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Interferon gamma (IFN-γ) disrupts energy expenditure and metabolic homeostasis by suppressing SIRT1 transcription

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ABSTRACT

Chronic inflammation impairs metabolic homeostasis and is intimately correlated with the pathogenesis of type 2 diabetes. The pro-inflammatory cytokine IFN-γ is an integral part of the metabolic inflammation circuit and contributes significantly to metabolic dysfunction. The underlying mechanism, however, remains largely unknown. In the present study, we report that IFN-γ disrupts the expression of genes key to cellular metabolism and energy expenditure by repressing the expression and activity of SIRT1 at the transcription level. Further analysis reveals that IFN-γ requires class II transactivator (CIITA) to repress SIRT1 transcription. CIITA, once induced by IFN-γ, is recruited to the SIRT1 promoter by hypermethylated in cancer 1 (HIC1) and promotes down-regulation of SIRT1 transcription via active deacetylation of core histones surrounding the SIRT1 proximal promoter. Silencing CIITA or HIC1 restores SIRT1 activity and expression of metabolic genes in skeletal muscle cells challenged with IFN-γ. Therefore, our data delineate an IFN-γ/HIC1/CIITA axis that contributes to metabolic dysfunction by suppressing SIRT1 transcription in skeletal muscle cells and as such shed new light on the development of novel therapeutic strategies against type 2 diabetes.

INTRODUCTION

Type 2 diabetes is characterized by the loss of response to insulin in peripheral organs including the skeletal muscle leading up to centric obesity. Animal studies have correlated type 2 diabetes with impaired mitochondrion biogenesis, defective oxidative phosphorylation and diminished synthesis of proteins involved in energy expenditure (1,2). Human epidemiology surveys also reveal a significant mitochondrial dysfunction with reduced energy consumption (3,4).

Energy expenditure is intricately programmed by the coordinate action of a network of transcriptional modulators *in vivo*; dysregulation of this process results in mitochondrial defect in the skeletal muscle and ultimately type 2 diabetes (5,6). FOXO1 is considered one of the master regulators of mitochondrion-dependent energy consumption (7). Nutrient withdrawal activates FOXO1, which in turn drives the transcription activation of several key genes involved in fatty acid oxidation, mitochondrial biosynthesis and oxidative phosphorylation such as *pdk4*, *mcad*, *ppargc-1a* and *cycs* (8). FOXO1 activity is tightly controlled post-translationally by posphorylation and acetylation. Importantly, deacetylation of FOXO1 by the class III deacetylase SIRT1 has been demonstrated to enhance its nuclear enrichment and target promoter binding (9,10). In addition, hypoacetylated FOXO1 mimics the effect of SIRT1 over-expression in adipocyte, suggesting the FOXO1 is a primary target of SIRT1 in balancing metabolic homeostasis (11).

SIRT1 is the mammalian ortholog of the yeast protein sir2 that links calorie restriction (CR) to longevity (12).

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The authors wish it to be known that, in their opinion, the first three authors should be regarded as joint First Authors.

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SIRT1 participates in cellular metabolism serving as an energy sensor by deacetylating a range of transcriptional modulators including FOXOs, peroxisome proliferator activated receptors (PPARs), liver X receptor (LXR) and PPAR coactivator 1 alpha (PGC-1α) (13). Notably, expression and/or activity of SIRT1 are altered during metabolic disorders. For instance, SIRT1 messages correlate with insulin sensitivity in patients with hyperglycemia whereas high levels of glucose and fatty acids are synonymous with low SIRT1 expression in cells (14,15). Despite these observations and the fact that SIRT1 agonists show potential in the treatment of type 2 diabetes (16), it remains undetermined the exact mechanism whereby SIRT1 expression is regulated.

Mounting evidence connects chronic inflammation to the disruption of cellular metabolism (17). Liu *et al.* have recently reported that mast cells contribute to the pathogenesis of type 2 diabetes by producing the pro-inflammatory cytokine interferon gamma (IFN-γ) (18). Several independent investigations have implicated IFN-γ in a host of disorders associated with mitochondrial defect. IFN-γ, through its downstream mediator JAK/IRF-1, disturbs the mitochondrial membrane potential and exacerbates liver injury induced by LPS (19). IFN-γ also desensitizes adipocyte to insulin and blocks the maturation of pre-adipocyte in a JAK1/STAT1 dependent manner (20). Here we report that IFN-γ down-regulates SIRT1 transcription and impairs energy expenditure in skeletal muscle cells by inducing the transcriptional modulators class II transactivator (CIITA) and hypermethylated in cancer 1 (HIC1). Our data highlight a previously unknown function for CIITA/HIC1 complex and provide novel insight into the pathogenesis of type 2 diabetes.

MATERIALS AND METHODS

Cell culture and treatment

HEK293, C2C12 and IMR-90 cells were grown in DMEM supplemented with 10% FBS. Cells were treated with human or murine recombinant IFN-γ (100 U/ml, R&D), nicotinamide (10 mM, Sigma), sirtinol (10 μM, Sigma), or TSA (100 nM, Sigma) for 12–36 h as indicated.

Plasmids, transient transfection and luciferase assay

FLAG-tagged CIITA (21), GFP-tagged HIC1 (22) and the promoter–luciferase constructs for the *SIRT1*, *mcad*, *ppargc-1a, cytc, cpt1* and *cox4* genes (23–27) have been described previously. Silencing of CIITA and HIC1 were mediated by small interfering RNA (siRNA) using the following sequences: for murine Ciita, #1: 5′-CUGGCA CAGUGCAAUGAAATT-3′ and #2: 5′-GAGUGAUAC AAUGGCAUUATT-3′; for human CIITA, 5′ UCUCCA GUAUAUUCAUCUATT-3′; for murine Hic1, 5′-GUCC CUUUGUAUAUUCUCUTT-3′; for human HIC1, 5′-U GAAGCGGACAUUUUACUUTT-3′. Transient transfections were performed with Lipofectamine 2000 (Invitrogen). Luciferase activities were assayed 24–48 h after transfection using a luciferase reporter assay system (Promega). Experiments were routinely performed in triplicate wells and repeated three times.

Protein extraction, immunoprecipitation and western blot

Whole-cell lysates were obtained by re-suspending cell pellets in RIPA buffer (50 mM Tris pH7.4, 150 mM NaCl, 1% Triton X-100) with freshly added protease inhibitor (Roche). Nuclear proteins were extracted essentially as described before (28). Specific antibodies or pre-immune IgGs (P.I.I.) were added to and incubated with cell lysate overnight before being absorbed by Protein A/G-plus Agarose beads. Precipitated immune complex was released by boiling with 1× SDS electrophoresis sample buffer. Alternatively, FLAG-conjugated beads (M2, Sigma) were added to and incubated with lysates overnight. Precipitated immune complex was eluted with 3× FLAG peptide (Sigma). Western blot analyses were performed with anti-FLAG, anti-GFP, anti-β-actin (Sigma), anti-acetyl lysine (Cell Signaling), anti-CIITA, anti-HIC1, anti-Brg1 and anti-SIRT1 (Santa Cruz) antibodies.

