Supplemental Data

Identification, structure, and functional requirement of the Mediator submodule Med7N/31

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Supplementary Table I Data collection and refinement statistics for Med7N/31

	Selenomethionine SAD Med7ΔC84/Med31	Native dataset, mutant construct 6 (Table S2)
Data collection		
Space group	R 32	R 32
Cell parameters		
a,b,c (Å)	174.6, 174.6, 117.8	174.2, 174.2, 115.5
α, β, γ (°)	90, 90, 120	90, 90, 120
Wavelength (Å)	0.98	0.92
Resolution range (Å)[a]	20-2.8 (2.97-2.80)	50-3.0 (3.18-3.00)
Completeness (%)	99.4 (98.0)	99.7 (98.2)
Unique reflections	32,854 (5275)	13576 (2138)
Redundancy	11.3 (9.1)	11.2 (11.0)
Rsym (%)	6.0 (61.8)	5.0 (57.4)
<I>/<σ>	27.8 (4.1)	32.7 (5.2)
Refinement		
Number of residues	282	278
Number of non-hydrogen atoms	2436	2402
rms bond deviation (Å)	0.007	0.008
rms angle deviation (°)	1.041	1.076
Ramachandran plot (%) (favored/allowed)	95.0/100.0	95.0/100.0
Rcryst (%)	19.5	17.8
Rfree (%)[b]	24.3	23.1

[a] Highest resolution shell is shown in parenthesis.

[b] 5% of the data were set aside for free R-factor calculation.

Supplementary Table II Summary of Med7N mutant constructs of the polyproline stretches 1 and 2.

No.	Med7N construct[a]	Mutation	Replaced sequence	Copurification with Med31
1	Med7 (1-83)	- (crystallized construct)		yes
2	Med7 (20-83)	N-terminus including pPS1 removed		no
3	Med7 (23-83)	N-terminus including pPS1 removed		no
4	Med7 (1-61)	C-terminus including pPS2 removed		no
5	Med7 (1-66)	C-terminus including pPS2 removed		no
6	Med7 (1-83) (12...18 YSPTSPS)	pPS1 replaced by CTD-repeat	LYPPPPP	yes
7	Med7 (1-83) (13...19 YSPTSPS)	pPS1 replaced by CTD-repeat	YPPPPPY	yes
8	Med7 (1-83) (66...72 YSPTSPS)	pPS2 replaced by CTD-repeat	IPPPMPK	no
9	Med7 (1-83) (65...71 YSPTSPS)	pPS2 replaced by CTD-repeat	LIPPPMP	no
10	Med7 (1-83) (12...18 SPSTPSY)	pPS1 replaced by reversed CTD-repeat	LYPPPPP	yes
11	Med7 (1-83) (12...18 YSPTDPS)	pPS1 replaced by mutated CTD-repeat	LYPPPPP	yes
12	Med7 (1-83) (12...18 YSPTRPS)	pPS1 replaced by mutated CTD-repeat	LYPPPPP	yes
13	Med7 (1-83) (12...18 YDPTSPS)	pPS1 replaced by mutated CTD-repeat	LYPPPPP	yes

[a] For interaction assays, Med7 (1-83), Med7 (1-61), Med7 (1-66), Med7 (20-83), Med7 (23-83) were cloned into pET21b using *Nde*I/*Sal*I, such that a N-terminal Strep-tag was introduced. With these constructs, copurification was analyzed both via the His-tag on Med31 and via the Strep-tag on the Med7 variants.

Supplementary Table III List of yeast strains used within this study.

