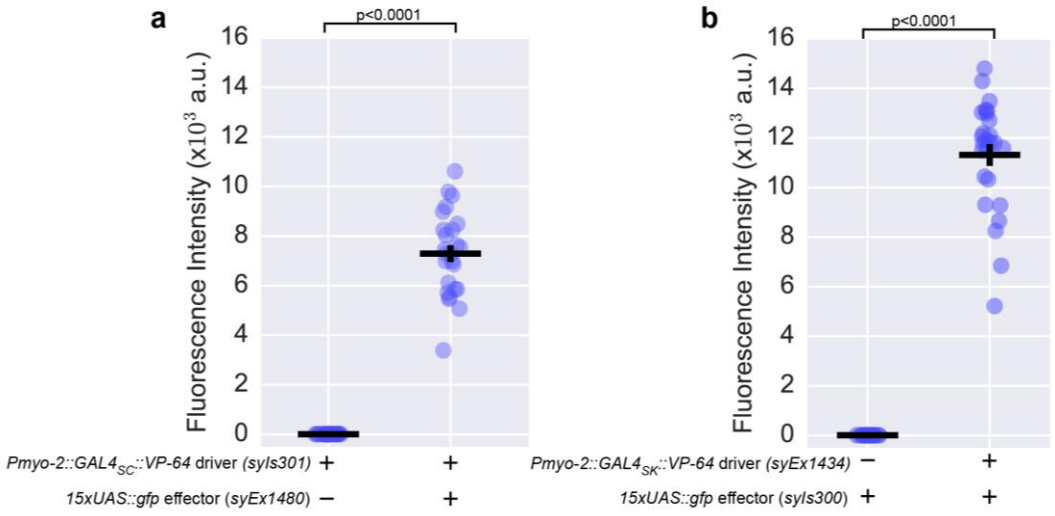
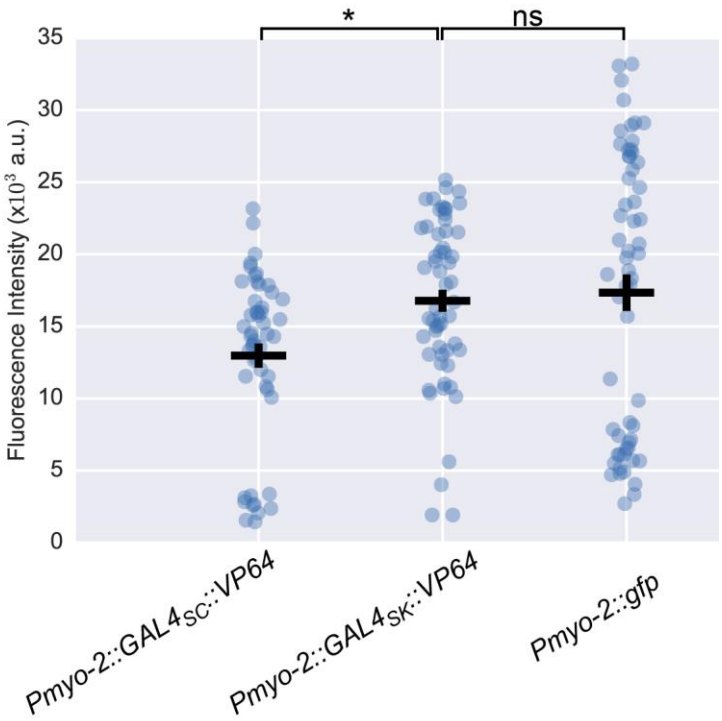


