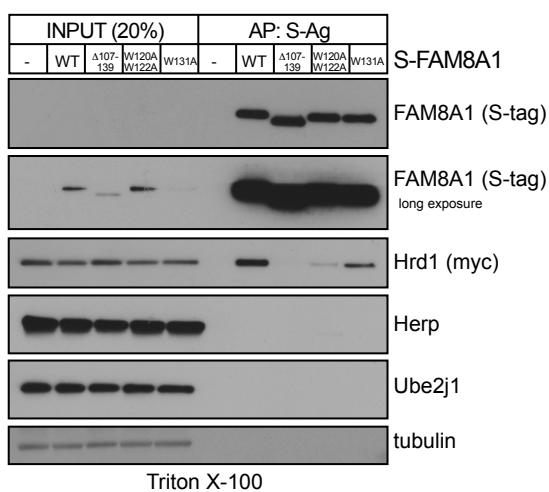
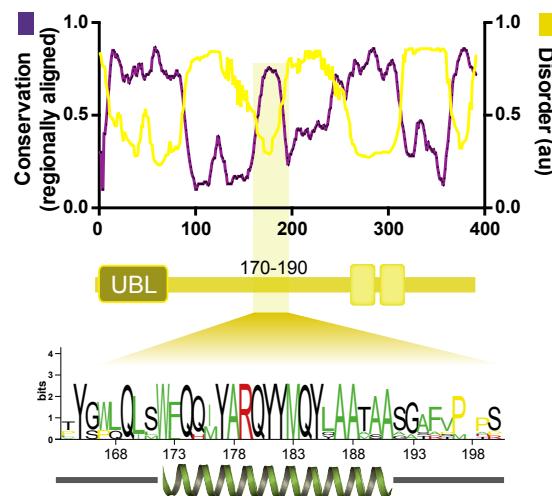
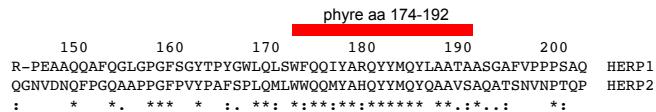
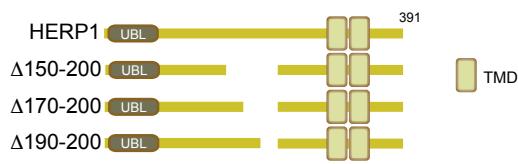
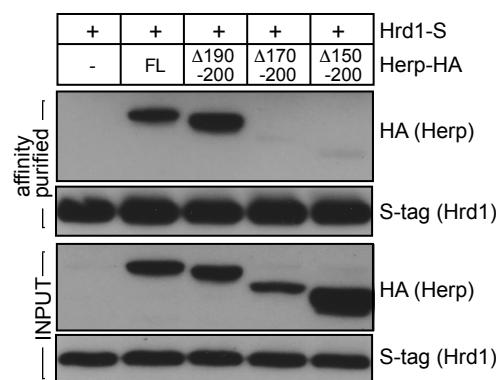
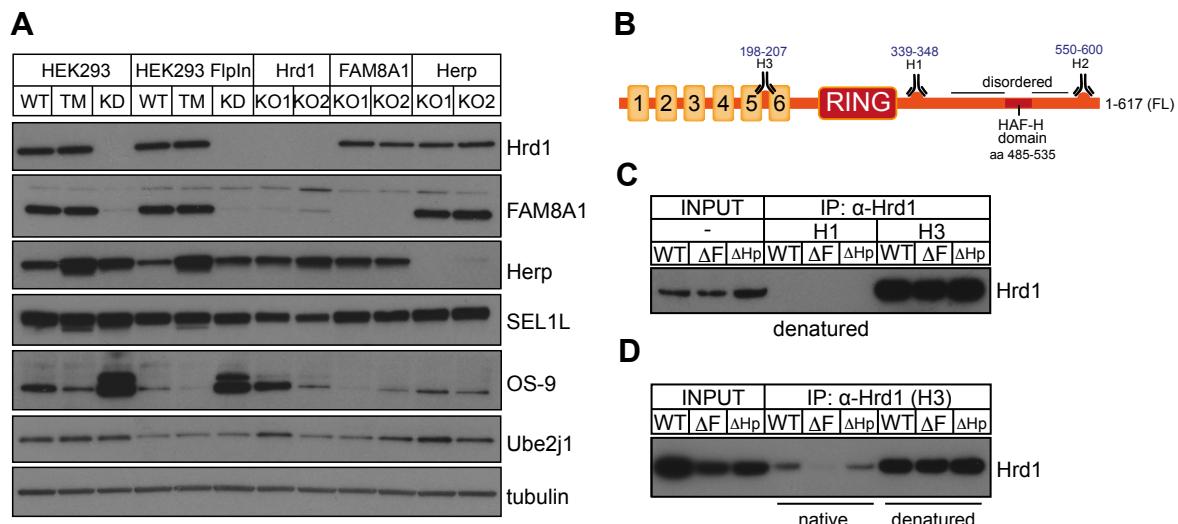


