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Local corticosterone activation by 11 β -hydroxysteroid dehydrogenase 1 in keratinocytes: the role in narrow-band UVB-induced dermatitis

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ABSTRACT

Keratinocytes are known to synthesize cortisol through activation of the enzyme 11 β -hydroxysteroid dehydrogenase 1 (11 β -HSD1). To confirm the function of 11 β -HSD1 in keratinocytes during inflammation *in vivo*, we created keratinocyte-specific-11 β -HSD1 knockout mice (*K5-Hsd11b1-KO* mice) and analyzed the response to narrow-band ultraviolet B (NB-UVB) irradiation. Firstly, we measured the mRNA and protein levels of 11 β -HSD1 following NB-UVB irradiation and found that the expression of 11 β -HSD1 in keratinocytes of mouse ear skin was enhanced at 3 and 24 hours after 250 mJ/cm², 500 mJ/cm², 1 J/cm², and 2 J/cm² NB-UVB irradiation. Next, we determined that 24 hours after exposure to 1 J/cm² NB-UVB irradiation, the numbers of F4/80-, CD45-, and Gr-1-positive cells were increased in *K5-Hsd11b1-KO* mice compared to wild type (WT) mice. Furthermore, the expression of the chemokine (C-X-C-motif) ligand 1 (CXCL1) and interleukin (IL)-6 was also significantly enhanced in NB-UVB-irradiated *K5-Hsd11b1-KO* mice compared with WT mice. In addition, activation of nuclear factor-kappa B (NF- κ B) after NB-UVB irradiation was enhanced in *K5-Hsd11b1-KO* mice compared to that in WT mice. Thus, NB-UVB-induced inflammation is augmented in *K5-Hsd11b1-KO* mice compared with WT mice. These results indicate that 11 β -HSD1 may suppress NB-UVB-induced inflammation via inhibition of NF- κ B activation.

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Introduction

Since the discovery of cortisone in the 1950s, glucocorticoid (GC) has been used as one of the most effective anti-inflammatory drugs for the treatment of inflammatory and autoimmune diseases and is also widely used as a topical applying for inflammatory dermatitis. In keratinocytes, GCs also affect proliferation, differentiation, and metabolism of cells in addition to exerting anti-inflammatory effects.¹

The anti-inflammatory effects of GCs are achieved at concentrations that are much higher than the physiological levels of GCs. At physiological doses, GCs have the potential to enhance or suppress the immune response depending on the concentration and duration of exposure.^{2,3} In keratinocytes, interleukin-1 β -induced interleukin-6 (IL-6) production is stimulated by low dose (10⁻¹⁰ M) cortisol but is suppressed with a high dose (10⁻⁵ M).⁴ The endogenous GCs in

humans and rodents are cortisol and corticosterone, respectively. Cortisol in skin cells was first demonstrated in human melanoma cells.⁵ Thereafter, cortisol in human melanocytes and fibroblasts was reported.^{6,7} Corticosterone was first reported in rodent skin.⁸ The stressors such as inflammation, viral infection, and trauma stimulate the hypothalamo-pituitary-adrenal (HPA) axis, which triggers the production of systemic cortisol. It is reported that HPA axis also exists in the skin and synthesizes cortisol.^{6,7,9-13} The skin is reported to have neuroendocrine capabilities that express elements of the HPA axis, key enzyme of corticosteroid glucocorticoids.^{14,15} In addition to cortisol production from the HPA axis, extra-adrenal cortisol production in various tissues, including colon, heart, and lung, has also been reported.¹⁶⁻²⁰

Skin cells are also known to synthesize cortisol²¹⁻²⁴ or corticosterone²⁵ through a *de novo* pathway

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involving an activating enzyme. The enzyme that catalyzes the intracellular conversion of hormonally-inactive cortisone into active cortisol is 11β -hydroxysteroid dehydrogenase 1 (11β -HSD1).⁴ Two iso-enzymes exist: 11β -HSD1 activates cortisol from cortisone, and 11β -HSD2 inactivates cortisol to cortisone.^{26,27} 11β -HSD1 is expressed in many tissues, with the highest levels found in the liver, lung, adipose tissue, ovaries, and central nervous system.²⁶ In the skin, 11β -HSD1 is expressed in epidermal keratinocytes, dermal fibroblasts, and root sheath cells of the outer hair follicle.²⁸⁻³¹

Our and other group previously reported a role for 11β -HSD1 in the skin and demonstrated that the expression of 11β -HSD1 increased with age in mouse skin and negatively regulated cutaneous wound healing by modulating proliferation of keratinocytes and fibroblasts.^{28,32,33} The expression of 11β -HSD1 was decreased in benign and malignant tumors of the skin, such as squamous cell carcinoma, basal cell carcinoma, and seborrheic keratosis, indicating the association of 11β -HSD1 with cell proliferation.³⁴ Skobowiat et al. reported that UVB and UVC, but not UVA irradiation, increased the expression of 11β -HSD1 in human keratinocytes. Conversely, irradiation with UVA, but not UVB and UVC, induced the expression of 11β -HSD2.³⁵ Alteration of cortisol by UV irradiation may alter the maintenance of homeostasis in keratinocytes.

In this study, we investigated the importance of 11β -HSD1 in keratinocytes during narrow-band (NB-UVB)-induced inflammation *in vivo* using keratinocyte-specific 11β -HSD1 knockout (*K5-Hsd11b1-KO*) mice. We previously tried to investigate the effects of 11β -HSD1 in skin using systemic 11β -HSD1 knockout mice; however, analysis of these mice was complicated, as these animals showed increased serum corticosterone levels probably due to some compensatory mechanism.^{32,36} Thus, in the current study, we generated *K5-Hsd11b1-KO* mouse to investigate the function of 11β -HSD1 in keratinocytes and to eliminate the increased serum corticosterone levels observed in systemic 11β -HSD1 knockout mice.

