

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 μ 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 μ m. (B) Keratinocyte layers grown on Transwell inserts were incubated with 2.5, 5, 10, or 20 μ 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.

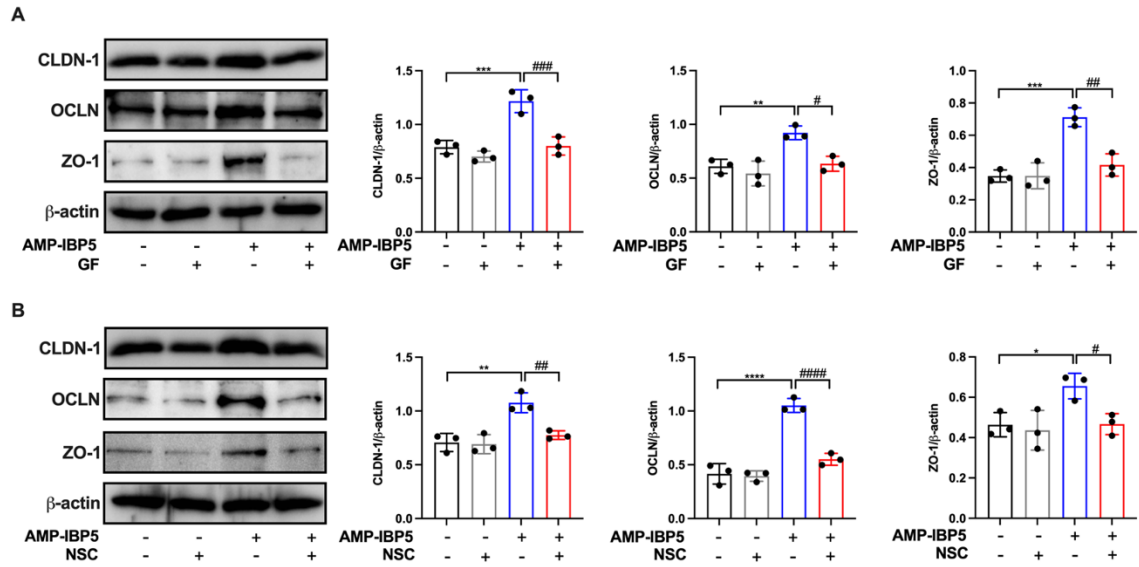


Figure S2. The expression of TJ-related proteins was blocked by treatment with inhibitors of PKC ζ and Rac1. Human primary keratinocytes were pretreated with 100 nM GF 109203X (A) and 20 μ M NSC23766 (B) for 24 hours, stimulated with 10 μ M AMP-IBP5 for 48 hours and subjected to Western blotting using 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.

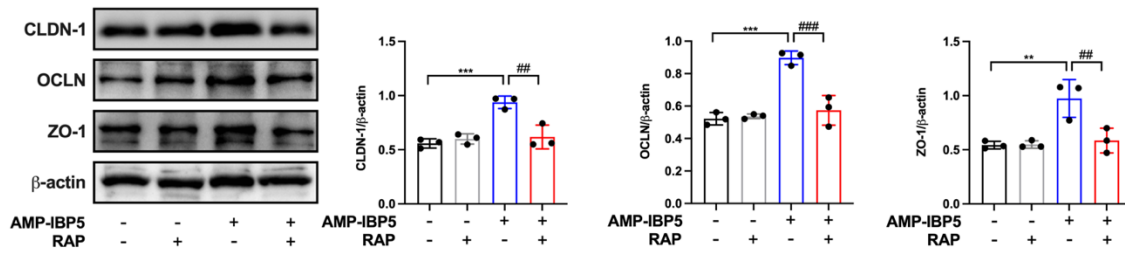


Figure S3. The expression of TJ-related proteins was blocked by treatment with an LRP1 antagonist. Human primary keratinocytes were pretreated with 1 μ g/ml RAP for 24 hours, stimulated with 10 μ M AMP-IBP5 for 48 hours and subjected to Western blotting using antibodies specific for claudin-1, occludin and ZO-1. Bands were quantified using densitometry; n=3/group. The data are presented as the means \pm SDs. ** P < 0.01, *** 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.

