Loss of Epidermal p38α Signaling Prevents UVR-Induced Inflammation via Acute and Chronic Mechanisms

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UVB is a component of solar radiation primarily responsible for causing damage and cancer in irradiated skin, and disrupting immune homeostasis. The immediate harm and long-term health risks of excessive sunlight exposure are affecting the lives of nearly all people worldwide. Inflammation is a key mechanism underlying UVB's various detrimental effects. Here we show that activation of the protein kinase p38α is restricted to the epidermis in UVB-exposed skin, and that p38α ablation targeted to the epithelial compartment is sufficient to suppress UVB-induced inflammation. Mechanistically, loss of epithelial p38α signaling attenuates the expression of genes required to induce vascular leakage and edema, and also increases the steady-state abundance of epidermal γδ T cells, which are known to promote the repair of damaged epidermis. These effects of p38α deficiency delineate a molecular network operating at the organism–environment interface, and reveal conditions crucial to preventing the pathology resulting from sun-damaged skin.

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INTRODUCTION

Sunlight affects health and disease in various ways, eliciting changes in metabolism, tissue homeostasis, immunity, and neuroendocrine function. Inadequacy of sunlight exposure can result in a vitamin D deficiency, whereas its excess poses a host of other health risks such as sunburn, impaired immune defense, premature skin aging, and skin cancer (Yaar and Gilchrest, 2007; Hart *et al.*, 2011; Elmets and Athar, 2013). UVR with a wavelength range of 290–315 nm, referred to as UVB, is a component of solar radiation that principally, if not exclusively, produces these deleterious effects (Pfeifer and Besaratinia, 2012). Owing to its limited penetration, UVB can only cause primary damage in the cells of the body surface. In sunlight-exposed skin, the molecular lesions directly induced by UVB are restricted mainly to the epidermis. UVB-inflicted epidermal damage is followed by inflammatory responses. Despite its inherently protective function, inflammation can exacerbate photodamage and, if recurring or long-lasting, promotes other, slow-developing forms of solar radiation–induced pathology. Ironically, controlled UVB exposure can ameliorate chronic inflammatory skin diseases, as in the setting of phototherapy, although the mechanistic basis for this remains unclear (Hönigsmann, 2013).

Photons of UV light drive chemical reactions in the epidermis, leading to the formation of photoproducts such as nucleic acids with cyclobutane pyrimidine dimers (Ley *et al.*, 1977), cholecalciferol (Holick, 1981), *cis*-urocanic acid (De Fabo and Noonan, 1983), and 6-formylindolo[3,2-*b*]carbazole (Fritsche *et al.*, 2007). In addition, UV light induces a rise in reactive oxygen species and intracellular calcium levels. These molecules signify the need for tissue repair, immunoregulation, and metabolic adaptation. A multitude of proteins have been identified as sensors for photoproducts, oxidized cell components, and other molecular signatures of UVR exposure. These include receptors of the innate immune system, such as the NLRP3 inflammasome (Feldmeyer *et al.*, 2007), Toll-like receptor 3 (Bernard *et al.*, 2012), Toll-like receptor 4 (Bald *et al.*, 2014), and the DNA-responsive cyclic dinucleotide receptor STING (Gehrke *et al.*, 2013); nuclear receptors for lipophilic ligands, such as the aryl hydrocarbon receptor (Fritsche *et al.*, 2007) and the vitamin D receptor (Biggs *et al.*, 2010); and a few other proteins with apparent functional heterogeneity, most notably the serotonin receptor 5-HT_{2A} (Walterscheid *et al.*, 2006) and the transient receptor potential ion channel TRPV4 (Moore *et al.*, 2013). Activation of these sensors initiates intracellular signaling cascades linked to UVR-induced physiological responses including inflammation. The transcription factor NF-κB and the protein kinases c-Jun N-terminal kinase and p38 are central to inflammatory signaling in general, and function downstream of several of the known UVB sensors. Activation of NF-κB,

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Abbreviations: COX, cyclooxygenase; DETC, dendritic epidermal T cell; LC, Langerhans cell; PG, prostaglandin; WT, wild type

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c-Jun N-terminal kinase, and p38 by UVB is readily observed in cultured cells (Bode and Dong, 2003; Herrlich *et al.*, 2008), yet their roles in triggering the immediate symptoms and mediating the long-term health effects of UVB exposure remain to be fully determined.

The epidermis is a unique tissue in the metazoan body where signaling pathway activation and gene expression changes occur as a cell-autonomous response to solar radiation. Keratinocytes are key contributors to the UVB response of mammalian skin. Numerous synthetic and natural compounds have been identified as targeting the signaling pathways in keratinocytes and thereby alleviating UVB-induced inflammation and tissue damage (Fisher *et al.*, 1996; Nichols and Katiyar, 2010; Lieder *et al.*, 2012; Conney *et al.*, 2013). However, few definitive studies have been conducted to determine the effect of genetic ablation of keratinocyte signaling on UVB-induced skin pathology. Moreover, the role of hematopoietic-derived epidermal cells in the UVB response remains incompletely understood. The mouse epidermis harbors distinct subpopulations of dendritic cells and γδ T cells, known as Langerhans cells (LCs) and dendritic epidermal T cells (DETCs), respectively. LCs and DETCs are known to have roles in immune defense, tissue repair, and tumorigenesis in the skin (Macleod and Havran, 2011; Romani *et al.*, 2012). Nevertheless, their contribution to sensing and responding to photodamage is as yet unclear.

