Controlling gene expression with the Q repressible binary expression system in *Caenorhabditis elegans*

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We established a transcription-based binary gene expression system in *Caenorhabditis elegans* using the recently developed Q system. This system, derived from genes in *Neurospora crassa*, uses the transcriptional activator QF to induce the expression of target genes. Activation can be efficiently suppressed by the transcriptional repressor QS, and suppression can be relieved by the nontoxic small molecule quinic acid. We used QF, QS and quinic acid to achieve temporal and spatial control of transgene expression in various tissues in *C. elegans*. We also developed a split Q system, in which we separated QF into two parts encoding its DNA-binding and transcription-activation domains. Each domain showed negligible transcriptional activity when expressed alone, but expression of both reconstituted QF activity, providing additional combinatorial power to control gene expression.

The capability to regulate the expression of engineered transgenes has revolutionized the study of biology in multicellular genetic model organisms. One popular and powerful strategy is using a binary expression system such as the tetracycline-regulated tTA–tetracycline response element system in mammals[1] and the GAL4–*UAS-GAL80* system in *Drosophila melanogaster*[2,3]. Despite its success in *D. melanogaster*, to our knowledge so far there is no transcription-based binary expression system reported in the nematode *C. elegans*.

An alternative method in *C. elegans* is to use DNA recombinase systems such as Flp-*FRT*[4] or Cre-*LoxP*[5] to remove regulatory elements in transgene constructs to control gene expression. However, the action of the recombinases is not reversible or repressible, and the expression pattern integrates the developmental history of the promoter that drives recombinase expression. Another strategy is to combine heat-shock control and a tissue-specific promoter; in an *hsf-1* mutant background that is defective for the heat-shock response, the combination of cell autonomous rescue of *hsf-1* and a heat-shock promoter–driven transgene can achieve spatial and temporal control of gene expression[6]. Although this method has many advantages, it requires the transgenes to be expressed in the *hsf-1* mutant background. In addition, because worms cannot tolerate extended heat shock, this method can only achieve gene expression with transient onset and offset.

Recently, a repressible binary expression system, the Q system, was established in *D. melanogaster* and mammalian cells based on regulatory genes from the *Neurospora crassa qa* gene cluster[7]. The transcriptional activator QF binds to a 16 base pair (bp) sequence (called *QUAS*) and activates expression of target genes under the control of *QUAS* sites. Expression can be efficiently suppressed by the transcriptional repressor QS, and the transcriptional suppression can be relieved by feeding worms quinic acid, a nontoxic small molecule (**Fig. 1a**). Here we adapted the Q system to *C. elegans* and demonstrated its utility for controlling transgene expression with temporal and spatial precision. We also developed the split Q system by separating the transcriptional activator QF into two parts, to achieve intersectional control of gene expression.

RESULTS

Characterization of the Q system in *C. elegans*

We used the Q system to label A-type motor neurons in *C. elegans* (**Fig. 1**). A-type motor neurons (DA and VA neurons) are cholinergic, excitatory and responsible for backward movement[8]. The cell bodies of both DA and VA neurons are located in the ventral nerve cord, and DA neurons send their axonal commissures to the dorsal nerve cord, whereas VA neurons extend their axons exclusively in the ventral nerve cord (**Fig. 1b**). We used the *unc-4* promoter (expressed in both DA and VA neurons)[9] to drive the expression of QF and monitored expression of QF with a *SL2::mCherry* cassette. The *trans*-spliced leader sequence *SL2* permits the bicistronic expression of QF and monomeric (m)Cherry under the control of the *unc-4* promoter, similarly to the internal ribosomal entry site in the vertebrate system[10].

The transcriptional machinery of *C. elegans* requires a minimal promoter to initiate transcription. After trying several such sequences (data not shown), we found that the *Δpes-10* minimal promoter[11] supported strong expression with the Q system in *C. elegans*. We created a transgenic strain (*wyEx3661*) in which the *QF* construct was expressed together with *QUAS::Δpes-10::GFP* (*S65C*) (called *QUAS::GFP* hereafter). As expected, GFP was robustly expressed in DA and VA neurons in worms that expressed both constructs (**Fig. 1c–f**) but not in lines that expressed the constructs individually (**Supplementary Fig. 1a–d**). The fraction of worms showing expression with the QF system was comparable to

