

## Supplemental material

York, J.M. Temperature activated transient receptor potential (TRP) ion channels from Antarctic fishes. *Open Biology*. DOI: 10.1098/rsob.230215

Raw data can be found at the United States Antarctic Program Data Center (<https://doi.org/10.15784/601695>).

## Supplemental Figure Captions

Figure supplement 1: Voltage current relationships for TRPA1b (A, top row) and TRPV1a (C, second row from bottom) across multiple temperatures. Blank, water-injected controls from oocytes incubated at the same temperatures for each respective channel are shown in the second and bottom rows. Colors indicate holding temperatures. Middle panels (B) show TRPA1b and blank curves at 25°C (left side) compared with staurosporine treated oocytes (center, green). Right center plot shows TRPA1b relative conductance curves at 25°C and then at 25°C treated with staurosporine; there was no significant difference between these curves. Reliable conductance curves could not be calculated for TRPV1a so these are not shown.

Figure supplement 2: Increasing osmolarity and pH changes leak and maximum current but not threshold or Q10. Panels show control conditions of 250 mOsm pH 7.4 in black compared to 500 mOsm pH 8.5 in purple for TRPA1b (left side) and TRPV1a (right side); p-values indicate the effect of experimental condition on the dependent variables of threshold (top), Q10 (second row), leak (third row), and maximum current (bottom). Grey bars indicate the percent of cells measured that had no distinct threshold. Note maximum current is not plotted for TRPV1a as it was not a robust measurement given that the threshold of this channel was close to the maximum tested temperature.

Figure supplement 3: Effects of the addition of trimethylamine oxide (TMAO) to the incubation solution. Panels compare oocytes incubated in control incubation solution (black) with incubation solution in which 15% of the osmolarity was from TMAO (orange) for TRPA1b (left side) and TRPV1a (right side); p-values indicate effect of TMAO on the dependent variables shown: threshold (top), Q10 (second row), leak (third row), and maximum current (bottom). Grey bars show the percent of oocytes measured that had no distinct threshold.

Figure supplement 4: TRP channel associated factors (TCAFs) in notothenioids. (A) Maximum likelihood gene tree for TCAFs. Reference TCAF sequences from Gkika et al. (2015) are in black, notothenioid sequences are in blue. Tip labels that are accession numbers refer to accessions from *H. antarcticus* transcriptome (PRJEB26835). Other notothenioid sequences are from NCBI. Support values are from 1000 bootstrap replicates. (B) Count data showing expression of the c40238\_g1\_i3 isoform that showed expression in the neural tissue but not non-neural tissue in *H. antarcticus* (count data from York and Zakon, 2022; PRJNA758918).

Figure supplement 5: Co-expression of TCAF does not change threshold, Q10, or maximum current of TRPA1b. Comparisons of TRPA1b (black) controls with TRPA1b co-injected with TCAF from *G. acuticeps* (pink) showing threshold (top left) and Q10 (middle left). P-values indicate significance of co-injection on the dependent variables. Maximum currents of water injected blank oocytes are compared with oocytes injected with TCAF alone and TRPA1b alone and co-injected (bottom left), p-values indicate pairwise comparisons. Example individual response curves are shown on the right, with temperature stimuli in red and current response in black, top panel is a water-injected control, second panel is TCAF alone, third panel is TRPA1b alone, and the bottom panel is TRPA1b co-injected with TCAF.

Figure supplement 6: Oxidation with 1 mM H<sub>2</sub>O<sub>2</sub> decreases threshold of activation for TRPV1a but not TRPV1a. Effects of 1500 nM (light blue), 100 μM (medium blue), and 1 mM H<sub>2</sub>O<sub>2</sub> (dark blue) are compared to control conditions (black) for TRPA1b (left side) and TRPV1a (right side). Significance of the experimental condition on the dependent variable is indicated by the p-values, the dependent variables shown are threshold (top), Q10 (second row), leak (third row), and maximum current (bottom). Data shown for first activation only.

Figure supplement 7: Kinase inhibitor staurosporine lowers threshold and Q10 of activation for TRPA1b but not TRPV1a. Controls (black) are compared with oocytes treated with staurosporine (light green) or DMSO only (dark green) for TRPA1b (left side) and TRPV1a (right side). Effect of treatment on the dependent variables or the pairwise significance are indicated by the p-values; dependent variables plotted are threshold (top), Q10 (second row), leak (third row), and maximum current (bottom). Grey bars indicate the percent of tested oocytes that showed no distinct threshold.

Figure supplement 8: Reducing cholesterol in the oocyte membrane lowers threshold for TRPA1b. Effects of cholesterol supplementation (dark red), cholesterol reduction with methyl-β-cyclodextrin (MBC; light red), and additive effects of MBC and oxidation (brown) are compared with control oocytes (black). P-values indicate significance of condition on the dependent variable or pairwise comparisons as indicated. Level of current at 10°C is also compared (bottom right). Grey bars indicate the percent of oocytes that show no distinct threshold.

## Sequences

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