

# Supplementary Information for

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

Ukjin Choi, Han Wang, Mingxi Hu, Sungjin Kim, and Derek Sieburth

Derek Sieburth Email: <u>sieburth@usc.edu</u>

# 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: <u>http://depts.washington.edu/jtlab/software/otherSoftware.html</u>) (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<sup>2</sup> 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(\Delta)::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(\Delta):::epp-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 vjls58 (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, vjls58, vjls64 (Pmyo-2::NLS::mCherry, Punc-47(mini)::GCaMP3), and vjls183 (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<sub>0</sub>) 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.



**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(A) 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- $25(\Delta)$  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(\Delta*)) 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 in durates. Means and standard errors are shown. Asterisks indicate significant differences: \* P<0.05 in one way ANOVA with Bonferroni's correction for multiple comparisons.

Genotype	Strain	Plasmid
Wild-type Bristol strain	N2	
nlp-40(tm4085) I	OJ794	
vjEx1530	OJ7346	pUC96 ( <i>Pflp-10::PH domain::miniSOG</i> ), 50 ng/µL
vjEx1578	OJ5350	pUC94 ( <i>Pflp-22::PH</i> domain::miniSOG), 50 ng/uL
vjEx2701	OJ7888	pUC94 ( <i>Pflp-22::PH</i> domain::miniSOG), 50 ng/µL + pUC96 ( <i>Pflp-10::PH</i> domain::miniSOG), 50 ng/µl
nlp-40(tm4085) l; vjEx1530	OJ5030	pUC96 ( <i>Pflp-10::PH</i> domain::miniSOG), 50 ng/μL
nlp-40(tm4085) l; vjEx1578	OJ7391	pUC94 ( <i>Pflp-22::PH</i> <i>domain::miniSOG</i> ), 50 ng/µL
inx-1(tm3524) X	OJ2446	
nlp-40(tm4085) I; inx-1(tm3524) X	OJ2323	
nlp-40(tm4085)	OJ2156	
nlp-40(tm4085) l; inx-1(tm3524) X; vjEx917	OJ2343	pUC08 ( <i>Pnlp-40::inx-1a</i> ), 25 ng/μL
nlp-40(tm4085) l; inx-1(tm3524) X; vjEx912	OJ2331	рНW175 ( <i>Punc-47(FL)::inx-1a</i> ), 25 ng/µL
nlp-40(tm4085) l; inx-1(tm3524) X; vjEx915	OJ2337	pŪĊ07 ( <i>Pmyo-3::inx-1a</i> ), 25 ng/μL
nlp-40(tm4085) I; inx-1(tm3524) X; vjEx916	OJ2342	pUC09 ( <i>Phsp-16.2∷inx-1a</i> ), 25 ng/µL
nlp-40(tm4085)	OJ2805	pUC30 ( <i>Pflp-22::inx-1a::GFP</i> ), 25 ng/µL
nlp-40(tm4085) I; inx-1(tm3524) X; vjEx1193	OJ7566	pŪČ34 ( <i>Pflp-10::inx-1a::GFP</i> ), 25 ng/µL
nlp-40(tm4085) \; inx-1(tm3524) X; vjEx1080	OJ2846	pUC30 ( <i>Pflp-22::inx-1a::GFP</i> ), 25 ng/µL + pUC34 ( <i>Pflp-10::inx-</i> <i>1a::GFP</i> ), 25 ng/µL
nlp-40(tm4085) I; inx-1(vj46) X	OJ1664	<i>,,</i> 31
nlp-40(tm4085) I; snta(vj48) IV	OJ1665	
nlp-40(tm4085) I; snta(vj49) ∨	OJ1667	
<i>vjls123</i>	OJ3005	pMH52 ( <i>Punc-25(Δ)::GFP::rab-3</i> ), 5ng/μL
vjls123 II; inx-1(tm2534) X	OJ3651	рЙН́52 ( <i>Punc-25(Δ)::GFP::rab-</i> 3), 5ng/µL
inx-1(tm3524) X; vjEx955; vjEx1187	OJ7359	vjEx955 [pUC06 ( <i>Punc-47(FL</i> )::inx- 1a::GFP(TFV)), 25 ng/µL], vjEx1187 [pUC55 ( <i>Punc-47(FL</i> )::unc- 10::mCherny), 25 ng/µL]
vjEx1260; vjEx1984	OJ7555	vjEx1260 [pUC57 ( <i>Punc-25(Δ</i> )::inx- 1a::GFP), 50 ng/μL], vjEx1984 [pUC93 ( <i>Pflp-10::inx-1a::mCherry</i> ), 50
inx-1(tm3524) X; vjEx955; vjEx1188	OJ3289	vjEx955 [pUC06 ( <i>Punc-47(FL)::inx- 1a::GFP(TFV</i> )), 25 ng/µL], vjEx1188 [pUC56 ( <i>Punc-</i> 47(FL)::Cx36::mCherry), 25 ng/uL1

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

Table S1. Cont.

Genotype	Strain	Plasmid
nlp-40(tm4085) l; unc-33(e204) IV	OJ6236	
nlp-40(tm4085) I; unc-33(e204) IV; viEx1828	OJ6418	pUC77 ( <i>Punc-47(FL)::unc-33c</i> ), 25 ng/ul
unc-33(e204) IV	CB204	
vjEx1478	OJ4594	рUC63 ( <i>Pflp-10::HisCl1</i> ), 50 ng/µL
inx-1(tm3524) X; vjEx1478	OJ4595	pUC63 ( <i>Pflp-10::HisCl1</i> ), 50 ng/µL
nlp-40(tm4085) I; inx-1(tm3524) X;	OJ2801	pUC18 ( <i>Punc-47(FL</i> )::PANX1), 25
vjEx1060		ng/µL
nlp-40(tm4085)	OJ3182	pUC56 ( <i>Punc-</i> 47(EL)::Cx36*::mCherny), 25 ng/ul
nln-40(tm4085) 1: inx-1(tm3524) X:	0 12819	nIC19 (Punc-47(EL)inx-1hGEP)
vjEx1067	002013	25 ng/µL
nlp-40(tm4085) I; inx-1(tm3524) X; viEx1068	OJ2820	pUC20 ( <i>Punc-47(FL)::inx-</i> 1(1~307)::linker::GFP), 25 ng/uL
nlp-40(tm4085) I: inx-1(tm3524) X:	OJ2821	pUC21 ( <i>Punc-47(FL</i> )::inx-
vjEx1069		1(1~338):: <i>linker::GFP</i> ), 25 ng/µL
nlp-40(tm4085)	OJ2803	pUC26 ( <i>Punc-47(FL)::inx-</i> <i>1(1~356)::linker::GFP</i> ), 25 ng/µL
nlp-40(tm4085)	OJ2818	pUC25 ( <i>Punc-47(FL)::inx-</i> <i>1(1~372)::linker::GFP</i> ), 25 ng/µL
nlp-40(tm4085) I; inx-1(tm3524) X;	OJ2822	pUC31 ( <i>Punc-47(FL</i> )::inx-
vjEx1070		1(1∼307)∷inx-1a(339∼428)∷GFP), 25 ng/µL
nlp-40(tm4085)	OJ2823	pŪC32 (Punc-47(FL)::inx- 1(1~307)::inx-1a(357~428)::GFP), 25
otls348 IV; vjEx1260	OJ7555	ng/μL pUC57 ( <i>Punc-25(Δ)::inx-1a::GFP</i> ), 50
otls348 IV; inx-1(tm3524) X; vjEx1193	OJ3820	pUC34 ( <i>Pflp-10::inx-1a::GFP</i> ), 25
vjls192 V	OJ4395	pMH212 ( <i>Punc-25(Δ)::ebp-1::GFP</i> ), 25 ng/ul
unc-33(e204) IV; vjls192 V	OJ4459	pMH212 ( <i>Punc-25(Δ)::ebp-1::GFP</i> ), 25 ng/μL
vjls192 V; inx-1(tm3524) X	OJ5160	pMH212 ( <i>Punc-25(Δ)::ebp-1::GFP</i> ), 25 ng/μL
nlp-40(tm4085)	OJ3679	
nlp-40(tm4085)	OJ2326	
nlp-40(tm4085)	OJ4155	
nlp-40(tm4085) I; unc-7(e5) X	OJ3713	
nlp-40(tm4085) I; unc-9(e101) X	OJ3714	
unc-13(s69) I; vjls58 IV; vjEx2554	OJ7242	vjls58 [pHW100 (Punc- 47(mini)::GCaMP3), 125ng/µl], vjEx2554 [pUC224 (Pnmur-3::Pegl- 18::GCaMP6), 25 pg/µl ]
unc-13(s69) I; vjls58 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
vjls187 V; aex-2(sa3) X	OJ7528	<i>vjls187</i> [pMH44 ( <i>Pflp-22∷aex-2</i> ), 25
		ng/µL]
vjls187 V; aex-2(sa3) inx-1(tm3524) X	OJ7529	<i>vjls1</i> 87 [pMH44 ( <i>Pflp-22::aex-2</i> ), 25
una 12/260) l: vila196 ll: vila192 \/: 224	014467	$ng/\mu L$ ]
2(co3) X	OJ4107	$V_{JIS} = 00 \text{ [PIVIP44 (PIIP-22aex-2), 25]}$
2(383) X		$10^{\circ}GCaMP3$ ) 50 ng/ul 1
unc-13(s69)  : vils186   : vils183 V: aex-	OJ4679	vils186 [pMH44 ( <i>Pflp-22::aex-2</i> ), 25
2(sa3) inx-1(tm3524) X		ng/µL], vj/s183 [pUC61 (Pflp-
		<i>10::GCaMP3</i> ), 50 ng/μL]
unc-13(s69) I; vjls58 IV	OJ1443	pHW100 ( <i>Punc-47(mini)::GCaMP3</i> ),
		125ng/µl
unc-13(s69) I; vjIs58 IV; inx-1(tm3524) X	OJ2161	pHW100 ( <i>Punc-47(mini)::GCaMP3</i> ),
	0 14 407	125ng/µl
nip-40(tm4085) unc-13(\$69) 1; VJI\$58 IV	OJ1467	pHvv100 (Punc-47(mini)::GCaMP3),
nln_40(tm4085) unc_13(s60) 1: vils58 \\/:	0 17567	125119/μ1 pHW/100 (Pupe-47(mini)···CCaMP3)
inx-1(tm3524) X	001001	125ng/ul
snt-2(tm1711)	0.11352	
snt-2(tm1711) III: inx-1(tm3524) X	0.12291	
aex-2(sa3) X	012834	
2(323) X	012007	
$acx-2(SaS)$ (11X-1((11)SS24) $\land$	014602	n WALLEA (Dunn AZ/EL) whin
VJIS76(PKA[DIN]) V	OJ1603	PHVV154 ( <i>Punc-47(FL)::Kin-</i> 2a(G310D)) 50 pg/ul
vils76(PKAIDNI) V· inx-1(tm3524) X	0.13382	pHW154 ( <i>Punc-47(FL</i> )::kin-
	000002	2a(G310D)). 50 ng/ul
egl-19(n582) IV	OJ1911	
egl-19(n582) IV; inx-1(tm3524) X	OJ3488	
eal-19(n582) IV: aex-2(sa3) inx-1(tm3524)	OJ6341	
Χ		
unc-2(lj1) X	OJ1526	
unc-2(lj1) inx-1(tm3524) X	OJ2290	
nlp-40(tm4085) I; unc-2(lj1) inx-1(tm3524)	OJ3682	
X		
unc-13(s69) I; vjls64 II; egl-19(n582) IV	OJ1916	pHW100 ( <i>Punc-47(mini)::GCaMP3</i> ),
	0 10 400	125ng/µl
unc-13(s69) I; vJIs64 II; egi-19(n582) IV;	OJ3428	pHVV100 (Punc-47(mini)::GCaMP3),
INX-1(IM3524) X unc-13(s69) I: vils58 IV: unc-2(li1) X	011010	nHW/100 (Punc-47(mini)::GCaMP3)
une-15(309) 1, vji330 1V, une-2(ij 1) X	001010	125ng/ul
unc-13(s69)	OJ3464	pHW100 ( <i>Punc-47(mini</i> )::GCaMP3).
1(tm3524) X		125ng/µl
egl-36(n2332 sa577) X	JT577	
egl-36(n728) X	OJ1897	
vjls103 l	OJ1858	pHW173 (Punc-47(FL)::kin-1a(H96R
		W205Q), 50ng/µl
vjls103 l; egl-36(n728) X	OJ1898	pHW173 (Punc-47(FL)::kin-1a(H96R
and 26(n720) It into 4 (to 2504) V	0 10700	W205Q), 50ng/μl
eyi-30(11/28) 1, 111x-1(tM3524) X	013108	

