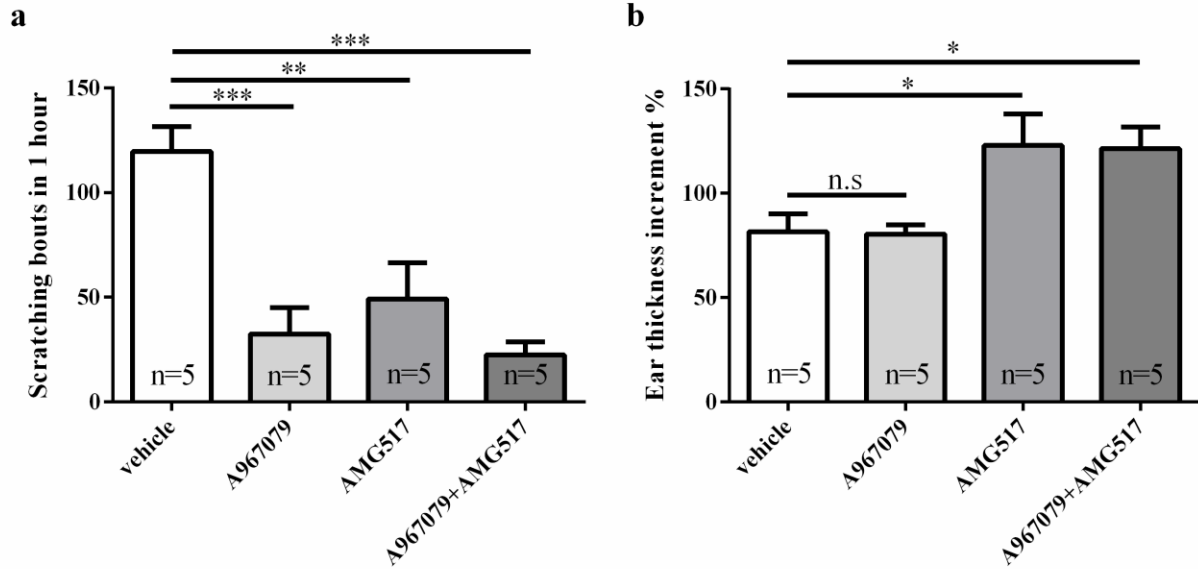
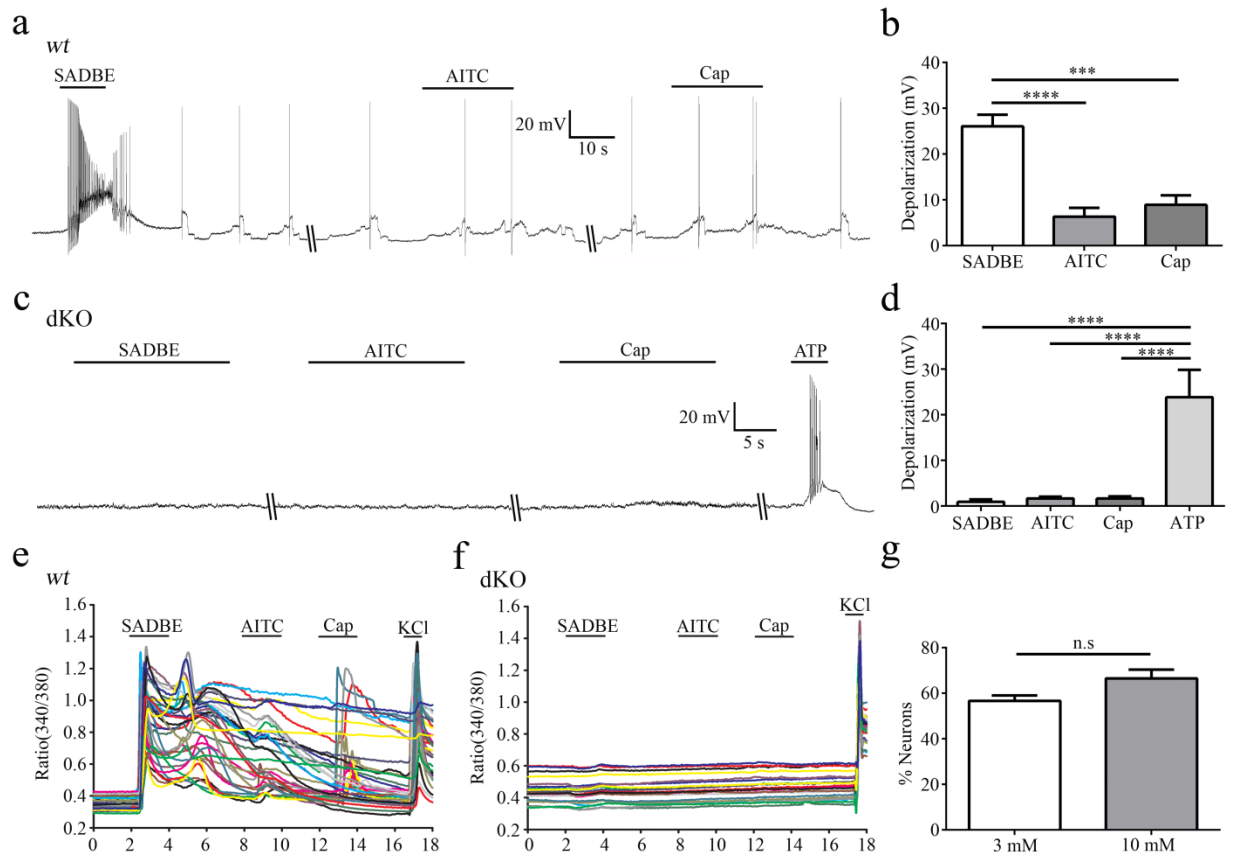


