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Appendix S1

SUPPLEMENTARY MATERIALS AND METHODS

siRNA transfection

Transfection of keratinocytes with a confluence of 60–70% was performed as described previously (20). The transfection was performed with human IκBζ siRNA (Dharmacon, Lafayette, CO, USA, L-013497-00-0005) and with siGenome control pool non-targeting siRNA (Dharmacon, Lafayette, CO, USA, D-001206-13-05) as a negative control. The formed transfection reagent complexes were added to the cultured cells at a final concentration of 20 nM. The cells were stimulated with TNFα (10 ng/ml) and IL-17A (100 ng/ml) 24 h after transfection.

RNA isolation

Punch biopsies were obtained from lesional and non-lesional plaque-type psoriatic skin as described previously (S1). The biopsies were transferred to 1 ml cold RNAlater-ICE (Life Technologies, Carlsbad, CA, USA) and stored at –80°C until 24 h before RNA purification when they were transferred to –20°C. The biopsies were removed from RNAlater-ICE and added 175 µl SV RNA lysis buffer (SV Total RNA Isolation System; Promega, Madison, WI, USA) and homogenized. For isolation of RNA from cultured human keratinocytes, cells were washed with PBS and 150 µL SV RNA lysis buffer were added each well in a 6-well plate. The remainder of the purification was performed according the manufacturer's instructions (Promega) and the RNA was stored at –80°C for further use.

Quantitative PCR

Reverse transcription was performed using TaqMan® Reverse Transcription Reagents (Applied Biosystems, Foster City, CA, USA). For qPCR, the mastermix used was Platinum® qPCR Super-mix-UDG (Invitrogen by Life Technologies). Primers/probes from Thermo Fisher Scientific against human (assay ID: Hs00205367, Hs00758166, Hs00219742, Hs01104220, respectively) or murine (assay ID: Mm00457645, Mm01337546, Mm00463327, Mm01333586, respectively) *IL36A, IL36B, IL36G, IL36RN* were used. As reference genes human RPLP0 (assay ID: Hs99999902) or murine *Actb* (assay ID: Mm02619580) (TaqMan Gene Expression assay, Thermo Fisher Scientific) were used for normalization. Each gene was analysed in triplets on the real-time qPCR machine Rotorgene 3000 (Corbett Research, Sydney, Australia). The relative gene expression levels were determined from a standard curve for each gene made from a 4-fold serial dilution.

Western blotting

The protein extracts from cultured human keratinocytes were isolated and western blotting analysis performed as described previously (S2). Equal amounts of protein were separated by SDS-PAGE and blotted onto a nitrocellulose membrane. The membrane was incubated with primary antibody: anti-IL-36α; anti- IL-36β; anti-IL-36γ; anti-IL-36Ra (cat. number 1078, 1099, 2320, 1275, R&D Systems, Abingdon, UK); anti-IκB-ζ (cat. number 9244, Cell Signaling Technology, Danvers, MA, USA) or anti-β-actin (cat. number A1978, Sigma-Aldrich, St Louis, MO, USA). The following secondary antibodies were used: Rabbit anti-goat (cat. number P0449, DAKO, Glostrup, Denmark); goat anti-rabbit (cat. number P0448, DAKO); goat anti-rabbit (cat. number 7074, Cell Signaling Technology); horse anti-mouse (cat. number 7076S, Cell Signaling Technology). The proteins were then visualized with Clarity™ Western ECL substrate (BIO-RAD, Herlev, Denmark) by LI-COR C-digit according to manufacturer's instructions.

Transfection and determination of promoter activity

Human keratinocytes were cultured in 24-well plates and transfected at 60–70% confluence. Cells were transfected with 0.5 µg of the indicated *IL36G* plasmid and 0.025 µg of the internal control Renilla luciferase expression plasmid (pRL-SV40, Promega, Madison, WI, USA) using Fugene 6 transfection reagent (Promega) according to the manufacturer's protocol. The cells were stimulated with TNFα (10 ng/ml) and/or IL-17A (100 ng/ml) 48 h after transfection. After 24 h of stimulation the luciferase assay was performed by using the dual luciferase assay system (Promega, Madison, WI, USA) on a fluoroskan Ascent F1 (BIE & Berntsen, Rødovre, Denmark). The relative promoter activity was calculated as the ratio between firefly luciferase activity by the *IL36G* promoter and Renilla luciferase activity in each sample.

REFERENCES

S1. Johansen C, Moeller K, Kragballe K, Iversen L. The activity of caspase-1 is increased in lesional psoriatic epidermis. J Invest Dermatol 2007; 127: 2857–2864.
S2. Kjellerup RB, Kragballe K, Iversen L, Johansen C. Pro-inflammatory cytokine release in keratinocytes is mediated through the MAPK signal-integrating kinases. Exp Dermatol 2008; 17: 498–504.