RNA isolation and real-time PCR

RNA was extracted with the RNeasy RNA isolation kit (Qiagen). Reverse transcriptase reactions were performed using a SuperScript First-strand Synthesis System (Invitrogen). Real-time PCR reactions were performed on an ABI Prism 7500 system. Primers and Taqman probes used for real-time reactions were purchased from Applied Biosystems.

Chromatin Immunoprecipitation

Chromatin Immunoprecipitation (ChIP) assays were performed essentially as described before (28). In brief, chromatin in control and treated cells were cross-linked with 1% formaldehyde. Cells were incubated in lysis buffer (150 mM NaCl, 25 mM Tris pH 7.5, 1% Triton X-100, 0.1% SDS, 0.5% deoxycholate) supplemented with protease inhibitor tablet and PMSF. DNA was fragmented into ~500-bp pieces using a Branson 250 sonicator. Aliquots of lysates containing 200 μg of protein were used for each immunoprecipitation reaction with anti-CIITA, anti-HIC1 (Santa Cruz), anti-acetyl histone H3, anti-acetyl histone H4 (Millipore), or pre-immune IgG. Precipitated genomic DNA was amplified by real-time PCR with primers surrounding the murine Sirt1 proximal promoter: forward, 5′-GCCA TCGCAAACTTGAACCACC-3′ and reverse, 5′-CGTCC GCCATCTTCCAACTGC-3′.

SIRT1 activity assay

Cells were treated with or without IFN-γ for 24 h. Whole-cell proteins were isolated with the NETN buffer (20 mM Tris pH 8.0, 100 mM NaCl, 1 mM EDTA, 0.5% NP-40) with freshly added protease inhibitor tablet and SIRT1 activities were measured with a SIRT1 assay kit (Sigma) according to vender's recommendations.

Statistical analysis

One-way ANOVA with *post hoc* Scheffe analyses were performed using an SPSS package. $P < 0.05$ were considered statistically significant (asterisks).

RESULTS

IFN-γ disrupts metabolic homeostasis

Mast cell-derived IFN-γ triggers inflammatory responses and contributes to the pathogenesis of type 2 diabetes (18). In order to probe the effect of IFN-γ on metabolic homeostasis, we examined the expression of several key genes involved in this process in response to IFN-γ treatment. IFN-γ significantly downregulated mRNA levels of a series of genes involved in energy metabolism in both skeletal muscle (C2C12, Figure 1A) and fibroblast cells (IMR-90, Figure 1B). The down-regulation of message levels of these genes likely stemmed from decreased transcription as IFN-γ directly repressed the promoter activities of these genes (Figure 1C).

The forkhead transcription factor FOXO1, whose functionalities among others include transcriptional activation of the aforementioned genes, is essential in maintaining the cellular metabolic balance. Therefore, we examined whether IFN-γ could antagonize the overall FOXO1 dependent transcriptional program. To this end, a luciferase construct driven by an array of generic forkhead response elements (FHRE) was introduced into C2C12 and IMR-90 cells. As shown in Supplementary Figure S1A, FOXO1 markedly activated luciferase activity whereas IFN-γ repressed both basal and FOXO1-stimulated activities, implicating IFN-γ as a potent suppressor of FOXO1 mediated transcription. Transcriptional activity of FOXO1 is regulated by a range of different post-translational modifications. It has been demonstrated that deacetylation of FOXO1 favors its nuclear accumulation and target occupancy (10), raising the possibility that IFN-γ may affect FOXO1 activity by targeting its acetylation levels. Indeed, exposure of cells to IFN-γ increased FOXO1 acetylation (Supplementary Figure S1B), decreased nuclear compartmentation of FOXO1 (Supplementary Figure S1C), and impeded FOXO1 recruitment to target promoter as evidenced by gel shift assay (Supplementary Figure S1D). Taken together, these data suggest that IFN-γ disrupts metabolic homeostasis in vitro in cultured skeletal muscle cells.

IFN-γ suppresses the expression of SIRT1

SIRT1 is a key mediator of cellular metabolism and energy expenditure by regulating the acetylation status of transcription factors involved in this process. Therefore, we hypothesized that IFN-γ might directly impact the expression and/or activity of SIRT1. As depicted in Figure 2A and B, both mRNA and protein levels of SIRT1 were attenuated in the presence of IFN-γ. In contrast, IFN-γ did not significantly affect the expression of the acetyltransferase CBP in either cells (Supplementary Figure S2A). Decreased SIRT1 expression was a direct result of reduced transcription rate as demonstrated by promoter reporter assay (Figure 2C). This was, however, not caused by altered stability of SIRT1 message (Supplementary Figure S2B). Moreover, IFN-γ treatment led to an inhibition of SIRT1 activity (Figure 2D). Collectively, our results indicate that IFN-γ suppresses SIRT1 expression and activity at the transcriptional level.

IFN-γ suppresses SIRT1 transcription through the induction of CIITA

We have previously demonstrated that CIITA is a key mediator of several IFN-γ-dependent transcriptional events (29–31). Therefore, we sought to determine whether repression of SIRT1 transcription by IFN-γ also depends on CIITA. CIITA expression was markedly stimulated by IFN-γ in C2C12 and IMR-90 cells, paralleling the down-regulation of SIRT1 expression (Figure 3A and B). Decrease of SIRT1 levels by CIITA likely occurred as a result of transcriptional inhibition of the SIRT1 promoter since CIITA directly repressed SIRT1 promoter activity in a dose response manner (Figure 3C). In accordance with decreased SIRT1 levels, transcriptional activity of a panel of metabolic genes as probed in Figure 1 was severely impaired (Figure 3D).

In order to verify whether CIITA is required for IFN-γ-induced repression of SIRT1 transcription, small interfering RNA (siRNA) targeting CIITA was employed. Knockdown of CIITA expression by siRNA attenuated the repression of SIRT1 expression levels (Figure 4A–C). More importantly, alleviation of SIRT1 repression by CIITA siRNA also normalized SIRT1 activity (Figure 4D). As a result, down-regulation of genes involved in energy expenditure by IFN-γ was relieved (Figure 4E). In order to verify that alleviation of repression of metabolic genes by CIITA knockdown was via restoration of SIRT1 activity, we treated the cells with two different types of SIRT1 inhibitors, nicotinamide (NAM) and sirtinol, in the presence of CIITA siRNA. As shown in Supplementary Figure S3, treatment of either SIRT1 inhibitor blocked the effect of CIITA silencing on the expression of metabolic genes. In aggregate, this line of data suggest that CIITA mediates the transcriptional repression of SIRT1 by IFN-γ.