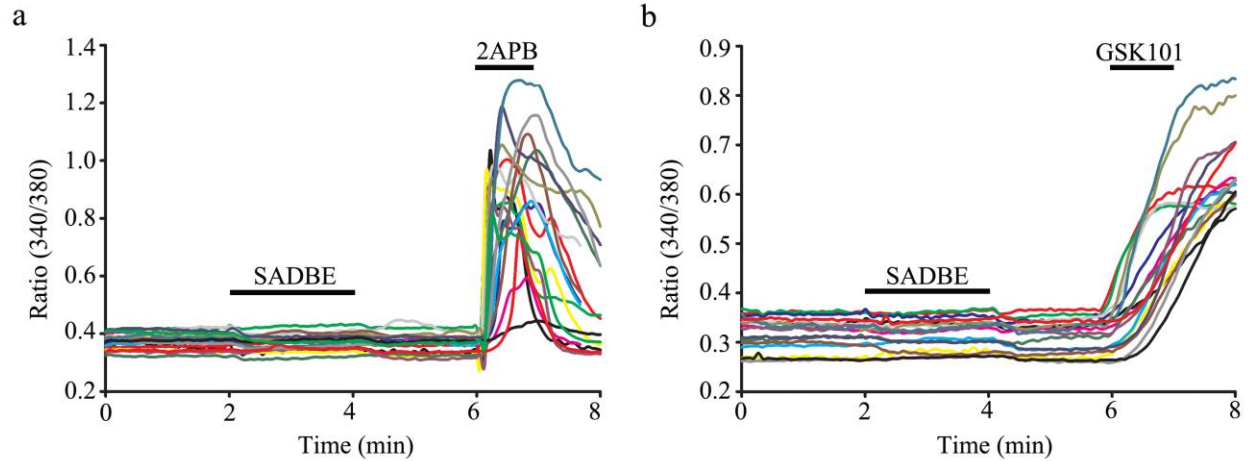
Supplementary Figure 1. Pharmacological inhibition of T cell egression with FTY720 affects neither skin inflammation nor spontaneous scratching. (a) Schematic protocol of FTY720 treatment in the induction of SADBE-induced CHS. (b-c) Ear thickness increment (b) and spontaneous scratching (c) in *wt* mice when compared with vehicle-treated animals. Data are presented as mean \pm SEM. n.s, not significant, Student's *t*-test.



