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Dual role of SIRT1 in UVB-induced skin tumorigenesis

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Abstract

The protein deacetylase SIRT1 regulates various pathways in metabolism, aging and cancer. However, the role of SIRT1 in skin cancer remains unclear. Here, using mice with targeted deletions of SIRT1 in their epidermis in both resistant B6 and sensitive SKH1 hairless backgrounds, we show that the role of SIRT1 in skin cancer development induced by ultraviolet B (UVB) radiation is dependent on its gene dose. Keratinocyte-specific heterozygous deletion of SIRT1 promotes UVB-induced skin tumorigenesis, whereas homozygous deletion of SIRT1 suppresses skin tumor development but sensitizes the B6 mice to chronic solar injury. In mouse skin, SIRT1 is haploinsufficient for UVB-induced DNA damage repair and expression of xeroderma pigmentosum C (XPC), a protein critical for repairing UVB-induced DNA damage. As compared with normal human skin, down-regulation of SIRT1 is in parallel with down-regulation of XPC in human cutaneous squamous cell carcinoma at both the protein and mRNA levels. In contrast, homozygous SIRT1 deletion in mouse skin augments p53 acetylation and expression of its transcriptional target Noxa, and sensitizes the epidermis to UVB-induced apoptosis *in vivo*, while heterozygous SIRT1 deletion has no such effect. The gene dosage-dependent function of SIRT1 in DNA repair and cell survival is consistent with the dual roles of SIRT1 in UVB-induced skin tumorigenesis. Our results reveal the gene dosage-dependent *in vivo* functions of SIRT1 in skin tumorigenesis and may shed light on the role of SIRT1 in epithelial cancer induced by DNA damage.

Keywords

SIRT1; UVB; skin tumorigenesis; DNA repair; apoptosis

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CONFLICT OF INTEREST

Keyoumars Soltani serves on the board of directors in Elorac Pharma and receives stocks from Elorac Pharma, DUSA, Winston and Gideon Pharmaceuticals, but none of them are relevant to this study. Other authors declare no conflicts of interest.

INTRODUCTION

Sirtuin 1 (SIRT1), a mammalian counterpart of the yeast silent information regulator 2 (Sir2) and a proto member of the sirtuin family, is an NAD-dependent protein deacetylase. It has been revealed that SIRT1 regulates important cellular processes including metabolism, cell survival, and stress response (1–4). However, the role of SIRT1 in cancer development is still under debate (4–6). It has been considered as a tumor promoter because of its increased expression in some types of cancers and its role in inactivating proteins that are involved in tumor suppression (6). It deacetylates and thus inhibits the tumor suppressor p53 (7–9). SIRT1 protein levels were found to be significantly elevated in human prostate cancer (10), acute myeloid leukemia (11), primary colon cancer (12) and skin cancer (13). However, SIRT1 has also been reported to mediate BRCA1 signaling and inhibit tumor growth through repressing transcription of oncogenes or activity of oncoproteins (14, 15). By regulating the DNA repair protein Ku70, SIRT1 was shown to promote cell survival (16, 17).

Data using genetic or spontaneous tumorigenesis mouse models support a role for SIRT1 as a tumor suppressor (18–23) and an oncogene (24). SIRT1 plays an important role in DNA damage repair, maintains genomic integrity, and suppresses spontaneous tumor development (18). It was found to promote DNA damage repair by positively regulating NBS1 (25). Furthermore, by promoting deacetylation of xeroderma pigmentosum protein A (XPA) (26) and promoting the expression of xeroderma pigmentosum C (XPC) (27), SIRT1 promotes nucleotide excision repair, which removes UVB-induced DNA damage. In addition, SIRT1 is down-regulated in UV-associated human skin cancers (27), suggesting that it is a tumor suppressor.

To elucidate the precise function of SIRT1 in human epithelial cancer development, we generated mice with a keratinocyte-specific SIRT1 deletion and monitored tumor development following chronic UVB irradiation. We found that, in mouse skin, SIRT1 acts as either a tumor suppressor or an oncogene, depending on the SIRT1 gene dose, which is consistent with its role in promoting UVB-induced DNA damage repair and suppressing UVB-induced p53-mediated apoptosis. These findings may provide new insights into the mechanistic role of SIRT1 in cancer development and in cutaneous homeostasis.

RESULTS

The function of SIRT1 in UVB-induced skin carcinogenesis in B6 mice is dependent on SIRT1 gene dose

To better understand the role of SIRT1 in skin tumorigenesis in which UVB is the major risk factor for humans, we first compared UVB-induced tumor formation among mice in a B6 background with normal SIRT1 (WT), heterozygous deletion (cHet) and homozygous deletion (cKO) specifically in their epidermis (Fig. 1A–C). UVB was found to induce tumor formation in SIRT1 WT and SIRT1 cHet mice, but not in SIRT1 cKO mice (Fig. 1D). SIRT1 cHet mice started to develop skin tumors at 35 weeks of UVB radiation; however, SIRT1 WT mice did not develop tumors until 42 weeks (Fig. 1D). A log-rank test showed that SIRT1 hemizygosity significantly accelerates skin tumorigenesis following UVB

radiation ($P< 0.01$). Histological analysis of representative tumors (Fig. s1A) showed that at the end of the study or when the mice were euthanized due to tumor burden, all the lesions were papillomas in WT mice and squamous cell carcinomas (SCCs) in cHet mice (Fig. s1B).

SIRT1 null deficiency predisposes B6 mouse skin to chronic UV injury

Although homozygous SIRT1 deletion prevented UVB-induced skin tumorigenesis (Fig. 1), the cKO mice showed reduced overall survival as compared with WT and cHet mice (Fig. 2A). When death mediated by tumor burden was excluded, the percentage of non-tumor (NT) survival in cKO mice was significantly lower than in either WT or cHet mice (Fig. 2B; $P< 0.05$; Log-Rank test). Following repeated UVB radiation, cKO mice developed chronic injury, which later evolved into an open lesion (Fig. 2C). These lesions shared several common histological features with wounds, including hyperplasia in surrounding skin, and edema, ulceration and loss of epidermis in the lesion sites (Fig. 2D), with the presence of proliferating cells that were positive for the proliferation marker Ki67 (Fig. 2E). These data demonstrate that SIRT1 null deletion sensitizes mouse skin to chronic UV injury.

