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Supplementary Figures:

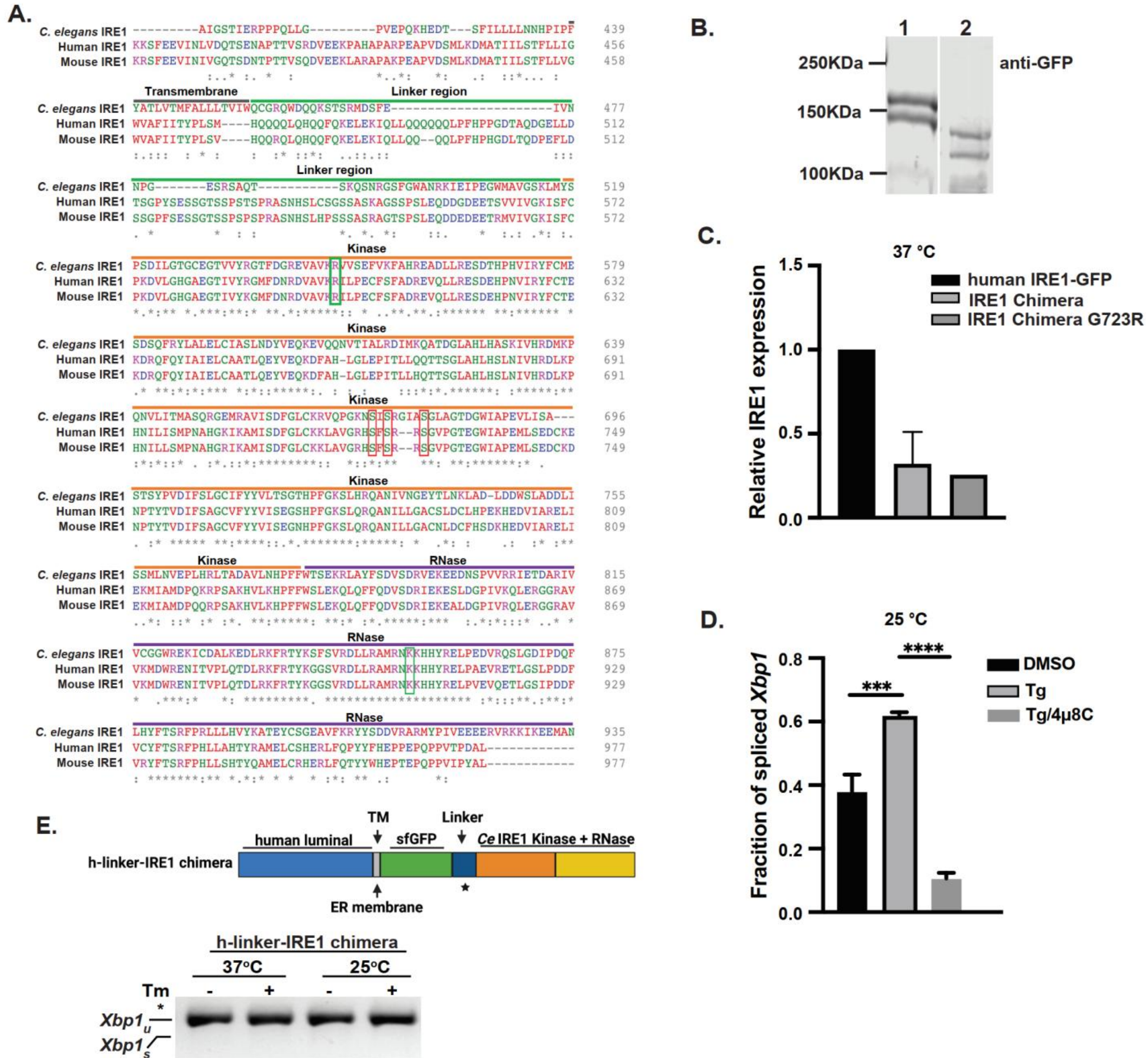

Figure S1. IRE1 chimera with *C. elegans* linker region is functional in human cells. Related to Figure 1. **(A)** Alignment of cytosolic domains of mammalian and *C. elegans* IRE1s (Clustal Omega[107]). *****, indicates fully conserved; **:**, highly similar; **·**, weakly similar. Partial transmembrane domain (grey), linker region (green), kinase domain (orange) and RNase domain (purple) of *C. elegans* IRE1 are indicated. Red boxes: serine residues in the activation loop of kinase domain; green boxes: 4μ8C contact sites. **(B and C)** Western blot (with anti-GFP) and quantitation (with anti-N-term IRE1) of transgenic IRE1 proteins stably expressed in HAP1 IRE1KO cells. Tubulin was used as a loading control. **(D)** IRE1 chimera is active in *Xbp1* splicing in human cells, and its basal activity is augmented by Tm and inhibited by IRE1-selective inhibitor 4μ8C. Cells were treated with Tm (4μg/ml) for 4hrs at 25°C; splicing activity was analyzed by RT-PCR assay.Anove with Tukey