Supplementary Material

For

Structural insights into the interaction of the nuclear exosome helicase Mtr4 with the pre-ribosomal protein Nop53

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Supplemental Figures

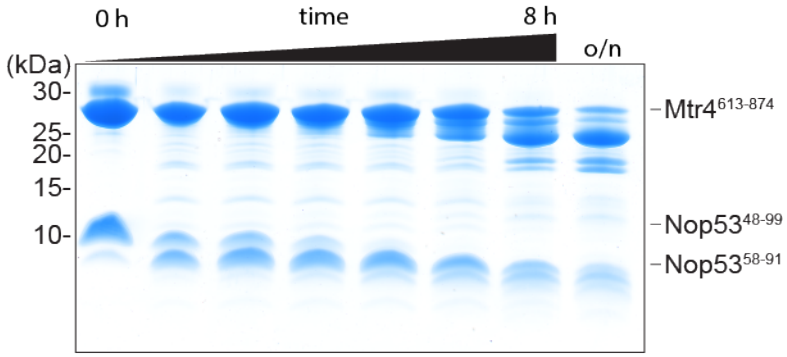


Figure S1. Limited proteolysis of the Mtr4 arch domain Nop53 complex.

The complex of the Mtr4 arch domain ($Mtr4^{arch}$: residues 613-874) and $Nop53^{AIM}$ (residues 48-99) was subjected to limited proteolysis with subtilisin. 10 µg of the $Mtr4^{arch}$-$Nop53^{AIM}$ complex was incubated with 30 ng of subtilisin at 0°C. Samples were taken at different time points and the reaction was stopped by the addition of SDS sample buffer and incubation at 95°C for 5 min. The samples were analysed on a 16% SDS-PAGE and proteins were visualized by Coomassie staining. To determine the sequence of the proteolysed Nop53 fragment, the reaction was upscaled to 500 µg $Mtr4^{arch}$-$Nop53^{AIM}$ and 1.5 µg subtilisin. The reaction was allowed to proceed for 30 min at 0°C, was subsequently quenched by the addition of 1 mM AEBSF (4-(2-Aminoethyl)benzensulfonylfluorid) and was then submitted to gelfiltration on a S75 (10/300) column in 20 mM Tris/HCl, 150 mM NaCl, 2 mM DTT, pH 7.5. Peak fractions were analyzed by ESI-MS to determine the mass and sequence of the Nop53 fragment from the proteolysis experiment.

