

Supplementary Materials for

UPR^{ER} promotes lipophagy independent of chaperones to extend life span

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This PDF file includes:

Fig. S1. ER remodeling of the intestine through UPR^{ER} activation requires neuronal nonautonomous signaling.

Fig. S2. LAMPro technology can be reliably used to quantify LDs using DHS-3::GFP.

Fig. S3. ER remodeling is coincident with more intestinal lysosomes in neuronal *xbp-1s* animals.

Fig. S4. Lipid depletion in neuronal *xbp-1s* is dependent on *xbp-1* and *ehbp-1*.

Fig. S5. Knockdown of lipophagic components does not affect chaperone induction by *xbp-1s*.

Table S1. Statistics for all life-span data.

Table S2. Organisms and strains used.

Sequences used for cloning

Supplemental Information

Supplemental Figures

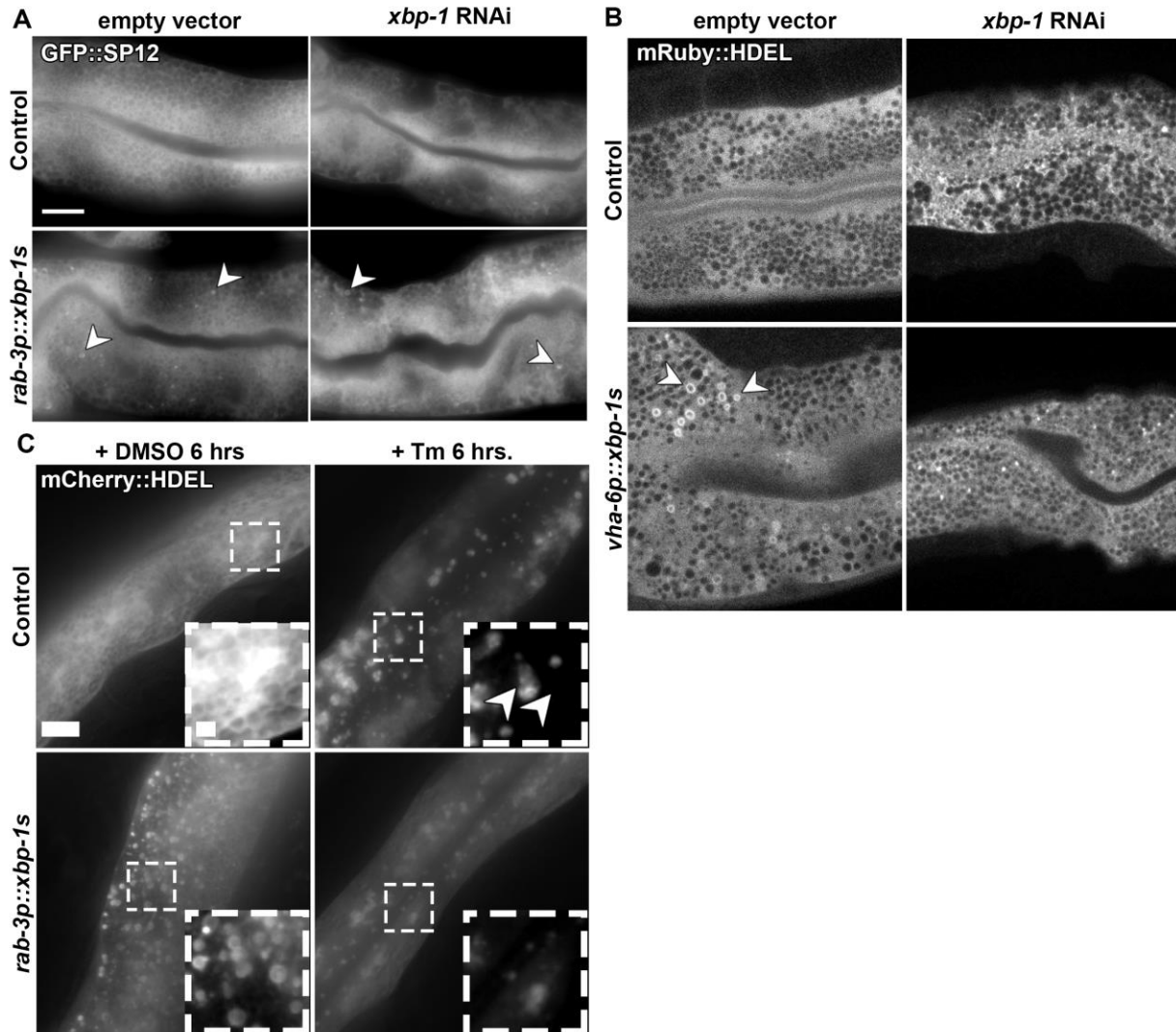


Fig. S1. ER remodeling of the intestine through UPR^{ER} activation requires neuronal nonautonomous signaling. (A) ER morphology in intestine of control and neuronal *xbp-1s* animals. An alternate ER transmembrane marker (VS25: *vha-6p::GFP::C34B2.10 (SP12)*) is used here to demonstrate that phenotypes depicted are not an artifact of luminal ER fluorophores. While the ER remodeling is much more subtle using