Supplementary material for

Kin28 regulates the transient association of mediator with core promoters

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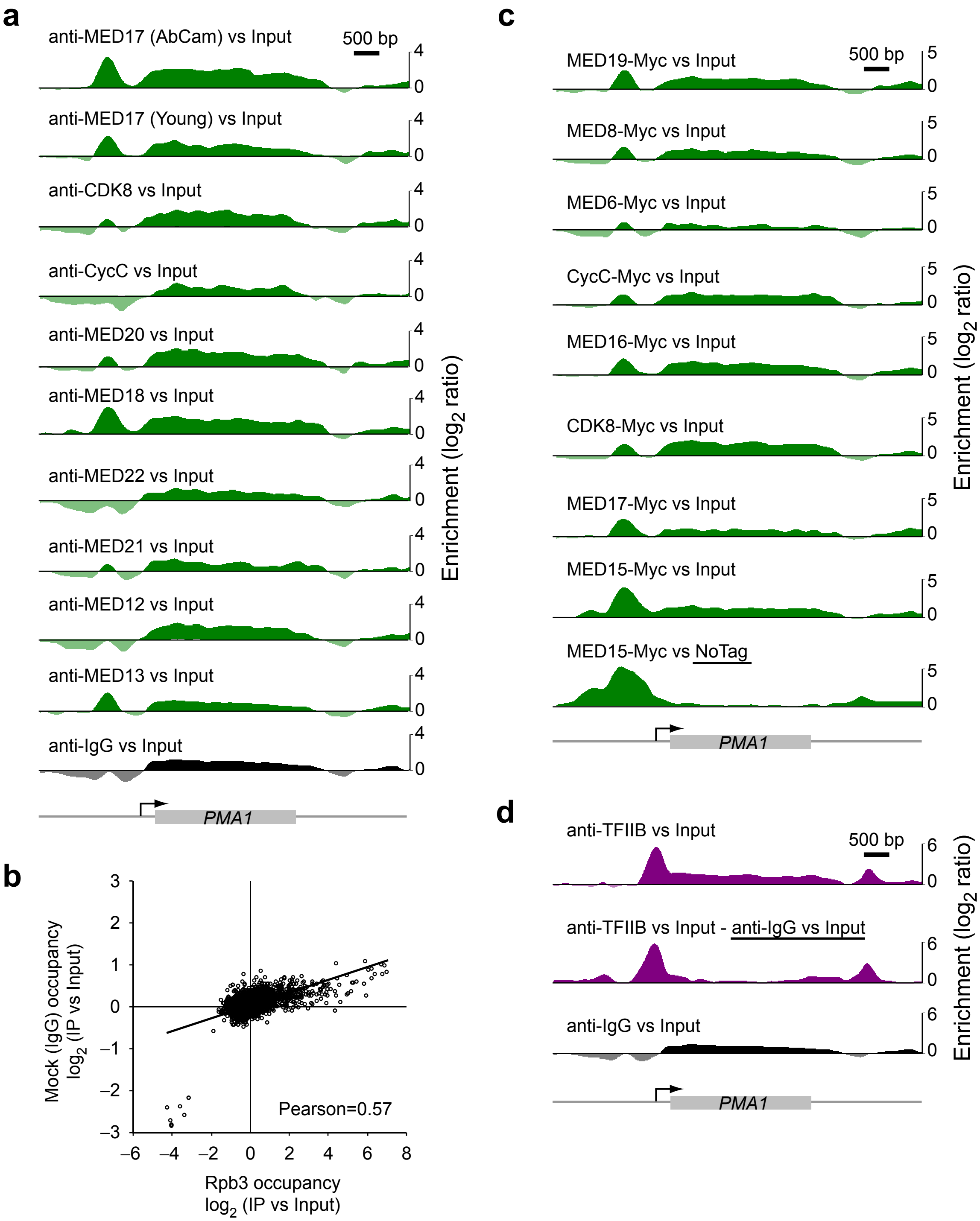
Supplementary Figure 1 Enrichment in highly transcribed coding regions is often observed in ChIP experiments and has to be controlled for.

