Supporting Information

Scutellarein Attenuates Atopic Dermatitis by Selectively Inhibiting Transient Receptor Potential Vanilloid 3

Supplementary Methods

Intracellular calcium measurements

Changes in intracellular Ca^{2+} levels were monitored as described previously (Yang et al., 2019). HEK-293 cells expressing hTRPV1, mTRPV2 and mTRPV3, respectively, were seeded in 96-well plates (Corning, NY, USA) at a density of 2×10^4 cells/well and incubated overnight. After removing the growth medium, cells were incubated with 4 μM Fluo-4/AM in 100 μL Locke's buffer (in mM: 8.6 HEPES, 5.6 KCl, 154 NaCl, 5.6 glucose, 1.0 $MgCl_2$, 2.3 $CaCl_2$, and 0.0001 glycine, pH 7.4) for 1 hr. After washing the cells with Locke's buffer, the plates were transferred into the FLIPR (Molecular Devices, LLC., CA, USA) chamber set to be 30 ℃. Cells were excited at 488 nm and the emitted fluorescence at 515–575 nm was recorded at 1 Hz. To test agonistic effects of scutellarein (Scu) on hTRPV1 and mTRPV2, 10 μM Scu was added at 60 s. Capsaicin (1 μM) and 2-APB (0.3 mM) were used as positive control to activate hTRPV1 and mTRPV2-mediated Ca^{2+} influx, respectively. To evaluate the effect of the test compound on agonist induced Ca^{2+} response, two additions at 60 s (test compound) and 360 s (agonist cocktail) were performed. The results are presented as F/F_0, where F is the fluorescence intensity at any time point whereas F_0 is the basal fluorescence intensity averaged from the initial 10 data points. The area under the curve (AUC) from an epoch of 5 min after agonist addition was calculated to quantify the effect of the test compound on agonist-induced Ca^{2+} response. Ruthenium red was used as a nonspecific TRPV3 inhibitor.

MTT assay

To evaluate the effects of Scu analogues on cell proliferation, an MTT assay was performed as previously described (Wang et al., 2021). Briefly, HaCaT cells were treated as described in BrdU-incorporation assay, followed by incubation with MTT at a final concentration of 0.5 mg mL^{-1} for another 1 hr at 37 ℃. The formazan crystals in

cells were dissolved in DMSO and the absorbance was determined at 570 nm using a spectrometer (Tecan Austria Gmbh, Grödig, Austria).

References

Wang, Y., Li, H., Xue, C., Chen, H., Xue, Y., Zhao, F., *et al.* Cao, Z. (2021). TRPV3 enhances skin keratinocyte proliferation through EGFR-dependent signaling pathways. *Cell Biol Toxicol* ***37***(2), 313-330. doi:10.1007/s10565-020-09536-2

Yang, G., Ma, H., Wu, Y., Zhou, B., Zhang, C., Chai, C., & Cao, Z. (2019). Activation of TRPC6 channels contributes to (+)-conocarpan-induced apoptotic cell death in HK-2 cells. *Food Chem Toxicol* ***129***, 281-290. doi:10.1016/j.fct.2019.04.061

Table S1: Primer sequences used for qPCR

Name	Forward	Reverse
mouse *Il-1β*	GAAATGCCACCTTTTGACAGTG	TGGATGCTCTCATCAGGACAG
mouse *Tnf-α*	GACGTGGAACTGGCAGAAGAG	TGCCACAAGCAGGAATGAGA
mouse *Il-4*	GGTCTCAACCCCCAGCTAGT	GCCGATGATCTCTCTCAAGTGAT
mouse *Il-6*	CTGCAAGAGACTTCCATCCAG	AGTGGTATAGACAGGTCTGTTGG
mouse *Cxcl15*	CAAGGCTGGTCCATGCTCC	TGCTATCACTTCCTTTCTGTTGC
mouse *Gapdh*	CATCTTCCAGGAGCGAGACC	GAAGGGGCGGAGATGATGAC
human IL-6	GTGTGAAAGCAGCAAAGAG	CTCCAAAAGACCAGTGATG
human IL-8	GTCCTTGTTCCACTGTGCCT	GCTTCCACATGTCCTCACAA
human TNF-α	CCTGTGAGGAGGACGAACAT	TTTGAGCCAGAAGAGGTTGAG
human TSLP	ATGTTCGCCATGAAAACTAAGGC	GCGACGCCACAATCCTTGTA
human GAPDH	AACGGATTTGGTCGTATTGGG	TCGCTCCTGGAAGATGGTGAT