Materials and methods

Cell culture

The isolation and culture of mouse keratinocytes was carried out as follows. Full-thickness skin

harvested from 2-month-old mice was treated with 4 mg/ml of dispase (Gibco; Invitrogen, Paisley, UK) for 1 hour at 37°C. Next, the epidermis was peeled from the dermis, trypsinised to prepare single cells, and incubated in EpiLife Medium with 60 μ M of calcium (Invitrogen) in EpiLife Human Keratinocyte Growth Supplement (Invitrogen) Medium for 24 hours at 37°C under an atmosphere of 5% CO₂. Non-adherent cells were removed by 2 washes with phosphate-buffered saline (PBS), and then cells were cultured for 2 d in EpiLife Medium including Keratinocyte Growth Supplement. Non-adherent cells were again removed with 2 washes with PBS, and then cells were cultured for one day in EpiLife medium without bovine pituitary extract and hydrocortisone prior to use in the experiments.

Mice

Animal care was in strict accordance with the institutional guidelines of Osaka University. All of the animal experiments were carried out with the approval of the Animal Experiments Committee of Osaka University (#20-003-0).

Generation of *K5-Hsd11b1-KO* mice is that *Hsd11b1^{tm1a/+}* ES cells were purchased from the Knockout Mouse Project (KOMP) Repository (Davis, CA, USA). The ES cells were injected into blastocysts collected from superovulated BALB/c female mice. The treated blastocysts were then transferred into the uterus of pseudopregnant ICR female mice (SLC Japan) to obtain chimeric mice. Male chimeras were mated with female C57BL/6 mice, resulting in germline transmission of the *Hsd11b1 tm1a* allele. *Hsd11b1^{tm1a/tm1a}* mice were obtained by intercrossing *Hsd11b1^{tm1a/+}* mice. To create *Hsd11b1^{flox/flox}* mice, *Hsd11b1^{tm1a/tm1a}* mice were crossed with B6.Cg-Tg (ACTFLPe) 9205Dym/J purchased from The Jackson Laboratory (Bar Harbor, ME, USA) to remove poly (A) signal cassette. Generation of K5-Cre transgenic mice was previously described.³⁷ K5-Cre transgenic mice were bred with *Hsd11b1^{flox/flox}* mice to generate mice carrying the K5-Cre transgene and a floxed *Hsd11b1* allele (*K5^{Cre/0}; Hsd11b1^{flox/+}*). These mice were then mated with each other. Offsprings carrying *K5^{Cre/0}; Hsd11b1^{flox/flox}* and *K5^{Cre/0}; Hsd11b1^{+/+}* were used for further analyses as *K5-Hsd11b1-KO* and wild type (WT), respectively.

UVB irradiation

The source of UV irradiation was M-DER-320 (Tokodenki, Tokyo, Japan). The lamps of UV irradiation were NB-UVB fluorescent lamp (TL-20 W/ 01; Philips, Rosenthal, Holland). 85% of the UVB emission was at 311 ± 2 nm. The dose of UVB was quantified using a UV radiometer (Topcon Corporation, Tokyo, Japan), and murine skin was irradiated with a single dose of 40 mJ/cm^2 (*in vitro*) or 250 mJ/cm^2 , 500 mJ/cm^2 , 1 J/cm^2 , or 2 J/cm^2 (*in vivo*) NB-UVB.

Histopathological analysis

The samples were fixed in 10% formaldehyde for 24 hours, followed by embedding in paraffin and microtome sectioning. The sections ($4 \mu\text{m}$ thick) were then stained with haematoxylin and eosin (H&E). For immunohistochemical analysis, the sections were hydrated via passage through xylene and graded ethanol. Following antigen retrieval for 10 minutes at 110°C in citric buffer (pH 6.0), the slides were blocked with serum-free protein block (Dako-Cytomation, Carpinteria, CA, USA) for 10 minutes, then incubated with the primary antibody overnight at 4°C (rabbit anti-11 β -HSD1 antibody 1:100 dilution, Abcam, Cambridge, UK). After washing with Tris-buffered saline (TBS) containing 0.05% Triton-X100, the slides were mounted using the Vectastain ABC kit (Vector Laboratories, Burlingame, CA, USA) followed by counterstaining with haematoxylin. For immunofluorescence analysis, the sections were hydrated as described above and incubated with the following primary antibodies: rat anti-F4/80 antibody diluted at 1:100 (AbD Serotec, Oxford, UK), rat anti-CD45 antibody diluted at 1:100 (BD Biosciences, San Jose, CA, USA), rat anti-Gr-1 antibody diluted at 1:100 (eBioscience, San Diego, CA, USA), and rabbit anti-nuclear factor kappaB (NF- κ B) antibody diluted at 1:100 (Cell Signaling Technology, Beverly, MA, USA). Sections were then incubated with the secondary antibody (anti-rat Alexa Fluor 555 or anti-rabbit Alexa Fluor 555, Invitrogen). Rabbit IgG and Rat IgG (DAKO-Cytomation, Carpinteria, CA) were used as an isotype control, respectively.

Myeloperoxidase (MPO) activity assessment

The MPO activity in tissue lysates derived from the skin samples was determined using the MPO Assay

Kit (Bio Vision, Mountain View, CA, USA) according to the manufacturer's instructions. Briefly, samples of mouse skin were cut into small pieces with scissors and crushed by sonication. Samples were then subjected to centrifugation at $1,500 \times g$ for 15 minutes, and the supernatants of the mouse skin tissue lysates were collected and diluted 5-fold. The MPO activity of each sample was subsequently determined and normalized to its respective protein concentration.

