Supplemental Materials

Functional interplay between Mediator and TFIIIB in preinitiation complex assembly in relation to promoter architecture

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Supplemental Materials and Methods

Yeast strains

All S. cerevisiae strains are listed in Supplemental Table S1. MED10 or MED14 were deleted in YPH499 (MATa ura3-52 his3-Δ200 ade2-101uaa trp1-Δ63 lys2-801uag leu2-Δ1) complemented with pVV208-MED10 or pVV208-MED14, respectively, and replaced by a KanMX6 marker using the standard one-step method (Longtine et al. 1998). med10 conditional mutant was obtained as previously described for med17 mutants (Soutourina et al. 2011). We mutagenized MED10 by error-prone PCR and introduced the mutated fragment in a pVV204 vector bearing the TRP1 marker by gap-repair in the strain carrying a med10 deletion complemented by wild-type MED10 borne on a URA3 vector (pVV208-MED10). After chasing the wild-type MED10 allele on 5-FOA, colonies growing at 30°C were replica plated at 37°C to identify thermosensitive colonies. The plasmids were isolated, re-transformed in med10Δ/pVV208-MED10 strain and sequenced. Med5-HA, Med15-HA, Med17-HA, Rad3-HA and Kin28-HA and Taf1-HA strains carrying C-terminal HA-tagged version of Med5, Med15 and Med17 Mediator subunits, Rad3 and Kin28 TFIIH subunits and Taf1 were constructed by inserting 3HA epitopes followed by His3MX6 marker using the standard one-step methods. The HA-TBP strain expressing N-terminal HA-tagged version of TBP was obtained by inserting 3HA epitopes preceded by LEU2 marker. Toa2-TAP, TFIIIB-TAP, Tfg1-TAP, Tfa2-TAP strains carrying C-terminal TAP-tagged versions of Toa2, TFIIB,
Tfg1 and Tfa2 were constructed by inserting TAP cassette followed by His3MX6 marker using the one-step standard method.

Double-tagged strains carrying Toa2-Myc and Med5-HA version of Toa2 and Med5 were constructing by first inserting 3HA epitope followed by His3MX6 marker and by inserting 13Myc epitope followed by a TRP1 marker using standard one step protocol. These double-tagged strains were then transformed by pAG415-MED10 or med10 mutant plasmid. Med10 mutants and a WT strain for Mediator purification were derived from protease deficient CA001 strain (MATa leu2 trp1 ura3-52 prb1-1122 pep4-3 prc1-407 gal2 MED22::10His-TEV-ProteinA::KTRP1 MED8-PreSci-3HA::KanR) carrying C-terminal HA-tagged version of Med8 and C-terminal 10xHis-TEV-ProteinA-tagged version of Med22 (Cai et al. 2009). MED10 was deleted in CA001 complemented with pVV208-MED10 and replaced by a natNT2 marker from pFA6a-natNT2 (Janke et al. 2004) using the standard one-step method, then transformed by pAG415-GPD-MED10 or med10-mutant plasmids. Finally, MED10 URA3 plasmid was chased on 5-FOA medium.

For synthetic phenotype tests between med10-196 and sua7 mutants, a strain was deleted for med10 by introduction of an ADE2 cassette and complemented by a centromeric plasmid carrying MED10 or med10-196 and a TRP1 marker. This strain was deleted for sua7 by insertion of a KanMx6 cassette and complemented by a plasmid carrying SUA7 and a URA3 marker. This double-deleted strain was transformed by the plasmids carrying either WT or mutated version of SUA7 with a HIS3 marker (Wu et al. 1999). Transformants were spotted on medium containing 5-FOA to chase SUA7 URA3 plasmid.

Unless otherwise stated, yeast strains were grown at 30°C in YPD rich medium containing 2% of glucose.
**Plasmid constructions and cloning**

All plasmids are listed in Supplemental Table S2. All cloning experiments were done using the Gateway Invitrogen cloning method. Wild-type *MED10* or *MED14* genes were amplified from YPH499 genomic DNA using oligonucleotides matching the gene sequence initiation codon and following codons for the 5’ forward primer and the stop codon and preceding codons for the 3’ reverse primer. The oligonucleotides were flanked with *attB1* or *attB2* sequences, respectively. For *med14* C-terminal truncations (*med14*-483, 686, 752), the corresponding fragments were amplified by the same procedure with appropriate oligonucleotides matching the gene sequence initiation codon and following codons for the 5’ forward primer and the stop codon at amino acid position 483, 686 or 752, respectively, and preceding codons for the 3’ reverse primer. The amplified sequence was cloned into pDONR201 (Invitrogen) using standard BP reaction. The recombinant plasmid was sequence verified. The cloned sequence was then transferred into pVV208 (CEN URA3), pVV204 (CEN TRP1) (Van Mullem et al. 2003), or pAG415-GPD vector (Alberti et al. 2007) by the LR reaction.

**Two hybrid assay**

Two-hybrid experiments were done as described previously (Werner et al. 1993). Briefly, the Y190 yeast strain was co-transformed by pVV213 and pVV212 carrying the bait protein fused with Gal4-DNA-Binding-Domain and the prey fused to Gal4-Activating-Domain, respectively. The activation of the *lacZ* or *HIS3* reporters was tested using an X-Gal overlay plate assay or 3AT plate assay.

For quantitative analysis, β-galactosidase was assayed according to the Miller method using Yeast β-galactosidase assay kit (Thermo Scientific) with o-nitrophenyl-β-D-galactopyranoside (ONPG) as a substrate, following the manufacturer's instructions.
Alternatively, when indicated, a more sensitive β-galactosidase assay using chlorophenol red-
β-galactopyranoside (CPRG, Roche) as a substrate was performed. Briefly, cells from 1.5 ml
of culture grown to mid-log phase were harvested by centrifugation, washed with 1 ml of
buffer A (10 mM HEPES, pH 7.3, 150 mM NaCl, 50 mM L-Aspartate, 1% BSA, 0.05% Tween 20) and resuspended in the 300 µl of the same buffer. The OD_{600} was measured at this
stage. 100 µl of cell suspension were transferred to a microfuge tube and flash-frozen in
liquid nitrogen. Frozen pellet was immediately thawed in a 37°C water bath. The freeze and
thaw cycle was repeated twice to break the cells. 700 µl of buffer B (2.23 mM of CPRG in
Buffer A) were added, mixed thoroughly and incubated at room temperature overnight. The
reaction was stopped by addition of 500 µl of 3 mM ZnCl$_2$. After centrifugation to pellet cell
debris, absorbance at 570nm was measured. Miller units were calculated according to (Miller
1972). The mean values and standard deviation (indicated by error bars) of three independent
experiments were calculated.

