

Split Fluorescent Proteins for *C. elegans*

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This project is maintained by [Maria Ingaramo](#) in the [York lab](#), and is funded by [Calico Life Sciences LLC](#)

Supplementary Text and Figures

Supplementary Materials and Methods

Mammalian cell culture.

HEK293T cells (ATCC # CRL-3216) were cultured in high-glucose DMEM supplemented with 10% FBS, 1 mM glutamine and 100 µg/mL penicillin/streptomycin (Gibco). A split-wrmScarlet₁₋₁₀ cDNA codon-optimized for mammalian expression was fused to the C-terminus of eGFP and cloned into a pCDH lentiviral expression vector (SFFV GFP-split-wrmScarlet₁₋₁₀). Lentivirus was prepared using standard protocols [[Kamiyama 2016](#)] and used to infect HEK293T cells. A polyclonal population of GFP-mScarlet₁₋₁₀ positive cells was isolated by FACS (using GFP fluorescence) and served as parental cell line for further experiments. For CLTA-N CRISPR engineering, *S. pyogenes* Cas9/sgRNA ribonucleoprotein complexes were prepared as in [[Leonetti 2016](#)], mixed with HDR donor templates and electroporated into of GFP-mScarlet₁₋₁₀ cells by nucleofection.

CLTA-N split-wrmScarlet₁₁ donor library.

A cDNA pool of degenerate split-wrmScarlet₁₁ sequences was generated by oligonucleotide synthesis (GeneScript) and homology arms for HDR-mediated insertion at CLTA N-terminus were appended by PCR (Supplementary Material – [Table S7](#) for sequences). Library diversity was verified by Illumina MiSeq deep-sequencing.

Supplementary Results

Split mScarlet screening in mammalian cells

We tested the applicability of the split-wrmScarlet₁₋₁₀ system for mammalian cell engineering but were surprisingly unsuccessful at detecting fluorescence. We designed a human codon-optimized split-wrmScarlet₁₋₁₀ cDNA and expressed it as a C-terminal GFP fusion in HEK293T cells by lentiviral transduction. Expression of GFP verified the successful expression of the fusion protein (Figure S10A). However, subsequent expression of split-wrmScarlet₁₁ fragments did not give rise to detectable red fluorescence despite numerous attempts. We reasoned that the split-wrmScarlet₁₁ amino-acid sequence might be sub-optimal for complementation in human cells and synthesized a library of degenerate split-wrmScarlet₁₁ sequences covering any possible single and double amino-acid mutants. Using an established assay for CRISPR-based knock-in of sequences at the CLTA N-terminus (a highly expressed gene in HEK293T cells [Leonetti 2016]), neither our original split-wrmScarlet₁₁ sequence nor its mutant library enabled detectable complementation (Figure S10B, left panels). By contrast, a control experiment using the GFP₁₋₁₀/GFP₁₁ system showed a high level of knock-in and complementation in HEK293T (Figure S10B, right panels). It is possible that split-wrmScarlet₁₋₁₀ is expressed in a non-functional form in human cells, or that its binding to split-wrmScarlet₁₁ is occluded by competing interactions (with cellular chaperones, for example). In addition, we did not attempt complementation on primary non-transformed cell lines, like WI-38 cells, whose different proteostasis network and chaperones could aid split mScarlet folding. At this point, more experiments will be required to fully test the portability of split-wrmScarlet to mammalian systems.

Experiments to investigate whether split-wrmScarlet₁₁ functions as a degron in *C. elegans*

After finalizing our experiments, a paper that shows that C-terminal gly-gly sequences might function as degrons in mammalian cells was brought to our attention [Koren 2018]. Since we were unable to obtain non-sterile positive clones of TOMM-20::split-wrmScarlet₁₁ that did not have a mutation on the last glycine, and we were also unable to obtain EAT-6 homozygotes, we were concerned that our split-wrmScarlet₁₁ might be recognized as a degron. To investigate this, we first labeled HIS-3, EAT-6, and TOMM-20 with the 24 a.a. split-wrmScarlet_{11(MDELYK)}, which adds the sequence MDELYK to the C-terminus of split-wrmScarlet₁₁. These worms were fertile, and at least as bright as those labeled with split-

wrmScarlet₁₁ (Figure S6). However, increased fluorescence could be due to increased molecular brightness rather than increased abundance. To address this, we compared the abundance of nuclear HIS-3, HIS-3::split-wrmScarlet₁₁, and HIS-3::split-wrmScarlet_{11(MDELYK)} by western blot, and were unable to detect a significant change in abundance (Figure S11). We also could not detect differences in abundance in *S. cerevisiae*, using a p416-GPD plasmid expressing a mTagBFP-mScarlet fusion or the same fusion truncated so that it ends with gly-gly (Figure S12). However, because HIS-3 is a nuclear protein, and expression in yeast was done from an overexpressing plasmid, we cannot exclude that a protein ending with two glycines might be recognized as a degron in other cellular compartments, or at different expression levels, nor that there is no DesCEND degron pathway in yeast and worms. For these reasons, we recommend using the 24 a.a. split-wrmScarlet_{11(MDELYK)} when labeling proteins at their C-termini.

Figure S1. Split-wrmScarlet sequence comparison to mScarlet.

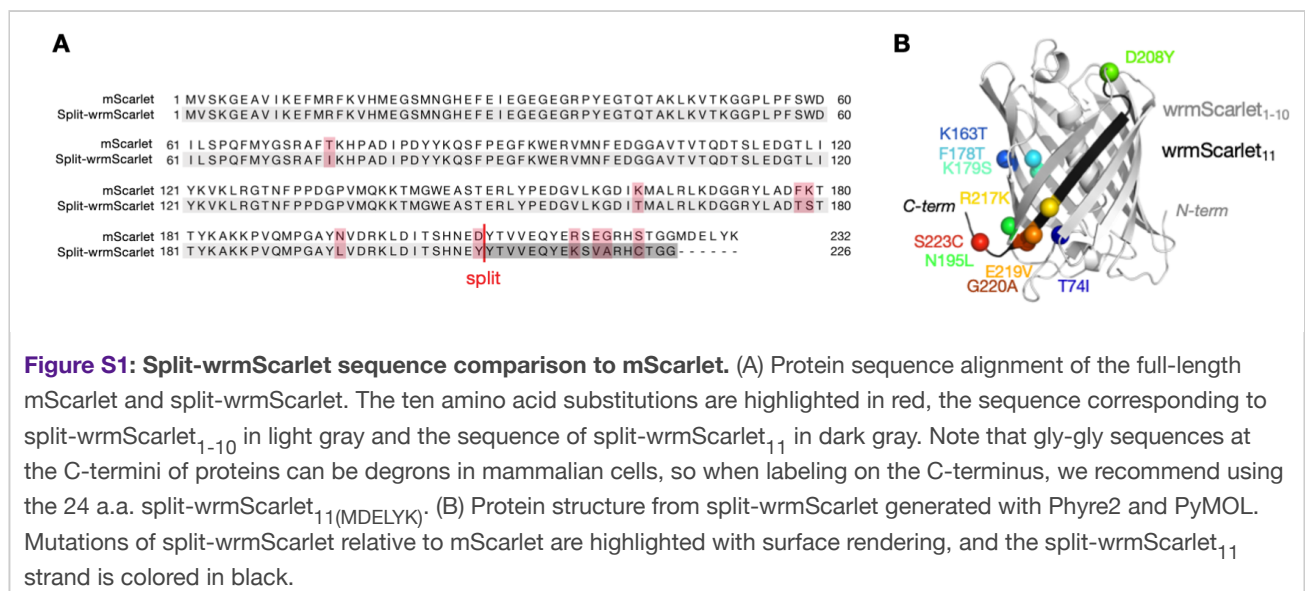
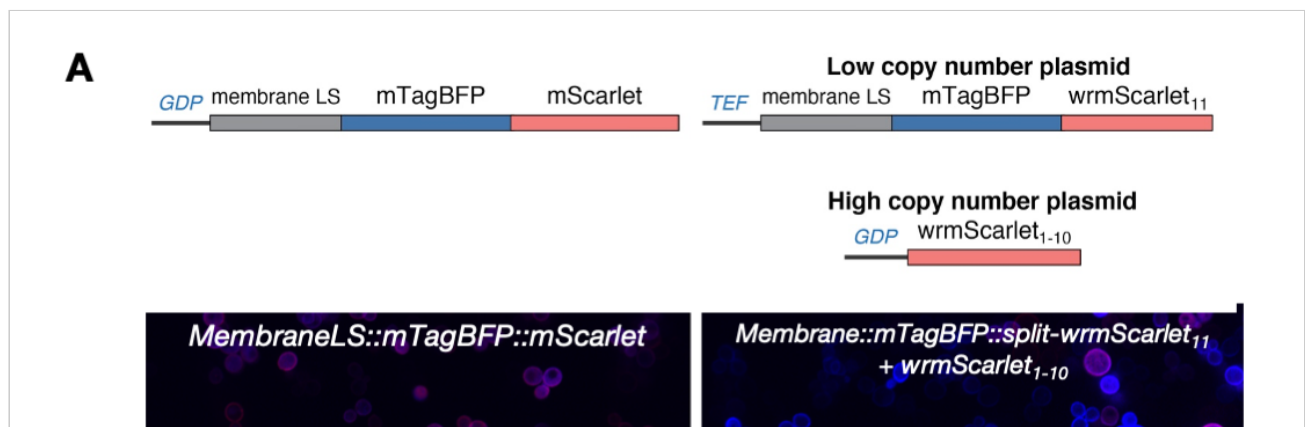
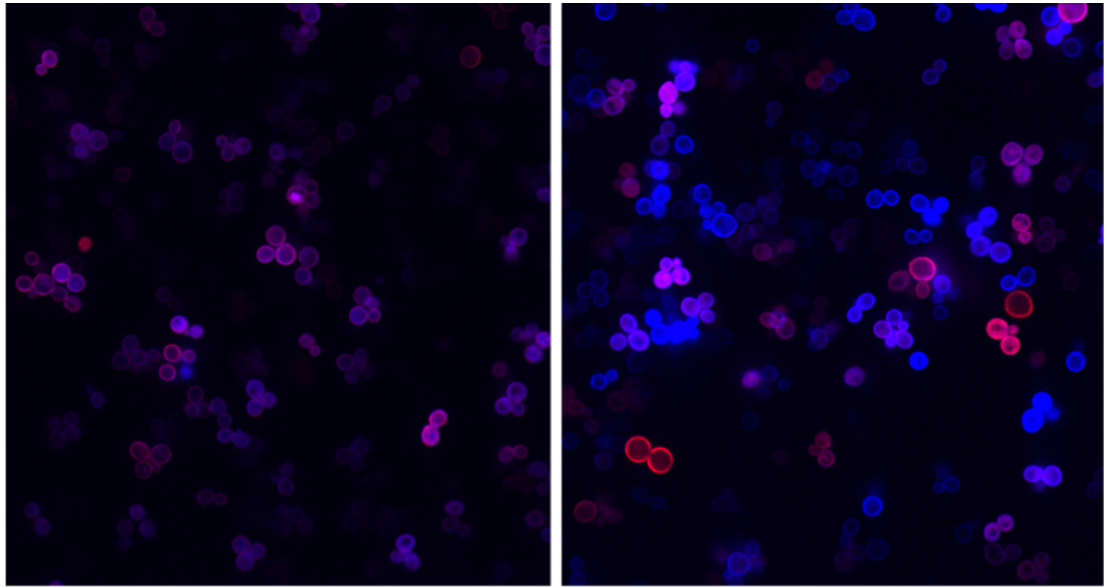


Figure S2. Split-wrmScarlet brightness in *S. cerevisiae*.





B

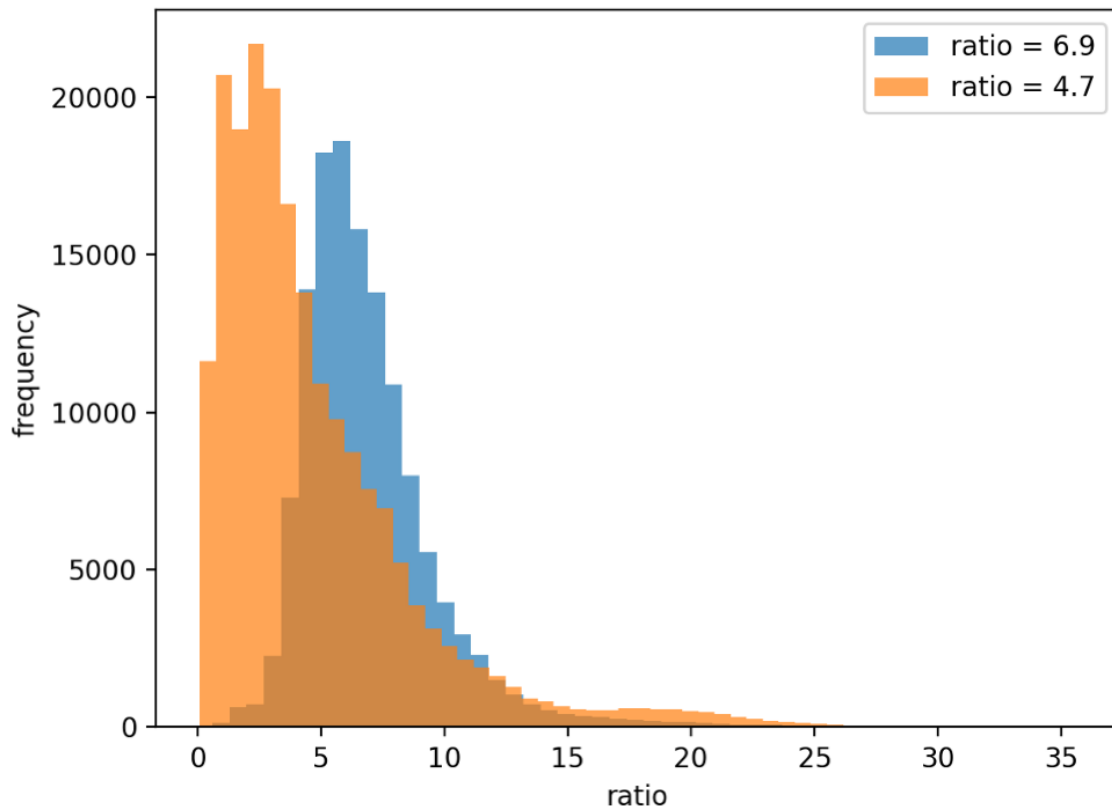


Figure S2: Split-wrmScarlet brightness in *S. cerevisiae*. (A) Composite display of red and blue channels for membrane-localized mTagBFP-mScarlet (wild-type) fusion or split-wrmScarlet₁₋₁₀ plus membrane localized mTagBFP-split-wrmScarlet₁ in yeast. Images were acquired and are displayed under identical conditions. Note that the heterogeneity inherent to expression from plasmids is large, but split-wrmScarlet is capable of brightness levels similar to the parent protein. A schematic of the plasmids transformed is presented above each image. (B) Histograms displaying the per-pixel ratio of red to blue fluorescence for background corrected, masked images. mScarlet/mTagBFP ratios are displayed in blue, and split-wrmScarlet/mTagBFP in orange. The inset displays the average red/blue ratio.

Figure S3. Developmental toxicity in worms expressing split-sfCherry3 in somatic nuclei.

