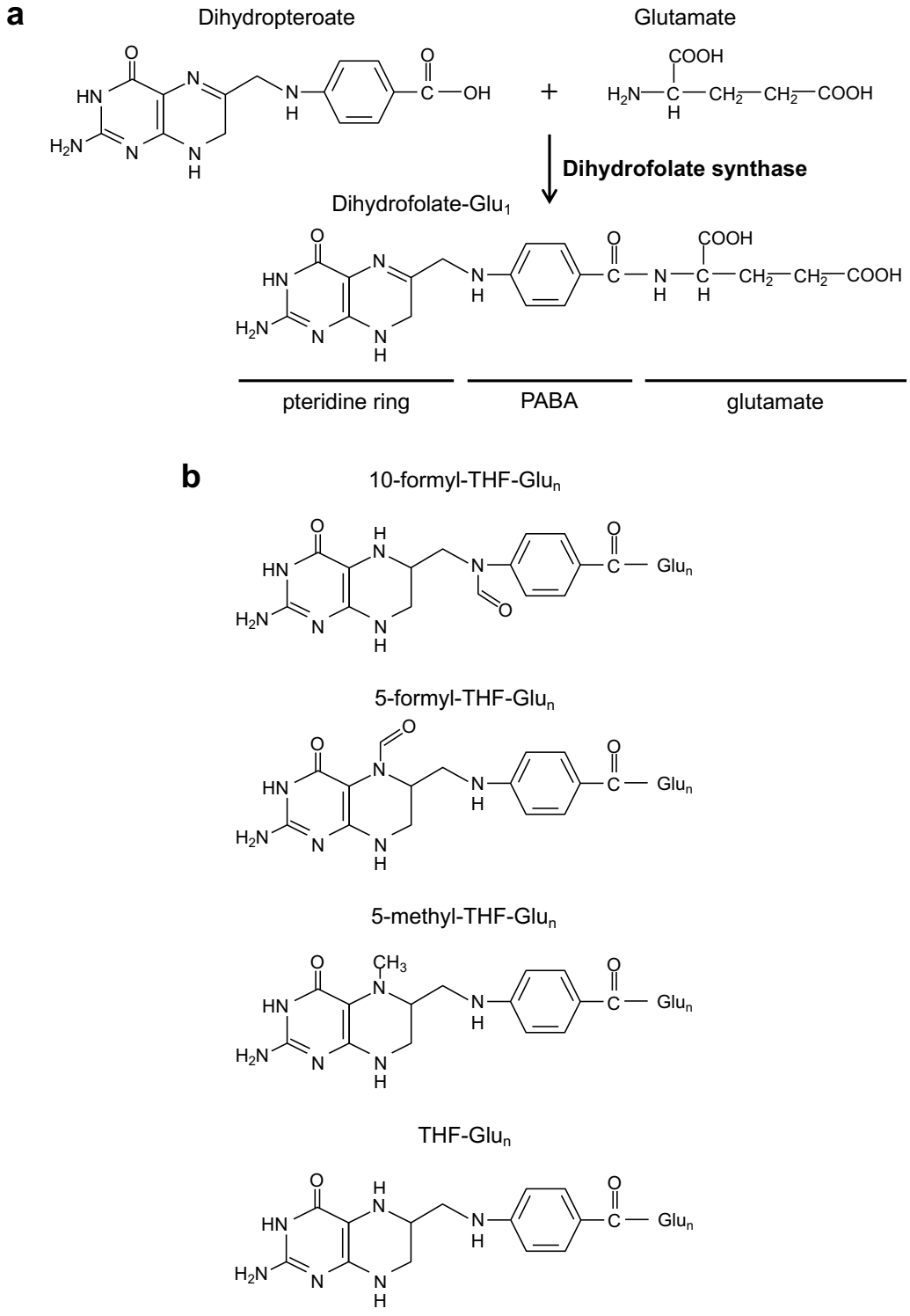
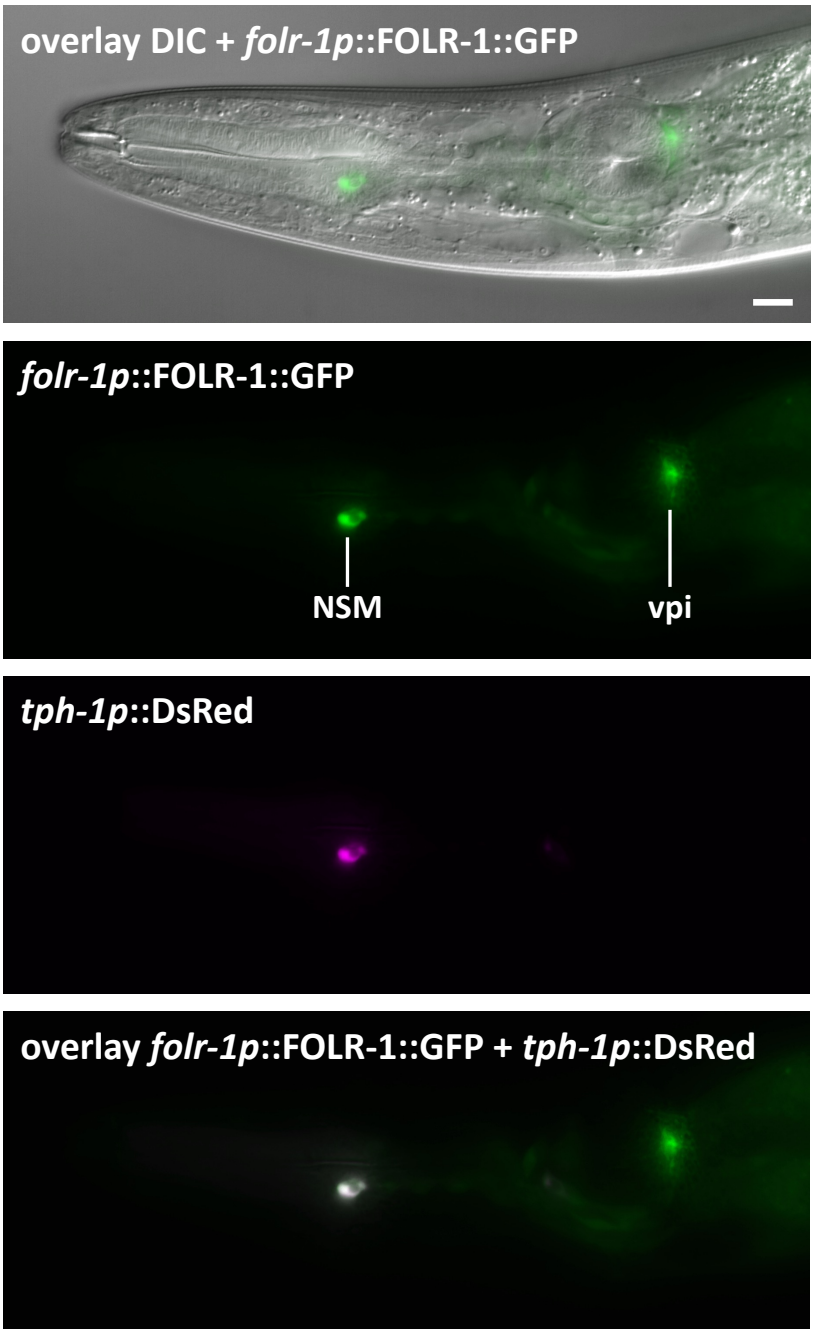
Supplementary Material

