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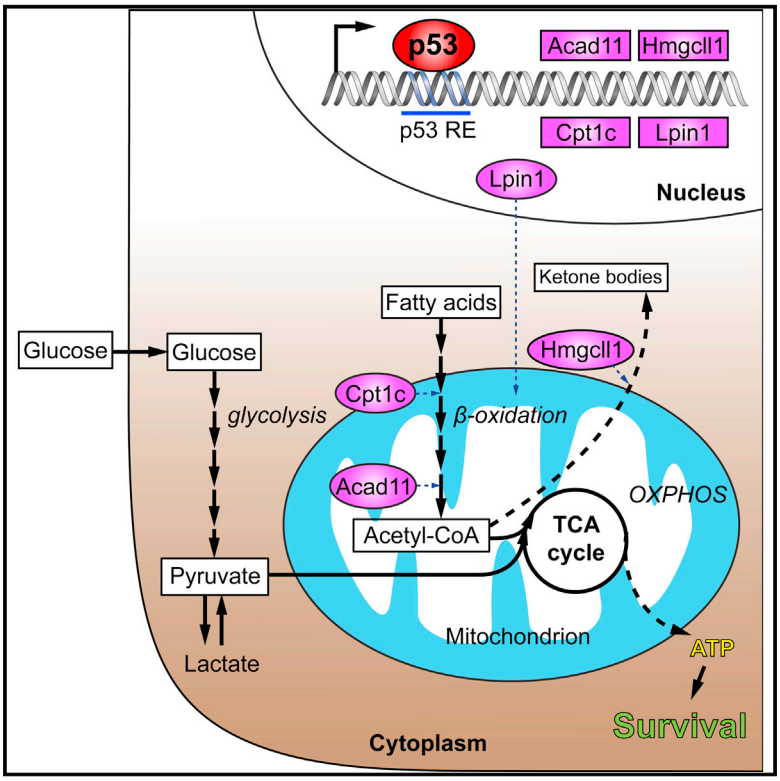
Analysis of p53 Transactivation Domain Mutants Reveals *Acad11* as a Metabolic Target Important for p53 Pro-Survival Function

Graphical Abstract



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In Brief

Jiang et al. show that in oncogenic Ras-expressing cells, p53 promotes oxidative phosphorylation and cell survival in response to glucose starvation. These p53 activities depend on p53 transcriptional activation function and on *Acad11*, a p53 target gene that encodes a protein involved in fatty acid oxidation.

Highlights

- p53 promotes OXPHOS to support cell survival upon glucose deprivation
- Promoting cell survival and OXPHOS depends on p53 transactivation function
- Both wild-type p53 and the p53^{25,26} mutant activate fatty acid metabolism genes
- Acad11 is a p53 target gene involved in OXPHOS and promoting cell survival

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Analysis of p53 Transactivation Domain Mutants Reveals *Acad11* as a Metabolic Target Important for p53 Pro-Survival Function

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SUMMARY

The p53 tumor suppressor plays a key role in maintaining cellular integrity. In response to diverse stress signals, p53 can trigger apoptosis to eliminate damaged cells or cell-cycle arrest to enable cells to cope with stress and survive. However, the transcriptional networks underlying p53 pro-survival function are incompletely understood. Here, we show that in oncogenic-Ras-expressing cells, p53 promotes oxidative phosphorylation (OXPHOS) and cell survival upon glucose starvation. Analysis of p53 transcriptional activation domain mutants reveals that these responses depend on p53 transactivation function. Using gene expression profiling and ChIP-seq analysis, we identify several p53-inducible fatty acid metabolism-related genes. One such gene, *Acad11*, encoding a protein involved in fatty acid oxidation, is required for efficient OXPHOS and cell survival upon glucose starvation. This study provides new mechanistic insight into the pro-survival function of p53 and suggests that targeting this pathway may provide a strategy for therapeutic intervention based on metabolic perturbation.

INTRODUCTION

As a critical tumor suppressor, *p53* is mutated in more than half of human malignancies (Olivier et al., 2010). In addition, mice lacking *p53* develop cancer with 100% penetrance, further underscoring the essential role for p53 in tumor suppression (reviewed in Kenzelmann Broz and Attardi, 2010). In response to diverse stress signals associated with tumor development, including oncogene activation, DNA damage, nutrient deprivation, and hypoxia, p53 is activated and induces transient G_1 cell-cycle arrest, cellular senescence, or apoptosis as measures to limit tumorigenesis (Brady and Attardi, 2010; Vousden and Prives, 2009). When damage is severe, terminal fates such as apoptosis or senescence can eliminate compromised cells. However, p53 can also play a pro-survival role by eliciting a reversible G_1 cell-cycle arrest in the presence of milder levels of DNA damage, allowing the cell to pause and repair the damage before proceeding through the cell cycle (Vousden and Prives, 2009). p53 induces these responses largely by serving as a transcriptional activator, a function crucial for various p53 cellular responses, as well as for tumor suppression (Bieging et al., 2014). p53 also directly represses specific target genes (Brady and Attardi, 2010; Hammond et al., 2006).

In recent years, an additional role for p53 in regulating cellular metabolism has been recognized. Reprogramming of cellular metabolism, characterized by enhanced aerobic glycolysis and the concomitant decrease in mitochondrial oxidative phosphorylation (OXPHOS), is a hallmark of cancer development vital for tumor cells to sustain energy production and support macromolecular biosynthesis needed for growth and proliferation (Hanahan and Weinberg, 2011). p53 counteracts these effects by limiting glycolytic flux and promoting OXPHOS through various mechanisms. For example, p53 suppresses glycolysis by directly repressing the expression of the GLUT1/4 glucose transporters (Schwartzenberg-Bar-Yoseph et al., 2004) and by inducing expression of TIGAR, which lowers the levels of fructose-2,6-bisphosphate, a key component of the glycolytic pathway (Bensaad et al., 2006). p53 also directly stimulates mitochondrial OXPHOS by inducing *SCO2*, whose encoded protein promotes assembly of the cytochrome *c* oxidase complex, and of *GLS2*, which encodes a protein that enhances glutamate and α-ketoglutarate production, thereby fueling OXPHOS (Hu et al., 2010; Matoba et al., 2006; Suzuki et al., 2010). In addition, p53 activates numerous target genes, including *GPX1*, *ALDH4*, and *TP53INP1*, encoding proteins involved in inhibiting reactive oxygen species (ROS) accumulation, which may also contribute to tumor suppression (Maddocks and Vousden, 2011). p53's ability to restrain

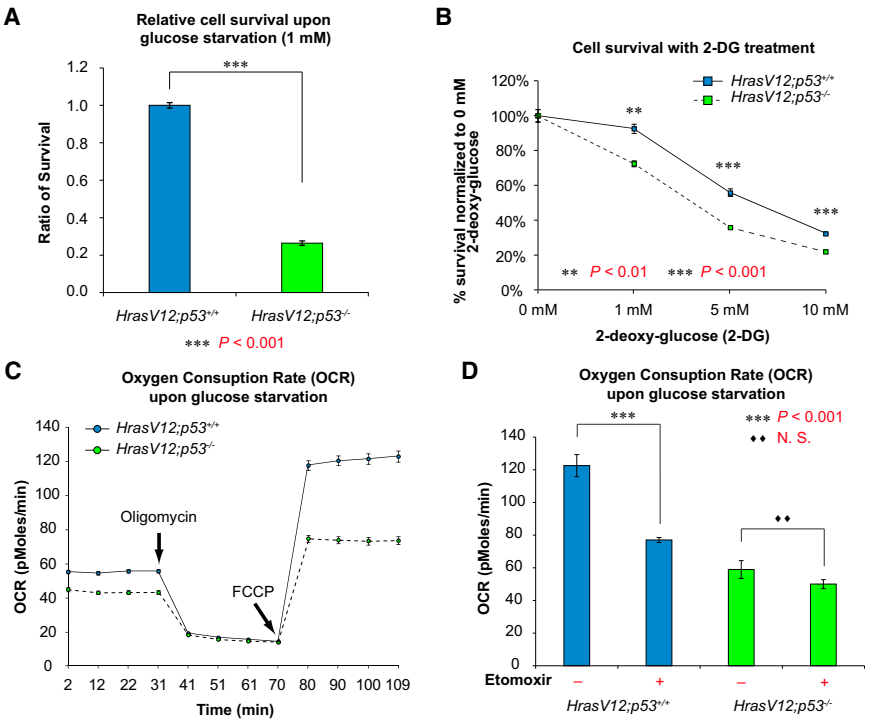

Figure 1. p53 Promotes Cell Survival and OXPHOS in HrasV12 MEFs upon Glucose Starvation

(A) Relative survival of *HrasV12;p53*$^{+/+}$ and *HrasV12;p53*$^{-/-}$ MEFs in 1 mM glucose normalized to cell survival in 25 mM glucose after 72 hr. The ratio of *HrasV12;p53*$^{+/+}$ MEFs is set to 1.0.

(B) Percentages of surviving *HrasV12;p53*$^{+/+}$ and *HrasV12;p53*$^{-/-}$ MEFs in the presence of varying concentrations of 2-DG relative to cell survival in 0 mM 2-DG, which is set to 100%, after 72 hr. For (A) and (B), results represent the mean ± SEM from direct cell counts with the Coulter counter.

(C) Average OCR ± SEM of *HrasV12;p53*$^{+/+}$ and *HrasV12;p53*$^{-/-}$ MEFs determined by the Seahorse XF assay.

