Supplementary Material to

Intercellular communication between keratinocytes and fibroblasts induces local osteoclast differentiation: a mechanism underlying cholesteatoma-induced bone destruction

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Supplementary figure legend

Supplementary Figure 1

Time-course analysis of epidermal cyst-like tissue.

(A) Confocal images of calvarial sections from WT mice injected with EGFP-expressing keratinocytes and CAG-EGFP-expressing fibroblasts at 2 and 4 days after injection. Scale bar; 250 mm. Representative images of three mice are shown. (B) The width and the thickness of epidermal cyst-like tissue at each time period.

Supplementary Figure 2

Expression of keratinocyte differentiation markers in normal mouse ear pinna and epidermal cyst-like tissue.

Immunohistochemical analysis of normal mouse ear pinna (upper) and epidermal cyst-like tissue (lower) using anti-CK14 (left) or anti-CK10 antibodies (right). No significant staining was observed in a specific isotype-matched negative control (left). Representative images of three mice are shown.

Supplementary Figure 3

Expression of RANKL in epidermal cyst-like tissue and normal dermal tissues.

(A) RANKL expression in normal dermla tissues, normal mouse skin (left) and ear pinna (right.) Isotype matched control (lower) and anti-RANKL antibody (upper). Red-boxed regions in the left

images are magnified in the right images. Arrowheads indicate RANKL-expressing cells. Arrows indicate melanocytes. (B) RANKL expression in epidermal cyst-like tissues at 2, 4 and 7 days after injection (right). No significant staining was observed in a specific isotype-matched negative control (left). Scale bar, 200 mm. Representative images of three mice are shown.

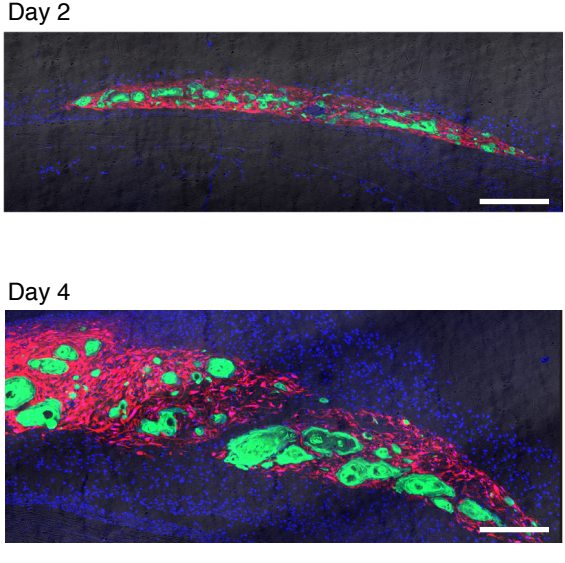
Supplementary Figure 4

Effect of cytokines secreted from keratinocytes on *Tnfsf11* mRNA expression in fibroblasts.

(A) Effect of LPS and supernatant from keratinocyte culture on *Tnfsf11* mRNA expression in ear pinna-derived fibroblasts, measured by RT-qPCR analysis. Relative expression to fibroblasts cultured in fresh media without LPS. (B) Relative mRNA expression of *Lgals3, Plau, Slpi and Vegfa*, *Grn* (encoding Progranulin), *Il9* (encoding IL-9), *Cxcl1* (encoding CXCL-1), *Csf3* (encoding G-CSF) and *Cxcl16* (encoding CXCL-16) in ear-pinna derived fibroblasts and keratinocytes. Data are presented as mean ± SD. (one-way ANOVA in A)

