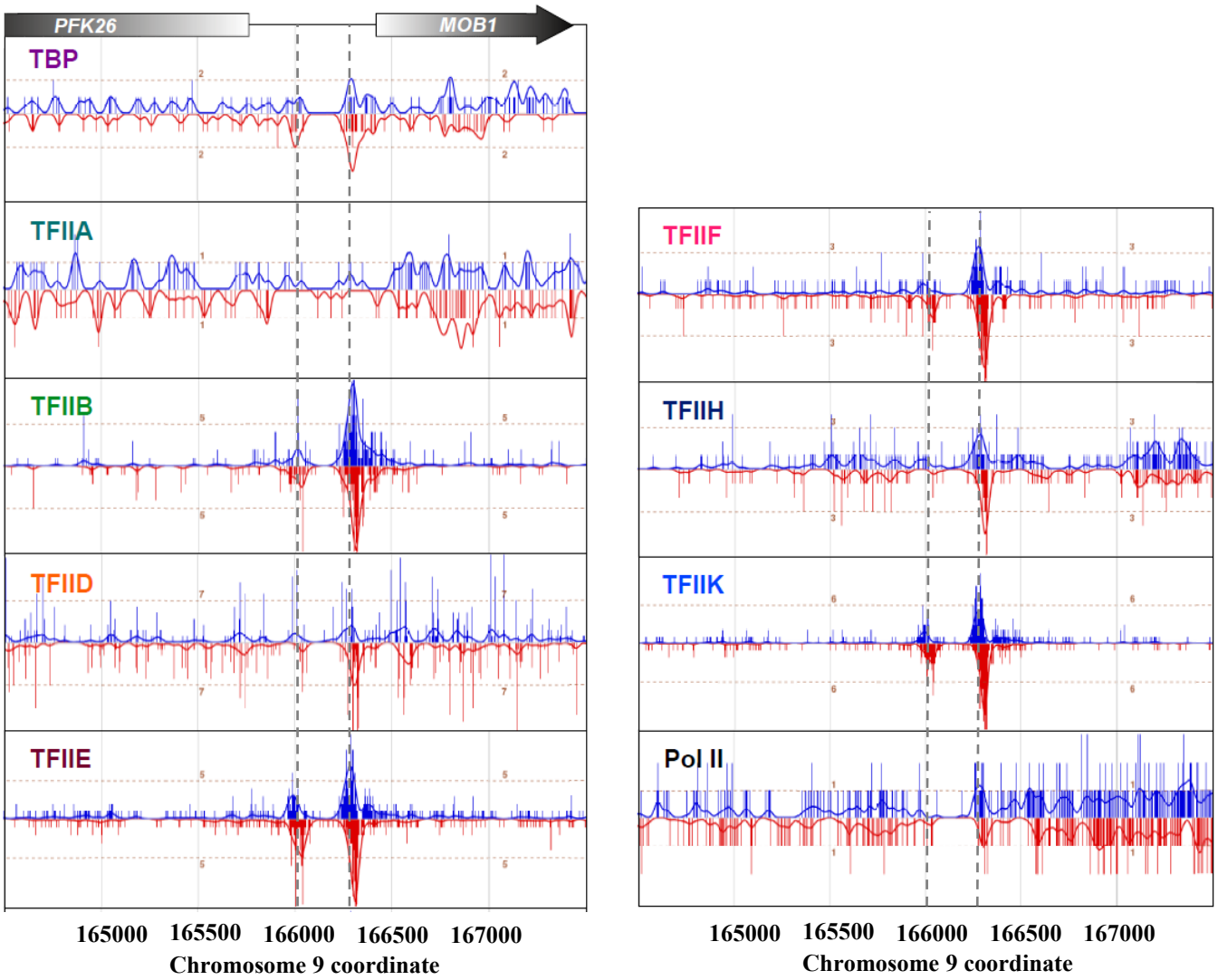

Figure S1. ChIP-Exo measurement of pre-initiation complex (PIC) on *PFK26-MOB1* promoter. All the data in this figure were extracted from the dataset reported in Rhee and Pugh, 2012. Two vertical dash lines mark the locations of TATA-like element of *PFK26* promoter (left) and *MOB1* promoter (right). The PIC forms symmetrically on the two TATA elements. No significant polII ChIP signal was detected between the two TATA elements.

