Supplemental data for

Preparation and topology of the Mediator middle module

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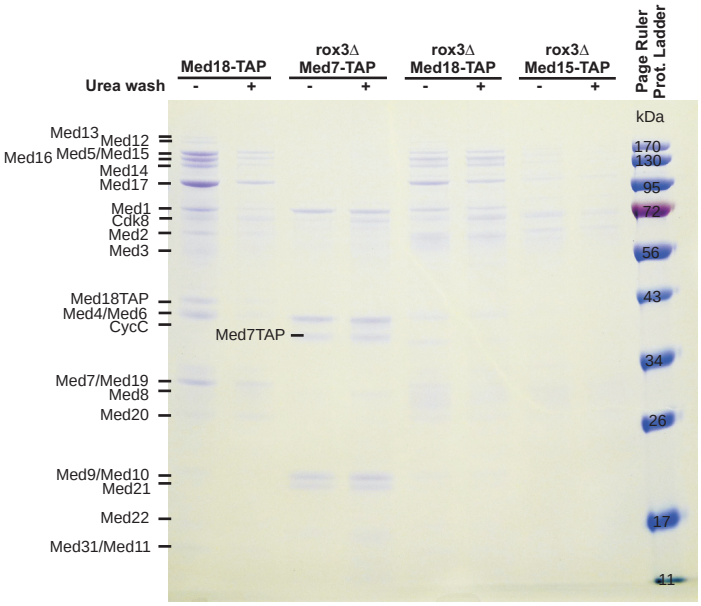
SUPPLEMENTARY METHODS

