

Split Fluorescent Proteins for *C. elegans*

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This project is maintained by Maria Ingaramo in the York lab, and was 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.

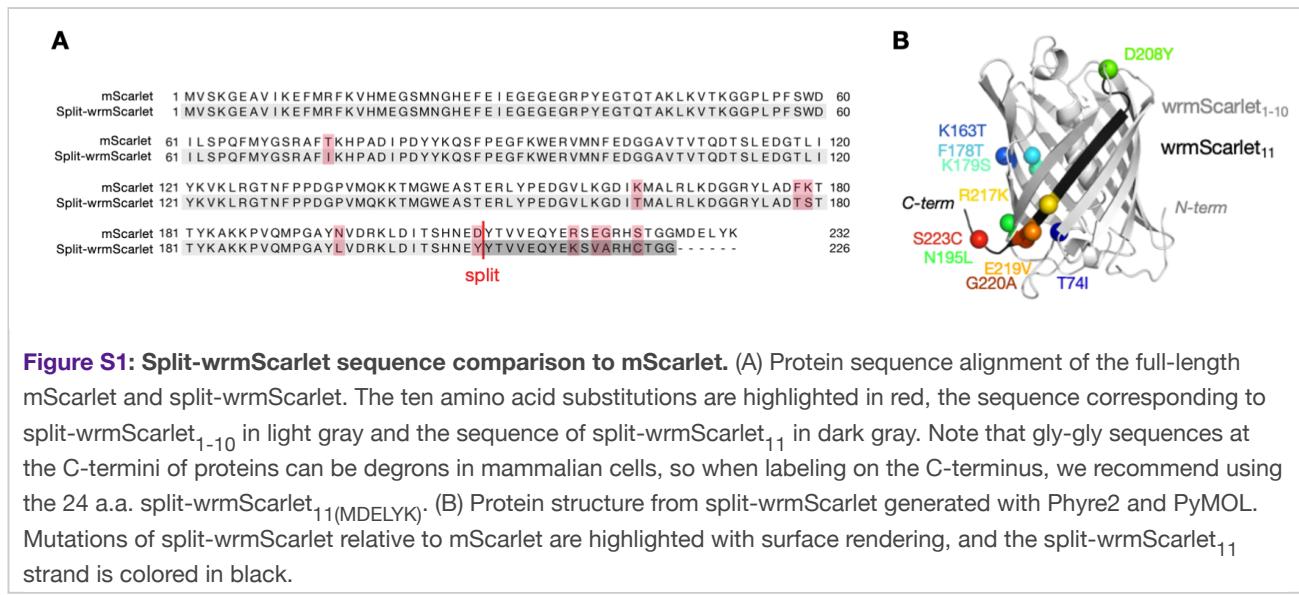
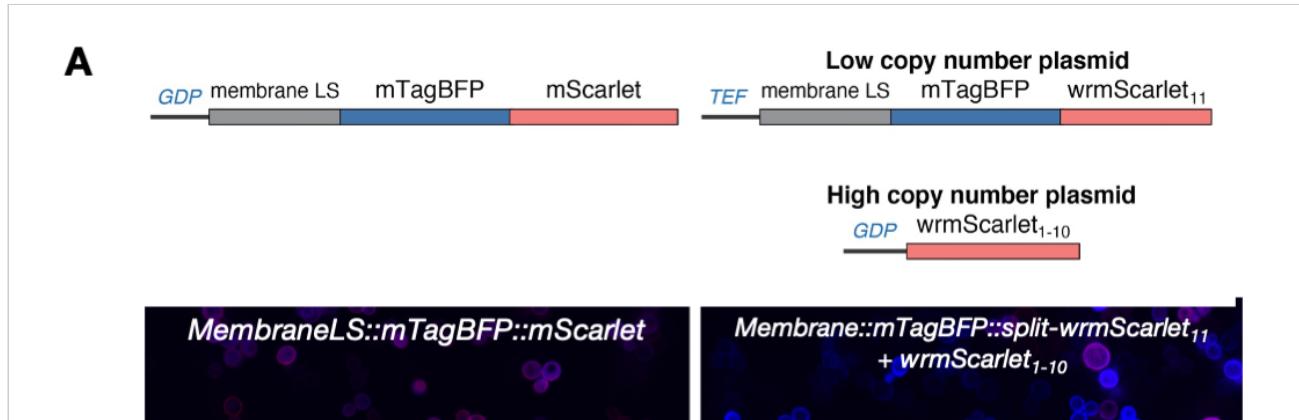
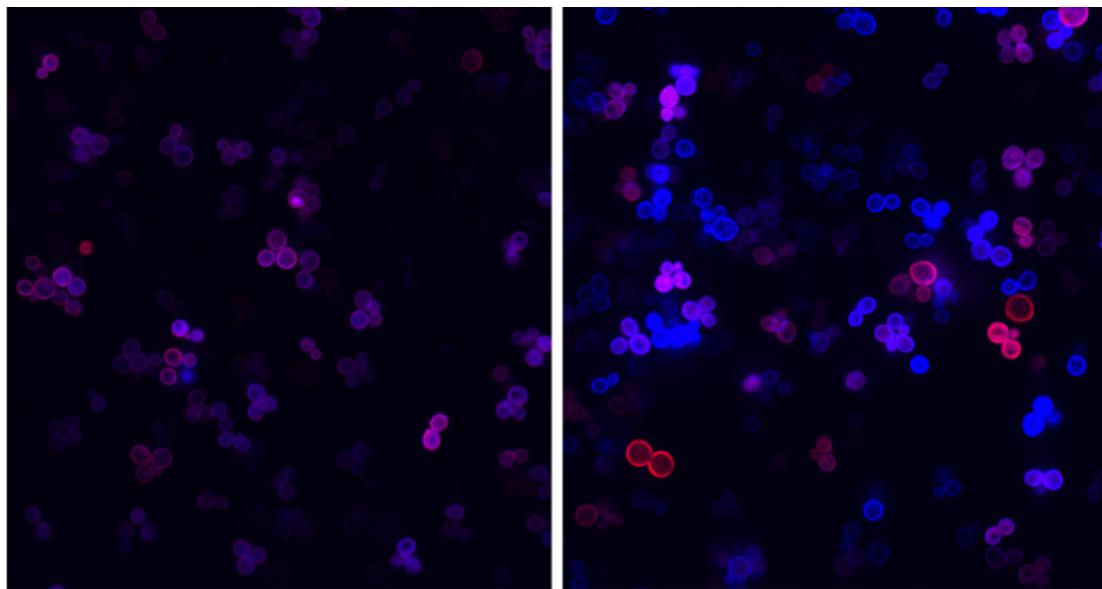


Figure S2. Split-wrmScarlet brightness in *S. cerevisiae*.





B

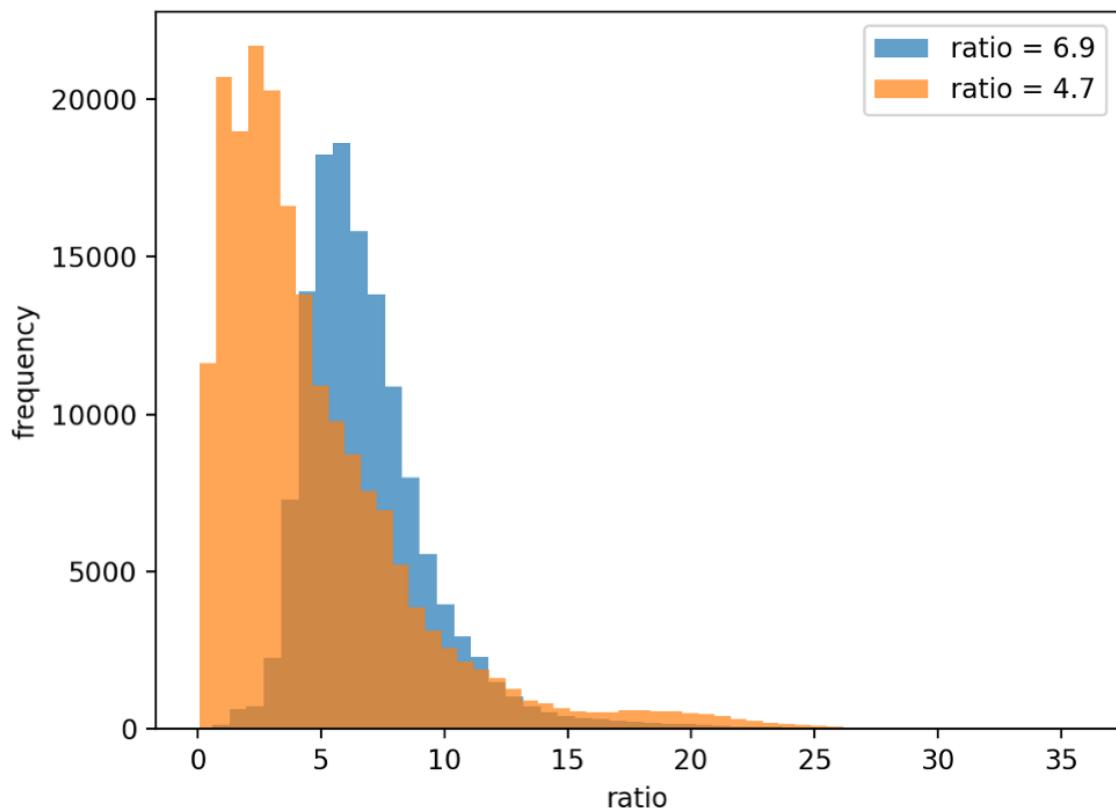


Figure S2: Split-wrmScarlet brightness in *S. cerevisiae*. (A) Composite display of red and blue channels for membrane-localized mTagBFP-mScarlet (wild-type) fusion or split-wrmScarlet₁₋₁₀ plus membrane localized mTagBFP-split-wrmScarlet₁₁ in yeast. Images were acquired and are displayed under identical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

