SUPPLEMENTAL DATA

Supplemental table 1. List of primers used for RT-PCR assays.

	5' forward 3'	5' reverse 3'	Expected Size (bp)	Accession number
TRPM8 F12/R15	GATTTTCACCAATGACCG CCG	CCCCAGCAGCATTGATG TCG	503	AY328400
TRPM8 F8/R9	CTGTCATGGACATCCCAC TG	GGGATCTTGCCACCATA GTT	102	AY328400
TRPM8 F21/R22	ATTCCGTTCGGTCATCTAC G	CACACACAGTGGCTTGG ACT	127	AY328400
AR	TTGAGCCAGGTGTAGTGT GTG	CTGGAGTTGACATTGGT GAAGG	241	NM_000044
PSA	CTCACCCTGTCCGTGACGT GGATT	AAGCTGTGGCTGACCTG AAATA	266	NM_001648.2
GAPDH	TTCACCACCATGGAGAAG GC	GGCATGGACTGTGGTCA TGA	237	NM_002046.3
HPRT	GGCGTCGTGATTAGTGAT GAT	CGAGCAAGACGTTCAGT CCT	134	NM_000194

Supplemental table 2: Compositions of bath and pipette solutions for electrophysiological recordings.

	$I_{MENTHOL}$		$I_{SOC/MENTHOL}$	
Salt	BATH	PIPETTE	BATH	PIPETTE
NaCl	140	8	145	-
CH_3O_3SCs	-	-	-	120
CsCl	-	-	-	10
KCl	5	145	5	-
$CaCl_2$	2	0.5	10	-
$MgCl_2$	2	0.1	1	6
Na_2HPO_4	0.4	-	-	-
KH_2PO_4	0.4	-	-	-
$NaHCO_3$	5	-	-	-
HEPES	10	10	10	10
BAPTA	-	-	-	10
EGTA	-	1	-	-
Glucose	5	-	5	-
pH adj	NaOH	KOH	NaOH	CsOH

Concentrations are given in mM, pH of all solutions was adjusted to 7.3. Osmolarity of the bath and pipettes solutions was approximately 300 and 280mosm/L, respectively.

Supplemental Methods

Creation of HEK-293 cell line stably expressing TRPM8 – was performed as described in (1).

Recombinant protein preparation – TRPM8 protein fused with COOH-terminal 6-histidine epitope tags were purified with the denaturing condition of Ni-NTA agarose kit (Invitrogen Inc) as recommended. Protein quantities in fractions were assessed by taking OD280 readings. Fractions containing proteins were finally concentrated with a cut-off at 10,000 MW.

RT-PCR – Total RNAs were isolated from PC-3 and HEK-TRPM8 cells as described (1). To detect TRPM8 cDNA, PCR was performed by adding 1µl of the RT template to a mixture of: 50 mM KCl, 10 mM Tris-HCl (pH 8.3), 2.5 mM $MgCl_2$, 200 µM of each dNTP, and 1 U AmpliTaq Gold (Applied Biosystems), in a final volume of 25 µl. DNA amplification conditions included an initial denaturation step of 7 min at 95°C, and 40 cycles of 30 s at 95°C, 30 s at 60°C, 40 sec at 72°C, and finally 7 min at 72°C. Primers used are listed in supplemental Table 1.

Immunohistochemistry – 1) PrPE were fixed with 4% formaldehyde-1X PBS for 15 minutes, washed three times, then permeabilized in PBS-gelatine (Phosphate buffer saline, gelatine 1.2%) complemented with 0.01% Tween 20 and 100mM glycin for 30 minutes at 37°C. 2) Resection specimens from human prostate were frozen in liquid nitrogen-cooled isopentane and kept in “Tissue-Tek”® at -80°C before 10µm sections were prepared at -20°C with a cryostat and mounted on glass slides. The sections were blocked with PBS-gelatine (Phosphate buffer saline, gelatine 1.2%) for 30 minutes at 37°C. Afterwards the cells were incubated with primary antibodies: (1/200) rabbit polyclonal anti-TRPM8 antibody (Abcam), (1/25) mouse monoclonal CD10 antibody (Abcam), (1/1,000) mouse monoclonal cytokeratin 18 antibody (Neomarkers), (1/1,000) mouse monoclonal cytokeratin 8 antibody (Neomarkers), (1/1,000) mouse monoclonal cytokeratin 14 antibody (Chemicon international) in PBS-gelatine at 37°C for 1.5 hours. After thorough washes, the slides were treated with the corresponding anti-rabbit or anti-mouse IgG, coupled with either Alexa fluor 546-labeled (Molecular probes, dilution:

1/4,000) or Alexa fluor 488-labeled (Molecular probes, dilution: 1/2,000) diluted in PBS-gelatine for 1 hour at room temperature. After two washes, the slides were mounted with Mowiol®.