Dual role of SIRT1 in UVB-induced skin tumorigenesis in SKH1 hairless mice

To further determine the role of SIRT1 in skin cancer, we generated WT, cHet, and cKO mice in a SKH1 hairless background, a tumor-sensitive strain that has been widely used in photocarcinogenesis. As compared with WT mice, cHet mice showed an accelerated tumor onset, increased tumor multiplicity, and increased tumor growth, whereas cKO mice yielded delayed onset, decreased multiplicity, and decreased tumor growth (Fig. 3A–C, s2). Histological analysis revealed that cHet mice developed more squamous cell carcinomas (SCC) and similar number of papillomas (PAP), while cKO mice developed similar number of SCCs and fewer papillomas, as compared with WT mice (Fig. 3D). The ratio of malignant conversion from papilloma to SCC was higher in both cHet and cKO mice than in WT mice (Fig. 3E). No chronic UV injury was detected in any group. These results demonstrate that SIRT1 has a dual role in UVB-induced skin tumorigenesis depending on its gene dose and suggest that SIRT1 inhibition increases malignant conversion.

SIRT1 is haploinsufficient for UVB-induced DNA damage repair and XPC expression

In addition to regulating XPA (26), we have recently demonstrated that, in HaCaT cells and mouse embryonic fibroblasts, SIRT1 promotes UVB-induced DNA damage repair through XPC (27), which supports the tumor suppressing role of SIRT1 in skin cancer. To determine whether SIRT1 plays the same role in normal human epidermal keratinocytes (NHEK), we measured the percentage of DNA repair at different intervals post-UVB in NHEK cells transfected with siRNA targeting SIRT1 (siSIRT1) or negative control siRNA (NC). Inhibition of SIRT1 reduced the expression of XPC but not XPA, and inhibited repair of cyclobutane pyrimidine dimers (CPDs), the major UVB-induced DNA damage product (Fig. 4A–B). To determine the function of SIRT1 in XPC expression and DNA repair *in vivo*, SIRT1 WT, cHet, and cKO mice were irradiated with UVB (350 mJ/cm^2) and skin was collected at 0 and 24 h post-UVB. XPC mRNA and protein levels were much lower in both cHet and cKO mouse skin than in WT skin (Fig. 4C–D, s3). The repair of CPDs was reduced in both cHet and cKO skin as compared with SIRT1 WT skin (Fig. 4E). Addition of

XPC increased the DNA repair capability in SIRT1-inhibited NHEK cells (Fig. 4F–G). These results indicate that SIRT1 is haploinsufficient for XPC expression and UVB-induced DNA damage repair. To assess the specific association of SIRT1 with XPC expression, we analyzed the SIRT1 and XPC protein levels in normal human skin tissue and SCC. We found that, at the protein and mRNA levels, down-regulation of SIRT1 is closely associated with reduced XPC expression in SCCs as compared with normal skin tissue (Fig. 4H–I), further supporting the essential role of SIRT1 in maintaining XPC availability.

SIRT1 null deletion activates p53 and promotes UVB-induced apoptosis

One of the first non-histone substrates of SIRT1 identified is the tumor suppressor p53 (7, 8, 28), leading to the proposed oncogenic role of SIRT1. SIRT1 deacetylates p53 and thus inhibits its transcriptional function to prevent apoptosis induced upon stress and DNA damage (7, 8, 28). However, *in vivo* p53 deacetylation by SIRT1 in mice (29) was not confirmed in another report (30). To quantify the effect of SIRT1 deletion on UVB-induced apoptosis *in vivo*, we used the TUNEL assay to measure the percentage (%) of apoptotic cells in WT, cHet and cKO mouse skin following sham or UVB radiation. SIRT1 deletion had no effect on apoptosis without UVB irradiation (Fig. 5A–B). However, following UVB irradiation, the percentage of TUNEL positive (+) cells was significantly higher in cKO mouse skin than in either WT or cHet mouse skin (Fig. 5A–B, $P< 0.05$). No significant difference was detected between WT and cHet mice.

To determine the regulation of p53 by SIRT1 upon UVB irradiation *in vitro* and *in vivo*, we analyzed the levels of acetylated p53 at K382, a known SIRT1 deacetylation site in NHEK cells and mouse skin following UVB irradiation. In NHEK cells, UVB increased p53 acetylation (Fig. 5C), consistent with previous reports in immortalized cell lines (7, 8, 28). Inhibition of SIRT1 by transfection with siRNA targeting SIRT1 increased p53 acetylation as compared with cells transfected with negative control (NC) siRNA (Fig. 5C). To determine the role of SIRT1 in p53 acetylation and apoptosis *in vivo*, we treated SIRT1 WT, cHet, cKO mice with UVB (350 mJ/cm^2) three times at intervals of every other day, and collected skin tissue at 24 h following the final UVB exposure. UVB irradiation upregulated p53 levels and the expression of the pro-apoptotic p53 target genes Noxa, Puma, and Bax in all groups (Fig. 5D–G). As compared with the WT group, however, acetylated p53 was only increased in the UVB-irradiated cKO group, not in the cHet group. In sham-treated mice, acetylation of p53 was elevated in some mice, whereas UVB radiation increased p53 acetylation in all mice (Fig. 5D). Intriguingly, SIRT1 inhibition significantly augmented Noxa induction following UVB irradiation (Fig. 5E; $P< 0.05$; Student's *t*-test), while it had no effect on UVB-induced Puma or Bax up-regulation (Fig. 5F–G). Knockdown of p53 reduced apoptosis in UVB-irradiated NHEK cells with SIRT1 knockdown, while it had no effect on cells transfected with negative control siRNA (siNC) (Fig. 5H–I). Similarly, overexpression of the p53K382R mutant (p53Mut) rescued apoptosis resulting from SIRT1 knockdown in UVB-irradiated NHEK cells (Fig. 5I). These data indicate that homozygous deletion of SIRT1 augmented p53 acetylation, and thereby sensitized mouse skin to UVB-induced apoptosis, which may eliminate cells that cannot repair their DNA damage effectively to prevent tumorigenic mutations.

