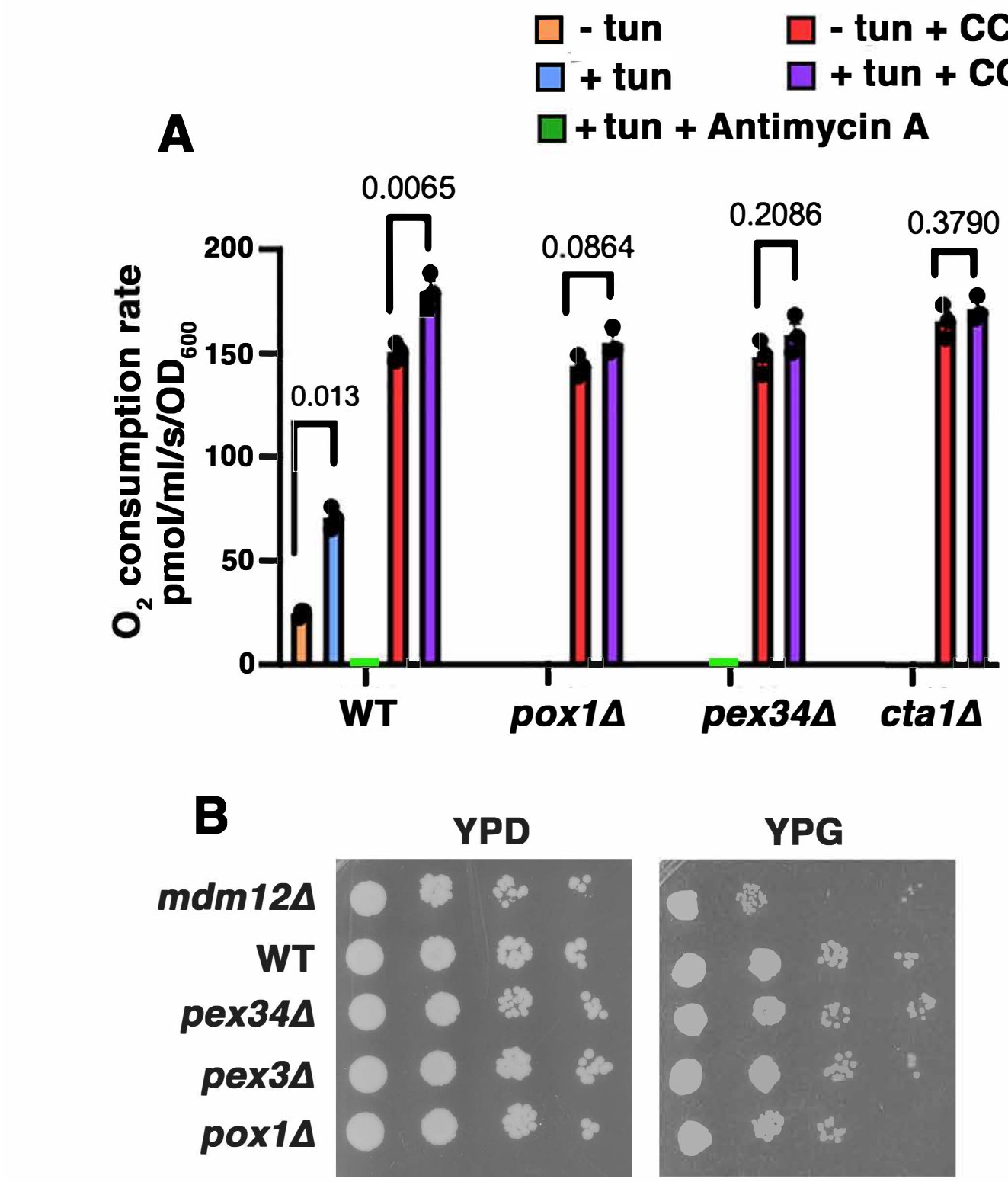

Fig. 1. (A) Mitochondrial oxygen consumption rate in cells exponentially growing in medium with abundant glucose. OCR was assayed in cells untreated or treated with tunicamycin (0.5 µg/ml) for 5h with a high resolution respirometer. To measure maximal OCR, 5 µM CCCP was added. To inhibit mitochondrial respiration, antimycin A (4 µM) was added. (B) *pex3*Δ*, pex34*Δ *and pox1*Δ mutants are not impaired in growth on a non-fermentable carbon source (3 % glycerol).