# Table S1. Cont.

Genotype	Strain	Plasmid
vjEx1272	OJ3735	pUC58 ( <i>Punc-47(FL)::egl-</i> 36( <i>af</i> )::GFP), 25 ng/uL
inx-1(tm3524) X; vjEx1272	OJ3751	pUC58 ( <i>Punc-47(FL</i> ):: <i>egl-</i> 36( <i>af</i> ):: <i>GFP</i> ), 25 ng/µL
unc-13(s69) I; vjls58 IV; egl-36(n728) X	OJ1906	pHW100 ( <i>Punc-47(mini)::GCaMP3</i> ), 125ng/ul
unc-13(s69) I; vjls58 IV; inx-1(tm3524) egl- 36(n728) X	OJ3332	рНW100 ( <i>Punc-47(mini)::GCaMP3</i> ), 125ng/µl

# Table S2. Oligos used in this study

flp-10 promoter forward ccccccGCATGCtactcgctatgattgtg   flp-22 promoter reverse ccccccCATGCtactcgctatagttgttg   unc-25(Δ) promoter forward ccccccCCCGGATCCATCTGGCCCTCGGGGC   nmur-3 promoter forward cccccCCCGGATGCtactcgttatagttgttgta   egl-18 basal promoter forward cccccCCGGGATCCCtcatagtagttatagttgta   inx-1a cDNA forward ccccccCCCGGGATGCtactagtagtagaca   inx-1a cDNA forward ccccccCCCGGGATGCtactagagagtaca   inx-1a cDNA forward ccccccCCCGGATGCcaaaatggccatcgtcaagagtatc   inx-1a cDNA forward ccccccCCCGGTGCcaaaatggaagatccagagagaattc   inx-3c cDNA forward ccccccCCCGTGCcaaaatgcaagcaatgg   inx-3c cDNA forward ccccccGCATGCcaaaatgcaagccaatcgccaatggaagtcc   inx-1a cDNA forward ccccccGCATCGCaaaatgGaagagatcaagagtcca   inx-1a cDNA forward ccccccGCGTGCCaaaaTGGCGACCTCATATGTATGCG   inx-1a cDNA forward cccccccCCGGTGCGAaaaTGGCGACTCCTATGGGAC   inx-1a cDNA forward cccccccCCGGTGCGAaaaTGGCGACCTCCTATGGCG   inx-1a cDNA forward cccccccCCGGTGCGAaaaTGGCGACCTCCTATGGCG   inx-10 cDNA forward cccccccCCGGTGCGAaaaTGGCGCGCTCCTAAGGCGACGTCG   inx-110 cDNA forward ccccccccCCGGTGCGAaaaATGGCGACACTCC   inx-11(1-307)<	Sequence		Oligos
reverseccccccGGATCCcctttgctgtatagttgattgfip-22 promoterforwardccccccCCGGTtCCqagcataagctcttcttgaattacunc-25(Δ) promoterforwardcccccCCCGGGtttggtatatctggtgnmur-3 promoterforwardcccccCCCGGGttCGqgttcaattagtgtgtaegl-18 basal promoterforwardforwardccccccGCATCCtccatagtagtacattttagtgtcaegl-18 basal promoterforwardccccccCCCGGGatagactgtggagacacinx-1a cDNAforwardccccccCCCGGGtacGAACAATGCTTCTATATTATCGGGPANX1 cDNAforwardccccccCCATGCaaatggagaatccgaaatggHisCl1forwardccccccCCCGGTAGCaaaatggaagcacaatggaagatccgaaatggunc-33 c DNAforwardccccccCCGGTCGCaaaaTGCGCAGCGAACGGAACFor 3' terminal fusionwith GFP, mCherry, orink-ris GFDinx-1b cDNAforwardccccccCGGTGGCaaaATGCAGCGTCGTATTGGGACreverseccccccGCGTGGCaaaATGCAGCGCCGTGGAGGAGCGGGGGGGGGG	flp-10 promoter	forward	ccccccGCATGCtactcggctaatgactagtg
#p-22 promoter   forward   ccccccGCATGCggcataagctcttcttgatta     unc-25(A) promoter   forward   ccccccCCGGGttttgtgtatacctgaataaaac     unc-25(A) promoter   forward   ccccccGCATGCCTCATTTCGCCCTCGGGC     nmur-3 promoter   forward   cccccCCCGGatagctttttagtgtatactgtg     egl-18 basal promoter   forward   cccccCCCGGGATCCgcttagtagtacttttaggt     inx-1a cDNA   forward   cccccCCCGGGatagctgtggagacac     inx-1a cDNA   forward   cccccCCCGGGatagaatggtggagaac     PANX1 cDNA   forward   ccccccCCCGGatagaatggtggagaatccagaagtct     HisCl1   forward   ccccccGCATGGaaaatggcaactggaagaatccagaagtct     HisCl1   forward   ccccccGCATGGaaaatgcaagagaatccagaagtcc     unc-33c cDNA   forward   ccccccGCATGGaaaatgGCAGACGTCGTATGGGAAC     reverse   cccccccGCGTCGCTAGGAaaatgCaCGTGGTAGGCA     reverse   ccccccCCCCGggccgCTACCAAAACCCTGTAGGCAC     inx-1a cDNA   forward   cccccccGCGTGGCGAGAGAGTCGTGTAGTAGGCG     inx-1a cDNA   forward   ccccccCCGGTGCGTGGCAGGAGTCCGTGGG     inx-10 cDNA   forward   cccccccCCGGTGCGCAGAAATGCCAACTTCAATATACTGGCG     inx-10 cDNA   forward   cccccccCCGGTGCGTGGCAAGGGGAGTCGTGGTGGGGGGGGGG	- •	reverse	ccccccGGATCCcctttgctgtatgagttgattg
reverseccccccCCCGGCttttgtgtatatctgaataaaacunc-25(A) promoterforwardccccccGCATGCCTCAAGGTCCTCGGGGCnmur-3 promoterforwardccccccGCATGCaacacagttcaattgttgegl-18 basal promoterforwardcccccCGGATCCGggttcaattagttgtgtaainx-1a cDNAforwardccccccGCATGCACGaatagtgcagaacainx-1a cDNAforwardcccccCGCATGCCaaaatggcatcaattgtPANX1 cDNAforwardcccccCGCTAGCaaaatggcatcaattgtPANX1 cDNAforwardcccccCGCATGCCaaaatggcatcaattgreversecccccCGCATGCCaaaatggcatcaatggreversecccccCGCATCGCaaaatgGaagacacaactgcaaattgreversecccccCGGATCGCaaaatgGaagccaaatggaagtccreversecccccCGGATCGCaaaatGGAAAACCCTGTATGGGAACreversecccccCGGGTGCCAaaaATGGCAGTCGTATGGGACreversecccccCGGGTGGCGAaaATGGCAGTCGTATGGGAACreversecccccCGCGGTGGCGAAAAATGCTTCTATATATCTGGCGinx-1a cDNAforwardccccccGCGGGGGGAGGAGTCGATGGGACinx-1a cDNAforwardccccccGCCGGTGGGGGAGCCGTGGATGGGACinx-1b cDNAforwardccccccGCCGGTGGGGGGGGCGCGCGGGGCTGGGATGGGA	flp-22 promoter	forward	cccccGCATGCgagcataagctcttcttgaattc
unc-25(Δ) promoter   forward   ccccccGCATGCCTATTCGCCCTCGGGGC     nmur-3 promoter   forward   ccccccGCATGCacacacgttCacatagtagt     egl-18 basal promoter   forward   ccccccGGATCCctcatagtagtacattgtggagacac     inx-1a cDNA   forward   ccccccGCTGGGaaaatggcGTGCTGCtGaaagtgtgtggagacac     inx-1a cDNA   forward   ccccccGCTGGGaaaatggcGAAAAATGCTTCTATATTATCTGGCG     PANX1 cDNA   forward   ccccccGCTGGGaaaatggcGaacactagctagaatgtgtgagacac     inx-1a cDNA   forward   ccccccGCTGGGaaaatggcGaacagagagtcC     HisCl1   forward   ccccccGCGATCGCaaaatggcaactgcGaacatagcaaatgg     unc-33c cDNA   forward   ccccccGCGTCCCtCataggaagttgtccaatagac     with GFP, mCherry, or   inx-1a cDNA   forward   ccccccGCGTGCGCAAGAAAATGCTTCTATATTATCTGGCG     inx-1a cDNA   forward   ccccccGCGTGCGCAAGAAAATGCTTCTATATTATCTGGCG   inx-1a cDNA   forward   ccccccGCGTGCGGAAGAGGACGGAGTCCGGTG     inx-10 cDNA   forward   ccccccGGATGCGaaaATGGCAACTCGAGACTGGAGACTGC   inx-1a cCCCCCCGCGGTGCGGAGGACTCCGCTGG     inx-11 c307)   forward   cccccccCGGATGGCGaaAATGGCGGAGTCCGGAG   inx-1(1~307)   forward   cccccccGCCGATGCGAAAAATGCTTCTATATTATCTGGCG     inx-1(1~338)   forward   cccccccdccggtGGCTGGTAGGAAGATGGAACTGG </th <th>, ,</th> <th>reverse</th> <th>cccccCCCGGGttttgtgtatatcctgaaataaaac</th>	, ,	reverse	cccccCCCGGGttttgtgtatatcctgaaataaaac
reverseCCCCCCgctagcCTCCAAGGGTCCTCctgaaatgnmur-3 promoterforwardcccccCGCATGCaacacacgttcaactagttgreversecccccCCCGGGATCCggcttcaattagttgtgagacainx-1a cDNAforwardcccccCCCGGGATCGCaacacgtgPANX1 cDNAforwardcccccCCCATGGCaacaatggcaactagatgreversecccccCCCATGGCaacaatggcaattagatggcccccCCCATGGCaaaatggcaattagatgaagtcareversecccccCCCATGGCaaaatggcaagtcgaagagreversecccccCCCATGGCaaaatgGaagagccaactagcaaattgreversecccccCGCATGCCaaaaATGGCACGTGATGGAACreversecccccCGCATGCCaaaaATGGCACCGTGAGGAACreversecccccCGGATCGCaaaaATGGCAGCGTGATGGGAACreversecccccCGGTGCGCAaaATGGCACCGTGAGGAACreverseccccccGGTGGCGGAGGCGAAAACCCTGTAGTAGCGreversecccccCGGTGGCGGAGGCGAACGTGAAGGAACCreverseccccccGCGTGGGTGGGTGGGTGAGGGATCCGTGinx-1a cDNAforwardccccccGCGGGGTGGGTGGGGACCACCCCGGGGreverseccccccGCGGGGGGGGGGGCCACCGCGGGGGGGCGCCCCGGGGGGG	<i>unc-25(Δ</i> ) promoter	forward	CCCCCGCATGCCTCATTTCGCCCTCGGGGC
