 ng/ml) for 3 hours and fixation with 4% paraformaldehyde. Triton-permeabilized cells were incubated with anti-V5 antibody overnight, followed by Alexa 488-conjugated secondary antibody to visualize proteins.

Cell fractionation

H2.35 cells transduced with adenovirus expressing control vector, PGC-1α1 or PGC-1α4 were starved overnight of dexamethasone prior to TNFα treatment (50 ng/ml) for 3 hours. Cell pellets were washed in PBS and resuspended in Lysis Buffer (10 mM Hepes (pH 7.5), 10 mM KCl, 3 mM $MgCl_2$, 0.35 M sucrose, 0.1% NP40, 3 mM 2-mercaptoethanol, 0.4 mM PMSF, 1 uM pepstatin A, 1 uM leupeptin and 5 ug/ml aprotinin). After centrifugation, supernatants were kept as cytoplasmic fraction. The pellet (nuclear fraction) was washed twice with lysis buffer, resuspended in Buffer A (3 mM EDTA, 0.2 mM EGTA, 1 mM dithiothreitol, 100 mM NaCl and 0.8% NP40) and sonicated for 10 minutes (cycles of 30 seconds ON and 30 seconds OFF). Equal amount of proteins were resolved by SDS-PAGE.

Microarray and Gene set enrichment analysis

mRNA was isolated from primary mouse hepatocytes infected with adenovirus expressing PGC-1α1, PGC-1α4 or vector control treated with 2 ng/mL TNFα or vehicle (PBS) for 2 hours (n = 3) and gene expression profiles generated using Affymetrix Mouse Genome 430 2.0 Arrays. Raw CEL

files were normalized using RMA [PMID: 12925520] and annotated using biomaRt [PMID: 16082012]. Raw data and sample annotation are available on GEO (GSE132458).

Gene set enrichment analysis was performed using javaGSEA software (version 3.0 – build: 01600) on chip data using the Gene Ontology processes (number of permutations = 1000, Permutation type = gene_set, Chip platform = Affy_430_2.0_mgi (version 2011) from the Mouse Genome Database. The *Ppargc1a* probe (1434099_at) was removed prior to analysis to eliminate over-expression bias. Full GSEA results are provided in Supplemental File 1. A MySQL database generated lists of genes significantly regulated (adj. p-value < 0.01, Log10 FC ≥ 0.3 or ≤ -0.3). Full lists are provided in Supplemental Files 2 (untreated samples) and 3 (TNFα–treated samples).

Clustering based on PGC-1α4-regulated genes was performed using dChip software. Over-representation analysis (ORA) of Gene Ontology processes was performed using ClusterProfiler and the mouse genome-wide annotation in R (www.r-project.org). The top 10 statistically over-represented processes were determined for each condition, merged in to one list, and represented as a dot plot (adj. p-value < 0.05, correction method = Bonferroni). For 175 genes regulated oppositely by the variants, ORA was performed using g:Profiler (adj. p-value < 0.05, correction method = g:SCS threshold). Gene lists were evaluated for enrichment of transcription factor signatures and binding sites in the proximal promoters and distant regulatory elements using iRegulon and DiRE (http://dire.code.org) with default analysis settings.

RNA isolation, PCR, and quantitative RT-PCR.

RNA was isolated from frozen tissue or cells using TRIzol (Invitrogen). 1 μg of RNA treated with DNase I was reverse-transcribed using the High Capacity Reverse Transcription Kit (Applied Biosystems). cDNA was quantified using SYBR Green PCR master mix (Bioline) and normalized to Hypoxanthine-guanine phosphoribosyltransferase (*Hprt*) mRNA using the ΔΔCt threshold cycle

method. Presence or absence of PGC-1α variants was confirmed using isoform-specific primers by conventional PCR and sequencing (Supplemental Table S3).

Patients and liver samples

Human liver samples were collected from 38 subjects age 33-81 years (Low 3 M: 4 W, NAFLD 10 M: 4 W, NASH 6 M: 3 W, Cirrhotic 4 M: 4 W) undergoing hepatic resection at the McGill University Health Centre after informed consent obtained. Samples were snap-frozen and stored at -80°C. Specimens were scored by a pathologist and classified based on NAFLD Activity Score (NAS: Low =<2, NAFLD =3-5, NASH =6-9, Cirrhotic =7-9) and fibrosis staging from 1A to 3. Study protocol was approved by the Research Ethics Boards of McGill and the Institut de Recherches Cliniques de Montréal (IRCM). M= men W= women.

Statistical analysis

Normal distribution and homoscedasticity of data were tested by Shapiro–Wilks and Bartlett tests, respectively. Parametric tests were used if distributions normal and variances equal. One-way or Two-way analysis of variance were followed by Tukey's (one-way) or Dunnett's multiple comparisons (two-way) post-hoc test.using GraphPad Prism software. Data are expressed as mean ± SEM unless otherwise indicated.

RESULTS

Loss of hepatic PGC-1α results in increased inflammatory damage to liver

Over-expression of PGC-1α inhibits NF-κB and increases anti-inflammatory cytokines (Buler et al., 2012; Eisele et al., 2013; Rao et al., 2014). Consistently, low PGC-1α increases hepatic inflammatory signaling in a mouse model of obesity and fatty liver disease (Besse-Patin

et al., 2017), but it is not known whether altered PGC-1α expression influences inflammatory liver damage. To investigate this, we subjected male mice with hepatocyte-specific deletion of the *Ppargc1a* (PGC-1α) gene (LKO mice) to 6-weeks of a methionine-choline-deficient (MCD) diet, a murine model of inflammatory steatohepatitis. LKO mice had higher circulating levels of alanine aminotransferase (ALT) 42 days after initiation of the diet compared to sex- and age-matched littermate wild-type (WT) controls (Fig 1A), suggesting that loss of hepatic PGC-1α aggravates liver damage within a setting of steatosis and inflammation.

PGC-1α1 and PGC-1α4 are expressed in inflamed liver

We next investigated whether hepatic PGC-1α expression associated with liver inflammation. PGC-1α proteins at molecular weights (MW) of ~110 kDa and ~37 kDa were immunoprecipitated at higher levels from mouse liver tissue fifteen days after initiation of the MCD diet compared to control diet-fed mice (Fig 1B), concurrent with an increase in inflammatory markers (Fig 1C). Multiple splice variants of PGC-1α have been identified that are transcribed from proximal or alternative promoters (Felder et al., 2011; Miura, Kai, Kamei, & Ezaki, 2008; Ruas et al., 2012; Yoshioka et al., 2009; Zhang et al., 2009). Since some of these isoforms have biological activity distinct from canonical PGC-1α (herein called PGC-1α1) (Martinez-Redondo, Pettersson, & Ruas, 2015), we sought to identify which variants were impacted in inflamed liver. In healthy fed mice, only *Pgc-1α1* transcripts are detected at appreciable levels in liver (Ruas et al., 2012). Since expression of alternative PGC-1α isoforms is often stimulus- and context-dependent (Chinsomboon et al., 2009; Norrbom et al., 2011; Popov, Bachinin, Lysenko, Miller, & Vinogradova, 2014; Tadaishi, Miura, Kai, Kawasaki, et al., 2011; Thom, Rowe, Jang, Safdar, & Arany, 2014; Wen et al., 2014; Ydfors et al., 2013) and qPCR cannot discern between certain variants, we first used variant-specific PCR (Martinez-Redondo et al.,

2015) to explore which are expressed in mouse hepatocytes treated or not with forskolin (to induce both proximal and alternative promoters). In untreated primary hepatocytes, we detected few transcripts for any variant. Forskolin induced expression of *Pgc-1α1* and *NT-Pgc-1α-a* from the proximal promoter, and *Pgc-1α-b* and *Pgc-1α4* from the alternative promoter (Supplemental Fig. S2).

Consistent with protein levels (Fig 1B), transcripts for *Pgc-1α1* and *Pgc-1α4* were increased in wild-type mouse livers after initiation of MCD diet and concurrent with increased inflammatory markers (Fig 1D). Interestingly, only transcripts from the alternative promoter (containing exons 1b and 1b') were increased in liver samples of human subjects with biopsy-confirmed inflammatory liver disease (i.e. NAFLD, NASH or cirrhosis) compared to livers with simple steatosis (low) (Supplemental Fig. S3). Of these, we found *PGC-1α4* transcript levels increased proportionally with the severity of inflammatory liver disease, with significantly higher mRNA levels detected in cirrhotic liver (Fig 1E). In contrast, transcripts from the proximal promoter trended downward in human samples (Supplemental Fig. S3, Fig 1E). Taken together, our data suggest that inflammation differentially regulates PGC-1α variant expression and that the alternative *PPARGC1A* promoter is activated in hepatic inflammatory disease, leading to increased PGC-1α4.

PGC-1α1 and PGC-1α4 have distinct roles in the hepatic response to TNFα

Since PGC-1α isoforms can have overlapping and distinct biological activity (Martinez-Redondo et al., 2015), we sought to determine whether PGC-1α1 and PGC-1α4 influence inflammatory signaling pathways. We first compared the transcriptome of primary mouse hepatocytes by microarray following over-expression of PGC-1α1, PGC-1α4, or vector alone in the absence or presence of the inflammatory cytokine, tumor necrosis factor (TNFα) (GEO#:TBD)

(Supplemental Fig. S4). More than 1000 genes changed by ≥2-fold following PGC-1α1 over-expression compared to vector alone ($p<0.05$, FDR<0.01), while only 24 were changed by PGC-1α4 and only 4 genes overlapped between the two lists (Fig 2A, Supplemental File 1). Following TNFα treatment, >4500 genes were changed ≥2-fold in hepatocytes over-expressing PGC-1α1 and >3000 for PGC-1α4, with 36% of the genes shared between isoforms (Fig 2A, Supplemental File 2). Clustering of PGC-1α4-modulated genes and comparison to levels in vector- or PGC-1α1-expressing hepatocytes suggested that the activity of PGC-1α4 relied heavily on the presence of TNFα (Fig 2B). Furthermore, within this inflammatory context, PGC-1α4 had both over-lapping and distinct activity from PGC-1α1. Of the 2051 genes shared by the variants in TNFα-treated cells, the majority (91.5%) were regulated in the same manner (positively or negatively, Supplemental File 2).

To gain a global impression of biological process regulated by the PGC-1α variants in hepatocytes, we performed gene set enrichment analysis (GSEA). Gene sets relating to mitochondrial respiration and substrate metabolism were enriched by both PGC-1α1 and PGC-1α4 (FDR q-value < 0.1). PGC-1α1 predominantly regulated mitochondrial respiration, and glucose, amino acid and fatty acid metabolism, regardless of TNFα treatment (Supplemental File 3). This is consistent with known roles of PGC-1α1 on mitochondrial metabolism and supported by qPCR (Supplemental Fig. S5). Although we saw no effect of PGC-1α4 on these PGC-1α1 target genes, PGC-1α1 and PGC-1α4 shared many overlapping gene sets (Supplemental File 3). GSEA for PGC-1α4 in untreated hepatocytes centered on lipid metabolism (fatty acids and triglycerides), sterol metabolism and mitochondrial respiration, but individual gene effects were mild and most did not reach the 2-fold cut-off. However, when TNFα was present, PGC-1α4-enriched pathways included regulation of transcription factor transport to the nucleus, innate immunity, responses to interferon/PAMP, TLR signaling, acute inflammation, and apoptosis. Overall, TNFα signaling

289 revealed isoform-specific responses and highlighted processes related to the innate immune
290 response and cell death unique to PGC-1α4.

