Supplemental material

Bae et al., https://doi.org/10.1083/jcb.201809027

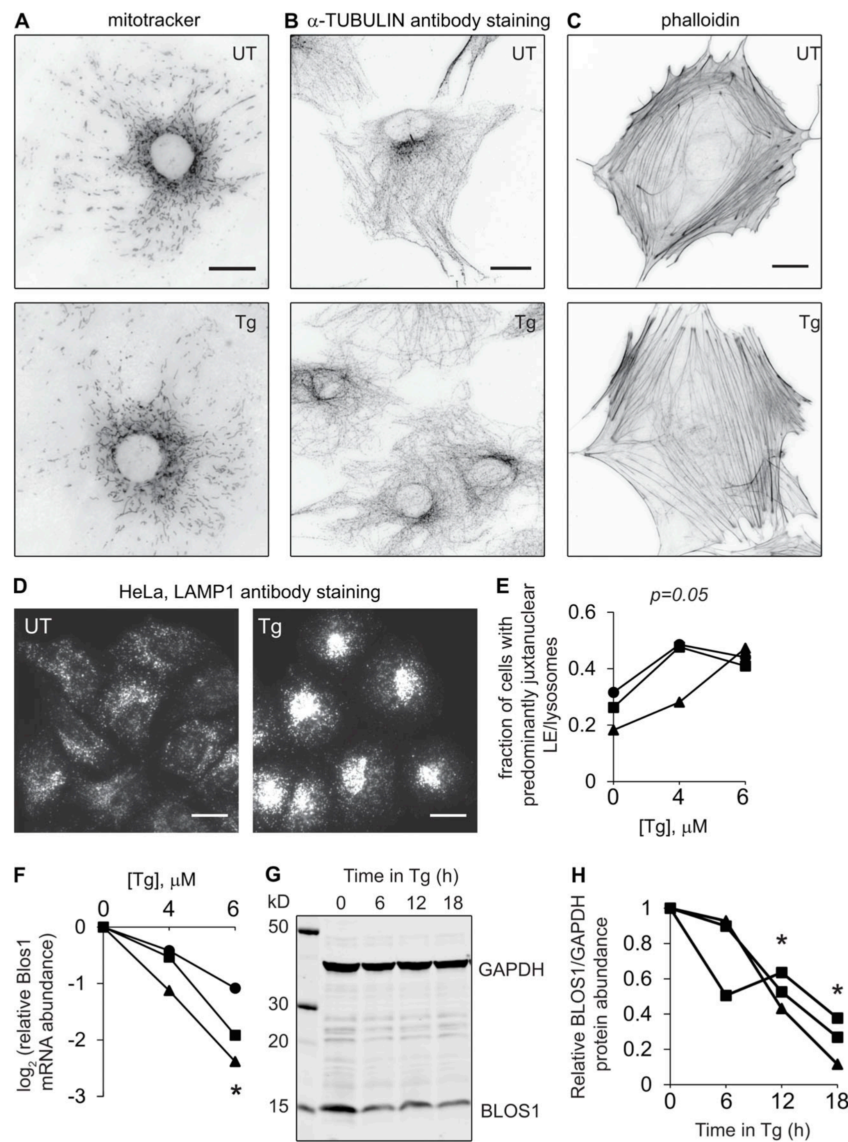

Figure S1. **Organelle labeling during ER stress and LE/lysosome repositioning in HeLa cells. (A–C)** We treated MC3T3-E1 cells with Tg (2 µM, 18 h), then stained with mitotracker (A) or phalloidin (C) or fixed and stained for α-tubulin (B). **(D–F)** We treated HeLa cells with Tg (18 h), and then fixed and stained with a LAMP1 antibody and collected RNA samples in parallel. We imaged and scored cells for juxtanuclear lysosome clustering (D and E) and measured *Blos1* mRNA levels by qPCR (F) for three independent experiments. **(G and H)** We treated HeLa cells with 6 µM Tg, collected samples over time, and analyzed by immunoblotting. G is a representative blot and H shows quantification of three independent experiments. Symbols represent independent experiments and are maintained between E and F; lines connect data from the same replicate experiment. We determined P values for E, F, and H using ANOVA followed by Tukey's honestly significant difference test, *n* = 3. *P < 0.05 for stressed versus UT cells. Scale bars, 10 µm. UT, untreated.

