Supporting Information

Wang et al. 10.1073/pnas.1720063115

SI Materials and Methods

Molecular Biology. The plasmid list, DNA and peptide sequences for the four adapters (intein gp41-1, intein *Npu* DnaE, SpyTag/SpyCatcher, and leucine zipper), and the oligo list are included in Tables S1–S3, respectively.

The coding sequences of split inteins gp41-1 (1) and *Npu* DnaE (2) were codon-optimized for *Caenorhabditis elegans* and chemically synthesized (Integrated DNA Technologies). Leucine zipper-NZ was amplified from the genomic DNA of the *C. elegans* strain PY7502; leucine zipper-CZ was amplified from the plasmid TU814 (Addgene 16083). miniSpyCatcher, a shorter but optimized version of the original SpyCatcher (3), was amplified from the plasmid *Phsp16.41*::*lin-3* signal sequence::*SpyCatcher-GFP* (4), and the short SpyTag (5) was obtained by annealing a pair of oligos (Integrated DNA Technologies).

To make the four constructs for *Pmyo-2::NLS::cGAL(DBD)::adapter::unc-54 3′UTR*, the vector pG4US19[*Pmyo-2::NLS::cGAL(DBD)::cGAL(AD)::unc-54 3′UTR*] (6) was digested with AvrII and KpnI, and the adapter (gp41-1-N-intein, DnaE-N-intein, leucine zipper-NZ, or SpyTag) was inserted in-frame at the C terminal of cGAL(DBD), replacing cGAL(AD). To make the four constructs for *Pmyo-2::NLS::adapter::cGAL(AD)::unc-54 3′UTR*, the vector pG4US19[*Pmyo-2::NLS::cGAL(DBD)-cGAL(AD)::unc-54 3′UTR*] (6) was digested with NheI and PstI, and the adapter (gp41-1-C-intein, DnaE-C-intein, leucine zipper-CZ, or miniSpyCatcher) was inserted in-frame at the N terminal of cGAL(AD), replacing cGAL(DBD). Various linker sequences were also included for each construct.

For split cGAL driver plasmids containing the *let-858* 3′UTR, the *NLS::cGAL(DBD)::gp41-1-N-intein* from the plasmid pHW510 and *NLS::gp41-1-C-intein::cGAL (AD)* from pHW511 sequences were subcloned into pHW393 [*Prab-3::NLS::cGAL(DBD)::cGAL(AD)::let-858 3′UTR*] (6) using the enzymes AscI and KpnI to replace "intact" cGAL with each fusion to generate split cGAL driver constructs with the *let-858* 3′UTR. *myo-2*, *hsp-16.41*, *eft-3*, *unc-17*, and *ceh-19b* promoter sequences were inserted into the split cGAL driver constructs with the *let-858* 3′UTR, in between FseI and AscI.

To make the effector construct pHW539 (*15xUAS::kin-2a(G310D)::SL2::gfp::let-858 3′UTR*) with dominant-negative PKA, the mutated *C. elegans* PKA regulatory subunit *kin-2a(G310D)* cDNA was amplified from the plasmid pHW154 (7), and inserted into the vector pJL046 (*15xUAS::HisCl1::SL2::gfp::let-858 3′UTR*) (6) digested with KpnI, replacing HisCl1.

Microscopy. For Fig. S3, animals were imaged with a Zeiss Imager Z2 equipped with an Apotome 2 and an Axiocam 506 Mono camera. Images were captured through a Plan Apochromat 63×/1.4 Oil DIC objective, using ZEN Blue 2.3 software.

Transgenes and Strains. For comparison, each pair of the four *Pmyo-2* split cGAL drivers were injected directly into the strain PS6932 at 10 ng/μL for each half, together with 50 ng/μL of the *unc-119(+)* rescue plasmid as the injection marker. The plasmid pBlueScript KS (+) was included as DNA carrier to bring the total DNA concentration of each injection solution to 100 ng/μL. Refer to Table S1 for the detailed information about the plasmids used in the transgenes. Some of the strains were described in our previous paper on cGAL (6).