The kinase p38 was discovered owing its stress- and cytokine-inducible activity and affinity for anti-inflammatory small molecules (Freshney *et al.*, 1994; Han *et al.*, 1994; Lee *et al.*, 1994; Rouse *et al.*, 1994). Activation of p38 signaling by UVR is conserved in eukaryotes ranging from yeast to mammals (Price *et al.*, 1996; Degols and Russell, 1997; Iordanov *et al.*, 1997; Han *et al.*, 1998). Pharmacological inhibition studies showed that blockade of p38 signaling reduced skin pathology in UVB-irradiated mice (Hildesheim *et al.*, 2004; Kim *et al.*, 2005). Interpretation of these results, however, was confounded by the off-target effects of the p38 inhibitors, the presence of multiple p38 isoforms in mammals, and cell type–specific differences in p38 function. Among the four mammalian p38 isoforms (p38α, p38β, p38γ, and p38δ), p38α is the most ubiquitously expressed in adult tissues (Jiang *et al.*, 1997). Here, we show that keratinocyte-specific ablation of p38α expression potently suppresses UVB-induced inflammation in mice. We identify two distinct effects of p38α deficiency that account for the reduction in inflammation: loss of p38α attenuates the expression of specific keratinocyte genes required for acute inflammatory responses and increases the steady-state abundance of DETCs. Our study thus reveals mechanisms and molecular mediators that can be targeted for preventing the detrimental health effects of solar radiation.

RESULTS

Skin inflammation and epidermal p38 activation induced by UVB

The magnitude and nature of UVB-induced inflammation vary according to the dose of radiation inflicted. Exposure to a minimal erythemal dose, the lowest dose of UVR that yields perceptible signs of skin irritation, leads to vasodilation and vascular leak in the dermis. These reactions last from only several hours to a few days depending on the skin type, and resolve without disrupting the integrity of the affected epidermis. Exposure to higher doses, on the other hand, can result in extensive cytotoxicity and epidermal erosion. In a previous experiment using such a high dose of UVB (160 mJ cm^{-2}), we observed that post-irradiation injury and inflammation were reduced in mice with keratinocyte-specific p38α gene ablation (*Mapk14*$^{fl/fl}$*;K14Cre*, or ΔK; Kim *et al.*, 2008). This observation, however, left unclear whether the inflammation developing in this condition was directly responsive to UVB or caused by loss of epithelial integrity and the resultant penetration of external irritants such as microbes. To circumvent this complication, we set up a new experiment involving exposure of shaved mouse back skin to UVB at 50 mJ cm^{-2}. This low-dose irradiation could still produce epidermal DNA lesions (Figure 1a) and, over 24–72 hours, resulted in

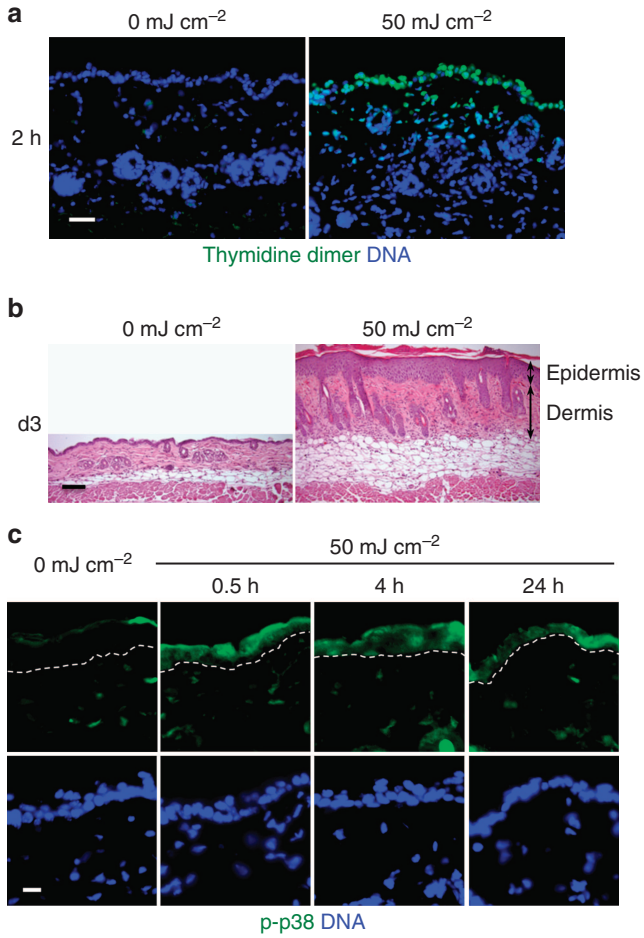

Figure 1. Skin exposure to low-dose UVB results in cyclobutane pyrimidine dimer (CPD) formation, inflammation, and epidermal p38 activation. The shaved back skin of C57BL/6 mice was left unirradiated or irradiated with UVB (50 mJ cm^{-2}). Skin tissue sections prepared at the indicated time points after irradiation were analyzed by immunostaining for thymidine dimers (**a**), hematoxylin and eosin staining (**b**), and immunostaining for phosphorylated (p-) p38 (**c**). Bar = 50 μm (**a**), 100 μm (**b**), and 10 μm (**c**).

epidermal and dermal thickening, indicative of acanthosis and edema formation, respectively (Figure 1b). There were, however, no pervasive epidermal erosions throughout the inflammatory response in the irradiated skin. UVB-exposed skin displayed mainly epidermis-restricted phosphorylation, and hence activation of p38 as early as 30 minutes and for up to 24 hours after irradiation (Figure 1c). As the low UVB dose we tested was sufficient to induce epidermal p38 signaling and inflammation without disrupting epidermal integrity, and was likely more relevant to human skin irritation by ambient solar radiation, we performed all subsequent *in vivo* experiments using the same irradiation protocol.

UVB-induced inflammation suppressed by keratinocyte-specific p38α ablation

The majority of small-molecule p38 inhibitors, including those shown to alleviate UVB-induced skin pathology in mice (Hildesheim *et al.*, 2004; Kim *et al.*, 2005), target both p38α and p38β as well as kinases in other signaling pathways (Godl *et al.*, 2003; Karaman *et al.*, 2008). We detected high expression of all p38 isoforms except p38β in cultured keratinocytes and epidermis from wild-type (WT) mice (Figure 2a). ΔK mice would therefore simulate the epidermal effects of p38 inhibitors; we confirmed efficient and keratinocyte-specific ablation of p38α expression in ΔK mice, and the deficiency of the two inhibitor-sensitive p38 isoforms in their keratinocytes (Figure 2a and Supplementary Figure S1 online).

