Figure S1: AFM imaging of wild type cells CEN.PK113-7D treated with ethanol. High resolution AFM image (z-range = 100 nm; scale bar = 200 nm) of an exponentially growing cell of strain CEN.PK113-7D on YPD not treated or treated for 5 h with 9 % ethanol is shown (a,b), with the measured roughness on different area on the untreated and ethanol-treated cell (e). Elasticity maps (z-range =800 kPa; scale bar = 200nm) recorded from the Force versus Distance curves (n=1064) illustrated a reduced stiffness on the cell surface of the ethanol treated cell (f) compared to untreated cells (c) which was reflected by a Young'modulus of these ethanol-treated cells 3,5 fold lower (g) than untreated cells (d)

Figure S1 (Schiavone *et al*.)

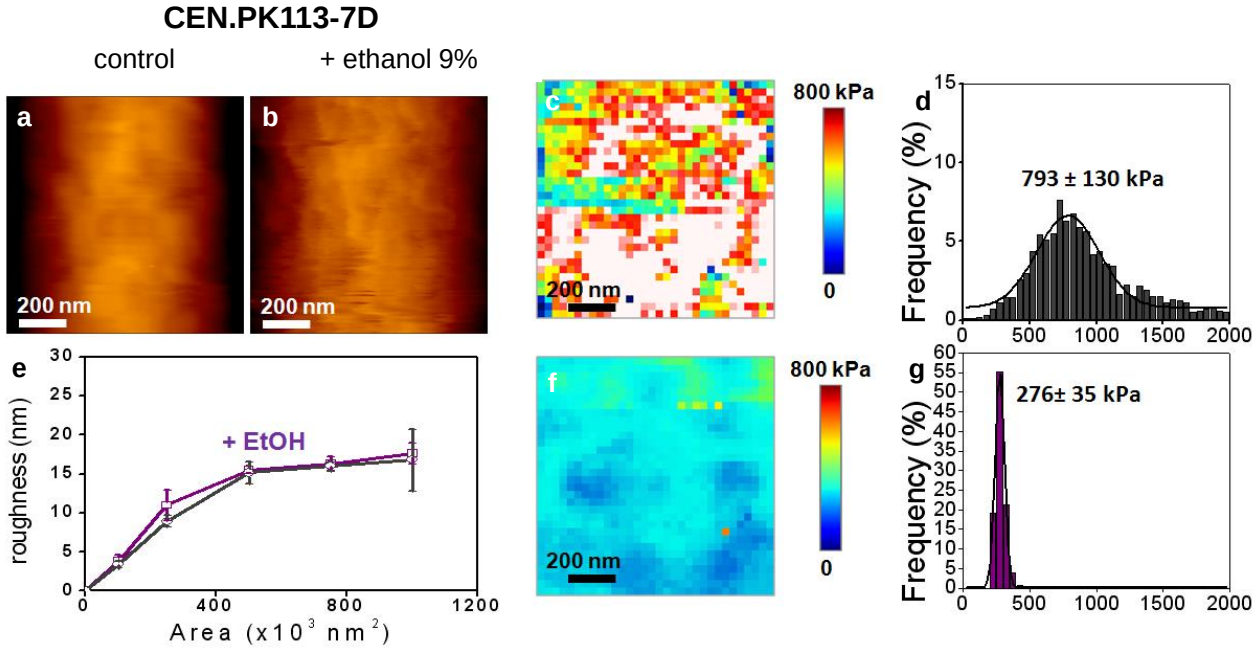
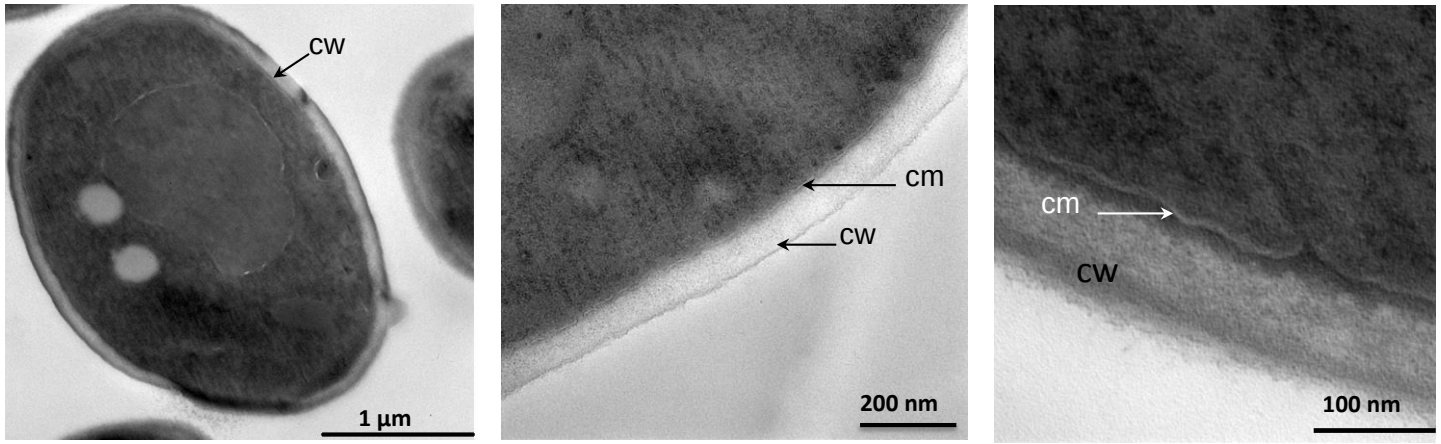