Western blotting

The skin samples were crushed in liquid nitrogen and solubilized at 4°C in lysis buffer (0.5% sodium deoxycholate, 1% Nonidet P40, 0.1% sodium dodecyl sulfate, $100 \mu\text{g/ml}$ phenylmethylsulfonyl fluoride, 1 mM sodium orthovanadate, and protease inhibitor cocktail). Proteins ($30 \mu\text{g}$) were separated on SDS-polyacrylamide gels and transferred onto polyvinylidene fluoride membranes (Bio-Rad, Hercules, CA, USA). Non-specific protein binding was blocked by incubating the membranes in 5% w/v non-fat milk powder in TBS-T (50 mM Tris-HCl [pH 7.6], 150 mM NaCl, and 0.1% v/v Tween-20), and then the membranes were incubated with rabbit anti-phospho-NF- κ B p65 antibodies or rabbit anti-NF- κ B p65 antibodies (Cell Signaling Technology) diluted 1:1,000 overnight at 4°C or with mouse anti- β -actin antibody (Sigma-Aldrich, St. Louis, MO, USA) diluted 1:10,000 for 30 minutes at room temperature. The membranes were then washed 3 times in TBS-T for 5 minutes each and finally incubated with either HRP-conjugated anti-mouse or anti-rabbit antibodies at a dilution of 1:10,000 for 60 minutes at room temperature. Protein bands were detected using the ECL Plus kit (GE Healthcare, Buckinghamshire, UK), and the intensity of the bands was quantified using the NIH Image J software program.

RNA isolation and quantitative real-time polymerase chain reaction (RT-PCR)

Total RNA was isolated from the cells using the SV Total RNA Isolation System (Promega, Madison, WI, USA). The product was reverse transcribed into first-strand cDNA (cDNA), and the expression levels of Hsd11b1, chemokine (C-X-C-motif) ligand1 (CXCL1), and IL-6 were measured using the Power SYBR Green PCR Master Mix (Applied Biosystems, Foster City, CA, USA), according to the manufacturer's protocol.

Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used to normalize the mRNA, and sequence-specific primers were designed as follows: Hsd11b1, sense 5'-aaaattacctctcccgatcct-3', antisense 5'-ggcagcgagacac-taccttc-3'; CXCL1, sense 5'-gcctatcgccaatgagctg-3', antisense 5'-tctccgttacttggggacac-3'; IL-6, sense 5'-tgggaaa tcgtggaaatgaga-3', antisense 5'-aagtgcatacatcgttggcagcag-3'; and GAPDH, sense 5'-tgatcactactggcaggttct-3', antisense 5'-catggcctccgtgttccta-3'. RT-PCR (40 cycles of denaturation at 92°C for 15 seconds followed by annealing at 60°C for 60 seconds) was run on an ABI 7000 Prism system (Applied Biosystems).

Enzyme-linked immunosorbent assay (ELISA)

ELISA was performed on the tissue lysates derived from mouse skin samples using Quantikine Immunoassay (R&D Systems, Minneapolis, MN, USA). The assay was performed according to the manufacturer's instructions. Samples of mouse skin were cut into small pieces with scissors and then crushed by sonication. Following centrifugation at 1,500× g for 15 minutes, the supernatants of the mouse skin tissue lysates were collected. The concentrations of CXCL1 and IL-6 were normalized to the respective protein concentration.

Statistical analysis

The data are expressed as the mean ± standard deviation (SD). The Student's *t*-test was used to determine the level of significance of the differences between the 2 groups. An analysis of variance (ANOVA) was performed followed by the Bonferroni-Dunn test for multiple comparisons to allow for pairwise testing of significant differences between the groups. Statistical significance was defined with a *P*-value of <0.05.

Results

Generation of K5-Hsd11b1-KO mice

To investigate the function of 11β-HSD1 in keratinocytes, we generated *K5-Hsd11b1-KO* mice by breeding K5-Cre transgenic mice with *Hsd11b1^{lox/flox}*. The expression of 11β-HSD1 was significantly decreased in epidermis of *K5-Hsd11b1-KO* mice (Fig. 1A). The mRNA expression level of 11β-HSD1 was also decreased in keratinocytes derived from *K5-Hsd11b1-KO* mice (Fig. 1B).

NB-UVB irradiation increased the expression of 11β-HSD1 in mouse skin

To examine the dynamics of 11β-HSD1 expression following NB-UVB irradiation, we first irradiated mouse ear skin with various doses of NB-UVB. The expression of 11β-HSD1 in epidermis increased following exposure to 250 mJ/cm², 500 mJ/cm², 1 J/cm², and 2 J/cm² NB-UVB irradiation (Fig. 1C). The mRNA expression of 11β-HSD1 also increased in a dose-dependent manner 24 hours after NB-UVB irradiation, and peak mRNA levels were observed following 1 J/cm² NB-UVB irradiation (Fig. 1D).

Thus, we irradiated mouse ear skin with 1 J/cm² of NB-UVB in subsequent experiments. The protein expression of 11β-HSD1 began to increase 3 hours after NB-UVB irradiation and continued to increase until 24 hours after NB-UVB irradiation (Fig. 1E). The mRNA expression of 11β-HSD1 also increased in skin irradiated with NB-UVB (Fig. 1F). In addition, increased expression of 11β-HSD1 was observed in NB-UVB-irradiated keratinocytes derived from ear and tail *in vitro* (Fig. 1G).

Inflammatory cell infiltration after NB-UVB irradiation was augmented in K5-Hsd11b1-KO mouse skin

Following NB-UVB irradiation of WT and *K5-Hsd11b1-KO* mice, the function of increased 11β-HSD1 in keratinocytes was evaluated. NB-UVB irradiation significantly increased the number of infiltrated inflammatory cells in the dermis (Fig. 2A). Specifically, the numbers of F4/80-, CD45-, and Gr-1-positive cells were significantly higher in irradiated *K5-Hsd11b1-KO* mice than in irradiated WT mice (Fig. 2A-D). Accordingly, myeloperoxidase (MPO) activity was significantly increased in the NB-UVB-irradiated *K5-Hsd11b1-KO* mice compared to that in the irradiated WT mice (Fig. 2E).

