INTRODUCTION

Atopic dermatitis (AD) and psoriasis (PSO) are common chronic inflammatory skin disorders. The data from the WHO Global Burden of Diseases initiative indicate that at least 230 and 125 million people worldwide have AD and PSO (lifetime prevalences of 10%-15% and 2%-3%, respectively), with AD being the leading cause of the non-fatal disease burden conferred by skin conditions on a global level.[1,2] Both diseases result from the complex interaction of genetic and environmental factors leading to epidermal dysfunction as well as cutaneous inflammation, which is driven by excessive T-cell activation of, however, differing polarity.[3,4] While PSO is largely driven by type 17 responses,[5] AD has a strong Th2 and Th22 component, but appears to be more heterogeneous with the involvement of multiple immune pathways, potentially with different disease features.[6] Further, in both diseases, prolonged presence of oxidative stress and a redox imbalance are postulated to promote inflammatory processes.[7] Oxidative stress not only enhances inflammation through upregulation of inflammatory genes, but can also damage cellular structures of the skin and weaken the skin barrier function.[8]

Redox imbalance in the skin can arise from excessive formation of reactive oxygen species (ROS) through pro-inflammatory stimuli such as pro-inflammatory cytokines, H₂O₂ or UV irradiation. Oxidative stress can lead to lipid peroxidation, protein oxidation and DNA damage, thus inducing several physiological dysfunctions.[9-11] Cells have therefore developed various antioxidative mechanisms. Antioxidative responses include activation of signalling pathways that depend on the strength of and damage induced by the stress

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to initialize either pro-survival gene expression programmes for continuous ROS detoxification (eg Nrf2, pHsp27, pERK) and DNA damage repair (eg ATM and p53) or cell-death–inducing programs (eg NF-κB, p53, p38). However, ROS can also lead to the activation of NADPH oxidase (NOX), which themselves generate ROS and therefore reinforce the initial ROS production. The family of NOX comprises of several members (NOX1, NOX2, NOX3, NOX4, NOX5, DUOX1, DUOX2). NOX catalyses the production of superoxide through reduction in NADPH. Via the generation of ROS, NOX mediates diverse functions such as host defense and inflammation, posttranslational processing of proteins, cellular signalling and regulation of gene expression. However, NOX also contributes to a wide range of diseases as a deficiency can lead to immunosuppression and increased activity will lead to excess ROS which is associated with cardiovascular changes, neurodegeneration and inflammatory skin diseases.

More evidence for an association between oxidative stress and AD and PSO has been provided by studies which have analysed oxidative stress markers and which have shown that in both AD and PSO malondialdehyde levels are increased, and antioxidant enzymes such as superoxide dismutase, catalase and GSH peroxidase are decreased.

Although extensive evidence exists suggesting an important role of oxidative stress in the pathology of inflammatory skin diseases, no comprehensive study into the molecular mechanisms has been conducted and data on the oxidative state of the skin remain sparse. Furthermore, the mechanism by which oxidative stress might influence pathophysiology has not been discovered yet. The association of oxidative stress with both AD and PSO leads to the question whether ROS play a role in the pathophysiology of both diseases and if yes, whether there are disease-specific differences in redox regulation.

2 | MATERIALS AND METHODS

2.1 | Cell culture

Normal human epidermal keratinocytes (NHEKs) (PromoCell, Lot number 407Z001) were cultured in Keratinocyte Growth Medium (KGM) + supplements + CaCl₂ + penicillin/streptomycin at 37°C and 5% CO₂. Cells were used at passages 4-10.

2.2 | Induction of a 2-D PSO and AD cell culture model

2D models of AD and PSO were induced by adding respective cytokine mixes to the culture dishes for 24 hours prior to experiments. We used neonatal human keratinocytes and adapted a protocol to stimulate with either an AD cytokine or a PSO cytokine mix as described previously. For PSO induction, 40 ng/mL IL-22, 40 ng/mL IL-17a and 10 ng/mL TNF-α were added. For AD induction, 40 ng/mL IL-22, 40 ng/mL IL-4, 40 ng/mL IL-13 and 10 ng/mL TNF-α were added.