Figure S2: Transmission electron micrographs of untreated and ethanol treated yeast

Yeast cells of the BY4741 strain was cultivated in YPD and collected in the exponential phase (OD_{600} around 1 unit) and treated or not with 9 % (v/v) ethanol. After 5 h, untreated and ethanol-treated cells were collected and processed for TEM analyses as described in Material & Methods Abbreviations are: CW: cell wall; cm: cell membrane

Figure S2 (Schiavone *et al*.)

A: untreated BY4741 cells



B: Ethanol treated BY4741 cells

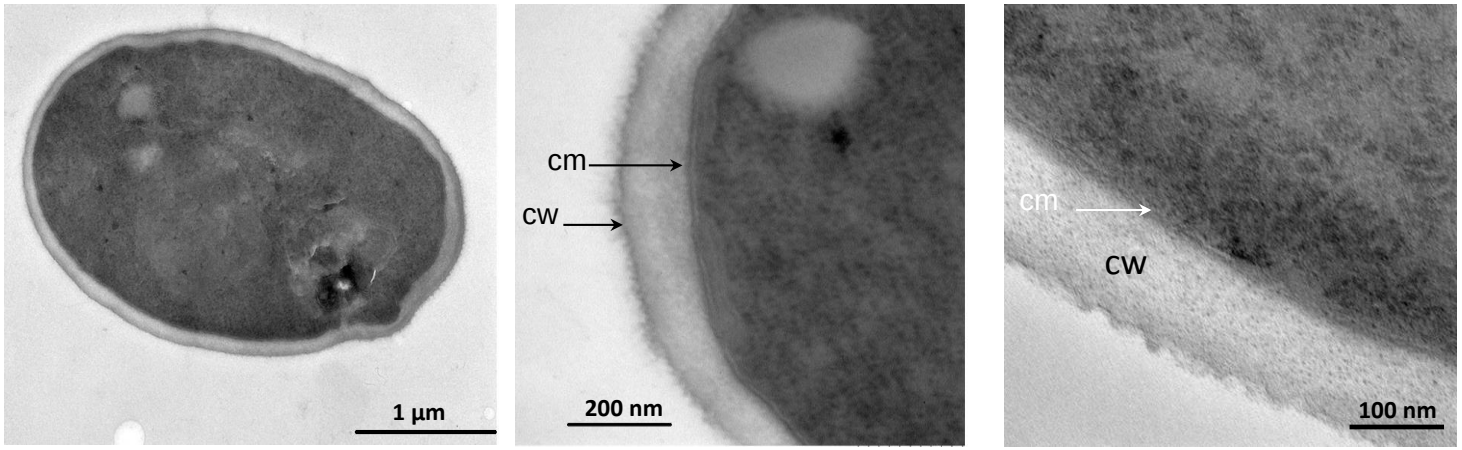
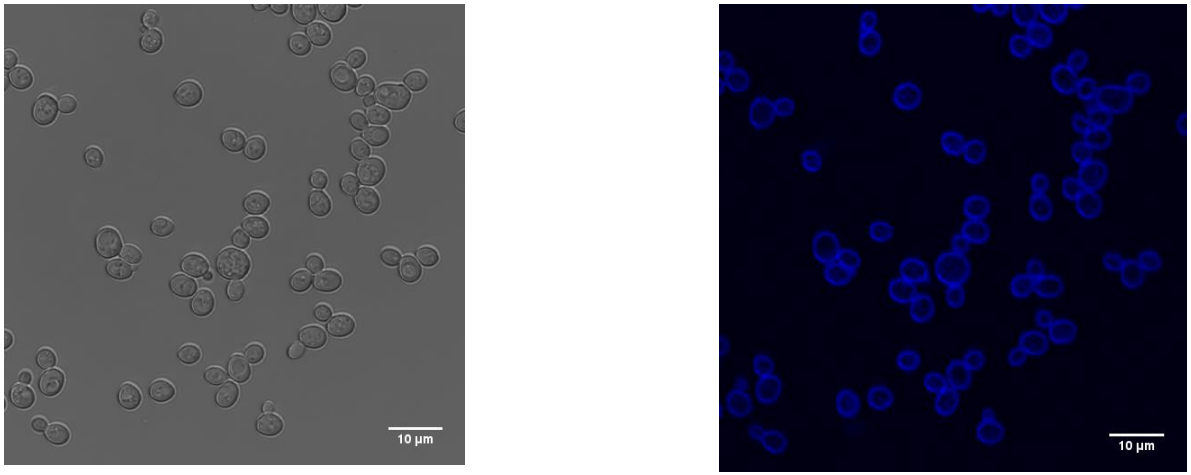


Figure S3: Labelling of untreated or ethanol-treated BY4741 cells with TMPA-DPH fluorophore. Cells were visualized with FV500 confocal laser microscope. In both strain, the fluorophore localizes in the cell membrane, but also diffuses in the ethanol-treated cells, labelling other internal structures

Figure S3(Schiavone *et al*.)

