

Supplementary Data

Mediator independently orchestrates multiple steps of preinitiation complex assembly *in vivo*

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Supplementary Materials and Methods

Plasmid constructions and cloning

Plasmids are listed in supplementary Table S2. All cloning experiments were done using the Gateway Invitrogen cloning method. Wild-type *MED17* gene was 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. 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) (1), pAG414-GPD-EGFP or pAG415-GPD vector (2) by the LR reaction.

Point mutations and M504P mutation in Med17 equivalent to human L371P mutation were obtained using QuikChange Lightning site-directed mutagenesis kit (Agilent), cloned using the Gateway standard method and transferred into pVV204.

Yeast strains

Strains are listed in supplementary Table S1. *MED17* was deleted in YPH499 (*MATa ura3-52 his3-Δ200 ade2-101uaa trp1-Δ63 lys2-80luag leu2-Δ1*) complemented with pVV208-*MED17* and replaced by a KanMX6 marker using the standard one-step method (3). *med17* conditional mutants were obtained as previously described (4).

Med5-HA, Med6-HA, Med15-HA, Rad3-HA and Kin28-HA strains carrying C-terminal HA-tagged version of Med5, Med6 and Med15 Mediator subunits, Rad3 and Kin28 TFIIH subunits 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.

Med17-EGFP Rpb3-Myc double-tagged strains were derived from Y4945 (*med17Δ/pVV208-MED17*) strain that was first tagged for Rpb3-Myc by insertion of 13Myc epitopes followed by a HISMX marker using the standard one-step method, then transformed by pAG414-GPD-EGFP-MED17 plasmid. Finally, *MED17 URA3* plasmid was chased on 5-FOA medium.

Med17 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::KITRPI MED8-PreSci-3HA::KanR*) carrying C-terminal HA-tagged version of Med8 and C-terminal 10xHis-TEV-ProteinA-tagged version of Med22 (5). *MED17* was deleted in CA001 complemented with pVV208-*MED17* and replaced by a natNT2 marker from pFA6a-natNT2 (6) using the standard one-step method, then transformed by pAG415-GPD-MED17 or *med17*-mutant plasmids. Finally, *MED17 URA3* plasmid was chased on 5-FOA medium.

Unless otherwise stated, yeast strains were grown at 30°C in YPD rich medium containing 2% of glucose. For galactose induction, yeast cells were grown in YP Raffinose rich medium containing 2% of raffinose. Galactose was then added to reach 2% final concentration.

Table S1. Yeast strains

Name	Tagged or Mutant protein	Genotype	Reference
YPH499	WT	<i>MATa ura3-52 his3-Δ200 ade2-101uaa trp1-Δ63 lys2-80luag leu2-Δ1</i>	(7)
Y4945	<i>MED17</i>	<i>MATa ura3-52 his3-Δ200 ade2-101uaa trp1-Δ63 lys2-80luag leu2-Δ1 med17::KanMX6 // * MED17 CEN URA3</i>	(4)
Y5084	<i>MED17</i>	<i>MATa ura3-52 his3-Δ200 ade2-101uaa trp1-Δ63 lys2-80luag leu2-Δ1 med17::KanMX6 // MED17 CEN TRP1</i>	(4)
Y 5304	<i>med17-8</i>	<i>MATa ura3-52 his3-Δ200 ade2-101uaa trp1-Δ63 lys2-80luag leu2-Δ1 med17::KanMX6 // med17-8 CEN TRP1</i>	This work
Y 5305	<i>med17-68</i>	<i>MATa ura3-52 his3-Δ200 ade2-101uaa trp1-Δ63 lys2-80luag leu2-Δ1 med17::KanMX6 // med17-68 CEN TRP1</i>	This work
Y 5306	<i>med17-95</i>	<i>MATa ura3-52 his3-Δ200 ade2-101uaa trp1-Δ63 lys2-80luag leu2-Δ1 med17::KanMX6 // med17-95 CEN TRP1</i>	This work
Y 5307	<i>med17-96</i>	<i>MATa ura3-52 his3-Δ200 ade2-101uaa trp1-Δ63 lys2-80luag leu2-Δ1 med17::KanMX6 // med17-96 CEN TRP1</i>	This work
Y5308	<i>med17-98</i>	<i>MATa ura3-52 his3-Δ200 ade2-101uaa trp1-Δ63 lys2-80luag leu2-Δ1 med17::KanMX6 // med17-98 CEN TRP1</i>	This work
Y5309	<i>med17-140</i>	<i>MATa ura3-52 his3-Δ200 ade2-101uaa trp1-Δ63 lys2-80luag leu2-Δ1 med17::KanMX6 // med17-140 CEN TRP1</i>	(8)
Y5310	<i>med17-157</i>	<i>MATa ura3-52 his3-Δ200 ade2-101uaa trp1-Δ63 lys2-80luag leu2-Δ1 med17::KanMX6 // med17-157 CEN TRP1</i>	This work
Y5311	<i>med17-158</i>	<i>MATa ura3-52 his3-Δ200 ade2-101uaa trp1-Δ63 lys2-80luag leu2-Δ1 med17::KanMX6 // med17-158 CEN TRP1</i>	This work
Y5312	<i>med17-187</i>	<i>MATa ura3-52 his3-Δ200 ade2-101uaa trp1-Δ63 lys2-80luag leu2-Δ1 med17::KanMX6 // med17-187 CEN TRP1</i>	This work
Y5313	<i>med17-208</i>	<i>MATa ura3-52 his3-Δ200 ade2-101uaa trp1-Δ63 lys2-80luag leu2-Δ1 med17::KanMX6 // med17-208 CEN TRP1</i>	This work
Y5314	<i>med17-218</i>	<i>MATa ura3-52 his3-Δ200 ade2-101uaa trp1-Δ63 lys2-80luag leu2-Δ1 med17::KanMX6 // med17-218 CEN TRP1</i>	This work
Y5315	<i>med17-233</i>	<i>MATa ura3-52 his3-Δ200 ade2-101uaa trp1-Δ63 lys2-80luag leu2-Δ1 med17::KanMX6 // med17-233 CEN TRP1</i>	(8)
Y5316	<i>med17-239</i>	<i>MATa ura3-52 his3-Δ200 ade2-101uaa trp1-Δ63 lys2-80luag leu2-Δ1 med17::KanMX6 // med17-239 CEN TRP1</i>	This work
Y5317	<i>med17-241</i>	<i>MATa ura3-52 his3-Δ200 ade2-101uaa trp1-Δ63 lys2-80luag leu2-Δ1 med17::KanMX6 // med17-241 CEN TRP1</i>	This work
Y5318	<i>med17-248</i>	<i>MATa ura3-52 his3-Δ200 ade2-101uaa trp1-Δ63 lys2-80luag leu2-Δ1 med17::KanMX6 // med17-248 CEN TRP1</i>	This work
Y5319	<i>med17-247</i>	<i>MATa ura3-52 his3-Δ200 ade2-101uaa trp1-Δ63 lys2-80luag leu2-Δ1 med17::KanMX6 // med17-247 CEN TRP1</i>	This work
Y5320	<i>med17-249</i>	<i>MATa ura3-52 his3-Δ200 ade2-101uaa trp1-Δ63 lys2-80luag leu2-Δ1 med17::KanMX6 // med17-249 CEN TRP1</i>	This work
Y5321	<i>med17-257</i>	<i>MATa ura3-52 his3-Δ200 ade2-101uaa trp1-Δ63 lys2-80luag leu2-Δ1 med17::KanMX6 // med17-257 CEN TRP1</i>	(8)
Y5322	<i>med17-299</i>	<i>MATa ura3-52 his3-Δ200 ade2-101uaa trp1-Δ63 lys2-80luag leu2-Δ1 med17::KanMX6 // med17-299 CEN TRP1</i>	This work
Y5323	<i>med17-326</i>	<i>MATa ura3-52 his3-Δ200 ade2-101uaa trp1-Δ63 lys2-80luag leu2-Δ1 med17::KanMX6 // med17-326 CEN TRP1</i>	This work
Y5327	<i>med17-327</i>	<i>MATa ura3-52 his3-Δ200 ade2-101uaa trp1-Δ63 lys2-80luag leu2-Δ1 med17::KanMX6 // med17-327 CEN TRP1</i>	This work
Y5328	<i>med17-348</i>	<i>MATa ura3-52 his3-Δ200 ade2-101uaa trp1-Δ63 lys2-80luag leu2-Δ1 med17::KanMX6 // med17-348 CEN TRP1</i>	This work
Y5324	<i>med17-370</i>	<i>MATa ura3-52 his3-Δ200 ade2-101uaa trp1-Δ63 lys2-80luag leu2-Δ1 med17::KanMX6 // med17-370 CEN TRP1</i>	This work
Y5329	<i>med17-396</i>	<i>MATa ura3-52 his3-Δ200 ade2-101uaa trp1-Δ63 lys2-80luag leu2-Δ1 med17::KanMX6 // med17-396 CEN TRP1</i>	This work
Y5331	<i>med17-404</i>	<i>MATa ura3-52 his3-Δ200 ade2-101uaa trp1-Δ63 lys2-80luag leu2-</i>	This work

