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**Supplementary Information for**

# Presynaptic coupling by electrical synapses coordinates a rhythmic behavior by synchronizing the activities of a neuron pair.

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**This PDF file includes:**

SI Materials and Methods
Figures S1 to S5
Tables S1 to S2
Legends for Movies S1 to S4

**Other supplementary materials for this manuscript include the following:**

Movies S1 to S4

## SI Materials and Methods

### *nlp-40* suppressor screening and *inx-1* cloning

The parental strain *nlp-40(tm4085)* was mutagenized with EMS for a standard non-clonal $F_2$ screen (1). $F_2$ progenies of ~ 8,400 mutagenized genomes were screened and three mutants that suppressed the constipated phenotype of *nlp-40(tm4085)* were identified. The *vj46* was mapped to LG X using SNP mapping (2). The lesion of *vj46* in the *inx-1* gene was identified by whole genome sequencing and the software MAQgene as previously described (3).

### Behavioral assays

The defecation motor program was analyzed as previously described (4, 5). Ten to fifteen worms were moved to fresh NGM plate seeded with OP50 bacterial lawn and allowed to settle for at least ten minutes for recovery. At least ten constitutive defecation cycles were observed from each worm. The pBoc and Exp steps were recorded using custom Etho software (James Thomas Lab website: http://depts.washington.edu/jtlab/software/otherSoftware.html) (5, 6). Five to ten worms were assayed, and the mean and the standard error of the mean (SEM) was calculated for each genotype.

### RNA Interference

RNAi plates were made using established protocols (7). Ten gravid adult animals were bleached on RNAi plates seeded with HT115 (DE3) bacteria that was transformed with the targeted gene insert in the L4440 vector for knockdown or empty L4440 vector as a control. Three to four days later, adult animals were assayed for the defecation motor program. RNAi clones were from the Ahringer or Vidal RNAi library.

### Fluorescence imaging

Fluorescence imaging was done by using a Nikon eclipse 90i microscope equipped with a Nikon Plan Apo 40x, 60x, and 100x oil objective (N.A.=1.40), and a Photometrics Coolsnap $ES^2$ camera or a Hamamatsu Orca Flash LT+ CMOS camera. Adult worms were paralyzed with 30 mg/ml 2, 3-Butanedione monoxime (BDM, Sigma) in M9 buffer, and then mounted on 2% agarose imaging pad. Metamorph 7.0 software (Universal Imaging) was used to capture image stacks and to obtain maximum intensity projections. All images were captured from left laterally positioned animals facing up. Fluorescence imaging in the GABAergic motor neurons were captured from the neuromuscular junction (NMJ) region of the AVL and DVB at the preanal ganglion. To analyze the synaptic structure in N2 and *inx-1* mutants, GFP::RAB-3 fusion protein was expressed in AVL (*Punc-25(Δ)::GFP::rab-3*) and the synaptic region of AVL was imaged in adult animals. For analyzing AVL axon process in N2, *unc-33*, and *inx-1* mutants, EBP-1::GFP fusion protein was expressed in AVL (*Punc-25(Δ)::ebp-1::GFP*) and adult animals were imaged from head to tail.

***In vivo* calcium live imaging**

Calcium imaging was performed as previously described (8, 9). For calcium imaging simultaneously in AVL NMJ and DVB soma, we used a transgenic line *vjIs58* (*Pmyo-2::NLS::mCherry*, *Punc-47(mini)::GCaMP3*); *vjEx2554* (*Pofm-1::mCherry*, *Pnmur-3::Pegl-18::GCaMP6*) to express GCaMP3 in DVB and GCaMP6 in AVL. For calcium imaging only in DVB NMJ, we used three transgenic lines, *vjIs58*, *vjIs64* (*Pmyo-2::NLS::mCherry, Punc-47(mini)::GCaMP3*), and *vjIs183* (*Pofm-1::mCherry*, *Pflp-10::GCaMP3*) which expresses GCaMP3 in DVB neurons to perform in vivo calcium imaging at the synaptic region. All four transgenic lines were imaged in the *unc-13(s69)* mutant background to immobilize animals for live imaging, and *unc-13* mutants carrying these transgenes had normal Exp frequency. Adult worms were transferred to NGM-agarose plates seeded with OP50 and the plates were topped with a cover slide. Live imaging was done using a Nikon eclipse 90i microscope equipped with a Nikon Plan Apo 40x oil objective (N.A.=1.0), a standard GFP filter and a Hamamatsu Orca Flash LT+ CMOS camera. The worms that were pumping and positioned laterally with the left side or right side facing the objective were selected for imaging. Metamorph 7.0 software (Universal Imaging) was used to obtain time lapse imaging. For each worm, the neuromuscular junction (NMJ) of AVL and DVB soma together or DVB NMJ was recorded for 250 s at 4 frames per second (2x2 or 3x3 binning with 30-90 ms exposure time depending on the baseline of GCaMP3 or GCaMP6 fluorescence in the NMJ region and DVB soma in each worm).

The GCaMP3 and GCaMP6 fluorescence intensity in the NMJ region of AVL/DVB or DVB soma was quantified using Metamorph 7.0 software (Universal Imaging). The average fluorescence (F) of GCaMP3 or GCaMP6 was calculated by the average fluorescence of a region of interest (ROI) in the NMJ region of the AVL/DVB or DVB soma minus the background fluorescence of a similar region near the tail. The baseline fluorescence ($F_0$) was defined by the average GCaMP3 or GCaMP6 fluorescence in the first 10 frames before the initiation of pBoc. The fluorescent change of the GCaMP3 or GCaMP6 for each frame was defined as $\Delta F/F_0 = (F-F_0)/F_0$.