291 To explore differential effects of the isoforms on inflammation, we performed gene
292 ontology (GO) analysis on gene changes occurring only in the presence of TNFα. Top 10 GO
293 pathways unique to each variant, or shared, are shown in Fig 2C. All of the top PGC-1α1-regulated
294 processes focused on energy metabolism and were shared with PGC-1α4. However, 6 of the top
295 pathways for PGC-1α4 were unique to this variant, including 6-carbon sugar metabolism,
296 proteolysis, immune signaling in response to pathogens, and regulation of cell death (Fig 2C).
297 Interestingly, GO terms associated with the 175 shared genes regulated in an opposite manner by
298 the variants (Supplemental File 4) centered mainly on cell death and apoptosis (Fig 2D). These
299 data suggest that apoptosis is an important effector pathway *differentially* regulated by these two
300 PGC-1α protein variants.

301

302 ***TNFα signaling influences localization of PGC-1α4 within liver cells***

303 TNFα treatment substantially increased the number of PGC-1α4 gene targets, revealing
304 that external signals such as inflammation might be necessary for PGC-1α4 activity. Over-
305 expressed PGC-1α4 localized primarily to the cytoplasm of H2.35 liver cells; therefore, nuclear
306 exclusion might explain why increased PGC-1α4 has little effect on basal gene expression in
307 untreated hepatocytes (Fig 2A). Following addition of TNFα to media, a significant proportion of
308 PGC-1α4 was observed in the perinuclear and nuclear compartments (Fig 3A). Cell fractionation
309 confirmed that PGC-1α4 protein was only detected in the nuclear pellet following TNFα
310 treatment (Fig 3B). In contrast, PGC-1α1 localized exclusively to the nucleus of liver cells
311 regardless of treatment condition (Fig 3A). Total levels of both PGC-1α1 and PGC-1α4 modestly
312 increased with short-term TNFα exposure.

313

314

315 ***Increased PGC-1α4 prevents hepatocyte apoptosis in response to inflammatory signaling***

316 Data so far suggested that different PGC-1α isoforms influence inflammatory and anti-
317 apoptotic signals in liver cells. Using gain- and loss-of-function models, we investigated whether
318 PGC-1α1 or PGC-1α4 impacted cell death downstream of inflammatory signals *in vitro* and *in vivo*.
319 Primary mouse hepatocytes over-expressing PGC-1α1 had increased cleaved caspase 3 (Fig 4A)
320 and nucleosome fragmentation (Fig 4B) in response to TNFα treatment compared to vector,
321 while over-expression of PGC-1α4 almost completely blocked apoptosis. To test this *in vivo* and
322 avoid potentially confounding effects of inflammatory and immune responses caused by viral
323 vectors, we created a transgenic mouse model permitting tissue-specific over-expression of PGC-
324 1α4 (Fig 4C). Recombination at LoxP sites using Albumin promoter-driven Cre-recombinase
325 removed a transcriptional Stop signal driving PGC-1α4 expression only in hepatocytes (PGC-
326 $1\alpha4^{HepTg}$, Fig 4D, E). A small increase in PGC-1α4 transcripts in the absence of Cre-recombinase
327 (^{LSL}PGC-1α4, Fig 4C) indicated a low level of leaky transgene expression, but an increase of ~50-
328 fold expression was observed in livers of PGC-$1\alpha4^{HepTg}$ mice. Supporting an anti-apoptotic role
329 for hepatic PGC-1α4, there were reduced levels of cleaved caspase 3 in livers of both male and
330 female PGC-$1\alpha4^{HepTg}$ mice following injection of LPS (Fig 4F).

331 Consistent with gain-of-function studies, mice lacking PGC-1α in liver had increased
332 cleaved caspase 3 levels when exposed to LPS (Fig 5A). However, this knockout model ablates all
333 *Ppargc1a* transcripts, making it impossible to discern roles for any specific isoform. Thus, we
334 created a mouse model where only the alternative promoter of *Ppargc1a* was disrupted in a
335 tissue-specific manner ($AltProm^{FL/FL}$), blunting expression of transcripts containing exon 1b and
336 1b' (including PGC-1α4), but not PGC-1α1 (Fig 5B). To assess efficiency of the promoter

knockout, primary hepatocytes from control and KO mice were treated with glucagon, which significantly induced expression of multiple PGC-1α transcripts (Fig 5C) and proteins (Fig 5D) from both the proximal and alternative promoter in control AltProm$^{FL/FL}$ cells. In contrast, ablation of the alternative promoter by crossing floxed mice with Alb-Cre^{Tg} mice (AlbPromKO) blunted induction of alternative transcripts in response to glucagon, yet increases in proximal transcripts were similar to (or even higher than) control cells (Fig 5C). The 37kD PGC-1α protein induced by glucagon was almost completed ablated by knockout of the alternative promoter, identifying PGC-1α4 as the predominant truncated PGC-1α variant responsive to glucagon in liver cells (Fig 5D). Consistent with PGC-1α4 being involved in prevention of apoptosis, hepatocytes from AlbPromKO mice had higher basal and TNFα-induced cleaved caspase 3 levels (Fig 5E) and increased fragmented nucleosomes in response to inflammatory signaling (Fig 5F) compared to cells from littermate controls. Taken together, PGC-1α4 appears to have the unique ability to prevent inflammatory-mediated apoptosis in liver cells.

PGC-1α isoforms differentially regulate pathways involved in inflammation and cell survival

In an attempt to identify transcription factors downstream of PGC-1α variants that might mediate these effects, we searched for transcription factor motifs and signatures enriched in gene sets significantly changed by PGC-1α1 or PGC-1α4 alone, or shared, when TNFα was present (Fig 2) using iRegulon (Supplemental Table S4) and DiRE (Supplemental Fig. S6). A significant number of genes unique to PGC-1α1 contained elements corresponding to ETV6 (TEL) binding sites, a member of the ETS transcription factor family not previously associated with PGC-1α activity. IRF4 motifs were enriched in genes shared by PGC-1α1 and PGC-1α4, consistent with previous findings (Kong et al., 2014). However, many other novel motifs were enriched in this

subgroup, including those for ELK4, NR1H2 (LXRβ), ZBTB33 (KAISO), ZFP143, and PITX2. In contrast, genes unique to PGC-1α4 were enriched in motifs for SP4, the NFY complex (NFYC/A), IRF6, GM7148 (TGIF2), PITX2, HSF4, and E2F1DP1. Among the 175 genes oppositely regulated by the variants, a unique set of motifs were identified, including binding sites for STAT, SPIB, NFATC2, and KLF4, transcription factors generally involved in cancer progression, apoptosis, and cell cycle. Narrowing our search to motifs within targets implicated in cell survival (Fig 2D) revealed one transcription factor, ST18 (also known as MYT3 or NZF3), whose predicted binding site was enriched in 16 of these genes.

Focusing on the transcription factors with links to apoptosis and cell death, we surveyed whether over-expression of the PGC-1α variants modulated expression of their target genes. SP4 and STAT targets were repressed by increased PGC-1α1, but not remarkably changed by over-expression of PGC-1α4 (Supplemental Fig. S7A,B). NFY target genes were regulated similarly by the PGC-1α variants, being generally repressed (Supplemental Fig. S7C). IRF4 targets *Tnfrsf17* and *Nip3* were increased by PGC-1α1, but not regulated by PGC-1α4 or TNFα treatment (Supplemental Fig. S7D). *Myc*, *Cdkn2a*, *Nfil3*, and *Casp3* expression were unchanged. PGC-1α1 alone increased ST18 targets *Gata3*, *Foxc1* and *Atad3a* and repressed most other target genes (Supplemental Fig. S7E).

So far, our data illustrated that PGC-1α1 and PGC-1α4 had a variety of effects on expression of multiple mediators of inflammation and apoptosis. However, gene effects seen could not explain opposing effects on cell death observed for the variants in our *in vitro* models. Searching the microarray for candidate anti-apoptotic genes downstream of PGC-1α4, we identified *Birc2* (*Ciap1*) and *Tnfaip3* (also known as A20) (Fig. 2A), two anti-apoptotic proteins that prevent cell death downstream of inflammatory signalling. In a separate experiment, we confirmed that their transcript levels were significantly higher in mouse primary hepatocytes

over-expressing PGC-1α4 only in the presence of TNFα (Fig 6A). Related *Birc3* (*Ciap2*) was also increased by TNFα/PGC-1α4, while *Birc5* expression did not change. In addition, transcripts for apoptosis inhibitors *Naip* and *Xiap* were significantly increased by PGC-1α4, regardless of TNFα treatment. In contrast, over-expression of PGC-1α1 decreased expression of *Birc3, Birc5*, and *Tnfαip3* (Fig 6A) and had no effect on *Naip* and *Xiap.*

Since these genes are all regulated by NF-κB, we hypothesized that PGC-1α4 might enhance NF-κB activity, contrasting with reported repressive effects of PGC-1α1 on this pro-inflammatory transcription factor. Basal expression of a 3x NF-κB response element reporter was increased when PGC-1α1 was co-expressed in primary hepatocytes (Fig 6B); yet consistent with previous findings (Alvarez-Guardia et al., 2010; Eisele et al., 2013), induction of the reporter by TNFα was significantly blunted by high PGC-1α1. PGC-1α4 had no effect on basal or TNFα-induced NF-κB reporter activity. Protein levels of p50 were decreased by both PGC-1α1 and PGC-1α4 in the presence of TNFα, and p65 remained unchanged in all conditions. Over-expression of PGC-1α1 modestly decreased IKKβ and IκBα proteins, which could relieve inhibition on NF-κB and possibly explain increased basal activity. PGC-1α4 over-expression had no effect on these proteins (Fig 6C). However, consistent with previous reports, increased PGC-1α1 significantly decreased basal and/or TNFα-induced levels of pro-inflammatory genes *Mcp-1*, *Tnfα*, *Iκbα* and *Ccl5* in primary hepatocytes (Fig 6D), demonstrating a strong inhibitory role on NF-κB target genes. In contrast, PGC-1α4 had little impact on these genes, except to potentiate the *Tnfα* response similar to the pattern seen on the anti-apoptotic targets (Fig 6A).

In summary, PGC-1α1 had generally repressive effects on transcription of genes involved in inflammation and cell death that were mostly independent of TNFα. In contrast, PGC-1α4 differentially enhanced a select program of anti-apoptotic factors in hepatocytes only in the presence of inflammatory signaling. While we identified multiple genes involved in cell survival

409 oppositely regulated by the variants, enrichment of transcription factor motifs did not reveal the
410 mechanism by which PGC-1α4 specifically enhances the anti-apoptotic gene program.

411 **DISCUSSION**

412 In the current study, we found that various non-canonical PGC-1α protein variants are
413 expressed in inflamed liver and differentially regulate hepatic inflammatory signaling. Gene set
414 enrichment analysis revealed that in the presence of the inflammatory cytokine TNFα, PGC-1α4
415 influences innate immunity and cell death, while PGC-1α1 remains primarily associated with
416 mitochondrial function and metabolic processes. Gene ontology (GO) analysis illustrated that
417 genes implicated in cell death and apoptosis appear to be oppositely regulated by these two
418 variants. In primary liver cells, PGC-1α4 significantly blunted apoptosis in response to TNFα, a
419 function that may be controlled by shuttling of PGC-1α4 between cytoplasm and nucleus. We
420 conclude that alternative forms of PGC-1α are induced in inflammatory environments, giving rise
421 to increased levels of the truncated PGC-1α4 isoform that attenuates apoptosis downstream of
422 inflammatory signaling. These findings give mechanistic insight into how PGC-1α, as a family of
423 proteins, facilitate parallel adaptation to metabolic demand and mitigation of inflammatory
424 damage in cells.

