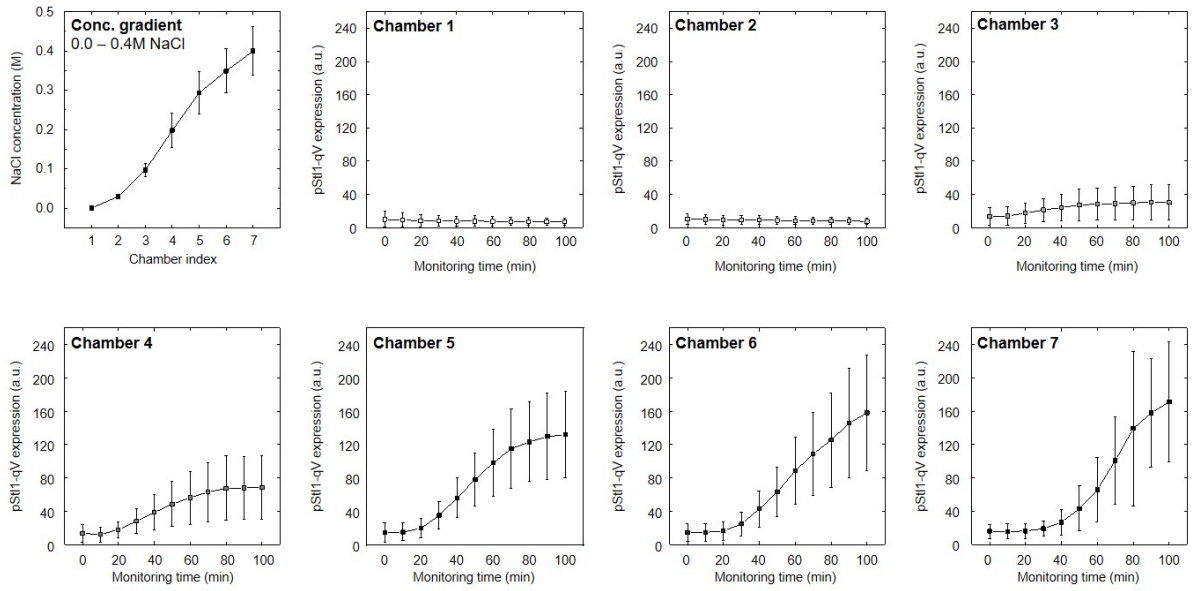
Supplementary Table 1. Yeast strains

Strain ID	Description	Source	Used in figure
BY4741	MATa his3Δ1; leu2Δ0; met15Δ0; ura3Δ0	OpenBiosystems	Parental
W303	leu2-3,112; trp1-1; can1-100; ura3-1; ade2-1; his3-11,15	Lab collection	Parental
	[BY4741] Yap1-GFP	OpenBiosystems	Fig.1E, Supplementary Fig. 3, 9
yMU53	[BY4741] Hog1-mCherry Hta2-CFP p*STL1*-qV	Lab collection	Fig. 2A-F, Fig. 4B, Supplementary Fig. 1, 2, 6
yBH100	[BY4741] pFIG1-qVenus	Lab collection	Fig. 3B and C, Fig. 4F, Supplementary Fig. 4, 5, 7, 8
yRM120	[BY4741] pkc1Δ::PKC1-GFP-HIS3MX6 ura3Δ::TMD-dCherry	Mishra et al, 2017	Fig. 5B
yRM124	[yRM120] mid2Δ::kanMx	Mishra et al, 2017	Fig. 5B
ySP336	[W303] Ste5(S185A) p*FIG1*-qVenus	Lab collection	Fig. 5C and D, Supplementary Fig. 11
ySP337	[W303] p*FIG1*-qVenus	Lab collection	Fig. 4F, Fig. 5C and D, Supplementary Fig. 11
ySSL114	[BY4741] Hog1-YFP Hta2-CFP SKAR	Durandau et al, 2017	Fig. 4C-D and G-H