CIITA is recruited to the SIRT1 promoter by HIC1

In order to tackle the mechanism whereby CIITA represses the transcription of SIRT1, we used SIRT1 promoter constructs harboring progressive deletions. CIITA repressed the activities of all the promoter constructs equally well and the region putatively targeted by CIITA was mapped to −115/+58 (Figure 5A). Indeed, IFN-γ stimulated the occupancy of CIITA to this region of the SIRT1 promoter in ChIP assay (Figure 5B). Close examination of this region unveiled a conservative binding site for the sequence-specific transcription factor hypermethylated in cancer 1 (HIC1) (32), suggesting that CIITA may be enlisted by HIC1 via protein-protein interaction. To this end, FLAG-tagged CIITA and GFP-tagged HIC1 were co-expressed in HEK293 cells.

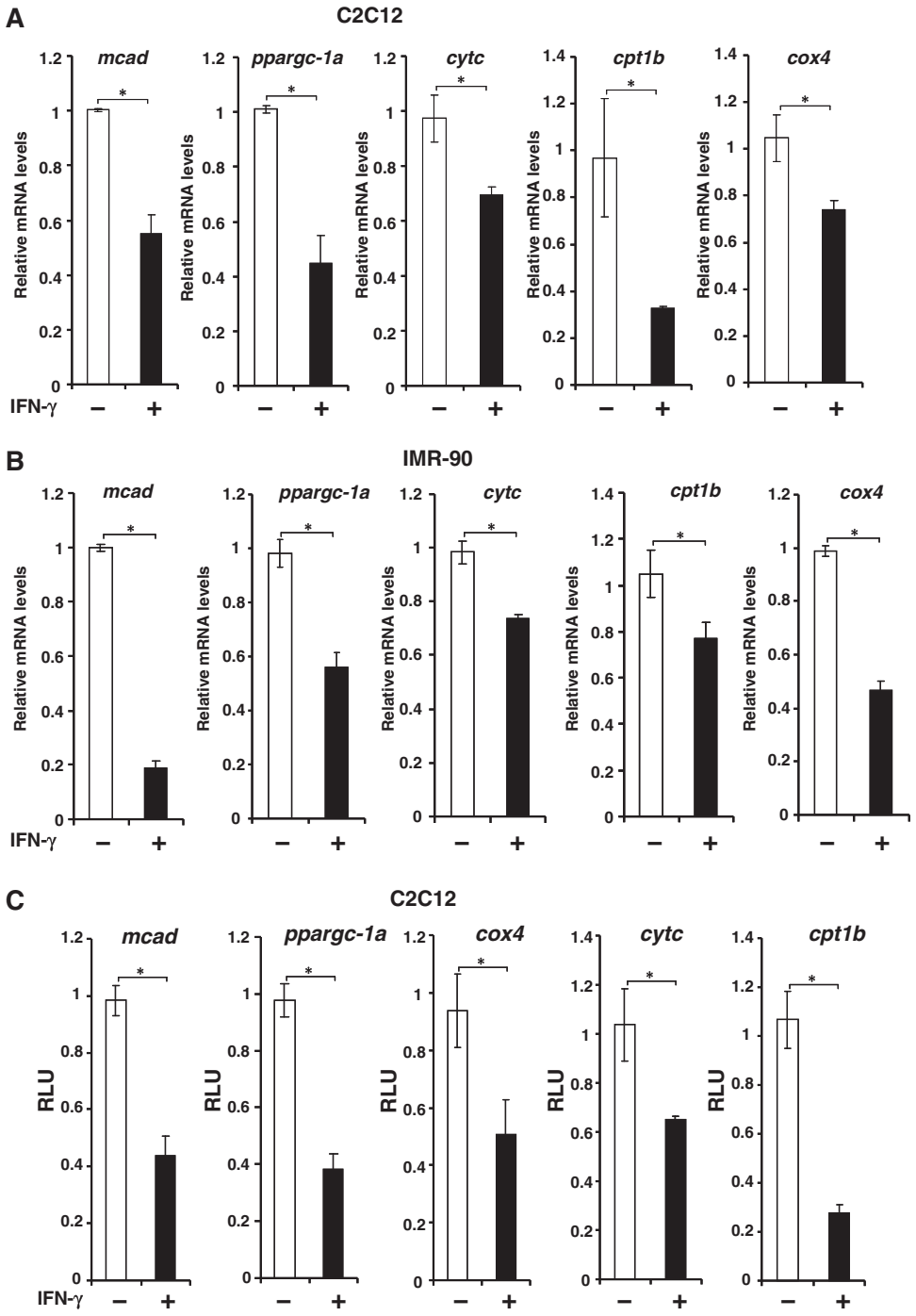

Figure 1. IFN-γ disrupts metabolic homeostasis in skeletal muscle cells. (**A** and **B**) C2C12 (A) or IMR-90 (B) cells were treated with IFN-γ. mRNA levels were measured by real-time qPCR. (**C**) C2C12 cells were transfected with different promoter luciferase constructs followed by treatment with IFN-γ. Luciferase activities were normalized to protein concentration and GFP fluorescence for transfection efficiency and expressed as relative luciferase unit (RLU).

HIC1 was detected in the immune complex along with CIITA when whole cell lysates were precipitated by anti-FLAG (Figure 5C). Reciprocal immunoprecipitation using anti-GFP antibody was able to pull down both CIITA and HIC1, confirming that these two proteins formed a complex *in vivo*. More importantly, CIITA and HIC1 synergistically suppressed the promoter activity of SIRT1 (Figure 5D) whereas depletion of HIC1 with siRNA blocked the recruitment of CIITA to the SIRT1 promoter (Figure 5E). In accordance, repression of SIRT1 promoter activity by CIITA was attenuated (Supplementary Figure S4). Together, these data suggest that CIITA interacts with and is recruited to the SIRT1 promoter by HIC1 to repress SIRT1 transcription.