NB-UVB-induced pro-inflammatory cytokines were augmented in the K5-Hsd11b1-KO mice

Next, we investigated the expression of pro-inflammatory cytokines in WT and *K5-Hsd11b1-KO* mice following NB-UVB irradiation. The mRNA and protein levels of CXCL1 and IL-6 were significantly higher in NB-UVB-irradiated *K5-Hsd11b1-KO* mouse skin than in irradiated WT mouse skin (Fig. 3A-D).

Furthermore, we examined the effect of various doses of cortisol on the inflammatory response of keratinocytes in culture. CXCL1 mRNA levels were significantly decreased in keratinocytes derived from WT mice exposed to higher doses of cortisol (1×10^{-7} M and 1×10^{-5} M) compared with WT keratinocytes cultured in cortisol-free media (Fig. 3E). Similarly, CXCL1 concentrations in culture media tended to

decrease following exposure to higher doses of cortisol (Fig. 3F).

NB-UVB-induced NF- κ B activation was augmented in the K5-Hsd11b1-KO mice

Subsequently, the activation of NF- κ B by NB-UVB irradiation was evaluated in *K5-Hsd11b1-KO* mice.

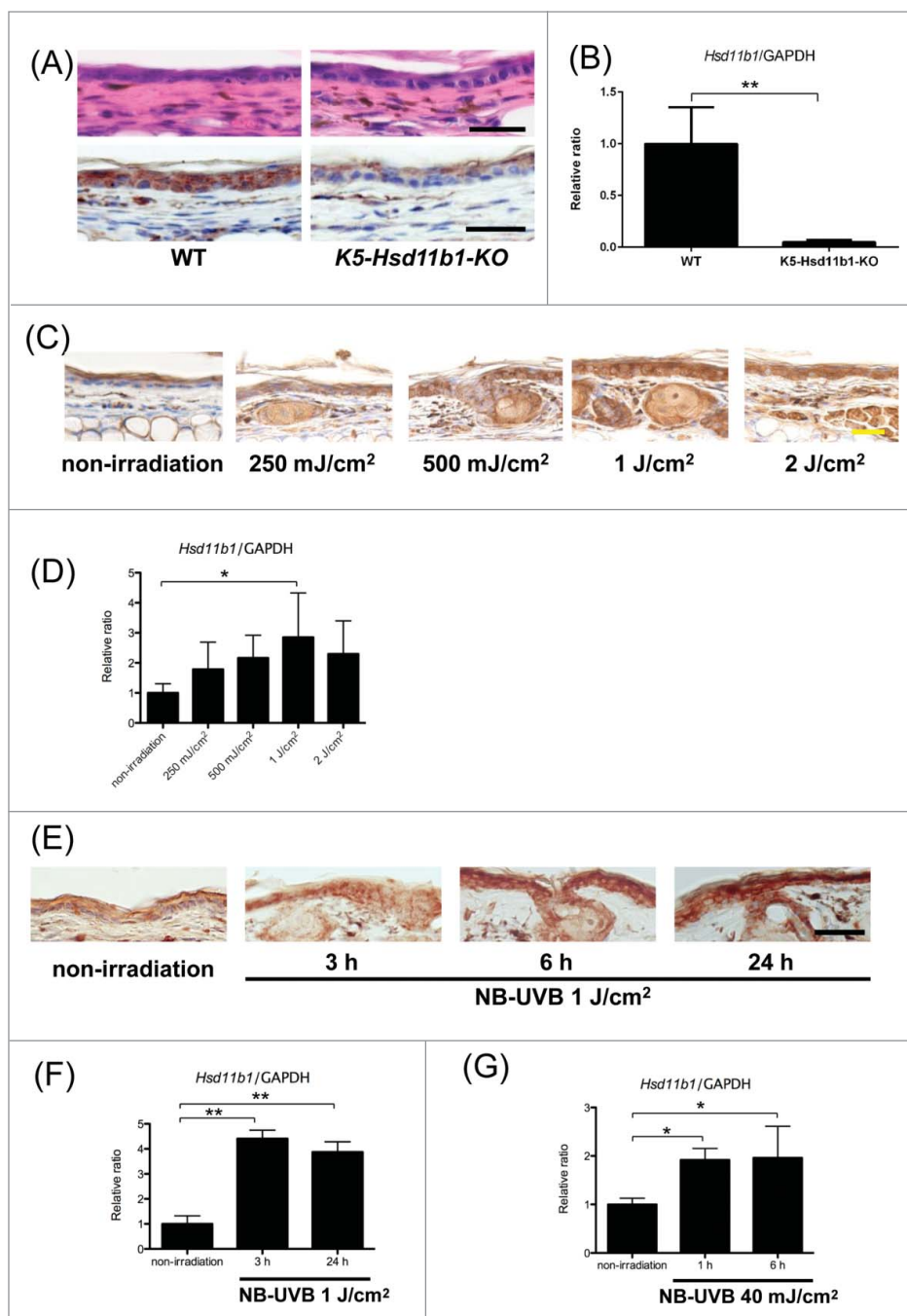


Figure 1. (For figure legend, see page 6.)

Immunohistochemical analysis revealed nuclear translocation of NF- κ B 3 hours after NB-UVB irradiation of keratinocytes. The number of keratinocytes with activated NF- κ B was higher in the irradiated *K5-Hsd11b1-KO* mouse skin than in the irradiated WT mouse skin (Fig. 4A). In addition, phosphorylation of NF- κ B was enhanced one hour after NB-UVB irradiation in the irradiated *K5-Hsd11b1-KO* mouse skin as evaluated by western blotting, and the amount of phosphorylated NF- κ B was significantly higher in *K5-Hsd11b1-KO* mouse skin extract than in WT mouse skin extract (Fig. 4B).

Discussion

In this study, we analyzed the inflammatory response to NB-UVB irradiation in *K5-Hsd11b1-KO* mice. The response to NB-UVB irradiation was enhanced in *K5-Hsd11b1-KO* mice compared with WT mice possibly through enhanced NF- κ B activation. Therefore, these results suggest that local cortisol activation in keratinocytes through 11 β -HSD1 is important for regulating NB-UVB-induced inflammation.