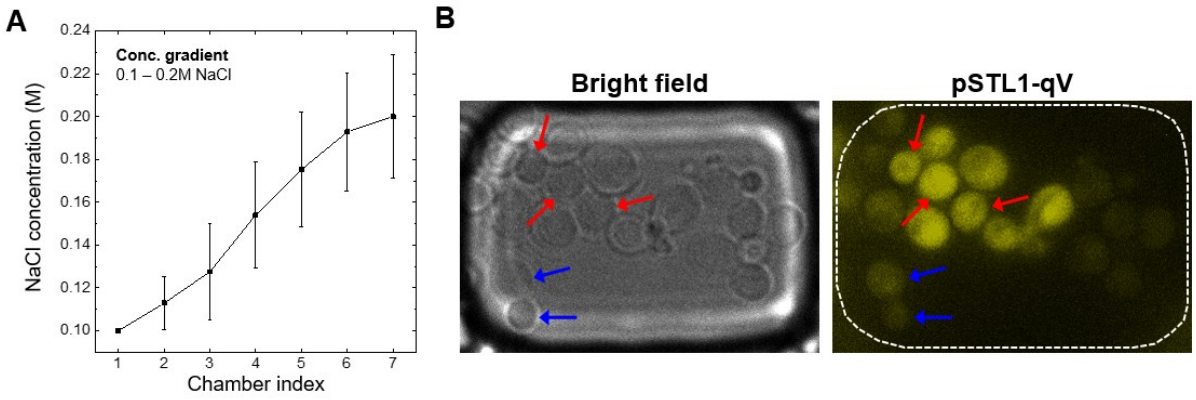
Supplementary Table 2. Plasmid

Plasmid ID	Description	Backbone	Source
pRM1	Msn2-GFP	pRS315	Görner et al., 2002

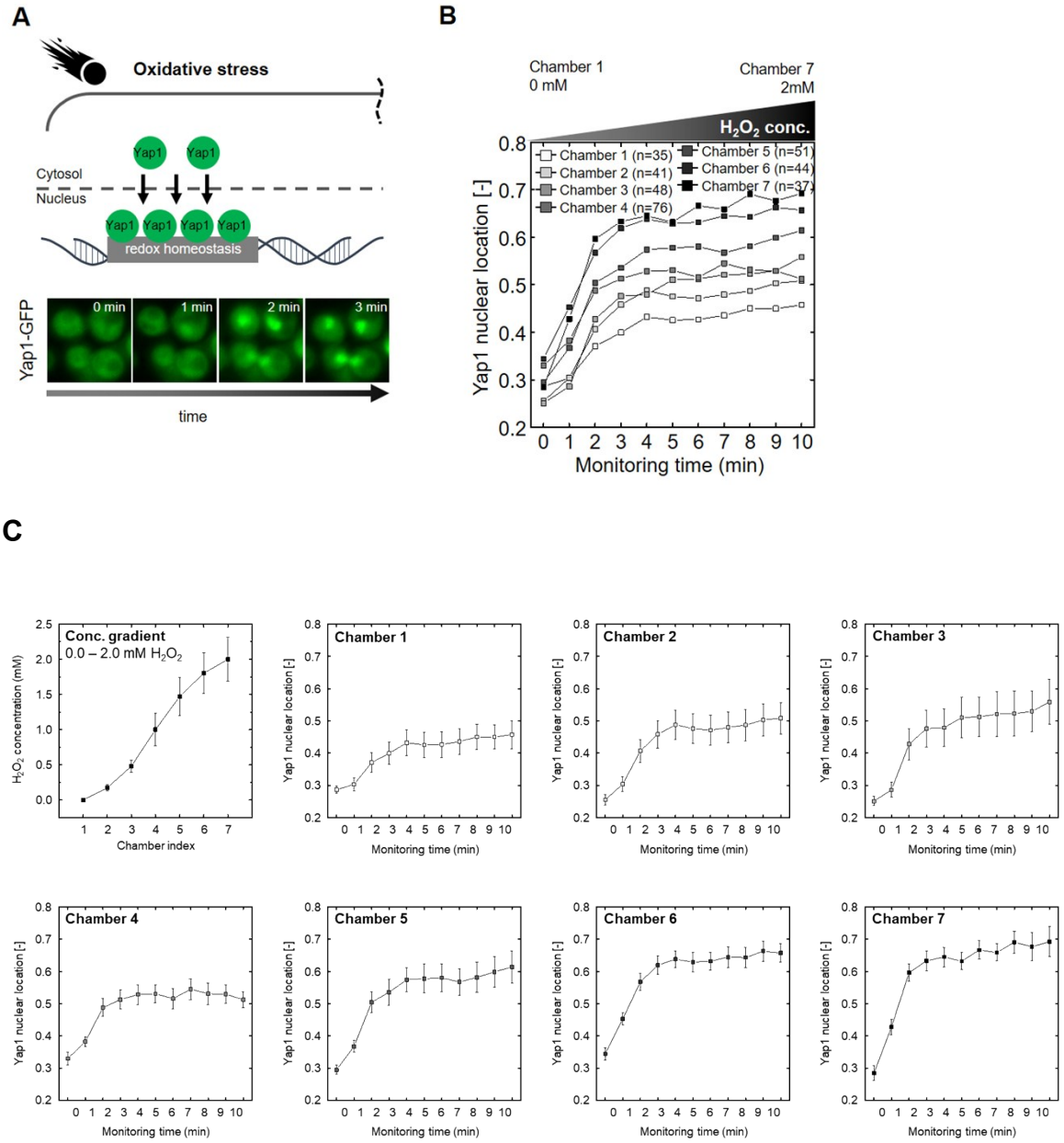
Durandau, E, Aymoz, D and Pelet, S. *BMC Biol*, (2015), **13**, 55.
Görner, W, Durchschlag, E., Wolf, J., Brown, E.L., Ammerer, G., Ruis, H. and Schüller, C. *EMBO J.* (2002) **21**,135-144.
Mishra, R., van Drogen, F., Dechant, R., Oh, S., Jeon, N. L., Lee, S. S. and Peter, M. *PNAS,* (2017) **114**, 13471-13476.