		<i>Δ1 med17::KanMX6 // med17-404 CEN TRP1</i>	
Y5325	<i>med17-444</i>	<i>MATa ura3-52 his3-Δ200 ade2-101uaa trp1-Δ63 lys2-80luag leu2-Δ1 med17::KanMX6 // med17-444 CEN TRP1</i>	This work
Y6106	<i>med17-504</i>	<i>MATa ura3-52 his3-Δ200 ade2-101uaa trp1-Δ63 lys2-80luag leu2-Δ1 med17::KanMX6 // med17-504 CEN TRP1</i>	This work
Y5326	<i>med17-510</i>	<i>MATa ura3-52 his3-Δ200 ade2-101uaa trp1-Δ63 lys2-80luag leu2-Δ1 med17::KanMX6 // med17-510 CEN TRP1</i>	This work
Y5327	<i>med17-546</i>	<i>MATa ura3-52 his3-Δ200 ade2-101uaa trp1-Δ63 lys2-80luag leu2-Δ1 med17::KanMX6 // med17-546 CEN TRP1</i>	(8)
Y5328	<i>med17-670</i>	<i>MATa ura3-52 his3-Δ200 ade2-101uaa trp1-Δ63 lys2-80luag leu2-Δ1 med17::KanMX6 // med17-670 CEN TRP1</i>	This work
Y5485	Med5-HA	<i>MATa ura3-52 his3-Δ200 ade2-101uaa trp1-Δ63 lys2-80luag leu2-Δ1 med17::KanMX6 // MED17 CEN TRP1 MED5::3HA::HIS3</i>	This work
Y5773	Med6-HA	<i>MATa ura3-52 his3-Δ200 ade2-101uaa trp1-Δ63 lys2-80luag leu2-Δ1 med17::KanMX6 // MED17 CEN TRP1 MED6::3HA::HIS3</i>	This work
Y5540	Med15-HA	<i>MATa ura3-52 his3-Δ200 ade2-101uaa trp1-Δ63 lys2-80luag leu2-Δ1 med17::KanMX6 // MED17 CEN TRP1 MED15::3HA::HIS3</i>	(8)
Y5551	HA-TBP	<i>MATa ura3-52 his3-Δ200 ade2-101uaa trp1-Δ63 lys2-80luag leu2-Δ1 LEU2::3HA::SPT15 med17::KanMX6 // MED17 CEN TRP1</i>	This work
Y5557	Rad3-HA	<i>MATa ura3-52 his3-Δ200 ade2-101uaa trp1-Δ63 lys2-80luag leu2-Δ1 RAD3::3HA::HIS3 med17::KanMX6 // MED17 CEN TRP1</i>	This work
Y5545	Kin28-HA	<i>MATa ura3-52 his3-Δ200 ade2-101uaa trp1-Δ63 lys2-80luag leu2-Δ1 KIN28::3HA::HIS3 med17::KanMX6 // MED17 CEN TRP1</i>	This work
Y5637	Med15-HA	<i>MATa ura3-52 his3-Δ200 ade2-101uaa trp1-Δ63 lys2-80luag leu2-Δ1 MED15::3HA::HIS3 med17::KanMX6 // med17-98 CEN TRP1</i>	This work
Y5643	Med15-HA	<i>MATa ura3-52 his3-Δ200 ade2-101uaa trp1-Δ63 lys2-80luag leu2-Δ1 MED15::3HA::HIS3 med17::KanMX6 // med17-257 CEN TRP1</i>	This work
Y5538	Med15-HA	<i>MATa ura3-52 his3-Δ200 ade2-101uaa trp1-Δ63 lys2-80luag leu2-Δ1 MED15::3HA::HIS3 med17::KanMX6 // med17-326 CEN TRP1</i>	This work
Y5639	Med15-HA	<i>MATa ura3-52 his3-Δ200 ade2-101uaa trp1-Δ63 lys2-80luag leu2-Δ1 MED15::3HA::HIS3 med17::KanMX6 // med17-444 CEN TRP1</i>	This work
Y5640	Med15-HA	<i>MATa ura3-52 his3-Δ200 ade2-101uaa trp1-Δ63 lys2-80luag leu2-Δ1 MED15::3HA::HIS3 med17::KanMX6 // med17-546 CEN TRP1</i>	This work
Y5641	Med15-HA	<i>MATa ura3-52 his3-Δ200 ade2-101uaa trp1-Δ63 lys2-80luag leu2-Δ1 MED15::3HA::HIS3 med17::KanMX6 // med17-670 CEN TRP1</i>	This work
Y6063	Med15-HA	<i>MATa ura3-52 his3-Δ200 ade2-101uaa trp1-Δ63 lys2-80luag leu2-Δ1 MED15::3HA::HIS3 med17::KanMX6 // med17-504 CEN TRP1</i>	This work
Y5491	Med5-HA	<i>MATa ura3-52 his3-Δ200 ade2-101uaa trp1-Δ63 lys2-80luag leu2-Δ1 MED5::3HA::HIS3 med17::KanMX6 // med17-98 CEN TRP1</i>	This work
Y5489	Med5-HA	<i>MATa ura3-52 his3-Δ200 ade2-101uaa trp1-Δ63 lys2-80luag leu2-Δ1 MED5::3HA::HIS3 med17::KanMX6 // med17-258 CEN TRP1</i>	This work
Y5520	Med5-HA	<i>MATa ura3-52 his3-Δ200 ade2-101uaa trp1-Δ63 lys2-80luag leu2-Δ1 MED5::3HA::HIS3 med17::KanMX6 // med17-326 CEN TRP1</i>	This work
Y5494	Med5-HA	<i>MATa ura3-52 his3-Δ200 ade2-101uaa trp1-Δ63 lys2-80luag leu2-Δ1 MED5::3HA::HIS3 med17::KanMX6 // med17-444 CEN TRP1</i>	This work
Y5496	Med5-HA	<i>MATa ura3-52 his3-Δ200 ade2-101uaa trp1-Δ63 lys2-80luag leu2-Δ1 MED5::3HA::HIS3 med17::KanMX6 // med17-546 CEN TRP1</i>	This work
Y5521	Med5-HA	<i>MATa ura3-52 his3-Δ200 ade2-101uaa trp1-Δ63 lys2-80luag leu2-Δ1 MED5::3HA::HIS3 med17::KanMX6 // med17-670 CEN TRP1</i>	This work
Y6060	Med5-HA	<i>MATa ura3-52 his3-Δ200 ade2-101uaa trp1-Δ63 lys2-80luag leu2-Δ1 MED5::3HA::HIS3 med17::KanMX6 // med17-504 CEN TRP1</i>	This work
Y5774	Med6-HA	<i>MATa ura3-52 his3-Δ200 ade2-101uaa trp1-Δ63 lys2-80luag leu2-Δ1 MED6::3HA::HIS3 med17::KanMX6 // med17-98 CEN TRP1</i>	This work