**Cell ablation by miniSOG**

Transgenic lines were generated by expressing membrane-targeted miniSOG in AVL (*Pflp-22::PH domain::miniSOG*) and in DVB (*Pflp-10::PH domain::miniSOG*), separately or together. To ablate AVL or DVB, 20 to 30 L4 stage transgenic animals were transferred to an OP50 seeded NGM plate. The plate was illuminated with blue light using an EXFO mercury light source for 10 minutes with the cover off. Blue light illuminated animals were recovered at 20°C for 24 hours, and then assayed for the defecation motor program.

**Histamine chloride inhibition**

One day before the defecation motor program assay, histamine containing plates were made by adding histamine dihydrochloride (TCI) into standard NGM agar to a final concentration of 10mM.

OP50 was seeded to the plates and was grown for overnight. Next day, transgenic animals expressing HisCl channels in DVB (*Pflp-10::HisCl1*) were transferred to histamine-containing NGM plates. After 30 minutes, behavioral assay was performed for the defecation motor program.

A

Suppressors of *nlp-40*

| Allele | Gene | Chromosome | Exp in *nlp-40* mutant background |
|---|---|---|---|
| *vj46* | *inx-1* | X | 33±6.98% ** |
| *vj48* | ? | IV | 31±4.69% *** |
| *vj49* | ? | V | 23±3.84% *** |

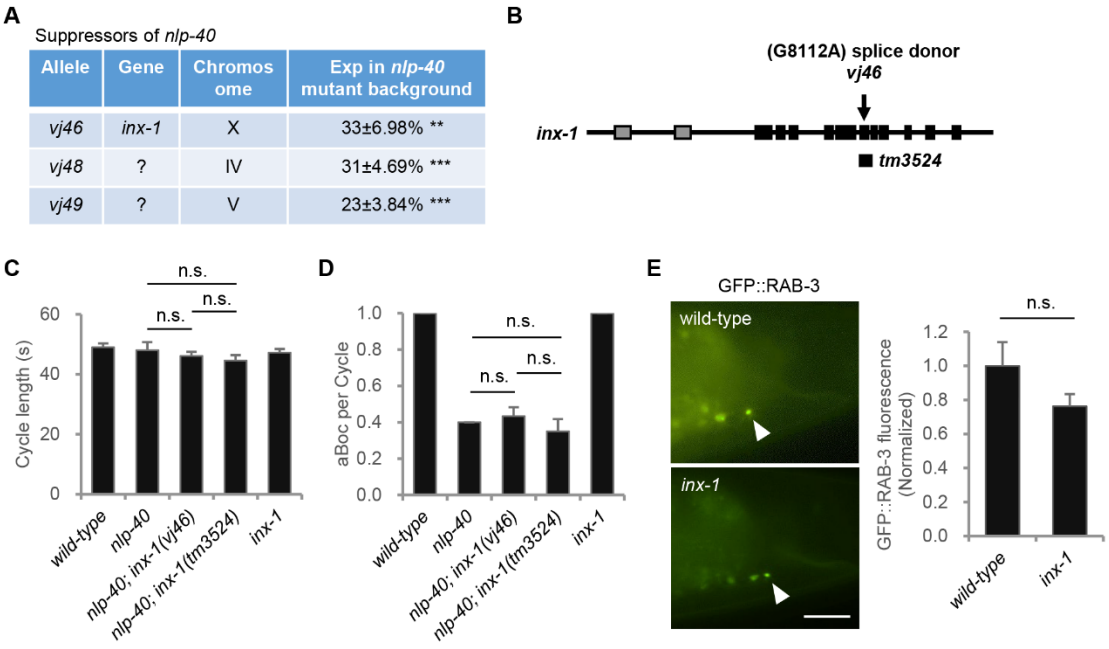


**Fig. S1.** *inx-1* does not affect anterior body wall contraction frequency, cycle length, or synaptic structure. (*A*) Table of the alleles identified in forward genetic screens for suppressors of the Exp defects of *nlp-40* mutants. (*B*) Genomic organization of the *inx-1* locus showing the locations and the lesions of the *tm3524* and *vj46* alleles. *tm3524* is a 238bp deletion that deletes all of exon 8 in *inx-1*. *vj46* is a guanine to adenosine (G to A) substitution in a splice donor site following exon 5 at position 8112 (from the 5' UTR of the unspliced *inx-1a* transcript) that leads to a truncated INX-1 that includes 1-250 amino acids with additional 30 amnio acids arising from the frameshift. (*C-D*) Quantification of the DMP cycle length and anterior body wall contraction (aBoc) frequency in adult worms with the indicated genotypes. One way ANOVA with Bonferroni's correction for multiple comparisons. (*E*) *Left*: representative images of GFP::RAB-3 fusion protein expressed at the NMJ of AVL in wild-type and *inx-1* mutants. Scale bar represents 10 µm. *Right*: quantification of average GFP::RAB-3 fluorescence at the NMJ of AVL in wild-type (20 animals) and *inx-1* mutants (16 animals). Means and standard errors are shown. Asterisks indicate significant differences: *** P<0.001 and ** P<0.01 in Student's t-test.

