UVB-dependent changes in the expression of fast-responding early genes is modulated by huCOP1 in keratinocytes

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Abstract

Ultraviolet (UV) B is the most prominent physical carcinogen in the environment leading to the development of various skin cancers. We have previously demonstrated that the human ortholog of the *Arabidopsis thaliana* constitutive photomorphogenesis 1 (COP1) protein, huCOP1, is expressed in keratinocytes in a UVB-regulated manner and is a negative regulator of p53 as a posttranslational modifier. However, it was not known whether huCOP1 plays a role in mediating the UVB-induced early transcriptional responses of human keratinocytes. In this study, we report that stable siRNA-mediated silencing of huCOP1 affects the UVB response of several genes within 2 h of irradiation, indicating that altered huCOP1 expression sensitizes the cells toward UVB. Pathway analysis identified a molecular network in which 13 of the 30 examined UVB-regulated genes were organized around three central proteins. Since the expression of the investigated genes was upregulated by UVB in the siCOP1 cell line, we hypothesize that huCOP1 is a repressor of the identified pathway. Several members of the network have been implicated previously in the pathogenesis of non-melanoma skin cancers; therefore, clarifying the role of huCOP1 in these skin diseases may have clinical relevance in the future.

Keywords: Constitutive photomorphogenic protein 1, photobiology, UVB, transcriptional regulation, keratinocyte

1. Introduction

Ultraviolet (UV) B light is one of the most important physical carcinogens in the environment, and the skin is the first and major barrier to protect the body from its harmful effects. At the molecular level, UVB causes DNA damage, transcriptional changes and apoptosis. Although epidermal keratinocytes, the main site of environmental UVB damage, provide a useful model system to study UVB-induced cellular responses, information on the molecular pathways mediating these processes is currently limited. UVB irradiation changes the expression of several genes in keratinocytes, including the p53 nuclear phosphoprotein [1-3]. This tumor suppressor functions as a transcription factor and has a central role in keratinocyte stress responses, including UV-induced responses [1-5]. The UVB-dependent molecular network in which p53 plays a pivotal role has not been revealed yet. One of the p53-interacting partners, the E3 ubiquitin ligase, COP1 (constitutive photomorphogenic 1), has been intensively studied in various organisms [6-11].

The COP1 protein was first identified as a central negative regulator of light-regulated development in *A. thaliana* [12]. *A. thaliana* COP1 (AtCOP1) contains three conserved structural domains: a RING finger at the amino terminus mediating ligase activity, a coiled-coil domain in the middle mediating dimerization and seven WD40 repeat domains at the carboxyl-terminal end of the protein implicated in the binding of target proteins [13-15]. AtCOP1 functions as an E3 ubiquitin ligase targeting selected proteins for proteosomal degradation in plants [15]. Among its substrates are important transcription factors, such as the key regulators of photomorphogenesis under all light conditions, including UVB [16]. In contrast the negative regulation observed for visible-light responses, AtCOP1 is a critical positive regulator of responses to low levels of UVB. According to the latest data, UVB triggers the physical and functional disassociation of the AtCOP1–SPA core complex from CUL4-DDB1 and the formation of a new complex containing the UVB photoreceptor, UV Resistance Locus 8. This UVB-induced machinery is associated with the positive role of AtCOP1 in facilitating the stability and activity of key transcription factors.[8]
Sequence analysis of COP1 orthologs from arabidopsis, human and mouse indicated that the COP1 domain structure is highly conserved in higher plants and vertebrates [16]. The mouse (MmCOP1) and human COP1 (huCOP1) sequences are located on chromosome 1 and the high degree of sequence conservation with AtCOP1 suggested functional conservation [12]. Both MmCOP1 and huCOP1 target bZIP transcription factors of the Jun family and p53 for degradation in a similar manner to AtCOP1 [6,17]. Lee *et al.* discovered that the constitutive photomorphogenesis 9 signalosome (CSN) plays a role in the control of DNA damage and carcinogenesis caused by UV light [18,19]. CSN components seem to act upstream of COP1 in

mammalian cells stimulated by UV. Based on this observation, CSN components might act through huCOP1 in the negative regulation of important cancer genes, including p53, MDM2, P27, c-JUN, NFKB, SMAD7, RUNX3, ID1, SKP2 and HIFI [18,19].

The function of huCOP1 has not yet been investigated in detail. We previously demonstrated that huCOP1 is expressed in keratinocytes in an UVB-regulated manner and is a negative regulator of p53 as a post-translational modifier [10]. The regulation of p53 by huCOP1 in keratinocytes is of particular importance, as this role suggests involvement in both cellular UV responses and carcinogenesis. MmCOP1 has also been shown to repress c-JUN mediated AP-1 transcription [11,20].

The mechanisms by which the skin protects against UV damage have been investigated in detail. DNA arrays have been used to examine the UV-induced change in expression of 6800 genes in epidermal keratinocytes. Differential expression of 198 genes was detected in three waves occurring at 0.5–2 h, 4–8 h and 16–24 h after UV irradiation [1]. Although it is evident from the available data that huCOP1 and orthologs are posttranslational regulators of late UV responses in a wide range of organisms, it was not known how early huCOP1 acts on transcriptional responses. Based on these data and on the results obtained from a UVB-dependent transcriptome analysis performed with arabidopsis [21], we raised the question whether huCOP1 plays a role in early UVB-induced signaling processes that lead to transcriptional changes in keratinocytes.

In this study we report that stable siRNA-mediated silencing of huCOP1 sensitises the keratinocyte cells toward UVB and affects the UVB response of several genes within 2 h of irradiation. Our results suggest that huCOP1 plays a role in modulating the early cellular UVB response at the transcriptional level in human keratinocytes.

