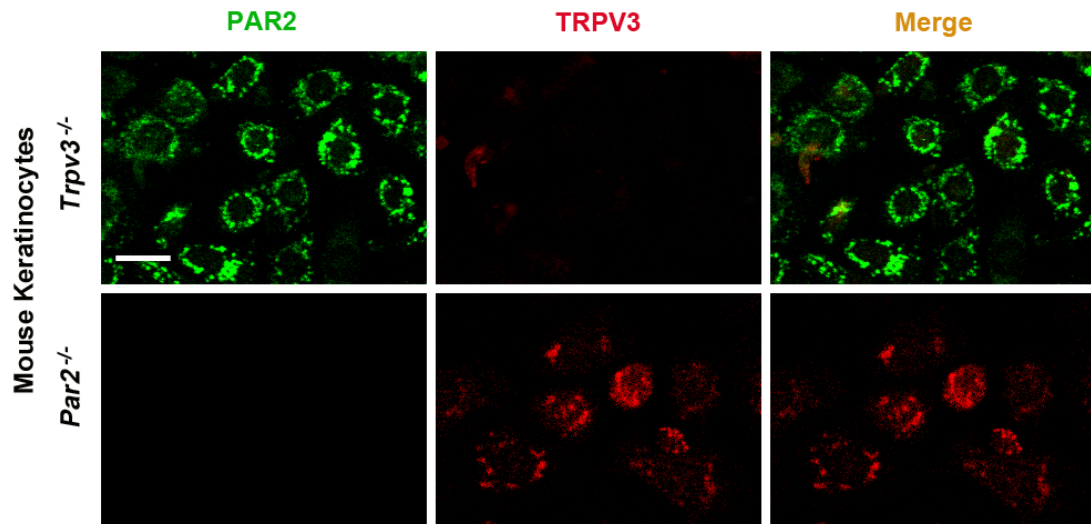


## **Supplementary Information for**

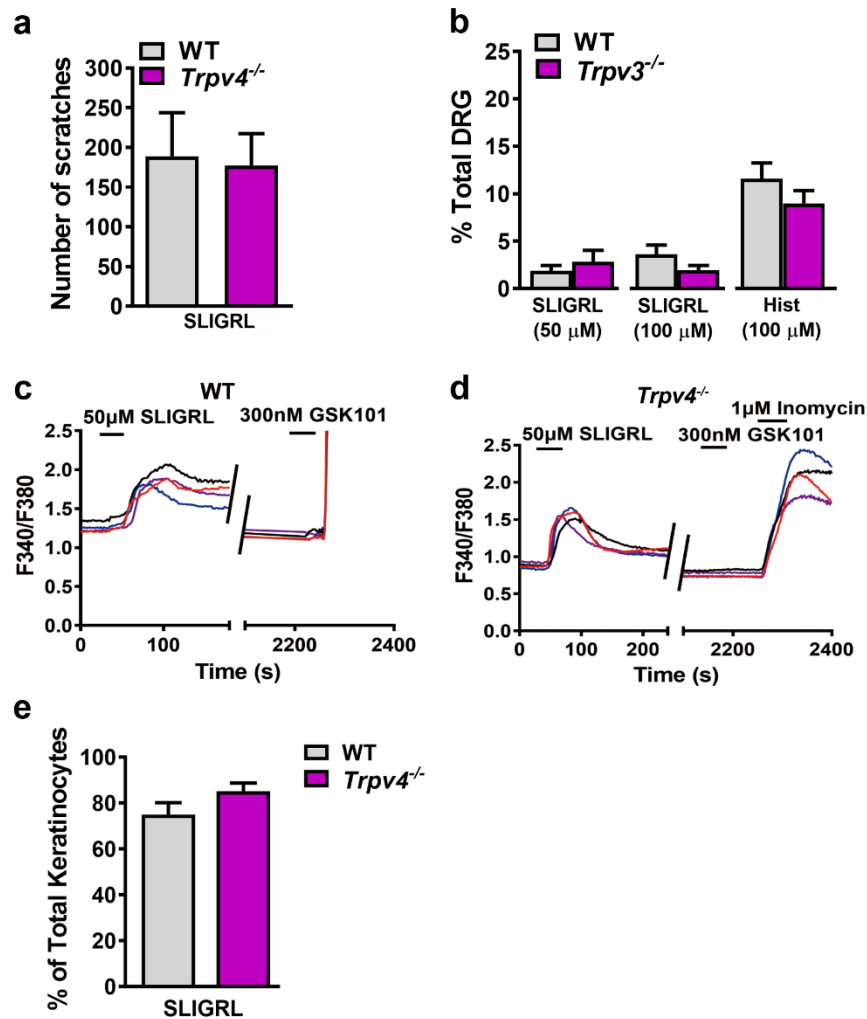
### **PAR2 mediates itch via TRPV3 signaling in keratinocytes**

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**Supplementary Figure S1. Anti-PAR2 and anti-TRPV3 antibodies are specific.**

