

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.

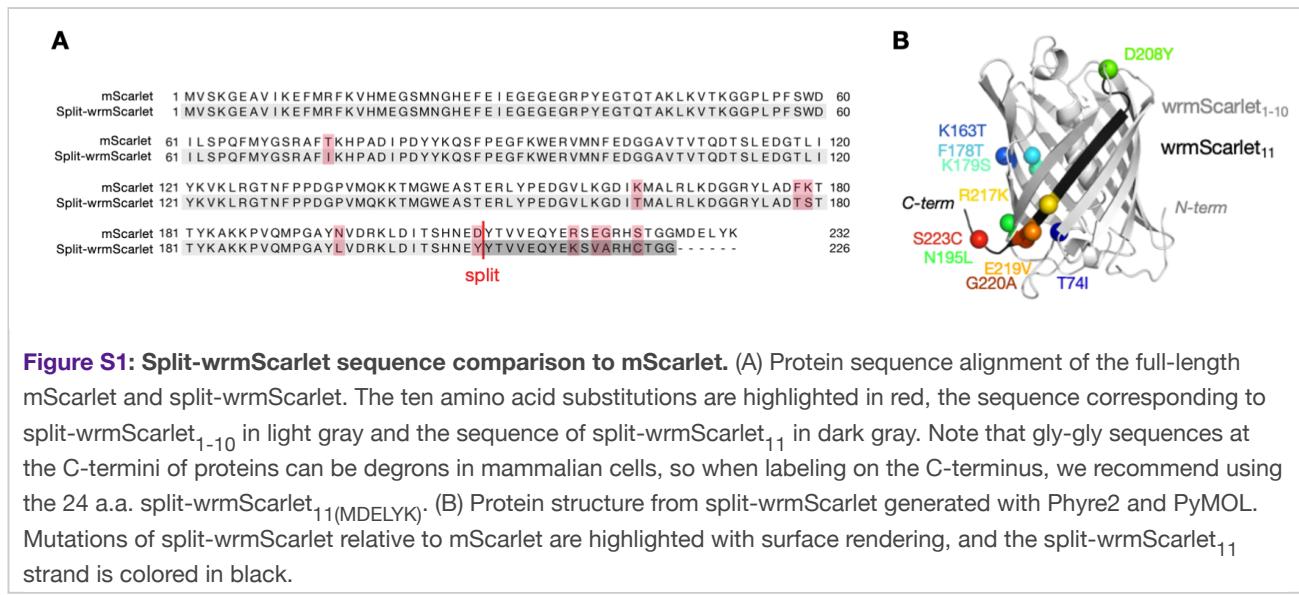
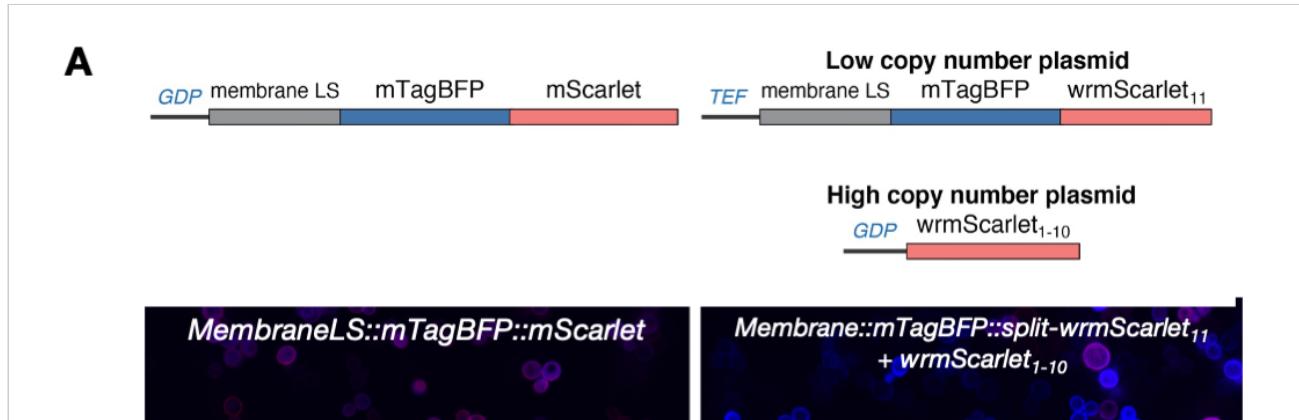
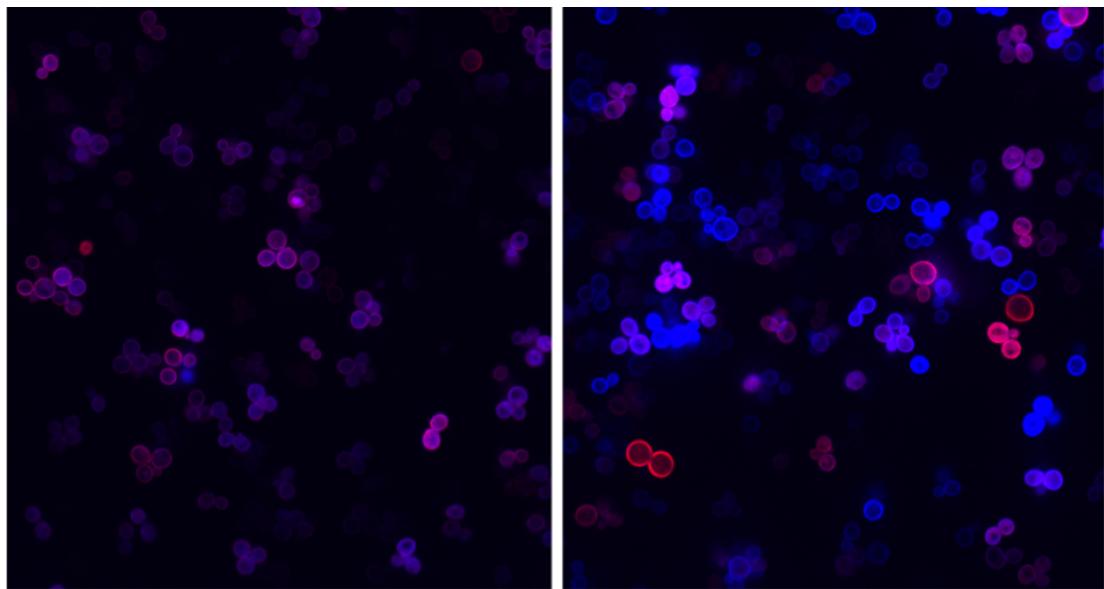


Figure S2. Split-wrmScarlet brightness in *S. cerevisiae*.





B

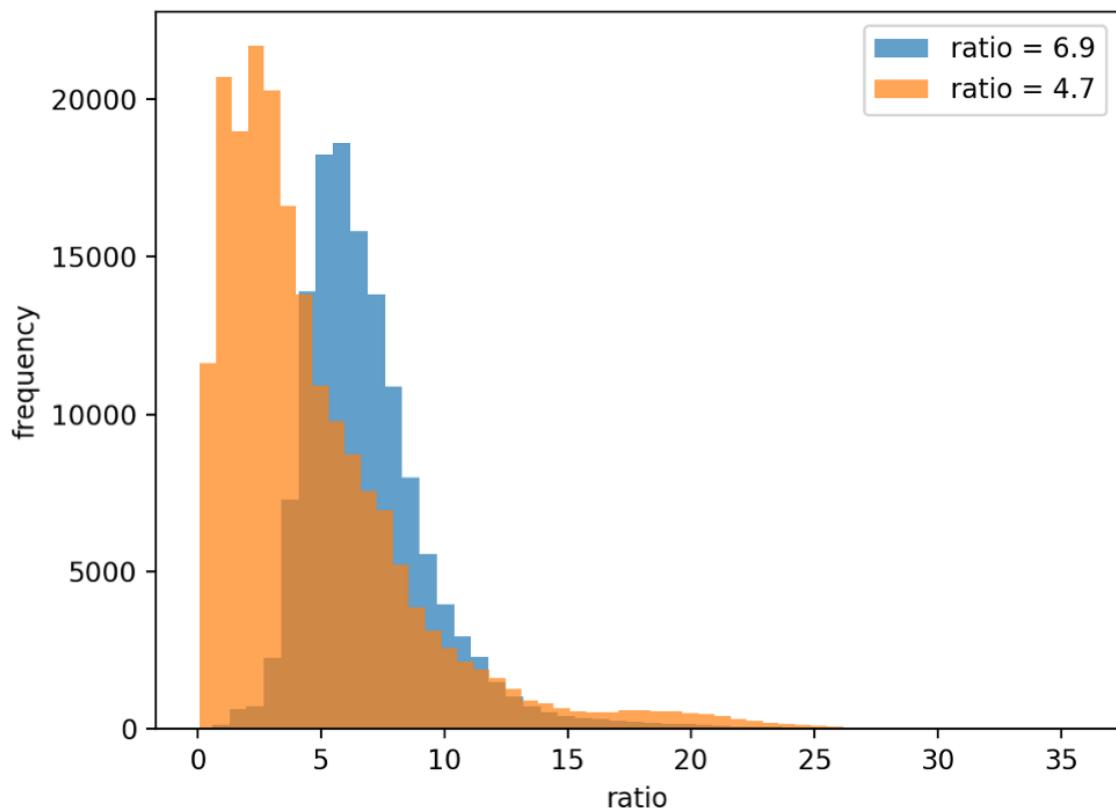


Figure S2: Split-wrmScarlet brightness in *S. cerevisiae*. (A) Composite display of red and blue channels for membrane-localized mTagBFP-mScarlet (wild-type) fusion or split-wrmScarlet₁₋₁₀ plus membrane localized mTagBFP-split-wrmScarlet₁₁ in yeast. Images were acquired and are displayed under identical 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.

