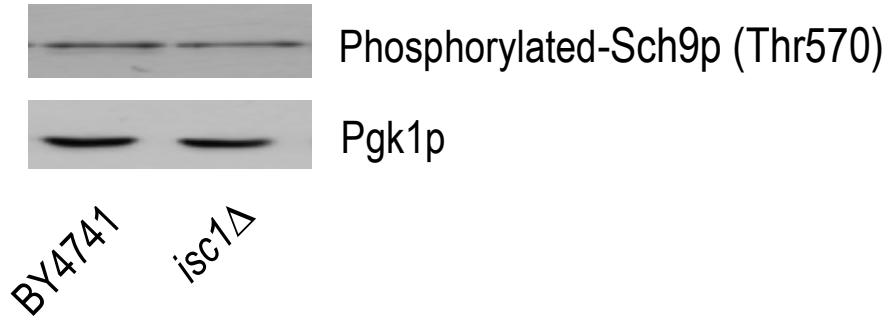

Figure S1. The Pkh1/2p-dependent Sch9p phosphorylation does not contribute to *isc1*Δ phenotypes. The Pkh1/2p-mediated phosphorylation of Sch9p at Thr570 was monitored in BY4741 and *isc1*Δ cells by immunoblotting, using anti-phospho-Thr570-Sch9p antibody (top panel) or anti-Pgk1p (loading control) as primary antibodies. A representative blot is shown.

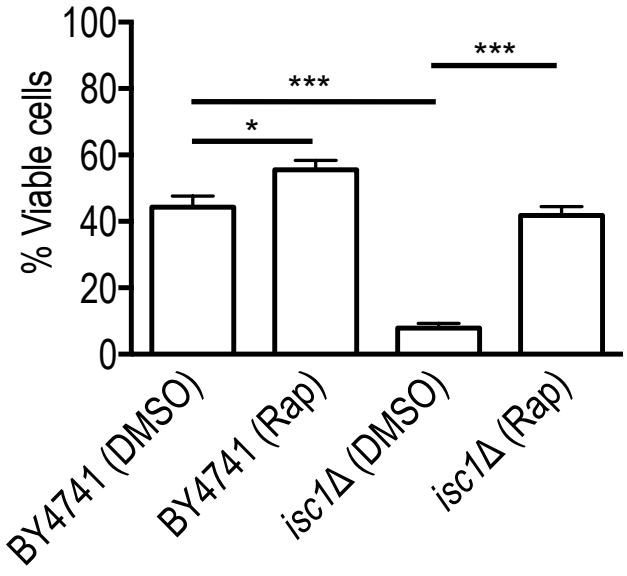

Figure S2. **Inhibition of TORC1 signaling with rapamycin suppresses oxidative stress sensitivity displayed by *isc1*Δ cells.** Yeast cells were grown in SC-medium to the early exponential phase (OD_{600}=0.3), pre-incubated with either 200 ng.mL^{-1} rapamycin (Rap) or vehicle (DMSO) for 3h and then treated with 1.5 mM H_2O_2 for 60 min. Cell viability was determined as described in Experimental Procedures. ***p<0.001; *p<0.05.