A: untreated yeast cells



B: Yeast cells after 5 hr treatment with ethanol 9%

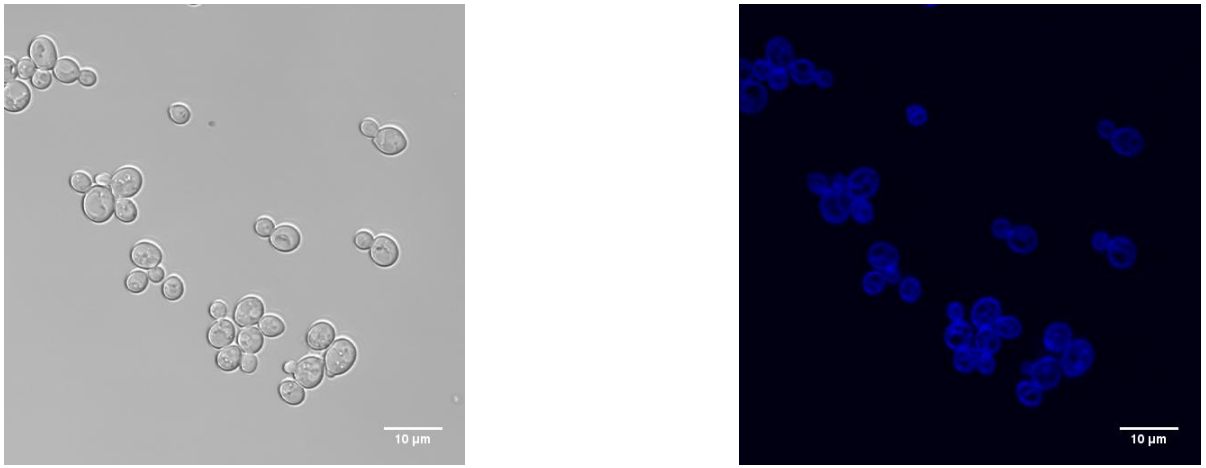


Figure S4: Quantitative RT-PCR of transcriptional changes of selected genes after treatment with ethanol. Exponential cells of BY4741 on YPD were treated (or not) for 5 h with 9 % ethanol. qRT-PCR was carried out on RNA extracted from untreated and treated cells as described in Material & Methods. The fold change is the ratio between transcript abundance of each gene measured in ethanol treated cells referred to reference genes (see Material & methods) in that condition to that of untreated cells referred to same reference genes in the untreated condition.

Figure S4 (Schiavone *et al*.)

