Supplementary Fig. 1. Dermal inflammatory cells are diminished by Tyk2 deficiency in IL-23-induced skin inflammation. Dermal cells were isolated from digested Tyk2 $^{+/+}$ and Tyk2 $^{-/-}$ ear skin on day 4, and subjected to flow cytometry. The absolute number of inflammatory cells from uniformly collected ear tissue by biopsy punch is shown. Data represents mean ± SD (n=5). **$p<0.01$, ***$p<0.001$ compared with BSA-injected mice group; ###$p<0.001$ compared with Tyk2 $^{-/-}$ mice group. Similar results were obtained in two independent experiments. (B) Tyk2 $^{+/+}$ and Tyk2 $^{-/-}$ mice were intradermally injected with 1 µg/ear of IL-23 or BSA for every day. mRNAs were prepared from the ear draining LN of Tyk2 $^{+/+}$ and Tyk2 $^{-/-}$ mice on day 4, and subjected to quantitative RT-PCR with the indicated primers. Gene expression level was normalized by GAPDH expression. Data represents mean ± SD (n=5). *$p<0.05$, **$p<0.01$ compared with BSA-injected mice group; º$p<0.05$, ##$p<0.01$ compared with Tyk2 $^{-/-}$ mice group. Similar results were obtained in two independent experiments.
Supplementary Fig. 2. Involvement of Tyk2 in IL-23-induced prolonged skin inflammation. (A) The ear pinna of Tyk2+/+ and Tyk2−/− mice was intradermally injected with 1 μg/ear of IL-23 or BSA on an intermissive schedule, 5-times in 10 days. Ear swelling was evaluated on the days as indicated. (B) Epidermal hyperplasia was evaluated by measuring the epidermal thickness by imaging analysis. Data represents mean ± SD (n=5). *p<0.05, **p<0.01, ***p<0.001 compared with BSA-injected mice group; ##p<0.01, ###p<0.001 compared with Tyk2−/− mice group. (C) Representative H&E stained histological features of IL-23-treated ear pinna from Tyk2+/+ and Tyk2−/− mice. Scale bar, 100 μm. (D) Flow cytometry analysis of the dermal cells isolated from pooled Tyk2+/+ and Tyk2−/− ear skin by the intermissive injection. Surface markers of major inflammatory cells were analyzed with fluorescent-labeled specific antibodies. Similar results were obtained in two independent experiments.
Supplementary Fig. 3. Tyk2 directly regulates IL-22-induced skin inflammation. (A) IL-22-dependent inflammatory molecules expression in ear pinna. IL-22 was injected into the ear pinna, then the pinna was harvested from sacrificed Tyk2\(^{+/+}\) and Tyk2\(^{-/-}\) mice at 1, 3 and 6 h after the single injection. Data represents mean ± SD (n=5). *p<0.05, **p<0.01, ***p<0.001 compared with Tyk2\(^{+/+}\) mice group. (B) IL-22-dependent keratinocyte proliferation marker K16 and antimicrobial peptide expressions in the ear pinna. The ear pinna from Tyk2\(^{+/+}\) and Tyk2\(^{-/-}\) mice was harvested at 1, 3 and 6 h after the single IL-22 injection. Similar results were obtained in two independent experiments. (C) Epidermal hyperplasia is not depend on IL-17A in IL-23-induced skin inflammation. Anti-IL-17A neutralization antibody (0.1 mg/mouse) was intraperitoneally injected on day 0, then the ear pinna of Tyk2\(^{+/+}\) mice was intradermally injected with 1 \(\mu\)g/ear of IL-23 or BSA for 4 consecutive days. Epidermal thickness were evaluated on day 4. Data represents mean ± SD (n=5). ***p<0.001 compared with BSA. Similar results were obtained in two independent experiments.