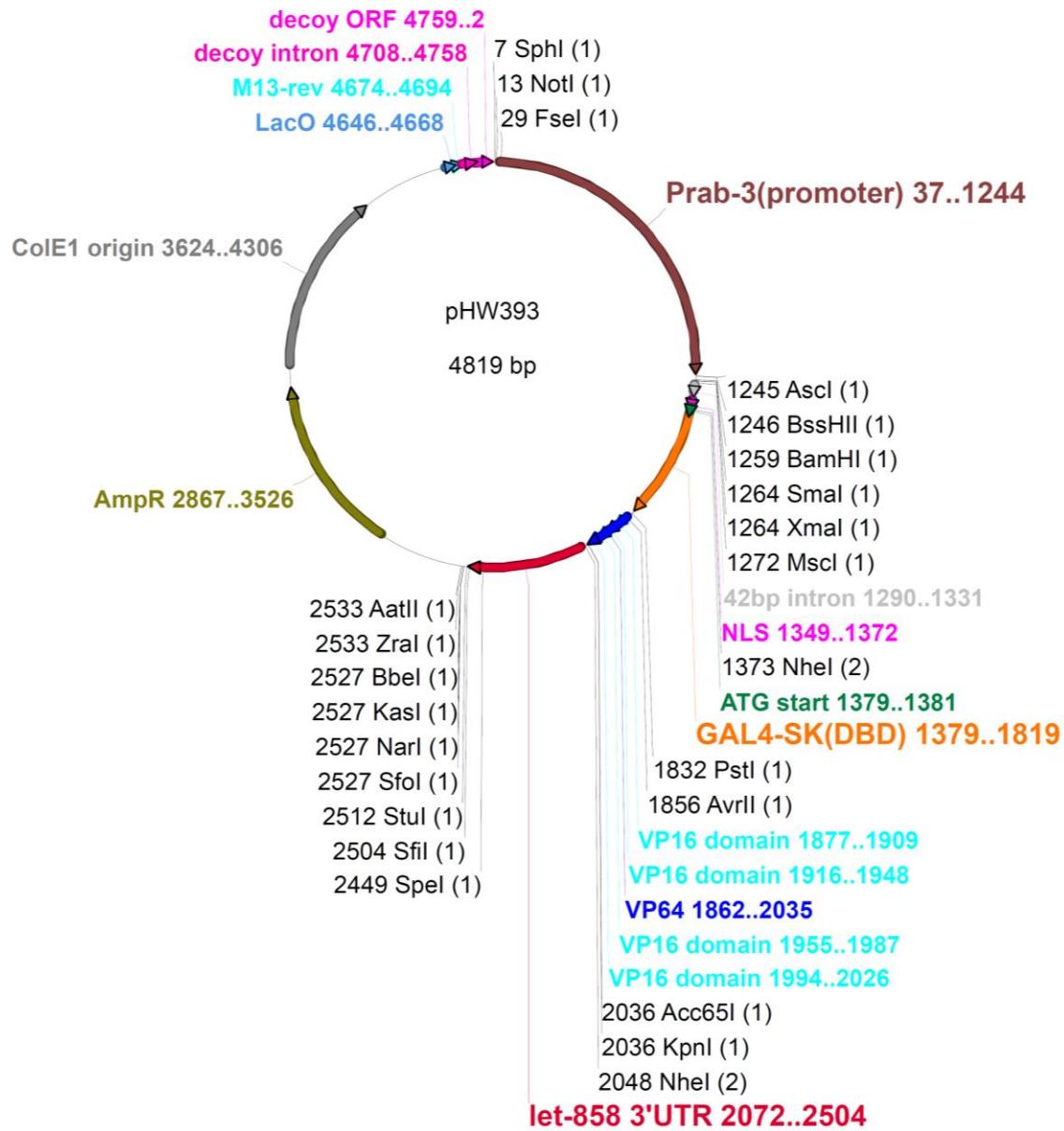


Supplementary Information

cGAL, a Temperature-Robust GAL4-UAS System for *C. elegans*