**Coimmunoprecipitation experiments**

Whole yeast extracts were prepared from 100 ml of cells grown to exponential phase
in YPD medium at 30°C. When indicated, the cells were then transferred for 90 min to 37°C.
Whole yeast extract preparation, immunoprecipitation (IP) in IP buffer (50 mM HEPES, pH
7.5, 100 mM NaCl, 20% glycerol, 1 mM dithiothreitol, 0.5 mM EDTA, 0.05% NP-40)
supplemented with a protease inhibitor cocktail (Complete, Roche) and 1 mM PMSF and
western blotting were performed as described previously (Soutourina et al. 2006).

In some cases, whole cell extract were extracted by disrupting yeast cells in lysis
buffer (50 mM Tris-HCl [pH 7.5], 15% glycerol, 5mM MgCl$_2$) in Mikro-Dismembrator S
(Sartorius Stedim Biotech S.A.). After centrifugation at 13,000 × g for 20 min, the resulting
supernatant plus 100 mM NaCl and Igepal 0.05% was used as input. HA-tagged proteins were
immunoprecipitated using HA isolation kit (Miltenyi Biotec), following the manufacturer's instructions. All washes were performed using the lysis buffer.

The 12CA5 anti-HA antibodies were used against HA-tagged proteins; the 9E10 monoclonal antibody was used against Myc-tagged proteins; rabbit-polyclonal anti-Sua7p (Abcam) was used against Sua7; rabbit polyclonal anti-Med14 and anti-Med17 antibodies against corresponding N-terminal peptides were used to detect Med14 and Med17 Mediator subunits, respectively.

The relative intensity of immune staining was quantified using ImageJ version 1.46r. The intensity of immune staining for coimmunoprecipitated TFIIB signals relative to the wild-type was normalized against immunoprecipitation signals. The mean values and standard deviation (indicated by error bars) of three independent experiments were calculated.

**Mediator purification**

Mediator purification was done as previously described (Eyboulet et al. 2015). *MED10* WT and *med10*-196 strains were grown in 10 liters of 2X YPD overnight to late exponential phase at 30°C or transferred for 90 min to 37°C.

**Mass spectrometry**

To analyze Mediator integrity in *med10* mutant, the purified Mediator complex was analyzed by mass spectrometry. A short migration of proteins from the Mediator complex was performed in NuPage 4–12% gel (Life Technologies), about 1 cm below the stacking gel. After coomassie blue staining, the 1 cm band was cut, and proteins were digested overnight using trypsin. Tryptic peptides were extracted with 60% acetonitrile and 0.1% (v/v) formic acid, vacuum dried and resuspended in 0.1% (v/v) formic acid prior to nanoLC–MS/MS mass spectrometry analyses.
The nanoLC–MS/MS analyses were performed with the Triple-TOF 4600 mass spectrometer (AB Sciex, Framingham, MA, USA) coupled to the nanoRSLC ultra performance liquid chromatography (UPLC) system (Thermo Scientific) equipped with a trap column (Acclaim PepMap100C18, 75 µm i.d. × 2 cm, 3 µm) and an analytical column (Acclaim PepMapRSLCC18, 75 µm i.d. × 25 cm, 2 µm, 100 Å). Peptide separation was performed with a 5–35% solvent B gradient for 40 min. Solvent A was 0.1% formic acid in water, and solvent B was 0.1% formic acid in 100% acetonitrile. The nanoLC–MS/MS experiments were conducted using the data-dependent acquisition method by selecting the 20 most intense precursors for CID fragmentation with Q1 quadrupole set at low resolution for better sensitivity. Raw data were processed using MS Data Converter software (AB Sciex) for generating .mgf data files. Protein identification was performed using the MASCOT database search engine (Matrix science, London, UK) against the Swissprot database (release 2016_05) with trypsin specificity (with 2 missed cleavages), carbamidomethylation of cysteines and oxidation of methionines set as fixed and variable modifications, respectively. Peptide and fragment tolerance were respectively set at 20 ppm and 0.05 Da. Results of three replicates were merged. Only proteins with at least two unique peptides with mascot ion scores above identity threshold (25) at less than 1% FDR were considered.

**In vitro assay of preinitiation complex assembly**

The *in vitro* analysis of preinitiation complex was done as previously described (Ranish et al. 1999). Briefly, a biotin-labeled-PCR-generated DNA fragment was used as a template and was bound to Streptavidin beads (Dynabeads). This matrix was incubated with purified Gal4-Gcn4 activator and a nuclear extract from WT or mutant strains. Activator was purified as described and nuclear extract was prepared with standard procedure (Ranish et al. 1999; Reeves and Hahn 2003). After assembly on the DNA matrix, the reactions were washed
and eluted from the beads by restriction enzyme digestion (PstI, Invitrogen). Condition without DNA was used as a control. Condition with DNA matrix but without activator was used as a control of basal transcription. When indicated, purified Mediator was added to the reaction. Eluates were analyzed by western blotting. Rabbit polyclonal anti-Med14 and anti-Med17 antibodies were used against corresponding N-terminal peptides to detect Med14 and Med17 Mediator subunits, respectively. 12CA5 monoclonal antibody was used to detect HA-tagged protein. Mouse monoclonal antibody 8WG16 and 1Y26 were used to detect Pol II subunits Rpb1 and Rpb3, respectively. Rabbit polyclonal anti-Sua7p (Abcam) was used to detect Sua7, rabbit polyclonal anti-Toa1 (Abcam) was used to detect Toa1, rabbit polyclonal antibody anti-Kin28 (Biolegend) was used to detect Kin28. Rabbit polyclonal antibody against H3 histone (Abcam) was used as a loading control of nuclear extract.

