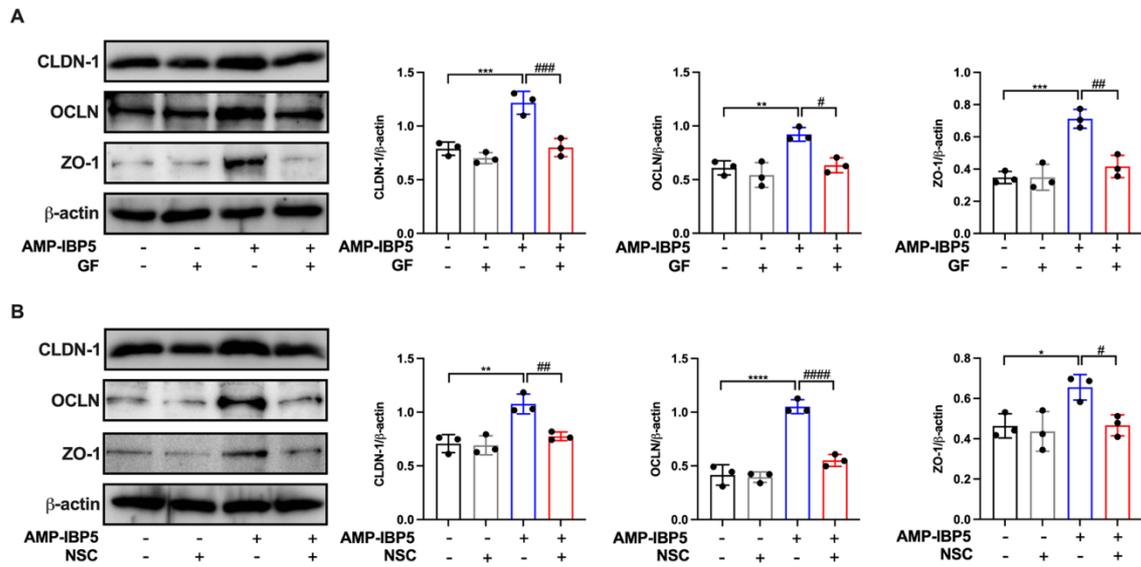
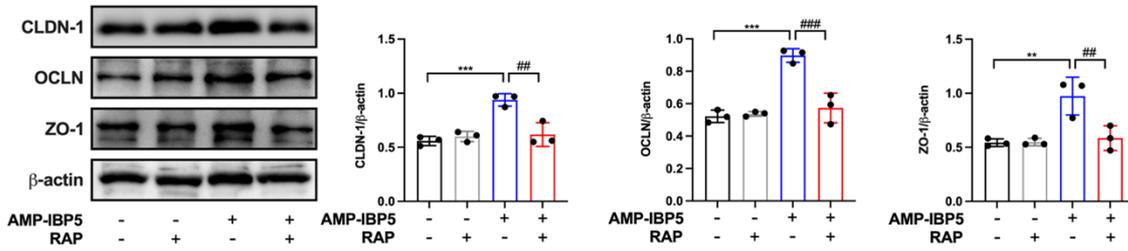


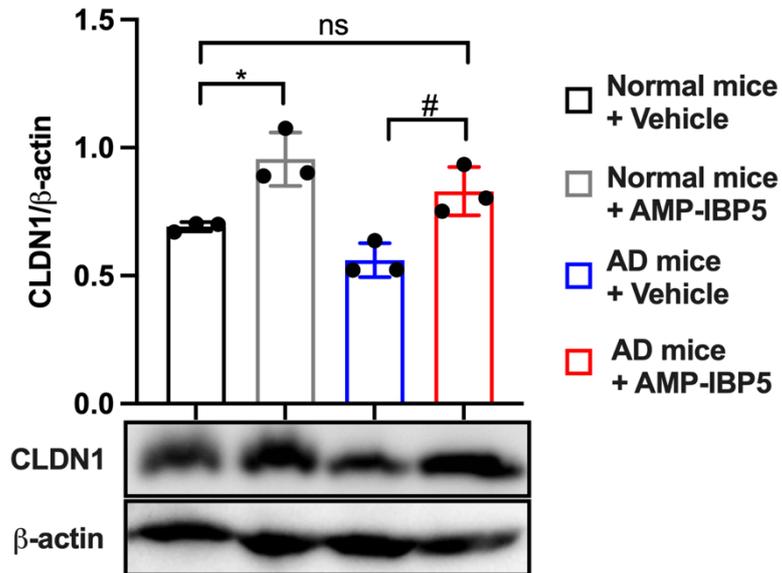
**Figure S1. AMP-IBP5 enhanced TJ barrier function in human keratinocytes.** (A) Human primary keratinocytes grown to confluence on collagen I-coated chamber slides were incubated with 10  $\mu$ M AMP-IBP5 for 48 hours, and immunofluorescence staining of TJ-related proteins, including claudin-1, -4 and -7, occludin, ZO-1 and isotype control, upon stimulation with AMP-IBP5 is shown. Scale bar: 50  $\mu$ m. (B) Keratinocyte layers grown on Transwell inserts were incubated with 2.5, 5, 10, or 20  $\mu$ M AMP-IBP5 for 120 hours, and TER was measured by CellZscope. The data are presented as the means  $\pm$  SDs. \* $P$  < 0.05, \*\* $P$  < 0.01, \*\*\* $P$  < 0.001. Statistical significance was determined by one-way ANOVA with Tukey's multiple comparisons test. All data are representative of three independent experiments.



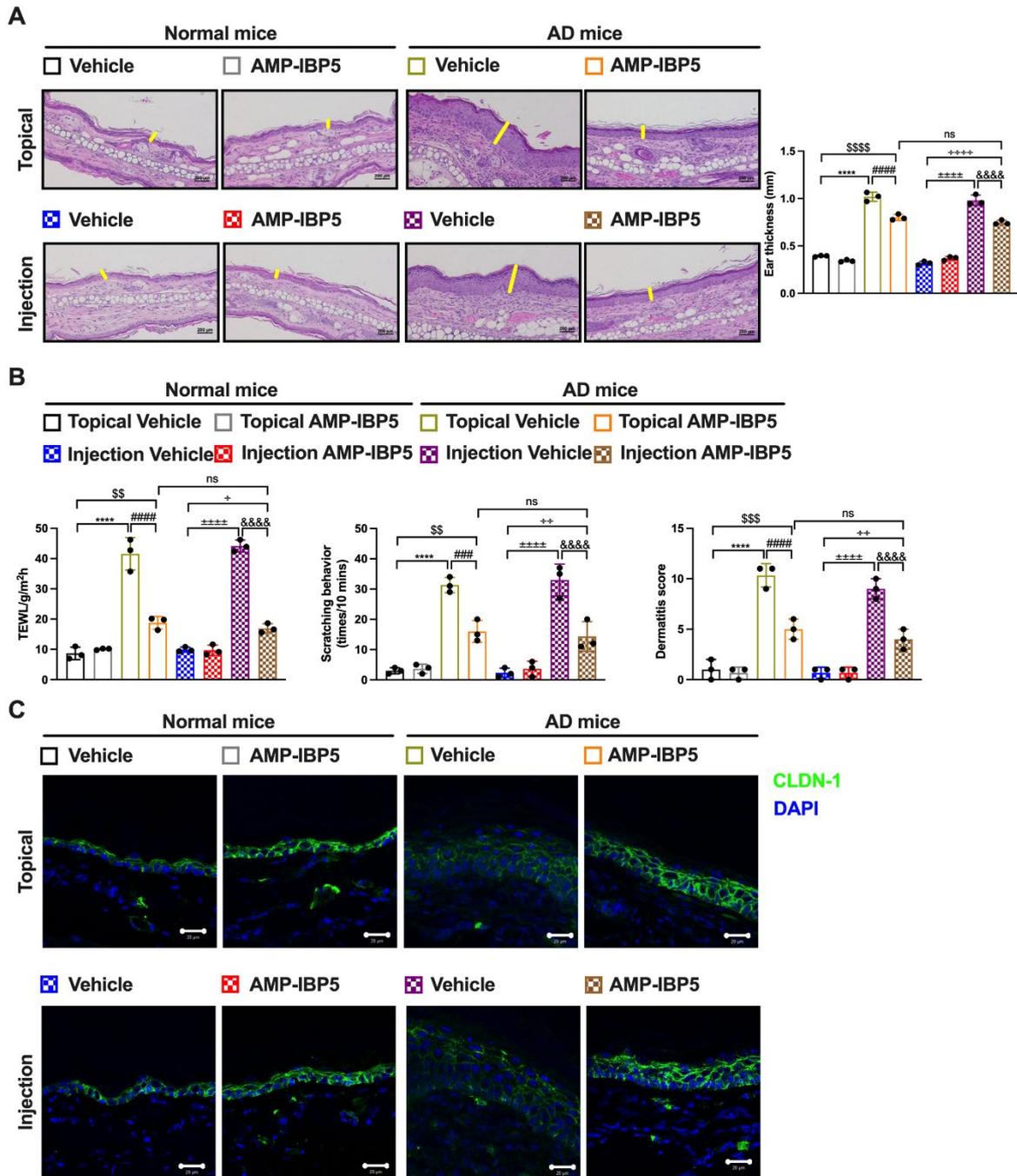
