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UV-B induces cytoplasmic survivin expression in mouse epidermis

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Letter to the Editor

Excessive exposure to ultra-violet (UV) light, the UV-B component (290-230 nm) in particular, represents the most harmful DNA damage-inducing condition that keratinocytes have to face on a regular basis. UV-B exposure induces survivin expression in keratinocytes *in vitro* (Dallaglio *et al.*, 2009) and *in vivo* (Aziz *et al.*, 2004). Survivin (BIRC5) is a member of the "inhibitor of apoptosis (IAP)" protein family that can directly or indirectly inhibit caspases, the proteases that mediate apoptosis. Unlike the other members of the family, survivin is also a chromosomal passenger that ensures proper chromosome segregation during cell mitosis. Survivin is often considered as a marker of malignancy, being virtually undetectable in most normal cells and over-expressed in cancer cells (Wheatley and McNeish, 2005). *In vitro* studies indicate that upon stress, survivin may translocate to the cytoplasm (Asumen *et al.*, 2010). The proposed anti-apoptotic mechanisms allowing survivin to protect cells rely on a cytoplasmic location of the protein where it can either inhibit pro-apoptotic proteins or stabilize anti-apoptotic proteins. However, the implication of survivin in anti-apoptotic responses is highly debated (Yue *et al.*, 2008). Nevertheless, there is a consensus that, if survivin does have anti-apoptotic functions, this results from its expression in the cytoplasm or its association to the mitochondria (Connell *et al.*, 2008). Indeed, preventing survivin translocation from the nucleus to the cytoplasm, while not affecting cell division, renders cells more sensitive to irradiation-induced apoptosis (Colnaghi *et al.*, 2006). However, no publication yet has reported the presence of survivin in the cytoplasm of stressed or damaged cells *in vivo* in non-pathological conditions and hence the physiological role of cytoplasmic survivin is still unclear. The aim of the present study was to define the

mode of survivin expression in mouse skin in response to UV-B exposure and determined whether there is an association between survivin expression and apoptosis.

Only a low percentage (about 2-3%) of keratinocytes and follicle cells in non-exposed skin expressed survivin (Figure 1A, left panel; quantitation shown in Figure 1C-D; supplementary material including detailed experimental procedures is available online). The majority of these cells displayed a nuclear survivin expression (filled arrowheads in Figure 1A; Figure 1B, upper panels; quantitation shown in Figure 1C-D). The percentage of survivin-positive cells increased in a statistically significant manner starting 24 hours post-UV-B irradiation in the epidermis (Figure 1B; quantitation shown in Figure 1C) and 48 hours post-UV-B irradiation in follicles (Figure 1D). Virtually no cells in the dermis were found to express survivin, whether the skin was exposed to UV-B or not (Figure 1A). Upon UV-B exposure, the location of survivin was mostly nuclear in follicle cells (Figure 1A, filled arrowhead in right panel), while it was mostly cytoplasmic in keratinocytes (Figure 1A, open arrowhead in right panel; Figure 1B, lower panel). Expression of cytoplasmic survivin in follicle cells and nuclear survivin in keratinocytes was not affected by UV-B irradiation (Figure 1C-D).

To assess which layers of UV-B-exposed epidermis express survivin, co-immuno-staining of survivin with either keratin 5 (a basal cell layer marker) or keratin 10 (a supra-basal layer marker) was performed. Nuclear survivin was exclusively found in the basal cell layer of the epidermis (Figure 1E). In contrast, upon UV-B exposure, survivin was expressed in the cytoplasm of keratinocytes of the basal layers of the epidermis, and to a limited extent, keratinocytes of the supra-basal layer (Figure 1E). There is evidence that cytoplasmic survivin is present in a few cells of the basal cell

layer in normal human epidermis (Marconi *et al.*, 2007). However, in the present study using mouse epidermis, we did not observe such a cytoplasmic staining for survivin in normal non-exposed conditions. Our results indicate that induction of cytoplasmic survivin upon UV-B irradiation mainly occurs in the proliferative layers of the epidermis, at least in mice.

To assess if there was a correlation between survivin expression in keratinocytes and apoptosis, skin sections of UV-B-irradiated mice were stained with an anti-survivin antibody and labeled with TUNEL to detect apoptotic cells (Figure 2A). UV-B irradiation increased by about 10 fold the percentage of apoptotic cells (Figure 2B). Upon UV-B-treatment, about 10% of the keratinocytes expressed survivin in their cytoplasm and about the same percentage of keratinocytes were undergoing apoptosis (Figure 2C). However, there was limited co-localization of the survivin and TUNEL signals. Indeed, only about 20% of the survivin-positive cells were apoptotic and conversely, only about 20% of the TUNEL-positive cells expressed cytoplasmic survivin (Figure 2D). Therefore, the majority of survivin-positive cells were not undergoing apoptosis; in agreement with data obtained in catagen-phase human hair follicles (Botchkareva *et al.*, 2007) indicating that translocation of survivin to the cytoplasm is not a consequence of cell death. Rather, UV-B-induced cytoplasmic survivin expression may exert anti-apoptotic functions. On the other hand, the percentage of apoptotic cells within the cytoplasmic survivin-positive keratinocyte population was not decreased (it was in fact higher) compared to survivin-negative keratinocytes (~20% vs ~10%). Possibly, survivin positive keratinocytes are the cells that experienced the highest damage in response to UV-B irradiation and cytoplasmic expression of survivin might represent an attempt to cope with this damage. Further studies would need to be conducted to test this assumption.