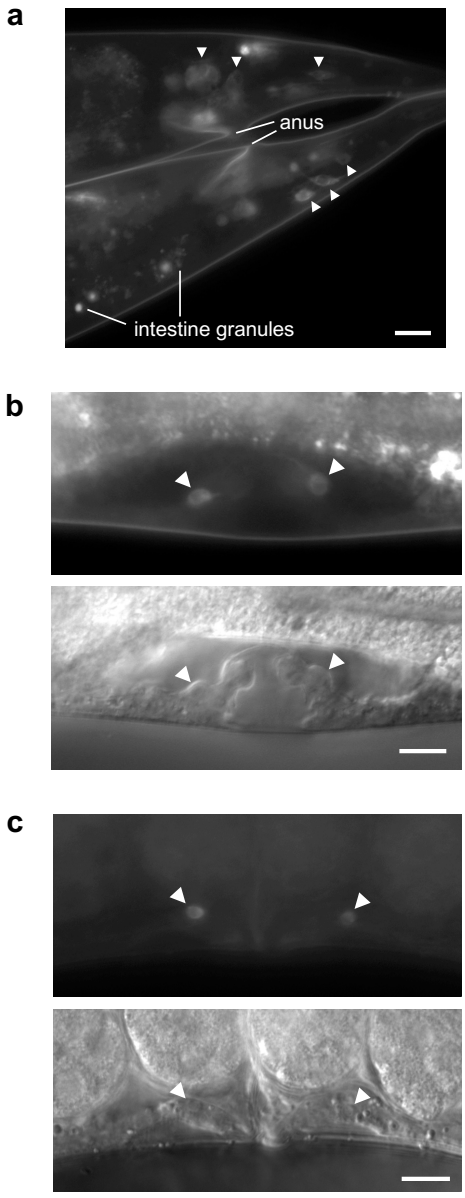
A specific folate activates serotonergic neurons to control *C. elegans* behavior



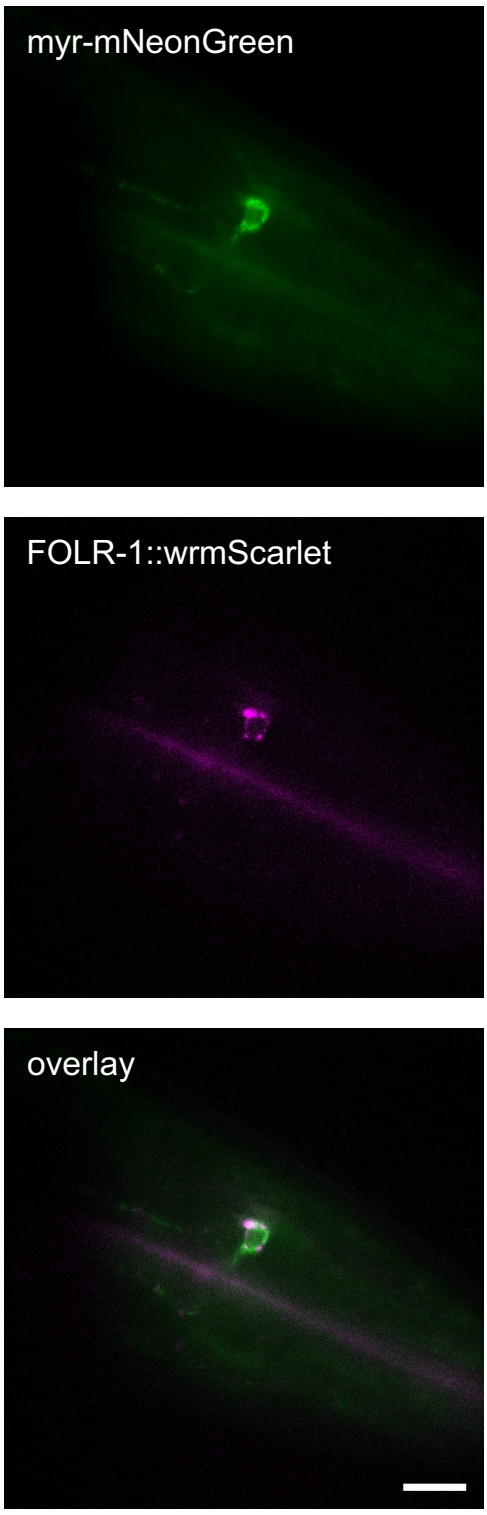
Supplementary Fig. 1. The structure of folates and dihydropteroate. **a** Diagram of how the enzyme dihydrofolate synthase converts dihydropteroate and glutamate to dihydrofolate, which has its pteridine ring, PABA, and glutamyl moieties labeled. **b** Structure of four folates (Glu_n denotes one or more glutamates). PABA, para-aminobenzoic acid.