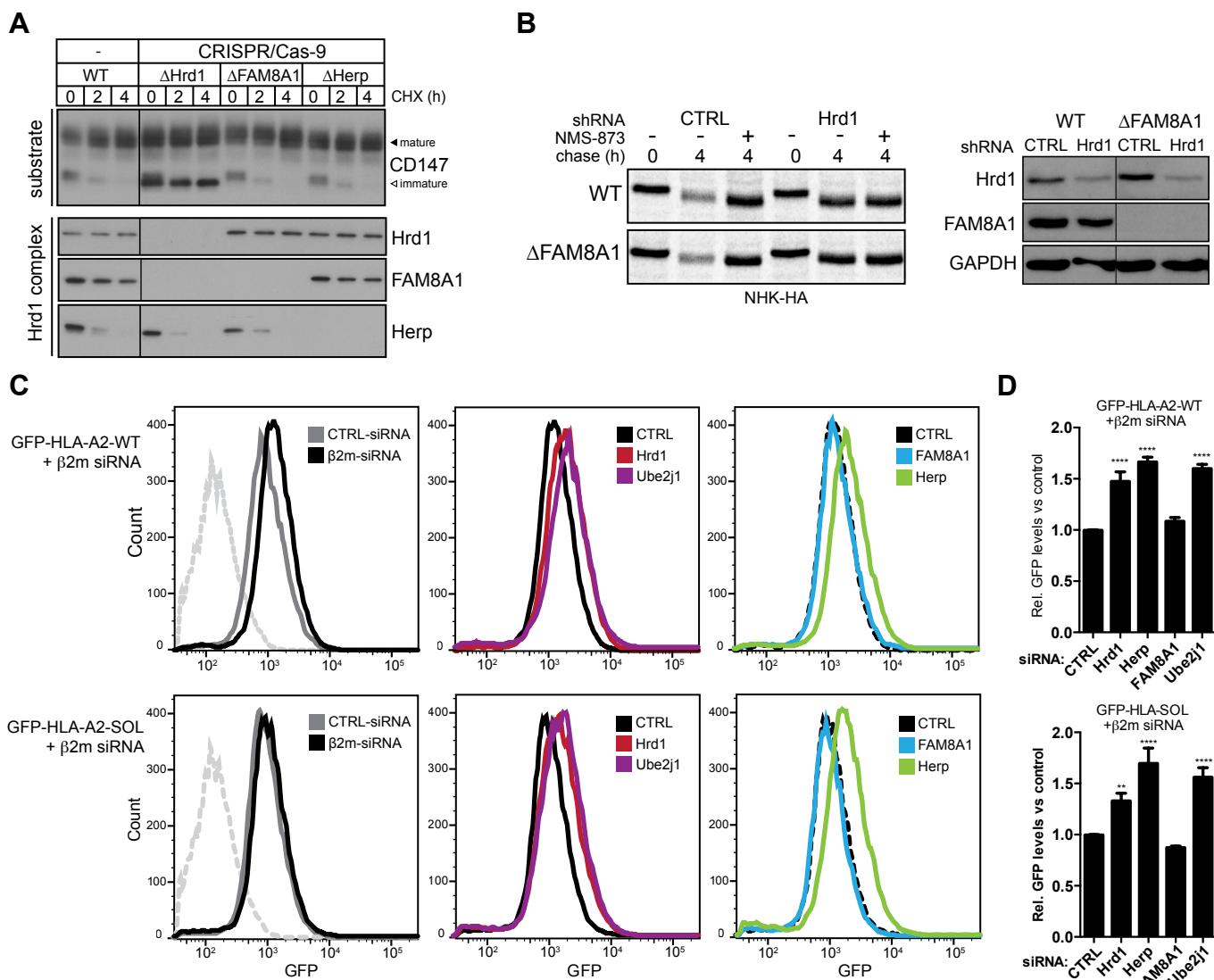
Supplemental Figure S1. (A) Determination of HRD1 mRNA levels in HEK293_{FlipIn/WT} and HEK293_{FlipIn/Hrd1-KD} by qRT-PCR. (B) Hrd1 protein levels in HEK293_{FlipIn/WT} and HEK293_{FlipIn/Hrd1-KD} cells detected by WB. Tubulin is included as a loading control. (C) Comparison of Hrd1-SEL1L complex stability when solubilized in different detergents. Complexes were IPed by anti-SEL1L and resulting WBs probed for the indicated cofactors. (D) Velocity sedimentation of lysates from HEK293_{FlipIn/WT} (top), HEK293_{Flip-In/Hrd1-KD} (middle) and HEK293_{Flip-In/Hrd1-KD} + Hrd1_{FL}-S (bottom) cells solubilized in 1% LMNG-containing buffer on 10-40% sucrose gradients. Individual fractions (1-13) precipitated by TCA were separated by SDS-PAGE with the resulting WBs probed using indicated antibodies. Input is 8% of total amount loaded on gradients. Red boxes indicate fractions where Hrd1 complexes natively migrate. (E) Western blots of lysate from HEK293_{Flip-In/Hrd1-KD} cells + Hrd1₁₋₄₉₉ treated with Usp2cc or Lambda Protein phosphatase (PP) and probed for Ube2j1 and tubulin. Phosphorylated Ube2j1 indicated by arrow (F) Translation shutoff assays of HEK293 (left) and HEK293_{Hrd1-KD} (right) cells using cycloheximide (CHX) for the indicated times and including MG132 (10 μ M, 4 h only). Western blots of 1% LMNG lysates separated by SDS-PAGE were probed for indicated antibodies. The (*) indicates a non specific band while the triangles indicate the 2 isoforms of OS-9 (OS-9.1, OS-9.2). (G) siRNA screen in CHX-treated HEK293_{Hrd1-KD} cells for ER-resident E3 ligases are shown. HEK293WT cells (untreated and TUNIC, 4 hrs.) serve as controls for ER stress and scramble siRNA pool (\pm MG132, 10 μ M) was included as a negative control. Western blots of the resulting lysates were probed with the indicated antibodies. The (*) indicates a non specific band.

