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Supplementary Materials for

Cold acclimation via the KQT-2 potassium channel is modulated by oxygen in *Caenorhabditis elegans*

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The PDF file includes:

Other Supplementary Material for this manuscript includes the following:

(available at advances.sciencemag.org/cgi/content/full/5/2/eaav3631/DC1)

Supplementary raw data file (Microsoft Excel format)

Supplementary Results and Discussion

KQT-2 indirectly influences fatty acid composition of total lipids

In many organisms, including nematodes, the proportion of different fatty acids in the body is an important aspect of cold acclimation (*7, 13, 16)*. We therefore measured the fatty acid composition of total lipids in the *kqt-2* mutant. We found that the fatty acid composition of total lipids was slightly different between wild-type animals and *kqt-2* mutants (fig. S3, A and B). These results indicate that KQT-2 indirectly affects the fatty acid composition of total lipids in the wild-type.

KQT-type potassium channel subunit KQT-1 is not involved in cold acclimation

C. elegans has three KQT-type potassium channels encoded by *kqt-1, kqt-2* and *kqt-3*. Of these, *kqt-1* is not expressed in ADL neurons (*25*), which is consistent with this study where *kqt-1* mutants showed normal temperature acclimation (fig. S5).

Supplementary Methods

Strains

The *C. elegans* N2 (Bristol) strain was used as the wild-type strain in all experiments in this study. In addition, the following mutant strains were also used:

N2 Bristol England, KHR181 *kqt-2(ok732)*, *kqt-2(tm642)*, CX4544 *ocr-2(ak47)*, CX4652 *ocr-2(ak47) osm-9(ky10)*, ZK64 *kqt-3(aw1)*, KHR182 *kqt-3(aw1);kqt-2(ok732)*,

KHR188 *kqt-3(aw1);kqt-2(ok732)*, CX4533 *ocr-1(ok132)*, KHR183 *ocr-1(ok132);kqt-2(ok732)*, KHR184 *ocr-2(ak47);kqt-2(ok732)*, CX6448 *gcy-35(ok769)*, KHR185 *gcy-35(ok769);kqt-2(ok732)*, KHR189 *gcy-35(ok769);kqt-2(ok732)*, RB1415 *catp-3(ok1612)*, VC693 *cgt-1(ok1045)*, RB2095 *clec-67(ok2770)*, RB1262 *cpr-1(ok1344)*, FX2418 *dhs-4(tm2418)*, RB1772 *dmd-7(ok2776)*, FX2468 *F58E6.7(tm2468)*, NL795 *gpa-7(pk610)*, RB1373 *gpdh-1(ok1558)*, RB883 *kqt-2 (ok732)*, CZ1758 *max-1(ju142)*, FX1770 *mtl-1(tm1770)*, FX830 *pgp-9(tm830)*, RB908 *pmp-1(ok773)*, *T28C12.4(tm1013)*, CX0010 *osm-9(ky10)*, FK127 *tax-4(p678)*, VC1149 *C25B8.4&kqt-1(ok413)*, FX05415 *gsp-4(tm5415)*, KHR187 *gsp-4(tm5415);kqt-2(ok732)*, KHR191 *gsp-4(tm5415);kqt-2(ok732)*, *kqt-2(ok732);[pKDK66, pAK62]*, *kqt-2(ok732);Ex[kqt-2 genomic gene, pKDK66, pAK62]*, *kqt-2(ok732);Ex[ges1-p::kqt-2, pKDK66, pAK62]*, *kqt-2(ok732);Ex[osm-6p::kqt-2, pKDK66, pAK62]*, *N2;Ex[*pMSK029 *kqt-2p(1kb)::kqt-2 genomic gene(1st to 12th exon)::gfp, pRF4]*, *N2;Ex[*pMSK030 *kqt-2p(2.3kb)::kqt-2 genomic gene(1st to 12th exon)::gfp, pRF4]*, *N2;Ex[*pMSK031 *kqt-2p(4.6kb)::kqt-2 genomic gene(1st to 12th exon)::gfp, pRF4]*, *N2;Ex[kqt-2cDNA::gfp]*, *kqt-2(ok732);Ex[sra-7p::kqt-2cDNA pAK62, pKDK66]*, *kqt-2(ok732);Ex[sre-1p::kqt-2cDNA, pAK62, pKDK66]*, *N2; Ex[sre-1p::yc3.60, pRF6]*, *kqt-2(ok732); Ex[sre-1p::yc3.60, pRF6]*, *kqt-2(ok732);Ex[sre-1p::kqt-2cDNA, sre-1p::yc3.60]*, *N2;Ex[sre-1p::kqt-2cDNA, sre-1p::yc3.60]*, *kqt-3(aw1);kqt-2(ok732) Ex[sre-1p::yc3.60]*, *kqt-3(aw1); Ex[sre-1p::yc3.60]*, *ocr-1(ok132);Ex[sre-1p::yc3.60]*, *ocr-2(ak47);Ex[sre-1p::yc3.60]*, *osm-9(ky10);ocr-1(ok132);Ex[sre-1p::yc3.60]*, *ocr-2(ak47);ocr-1(ok132);Ex[sre-1p::yc3.60]*, *ocr-1(ok132);kqt-2(ok732);Ex[sre-1p::yc3.60]*, *osm-9(ky10)ocr-2(ak47);Ex[sre-1p::yc3.60]*.

