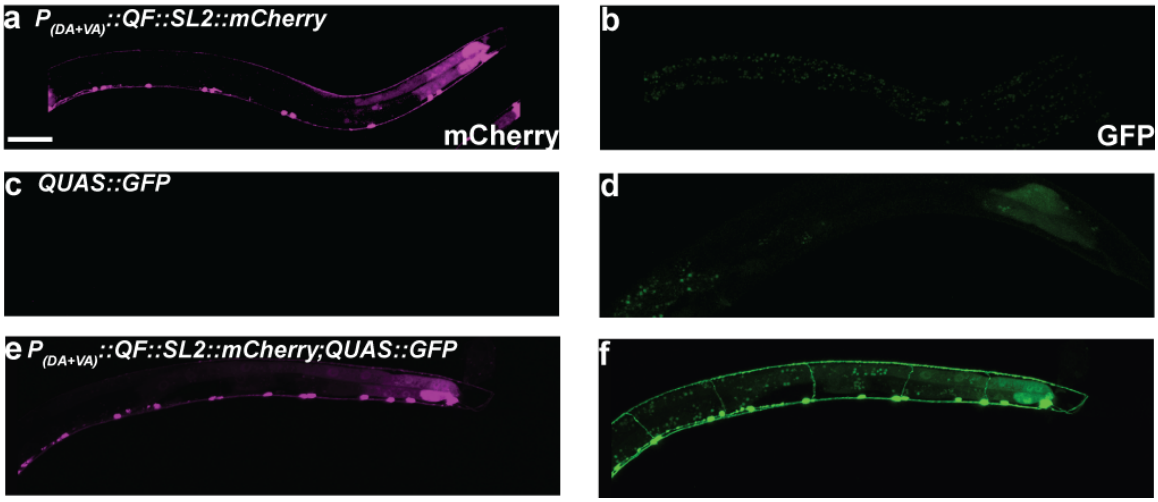
Supplementary Figure 1. Both QF and QUAS are required for binary expression.

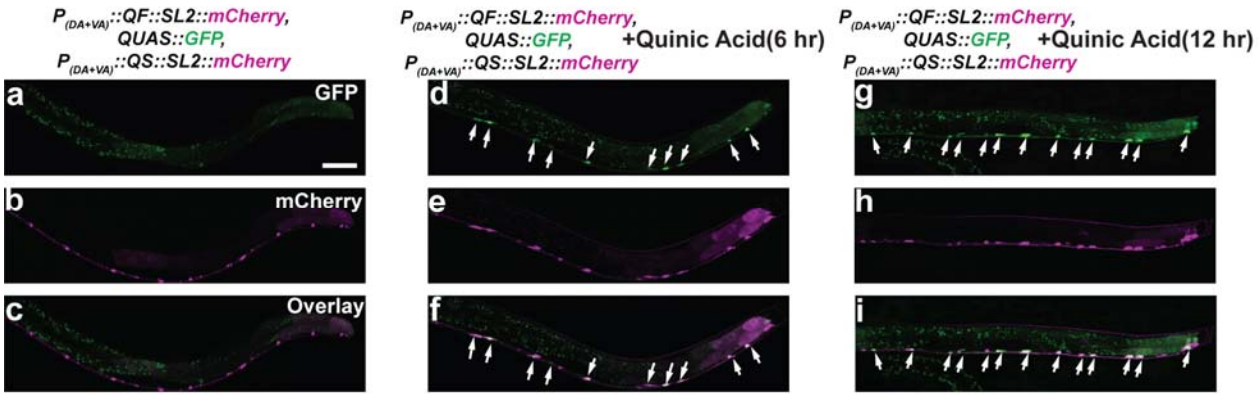


(**a-b**) No GFP expression in transgenic strain containing only QF (*wyEx3632*). (**a**) QF is expressed in VA and DA neurons driven by the *unc-4* promoter labeled by mCherry. (**b**) No GFP expression in these neurons.

(**c-d**) No GFP expression in transgenic strains containing only *QUAS::GFP* (*wyEx3670*). (**c**) No QF is expressed in DA and VA neurons. (**d**) No expression of *QUAS::GFP* in these neurons.