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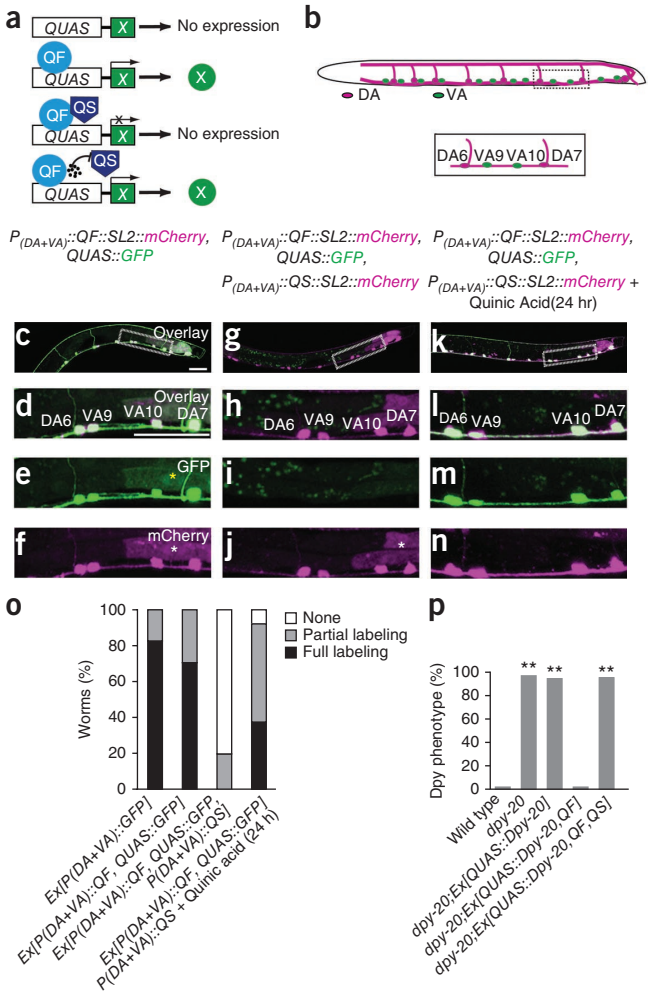

Figure 1 | The repressible Q binary system functions effectively in *C. elegans*. (**a**) Schematic of the Q system. *X* indicates transgene. Black dots at the bottom indicate quinic acid. (**b**) Schematic diagram of VA and DA motor neurons in the ventral nerve cord. The boxed region is magnified to show DA6, VA9, VA10 and DA7 neurons. (**c**–**n**) The micrographs show transgenic worms (L3 larvae) expressing the indicated transgenes, with or without treatment with quinic acid for 24 h. An overview of the ventral nerve cord (**c**,**g**,**k**) and magnification of the boxed region (**d**–**n**) are shown. *P*, promoter; *SL2*, trans-spliced leader sequence. White asterisks denote ectopic gut fluorescence caused by *SL2::mCherry* cassette (X.W. and K.S., unpublished results). Yellow asterisk denotes occasional ectopic gut fluorescence resulting from *QUAS::GFP*. Scale bars, 20 µm. (**o**) Quantification of labeling efficiency in **c**–**n**. Late L3 or early L4 stage larvae were scored ($n > 200$ for each strain). Worms were divided into three categories: none (no A-type neurons labeled), full labeling (all A-type neurons labeled) and partial-labeling (between no and full labeling). (**p**) Quantification of Dpy rescue efficiency with Q system in **Supplementary Figure 4** ($n = 40$ for each group; $^{**}P \leq 0.0001$ versus wild-type worms, χ^2 test). Worms with a body length >972 µm were scored as wild type. Worms with a body length <972 µm were scored as Dpy mutants.

that seen with direct promoter fusion (**Fig. 1o**). When we crossed the two single construct–expressing transgenic strains, we also obtained robust GFP expression in both DA and VA neurons in worms with both transgenes (**Supplementary Fig. 1e**,**f**); this excluded the possibility that GFP fluorescence was due to recombination between the *unc-4* promoter and *QUAS::GFP* during the generation of the extrachromosomal array[12].

To test whether the action of QF is repressible, we generated a transgenic line (*wyEx4048*) that expressed *QS*, *QF* and *QUAS::GFP* in A-type neurons. In these worms, GFP expression in DA and VA neurons was efficiently suppressed (**Fig. 1g**–**j**,**o**). Finally, to test whether quinic acid can derepress the QS inhibition of QF, we applied the drug to the same *QS* transgenic strain. Transgenic larvae fed on quinic acid showed detectable GFP signal after 6 h, which increased over time (**Supplementary Fig. 2**) and was saturated after 24 h of drug application (**Fig. 1k**–**o**). The effective concentration of quinic acid (7.5 mg ml^{-1}, similar to the doses used in *D. melanogaster* and *Neurospora crassa*) did not cause noticeable abnormalities in transgenic worms (Online Methods), and was lower than the concentration of quinic acid naturally present in cranberry juice (>1%)[13]. The derepression effect of quinic acid in nematodes was more rapid than in flies[7] and may be useful for temporally regulating QF-driven transgene expression.

Application of Q system in various tissues

We expressed QF in body-wall muscles of *QUAS::GFP* transgenic worms using the *myo-3* promoter, and it robustly activated the expression of GFP in this tissue (**Supplementary Fig. 3a**). GFP expression was effectively suppressed by also expressing QS in body wall muscles, and the suppression was relieved when we fed the worms with quinic acid (**Supplementary Fig. 3b**,**c**).