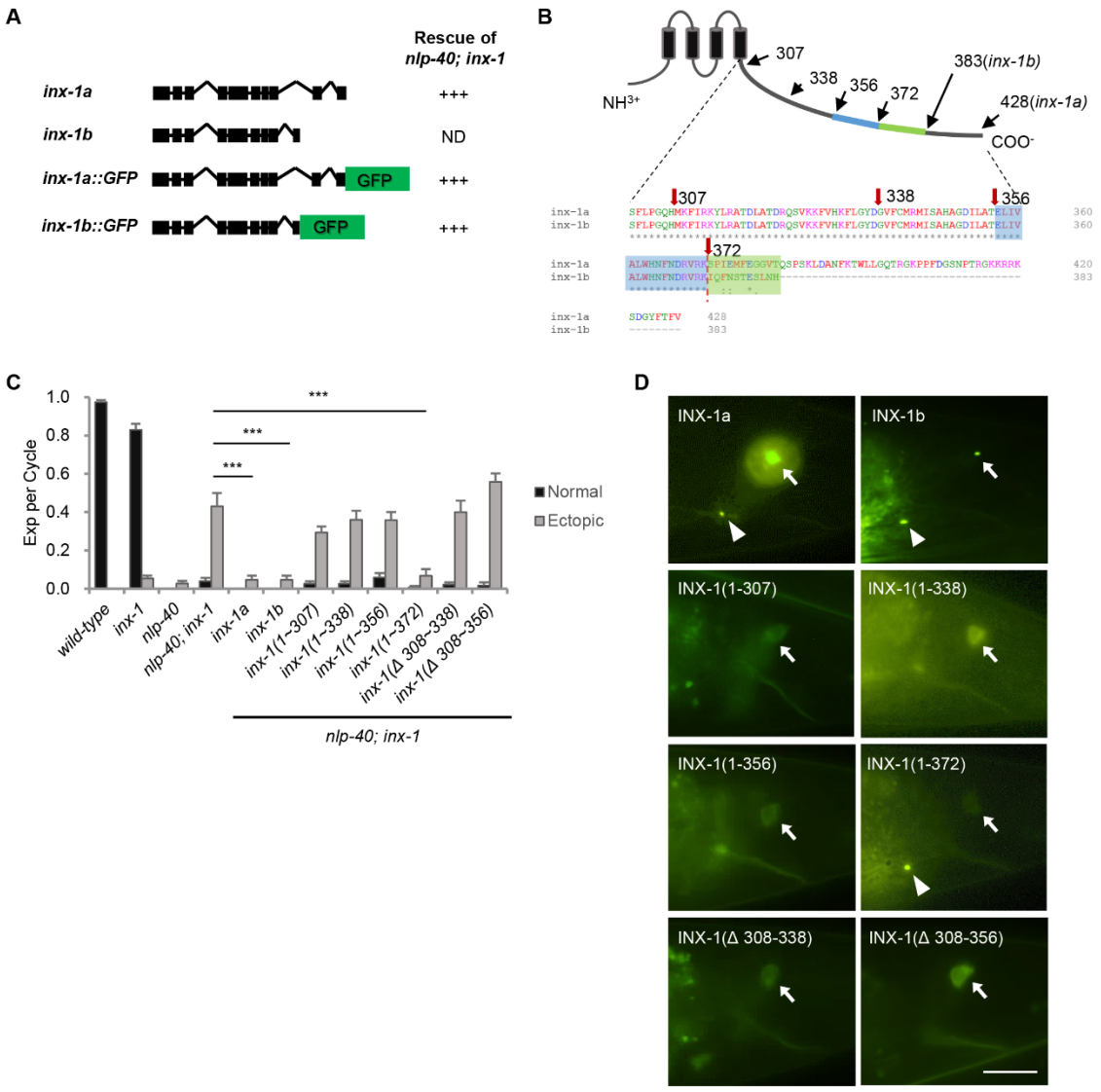


**Fig. S2.** INX-1 regulates Exp at NMJs. (*A*) The gene structure of *inx-1a* and *b* isoforms and location of GFP tags used for rescue experiments. +++ denotes full rescue (5% Exp frequency) of *nlp-40; inx-1* double mutants expressing the indicated *inx-1* transgenes under the GABAergic neuron-specific (*Punc-47*) promoter. (*B*) Diagram showing the structure of INX-1 protein and sequences of the INX-1 truncations generated and tested. The positions of the various truncated proteins (red arrow) are indicated. Blue box indicates the motif necessary for the localization of INX-1 at the NMJ of AVL/DVB. Green box indicates where the sequence is different between *inx-1a* and *inx-1b*. (*C*) Quantification of the Exp frequency in adults expressing the indicated truncated INX-1 proteins. (*D*) Representative images of the AVL/DVB NMJs (arrowhead) and DVB soma (arrow) in young adults expressing the indicated INX-1::GFP fusion proteins under the *unc-47* promoter. Scale bar represents 10 μm. Means and standard errors are shown. Asterisks indicate significant differences: *** P<0.001 in Student's t-test.

