Expanded View Figures

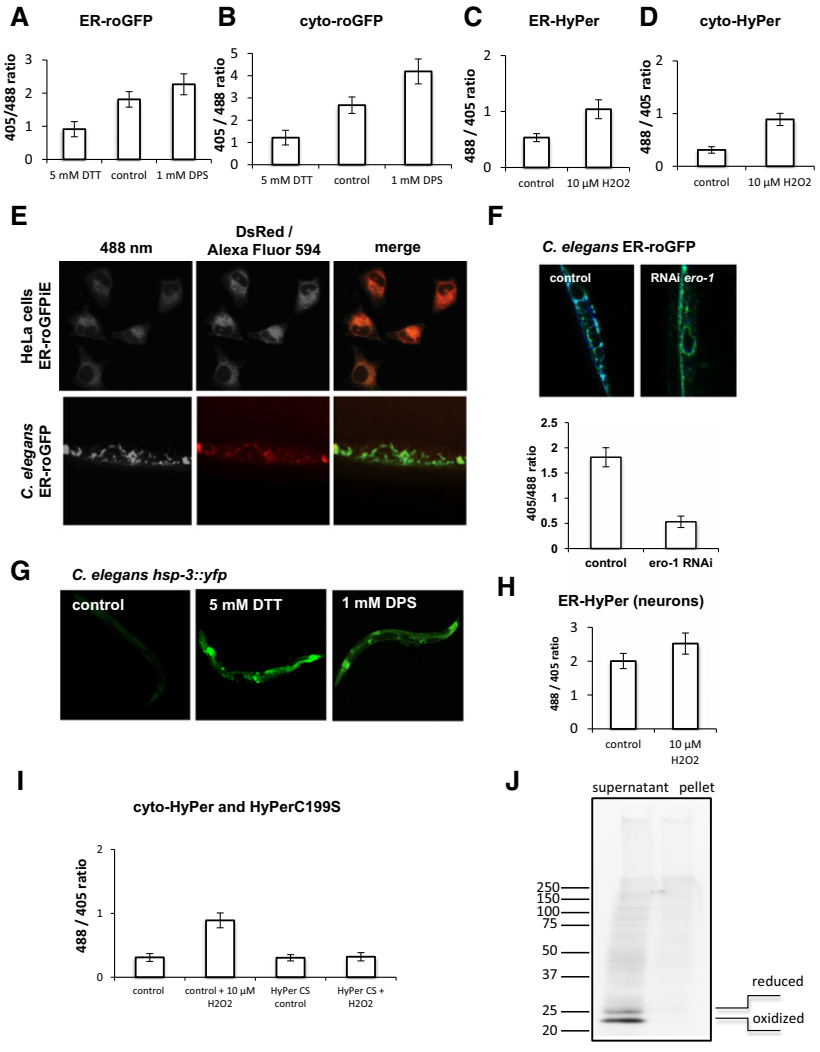


Figure EV1. Validation of roGFP and HyPer sensors.

A–D The graphs depict the ratio-metric analysis of the images shown in Fig 1A, C, F and G, respectively. The statistical data of the ANOVAs and the *post hoc* Tukey's HSD test of (A) and (B) are presented in the legend for Fig 1. All ratio-metric data are represented as mean ± SD.

E Co-localization analysis of HeLa ER-roGFPiE using ER-targeted DsRed as reference and of *C. elegans* ER-roGFP using an immunostaining with Alexa Fluor 594-coupled antibodies directed against KDEL antibodies as reference. The 488-nm channel is depicted on the left, the red channel in the middle and the overlay of both on the right.

F RNAi-mediated knockdown of *ero-1* results in a strong reduction in the ER redox state. The knockdown was performed at the L1 stage, and the analysis was performed on day 4 of life (young adults). Representative images are shown on the top and the quantification of the ratio-metric analysis is shown underneath. Twenty nematodes each were analysed. The ratio-metric data are represented as means ± SD.

G Exposure of nematodes to 5 mM DTT or 1 mM DPS results in UPR^{ER} induction as judged by the expression of *hsp-3::yfp* reporter. The non-treated control is shown on the left.

H The graph depicts the ratio-metric analysis of *C. elegans* neuronal ER-HyPer. Analogous to the muscle reporter, the sensor-expressing nematodes were treated with 10 µM H_2O_2. The ratio-metric data are represented as means ± SD.

I Validation of the HyPer sensor for its specificity towards H_2O_2. Depicted are the ratio-metric analyses of nematodes expressing cytosolic HyPer or HyPerC199S (HyPerCS) in the presence and absence of H_2O_2. Twenty animals were analysed for each condition. The ratio-metric analyses between the control, control + H_2O_2, HyPer CS control and HyPer CS + H_2O_2 passed the ANOVA ($F = 294$; $P > 0.0001$) and the *post hoc* Tukey's HSD test confirmed a significant difference between control versus control + H_2O_2, control + H_2O_2 versus HyPer CS control, control + H_2O_2 versus HyPer CS + H_2O_2. The difference between control versus HyPer CS control, control versus HyPer CS + H_2O_2 and HyPer CS control versus HyPer CS + H_2O_2 is non-significant. All ratio-metric data are represented as means ± SD.