this marker, clear spherical structures can be detected as marked by arrowheads. Animals were grown from hatch on ev or *xbp-1* RNAi and live-imaged using a compound microscope. Scale = 10 μ m. (B) Representative micrographs of ER morphology (mRuby::HDEL) in intestine of control and intestinal *xbp-1s* (*vha-6p::xbp-1s*) animals. ER remodeling is depicted by arrowheads. (C) Representative compound micrographs of ER (mCherry::HDEL) with and without 6 hours treatment with 25 ng/ μ L tunicamycin. Briefly, animals were treated in tunicamycin in M9 liquid spinning at 20°C or with 1% DMSO as a control. Drugs were washed out and animals were live-imaged immediately

on a compound microscope. Scale bar is 10 μ m. For A-C, animals are imaged at late Day 2 of adulthood.

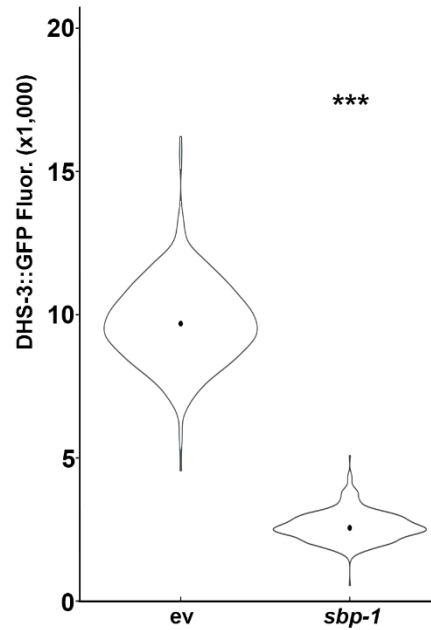


Fig. S2. LAMPro technology can be reliably used to quantify LDs using DHS-3::GFP.

Quantification of lipid droplets (LDs) (via DHS-3::GFP fluorescence) in animals grown on *ev* or *sbp-1* RNAi from hatch. *sbp-1* is used as a control for decreased lipids. *** = $p < 0.001$ using non-parametric Mann-Whitney testing. Plots are representative of three biological replicates and $n = 921$ (*ev*), 435 (*sbp-1*). Animals were imaged at late Day 2 of adulthood.

