Supplementary Information

The molecular basis of protein toxin HicA–dependent binding of the protein antitoxin HicB to DNA

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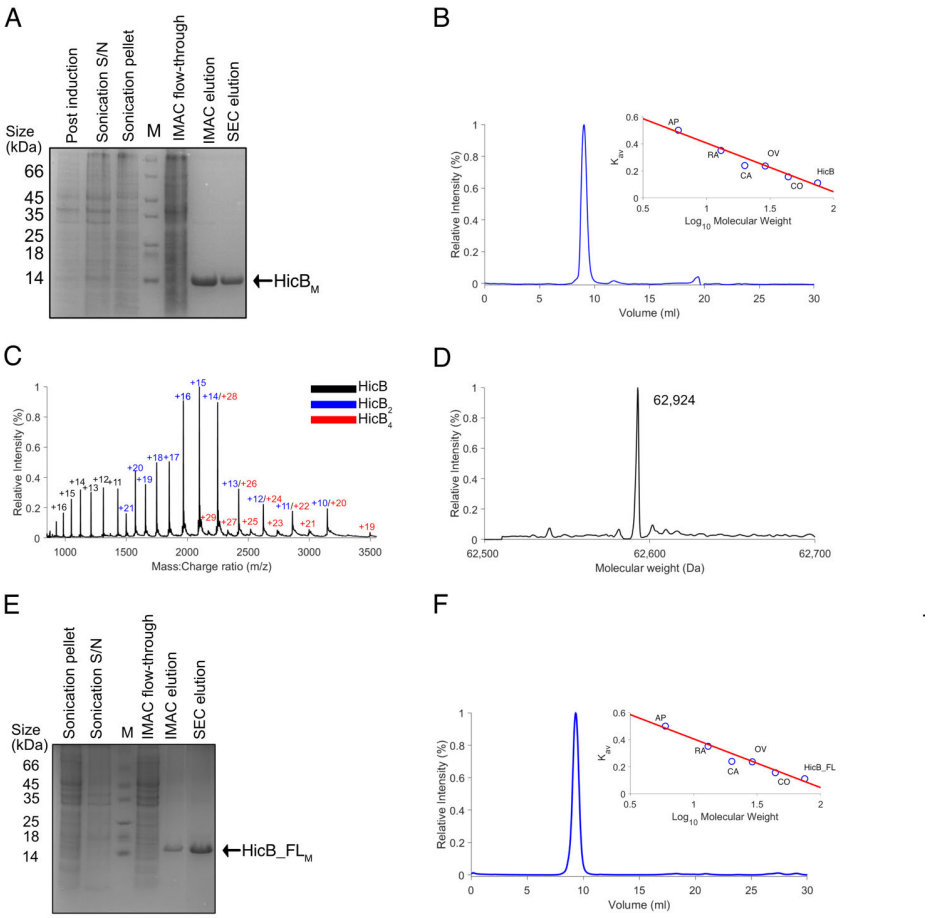

Figure S1. Characterisation of HicB and HicB_FL.
(A) SDS-PAGE following the expression and purification of HicB (lacking C-terminal residues 135-138, Table S1) from post induction to isolation via IMAC and SEC. (B) SEC of HicB, eluting as a single oligomeric species. (C) Native mass spectrometry of HicB. Peaks were assigned based on their charge state: monomeric (black), dimeric (blue) or tetrameric (red). (D) De-convoluted spectrum of the m/z envelope shown in e yielding tetrameric HicB. Expected molecular weight: 62,952 Da. (E) SDS-PAGE following the expression and purification of full length HicB (HicB_FL, see Experimental procedures and Table S1) from post induction to isolation via IMAC and SEC. ESMS (not shown) (expected 16,201 Da, observed 16,200 Da). (F) SEC of HicB_FL eluting as a single oligomeric species calculated as 64,800 Da (tetramer). Each SEC profile contains a calibration curve (inset) using known standards: Aprotinin (AP), Ribonuclease A (RA), Carbonic Anhydrase (CA), Ovalbumin (OV) and Conalbumin (CO).

