Supplemental Materials

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

Thomas Eychenne, Elizaveta Novikova, Marie-Bénédicte Barrault, Olivier Alibert, Claire Boschiero, Nuno Peixeiro, David Cornu, Virginie Redeker, Laurent Kuras, Pierre Nicolas, Michel Werner and Julie Soutourina

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 *med10*Δ/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, TFIIB-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::KlTRP1 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 ade2-101uaa trp1-Δ63 lys2-801uag leu2-Δ1 med10::KanMX6 LEU2::3HA::SPT15 // MED10 CEN URA3*	This work
Y6581	Taf1-HA	*MATa ura3-52 his3-Δ200 ade2-101uaa trp1-Δ63 lys2-801uag leu2-Δ1 med10::KanMX6 TAF1::3HA::HIS3 // MED10 CEN URA3*	This work
Y5859	Med5-HA	*MATa ura3-52 his3-Δ200 ade2-101uaa trp1-Δ63 lys2-801uag leu2-Δ1 med10::KanMX6 MED5::3HA::HIS3 // MED10 CEN TRP1*	This work
Y5860	Med5-HA *med10-196*	*MATa ura3-52 his3-Δ200 ade2-101uaa trp1-Δ63 lys2-801uag leu2-Δ1 med10::KanMX6 MED5::3HA::HIS3 // med10-196 CEN TRP1*	This work
Y5861	Med15-HA	*MATa ura3-52 his3-Δ200 ade2-101uaa trp1-Δ63 lys2-801uag leu2-Δ1 med10::KanMX6 MED15::3HA::HIS3 // MED10 CEN TRP1*	This work
Y5862	Med15-HA *med10-196*	*MATa ura3-52 his3-Δ200 ade2-101uaa trp1-Δ63 lys2-801uag leu2-Δ1 med10::KanMX6 MED15::3HA::HIS3 // med10-196 CEN TRP1*	This work
Y5863	Med17-HA	*MATa ura3-52 his3-Δ200 ade2-101uaa trp1-Δ63 lys2-801uag leu2-Δ1 med10::KanMX6 MED17::3HA::HIS3 // MED10 CEN TRP1*	This work
Y5864	Med15-HA *med10-196*	*MATa ura3-52 his3-Δ200 ade2-101uaa trp1-Δ63 lys2-801uag leu2-Δ1 med10::KanMX6 MED17::3HA::HIS3 // med10-196 CEN TRP1*	This work
Y5865	Rad3-HA	*MATa ura3-52 his3-Δ200 ade2-101uaa trp1-Δ63 lys2-801uag leu2-Δ1 med10::KanMX6 RAD3::3HA::HIS3 // MED10 CEN TRP1*	This work
Y5866	Rad3-HA *med10-196*	*MATa ura3-52 his3-Δ200 ade2-101uaa trp1-Δ63 lys2-801uag leu2-Δ1 med10::KanMX6 RAD3::3HA::HIS3 // med10-196 CEN TRP1*	This work
Y5867	Kin28-HA	*MATa ura3-52 his3-Δ200 ade2-101uaa trp1-Δ63 lys2-801uag leu2-Δ1 med10::KanMX6 KIN28::3HA::HIS3 // MED10 CEN TRP1*	This work
Y5868	Kin28-HA *med10-196*	*MATa ura3-52 his3-Δ200 ade2-101uaa trp1-Δ63 lys2-801uag leu2-Δ1 med10::KanMX6 KIN28::3HA::HIS3 // med10-196 CEN TRP1*	This work
Y5869	HA-TBP	*MATa ura3-52 his3-Δ200 ade2-101uaa trp1-Δ63 lys2-801uag leu2-Δ1 med10::KanMX6 LEU2::3HA::SPT15 // MED10 CEN TRP1*	This work
Y5870	HA-TBP *med10-196*	*MATa ura3-52 his3-Δ200 ade2-101uaa trp1-Δ63 lys2-801uag leu2-Δ1 med10::KanMX6 LEU2::3HA::SPT15 // med10-196 CEN TRP1*	This work
Y6596	Taf1-HA	*MATa ura3-52 his3-Δ200 ade2-101uaa trp1-Δ63 lys2-801uag leu2-Δ1 med10::KanMX6 TAF1::3HA::HIS3 // MED10 CEN TRP1*	This work