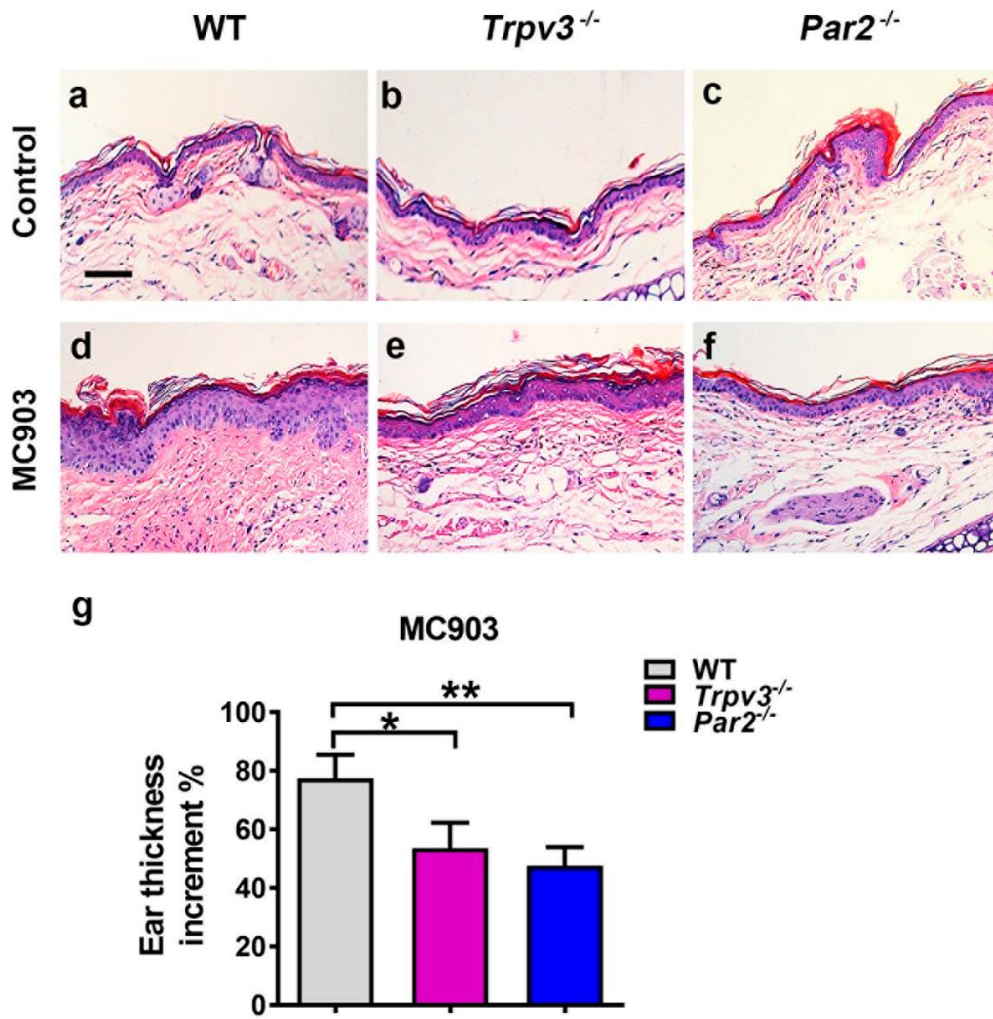
The immunoreactivity to anti-TRPV3 and anti-PAR2 antibodies was positive in keratinocytes from *Par2*<sup>-/-</sup> and *Trpv3*<sup>-/-</sup> mice and was absent in keratinocytes from *Trpv3*<sup>-/-</sup> and *Par2*<sup>-/-</sup> mice, respectively. Scale bar, 20  $\mu$ M.



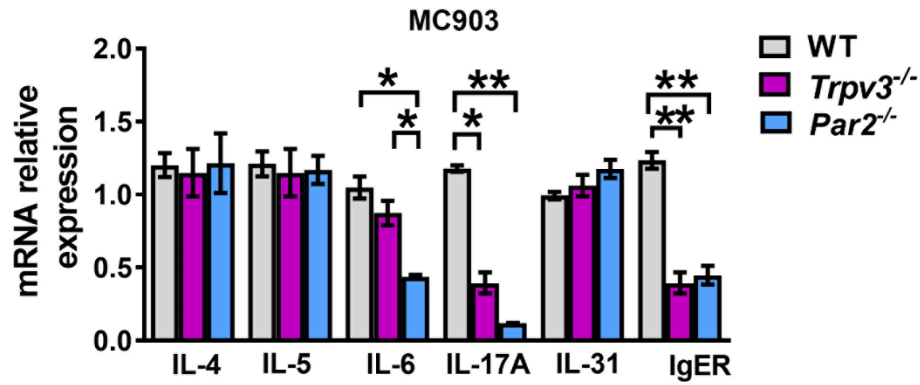
**Supplementary Figure S2. TRPV4 in keratinocytes and TRPV3 in DRG were not involved in PAR2 activation-induced itch signaling.**

