**Supplementary Materials and Methods**

**Strains**

*C. elegans* strains were grown at 20°C with OP50 bacteria as food. N2 (Bristol) is the reference wild type strain. Strains used in this study include:

CSM660 *unc-79(mac383) III* (backcrossed 6 times, reference lf allele)

CSM555 *unc-79(mac389)* *III*

CSM559 *unc-79(mac393)* *III*

CB1291 *unc-79(e1291) III* (Jospin et al., 2007)

VC9 *nca-2(gk5) III* (Jospin et al., 2007; Yeh et al., 2008)

VC12 *nca-1(gk9)* *IV* (Jospin et al., 2007; Yeh et al., 2008)

DR1089 *nca-1(e625) IV* (Brenner, 1974; Yeh et al., 2008)

CB1272 *unc-80(e1272) V* (Jospin et al., 2007)

CSM659 *unc-80*(*mac379)* *V* (backcrossed 6 times, reference lf allele)

CSM546 *unc-80(mac380)* *V*

CSM547 *unc-80(mac381)* *V*

CSM548 *unc-80(mac382)* *V*

CSM550 *unc-80(mac384)* *V*

CSM551 *unc-80(mac385)* *V*

CSM552 *unc-80(mac386)* *V*

CSM554 *unc-80(mac388)* *V*

CSM556 *unc-80(mac390)* *V*

CSM557 *unc-80(mac391)* *V*

CSM560 *unc-80(mac394)* *V*

CSM710 *nlf-1(mac408) X*

CSM720 *nlf-1(mac409) X* (backcrossed 3 times, reference lf allele)

CSM712 *nlf-1(mac410) X*

CSM553 *mac387*

CSM661 *unc-79(mac383)* *III; unc-80*(*mac379)* *V*

CSM699 *unc-79(mac383)* *III; nca-1(e625)* *IV*

CSM662 *nca-2(gk5) III; nca-1(gk9)* *IV*

CSM813 *nca-2(gk5) III; nlf-1(mac409) X*

CSM812 *nca-1(gk9) IV; nlf-1(mac409) X*

CSM700 *nca-1(e625)* *IV; unc-80*(*mac379)* *V*

CSM730 *nca-1(e625) IV; nlf-1(mac409) X*

CSM1227 *unc-79(mac383) III; macEx668[PLunc-79a::unc-79a\_gDNA; Pmyo-3::GFP] #1*

CSM1228 *unc-79(mac383) III; macEx698[PLunc-79a::unc-79a\_gDNA; Pmyo-3::GFP] #2*

CSM731 *unc-79(mac383) III; macEx427[PSunc-79a::unc-79a\_gDNA; Pmyo-3::GFP] #1*

CSM732 *unc-79(mac383) III; macEx428[PSunc-79a::unc-79a\_gDNA; Pmyo-3::GFP] #2*

CSM736 *unc-79(mac383) III; macEx430[Punc-79b::unc-79b\_gDNA; Pmyo-3::GFP] #1*

CSM737 *unc-79(mac383) III; macEx431[Punc-79b::unc-79b\_gDNA; Pmyo-3::GFP] #2*

CSM936 *unc-80(mac379) V; macEx511[Punc-80::unc-80\_gDNA; Pmyo-3::GFP] #1*

CSM937 *unc-80(mac379) V; macEx512[Punc-80::unc-80\_gDNA; Pmyo-3::GFP] #2*

CSM1103 *nca-2(gk5) III; nca-1(gk9) IV; macEx582[Pnca-1::nca-1\_gDNA; Pmyo-3::GFP] #1*

CSM1104 *nca-2(gk5) III; nca-1(gk9) IV; macEx583[Pnca-1::nca-1\_gDNA; Pmyo-3::GFP] #2*

CSM1105 *nca-2(gk5) III; nca-1(gk9) IV; macEx584[Pnca-2::nca-2\_gDNA; Pmyo-3::GFP] #1*

CSM1106 *nca-2(gk5) III; nca-1(gk9) IV; macEx585[Pnca-2::nca-2\_gDNA; Pmyo-3p::GFP] #2*