Supplemental Table S1. Yeast strains

| Name       | Tagged or Mutant protein | Genotype                           | Reference         |
|------------|--------------------------|------------------------------------|-------------------|
| YPH499     | WT                       | MATa ura3-52 his3-Δ200 ade2-101uaa trp1-Δ63 lys2-801uag leu2-Δ1 | (Sikorski and Hieter 1989) |
| Y4987      | WT                       | MATa ura3-52 his3-Δ200 ade2-101uaa trp1-Δ63 lys2-801uag leu2-Δ1 med10::KanMX6//MED10 CEN URA3 | This work         |
| Y5466      | WT                       | MATa ura3-52 his3-Δ200 ade2-101uaa trp1-Δ63 lys2-801uag leu2-Δ1 med10::KanMX6//MED10 CEN TRP1 | This work         |
| Y5467      | med10-196                | MATa ura3-52 his3-Δ200 ade2-101uaa trp1-Δ63 lys2-801uag leu2-Δ1 med10::KanMX6//med10-196 CEN TRP1 | This work         |
| Y5801      | Med5-HA                  | MATa ura3-52 his3-Δ200 ade2-101uaa trp1-Δ63 lys2-801uag leu2-Δ1 med10::KanMX6 MED5::3HA::HIS3 // MED10 CEN URA3 | This work         |
| Y5802      | Med15-HA                 | MATa ura3-52 his3-Δ200 ade2-101uaa trp1-Δ63 lys2-801uag leu2-Δ1 med10::KanMX6 MED15::3HA::HIS3 // MED10 CEN URA3 | This work         |
| Y5803      | Med17-HA                 | MATa ura3-52 his3-Δ200 ade2-101uaa trp1-Δ63 lys2-801uag leu2-Δ1 med10::KanMX6 MED17::3HA::HIS3 // MED10 CEN URA3 | This work         |
| Y5804      | Rad3-HA                  | MATa ura3-52 his3-Δ200 ade2-101uaa trp1-Δ63 lys2-801uag leu2-Δ1 med10::KanMX6 RAD3::3HA::HIS3 // MED10 CEN URA3 | This work         |
| Y5805      | Kin28-HA                 | MATa ura3-52 his3-Δ200 ade2-101uaa trp1-Δ63 lys2-801uag leu2-Δ1 med10::KanMX6 KIN28::3HA::HIS3 // MED10 CEN URA3 | This work         |
| Y5806 | HA-TBP | MATa ura3-52 his3-Δ200 ade2-101 uaa trp1-Δ63 lys2-801 uag leu2-Δ1 med10::KanMX6 LEU2::3HA::SPT15 // MED10 CEN TRP1 | This work |
| Y6581 | Taf1-HA | MATa ura3-52 his3-Δ200 ade2-101 uaa trp1-Δ63 lys2-801 uag leu2-Δ1 med10::KanMX6 TAF1::3HA::HIS3 // MED10 CEN URA3 | This work |
| Y5859 | Med5-HA | MATa ura3-52 his3-Δ200 ade2-101 uaa trp1-Δ63 lys2-801 uag leu2-Δ1 med10::KanMX6 MED5::3HA::HIS3 // MED10 CEN TRP1 | This work |
| Y5860 | Med5-HA med10-196 | MATa ura3-52 his3-Δ200 ade2-101 uaa trp1-Δ63 lys2-801 uag leu2-Δ1 med10::KanMX6 MED5::3HA::HIS3 // med10-196 CEN TRP1 | This work |
| Y5861 | Med15-HA | MATa ura3-52 his3-Δ200 ade2-101 uaa trp1-Δ63 lys2-801 uag leu2-Δ1 med10::KanMX6 MED15::3HA::HIS3 // MED10 CEN TRP1 | This work |
| Y5862 | Med15-HA med10-196 | MATa ura3-52 his3-Δ200 ade2-101 uaa trp1-Δ63 lys2-801 uag leu2-Δ1 med10::KanMX6 MED15::3HA::HIS3 // med10-196 CEN TRP1 | This work |
| Y5863 | Med17-HA | MATa ura3-52 his3-Δ200 ade2-101 uaa trp1-Δ63 lys2-801 uag leu2-Δ1 med10::KanMX6 MED17::3HA::HIS3 // MED10 CEN TRP1 | This work |
| Y5864 | Med15-HA med10-196 | MATa ura3-52 his3-Δ200 ade2-101 uaa trp1-Δ63 lys2-801 uag leu2-Δ1 med10::KanMX6 MED17::3HA::HIS3 // med10-196 CEN TRP1 | This work |
| Y5865 | Rad3-HA | MATa ura3-52 his3-Δ200 ade2-101 uaa trp1-Δ63 lys2-801 uag leu2-Δ1 med10::KanMX6 RAD3::3HA::HIS3 // MED10 CEN TRP1 | This work |
| Y5866 | Rad3-HA med10-196 | MATa ura3-52 his3-Δ200 ade2-101 uaa trp1-Δ63 lys2-801 uag leu2-Δ1 med10::KanMX6 RAD3::3HA::HIS3 // med10-196 CEN TRP1 | This work |
| Y5867 | Kin28-HA | MATa ura3-52 his3-Δ200 ade2-101 uaa trp1-Δ63 lys2-801 uag leu2-Δ1 med10::KanMX6 KIN28::3HA::HIS3 // MED10 CEN TRP1 | This work |
| Y5868 | Kin28-HA med10-196 | MATa ura3-52 his3-Δ200 ade2-101 uaa trp1-Δ63 lys2-801 uag leu2-Δ1 med10::KanMX6 KIN28::3HA::HIS3 // med10-196 CEN TRP1 | This work |
| Y5869 | HA-TBP | MATa ura3-52 his3-Δ200 ade2-101 uaa trp1-Δ63 lys2-801 uag leu2-Δ1 med10::KanMX6 LEU2::3HA::SPT15 // MED10 CEN TRP1 | This work |
| Y5870 | HA-TBP med10-196 | MATa ura3-52 his3-Δ200 ade2-101 uaa trp1-Δ63 lys2-801 uag leu2-Δ1 med10::KanMX6 LEU2::3HA::SPT15 // med10-196 CEN TRP1 | This work |
| Y6596 | Taf1-HA | MATa ura3-52 his3-Δ200 ade2-101 uaa trp1-Δ63 lys2-801 uag leu2-Δ1 med10::KanMX6 TAF1::3HA::HIS3 // MED10 CEN TRP1 | This work |
| Y6597 | Taf1-HA med10-196 | MATa ura3-52 his3-Δ200 ade2-101 uaa trp1-Δ63 lys2-801 uag leu2-Δ1 med10::KanMX6 TAF1::3HA::HIS3 // med10-196 CEN TRP1 | This work |
| CA001 | Med22-10HisTEVProteinA Med8-HA | MATa leu2 trp1 ura3-52 prb1-1122 pep4-3 prcl-407 gal2 MED22 ::10His-TEV-ProteinA::KlTRP1 MED8-PreSci-3HA::KanR | (Cai et al. 2009) |
| Y6464 | Med22-10HisTEVProteinA Med8-HA MED10 | MATa leu2 trp1 ura3-52 prb1-1122 pep4-3 prcl-407 gal2 MED22 ::10His-TEV-ProteinA::KlTRP1 MED8-PreSci-3HA::KanR // MED10 CEN URA3 | This work |
| Y6491 | Med22-10HisTEVProteinA Med8-HA MED10 | MATa leu2 trp1 ura3-52 prb1-1122 pep4-3 prcl-407 gal2 MED22 ::10His-TEV-ProteinA::KlTRP1 MED8-PreSci-3HA::KanR // MED10 CEN LEU | This work |
Y6492 Med22-10HisTEVProteinA Med8-HA med10-196 MATa leu2 trp1 ura3-52 prb1-1122 pep4-3 prcl-407 gal2 MED22::10His-TEV-ProteinA::KITRP1 MED8::PreSci-3HA::KanR::MED10-196 CEN LEU
This work