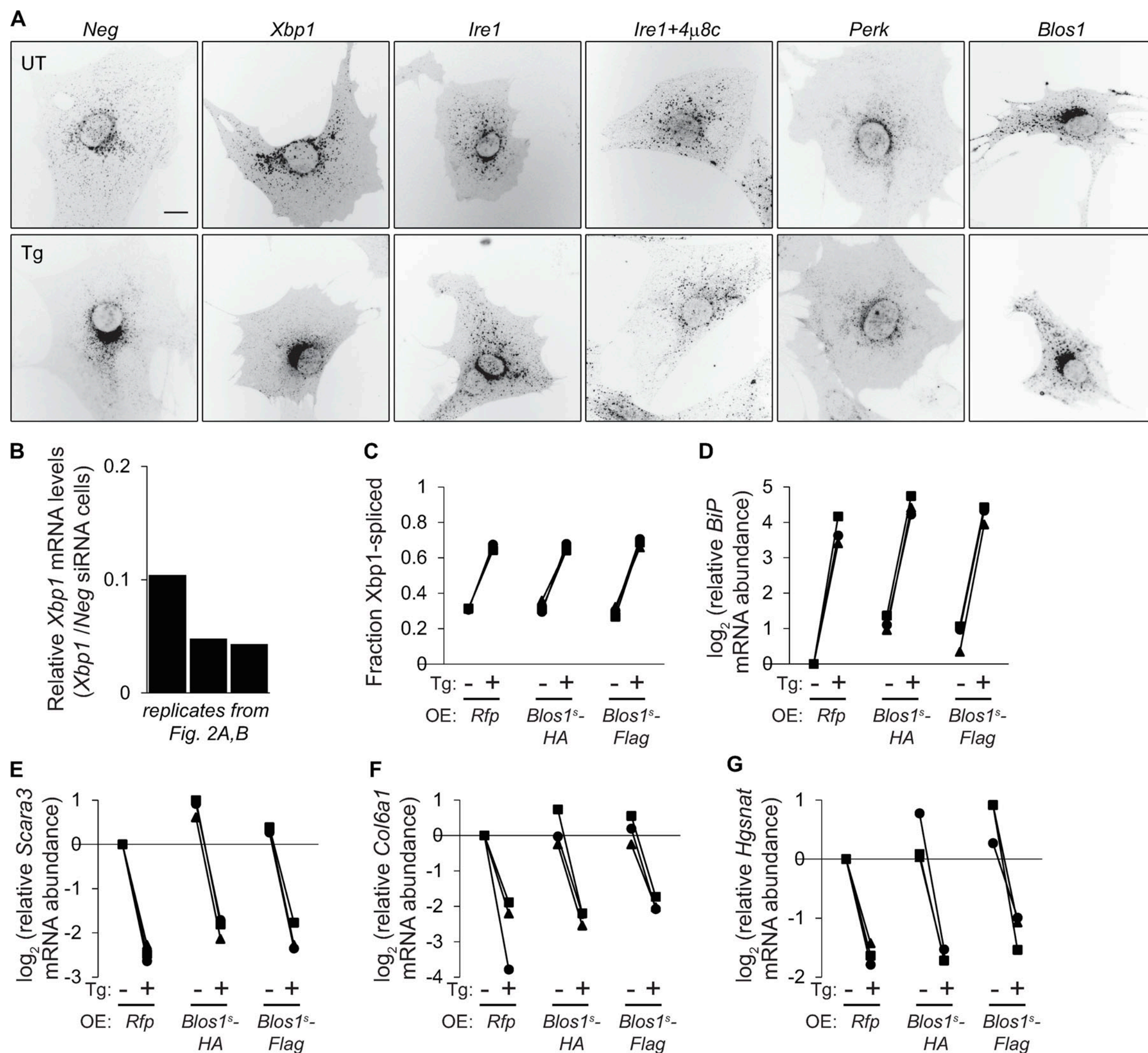

Figure S2. **LE/lysosome repositioning during ER stress is dependent on the degradation of *Blos1* mRNA by RIDD. (A)** Representative images from the experiments described in Fig. 2, A and B. Scale bar, 10 µm. **(B)** We used RNA samples from Fig. 2 B to measure relative *Xbp1* mRNA levels by qPCR. **(C)** We used RNA samples from Fig. 2 D to measure *Xbp1* splicing by PCR, as described in Materials and methods. **(D–G)** We used RNA samples from Fig. 2 D to measure mRNA levels of various ER stress–responsive genes by qPCR. B–G show three independent experiments. UT, untreated; OE, overexpressed.