1. Dassa B, London N, Stoddard BL, Schueler-Furman O, Pietrokovski S (2009) Fractured genes: A novel genomic arrangement involving new split inteins and a new homing endonuclease family. *Nucleic Acids Res* 37:2560–2573.
2. Iwai H, Züger S, Jin J, Tam P-H (2006) Highly efficient protein trans-splicing by a naturally split DnaE intein from *Nostoc punctiforme*. *FEBS Lett* 580:1853–1858.
3. Li L, Fierer JO, Rapoport TA, Howarth M (2014) Structural analysis and optimization of the covalent association between SpyCatcher and a peptide tag. *J Mol Biol* 426:309–317.
4. Bedbrook CN, et al. (2015) Genetically encoded spy peptide fusion system to detect plasma membrane-localized proteins in vivo. *Chem Biol* 22:1108–1121.
5. Zakeri B, et al. (2012) Peptide tag forming a rapid covalent bond to a protein, through engineering a bacterial adhesin. *Proc Natl Acad Sci USA* 109:E690–E697.
6. Wang H, et al. (2017) cGAL, a temperature-robust GAL4-UAS system for *Caenorhabditis elegans*. *Nat Methods* 14:145–148.
7. Wang H, Sieburth D (2013) PKA controls calcium influx into motor neurons during a rhythmic behavior. *PLoS Genet* 9:e1003831.

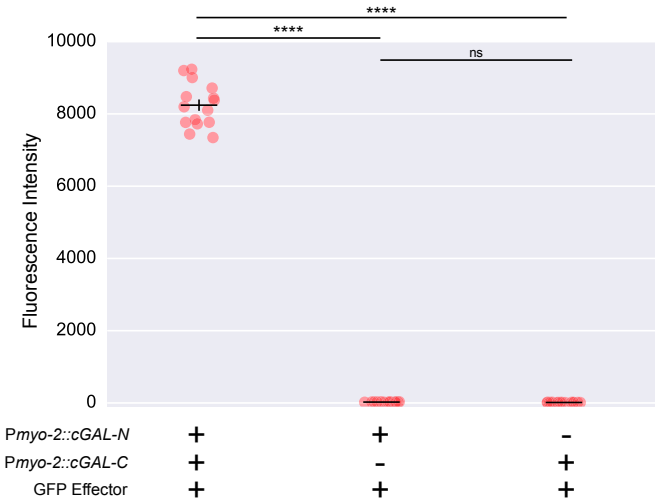

Fig. S1. Activation of the GFP effector is dependent on both components of the split cGAL drivers. Quantification of fluorescence in the pharynx of animals with indicated genotypes. All transgenes are integrated into the genome (*syIs431* for P*myo-2::cGAL-N*; *syIs433* for P*myo-2::cGAL-C*; *syIs300* for GFP effector). +, heterozygote for indicated transgene; −, no indicated transgene. Bars are mean ± SEM $n = 15$ for all three genotypes. ****$P < 0.0001$. ns, not significant. One-way ANOVA with Tukey's correction for multiple comparisons. cGAL-N and cGAL-C represent the two halves of the gp-41-1–mediated split cGAL driver. ns, not significant.