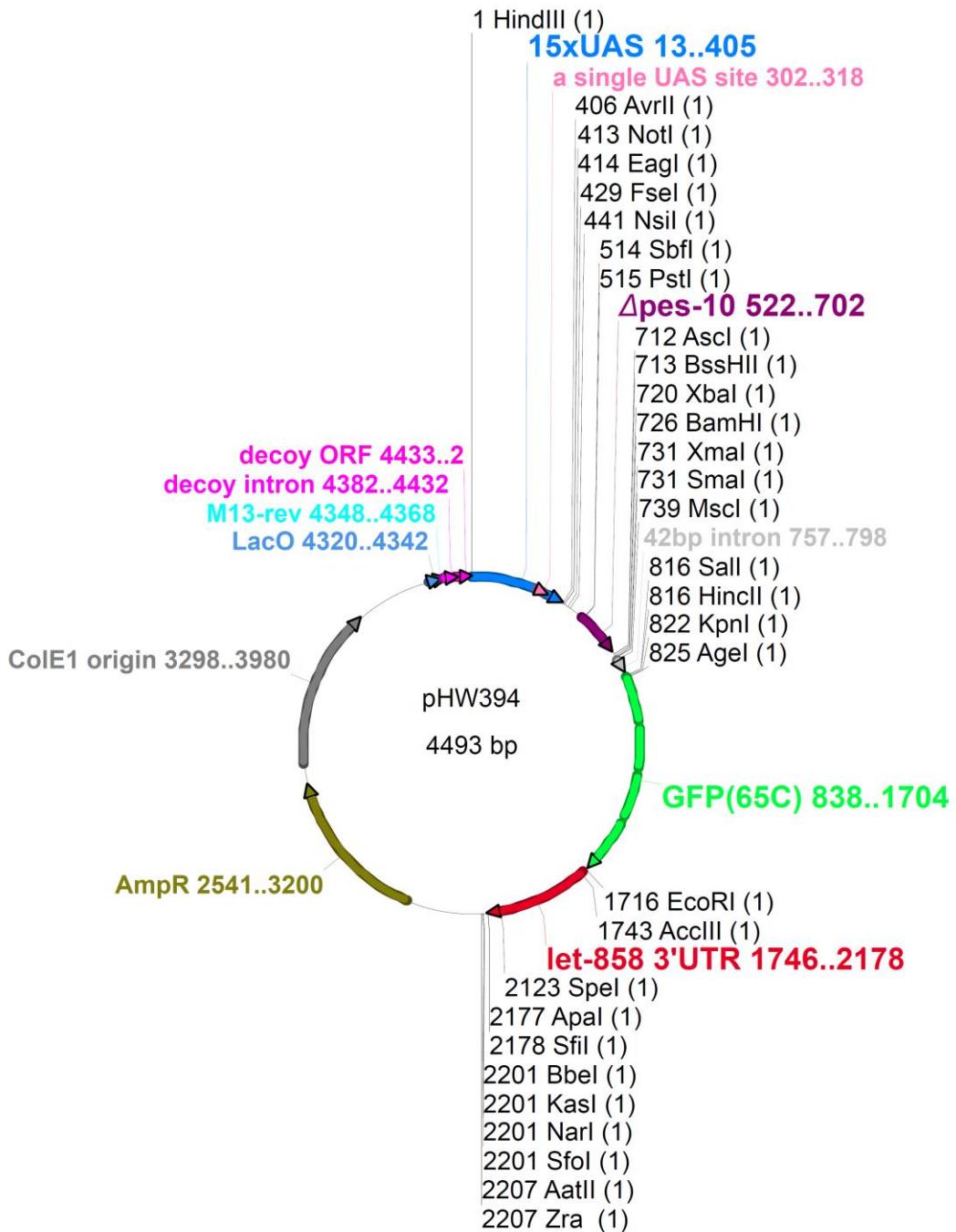
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*These authors contributed to this work equally.