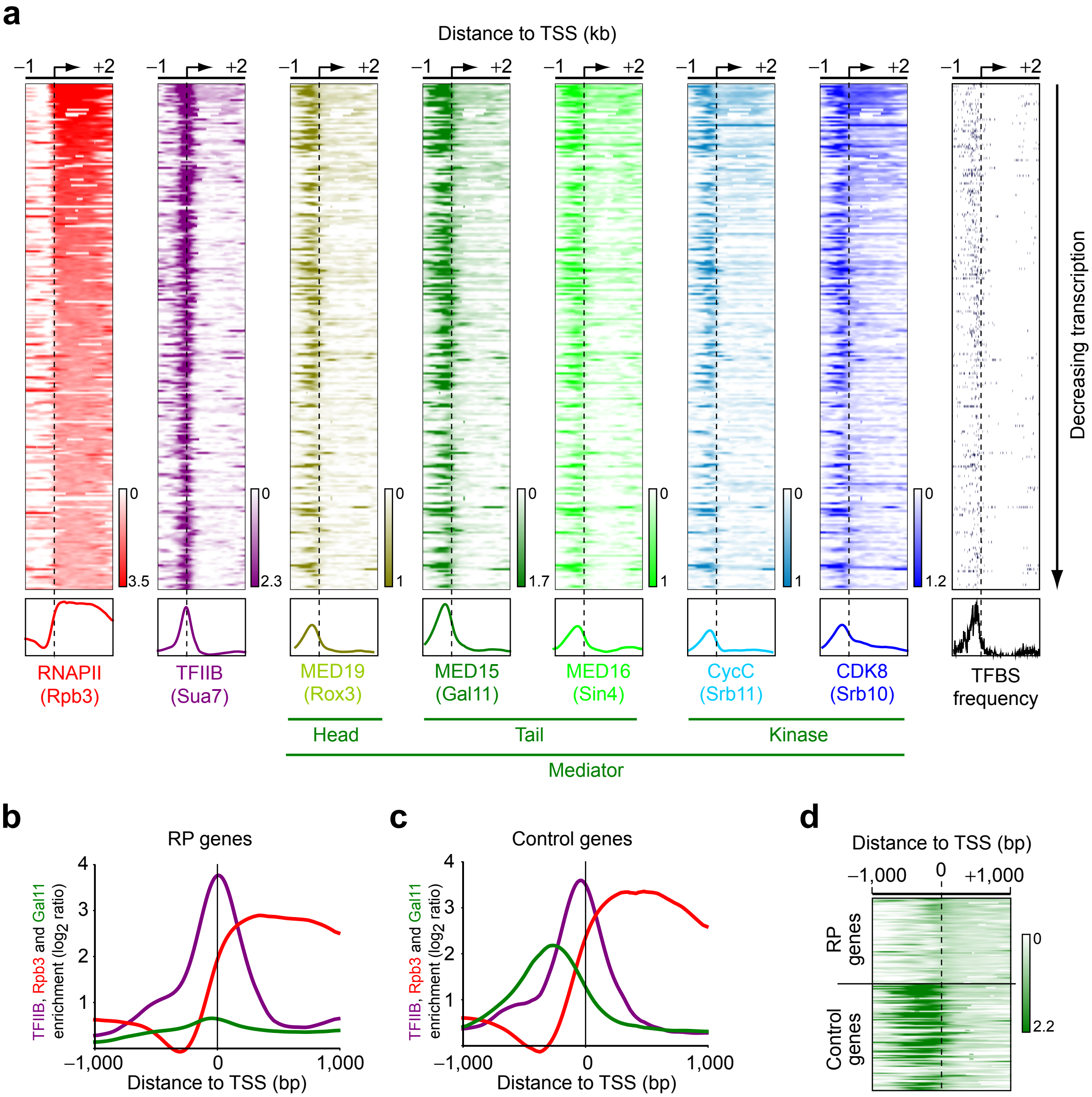
Supplementary Figure 2 A complement to Figure 1.

Supplementary Figure 3 A complement to Figure 3.