Y5779	Med6-HA <i>med17-257</i>	<i>MATa ura3-52 his3-Δ200 ade2-101uaa trp1-Δ63 lys2-80luag leu2-Δ1 MED6::3HA::HIS3 med17::KanMX6 // med17-257 CEN TRP1</i>	This work
Y5775	Med6-HA <i>med17-326</i>	<i>MATa ura3-52 his3-Δ200 ade2-101uaa trp1-Δ63 lys2-80luag leu2-Δ1 MED6::3HA::HIS3 med17::KanMX6 // med17-326 CEN TRP1</i>	This work
Y5776	Med6-HA <i>med17-444</i>	<i>MATa ura3-52 his3-Δ200 ade2-101uaa trp1-Δ63 lys2-80luag leu2-Δ1 MED6::3HA::HIS3 med17::KanMX6 // med17-444 CEN TRP1</i>	This work
Y5777	Med6-HA <i>med17-546</i>	<i>MATa ura3-52 his3-Δ200 ade2-101uaa trp1-Δ63 lys2-80luag leu2-Δ1 MED6::3HA::HIS3 med17::KanMX6 // med17-546 CEN TRP1</i>	This work
Y7778	Med6-HA <i>med17-670</i>	<i>MATa ura3-52 his3-Δ200 ade2-101uaa trp1-Δ63 lys2-80luag leu2-Δ1 MED6::3HA::HIS3 med17::KanMX6 // med17-670 CEN TRP1</i>	This work
Y6061	Med6-HA <i>med17-504</i>	<i>MATa ura3-52 his3-Δ200 ade2-101uaa trp1-Δ63 lys2-80luag leu2-Δ1 MED6::3HA::HIS3 med17::KanMX6 // med17-504 CEN TRP1</i>	This work
Y5558	Rad3-HA <i>med17-98</i>	<i>MATa ura3-52 his3-Δ200 ade2-101uaa trp1-Δ63 lys2-80luag leu2-Δ1 RAD3::3HA::HIS3 med17::KanMX6 // med17-98 CEN TRP1</i>	This work
Y5602	Rad3-HA <i>med17-257</i>	<i>MATa ura3-52 his3-Δ200 ade2-101uaa trp1-Δ63 lys2-80luag leu2-Δ1 RAD3::3HA::HIS3 med17::KanMX6 // med17-257 CEN TRP1</i>	This work
Y5559	Rad3-HA <i>med17-326</i>	<i>MATa ura3-52 his3-Δ200 ade2-101uaa trp1-Δ63 lys2-80luag leu2-Δ1 RAD3::3HA::HIS3 med17::KanMX6 // med17-326 CEN TRP1</i>	This work
Y5560	Rad3-HA <i>med17-444</i>	<i>MATa ura3-52 his3-Δ200 ade2-101uaa trp1-Δ63 lys2-80luag leu2-Δ1 RAD3::3HA::HIS3 med17::KanMX6 // med17-444 CEN TRP1</i>	This work
Y5561	Rad3-HA <i>med17-546</i>	<i>MATa ura3-52 his3-Δ200 ade2-101uaa trp1-Δ63 lys2-80luag leu2-Δ1 RAD3::3HA::HIS3 med17::KanMX6 // med17-546 CEN TRP1</i>	This work
Y5562	Rad3-HA <i>med17-670</i>	<i>MATa ura3-52 his3-Δ200 ade2-101uaa trp1-Δ63 lys2-80luag leu2-Δ1 RAD3::3HA::HIS3 med17::KanMX6 // med17-670 CEN TRP1</i>	This work
Y6025	Rad3-HA <i>med17-504</i>	<i>MATa ura3-52 his3-Δ200 ade2-101uaa trp1-Δ63 lys2-80luag leu2-Δ1 RAD3::3HA::HIS3 med17::KanMX6 // med17-504 CEN TRP1</i>	This work
Y5546	Kin28-HA <i>med17-98</i>	<i>MATa ura3-52 his3-Δ200 ade2-101uaa trp1-Δ63 lys2-80luag leu2-Δ1 KIN28::3HA::HIS3 med17::KanMX6 // med17-98 CEN TRP1</i>	This work
Y5600	Kin28-HA <i>med17-257</i>	<i>MATa ura3-52 his3-Δ200 ade2-101uaa trp1-Δ63 lys2-80luag leu2-Δ1 KIN28::3HA::HIS3 med17::KanMX6 // med17-257 CEN TRP1</i>	This work
Y5547	Kin28-HA <i>med17-326</i>	<i>MATa ura3-52 his3-Δ200 ade2-101uaa trp1-Δ63 lys2-80luag leu2-Δ1 KIN28::3HA::HIS3 med17::KanMX6 // med17-326 CEN TRP1</i>	This work
Y5548	Kin28-HA <i>med17-444</i>	<i>MATa ura3-52 his3-Δ200 ade2-101uaa trp1-Δ63 lys2-80luag leu2-Δ1 KIN28::3HA::HIS3 med17::KanMX6 // med17-444 CEN TRP1</i>	This work
Y5549	Kin28-HA <i>med17-546</i>	<i>MATa ura3-52 his3-Δ200 ade2-101uaa trp1-Δ63 lys2-80luag leu2-Δ1 KIN28::3HA::HIS3 med17::KanMX6 // med17-546 CEN TRP1</i>	This work
Y5550	Kin28-HA <i>med17-670</i>	<i>MATa ura3-52 his3-Δ200 ade2-101uaa trp1-Δ63 lys2-80luag leu2-Δ1 KIN28::3HA::HIS3 med17::KanMX6 // med17-670 CEN TRP1</i>	This work
Y6066	Kin28-HA <i>med17-504</i>	<i>MATa ura3-52 his3-Δ200 ade2-101uaa trp1-Δ63 lys2-80luag leu2-Δ1 KIN28::3HA::HIS3 med17::KanMX6 // med17-504 CEN TRP1</i>	This work
Y5552	HA-TBP <i>med17-98</i>	<i>MATa ura3-52 his3-Δ200 ade2-101uaa trp1-Δ63 lys2-80luag leu2-Δ1 LEU2::3HA::SPT15 med17::KanMX6 // med17-98 CEN TRP1</i>	This work
Y5601	HA-TBP <i>med17-257</i>	<i>MATa ura3-52 his3-Δ200 ade2-101uaa trp1-Δ63 lys2-80luag leu2-Δ1 LEU2::3HA::SPT15 med17::KanMX6 // med17-257 CEN TRP1</i>	This work
Y5553	HA-TBP <i>med17-326</i>	<i>MATa ura3-52 his3-Δ200 ade2-101uaa trp1-Δ63 lys2-80luag leu2-Δ1 LEU2::3HA::SPT15 med17::KanMX6 // med17-326 CEN TRP1</i>	This work
Y5554	HA-TBP <i>med17-444</i>	<i>MATa ura3-52 his3-Δ200 ade2-101uaa trp1-Δ63 lys2-80luag leu2-Δ1 LEU2::3HA::SPT15 med17::KanMX6 // med17-444 CEN TRP1</i>	This work
Y5555	HA-TBP <i>med17-546</i>	<i>MATa ura3-52 his3-Δ200 ade2-101uaa trp1-Δ63 lys2-80luag leu2-Δ1 LEU2::3HA::SPT15 med17::KanMX6 // med17-546 CEN TRP1</i>	This work
Y5556	HA-TBP <i>med17-670</i>	<i>MATa ura3-52 his3-Δ200 ade2-101uaa trp1-Δ63 lys2-80luag leu2-Δ1 LEU2::3HA::SPT15 med17::KanMX6 // med17-670 CEN TRP1</i>	This work
Y6064	HA-TBP <i>med17-504</i>	<i>MATa ura3-52 his3-Δ200 ade2-101uaa trp1-Δ63 lys2-80luag leu2-Δ1 LEU2::3HA::SPT15 med17::KanMX6 // med17-504 CEN TRP1</i>	This work
Y5563	Rpb3-Myc MED17	<i>MATa ura3-52 his3- Δ200 ade2-101uaa trp1- Δ63 lys2-80luag leu2-Δ1 med17::KanMX6 RPB3::13Myc::HIS3MX // MED17 CEN URA3</i>	This work
Y5651	Rpb3-Myc MED17- GFP	<i>MATa ura3-52 his3- Δ200 ade2-101uaa trp1- Δ63 lys2-80luag leu2-Δ1 med17::KanMX6 RPB3::13Myc::HIS3MX // EGFP-MED17 CEN TRP1</i>	This work
Y5739	Rpb3-Myc	<i>MATa ura3-52 his3- Δ200 ade2-101uaa trp1- Δ63 lys2-80luag leu2-</i>	This work