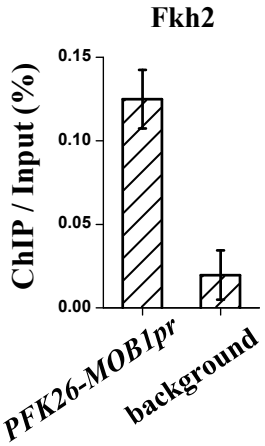

Figure S2. Fkh2 binds to wild-type *PFK26-MOB1* promoter. ChIP measurements of Fkh2 binding on wild-type *PFK26-MOB1* promoter and a control region (background). The error bars in the plot represent the standard errors in the ChIP signal among three biological replicates.

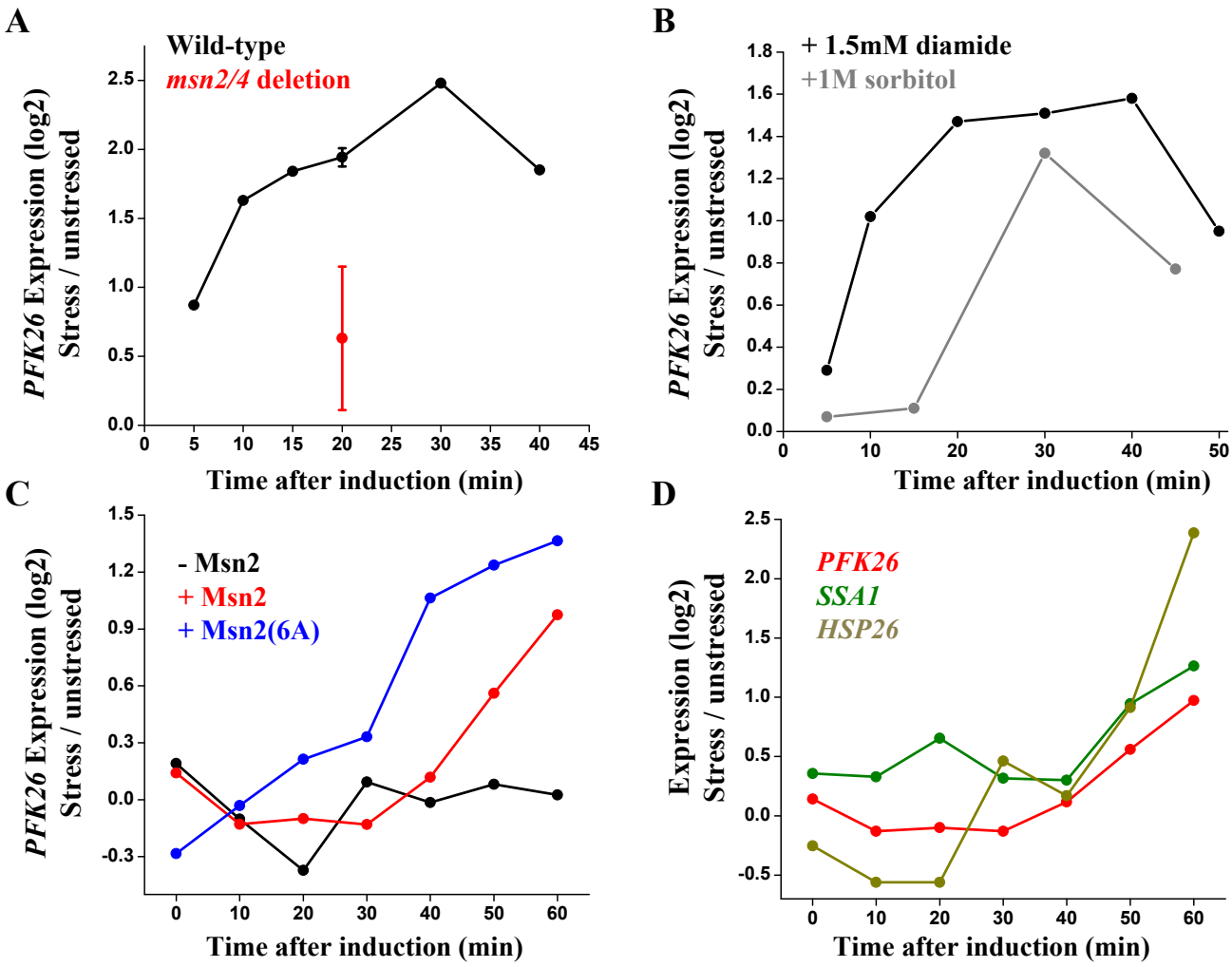

Figure S3. Msn2/4 is at least partially responsible for the stress response of *PFK26*. A) Mutation in Msn2 reduces the heat shock activation of *PFK26*. *PFK26* is induced upon heat shock in wild-type cells (black curve), and msn2 mutation significantly reduced this heat shock response (measured at a single time point; red dot). Data are from Gasch et al., 2000. **B)** *PFK26* induction by diamide and sorbitol. Besides heat shock, *PFK26* is induced under many stress conditions, including oxidative stress (diamide and H2O2), reductive stress (DTT), nitrogen depletion, osmotic stress (1M sorbitol), and diauxic shift (Gasch et al., 2000). These observations are consistent with the idea that *PFK26* is under the regulation of the general stress response activator