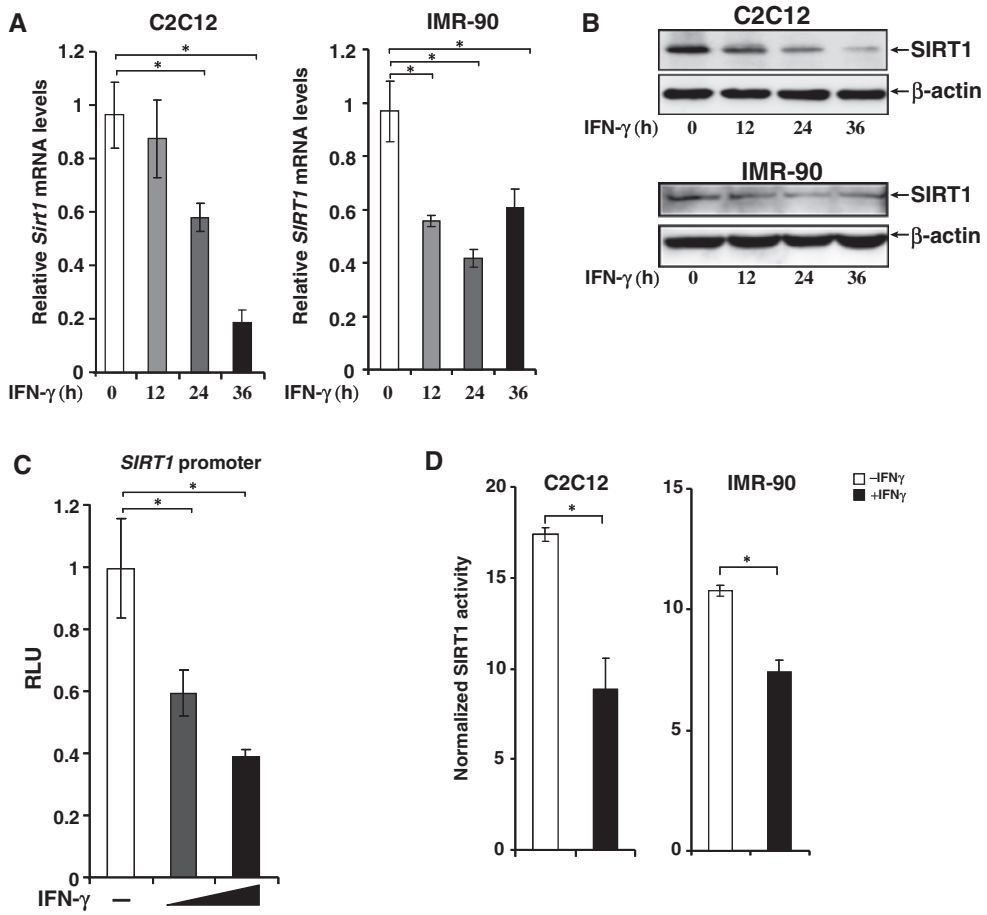

Figure 2. IFN-γ suppresses expression and activity of SIRT1. (**A** and **B**) C2C12 and IMR-90 cells were treated with IFN-γ and harvested at different time points as indicated. SIRT1 mRNA (A) and protein (B) levels were measured by real-time qPCR and Western. (**C**) C2C12 cells were transfected with a SIRT1 promoter luciferase construct followed with IFN-γ treatment of various doses (50 U/ml and 100 U/ml) for 24 h. Promoter and Western activities were expressed as RLU. **(D)** C2C12 and IMR-90 cells were treated with IFN-γ for 24 h and SIRT1 activity was measured as described under 'Materials and Methods' section.

HIC1 expression is induced by IFN-γ and is necessary for SIRT1 repression

Next, we examined how HIC1 contributed to the down-regulation of SIRT1 transcription by IFN-γ. IFN-γ stimulated both mRNA and protein levels of HIC1 (Figure 6A and B). More importantly, occupancy of HIC1 on the SIRT1 proximal promoter was enhanced by IFN treatment (Figure 6C). On the contrary, siRNA mediated knockdown of HIC1 alleviated the repression of SIRT1 mRNA (Figure 6D) and protein (Figure 6E) expression and normalized SIRT1 activity in C2C12 cells treated with IFN-γ (Supplementary Figure S5A). Finally, depletion of HIC1 restored expression of genes involved in energy expenditure in response to IFN-γ (Figure 6F). Similarly, treatment of either SIRT1 inhibitor blocked the effect of HIC1 silencing on the expression of metabolic genes, indicating that alleviation of repression of metabolic genes by HIC1 knockdown was via restoration of SIRT1 activity (Supplementary Figure S5B). In conclusion, HIC1 is necessary for IFN-γ induced repression of SIRT1 transcription and disruption of metabolic equilibrium.

Repression of SIRT1 transcription by IFN-γ requires histone deacetylation

CIITA has been reported to actively engage the epigenetic machinery, histone deacetylases (HDACs) in particular, in transcriptional repression (30,33). Thus, we set out to determine whether histone deacetylation might be involved in the repression of SIRT1 transcription by IFN-γ. Twenty-four hours after IFN-γ treatment, significant amounts of acetylated histones H3 and H4 were removed from the proximal SIRT1 promoter region as shown by ChIP assays (Figure 7A), alluding to a possible scenario wherein certain HDAC is recruited to the SIRT1 promoter to actively deacetylate core histones. Intriguingly, there was a marked restitution of acetylated core histones once CIITA was silenced by siRNA (Figure 7A), indicating that enlistment of HDACs to the SIRT1 promoter relies on CIITA. Consistently, pre-treatment with trichostatin A (TSA), a universal inhibitor of class I and II HDACs, relieved down-regulation of SIRT1 promoter activity by CIITA (Figure 7B) and IFN-γ (Figure 7C). Thus, IFN-γ represses SIRT1 transcription possibly through CIITA-dependent histone deacetylation of the SIRT1 promoter.