2. Materials and methods

2.1. Keratinocyte transformation

HPV-immortalized human keratinocytes (HPV-KER clone II/15) were maintained as described [22]. Stable transformation of HPV-KER II/15 was performed at approximately 70% confluency in supplement-free medium with either the empty vector (pSuperior.puro vector) or a vector harboring huCOP1 silencing sequences [10]. Plasmid DNA for transfection was purified with the QIAGEN Plasmid Maxi Kit (QIAGEN, Hilden, Germany). Transfection was carried out with the X-tremeGENE 9 DNA Transfection Reagent (Roche Applied Science, Mannheim, Germany) and the Human Keratinocyte Nucleofector Kit (Lonza Cologne AG, Cologne, Germany) according to the manufacturer's instruction.

2.2. Western blot analysis

HuCOP1 protein was detected using previously described standard procedures [10] with the exception that anti-mouse IgG-HRP (Dako Thermo Scientific Waban, MA, USA) was used as a secondary antibody at a dilution of 1:1000. Membranes were incubated overnight at 4ºC with purified rabbit polyclonal anti-huCOP1 antibody. After treating the membrane with the Immobilon Western Chemiluminescent HRP substrate (Merck Millipore Corporation, Billerica, MA, USA), luminescent signals were detected using a liquid-nitrogen-cooled charge-coupled-device camera (Micromax; Roper Scientific Canada).

2.3. Cell viability measurements

Keratinocytes were seeded in 96-well plates at 10,000 cells per well. Growth of the cells was followed for 6 days. Cell viability of the established keratinocyte cell lines was measured with the xCELLigence RTCA System [39] following the manufacturer's instructions (Roche Applied Science, Mannheim, Germany). See Supplementary Methods online for details.

2.4. Immunocytochemistry

Unirradiated and UVB-irradiated control and siCOP1 cells were immunostained 24 hours after UVB exposure. DNA was stained with 4,6-diamidino-2-phenylindole (DAPI). The primary antibody used for immunohistochemistry was rabbit polyclonal anti-huCOP1 (Bethyl Laboratories, Montgomery, USA). A Zeiss AxioImager fluorescent light microscope (Carl Zeiss MicroImaging, Thornwood, NY, USA) fitted with a PixeLINK CCD camera (PixeLINK, Ottawa, ON, Canada) was used for detection. The subsequent semiquantitative analysis was carried out using Metamorph software (Universal Imaging Corp., Sunnyvale, California, United States). See Supplementary Methods online for details.

2.5. UVB irradiation

For irradiation, the medium covering the keratinocytes was replaced with phosphate-buffered saline (PBS). In preliminary experiments, 10, 20, 40 $mJcm^{-2}$ UVB doses were used to determine the most appropriate dose for further studies. The 40 $mJcm^{-2}$ dose resulted in a decrease in cell viability, whereas the 20 $mJcm^{-2}$ did not affect cell viability (data not shown). It was also demonstrated that the 20 $mJcm^{-2}$ dose effected the expression of well known UVB-inducible genes

(*G0S2*, *JUNB*, *JUND*, data not shown). Cells were irradiated with a 20 $mJcm^{-2}$ dose of 312 nm UVB [40] from an FS20 lamp (Westinghouse, Pittsburgh, PA). After UVB treatment, PBS was replaced with fresh medium. Control cells were subjected to the identical procedure without UVB treatment.

2.6. Real-time RT-PCR array and validation experiments

Real-time RT-PCR was performed with a custom-made StellARray™ Gene Expression System (Bar Harbor BioTechnology, Trenton, ME) carrying 30 UVB-regulated genes. The validation real-time RT-PCR experiments were carried out with the Universal Probe Library system (F. Hoffmann-La Roche AG, Basel, Switzerland). Sequences of the primers used for PCR amplification of the FOS, JUND ZFP36 and SIK1 genes are listed in Table S1. The expression of each gene was normalized to the signal from the 18S ribosomal RNA gene. Results are averages of three parallel experiments. The relative mRNA expression levels were calculated by the $\Delta\Delta C_t$ method [41]. See Supplementary Methods online for details.

2.7. Pathway analysis

Pathway analysis was performed using Ingenuity Pathway Analysis software (IPA, Ingenuity Systems, Stanford, USA). See Supplementary Methods online for details.

3. Results

3.1. Characterization of siCOP1 keratinocyte cell lines

To determine the role of huCOP1 in the early UVB response, we created keratinocyte cell lines in which the expression level of huCOP1 was stably decreased. For this purpose, we used an HPV-immortalized keratinocyte cell line in which the UVB response of p53 is intact: its UVB inducibility resembles that of normal human cultured keratinocytes. However, sequence analysis revealed that the p53 gene in this cell line harbours the codon 72 polymorphism of the protein (manuscript in preparation). G-banding analyses of the cell line revealed normal human karyogram (46, XX) [22]. Four cell lines harboring the empty vector and three cell lines carrying the huCOP1 silencing sequences were established. To select the most appropriate cell lines for further investigation, the degree of huCOP1 silencing and the proliferation of the cell lines were compared. Based on the results of these preliminary experiments (data not shown), two cell lines

— control and siCOP1 — were selected. Semiquantitative analysis of chemiluminescent western blot experiments demonstrated that the expression of huCOP1 in the siCOP1 cell line was decreased by 70% compared to the control line (Fig. 1a and b). The silencing of huCOP1 did not affect cell proliferation (Fig. 2).

3.2. UVB irradiation decreased huCOP1 protein abundance in transformed cell lines

We previously reported that huCOP1 levels decrease in human keratinocytes after UVB irradiation [10]. To confirm that the stably transformed cell lines react similarly to UVB irradiation, we compared unirradiated and UVB-irradiated control and siCOP1 cells using the semiquantitative immunocytochemical approach to detect huCOP1 expression. The applied UVB (20 $mJcm^{-2}$) did not affect the viability or proliferation of the cells (data not shown). An approximately 50% decrease in huCOP1 expression was detected in both cell lines 24 hours after UVB irradiation (Fig. 3).