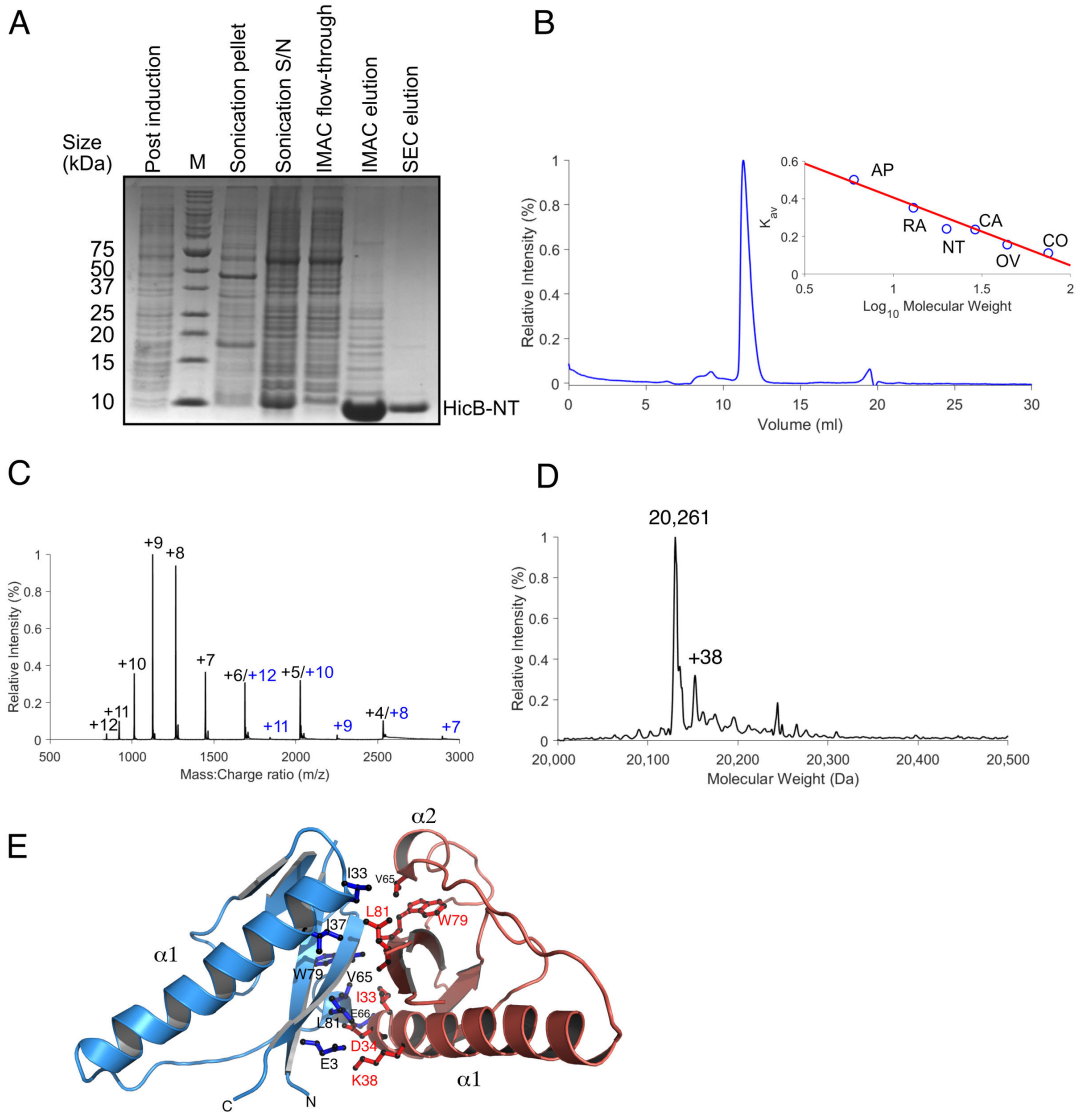

Figure S2. Characterisation of HicB-NT.