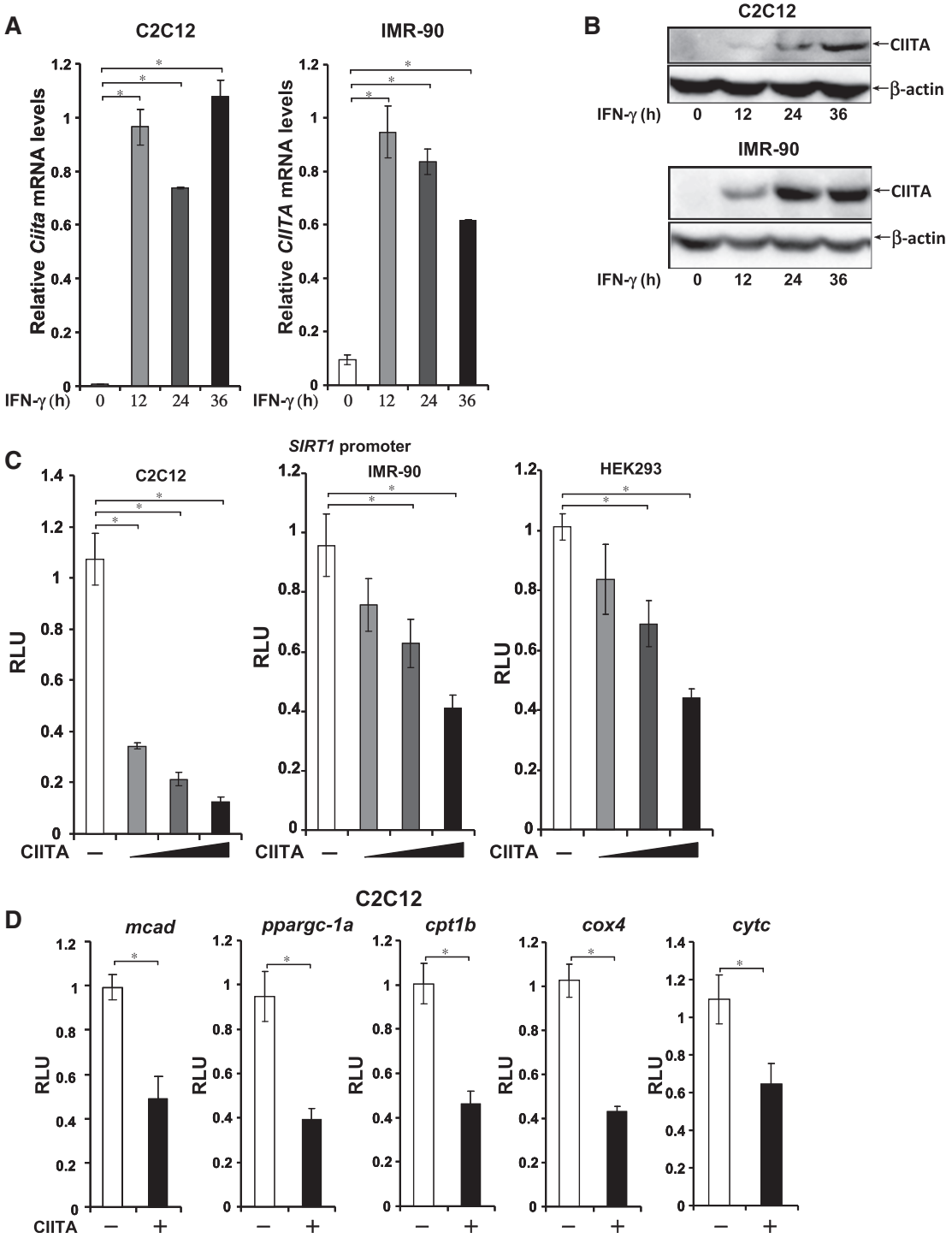

Figure 3. CIITA is induced by IFN-γ and directly suppresses SIRT1 transcription. (**A** and **B**) C2C12 and IMR-90 cells were treated with IFN-γ, harvested at different time points and probed for CIITA mRNA (A) and protein (B) levels with real-time qPCR and Western. (**C**) C2C12 and IMR-90 cells were transfected with a SIRT1 promoter luciferase construct with increasing amount of CIITA plasmid. Promoter activities were expressed as RLU. (**D**) C2C12 cells were transfected with different promoter luciferase constructs (*pdk4*, *mcad*, *ppargc-1a, cpt1, cytc* and *cox4*) with or without CIITA. Luciferase activities were expressed as relative luciferase unit (RLU).

DISCUSSION

When challenged with a demanding need for fuel in such situations as exercising and fasting, skeletal muscle cells switch to fatty acid oxidation and ramp up oxidative phosphorylation with accelerated rate of energy expenditure. Disruption of metabolic adaptation in skeletal muscle cells is associated with insulin resistance and type 2 diabetes (1,3,4). The NAD+ dependent deacetylase SIRT1 has emerged as a critical coordinator of cellular metabolism primarily by impacting deacetylation levels and thus

