Supplementary Table 1

Residue	Score
S267[a]	0.967
S134[a]	0.901
S130	0.879
S179[a]	0.858
S73[b]	0.832
S252	0.798
S21	0.777
S244	0.766
S143	0.713
S15	0.694
S289[b]	0.694
T3	0.691
S72	0.648
S278	0.647
S149	0.608
T75	0.604
S76	0.588
S265	0.576

Predicted Sae2 phosphorylation sites obtained with NetPhosYeast 1.0 (http://www.cbs.dtu.dk/services/NetPhosYeast/)[1]. [a] Three putative CDK phosphorylation sites on Sae2. [b] Two of the five residues proposed to be phosphorylated by Mec1 and Tel1[2].

Supplemental figure 1: Conservation of Sae2 proteins within the *Saccharomyces* genus. Sae2 orthologue sequences from *Saccharomyces cerevisiae* (Scer), *S. paradoxus* (Spar), *S. mikatae* (Smik), *S. bayanus* (Sbay), *S. kudriavzevii* (Skud), *S. castellii* (Scas) and *S. kluyveri* (Sklu) were aligned with the ClustalW program (http://www.ebi.ac.uk/clustalw/index.html). Previously proposed Mec1 and Tel1 phosphorylation sites are highlighted in blue and indicated with a blue asterisk. The three putative CDK1 phosphorylation sites are labelled with a red dot and highlighted in red. A putative cyclin-binding motif (RXL) is highlighted in red and indicated with a bar.

Supplemental figure 2: Sae2 phosphorylation controls sensitivity towards DNA damage generated in S-phase. a, G2-arrested TAP-purified Sae2 was treated with lambda phosphatase as indicated. Other details as in Fig. 1a. **b,** 5-fold serial dilutions of saturated overnight cultures of *sae2*Δ cells transformed with plasmids harbouring the indicated *sae2* mutants were plated as described in Fig 1c. **c**, Sensitivity to camptothecin of *sae2*Δ cells transformed with a plasmid containing *SAE2*, *sae2-S267A*, *sae2-S267E* or an empty vector was measured by plotting the values obtained when the slopes of growth curves in the presence of the indicated doses of camptothecin ($Slope_c$) were normalized to the corresponding slopes obtained with a DMSO control ($Slope_{c=0}$), as previously described[3]. **d**, Cells were grown and plated as described in Fig. 1d but in the presence of 0.018% MMS instead of camptothecin. **e**, Expression levels of GFP-CtIP fusions before (- siCtIP) and after (+ siCtIP) downregulation of the endogenous CtIP. Protein extracts were separated by electrophoresis on 8% SDS-PAGE and blotted with either anti-CtIP mouse monoclonal antibody or anti-Mre11 as loading control.

Supplemental figure 3: DNA resection in *sae2* mutants. a, Representative neutral and denatured dot blots of the data shown in Fig. 3a. **b,**

Representative neutral and denatured dot blots of the data shown in Fig. 3b. Asterisk marks unspecific hybridization.

Supplemental figure 4: Recombination between sister chromatides in *sae2* mutants. Representative Southern blots of the data plotted in Fig. 4e. Experiments were performed as described[4]. Mid-log phase cultures of yeast cells grown on 3% glycerol-2% lactate medium were supplemented with 2% galactose to induce HO expression. Samples were taken at the times indicated, DNA was isolated for Southern analysis and the total DNA was digested with SpeI and XhoI, then probed with the ^{32}P-labeled 0.6 kb ClaI-EcoRV internal fragment of the *LEU2* repeats. The positions of the uncut fragment, the two fragments that appear after HO cleavage (DSB) and the recombination intermediates (SCE; sister-chromatid exchange) are shown. A schematic representation of how the different bands appear after conversion of the single-strand break (SSB) to a DSB after replication and after recombination takes place is shown above. As the 2.9 kb band (SpeI-SpeI site) can arise from other types of recombination[5] but the 4.7 kb (XhoI-XhoI) only appears after sister chromatid exchange, SCE levels were calculated as the ratio between the 4.7 kb band and the total plasmid DNA (presented in Fig. 4e). All experiments were performed at least two times.