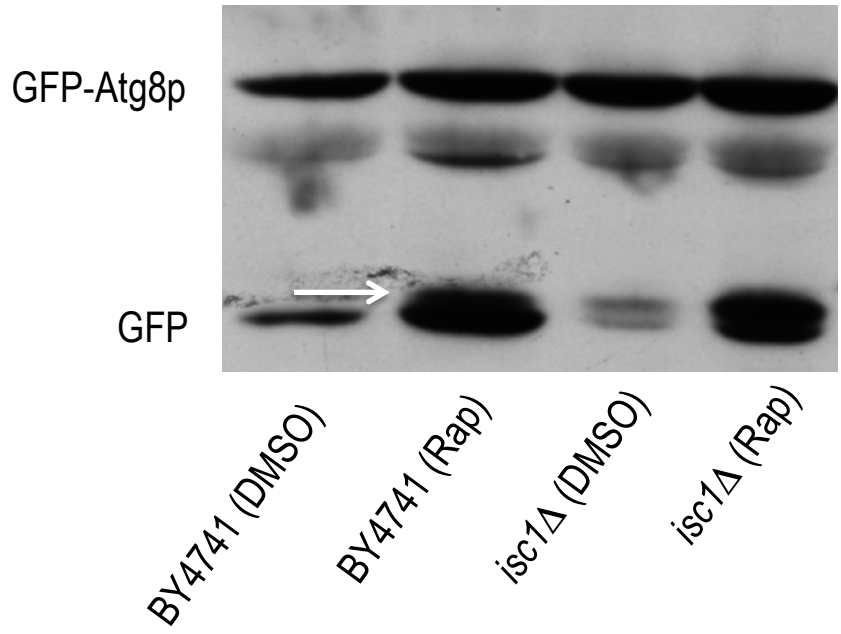

Figure S3. **The autophagic flux decreases in *isc1*Δ cells.** *S. cerevisiae* BY4741 and *isc1*Δ cells carrying pRS416 GFP-*ATG8* were grown to the exponential phase in SC-medium and treated with either 200 $ng.mL^{-1}$ rapamycin (Rap) or DMSO (vehicle) for 3 hours. Proteins were analyzed by immunoblotting, using anti-GFP antibody. The Western blot shown here is a replicate of that shown in Fig. 4C (only for BY4741 and *isc1*Δ cells) with a longer exposure time.

A slower migrating band (above free GFP) was observed in *isc1*Δ cells, both under basal conditions and upon rapamycin treatment. A similar upper band (marked with an arrow) was observed in parental cells upon treatment with rapamycin. Notably, this upper band was more abundant in *isc1*Δ cells when compared to the one observed in parental cells. It probably results from impaired or aberrant GFP-Atg8p processing, possibly due to vacuolar dysfunction (defective Pep4p-dependent proteolytic activity) or alterations in vacuolar morphology upon deletion of *ISC1*, as mentioned in the main text. Thus, we have considered only the higher migrating band as free GFP (generated from processing of GFP-Atg8p) for autophagic flux quantification (Fig. 4D).