Supplementary Figure 1

Neither driver nor effector alone displays expression of GFP

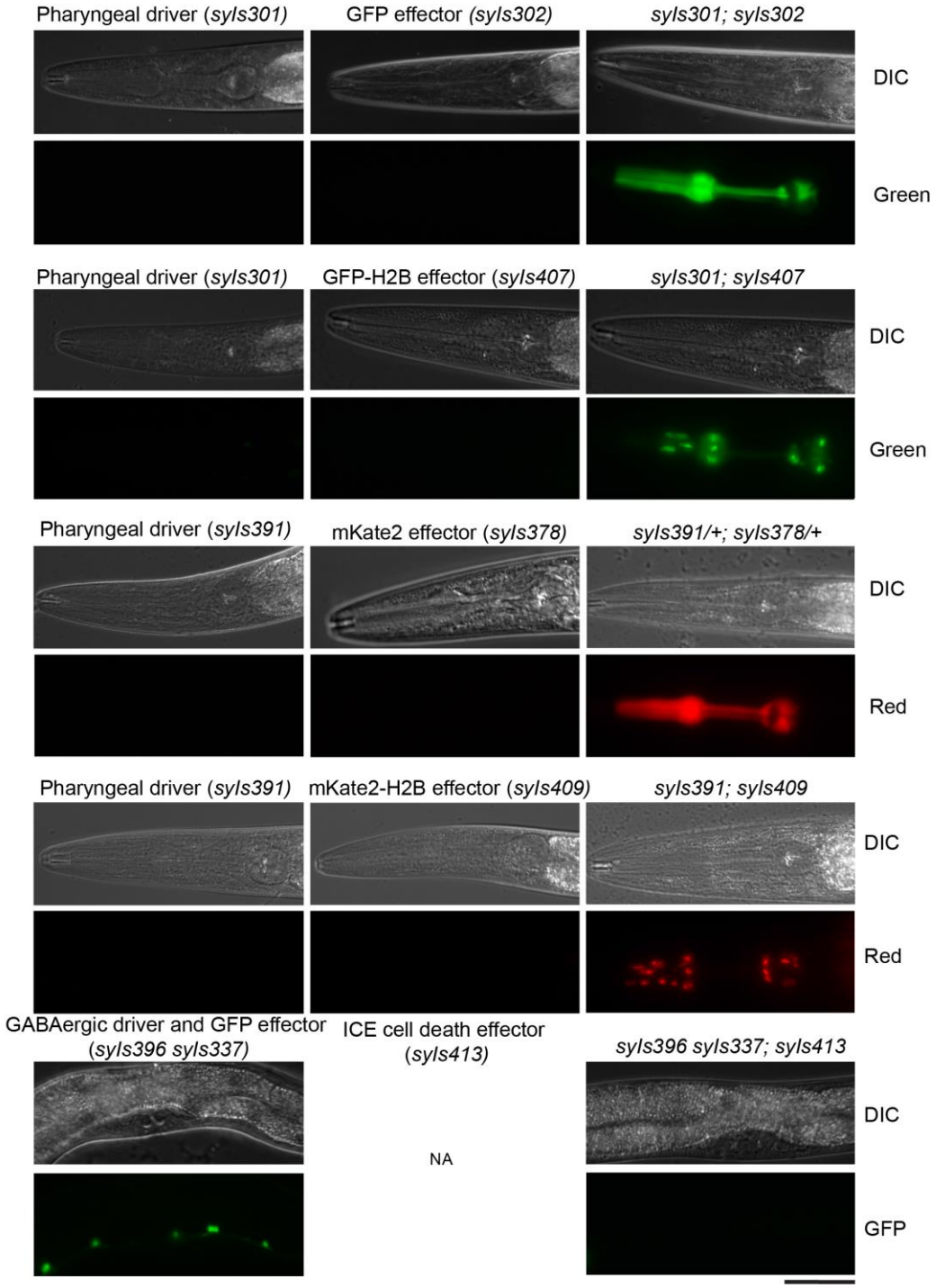
Comparison of GFP fluorescence in a driver- only strain (**a**) or an effector-only strain (**b**) to their driver + effector combination controls. One-tailed t-test with Welch's correction.