We investigated whether we could use this system to express nonfluorescent transgenes. The *dpy-20* gene encodes a nematode-specific zinc-finger protein, which is expressed and required in hypodermal cells for normal body morphology[14]. *dpy-20 (e1282ts)* mutant worms raised at a restrictive temperature (25 °C) exhibited a Dpy (shortened body length) phenotype (body length: wild-type, 1,052 µm ± 40 µm; *dpy-20*, 922 µm ± 14 µm; (±s.e.m.) $n = 40$)[14]. *dpy-20 (e1282ts)* mutants carrying only the *QUAS::dpy-20* transgene still showed the Dpy phenotype (**Supplementary Fig. 4a**; body length: 920 µm ± 17 µm; $n = 40$), but additional expression of QF in hypodermal cells using *dpy-7* promoter rescued the phenotype (**Supplementary Fig. 4b**; body length: 1,085 µm ± 94 µm). Furthermore, rescue was suppressed when we expressed QS in hypodermal cells (**Supplementary Fig. 4c** and **Fig. 1p**; body length: 917 µm ± 25 µm) and the suppression was relieved when we allowed the worms to develop in the presence of quinic acid (data not shown).

Refining expression patterns with a 'not' gate

In addition to permitting precise spatial and temporal control of transgene expression in various tissues, the Q system can also be used to refine spatial control. In *C. elegans,* although some promoter elements are highly specific for a single cell or few cells, most promoters are expressed in many cells[15]. It is desirable to develop specific labeling schemes for the reproducible marking of small subsets of cells. The repressible Q system can meet this need by combining QF and QS into a 'not' gate. For instance, for A-type motor neurons, DA and VA neurons are both labeled when using the *unc-4* promoter, whereas a truncated *unc-4* promoter (*unc-4c*) drives expression only in DA neurons in the ventral nerve cord (M. Vanhoven and K.S., unpublished results; **Supplementary Fig. 5**). However, there is no available promoter to only label

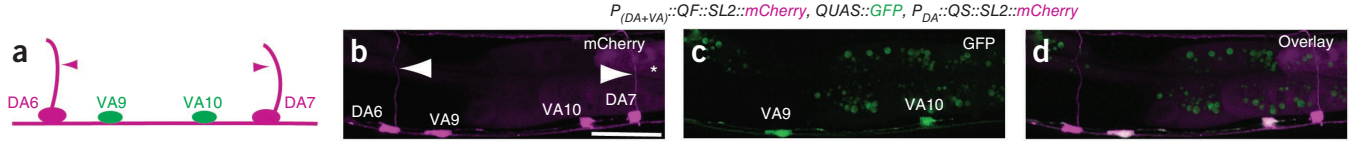

Figure 2 | Refining expression patterns in VA motor neurons with a 'not' gate. (**a**) Schematic diagram of VA and DA motor neurons in the posterior region of the ventral nerve cord. DA neurons extend axonal commissures to the dorsal nerve cord. The *unc-4* promoter was expressed in VA and DA neurons whereas the *unc-4c* promoter was only expressed in DA neurons. (**b**–**d**) Micrographs show the region depicted in **a** in an early L4 larva expressing the indicated transgenes. Scale bar, 20 μm.

VA neurons (**Fig. 2a**). To achieve specific expression in VA neurons, we created transgenic strains that express simultaneously *unc-4::QF* (expression in VA and DA neurons), *unc-4c::QS* (DA neurons only) and *QUAS::GFP*. In the same line, we also used an *SL2::mCherry* cassette fused to *QF* and *QS* to label both DA and VA neurons. In 96% (98/102) of worms of these transgenic strains, we detected GFP only in VA neurons, evident from the lack of commissures in the GFP channel, whereas we detected mCherry signals in both VA and DA neurons (**Fig. 2b**–**d**). Therefore, expression of the QS in DA neurons limited activity of QF to only VA neurons.

Refining expression patterns with an 'and' gate

In *D. melanogaster*, the DNA-binding domain (BD) and transcription-activation domain (AD) domains from GAL4 can be independently expressed using different promoters, and transcriptional activity can be reconstituted in the intersectional subset of two promoters[16]. QF has an analogous organization and contains a BD, a putative dimerization domain (DM) and an AD[17] (**Fig. 3a**). We tested whether QF can be similarly divided into two modules that can be used for intersectional labeling (**Fig. 3b**). We fused a heterodimerizing leucine zipper fragment to each domain to enhance the reconstitution efficiency of active QF[18]. We found that the putative DM domain was required for reconstituted activity (**Supplementary Table 1**). The reconstituted activity of the optimal pair was 42% of intact QF driven by the same promoter, measured as the expression of *QUAS::GFP* in these transgenic strains. Transgenic lines only expressing the individual domains of QF had minimal transcriptional activity (**Fig. 3c**).

To test whether the split Q system can be applied to label the intersectional group of two different promoters, we expressed *QF-AD* from the *mig-13* promoter and *QF-BD-DM* from the *unc-4c* promoter (*wyEx4355*). In the tail region, the construct driven by the *mig-13* promoter was expressed in DA9 and VA12 neurons[19] whereas the construct driven by the *unc-4c* promoter was expressed in DA7, DA8 and DA9 neurons (**Fig. 3d**). All the neurons were labeled by the *SL2::mCherry* cassette fused to sequences encoding the two halves of QF. We found that *QUAS::GFP* was only expressed in the DA9 neuron (61%, 62/103 in this line), which is at the intersection of the expression patterns of *mig-13* and *unc-4c* promoters (**Fig. 3e**–**g**), and no worms (0/103) showed GFP labeling in other tail neurons. Transgenic lines only expressing *mig-13::QF-AD* displayed no GFP signal in DA9 neurons (data not shown). Moreover, the reconstituted activity of QF was suppressed completely in 98% of worms (202/207) by also expressing *unc-4c::QS* introduced by sequential injection (*wyEx4355;wyEx4409*).