2.3 | Inhibition of NADPH oxidases

In experiments where diphenyleneiodonium (DPI) was used, DPI was added at a final concentration of 10 µmol/L for 24 hours (Santa Cruz sc-202584) prior to H₂O₂ treatment. In experiments where NOX1 inhibitor (Nox1i) was used, ML171 was added at a final concentration of 10 µmol/L for 24 hours (ML171-CAS 6631-94-3-Calbiochem) prior to H₂O₂ treatment, and in experiments where NOX1/4 inhibitor (Nox1/4i) was used, GKT136901 was added at a final concentration of 10 µmol/L for 24 hours (GKT136901-CAS 955272-06-7-Calbiochem) prior to H₂O₂ treatment. All inhibitors were dissolved in DMSO. Control cells were treated accordingly with 10 µmol/L DMSO.

2.4 | Induction of oxidative stress

To induce oxidative stress, cells were treated with hydrogen peroxide (H₂O₂) (Merck, 31642). For H₂O₂ treatment, media were replaced with freshly prepared media containing 250 µmol/L H₂O₂. In control cells, the media were switched to fresh media without H₂O₂. For DCF assays, H₂O₂ was added for 10 minutes, and for immunofluorescence and Western blotting, H₂O₂ was added for 1 hour.

2.5 | DCFDA assay

Intracellular ROS levels were measured using the DCFDA assay (Abcam, AB113851) according to the manufacturer’s instructions. Briefly, cells were seeded in a 96-well plate until ~80% confluency. Cells were incubated with 10 µmol/L 2’,7’-dichlorofluorescein (DCF) and then washed twice. Oxidative stress was induced by treatment of cells with 250 µmol/L H₂O₂. Cells were washed twice with PBS, and ROS levels were quantified by measuring fluorescence at excitation/emission of 495 nm/520 nm.

2.6 | Western blotting

For Western blot analysis, cells were lysed in RIPA buffer (50 mmol/L Tris pH 8.0, 150 mmol/L NaCl, 1% Triton X-100, 0.5% natriumdeoxycholate, 0.1% SDS) plus protease and phosphatase inhibitors (Roche, 04906845001 and 04693124001, respectively). The determination of the protein concentration was performed by using the Pierce 600 nm Assay and the Tecan Reader according to the manufacturer’s instructions. Western blotting was performed using WES™ according to the manufacturer’s instructions. Antibodies used were as follows: Actin (CST #8457), ERK (CST #9102), pERK (CST #9101), p38 (CST #9212), pP38 (CST #9216), NOX1 (Santa Cruz sc-518023), NOX4 (Abcam 133303), pHsp27 (CST #2406) and p22phox (Santa Cruz sc-518023).
Cruz sc-271968). All primary antibodies were used at a concentration of 1:30. WES-supplied secondary antibodies were used according to the manufacturer’s instruction at a final concentration of 1:1.

2.7 Immunofluorescence

Cells grown on coverslips were fixed with 3.7% paraformaldehyde solution (PFA) for 30 minutes and washed three times with PBS. To permeabilize the cells, a 0.1% Triton X-100 in PBS solution was added for 15 minutes and followed by 30 minutes of blocking with blocking solution (1% BSA in combination with 10% goat serum in PBS). The incubation with primary antibodies (pH2AX, CST #2577; NOX1 Santa Cruz sc-518023, NOX4 Abcam 133303, p22phox Santa Cruz sc-271) was at room temperature for 1 hour in blocking buffer. All primary antibodies were used at a 1:200 final concentration. Cells were washed twice with PBS and incubated with the secondary antibodies (Alexa Fluor anti-rabbit 488, Thermo Fischer) at a 1:400 concentration for 90 minutes at room temperature in blocking buffer. Secondary antibodies were used at a final concentration of 1:400. After a final washing step with PBS, mounting medium with DAPI was added to the cells. Images were captured using a Zeiss microscope (serial number: 551 095) using a 40× or 63× objective. For each biological replicate, at least three images were taken and a total of at least 30 cells each per condition were analysed.

For quantification of pH2AX, cell nuclei were manually selected via the DAPI staining in ImageJ to create a mask. The mask was then used to measure fluorescence intensity of both the DAPI and the 488 channels. pH2AX intensity was normalized to the DAPI signal for each image. For fluorescence quantification of NOX1, NOX4 and p22phox, the fluorescence intensity of the total 488 channel signal per image normalized to a blank ROI was measured and then normalized to the total DAPI channel signal normalized to a blank ROI.

