Supplemental Methods

Yeast strains

Strains (complete list provided in Supplementary **Table** S6) were generated by homology-driven genomic integration of tagging or disruption cassettes (Longtine et al., 1998; Rigaut et al., 1999) and/or by genetic crosses.

Yeast growth conditions

Most experiments were performed with exponential phase cells harvested between OD_{600} 0.4 and 0.6. Overnight cultures were diluted to OD_{600} = 0.1, grown at 30°C to exponential phase (OD_{600} = 0.4 -0.6), and then treated with rapamycin at 1µg/ml (from a 1 mM stock solution in 90% ethanol, 10% Tween-20) for anchor-away experiments. Genome-wide localization of Sfp1-TAP by ChIP-seq was performed on cultures grown in yeast extract, peptone, adenine medium (YPA) with 2% galactose, 2% raffinose, or 2% glucose (w/v). The untagged wild-type (WT) strain was used as a control (YDS2). The strain expressing *pGAL1-SFP1*-TAP was grown in YPA raffinose-containing medium for two generations and subsequently treated for 1 hr with 2% galactose to induce *SFP1* expression. For glucose-pulse experiments, WT strains were grown in YP glycerol (3%) plus glucose (0.05%) and shifted to YPAD (YPA with 2% glucose/dextrose).

ChIP-seq

Cultures of 200 ml were collected at OD_{600} = 0.5-0.8 for each condition. The cells were crosslinked with 1% formaldehyde for 10 min at room temperature and quenched by adding 125 mM glycine for 5 min at RT. Cells were washed with ice-cold HBS and resuspended in 3.6 ml of ChIP lysis buffer (50 mM HEPES-Na pH 7.5, 140 mM NaCl, 1mM EDTA, 1% NP-40, 0.1%

sodium deoxycholate) supplemented with 1mM PMSF and 1x protease inhibitor cocktail (Roche). Samples were aliquoted in 6 Eppendorf tubes and frozen. After thawing, the cells were broken using Zirconia/Silica beads (BioSpec). Lysates were spun at 13'000 rpm for 30 min at 4°C. The pellet was resuspended in 300 µl ChIP lysis buffer + 1mM PMSF and sonicated for 15 min (30" on - 60" off) in a Bioruptor (Diagenode). Sonicated lysates were then spun at 7'000 rpm for 15 min at 4°C. Sfp1-TAP, RNAPII, and TBP-Myc binding were analyzed using TAP-specific, Rpb1, and anti-Myc antibody, respectively (Thermo Fisher CAB1001, Abcam 5131, Myc epitope 9E10). The antibody (1 µg per 300 µl of lysate) was added to the supernatant and incubated for 1h at 4°C. The magnetic beads were washed three times with PBS plus 0.5% BSA and added to the lysates (30 µl of beads per 300 µl of lysate). The lysate/bead mixes were then incubated for 2 hr at 4°C. Beads were then washed twice with 50 mM HEPES-Na pH 7.5, 140 mM NaCl, 1mM EDTA, 0.03% SDS, once with AT2 buffer (50 mM HEPES-Na pH 7.5, 1 M NaCl, 1mM EDTA), once with AT3 buffer (20 mM Tris-Cl pH 7.5, 250 mM LiCl, 1mM EDTA, 0.5% NP-40, 0.5% sodium deoxycholate) and twice with TE. Chromatin was eluted from the beads by resuspending them in TE + 1% SDS and incubation at 65°C for 10 min. The eluate was transferred to an Eppendorf tube and incubated overnight at 65 °C to reverse the crosslinks. DNA was purified using the High Pure PCR Cleanup Micro Kit (Roche) and libraries were prepared for sequencing using TruSeq ChIP Sample Preparation Kit (Illumina) according to the manufacturer's instructions. The libraries were sequenced on a HiSeq 2500 machine and the reads were mapped to the sacCer3 genome assembly using HTSstation (David et al. 2014).

ChIP-seq peaks of Sfp1 binding were defined by shifting the plus and minus strand ChIP-seq profiles towards each other by 150 bp and extending each read by 40 bp. To quantify ChIP-seq signals for each promoter, a ratio between the total number of reads from each sample in a 400 bp region upstream the transcription start site (TSS; (Jiang and Pugh 2009)) of each

ORF and the total number of reads from the same region obtained with mock IP of the control untagged strain. The same logic was applied to quantify signals within ORFs.

ChIP-chip and ChIP-seq data from Harbison et al. (2004) and Knight et al. (Knight et al. 2014) was used to define Swi4 and Ifh1 binding, respectively. The ChIP-seq peaks were defined by shifting the plus and minus strand ChIP-seq profiles towards each other by 150 bp and extending each read by 40 bp. To quantify ChIP-seq signals for each promoter, the total number of reads from each sample in a 400 bp region upstream the TSS of each ORF was determined.

To quantify Rpb1 ChIP-seq signals for each gene, a ratio was calculated of the total number of reads in each ORF before and after treatment (either rapamycin or vehicle, or after 1h +/- galactose in the case of the p*GAL1-SFP1*-TAP strain.

