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Supplemental Information

Growth at Cold Temperature Increases the Number of Motor Neurons to Optimize Locomotor Function

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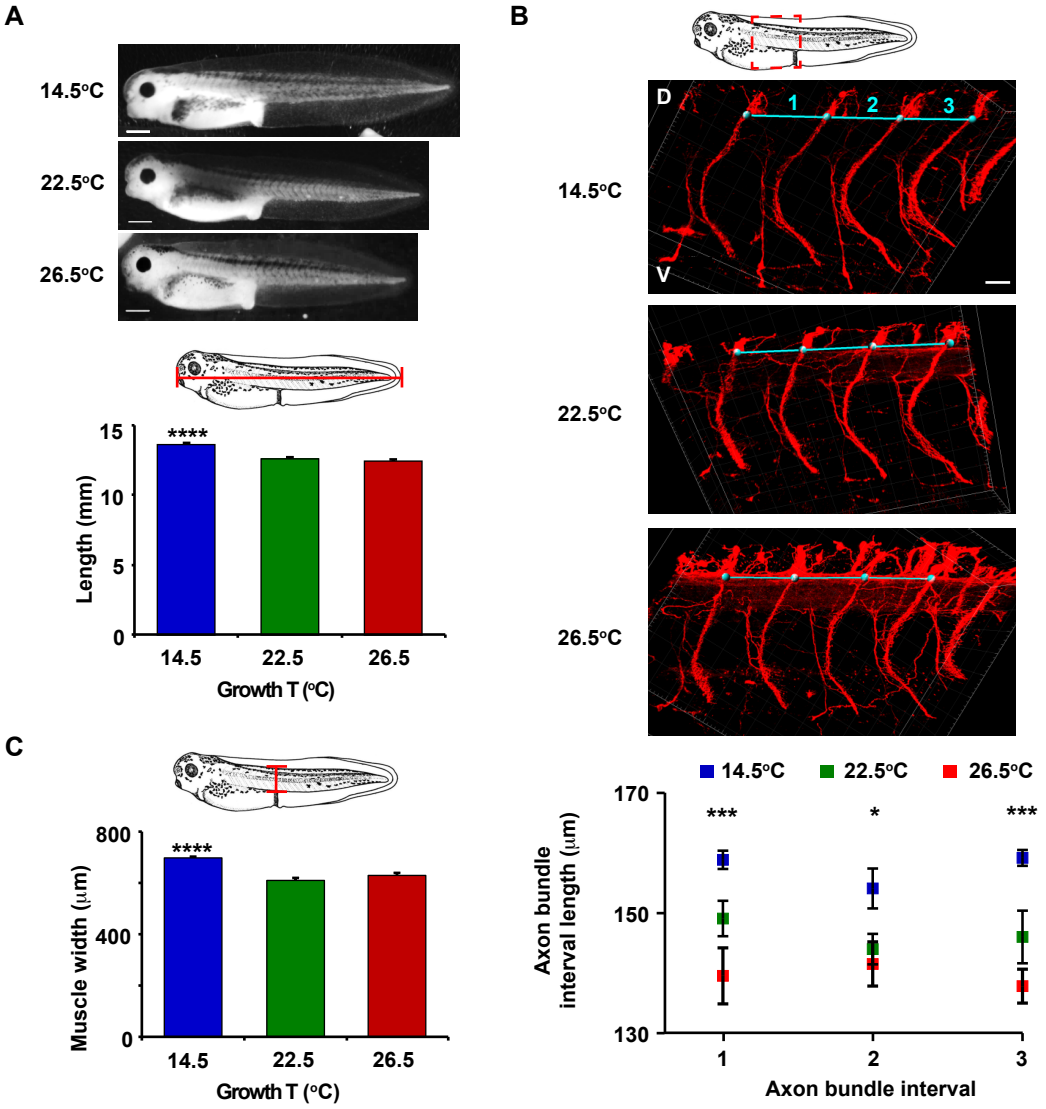