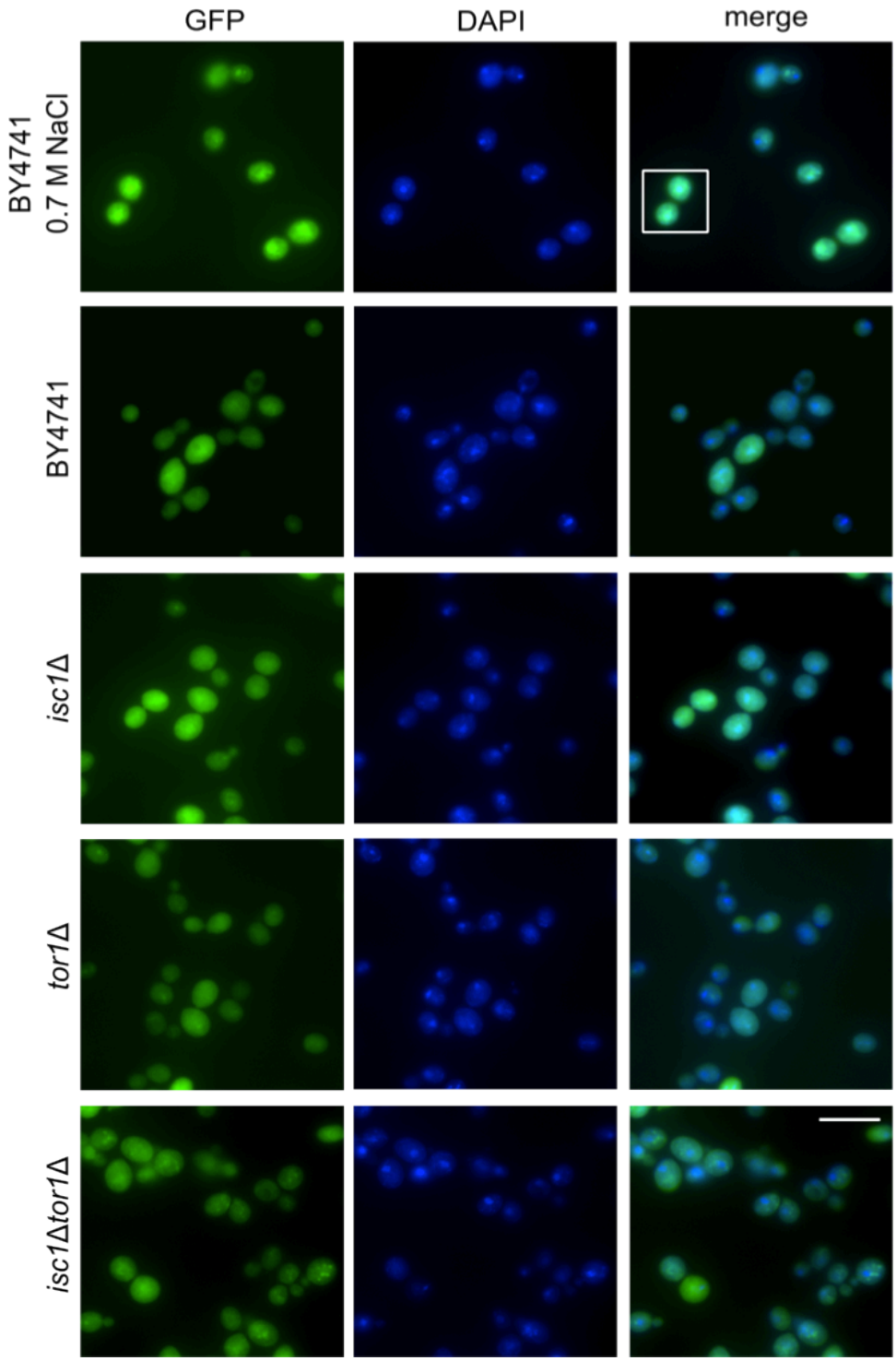

Figure S4. The deletion of *TOR1* does not alter Hog1p localization in *isc1*Δ cells. *S. cerevisiae* BY4741, *isc1*Δ, *tor1*Δ and *isc1*Δ*tor1*Δ cells transformed with pRS416-*HOG1-GFP* were grown to the exponential phase and live cells were visualized by fluorescence microscopy, as described in *Experimental Procedures*. BY4741 cells treated with 0.7 M NaCl for 20 min were used as positive control to monitor Hog1p translocation to the nucleus. Scale bar: 5 μm.

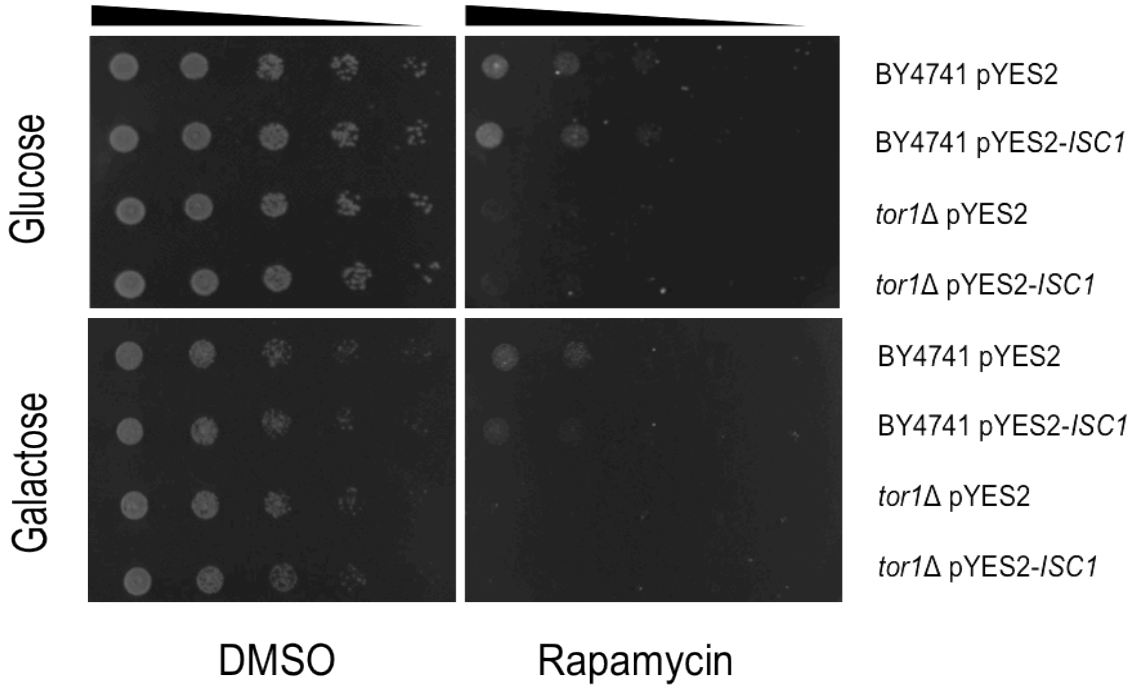

Figure S5. The rapamycin sensitivity of the *tor1*Δ mutant was not suppressed by *ISC1* overexpression. *S. cerevisiae* BY4741 and *tor1*Δ cells carrying pYES2 or pYES2-*ISC1* were grown in SC-medium to the exponential phase and then diluted to OD_{600}=0.1. Fivefold dilution series were spotted on glucose or galactose media containing either rapamycin (50 $ng.mL^{-1}$, dissolved in DMSO) or DMSO (vehicle) and cells were grown at 26°C for 5 days.