In WT mouse-derived keratinocytes *in vitro*, a higher dose of cortisol suppressed the immune response. We previously reported that 1×10^{-5} M cortisol attenuated IL-1 β -induced IL-6 production in normal human epidermal keratinocytes.⁴ Additionally, 10^{-7} M and 10^{-5} M concentrations of corticosterone have been shown to suppress the expression of pro-inflammatory cytokines in mouse peritoneal macrophages,³⁸ and these results are consistent with our findings in mouse-derived keratinocytes.

Association of 11 β -HSD1 in various inflammatory diseases such as inflammatory bowel diseases, and rheumatoid arthritis has been reported.^{20,39-42} In Crohn's disease and ulcerative colitis in humans and in dextran sodium sulfate-induced colitis in rats, expression of 11 β -HSD1 is upregulated whereas, the expression of 11 β -HSD2 was downregulated, indicating GC activation is strongly increased in inflammatory bowel diseases.^{40,41} Local GC activation in synovial fluids in patients with rheumatoid arthritis has also been reported, and systemic 11 β -HSD1 activity was found to be higher in untreated rheumatoid arthritis patients.³⁹ These results raise the possibilities that local GC activation was increased in these inflammatory diseases to either attenuate or to augment the local inflammation. A study using 11 β -HSD1-deficient mice in a murine model of arthritis and peritonitis may provide an answer to the question of whether GC activation attenuates or augments local inflammation.⁴³ In this study, the 11 β -HSD1-deficient mice but not the 11 β -HSD2-deficient mice exhibited earlier onset and slower resolution of inflammation than the WT mice in the K/BxN serum transfer model of arthritis, suggesting that increased 11 β -HSD1 activity is involved in resolving inflammation. Similarly, in thioglycollate-induced sterile peritonitis, 11 β -HSD1-deficient mice had more inflammatory cells in the peritoneum than 11 β -HSD2-deficient mice.⁴³ The results obtained in these arthritis and peritonitis models are in agreement with our finding that the response to NB-UVB irradiation was enhanced in *K5-Hsd11b1-KO* mice. In addition, our results demonstrated the importance of local corticosterone activation in

Figure 1 (see previous page). Generation of *K5-Hsd11b1-KO* mice. The expression of 11 β -HSD1 in the skin was enhanced following NB-UVB irradiation in WT mice. (A) Representative H&E staining (upper panel) and 11 β -HSD1 staining (lower panel) of 2-month-old WT and *K5-Hsd11b1-KO* mouse skin. Bar = 50 μ m. (B) The relative expression levels of 11 β -HSD1 in keratinocytes derived from WT and *K5-Hsd11b1-KO* mice were assessed using RT-PCR. GAPDH was used as an internal control. The bars indicate the mean \pm SD (N = 5; *P < 0.05, Student's *t*-test). (C) Expression of 11 β -HSD1 in 2-month-old WT mice irradiated with 250 mJ/cm², 500 mJ/cm², 1 J/cm², and 2 J/cm² NB-UVB. Skin specimens were harvested 24 hours after NB-UVB irradiation. Rabbit IgG was used as isotype control. Bar = 20 μ m (N = 6 per group). (D) The relative expression levels of 11 β -HSD1 in WT mice irradiated with 250 mJ/cm², 500 mJ/cm², 1 J/cm², and 2 J/cm² NB-UVB were assessed using RT-PCR. Skin samples were collected 24 hours after NB-UVB irradiation. GAPDH was used as an internal control. The bars indicate the mean \pm SD (N = 6; **P < 0.01, one-way ANOVA followed by the Bonferroni-Dunn test for multiple comparisons). (E) Expression of 11 β -HSD1 in 2-month-old WT mice irradiated with 1 J/cm² of NB-UVB. Skin specimens were harvested 3, 6, and 24 hours after NB-UVB irradiation. Rabbit IgG was used as isotype control. Bar = 50 μ m (N = 6 per group). (F) The relative expression levels of 11 β -HSD1 in WT mice irradiated with 1 J/cm² NB-UVB were assessed using RT-PCR. Skin samples were collected at the indicated time points for unirradiated mice and 3 and 24 hours after NB-UVB irradiation. GAPDH was used as an internal control. The bars indicate the mean \pm SD (N = 6; **P < 0.01, one-way ANOVA followed by the Bonferroni-Dunn test for multiple comparisons). (G) The relative expression levels of 11 β -HSD1 in WT mouse-derived keratinocytes after NB-UVB irradiation with 40 mJ/cm² NB-UVB were assessed using RT-PCR. Keratinocytes were collected at the indicated time points for unirradiated mice and 1 and 6 hours after NB-UVB irradiation. GAPDH was used as an internal control. The bars indicate the mean \pm SD (N = 6; *P < 0.05, one-way ANOVA followed by the Bonferroni-Dunn test for multiple comparisons).