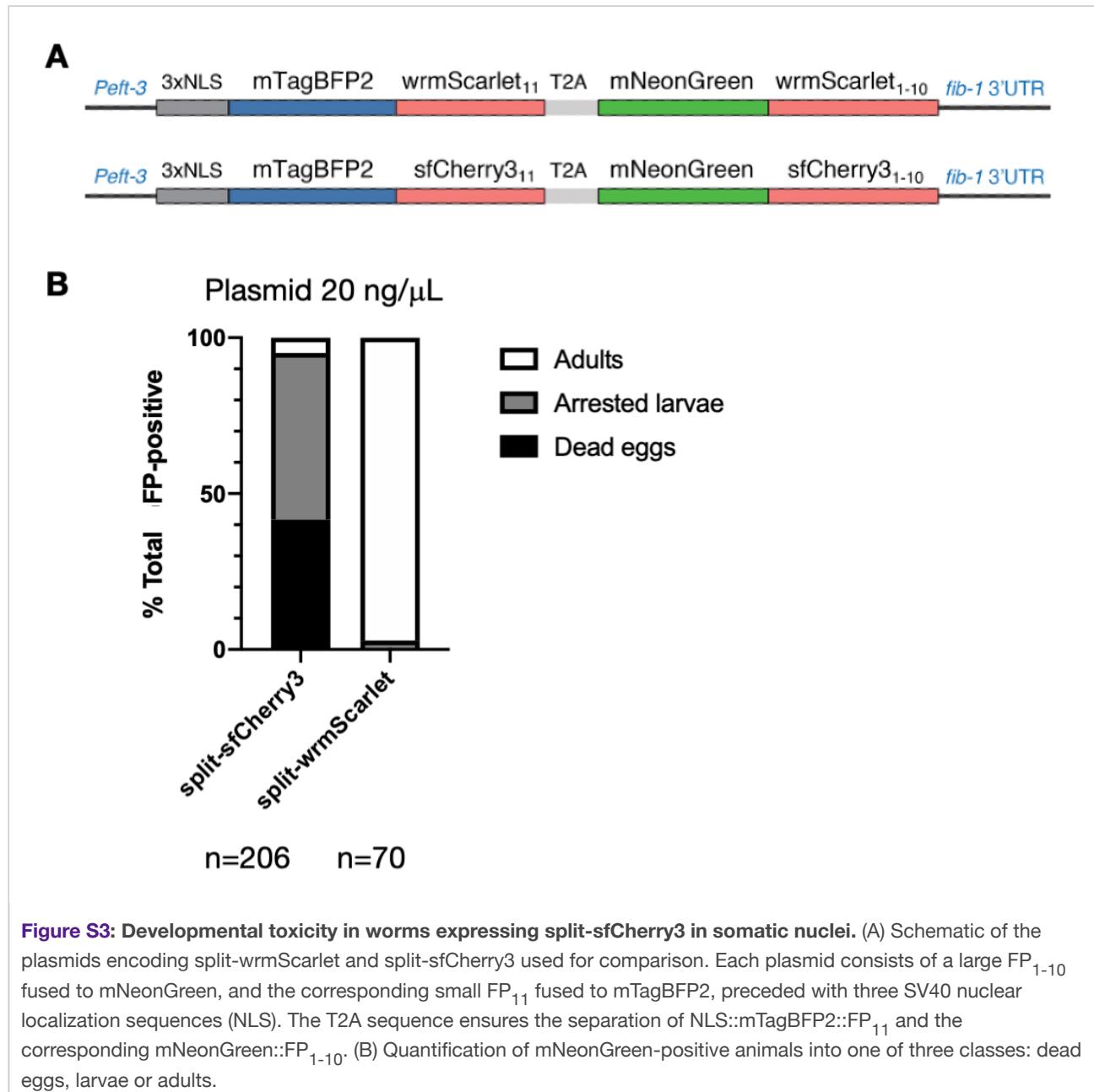


Figure S4. Split-wrmScarlet and split-sfCherry3 comparison.

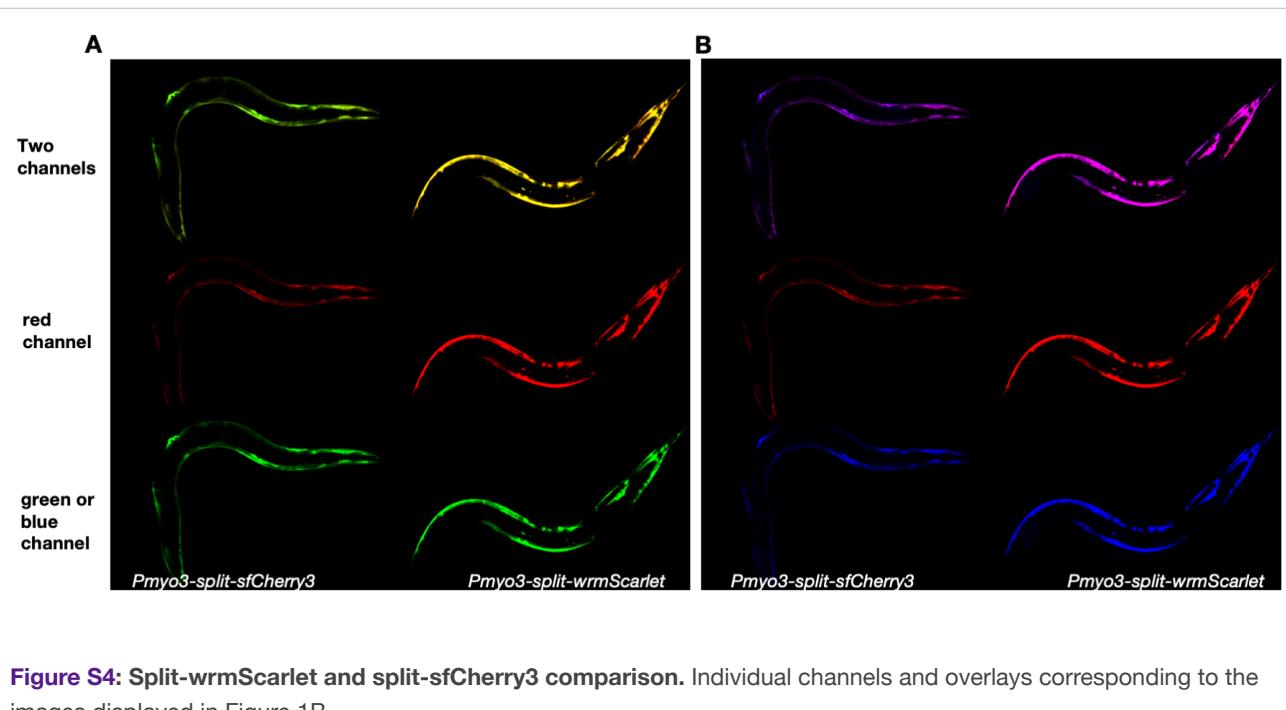


Figure S4: Split-wrmScarlet and split-sfCherry3 comparison. Individual channels and overlays corresponding to the images displayed in Figure 1B.

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

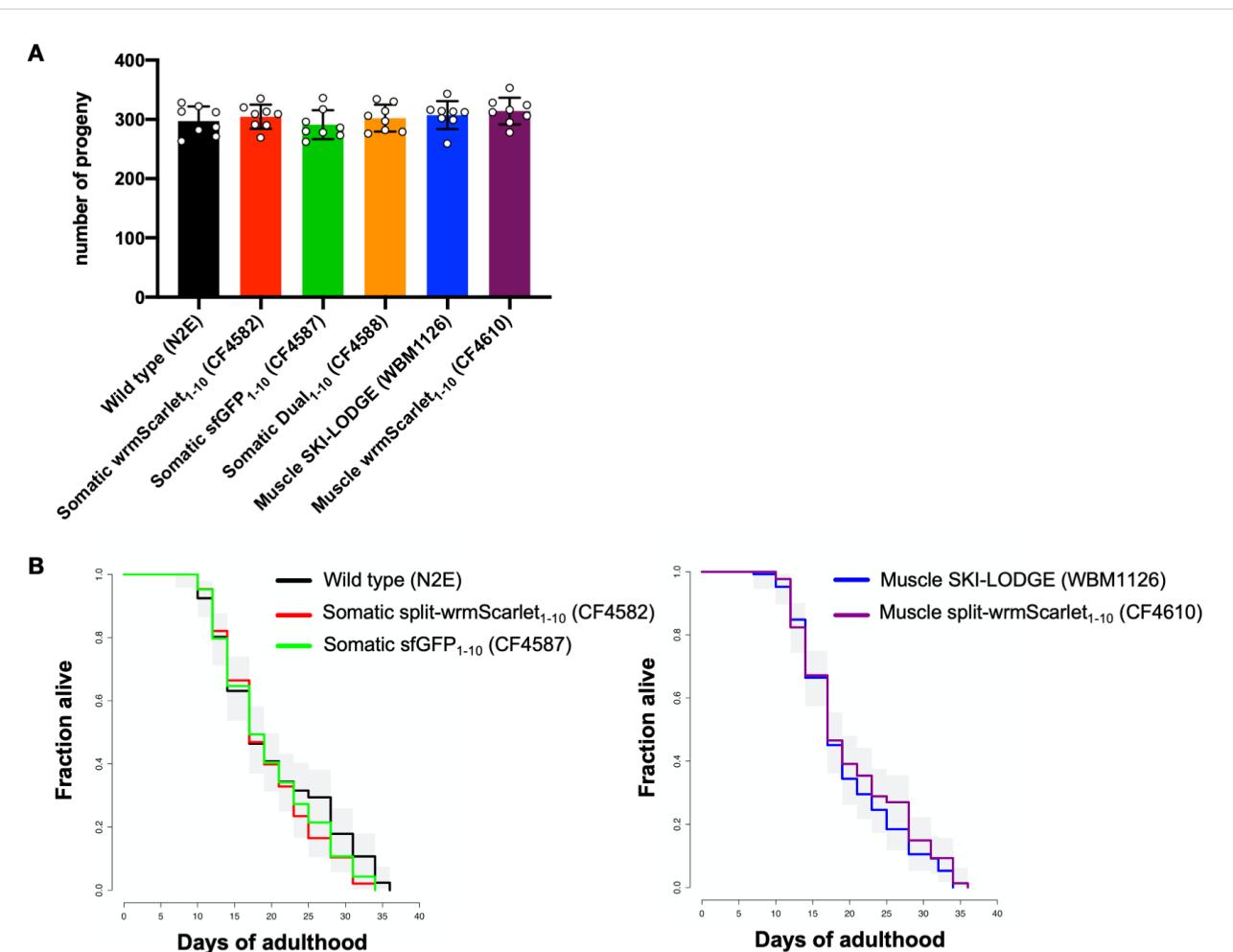


Figure S5: Brood size and lifespan of split-wrmScarlet₁₋₁₀ and sfGFP₁₋₁₀ lines. Split-wrmScarlet₁₋₁₀ and split-sfGFP₁₋₁₀ lines produced wild-type numbers of progeny (A) and a wild-type lifespan (B). Genotypes: N2E (wild-type), CF4582 (muls252[Peft-3::split-wrmScarlet₁₋₁₀::unc-54 3'UTR, Cbr-unc-119(+)]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

