Supporting Information

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SI Materials and Methods

Animals. *C. elegans* strains were grown at 20 °C under standard conditions (1): WT N2, *che-3(e1124)*, *osm-3(p802)*, *odr-3(n2105)*, *unc-79(tm1979)*, *unc-79(ec1)*, *unc-79(e1068)*, *unc-80(eg684)*, *unc-80(e1272)*, *unc-80(e1069)*, *nca-1(tm1851)*, *nca-2(tm377)*, *nca-2(tm1305)*, *nca-1(tm1851)*;*nca-2(tm377)*, *cca-1(e625)*, *cca-1*;*nca-1(tm1851)*, *cca-1*;*nca-2(tm377)*, *cca-1*;*unc-80(eg684)*, *cca-1*;*unc-79(tm1979), stn-1(n2665), unc-2(e156),* and *egl-19(n582)*. GFP lines Punc-4::gfp (*uIs33*) from M. Chalfie and Pnmr-1::gfp (*akIs3*) from V. Maricq were used to access gross neuronal morphology.

Muscle Imaging. A single worm was inserted into a micro-fluidic chip made of transparent polydimethylsiloxane. To study crawling, the worm was sucked via buffer fluid into a narrow channel on the chip (900 μm length $\times$ 70 μm width $\times$ 28 μm depth). Although the worm would clearly pass multiple cycles of sinusoidal crawling bends through its body, it would make very little forward or backward progress as a result of slippage. To study swimming, the worm was sucked further into the open reservoir of the chip with the same depth at which motion was unrestrained in two dimensions. Worms displayed typical crawling motion in the liquid-filled channel and typical swimming motion in the liquid-filled reservoir.

Note that, because the worm moves in a two-dimensional plane in which it lies on its side and makes DV bends, fluorescence was indistinguishable in left and right muscle quadrants when imaged from above at low magnification. Depending on the imaging session, we would focus on either the left or right rows of ventral and dorsal muscles. The patched expression of YC2 resulting from mosaicism aided in image alignment across subsequent frames.

Cloning and gfp Reporter Fusion Experiments. We localized *unc-80(eg684)* to the right arm of LGV with snipSNP mapping (2). The entire predicted *F25C8.3*/*unc-80* ORF and 2.7 kb upstream region was amplified via PCR with the primers F25C8_A1 (CAGATTGCTTGCATACCGTCGC) and F25C8_3B (CAGCGGGTTATACTTTGCATTTCTAC) using genomic DNA and injected with Pmyo-3::gfp as co-injection marker (25 μg/ml) to obtain transformation rescue in 4/4 independent lines. The line *unc-80(eg684)*;*egEx40*[*unc-80*(+) + Pmyo-3::gfp] was used for quantitative analysis. Promotor GFP fusions were made to determine probable expression pattern of *unc-80* and *unc-79* (3). Briefly, initial PCR products were made with forward primers F25C8_A1 or F25C8_B1, (CTCATCACGATGGGTCTCGCCAC) with reverse primer F25C8_C (AGTCGACCTGCAGGCATGCAAGCTTCGAACATG*CAT*TCTTTATAATTTTCGG) to generate 2.7 kb and 4.5 kb products, respectively. For Punc-79::gfp, the initial product was made with forward primer unc_79_A1 (CAGTCTCACTTCTCTCACCGC) and reverse primer unc_79_C (AGTCGACCTGCAGGCATGCAAGCTTAGTAGTGTTTT*CAT*GTTTGAACTGA) to generate a 2.7 kb product. These three products were then separately fused to DNA that encodes GFP in secondary reactions, isolated and injected into WT animals (30 μg/ml) to create strains *egEx34* and *egEx39* for Punc-80::gfp and *egEx51* for Punc-79::gfp. As coelomocyte-specific promotor fragment Pofm-1::gfp was included as a co-injection marker for *unc-79*, we could not rule out the possibility of coelomocyte expression for *unc-79*.

Chemotaxis in Liquid Assay. Chemotaxis assay plates were constructed as described (4). Control quadrant plates were made the same using agar without NaCl. Once the agar solidified, WT animals (n = ≈30) were rinsed from their growth plates with chemotaxis assay buffer and transferred by dropping 5 μl centered on the border between two quadrants of an assay plate.