Molecular biology techniques

A PCR-amplified DNA fragment containing 3.4 kb upstream of *kqt-2*, and all exons

and introns of the *kqt-2* genomic sequence was used for the transgenic rescue experiment. pMSK008 contains a Kozak sequence, a *kqt-2* cDNA and the 3′-UTR of the *unc-54* gene. The promoter sequences, *ges-1p* (3.3 kb), *osm-6p* (2.1 kb), *sra-7p* (4.2 kb) and *sre-1p*(1.2 kb) were inserted upstream of pMSK008 *kqt-2cDNA,* to create respectively, pMSK004, pMSK005, pMSK007, and pMSK024 plasmids for transgenic expression. As previously reported, *kqt-2::gfp* includes exons 1 to 12 of the *kqt-2* gene and 9 kb of upstream 5′ non-coding sequence (*25*). *kqt-2cDNA::gfp* (pMSK028), a full-length GFP fusion expression construct was generated by inserting a *kqt-2cDNA* and a 9 kb of *kqt-2* 5′-upstream promoter sequence into pPD95.75 containing GFP.

Statistical analysis

The cold acclimation tests were performed on more than three plates. All error bars in the figures indicate the standard error of the mean (SEM). Assuming that the distributions of all data follow normal distribution, all statistical analysis were performed with a parametric test, the Tukey-Kramer method, Dunnett's test or the unpaired t test (Welch). Multiple comparisons were performed using one-way ANOVA with comparisons tested using the Tukey-Kramer method and Dunnett's test. Dunnett's test was performed to compare the left-most groups of the bar graphs with other groups. Comparisons between two groups were performed using the unpaired t test (Welch). *$p < 0.05$; **$p < 0.01$. The tests were performed using Mac statistical analysis ver. 2 (Esumi, Japan).

Chemotaxis to Attractive Volatile Odorant Assay

Chemotaxis to volatile odorants was assayed according to a previous report (*32*).

Fatty acid composition

Lipids were extracted from synchronized cultures of well-fed adults and transmethylated, as described previously (*13, 16*). Fatty acid methyl esters were analyzed by gas–liquid chromatography and mass spectrometry analysis, and identified by comparing peak retention times with authentic standards. Fatty acid compositions are presented on a percentage weight basis.