Y6826 Toa2-TAP MATa ura3-52 his3-D200 ade2-101 uaa trpl-Δ63 lys2-801 uag leu2-Δ1 med10::KanMX6 TOA2::TAP::HIS3::MED10 CEN URA3 This work

Y6600 Tfg1-TAP MATa ura3-52 his3-D200 ade2-101 uaa trpl-Δ63 lys2-801 uag leu2-Δ1 med10::KanMX6 TFG1::TAP::HIS3::MED10 CEN URA3 This work

Y6603 Tfa2-TAP MATa ura3-52 his3-D200 ade2-101 uaa trpl-Δ63 lys2-801 uag leu2-Δ1 med10::KanMX6 TFA2::TAP::HIS3::MED10 CEN URA3 This work

Y6493 TFIIB-TAP MATa ura3-52 his3-D200 ade2-101 uaa trpl-Δ63 lys2-801 uag leu2-Δ1 med10::KanMX6 SUA7::TAP::HIS3::MED10 CEN URA3 This work

Y6665 Toa2-TAP MED10 MATa ura3-52 his3-D200 ade2-101 uaa trpl-Δ63 lys2-801 uag leu2-Δ1 med10::KanMX6 TOA2::TAP::HIS3::MED10 CEN TRP1 This work

Y6666 Toa2-TAP med10-196 MATa ura3-52 his3-D200 ade2-101 uaa trpl-Δ63 lys2-801 uag leu2-Δ1 med10::KanMX6 TOA2::TAP::HIS3::MED10-196 CEN TRP1 This work

Y6608 Tfg1-TAP MED10 MATa ura3-52 his3-D200 ade2-101 uaa trpl-Δ63 lys2-801 uag leu2-Δ1 med10::KanMX6 TFG1::TAP::HIS3::MED10 CEN TRP1 This work

Y6609 Tfg1-TAP med10-196 MATa ura3-52 his3-D200 ade2-101 uaa trpl-Δ63 lys2-801 uag leu2-Δ1 med10::KanMX6 TFG1::TAP::HIS3::MED10-196 CEN TRP1 This work

Y6606 Tfa2-TAP MED10 MATa ura3-52 his3-D200 ade2-101 uaa trpl-Δ63 lys2-801 uag leu2-Δ1 med10::KanMX6 TFA2::TAP::HIS3::MED10 CEN TRP1 This work

Y6607 Tfa2-TAP med10-196 MATa ura3-52 his3-D200 ade2-101 uaa trpl-Δ63 lys2-801 uag leu2-Δ1 med10::KanMX6 TFA2::TAP::HIS3::MED10-196 CEN TRP1 This work

Y6667 TFIIB-TAP MED10 MATa ura3-52 his3-D200 ade2-101 uaa trpl-Δ63 lys2-801 uag leu2-Δ1 medi10::KanMX6 SUA7::TAP::HIS3::MED10 CEN TRP1 This work

Y6668 TFIIB-TAP med10-196 MATa ura3-52 his3-D200 ade2-101 uaa trpl-Δ63 lys2-801 uag leu2-Δ1 medi10::KanMX6 SUA7::TAP::HIS3::MED10-196 CEN TRP1 This work

BY4741 WT MATa uraD0 his3D1 lysD0 leu2D0 (Brachmann et al. 1998)

Y6809 WT MATa uraD0 his3D1 lysD0 leu2D0 med10::KanMX6::MED10 CEN URA3 This work

Y6810 Rad3-HA MED10 MATa uraD0 his3D1 lysD0 leu2D0 med10::KanMX6 RAD3::3HA::HIS3Mx6::MED10 CEN URA3 This work

Y6829 Rad3-HA MED10 MATa uraD0 his3D1 lysD0 leu2D0 med10::KanMX6 RAD3::3HA::HIS3Mx6::MED10 CEN URA3 This work

Y6830 Rad3-HA med10-196 MATa uraD0 his3D1 lysD0 leu2D0 med10::KanMX6 RAD3::3HA::HIS3Mx6::MED10-196 CEN LEU2 This work

Y6972 Toa2-Myc Med5-HA MATa ura3-52 his3-D200 ade2-101 uaa trpl-Δ63 lys2-801 uag leu2-Δ1 medi10::KanMX6 MED5::3HA::HIS3 TOA2::Myc::TRP1::MED10 CEN URA3 This work

Y6991 Toa2-Myc Med5-HA MED10 MATa ura3-52 his3-D200 ade2-101 uaa trpl-Δ63 lys2-801 uag leu2-Δ1 medi10::KanMX6 MED5::3HA::HIS3 TOA2::Myc::TRP1::MED10 CEN URA3 This work
Y6992  Toa2-Myc Med5-HA med10-196  MATa ura3-52 his3-Δ200 ade2-101 uaa trp1-Δ63 lys2-801 uag leu2-Δ1 med10::KanMX6 MED5::3HA::HIS3 TOA2::13Myc::TRP1 // med10-196 CEN LEU2  This work