1. Brenner S (1974) The genetics of *Caenorhabditis elegans. Genetics* 77:71–94.
2. Wicks SR, Yeh RT, Gish WR, Waterston RH, Plasterk RH (2001) Rapid gene mapping in Caenorhabditis elegans using a high density polymorphism map. *Nat Genet* 28:160–164.
3. Boulin T, Etchberger JF, Hobert O (2006) Reporter gene fusions. *WormBook*, 10.1895/wormbook.1.106.1.
4. Miller AC, Thiele TR, Faumont S, Moravec ML, Lockery SR (2005) Step-response analysis of chemotaxis in *Caenorhabditis elegans. J Neurosci* 25:3369–3378.

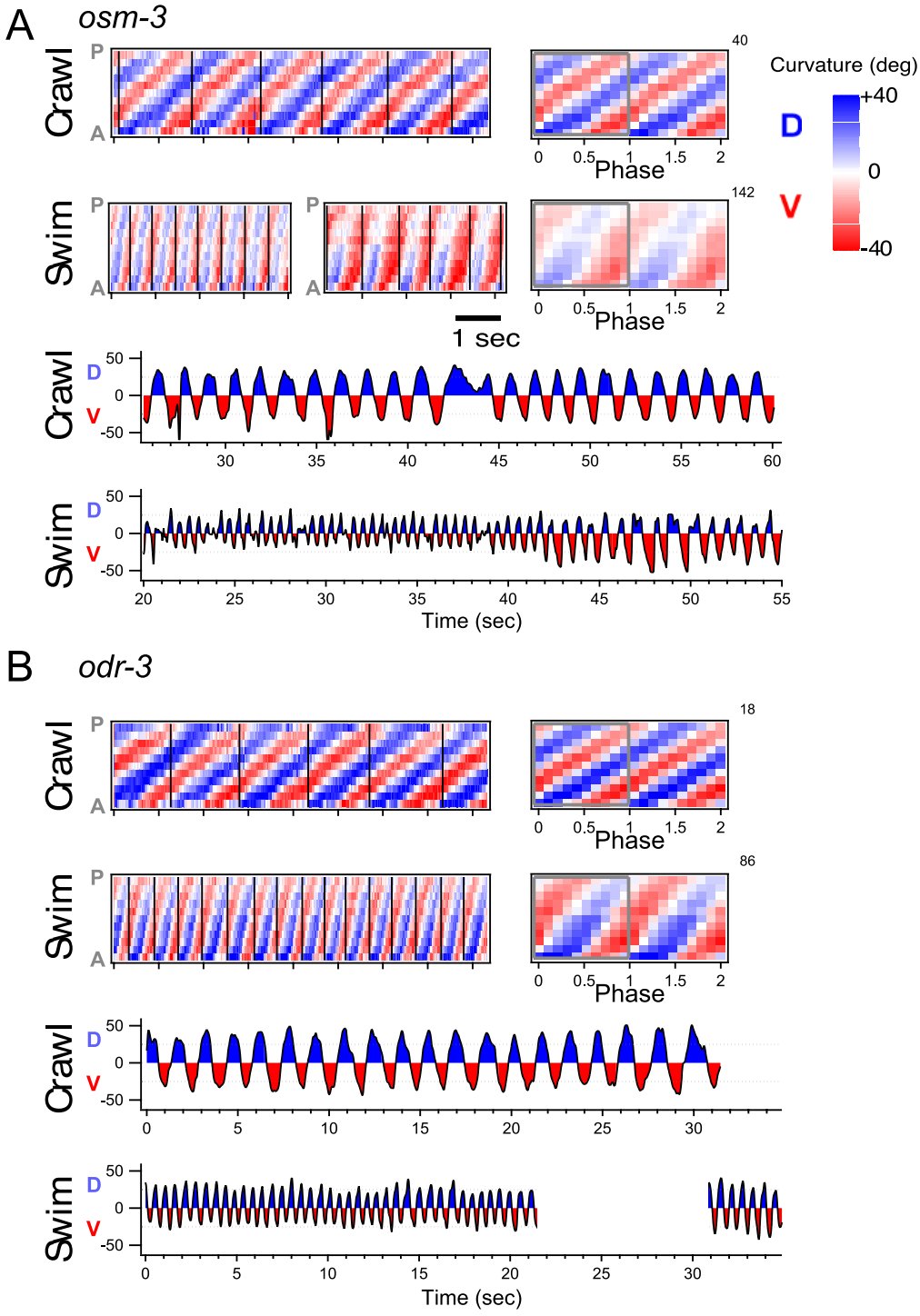

Fig. S1. Analysis of forward crawl and swim behavior for *osm-3* (A) and *odr-3* (B) mutants. Curvature matrices for mutant individuals for 8.25 sec of crawl and swim behaviors are shown (*Left*). Average curvature matrix for DV head bend cycles for all cycles of forward motion are displayed within 1 min for a single individual (*Right*). Plots of neck curvature versus time for the same individual