PNAS

Supporting Information for

Voltage-induced calcium release in *C. elegans* body muscles

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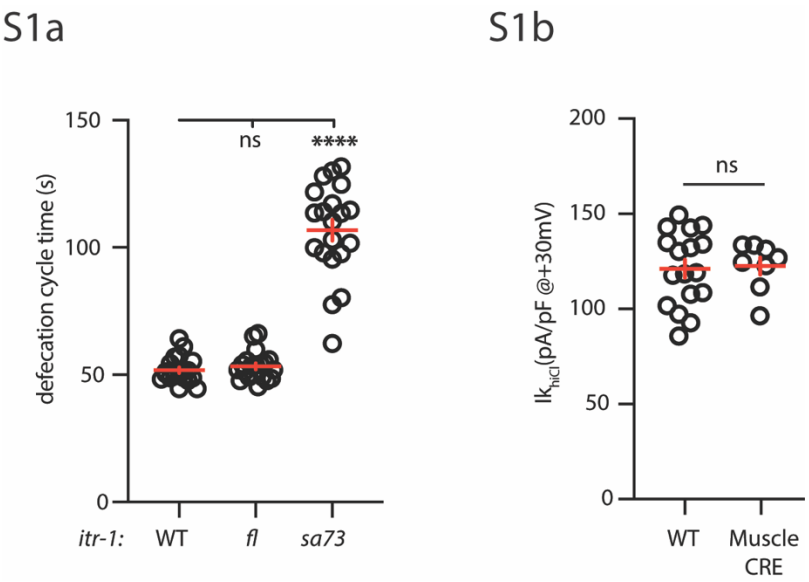
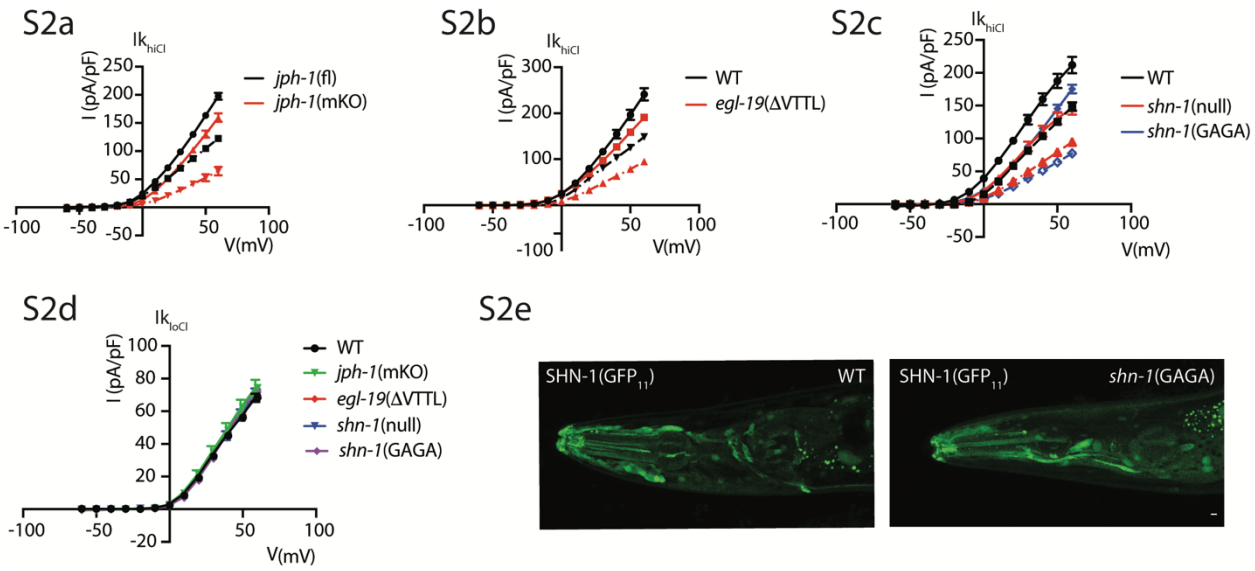
Other supporting materials for this manuscript include the following:

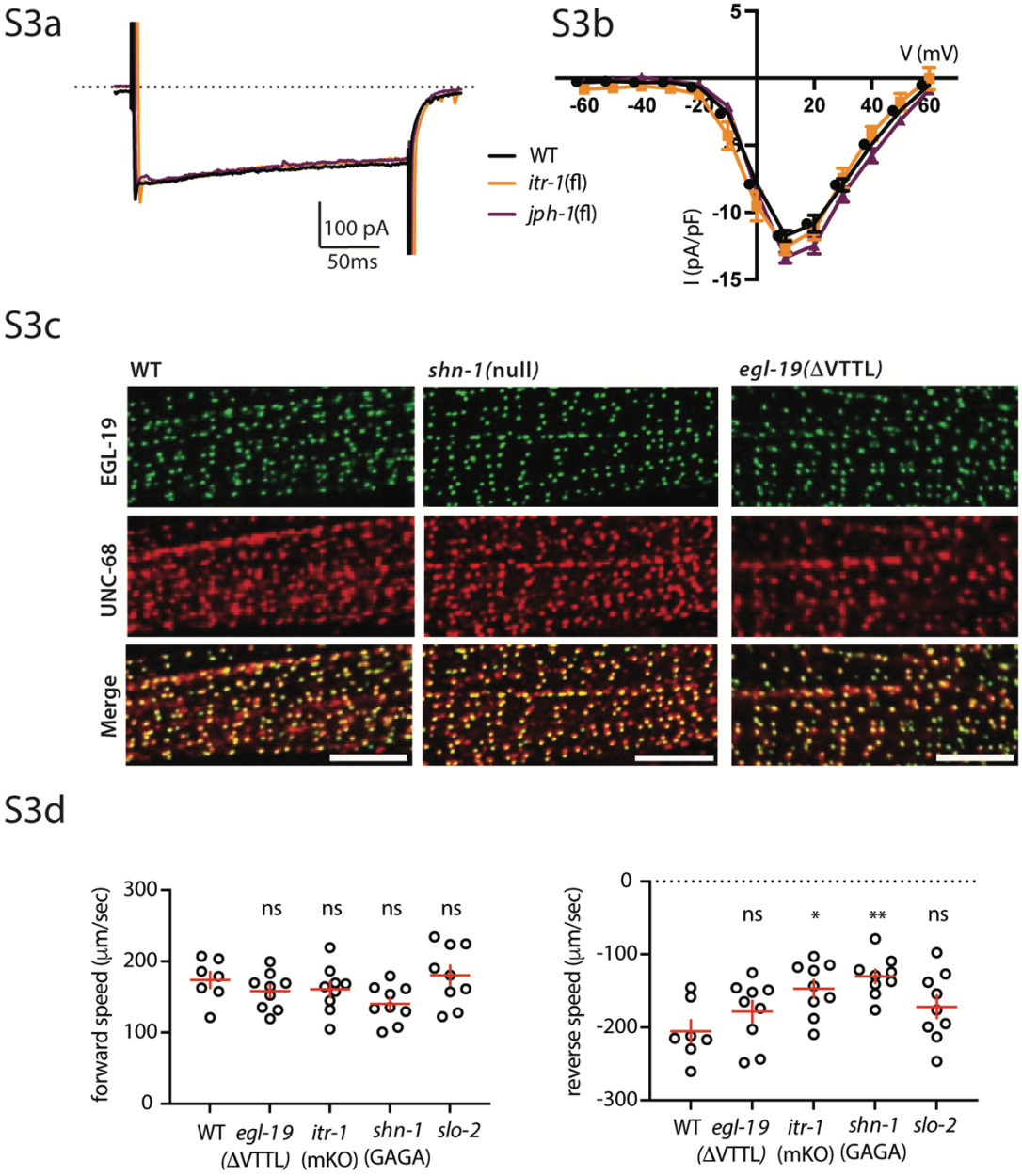
Dataset S1



Supplemental Figure 1. Supplementary data for Figure 1. (A) Mean defecation cycle duration is plotted in the indicated genotypes. Defecation cycles are significantly longer in a hypomorphic *itr-1(sa73)* mutant but were unaffected in the non-excised *itr-1(fl)* mutants. These results suggest that ITR-1 function was not significantly impaired by insertion of the LoxP sites. (B) Ik_{hiCl} was unaffected by CRE expression in WT body muscles. Mean Ik_{hiCl} current density at +30 mV is plotted for WT and *nuSi572* Pmyo-3::CRE animals. These results suggest that SLO-2 defects observed in muscle knockout strains are unlikely to results from toxicity associated with CRE expression. Values that differ significantly are indicated (ns, not significant; ****, p <0.0001). Error bars indicate SEM.