425 Immune responses to danger signals are metabolically challenging and can lead to a trade-
426 off between maintaining highly energy demanding processes of nutrient metabolism versus
427 adaptation to inflammatory stimuli (Ganeshan et al., 2019). Inflammation itself may also inhibit
428 metabolism and impede mitochondrial function. Here, we show that signaling via TNFα or LPS
429 leads to a shift in the PGC-1α gene program downstream of PGC-1α1 and PGC-1α4, ensuring that
430 concurrent inflammatory signaling does not impede the ability to respond to metabolic need.
431 This mechanism represents an additional layer by which the family of PGC-1α proteins help
432 balance an integrated metabolic response modulated by the inflammatory status of the liver.

It is now well established that PGC-1α is a family of proteins created by alternative splicing of the *PPARGC1A* gene in many tissues including skeletal muscle (Martinez-Redondo et al., 2015; Ruas et al., 2012; Yoshioka et al., 2009), brown adipose tissue (Chang et al., 2010; Zhang et al., 2009), and liver (Felder et al., 2011). However, a functional role for many of the alternative isoforms remains unknown. While some PGC-1α variants share overlapping functions with canonical PGC-1α1 (Chang, Jun, & Park, 2016; Miura et al., 2008; Tadaishi, Miura, Kai, Kano, et al., 2011; Thom et al., 2014; Wen et al., 2014), PGC-1α4 has distinct effector pathways in muscle and brown adipose tissue (Chang et al., 2012; Ruas et al., 2012). We show here that PGC-1α1 and PGC-1α4 also have differential effects on cell death downstream of inflammatory signals. PGC-1α4 almost completely blocks apoptosis *in vitro* and *in vivo*, while PGC-1α1 decreases expression of a broad program of inflammatory genes, but does not inhibit cell death in response to TNFα. Mechanisms linking PGC-1α, at least the canonical protein, to apoptosis have been proposed. PGC-1α1 can induce apoptosis through PPARγ, TFAM, generation of reactive oxygen species, or Ca^{2+} signaling (Adhihetty et al., 2009; Bianchi et al., 2006; D'Errico et al., 2011; Onishi et al., 2014; Zhang et al., 2007) or attenuate cell death through a p38/GSK3B/Nrf-2 axis or activation of p53 (Choi et al., 2017; Sen, Satija, & Das, 2011). Several splice variants coming from differentially regulated promoters adds a layer of complexity, but also may explain existing (and often conflicting) previous reports.

An obvious candidate effector in inflammation-mediated apoptosis is NF-κB. Consistent with previous studies (Eisele & Handschin, 2014; Zhang et al., 2017), we show that PGC-1α1 represses NF-κB activity. However, unlike PGC-1α1, our evidence suggests no impact of PGC-1α4 on this transcription factor. Although PGC-1α4 shares the complete activation domain of PGC-1α1, its alternative exon 1 and significant C-terminal truncation may lead PGC-1α4 to regulate a different set of DNA-binding proteins. Our microarray identifies multiple pathways differentially

457 regulated by the two variants, including those targeted by NF-κB, SP4, NF-Y, ST18, STAT and
458 IRF4. However, in our model system, PGC-1α4 did not appear to act as a traditional
459 transcriptional coregulator for many of their gene targets. One possible explanation could be that
460 PGC-1α4 instead promotes novel splicing events to create alternative gene products, similar to
461 the function of related PGC-1α2 and PGC-1α3 variants (Martinez-Redondo et al., 2016). Aberrant
462 alternative splicing can substantially affect cellular function and is associated with disease. For
463 example, alternative splicing of TNFα-regulated genes (such as *Tnfaip3*) produces protein
464 variants with distinct roles in cell death and cell survival (Lopez-Urrutia, Campos-Parra, Herrera,
465 & Perez-Plasencia, 2017).

466 While the exclusive nuclear localization of PGC-1α1 supports its function as a
467 transcriptional coactivator, the ability of PGC-1α4 to shuttle between compartments suggests
468 that it might interact with transcription factors in the cytoplasm and/or regulate their entry into
469 the nucleus, a possibility also supported by our GSEA analysis. Interferon (INF) regulatory factors
470 (IRFs) are well-known transcription factors that shuttle in response to inflammatory stimuli
471 (Reich, 2002) and our data suggest that both PGC-1α1 and PGC-1α4 converge on interferon
472 signaling in liver cells. Canonical PGC-1α has been associated with interferon response in the
473 contexts of HCV infection and thermogenesis (Kong et al., 2014; Shlomai et al., 2012).
474 Interestingly, three interferon regulatory factors (IRF1, IRF4, IRF6) were identified in our motif
475 enrichment analysis and numerous studies implicate interferons as critical regulators of
476 apoptosis (Chattopadhyay et al., 2010; Kim et al., 2004). Although we focused on TNF signaling,
477 our data suggest that PGC-1α1 and PGC-1α4 might also regulate the interferon response;
478 however, further studies are necessary to confirm this hypothesis.

479 PGC-1α4 shares many similarities to another isoform, NT-PGC-1α, which is transcribed
480 from the proximal promoter. Both have two N-terminal nuclear exclusion signals and three

putative phosphorylation (S190, S237, and T252) sites, which regulate nuclear shuttling of NT-PGC-1α (Chang et al., 2010). Our data are consistent with reports describing cytoplasmic to nuclear movement of other truncated variants of PGC-1α (Chang et al., 2010; Zhang et al., 2009). Given similarities between these two proteins, it is possible that NT-PGC-1α localization is also regulated by inflammation similar to PGC-1α4, and while likely, it remains to be seen whether PGC-1α4 and NT-PGC-1α have overlapping functions.

We note that only transcripts from the alternative promoter were increased in human NASH and cirrhotic livers, and proximal transcripts appeared to decrease in cirrhosis. This would suggest that inflammatory signals shift preference from the proximal to the alternative PGC-1α promoter and imply that PGC-1α4 (from the alternative promoter) could be the predominant truncated isoform influencing apoptosis in inflamed human liver. This shift in *PPARGC1A* promoter usage is consistent with previous studies showing a shift to the proximal promoter upon cold exposure in brown adipose tissue and to the alternative promoter upon exercise in skeletal muscle (Chinsomboon et al., 2009; Norrbom et al., 2011; Popov et al., 2014; Popov et al., 2015; Tadaishi, Miura, Kai, Kawasaki, et al., 2011; Thom et al., 2014; Wen et al., 2014; Ydfors et al., 2013). Our data also imply boosting expression of multiple PGC-1α isoforms could allow liver cells to more efficiently respond to energy demand when faced with both high metabolic and inflammatory challenges associated with metabolic disease.

In summary, we show that PGC-1α4 is present in mouse and human liver, and is induced within the context of inflammation. TNFα dynamically regulates localization of PGC-1α4 in liver cells and this isoform plays a role in the prevention of liver cell apoptosis downstream of this inflammatory cytokine and LPS. We also show that PGC-1α1 and PGC-1α4 influence TNFα signaling in liver cells in different, yet complementary ways. Increased PGC-1α1 generally represses expression of inflammatory genes, while PGC-1α4 activity promotes pathways that

505 inhibit apoptosis. Mechanisms underlying the anti-apoptotic role of hepatic PGC-1α4 appear
506 complex, possibly involving interaction with cytoplasmic proteins, dominant-negative effects on
507 other PGC-1α variants, or regulation of alternative splicing of genes implicated in apoptosis. In
508 conclusion, coordinated activity of these PGC-1α isoforms allows fine-tuning of metabolic and
509 inflammatory networks, supporting efficient adaptation to energy demand within the highly
510 dynamic and inflammatory environment of the liver. Offsetting this balance could result in
511 inefficient nutrient metabolism and/or inappropriate responses to inflammatory stimuli, which
512 may play a role in the pathogenesis of NAFLD or NASH.
513

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521 ***Author contributions:***

522 ML, ABP, NJ, SJ, JLR and JLE designed concept and experiments. ML, ABP, NJ, SJ, NPK, SS, CB, AD,
523 JC, JB and PJ performed and analyzed experiments. SKP, AL, and PM created the human liver
524 biobank, characterized samples, and contributed to analysis design. ML, ABP, NJ, SJ, JLR and JLE
525 wrote the manuscript. All authors reviewed the manuscript.
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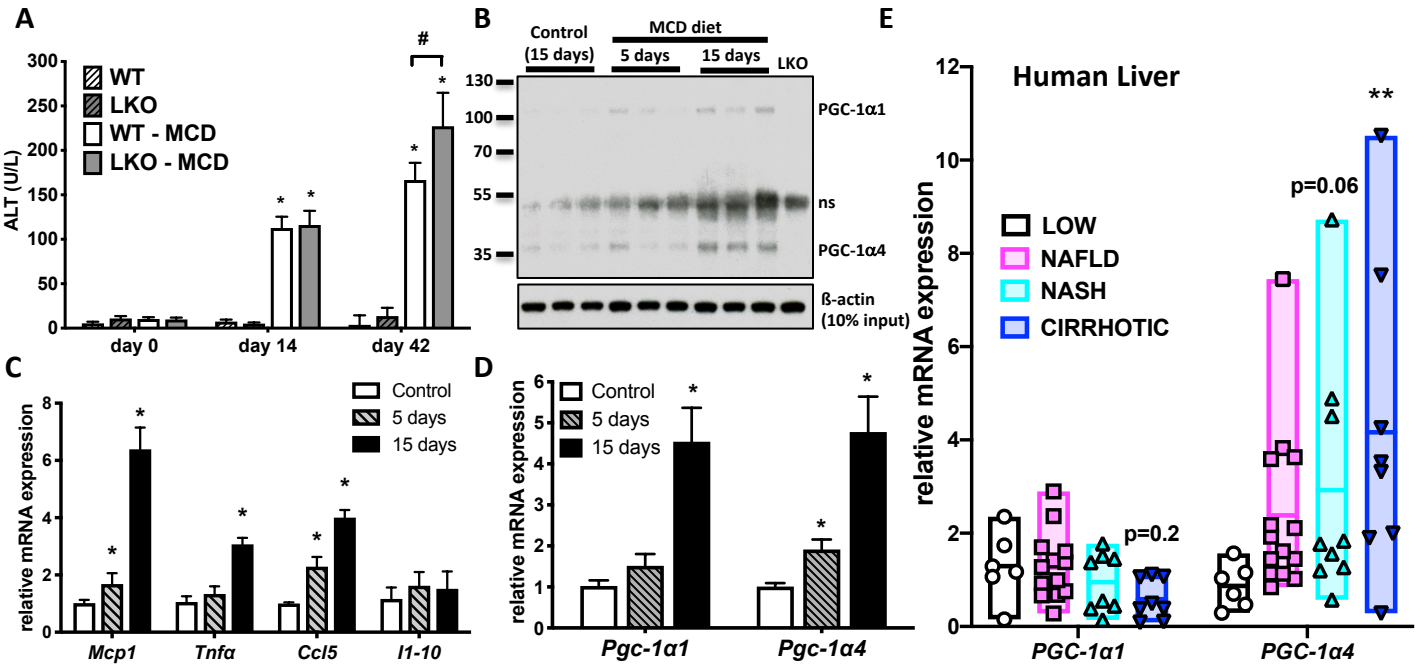


Figure 1: PGC-1α expression is increased in the presence of inflammatory liver damage. A) Serum ALT levels in male WT or LKO mice on MCD (or matched control) diet for 42 days (n = 10-11 mice). *p<0.05 Control versus MCD diet, #p<0.05 WT versus LKO. B) Western blot of immunoprecipitated PGC-1α proteins (n = 3 mice). Loading control (β-actin) represents 10% of input proteins used for immunoprecipitation. C, D) mRNA levels of detectable PGC-1α isoforms (n = 4 mice) from livers of mice fed control or MCD diet for 5 or 15 days. *p<0.05 versus control levels. Data are representative of 2 independent experiments. E) mRNA levels of PGC-1α isoforms in human liver tissues. NAS values: Low ≤ 2 (n = 6), NAFLD = 3-5 (n = 14), NASH = 6-9 (n = 9), Cirrhotic = 7-9 + fibrosis (n = 8). Bars represent max. to min., line represents mean.

