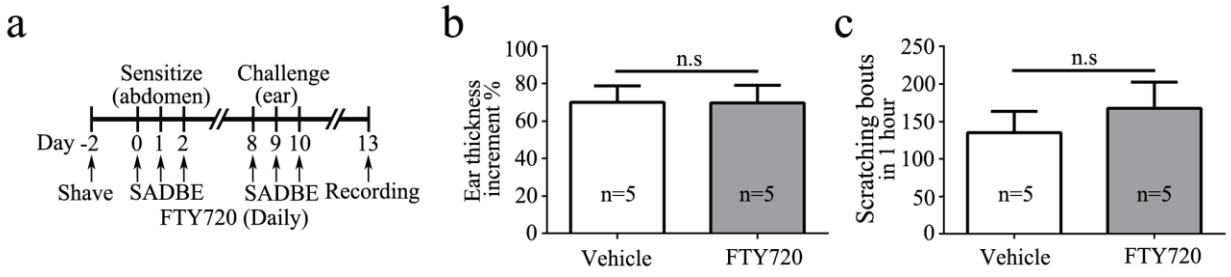
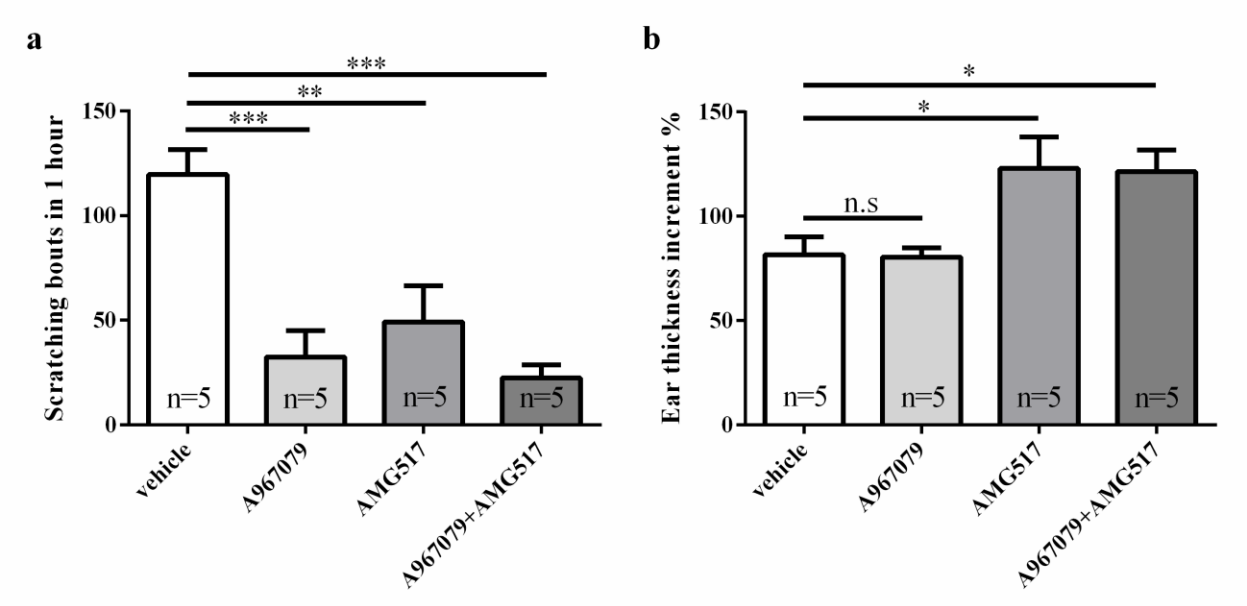
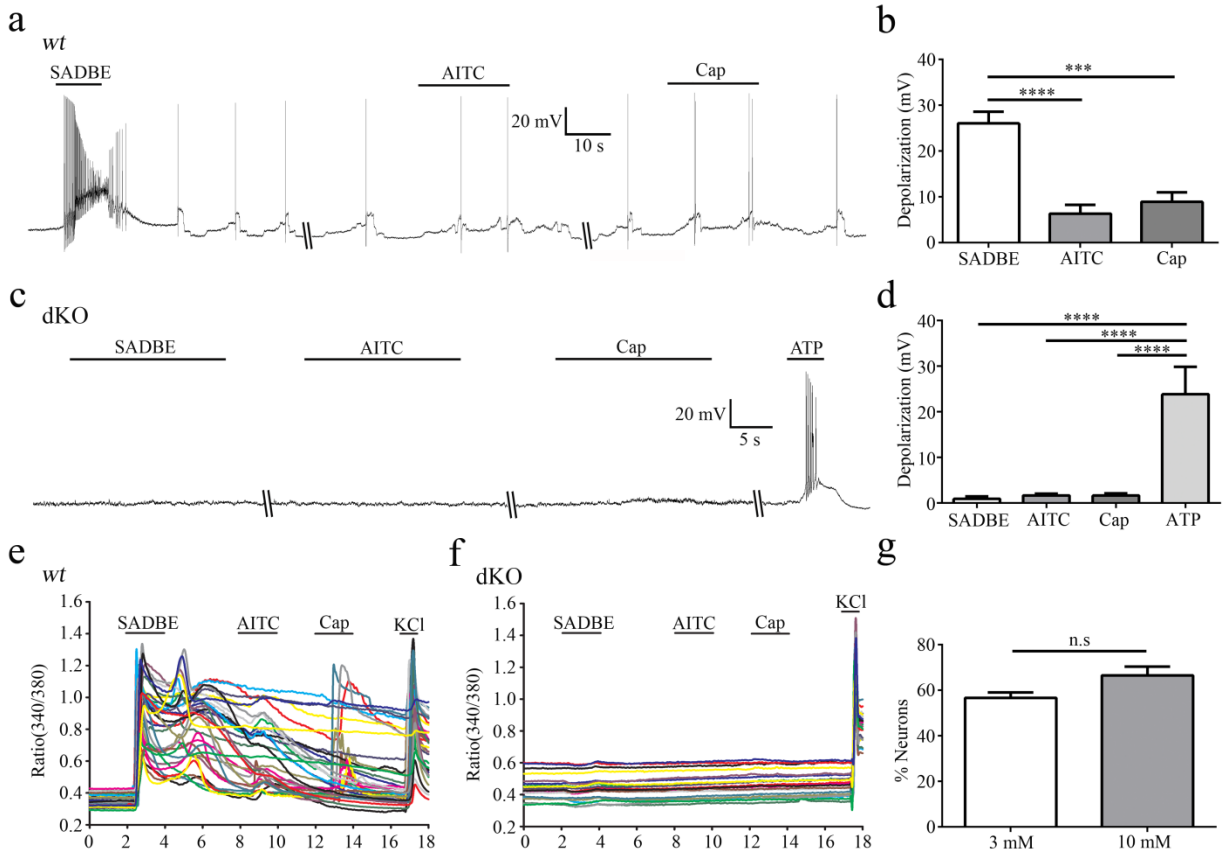