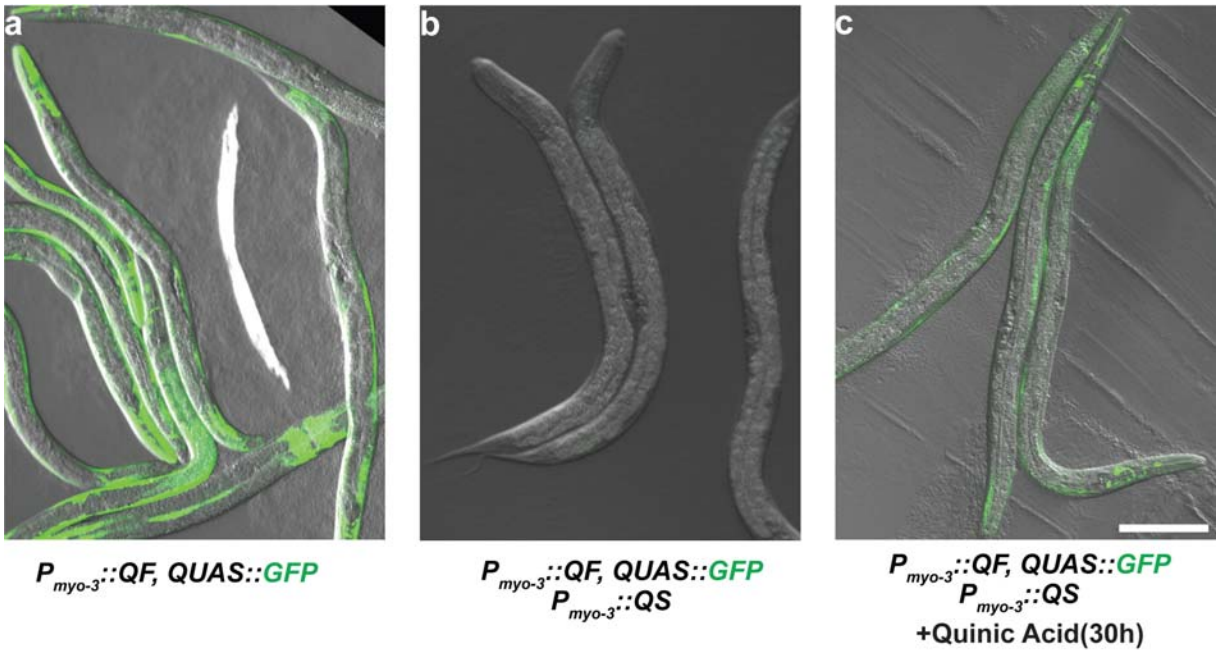
(**e-f**) Animals carrying both *QF* and *QUAS::GFP* (*wyEx3632; wyEx3670*) show GFP labeling in A-type neurons. (**e**) QF is driven by the *unc-4* promoter in VA and DA neurons (co-labeled by mCherry). (**f**) Activated expression of *QUAS::GFP* in VA and DA neurons. Late L3 stage larvae were chosen for imaging. Scale bar, 20 μm.

Supplementary Figure 2. The time course (6 hr and 12 hr) of derepression of QS by quinic acid**.**



In the same strain containing *QF*, *QS* and *QUAS::GFP* (*wyEx4048*), GFP is re-expressed in VA and DA neurons after treatment with quinic acid. Transgenic larvae were transferred to NGM plates containing quinic acid for 6 hr or 12 hr. (**a-c**) Expression of the *QUAS::GFP* reporter is suppressed by QS in these neurons without treatment with quinic acid. (**a**) No GFP expression. (**b**) mCherry expression (**c**) Merge. (**d-f**) Treatment with quinic acid for 6 hr begins to relieve the expression of the *QUAS::GFP* reporter in these neurons. (**d**) Detectable GFP expression in A-type neurons. (**e**) mCherry expression (**f**) Merge. (**g-i**) Treatment of quinic acid for 12 hr strongly relieves the expression of the *QUAS::GFP* reporter in these neurons. (**g**) Strong GFP expression. (**h**) mCherry expression (**i**) Merge. Scale bar, 20 μm. White arrows indicate the A-type neuron cell bodies labeled by GFP.

Supplementary Figure 3. Q system functions in *C. elegans* body wall muscles.