Supplementary Figure 2

Performance of different DBDs from Gal4 proteins at room temperature

Quantification of GFP fluorescence in the pharynx of transgenic worms with either *Pmyo-2::GAL4$_{SC}$::VP64* or *Pmyo-2::GAL4$_{SK}$::VP64* drivers injected into a strain carrying an integrated *15xUAS::gfp* transgene (*syIs300*) at room temperature (22-23°C). The drivers were both injected at 10 ng/µL. Strains with a direct *Pmyo-2::gfp* fusion array at 10 ng/µL was measured for comparison. Two independent lines were imaged for each genotype. n = 20 - 30 for each line. Bars are mean ± SEM. * $p<0.05$. ns, not significant. One-way ANOVA with Tukey's post-test. a.u., artificial units.



Supplementary Figure 3

Functional verification of integrated effectors

Expression of integrated drivers (left column) or integrated effectors alone (middle column) shows no basal expression. Only the combination (right column) show expression of cytoplasmic or nuclear-localized reporters, or death of appropriate cells. DIC, Differential interference contrast; Green, green filter; Red, red filter. Scale bar is 20 μm.

Supplementary Information

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

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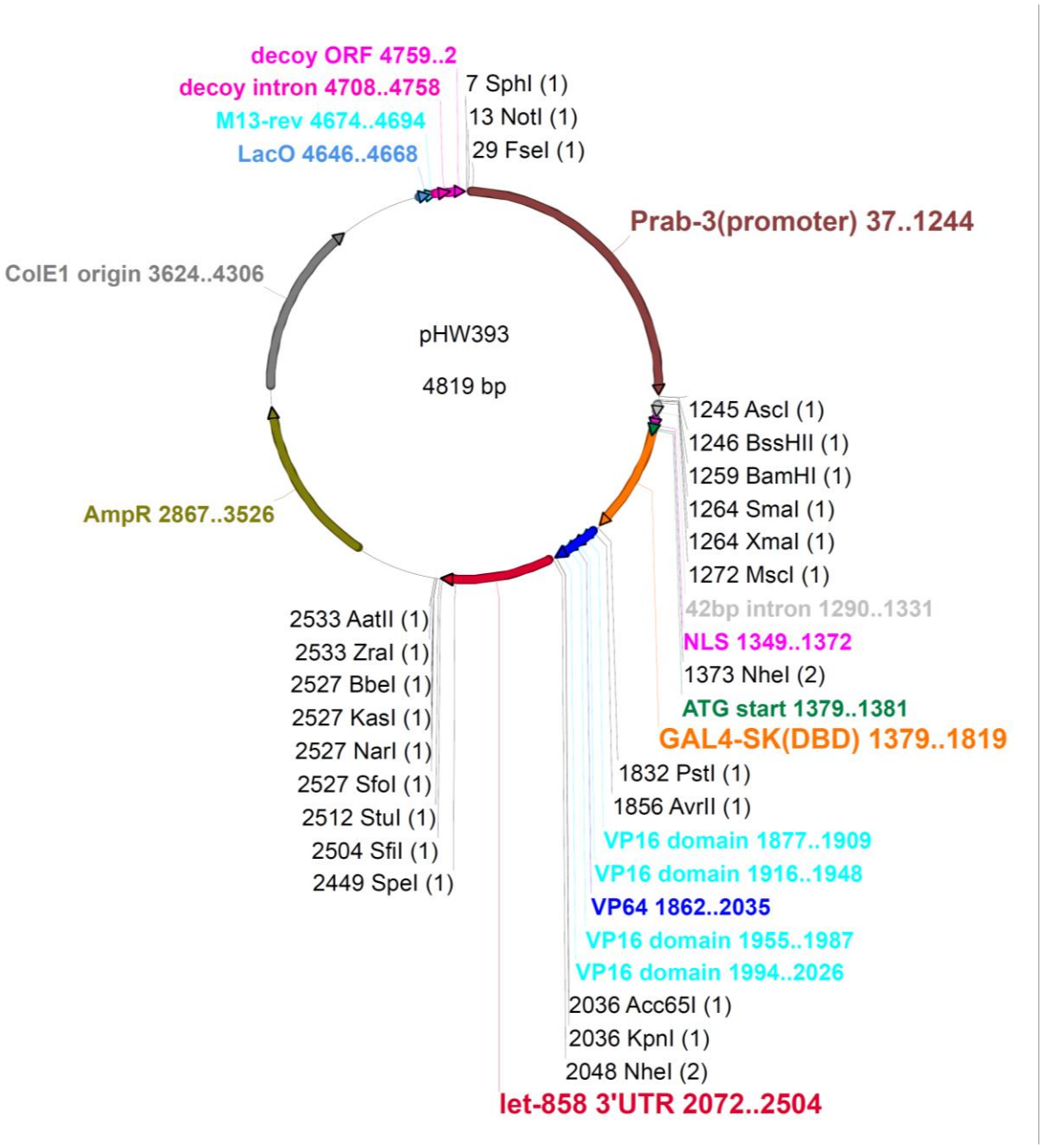
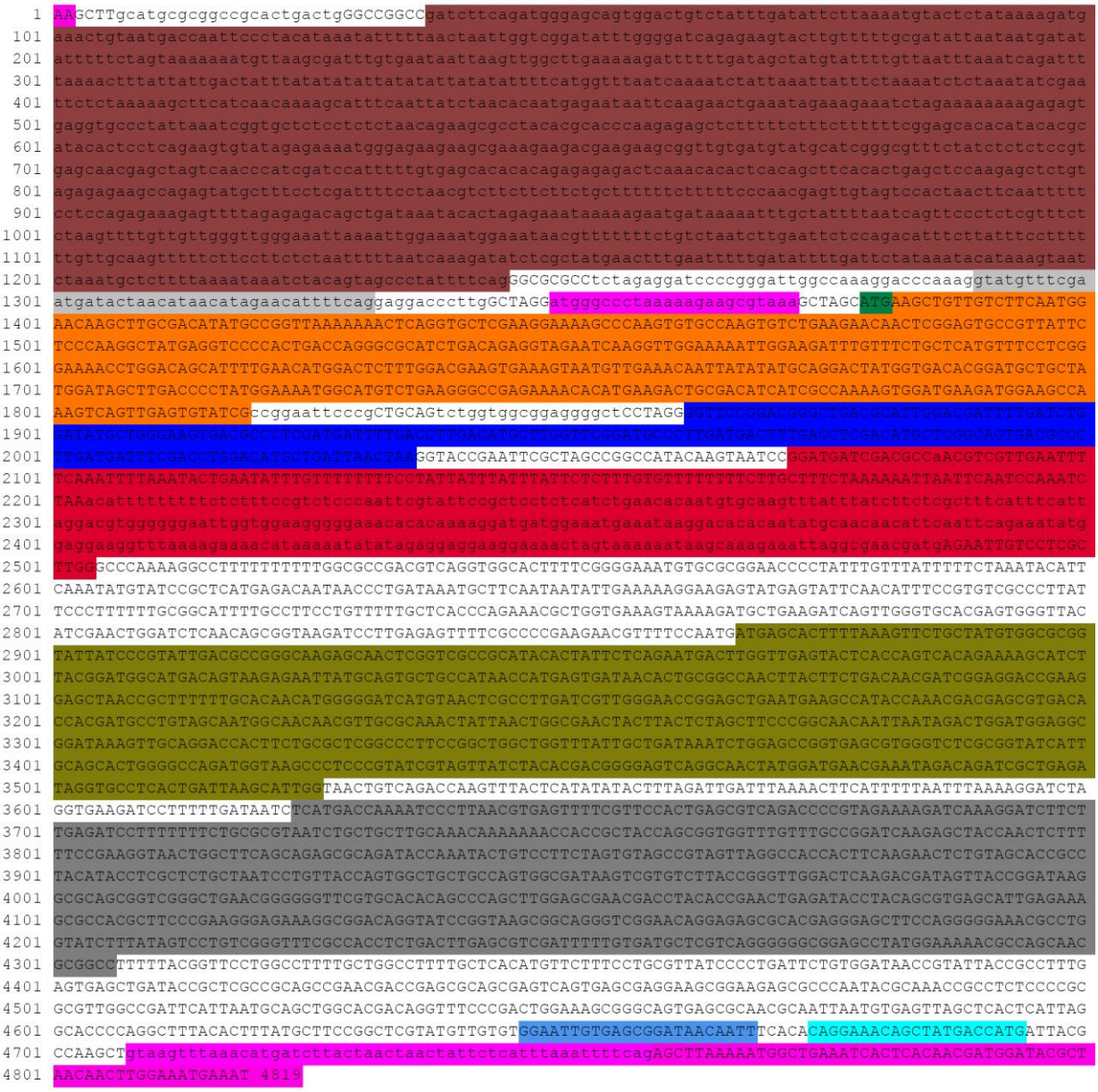
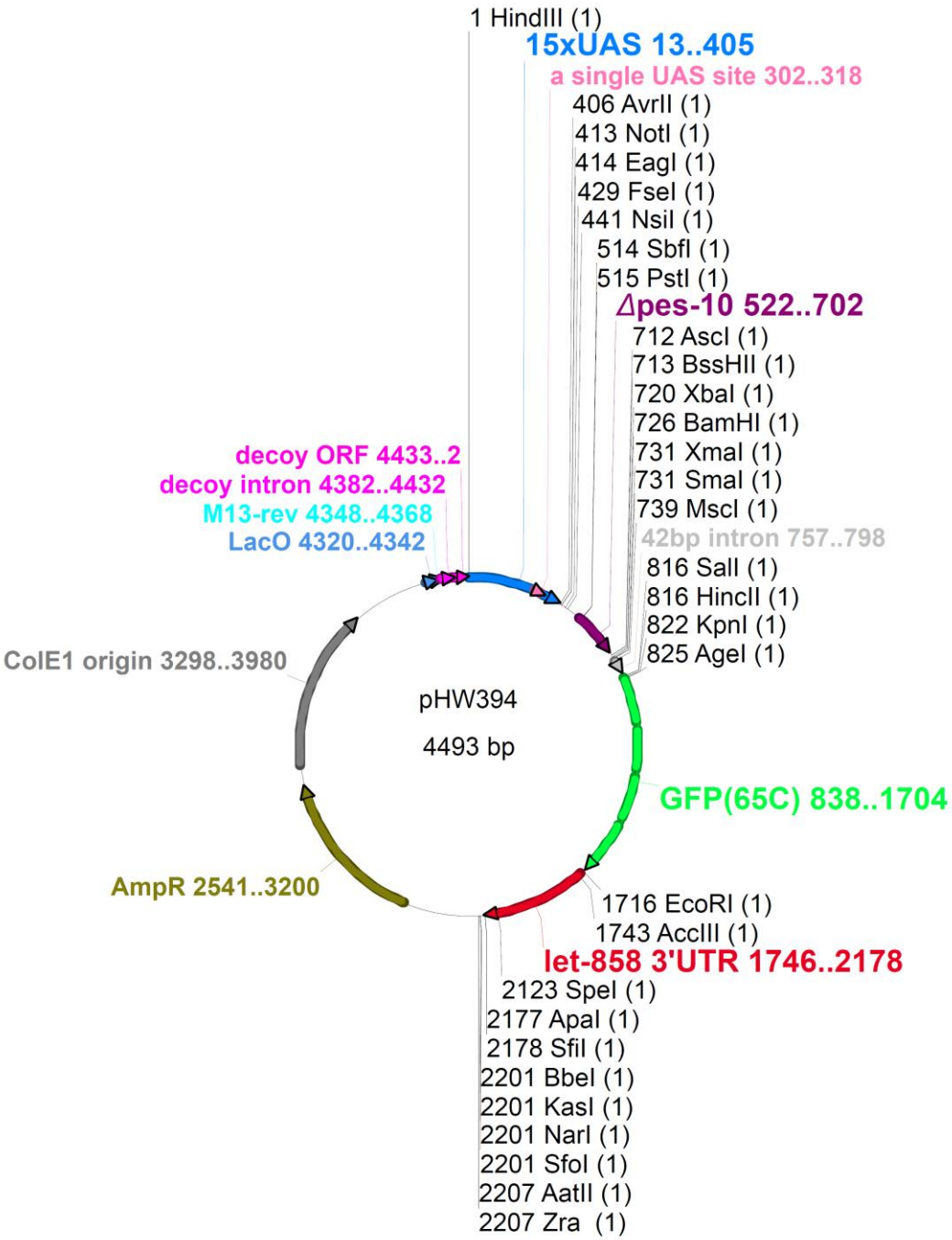
* These authors contributed to this work equally.



