SUPPLEMENTARY INFORMATION

for

Transcription is regulated by NusA:NusG interaction

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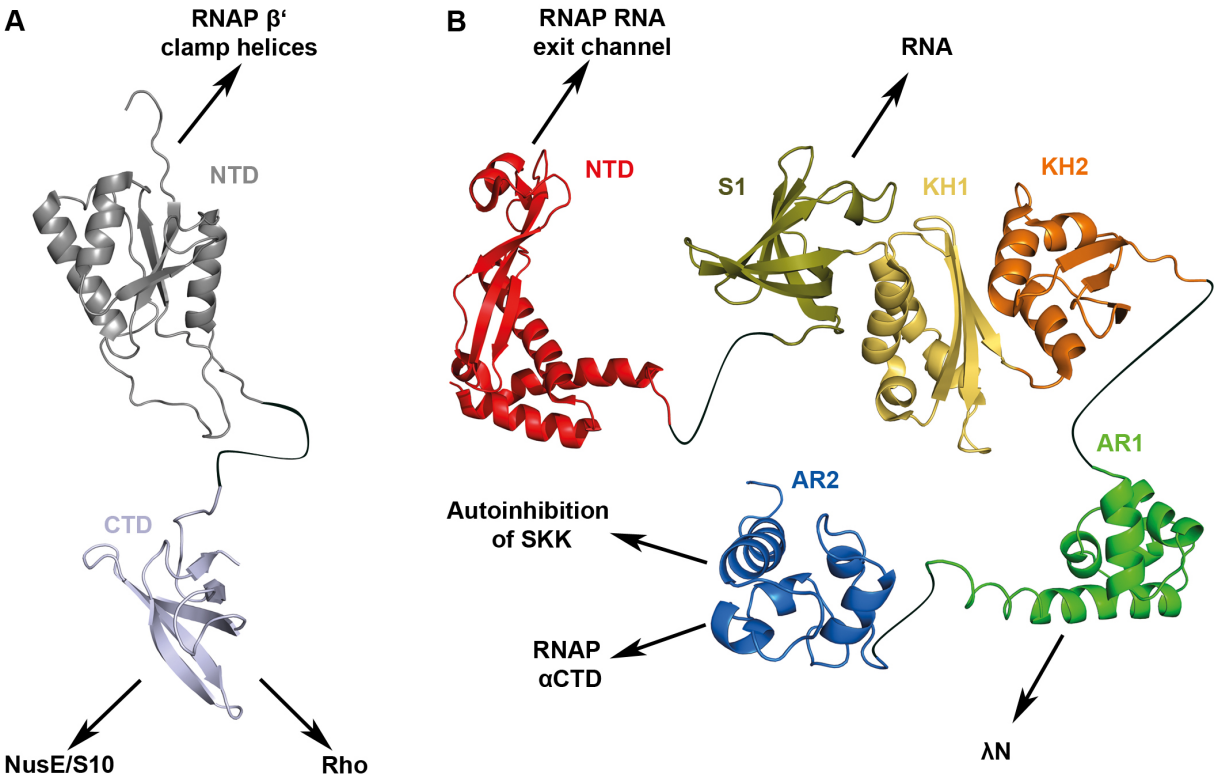
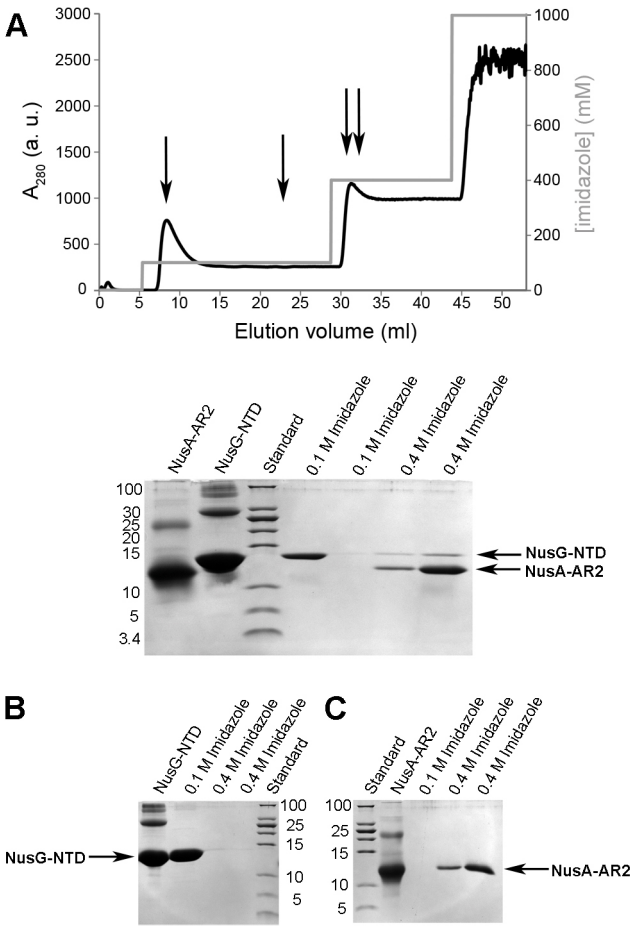
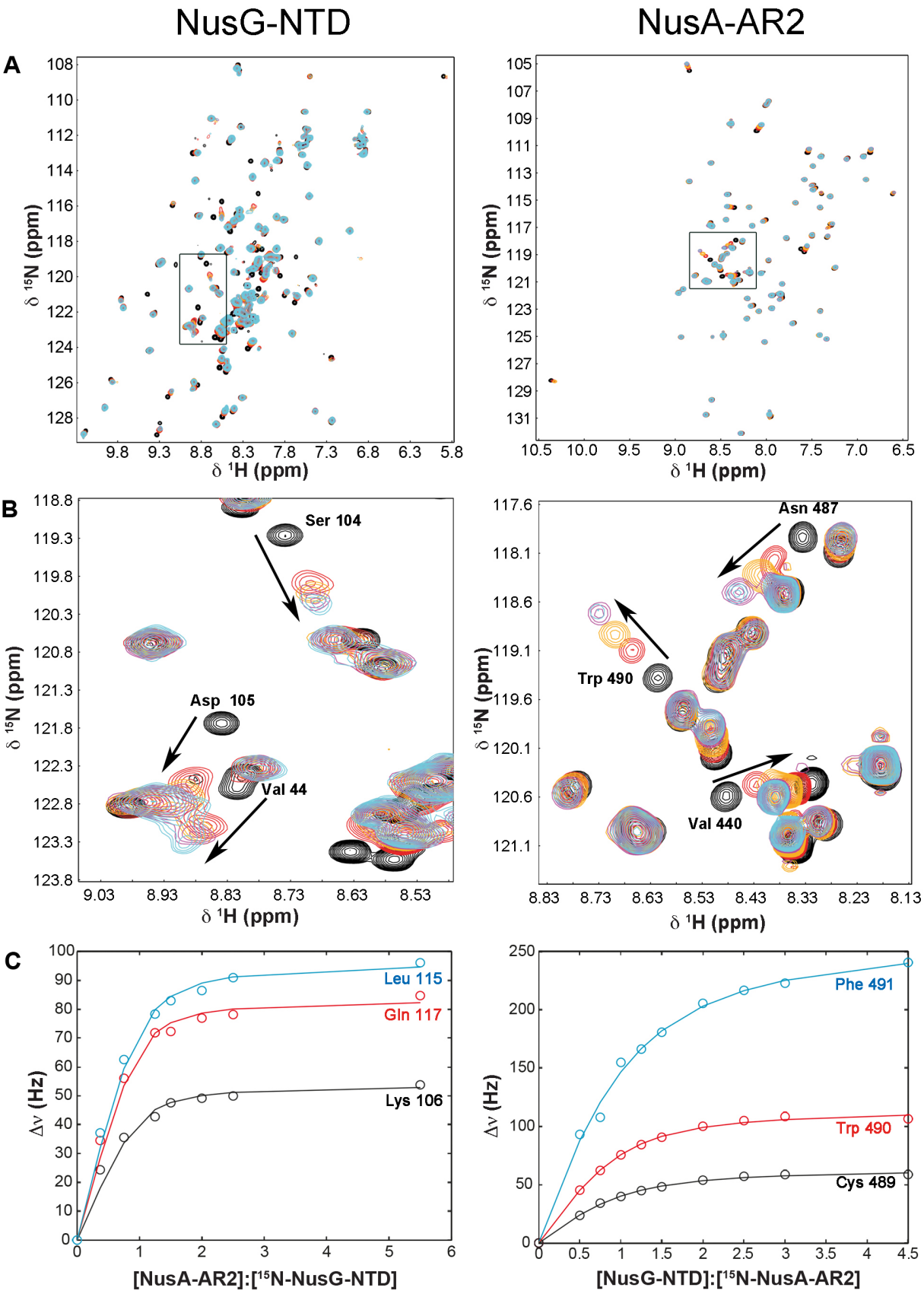