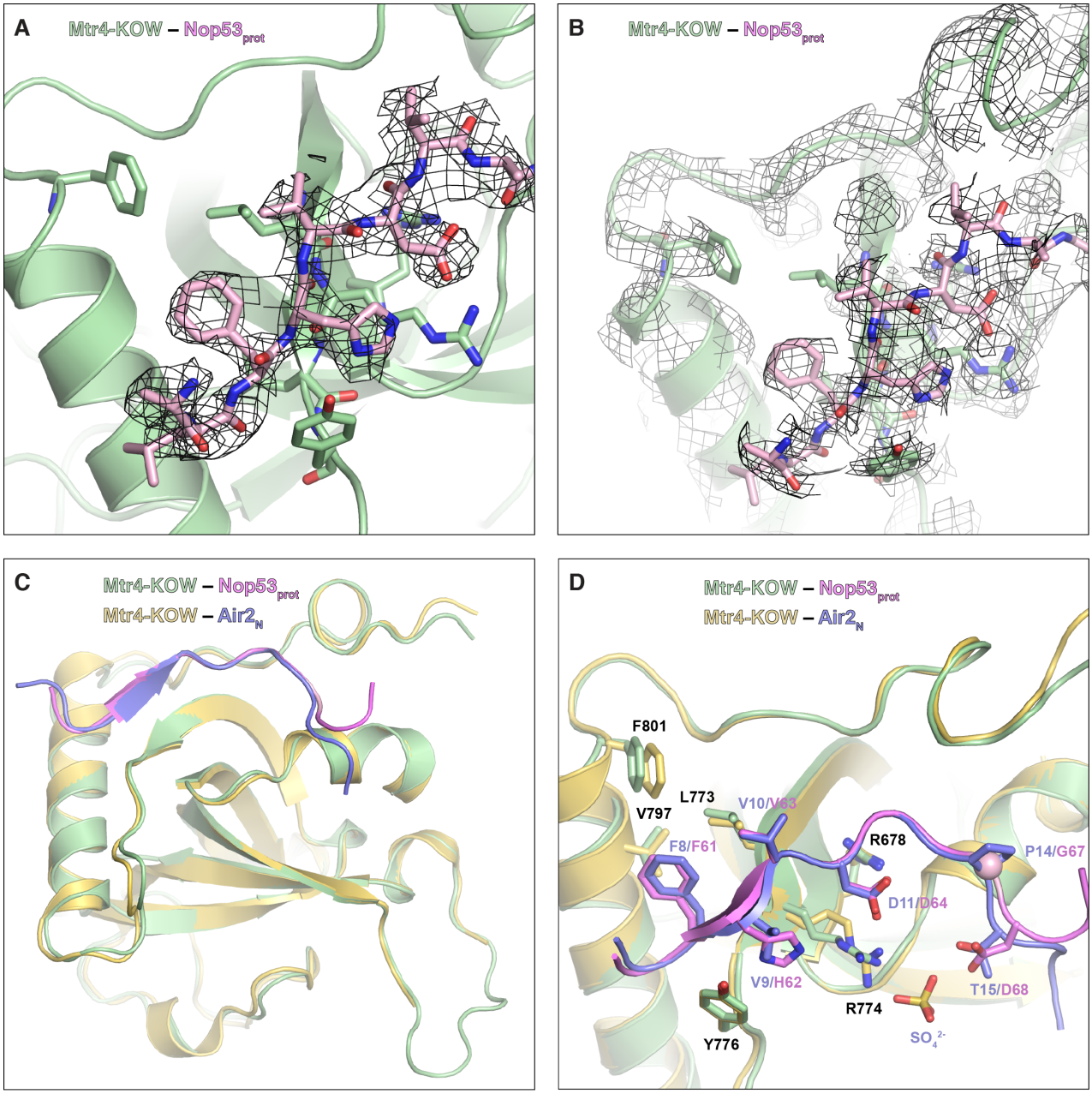


Figure S2. Comparison of the Nop53 AIM motif and the Air 2 AIM-mimicking motif.

(*A*) Snapshot of the refined electron density from the Mtr4-**Δ**N – Nop53 AIM interface. The refined 2mFo-DFc map around the Nop53 AIM peptide (sharpened with -90 Å^2 B-factor) is contoured at 1.0 σ and superposed with the final model. Mtr4 is colored green and the Nop53 AIM peptide in pink. (*B*) Snapshot of the simulated annealing composite omit map (calculated with Phenix) from the Mtr4-**Δ**N – Nop53 AIM interface. The 2mFo-DFc map is contoured at 1.0 σ and superposed with the final model. Mtr4 is colored green and the Nop53 AIM peptide in pink. (*C*)

Superposition of the KOW domains in the Nop53-bound structure and in the Trf4-Air2-bound structure. The AIM-mimicking motif from Air2 that forms a crystal lattice contact in the Trf4-Air2-bound structure (in blue) superposes well with the Nop53 AIM motif (in pink). The Mtr4 KOW domain from the Trf4-Air2-bound structure is shown in yellow the Mtr4 KOW domain of the $Nop53_{prot}$ bound structure in green. (*D*) Zoom in of the superposition showing the details of the interacting residues. The sulfate residue is part of the Trf4-Air2-bound structure and coordinated by Arg774 of Mtr4 and Thr15 of Air2. (*E*) Sequence alignment of the Nop53 and Utp18 AIM motifs with the Air2 AIM-mimicking motif from *S. cerevisae*.