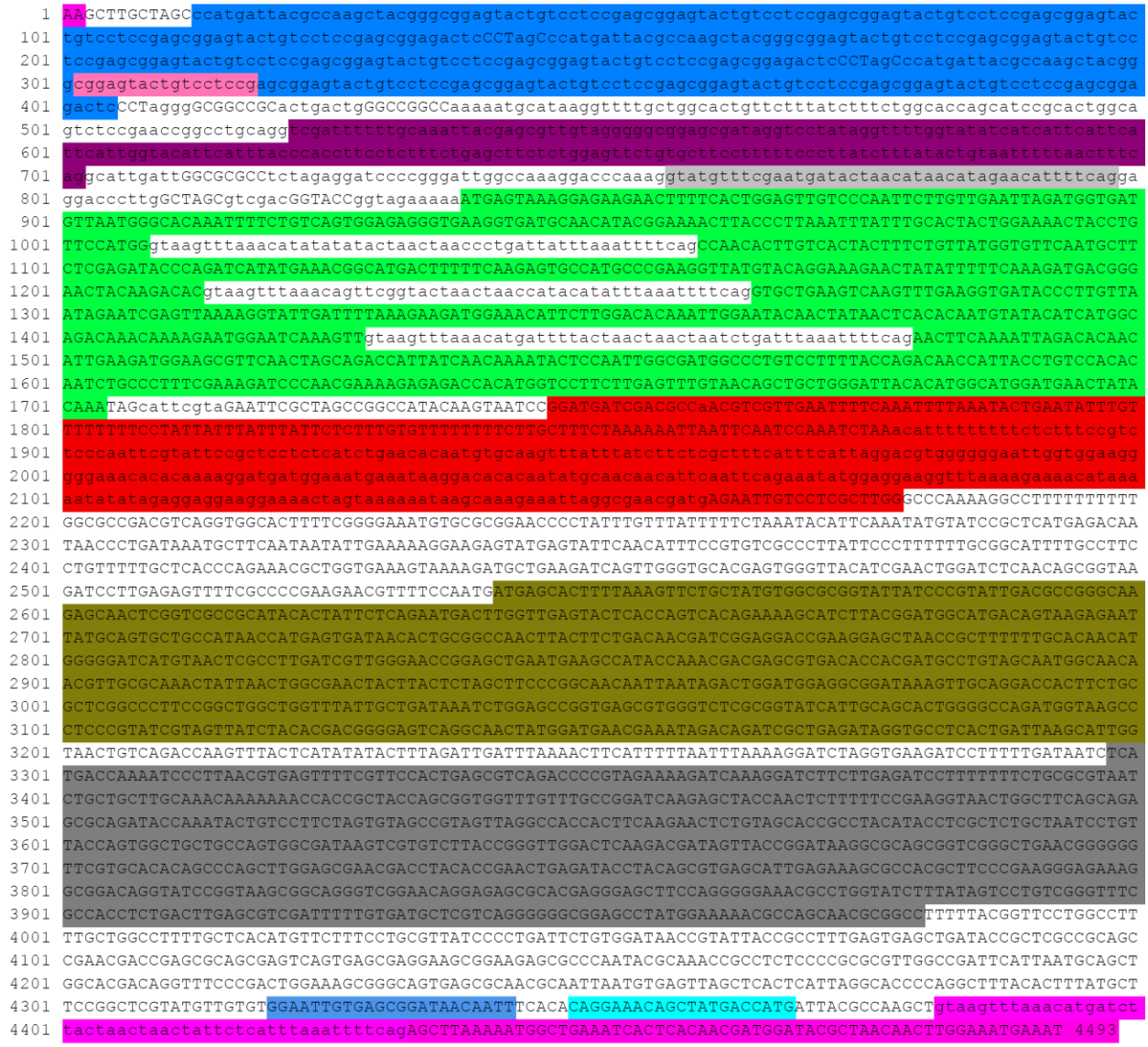
Supplementary Note 1 | Vector Map of the driver construct pHW393 (pHW393) *Prab-3::nls::GAL4-SK(DBD)::VP64::let-858 3'UTR.* New driver constructs can be generated by cloning new promoters into the MCS, between SphI/NotI/FseI and AscI/BssHII/BamHI/SmaI/XmaI/MscI. The backbone of pHW393 (between SphI and 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 2 | Sequence of the driver construct pHW393 (pHW393) *Prab-3::nls::GAL4-SK(DBD)::VP64::let-858 3'UTR*, is available from Addgene (www.addgene.org; plasmid no. 85583). The color scheme here matches the vector map of pHW393 shown in Supplementary Note 1.