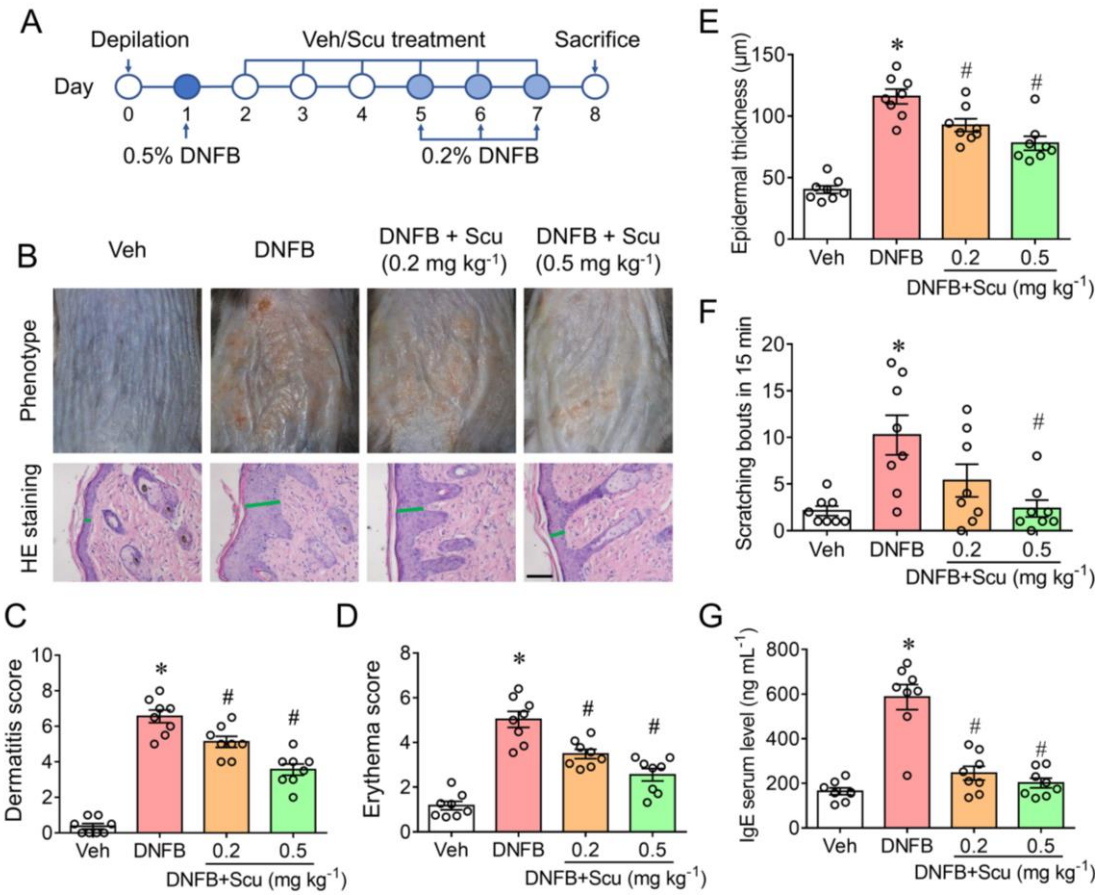

Figure S1. Scu alleviates DNFB-induced dermatitis

(**A**) Schematic illustration depicting the establishment of DNFB atopic dermatitis mouse model and the administration of Scu (0.2 mg kg^{-1} and 0.5 mg kg^{-1}). (**B**) Representative images of the gross appearance (upper panel) and H&E-stained sections (bottom panel) of the back skins of WT C57BL/6J mice treated with vehicle (Veh, acetone/olive oil and saline containing 10% tween-80), DNFB, and DNFB plus Scu. Black scale bar: 100 μm. Green lines indicate the thickness of epidermis. (**C-G**) Quantification of dermatitis score (**C**), erythema score (**D**), epidermal thickness (**E**), scratching bouts (**F**), and serum IgE level (**G**) in mice treated with Veh, DNFB, and DNFB plus Scu. All bar graphs represent the mean ± SEM (N = 8 mice). * $P < 0.05$, versus Veh; $^{\#}$ $P < 0.05$ versus DNFB, by one-way ANOVA followed by Dunnett's multiple comparison tests.