Germline transformation

Germline transformations were performed with co-injection mixes consisting of experimental plasmid DNAs at various concentrations (5.0–100 ng/µL) and pAK62 *AIYp::GFP*, pKDK66 *ges-1p::NLS::GFP* or pRF4 *rol-6gf* as transgenic markers at 30–50 ng/µL.

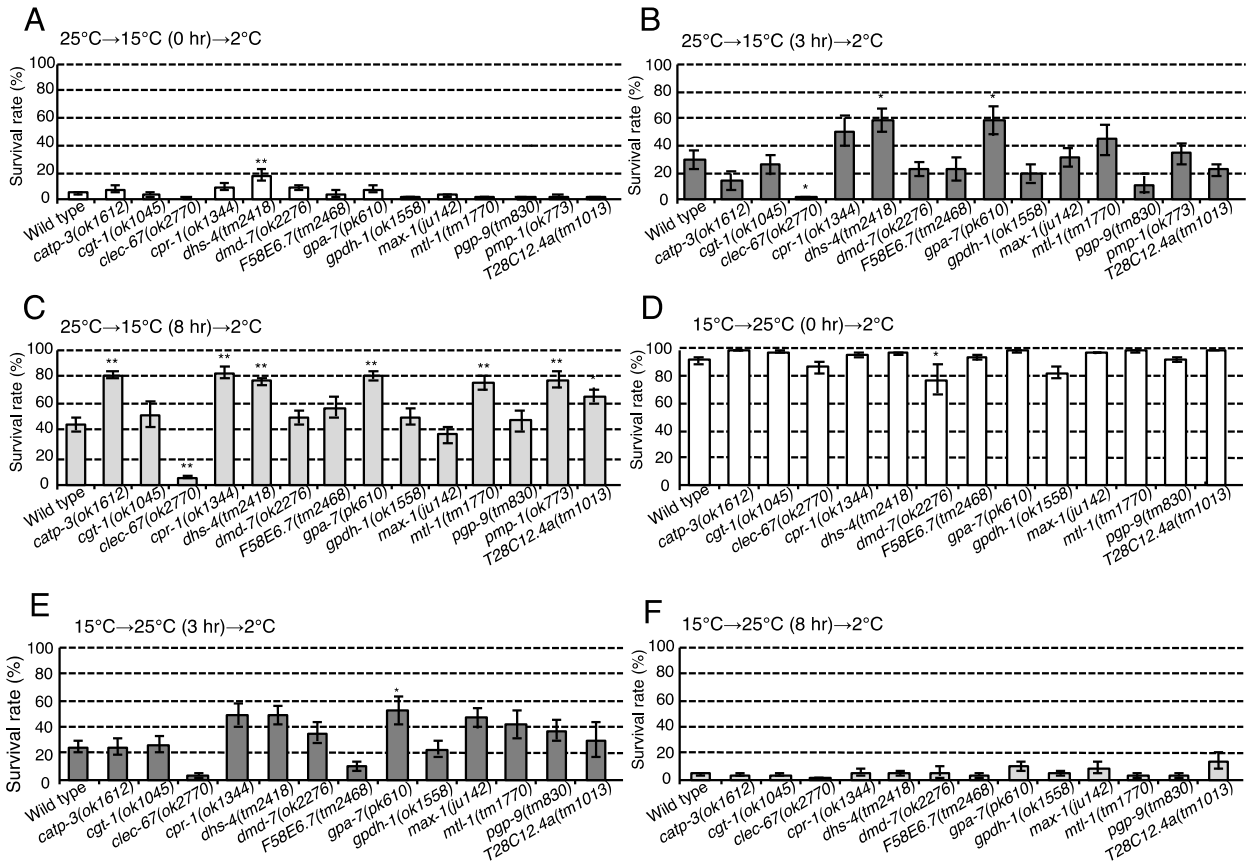

Fig. S1. Cold acclimation assays of selected mutant animals defective in genes identified by previous DNA microarray analysis. When animals were transferred to 17°C for 4 hours after cultivation at 23°C, expression levels of 79 genes were changed (*35*). Cold acclimation was tested in a subset of 14 mutants of these genes. (A to C) Animals grown at 25°C were transferred to 15°C and maintained at 15°C for 0, 3, or 8 hours, and then transferred to 2°C for 48 hours [25→15 (0, 3, 8 hrs)→2°C]. *clec-67(ok2770), dhs-4(tm2418)* and *gpa-7(pk610)* mutants showed