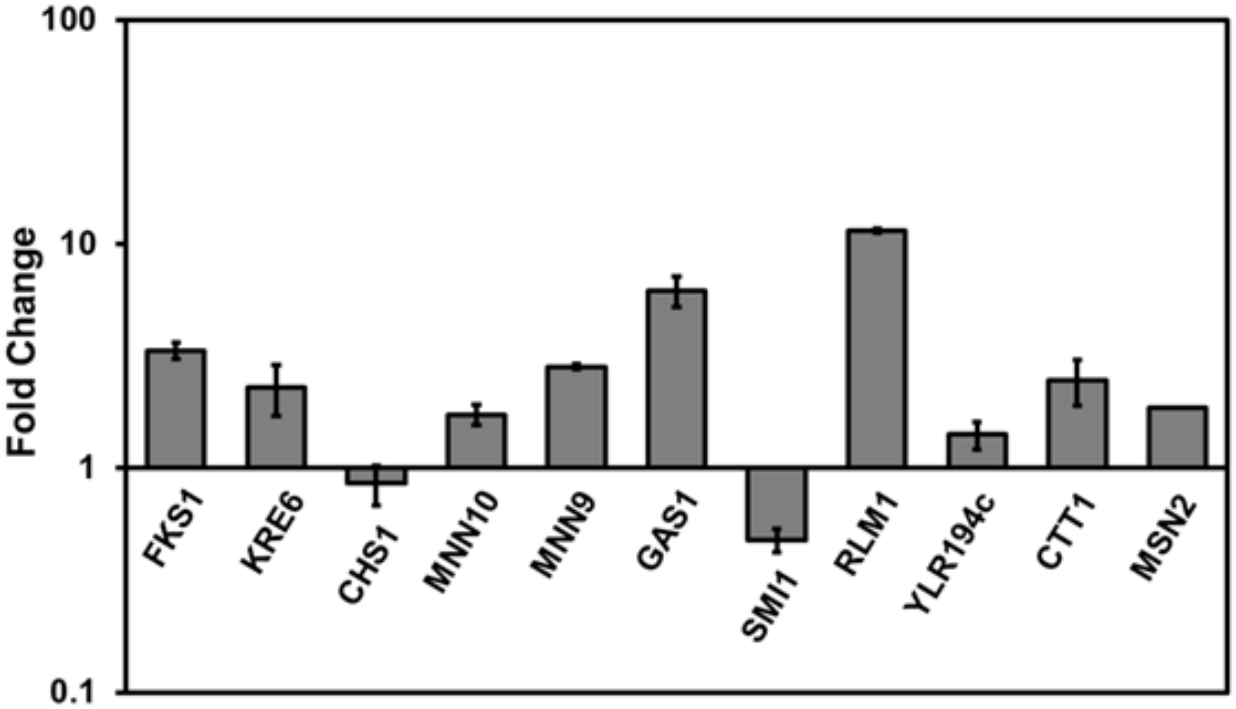


Figure S5: Cell viability and trehalose levels in mutant strain either in *MSN2/MSN4*, *YAP1* or expressing constitutively *MSN2* compared to wild type. Cells viability was assessed by flow cytometry on exponential cells cultivated in YPD before and after 5 hr with 9 % ethanol. Trehalose content was determined on these untreated and treated cells by the method described in Material & Methods. The values shown are the mean ± SD of three independent experiments.

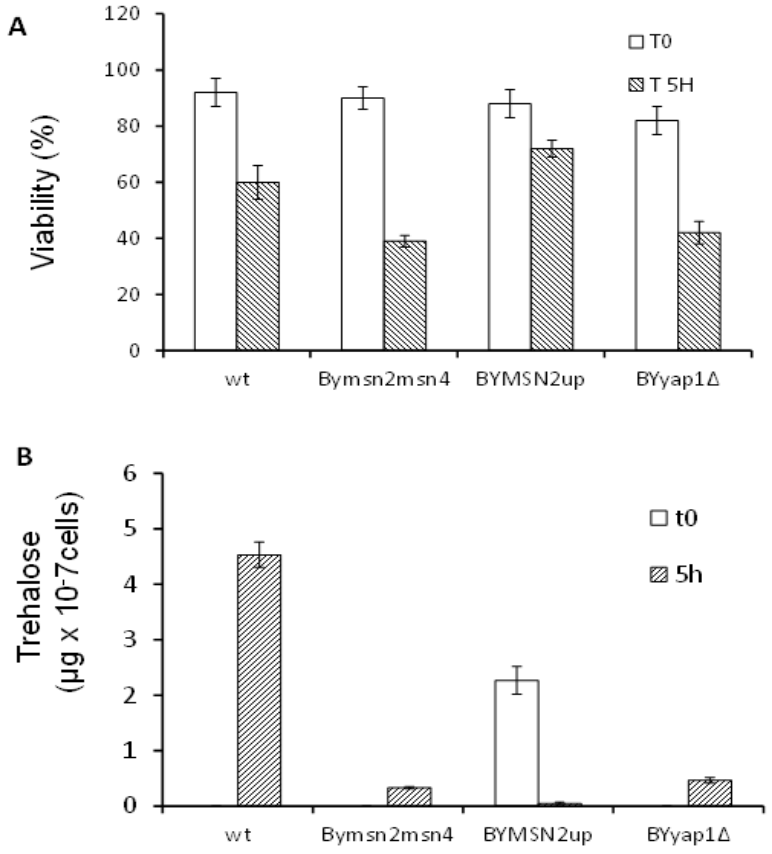


Figure S6: Sensitivity assays wild type and mutants strains to ethanol and cell wall interfering drugs. S BY4741 (wild type), BY*msn2Δmsn4Δ*, BY*yap1Δ* *or* BYMSN2up strain cultivated in YPD to exponential phase (OD_{600} around 2 were collected, resuspended in sterile water at 5 OD_{600} and then aliquots (5 µl) diluted 10, 10^2, 10^3 and 10^4 cells/ml (right to left on each picture) were spotted on YPD plates containing ethanol, Calcofluor White or Congo Red at the concentration indicated in the Figure. The growth was scored after 48 hr at 30°C.

Figure S6 (Schiavone et al.)