Y6864  MED10 WT  MATa ura3-52 his3-Δ200 ade2-101 uaa trp1-Δ63 lys2-801 uag leu2-Δ1 med10::ADE2 sua7::KanMX6// MED10 CEN TRP1 SUA7 CEN URA3  This work

Y6865  med10-196  MATa ura3-52 his3-Δ200 ade2-101 uaa trp1-Δ63 lys2-801 uag leu2-Δ1 med10::ADE2 sua7::KanMX6// med10-196 CEN TRP1 SUA7 CEN URA3  This work

Y5373  MED14 WT  MATa ura3-52 his3-Δ200 ade2-101 uaa trp1-Δ63 lys2-801 uag leu2-Δ1 med14::KanMX6// MED14 CEN LEU2  This work

Y5669  MED14 WT  MATa ura3-52 his3-Δ200 ade2-101 uaa trp1-Δ63 lys2-801 uag leu2-Δ1 med14::KanMX6// MED14 CEN TRP1  This work

Y5665  med14-483  MATa ura3-52 his3-Δ200 ade2-101 uaa trp1-Δ63 lys2-801 uag leu2-Δ1 med14::KanMX6// med14::ADE2 sua7::KanMX6// MED14 CEN TRP1  This work

Y5666  med14-686  MATa ura3-52 his3-Δ200 ade2-101 uaa trp1-Δ63 lys2-801 uag leu2-Δ1 med14::KanMX6// med14::ADE2 sua7::KanMX6// MED14 CEN TRP1  This work

Y5668  med14-752  MATa ura3-52 his3-Δ200 ade2-101 uaa trp1-Δ63 lys2-801 uag leu2-Δ1 med14::KanMX6// med14::ADE2 sua7::KanMX6// MED14 CEN TRP1  This work

Y7057  MED14 WT  MATa ura3-52 his3-Δ200 ade2-101 uaa trp1-Δ63 lys2-801 uag leu2-Δ1 med14::ADE2 sua7::KanMX6// MED14 CEN TRP1 SUA7 CEN URA3  This work

Y7058  med14-483  MATa ura3-52 his3-Δ200 ade2-101 uaa trp1-Δ63 lys2-801 uag leu2-Δ1 med14::ADE2 sua7::KanMX6// med14::ADE2 sua7::KanMX6// MED14 CEN TRP1 SUA7 CEN URA3  This work

Y7059  med14-686  MATa ura3-52 his3-Δ200 ade2-101 uaa trp1-Δ63 lys2-801 uag leu2-Δ1 med14::ADE2 sua7::KanMX6// med14::ADE2 sua7::KanMX6// MED14 CEN TRP1 SUA7 CEN URA3  This work

Y7060  med14-752  MATa ura3-52 his3-Δ200 ade2-101 uaa trp1-Δ63 lys2-801 uag leu2-Δ1 med14::ADE2 sua7::KanMX6// med14::ADE2 sua7::KanMX6// MED14 CEN TRP1 SUA7 CEN URA3  This work

* Plasmid descriptions are separated from chromosomal genotype by //.
## Supplemental Table S2. Plasmids

| Name        | Genotype                                      | Reference      |
|-------------|-----------------------------------------------|----------------|----------------|
| pVV208-MED10| Amp CEN URA3 MED10                            | This work      |
| pVV204-MED10| Amp CEN TRP1 MED10                            | This work      |
| pVV204-med10-196| Amp CEN TRP1 med10-196                    | This work      |
| pAG-GPD-MED10| Amp CEN LEU2 MED10                            | This work      |
| pAG-GPD-med10-196| Amp CEN LEU2 med10-196            | This work      |
| pVV212      | Amp 2µ TRP1 pADH1 GAL4-BD tADH1              | This work      |
| pVV213-MED1 | Amp 2µ LEU2 pADH1 GAL4-AD tADH1 MED1         | This work      |
| pVV213-MED4 | Amp 2µ LEU2 pADH1 GAL4-AD tADH1 MED4         | This work      |
| pVV213-MED7 | Amp 2µ LEU2 pADH1 GAL4-AD tADH1 MED7         | This work      |
| pVV213-MED9 | Amp 2µ LEU2 pADH1 GAL4-AD tADH1 MED9         | This work      |
| pVV213-MED10| Amp 2µ LEU2 pADH1 GAL4-AD tADH1 MED10        | This work      |
| pVV213-med10-196| Amp 2µ LEU2 pADH1 GAL4-AD tADH1 med10-196  | This work      |
| pVV213-MED14| Amp 2µ LEU2 pADH1 GAL4-AD tADH1 MED14        | This work      |
| pVV213-MED21| Amp 2µ LEU2 pADH1 GAL4-AD tADH1 MED21        | This work      |
| pVV213-MED31| Amp 2µ LEU2 pADH1 GAL4-AD tADH1 MED31        | This work      |
| pVV212-SUA7 | Amp 2µ TRP1 pADH1 GAL4-BD tADH1 SUA7         | This work      |
| pVV212-sua7-11| Amp 2µ TRP1 pADH1 GAL4-BD tADH1 sua7-11     | This work      |
| pVV212-sua7-34| Amp 2µ TRP1 pADH1 GAL4-BD tADH1 sua7-34    | This work      |
| pVV212-sua7-36| Amp 2µ TRP1 pADH1 GAL4-BD tADH1 sua7-36    | This work      |
| pVV208-MED14| Amp CEN URA3 MED14                            | This work      |
| pVV204-MED14| Amp CEN TRP1 MED14                            | This work      |
| pVV204-med14-483| Amp CEN TRP1 med14-483                | This work      |
| pVV204-med14-686| Amp CEN TRP1 med14-686              | This work      |
| pVV204-med14-752| Amp CEN TRP1 med14-752            | This work      |
| pDW11       | Amp CEN SUA7 URA3                            | This work      |
| pM299       | Amp CEN SUA7 HIS3                            | (Wu et al. 1999)|
| pM326       | Amp CEN sua7-1 HIS3                          | (Wu et al. 1999)|
| pM365       | Amp CEN sua7-8 HIS3                          | (Wu et al. 1999)|
| pM376       | Amp CEN sua7-11 HIS3                         | (Wu et al. 1999)|
| pM377       | Amp CEN sua7-12 HIS3                         | (Wu et al. 1999)|
| pM378       | Amp CEN sua7-13 HIS3                         | (Wu et al. 1999)|
| pM392       | Amp CEN sua7-18 HIS3                         | (Wu et al. 1999)|
| pM401       | Amp CEN sua7-24 HIS3                         | (Wu et al. 1999)|
| pM404       | Amp CEN sua7-28 HIS3                         | (Wu et al. 1999)|
| pM415       | Amp CEN sua7-20 HIS3                         | (Wu et al. 1999)|
| pM502       | Amp CEN sua7-31 HIS3                         | (Wu et al. 1999)|
| pM505       | Amp CEN sua7-34 HIS3                         | (Wu et al. 1999)|
| pM506       | Amp CEN sua7-35 HIS3                         | (Wu et al. 1999)|
| pM507       | Amp CEN sua7-36 HIS3                         | (Wu et al. 1999)|
| pM512       | Amp CEN sua7-41 HIS3                         | (Wu et al. 1999)|
| pM513       | Amp CEN sua7-42 HIS3                         | (Wu et al. 1999)|
| pM515       | Amp CEN sua7-44 HIS3                         | (Wu et al. 1999)|
## Supplemental Table S3. Oligonucleotides