3.3. Array analysis of selected genes differentially expressed in early UVB response revealed a transcriptional regulatory role for huCOP1 in keratinocytes

To understand the role of huCOP1 in the UVB-induced early signaling processes of human keratinocytes, we performed an expression profile analysis. For this purpose, the siCOP1 and control cell lines were evaluated using the StellArray™ Gene Expression System. Based on published data [1-3], we selected a set of genes showing a 2–4 fold change in gene expression within 2 h after UVB irradiation (14 up- and 16 down-regulated genes). The expression of the selected genes was compared in unirradiated and UVB-irradiated cells 2 hours after treatment. Changes in gene expression in the control cells were in good agreement with published results for most genes. The decreased abundance of huCOP1 protein in the siCOP1 cells further modulated this UVB effect, resulting in an overall higher gene expression level. Expression of the genes was similar in unirradiated siCOP1 and control lines, indicating that the silencing of COP1 had no direct effect on the expression of these genes in the absence of UVB (Table 1).

To verify the array results, we performed real-time RT-PCR analyses of four genes, FBJ murine osteosarcoma viral oncogene homolog (FOS), JUND, zinc finger transcriptional regulator (ZFP36), and SIK1 (salt-inducible kinase 1) based on the robust UVB-induced changes in gene expression observed in the array experiment. The real-time RT-PCR assay of independent biological samples confirmed that the detected differential expression was a transcriptional consequence of huCOP1 silencing (Supplementary Fig. S1).

3.4. A UVB regulatory network identified by pathway analysis was modified by huCOP1 expression

Using the Ingenuity Pathway Analysis software (IPA, Ingenuity Systems, Stanford, USA), we identified a regulatory network of UVB-regulated genes in which 13 of the selected 30 genes participated (Fig. 4a). Mitogen-activated protein kinase (ERK1/2), cAMP responsive element binding protein (CREB) and ubiquitin (not included in the array experiment) were identified as central organizers of this network. Expression of all 13 selected genes increased after UVB irradiation in the siCOP1 cell line, indicating that huCOP1 modulates the expression of these genes. To clarify whether the gene expression of ERK1/2 and CREB is UVB-regulated, we carried out real-time RT-PCR experiments. UVB irradiation reduced the mRNA level of the central organizers in the control cell line and this affect was moderated by huCOP1 silencing (Fig. 4b).
The pathway analysis also revealed the following upstream regulators of the identified network: epidermal growth factor (EGF), nerve growth factor, fibroblast growth factor 2, interferon gamma, interleukin-1 beta, interleukin 6 cytokines and nuclear factor of kappa beta (NFKB) (Fig. 5a). According to the analysis, these regulators are in an activated state to trigger the UVB response through the identified network but the relationships among the upstream regulators were not assessed. We choose three upstream regulator molecules (IL1B, IL6 and NFKB) to test wheather the corresponding genes exhibit UVB-induced changes in expression. In agreement with published data [2,4,23], our results demonstrated that the transcription of these genes is UVB sensitive and that huCOP1 silencing had an effect on their transcription levels after UVB irradiation (Fig. 5b).

4. Discussion

UVB light is undoubtedly the most important carcinogenic environmental stressor of human skin, and UV-induced changes in keratinocytes have been widely studied [3,24]. HuCOP1 has been implicated in the negative regulation of important cancer-related genes acting in the cellular response to UVB [11,25]. Nevertheless, the possible role of huCOP1 in the keratinocyte UVB response has not yet been investigated in detail.

To address these issues we produced transgenic cell lines in which the expression of huCOP1 was stably silenced (siCOP1). For this purpose, we used an HPV-immortalized keratinocyte cell line carrying the codon 72 polymorphism of the p53 gene, a well-known and common polymorphism with yet unconfirmed significance in cancer risk. Since this cell line exhibited a normal p53 UVB response, we considered it suitable for our purposes. After

establishing the huCOP1 silenced transgenic keratinocyte cell line, we examined the expression of selected UVB-regulated genes with or without UVB irrradiation. Our experiments revealed that (i) the silencing of huCOP1 did not affect cell viability, (ii) the expression level of the selected genes was not affected by huCOP1 silencing in unirradiated cells, and (iii) DNA array and validating real-time RT-PCR experiments confirmed that transcript levels of the selected genes exhibited changes as early as 2 hours after UVB irradiation and that these changes were in good agreement with previously published data [1-4]. However, very importantly, we found that (i) the residual huCOP1 level was further reduced by UVB and (ii) the significantly reduced huCOP1 level resulted in more pronounced UVB-induced changes in the expression of genes as compared to non-transgenic keratinocytes. These data demonstrate that this cell line is a particularly suitable tool for studying huCOP1-dependent UVB-induced changes in early gene expression responses.

The possible interactions among the examined genes were analyzed using Ingenuity Pathway Analysis software. This software uses published interaction data for composing potential new networks based on novel experimental data. The pathway analysis identified a network in which 13 of the 30 examined genes were organized around three central molecules, ERK1/2, CREB and ubiquitin.

Functional connections between certain members of the identified network have already been described [26-29]. All 13 genes were differentially expressed after UVB irradiation, and their expression was increased in siCOP1 cells compared to control. Similar changes have been detected in ERK1/2 and CREB gene expression, affirming their central role in the identified network.

Some of the components of the identified network have already been implicated in huCOP1-mediated processes: Liu *et al.* [30] have recently demonstrated that huCOP1 promotes the ubiquitilation and degradation of the cAMP responsive CREB-regulated transcription coactivator 2. COP1 has also been shown to play a role in the degradation of the c-Jun protein [9,11,20,25].