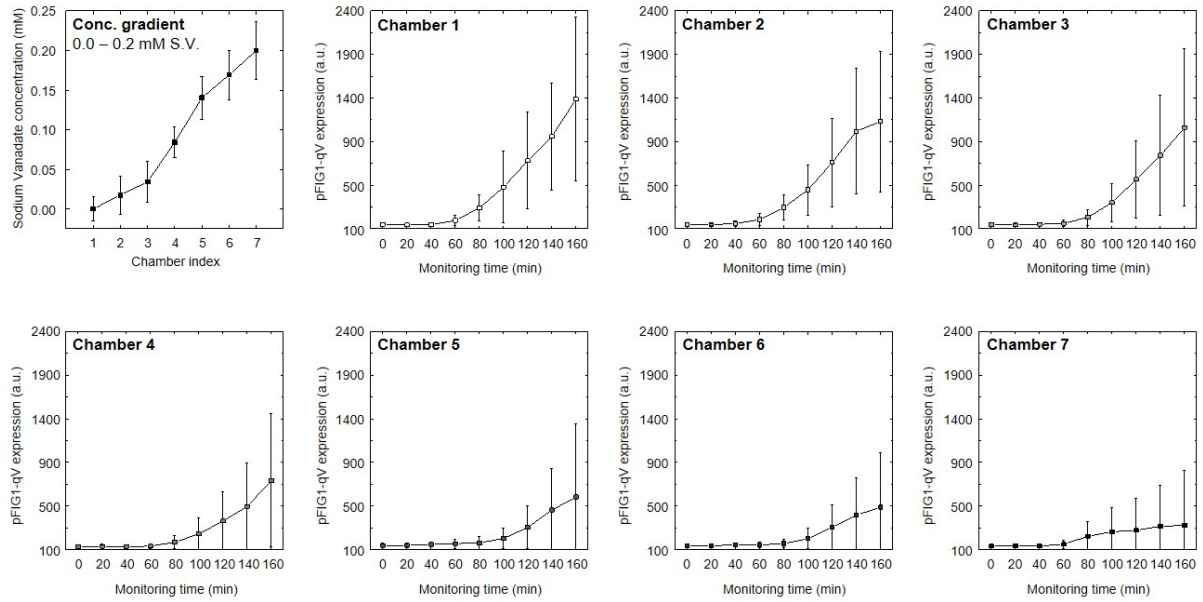
Supplementary Figure 1. NaCl concentration profile, and p*STL1*-qV expression in each chamber of the experiment shown in Figure 2B.