A**B****C****D****E**

Supplemental Figure S2. (A) S-tagged FAM8A1 variants (FL, Δ 107-139, W120A/W122A and W131A were transiently co-expressed along with Hrd1-myc (FL) in HEK293_{Fip-In/Hrd1-KD} cells. Following isolation by S-Ag from cells lysed in 1% TritonX-100 containing buffer, the resulting WBs of lysates (20% of AP) and APs were probed for Hrd1 (myc), FAM8A1 (S-tag), Herp, Ube2j1 and tubulin. (B) Secondary structure predictions of protein disorder (MetaDisorder, yellow) and evolutionary conservation (ConSurf, purple) for Herp. Consensus sequence for Herp (aa 170-190) generated by WebLogo 3.0 is shown below. (C) Pairwise alignment of Herp (148-203) and Herp2 (146-202). α -helical content predicted by Phyre2 (red) is shown above sequence. (D) Diagram of Herp nested truncations. The UBL and TM domains are indicated. (E) Hrd1-S and Herp-HA truncations (Δ 150-, Δ 170-, Δ 190-200) transiently co-expressed in HEK293_{FipIn/Hrd1-KD} were affinity purified by S-Ag, separated by SDS-PAGE and resulting WBs probed with antibodies to the S-tag (Hrd1) and HA (Herp).