Our array identified two other functional components of the activator protein-1 (AP-1) transcription factor complex: JUNB and JUND. HuCOP1 might regulate these two genes through an as yet unidentified mechanism. On the basis of these data, we hypothesize that huCOP1 might contribute to the regulation of the AP-1 transcription complex at different levels: by altering the expression of the JUNB and JUND genes and by promoting the degradation of c-Jun *via* its E3 ubiquitin ligase activity. Such functional interactions were suggested as early as 2003, when Bianchi and co-workers identified huCOP1 as the human ortholog of AtCOP1 and performed the primary functional characterization of the gene and its protein product [20]. Subsequently Migliorini *et al.* (2011) demonstrated the direct c-Jun-COP1 interaction.

AP-1-related transcription factors have long been implicated in tumorigenesis; therefore, the putative role of huCOP1 in the pathogenesis of human cancers emerged early [11,20]. Much

evidence demonstrating that COP1 plays a major role in tumorigenesis has since accumulated [25]. However, it is still unclear whether COP1 is a tumor suppressor or an oncogene. Marine speculated in this recent review that COP1 may have a "dual face," functioning either as an oncogene or as a tumor suppressor, depending on the cellular context.

Our results and the data available in the literature indicate that decreased huCOP1 levels sensitize the cells to UVB damage or oxidative stress and modify the UVB-induced stress response of keratinocytes. The loss of some members in the identified network increases the sensitivity of the cells to UVB. Maeda *et al.* [31], for example, demonstrated that GADD45A -/- keratinocytes are more sensitive to UVB than GADD45A +/+ cells due to reduced DNA repair and lack of G2/M arrest. It is also well known that the symptoms of systemic lupus erythematosus are exacerbated by sun exposure. Pflegerl *et al.* [32] reported that challenging $JUNB^{\Delta ep}$ mice with UVB irradiation enhanced the severity of their lupus-like lesions. It has also been published that DAXX-depleted fibroblasts are resistant to UVB- and oxidative-stress-induced cell death [33]. Presumably, the absence of certain members of the huCOP1-mediated transcriptional cascade leads to an abnormal UVB response.

Several members of the identified huCOP1-mediated UVB-responsive network have already been correlated with non-melanoma skin cancers, such as basal cell carcinoma (BCC) and squamous cell carcinoma (SCC). Immunohistochemical studies recently performed by our workgroup [34] indicated that huCOP1 expression is altered in both non-melanoma skin cancers. Moreover, well established data are available on the differential expression of several members of the identified huCOP1-mediated UVB-responsive network both in BCC and SCC ([34-38]).

We demonstrated that huCOP1 contributes to the transcriptional regulation of the keratinocyte UVB response by the downregulation of an UVB inducible network operating through three newly identified central organizers. This network is under the control of upstream regulators also showing UVB response. We hypothetise that huCOP1 operates as a negative factor of the identified transcriptional network by modifying the function of the upstream regulators. Mechanism underlying the UVB-induced cellular events may include a re-distribution of huCOP1 to different molecular complexes. Such a mechanism has recently been demonstrated for arabidopsis [8]. The expression of huCOP1 is altered in non-melanoma skin cancers [34] and several members of the identified regulatory network have been implicated in the pathogenesis of these skin diseases [35-38]. Thus, huCOP1 emerges as a potential target molecule for BCC and/or SCC. Since COP1 possibly acts both as an oncogene and as a tumor suppressor, further studies should clarify the role of huCOP1 for human keratinocytes in photo-damaged skin and these skin diseases.

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Figure captions

Fig. 1. HuCOP1 protein expression in keratinocytes. a: HuCOP1 protein levels from control and siCOP1 cell lines were detected by chemiluminescent western blot analysis; b: The huCOP1 expression was subjected to semiquantitative analysis. The expression of huCOP1 was normalized to the expression of α-actin. The average of three independent experiments is shown.

Fig. 2. Dynamic proliferation curves for siCOP1 and control cells. Cell viability of the established keratinocyte cell lines was measured with the xCELLigence RTCA System. Black line: control cells; gray line: siCOP1 cells.

Fig. 3. UVB irradiation decreases huCOP1 protein level in keratinocytes. a: Unirradiated and UVB-irradiated control and siCOP1 cells 24 hours after treatment. b: Semiquantitativ analysis of huCOP1 protein level detected by immunocytochemistry before and at 24 hours after UVB irradiation. Results are the averages of the *huCOP1* expression after UVB irradiation from 30 independent cells normalized to the control cell line.

Fig. 4. The UVB-regulatory network identified by the Ingenuity Pathway Analysis. a: Arrows with continuous lines indicate well established direct regulatory connection between the members of the network. Arrows with dashed lines refer to well established indirect connections between the members of the network. Grey ovals: UVB-upregulated genes; white ovals: UVB-downregulated genes; white rectangles: predicted key regulatory molecules. Single-headed arrows: one-way connection. Double-headed arrows: determined interaction. Lines without arrows: connection with yet undetermined direction; b: Relative transcript levels of ERK and CREB are compared for UVB-irradiated siCOP1 (white bars) and control (black bars) cells. Expression levels were normalized to the 18S ribosomal RNA gene. Values reflect the fold change between the untreated and irradiated control and siCOP1 cells from three independent irradiation experiments. The applied statistical analysis (Student's two-tailed heteroscedastic *t* test) did not reveal significant difference in UVB-induced gene expression between the control and siCOP1 cells, however the tendency is clearly demonstrated.