nmur-3 promoterforwardccccccCCATGCaacacacgttcaattagttgtgtcaegl-18 basal promoterforwardccccccCCGGGATCCggttcaattagttgtgtcainx-1a cDNAforwardccccccgGATCCGaacatCGGTGATGAGTAACPANX1 cDNAforwardccccccGCATGCaacaatggccaactaggagactcHisCl1forwardccccccGCGATGCCaacaatggccaactaggagactcunc-33c cDNAforwardccccccGGATGCCaacaatgGCaactggcaactagacreverseccccccGCGATGCaacaatgGCaactgGCaactagcaattgunc-33c cDNAforwardccccccGGATGCCaacaatGCAGCGGACFor 3' terminal fusionreverseccccccGGTGACGAAAATGCTTCTATATTATCTGGCGwith GFP, mCherry, orreverseccccccGGTGACGAAAATGCTTCTATATTATCTGGCGinx-1a cDNAforwardccccccGCGTGACGAACGTGAAGTAACCinx-1b cDNAforwardccccccGCGTGGAGCAACCTCAACTGAGCACCCGTGunc-10 cDNAforwardccccccGCGATGGCGACGTGGAGCACTCCCAACTGreverseccccccGCCACCGGTGGTGGTTGAGGAACCTCCAACTGcx36forwardccccccgCCGCGCGGAGCAGCACTCCAACTGinx-1(1~307)forwardccccccgctagcAAAATGCTTCTATATTATCTGGCGinx-1(1~338)forwardccccccgctagcAAAATGCTTCTATATTATCTGGCGinx-1(1~372)forwardccccccgctagcAAAATGCTTCTATATTATCTGGCGinx-1(1~372)forwardccccccgctagcAAAATGCTTCTATATTATCTGGCGinx-1(1~372)forwardccccccgctagcAAAATGCTTCTATATTATCTGGCGinx-1(1~372)forwardccccccgctagcAAAATGCTTCTATATTATCTGGCGinx-1(1~372)forwardccccccgCCCGGTGCTGCTGCATGAGAGACTTCCinx-1(1~372)forwardcccccccCCCCCCCCGGTGACACAGAGACTTGT <th></th> <th>reverse</th> <th>CCCCCCgctagcCTCCAAGGGTCCTCctgaaaatg</th>		reverse	CCCCCCgctagcCTCCAAGGGTCCTCctgaaaatg
reverse egl-18 basal promoterreverse forward ccccccGCAGCCCCGGGATCCggcttcaattagtgacact cccccCCCGGGatagactgtggagacac cccccCCCGGGatagactgtggagacac cccccCCCGGGatagactgtggagacac cccccCCCGGTaGCaaaatggccaactggcaactgg ccaataga cccccCGCATGGtcagaagagatccagaagtctcPANX1 cDNAforward forward cccccCGCATGGtcagaagagaatccagaagtct cccccCGCATGGtcagaagagatccagaagtct cccccCGCATGGtcagaagagatccagaagtct cccccCGCATGGtcagaagagatccagaagtc cccccGGATCGCaaaatggcaactggccaatagc cccccGGATCGCaaaatgGCAGTCGTATGGGAAC reverse cccccCGCATGGtcaggagagtgtgtccaataga cccccGGATCGCaaaatgGCAGTCGTATGGGAAC reverse cccccCGCGTGCGTGAGGAACCTGTAGGCAC reverse cccccCGCGTGGGTGAGGAACGTGAAAAATGCTTCTATATTATCTGGCG reverse cccccCGCGATGGCAAAAATGCTTCTATATTATCTGGCG reverse ccccccGCGATGGCGAACGTGAGGAATCCGTCGATGA reverse ccccccGCGATGGCGAACGTGAGGAATCCGTCG reverse ccccccGCGATGGCGAACGTGAGAAAATGCTTCTATATTATCTGGCG reverse ccccccGCGATGGCGAACGTGGAGAGTGGACCATCC reverse ccccccGCGAGCGGAGAGTGGAGCAGTGGACCATCC reverse ccccccGCTACGCaaaATGGACGATCCGACGA reverse ccccccGCTACGCAGAAAATGCTTCTATATTATCTGGCG reverse ccccccGCTACGCAGAAAATGCTTCTATATTATCTGGCG reverse ccccccGCTACGAAAATGCTTCTATATTATCTGGCG reverse ccccccGCTACGAGAAAATGCTTCTATATTATCTGGCG reverse ccccccACCGGTGTGGCGCTGCTGGAAAAATGCTTCTATATTATCTGGCG reverse ccccccACCGGTGTGGCAGAGAGAGAGAAAAAGCCTGGTG reverse ccccccACCGGTGTGGAGAAAATGCTTCTATATTATCTGGCG reverse ccccccACCGGTGTGGCAGAAAATGCTTCTATATTATCTGGCG reverse ccccccACCGGTGTTGGCAAAAATGCTTCTAAGAAGA cccCCCCCCCCCCCGCGTGTTGGCAGAGAAAATGCTTCTAAGTTAATTGTGGCG reverse ccccccACCGGTGTTGGCATAAAATGCTTCTAAGTTAATTGTGGCG reverse ccccccACCGGTGTGGAAAAATGCTTCTATTATTGTGGCG reverse ccccccACCGGTGTGGAAAAATGCTTCTAAGTTAAGAG ccccccCCCCCCCCCCCCCCCCCCCCCCCCCCCCGCGTGTTGGCATAAAAGCCTTCCTT	<i>nmur-3</i> promoter	forward	ccccccGCATGCaacacacgttcaactcgttg
egl-18 basal promoter   forward   ccccccCGGATCCctccatagtagtagtacattttaaggt     inx-1a cDNA   forward   ccccccCCGGGatagactgtgtggagacac     PANX1 cDNA   forward   ccccccCCGGGatagactgtgtggagacac     PANX1 cDNA   forward   ccccccCCGGGatagactgtgtggagatccagagtct     HisCl1   forward   ccccccCCGGTAGCaaaatggcatcagtgtcaatggcaattgcaatggcaattg     wnc-33c cDNA   forward   ccccccGGTACCtcataggagatccagagtgtgcaatggcaattgcaatggc     wnc-33c cDNA   forward   ccccccGCGATCGCaaaaATGGCAGCGTATGGGAAC     reverse   ccccccGCGTACCtcataggagatcagtgtgtcaatggc     wnc-33c cDNA   forward   ccccccGCGTACCtcataggagatcagtgtgtcaatggc     inx-1a cDNA   forward   ccccccGCGGTGGGGACGGAACGTGAGAAAACCCTGTAGGCA     reverse   ccccccGCGGTGGGGGACCGTCCAACAGTGAGTACC   ccccccGCGGTGGGGAACCTCAAAAATGCTTCTATATTATCTGGCG     inx-10 cDNA   forward   ccccccGCGTGCGGAGAAAAATGCTTCTATATTATCTGGCG     wnc-10 cDNA   forward   ccccccGCGTGCGGAGAAAAATGCTTCTATATTATCTGGCG     inx-1(1~307)   forward   ccccccgccggtGCCTGCGGAGACGGAGCCGACC     inx-1(1~338)   forward   ccccccgccggtGGCGTCTCTGTATATTATCTGGCG     inx-1(1~372)   forward   cccccccccGCGTGCGGCTCTCTAAAAATGCTTCTATATTATCTGGCG     inx		reverse	cccccCCCGGGATCCggcttcaattagttgtgtca
inx-1a cDNAreverse forward ccccccgCCCGGGatagactgtgggagacac ccccccgCTAGCaaaatggcatcgtcaactgg ccccccGCTAGCaaaatggcatcgtcaactgg ccccccGCTAGCaaaatggcatcgaagagaatccagaagtc everse ccccccGCTAGCaaaatggcaagagaatccagaagtct everse ccccccGCTACCtataggacgaagagaccaatgg cccccCGGTCGCaaaatggcaagagaatcagaagatc caaatggcaaggcgcactgccaatagac cccccCGGTCGCaaaatgGCAGTCGCaaaatgGCAGTCGCaaaatg geverse ccccccGCGATCGCaaaaTGGCAGTCGTATGGGAAC reverse ccccccGCGATCGCaaaaTGGCAGTCGTATGGGAAC reverse cccccCGCGTCGCAAAAATGCTTCTATATTATCTGGCG reverse ccccccGCGTGCGCAACAAAATGCTTCTATATTATCTGGCG reverse ccccccGCGTGGCGAAAAATGCTTCTATATTATCTGGCG reverse ccccccGCGTGGCGAAAAATGCTTCTATATTATCTGGCG reverse ccccccGCGCGCGCGCGCGCGCGCGCGCGCGCGCGCGCG	<i>eql-18</i> basal promoter	forward	ccccccGGATCCctccatagtagtacattttaaggt
inx-1a cDNAforwardccccccgctagcAAAAATGCTTCTATATTATCTGGCGPANX1 cDNAforwardccccccGCTAGCaaaaatggcatcgtcaactggPANX1 cDNAforwardccccccGCTAGCaaaatggcatcgtcaactggHisCl1forwardccccccGCATCGCaaaatggcaatgtccaatagaaunc-33c cDNAforwardccccccGCATCGCaaaatGGCAGTGGTAGGAACTGGGAACFor 3' terminal fusionreverseccccccGCATCGCaaaatGGCAGTGGTAGTGGAACreverseccccccGCATCGCaaaatGGCAGTGGAACCreverseccccccGCATCGCaaaaTGGCAGTGAAACCinx-1a cDNAforwardccccccGCGTGCGACGAACGTGAAGTAACCinx-1b cDNAforwardccccccGCGATCGCaaaaTGGACGATCCGTGunc-10 cDNAforwardccccccGCGATCGCaaaaTGGACGACCGTGATGGreverseccccccGCGATCGCaaaaTGGACGACCGTGATGGinx-1(1~307)forwardccccccGCGATCGCaaaaTGGACGACCGGAreverseccccccGCCACCGGTGTGCTGAGGACCATCCreverseccccccGCCACCGGTGTGCTGAGGACCGGAGinx-1(1~307)forwardccccccgctagcAAAAATGCTTCTATATTATCTGGCGreverseccccccgctagcAAAAATGCTTCTATATTATCTGGCGinx-1(1~372)forwardccccccgctagcAAAAATGCTTCTATATTATCTGGCGinx-1(1~372)forwardccccccgCGGTGCTGCTCGAGCACTGTGTGAGAGAGGAGGAGCACTCCegl-36(gf)reverseccccccACCGGTGAAAAATGCTTCTATATTATCTGGCGegl-36(gf)forwardcccccccaccgtGGAAAATGCTCTTACGAGAAGGCFor internal deletion ofinx-1 cDNA	3	reverse	cccccCCCGGGatagactgtgtggagacac