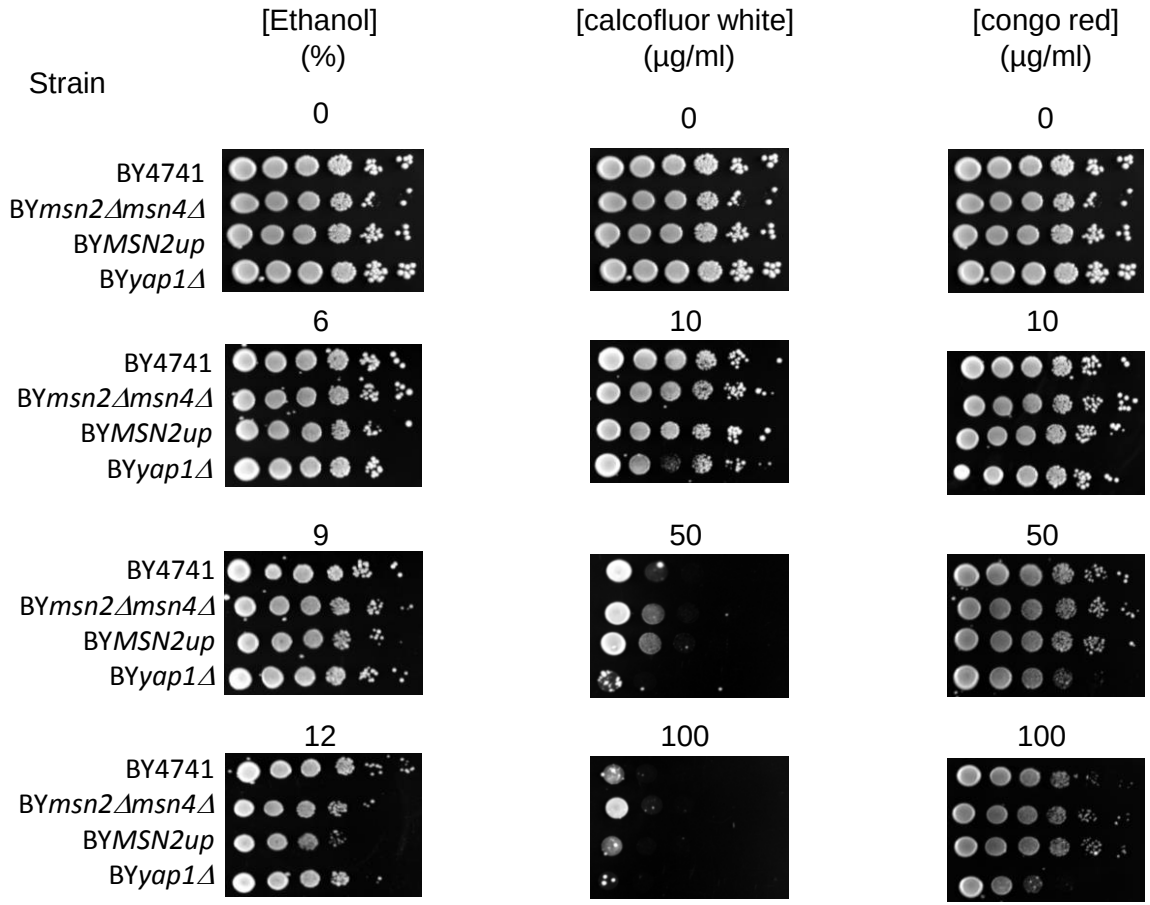


Figure S7: AFM height images of (z-range = 3 µm; scale bar = 1) of a yeast cell untreated (a, c, e) and treated cells of the BY*yap1Δ* , BY*msn2Δmsn4Δ* and BYMSN2up strain (b,d, f) for 5 h with 9% ethanol