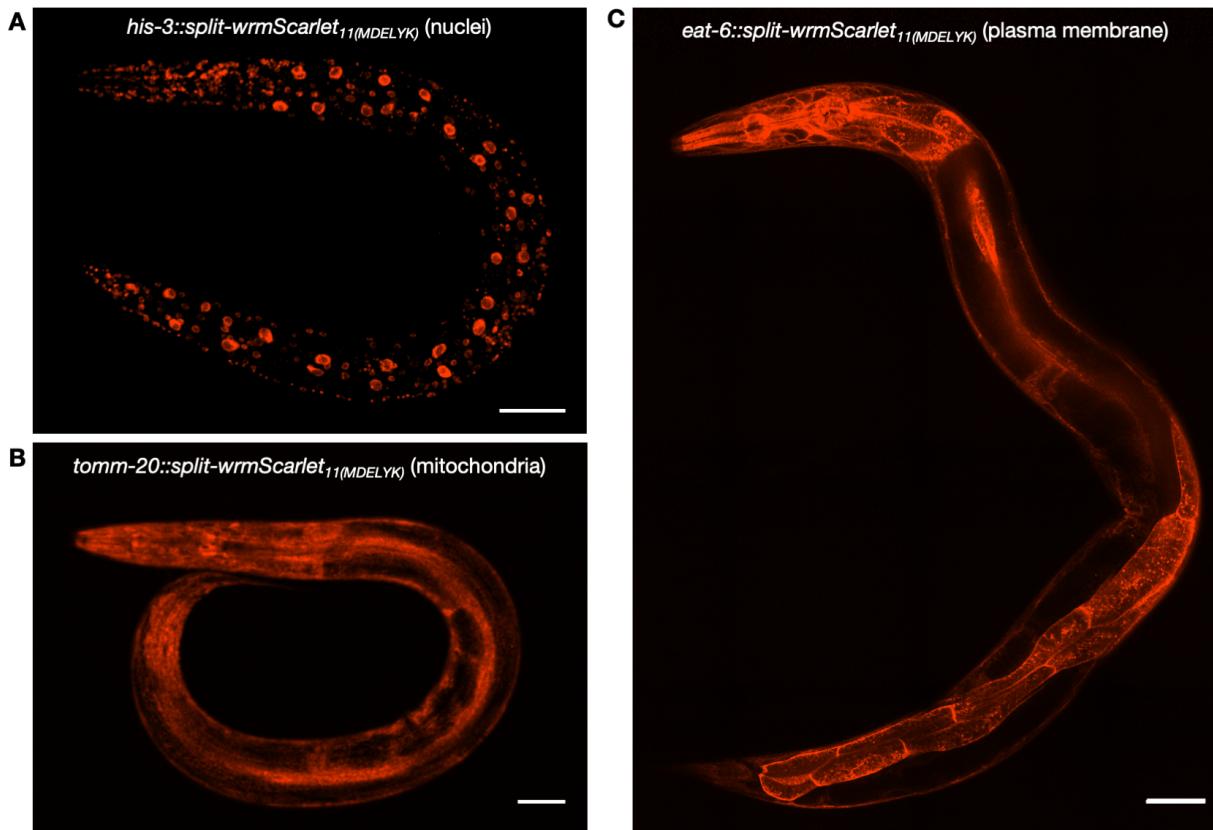


Figure S6: Proteins tagged at their C-terminus with the 24 a.a. split-wrmScarlet_{11(MDELYK)}. Endogenous proteins tagged with split-wrmScarlet_{11(MDELYK)} in animals expressing split-wrmScarlet₁₋₁₀ in somatic tissues. (A-C) Confocal images of worms expressing somatic split-wrmScarlet₁₋₁₀ and (A) HIS-3::split-wrmScarlet_{11(MDELYK)} (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.

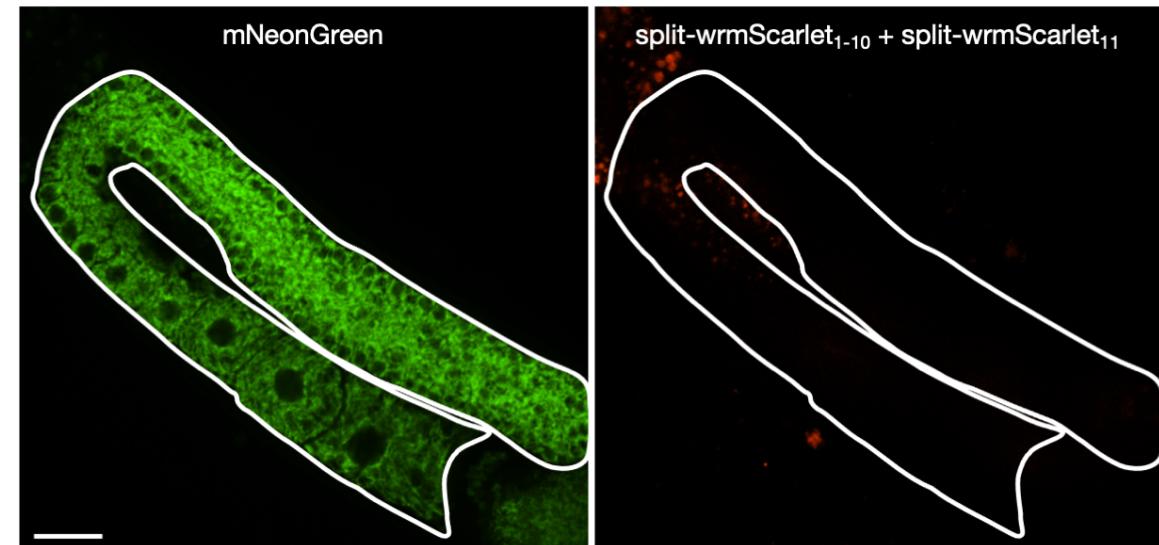
A**B**

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.

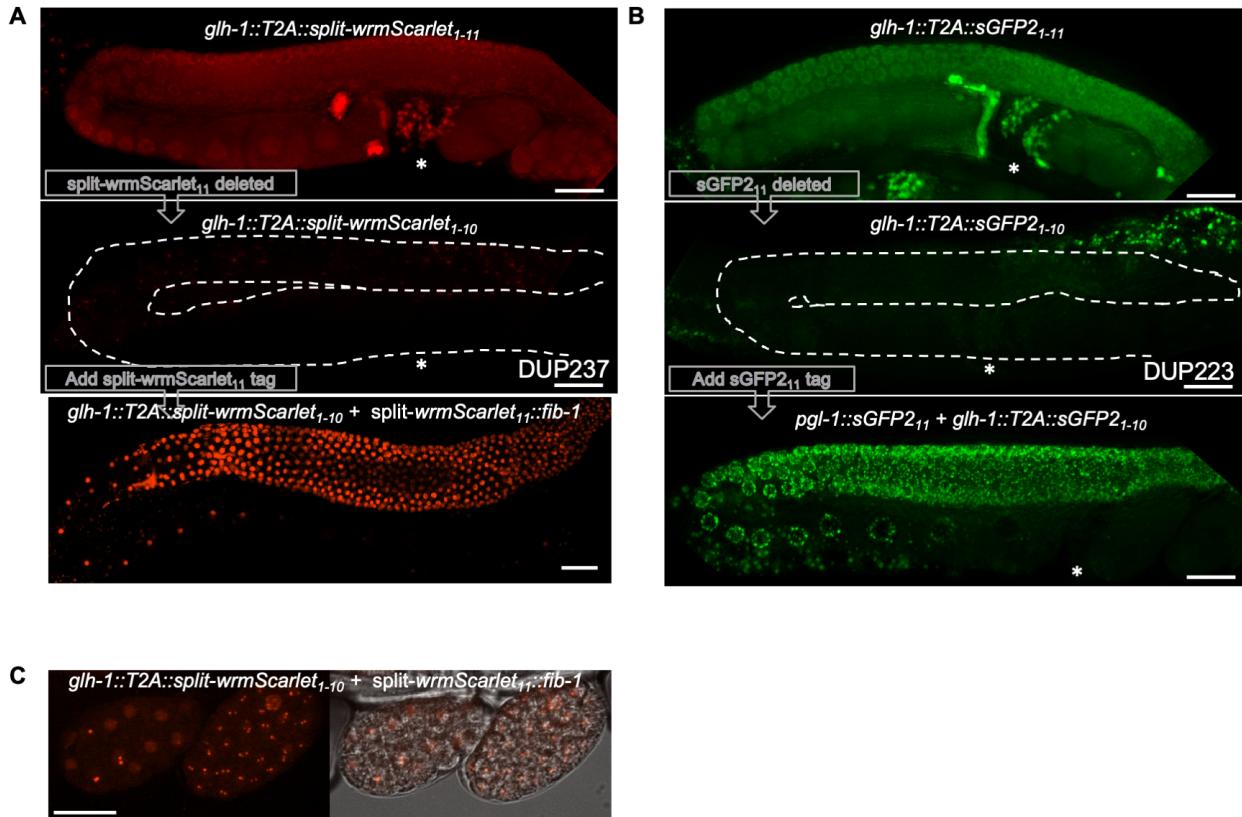


Figure S8: Generation and validation of germline-specific split-wrmScarlet₁₋₁₀ and sGFP2₁₋₁₀ strains. In order to generate germline-specific split-fluorescent strains, we first tagged the C-terminus of *glh-1* with T2A::split-wrmScarlet₁₋₁₁ (A) or T2A::sGFP2₁₋₁₁ (B). The T2A separates GLH-1 post-translationally 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.