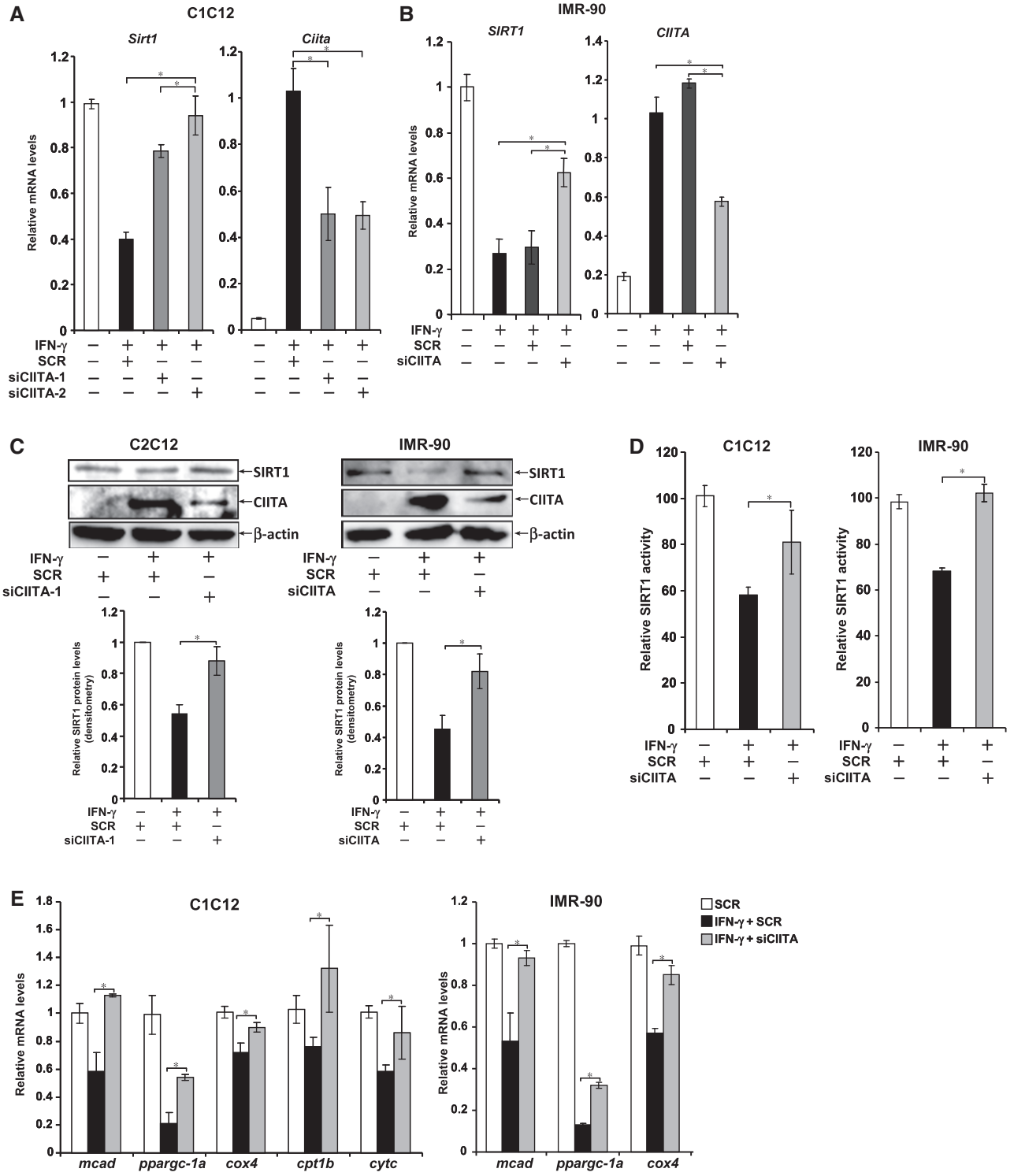

Figure 4. CIITA is indispensible for IFN-γ induced suppression of SIRT1 expression. (**A–D**) C2C12 and IMR-90 cells were transfected with siRNA targeting CIITA (siCIITA) or random sequence (SCR) followed by treatment with IFN-γ for 36 h. mRNA (A and B) and protein (C) levels of SIRT1 were measured by real-time qPCR and Western. Densitometry of SIRT1 blot was normalized to β-actin blot and expressed as relative unit compared to the control group which is set arbitrarily as 1. SIRT1 activity (D) was measured as described under 'Materials and Methods' section. (**E**) C2C12 and IMR-90 cells were transfected with siCIITA or SCR followed by treatment with IFN-γ. mRNA levels of *pdk4*, *mcad*, *ppargc-1a, cpt1, cytc* and *cox4* were measured by qPCR.

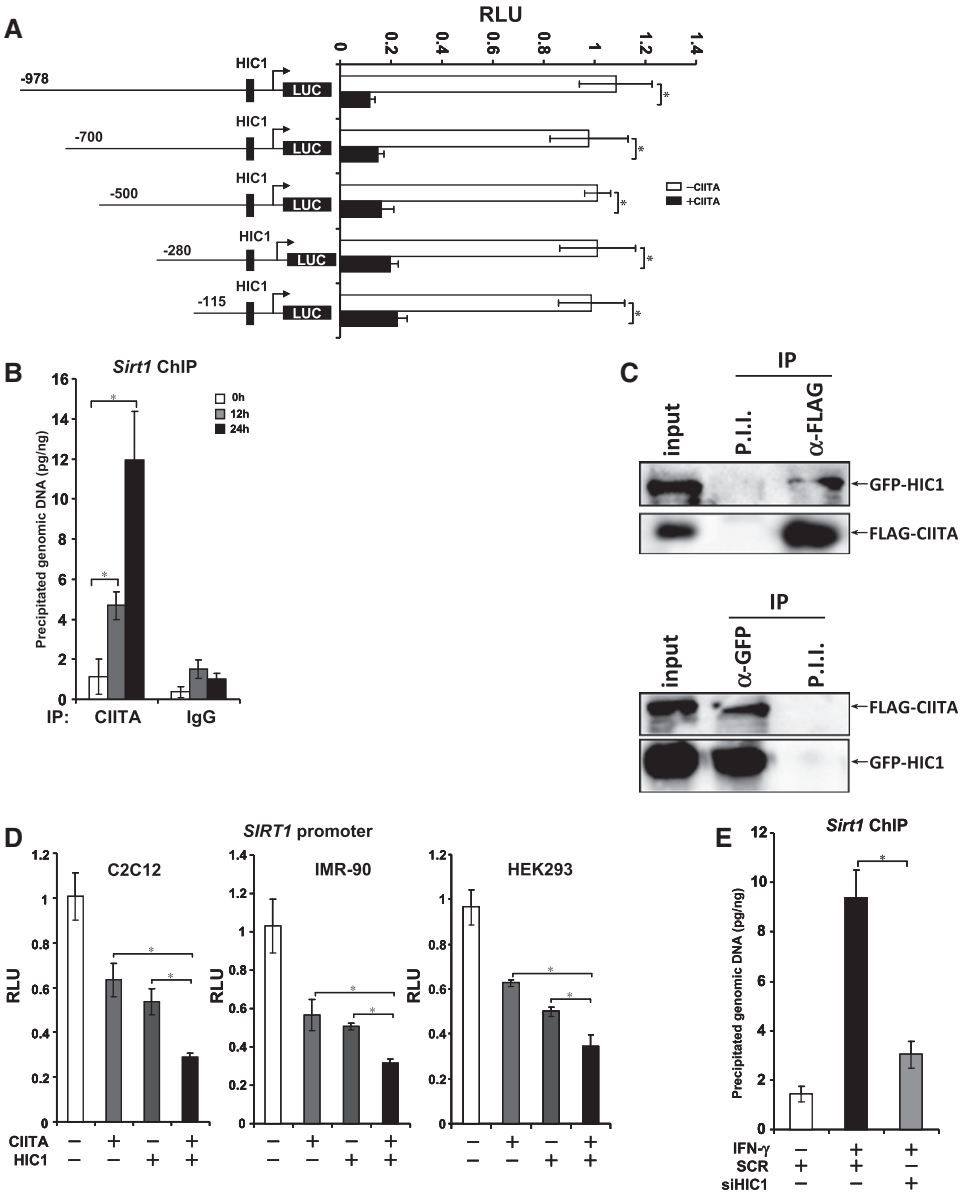

Figure 5. CIITA is recruited to the SIRT1 promoter by HIC1. **(A)** SIRT1 promoter constructs of various lengths were transfected into C2C12 cells with or without CIITA. Promoter activities were expressed as RLU. (**B**) C2C12 cells were treated with IFN-γ. Cells were harvested at different time points after treatment as indicated and ChIP was preformed with anti-CIITA. Data were expressed as fold enrichment. (**C**) FLAG-tagged CIITA and GFP-tagged HIC1 were co-transfected into HEK293 cells. Immunoprecipitation assays were performed with anti-FLAG, anti-GFP, or pre-immune IgG (P.I.I.) as indicated. Eluates were analyzed by western with anti-FLAG or anti-GFP. (**D**) A SIRT1 promoter luciferase construct was transfected into C2C12, IMR-90 or HEK293 cells with HIC1 and/or CIITA as indicated. Promoter activities were expressed as RLU. (**E**) C2C12 cells were transfected with HIC1 siRNA or scrambled siRNA followed by treatment with IFN-γ. ChIP was preformed with anti-CIITA. Data were expressed as fold enrichment.

fine-tuning the activities of key transcription factors involved in this process (5,34). We report here that a CIITA:HIC1 complex is responsible for SIRT1 repression and impairment of energy homeostasis in vitro in cultured skeletal muscle cells by IFN-γ.

