Supplementary materials for Gaddam et al, Comparison of mRNA localization and regulation during endoplasmic reticulum stress in *Drosophila* cells

Table S1. Statistical analysis of mRNA decay changes during ER stress. We conducted paired t-tests (identical spots were paired) for differences between actinomycin treated samples in the presence vs. absence of ER stress and between control vs. Ire1 RNAi samples in the presence of ER stress. Shown are the p-values from t-tests for spots within a given window of FM values, defined by a sliding window of 50 spots. Similar results were obtained using a sliding window of 25 spots or with a defined range of FM values (for example, an FM range of 0.2).

range of FM values	pvalue for paired t-test, control –DTT vs. control +DTT	pvalue for paired t-test, control +DTT vs. Ire1 RNAi +DTT
above 0.85	$<10^{-5}$	$<10^{-5}$
0.82-0.85	$<10^{-5}$	$<10^{-5}$
0.80-0.82	$<10^{-5}$	$<10^{-5}$
0.76-0.80	$<10^{-5}$	$<10^{-5}$
0.74-0.76	$<10^{-5}$	$<10^{-5}$
0.71-0.74	$<10^{-5}$	$<10^{-5}$
0.68-0.71	$<10^{-5}$	$<10^{-5}$
0.66-0.68	$<10^{-5}$	$<10^{-5}$
0.63-0.66	$<10^{-5}$	$<10^{-5}$
0.60-0.63	$<10^{-5}$	$<10^{-5}$
0.58-0.60	0.0003	$<10^{-5}$
0.57-0.58	0.0003	0.0009
0.55-0.57	0.00001	$<10^{-5}$
0.54-0.55	0.0006	0.00006
0.53-0.54	0.35	0.11
0.52-0.53	0.02	0.02
0.51-0.52	0.39	0.17
0.50-0.51	0.33	0.48

Table S2. mRNAs displaying weak RIDD (two-fold less degradation than predicted based on FM), for which the expected RIDD score is <-1.0 (i.e., two-fold degradation is expected based on FM). The expected scores were determined by a linear fit of the RIDD score vs. FM for all mRNAs with FM>0.54. GO terms enriched in this list, compared to all RNAs from the array data with expected RIDD scores of <-1.0, included 5 terms associated with neuron development and differentiation (N=3, p-value< 0.025, calculated using the DAVID Bioinformatic database (Huang da *et al.*, 2009)). NA=not annotated.

gene ID	gene name	RIDD factor	FM	function	signal sequence
CG7013	Mesencephalic astrocyte-derived neurotrophic factor (ManF)	0.37	0.87	neuron homeostasis	Y
CG13388	A kinase anchor protein 200 (Akap200)	0.59	0.80	signaling	N
CG10497	Syndecan	0.13	0.79	axon guidance	Y
CG5885	-	-0.05	0.83	protein trafficking	N
CG10811	eukaryotic translation initiation factor 4G	0.07	0.79	translation	N
CG12918	-	0.00	0.80	NA	Y
CG11081	plexin A (plexA)	-0.13	0.83	axon guidance	Y
CG7523	-	-0.07	0.81	NA	N
CG10470	-	-0.13	0.82	NA	Y
CG1291	-	0.00	0.77	glycolipid transferase	N

Table S3. mRNAs displaying strong RIDD (two-fold more degradation than predicted based on FM), for which the expected RIDD score is <-1.0 (i.e., two-fold degradation is expected based on FM). The expected scores were determined by a linear fit of the RIDD score vs. FM for all mRNAs with FM>0.54. GO terms enriched in this list, compared to all RNAs from the array data with expected RIDD scores of <-1.0, included 4 terms associated with the extracellular matrix and cell adhesion (N=3-5, p-value< 0.025, calculated using the DAVID Bioinformatic database (Huang da *et al.*, 2009)). NA=not annotated.

