Supplementary data

Material and Methods

RT-PCR – Total mRNA was isolated from cells as described (1). DNA amplification conditions included an initial denaturation step of 7 min at 95° C, and 36 cycles of 30 sec at 95° C, 30 sec at 60° C, 30 sec at 72° C, and finally 7 min at 72° C. Primers used are listed in the Table 2.

Table 2. Primers used in RT-PCR

Gene	Forward primer 5'-3'	Reverse primer 5'-3'
Human α2A-AR	AACGAGGTCATGGGCTACTG	CTGGTAGATGCGCACGTAGA
Rat TRPM8	AGTACCACATATGACTTCTCCCAC	ACAAGGTAATTCTCCTTCATGACG
Human HPRT	GGCGTCGTGATTAGTGATGAT	CGAGCAAGACGTTCAGTCCT
Rat α2A-AR	ATTTTCAACCACGACTTCCG	TGTCCCTCTCAGCCAGAACT
Rat HPRT	TAAGTTCTTTGCTGACCTGCTG	CCCGTTGACTGGTCATTACA

Biotinylation assay and immunoblotting – HEK-293_{M8} cells were subjected to cell surface biotinylation as described in (2). Next, cells were precipitated after lysis with neutravidin-agarose beads (Pierce Rockford, IL, USA). Immunoblot analysis was performed for TRPM8 expression in the plasma membrane fraction ($TRPM8_{PM}$) and in the total cell lysates ($TRPM8_{TL}$). As negative control, non-induced (NI) HEK-293_{M8} or non-transfected HEK-293 (NT) cells were used.
For the immunoblot analysis we have used the anti-TRPM8 antibody (1/2000, Abcam), Anti-a2A adrenergic receptor (1:250, sc-28983, Santa Cruz Biotechnology) and Anti-calnexin (1:1000, MAB3126, Milippore).

Legend

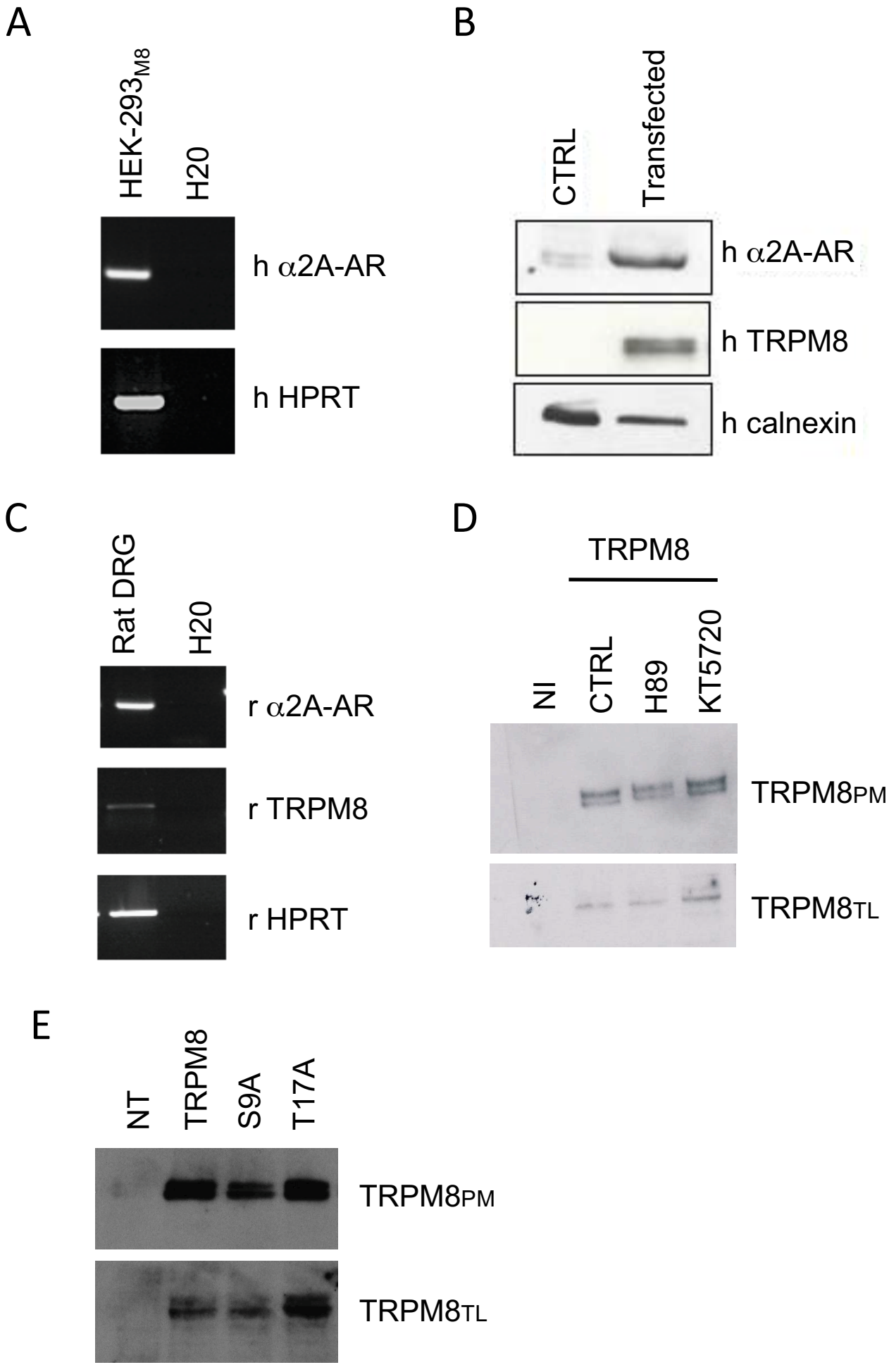
Supplementary data figure S1. *A.* RT-PCR analysis of the α2A-ARs (h α2A-AR) expression in HEK-293_{M8} cells. Human form of Hypoxanthine-Guanine-Phospho-Ribosyl- Transferase (h HPRT) is used as control. *B.* Immunoblotting showing co-detection of human forms of α2A-ARs (h α2A-AR) and TRPM8 (h TRPM8) in HEK-293_{M8} cells transfected with α2A-AR plasmid. The human form of calnexin (h calnexin) is used as expression control. *C.* RT-PCR analysis of TRPM8 and α2A-ARs expression in cultured rat DRG neurons. *D,* Biotinylation assay showing plasma membrane expression of TRPM8 channels ($TRPM8_{PM}$) in HEK-293_{M8} cells non induced by tetracycline (NI) or induced (TRPM8), in presence or not (CTRL) of PKA inhibitors H89 and KT5720. *E,* Biotinylation assay showing plasma membrane expression of TRPM8 channels ($TRPM8_{PM}$) in HEK-293 cells transfected with TRPM8 (TRPM8) or mutants S9A and T17A. Non-transfected HEK-293 (NT) cells were used as negative control. Expression of TRPM8 in total cell lysates ($TRPM8_{TL}$) is shown below the $TRPM8_{PM}$ blot for both D and E panels.