ChEC-seq

ChEC-seq experiments were performed essentially as described (Zentner et al. 2015) with the following modifications. Cells in which MNase was fused at the C-terminus of the endogenous *SFP1* gene were used to determine Sfp1 binding. Cells in which MNase was placed under the control of *REB1* promoter were used as a control. One sample corresponds to 12 ml of culture at OD600 = 0.7. Cells were washed twice with buffer A (15 mM Tris 7.5, 80 mM KCl, 0.1 mM EGTA, 0.2 mM spermine, 0.5 mM spermidine, 1xRoche EDTA-free mini protease inhibitors, 1 mM PMSF) and resuspended in 200 µl of buffer A with 0.1% digitonin. The cells were incubated for 5 min at 30°C. Then, MNase action was induced by addition of 5 mM $CaCl_2$ and stopped at the desired timepoint by adding EGTA to a final concentration of 50 mM. DNA was purified using MasterPure Yeast DNA purification Kit (Epicentre) according to the manufacturer's instruction. Large DNA fragments were removed by a 5-min incubation with 2.5x volume of AMPure beads (Agencourt) after which the supernatant was kept, and MNase-

digested DNA was precipitated using isopropanol. Libraries were prepared using NEBNext kit (New England Biolabs) according to the manufacturer's instructions. Before the PCR amplification of the libraries small DNA fragments were selected by a 5-minute incubation with 0.9x volume of the AMPure beads after which the supernatant was kept and incubated with the same volume of beads as before for another 5 min. After washing the beads with 80% ethanol the DNA was eluted with 0.1x TE and PCR was performed. Adaptor dimers were removed by a 5-min incubation with 0.8x volume of the AMPure beads after which the supernatant was kept and incubated with 0.3x volume of the beads. The beads were then washed twice with 80% ethanol and DNA was eluted using 0.1x TE. The quality of the libraries was verified by running an aliquot on a 2% agarose gel. Libraries were sequenced using a HiSeq 2500 machine in single-end mode. Reads were extended by the read length. To analyze the Sfp1-MNase binding pattern, read ends were considered to be MNase cuts and were mapped to the genome (sacCer3 assembly) using HTSstation (David et al. 2014). For peak analysis MACS software was used through HTSstation, using free-MNase signal as background. Motifs were detected using MEME (Bailey et al. 2009) with sequences from each identified ChEC signal peak as input.

Microscopy

Cells were grown overnight at 30°C in SC liquid medium (0.67% nitrogen base without amino acids [BD], 2% Glucose, Raffinose or Galactose, supplemented with amino acids mixture [AA mixture; Bio101], adenine, and uracil). Cells were diluted into fresh medium to OD_{600} = 0.1 and grown until they reached an OD_{600} of 0.4. Cells were then spread onto slides coated with an SC medium patch containing 2% agarose and 2% glucose. Stacked images were recorded on a spinning disc confocal microscope (Intelligent Imaging Innovations) mounted on an inverted microscope (Leica DMIRE2) using a 100x oil objective and an Evolve EMCCD Camera (Photometrics). Images were processed using FIJI (Schindelin et al. 2012).

Data and software availability

All sequencing and microarray data generated in this study was submitted to the GEO database as SuperSeries. GSE118561.

References

Bailey TL, Boden M, Buske FA, Frith M, Grant CE, Clementi L, Ren J, Li WW, Noble WS. 2009. MEME SUITE: tools for motif discovery and searching. *Nucleic Acids Res* **37**: W202-208.

David FP, Delafontaine J, Carat S, Ross FJ, Lefebvre G, Jarosz Y, Sinclair L, Noordermeer D, Rougemont J, Leleu M. 2014. HTSstation: a web application and open-access libraries for high-throughput sequencing data analysis. *PLoS One* **9**: e85879.

Harbison CT, Gordon DB, Lee TI, Rinaldi NJ, Macisaac KD, Danford TW, Hannett NM, Tagne JB, Reynolds DB, Yoo J et al. 2004. Transcriptional regulatory code of a eukaryotic genome. *Nature* **431**: 99-104.

Jiang C, Pugh BF. 2009. A compiled and systematic reference map of nucleosome positions across the Saccharomyces cerevisiae genome. *Genome Biol* **10**: R109.

Knight B, Kubik S, Ghosh B, Bruzzone MJ, Geertz M, Martin V, Denervaud N, Jacquet P, Ozkan B, Rougemont J et al. 2014. Two distinct promoter architectures centered on dynamic nucleosomes control ribosomal protein gene transcription. *Genes Dev* **28**: 1695-1709.

Schindelin J, Arganda-Carreras I, Frise E, Kaynig V, Longair M, Pietzsch T, Preibisch S, Rueden C, Saalfeld S, Schmid B et al. 2012. Fiji: an open-source platform for biological-image analysis. *Nat Methods* **9**: 676-682.

Zentner GE, Kasinathan S, Xin B, Rohs R, Henikoff S. 2015. ChEC-seq kinetics discriminates transcription factor binding sites by DNA sequence and shape in vivo. *Nat Commun* **6**: 8733.