

Supplementary Materials

Title: A topical Chinese herbal inhibits pruritus and skin inflammation via neural TRPM8 in atopic dermatitis

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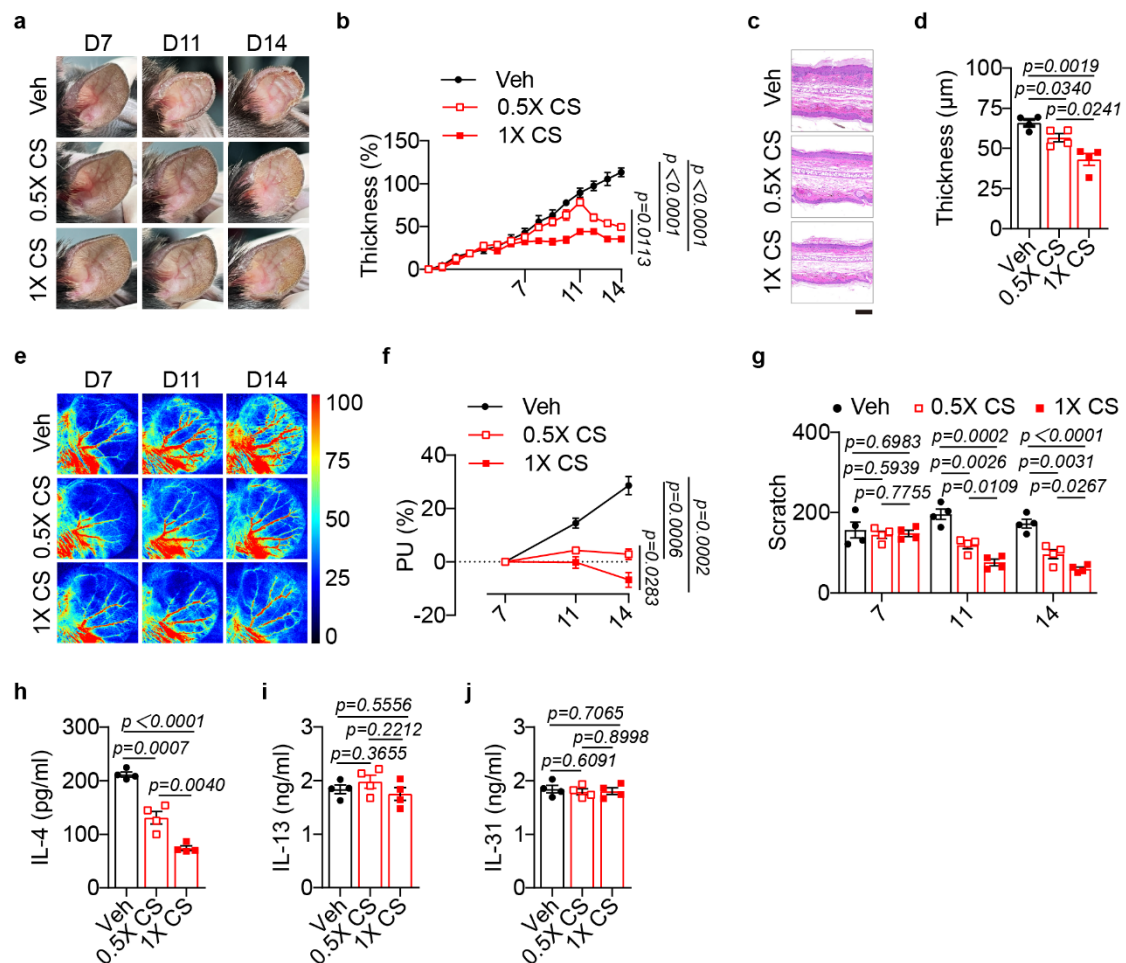