abnormal cold acclimation at 15°C after 3 or 8 hours. Number of assays ≥ 8. Error bar indicates SEM. Comparisons were performed using Dunnett's test. *$p < 0.05$; **$p < 0.01$. (D to F) Animals grown at 15°C were transferred to 25°C and maintained at 25°C for 0, 3, or 8 hours, then transferred to 2°C for 48 hours [15→25 (0, 3, 8 hrs)→2°C]. *gpa-7(pk610)* mutants exhibited abnormal cold acclimation after conditioning at 25°C for 3 hours. Number of assays ≥ 3. Error bar indicates SEM. Comparisons were performed using Dunnett's test. *$p < 0.05$; **$p < 0.01$

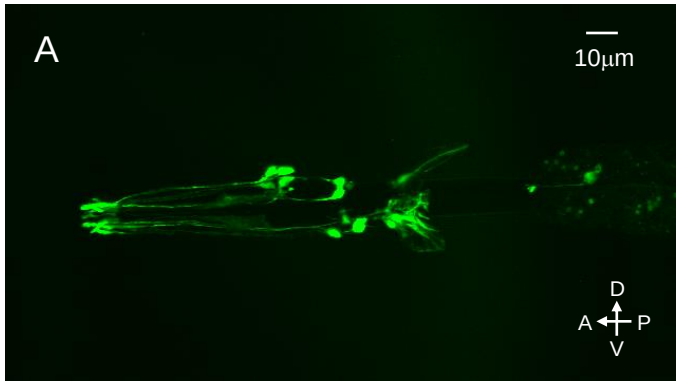

Fig. S2. Localization analysis of KQT-2. (A) Wild-type animal expressing *kqt-2cDNA::gfp* (100 ng/μl) using a 9.0 kb *kqt-2* promoter. KQT-2::GFP is observed in whole sensory neurons and is especially localized to sensory endings and cell bodies of head sensory neurons.