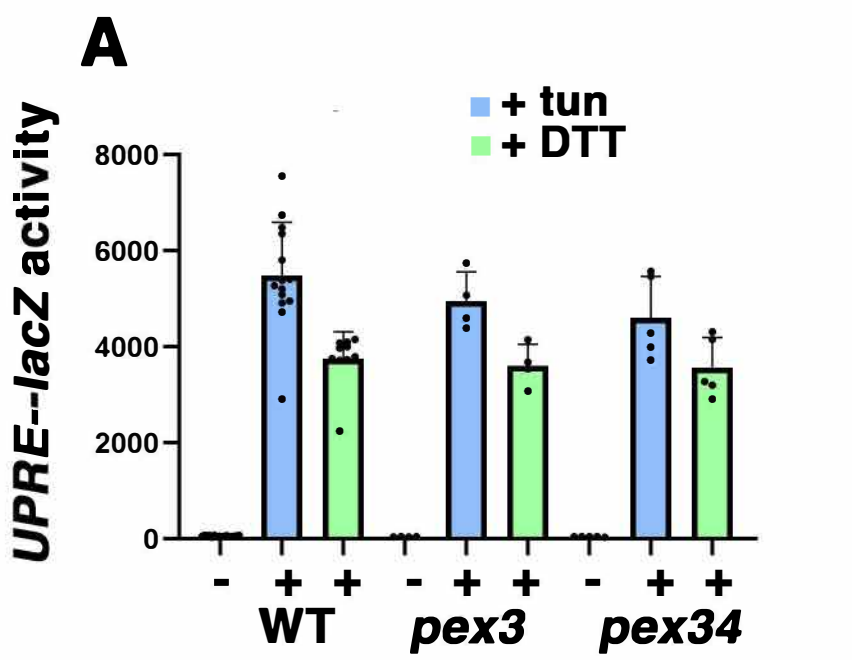

Fig. 2. *pex*Δ mutants are competent to induce UPR and OSR in response to ER stress. To assay UPR, exponentially growing cells bearing a *UPRE-lacZ* reporter (pJC104) were treated with tunicamycin (0.5 µg/ml) and dithiothreitol (1 mM) for 5h (A). To assay OSR, exponentially growing cells transformed with *TRX2-lacZ* were treated with tunicamycin for 5h (B). Cell lysates were assayed for ß galactosidase activity, expressed as µmol/min/mg.