Supplementary Figure 2. Differential effects of pharmacological inhibition of TRPA1 and/or TRPV1 on SADBE-induced skin inflammation and spontaneous scratching. (a-b) SADBE-elicited spontaneous scratching (**a**) and skin inflammation (**b**) in *wt* mice treated with selective TRPA1 and/or TRPV1 antagonists. Mice were given 200 μ l of 10 mg/kg A967079 (a potent and selective TRPA1 antagonist) and/or 30 mg/kg AMG517 (a potent and selective TRPV1 antagonist) by oral gavage daily starting 3 days before the first SADBE challenge. Itch behavior and ear edema were measured 3 days after the last SADBE challenge. Data are presented as mean \pm SEM. n.s, not significant, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, ANOVA.

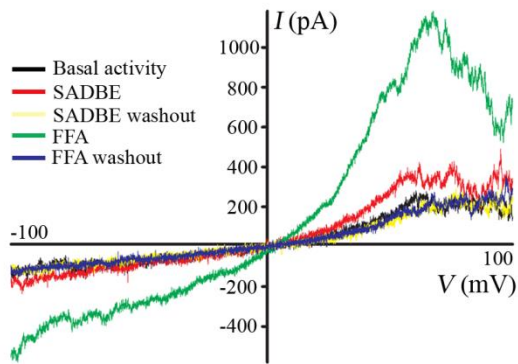


Supplementary Figure 3. SADBE at 10 mM does not activate DRG neurons isolated from the *Trpa1*^{-/-}/*Trpv1*^{-/-} dKO mice. (a) SADBE-induced depolarization of membrane potential and action potential firing in DRG neurons isolated from *wt* mice; (b) Quantification of membrane depolarization induced by SADBE, AITC and Cap. Data are presented as mean ± SEM. n=5, *** $p < 0.001$, **** $p < 0.0001$, ANOVA; please note that AITC- and Cap-activated responses were markedly reduced when applied after SADBE. (c) SADBE did not induce depolarization of membrane potential and action potential firing in DRG neurons isolated from the *Trpa1*^{-/-}/*Trpv1*^{-/-} dKO mice; ATP was used as a positive control; (d) Quantification of depolarization of membrane potentials induced by SADBE, AITC, Cap and ATP. Data are presented as mean ± SEM. n=5, **** $p < 0.0001$, ANOVA; (e-f) SADBE-induced calcium influx in *wt* (n=5 coverslips, 689 neurons) and *Trpa1*^{-/-}/*Trpv1*^{-/-} dKO (n=5 coverslips, 752 neurons) DRG neurons; (g) Percentages of *wt* DRG neurons responded to 3 mM and 10 mM SADBE. Data are presented as mean ± SEM. n.s, not significant. Student's *t*-test.

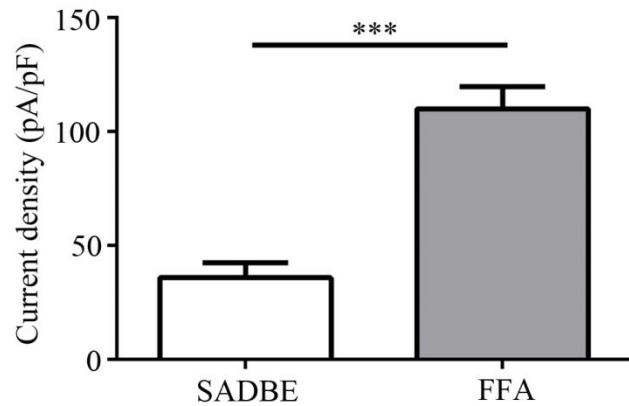


Supplementary Figure 4. SADBE does not activate HEK293 cells transfected with mouse TRPV3 or rat TRPV4 construct. (a) 3 mM SADBE did not induce calcium influx in HEK293 cells transfected with mouse TRPV3; the TRPV4 activator 300 μ M 2-Aminoethoxydiphenylborane (2APB) was used as a positive control; (b) 3 mM SADBE did not induce calcium influx in HEK293 cells transfected with rat TRPV4; the TRPV4 activator GSK1016790A (GSK101, 0.3 μ M) was used as a positive control.