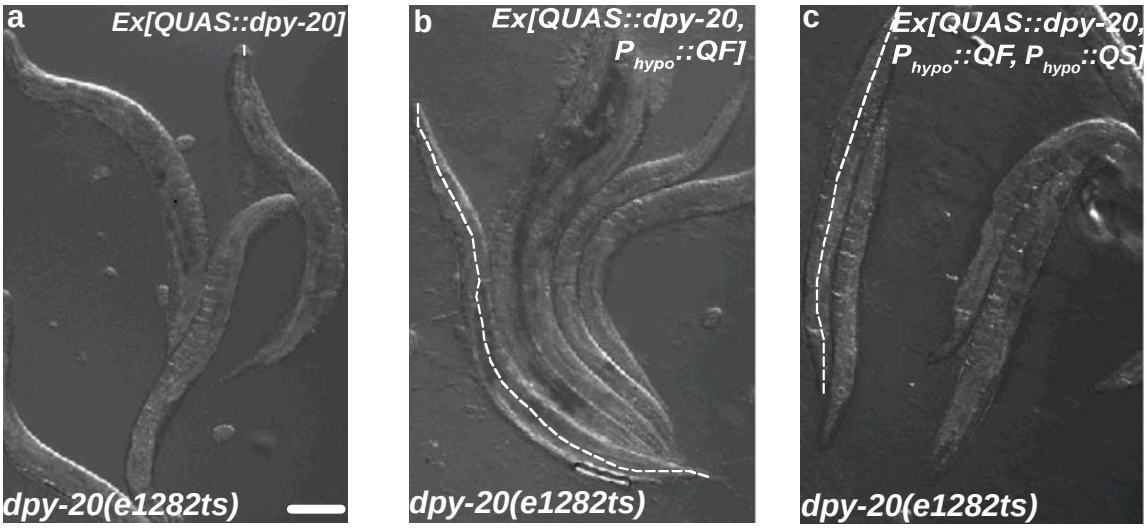
(**a**) QF expressed in body wall muscles can activate expression of *QUAS::GFP* (*wyEx4698*)

(**b**) The activation of *QUAS::GFP* is suppressed by QS expressed in muscles (*wyEx4697*).

(**c**) In the same strain (*wyEx4697*), GFP is expressed in body wall muscles after treatment with quinic acid for 30 hr.

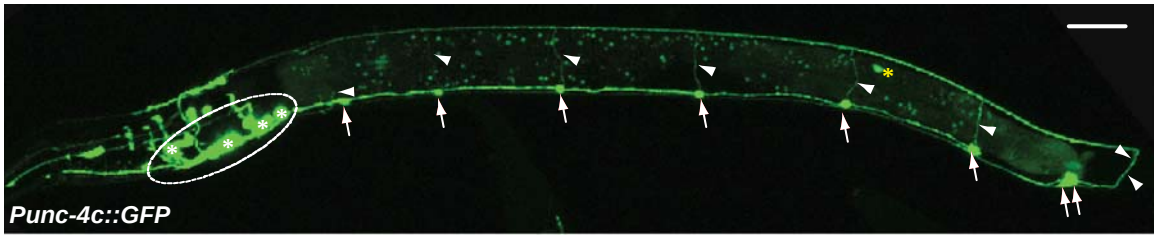
L4 stage larvae were chosen for imaging and each animal shown in these images contains co-injection marker (*Podr-1::RFP*). Scale bar, 100 μm.

Supplementary Figure 4. Q system for functional rescue in hypodermal cells.