Supplementary Fig. 2. Localization of *folr-1p*::FOLR-1::GFP to the NSM neuron in an adult hermaphrodite. The first image (top) shows an overlay of DIC and FOLR-1::GFP expressed from its own promoter (green). The second image shows FOLR-1::GFP with localization to NSM and vpi cells marked. The third image shows DsRed expressed in the NSM neuron using the *tph-1* promoter (magenta). The fourth image shows an overlay of FOLR-1::GFP and NSM-localized DsRed. Note the overlap (white) of FOLR-1::GFP and DsRed in the NSM neuron. Similar localization was observed in n = 29 animals from n = 7 independent experiments. vpi, pharyngeal-intestinal valve cells.



Supplementary Fig. 3. Expression of *folr-1p*::FOLR-1::GFP in cells in the tail and the uterus. ***a*** Image of *folr-1p*::FOLR-1::GFP expression in the tails of two L4-stage hermaphrodites. The top animal shows the left lateral side, and the bottom animal shows the right lateral side. **b**, **c** There is transient expression of *folr-1p*::FOLR-1::GFP in uterine cells in L4-stage larvae and young adults, including in uv2 and uv3 uterine cells. **b** L4-stage vulva region showing the two uv3 uterine cells with *folr-1p*::FOLR-1::GFP expression. **c** Young adult-stage vulva region showing the two uv2 uterine cells with *folr-1p*::FOLR-1::GFP expression (at this stage, uv3 cells are ventrally located nearer to the vulval opening). Arrowheads denote cells with *folr-1p*::FOLR-1::GFP expression. (**a**), (**b**), (**c**) similar expression patterns were observed in at least n = 18 animals from at least n = 2 experiments. Scale bars are 10 μm.



Supplementary Fig. 4. Localization of *tph1pDE*::FOLR-1::wrmScarlet and *tph1pDE*::myr-mNeonGreen in the NSM neuron. Maximum image projection of confocal z sections of an NSM neuron of an adult hermaphrodite expressing both fluorescent proteins. Similar subcellular localization was observed in n = 32 animals from n = 6 experiments. Scale bar is 10 μm.

a

ATGAGATTCAAACTTGTTTGTCTTTTTTTAAGTCTATTTGCTCTTCCGAGTGTGTCCCTCACAT
GGGACATGAGCAAGTGGATGCCAGACACCCGGACTCACTCGATGGTGAAGCGGCAATGCGCAAT
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CGAAAAAATAAAGATGATCGTGAGCCAGTGGTTAGCTACTGTCGTGACATTAAAGAGCATATTG
CAAGAATGGAACGCGGGCAAATTAAGCATGGGAAAGAGTTTAACAAGAACGTAGAGAATGTCCA
AGACAATTTCACCGACAAGGATGATGCACACATGACGGACAATGAGTGTAATATGAACATCACT
GATAATCAACAGCCTAAATAA

b

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FOLR-1:       MRFKLVCLFLSLFALPSVSLTWDMSKWMPDTRTHSMVKRQCAMCIAGDFLDKKDLM
FOLR-1(ek44): MRFKLVCLFLSLFALPSVSLTWDMSKWMPDTRTHSMVKRQCAMCIAGDFLDKKDLM

VDVSIGQMIVWNDEWDLTDCWTGDVVIKECASACVSIYTKSKTREGWFWTSVLMDCSEALIWSSPDLSLK
VDVSIGQMIVWNDEWDLTDCWTGDVVIKECAPYTPSRKPVKDGFGQVSLWIVPRH*

PTTMVLLDGVYVASRKGHDIKYIFSTNNNNSTLDIREHIFSFMVPGIRQKRTTFGTKVLLASIAVIMIFV

VLALLKIVYKEERSRKNKDDREPVVSYCRDIKEHIARMERGQIKHGKEFNKNVENVQDNFTDKDDAHMTD

NECNMNITDNQQPK*
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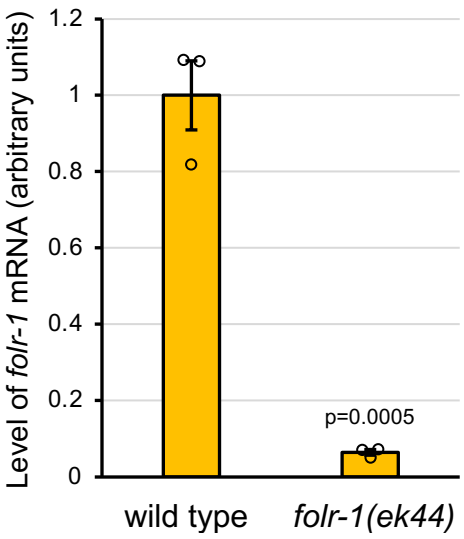
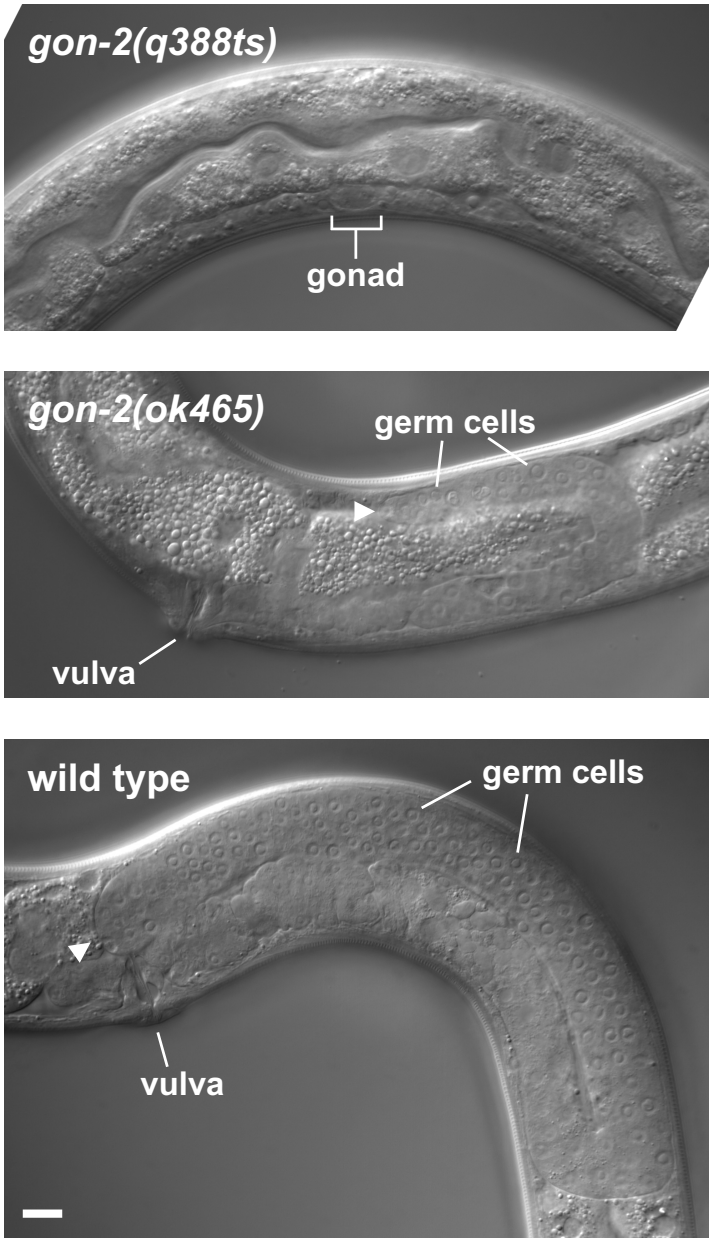
280 aa wild-type FOLR-1 sequence
87 aa ek44 FOLR-1 sequence + 24 out-of-frame aa

