Supplemental Fig S1. The increased SBF binding in a *sin4* mutant persists during the cell cycle.

Wild type (solid lines) and *sin4* (dotted lines) cells with a *GALp:CDC20* allele and a Swi4-V5 epitope tag were synchronized by galactose withdrawal and re-addition, and factor binding was measured by ChIP during the cell cycle. The ChIP signal is plotted as a function of time after release from the G2/M arrest. SBF binding was measured to the left (blue) or the right (red) parts of URS2, using the primers indicated on the diagram.

Supplemental Fig S2. *sin4* and *ash1* mutations additively increase *HO* expression.

*HO* mRNA levels were measured for the various mutant *HO* promoters indicated on the left, in either wild type or *sin4* mutants. The error bars reflect the standard deviation of two biological samples.

Supplemental Fig S3. The Srb4 subunit of the Head module and the Nut1 subunit of the Middle module of Mediator are not recruited to *HO* URS1 and URS2 in a *sin4* mutant.

**A.** ChIP assays were used to measure binding of Gal11-V5, Nut1-Flag, and Srb4-Myc to *HO* URS1 and URS2 in wild type and a *sin4* mutant. The error bars reflect the standard deviation of three biological samples. \**p* < 0.05, \*\**p* < 0.01.

**B.** ChIP assays were used to measure binding of Nut1-Flag and Srb4-Myc to the *HO* TATA region in wild type and a *sin4* mutant. The error bars reflect the standard deviation of three biological samples. \**p* < 0.05.

Supplemental Fig S4. The *sin4* suppression of *HO* expression is dependent on the *PGD1* and *MED2* tail subunits.

*HO* mRNA levels were measured in the indicated strains. The error bars reflect the standard deviation of two biological samples.

Supplemental Fig S5. Movies of fluorescently labeled cells used for single cell analysis.

Movies showing expression of a destabilized GFP protein from the *HO* promoter (*HO-GFP* in green) in live yeast cells over time (2 movies for each strain). Myo1-mCherry (red) forms a ring at the bud neck between bud emergence and cytokinesis, and serves as a cell cycle progression marker. Different movies can have different excitation light settings, and the intensity of the light source changes over time, making it difficult to judge the intensity of expression visually. Importantly, for each mutant strain imaged, a WT control was imaged at the same time and used to normalize the expression level. We also varied the frame rate for each strain: for WT strain, we took one image every 4 min; for *gcn5*, *gcn5 sin4*, *sin4*, *swi2*, and *swi2 sin4* strains, this number is 5, 6, 6, 4, and 4 min respectively.  For some slow-growing strains, we reduced the frame rate to minimize the light damage to the cells.