Supplementary Information

Mediator head subcomplex Med11/22 contains a common helix bundle building block with a specific function in transcription initiation complex stabilization

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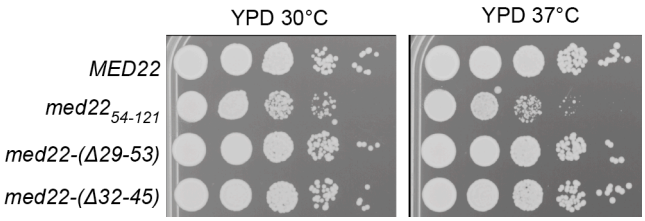
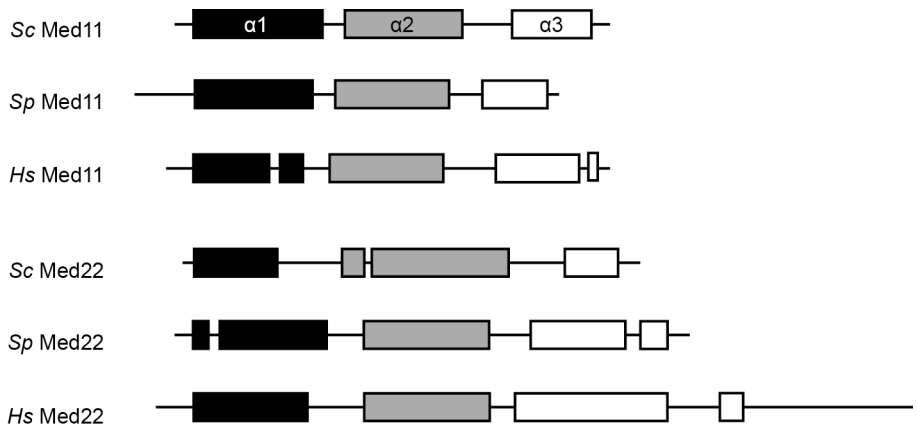
Supplementary Figure 1



Deletion of unstructured Med22 linker has no phenotype *in vivo*.

Yeast complementation assays as in Figure 2. MED22, *med22*$_{54\text{-}121}$, *med22-(Δ29-53)* and *med22-(Δ32-45)* yeast strains on YPD plates incubated at 30°C and 37°C.

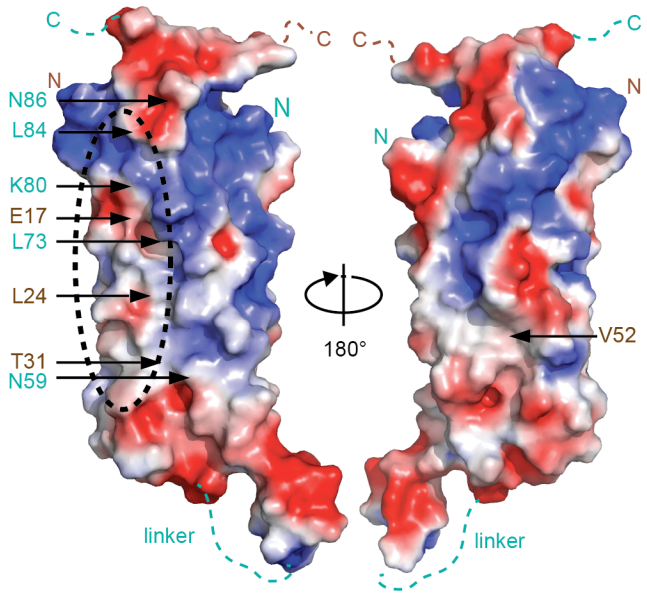
Supplementary Figure 2



C-terminal helices in Med11/22 are conserved across species.

Conservation of secondary structure elements in Med11 and Med22 across species (*Saccharomyces cerevisiae* (*Sc*), *Schizosaccharomyces pombe* (*Sp*) and *Homo sapiens* (*Hs*)). All secondary structure elements from crystal structures (when available) or from consensus secondary structure predictions are drawn to scale (boxes, α-helices; lines, ordered but without secondary structure). Helix α1, α2 and C-terminal helical extensions are colored in black, grey and white, respectively.

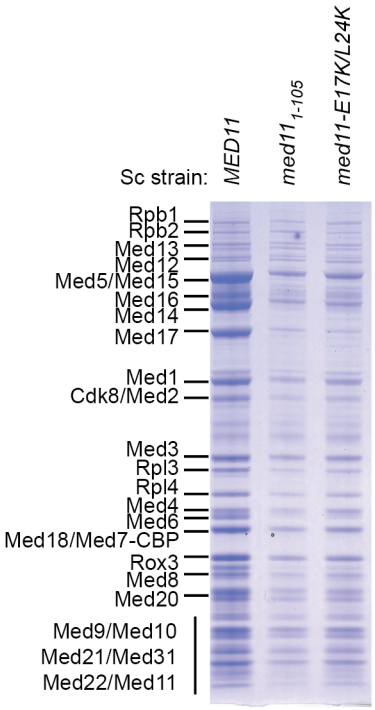
Supplementary Figure 3



Med11/22 surface charge distribution.