	<i>med17-98-GFP</i>	<i>Δ1 med17::KanMX6 RPB3::13Myc::HIS3MX // EGFP-med17-98 CEN TRP1</i>	
Y5652	Rpb3-Myc <i>med17-257-GFP</i>	<i>MATa ura3-52 his3- Δ200 ade2-101uaa trp1- Δ63 lys2-80luag leu2- Δ1 med17::KanMX6 RPB3::13Myc::HIS3MX // EGFP-med17-257 CEN TRP1</i>	This work
Y5740	Rpb3-Myc <i>med17-326-GFP</i>	<i>MATa ura3-52 his3- Δ200 ade2-101uaa trp1- Δ63 lys2-80luag leu2- Δ1 med17::KanMX6 RPB3::13Myc::HIS3MX // EGFP-med17-326 CEN TRP1</i>	This work
Y5741	Rpb3-Myc <i>med17-444-GFP</i>	<i>MATa ura3-52 his3- Δ200 ade2-101uaa trp1- Δ63 lys2-80luag leu2- Δ1 med17::KanMX6 RPB3::13Myc::HIS3MX // EGFP-med17-444 CEN TRP1</i>	This work
Y5742	Rpb3-Myc <i>med17-546-GFP</i>	<i>MATa ura3-52 his3- Δ200 ade2-101uaa trp1- Δ63 lys2-80luag leu2- Δ1 med17::KanMX6 RPB3::13Myc::HIS3MX // EGFP-med17-546 CEN TRP1</i>	This work
Y5743	Rpb3-Myc <i>med17-670-GFP</i>	<i>MATa ura3-52 his3- Δ200 ade2-101uaa trp1- Δ63 lys2-80luag leu2- Δ1 med17::KanMX6 RPB3::13Myc::HIS3MX // EGFP-med17-670 CEN TRP1</i>	This work
Y6341	Rpb3-Myc <i>med17-504-GFP</i>	<i>MATa ura3-52 his3- Δ200 ade2-101uaa trp1- Δ63 lys2-80luag leu2- Δ1 med17::KanMX6 RPB3::13Myc::HIS3MX // EGFP-med17-504 CEN TRP1</i>	This work
CA001	Med22-10His-TEV-ProteinA Med8-HA	<i>MATa leu2 trp1 ura3-52 prb1-1122 pep4-3 prc1-407 gal2 MED22 ::10His-TEV-ProteinA::KITRP1 MED8-PreSci-3HA::KanR</i>	(5)
Y6704	Med22-10His-TEV-ProteinA Med8-HA <i>MED17</i>	<i>MATa leu2 trp1 ura3-52 prb1-1122 pep4-3 prc1-407 gal2 MED22 ::10His-TEV-ProteinA::KITRP1 MED8-PreSci-3HA::KanR med17::natNT2 // MED17 LEU2 CEN</i>	This work
Y6705	Med22-10His-TEV-ProteinA Med8-HA <i>med17-98</i>	<i>MATa leu2 trp1 ura3-52 prb1-1122 pep4-3 prc1-407 gal2 MED22 ::10His-TEV-ProteinA::KITRP1 MED8-PreSci-3HA::KanR med17::natNT2 // med17-98 LEU2 CEN</i>	This work
Y6706	Med22-10His-TEV-ProteinA Med8-HA <i>med17-444</i>	<i>MATa leu2 trp1 ura3-52 prb1-1122 pep4-3 prc1-407 gal2 MED22 ::10His-TEV-ProteinA::KITRP1 MED8-PreSci-3HA::KanR med17::natNT2 // med17-444 LEU2 CEN</i>	This work
Y6707	Med22-10His-TEV-ProteinA Med8-HA <i>med17-504</i>	<i>MATa leu2 trp1 ura3-52 prb1-1122 pep4-3 prc1-407 gal2 MED22 ::10His-TEV-ProteinA::KITRP1 MED8-PreSci-3HA::KanR med17::natNT2 // med17-504 LEU2 CEN</i>	This work
Y6708	Med22-10His-TEV-ProteinA Med8-HA <i>med17-670</i>	<i>MATa leu2 trp1 ura3-52 prb1-1122 pep4-3 prc1-407 gal2 MED22 ::10His-TEV-ProteinA::KITRP1 MED8-PreSci-3HA::KanR med17::natNT2 // med17-670 LEU2 CEN</i>	This work

* Plasmid descriptions are separated from chromosomal genotype by //.

Table S2. Plasmids

Name	Genotype	Reference
pVV208-MED17	<i>Amp CEN URA3 MED17</i>	(4)
pVV204-MED17	<i>Amp CEN TRP1 MED17</i>	(4)
pVV204-med17-8	<i>Amp CEN TRP1 med17-E123G, L510P</i>	This work
pVV204-med17-68	<i>Amp CEN TRP1 med17-P370S, L441P</i>	(4)
pVV204-med17-95	<i>Amp CEN TRP1 med17-P104T, L510P</i>	This work
pVV204-med17-96	<i>Amp CEN TRP1 med17-L674P</i>	This work
pVV204-med17-98	<i>Amp CEN TRP1 med17-L86Q, E186G, E448Q</i>	This work
pVV204-med17-140	<i>Amp CEN TRP1 med17-M442L, Q444P</i>	(4)
pVV204-med17-157	<i>Amp CEN TRP1 med17-R352K, C449F, K517R, N534Y, K618Q</i>	This work
pVV204-med17-158	<i>Amp CEN TRP1 med17-F159Y, S226T, K280M, K377N, E438G, V465E</i>	(4)
pVV204-med17-187	<i>Amp CEN TRP1 med17-N35S, K247E, L254I, K266R, N356D, E400G, L617P</i>	This work
pVV204-med17-208	<i>Amp CEN TRP1 med17-L441P, N611H, A655T, E669D</i>	(4)
pVV204-med17-218	<i>Amp CEN TRP1 med17-I397N, Y554F, L617P</i>	This work
pVV204-med17-233	<i>Amp CEN TRP1 med17-K387I, A450T, L674P</i>	(4)
pVV204-med17-239	<i>Amp CEN TRP1 med17-M207K, N276Y, L302S, R543C</i>	This work
pVV204-med17-241	<i>Amp CEN TRP1 med17-F295L, L546P</i>	This work
pVV204-med17-248	<i>Amp CEN TRP1 med17-I393T, L469Q, I647N</i>	This work
pVV204-med17-247	<i>Amp CEN TRP1 med17-S230P, K433I, L508W</i>	This work
pVV204-med17-249	<i>Amp CEN TRP1 med17-L558P</i>	This work
pVV204-med17-257	<i>Amp CEN TRP1 med17-L520S, I541K, E669G</i>	(4)
pVV204-med17-299	<i>Amp CEN TRP1 med17-N76I, I467T, D568Y</i>	This work
pVV204-med17-326	<i>Amp CEN TRP1 med17-F388L, L546P</i>	This work
pVV204-med17-327	<i>Amp CEN TRP1 med17-M442V, V670E</i>	(4)
pVV204-med17-348	<i>Amp CEN TRP1 med17- D498N, I545F, V670E</i>	This work
pVV204-med17-370	<i>Amp CEN TRP1 med17-P370S</i>	This work
pVV204-med17-396	<i>Amp CEN TRP1 med17-S12G, I60V, S283G, Y348F, I476T, L609Q</i>	This work
pVV204-med17-404	<i>Amp CEN TRP1 med17- I404V I429N E472V K517E</i>	This work
pVV204-med17-444	<i>Amp CEN TRP1 med17-Q444P</i>	This work
pVV204-med17-504	<i>Amp CEN TRP1 med17-M504P</i>	This work
pVV204-med17-510	<i>Amp CEN TRP1 med17-L510P</i>	This work
pVV204-med17-546	<i>Amp CEN TRP1 med17-L546P</i>	(4)
pVV204-med17-670	<i>Amp CEN TRP1 med17-V670E</i>	This work
pAG414GPD-EGFP-MED17	<i>AmpR MED17 CEN TRP1</i>	This work
pAG414GPD-EGFP-med17-98	<i>AmpR med17-98 CEN TRP1</i>	This work
pAG414GPD-EGFP-med17-444	<i>AmpR med17-444 CEN TRP1</i>	This work
pAG414GPD-EGFP-med17-670	<i>AmpR med17-670 CEN TRP1</i>	This work
pAG414GPD-EGFP-med17-504	<i>AmpR med17-504 CEN TRP1</i>	This work
pAG415GPD-MED17	<i>AmpR MED17 CEN LEU2</i>	This work
pAG415GPD-med17-98	<i>AmpR med17-98 CEN LEU2</i>	This work
pAG415GPD-med17-444	<i>AmpR med17-444 CEN LEU2</i>	This work
pAG415GPD-med17-670	<i>AmpR med17-670 CEN LEU2</i>	This work
pAG415GPD-med17-504	<i>AmpR med17-504 CEN LEU2</i>	This work