Y6597	Taf1-HA *med10-196*	*MATa ura3-52 his3-Δ200 ade2-101uaa trp1-Δ63 lys2-801uag leu2-Δ1 med10::KanMX6 TAF1::3HA::HIS3 // MED10 CEN TRP1*	This work
CA001	Med22-10HisTEVProteinA Med8-HA	*MATa leu2 trp1 ura3-52 prb1-1122 pep4-3 prc1-407 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 prc1-407 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 prc1-407 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 prc1-407 gal2 MED22 ::10His-TEV-ProteinA::KlTRP1 MED8-PreSci-3HA::KanR // med10-196 CEN LEU*	This work
Y6826	Toa2-TAP	*MATa ura3-52 his3-Δ200 ade2-101uaa trp1-Δ63 lys2-801uag leu2-Δ1 med10::KanMX6 TOA2::TAP::HIS3 // MED10 CEN URA3*	This work
Y6600	Tfg1-TAP	*MATa ura3-52 his3-Δ200 ade2-101uaa trp1-Δ63 lys2-801uag leu2-Δ1 med10::KanMX6 TFG1::TAP::HIS3 // MED10 CEN URA3*	This work
Y6603	Tfa2-TAP	*MATa ura3-52 his3-Δ200 ade2-101uaa trp1-Δ63 lys2-801uag leu2-Δ1 med10::KanMX6 TFA2::TAP::HIS3 // MED10 CEN URA3*	This work
Y6493	TFIIB-TAP	*MATa ura3-52 his3-Δ200 ade2-101uaa trp1-Δ63 lys2-801uag leu2-Δ1 med10::KanMX6 SUA7::TAP::HIS3 // MED10 CEN URA3*	This work
Y6665	Toa2-TAP *MED10*	*MATa ura3-52 his3-Δ200 ade2-101uaa trp1-Δ63 lys2-801uag leu2-Δ1 med10::KanMX6 TOA2::TAP::HIS3 // MED10 CEN TRP1*	This work
Y6666	Toa2-TAP *med10-196*	*MATa ura3-52 his3-Δ200 ade2-101uaa trp1-Δ63 lys2-801uag leu2-Δ1 med10::KanMX6 TOA2::TAP::HIS3 // med10-196 CEN TRP1*	This work
Y6608	Tfg1-TAP *MED10*	*MATa ura3-52 his3-Δ200 ade2-101uaa trp1-Δ63 lys2-801uag leu2-Δ1 med10::KanMX6 TFG1::TAP::HIS3 // MED10 CEN TRP1*	This work
Y6609	Tfg1-TAP *med10-196*	*MATa ura3-52 his3-Δ200 ade2-101uaa trp1-Δ63 lys2-801uag leu2-Δ1 med10::KanMX6 TFG1::TAP::HIS3 // med10-196 CEN TRP1*	This work
Y6606	Tfa2-TAP *MED10*	*MATa ura3-52 his3-Δ200 ade2-101uaa trp1-Δ63 lys2-801uag leu2-Δ1 med10::KanMX6 TFA2::TAP::HIS3 // MED10 CEN TRP1*	This work
Y6607	Tfa2-TAP *med10-196*	*MATa ura3-52 his3-Δ200 ade2-101uaa trp1-Δ63 lys2-801uag leu2-Δ1 med10::KanMX6 TFA2::TAP::HIS3 // med10-196 CEN TRP1*	This work
Y6667	TFIIB-TAP *MED10*	*MATa ura3-52 his3-Δ200 ade2-101uaa trp1-Δ63 lys2-801uag leu2-Δ1 med10::KanMX6 SUA7::TAP::HIS3 // MED10 CEN TRP1*	This work
Y6668	TFIIB-TAP *med10-196*	*MATa ura3-52 his3-Δ200 ade2-101uaa trp1-Δ63 lys2-801uag leu2-Δ1 med10::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 LEU2*	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-Δ200 ade2-101uaa trp1-Δ63 lys2-801uag leu2-Δ1 med10::KanMX6 MED5::3HA::HIS3 TOA2::13Myc::TRP1 // MED10 CEN URA3*	This work
Y6991	Toa2-Myc Med5-HA *MED10*	*MATa ura3-52 his3-Δ200 ade2-101uaa trp1-Δ63 lys2-801uag leu2-Δ1 med10::KanMX6 MED5::3HA::HIS3 TOA2::13Myc::TRP1 //*	This work

