Online supplementary data

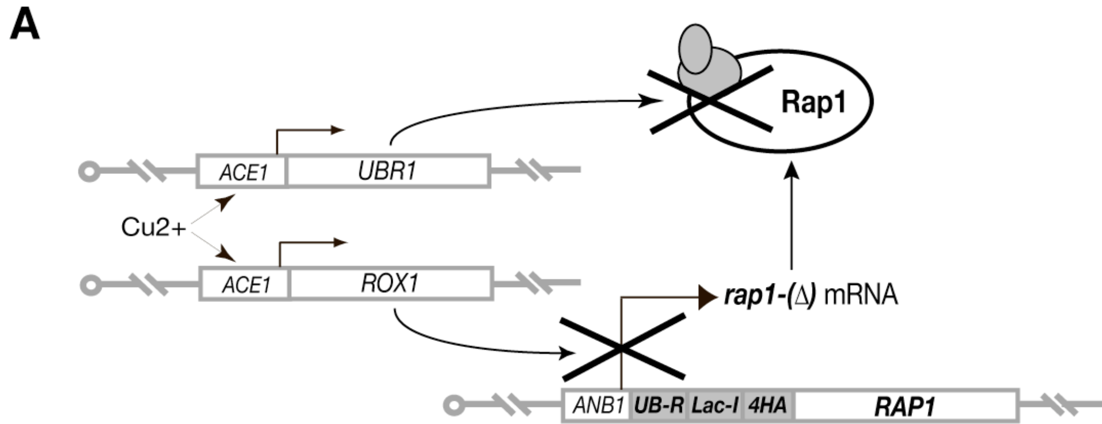
Copper-induced Rap1 loss causes telomere lengthening and fusions

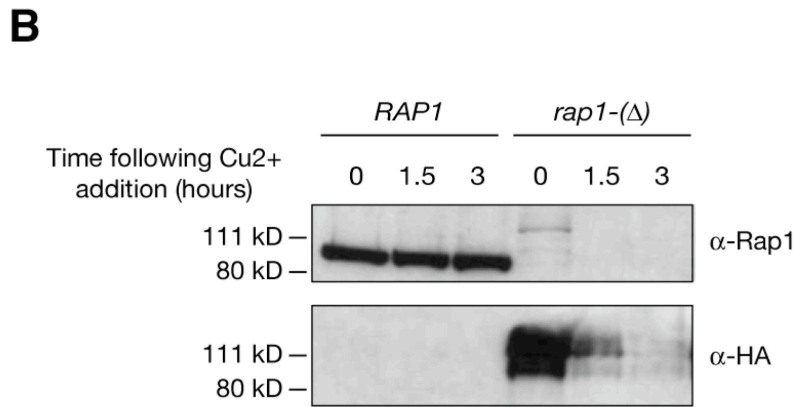
rap1-(Δ) is a "double shut-off" allele designed to allow rapid Rap1 loss upon copper addition to the medium (Supplementary Figure S1A, B). In cells growing exponentially, induction of Rap1 loss by copper results in cell growth arrest after two to three generations following copper addition (data not shown). This block is not reversed by washing out copper and still occurs in a *rap1-(Δ) lif1-*Δ double mutant defective for NHEJ (data not shown). The southern shown in Supplementary Figure S1C reveals the length of the telomeres with a Y' element, which are about 50 bp shorter in uninduced *rap1-(Δ)* cells compared to wild-type cells (see the Method section). Copper-induced Rap1 loss causes telomere elongation. The mean length increases by about 130-bp. In wild-type cells and in *rap1-(Δ)* cells progressing toward stationary phase without added copper, telomere length remains constant. In a *rap1-(Δ) tel1-*Δ double mutant induced by copper, telomere length remains constant, suggesting that the telomere elongation observed in *rap1-(Δ)* cells is telomerase-dependent (data not shown).