In conclusion, this work demonstrates that UV-B irradiation leads to the expression of survivin in the cytoplasmic compartment of keratinocytes located in the basal layer of the mouse epidermis. Earlier work had shown that UV-B can induce survivin expression in mouse skin (Chun and Langenbach, 2011) but in which subcellular compartment this occurred had not been investigated. Data shown here provide the first demonstration that survivin can be induced in the cytoplasm of non-cancer cells in conditions where survivin may exert anti-apoptotic functions (i.e. when cells are stressed or damaged). Since cytoplasmic survivin has been shown to counteract apoptosis, this is expected to induce a protective signal in UV-B-exposed keratinocytes. However, no evidence for increased resistance to apoptosis in cytoplasmic survivin expressing keratinocytes could be demonstrated and the exact role played by survivin in the cytoplasm of keratinocytes remains to be defined.

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FIGURE LEGENDS

Figure 1: UV-B induces expression of survivin in the cytoplasm of basal epidermal cells.

A. Representative images of control mouse skins (0.0 J/cm^2) and mouse skins 24 hours post UV-B (0.3 J/cm^2) irradiation. The expression of survivin was visualized by immuno-fluorescence (red staining). Nuclei were stained in blue with the Hoechst 33342 dye. Filled arrowheads, nuclear survivin; open arrowhead, cytoplasmic survivin; D, dermis; arrow, non-specific staining of a hair shaft. **B**. Representative images of nuclear versus cytoplasmic survivin expression in epidermal cells 24 hours after exposure with the indicated UV-B doses. Insets show enlarged sections with the upper one depicting a cell with nuclear survivin and the lower one depicting a cell with cytoplasmic survivin. **C**. Skins of mice were treated as in panel A for the indicated time periods following UV-B (0.3 J/cm^2) irradiation. The percentage of epidermal cells expressing survivin in the nucleus or in the cytoplasm was then quantitated. Results correspond to the mean ± 95% confidence interval (CI) of 3 mice (6, 12, and 48 hour-time points) and 8 mice (0 and 24 hour-time points). The percentage of keratinocytes expressing cytoplasmic survivin increased significantly over time (conditions with different letters are statistically different), while the percentage of keratinocytes expressing nuclear survivin did not. **D**. Alternatively, the percentage of follicle cells expressing survivin in the nucleus or in the cytoplasm was quantitated and analyzed as in panel C. Results correspond to the mean ± 95% CI of 3 mice (the percentages of follicle cells expressing cytoplasmic survivin did not statistically differ over time after UV-B exposure). **E**. Mouse skin were exposed or not to UV-B (0.3 J/cm^2) and isolated 24 hours later. Survivin (red staining), nuclei (blue

staining), keratin 5 (green staining, left panel) and keratin 10 (green staining, right panel) were visualized on skin sections by immunofluorescence. Insets represent enlargement of the indicated areas. Open arrowheads, nuclear survivin; white-filled arrowheads, cytoplasmic survivin in basal layer keratinocytes; orange-filled arrowheads, cytoplasmic survivin in supra-basal layer keratinocytes. The graph represents the quantitation of cells expressing nuclear or cytoplasmic (Cyt.) survivin in the basal and supra-basal layers. The results correspond to the mean ± 95% CI of 4 mice. Scale bar for all images: 20 μM.

Figure 2: TUNEL and survivin labeling of UV-B-exposed mouse skin

Mice were treated as in Figure 1A. **A**. Skin sections were labeled with an anti-survivin antibody (red staining), with TUNEL (green staining), and with Hoechst 33342 (blue staining). An example of an apoptotic cell expressing cytoplasmic survivin is indicated with an arrow and enlarged in the inset. Scale bar: 20 μm. **B**. Quantitation of apoptotic keratinocytes 24 hours following UV-B (0.3 J/cm^2) exposure or not. The results correspond to the mean ± 95% CI of 7 mice. **C**. Quantitation of cells expressing cytoplasmic survivin or positive for TUNEL labeling. **D**. The graph on the left hand side only considers TUNEL-positive cells and whether these cells express or not survivin. The right-hand side graph only considers cytoplasmic survivin-positive cells and whether they are apoptotic or not. The results correspond to the mean ± 95% CI of 4 mice. *, statistically different.