Q system functions effectively with single-copy transgenes

Standard transgenic technique in *C. elegans* involves microinjection of exogenous DNA into the gonad, which results in complex extrachromosomal arrays containing high-copy-number transgenes. Although this method is convenient and frequently used, it does not provide stable transgenes or reliable expression because of silencing effects[20]. An alternative transgenic method, *Mos1*-mediated single-copy insertion (MosSCI), uses the transposon *Mos1* to introduce a single-copy, stably inherited transgene[21]. We tested whether the Q system was functional when its

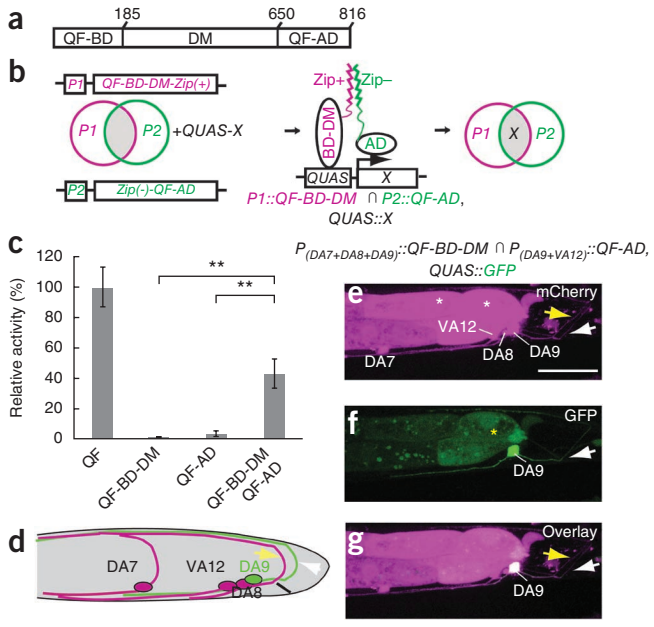

Figure 3 | The split Q system. (**a**) Schematic of QF-BD, putative DM and QF-AD with amino acid positions for each domain indicated. (**b**) Schematic of the split Q system. The QF-BD-DM and QF-AD are driven using promoters *P1* and *P2*. Zip+ and Zip– are leucine zippers that heterodimerize. The transgene *X* is expressed only at the intersection of *P1* and *P2* promoters. ∩ denotes the intersection of *P1* and *P2*. (**c**) Relative transcriptional activities (measured as GFP fluorescence intensity) of the indicated split Q constructs, normalized to the activity measured in strains containing intact QF. All constructs were driven by the same promoter (*mig-13*). Error bars, s.e.m. $^{**}P < 0.01$, $n = 40$, one-tailed *t*-test. (**d**) Schematic of DA and VA neurons in the tail region (left view). The *mig-13* promoter is expressed in DA9 and VA12 neurons, and the *unc-4c* promoter is expressed in DA7, DA8 and DA9 neurons. Yellow and white arrows indicate the commissures of DA8 and DA9, respectively. (**e**–**g**) Micrographs of an early stage-4 larva expressing the indicated transgenes. Yellow and white arrows indicate the commissures of DA8 and DA9, respectively. White asterisks denote ectopic gut fluorescence caused by *SL2::mCherry*. Yellow asterisk denotes occasional ectopic gut fluorescence resulting from *QUAS::GFP*. Scale bar, 20 μm.