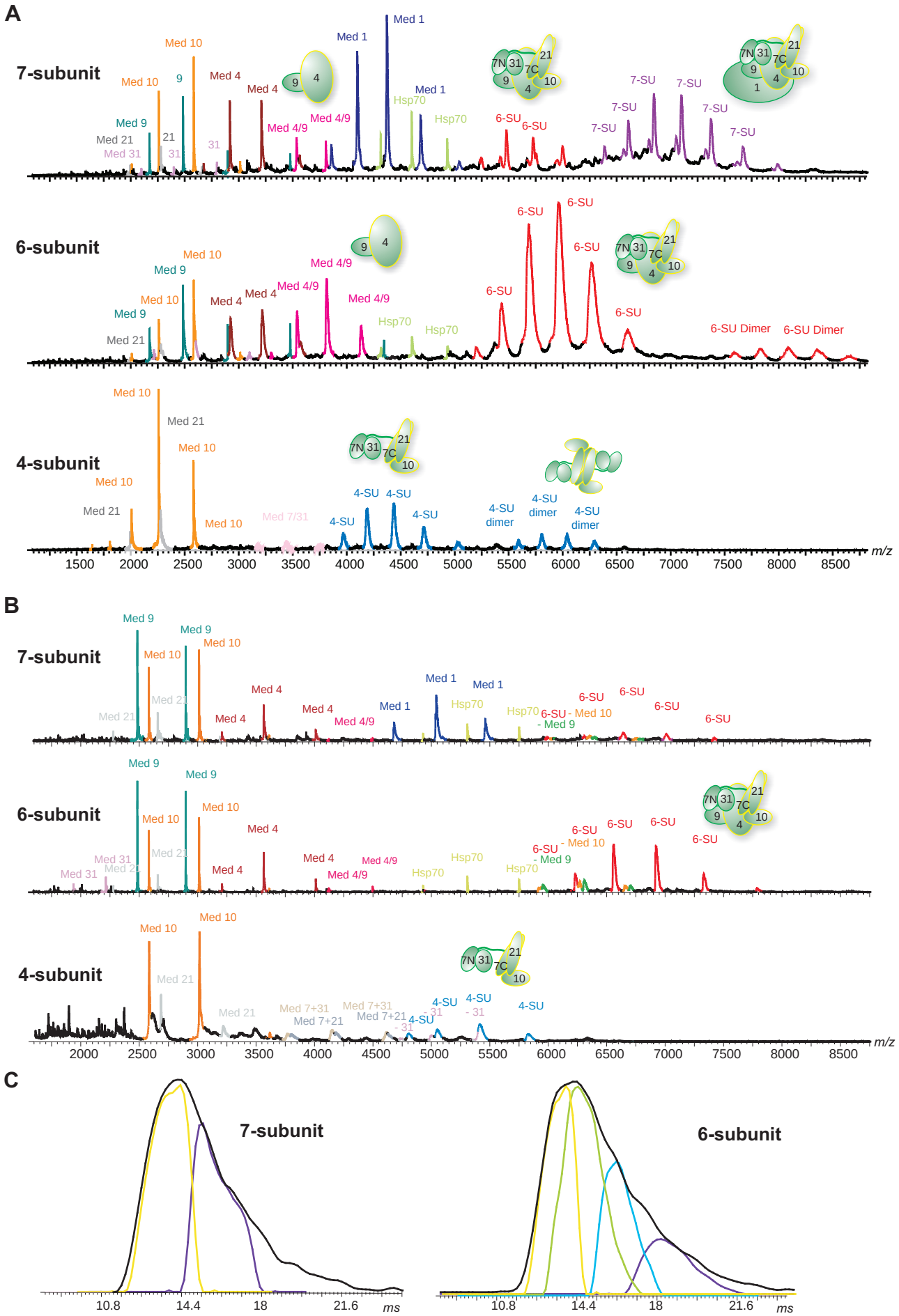
Static light scattering analysis

Static light scattering analysis was performed using a Superose 6 gel filtration column (GE Healthcare) combined with a triple detector TDA (Viscotek). Med7C/21 was analyzed in buffer E (150 mM KCl, 10 mM Hepes pH 7.3, 5 mM DTT) at a concentration of 1.8 mg/ml, Med7N/31 and Med4/7/9/10/21/31 were analyzed in buffer A at 1.0 mg/ml. Data analysis was performed with OmniSEC software (Viscotek).

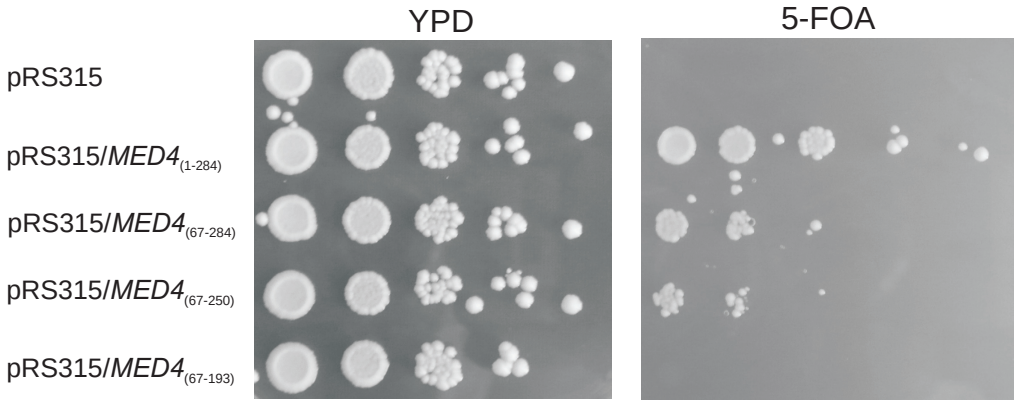
SUPPLEMENTARY FIGURES AND TABLES



Supplementary Figure 1. Destabilization of the Mediator complex. Mediator has been purified in med19Δ background strains via a C-terminal TAP-tag on Med7 from the middle module, Med18 from the head module and Med15 from the tail module. Copurifying proteins were separated on a 12% NuPAGE gel (Invitrogen), and bands were stained with Coomassie blue. Using our strain background, we did not observe loss of the middle module for Med18-TAP/med19Δ or Med15-TAP /med19Δ strains even after 1M urea washing during purification (using 2 L yeast culture per lane). However, in a Med7-TAP /med19Δ strain, the remaining modules are lost during purification even without urea treatment and exclusively the middle module is purified.