Supplemental Figure 2. Supplementary data for Figure 4. (A-C) Ik_{hiCl} currents were recorded from adult body wall muscles at holding potentials of -60 to +60 mV with 5 mM (solid lines) and 0 mM (dashed lines) external calcium. Mean current density is plotted as a function of membrane potential. (D) SHK-1 (Ik_{loCl}) currents were unaffected in *jph-1(*mKO*)*, *egl-19*(ΔVTTL), *shn-1(*null*)*, and *shn-1(*GAGA*)* mutants. Mean Ik_{loCl} current density is plotted as a function of membrane potential. (E) Expression of endogenous SHN-1 is unaffected in *shn-1(*GAGA*)* mutants. SHN-1(GFP_{11}) fluorescence was reconstituted by expressing GFP_{1-10} with a ubiquitous promoter (*eft-3*). Representative images are shown. Error bars indicate SEM. Scale bar indicates 4 μm.



Supplemental Figure 3. Supplementary data for Figure 5. EGL-19/CaV1 currents were recorded from adult body wall muscles at holding potentials of -60 to +60 mV in the indicated genotypes. Representative traces (at +20 mV) (A) and mean current density as a function of membrane potential (B) are shown. EGL-19 current was unaffected in strains containing the *itr-1(nu774 FLOX)* and *jph-1(nu733 FLEX)* alleles. (C) Representative images of EGL-19(nu674 GFP_{11}) and UNC-68(nu628 $Cherry_{11}$) co-localization are shown in WT, *egl-19(*ΔVTTL*)*, and *shn-1(nu712* null*)*

mutants. GFP_{11} and $Cherry_{11}$ fluorescence were reconstituted by expressing GFP_{1-10} and $Cherry_{1-10}$ in body muscles. (D) Forward and reverse locomotion rate of adults was compared for the indicated genotypes. Error bars indicate SEM. Values that differ significantly from WT controls are indicated (ns, not significant; *, p <0.05; **, p<0.01). Scale bars indicate 5 μm.

Supplemental Table 1. Key resources table

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Chemicals, peptides, and recombinant proteins		
Nemadipine-A: L-type calcium channel protein inhibitor	ABCAM	Catalog #:ab145991
Cyclopiazonic acid: SERCA pump inhibitor	Sigma-Aldrich	Catalog #:C1530
Experimental models: Organisms/strains		
strain (*C. elegans*) N2	CGC	Wormbase ID:N2
C. elegans: Strain LY100 *slo-2(nf100)*	CGC	LY100
C. elegans: Strain TR2171 *unc-68(r1162)*	CGC	TR2171
strain (*C. elegans*) KP10950 *itr-1(nu774 FLOX)*	This study	KP10950
strain (*C. elegans*) KP10696 nuSi572 myo-3::CRE	(1)	KP10696
strain (*C. elegans*) KP10972 *itr-1(nu774 FLOX);* nuSi572	This study	KP10972
strain (*C. elegans*) KP11233 *unc-68(r1162)*; *itr-1(nu774 FLOX);* nuSi572	This study	KP11233
strain (*C. elegans*) KP10407 *jph-1(nu733 FLEX)*	This study	KP10407
strain (*C. elegans*) KP11152 *jph-1(nu733 FLEX);* nuSi572	This study	KP11152
strain (*C. elegans*) KP7992 *egl-19(nu496 ΔVTTL)*	(2)	KP7992
strain (*C. elegans*) KP10151 *shn-1(nu712)*	(1)	KP10151
strain (*C. elegans*) KP9232 *shn-1(nu604 GFP11);nuSi205* Peft-3::GFP1-10	(1)	KP9232
strain (*C. elegans*) KP11704 *shn-1(nu793* GAGA *nu604* GFP11);*nuSi205* Peft-3::GFP1-10	This study	KP11704
strain (*C. elegans*) KP10373 *nuSi456* pat-10::Cherry1-10 SL2 GFP1-10	(3)	KP10373
strain (*C. elegans*) KP11234 *egl-19(nu674 GFP11);nuSi456*	This study	KP11234
strain (*C. elegans*) KP11235 *unc-68(r1162); egl-19 (nu674 GFP11);nuSi456*	This study	KP11235
strain (*C. elegans*) JT73 *itr-1(sa73)*	CGC	JT73
strain (*C. elegans*) KP10405 *egl-19(nu674 GFP11);unc-68(nu628 Cherry11);nuSi456*	(3)	KP10405
strain (*C. elegans*) KP11253 *egl-19(nu496 ΔVTTL nu674 GFP11);unc-68(nu628 Cherry11);nuSi456*	This study	KP11253
strain (*C. elegans*) KP11260 *shn-1(nu712); egl-19(nu674 GFP11);unc-68(nu628 Cherry11);nuSi456*	This study	KP11260
Oligonucleotides		
ttacctgacatgatggacacAGG	IDT	guide RNA for x7 GFP11 insertion at egl-19 codon 2
gatgctgcagccacgggcggTGG	IDT	guide RNA for x4 CherryFP11 insertion at unc-68A codon 5202
ccacgtggtgtcaagggattCGG	IDT	guide RNA for mutation of the shn-1 PDZ domain
gtttagcaccgatcctttgcTGG	IDT	Guide RNA for insertions into the 1st exon of jph-1
caaagttagcgttataggtcCGG	IDT	Guide RNA for insertion 5' loxP site into the 8th intron of itr-1A
aataaaaatacgttgaagacAGG	IDT	Guide RNA for insertion of 3' loxP site into the 3' UTR of itr-1A