Msn2/4. **C)** *PFK26* is induced by ectopic expression of Msn2 or Msn2(6A), an Msn2 with mutations in the phosphorylation sites so that it remains inside the nucleus (Elfving et al., 2014). Note that Elfving et al. used a highly specific synthetic biology approach to control Msn2 expression, and any gene rapidly induced in this assay is most likely to be under the direct regulation of Msn2. **D)** The induction rate of *PFK26* in panel C is similar to other well-known Msn2/4 target genes, such as *SSA1* and *HSP26* (Venters et al., 2011; Macisaac et al., 2006). All evidence above supports that Msn2/4 is at least partially responsible for *PFK26* activation.

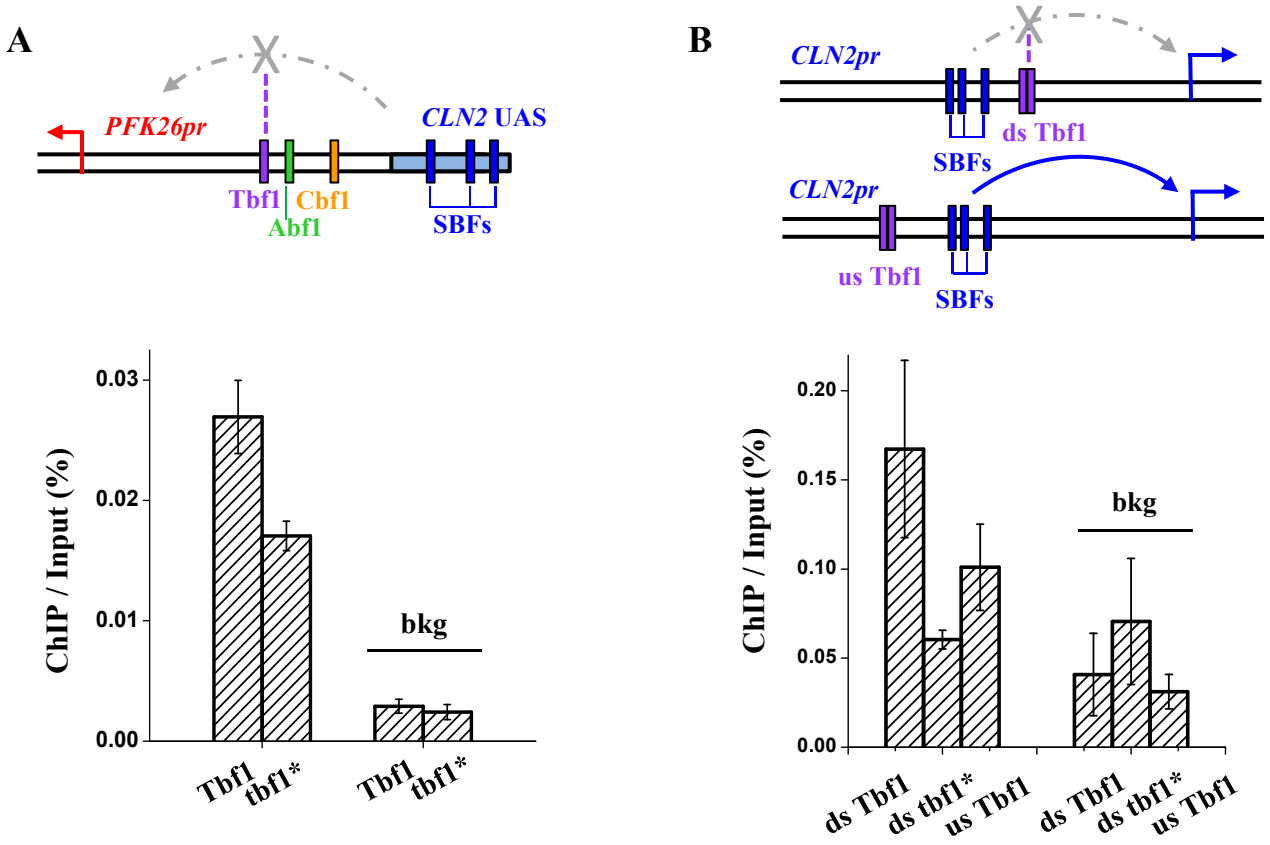

Figure S4. Tbf1 ChIP on Synthetic promoters. A) Tbf1 can bind to the synthetic promoter containing wild type Tbf1 binding site, and this binding is significantly reduced with Tbf1 binding site mutation (tbf1*). Bkg: background in theYER129W ORF (same as in B). **B)** Tbf1 can bind to *CLN2* promoter with wild type tbf1 binding sites (Tbf1) both upstream and downstream of SBFs but not the one with mutant tbf1 binding sites (tbf1*).

