*Appendix S1*

## METHODS

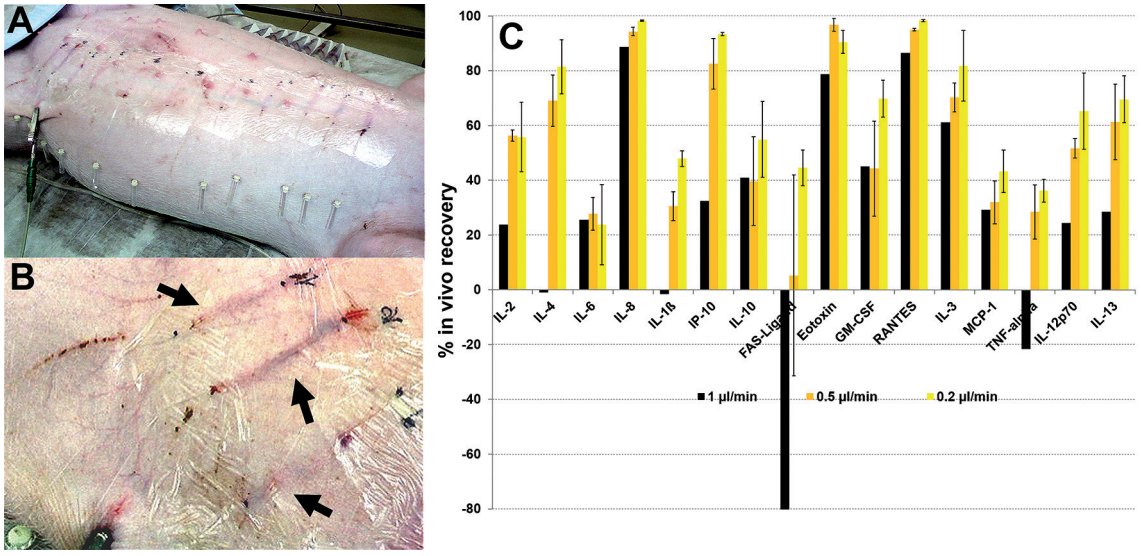
### In vitro *cell culture experiments*

The contribution of isolated keratinocytes to the kinetic profile of inflammatory markers, especially of prostanoids and cytokines by irradiation of keratinocytes *in vitro* was tested using an established *in vitro* model of UVB-irradiated HaCaT keratinocytes, which are known to release easily detectable amounts of prostanoids *in vitro* (27). From the panel of cytokines, we chose those that are most sensitive to UVB irradiation: IL-6, IL-8, and TNF-α. For this, we performed 2 experiments, in which 2×107 HaCaT cells were seeded on dishes 25 cm diameter, surface area 148 $cm^2$, overnight. The next day, in the first experiment, cells were irradiated with 50 $mJ/cm^2$ UVB in phosphate-buffered saline (PBS) (Waldmann UV 3003K, Herbert Waldmann GmbH & Co. KG, Villingen-Schwenningen, Germany), which is a sublethal dose known to induce apoptosis of HaCaT cells as well as the release of the prostanoids 9α,11α-$PGF_{2α}$ and 8-iso-$PGF_{2α}$ (4, 13, 14). The cells were then fed with fresh Dulbecco's modified Eagle's medium (DMEM) medium (10% foetal calf serum (FCS), 2% glutamine, 1% penicillin/streptomycin), this procedure was continued for up to 48 h after irradiation. The supernatant was supplemented with 0.01% of the antioxidant butylhydroxytoluene (BHT) and stored immediately after collection in liquid nitrogen. In the second experiment, to compare prostanoid release into the surrounding medium with intracellular generation, 12 parallel dishes were used, seeded with $2×10^7$ HaCaT cells and cultured overnight. The next day, cells were irradiated with a higher dose of UVB, 100 $mJ/cm^2$ (Waldmann UV 3003K, Herbert Waldmann GmbH & Co. KG, Villingen-Schwenningen, Germany) in PBS to increase the number of inflammatory markers. In contrast to the previous experiment, every 4 h, HaCaT cells from 1 dish were harvested, and the supernatant collected up to 48 h. HaCaT cells were harvested to analyse intracellular generation of F2-isoprostanes (5- and 8-iso-$PGF_{2α}$) that are not released into the surrounding medium. For all experiments, 3 independent biological experiments were performed. The supernatants were analysed for their levels of the cytokines IL-6 and TNF-α and the chemokine IL-8 (15, 16) using cytokine-specific enzyme-linked immunoassays (ELISAs) (RayBio® ELISA kits, RayBiotech Inc., Norcross, GA, USA). The supernatants and cellular levels of the lipid mediators F2-isoprostanes (5- and 8-iso-$PGF_{2α}$) and prostaglandins (9α,11α-$PGF_{2α}$ and PGE2) were analysed by gas chromatography-mass spectrometry (GC/MS) /negative-ion chemical ionization, as described previously (9).

### *Animal model:* ex vivo *recovery experiments*

Recovery of cytokines was determined by *in vivo* retrodialysis in an animal model, in anaesthetized domestic pigs (SFig. 1A and B).

Porcine skin, due to its similarity to human skin regarding permeability, density, diameter of hair follicles, epidermal and dermal skin structure (thickness, lipids, elastic fibres), is a good *in vivo* model to study recovery with microdialysis (17). However, both, retrodialysis and porcine skin are models that are approaching the human *in vivo* situation, but do not completely reproduce it. Four microdialysis catheters (100 kDa cut-off membranes CMA 66/30 membranes, from CMA Microdialysis, Sweden) were placed in the dermal porcine skin (17), which has the best dermal properties of all animals in relation to human skin (17). The microdialysis catheters were placed at a depth of 0.9–1.2 mm and were controlled by a 22 MHz ultrasound (Taberna Pro Medicum, Luneburg, Germany). Microdialysis catheters were perfused at 0.2, 0.5 or 1 µl/min with 1,600 pg/ml mixed cytokine standards (IL-1β, IL-2, IL-3, IL-4, IL-6, IL-8, IL-10, TNF-α, FasL, IP-10, monocyte chemoattractant protein 1 (MCP-1), RANTES, eotaxin, and GM-CSF; all standards from BD Biosciences Cytometric Bead Array (CBA) Flex Sets, Heidelberg, Germany) in Dextran 60 (Deltadex 60®, Deltaselect, Germany). Recovery was calculated as the difference between the starting concentration and the concentration found in the dialysate, which was the percentage of mediators that passed through the membrane into the surrounding dermal tissue. Recovery of prostanoids using the same animal model and method has been reported previously by our group (18). Using a 20 kDa microdialysis membrane, which was > 80% for all mediators at a flow rate of 0.5 µl/min and was consistently higher than the flow rate of 1 µl/min. Therefore, a flow rate of 0.5 µl/min was further used for clinical dermal microdialysis experiments (18).




*SFig. 1. In vivo* recovery of cytokines from porcine skin using retrodialysis. (A) Microdialysis catheters were placed in porcine skin and flushed with a solution of all cytokines of interest (1,600 pg/ml) resulting in (B) skin erythema and oedema (black arrow) where the catheters are located.