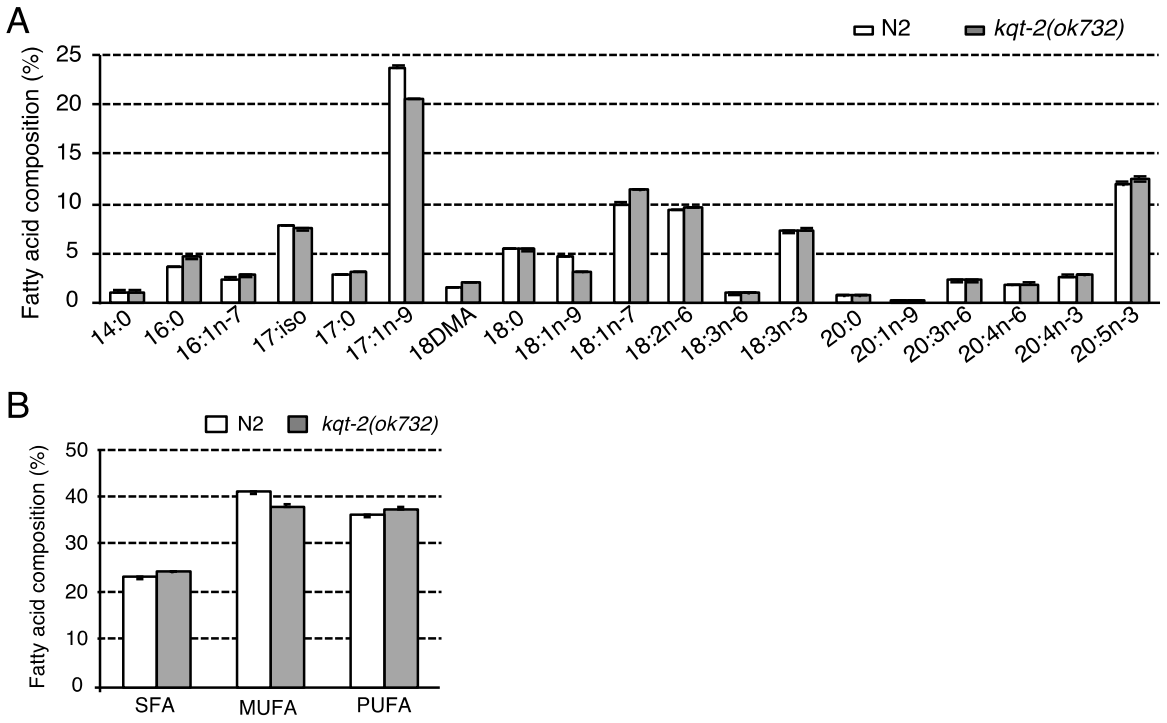

Fig. S3. Lipid composition of wild-type and *kqt-2(ok732)*. (A and B) The fatty acid composition of wild type and *kqt-2(ok732)*. Comparison of: (A) chain lengths; and (B) saturated fatty acids (SFA), mono-unsaturated fatty acids (MUFA) and poly-unsaturated fatty acids (PUFA). N=3 for each assay.

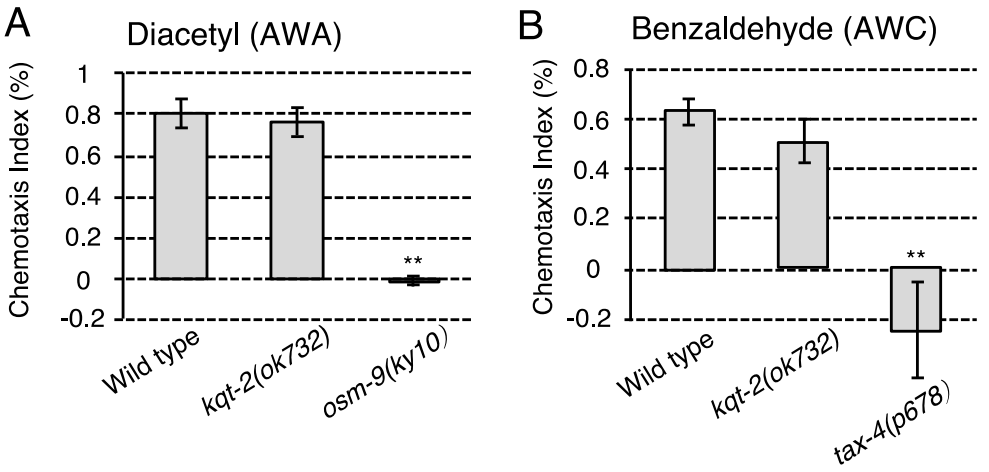

Fig. S4. Behavioral assays of *kqt-2(ok732)*. (A) Chemotaxis to diacetyl sensed by AWA neurons (*32*). *kqt-2* mutants showed normal chemotaxis to diacetyl. Number of assays ≥ 9. Error bar indicates SEM. Comparisons were performed using Dunnett's test. *p < 0.05; **p < 0.01. (B) Chemotaxis to benzaldehyde sensed by AWC neurons. *kqt-2* mutants showed normal chemotaxis to benzaldehyde. Number of assays ≥ 9. Error bar indicates SEM. Comparisons were performed using Dunnett's test. *p < 0.05; **p < 0.01.