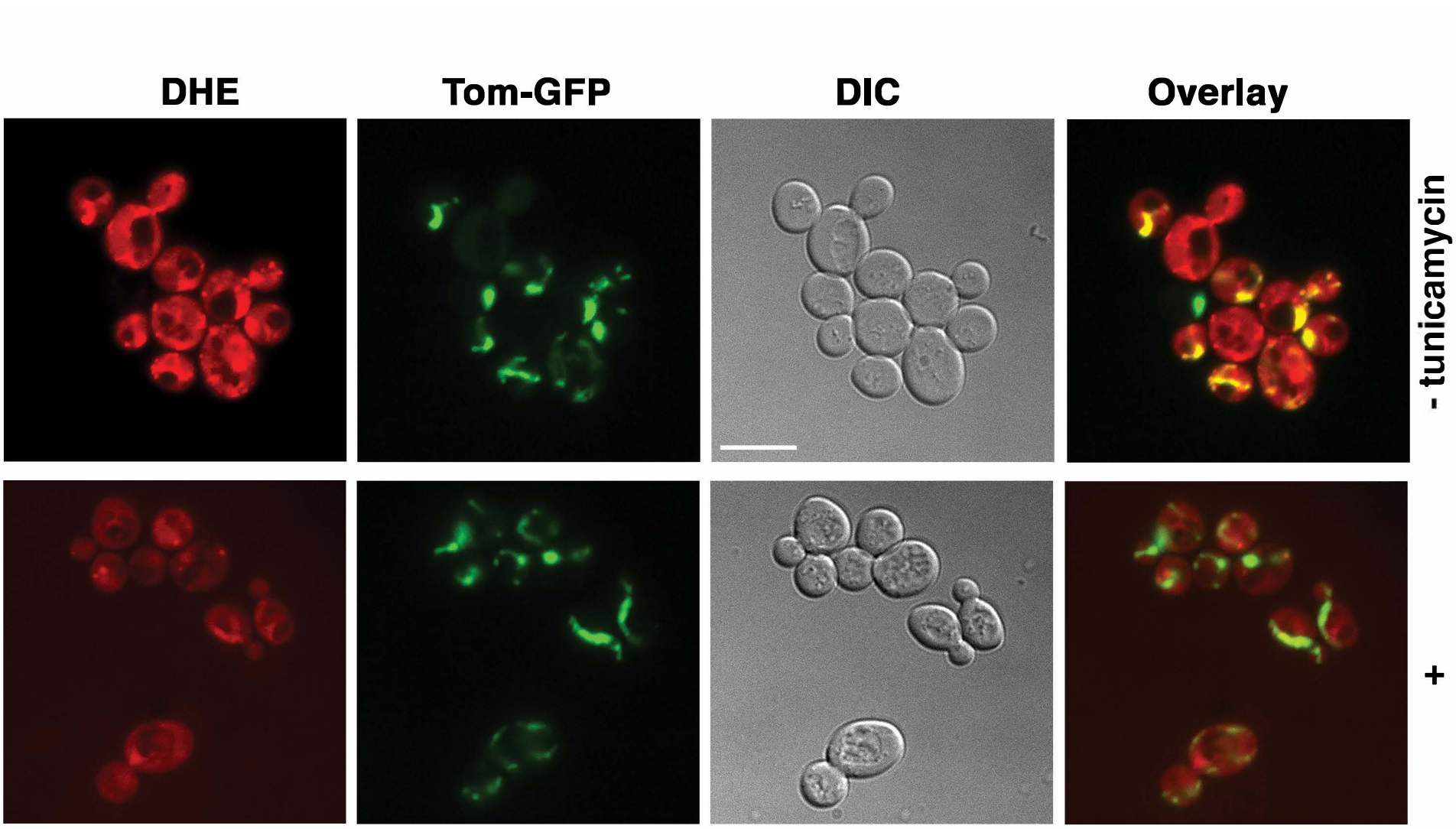

Fig. 3. Mitochondrial ROS detected by DHE staining. Wild-type cells with the mitochondrial outer membrane protein Tom40 tagged with GFP at the chromosomal locus were untreated or treated with 0.5 μg/ml tunicamycin for 5h. The cells were then stained with 2 μg/ml DHE for 15 min, and fluorescence images were collected. Scale bar = 10 μm.