Supplemental Figure S3. (A) Validation of stable Flp-In™T-REx™ 293 cell lines genomically edited by CRISPR/Cas-9 to disrupt expression of Hrd1, FAM8A1 and Herp. HEK293_{Hrd1-KD} and HEK293_{Flp-In/Hrd1-KD} treated with tunicamycin (TM) are shown alongside 2 independent clones (KO1, KO2) for each target. Western blots of resulting lysates were probed with antibodies towards the indicated targets (Hrd1, FAM8A1, Herp, SEL1L, Ube2j1 and OS-9) with tubulin serving as the loading control. (B) Diagram of Hrd1 topology indicating sites of antibody recognition. (C) Immunoprecipitation of Hrd1 by the indicated antibodies (H1, H3) from lysates of wild-type (WT), Δ FAM8A1 (Δ F) or Δ Herp (Δ Hp) Flp-In™T-REx™ 293 cell lines denatured by 1% SDS. SDS was diluted to 0.1% v/v with 1% Triton X-100 to enable antibody recognition. Input (20%) and IPs were probed by western blot with anti-Hrd1. Denaturation disrupted the epitope recognised by the H1 antibody (Hrd1₃₃₉₋₃₄₈) for IP, while exposing the epitope recognised by H3 (Hrd1₁₉₈₋₂₀₇). (D) Comparison of IP by anti-Hrd1 H3 antibody from cell lines used in (C) and solubilised with Triton X-100 (native) or SDS/Triton X-100 (denatured).



Supplemental Figure S4. (A) Degradation of CD147 monitored by CHX chase (0, 2, 4 hrs; 10 μ g/mL) and WB in Δ Hrd1, Δ FAM8A1 and Δ Herp Flp-In™T-REx™ 293 cells. Resulting WBs probed with antibodies to CD147, Nrf1, Hrd1, FAM8A1 and Herp. (B) Left panel shows a representative 35 S-Met/Cys pulse-chase assays of NHK-HA expressed in WT and Δ FAM8A1 Flp-In™T-REx™ 293 cells (0, 4 hrs) and co-transfected with CTRL or Hrd1 shRNA plasmids (Christianson et al., 2012). Cells were also treated \pm NMS-873 (5nM). Right panel shows western blots for Hrd1, FAM8A1 and GAPDH from lysates of same cells to validate knockdown by shRNA. (C) Representative fluorescence histograms of reporter HeLa cells stably expressing GFP-HLA-A2 WT (left) and GFP-HLA-A2 SOL (right) and transfected with β 2-microglobulin (β 2-m) siRNA along with Hrd1, Ube2j1, FAM8A1 or Herp siRNA. Parental cells are shown (light grey), as are cells expressing a control/scrambled siRNA (dark grey). (D) Quantification of (C). Median values \pm s.e.m. for 3 biological replicates are shown (n=3). Significance is determined by one-way ANOVA, **p>0.01, ***p>0.0001.