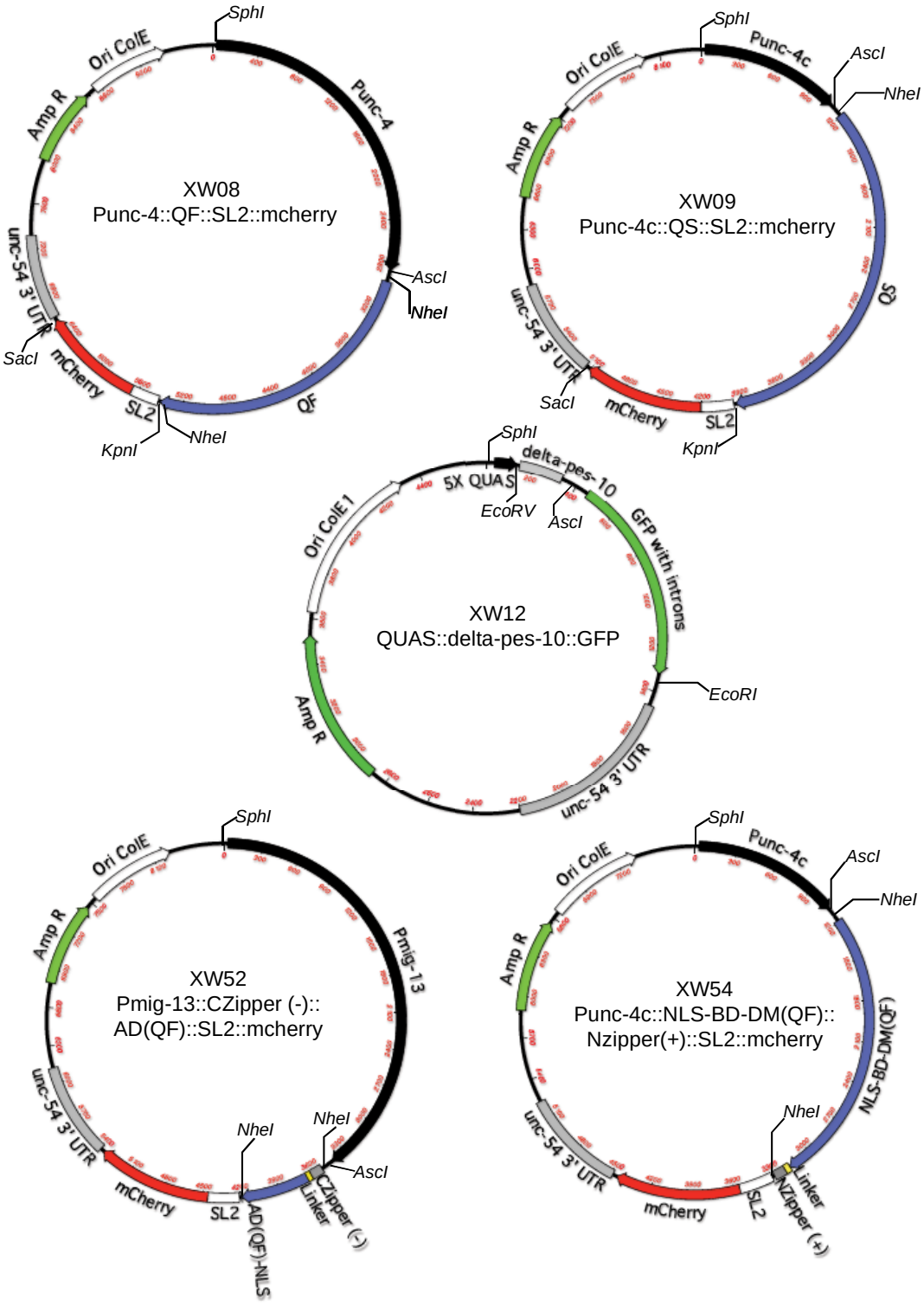
(**a-c**) The micrographs show *dpy-20 (e1282ts)* mutant worms at the restrictive temperature (25°C) and carrying the indicated transgenes as extrachromosomal arrays. "P_{hypo}" means hypodermal cell promoter**.** The white dashed lines in **a**, **b** and **c** depict the measurement of body length in the mid body from the tip of the head to the tail. Each animal shown in these images is adult and contains the co-injection marker (*Podr-1::DsRed*). Scale bar, 100 μm.

Supplementary Figure 5. The expression pattern of the *unc-4c* promoter.



GFP is driven by the *unc-4c* promoter (*wyEx1817*). The cell bodies of DA neurons (DA2-DA9) in the ventral nerve cord are indicated by white arrows. White arrowheads indicate the commissures of these DA neurons. In the dashed oval region, SABs and I5 in the retrovesicular ganglion (RVG) near pharynx are also labeled, and their cell bodies are depicted by white asterisks. The yellow asterisk denotes one unidentified neuron in the dorsal side. Late L3 stage larva was chosen for imaging. Scale bar, 20 μm.

Supplementary Figure 6. Vector maps containing components of Q system and Split Q system with available cloning sites. See details in **Supplementary Note 1**.



Supplementary Table 1. Relative transcriptional activities of primary Split Q constructs tested during design optimization.