Fig. 5. Predicted upstream regulators of the identified network. a: White ovals: upstream regulators; gray rectangles: UVB-upregulated genes of identified network; white rectangles: UVB-downregulated genes of the identified network. All of the upstream regulators were in an activated state, but the relationships among them were not investigated; b: Relative transcript levels of IL1B,

IL6 and NFKB are compared for UVB-irradiated siCOP1 (white bars) and control (black bars) cells. Expression levels were normalized to the 18S ribosomal RNA gene. Values reflect the fold change between the untreated and irradiated control and siCOP1 cells in three independent irradiation experiments. The applied statistical analysis (Student's two-tailed heteroscedastic *t* test) revealed a significant ($p<0.05$) difference in the expression of the *IL6* gene after UVB irradiation of control and siCOP1 cells and demonstrated a non-significant but tendencious difference in the case of *IL1B* and *NFKB* gene expression.

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Figure 1

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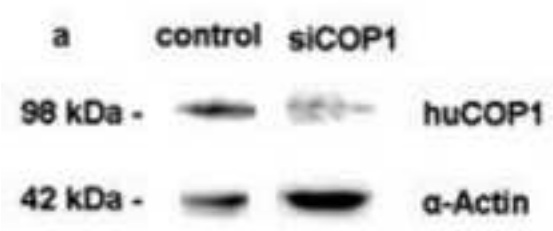

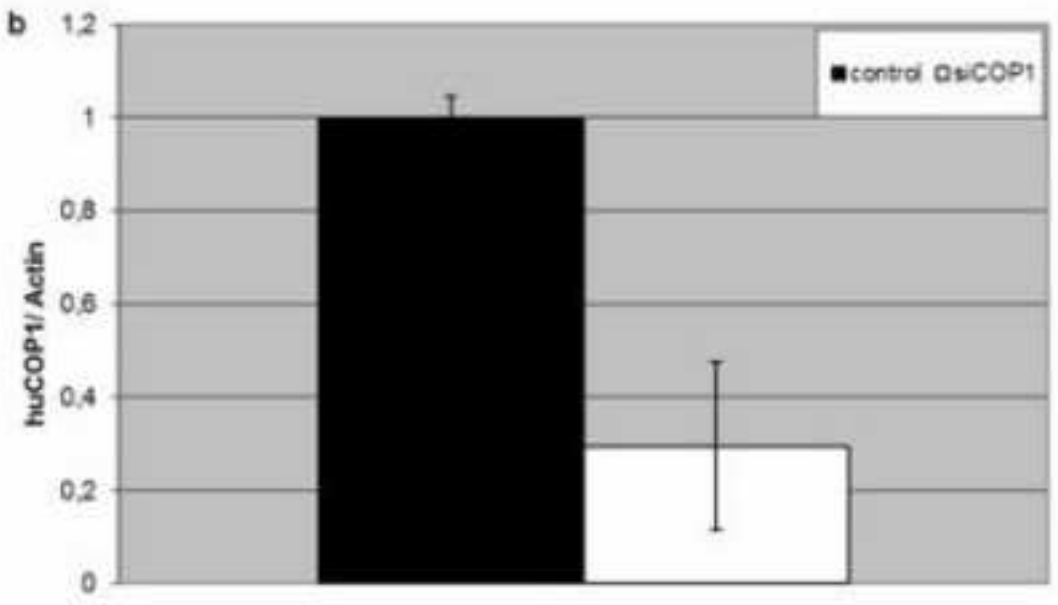

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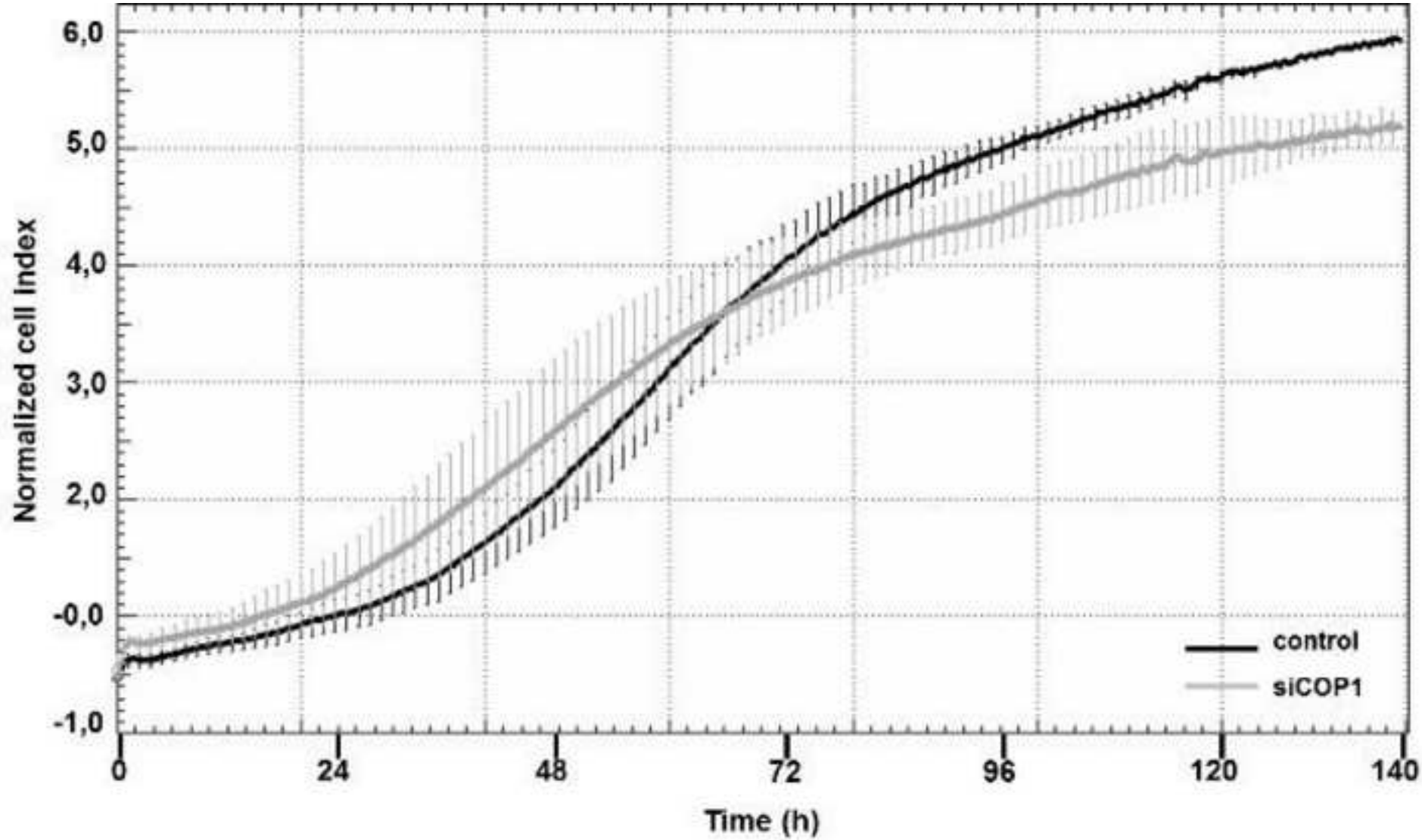