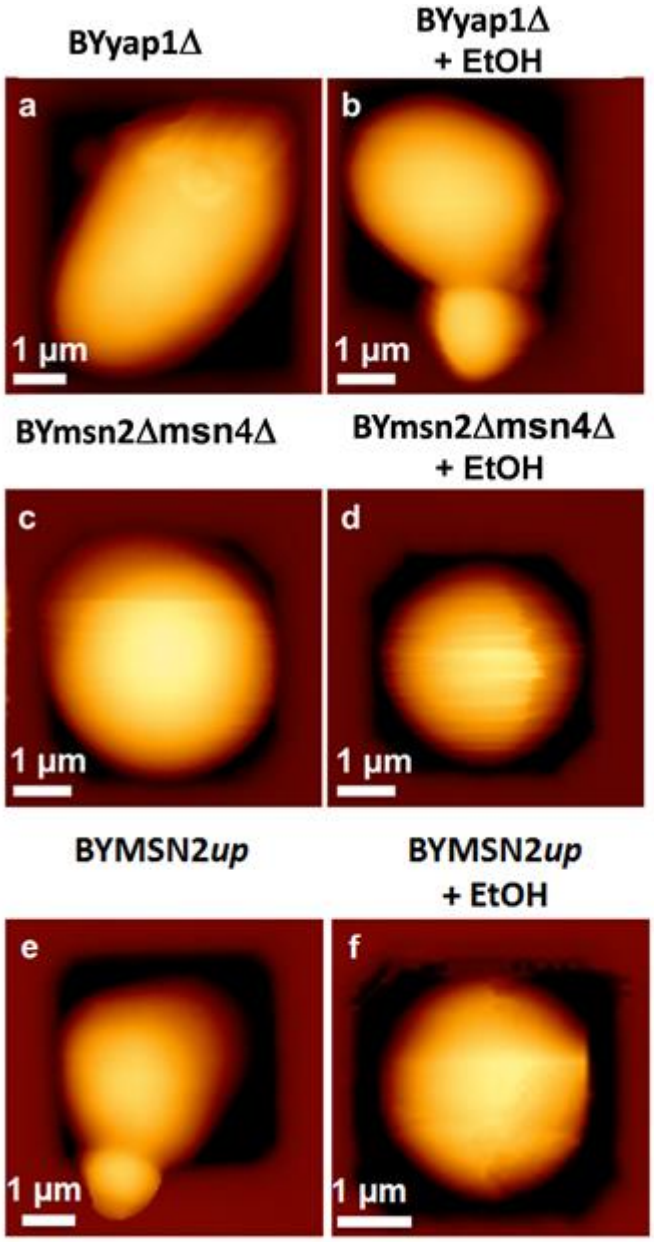


Table S1 : Primers used in this work.

constructs	PCR name	sequence
YipLac33-MSN2-CYC1	Msn2_BamH1_S	5'- GCCGGATCCATGACGGTCGACCATG-3'
	Msn2_HindII_AS	5'- GCCAAGCTTGTCTATTAAATGTCTCCATG-3'
YipLac211PGK1-MSN2-CYC1	YIplac211_PGK_S	5'- GATTACGCCAAGCTTCTTCTCAAGCAAGGTTTTCAGTATAATG-3'
	YIplac211_CYC1_AS	5'-ACGGCCAGTGAATTCCCCGACTCTTTTCTTCTAACCAAGG-3'.
CDRE-lacZ	Sens PCR1	TTTGAACGAAGAAAGGAAAGCAGG
	Antisens PCR2	GCTGCAAAGGTCCTAATGTATAAGG
pSG2	Antisens PCR1(StuI)	GTCGTCTCAAGGCCTGAGGCTGAGGCTGAGGCTGAGGCTGAGGCTGA GGCTCACGGAAATATAATTCCATTTCTTGG
	Sens PCR2 (StuI)	AGCCTCAGCCTCAGCCTCAGCCTCAGCCTCAGCCTCAGGCCTTGAGACG ACATCGTCGAATATGATT