(D) Average OCR ± SEM by the Seahorse XF assay with or without the specific FAO inhibitor etomoxir (100 μM) in the presence of uncoupler FCCP. The decrease in OCR upon etomoxir treatment represents the proportion of the OCR due to FAO.

The p values from Student's t test are indicated. N.S., not significant. See also Figures S1 and S2.

metabolic reprogramming is thought to be important for tumor suppression in vivo, as suggested by studies of two *p53* knockin mutant mouse strains, expressing p53^{E177R} or p53^{3KR}, mutants altered in the DNA binding domain and in the ability to activate certain p53 target genes but not others (Li et al., 2012; Timofeev et al., 2013). Although the p53^{E177R} mutant is defective in inducing apoptosis and the p53^{3KR} mutant in inducing cell-cycle arrest, senescence, and apoptosis, in response to stress signals, both mutants retain the capacity to inhibit glucose uptake, glycolysis, and ROS accumulation, as well as to suppress spontaneous tumorigenesis in mice. These findings suggest the importance of p53 activity in suppressing metabolic reprogramming for its tumor suppressor function in vivo.

The participation of p53 in metabolic pathways, both in responding to metabolic stress and in regulating metabolism, can, in some contexts, promote enhanced cell survival. For example, upon glucose limitation, AMPK, a major sensor of ATP depletion in the cell, promotes a reversible G_1 cell-cycle arrest and cell survival in primary fibroblasts with wild-type p53, whereas *p53*$^{-/-}$ fibroblasts fail to arrest or maintain viability (Jones et al., 2005). The p53 target gene *TIGAR* can also promote cell survival by increasing flux through the pentose phosphate pathway, leading to the generation of NADPH, which promotes an antioxidant environment (Bensaad et al., 2006). p53 can also induce G_1 arrest and direct de novo serine synthesis to GSH production to protect cells from oxidative damage and enhance survival upon serine starvation (Maddocks et al., 2013). Thus, p53 can promote cell survival through multiple mechanisms.

Although p53 clearly promotes cell survival in response to nutrient starvation, the transcriptional programs underlying p53 pro-survival function remain incompletely understood. Here, we leverage a panel of previously generated p53 transcriptional activation domain (TAD) mutant knockin mouse strains to study p53 pro-survival transcriptional programs. In particular, a mutant in the first p53 TAD, known as p53^{25,26}, is especially useful, as it is severely compromised for the activation of most p53 target genes but activates a small subset of p53 targets efficiently and retains many p53 functions. We discover that promoting cell survival upon nutrient deprivation is an activity retained by p53^{25,26}, in conjunction with the activation of a cohort of direct metabolism-associated p53 target genes. Characterization of these genes reveals that *Acad11*, encoding an acyl-CoA dehydrogenase family member (He et al., 2011), is a key cell survival gene, lending new insight into how p53 allows cells to cope with metabolic stress.

RESULTS

p53 Promotes Cell Survival upon Glucose Deprivation by Stimulating OXPHOS

To investigate the mechanisms underlying the p53 response to metabolic stress, we used mouse embryonic fibroblasts (MEFs) expressing oncogenic HrasV12 as a model for p53 function in neoplastic cells under metabolic stress (Serrano et al., 1997). In this model, HrasV12 constitutively activates p53, which in turn restrains proliferation. To examine the effects of a metabolic stress encountered by incipient tumors, we studied the consequences of nutrient starvation, specifically glucose starvation, on the viability of *HrasV12;p53*$^{+/+}$ and *HrasV12;p53*$^{-/-}$ MEFs. Consistent with previous observations in primary MEFs (Jones et al., 2005), *HrasV12;p53*$^{-/-}$ MEFs displayed decreased survival compared with *HrasV12;p53*$^{+/+}$ MEFs upon glucose starvation (Figure 1A). Similarly, treatment of HrasV12-expressing MEFs

with 2-deoxyglucose (2-DG), a glycolysis inhibitor (Wick et al., 1957), led to diminished survival of *HrasV12;p53*$^{-/-}$ MEFs relative to *HrasV12;p53*$^{+/+}$ counterparts (Figure 1B). Thus, in the context of oncogenic Hras, p53 promotes cell survival upon glucose deprivation.

Because glucose is a major source of cellular energy, the decrease in cell viability upon glucose starvation is likely due to a deficit in energy production. In response to such stress, the cell may use other sources of nutrients, including fatty acids and certain amino acids, to maintain energy homeostasis through OXPHOS (Berkers et al., 2013). To measure the cellular oxygen consumption rate (OCR), a reflection of OXPHOS activity, in living cells in real time, we used the Seahorse Extracellular Flux (XF) assay under conditions of glucose starvation. Relative to *HrasV12;p53*$^{-/-}$ MEFs, *HrasV12;p53*$^{+/+}$ MEFs have a higher OCR, both at the basal level and under conditions of maximum respiratory capacity triggered by the mitochondrial uncoupler FCCP (Figure 1C) (Brand and Nicholls, 2011). The use of oligomycin, an ATP synthase inhibitor, establishes the amount of OCR devoted to ATP production. Because p53 has been reported to regulate fatty acid oxidation (FAO) (Assaily et al., 2011), which itself can fuel OXPHOS, we examined whether differences in FAO levels between *HrasV12;p53*$^{+/+}$and *HRasV12;p53*$^{-/-}$ MEFs could account for differences in OXPHOS. Treatment of *HrasV12;p53*$^{+/+}$ MEFs, but not *HRasV12;p53*$^{-/-}$ MEFs, with the FAO inhibitor etomoxir provoked a significant decrease in OCR under conditions of glucose starvation, indicating that *HrasV12;p53*$^{+/+}$ MEFs have higher FAO levels than *HRasV12;p53*$^{-/-}$ MEFs and that upon glucose depletion, p53 promotes OXPHOS through FAO, thus maintaining energy homeostasis to stimulate cell survival. The pro-survival effect by p53 is abolished under hypoxic conditions (0.5% O_2), which also inhibit OXPHOS, suggesting further that OXPHOS is required for the pro-survival function of p53 (Figure S1). Therefore, in the context of oncogenic Hras, p53 promotes cell survival upon glucose starvation at least in part by promoting FAO and sustaining cellular energy homeostasis through OXPHOS.

In addition to stimulating OXPHOS, the ability of p53 to regulate cell-cycle progression could also contribute to the enhanced survival in p53-expressing cells, as described previously (Jones et al., 2005; Maddocks and Vousden, 2011). We therefore examined the proliferation rates of *HrasV12;p53*$^{+/+}$ and *HrasV12;p53*$^{-/-}$ MEFs upon glucose starvation. Consistent with the previously reported growth arrest and senescence responses in *HrasV12;p53*$^{+/+}$ MEFs, low BrdU incorporation (~15% BrdU+) is observed under normal and low-glucose conditions, whereas *HrasV12;p53*$^{-/-}$ MEFs remain highly proliferative (>50% BrdU+) under both normal and low-glucose conditions (Figure S2A). Thus, as suggested previously (Jones et al., 2005), induction of cell-cycle arrest by p53 may contribute to survival in *HrasV12;p53*$^{+/+}$ MEFs under glucose starvation, likely by limiting the energy consumption associated with cell proliferation. In contrast, *HrasV12;p53*$^{-/-}$ MEFs undergo unchecked proliferation upon glucose starvation and eventually die. These findings suggest further that glucose limitation does not alter the biological p53 response of cell-cycle arrest in HrasV12 MEFs but that this HrasV12-activated, p53-dependent response may still help protect against the decreased survival triggered by glucose starvation. We also noted a higher induction of apoptosis in *HrasV12;p53*$^{-/-}$ MEFs than in *HrasV12;p53*$^{+/+}$ MEFs upon glucose deprivation (Figure S2B). Thus, the decreased cell survival of *HrasV12;p53*$^{-/-}$ MEFs upon glucose starvation can also be explained in part by elevated apoptosis.

Selective p53 Transactivation Function Suffices for Promoting Cell Survival and OXPHOS upon Glucose Starvation

To gain insight into the molecular basis of p53 pro-survival function, we sought to define the transcriptional programs associated with this response. We took advantage of *p53* knockin mouse strains expressing mutants altered in the first (p53^{25,26}) or both TADs (p53^{25,26,53,54}) that we generated previously and that display different transactivation potentials that could help define transcriptional programs involved in cell survival (Figure 2A) (Brady et al., 2011; Johnson et al., 2005). Specifically, the p53^{25,26} mutant is drastically impaired for the activation of most p53 target genes but retains the capacity to efficiently induce a select set of p53 target genes, while the p53^{25,26,53,54} mutant is transactivation-dead. Notably, mutation of the 53,54 residues alone does not compromise p53 transcriptional activity. Given that the p53^{25,26} mutant efficiently activates only a small subset of p53 targets, it provides an ideal molecular tool to pinpoint essential p53 transcriptional targets involved in the responses where p53^{25,26} is active.