Supplementary Note 1 | Vector Map of the driver construct pHW393

(pHW393) *Prab-3::nls::GAL4-SK(DBD)::VP64::let-858 3'UTR*. New driver constructs can be generated by cloning new promoters into the MCS, between SphI/NotI/FseI and Ascl/BssHII/BamHI/SmaI/XmaI/Mscl. The backbone of pHW393 (between SphI and KpnI) is derived from the plasmid pPD117.01 from the Fire lab *C. elegans* Vector Kit 1997. pPD117.01 has a 5' decoy and the *let-858* 3'UTR. The 5' decoy contains a splice acceptor (decoy intron) and a short terminated coding region (decoy ORF) upstream of the MCS, which was designed to catch any upstream transcription with a 3' splice site. Adding this 5' decoy sequence was shown to decrease ectopic expression in the posterior gut (A. Fire, personal communication). We highly recommend using the pPD117.01 vector for new neuronal driver constructs.



Supplementary Note 3 | Vector map of the effector construct pHW394

(pHW394) 15xUAS::Δpes-10::gfp::let-858 3'UTR. New effector constructs can be generated by cloning new effector genes into the MCS, between Sall/Hincll/KpnI/Agel and EcoRI/Acclll. The backbone of pHW393 (between Spel and KpnI) is derived from the plasmid pPD117.01 from the Fire lab C. elegans Vector Kit 1997. pPD117.01 has a 5' decoy and the let-858 3'UTR. The 5' decoy contains a splice acceptor (decoy intron) and a short terminated coding region (decoy ORF) upstream of the MCS, which was designed to catch any upstream transcription with a 3' splice site. Adding this 5' decoy sequence was shown to decrease ectopic expression in the posterior gut (A. Fire, personal communication).