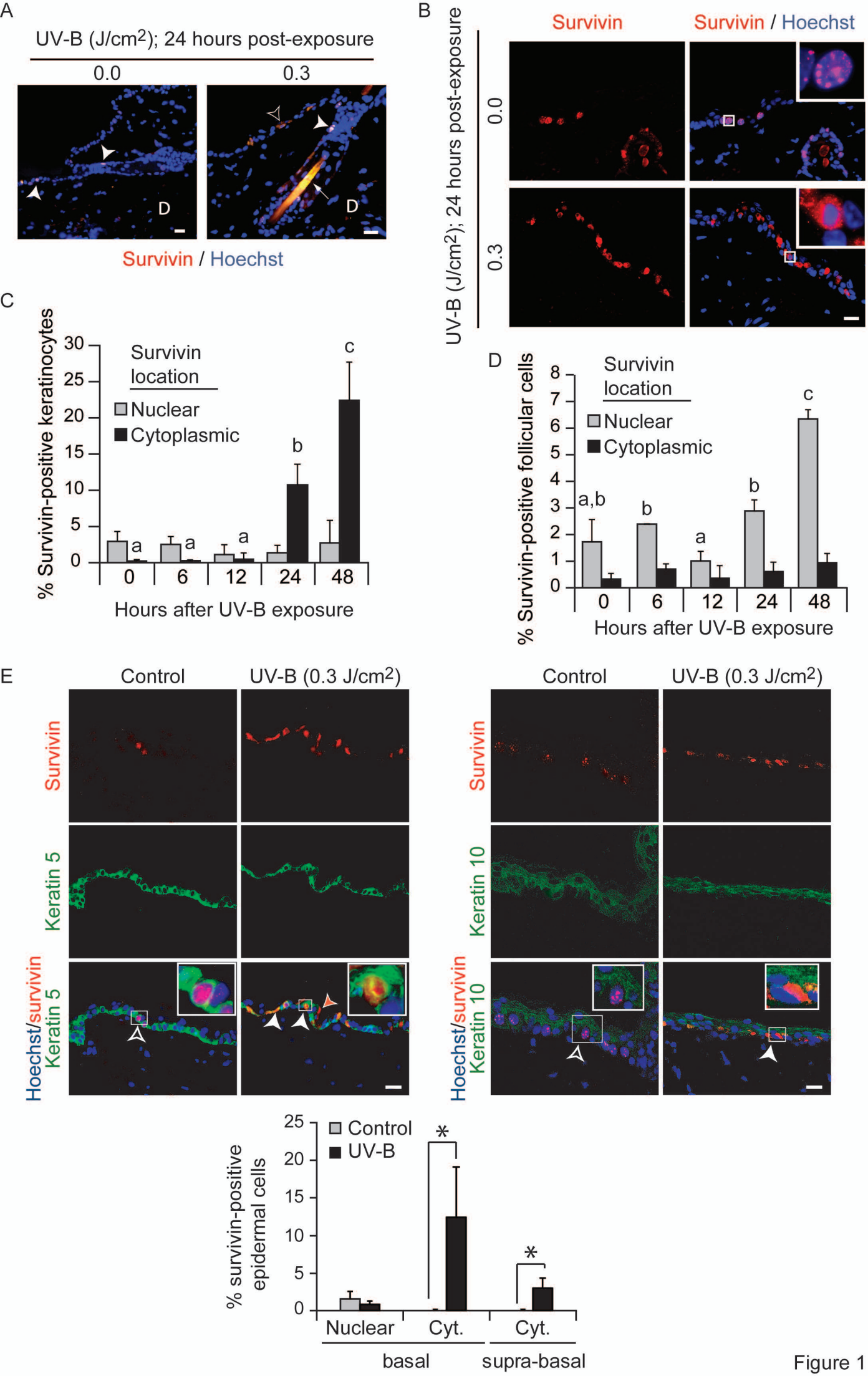
A
UV-B (J/cm2); 24 hours post-exposure
0.0
0.3
D
D
Survivin / Hoechst
B
Survivin
Survivin / Hoechst
UV-B (J/cm2); 24 hours post-exposure
0.0
0.3
C
% Survivin-positive keratinocytes
Survivin location
Nuclear
Cytoplasmic
a
a
a
b
c
0
6
12
24
48
Hours after UV-B exposure
D
% Survivin-positive follicular cells
Survivin location
Nuclear
Cytoplasmic
a,b
b
a
b
c
0
6
12
24
48
Hours after UV-B exposure
E
Control
UV-B (0.3 J/cm2)
Survivin
Keratin 5
Hoechst/survivin Keratin 5
Control
UV-B (0.3 J/cm2)
Survivin
Keratin 10
Hoechst/survivin Keratin 10
% survivin-positive epidermal cells
Control
UV-B
*
*
Nuclear
Cyt.
Cyt.
basal
supra-basal