To test the dependence of p53 pro-survival function on the transactivation activity of p53, we examined the ability of the p53 TAD mutants to promote cell survival in HrasV12 MEFs upon glucose starvation. In these MEFs, expression of the different p53 TAD mutants is silenced by an upstream *Lox-Stop-Lox* (*LSL*) transcriptional stop element, until Cre recombinase introduction through adenoviral-Cre (Ad-Cre) infection drives recombination of the stop element and *p53* expression. Here and in all experiments, highly efficient Ad-Cre-induced p53 expression (>90% of cells) was confirmed by immunofluorescence and/or western blotting (Figure 2B). Similarly to *HrasV12;p53*$^{-/-}$ MEFs, *HrasV12;p53*$^{25,26,53,54/25,26,53,54}$ MEFs showed compromised survival in response to glucose starvation, indicating that p53 transactivation function is indeed critical for preserving viability upon glucose deprivation (Figure 2C). Consistent with this idea, *HrasV12;p53*$^{R172H/R172H}$ MEFs, expressing the mouse ortholog of a human tumor-derived mutant, p53^{R175H}, with a DNA binding domain mutation rendering it unable to bind p53 response elements, failed to survive efficiently upon glucose starvation, like *HrasV12;p53*$^{-/-}$ MEFs (Figure 2D). Interestingly, the protection conferred by the p53^{25,26} mutant is similar to that observed with wild-type p53, suggesting that the selective transactivation potential of the p53^{25,26} mutant is sufficient for the p53-dependent pro-survival function upon glucose deprivation (Figure 2C).

To determine whether the upregulation of OXPHOS by p53 depends on its transactivation function, we measured the OCR upon glucose starvation in the same panel of cells. Although the capacity of the p53^{25,26} mutant to transcriptionally activate most p53 target genes is severely compromised, it can enhance OXPHOS to an extent similar to that induced by wild-type p53 and significantly better than p53^{25,26,53,54} (Figure 2E). Together,

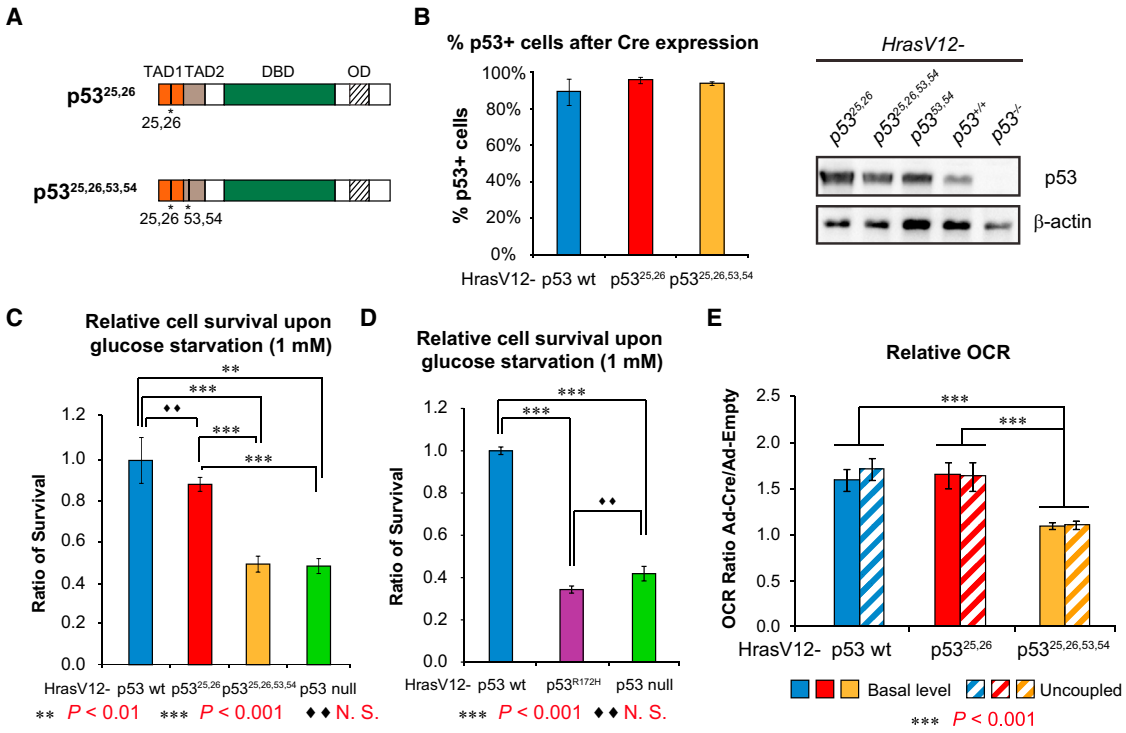

Figure 2. p53 Transactivation Potential Is Critical for Cell Survival and OXPHOS under Metabolic Stress

(A) Schematic view of p53 TAD mutants used in this study. DBD, DNA-binding domain; OD, oligomerization domain.

(B) Left: in HrasV12 MEFs homozygous for each *p53* allele, efficiency of Ad-Cre-mediated recombination of the *LSL* element to induce wild-type or mutant p53 expression was determined by p53 immunofluorescence staining and counting the percentage of p53-positive cells out of 200 DAPI-positive cells in each experiment. Right: p53 protein levels in HrasV12-expressing MEFs of different p53 genotypes. β Actin served as loading control.

(C) Relative survival by direct cell counts of HrasV12 MEFs expressing wild-type p53, p53 TAD mutants, or no p53 in 1 mM glucose normalized to cell survival in 25 mM glucose after 72 hr. The ratio of *HrasV12;p53*$^{+/+}$ MEFs is set to 1.0.

(D) Relative survival by SRB staining of *HrasV12;p53*$^{+/+}$, *HRasV12;p53*$^{-/-}$, and *HRasV12;p53*$^{R172H/R172H}$ MEFs cultured in 1 mM glucose normalized to cell survival in 25 mM glucose after 72 hr. The ratio of *HrasV12;p53*$^{+/+}$ MEFs is set to 1.0.

(E) Relative OCRs of HrasV12 MEFs expressing wild-type p53 or different p53 TAD mutants, determined by the Seahorse XF assay. Results represent normalized OCRs (OCR of Ad-Cre-infected *HrasV12;p53*$^{LSL/LSL}$ MEFs to OCR of the same Ad-Empty-infected *HrasV12;p53*$^{LSL/LSL}$ MEF line). Solid bars show basal OCR measurements and hatched bars show OCR measurements after adding FCCP.

Histogram results in all panels represent the mean ± SEM. p values from Student's t test are indicated. N.S., not significant.

these findings suggest that transcriptional activation is critical for p53-mediated cell survival and for OXPHOS induction upon glucose deprivation and that the selective transactivation retained by the p53^{25,26} mutant suffices for promoting these responses.

Gene Expression Profiling Uncovers Key p53 Target Genes Involved in Metabolic Regulation

Because p53^{25,26} activates only a small subset of p53-dependent genes, yet retains full biological activity in pro-survival and OXPHOS regulation, we used it to hone in on the most relevant p53 target genes for metabolic homeostasis. We performed transcriptomic analysis of oncogenic HrasV12-expressing MEFs expressing each of the TAD mutants (Figure 3A) (Brady et al., 2011). To identify the small subset of p53 target genes still activated efficiently by p53^{25,26}, we used significance analysis of microarrays (SAM) with a false discovery rate of 1% (Tusher et al., 2001) to compare the gene expression profiles of *HrasV12;p53*$^{25,26/25,26}$ and *HrasV12;p53*$^{-/-}$ MEFs, as well as those of *HrasV12;p53*$^{+/+}$ and *HrasV12;p53*$^{-/-}$ MEFs. We derived lists of genes induced at least two-fold by p53^{25,26} and wild-type p53 and then interrogated which genes were activated by p53^{25,26} to an extent at least 70% of that seen with wild-type p53. We thus identified a list of 50 unique genes.

We performed Gene Ontology-based functional enrichment analysis using the PANTHER Classification System. Intriguingly, the most enriched biological processes were the energy homeostasis-related Acyl-CoA metabolic process (p = 0.001), with multiple other metabolism-related functional groups also being significantly enriched (Figure 3B). Acyl-CoA is an intermediate formed during fatty acid catabolism, upon attachment of coenzyme A to long-chain fatty acids. During FAO, acyl-CoA is broken down in the mitochondria through β oxidation to produce acetyl-CoA, NADH, and $FADH_2$, which can be channeled into the tricarboxylic acid cycle and electron transport chain for ATP production, thus providing an important energy-generating strategy upon glucose starvation. p53 has a documented

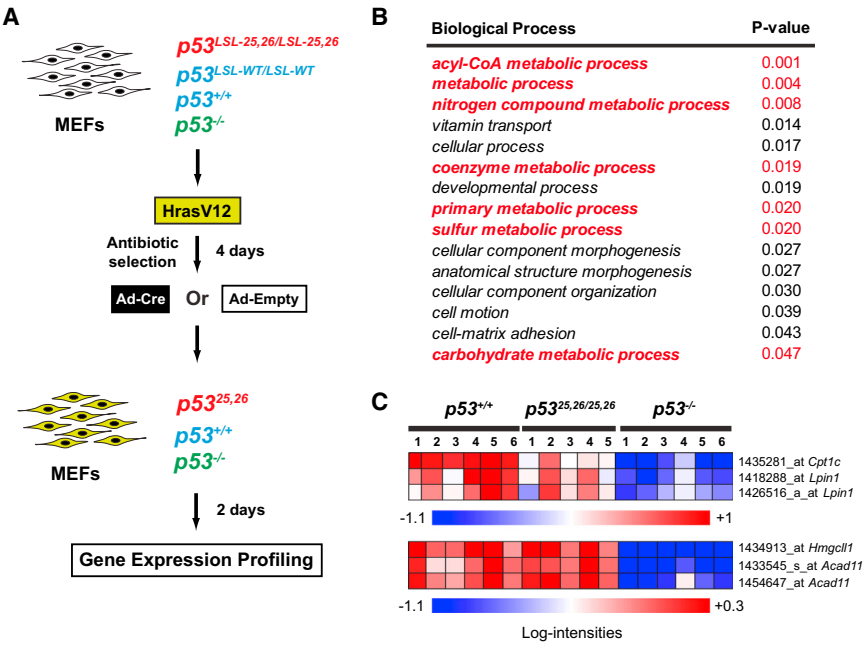

Figure 3. Gene Expression Profiling to Identify Genes Activated by Both Wild-Type p53 and $p53^{25,26}$ in HrasV12 MEFs

(A) Experimental scheme. Primary MEFs homozygous for the various *LSL p53* TAD mutant alleles were retrovirally transduced with HrasV12, then infected with Ad-Cre to recombine the LSL element and express the *p53* alleles. Empty adenoviruses (Ad-Empty) were used to generate *p53* null control MEFs. Wild-type ($p53^{+/+}$) and $p53^{-/-}$ MEFs provided additional controls.