PANX1 cDNAreverse forward ccccccGCTAGCaaaatggccatcgtcaactgg reverse ccccccGCATGCaaaatggccatcgtcaactgg cccccGGTAGCaaaatggcaagaatccagaagtct cccccGGTAGCCaaaatgccaactagcaattg reverse cccccGGTAGCCaaaatgGCAGTGGTAAGAAC reverse cccccGGTAGCCaaaatgGCAGTGGTAGGAAC reverse cccccGGTAGCCaaaatgGCAGTGGTAGGAAC reverse cccccGGTAGCGAAAAATGGCTTCTATATTATCTGGGG inx-1a cDNAforward reverse ccccccGGTAGCGAACAATGGCTGTAAGGAATCGTGG cccccGGGTAGCGAACGTGAAGAAAATGCTTCTATATTATCTGGGG cccccGGGAGGTGGGTGAGGAACGTGGAAGTAACC cccccGGGAGGTGGGTGAGGAGCGTGGAGTAGGGA forward ccccccGGGATGGCAaCGTGGAAGTAGCG cccccGGGAGGTGGGGGGAGCGTGGGAGTGGGGG feverse ccccccGCGGAGCGAAGAAAGGTTCGATG forward ccccccGGGAGCGAGGAGGGGAGCGGAG feverse cccccGCGGAGCGAAGAAAGGCTCGAACGTGAAGG forward ccccccGGGAGCGAGGGGAGGGGAGCATCC reverse ccccccGCGGGGAGCAAGGGGAGGGGGACCATCC reverse ccccccGCGGGGAGCAAGAGGGGAGCGGAG forward ccccccgGtGGCGTGGTGGGGGAGCGGAG forward ccccccgGtGGCGAGGAGGGGGAGCGGAG forward ccccccgGtGGCGAGAAAATGCTTCTATATTATCTGGGG reverse cccccCGCGGGGAAAAATGCTTCTATATTATCTGGGG forward ccccccgGtGGCGAGAAAATGCTTCTATATTATCTGGGG reverse cccccCACGGTGTAGCAAGAATAGCTTCTATATTATCTGGGG forward ccccccgCGGGGAAAAATGCTTCTATATTATCTGGGG reverse cccccCACGGTGTAGCAAGAATAGCTTCTATATTATCTGGGG forward ccccccgCGCGGCGTTCCGACAGAGAATATCTCCAGC forward ccccccGCGGGGAAAAATGGTCTTATATTATCTGGGG feverse cccccCACGGGTGTAGCAAGAATATCTCGAGGAACTGTG forward ccccccgCGCGGCGTTTCCGACGAGAGAATATCTCCAGC forward ccccccGCGCGGGGAATTCTTCGGCGCTCTCCTGCTC feverse feverse forward ccccccGCGGGGAATTCTTCGGCGCTCTCCCCC forward ccccccGCGCGGGGAATTCTTCGGCGCTCTCCTGCTC feverse feverse feverse feverse forward ccccccccGCGGGGAAAATGCTCGCGGGGCTCTTCGGAGAG forward cccccccCCCGCGGGGAATTCTTCGGCGCTCTCCTC feverse feverse forward for	<i>inx-1a</i> cDNA	forward	CCCCCCqctaqcAAAATGCTTCTATATTATCTGGCG
PANX1 cDNAforwardccccccGCTAGCaaaaatggccatcgtcaactggHisCl1reverseccccccGGTACtCaaaaatggcaatccagaagtccHisCl1forwardcccccGGTACtCaaaaatgcaagccaactagcaaattgunc-33c cDNAforwardcccccGGTACtCataggaagtctaatgaaunc-33c cDNAforwardcccccGGTACtCAAAACCCTGTAGGAACreverseCCCCCCgcggccgCTACCAAAACCCTGTAGGGAACreverseccccccGGTACCCAAAACCCTGTAGGCGreverseccccccGGTACCCAAAAACCCTGTAGTGGGGinx-1a cDNAforwardccccccgctagcAAAAATGCTTCTATATTATCTGGCGinx-1b cDNAforwardccccccGCGGTGGTGGTGAGGAAGTGACCGGGGunc-10 cDNAforwardccccccGCGATCGCaaaaATGGAGATGCGCCATCGforwardccccccgCGGTGCGGAGGAGTGGACCATCGcx36forwardccccccgCGGTGCGGAGGAGTCGGACGACCTCCAACTGcx36forwardccccccgCCGCGGAGGGGAGTCGGACGACCGGGGinx-1(1~307)forwardccccccgctagcAAAAATGCTTCTATATTATCTGGCGreverseCCCCCCaccggtGCGCTGCTGGAGAACTGGACinx-1(1~338)forwardccccccgctagcAAAAATGCTTCTATATTATCTGGCGreverseccccccgctagcAAAAATGCTTCTATATTATCTGGCGreverseccccccgctagcAAAATGCTTCTATATTATCTGGCGreverseccccccdCGGTTCTCTGACAAGAATTTCCGAGCinx-1(1~372)forwardccccccdCGGTCTCTGCAAGAAATGCTTCTATATTATCTGGCGreverseccccccdCCGGTCTCTCTGCCegl-36(gf)forwardcccccdCCGGTCTTTTGTGTCTTTACCATGAGGreversecccccdCCGGTCTTTTGTGTCTTTACCATGAGforwardcccccccccccccgtgtGGAAATTTTTGGGTGTAATGGCFor internal deletion ofinx-1 cDNA<		reverse	CCCCCCggtaccTTAGACGAACGTGAAGTAAC
HisCl1reverse forwardccccccGCATGGtcagcaagaagatccagaagtctc cccccGGATCGCaaaatgcaaatgccaatagcaattg reverse cccccGGATCGCaaaatgcaaatgccaatagcaattg reverse cccccGGATCGCaaaatGGCAGTCGTATGGGAAC reverseFor 3' terminal fusion with GFP, mCherry, or linker::GFPccccccGCGCATCGCaaaaTGGCAGTCGTATGGGAAC ccCCCCGgggccgCTACCAAAACCCTGTAGTCGGinx-1a cDNAforward reverseccccccGCGATCGCAACGTGAAGTAACC cccccCGCGTGGGGAACGTGAAGTAACC cccccCGCGTGGGTGGGGGAACGTCCGTTG unc-10 cDNAforward ccccccGCGATCGCaaaATGGACGATCCCTCGATGATG reverse ccccccGCGATCGCaaaATGGACGATCCCTCGATGATG reverse ccccccGCGATCGCaaaATGGACGATCCCTCCAACTG cccccGCGATGGCGAGGACTCCGAGGAGCCCCCACACTG cccccGCTAGCaaaATGGACGCTCCAACTG creverse ccccccgctagcAAAAATGCTTCTATATTATCTGGCG reverse cccccccgctagcAAAAATGCTTCTATATTATCTGGCG reverse cccccccCCCCCcccggtGCCGTGCTGAGGAACTTGTG inx-1(1~338)forward reverse cccccccCCCCCcccggtGCCTCCAACTGG reverse ccccccCCCCCCcccggtGCCTCCTAATATTATCTGGCG reverse ccccccCCCCCCcccggtACAAAATGCTTCTATATTATCTGGCG reverse ccccccCCCCCCcccggtACCATCCAACTGG inx-1(1~372)forward ccccccgCCCCCCcccggtACCATCCTAAATATCCCAGC reverse ccccccACCGGTCTTCCTGACACGATCATTGAAG ecccccCCCCCCCCCCCCGCGTCTCCTGACACGATCATTGAAG ecccccCCCCCCCCCCCCCCGCTCCTCTAATATTATCTGGCG reverse ccccccACCGGTCTTCCTGACACGATCATTGAAG eccccCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCC	PANX1 cDNA	forward	ccccccGCTAGCaaaaatggccatcgctcaactgg
HisCl1forward reverseccccccGCGATCGCaaaaatgcaaagcccaactagcaattg cccccGGTACCtcataggacgttgtccaatagac cccccGGTACCtataggacgttgtccaatagac cccccGGTACCCaaaaATGGCAGTCGTATGGGAAC reverseFor 3' terminal fusion with GFP, mCherry, or linker::GFPforward ccccccGCGGTGCGAAAAATGCTTCTATATTATCTGGCG reverseinx-1a cDNAforward reverseccccccqCGGTGACGAACGTGAAGAAACC ccccccACCGGTGACGAACGTGAAGAACC ccccccACCGGTGACGAACGTGAAGAACC inx-1b cDNAinx-1b cDNAforward reverseccccccqCGGTGGTGAGGAACGTCGTGG ccccccGCGATCGCaaaaATGGACGATCCGTTG ccccccGCGATCGCaaaATGGACGATCCGTCG reverseunc-10 cDNAforward reverseccccccGCGATCGCaaaaATGGAGAGGACCATCC reversecx36forward ccccccggtGagCTGCTGCGGAGGAGCGACCCCAACTG reverseccccccggtGaaaATGGGAGAGGAGCGACCCC reverseinx-1(1~307)forward ccccccggtagcAAAAATGCTTCTATATTATCTGGCG reverseccccccggtagcAAAAATGCTTCTATATTATCTGGCG reverseinx-1(1~338)forward ccccccggtagcAAAAATGCTTCTATATTATCTGGCG reverseccccccggtagcAAAAATGCTTCTATATTATCTGGCG reverseinx-1(1~372)forward ccccccgctagcAAAAATGCTTCTATATTATCTGGCG reverseccccccGCTAGCaaaATGGTCAACGATCATTATC reverseegl-36(gf)forward forwardcccccCGCTAGCaaaATGCTCGTCTTTGGATC reverseFor internal deletion of inx-1 cDNAforward inx-1 cDNA		reverse	cccccCCATGGtcagcaagaagaatccagaagtctc