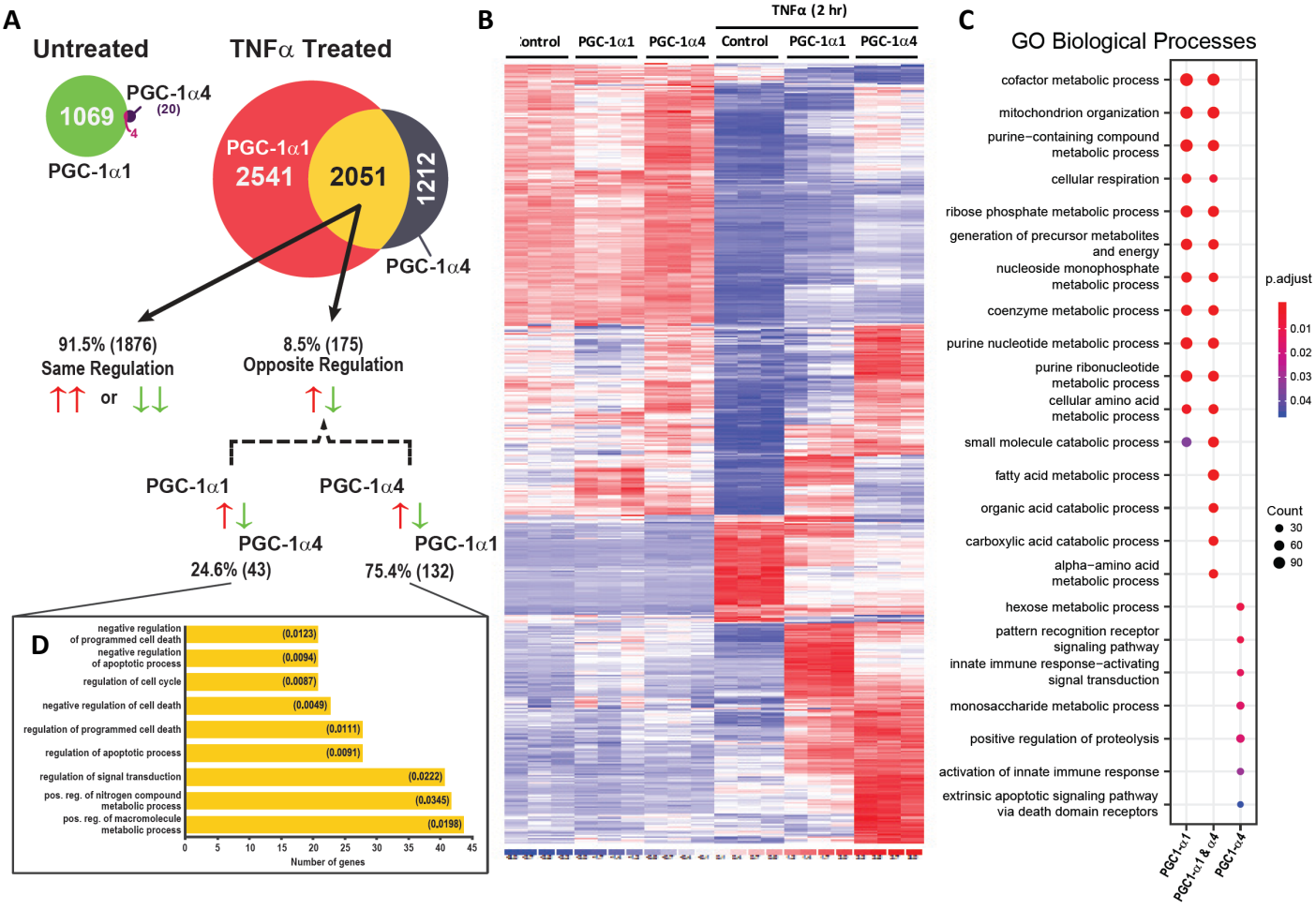


Figure 2: PGC-1α isoforms differentially regulate inflammatory and metabolic signaling pathways downstream of TNFα. Gene expression microarrays of mRNA isolated from primary mouse hepatocytes over-expressing either PGC-1α1, PGC-1α4, or vector control by adenoviral infection. A) Number of genes changed greater than 2-fold 48 hr following transduction in the absence or presence of 2 ng/mL TNFα (2 hr) (n = 3 biological replicates, p<0.5, FDR: 1%). B) Clustering of genes significantly changed by over-expression of PGC-1α4 in primary hepatocytes in the presence of TNFα. C) Top 10 gene ontology pathways were identified from each list generated from TNFα-treated samples in A and listed on x-axis. Size of dot represents number of genes identified in each pathway, in comparison to other genotypes. D) GO analysis of terms associated with 175 genes regulated in the opposite direction.

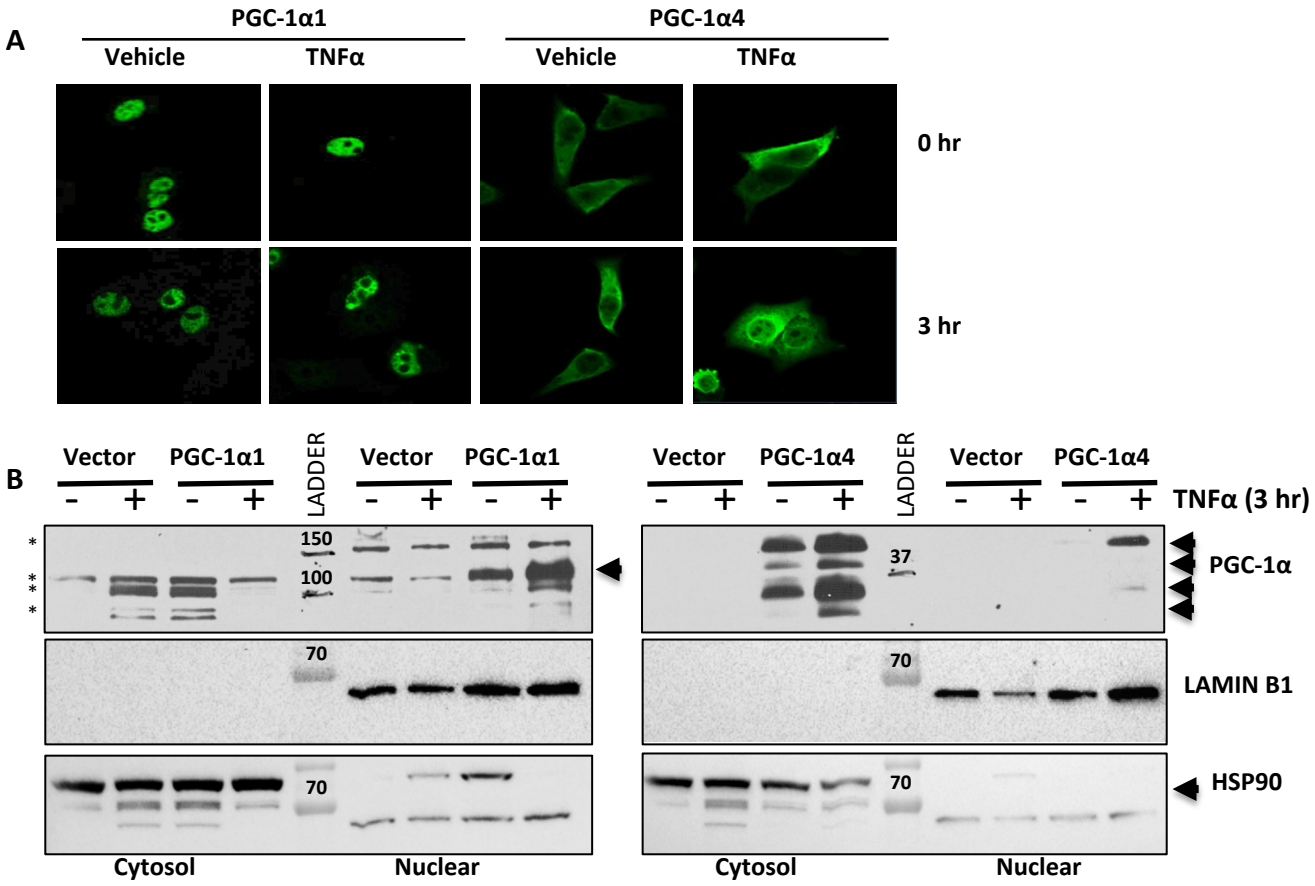


Figure 3: TNFα signaling promotes mobilization of cytoplasmic PGC-1α4 to nuclear and perinuclear regions. A) Confocal imaging of H2.35 mouse liver cells transfected with plasmids expressing V5-tagged PGC-1α1 or PGC-1α4 treated with 20 ng/mL TNFα or vehicle (PBS) for 3 hours. B) Cell fractionation of H2.35 mouse liver cells transduced with adenovirus expressing control vector, PGC-1α1 or PGC-1α4. Images are representative of at least 3 independent experiments. *non-specific bands.