In contrast to the strong inflammatory response of WT mice following UVB exposure, ΔK skin showed markedly reduced edema and epidermal hyperplasia (Figure 2 b–d). There were no delayed-onset responses in the mutant mice. We next investigated whether UVB-induced inflammation was dependent on p38α signaling in other epidermis-resident cell types. To this end, we examined the response of two additional p38α conditional–knockout lines, *Mapk14*$^{fl/fl}$*;CD11cCre* (ΔD) and *Mapk14*$^{fl/fl}$*;LckCre* (ΔT), which had the p38α gene deleted in LCs and DETCs, respectively (Ritprajak *et al.*, 2012). UVB-induced inflammatory responses were intact in these mice (Figure 2 e–g). Therefore, it is in the epithelial compartment of UVB-exposed epidermis that p38α serves to initiate inflammation.

The rate of forming and repairing DNA lesions, apoptotic sensitivity, and epidermal differentiation state can affect the strength of inflammatory responses in photodamaged skin. UVB-exposed WT and ΔK epidermis, however, exhibited no differences in cyclobutane pyrimidine dimer frequency or apoptosis rate (Supplementary Figure S2 online). The expression of epidermal differentiation markers was comparable between WT and ΔK skin in areas where acanthosis developed to similar degrees (Supplementary Figure S2 online). These findings indicate that the lack of strong inflammatory responses in ΔK skin was not due to changes in epidermal processes influencing DNA damage, cell death, or differentiation state. Consistent with the drastic reduction in edema formation, dermal vascular dilation and leakage were only weakly induced by UVB irradiation in ΔK skin (Figure 3a and b). UVB-induced neutrophil infiltration was also substantially reduced in ΔK dermis compared with WT dermis (Figure 3c and d). These observations suggested that UVB-activated p38α signaling in keratinocytes was essential for producing inflammatory mediators, inducing dermal vascular changes and infiltration, thereby initiating inflammatory responses in the skin.

Inflammation driven by p38α-dependent gene expression

Signaling by p38 mediates cellular responses to external stimuli partly by affecting changes in gene expression. To determine how p38α contributes to UVB-induced inflammation, we compared the genome-wide expression profiles of keratinocytes from WT with that of ΔK mice by DNA microarray analysis. For this experiment, RNA was isolated from each group of cultured keratinocytes before and 4 hours after UVB irradiation, an interval permitting investigation of the primary transcriptional response to UVB. The data thus obtained revealed the multi-faceted impacts of UVB exposure and p38α ablation on keratinocyte mRNA abundance: the expression of some genes increased and that of others precipitously declined after irradiation; loss of p38α led to diminished and augmented expression of distinct subsets of genes (Supplementary Table S1 online). The two largest clusters of genes sharing expression patterns comprised p38α-dependent genes showing UVB-induced and constitutive expression, respectively (Figure 4a). The differential expression of several of the p38α-dependent and p38α-repressed genes was verified by real-time quantitative PCR analysis (Figure 4b).

The p38α-dependent genes identified represented functions related to eicosanoid biosynthesis (*Ptgs2*, *Alox12b*), cytokine and growth factor signaling (*Csf2*, *Il1a*, *Il1f5*, *Il1f6*, *Areg*, *Epeg*, *Hbegf*), leukocyte recruitment (*Cxcl2*, *Cxcl3*, *Ccl3*), antimicrobial defense (*Defb3*, *Lcn2*, *S100a8*, *S100a9*), cornified envelope formation (*Lce* and *Sprr* genes, *Cnfn*, *Flg*, *Lor*, *Tgm1*), and proteolysis (*Mmp13*, *Spink5*, *Slpi*). *Ptgs2* and *Csf2* encode cyclooxygenase (COX)-2 and GM-CSF, respectively. COX-2 is responsible for the synthesis of prostaglandins (PGs) such as PGE_2. PGs and GM-CSF serve pleiotropic functions in promoting as well as resolving inflammation. Consistent with the amounts of *Ptgs2* and *Csf2* mRNA, the production of COX-2, GM-CSF, and PGE_2 was lower in ΔK compared with WT keratinocytes (Figure 4c–e). GM-CSF promotes differentiation of bone marrow progenitors into mature myeloid cells. Medium conditioned by UVB-irradiated but not unirradiated keratinocytes supported the formation of bone marrow–derived cells whose morphology and gene expression signatures were indicative of myeloid cells (Supplementary Figure S3 online). Conditioned medium of UVB-irradiated ΔK keratinocytes, however, did not have such activity (Figure 4f).

We examined p38α-dependent gene expression in the skin of WT and ΔK mice. The intensities of immunostaining of COX-2 and the protein products of other select p38α-related genes, such as S100A8, S100A9, and MGST3, in UVB-exposed WT and ΔK epidermis paralleled with the gene expression data obtained from cultured keratinocytes (Figure 5a). The defect of ΔK mice in UVB-induced COX-2 production was also evident in immunoblot analysis of epidermal extracts