Construct(s) Tested		Activity
QF-BD::DM::AD (full length 1-816)		++++
NLS-QF-AD(650-816aa)::Zip(+)		-
Zip(-)::QF-BD(1-185aa)	NLS-QF-AD(650-816aa)::Zip(+)	-
Zip(-)::QF-BD(1-185aa)	QF-DM::AD(185-816aa)::Zip(+)	-
QF-BD(1-185aa)::Zip(+)	Zip(-)::QF-DM::AD(185-816aa)	+
QF-BD::DM(1-650aa)::Zip(+)	Zip(-)::QF-AD(650-816aa)	++
QF-BD::DM(1-650aa)::Zip(+)	Zip(-)::QF-AD-NLS(650-816aa)	+++

As described in the **Fig. 3a** legend, three domains from the QF were tested. The putative dimerization domain (DM) is required for reconstituted activity, and the sites of fusion (N or C terminus) for leucine zippers are also important. Leucine zipper domains are fused to either the N-terminus (Zip-) or C-terminus (Zip+). NLS denotes SV40 nuclear localization sequence. Relative transcriptional activities of the Split Q constructs were compared when driven by the same promoter (*mig-13*). Transcriptional activity was quantified by measuring the fluorescent GFP intensity in the cell body of each neuron in the ventral nerve cord in transgenic strains co-expressing indicated Split QF constructs with a *QUAS::GFP* reporter. Fluorescence intensity was scored subjectively on a scale of 0 (-) to 4 (++++). The optimal pair of constructs is indicated by red color.

Supplementary Note 1. Constructs information.

XW08 (Punc-4::QF::SL2::mCherry): gpd-2 SL2::mCherry was PCR amplified from pBALU12[23] in which the kanamycin cassette and N-terminus nuclear localization sequence (NLS) were removed, using primers 5'-AACTGGTACCGCTGTCTCATCCTA CTTTCACC and 3'-AGGCGAGCTCTTACTTATACAATTCATCCATGCC. The fragment was inserted into the KpnI and SacI sites of pSM and then the *unc-4* promoter (4kb)[9] was cloned into SphI and AscI sites of pSM-SL2::mCherry. The QF was amplified from pCaSpeR4[7] with the primers 5'-CATAGCTAGCATGCCGCCTAAACGCA and 3'-CATAGCTAGCTATTGCTCATACGTGTTGATATC, and the fragment was cloned into NheI site of pSM-Punc-4::SL2::mCherry. Cloning sites are denoted in **Supplementary Figure 6**

XW09 (Punc-4c::QS::SL2::mCherry): the *unc-4c* promoter (bashed *unc-4* promoter, ~1kb 3' fragment of *unc-4* promoter, M. Vanhoven and K. Shen, unpublished results) from **VMC63** (Punc-4c::GFP), was subcloned into SphI and AscI sites of pSM-SL2::mCherry. The QS was amplified from pACPL-QS[7] with the primers 5'-CATAGCTAGCATGAACACCATCCCGGC and 3'- AACTGGTACCTCAAGATA TTTGCGTTGCAA, and the fragment was cloned into NheI and KpnI sites of pSM-Punc-4c::SL2::mCherry. Cloning sites are denoted in **Supplementary Figure 6**

XW12 (QUAS-Δpes-10-GFP): 5xQUAS-hsp70 was amplified from pQUAS-CD8-GFP[7] with primers 5'- ACTTACTTGCATGCGGATCCGGGTAATCGCTTA and 3'-AGTGGCGCGCCCAATTCCCTATTCAGAGTTC, and the fragment was inserted into SphI and AscI sites of pSM-GFP. Δpes-10 minimal promoter was amplified from pPD97.78 (A. Fire) with primers 5'- GCAAGTGATATCCCTGCAGGATCGATTTT

TTGCA and 3'- GATGGCGCGCCCTGAAAGTTAAAAATTACAGTATAAAGATA AGGGA, and was subcloned into the EcoRV-AscI fragment from P5xQUAS-hsp70-GFP, replacing the hsp-70 minimal promoter. Cloning sites are denoted in **Supplementary Figure 6**

XW25 Punc-4::QS::SL2::mCherry: the *unc-4* promoter (4kb) was subcloned into the SphI-AscI fragment from XW09, replacing the *unc-4c* promoter.

XW43 Pmig-13::QF::SL2::mCherry: The endogenous SphI, SacI and KpnI sites in QF in XW08 were removed by QuickChange (Stratagene) to construct XW17. *mig-13* promoter (3.4kb) [19]was subcloned into the SphI and AscI sites of XW17, and replaced the *unc-4* promoter.