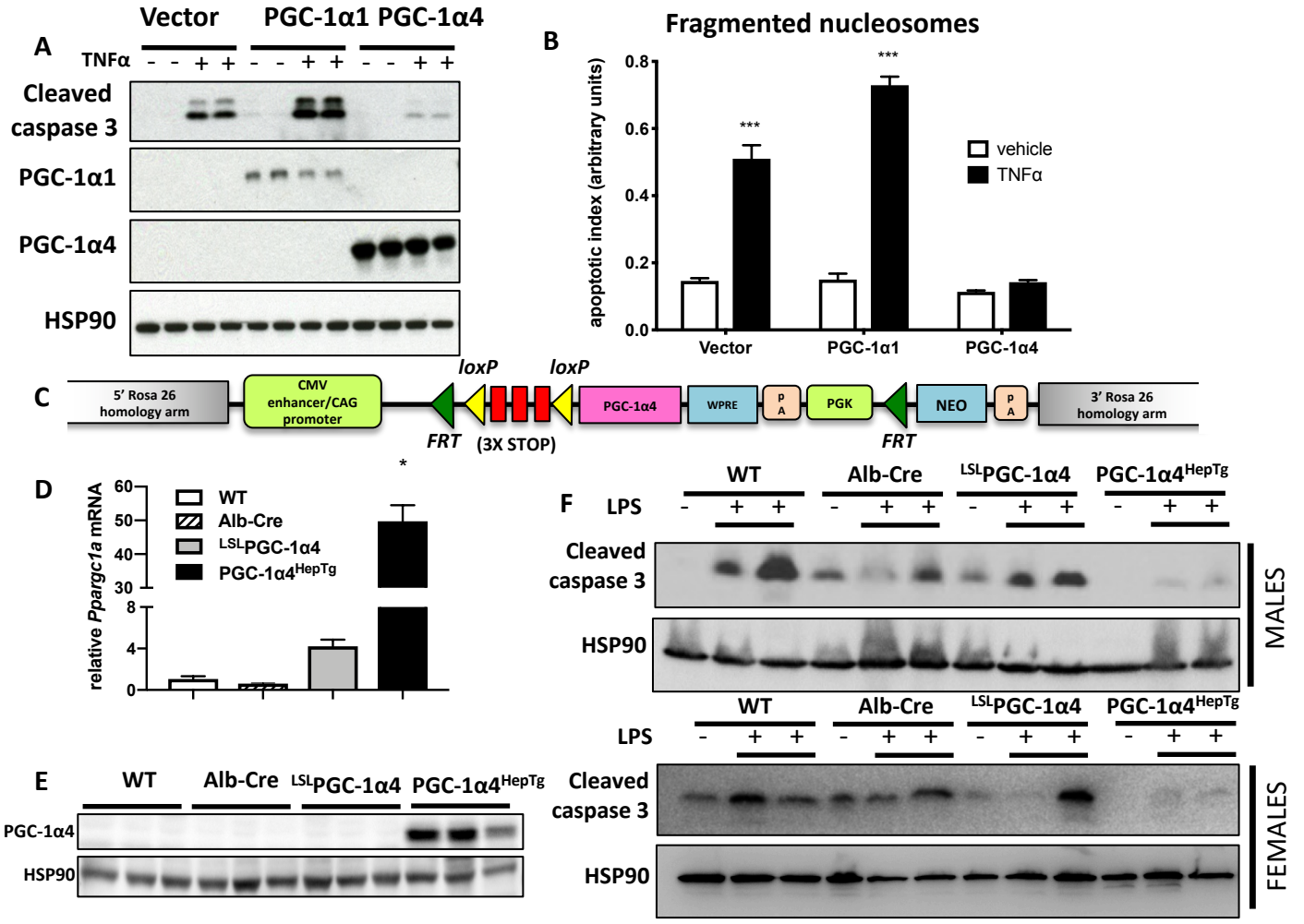


Figure 4: Over-expression of PGC-1α4 protects against liver cell apoptosis induced by inflammatory signals. A) Western blot and B) fragmented nucleosomes in primary mouse hepatocytes over-expressing either PGC-1α1, PGC-1α4, or vector control by adenoviral infection, treated with or without 20 ng/mL TNFα for 8 hours. ***p<0.001 versus vehicle. Data are representative of 3 independent experiments. C) Targeting construct for transgenic mouse allowing tissue-specific over-expression of PGC-1α4 D) mRNA and E) protein from livers of transgenic mice (n = 3) following cross with Albumin-Cre Tg mice to drive PGC-1α4 only in hepatocytes. *p<0.05 versus WT control. F) Western blot of liver protein from male and female mice 6 hours following tail-vein injection of 2 mg/kg LPS (n = 6) or vehicle (PBS) (n = 3).

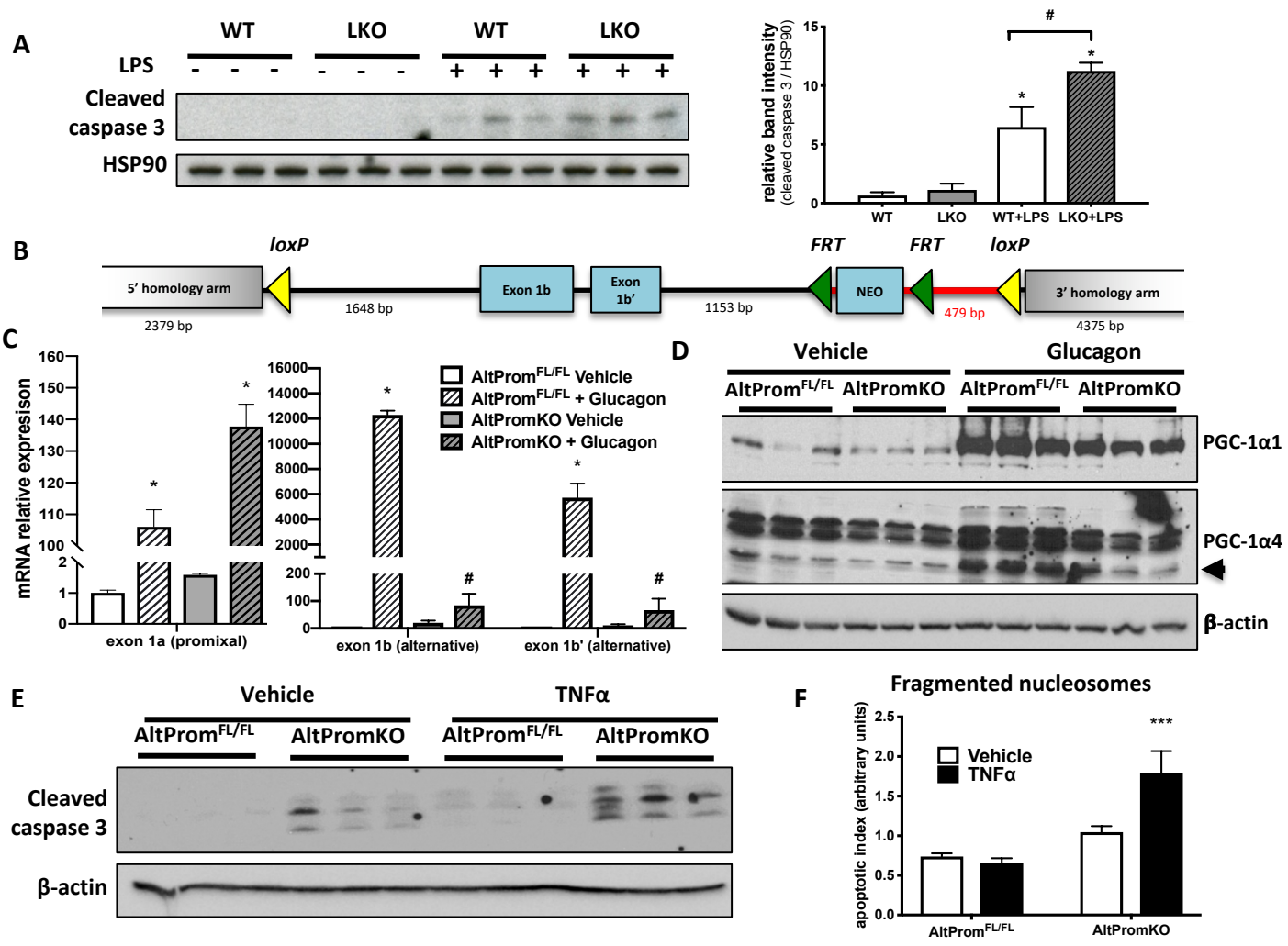


Figure 5: Loss of PGC-1α4 expression enhances apoptosis in response to TNFα. A) Western blot of liver protein from male WT or LKO mice (n = 3) 6 hours following injection of LPS (2 mg/kg) or vehicle (PBS). *p<0.05 versus WT control levels. # p<0.05 versus WT + LPS levels. B) Targeting construct for creation of mouse allowing tissue-specific ablation of the alternative *Ppargc1α* promoter (AltProm$^{FL/FL}$). C) mRNA of proximal and alternative *Pgc-1α* transcripts and D) western blot of proteins from primary mouse hepatocytes treated with 50 nM glucagon or vehicle. *p<0.05 versus AltProm$^{FL/FL}$ Vehicle. #p<0.05 versus AltPromKO Vehicle. E) Western blot and F) fragmented nucleosomes from primary mouse hepatocytes treated with 20 ng/mL TNFα or vehicle for 6 hours. ***p<0.001 versus AltProm$^{FL/FL}$ Vehicle. Data are representative of at least 3 independent experiments.