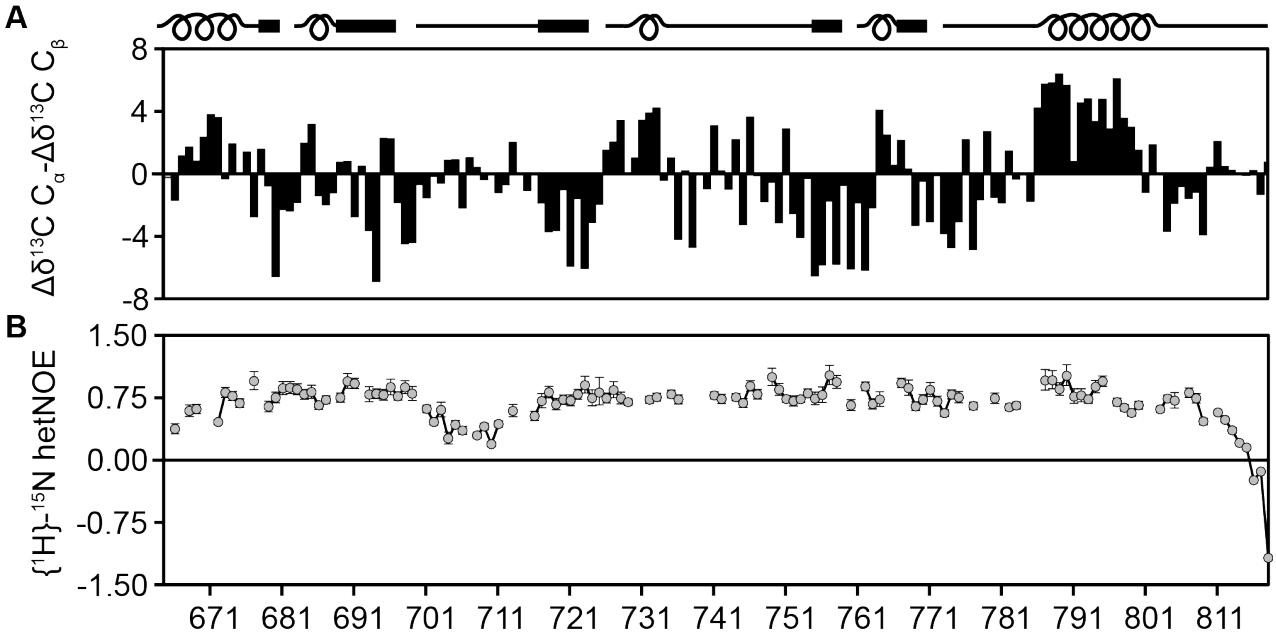
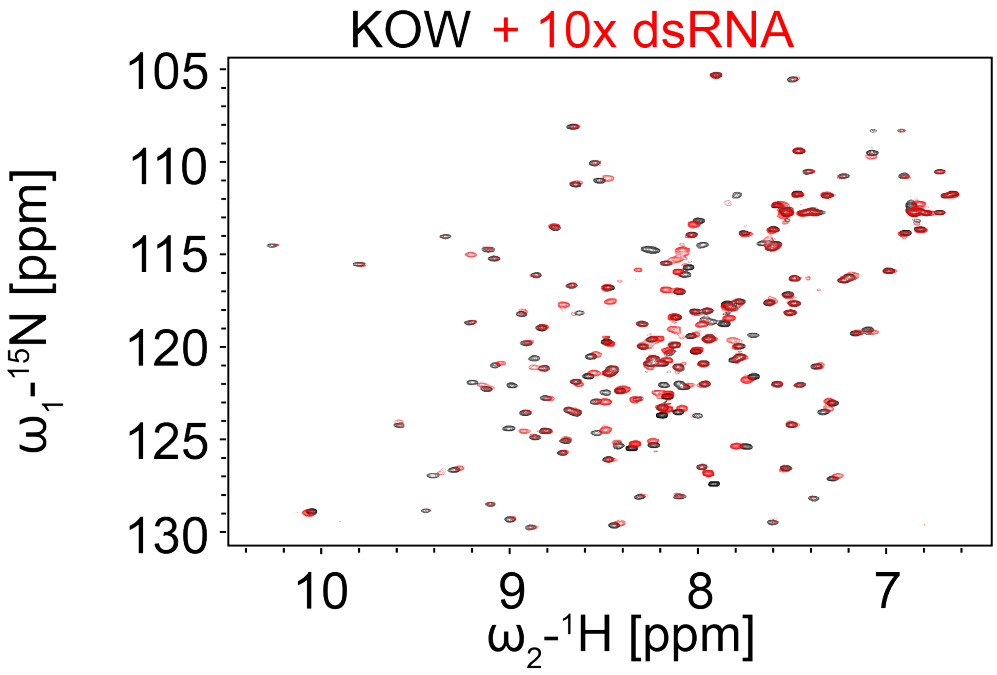