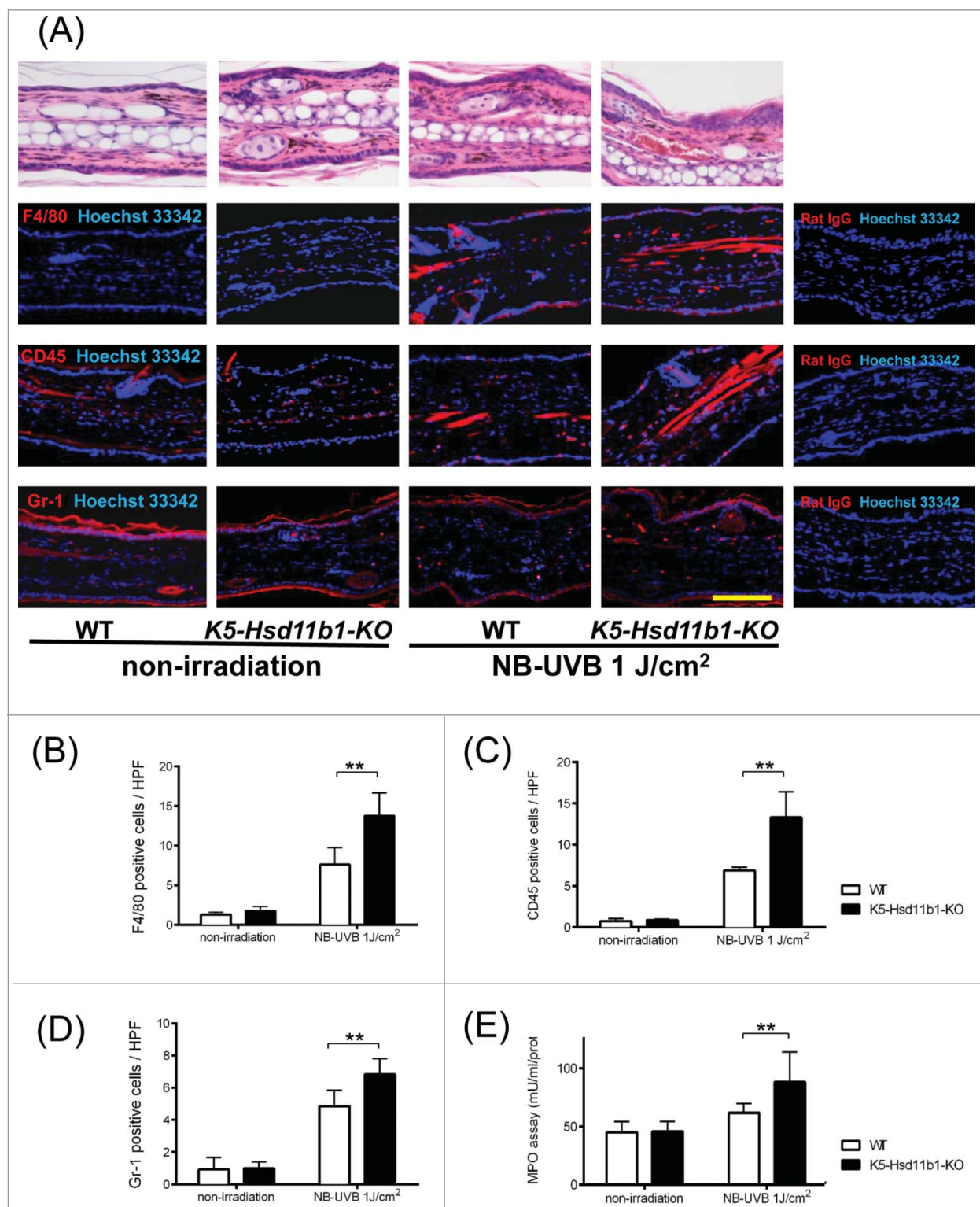


Figure 2. NB-UVB-induced infiltration of inflammatory cells was increased in the *K5-Hsd11b1-KO* mouse skin. (A) H&E staining (upper panel) and immunofluorescence staining of F4/80, CD45, and Gr-1 (red) and nuclei (Hoechst 33342, blue) (lower panel) in the 2-month-old WT and *K5-Hsd11b1-KO* mouse skin 24 hours after NB-UVB irradiation at 1 J/cm² (N = 8 per group). Rat IgG was used as isotype control. Bar = 100 μ m. (B-D) Numbers of F4/80- (B), CD45- (C), Gr-1-positive (D) cells per each high power field in the 2-month-old WT (white bar) and *K5-Hsd11b1-KO* (black bar) mouse skin after NB-UVB irradiation at 1 J/cm². Three sections from each mouse were evaluated. The bars indicate the mean \pm SD (N = 8; **P < 0.01, two-way ANOVA followed by the Bonferroni-Dunn test for multiple comparisons). (E) Myeloperoxidase (MPO) activity in skin from 2-month-old mice 24 hours after NB-UVB irradiation at 1 J/cm². The bars indicate the mean \pm SD (N = 6; **P < 0.01, 2-way ANOVA followed by the Bonferroni-Dunn test for multiple comparisons).