DISCUSSION

Over the past decade, SIRT1 has attracted enormous attention due to its beneficial role in cell metabolism and survival *in vitro* and *in vivo* animal studies (1–4). However, accumulating evidence indicates that the role of SIRT1 in cancer is complex. It remains under debate whether SIRT1 acts as a tumor suppressor or as an oncogene (4–6). In this study, using a keratinocyte-specific SIRT1 deletion and UVB-induced skin tumorigenesis model, we demonstrated that the function of SIRT1 in carcinogenesis is dependent on its gene dose. Heterozygous deletion promotes UVB tumorigenesis, whereas homozygous deletion inhibits tumorigenesis. At the molecular level in mouse skin, we found that SIRT1 is haploinsufficient for UVB-induced DNA damage repair and XPC expression. However, only homozygous SIRT1 deletion increased p53 activation and UVB-induced apoptosis *in vivo*, while heterozygous deletion had no such effect. Consequently, following chronic UVB radiation, mice with homozygous SIRT1 deletion developed severe solar injury, although they were protected from tumor formation, as compared with mice with wild type or heterozygous SIRT1 deletion.

Intriguingly, we found that SIRT1 is haploinsufficient for UVB-induced DNA damage repair and tumorigenesis. Using HaCaT and MEF cells, our previous studies had shown that SIRT1 is required for efficient repair of UVB-induced DNA lesions. In a wide spectrum of UV-associated keratinocyte-derived neoplasms, SIRT1 expression is significantly reduced as compared with normal skin, suggesting that SIRT1 is a potential tumor suppressor in skin cancer (31). Increasing evidence has suggested that SIRT1 plays an important role in DNA repair of UV lesions and double stand breaks (32–34). It is noteworthy that XPC abundance is critical for efficient GG-NER, and thus for preventing UVB-induced skin carcinogenesis (35, 36). Haploinsufficiency of the XPC gene is a risk factor for UVB-induced skin cancer in mice (37), suggesting that XPC down-regulation by SIRT1 inhibition plays an active role in increased skin cancer susceptibility by SIRT1 deletion. In mouse skin, heterozygous SIRT1 deletion inhibited XPC expression and UVB-induced DNA repair, and increased skin tumorigenesis. These results suggest that SIRT1 plays an important role in maintaining genome integrity *in vivo* and acts as a haploinsufficient tumor suppressor in mice.

In contrast, SIRT1 is haplosufficient for cell survival *in vivo* following UVB damage. We found that homozygous but not heterozygous SIRT1 deletion enhances UVB-induced p53 acetylation and activation as well as apoptosis *in vivo*. SIRT1 has been shown to deacetylate and inactivate p53 to promote cell survival in MEF cells, fibroblasts and cell lines (7, 8). A variety of posttranslational modifications can regulate p53 activity, including phosphorylation, acetylation, methylation, and sumoylation (38). Since p53 is a well-known tumor suppressor, its inhibition by SIRT1 had categorized SIRT1 as an oncogene. However, recent studies have shown that while SIRT1 deacetylates p53, there is no biological outcome of this deacetylation (30, 39). Our results indicate that, in mouse skin, SIRT1 inhibition increased p53 acetylation and expression of some p53 target genes, such as Noxa, but not Puma or Bax. Deacetylation of p53 via SIRT1 may play an important role in preventing UVB-induced apoptosis in mice. The role of XPA deacetylation by SIRT1 in UV-induced DNA repair and survival may play a role (26). However, we were unable to detect endogenous XPA acetylation or the effect of SIRT1 deletion in either mouse skin or NHEK

cells. Nevertheless, the increased apoptosis may facilitate elimination of unrepaired cells and thus reduce malignant transformation. This is consistent with the gene-dose-dependent role of SIRT1 in UVB-induced skin tumorigenesis. As compared with mice with wild-type SIRT1, mice with heterozygous SIRT1 deletion exhibit reduced DNA repair with cell survival unaffected, supporting increased tumorigenesis, whereas mice with homozygous SIRT1 deletion exhibit both reduced DNA repair and cell survival, supporting reduced tumorigenesis as detected. The distinct dose-dependent role of SIRT1 underscores its opposing complex functions in epithelial tumorigenesis induced by DNA damage.

The role of SIRT1 in cancer is complex. While *in vivo* studies have overwhelmingly supported the tumor-suppressing role of SIRT1 in genetic or spontaneous tumorigenesis mouse models, including lymphoma, sarcoma, teratoma, carcinoma of the salivary gland and mammary gland (18), intestinal malignancies (19), liver cancer (21), and prostate neoplasia (22), SIRT1 has been demonstrated to act as an oncogene in thyroid carcinogenesis driven by PTEN deficiency (24). In contrast, in whole-body SIRT1 knockout mice in combination with a two-stage chemical carcinogenesis model, SIRT1 was found not to affect the incidence or tumor load but to be required for the antitumor activity of resveratrol (40). The discrepancy is likely due to the mixed genetic background of the mice used in this study, which can significantly affect susceptibility to tumorigenesis, and the different carcinogens used. The genetic background also determines the severity of the phenotype of SIRT1-null mice. In most cases, the loss of SIRT1 was embryonic lethal (18). A very small proportion of SIRT1-null mice were born viable but failed to survive more than a few months beyond birth (29, 41). Chemical carcinogenesis protocols provide a defined initiation-promotion model to study tumorigenesis in rodents over a relatively short time period. However, chronic exposure to UV light, particularly UVB, which causes DNA damage, is the major environmental risk factor in human skin carcinogenesis. Different or opposing functions of genes including DDB2 (42) and phospholipase Cε (43) have been detected in a UV tumorigenesis model verses a chemical tumorigenesis model. Indeed our data is consistent with recent studies in whole body heterozygous SIRT1 deletion mice with or without p53 heterozygosity (18), supporting a tumor-suppressing role for SIRT1 heterozygosity. Furthermore, our UVB tumorigenesis model using keratinocyte-specific SIRT1 deletion permitted us to elucidate the complex consequences of both heterozygous and homozygous SIRT1 deletion following DNA damage. Although SIRT1 homozygous deletion suppressed tumorigenesis, it increased the ratio of malignant conversion from benign papilloma to SCC. These further support the multifaceted role of SIRT1 in cancer. The underlying mechanism is under investigation in our laboratory.

