Supplementary Material:

Fig. s1

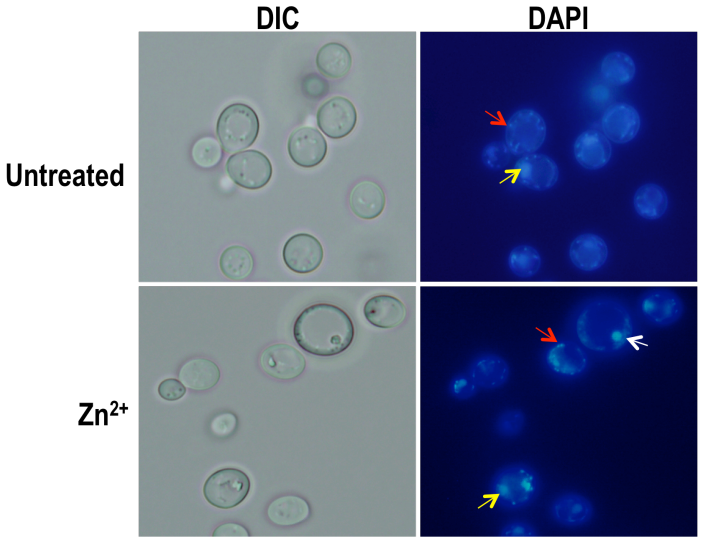

Figure s1. Vacuolar inclusions in Zn^{2+}-treated cells bound DAPI. BY4741 PS cells were grown to an OD_{600} = 0.2, treated with 13 mM $ZnSO_4$ for 6 h, and stained with 3 μM DAPI. The photograph shows an example of the results obtained in 3 independent experiments. Yellow arrows point to the stained nuclei, red arrows point to the stained mitochondria, and white arrows point to stained vacuolar inclusions, in representative cells. Both the location and the fluorescence of the material were characteristic of polyphosphates. [1] Moreover, *Δvtc4* strains, which are characteristically defective in polyphosphate accumulation in vacuoles, accumulated fewer inclusions.

Fig. s2

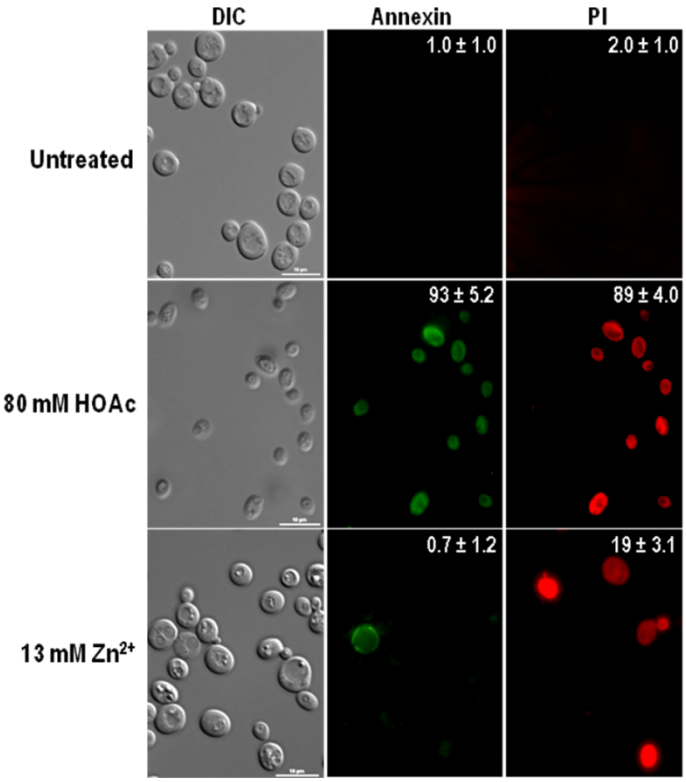

Figure s2. Zn^{2+} treated cells failed to expose annexin-binding sites. BY4741 PS cells were grown to an OD_{600} = 0.2, treated with 13 mM $ZnSO_4$ for 6 h, and permeabilized as described in Materials and Methods. The wall-less cells were subsequently stained with both annexin-FITC (green fluorescence) and PI (red fluorescence) following standard procedures. [2] Acetic acid treatments [3] were included to induce standard apoptotic responses. The numbers in the upper right corner of each panel represent the average number ± SD of necrotic (PI-staining) or apoptotic (annexin-staining) cells based on 3 independent experiments, with 44-72 cells/ treatment.