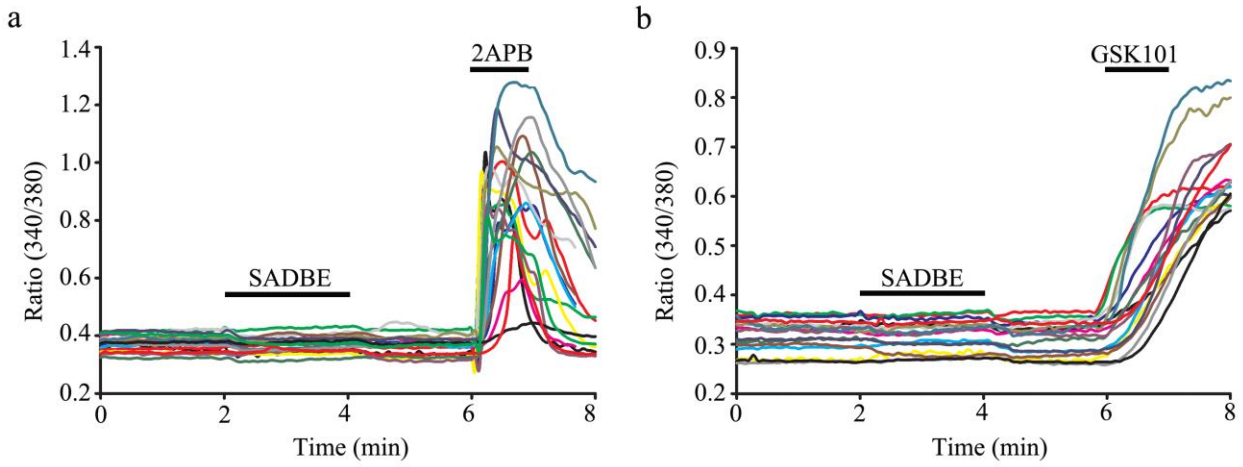

**Supplementary Figure 1. Pharmacological inhibition of T cell egression with FTY720 affects neither skin inflammation nor spontaneous scratching.** (**a**) Shematic protocol of FTY720 treatment in the induction of SADBE-induced CHS. (**b-c**) Ear thickness increment (**b**) and spontaneous scratching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



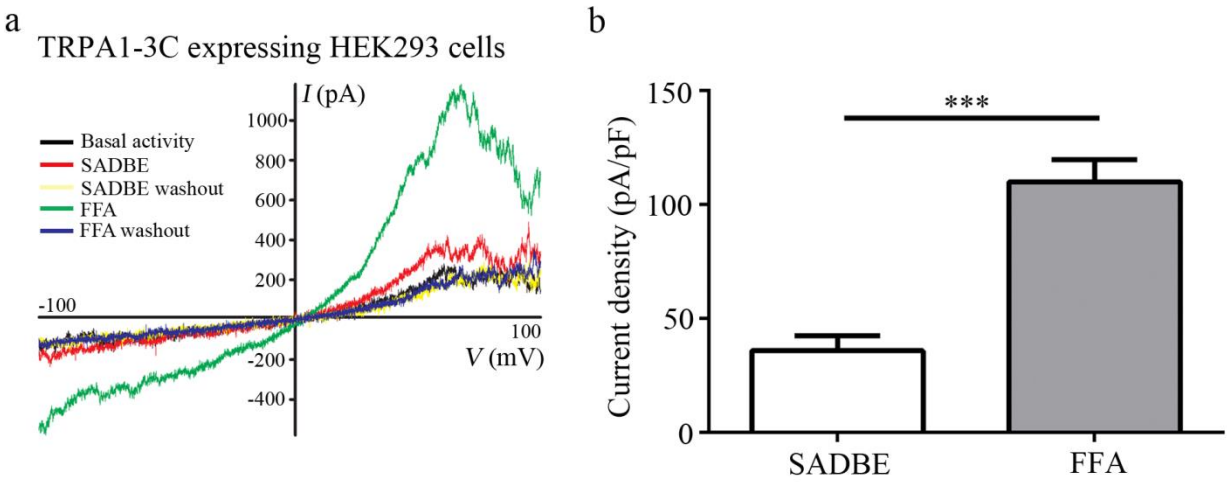

**Supplementary Figure 2. Differential effects of pharmacological inhibition of TRPA1 and/or TRPV1 on SADBE-induced skin inflammation and spontaneous scratching.** (**a-b**) SADBE-elicited spontaneous scratching (**a**) and skin inflammation (**b**) in *wt* mice treated with selective TRPA1 and/or TRPV1 antagonists. Mice were given 200 µl 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 ± SEM. n.s, not significant, * $p<0.05$, ** $p<0.01$, *** $p<0.001$, ANOVA.