(B) Top enriched biological processes ($p < 0.05$) by PANTHER analysis of the top 50 unique genes identified as being efficiently activated by both wild-type p53 and $p53^{25,26}$. The p values are calculated by the binominal statistic.

(C) Heatmap analysis of *Acad11* (two probe sets) and *Hmgcll1* (one probe set), genes within the Acyl-CoA metabolic process category (bottom). The numbers above the heatmaps represent the different biological replicates within each genotypic group of MEFs. *Cpt1c* (one probe set) and *Lpin1* (two probe sets), which fail to meet the stringent cutoff, are shown (top). Red and blue represent higher and lower expression, respectively.

role in FAO (Goldstein and Rotter, 2012), and p53 activates genes involved in FAO, including *Cpt1c* (carnitine palmitoyltransferase 1C), encoding a protein that stimulates the import of fatty acids into mitochondria for β oxidation (Kerner and Hoppel, 2000), and *Lpin1*, encoding Lipin 1, which cooperates with peroxisome proliferator-activated receptor (PPAR) α and PPARγ coactivator-1α to activate FAO (Assaily et al., 2011). Because FAO is a complex catabolic process, we reasoned that Cpt1c and Lpin1 might not account for the full effect of p53 on FAO. Moreover, although we found that *Cpt1c* and *Lpin1* exhibited a similar expression pattern to the top genes identified by microarray analysis, displaying reasonably efficient induction in HrasV12 MEFs by wild-type p53 and $p53^{25,26}$ relative to p53-deficient cells, they did not pass the stringent filters to be listed in the top 50 genes (Figure 3C, top). Interestingly, one of the genes we identified in the Acyl-CoA metabolic process group is *Acad11*, encoding a member of the acyl-CoA dehydrogenase family (He et al., 2011), a group of enzymes involved in β oxidation of fatty acids, suggesting that it could contribute to p53 induction of FAO (Figure 3C, bottom). In addition, although not directly involved in FAO, the other gene in this category, *Hmgcll1*, encodes an extramitochondrial hydroxymethylglutaryl-CoA lyase, a key enzyme in ketogenesis, a process by which acetyl-CoA is converted into ketone bodies mainly in the liver, which are then dispersed to other tissues in the body under conditions of starvation for energy production (Montgomery et al., 2012).

Because these four genes are involved in fatty acid metabolism, analysis of their p53-dependent regulation may help better understand the pro-survival function of p53. We validated the differential expression of *Acad11*, *Hmgcll1*, *Cpt1c* and *Lpin1*, using quantitative RT-PCR (qRT-PCR) to examine their relative expression levels in a panel of HrasV12 MEFs expressing different p53 TAD mutants. MEFs with wild-type p53 showed robust induction of all these genes relative to p53-deficient MEFs (Figure 4A). Moreover, $p53^{25,26}$ activated *Acad11* as potently as wild-type p53 and efficiently induced *Hmgcll1*, *Cpt1c*, and *Lpin1* expression, albeit not quite to the same extent as wild-type p53. In contrast, the transcriptionally dead $p53^{25,26,53,54}$ mutant failed to induce the expression of these genes, indicating that their induction indeed depends on p53 transactivation.

Metabolic Target Genes Are Induced by p53 in Different Cellular Contexts

To assess how generally these genes are regulated by p53, we tested whether these genes are induced by p53 in other contexts. To assess p53 activation triggered by another type of stress signal, we examined the expression of these genes in wild-type p53 and *p53* null MEFs treated with doxorubicin, a genotoxic agent that activates p53 (Figure 4B). Doxorubicin treatment robustly increased the expression of *Acad11*, *Hmgcll1*, and *Cpt1c* in wild-type p53 MEFs compared with *p53* null MEFs and in normal human fibroblasts expressing a control small hairpin RNA (shRNA) relative to those expressing a *p53* shRNA (Figure 4C). *Lpin1* induction was not different in wild-type and p53-deficient fibroblasts treated with DNA damage, in contrast to the previously reported p53-dependent *Lpin1* induction by γ irradiation in mouse hematopoietic cells (Assaily et al., 2011), suggesting that the regulation of *Lpin1* by p53 is context dependent.

To determine whether *Acad11* is induced by wild-type p53 and $p53^{25,26}$ in a different tumor cell type, we examined its expression in cells derived from non-small-cell lung cancers (NSCLCs) arising in $Kras^{G12D};p53^{LSL\text{-}wt/LSL\text{-}wt}$ or $Kras^{G12D};p53^{LSL\text{-}25,26/LSL\text{-}25,26}$ mice harboring a latent $Kras^{G12D}$ allele (Johnson et al., 2001). Expression of the *p53* alleles was achieved by Ad-Cre infection in vitro. *Acad11*, *Cpt1c*, and *Hmgcll1* levels were efficiently

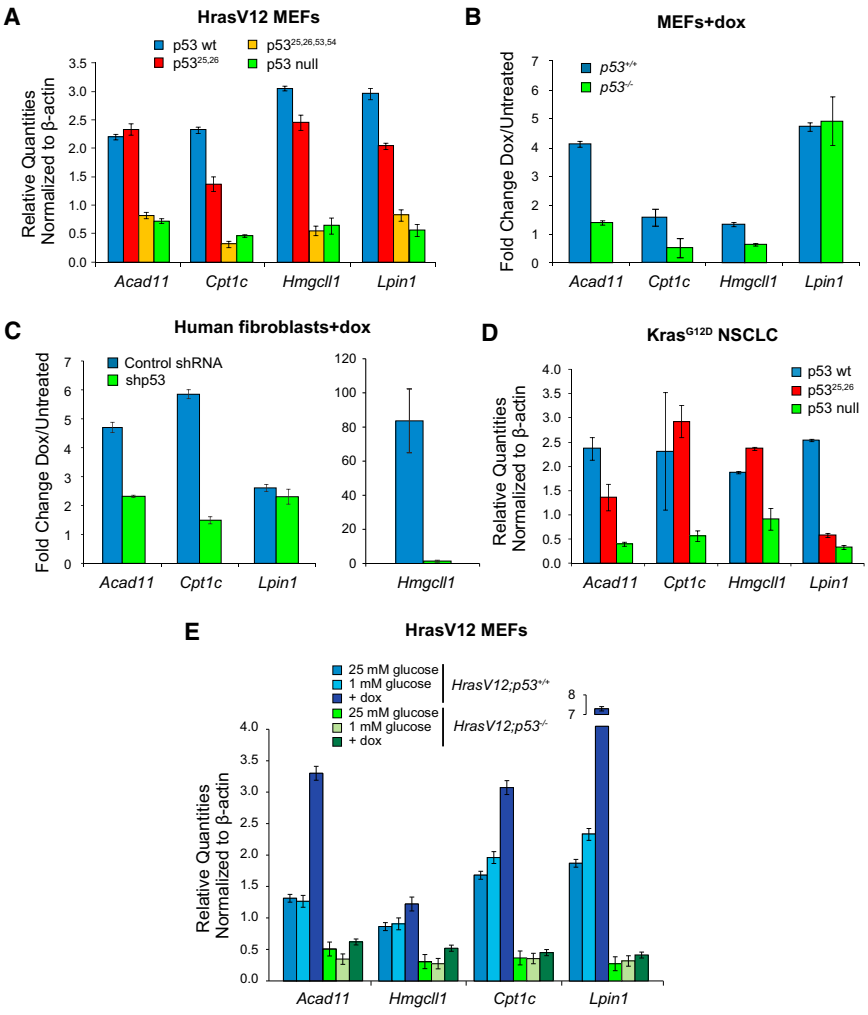

Figure 4. Induction of Metabolic Target Genes Depends on p53 Transactivation Function

(A) Validation of *Acad11*, *Cpt1c*, *Hmgcll1*, and *Lpin1* expression levels using qRT-PCR analysis on HrasV12 MEFs homozygous for *p53* mutant alleles.

(B) qRT-PCR analysis of *Acad11*, *Cpt1c*, *Hmgcll1*, and *Lpin1* expression levels in $p53^{+/+}$ and $p53^{-/-}$ MEFs either left untreated or treated with 0.2 μg/ml doxorubicin (dox) for 6 hr.

(C) qRT-PCR analysis of *Acad11*, *Cpt1c*, *Hmgcll1*, and *Lpin1* expression levels in normal human fibroblasts expressing a *p53* shRNA or control shRNA. Cells were left untreated or treated with 0.2 μg/ml dox for 6 hr.