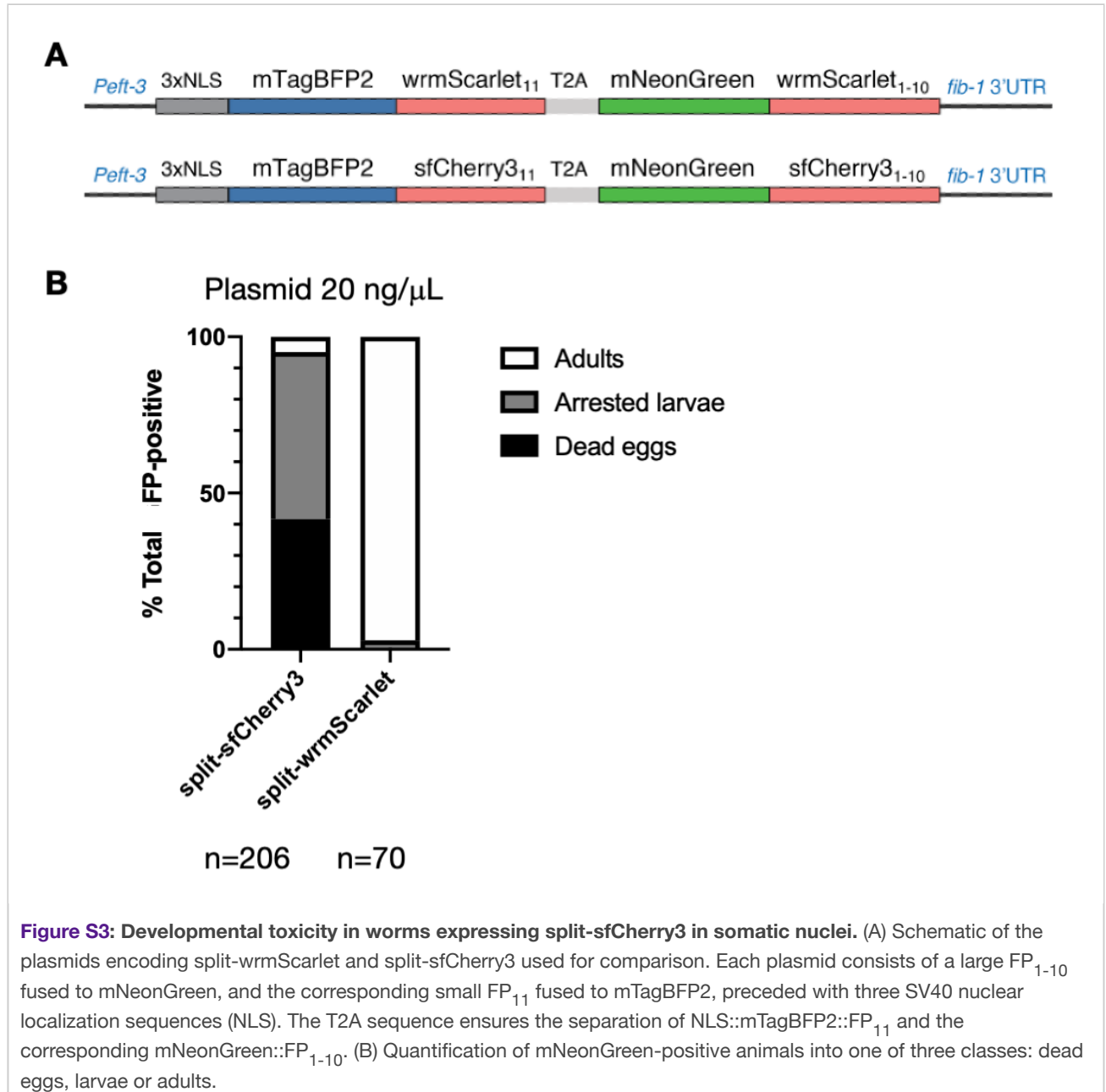


Figure S4. Split-wrmScarlet and split-sfCherry3 comparison.

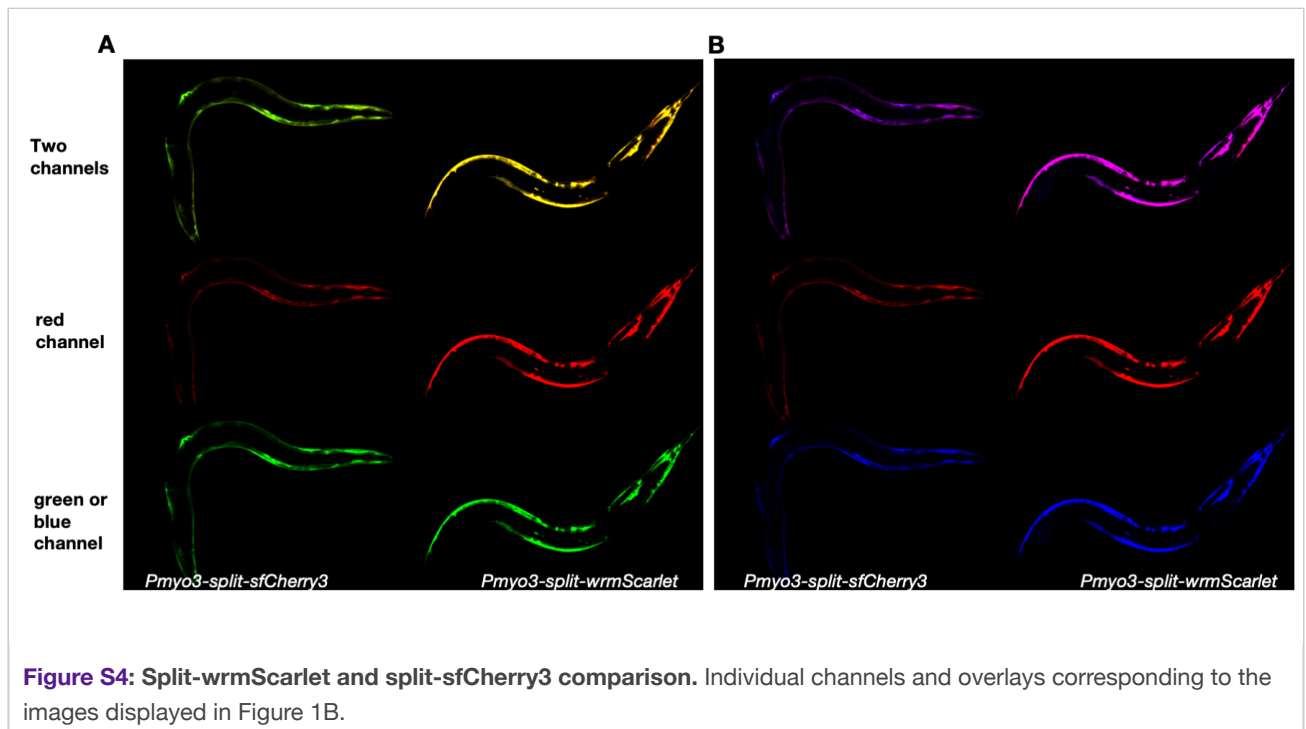


Figure S5. Brood size and lifespan of split-wrmScarlet₁₋₁₀ and sfGFP₁₋₁₀ lines.

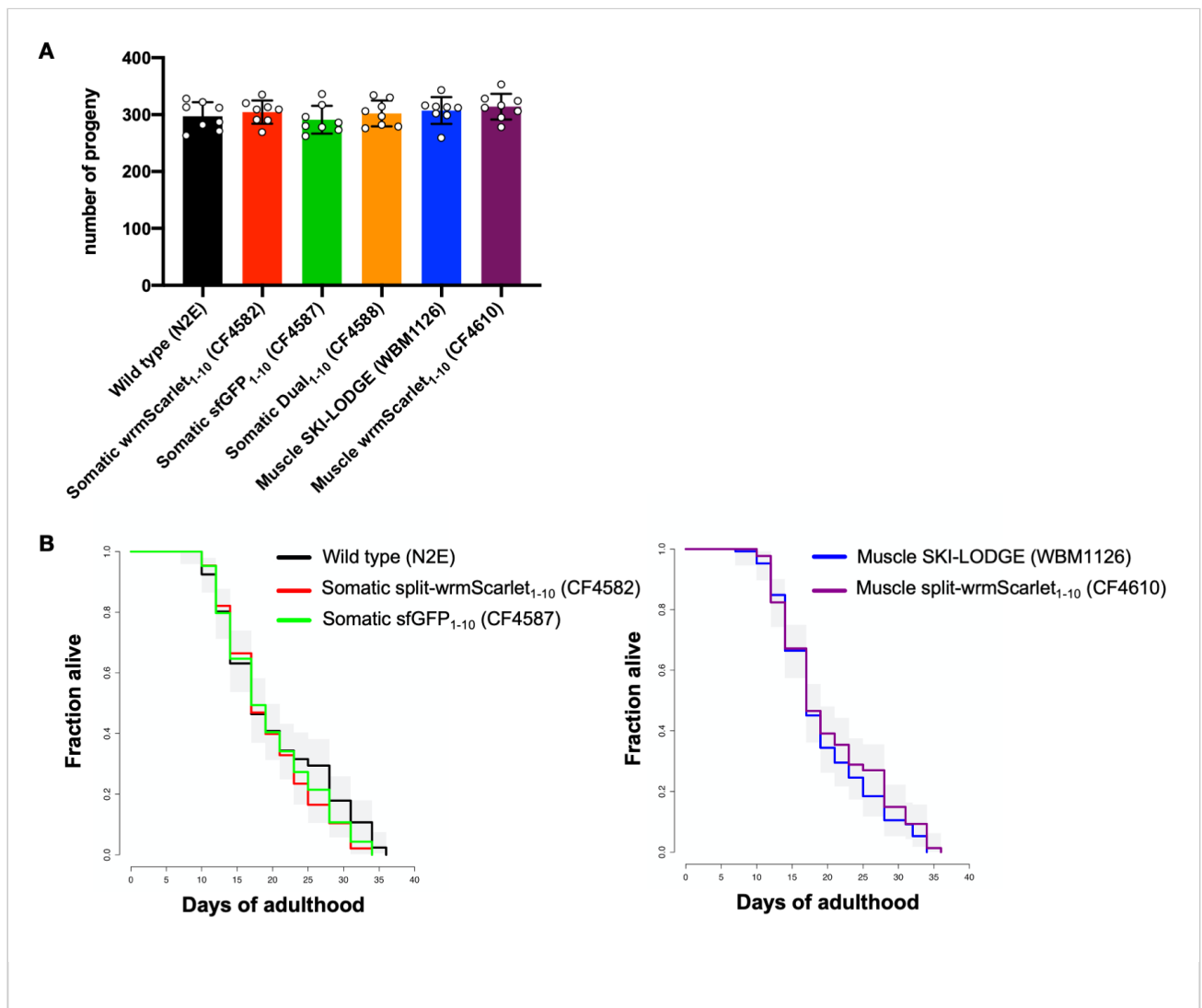


Figure S5: Brood size and lifespan of split-wrmScarlet₁₋₁₀ and sfGFP₁₋₁₀ lines. Split-wrmScarlet₁₋₁₀ and split-sfGFP₁₋₁₀ lines produced wild-type numbers of progeny (A) and a wild-type lifespan (B). Genotypes: N2E (wild-type), CF4582 (muls252[Peft-3::split-wrmScarlet₁₋₁₀::unc-54 3'UTR, Cbr-unc-119(+)] II; unc-119(ed3) III), CF4587 (muls253[[Peft-3::sfGFP₁₋₁₀::unc-54 3'UTR, Cbr-unc-119(+)] II; unc-119(ed3) III), CF4588 (muls253[[Peft-3::sfGFP₁₋₁₀::unc-54 3'UTR, Cbr-unc-119(+)], muls252[[Peft-3::split-wrmScarlet₁₋₁₀::unc-54 3'UTR, Cbr-unc-119(+)] II; unc-119(ed3) III), CF4610 (muls257[Pmyo-3::split-wrmScarlet₁₋₁₀::unc-54 3'UTR] I) and WBM1126 (wbmls61[myo-3p::3XFLAG::dpy-10 crRNA::unc-54 3'UTR] I). Supplementary table S6 show survival statistics for all lifespan experiments.

Figure S6. Proteins tagged at their C-terminus with the 24 a.a. split-wrmScarlet_{11(MDELYK)}*

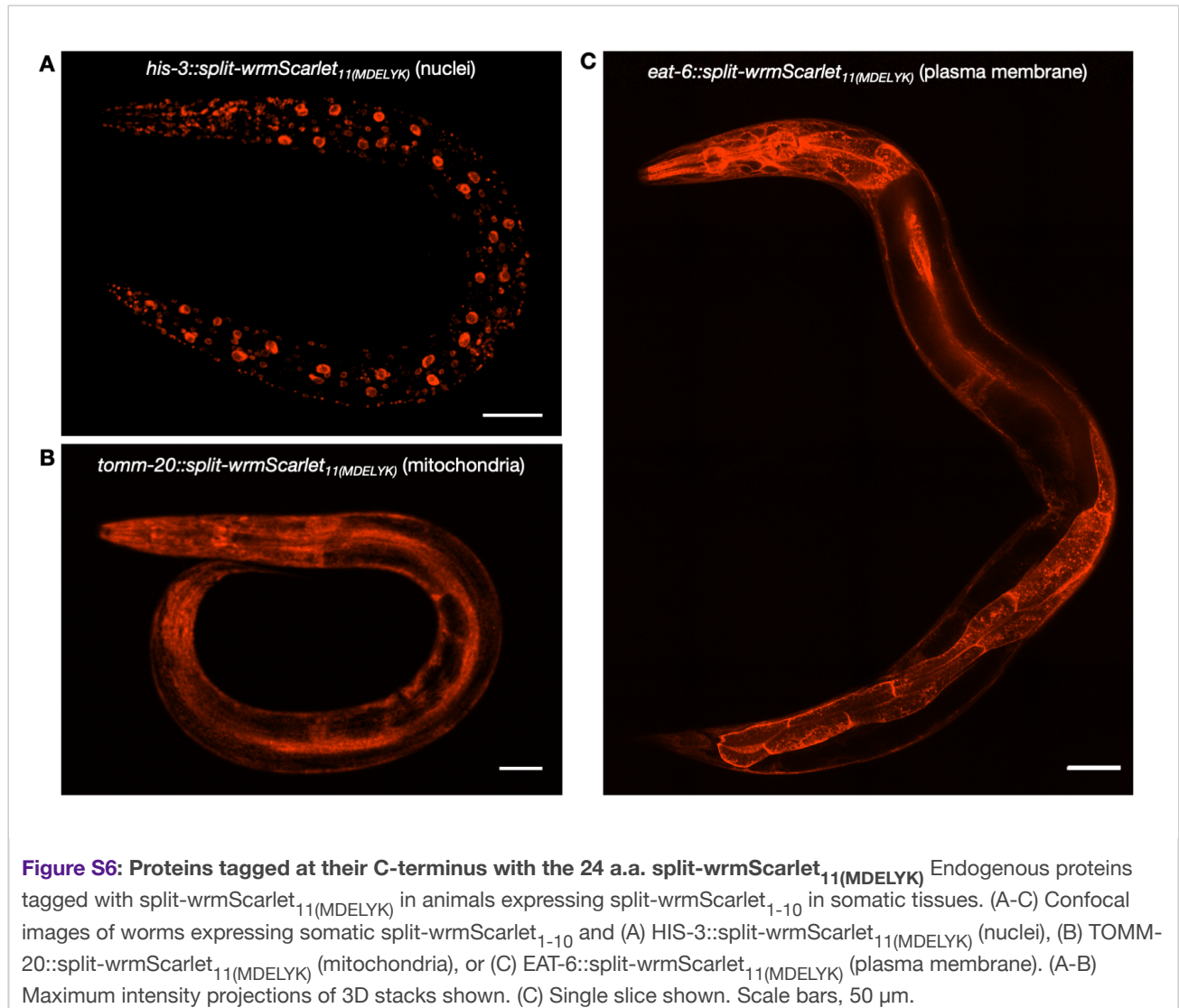


Figure S7. Tissue-specific split-wrmScarlet fluorescence in the germline is undetectable when split-wrmScarlet₁₋₁₀ is integrated using a single-copy transgene via MosSCI.