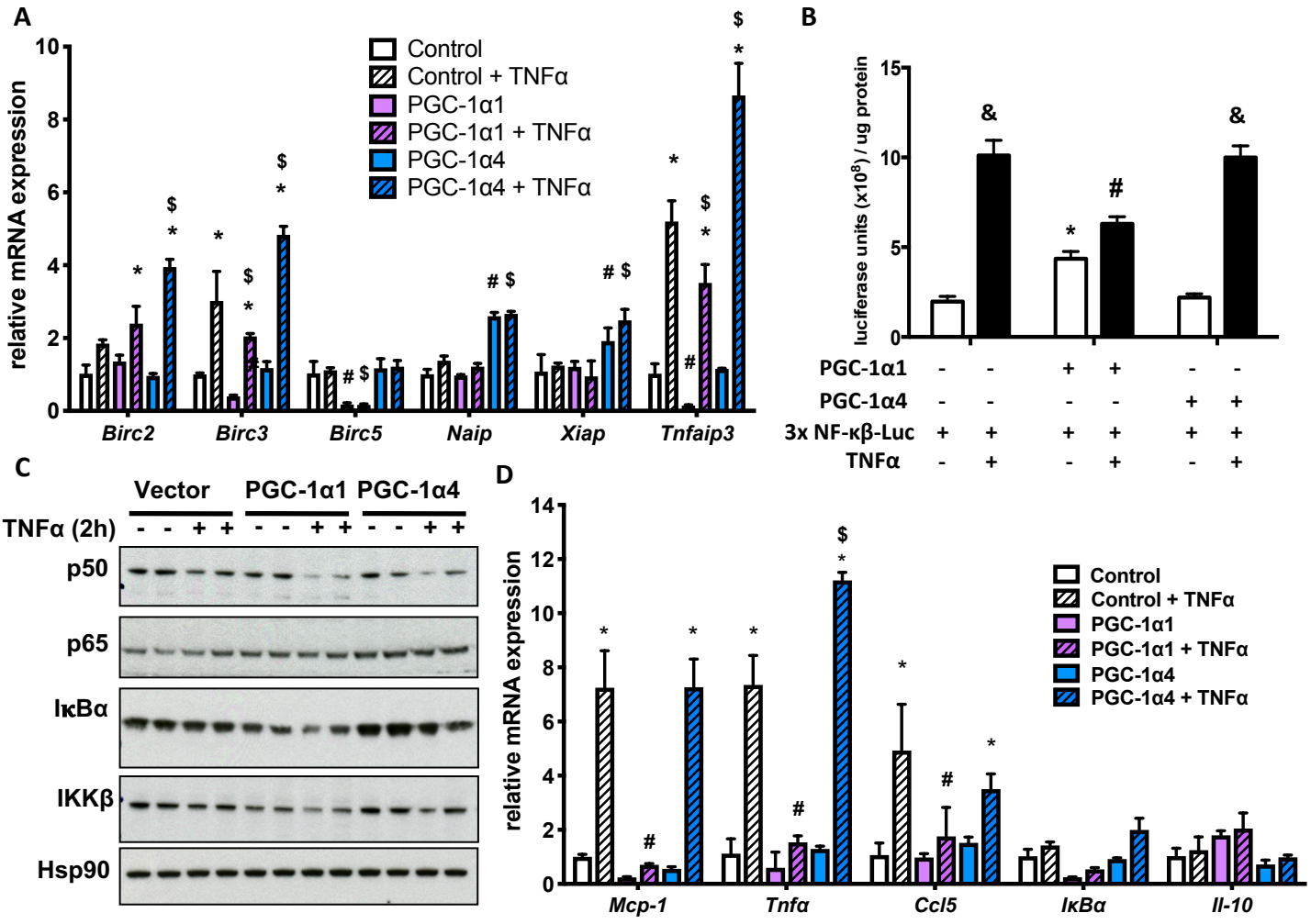
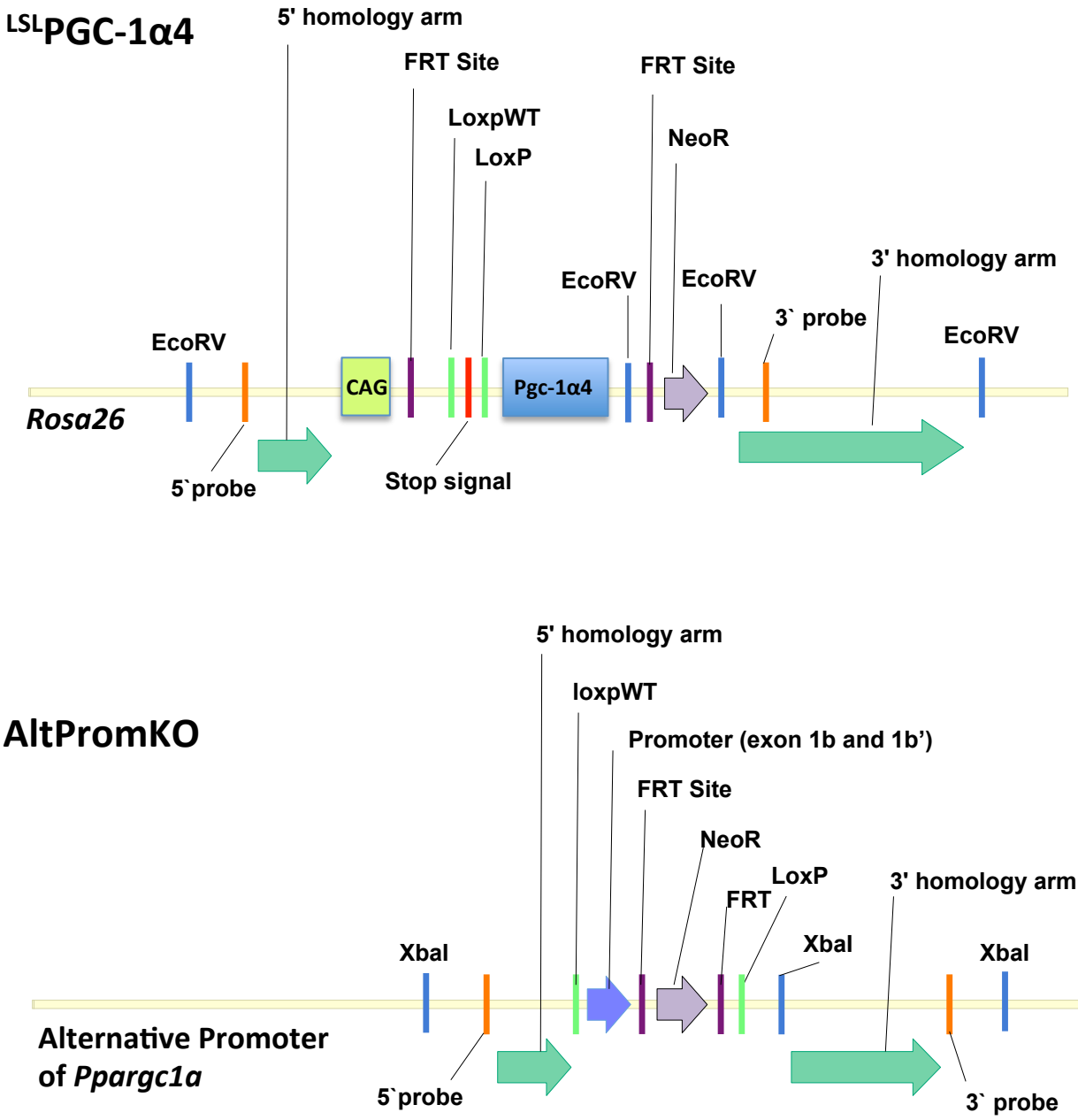
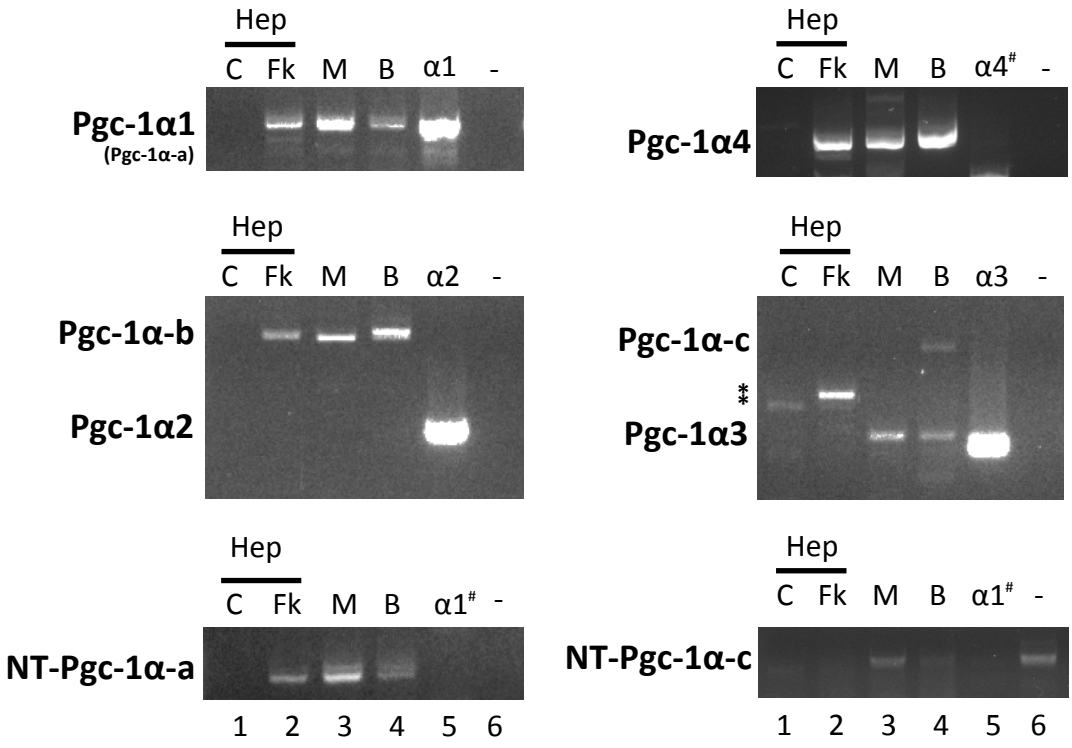


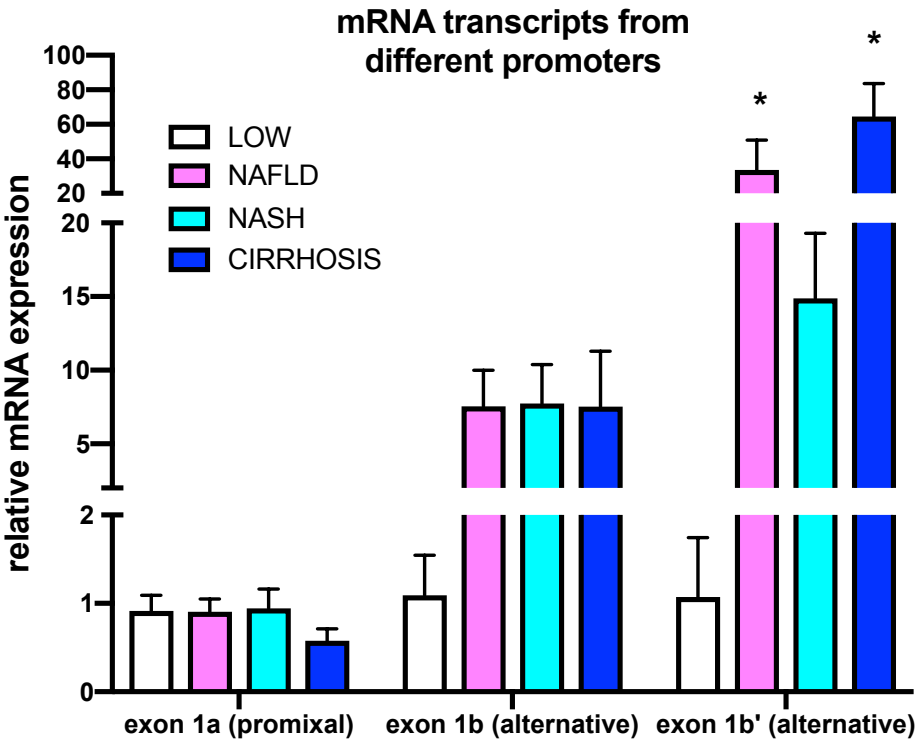
Figure 6: PGC-1α4 potentiates anti-apoptotic gene expression independently of NF-κβ transcriptional coactivation. A) mRNA expression of primary mouse hepatocytes over-expressing PGC-1α1, PGC-1α4 or vector alone following 2-hr treatment with 2 ng/mL TNFα or vehicle (n=3). B) Luciferase activity in primary mouse hepatocytes treated with 2 ng/ml TNFα or vehicle 48 hours following transfection with a 3x NF-κB reporter and constructs for PGC-1α1 or PGC-1α4 (or vector alone, n=3). *p<0.05 genotype effect compared to reporter vector, $^{\&}$p<0.05 TNFα response compared to vehicle, $^{\#}$p<0.05 TNFα response compared to vector + TNFα. C) Western blot and D) mRNA expression of primary mouse hepatocytes over-expressing PGC-1α1, PGC-1α4 or vector following 2-hr treatment with 2 ng/mL TNFα or vehicle (n=3). *p<0.05 effect of TNFα within each genotype. $^{\#}$p<0.05 TNFα response compared to Control + TNFα. $^{\$}$p<0.05 TNFα response compared to PGC-1α1 + TNFα. Data are representative of 2-3 independent experiments.