3 RESULTS

3.1 Treatment of a 2D atopic dermatitis and psoriasis skin model with pro-inflammatory cytokines leads to increased oxidative stress

In order to determine the role of oxidative stress in atopic dermatitis (AD) and psoriasis (PSO), we established an adapted 2D in vitro model of AD and PSO keratinocytes. AD, PSO and unstimulated control (Ctrl) keratinocytes were treated with a disease-specific cytokine mix for 24 hours to stimulate the Th1 or Th2 environment, respectively. Treatment with the cytokine mixes had no impact on proliferation or viability (data not shown). To find out whether oxidative stress plays a role in the pathogenesis of inflammatory skin diseases, we first investigated whether stimulation of cells with inflammatory cytokines leads to increased oxidative stress. We induced oxidative stress by treating the cells with 250 µmol/L H2O2 and measured intracellular reactive oxygen species (ROS) using a H2DCFDA assay. Treatment with H2O2 led to an increase in ROS production in all three models, which, however, were significantly greater in the AD and PSO models compared with the control (Figure 1A; P Value (Ctrl with H2O2 vs AD with H2O2) < .0001; P Value (Ctrl with H2O2 vs PSO with H2O2) = .0001). The data show that both the AD and PSO models are characterized by an increased oxidative state. To unravel whether the differences in oxidative capacity translate into downstream detrimental biological processes, we analysed DNA damage, as ROS are well-recognized mediators of DNA damage.[27,28]

The results showed that H2O2 induced increased DNA damage in the control, as well as in the cytokine-stimulated cells (Figure 1B, Figure S1; P Value (Ctrl without H2O2 vs Ctrl with H2O2) = .0582; P Value (AD without H2O2 vs AD with H2O2) < .05; P Value (PSO without H2O2 vs PSO with H2O2) not significant). Interestingly, DNA damage was highest in the AD model (Figure 1B, Figure S1; P Value (Ctrl with H2O2 vs AD with H2O2) < .05; P Value (Ctrl with H2O2 vs PSO with H2O2) not significant). These data recapitulate the finding that the AD-like stimulation leads to increased ROS levels, which translate into more DNA damage.

3.2 Inhibition of NADPH oxidase activity leads to abrogation of elevated oxidative stress levels in the AD and PSO models

In order to find out whether the elevated ROS levels in the AD and PSO models are due to increased ROS production or impaired ROS clearance, we measured intracellular ROS after inhibition of NADPH oxidase activity. DPI addition successfully reduced ROS levels after stress induction in all three models (Figure 2A; P Value (Ctrl with H2O2 without DPI vs Ctrl with H2O2 with DPI) = .1681; P Value (AD with H2O2 without DPI vs AD with H2O2 with DPI) < .001, P Value (PSO with H2O2 without DPI vs PSO with H2O2 with DPI) < .001), but inhibition of ROS production was significantly greater in the AD model compared with the Ctrl (Figure 2A; P Value (Ctrl with H2O2 without DPI/ Ctrl with H2O2 with DPI vs AD with H2O2 without DPI/ AD with H2O2 with DPI) < .05). Inhibition of NOX activity also rescued AD cells from increased DNA damage accumulation (Figure 2B). Analysis of DNA damage accumulation showed that the inhibition of NOX activity significantly reduced DNA damage accumulation after stress in AD and PSO, but reduction in DNA damage accumulation was not significant in control cells.

3.3 Increased oxidative stress in the AD and PSO models is mainly due to NADPH oxidase 1 activity

To further characterize the molecular mechanisms involved in increased oxidative stress sensitivity in the AD and PSO models, we analysed NOX family member expression and localization in keratinocytes. Western blot analysis revealed that only NOX1 and NOX4 can be detected in keratinocytes (Figure 3A). There was no difference in the expression of NOX1 and NOX4 between AD- and
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PSO-stimulated cells. DPI treatment reduced NOX1 but not NOX4 expression after DPI and H$_2$O$_2$ treatment in control, and AD and PSO model keratinocytes. Detailed immunofluorescence analysis showed that as expected, there is no difference in expression or localization of either NOX1 or NOX4 in the AD and PSO models (Figures S2 and S3), and that neither expression nor localization of the regulatory p22phox subunit of NOX family members is altered, indicating that DPI reduced NOX activity, but not its abundance (Figure S4).