CSM1107 *nca-2(gk5) III; nca-1(gk9) IV; macEx586[Pnca-1::nca-1\_gDNA; Pnca-2::nca-2\_gDNA; Pmyo-3::GFP] #1*

CSM1108 *nca-2(gk5) III; nca-1(gk9) IV; macEx587[Pnca-1::nca-1\_gDNA; Pnca-2::nca-2\_gDNA; Pmyo-3::GFP] #2*

CSM1223 *macEx664[PLunc-79a::GFP]*

CSM749 *macEx436[PSunc-79a::GFP]*

CSM931 *macEx506[Punc-80::GFP]*

CSM1247 *macEx664[PLunc-79a::GFP]; macEx685[Punc-80::mCherry]*

CSM950 *macEx506[Punc-80::GFP]; macEx524[PSunc-79a::mCherry]*

CSM913 *macEx436[PSunc-79a::GFP]; macEx502[Pssu-1::mCherry]*

CSM932 *macEx436[PSunc-79a::GFP]; macEx507[Pglr-3::mCherry]*

CSM953 *macEx436[PSunc-79a::GFP]; macEx527[Psra-6::mCherry]*

CSM972 *macEx528[Pnlf-1::mCherry]*

CSM1004 *macEx506[Punc-80::GFP]; macEx539[Psra-11::mCherry]*

CSM1005 *macEx506[Punc-80::GFP]; macEx540[Pmgl-1::mCherry]*

CSM1006 *macEx506[Punc-80::GFP]; macEx541[Pnmr-1::mCherry]*

CSM1038 *macEx506[Punc-80::GFP]; macEx545[Pnlf-1::mCherry]*

CSM1248 *macEx686[PLunc-79a::Cas9; PU6::unc-79\_sgRNA; Pmyo-3::GFP] #1*

CSM1249 *macEx687[PLunc-79a::Cas9; PU6::unc-79\_sgRNA; Pmyo-3::GFP] #2*

CSM1079 *macEx562[PSunc-79a::Cas9; PU6::unc-79\_sgRNA; Pmyo-3::GFP] #1*

CSM1080 *macEx563[PSunc-79a::Cas9; PU6::unc-79\_sgRNA; Pmyo-3::GFP] #2*

CSM1095 *macEx574[Punc-119::Cas9; PU6::unc-79\_sgRNA; Pmyo-3::GFP] #1*

CSM1096 *macEx575[Punc-119::Cas9; PU6::unc-79\_sgRNA; Pmyo-3::GFP] #2*

CSM1097 *macEx576[Punc-119::Cas9; PU6::unc-80\_sgRNA; Pmyo-3::GFP] #1*

CSM1098 *macEx577[Punc-119::Cas9; PU6::unc-80\_sgRNA; Pmyo-3::GFP] #2*

CSM1155 *macEx617[Pnmr-1::Cas9; PU6::unc-80\_sgRNA; Pmyo-3::GFP] #1*

CSM1156 *macEx618[Pnmr-1::Cas9; PU6::unc-80\_sgRNA; Pmyo-3::GFP] #2*

CSM939 *unc-80(mac379) V; macEx514[Punc-80::unc-80\_cDNA; Pmyo-2::mCherry] #1*

CSM940 *unc-80(mac379) V; macEx515[Punc-80::unc-80\_cDNA; Pmyo-2::mCherry] #2*

CSM1250 *unc-80(mac379) V; macEx688[PLunc-79a::unc-80\_cDNA; Pmyo-3::GFP] #1*

CSM1251 *unc-80(mac379) V; macEx689[PLunc-79a::unc-80\_cDNA;*

*Pmyo-3::GFP] #2*

CSM942 *unc-80(mac379) V; macEx517[Pnlf-1::unc-80\_cDNA; Pmyo-3::GFP] #1*

CSM943 *unc-80(mac379) V; macEx518[Pnlf-1::unc-80\_cDNA; Pmyo-3::GFP] #2*

CSM1040 *unc-80(mac379) V macEx547[Pnmr-1::unc-80\_cDNA; Pmyo-2::mCherry] #1*

CSM1041 *unc-80(mac379) V macEx548[Pnmr-1::unc-80\_cDNA; Pmyo-2::mCherry] #2*

CSM1122 *unc-80(mac379) V; macEx594[Pglr-1::unc-80\_cDNA; Pmyo-3::GFP] #1*

CSM1123 *unc-80(mac379) V; macEx595[Pglr-1::unc-80\_cDNA; Pmyo-3::GFP] #2*

CSM1205 *unc-80(mac379) V; macEx660[Pflp-18::unc-80\_cDNA; Pflp-1::unc-80\_cDNA; Punc-7b::unc-80\_cDNA; Pmyo-2::mCherry] #1*

CSM1206 *unc-80(mac379) V; macEx661[Pflp-18::unc-80\_cDNA; Pflp-1::unc-80\_cDNA; Punc-7b::unc-80\_cDNA; Pmyo-2::mCherry] #2*