Fig. s3

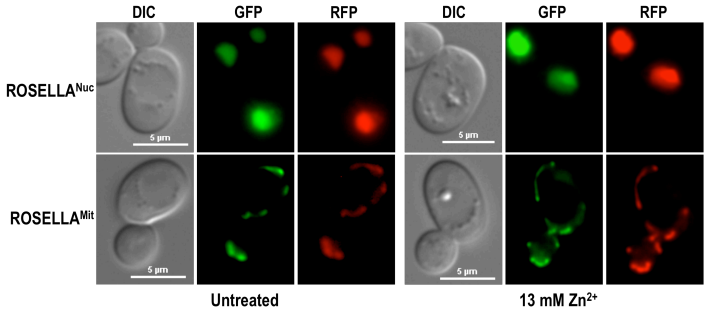

Figure s3. Zn^{2+} did not promote the harvesting of either nuclear- or mitochondrially-targeted ROSELLA. BY4741 PS cells transformed with plasmids encoding either *NAB35-ROSELLA* ($ROSELLA^{Nuc}$; a reporter targeted to the nucleus) or *CIT2-ROSELLA* ($ROSELLA^{Mit}$; a reporter targeted to the mitochondrion) [4], were grown to an OD_{600} = 0.2 and then treated with1 mM PSMF and 13 mM $ZnSO_4$ for 6 h. Vacuolar inclusions are prominent in both images at this time but neither the encoded proteins, nor a ribosome-targeted reporter, RPL25-GFP [5] (data not shown), accumulated in vacuoles, even after 24 h (data not shown).

Fig. s4

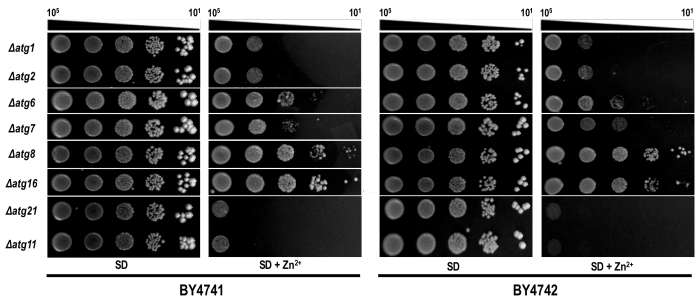

Figure s4. Autophagic mutants were differentially inhibited by Zn^{+2}. BY4741 PS cells together with 8 knockout mutants were grown in SD + 0.002 % uracil, diluted by factors of 10, and replica plated onto SD medium containing 0.002% uracil with or without 13 mM $ZnSO_4$. For the sake of consistency, the same experiments were carried on mutations in a BY4742 background. Pictures were taken after 3 d. The white spaces were introduced to show that figures were constructed from tests carried out on different plates containing the same media.

Fig. s5

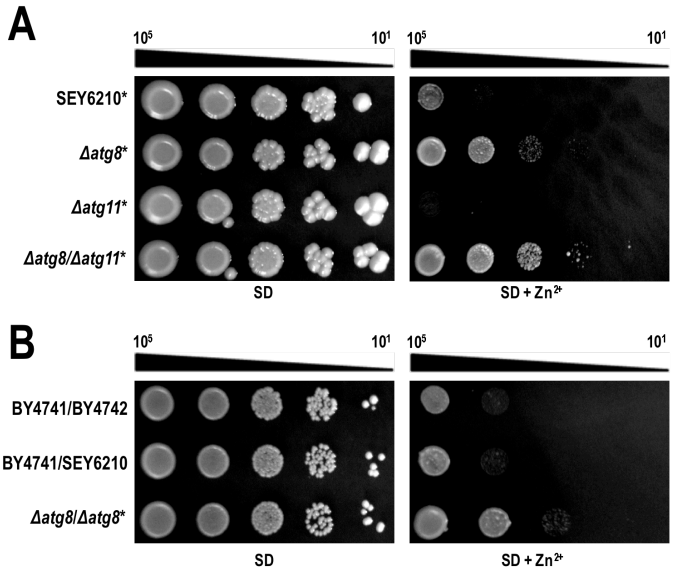

Figure s5. Mutants of SEY6210 behaved qualitatively like those of BY4741. A. SEY6210 and 3 autophagy mutants were cultured on SD medium supplemented with 0.002% histidine, 0.01% leucine, 0.003% lysine, 0.002% tryptophan, and 0.002% uracil for 5 d. The asterisk denotes strains derived from a SEY6210 parent. Note that SEY6210 was more inhibited by 13 mM Zn^{+2} than BY4741 grown for only 3 d (Fig s4). Note too, that strains missing both *ATG8* and *ATG11* grew better than strains missing either of the genes alone. B. Diploid strains were grown for 3 d on the same medium as in Fig s5A. Note that the sensitivity of SEY6210 was recessive to the tolerance of BY4741.

1. Puchkov EO. Brownian motion of polyphosphate complexes in yeast vacuoles: characterization by fluorescence microscopy with image analysis. Yeast 2010; 27:309-15.
2. Madeo F, Frohlich E, Frohlich KU. A yeast mutant showing diagnostic markers of early and late apoptosis. J Cell Biol 1997; 139:729-34.
3. Ludovico P, Sousa MJ, Silva MT, Leao C, Corte-Real M. Saccharomyces cerevisiae commits to a programmed cell death process in response to acetic acid. Microbiology 2001; 147:2409-15.
4. Rosado CJ, Mijaljica D, Hatzinisiriou I, Prescott M, Devenish RJ. Rosella: a fluorescent pH-biosensor for reporting vacuolar turnover of cytosol and organelles in yeast. Autophagy 2008; 4:205-13.
5. Kraft C, Deplazes A, Sohrmann M, Peter M. Mature ribosomes are selectively degraded upon starvation by an autophagy pathway requiring the Ubp3p/Bre5p ubiquitin protease. Nature cell biology 2008; 10:602-10.