Supplementary data figure S2. *A.* Averaged I-V relationships of menthol (triangles) and cold (circles)-activated I_{TRPM8} before (black) and after (open) exposure to a short pretreatment of clonidine (1-3 minutes, mean ± s.e.m., n = 8). *B.* quantification of the effects of a short incubation of clonidine on the density of menthol and cold-activated I_{TRPM8} at + 100 mV (mean ± s.e.m., n = 8 for each condition). *C.* Quantification of the inhibitory effect of clonidine in HEK-$293_{M8\text{-}\alpha2A\text{-}AR}$ cells with TRPM8-activating stimuli perfused successively (mean ± s.e.m., n = 5-10 for each condition); (*), (**) and (***) denote statistically significant differences with $P<0.05$, $P<0.02$ and $P<0.01$, respectively.

References

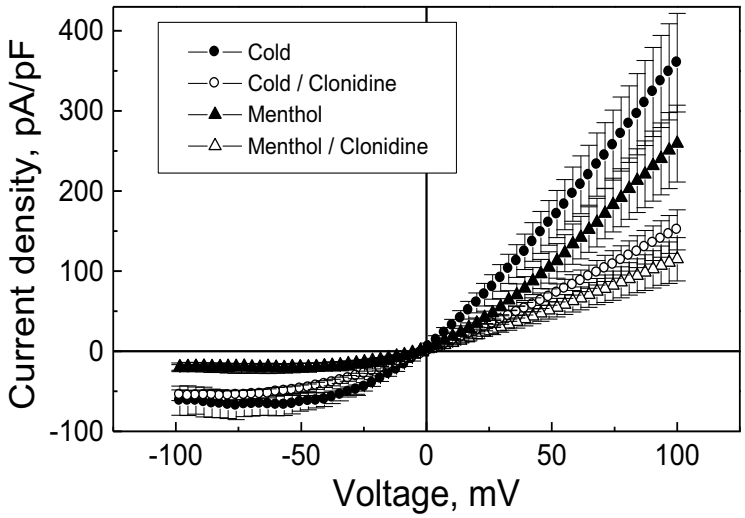
1. Thebault, S., Lemonnier, L., Bidaux, G., Flourakis, M., Bavencoffe, A., Gordienko, D., Roudbaraki, M., Delcourt, P., Panchin, Y., Shuba, Y., Skryma, R., and Prevarskaya, N. (2005) *J Biol Chem* 280(47), 39423-39435
2. Gkika, D., Topala, C. N., Chang, Q., Picard, N., Thebault, S., Houillier, P., Hoenderop, J. G., and Bindels, R. J. (2006) *Embo J* 25(20), 4707-4716

Supplementary data S1



Supplementary data S2

A



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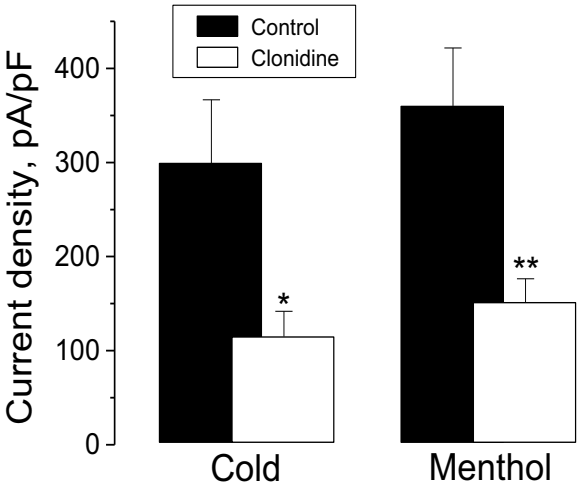

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