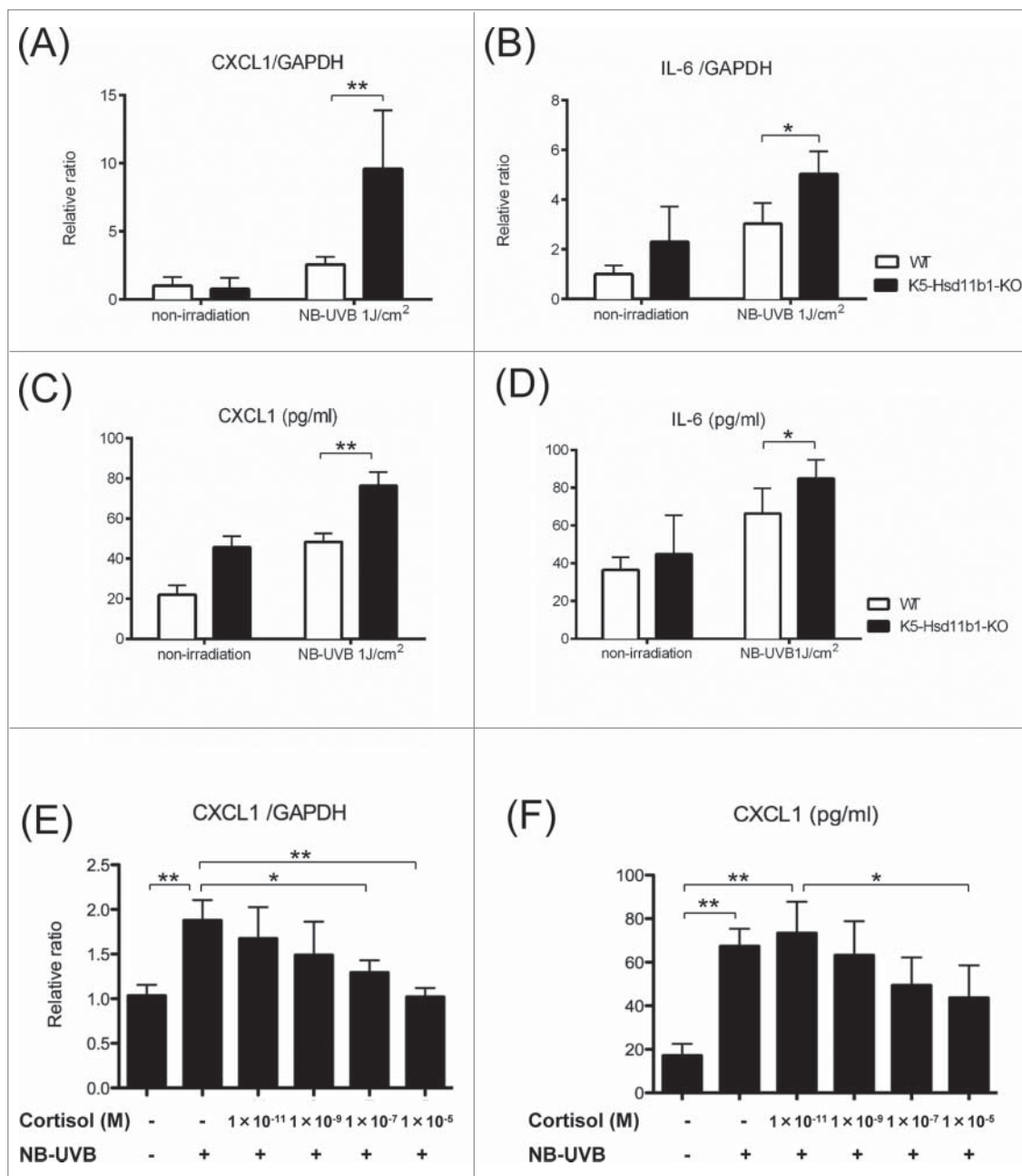


Figure 3. Inflammatory response to NB-UVB irradiation was enhanced in the *K5-Hsd11b1-KO* mouse skin. (A, B) Relative expression levels of CXCL1 (A) and IL-6 (B) in skin from 2-month-old mice 3 hours after NB-UVB irradiation at 1 J/cm². GAPDH was used as an internal control. The bars indicate the mean ± SD (N = 6; **P < 0.01, *P < 0.05, two-way ANOVA followed by the Bonferroni-Dunn test for multiple comparisons). (C, D) The levels of CXCL1 (C) and IL-6 (D) in the skin of 2-month-old WT mice 10 hours after NB-UVB irradiation at 1 J/cm² were measured using ELISA. The bars indicate the mean ± SD (N = 6; **P < 0.01, *P < 0.05, two-way ANOVA followed by the Bonferroni-Dunn test for multiple comparisons). (E) The indicated dose of cortisol was added to the cultured media of the WT mice-derived keratinocytes for 24 hours, and then cells were treated with or without 40 mJ/cm² NB-UVB irradiation. Cells were harvested after 1 hour, and CXCL1 expression was investigated by rtPCR. GAPDH was used as an internal control. The bars indicate the mean ± SD (N = 6; **P < 0.01, *P < 0.05, one-way ANOVA followed by the Bonferroni-Dunn test for multiple comparisons). (F) The indicated dose of cortisol was added to the cultured media of WT mice-derived keratinocytes for 24 hours, and then cells were treated with or without 40 mJ/cm² NB-UVB irradiation. Culture media was collected 10 h later, and concentrations of CXCL1 were measured by ELISA. The bars indicate the mean ± SD (N = 6; **P < 0.01, *P < 0.05, one-way ANOVA followed by the Bonferroni-Dunn test for multiple comparisons).

keratinocytes during skin inflammation in *K5-Hsd11b1-KO* mice. As keratinocytes are located on the superficial surface of the skin and are exposed daily to

various mechanical and chemical agents, this local cortisol activation mechanism may play a pivotal role to combat daily skin inflammation.