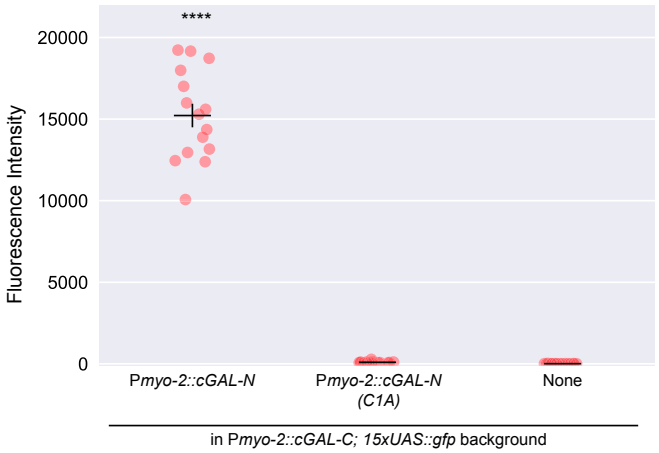

Fig. S2. Successful reconstitution of cGAL requires gp41-1–mediated protein splicing. Quantification of fluorescence in the pharynx of animals with indicated genotypes. Mutating the first cysteine of gp41-1 N-intein to alanine (referred as C1A) disrupts gp41-1–mediated protein splicing (1). The cGAL-C driver and GFP effector are integrated into the genome (*syIs433* for P*myo-2::cGAL-C*; *syIs300* for GFP effector), where P*myo-2::cGAL-N (syEx1589)* and P*myo-2::cGAL-N(C1A) (syEx1590)* are extrachromosomal arrays. Bars are mean ± SEM. For columns from left to right, $n = 15$, 16, 17. ****$P < 0.0001$. One-way ANOVA with Tukey's correction for multiple comparisons. cGAL-N and cGAL-C represent the two halves of the gp-41-1–mediated split cGAL driver.

1. Carvajal-Vallejos P, Pallissé R, Mootz HD, Schmidt SR (2012) Unprecedented rates and efficiencies revealed for new natural split inteins from metagenomic sources. *J Biol Chem* 287: 28686–28696.

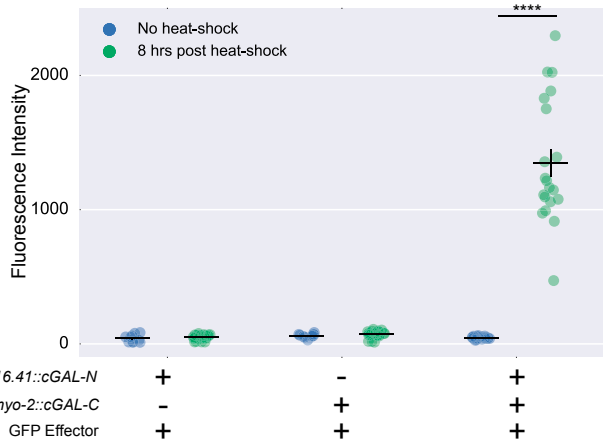

Fig. S3. The conditional expression of GFP in pharyngeal muscles required both *hsp-16.41* and *myo-2* split cGAL drivers, in addition to heat shock. Quantification of fluorescence in the pharynx of animals with indicated genotypes, both with and without heat shock. All transgenes are integrated into the genome (*syIs435* for P*hsp16.41::cGAL-N*; *syIs433* for P*myo-2::cGAL-C*; *syIs300* for GFP effector). +, heterozygote for indicated transgene; –, no indicated transgene. Bars are mean ± SEM $n = 10$, 20, 10, 19, 21, and 20, from left to right. ****$P < 0.0001$. Two-way ANOVA with Sidak's correction for multiple comparisons. cGAL-N and cGAL-C represent the two halves of the gp-41-1–mediated split cGAL driver.

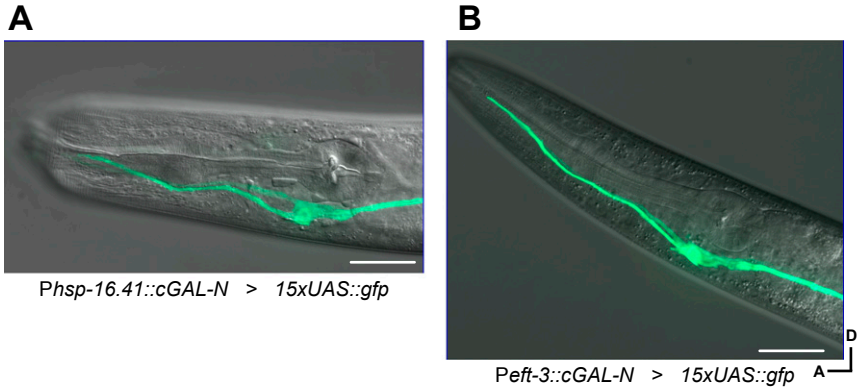

Fig. S4. Nonspecific expression of GFP in the excretory canal cell. (*A*) Merged image of transgenic worms with P*hsp-16.41::cGAL-N; 15xUAS::gfp* (*syIs435; syIs300*), showing GFP expression in the excretory cell 24 h after heat shock treatment. (*B*) Merged image of transgenic worms with P*eft-3::cGAL-N; 15xUAS::gfp (syEx1581; syIs300)*, showing GFP expression in the excretory cell. (Scale bar, 20 μm.) cGAL-N represents the split cGAL half cGAL(DBD)-gp41-1-N-intein.