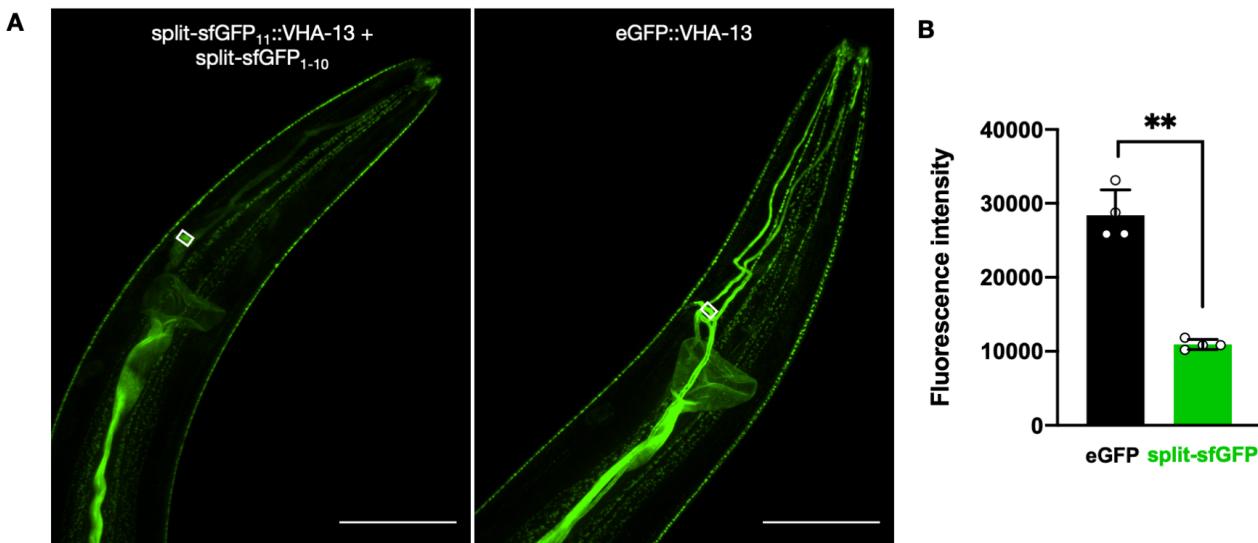


Figure S9: Somatic sfGFP₁₁ compared to full-length eGFP at the endogenous vha-13 locus. (A) Representative images of animals expressing sfGFP₁₋₁₀ in somatic tissues with endogenous VHA-13 tagged with sfGFP₁₁ (left panel), or endogenous VHA-13 tagged with eGFP in a wild-type background (right panel).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

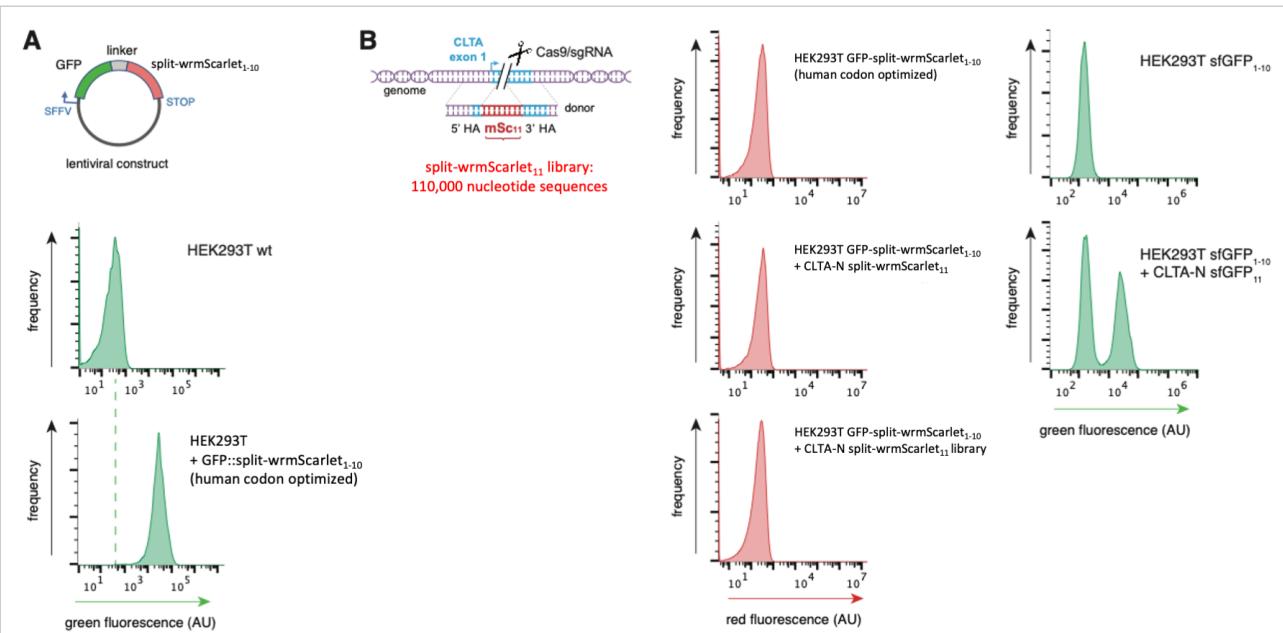


Figure S10: Screen for split-wrmScarlet fluorescence in mammalian cells. (A) FACS histograms of human codon-optimized split-wrmScarlet₁₋₁₀ expressed as a C-terminal GFP fusion. GFP expression verifies successful expression of the fusion protein in HEK293T cells by lentiviral transduction. (B) Schematic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

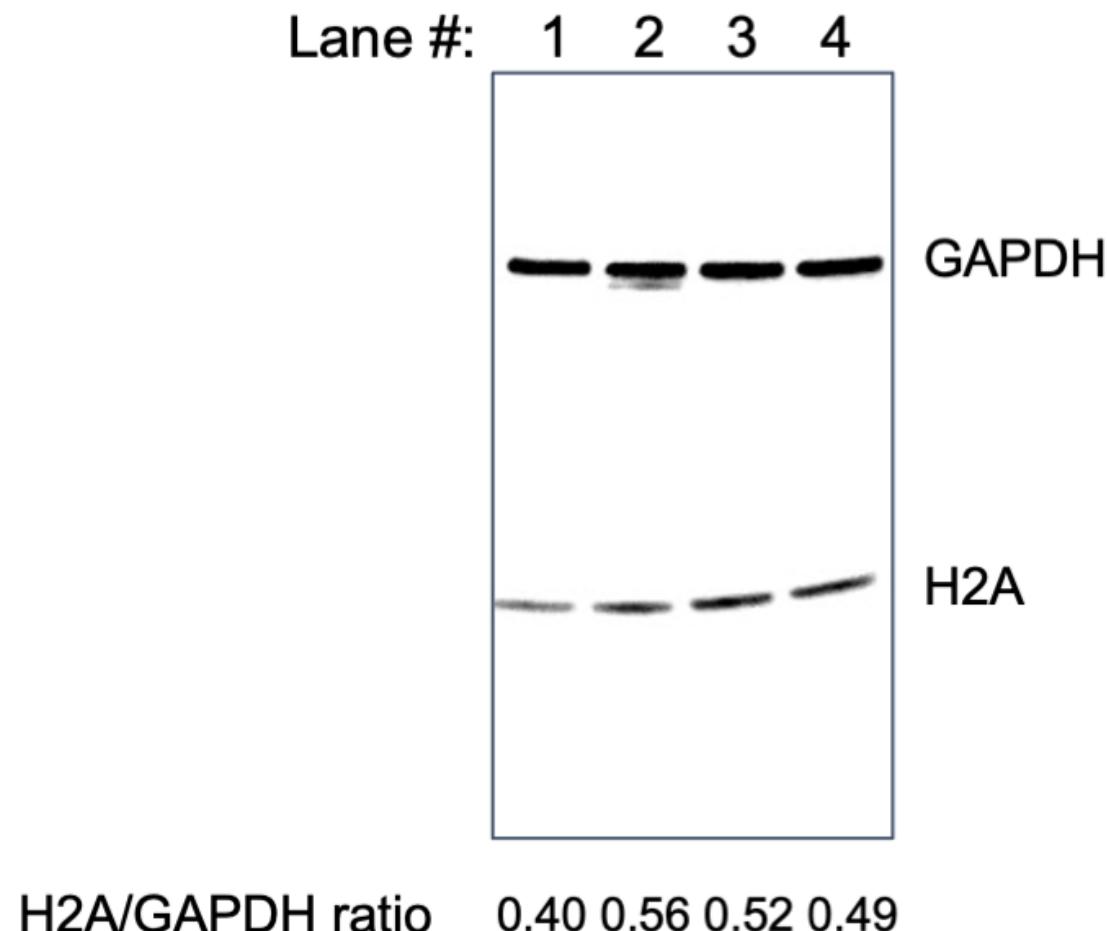


Figure S11: Split-wrmScarlet₁₁ C-terminal amino acids did not affect H2A abundance. Western-blot of histone H2A (his-3) with split-wrmScarlet₁₁+/- MDELYK. Western blot targeting HIS-3 in wild-type animals (lane 1), somatic split-wrmScarlet₁₋₁₀ expressing animals (lane 2), somatic split-wrmScarlet₁₋₁₀ 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.