Figure S1. Xenopus laevis larvae follow the Temperature-Size Rule. Related to Figure 2.
Larvae were raised in cold (14.5°C) or warm (22.5 and 26.5°C) temperatures until stage 40.
(A) Representative bright field images of larvae grown in different temperatures. Scale bar, 1 mm. Graph shows anteroposterior larva length per growth temperature, mean±SEM, n=43-54 larvae per condition, ****p<0.0001, 1-way ANOVA, post-hoc Tukey test.
(B) Immunostaining with HNK-1 (membrane glycoprotein) antibody of axons in whole mount larvae. Dashed rectangle indicates region imaged. D= Dorsal, V= Ventral; scale bar, 50 μm; blue lines indicate the distance measured between bundles of HNK-1-labeled axons. Graph shows distance between HNK-1+ bundles, mean±SEM, n=5 larvae per condition, *p<0.05, ***p<0.001 for 14.5 compared to 26.5°C, 1-way ANOVA, post-hoc Tukey test.
(C) Graph shows width of axial musculature at the proctoderm, mean±SEM, N≥5 independent cohorts of larvae, n>20 larvae per condition per cohort, ****p<0.0001, 1-way ANOVA, post-hoc Tukey test.

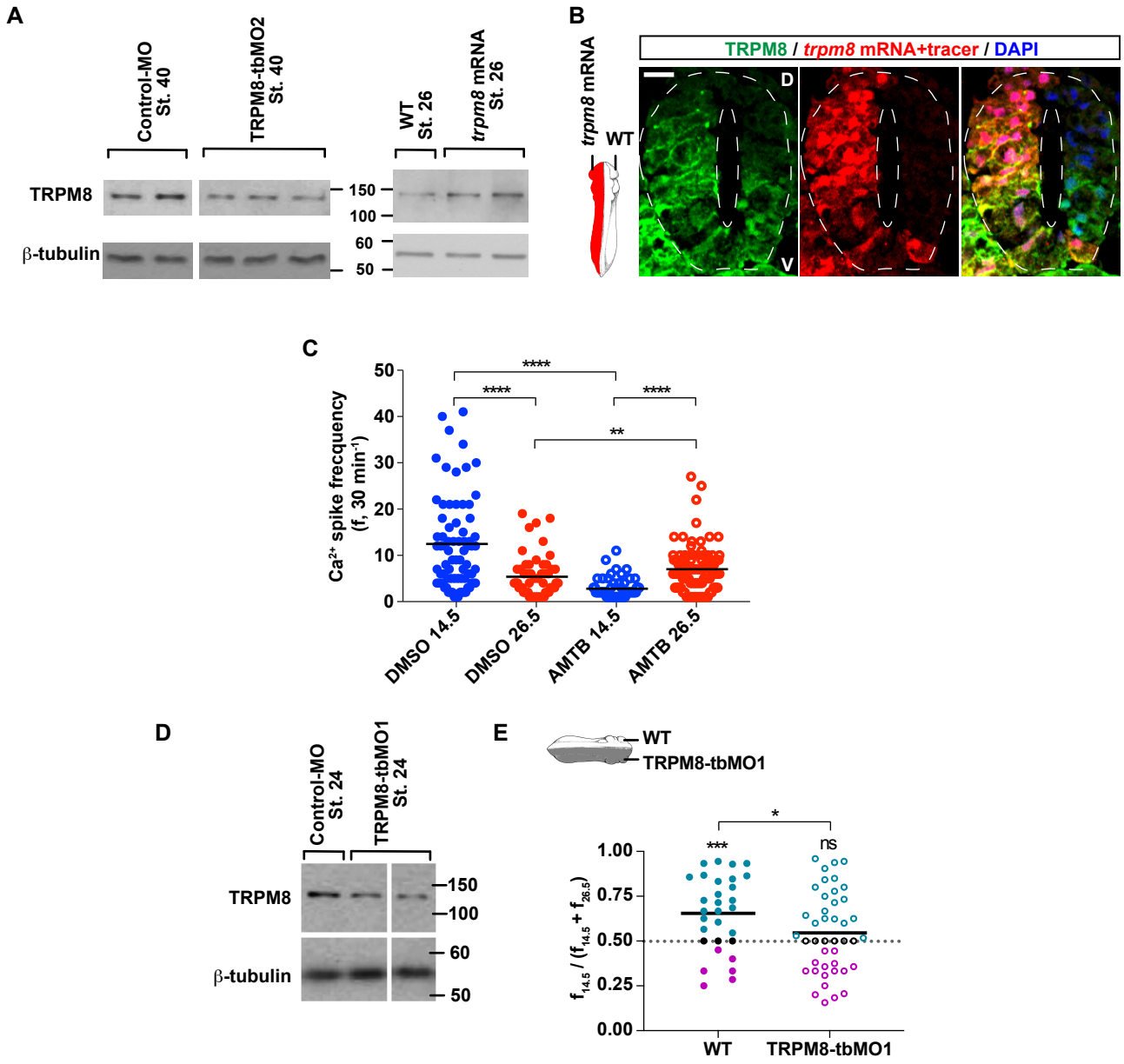

Figure S2. Validity of TRPM8 antibody and Ca2+ spike frequency dependence on TRPM8 in the ventral embryonic spinal cord. Related to Figure 4.

(A-B, D-E) Two-cell stage embryos were bilaterally (A,D) or unilaterally (B,E) injected with 2.5 pmol TRPM8-tbMO2 (A), TRPM8-tbMO1 (D,E) or Control-MO (A,D), or with 500 pg trpm8 mRNA (A,B) and allowed to develop at room temperature (22.5°C).

(A,D) Stage 40 larva (A) or stage 24-26 embryos (A,D) were collected for Western blot assays. Shown are representative examples of one of 3 independent experiments. β-tubulin was used as a loading control.