Table S3. Oligonucleotides

Name	Forward	Reverse
ATP1-O	TCTTGCAGTCGGTGATGGTA	TATCGGAACCGAAAAGAACG
ALD6-P	AGCCGACAAAAGAAAAACGA	CGTTCCCAAGAGGAGATCAA
ALD6-O	CCCATTTGGTCTTTGACGAT	GCCTTGAAAGCAGCCAATAG
SRM1-P	GGCAAGGCAGGATGAATAAA	ATTGGTGGCGACTGTTCTTT
PHO84-P	CCTCATCTCTTGATAAAGTAAAGTAAAGTTCTAAGTTC	TTGTGTGCCCTGGTGATCTACG
PHO84-O	TTGGGTTGGGTACCGTTCTA	TTCCGCATCTTGTCTTGTG
QCR6-O	GATGACGATAACGAGCAGCA	TACCCTCCTCCGTGTTCTTG
PIL1-O	GATTCTTTGGGAAGGGTGGT	ATCGTCATCGTTTTCCAACC
MGR1-P	TGCATGGCATCGTCTTTTAC	GTATGGTCGGTGGAATTGCT
RPA34-P	CATCAGGGCCAATCAATTCT	TTTCGACTTCAGCATTGCAC
PSA1-P	CTGCACCCGATCCTTCTTAC	TTCTGTTTTCGCGTTCCTCA
ALD6-P	ATGCGATATAGCACCGACCA	CTTGACCTCGAACGGTGTTT
ACT1-P	TCCACGTCCTCTTGATAAAA	GGTTTGAGTAGAAAAGGGGAAGG
APA1-P	GAGCGGCCTGAAATACTGTC	AACGTTCCGAAACAGGACAC
PIM1-P	GCCACCGGAAGGAAATAAGT	GTTCAAGCACCTCGAAAACC
STI1-P	CCAAAAGTCTGCTCCCAAAT	TGCAGCGTTACCTTGTTGTT
YIP5-P	CAACGTCCTACGCTCAAGGT	CCCTCGAGATCATCGTCAAT
PSA1-P1	CTGCACCCGATCCTTCTTAC	TTCTGTTTTCGCGTTCCTCA
PSA1-P2	GGAGCCACCACGTTTATTTT	GTTTACAAGAGCGCCACGAG
PRB1-P	CCGGGTTAGCAGAGTAGCAG	CTCTTATCGCGGGTGTGTT
ARG3-P	GTGAATTTTCGAGGGTCACG	AAAATTTGTCCGGGCATTTT
FLC1-P	GCGGAGAGGAGGAGAGAGAC	CGTAGGAAAACGCATGTGGTA
HSP42-P	GGGAGGCCTCTGTGAAGTTA	GCCTGAACGTGTCCCTATGT
HSP42-O	CACCTGGTAACGCAAGAACA	CATCGTTCGCTCACCTACAA
HSP30-P1	ATCCCAGTCCCGACTCTTAT	GGCCAATTAGGGAGAGGAAA
HSP30-P2	ATTTTGTGGCCATTTTCCA	CGGGATATGGCTTTGCTTAC
HSP30-O	CAAGGTGTGATATGCCAACG	ATAGCCTCACCGTCTGGTTG
HSP150-P1	GGAACACTTGAAGTCTAACGACA	AAGCAGAGGCAACCAAAGTC
HSP150-P2	ATTATCCTGGCCGCTAATCC	GTGGCAAAGCAGTGAGATTG
HSP150-O	CCGTTCAACCAATCTCCACT	GGCAGCGGTACTCTTGGTAG
HSP104-P1	CCCATCTCAAAGAACTGCAA	GGAACAAGTGACAAAGGAACG
HSP104-O	GGTACCACGCGAGGTTATGT	CCGTCATCCAACATTTGTAGC
HSP12-O	CAAGGGTGTCTTCCAAGGTG	CGACCGGAAACATATTCGAC
ADH1-P1	ATAGGCGCATGCAACTTCTT	CATCAGCTCTGGAACAACGA
ADH1-P2	TTCCTTCATTCACGCACACT	AGGGAACGAGAACAATGACG
ADH1-O	GGGTATTGACGGTGGTGAAG	AAACGTTGATGACACCGTGA
PYK1-P1	CGCACCGTCACAAAGTGTT	TGGGAAGGAAAGGAAATCAC
PYK1-P2	CCTTTCTTCCCATATGATGC	ACTTTGAAAGGGGACCATGA
PYK1-O	TGCTTTGAGAAAGGCTGGTT	TCTGATTTCTGGACCCTTGG
PYK1-ATG	GGTCCCCTTTCAAAGTTATTCTCTA	CTTGAGAAGAACCTCCATCATTG
PMA1-O	GGTTTTGGTCATTGCCACTT	ACGGCCATAGTGGTGTAAC
PMA1-P1	AACAAACCCGGTCTCGAAG	GAAGTGCCGCATTAGGAAAT
PMA1-P2	GATGGTGGGTACCGCTTATG	TTGGTGTTATAGGAAAGAAAGAG
GAL1-P	ACGCTTAACTGCTCATTGCT	TGTTCCGGAGCAGTGCGGCGC
GAL1-O1	AAAGAACTTGCACCGGAAA	GGCCCATATTCGCTTTAACA
GAL1-O2	ACATTTCCACACCCTGGAAC	TTCTTCGCGAGAACAATTCA
rDNA-25S	GGTTATATGCCGCCCGTCTTGA	CCCAACAGCTATGCTCTTACTC
rDNA-18S	GGTCTGTGATGCCCTTAGACG	AGTTTCACAAGATTACCAAGACC
SCR1	GTCTGGGCAGAGCTGTCT	AAGGTGGAGCCCCTAAGGA
RPN2	GCGGATACAGGCACATTGGATACC	TGTTGCTACCTTCTACCTCCTT
TAF10	ATATTCCAGGATCAGGTCTCCGTAGC	GTAGTCTTCTCATTCTGTTGATGT
PMA1-O2	TCTCCAAAGCCCGTTAAATG	CCGTTTCATAGCACCGAAGTT

Table S4. Total number of mapped reads for ChIP-seq experiments

Sample	Million of mapped reads
INPUT-MED17	2.9
INPUT-med17-98	3.0
INPUT-med17-444	4.0
INPUT-med17-670	3.5
POL2-MED17	7.9
POL2- med17-98	8.7
POL2- med17-444	10.4
POL2- med17-670	7.8
TBP-WT	2.8
TBP- med17-98	2.8
TBP- med17-444	3.2
TBP- med17-670	3.6
RAD3-WT (core TFIIH)	5.0
RAD3- med17-98	3.4
RAD3- med17-444	11.3
RAD3- med17-670	3.9
KIN28-WT (TFIIH kinase module)	2.9
KIN28- med17-98	3.0
KIN28- med17-444	4.0
KIN28- med17-670	3.5
MED15-WT (Mediator)	3.5
MED15- med17-98	9.2
MED15- med17-444	3.0
MED15- med17-670	3.7
INPUT- med17-504	15.2
POL2-WT	18.7
POL2- med17-504	16.2
TBP-WT	9.9
TBP- med17-504	11.7
RAD3-WT	10.6
RAD3- med17-504	8.0
KIN28-WT	5.0
KIN28- med17-504	3.2
MED15-WT	6.3
MED15- med17-504	3.5
NT-WT	1.7

Table S5. Values used to normalize the ChIPseq data from untagged strain sample before subtraction

Sample	Normalization value for untagged strain sample
TBP-WT	0.60
TBP- med17-98	0.53
TBP- med17-444	0.20
TBP- med17-670	0.86
RAD3-WT (core TFIIH)	0.57
RAD3- med17-98	0.38
RAD3- med17-444	2.69
RAD3- med17-670	0.25
KIN28-WT (TFIIH kinase module)	0.70
KIN28- med17-98	0.80
KIN28- med17-444	0.20
KIN28- med17-670	0.62
MED15-WT (Mediator)	2.10
MED15- med17-98	1.43
MED15- med17-444	0.77
MED15- med17-670	1.65
TBP-WT-2	2.45
TBP- med17-504	1.45
RAD3-WT-2	2.04
RAD3- med17-504	2.04
KIN28-WT-2	2.46
KIN28- med17-504	1.42
MED15-WT-2	4.94
MED15- med17-504	3.53

Table S6. Regions used for ChIP-seq data normalization

Protein	Regions
Pol II	ADH1-O, ATP1-O, GAL1-O2, HSP104-O, HSP12-O, HSP150-O, HSP30-O, HSP42-O, PMA1-O, PYK1-O, PHO84-O, QCR6-O, PIL1-O
Kin28, Rad3	ADH1-P2, GAL1-O2, HSP104-P1, HSP30-P1, HSP42-P, MGR1-P, PMA1-P2, PYK1-P2, RPA34-P, SRM1-P, PSA1-P2, ALD6-P, PHO84-P, PSA1-P2, ACT1-P, APA1-P
TBP	ADH1-P2, GAL1-O2, HSP104-P1, HSP12-P1, HSP150-P1, HSP42-P, PIM1-P, PMA1-P2, PYK1-P2, 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

Table S7. Normalization coefficients for ChIP-seq data

Protein	Mutant			
	<i>med17-98</i>	<i>med17-444</i>	<i>med17-670</i>	<i>med17-504</i>
Pol II	0.83	0.38	0.70	0.88
Kin28	1.26	0.28	0.88	1.42
Rad3	1.25	0.85	0.59	1.09
TBP	1.24	0.62	0.89	0.82
Med15	0.31	0.72	0.73	1.96