Supplementary Figure S1. Three-dimensional structures of (A) NusG and (B) NusA. Protein structures are in cartoon representation. Arrows indicate the interaction partner(s) of individual domains. (**A**) NusG from *E. coli.* NusG-NTD, gray, PDB ID: 2K06; NusG-CTD, light blue, PDB ID: 2JVV; flexible linker, black line. (**B**) NusA from *E. coli.* NusA-NTD, red, PDB ID: 2KWP; NusA-S1, olive; NusA-KH1, yellow; NusA-KH2, orange (as no structure of *E. coli* NusA-SKK is available the structure of *Thermotoga maritima* NusA-SKK is shown, PDB ID: 1HH2); NusA-AR1, green, PDB ID: 1WCL; NusA-AR2, blue, PDB ID: 1WCN; linker, black line.

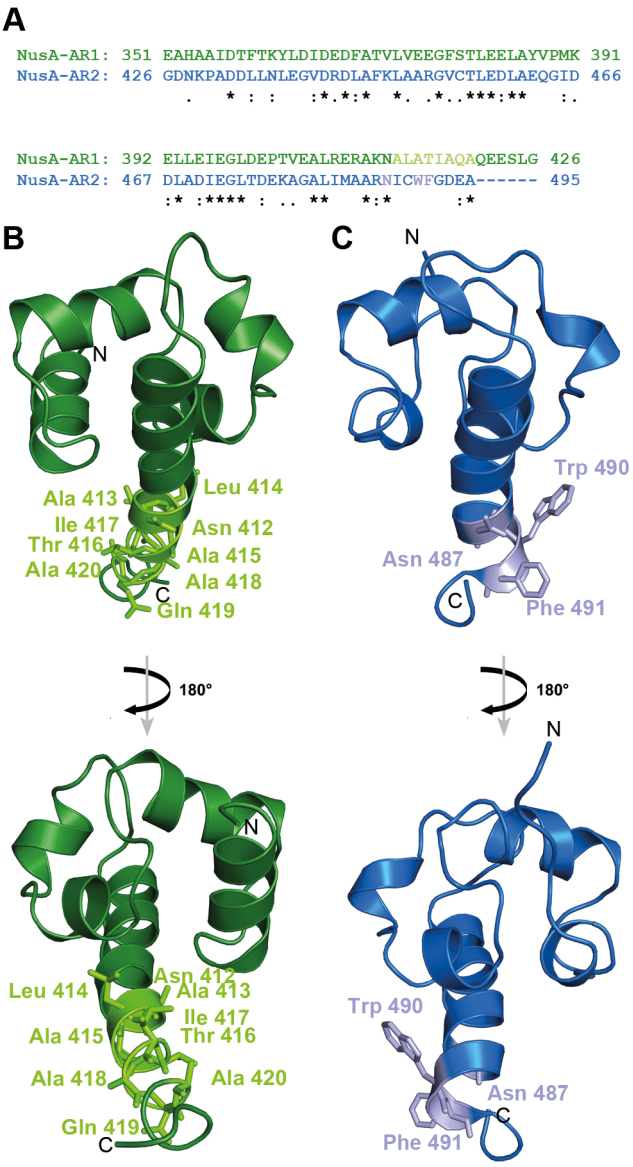


Supplementary Figure S2. Pull-down of NusG-NTD with His_{10}-NusA-AR2. (**A**) His_{10}-NusA-AR2 (200 μM) and NusG-NTD (400 μM) were preincubated for 15 min and then applied to a 1 ml HisTrap column. After washing, stepwise