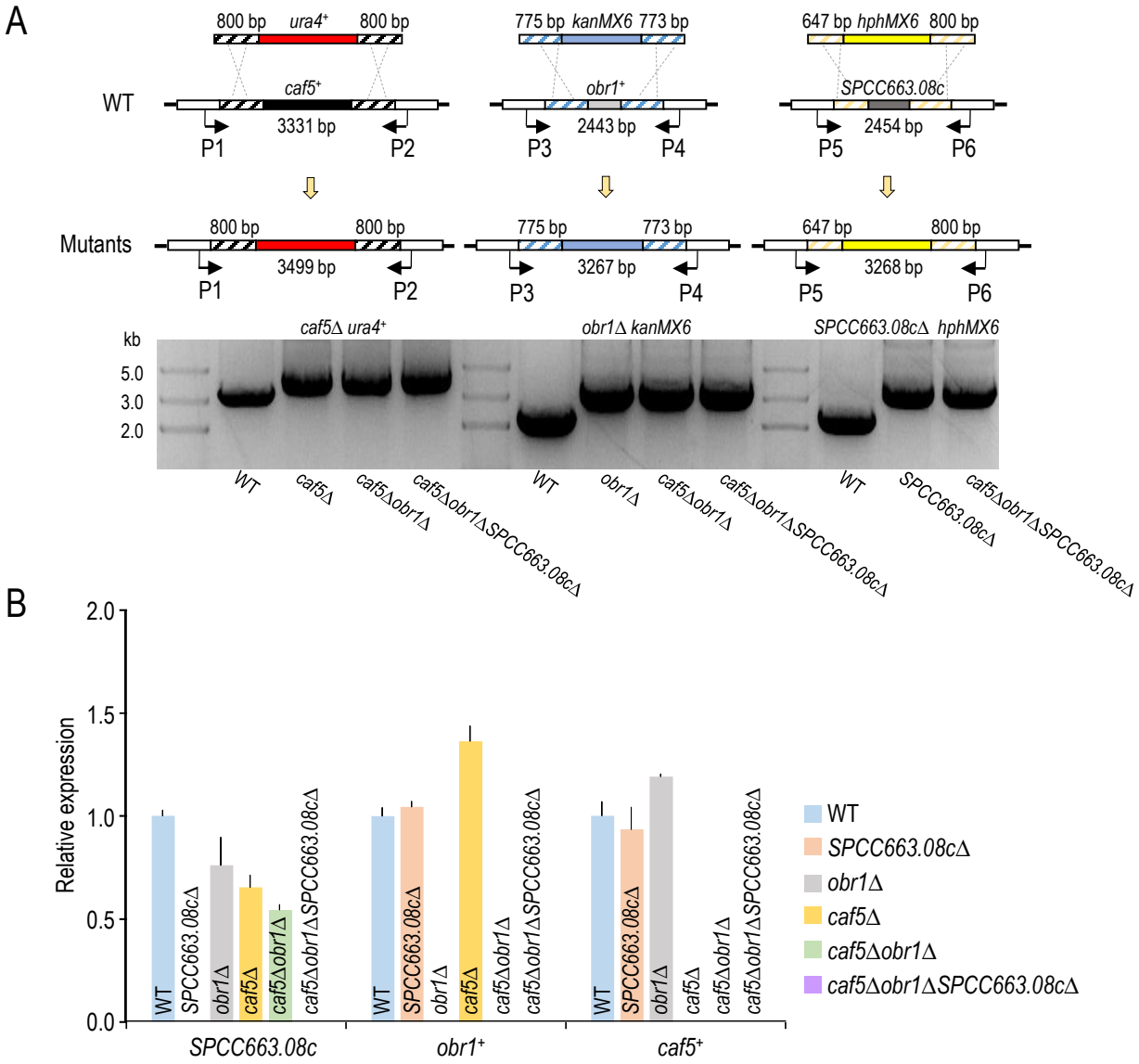

Figure S1 Construction of *caf5*Δ, *obr1*Δ, *SPCC663.08c*Δ single, double or triple mutants. (A) Schematic illustration of gene replacement by homologous recombination. DNA flanking *caf5*$^{+}$, *obr1*$^{+}$ or *SPCC663.08c* shown as hatched boxes (upstream of ATG and downstream of TAA) were PCR amplified from genomic DNA and linked respectively to selectable marker *ura4*$^{+}$, *kanMX6* or *hphMX6* by overlapping PCR, resulting in linear cassettes for homologous recombination in wild type cells. The *caf5*Δ, *obr1*Δ or *SPCC663.08c*Δ mutants were selected respectively on EMM medium with leucine but without uracil, YES medium with 50 μg/ml G418 or YES medium with 75 μg/ml hygromycin B. Similarly, *caf5*Δ*obr1*Δ was derived from *caf5*Δ and the triple mutant from *caf5*Δ*obr1*Δ. Mutants were genotyped by genomic