Next, we used the selective NOX1 and NOX4 inhibitors, respectively, to find out which NOX family member is responsible for induction of elevated ROS levels in the AD and PSO models. DCFDA analysis revealed that the inhibition of NOX1 and NOX4 by NOX1/4 inhibitor (Nox1/4i) (GKT136901) led to a significant but slight decrease in ROS levels in H$_2$O$_2$-stressed AD model keratinocytes, but had no effect on control or PSO model keratinocytes (Figure 3B; $P$ Value (Ctrl with H$_2$O$_2$ without Nox1/4i vs Ctrl with H$_2$O$_2$ with Nox1/4i) = n.s.; $P$ Value (AD with H$_2$O$_2$ without Nox1/4i vs AD with H$_2$O$_2$ with Nox1/4i) < .05; $P$ Value (PSO with H$_2$O$_2$ without Nox1/4i vs PSO with H$_2$O$_2$ with Nox1/4i) = n.s.)).

Inhibition of NOX1 with the NOX1 inhibitor (NOX1i) ML171 on the other hand completely abolished increased ROS production after oxidative stress in AD model keratinocytes and led to a significant reduction in ROS in control cells, but not in PSO model keratinocytes (Figure 3B, $P$ Value (Ctrl with H$_2$O$_2$ without NOX1i vs Ctrl with H$_2$O$_2$ with NOX1i) < .01; $P$ Value (AD with H$_2$O$_2$ without NOX1i vs AD with H$_2$O$_2$ with NOX1i) < .01; $P$ Value (PSO with H$_2$O$_2$ without NOX1i vs PSO with H$_2$O$_2$ with NOX1i) = n.s.).

3.4 | Inhibition of NADPH oxidase activity rescues elevated stress signalling in keratinocytes in the AD and PSO models and promotes survival

For the identification of signalling cascades involved in redox balance in the AD and PSO models, we analysed the stress-related signalling cascades via Western blot. Treatment with H$_2$O$_2$ led to an activation of well-known stress-related signalling cascades such pERK, pP38 and pHsp27 in all three cell models (Figure 4). While P38 signalling is considered to be a pro-apoptosis signal,\[10,29\] activation of ERK signalling and of the heat shock response via phosphorylation of Hsp27 are considered to be survival-related signalling pathways.\[9,10,30,31\] Our Western blot analysis revealed activation of both pro-apoptotic and pro-survival signalling pathways.\[9,10,30,31\] Analysis of cell viability and caspase cleavage showed no significant differences between Ctrl, AD, PSO or H$_2$O$_2$ treatment (data not shown).

We investigated whether stress induction impacts long-term survival by conducting a colony formation assay (CFA). For the CFA, cells were plated very sparsely on a 10-cm cell culture dish, stimulated

FIGURE 1 Atopic dermatitis (AD) and psoriasis (PSO) model keratinocytes react more sensitive to oxidative stress. A, Cells were treated with H$_2$O$_2$ to induce oxidative stress, and reactive oxygen species (ROS) were measured by using a DCFDA assay. AD model keratinocytes exhibit significantly more ROS than untreated control cells. B, DNA damage in the AD and PSO models. Cells were stressed with H$_2$O$_2$, and DNA damage was measured by analysing levels of pH2AX via immunofluorescence staining (IF). B, shows representative images as well as the quantification of nuclear pH2AX fluorescence intensity normalized to nuclear DAPI. Results represent the means and s.e.m. from more than five independent experiments. *$P$ < .05: statistically significant difference from control value after paired Student t test.
with the AD or PSO mix for 24 hours, respectively, and then stressed by addition of 250 µmol/L H₂O₂ for 24 hours. Colony formation was measured after 14 days, thus indicating long-term survival of colonies. Figure 5 shows that treatment of Ctrl, AD and PSO keratinocytes with H₂O₂ led to a reduction in colony-forming units (CFUs). Inhibition of NOX activity only led to a better survival rate after oxidative stress in AD and PSO cells models (Figure 5; P Value (Ctrl with H₂O₂ without DPI vs Ctrl with H₂O₂ with DPI) = n.s.; P Value (AD with H₂O₂ without DPI vs AD with H₂O₂ with DPI) < .05; P Value (PSO with H₂O₂ without DPI vs PSO with H₂O₂ with DPI) < .05).