Table S8. *med17* mutations and their phenotypes

Name	Mutations	Phenotype ¹
Single mutations		
<i>med17-96</i>	L674P	N 30 / S 37
<i>med17-249</i>	L558P	S 30 / S 37
<i>med17-370</i> ^{*2}	P370S	N 30 / S 37
<i>med17-444</i> [*]	Q444P	S 30 / L 37
<i>med17-504</i> [*]	M504P	S 30 / L 37
<i>med17-510</i> [*]	L510P	S 30 / L 37
<i>med17-546</i> [*]	L546P	S 30 / S 37
<i>med17-670</i> [*]	V670E	S 30 / S 37
Double mutations		
<i>med17-8</i>	E123G L510P	N 30 / L 37
<i>med17-68</i>	P370S L441P	S 30 / L 37
<i>med17-95</i>	P104T L510P	S 30 / L 37
<i>med17-140</i>	M442L Q444P	S 30 / L 37
<i>med17-241</i>	F295L L546P	N 30 / S 37
<i>med17-326</i>	F388L L546P	N 30 / S 37
<i>med17-327</i>	M442V V670E	S 30 / L 37
Triple mutations		
<i>med17-98</i>	L86Q E186G E448Q	N 30 / S 37
<i>med17-218</i>	I397N Y554F L617P	N 30 / L 37
<i>med17-247</i>	S230P K433I L508W	N 30 / S 37
<i>med17-233</i>	K387I A450T L674P	S 30 / L 37
<i>med17-257</i>	L520S I541K E669G	S 30 / L 37
<i>med17-248</i>	I393T L469Q I647N	N 30 / S 37
<i>med17-299</i>	N76I I467T D568Y	S 30 / S 37
<i>med17-348</i>	D498N I545F V670E	S 30 / L 37
Multiple mutations		
<i>med17-157</i>	R352K C449F K517R N534Y K618Q	N 30 / L 37
<i>med17-158</i>	F159Y S226T K280M K377N E438G V465E	S 30 / L 37
<i>med17-187</i>	N35S K247E L254I K266R N356D E400G L617P	N 30 / S 37
<i>med17-208</i>	L441P N611H A655T E669D	S 30 / L 37
<i>med17-239</i>	M207K N276Y L302S R543C	N 30 / L 37
<i>med17-396</i>	S12G I60V S283G Y348F I476T L609Q	N 30 / L 37
<i>med17-404</i>	I404V I429N E472V K517E	N 30 / L 37

¹ Normal (N), slow growth (S) and lethal (L) phenotypes at 30°C (30) or 37°C (37) are indicated.

² Mutations obtained by site-directed mutagenesis are indicated by asterisk. The four mutants chosen for a detailed analysis are shown in red.

Table S9. Mediator integrity in *med17* mutants¹

Mutant	Head					Tail	Middle/Tail			Tail
Co-IP	Med6	Med20	Med8-HA			Med5-HA	Med14			Med2-HA
IP	Med8-HA	Med5-HA	Med8-HA	Med2-HA	Med8-HA	Med5-HA	Med5-HA	Med2-HA	Med8-HA	Med2-HA
<i>med17-8</i>	+	+	+	+	+	+	+	+	+/-	+
<i>med17-68</i>		+/-		+	+	+/-	+	++		+
<i>med17-95</i>	+	-	+	+	+	+	+	+	+	+
<i>med17-96</i>	+	+/-	+/-	+	+	+	+	+	+	+
<i>med17-98</i>	+	+	+	+	+	+	+	+	++	+
<i>med17-140</i>	+/-	+/-	+	+	+/-	+	+	+	+	+
<i>med17-157</i>	+/-	+/-	+	+	+	+	+	+	+	+
<i>med17-158</i>	++	+/-	+	+	+	+/-	+	++	++	+
<i>med17-187</i>	+	+	+	+	+	+	+	++	+	+
<i>med17-208</i>		+		+	+	+	+	+		+
<i>med17-218</i>	+	+/-	+	+	+	+/-	+	+	+	+
<i>med17-233</i>	+/-	+/-	+	+	+	+/-	+/-	+/-	+/-	+/-
<i>med17-239</i>	+/-	+/-	+	+	+/-	+	+	+/-	+	+/-
<i>med17-241</i>	+/-	+/-	+/-	+/-	+/-	+	+	+	+	+
<i>med17-247</i>	+	+/-	+/-	+	+	+	+	+	++	+
<i>med17-248</i>	+	+/-	+	+	+	+/-	+	+	++	+
<i>med17-249</i>	+	+/-	+	+	+	+	+	+/-	+	+
<i>med17-257</i>	-	+/-	+/-	+	+/-	+	+	+/-	+/-	+
<i>med17-299</i>	+/-	+	+/-	+	+/-	+	+	+/-	+	+
<i>med17-326</i>	+	+/-	+	+	+	+	+	+	++	+
<i>med17-327</i>	-	+/-	+/-	+	+/-	+	+	+/-	+	+
<i>med17-348</i>	-	-	+/-	+/-	+/-	+	+	+/-	+	+
<i>med17-370</i>	++	+	+	+	++	+	+	+	+	+
<i>med17-396</i>	+	+/-	+	+	+	+	+	+	+	+
<i>med17-404</i>	+/-	+/-	+	+	+	+/-	+	+	+	+
<i>med17-444</i>	+	+/-	+	+	+	+	+	+	+	+
<i>med17-510</i>	+	+	+	+	+	+	+	+	+	+
<i>med17-546</i>	+	+/-	+	+	+	+	+	+	+	+
<i>med17-670</i>	+	+/-	+	+	++	+	+	+/-	+	+

¹ Yeast strains carrying a HA-tag on Med8, Med5 or Med2 Mediator subunits were used for CoIP experiments. Med6, Med20, Med8-HA, Med5-HA, Med14 and Med2-HA subunits were detected by western blotting with corresponding antibodies. Levels of western blotting signals compared to the control IP with a wild-type strain are indicated as follows: similar (+), slightly decreased (+/-), decreased (-) and slightly increased (++) levels. It should be noted that lower IP signals could explain lower CoIP signals.

Supplementary figure legends

Figure S1. Growth phenotypes of *med17* mutants.

Cultures of wild-type (WT) and mutant *med17* yeast strains were serially diluted, spotted on YPD agar plates and incubated for 3 days at permissive (30°C) or non-permissive (37°C) temperature. *med17* mutants selected for a detailed analysis are indicated in red.

Figure S2. Schematic representation of the localization of selected Med17 mutations.

(A) Multiple alignment of the Med17 conserved domain 11 (signature specific motif, SSM#11) from *Homo sapiens sapiens* (Hs), *Mus musculus* (Mm), *Gallus gallus* (Gg), *Xenopus tropicalis* (Xt), *Danio rerio* (Dr), *Tetraodon nigroviridis* (Tn), *Ciona savignyi* (Cs), *Drosophila melanogaster* (Dm), *Drosophila pseudoobscura* (Dp), *Anopheles gambiae* (Ag), *Apis mellifera* (Am), *Bombyx mori* (Bo), *Tribolium castaneum* (Tc), *Caenorhabditis elegans* (Ce), *Caenorhabditis briggsae* (Cb), *Brugia malayi* (Bm), *Schistosoma mansoni* (Sm), *Rhizopus oryzae* (Ro), *Cryptococcus neoformans JEC21* (Cn), *Ustilago maydis* (Um), *Coprinus cinereus/Coprinopsis cinerea* (Cc), *Phanerochaete chrysosporium* (Pc), *Neurospora crassa* (Nc), *Magnaporthe grisea* (Mg), *Gibberella zeae/Fusarium graminearum* (Gz), *Aspergillus nidulans* (An), *Aspergillus fumigatus* (Af), *Coccidioides posadasii* (Co), *Schizosaccharomyces pombe* (Sp), *Yarrowia lipolytica* (Yl), *Candida albicans* (Ca), *Debaryomyces hansenii* (Dh), *Kluyveromyces lactis* (Kl), *Kluyveromyces waltii* (Kw), *Eremothecium gossypii* (Eg), *Candida grabata* (Cg) and *Saccharomyces cerevisiae* (Sc) was modified from (9). The consensus SSM generated by WebLogo is shown above the alignment. Location of L371P mutation in human Med17 and M504P mutation in yeast *S. cerevisiae* Med17 is indicated with red dotted line.

(B) Location of Med17 mutations is indicated for selected mutants: *med17-98* (L86Q, E186G, E448Q) in blue, *med17-444* (Q444P) in orange, *med17-504* (M504P) in green, *med17-670*

(V670E) in red.

The grey boxes indicate the positions of the conserved domains (signature specific motifs, SSMs) (9). The location of conserved regions #12 and #13 is not indicated on Figure since it is less obvious and depends on the number of sequences in alignments.