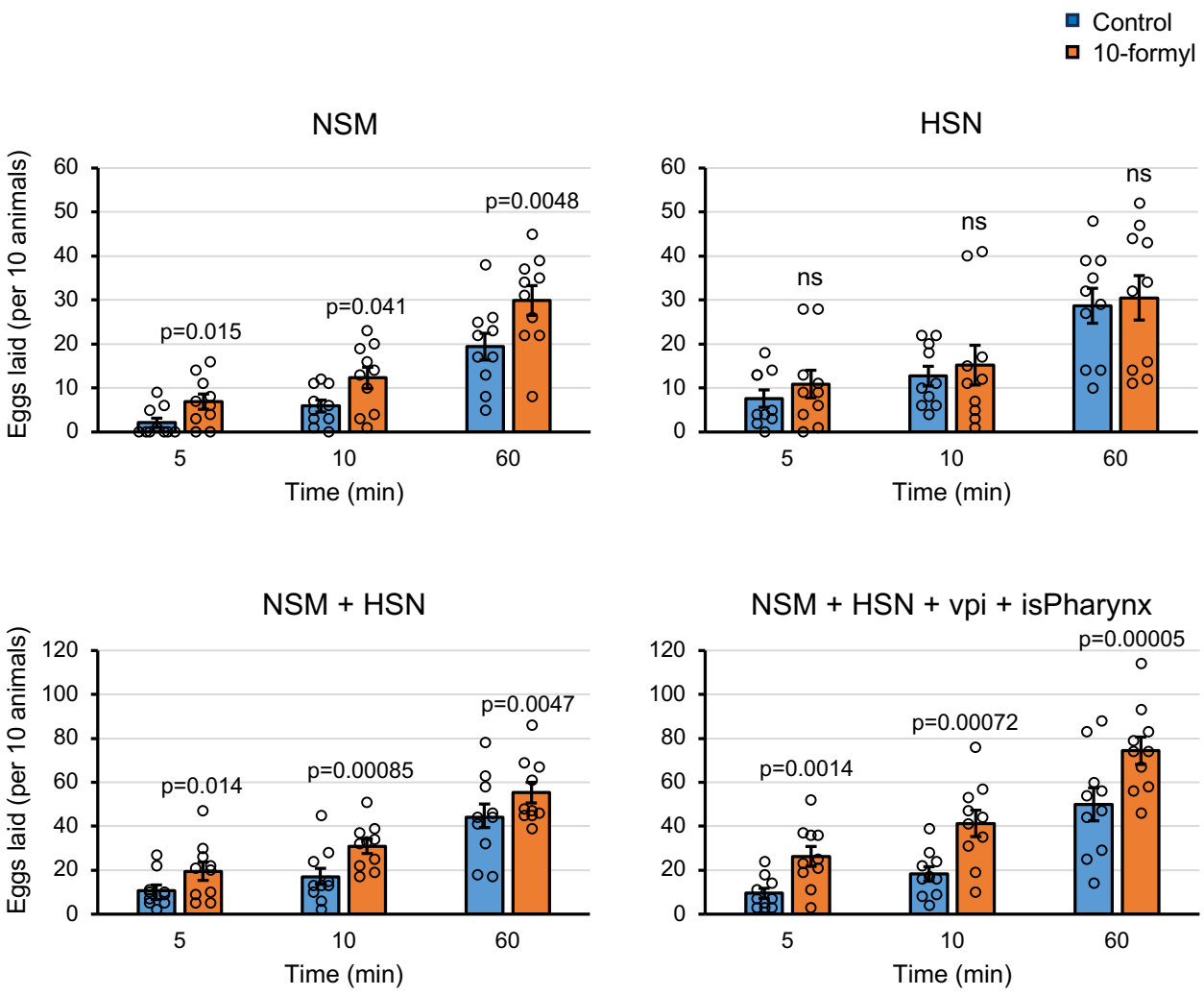
Supplementary Fig. 5. Effect of the *folr-1(ek44)* deletion mutation on the *folr-1* coding sequence and FOLR-1 protein. **a** The *folr-1(ek44)* mutation in the *folr-1* genomic coding region. Exons are shown in uppercase with yellow highlight; introns are lowercase without highlight. The 13 base pairs that are deleted in the *folr-1(ek44)* mutation are highlighted in magenta. **b** The FOLR-1(ek44) truncated protein. Wild-type FOLR-1 and the FOLR-1(ek44) truncated proteins are aligned. Residues that are identical to mammalian FOLR1 residues that bind folate[1] are highlighted in blue. The predicted transmembrane domain is highlighted in olive. The out-of-frame sequence for FOLR-1(ek44) is highlighted in red. Asterisks denote stop codons.