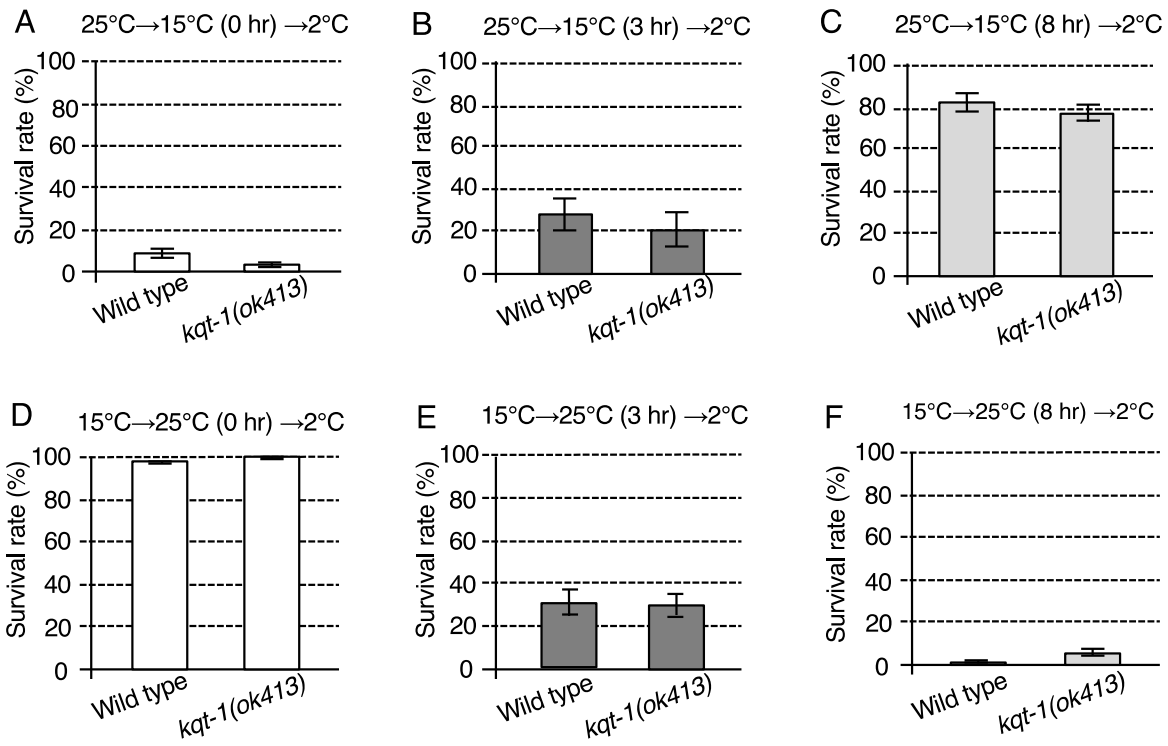

Fig. S5. Cold acclimation assays of *kqt-1(ok413)*. (A to C) Animals grown at 25°C were transferred to 15°C and maintained at 15°C for 0, 3, or 8 hours, and then transferred to 2°C for 48 hours [25→15 (0, 3, 8 hrs)→2°C]. The *kqt-1(ok413)* mutant exhibited normal cold acclimation. Number of assays ≥ 11. Error bar indicates SEM. Comparisons were performed using the unpaired t test (Welch). (D to F) Animals grown at 15°C were transferred to 25°C and maintained at 25°C for 0, 3, or 8 hours, then transferred to 2°C for 48 hours [15→25 (0, 3, 8 hrs) →2°C]. The *kqt-1(ok413)* mutant exhibited normal cold acclimation. Number of assays ≥ 7. Error bar indicates SEM. Comparisons were performed using the unpaired t test (Welch).