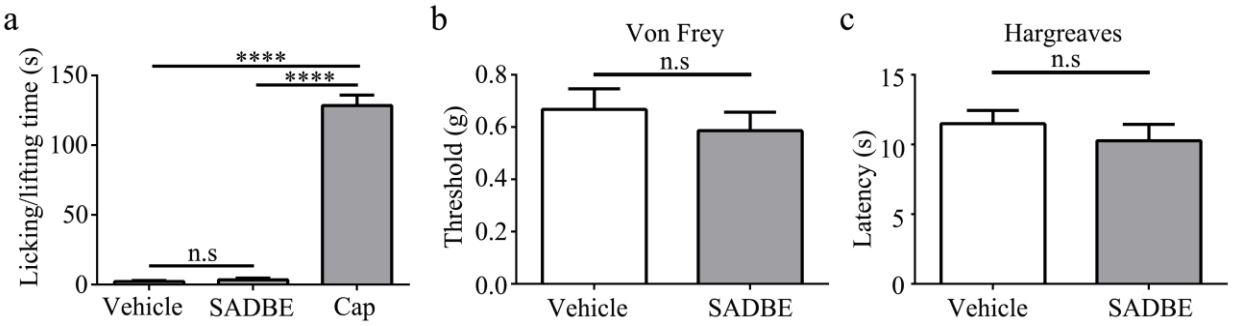

**Supplementary Figure 3. SADBE at 10 mM does not activate DRG neurons isolated from the *Trpa1*$^{-/-}$/*Trpv1*$^{-/-}$ dKO mice.** (**a**) SADBE-induced depolarization of membrane potential and action potential firing in DRG neurons isolated from *wt* mice; (**b**) Quantification of membrane depolarization 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.