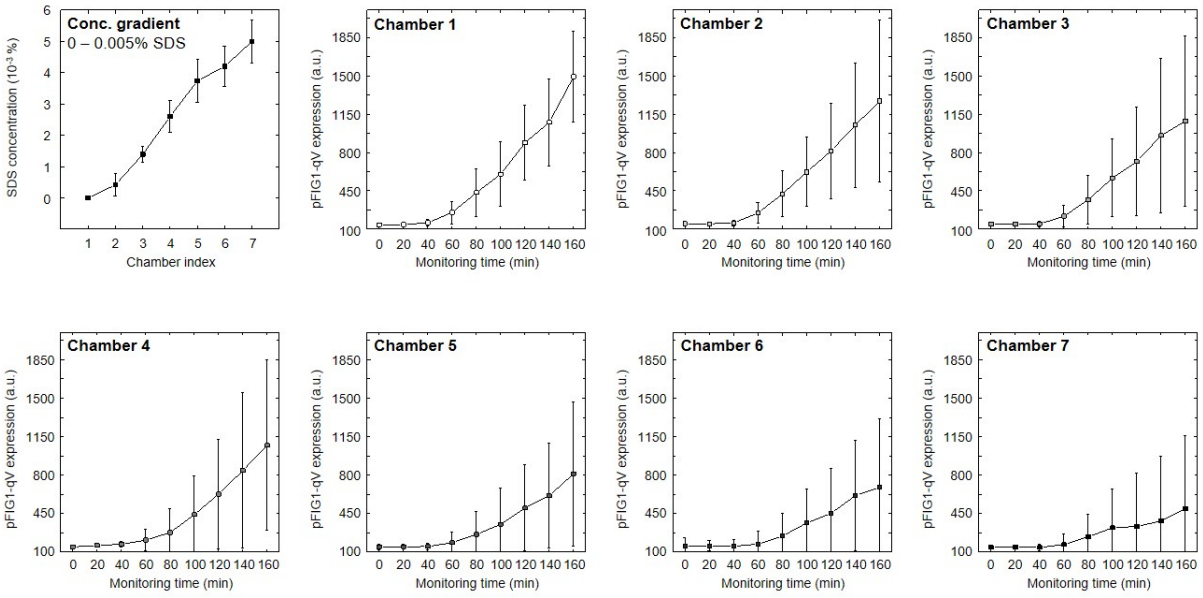
Supplementary Figure 2. **(A)** NaCl concentration profile of the experiment shown in Figure 2C-F **(B)** Bimodal expression of the p*STL1*-qV reporter in cells exposed to 0.13 M NaCl. Cells under a single pad were imaged by bright field and GFP-microscopy 100 min after NaCl addition. The red arrows indicate cells closely surrounded by neighboring cells with strong expression, while the blue arrows mark cells freely exposed to the environment with weak expression.