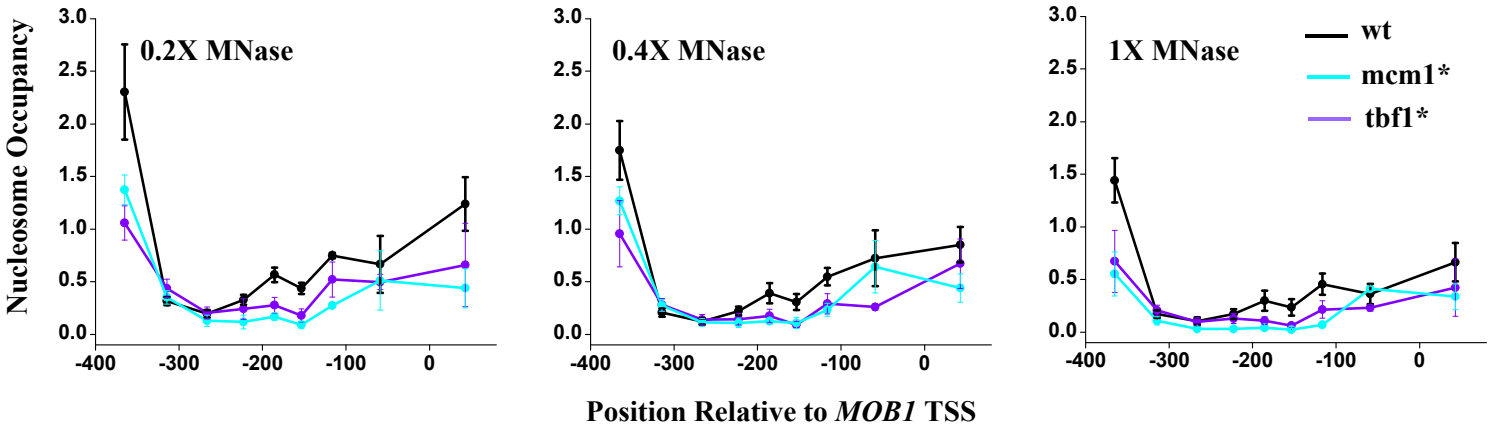

Figure S5. Nucleosome mapping on *PFK26-MOB1* promoter using different MNase concentration. From left to right: we digested the chromosome with 0.2X, 0.4X or 1X MNase concentrations, which converts ~20%, 50%, and 80% of chromosomal DNAs into mono-nucleosomes, respectively. We then purified the mono-nucleosome DNA and measured nucleosome occupancy using qPCR (Methods). The measurements were performed on the wild-type promoter (black), or with Mcm1/Tbf1 binding site mutation (cyan/purple). The error bars represent the standard errors in three biological replicates.

References:

Elfving N, Chereji RV, Bharatula V, Bjorklund S, Morozov AV, Broach JR. 2014. A dynamic interplay of nucleosome and Msn2 binding regulates kinetics of gene activation and repression following stress. *Nucleic acids research* **42**: 5468-5482.

Gasch AP, Spellman PT, Kao CM, Carmel-Harel O, Eisen MB, Storz G, Botstein D, Brown PO. 2000. Genomic expression programs in the response of yeast cells to environmental changes. *Molecular biology of the cell* **11**: 4241-4257.

MacIsaac KD, Wang T, Gordon DB, Gifford DK, Stormo GD, Fraenkel E. 2006. An improved map of conserved regulatory sites for Saccharomyces cerevisiae. *BMC bioinformatics* **7**: 113.

Rhee HS, Pugh BF. 2012. Genome-wide structure and organization of eukaryotic pre-initiation complexes. *Nature* **483**: 295-301.

Venters BJ, Wachi S, Mavrich TN, Andersen BE, Jena P, Sinnamon AJ, Jain P, Rolleri NS, Jiang C, Hemeryck-Walsh C et al. 2011. A comprehensive genomic binding map of gene and chromatin regulatory proteins in Saccharomyces. *Molecular cell* **41**: 480-492.