ATGATTGCAGCTGGACACGAGACAAATATCGCTCGATTCTGGTG ATTCCGCGGGGAGTGAAAGGAGCTGGTGCAATTGCTAGAGGAG CTAAACGTAAATTCCTTTTTCTTATGTAACTGCTCAATAATT	IDT	Repair oligonucleotide to make shn-1(nu793 GAGA) mutation
tcaaacaaaacttagtttacttgcatgttgataataattgctgctgtccgATAACTTC GTATAgcatacatTATACGAAGTTATcggccgacctataacgctaactttgct attactaatttttgaaacacaacgggatccc	IDT	Repair oligonucleotide to insert loxP site into the 8th intron of itr-1A
aacctgtttagtcgttttcaaaaccggtatttcaatgtttttttttacctATAACTTCG TATAgcatacatTATACGAAGTTATcggccgtcttcaacgtatttttatttttttcg tctttatctaagtttgattttcaa	IDT	Repair oligonucleotide to insert loxP site into the 3' UTR of itr-1A
ttacctgacatgatggacacAGG	IDT	guide RNA for x7 GFP11 insertion at egl-19 codon 2
Recombinant DNA		
Plasmid: KP#4527 Pmyo-3 NLS CRE SL2 NLS BFP miniMOS hyg	This study	Expresses nuclear localized CRE recombinase and BFP in body wall muscles
Plasmid: KP#4522 Ppat-10 Cherry 1-10 SL2 GFP 1-10 G418	(3)	Expresses Cherry 1-10 and GFP 1-10 in body wall muscles
Plasmid KP#3323 jph-1 1st intron FLEX ON to OFF repair	This study	Repair template for insertion of FLEX cassette into the 1st intron of jph-1
Software and algorithms		
ImageJ /Fiji	NIH	https://fiji.sc/
pClamp 10	Molecular Devices	https://www.moleculardevices.com
Prism9	GraphPad	https://www.graphpad.com
OriginLab	OriginLab	https://www.originlab.com/
Adobe illustrator 2020	Adobe	Adobe.com
Tierpsy Worm Tracker	(4)	https://github.com/Tierpsy/tierpsy-tracker
NIS Elements	Nikon	https://www.microscope.healthcare.nikon.com/
Other		
Polybead Microspheres 0.10µm	Polysciences Inc	Catalog #: 00876-15

SI References

1. L. Gao *et al.*, Shank promotes action potential repolarization by recruiting BK channels to calcium microdomains. *Elife* **11** (2022).
2. E. Pym *et al.*, Shank is a dose-dependent regulator of Cav1 calcium current and CREB target expression. *Elife* **6** (2017).
3. C. A. Piggott *et al.*, Caenorhabditis elegans Junctophilin has tissue-specific functions and regulates neurotransmission with extended-synaptotagmin. *Genetics* 10.1093/genetics/iyab063 (2021).
4. A. Javer *et al.*, An open-source platform for analyzing and sharing worm-behavior data. *Nat Methods* **15**, 645-646 (2018).