elution was carried out with 100 and 400 mM imidazole. (Upper panel) Chromatogram of the pull-down assay. Arrows indicate the fractions analyzed by sodium dodecylsulfate (SDS) polyacrylamide gel electrophoresis. (lower panel) 20 % SDS polyacrylamide gel of samples taken during the pull-down assay. NusA-AR2, pure His_{10}-NusA-AR2; NusG-NTD, pure NusG-NTD; 0.1 M imidazole, elution with 100 mM imidazole; 0.4 M imidazole, elution with 400 mM imidazole. (**B,C**) Control experiments with (**B**) NusG-NTD and (**C**) His_{10}-NusA-AR2. Isolated NusG-NTD (400 μM) or His_{10}-NusA-AR2 (200 μM) was applied to the column and treated like in (**A**).

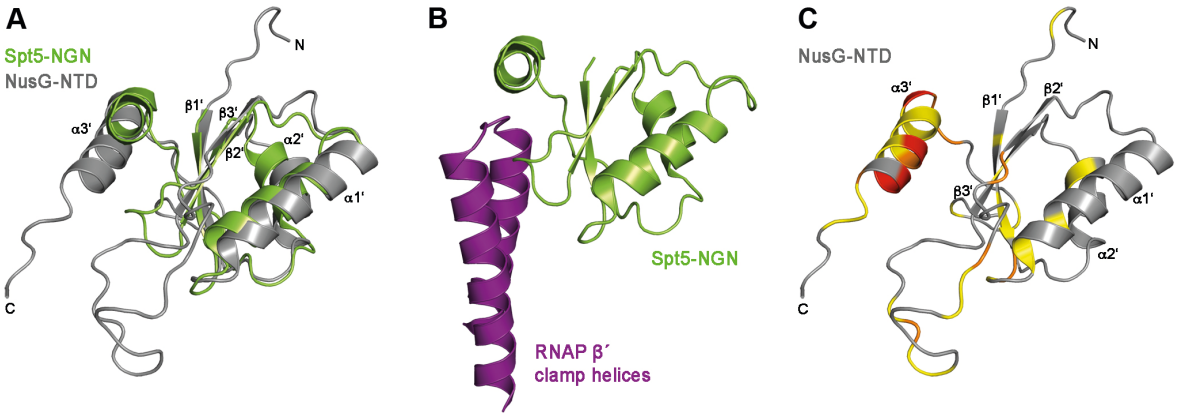


Supplementary Figure S3. Determination of K_D values of the NusA-AR2:NusG-NTD complex. (**A**, left) [^{1}H,^{15}N]-HSQC titration of ^{15}N-NusG-NTD (140 μM) with NusA-AR2. NusA-AR2 was added in

