PNAS

Supporting Information for

KCNQ1 is an essential mediator of the sex-dependent perception of moderate cold temperatures

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Figures S1 to S3
SI References

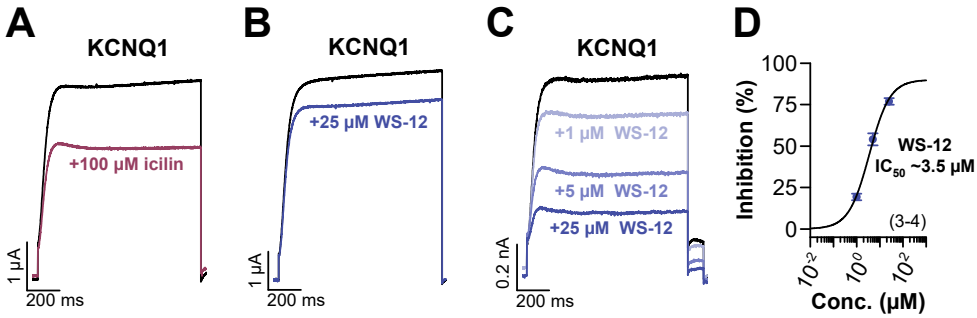

Figure S1. The super-cooling agent icilin and the menthol derivative WS-12 inhibit KCNQ1 channels. (*A*) Representative current traces of two-electrode voltage-clamp recordings from human KCNQ1 channels before (black) and after (red) application of 100 µM icilin. (*B*) Representative current traces of KCNQ1 expressed in oocytes, before (black) and after (blue) application of 25 µM WS-12. Note that due to solubility problems, we could not apply higher concentrations of WS-12 to induce a stronger inhibition in the oocyte expression system. (*C*) Since channel blockers tend to be weaker in the oocyte expression system (about 7-fold higher IC_{50}), acting as a lipophilic sink, and since we could not apply higher concentrations of WS-12, we aimed to asses the WS-12 affinity of KCNQ1 in mammalian cells. Illustrated are representative current traces of whole-cell patch-clamp recordings from HeLa cells transfected with human KCNQ1 channel before (black) and after application of WS-12 in increasing concentrations. (*D*) A dose-response curve of WS-12 on KCNQ1 currents. The IC_{50} of WS-12 for KCNQ1 was 3.5 µM and calculated by fitting the data to a Hill equation. The number of biological replicates (n) is indicated within the graph. Data are presented as mean ± S.E.M..

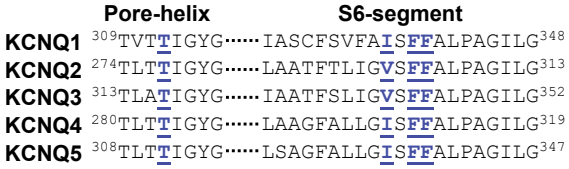

Figure S2. Sequence alignment of KCNQ channels. Blue bold and underlined letters indicate the menthol binding site in the KCNQ1 channel.

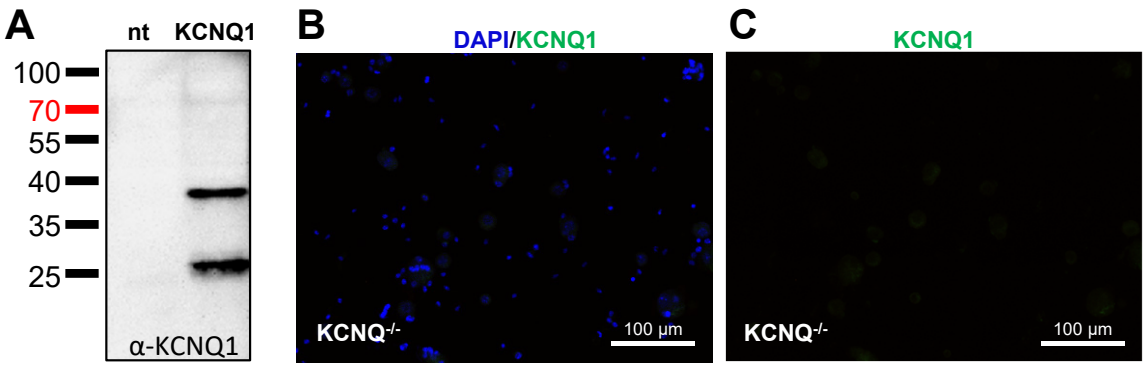

Figure S3. Validation of the KCNQ1 antibody. (*A*) Western blot of HeLa cells transfected or non-transfected (nt) with KCNQ1. Please note that three KCNQ1-specific bands appeared. In addition to the very weak signal at ~70 kDa for the full-length channel, two stronger signals were detected at ~40 and ~28 kDa, reflecting the previously described caspase-mediated proteolysis of KCNQ1 (1). (*B*) Immunocyto-chemistry of dissociated DRG neurons from a male KCNQ1$^{-/-}$ mice with DAPI or (*C*) without DAPI.

SI References

1. A. Strigli *et al.*, Doxorubicin induces caspase-mediated proteolysis of Kv7.1. *Communications Biology* **1** (2018).