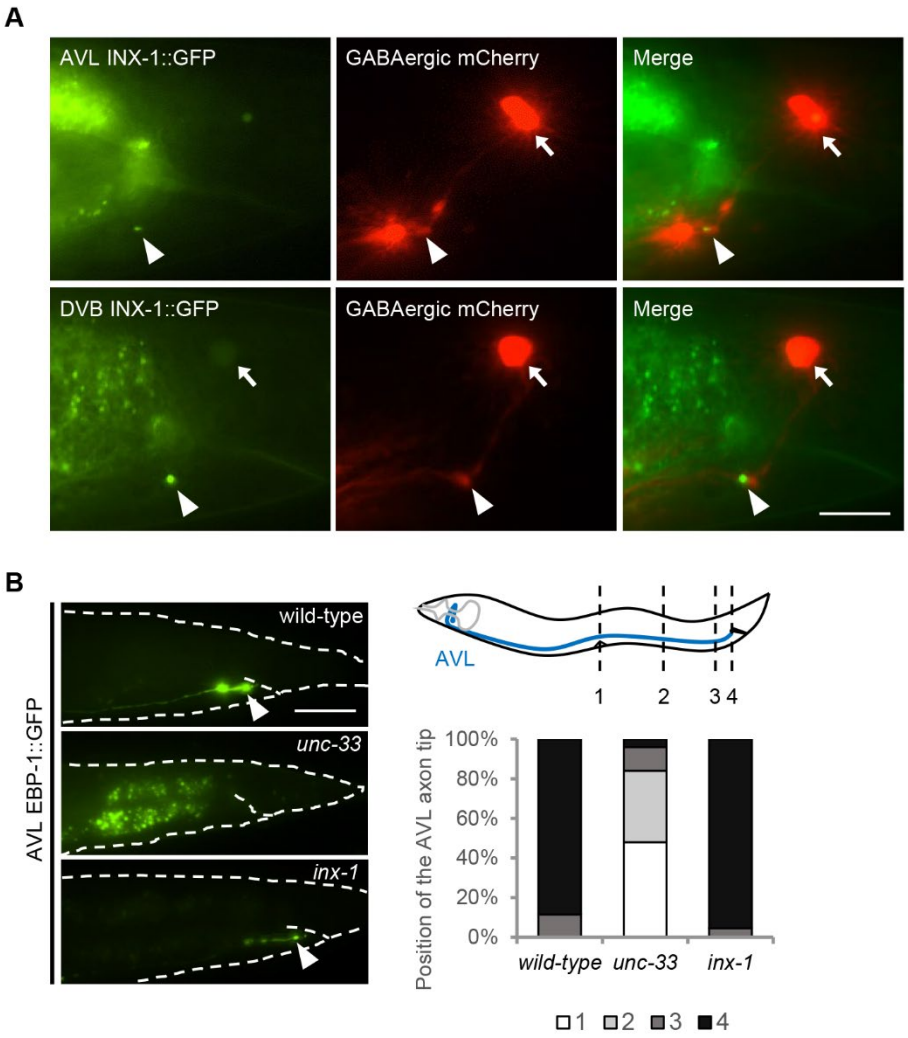


**Fig. S3**. Localization of INX-1::GFP and AVL axon outgrowth defects in *unc-33* mutants. (*A*) Representative images of the AVL/DVB NMJs (arrowhead) and DVB soma (arrow) in young adults expressing the indicated INX-1::GFP fusion proteins. "AVL INX-1::GFP" denotes INX-1::GFP fusion protein expressed under the *unc-25(Δ)* promoter, "DVB INX-1::GFP" denotes INX-1::GFP expressed under the *flp-10* promoter, and "GABAergic mCherry" denotes expressing mCherry under the *unc-47* promoter. Both INX-1::GFP fusion proteins localized to the bend of DVB where the NMJ is located (white arrow head). Scale bar represents 10 μm. (*B*) *Left*, representative images of adult animals showing the position of the AVL axon tip with the indicated genotypes. AVL axon tip was labeled with GFP tagged EBP-1, a microtubule plus-end binding protein, under the *unc-25(Δ)* promoter. Wide-type: 26 animals (exposure time of 10 ms), *unc-33*: 25 animals (exposure time of 200 ms), and *inx-1*: 22 animals (exposure time of 30 ms). Scale bar represents 20 μm. "AVL EBP-1::GFP" denotes EBP-1::GFP expressed under AVL neuron-specific ((*Punc-25(Δ)*) promoter. *Right*, *top*, diagram showing the position of the AVL axon tip. "1" denotes tip ending around the vulva, "2" denotes tip ending around half way between the vulva and the NMJ, "3" denotes tip ending one third of the way between 2 and 4 from 4, and "4" denotes tip ending around the NMJ. *Right*, *bottom*, the frequency of the position of the AVL axon tip is quantified for the indicated genotypes.

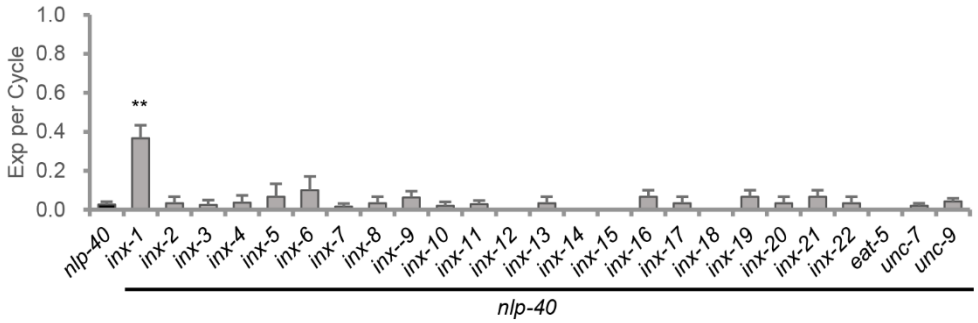


**Fig. S4.** INX-1 negatively regulates Exp in the absence of *nlp-40*. Quantification of the number of Exp per defecation cycle in *nlp-40* adults with knockout or knockdown of the in indicated innexins. Innexins were knocked down by feeding of *nlp-40; eri-1; lin-15* mutants, to enhance neuronal RNAi. *eat-5*, *unc-7*, and *unc-9* were tested as mutants. Means and standard errors are shown. Asterisks indicate significant differences: ** P<0.01 in Student's t-test.