In summary, our results identified an essential gene dosage-dependent role of SIRT1 in regulating tumorigenesis and epithelial homeostasis under genotoxic UVB stress. Heterozygous loss of SIRT1 function increases UVB tumorigenesis. Null deletion of SIRT1 suppresses tumorigenesis, while it sensitizes mouse skin to UVB-induced injury and to malignant conversion. SIRT1 is haploinsufficient for UVB-induced DNA damage repair, but haplosufficient for cell survival following UVB damage. Our results underscore the gene dosage-dependent dual roles of SIRT1 in UVB skin tumorigenesis.

MATERIALS AND METHODS

Animal treatments and UVB irradiation

All animal resources have been approved by the University of Chicago Institutional Animal Care and Use Committee. The *Sirt1* allele with floxed (Jackson Laboratory) (44) was backcrossed into the C57BL/6 background and then bred with mice expressing Cre recombinase driven by the K14 (Jackson Laboratory) promoter to generate skin keratinocyte-specific heterozygous SIRT1 deletion (cHet) and homozygous SIRT1 knockout (cKO) mice. Sirt1flox/flox (Wild-type, WT) mice were used as comparison controls. Mice (n=15) were shaved one day prior to the initial UVB radiation and later as needed. Shaved mice were exposed to UVB (350mJ/cm^2, a dose selected to avoid visible sunburn in WT mice) dorsally or sham-irradiated, three times a week for 42 weeks and then kept for an additional 20 weeks to monitor tumor formation and growth. To generate WT, cHet, and cKO mice in SKH1 hairless background, mice were backcrossed with SKH1 female for at least five times. The SKH1 mice were irradiated with UVB (100 mJ/cm^2) three times a week for 25 weeks as described in our recent studies (45). Mice were housed five animals per cage, and there was no evidence of dorsal wounds caused by fighting or sunburn.

Human normal and tumor samples

All human specimens were studied after approval by the University of Chicago Institutional Review Board. Frozen tissues were obtained under consent at the Section of Dermatology, Department of Medicine, University of Chicago as in our previous studies (46).

Cell culture

Normal human epidermal keratinocytes (NHEK) were obtained from Clonetics (Lonza) and cultured in KGM Gold BulletKit medium (Clonetics, Lonza) according to the manufacturer's instructions. NHEK cells were cultured for less than 4 passages. No authentication was done.

Statistical analyses

Statistical analyses were performed using Prism 5 (GraphPad software, San Diego, CA, USA). Data were expressed as the mean of at least three independent experiments, and analyzed by Student's *t*-test. Error bars indicate standard error of means (s.e.). Log-rank tests were used to evaluate tumor onset. A *P*-value of <0.05 was considered statistically significant.

The details for siRNA transfection, Western blotting, real-time PCR, Determination of two major forms of UVB-induced DNA damage in genomic DNA by slot blot assay, TUNEL assay and sub-G1 flow cytometric analysis can be found in the supplementary information.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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ABBREVIATIONS

cHet	keratinocyte-specific heterozygous SIRT1 deletion
cKO	keratinocyte-specific homozygous SIRT1 deletion
CPD	cyclobutane pyrimidine dimers
NHEK	normal human epidermal keratinocytes
NMSC	non-melanoma skin cancer
PAP	papilloma
SCC	squamous cell carcinoma
SIRT1	sirtuin 1
UVB	Ultraviolet B
WT	wild-type
XP	xeroderma pigmentosum
XPC	xeroderma pigmentosum group C

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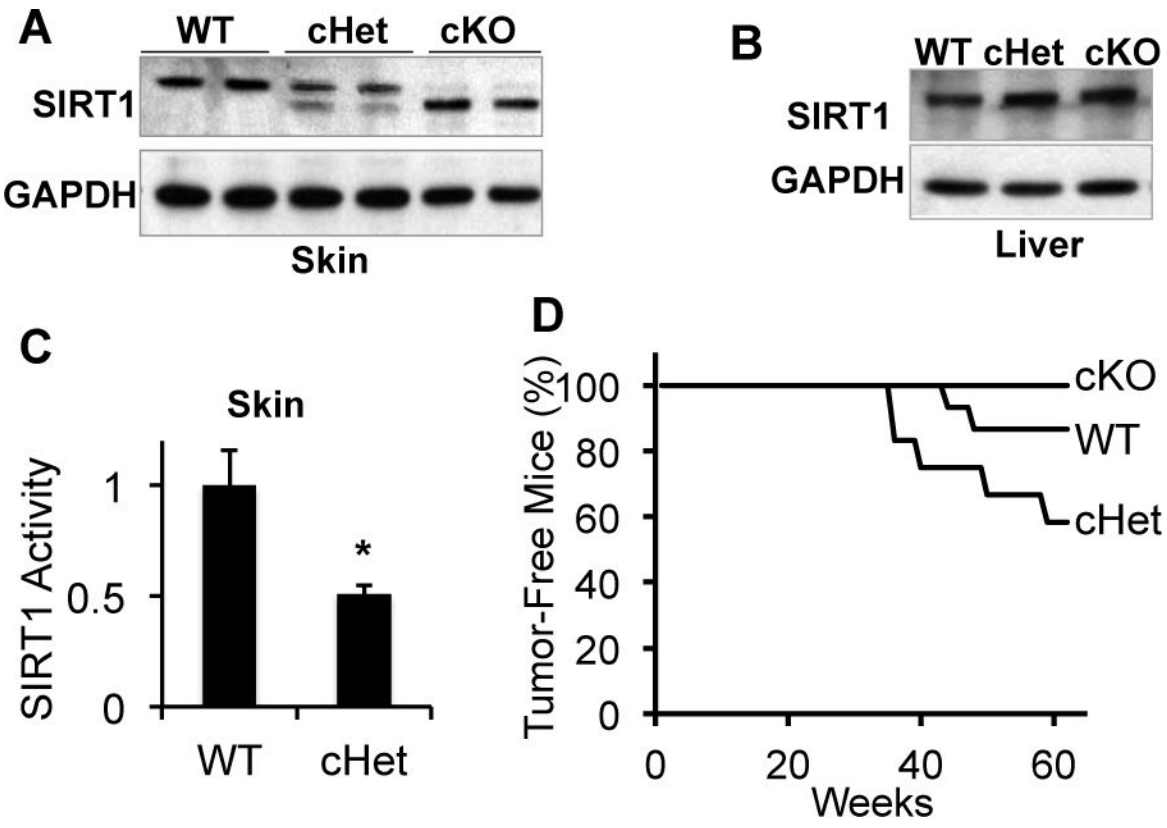


Figure 1.