		MED10 CEN LEU2	
Y6992	Toa2-Myc Med5-HA *med10-196*	*MATa ura3-52 his3-Δ200 ade2-101uaa trp1-Δ63 lys2-801uag leu2-Δ1 med10::KanMX6 MED5::3HA::HIS3 TOA2::13Myc::TRP1 // med10-196 CEN LEU2*	This work
Y6864	*MED10 WT*	*MATa ura3-52 his3-Δ200 ade2-101uaa trp1-Δ63 lys2-801uag leu2-Δ1 med10::ADE2 sua7::KanMX6// MED10 CEN TRP1 SUA7 CEN URA3*	This work
Y6865	*med10-196*	*MATa ura3-52 his3-Δ200 ade2-101uaa trp1-Δ63 lys2-801uag leu2-Δ1 med10::ADE2 sua7::KanMX6// med10-196 CEN TRP1 SUA7 CEN URA3*	This work
Y5373	*MED14 WT*	*MATa ura3-52 his3-Δ200 ade2-101uaa trp1-Δ63 lys2-801uag leu2-Δ1 med14::KanMX6// MED14 CEN URA3*	This work
Y5669	*MED14 WT*	*MATa ura3-52 his3-Δ200 ade2-101uaa trp1-Δ63 lys2-801uag leu2-Δ1 med14::KanMX6// MED14 CEN TRP1*	This work
Y5665	*med14-483*	*MATa ura3-52 his3-Δ200 ade2-101uaa trp1-Δ63 lys2-801uag leu2-Δ1 med14::KanMX6// med14-Δ483 CEN TRP1*	This work
Y5666	*med14-686*	*MATa ura3-52 his3-Δ200 ade2-101uaa trp1-Δ63 lys2-801uag leu2-Δ1 med14::KanMX6//med14-Δ686 CEN TRP1*	This work
Y5668	*med14-752*	*MATa ura3-52 his3-Δ200 ade2-101uaa trp1-Δ63 lys2-801uag leu2-Δ1 med14::KanMX6// med14-Δ752 CEN TRP1*	This work
Y7057	*MED14 WT*	*MATa ura3-52 his3-Δ200 ade2-101uaa trp1-Δ63 lys2-801uag leu2-Δ1 med14::ADE2 sua7::KanMX6// MED14 CEN TRP1 SUA7 CEN URA3*	This work
Y7058	*med14-483*	*MATa ura3-52 his3-Δ200 ade2-101uaa trp1-Δ63 lys2-801uag leu2-Δ1 med14::ADE2 sua7::KanMX6// med14-Δ483 CEN TRP1 SUA7 CEN URA3*	This work
Y7059	*med14-686*	*MATa ura3-52 his3-Δ200 ade2-101uaa trp1-Δ63 lys2-801uag leu2-Δ1 med14::ADE2 sua7::KanMX6//med14-Δ686 CEN TRP1 SUA7 CEN URA3*	This work
Y7060	*med14-752*	*MATa ura3-52 his3-Δ200 ade2-101uaa trp1-Δ63 lys2-801uag leu2-Δ1 med14::ADE2 sua7::KanMX6// med14-Δ752 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*	
pVV213	*Amp 2μ LEU2 pADH1 GAL4-AD tADH1*	
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	TCTTGCAGTCGGTGATGGTA	TATCGGAACCGAAAAGAACG
ALD6-P	AGCCGACAAAAGAAAAACGA	CGTTCCCAAGAGGAGATCAA
ALD6-O	CCCATTTGGTCTTTGACGAT	GCCTTGAAAGCAGCCAATAG
SRM1-P	GGCAAGGCAGGATGAATAAA	ATTGGTGGCGACTGTTCTTT
QCR6-O	GATGACGATAACGAGCAGCA	TACCCTCCTCCGTGTTCTTG
PIL1-O	GATTCTTTGGGAAGGGTGGT	ATCGTCATCGTTTTCCAACC
MGR1-P	TGCATGGCATCGTCTTTTAC	GTATGGTCGGTGGAATTGCT
RPA34-P	CATCAGGGCCAATCAATTCT	TTTCGACTTCAGCATTGCAC
PSA1-P	CTGCACCCGATCCTTCTTAC	TTCTGTTTTCGCGTTCTTCA
ALD6-P	ATGCGATATAGCACCGACCA	CTTGACCTCGAACGGTGTTT
ACT1-P	TCCACGTCCTCTTGCATAAA	GGTTTGAGTAGAAAGGGGAAGG
APA1-P	GAGCGGCCTGAAATACTGTC	AACGTTCCGAAACAGGACAC
PIM1-P	GCCACCGGAAGGAAATAAGT	GTTCAAGCACCTCGAAAACC
STI1-P	CCAAAAGTCTGCTCCCAAAT	TGCAGCGTTACCTTGTTGTT
YIP5-P	CAACGTCCTACGCTCAAGGT	CCCTCGAGATCATCGTCAAT
PSA1-P1	CTGCACCCGATCCTTCTTAC	TTCTGTTTTCGCGTTCTTCA
PSA1-P2	GGAGCCACCACGTTTATTTT	GTTTACAAGAGCGCCACGAG
PRB1-P	CCGGGTTAGCAGAGTAGCAG	CTCTTTATCGCGGGTGTGTT
HSP42-P	GGGAGGCCTCTGTGAAGTTA	GCCTGAACGTGTCCCTATGT
HSP42-O	CACCTGGTAACGCAAGAACA	CATCGTTCGCTCACCTACAA
HSP30-P1	ATCCCGATCCCGACTCTTAT	GGCCAATTAGGGAGAGGAAA
HSP30-P2	ATTTTGTTGGCCATTTTCCA	CGGGATATGGCTTTGCTTAC
HSP30-O	CAAGGTGTGATATGCCAACG	ATAGCCTCACCGTCTGGTTG
HSP150-P1	GGAACACTTGAAGTCTAACGACA	AAGCAGAGGCAACCAAAGTC
HSP150-P2	ATTATCCTGGCCGCTAATCC	GTGGCAAAGCAGTGAGATTG
HSP104-P1	CCCATCTCAAAGAACTGCAA	GGAACAAGTGACAAAGGAACG
HSP104-O	GGTACCACGGCAGGTTATGT	CCGTCATCCAACATTTGTAGC
HSP12-O	CAAGGGTGTCTTCCAAGGTG	CGACCGGAAACATATTCGAC
ADH1-P1	ATAGGCGCATGCAACTTCTT	CATCAGCTCTGGAACAACGA
ADH1-P2	TTCCTTCATTCACGCACACT	AGGGAACGAGAACAATGACG
ADH1-O	GGGTATTGACGGTGGTGAAG	AAACGTTGATGACACCGTGA
PYK1-P1	CGCACCGTCACAAAGTGTT	TGGGAAGGAAAGGAAATCAC
PYK1-P2	CCTTTCCTTCCCATATGATGC	ACTTTGAAAGGGGACCATGA
PYK1-O	TGCTTTGAGAAAGGCTGGTT	TCTGATTTCTGGACCCTTGG
PMA1-O	GGTTTTGGTCATTGCCACTT	ACGGCCATAGTGGTGGTAAC
PMA1-P1	AACAAACCCGGTCTCGAAG	GAAGTGCCGCATTAGGAAAT
PMA1-P2	GATGGTGGGTACCGCTTATG	TTGGTGTTATAGGAAAGAAAGAG
GAL1-P	ACGCTTAACTGCTCATTGCT	TGTTCGGAGCAGTGCGGCGC
GAL1-O1	AAAGAAACTTGCACCGGAAA	GGCCCATATTCGCTTTAACA
GAL1-O2	ACATTTCCACACCCTGGAAC	TTCTTCGCGAGAACAATTCA
PMA1-O2	TCTCCAAAGCCCGTTAAATG	CCGTTCATAGCACCGAAGTT