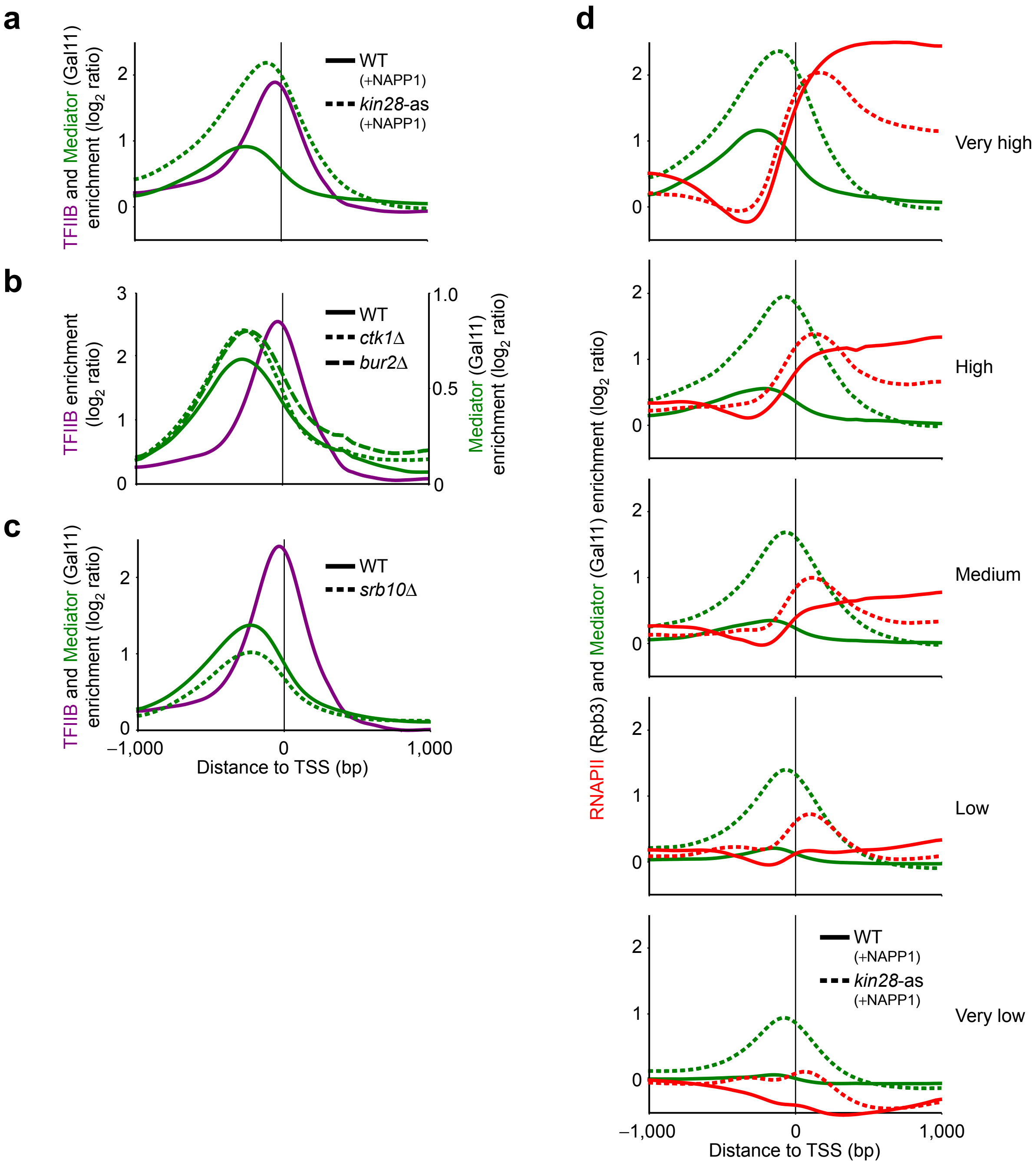
Supplementary Table 1 Yeast strains used in this study.



Supplementary Figure 1 Enrichment in highly transcribed coding regions is often observed in ChIP experiments and has to be controlled for. **(a)** Enrichment of various Mediator subunits at the highly transcribed *PMA1* gene as determined by ChIP-chip using a panel of rabbit polyclonal antibodies (green), as well as using a control IgG antibody (black). All ChIPs were hybridized (and therefore normalized) against input DNA. **(b)** A scatter plot of the enrichment over coding regions in a control IgG ChIP and an Rpb3 ChIP. **(c)** Enrichment of various Mediator subunits as determined by ChIP-chip using epitope (Myc)-tagged Mediator subunits (green). Except for the last trace, all ChIPs were hybridized (and therefore normalized) against input DNA as in **a**. The last profile, however, shows an experiment were the MED15-Myc ChIP sample was hybridized (and therefore normalized) against a control ChIP sample performed using the same anti-Myc antibody but from an isogenic non-tagged strain. **(d)** TFIIB enrichment as determined by ChIP-chip using a rabbit polyclonal anti-TFIIB antibody (purple) as well as using a control IgG antibody (black) as in **a**. The middle panel shows TFIIB enrichment after subtracting the signal from the control (black) experiment. Overall, these experiments show that ChIP experiments tend to enrich highly transcribed coding regions regardless of the antibody used. It also shows that most of this systematic error can be removed either by normalizing against controls ChIPs performed in a non-tagged strain (in the case where a tagged protein is tested) or against a control ChIP performed using IgG (when a polyclonal antibody is used). Because each antibody varies with regards to the amount of noise it generates, we favor, when possible, using the "tagged versus non-tagged" strategy.