(C) Location of sites of *med17* (red spheres) mutations is indicated on the model of *S. cerevisiae* Mediator head module from Larivière et al. (10) for *med17-98*, -444, -504 and -670 when the corresponding residues are present on the model. The residues L86 and E186 mutated in *med17-98* are absent in the model. Mediator subunits are indicated in different colours.

Figure S3. Mediator integrity in *med17* mutants.

(A) Wild-type (WT and WT Med5-3HA) strains and *med17* mutant strains carrying a Med5-HA tag were grown to exponential phase at 30°C or transferred to 37°C for 45 min. The presence of a Med5-HA tag is indicated by a line above the western blots. 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. *MED17* strain carrying non-tagged Mediator subunit was used as a negative control. Coimmunoprecipitated Mediator subunits were detected by western blotting using anti-Med14 and -Med17 antibodies.

(B) The presence of Med21 subunit in purified Mediator complex from *med17-98* mutant compared to the WT strain was confirmed by western blotting using anti-Med21 antibody.

Figure S4. Interaction between Pol II and Mediator in *med17* mutants.

Interaction between Pol II and Mediator in *med17-98*, 444 and 670 mutants compared to the WT strain is shown. Rpb3-Myc Med17-EGFP strains with WT *MED17* or *med17* mutations were grown at 30°C in YPD medium and cross-linked or not with formaldehyde (FA), as

indicated. Med17-EGFP was immunoprecipitated (IP) with anti-EGFP antibody from crude extracts (Input) and analysed by western blotting with anti-Myc antibody (CoIP) against Rpb3. The cross-linked Rpb3-Med17 band is indicated in red. The position of unidentified cross-linked proteins with the tagged Med17 or Rpb3 subunits is indicated by a vertical bar.

Figure S5. *med17* mutations influence mRNA abundance after a shift at 37°C.

Yeast strains were grown at 30°C in YPD medium and then shifted for the indicated time at 37°C. mRNA levels were determined by quantitative RT-PCR. qPCR results were normalized to the WT signal. *25S*, *18S rRNA* and *SCR1* genes were used as internal controls for normalization.

Figure S6. Conservation of Med17 Mediator subunit from yeast *S. cerevisiae* to human.

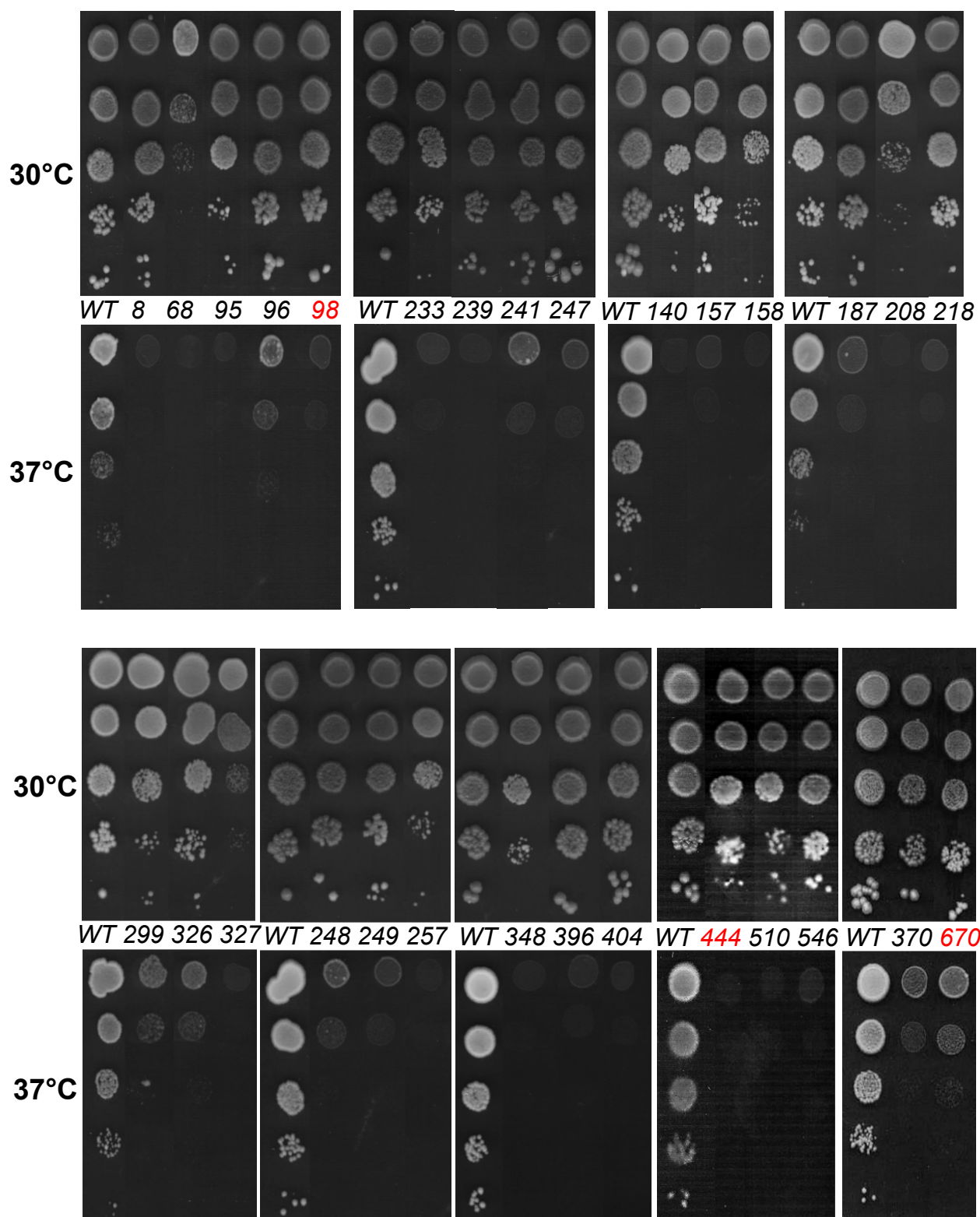
Alignment of Med17 Mediator subunit from yeast *S. cerevisiae* (Sc) and human (Hs) was modified from (9). Signature specific motifs (SSMs) are indicated (9). Localization of Med17 mutations is indicated for mutants characterized in this study: *med17-98* (L86Q, E186G, E448Q), *med17-444* (Q444P), *med17-504* (M504P), *med17-670* (V670E).

Supplementary references

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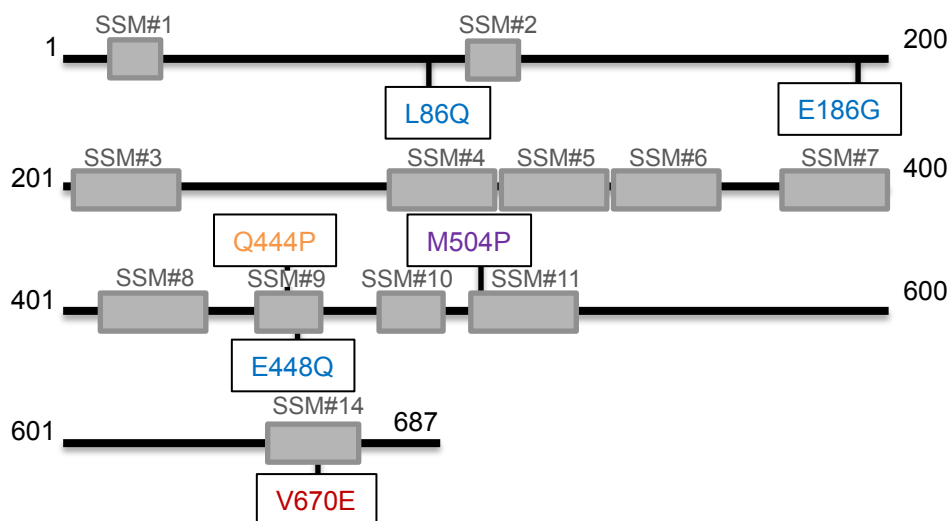
Figure S1