correction, n=3. **(F)** IRE1 chimera with human linker is not functional. Protein with human IRE1 linker region (star) was expressed in HAP1 IRE1KO cells. Cells were treated and analyzed at indicated temperatures, as in D.

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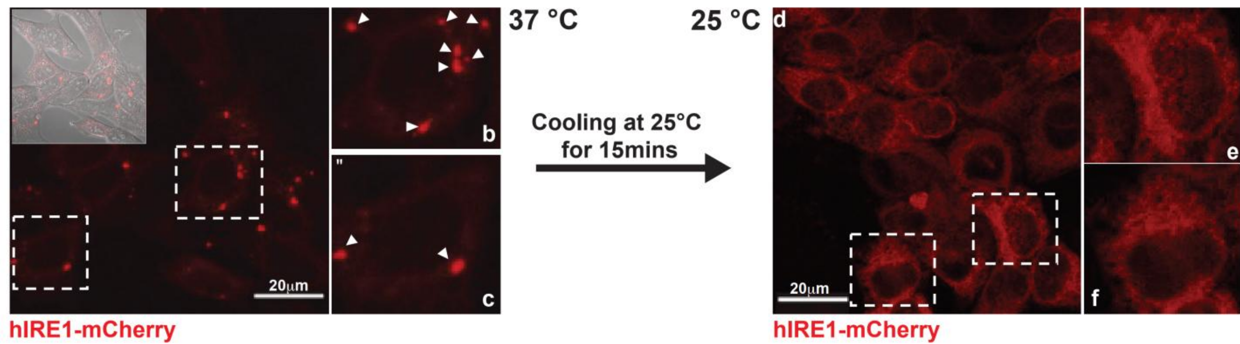

Figure S2. IRE1 clusters are rapidly dispersed upon temperature downshift. Related to Figure 1. HAP-1 IRE1KO cells expressing human IRE1-mCherry were treated with Tm (4µg/ml) at 37°C and then cooled for 15 minutes at 25°C . Maximum intensity projections of confocal z-stacks are shown; scale bars = 20µm. Arrowheads point to clusters; inset in 37°C image shows DIC image; close-ups are of individual cells.

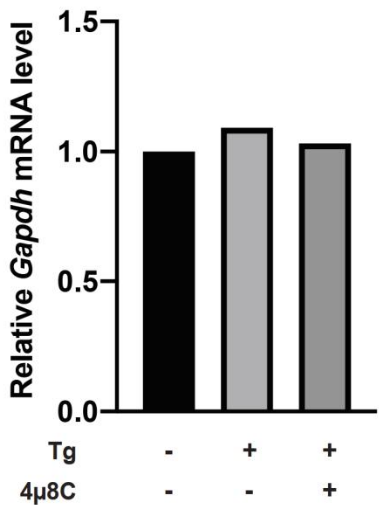

Figure S3. IRE1 activation does not cause general downregulation of mRNA. Related to Figure 2. RT-qPCR analysis of non-RIDD substrate *Gapdh* mRNA levels in the HAP1 IRE1KO cells expressing IRE1 chimeras. Cells were treated with 2.5µM Tg and 16µM 4µ8C for 4hrs at 25°C. *Gapdh* mRNA was normalized to *Rpl19*. Means of 2 independent experiments.