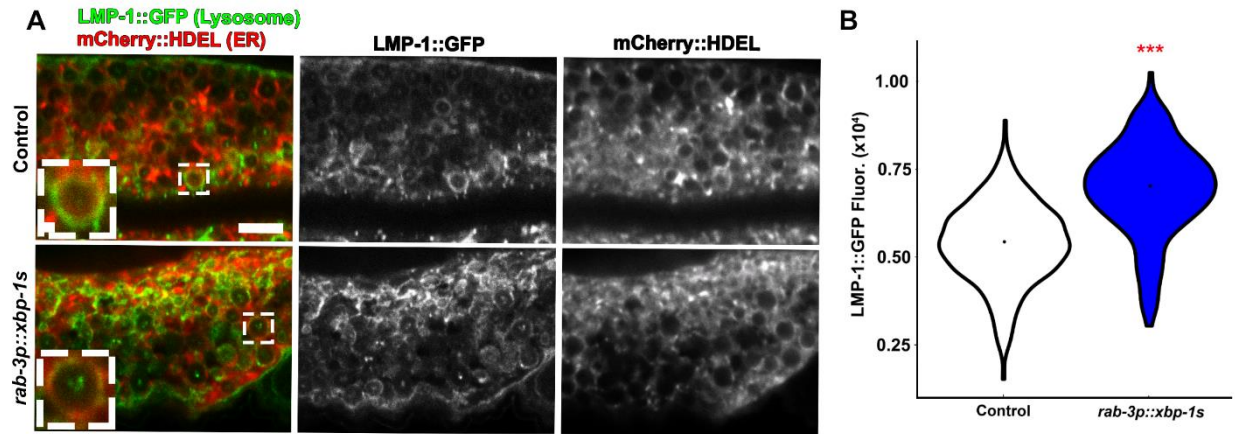


Fig. S3. ER remodeling is coincident with more intestinal lysosomes in neuronal *xbp-1s* animals. (A) Confocal micrographs of intestinal ER (*vha-6p::mCherry::HDEL*) and lysosomes (*vha-6p::lmp-1::GFP*), in fixed animals, with and without *rab-3p::xbp-1s*. Scale = 5 μ m (and 0.5 μ m for inset). (B) Quantification of lysosomes (via LMP-1::GFP) in control and *rab-3p::xbp-1s* animals. *** = $p < 0.001$ using non-parametric Mann-Whitney testing. $n > 50$ animals per condition. For A-B, animals are imaged at late Day2 of adulthood.

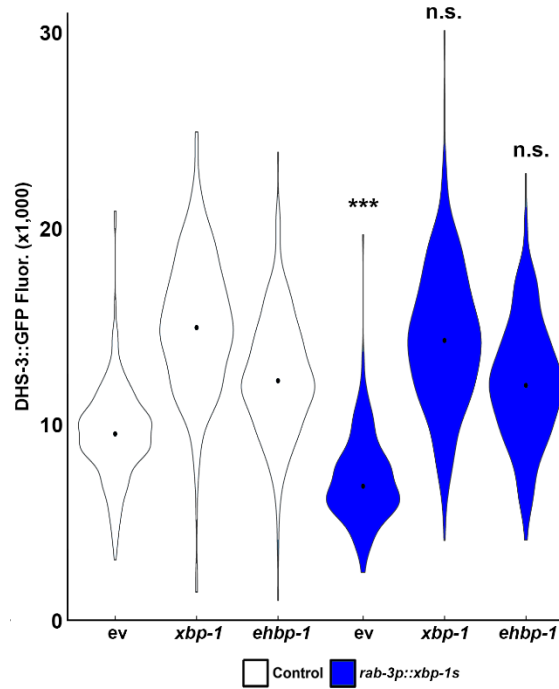


Fig. S4. Lipid depletion in neuronal *xbp-1s* is dependent on *xbp-1* and *ehbp-1*.

Quantification of LDs (via DHS-3::GFP) in control and *rab-3p::xbp-1s* animals grown on *ev*, *xbp-1*, or *ehbp-1* RNAi from hatch. *** = $p < 0.001$, n.s. = $p > 0.10$ using non-parametric Mann-Whitney testing. Plots are representative of four biological replicates and $n = 320-484$ animals per strain. Animals are imaged at late Day 2 of adulthood.