Supplementary Figure 2. Mass spectrometry analyses of Mediator middle modules. (A) Native mass spectrometry analyses of Mediator middle modules. Shown are spectra of 4-, 6-, and 7-subunit (SU) middle subcomplexes, respectively and of their subcomponents. All subunits are present in monomeric stoichiometry. The composition of each complex and subcomplex has been confirmed by tandem MS. Hsp70 (DnaK) is a common contaminant in purifications, it has been confirmed by proteomics experiments that the protein with the mass of 69 kDa is DnaK. (B) DMSO Spectra of the Mediator middle modules. Shown are spectra of 4-, 6-, and 7-subunit middle subcomplexes, respectively and of their subcomponents. The marked complexes and subcomplexes have been confirmed by Tandem MS experiments. (C) Drift time plots for 7- and 6-subunit middle modules (charge state 27^{+} and 20^{+}, respectively) analogous to Figure 6D.



Supplementary Figure 3. Yeast complementation assays. Plasmids encoding full-length MED4 or N- and C-terminal truncations of MED4 were cloned into the SalI/NotI restrictions sites of a pRS315 vector. Individual plasmids were transformed into a MED4 shuffle strain (EuroScarf Y22430 sporulated; MATa or MATα; YOR174W::kanMX4 /pRS316-MED4) and streaked onto 5-FOA-containing plates to shuffle out the MED4 encoding URA3 plasmid. Yeast cells carrying only MED4 (67-250) are viable, whereas the Med4 C-terminal part including residues 193-250 was required to rescue cell growth.

Supplementary Table I. Expression vectors for recombinant Mediator middle module subunits and interaction assays.

Vector	Inserts	Type	Restriction sites	Reference
pSB45	MED7 (103-222) MED21-His_6	pET21b	Nhe I, EcoR I Sal I, Not I	Baumli et al. (1)
pSB48	MED7 (103-222) MED21-His_6	pET24b	Nhe I, EcoR I Sal I, Not I	Baumli et al. (1)
pSB60	MED10	pET21b	Nde I, EcoR I	This work
pSB77	MED10-His_6	pET24b	Nde I, Not I	This work
pSB91	MED4	pET21b	Nde I, Not I	This work
pSB102	MED31-His_6	pET24d	Nco I, EcoR I	This work
pSB104	MED10 MED7 MED21	pET21b	Nhe I, EcoR I Nco I, Sal I Nde I, Xho I	This work
pSB118	MED9 MED4	pET21b	Nde I, BamH I Nde I, Xho I	This work
pTK24	MED31-His_6 MED7 (1-101)	pET24d	Nco I, EcoR I Sal I, Not I	This work
pTK26	His_6-thrombin-MED31	pET28b	Nde I, Not I	This work
pTK69	MED4 (19-250)	pET21b	Sal I, Xho I	This work
pTK70	His_6-thrombin-MED9	pET28b	Nde I, Sal I	This work
pTK74	His_6-thrombin-MED9 Δ(19-63)	pET28b	Nde I, Sal I	This work
pTK97	MED10 (1-73)	pET21b	Nde I, EcoR I	This work
pTK98	MED10 (74-157)	pET21b	Nde I, EcoR I	This work
pTK99	MED31-His_6 StrepII-MED7 (1-84)	pET24d	Nco I, EcoR I Sal I, Not I	This work
pTK101	StrepII-MED14 (1-259)	pET24d	Nco I, Xho I	This work
pTK104	StrepII-MED1 MED14 (1-259)	pET24d	Nco I, Sal I Sal I, Not I	This work
pTK107	MED1	pET24d	Nco I, Sal I	This work
pTK114	StrepII-MED1 MED9 MED4	pCDFDuet-1	Nco I, Not I in MCS1 Asi SI, BamH I in MCS2 Nde I, Xho I in MCS2	This work
pTK133	MED31-His_6 StrepII-MED7 (1-101)	pET24d	Nco I, EcoR I Sal I, Not I	This work

Supplementary Table II. Mediator middle module interaction assays[a].