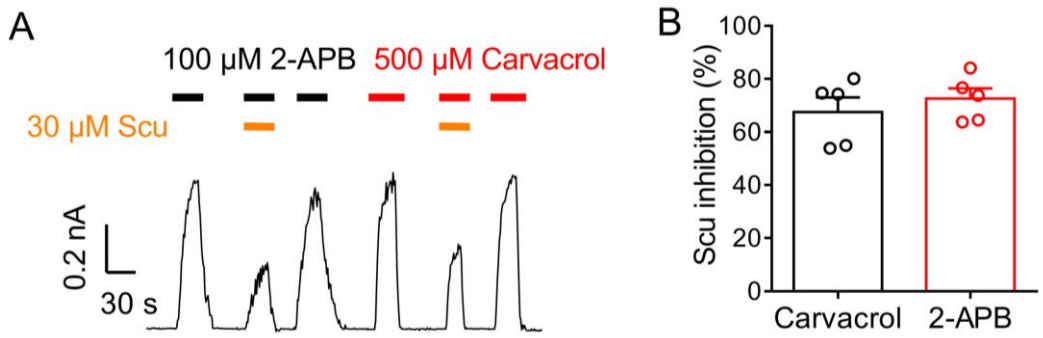

Figure S2. Influence of Scu (30 μM) on mTRPV3 currents induced by carvacrol (500 μM) and 2-APB (100 μM)

(A) Representative traces of mTRPV3 mediated whole-cell outward currents induced by carvacrol or 2-APB in the absence and presence of scutellarein (Scu). **(B)** Quantification of Scu inhibition on carvacrol or 2-APB-induced outward currents recorded at + 100 mV. Each data point represents Mean ± SEM, N=5 cells.

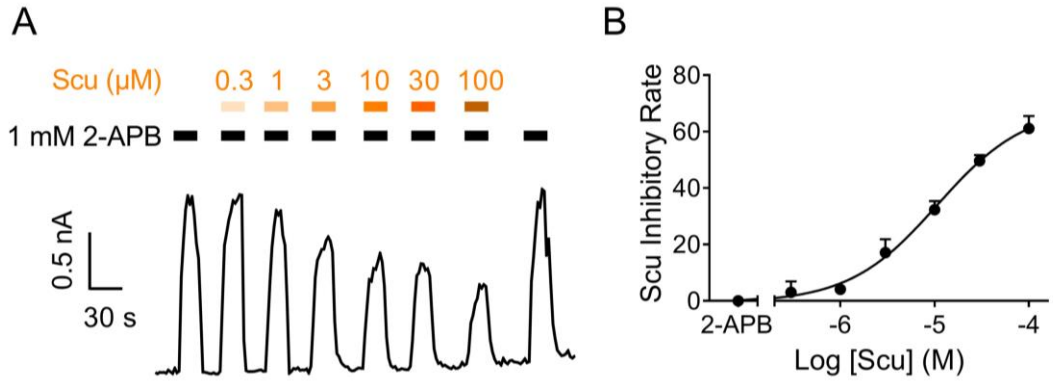

Figure S3. Scu weakly inhibits TRPV2 activity

(A) Representative traces of whole-cell currents at + 100 mV activated by 1 mM 2-APB in HEK-293 cells expressing mTRPV2. Scu at different concentrations was applied sequentially as indicated. **(B)** Concentration-response curve of Scu suppression of 2-APB-induced mTRPV2 currents. Each data point represents mean ± SEM (N = 5 cells).