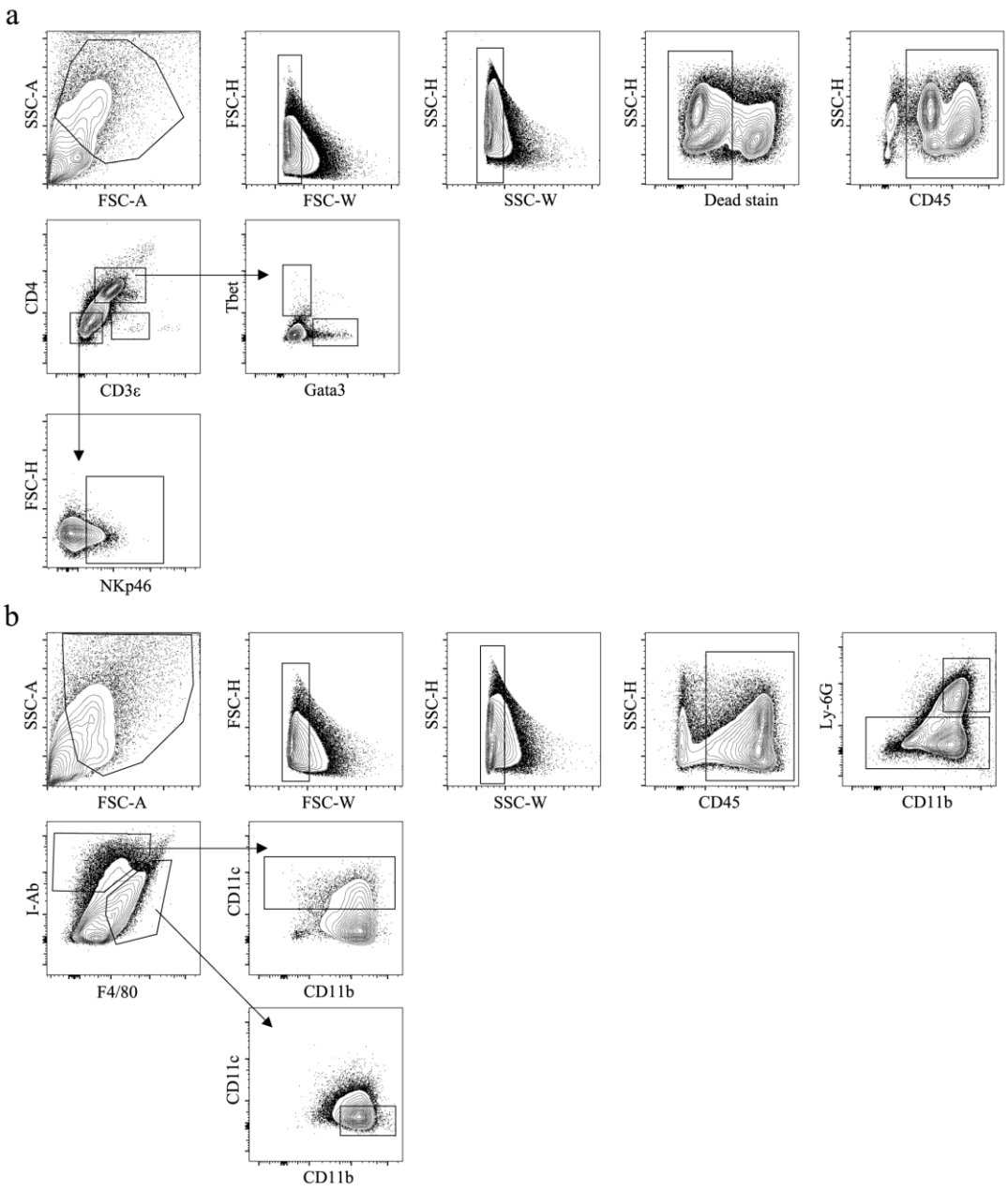

**Supplementary Figure 4. SADBE does not activate HEK293 cells transfected with mouse TRPV3 or rat TRPV4 construct.** (**a**) 3 mM SADBE did not induce calcium influx in HEK293 cells transfected with mouse TRPV3; the TRPV4 activator 300 µM 2-Aminoethoxydiphenylborane (2APB) was used as a positive control;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



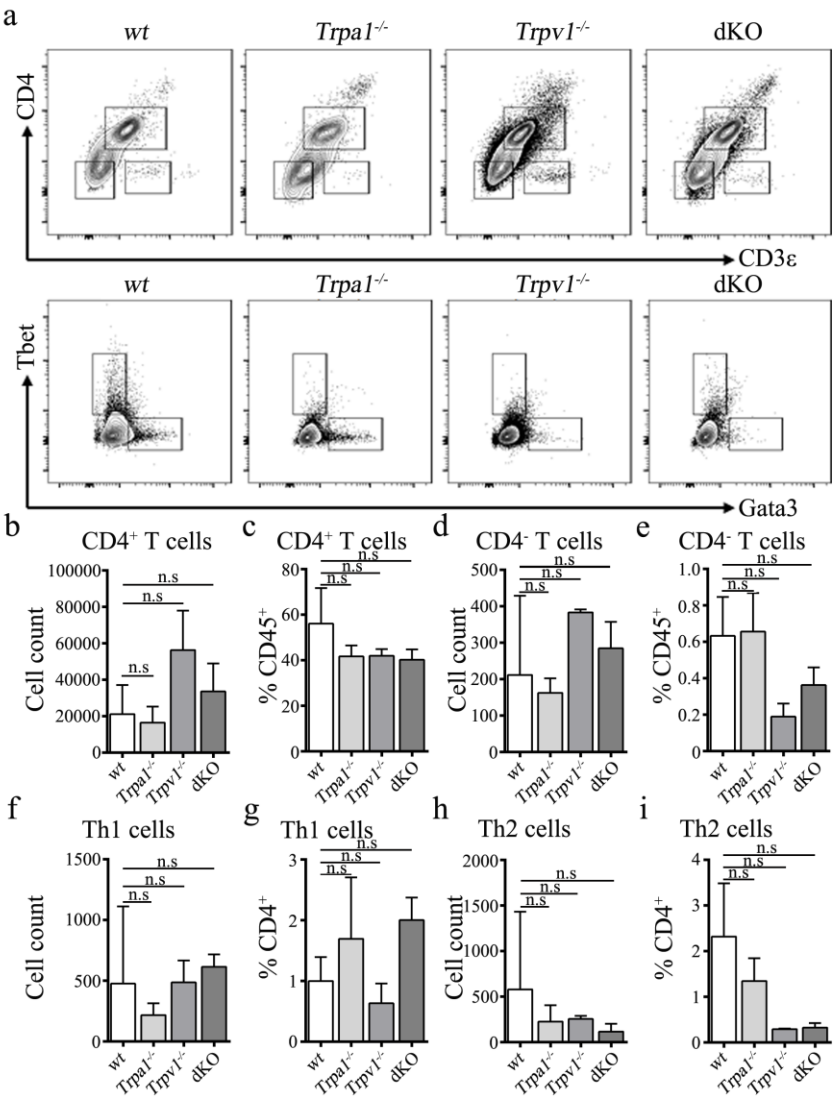

**Supplementary Figure 5. SADBE-activated whole-cell membrane currents are severely attenuated in TRPA1 cysteine mutants expressed in HEK293 cells**. (**a**) Representative I-V curves of TRPA1-3C currents in response to 3 mM SADBE and 100 µM FFA; (**b**) Quantification of SADBE- and FFA-induced TRPA1-3C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