SIRT1 plays an important role in UVB-induced skin tumorigenesis. A–B, Immunoblot analysis of SIRT1 and GAPDH in skin (A) and liver (B) from WT, cHet and cKO mice in a B6 background. The upper band in the SIRT1 blot in A shows the wild-type SIRT1 protein, whereas the lower band shows the nonfunctional truncated protein results from the excision of the conserved Sir2 motif of the SIRT1 catalytic domain. C, Fluorometric assay of SIRT1 activity in the skin from WT and cHet mice (n =3). D, percent (%) of tumor-free mice in WT, cHet and cKO groups (n = 15) following chronic UVB radiation.

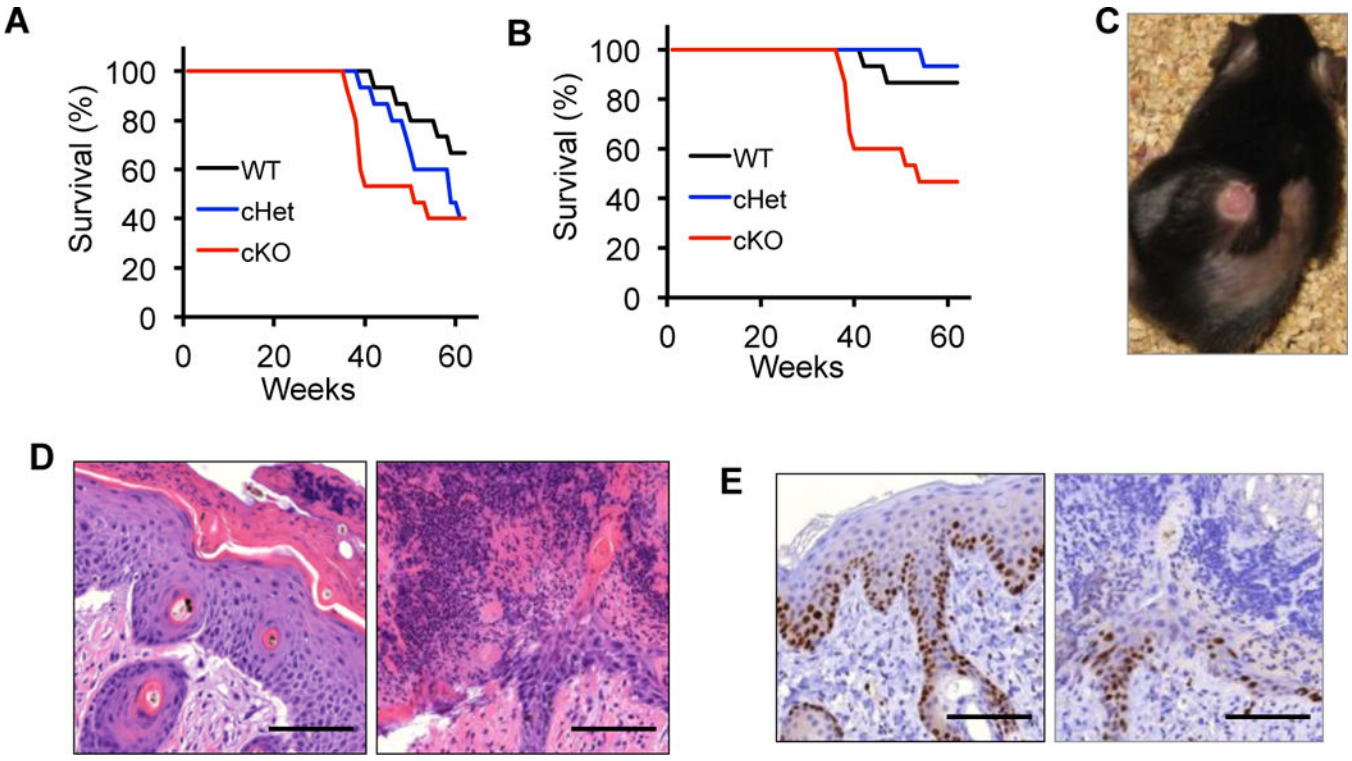


Figure 2.

SIRT1 deficiency sensitizes mouse skin to UV-induced injury. A, Overall survival of SIRT1 WT, cHet and cKO B6 mice following UVB damage. B, non-tumor survival of SIRT1 WT, cHet and cKO mice following UVB damage. C, representative skin lesions from UVB-irradiated cKO mice. D, histological analysis of adjacent and lesional epidermis from SIRT1 cKO mice. E, Immunohistochemical analysis of Ki67 positive cells in adjacent and lesional epidermis from SIRT1 cKO mice. Scale bar, 100 μm.