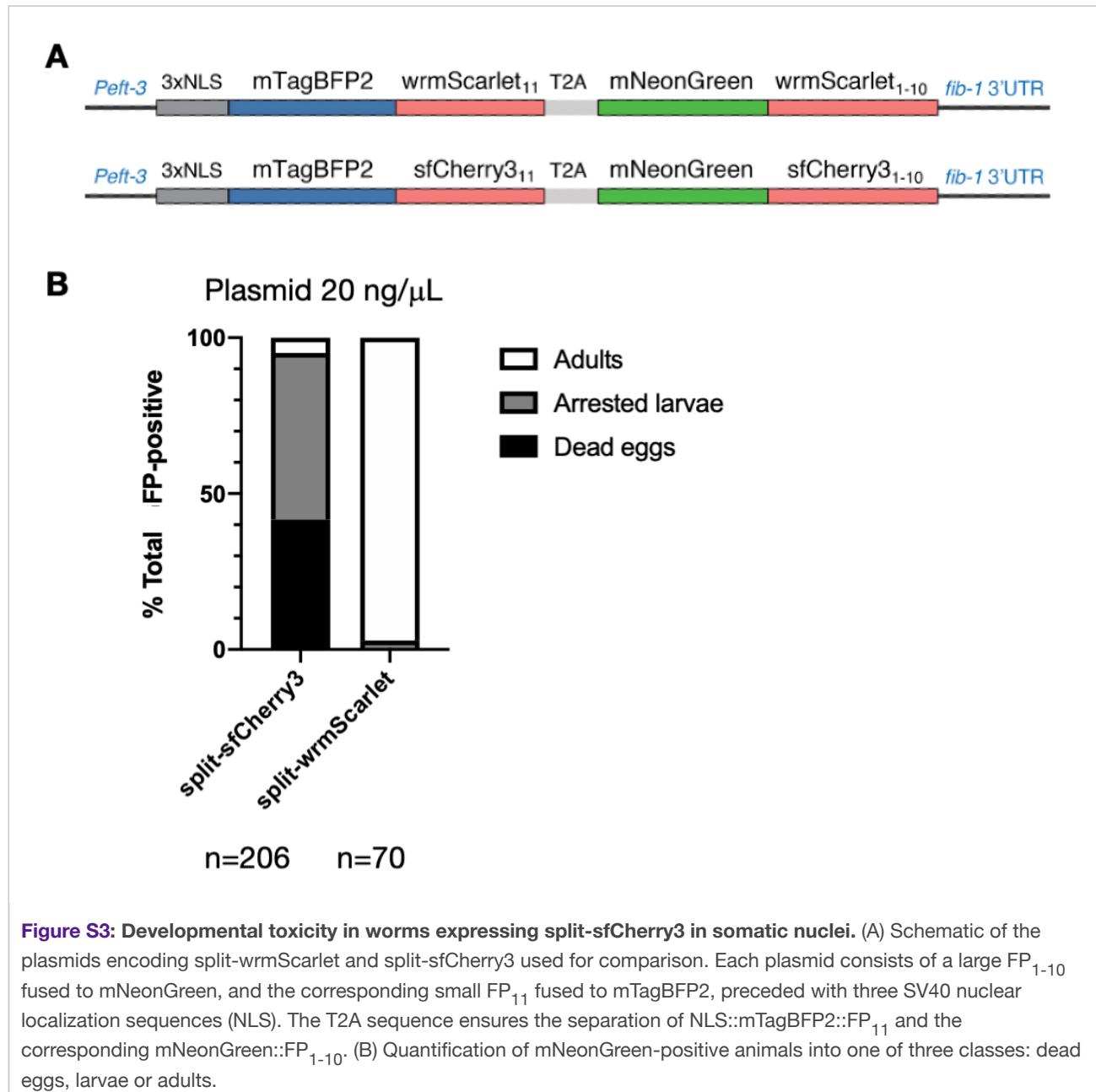


Figure S4. Split-wrmScarlet and split-sfCherry3 comparison.

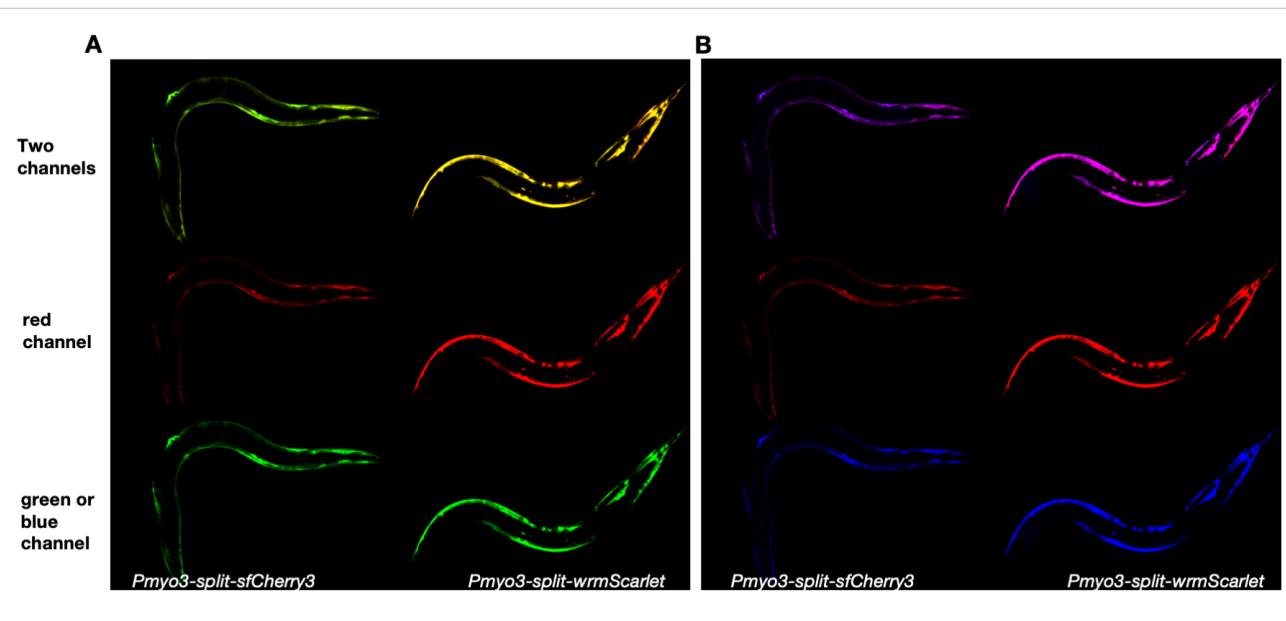


Figure S4: Split-wrmScarlet and split-sfCherry3 comparison. Individual channels and overlays corresponding to the images displayed in Figure 1B.

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

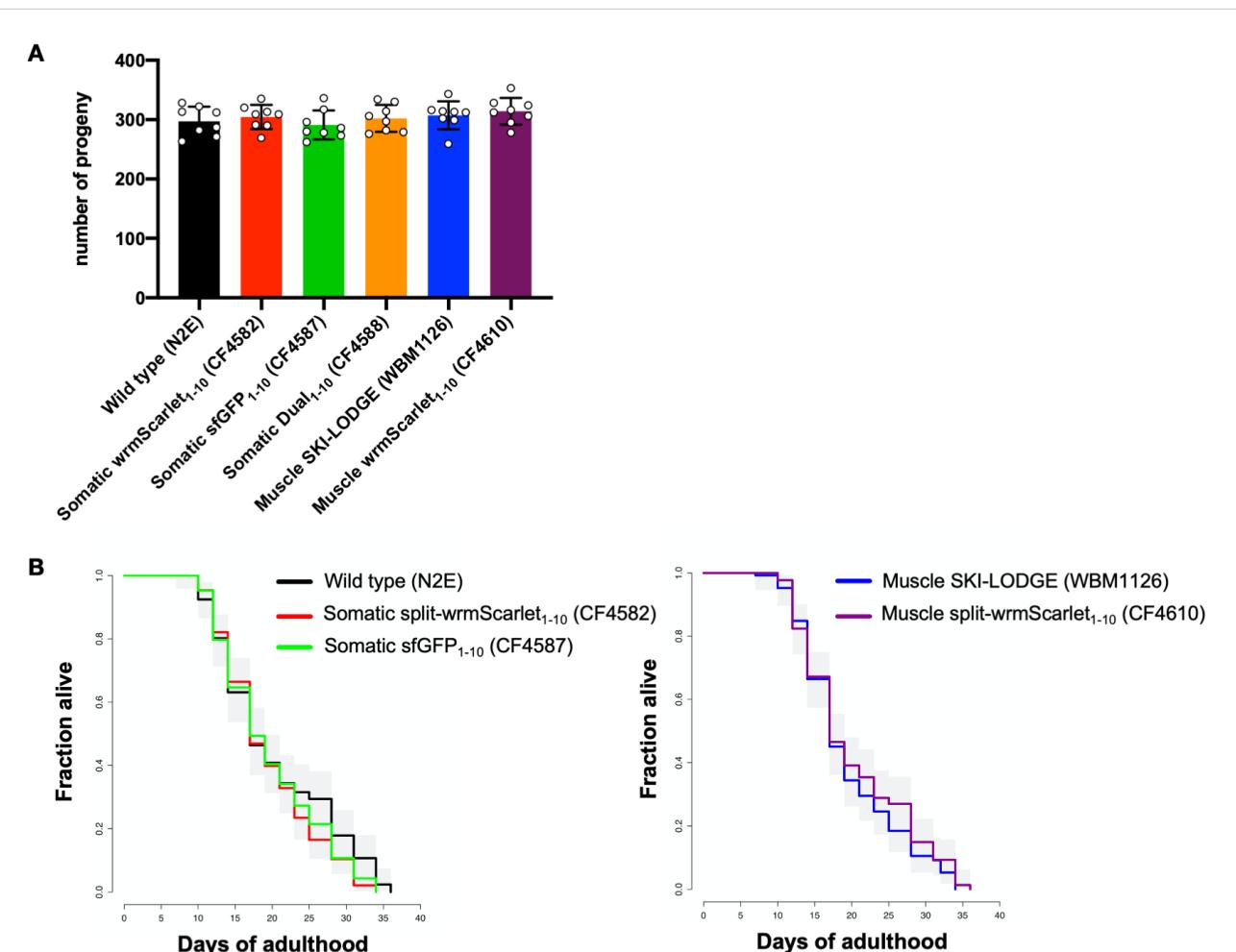


Figure S5: Brood size and lifespan of split-wrmScarlet₁₋₁₀ and sfGFP₁₋₁₀ lines. Split-wrmScarlet₁₋₁₀ and split-sfGFP₁₋₁₀ lines produced wild-type numbers of progeny (A) and a wild-type lifespan (B). Genotypes: N2E (wild-type), CF4582 (muls252[Peft-3::split-wrmScarlet₁₋₁₀::unc-54 3'UTR, Cbr-unc-119(+)] 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(+)] 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)}.