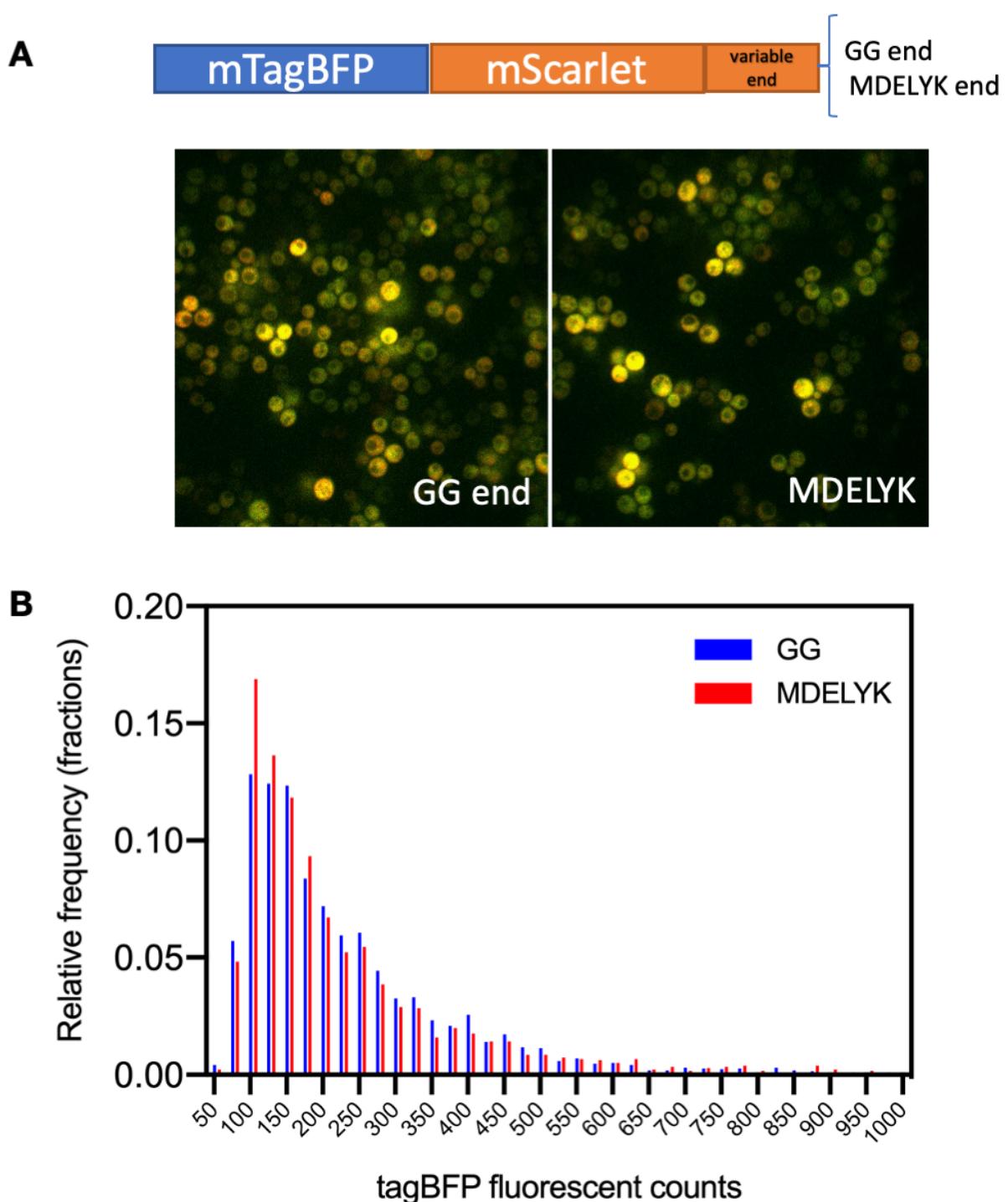


Figure S12: mScarlet ending with GG or MDELYK yields similar protein abundance in yeast. (A) Representative images of yeast expressing mTagBFP::mScarlet fusion truncated to end with gly-gly (GG end) or MDELYK from a p416-GPD promoter plasmid. mTagBFP fluorescence is pseudocolored in green, and mScarlet 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	ATGGTATCGAAGGGAGAACAGTAATCAAGGAGTTCAT GCCTTCAGGTCCACATGGAGGGATCCATGAACGGAA CACGAGTTGAGATCGAGGGAGAGGGAGAGGGACGT CCATACGAGGAACCCAAACCGCCAAGCTCAAGGTCA CCAAAGgttaagttaaacatataactaaactaccctgatttttttt cagGGAGGCCACTCCCATTCTCTGGGACATCCTCTC CCCAAATTATGTACGGATCCCGTGCCTCATCAAGC ACCCAGCCGACATCCCAGACTACTACAAGCAATCCTC CCAGAGGGATTCAAGTGGGAGCGTGTATGAACCTCG AGGACGGAGGAGCCGTACCGTACCCAAAGACACCT CCCTCGAGGACGGAACCCCTCATCTACAAGGtaagttaaac agtcggtaactaactaacatataatttttttttttttttttttt GTGGAAACCAACTTCCCACAGACGGACCAGTATGCA AAAGAAGACCATGGGATGGGAGGGCTCCACCGAGCG TCTCTACCCAGAGGACGGAGTCCTCAAGGtaagttaaca tgattttactaactaactaatctgatttttttttttttttttttt tagGGAGACATCACC ATGGCCCTCCGTCTCAAGGACGGAGGACGTTACCTCG CCGACACCTCCACCCACCTACAAGGCCAAGAACGCCAG TCCAAATGCCAGGAGCCTACCTCGTGCACCGTAAGCT CGACATCACCTCCCACAACGAGTAC TACACCGTCGTCGAGCAATACGAGAACGTTCCGTCGCC GTCACTGCAACGGAGGA TACACCGTCGTCGAGCAATACGAGAACGTTCCGTCGCC GTCACTGCAACGGAGGAATGGATGAGTTATAACAG ATGCTCAAGGGAGAACGAGTTATTACTGGAGTTGTGCC GATCCTCGTCGAGCTCGACGGAGACGTCAACGGACAA CAAGTTCTCCCGTGGAGAGGGAGAGGGAGACGCC CACCATCGGAAAGCTCACCCTCAAGTTCATGTCACC ACCGGAAAGCTCCAGTCCCATGGCAACCCCTCGTC ACCACCTCACCTACGGAGTCCAATGCTCTCCGTTA CCAGACCATGAGCGTCACGACTTCTCAAGTCC GCCATGCCAGAGGGATACTGCTCAAGGAGCGTACCATCT CCTTCAGGACGGACGGAAAGTACAAGGtaagttaacatata atataactaactaacctgatttttttttttttttttttttttt TCAAGTTGAGGGAGACACCCTCGTCAACCGTATCGA GCTCAAGGGAAACCGACTTCAAGGAGGACGGAAACAT CCTCGGACACAAGCTCGAGTACAACCTCAACTCCAC AACGTCTACATCACC CGCGACAAGCAAAGAACCGGAA TCAAGGCCAACTTCAACCGTCCGTACAACGTCGAGGA CGGATCCGTCAACTCGCCGACCAACTACCAACAAAC ACCCCAATCGAGACGGACCGACTTCCCTCCAGAC AACCAACTACCTCTCCACCCAAACCGTCTCTCCAAGG ACCCAAACGAGAAG CGTGACCACATGGTCCTCATGAGTATGTAATGCTGC TGGGATTACA ATGGTTCCAAGGGAGAGGGCTGTTATCAAGGAATTAT GCCTTCAGGTTCACATGGAAAGGATCTATGAACGGAA CACGAATTCTGAAATCGAAGGAGAACGGAGAACGGACGCC CATACGAGGGAAACTCAAACGCTAAGCTTAAGGTTACT AAAGGAGGACCACTTCAATTCTCTGGGATATCCTTCT CCACAGTTCATGTACGGATCTCGCGCTTCATCAAGCA CCCAGCTGATATCCCAGATTACTACAAGCAGTCTTCC CAGAAGGATTCAAATGGGAGCGCGTTATGAACCTCGAA GATGGAGGAGCTGTTACCGTACCCAAAGATACTCTCC TGAGGATGGAACCTTATCTACAAGGtaagttaacatata actaactaactaacctgatttttttttttttttttttttttt CTAATTTCCCACCAAGATGGACCAGTTATGCAGAAGAAG ACTATGGGATGGGAAGCTTACCGAGCGCCTTACCC AGAGGATGGAGCTTAAGGGAGATATCACCAGTGCTC TTCGCTTAAGGATGGAGGAGCTTACCTTGCTGATACC TCTACTACTACAAGGCTAAGAACGCCAGTTCAAGATGCC AGGAGCTTACCTTGTCGATCGTAAGCTTGATATCACTTC TCATAACGAATAC ATGAGTAAAGGAGAACATTGTTACTGGAGTTGTCCC AATCTCGTCGAGCTCGACGGAGACGTCAACGGACAA CAAGTTCTCCCGTGGAGAGGGAGAGGGAGACGCC CACCATCGGAAAGCTCACCCTCAAGTTCATGTCACC ACCGGAAAGCTCCAGTCCCATGGCAACCCCTCGTC ACCACCTCACCTACGGAGTCCAATGCTTCCGCCCC ACCCAGACCATGAAGCGTCACGACTTCTCAAGTC CGCCATGCCAGAGGGATACTGCTCAAGAGCGTACCATC TCCTCAAGGtaagttaacatataactaactactgatttttttt ttagGACGACGGAAAGTACAAGAACGCCGTGCCGTGTC AGTTGAGGGAGAACCCCTCGTCAACCGTATCGAGCT
Codon-optimized split-wrmScarlet ₁₁	
Codon-optimized split-wrmScarlet _{11(MDELYK)}	
Codon-optimized sfGFP ₁₋₁₀ with 1 intron	
Codon-optimized sfGFP ₁₁	
Codon-optimized split-wrmScarlet ₁₋₁₀ with 1 intron for germline expression	
Codon-optimized sGFP2 ₁₋₁₀ with 2 intron for germline expression	

Codon-optimized sequence of split-wrmScarlet₁₋₁₀ with 3 introns, engineered to avoid piRNA recognition transgene silencing. (Undetectable fluorescence in the MosSCI strain PHX1797)

CAAGGGAAACCGACTTCAAGGAGGGACGGAAACATCCTC
GGACACAAGCTCGAGTACAACCTCAACTCCACAACG
TCTACATCACCAGCCGACAAGCAAAGAACGGAATCAA
GGCCAACCTTACCGtaagttaaacatgatttactaactaaactct
gattttaaatttcagACCGTCAACACGTCGAGGACGGATCC
GTCCAACCTCGCCGACCACCTACCAACAAAACACCCCCAA
TCGGAGACGGACCGAGTCCTCTCCAGACAAACCACTA
CCTCTCACCCAAACCGTCTCTCCAAGGACCCAAAC
GAGAAG
ATGGTATCGAAGGGAGAAGCAGTCATCAAGGAGTTCAT
GCGTTCAAGGTCCACATGGAGGGATCCATGAACCGGA
CACGAGGTCGAGATGCAGGGAGAGGGAGAGGGACG
CCATACGAGGGAAACCCAAACCGCCAAGCTCAAGGTCA
CCAAGGtaagttaaacatataactaactaacccgttattttaaatttt
cagGGAGGGACCACTCCCATTCTCTGGGACATCCTCTC
CCCACAAATTATGTACGGATCCCGTGCCCTCATCAAGC
ACCCAGCCGACATCCCAGACTACTACAAGCAATCC
CCAGAGGGATTCAAGTGGGAGCGTGTGATGAACCTCG
AGGACGGAGGGAGCGTCAACCGTCAACCAAGACACCT
CCCTCGAGGACGGAACCCCTCATACAAAGGtaagttaaac
agttcgtaactaactaacccatataattttttcagGTCAAGCTCC
GTGGAACCAACTTCCCACCCAGACGGACCGAGTCATGCA
AAAGAAGACCATGGATGGAGGCCTCCACCGAGCG
TCTTACCCAGAGGACGGAGTCCTCAAGGtaagttaaca
tgatttactaactaactaactatgtttaattttcagGGAGACATC
ATGGCCCTCCGTCACAGGACGGAGGACGTTACCTCG
CCGACACCTCCACCCACCTACAAAGGCCAAGAACGGCAG
TCCAAATGCCAGGAGGCACCTACCTCGTGCACCGTAAGCT
CGACATCACCTCCCACAACGAGTAC