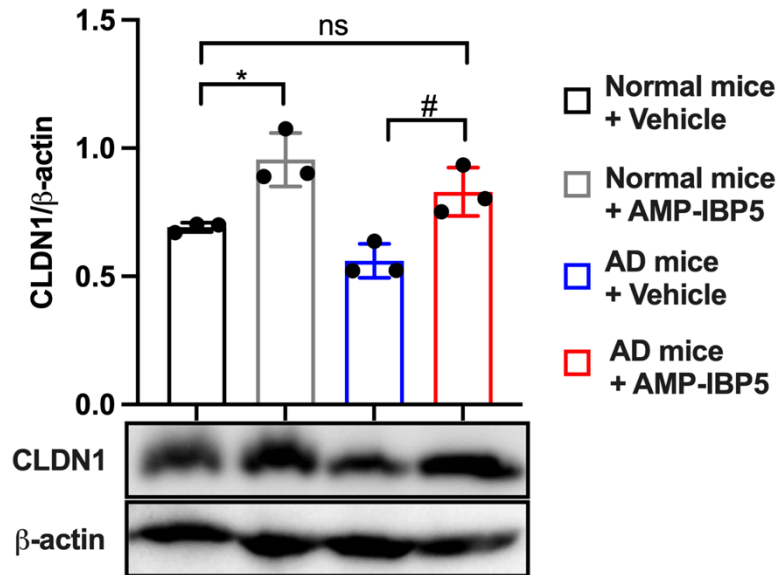


Figure S4. Administration of AMP-IBP5 restored the expression of claudin-1 in AD mice. The ear skin of the AD mice was subcutaneously injected with 25 μ l of 25 μ M AMP-IBP5 on Days 15, 17, and 18. On Day 19, the skin biopsies were harvested and subjected to Western blotting using antibodies specific 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.