Figure 1

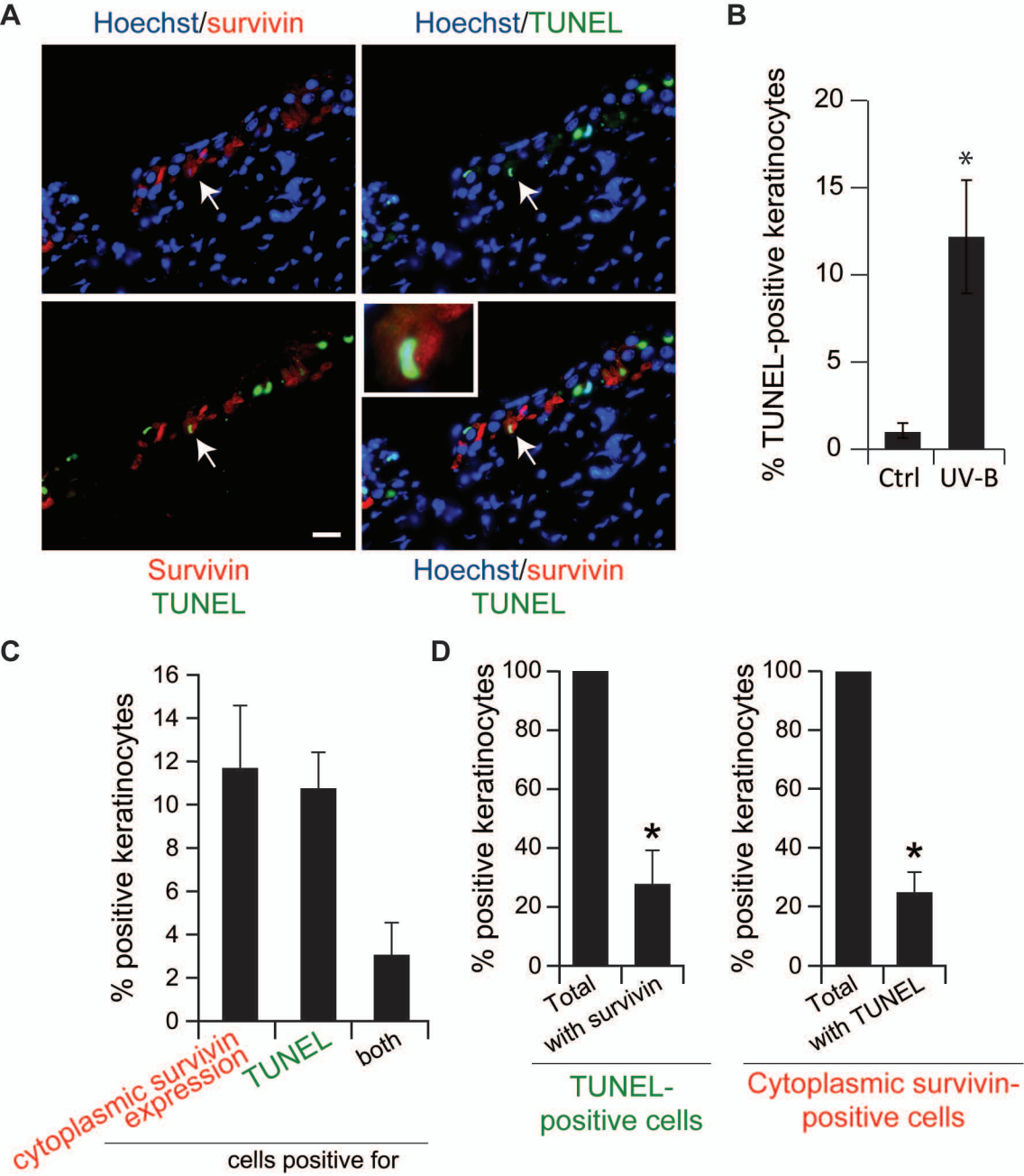
A
Hoechst/survivin
Hoechst/TUNEL
Survivin
TUNEL
Hoechst/survivin
TUNEL
B
% TUNEL-positive keratinocytes
20
15
10
5
0
*
Ctrl
UV-B
C
% positive keratinocytes
16
14
12
10
8
6
4
2
0
cytoplasmic survivin expression
TUNEL
both
cells positive for
D
% positive keratinocytes
100
80
60
40
20
0
*
Total
with survivin
TUNEL-positive cells
% positive keratinocytes
100
80
60
40
20
0
*
Total
with TUNEL
Cytoplasmic survivin-positive cells

Figure 2