PCR with primers outside of the integration cassettes using primers P1/P2, P3/P4 or P5/P6 and confirmed by sequencing the PCR products. (B) Transcript levels of *caf5*$^{+}$, *obr1* $^{+}$or *SPCC663.08c* mRNA determined by qRT-PCR from indicated strains.

Figure S2

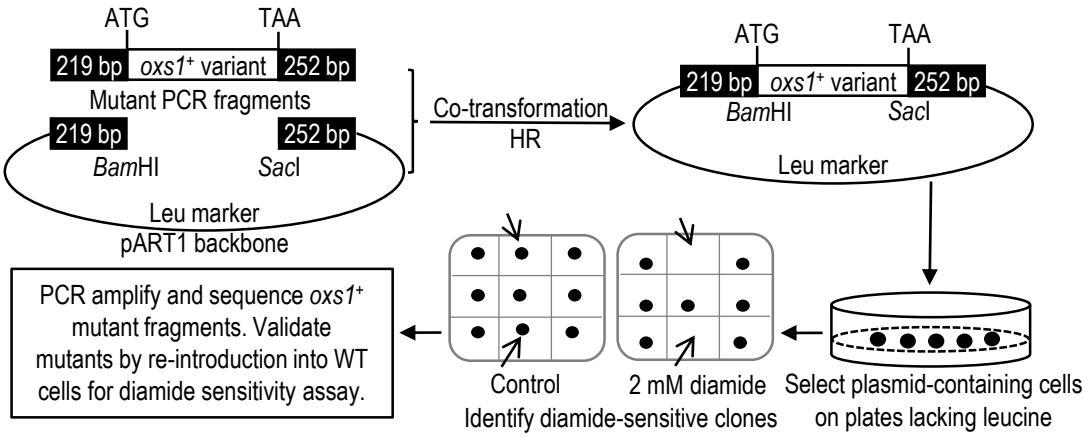

Figure S2 Schematic representation of the screen for base change mutations in *oxs1*$^{+}$. Mutagenized *oxs1*$^{+}$ fragments were generated by error-prone PCR, mixed with *Bam*HI and *Sac*I linearized pART1 vector, transformed into WT cells and plated on media lacking leucine. Circularized plasmids were formed *in vivo* by homologous recombination between the PCR product and gapped vector. Diamide sensitive clones were identified by a spot assay. Mutant *oxs1*$^{+}$ fragments were PCR amplified and sequenced to determine the nucleotide alteration(s).