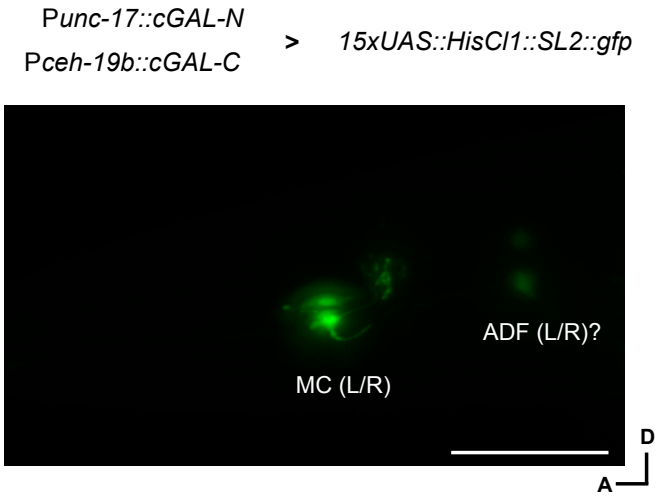

Fig. S5. The split cGAL drivers for MC neurons weakly drive expression in ADF. Fluorescence imaging showing transgenic worms with P*unc-17::cGAL-N,* P*ceh-19b:: cGAL-C; 15xUAS::HisCl1::SL2::gfp (syIs483; syIs371),* had strong GFP expression in the MC neurons and weak GFP expression in suspected ADF neurons. (Scale bar, 20 μm.) cGAL-N and cGAL-C represent the two halves of the gp-41-1–mediated split cGAL driver.

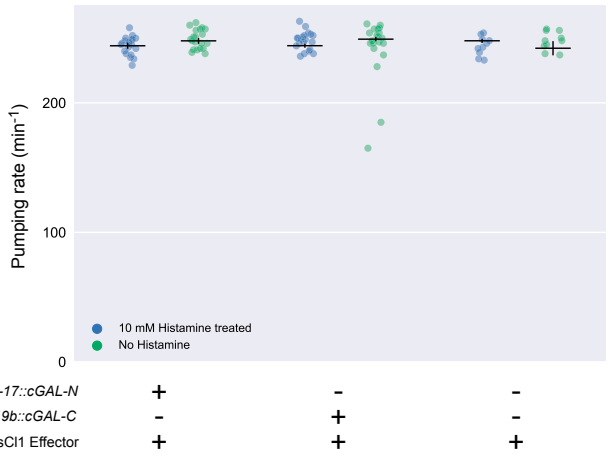

Fig. S6. Neither split cGAL drivers for MC neurons alone is sufficient to reduce pumping rate. Quantification of pumping rate of animals with indicated genotype, treated with or without 10 mM histamine. P*unc-17::cGAL-N* (*syEx1601* and *syEx1602*) and P*ceh-19b::cGAL-C* (*syEx1603* and *syEx1604*) are extrachromosomal arrays, and HisCl1 effector is integrated line (*syIs371*). +, presence of indicated transgene; −, absence of indicated transgene. Bars are mean ± SEM $n = 20$, 20, 20, 20, 10, and 10, for columns from left to right. Results are not significant by two-way ANOVA with Bonferroni correction. cGAL-N and cGAL-C represent the two halves of the gp-41-1–mediated split cGAL driver.