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

DUP237	glh-1(sam140[glh split-wrmScarlet-1::T2A::split-wrmScarlet, 10]) I	Germline	I: 6.85 MB	I: 1.41
				TCGACAAGATTGGAGCTGCAAACAAGTGC GTCCTACAGGAATTGAGAGATGCGAAAG AAGCGAGAAGAAGGACAAACTCTAGAGC TTCTGGGAATCGATATCGACAGTTACACGA CCGAGAAAAGtgcgttttcgtttctatttgatgaaata aattcaatattcagGTGCCGAAGTTACACAAA GAAAACCATGGTCTCGTTCTCAAAGAGC AATGGCTGATACACTGGCTTCAATTGTCA TCGGCTCAAGTTCAGCTATCACGgttgtat atttcattttgaccgcgttttaattcaaaatgtacagATCCA TGGTGCCCCGTGAGCAGAGAGAGCGTTC GAAGCTTGGAGACAATTCCGAAATGGATCG AACACCTGTTCTATTGCTACTGCGGTCGCT GAACGTGGACTTGATATCAAAGGAGTGGAT CATGTCATCAACTATGACATGCCAGACAAAC ATTGATGACTATATCCATCGTACGAAAGGtc agttatattttatattcaataatgcgaactgttttcag AACTGGAAGAGTTGAAACTCTGGAAGAG CTACAAGCTTCATCTCGGAGGATTGAGTC TTCTGTCCGAACTGTTGGTCTCGCCG ACGCACAAACAGATTGTCGAACTGGATG CAAGGTGCTGCTGGAGGCAATTACGGAGC TAGTGGATTGGTCCAGTGACCAACTCA AGTCCCAGGACGAGGAGGGTGG GGATCGGGA GAGGGACGTGGATCCCTCTTACCTGCGG AGACGTGAGGAGAACCGAGGACCA GGAGCATCGGGAGCCTCAGGAGCATCG ATGAGTAAGGAGAAGAATTGTTCACTGGA GTTGTCCCAATCCTCGTCGAGCTCGACCG AGACGTCAACGGACACAAGTTCTCGTCC GTGGAGAGGGAGAGGGAGACGCCACCAT CGGAAAGCTCACCCCTCAAGTTCATCTGCA CCACCGGAAAGCTCCAGTCCATGGCC AACCCCTCGTCACCACCCCTCACCTACGGAG TCCAATGTTGCCCCGTTACCCAGACCCAC ATGAAGCGTCACGACTTCTCAAGTCCGC CATGCCAGAGGGATACGTCCAAGAGCGTA CCATCTCCTCAAGgtaaactatataact aactactgattttaaatttcagGAGCAGCAGGAAAGT ACAAGACCCGTGCGTCAAGTTGAG GGAGACACCCCTCGTCAACCGTATCGAGCT CAAGGGAAACCGACTTCAAGGAGGGAGCGGA AACATCTCGGACACAAGCTCGAGTACAA CTTCAACTCCCACACGTCACATCACCGC CGACAAGCAAAAGAACGGAATCAAGGCCA ACTTCACCgtaaactttaactgatttactaactaacta atctgattttaaatttcagACCCGTCAACACGTCGA GGACGGATCCGTCAACTCGCCGACCAACT ACCAACAAAACACCCCAATCGGAGACGGGA CCAGTCCTCCTCCAGACAACCAACTACCT CTCACCCCAACCGTCTCTCCAAGGACC CAAACGAGAAGTAG aaaaccgaccaattgtatgtttcgcatattttatgtgtc agttcccccataattttatctgccccgttattttatgttatt ttgtgtgtgtgtgtatagtctcgcgcataactct gttccgcacgcgcggccaaactacagtaacctcgacacac tcatctactaaattttggacagtcctaatttttgcgtttt caactcaattttcgaaaaaatcttaattttcgcaaaATG TCTGATGGTTGGAGTGTAGCGAAAGTGTCT GCTAAGGgttagtttattttgtatgttttttttt ttgattaaaaacttttttcagCCAAACTGGATTG GTAGTGGAGGCGGTTCGGTGGTGGTAAC AATGGAGGATCTGGTTGGTGGTAAC AATGGAGGATCTGGATTGGAGGAGGAGGAA CACTGGGGATCTGGATTGGAGGAGGAGGAA ACACTGGCGGATCTGGATTGGAGGAGGAGGAA AAGACTGGCGGTTCTGGATTGGAGGAGGAGGAA AAATACTGTGGATCCGGCTTGGAGGAGGAGGAA GCAGTACAGGAGGATCGCCGTATGGAGGAA GCCAGTCTGGATTGGAGGAGTACTGC CACATCTGGATTGGAGGATCAGGTGGCTTGG AAGTGCATTGGAGGATCAGGTGGCTTGG GAGGTAGTGCACACTGGATTGGAGGAGTGGAA GGAGGATCCTTGAGGTGGCAACTCTGG TTTGGGAAAGGAGGACATGGCGCGGA GAGAGAAACAATgttcgtttttaaattgtacttata attacgttttcagATTGTTCAATTGCAACAG CCAGGACATCGATCGAGTGAATGTC GCCGAGAAGGAAAGAGAGAGGCCGAGAGGttt tatttgatataactttattggcgtatgttttttttcagTG TGCTACAATTGCCAGCAACCCGGGCACAC

CTCTCGTGAATGTACAGAAGAACGCAAGC
CGCGTGAGGGTCGCACTGGTGATTGG
GGCGGGAGCTGGATTGGAAACAATGGAG
GAAATGACGGTTCTGGTGGGACGGTGGT
TTGGTGGAGGCGAAGAACGTGGTCCAAT
GAAATGTTCAACTGTAAAGGCAGGGGACA
TCGCTCTGCTGAATGTCCGGAGGCCACCCC
GTGGATGTTCAATTGTGGCAGCAAGGT
CATCGCTCGAATGAGTGCCCCAATCCAGC
CAAGCCAAGGGAAAGGTGTTGAAGGAGAA
GGACCTAAGGCAGACATACGTGCACTGCA
AGACAAACATGGAGGACGTTCAACATGCA
GAAAATTCCGAGGGCTTATGTTCAACAA
GTTTTCGATGCCAGTAAACTGACTTC
ATCCGAGAAAGACTGTGGTATCAAACCTTG
CAAGACATTGCCAGAAGCTAATCTCACGG
AGACCATGCAAGAAAAACGTTGCTCATGCT
GGATACTCCAAGACCACCTCAATTGAGCAA
TATGCTCTTCACTTGTTCATCAGGGATATG
ATATCATGGCTTGTGCTCAAACCTGGATCAG
GAAAACCGCTGCATTCTCTGCCTATCA
TGACTCGTCTCATGACGATAATAATCTGAA
CACTGCCGGAGAAGGGGGTGTATCCCC
GTTGCATCATCTGACTCCAACCTGCCAAC
TCGCTGATCAAATTACAACGAGGGAGAA
AGTTGCTTACAAACATGATGGAGATCAA
ACCAGTTACGGAGGATGGCTGTCGGTTA
TAATAAGGGTCAGATCGAAAAGGGAGCCA
CGATCATTGTCGGAACTGTCGGAAAGATCA
AGCACTCTGTGAAAGAGGGTACCATCAAG
CTTGACAAATGCCCTCTTGTCTTGT
GAGGCTGATCGTATGATCGATGCTATGGGA
TTCGGAACTGACATCGAAACTATTGTCATT
ATGACAGTATGCCAGAGAAAGAAAATCGC
CAGACACTCATGTTCACTGCCACTTCCC
CGATTCTGTACAGGAAGCAGCTCGCGCTT
TTCAGAGAAAACACTCGTGTGATTGCA
TCGACAAGATTGGAGCTGCAAACAAGTGC
GTCCTACAGGAATTGAGAGATGCGAAAG
AAGCGAGAAGAAGGGACAAACTTAGAGC
TTCTGGGAATCGATATCGACAGTTACACGA
CCGAGAAAAGttagtttcgtttctatttgatgaaata
aattcaatatttcagGTGCCAGTTCACAAA
GAAAACCATGGCTTCTCAAAGAGC
AATGGCTGATACACTGGCTCAATTGTC
TCGGCTCAAGTTCCAGCTATCACGtttgttat
atttcattttgaccgttttaattcaaatgtacagATCCA
TGGTGCCCGTGAGCAGAGAGAGCGTTCA
GAAGCTTGAGAGACAATTCCGAAATGGATCG
AAACCTGTTCTATTGCTACTGCCAGACAAAC
ATTGATGACTATATCCATCGTATCGGAAGgtc
agtatattttataatgttcaataatgtcaaggatgtttcag
AACTGGAAAGAGTTGGAAACTCTGGAAAGAG
CTACAAGCTTCACTCGGAGGATGCACTG
TTCTGTCCGAACCTGTTGGTGTCTCGCCG
ACGCACAAACAGATTGTTCCAGACTGGATG
CAAGGTGCTGCTGGAGGCAATTACGGAGC
TAGTGGATTGGGTCCAGTGTACCAACTCA
AGTCCCAGGACGAGCAGGAGGGTGG
GGATCGGG
GAGGGACGTGGATCCCTTACCTGGG
AGACGTCGAGGAGAACCCAGGACCA
GGAGCATCGGGAGCCTCAGGAGCATCG
ATGGTTCCAAGGGAGAGGCTGTTATCAAG
GAATTCTGCGCTTCAAGGTTACATGGAA
GGATCTATGAACGGACACGAATTGCGAAATC
GAAGGAGAAGGAGAAGGGACGCCATACG
AGGGAACTCAAACCTGCTAACGTTAAGGTTA
CTAAAGGAGGACCACCTCCATTCTTGGG
ATATCCTTCTCCACAGTTCATGTCAGGATC
TCGCGCTTCTCATCAAGCACCCAGCTGATAT
CCAGATTACAAGCAGTCTTCCAGA
AGGATTCAAATGGGAGCGCGTTATGAACCT
CGAAGATGGAGGAGCTGTTACCGTTACCC
AAGATACTCCCTGAGGATGGAACCCCTTA
TCTACAAGgtaaatgttacatataactaacc
ctgattatttaatttcagGTTAAGCTTGGCGGAAC
TAATTCCCACCAAGATGGACCAAGTTATGCA
GAAGAAGACTATGGGATGGGAAGCTTCTAC
CGAGCGCCCTTACCCAGAGGATGGAGTCC
TTAAGGGAGATATCACCAGTGGCTTCTCGTC