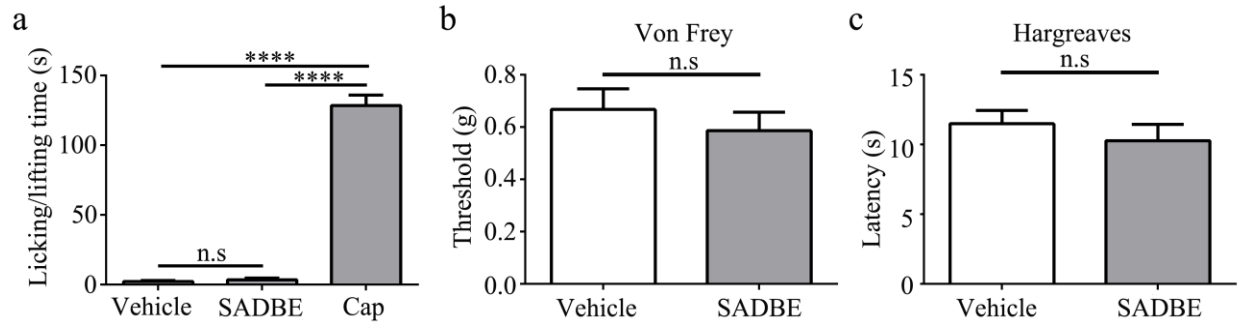
a TRPA1-3C expressing HEK293 cells



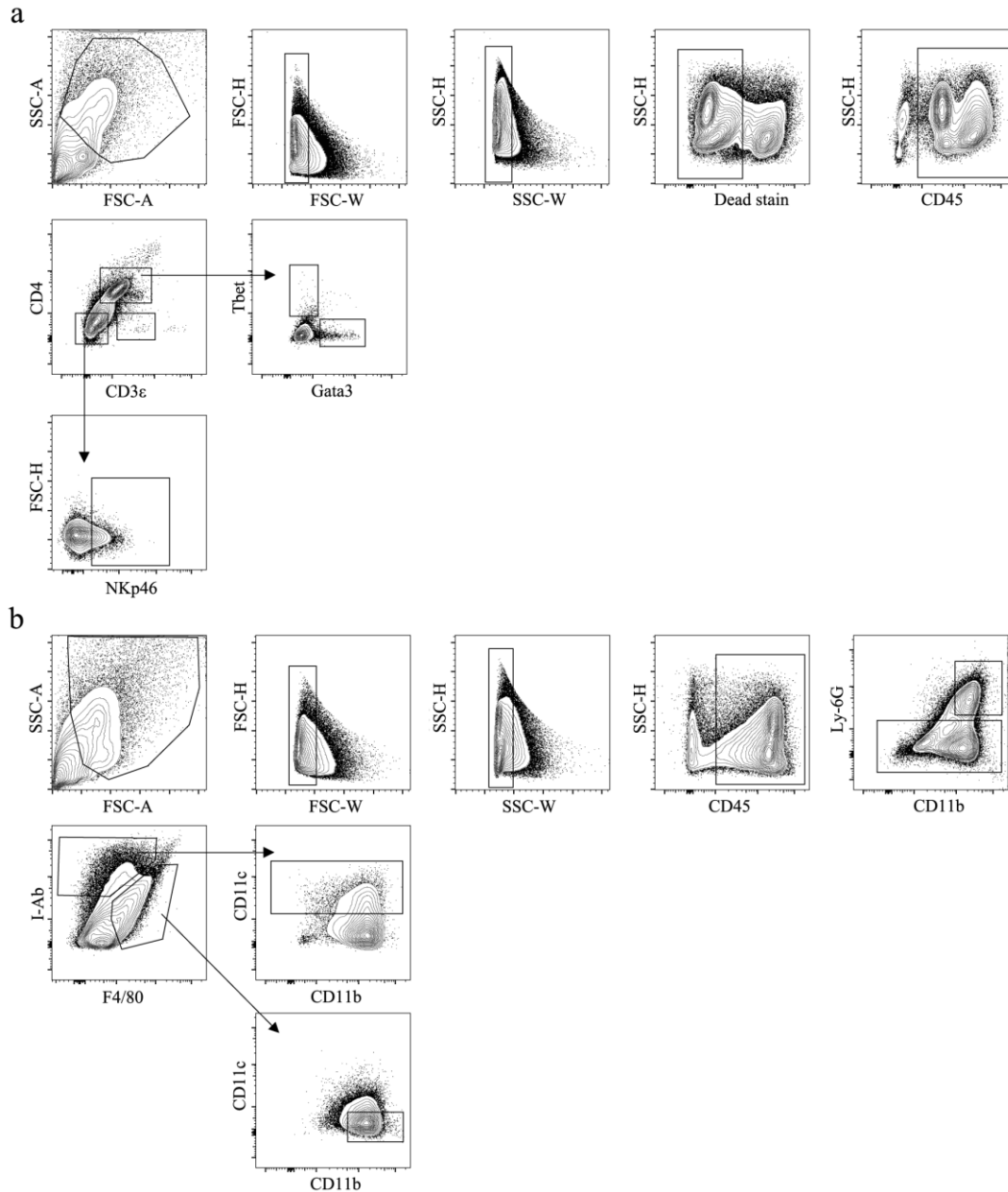
b



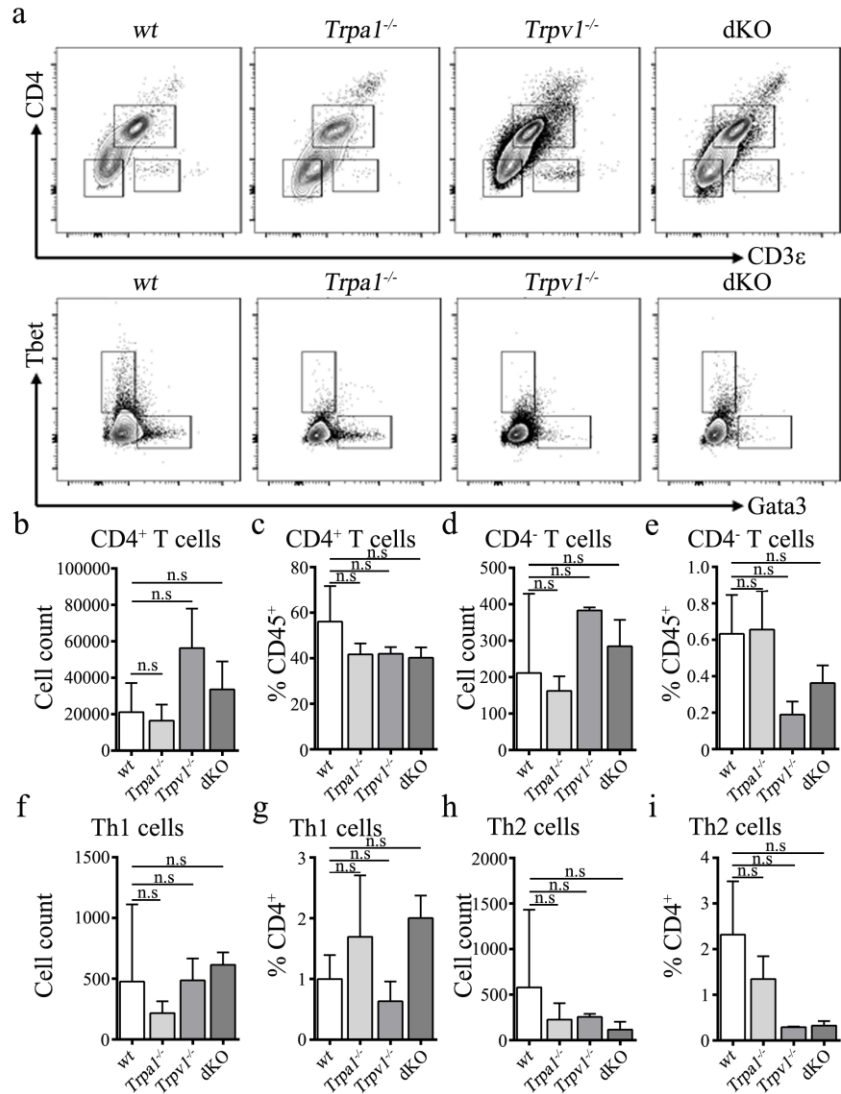
Supplementary Figure 5. SADBE-activated whole-cell membrane currents are severely attenuated in TRPA1 cysteine mutants expressed in HEK293 cells. (a) Representative I-V curves of TRPA1-3C currents in response to 3 mM SADBE and 100 μ M FFA; (b) Quantification of SADBE- and FFA-induced TRPA1-3C currents measured at +60 mV. FFA is a non-electrophilic TRPA1 activator. Data are presented as mean \pm SEM. n=6. *** p <0.001, Student's t -test.