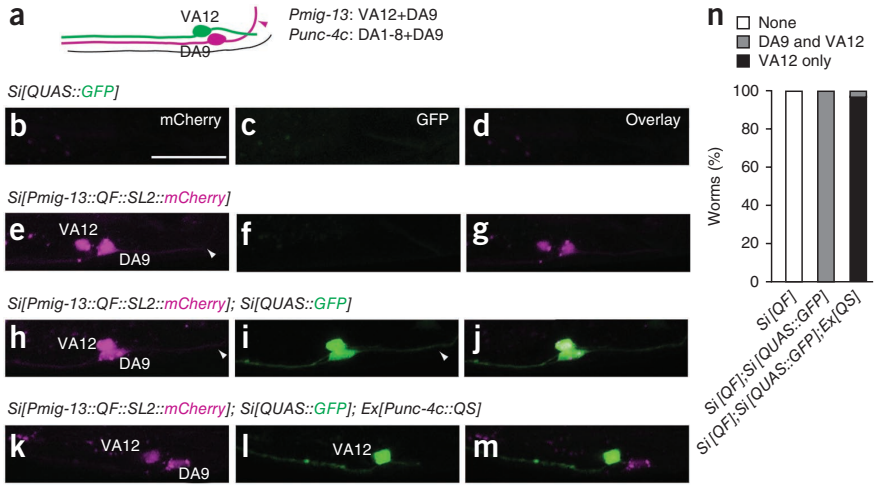

Figure 4 | The Q system functions effectively with single-copy transgene. (**a**) Schematic diagram of DA9 and VA12 neurons in the tail region. The *mig-13* promoter is expressed in DA9 and VA12 neurons whereas the *unc-4c* promoter expresses in the DA9 neuron. Arrowhead, commissure of DA9. (**b**–**m**) The images show the same region as schematically depicted in **a** in transgenic worms (L4 larvae) containing the indicated transgenes. Si, single insertion. Ex, extrachromosomal array. Arrowheads, commissures of DA9. Scale bar, 20 µm. (**n**) Quantification of labeling efficiency in **b**–**m**. Late L3 or early L4 stage larvae were scored ($n > 100$ for each strain). Worms were divided into three categories: none (neither DA9 nor VA12 neurons are labeled by GFP), DA9 and VA12 (both neurons are labeled), and VA12 only (only VA12 neuron was labeled).

components were integrated as MosSCI transgenes. We created two MosSCI transgenes that contain *QUAS::GFP* (*wySi374*) and *Pmig-13::QF::SL2::mCherry* (*wySi377*), respectively. Neither transgene alone yielded a GFP signal (**Fig. 4a**–**g**). However, worms carrying both transgenes consistently showed robust expression of GFP in VA12 and DA9 neurons (100%, 120/120), reflecting the promoter activity of *Pmig-13* (**Fig. 4h**–**j**). In addition, when *QS* driven by the *unc-4c* promoter (expressed in all DA neurons including DA9) was introduced into this strain, the transcriptional activation in DA9 neuron was specifically suppressed by QS, leaving VA12 neuron as the only GFP-expressing neuron in 97% of worms (126/130) (**Fig. 4k**–**n**). These results demonstrate that Q system components are functional when expressed from both array transgenes as well as from integrated, single-copy transgenes.

DISCUSSION

Compared to transgene expression driven directly by promoters, the repressible Q binary system offers several advantages. First, it is difficult to 'turn off' or 'turn down' gene expression by direct promoter-fusion methods. In contrast, QS expression provides an efficient approach to suppress gene expression. With the regulatory role of quinic acid, the Q system can 'turn on' gene expression at any desired time, and combines spatial and temporal control. Second, using a combination of promoters, the Q system can refine transgene expression to more specific subsets of cells. Third, although it is routine to generate transgenic *C. elegans* lines with direct promoter-fusion methods, using new promoters or effectors in each case necessitates that the transgenes must be transformed (and possibly integrated) again. By comparison, a library of single-copy insertion strains containing transgenes expressing various QF drivers and/or *QUAS* effectors can be systematically combined by genetic crosses to generate reproducible expression patterns. The Q system could become an important pillar of the *C. elegans* toolbox as more and more strains containing *QF* and *QUAS*-effector transgenes become available.

Finally, our split Q system affords a high degree of control and can achieve expression even at single-cell resolution. Owing to the complexity and heterogeneity of the nervous system, one bottleneck in understanding neural circuits and behavior is genetic access to specific neurons or groups of neurons, such that one can reproducibly label them with anatomical or developmental markers, express genetically encoded indicators of activity, or selectively silence or activate specific neurons[22]. The split Q system should greatly increase the precision of genetic access to specific neuronal populations. This system could also be applied to other model organisms to more precisely control transgene expression.

METHODS

Methods and any associated references are available in the online version of the paper at http://www.nature.com/naturemethods/.

Note: Supplementary information is available on the Nature Methods website.

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AUTHOR CONTRIBUTIONS

X.W. and K.S. designed the experiments and wrote the paper. X.W. performed all experiments and data analysis. L.L. and C.J.P. provided unpublished information on the Q system and guided experimental design.

COMPETING FINANCIAL INTERESTS

The authors declare no competing financial interests.

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ONLINE METHODS

Expression constructs. Expression clones were made in the pSM vector, a derivative of pPD49.26 (A. Fire; Stanford University) with extra cloning sites (S. McCarroll and C.I. Bargmann; Rockefeller University. *gpd-2 SL2∷mCherry* was PCR-amplified from pBALU12 (ref. 23) in which the kanamycin cassette and N-terminal nuclear localization sequence (NLS) were removed. *QF* was amplified from pCaSpeR4, *QS* was amplified from pACPL-QS and *5xQUAS* was amplified from pQUAS-CD8-GFP[7]. Δ*pes-10* minimal promoter was amplified from pPD97.78 and *myo-3* promoter was from pPD122.66 (A. Fire). The *unc-4* promoter (4 kb)[9], the *unc-4c* promoter (bashed *unc-4* promoter, ~1 kb, M. Vanhoven and K.S., unpublished results), *mig-13* promoter (3.4 kb)[19] and *dpy-7* promoter (218 bp)[24] were amplified from N2 genomic DNA. The *dpy-20* genomic DNA (3 kb, including the entire *dpy-20* gene from initial ATG to stop codon) was amplified from fosmid WRM0616CH07. QF-AD was the C-terminal part (amino acids 650–816) of QF[17], adding GSGSGSGSGSGT linker sequence at N terminus and SV40 NLS at the C terminus. The sequence encoding Zip–antiparallel leucine zipper (AQLEKKLQALEK KLAQLEWKNQALEKKLAQ) was amplified from *CZ-CED-3* (a gift from M. Chalfie; Columbia University)[25], and was fused with *QF-AD* fragment by overlapping PCR, adding NheI sites at 5′ and 3′ ends. QF-BD-DM was the N terminus (amino acids 1–650) of QF, with the addition of SV40 NLS at the N terminus and GSGSGSGSGSGSA linker sequence at the C terminus. The sequence encoding Zip+ antiparallel leucine zipper (ALKKELQ ANKKELAQLKWELQALKKELAQ) was amplified from *CED-3-NZ* (gift from M. Chalfie)[25], and was fused with *QF-BD-DM* fragment by overlapping PCR, adding NheI sites at 5′ and 3′ ends. Details about primer sequences and constructs are available in **Supplementary Note 1**. All plasmids are available upon request.