Table S1. Plasmids used in this study

Plasmid name	Plasmid information
pG4US19	*Pmyo-2::NLS::cGAL(DBD)::cGAL(AD)::unc-54 3′UTR*
pHW375	*Pmyo-2::NLS::cGAL(DBD)::SpyTag::unc-54 3′UTR*
pHW378	*Pmyo-2::NLS::miniSpyCatcher::cGAL(AD)::unc-54 3′UTR*
pHW438	*Pmyo-2::NLS::cGAL(DBD)::DnaE-N-intein::unc-54 3′UTR*
pHW439	*Pmyo-2::NLS::DnaE-C-intein::cGAL(AD)::unc-54 3′UTR*
pHW508	*Pmyo-2::NLS::cGAL(DBD)::leucine-zipper-NZ::unc-54 3′UTR*
pHW509	*Pmyo-2::NLS::leucine-zipper-CZ::cGAL(AD)::unc-54 3′UTR*
pHW510	*Pmyo-2::NLS::cGAL(DBD)::gp41-1-N-intein::unc-54 3′UTR*
pHW511	*Pmyo-2::NLS::gp41-1-C-intein::cGAL(AD)::unc-54 3′UTR*
pHW522	*Prab-3::NLS::gp41-1-C-intein::cGAL(AD)::let858 3UTR*
pHW530	*Prab-3::NLS::cGAL(DBD)::gp41-1-N-intein::let-858 3′UTR*
pHW531	*Peft-3::NLS::gp41-1-C-intein::cGAL(AD)::let-858 3′UTR*
pHW533	*Peft-3::NLS::cGAL(DBD)::gp41-1-N-intein::let-858 3′UTR*
pHW539	*15xUAS::kin-2a(G310D)::SL2::gfp::let-858 3′UTR* *PKA dominant negative effector construct*
pHW564	*Pmyo-2::NLS::cGAL(DBD)::gp41-1-N-intein(C1A)::let-858 3′UTR*
pAH34	*Phsp-16.41::NLS::cGAL(DBD)::gp41-1-N-intein::let-858 3′UTR*
pAH35	*Pmyo-2::NLS::cGAL(DBD)::gp41-1-N-intein::let-858 3′UTR*
pAH36	*Pmyo-2::NLS::gp41-1-C-intein::cGAL(AD)::let-858 3′UTR*
pJL080	*Punc-17::NLS::cGAL(DBD)::gp41-1-N-intein::let-858 3′UTR*
pJL081	*Pceh-19b::NLS::gp41-1-C-intein-cGAL(AD)::let-858 3′UTR*
unc-119(+) rescue plasmid	*Injection marker, used to rescue the Unc phenotype of the unc-119(ed3)*
KP708	*Pttx-1::rfp, injection maker, red fluorescence in the AIY neurons*
KP1368	*Pmyo-2::NLS::mCherry, injection marker, red fluorescence in the nuclei of pharyngeal muscles*
Punc-112::rfp	*Injection marker, red fluorescence in coelomocytes*
Punc-122::gfp	*Injection marker, green fluorescence in coemolocytes*
pBlueScript KS(+)	*DNA carrier for injection solution*

Notes: (*i*) For new cGAL drivers and effectors, we recommend using backbones with the *let-858* 3′UTR. (*ii*) The sequences of cGAL(DBD), the DNA binding domain of Gal4p from *Saccharomyces kudriavzevii* and cGAL(AD), the synthetic transcription activation domain VP64, were described previously (1). (*iii*) cGAL-N, cGAL-C in the paper denote NLS::cGAL(DBD)::gp41-1-*N*-intein and NLS::gp41-1-C-intein::cGAL(AD), respectively. (*iv*) pHW522, pHW530, pHW531, pHW533, pHW539, pHW564, pAH34, pAH35, and pAH36 are deposited in Addgene (https://www.addgene.org).

1. Wang H, et al. (2017) cGAL, a temperature-robust GAL4-UAS system for *Caenorhabditis elegans*. *Nat Methods* 14:145–148.