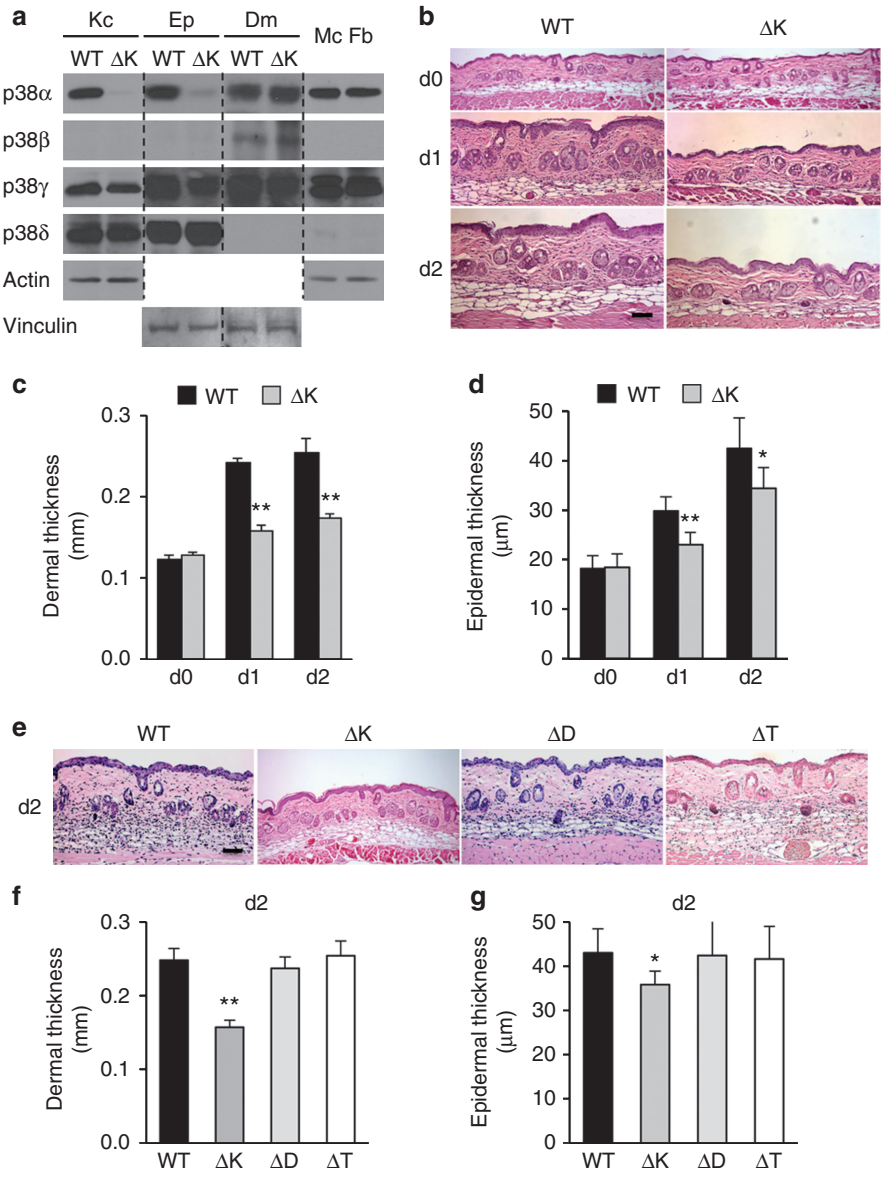

Figure 2. Keratinocyte-specific p38α ablation suppresses UVB-induced inflammation. (**a**) Lysates of cultured keratinocytes, epidermal sheets, and dermal tissue (Kc, Ep, and Dm, respectively) from wild-type (WT) and ΔK newborn skin were analyzed by immunoblotting. Lysates of WT bone marrow–derived macrophages (Mc) and fibroblasts (Fb) were analyzed in parallel. (**b**–**g**) Back skin sections from the indicated mice were prepared at the indicated time points after UVB irradiation (50 mJ cm^{-2}) and analyzed by hematoxylin and eosin (H&E) staining (**b** and **e**). Bar = 100 μm. Dermal and epidermal thickness was determined from images of H&E-stained skin (n = 4–6) and is shown as mean ± SEM (**c**, **d**, **f**, **g**). $^{**}P < 0.01$ (relative to WT).

(Figure 5b). Given the previous finding that pharmacological COX-2 inhibition suppressed UVB-induced skin inflammation (Wilgus *et al.*, 2000), the blunted inflammatory response of ΔK skin following UVB exposure may be attributable to attenuated COX-2 expression. To assess the contribution of COX-2 to the p38α-dependent UVB response, we investigated COX-2-knockout mice. Genetic COX-2 deficiency prevented edema formation and substantially reduced acanthosis in UVB-irradiated skin (Figure 5c–e). Taken together, our gene expression analysis and loss-of-function study highlight the role of keratinocyte p38α in linking UVR exposure to the induction of genes with functional relevance to inflammation.

DETC abundance regulated by keratinocyte p38α

Although mice lacking p38α in LCs or DETCs did not show noticeable differences in UVB-induced inflammation, it was still possible that these epidermal cells participated in the UVB response independently of p38α or via mechanisms involving non-cell-autonomous p38α functions. Importantly, we observed an increase in the abundance of DETCs but not LCs in steady-state ΔK skin (Figure 6a–e). Greater numbers of cells expressing high amounts of TCRγδ and CD3 were detected in whole-mount immunostaining and flow cytometry analysis of the epidermal sheet of ΔK pinnae relative to WT counterparts (Figure 6a and b). WT epidermis contained a