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TTAAGGATGGAGGACGTTACCTTGCTGATA
CCTCTACTACTTACAAGGCTAACAGGCCAG
TTCAGATGCCAGGAGCTTACCTTGTCGATC
GTAAGCTTGATATCACTTCTCATAACGAATA
CTAG
aaaaccgaccaattgtatgtttcgccattttaaatgtcgtc
agtcccccatatttatctgccttgattttaaatgtatt
tgggttgtgtgtcgatgtcctccgcgcataaactct
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	cgacgagatccgtcgttcttgattcgagatatccaggag gatgggtcgaggcgtagacactac GGAGGAGGATCC TACACCGTCGTCGAGCAATACGAGAAGTC CGTCGCCCGTCACTGCACCGGAGGA taaagaacagcttgtaatcttttgcataatttttatc ttatgtttttatgtttccat	CCTGGTT CATGTGC TATTGCC	CGACGAC AGAAAGT AGCATCA C
fib-1	T01C3.7	N	AUAAUCG AUUUUU GUAGUAU	aatacggaaaaatctgtaaattcagttaccctccgcgc qccggccacqattqaactctggacgtcc GGATCCTCC TCCTCCGGTGCAGTGACGGGGACGGAC TTCTCGTATTGCTCGACGACGGTGTA catactacaaaaatcgattttaaacaaaacgaaaagcg aaattacggaaaaatacatacccgc	TTCTAGTC GATTCA ATCGACT	CGAAACT GCCACGA TCACC GG
his-3	T10C6.12	C	GAAGAAA ACCGGA GGAGACA	gattttaaatattgtggccctaagaggggccgtgggttc gtaaaaatgtttaaagaaggcatca TCTCCGGTGCAGTGACGGGGACGGAC TTCTCGTATTGCTCGACGACGGTGTA GGATCCTCC ttc I tgtctccctccgt I ttct I ggaaaaaaacagctt ggatatttggaaaaactcctccctgg	CCAAGGA GGAGTT TTCAAAT ATC	GATTTAA ATATTGT GCCCTA AAG
tbb-2	C36E8.5	N	CGCAUU GUCCGG CUUGCA CG	tcttcctatctaaatatagttttcaattcattacgcaccc gcaaaaaatg TACACCGTCGTCGAGCAATACGAGAAGTC CGTCGCCCGTCACTGCACCGGAGGA GGAGGAGGATCCGGAGGAGGATCCGGAG GAGGATCC agagagatcgt I cacgtcagccgacaatgcggaaa ccaaatcgatccaat	CAATATCG ACCATGA CGTGTTC TC	CTTGAAG GTTCCGT CTGGC AAG
tommm-20	F23H12.2	C	ACACCGA CGACUU GGAGUAA	gttggtaacggaaaaatacgaagaattaattggaaatgtt ataaaaactttaaatcattatccatca TCTCCGGTGCAGTGACGGGGACGGAC TTCTCGTATTGCTCGACGACGGTGTA GGATCCTCC ctccaagtgcgtgtcatcgataagcttggatttg tggggagggtccgtctccgaggat	GAGCGAA AGCAGAT GAGGC	CCCGTGA GGAGGAA AACACC
actual tommm-20 mutation recovered	F23H12.2	C	ACACCGA CGACUU GGAGUAA	gttggtaacggaaaaatacgaagaattaattggaaatgtt ataaaaactttaaatcattatccatca TATCCATTGCAAGTGACGGGGACGGACT TCTCGTATTGCTCGACGACGGTGTA GGATCCTCC ctccaagtgcgtgtcatcgataagcttggatttg tggggagggtccgtctccgaggat	GAGCGAA AGCAGAT GAGGC	CCCGTGA GGAGGAA AACACC
vha-13	Y49A3A.2	N	AUUCUG CGGCCA UCUUUU CC	gtttttttgtttttcgattccatatacgttctaaattc attcattccaggaaaaatgt TACACCGTCGTCGAGCAATACGAGAAGTC CGTCGCCCGTCACTGCACCGGAGGA GGAGGAGGATCC gccgcagaatctcgatcgattcggttacggagtgcc gacctgtcgacagccgagaagatgg	GGTTTATT TTGATTT CTTTTCG	CCATCTT CTCGGCT GTGAC ATTTC

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	atagaggatttaaatattgtggccctaaagaggcccgtt gggttcggtaaaatgttttaagaaggcatcta GGTGATTCCGGCGGCCTGACGTACTCGT GGAGGACCATGTTGTCACG TCCTCCTCC ttc I tgtctccgtt C tt I ggcaaaaagaacagctt ggatatttggagaactc c ttggcg	CCAAGGA GGAGTTC TTCCAAAT ATC	GATTTAA ATATTGTT GCCCTA AAG
vha-13	Y49A3A.2	N	AUUCUG CGGCCA UCUUUU CC	ggttatattgttttcgttccatatacgcttcaaattc attcattccaggaaaatggatg CGTGACCACATGGTCCTCCACGAGTACGT CAACGCCGCCGAATCACC GGAGGAGGATCC gccgcagaatcttcgtacggattcgittacggagtgtccg gacctgtcgtacccggagaatggatgg ggatatttggagaactc c ttggcg	GGTTTATT TTGATTT CTCGGCT CTTTTCG ATTTC	CCATCTT CTCGGCT GTGAC ATTTC
pgl-1	ZK381.4	C	GGTGGTT ACGGGG GTCGTGG	tacggggagatcggtacgttggttacggggaaag aggaggtagatggatgg GGAGCATCGGGAGCCTCAGGAGCATCG CGTGACCACATGGTCCTCCACGAGTACGT CAACGCCGCCGAATCACC taaactccaactattgaatgttaatttgtttttta	CCAAAGT TGCAAAA GGATTG GTCAATT	CATTAC GGGAACA AGGAAA ACAGGTT
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	cgacgagatccgtcgttgcattcgacatattcaggag gatgggtcggcgatcactac GGAGGAGGATCC TACACCGTCGTCGAGCAATACGAGAAC CGTCCCGTCACTGCACCGGAGGAATG GATGAGTTATACAAG taaagaacagctgtgaatctttagaaatttctattttatc ttatgtttttatgtttccat	CCTGGTT CATGTGC TATTGCC	CGACGAC AGAAAGT AGCATCA
his-3	T10C6.12	C	GAAGAAA ACCGGA GGAGACA	gattttaaatattgtggccctaaagaggcccgttgggttc gtaaaatgttttaagaaggcatcta CTTGATAACTCATCCATTCCCTCCGGTGCA GTGACGGGCACGGACTCTCGTATTGCT CGACGACGGTGTA GGATCCTCCTCC ttc I tgtctccgtt C tt I ggcaaaaagaacagctt ggatatttggagaactc c ttggcg	CCAAGGA GGAGTTC TTCCAAAT ATC	GATTTAA ATATTGTT GCCCTA AAG
tomm-20	F23H12.2	C	ACACCGA CGACUU GGAGUAA	gttggtaacgaaaaatacgaagaattaaattgtaaatgg ataaaaactttaaatcattatccattta CTTGATAACTCATCCATTCCCTCCGGTGCA GTGACGGGCACGGACTCTCGTATTGCT CGACGACGGTGTA GGATCCTCCTCC ctccaagtgcgtcggtcatcgataagcttggatttg tggtgagggtcgctccggatgtat	GAGCGAA AGCAGAT GAGGC	TCCGTGA GGAGGAA AACACC
actual tomm-20 mutation recovered	F23H12.2	C	ACACCGA CGACUU GGAGUAA	gttggtaacgaaaaatacgaagaattaaattgtaaatgg ataaaaactttaaatcattatccattta CTTGATAACTCATCCATTCCCTCCGGTGCA GTGACGGGCACGGACTCTCGTATTGCT CGACGACGGTGTA GGATCCTCCTCC ctccaagtgcgtcggtcatcgataagcttggatttg tggtgagggtcgctccggatgtat	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-N		ggtttatattgttttcgttccatatacgcttcaaattcattccaggaaaatggat TACACCGTCGTCGAGCAATACGAGAAC GTCCGTCGCCCCACTGCACCGGAGGA

his-3::split-wrmScarlet ₁₁ (x3)	C	<pre> GGTGGCTCTGGAGGT TACACCGTTGAGCAATACGAGAAGTCTGGCTCGTCAGCACCAGGAGGC GGAGGAGGATCC ggcgagaatctcgacggattcggtacggagtgtccggacgtcgtaacagccgagaagatgg gattttaaatattgtggccctaaagaggccgtgggtcgtaaatgtttaagaaggcatcta TCCTCCGGTGCAGTGACGGGCACGGACTTCTGTATTGCTGACGACGGTGTA TCCTCCACTACCGCC TCCTCCGGTGCAGTGACGGGCACGGACTTCTGTATTGCTGACGACGGTGTA ACCTCCAGAGGCCACC TCCTCCGGTGCAGTGACGGGCACGGACTTCTGTATTGCTGACGACGGTGTA GGATCCTCCTCC ttc<u>T</u>tgtctccctccgt<u>C</u>tt<u>T</u>ggaaaagaacagctggatatttggagaagaactcccttgg </pre>
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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	GGTTTATTTGATTTCTTTCGATTCC
vvha-13_R	CCATCTCTCGGCTGTGAC