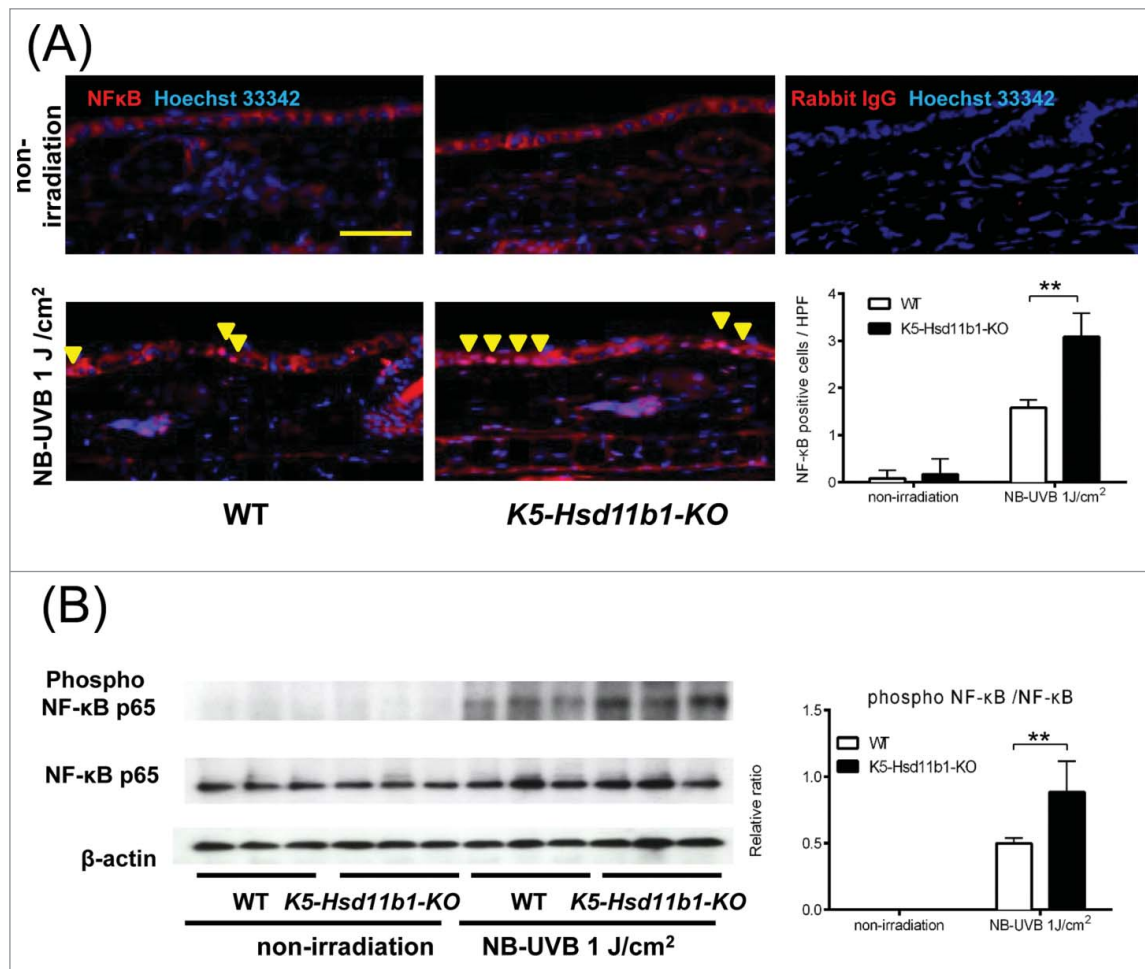


Figure 4. NB-UVB-induced activation of NF- κ B in the *K5-Hsd11b1-KO* mouse skin was enhanced. (A) Immunofluorescence staining of NF- κ B p65 (red) and nuclei (Hoechst 33342, blue) in skin of 2-month-old WT and *K5-Hsd11b1-KO* mice 3 hours after NB-UVB irradiation at 1 J/cm². Rabbit IgG was used as isotype control. Bar = 100 μ m. Three sections from each mouse were evaluated. The bars indicate the number of NF- κ B p-65-positive cells translocated into the nucleus after NB-UVB irradiation (mean \pm SD; N = 6; ***P* < 0.01, two-way ANOVA followed by the Bonferroni-Dunn test for multiple comparisons). (B) Western blot analysis of the phospho-NF- κ B p65 expression in skin from 2-month-old WT and *K5-Hsd11b1-KO* mice 1 hour after NB-UVB irradiation at 1 J/cm². The intensity of the bands was quantified using the NIH Image J software program. The phospho-NF- κ B p65/NF- κ B p65 was calculated. The bars indicate the mean \pm SD (N = 3; **P* < 0.05, 2-way ANOVA followed by the Bonferroni-Dunn test for multiple comparisons).

Recently, a mouse model of 11 β -HSD1 deficiency in liver and adipose tissue has been reported.^{44,45} Adipose-specific-11 β -HSD1-deficient mice were protected from hepatic steatosis and circulating fatty acid excess induced by excess corticosterone⁴⁴; however, whether an increased circulating GC excess occurred in liver-specific 11 β -HSD1-deficient mice was unclear from 2 studies.^{44,45} One study demonstrated that cortisone and 11-DHC-induced hepatic steatosis were not different between liver-specific-11 β -HSD1-deficient mice and WT mice,⁴⁴ whereas another study showed that liver-specific 11 β -HSD1-deficient mice were protected from 11-DHC-induced metabolic effects.⁴⁵ This difference may stem from the difference

in the dose of 11-DHC given to mice, as the anti-inflammatory effect of GCs depends on its dose.

We lastly investigated the suppression of NB-UVB-induced inflammatory response in keratinocytes by 11 β -HSD1. We focused on NF- κ B, as this transcription factor regulates a variety of genes encoding proinflammatory cytokines. UVB irradiation phosphorylated NF- κ B, which translocated from the cytosol to the nucleus. GCs are known to suppress the expression of inflammatory gene transcription factors, such as NF- κ B.^{46,47} In our study, the activation of NF- κ B after NB-UVB irradiation was enhanced in the *K5-Hsd11b1-KO* mice. Furthermore, previous research has also demonstrated that 11 β -HSD1 regulates

NF- κ B activation by modulating the coordinated action of GC receptors and mineralocorticoid receptor.⁴⁸ Therefore, we conclude that corticosterone activation by 11 β -HSD1 in keratinocytes represses local inflammation induced by NB-UVB via suppression of NF- κ B activation. In summary, we demonstrated that NB-UVB-induced inflammation was augmented in *K5-Hsd11b1-KO* mice compared with WT mice. Activation of corticosterone in keratinocytes is considered important for the suppression of local inflammation via the suppression of NF- κ B activation. These findings indicate the importance of corticosterone production in keratinocytes as a local first-line defense to protect the skin from external stimuli.

Abbreviations

11 β -HSD1	11 β -hydroxysteroid dehydrogenase 1
cDNA	complementary DNA
CXCL1	chemokine (C-X-C-motif) ligand1
ELISA	enzyme-linked immunosorbent assay
GAPDH	glyceraldehyde-3-phosphate dehydrogenase
GC	glucocorticoid
HPA	hypothalamo-pituitary-adrenal
IL-6	interleukin-6
<i>K5-Hsd11b1-KO</i>	keratinocyte-specific 11 β -HSD1 knockout
MPO	myeloperoxidase
NB-UVB	narrow-band UVB
NF- κ B	nuclear factor-kappa B
PBS	phosphate-buffered saline
SD	standard deviation
TBS	Tris-buffered saline
WT	wildtype

Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

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