gene ID	gene name	RIDD factor	FM	function	signal sequence
CG3979	I´m not dead yet	-4.82	0.83	transmembrane transport	N
CG8947	26-29kD-proteinase	-4.41	0.90	NA (protease)	Y
CG6453	-	-3.67	0.87	NA	Y
CG7123	LanB1	-3.36	0.89	embryonic morphogenesis	Y
CG6378	BM-40-SPARC (sparc)	-3.30	0.91	cell adhesion	Y
CG10449	Catecholamines up	-3.13	0.86	transmembrane transport	Y
CG3984	-	-3.12	0.85	NA	Y
CG3322	Laminin B2	-3.05	0.89	embryonic morphogenesis	Y
CG4821	Tequila	-3.04	0.90	NA (protease)	Y
CG14464	-	-2.95	0.85	NA	N
CG2915	-	-2.83	0.79	NA (protease)	Y
CG4572	-	-2.69	0.85	RNA transport	Y
CG1471	Ceramidase	-2.66	0.84	synaptic transmission	Y
CG3488	alpha/beta hydrolase 2 (Hydr2)	-2.47	0.88	lipid metabolism	Y
CG9302	-	-2.45	0.86	redox homeostasis	Y
CG11527	Tiggrin	-2.43	0.87	axon guidance	Y
CG2206	lethal (1) G0193	-2.32	0.85	NA	Y
CG1275	-	-2.32	0.82	NA	Y
CG12369	Lachesin	-2.18	0.77	tracheal development	Y

Table S4. Confirmed RIDD targets from mammalian cells. Confirmed RIDD targets from cell lines displayed increased decay rates during ER stress in wildtype cells (not overexpressing Ire1); for liver samples mRNAs displayed decreased abundance in response to tunicamycin-injection.

RIDD target	Sequence ID	Cell type or organism	Xbp1-like cleavage site	Reference
Heparan-alpha-glucosaminide N-acetyltransferase	NM_029884	Mouse embryonic fibroblasts	yes	(Hollien *et al.*, 2009)
Biogenesis of lysosome-related organelles complex-1, subunit 1	NM_015740	Mouse embryonic fibroblasts	yes	(Hollien *et al.*, 2009)
Scavenger receptor class A, member 3	NM_172604	Mouse embryonic fibroblasts	yes	(Hollien *et al.*, 2009)
Platelet derived growth factor receptor, beta polypeptide	NM_001146268	Mouse embryonic fibroblasts	yes	(Hollien *et al.*, 2009)
Peripheral myelin protein	NM_008885	Mouse embryonic fibroblasts	yes	(Hollien *et al.*, 2009)
Collagen, type VI, alpha 1	NM_009933	Mouse embryonic fibroblasts	yes	(Hollien *et al.*, 2009)
Insulin (Ins1)	NM_019129	INS-1 (rat insulinoma)	no	(Han *et al.*, 2009)
Glycosyltransferase-like 1B	NM_199107	INS-1 (rat insulinoma)	yes	(Han *et al.*, 2009)
BiP	NM_013083	INS-1 (rat insulinoma)	yes	(Han *et al.*, 2009)
Cytochrome P450 family 1, subfamily a, polypeptide 2	NM_009993	mouse liver	yes	(Hur *et al.*, 2012)
Cytochrome P450 family 2, subfamily e, polypeptide 1	NM_021282	mouse liver	no	(Hur *et al.*, 2012)

Table S5. Primers used for qPCR.