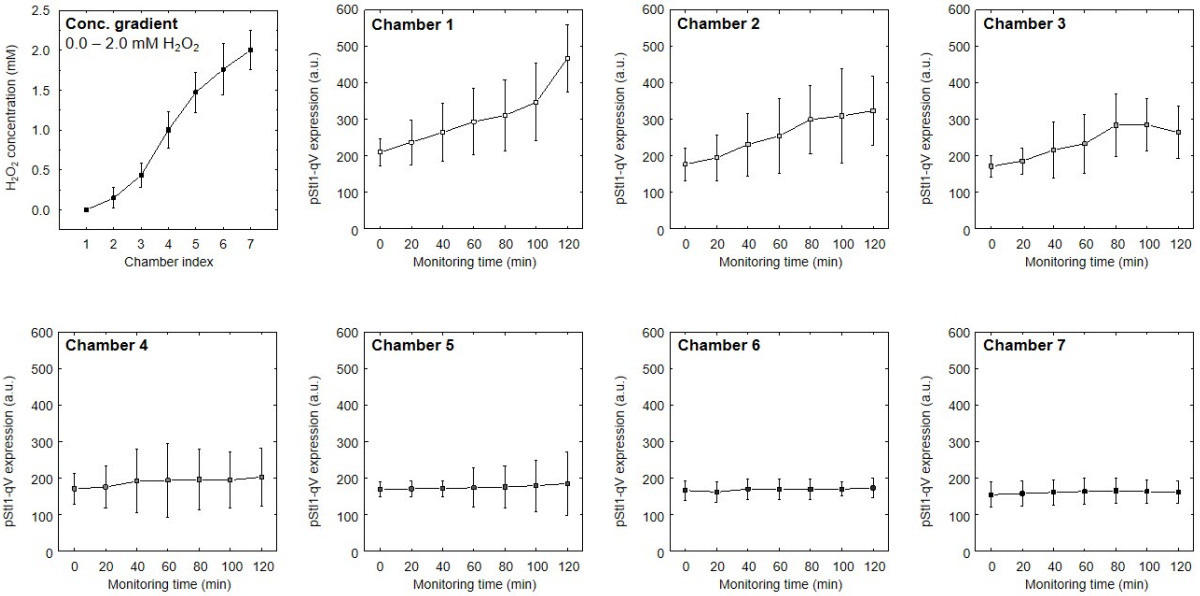
Supplementary Figure 3. Microfluidic-based single cell analysis of oxidative stress signaling pathway (A) Schematic drawing and microscopic images of cells expressing Yap1-GFP, showing its rapid nuclear translocation in response to oxidative stress conditions. Cells were exposed to 2 mM H_2O_2 (at time 0) and nuclear translocation of Yap1-GFP was analyzed microscopically at the times indicated **(B)** Nuclear translocation of Yap1-GFP was quantified by normalized standard deviation of GFP intensity in segmented cells and plotted against different H_2O_2 concentrations (0 to 2 mM) obtained by microfluidic dilution in each chamber. **(C)** Plots depicting the H_2O_2 concentration profile and the nuclear translocation of Yap1-GFP in each chamber of the experiment shown in panel B.



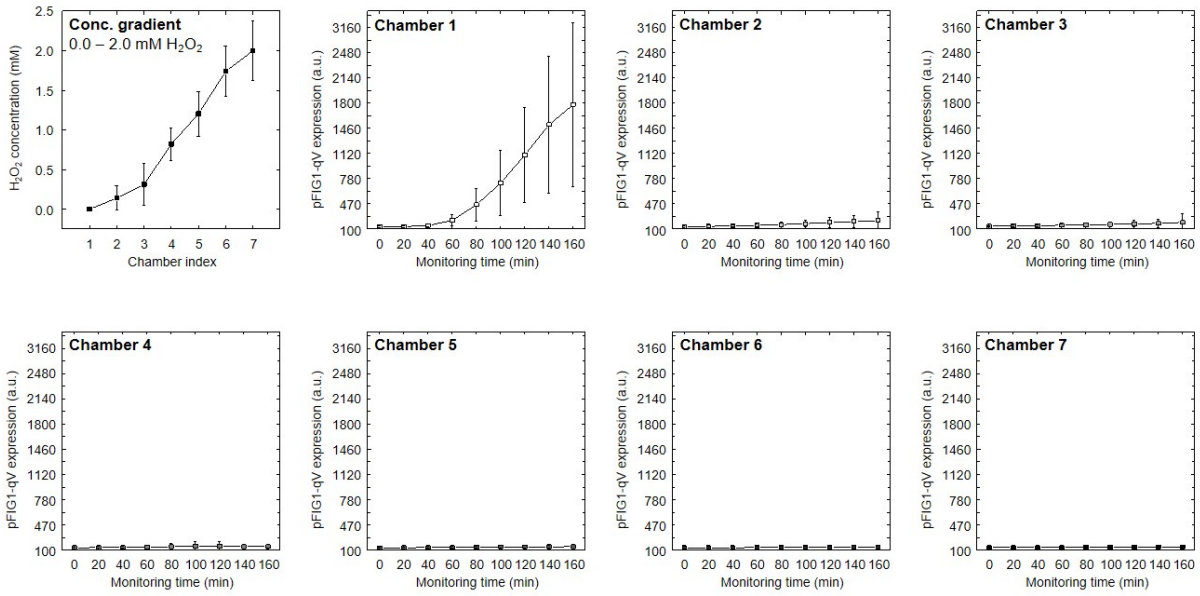
Supplementary Figure 4. Sodium Vanadate concentration profile, and p*FIG1*-qV expression in each chamber of the experiment shown in Figure 3B.