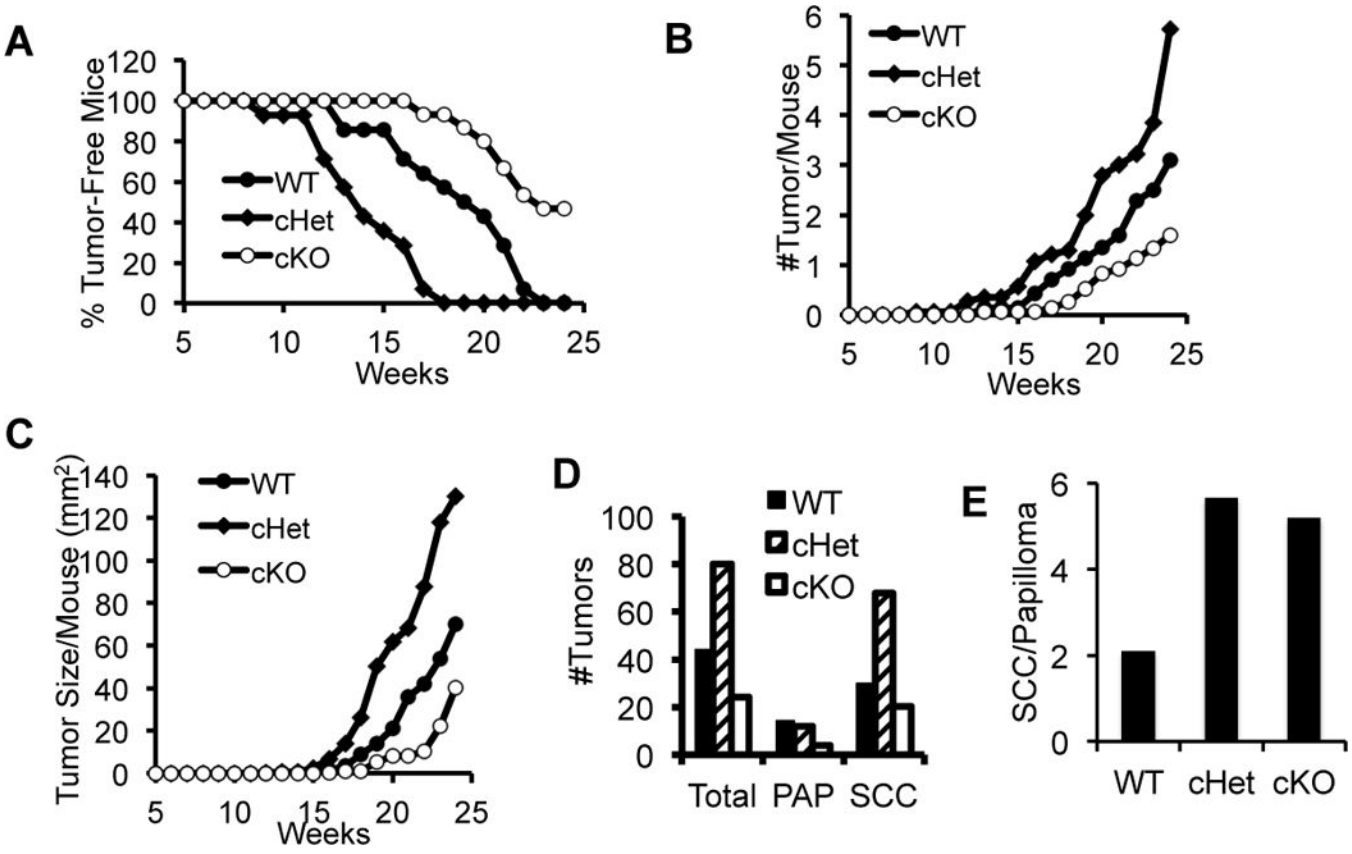


Figure 3.

SIRT1 has a dual role in UVB-induced skin tumorigenesis in SKH1 mice. A, percent (%) of tumor-free mice in WT (n = 14), cHet (n = 14), and cKO (n = 15) groups following chronic UVB radiation. B, average tumor number (#) per mouse in WT, cHet, and cKO mice at different weeks following UVB radiation. C, tumor size (mm^2) per mouse in WT, cHet, and cKO mice at different weeks following UVB radiation. D, number (#) of total tumors, papilloma (PAP), and SCC in WT, cHet, and cKO at the end of study. E, Ratio of SCC to papilloma in WT, cHet, and cKO mice.