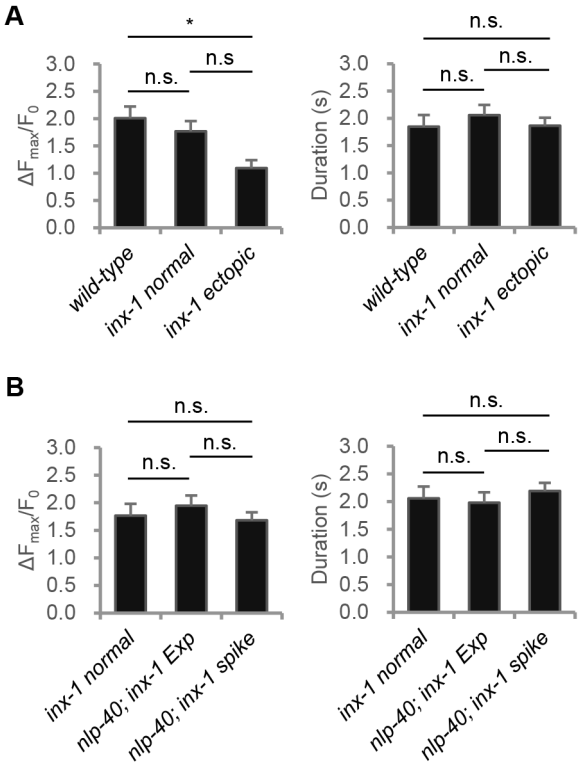


**Fig. S5.** Calcium spike duration and intensity in *inx-1* mutants. (*A* and *B*) *Left*, average GCaMP3 fluorescence peak amplitude in DVB NMJs in the indicated genotypes expressing GCaMP3 under the *unc-47(mini)* promoter. *Right*, average GCaMP3 fluorescence duration in DVB NMJs in the indicated genotypes. "*inx-1* normal" denotes calcium spikes at the right time followed by Exp and "*inx-1* ectopic" denotes ectopic calcium spikes followed by no Exp in *inx-1* mutants. "*nlp-40; inx-1* Exp" denotes ectopic calcium spikes followed by Exp and "*nlp-40; inx-1*" denotes ectopic calcium spikes with no Exp *nlp-40; inx-1* double mutants. Means and standard errors are shown. Asterisks indicate significant differences: * P<0.05 in one way ANOVA with Bonferroni's correction for multiple comparisons.

**Table S1. Strains, transgenic lines, and plasmids used in this study**

| Genotype | Strain | Plasmid |
|---|---|---|
| Wild-type Bristol strain | N2 | |
| *nlp-40(tm4085)* I | OJ794 | |
| *vjEx1530* | OJ7346 | pUC96 (*Pflp-10::PH domain::miniSOG*), 50 ng/µL |
| *vjEx1578* | OJ5350 | pUC94 (*Pflp-22::PH domain::miniSOG*), 50 ng/µL |
| *vjEx2701* | OJ7888 | pUC94 (*Pflp-22::PH domain::miniSOG*), 50 ng/µL + pUC96 (*Pflp-10::PH domain::miniSOG*), 50 ng/µL |
| *nlp-40(tm4085)* I*; vjEx1530* | OJ5030 | pUC96 (*Pflp-10::PH domain::miniSOG*), 50 ng/µL |
| *nlp-40(tm4085)* I*; vjEx1578* | OJ7391 | pUC94 (*Pflp-22::PH domain::miniSOG*), 50 ng/µL |
| *inx-1(tm3524)* X | OJ2446 | |
| *nlp-40(tm4085)* I*; inx-1(tm3524)* X | OJ2323 | |
| *nlp-40(tm4085)* I*; wpSi1* II*; eri-1(mg366)* IV*; rde-1(ne219)* V*; lin-15B(n744)* X | OJ2156 | |
| *nlp-40(tm4085)* I*; inx-1(tm3524)* X*; vjEx917* | OJ2343 | pUC08 (*Pnlp-40::inx-1a*), 25 ng/µL |
| *nlp-40(tm4085)* I*; inx-1(tm3524)* X*; vjEx912* | OJ2331 | pHW175 (*Punc-47(FL)::inx-1a*), 25 ng/µL |
| *nlp-40(tm4085)* I*; inx-1(tm3524)* X*; vjEx915* | OJ2337 | pUC07 (*Pmyo-3::inx-1a*), 25 ng/µL |
| *nlp-40(tm4085)* I*; inx-1(tm3524)* X*; vjEx916* | OJ2342 | pUC09 (*Phsp-16.2::inx-1a*), 25 ng/µL |
| *nlp-40(tm4085)* I*; inx-1(tm3524)* X*; vjEx1064* | OJ2805 | pUC30 (*Pflp-22::inx-1a::GFP*), 25 ng/µL |
| *nlp-40(tm4085)* I*; inx-1(tm3524)* X*; vjEx1193* | OJ7566 | pUC34 (*Pflp-10::inx-1a::GFP*), 25 ng/µL |
| *nlp-40(tm4085)* I*; inx-1(tm3524)* X*; vjEx1080* | OJ2846 | pUC30 (*Pflp-22::inx-1a::GFP*), 25 ng/µL + pUC34 (*Pflp-10::inx-1a::GFP*), 25 ng/µL |
| *nlp-40(tm4085)* I; *inx-1(vj46)* X | OJ1664 | |
| *nlp-40(tm4085)* I; *snta(vj48)* IV | OJ1665 | |
| *nlp-40(tm4085)* I; *snta(vj49)* V | OJ1667 | |
| *vjIs123* II | OJ3005 | pMH52 (*Punc-25(Δ)::GFP::rab-3*), 5ng/µL |
| *vjIs123* II*; inx-1(tm2534)* X | OJ3651 | pMH52 (*Punc-25(Δ)::GFP::rab-3*), 5ng/µL |
| *inx-1(tm3524)* X*; vjEx955; vjEx1187* | OJ7359 | *vjEx955* [pUC06 (*Punc-47(FL)::inx-1a::GFP(TFV)*), 25 ng/µL], *vjEx1187* [pUC55 (*Punc-47(FL)::unc-10::mCherry*), 25 ng/µL] |
| *vjEx1260; vjEx1984* | OJ7555 | *vjEx1260* [pUC57 (*Punc-25(Δ)::inx-1a::GFP*), 50 ng/µL], *vjEx1984* [pUC93 (*Pflp-10::inx-1a::mCherry*), 50 ng/µL] |
| *inx-1(tm3524)* X*; vjEx955; vjEx1188* | OJ3289 | *vjEx955* [pUC06 (*Punc-47(FL)::inx-1a::GFP(TFV)*), 25 ng/µL], *vjEx1188* [pUC56 (*Punc-47(FL)::Cx36::mCherry*), 25 ng/µL] |