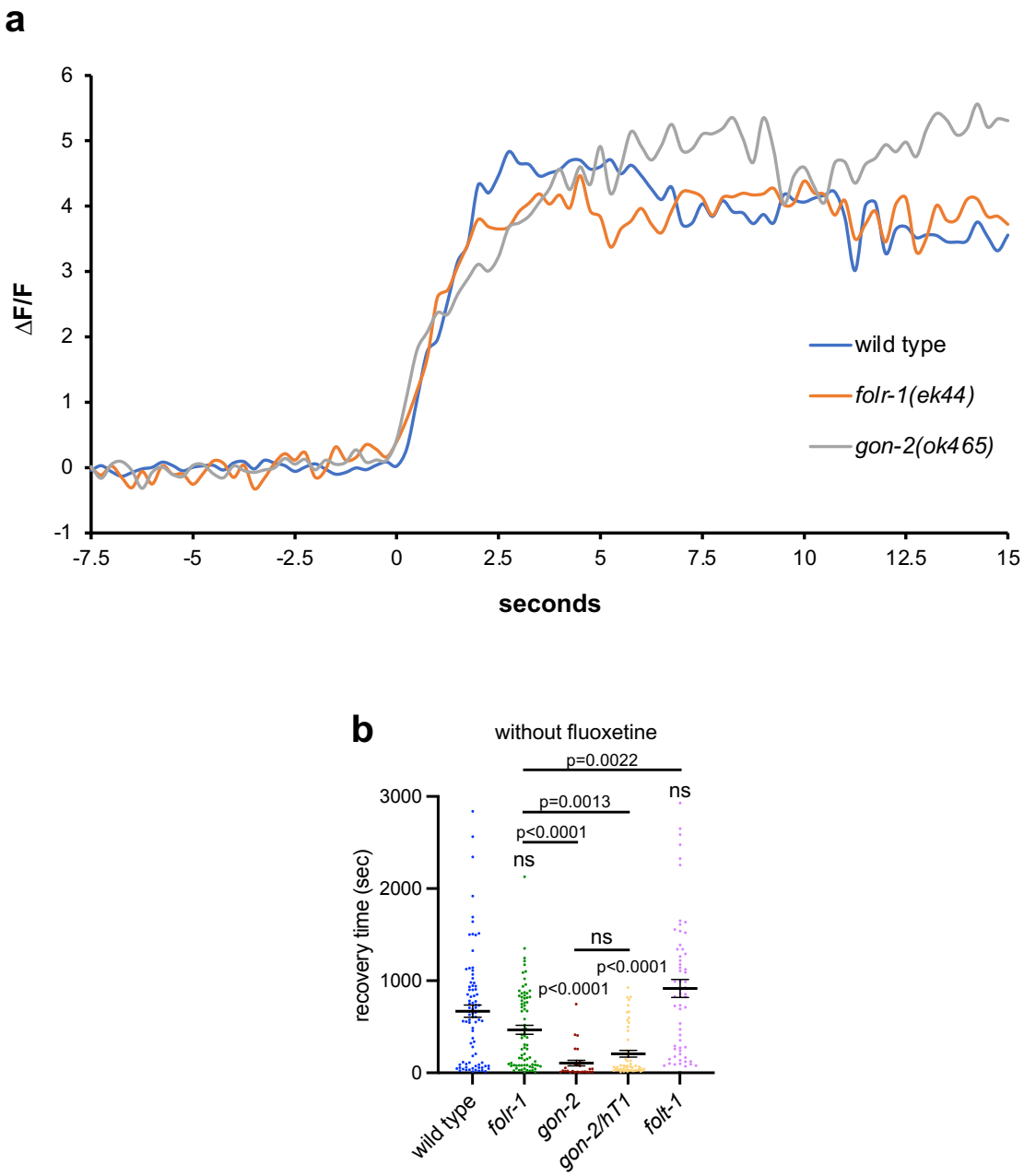
Supplementary Fig. 6. *folr-1* mRNA levels are reduced in *folr-1(ek44)* mutants. Real-time quantitative reverse transcription PCR (qRT-PCR) of *folr-1* mRNA levels in L4-stage wild type and *folr-1(ek44)* mutants. The RPL19 ribosome subunit-encoding gene *rpl-19* was used as the normalization control. The wild-type *folr-1* mRNA level is set to 1.0. Data are presented as mean ± SEM. Unpaired two-tailed Student's t-test were conducted for statistical analysis. n = 3 biological replicates. Source data are provided as a Source Data file.