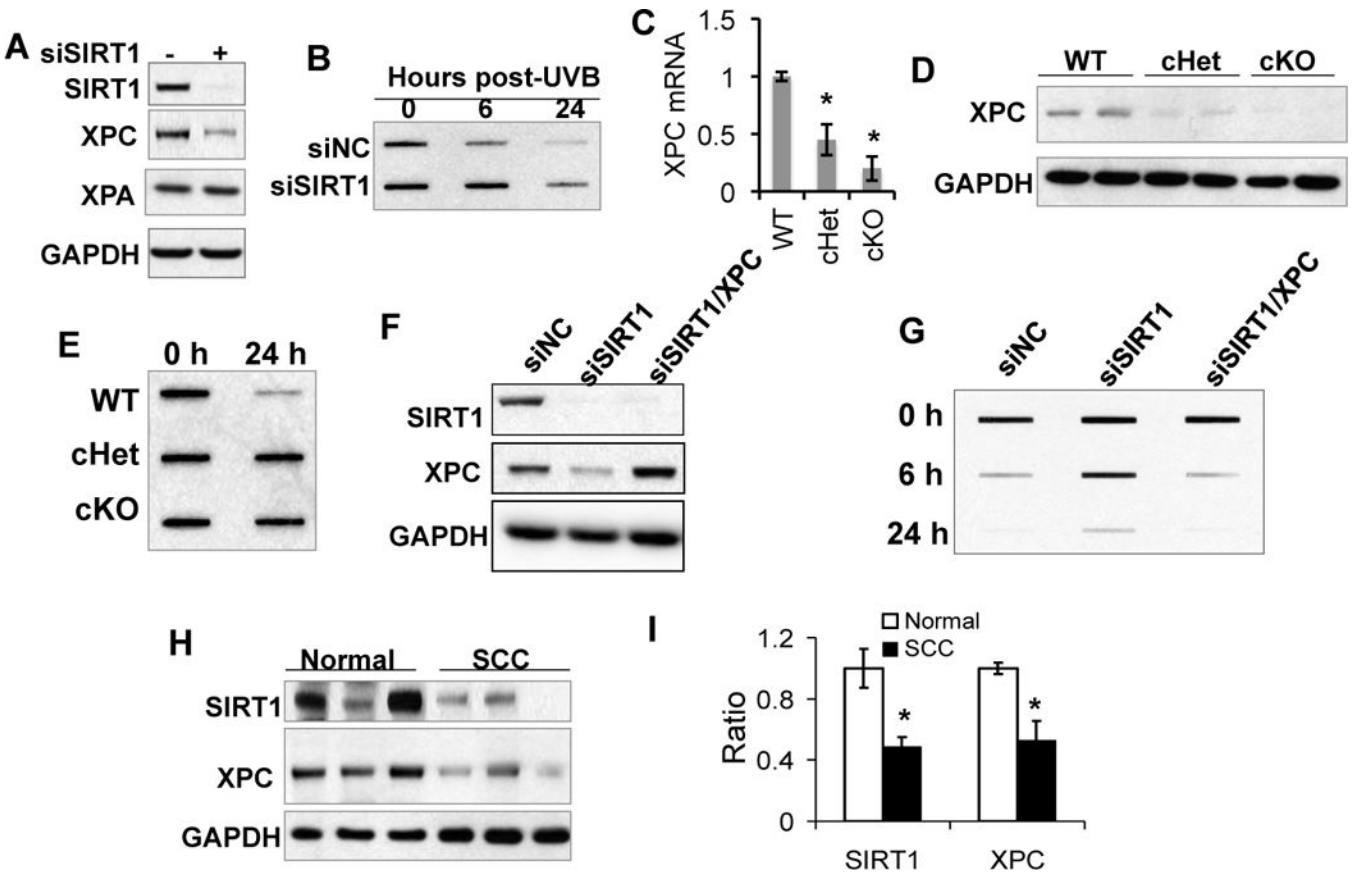
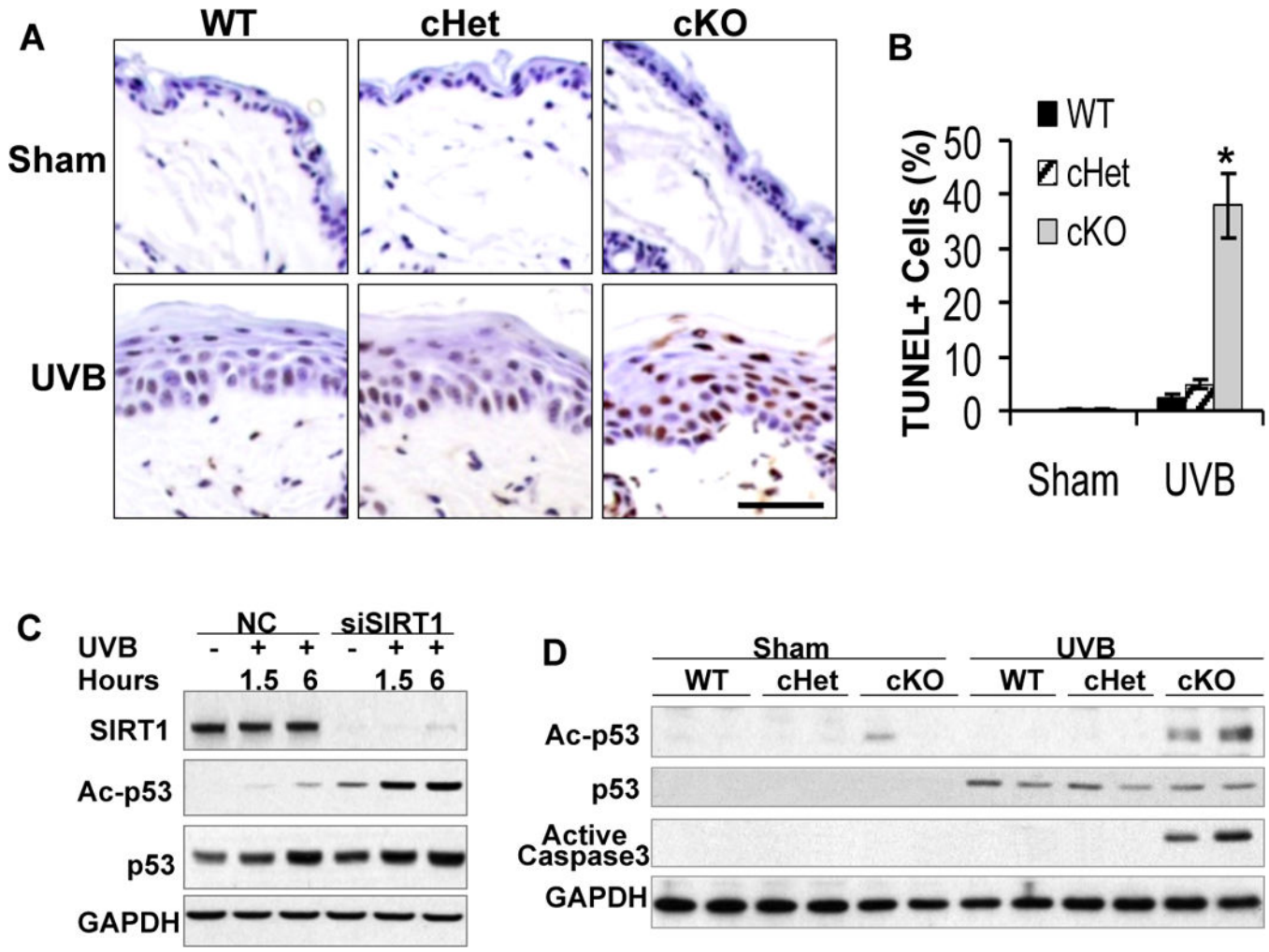


Figure 4.

SIRT1 is haploinsufficient for UVB-induced DNA repair and XPC expression. A, immunoblot analysis of SIRT1, XPC, XPA, and GAPDH in NHEK cells transfected with siRNA targeting SIRT1 (siSIRT1) or negative control (NC) siRNA. B, slot blot analysis of CPD levels in NHEK cells transfected with siSIRT1 or NC. C, real-time PCR analysis of XPC mRNA levels in WT, cHet and cKO mouse skin (n=5). *, $P < 0.05$, significant differences between cHet or CKO and WT mouse skin. D, immunoblot analysis of XPC and GAPDH in WT, cHet and cKO B6 mouse skin (n=5). E, slot blot analysis of the levels of CPD in WT, cHet, and cKO B6 mouse skin at 0 and 24 h following UVB radiation. F, immunoblot analysis of SIRT1, XPC and GAPDH in NHEK cells transfected with siNC, siSIRT1, and siSIRT1/XPC. G, slot blot analysis of the levels of CPD in NHEK cells transfected with siNC, siSIRT1, and siSIRT1/XPC at 0, 6 and 24h following UVB radiation. H, immunoblot analysis of SIRT1, XPC, and GAPDH in normal human skin and SCC samples. I, real-time PCR analysis of SIRT1 and XPC mRNA levels in normal human skin and SCCs. *, $P < 0.05$, significant differences between SCCs and normal skin.