**Table S1. Cont.**

| Genotype | Strain | Plasmid |
|---|---|---|
| *nlp-40(tm4085)* I*; unc-33(e204)* IV | OJ6236 | |
| *nlp-40(tm4085)* I*; unc-33(e204)* IV; *vjEx1828* | OJ6418 | pUC77 (*Punc-47(FL)::unc-33c*), 25 ng/µL |
| *unc-33(e204)* IV | CB204 | |
| *vjEx1478* | OJ4594 | pUC63 (*Pflp-10::HisCl1*), 50 ng/µL |
| *inx-1(tm3524)* X*; vjEx1478* | OJ4595 | pUC63 (*Pflp-10::HisCl1*), 50 ng/µL |
| *nlp-40(tm4085)* I*; inx-1(tm3524)* X*; vjEx1060* | OJ2801 | pUC18 (*Punc-47(FL)::PANX1*), 25 ng/µL |
| *nlp-40(tm4085)* I*; inx-1(tm3524)* X*; vjEx1188* | OJ3182 | pUC56 (*Punc-47(FL)::Cx36*::mCherry*), 25 ng/µL |
| *nlp-40(tm4085)* I*; inx-1(tm3524)* X*; vjEx1067* | OJ2819 | pUC19 (*Punc-47(FL)::inx-1b::GFP*), 25 ng/µL |
| *nlp-40(tm4085)* I*; inx-1(tm3524)* X*; vjEx1068* | OJ2820 | pUC20 (*Punc-47(FL)::inx-1(1~307)::linker::GFP*), 25 ng/µL |
| *nlp-40(tm4085)* I*; inx-1(tm3524)* X*; vjEx1069* | OJ2821 | pUC21 (*Punc-47(FL)::inx-1(1~338)::linker::GFP*), 25 ng/µL |
| *nlp-40(tm4085)* I*; inx-1(tm3524)* X*; vjEx1062* | OJ2803 | pUC26 (*Punc-47(FL)::inx-1(1~356)::linker::GFP*), 25 ng/µL |
| *nlp-40(tm4085)* I*; inx-1(tm3524)* X*; vjEx1066* | OJ2818 | pUC25 (*Punc-47(FL)::inx-1(1~372)::linker::GFP*), 25 ng/µL |
| *nlp-40(tm4085)* I*; inx-1(tm3524)* X*; vjEx1070* | OJ2822 | pUC31 (*Punc-47(FL)::inx-1(1~307)::inx-1a(339~428)::GFP*), 25 ng/µL |
| *nlp-40(tm4085)* I*; inx-1(tm3524)* X*; vjEx1071* | OJ2823 | pUC32 (*Punc-47(FL)::inx-1(1~307)::inx-1a(357~428)::GFP*), 25 ng/µL |
| *otIs348* IV*; vjEx1260* | OJ7555 | pUC57 (*Punc-25(Δ)::inx-1a::GFP*), 50 ng/µL |
| *otIs348* IV*; inx-1(tm3524)* X*; vjEx1193* | OJ3820 | pUC34 (*Pflp-10::inx-1a::GFP*), 25 ng/µL |
| *vjIs192* V | OJ4395 | pMH212 (*Punc-25(Δ)::ebp-1::GFP*), 25 ng/µL |
| *unc-33(e204)* IV*; vjIs192* V | OJ4459 | pMH212 (*Punc-25(Δ)::ebp-1::GFP*), 25 ng/µL |
| *vjIs192* V*; inx-1(tm3524)* X | OJ5160 | pMH212 (*Punc-25(Δ)::ebp-1::GFP*), 25 ng/µL |
| *nlp-40(tm4085)* I*; inx-7(ok2319)* IV | OJ3679 | |
| *nlp-40(tm4085)* I*; inx-10(ok2714)* V | OJ2326 | |
| *nlp-40(tm4085)* I*; inx-11(ok2783)* V | OJ4155 | |
| *nlp-40(tm4085)* I*; unc-7(e5)* X | OJ3713 | |
| *nlp-40(tm4085)* I*; unc-9(e101)* X | OJ3714 | |
| *unc-13(s69)* I*; vjIs58* IV*; vjEx2554* | OJ7242 | *vjIs58* [pHW100 (*Punc-47(mini)::GCaMP3*), 125ng/µl], *vjEx2554* [pUC224 (*Pnmur-3::Pegl-18::GCaMP6*), 25 ng/µL] |
| *unc-13(s69)* I*; vjIs58* IV*; inx-1(tm3524)* X*; vjEx2554* | OJ7243 | *vjIs58* [pHW100 (*Punc-47(mini)::GCaMP3*), 125ng/µl], *vjEx2554* [pUC224 (*Pnmur-3::Pegl-18::GCaMP6*), 25 ng/µL] |