(B) Immunostained transverse section of stage 26 spinal cord (outlined). D: dorsal, V: ventral; scale bar 20 µm. DAPI used as counterstaining.

(C) Ca^{2+} imaging of the ventral spinal cord from stage 24 embryos was done in the same sample for 30-min intervals at 14.5°C and 26.5°C in the absence (vehicle, 0.1% DMSO) or presence of 10 µM AMTB, TRPM8 inhibitor. Scattered graph shows Ca^{2+} spike frequency in individual spinal neurons and geometric mean (black lines) at both temperatures from N=3 embryos per condition (n of neurons analyzed: 14.5°C DMSO, 77; 26.5°C DMSO, 65; 14.5°C AMTB, 76; 26.5°C AMTB, 102), ****p<0.0001, **p<0.01, comparison within treatments Wilcoxon matched-pairs signed rank, two-tailed test; comparison between treatments Kruskal-Wallis test, Dunn's multiple comparisons.

(E) Ca^{2+} imaging of the ventral spinal cord from stage 24 embryos was done in the same sample for 30-min intervals at 14.5°C and 26.5°C. Scatter plots show changes in Ca^{2+} spike frequency (f) for the same ventral neuron and mean (black lines) at pairs of temperature in wild-type (WT) and TRPM8-tbMO1 containing cells from N=3 ventral spinal cords (n of neurons analyzed: WT, 30; TRPM8-tbMO1, 43). Teal circles represent neurons with higher spike frequency at 14.5°C, magenta circles represent neurons with higher spike frequency at 26.5°C, black circles represent neurons with no change in spike frequency across temperatures, ***p<0.001, *p<0.05, ns: not significant, comparison within treatments two-tailed paired t-test; comparison between treatments two-tailed unpaired t-test.