A



B



C

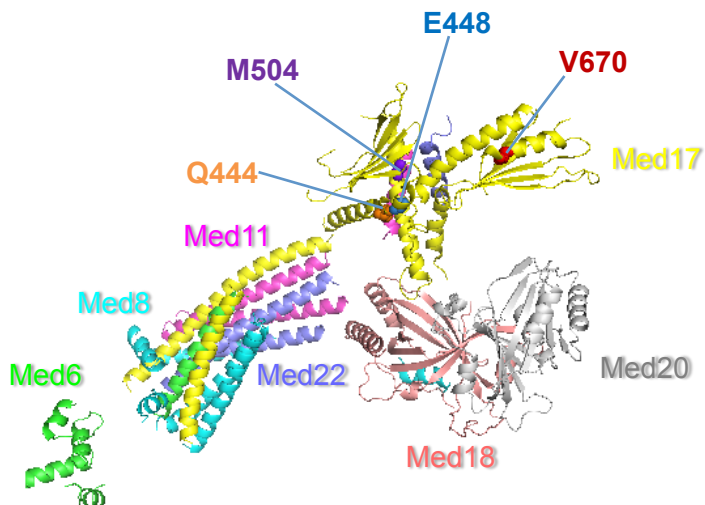
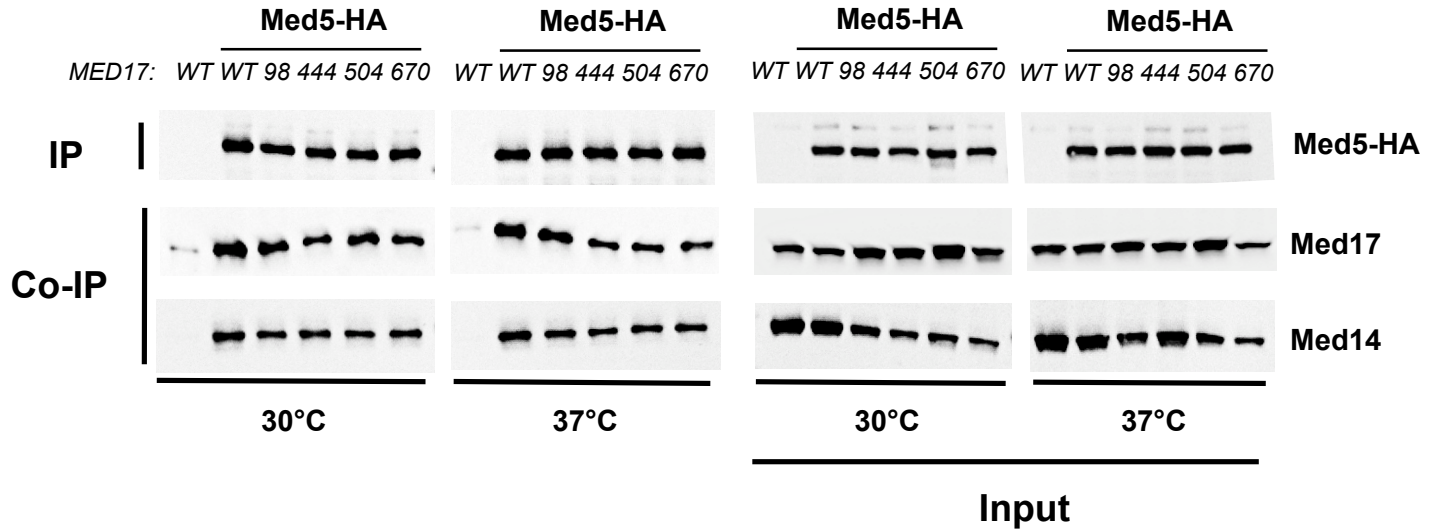


Figure S3

A



B



Figure S4

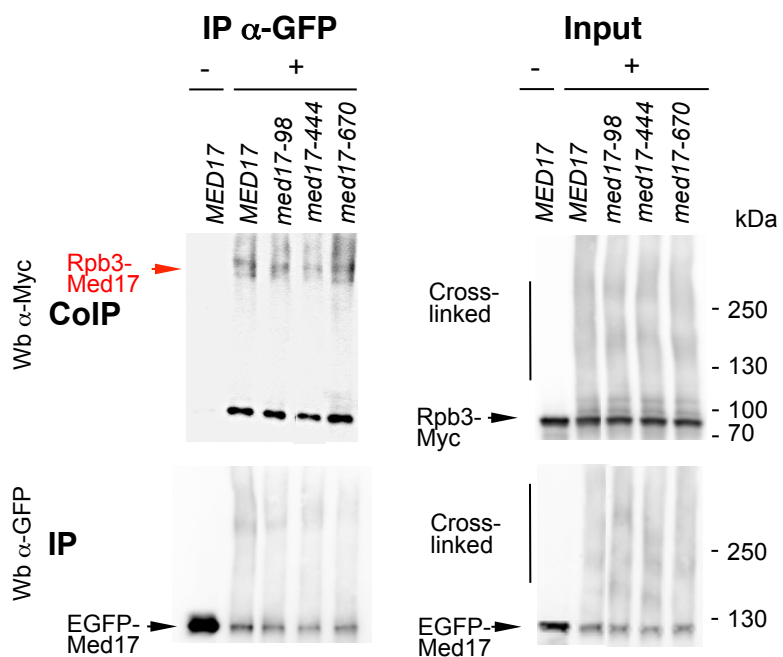


Figure S5

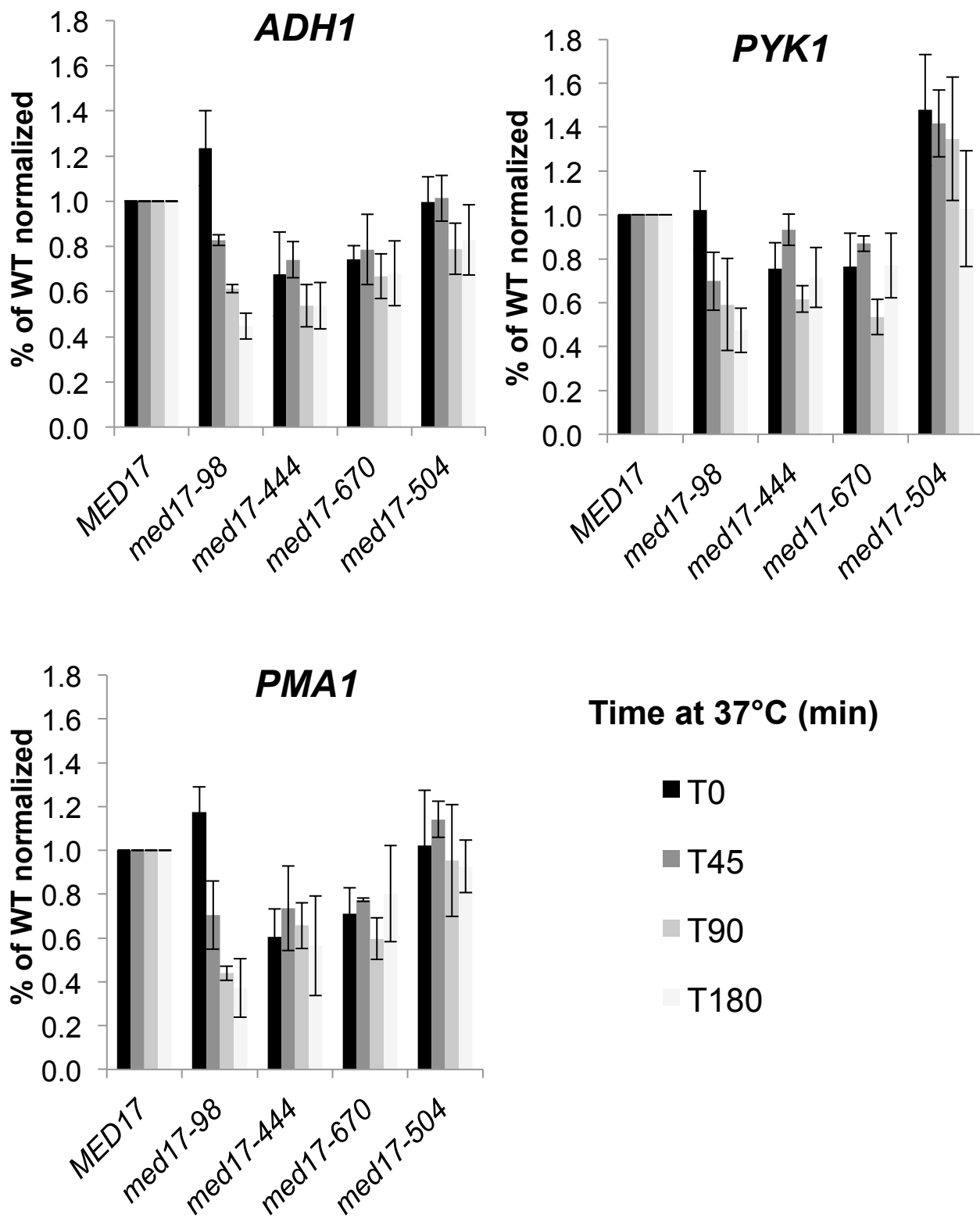


Figure S6