Gene Name	Annotation symbol	Primer1	Primer 2
Act5C (actin)	CG4027	ATGTGTGACGAAGAAGTTGCT	GAAGCACTTGCGGTGCACAAT
sparc	CG6378	AAAATGGGCTGTGTCCTAACC	TGCAGCACAATCTACTCAATCC
Hydr2	CG3488	CGCATACACGACTATTTAACGC	TTTGGTTTCTCTTTGATTTCCG
Akap200	CG13388	AACAACAAAAGAACGCAACG	TGGATGTGTTTTTGGGGACT
Manf	CG7013	GAGGAGGACTGCGAAGTTTG	GTGCGTCCTTCTTCTTCAGC
CG5885	CG5885	TCCTGTTCGTCCTGGTGAC	CGTAGTCGGCTACCTCGTTC
plexA	CG11081	GGCAAAGACTCACCAAGCTC	TTTAAAAGCCAATCGCTGCT
Atf4/crc	CG8669	AGACGCTGCTTCGCTTCCTTC	GCCCGTAAGTGCGAGTACGCT
Gadd34	CG3825	CGGTGAGGATGAAAATACCG	GTCAGGACGGCATTGAGAAT
Ribosomal Protein L19 (Rpl19)	CG2746	AGGTCGGACTGCTTAGTGACC	CGCAAGCTTATCAAGGATGG
sparc reporters		TGCAGCACAATCTACTCAATCC	GTAGAATCGAGACCGAGGAGAG
GFP reporter		CCTGAAGTTCATCTGCACCA	TGCTCAGGTAGTGGTTGTCG

References for Supplementary Tables

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Hollien, J., Lin, J.H., Li, H., Stevens, N., Walter, P., and Weissman, J.S. (2009). Regulated Ire1-dependent decay of messenger RNAs in mammalian cells. J Cell Biol *186*, 323-331.

Huang da, W., Sherman, B.T., and Lempicki, R.A. (2009). Systematic and integrative analysis of large gene lists using DAVID bioinformatics resources. Nat Protoc *4*, 44-57.

Hur, K.Y., So, J.S., Ruda, V., Frank-Kamenetsky, M., Fitzgerald, K., Koteliansky, V., Iwawaki, T., Glimcher, L.H., and Lee, A.H. (2012). IRE1alpha activation protects mice against acetaminophen-induced hepatotoxicity. J Exp Med *209*, 307-318.