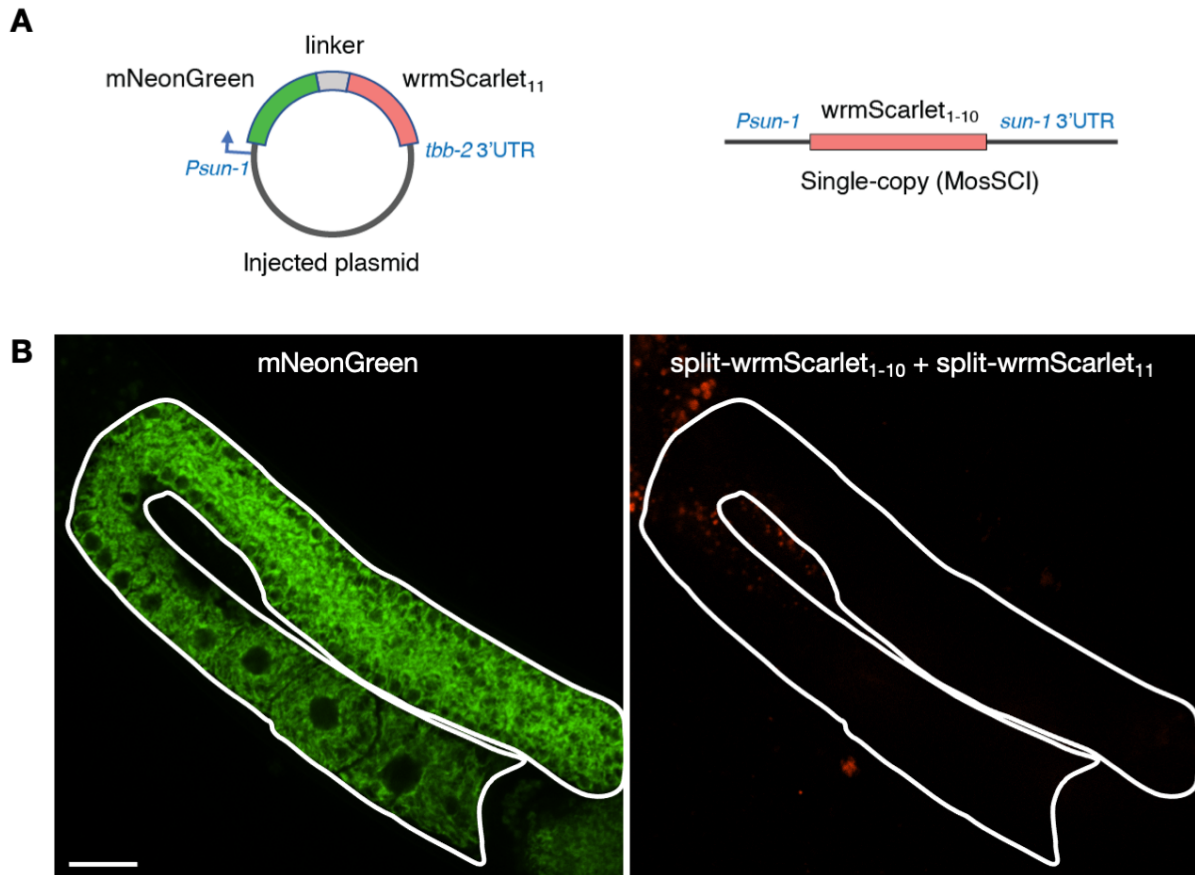


Figure S7: Tissue-specific split-wrmScarlet fluorescence in the germline is undetectable when split-wrmScarlet₁₋₁₀ is integrated using a single-copy transgene via MosSCI. (A) Schematic of the plasmid encoding *Psun-1::mNeonGreen::linker::split-wrmScarlet₁₁::tbb-2* 3'UTR (left), which was injected into the MosSCI strain PHX1797 carrying a single, integrated copy of *Psun-1::split-wrmScarlet₁₋₁₀::sun-1* 3'UTR (right). (B) Images of animal expressing mNeonGreen::linker::split-wrmScarlet₁₁ and split-wrmScarlet₁₋₁₀ in the germline. Despite detecting mNeonGreen fluorescence, split-wrmScarlet was undetectable in this MosSCI strain, potentially due to compromised expression, folding or maturation of split-wrmScarlet₁₋₁₀. Scale bar, 20 μ m.

Figure S8. Generation and validation of germline-specific split-wrmScarlet₁₋₁₀ and sGFP2₁₋₁₀ strains.

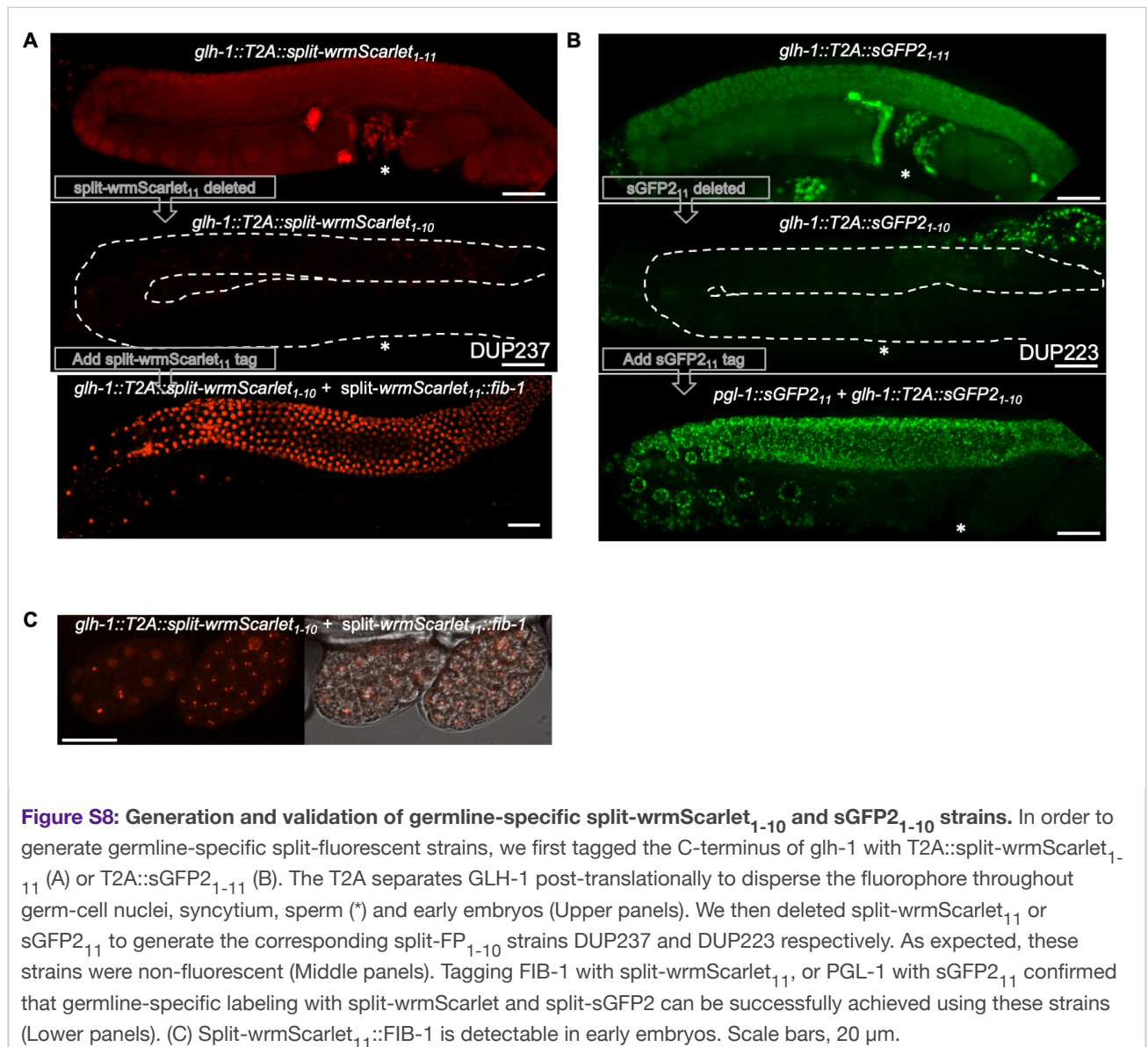


Figure S8: Generation and validation of germline-specific split-wrmScarlet₁₋₁₀ and sGFP2₁₋₁₀ strains. In order to generate germline-specific split-fluorescent strains, we first tagged the C-terminus of *glh-1* with T2A::split-wrmScarlet₁₋₁₁ (A) or T2A::sGFP2₁₋₁₁ (B). The T2A separates GLH-1 post-translationally to disperse the fluorophore throughout germ-cell nuclei, syncytium, sperm (*) and early embryos (Upper panels). We then deleted *split-wrmScarlet₁₋₁₁* or *sGFP2₁₋₁₁* to generate the corresponding split-FP₁₋₁₀ strains DUP237 and DUP223 respectively. As expected, these strains were non-fluorescent (Middle panels). Tagging *FIB-1* with *split-wrmScarlet₁₋₁*, or *PGL-1* with *sGFP2₁₋₁* confirmed that germline-specific labeling with *split-wrmScarlet* and *split-sGFP2* can be successfully achieved using these strains (Lower panels). (C) *Split-wrmScarlet₁₋₁::FIB-1* is detectable in early embryos. Scale bars, 20 μ m.

Figure S9. Somatic sfGFP₁₁ compared to full-length eGFP at the endogenous *vha-13* locus.

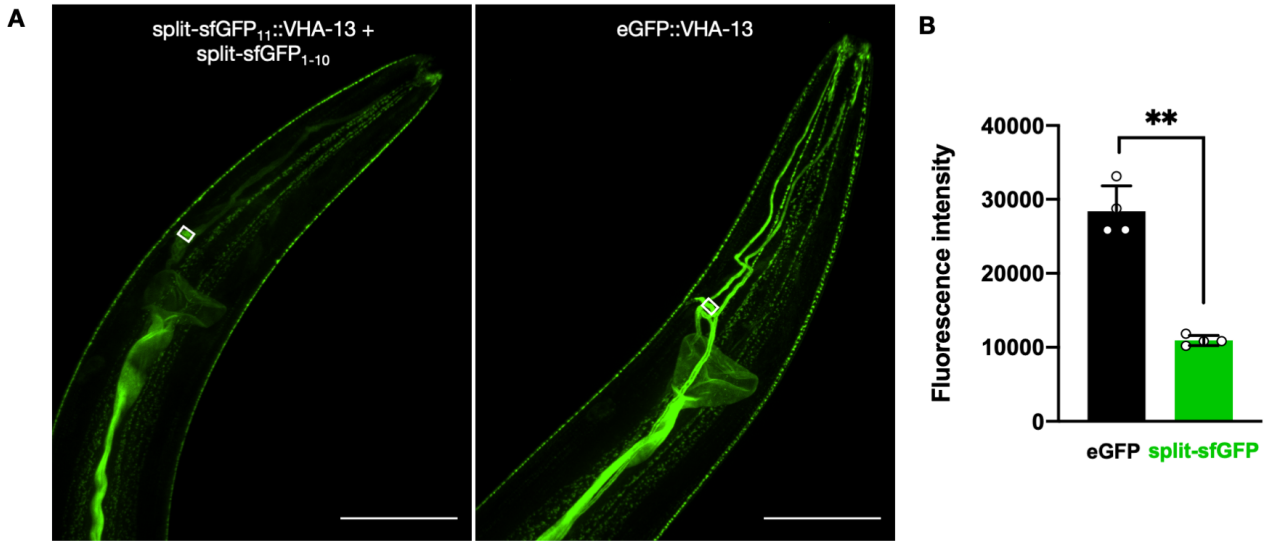


Figure S9: Somatic sfGFP₁₁ compared to full-length eGFP at the endogenous vha-13 locus. (A) Representative images of animals expressing sfGFP₁₋₁₀ in somatic tissues with endogenous VHA-13 tagged with sfGFP₁₁ (left panel), or endogenous VHA-13 tagged with eGFP in a wild-type background (right panel). Maximum intensity projections of 3D stacks shown. Scale bars, 50 μ m. (B) Emission intensities from somatic sfGFP::VHA-13 and eGFP::VHA-13. Quantification was performed in the cell body, as quantifications in the excretory canal had higher variance. Mean \pm s.d. Circles are individuals (n=4 for each condition). **P < 0.005.

Figure S10. Screen for split-wrmScarlet fluorescence in mammalian cells.

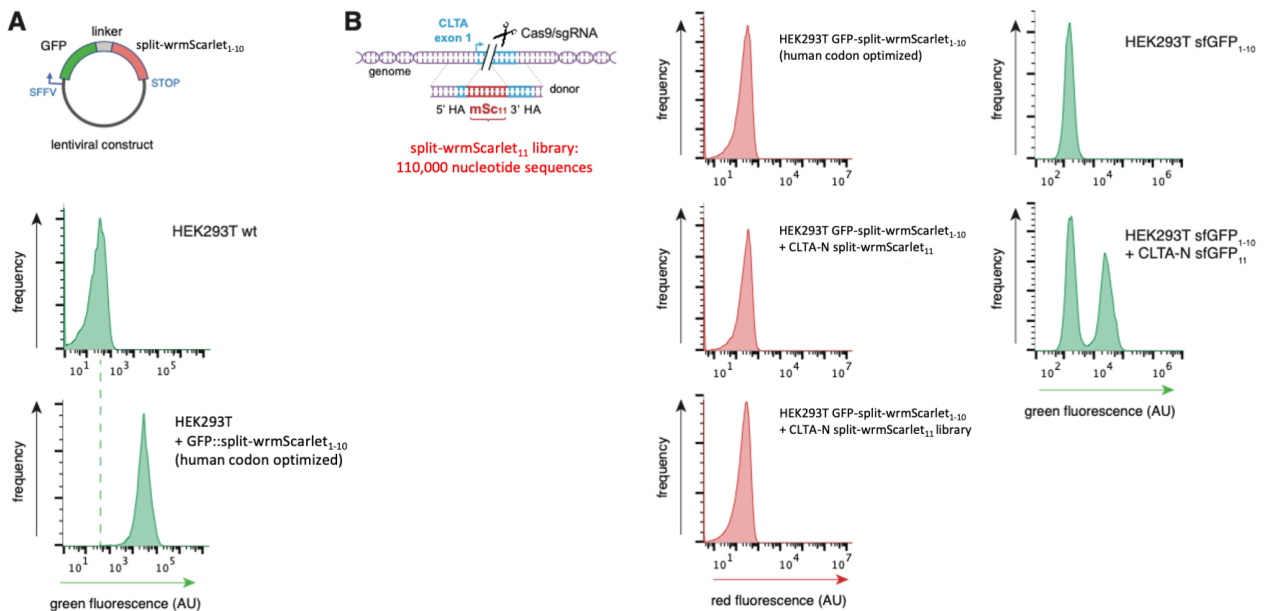


Figure S10: Screen for split-wrmScarlet fluorescence in mammalian cells. (A) FACS histograms of human codon-optimized split-wrmScarlet₁₋₁₀ expressed as a C-terminal GFP fusion. GFP expression verifies successful expression of the fusion protein in HEK293T cells by lentiviral transduction. (B) Schematic of the CRISPR-based knock-in design for screening single and double mutants of split-wrmScarlet₁₁. Left panel shows that neither our original split-wrmScarlet₁₁ sequence nor its mutant library enabled detectable complementation as detected by FACS. Right panel shows that the control experiment using the sfGFP₁₋₁₀/sfGFP₁₁ system displays high levels of knock-in and complementation in HEK293T cells.