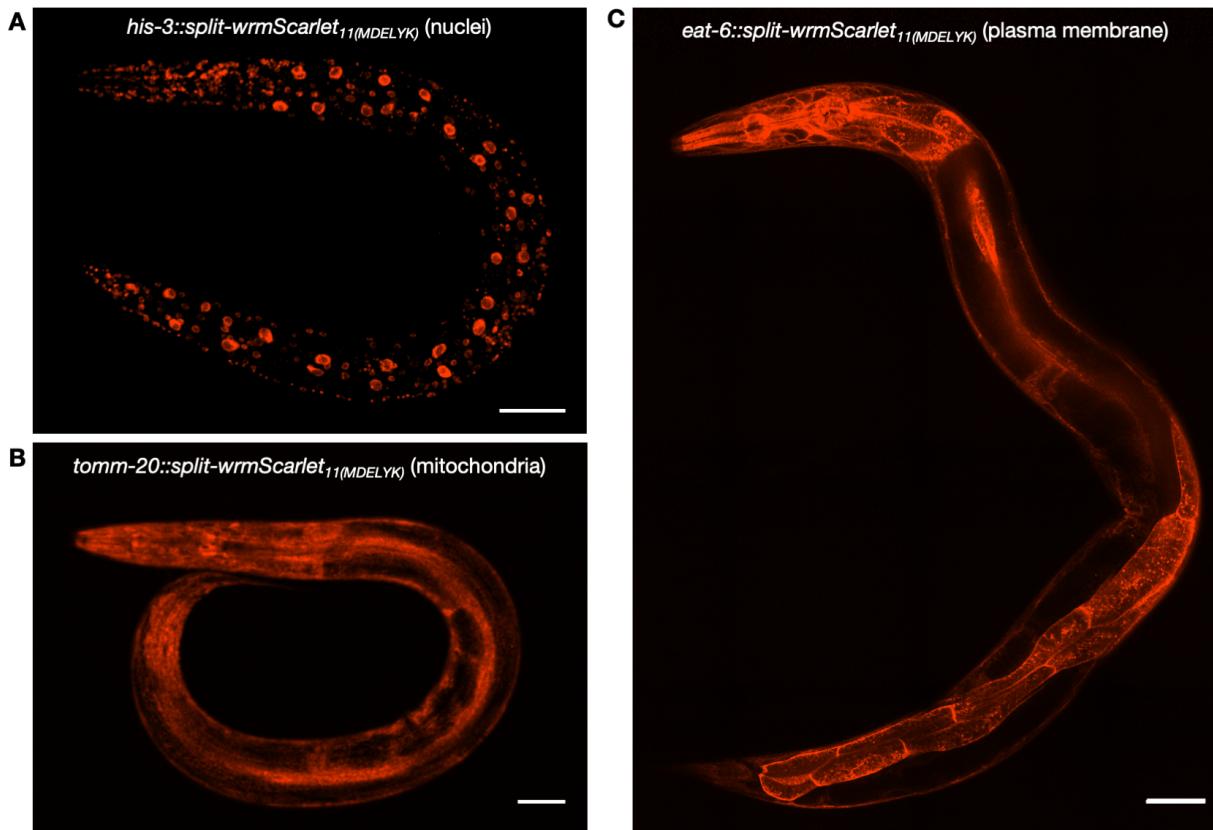


Figure S6: Proteins tagged at their C-terminus with the 24 a.a. split-wrmScarlet_{11(MDELYK)}. Endogenous proteins tagged with split-wrmScarlet_{11(MDELYK)} in animals expressing split-wrmScarlet₁₋₁₀ in somatic tissues. (A-C) Confocal images of worms expressing somatic split-wrmScarlet₁₋₁₀ and (A) HIS-3::split-wrmScarlet_{11(MDELYK)} (nuclei), (B) TOMM-20::split-wrmScarlet_{11(MDELYK)} (mitochondria), or (C) EAT-6::split-wrmScarlet_{11(MDELYK)} (plasma membrane). (A-B) Maximum intensity projections of 3D stacks shown. (C) Single slice shown. Scale bars, 50 μ m.

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.

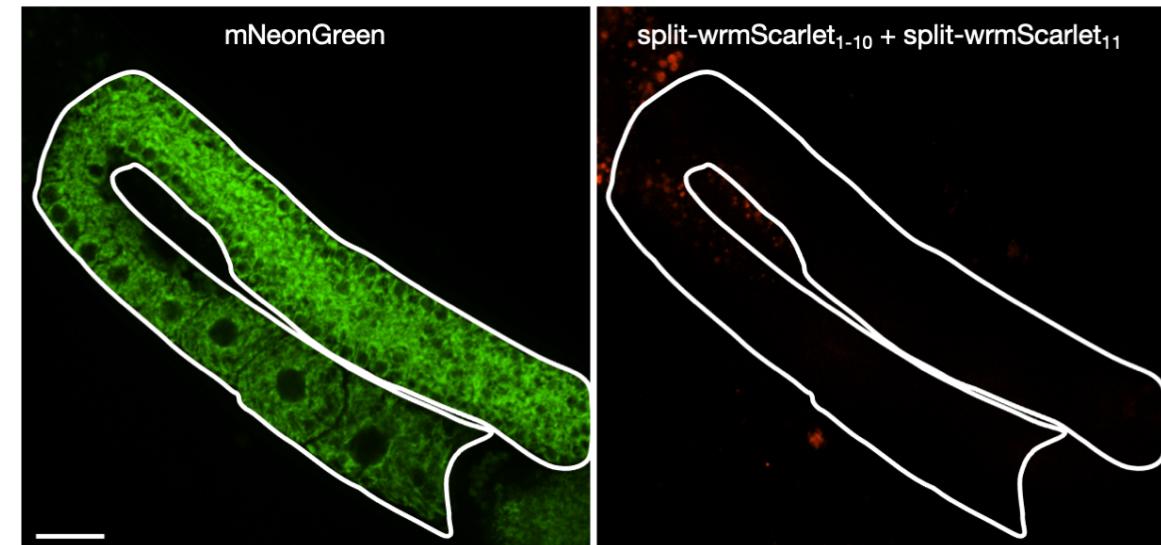
A**B**

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.

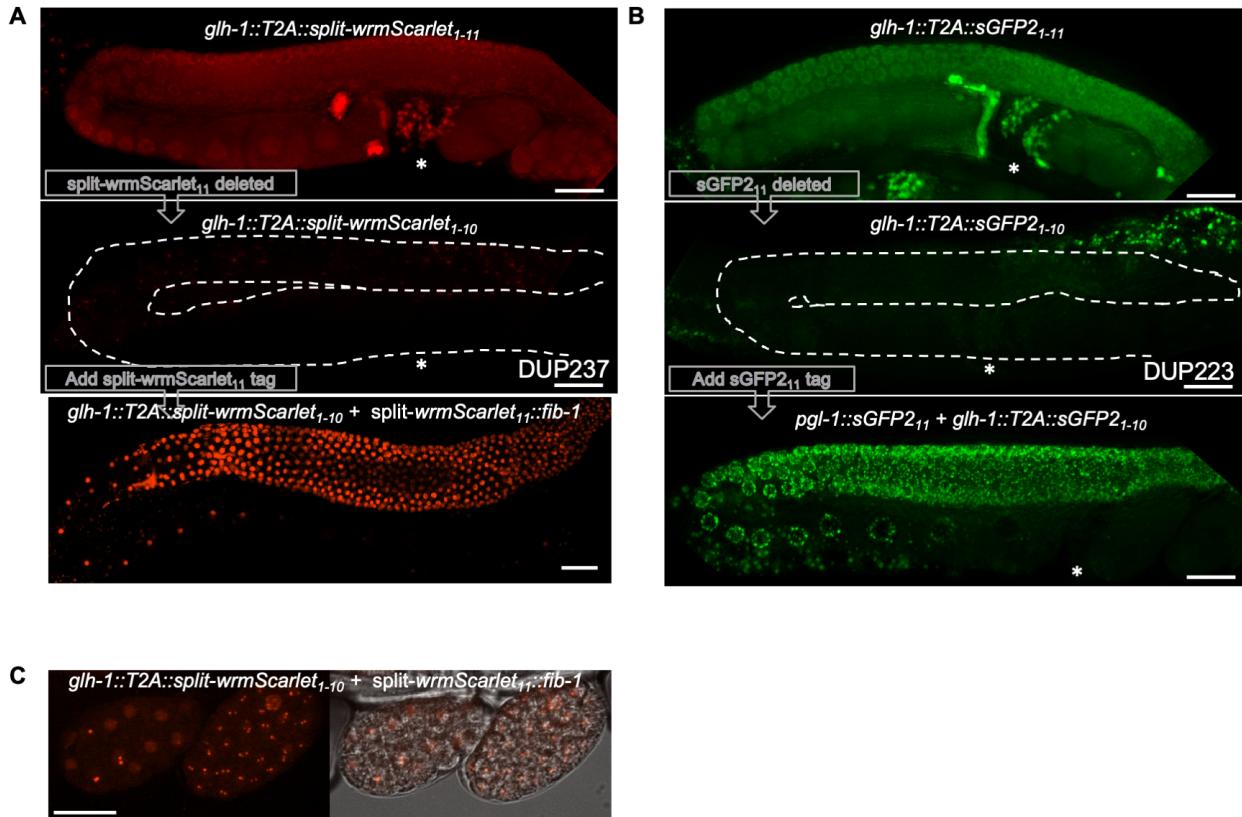


Figure S8: Generation and validation of germline-specific split-wrmScarlet₁₋₁₀ and sGFP2₁₋₁₀ strains. In order to generate germline-specific split-fluorescent strains, we first tagged the C-terminus of *glh-1* with T2A::split-wrmScarlet₁₋₁₁ (A) or T2A::sGFP2₁₋₁₁ (B). The T2A separates GLH-1 post-translationally to disperse the fluorophore throughout germ-cell nuclei, syncytium, sperm (*) and early embryos (Upper panels). We then deleted split-wrmScarlet₁₁ or sGFP2₁₁ 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-wrmScarlet₁₁, or PGL-1 with sGFP2₁₁ confirmed that germline-specific labeling with split-wrmScarlet and split-sGFP2 can be successfully achieved using these strains (Lower panels). (C) Split-wrmScarlet_{11::FIB-1} is detectable in early embryos. Scale bars, 20 μm.

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

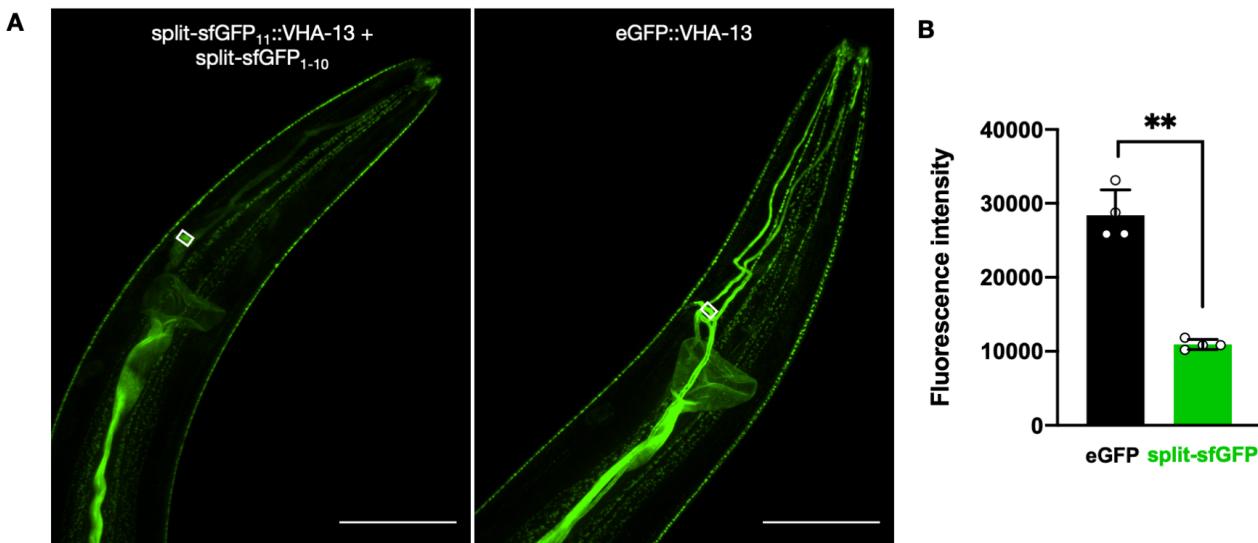


Figure S9: Somatic sfGFP₁₁ compared to full-length eGFP at the endogenous vha-13 locus. (A) Representative images of animals expressing sfGFP₁₋₁₀ in somatic tissues with endogenous VHA-13 tagged with sfGFP₁₁ (left panel), or endogenous VHA-13 tagged with eGFP in a wild-type background (right panel). 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.

