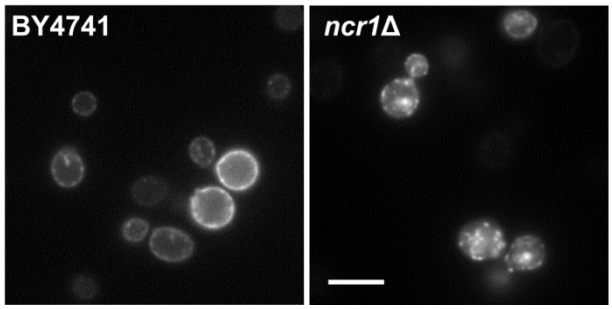

Fig. S1. Accumulation of intracellular sterol in Ncr1p deficient cells. *S. cerevisiae* BY4741 and *ncr1*Δ*::KanMX4* cells were fixed and treated with filipin complex as previously described (Beh & Rine, 2004). Briefly, $1.5x10^8$ cells were fixed with 3.75 % formaldehyde for 10 min with agitation at 26 ºC .The fixed cells were washed and ressupended in sterile phosphate buffer and filipin complex (Sigma) was added to a final concentration of 0.1 mg ml^{-1}. After incubation for 15 minutes in the dark, cells were washed with phosphate buffer and mounted in poly-lysine coated slides, sealed under coverslips with nail polish, and imaged on Zeiss AxioImager Z1 microscope (Carl Zeiss) using an Axiocam v3.0 (Carl Zeiss). Equal exposure times were used to compare cellular fluorescence. Images were analyzed with ImageJ 1.47n software. Scale bar: 5 µm.

References

Beh, C.T. & Rine, J. (2004) A role for yeast oxysterol-binding protein homologs in endocytosis and in the maintenance of intracellular sterol-lipid distribution. *J Cell Sci* **117**: 2983-2996.

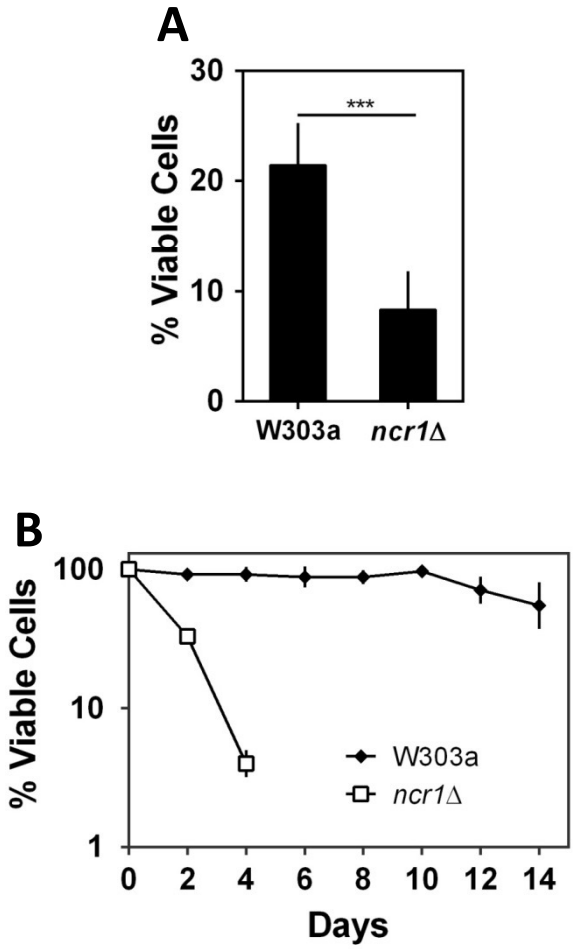

Fig. S2. Ncr1p deficiency decreases chronological lifespan and oxidative stress resistance in W303a (Matα, *ade2-1*, *can1-100*, *trp1-1*, *ura3-1*, *his3-11,15*, *leu2-3,112*) background. (A) *S. cerevisiae* W303 and W303 *ncr1*Δ*::KanMX4* cells were grown to exponential phase and exposed to 1.5 mM H_2O_2 for 1 hour. Cellular viability was measured as the percentage of the colony-forming unit (treated cells vs non-stressed cells). ****p*<0.001, unpaired Student's t-test. (B) Yeast cells were grown in SC-glucose medium at 26 ºC to PDS phase. Cellular viability was measured at 2 to 3 days intervals and expressed as % colony forming units (aged vs day 0). Values are mean ± SD of at least three independent experiments.