(a) No significant difference in SLIGRL-induced scratching number was found between *Trpv4*<sup>-/-</sup> mice and their WT littermates (n=6-8). (b) There were no statistical differences in the percentage of DRG with Ca<sup>2+</sup> fluorescence after stimulation with 50 μM SLIGRL, 100 μM SLIGRL and 100 μM histamine between DRG from WT and *Trpv3*<sup>-/-</sup> mice. n = 3 mice per group. The total number of neurons is 917 and 1078 for WT and *Trpv3*<sup>-/-</sup> group, respectively. (c, d) Keratinocytes were incubated with 50 μM SLIGRL, and the intensity of SLIGRL-induced Ca<sup>2+</sup> fluorescence was similar between keratinocytes from WT and *Trpv4*<sup>-/-</sup> mice. The subsequent high responses to GSK101 (TRPV4 agonist) and inomycin confirmed that the SLIGRL-induced changes were

obtained from reactive cells. **(e)** Percentage of Trpv4<sup>-/-</sup> positive keratinocytes after SLIGRL stimulation relative to WT controls (Student's unpaired t test, n=80-100 cells per experiment, 3 independent experiments).



**Supplementary Figure S3. The ear inflammation reduced in *Trpv3*<sup>-/-</sup> and *Par2*<sup>-/-</sup> mice compared with WT samples in MC903-induced AD-like skin lesion. (a-c)** Hematoxylin-eosin staining of healthy controls in the ear of WT, *Trpv3*<sup>-/-</sup> and *Par2*<sup>-/-</sup> mice. **(d-f)** Pathological appearance showing changes in hyperkeratosis and lymphocytes infiltration in the ears of corresponding mice in panels a-c, respectively. **(g)** The ear thickness increment of WT, *Trpv3*<sup>-/-</sup> and *Par2*<sup>-/-</sup> mice after MC903 treatment for 7 days. \*p < 0.05, \*\*p < 0.01, one-way ANOVA followed by Tukey's post hoc. n = 4 mice per group. Scale bar, 50  $\mu$ M.



**Supplementary Figure S4. IL-6, IL-17A and IgER mRNA levels was significantly reduced in the ear samples from AD models of *Par2*<sup>-/-</sup> and *Trpv3*<sup>-/-</sup> mice.** The ear sections were collected from the AD models of WT, *Par2*<sup>-/-</sup> and *Trpv3*<sup>-/-</sup> mice after topical application of MC903 for 7 days. \*p < 0.05, \*\*p < 0.01, one-way ANOVA followed by Tukey's post hoc. n = 3-4 mice per group.

**Supplementary Table S1. Primer sequences for quantitative RT-PCR**

	Sense Primer ( 5'-3' )	Antisense Primer ( 5'-3' )
<b>mIL-4</b>	5'-GGTCTCAACCCCAGCTAGT-3'	5'-GCCGATGATCTCTCTCAAGTGAT-3'
<b>mIL-5</b>	5'-CGCTCACCGAGCTCTGTTG-3'	5'-CCAATGCATAGCTGGTGATT-3'
<b>mIL-6</b>	5'-GAGGATACCACTCCCAACAGACC-3'	5'-AAGTGCATCATCGTTGTTTCATACA-3'
<b>mIL-17A</b>	5'- GGCTGCCTAAACCACGTAATG -3'	5'- CCCGTTGAATGAGAATCGTGT -3'
<b>mIL-31</b>	5'- TCAGCAGACGAATCAATACAGC -3'	5'- TCGCTCAACACTTTGACTTTCT -3'
<b>mFcer1a</b>	5'-GAGTGCCACCGTTCAAGACA-3'	5'-GTAGATCACCTTGCGGACATTC-3'
<b>mTrpv3</b>	5'-CTCAAACCTCCAAGATGGTCCT-3'	5'-TCACCTATGTCATCCTCACCT-3'
<b>mPar2</b>	5'-ATGCGAAGTCTCAGCCTGGC -3'	5'-AGGTCATGAAGAGGATGGAGCAATAC-3'
<b>mTslp</b>	5'-GCCAGGGATAGGATTGAGAGTA-3'	5'- GGCTAAGTTCGAGCAAATCG -3'
<b>mGapdh</b>	5'- CCCAGCAAGGACACTGAGCAA -3'	5'- TTATGGGGGTCTGGGATGGAAA -3'
<b>hPAR2</b>	5'-CGTTCTTTGCATGATCCCTGA-3'	5'-GCCATGTCTATGCCCTGTA-3'
<b>hTRPV3</b>	5'-GCGTCTTCCATTCAGTCCA-3'	5'-CAGGACCATCTTGGAGTTTGAG-3'
<b>hGAPDH</b>	5'-TGTAGTTGAGGTCAATGAAGGG-3'	5'-ACATCGCTCAGACACCATG-3'