unc-33c cDNAreverse forward reverseccccccGGTACCtcataggaacgttgtccaatagac cccccGGATCGCaaaATGGCAGTCGTATGGGAAC reverseFor 3' terminal fusion with GFP, mCherry, or linker::GFPccccccgctagcAAAAATGCTTCTATATTATCTGGCG reverseinx-1a cDNAforward reverseccccccgctagcAAAAATGCTTCTATATTATCTGGCG reverseinx-1b cDNAforward reverseccccccgCGTGGTGAGGAGGATCCCGTGGunc-10 cDNAforward reverseccccccGCGTGGTGGTGGGGAGGACCGTGGTGGTGGTGGAGGAGCGATCC reverseccccccGCTAGCaaaATGGAGGATCCCGTCGATGATG reverseccccccGCTAGCaaaATGGAGGAGCGACCATCC reverseccccccGCTAGCaaaATGGGAGAGGAGCGACCATCC reverseccccccggtGCGCTGCTGAGGAGCGAGGAG ccccccggtGGCGCTGCTGCTGTAAGAAAG ccccccggtGGCGCTGCTGTCTGTATATTATCTGGCG reverseinx-1(1~307)forward ccccccggtGGCGCTGCGGAGGAGGAGGAGGAGGAG reverseinx-1(1~307)forward ccccccggtGGCGCTGCTGTGTAAGAAAG forward ccccccggtGGCGCTGCTGCTGTAAGAAAG inx-1(1~356)forward ccccccgctagcAAAAATGCTTCTATATTATCTGGCG reverse ccccccACCGGTCTTCCTGACAAAATGCTTCTATATTATCTGGCG reverse ccccccACCGGTCTTCCTGACAGAGAATATCTCCCAGC forward ccccccGCTAGCAaAAATGCTTCTATATTATCTGGCG reverse ccccccACCGGTCTTCCTGACACGATCATTGAAG ebp-1 cDNA forwardforward cccccGCTAGCaaaATGCTCGACGCTTCTGGACTCTCTGGCTTC reverse cccccCACCGGTCTTCTGGAATAATGGCTACAAGAGAG forward AGACACACAAAAAGCCCTTGCTGTTTTGGATC reverse ccccccaccggtGGAATTATTGTGGTGCTTTAGCATGAG forward AGACACACAAAAAGCCCTTGCTGTTTTGGATC reverse ccccccaccggtGGAATTATTGTGGTGTTTTGGATC reverse cccccccaccggtGGAATTATTGTGGTGTTTTGGATC reverse ccccccaccggtGGAATTATTGTGGTGTTTTGGATC reverse ccccccaccggtGGAATTATTGTGGTGTTTTGGATC	HisCl1	forward	cccccGCGATCGCaaaaatgcaaagcccaactagcaaattg
unc-33c cDNAforward reverseccccccGCGATCGCaaaATGGCAGTCGTATGGGAAC reverseFor 3' terminal fusion with GFP, mCherry, or linker::GFPccccccgctagcAAAAATGCTTCTATATTATCTGGCG reverseinx-1a cDNAforward reverseccccccgCtagcAAAAATGCTTCTATATTATCTGGCG ccccccACCGGTGGCGAACGTGAAGTAACCinx-1b cDNAforward reverseccccccGCGATCGCGAAGGGATTCCGTTG cccccCGCGATCGCGAAGGGACCTCCAACTGunc-10 cDNAforward reverseccccccGCGATCGCaaaATGGAGACTCCGTCGATGATG cccccGCGATCGCGAGGACCTCCAACTGCX36forward ccccccgcggcgcgCTGCTGAGCAGCCTCCAACTG reverseccccccggtGACGTAGGAGAGTGGACCATCC reverseccccccggtGACGTAGCGAGAGTGGACATCC reverseccccccggtGACGTAGCGGAGGGGAG cCCCCCCaccggtGCTGCTGCTGGTAGAAAG ccccccgctagcAAAATGCTTCTATATTATCTGGCG reverseinx-1(1~307)forward cccccccgctagcAAAATGCTTCTATATTATCTGGCG reverseinx-1(1~338)forward cccccccgctagcAAAATGCTTCTATATTATCTGGCG reverseinx-1(1~372)forward cccccccCCCCCCcccggtGAAAATGCTTCTATATTATCTGGCG reverse ccccccACCGGTGTAGCAAGAATATCTCCAGCegl-36(gf)forward forward cccccccaCccgctagcAAAATGCTCCGACGATCATGAAG AGACACAAAAAGACCCTTGCTGTTTTGGATC reverse ccccccACCGGTGAATCTTCGGCTGTCTGGATATGGCFor internal deletion of inx-1 cDNAforward inx-1 cDNA		reverse	ccccccGGTACCtcataggaacgttgtccaatagac
reverseCCCCCCgcgggcgcgcCTACCAAAACCCTGTAGTCCGFor 3' terminal fusion with GFP, mCherry, or linker::GFPccccccgctagcAAAAATGCTTCTATATTATCTGGCGinx-1a cDNAforward reverseccccccqCtagcAAAAATGCTTCTATATTATCTGGCGinx-1b cDNAforward reverseccccccdCCGGTGGTGGTGAGGAGTACCunc-10 cDNAforward reverseccccccGCGATCGCaaaATGGACGACCGTCGATGATGcx36forward reverseccccccgtagcAAAAATGCTTCTATATTATCTGGCGreversecccccccGCGTAGCaaaATGGAGAGCACCCCCAACTGcx36forward reverseccccccggtgCGCGTGCTGGCGAGGAGCCACCCreverseCCCCCCaccggtGACGTAGCGAGCGAGCinx-1(1~307)forward reverseccccccgtagcAAAAATGCTTCTATATTATCTGGCGinx-1(1~338)forward reverseccccccgtagcAAAAATGCTTCTATATTATCTGGCGinx-1(1~356)forward reverseccccccgtagcAAAAATGCTTCTATATTATCTGGCGinx-1(1~372)forward reverseccccccdCCGGTTGTAGCAAGAAGACTCTGTGinx-1(1~372)forward reverseccccccACCGGTCTTCTGAGCAGTAATGGCebp-1 cDNAforward reversecccccCACCGGTCAAAAATGCTTCTATATTATCTGGCGreverse reversecccccCACCGGTGAATCTTGGCTCTGCTC reversecccccCACCGGTGAAAAATGCTTCTAGAGAGAAGAegl-36(gf)forward reverseccccccaccggtGGAAATTCTTGGGTGGTAAGAGforward reverseccccccaccggtGGAAAATGCTTCTGGTGTTTGGATC reverseccccccaccggtGGAAATTCTTGGGTGGTAAGAGforward reverseccccccccccccccgtgtGGAAATTCTTGGCTCCTC reversecccccccccccCCCGGTGGTTTGGTGTTTGGATC reversereverse reverseccccccccccccccccgtgtGGAAATTCTTGGCTCCCCCCCCCCCCCCCCCCCCCCCC	unc-33c cDNA	forward	CCCCCGCGATCGCaaaaATGGCAGTCGTATGGGAAC
For 3' terminal fusion with GFP, mCherry, or linker::GFPCccccccgctagcAAAAATGCTTCTATATTATCTGGCG reverse ccccccACCGGTGACGAAGTGAAGCA ccccccGCGTGGTGAGGAAGTGAACC inx-1b cDNAinx-1a cDNAforward reverse cccccccACCGGTGGTGGTGAGGAGTAACC reverse ccccccCGCGACGGTGGTGGAGGAGTCCGTGG reverse ccccccGCGATCGCaaaAATGGAGCATCCCGTGG reverse ccccccGCGACGGTGGTGAGGAGGGGACCATCC reverse cccccccgctagcAAAAATGCTTCTATATTATCTGGCG reverse ccccccgggcgcCTGCTGAGCACCTCCAACTG ccccccgctagcAAAAATGCTTCTATATTATCTGGCG reverse cccccccgctagcAAAAATGCTTCTATATTATCTGGCG reverse cccccccgctagcAAAAATGCTTCTATATTATCTGGCG reverse cccccccgctagcAAAAATGCTTCTATATTATCTGGCG reverse cccccccdCCGGTGTGAGCAAGAAGATATCTCCAGC inx-1(1~338)forward inx-1(1~356)forward ccccccgctagcAAAAATGCTTCTATATTATCTGGCG reverse ccccccACCGGTTGTAGCAAGAATATCTCCAGC inx-1(1~372)forward cccccccACCGGTCTCTCTGGCTCTTATATTATCTGGCG reverse ccccccACCGGTCTCTGGCAACAATGCTTCTATATTATCTGGCG reverse ccccccACCGGTCTTCCTGACACGATCATTGAG ccccccACCGGTCTTCTGGCTCTTACGTAGTAATG reverse ccccccACCGGTCTTCTGGCGTCTTGGCG reverse ccccccACCGGTCATCCGGCGTCTTTGGCTCTTAGCATGAGA forward ccccCCCCCGCCGGCGAATTCTTCGGCGCTCCTGCTC reverse ccccccACCGGTGAATTCTTCGGCGCTCCTGCTC reverse ccccccACCGGTGAATTCTTGGTGCTTTAGCATGAAG forward ccccCCCCGCCGCGGCAAAAATGCCCTTGCTGCTC reverse ccccccCCCCGCGGCAAAAAGCCCTTGCTGTCTTAGCATGAAG forward ccccccccccccggtGGAAATATTTGTGGTGCTTTAGCATGAAG forward ccccccccccccggtGGAAATTATTGTGGTGCTTTAGCATGAAG forward ccccccccccccggtGGAAATTATTGTGGTGGTAATGGCFor internal deletion of inx-1 cDNAForward inx-1 cDNA		reverse	CCCCCCqcqqccqcCTACCAAAACCCTGTAGTCCG
with GFP, mCherry, or linker::GFPinx-1a cDNAforwardccccccqctagcAAAAATGCTTCTATATTATCTGGCGinx-1b cDNAforwardccccccqctagcAAAAATGCTTCTATATTATCTGGCGunc-10 cDNAforwardccccccGCGTGCGGTGGGGAGCGACGACGTGGATGunc-10 cDNAforwardccccccGCGTCGCGAGCGACCTCCAACTGCx36forwardccccccGCTAGCaaaATGGAGAGTGGACCATCCreversecCCCCCCaccggtGACGTAGGCGAGTCGGAGinx-1(1~307)forwardccccccgctagcAAAAATGCTTCTATATTATCTGGCGinx-1(1~338)forwardccccccgctagcAAAAATGCTTCTATATTATCTGGCGinx-1(1~356)forwardccccccgctagcAAAAATGCTTCTATATTATCTGGCGinx-1(1~372)forwardcccccccdCGGTGTGTGCCTGGACACATGTGinx-1(1~372)forwardccccccdCCGGTCTTCCTGACAGAAATGCTTCTATATTATCTGGCGreverseccccccdCCGGTCTTCCTGACCAGTAGTAAGAebp-1 cDNAforwardcccccCCCCGccgGGGAATTCTTGGCTTCTATATTATCGGCGreversecccccCCCGCGGGGAATTCTTCGGCTCTCTGCTCegl-36(gf)forwardccccccaccggtGGAAAAATGCCTTGTAGCAAGAGforwardAGACACACAAAAGACCCTTGCTGTTTTGGGTGCTTTAGCATGAAGforwardAGACACACAAAAAGACCCTTGCTGTTTTGGGTGCTTTAGCATGAGforwardAGACACACAAAAAGACCCTTGCTGCTTTTGGGTGGTAATGCreverseccccccaccggtGGAAATATTTGGGTGGTAATGCCforwardAGACACACAAAAAGACCCTTGCTGCTTTAGCATGAAGforwardAGACACACAAAAAGACCCTTGCTGGTAATGGCFor internal deletion ofinx-1 cDNA	For 3' terminal fusion		