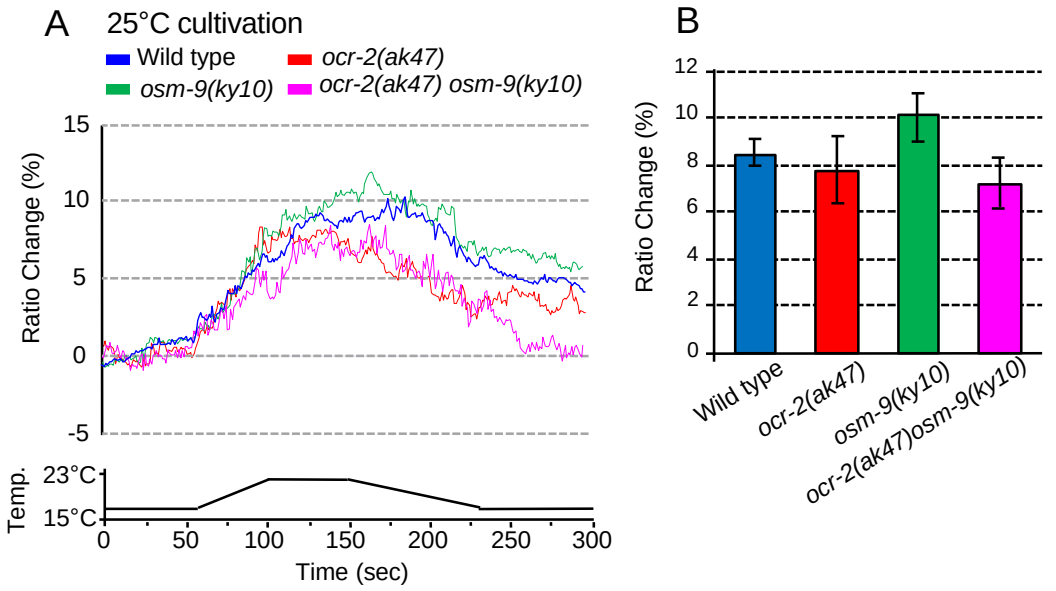

Fig. S6. Ca^{2+} imaging of ADL neurons in mutants defective in TRP channels expressed in ADL neurons. (A) Ca^{2+} imaging of ADL neurons in *osm-9(ky10)*, *ocr-2(ak47)* and *osm-9(ky10);ocr-2(ak47)* mutants, in response to temperature stimuli. All mutants exhibited normal Ca^{2+} thermal responses, relative to the wild type. (B) The bar graph indicates the average ratio change from 140 to 150 sec. N ≥ 17. Error bar indicates SEM. Comparisons were performed using the Tukey-Kramer method. Data from wild-type animals cultivated at 25°C in fig. S6A are shared with Fig. 2C, E, I, K, Fig. 3D, F, H and Fig. 4D given that the experiments were conducted simultaneously. Data from *ocr-2(ak47)* animals cultivated at 25°C in fig. S6A are shared with Fig. 3D given that the experiments were conducted simultaneously.