**Table S1. Cont.**

| Genotype | Strain | Plasmid |
|---|---|---|
| *vjIs187* V*; aex-2(sa3)* X | OJ7528 | *vjIs187* [pMH44 (*Pflp-22::aex-2*), 25 ng/µL] |
| *vjIs187* V*; aex-2(sa3) inx-1(tm3524)* X | OJ7529 | *vjIs187* [pMH44 (*Pflp-22::aex-2*), 25 ng/µL] |
| *unc-13(s69)* I*; vjIs186* II*; vjIs183* V*; aex-2(sa3)* X | OJ4167 | *vjIs186* [pMH44 (*Pflp-22::aex-2*), 25 ng/µL], *vjIs183* [pUC61 (*Pflp-10::GCaMP3*), 50 ng/µL] |
| *unc-13(s69)* I*; vjIs186* II*; vjIs183* V*; aex-2(sa3) inx-1(tm3524)* X | OJ4679 | *vjIs186* [pMH44 (*Pflp-22::aex-2*), 25 ng/µL], *vjIs183* [pUC61 (*Pflp-10::GCaMP3*), 50 ng/µL] |
| *unc-13(s69)* I*; vjIs58* IV | OJ1443 | pHW100 (*Punc-47(mini)::GCaMP3*), 125ng/µl |
| *unc-13(s69)* I*; vjIs58* IV*; inx-1(tm3524)* X | OJ2161 | pHW100 (*Punc-47(mini)::GCaMP3*), 125ng/µl |
| *nlp-40(tm4085) unc-13(s69)* I*; vjIs58* IV | OJ1467 | pHW100 (*Punc-47(mini)::GCaMP3*), 125ng/µl |
| *nlp-40(tm4085) unc-13(s69)* I*; vjIs58* IV*; inx-1(tm3524)* X | OJ7567 | pHW100 (*Punc-47(mini)::GCaMP3*), 125ng/µl |
| *snt-2(tm1711)* III | OJ1352 | |
| *snt-2(tm1711)* III*; inx-1(tm3524)* X | OJ2291 | |
| *aex-2(sa3)* X | OJ2834 | |
| *aex-2(sa3) inx-1(tm3524)* X | OJ2327 | |
| *vjIs76(PKA[DN])* V | OJ1603 | pHW154 (*Punc-47(FL)::kin-2a(G310D)*), 50 ng/µl |
| *vjIs76(PKA[DN])* V*; inx-1(tm3524)* X | OJ3382 | pHW154 (*Punc-47(FL)::kin-2a(G310D)*), 50 ng/µl |
| *egl-19(n582)* IV | OJ1911 | |
| *egl-19(n582)* IV*; inx-1(tm3524)* X | OJ3488 | |
| *egl-19(n582)* IV*; aex-2(sa3) inx-1(tm3524)* X | OJ6341 | |
| *unc-2(lj1)* X | OJ1526 | |
| *unc-2(lj1) inx-1(tm3524)* X | OJ2290 | |
| *nlp-40(tm4085)* I*; unc-2(lj1) inx-1(tm3524)* X | OJ3682 | |
| *unc-13(s69)* I*; vjIs64* II*; egl-19(n582)* IV | OJ1916 | pHW100 (*Punc-47(mini)::GCaMP3*), 125ng/µl |
| *unc-13(s69)* I*; vjIs64* II*; egl-19(n582)* IV*; inx-1(tm3524)* X | OJ3428 | pHW100 (*Punc-47(mini)::GCaMP3*), 125ng/µl |
| *unc-13(s69)* I*; vjIs58* IV*; unc-2(lj1)* X | OJ1919 | pHW100 (*Punc-47(mini)::GCaMP3*), 125ng/µl |
| *unc-13(s69)* I*; vjIs58* IV*; unc-2(lj1) inx-1(tm3524)* X | OJ3464 | pHW100 (*Punc-47(mini)::GCaMP3*), 125ng/µl |
| *egl-36(n2332 sa577)* X | JT577 | |
| *egl-36(n728)* X | OJ1897 | |
| *vjIs103* I | OJ1858 | pHW173 (Punc-47(FL)::kin-1a(H96R, W205Q), 50ng/µl |
| *vjIs103* I*; egl-36(n728)* X | OJ1898 | pHW173 (Punc-47(FL)::kin-1a(H96R, W205Q), 50ng/µl |
| *egl-36(n728)* I*; inx-1(tm3524)* X | OJ3709 | |

**Table S1. Cont.**

| Genotype | Strain | Plasmid |
|---|---|---|
| *vjEx1272* | OJ3735 | pUC58 (*Punc-47(FL)::egl-36(gf)::GFP*), 25 ng/μL |
| *inx-1(tm3524)* X*; vjEx1272* | OJ3751 | pUC58 (*Punc-47(FL)::egl-36(gf)::GFP*), 25 ng/μL |
| *unc-13(s69)* I*; vjIs58* IV*; egl-36(n728)* X | OJ1906 | pHW100 (*Punc-47(mini)::GCaMP3*), 125ng/μl |
| *unc-13(s69)* I*; vjIs58* IV*; inx-1(tm3524) egl-36(n728)* X | OJ3332 | pHW100 (*Punc-47(mini)::GCaMP3*), 125ng/μl |