SUPPLEMENTAL TABLES**TABLE S1- PCR primer sequences**

PRIMER NAME	SEQUENCE (5' to 3')
Hrd1	
Hrd1 1-617 forward	TATCTAGAGCCACCATGTTCCGCACGGCAGTGATGA
Hrd1 1-617 reverse	TTAGGTACCGTGGCAACAGGAGACTC
Hrd1 1-540 reverse	ATAGGTACCGACTGAAGTGGCAGGCCG
Hrd1 1-499 reverse	TATAGGTACCGTGTGCCGCTCATGGC
Hrd1 1-282 reverse	TTAGGTACCCCTCCTCTGGGTGGCATCTG
Hrd1 1-251 reverse	TTAGGTACCGGCCAGGTACATGGCCGGAT
Hrd1 252-617 forward	TATCTAGAGCCACCATGAGACAGTTAAGAAAGCTG
Hrd1 Δ71-251 forward	AAAGCTCTCTAGACAGTTCAAGAAAGCTGTGACA
Hrd1 Δ71-251 reverse	AAAGCTCTCTCTCAGTTGCCAAAGAACACCTT
Hrd1 Δ1-84 forward	TATCTAGAGCCACCATGGTCACAGAGACTTGTCTGGCCTT
Hrd1 Δ41-124 forward	AAGCTCTTAGCCGATTTGTCTCTTATGTTCTC
Hrd1 Δ41-124 reverse	AAGCTCTTCTGCTGGACTTGGTCAGGTACACCCAC
Hrd1 Δ165-251 forward	AAGCTCTCTAGACAGTTCAAGAAAGCTGTGACA
Hrd1 Δ165-251 reverse	AAGCTCTCTCTGGTCAGGTGCTGTGATAGGC
Hrd1 Δ485-528 forward	CTGACCGCCCTCTGGGGCCCCCCCAGCCTG
Hrd1 Δ485-528 reverse	GGAGGCGGTACAGCCCAGCAAAGCCCGC
Hrd1 L489A forward	GAGCTCTGGAGGGCCATGAGCGGCAG
Hrd1 L489A reverse	TGGCCCTCCAGAGCTGTCGCTCCTCCTC
Hrd1 R503L forward	GGCAGCACCTGGAGGCCCTGCTGCAG
Hrd1 R503L reverse	GCCTCCAGGTGCTGCCGCTCATGGCC
FAM8A1	
FAM8A1 1-413 forward	ACAGCTGCCGGCATCAGCACCCCTGCTCCAGT
FAM8A1 1-413 reverse	CCTCTAGATTATCTGACCCCCATTCTTTTACCC
FAM8A1 1-256 reverse	CCTCTAGATTACACCACATCTGCCATAATCTG
FAM8A1 229-413 forward	TCTGACCGGTGGTACCATCAGTCGATTGCGCG
FAM8A1 Δ107-139 forward	AAGCTCTCTAGCTGGTCTCTCTCGTGGCGCC
FAM8A1 Δ107-139 reverse	AAGCTCTCTGCTTAAGCAGCCCCCAGCCCC
FAM8A1 W120A/W122A TOP	CGCGCAAGTGCACGAGGGCGCTGGCGCAGTCCTACTGCGG
FAM8A1 W120A/W122A BOT	CCGCAGTAGGACTGCGCAGCGCCTGCACTTGCAGG
FAM8A1 W131A TOP	CGGCTACCTCACCGCGCACAGCGGCC
FAM8A1 W131A BOT	CAGGCCGCTGTGCGCGGTGAGGTAGCCG
FAM8A1 100 for	ATAACCGGTCCACGAGAGAGACCAGCTGG
FAM8A1 140 rev EcoRI	TAAGAATTCTGCTAGGCTGGGAAGGCGGCCAG
HERP	
Herp Δ150-200 forward	CCCAAGCAGGCACAAGAGATAACCTGTGGTC
Herp Δ150-200 reverse	TCTTGTCCTGCTGGCAGCTTCAGG
Herp Δ170-200 forward	CTTCAGCTTGACAAGAGATAACCTGTGGTC
Herp Δ170-200 reverse	TCTTGTCAGCTGAAGGCCACCATAG
Herp Δ190-200 forward	CCACTGCTGACAAGAGATAACCTGTGGTC
Herp Δ190-200 reverse	TCTTGTCAGTGGCTGCTAAATATTG

TABLE S2 – sgRNA sequences

Gene	Vector	Selection	Function	sgRNA name	Target site	Vector name	Vector #	Used in study
FAM8A1	pX459	Puro	Frame shift mutation	JC_hFAM8A1_1	GCGCGGCGCTCCAATTGTCGG	pX459-JC_hFAM8A1_1	395	
	pX459	Puro		JC_hFAM8A1_2	TTCCGCGTGGGGTGTGCGGG	pX459-JC_hFAM8A1_2	396	
	pX459	Puro		JC_hFAM8A1_3	CACTGCCGGAGTACTGCGGG	pX459-JC_hFAM8A1_3	397	*
HERPUD1	pX459	Puro	Frame shift mutation	JC_hHERP_1	CCGCTCTCACCGGACGCTCGGGG	pX459-JC_hHERP_1	398	*
	pX459	Puro		JC_hHERP_2	AAGTCGGTGGCGCTGGTGGG	pX459-JC_hHERP_2	399	
	pX459	Puro		JC_hHERP_3	TTTCATGATACCGCTCAGAGG	pX459-JC_hHERP_3	400	
SYVN1	pX459	Puro	Frame shift mutation	JC_hSYVN1_1	GGCCAGGGCAATGTCGCACGG	pX459-JC_hSYVN1_1	407	*
	pX459	Puro		JC_hSYVN1_2	CTTGGTCAGGTACACCACAGTGG	pX459-JC_hSYVN1_2	408	
	pX459	Puro		JC_hSYVN1_3	GTGATGGCAAGGTGTTCTTGG	pX459-JC_hSYVN1_3	409	

TABLE S3 – qRT-PCR primers

PRIMER NAME	DIRECTION	sequence (5'-3')
FAM8A1_2F	FORWARD	AAAATGATGGTTGTGGCACCTTA
FAM8A1_2R	REVERSE	TGTATCACATGTACAACCTCGAA
Hrd1_1F	FORWARD	TCTTCCTCAAATGTTCCACTG
Hrd1_1R	REVERSE	TCGTCACTCAGGATGGCATAA
GAPDH_F	FORWARD	ACCCACTCCTCCACCTTTGA
GAPDH_R	REVERSE	CATACCAGGAAATGAGCTTGACAA