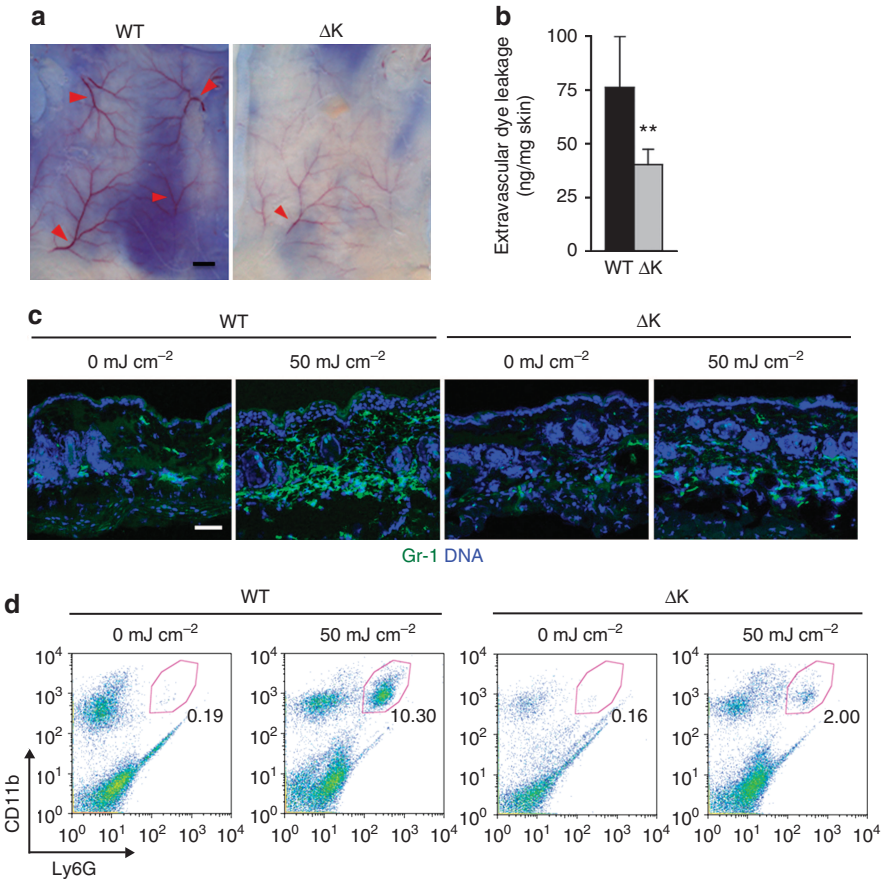

Figure 3. Loss of epidermal p38α signaling prevents dermal vascular leakage and neutrophil infiltration after UVB irradiation. Wild-type (WT) and ΔK back skin was analyzed 48 hours after irradiation with UVB (50 mJ cm^{-2}). (**a**, **b**) Evans Blue was injected intravenously into mice. The inner surfaces of back skin were photographed 30 minutes after dye injection (**a**). Red arrowheads indicate dilated blood vessels. Dye extravasation was quantified by colorimetry ($n=4$), and is shown as mean ± SEM (**b**). $^{**}P<0.01$. (**c**) Skin sections were analyzed by immunostaining for the neutrophil marker Gr-1 and counter stained for DNA. Bar = 50 μm. (**d**) The percentage of CD11b$^+$Ly6G$^+$ neutrophils among skin cells was determined by flow cytometry.

subpopulation of cells with low expression of TCRγδ and CD3, whereas in ΔK epidermis the majority of TCRγδ$^+$CD3$^+$ cells displayed uniformly high expression of the two markers. Similar increases in the abundance and homogeneity of DETCs were also observed when ΔK epidermal cells were stained for TCR with the epidermis-specific invariant Vγ3 chain (Figure 6c). The back skin of ΔK mice, too, harbored higher densities of DETCs, as shown by immunostaining of skin sections (Figure 6d and e).

DETCs are known to afford surveillance and protection against mechanical and chemical damage to the skin, yet their role in UVB-exposed skin remains unclear. We examined UVB-induced inflammation in TCRδ chain–deficient mice, which were devoid of γδ T cells including DETCs. Skin edema in these mice was comparable to that in WT mice during the first 48 hours after irradiation, but became more severe later on (Figure 6f and g). UVB-induced inflammation in TCRδ-knockout skin eventually entered a resolution phase as in WT skin. The development of excessive inflammation and its delayed manifestation in the absence of DETCs suggested a role for these cells in limiting photodamage and dampening inflammatory responses secondary to persistent epidermal injury. Conceivably, DETCs present in greater abundance in ΔK mice may contribute to attenuating inflammatory responses.

DISCUSSION

The epidermal barrier is indispensable for life processes in the inner tissues, and needs to be constantly repaired and regenerated in the face of deleterious solar radiation. UVB-induced inflammation is thought to mobilize humoral and cell-mediated mechanisms that defend and heal damaged epidermis, yet its excess and chronicity produce pernicious effects such as disrupting immune homeostasis and promoting skin cancer. Controlling inflammation therefore has implications beyond treating sun-irritated skin. The investigation of ΔK mice allowed us to discern two distinct attributes that accounted for their reduced inflammatory responses to UVB exposure relative to those in WT mice: attenuated expression of COX-2 and other inflammatory mediators in keratinocytes, and greater abundance of DETCs. With these changes simultaneously in effect, UVB-irradiated ΔK skin could not evoke