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,STI1-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, STI1-P
Tfg1	ADH1-P, ALD6-P, APA1-P, ATP1-P, GAL1-P, PMA1-P, PRB1-P, PSA1-P,PYK1-P, RPA34-P, SRM1-P, STI1-P
Tfa2	ADH1-P, ALD6-P, APA1-P, ATP1-P, GAL1-P, PMA1-P, PRB1-P, PSA1-P, PYK1-P, RPA34-P, SRM1-P, STI1-P
Toa2	ADH1-P, ALD6-P, APA1-P, ATP1-P, GAL1-P, PMA1-P, PRB1-P, PSA1-P, PYK1-P, RPA34-P, SRM1-P, STI1-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

	Mutant
Protein	***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. Coimunoprecipitation 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.

(**C**) 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 (G_{DB}-Sua7, Sua7-L136P), and Med10, 14, 21 were fused to the Gal4 activation domain (G_{AD}-Med10, 14, 21). A control corresponds to the empty vectors ($G_{DB,}$ G_{AD}). 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 colethal 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 colethal 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 TFIIH, TFIIK, 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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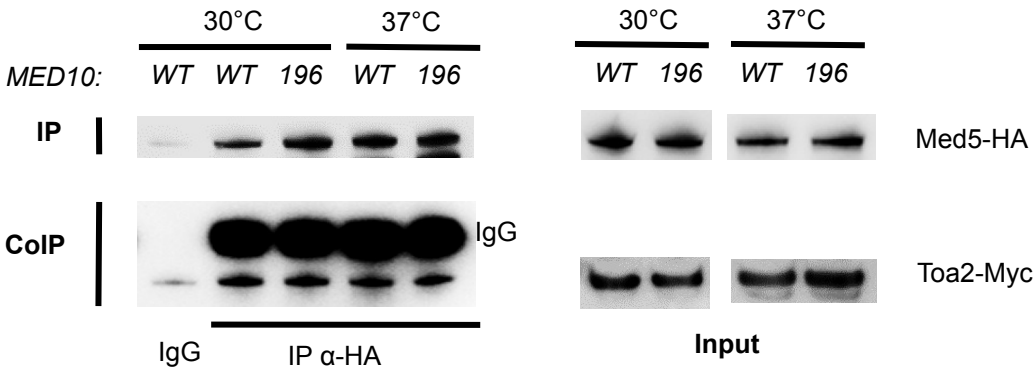