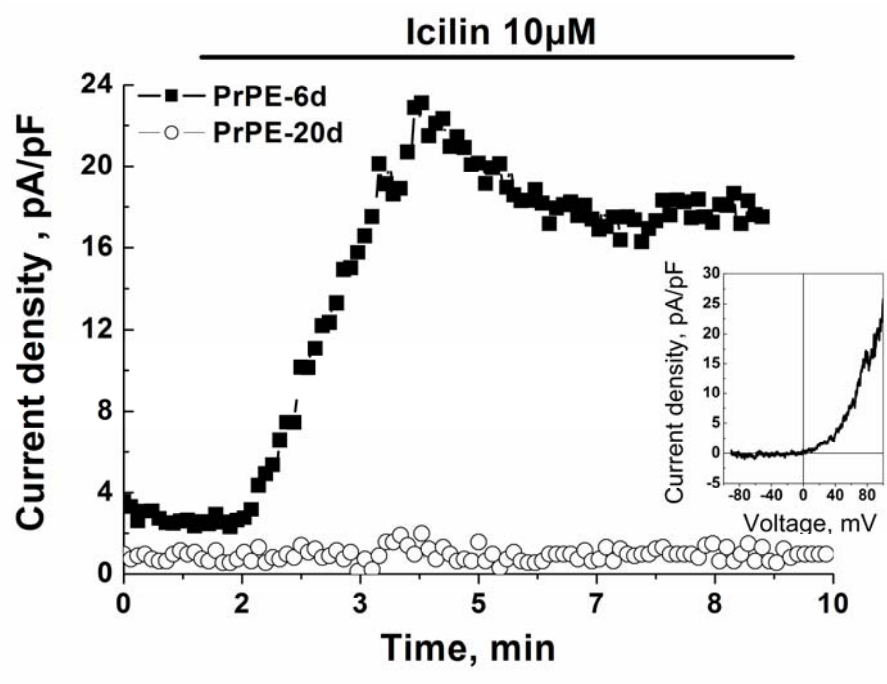
Fluorescence analysis was carried out using a Zeiss LSM 510 confocal microscope and analysis software (AIM 3.2, Zeiss), as previously described (1).

Supplemental References

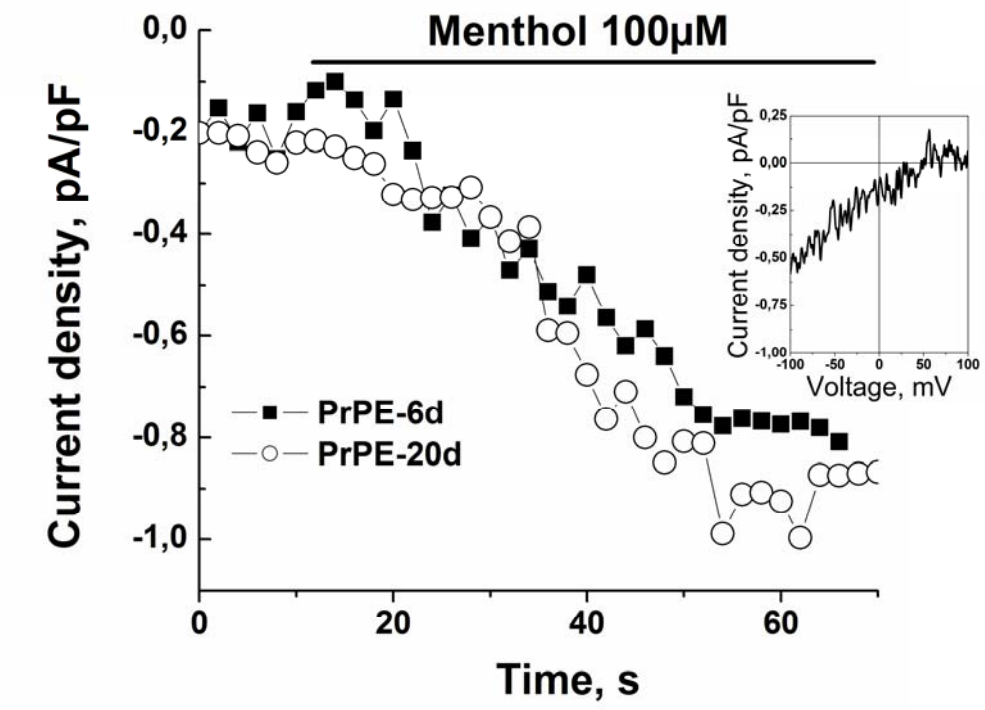
1. Thebault, S., Lemonnier, L., Bidaux, G., Flourakis, M., Bavencoffe, A., Gordienko, D., Roudbaraki, M., Delcourt, P., Panchin, Y., Shuba, Y., et al. 2005. Novel role of cold/menthol-sensitive transient receptor potential melastatine family member 8 (TRPM8) in the activation of store-operated channels in LNCaP human prostate cancer epithelial cells. *J Biol Chem* 280:39423-39435.