Supplementary Figure 2 A complement to Figure 1. **(a)** A heat map representation of the data from Figure 1a. Genes were sorted by decreasing Rpb3 occupancy and the data were aligned on the TSS. **(b)** The average TFIIB (purple), RNAPII (Rpb3, red) and Mediator (Gal11, green) occupancies are shown around the TSS of RP genes. **(c)** Same as in **b** but for a group of control genes chosen based on the fact that they exhibit similar transcription rates as the RP genes from **b**. **(d)** A heat map representation of Mediator (Gal11) occupancy over the same genes as in **b** and **c**.



Supplementary Figure 3 A complement to Figure 3. **(a-c)** Average Mediator (Gal11, green) occupancy in wild type (WT, solid traces) and the indicated CTD kinase mutants (dotted and dashed traces) around the TSS of all genes with an Rpb3 average ORF occupancy >1 in WT cells. TFIIB (purple) from WT cells is shown as a place-holder for core promoters. Data are shown for *kin28*-as mutant treated with NAPP1 (n=266) **(a)**, *ctk1*Δ and *bur2*Δ mutants (n=299) **(b)** and *srb10*Δ mutant (n=295) **(c)**. **(d)** Average Mediator (Gal11, green) and RNAPII (Rpb3, red) occupancy in wild type (WT, solid traces) and *kin28*-as mutant (*kin28*-as, dotted traces) cells, both treated with NAPP1, around the TSS for groups of genes with different RNAPII occupancies. The genes were grouped based on the average Rpb3 enrichment ($\log_2$ ratio) over their ORF (>1.5, Very high n=142; 1-1.5, High n=135; 0.5-1, Medium n=337; 0-0.5, Low n=827; <0, Very low n=1764).

Supplementary Table 1 Yeast strains used in this study.