(D) qRT-PCR analysis of *Acad11*, *Cpt1c*, *Hmgcll1*, and *Lpin1* expression levels in $Kras^{G12D}$-expressing mouse NSCLC cells of different *p53* genotypes. For all panels, colors represent the different p53 genotypes.

(E) qRT-PCR analysis of *Acad11*, *Cpt1c*, *Hmgcll1*, and *Lpin1* expression levels in *HrasV12;*$p53^{+/+}$ and *HRasV12;*$p53^{-/-}$ MEFs cultured in normal glucose (25 mM), low glucose (1 mM), or 0.2 μg/ml dox as a positive control for p53 induction, for 6 hr.

For (A), (D), and (E), values are average quantities of technical triplicates normalized to β actin ± c.v. (coefficient of variation). For (B) and (C), the ratios of treated/untreated normalized to β actin ± c.v. are graphed. See also Figure S3.

induced by both wild-type p53 and $p53^{25,26}$ in $Kras^{G12D}$-expressing NSCLC cells (Figure 4D). In contrast, *Lpin1* was not efficiently induced by $p53^{25,26}$ in this setting. Together, these data show that a group of genes involved in fatty acid metabolism—*Acad11*, *Hmgcll1*, and *Cpt1c*—are regulated by p53 in different cell types and in response to both oncogenic stress and DNA damage, suggesting their general importance for p53 function.

To link the p53 transactivation-dependent induction of these target genes to pro-survival function upon glucose starvation, we examined whether glucose deprivation also induces expression of these genes. qRT-PCR analysis of *HrasV12;*$p53^{+/+}$ and *HrasV12;*$p53^{-/-}$ MEFs under both normal and low-glucose conditions revealed that the p53-dependent induction of all four metabolic target genes was comparable between the two glucose conditions, indicating that glucose deprivation of oncogenic Ras-expressing cells does not further induce metabolic gene expression (Figure 4E). Accordingly, total p53 protein levels and p53 serine 18 phosphorylation (serine 15 in humans) were not altered by either low glucose or 2-DG treatment, as they were by doxorubicin (Figure S3). Together, these findings indicate that oncogenic Hras triggers p53-dependent expression of these metabolic target genes and that glucose deprivation does not further augment this induction. Thus, p53 is poised to respond to glucose limitation yet glucose deprivation does not itself directly affect p53 activity but instead some other cooperating pathway, lending important insight into how p53 responds to nutrient starvation.

p53-Regulated Metabolism Genes Are Direct p53 Target Genes

To determine whether these metabolism-related genes are direct targets of p53, we leveraged a chromatin immunoprecipitation (ChIP)-sequencing (ChIP-seq) data set we generated previously using wild-type MEFs treated with doxorubicin (Kenzelmann Broz et al., 2013). We associated p53-binding regions with specific genes if within 10 kb of the gene, and these regions were subjected to sequence analysis to identify p53 binding elements (Menendez et al., 2009). These analyses revealed p53 binding sites matching the RRRCWWGYYY(0-13bp) RRRCWWGYYY consensus binding sequence in all four genes, with *Acad11*, *Cpt1c*, and *Lpin1* each bearing two sites and *Hmgcll1* bearing one (Figure 5A). Site 1 of *Acad11*, site 2 of *Cpt1c*, and both sites of *Lpin1* significantly match the p53 consensus binding sequence, with at least 7 of 10 matching base pairs in each half-site and minimal spacers (0 or 1 base pair) between half-sites. Notably, site 2 of *Cpt1c* and site 1 of

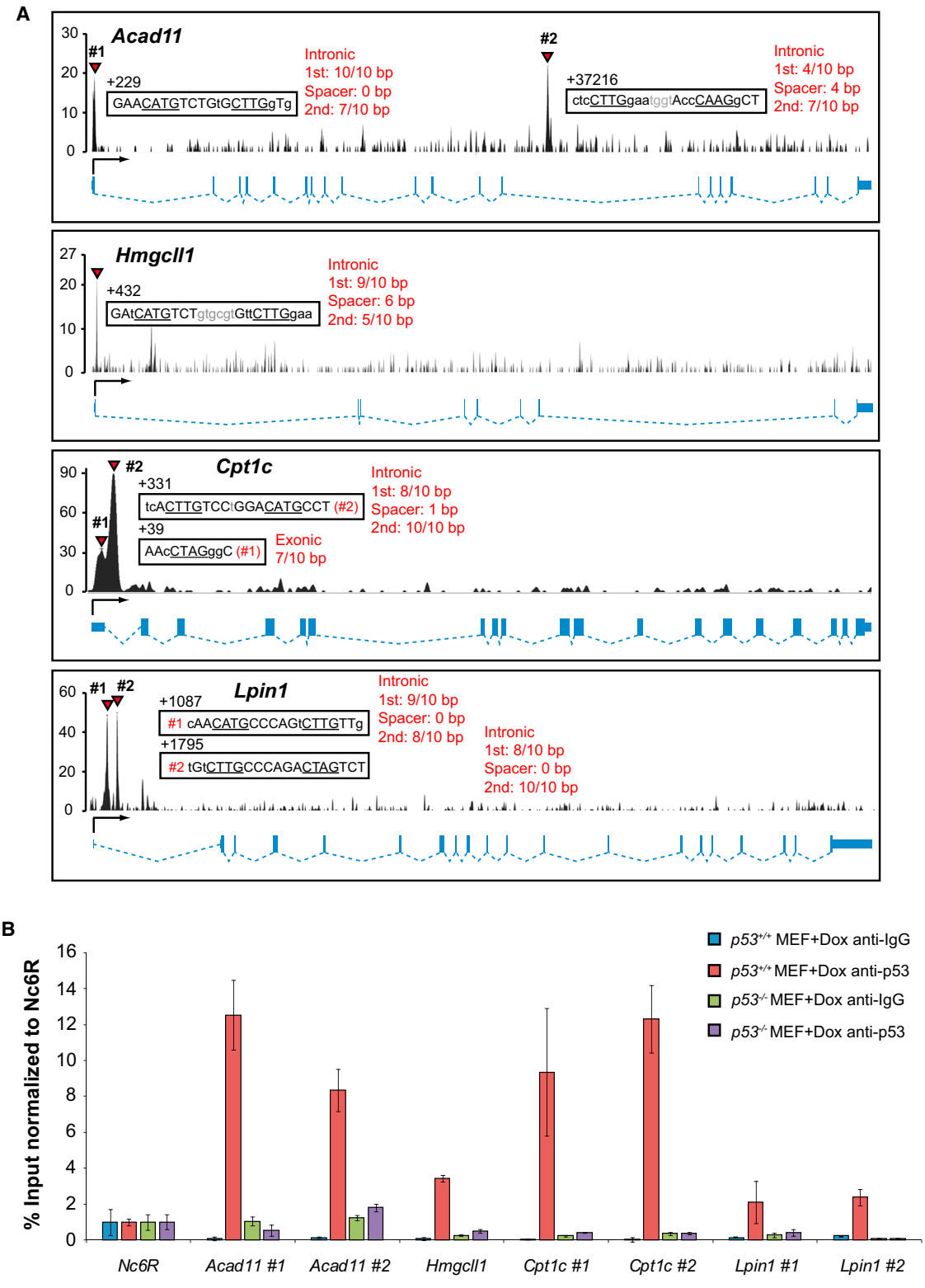

Figure 5. ChIP Analysis of Direct p53 Binding to Metabolic Genes

(A) ChIP-seq profiles and identified peak-associated p53 binding sites for each metabolism-related gene. Exons are shown as blue boxes, and introns are marked by blue dashed lines. Inverted red triangles point to the called peaks. Arrows indicate the transcription start site (TSS). Uppercase letters in binding site represent bases matching the consensus p53-binding sequence, and lowercase letters represent mismatches. Underlined letters highlight the critical bases in the p53

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Lpin1 match the previously published sites (site RE-A of *Cpt1c* and 1A of *Lpin1*; Assaily et al., 2011; Sanchez-Macedo et al., 2013), further validating our results. The other sites were more degenerate relative to the consensus. Interestingly, within the first ChIP-seq peak in *Cpt1c*, we only identified a half-site with 7 of 10 base pairs matching the consensus, consistent with reports that half-sites can be bound and regulated by p53 (Menendez et al., 2009). p53 binding to all regions in these genes containing predicted sites was confirmed by individual ChIP-qPCR analyses in doxorubicin-treated wild-type MEFs (Figure 5B). Collectively, our findings demonstrate that *Acad11*, *Hmgcll1*, *Cpt1c*, and *Lpin1* are direct p53 targets.