Inker::GFPinx-1a cDNAforwardccccccccccccccccccccccccccccccccc	with GFP, mCherry, or		
inx-1a cDNAforwardccccccgctagcAAAATGCTTCTATATTATCTGGCG reverseinx-1b cDNAforwardccccccgctagcAAAATGCTTCTATATTATCTGGCG reverseunc-10 cDNAforwardccccccGCGTGCGAGGACGTCCCATCGATGATG reversecCx36forwardccccccGCTAGCaaaATGGACGATCCGACGAC reverseinx-1(1~307)forwardccccccgctagcAAAAATGCTTCTATATTATCTGGCG reverseinx-1(1~338)forwardccccccgctagcAAAAATGCTTCTATATTATCTGGCG reverseinx-1(1~356)forwardccccccgctagcAAAAATGCTTCTATATTATCTGGCG reverseinx-1(1~372)forwardccccccgctagcAAAAATGCTTCTATATTATCTGGCG reversecccccdgtagcAAAAATGCTTCTATATTATCTGGCG reversecccccgctagcAAAAATGCTTCTATATTATCTGGCG reverseinx-1(1~372)forwardcccccgctagcAAAAATGCTTCTATATTATCTGGCG reversecccccdgtagcAAAAATGCTTCTAACAAGA reversecccccgctagcAAAAATGCTTCTATATTATCTGGCG reversecccccdgtagcAAAAATGCTTCTATATTATCTGGCG reversecccccgctagcAAAATGCTTCTATATTATCTGGCG reversecccccdgtagcAAAATGCTTCTATATTATCTGGCG reversecccccgctagcAAAATGCTTCTATATTATCTGGCG reversecccccdcCGGTGTTCCTGACACGGTCTTTAAGA reversecccccgctagcAAAATGCTCCAACGATCATGAAG reverseebp-1 cDNAforwardCCCCCgctagcAAAATGGCTCTTAGCATGAAG reverseegl-36(gf)forwardccccCCCCGCGGGAATTCTTGGGTGCTTAGCAAGA reverseFor internal deletion of inx-1 cDNAforward	linker::GFP		
InterfactionInterfactorinx-1a cDNAforwardccccccdCCGGTGACGAACCTGAAGTAACCinx-1b cDNAforwardccccccdCCGTGTGGTTGAGGGATTCCGTTGunc-10 cDNAforwardccccccdCCGCGACGCGAAAAATGCTCCAACTGCx36forwardccccccdCCGCTAGCaaaaATGGAAGATGCACCATCCreversecCCCCCaccggtGACGTAGCGAGGAGTGGACCATCCreverseCCCCCCaccgtGTGTGTGTCTTATATTATCTGGCGinx-1(1~307)forwardccccccgctagcAAAATGCTTCTATATTATCTGGCGinx-1(1~338)forwardccccccgctagcAAAATGCTTCTATATTATCTGGCGinx-1(1~356)forwardccccccgctagcAAAATGCTTCTATATTATCTGGCGinx-1(1~372)forwardccccccgctagcAAAATGCTTCTATATTATCTGGCGinx-1(1~372)forwardccccccgctagcAAAATGCTTCTATATTATCTGGCGegl-36(gf)forwardccccccdCGGTGTACCAGGATCATTGAAGforwardccccccdCCGGTGAAAAATGCTCCTTTAGCATGAAGAreverseccccccdCCGGTGTACCAGGATCATTGAAGreverseccccccdCCGGTGTACCAGGATCATTGAAGreverseccccccdCCGGTGTATCAAGTAATGGCTGTCTreverseccccccdCCGGTGTATCAAGTAGTTAATGreverseccccccdCGGTGAATTCTTCGGCTGTCCegl-36(gf)forwardccccccdccggtGGAAATTCTTGGGTGGTAATGGCFor internal deletion ofinx-1 cDNA	inv-1a cDNA	forward	CCCCCCCCCT ACCAAAAATGCTTCTATATTATCTGCCG
inx-1b cDNAforwardccccccgctagcAAAAATGCTTCTATATTATCTGGCGunc-10 cDNAforwardccccccGCGATCGCaaaAATGGACGATCCGTCGATGATGunc-10 cDNAforwardccccccGCGATCGCaaaATGGACGATCCGTCGATGATGcx36forwardccccccGCTAGCaaaATGGGAGACCATCCreverseCCCCCCaccggtGACGTAGGCGAGACCGGAGinx-1(1~307)forwardccccccgctagcAAAAATGCTTCTATATTATCTGGCGreverseCCCCCCaccggtGTGCTGCTGGTAAGAAAGinx-1(1~338)forwardccccccgctagcAAAAATGCTTCTATATTATCTGGCGinx-1(1~356)forwardccccccgctagcAAAAATGCTTCTATATTATCTGGCGinx-1(1~372)forwardccccccgctagcAAAAATGCTTCTATATTATCTGGCGinx-1(1~372)forwardccccccgctagcAAAAATGCTTCTATATTATCTGGCGebp-1 cDNAforwardcccccCCCCGGTGTAACAAATGGCTACTAAGTAGTAATGreversecccccCACCGGTGAATCTTCGGCTTCTGCTCegl-36(gf)forwardccccccaccggtGGAAATTCTTCGGCTCCTGTCFor internal deletion ofinx-1 cDNA		reverse	
Inx ho optioninvaluecoccector opticationunc-10 cDNAforwardccccccCGGTGTGGTGAGGAGTCCGTTGunc-10 cDNAforwardccccccGCATCGCaaaaATGGACGATCCGTCGATGATGcx36forwardccccccGCTAGCaaaaATGGAGAGTGGACCATCCreverseCCCCCCaccggtGACGTAGGCGAGTCGGAGinx-1(1~307)forwardccccccgctagcAAAAATGCTTCTATATTATCTGGCGreverseCCCCCCaccggtGTGCTGTCCTGGTAAGAAAGinx-1(1~338)forwardccccccgctagcAAAAATGCTTCTATATTATCTGGCGinx-1(1~356)forwardccccccgctagcAAAAATGCTTCTATATTATCTGGCGinx-1(1~372)forwardccccccgctagcAAAAATGCTTCTATATTATCTGGCGebp-1 cDNAforwardccccccdCCGGTGAAAAATGCTTCTATATTATCAGCGegl-36(gf)forwardcccccGCTAGCaaaATGCTCGACGATCATGAAGforwardccccCGCTAGCaaaATGCTCGACGGTCCTGTCreversecccccCACCGGTCTTTGTGGTGCTCTTGGATCreverseccccccACCGGTCAGCAAAAATGGCTTCTGGCTCreverseccccccACCGGTCAAGTAATCGCCTCGTCCreversecccccCACCGGTCTTTGTGGTGCTTTAGCATGAAGinx-1(1~372)forwardcccccCACCGGTGAATTCTCGGCTACTGGCCreversecccccCACCGGTGAATTCTCGGCTACTGGCCreverseccccCCCCCCCCCCCGCTGAAAAATGGCCTTCTGCTCreversecccccCCCCCCGTAGCAaaATGCTCGACGCGTGCTCCTGCTCreverseccccccCCCCCCCCCGTGCTGTTTGGATCreverseccccccCCCCCCCCCGTGCTGTTTTGGGTGGTAATGGCFor internal deletion ofinx-1 cDNA	inx-1h cDNA	forward	
unc-10 cDNAforwardccccccGCGATCGCaaaATGGACGATCCGTCGATGATGcx36forwardccccccGCTAGCaaaATGGAGAGTGGACCATCCreverseCCCCCCaccggtGACGTAGGCGAGTCGGAGinx-1(1~307)forwardccccccgctagcAAAAATGCTTCTATATTATCTGGCGinx-1(1~338)forwardccccccggtagcAAAAATGCTTCTATATTATCTGGCGinx-1(1~356)forwardccccccggtagcAAAAATGCTTCTATATTATCTGGCGinx-1(1~372)forwardccccccggtagcAAAAATGCTTCTATATTATCTGGCGinx-1(1~372)forwardccccccggtagcAAAAATGCTTCTATATTATCTGGCGebp-1 cDNAforwardccccccgctagcAAAAATGCTTCTATATTATCTGGCGegl-36(gf)forwardccccccGCTAGCAaaAATGCTCGACGAGTCTTCGACGFor internal deletion ofinx-1 cDNA		reverse	
Internal deletion ofIonwardCccccccgcggccgcCTGCTGAGCACCTCCAACTGreversecccccccGCTAGCaaaaATGGGAGAGTGGACCATCCreverseCCCCCCaccggtGACGTAGGCGAGAGGTCGGAGinx-1(1~307)forwardccccccgctagcAAAAATGCTTCTATATTATCTGGCGinx-1(1~338)forwardccccccgctagcAAAAATGCTTCTATATTATCTGGCGinx-1(1~356)forwardccccccgctagcAAAAATGCTTCTATATTATCTGGCGinx-1(1~372)forwardccccccgctagcAAAAATGCTTCTATATTATCTGGCGinx-1(1~372)forwardccccccdCCGGTGTAGCAAGAATATCTCCAGCreverseccccccdCCGGTCTTCCTGACACGATCATTGAAGebp-1 cDNAforwardcccccCCCCGCTAGCaaaATGCTCGACGATGTTCAAGTAATGreverseccccccCACCGGTGAATCTTCGGCTTCegl-36(gf)forwardccccccaccggtGGAAATTATTGTGGTGTCTTTAGAAGforwardAGACACACAAAAAGACCCTTGCTGTTTTGGATCreverseccccccaccggtGGAAATTATTGTGGTGGTAATGGCFor internal deletion ofinx-1 cDNAforward	unc-10 cDNA	forward	
Cx36forwardccccccGCTAGCaaaaATGGGAGAGTGGACCATCC reverseinx-1(1~307)forwardccccccgctagcAAAAATGCTTCTATATTATCTGGCGinx-1(1~338)forwardccccccgctagcAAAAATGCTTCTATATTATCTGGCGinx-1(1~338)forwardccccccgctagcAAAAATGCTTCTATATTATCTGGCGinx-1(1~356)forwardccccccgctagcAAAAATGCTTCTATATTATCTGGCGinx-1(1~372)forwardccccccgctagcAAAAATGCTTCTATATTATCTGGCGinx-1(1~372)forwardccccccgctagcAAAAATGCTTCTATATTATCTGGCGebp-1 cDNAforwardccccccdCCGGTGAATTCTCGACCAGATCATTGAAGebp-1 cDNAforwardcccccCCCGGTGAATTCTTCGGCTTCTGTCegl-36(gf)forwardcccccccaccggtGGAAATTCTTCGACGGTGTCCTGAAGFor internal deletion of inx-1 cDNAccccccaccggtGGAAATTATTGTGGTGGTAATGGC		reverse	