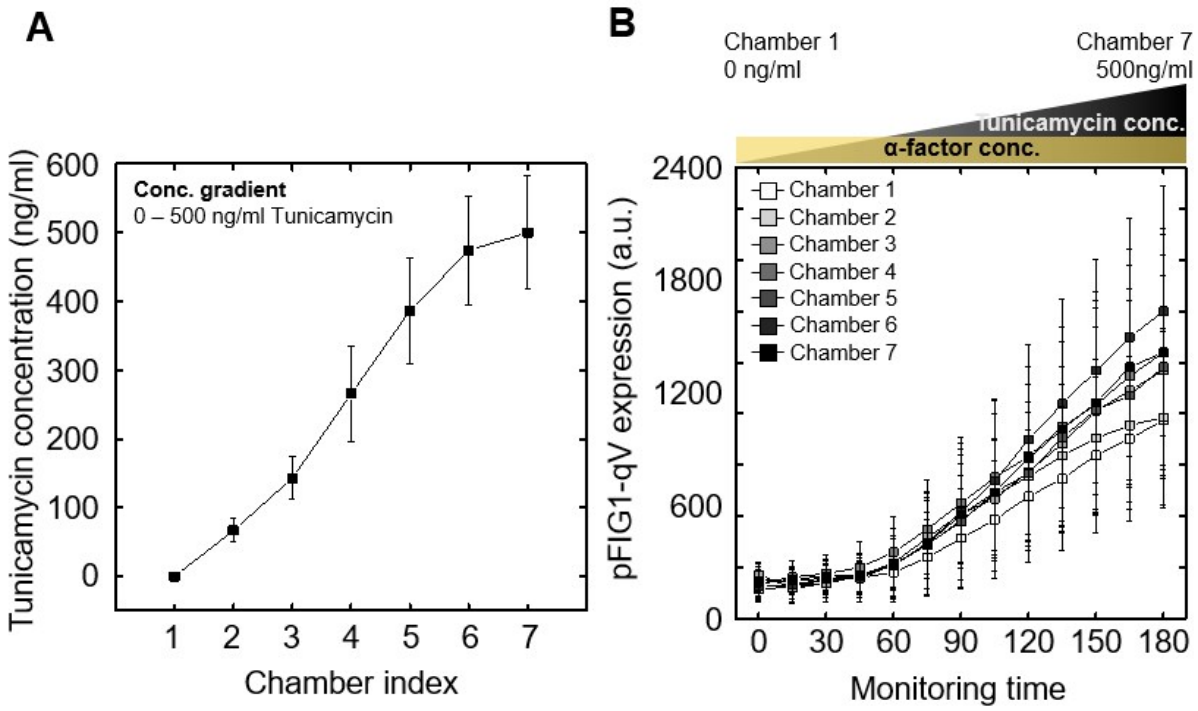
Supplementary Figure 5. SDS concentration profile, and p*FIG1*-qV expression in each chamber of the experiment shown in Figure 3C.