Subunit 1	Subunit 2	Subunit 3	Subunit 4	Technique	Reference
Med7 (101-222)	Med21 fl[b]	-	-	Structure	(1)
Med4 fl	Med21-His_6 fl	-	-	Gel filtration	(1)
Med10 fl	Med21-His_6 fl	-	-	Gel filtration	(1)
Med4-His_6 fl	Med21 fl	Med7 (104-222)	-	Gel filtration	(1)
Med7 (1-84)	Med31 fl	-	-	Structure	(2)
StrepII-Med7 (1-101)	Med31-His_6	Med4 fl	Med9 fl	Coexpr. & pull-down	This work
StrepII-Med7 (1-84)	Med31-His_6	Med4 fl	Med9 fl	Coexpr. & pull-down	This work
StrepII-Med7 (1-84)	Med31 fl	Med7 (104-222)	Med21 fl	Coexpr. & pull-down	This work
StrepII-Med14 (1-259)	Med10 fl	-	-	Coexpr. & pull-down	This work
StrepII-Med14 (1-259)	Med4 fl	Med9 fl	-	Coexpr. & pull-down	This work
Med21-**His_6** fl	Med7 (104-222)	Med10 (74-157)	-	Coexpr. & pull-down	This work
Med10-**His_6**	Med4*			Coexpr. & pull-down	This work
Med10-**His_6**	Med4 fl*	Med9 fl*		Coexpr. & pull-down	This work
StrepII-Med1 fl	Med4 fl	Med9 fl	-	Coexpr. & pull-down	This work
StrepII-Med1 fl	Med14 (1-259)*	-	-	Coexpr. & pull-down	This work
StrepII-Med1 fl	Med14 (1-259)*	Med10 fl*	-	Coexpr. & pull-down	This work
StrepII-Med1 fl	Med14 (1-259)	Med10 (1-73)	-	Coexpr. & pull-down	This work
StrepII-Med1 fl	Med14 (1-259)	Med10 (74-157)	-	Coexpr. & pull-down	This work
Med31-**His_6**	Med7 (1-84)	Med10 fl	-	Coexpr. & pull-down	This work
His_6-Med9 fl	Med4 (19-250)	-	-	Coexpr. & pull-down	This work
His_6-Med9 Δ(19-63)	Med4 fl	-	-	Coexpr. & pull-down	This work

[a] Bold letter indicated the protein purification tags which have been used in the assay. Red subunits were not detected in pull-down experiments. Weak interactions are indicated by a star.

[b] full-length

Supplementary Table III Static light scattering (SLS) and Small-angle X-ray scattering (SAXS) measurements.

Technique	Sample	Hydro-dynamic radius R_H (nm)	Radius of gyration R_G (nm)	Dimensions from X-ray structure (nm)	Determined molecular weight (kDa)	Theoretical molecular weight (kDa)	Oligo-meric state	Conc. (mg/ ml)
SLS	Med7C/21	4.9	-	11.1 x 3.0 x 3.0	60	32	Hetero-tetramer	1.8
SLS	Med7N/31	2.6	-	4.0 x 4.5 x 3.0	30	27	Hetero-dimer	1.0
SLS	Med4/7/9/ 10/21/31	6.6	-	-	130	125	Hetero-hexamer	1.0
SAXS	Med4/7/9/ 10/21/31	-	6.6	-	(607)	125	-	5.0

Supplementary Table IV Calculations of collisional cross sections.
Please note the according Excel table.

References

1. Baumli, S., Hoeppner, S. and Cramer, P. (2005) A conserved mediator hinge revealed in the structure of the MED7.MED21 (Med7.Srb7) heterodimer. *The Journal of biological chemistry*, **280**, 18171--18178.
2. Koschubs, T., Seizl, M., Lariviere, L., Kurth, F., Baumli, S., Martin, D.E. and Cramer, P. (2009) Identification, structure, and functional requirement of the Mediator submodule Med7N/31. *The EMBO journal*, **28**, 69-80.