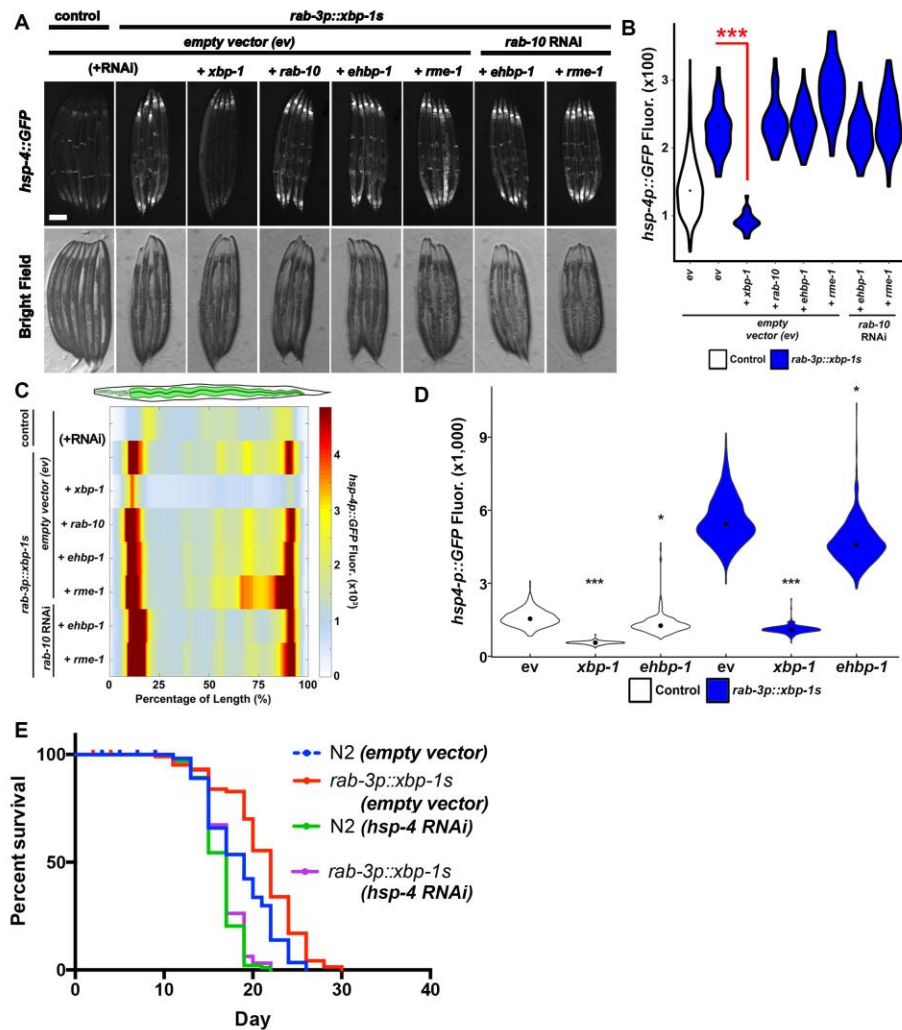


Fig. S5. Knockdown of lipophagic components does not affect chaperone induction by *xbp-1s*. (A) Fluorescent light micrographs of *hsp-4p::GFP* UPR^{ER} reporter animals, imaged as Day 2 adults, and treated with the indicated RNAi(s) from hatch. Scale = 100 μ m. (B) Quantification of *hsp-4p::GFP* fluorescence in control (white) and *rab-3p::xbp-1s* (blue) nematodes, using a Biosorter, following various RNAi treatments. *** = $p < 0.001$ by Mann-Whitney. Wild-type controls on *xbp-1* and *ehbp-1* RNAis can be found in panel (d). (C) Median profiles of GFP fluorescence of the *hsp-4p::GFP* animals, under various RNAi treatments, in a *rab-3p::xbp-1s* background, following alignment. Plots are representative of three biological replicates and derived from $n = 28-71$ per strain. (D) Quantification of *hsp-4p::GFP* fluorescence in control (white) and *rab-3p::xbp-1s* (blue) nematodes, using a Biosorter, following various RNAi treatments. *** = $p < 0.001$, * = $p < 0.10$ using non-parametric Mann-Whitney testing. p -values are in comparison to their corresponding ev controls. Data is representative of two independent trials with $n = 224-573$ per strain. (E) Lifespan data for wild-type N2 animals or neuronal *xbp-1s* (*rab-3p::xbp-1s*) animals treated with EV or *hsp-4* RNAi from hatch. RNAi knockdown of *hsp-4* is sufficient to eliminate the lifespan extension normally seen in neuronal

xbp-1s animals. See **table S1** for lifespan statistics. For A-D, animals are imaged at Day 1 of adulthood.

Table S1. Statistics for all life-span data.