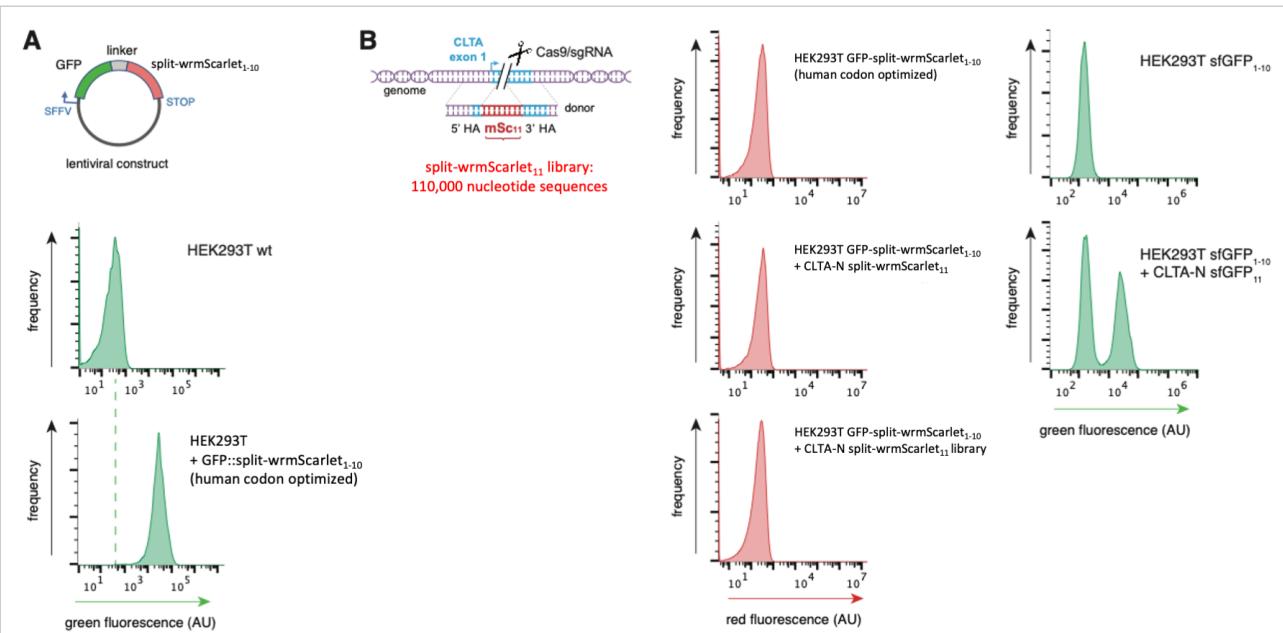


Figure S10: Screen for split-wrmScarlet fluorescence in mammalian cells. (A) FACS histograms of human codon-optimized split-wrmScarlet₁₋₁₀ expressed as a C-terminal GFP fusion. GFP expression verifies successful expression of the fusion protein in HEK293T cells by lentiviral transduction. (B) Schematic 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.

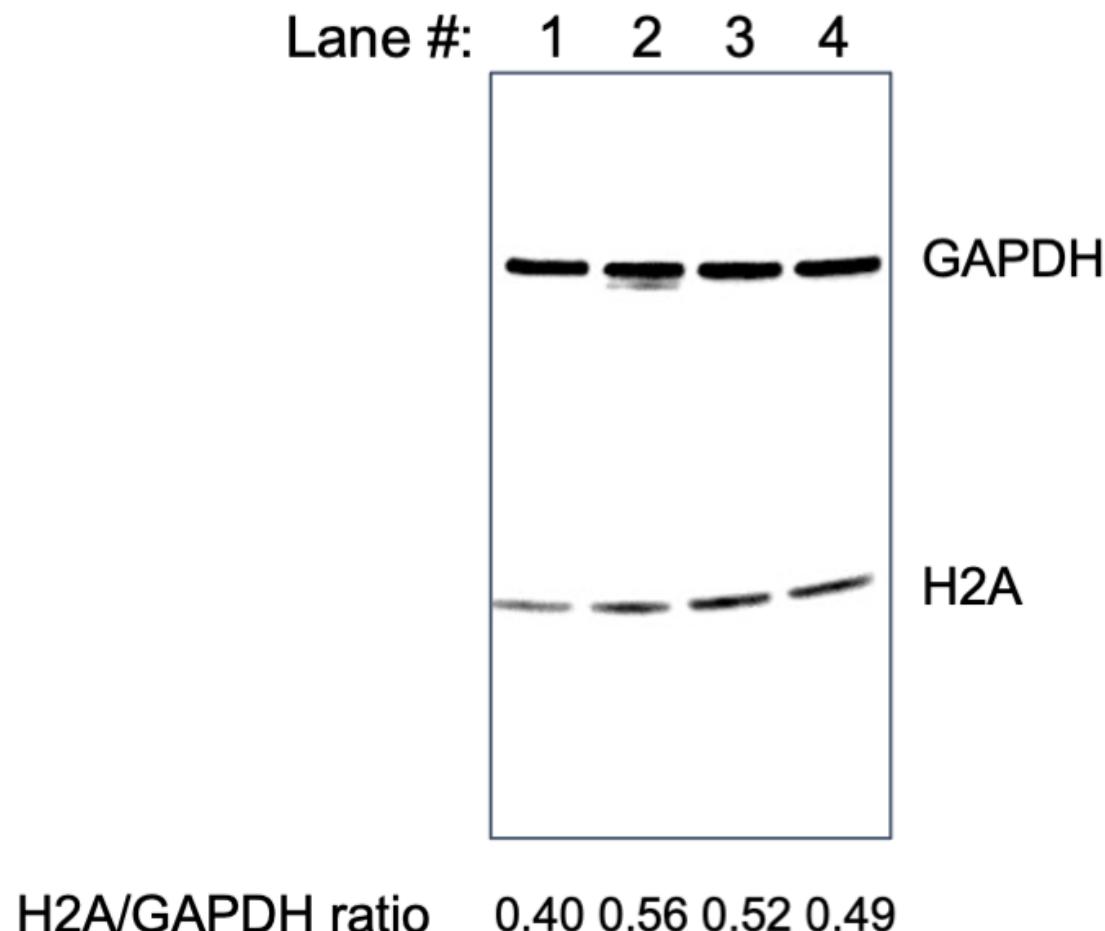


Figure S11: Split-wrmScarlet₁₁ C-terminal amino acids did not affect H2A abundance. Western-blot of histone H2A (his-3) with split-wrmScarlet₁₁+/- MDELYK. Western blot targeting HIS-3 in wild-type animals (lane 1), somatic split-wrmScarlet₁₋₁₀ expressing animals (lane 2), somatic split-wrmScarlet₁₋₁₀ strain with HIS-3::split-wrmScarlet₁₁ ending with two glycines (lane 3), or somatic split-wrmScarlet₁₋₁₀ + HIS-3::split-wrmScarlet_{11(MDELYK)}. GAPDH was used as a loading control, and the HIS-3/GAPDH ratios are displayed under each lane.