Red, blue and white areas indicate negative, positive and neutral charges, respectively. Residues of Med11 (brown) and Med22 (cyan) targeted by mutagenesis in this study and in previous reports are indicated with arrows. The two views are related by a 180° rotation around the vertical axis.

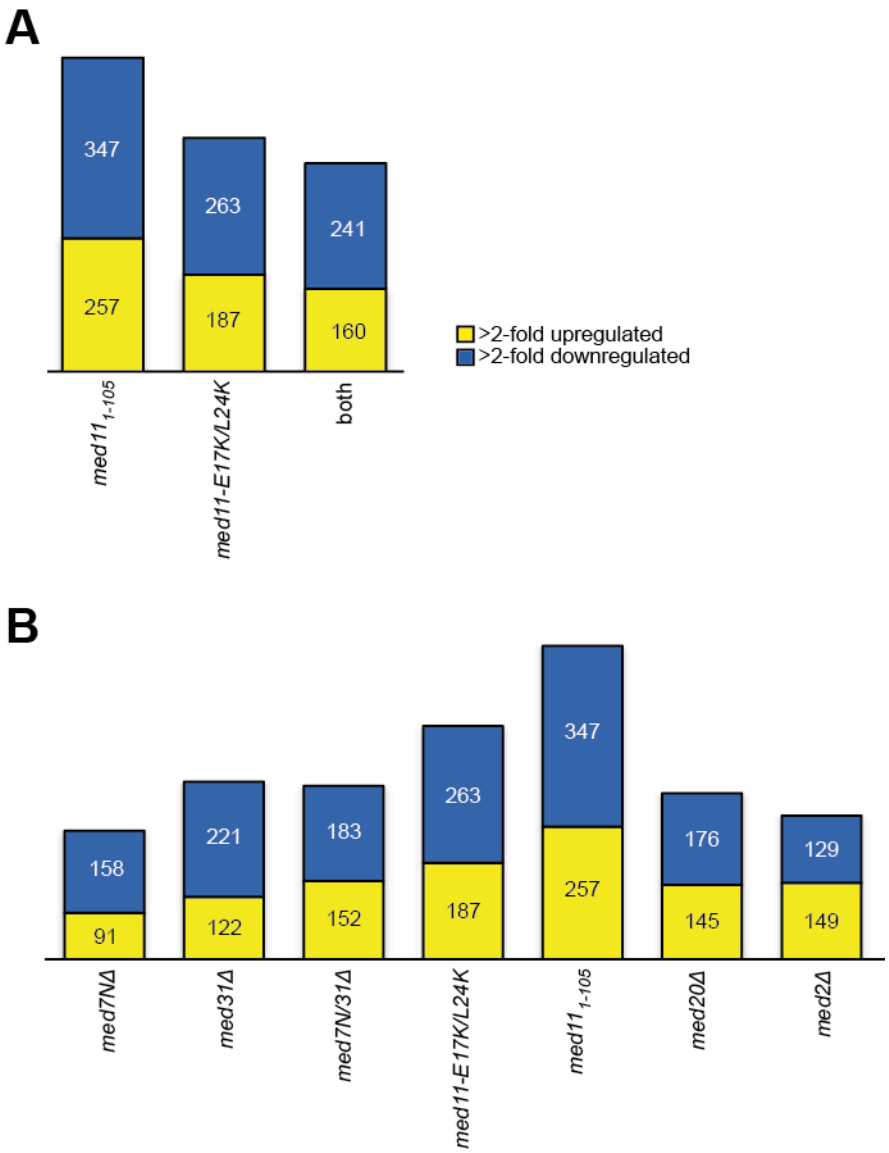
Supplementary Figure 4



Co-immunoprecipitation of Mediator from wild-type and mutant strains

Mediator tandem affinity purification through Med7-TAP from wild-type, *med11*$_{1\text{-}105}$ and *med11-E17K/L24K* yeast strains. Co-purifying proteins were separated on a 5-12% gradient gel (Invitrogen) and bands were stained with Coomassie blue. Mediator subunits and common co-purifying contaminants are labeled. Med7-CBP marks Med7 after tandem-affinity purification still carrying the calmodulin binding protein-tag but lacking the cleaved protein A-tag.

Supplementary Figure 5



Genome-wide gene-expression profiling.

(A) Number of genes significantly (fold change > 2.0, p-value < 0.05) up-regulated (yellow) and down-regulated (blue) in *med11*$_{1-105}$, *med11-E17K/L24K* and in both strains.

(B) Comparison of gene expression changes of all mutants analyzed in this study. Number of genes significantly (fold change > 2.0, p-value < 0.05) up-regulated (yellow) and down-regulated (blue) is shown.