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37°C
30°C
37°C
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WT
WT
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WT
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WT
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Med5-HA
IgG
Toa2-Myc
IgG
IP α-HA
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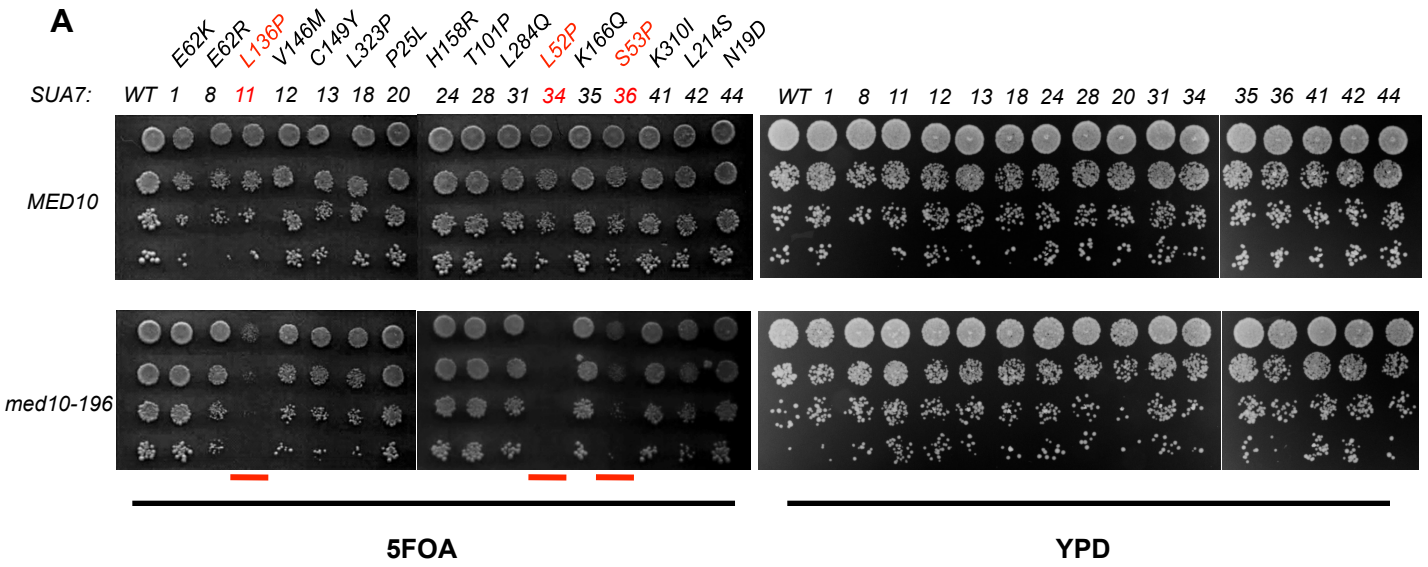
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L136P
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S53P
K310I
L214S
N19D
SUA7:
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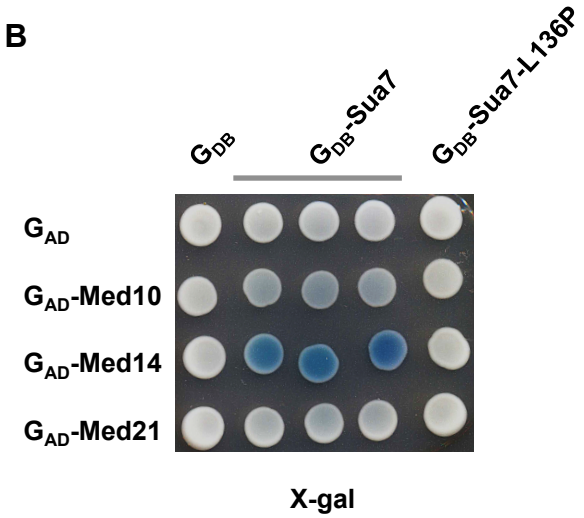
MED10
med10-196
5FOA
YPD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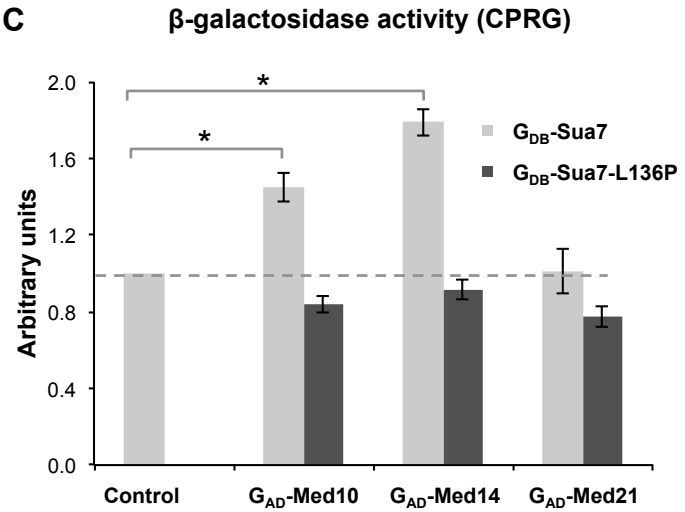
X-gal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Arbitrary units
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1.2
0.8
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*
*
GDB-Sua7
GDB-Sua7-L136P
Control
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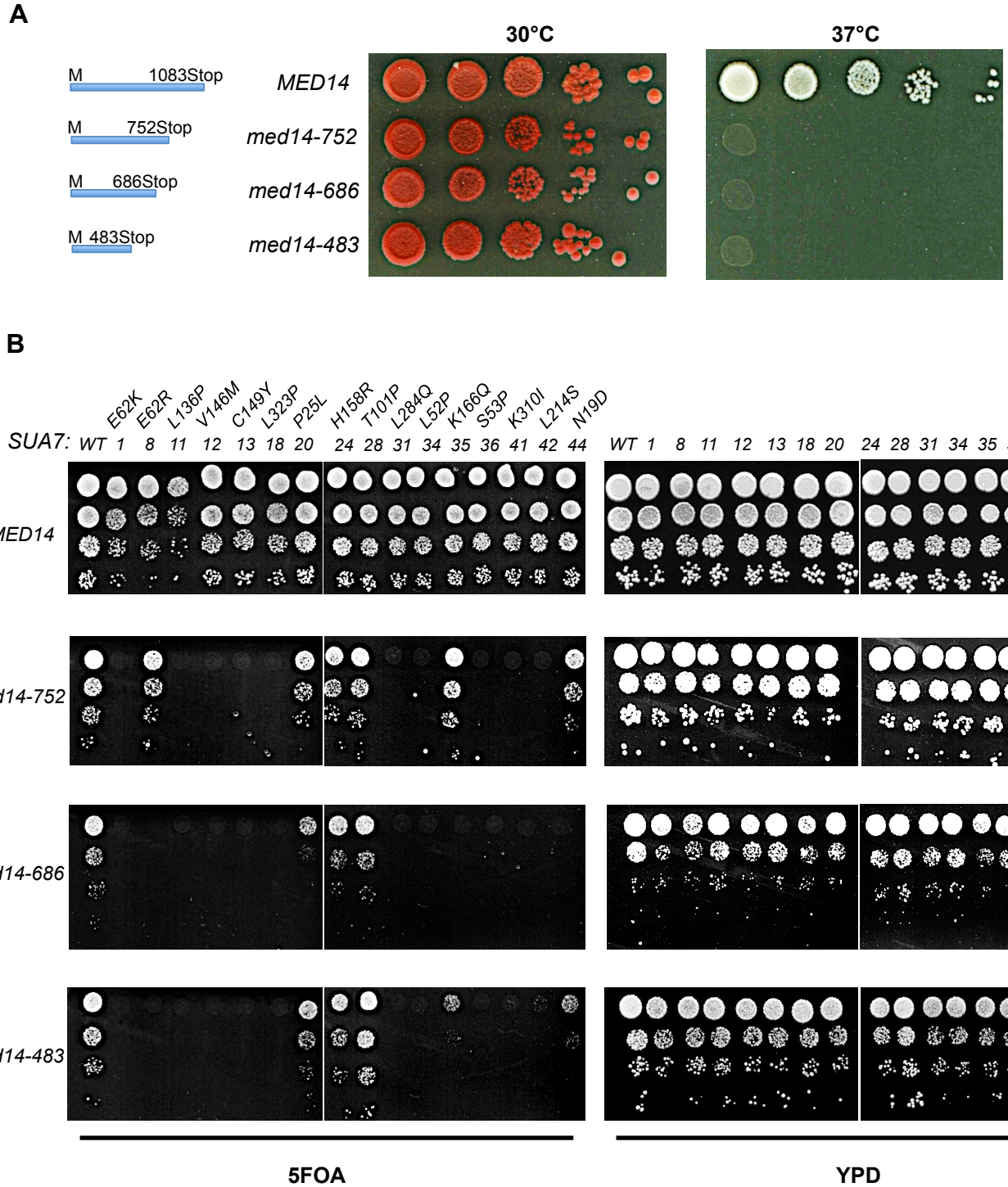
GAD-Med21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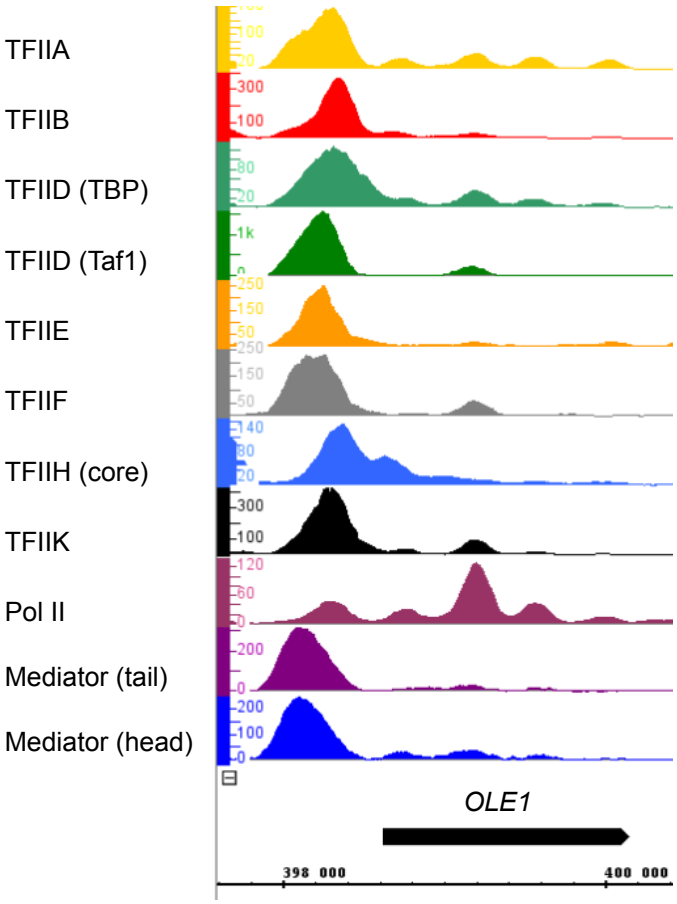
YPD