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

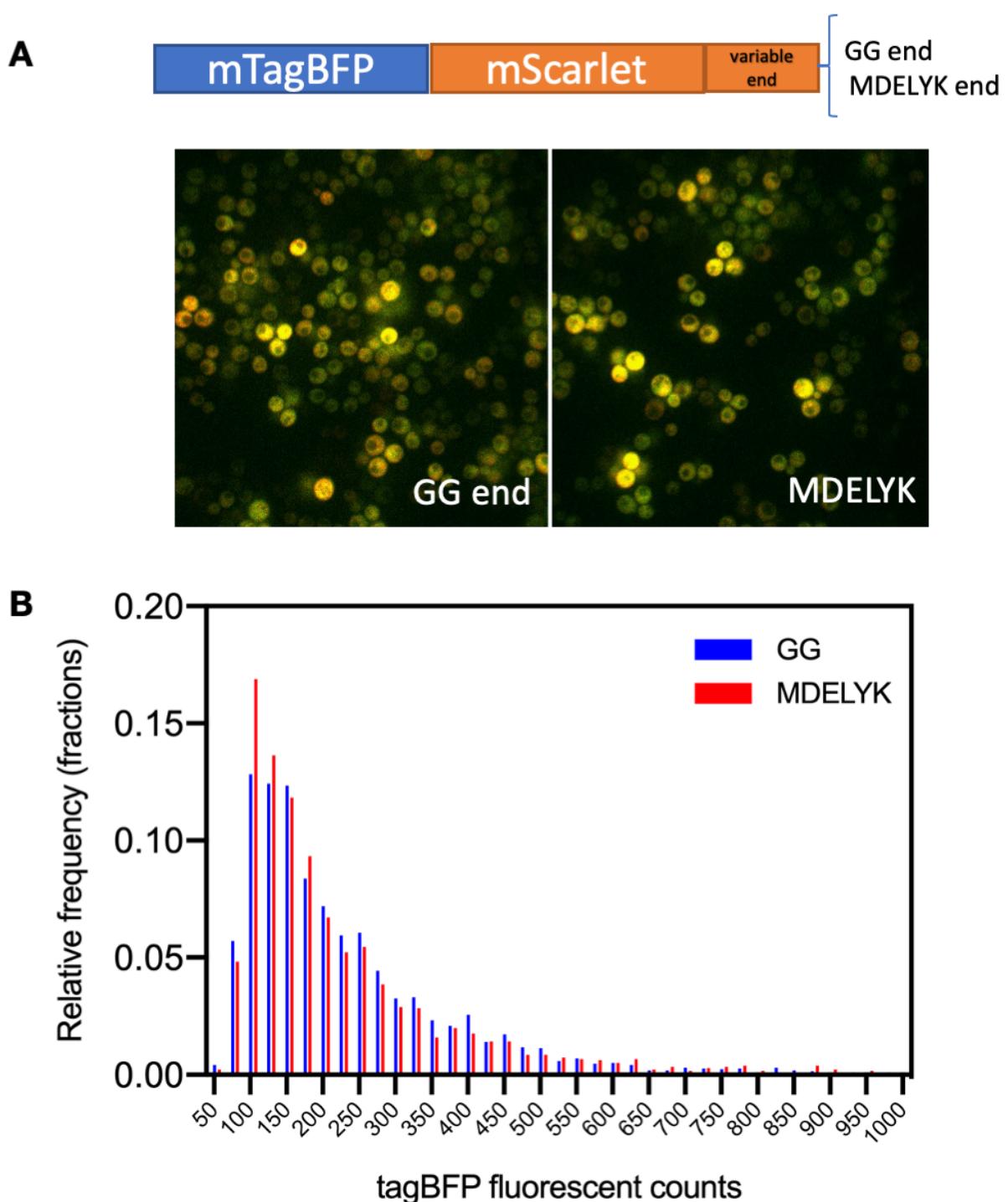


Figure S12: mScarlet ending with GG or MDELYK yields similar protein abundance in yeast. (A) Representative images of yeast expressing mTagBFP::mScarlet fusion truncated to end with gly-gly (GG end) or MDELYK from a p416-GPD promoter plasmid. mTagBFP fluorescence is pseudocolored in green, and mScarlet 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	ATGGTATCGAAGGGAGAACGAGTAATCAAGGAGTTCAT GCGTTCAAGGTCCACATGGAGGGATCCATGAACGGAA CACGAGTTGAGATCGAGGGAGAGGGAGAGGGACGT CCATACGAGGGAAACCCAAACCGCCAAGGCTCAAGGTCA CCAAAGgttaagttaaacatataactaaactaaccctgattttaaatttt cagGGAGGACCACTCCCATTCTCTGGGACATCCTCTC CCACAATTATGTACGGATCCCCTGCCTTCATCAAGC ACCCAGCCGACATCCCAGACTACTACAAGCAATCCTC CCAGAGGGATTCAAGTGAGCGTGTATGAACCTCG AGGACGGAGGAGCCGTACCGTACCCAAAGACACCT CCCTCGAGGACGGAACCCCTCATCTACAAGGttaaac agtcggtaactaactaaccatataatttttagtGTCAGTCTC GTGGAAACCAACTTCCCACAGACGGACCAGTATGCA AAAGAAGACCATGGGATGGGAGGGCTCCACCGAGCG TCTCTACCCAGAGGACGGAGTCCTCAAGgttaaca tgattttactaactaactaatctgatttttagGGAGACATCACC ATGCCCTCCGTCTCAAGGACGGAGGACGTTACCTCG CCGACACCTCACCACCTACAAGGCCAAGAACCGAG TCCAAATGCCAGGAGCCTACCTCGTGACCGTAAGCT CGACATCACCTCCCACAACGAGTAC
Codon-optimized split-wrmScarlet ₁₁	TACACCGTCGTCGAGCAATACGAGAACGTTCCGTCGCC GTCACTGCACCGGAGGA
Codon-optimized split-wrmScarlet _{11(MDELYK)}	TACACCGTCGTCGAGCAATACGAGAACGTTCCGTCGCC GTCACTGCACCGGAGGAATGGATGAGTTATAACAAG
Codon-optimized sfGFP ₁₋₁₀ with 1 intron	ATGCTAAAGGGAGAACGAGTTATTAACGGAGTTGTGCC GATCCTCGTCGAGCTGACGGAGACGTCAACGGACA CAAGTTCTCCCGTGGAGAGGGAGAGGGAGACCG CACCATCGGAAAGCTCACCCTCAAGTTCATGTCACC ACCGGAAAGCTCCAGTCCCCTGGCAACCCCTCGTC ACCACCTCACCTACGGAGTCCAATGCTCTCCGTTA CCCAGACCACATGAAGCGTCACGACTTCTCAAGTCC GCCATGCCAGAGGGATACTGCTCAAGGAGCGTACCATCT CCTTCAGGACGGACGGAAAGTACAAGGttaaacatata atataactaactaaccctgatttttagGACCGTGTGCG TCAAGTTGAGGGAGACCCCTCGTCAACCGTATCGA GCTCAAGGGAAACCGACTTCAAGGAGGACGGAAACAT CCTCGGACACAAGCTCGAGTACAACCTCAACTCCAC AACGTCTACATCACCAGCGACAAGCAAAGAACGGAA TCAAGGCCAACTTCAACCGTGTGTCACAACGTCGAGGA CGGATCCGTCAACTCGCCGACCAACTACCAACAAAAC ACCCCAATCGAGACGGACCGACTTCCCTCCCCAGAC AACCAACTACCTCTCCACCCAAACCGTCTCTCCAAGG ACCCAAACGAGAAG
Codon-optimized sfGFP ₁₁	CGTGACCACATGGTCCTCATGAGTATGTAATGCTGC TGGGATTACA
Codon-optimized split-wrmScarlet ₁₋₁₀ with 1 intron for germline expression	ATGGTTCCAAGGGAGAGGGCTGTTATCAAGGAATTCA GCGCTTCAGGTTCACATGGAAGGATCTATGAACGGAA CACGAATTCTGAAATCGAAGGAGAACGGAGAACGGC CATACGAGGGAACTCAAACGCTAAGCTTAAGGTTACT AAAGGAGGACCACTTCAATTCTCTGGGATATCCTTCT CCACAGTTCATGTACGGATCTCGCGTTTCATCAAGCA CCCAGCTGATATCCCAGATTACTACAAGCAGTCTTCC CAGAAGGATTCAAATGGGAGCGCGTTATGAACCTCGAA GATGGAGGAGCTGTTACCGTACCCAAAGATACTCTCC TGAGGATGGAACCTTATCTACAAGGttaaacatata actaactaaccctgatttttagGTTAGCTCGGGAA CTAATTTCCCACCAAGATGGGACCAAGTTATGCAAGAAG ACTATGGGATGGGAAAGCTTCTACCGAGCGCCTTACCC AGGGATGGAGCTTAAGGGAGATATCACCATGGCTC TTCGCTTAAGGATGGAGGAGCTTACCTTGCTGATACC TCTACTACTACAAGGCTAAGAAGGCCAGTTCAAGATGCC AGGAGCTTACCTTGTGATCGTAAGCTTGATATCACTTC TCATAACGAATAC
Codon-optimized sGFP2 ₁₋₁₀ with 2 intron for germline expression	ATGAGTAAAGGAGAACGAGATTGTTACTGGAGTTGCC AATCCTCGTCGAGCTGACGGAGACGTCAACGGACA CAAGTTCTCCCGTGGAGAGGGAGAGGGAGACGC CACCATCGGAAAGCTCACCCTCAAGTTCATGTCACC ACCGGAAAGCTCCAGTCCCCTGGCAACCCCTCGTC ACCACCTCACCTACGGAGTCCAATGCTTCCGCCCC ACCCAGACCACTGAAGCGTCACGACTTCTCAAGTC CGCCATGCCAGAGGGATACTGCTCAAGAGCGTACCATC TCCTCAAGGttaaacatataactaactactgatttttag tcagGACGACGGAAAGTACAAGACCCGTGCGCTGTC AGTTGAGGGAGAACCCCTCGTCAACCGTATCGAGCT

Codon-optimized sequence of split-wrmScarlet ₁₋₁₀ with 3 introns, engineered to avoid piRNA recognition transgene silencing. (Undetectable fluorescence in the MosSCI strain PHX1797)	<pre> CAAGGGAAACCGACTTCAAGGAGGGACGGAAACATCCCTC GGACACAAGCTGAGTACAACCTCAACTCCCACAACG TCTACATCACCGCCGACAAGCAAAGAACGGAATCAA GGCCAACCTTACCGtaagttaaacatgattttactaactaactaatct gattttaaattttcagACCCGTACAACAGTCGAGGGACGGATCC GTCCAACCTGCCGACCATAACAAACAAAACACCCCAA TCGGAGACGGACCAGTCCTCTCCAGACAAACCAACTA CCTCTCCACCCAAACCGTCCTCTCCAAGGACCCAAAC GAGAAG </pre>
	<pre> ATGGTATCGAAGGGAGAAGCAGTCATCAAGGAGTTCAT GCGTTCAAGGTCCACATGGAGGGATCCATGAACCGA CACGAGTCGAGATCGAGGGAGAGGGAGAGGGACGT CCATACGAGGGAAACCCAAACCGCCAAGCTAACAGGTCA CAGGtaagttaaacatataactaactaaccgttattttaaattt cagGGAGGGACCACTCCCATTCTCTGGGACATCCTCTC CCCACAATTATGTACGGATCCCGTGCCTCATCAAGC ACCCAGCCGACATCCCAGACTACTACAAGCAATCCCTC CCAGAGGGATTCAAGTGGAGCGTGTGATGAACTCTG AGGACGGAGGGAGGCCGTACCGTCACCCAGACAC CCCTCGAGGAGCGGAACCCATCTACAAAGtaagttaaac agtccgtactaactaaccatataattttcagGTCAAGCTC GTGGAACCAACTTCCCACCAAGACGGACCGAGTCATGCA AAAGAAGACCATGGGATGGGAGGCCTCACCGAGCG TCTTACCCAGAGGGACGGAGTCCTCAAGGtaagttaaca tgattttactaactaactatctgatttttcagGGAGACATCACC ATGGCCCTCCGTCTCAAGGAGCGGAGCGTACCTCTG CCGACACCTCCACCAACCTACAAGGCGAACAGCCAG TCCAAATGCCAGGAGCCTACCTCGTCGACCGTAAGCT CGACATCACCTCCCCACAACGAGTAC </pre>

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	gcaccctttgttattgtcaactccatggttctccattgttctgttaataatgtaaatttcataaaaaataaagacattatacaataaaaaataaagaaatattgtaaaataaaactgcccagagaaaaaaatgtcaacactcccgccgagagtgtttgaatgggtacggatcatttcgtctaggatgttagatgtgcaggcagcaacgagagggggagagatttttggccctgtgaaattaacgtgatgttctggatctgactaatacatgttggttttttgtggtttatttgttttatcttgttttatccagattaggaatttaaattttatgtaaatgtaaatgggtcaaaacattcgtccagctttttccctgttcactgttactgtcaattttattttaggtctcaacaatgttctaactgtcttattgtgacccacttttataatttttatttaaaaatattgtaaatgggtcaattttattttaggtctgacttttattctctaccgtccgactcttctactttttaaattaaatgtttttttcagttggaaacactttgtcaactccgtacgcagccATGGTATCGAAGGGAGAAGCAGTAA TCAAGGAGTTCATGCTTCAAAGGTCCACA TGGAGGGATCCATGAACGGACACGAGGTC GAGATCGAGGGAGAGGGAGAGGGAGCTC CATACGAGGGACCCAAACCGCCAAGCTC AAGGTCACCAAGGtaatggatttaaacatataactaa ctaaccctgattttaaatttcagGGAGGACCAACTC CCATTCTCCTGGGACATCCTCTCCCCACAA TTCATGTACGGATCCGTGCCTTCATCAAG CACCCAGCCGACATCCCAGACTACTACAA GCAATCCTCCCAGAGGGATTCAAGTGGG AGCGTGTCACTGAACCTCGAGGACGGAGGA GCCGTACCGTCACCCAAGACACCTCCCT CGAGGACGGAACCCCTCATCTACAAGGtaatgt ttaaacagttcggtactaactaaaccatacattttttt agGTCAAGCTCGTGGAAACCAACTTCCCA CCAGACGGACCAGTCATGCAAAGAAGAC CATGGGATGGGAGGGCTCCACCGAGCGT CTCTACCCAGAGGACGGAGTCCTCAAGGtaatgt agttttaaacatgtttactaactaaactatgttttt cagGGAGACATCACCAGTGGCCCTCCGTCTC AAGGGACGGAGGACGTTACCTCGCCGACA CCTCCACCCACCTACAAGGCCAAGAAGCC GTCCAAATGCCAGGAGCCTACCTCGTCGA CCGTAAGCTCGACATCACCTCCACAACG AGTACTAA taagtccaaattacttcaacatccctcatatgttattatcaa cttttccctgtgtccacccttattttgttattatcaa