Strains and transformation. Wild-type worms were *C. elegans* Bristol strain N2. All mutants used in the paper were provided by Caenorhabditis Genetics Center. Strains were maintained using standard methods[26], and worms were grown at 20 °C except lines containing split Q constructs and *dpy-20 (e1282ts)*, which were grown at 25 °C. Normal transgenic lines were made using standard protocols[12]. Transgenic arrays were generated in N2 background except *dpy-20 (e1282ts)* used for Dpy rescue experiments. For each transformation, at least two transgenic lines were obtained showing similar results. MosSCI transformation was performed based on the protocol described in http://sites.google.com/site/jorgensenmossci/[21]. The MosSCI insertion strains EG4322 or EG5003 were used for injection. These single-copy insertions were verified by following the protocol of long-fragment PCR provided by M. Nonet (http://thalamus.wustl.edu/nonetlab/ResourcesF/PCR%20of%20MosSCI%20transgenes.pdf). Strain information is available in **Supplementary Note 2**. Vector maps are available in **Supplementary Note 3**.

Quinic acid desuppression treatment. The fresh quinic acid stock solution (300 mg/ml) was prepared from D-(–)-quinic acid (Sigma-Aldrich, 98%) in sterile Milli-Q water. The stock can be kept in 4 °C for at least one month. Neutralized with 5 M NaOH to pH 6–7, the quinic acid stock solution can be added into nematode growth medium (NGM) agar (7.5 mg/ml) before pouring into Petri plates or onto NGM plates seeded with OP50 directly (per 60 mm × 15 mm Petri dish, 40 µl M9 buffer with ~300 µl quinic acid stock solution, adding ~60–70 µl 5 M NaOH to pH 6.0–7.5. pH was tested by EMD pH-indicator strips, pH 5.0–10.0). Worms were synchronized by hypochlorite bleaching and were cultured on NGM plates with OP50. They were transferred onto quinic acid plates with OP50 6 h, 12 h, 24 h or 30 h before taking images. Worms kept on seeded NGM plates containing quinic acid for five generations exhibited no noticeable abnormalities of morphology, development, brood size, egg-laying behavior and touch response.

Confocal imaging and image quantification. Images of fluorescent proteins were captured in live worms using Plan-Apochromat 40×, 1.3 numerical aperture (NA) objective except the images in **Figure 3e–g** (using Plan-Apochromat 63×, 1.4 NA objective), and images in **Supplementary Figures 3** and **4** (Plan-Apochromat 10×, 0.47 NA objective) on a Zeiss LSM710 confocal microscope (Carl Zeiss). Worms were immobilized on 2% agarose pad using 10 mM levamisole (Sigma-Aldrich) and oriented anterior to the left and dorsal up. For imaging and measuring body length, 0.3 M 2,3-butanedione monoxime (Sigma-Aldrich) was used. Images in **Supplementary Figure 3** are overlays of single fluorescent single plane image and differential interference contrast (DIC) image, and images in **Supplementary Figure 4** are DIC images. All images were taken using Zen2009 (Carl Zeiss) and confocal images were rendered in three dimensions by maximum intensity projection method. Images were adjusted as necessary in Photoshop (Adobe) using cropping and thresholding tools, and assembled into figures using Illustrator (Adobe). For quantification of fluorescence intensity in **Figure 3c**, fluorescence images of ventral nerve cord neurons labeled by *mig-13* promoter (DD and DA neurons) were captured using the same parameters across groups with a 40× objective. The total fluorescence intensity of GFP in each cell body was determined using Image J (US National Institutes of Health) and Excel (Microsoft) by integrating pixel intensity across the cell body region. Forty neurons from 20 early L4 larvae were used to calculate the average fluorescence intensity for each group. The activity was normalized to the percentage of the activity measured in strains containing intact QF (*wyEx4212*).

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Nature Methods

Controlling gene expression with the Q repressible binary expression system in *Caenorhabditis elegans*

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Supplementary Figure 1	Both QF and QUAS are required for binary expression.
Supplementary Figure 2	The time course (6 h and 12 h) of derepression of QS by quinic acid.
Supplementary Figure 3	Q system functions in *C. elegans* body wall muscles.
Supplementary Figure 4	Q system for functional rescue in hypodermal cells.
Supplementary Figure 5	The expression pattern of the *unc-4c* promoter.
Supplementary Table 1	Relative transcriptional activities of primary Split Q constructs tested during design optimization.
Supplementary Note 1	Constructs information.
Supplementary Note 2	Strains information.
Supplementary Note 3	Vector maps containing components of Q system and Split Q system with available cloning sites.