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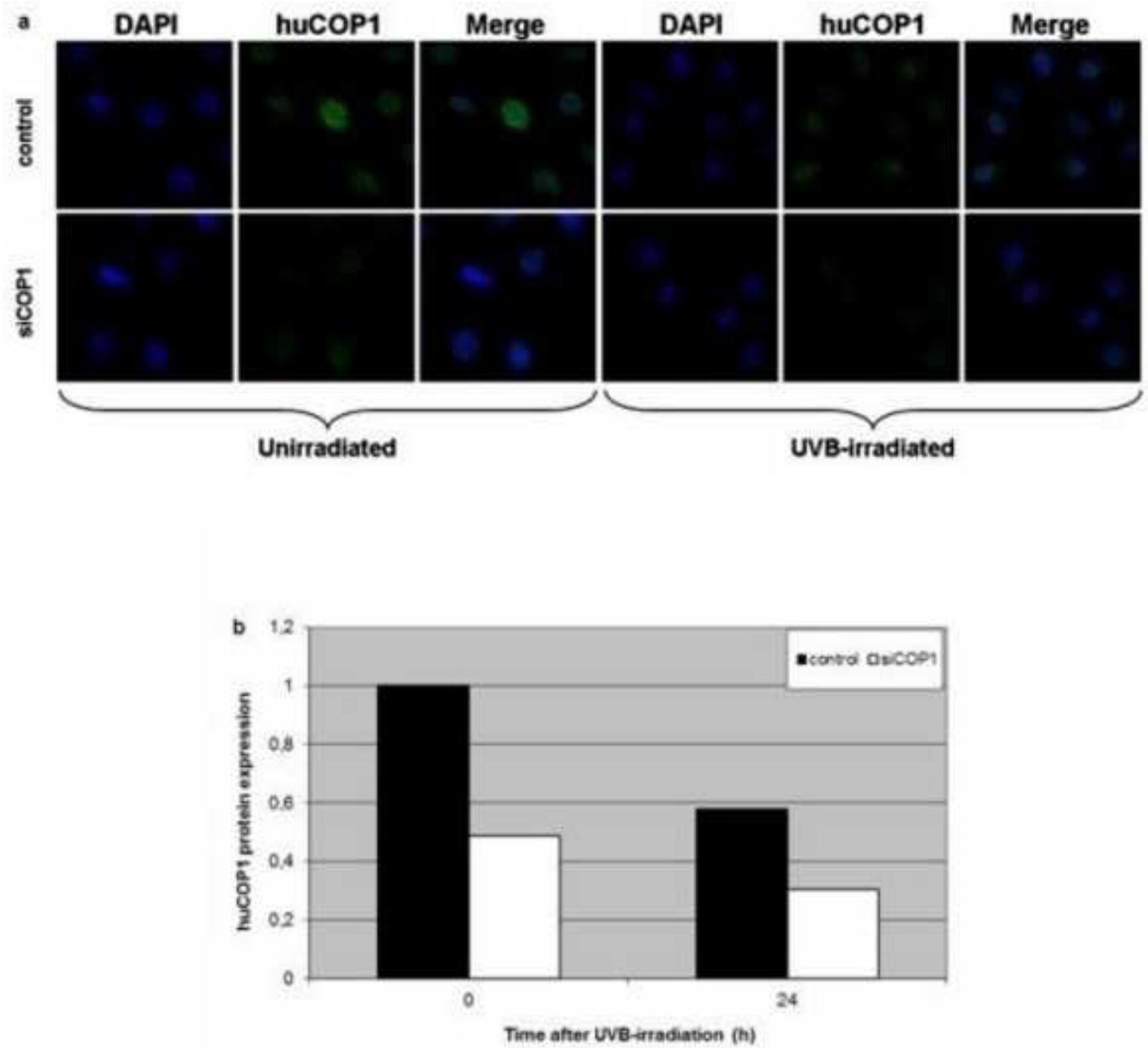

