Supplemental Figure 1. Expression of NGF in BMCMCs and keratinocytes after TNF stimulation

A: Bone marrow-derived cultured mast cells (BMCMCs) derived from C57BL/6J mice were sensitized with 10 μg/ml anti-dinitrophenyl (DNP)-IgE (H1-ε-26) at 37°C overnight and then washed. For antigen stimulation, IgE-sensitized BMCMCs were cultured in the presence of 20 ng/ml DNP-human serum albumin (DNP-HSA; Sigma, St Louis, MO). Naïve, IgE-sensitized, and IgE-sensitized and antigen-stimulated mouse BMCMCs were maintained in the presence or absence of various concentrations of recombinant mouse (rm)TNF (PeproTech, Rockey Hill, NJ) for 24 h or 48 h (data not shown). NGF and IL-6 concentrations in culture supernatants were measured by NGF Emax ImmunoAssay system (Promega, Madison, WI) and Mouse IL-6 BD OptEIA ELISA Set (BD PharMingen, San Diego, California), respectively. Neonatal mouse brain homogenates were used as a positive control for NGF. Data are the average ± SD of triplicate samples from one of three independent experiments (using three different batches of BMCMCs), each of which gave similar results.

B: Keratinocytes (Ks) were prepared from epidermal sheets as described by Nakae et al.[60] Briefly, C57BL/6J female mice (6-8 weeks) were shaved in dorsal and abdominal areas and the hair was completely removed with a hair-remover cream 2 days before experiments. The mice were sacrificed, the depilated skin was harvested, and the hypodermal tissue was removed. The skin was incubated with 0.25% trypsin (Sigma) and 50 U/ml of dispase (Roche, Indianapolis, IN) in PBS for 1 h at 37˚C, and epidermal sheets were prepared. Epidermal sheets were stirred in PBS containing 2% FCS for 15 min at room temperature. After filtration, epidermal cells were collected, and the cells ($2.5x10^5$ cells/ml) were cultured in the absence (-) or presence (+) of 10 ng/ml rmTNF for 3 h. After treatment with or without TNF, total RNA from cells was extracted with RNeasy® kit (QIAGEN, Valencia, CA) according to the manufacturer's instructions. Expression of mRNA for bNGF (the beta chain of NGF) in keratinocytes was determined by RT-PCR using bNGF PCR primers obtained from R&D systems (Minneapolis, MN). Whole brain RNA was used as a positive control for bNGF mRNA expression, and GAPDH mRNA expression was used as a housekeeping control gene.

Supplemental Figure 1 Kakurai et al.