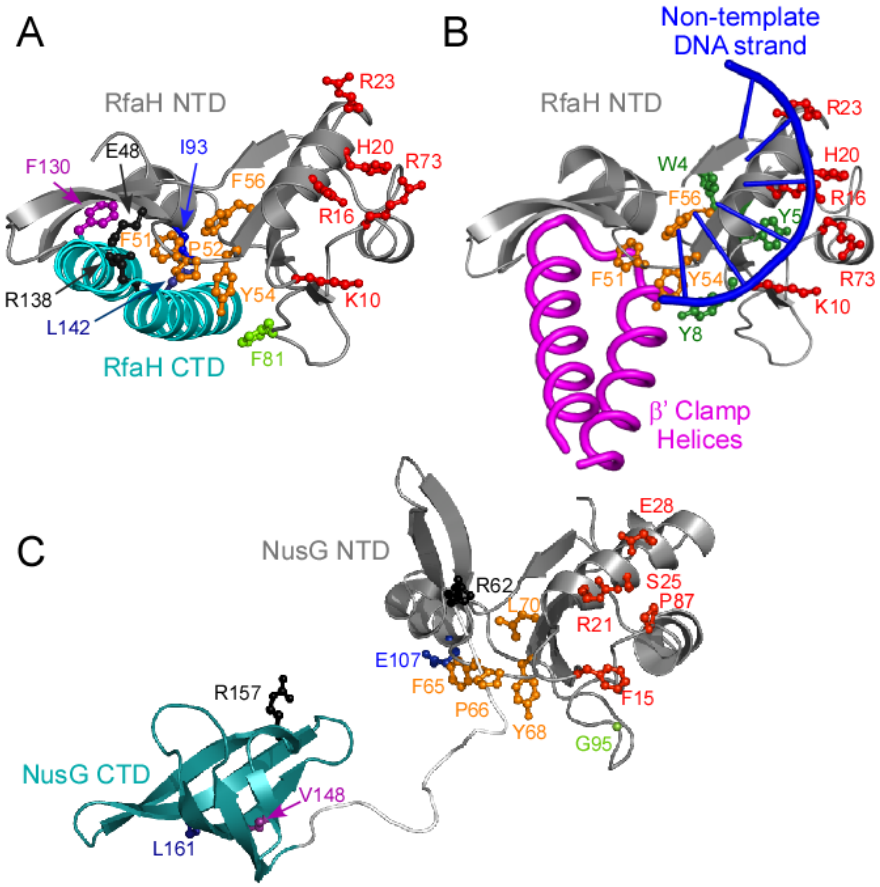

Supplementary Figure 1. Different states of RfaH and NusG proteins. (A) The closed state of RfaH; PDB ID 2OUG, (B) a model of the open, RNAP-bound state of RfaH, and (C) the open state of NusG; PDB ID 2K06 and 2KVQ. Key residues are shown as ball-and-stick models; the residues at corresponding positions in RfaH and NusG are colored similarly to facilitate comparison. An ionic pair between Glu48 in the NTD and Arg138 in the CTD (in black) is essential for maintaining the closed state of RfaH (A); Glu48 corresponds to Arg62 in NusG (C). In the TEC (B), RfaH interacts with the non-template DNA (blue) and the β' Clamp Helices domain of RNAP (magenta); this model is supported by *in vivo* and *in vitro* analyses (Ref. 19 in the main text). Residues in red are proposed to interact with the *ops* DNA element, and their substitutions compromise recruitment; among these, only one (Arg21; red) is identical in NusG. Substitutions of Trp4, Tyr5, and Tyr8 (dark green) and Phe51, Tyr54 and Phe56 (orange) lead to defects in RNAP binding; Phe51, Tyr54 and Phe56 are located at the RfaH IDI. The CTD folds as a β-barrel whenever its contacts with the NTD are destabilized (*e.g.*, by E48A substitution) or abolished (*e.g.*, by cleavage of the interdomain linker or RNAP binding). This figure was prepared with Pymol 1.8.2.3 (Schrodinger, LLC).