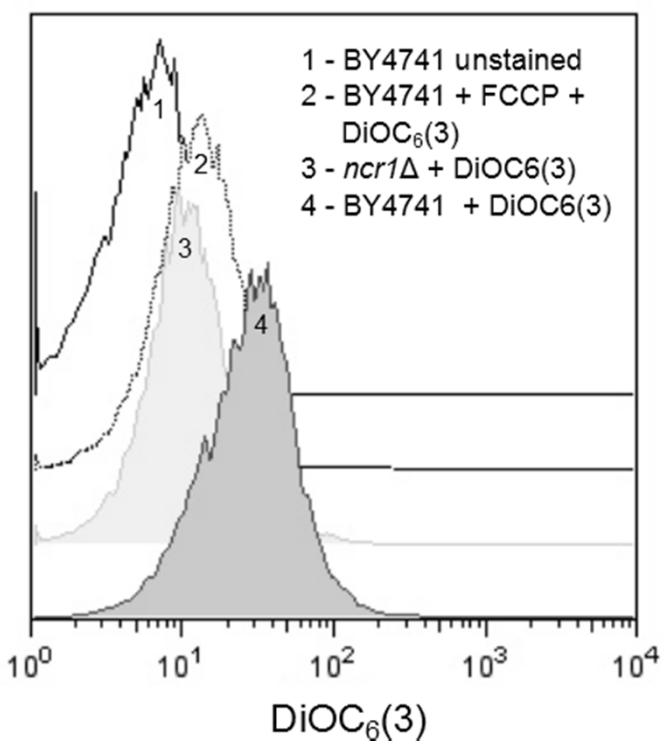

Fig. S3. *S. cerevisiae* BY4741 and *ncr1*Δ*::KanMX4* cells were grown in SC-glucose medium to PDS phase. Mitochondrial membrane potential was determined by flow cytometry using cells unlabeled (auto fluorescence) or labeled with $DiOC_6(3)$, as described in Experimental procedures. Representative histograms for each condition are shown. As a positive control, mitochondrial membrane potential was measured in BY4741 cells pre-incubated with 10 μM FCCP for 10 minutes prior to the addition of $DiOC_6(3)$.

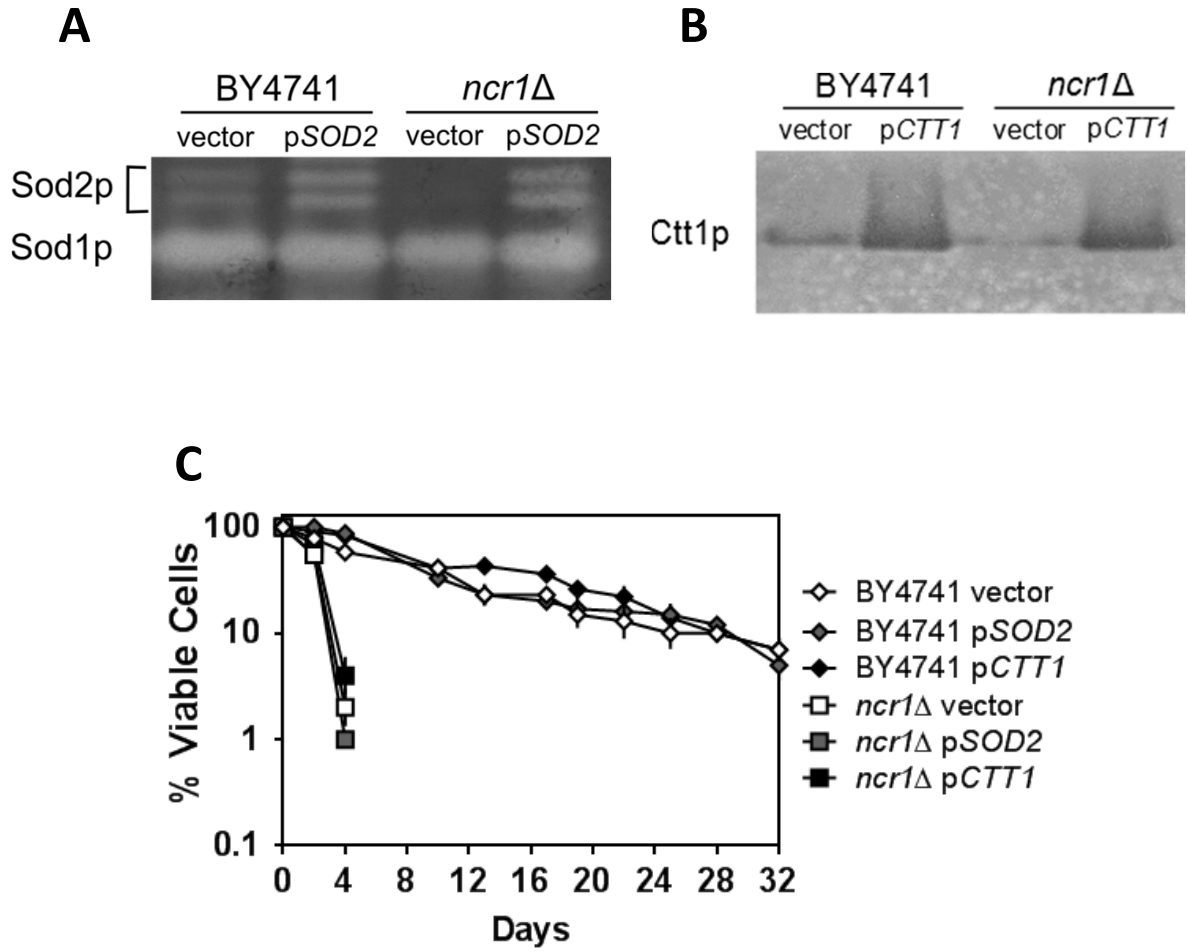

Fig. S4. *S. cerevisiae* BY4741 and *ncr1*Δ*::KanMX4* cells were transformed with plasmids expressing *CTT1* or *SOD2* and grown in SC-glucose medium without uracil to PDS phase. (A, B) The activity of Sod2p and Ctt1p was assessed after native-PAGE, as described in methods. One representative experiment out of two is shown. (C) Cellular viability was measured at 2 to 3 days intervals and was expressed as % colony forming units (aged vs day 0). Values are mean ± SD of two independent experiments.