https://doi.org/10.1083/jcb.201809027

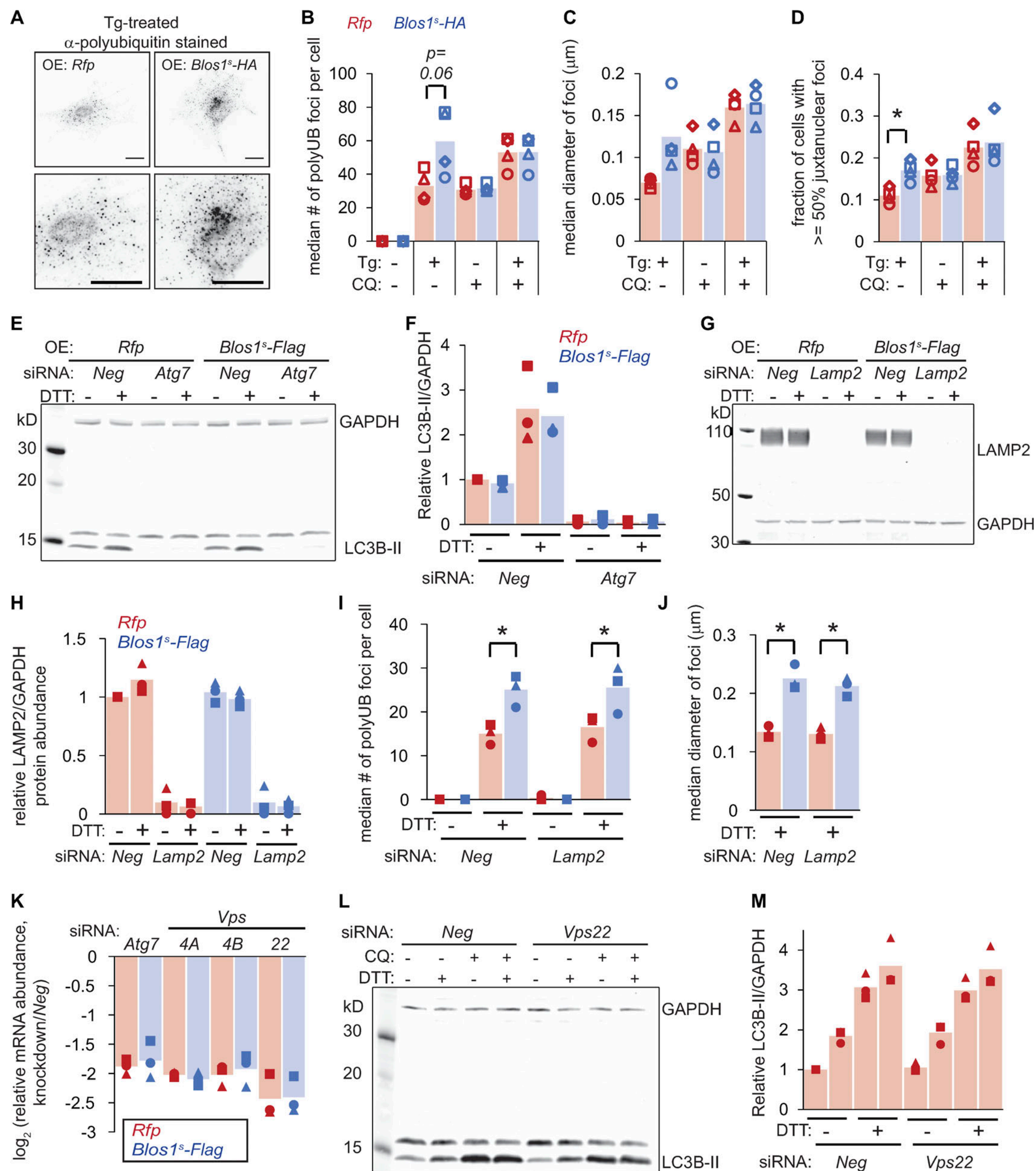

Figure S3. **Mechanisms of protein aggregate clearance during ER stress. (A–D)** We treated MC3T3-E1 cells stably expressing the indicated mRNAs with Tg (2 µM, 18 h) ± CQ (120 µM) for the final 2 h of treatment. We then fixed and stained cells with an antibody for polyubiquitin and analyzed foci as described in Materials and methods. Panel A shows representative images (inverted, scale bar = 10 µm), and B–D show four independent experiments. *P < 0.05, paired *t* test comparing *Rfp*- versus *Blos1s*-expressing cell lines, corrected for multiple comparisons within each panel (P values between 0.05 and 0.15 are also shown). **(E and F)** We transfected cells with *Atg7* or *Neg* control siRNAs, then treated with DTT (2 mM, 4 h) and analyzed LC3B-II by immunoblotting. E shows a representative blot and F shows quantification of three independent experiments. **(G–J)** We transfected cells with *Lamp2* or *Neg* control siRNAs, then treated with DTT (2 mM, 4 h) and analyzed LAMP2 levels by immunoblotting (G and H) or polyubiquitin foci by immunostaining (I and J) The legend in H also applies to I and J. **(K)** We collected RNA samples from knockdown experiments in Fig. 5 I and measured target mRNA levels by qPCR. **(L and M)** We transfected *Rfp*-expressing controls cells with *Vps22* or *Neg* control siRNAs, then treated with 2 mM DTT ± 120 µM CQ (4 h) and analyzed LC3B-II as in E and F. For F–M: *P < 0.05 for *Rfp* versus *Blos1s* cells, using paired *t* tests with corrections for multiple comparisons; *n* = 3. polyUB, polyubiquitin; OE, overexpressed.

 https://doi.org/10.1083/jcb.201809027