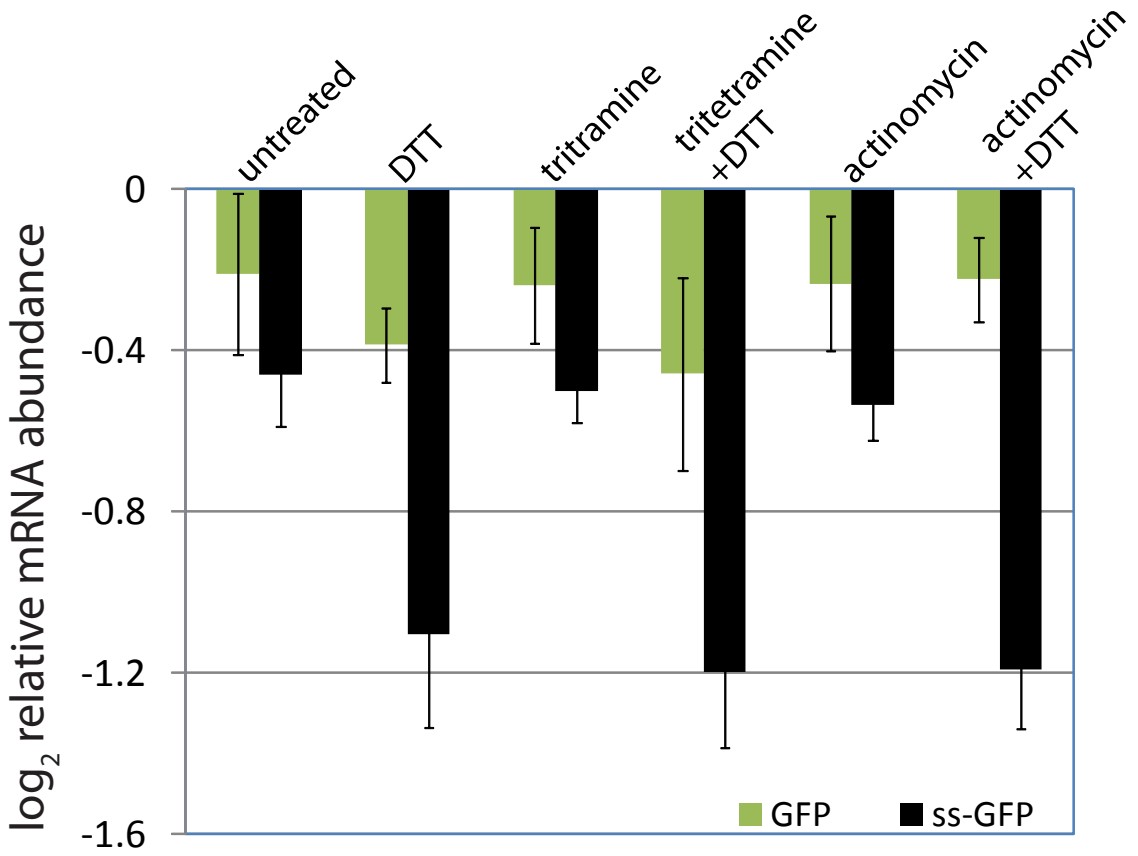

Figure S1. Copper-washout is an effective method of turning off transcription of reporter mRNAs. GFP and ss-GFP (from Figure 1) were placed under the control of the copper-inducible metallothionein promoter and stably transfected into *Drosophila* S2 cells. We induced expression for 3 hours, removed copper and added either triethylenetretramine (200 uM) to chelate remaining copper or actinomycin (1 ug/mL) to block transcription. We then measured mRNA levels after 3 hours in the absence and presence DTT (2 mM), by qPCR. GFP mRNA levels were normalized to that of Rpl19. Shown is the average and SD of 3 independent experiments.