Strain	Genotype	Source
wt	BY4741; *MATa*; *his3*Δ1; *leu2*Δ0; *met15*Δ0; *ura3*Δ0	Euroscarf Y00000
MED7/*med7*Δ	BY4743; *MATa/α*; *his3*Δ1/*his3*Δ1; *leu2*Δ0/*leu2*Δ0; *lys2*Δ0/*LYS*2; *MET15*/*met15*Δ0; *ura3*Δ0/*ura3*Δ0; *YOL135C*::*kanMX4*/*YOL135C*	Euroscarf Y26285
MED7 shuffle	Y26285 sporulated; *MATa* or *MATα*; *YOL135C*::*KanMX4*/p20062 (cognate clone plasmid)	this work
*med31*Δ	BY4741; *YGL127C*::*kanMX4*	Euroscarf Y04494
*med7N*Δ	BY4741; *YOL135C*::*YOL135*ΔN84-*ClonNAT*	this work
*med7N/31*Δ	BY4741; *YOL135C*::*YOL135*ΔN84-*ClonNAT*; *YGL127C*::*kanMX4*	this work
wt/ *Med18-TAP*	BY4741; *YGR104C*::*YGR104C-TAP-URA3*	this work
*med31*Δ/ *Med18-TAP*	BY4741; *YGL127C*::*kanMX4*; *YGR104C*::*YGR104C-TAP-URA3*	this work
*med7N*Δ/ *Med18-TAP*	BY4741; *YOL135C*::*YOL135*ΔN84-*ClonNAT*; *YGR104C*::*YGR104C-TAP-URA3*	this work
*med7N/31*Δ / *Med18-TAP*	BY4741; *YOL135C*::*YOL135*ΔN84-*ClonNAT*; *YGL127C*::*kanMX4*; *YGR104C*::*YGR104C-TAP-URA3*	this work
*dst1*Δ	BY4741; *YGL0143W*::*kanMX4*	Euroscarf Y04411
*med18*Δ	Y24734 sporulated; MATa or MATα; *YGR104C*::*kanMX4*	(Larivière et al, 2008)
*med8C*Δ	Y23333 sporulated; MATa or MATα; *YBR193C*::*kanMX4*	(Larivière et al, 2008)
*cycC*Δ	BY4741; *YNL025C*::*kanMX4*	Euroscarf Y05351
*med1*Δ	BY4741; *YPR070W*::*kanMX4*	Euroscarf Y05489
*med9*Δ	BY4741; *YNR010W*::*kanMX4*	Euroscarf Y05385
*med2*Δ	BY4742; *YDL005C*::*kanMX4*	Euroscarf Y13701
*med3*Δ	BY4741; *YGL025C*::*kanMX4*	Euroscarf Y04393
*med5*Δ	BY4741; *YGL151W*::*kanMX4*	Euroscarf Y04518

Supplementary Table IV Analysis of transcriptome profiling. **(A)** List of up- and down-regulated genes in the yeast deletion strains *med31*Δ, *med7N*Δ, *med7N/31*Δ and *dst1*Δ. **(B)** Overrepresented biological processes and associated genes detected in the GO analysis of *med31*Δ, *med7N*Δ, *med7N/31*Δ and *dst1*Δ profiles. **(C)** GO-analysis for the *med7N/31*Δ, *med8C/18/20*Δ and *med2/3*Δ submodule profiles. **(D)** GO-comparison table and visualization of the three submodule deletion profiles. The data was split into up- and down-regulated genes (red and green bars, respectively). **(E)** Complete list of expression values of the yeast deletion strains *med31*Δ, *med7N*Δ, *med7N/31*Δ and *dst1*Δ. **(F)** Ranking of transcription factors based on Fisher's exact test which are potentially regulating the differentially expressed genes in *med31*Δ, *med7N*Δ, *med7N/31*Δ and *dst1*Δ strains. **(G)** Ranking of transcription factors based on Fisher's exact test which are potentially regulating the differentially expressed genes in Med7N/31, Med8C/18/20 and Med2/3 submodule deletion strains.

Supplementary Table V Mass spectrometry analysis of Mediator TAP-tag purification of Figure 3E.

Supplementary Figure 1 *In vitro* transcription assay using Gal4-Gal4activation-helix (Gal4-Gal4AH) as activator (Carey et al, 1990). A med7N/31Δ nuclear extract was also defective in activated transcription using a Gal4-Gal4AH as activator (lane 1) but could be rescued by addition of recombinant Med7N/31 (lane 2, 200 pmol). Similarly as shown in Figure 3D, the reconstituted complex could be further stimulated by addition of recombinant TFIIS (lane 3-5, 20-60 pmol). The assay was performed using 100 µg of nuclear extract.

Supplementary Figure 2 Analysis of published genetic interactions of Med31 and TFIIS. **(A)** Common and disparate synthetic lethality partners of Med31 and TFIIS are depicted as nodes. **(B)** Common and disparate phenotypic suppression partners of Med31 and TFIIS. Figures were prepared using the Osprey software (Breitkreutz et al, 2003) with interaction data (compare interaction references) downloaded from BioGrid database (Stark et al, 2006).

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