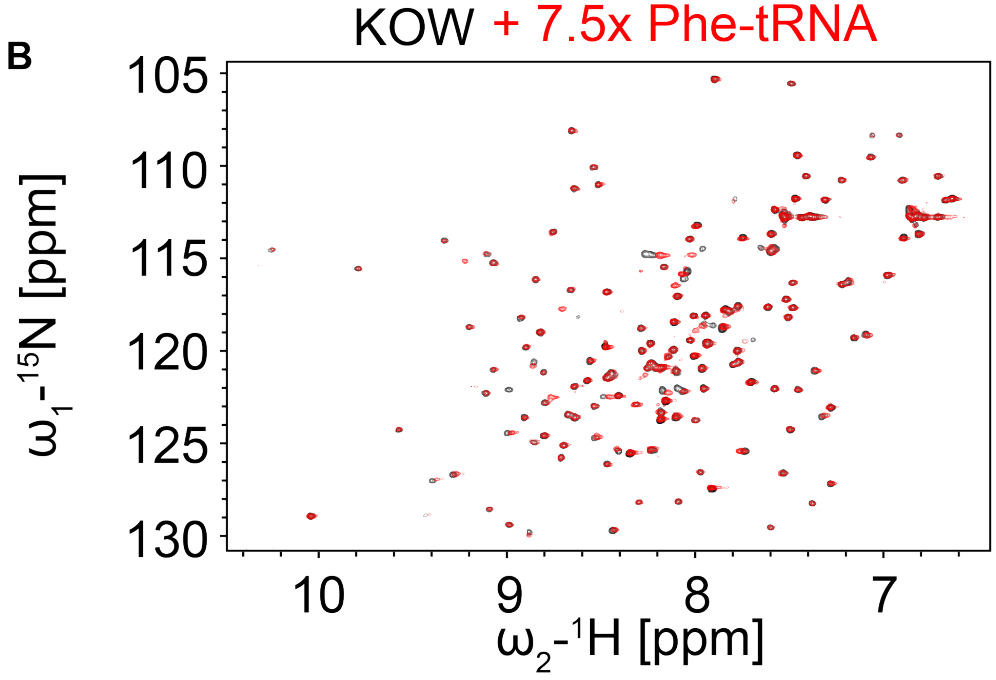
Figure S3. NMR structural analysis.

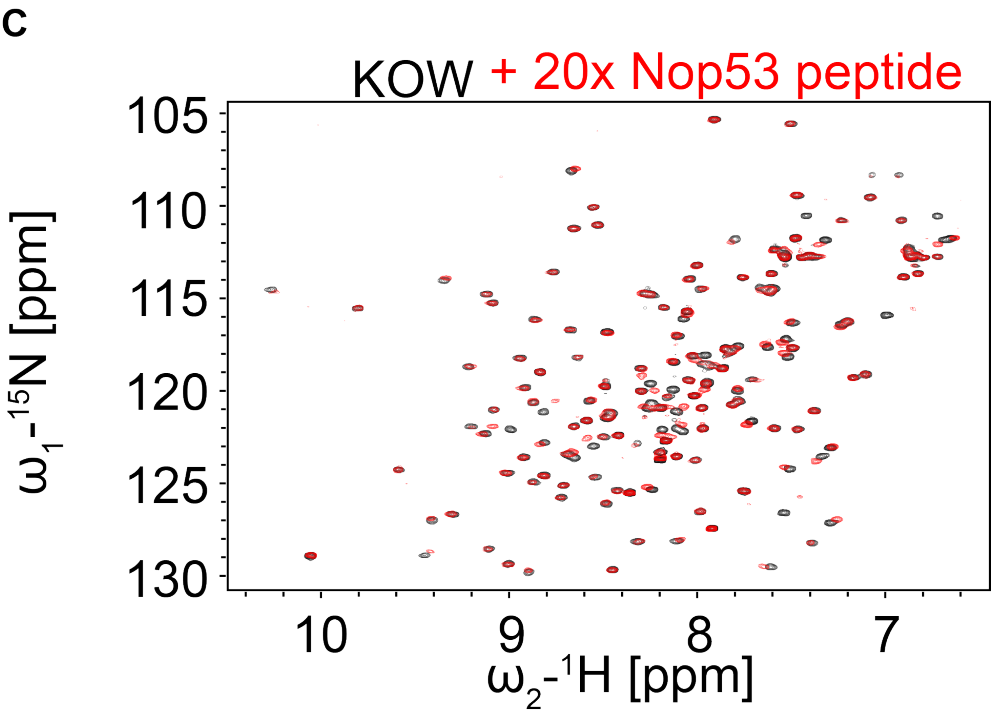
(*A*) ^{13}C secondary chemical shifts recorded for the KOW domain plotted against residue numbers of the full-length Mtr4 protein. Positive values indicate α–helical, negative β–strand regions. The secondary structure elements based on the crystal structure are plotted above as cartoon. (*B*)$\{^1H\}$-^{15}N-heteronuclear NOE data for the free KOW domain. Lower values for residues 701-711 indicate a higher flexibility observed for the loop.