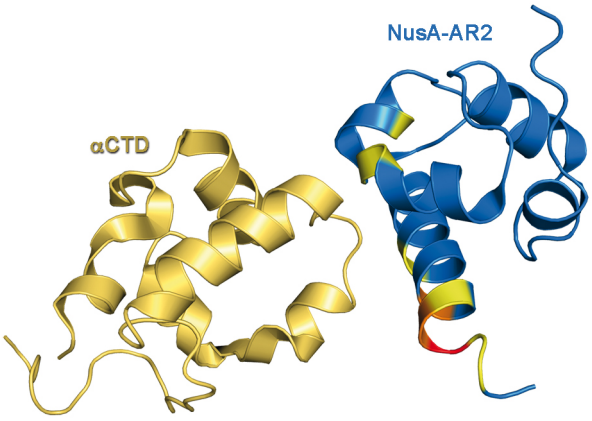
molar ratios of 1:0, black, 1:0.75, red, 1:1.25, orange, 1:2.5, magenta, and 1:3.5, cyan. (right) [^{1}H,^{15}N]-HSQC titration of ^{15}N-NusA-AR2 with NusG-NTD. Spectra corresponding to molar ratios 1:0, 1:0.5, 1:1, 1:2.5, and 1:3 are in black, red, orange, magenta, and cyan, respectively. (**B**) Magnifications of (**A**). Selected signals are labeled. (**C**) Backbone amide chemical shift perturbations for selected residues obtained form (**A**) *vs.* molar ratio of the titration partners. (Left) ^{15}N-NusG-NTD+NusA-AR2; (right) ^{15}N-NusA-AR2 + NusG-NTD. The lines represent nonlinear least squares best fits of the normalized changes in the ^{1}H and ^{15}N chemical shifts, based on a bimolecular equilibrium binding model. The optimized average K_D values are 13 μM for ^{15}N-NusG-NTD + NusA-AR2 and 35 μM for ^{15}N-NusA-AR2 + NusG-NTD, yielding an overall K_D of approximately 22 μM for the NusA-AR2:NusG-NTD interaction.



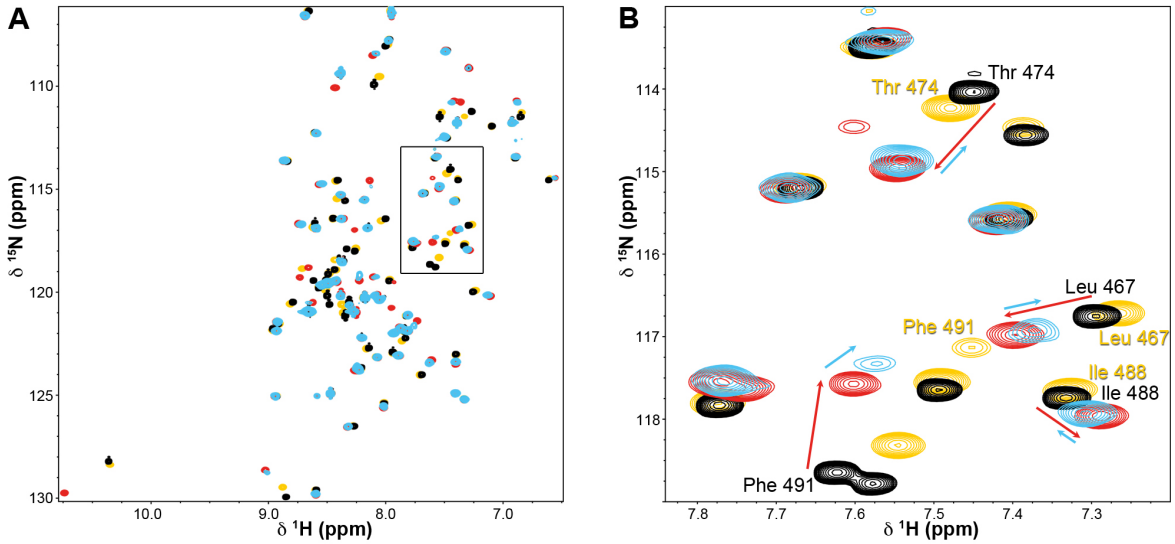
Supplementary Figure S4. Comparison of NusA-AR1 and NusA-AR2. (**A**) Amino acid sequence alignment of NusA-AR1 and NusA-AR2. Asterisk, identical amino acids; colon, conservation between groups of strongly similar properties; dot, conservation between groups of weakly similar properties. (**B,C**) Structures of (**A**) NusA-AR1, green, and (**B**) NusA-AR2, blue, both in cartoon representation. Residues of NusA-AR2 which are strongly affected by NusG-NTD binding ($\Delta\delta_{norm}$ > 0.12 ppm) as well as corresponding residues in NusA-AR1 are shown as sticks in light colours and labelled. PDB IDs: NusA-AR1, 1WCL; NusA-AR2, 1WCN. .