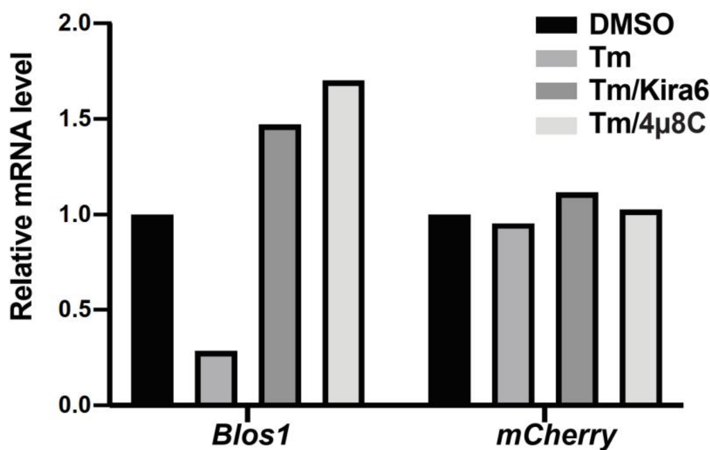

Figure S4. *mCherry* is not a RIDD target. Related to Figure 3. RT-qPCR analysis in HAP1 IRE1KO cells expressing human IRE1-GFP. Cells were treated with Tm (4µg/ml) and IRE1-specific inhibitors KIRA6[73] (1µg/ml) and 4µ8C (16µM) for 6hrs. Normalized to *Rpl19*

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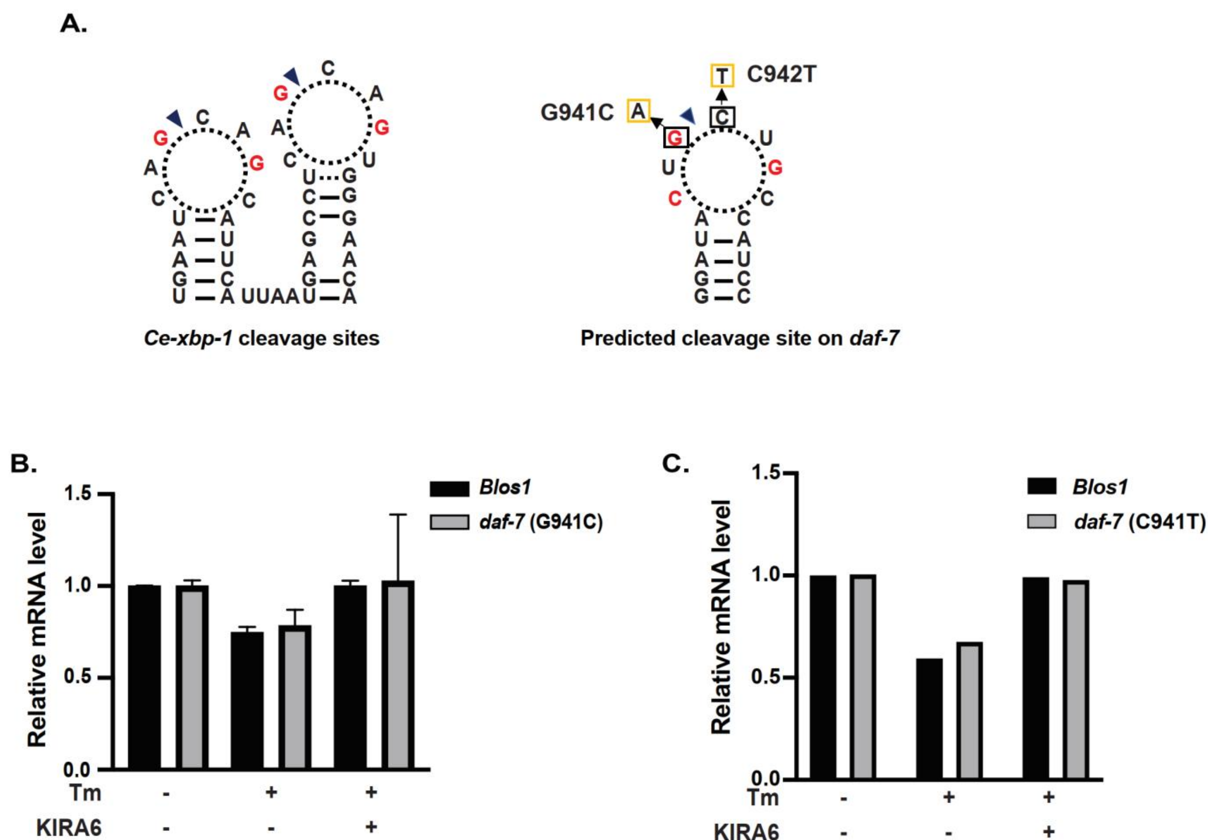

Figure S5. *daf-7* mRNA likely contains IRE1 cleavage sites that are different from the canonical one. Related to Figure 3. **(A)** Predicted IRE1 recognition and cleavage sites on *daf-7* mRNA. Left, double-stem loop and cleavage site(s) (arrowheads) in the known *C. elegans* IRE1 substrate *xbp-1* mRNA; right, stem loop with a predicted recognition and cleavage (arrowhead) site on *daf-7* mRNA. The potential cleavage site (dark triangle) is shown on the right, mutations are indicated. (**B and C)** Mutating predicted IRE1 cleavage site does not affect *daf-7* downregulation. RT-qPCR analysis of *Blos1* and mutated *daf-7* mRNA in WT HAP1. Cells treated as in Fig. S4. 3 independent experiments for *daf-7* with G941C mutation, error bars: mean+/-SD.