Figure S11. Split-wrmScarlet₁₁ C-terminal amino acids did not affect H2A abundance.

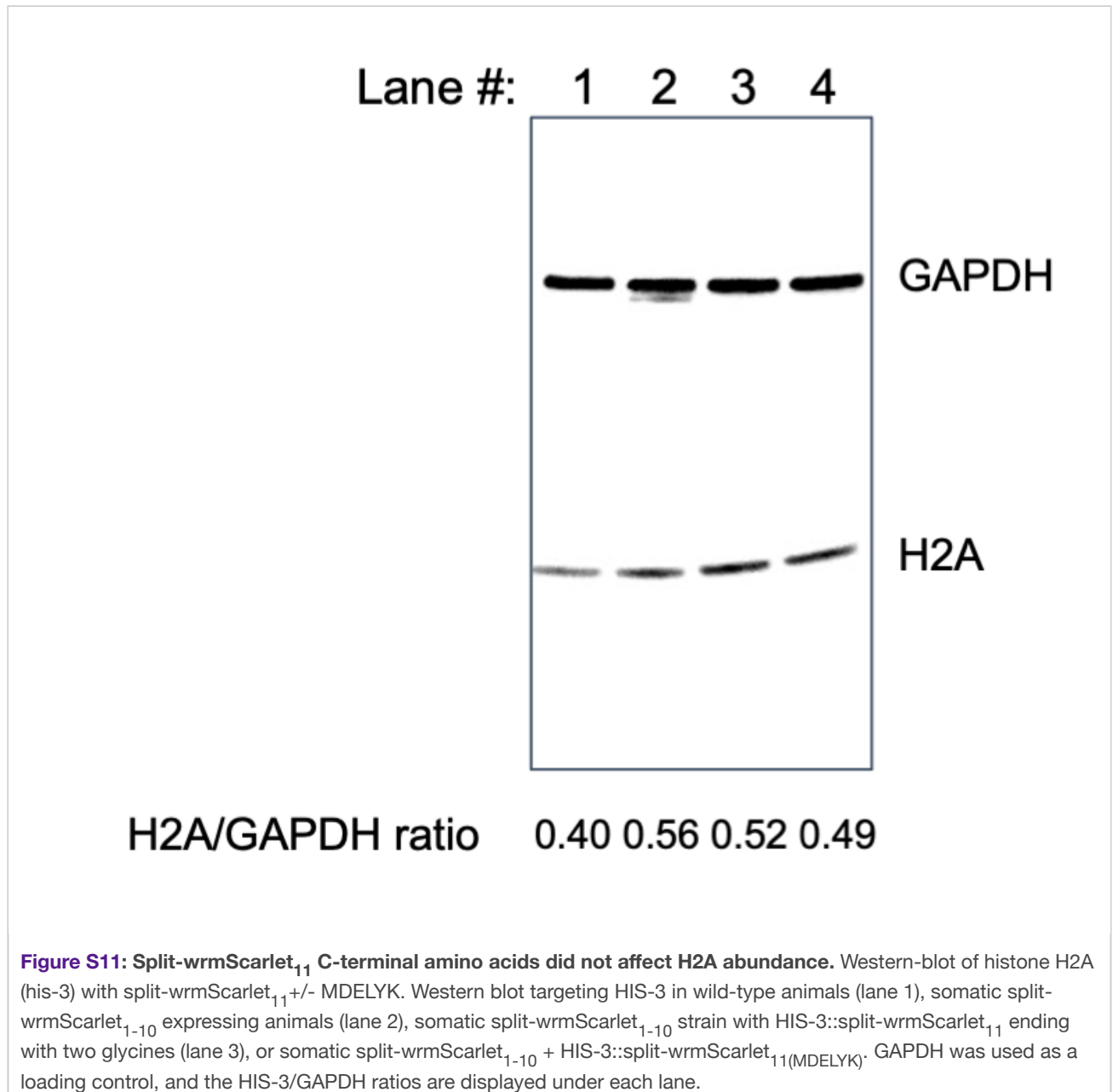


Figure S12. mScarlet ending with GG or MDELYK yields similar protein abundance in yeast.

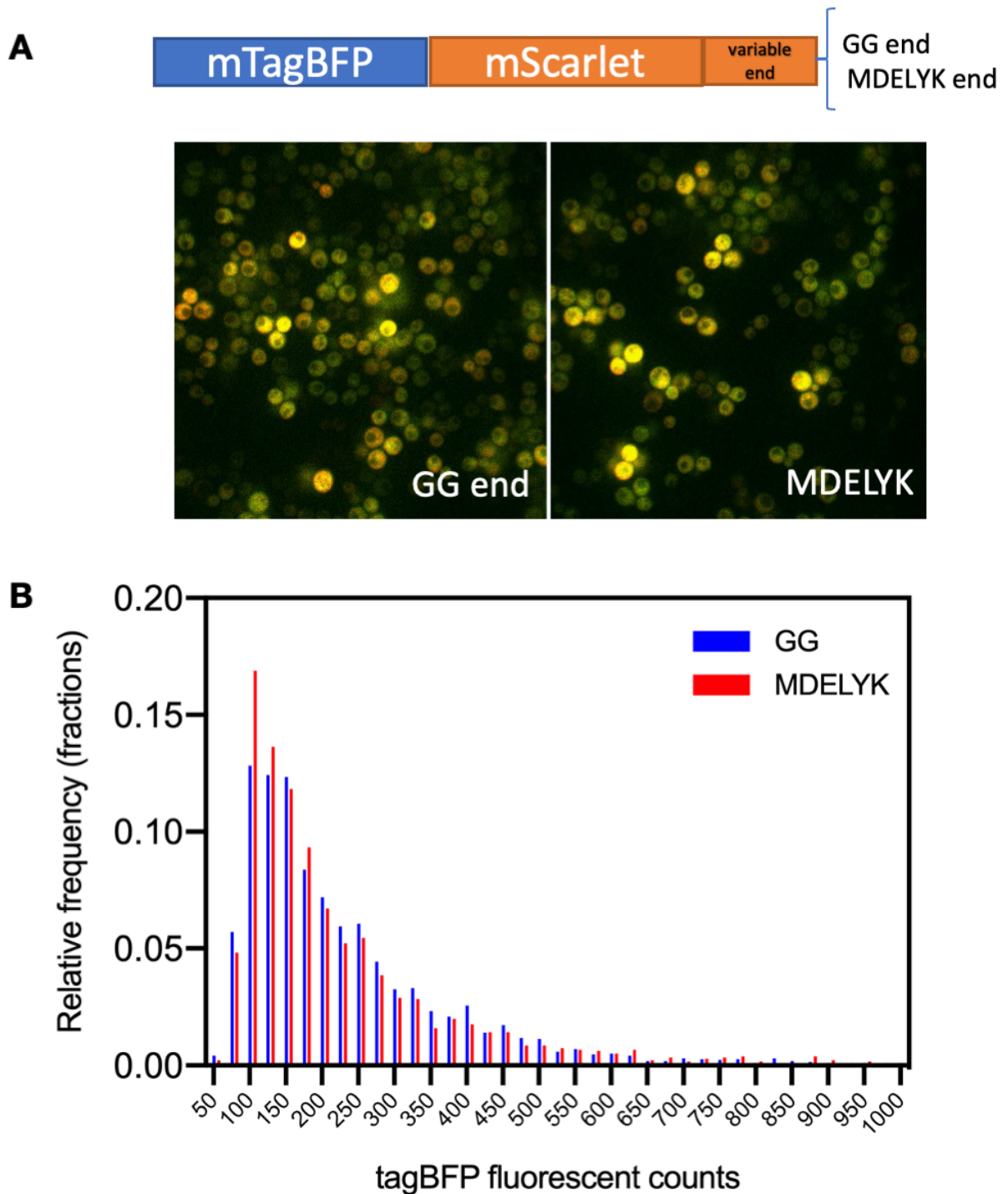


Figure S12: mScarlet ending with GG or MDELYK yields similar protein abundance in yeast. (A) Representative images of yeast expressing mTagBFP::mScarlet fusion truncated to end with gly-gly (GG end) or MDELYK from a p416-GPD promoter plasmid. mTagBFP fluorescence is pseudocolored in green, and mScarlet in red. (B) Histogram of mTagBFP fluorescence from 2556 yeasts expressing the truncation after GG and 1777 yeasts expressing the MDELYK end.

Supplementary Tables

Table S1. DNA Sequences of split-wrmScarlet₁₋₁₀, split-wrmScarlet₁₁, sfGFP₁₋₁₀, sfGFP₁₁, sGFP2₁₋₁₀ and sGFP2₁₁

Gene	DNA sequence
Codon-optimized split-wrmScarlet ₁₋₁₀ with 3 introns	ATGGTATCGAAGGGGAGAAGCAGTAATCAAGGAGTTCAT GCGTTTCAAGGTCCACATGGAGGGATCCATGAACGGA CACGAGTTCGAGATCGAGGGAGAGGGAGAGGACGT CCATACGAGGGAAACCCAAACCGCCAAGCTCAAGGTCA CCAAGgtaagtttaaacatatataactaactaacctgattattaaat cagGGAGGACCACTCCCATTCTCCTGGGACATCCTCTC CCCACAATTCATGTACGGATCCCGTGCCTTCATCAAGC ACCCAGCCGACATCCAGACTACTACAAGCAATCCTTC CCAGAGGGATTCAAGTGGGAGCGTGTTCATGAACTTCG AGGACGGAGGAGCCGTCACCGTCACCCAAGACACCT CCCTCGAGGACGGAACCTCATCTACAAGgtaagtttaaac agttcggtaactaactaacatataattaaatcagGTCAAGCTCC GTGGAACCAACTTCCCACCAGACGGACCAAGTCATGCA AAAGAAGACCATGGGATGGGAGGCCTCCACCGAGCG TCTTACCCAGAGGACGGAGTCTCAAGgtaagtttaaac tgatttactaactaactaatctgattaaatcagGGAGACATCAC ATGGCCCTCCGTCTCAAGGACGGAGGACGTTACCTCG CCGACACCTCCACCACCTACAAGGCCAAGAAGCCAG TCCAATGCCAGGAGCCTACCTCGTCGACCGTAAGCT CGACATCACCTCCCACAACGAGTAC
Codon-optimized split-wrmScarlet ₁₁	TACACCGTTCGTCGAGCAATACGAGAAGTCCGTCGCC GTCACTGCACCGGAGGA
Codon-optimized split-wrmScarlet ₁₁ (MDELYK)	TACACCGTTCGTCGAGCAATACGAGAAGTCCGTCGCC GTCACTGCACCGGAGGAATGGATGAGTTATACAAG
Codon-optimized sfGFP ₁₋₁₀ with 1 intron	ATGTCTAAGGGGAGAAGAGTTATTTACTGGAGTTGTGCC GATCCTCGTCGAGCTCGACGGAGACGTCAACGGACA CAAGTTCTCCGTCCGTGGAGAGGGAGAGGGAGACGC CACCATCGGAAAGCTCACCTCAAGTTCATCTGCACC ACCGGAAAGCTCCCAGTCCCATGGCCAACCCCTCGTC ACCACCCTCACCTACGGAGTCCAATGCTTCTCCCGTTA CCCAGACCACATGAAGCGTCCGACGTTCTTCAAGTCC GCCATGCCAGAGGGATACGTCCAAGAGCGTACCATCT CCTTCAAGGACGACGGAAAGTACAAGgtaagtttaaacatat ataactaactaacctgattattaaatcagACCCGTGCCGTCCG TCAAGTTCGAGGGAGACACCCTCGTCAACCGTATTCGA GCTCAAGGGAAACCGACTTCAAGGAGGACGGAAACAT CCTCGGACACAAGCTCGAGTACAACCTCAACTCCCAC AACGTCTACATCACCGCCGACAAGCAAAAAGAACGGAA TCAAGGCCAACTTCCCGTCCGTCACAACGTCGAGGA CGGATCCGTCCAACCTCGCCGACCACTACCAACAAAAC ACCCCAATCGGAGACGGACCAAGTCCCTCCTCCAGAC AACCACTACCTCTCCACCCAAACCGTCTCTCCAAGG ACCCAACGAGAAG
Codon-optimized sfGFP ₁₁	CGTGACCACATGGTCCTTCATGAGTATGTAATGCTGC TGGATTACA
Codon-optimized split-wrmScarlet ₁₋₁₀ with 1 intron for germline expression	ATGGTTTCCAAGGGGAGAGGCTGTTATCAAGGAATTCAT GCGCTTCAAGGTTACATGGAAGGATCTATGAACGGA CACGAATTCGAAATCGAAGGAGAAGGAGAAGGACGCC CATACGAGGGAACTCAAACCTGCTAAGCTTAAGGTTACT AAAGGAGGACCACTTCCATTCTCTTGGGATATCCTTCT CCACAGTTCATGTACGGATCTCGCGCTTTCATCAAGCA CCCAGCTGATATCCAGATTACTACAAGCAGTCTTTCC CAGAAGGATTCAAATGGGAGCGCGTTATGAACTTCGAA GATGGAGGAGCTGTTACCGTTACCCAAGATACCTCCCT TGAGGATGGAACCCCTTATCTACAAGgtaagtttaaacatatata actaactaacctgattattaaatcagGTTAAGCTTCGCGGAA CTAATTTCCCACCAGATGGACCAAGTATGCAGAAGAAG ACTATGGGATGGGAAGCTTCTACCGAGCGCCTTTACCC AGAGGATGGAGTCCCTAAGGGAGATATCACCATGGCTC TTCGTCTTAAGGATGGAGGACGTTACCTTGCTGATACC TCTACTACTTACAAGGCTAAGAAGCCAGTTTCAAGTACC AGGAGCTTACCTTGTGATCGTAAGCTTGATATCACTTC TCATAACGAATAC
Codon-optimized sGFP2 ₁₋₁₀ with 2 intron for germline expression	ATGAGTAAAGGGAGAAGAATTGTTCACTGGAGTTGTCCC AATCCTCGTCGAGCTCGACGGAGACGTCAACGGACA CAAGTTCTCCGTCCGTGGAGAGGGAGAGGGAGACGC CACCATCGGAAAGCTCACCTCAAGTTCATCTGCACC ACCGGAAAGCTCCCAGTCCCATGGCCAACCCCTCGTC ACCACCCTCACCTACGGAGTCCAATGCTTCGCCCGTT ACCCAGACCACATGAAGCGTCCAGACTTCTTCAAGTC CGCCATGCCAGAGGGATACGTCCAAGAGCGTACCATC TCTTCAAGgtaagtttaaacatatataactaactactgattattaaat tcagGACGACGGAAAGTACAAGACCCGTGCCGTGCTCA AGTTCGAGGGAGACACCCTCGTCAACCGTATCGAGCT