					aaacctcttaattcttgggttttagctctttaagtccaccc taacaatgaaattgttagattcaaaaatagaattaatcg aataaaaaatgcggaaaaaatggctcccccattaa ataataattctatccaaaatctacacaatgtctgtaca cttcattatgttttactctgataaaatttttgaaacatcataga aaaaaccgcacacaaaataccatcatatgttgcgg gtttatgaccgcaatttatctcgacgtctggccctc atgacgtcaaatcatgtctgcaatggggat tttggaaatttcaatcaagtggaaagttatgaaatatttc ctgcttgcattttgggttccctattgttgcagatttc aggacggcggttctgctaaaatcacaagtattgtgac acgatgcaagaaagatcgagaagggttgggttgg ctcagtggaaagggttagaagtggataattgaaatgg agtatgtctatgggttttgcctaaatgacagaatacatt cccaatataccaaacataactgttt
CF4587	muls253[(Pef t-3::sfGFP 1-10::unc-54 3'UTR, Cbr- unc-119(+)] II; unc- 119(ed3) III	Somatic sfGFP ₁₋₁₀ promoter)	II: 8.42 MB	II:0.77	gcaccttggctttatgtcaactccattggcttccattgt ttctgttaaattaaatgaattttatcataaaaataagacattatac aatataaaaatgaagaattttatgaaaataaactgccagag agaaaaaagtatgcaacactcccccggagagtgtttgaat ggtgtacggatctttatctgtctggatgtatgtcagg cagcaacggagggggggagagatttttggccctgtgaa attaaacgtgatgtttatccatgtactaataatcatgtttt tgggtttatgtttatctgtttatccagatttagaaat ttaaattttatgaaatttataatgaggtcaaacattcagtccca gcgttttccctgttcaactgtttatgtcaattttttaggttt tcaacaatgttcaactgtttatgtgaccctactttata ttttttatattttaaaatagaagtttcttaggaaatttttc gacttttattctctaccgtccgactcttcttactttaaatt aaatgttttttcaagtggaaacacttgc aaaa ATGTCTAAGGGAGAAGAGTTATTACTGGA GTTGTGCCGATCCTCGTCGAGCTCGACGG AGACGTCAACGGACACAAGTTCCCGTCC GTGGAGAGGGAGAGGGAGACGCCAACCAT CGGAAAGCTCACCTCAAGTTCATCTGCA CCACCGGAAAGCTCCAGTCCCAGTGGCC AACCCCTCGTCAACCACCTCACCTACGGAG TCCAATGCTCTCCCGTTACCCAGACCACA TGAAGCGTACGACTTCTCAAGTCCG ATGCCAGAGGGATACTGCCAAGAGCGTAC CATCTCCTCAAGGACGACGGAAAGTACA AGtaagttaaacataataactaactaaccctgattatt aaatttcagACCCGTGCCCGTCAAGTTCG AGGGAGACACCCCTCGTCAACCGTATCGAG CTCAAGGGAAACCGACTTCAAGGAGGAGC GAAACATCCTCGGACACAAGCTCGAGTAC AACTCACTCCCACAACGTCTACATCACC GCCGACAAGCAAAGAACGGAATCAAGGC CAACTTCACCGTCCGTACAACGTCGAGG ACGGATCCGTCACACTGCCGACCACTAC CAACAAAACACCCCAATCGGAGACGGACC AGTCCTCCTCCAGACAACCAACTACCTCT CCACCCAAACCGTCTCTCCAAGGACCCA AACGAGAAGTAA atatccaccgtgtggcgggagggtcgc catctcgccccgtgcctctgacttcaagtccaaattactct tcaacatccctactgtctttccctgtgcctccacccccc tattttgttattatcaaaaaacttcttaatttttgcatttttag cttctttaagtccacttcaataatgaaattgttagatca aaatagaattaatcgtaataaaaaatcgaaaaaaatgtg ctccctccccccatataataattctatccaaaatctaca caatgttctgttacacttcttgcattttactctgataaaattt tttggaaacatcatagaaaaaccgcacacaaaatcccta tcatatgttacgtttcagttatgaccgcattttatctcg acgtctgggcctcatgtacgtcaaatcatgtctatcg aaaatgtttggaggatattttggaaattttcaatcaagtggaaat ttatgaaatttaatttctgttttgcattttggggttccctatt gtttgtcaagatttgcaggacggcgtttctgtctaaatca caagtattgtgacgcacatgtcaagaaatcgaaagaa gttggggtttggaggctcagttggaaagggttagatca taatttggaaatgtggaggtagtgcattatggggtttgccttaat gacagaatacattcccaatataccaaacataactgttt
CF4588	muls253[Peft -3::sfGFP 1-10::unc-54 3'UTR, Cbr- unc-119(+)], muls252[Peft -3::split- wrmscarlet ₁₋₁₀ 1-10::unc-54 3'UTR, Cbr- unc-119(+)]	Somatic sfGFP ₁₋₁₀ and somatic split- wrmscarlet ₁₋₁₀ (eft-3 promoter)	II: 8.42 MB, 9.83 MB	II:0.77, II:1.73	Sequences from CF4582 and CF4587

					TCGACAAAGATTGGAGCTGCAAACAAAGTGC GTCCTACAGGAATTGAGAGATGCGAAAG AAGCGAGAAGAAGGACAACCTCTAGAGC TTCTGGGAATCGATATCGACAGTACACGA CCGAGAAAAGttaggtttcgatatttgatgaaa aattcaatattcaqTGCGCAAGTTACACAAA GAAAACCATGGCTTCGTTCTCAAAGAGC AATGGCTGATAACTGGCTCAATTGTCA TCGGCTCAAGTTCAGCTACGgttgtat atttcattttgaccgccttttaattccaaaatgtacagATCCA TGGTGCCCGTGCAGAGAGAGGAGCGTTC GAAGCTTTGAGACAATTCCGAAATGGATCG AACACTGTTCTATTGCTACTGCGGGTGCCT GAACGTGGACTTGATATCAAAGGAGTGGAT CATGTCATCAACTATGACATGCCAGACAAC ATTGATGACTATATCCATCGTATCGGAAGGtc agttagtattttataatgttcaataatgcagaacgtttcag AACTGGAAGAGTTGGAAACTCTGGAAAG CTACAAGCTTCATCTCGGAGGATTGAGTC TTCTGTCCGAACCTGTTGGTCTCGCCG ACGCACAACAGATTGTTCCAGACTGGATG CAAGGGTCTGCTGGAGGCAATTACGGAGC TAGTGGATTGGGTCAGTGTACCAACTCA AGTCCCCAGGACGAGGAGGGTGG GGATCGGGA GAGGGACGTGGATCCCTCTTACCTGCGG AGACGTCGAGGAGAACCCAGGACCA GGAGCATCGGGAGCCTCAGGAGCATCG ATGAGTAAGGAGAAGAATTGTCACTGGA GTTGTCCCACCTCTCGTCAGGCTCGACGG AGACGTCACGGACACAAGTTCTCGTCC GTGGAGAGGGAGAGGGAGACGCCACCAT CGGAAAGCTCACCCCTCAAGTTCATCTGCA CCACCGGAAAGCTCCAGTCCATGGCC AACCCCTCGTCAACCCCTCACCTACGGAG TCCAATGCTCGCCCGTACCCAGACCAC ATGAAGCGTCACGACTTCTCAAGTCC CATGCCAGAGGGATACGTCCAAGAGCGTA CCATCTCCTTCAAGgttaacttataact aactactgattttaaatttcagGACGACGGAAAGT ACAAGACCCGTGCGTCGTCAGATTGAG GGAGACACCCCTCGTCAACCGTATCGAGCT CAAGGGAAACCGACTTCAAGGAGGAGCGGA AACATCCTCGGACACAAGCTCGAGTACAA CTTCAACTCCCACACGTCTACATCACC CGACAAGCAAAGAACGGAATCAAGGCCA ACTTCACCGtaagttaaacatgattactaact atctgattttaaatttcagACCCGTCAACACGT GGACGGATCCGTCAACTCGCCGACCACT ACCAACAAAACACCCCAATCGGAGACGG CCAGTCCCTCCCTCCAGACAACCAACTACCT CTCCACCCAAACCGTCCTCTCCAAGGACC CAAACGAGAAGTAG aaaaccggaccaattgtatgtttcgcatatttaatgtgtc agttccccatatttaccccccgttattttatgtatt tggtttgtttgtgtgtatagtccctccgcataactct gttc
DUP237	glh-1(sam140[glh-1::T2A::split-wrmScarlet ₁₋₁₀]) I	Germline split-wrmScarlet ₁₋₁₀	I: 6.85 MB	I: 1.41	gttcggccacgcgcccccaactacagtaacctcgacacac tcatctactaaattttggacagtcctaatttttgcgtttt caactcaatttctggaaaaaatctaattttctgcggaaaATG TCTGATGGTTGGAGTATGCGAAAGTGTCT GCTAAGGGtgatattttgaaactttccaccgggtttatt ttgattttaaacttttcagCCAAACTGGATTG GTAGTGGAGGCCTTCGGTGGTGGTAAC AATGGAGGATCTGGTTGGTGGTGGTAAC AATGGAGGTACTGAGTCTGGTGGAGGAAA CACTGGCGGATCTGGATTCTGGTGGAGGAAA ACACTGGCGGATCTGGATTCTGGTGGAGGAAA AAGACTGGCGGTTCTGGATTGGAGGTTGG AAATACTTGTGGATCCGGCTTCTGGTGGAG GCAGTACAGGAGGATGCCGTATGGAGGA GCCAGTTCTGGATTCTGGTGGTAGTACTGC CACATCTGGATTGGAGCAGCGGTGAAAAAC AAGTGCATTTGGAGGATCAGGTGGCTT GAGGTAGTGCACCTGGATTCTGGAGTGG GGAGGATCCTTGAGGTGGCAACTCTGG TTTGGGAAAGGAGGACATGGCGGCGGA GAGAGAAAACAATAGttcgatatttgatca attacgttttcagATTGTTCAATTGCCAACAG CCAGGACATCGATCGAGTGTACTGCCAG GCCGAGAAAAGGAAAGAGAGGCCGAGAGGt tatttgatataactttattggcgtatatttgatatttcagTG TGCTACAATTGCCAGCAACCCGGGCACAC

