Supplementary information, Fig. S7

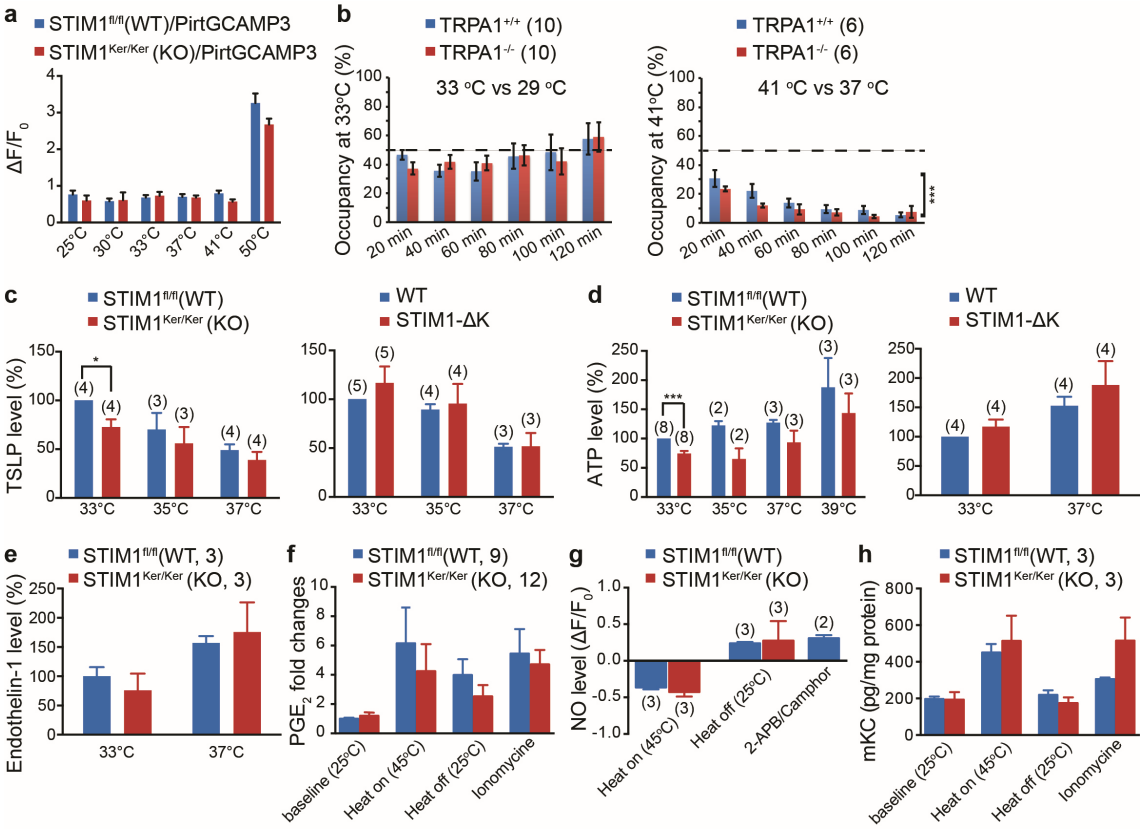

Fig. S7 Investigation of the signal transduction pathway that couples STIM1-dependent temperature responses of keratinocytes to DRG activation. a In vivo recorded GCAMP3 fluorescence changes of DRG neurons of the STIM1$^{fl/fl}$ (WT) and STIM1$^{Ker/Ker}$ (KO) mice in response to the indicated temperature simulation. **b** Two-temperature choice assays show no difference of *TRPA1*$^{+/+}$ and *TRPA1*$^{-/-}$ mice in the indicated test temperatures. *indicates paired Student's *t*-test of either genotypes for preference between the two tested temperatures under the indicated test paradigm, ***P < 0.001. **c, d** TSLP (**c**) and ATP (**d**) level measured from medium of keratinocytes derived from the indicated mice treated with the indicated temperatures. The STIM1$^{fl/fl}$ (WT) (left panel) or WT (right panel) sample treated at 33 °C was used for

normalization. The number of independent experiments is labeled on top of the bar. Unpaired Student's *t*-test, **P* < 0.05, ****P* < 0.001. **e** Endothelin-1 mRNA level from $STIM1^{fl/fl}$ (WT) and $STIM1^{Ker/Ker}$ (KO) keratinocytes treated with the indicated temperatures detected via RT-PCR. The $STIM1^{fl/fl}$ (WT) sample treated at 33 °C was used for normalization. **f, g** Medium from cultured keratinocytes derived from $STIM1^{fl/fl}$ (WT) and $STIM1^{Ker/Ker}$ (KO) mice were sequentially collected at the indicated treatment conditions and measured for the level of PGE_2 (normalized to the baseline sample of $STIM1^{fl/fl}$ (WT)) (**f**) and mKC (**g**). 10 μM ionomycine, which increases intracellular Ca^{2+}, was used as a positive control. The number of samples is shown. **h** The fluorescence change of the NO indicator dye, DAF-FM, from the cultured $STIM1^{fl/fl}$ (WT) and $STIM1^{Ker/Ker}$ (KO) keratinocytes during heat on and heat off. The negative value of the fluorescence change during heat on is due to the heating effect on the NO indicator dye. 100 μM 2-APB/1 mM Camphor, which activates TRPV3 expressed in keratinocytes, was used as a positive control. The number of imaged coverslips is shown.