To evaluate whether p53 binding to *Acad11* sites is retained in HrasV12 MEFs under different glucose conditions, we performed p53 ChIP on both *HrasV12;p53*$^{+/+}$ and *HrasV12;p53*$^{25,26/25,26}$ MEFs. qPCR analysis demonstrated binding to both sites by both wild-type p53 and p53^{25,26} in both normal and low-glucose conditions (Figure S4). The 25,26 mutation quantitatively affects binding to these sites, particularly binding site 1, but without compromising the induction of *Acad11*, suggesting that binding site 2 may be more important for *Acad11* induction in this setting (Figure 4). These findings suggest that p53 directly binds to the *Acad11* genomic locus to induce its expression in the context of oncogenic Hras expression, and that p53 binding is not significantly altered by glucose starvation, in keeping with the observation that *Acad11* induction by p53 is not altered by glucose starvation in this context (Figure 4E).

p53-Regulated Metabolic Genes Are Evolutionarily Conserved

Evolutionary conservation of a gene product provides key evidence for an essential role in supporting normal cellular activities. Because *Acad11* is a previously undescribed p53 target gene encoding a component involved in FAO, a process clearly implicated in survival under metabolic stress, we analyzed its evolutionary conservation. Interestingly, protein sequence alignment revealed Acad11 orthologs, with two conserved regions, in *D. melanogaster* and *C. elegans* (Figures 6A and 6B). DNA sequence analysis also revealed the presence of putative p53 binding sites in the promoters of the fly and worm genes, supporting the notion that these genes are regulated by p53 in simpler eukaryotes (Figure 6C). Indeed, the *CG6638* transcript is significantly induced in flies expressing wild-type Dmp53, the fly p53 ortholog, compared with those carrying a *Dmp53* null allele (Figure 6D) (Sogame et al., 2003). This observation suggests that the regulation of *Acad11* expression by p53 is evolutionarily conserved, highlighting its functional relevance. Similarly, we identified sequence conservation of Hmgcll1, Cpt1c, and Lpin1 proteins across species as well as putative p53-binding sites in these genes in flies and worms, again suggesting the importance of these genes for p53 function (Figure S5).

Acad11 Is a Component of p53 Pro-Survival Program

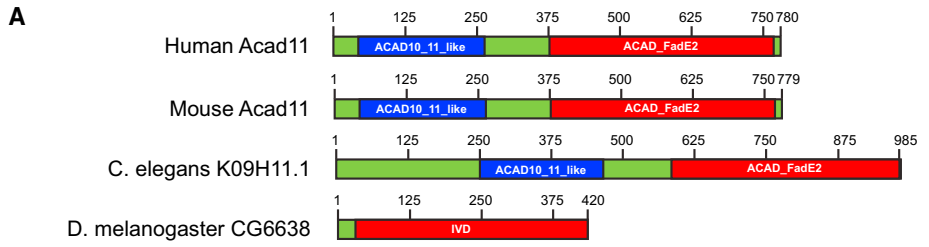
To gain new insight into p53 pro-survival function, we tested whether Acad11 is required for survival upon glucose deprivation by comparing the survival of *HrasV12;p53*$^{+/+}$ MEFs expressing *Acad11* shRNAs with those expressing shGFP shRNAs upon 2-DG treatment. Acad11 knockdown rendered the cells more sensitive to 2-DG treatment, indicating that Acad11 does in fact contribute to the pro-survival function of p53 in this setting (Figures 7A and 7B). In addition, as we showed that OXPHOS is associated with p53 pro-survival function, we characterized the dependence of OXPHOS on Acad11. The OCR of *HrasV12;p53*$^{+/+}$ MEFs with attenuated *Acad11* expression was significantly diminished relative to the OCR in shGFP-expressing cells, suggesting that Acad11 is a downstream mediator of p53 in promoting OXPHOS (Figure 7C). As a complementary approach, we tested whether enforced expression of Acad11 is sufficient to protect cells from metabolic stress, by comparing the survival of *HrasV12;p53*$^{-/-}$ MEFs overexpressing *Acad11* with the survival of *HrasV12;p53*$^{-/-}$ MEFs overexpressing GFP upon glucose starvation. Indeed, upon glucose starvation, *HrasV12;p53*$^{-/-}$ MEFs overexpressing Acad11 displayed significantly improved survival relative to GFP-expressing control cells (Figures 7D and 7E). *Hmgcll1*, but not *Cpt1c*, also promoted cell survival in this context. Interestingly, combined overexpression of *Acad11* with other target gene(s) did not further improve survival, suggesting that these gene products may function in a redundant fashion. Overexpression of Acad11 in HT1080 human fibrosarcoma cells also significantly improved survival upon glucose starvation (Figure S6). Collectively, these observations suggest that Acad11 is an important component of the p53 pro-survival program.

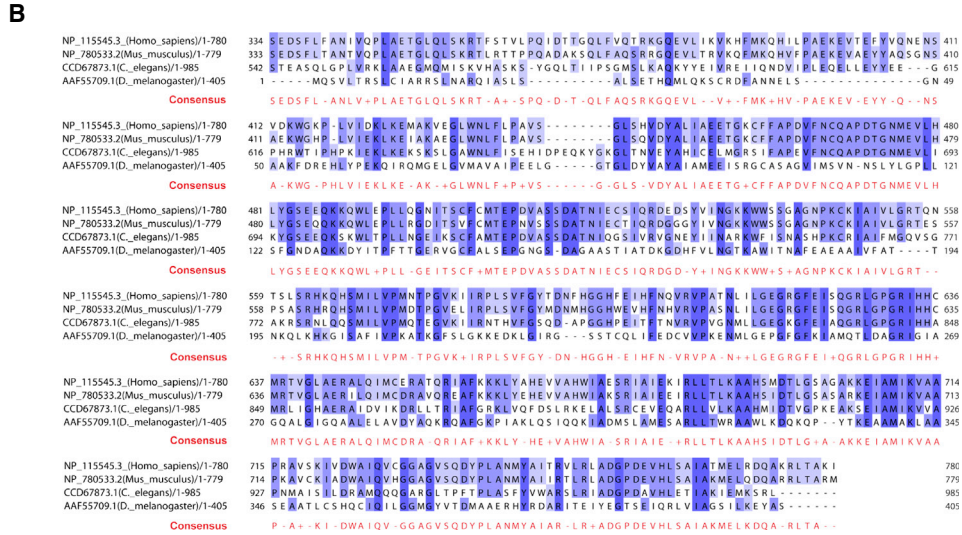
In certain contexts, the p53-mediated pro-survival function may promote tumor growth by leading to resistance to metabolic stresses characteristic of tumor microenvironment. In support of this notion, *p53*$^{-/-}$ HCT116 xenograft tumors display heightened sensitivity to serine and glycine deprivation relative to *p53*$^{+/+}$ HCT116 tumors (Maddocks et al., 2013). To examine whether p53-deficient tumors are similarly sensitive to glucose limitation in vivo, we established subcutaneous *HrasV12;p53*$^{-/-}$ MEF xenograft tumors in nude mice and then compared tumor growth upon maintaining these mice on a standard diet (44.2% carbohydrate) or on a low-carbohydrate, high-fat ketogenic diet (3.2% carbohydrate), thus mimicking glucose starvation. We found that the ketogenic diet significantly delayed early tumor growth (Figure 7F). The difference in tumor growth was not due to

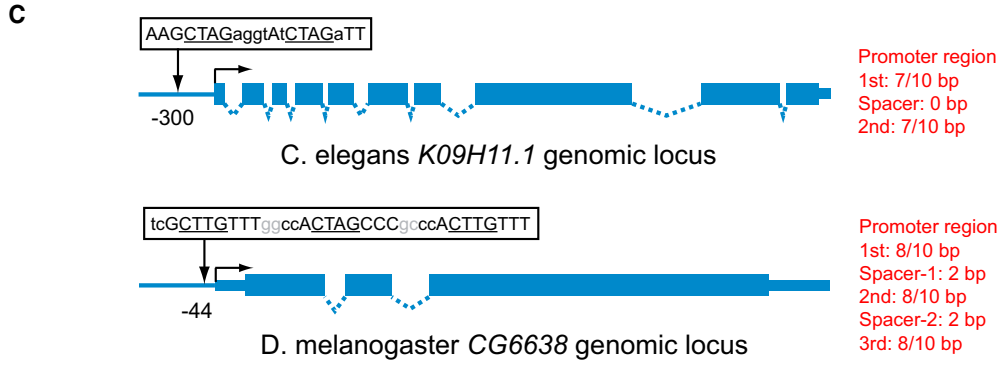
response element. Gray lowercase letters represent spacers between the two half-sites. The position of the site (intronic or exonic), the number of base pairs in individual half-sites matching the consensus sequence, and the length of the spacers between half-sites are summarized in red. The numbers above the sites show the distance in base pairs from the TSS.

(B) qPCR analysis confirming enrichment of p53 binding at the sites shown in (A) after ChIP using either a p53-specific antibody or immunoglobulin G control, from dox-treated *p53*$^{+/+}$ (red and blue bars, respectively) and *p53*$^{-/-}$ MEFs (negative control, purple and green bars, respectively). Percentages of ChIP relative to input were calculated for individual sites, then normalized to that of Nc6R, which is set to 1%. The results are then plotted ± SEM. Nc6R represents a random "gene desert" region selected as a negative control.

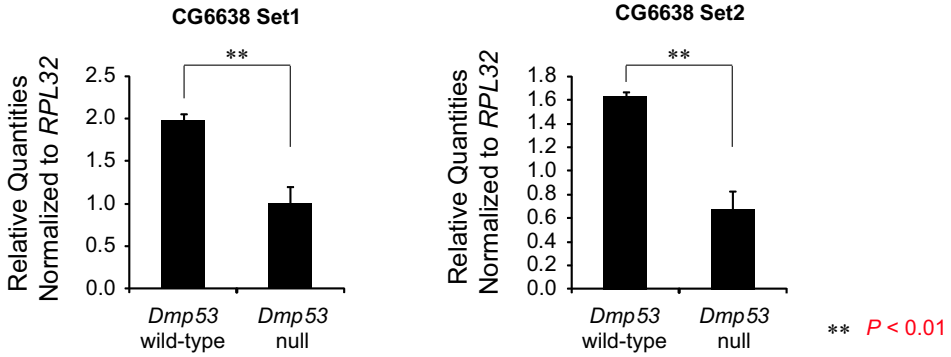
See also Figure S4.