CTCTCGTGAATGTACAGAAGAACGCAAGC
CGCGTGGGGTCGCACTGGTGATTGG
GGCGGGAGCTGGATTGGAAACAATGGAG
GAAATGACGGTTCTGGTGGGACGGTGGT
TTGGTGGAGGCGAAGAACGTGGTCCAAT
GAAATGTTCAACTGTAAGGGCAGGGGACA
TCGCTCTGCTGAATGTCGGAGGCCACCCC
GTGGATGTTCAATTGTGGCAGCAAGGT
CATCGCTCGAATGAGTGCCCCAATCCAGC
CAAGCCAAGGGAAAGGTGTTGAAGGAGAA
GGACCTAAGGCAGACATACGTGCAAGTCGA
AGACAAACATGGAGGACGTTCAACATGCA
GAAAATTCCGAAGGGCTTATGTTCAACAA
GTTTTCGATGCCAGTAAACTGACTTC
ATCCGAGAAAGACTGTGGTATCAAACCTTG
CAAGACATTGCCAGAAGCTAATCTCACGG
AGACCATGCAAGAAAAACGTTGCTCATGCT
GGATACTCCAAGACCACCTCAATTGAGCAA
TATGCTCTTCACTTGTGCTCAAACCTGGATAG
ATATCATGGCTTGTGCTCAAACCTGGATAG
GAAAACCGCTGCATTCTCTGCCTATCA
TGACTCGTCTCATGACGATAATAATCTGAA
CACTGCCGGAGAAGGGGGTGTATCCCC
GTTGCATCATCTGACTCCAACCTCGCGAAC
TCGCTGATCAAATTACAACGAGGGAGAA
AGTTGCTTACAAACATGATGGAGATCAA
ACCAGTTACGGAGGATTGGCTGTCGGTTA
TAATAAGGGTCAGATCGAAAAGGGAGCCA
CGATCATTGCGGAACTGTCGGAGAACATCA
AGCACTCTGTGAAGAGGGTACCATCAAG
CTTGACAAATGCCGCTCTTGTCTTGT
GAGGCTGATCGTATGATCGATGCTATGGGA
TTCGGAACTGACATCGAAACTATTGTCATT
ATGACAGTATGCCAGAGAAAGAAAATCGC
CAGACACTCATGTTCACTGCCACTTCCC
CGATTCTGTACAGGAAGCAGCTCGCGCTT
TTCTCAGAGAAAACACTCGTGTGATTGCA
TCGACAAGATTGGAGCTGCAAACAAGTGC
GTCCTACAGGAATTGAGAGATGCGAAAG
AAGCGAGAAGAAGGGACAAACTTAGAGC
TTCTGGGAATCGATATGACAGTTACACGA
CCGAGAAAAGttagtttcgtttttatgtgaaata
aattcaatattcagGTGCCAGTTCACAAA
GAAAACCATGGTCTCGTTCTCAAAGAGC
AATGGCTGATACACTGGCTCAATTGTC
TCGGCTCAAGTTCCAGCTATCACGtttgat
atttcattttgaccgttttaattcaaatgtacagATCCA
TGGTCCCCGTGAGCAGAGAGAGCGTTCA
GAAGCTTGAGAGACAATTCCGAAATGGATCG
AAACCTGTTCTATTGCTACTGCCAGACAAAC
ATTGATGACTATATCCATCGTATCGGAAGgtc
agtatattttatgtttcaataatgtcaaggatgtttcag
AACTGGGAAGAGTTGGAAACTCTGGAGAG
CTACAAGCTTCACTCGGAGGATGCACTG
TTCTGTCGAACCTGTTGGTGTCTCGCC
ACGCACAAACAGATTGTCAGACTGGATG
CAAGGTGCTGCTGGAGGCAATTACGGAGC
TAGTGGATTGGGTCCAGTGTACCAACTCA
AGTCCCAGGACGAGCAGAGGGGGTGG
GGATCGGG
GAGGGACGTGGATCCCTTACCTGGG
AGACGTCGAGGAGAACCCAGGACCA
GGAGCATCGGGAGCCTCAGGAGCATCG
ATGGTTCCAAGGGAGAGGCTGTTATCAAG
GAATTGATGCGCTTCAAGGTTACATGGAA
GGATCTATGAACGGACACGAATTGCGAAATC
GAAGGAGAAGGAGAAGGAGCAGCCCACAG
AGGGAACTCAAACCTGCTAACGTTAAGGTTA
CTAAAGGAGGACACTTCCATTCTCTGGG
ATATCCTTCTCCACAGTTGATGACGGATC
TCGCGCTTCAAGCAGCTGGATAT
CCCAGATTACAAGCAGTCTTCCAGA
AGGATTCAAATGGGAGGCGTTATGAACCT
CGAAGATGGAGGAGCTGTTACCGTTACCC
AAGATACTCCCTGAGGATGGAACCTTA
TCTACAAGgtaaactatataactaactaacc
ctgattttaaatttcagGTTAAAGCTTGGGAAAC
TAATTCCCACCAAGATGGACCAAGTTATGCA
GAAGAAGACTATGGGATGGGAAGCTTCTAC
CGAGCGCCCTTACCCAGAGGATGGAGTCC
TTAAGGGAGATATCACCAGTGGCTTCTCGTC

				TTAAGGATGGAGGACGTTACCTTGCTGATA CCTCTACTACTTACAAGGCTAACAGGCCAG TTCAGATGCCAGGAGCTTACCTTGTCGATC GTAAGCTTGATATCACTTCTCATAACGAATA CTAG aaaaccgaccaattgtatgtttcgcatattaaatgtgtc agtccccatatttatctgccttgtgattttaaatgtatt tggttgttggtgtcgatagtccctccgcataaactct 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 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	atagaggatttaaatattgtggccctaaagaggccgtt gggttcggtaaaatgttttaagaaggcatcta GGTGATTCCGGCGGCCTGACGTACTCGT GGAGGACCATGTGGTCACG TCCTCCTCC ttc I tgtctccgtt C tc I ggcaaaaagaacagctt ggatatttggagaagaactc c tc c ttggcg	CCAAGGA GGAGTTC TTCCAAAT ATC	GATTTAA ATATTGT GCCCTA AAG
vha-13	Y49A3A.2	N	AUUCUG CGGCCA UCUUUU CC	ggtttattttgatttcttcgatttccatatacgcttcaaattc atccatccaggaaaaatag CGTGACCACATGGTCCTCCACGAGTACGT CAACGCCGCCGAATCACC GGAGGAGGATCC gccgcagaatcttcgtacggattcgittacggagtgtccg gaccgtcgtcacccggagaatgttgc taaactccaactattgaatgttaatttgtttttta	GGTTTATT TTGATTT CTTTTCG ATTTC	CCATCTT CTCGGCT GTGAC
pgl-1	ZK381.4	C	GGTGGTT ACGGGG GTCGTGG	tacggcgagatcggtacgttggttacggggaaag aggaggtagatggattt GGAGCATCGGGAGCCTCAGGAGCATCG CGTGACCACATGGTCCTCCACGAGTACGT CAACGCCGCCGAATCACC taaactccaactattgaatgttaatttgtttttta	CCAAAGT TGCAAAA GGATTCTG GTCAATT G	CATTTAC GGGAACA AGGAAA 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	cgacgagatccgtcgtttttgttgcagatattccaggag gtgggtcgagcgtagacactac GGAGGAGGATCC TACACCGTCGTCGAGCAATACGAGAACGTC CGTCCCCGTACTGCACCGGAGGAATG GATGAGTTATACAAG taaagaacagctgtgaatctttagaaatttttatattttatc ttatgtttttatgtttccat	CCTGGTT CATGTGC TATTGCC	CGACGAC AGAAAGT AGCATCA C
his-3	T10C6.12	C	GAAGAAA ACCGGA GGAGACA	gattttaaatattgtggccctaaagaggccgtgggttcg gtaaaatgttttaagaaggcatcta CTTGATAACTCATCCATTCCCTCCGGTGCA GTGACGGGCACGGACTCTCGTATTGCT CGACGACGGTGTA GGATCCTCCTCC ttc I tgtctccgtt C tc I ggcaaaaagaacagctt ggatatttggagaagaactc c tc c ttggcg	CCAAGGA GGAGTTC TTCCAAAT ATC	GATTTAA ATATTGT GCCCTA AAG
tommm-20	F23H12.2	C	ACACCGA CGACUU GGAGUAA	gttggtaacgaaaaatacgaagaattaaattgttggat ataaaaactttaaatcattatccattta CTTGATAACTCATCCATTCCCTCCGGTGCA GTGACGGGCACGGACTCTCGTATTGCT CGACGACGGTGTA GGATCCTCCTCC ctccaagtgcgtcggtcatcgataagcttggattttgtgc ttggggagggtcgctccggatgtat	GAGCGAA AGCAGAT GAGGC	TCCGTGA GGAGGAA AACACC
actual tomm-20 mutation recovered	F23H12.2	C	ACACCGA CGACUU GGAGUAA	gttggtaacgaaaaatacgaagaattaaattgttggat ataaaaactttaaatcattatccattta CTTGATAACTCATCCATTCCCTCCGGTGCA GTGACGGGCACGGACTCTCGTATTGCT CGACGACGGTGTA GGATCCTCCTCC ctccaagtgcgtcggtcatcgataagcttggattttgtgc ttggggagggtcgctccggatgtat	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	ggtttattttgatttcttcgatttccatatacgcttcaaatttattccaggaaaaatag TACACCGTCGTCGAGCAATACGAGAACGTC CGACGACGGTGTA GGATCCTCCTCC

		<p>GGTGGCTCTGGAGGT TACACCGTTGAGCAATACGAGAAGTCTGGCTCGTCACTGCACCGGAGGC GGAGGAGGATCC gcccagaatctcgatcgattacggactgtccggacgtcgtcacagccgagaagatgg</p>
his-3::split-wrmScarlet ₁₁ (x3)	C	<p>gatttaaatattgtggccctaaagaggccgtgggtcgtaaatgtttaagaaggcatcta TCCTCCGGTGCAGTGACGGCGACGGACTTCTGTATTGCTGACGACGGTGTA TCCTCCACTACCGCC TCCTCCGGTGCAGTGACGGCGACGGACTTCTGTATTGCTGACGACGGTGTA ACCTCCAGAGGCCACC TCCTCCGGTGCAGTGACGGCGACGGACTTCTGTATTGCTGACGACGGTGTA GGATCCTCCTCC ttc<u>T</u>tgtctccctccgt<u>C</u>tctt<u>G</u>ggaaaagaacagctggatatttggagaagaactcccttgg</p>

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

Primer Name	Sequence of DNA oligo
his-3_F	CCAAGGAGGAGTTCTTCAAATATC
his-3_R	GATTTAAATATTGTGGCCCTAAAG
vha-13_F	GGTTTATTTGATTTCTTTGATTTC
vvha-13_R	CCATCTTCTCGGCTGTGAC

S4D. Sequences of crRNA and HDR template used to generate split-wrmScarlet₁₋₁₀ 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	UACUUUCUUCUG GAAACGACA	AAGUUCGCUGG ACUUGGAGG	tagaaggttcttaggataattttcgactttattctcttaccgtccgcacttt cttacttttaaattaaatttttttcgatgtggaaaacacttgcactccgt agcagccATGGTATCGAAGGGAGAACGAGTAATCAAGGA GTTCATGCGTTCAAGGTCCACATGGAGGGATCCATG AACGGACACGAGTTCGAGATCGAGGGAGAGGGAGAG GGACGTCCATACGAGGGAACCCAAACCGCCAAGCTC AAGGTCACCAAGgtaaatccatataactaactaaccctgat tatttaatttcagGGAGGACCACTCCCATTCTCCTGGAC ATCCTCTCCCCACAATTATGTACGGATCCCGTGCCTT CATCAAGCACCCAGCCGACATCCAGACTACTACAAG CAATCCTCCAGAGGGATTCAAGTGGAGCGTGTCA TGAACCTCGAGGACGGAGGAGCCGTACCGTCACCC AAGACACCTCCCTCGAGGACGGAACCTCATCTACAA GgtaaatccatactaactaactaaccatacatattttcagG TCAAGCTCGTGGAACCAAACCTCCCACCAAGACGGAC CAGTCATGAAAAGAACCATGGGATGGGAGGGCTC CACCGAGCGTCTCACCCAGAGGGACGGAGTCCTCAA GgtaaatccatactaactaactaactaactatgtttttcagGG AGACATCACCATGGCCCTCGTCTCAAGGACGGAGG ACGTTACCTCGCCGACACCTCCACCAACCTACAAGGCC AAGAAGCCAGTCAAATGCCAGGAGCCTACCTCGTCA ACCGTAAGCTCGACATCACCTCCCACAAACGAGTACTA