Residue number	Residue name	Conservation score	IDI contact area (A^2)	Entropy	$\Delta\Delta G_{bind}$ (kcal/mol)
2	Gln	0.55	0.00	2.13	-8.61
3	Ser	0.52	0.00	2.18	0.97
4	Trp	0.97	0.00	0.00	0.88
5	Tyr	0.94	0.00	0.27	0.80
6	Leu	0.92	31.10	0.44	1.06
7	Leu	0.87	0.00	1.18	0.98
8	Tyr	0.71	7.11	0.90	0.74
9	Cys	0.79	0.00	0.49	0.82
10	Lys	0.98	0.00	0.14	0.86
11	Arg	0.65	0.00	1.00	0.89
12	Gly	0.64	0.00	1.35	0.00
13	Gln	0.86	0.00	0.68	0.78
14	Leu	0.59	0.00	1.83	0.98
15	Gln	0.48	0.00	2.31	0.98
16	Arg	0.95	0.00	0.25	0.98
17	Ala	0.98	0.00	0.01	0.99
18	Gln	0.64	0.00	1.86	0.98
19	Glu	0.73	0.00	1.63	0.93
20	His	0.83	0.00	0.46	1.01
21	Leu	0.99	0.00	0.06	0.90
22	Glu	0.65	0.00	1.93	0.92
23	Arg	0.72	0.00	1.05	1.04
24	Gln	0.99	0.00	0.01	1.02
25	Ala	0.52	0.00	2.07	0.94
26	Val	0.72	0.00	1.37	1.06
27	Asn	0.59	0.00	2.06	0.92
28	Cys	0.79	0.00	0.50	0.89
29	Leu	0.70	0.00	1.55	0.96
30	Ala	0.47	0.00	2.01	0.99
31	Pro	0.98	0.00	0.05	1.06
32	Met	0.57	0.00	1.76	0.91
33	Ile	0.67	40.43	2.05	-0.21
34	Thr	0.50	0.00	2.33	0.95
35	Leu	0.60	38.35	2.34	1.02
36	Glu	0.79	0.00	1.31	0.97
37	Lys	0.84	0.00	0.81	1.41
38	Ile	0.69	0.00	1.72	0.90
39	Val	0.58	0.00	2.03	0.82
40	Arg	0.74	0.00	1.23	0.80
41	Gly	0.81	0.00	1.00	0.00
42	Lys	0.83	0.00	1.17	0.89
43	Arg	0.60	0.00	0.77	0.86
44	Thr	0.54	0.00	1.94	0.68
45	Ala	0.44	0.00	2.53	0.83
46	Val	0.63	0.00	1.87	1.01
47	Ser	0.49	0.00	2.56	0.95
48	Glu	0.91	12.97	0.57	1.61
49	Pro	0.79	5.07	0.71	0.91