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 SalI/HincII/KpnI/AgeI and EcoRI/AccIII. 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).



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 (Pttx-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 *(**syIs####**)* 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 *[15xUAS::Δpes-10::gfp::unc-54 3'UTR, 25ng/μL; Pttx-3::rfp, 40ng/μL; pBlueScript, 35 ng/μL]*, injected into N2, used to generate ***syIs300*** and ***syIs302***.

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); syIs300*.

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); syIs300.*

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); syIs300.*

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 *syIs302. syEx1449* was used to generate ***syIs318***, ***syIs319*** and ***syIs320*** 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 *syIs302. syEx1451* was used to generate ***syIs321*** 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 ***syIs322***, ***syIs323***, ***syIs324*** and ***syIs325***, 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*** *[5xUAS::Δpes-10::gfp::unc-54 3'UTR, 25ng/μL; unc-119(+), 50ng/μL; pBlueScript, 25 ng/μL]*, injected into the strain *unc-119(ed3); syIs301*.

syEx1478 and ***syEx1479*** *[10xUAS::Δpes-10::gfp::unc-54 3'UTR, 25ng/μL; unc-119(+), 50ng/μL; pBlueScript, 25 ng/μL],* injected into the strain *unc-119(ed3); syIs301*.

syEx1480 and ***syEx1481*** *[15xUAS::Δpes-10::gfp::unc-54 3'UTR, 25ng/μL; unc-119(+), 50ng/μL; pBlueScript, 25 ng/μL],* injected into the strain *unc-119(ed3); syIs301.*

syEx1482 and ***syEx1483*** *[20xUAS::Δpes-10::gfp::unc-54 3'UTR, 25ng/µL; unc-119(+), 50ng/µL; pBlueScript, 25 ng/µL],* injected into the strain *unc-119(ed3); syIs301.*

syEx1443 and ***syEx1444*** *[15xUAS::Δpes-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 ***syIs334***, ***syIs335*** *and* ***syIs336*** 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 ***syIs301*** as the pharyngeal muscle driver.