Initially identified as the master regulator of MHC II transactivation, the role of CIITA has been greatly expanded; recent evidence points to a key role for CIITA in a variety of pathologies including atherosclerosis, pulmonary fibrosis, scleroderma and cancer (35). Based on homology, CIITA can be placed into the nucleotide-binding leucine-rich repeat (NLR) family of proteins that form the cytokine-processing inflammasome and constitute an integral part of the innate immune system, being the only member of this family that directly regulates transcription (36). Other members, especially NLRP3, have recently been implicated in the pathogenesis of a myriad of metabolic disorders (37). In support of this notion, it has been found that improved glucose tolerance is associated with a reduction of NLRP3 mRNA in patients with type 2 diabetes (38). Our new findings presented here illustrate that CIITA links the

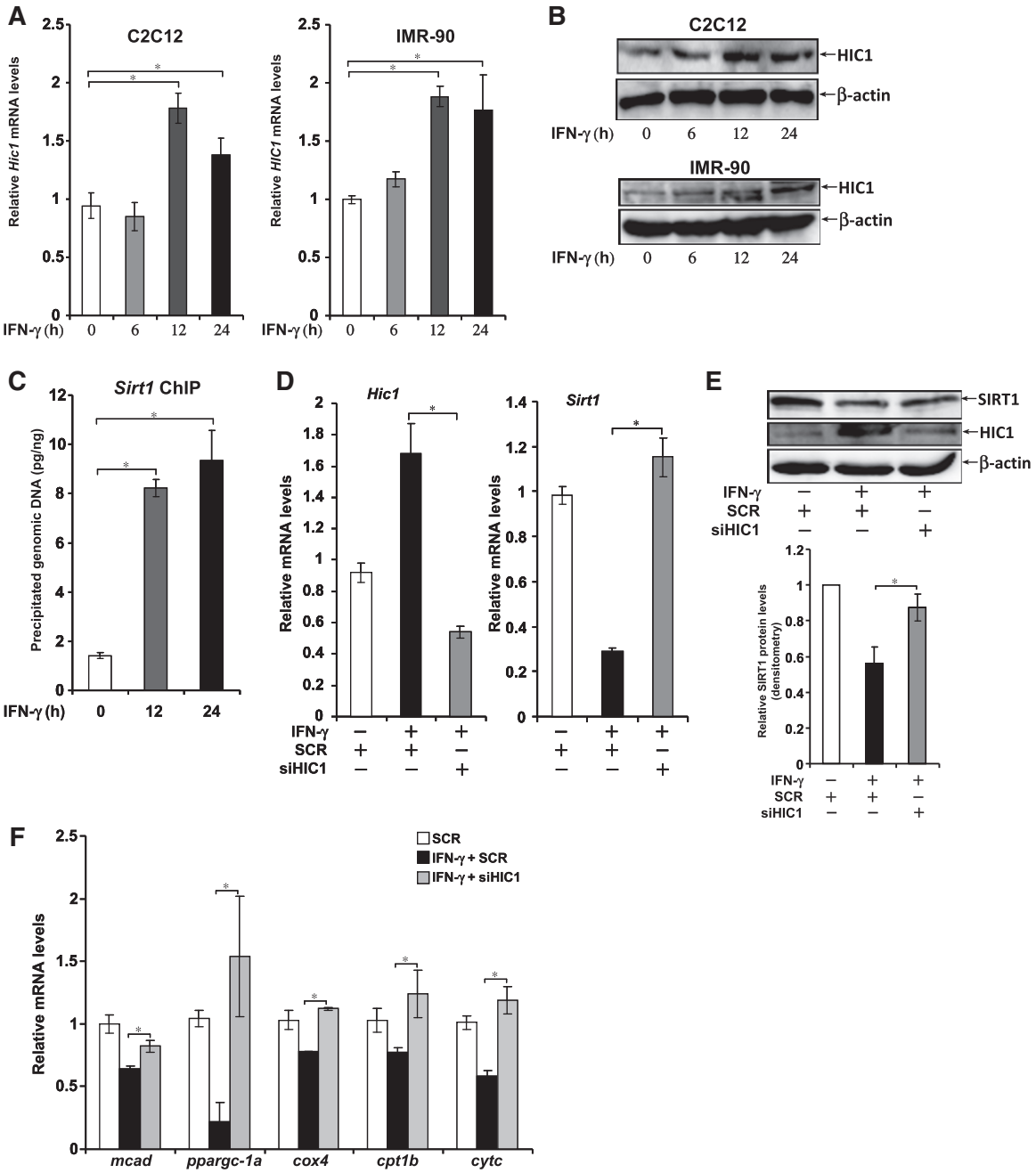

Figure 6. HIC1 expression is induced by IFN-γ and is necessary for SIRT1 repression. (**A** and **B**) C2C12 and IMR-90 cells were treated with IFN-γ. HIC1 mRNA (A) and protein expression (B) was probed by qPCR and Western. (**C**) C2C12 cells were treated with IFN-γ and harvested at indicated time points. ChIP was preformed with anti-HIC1. Data were expressed as fold enrichment. (**D–F**) C2C12 cells were transfected with siHIC1 or SCR followed by treatment with IFN-γ for 36 h. SIRT1 mRNA (D) and protein (E) levels were evaluated by qPCR and Western. (F) mRNA levels of *pdk4*, *mcad*, *ppargc-1a, cpt1, cytc* and *cox4* were measured by qPCR.

pro-inflammatory signal triggered by IFN-γ to reduced SIRT1 transcription and crippled energy expenditure in skeletal muscle cells, alluding to a decompensatory role in cellular metabolism and human disease that is shared within the NLR family members although the underlying mechanisms differ significantly.

HIC1 is considered a tumor suppressor gene frequently silenced in human cancers (39). SIRT1 remains one of a handful of direct targets identified for HIC1 so far (40). Acting primarily as a transcriptional repressor, HIC1 forges a complex with several co-repressors including CtBP and NuRD (32,41). A recent investigation by Zhang *et al*. reveals a delicate balance between intracellular energy status and SIRT1 transcription in a HIC1 dependent manner (42); blockade of glycolysis by 2-deoxyglucose (2-DG) prevents the formation of a HIC1:CtBP complex on the SIRT1 promoter and relieves SIRT1 repression. Our data (Figure 5) define a novel HIC1-binding protein