Supplemental figure 1

A

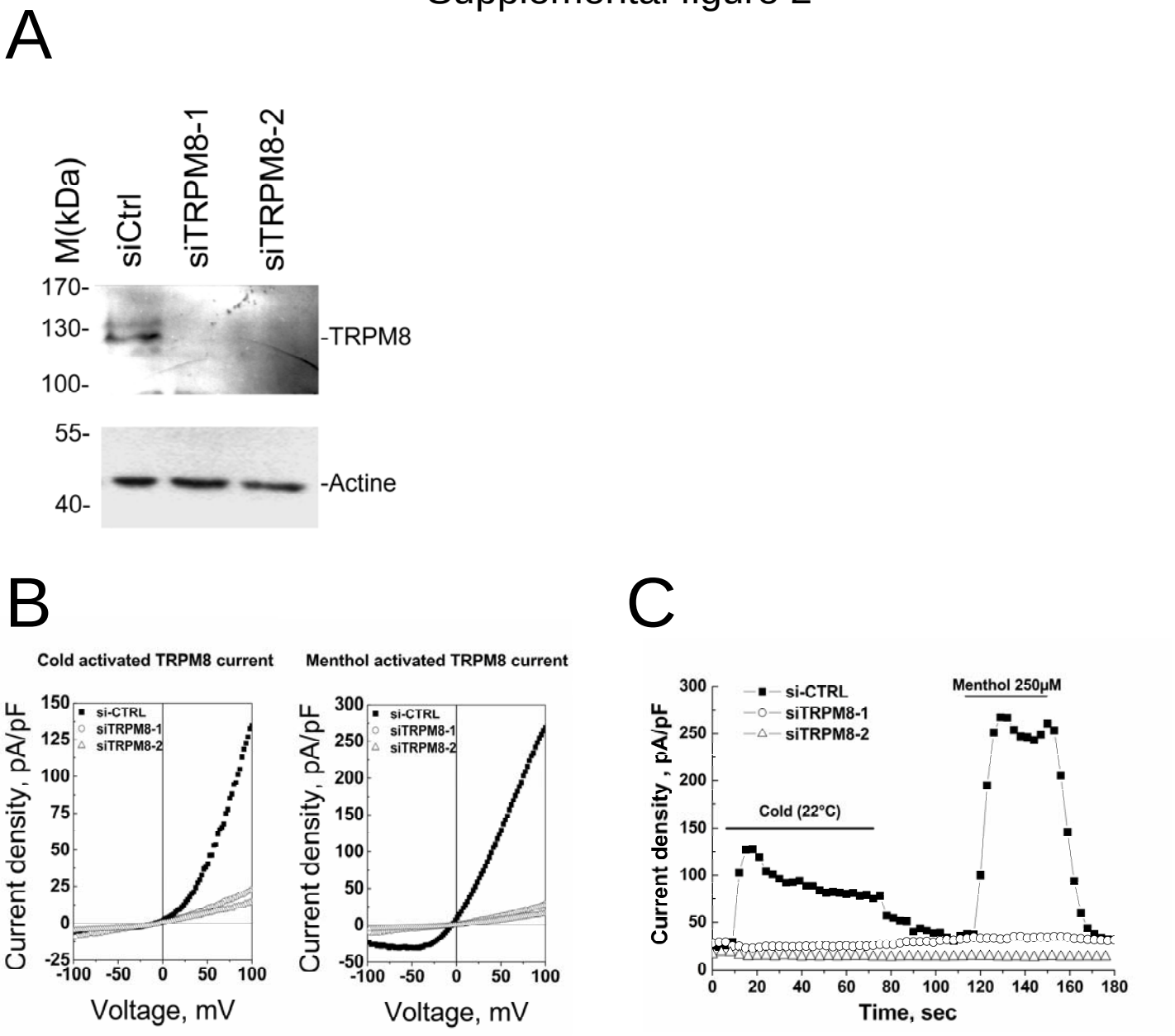


B



(A), Icilin (10μM) evoked I_{TRPM8} (22.89 ± 3.4 pA/pF at +100 mV, n=11) strictly restricted to PrPE-6d. Currents were recorded from voltage ramps at +100mV. Inset shows the representative I-V relationships of I_{TRPM8}. **(B)**, Representative time courses of whole-cell $I_{SOC/MENTHOL}$ induced by 100μM menthol in PrPE-6d and PrPE-20d at 36°C. Currents obtained from voltage ramps at -100mV. The inset shows the representative I-V relationships of $I_{SOC/MENTHOL}$.

Supplemental figure 2



Specific siRNA-mediated ablation of either classical TRPM8 or total TRPM8 mRNAs has different effects on menthol-evoked Ca2+ release. **(A)** Immunoblotting showing detection of TRPM8 proteins in HEK-TRPM8 cells with 100 nM of either control siRNA or siRNA anti-TRPM8. **(B)** Representative I-V relationships of the cold or menthol activated I_{TRPM8} in HEK-TRPM8 cells with either control siRNA or siRNA anti-TRPM8. **(C)** Typical traces of the cold or menthol activated ITRPM8 in HEK-TRPM8 cells with either control siRNA or siRNA anti-TRPM8 (currents recorded from voltage ramps at +100mV).