Supplemental methods:

Sae2 orthologues in higher eukaryotes: The Sae2 orthologues shown in Fig. 1b correspond to the following species: *Saccharomyces cerevisiae* (Sae2), *Ashbya gossypii* (*A. gossypii*), *Yarrowia lipolytica* (*Y. lipolytica*), *Cryptococcus neoformans* (*C. neoformans*), *Neurospora crassa* (*N. crassa*), *Chaetomium globosum* (*C. globosum)*, *Phaeosphaeria nodorum* (*P. nodorum*), *Caenorhabditis elegans* (*C. elegans* Com-1), *Arabidopsis thaliana* (*A. thaliana* Com1), *Xenopus laevis* (*Xenopus*), *Gallus gallus* (*Chicken CtIP*), *Homo sapiens* (*Human CtIP*).

Supplementary references:

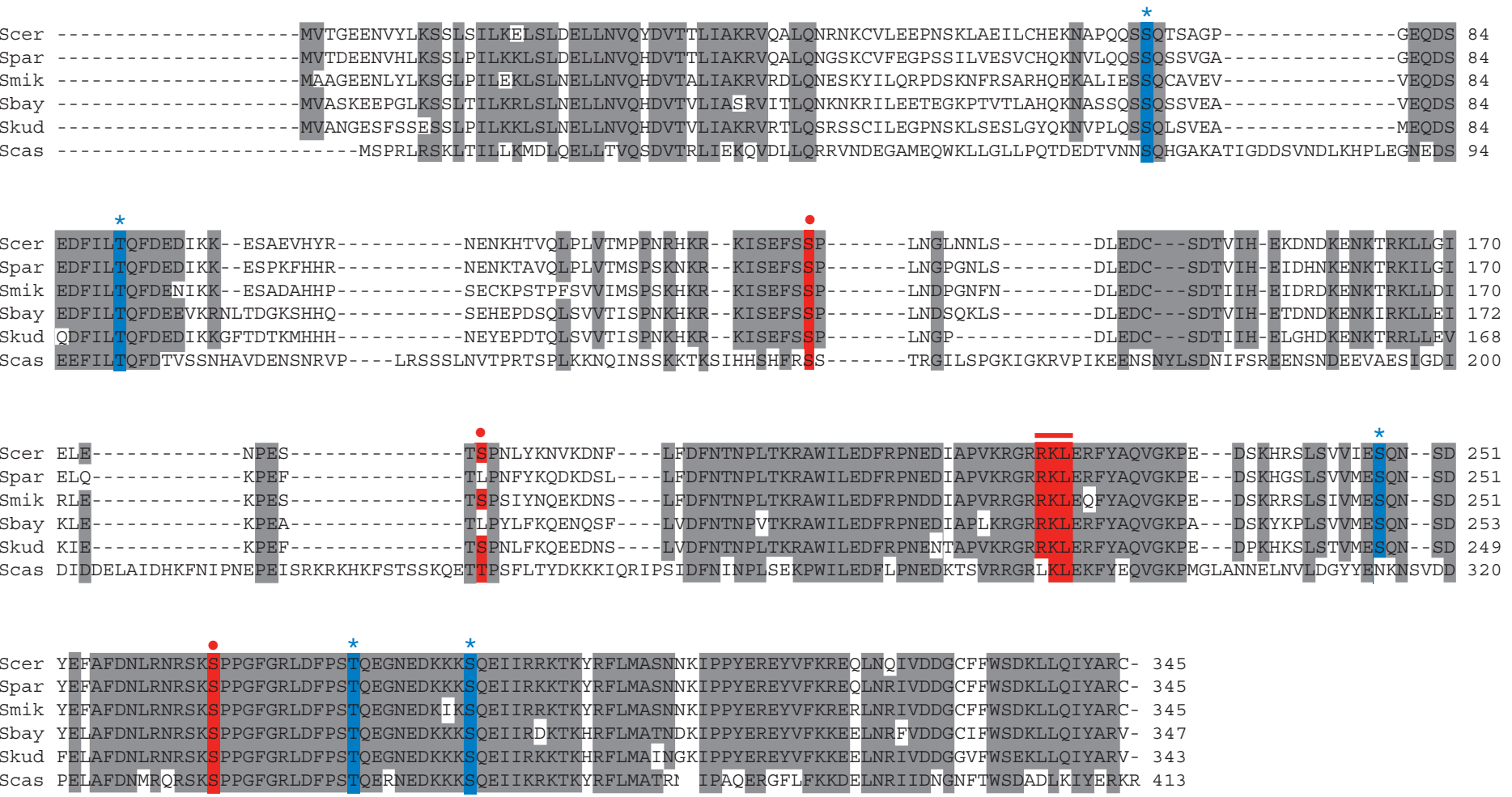
1. Ingrell, C. R., Miller, M. L., Jensen, O. N. & Blom, N. NetPhosYeast: prediction of protein phosphorylation sites in yeast. *Bioinformatics* **23**, 895-7 (2007).