syEx1488 *[15xUAS::Δpes-10::gfp::let-858 3'UTR, 25 ng/µL; Pttx-3::rfp, 50 ng/µL; 1 kb ladder (NEB), 125 ng/µL],* injected into N2, used to generate ***syIs337*** and ***syIs390*** for *15xUAS::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 *syIs390; 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 *syIs390; 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 *syIs390; unc-119(ed3)*.

syEx1460 *[15xUAS::Δpes-10::hChR2(H134R)::eyfp::let-858 3'UTR, 25ng/µL; Pttx-3::rfp, 40ng/µL; pBlueScript, 35 ng/µL],* injected into N2, used to generate ***syIs340***, ***syIs341*** and ***syIs342*** for *15xUAS::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 *syIs341*.

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 *syIs300* V
PS6932 *unc-119(ed3); syIs300*
PS6900 *syEx1431; unc-119; syIs300*
PS6901 *syEx1432; unc-119(ed3); syIs300*
PS6902 *syEx1433; unc-119(ed3); syIs300*
PS6903 *syEx1434; unc-119(ed3); syIs300*

Figure 1d, 1e and Supplementary Figure 1b

PS6844 *syIs301* V
PS6964 *unc-119(ed3); syIs301*
PS7007 *syEx1475; unc-119(ed3); syIs301*
PS7008 *syEx1476; unc-119(ed3); syIs301*
PS7009 *syEx1477; unc-119(ed3); syIs301*
PS7010 *syEx1478; unc-119(ed3); syIs301*
PS7012 *syEx1480; unc-119(ed3); syIs301*
PS7013 *syEx1481; unc-119(ed3); syIs301*
PS7014 *syEx1482; unc-119(ed3); syIs301*
PS7015 *syEx1483; unc-119(ed3); syIs301*

Figure 2 and Supplementary Figure 2:

PS6902 *syEx1433; unc-119(ed3); syIs300*
PS6903 *syEx1434; unc-119(ed3); syIs300*
PS6904 *syEx1435; unc-119(ed3); syIs300*
PS6905 *syEx1436; unc-119(ed3); syIs300*
PS6906 *syEx1437; unc-119(ed3)*
PS6907 syEx1438; unc-119(ed3)

Figure 3a-3f:

PS6933 *syIs318 syIs302 III*
PS7067 *syIs321; syIs300*
PS6987 *syIs337; syIs334*
PS7149 *syIs390*
PS7184 *syIs390; unc-119(ed3)*
PS7018 *syEx1484; syIs390; unc-119(ed3)*

PS7019 *syEx1485; syIs390; unc-119(ed3)*
PS7020 *syEx1486; syIs390; unc-119(ed3)*

Figure 3h:

JT3 *aex-2(sa3)* X
PS6975 *syEx1443; aex-2(sa3)*
PS6976 *syEx1444; aex-2(sa3)*
PS6936 *syIs321*
PS6935 *syIs320*
PS6938 *syIs323*

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)
syIs323/+; aex-3(sa3)
syEx1444; syIs323/+; aex-2(sa3)
syEx1444; syIs321/+; aex-2(sa3)
syEx1444; syIs320/+; aex-2(sa3)

Figure 3i and Supplementary Video 1-2:

PS7021 *syEx1487; syIs341*
PS7044 *syIs341*

Supplementary Figure 3 and Supplementary Video 3-5:

PS6872 *syIs302* III
PS6844 *syIs301* V
PS6965 *syIs301; syIs302*
PS7186 *syIs407*
PS7154 *syIs391* IV
PS7136 *syIs378* V
PS7190 *syIs409* X
PS7167 *syIs396 syIs337* III
PS7192 *syIs413* IV
PS6936 *syIs321*
PS7205 *syIs424* III
PS7199 *syIs371*
PS7201 *syIs421*