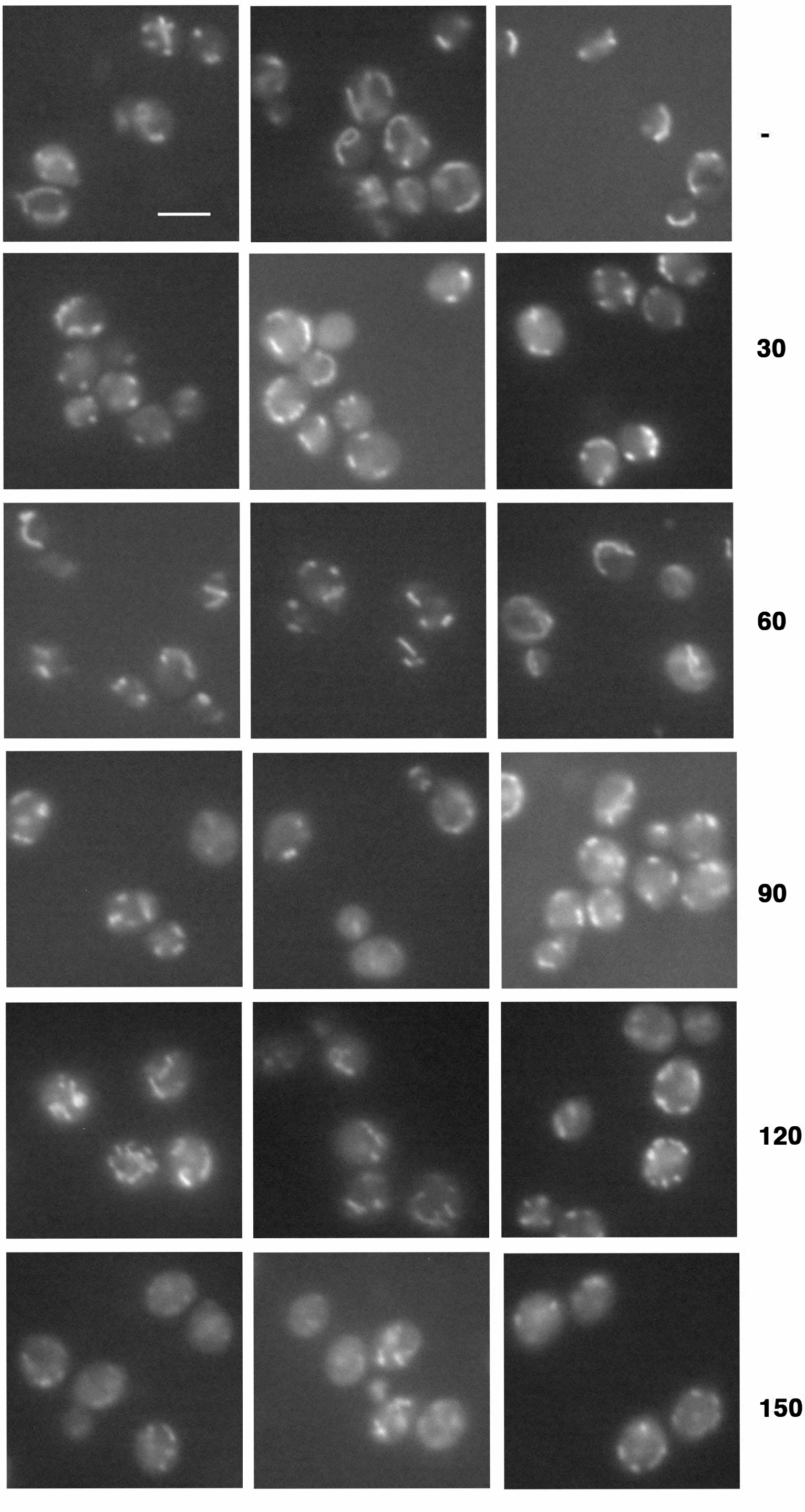

Fig. S4. Time course of ROS staining after ER stress. Wild-type cells untreated and treated for various times with 0.5 µg/ml tunicamycin were stained with DHE and fluorescence images were collected at the same exposure and adjusted with the same Photoshop settings. The three representative images in each row are representative of each time point. Scale bar = 10 µm.