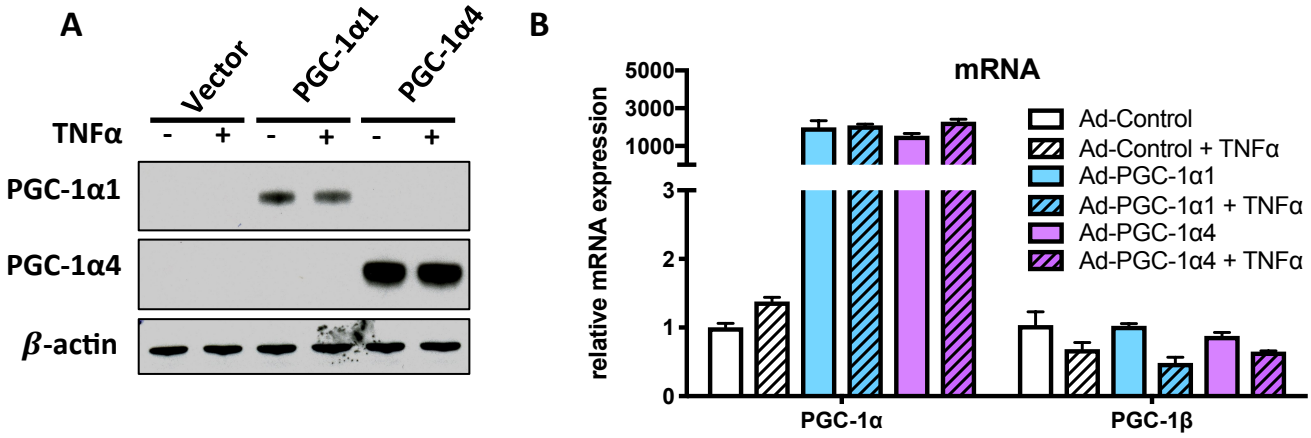
Supplemental Figure S1: Targeting strategies for LSLPGC-1α4 and AltPromKO mouse lines. Schematic of genomic locus for each mouse line, following recombination. Restriction sites (blue) and DNA probes (orange) used for Southern blot screening of ES clones are indicated. Complete diagrams of regulatory and cDNA elements inserted into genome can be found in Figures 4 and 5.



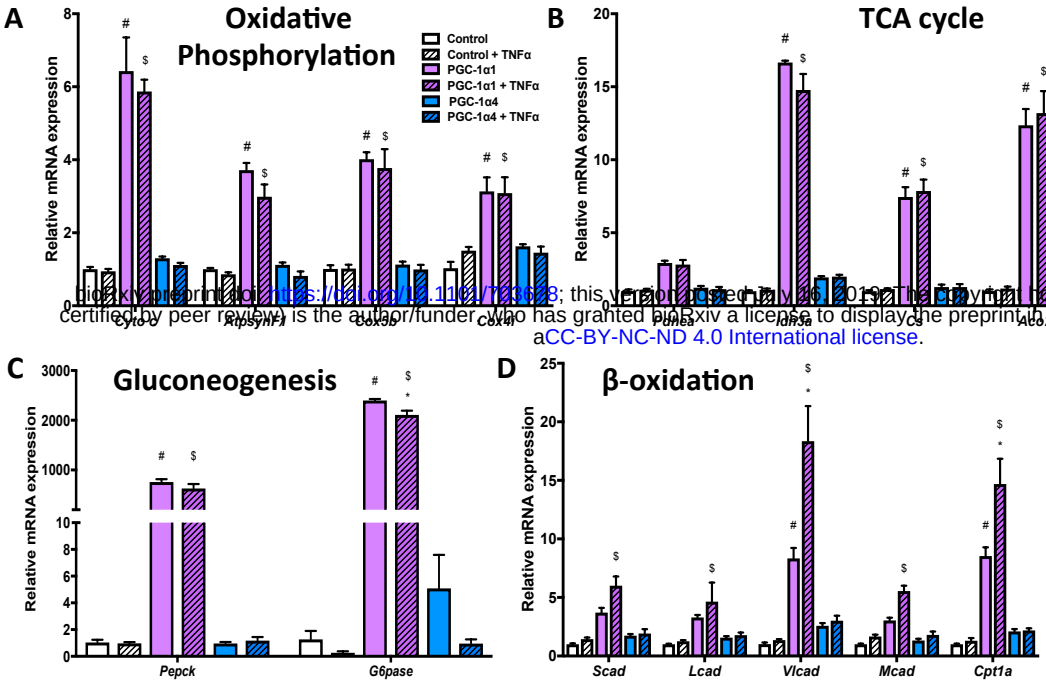
Supplemental Figure S2: Expression levels of all known *Ppargc1a* transcripts in mouse tissues. Bands represent PCR products specific to each known PGC-1α isoform, amplified from cDNA. Lanes 1 and 2: Primary mouse hepatocytes treated with 10 nM forskolin or control vehicle (DMSO) for 3 hours. C − vehicle control, Fk − forskolin treated. Lane 3: Mouse muscle (M), Lane 4: Mouse brown adipose tissue (B). Lane 5: Primary mouse hepatocytes over-expressing the indicated PGC-1α isoform (positive and negative controls). Control vectors for some variants were not available, in this case, # represents control performed on cells over-expressing a structurally similar variant to demonstrate specificity and non-cross-reactivity of primer sets. Lane 6: Water control.



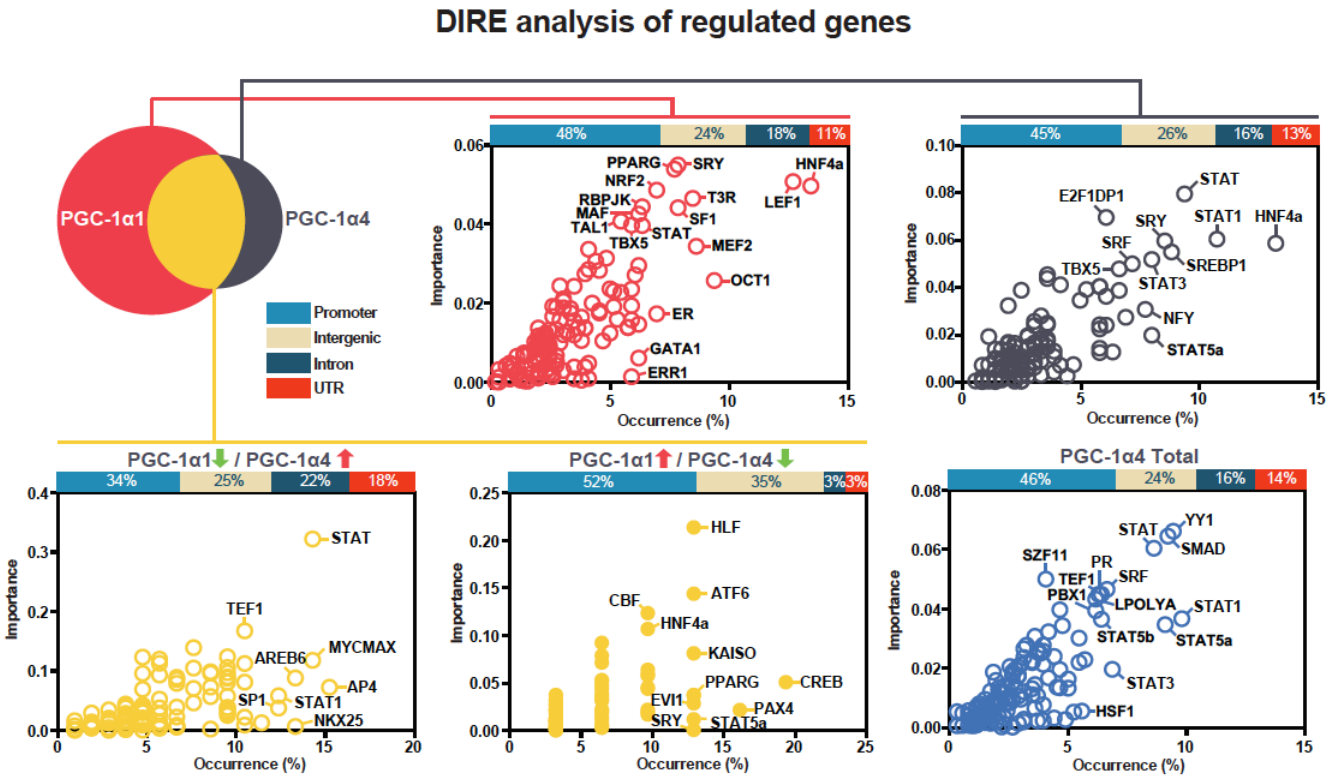
Supplemental Figure S3: Expression of mRNA transcripts driven by proximal and alternative promoters of the *PPARGC1A* gene in human liver sample. Quantification of mRNA transcripts amplified from human liver samples provided by the McGill Liver Disease Biobank. PCR products were specific for transcripts expressing either exon 1a of the proximal promoter, or exon 1b or exon 1b' of the alternative promoter. NAS values: LOW ≤ 2 (n=6), NAFLD = 3-5 (n=14), NASH = 6-9 (n=9), CIRRHOSIS = 7-9 + fibrosis (n = 8). *p<0.05 compared to levels in human liver with low steatosis (LOW).



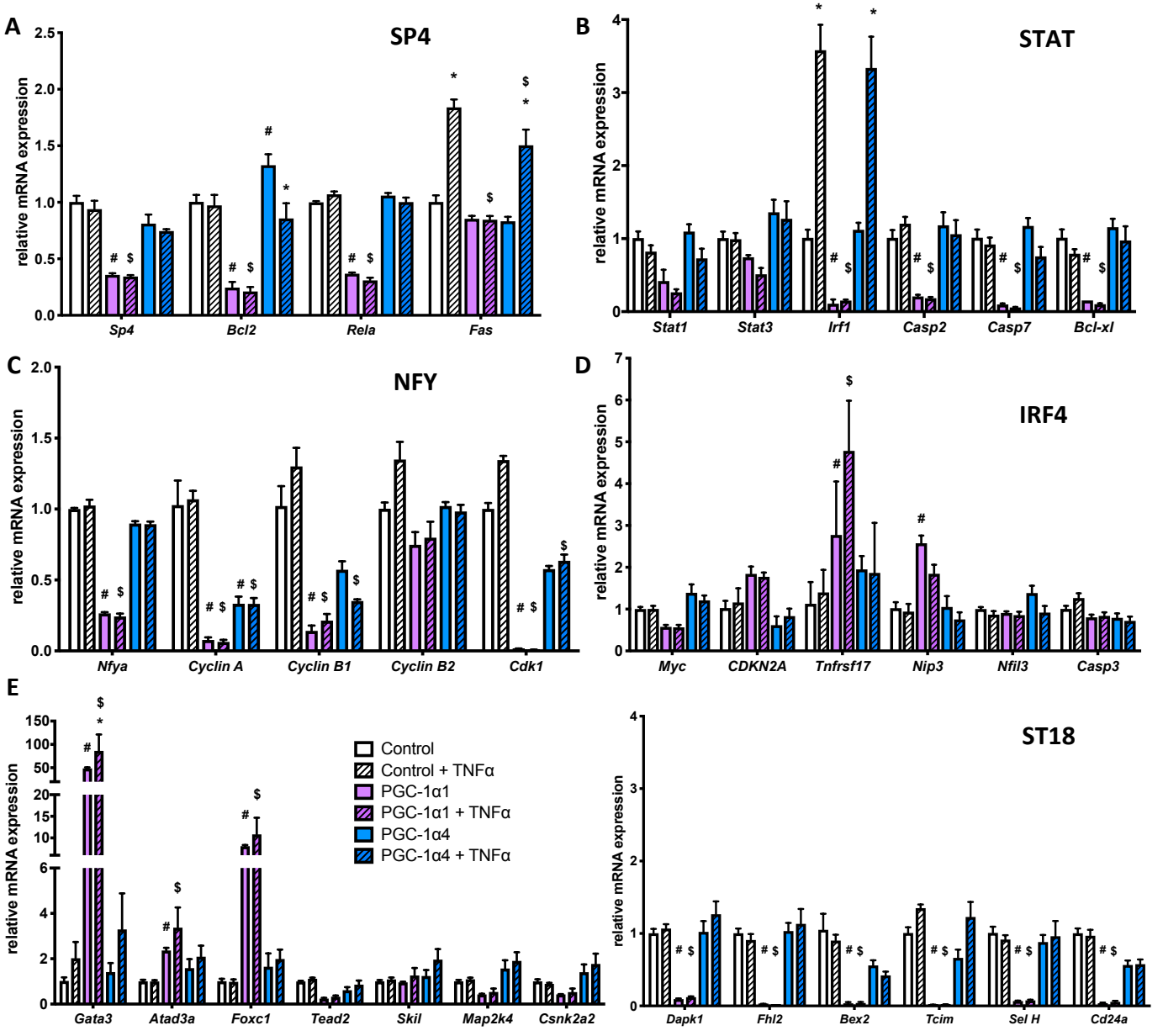
Supplemental Figure S4: Relative levels of PGC-1 mRNA and protein following over-expression in primary mouse hepatocytes and TNFα treatment. A) Western blot of proteins and B) relative mRNA levels in primary hepatocytes 48 hours following transduction with an adenovirus expressing cDNA for PGC-1α1, PGC-1α4 or vector alone (Ad-CMV-GFP). Prior to harvest, cells were treated for 2 hours with 20 ng/mL TNFα or vehicle alone (PBS).