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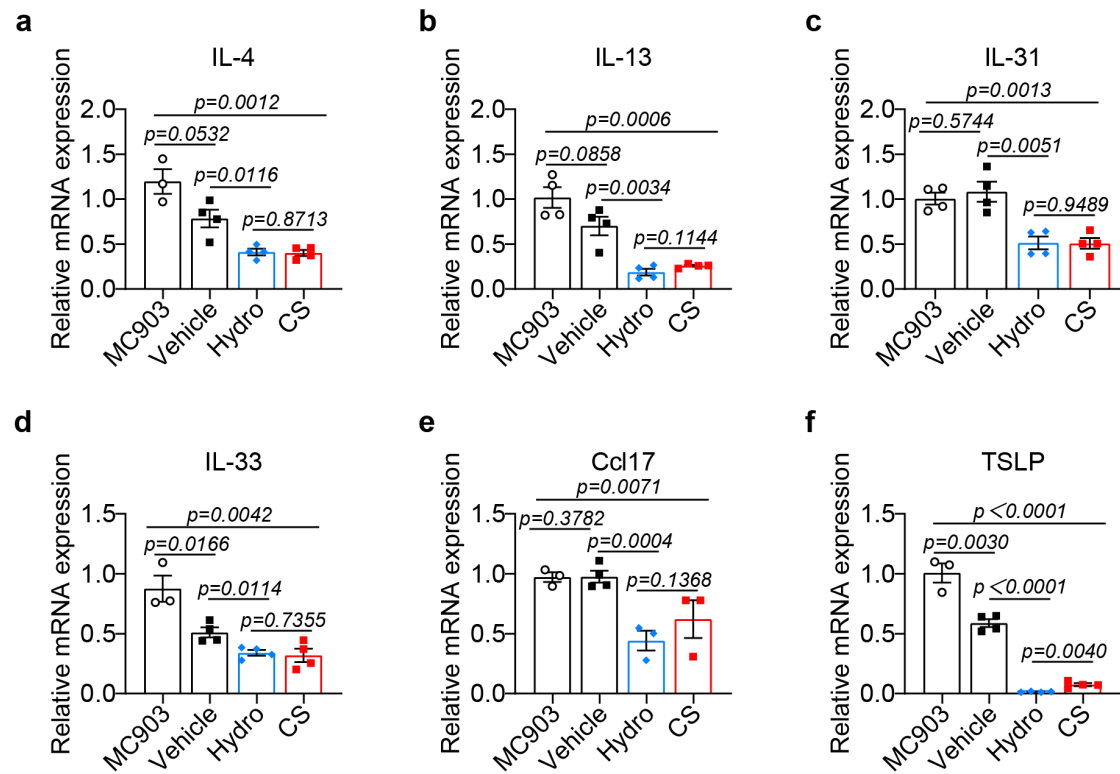
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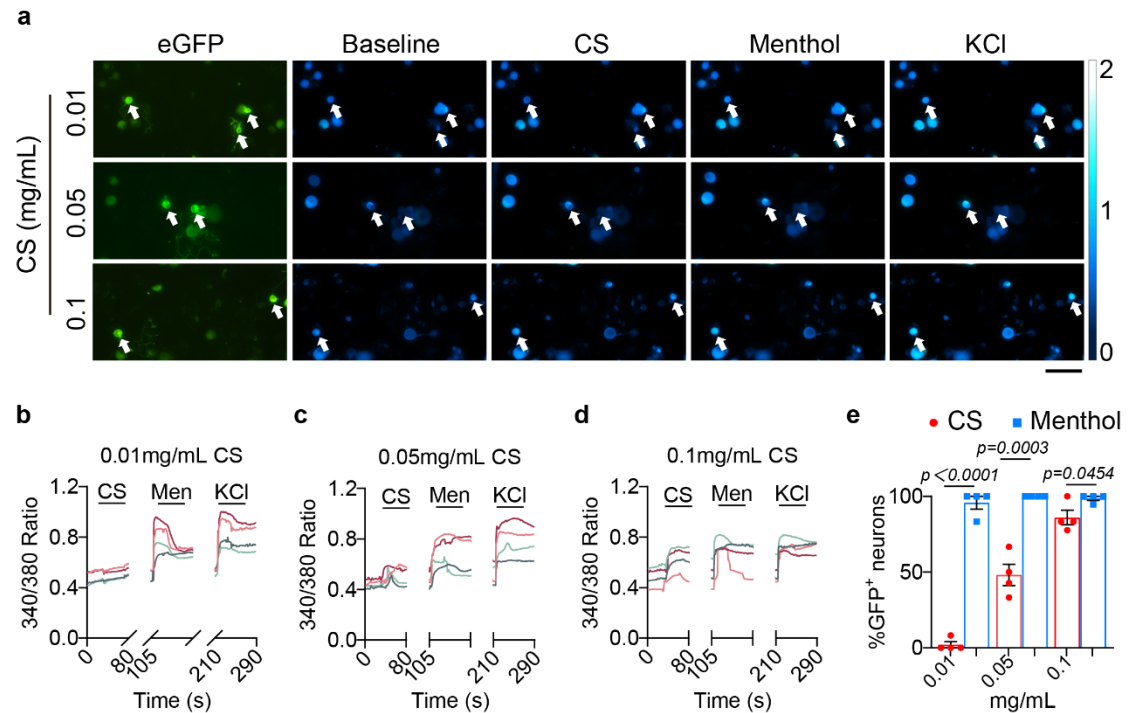
Supplementary Figures:



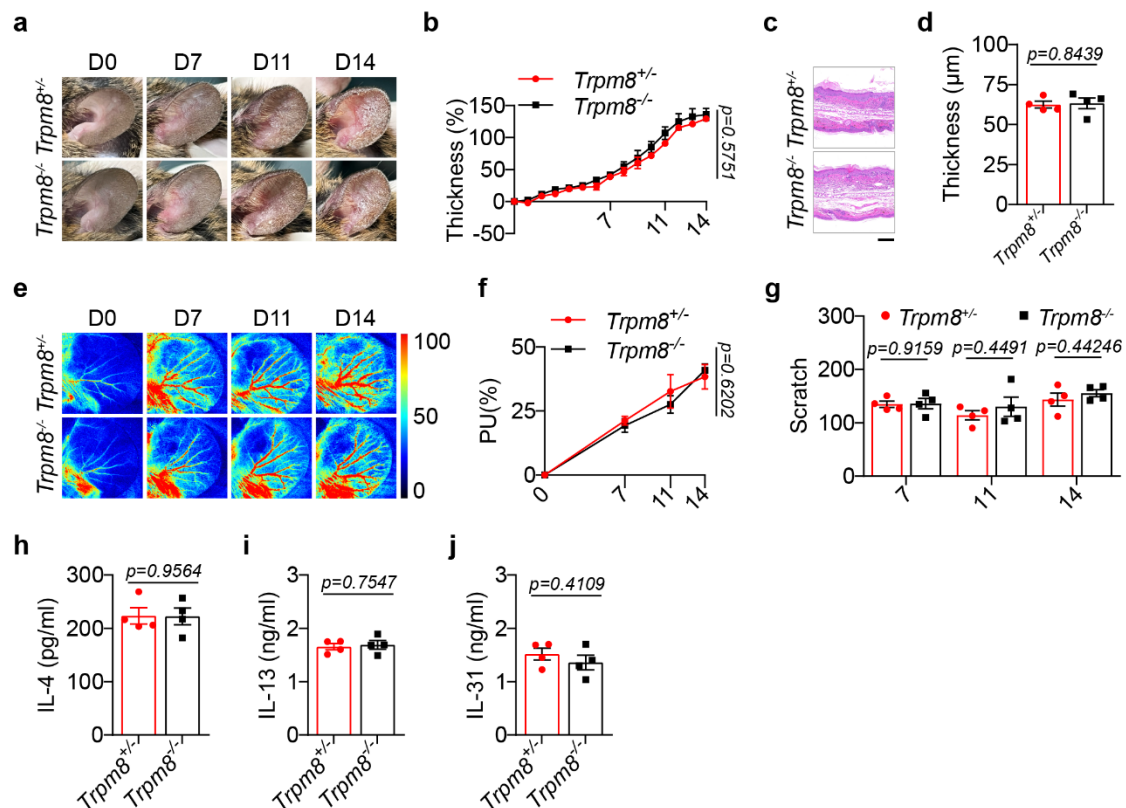
Supplementary Figure 1. CS ointment reduces inflammation and itching in AD-like mice in a dose-dependent manner. (a) Representative images showing the appearance of mouse ears after treatment with Vehicle, 0.5X CS, and 1X CS. (b) Ear thickness measurements were quantified in mice treated with vehicle, 0.5X CS and 1X CS (n = 4 mice per group). (c) Histological examination of ear skin sections obtained on day 14 after treatment with vehicle, 0.5X CS and 1X CS reveals changes in tissue morphology (scale bar = 100 μm). (d) The epidermal thickness of mouse ears was measured (n = 4 mice per group). (e-f) The laser speckle contrast imaging method detected decreased perfusion velocity in mice treated with 0.5X CS and 1X CS, but not with vehicle (e, quantified in f, n = 4 mice per group). (g) Scratching behavior was quantified in treated mice over a 30-minute period. (n = 4 mice per group). (h-j) Lysate assays analyzed the expression of inflammatory cytokines IL-4, IL-13 and IL-31 in ear tissue samples collected on day 14 from mice treated with vehicle, 0.5X CS and 1X CS (n = 4 mice per group). All representative images represent multiple mice (minimum of three) per condition. Data are expressed as mean ± SEM. Statistical significance is determined by using a two-tailed Student's t-test (b, d, f, g, h, i, j).



Supplementary Figure 2. In MC903-treated mice, CS and Hydro treatment significantly reduced the expression level of pro-inflammatory factors. IL-4 (a), IL-13 (b), IL-31 (c), IL-33 (d), Ccl17 (e) and TSLP (f) were detected by RT-PCR. Data are expressed as mean $\pm$ SEM. Statistical significance is determined by using a two-tailed Student's t-test.