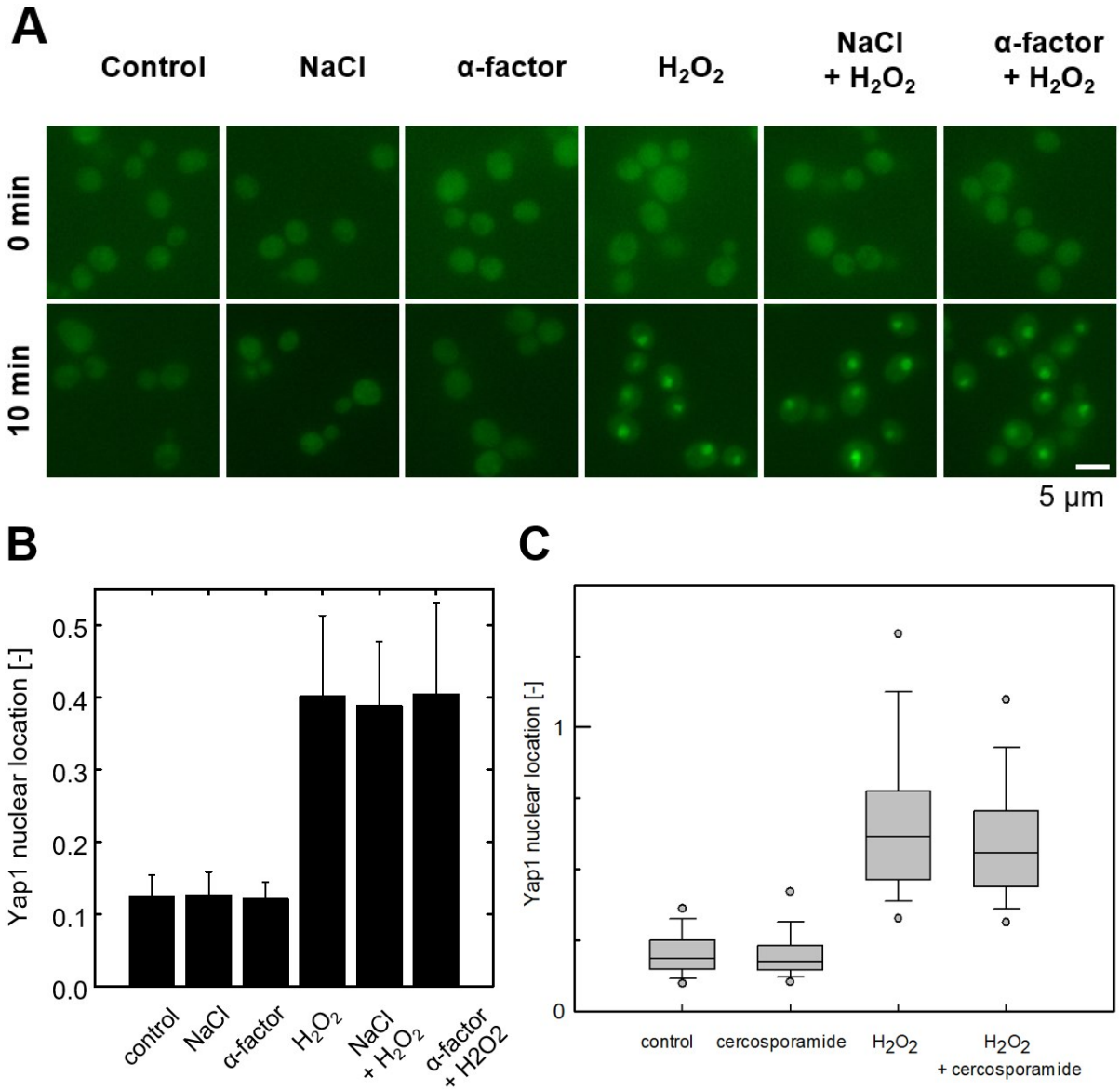
Supplementary Figure 6. H_2O_2 concentration profile, and p*STL1*-qV expression in each chamber of the experiment shown Figure 4B.



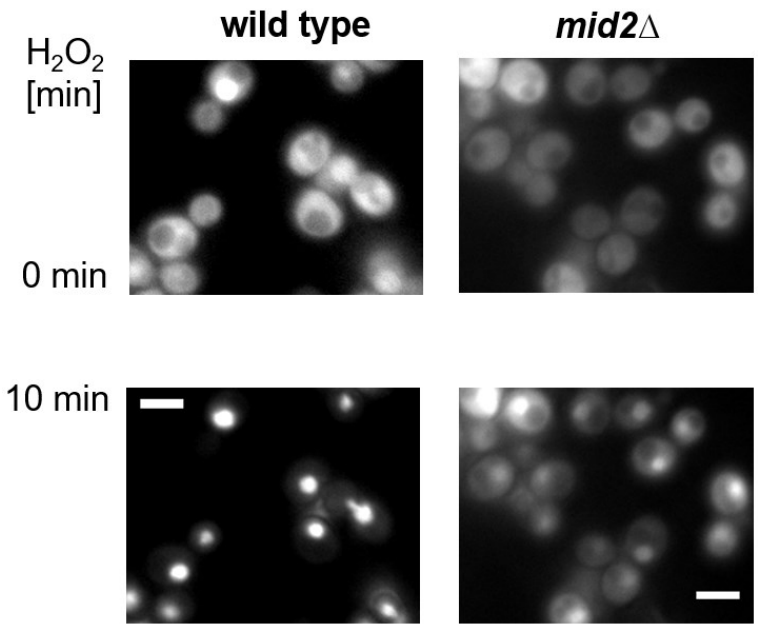
Supplementary Figure 7. H_2O_2 concentration profile and p*STL1*-qV expression in each chamber of the experiment shown in Figure 4C.