**Figure S2. The expression of TJ-related proteins was blocked by treatment with inhibitors of PKC $\zeta$  and Rac1.** Human primary keratinocytes were pretreated with 100 nM GF 109203X (A) and 20  $\mu$ M NSC23766 (B) for 24 hours, stimulated with 10  $\mu$ M AMP-IBP5 for 48 hours and subjected to Western blotting using antibodies specific for claudin-1, occludin and ZO-1. Bands were quantified using densitometry;  $n=3$ /group. The data are presented as the means  $\pm$  SDs. \* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$ , \*\*\*\* $P < 0.0001$ , # $P < 0.05$ , ## $P < 0.01$ , ### $P < 0.001$ , #### $P < 0.0001$ . Statistical significance was determined by one-way ANOVA with Tukey's multiple comparisons test. All data are representative of three independent experiments.



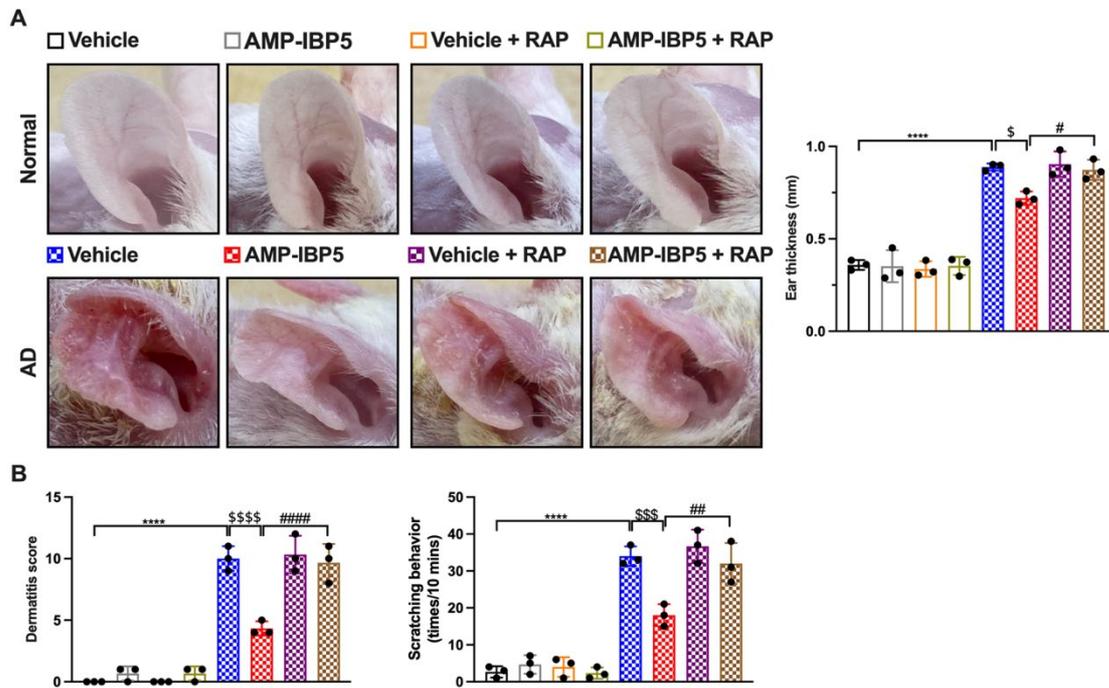
**Figure S3. The expression of TJ-related proteins was blocked by treatment with an LRP1 antagonist.** Human primary keratinocytes were pretreated with 1  $\mu\text{g/ml}$  RAP for 24 hours, stimulated with 10  $\mu\text{M}$  AMP-IBP5 for 48 hours and subjected to Western blotting using antibodies specific for claudin-1, occludin and ZO-1. Bands were quantified using densitometry;  $n=3/\text{group}$ . The data are presented as the means  $\pm$  SDs.  $**P < 0.01$ ,  $***P < 0.001$ ,  $##P < 0.01$ ,  $###P < 0.001$ . Statistical significance was determined by one-way ANOVA with Tukey's multiple comparisons test. All data are representative of three independent experiments.



**Figure S4. Administration of AMP-IBP5 restored the expression of claudin-1 in AD mice.** The ear skin of the AD mice was subcutaneously injected with 25  $\mu$ l of 25  $\mu$ M AMP-IBP5 on Days 15, 17, and 18. On Day 19, the skin biopsies were harvested and subjected to Western blotting using antibodies specific for claudin-1. Bands were quantified using densitometry; n=3 mice/group. The data are presented as the means  $\pm$  SDs. \* $P$  < 0.05, # $P$  < 0.05. Statistical significance was determined by one-way ANOVA with Tukey's multiple comparisons test. All data are representative of three independent experiments.



**Figure S5.** Topical application and injection of AMP-IBP5 alleviated dermatitis-like symptoms in AD mice to similar degrees. (A) Representative histological sections of mouse ears stained with H&E. The yellow lines indicate the epidermis (left panel, scale bars: 200  $\mu$ m). Ears thickness of mice are shown (right panel,  $n=3$  mice/group). (B) The dermatitis score, scratching behavior and transepidermal water loss (TEWL) of mice are shown. (C) Representative immunofluorescence images of claudin-1 in the mouse epidermis. Scale bar: 20  $\mu$ m;  $n=3$ /group. \*\*\*\* $P < 0.0001$ , ### $P < 0.001$ ,#### $P < 0.0001$ , §§ $P < 0.01$ , \$\$\$ $P < 0.001$ , ssss $P < 0.0001$ , ++++ $P < 0.0001$ , + $P < 0.05$ , ++ $P < 0.01$ , +++ $P < 0.0001$ . Statistical significance was determined by one-way ANOVA with Tukey's multiple comparisons test. All data are representative of three independent experiments.



**Figure S6. The restorative effect of AMP-IBP5 on skin barrier function in mice is blocked by RAP injection.** (A) Representative images of the ears from mice (left) and the ear thickness (right) are depicted; n=3 mice/group. (B) The dermatitis scores and scratching behavior of AD mice are shown. The data are presented as the means  $\pm$  SDs. \*\*\*\* $P < 0.0001$ , # $P < 0.05$ , ## $P < 0.01$ , #### $P < 0.0001$ , \$ $P < 0.05$ , \$\$\$ $P < 0.001$ , \$\$\$\$ $P < 0.0001$ . Statistical significance was determined by one-way ANOVA with Tukey's multiple comparisons test.