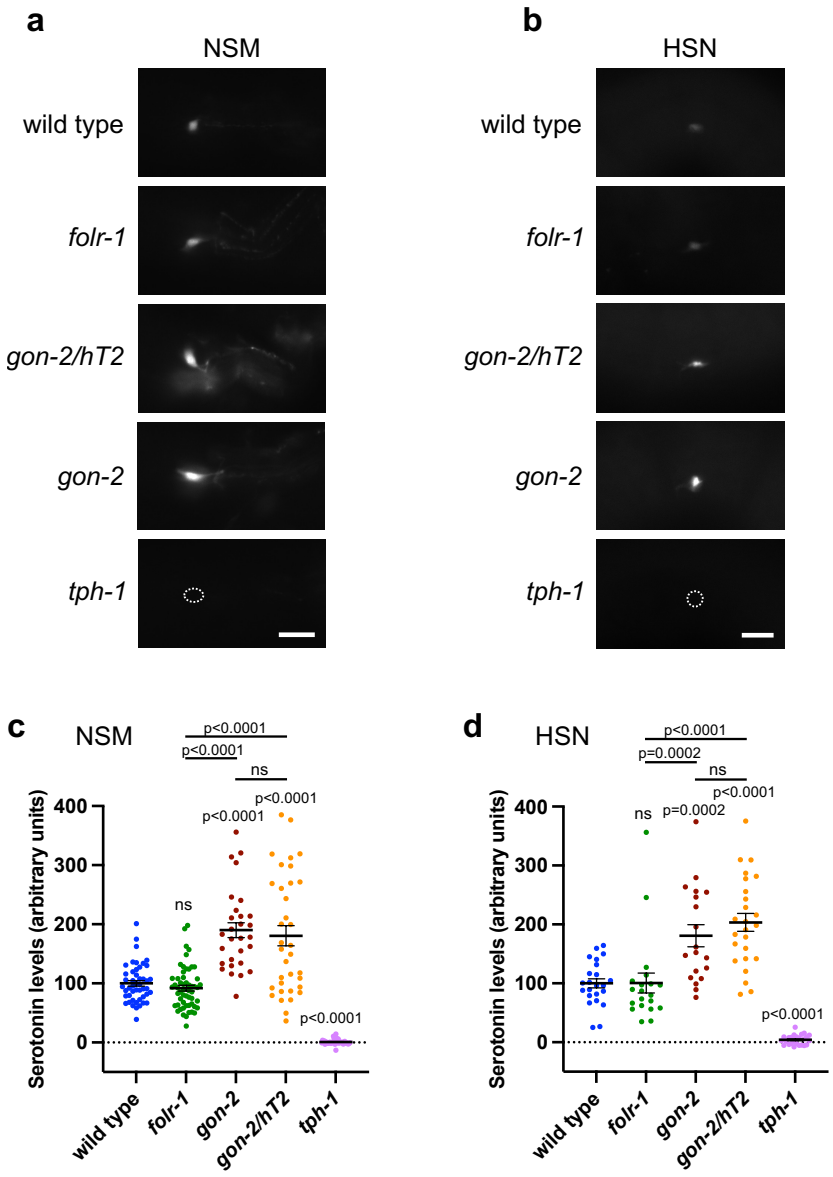
Supplementary Fig. 7. The *gon-2(ok465)* null allele develops the somatic gonad structure but has less germ cells. DIC micrographs of the germline of young adult hermaphrodites. **top)** A *gon-2(q388ts)* mutant grown at the non-permissive temperature of 25°C, with a stunted somatic gonad that did not enlarge. Because the somatic gonadal precursor cells did not divide to generate an anchor cell, the anchor cell was not present to initiate vulva formation[2], and so the vulva is missing. **middle)** *gon-2(ok465)* null mutant, showing a reflexed gonad arm with a normal structure but with few germ cells. **bottom)** Wild-type hermaphrodite showing a normal reflexed gonad arm with the normal number of germ cells. Similar images were obtained in at least n = 28 animals from at least n = 3 experiments per genotype. The distal end of the gonad arm is marked by an arrowhead. Scale bar is 10 μm.



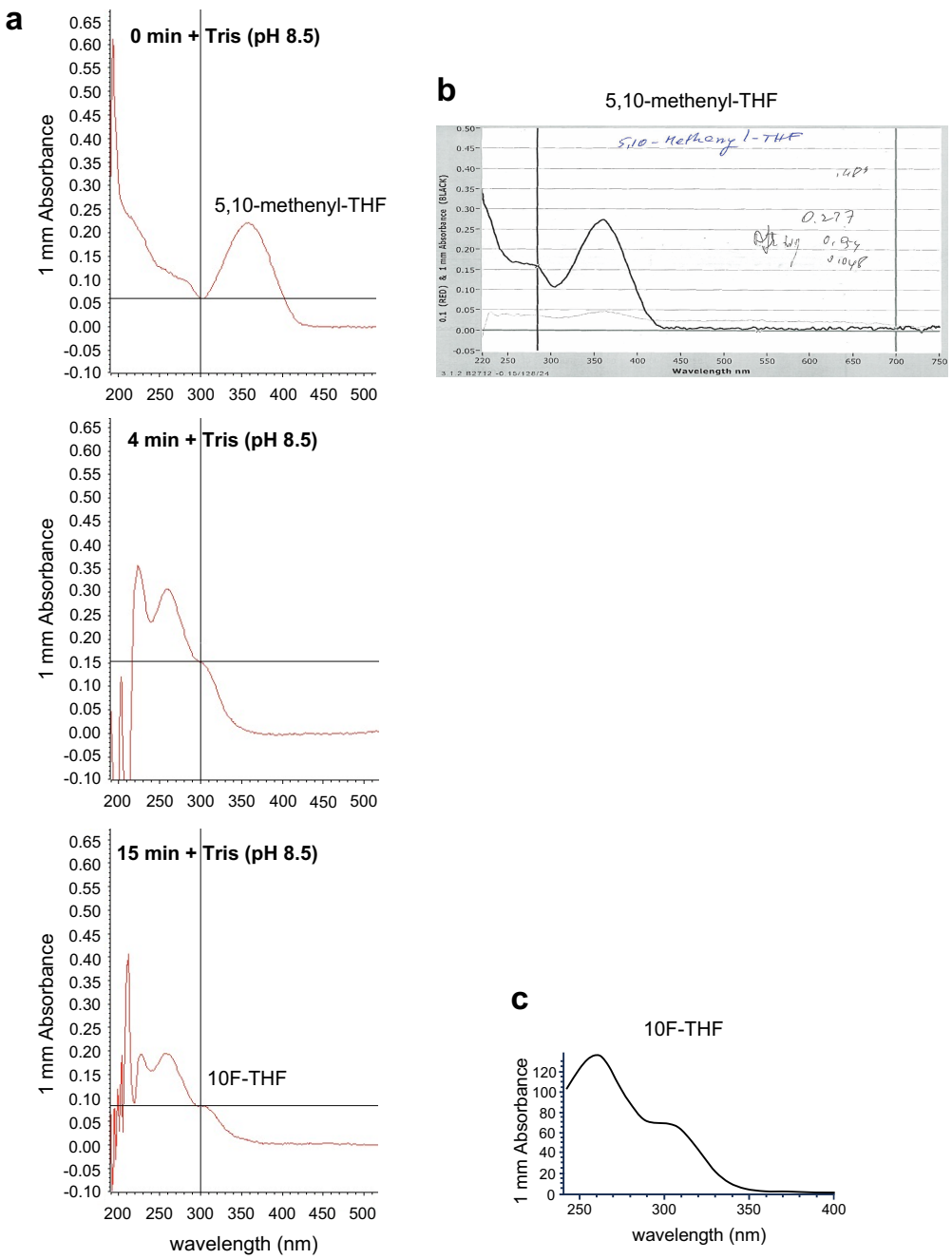
Supplementary Fig. 8. Tissue-specific expression of GON-2 to rescue *gon-2/hT2* heterozygous mutants for egg laying response to 10F-THF. The number of eggs laid by *gon-2(ok465)/hT2* heterozygous mutant strains with GON-2 expressed in the indicated tissues in response to 10F-THF or control buffer (n = 10). n = groups of 10 animals. Data are presented as mean ± SEM. Paired two-tailed Student's t-test were conducted for statistical analyses. The experiments were repeated two times with similar results. Source data are provided as a Source Data file. vpi, pharyngeal-intestinal valve cells; isPharynx, the isthmus of the pharynx