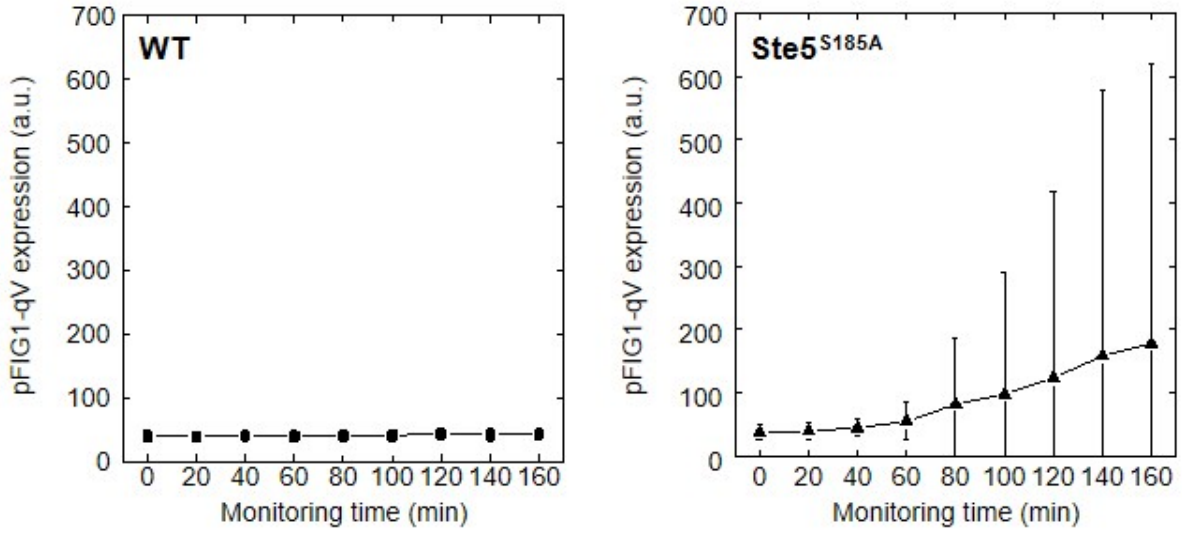
Supplementary Figure 8. (A) Tunicamycin concentration profile, and **(B)** up-regulation of p*FIG1*-qV expression upon tunicamycin treatment (ER stress). The error bars indicate the standard deviation after analyzing at least 74 cells.



Supplementary Figure 9. Yap1-GFP nuclear relocation upon stress (**A**) Yap1-GFP images before and 10 min after exposing cells to the indicated stress conditions (**B**) Quantification of Yap1-GFP nuclear relocation upon stress. Nuclear relocation was quantified by normalized standard deviation of the 50% brightest pixels in at least 86 segmented cells, and the error bar shows the standard deviation (**C**) Pkc1-dependence of Yap1-GFP nuclear relocation. The box- and whisker plots show the median, and first and third quartiles, with the outlier 5th and 95th percentiles indicated with filled circles. The difference in the median values between H_2O_2 alone and H_2O_2 with the Pkc1-inhibitor cercosporamide is greater than would be expected by chance. Statistical significance was determined by the Mann-Whitney Rank sum test (p=0.011). At least 210 cells were analyzed for each condition.



Supplementary Figure 10. Nuclear translocation of Msn2-GFP upon oxidative stress triggered by H_2O_2. Wild type or *mid2Δ* cells expressing Msn2-GFP were imaged before (0 min) or 10 min after addition of 2 mM H_2O_2. Note that the nuclear translocation of Msn2-GFP induced by oxidative stress is dampened in the absence of Mid2. Scale bar: 5 µm.



Supplementary Figure 11. p*FIG1*-qV expression with error bars of the experiment shown in Figure 5D, quantified microscopically in wild type (WT) and *ste5*^{S185A} cells at the indicated time points.