

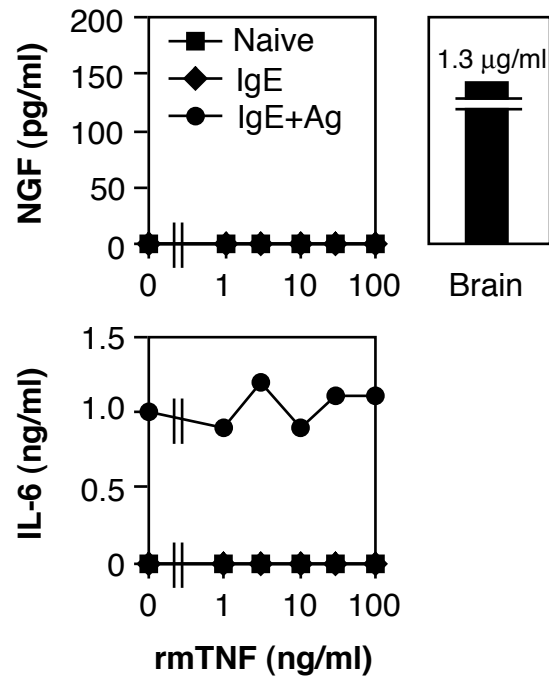
Supplemental Figure 1. Expression of NGF in BMCMCs and keratinocytes after TNF stimulation

A: Bone marrow-derived cultured mast cells (BMCMCs) derived from C57BL/6J mice were sensitized with 10 $\mu\text{g/ml}$ 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, Rocky 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 \pm 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 (2.5×10^5 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.

A



B