	<p>CAAGGGAACCGACTTCAAGGAGGACGGAAACATCCTC GGACACAAGCTCGAGTACAACCTCAACTCCCACAACG TCTACATCACCGCCGACAAGCAAAGAACCGGAATCAA GGCCAACCTTACCgtaagtttaaacatgattttactaactaact gatttaaatctcagACCCGTCACAACGTCGAGGACGGATCC GTCCAACTCGCCGACCACTACCAACAAAACACCCCAA TCGGAGACGGACCAAGTCTCTCTCCAGACAACCACTA CCTCTCCACCCAAACCGTCTCTCAAGGACCCAAAC GAGAAG</p>
<p>Codon-optimized sequence of split-wrmScarlet₁₋₁₀ with 3 introns, engineered to avoid piRNA recognition transgene silencing. (Undetectable fluorescence in the MosSCI strain PHX1797)</p>	<p>ATGGTATCGAAGGGAGAAGCAGTCATCAAGGAGTTCAT GCGTTTCAAGGTCCACATGGAGGGATCCATGAACGGA CACGAGTTCGAGATCGAGGGAGAGGGAGAGGGACGT CCATACGAGGGAACCCAAACCGCCAAGCTCAAGGTCA CAAAGgtaagtttaaacatataataactaactaaccctgatttt cagGGAGGACCACTCCCATTCTCTCTGGGACATCCTCTC CCCACAATTCATGTACGGATCCCGTGCCTTCATCAAGC ACCCAGCCGACATCCCAGACTACTACAAGCAATCCTTC CCAGAGGGATTCAAGTGGGAGCGTGTGATGAACCTCG AGGACGGAGGAGCCGTCACCGTCACCCAAGACACCT CCCTCGAGGACGGAACCCCTCATCTACAAGgtaagtttaaac agttcggtaactaactaaccatacatatttaaatctcagGTCAAGCTCC GTGGAACCAACTTCCCACCAGACGGACCAAGTCATGCA AAAGAAGACCATGGGATGGGAGGCCCTCCACCGAGCG TCTCTACCCAGAGGACGGAGTCTCAAGgtaagtttaaac tgattttactaactaactaactgatttaaatctcagGGAGACATCACC ATGGCCCTCCGTCCTCAAGGACGGAGGACGTTACCTCG CCGACACCTCCACCACCTACAAGGCCAAGAAGCCAG TCCAAATGCCAGGAGCCTACCTCGTCGACCGTAAGCT CGACATCACCTCCCACAACGAGTAC</p>

Table S2. *C. elegans* lines expressing single-copy of split-wrmScarlet₁₋₁₀ and/or sfGFP₁₋₁₀

Strain	Genotype	Description	Genomic Position	Genetic Position	Sequence
CF4582	muls252[Peft-3::split-wrmScarlet ₁₋₁₀ ::unc-54 3'UTR, Cbr-unc-119(+)] II; unc-119(ed3) III	Somatic split-wrmScarlet ₁₋₁₀ (eft-3 promoter)	II: 9.83 MB	II: 1.73	<p>gcaccttggcttttattgtcaactccattggttcttccattgt ttctgttaaatgaattttcataaaataaagacattatac aatataaaaatgaagaattttgaaaaaaactgccagag agaaaaagtatgcaaacctcccgccgagagtgttgaat ggtgtacggtacattttctgtgctaggagttagatgtgcagg cagcaacgagagggggagagatttttggcctgtgaa attaacgtgagtttctggtcatctgactaatcatgttggtttt tgttggttatttgttttatctttgttttatccagattaggaat tfaaatttatgaattataatgaggtcaaacattcagtccca cgcttttccgttctcactgttttagtcaattttatttaggctt tcaacaaatgttcaactgtcttattgtgacctcacttttata tttttaattttfaaaaataitagaagttctaggataattttc gacttttattctctaccgtccgactctcttacttttaatt aaattgttttttccagttgggaacacittgctcactccgta gcagccATGGTATCGAAGGGAGAAGCAGTAA TCAAGGAGTTCATGCGTTTCAAGGTCCACA TGGAGGGATCCATGAACGGACACGAGTTC GAGATCGAGGGAGAGGGAGAGGGACGTC CATACGAGGGAACCCAAACCGCCAAGCTC AAGGTCACCAAGgtaagtttaaacatataataactaa ctaaccctgattttaaatctcagGGAGGACCACTC CCATTCTCTGGGACATCCTCTCCCCACAA TTCATGTACGGATCCCGTGCCTTCATCAAG CACCCAGCCGACATCCCAGACTACTACAA GCAATCCTTCCCAGAGGGATTCAAGTGGG AGCGTGTATGAACCTTCGAGGACGGAGGA GCCGTCACCGTCACCCAAGACACCTCCCT CGAGGACGGAACCCCTCATCTACAAGgtaagtt ttaaacagttcggtaactaactaaccatacatatttaaatctc agGTCAAGCTCCCGTGGAAACCAACTTCCCA CCAGACGGACCAAGTCATGCAAAAAGAAGAC CATGGGATGGGAGGCCCTCCACCGAGCGT CTCTACCCAGAGGACGGAGTCTCAAGgta agtttaaacatgattttactaactaactaactgatttaaatctc cagGGAGACATCACCATGGCCCTCCGTC AAGGACGGAGGACGTTACCTCGCCGACA CCTCCACCACCTACAAGGCCAAGAAGCCA GTCCAAATGCCAGGAGCCTACCTCGTCGA CCGTAAGCTCGACATCACCTCCACAACG AGTACTAAtaagtcattactctcaacatccctacatg ctctttctccctgtgctcccaccctattttgttattatcaaa</p>

					<pre> aaacttctctaattcttgttttttagctctttaaagtcacctc taacaatgaaattgtgtagattcaaaaatagaattaattcgt aataaaaagtcgaaaaaaatgtgctccctccccccatta ataataattctatccaaaatctacacaatgttctgtgtaca cttctatgtttttacttctgataaaatgttgaacatcataga aaaaccgcacacaaaataccttatcatatgttacgtttca gtttatgaccgcaattttatttctcgacgtctgggacctc atgacgtcaaatcatgctcatcgtgaaaaagtttgagat tttggaaatccaatcaagtgaagttatgaaattaatttc ctgcttttgcttttggggttcccctattgttgcaagatttcg aggacggcgttttctgtcaaaaacacaagattgatgagc acgatgcaagaagatcggaagaagtttggggttgagg ctcagtggaaggtgagtagaagttgataattgaaagtg agtagtctatggggtttgccttaaatgacagaatacatt ccaatataccaacaataactggtt </pre>
CF4587	<p>muls253[(Pef t-3::sfGFP₁₋₁₀ ::unc-54₁₋₁₀ 3'UTR, Cbr- unc-119(+)] II; unc- 119(ed3) III</p>	<p>Somatic sfGFP₁₋₁₀ (eft-3 promoter)</p>	II: 8.42 MB	II:0.77	<pre> gcaccttggctttttattgtcaactcattggttctccattgt ttctgttaaattaatgaattttcataaaataaagacattatac aatataaaaaatgaagaattattgaaaataaactgccagag agaaaaagatgcaaacactcccgcggagaggtgttgaat gggtgacggtacattttcgtgctaggagtagatgagcagg cagcaacgagagggggagagatttttggcctgtgaa attaactgagttttctggtcatctgactaatcatgttggtttt tgttggttatttgttttacttgtttatccagattagaaat tfaaatttatgaattataatgaggtcaaacattcagtcca gcgttttctgttctcactgttagtgaatttttttaggctt fcaacaaatgttcaactgtcttattgtgacctcacttttata tttttaattttfaaaaatitagaagttctaggataatttttc gacttttattctctaccgctccgactcttctacttttaatt aaatgtttttttcagttgggaacactttgctc aaaa ATGTCTAAGGGGAGAAGAGTTATTTACTGGA GTTGTGCCGATCCTCGTCGAGCTCGACGG AGACGTCAACGGACACAAGTTCTCCGTCC GTGGAGAGGGAGAGGGAGACGCCACCAT CGGAAAGCTCACCTCAAGTTCATCTGCA CCACCGGAAAGCTCCCAGTCCCATGGCC AACCTCGTCACCACCCTCACCTACGGAG TCCAATGCTTCTCCGTTACCCAGACCACA TGAAGCGTCACGACTTCTTCAAGTCCGCC ATGCCAGAGGGATACGTCCAAGAGCGTAC CATCTCCTTCAAGGACGACGGAAAGTACA AGGtaagtttaacatataataactaactaacctgattattt aattttcagACCCGTGCCGTGTCAGTTTCG AGGGAGACACCCTCGTCAACCGTATCGAG CTCAAGGGAACCGACTTCAAGGAGGACG GAAACATCTCGGACACAAGCTCGAGTAC AACTTCAACTCCCACAACGTCTACATACC GCCGACAAGCAAAAGAACGGAATCAAGGC CAACTTACCGTCCGTCAACAACGTCGAGG ACGGATCCGTCCAACCTCGCCGACCACTAC CAACAAAACACCCCAATCGGAGACGGACC AGTCTCCTCCAGACAACCACTACCTCT CCACCCAAACCGTCTCTCCAAGGACCCA AACGAGAAGTAA atataccaccgtgctggcgggaggtcgc catctcggcccgtgacctgacttcaagtccaattactct tcaacatcccctacatgcttctccctgtgctcccacccc tattttgtattatcaaaaaactctctaatttcttgttttttag cttcttttaagtcaccttaacaatgaaattgtgtagattcaa aaatagaattaatcgaataaaaagtcgaaaaaaattgtg ctcccctccccattaataataattctatccaaaatctaca caatgtctgtgtacacttctatgttttacttctgataaattt ttgaaacatcatagaaaaaacgcacacaaaatacctta tcatatgttacgtttcagtttatgaccgcaattttatttctcgc acgtctgggacctcatgacgtcaaatcatgctcatcgtga aaaagtttgagatattttggaattttcaatcaagtgaaggt ttatgaaattaattttctgcttttggggttcccctatt gtttgcaagatttcgaggacggcgttttctgctaaaatca caagattgatgagcagatgcaagaagatcggaagaa ggtttgggttgaggctcagtggaaggtgagtagaagttga taattgaaagtgagtagtctatggggtttgccttaaat gacagaatacattccaatataccaacaataactggtt </pre>
CF4588	<p>muls253[(Pef -3::sfGFP₁₋₁₀ ::unc-54₁₋₁₀ 3'UTR, Cbr- unc-119(+)] muls252[(Pef -3::split- wrmScarlet₁₋₁₀ ::unc-54₁₋₁₀ 3'UTR, Cbr- unc-119(+)]</p>	<p>Somatic sfGFP₁₋₁₀ and somatic split- wrmScarlet₁₋₁₀ (eft-3 promoter)</p>	II: 8.42 MB, 9.83 MB	II:0.77, II:1.73	<p>Sequences from CF4582 and CF4587</p>

	II; unc-119(ed3) III				
CF4610	muls257[Pmyo-3::split-wrmScarlet ₁₋₁₀ ::unc-54 ₃ UTR] I	Muscle split-wrmScarlet ₁₋₁₀ (myo-3 promoter)	I: 2.85 MB	I:-5.32	<p> agtgattatagctctgtttcgtaatttgaatttggcttgata aggctgcaacaaagatcaggttgacatattttcagtaatt tafttaacctgfactctatcacctgccggctataaagttctt gaataaaataattttccgcacaaaacatgagttttcttcg aaaataaaagtgccaggtaattagagattttctgtaattaa ctgcataatttgcacgtgcatagttttacattccactacgt catagttctaaaactaactctcctgaaaatagaagtaggt gaagaaagtttaattatcagttctaaaatgacaattgatctt ggaatatgttctgaaactaccgatcattgaacagatgctatt tgaatgatatagaattgtatatttgaatttctgaacgcgtt ctaaaggcacacagattaattcaaaaagtaagctctcaca cctttctctggtatggtggccgattttgagtttgggtgatt gcttttcacaatcagttttcaggattatgtgatgaactag atctcaagtttctgtaacttcatatgttttcggaactcaga agtacatattgggtattgtgctcaaaaaattcagcaatcag cttcgctccgctgactttagaaccccaaaaaatagtaggc caactgactgtgtacgatcatttcaattttcaatacatatt taagatttcaagagtaagaaggcacaacgttctggaa tacatataattttcagggtacaattagcaaaaagtgcaact gaaatatacgttttaatttcacgaataacccaattagttcaat gtattttggtaaccaacgttaagtttggctccaaccaat tatcatttctgatcaaccacaatgtttttctttatctgaagtt aattttttttatccagatgtttggcatattttcaattctca ctagcgcctcactcttgcactccggcgccctgaactaat gcatctgtgcaagaattgaaagaccaatcaacacattgtt ttctcagagatactgaagaaaatgaataaaaacagaga aaaagagccatgtgattagtgacaactgttctaacagat accatagcttggacttggatcgtgatggcaactatgggtc aacaataatgattgcagaggggtgcaaacagtcaggt cgagaaaatataaaaaacagaaaaaagaacagaaaa aatgggttggagagtcagataattataaaagaaaaattgt acatagaataaacattttgtagaagaagttattttcaag catcgttaaaaatttcaagcaccttattcatatttaatttt aaacatggttaaatgaacaacacgggtgcgcaatcaggaa aacttgaaatctgaaactgttgggtgatcttctcgcaactg ttcagatagcactagtgtaattgtaagagtgccgcaatata atggaatataatggatcacacctcctgccatcaggtaaac gtctctgttatcacatatttccaactattaaattttacctttac agttttacattttttgaaaaaagtaactttttgcttcaaaatc cctgacgaaaatatacaatatttatacagactgcagag gaaccgattgatgattggaaaatccagcttaccctgtgaa gaaactgaaaagttcataaccctagggtattcccagttaca ttcccactggctaaacaatgacaccagtttttcatcacctt tctcaaatctcggcgatttgttaaaacaaaatttggctcc cttctctgatatctctatgtctctaaacacaagttcatcggaa aacgaaggagggtaggtgttgggtggctcccgaagtga aaatagaagagcaagaatagaatattagagagagagtg agagagggcgggatagctcccgggattccgtttctctc ttatctcaacgatgatgtgtgctggtgtatagattctgtt gctccccacaactcgtccgaaggctcaatacaattca attgatattggaggagagcctaccggagtgaggagataa gaagaaacataagaagaagaagaagaagaagcatgctt ctggttttgatgctatgaaaacgggcacaaaagatgattg aggctccctttcaataccttctctcatcttcaaatcccattga aacctaaaacttccaccacgctttaccattgttctccaaaa acttatagcaatgtctataactttttatctctgaaaagcagtg ttccattttcttttctattttatttcaattgttctcacattcgtt tggattcttgcctgcaaccagcttcttctccactttaccgt ctaattttcagggcaggagccatcaaacccacgaccac tagatccat ATGGTATCGAAGGGAGAAGCAGTAATCAAG GAGTTCATGCGTTTCAAGGTCCACATGGA GGGATCCATGAACGGACACGAGTTTCGAGA TCGAGGGAGAGGGAGAGGGACGTCCATA CGAGGGAACCCAAACCGCCAAGCTCAAG GTCACCAAGGtaagtttaaacatatataactaa ccctgatttttaattttcagGGAGGACCACTCC ATTCTCCTGGGACATCCTCTCCCCACAATT CATGTACGGATCCCGTGCCCTTCAAGCA CCCAGCCGACATCCCAGACTACTACAAGC AATCCTTCCCAGAGGGATTCAAGTGGGAG CGTGTGATGAACCTTCGAGGACGGAGGAGC CGTCACCGTACCCAAAGACACCTCCCTCG AGGACGGAACCCTCATCTACAAGGtaagttta aacagttcggtaactaactaacatacatatttaatttcag GTCAAGCTCCGTGGAACCAACTTCCCACC AGACGGACCAGTCATGCAAAAAGAACCA TGGGATGGGAGGCCTCCACCGAGCGTCT CTACCCAGAGGACGGAGTCCCTCAAGGtaag tttaaacatgattttactaactaactatctgattaaatttca gGGAGACATCACCATGGCCCTCCGTCTCA </p>