Supplementary Fig. 9. Calcium levels in starved wild-type, *folr-1(ek44)*, and *gon-2(ok465)* adult hermaphrodites as they encounter bacteria and their recovery times. **a** ΔF/F traces of fluorescence from GCaMP7b transgenic strains as starved animals encounter bacteria at time 0. Average ΔF/F values are shown for n = 16 wild type, n = 14 *folr-1(ek44)*, and n = 13 *gon-2(ok465)*. **b** The recovery times after encountering bacteria for the indicated genotypes without fluoxetine pretreatment (from left to right, n = 84, 78, 29, 53, 59). Data are presented as mean ± SEM (**b**). Kruskal-Wallis test with Dunn's multiple comparisons were used for statistical analyses (**b**). n = individual animals analyzed for movement (**a**, **b**). (**a**) and (**b**) were repeated two times with similar results. Source data are provided as a Source Data file.



Supplementary Fig. 10. Serotonin levels in NSM and HSN neurons in wild type, *folr-1(ek44)*, *gon-2(ok465)*, *gon-2(ok465)/hT2* heterozygotes, and *tph-1(n4622)* mutants. a, **b** Images of the NSM neuron (**a**) and HSN neuron (**b**) stained by immunofluorescence with anti-serotonin antibody in the indicated genotypes. *tph-1(n4622)* mutants do not synthesize serotonin, and so are negative controls for the anti-serotonin immunofluorescence. Scale bars are 10 µm. **c** The levels of anti-serotonin staining in the NSM neurons for the indicated genotypes (from left to right, n = 47, 55, 29, 35, 44). **d** The levels of anti-serotonin staining in the HSN neurons for the indicated genotypes (from left to right, n = 23, 20, 19, 25, 25). Data are presented as mean ± SEM (**c**, **d**). One-way ANOVA followed by Sidak Holm multiple comparisons was used for statistical analyses (**c**, **d**). n = individual neurons analyzed. (**c**) and (**d**) were repeated two times with similar results. Source data are provided as a Source Data file.



Supplementary Fig. 11. Absorbance spectra to follow conversion of 5,10-methenyl-THF to 10F-THF. **a** Time course of absorbance spectra for the conversion of 10 µM 5,10-methenyl-THF to 10F-THF in the presence of 50 mM Tris (pH 8.5). Absorbance spectra for 0 min (top), 4 min (middle), and 15 min (bottom) in the presence of 50 mM Tris (pH 8.5) are shown. **b** The absorbance spectrum of 5,10-methenyl-THF is from the notes of Dr. Jacob Selhub. **c** A reproduction of the absorbance spectra from Selhub, 1989[3]. Similar results were obtained in n = 10 independent experiments.

Supplementary Table 1. *C. elegans* strains used.