(A) SDS-PAGE following the expression and purification of HicB-NT from initial induction to isolation via IMAC and SEC. (B) SEC of HicB-NT, eluting as a single oligomeric species. (inset) calibration curves using known standards: Aprotinin (AP), Ribonuclease A (RA), Carbonic Anhydrase (CA), Ovalbumin (OV) and Conalbumin (CO). (C) Native mass spectrometry of HicB-NT. Peaks were assigned on their charge state: monomeric (black) or dimeric (blue). (D) De-convoluted spectrum (spanning 20-20.5 kDa) of the m/z envelope shown in c yielding dimeric HicB-NT: Expected molecular weight: 20,266 Da. (E) Structural representation of dimeric HicB-NT with subunits coloured blue and red respectively, highlighting the hydrophobic dimerization interface. Adjacent subunits associate via parallel packing of the β4 strands (V77-S82), flanked by the α2 helix (V64-L67) of each subunit. Adjacent subunits form a hydrogen bond and salt-bridge network between the side chains of β4-β4 (V77-L80) β1-α1 (E2-K37), α2-α1 (E65-I32, V64-D33, E65-D33), together burying 1,271 $Å^2$ of the total surface area (10,466 $Å^2$). The final HicB-NT model comprises 1-85 residues.

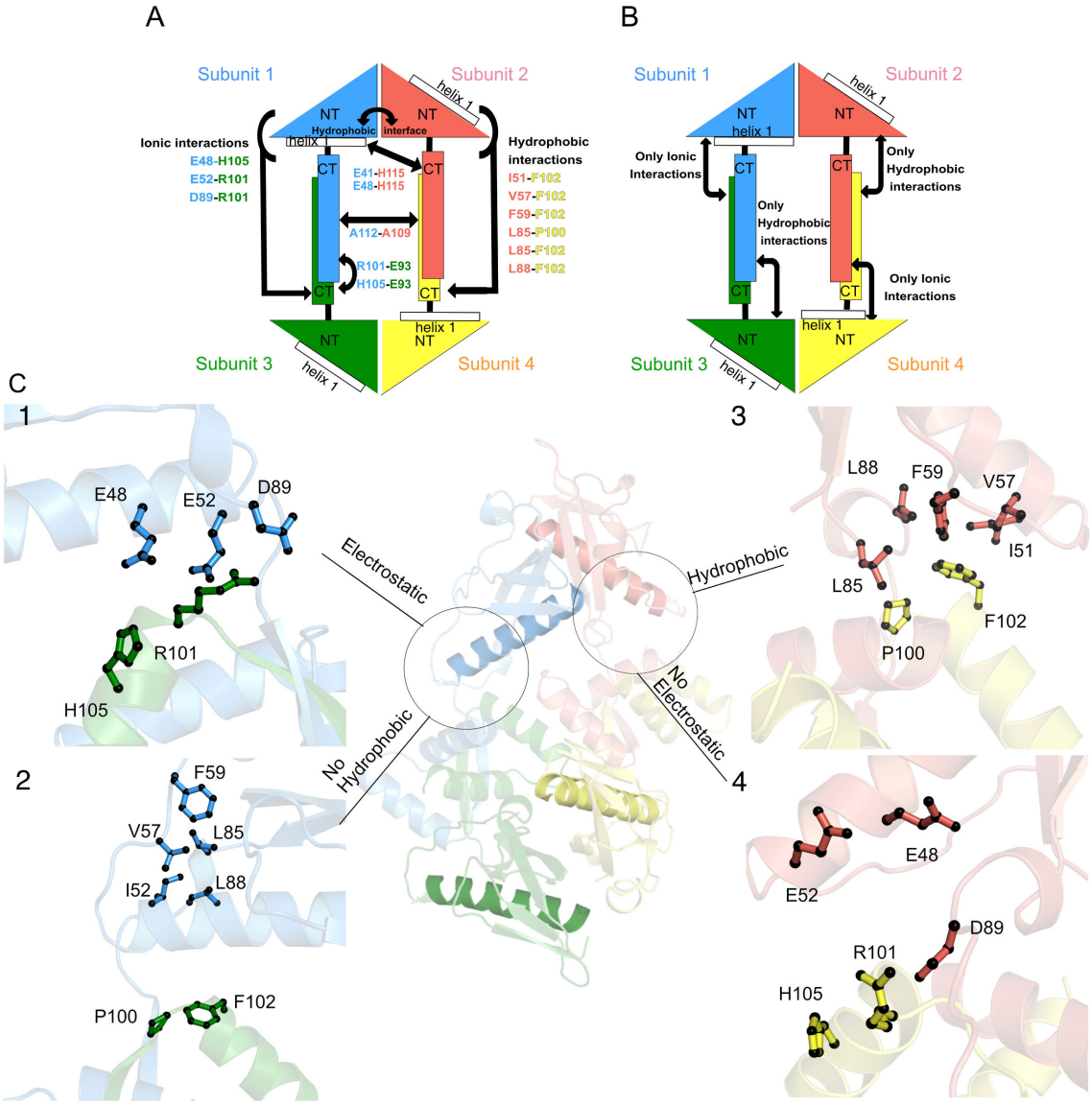

Figure S3 Tethering interactions between adjacent N-terminal and C-terminal domains of HicB in the unbound conformation.

(A) Cartoon representation of the hydrophobic and ionic interactions between adjacent C-terminal domains and N-terminal/hinge region. (B) Cartoon representation of the location of hydrophobic and ionic interaction sites that are dependent on the placement of the α1 helix. (C) Interaction sites within the tetramer. Box 1 highlight electrostatic interaction sites (Residues E48, E52, D89, R101 and H105) and box 2 indicates the absence of hydrophobic interaction sites (Residues I51, V57, F59, L85, L88 with P100 and F102) between adjacent subunits electrostatic sites. Box 3 and 4 show the respective presence and absence of hydrophobic interaction sites (Residues I51, V57, F59, L85, L88 with P100 and F102) and electrostatic sites (Residues E48, E52, D89 with R101 and H105) between adjacent subunits.