Supplementary Figure 1

A



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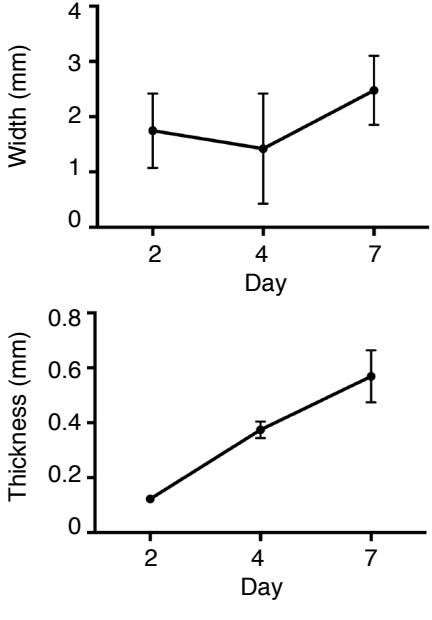
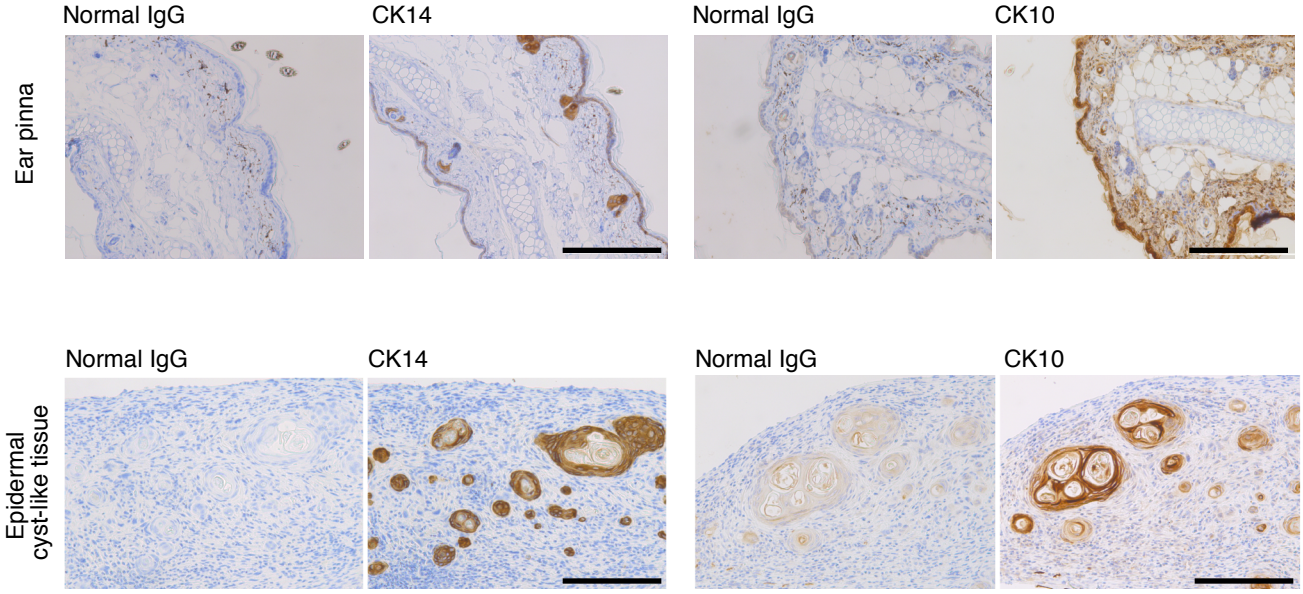
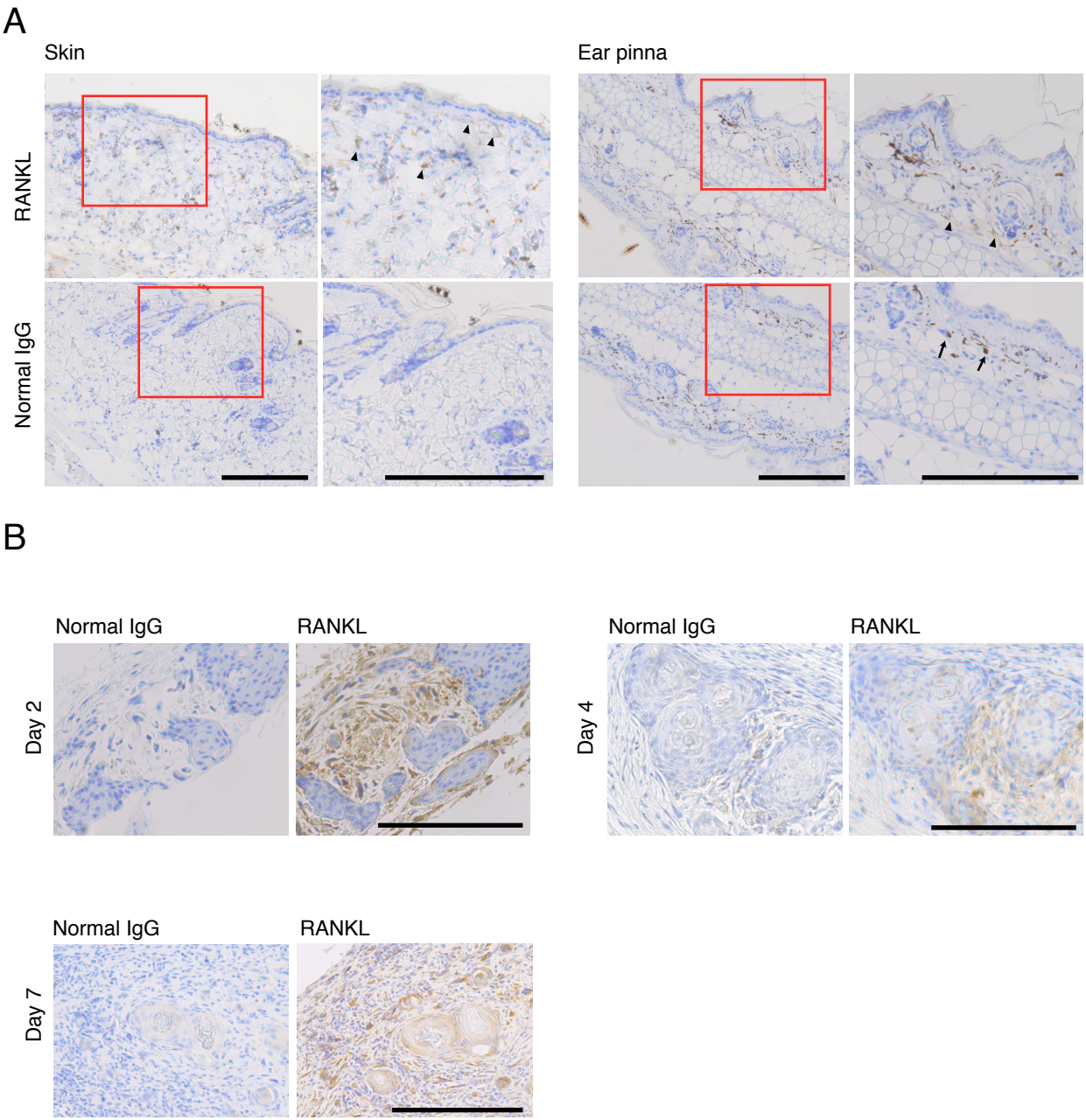


Supplementary Figure 2



Supplementary Figure 3



Supplementary Figure 4