1 AAGCTTGCTAGCccatgattacggcaagctacggcgaggactgtcctccgagcggaggactgtcctccgagcggaggactgtcctccgagcggaggact
101 tgcggatcccggcggatgttcgcgagcgaggactgtcctccgagcggaggactgtcctccgagcggaggactgtcctccgagcggaggactgtc
201 tccgagcgaggactgtcctccgagcgaggactgtcctccgagcggaggactgtcctccgagcggaggactgtcctccgagcggaggactgtc
301 gcgaggactgtcctccgagcgaggactgtcctccgagcggaggactgtcctccgagcggaggactgtcctccgagcggaggactgtcctccgagcg
401 gactCCATGGGCAGGCGCActgactGGCCGGCCaaaatgcataagggttttgtcggactgttttatcttcgtgcaccacatccgactggca
501 gtctccgaacggcctgcaggcgatgttttgcatttgcgcattttttgcatttgcgcatttttgcatttgcgcattttttgcatttgcgcattttgc
601 ttcatgttgcatttttttgcattttttgcattttttgcatttgcgcatttttgcatttgcgcatttttgcatttgcgcattttttgcatttgc
701 aggcatttgcgcgcctcttagggatccccggattggccaaaggccaaaggatgtttgcattttgcattttgcatttgcgcatttttgcatttgc
801 ggaccccttgCTAGCgtcgcacGGTACCCggtagaaaaaaATGAGTAAGGAGAAGAACCTTTACTGGAGTTGTCCAATTCTTGATGGATGGT
901 GTTAATGGGCACAAAATTTCCTGTCAGTGAGGAGGGTGAAGGTGATGCAACATACGGAAAATCTACCTTAAATTATTTGCACTACTGGAAA
1001 ACCTGTTGTGATACTTCAACATGGGAGAACTTATTTGCACTACTGGAAAATCTACCTTAAATTATTTGCACTACTGGAAAATCTACCTG
1101 TTCCATGGGataagtttacatataactaactaacccatttgcattttttgcattttttgcattttttgcattttttgcattttttgcatttttgc
1201 CTCGAGATACCCAGATCATATGAAACCGCATGACTTTCAAGAGTGCCTGGCAGGAGTATGACAGAAAACTATTTCAAAAGATGACGG
1301 AACTCACAGACAcgttaatgcatt
1401 ATAGAACATGAGTAAAGGTATTGATTTAAAGAAAGATGGAAAATCTTGACAAACTATACACAAATGTATACATCATGGC
1501 AGACAAACAAAAGAATGGAATCAAAGGTTaaatgcatt
1601 ATTGAAGATGGAAGCGTCAACTAGCAGACCATTTACACAAAATCTCAACATTGGCAGTGGCTCTTACCATCAGACAACCATTACCTG
1701 CCATGGCCTTCGAAAGATCCACGAAAAGAGAGACACATGGCTCTTCTGGAGTTGAAACAGCTGCTGGGATTACACATGGCATGGAT
1801 GAAATTAGCatttgcatt
1901 CAAATTAGCTTATTATTTATTTCTCTTGTGTTTTTTCTCTCTAAATTAAATTCATCCAAATCTAAATTTTAAATTACTGAAATT
2001 ggaaacacacacaaaggatgttttgcatt
2101 ttatatatagaggaggaggaaactagttt
2201 tt
2301 GGGCGCGACGTAGGTGCACTTTGGGAAATGCGCGAACCCCATTGTTATTTCTAAATACATCCTAAATATGTTACCGCTCATG
2401 GAACTGCGTCAATATTTGAAAGAGATGAGTATTCAACATTCGCGCTTATCCCTTTTGCGGCATTTGCCTTC
2501 CTGTTTGTCACCCAGAACAGCTGGTAAAGTAAAAGATGCTGAAGATCAGTGGGTGACAGTGGTACATCGAACTGGATCTCA
2601 AACAGCCTTCGCCCCGAAAGAACGTTTCAATGAGGAGCTGGTTGAGTACTCACCAGTCACAGAAAAGCATCTACGGATGC
2701 GAGCAACTCGGTGCCATAACCATGAGTAAACACTCGGCCACTTACTCTGACAAACGATCGGAGGACGAAGGAGCTAAC
2801 GGGGATCATGTAACCTGCCTTGATCTGGGAAACCGGAGTGAAGATCAGTGGGTGACACCCAGTGCCTGAGCAAC
2901 GACGTTGGCAGGAAACTTAACTGGGAGACTACTACACGGGACTACGCTTCGGGAAACAAATTAATGAGCTGGGAG
3001 TGGGGGGGGAGGCGGG
3101 GCTCGGCCCTTCCGCGCTGGTTATTGCTGATAAAATCTGGGAGCTGGGGGTGAGCTGCTGATTCGGGAGTAC
3201 CGCCCGTATGTTACTACACGACGGGAGTCAGGCAACTATGGTGAACGAAATAGACAGATCTGGGAGATGGCTCACT
3301 TAATGCAAGCTGG
3401 GGCACGCTCTGCGGGCTTCCGGGGGAGGG
3501 CGCGACAGGAAACGATCTGGCTTCTGG
3601 TACCAACTGCTGGGAGTGGGCTTCCGG
3701 TTCGGTACACAGCCGACTTGAGGCGAAGCCTACACCGGAACTGAGGAGATACTACAGCGTGGAGAACGGG
3801 CGGGGACGGTAGTGGCTTCCGGGTGGGAGGG
3901 CGCCACCTCTGACTGGCTGCTACATGTTCTGTGCTGCTGG
4001 GGCGGGCTTTTGCTACATGTTCTGTGCTTACCTGGGTTATTCTGTTGGGGGATACCTGATTCGGCTTGTGGGGGGGG
4101 CGAACGACCGAGCGCAGCGAGTGGAGGGAGGAGGG
4201 GGCACGACAGGGTTCCCGACTGGAAAGCGGGGAGTCAGGAGCGAACGCAATTAGTGTAGCTCACTCATTAG
4301 CGCGCTCGTATGTTGTTGGGGATAACAAATTTCACACAGGAAACAGCTATGACCATGATTACGCCAACGCTTAC
4401 tactaacttaactattctcatt