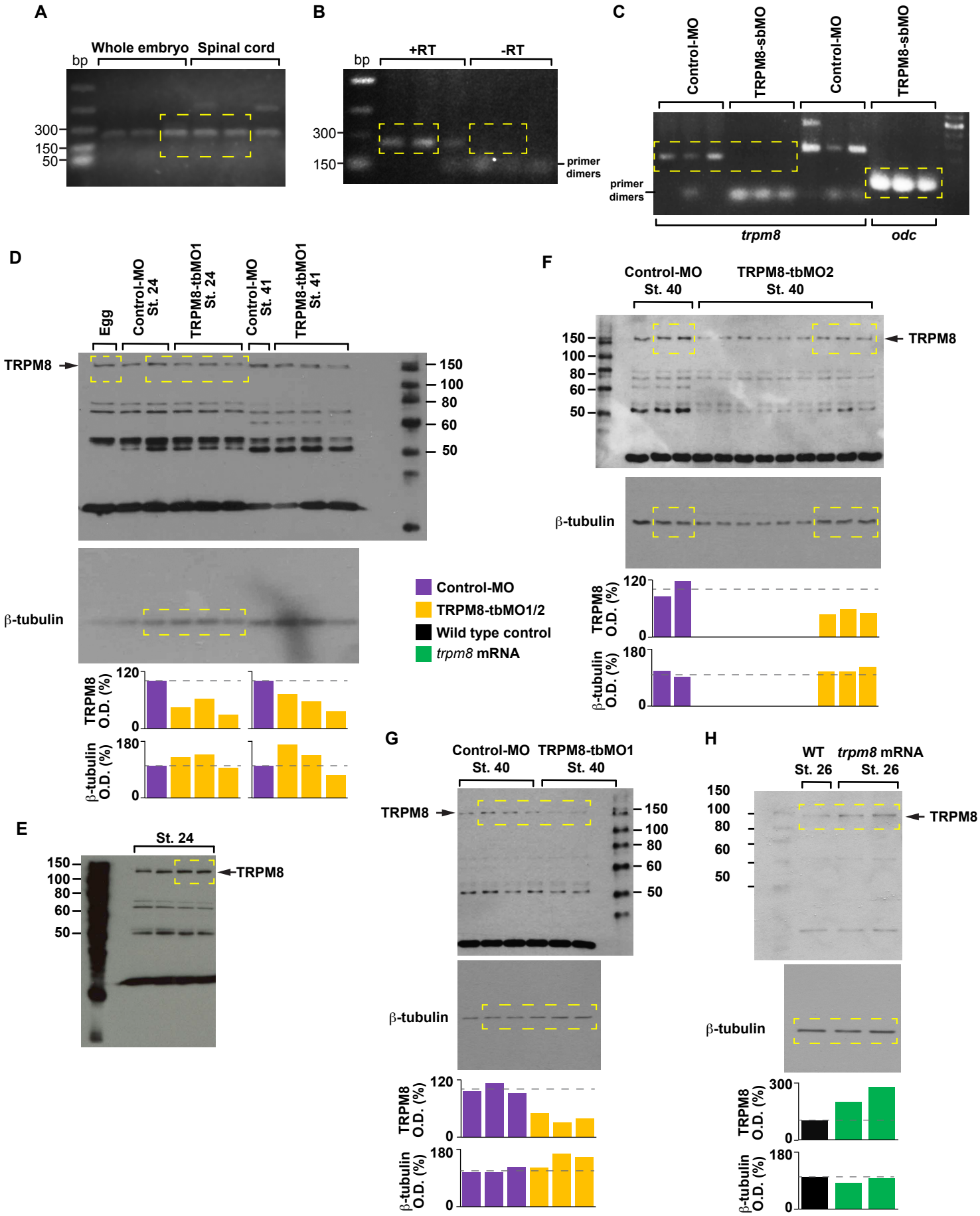

Figure S3. Full size gels. Selections presented in Figure 4A,C,G and Figure S2A,D.

Dashed rectangles indicate sections used in figures. Procedures are indicated in Methods and Figure Legends.

(A-C) RT-PCR for *trpm8* (A-C) and *odc* (C). Presented in Figure 4A (A,B) and 4G (C). +/-RT: presence or absence of reverse transcriptase, respectively.

(D-H) Western blot assays for TRPM8 and β-tubulin (as loading control). Presented in Figure 4C (D,E,G), Figure S2A (F,H) and Figure S2D (D). Bar graphs represent relative optical density (O.D.) measured with Image J (Gels analysis tool) of band between 100 and 150 kDa for membranes probed with anti-TRPM8 and band between 50 and 60 kDa for membranes probed with anti-β-tubulin. Dashed lines in graphs indicate 100% O.D.