J Western blot analysis of soluble and insoluble samples of nematodes expressing ER-roGFP. The left lane depicts the soluble and the right lane the insoluble fraction. The Western blot was analysed using antibodies directed against GFP (Roche) and the corresponding signal for the reduced and oxidized version of GFP is indicated on the right.

Source data are available online for this figure.

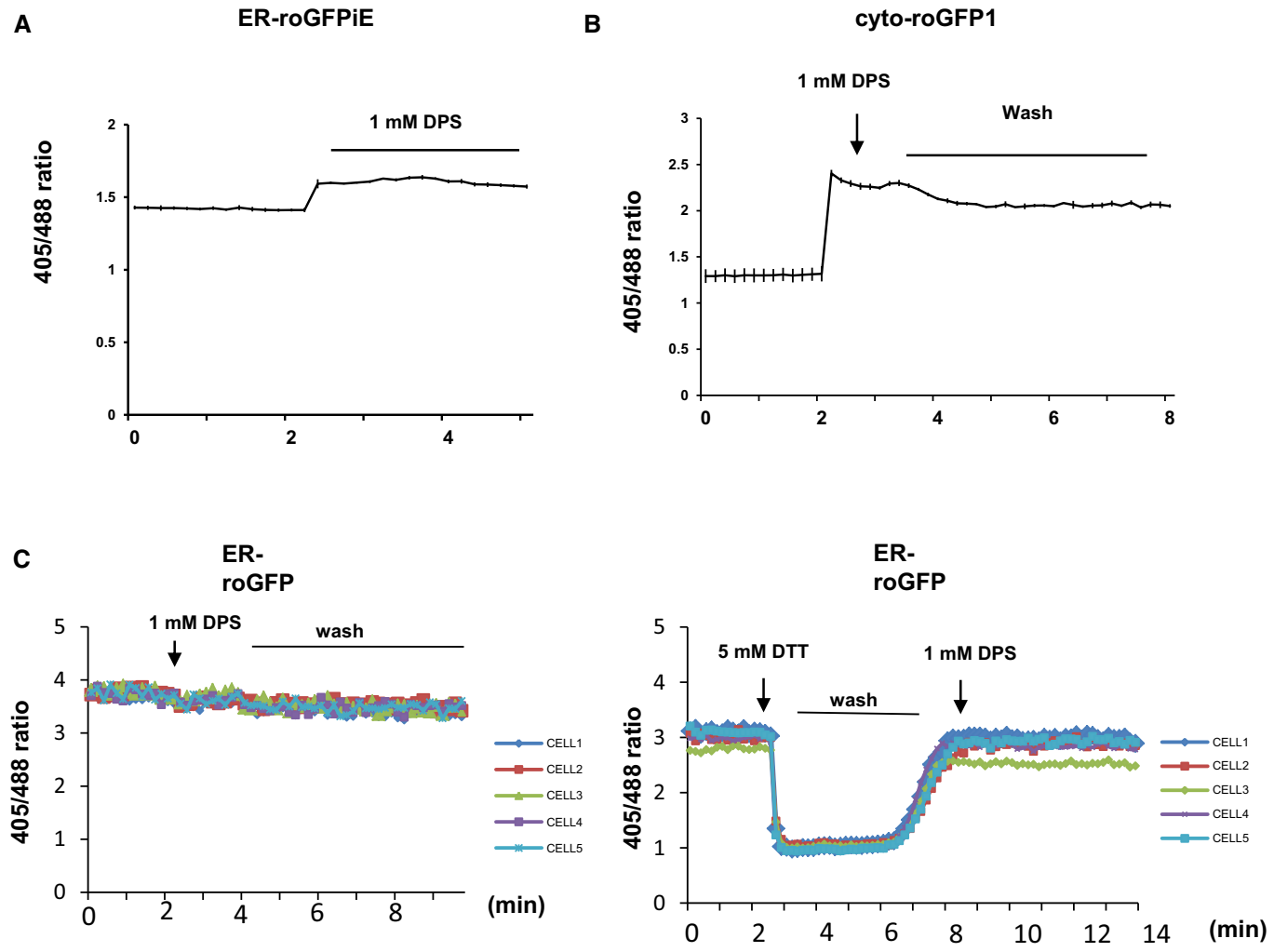


Figure EV2. Validation of ER and cytosolic roGFP in HeLa towards more oxidative conditions.

A Ratio traces of ER-roGFPiE expressed in the ER in HeLa cells. 1 mM DPS was added at 2 min.