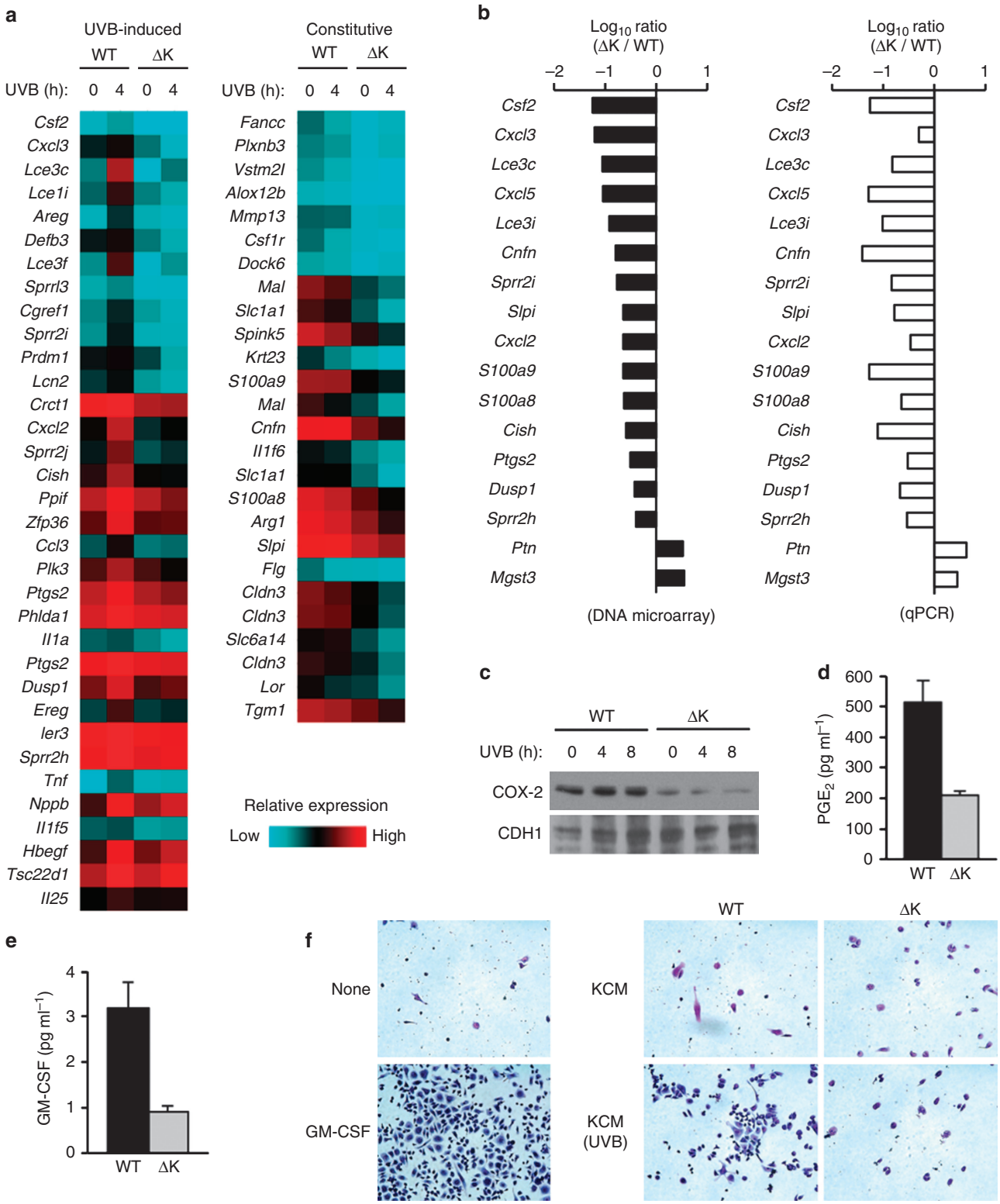

Figure 4. Signaling by p38α is required for keratinocyte gene expression. (**a**, **b**) Wild-type (WT) and ΔK keratinocytes before and 4 hours after UVB irradiation were subjected to DNA microarray analysis (**a**). The ratios of mRNA amount at 4 hours post UVB were analyzed by quantitative PCR and are presented together with the DNA microarray data (**b**). (**c**–**e**) WT and ΔK keratinocyte lysates prepared at the indicated time points after irradiation were analyzed by immunoblotting (**c**). Prostaglandin E_2 (PGE_2) and GM-CSF amounts in culture supernatants 8 hours post UVB ($n = 3$) were determined by ELISA, and are shown as mean ± SD (**d, e**). (**f**) Mouse bone marrow was incubated in medium supplemented with GM-CSF (20 ng ml^{-1}), and keratinocyte-conditioned medium (KCM; 50%, v/v). Cells generated after 7 days of incubation were stained with crystal violet.

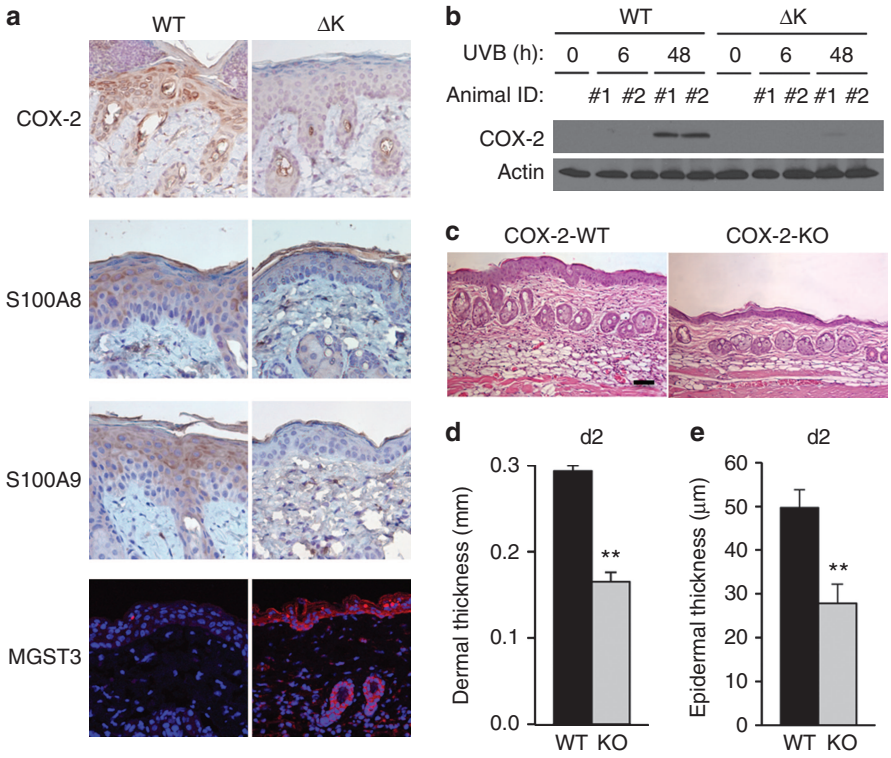

Figure 5. Cyclooxygenase (COX)-2 is essential for UVB-induced skin inflammation. The shaved back skin of the indicated mice was irradiated with UVB (50 mJ cm^{-2}). (**a**) Skin tissue sections were prepared 48 hours after irradiation and analyzed by immunostaining for the proteins indicated on the left. MGST3 staining (red) was shown together with the counter staining of DNA (blue). (**b**) Skin lysates were prepared at the indicated time points after irradiation and analyzed by immunoblotting with COX-2 and actin antibodies. (**c**–**e**) Skin tissue sections were prepared 48 hours after irradiation and analyzed by hematoxylin and eosin (H&E) staining (**c**). Bar = 100 μm. Dermal and epidermal thickness was determined from images of H&E-stained skin ($n = 6$) and is shown as mean ± SEM (**d, e**). KO, knockout; WT, wild type. $^{**}P < 0.01$.