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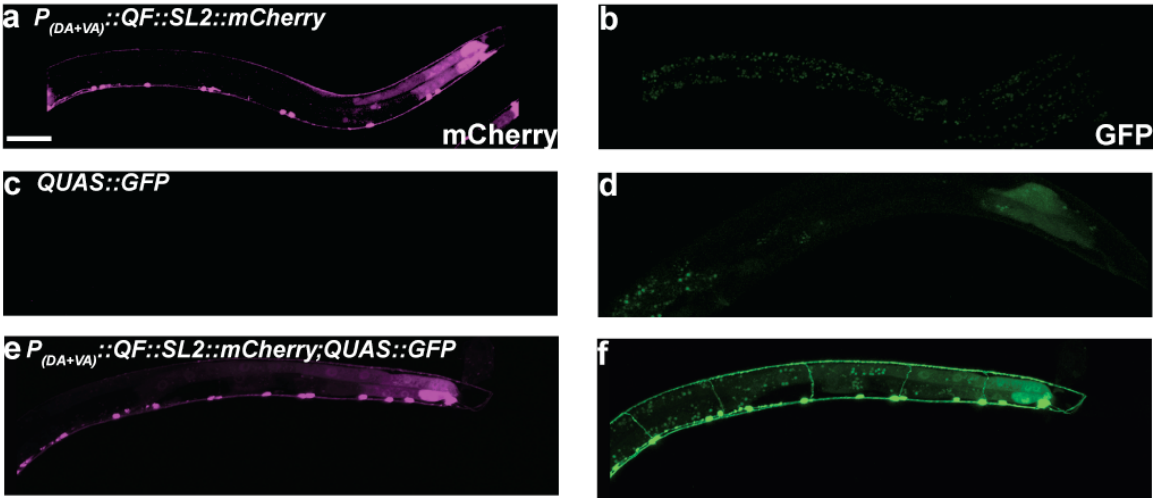
Supplementary Figure 1. Both QF and QUAS are required for binary expression.



(**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.

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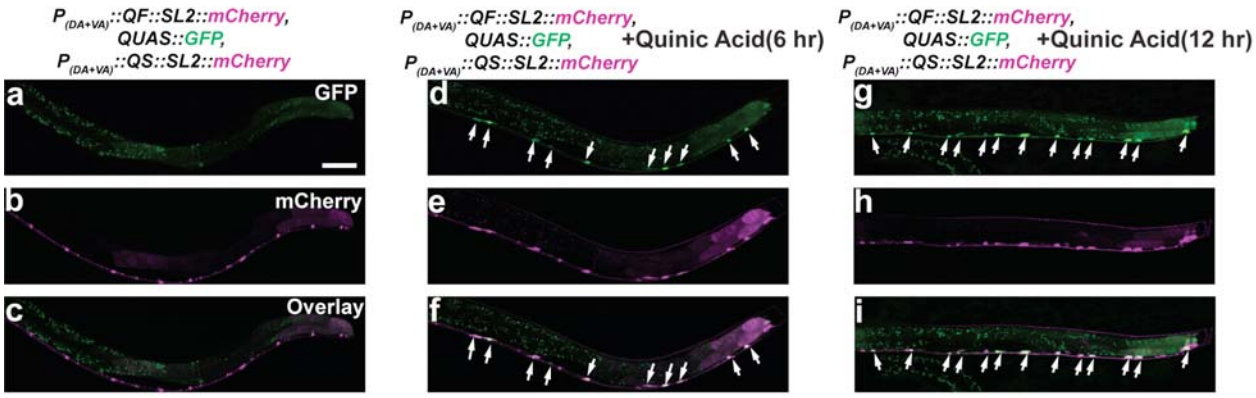
Supplementary Figure 2. The time course (6 hr and 12 hr) of derepression of QS by quinic acid**.**



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.