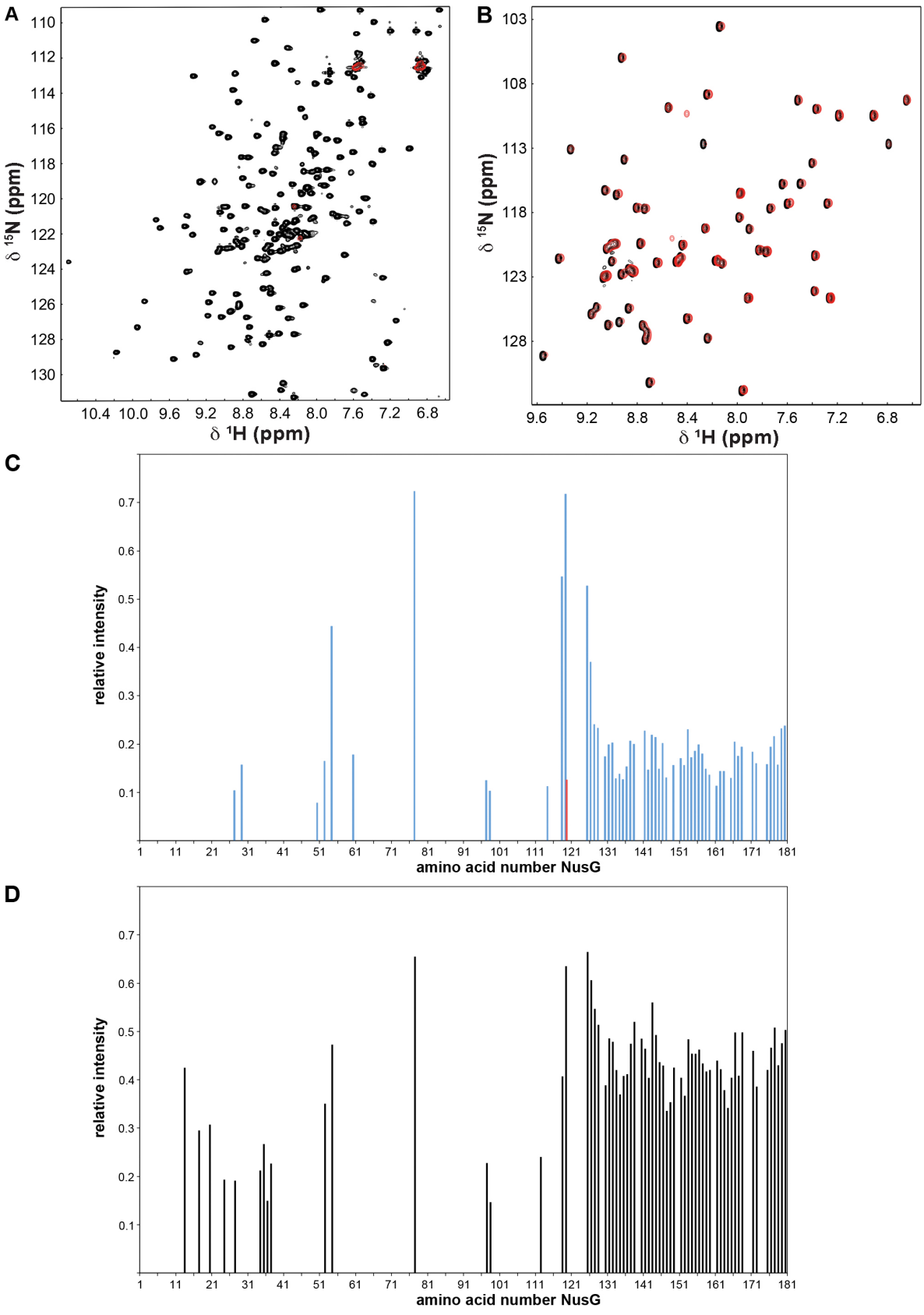
Supplementary Figure S5. Binding of NusG proteins to RNAP β'CH. (**A**) Superposition of Spt5-NusG N-terminal domain (NGN) from *Pyrococcus furiosus* (*P. furiosus*, green, PDB ID: 3QQC) and NusG-NTD from *E. coli* (grey, PDB ID: 2K06), both in cartoon representation. (**B**) Spt5-NGN bound to the β'CH (purple) in *P. furiosus* (PDB ID: 3QQC). (**C**) NusG-NTD in the same orientation as in (**A**). Residues that are affected by the interaction with NusA-AR2 are in red (strongly affected), orange (moderately affected), and yellow (slightly affected), see Figure 3.