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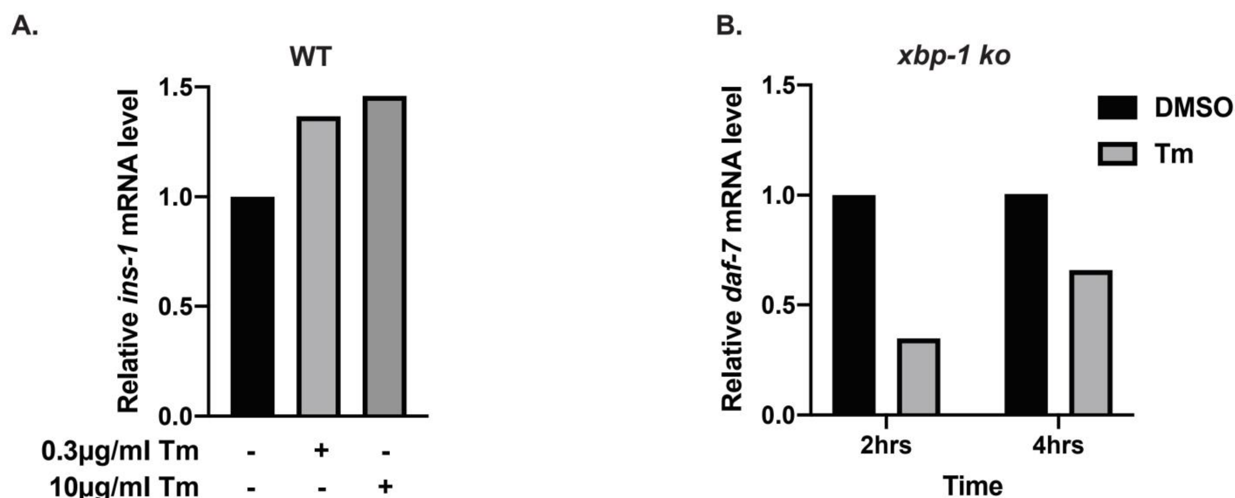

Figure S6. RIDD in *C. elegans* is XBP-1 independent and substrate selective. Related to Figure 4. **(A)** *ins-1* mRNA is not downregulated by IRE1 in *C. elegans.* Late L1 larvae were treated with either 0.3µg/ml or 10µg/ml Tm for 4hrs and analyzed by RT-qPCR. The *ins-1* transcript level change was normalized to *actin*. **(B)** Downregulation of *daf-7* mRNA by activated IRE1 is XBP-1-independent. Late L1 animals were treated with 10µg/ml Tm for 2hrs or 4hrs. *daf-7* transcripts were normalized to *actin*.