edema and dermal neutrophil infiltration, and manifested only mild acanthosis. These responses were intact in mice lacking p38α in LCs or DETCs, indicating that it was in the keratinocyte that UVB-activated p38α signaled to initiate inflammation. This may, however, simply reflect the small population sizes of LCs and DETCs compared with that of the keratinocytes. Hence, we do not exclude the possibility that p38α function in the three epidermal cell compartments may be qualitatively similar. Either way, our findings point to p38α and p38α-dependent genes in keratinocytes as crucial links between UVB sensing and inflammatory responses, and as important targets for the prevention and treatment of clinical conditions caused by excessive sunlight exposure. The long-term effects of reduced inflammation in UVB-exposed ΔK skin are as yet unknown. Acute inflammatory responses may critically promote tissue repair or prevent autoimmunity. Loss of these functions could conceivably produce adverse clinical effects that outweigh short-term benefits.

Transgenic mice with epidermal expression of cyclobutane pyrimidine dimer photolyase (Schul *et al.*, 2002) or the antioxidant transcription factor NF-E2-related factor-2 (Schäfer *et al.*, 2010) were found resistant to UVB-induced skin pathology, a corollary of diminished cyclobutane pyrimidine dimer formation and apoptosis. By contrast, ΔK skin irradiated with UVB was as prone to these lesions as WT skin, but defective in inducing inflammation. In this regard, ΔK mice will serve as a unique experimental model in which UVB-inflicted epidermal damage is dissociated from inflammatory responses, enabling analysis of the effects of the former without the influence of the latter.

The p38 signaling pathway is essential for protecting single-celled eukaryotes against physical and chemical stress, and appears to have been co-opted to deal with other types of stress in multicellular life. In the nematode *Caenorhabditis elegans*, p38 has a central role in linking microbial infection and tissue injury to antimicrobial gene expression, and this function depends, at least in part, on p38 signaling in the epidermis (Kim *et al.*, 2002; Pujol *et al.*, 2008). It has not been established, however, whether p38 in mammalian epidermis has cell-autonomous functions in immune and inflammatory responses. On the basis of approaches involving low-dose UVB irradiation and keratinocyte-specific gene ablation, we investigated p38α signaling in experimental conditions where p38α was activated in and its ablation was targeted to the skin epithelium. The results thus obtained revealed that the mammalian skin too required epithelial p38α signaling for inflammatory responses and the expression of