Consensus **5'- PuPuPu C(A/T)(T/A)G PyPyPy -3' (0~13) 5'- PuPuPu C(A/T)(T/A)G PyPyPy -3'**



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general nutrient deficiency, as the average weights of the mice in the two cohorts were similar during the dietary treatment (Figure 7G). Together, these findings suggest that because of their compromised FAO-promoting capacity, p53-deficient tumors may be sensitive to metabolic insults inherent to the tumor microenvironment.

DISCUSSION

Here, we examine the mechanisms underlying p53 pro-survival function in neoplastic cells in response to glucose deprivation. We find that oncogenic HrasV12-expressing MEFs with wild-type p53 survive better than their *p53* null counterparts upon glucose starvation. In this context, p53 promotes OXPHOS by promoting FAO, suggesting that the pro-survival effect is due at least in part to positive regulation of the cellular energy supply by p53. Consistent with its critical role in other p53-regulated processes, including acute DNA damage responses and tumor suppression, p53 transactivation function is also required for promoting survival and OXPHOS upon glucose deprivation. To gain mechanistic insight into p53 pro-survival function, we performed microarray analysis on HrasV12-expressing MEFs derived from knockin mice expressing various p53 TAD mutants. In particular, we leveraged cells expressing $p53^{25,26}$, a TAD mutant capable of promoting survival and OXPHOS upon glucose starvation, despite being deficient for efficient transactivation of most p53 target genes. This mutant remains capable, however, of robustly activating a small subset of p53 genes and therefore provides a useful tool for honing in on p53 target genes involved in particular p53 biological functions retained by this mutant (Brady et al., 2011; Jiang et al., 2011). Interestingly, we found that metabolic regulation was the top enriched function in the list of genes significantly induced by both wild-type p53 and $p53^{25,26}$. Within this category, we identified a group of lipid metabolism-related genes, including both new (*Acad11* and *Hmgcll1*) and known (*Cpt1c* and *Lpin1*) transcriptional targets of p53. As a new p53 target gene with a known function in FAO, we analyzed *Acad11* further and demonstrated that Acad11 contributes to the p53-dependent functions of promoting cell survival and OXPHOS upon glucose starvation. Our studies thus reveal that *Acad11* is an important p53 target gene involved in p53 pro-survival function under metabolic stress.

To survive deprivation of glucose, cells can use other sources of energy-producing nutrients, such as fatty acids and glucogenic and ketogenic amino acids (Wolfe, 1998). FAO is a complex process involving substrate activation, transport into the mitochondria, and sequential removal of acetyl-CoA molecules, which can enter the citric acid cycle for ATP production. Although a role for p53 in FAO upon glucose starvation has been shown (Assaily et al., 2011), the mechanisms by which p53 regulates FAO are continually emerging (Goldstein and Rotter, 2012), as exemplified by our identification of *Acad11* as a p53 target gene. *Acad11* encodes a member of the acyl-CoA dehydrogenase family and is highly expressed in adult human brain, liver, heart, and kidney (He et al., 2011). Acad11 preferentially uses very long-chain fatty acids as substrates, which are abundant in neuronal tissues and are important for myelin synthesis (He et al., 2011; Svennerholm and Ställberg-Stenhagen, 1968). Thus, Acad11 is likely important for energy homeostasis not only in neoplastic cells, as we have shown, but also potentially in these normal tissues. Interestingly, combined overexpression of *Acad11* and other p53 target genes involved in fatty acid metabolism did not further increase survival upon glucose starvation, suggesting that these specific genes may function in a redundant fashion and that that genes in other metabolic pathways may also be important for mediating the full pro-survival effects of p53. Future analyses will further elaborate the intricate network activated by p53 to protect cells from glucose starvation.

Interestingly, our study suggests that the ability of p53 to positively regulate FAO by transactivating specific targets could also contribute to the survival of tumor cells in certain circumstances. Indeed, a pro-survival function for p53 in cancer has been suggested by several recent studies. For example, by directing de novo serine synthesis to GSH production upon serine starvation and consequently limiting oxidative stress, p53 can promote human colon carcinoma cell survival both in vitro and in a xenograft model (Maddocks et al., 2013). Analysis of mice lacking the p53 antioxidant target gene *Tigar* showed that Tigar supports intestinal adenoma development, an observation supported by TIGAR overexpression during human colon cancer progression (Cheung et al., 2013). Furthermore, when challenged by prolonged nutrient starvation, p53 promotes a reduced but sustainable level of autophagic flux by downregulating LC3 expression, facilitating tumor cell

Figure 6. Regulation of Acad11 by p53 Is Evolutionarily Conserved

(A) The organization of the domains in the ACAD proteins is conserved across species, as determined by the NCBI Conserved Domain Database. Blue denotes the ACAD10_11_like domain, which is unique to the Acad10 and Acad11 members of the ACAD protein family and has similarity to phosphotransferases catalyzing intramolecular transfer of phosphate groups. Red denotes the ACAD_FadE2 FAD-binding domain identified in FadE2-like Acyl-CoA dehydrogenases within the ACAD family of proteins in *Homo sapiens*, *Mus musculus*, and *C. elegans* and the IVD FAD-binding domain (identified in isovaleryl-CoA dehydrogenase [IVD]) within the *D. melanogaster* homolog. The numbers above each protein denote amino acid positions.

(B) Protein sequence alignment of the ACAD_FadE2 domains in *H. sapiens*, *M. musculus*, and *C. elegans* and the IVD domain in *D. melanogaster* using the ClustalW2 program. Blue shading indicates the identities of residues between proteins of different species, generated by the percentage identity option in Jalview, with darker blue signifying identity in more species.

(C) The genomic organization of the *K09H11.1* (*C. elegans Acad11* homolog) and *CG6638* (*D. melanogaster Acad11* homolog) loci and the sequences of the identified p53-binding sites within these loci. The number of base pairs in individual half-sites matching the consensus sequence and the length of the spacers between the two half-sites are summarized in red. The consensus p53-binding sequence is shown below.

(D) qRT-PCR analysis of *CG6638* expression in cells from wild-type or *Dmp53* null *D. melanogaster* embryos.

Levels represent mean quantities of technical triplicates from two independent sets of total RNAs normalized to *RPL32* ± c.v. The p values from Student's t test are indicated. See also Figure S5.

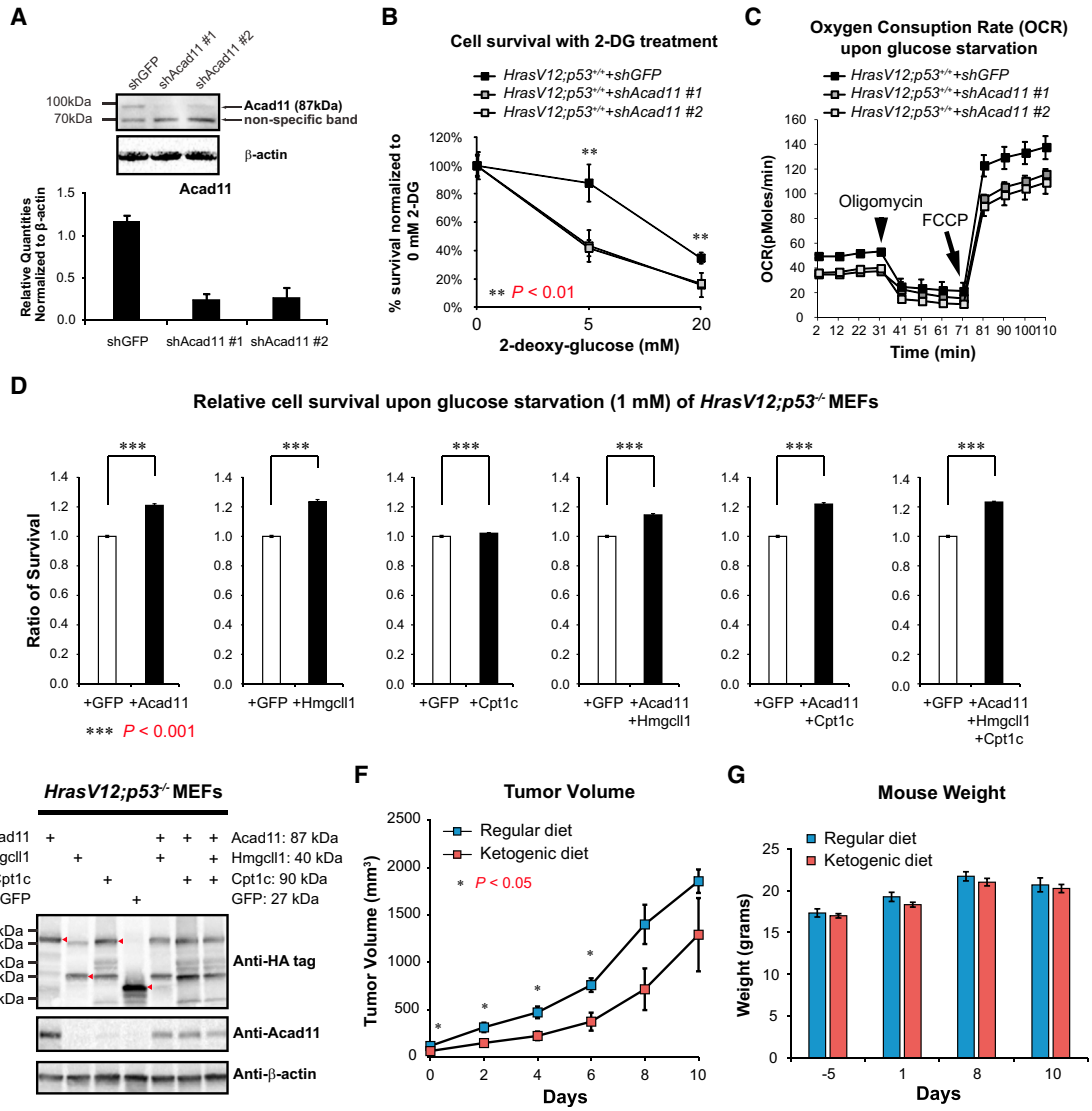

Figure 7. Acad11 Is Required for the Pro-Survival and OXPHOS-Promoting Functions of p53

(A) Knockdown of Acad11 was confirmed by western blotting (top) and qRT-PCR (bottom). β Actin served as loading control. qRT-PCR data represent the mean quantities from technical triplicates normalized to β actin ± c.v.