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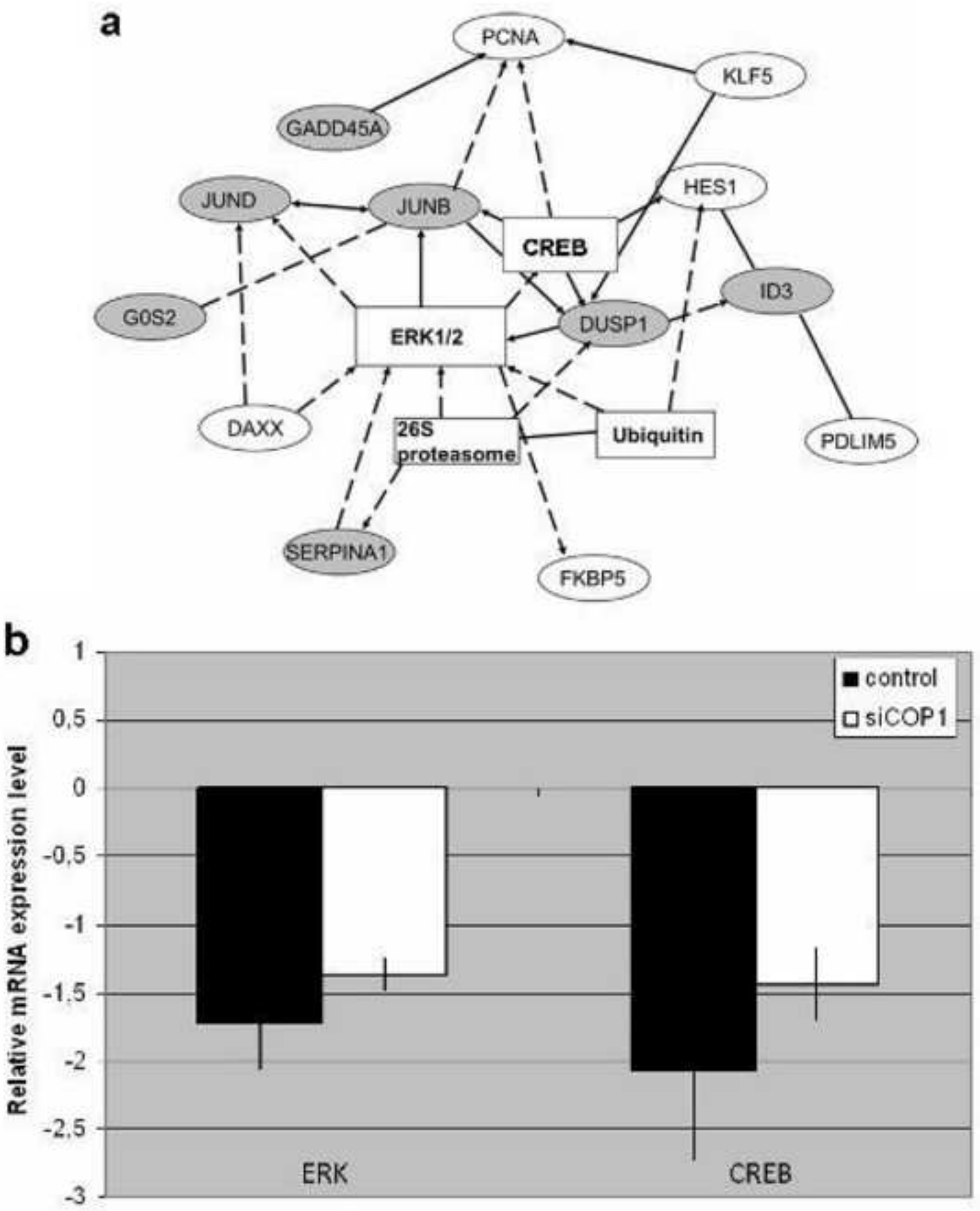

Figure 5

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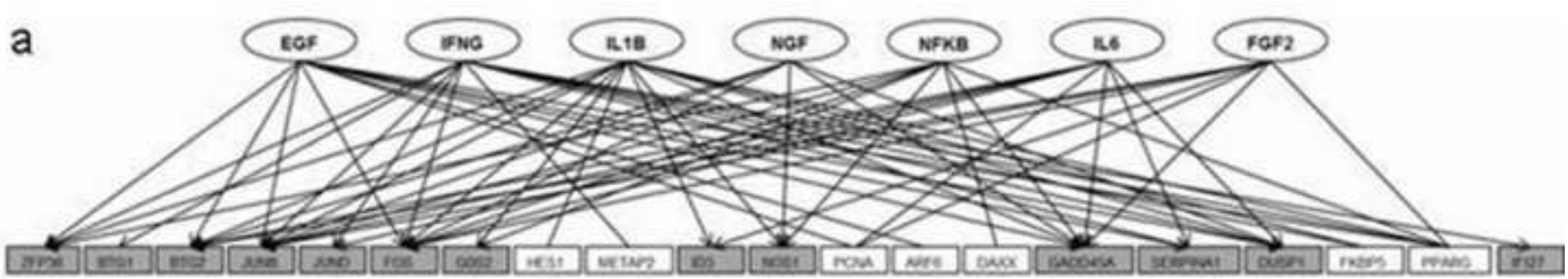