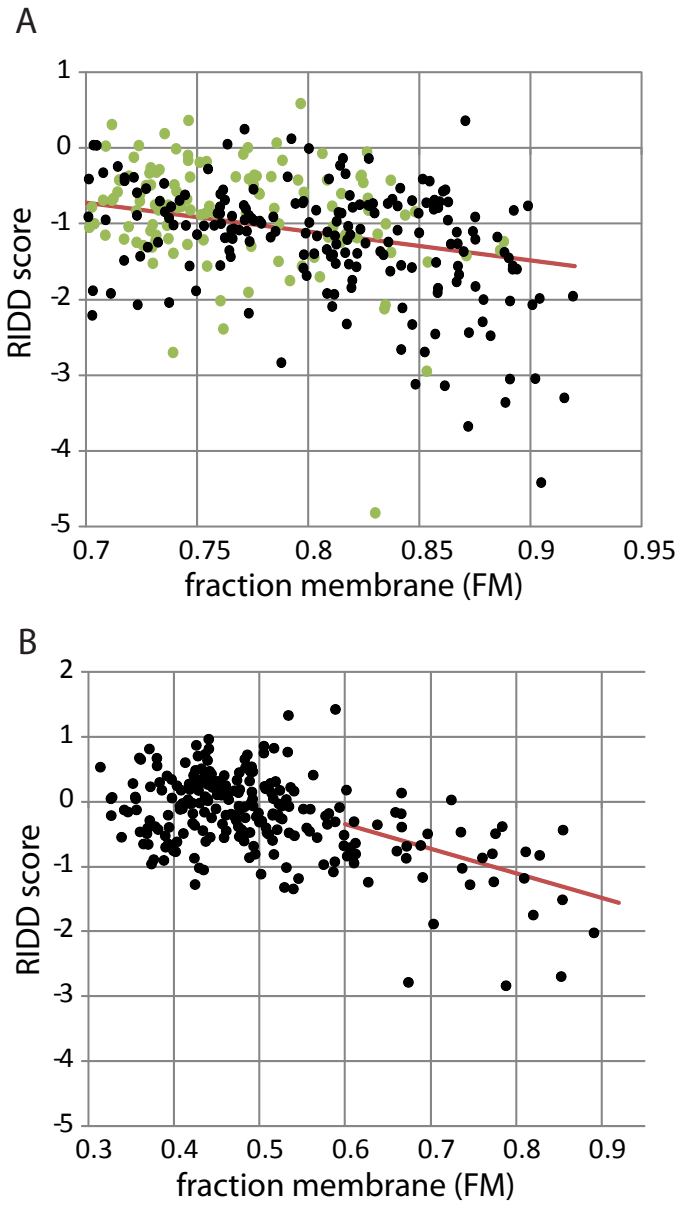

Figure S2. RIDD depends on membrane association of the target mRNA and not on the presence of a predicted signal sequence or a canonical Ire1 cleavage site. (A) RIDD score vs. FM as in Figure 2B-C for mRNAs with FM>0.70 and containing predicted signal sequence coding regions (black) or lacking both predicted signal sequence and transmembrane domain coding regions (green). (B) RIDD score vs. FM for mRNAs containing predicted Ire1 cleavage sites, defined as a stem-loop structure with a loop sequence of CnGCnGn and a 5 base-pair stem, allowing for GC, AU, and GU pairs. For all, the red line indicates a linear fit of the entire dataset with FM>0.54, as in Figure 2.