below each matrix. Curvature traces are filled in with solid blue (dorsal) or red (ventral) to aid interpretation. Areas in neck curvature blanked during reversal motion to emphasize forward motion. Note *osm-3* mutant displays abnormal swim motion relative to WT (Fig. 1) but much less severe than *che-3* mutant (Fig. 4). The *odr-3* mutant displays essentially normal crawl and swim kinematics. *osm-3* and *odr-3* are expressed in non-overlapping subsets of *che-3*-expressing sensory neurons.

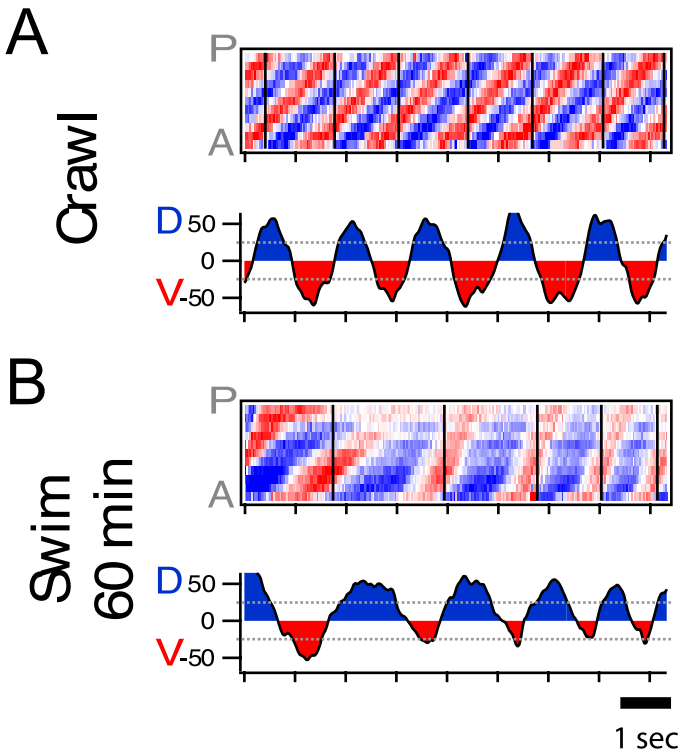

Fig. S2. Rare WT crawl-like behavior in liquid. (*A*) Example of WT animal crawling. Curvature matrix data repeated from Fig. 5*A*. Corresponding neck curvature vs. time below. (*B*) Example of WT animal exhibiting crawl-like motion after being in liquid for 60 min. Curvature matrix data (*Top*) and corresponding neck curvature versus time (*Bottom*). Note crawl-like amplitude and frequency of neck bending.