Supplemental Table S1. Statistical Analyses of Lifespan Data

Corresponding Figure	Strain, Treatment	Median Lifespan [days]	# Deaths/# Total	% Change in Median Lifespan	p-value Log-rank (Mantel-Cox)
2f	N2, vector RNAi, 1% DMSO	22	95/120	n/a	n/a
	<i>rab-3p::xbp-1s</i> , vector RNAi, 1% DMSO	26	94/120	1.2	<0.0001
	N2, <i>xbp-1</i> RNAi, 1% DMSO	14	92/120	0.6	<0.0001
	<i>rab-3p::xbp-1s</i> , <i>xbp-1</i> RNAi, 1% DMSO	15	95/120	0.7	<0.0001
	N2, <i>ehbp-1</i> RNAi, 1% DMSO	16	92/120	0.7	<0.0001
	<i>rab-3p::xbp-1s</i> , <i>ehbp-1</i> RNAi, 1% DMSO	15	96/120	0.7	<0.0001
2g	N2, vector RNAi, 25ng/μL tunicamycin	14	97/120	n/a	n/a
	<i>rab-3p::xbp-1s</i> , vector RNAi, 25ng/μL tunicamycin	18	109/120	1.3	<0.0001
	N2, <i>xbp-1</i> RNAi, 25ng/μL tunicamycin	12	98/120	0.9	<0.0001
	<i>rab-3p::xbp-1s</i> , <i>xbp-1</i> RNAi, 25ng/μL tunicamycin	12	101/120	0.9	<0.0001
	N2, <i>ehbp-1</i> RNAi, 25ng/μL tunicamycin	14	111/120	1.0	0.2271
	<i>rab-3p::xbp-1s</i> , <i>ehbp-1</i> RNAi, 25ng/μL tunicamycin	14	111/120	1.0	0.8208
S5e	N2, vector RNAi	19	100/120	n/a	n/a
	<i>rab-3p::xbp-1s</i> , vector RNAi	22	88/120	1.2	<0.0001
	N2, <i>hsp-4</i> RNAi	17	102/120	0.9	<0.0001
	<i>rab-3p::xbp-1s</i> , <i>hsp-4</i> RNAi	17	96/120	0.9	<0.0001
3d	N2, vector RNAi	17	101/120	n/a	n/a
	<i>vha-6p::ehbp-1</i> , vector RNAi	21	98/120	1.2	0.0221

Table S2. Organisms and strains used.

BACTERIAL AND VIRUS STRAINS		
OP50	CGC	N/A
HT115	CGC	N/A
DH5 α	Invitrogen	
EXPERIMENTAL MODELS: ORGANISMS/STRAINS		
<i>C. elegans</i> : Bristol (N2) strain as wild type (WT)	CGC	N2
<i>C. elegans</i> : AGD927: uthIs270 [rab-3p::Xbp-1s, myo-2p::tdTomato]	(Taylor and Dillin, 2013)	N/A
<i>C. elegans</i> : AGD928: uthIs270[rab-3p::Xbp-1s, myo-2p::tdTomato]; zcls4[hsp-4p::GFP]	(Taylor and Dillin, 2013)	N/A
<i>C. elegans</i> : AGD1289: N2, uthIs368[rab-3p::HSF-1 FL, myo-2p::tomato]	(Douglas et al., 2015)	N/A
<i>C. elegans</i> : AGD1376: hjSi158[vha-6p::SEL-1(1-79)::mCherry::HDEL]; uthIs270[rab-3p::Xbp-1s, myo-2p::tdTomato]	This study	N/A
<i>C. elegans</i> : AGD1415: pwIs23[vit-2::GFP]	This study	N/A
<i>C. elegans</i> : AGD1771: N2, uthIs464[rgef-1p::Xbp-1s, myo-2p::tdTomato]	This study	N/A
<i>C. elegans</i> : AGD2048: dhs-3p::dhs-3::GFP; hjSi158 [vha-6p::SEL-1(1-79)::mCherry::HDELlet-858 3' UTR]	This study	N/A
<i>C. elegans</i> : AGD2065: dhs-3p::dhs-3::GFP; hjSi158 [vha-6p::SEL-1(1-79)::mCherry::HDELlet-858 3' UTR]; uthIs270[rab-3p::Xbp-1s, myo-2p::tdTomato]	This study	N/A
<i>C. elegans</i> : AGD2129: pwIs23[vit-2::GFP]; uthIs270 [rab-3p::Xbp-1s, myo-2p::tdTomato]	This study	N/A
<i>C. elegans</i> : AGD2192: unc-119(ed3) III; uthSi60[vha-6p::ERss::mRuby::HDEL::unc-54 3'UTR cb-unc-119(+)] IV	This study	N/A
<i>C. elegans</i> : AGD2230: uthIs488[vha-6p::xbp-1s, myo-2p::BFP]; unc-119(ed3) III; uthSi60[vha-6p::ERss::mRuby::HDEL::unc-54 3'UTR cb-unc-119(+)] IV	This study	N/A
<i>C. elegans</i> : AGD2267: uthIs270 [rab-3p::xbp-1s, myo-2p::tdTomato]; unc-119(ed3)III; uthSi60 [vha-6p::Erss::mRuby::HDEL::unc-54 3'UTR cb-unc-119(+)]	This study	N/A
<i>C. elegans</i> : AGD2349: uthIs270[rab-3p::Xbp-1s, myo-2p::tdTomato]; vha-6p::GFP::C34B2.10(SP12)+unc119(+); unc-119(ed3) III; uthSi60[vha-6p::ERss::mRuby::HDEL::unc-54 3'UTR cb-unc-119(+)] IV	This study	N/A
<i>C. elegans</i> : AGD2363: N2, uthEx911[vha-6p::ehbp-1::unc-54 3'UTR; myo-2p::GFP]	This study	N/A
<i>C. elegans</i> : AGD2364: N2, uthEx912[vha-6p::ehbp-1::unc-54 3'UTR; myo-2p::GFP]	This study	N/A

