

Expanded View Figures

Figure EV1. Sir3 overexpression limits resection and increases error-prone NHEJ.

- A Survival frequencies and characterization of the repair events after induction of a DSB at LYS2 in absence of recombination substrate.
- B Survival frequencies and characterization of the repair events after induction of a DSB at TEL6R in absence of recombination substrate.
- C DNA levels measured at 1 kb from the I-Scel cut site at LYS2 after 2 h DSB induction by qPCR in WT and sae2∆ cells expressing or not high levels of Sir3p (oeSir3 and WT, respectively). DNA levels were normalized to DNA levels at the OGG1 locus and corrected for differences in DSB cleavage efficiency (see Materials and Methods for details). Error bars represent the standard deviation (SD) of three independent experiments.

Data information: (A, B) NHEJ stands for error-prone end-joining events detected by a PCR product that cannot be cleaved *in vitro* by I-Scel. No DSB corresponds to survivors giving a PCR product that can be cleaved by I-Scel *in vitro* showing that they failed to induce I-Scel. Other gathers survivors in which no PCR product was obtained suggesting that repair occurred through other mechanisms. PCR products corresponding to NHEJ events were sequenced and exhibit patterns typical of NHEJ repair (rejoining with 1- to 9-bp deletion between sequences showing no or limited homology).



Figure EV2. Sir3 overexpression does not affect Sae2 levels.

Western blot analysis with anti-GFP antibodies of whole-cell protein extracts prepared from stationary phase cells. The 80 kDa band detected by mab414 is used as a loading control.

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Figure EV3. Protein sequence alignment of Sae2 and Sir3 interacting domains.

A Multiple sequence alignment of the Sir3^{SaID} domain of the Saccharomycetaceae family. NCBI RefSeq identifiers are given in parentheses. Below, ribon representation of the X-ray structure of the Sir3^{SaID} domain (rainbow colours) and a surface projection of the conservation as calculated by the rate4site algorithm (Pupko *et al*, 2002) with a white-yellow-red colour gradient highlighting the most conserved region in red.

B Multiple sequence alignment of the Sae2^C domain of the Saccharomycetaceae family. NCBI RefSeq identifiers are given in parentheses.





Figure EV4. The T557I mutation does not affect Sir3 protein levels.

A, B Western blot analysis with Sir3 antibodies of protein extracts prepared from stationary phase cells of the indicated strains. Dps1 is used as a loading control. *Asterisk marks cross-reacting Orc1 detected by the Sir3 antibody.





Figure EV5. Telomeric silencing is not affected upon Sae2 overexpression.

Telomeric silencing assay at TEL7L as depicted in the upper scheme, in WT and sir4 Δ cells overexpressing SAE2 (2 μ -Sae2) or not (2 μ). Increased growth on 5-FOA or decreased growth on -URA plates reflects an increase in telomeric silencing.