			Ataagtccaattactcttcaacatccctacatgctttccctgtgtccca ccccctattttgttattatcaaaaaacttcctaattcttggttttagcttta taagtc
COP1795 -> CF4587	GACCAGCUGGG CGCAUAGGG	GCCGCCACG AGGGCCAGG	aactatttcaattcaactgaaagattttcattagagaatgtctagaacta ggcccggtctacgtaatacgactcacttaaggcctaattgggtctggctg catccaggaggtGAACCTTGGTCTTTATTGTCAACTTC CATTGGTTCTTCATTGTTCTGTTAAATTAATGAATT CATAAAATAAAGACATTATAACAATATAAAAAATGAAGAATT ATTGAAAATAAACTGCCAGAGAGAGAAAAAGTATGCAACA CTCCCAGCCAGAGTGTTGAAATGGTGTACGGTACATT TTCGTGCTAGGAGTTAGATGTGCAGGCAGCAACGAGA GGGGGAGAGATTTGGGCCTTGAAATTAAACGTG AGTTTCTGGTCACTGACTAATCATGTTGGTTTTGTT GGTTTATTTGTTTATCTTGTGTTATCCAGATTAGGA AATTAAATTTATGAATTATAATGAGGTCAAACATTCA TCCCAGCGTTTCTGTTCTCACTGTTAGTCA TTATTAGGCTTCAACAAATGTTCAACTGTCTTATT GTGACCTCACTTTATATTAAATTAAATTAAATTAA GAAGTTCTAGGATAATTTCGACTTTATTCTCTCTA CCGTCGCACTCTTCACTTTAAATTAAATTGTTT TTTCAGTTGGAAACACTTGCTCaaaaatgtctaaggaga agaggattactggagtgtgccatcctcgtcagctcgcacggagacgt caacggacacaagtccctccgtggagagggagagggagacgccac catcg
WBM1126 -> CF4610	GCUACCAUAGG CACCACGAG		cactttaccgtctaatttcaggcgaggcgcataaaccacgaccac tagatccatATGGTATCGAAGGGAGAACAGCTAATCAAGG AGTCATGCGTTCAAGGTCCACATGGAGGGATCCAT GAACGGACACGAGTCGAGATCGAGGGAGAGGGAGA GGGACGTCCATACGAGGGAAACCCAAACCGCCAAGCT CAAGGTCAACAAAGgtaaatccatataactaactaaccctg attttaaatttcagGGAGGACCACTCCCATTCTCCTGGGA CATCCTCTCCCCACAATTCTACATGTCGGATCCCGTGCCT TCATCAAGCACCCAGCCACATCCCAGACTACTACAA GCAATCCTCCCAGAGGGATTCAAGTGGAGCGTGTG ATGAACCTCGAGGACGGAGGGAGCCGTACCGTCACC CAAGACACCTCCCTCGAGGACGGAAACCTCATCTACA AGgtaaatccatactaactaactaactaactaactaaccatattttcag GTCAAGCTCGTGGAAACCAACTTCCCACCGACGGA CCAGTCATGCAAAAGAACGACATGGATGGGAGGGCCT CCACCGAGCGCTCTACCCAGAGGGACGGAGTCCTCA AGgtaaatccatactaactaactaactaactaacttgcattttcagG GAGACATCACCATGGCCCTCCGTCCTCAAGGACGGAG GACGTTACCTCGCCGACACCTCCACCCACCTACAAGG CCAAGAAGCCAGTCAAATGCCAGGAGCCTACCTCGT CGACCGTAAGCTCGACATCACCTCCCACAACGAGTAC TAActctcgcccgccgtcctgtactctaatttcaattacttcaacat ccctacatgt

S4E. Primers long and short

Primers tomplify split-wrmScarlet for CF4582	Sequence
eft3p_S110(A19)_F	tagaagttctaggataattttcgactttattctctaccgtccgcactttc ttactttaaatccatgttttttcagttggaaacacttgctcactccgtag cagccATGGTATCGAAGGGAGAACG
unc54_S110(A19)_R	tgactttaaaagaagctaaaaacaaagaaattaagagaagttttgataat aacaaaaataggggtggagcacagggagaaagagcatgttagggatg tgaagagaattggacTTATTAGTACTCGTTGTGGGAGG

S1-10_A19_F	ATGGTATCGAAGGGAGAACG
S1-10_A19_R	TTAGTACTCGTTGTGGGAGGTG

Primers to amplify Peft-3 for CF4587	Sequence
E7_eft-3p_F-Long	aactcatttcaattcaactgaaagattttcattagagaatgtctagaacta ggcccccgggtacgttaatacgactcacttaaggcctaattgggtctggctg catgccaggaggtGCACCTTGGTCTTTATTGTCAAC
E8_eft-3p_R-Long	cgttgtggcgtccctccctccacggacggagaacttgtccgtt gacgtctccgtcgagctcgacgaggatcggcacaactccagtaataact cttcccttagacatttGAGCAAAGTGTTCACCACTG
E5_eft-3p_F	GCACCTTGGTCTTTATTGTCAAC
E6_eft-3p_R	GAGCAAAGTGTTCACCACTG

Primers to amplify split-wrmScarlet for CF4610	Sequence
PrimerS1-10_myo3F	cactttaccgtctaatttcagggcagggagccatcaaaccacgaccac tagatccatATGGTATCGAAGGGAGAACG
PrimerS1-10_myo3R	agcatgtaggatgtgaagagtaattggacttagaagtgcagaggcacgg gcgcgagatgTTAGTACTCGTTGTGGGAGGTG

Table S5. Plasmid sequences

Name	Sequence
Peft-3::3NLS::mTagBFP2::split-wrmScarlet ₁ ::T2A::mNeonGreen::split-wrmScarlet ₁₀ ::fib-1 UTR (C. elegans)	gcaccttggctttattgtcaactccattggtttccattgtttctgttaatt aatgaattttcataaaaataaagacattatacaatataaaaaatgaagaatttt gaaaataaaactgcacagagagaaaaagtatgcacactccccccggagat gttggaaatgggtacggcatatttcgtctgtagggatgtgcaggcag caacgagagggggagagatttttggccctgtgaaattaacgtgagtttc tggtcatctgactaatcatgttggttttgcgttattttatcttgcgtttta tccagatttagaaatttaatttatgaatttataatgaggtaaacattcagtc ccagcgtttctgtctactgttagtgcattttattttaggtttcaacaa atgttctcaactgtctattttgtgaccctacttttatatttttaatttttaatt agaagtttctaggataatttttcgacttttattctctaccgtccgcactctt tacttttaattaaatttttttcgatgtggaaaacttgcctATGCCAA AAAAGAACGTAAGGTTGATCCGAAGAAAAAGCGAAAA GTTGATCCTAAAAAAAGCGTAAAGTCGTCTCCAAGGG AGAGGAGCTCATCAAGGAGAACATGCACATGAAGCTC TACATGGAGGGAAACCGTCGACAACCACCACTTCAGT GCACCTCCGAGGGAGAGGGAAAGCCATACGAGGGAA CCCCAAACCATCGTATCAAGGTGTCGAGGGAGGGACC ACTCCCATTGCCCTCGACATCCTGCCACCTCCTTC CTCTACGGCTCCAAGACCTTCATCAACCACACCCAAAG GAATCCCAGACTTCTCAAGCAATCCTTCCAGAGGGGA TTCACCTGGGAGCGTGTGACCACCTACGAGGGACGGA GGAGTCCTCACGCCACCCAAGACACCTCCCTCCAA GACGGATGCCATCTACAAACGTCAAAGgttaagttaacat atatactaactaaccctgattattaaatttcagatCCGTGGAGTC CTTCACCTCCAACGGGACCAGTCATGCAAAGAAGACC CTCGGATGGGAGGCCTTCACCGAGAACCCCTACCCA GCCGACGGAGGGACTGGAGGGACGTAACGACATGGCC

CTCAGCTCGCAGGGTTCCCACCTCATGCCAACG
 CCAAGACCACCTACCGTCCAAGAAGCCAGCCAAGAA
 CCTCAAGATGCCAGGAGTCACTACGTCGACTACCGT
 CTGGAGCGTATCAAGGAGGCCAACAACGAGACCTACG
 TCGAGCAACACGAGGTGCGCGTCGCCCCGTTACTGCG
 ACCTCCCATCAAGCTCGGACACAAGCTTAACGGCGG
 AGGGTCTTACCGTTGAGCAATATGAAAAGTCAGT
 GGCGAGGCAGTGTACAGGGGGAGGATCCGGCGAGG
 GACGTGGCTCCCTCACCTGCGGAGACGTCGAGG
 AGAACCCAGGACCAGTCTCAAGGGAGAGGGAGGACA
 ACATGGCCTCCCTCCCAGCCACCACGAGCTCCACAT
 CTTGGGTCACCGAGTCGACTCGACATGGTC
 GGACAAGGAACCGGAAACCAAACGACGGATACGAG
 GAGCTCAACCTCAAGTCCACCAAGGtaagttaaacagtcg
 gtactaactaaccatacatattaaatttcagGGAGACCTCAATT
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 CCACCAATACCTCCCATAACCCAGACGGAATGTCCCCAT
 TCCAAGCCGCATGGTCGACGGCTCCGGATACCAAGT
 CCACCGTACCATGCAATTGAGGGACGGAGCCTCCCTC
 ACCGTCAACTACCGTTACACCTACGAGGGTTCCCACAT
 CAAGGGAGAGGCCAACGTCAAGGGAACCGGAACTCCC
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 TCAAGAACCAACCAATGTACGTCTCCGTAAGACCGAG
 CTCAGCACTCCAAGACCGAGCTCAACTCAAGGAGT
 GGCAAAAGGCCTTACCGACGTCATGGGAATGGACGA
 GCTCTACAAGACTAGTATGGCTCTAAGGGTGAAGGCCG
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 GTTCATGAACCGGACATGAGTTGAGATAGAAGGAGA
 GGGGGAGGGGAGACCTATGAGGGGACCCAGACGGC
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 TATAAAGTAAAATTGAGGGGACGAACCTTCCCCCGA
 CGGTCCAGTAATGCAAAAAAAACATGGGCTGGGAA
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 cctgcccgtatattctacttaattgtaaaccttatttgttcttca
 attattacagggt

Peft-
 3::3NLS::mTagBFP2::sfCherry3₁₁::T2A::mNeonGreen::sf
 Cherry31-10::fib-1 UTR (C. elegans)

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 GCACCTCCGGGGAGAGGGAAAGCCATACGAGGGAA
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 CTGGAGCGTATCAAGGAGGCCAACACGAGACCTACG

Pmyo-3::mTagBFP2::split-wrmScarlet₁₁::T2A::mNeonGreen::split-wrmScarlet₁₋₁₀::fib-1 UTR (C. elegans)

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Pmyo-
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31-10::fib-1 UTR (C. elegans)

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GCTCCAAGACCTCATCAACCACACCCAAAGGAATCCC
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pRSET-split-wrmScarlet₁₋₁₀

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Table S6. Adult lifespans of strains in this study

Strain	Events / n initial	Mean lifespan +/- SEM (Days)	Median lifespan	% mean lifespan change vs. N2	P-value (log-rank) vs. N2
N2E	105 / 134	20.20 +/- 0.74	17		
CF4582	118 / 136	19.27 +/- 0.57	17	-4.60	0.19
CF4587	109 / 129	19.56 +/- 0.64	17	-3.17	0.29
WBM1126	112 / 127	19.31 +/- 0.61	17	-4.41	0.28
CF4610	108 / 128	20.18 +/- 0.69	17	-0.10	0.92

Strain	Events / n initial	Mean lifespan +/- SEM (Days)	Median lifespan	% mean lifespan change vs. WBM1126	P-value (log-rank) vs. N2
WBM1126	112 / 127	19.31 +/- 0.61	17		
CF4610	108 / 128	20.18 +/- 0.69	17	4.51	0.33

Table S7. Mammalian cell screen oligo pool sequences

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