B Ratio traces of roGFP expressed in cytosol in HeLa cells. 1 mM DPS was added at 2 min and wash starts at 3 min.

C Ratio traces of wild-type version of roGFP targeted to the ER lumen (ER-roGFP), showing ER-roGFP in HeLa cells can sense reducing, but not oxidative challenge. 1 mM DPS was treated at 2 min (left). 5 mM DTT was added at 3 min, wash was done from 4 to 7 min, and 1 mM DPS was added at 7 min (right).

Source data are available online for this figure.

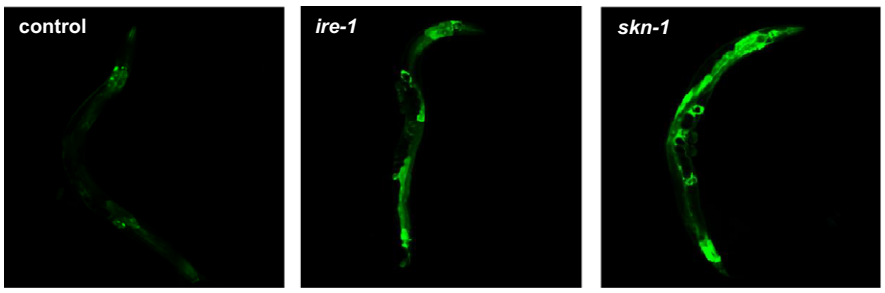
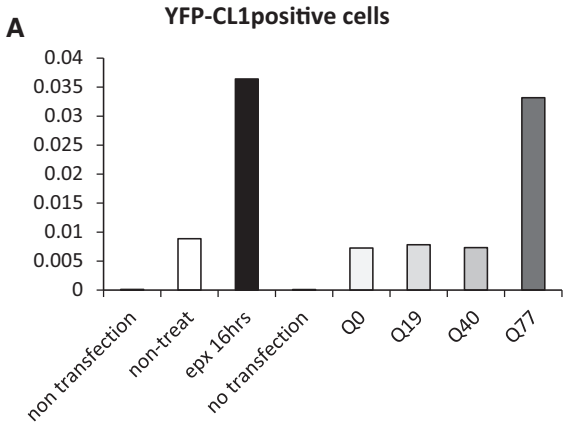


Figure EV3. Effect of knockdown of *ire-1* and *skn-1* on UPR^{ER}.

The pictures show representative images of nematodes expressing *hsp-3::yfp* in response to RNAi-mediated knockdown of either *ire-1* or *skn-1*.



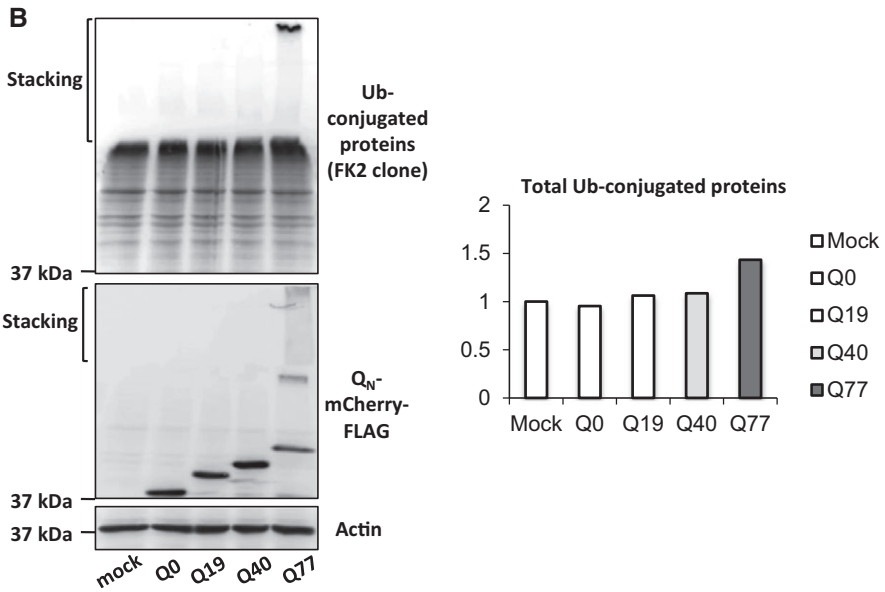


Figure EV4. Proteasome inhibition by polyQ.

A Activity of proteasome is decreased in epoxomicin-treated or Q77-mCherry-expressing HeLa cells. Proteasome activity was monitored by the intensity of degradation signal of YFP-tagged CL1(YFP-CL1). The fraction of YFP-CL1-positive cells was determined by FACS analysis.

B Accumulation of ubiquitinated proteins in HeLa cells expressing Q77, but not Q0, 19 and 40. Abundance of ubiquitination was analysed by Western blot (left).

Source data are available online for this figure.