XW52 Pmig-13::Zip(-)::QF-AD::SL2::mCherry: QF-AD was the C-terminus part (650-816aa) of QF[17], and was amplified from XW42 by PCR, adding GSGSGSGSGSGT linker sequence at 5' and SV40 NLS at 3'. The sequence of Zip(-) antiparallel leucin zipper (AQLEKKLQALEKKLAQLEWKNQALEKKLAQ) was amplified from CZ-CED-3 (a gift from M. Chalfie)[24], and was fused with QF-AD fragment by overlapping PCR, adding NheI sites at 5' and 3'ends. The Zip(-)::QF-AD was subcloned into NheI of XW42, replacing the full length QF. Cloning sites are denoted in **Supplementary Figure 6.**

XW54 Punc-4c::QF-BD-DM::Zip(+)::SL2::mCherry: QF-BD-DM was the N-terminus part (1-650aa) of QF, and was amplified from XW42 by PCR, adding SV40 NLS at 5' and GSGSGSGSGSGSA linker sequence at 3'. The sequence of Zip(+) antiparallel leucin zipper (ALKKELQANKKELAQLKWELQALKKELAQ) was amplified from CED-3-NZ (a gift from M. Chalfie)[24], and was fused with QF-BD-DM fragment by overlapping

PCR, adding NheI sites at 5' and 3' ends. The QF-BD-DM::Zip(+) was subcloned into NheI of XW09, replacing the QS. Cloning sites are denoted in **Supplementary Figure 6.**

XW55 Pmig-13::QF-BD-DM::Zip(+)::SL2::mCherry: *mig-13* promoter was inserted into SphI and AscI sites of XW54 to replace *unc-4c* promoter.

XW66 Pmyo-3::QF:. *myo-3* promoter from pPD122.66 (Pmyo-3::GFP A.Fire) was subcloned into the HindIII and BamHI sites of XW42, and replaced the *mig-13* promoter.

XW67 Pmyo-3::QS ::SL2::mCherry: QS::SL2::mCherry from XW25 (NheI and ApaI) was subcloned into the XbaI and ApaI sites of pPD122.66, and replaced the GFP fragment.

XW71 QUAS-Δpes-10-dpy-20: The *dpy-20* genomic DNA (3kb, including the whole *dpy-20* gene from initial ATG to stop codon) was amplified from fosmid WRM0616CH07 with the primers 5'ACATAGCTAGCATGGAAGGGCATAGTAATA CTTCT and 3'- GTACCATTGTTTAAACTTATTTAACGCTGAAAGTTGTCTG, and the fragment was cloned into NheI and PmeI sites of XW12, and replaced the GFP fragment.

XW74 Pdpy-7::QS::SL2::mCherry: The *dpy-7* upstream fragment (218bp) was amplified from N2 genomic DNA, was subcloned into the SphI and AscI sites of XW25, and replaced the *unc-4* promoter.

XW75 Pdpy-7::QF: The *dpy-7* upstream fragment (218bp) was amplified from N2 genomic DNA, was subcloned into the SphI and AscI sites of XW17, and replaced the *unc-4* promoter.

XW82 MosSCI-QUAS-Δpes-10-GFP: QUAS-Δpes-10-GFP fragment from XW12 was subcloned into SphI and PacI sites of CM224 (a derivative of pCFJ151[21] with extra

cloning sites, a kind gift from C. Maeder), which is the chromosome II *ttTi5605* site MosSCI targeting vector.

XW83 MosSCI-Pmig-13::QF::SL2::mCherry: Pmig-13::QF::SL2::mCherry fragment from XW43 was subcloned into SphI and PacI sites of CM290 (a derivative of pCFJ178[21] with extra cloning sites, a kind gift from C. Maeder), which is the chromosome IV *cxTi10882* site MosSCI targeting vector.