Strain	Alias	Background	Genotype	Source
yFR116	Z1256	W303	MATa ade2-1 trp1-1 can1-100 leu2-3,112 his3-11,15 ura3	REF[1]
yFR117		S288C	MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0	Open Bio.
yFR119		W303	MATa ade2-1 trp1-1 can1-100 leu2-3,112 his3-11,15 ura3 ROX3-18MYC::klTRP1	This work
yFR121		W303	MATa ade2-1 trp1-1 can1-100 leu2-3,112 his3-11,15 ura3 MED8-9MYC::klTRP1	This work
yFR150		W303	MATa ade2-1 trp1-1 can1-100 leu2-3,112 his3-11,15 ura3 MED6-18MYC::klTRP1	This work
yFR167		W303	MATa ade2-1 trp1-1 can1-100 leu2-3,112 his3-11,15 ura3 SRB11-9MYC::klTRP1	This work
yFR169		W303	MATa ade2-1 trp1-1 can1-100 leu2-3,112 his3-11,15 ura3 SIN4-18MYC::klTRP1	This work
yFR178		W303	MATa ade2-1 trp1-1 can1-100 leu2-3,112 his3-11,15 ura3 SRB10-9MYC::klTRP1	This work
yFR399	KLY091	W303	MATa ade2-1 trp1-1 can1-100 leu2-3,112 his3-11,15 ura3 GAL11-9MYC::TRP1	REF[2]
yFR401	KLY097	W303	MATa ade2-1 trp1-1 can1-100 leu2-3,112 his3-11,15 ura3 SRB4-9MYC::TRP1	REF[2]
yFR551		S288C	MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 ctk1Δ::kanMX4	Open Bio.
yFR682		S288C	MATa his3Δ1 leu2Δ0 met15Δ0 ura3D0 bur2Δ::kanMX4	Open Bio.
yFR762	SHY278	S288C	MATα ade2::hisG his3Δ200 leu2Δ0 lys2Δ0 met15Δ0 trp1Δ63 ura3Δ0	REF[3]
yFR763	SHY508A	S288C	MATα ade2::hisG his3Δ200 leu2Δ0 lys2Δ0 met15Δ0 trp1Δ63 ura3Δ0 kin28::kin28-as (L83G) [pSH579, CEN URA3 kin28-as (L83G)]	REF[3]
yFR999		W303	MATa ade2-1 trp1-1 can1-100 leu2-3,112 his3-11,15 ura3 srb10Δ::URA3	REF[4]
yFR1321	HHY168	W303	MATα ade2-1 trp1-1 can1-100 leu2-3,112 his3-11,15 ura3 tor1-1 fpr1::NAT RPL13A-2xFKBP12::TRP1	Euroscarf
yFR1324	HHY170	W303	MATα ade2-1 trp1-1 can1-100 leu2-3,112 his3-11,15 ura3 tor1-1 fpr1::NAT RPL13A-2xFKBP12::TRP1 RPB1-FRB::kanMX6	Euroscarf
yFR1325	HHY178	W303	MATα ade2-1 trp1-1 can1-100 leu2-3,112 his3-11,15 ura3 tor1-1 fpr1::NAT RPL13A-2xFKBP12::TRP1 SRB5-FRB::kanMX6	Euroscarf
yFR1499		W303	MATα ade2-1 trp1-1 can1-100 leu2-3,112 his3-11,15 ura3 tor1-1 fpr1::NAT RPL13A-2xFKBP12::TRP1 RPB1-FRB::kanMX6 [pFR482, CEN HIS3 RPB1-CTDwt-3FLAG]	This work
yFR1501		W303	MATα ade2-1 trp1-1 can1-100 leu2-3,112 his3-11,15 ura3 tor1-1 fpr1::NAT RPL13A-2xFKBP12::TRP1 RPB1-FRB::kanMX6 [pFR486, CEN HIS3 RPB1-CTDS2A-3FLAG]	This work
yFR1503		W303	MATα ade2-1 trp1-1 can1-100 leu2-3,112 his3-11,15 ura3 tor1-1 fpr1::NAT RPL13A-2xFKBP12::TRP1 RPB1-FRB::kanMX6 [pFR490, CEN HIS3 RPB1-CTDS5A-3FLAG]	This work
yFR1526		W303	MATα ade2-1 trp1-1 can1-100 leu2-3,112 his3-11,15 ura3 tor1-1 fpr1::NAT RPL13A-2xFKBP12::TRP1 RPB1-FRB::kanMX6 [pFR494, CEN HIS3 RPB1-CTDS7A-3FLAG]	This work
yFR1544		W303	MATα ade2-1 trp1-1 can1-100 leu2-3,112 his3-11,15 ura3 tor1-1 fpr1::NAT RPL13A-2xFKBP12::TRP1 SRB4-FRB::kanMX6	This work
yFR1636		S288C	MATα ade2::hisG his3Δ200 leu2Δ0 lys2Δ0 met15Δ0 trp1Δ63 ura3Δ0 GAL11-9MYC::klTRP1	This work
yFR1638		S288C	MATα ade2::hisG his3Δ200 leu2Δ0 lys2Δ0 met15Δ0 trp1Δ63 ura3Δ0 kin28::kin28-as (L83G) GAL11-9MYC::klTRP1 [pSH579, CEN URA3 kin28-as (L83G)]	This work
yFR1640		W303	MATa ade2-1 trp1-1 can1-100 leu2-3,112 his3-11,15 ura3 srb10Δ::URA3 GAL11-9MYC::klTRP1	This work
yFR1642		W303	MATα ade2-1 trp1-1 can1-100 leu2-3,112 his3-11,15 ura3 tor1-1 fpr1::NAT RPL13A-2xFKBP12::TRP1 RPB1-FRB::kanMX6 GAL11-18MYC::klURA3	This work
yFR1645		W303	MATα ade2-1 trp1-1 can1-100 leu2-3,112 his3-11,15 ura3 tor1-1 fpr1::NAT RPL13A-2xFKBP12::TRP1 RPB1-FRB::kanMX6 GAL11-18MYC::klURA3 [pFR482, CEN HIS3 RPB1-CTDwt-3FLAG]	This work
yFR1649		W303	MATα ade2-1 trp1-1 can1-100 leu2-3,112 his3-11,15 ura3 tor1-1 fpr1::NAT RPL13A-2xFKBP12::TRP1 RPB1-FRB::kanMX6 GAL11-18MYC::klURA3 [pFR490, CEN HIS3 RPB1-CTDS5A-3FLAG]	This work
yFR1651		W303	MATα ade2-1 trp1-1 can1-100 leu2-3,112 his3-11,15 ura3 tor1-1 fpr1::NAT RPL13A-2xFKBP12::TRP1 RPB1-FRB::kanMX6 GAL11-18MYC::klURA3 [pFR494, CEN HIS3 RPB1-CTDS7A-3FLAG]	This work
yFR1658		S288C	MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 GAL11-18MYC::klURA3	This work
yFR1660		S288C	MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 ctk1Δ::kanMX4 GAL11-18MYC::klURA3	This work
yFR1662		S288C	MATa his3Δ1 leu2Δ0 met15Δ0 ura3D0 bur2Δ::kanMX4 GAL11-18MYC::klURA3	This work

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