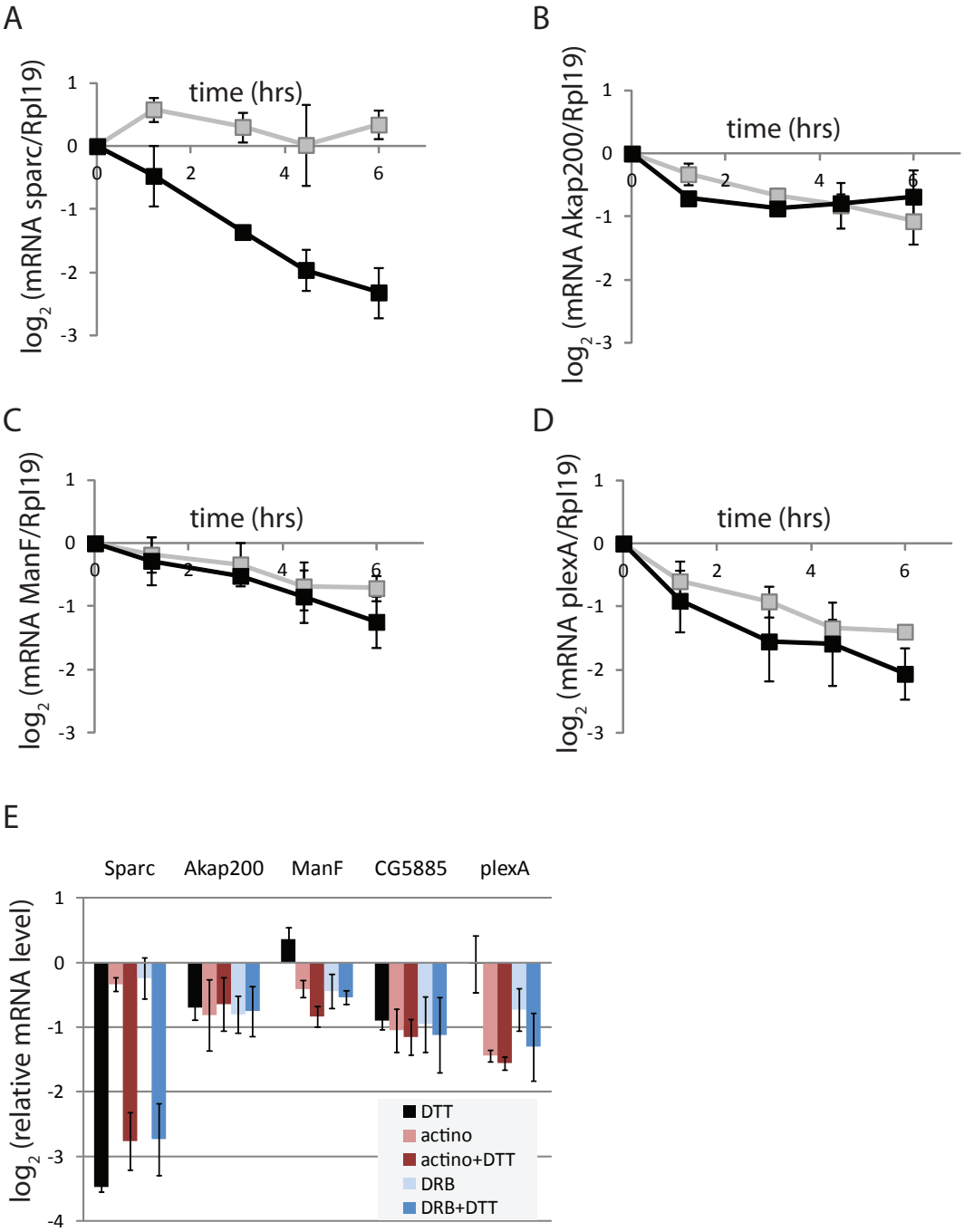

Figure S3. Confirmation of the protection from RIDD. (A-D) We inhibited transcription in *Drosophila* S2 cells (1 μg/mL actinomycin), induced ER stress (2 mM DTT, black squares, compared to no treatment, gray squares), and collected RNA samples over time. We measured mRNA levels compared to Rpl19 by qPCR; shown are the averages and SDs for 2 independent experiments. (E) We either left cells untreated or inhibited transcription using 5 ug/mL actinomycin (actino) or 100 uM DRB, and collected cells following 4.5 hrs in the absence or presence of ER stress (2 mM DTT). Shown are the mRNA levels relative to Rpl19 and normalized to RNA from untreated cells, with averages and SDs from 3 independent experiments.

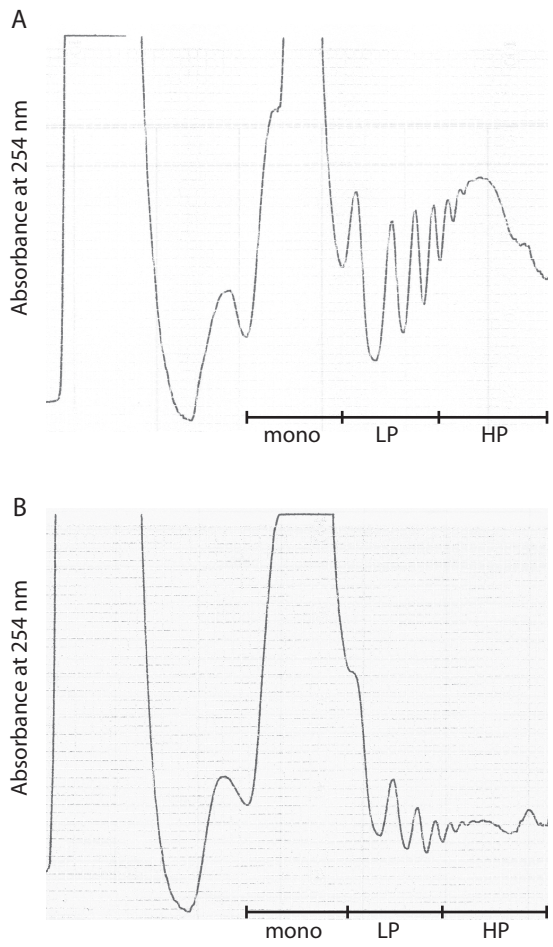

Figure S4. Polysome profiles of S2 cells in the absence (A) and presence (B) of ER stress (2 mM DTT, 20 min). Fractions containing monosomes (mono), low density polysomes (LP) or high density polysomes (HP) were collected for analysis.