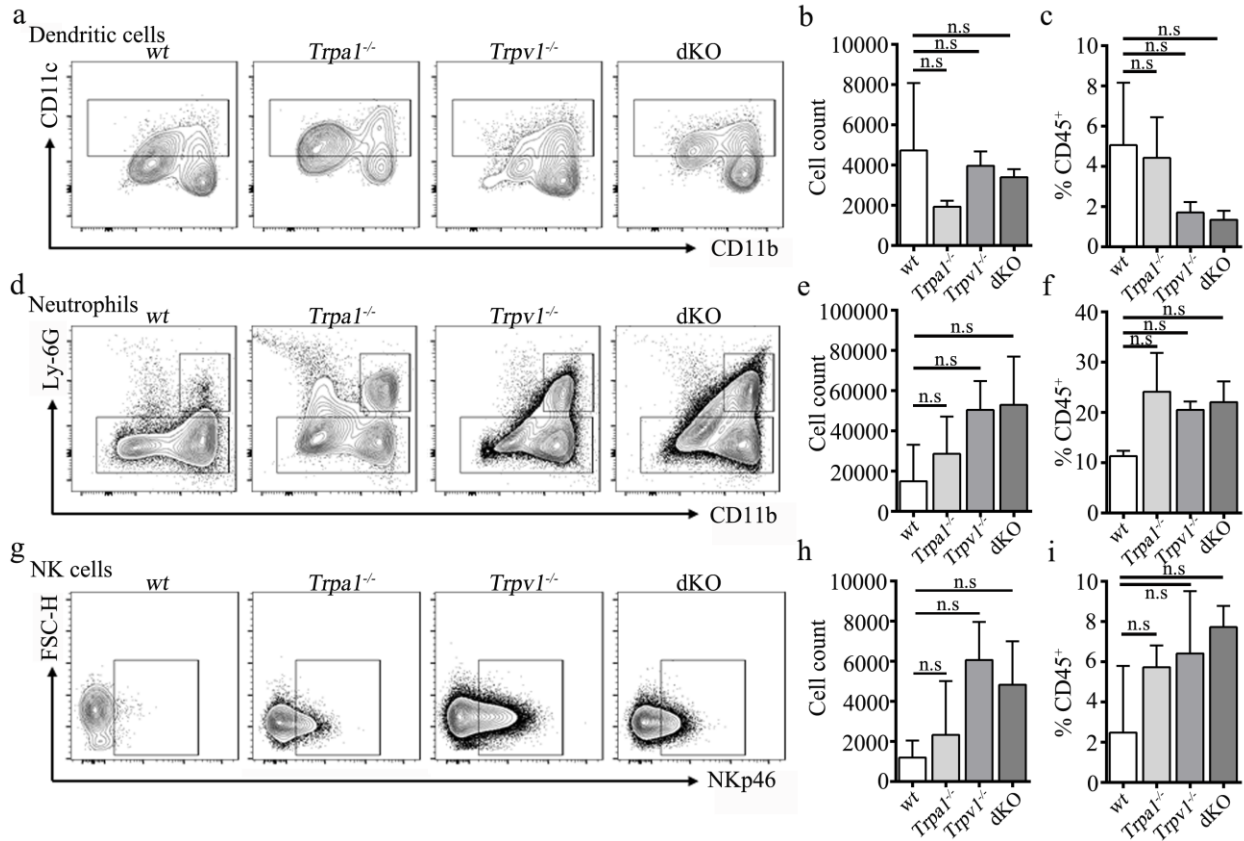
Supplementary Figure 6. Intraplantar injections of SADBE does not elicit a pain-like behaviors in mice. (a) Paw licking/lifting nocifensive response after intradermal injection of vehicle, SADBE (30mM) or capsaicin (Cap, 0.5 μ g) into a hindpaw of *wt* mice. Data are presented as mean \pm SEM. $n=5$ per group. Asterisks indicate statistical significance. **** $p<0.0001$, ANOVA. n.s, not significant. (b-c) Paw withdrawal threshold in response to mechanical stimuli (b) and thermal stimuli (c) after intraplantar injections of 10 μ l vehicle or 30 mM SADBE in *wt* mice. Data are presented as mean \pm SEM. $n=5$ per group. n.s, not significant, Student's *t*-test;