| Name     | Forward                          | Reverse                          |
|----------|----------------------------------|----------------------------------|
| ATP1-O   | TCTTCGCACTCGGGTGATGGTA          | TATCGGAACCGAAAAGAACG             |
| ALD6-P   | AGCGCAACAAAGAAAGAAAACGA         | CGTTCACCAAGAGGAGACATCAA          |
| ALD6-O   | CCCATGTGTTTGGATGCGATGGTA        | GGCTGGAGAACGCACTGCAATGGAAGAC     |
| SRM1-P   | GGCAAGGAGGATACTGCTTTTAC         | ATTTTGCCACCTGCTTTTAC             |
| QCR6-O   | GATGCGATAACAGGAGCAGCA           | TACCTGCTCTCGTGTCTTGTG            |
| PIL1-O   | GATTCTTTGGGAGGGTGGTA            | ATGGCGATCTCGCTTTTAC              |
| MGR1-P   | TGATCGATGACGTTAAATGTTGTA        | GATGGGGAGGAGTACGCTTTTAC          |
| RPA34-P  | CATCACGTGACGTTAAATGTTGTA        | TCGTATTTCGCTTTTAC                |
| PSA1-P   | CTGAGCGACGTTAAATGTTGTA          | GTCGCTGCGGTAGCTTTTAC             |
| ALD6-P   | ATGGCGATGACGTTAAATGTTGTA        | GTCGCTGCGGTAGCTTTTAC             |
| ACT1-P   | TCCAGGTGTCGCTTCTGCTAAAT         | GTCGCTGCGGTAGCTTTTAC             |
| APA1-P   | GAGCGAGGCTGAAATGTTGTA           | GAAGGAGGAGGAGGAGGAGAAAGAGAGAGAG|
| PIM1-P   | GCCACGGAAGGAAATGTTGTA           | GATGGGGAGGAGTACGCTTTTAC          |
| STI1-P   | CCAAAAGTCTGCTTCTCCAAAAT         | TGGGATTCGTTTCTTCTTCTT            |
| YIP5-P   | CAACGCAGCCTGCTAAGGTA            | GTTGAATGGGAACGCGGACG             |
| PSA1-P1  | CTGACGAGGATGTCGCTTCTTAC         | TCTTGAATGGGAACGCGGACG             |
| PSA1-P2  | GGAGCGAGGCTGCTTCTTAC            | TCTTGAATGGGAACGCGGACG             |
| PRB1-P   | CCGTGTTGACGAGTAGCTGCA           | TCTTGAATGGGAACGCGGACG             |
| HSP42-P  | GGAGCGAGGCTGCTTCTTAC            | TCTTGAATGGGAACGCGGACG             |
| HSP42-O  | CATCGTATGACGTTAAATGTTGTA        | GTCGCTGCGGTAGCTTTTAC             |
| HSP30-P1 | ATCCCGATCCCGACTCTTTAT           | GGCATTTAGGGAGGAGGAGAA            |
| HSP30-P2 | ATTTTGTTGAGCTTACCTCCCAAT        | GGATGCTGCTTCTTCTTCTT             |
| HSP30-O  | CAATTTGACGCTGCTTCTTCTT          | ATAGGCTGCTGCTTCTTCTT             |
| HSP150-P1| GGAACACCTGGAAGTCTAAGGTA         | AAGCAAGAAGGAAACAAATGCT           |
| HSP150-P2| ATTATCTCTGCTGCTTCTTCTT          | TGGGAGGAGGAGGAGGAGGAGAAGGAGAGAG|
| HSP104-P | CCCATGACAGGATGTTGAAGTGTA        | GAGCAAGAAGGAAACAAATGCT           |
| HSP104-O | GTATCGACAGGATGTTGAAGTGTA        | GAGCAAGAAGGAAACAAATGCT           |
| HSP12-O  | CAAGGGTGTCGTCTTCAAGGTA          | CAAGGGGAAACATATTGGC              |
| ADH1-P1  | ATAGGCGGTGTTACGTTCTGT          | CATCGTGCTGCTGCTTCTT             |
| ADH1-P2  | TCTTCTTCTTCTTCTTCTT             | CATCGTGCTGCTGCTTCTT             |
| ADH1-O   | GGATTTGTGAGGTTGAGGTA            | AAGCAGAAGGAAACAAATGCT           |
| PYK1-P1  | CGCAGGCTAGAGGAGTGTGA            | TGGGAGGAGGAGGAGGAGGAGAAGGAGAGAG|
| PYK1-P2  | CCTTTGTTGAGGAGTGTGA             | TGGGAGGAGGAGGAGGAGGAGAAGGAGAGAG|
| PYK1-O   | TCTTTGTTGAGGAGTGTGA             | TGGGAGGAGGAGGAGGAGGAGAAGGAGAGAG|
| PMA1-O   | GTGGGAAAGGAGGAGGAGGAGAAGGAGAGAG| GAGCAAGAAGGAAACAAATGCT           |
| PMA1-P1  | AACAAACCGGCTGCTGCTGCAAGG   | GATGGGAGGAGGAGGAGGAGAAGGAGAGAG|
| PMA1-P2  | GATGCGTATGACGTTAAATGTTGTA       | GATGGGAGGAGGAGGAGGAGAAGGAGAGAG|
| GAL1-P   | ACGCTTTTCTTCTTCTTCTT            | TGGGAGGAGGAGGAGGAGGAGAAGGAGAGAG|
| GAL1-O1  | AACATGACAGGATGCTTCTT            | TGGGAGGAGGAGGAGGAGGAGAAGGAGAGAG|
| GAL1-O2  | ACATTTTGCTGCTGCTTCTT            | TGGGAGGAGGAGGAGGAGGAGAAGGAGAGAG|
| PMA1-O2  | TCTTTAAGGCGGCTGCTTCTT          | TGGGAGGAGGAGGAGGAGGAGAAGGAGAGAG|
## Supplemental Table S4. Total number of mapped reads for ChIP-seq experiments