A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Mediator (head)
OLE1
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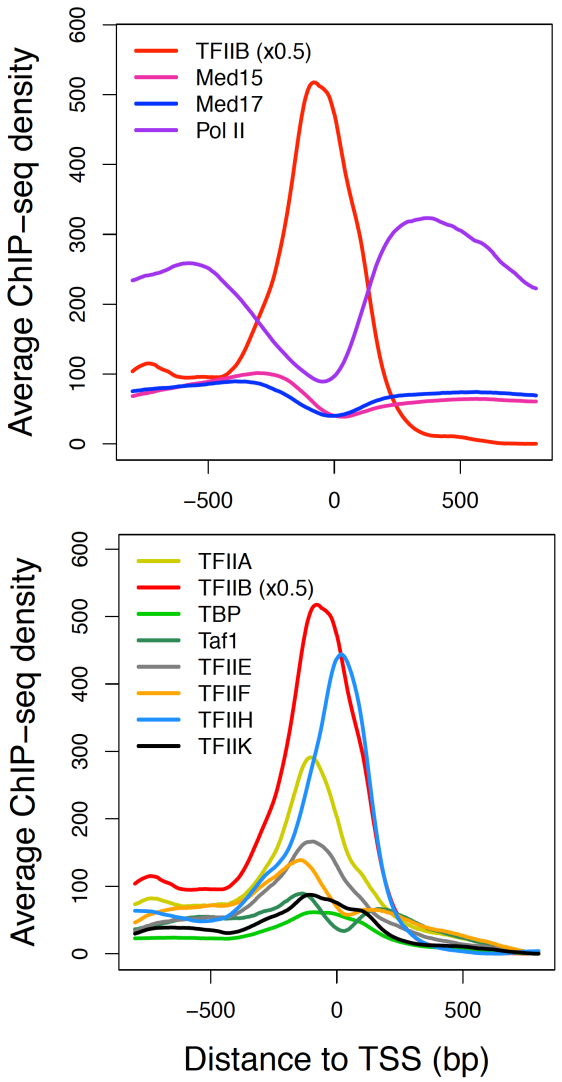
400 000