CSM1261 *unc-79(mac383) III; macEx690[PLunc-79a::unc-79a\_cDNA::GFP; Pmyo-2::mCherry] #1*

CSM1262 *unc-79(mac383) III; macEx691[PLunc-79a::unc-79a\_cDNA::GFP; Pmyo-2::mCherry] #2*

**Molecular biology**

The 21.2 kb *PLunc-79a::unc-79a\_gDNA* including an *unc-79a* longpromoter (5056 bp upstream of the ATG start codon) and the full-length genomic fragment was amplified by PCR with the fosmid WRM0625cH11 (kindly provided by Yingchuan Qi) as template.

The 17.9 kb *PSunc-79a::unc-79a\_gDNA* including an *unc-79a* short promoter (1946 bp upstream of the ATG start codon and 2 bp of the first exon) and the full-length genomic fragment was amplified by PCR with the fosmid WRM0625cH11 (kindly provided by Yingchuan Qi) as template.

The *Punc-79b::unc-79b\_gDNA* consists of the 16 kb *unc-79a* full-length genomic fragment without the *unc-79a* promoter.

The 22.5 kb *Punc-80::unc-80\_gDNA* including an *unc-80* promoter (3595 bp upstream of the ATG start codon and 54 bp of the first exon) and the full-length genomic fragment was amplified by PCR with the fosmid WRM0616bC05 (kindly provided by Yingchuan Qi) as template.

The 19.2 kb *Pnca-1::nca-1\_gDNA* including an *nca-1* promoter (3142 bp upstream of the ATG start codon) and the full-length genomic fragment was amplified by PCR with the fosmid WRM0613dC08 (kindly provided by Yingchuan Qi) as template.

The 17.3 kb *Pnca-2::nca-2\_gDNA* including an *nca-2* promoter (3062 bp upstream of the ATG start codon) and the full-length genomic fragment was amplified by PCR with the fosmid WRM065cA04 (kindly provided by Yingchuan Qi) as template.

For *PLunc-79a::GFP* construct, the *unc-79a* longpromoter was amplified by PCR and subcloned to pPD95\_79 vector using *Sbf*I/*Xma*I restriction sites.

For *PSunc-79a::GFP* construct, the *unc-79a* shortpromoter was amplified by PCR and subcloned to pPD95\_79 vector using *Sph*I/*Xma*I restriction sites.

For *Pssu-1::mCherry* construct, the *mCherry gDNA* was amplified by PCR using pCFJ90 as template and subcloned to pPD95\_79 vector by replacing *GFP* using *Age*I/EcoRI restriction sites. The *ssu-1* promoter (543 bp upstream of the ATG start codon) (Carroll et al.) was amplified by PCR and subcloned to pPD95\_79*-mCherry* using *Sph*I/*Xma*I restriction sites.

For *Psra-6::mCherry* construct, a *sra-6* promoter (3734 bp upstream of the ATG start codon and 33 bp of the first exon) (Troemel et al., 1995) was amplified by PCR and subcloned to pPD95\_79*-mCherry* in frame with *mCherry* using *Sbf*I/*Xma*I restriction sites.