Supplementary Note 4 | Sequence of the effector construct pHW394

(pHW394) 15xUAS::Δpes-10::gfp::let-858 3'UTR is available from Addgene (www.addgene.org; plasmid no. 85584). The color scheme here matches the vector map of pHW394 shown in Supplementary Note 3.

Supplementary Note 5 | Information for extrachromosomal arrays and integrants

The co-injection markers used include *KP708* (*Ptx-3::rfp*), *KP1369* (*Pmyo-2::nls::mCherry*), *KP1106* (*Pmyo-2::nls::gfp*), *unc-119(+)* rescue plasmid, *Pofm-1::rfp* and *Punc-122::gfp*.

All initial descriptions of extrachromosomal arrays (**syEx####**) and integrants (**syls####**) are highlighted for convenience. All integrants were generated by X-ray irradiation.

A full list of the available integrated drivers and effectors are listed in Supplementary Table 1.

syEx1452 [$15 \times UAS::\Delta pes-10::gfp::unc-54$ 3'UTR, 25ng/ μL ; *Ptx-3::rfp*, 40ng/ μL ; *pBlueScript*, 35ng/ μL], injected into N2, used to generate **syls300** and **syls302**.

syEx1431 and **syEx1432** [*Pmyo-2::GAL4_{SC}::VP16::unc-54* 3'UTR, 10ng/ μL ; *unc-119(+)*, 50ng/ μL ; *pBlueScript*, 40 ng/ μL], injected into the strain *unc-119(ed3)*; **syls300**.

syEx1433 and **syEx1434** [*Pmyo-2::GAL4_{SC}::VP64::unc-54* 3'UTR, 10ng/ μL ; *unc-119(+)*, 50ng/ μL ; *pBlueScript*, 40 ng/ μL], injected into the strain *unc-119(ed3)*; **syls300**.

syEx1435 and **syEx1436** [*Pmyo-2::GAL4_{SK}::VP64::unc-54* 3'UTR; 10ng/ μL , *unc-119(+)*, 50ng/ μL ; *pBlueScript*, 40 ng/ μL], injected into the strain *unc-119(ed3)*; **syls300**.

syEx1437 and **syEx1438** [*Pmyo-2::gfp::unc-54* 3'UTR, 10ng/ μL ; *unc-119(+)*, 50ng/ μL ; *pBlueScript*, 40 ng/ μL], injected into the strain *unc-119(ed3)*.

syEx1448 and **syEx1449** [*Pnlp-40::GAL4_{SK}::VP64::unc-54* 3'UTR, 10ng/ μL ; *Pmyo-2::nls::mCherry*, 10ng/ μL ; *pBlueScript*, 80ng/ μL], injected into **syls302**. **syEx1449** was used to generate **syls318**, **syls319** and **syls320** as intestine drivers.

syEx1450 and **syEx1451** [*Pmyo-3::GAL4_{SK}::VP64::unc-54* 3'UTR, 10ng/ μL ; *Pmyo-2::nls::mCherry*, 10ng/ μL ; *pBlueScript*, 80ng/ μL], injected into **syls302**. **syEx1451** was used to generate **syls321** as the body wall muscle driver.

syEx1471 [*Punc-47::GAL4_{SK}::VP64::unc-54* 3'UTR, 60ng/ μL ; *Pofm-1::rfp*, 40ng/ μL], **syEx1471** was used to generate **syls322**, **syls323**, **syls324** and **syls325**, as GABAergic neuron drivers (These GABAergic drivers were weak, we suggest using drivers built in the pPD117.01 backbone with the *let-858* 3'UTR).