inx-1(1~307)forwardCCCCCCaccggtGACGTAGGCGAGTCGGAGinx-1(1~338)forwardccccccgctagcAAAAATGCTTCTATATTATCTGGCGinx-1(1~338)forwardccccccgctagcAAAAATGCTTCTATATTATCTGGCGinx-1(1~356)forwardccccccgctagcAAAAATGCTTCTATATTATCTGGCGinx-1(1~372)forwardccccccgctagcAAAAATGCTTCTATATTATCTGGCGinx-1(1~372)forwardcccccccccCGGTGTACAACGATCATTGAAGebp-1 cDNAforwardCCCCCCgctagcAAAAATGGCTTCTATATTATCTGGCGegl-36(gf)forwardcccccCGCTAGCaaaATGCTCGACGGTGCTCGTCFor internal deletion of inx-1 cDNAcccccccaccggtGGAAATTATTGTGGGTGGTAATGGC	Cx36	forward	CCCCCCGCTAGCaaaaATGGGAGAGTGGACCATCC
inx-1(1~307)forwardccccccgctagcAAAAATGCTTCTATATTATCTGGCGinx-1(1~338)forwardccccccgctagcAAAAATGCTTCTATATTATCTGGCGinx-1(1~356)forwardccccccgctagcAAAAATGCTTCTATATTATCTGGCGinx-1(1~372)forwardccccccgctagcAAAAATGCTTCTATATTATCTGGCGinx-1(1~372)forwardccccccgctagcAAAAATGCTTCTATATTATCTGGCGebp-1 cDNAforwardcccccccGGTGACCGGTCTTCCTGACACGATCATTGAAGebp-1 cDNAforwardcccccCCCCCGGTGAAAAATGGCTATCAAGTAGTTAATGreverseccccccCACCGGTGAATTCTTCGGCTTCTGCTCegl-36(gf)forwardccccCCCCCGCTGTGTGTGTGTGTGTCTTTGGATCFor internal deletion of inx-1 cDNAforwardcccccccaccggtGGAAATTATTGTGGTGGTAATGGC	0,00	reverse	
ink 1(1-001)IdikitaCCCCCaccggtGTGCTGTCTGGTAAGAAGinx-1(1~338)forwardccccccgctagcAAAAATGCTTCTATATTATCTGGCGinx-1(1~356)forwardccccccgctagcAAAAATGCTTCTATATTATCTGGCGinx-1(1~372)forwardccccccgctagcAAAAATGCTTCTATATTATCTGGCGebp-1 cDNAforwardccccccGCGTGGAATCATCGGCTCTGCTGAGAAGGegl-36(gf)forwardccccccccccgctagcAAAAATGCTCTGACGGTGCTCTGTCFor internal deletion ofinx-1 cDNA	inx-1(1~307)	forward	CCCCCCGCtagCAAAAATGCTTCTATATTATCTGGCG
inx-1(1~338)forwardccccccgctagcAAAAATGCTTCTATATTATCTGGCGinx-1(1~356)forwardccccccgctagcAAAAATGCTTCTATATTATCTGGCGinx-1(1~372)forwardccccccgctagcAAAAATGCTTCTATATTATCTGGCGebp-1 cDNAforwardccccccdCCGGTGAATGGCTATCAAGTAGTAATGegl-36(gf)forwardcccccdCCGGTCTTCTGTGTGCTCTGCTCegl-36(gf)forwardccccccdcCCGGTCTTCTTTGGGTGGTGAAGAFor internal deletion ofinx-1 cDNAinx-1 cDNA		reverse	CCCCCCaccggtGTGCTGTCCTGGTAAGAAAG
intractreverseCCCCCCaccgtATCATATCCGAGGAACTTGTGinx-1(1~356)forwardccccccqctagcAAAAATGCTTCTATATTATCTGGCGinx-1(1~372)forwardccccccqctagcAAAAATGCTTCTATATTATCTGGCGebp-1 cDNAforwardCCCCCCgctagcAAAAATGGCTACAAGTAGTTAATGedp-1 cDNAforwardCCCCCCgctagcAAAAATGGCTATCAAGTAGTTAATGegl-36(gf)forwardccccGCTAGCaaaaATGCTCGACGGTGCTCGTTCFor internal deletion ofinx-1 cDNA	inx-1(1~338)	forward	CCCCCCGCtagCAAAAATGCTTCTATATTATCTGGCG
inx-1(1~356)forwardccccccgctagcAAAAATGCTTCTATATTATCTGGCGinx-1(1~372)forwardccccccgctagcAAAAATGCTTCTATATTATCTGGCGebp-1 cDNAforwardccccccCGGTCTCCTGACACGATCATTGAAGebp-1 cDNAforwardCCCCCCgctagcAAAAATGGCTATCAAGTAGTTAATGreverseccccccACCGGTGAATTCTTCGGCTTCTGCTCegl-36(gf)forwardccccCGCTAGCAAAAAGCCCTTGCTGTGTGTGTCTTTAGCATGAAGFor internal deletion ofinx-1 cDNA		reverse	
inx 1(1~000)   intrail   intrail   reverse   ccccccACCGGTTGTAGCAAGAATATCTCCAGC     inx-1(1~372)   forward   ccccccgctagcAAAAATGCTTCTATATTATCTGGCG     ebp-1 cDNA   forward   CCCCCCgctagcAAAAATGGCTATCAAGTAGTTAATG     egl-36(gf)   forward   cccccGCTAGCAGAATTCTTCGGCTCTCGTC     egl-36(gf)   forward   cccccCCCCGGTGTCTTGTGTGTCTTTAGCATGAAG     For internal deletion of   inx-1 cDNA	inx-1(1~356)	forward	
inx-1(1~372)   forward   ccccccgctagcAAAAATGCTTCTATATTATCTGGCG     ebp-1 cDNA   forward   CCCCCCgctagcAAAAATGGGCTATCAAGTAGTTAATG     egl-36(gf)   forward   ccccGCTAGCaaaaATGCTCGACGGCGTCTCTGTC     egl-36(gf)   forward   ccccGCTAGCAAAAAGCCCTTGCTGAAG     For internal deletion of   inx-1 cDNA   inx-1 cDNA		reverse	CCCCCCACCGGTTGTAGCAAGAATATCTCCAGC
ebp-1 cDNA   reverse   ccccccACCGGTCTTCCTGACACGATCATTGAAG     egl-36(gf)   forward   CCCCCCGctagcAAAAATGGGCTATCAAGTAGTTAATG     reverse   cccccCACCGGTGAATTCTTCGGCTTCTGCTC     egl-36(gf)   forward   ccccGCTAGCaaaaATGCTCGACGCGTGCTCGTTC     reverse   GCAAGGGTCTTTTGTGTGTCTTTAGCATGAAG     forward   AGACACACAAAAGACCCTTGCTGTTTTGGATC     reverse   cccccccaccggtGGAAATTATTGTGGTGGTAATGGC     For internal deletion of   inx-1 cDNA	inx-1(1~372)	forward	CCCCCCGCtagCAAAAATGCTTCTATATTATCTGGCG
ebp-1 cDNA   forward   CCCCCCgctagcAAAAATGGGCTATCAAGTAGTTAATG     egl-36(gf)   forward   ccccGCTAGCaaaaATGCTCGACGCGTGCTCGTC     egl-36(gf)   forward   ccccGCTAGCaaaaATGCTCGACGCGTGCTCGTCC     reverse   GCAAGGGTCTTTTGTGTGTCTTTAGCATGAAG     forward   AGACACACAAAAAGACCCTTGCTGTTTTGGATC     reverse   ccccccccccggtGGAAATTATTGTGGGTGGTAATGGC     For internal deletion of   inx-1 cDNA		reverse	CCCCCCACCGGTCTTCCTGACACGATCATTGAAG
reverse   ccccccACCGGTGAATTCTTCGGCTTCTGCTC     egl-36(gf)   forward   ccccGCTAGCaaaaATGCTCGACGCGTGCTCGTTC     reverse   GCAAGGGTCTTTTGTGTGTCTTTAGCATGAAG     forward   AGACACACAAAAGACCCTTGCTGTTTTGGATC     reverse   ccccccaccggtGGAAATTATTGTGGGTGGTAATGGC     For internal deletion of inx-1 cDNA   inx-1	<i>ebp-1</i> cDNA	forward	CCCCCCgctagcAAAAATGGGCTATCAAGTAGTTAATG
egl-36(gf)   forward   ccccGCTAGCaaaaATGCTCGACGCGTGCTCGTTC     reverse   GCAAGGGTCTTTTGTGTGTCTTTAGCATGAAG     forward   AGACACACAAAAGACCCTTGCTGTTTTGGATC     reverse   ccccccaccggtGGAAATTATTGTGGGTGGTAATGGC     For internal deletion of inx-1 cDNA   inx-1	<i>i</i> -	reverse	CCCCCCACCGGTGAATTCTTCGGCTTCTGCTC
reverse GCAAGGGTCTTTTGTGTGTCTTTAGCATGAAG forward AGACACACAAAAGACCCTTGCTGTTTTGGATC reverse ccccccaccggtGGAAATTATTGTGGTGGTAATGGC For internal deletion of inx-1 cDNA	eql-36(qf)	forward	CCCCGCTAGCaaaaATGCTCGACGCGTGCTCGTTC
forward AGACACACAAAAGACCCTTGCTGTTTTGGATC reverse ccccccaccggtGGAAATTATTGTGGTGGTAATGGC For internal deletion of <i>inx-1</i> cDNA		reverse	GCAAGGGTCTTTTGTGTGTCTTTAGCATGAAG
reverse ccccccccggtGGAAATTATTGTGGTGGTAATGGC For internal deletion of <i>inx-1</i> cDNA		forward	AGACACAAAAAGACCCTTGCTGTTTTGGATC
For internal deletion of inx-1 cDNA		reverse	cccccaccqqtGGAAATTATTGTGGTGGTAATGGC
inx-1 cDNA	For internal deletion of		
	inx-1 cDNA		
<i>inx-1(1~307)</i> forward ccccccgctagcAAAAATGCTTCTATATTATCTGGCG	inx-1(1~307)	forward	CCCCCCGCtagCAAAAATGCTTCTATATTATCTGGCG
reverse CCCCCCaccggttctagaGTGCTGTCCTGGTAAGAAAG		reverse	CCCCCCaccggttctagaGTGCTGTCCTGGTAAGAAAG
inx-1a(339~428)::GFP forward CCCCCCtctagaGGAGTGTTTTGTATGAGAATGATTTCG	inx-1a(339~428)::GFP	forward	CCCCCCtctagaGGAGTGTTTTGTATGAGAATGATTTCG
reverse ggggggggtaccttaTTTGTATAGTTCATCCATGCC		reverse	qqqqqqqqtaccttaTTTGTATAGTTCATCCATGCC
inx-1a(357~428)::GFP forward ccccctctagaGAACTAATTGTTGCTCTGTGGC	inx-1a(357~428)::GFP	forward	ccccctctagaGAACTAATTGTTGCTCTGTGGC
reverse ggggggggtaccttaTTTGTATAGTTCATCCATGCC		reverse	ggggggggtaccttaTTTGTATAGTTCATCCATGCC