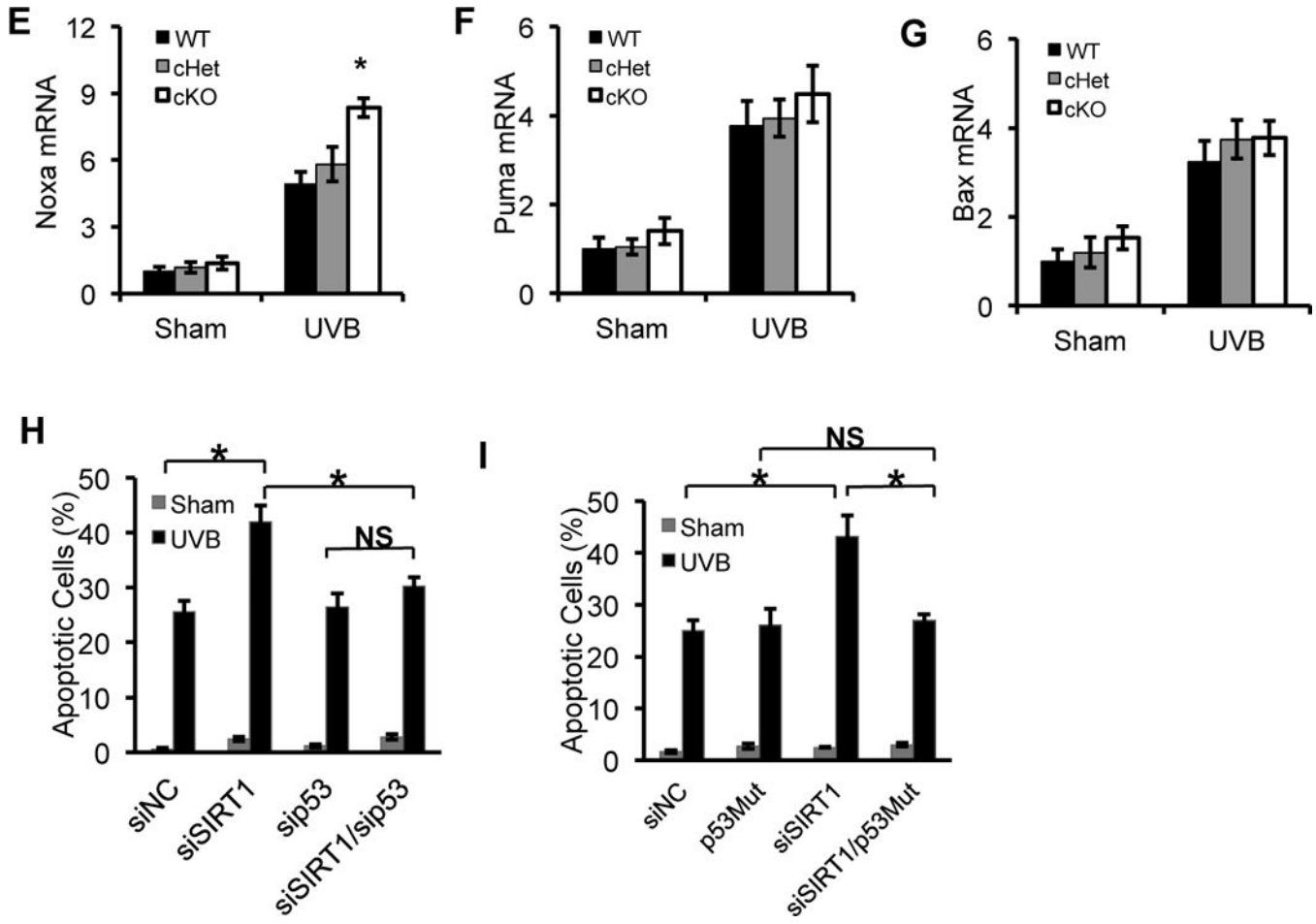


Figure 5.

SIRT1 deficiency can promote UV-induced apoptosis by activation of p53. A, TUNEL analysis of UVB-induced apoptosis in the epidermis from WT, cHet and cKO B6 mice (n=5). Scale bar, 50 μm. B, quantification of percent (%) of TUNEL-positive (+) cells in A. **P*<0.05, significant differences between cKO, and WT mice. C, immunoblot analysis of SIRT1, Ac-p53, p53, and GAPDH in NHEK cells transfected with siSIRT1 or NC at 1.5 h and 6 h post-UVB. D, immunoblot analysis of Ac-p53, p53, active Caspase3, and GAPDH from WT, cHet and cKO mouse skin (n=5) at 24 h post-the final UVB. E–G, real-time PCR analysis of Noxa (E), Puma (F) and Bax (G) in WT, cHet and cKO B6 mouse skin tissue as treated as in B. **P*<0.05, significant differences between cKO and WT mice. H, apoptosis analysis of NHEK cells at 24 h post-UVB (50 mJ/cm^2) or sham by flow cytometric analysis of sub-G1 cells (percentage, %). NHEK cells were transfected with siRNA targeting SIRT1 (siSIRT1), p53 (sip53), or the combination. Negative control siRNA (siNC) were used as transfection control. I. apoptosis analysis of NHEK cells at 24h post-UVB (50 mJ/cm^2) or sham by flow cytometric analysis of sub-G1 cells (percentage, %). NHEK cells were transfected with siNC, p53 K382R (p53Mut), siSIRT1, or siSIRT1/p53Mut. Empty vector was used as a control for p53Mut. **P*<0.05, significant differences. NS, P>0.05, not statistically significant.