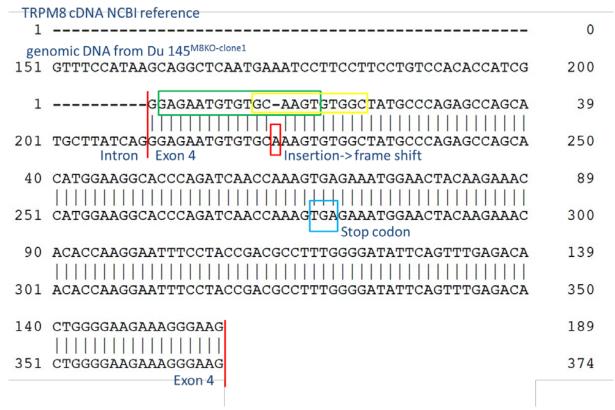
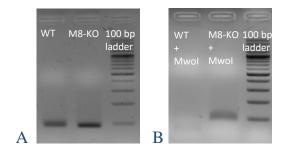
*Menthol-evoked Ca*<sup>2+</sup> responses

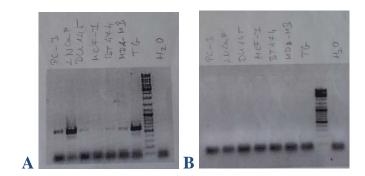
## **Supplementary Material**



**Fig S1. Detection of a CRISPR/Cas9-induced mutation in the** *TRPM8* **gene.** Alignment of *TRPM8* cDNA sequence (NCBI Reference Sequence: NM\_024080.4); upper row) and the *TRPM8* DNA sequence from genomic DNA isolated from Du 145<sup>M8KO-clone1</sup> cells (lower row). The sequence of the small guidance sgRNA, where CRISPR/Cas9 is expected to produce mutations is boxed in in green. The observed 1-nt A insertion by the CRISPR/Cas9 system is marked by a red box. The insertion results in a frame shift and a pre-terminal stop of TRPM8 translation (marked in blue box). The nucleotides comprising the *MwoI* site are in yellow box.



**Fig S2.** Confirmation that all *TRPM8* alleles are mutated in Du 145<sup>M8KO-clone1</sup> cells. A) Isolated genomic DNA was amplified with the following primer pairs: 5'-TTC CTT CCT GTC CAC ACC ATC-3' and reverse 5'-TTG ATC TGG GTG CCT TCC AT-3'. This produces a 96-bp product in wild type Du 145<sup>WT</sup> and a 97-bp product in knockout Du 145<sup>M8KO-clone1</sup> cells. **B)** After digestion with the restriction enzyme *MwoI* (recognition sequence: GCnn\_nnn'nnGC) the amplicon from WT cells is digested, but not the amplicon from the mutated cell line.



**Fig S3. The complete RT-PCR blots. A)** With reverse transcriptase enzyme. Lanes are the following: 3 prostate and 3 breast cancer cell lines, followed by an extract from human trigeminal ganglia (TG) as a positive control, a bp ladder and a water (negative) control  $(H_2O)$ . **B)** The same order but without addition of the reverse transcriptase enzyme to the PCR reaction.