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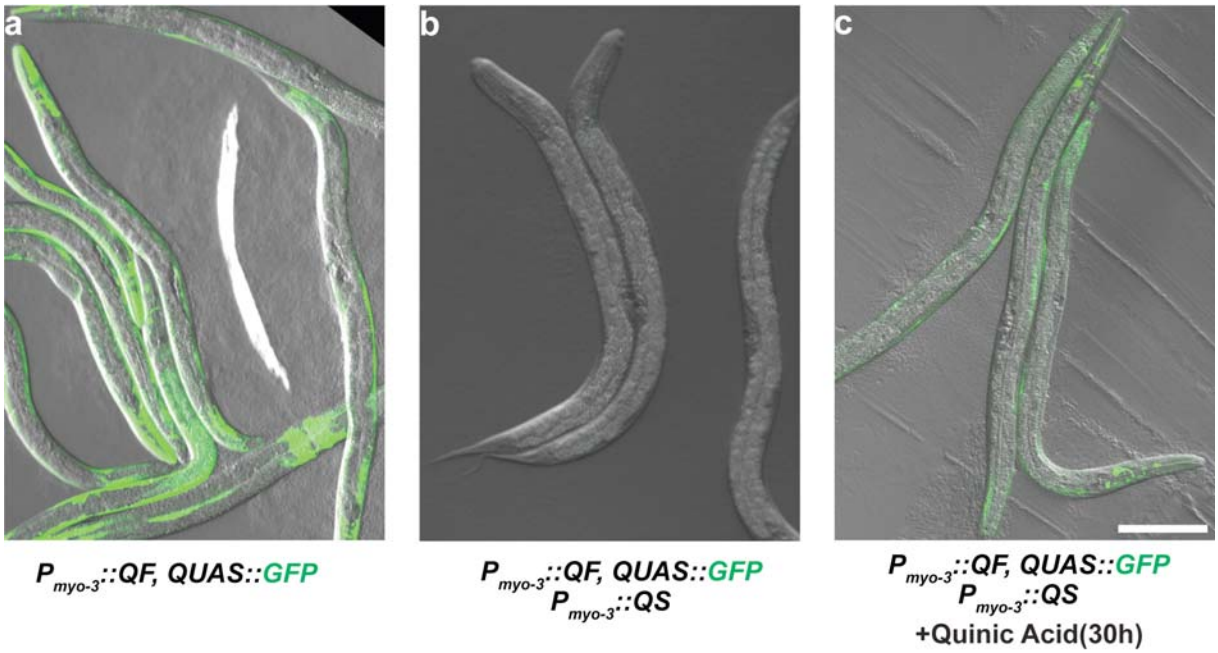
Supplementary Figure 3. Q system functions in *C. elegans* body wall muscles.



(**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.

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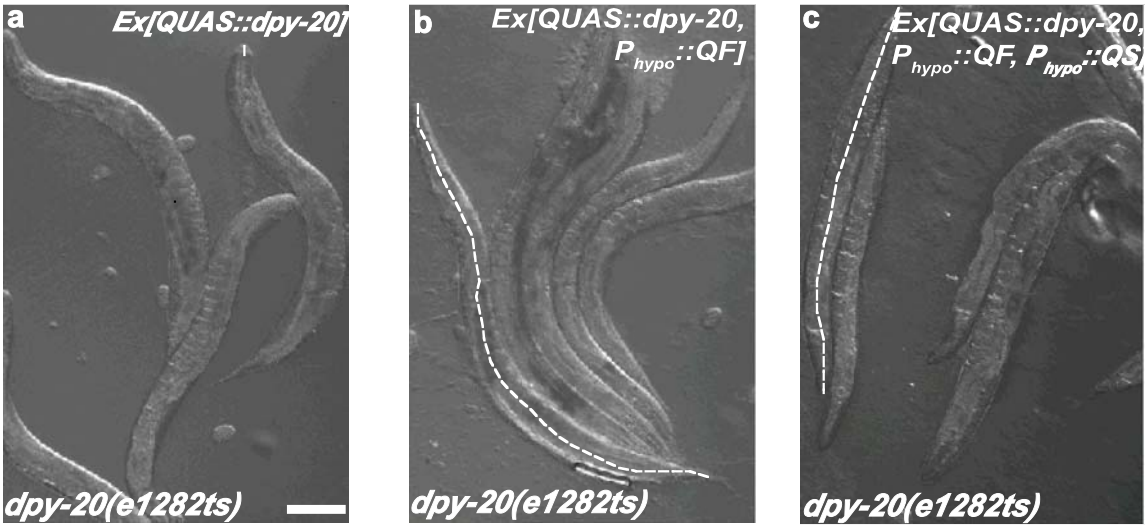
Supplementary Figure 4. Q system for functional rescue in hypodermal cells.



(**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.

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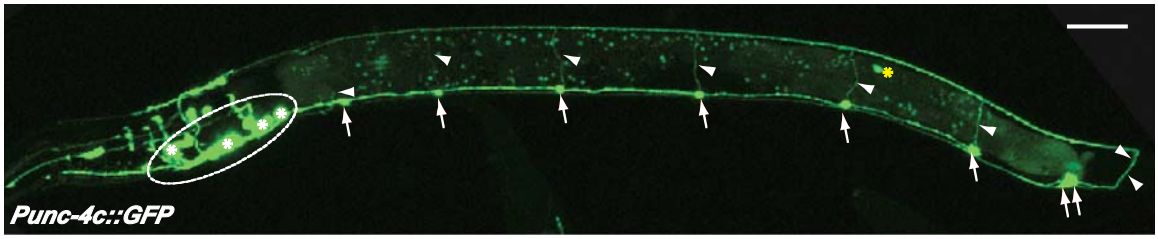
Supplementary Figure 5. The expression pattern of the *unc-4c* promoter.



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.

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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.

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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 Note 3**.

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 Note 3**.

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 Note 3**.

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 Note 3**.

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 Note 3**.

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.

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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*).

Supplementary Note 3. Vector maps containing components of Q system and Split Q system with available cloning sites. See details in **Supplementary Note 1**.