<i>C. elegans</i> : AGD2365: N2, uthEx911[vha-6p::ehbp-1::unc-54 3'UTR; myo-2p::GFP]; unc-119(ed3) III; uthSi60[vha-6p::ERss::mRuby::HDEL::unc-54 3'UTR cb-unc-119(+)] IV	This study	N/A
<i>C. elegans</i> : AGD2366: N2, uthEx911[vha-6p::ehbp-1::unc-54 3'UTR; myo-2p::GFP]; dhs-3p::dhs-3::GFP; hjSi158[vha-6p::SEL-1(1-79)::mCherry::HDELlet-858 3' UTR]	This study	N/A
<i>C. elegans</i> : AGD2367: N2, uthEx911[vha-6p::ehbp-1::unc-54 3'UTR; myo-2p::GFP]; zcls4(hsp-4p::GFP)V	This study	N/A
<i>C. elegans</i> : EG6703: unc-119(ed3) III; cxTi10816 IV; oxEx1582	CGC	EG6703
<i>C. elegans</i> : SJ4005: zcls4(hsp-4p::GFP)V	(Taylor and Dillin, 2013)	SJ4005
<i>C. elegans</i> : VS25: vha-6p::GFP::C34B2.10(SP12)+unc119(+); unc-119(ed3) III	CGC	VS25
<i>C. elegans</i> : VS30: hjSi158[vha-6p::SEL-1(1-79)::mCherry::HDELlet-858 3' UTR]	CGC	VS30
OLIGONUCLEOTIDES – ALL OLIGOS ORDERED FROM IDT		
Primer Name	Primer Sequence	Primer Purpose
JRD260 F_all_xbp1s_vectors	TAGCATTTCGTAGA ATTCCAACCTGAGC	forward primer to clone vector side of pJRD16
JRD262 R_myo3_vector_xbp1s	ctagatggatctagtggtc gtggg	reverse primer to clone vector side of pJRD16
JRD255 R_all_xbp1s_inserts	GCGCTCAGTTGGA ATTCTACGAATGC TATTAATTTTGGAA GAAATTTAGGTCG ATTG	Reverse primer to clone xbp-1s insert for pJRD16
JRD257 F_xbp1s_insert_myo3	ctagatggatctagtggtc gtggg	Forward primer to clone xbp-1s insert for pJRD16
gg288F	ATAGCATTTCGTAG CGAATGCGTATTG TaccggaccagtcAAC TCTCTTATCAAGG AAAAC	Forward primer to PCR ERSS::mRuby for pGIL122
gg22R	aagtcagaggcacgggc gagatgTTAaagttc gtcatgTCCTCCTCC AAGTCCAG	Reverse primer to PCR ERSS::mRuby for pGIL122
gg153F	catctcgcgcccggtg	Forward primer to PCR PCR vector for pGIL122