Table S2. Sequences of the four pairs of adapters used in this study

Adapter	DNA sequence	Protein sequence
gp41-1-N-intein	TGCCTCGACCTCAAGACCCAAGTCCAAACCCCACAAGGAATGAAGG- AGATCTCCAACATCCAAGTCGGAGACCTCGTCCTCTCCAACACC- GGATACAACGAGGTCCTCAACGTCTTCCCAAAGTCCAAGAAGAA- GTCCTACAAGATCACCCTCGAGGACGGAAAGGAGATCATCTGCT- CCGAGGAGCACCTCTTCCCAACCCAAACCGGAGAGATGAACATC- TCCGGAGGACTCAAGGAGGGAATGTGCCTCTACGTCAAGGAG	CLDLKTQVQTPQGMKEISNIQVGDLVLSNTG- YNEVLNVFPKSKKKSYKITLEDGKEIICS- EEHLFPTQTGEMNISGGLKEGMCLYVKE
gp41-1-C-intein with "SSDV" extein sequence	ATGATGTTGAAGAAGATTCTCAAAATTGAAGAACTGGATGAGCGTG- AGCTCATCGACATCGAGGTCTCCGGAAACCACCTCTTCTACGCC- AACGACATCCTCACCCACAACTCCTCCTCCGATGTA	MMLKKILKIEELDERELIDIEVSGNHLFYA- NDILTHNSSSDV
Npu DnaE-N-intein	TGCCTTTCCTACGAAACTGAAATTTTAACTGTTGAATATGGACTCC- TCCCAATCGGAAAGATCGTCGAGAAGCGTATCGAGTGCACCGTC- TACTCCGTCGACAACAACGGAAACATCTACACCCAACCAGTCGC- CCAATGGCACGACCGTGGAGAGCAAGAGGTCTTCGAGTACTGCC- TCGAGGACGGATCCCTCATCCGTGCCACCAAGGACCACAAGTTC- ATGACCGTCGACGGACAAATGCTCCCAATCGACGAGATCTTCGA- GCGTGAGCTCGACCTCATGCGTGTCGACAACCTCCCAAAC	CLSYETEILTVEYGLLPIGKIVEKRIECTV- YSVDNNGNIYTQPVAQWHDRGEQEVFEY- CLEDGSLIRATKDHKFMTVDGQMLPIDE- IFERELDLMRVDNLPN
Npu DnaE-C-intein with "CFN" extein sequence	ATGATTAAGATTGCTACGAGGAAATATTTGGGAAAACAAAACGTCT- ACGACATCGGAGTCGAGCGTGACCACAACTTCGCCCTCAAGAAC- GGATTCATCGCCTCCAACTGCTTCAAC	MIKIATRKYLGKQNVYDIGVERDHNFALKN- GFIASNCFN
GGGGS-linker- SpyTag	GGTGGAGGTGGATCAGCCCACATCGTGATGGTGGACGCCTACAAGC- CCACCAAG	GGGGSAHIVMVDAYKPTK
MiniSpyCatcher-GGGGS-linker	GATAGTGCTACCCATATTAAATTCTCAAAACGTGATGAGGACGGCA- AAGAGTTAGCTGGTGCAACTATGGAGTTGCGTGATTCATCTGGT- AAAACTATTAGTACATGGATTTCAGATGGACAAGTGAAAGATTT- CTACCTGTATCCAGGAAAATATACATTTGTCGAAACCGCAGCAC- CAGACGGTTATGAGGTAGCAACTGCTATTACCTTTACAGTTAAT- GAGCAAGGTCAGGTTACTGTAAATGGCGGAGGAGGCGGAAGT	DSATHIKFSKRDEDGKELAGATMELRDSSGK- TISTWISDGQVKDFYLYPGKYTFVETAAP- DGYEVATAITFTVNEQGQVTVNGGGGGS
GSGSG-linker-leucine zipper-NZ	GGCTCTGGCTCTGGCGCTCAGCTTAAGAAAGAGCTGCAGGCAAACA- AGAAAGAGCTGGCTCAGCTGAAGTGGGAACTGCAGGCACTGAAG- AAAGAACTGGCTCAG	GSGSGAQLKKELQANKKELAQLKWELQALK- KELAQ
leucine zipper-CZ-GGSG-linker	GCACAGCTGGAGAAGAAACTGCAGGCTCTGGAGAAGAAACTGGCAC- AGCTGGAGTGGAAAAACCAGGCACTGGAGAAGAAACTGGCACAG- GGTGGAAGCGGT	AQLEKKLQALEKKLAQLEWKNQALEKKLAQ- GGSG

The underlined text indicates the linker sequences (GGGGS, GSGSG or GGSG), the extein sequence (SSDV) from gp41-1, and the extein sequence (CFN) from *Npu* DnaE.