Figure S3

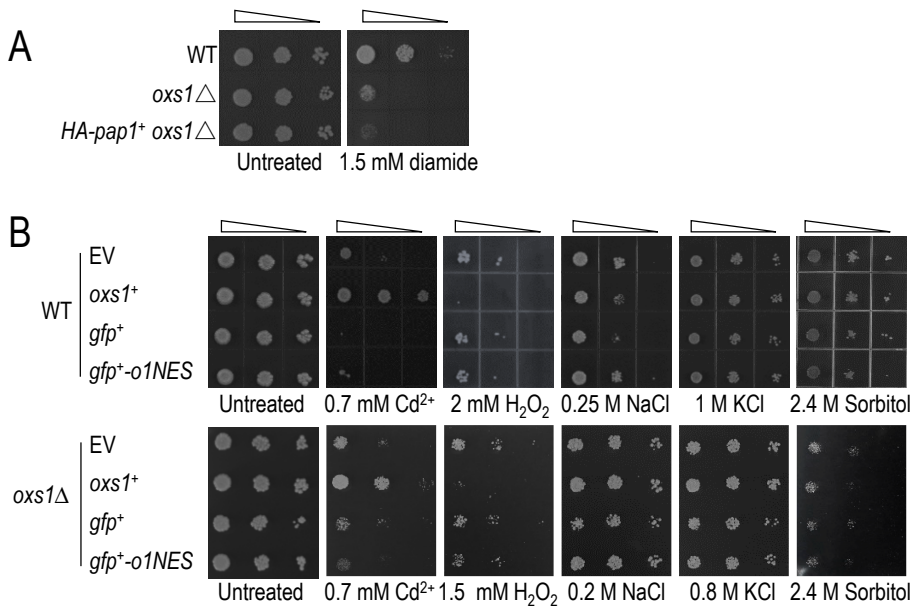

Figure S3 Phenotyping of indicated strains to different stresses. Tolerance of WT, *oxs1*Δ or *HA-pap1*$^{+}$ *oxs1*Δ cells to diamide (A); of WT or *oxs1*Δ cells overproducing Oxs1, GFP or GFP-o1NES to other stresses (B). Serial dilutions of indicated strains spotted onto EMM+Ura plates containing the indicated compounds.

Figure S4

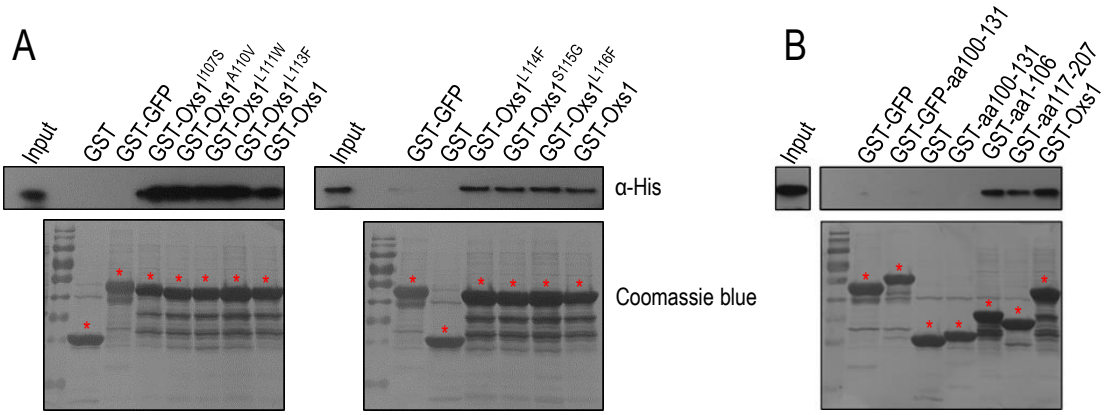

Figure S4 Oxs1 or Oxs1 variants interact with Pap1 *in vitro*. (A) *In vitro* binding of Pap1 to GST-fused to Oxs1 or Oxs1 variants with single amino acid substitution in the NES motif. His-Pap1 was incubated with GST beads bound to the GST-Oxs1 or GST-Oxs1 with indicated mutations (in NES). GST-GFP and GST alone serve as controls. Proteins bound to the beads analyzed by SDS-PAGE and subjected to western blotting with α-His antibody and Coomassie Brilliant Blue staining. Input represents 2% of the total amount of His-Pap1 used in each binding reaction. (B) Binding of GST-Oxs1 amino terminus (aa1-106), carboxyl terminus (aa117-207) or the NES-containing peptide (aa100-131) with Pap1 *in vitro*. In all lanes, asterisk denotes expected band.

Figure S5

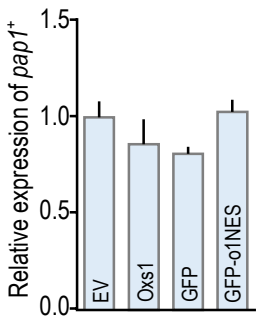

Figure S5 qRT-PCR analysis show that overexpression of the o1NES-conjugate does not alter the mRNA level of *pap1*$^{+}$. Mitotically growing WT cells overproducing Oxs1, GFP or GFP-o1NES were compared to the empty vector (EV) control set as 1.