Supplementary Figure 3. TRPM8 neurons are activated dose-dependently by CS ointment. (a) TRPM8-eGFP labelled TRPM8⁺ sensory neurons cultured from *Trpm8*^{EGFP/+} (*Trpm8*^{+/-}) mice exhibited responses to CS dose-independently, menthol (100 μ M) and KCl (50 μ M). White arrows indicate TRPM8-eGFP neurons (Scale bar = 100 μ m). (b-d) Representative calcium transients of sensory neurons from *Trpm8*^{+/-} mice in response to CS, menthol (Men) and KCl are shown. Each colored trace represents one TRPM8-eGFP neuron. The images are representative of five independent experiments using dorsal root ganglion and trigeminal ganglion from at least three individual mice. (b-d, quantified in e). Data are expressed as mean $\pm$ SEM. Statistical significance is determined using two tailed Student's t-test (e).



Supplementary Figure 4. *Trpm8*^{+/-} and *Trpm8*^{-/-} mice after MC903 treatment showed no difference in AD-like symptoms. (a) Representative images showing the appearance of mouse ears after application of MC903. (b) Ear thickness measurements were quantified in *Trpm8*^{+/-} and *Trpm8*^{-/-} mice induced by MC903 (n = 4 mice per group). (c) Histological examination of ear skin sections obtained on day 14 after MC903 (scale bar = 100 μm). (d) The epidermal thickness of mouse ears was measured (n = 4 mice per group). (e-f) The laser speckle contrast imaging method detected decreased perfusion velocity in both *Trpm8*^{+/-} and *Trpm8*^{-/-} mice treated with MC903 (e, quantified in f, n = 4 mice per group). (g) Scratching behavior was quantified in treated mice over a 30-minute period. (n = 4 mice per group). (h-j) Lysate assays analyzed the expression of inflammatory cytokines IL-4, IL-13 and IL-31 in ear tissue samples collected on day 14 from mice induced by MC903 on day 14 (n = 4 mice per group). All representative images represent multiple mice (minimum of three) per condition. Data are expressed as mean ± SEM. Statistical significance is determined by using a two-tailed Student's t-test (b, d, f, g, h, i, j).

Supplementary Table 1. Information of herb used in CS ointment.

Latin name	English name	Part of traditional Chinese medicines
<i>Cnidii Fructus</i>	Common Cnidium Fruit	Dried mature fruit of <i>Cnidium monnieri</i> (L.) Cuss.
<i>Coptidis Rhizoma</i>	Coptis Root,Chinese Goldthread	Dried rhizome of <i>Coptis chinensis</i> Franch.
<i>Phellodendri Chinensis Cortex</i>	Amur Cork-tree Bark	Dried bark of <i>Phellodendron chinense</i> Schneid.
<i>Dictamni Cortex</i>	Densefruit Pittany Root- bark	Dried root bark of <i>Dictamnus dasycarpus</i> Turcz.
<i>Sophorae Flavescentis Radix</i>	Lightyellow Sophora Root	Dried roots of the leguminous plant <i>Sophora flavescens</i> Ait.
<i>Polygoni Cuspidati Rhizoma et Radix</i>	Giant Knotweed Rhizome	Dried rhizome and roots of <i>Polygonum cuspidatum</i> Sieb.et Zucc.
<i>Violae Herba</i>	Violae Herba	Dried whole herb of <i>Viola yedoensis</i> Makino, family Corydaceae.
<i>Kochiae Fructus</i>	<i>Kochiae Fructus</i>	Dried mature fruit of <i>Quinoa Kochia scoparia</i> (L.) Schrad.
<i>Polygoni Avicularis Herba</i>	/	Dried above-ground part of <i>Polygonum aviculare</i> L.
<i>Artemisiae Scopariae Herba</i>	Capillary Wormwood Herb	Dried above-ground parts of <i>Artemisia scoparia</i> Waldst. et Kit. or <i>A. capillaris</i> Thunb.
<i>Atractylodis Rhizoma</i>	Rhizoma Atractylodis	Dried rhizome of <i>Atractylodes lancea</i> (Thunb.) DC. or <i>Atractylodes chinensis</i> (DC.) Koidz.
<i>Zanthoxylum bungeanum</i>	/	The pericarp of peppercorns and green peppercorns of the family Rutaceae.
<i>Borneolum Syntheticum</i>	Synthetic Borneol	Crystals chemically synthesised from camphor, turpentine, etc.