A


KOW + 10x dsRNA
105
110
115
120
125
130
10
9
8
7
ω1-15N [ppm]
ω2-1H [ppm]

B


KOW + 7.5x Phe-tRNA
105
110
115
120
125
130
10
9
8
7
ω1-15N [ppm]
ω2-1H [ppm]



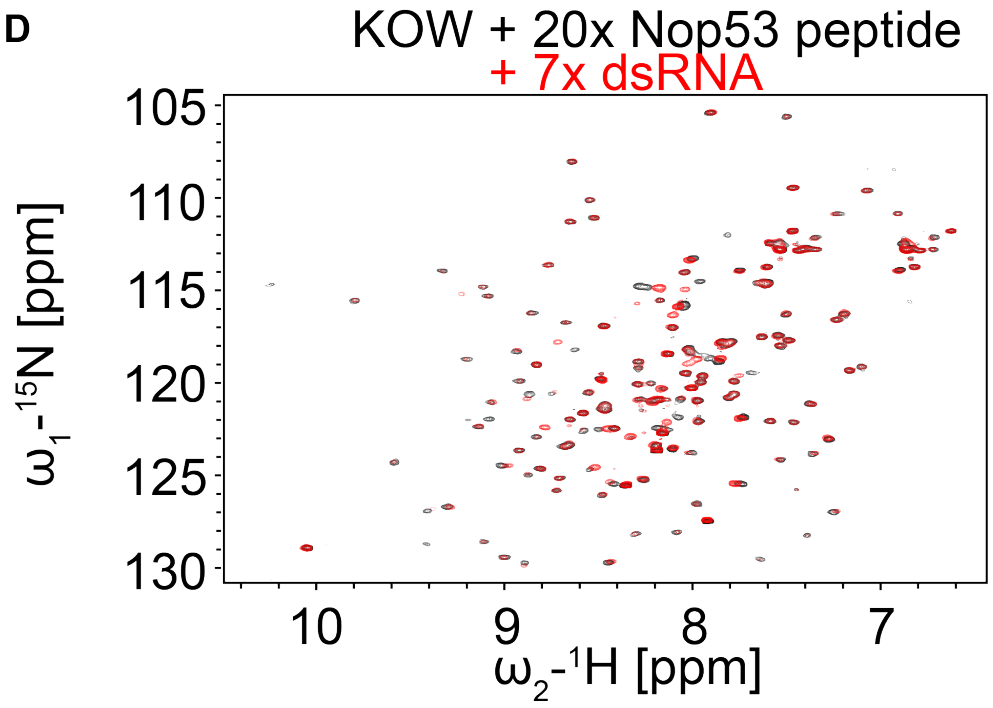


Figure S4. Mtr4 KOW domain titration with various ligands.

^{1}H,^{15}N-HSQC of free KOW (black) and in presence of a ligand (red): (*A*) dsRNA, (*B*) Phe-tRNA, (*C*) Nop53 peptide, (*D*) Nop53 peptide and dsRNA.