S4D. Sequences of crRNA and HDR template used to generate split-wrmScarlet₁₀ 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	AAGUUCGCUUG ACUUGGAGG	tagaaggttcttaggataattttcgactttattctcttaccgtccgcacttt cttactttaaattaaatttttttcagttggaaaacacttgcactccgt agcacccATGGTATCGAAGGGAGAACGAGTAATCAAGGA GTTCATGCGTTCAAGGTCCACATGGAGGGATCCATG AACGGACACGAGTTCGAGATCGAGGGAGAGGGAGAG GGACGTCCATACGAGGGAACCCAAACCGCCAAGCTC AAGGTCACCAAGgtaaatccatataactaactaaccctgtat tattttaaatttcagGGAGGACCACTCCCATTCTCCTGGGAC ATCCTCTCCCCACAATTATGTACGGATCCCGTGCCTT CATCAAGCACCCAGCCGACATCCAGACTACTACAAG CAATCCTCCAGAGGGATTCAAGTGGGAGCGTGTCA TGAACCTCGAGGACGGAGGAGCCGTACCGTCACCC AAGACACCTCCCTCGAGGACGGAACCCCTCATCTACAA GgttaagttaaacagttcggtactaactaaccatacatattttcagG TCAAGCTCCGTGGAACCAACTTCCCACCGAGCGAC CAGTCATGAAAAGAACCATGGGATGGGAGGGCTC CACCGAGCGTCTCACCCAGAGGGACGGAGTCCTCAA GgttaagttaaacatgtttactaactaactaacttgcattttcagGG AGACATCACCATGGCCCTCGTCTCAAGGACGGAGG ACGTTACCTCGCCGACACCTCCACCCACCTACAAGGCC AAGAAGCCAGTCAAATGCCAGGAGCCTACCTCGTCA ACCGTAAGCTCGACATCACCTCCCACAACGAGTACTA

COP1795 -> CF4587	GACCAGCUGGG CGCAUAGGG	GCCGCCACG AGGGCCAGG	Ataagtccaaattactctcaacatccctacatgctttccctgtgtccca ccccctattttgttattatcaaaaaacttcttaattcttgtttagcttta taagtca aactctttcaattcaactgaaagattttcattagagaatgtctagaacta ggcccggtacgtaatcgtactaaggcctaattgggtctggctg catgccaggaggtaGCACCTTGGTCTTTATTGTCAACTTC CATTGGTCTTCCATTGTTCTGTTAAATTATGAATT CATAAAATAAAGACATTATACAATATAAAAATGAAGAATT ATTGAAAATAAAGTGCAGAGAGAAAAAGTATGCAACA CTCCCGCCGAGAGTGTTGAAATGGTGTACGGTACATT TTCGTGCTAGGAGTTAGATGTGCAGGCAGCAACGGAGA GGGGGAGAGATTTGGGCTTGTGAAATTAAACGTG AGTTTTCTGGTCATCTGACTAATCATGTTGGTTTTGTT GGTTTATTGTTTATCTTGTGTTTATCCAGATTAGGA AATTAAATTTATGAATTATAATGAGGTCAAACATTCA TCCCAGCGTTTCTGTTCACTGTTAGTCGAATT TTATTTAGGCTTCAACAAATGTTCAACTGTCTTATT GTGACCTCACTTTTATATTTTTAATTAAAATTTAA GAAGTTCTAGGATAATTTCGACTTTATTCTCTCTA CCGTCCGCACTCTTACTTTAAATTAAATTGTTTT TTTCAGTTGGAAACACTTGCTCaaaaatgtctaaggaga agagttaattactggagttgcgcgtccgtcgactcgacggagacgt caacggacacaagttccgtccgtggagagggagagggagacccac catcg cactttaccgtctaatttcagggcagggagccatcaaaccacgaccac tagatccatATGGTATCGAAGGGAGAACAGTAATCAAGG AGTCATCGTTCAGGTCCACATGGAGGGATCCAT GAACGGACACGAGTCGAGATCGAGGGAGAGGGAGA GGGACGTCCATCGAGGGAACCCAAACCGCCAAGCT CAAGGTACCAAGgttaagttaaacatataactaactaaccctg attttaaatttcagGGAGGACCACCTCCATTCTCTGGGA CATCCTCTCCCCACAATTCTGACGGATCCCGTGCCT TCATCAAGCACCCAGCCGACATCCCAGACTACTACAA GCAATCCTTCCCAGAGGGATTCAAGTGGGAGCGTGTC ATGAACCTCGAGGACGGAGGGAGCCGTACCGTCACC CAAGACACCTCCCTCGAGGACGGAACCCCATCTACA AGgttaagttaaacagttcgactaactaaccatataatttcag GTCAAGCTCCGTGGAACCAACTTCCCACCAAGACGGA CCAGTCATGCAAAAGAAGACCATGGGATGGGAGGGCT CCACCGAGCGTCTTACCCAGAGGACGGAGTCTCA AGgttaagttaaacatgatattactaactaactaatttcag GAGACATACCATGGCCCTCGTCTCAAGGACGGAG GACGTTACCTCGCCGACACCTCCACCAACCTACAAGG CCAAGAAGCCAGTCCAAATGCCAGGAGGCCTACCTCGT CGACCGTAAGCTCGACATCACCTCCCACAACGAGTAC TAAcatctcgccgtccgtactaacttcaattactctcaacat ccctacatgt
WBM1126 -> CF4610	GCUACCAUAGG CACCACGAG		

S4E. Primers long and short

Primers to mplify split-wrmScarlet	Sequence
eft3p_S110(A19)_F	tagaagttctaggataattttcgactttattctctaccgtccgactcttc ttactttaaattaaattgttttttcagttggaaacacttgctactccgtag cagccATGGTATCGAAGGGAGAACG
unc54_S110(A19)_R	tgacttaaaaagaagctaaaaacaagaattaaagagaagttttgataat aacaaaaatagggggtgggagcacagggagaaagagagcatgttagggatg ttgaagagtaattggacTTATTAGTACTCGTTGGGAGG

S1-10_A19_F

ATGGTATCGAAGGGAGAAGC

S1-10_A19_R

TTAGTACTCGTTGTGGGAGGTG

Primers to amplify Peft-3 for CF4587

E7_eft-3p_F-Long

aactcatttcaattcaactgaaagattttcattagagaatgtctagaacta
ggcccccggctacgtaatacgactacttaaggcctaattgggtctggctg

E8_eft-3p_R-Long

catgccaggaggtGCACCTTGGTCTTTATTGTCAAC
cgatggtggcgtccctccctccacggacggagaacttgtccgtt

E5_eft-3p_F

GCACCTTGGTCTTTATTGTCAAC

E6_eft-3p_R

GAGCAAAGTGTTCACCAACTG

Primers to amplify split-wrmScarlet for CF4610

PrimerS1-10_myo3F

cactttaccgtctaatttcagggcagggagccatcaaaccacgaccac
tagatccatATGGTATCGAAGGGAGAAGC

PrimerS1-10_myo3R

agcatgtaggatgtgaagagtaattggacttagaagtgcagaggcacgg
gcgcgagatgTTAGTACTCGTTGTGGGAGGTG

Table S5. Plasmid sequences

Name

Peft-3::3NLS::mTagBFP2::split-wrmScarlet₁::T2A::mNeonGreen::split-wrmScarlet₁-10::fib-1 UTR (C. elegans)

Sequence

gcaccttggctttattgtcaactccattggtttccattgtttctgttaatt
aatgaattttcataaaaataaaagacattatacaatataaaaatgaagaatttt
gaaaataaaactgcacagagaaaaagtatgcacacactcccccggagat
gttggaaatgggtacggcattttcgtgtcattggaggttagatgtgcaggcag
caacgcaggggggagagattttggccctgtgaaattaacgtgagtttc
tggtcatctgactaatcatgttggttttgcatttttatcttgcattttt
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GCACCTCCGAGGGAGAGGGAAAGCCATACGAGGGAA
CCCCAAACCATGCGTATCAAGGTCGTGAGGGAGGGAC
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CTCTACGGCTCCAAGACCTTCATCAACCACACCCAAAG
GAATCCCAGACTTCTCAAGCAATCCTCCAGAGGGGA
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GGAGTCCTCACGCCACCCAAAGACACCTCCCTCCAA
GACGGGATGCCCATCTACAAACGTCAAAGgttaagttaaacat
atatactaactaaccctgattattaaatttcagatccgtggagtcaa
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CTCGGATGGGAGGCCTTCACCGAGGACCCCTTACCCA
GCCGACGGAGGGACTGGAGGGACGTAACGACATGGCC

CTCAAGCTCGTCGGAGGTTCCCACCTCATGCCAACG
CCAAGACCACCTACCGTCCAAGAAGCCAGCCAAGAA
CCTCAAGATGCCAGGAGTCACTACGTCGACTACCGT
CTGGAGCGTATCAAGGAGGCCAACAACGAGACCTACG
TCGAGCAACACGAGGTGCGCGTCGCGCTTACTGCG
ACCTCCCATCAAAGCTCGGACACAAGCTTAACGGCG
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GGCGAGGCAGTGTACAGGGGGAGGATCCGGCGAGG
GACGTGGCTCCCTCACCTGCGGAGACGTCGAGG
AGAACCCAGGACCAGTCTCAAGGGAGAGGGAGGACA
ACATGGCCTCCCTCCAGCCACCCACGAGCTCCACAT
CTTCGGGTCATCAAACGGAGTCGACTTCGACATGGTC
GGACAAGGAACCGGAAACCCAAACGACGGATACGAG
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attattacagggt

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

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CTGGAGCGTATCAAGGAGGCCAACACGAGACCTACG

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

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cagggt

Pmyo-
3::mTagBFP2::sfCherry3₁₁::T2A::mNeonGreen::sfCherry
31-10::fib-1 UTR (C. elegans)

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AGTACGACTGAATCCGGTGAGAATGGCAAAAGTTAT

pD881MR-mtagBFP-split-wrmScarlet₁₁

pRSET-split-wrmScarlet₁₋₁₀

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Table S6. Adult lifespans of strains in this study

Strain	Events / n	Mean lifespan +/- initial	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	Mean lifespan initial	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

Sequence

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