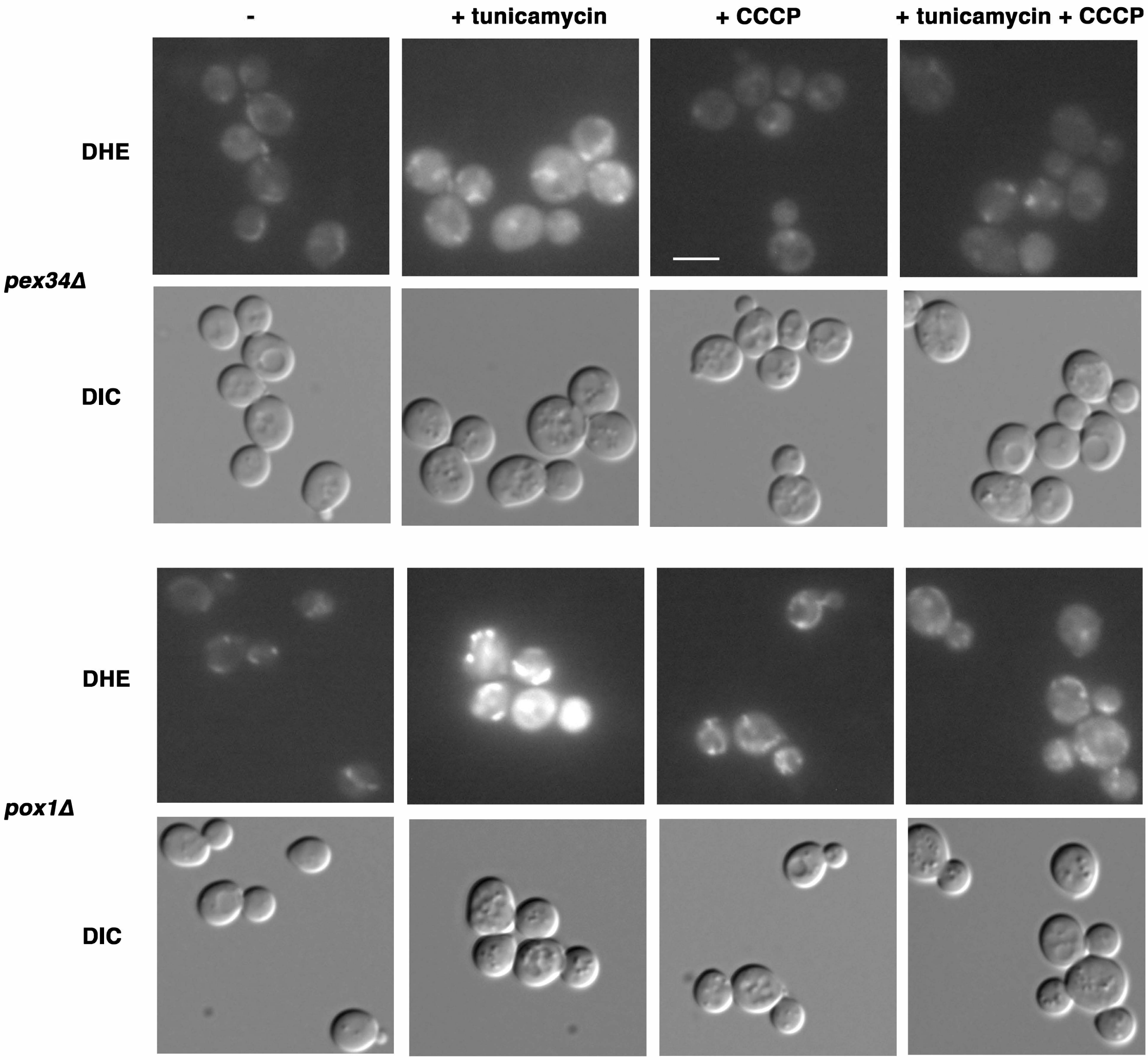

Fig. S5. Mitochondrial ROS accumulation in *pex34*Δ (top panels) *and pox1*Δ *cells* (bottom panels*).* Cells were treated with or without 0.5 µg/ml tunicamycin for 5h with or without CCCP (5 µM). Cells were then stained with DHE (2 µg/ml) for 15 min and then visualized by fluorescence microscopy and DIC. Scale bar = 10 µm.

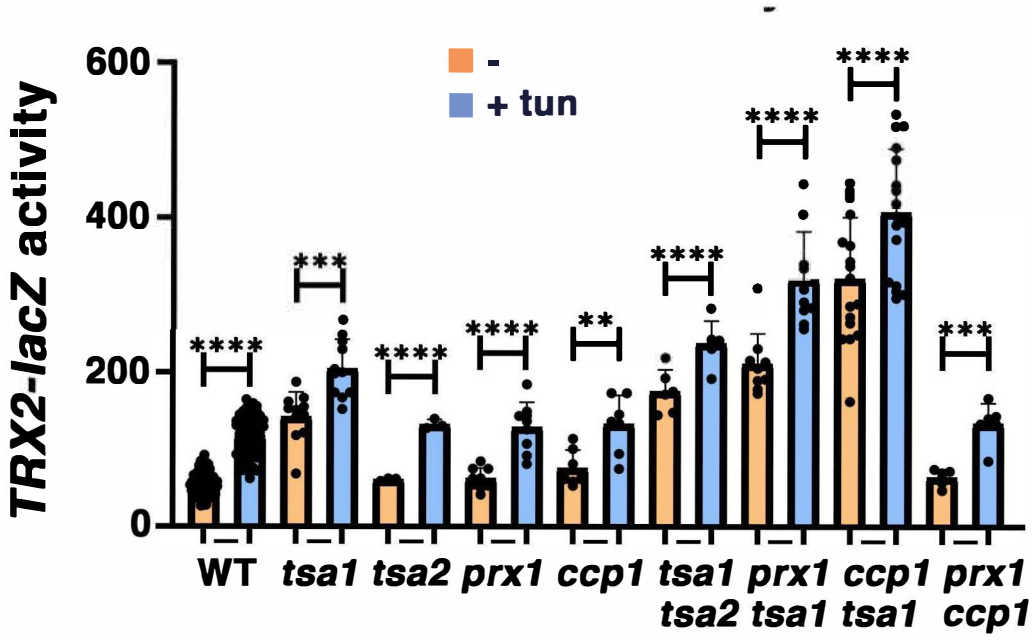

Fig. S6. Oxidative stress in peroxiredoxin mutants. Oxidative stress response was assayed using a *TRX2-lacZ* reporter. Cells were treated with 0.5 µg/ml tunicamycin. ß galactosidase activity is expressed as µmol/min/mg lysate protein. n ≥ 3. P values as indicated in Fig. 4 legend.

Table S1. Excel file with all proteins identified by LC-MS/MS analysis of mitochondria isolated from cells untreated and treated with tunicamycin (0.5 µg/ml) for 5h. Explanations are in Sheet 1, proteins are in Sheet 2.

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