

Appendix S1

Materials and methods

Ex vivo full-thickness skin organ cultures. Skin specimens from healthy donors were obtained for *ex vivo* assays. Biopsies were cultured as follows: A hole was punched in a Transwell filter (pore size 1 μm ; Beckton Dickinson Labware). The biopsy was inserted into the hole, and the filter containing the biopsy was placed in a 12-well culture plate (Beckton-Dickinson Labware) with Dulbecco's modified Eagle's medium (DMEM; Gibco) containing 10% fetal bovine serum (FBS; Gibco), 2 mM L-glutamine (Gibco) and antibiotics (100 IU/ml penicillin G, 100 $\mu\text{g/ml}$ streptomycin, Gibco). In this system, the epidermis faces upwards at the liquid-air interface whereas the dermis is suspended in the culture medium. The tissue was incubated at 37°C in a humidified atmosphere containing 5% CO₂. Healthy skin biopsies were stimulated with recombinant human TNF- α protein (R&D Systems), at 20 ng/ml for 24 h.

Normal human epidermal sheets and dermis. Normal human epidermal sheets and dermis were isolated from healthy donors as previously described (1). Epidermal sheets were cultured in DMEM (Gibco) containing 10% fetal bovine serum (FBS; Gibco), 2 mM L-glutamine (Gibco) and antibiotics (100 IU/ml penicillin G, 100 $\mu\text{g/ml}$ streptomycin; Gibco) and stimulated with TNF- α (R&D Systems) at 20 ng/ml for 24 h.

RNA extraction, cDNA synthesis and qRT-PCR. *Ex vivo* full-thickness skin organ cultures, normal human epidermal sheets were snap-frozen in liquid nitrogen and stored at -70°C until RNA extraction.

Reference

1. Kitano Y and Okada N: Separation of the epidermal sheet by dispase. Br J Dermatol 108: 555-560, 1983.

Figure S1. Gene expression of (A) Tyr and (B) BMP-4 in normal human epidermal sheet cultures treated with TNF- α (20 ng/ml) or untreated for 24 h. Gene expression of (C) Tyr and (D) BMP-4 in *ex vivo* healthy skin organ cultures treated with TNF- α (20 ng/ml) or untreated for 24 h. Data are presented as the mean \pm standard deviation. *P<0.05 vs. matched controls. Tyr, tyrosinase; BMP, bone morphogenetic protein; TNF- α , tumor necrosis factor- α ; EP, epidermal sheet; OC, organ culture.