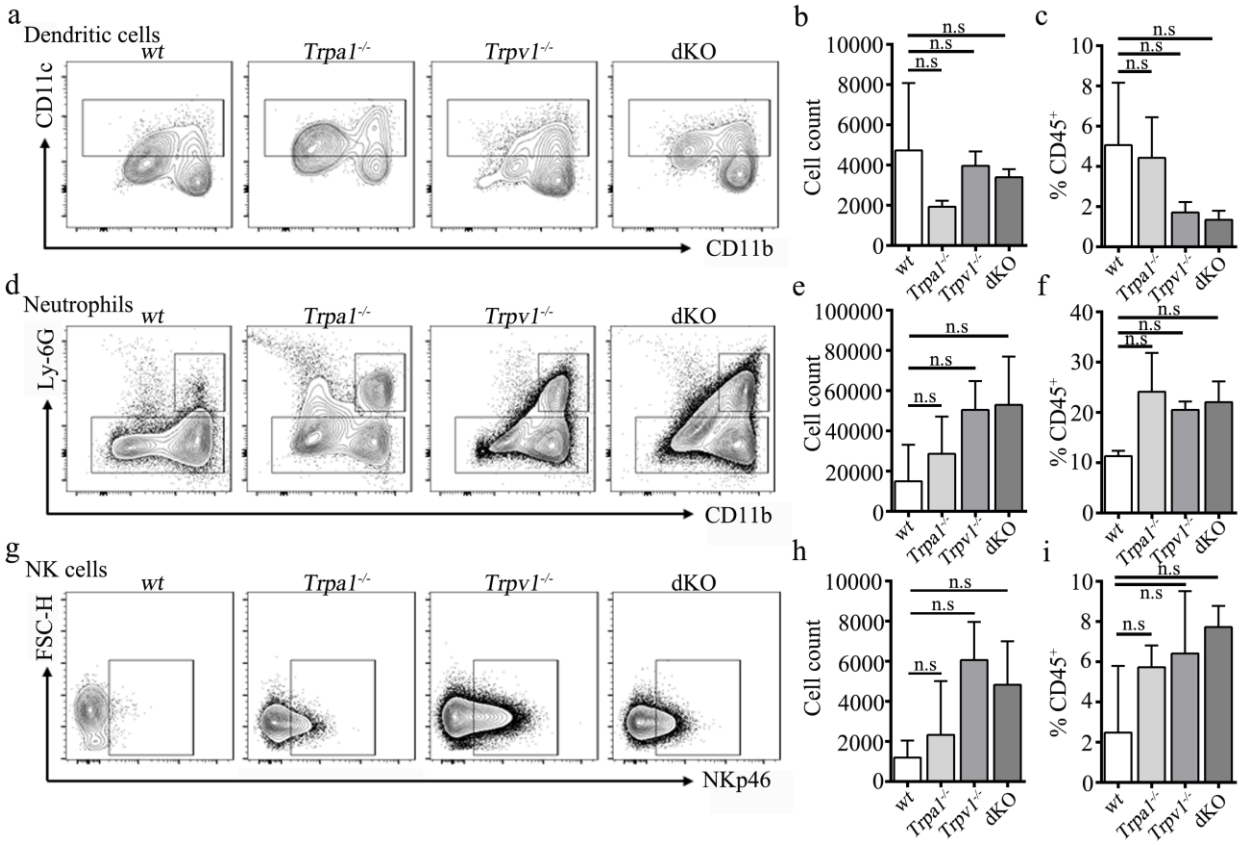

**Supplementary Figure 6. Intraplantar injections of SADBE does not elicit a pain-like behaviors in mice.** (**a**) Paw licking/lifting nocifensive response after intradermal injection of vehicle, SADBE (30mM) or capsaicin (Cap, 0.5 µg) into a hindpaw of *wt* mice. Data are presented as mean ± SEM. n=5 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 µl vehicle or 30 mM SADBE in *wt* mice. Data are presented as mean ± SEM. n=5 per group. n.s, not significant, Student's *t*-test;