TABLE S4 - Cumulative worm lifespans n=4

<i>C. elegans</i> strain	Temp °C	Mean lifespan ± S.E.M. (Days)	N (number of animals assayed)	% mean lifespan change compared to WT	P-value vs. WT (t-test)
Wt	20	18.50 ± 0.35	180		
<i>cup-2&tag-353(gk443)l</i>	20	18.94 ± 0.35	183	2.38	0.3790 (ns)
<i>F48B9.8</i> (gk272969)	20	20.17 ± 0.50	183	9.03	0.0072 (**)
<i>sel-11 (nDf59) V.</i>	20	10.47 ± 0.19	198	-43.4	< 0.0001 (***)
<i>sqt-3(sc8) sel-1 (e1948)V</i>	20	10.12 ± 0.16	193	-45.29	< 0.0001 (***)

Lifespan assay 1

<i>C. elegans</i> strain	Temp °C	Mean lifespan ± S.E.M. (Days)	N (number of animals assayed)	% mean lifespan change compared to WT	P-value vs. WT (t-test)
Wt	20	18.76 ± 0.67	45		
<i>cup-2&tag-353(gk443)l</i>	20	18.47 ± 0.61	45	-1.55	0.7513 (ns)
<i>F48B9.8</i> (gk272969)	20	21.53 ± 1.02	45	14.77	0.0256 (*)
<i>sel-11 (nDf59) V.</i>	20	9.7 ± 0.28	50	-48.3	< 0.0001 (****)
<i>sqt-3(sc8) sel-1 (e1948)V</i>	20	9.90 ± 0.32	49	-47.23	< 0.0001 (****)

Lifespan assay 2

<i>C. elegans</i> strain	Temp °C	Mean lifespan ± S.E.M. (Days)	N (number of animals assayed)	% mean lifespan change compared to WT	P-value vs. WT (t-test)
Wt	20	18.11 ± 0.72	45		
<i>cup-2&tag-353(gk443)l</i>	20	19.22 ± 0.71	50	6.13	0.2774 (ns)
<i>F48B9.8</i> (gk272969)	20	19.73 ± 1.00	48	8.95	0.1978 (ns)
<i>sel-11 (nDf59) V.</i>	20	10.71 ± 0.40	45	-40.86	< 0.0001 (****)
<i>sqt-3(sc8) sel-1 (e1948)V</i>	20	9.83 ± 0.28	46	-45.72	< 0.0001 (****)

Lifespan assay 3

<i>C. elegans</i> strain	Temp °C	Mean lifespan ± S.E.M. (Days)	N (number of animals assayed)	% mean lifespan change compared to WT	P-value vs. WT (t-test)
Wt	20	18.61 ± 0.70	46		
<i>cup-2&tag-353(gk443)l</i>	20	19.29 ± 0.74	45	3.65	0.5031 (ns)
<i>F48B9.8</i> (gk272969)	20	19.7 ± 1.016	44	5.86	0.3720 (ns)
<i>sel-11 (nDf59) V.</i>	20	10.46 ± 0.31	50	-43.79	< 0.0001 (****)
<i>sqt-3(sc8) sel-1 (e1948)V</i>	20	10.35 ± 0.34	48	-44.38	< 0.0001 (****)

Lifespan assay 4

<i>C. elegans</i> strain	Temp °C	Mean lifespan ± S.E.M. (Days)	N (number of animals assayed)	% mean lifespan change compared to WT	P-value vs. WT (t-test)
Wt	20	18.52 ± 0.76	44		
<i>cup-2&tag-353(gk443)l</i>	20	18.74 ± 0.77	43	1.19	0.8383 (ns)
<i>F48B9.8</i> (gk272969)	20	19.74 ± 1.00	46	6.59	0.3397 (ns)
<i>sel-11 (nDf59) V.</i>	20	10.35 ± 0.36	49	-44.11	< 0.0001 (****)
<i>sqt-3(sc8) sel-1 (e1948)V</i>	20	10.15 ± 0.35	48	-45.19	< 0.0001 (****)