Figure S6

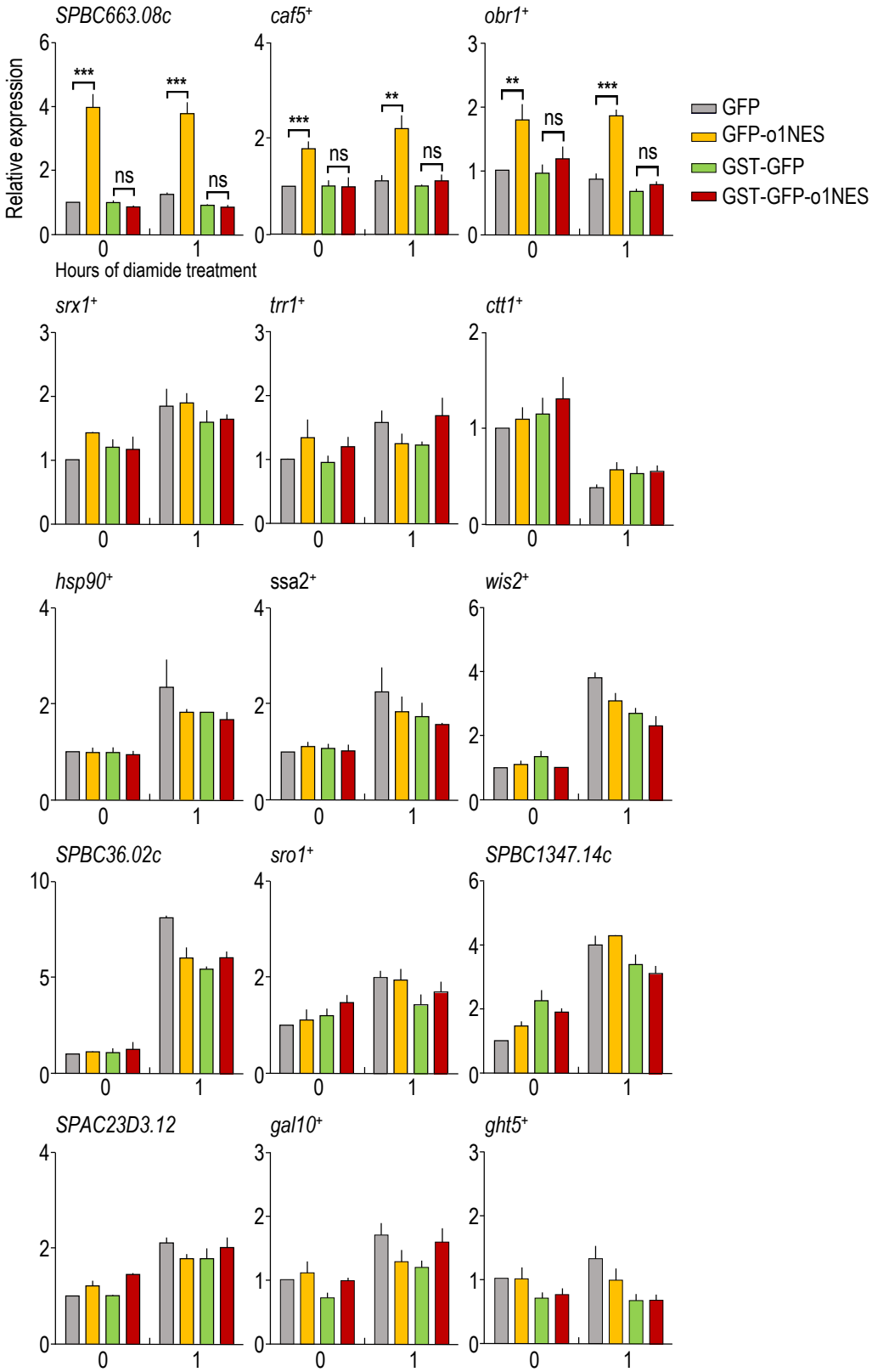

Figure S6 GST-GFP-o1NES fails to activate *SPCC663.08c*, *caf5*$^{+}$ or *obr1*$^{+}$ before or after 1 hour of diamide treatment. *HA-pap1*$^{+}$ *oxs1*Δ cells overproducing GFP, GFP-o1NES, GST-GFP or GST-GFP-o1NES. Data normalized to *act1*$^{+}$ mRNA of the strain overproducing GFP. Data represent mean +SEM from ≥ 3 independent experiments. Significant differences from unpaired Student's t-test (*P<0.05; **P<0.01; ***P<0.001; ****P<0.0001; ns, not significant).