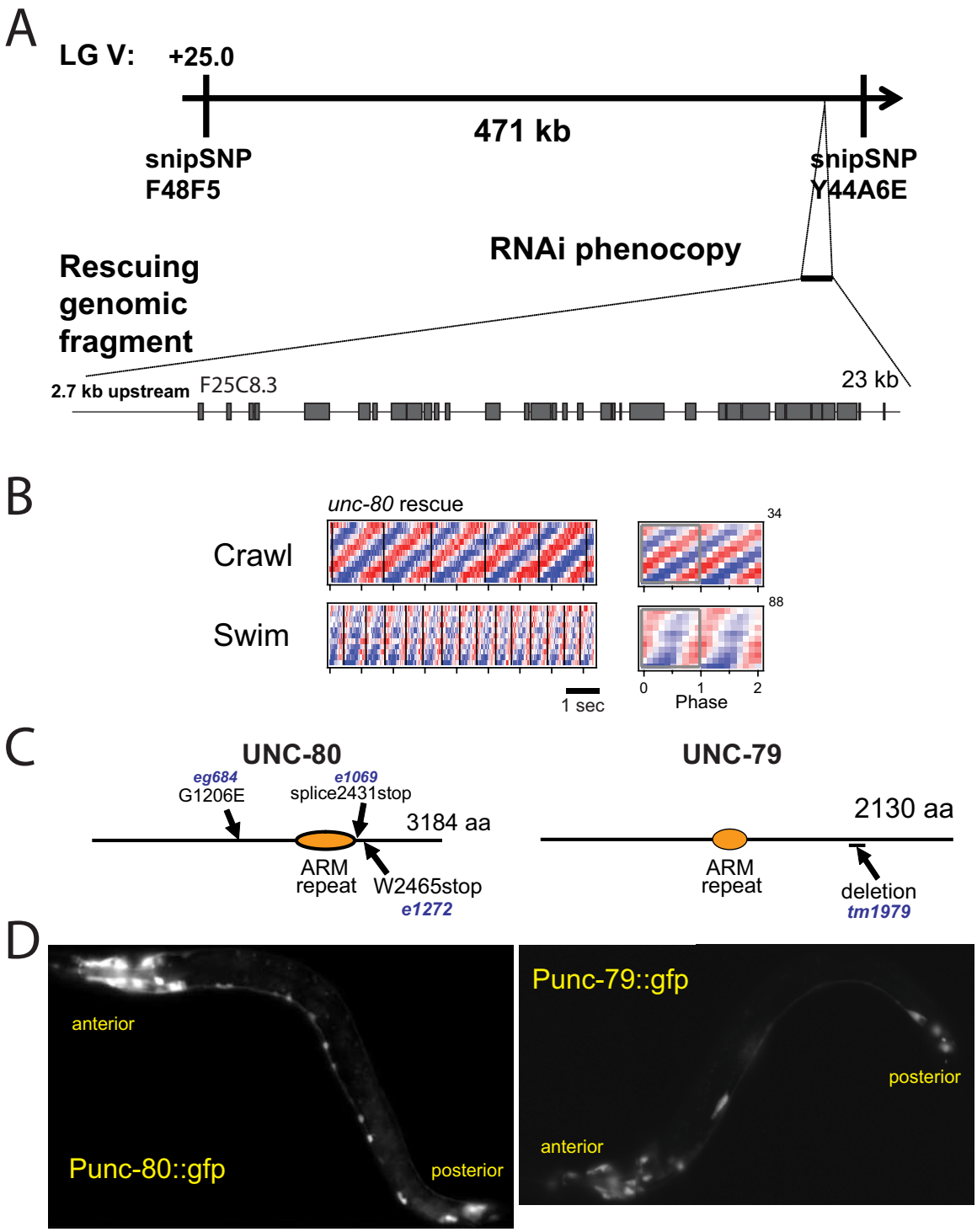

Fig. S3. *unc-80* and *unc-79* encode large conserved neuronal proteins expressed in the locomotion circuit. (*A*) Cloning *unc-80*. *eg684* was mapped within indicated boundaries. RNAi that targets ORF *F25C8.3* recapitulated the Unc-80 phenotypes in WT. Transformation with indicated 23 kb genomic fragment rescued *unc-80* behavioral defects. (*B*) Rescue of *unc-80* swimming displayed in curvature matrix and average matrix. (*C*) Predicted protein structure of UNC-80 and UNC-79 and mutations. (*D*) Expression pattern of Punc-79::gfp and Punc-80::gfp constructs. Expression was observed in overlapping regions throughout the nervous system including head ganglia, VA, and VB motor neurons, and tail ganglia from L1 larvae through adult stages with representative L3 stage animals shown. Ventral (*Top*) and dorsal (*Bottom*) aspects are shown.

Movie S1. Example of WT crawling. Animal displays persistent S-shaped waveform with low-frequency bends.

Movie S1 (AVI)

Movie S2. Example of WT swimming in the same animal from Movie S1, which responds to fluid immersion by backing and then moving forward with C-type kinematics with high-frequency bends.

Movie S2 (WMV)

Movie S3. Example of *che-3* mutant crawling. The *che-3* mutant animal displays similar crawling as WT.

Movie S3 (AVI)

Movie S4. Example of *che-3* mutant in liquid. Note that same mutant animal from Movie S3 moves with crawl-like S-type kinematics with low-frequency bends.

Movie S4 (AVI)

Movie S5. Example of *unc-80* mutant crawling. The *unc-80* mutant animal displays similar crawling as in WT.

Movie S5 (AVI)

Movie S6. Example of *unc-80* mutant in liquid. The same mutant animal from Movie S4 displays initial normal backing response to fluid immersion before suddenly ''fainting.''

Movie S6 (WMV)