B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Med17
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TFIIK
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0
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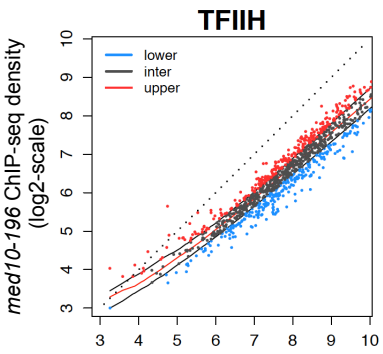
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lower
inter
upper
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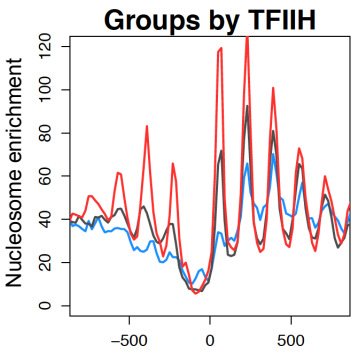
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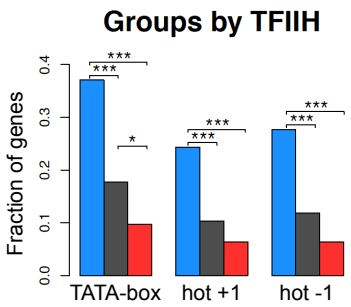
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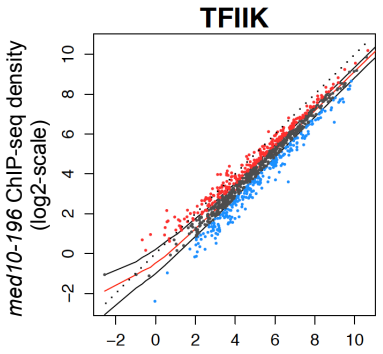
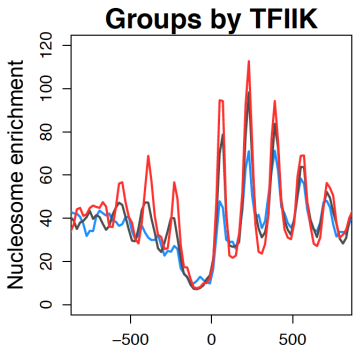
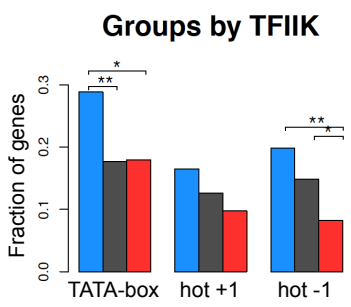
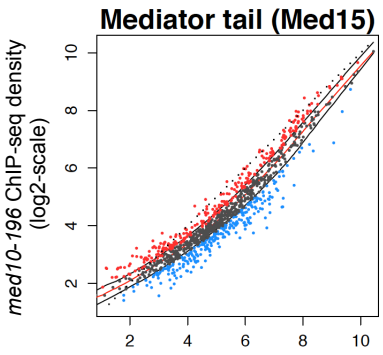
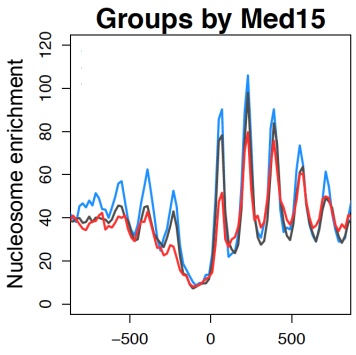
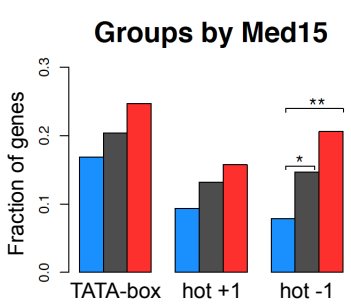

TFIIK
med10-196 ChIP-seq density (log2-scale)

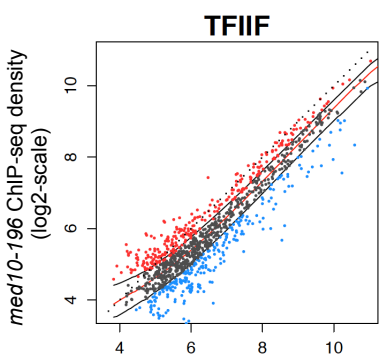

Groups by TFIIK
Nucleosome enrichment
−500
0
500

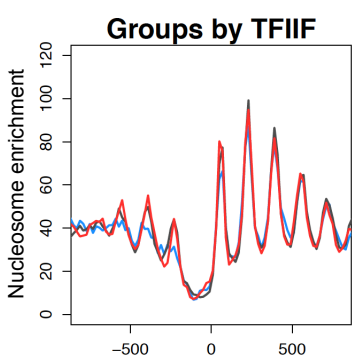

Groups by TFIIK
Fraction of genes
TATA-box
hot +1
hot -1

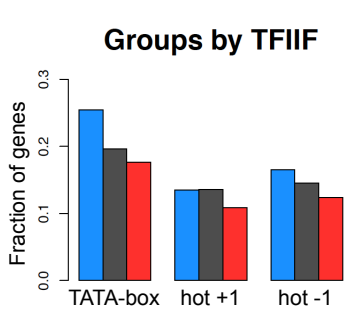

Mediator tail (Med15)
med10-196 ChIP-seq density (log2-scale)