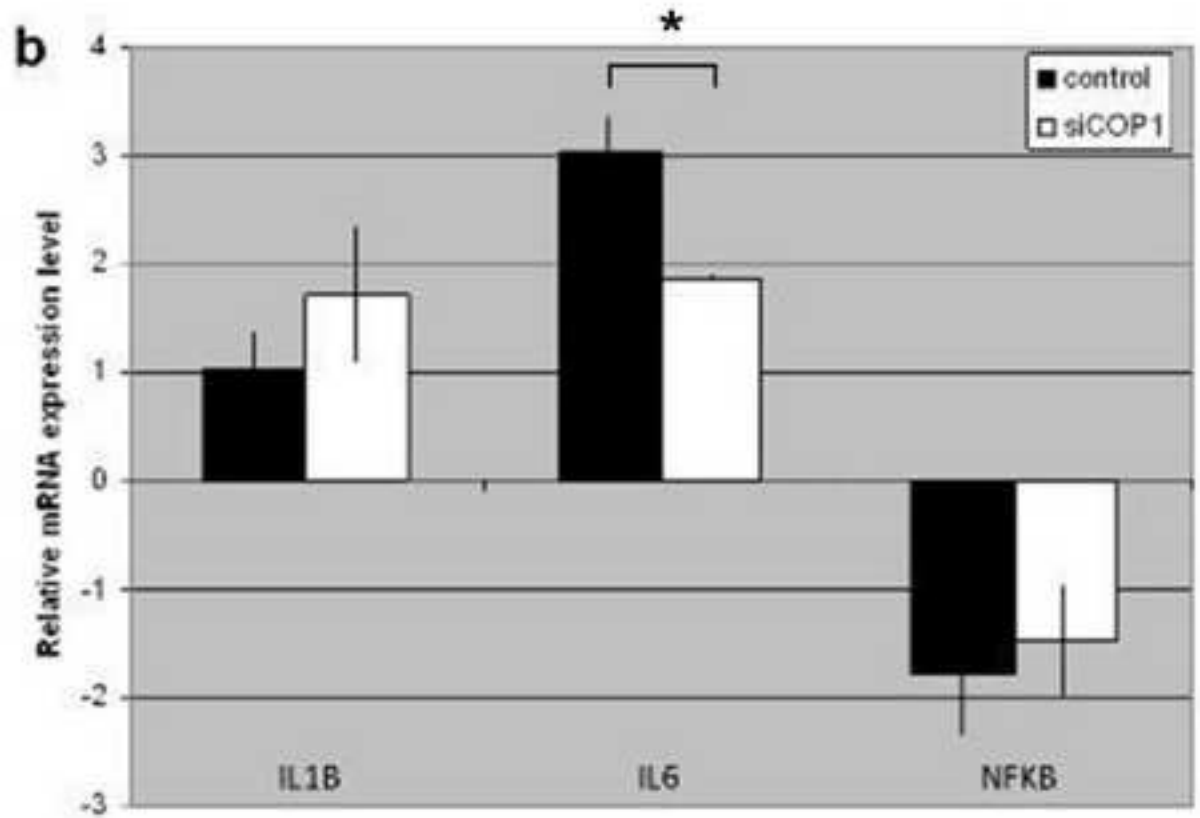

Table(s)

Table 1. Changes in gene expression of unirradiated and UVB-irradiated control and siCOP1 keratinocytes

Gene symbol	Entrez Gene ID	Gene name	Without UVB		UVB irradiated Change, fold		P-values	
Upregulated genes			**Control**	**siCOP1**	**Control**	**siCOP1**	**Control**	**siCOP1**
FOS	2353	FBJ murine osteosarcoma viral oncogene homolog	13,53	14,20	4,95	10,14	0,004	0,006
DUSP1	1843	Dual specificity phosphatase 1	11,99	11,94	6,62	6,35	0,004	0,020
BTG2	7832	Antiproliferative DNA damage response	11,77	12,28	3,43	5,65	0,038	0,021
BTG1	694	Antiproliferative DNA damage response	16,13	16,45	2,27	3,35	0,047	0,084
ZFP36	7538	Zinc finger transcriptional regulator	14,02	14,33	2,39	4,15	0,050	0,073
JUNB	3726	Jun B proto-oncogene	10,42	11,30	2,05	2,58	0,058	NS
JUND	3727	Jun D proto-oncogene	10,54	11,32	1,85	4,92	NS	NS
G0S2	50486	G0/G1 switch gene	10,38	10,52	1,56	2,68	NS	NS
GADD45A	1647	Growth arrest and DNA-damage-inducible	12,10	11,28	2,39	2,19	NS	NS
ID3	3399	HLH1R21 helix-loop-helix protein	12,63	13,08	1,41	1,91	NS	NS
NOS1	4842	Neuronal nitric oxide synthase	19,78	19,51	1,23	1,65	NS	NS
SERPINA1	5265	Serine (or cysteine) proteinase inhibitor A1	12,65	11,75	1,10	1,60	NS	NS
IFI27	3429	IFN-inducible	18,14	18,41	1,04	2,14	NS	NS
TAF10	6881	Transcription factor tafII30	10,78	10,70	1,08	1,58	NS	NS
Downregulated genes								
SIK1	150094	SWI/SWF complex 170 kDa subunit (BAF170)	12,17	12,54	-2,59	-1,48	0,019	0,055
PDLIM5	10611	LIM domain	11,33	12,13	-1,98	1,04	0,043	NS
KLF5	688	GC box binding protein BTEB2	9,92	11,00	-1,96	1,40	0,057	NS
SRF	6722	Serum response factor	12,99	13,37	-1,75	-1,10	NS	0,061
MLL	4297	Translocation T(4:11) of ALL-1 gene to chr,4	13,50	13,56	-1,75	-1,20	NS	NS
FKBP5	2289	Progesteron receptor-associated FJBP54	11,28	11,40	-1,67	1,08	NS	NS
CSNK2A1	1457	Casein kinase II A	11,13	10,82	-1,58	1,07	NS	NS
CSNK1A1	1452	Casein kinase 1A	11,31	11,16	-1,52	1,05	NS	NS
METAP2	10988	Methionin aminopeptidase, translation inhibitor	10,57	10,50	-1,48	1,22	NS	NS
HES1	3280	Transcription factor HRY	10,21	10,70	-1,09	1,67	NS	NS
PPARG	5468	PPAR gamma	14,31	13,80	-1,21	1,47	NS	NS
PKMYT1	9088	Myt1 kinase (preferentially phosphhorylates Cdc2)	14,48	14,36	-1,13	1,44	NS	NS
DAXX	1616	FAS binding protein	12,54	12,67	-1,26	1,51	NS	NS
H2AFZ	3015	Histone	8,36	8,03	-1,07	1,70	NS	NS
PCNA	5111	Proliferating cell nuclear antigen	9,23	9,49	-1,15	1,89	NS	NS
ARF6	382	ADP ribosilation factor 6 GTP binding	10,64	10,23	-1,20	1,12	NS	NS

*NS: not significant