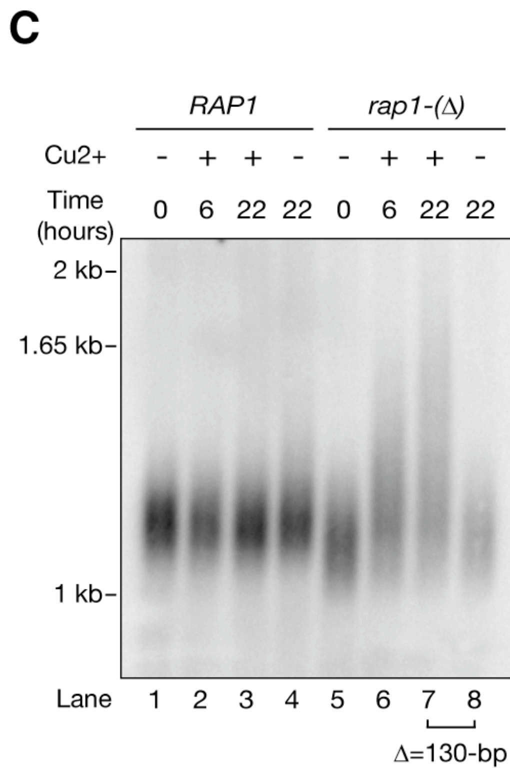
As shown in Supplementary Figure S1D, telomere fusions appears in *rap1-(Δ)* cells after copper addition (lanes 6 and 7). Fusions also appears in uninduced *rap1-(Δ)* cells that have exhausted the medium and exited exponential phase (lane 8) but not in wild-type cells nor in *rap1-(Δ)* cells prior to copper addition (lanes 1 to 5). The PCR signal from *rap1-(Δ)* cells induced with copper is at a higher molecular weight compared to the one obtained from cells progressing toward stationary phase without copper (Supplementary Figure S1D, lanes 7 and 8). The size difference between the two PCR smears is about 50-bp. Considering the 130-bp telomere elongation, this more modest difference suggests that the

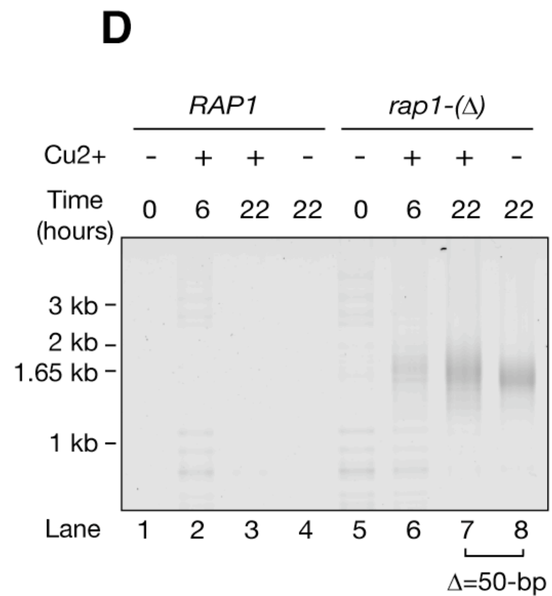
short telomeres are more prone to fuse or that the fusions between long telomeres are inefficiently amplified by the PCR.

Figure S1: Rap1 loss in growing cells. **A**, Schematic representation of the *rap1-(Δ)* "double shut off" allele in strain Lev391. The *UBR1* and *ROX1* genes are under a copper-inducible *ACE1* promoter. At its endogenous locus, *RAP1* is fused to an *ANB1* promoter and to a sequence encoding a UB-R tag to generate a N-end with an arginine residue and 4 HA epitopes. **B**, Immunoblot showing Rap1 level in wild-type and *rap1-(Δ)* cells following copper addition to exponentially growing cultures. **C**, Telomere elongation upon Rap1 loss in growing cells. Yeast strains ZMY60 (wild-type) and Lev391 (*rap1-(Δ)*) were maintained at 30°C in exponential phase by successive dilutions in synthetic medium. At time 0, the cultures (at a density of 2 to 4 x 10^6 cells per ml) were divided in two and one was supplemented with copper at a final concentration of 500μM. The cells were then allowed to exhaust the medium and eventually exit exponential phase. Genomic DNA were digested by *Xho*I, separated by agarose-gel electrophoresis, blot onto a membrane and hybridized with a probe complementary to the telomere proximal end of the Y' elements. The signal for each lane was quantified on a Typhoon™ imager by ImageQuant™ 5.0 to determine the length of the terminal restriction fragments. **D**, Detection by PCR of fusions between X and Y' telomeres in *rap1-(Δ)* after copper addition. The PCR were performed on the same genomic DNA samples that were used for Southern analysis. The signal for each lane was quantified on a Typhoon™ imager by ImageQuant™ 5.0 to determine the length of the amplified molecules.









Supplementary Figure S1