gg45R	ttttatggggtttgtagggttt ag	Reverse primer to PCR vector for pGIL122
SM8 vha6p-ehbp F1	cctaccaaaccataaa aaatggcgggtattctcgt cg	Forward primer to PCR ehbp-1 for pSM1
SM9 vha-6p-ehbp R1	ATTATCAACTTCTA CTCACtctttttcgaatg aatttttgctgaaca	Reverse primer to PCR ehbp-1 for pSM1
SM10 vha6-ehbp1 F2	atcattcgaaaaagaG TGAGTAGAAGTTG ATAATTTGAAAGT G	Forward primer to PCR vector for pSM1
SM11 vha6-ehbp1 R2	cgacgaagaataccgc catttttatggggtttgtag gtttagtc	Reverse primer to PCR vector for pSM1
RECOMBINANT DNA		
pCFJ356: Integration vector for EG6703 MOSCI: contains Right xcTi10816, cb-unc-119(+), and Left cxTi10816	Addgene	34871
pCFJ601: MOS-1 transposase	Addgene	34874
pCFJ90: myo-2p::mCherry::unc-54 3' UTR	Addgene	19327
pCFJ104: myo-3p::mCherry::unc-54 3' UTR	Addgene	19328
pGH8: rab-3p::mCherry::unc-54 3' UTR	Addgene	19359
pEK1: myo-2p::GFP::unc-54 3' UTR	This study	N/A
pEK2: myo-2p::tdtomato::unc-54 3' UTR	This study	N/A
pRT5: pAD1 rgef-1p::xbp-1s cDNA::unc-54 3'UTR	(Taylor and Dillin, 2013)	N/A
pSM1: vha-6p::ehbp-1::ehbp-1 3'UTR	This study	N/A
pGIL122: pCFJ356::vha-6p::hsp-4 ERSS::mRuby::HDEL::unc-54 3' UTR	This study	N/A

Sequences used for cloning

pSM1 (cloned into pCFJ90 backbone replacing myo-2p::mCherry with vha-6p::ehbp-1 sequence using Gibson)

vha-6p:

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tcggatattacagcacaagccgatgtaagattctgagaattaaaggattaattcagtttggnttttcagggtattcaagacaagatcgt
gaaacagaagttggaagttcagaagaagaaatgttgactgcacatatacttgagttgacaaatgaacggaacactctgtacatcgac
aggaatactataatcattgagacaattcgtcaggttacttcggaaattgaccaattgggaaaaacaaatcaatgaagttcctgatgatt
ccctcgttctgatgagaataagacggcaacggataaactgattgaacaatatagtgatgctatgaaaacaaaaagtaacttggttcaa
aagttatnttgcaactgaggacgaaatgtaagtttattgctttgtgaaccacgtggtgacaggctgtcccacacggttgatctacaaaa
aatgctggaatntttgcctaagaaagtgccgtcagcacgttctaaccatgcgaaatcagttgagaactcttcgtctcaacttccgaatntt
ttgtagatctacgtagatcaaacggaatgggacattctgacaccacgtaataaactactttcagtgaaactaatgaaacaacattcgaa
atnttcagcgaagaagattcggatcgttgaagaaccttactttggatagggtactcgttggctgtggaatagatcagcctgtttcagca
tcgaagcgacttattcaatgggtggcgccgtaa

ehp-1 3' UTR (predicted):

tacgcttcaacgttatttactcaatntaaaactacgggtcttcttcttctgaaaacaaagtttctccagtgctcctttgttatcttctatca
tttttctcctttccataattcactcttcccggtttttggcaatnttttaggagttaatcaaaacatcaaatntttctctatctttctatctatgtgat
atntttgattcagtttcttctcctcgggtgcacacaaatcctcagcttcaataaatcaattcagagttntaaatgatgttcagcaa
aaattcattcgaaaaaaga

pGIL122 (cloned into pCFJ356 multiple cloning site using Gibson):

vha-6p:

gcatgtaccctttataggtgcgctctacgcaattcaccagctgaacaatggagttgagcctaataactaaaatnttgaatgctttac
aaaaatattatnttcagatcttcgagatcatgaaaactatcaaacagcagcgccctggagcaatcagatcgttccacaatattcagggtg
atatgcaatcgttttagactacatttcggaatgtgacttcagagataaactgtaattatnttaaatcagcgcgaacgcggagaaag
tctgatcctgttaacaataacataaatcgttctcgtgatctcacggagatattgccagcatgctcaacggttcagtgatgaatcaa
gcacagaactgcattaagtatactatnttactcgatactttgttcacataggtntntaaatcattnttatgcatcatttatcatattcaatgca
tcattcatatcatagcaataaaaaggtgatttctcatgttctggnttcaaatgctgactttggtaaaaagaacgcgtgcctgcctattgcct
atcttggcattnttctcgataaattntaaatgtaggttcgatcttatgagattgtagtcaaaagagctcatatgattcaggtaggctggtgag
cgagaccaacttaatgcatgacaagcattntcaattgcccctggagcgaattggtnttattcgaataatgcacattctgttcccata
atataaaatnttcaggacgatataattacattcttcacaaaatattgcattacagacaccgacaaaagaatctccacctgatgaaaac
aatgagccaacaatgttatctgtattgccaccaccacattcctagtcattcagatataatgnttcaattgaatcattgcaggtatatacga
attgaactgtaaggctcattctcatttcaatacatcatccattccagagcagctccggccacacaaaatgggtggcggtctgat
attgataatcgaacttcttgacgtgcctgacggagcagcaaaagcggagcactgataagacaatgaagaactaaaaatgtcttcggtt
ttcagcttttagttctgcagcactnttntttgttctcctatntttccgcatnttctcaacttctgatgtccatttcaaatgattnttataaaaatgntt
aattcagggcgactaaaacctacaaaacccataaaaa