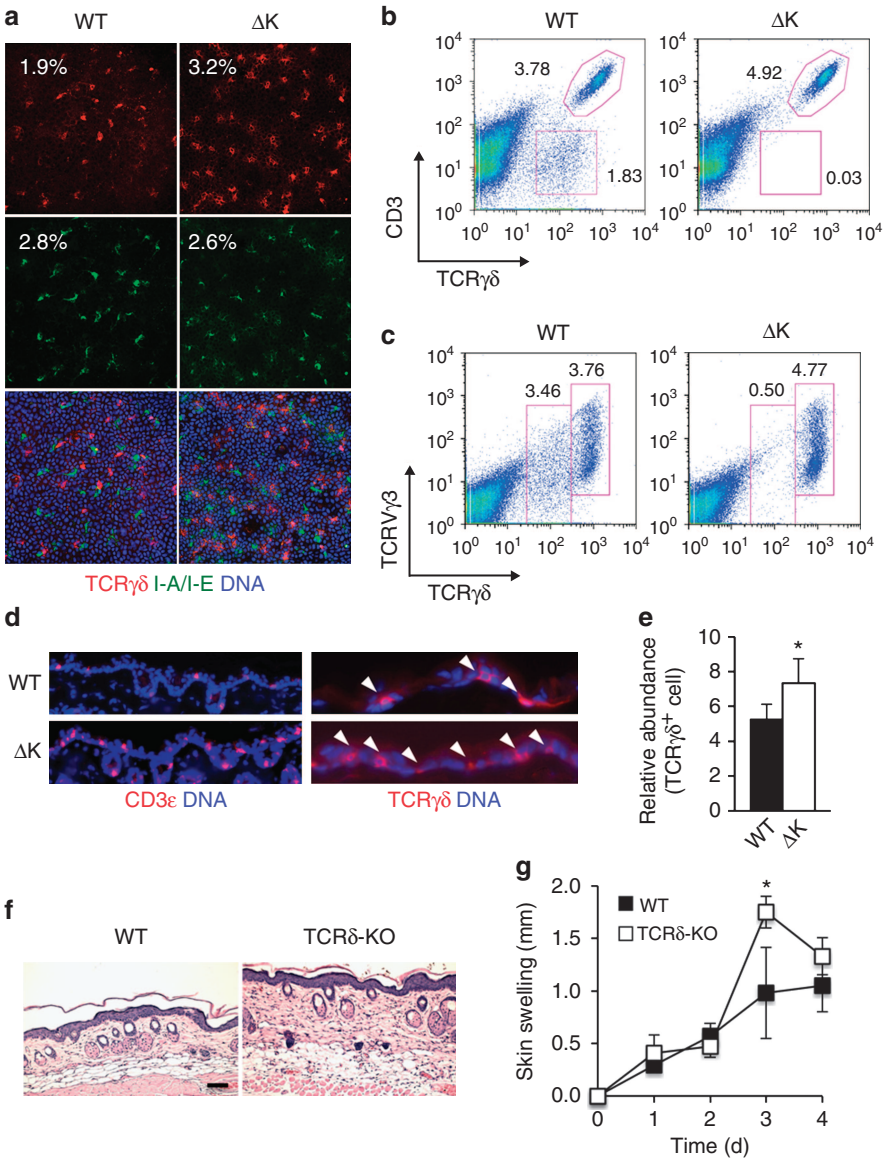

Figure 6. TCRγδ cells contribute to attenuating UVB response in the skin. (**a**) The epidermal sheet from wild-type (WT) and ΔK pinnae were immunostained for TCRγδ and I-A/I-E, and counter stained for DNA. (**b**, **c**) Epidermal cells from WT and ΔK pinnae were analyzed by flow cytometry. (**d**, **e**) Back skin sections from WT and ΔK mice were immunostained for the indicated proteins, and counter stained for DNA (**d**). TCRγδ$^+$ cell abundance is shown as mean ± SEM (**e**). *$P<0.05$. (**f**, **g**) Back skin sections from WT and TCRδ-knockout (KO) mice were prepared 4 days after UVB irradiation (50 mJ cm^{-2}) and analyzed by hematoxylin and eosin staining (**f**). Bar = 100 μm. Skin swelling was determined at the indicated time points after irradiation and is shown as mean ± SEM (**g**). *$P<0.05$.

genes with immune function. The UVB-inducible and p38α-dependent keratinocyte genes identified in this study may have functional implications in antimicrobial defense and immune disorders in the skin (Swamy *et al.*, 2010; Gallo and Hooper, 2012).

Little is known about how DETC recruitment to and maintenance in the skin are achieved and regulated. A higher density of DETCs in ΔK epidermis suggested that loss of keratinocyte p38α function created an epidermal environment conducive for promoting the recruitment, proliferation, or survival of DETCs. Besides ΔK mice, there are other mutant mouse lines in which a genetic alteration in keratinocytes leads to changes in DETC abundance: mice with keratinocyte-restricted ablation of fibroblast growth factor receptor 2b and the Wnt cargo receptor Evi/Wls were shown to have more and fewer DETCs, respectively (Grose *et al.*, 2007; Augustin *et al.*, 2013). It remains to be determined whether there are hierarchical relationships between the signaling pathways affected in these mutant mice and p38α function.

MATERIALS AND METHODS

Animals

ΔK, ΔD, and ΔT mice were bred in specific pathogen-free conditions as described (Kim *et al.*, 2008; Ritprajak *et al.*, 2012), and had mean

body weights comparable to WT. COX-2 (*Ptgs2*)-knockout and TCRδ (*Tcrd*)-knockout mice were obtained from Taconic (Germantown, NY) and the Jackson Laboratory (Bar Harbor, ME), respectively. All animals were on a C57BL/6J background except COX-2-knockout mice, which were on a mixed B6;129P2 background. Euthanasia was performed by carbon dioxide asphyxiation. All animal experiments were conducted under Institutional Animal Care and Use Committees-approved protocols.

UVB irradiation

Shaved and depilated back skin of 2–3-month-old mice was exposed to 50 mJ cm^{-2} UVB with UVB bulbs (Southern NE Ultraviolet, Branford, CT) and a Kodacel filter (Eastman Kodak, Rochester, NY). UVB dose was monitored with a radiometer (International Light, Peabody, MA). Primary keratinocytes were exposed to 75 mJ cm^{-2} UVB at 70–80% confluency.

Analysis of skin response to UVB irradiation

Dermal and epidermal thickness was measured from images of hematoxylin and eosin-stained skin and extrapolated by comparing with the scale bar. Skin swelling was determined by measuring the thickness of lifted back skin with a caliper (Mitutoyo, Kawasaki, Japan). To determine vascular permeability, 100 μl of 1% Evans Blue in phosphate-buffered saline was injected intravenously into the lateral tail vein 6 hours after UVB irradiation, and back skin samples were collected 30 minutes after dye injection for photography. Extravascular dye content was quantified as described (Kim *et al.*, 2008).

Histology and immunofluorescence

Skin sections and epidermal sheets were prepared as described (Kim *et al.*, 2008; Li *et al.*, 2011), and stained with hematoxylin and eosin or with antibodies against specific markers. TUNEL staining was performed with the *In Situ* Cell Death Detection kit (Roche Applied Science, Indianapolis, IN).

Flow cytometry

Minced back skin was incubated for 30 minutes at 37 °C in 1 mg ml^{-1} dispase and 1.5 mg ml^{-1} of collagenase. Pinnae were split into halves along the cartilage, placed dermal side down on 25% trypsin, and incubated for 1 hour at 37 °C; epidermal sheets were isolated, and rinsed in Hank's balanced salt solution with 1% fetal bovine serum. Cells obtained from back skin and pinna epidermis were passed through a strainer with 70-μm pores and subjected to flow cytometry using fluorescent dye–conjugated antibodies. Stained cells were analyzed using FACSCanto, BD Biosciences (San Jose, CA) and FlowJo software (Tree Star, Ashland, OR).

Protein and RNA analysis

Cell and tissue lysates were analyzed by immunoblotting using antibodies against specific proteins. PGE_2 and GM-CSF amounts were determined by ELISA (eBioscience, San Diego, CA). Real-time quantitative PCR was performed using the primers listed in Supplementary Table S2 online. DNA microarray analysis was performed using GeneChip Mouse Genome 430 2.0 Array (Affymetrix, Santa Clara, CA). All microarray data are available in the NCBI GEO database under the accession number GSE51206.

Statistical analysis

Data values in cell culture experiments and histology analysis are expressed as mean ± SD and mean ± SEM, respectively. *P*-values were obtained from the unpaired, two-tailed Student's *t*-test.

CONFLICT OF INTEREST

The authors state no conflict of interest.

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SUPPLEMENTARY MATERIAL

Supplementary material is linked to the online version of the paper at http://www.nature.com/jid

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