syEx1475, **syEx1476**, and **syEx1477** [$5 \times UAS::\Delta pes-10::gfp::unc-54$ 3'UTR, 25ng/ μL ; *unc-119(+)*, 50ng/ μL ; *pBlueScript*, 25 ng/ μL], injected into the strain *unc-119(ed3)*; **syls301**.

syEx1478 and **syEx1479** [$10 \times UAS::\Delta pes-10::gfp::unc-54$ 3'UTR, 25ng/ μL ; *unc-119(+)*, 50ng/ μL ; *pBlueScript*, 25 ng/ μL], injected into the strain *unc-119(ed3)*; **syls301**.

syEx1480 and **syEx1481** [$15 \times UAS::\Delta pes-10::gfp::unc-54$ 3'UTR, 25ng/ μL ; *unc-119(+)*, 50ng/ μL ; *pBlueScript*, 25 ng/ μL], injected into the strain *unc-119(ed3)*; **syls301**.

syEx1482 and **syEx1483** [$20\times UAS::\Delta pes-10::gfp::unc-54$ 3'UTR, 25ng/ μL ; $unc-119(+)$, 50ng/ μL ; $pBlueScript$, 25 ng/ μL], injected into the strain $unc-119(ed3)$; **syls301**.

syEx1443 and **syEx1444** [$15\times UAS::\Delta pes-10::aex-2(+)$ cDNA:: $unc-54$ 3'UTR, 25ng/ μL ; $Pmyo-2::nls::gfp$, 10ng/ μL ; $pBlueScript$, 65 ng/ μL], injected into the strain $aex-2(sa3)$.

syEx1433 and **syEx1447** [$Prab-3::GAL4_{SK}::VP64::let-858$ 3'UTR, 10 ng/ μL ; $Pofm-1::rfp$, 40ng/ μL ; $pBlueScript$, 50 ng/ μL], injected into N2. **syEx1447** was used to generate **syls334**, **syls335** and **syls336** as pan-neuronal driver lines.

syEx1430 [$Pmyo-2::GAL4_{SC}::VP64::unc-54$ 3'UTR; 10ng/ μL , $Pofm-1::rfp$ 40ng/ μL ; 1kb DNA ladder(NEB), 150 ng/ μL], also used to generate the **syls301** as the pharyngeal muscle driver.

syEx1488 [$15\times UAS::\Delta pes-10::gfp::let-858$ 3'UTR, 25 ng/ μL ; $Ptx-3::rfp$, 50 ng/ μL ; 1 kb ladder (NEB), 125 ng/ μL], injected into N2, used to generate **syls337** and **syls390** for $15\times UAS::gfp::let-858$ 3'UTR effector lines.

syEx1484 [$Punc-17::GAL4_{SK}::VP64::let-858$ 3'UTR, 25 ng/ μL ; $Punc-17::mCherry$, 25 ng/ μL ; $unc-119(+)$, 50 ng/ μL], injected into the strain **syls390**; $unc-119(ed3)$.

syEx1485 [$Punc-47::GAL4_{SK}::VP64::let-858$ 3'UTR, 25 ng/ μL ; $Punc-47::mCherry$, 25 ng/ μL ; $unc-119(+)$, 50 ng/ μL], injected into the strain **syls390**; $unc-119(ed3)$.

syEx1486 [$Peat-4::GAL4_{SK}::VP64::let-858$ 3'UTR, 25 ng/ μL ; $Peat-4::mCherry$, 25 ng/ μL ; $unc-119(+)$, 50 ng/ μL], injected into the strain **syls390**; $unc-119(ed3)$.

syEx1460 [$15\times UAS::\Delta pes-10::hChr2(H134R)::eyfp::let-858$ 3'UTR, 25ng/ μL ; $Ptx-3::rfp$, 40ng/ μL ; $pBlueScript$, 35 ng/ μL], injected into N2, used to generate **syls340**, **syls341** and **syls342** for $15\times UAS::hChr2(H134R)::eyfp::let-858$ 3'UTR effector lines.

syEx1487 [$Punc-47::GAL4_{SK}::VP64::let-858$ 3'UTR, 25 ng/ μL ; $Pofm-1::rfp$, 40 ng/ μL ; 1 kb ladder (NEB), 35 ng/ μL], injected into the strain **syls341**.