Supplementary Note 2. Strains information.

wyEx1817 (M. Vanhoven, unpublished strain) [Punc-4c::GFP(10ng/μl), Podr-1::dsRED (40ng/μl)];

wyEx3574[Punc-4::QF::SL2::mCherry (10ng/μl), QUAS-Δpes-10-GFP (5ng/μl), Punc-4c::QS::SL2::mcherry (15ng/μl), Podr-1::dsRED (60ng/μl)];

wyEx3632[Punc-4::QF::SL2::mCherry (5ng/μl), Podr-1::dsRED (60ng/μl)];

wyEx3661[Punc-4::QF::SL2::mCherry (5ng/μl), QUAS-Δpes-10-GFP (5ng/μl), Podr-1::dsRED (60ng/μl)];

wyEx3670 [QUAS-Δpes-10-GFP (10ng/μl), Podr-1::GFP (40ng/μl)];

wyEx4048[Punc-4::QF::SL2::mCherry (5ng/μl), QUAS-Δpes-10-GFP (5ng/μl), Punc-4::QS::SL2::mcherry (5ng/μl), Podr-1::dsRED (60ng/μl)];

wyEx4212[Pmig-13::QF::SL2::mCherry (5ng/μl), QUAS-Δpes-10-GFP (10ng/μl), Podr-1::dsRED(80ng/μl)];

wyEx4302[Pmig-13::Zip(-)::QF-AD::SL2::mCherry (7.5ng/μl), QUAS-Δpes-10-GFP (10ng/μl), Podr-1::dsRED (80ng/μl)];

wyEx4355[Punc-4c::QF-BD-DM::Zip(+)::SL2::mCherry (30ng/μl), Pmig-13::Zip(-)::QF-AD::SL2::mCherry (7.5ng/μl), QUAS-Δpes-10-GFP (15ng/μl), Podr-1::dsRED (80ng/μl)];

wyEx4394 [Pmig-13::QF-BD-DM::Zip(+)::SL2::mCherry (5ng/μl), Pmig-13::Zip(-)::QF-AD::SL2::mCherry (5ng/μl), QUAS-Δpes-10-GFP (10ng/μl), Podr-1::dsRED (80ng/μl)];

wyEx4397 [Pmig-13::QF-BD-DM::Zip(+)::SL2::mCherry (5ng/μl), QUAS-Δpes-10-GFP (10ng/μl), Podr-1::dsRED (80ng/μl)];

wyEx4409 [Punc-4c::QS (12ng/µl), Podr-1::GFP (40ng/µl), pBluescript (50ng/µl)], sequentially injected into animals containing wyEx4355;

wyEx4570 [Punc-4::GFP (5ng/µl), Podr-1:: dsRED (60ng/µl), pBluescript (50ng/µl)];

wyEx4697 [Pmyo-3::QF (10ng/µl), QUAS-Δpes-10-GFP (10ng/µl), Pmyo-3::QS ::SL2::mCherry (10ng/µl), Podr-1::dsRED (60ng/µl), pBluescript (50ng/µl)]

wyEx4698 [Pmyo-3::QF (10ng/µl), QUAS-Δpes-10-GFP (10ng/µl), Podr-1::dsRED (60ng/µl), pBluescript (50ng/µl)];

wyEx4701 [QUAS-Δpes-10-dpy-20 (15ng/µl), Podr-1::dsRED (60ng/µl), pBluescript (50ng/µl)];

wyEx4704 [Pdpy-7::QF (5ng/µl) QUAS-Δpes-10-dpy-20 (5ng/µl), Podr-1::dsRED (60ng/µl), pBluescript (50ng/µl)];

wyEx4710 [Pdpy-7::QF (5ng/µl) QUAS-Δpes-10-dpy-20 (5ng/µl), Pdpy-7::QS ::SL2::mCherry (5ng/µl), Podr-1::dsRED (60ng/µl), pBluescript (50ng/µl)];

wySi374 The plasmid mixture containing pJL43.1 (50 ng/µl), pCJF90 (1.5 ng/µl), Podr-1::dsRED (50 ng/µl), and XW82 (50 ng/µl) was injected into stain EG4322 (*ttTi5605 II; unc-119(ed3) III*). Homozygous single-copy insertion lines were generated and verified by PCR;

wySi377 The plasmid mixture containing pJL43.1 (50 ng/µl), pCJF90 (1.5 ng/µl), Podr-1::dsRED (50 ng/µl), and the respective QF expression clone XW83 (40 ng/µl) was injected into stain EG5003 (*unc-119(ed3) III; cxTi10882 IV*). Homozygous single-copy insertion lines were generated and verified by PCR;

wyEx5031 [Punc-4c::QS (15 ng/µl), Podr-1::dsRED (60 ng/µl), pBluescript (50ng/µl)] The plasmids were injected into strain TV12353 (*wySi374 II; wySi377 IV*).