| Sample           | Million of mapped reads |
|------------------|-------------------------|
| INPUT-MED10      | 13.6                    |
| INPUT-med10-196  | 12.8                    |
| NT               | 1.9                     |
| PolII-MED10      | 9.0                     |
| PolII-med10-196  | 10.0                    |
| TFIIB-MED10      | 15.9                    |
| TFIIB-med10-196  | 8.8                     |
| Rad3-MED10       | 6.4                     |
| Rad3-med10-196   | 7.7                     |
| Kin28-MED10      | 2.5                     |
| Kin28-med10-196  | 3.4                     |
| Med15-MED10      | 3.5                     |
| Med15-med10-196  | 4.0                     |
| Med17-MED10      | 4.3                     |
| Med17-med10-196  | 4.5                     |
| TBP-MED10        | 5.7                     |
| TBP-med10-196    | 3.4                     |
| Toa2-MED10       | 10.2                    |
| Toa2-med10-196   | 10.5                    |
| Tfa2-MED10       | 5.5                     |
| Tfa2-med10-196   | 5.1                     |
| Tfg1-MED10       | 6.5                     |
| Tfg1-med10-196   | 3.5                     |
| Taf1-MED10       | 5.1                     |
| Taf1-med10-196   | 3.8                     |
### Supplemental Table S5. Regions used for ChIP-seq data normalization

| Protein | Regions |
|---------|---------|
| **Pol II** | ADH1-O, ATP1-O, ALD6-O, GAL1-O2, HSP150-O, HSP30-O, HSP42-O, UTP20-O, PMA1-O, PYK1-O, PHO84-O |
| **Kin28, Rad3** | ADH1-P2, GAL1-O2, HSP104-P1, HSP30-P1, HSP42-P, MGR1-P, PMA1-P2, PYK1-P2, MGR1-P, RPA34-P, SRM1-P |
| **TBP** | ADH1-P2, GAL1-O2, PIM1-P, PMA1-P2, PRB1-P, PYK1-P2, RPA34-P, STII-P, YIP5-P, PSA1-P2 |
| **Med15** | ADH1-P1, ARG3-P1, FLC1-P, GAL1-O1, PMA1-P1, PYK1-P1, HSP42-P, HSP150-P2, HSP30-P2, PSA1-P1, PRB1-P |
| **Med17** | ADH1-P1, ARG3-P1, FLC1-P, GAL1-O1, PMA1-P1, PYK1-P1, HSP42-P, HSP150-P2, HSP30-P2, PSA1-P1, PRB1-P |
| **TFIIB** | ADH1-P, ALD6-P, APA1-P, ATP1-P, GAL1-P, PMA1-P, PRB1-P, PSA1-P, PYK1-P, RPA34-P, SRM1-P, STII-P |
| **Tfg1** | ADH1-P, ALD6-P, APA1-P, ATP1-P, GAL1-P, PMA1-P, PRB1-P, PSA1-P, PYK1-P, RPA34-P, SRM1-P, STII-P |
| **Tfa2** | ADH1-P, ALD6-P, APA1-P, ATP1-P, GAL1-P, PMA1-P, PRB1-P, PSA1-P, PYK1-P, RPA34-P, SRM1-P, STII-P |
| **Toa2** | ADH1-P, ALD6-P, APA1-P, ATP1-P, GAL1-P, PMA1-P, PRB1-P, PSA1-P, PYK1-P, RPA34-P, SRM1-P, STII-P |
| **Taf1** | HSP150-P1, ALD6-P, YIP5-P, PIM1-P, HSP12-P, PSA1-P, ACT1-P, QCR6-P, PRB1-P, PMA1-P, ADH1-P, GAL1-O2 |

### Supplemental Table S6. Normalization coefficients for ChIP-seq data

| Protein | Mutant med10-196 |
|---------|------------------|
| **Pol II** | 0.45 |
| Med15    | 0.46 |
| Med17    | 0.45 |
| TFIIB    | 0.57 |
| Toa2     | 0.97 |
| Tfg1     | 1.2 |
| Tfa2     | 0.66 |
| Rad3     | 0.42 |
| Kin28    | 0.48 |
| TBP      | 1.93 |
| Taf1     | 1.04 |
**Supplemental Figures legends**

**Supplemental Figure S1. Coimmunoprecipitation between Mediator and TFIIA in med10-196 mutant compared to the wild-type.**

Wild-type and med10 mutant strains carrying a Med5-HA tag and a Toa2-Myc tag were grown to exponential phase at 30°C or transferred to 37°C for 90 min. Mediator was immunoprecipitated (IP) through Med5-HA from crude extracts (Input) of wild-type and mutant strains using magnetic beads coupled to anti-HA antibodies. Control immunoprecipitation with IgG magnetic beads only was also performed (IgG). Coimmunoprecipitated Toa2-Myc was detected by western blotting using anti-Myc antibodies.

**Supplemental Figure S2. Functional interaction between Mediator and TFIIB.**

(A) Specific sua7 mutants have synthetic phenotypes in combination with med10-196.