4 | DISCUSSION

Both AD and PSO are common inflammatory skin diseases characterized by epidermal barrier dysfunction and excessive T-cell activation, however, differing polarity. While AD is driven by the type 2 cytokines, PSO is a Th17-mediated disease.[34-36] In both diseases, the increased release of other pro-inflammatory cytokines such as TNF-α and IL-1 in both AD and PSO may cause chronic low-grade systemic inflammation.[34] Our results indicate that the different cytokine milieus associated with AD and PSO have different effects on NOX activity, which might explain why our study revealed differences in the oxidative stress sensitivity of the AD and PSO models in vitro. It is well known that oxidative stress promotes tissue inflammation through upregulation of genes that code pro-inflammatory cytokines, and thus that inflammatory signalling is closely linked to oxidative stress.[37,38] Inflammatory cells in turn release free radicals when activated. Given its prominent inflammatory component, it is conceivable that oxidative stress may play a role in the pathogenesis of inflammatory skin diseases.

As both AD and PSO are inflammatory diseases, inflammatory cells such as T cells and dendritic cells could be the source of elevated oxidative stress marker, but pro-inflammatory signalling in keratinocytes has also been discussed as a possible cause.[8] Our study reveals for the first time that in fact keratinocytes are characterized by high intracellular levels of ROS after treatment with previously described AD and PSO signature cytokines,[26] with IL-4 and IL-13 having a greater effect than IL-17. These differences in oxidative burden translate into downstream detrimental biological processes, such as accumulation of DNA damage and reduced survival rate in the AD and PSO models.

Another important question when regarding impaired redox homeostasis mechanisms that might contribute to disease pathology is whether elevated oxidative stress is due to increased ROS production or due to reduced antioxidative capacity. By utilizing the pan-NADPH oxidase inhibitor DPI, which inhibits the intracellular production of ROS due to environmental stress induction, we were able to show that elevated oxidative stress in the AD and PSO models arises from increased intrinsic ROS production and not impaired antioxidant capacity as hypothesized previously elsewhere.[20,22-25] The results indicate a beneficial effect of NOX inhibition on reducing ROS in an AD and PSO models, however, whether these findings translate to in vivo remain to be investigated. Given the multifactorial nature of both AD and PSO, and the high level of complexity of measured after 14 days, thus indicating long-term survival of colonies. Figure 5 shows that treatment of Ctrl, AD and PSO keratinocytes with H₂O₂ led to a reduction in colony-forming units (CFUs). Inhibition of NOX activity only led to a better survival rate after oxidative stress in AD and PSO cells models (Figure 5; P Value (Ctrl with H₂O₂ without DPI vs Ctrl with H₂O₂ with DPI) = n.s.; P Value (AD with H₂O₂ without DPI vs AD with H₂O₂ with DPI) < .05; P Value (PSO with H₂O₂ without DPI vs PSO with H₂O₂ with DPI) < .05).
the underlying disease mechanisms, counteracting oxidative stress in vivo might not lead to similarly reduced cell damage as observed in vitro.

Interestingly, AD model keratinocytes reacted more sensitive to mild \( \text{H}_2\text{O}_2 \) stimulation than PSO model keratinocytes and had better responsiveness to treatment with NOX inhibitors. This is in line with the observation that while AD skin lesions are characterized by apoptosis of keratinocytes,[39] PSO lesions are characterized by hyperproliferation of keratinocytes.[40,41] It is tempting to speculate that the disease-specific cytokine profile leads to elevated ROS levels in both AD and PSO, but the unique concentration of the key cytokine mediators leads to alternate stress-induced outcomes of cell faith.

As oxidative stress resulting from excess ROS plays an important role in several diseases including AD, PSO, urticaria and allergic diseases,[7] there is a strong need to find strategies lowering ROS in keratinocytes. Possible strategies could include the use of antioxidants or the inhibition of ROS production. Despite many antioxidant therapies that have been evaluated in clinical trials involving tens of thousands of patients, most clinical trial results failed to show effectiveness.[42,43] One of the possible reasons is that in many clinical trials, antioxidants were not chosen because they proved to be the most effective antioxidants, but rather because of their easy availability. This led to the use of antioxidants that were unspecific, ineffective at the doses given or simply also had pro-oxidative effects (e.g., vitamin E).[42] Due to the clinical fail of many trials dealing with antioxidants, NOX inhibitors are more promising for diseases associated with excess oxidative stress, as NADPH oxidases are the only enzymes solely dedicated to ROS generation and thus prove to be a selective target.[44]