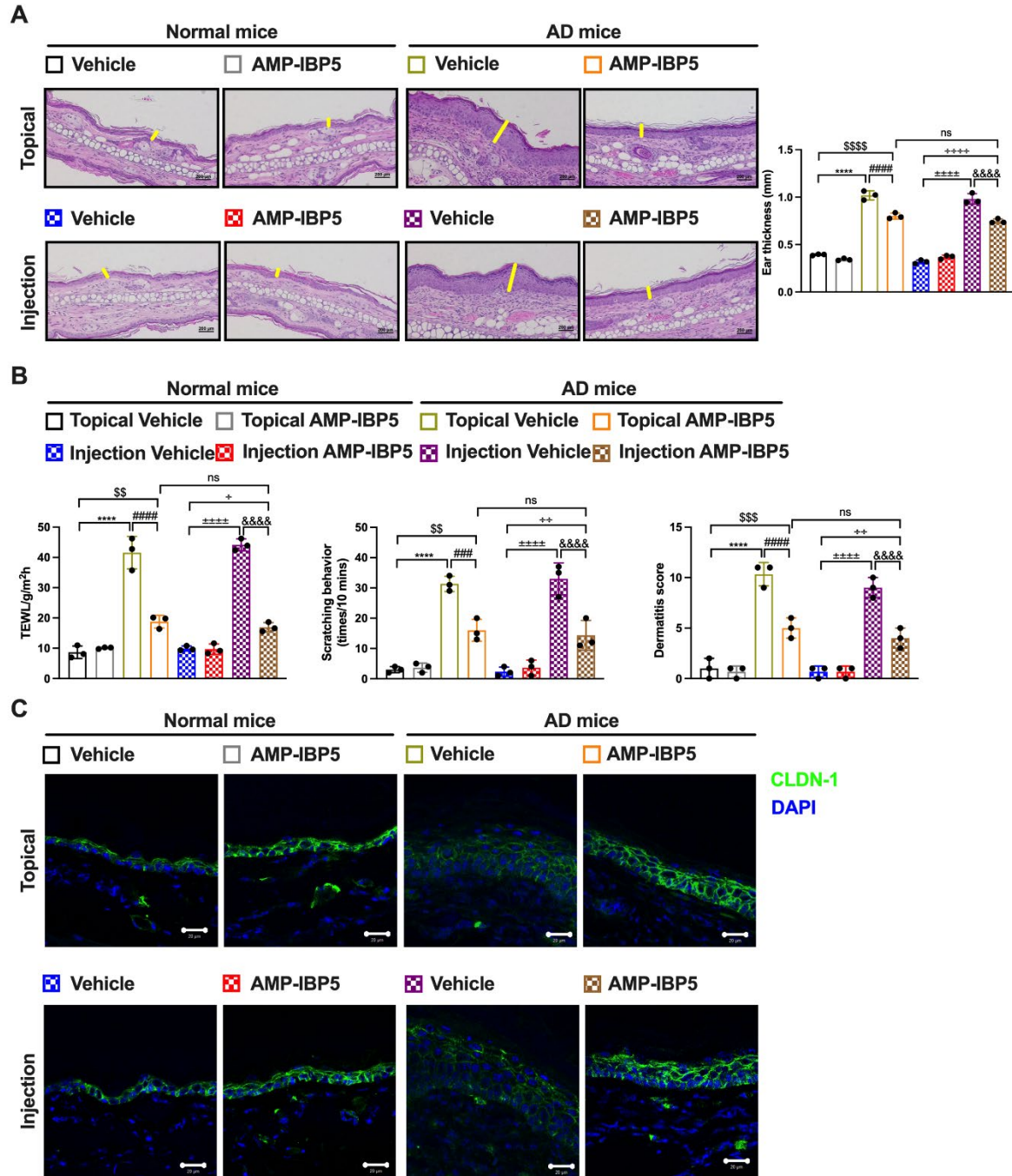


Figure S5. Topical application and injection of AMP-IBP5 alleviated dermatitis-like symptoms in AD mice to similar degrees. (A) Representative histological sections of mouse ears stained with H&E. The yellow lines indicate the epidermis (left panel, scale bars: 200 μm). Ears thickness of mice are shown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

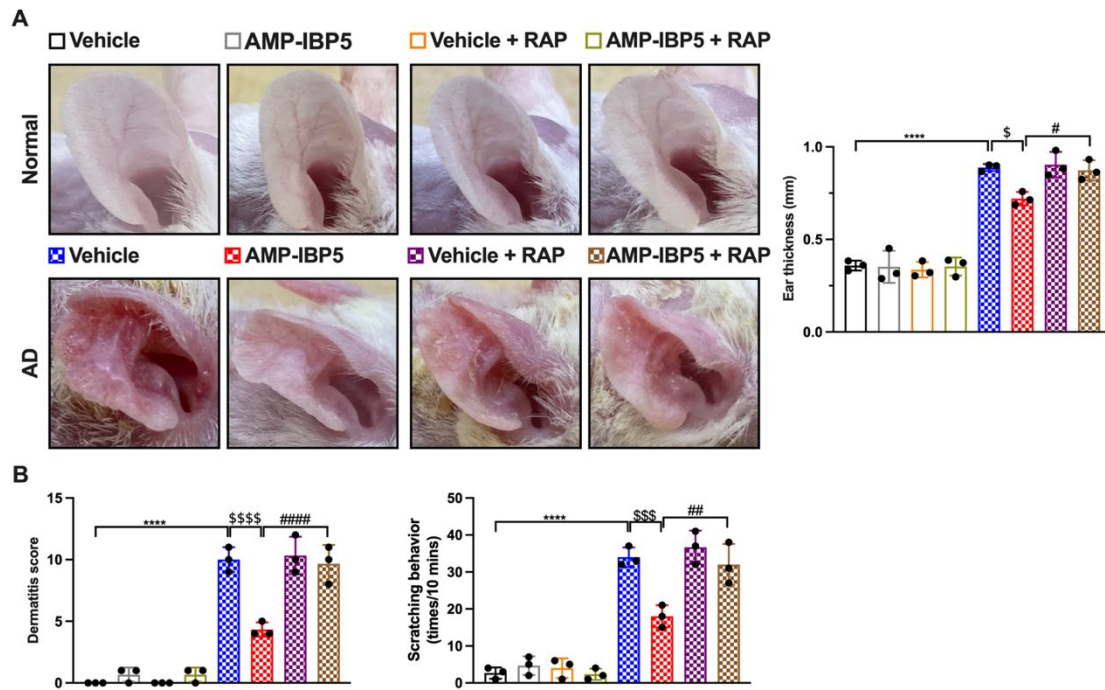


Figure S6. The restorative effect of AMP-IBP5 on skin barrier function in mice is blocked by RAP injection. (A) Representative images of the ears from mice (left) and the ear thickness (right) are depicted; $n=3$ mice/group. (B) The dermatitis scores and scratching behavior of AD mice are shown. The 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.