Residue number	Residue name	Conservation score	IDI contact area (A^2)	Entropy	$\Delta\Delta G_{bind}$ (kcal/mol)
50	Leu	0.98	57.80	0.45	1.37
51	Phe	1.00	79.82	0.01	3.06
52	Pro	0.99	55.46	0.02	1.71
53	Asn	0.63	1.81	1.42	1.02
54	Tyr	1.00	57.46	0.01	1.49
55	Leu	0.92	0.00	1.07	0.97
56	Phe	1.00	12.44	0.01	1.12
57	Val	0.90	0.00	0.93	1.09
58	Glu	0.59	0.00	2.01	1.13
59	Phe	0.79	0.00	1.05	0.85
60	Asp	0.87	0.00	0.80	0.97
61	Pro	0.54	0.00	1.88	1.02
62	Glu	0.62	0.00	1.76	1.00
63	Val	0.31	0.00	2.40	0.97
64	Ile	0.54	0.00	1.71	0.88
65	His	0.65	0.00	1.15	0.94
66	Thr	0.59	0.00	1.16	0.94
67	Thr	0.66	0.00	1.60	0.92
68	Thr	0.67	0.00	1.29	0.97
69	Ile	0.93	0.00	0.79	1.11
70	Asn	0.65	0.00	1.48	0.86
71	Ala	0.86	0.00	0.82	0.88
72	Thr	0.99	0.00	0.05	1.02
73	Arg	0.97	0.00	0.22	0.79
74	Gly	1.00	0.00	0.01	0.00
75	Val	0.98	0.00	0.31	0.96
76	Ser	0.69	0.00	1.62	0.77
77	His	0.58	0.00	1.28	0.89
78	Phe	0.77	0.00	0.97	0.78
79	Val	0.97	16.59	0.53	0.75
80	Arg	0.65	0.00	1.45	0.96
81	Phe	0.88	96.40	0.75	3.88
82	Gly	0.90	0.00	0.66	0.00
83	Ala	0.41	0.00	2.25	0.98
84	Ser	0.47	0.00	2.15	0.98
85	Pro	0.87	0.00	0.79	0.96
86	Ala	0.56	0.00	2.04	0.82
87	Ile	0.45	36.80	2.15	0.07
88	Val	0.94	8.46	0.74	0.92
89	Pro	0.59	15.55	1.53	0.48
90	Ser	0.54	14.15	2.18	0.29
91	Ala	0.49	0.00	2.42	0.59
92	Val	0.86	22.81	1.10	1.20
93	Ile	0.97	90.36	0.48	2.26
94	His	0.52	0.00	2.04	0.79
95	Gln	0.64	0.00	1.78	0.91
96	Leu	0.83	72.29	1.00	1.83
97	Ser	0.50	22.94	1.87	0.25

Residue number	Residue name	Conservation score	IDI contact area (A^2)	Entropy	$\Delta\Delta G_{bind}$ (kcal/mol)
98	Val	0.37	0.00	2.46	0.98
99	Tyr	0.26	0.00	2.16	0.88
100	Lys	0.24	21.65	2.42	1.55
115	Lys	0.48	0.00	2.51	-8.43
116	Val	0.95	0.00	0.51	0.95
117	Ile	0.51	0.00	2.41	0.95
118	Ile	0.92	20.21	0.83	1.41
119	Thr	0.62	0.00	1.81	1.09
120	Glu	0.61	0.00	2.14	0.98
121	Gly	0.91	0.00	0.54	0.00
122	Ala	0.47	27.99	2.16	1.64
123	Phe	0.89	20.67	0.75	1.57
124	Glu	0.54	0.00	2.13	0.98
125	Gly	0.73	0.00	1.12	0.00
126	Phe	0.82	80.34	1.63	3.11
127	Gln	0.75	0.00	1.67	0.97
128	Ala	0.92	0.00	0.40	0.95
129	Ile	0.97	15.36	0.37	0.29
130	Phe	0.90	99.58	0.81	2.77
131	Thr	0.48	1.46	2.21	0.95
132	Glu	0.69	2.73	1.56	0.83
133	Pro	0.55	22.76	1.92	1.15
134	Asp	0.88	0.00	0.83	0.95
135	Gly	0.96	21.35	0.20	0.00
136	Glu	0.81	39.86	1.23	0.91
137	Ala	0.54	0.00	2.02	0.99
138	Arg	0.98	78.08	0.01	4.59
139	Ser	0.75	44.41	1.18	0.43
140	Met	0.78	0.55	1.41	0.97
141	Leu	0.86	0.00	1.31	0.93
142	Leu	0.97	60.64	0.33	1.63
143	Leu	0.86	57.53	1.33	1.28
144	Asn	0.76	0.00	1.21	1.00
145	Leu	0.84	6.74	1.28	0.67
146	Ile	0.90	81.28	1.04	1.01
147	Asn	0.66	16.85	1.35	1.22
148	Lys	0.77	0.00	1.35	1.06
149	Glu	0.67	23.32	1.50	1.89
150	Ile	0.64	63.75	1.53	0.05
151	Lys	0.46	19.18	2.39	2.18
152	His	0.53	0.00	1.97	0.73
153	Ser	0.63	1.04	1.61	1.22
154	Val	0.75	15.03	1.30	1.12
155	Lys	0.50	0.00	1.68	0.83
156	Asn	0.63	0.00	1.07	0.89

Table S1. Properties of RfaH residues.