					<p>AGGACGGAGGACGTTACCTCGCCGACAC CTCCACCACCTACAAGGCCAAGAAGCCAG TCCAAATGCCAGGAGCCTACCTCGTCGAC CGTAAGCTCGACATCACCTCCCACAACGA GTAATAA</p> <p>catctcgccccgctgacctgaactcaagccaattactct tcaacatcccacatgctcttctccctgctccacccccc tattttgtattatcaaaaaactctcttaattctttgitttag ctcttttaagtcacctcaacaatgaaattgtgtagattcaa aaatagaatttaattcgtataaaaaagtcgaaaaaattgtg ctccctccccccattaataataattctatccaaaatctaca caatgttctgtgtacacttctatgtttttacttctgataaattt ttgaaacatcatagaaaaaacgcacacaaaatacctta tcatatgttacgttcagtttatgaccgcaattttatttcttccg acgtctggccctctcatgacgtcaaatcatgctcatcgtga aaaagtttggagatttttggaaattttcaatcaagtgaaagt ttatgaaattaatttctgcttttcttttgggtttccctatt gtttgtcaagatttcgaggacggcgttttctgctaaaatca caagtattgatgacacgatgcaagaagatcggaagaa ggtttgggttgaggctcagtggaag</p>
DUP223	glh- 1(sam129[glh - 1::T2A::sGFP 2 ₁₋₁₀)]	Germline sGFP2 ₁₋₁₀	l: 6.85 MB	l: 1.41	<p>gtttcgccacgcccccaactacagtaacctcgacacac tcatctactaaatttgggacagttcctaattcttttgcgtttt caactcaatttctggaaaaacttaatttctgcgaaaATG TCTGATGGTTGGAGTGATAGCGAAAGTGCT GCTAAGGgtgagtttatttgaacttccaccggtttatt ttgataaaaaactttatttcagCCAAAAC TGGATTGG GTAGTGGAGGCGGTTTCGGTGGTGGTAAC AATGGAGGATCTGGGTTTGGTGGTGGTAAA AATGGAGGTACTGGATTCCGGTGGAGGAAA CACTGGCGGATCTGGATTCCGGTGGAGGAA ACACTGGCGGATCTGGATTCCGGTGGAGGA AAGACTGGCGGTTCTGGATTGGAGGTGG AAATACTTGTGGATCCGGCTTCGGTGGAG GCAGTACAGGAGGATCGCCGTATGGAGGA GCCAGTTCTGGATTCCGGTGGTAGTACTGC CACATCTGGATTGGAAAGCGGTGAAAAATC AAGTGCATTTGGAGGATCAGGTGGCTTTG GAGGTAGTGCAACTGGATTCCGGAAGTGGGA GGAGGATCCTTTGGAGGTGGCAACTCTGG TTTTGGGGAAGGAGGACATGGCGGCGGA GAGAGAAACAATAgttcgtttttaaattgactttatata attacgtttgttcagATTGTTTCAATTGCCAACAG CCAGGACATCGATCGAGTGACTGTCCAGA GCCGAGAAAGGAAAGAGAGCCGAGAGgttt tattttgatataactttattgacagtaattgtttatttcagTG TGTACAATTGCCAGCAACCCGGGCACAC CTCTCGTGAATGTACAGAAGAACGCAAGC CGCGTGAGGGTTCGCACTGGTGGATTCCGG GGGCGGAGCTGGATTGGAAACAATGGAG GAAATGACGGTTTCGGTGGGGACGGTGGT TTTGGTGGAGGCGAAGAACGTGGTCCAAT GAAATGTTTCAACTGTAAAGGCGAGGGACA TCGCTCTGCTGAATGTCCGGAGCCACCCC GTGGATGTTTCAATTGTGGCGAGCAAGGT CATCGCTCGAATGAGTGCCCCAATCCAGC CAAGCCAAGGGAAGGTGTTGAAGGAGAA GGACCTAAGGCGACATACGTGCCAGTCCA AGACAACATGGAGGACGTTTTCAACATGCA GAAAATTTCCGAAGGCCTTATGTTCAACAA GTTTTTCGATGCCGAAGTTAAACTGACTTC ATCCGAGAAGACTGTCGGTATCAAACCTTG CAAGACATTCCGCGGAAGCTAATCTCACGG AGACCATGCAGAAAAACGTTGCTCATGCT GGATACTCCAAGACCACTCCAATTCAGCAA TATGCTCTTCCACTTGTTTCATCAGGGATATG ATATCATGGCTTGTGCTCAAACCTGGATCAG GAAAAACCGCTGCATTCTTCTGCCTATCA TGACTCGTCTCATTGACGATAATAATCTGAA CACTGCCGGAGAAGGCGGTTGCTATCCCC GTTGCATCATCTTGACTCCAACCTCGCGAAC TCGCTGATCAAATTTACAACGAGGGAAGAA AGTTTGCTTATCAAACAATGATGGAGATCAA ACCAGTTTACGGAGGATTGGCTGTGCGTTA TAATAAGGGTCAGATCGAAAAGGGAGCCA CGATCATTGTCCGAACTGTCCGGAAGAATCA AGCACTTCTGTGAAGAGGGTACCATCAAG CTTGACAAAATGCCGCTTCTTTGTTCTTGAC GAGGCTGATCGTATGATCGATGCTATGGGA TTCCGAACTGACATCGAACTATTGTCAATT ATGACAGTATGCCGAGGAAAGAAAATCGC CAGACACTCATGTTTCAGTGCCACTTTCCC CGATTCTGTACAGGAAGCAGCTCGCGCTT TTCTCAGAGAAAAC TACGTGATGATTGCAA</p>

					<p>TCGACAAGATTGGAGCTGCAAAACAAGTGC GTCCTACAGGAATTCGAGAGATGCGAAAAG AAGCGAGAAGAAGGACAAACTTCTAGAGC TTCTGGGAATCGATATCGACAGTTACACGA CCGAGAAAAGtgagttttcgtttctattgatgaaata aattcaatattcagGTGCCGAAGTTTACACAAA GAAAACCATGGTCTTCGTTTCTCAAAGAGC AATGGCTGATACACTGGCTTCAATTTTGTCA TCGGCTCAAGTCCAGCTATCACGgttgat attcattttgaccgcttttaattcaaaatgtacagATCCA TGGTGCCCGTGAGCAGAGAGAGCGTTCA GAAGCTTTGAGACAATTCGAAATGGATCG AAACCTGTTCTTATTGCTACTGCGGTGCT GAACGTGGACTTGATATCAAAGGAGTGGAT CATGTCATCAACTATGACATGCCAGACAAC ATTGATGACTATATCCATCGTATCGGAAGgtc agttatatttataatgtttcaataatgaagcattgtttcag AACTGGAAGAGTTGGAAACTCTGGAAGAG CTACAAGCTTCATCTCGGAGGATTGCAGTC TTCTGTCCGAAC TTGTTGGTGTTCGCGG ACGCACACAGATTGTTCCAGACTGGATG CAAGGTGCTGCTGGAGGCAATTACGGAGC TAGTGGATTTGGGTCCAGTGTACCAACTCA AGTCCCGCAGGACGAGGAGGGGTGG GGATCGGGA GAGGGACGTGGATCCCTTCTTACCTGCGG AGACGTGAGGAGAACCAGGACCA GGAGCATCGGGAGCCTCAGGAGCATCG ATGAGTAAAGGAGAAGAATTGTTCACTGGA GTTGTCCCAATCCTCGTCGAGCTCGACGG AGACGTCAACGGACACAAGTTCTCCGTCC GTGGAGAGGGAGAGGGAGACGCCACCAT CGGAAAGCTCACCTCAAGTTTCACTGCA CCACCGGAAAGCTCCCAGTCCCATGGCC AACCTCGTCACCACCCTCACCTACGGAG TCCAATGCTTCGCCCGTTACCCAGACCAC ATGAAGCGTCACGACTTCTTCAAGTCCGC CATGCCAGAGGGATACGTCCAAGAGCGTA CCATCTCCTTCAAgttaagttaacatatataact aactactgattattaaatttcagGACGACGGAAAAGT ACAAGACCCGTGCCGTGTCAGTTTCGAG GGAGACACCCTCGTCAACCGTATCGAGCT CAAGGGAACCGACTTCAAGGAGGACGGA AACATCCTCGGACACAAGCTCGAGTACAA CTTCAACTCCCACAACGTCTACATCACCGC CGACAAGCAAAAAGAACGGAATCAAGGCCA ACTTACCgtaagttaaacatgattttactaactaacta atctgattaaatttcagACCCGTACAACGTGCA GGACGGATCCGTCCAACCTCGCCGACCACT ACCAACAAAACACCCCAATCGGAGACGGA CCAGTCTCCTCCCAGACAACCACTACCT CTCCACCCAAACCGTCTCTCCAAGGACC CAAACGAGAAGTAG aaaaccgaccaattgatagtttcgacatttataatgctgtc agttcccccattttatcctgccctgtgatttttaattgatt tgttggttggtgtcgttatagtcctcgccgcataaactct gttc</p>
DUP237	glh-1(sam140[glh-1::T2A::split-wrmScarlet_1-10]) I	Germline split-wrmScarlet_1-10	l: 6.85 MB	l: 1.41	<p>gtttcgccacgcgccccactacagtaacctcgacacac tcatctactaaatttgggacagttcctaattcttttgcgtgttt caactcaattttctggaaaaatctaattttctgcgaaaATG CTGATGGTTGGAGTGATAGCGAAAAGTGTCT GCTAAGGgtgagttttattttgaactttccaccggtttatt tgattaaaactttatttcagCCAAAAC TGGATTCTG GTAGTGGAGGCGGTTTCGGTGGTGGTAAAC AATGGAGGATCTGGGTTTGGTGGTGGTAAA AATGGAGGTACTGGATTCTGGTGGAGGAAA CACTGGCGGATCTGGATTCTGGTGGAGGAA ACACTGGCGGATCTGGATTCTGGTGGAGGA AAGACTGGCGGTTCTGGATTTGGAGGTGG AAATACTTGTGGATCCGGCTTCGGTGGAG GCAGTACAGGAGGATCGCCGTATGGAGGA GCCAGTTCTGGATTCTGGTGGTAGTACTGC CACATCTGGATTTGGAAGCGGTGAAAAATC AAGTGCATTTGGAGGATCAGGTGGCTTTG GAGGTAGTGCAACTGGATTCTGGAAGTGGGA GGAGGATCCTTTGGAGGTGGCAACTCTGG TTTTGGGGAAAGGAGGACATGGCGGCGGA GAGAGAAACAATAgttcgttttttaattgactttatata attacgtttgttcagATTGTTTCAATTGCCAACAG CCAGGACATCGATCGAGTGACTGTCCAGA GCCGAGAAAGGAAAAGAGAGCCGAGAGgttt tatttgatataactttatggcagtaattgtttatttcagTG TGCTACAATTGCCAGCAACCCGGGCACAC</p>

CTCTCGTGAATGTACAGAAGAACGCAAGC
CGCGTGAGGGTTCGCACTGGTGGATTCCGG
GGGCGGAGCTGGATTTGAAACAATGGAG
GAAATGACGGTTTCGGTGGGGACGGTGGT
TTTGGTGGAGGCGAAGAACGTGGTCCAAT
GAAATGTTTCAACTGTAAAGGCGAGGGACA
TCGCTCTGCTGAATGTCCGGAGCCACCCC
GTGGATGTTTCAATTGTGGCGAGCAAGGT
CATCGCTCGAATGAGTGCCCCAATCCAGC
CAAGCCAAGGGAAGGTGTTGAAGGAGAA
GGACCTAAGGCGACATACGTGCCAGTCSGA
AGACAACATGGAGGACGTTTTCAACATGCA
GAAAATTTCCGAAGGCCTTATGTTCAACAA
GTTTTTCGATGCCGAAGTTAAACTGACTTC
ATCCGAGAAGACTGTCGGTATCAAACCTTG
CAAGACATTCGCGGAAGCTAATCTCACGG
AGACCATGCAGAAAAACGTTGCTCATGCT
GGATACTCCAAGACCACTCCAATTCAGCAA
TATGCTCTTCCACTTGTTTCATCAGGGATG
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GAAAAACCGCTGCATTCTTCTGCCTATCA
TGACTCGTCTCATTGACGATAATAATCTGAA
CACTGCCGGAGAAGGCGGTTGCTATCCCC
GTTGCATCATCTTGACTCCAACCTCGCGAAC
TCGCTGATCAAATTTACAACGAGGGAAGAA
AGTTTGCTTATCAAACAATGATGGAGATCAA
ACCAGTTTACGGAGGATTGGCTGTCGGTTA
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CGATCATTGTCGGAACGTGTCGGAAGAATCA
AGCACTTCTGTGAAGAGGGTACCATCAAG
CTTGACAAAATGCCGCTTCTTTGTTCTTGAC
GAGGCTGATCGTATGATCGATGCTATGGGA
TTCGGAACCTGACATCGAAACTATTGTCAATT
ATGACAGTATGCCGAGGAAAGAAAATCGC
CAGACACTCATGTTCAAGTCCCACTTTCCC
CGATTCTGTACAGGAAGCAGCTCGCGCTT
TTCTCAGAGAAAACCTACGTGATGATTGCAA
TCGACAAGATTGGAGCTGCAAAACAAGTGC
GTCCTACAGGAATTCGAGAGATGCGAAAAG
AAGCGAGAAGAAGGACAAACTTCTAGAGC
TTCTGGGAATCGATATCGACAGTTACACGA
CCGAGAAAAgtgagtttttcgtttctatttgatgaaata
aatttcaatatttcagGTGCCGAAGTTTACACAAA
GAAAACCATGGTCTTTCGTTTTCTCAAAGAGC
AATGGCTGATACACTGGCTTCAATTTTGTCA
TCGGCTCAAGTTCCAGCTATCACGgttgat
atttcattttgaccgcttttaattcaaaatgtacagATCCA
TGGTGCCCGTGAGCAGAGAGAGCGTTCA
GAAGCTTTGAGACAATTCCGAAATGGATCG
AAACCTGTTCTIATTGCTACTGCGGTGCT
GAACGTGGACTTGATATCAAAGGAGTGGAT
CATGTCATCAACTATGACATGCCAGACAAC
ATTGATGACTATATCCATCGTATCGGAAGgtc
agttatatttattaatgtttcaataatgaagcattgtttcag
AACTGGAAGAGTTGGAACTCTGGAAAGAG
CTACAAGCTTCATCTCGGAGGATTGCAGTC
TTCTGTCCGAACCTGTTGGTGTCTCGCCG
ACGCACAACAGATTGTTCCAGACTGGATG
CAAGGTGCTGCTGGAGGCAATTACGGAGC
TAGTGGATTTGGGTCCAGTGTACCAACTCA
AGTCCCGCAGGACGAGGAGGGGTGG
GGATCGGA
GAGGGACGTGGATCCCTTCTTACCTGCGG
AGACGTGAGGAGAACCAGGACCA
GGAGCATCGGGAGCCTCAGGAGCATCG
ATGGTTTCCAAGGGAGAGGCTGTTATCAAG
GAATTCATGCGCTTCAAGGTTACATGGAA
GGATCTATGAACGGACACGAATTCGAAATC
GAAGGAGAAGGAGAAGGACGCCATACG
AGGGAACCTCAAACCTGCTAAGCTTAAGGTTA
CTAAAGGAGGACCATTCCATTCTCTGGG
ATATCCTTTCTCCACAGTTCATGTACGGATC
TCGCGCTTTCATCAAGCACCCAGCTGATAT
CCCAGATTACTACAAGCAGTCTTTCCAGA
AGGATTCAAATGGGAGCGCGTTATGAACTT
CGAAGATGGAGGAGCTGTTACCGTTACCC
AAGATACCTCCCTTGAGGATGGAACCCCTTA
TCTACAAgtaagtttaaacatataataactaacc
ctgattatttaaatttcagGTTAAGCTTCGCGGAAC
TAATTTCCCACCAGATGGACCAGTTATGCA
GAAGAAGACTATGGGATGGGAAGCTTCTAC
CGAGCGCTTTACCCAGAGGATGGAGTCC
TTAAGGGAGATATCACCATGGCTCTTCGTC