Supplemental Figure S5: PGC-1α isoforms differentially regulate metabolic genes downstream of TNFα. A-D) mRNA expression of primary mouse hepatocytes over-expressing PGC-1α1, PGC-1α4 or vector alone following 2-hr treatment with 2 ng/mL TNFα or vehicle (n=3). *p<0.05 effect of TNFα within each genotype. #p<0.05 Effect of PGC-1α1 or PGC-1α4 expression compared to Control. $p<0.05 TNFα response compared to Control + TNFα.



Supplementary Figure S6: DiRE analysis of regulatory regions for enrichment of transcription factor binding sites. Plotted are the occurrence of each binding motif and its importance metric, which reflects binding site specificity to the input gene set, compared to a background random set of 5000 genes. The top horizontal bars depict relative distribution of identified regulatory elements in promoters, intergenic, intronic, or untranslated regions.



Supplemental Figure S7: Target genes downstream of transcription factors identified by iRegulon and DiRE. A-E) mRNA expression of primary mouse hepatocytes over-expressing PGC-1α1, PGC-1α4 or vector alone following 2-hr treatment with 2 ng/mL TNFα or vehicle (n=3). *p<0.05 effect of TNFα within each genotype. #p<0.05 Effect of PGC-1α1 or PGC-1α4 expression compared to Control. $p<0.05 TNFα response compared to Control + TNFα. Data are representative of at least 2 different experiments.

Supplemental Table S1: Primers used for conventional PCR (listed in 5' – 3' direction)

Gene	Forward Primer	Reverse Primer	Band size
Mouse Primers			
PGC-1α1	GACATGTGCAGCCAAGACTC	CTCAAATGGGGAACCCTTGG	816
PGC-1α2 and *PGC-1α-b*	GATTGTCATCCATGGATTC	GTTCGCTCAATAGTCTTGTTC	325 / 826
PGC-1α3 and *PGC-1α-c*	CTCAGACCCACTATGCTGCTG	GTTCGCTCAATAGTCTTGTTC	302 / 818
PGC-1α4	GATTGTCATCCATGGATTC	CTGGAAGATATGGCACAT	812
NT-PGC-1α-a	GACATGTGCAGCCAAGACTC	CTGGAAGATATGGCACAT	822
NT-PGC-1α-c	CTCAGACCCACTATGCTGCTG	CTGGAAGATATGGCACAT	803

Genotyping primers			
Alb-Cre^{Tg}	Forward (Albumin promoter) TTAGAGGGGAACAGCTCCAGATGG	Reverse (Cre-recombinase) GTGAAACAGCATTGCTGTCACTT	
^{LSL}PGC-1α4	Forward (*Ppargc1a* exon 6) CCAAACCAACAACTTTATCTC	Reverse 1 (*Ppargc1a* intron 7) CCTTCTGATAAAGAGTCAACGC	Reverse 2 (*WPRE*) GGAGAAAATGAAAGCCATACGG
Ppargc1a$^{fl/fl}$	Forward (*Ppargc1a* intron 2) GGAGAGGTGTCAGGGAGAG	Reverse (*Ppargc1a* intron 2) CACAGCAGAGCACAAAGGA	
AltProm$^{fl/fl}$	Forward AGAGTCAGCAGAACAAGCGT	Reverse TGCTTTGCAGAGGTGCTCAT	

Supplemental Table S2: Antibodies and Dilutions (Target, company, catalog number, dilution)

Target	**Company**	**Catalog Number**	**Dilution**
PGC-1α	Millipore	ST1202	1:500
V5	Thermo Scientific	MA5-15253	1:500
Hsp90	Cell Signaling	4874	1:2000
Cleaved Caspase 3 (Asp175)	Cell Signaling	9661	1:500
NFκB p50/p150	Abcam	ab32360	1:500
NFκB p65	Abcam	ab7970	1:500
IκBα	Abcam	ab32518	1:500
IKKβ	Abcam	ab32135	1:1000
Lamin B1	BioVision	3807	1:200
β-actin	Sigma	A5441	1:5000

Supplemental Table S3: Primers used for quantitative real-time PCR (listed in 5' – 3' direction)

Gene	Forward Primer	Reverse Primer
Mouse Primers		
Birc2 (Ciap1)	TCTGCTGTGGCCTGATGTTGGATA	ATGGAGACTGCAGACTGGCTGAAA
Birc3 (Ciap2)	AACTCCCTTCGGGAAATTGACCCT	TTCTTTCCTCCT GGAGTTTCCGCA
Birc5 (survivin)	TGGACAGACAGAGAGCCAAGAACA	AGCTGCTCAATTGACTGACGGGTA
Ccl5 (Rantes)	GCTGCTTTGCCTACCTCTCC	TCGAGTGACAAACACGACTGC
IkBa (Nfkbia)	AGACATCCTTCCGCAAACTC	TAGGTCCTTCCTGCCCATAA
Il-10	GCTCTTACTGACTGGCATGAG	CGCAGCTCTAGGAGCATGTG
Mcp1 (Ccl2)	TCACCTGCTGCTACTCATTCACCA	TACAGCTTCTTTGGGACACCTGCT
Naip	AGATGAAGAGCTCACCACCTGCTT	AGTTCAGTCAGTCTCATGGCAGCA
Pgc-1α1/NT-PGC-1α-a	GGACATGTGCAGCCAAGACTCT	CACTTCAATCCACCCAGAAAGCT
Pgc-1α4/NT-PGC-1α-a,c	TCACACCAAACCCACAGAAA	CTGGAAGATATGGCACAT
Tnfα	CCCTCACACTCAGATCATCTTCT	GCTACGACGTGGGCTACAG
Tnfaip3 (A20)	AGCCAGAAGAAGCTCAACTGGTGT	TGCATGCATGAGGCAGTTTCCATC
Xiap	CCAGCCATGGCAGAATATGA	TCGCCTTCACCTAAAGCATAAA
Nfya	CTCTGTGCCTGCTATCCAAA	CCTCTTAAGGATGCGGTGATAC
Cyclin A	CACTGACACCTCTTGACTATCC	CGTTCACTGGCTTGTCTTCTA
Cyclin B1	GGTCGTGAAGTGACTGGAAA	GTCTCCTGAAGCAGCCTAAAT
Cyclin B2	CTCTGCAAGATCGAGGACATAG	TGCCTGAGGTACTGGTAGAT
Cdk1	CAGACTTGAAAGCGAGGAAGA	TCCTGCAGGCTGACTATATTTG
Cdc25c	TGCACAGTCAGAAGGAACTG	GGAGGAGAATTCACAGAGGAAC
Atad3a	GACAGGACAGCACAGTAGTAAG	AGCAGACCATCTCGTCAATG
Pim1	TTCAGGCAAACGGTCTCTTC	CCACGGATGGTTCTGGATTT
Csnk2a2	CACATAGACCTAGATCCACACTTC	CAAGGTGCCTGTTCTCACTAT
Btg2	CGCACTGACCGATCATTACAA	GGGTCCATCTTGTGGTTGATAC
Myc	CTC CGT ACA GCC CTA TTT CAT C	TGG GAA GCA GCT CGA ATT T
CDKN2A (p16)	CAT GTT GTT GAG GCT AGA GAG G	CAC CGT AGT TGA GCA GAA GAG
Tnfrsf17	GCCTGGAGTATACAGTGGAAGA	CGGGAAGAAATGGTCAGAATCC
Nip3	GACGAAGTAGCTCCAAGAGTTC	CCAAAGCTGTGGCTGTCTAT
Nfil3	GGTTTCCGAAGCTGAGAATTTG	AGATCGGTTGTGTGGCTATG
Casp3	AGTGGGACTGATGAGGAGAT	GTAACCAGGTGCTGTAGAGTAAG
Sp4	TTTCTCAGCCAGCTTCTAGTTC	GGGTGGAAGGATTACCTGATTT
Bcl2	GGAGGATTGTGGCCTTCTTT	GTTCAGGTACTCAGTCATCCAC
RelA (p65)	GAGAAGCACAGATACCACCAAG	GAGATTCGAACTGTTCCTGGTC
Fas	CCAAGTGCAAGTGCAAACCAGACT	AGGATGGTCAACAACCATAGGCGA
Human Primers		
HPRT	GGCCAGACTTTGTTGGATTTG	TGCGCTCATCTTAGGCTTTGT
Pgc-1α1/NT-PGC-1α-a	GGACATGTGCAACCAGGACT	CACTTGAGTCCACCCAGAAAGCT
Pgc-1α4/NT-PGC-1α-a,c	TCACACCAAACCCACAGA	CTGGAAGATATGGCACAT

Supplemental Table S4. Enriched Transcription Factor Motifs (iRegulon)

Genes unique to PGC-1α1 (set of 2571 genes)

TF	NES	#Targets	#Motifs/Tracks
Etv6	3,93	1439	64
Mef2c	3,625	295	3
Jun	3,554	299	7

Genes unique to PGC-1α4 (set of 1208 genes)

TF	NES	#Targets	#Motifs/Tracks
Sp4	5,877	515	10
Nfyc	5,151	206	9
Nfya	4,96	295	13
Irf6	4,333	453	6
Gm7148	4,25	363	3
Pitx2	4,014	330	4
Irf4	3,951	118	2
Hsf4	3,932	336	4
Rela	3,588	93	4
Atf4	3,48	136	3
Srf	3,433	63	1
Tbx5	3,233	88	1
Ebf1	3,217	41	1

Genes shared by PGC-1α1 and PGC-1α4 (set of 2166 genes)

TF	NES	#Targets	#Motifs/Tracks
Irf4	5,143	996	83
Elk4	5,109	990	24
Nr1h2	3,992	412	4
Atf3	3,967	67	2
Zbtb33	3,731	577	3
Zfp143	3,52	239	2
Pitx2	3,275	322	1
Irf1	3,002	52	1

Shared by PGC-1α1 and PGC-1α4, opposing regulation (set of 175 genes)

TF	NES	#Targets	#Motifs/Tracks
Spib	5,472	40	4
Nfatc2	4,794	30	3
Klf4	4,716	23	7
Srf	4,423	31	7
Yeats4	4,131	15	2
Mef2a	4,115	26	3
Tead1	4,077	18	8
Prkaa2	3,652	25	2
Srebf2	3,331	15	3
Jund	3,293	14	1
Fezf2	3,223	7	1