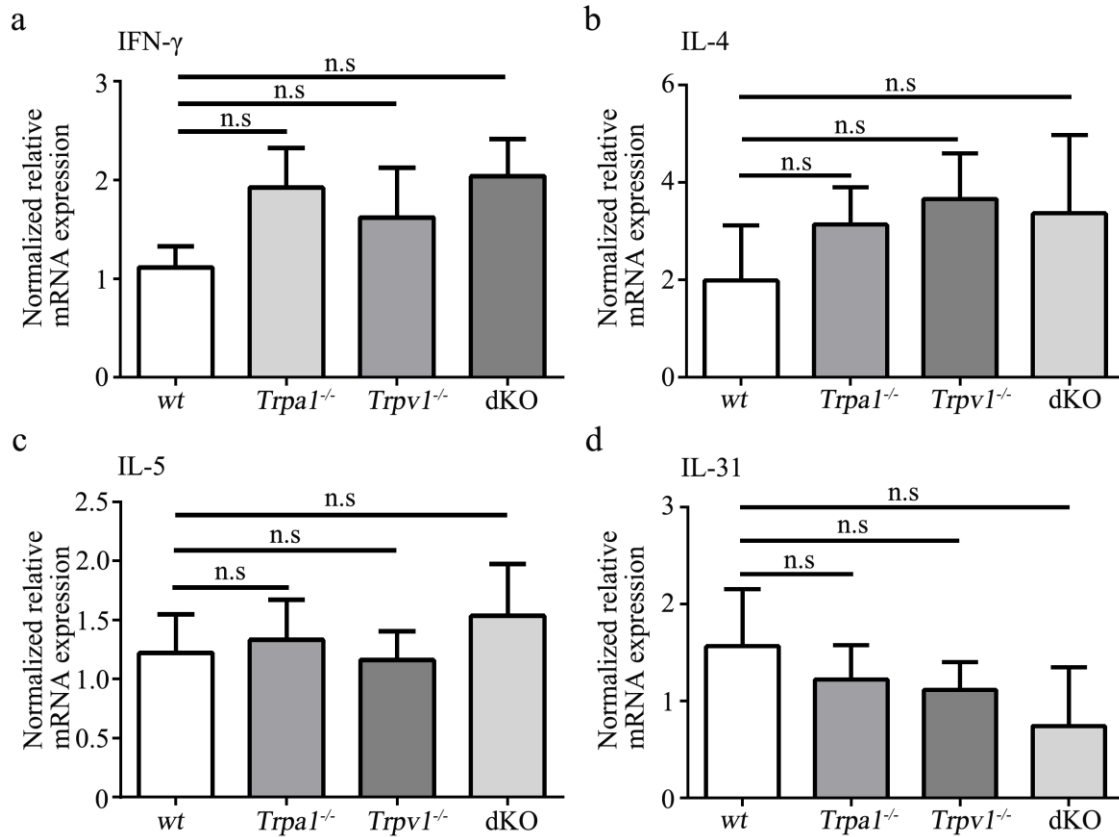
Supplementary Figure 7. Gating strategy for flow cytometry using single-cell suspensions from mouse ear skin preparations. (a) Cells were gated on size and granularity, doublet events and dead cells removed, and then CD45⁺ cells were selected. CD11b⁺ Ly-6G⁺ cells were used to define neutrophils. Ly-6G⁻ cells were further divided into F4/80^{hi} I-Ab^{low} macrophages and F4/80^{low/-} I-A^{b-hi} cells dendritic cells. Macrophages and dendritic cells were further classified as CD11c⁻ and CD11c⁺, respectively; (b) Helper T cells were defined as CD3ε⁺ CD4⁺ lymphocytes expressing either Gata3 (Th2) or Tbet (Th1) transcription factors by intracellular staining. NK cells were defined as CD3ε⁻ CD4⁻ lymphocytes that were NKp46⁺.