Groups by Med15
Nucleosome enrichment
−500
0
500

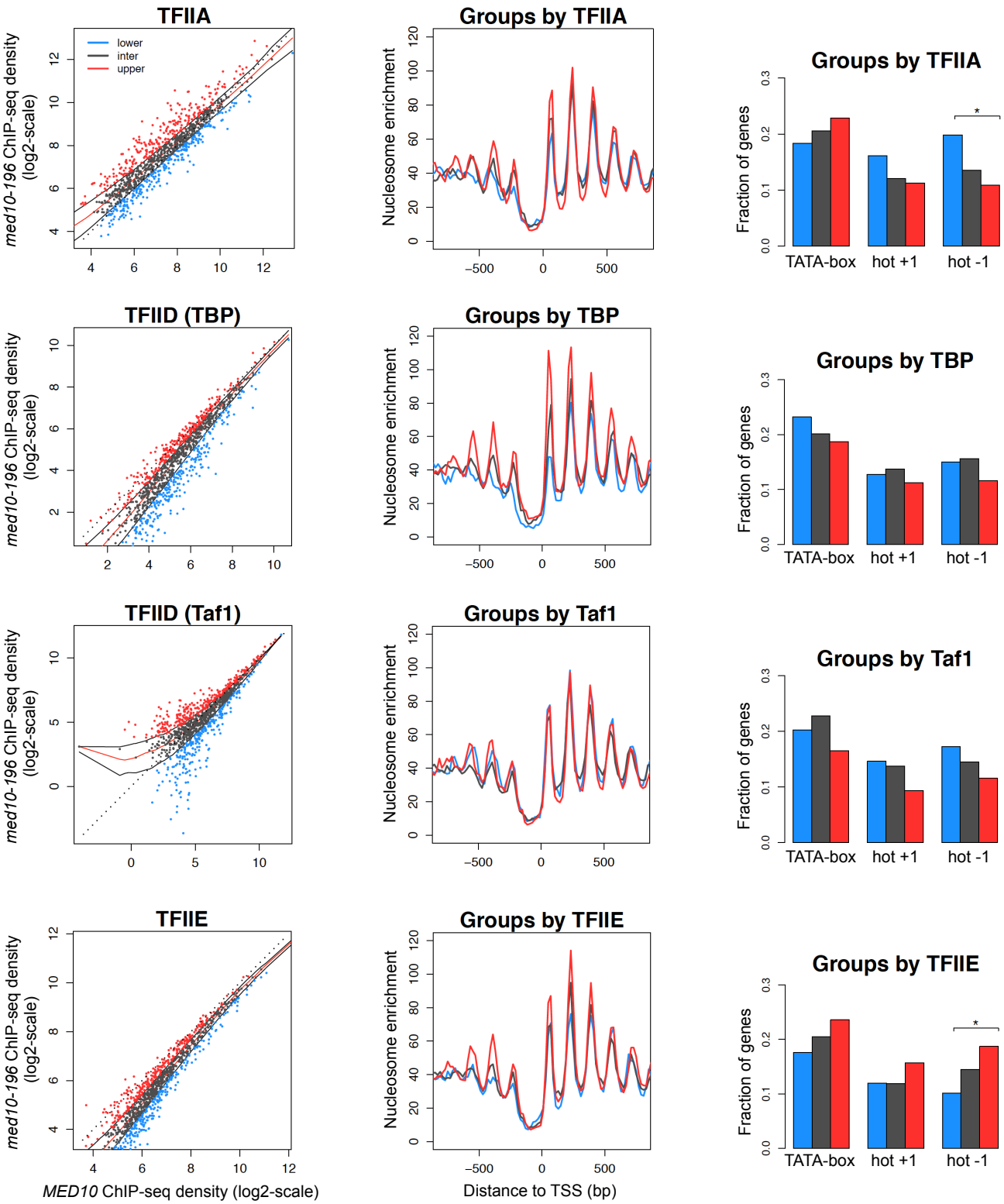

Groups by Med15
Fraction of genes
TATA-box
hot +1
hot -1


TFIIF
med10-196 ChIP-seq density (log2-scale)
MED10 ChIP-seq density (log2-scale)


Groups by TFIIF
Nucleosome enrichment
−500
0
500
Distance to TSS (bp)


Groups by TFIIF
Fraction of genes
TATA-box
hot +1
hot -1

(continued)


TFIIA
lower
inter
upper
med10-196 ChIP-seq density (log2-scale)
Groups by TFIIA
Nucleosome enrichment
Fraction of genes
TATA-box
hot +1
hot -1
*
TFIID (TBP)
Groups by TBP
TFIID (Taf1)
Groups by Taf1
TFIIE
Groups by TFIIE
MED10 ChIP-seq density (log2-scale)
Distance to TSS (bp)