					TTAAGGATGGAGGACGTTACCTTGCTGATA CCTCTACTACTTACAAGGCTAAGAAGCCAG TTCAGATGCCAGGAGCTTACCTTGTGCGATC GTAAGCTTGATATCACTTCTCATAACGAATA CTAG aaaaccgaccaattgatagtgttcgcatttataatgctgtc agttccccatattttatcctgccctgttgattttaaattgtatt tgttggttggtgtcgttatagtcctcgcgcgataaactct gttc
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Table S3. *C. elegans* strains, genotypes and sources

Strain	Genotype	Source
N2E	wild type	Kenyon Lab
CF4582	muls252[Peft-3::split-wrmScarlet ₁₋₁₀ ::unc-54 3'UTR, Cbr-unc-119(+)] II; unc-119(ed3) III	Kenyon Lab
CF4586	muls252[Peft-3::split-wrmScarlet ₁₋₁₀ ::unc-54 3'UTR, Cbr-unc-119(+)] II; unc-119(ed3) III; vha-13(muls262[split-wrmScarlet ₁₁ ::vha-13]) V	Kenyon Lab
CF4587	muls253[Peft-3::sfGFP ₁₋₁₀ ::unc-54 3'UTR, Cbr-unc-119(+)] II; unc-119(ed3) III	Kenyon Lab
CF4588	muls253[Peft-3::sfGFP ₁₋₁₀ ::unc-54 3'UTR, Cbr-unc-119(+)], muls252[Peft-3::split-wrmScarlet ₁₋₁₀ ::unc-54 3'UTR, Cbr-unc-119(+)] II; unc-119(ed3) III	Kenyon Lab
CF4589	muls253[Peft-3::sfGFP ₁₋₁₀ ::unc-54 3'UTR, Cbr-unc-119(+)] II; unc-119(ed3) III; vha-13(muls268[sfGFP ₁₁ ::vha-13]) V	Kenyon Lab
CF4592	muls253[Peft-3::sfGFP ₁₋₁₀ ::unc-54 3'UTR, Cbr-unc-119(+)] II; unc-119(ed3) III; his-3(muls255[his-3::sfGFP ₁₁]) V	Kenyon Lab
CF4594	muls252[Peft-3::split-wrmScarlet ₁₋₁₀ ::unc-54 3'UTR, Cbr-unc-119(+)] II; unc-119(ed3) III; his-3(muls258[his-3::split-wrmScarlet ₁₁]) V	Kenyon Lab
CF4601	muls252[Peft-3::split-wrmScarlet ₁₋₁₀ ::unc-54 3'UTR, Cbr-unc-119(+)] II; unc-119(ed3) III; fib-1(muls254[split-wrmScarlet ₁₁ ::fib-1]) V	Kenyon Lab
CF4602	muls253[Peft-3::sfGFP ₁₋₁₀ ::unc-54 3'UTR, Cbr-unc-119(+)], muls252[Peft-3::split-wrmScarlet ₁₋₁₀ ::unc-54 3'UTR, Cbr-unc-119(+)] II; unc-119(ed3) III; fib-1(muls254[split-wrmScarlet ₁₁ ::fib-1]), his-3(muls255[his-3::sfGFP ₁₁]) V	Kenyon Lab
CF4603	muls252[Peft-3::split-wrmScarlet ₁₋₁₀ ::unc-54 3'UTR, Cbr-unc-119(+)] II; unc-119(ed3) III; eat-6(muls269[eat-6::split-wrmScarlet ₁₁]/+) V	Kenyon Lab
CF4608	muls252[Peft-3::split-wrmScarlet ₁₋₁₀ ::unc-54 3'UTR, Cbr-unc-119(+)] II; unc-119(ed3) III; his-3(muls267[his-3::split-wrmScarlet ₁₁ (x3)]) V	Kenyon Lab
CF4610	muls257[Pmyo-3::split-wrmScarlet ₁₋₁₀ ::unc-54 3'UTR] I	Kenyon Lab
CF4611	muls257[myo-3p::split-wrmScarlet ₁₋₁₀ ::unc-54 3'UTR] I; fib-1(muls254[split-wrmScarlet ₁₁ ::fib-1]) V	Kenyon Lab
CF4612	muEx690[Pmyo-3::mTagBFP2::sfCherry3 ₁₁ ::T2A::mNeonGreen::sfCherry3 ₁₋₁₀ ::fib-1 3'UTR]	Kenyon Lab
CF4613	muEx691[Pmyo-3::mTagBFP2::split-wrmScarlet ₁₁ ::T2A::mNeonGreen::split-wrmScarlet ₁₋₁₀ ::fib-1 3'UTR]	Kenyon Lab
CF4614	muls252[Peft-3::split-wrmScarlet ₁₋₁₀ ::unc-54 3'UTR, Cbr-unc-119(+)] II; tbb-2(muls260[split-wrmScarlet ₁₁ ::tbb-2]), unc-119(ed3) III	Kenyon Lab
CF4615*	muls252[Peft-3::split-wrmScarlet ₁₋₁₀ ::unc-54 3'UTR, Cbr-unc-119(+)] II; unc-119(ed3) III; tomm-20(muls261[tomm-20::split-wrmScarlet ₁₁]) V	Kenyon Lab
CF4616	muls252[Peft-3::split-wrmScarlet ₁₋₁₀ ::unc-54 3'UTR, Cbr-unc-119(+)] II; unc-119(ed3) III; vha-13(muls264[split-wrmScarlet ₁₁ (x2)::vha-13]) V	Kenyon Lab
COP1795	knuSi785 [pNU1687(Plet-858::sfGFP ₁₋₁₀ ::unc-54 3'UTR, Cbr-unc-119(+)] II; unc-119(ed3) III	Nemametrix
DUP218	glh-1(sam124[glh-1::T2A::sGFP2 ₁₋₁₁]) I	Updike Lab
DUP223	glh-1(sam129[glh-1::T2A::sGFP2 ₁₋₁₀]) I	Updike Lab
DUP225	glh-1(sam129[glh-1::T2A::sGFP2 ₁₋₁₀]) I; pgl-1(sam126[pgl-1::GFP ₁₁]) IV	Updike Lab
DUP236	glh-1(sam139[glh-1::T2A::split-wrmScarlet ₁₋₁₁]) I	Updike Lab
DUP237	glh-1(sam140[glh-1::T2A::split-wrmScarlet ₁₋₁₀]) I	Updike Lab
PHX731	vha-13(syb731[wrmScarlet::vha-13]) V	SunyBiotech
PHX1049	vha-13(syb1049[gfp::vha-13]) V	SunyBiotech
PHX1797	sybSi66[Psun-1::split-wrmScarlet ₁₋₁₀ ::sun-1 3'UTR, Cbr-unc-119(+)] II; unc-119(ed3) III	SunyBiotech

CA1200	ieSi57[eft-3p::TIR1::mRuby::unc-54 3'UTR + Cbr-unc-119(+)] II; unc-119(ed3) III	CGC
WBM1126	wbmls61[myo-3p::3XFLAG::dpy-10 crRNA::unc-54 3'UTR] I	CGC

* Mutation present - see table S4 for the corresponding sequence

Table S4. crRNAs, HDR templates and oligonucleotide sequences

S4A. Sequences of crRNA and HDR template used for split-wrmScarlet₁₁ and sfGFP₁₁ knock-in experiments

Gene name	Gene ID	Tagged term	gene-specific crRNA sequence	1x split-wrmScarlet ₁₁ HDR donor sequence - Ultramer ssDNA (lower case: homology arms; red: split-wrmScarlet ₁₁ ; blue: linker)	Sequencing Primer (Forward)	Sequencing Primer (Reverse)
eat-6	B0365.3	C	ACAAGCU GUUCUU UAGUAGU	cgacgagatccgctcgtttcttgattcgcagatatccaggag gatgggctcgagcgtgagacctactac GGAGGAGGATCC TACACCGTCGTCGAGCAATACGAGAAGTC CGTCGCCCGTCACTGCACCGGAGGA taaagaacagcttgtaactttgtagaattttctattttatc ttagttttattgtttcccat	CCTGGTT CATGTGC TATTGCC	CGACGAC AGAAAGT AGCATCA C
fib-1	T01C3.7	N	AJAAUCG AUUUUU GUAGUAAU	aatacgaaaaaaatcctgaaattcagtttacctcgcgccc gcccgcacgattgaactctggacgtcc GGATCCTCCTCC TCCTCCGGTGCAGTGACGGGCGACGGAC TTCTCGTATTGCTCGACGACGGTGTA catactacaaaaatcgattatttaacaaaaacgaaaagcg aaattacgaaaaatacatacctcagcc	TTCTAGTC GATTCAG ATCGACT GG	CGAAACT GCCACGA TCACC
his-3	T10C6.12	C	GAAGAAA ACCGGA GGAGACA	gattttaaattttgtggcctaagaagggcgttggttcg gtaaaatgtttaagaagccatcta TACACCGTGCAGTGACGGGCGACGGAC TTCTCGTATTGCTCGACGACGGTGTA GGATCCTCCTCC ttcTttgtctcctccggtCttctTggcaaaagaacagctt ggatattggaagaactcctccttg	CCAAGGA GGAGTTC TTCCAAAT ATC	GATTTTAA ATATTTGT GGCCCTA AAG
tbb-2	C36E8.5	N	CGCAUU GUCCGG CUUGCA CG	tcttctctataaatatagttttcaattcattacgacctttca gcaaaaatg TACACCGTGCAGTGACGGGCGACGGAC CGTCGCCCGTCACTGCACCGGAGGA GGAGGAGGATCCGGAGGAGGATCCGGAG GAGGATCC agagagatcgtTcacgtgcaagcggacaatcgcgaaa ccaaatcggatccaaat	CAATATCG ACCATGA CGTGTC TC	CTTGAAG GTTCCGT CTGGC
tomm-20	F23H12.2	C	ACACCGA CGACUU GGAGUAA	gttggtgaacgaaaaatcgaagaattaattgaaagtta ataaaacttttaaatcattatccatta TACACCGTGCAGTGACGGGCGACGGAC TTCTCGTATTGCTCGACGACGGTGTA GGATCCTCCTCC ctccaagtcgctcgtgtcatcgataagctcttgattgtgc tggggagggtccgctcagaggat	GAGCGAA AGCAGAT GAGGC	TCCGTGA GGAGGAA AACACC
actual tomm-20 mutation recovered	F23H12.2	C	ACACCGA CGACUU GGAGUAA	gttggtgaacgaaaaatcgaagaattaattgaaagtta ataaaacttttaaatcattatccatta TATCCATTGCAGTGACGGGCGACGGACT TCTCGTATTGCTCGACGACGGTGTA GGATCCTCCTCC ctccaagtcgctcgtgtcatcgataagctcttgattgtgc tggggagggtccgctcagaggat	GAGCGAA AGCAGAT GAGGC	TCCGTGA GGAGGAA AACACC
vha-13	Y49A3A.2	N	AUUCUG CGGCCA UCUUUU CC	ggttatttgatttctttcgtatttccatagctttctaaattc attcattccaggaaaagatg TACACCGTGCAGTGACGGGCGACGGACT CGTCGCCCGTCACTGCACCGGAGGA GGAGGAGGATCC gccgcagaatctctgtacggattcgtttacggagtgtccg gacctgtcgtcacagccgagaagatgg	GGTTTATT TTGATTTT CTTTTCG ATTTCC	CCATCTT CTCGGCT GTGAC

Gene name	Gene ID	Tagged term	gene-specific crRNA sequence	1x sfGFP ₁₁ HDR donor sequence - Ultramer ssDNA (lower case: homology arms; green: sfGFP ₁₁ ; blue: linker)	Sequencing Primer (Forward)	Sequencing Primer (Reverse)
his-3	T10C6.12	C	GAAGAAA ACCGGA GGAGACA	atagaggattttaaattttgtggccctaagagggccggt gggttcggtaaaatgttttaagaaggcatcta GGTGATTCCGGCGGCGTTGACGTACTCGT GGAGGACCATGTGGTCAGC TCCTCCTCC ttcTttgtctcctccggtCttctTggcaaaagaacagctt ggatatttgaagaactcctccttgggcg	CCAAGGA GGAGTTC TTCCAAAT ATC	GATTTTAA ATATTTGT GGCCCTA AAG
vha-13	Y49A3A.2	N	AUUCUG CGGCCA UCUUUU CC	ggttattttgattttcttttcgattccatataagctttctaattc attcattccaggaaaagatg CGTGACCACATGGTCTCCACGAGTACGT CAACGCCGCCGGAATCACC GGAGGAGGATCC gccgcagaatcttctgacggattcgtttacggagtgccg gacctgtcgtcacagccgagaagatgg	GGTTTATT TTGATTTT CTTTTCG ATTTC	CCATCTT CTCGGCT GTGAC
pgl-1	ZK381.4	C	GGTGGTT ACGGGG GTCGTGG	tacggcggagatcgtggacgtggtggttacgggggaag aggaggtagaggtggatt GGAGCATCGGGAGCCTCAGGAGCATCG CGTGACCACATGGTCTCCACGAGTACGT CAACGCCGCCGGAATCACC taaactccaactattgaatgtttaattgttttta	CCAAAGT TGCAAAA GGATTTCG GTCAATTT G	CATTTACC GGGAACA AGGAAAA ACAGGTT G
Gene name	Gene ID	Tagged term	gene-specific crRNA sequence	1x split-wrmScarlet _{11(MDELYK)} HDR donor sequence - Ultramer ssDNA (lower case: homology arms; red: split-wrmScarlet _{11(MDELYK)} ; blue: linker)	Sequencing Primer (Forward)	Sequencing Primer (Reverse)
eat-6	B0365.3	C	ACAAGCU GUUCUU UAGUAGU	cgacgagatccgctggtttctgattcgcagatatccaggag gatgggtcagcgtgagacctactac GGAGGAGGATCC TACACCGTCGTCGAGCAATACGAGAAGTC CGTCGCCCGTCACTGCACCGGAGGAATG GATGAGTTATACAAG taaagaacagcttgaatctttgtagaattttctatttttatc ttagtttttattgtttccat	CCTGGTT CATGTGC TATTGCC	CGACGAC AGAAAGT AGCATCA C
his-3	T10C6.12	C	GAAGAAA ACCGGA GGAGACA	gattttaaattttgtggccctaagagggccggtgggttcg gtaaaatgttttaagaaggcatcta CTTGATAACTCATCCATTCTCCGGTGCA GTGACGGGCGACGGACTTCTCGTATTGCT CGACGACGGTGTA GGATCCTCCTCC ttcTttgtctcctccggtCttctTggcaaaagaacagctt ggatatttgaagaactcctccttgg	CCAAGGA GGAGTTC TTCCAAAT ATC	GATTTTAA ATATTTGT GGCCCTA AAG
tomm-20	F23H12.2	C	ACACCGA CGACUU GGAGUAA	gttggtgaacgaaaaatacgaagaattaattgaaagtt ataaaacttttaaatcattatccatta CTTGATAACTCATCCATTCTCCGGTGCA GTGACGGGCGACGGACTTCTCGTATTGCT CGACGACGGTGTA GGATCCTCCTCC ctccaagtcgctggtgcatcgataagctcttgattgtgc tggaggaggtccgctcctccagggtat	GAGCGAA AGCAGAT GAGGC	TCCGTGA GGAGGAA AACACC
actual tomm-20 mutation recovered	F23H12.2	C	ACACCGA CGACUU GGAGUAA	gttggtgaacgaaaaatacgaagaattaattgaaagtt ataaaacttttaaatcattatccatta CTTGATAACTCATCCATTCTCCGGTGCA GTGACGGGCGACGGACTTCTCGTATTGCT CGACGACGGTGTA GGATCCTCCTCC ctccaagtcgctggtgcatcgataagctcttgattgtgc tggaggaggtccgctcctccagggtat	GAGCGAA AGCAGAT GAGGC	TCCGTGA GGAGGAA AACACC