**Table S2. Oligos used in this study**

| Sequence | | Oligos |
|---|---|---|
| *flp-10* promoter | forward | ccccccGCATGCtactcggctaatgactagtg |
| | reverse | ccccccGGATCCcctttgctgtatgagttgattg |
| *flp-22* promoter | forward | ccccccGCATGCgagcataagctcttcttgaattc |
| | reverse | ccccccCCCGGGttttgtgtatatcctgaaataaaac |
| *unc-25(Δ)* promoter | forward | ccccccGCATGCCTCATTTCGCCCTCGGGGC |
| | reverse | CCCCCCgctagcCTCCAAGGGTCCTCctgaaaatg |
| *nmur-3* promoter | forward | ccccccGCATGCaacacacgttcaactcgttg |
| | reverse | ccccccCCCGGGATCCggcttcaattagttgtgtca |
| *egl-18* basal promoter | forward | ccccccGGATCCctccatagtagtacattttaaggt |
| | reverse | ccccccCCCGGGatagactgtgtggagacac |
| *inx-1a* cDNA | forward | cccccccgctagcAAAAATGCTTCTATATTATCTGGCG |
| | reverse | CCCCCCggtaccTTAGACGAACGTGAAGTAAC |
| *PANX1* cDNA | forward | ccccccGCTAGCaaaaatggccatcgctcaactgg |
| | reverse | ccccccCCATGGtcagcaagaagaatccagaagtctc |
| *HisCl1* | forward | ccccccGCGATCGCaaaaatgcaaagcccaactagcaaattg |
| | reverse | ccccccGGTACCtcataggaacgttgtccaatagac |
| *unc-33c* cDNA | forward | ccccccGCGATCGCaaaaATGGCAGTCGTATGGGAAC |
| | reverse | CCCCCCgcggccgcCTACCAAAACCCTGTAGTCCG |
| **For 3' terminal fusion with *GFP*, *mCherry*, or *linker::GFP*** | | |
| *inx-1a* cDNA | forward | cccccccgctagcAAAAATGCTTCTATATTATCTGGCG |
| | reverse | ccccccACCGGTGACGAACGTGAAGTAACC |
| *inx-1b* cDNA | forward | cccccccgctagcAAAAATGCTTCTATATTATCTGGCG |
| | reverse | ccccccACCGGTGTGGTTGAGGGATTCCGTTG |
| *unc-10* cDNA | forward | ccccccGCGATCGCaaaaATGGACGATCCGTCGATGATG |
| | reverse | ccccccgcggccgcCTGCTGAGCACCTCCAACTG |
| *Cx36* | forward | ccccccGCTAGCaaaaATGGGAGAGTGGACCATCC |
| | reverse | CCCCCCaccggtGACGTAGGCGGAGTCGGAG |
| *inx-1(1~307)* | forward | cccccccgctagcAAAAATGCTTCTATATTATCTGGCG |
| | reverse | CCCCCCaccggtGTGCTGTCCTGGTAAGAAAG |
| *inx-1(1~338)* | forward | cccccccgctagcAAAAATGCTTCTATATTATCTGGCG |
| | reverse | CCCCCCaccggtATCATATCCGAGGAACTTGTG |
| *inx-1(1~356)* | forward | cccccccgctagcAAAAATGCTTCTATATTATCTGGCG |
| | reverse | ccccccACCGGTTGTAGCAAGAATATCTCCAGC |
| *inx-1(1~372)* | forward | cccccccgctagcAAAAATGCTTCTATATTATCTGGCG |
| | reverse | ccccccACCGGTCTTCCTGACACGATCATTGAAG |
| *ebp-1* cDNA | forward | CCCCCCgctagcAAAAATGGGCTATCAAGTAGTTAATG |
| | reverse | ccccccACCGGTGAATTCTTCGGCTTCTGCTC |
| *egl-36(gf)* | forward | ccccGCTAGCaaaaATGCTCGACGCGTGCTCGTTC |
| | reverse | GCAAGGGTCTTTTGTGTGTCTTTAGCATGAAG |
| | forward | AGACACACAAAAGACCCTTGCTGTTTTGGATC |
| | reverse | ccccccaccggtGGAAATTATTGTGGTGGTAATGGC |
| **For internal deletion of *inx-1* cDNA** | | |
| *inx-1(1~307)* | forward | cccccccgctagcAAAAATGCTTCTATATTATCTGGCG |
| | reverse | CCCCCCaccggttctagaGTGCTGTCCTGGTAAGAAAG |
| *inx-1a(339~428)::GFP* | forward | CCCCCCtctagaGGAGTGTTTTGTATGAGAATGATTTCG |
| | reverse | gggggggtaccttaTTTGTATAGTTCATCCATGCC |
| *inx-1a(357~428)::GFP* | forward | ccccccctctagaGAACTAATTGTTGCTCTGTGGC |
| | reverse | gggggggtaccttaTTTGTATAGTTCATCCATGCC |

**Table S2. Cont.**

| Sequence | | Oligos |
|---|---|---|
| **For internal *GFP* fusion to *inx-1a* cDNA** | | |
| *GFP(TFV)* | forward | cccccacgcgtAGTAAAGGAGAAGAACTT |
| | reverse | cccccacgcgtTTTGTATAGTTCATCCATGCC |

Notes: *egl-36(gf)* was generated by overlapping PCR to introduce a point mutation.

**Movie S1.** Calcium live imaging of wild-type day 1 adults co-expressing GCaMP6 in AVL (under the *nmur-3* promoter) and GCaMP3 in DVB (under the *unc-47(mini)* promoter). Movie shows one cycle of the DMP in which a calcium spike in AVL (including the NMJ) and in the DVB cell body were observed at the same time (within 250ms of each other) about 3 seconds after pBoc and right before Exp.

**Movie S2.** Calcium live imaging of *inx-1* mutant day 1 adults co-expressing GCaMP6 in AVL (under the *nmur-3* promoter) and GCaMP3 in DVB (under the *unc-47(mini)* promoter). Movie shows one cycle of the DMP in which the calcium spike occurred at the normal time at the AVL NMJ, and occurred in the DVB cell body 2 seconds later. The Exp occurred during the calcium spike in DVB.

**Movie S3.** Calcium live imaging of day 1 *aex-2* mutants co-expressing *aex-2* cDNA in AVL (under the *flp-22* promoter) and GCaMP3 in DVB (under the *flp-10* promoter). Movie shows one cycle of the DMP in which a calcium spike was observed at the DVB NMJ right before Exp.

**Movie S4.** Calcium live imaging of day 1 *aex-2 inx-1* double mutants co-expressing *aex-2* cDNA in AVL (under the *flp-22* promoter) and GCaMP3 in DVB (under the *flp-10* promoter). Movie shows one cycle of the DMP in which no calcium spike was observed at the DVB NMJ right before Exp.

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