Strain	Genotype
ET562	*unc-119(ed3)* III; *ekEx38*[pCFJ350/*folr-1p*::FOLR-1::GFP::*tbb-2* 3'UTR]
ET566	*unc-119(ed3)* III; *ekIs27*[pCFJ350/*tph-1pDE*::GCaMP7s::P2A::mScarlet::*unc-54* 3'UTR]
ET567	*gon-2(ok465)* I/*hT2* [*bli-4(e937)* l*et-?(q782) qIs48*] (I;III); *ekIs27*[pCFJ350/*tph-1pDE*::GCaMP7s::P2A::mScarlet::*unc-54* 3'UTR]
ET685	*folr-1(ek44)* X; *ekIs27*[pCFJ350/*tph-1pDE:*:GCaMP7s::P2A::mScarlet::*unc-54* 3'UTR]
ET571	*lite-1(ce314)* X; *ekIs26*[pCFJ350/*tph-1pDE*::GCaMP7b::*unc-54* 3'UTR] II
ET588	*folr-1(ek44)* X; *lite-1(ce314)* X; *ekIs26*[pCFJ350/*tph-1pDE*::GCaMP7b::*unc-54* 3'UTR] II
ET590	*gon-2(ok465)* I/*hT2* [*bli-4(e937) let-?(q782) qIs48*] (I;III); l*ite-1(ce314)* X; *ekIs26*[pCFJ350/*tph-1pDE*::GCaMP7b::*unc-54* 3'UTR] II
ET645	*gon-2(ok465)* I/*hT2* [*bli-4(e937) let-?(q782) qIs48*] (I;III); *lite-1(ce314)*; *vsIs183*[*nlp-3p*::GCaMP5::*nlp-3* 3'UTR + *nlp-3p*::mCherry::*nlp-3* 3'UTR + *lin-15(+)*] X
ET649	*folr-1(ek44)* X; *lite-1(ce314)* X; *vsIs183*[*nlp-3p*::GCaMP5::*nlp-3* 3'UTR + *nlp-3p*::mCherry::*nlp-3* 3'UTR + *lin-15(+)*] X
ET865	*unc-119(ed3)* III; *folr-1(ek44)* X; *ekEx51*[*flr-4p*::FOLR-1 + *cdf-1p*::FOLR-1 + *tph-1pDE*::FOLR-1 + pCFJ350]
ET869	*unc-119(ed3)* III; *folr-1(ek44)* X; *ekEx54*[*flr-4p*::FOLR-1 + *cdf-1p*::FOLR-1 + *tph-1pDE*::FOLR-1 + pCFJ350]
ET661	*unc-119(ed3)* III; *folr-1(ek44)*; *ekEx71*[*egl-6p*::FOLR-1 + *flr-4p*::FOLR-1 + *cdf-1p*::FOLR-1 + *tph-1pDE*::FOLR-1 + pCFJ350]
ET662	*unc-119(ed3)* III; *folr-1(ek44)*; *ekEx72*[*egl-6p*::FOLR-1 + *flr-4p*::FOLR-1 + *cdf-1p*::FOLR-1 + *tph-1pDE*::FOLR-1 + pCFJ350]
ET663	*unc-119(ed3)* III; *folr-1(ek44)* X; *ekEx73*[*egl-6p*::FOLR-1 + *tph-1pDE*::FOLR-1 + pCFJ350]
ET664	*unc-119(ed3)* III; *folr-1(ek44)* X; *ekEx74*[*egl-6p*::FOLR-1 + *tph-1pDE*::FOLR-1 + pCFJ350]
ET668	*unc-119(ed3)* III; *folr-1(ek44)* X; *ekEx76*[*egl-6p*::FOLR-1 + *cdf-1p*::FOLR-1 + *tph-1pDE*::FOLR-1 + pCFJ350]
ET669	*unc-119(ed3)* III; *folr-1(ek44)* X; *ekEx77*[*egl-6p*::FOLR-1 + *cdf-1p*::FOLR-1 + *tph-1pDE*::FOLR-1 + pCFJ350]
ET672	*unc-119(ed3)* III; *folr-1(ek44)* X; *ekEx78*[*egl-6p*::FOLR-1 + *flr-4p*::FOLR-1 + *tph-1pDE*::FOLR-1 + pCFJ350]
ET673	*unc-119(ed3)* III; *folr-1(ek44)* X; *ekEx79*[*egl-6p*::FOLR-1 + *flr-4p*::FOLR-1 + *tph-1pDE*::FOLR-1 + pCFJ350]
ET676	*unc-119(ed3)* III; *folr-1(ek44)* X; *ekEx82*[*egl-6p*::FOLR-1 + *flr-4p*::FOLR-1 + *cdf-1p*::FOLR-1 + pCFJ350]
ET677	*unc-119(ed3)* III; *folr-1(ek44)* X; *ekEx83*[*egl-6p*::FOLR-1 + *flr-4p*::FOLR-1 + *cdf-1p*::FOLR-1 + pCFJ350]
ET828	*unc-119(ed3)* III; ekEx70[*tph-1pDE*::FOLR-1::GFP + *tph-1pDE*::myr-mNeonGreen]
ET694	*unc-119(ed3)* III; *gon-2(ok465)* I/*hT1*[*myo-2p::mKate2 + NeoR, I*] (I;V); *ekEx86*[*tph-1p::GON-2-3xFLAG + egl-6p::GON-2-3xFLAG* + pCFJ350]
ET695	*unc-119(ed3)* III*; gon-2(ok465)* I/*hT1*[*myo-2p::mKate2 + NeoR*] (I;V); *ekEx87*[*tph-1p::GON-2-3xFLAG + egl-6p::GON-2-3xFLAG + flr-4p::GON-2-3xFLG + cdf-1p::GON-2-3xFLAG* + pCFJ350]

ET696	*unc-119(ed3)* III*; gon-2(ok465)* I*/hT1*[*myo-2p::mKate2 + NeoR*] (I;V); *ekEx88*[*egl-6p::GON-2-3xFLAG* + pCFJ350]
ET697	*unc-119(ed3)* III*; gon-2(ok465)* I*/hT1*[*myo-2p::mKate2 + NeoR*] (I;V); *ekEx89[tph-1p::GON-2-3xFLAG* + pCFJ350]
LX2004	*lite-1(ce314) lin-15*&*lin-15A(n765)*; *vsIs183*[*nlp-3p*::GCaMP5::*nlp-3* 3'UTR + *nlp-3p*::mCherry::*nlp-3* 3'UTR + *lin-15(+)*] X
EJ938	*gon-2(q388)* I; *dxIs1*[*gon-2p*::GON-2::GFP], the kind gift of Eric Lambie
EJ1158	*gon-2(q388 ts)* I
ET625	*gon-2(ok465)* I*/hT2* [*bli-4(e937) let-?(q782) qIs48*] (I;III), outcrossed 6x
ET627	*folr-1(ek44)* X, outcrossed 6 times
ET582	*unc-119(ed3)* III; *folr-1(ek44)* X
MT1082	*egl-1(n487)* V
MT15434	*tph-1(mg280)* II
MT14984	*tph-1(n4622)* II
N2	wild type, Bristol

Supplementary References

1 Chen, C. *et al.* Structural basis for molecular recognition of folic acid by folate receptors. *Nature* **500**, 486-489 (2013).

2 Schmid, T. & Hajnal, A. Signal transduction during *C. elegans* vulval development: a NeverEnding story. *Curr. Opin. Genet. Dev.* **32**, 1-9 (2015).

3 Selhub, J. Determination of tissue folate composition by affinity chromatography followed by high-pressure ion pair liquid chromatography. *Anal. Biochem.* **182**, 84-93 (1989).