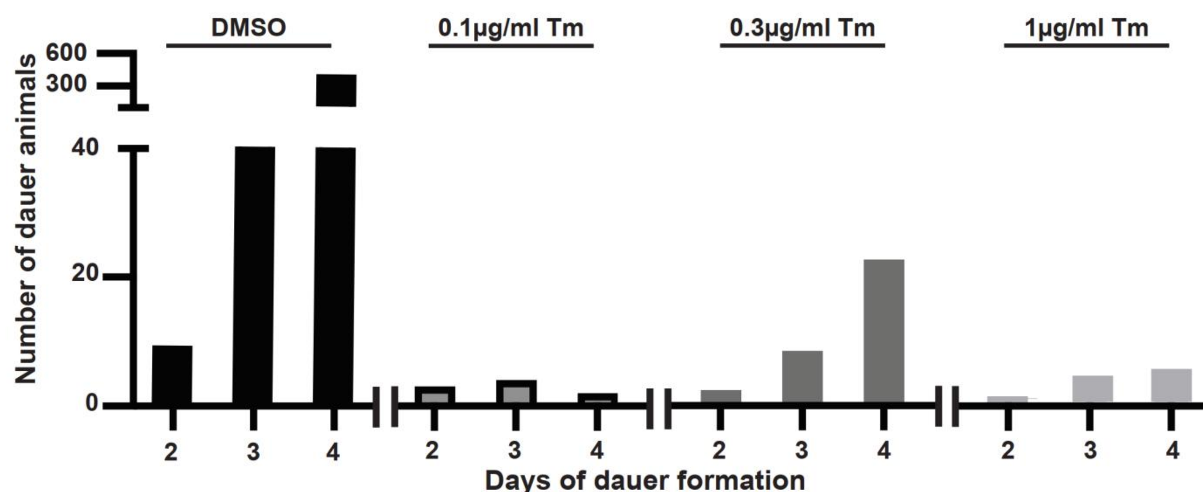

Figure S7. Tm treatments inhibit dauer transition even at low concentrations. Related to Figure 5. Two wild type L4 animals were placed onto small plates freshly seeded with bacteria and containing indicated concentration of Tm at 25°C. The animals were allowed to grow until all food was exhausted, and number of dauers was determined after 2, 3 and 4 days of starvation.

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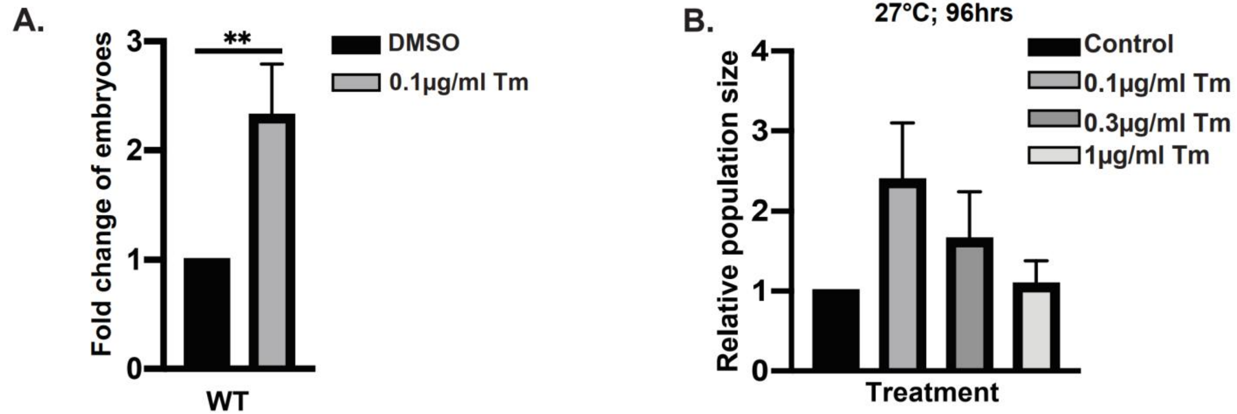

Figure S8. Pre-treatment with low Tm concentrations improves reproduction and population growth under environmental stress. Related to Figure 5. (**A**) Wild type embryos were plated as in Figure 5A and grown at 27°C with or without 0.1µg/ml Tm. After 72hrs, the number of embryos present on plates was counted. 3 independent experiments, error bars are means with SD, *t*-test, significance as described in material and method. **(B)** Low Tm concentrations are still protective when animals are allowed to grow at 27°C for extended times, reaching complete starvation 96hrs. Plates were set up as in Figure 5A, the data shown are from 3 independent experiments.

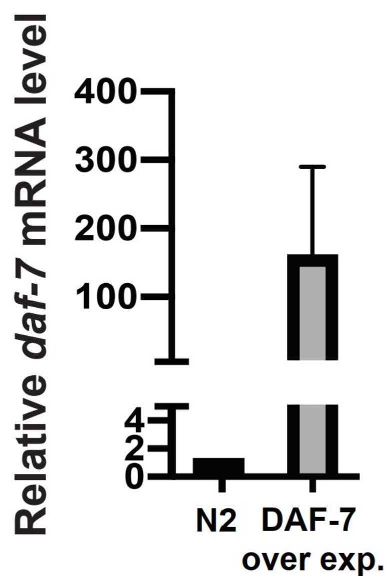

Figure S9. Quantitation of *daf-7* overexpression in transgenic animals. Related to Figure 5. RT-qPCR analysis of *daf-7* mRNA level in late L1 larvae, normalized to actin. DAF-7 over exp.: p*daf-7::mcherry::daf-7*) . 3 independent experiments, error bars: mean+/-SD.