

Supplementary Legends

Fig. S1. RtcB protein alignment.

Protein alignment generated using ClustalOmega and BoxShade. Protein accessions are denoted after the species name. Site of H428A point mutation used in Fig. 3 is denoted by a star.

Fig. S2. RtcB genomic locus.

Representation of the *C. elegans* *rtcB-1/RtcB* genomic locus (F16A11.2) on chromosome I. Teal bars represent exons, connecting lines are introns. The *gk451* deletion used in this research is represented by the yellow bar. The peach bar indicates the genomic region used to obtain single-copy insertion (MosSCI) rescue of the *gk451* allele. Modified from WormBase.

Fig. S3. RtcB splices tRNA^{Tyr(GUA)} in *C. elegans*.

Northern blots of RNA extracted from wild type and RtcB mutant worms, probed for tRNA^{Tyr(GUA)}. **(a)** tRNA halves accumulate in RtcB mutants (red arrowheads). **(b)** Levels of spliced mature tRNA^{Tyr(GUA)} are reduced in RtcB mutants (green arrowheads). **(c)** Untrimmed, spliced pre- tRNA^{Tyr(GUA)} intermediates are observed in wild type but not RtcB mutants (yellow arrowheads). **(d)** Unspliced pre- tRNA^{Tyr(GUA)} accumulates in RtcB mutants (blue arrowheads).

Fig. S4. Prespliced tRNAs do not rescue defective UPR activation in RtcB mutants.

Brightfield (top panels) and fluorescence micrographs (middle and bottom panels) of animals expressing prespliced tRNAs. Animals were either untreated (UT) or tunicamycin-treated (Tm), and carry the *Phsp-4::GFP* UPR reporter. Control animals (*RtcB/+*) express GFP in response to ER stress, but *RtcB* null mutants do not, and this is not rescued by expression of prespliced tRNAs (C). Arrowheads indicate GFP expression in the pharynx from the injection marker. Upper and middle panels scale bars, 100 μm ; bottom panels scale bars, 20 μm .

Fig. S5. *RtcB* mutants have defects in vulval development.

Representative DIC images of vulvae from wild type animals, *RtcB* null mutants, *RtcB* null mutants expressing wild type *RtcB* (+ rescue), *RtcB* null mutants expressing prespliced tRNAs (+ tRNA), *ire-1* mutants, and *xbp-1* mutants. Arrowheads point to the location of the vulva. Stars indicate embryos. Phenotypes were consistent across N=20 animals per genotype.

Fig. S6. *RtcB* null mutants display defective germlines.

Representative DIC images of germlines in wild type, *RtcB* null, and tRNA-rescued *RtcB* null worms. Asterisks mark developing oocytes. A few enlarged oocyte-like cells are observed in some tRNA rescued worms, but they remain completely sterile.

Table S1. Analysis of *RtcB*-dependent codons in *C. elegans* genes.

Twenty-seven *C. elegans* genes contain 4 or more *RtcB*-dependent codons in a row. The total number of codons, *RtcB*-dependent codons, and gene information are included for

each.

Table S2. Genes upregulated in wild type worms upon tunicamycin treatment.

121 genes had a fold change greater than or equal to 1.75 in wild type worms comparing tunicamycin treatment to untreated. ‘Gene’ is WormBase gene name. ‘Control RPKM’ is reads per kilobase per million (RPKM) for control animals under normal conditions. ‘Control+Tm RPKM’ is reads per kilobase per million (RPKM) for control animals under ER stress induced by tunicamycin. ‘Control+Tm/Control Log2 Fold Change’ is Log2 of the ratio of RPKM of control animals under stress compared to control animals under normal conditions. ‘RtcB RPKM’ is reads per kilobase per million (RPKM) for RtcB animals under normal conditions. ‘RtcB+Tm RPKM’ is reads per kilobase per million (RPKM) for RtcB animals under ER stress induced by tunicamycin. ‘RtcB+Tm/RtcB Log2 Fold Change’ is Log2 of the ratio of RPKM of RtcB animals under stress compared to RtcB animals under normal conditions. Data for all genes were sorted by the Log2 ration in control animals (‘Control+Tm/Control Log2 Fold Change’), and the table shows all genes for which this value was > 1.75 .

Table S3. Genes upregulated in RtcB null worms upon tunicamycin treatment.

135 genes had a fold change greater than or equal to 1.75 in RtcB null worms comparing tunicamycin treatment to untreated. Same data as Extended Data Table 2, but data for all genes were sorted by the Log2 ration in RtcB animals (‘RtcB+Tm/RtcB Log2 Fold Change’), and the table shows all genes for which this value was > 1.75 .