Clustered regularly interspaced short palindromic repeats (CRISPR)/Cas9-mediated gene editing

TRPM4-knockout HaCaT cell lines were generated utilizing the CRISPR/Cas9-mediated gene editing system. The CRISPR vector pCAS-Guide-EF1a-GFP (GE100018; OriGene Technologies, Inc., Rockville, MD, USA) expressing single-guide RNAs (gRNAs) containing the inserted target sequence for TRPM4 was transiently introduced into cells using Lipofectamine 2000 transfection reagent (ThermoFisher Scientific) in Opti-MEM. The target sequence of the complementary gRNA was as follows: gRNA 5′-GTCAACTATGAACGTCGTGC-3′ (S1 Fig). Subcloning was initiated after an expression period of 24 h by sorting single green fluorescent protein (GFP)-positive cells using a modified LSRII flow cytometer (BD Biosciences). Limiting dilution was performed for single-cell cloning. The medium was renewed every third day for 4 weeks. Genomic DNA was extracted using a standard phenol-chloroform DNA extraction protocol and sent for Sanger sequencing with an area of 500 base pairs around the target site. Single cells were picked from a mixed cell culture using a Unipick micro-pick and place system (Nepa Gene, Chiba, Japan) until sequence analysis showed that the single cell was cloned.