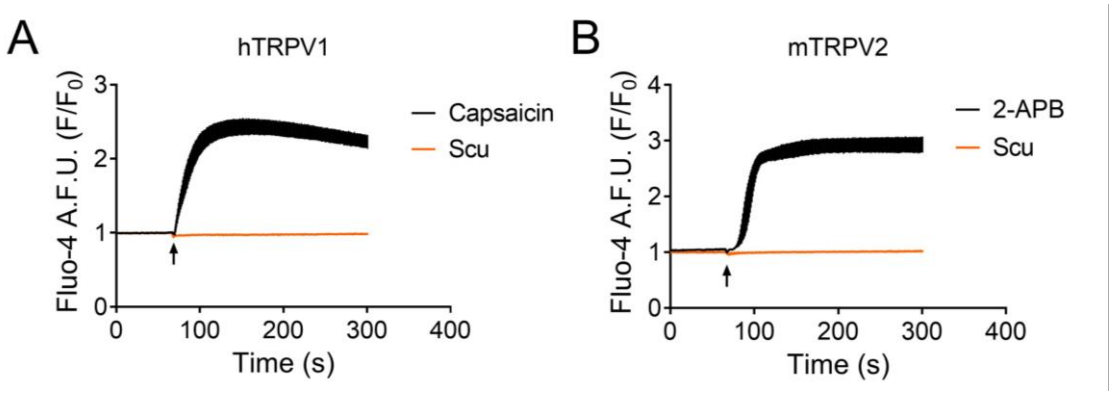

Figure S4. Scu doesn't activate hTRPV1 and mTRPV2

Representative traces showing lack of effect of Scu to stimulate Ca^{2+} influx in HEK-293 cells expressing hTRPV1 **(A)** and mTRPV2 **(B).** Capsaicin (1 μM) and 2-APB (0.3 mM) were used as positive controls for hTRPV1 and mTRPV2, respectively. The arrowhead indicates the addition of compounds.

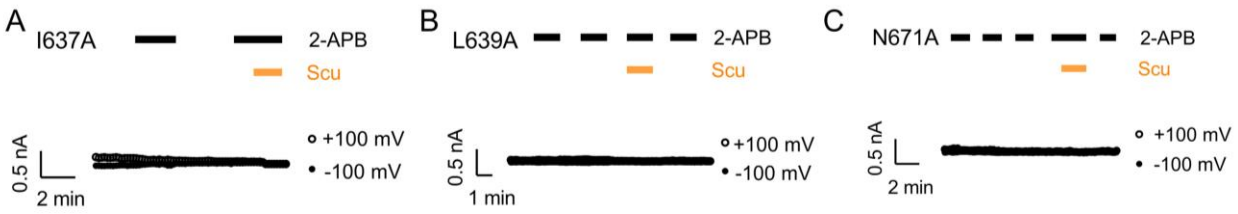

Figure S5. Some mTRPV3 mutants do not respond to 2-APB

Representative traces (at ± 100 mV) of whole-cell currents challenged by 2-APB (100 μM) or 2-APB plus Scu (10 μM) in HEK-293 cells transiently expressing I637A **(A)**, L639A **(B)**, and N671A **(C)** mutants of mTRPV3, respectively.