For *Pglr-3::mCherry* construct, a *glr-3* promoter (2182 bp upstream of the ATG start codon and 30 bp of the first exon) (Brockie et al., 2001a) was amplified by PCR and subcloned to pPD95\_79*-mCherry* in frame with *mCherry* using *Sph*I/*Kpn*I restriction sites.

For *Punc-80::GFP* construct, the *unc-80* promoter was amplified by PCR and subcloned to pPD95\_79 vector using *Sph*I/*Xma*I restriction sites.

For *Pnmr-1::mCherry* construct, an *nmr-1* promoter (5035 bp upstream of the ATG start codon) (Brockie et al., 2001b) was amplified by PCR and subcloned to pPD95\_79*-mCherry* using *Sph*I/*Xma*I restriction sites.

For *Pmgl-1::mCherry* construct, an *mgl-1* promoter (3956 bp upstream of the ATG start codon) was amplified by PCR and subcloned to pPD95\_79*-mCherry* using *Sbf*I/*Xma*I restriction sites. *mgl-1* was previously reported to be expressed in AIA, AIY, NSM and RMD neurons (Greer et al., 2008; Wenick and Hobert, 2004). In this study, we found that pharyngeal interneurons I3 and I4 were also labeled by the *Pmgl-1::mCherry* reporter.

For *Punc-80::mCherry* construct, the *unc-80* promoter was amplified and subcloned to pPD95\_79*-mCherry* using *Sph*I/*Xma*I restriction sites.

For *PSunc-79a::mCherry* construct, the *unc-79a* shortpromoter was amplified and subcloned to pPD95\_79*-mCherry* using *Sph*I/*Xma*I restriction sites.

For *Pnlf-1::mCherry* construct, an *nlf-1* promoter (5824 bp upstream of the ATG start codon from -40 to -5863 nt) was amplified by PCR and subcloned to pPD95\_79*-mCherry* using *Sbf*I/*Xma*I restriction sites. (Xie et al., 2013)

For *Psra-11::mCherry* construct, a *sra-11* promoter (3352 bp upstream of the ATG start codon and 104 bp of the first exon) (Altun-Gultekin et al., 2001) was amplified by PCR and subcloned to pPD95\_79*-mCherry* in frame with *mCherry* using *Sph*I/*Xma*I restriction sites.

For *PSunc-79a::Cas9::NLS::3’UTR* construct, the *Cas9::NLS::3’UTR* fragment was amplified by PCR using the pPD162 plasmid (Dickinson et al., 2013) as template and subcloned to pPD95\_79-*PSunc-79a* with In-fusion HD Cloning Kit (Clontech) using *Xma*I restriction sites.

For *PLunc-79a::Cas9::NLS::3’UTR* construct, the *unc-79a* long promoter was amplified and subcloned to pPD95\_79-*PSunc-79a::Cas9::NLS::3’UTR* by replacingthe *unc-79a* short promoter using *Sph*I/*Xma*I restriction sites*.*

For *Punc-80::Cas9::NLS::3’UTR* construct, the *unc-80* promoter was amplified and subcloned to pPD95\_79-*PSunc-79a::Cas9::NLS::3’UTR* by replacingthe *unc-79a* short promoter using *Sph*I/*Xma*I restriction sites*.*

For *Pnmr-1::Cas9::NLS::3’UTR* construct, the *nmr-1* promoter (Brockie et al., 2001b) was amplified and subcloned to pPD95\_79-*PSunc-79a::Cas9::NLS::3’UTR* by replacing the *unc-79a* short promoter using *Sph*I/*Xma*I restriction sites*.*

For *Punc-119::Cas9::NLS::3’UTR* construct, an *unc-119* promoter (1995 bp upstream of the *unc-119b* start codon and 49 bp of the first exon) (Maduro and Pilgrim, 1995) was amplified by PCR and subcloned to pPD95\_79-*PSunc-79a::Cas9::NLS::3’UTR* by replacing the *unc-79a* short promoter using *Sph*I/*Xma*I restriction sites.