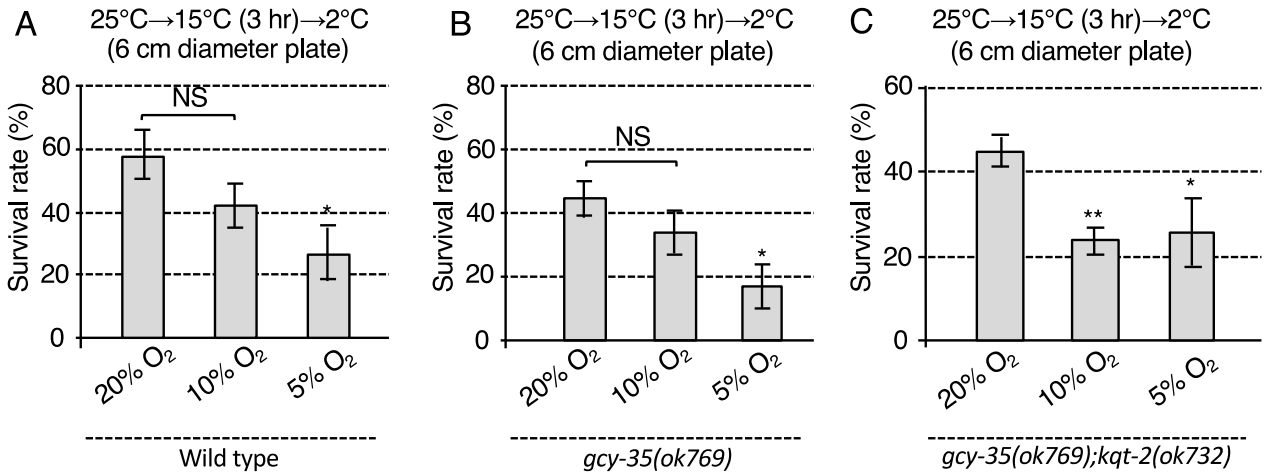

Fig. S7. Cold acclimation of animals cultivated with O_2 concentrations of 20, 10, and 5%. (A to C) Wild type and *gcy-35(ok769)* cultivated at 5% O_2 concentration showed decreased cold acclimation. *gcy-35(ok769);kqt-2(ok732)* showed decreased cold acclimation when cultivated at O_2 concentrations of 10% and 5%. Number of assays ≥ 9. Error bar indicates SEM. Comparisons were performed using Dunnett's test. *$p < 0.05$; **$p < 0.01$.

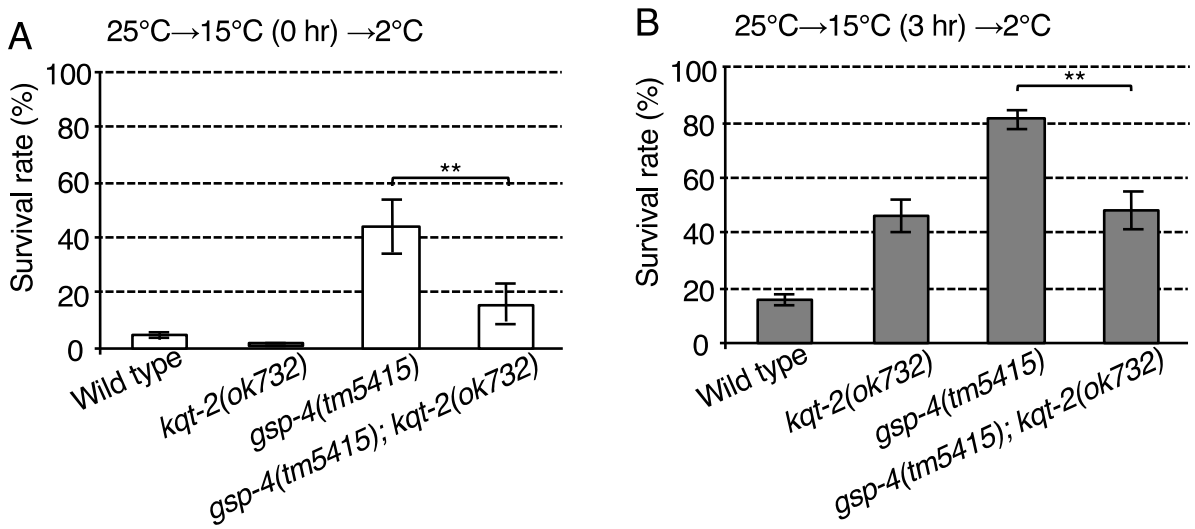

Fig. S8. Cold acclimation assays with mutants defective in GSP-4, a sperm-specific protein phosphatase (PP1). (A and B) Cold acclimation assays with *gsp-4(tm5415)* and *gsp-4(tm5415);kqt-2(ok732)* mutant animals. Number of assays ≥ 8. Error bar indicates SEM. Comparisons were performed the using the Tukey-Kramer method. *$p < 0.05$; **$p < 0.01$.