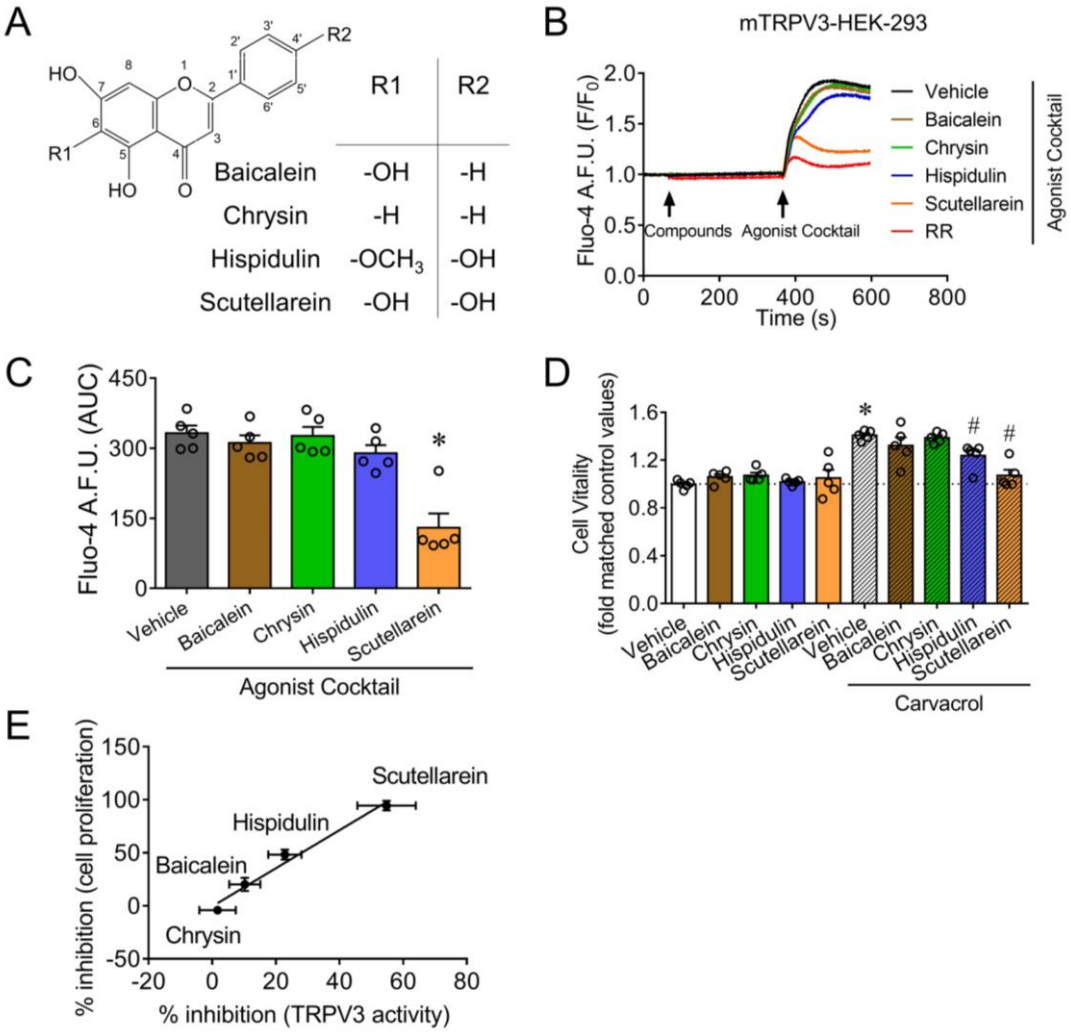

Figure S6. Effects of scutellarein analogs on TRPV3-mediated Ca^{2+} influx and cell vitality

(A) Chemical structures of scutellarein and its analogs, baicalein, chrysin, and hispidulin. **(B)** Representative traces showing the effects of scutellarein and its analogs on agonist cocktail-induced Ca^{2+} response in mTRPV3-expressing HEK-293 cells as a function of time. The first arrowhead indicates the addition of the flavonoids (10 μM) or ruthenium red (RR, 10 μM) and the second arrowhead indicates the addition of agonist cocktail (10 μM 2-APB plus 10 μM carvacrol). **(C)** Quantification of the effects of flavonoids on TRPV3-mediated Ca^{2+} response derived from experiment in (B). Data are presented as mean ± SEM (N = 5) and the statistical significance was analyzed using one-way ANOVA followed by Dunnett's multiple comparison test. * $P < 0.05$, versus agonist cocktail only group. **(D)** Influences of scutellarein, baicalein, chrysin and hispidulin on cell vitality in the absence and presence of 100 μM carvacrol. Data were

presented as mean ± SEM (N = 5) and the statistical significance was analyzed using one-way ANOVA followed by Dunnett's multiple comparison test. * $P < 0.05$, versus Vehicle; $^{\#}$ $P < 0.05$, versus Vehicle + carvacrol. (**E**) Correlation analysis between inhibition of TRPV3-mediated Ca^{2+} response in mTRPV3-expressing HEK-293 cells and suppression of HaCaT cell proliferation by Scu analogues. The points were evaluated with a linear correlation function.