Supplementary Note 6 | Strains used in the study

For detailed information about arrays and integrants, see **Supplementary Note 5** and **Supplementary Table 1**.

Wild type N2

PS6041 *unc-119(ed3)* III

Figure 1b, 1c and Supplementary Figure 1a:

PS6843 *syls300* V

PS6932 *unc-119(ed3); syls300*

PS6900 *syEx1431; unc-119; syls300*

PS6901 *syEx1432; unc-119(ed3); syls300*

PS6902 *syEx1433; unc-119(ed3); syls300*

PS6903 *syEx1434; unc-119(ed3); syls300*

Figure 1d, 1e and Supplementary Figure 1b

PS6844 *syls301* V

PS6964 *unc-119(ed3); syls301*

PS7007 *syEx1475; unc-119(ed3); syls301*

PS7008 *syEx1476; unc-119(ed3); syls301*

PS7009 *syEx1477; unc-119(ed3); syls301*

PS7010 *syEx1478; unc-119(ed3); syls301*

PS7012 *syEx1480; unc-119(ed3); syls301*

PS7013 *syEx1481; unc-119(ed3); syls301*

PS7014 *syEx1482; unc-119(ed3); syls301*

PS7015 *syEx1483; unc-119(ed3); syls301*

Figure 2 and Supplementary Figure 2:

PS6902 *syEx1433; unc-119(ed3); syls300*

PS6903 *syEx1434; unc-119(ed3); syls300*

PS6904 *syEx1435; unc-119(ed3); syls300*

PS6905 *syEx1436; unc-119(ed3); syls300*

PS6906 *syEx1437; unc-119(ed3)*

PS6907 *syEx1438; unc-119(ed3)*

Figure 3a-3f:

PS6933 *syls318 syls302* III

PS7067 *syls321; syls300*

PS6987 *syls337; syls334*

PS7149 *syls390*

PS7184 *syls390; unc-119(ed3)*

PS7018 *syEx1484; syls390; unc-119(ed3)*

PS7019 *syEx1485; syls390; unc-119(ed3)*
PS7020 *syEx1486; syls390; unc-119(ed3)*

Figure 3h:

JT3 *aex-2(sa3)* X
PS6975 *syEx1443; aex-2(sa3)*
PS6976 *syEx1444; aex-2(sa3)*
PS6936 *syls321*
PS6935 *syls320*
PS6938 *syls323*

The exact genotypes used for the quantification of the defecation assay in Figure 3h are (from left to right):

N2
aex-2(sa3)
syEx1444; aex-2(sa3)
syls323/+; aex-3(sa3)
syEx1444; syls323/+; aex-2(sa3)
syEx1444; syls321/+; aex-2(sa3)
syEx1444; syls320/+; aex-2(sa3)

Figure 3i and Supplementary Video 1-2:

PS7021 *syEx1487; syls341*
PS7044 *syls341*

Supplementary Figure 3 and Supplementary Video 3-5:

PS6872 *syls302* III
PS6844 *syls301* V
PS6965 *syls301; syls302*
PS7186 *syls407*
PS7154 *syls391* IV
PS7136 *syls378* V
PS7190 *syls409* X
PS7167 *syls396 syls337* III
PS7192 *syls413* IV
PS6936 *syls321*
PS7205 *syls424* III
PS7199 *syls371*
PS7201 *syls421*