S4B DNA template for split-wrmScarlet tandems HDR donor sequence - plasmids

Template name	Tagged term	DNA template for split-wrmScarlet tandems - dsDNA (lower case: homology arms; red: split-wrmScarlet ₁₁ ; blue: linkers)
wrmScarlet ₁₁ (x2)::vha-13	N	ggttattttgattttcttttcgattccatataagctttctaattcattcattccaggaaaagatg TACACCGTCGTCGAGCAATACGAGAAGTCCGTCGCCCGTCACTGCACCGGAGGA

		GGTGGCTCTGGAGGT TACACCGTTGTTGAGCAATACGAGAAGTCTGTTGCTCGTCACTGCACCGGAGGC GGAGGAGGATCC gccgcagaatcttcgtacggattcgtttacggagtggtccggacctgctgcacagccgagaagatgg
his-3::split-wrmScarlet ₁ (x3)	C	gattttaaatatttggtgccctaaagagggccgtgggttcggtaaaatgtttaagaagcactcta TCCTCCGGTGCAGTGACGGGCGACGGACTTCTCGTATTGCTCGACGACGGTGT TCCTCCACTACCGCC TCCTCCGGTGCAGTGACGGGCGACGGACTTCTCGTATTGCTCGACGACGGTGT ACCTCCAGAGCCACC TCCTCCGGTGCAGTGACGGGCGACGGACTTCTCGTATTGCTCGACGACGGTGT GGATCCTCCTCC ttcTttgtctcctccggTcttctTggcaaaagaacagcttggatatttgaagaactcctcctgg

S4C. Primers used to PCR split-wrmScarlet tandems HDR donor sequence from plasmid

Primer Name	Sequence of DNA oligo
his-3_F	CCAAGGAGGAGTTCTTCCAAATATC
his-3_R	GATTTTAAATATTTGTGGCCCTAAAG
vha-13_F	GGTTTATTTGATTTTCTTTTCGATTTCC
vvha-13_R	CCATCTTCTCGGCTGTGAC

S4D. Sequences of crRNA and HDR template used to generate split-wrmScarlet₁ and sfGFP₁₋₁₀ strains

Strain edited -> Final strain	crRNA#1 sequence	crRNA#2 sequence (if applicable)	DNA template - Hybrid PCR amplicon (lower case: homology arms, upper case: insert)
CA1200 -> CF4582	UACUUCUUCUG GAAACGACA	AAGUUCGCUGG ACUUGGAGG	tagaagttctaggataatTTTTcgactttattctctctaccgtccgcactctt cttacttttaaattaaatgTTTTtttcagttgggaacactttgctcactccgt agcagccATGGTATCGAAGGGAGAAGCAGTAATCAAGGA GTTTCATGCGTTTCAAGGTCCACATGGAGGGATCCATG AACGGACACGAGTTTCGAGATCGAGGGAGAGGGAGAG GGACGTCCATACGAGGGAACCCAAACCGCCAAGCTC AAGGTCACCAAGgtaagtttaaacatatataactaactaacctgat tatttaaattttcagGGAGGACCACTCCCATTCTCCTGGGAC ATCCTCTCCCCACAATTCATGTACGGATCCCGTGCCTT CATCAAGCACCCAGCCGACATCCCAGACTACTACAAG CAATCCTTCCCAGAGGGATTCAAGTGGGAGCGTGTCA TGAACCTCGAGGACGGAGGAGCCGTACCCGTACCC AAGACACCTCCCTCGAGGACGGAACCCTCATCTACAA GgtaagtttaaacagttcggactaactaaccatacatatttaaatttcagG TCAAGCTCCGTGGAACCAACTTCCCACCAGACGGAC CAGTCATGCAAAAAGAAGACCATGGGATGGGAGGCCTC CACCGAGCGTCTTACCCAGAGGACGGAGTCCCTCAA GgtaagtttaaacatgattttactaactaactaatctgatttaaatttcagGG AGACATACCATGGCCCTCCGTCTCAAGGACGGAGG ACGTTACCTCGCCGACACCTCCACCACCTACAAGGCC AAGAAGCCAGTCCAATGCCAGGAGCCTACCTCGTCG ACCGTAAGCTCGACATCACCTCCCACAACGAGTACTA

			Ataagtccaattactcttcaacatccctacatgctctttccctgtgctcca ccccctattttgtattatcaaaaaacttctttaattcttgttttagctcttt taagtca
COP1795 -> CF4587	GACCAGCUGGG CGCAUAGGG	GCCGCCCCACG AGGGCCAGG	aactcattttcaattcaactgaaagatttttcattagagaatgtctagaacta ggccccgggctacgtaatacgaactcacttaaggcctaatttgggtctggctg catgccaggaggaGCACCTTTGGTCTTTTATTGTCAACTTC CATTGGTTCTTCCATTGTTTCTGTAAATTAATGAATTTTT CATAAAATAAAGACATTATACAATATAAAAATGAAGAATTT ATTGAAAATAAACTGCCAGAGAGAAAAAGTATGCAACA CTCCC GCCGAGAGTGTGAAATGGTGTACGGTACATT TTCGTGCTAGGAGTTAGATGTGCAGGCAGCAACGAGA GGGGGAGAGATTTTTTGGGCCTTGTGAAATTAACGTG AGTTTTCTGGTCATCTGACTAATCATGTTGGTTTTTTGTT GGTTTTATTTGTTTTATCTTTGTTTTATCCAGATTAGGA AATTTAAATTTTATGAATTTATAATGAGGTCAAACATTGAG TCCCAGCGTTTTTCTGTTCTCACTGTTTAGTCGAATTT TTATTTTAGGCTTTCAACAAATGTTCTAACTGTCTTATTT GTGACCTCACTTTTTATTTTTTAATTTTTAAAAATATTA GAAGTTTCTAGGATAATTTTTTCGACTTTTATTCTCTCTA CCGTCCGCACTCTTCTTACTTTTTAAATTAATTTGTTTTTT TTTCAGTTGGGAAACACTTTGCTCaaaaatgtctaagggaga agagtatttactggagttgtgccgatcctcgtcagctcgacggagacgt caacggacacaagttctccgtccgtggagaggagagggagacgccac catcg
WBM1126 -> CF4610	GCUACCAUAGG CACCACGAG		cacttttaccgtctaattttcagggcagggagccatcaaaccacgaccac tagatccatATGGTATCGAAGGGAGAAGCAGTAATCAAGG AGTTCATGCGTTTTCAAGGTCACATGGAGGGATCCAT GAACGGACACGAGTTTCGAGATCGAGGGAGAGGGAGA GGGACGTCCATACGAGGGAAACCCAAACCGCCAAGCT CAAGGTCACCAAGGtaagtttaacatatataactaactaacctg attatttaattttcagGGAGGACCACTCCATTCTCCTGGGA CATCCTCTCCCCACAATTCATGTACGGATCCCGTGCCT TCATCAAGCACCCAGCCGACATCCAGACTACTACAA GCAATCCTTCCAGAGGGATTCAAGTGGGAGCGTGTG ATGAACCTCGAGGACGGAGGAGCCGTCACCGTCACC CAAGACACCTCCCTCGAGGACGGAACCCTCATCTACA AGGtaagtttaacagttcggtaactaactaacatataattttcag GTCAAGCTCCGTGGAACCAACTTCCCACCAGACGGA CCAGTCATGCAAAAGAAGACCATGGGATGGGAGGCCT CCACCGAGCGTCTCTACCCAGAGGACGGAGTCTCTA AGGtaagtttaacatgattttactaactaactaatctgatttaatttcagG GAGACATCACCATGGCCCTCCGTCTCAAGGACGGAG GACGTTACCTCGCCGACACCTCCACCACCTACAAGG CCAAGAAGCCAGTCCAAATGCCAGGAGCCTACCTCGT CGACCGTAAGCTCGACATCACCTCCACAACGAGTAC TAAcatctcgcgcccgtgcctctgacttctaagtccaattactctcaacat ccctacatgct

S4E. Primers long and short

Primers to mplify split-wrmScarlet for CF4582	Sequence
eft3p_S110(A19)_F	tgaagtttctaggataatTTTTcgaactttattctctaccgtccgcactctc ttacttttaataaattgtttttttcagttgggaaacactttgctcactccgtag cagccATGGTATCGAAGGGAGAAGC
unc54_S110(A19)_R	tgacttaaagaagctaaaaacaagaaattaagagaagtttttgataat aacaaaaataggggtgggagcacagggagaaagagcatgtagggatg ttgaagagtaattggacTTATTAGTACTCGTTGTGGGAGG

S1-10_A19_F	ATGGTATCGAAGGGAGAAGC
S1-10_A19_R	TTAGTACTCGTTGTGGGAGGTG

Primers to amplify Pef3 for CF4587	Sequence
E7_eft-3p_F-Long	aactcattttcaatttcaactgaaagatttttcattagagaatgtctagaacta ggcccgggctacgtaatacgaactcacttaaggcctaatttgggtctggctg catgccaggaggtaGCACCTTTGGTCTTTTATTGTCAAC
E8_eft-3p_R-Long	cgatggtggcgtctccctctccctctccacggacggagaacttgttccggt gacgtctccgtcgagctcgacgaggatcggcacaactccagtaaataact cttctcccttagacattttGAGCAAAGTGTTTCCCAACTG
E5_eft-3p_F	GCACCTTTGGTCTTTTATTGTCAAC
E6_eft-3p_R	GAGCAAAGTGTTTCCCAACTG

Primers to amplify split-wrmScarlet for CF4610	Sequence
PrimerS1-10_my03F	cactttaccgtctaattttcagggcagggagccatcaaaccacgaccac tagatccatATGGTATCGAAGGGAGAAGC
PrimerS1-10_my03R	agcatgtagggatgtgaagagtaattggacttagaagttagagggcagcg gcgcgagatgTTAGTACTCGTTGTGGGAGGTG

Table S5. Plasmid sequences

Name	Sequence
Pef3::3NLS::mTagBFP2::split-wrmScarlet ₁₋₁₀ ::T2A::mNeonGreen::split-wrmScarlet ₁₋₁₀ ::fib-1 UTR (C. elegans)	gcaccttggctctttatgtcaacttccattgggtcttccattggttctgtaaat aatgaattttcataaataaagacattatacaatataaaaatgaagaatttatt gaaaataaactgccagagagaaaagatgcaacactcccgcgagagat gttgaaatgggtgacggtagcatttctgctaggagtagatgtgcaggcag caacgagagggggagagattttttggcctgtgaaatcaactgagtttct tggatcctgactaatcatgttgggtttttgtggttattttgttttatcttgtttta tccagattaggaaattaaatttatgaattataatgaggtcaaacatcagtc ccagcgttttctgttctcactgttagtcgaattttattttaggcttcaacaa atgttctaactgtctatttggacactctttatatttttaatttttaaaatatt agaagtttctaggataaatttttgcacttttattctctaccgtccgactcttct tactttaaattaaattgtttttttcagttgggaaacactttgctcATGCCAA AAAAGAAACGTAAAGGTTGATCCGAAGAAAAAGCGAAAA GTTGATCCTAAAAAAAAGCGTAAAGTCGTCTCCAAGGG AGAGGAGCTCATCAAGGAGAATGCACATGAAGCTC TACATGGAGGGAACCGTCGACAACCACCACTTCAAGT GCACCTCCGAGGGAGAGGGAAAGCCATACGAGGGAA CCCAAACCATGCGTATCAAGGTCGTCCGAGGGAGGACC ACTCCCATTCGCCTTCGACATCCTCGCCACCTCCTTC CTCTACGGCTCCAAGACCTTCATCAACCACACCCAAAG GAATCCCAGACTTCTTCAAGCAATCCTTCCCAGAGGGGA TTCACCTGGGAGCGTGTCAACCACTACGAGGACGGA GGAGTCCCTCACCGCCACCCAAGACACCTCCCTCCA GACGGATGCCTCATCTACAACGTCAAAGGtaagtttaaacatat ataataaactaacctgatttttaattttcagATCCGTGGAGTCAA CTTACCTCCAACGGACCAGTCATGCAAAAAGAACCC CTCGGATGGGAGGCCTTCAACGAGACCTCTACCCA GCCGACGGAGGACTGGAGGGACGTAACGACATGGCC

CTCAAGCTCGTCGGAGGTTCCACCTCATCGCCAACG
CCAAGACCACCTACCGTTCGAAGAAGCCAGCCAAGAA
CCTCAAGATGCCAGGAGTCTACTACGTCGACTACCGT
CTGGAGCGTATCAAGGAGGCCAACAACGAGACTACG
TCGAGCAACACGAGGTCGCCGTCGCCCGTTACTGCG
ACCTCCCATCCAAGCTCGGACACAAGCTTAACGGCGG
AGGGTCTTATACCGTTGTGCGAGCAATATGAAAAGTCAGT
GGCGAGGCACTGTACAGGGGGAGGATCCGGCGAGG
GACGTGGCTCCCTCCTCACCTGCGGAGACGTGCGAGG
AGAACCCAGGACCAGTCTCCAAGGGAGAGGAGGACA
ACATGGCCTCCCTCCCAGCCACCCACGAGCTCCACAT
CTTCGGGTCCATCAACGGAGTCGACTTCGACATGGTC
GGACAAGGAACCGGAAACCCAAACGACGGATACGAG
GAGCTCAACCTCAAGTCCACCAAGGtaagtttaaacagttcg
gtactaactaaccatacatatttaattttcagGGAGACCTCCAATTC
TCCCATGGATTCTCGTCCCACACATCGGATACGGATT
CCACCAATACCTCCCATAACCCAGACGGAATGTCCCAT
TCCAAGCCGCCATGGTCGACGGCTCCGGATACCAAGT
CCACCGTACCATGCAATTCGAGGACGGAGCCTCCACTC
ACCGTCAACTACCGTTACACCTACGAGGGTTCCACAT
CAAGGGAGAGGCCCAAGTCAAGGGAACCGGATTCCC
AGCCGACGGACCAGTCATGACCAACTCCCTCACCGC
CGCCGACTGGTGCCGTTCCAAGAAGACCTACCCAAAC
GACAAAGtaagtttaaacatgattttactaactaactatctgatttaatttt
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Pmyo-3::mTagBFP2::split-
wrmScarlet₁::T2A::mNeonGreen::split-wrmScarlet₁
10::fib-1 UTR (C. elegans)

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Pmyo-
3::mTagBFP2::sfCherry3₁::T2A::mNeonGreen::sfCherry
31-10::fib-1 UTR (C. elegans)

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Table S6. Adult lifespans of strains in this study

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