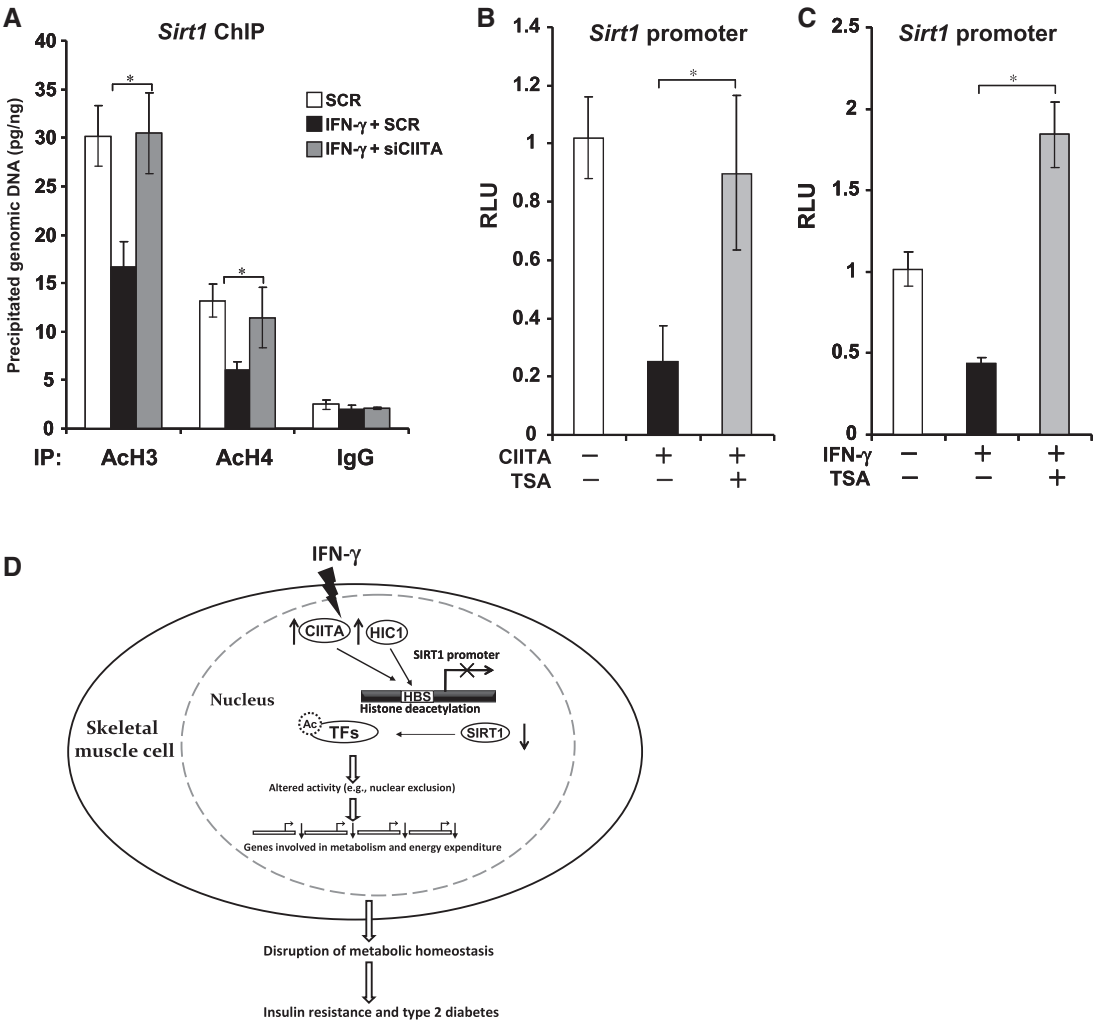

Figure 7. Repression of SIRT1 transcription by IFN-γ requires histone deacetylation. (**A**) C2C12 cells were transfected with siCIITA or SCR followed by treatment with IFN-γ. ChIP assays were performed with anti-acetyl histones H3 (AcH3) and H4 (AcH4). (**B**) A SIRT1 promoter luciferase construct was transfected into C2C12 cells with CIITA followed by treatment with TSA (100 nM). Luciferase activities were expressed as RLU. (**C**) A SIRT1 promoter luciferase construct was transfected into C2C12 followed by treatment with IFN-γ and TSA. Luciferase activities were expressed as RLU. (**D**) A schematic model illustrating the mechanism whereby IFN-γ may impact metabolic homeostasis of skeletal muscle cells. HBS, HIC1-binding site; TFs, transcription factors; Ac, acetyl group.

and assign a potential role for the HIC1:CIITA complex in the pathogenesis of type 2 diabetes. In addition, CIITA repressed the promoter activity driven by tandem copies of the HIC1-binding site (HBS, Li,P. and Xu,Y., unpublished observation), indicating that CIITA could indeed serve as a genuine corepressor for HIC1 *in vivo*. Recently, it has been demonstrated that patients with type 2 diabetes have higher levels of circulating IFN-γ (43). Since both CIITA (Figure 3) and HIC1 (Figure 6) can be up-regulated by IFN-γ in cultured skeletal muscle cells, it is of great interest to evaluate whether this phenomenon can be extrapolated *in vivo* to human subjects with or without type 2 diabetes.

Both CIITA and HIC1 are intimately wired to the epigenetic machinery for their transcriptional activities (32,44). Our data suggest that CIITA dependent histone deacetylation may play a role in IFN-γ induced repression of SIRT1 transcription (Figure 7) although the specific HDAC that is responsible for SIRT1 repression remains elusive. Jin *et al*. (45) recently reported that aging dampens SIRT1 transcription in the liver by promoting the binding of a C/EBPβ:HDAC1 complex on the distal SIRT1 promoter, implicating histone deacetylation as a potential mechanism shared in the repression of SIRT1 transcription. Our preliminary experiments showed that knockdown of HDAC3, but not HDAC1 or HDAC2, abrogated repression of SIRT1 promoter activity by IFN-γ (Li,P. and Xu,Y., unpublished data). We and others have previously identified HDAC1 and HDAC2 as binding partners for CIITA in the downregulation of MHC II and collagen type I genes, respectively (30,33). Unlike HDAC1 and HDAC2 that are usually found in large complexes containing Sin3, HDAC3 is believed to primarily mediate transcriptional repression by the nuclear receptor corepressors including NCoR/SMRT (46). Recently, Lazar and colleagues have reported that

disruption of HDAC3 activity by over-expressing a dominant negative form of NCoR in mice enhances energy expenditure and insulin sensitivity (47). Of note, class II HDACs, which include HDAC4, 5, 6, 7 and 10, are enriched and steer physiologically important programs in skeletal muscle cells (48). In perspective, future investigations should aim at determining the individual class I and II HDACs that interact with CIITA and/or HIC1 on the SIRT1 promoter and their relevance in insulin resistance and type 2 diabetes.

In summary, our findings (Figure 7D) allude to a scheme wherein upon challenge with IFN-γ, CIITA accumulates in the nucleus, is recruited by HIC1 to the HBS on the proximal SIRT1 promoter, and represses SIRT1 transcription through active histone deacetylation. Reduced cellular SIRT1 levels lead to a decrease in deacetylation of certain transcription factors, altering their activities. Consequently, transcription of a panel of genes that are involved in energy expenditure is down-regulated. Eventually, metabolic homeostasis in skeletal muscles is impaired with ensuing insulin resistance and type 2 diabetes.

SUPPLEMENTARY DATA

Supplementary Data are available at NAR Online: Supplementary Methods, Supplementary Figures 1–5.

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Conflict of interest statement. None declared.

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