Supplementary Figure S6. The NusA-AR2 binding sites for αCTD and NusG-NTD overlap. Solution structure of the NusA-AR2:αCTD complex (PDB ID: 2JZB, cartoon representation). Dark yellow, αCTD; blue, NusA-AR2. Residues of NusA-AR2 that are affected by the interaction with NusG-NTD are in red (strongly affected), orange (moderately affected), and yellow (slightly affected), see Figure 3.



Supplementary Figure S7. NusG-NTD:NusA-AR2 interaction in the presence of RNAP. (**A**) [^{1}H,^{15}N]-HSQC displacement experiment of ^{15}N-NusA-AR2 from αCTD by NusG-NTD. Black, ^{15}N-NusA-AR2 (100 μM); red, ^{15}N-NusA-AR2:αCTD = 1:1 (100 μM each); blue, ^{15}N-NusA-AR2:αCTD:NusG-NTD = 1:1:5; yellow, ^{15}N-NusA-AR2:NusG-NTD = 1:3. (**B**) Detail of (**A**). Red arrows, chemical shift changes of ^{15}N-NusA-AR2 upon ^{15}N-NusA-AR2:αCTD complex formation; blue arrows, chemical shift changes of ^{15}N-NusA-AR2 upon addition of NusG-NTD.



Supplementary Figure S8. NusG:RNAP *vs.* NusG:NusA *vs.* NusA:NusG. (**A**) [^{1}H,^{15}N]-HSQC spectrum of 50 μM ^{15}N-NusG in the absence, black, or presence, red, of RNAP in equimolar

concentration. (**B**) [^{1}H,^{15}N]-HSQC spectrum of 50 μM ^{15}N-NusG-CTD in the absence, black, or presence, red, of RNAP in equimolar concentration. (**C**) NusG binds to NusA in the presence of RNAP. Intensity plots of the titration of Fig. 4C. Relative intensities were calculated in respect to free NusG. Blue, ^{15}N-NusG:NusA = 1:1; red, ^{15}N-NusG:NusA:RNAP = 1:1:1 (**D**) NusG:NusA-AR2 complex remains intact in the presence of RNAP. Intensity plot of the displacement experiment in Fig. 4D. Relative intensities were calculated as ratio of intensities of ^{15}N-NusG signals in the presence of NusA-AR2 and RNAP (1:1:1) and ^{15}N-NusG signals in the presence of NusA-AR2 (1:1).