For *Punc-80::unc-80\_cDNA* construct, the full-length *unc-80\_cDNA* synthesized at Sangon Biotech (Shanghai) was subcloned to pPD95\_79 vector using *Xma*I/*Kpn*I sites. The *unc-80* promoter was amplified and subcloned to pPD95\_79-*unc-80\_cDNA* using *Sph*I/*Xma*I restriction sites.

For *PLunc79a::unc-80\_cDNA* construct, the *unc-79a* long promoter was amplified and subcloned to pPD95\_79-*Punc-80::unc-80\_cDNA* by replacing the *unc-80* promoter using *Sph*I/*Xma*I restriction sites.

For *PSunc79a::unc-80\_cDNA* construct, the *unc-79a* short promoter was amplified and subcloned to pPD95\_79-*Punc-80::unc-80\_cDNA* by replacing the *unc-80* promoter using *Sph*I/*Xma*I restriction sites.

For *Pnmr-1::unc-80\_cDNA* construct, the *nmr-1* promoter (Brockie et al., 2001b) was amplified and subcloned to pPD95\_79-*Punc-80::unc-80\_cDNA* by replacing the *unc-80* promoterusing *Sph*I/*Xma*I restriction sites.

For *Pnlf-1::unc-80\_cDNA* construct, the *unc-80 cDNA* was amplified and subcloned to pPD95\_79- *Pnlf-1::mCherry* using *Xma*I/*Kpn*I restriction sites.

For *Pglr-1::unc-80\_cDNA* construct, a *glr-1* promoter (2996 bp upstream of the ATG start codon) (Brockie et al., 2001) was amplified by PCR and subcloned to pPD95\_79-*Pnlf-1::unc-80\_cDNA* by replacing the *nlf-1* promoter using *Sbf*I/*Xma*I restriction sites.

For *Pflp-1::unc-80\_cDNA* construct, a *flp-1* promoter (1574 bp upstream of the ATG start codon) (Nelson et al., 1998) was amplified by PCR and subcloned to pPD95\_79-*Pnlf-1::unc-80\_cDNA* by replacing the *nlf-1* promoter using *Sbf*I/*Xma*I restriction sites.

For *Pflp-18::unc-80\_cDNA* construct, the *flp-18* promoter (Rogers et al., 2003) was amplified and subcloned to pPD95\_79-*Punc-80::unc-80\_cDNA* by replacing the *unc-80* promoterusing *Sph*I/*Xma*I restriction sites.

For *Punc-7b::unc-80\_cDNA* construct, an *unc-7b* promoter (3122 bp upstream of the *unc-7b* start codon) (Altun et al., 2009) promoter was amplified by PCR and subcloned to pPD95\_79-*Pnlf-1::unc-80\_cDNA* by replacing the *nlf-1* promoter using *Sbf*I/*Xma*I restriction sites.

For *Pmgl-1::unc-80\_cDNA* construct, the *mgl-1* promoter was amplified and subcloned to pPD95\_79-*Pnlf-1::unc-80\_cDNA* by replacing the *nlf-1* promoter using *Sbf*I/*Xma*I restriction sites.

For *Psra-11::unc-80\_cDNA* construct, the *sra-11* promoter was amplified and subcloned to pPD95\_79-*Punc-80::unc-80\_cDNA* by replacing the *unc-80* promoterusing *Sph*I/*Xma*I restriction sites.

For *PLunc-79a::unc-79a\_cDNA::GFP* construct, the full-length *unc-79a\_cDNA* was amplified by PCR and subcloned to pPD95\_79- *PLunc-79a* using *XmaI/KpnI* restriction sites.

All plasmids were verified by restriction digestion and sequencing. Primers are listed in Table S5.

**Generation of *nlf-1* deletion alleles using the CRISPR/Cas9 method**

For the generation of *nlf-1* deletions using a CRISPR/Cas9 method (Friedland et al., 2013), the transgenic mixture containing 50 ng/μl pPD162 (*Peft-3::Cas9::NLS::3’UTR*), 25 ng/μl *PU6::nlf-1\_sgRNA*, and 20 ng/μl pPD95\_86 (*Pmyo-3::GFP*) as co-injection marker was injected to wild type animals and GFP-positive F1 progeny were picked to individual plates and genotyped.

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