hsp-4 ERSS:

ATGAAAGTTTTCTCGCTGATTCTGATAGCATTTCGTAGCGAATGCGTATTGT

mRuby:

AACTCTTATCAAGGAAAACATGCGCATGAAGGTTGTTCTTGAGGGATCTGTTAACGGAC
ATCAGTTCAAGTGCACACTGGAGAAGGAGAAGGAAATCCATACATGGTATGTGATACATGGAA
ATAGGCACATTCATTAATATTTGTATTTTCAGGGAACCTCAACTATGCGCATCAAGGTTAT
CGAAGGAGGACCACTTCCATTCGCTTTTCGACATCCTTGCTACCTCTTTCATGTACGGATCT
CGCACTTTCATTAAGTATCCAAAGGGAATTCCAGATTTCTTCAAGCAGTCTTTCAGGAAAGG
ATTCACCTGGGAGCGCTTACCCGCTACGAGGATGGAGGAGTTATCACCGTTATGCAAGA
TACCTCCCTTGAGGATGGATGCCTTGTTTACCACGCTCAAGTTCGTGGAGTTAACTTCCA

TCTAACGGAGCTGTTATGCAGAAGAAGACTAAGGGATGGGAACCAAACACTGAAATGATGT
ACCCAGCTGATGGAGGACTTCGCGGATACACCCACATGGCTCTTAAGGTTGATGGAGGAG
GACACCTTTCTTGCTCTTTCGTTACCACCTACCGCTCTAAGAAGACTGTTGGAAATATTAAG
ATGCCAGGAATCCATGCTGTTCGATCACCGTCTTGAGCGTCTTGAGGAGTCTGATAACGAGA
TGTTTCGTTGTTCAACGTGAGCACGCTGTTCGCTAAGTTCGCTGGACTTGGAGGAGGA

unc-54 3'UTR:

AACTCTCTTATCAAGGAAAACATGCGCATGAAGGTTGTTCTTGAGGGATCTGTTAACGGAC
ATCAGTTCAAGTGCCTGGAGAAGGAGAAGGAAATCCATACATGGTATGTGATACATGGAA
ATAGGCACATTCATTAATATTTGTATTTTCAGGGAACCTCAAACATGCGCATCAAGGTTAT
CGAAGGAGGACCCTTCCATTCGCTTTTCGACATCCTTGCTACCTCTTTCATGTACGGATCT
CGCACTTTTATTAAGTATCCAAAGGGAATTCCAGATTTCTTCAAGCAGTCTTTCCAGAAGG
ATCACTTGGGAGCGCGTACCCGCTACGAGGATGGAGGAGTTATCACCGTTATGCAAGA
TACCTCCCTTGAGGATGGATGCCTTGTTTACCACGCTCAAGTTCGTGGAGTTAACTTCCA
TCTAACGGAGCTGTTATGCAGAAGAAGACTAAGGGATGGGAACCAAACACTGAAATGATGT
ACCCAGCTGATGGAGGACTTCGCGGATACACCCACATGGCTCTTAAGGTTGATGGAGGAG
GACACCTTTCTTGCTCTTTCGTTACCACCTACCGCTCTAAGAAGACTGTTGGAAATATTAAG
ATGCCAGGAATCCATGCTGTTCGATCACCGTCTTGAGCGTCTTGAGGAGTCTGATAACGAGA
TGTTTCGTTGTTCAACGTGAGCACGCTGTTCGCTAAGTTCGCTGGACTTGGAGGAGGA