Figure S7

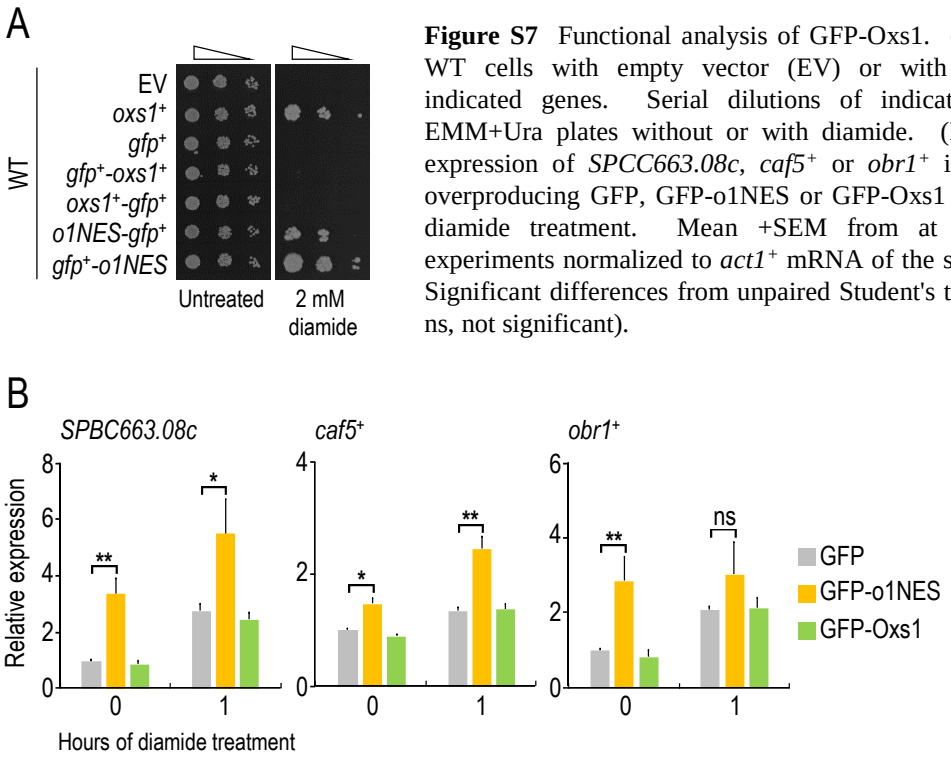

Figure S7 Functional analysis of GFP-Oxs1. (A) Diamide tolerance of WT cells with empty vector (EV) or with plasmid overexpressing indicated genes. Serial dilutions of indicated strains spotted onto EMM+Ura plates without or with diamide. (B) qRT-PCR analysis of expression of *SPCC663.08c*, *caf5*$^{+}$ or *obr1*$^{+}$ in *HA-pap1*$^{+}$ *oxs1*Δ cells overproducing GFP, GFP-o1NES or GFP-Oxs1 before or after 1 hour of diamide treatment. Mean +SEM from at least three independent experiments normalized to *act1*$^{+}$ mRNA of the strain overproducing GFP. Significant differences from unpaired Student's t-test (*P<0.05; **P<0.01; ns, not significant).

Figure S8

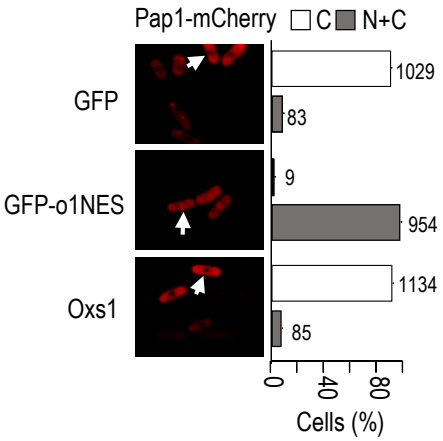

Figure S8 Pap1-mCherry is cytoplasmic-only when co-overproduced with Oxs1. Subcellular localization of Pap1-mCherry by fluorescence microscopy on WT cells co-overproducing indicated proteins. Pap1 displays observable nuclear localization in cells overproducing GFP-o1NES, but mainly cytoplasmic in strains overproducing Oxs1 or the control GFP. Arrows point to nuclei. Frequency of cells with Pap1-mCherry signals shown on the right: C = exclusively cytoplasmic; N+C = nuclear and cytoplasmic. Bar graph data represent mean +SEM from 3 independent experiments, with total number of cells scored shown (from Table S4).