(B) Fractions of surviving *HrasV12;p53*$^{+/+}$ MEFs expressing *Acad11* or *GFP* control shRNAs in the presence of 5 or 20 mM 2-DG compared with cell survival in 0 mM 2-DG, which is set to 100%, after 72 hr. Results represent the mean ± SEM of direct cell counts.

(C) Average OCR ± SEM of *HrasV12;p53*$^{+/+}$ MEFs expressing *Acad11* or *GFP* control shRNAs, by the Seahorse XF assay. (D) Relative survival of *HrasV12;p53*$^{-/-}$ cells overexpressing Acad11, Hmgcll1, Cpt1c, (black boxes), GFP (open boxes), or the combination of Acad11 and another gene (black boxes) in 1 mM glucose normalized to 25 mM glucose after 48 hr. Data are relative to the GFP control, which is set to 1.0. Results represent the mean ± SEM by SRB staining.

(E) Overexpressed protein levels were assessed by western blotting using anti-HA and anti-Acad11 antibodies. β Actin serves as a loading control. Red triangles point to the bands corresponding to the overexpressed proteins based on their molecular weight.

(F) Effect of glucose starvation on *HrasV12;p53*$^{-/-}$ xenograft tumor growth. Two weeks after tumor cell injection, mice were maintained on a ketogenic diet (glucose-starved) or a regular diet. Average tumor volume ± SEM was plotted as a function of time starting from the first measurement.

(G) Average mouse weights ± SEM in both dietary groups on specific days are graphed.

The p values from Student's t test are indicated. See also Figure S6.

survival (Scherz-Shouval et al., 2010). In addition, by inducing autophagy and FAO, p53 protects xenograft tumors from metabolic stress induced by metformin, an anti-diabetic agent that lowers blood glucose levels (Buzzai et al., 2007). Finally, Cpt1c also promotes tumor growth in breast and colon cancer xenograft models and in a neurofibromatosis type I genetically engineered mouse model (Sanchez-Macedo et al., 2013; Zaugg et al., 2011). Thus, multiple lines of evidence suggest that p53 may promote tumor cell survival under metabolic stress conditions. Indeed, the retention of wild-type p53 in some human

cancers suggests that p53 may promote tumor progression in certain contexts (Kim et al., 2009; Lill et al., 2011).

Although a key function of p53 across evolution is in maintaining germline integrity in response to genotoxic stress (Levine et al., 2011), there is also evidence that the pro-survival activity of p53 is an ancestral function. For example, the worm p53 ortholog CEP-1 is negatively regulated by AKT-1 and ABL-1 and is thus integrated into pathways for sensing glucose and oxygen availability (Belyi et al., 2010; Levine et al., 2011). Moreover, akin to mammalian cells, ATP deficiency activates the AMPK-p53 pathway in flies, promoting G_1 cell-cycle arrest (Mandal et al., 2005). We recently also showed that p53 and its family members p63 and p73, the ancestors of the family, participate together to induce an autophagy gene expression program, suggesting that metabolic regulation is an ancient function of the family that may have arisen to help cope with nutrient stress (Kenzelmann Broz et al., 2013). Accordingly, a recent study showed that TAp63, a p63 isoform that retains a full transactivation domain, transcriptionally activates the metabolic regulators *Sirt1*, *AMPKa2*, and *LKB1* and that TAp63-deficient MEFs displayed defects in FAO (Su et al., 2012). Furthermore, TAp73 transactivates *COX4*, encoding cytochrome *c* subunit 4, and TAp73 deficiency leads to attenuated oxygen consumption and ATP production (Rufini et al., 2012). These observations collectively suggest that a metabolic stress response program was built into the intricate functional network regulated by the p53 family during evolution. The fact that Acad11 is evolutionarily conserved in flies and worms suggests its importance in this metabolic network.

There are critical clinical implications of p53 pro-survival activity. The ability of p53 to limit ROS accumulation, through mechanisms such as inducing *TIGAR* and channeling serine metabolism toward GSH synthesis, highlights the increased fitness and survival potential of tumor cells maintaining wild-type p53 under metabolic stress. Thus, p53 deficiency, or inhibition of its downstream target genes involved in cell survival, such as *Acad11*, may create a specific vulnerability that can be targeted in therapies based on metabolic stress. Future investigation into the precise role of the p53 pro-survival program in different tumor types and stages will further illuminate the biological functions of p53 and reveal new opportunities for therapeutic intervention.

EXPERIMENTAL PROCEDURES

Cell Culture

Cells were prepared and cultured as described (Johnson et al., 2005; Kenzelmann Broz et al., 2013). Infections of adenovirus (University of Iowa GTVR) were performed as described (Brady et al., 2011). Lentiviral overexpression vectors (pLEX-Acad11/Hmgcll1/Cpt1c/GFP) were constructed using the pLEX-MCS vector (Thermo Scientific).

Microarray Analysis

Microarray experiments were performed and analyzed as described (Brady et al., 2011). Class comparison analysis was performed using SAM (Tusher et al., 2001).

qRT-PCR, ChIP, and Western Blotting

Total RNA isolation with Trizol reagent (Invitrogen) and qRT-PCR using SYBR green (SA-Biosciences) and a 7900HT Fast Real-Time PCR machine (Applied Biosystems) were performed as described (Brady et al., 2011). *Drosophila* mRNA was prepared as described (Link et al., 2013). ChIP and qPCR quantification was performed as described (Kenzelmann Broz et al., 2013). qPCR primer sequences are listed in the Supplemental Information. Western blotting was performed using standard protocols, with anti-p53 (CM5, Vector Labs, 1:1000), anti-phospho-p53Ser15 (9284, Cell Signaling, 1:1000), anti-Acad11 (E-15, Santa Cruz, 1:1000), anti-HA (HA.11, Covance, 1:2000) and anti-actin (C-11, Santa Cruz, 1:1000) antibodies.

Protein Sequence Analysis

To analyze protein orthologs in different organisms, BLASTP (http://blast.ncbi.nlm.nih.gov) searches using the human protein sequences as query sequences were performed. The protein sequences of the top hits were aligned using the ClustalW2 tool.

Cell Survival Assays

For cell survival assays, 0.15×10^6 cells/well (24-well plates) or 5×10^3 to 2×10^4 cells/well (96-well plates) were plated in DMEM High Glucose (Invitrogen) plus 10% fetal calf serum. Twelve to 24 hr later, cells were washed with PBS and placed in DMEM No Glucose (Invitrogen) plus 10% dialyzed fetal bovine serum (FBS) (Invitrogen) and different concentrations of glucose. A Coulter Z1 particle counter (Beckman Coulter) (24-well) or sulforhodamine B (SRB) staining (96-well) was used to quantitate cell number.

Seahorse XF Assay

Twenty thousand cells were plated into each well of XF 96-well plates (Seahorse Biosciences) in unbuffered DMEM without glucose. XF analyses were carried out using an XF96 extracellular analyzer (Seahorse Biosciences). After measurement of basal OCR, oligomycin (2.5 μg/mL; Sigma-Aldrich) and FCCP (5 μM; Sigma-Aldrich) were sequentially added. The raw OCRs were normalized by plating efficiency. To determine the fraction of OCR due to FAO, the FAO-specific inhibitor etomoxir (100 μM; Sigma-Aldrich) was added at the beginning of the assay.

In Vivo Xenograft Tumor Model

Growth of xenograft tumors from 2×10^6 *HrasV12;p53*$^{-/-}$ MEFs injected into the flanks of nude mice was monitored by caliper measurements once tumors became palpable (~2 weeks after cell injection). At the first tumor measurement, half of the mice (n = 5; 10 tumors) were fed a regular diet and the other half a ketogenic diet (Bio-Serv). All animal studies and care were performed under the guidelines of the Stanford University Administrative Panel on Laboratory Animal Care.

ACCESSION NUMBERS

The GEO accession numbers for the microarray and ChIP-seq data used in this paper are GSE27901 and GSE46240, respectively.

SUPPLEMENTAL INFORMATION

Supplemental Information contains Supplemental Experimental Procedures and six figures and can be found with this article online at http://dx.doi.org/10.1016/j.celrep.2015.01.043.

AUTHOR CONTRIBUTIONS

D.J. and L.D.A. designed the study. C.A.B. and L.D.A. generated the p53 knockin mice. D.J., E.L.L., D.K.B., K.T.B., and N.L. performed the experiments. D.J. and L.D.A. wrote the manuscript, with feedback from J.M.A. and A.J.G.

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