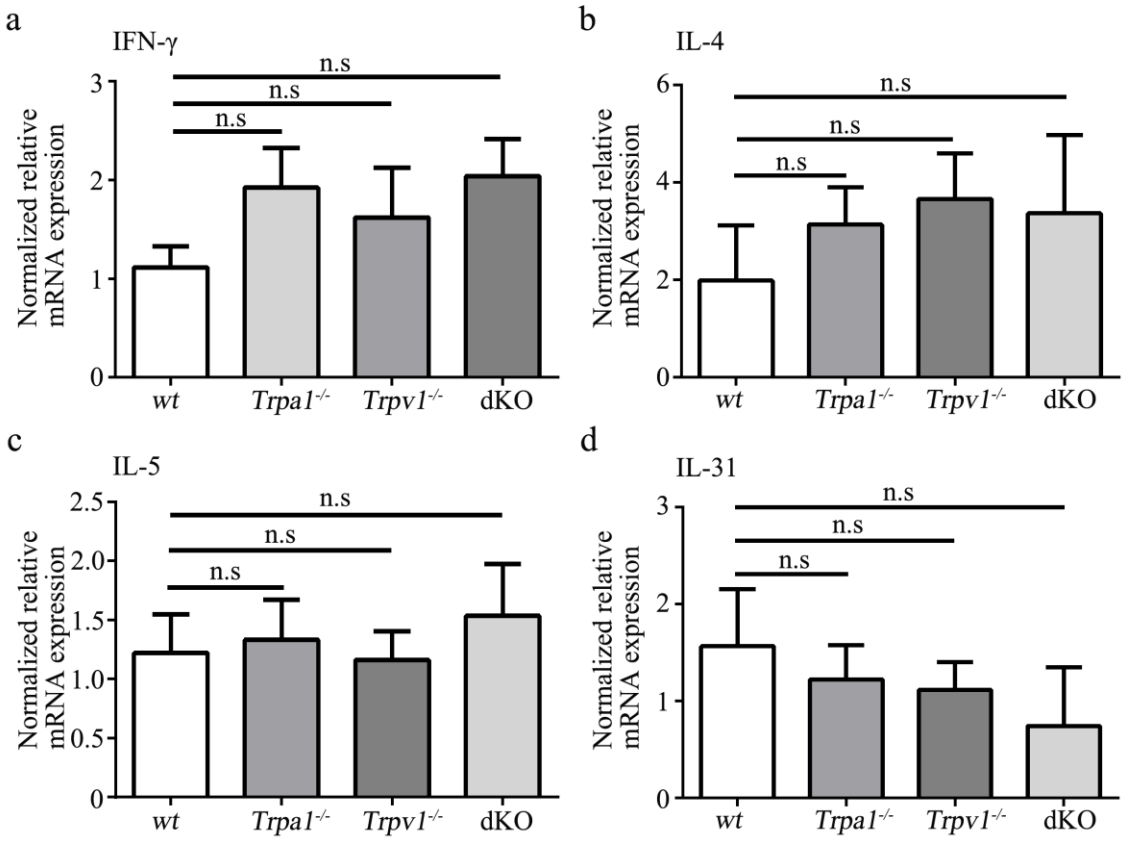

**Supplementary Figure 7. Gating strategy for flow cytometry using single-cell suspensions from mouse ear skin preparations**. (a) Cells were gated on size and granularity, doublet events and dead cells removed, and then $CD45^{+}$ cells were selected. $CD11b^{+}$ $Ly\text{-}6G^{+}$ cells were used to define neutrophils. $Ly\text{-}6G^{-}$ cells were further divided into $F4/80^{hi}$ $I\text{-}Ab^{low}$ macrophages and $F4/80^{low/-}$ $I\text{-}A^{b\text{-}hi}$ cells dendritic cells. Macrophages and dendritic cells were further classified as $CD11c^{-}$ and $CD11c^{+}$, respectively; (b) Helper T cells were defined as $CD3\varepsilon^{+}$ $CD4^{+}$ lymphocytes expressing either Gata3 (Th2) or Tbet (Th1) transcription factors by intracellular staining. NK cells were defined as $CD3e^{-}$ $CD4^{-}$ 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$^{b\text{-}hi}$ 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 ± SEM. n=3 for each group. n.s, not significant, ANOVA.

Supplementary Table 1

$EC_{50}$ 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

$EC_{50}$ 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.