Table S3. Oligos used in this study

Targets	Oligos
gp41-1-N-intein	oHW383f: cgCTGCAGtctggtggcggaggggctCCTAGGTGCCTCGACCTCAAGACCCAAG
	oHW384r: gatgcgGAGCTCagatatcaataccatGGTACCTTACTCCTTGACGTAGAGGCACATTC
gp41-1-C-intein with "SSDV" extein sequence	oHW385f: atgggccctaaaaagaagcgtaaaGCTAGCATGATGTTGAAGAAGATTCTC
	oHW386r: CTAGGagcccctccgccaccagaCTGCAGTACATCGGAGGAGGAGTTGTGGGTGAGGATG
Npu DnaE-N-intein	oHW305f: cgCTGCAGtctggtggcggaggggctCCTAGGTGCCTTTCCTACGAAACTGA
	oHW306r: gatgcgGAGCTCagatatcaataccatGGTACCTTAGTTTGGGAGGTTGTCGACACG
Npu DnaE-C-intein with "CFN" extein sequence	oHW307f: atgggccctaaaaagaagcgtaaaGCTAGCATGATTAAGATTGCTACGAGGAA
	oHW308r: GAACCCCTAGGagcccctccgccaccagaCTGCAGGTTGAAGCAGTTGGAGGCGATG
GGGGS-linker SpyTag	oHW195f: ctaggGGTGGAGGTGGATCAGCCCACATCGTGATGGTGGACGCCTACAAGCCCACCAAGTAAggtac
	oHW196r: cTTACTTGGTGGGCTTGTAGGCGTCCACCATCACGATGTGGGCTGATCCACCTCCACCc
MiniSpyCatcher-GGGGS-linker	oHW193f: ccccGCTAGCGATAGTGCTACCCATATTAAATTC
	oHW194r: ccccCTGCAGACTTCCGCCTCCTCCGCCATTTACAGTAACCTGAC
GSGSG-linker-leucine zipper-NZ	oHW387f: gCTGCAGtctggtggcggaggggctCCTAGGGGCTCTGGCTCTGGCGCTCAGCTTAAG
	oHW388r: atgcgGAGCTCagatatcaataccatGGTACCTCACTGAGCCAGTTCTTTCTTCAG
Leucine zipper-CZ-GGSG-linker	oHW389f: atgggccctaaaaagaagcgtaaaGCTAGCATGGCACAGCTGGAGAAGAAACTGC
	oHW390r: CTAGGagcccctccgccaccagaCTGCAGACCGCTTCCACCCTGTGCCAG
myo-2 promoter	Forward: ccccGGCCGGCCgtagtatcctttgctttaaatgtccata
	Reverse: aaaaGGCGCGCCttctgtgtctgacgatcgagggt
hsp-16.41 promoter	Forward: gaaGGCCGGCCACGTTGAGCTGGACGGAAATAG
	Reverse: gtgGGCGCGCCTTTCGAAGTTTTTTAGATGCACTAG
unc-17 promoter	oJL085: aaacGGCCGGCCgttcacatcccccgaaatttcc
	oJL086: tttgGGCGCGCCctgaaaattaaatattttagtgtaaaacttt
ceh-19b promoter	ceh-19bpF: cacaGGCCGGCCgcatcacacaaaccttcaaagtata
	ceh-19bpR: aaacGGCGCGCCttttcaatagtttttatttaaaagactttaagaaaatg
rab-3 promoter	oHW147f: aaaaGGCCGGCCgatcttcagatgggagcagtg
	oHW133r: aaaaGGCGCGCCctgaaaatagggctactgtag
eft-3 promoter	oHW406f: gcatgcgcggccgcactgactgGGCCGGCCGCACCTTTGGTCTTTTATTGTC
	oHW112r: aaaaGGCGCGCCTGAGCAAAGTGTTTCCCAACTG
kin-2a(G310D) cDNA	oHW435f: gacccttgGCTAGCgtcgacGGTACCggtaaaaATGTCGGGTGGAAACGAAG
	oHW436r: gaaagtaggatgagacagcTACGGTACCTTAGGTCATCAGTTTGACG

Other Supporting Information Files

SI Appendix (PDF)