Supplementary Figure 8. Quantification of T cell populations in SADBE-treated ear preparations from *wt*, *Trpa1*^{-/-}, *Trpv1*^{-/-} and *Trpa1*^{-/-}/*Trpv1*^{-/-} dKO mice. (a) Gating strategy for the identification of Th1 and Th2 cells. Th1 cells were defined as CD3ε⁺ CD4⁺ Tbet⁺, Th2 cells were defined as CD3ε⁺ CD4⁺ Gata3⁺; (b, d, f, h) Comparison of cell numbers of CD4⁺ T cells (b), CD4⁻ T cells (d), Th1 cells (f) and Th2 cells (h) sorted from the single-cell suspensions from mouse ear skin preparations of *wt*, *Trpa1*^{-/-}, *Trpv1*^{-/-} and *Trpa1*^{-/-}/*Trpv1*^{-/-} dKO mice; (c, e) Comparison of the percentage of CD4⁺ T cells (c) and CD4⁻ T cells (e) in the CD45⁺ population from the ear preparations of *wt*, *Trpa1*^{-/-}, *Trpv1*^{-/-} and *Trpa1*^{-/-}/*Trpv1*^{-/-} dKO mice; (g, i) Comparison of the percentages of Th1 cells (g) and Th2 cells (i) in the CD4⁺ population from the ear preparations of *wt*, *Trpa1*^{-/-}, *Trpv1*^{-/-} and *Trpa1*^{-/-}/*Trpv1*^{-/-} dKO mice. All data are presented as mean ± SEM. n=3 for each group. n.s., not significant, ANOVA.



Supplementary Figure 9. Quantification of different types of myeloid cells in single-cell suspensions from SADBE-treated ear skin preparations of *wt*, *Trpa1*^{-/-}, *Trpv1*^{-/-} and *Trpa1*^{-/-}/*Trpv1*^{-/-} dKO mice. (a, d, g) Representative FACS plots of dendritic cells (a), neutrophils (d) and NK cells (g). Dendritic cells were defined as I-A^{b-hi} F4/80^{+/+} CD11b^{+/+} CD11c⁺, neutrophils were defined as CD11b⁺ Ly6-G⁺ I-A^b F4/80⁻, NK cells were defined as CD3ε⁻ CD4⁻ NKp46⁺; (b, e, h) Comparison of cell number of dendritic cells (b), neutrophils (e) and NK cells (h) sorted from the ear preparations of *wt*, *Trpa1*^{-/-}, *Trpv1*^{-/-} and *Trpa1*^{-/-}/*Trpv1*^{-/-} dKO mice. All data are presented as mean ± SEM. n=3 for each group, n.s, not significant, ANOVA; (c, f, i) Comparison of the percentage of dendritic cells (c), neutrophils (f) and NK cells (i) in the CD45⁺ population from the inflamed ear preparations of *wt*, *Trpa1*^{-/-}, *Trpv1*^{-/-} and *Trpa1*^{-/-}/*Trpv1*^{-/-} dKO mice. All data are presented as mean ± SEM. n=3 for each group. n.s, not significant, ANOVA.



Supplementary Figure 10. Expression levels of Th1 and Th2 cytokines following SADBE treatments are not affected by genetic ablation of TRPA1 and/or TRPV1. (a-d) Expression of proinflammatory cytokines IFN- γ (a), IL-4 (b), IL-5 (c), and IL-31 (d) in the SADBE-treated ear preparations of *wt*, *Trpa1*^{-/-}, *Trpv1*^{-/-} and *Trpa1*^{-/-}/*Trpv1*^{-/-} dKO mice. All data are presented as mean \pm SEM. n=3 for each group. n.s, not significant, ANOVA.

Supplementary Table 1

EC₅₀ values of SADBE-activated responses in wild-type and TRPA1 mutants.

	EC ₅₀ (mM)	n
TRPA1	1.30±0.02	5
TRPA1-K710A	6.03±0.01*	5
TRPA1-3C	N.D	5
TRPA1-3C+K710A	N.D	5

N.D, not determined; * $p < 0.05$, *versus wt*, Student's *t*-test.

Supplementary Table 2

EC₅₀ values of SADBE-activated responses in wild-type and TRPV1 mutants.

	EC ₅₀ (mM)	n
TRPV1	7.26±0.01	5
TRPV1-R115A	13.79±0.03 ^{n.s}	5
TRPV1-Y512A	N.D	5
TRPV1-S513A	N.D	5
TRPV1-M548L	2.41±0.01 ^{**}	5
TRPV1-T551A	N.D	5

N.D, not determined; n.s, not significant; ** $p < 0.01$, *versus wt*, Student's *t*-test.