Diphenyleneiodonium is a non-specific flavin binder abstracting an electron from an electron transporter forming a radical which
then acts as an inhibitor of the respective electron transporter through a covalent binding step.\[^{45}\] It is commonly used as a NOX inhibitor not only in chronic inflammatory skin diseases as excess ROS production is associated with many different diseases. A major advantage of DPI is the incomplete suppression of ROS production maintaining a basal ROS level which is needed for physiological processes.\[^{44}\] DPI has been shown to be a potent and reliable NOX inhibitor; however, DPI has several drawbacks. As it is a flavoprotein inhibitor, it also inhibits CYP450, NO synthase and the mitochondrial electron chain, thus representing an unspecific and toxic inhibitor.\[^{46}\] In this study, the possible toxic effect of DPI on survival has been addressed via a colony formation assay. We observed reduced colony formation in cells treated with DPI, however, and most importantly, we showed that the combined treatment with \( \text{H}_2\text{O}_2 \) and DPI leads to increased colony formation as compared to \( \text{H}_2\text{O}_2 \) alone and thus rescues cells from oxidative damage. Due to the lack of specificity and its toxicity, DPI remains a problematic candidate for drug usage, but is useful as a reference compound for NOX inhibition in vitro.\[^{46}\]

Studies have showed NOX1 and NOX4 KO mice have no significant spontaneous pathologies making the respective selective inhibition of NOX1 and NOX4 more feasible. In addition to the unspecific NOX inhibitor DPI, novel selective NOX1 and NOX4 inhibitors are currently tested in clinical trials. The specific inhibition of respective NOX would lower off-target effects and would make the treatment more effective. The novel inhibitors ML171 and GKT136901 selectively inhibit NOX1 and NOX1/NOX4, respectively.\[^{46-50}\] Clinical trials with 170 patients have demonstrated that the novel inhibitors are well-tolerated and non-toxic, and thus provide a good alternative for DPI.\[^{50}\] In this study, we controlled for the possible unspecific effects of DPI by additionally analysing intracellular ROS levels after inhibition of NOX1 and NOX1/4 by the selective inhibitors. Both ML171 and GKT136901 significantly reduced ROS production in AD.
increased apoptosis of keratinocytes, the results of this study directly support a link between increased oxidative stress and reduced survival of keratinocytes and lesion formation in patients. In PSO, lesions are characterized by hyperproliferation of keratinocytes instead of apoptosis, but apopotic features as determined by TUNEL method are still prominent in lesions despite PSO being a hyperproliferative disorder.

Taken together, the data of this study demonstrate the possible use of NOX inhibitors to counteract detrimental effects of high levels of AD and PSO signature cytokines, but further in vivo experiments are warranted to further elucidate whether NOX inhibition may represent a promising treatment strategy for inflammatory skin diseases. In conclusion, it is important to focus further research on this field in order to characterize the mechanisms of NOX enzymes as major producer of ROS and in order to develop a safe and effective approach to attenuate the ROS production in AD and PSO.

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HE designed the experiments; HE, MF, Anke Rose and Steffen Bachmann acquired the data. HE and MF analysed the data, and HE and SW interpreted the data. HE, ER and SW drafted the article and revised it critically. All authors approved the final version.

CONFLICT OF INTEREST
The authors have declared no conflicting interests.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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**Figure S1.** Immunofluorescence staining of pH2AX in atopic dermatitis (AD)- and psoriasis (PSO)- model keratinocytes treated with H2O2 and diphenyleneiodonium chloride (DPI).

**Figure S2.** Immunofluorescence staining of NADPH oxidase 1 (NOX1) in atopic dermatitis (AD)- and psoriasis (PSO)- model keratinocytes treated with H2O2 and diphenyleneiodonium chloride (DPI).

**Figure S3.** Immunofluorescence staining of NADPH oxidase 4 (NOX4) in atopic dermatitis (AD)- and psoriasis (PSO)- model keratinocytes treated with H2O2 and diphenyleneiodonium chloride (DPI).

**Figure S4.** Immunofluorescence staining of p22phox in atopic dermatitis (AD)- and psoriasis (PSO)- model keratinocytes treated with H2O2 and diphenyleneiodonium chloride (DPI).

**App S1.** Supplementary figures.

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