2. Baroni, E., Viscardi, V., Cartagena-Lirola, H., Lucchini, G. & Longhese, M. P. The functions of budding yeast Sae2 in the DNA damage response require Mec1- and Tel1-dependent phosphorylation. *Mol. Cell. Biol.* **24**, 4151-65 (2004).

3. Vance, J. R. & Wilson, T. E. Yeast Tdp1 and Rad1-Rad10 function as redundant pathways for repairing Top1 replicative damage. *Proc. Natl. Acad. Sci. USA* **99**, 13669-74 (2002).

4. Gonzalez-Barrera, S., Cortes-Ledesma, F., Wellinger, R. E. & Aguilera, A. Equal sister chromatid exchange is a major mechanism of double-strand break repair in yeast. *Mol. Cell.* **11**, 1661-71 (2003).

5. Cortes-Ledesma, F. & Aguilera, A. Double-strand breaks arising by replication through a nick are repaired by cohesin-dependent sister-chromatid exchange. *EMBO Rep.* **7**, 919-26 (2006).



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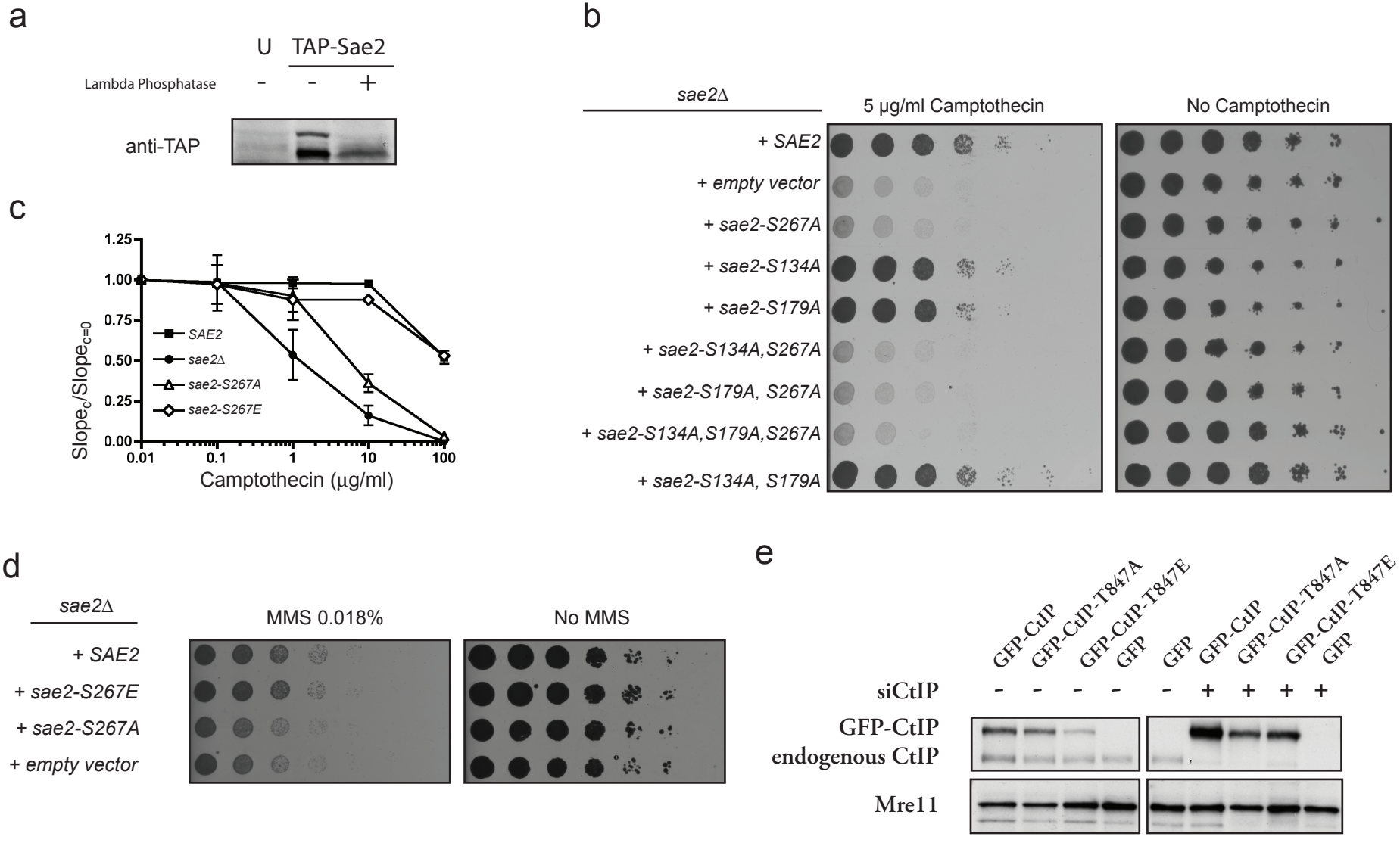
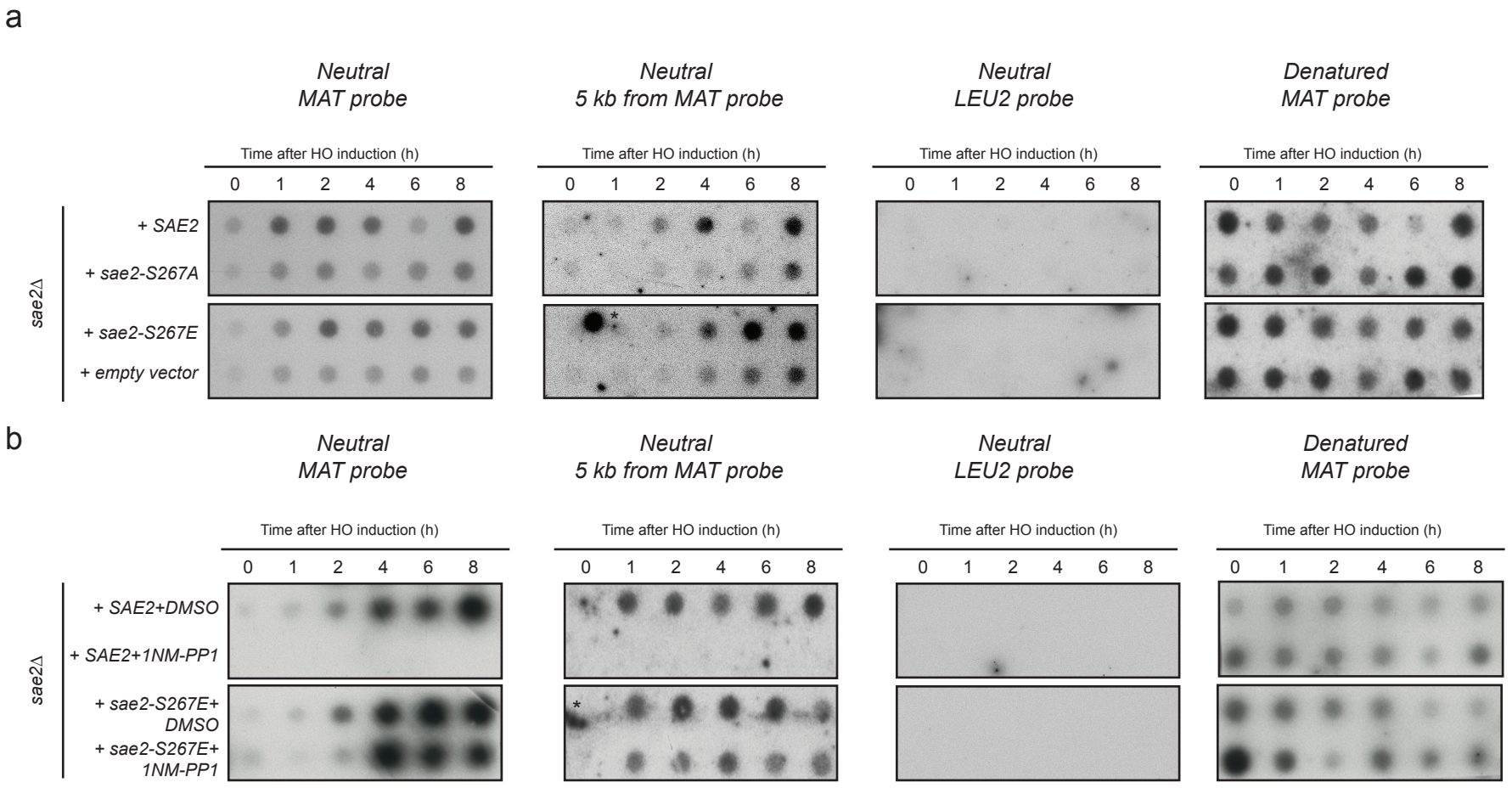
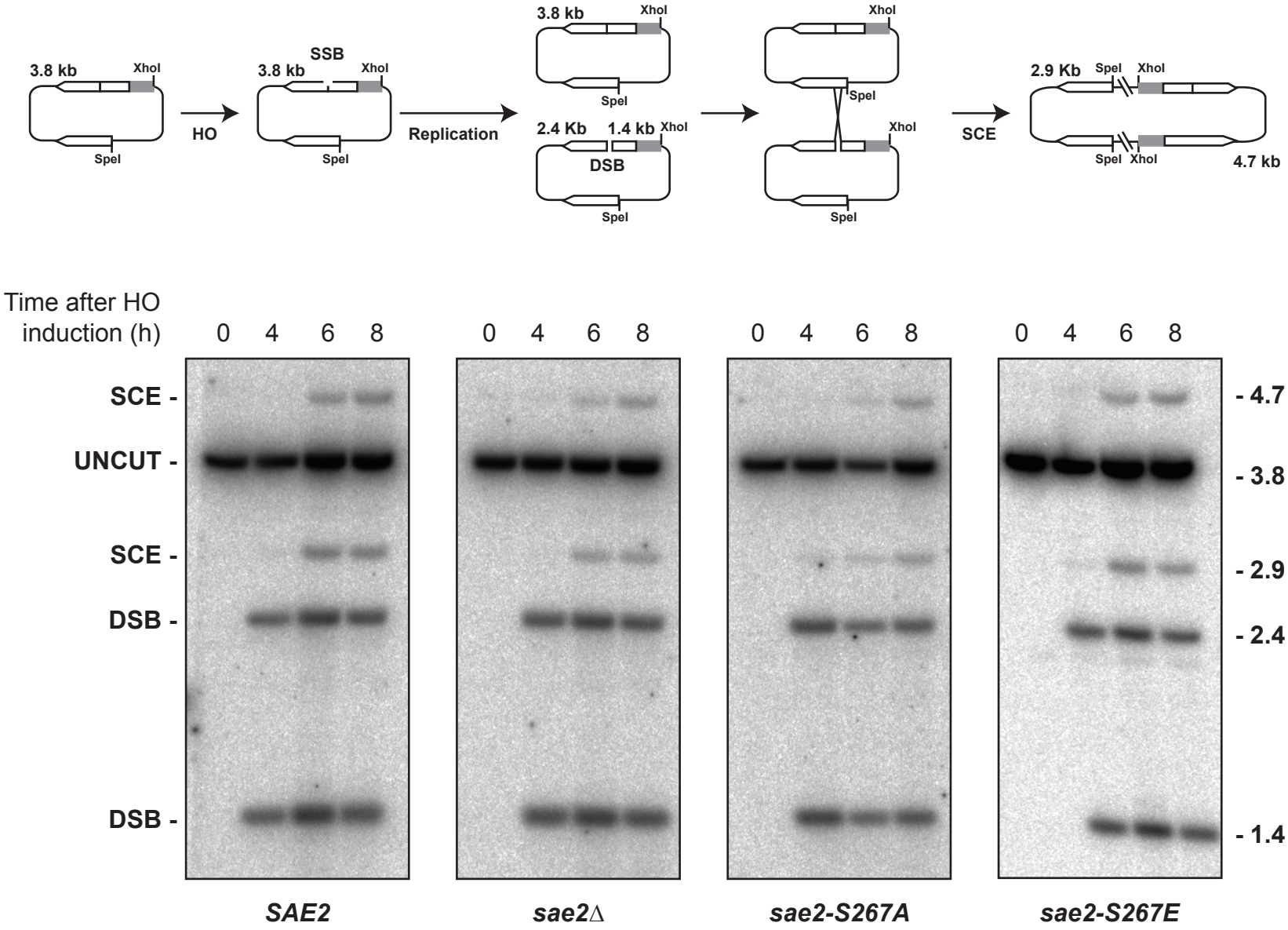


Huertas *et al*. Supplemental figure 2



Huertas *et al*. Supplemental Figure 3



Huertas *et al*. Supplemental figure 4