The strain deleted for med10 and complemented by a TRP1 plasmid carrying MED10 or med10-196, and also deleted for sua7 and complemented by a URA3 plasmid carrying SUA7, was transformed by the HIS3 plasmids carrying either WT or mutated version of SUA7. Transformants were serially diluted, spotted on 5-FOA-containing agar plates to counterselect the WT SUA7-bearing plasmid (see Supplemental Materials and Methods), and incubated at 30°C for 3 days, or on YPD agar plates and incubated at 30°C for 3 days. The sua7 mutants (sua7-11 (L136P), -34 (L52P) and 36 (S53P)) showing synthetic phenotypes with med10-196 are indicated in red.

(B) Two-hybrid interaction between Med10 and Med14 Mediator subunits and Sua7. Wild-type or mutant Sua7 was fused to the Gal4 DNA-binding domain (G_DB-Sua7, Sua7- L136P), and Med10, 14, 21 were fused to the Gal4 activation domain (G_AD-Med10, 14, 21). Three independent clones for G_DB-Sua7 were included to allow evaluation of the reproducibility of the experiments.
Quantitative analysis of two-hybrid interactions between Med10 and Med14 Mediator subunits and TFIIB. Wild-type or mutant Sua7 was fused to the Gal4 DNA-binding domain (GDB-Sua7, Sua7-L136P), and Med10, 14, 21 were fused to the Gal4 activation domain (GAD-Med10, 14, 21). A control corresponds to the empty vectors (GDB, GAD). β-galactosidase was assayed using CPRG as a substrate as described in Supplemental Materials and Methods. The values were normalized to the control. The mean values and standard deviation (indicated by error bars) of three independent experiments are shown. Asterisk represents a significant difference compared to the control for Med10-Sua7 and Med14-Sua7 at p-value <0.005 in a Student t-test. The background level was represented by a dotted line.

Supplemental Figure S3. Specific sua7 mutants are co-lethal with med14 mutants.

(A) Thermo-sensitive growth phenotype of the med14 mutants. med14-752, 686 and 483 correspond to C-terminal truncations with a stop codon at position 752, 686 or 483, respectively. Cultures of WT and mutant med14 yeast strains were serially diluted, spotted on YPD agar plates and incubated for 3 days at permissive (30°C) or non-permissive (37°C) temperatures.

(B) Specific sua7 mutants are co-lethal with med14 mutants.

The strain deleted for med14 and complemented by a TRP1 plasmid carrying MED14 or med14 mutants, and also deleted for sua7 and complemented by a URA3 plasmid carrying SUA7, was transformed by the HIS3 plasmids carrying either WT or mutated version of SUA7. Transformants were serially diluted, spotted on 5-FOA-containing agar plates to counterselect the WT SUA7-bearing plasmid, and incubated at 30°C for 3 days, or on YPD agar plates and incubated at 30°C for 3 days.
Supplemental Figure S4. ChIP-seq profiles of preinitiation complex components on yeast genome.

Cells were grown at 30°C in YPD medium and then transferred for 90 minutes to 37°C.

(A) Example of ChIP-seq profiles of PIC components on chromosome VII from 397,758 to 400,300 for the wild-type and med10-196 mutant. Densities of sequence tags were assessed from ChIP-seq experiments and displayed using the IGB yeast genome browser. Input DNA and DNA from ChIP with an untagged strain were used as negative controls. Densities of sequence tags were displayed after subtraction of the normalized control of an untagged strain.

(B) Distribution of PIC components ChIP-seq densities around TSS. Pol II, Mediator (Med15 and Med17 subunits) and TFIIB profiles are displayed on the upper graph. TFIIA, B, D (TBP and Taf1), E, F, H and K profiles are shown on the lower graph. The tag density values for TFIIB were rescaled (x0.5) as indicated. Intergenic regions encompassing Pol III-transcribed genes and divergent genes were excluded. The tag density was determined for each protein in a 1600-bp window centered on the TSS. Mean tag density for each nucleotide position was then calculated and plotted over the window.

Supplemental Figure S5. Clustering analysis of genome-wide PIC occupancy ratios between med10-196 and the wild type.

Clustering analysis was performed for TFIH, TFIK, Med15 Mediator, TFIIA, TFIID (TBP), TFIID (Taf1), TFIIE and TFIIF as described in Figure 5 legend and Materials and Methods.

The groups determined by indicated PIC component (left graph) were analyzed for the nucleosome occupancy in a 1600-bp window centered on the TSS (central graph) and for the presence of the TATA-box, for dynamic (hot) nucleosomes -1 and +1 (right graph).
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| MED10 | 30°C | WT  | WT  | 196 |
|-------|------|-----|-----|-----|
| CoIP  | IgG  | IP α-HA |
| IP    |      |        |

| 30°C | 37°C |
|------|------|
| WT   | 196  |
| Med5-HA |

| 30°C | 37°C |
|------|------|
| WT   | 196  |
| Toa2-Myc |

| Input |
|-------|
Eychenne et al._FigS2

A

|    | SUA7: WT | 1 | 8 | 11 | 12 | 13 | 18 | 20 | 24 | 28 | 31 | 34 | 35 | 36 | 41 | 42 | 44 |
|----|----------|---|---|----|----|----|----|----|----|----|----|----|----|----|----|----|----|
| MED10 |          |   |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |
| med10-196 |        |   |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |

5FOA

YPD

B

G_{AD}

G_{AD}-Med10

G_{AD}-Med14

G_{AD}-Med21

X-gal

C

β-galactosidase activity (CPRG)

|                | Control | G_{AD}-Med10 | G_{AD}-Med14 | G_{AD}-Med21 |
|----------------|---------|--------------|--------------|--------------|
| Arbitrary units|         | 0.8          | 1.2          | 0.8          |

* p < 0.05
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A

30°C

37°C

MED14

med14-752

med14-686

med14-483

B

SUA7: WT 1 8 11 12 13 18 20 24 28 31 34 35 36 41 42 44

MED14

med14-752

med14-686

med14-483

5FOA

YPD
A

TFIIA
TFIIB
TFIID (TBP)
TFIID (Taf1)
TFIIE
TFIIF
TFIIH (core)
TFIIK
Pol II
Mediator (tail)
Mediator (head)

B

Average ChIP-seq density

Distance to TSS (bp)
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