Table S2. Cont.		
Sequence		Oligos
For internal GFP		
fusion to <i>inx-1a</i> cDNA		
GFP(TFV)	forward	CCCCCCCCGCGTAGTAAAGGAGAAGAACTT
	reverse	CCCCCCaCgCgtTTTGTATAGTTCATCCATGCC

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.

# SI References

- 1. Jorgensen EM & Mango SE (2002) The art and design of genetic screens: Caenorhabditis elegans. *Nature Reviews Genetics* 3(5):356-369.
- 2. Davis MW, *et al.* (2005) Rapid single nucleotide polymorphism mapping in C. elegans. *BMC genomics* 6:118-118.
- 3. Bigelow H, Doitsidou M, Sarin S, & Hobert O (2009) MAQGene: software to facilitate C. elegans mutant genome sequence analysis. *Nature methods* 6(8):549-549.
- 4. Thomas JH (1990) Genetic analysis of defecation in Caenorhabditis elegans. *Genetics* 124(4):855-872.
- 5. Liu DWC & Thomas JH (1994) Regulation of a Periodic Motor Program in C-Elegans. *J Neurosci* 14(4):1953-1962.
- 6. Mahoney TR, *et al.* (2008) Intestinal signaling to GABAergic neurons regulates a rhythmic behavior in Caenorhabditis elegans. *Proc Natl Acad Sci U S A* 105(42):16350-16355.
- 7. Kamath RS & Ahringer J (2003) Genome-wide RNAi screening in Caenorhabditis elegans. *Methods* 30(4):313-321.
- 8. Wang H, et al. (2013) Neuropeptide secreted from a pacemaker activates neurons to control a rhythmic behavior. *Curr Biol* 23(9):746-754.
- 9. Wang H & Sieburth D (2013) PKA Controls Calcium Influx into Motor Neurons during a Rhythmic Behavior. *PLOS Genetics* 9(9):e1003831.