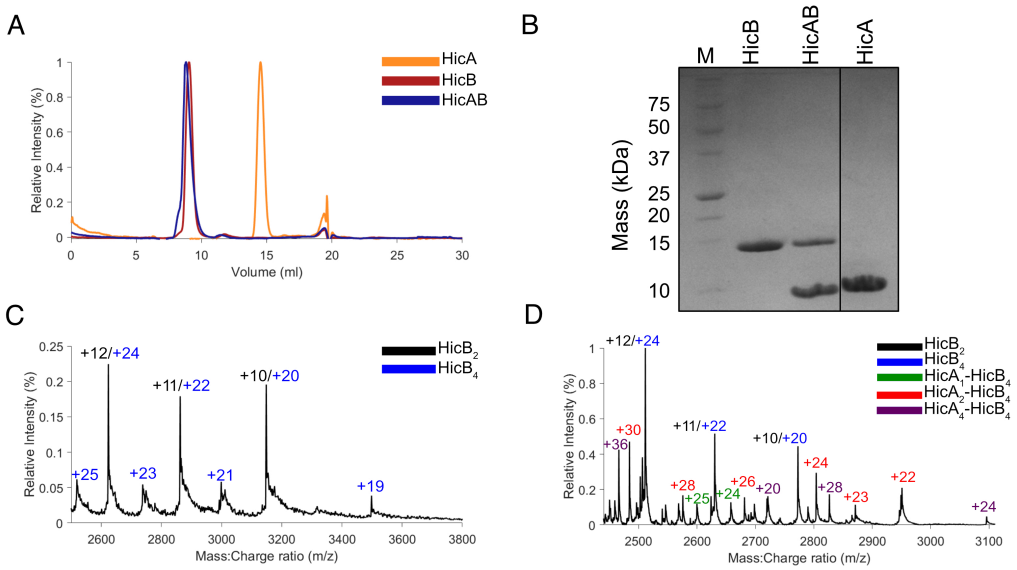

Figure S4. **Binding of HicA to HicB.**

(A) Analytical SEC profiles of HicB, HicAB and HicA. (B) SDS-PAGE of isolated fractions for each analytical S75 profile. The vertical black line indicates where the gel was spliced to remove two unwanted lanes and improve clarity. (C) Native mass spectrometry of HicB within the region 2500-3800 m/z with oligomeric states: dimeric (black) and tetrameric (blue) highlighted based on their charge state value. (D) Native mass spectrometry of HicAB within the region 2450-3150 with oligomeric states of $HicB_4$ (blue) and $HicA_4$-$HicB_4$ (purple) highlighted. Sub-stoichiometric complexes of $HicA_1$-$HicB_4$ (green), $HicA_2$-$HicB_4$ (red) and $HicB_2$ were also generated in the gas phase.

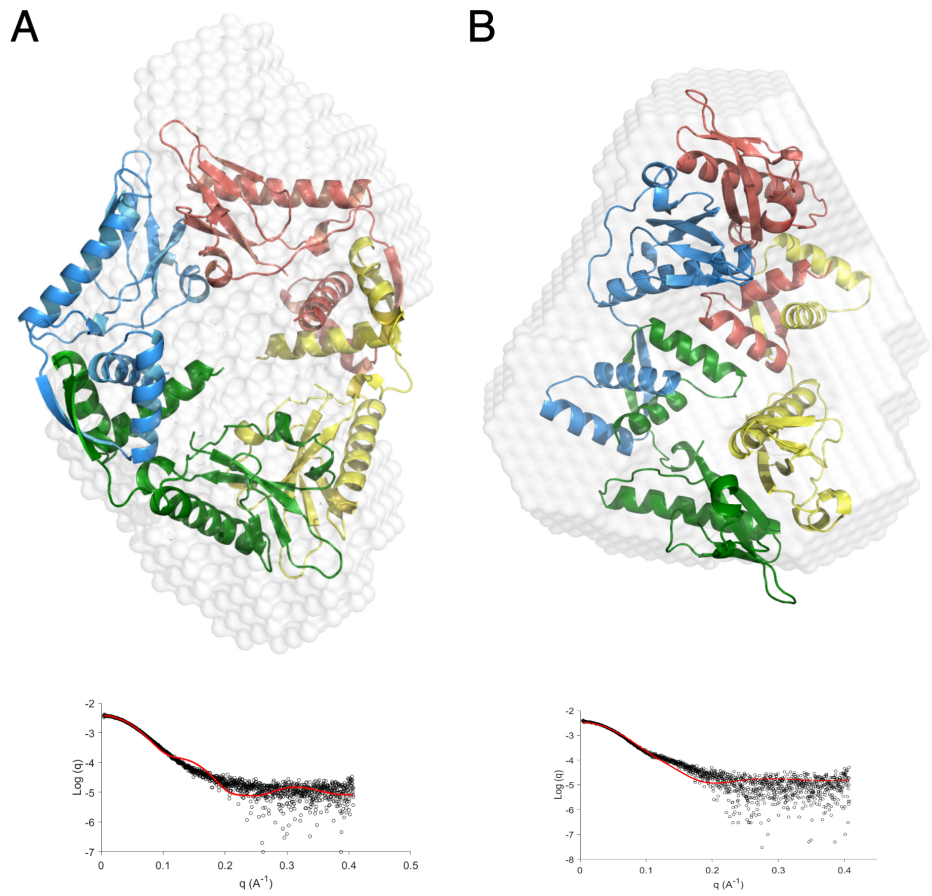

Figure S5. Small angle X-ray scattering of HicB and HicAB.

This figure uses the same scattering and shape data as shown in Figure 4, but is demonstrating the poor FoXS fit when the HicB crystal structures used to fit the data are swapped. (A) *Ab initio* modelling of HicB component of the HicAB crystal structure into the shape envelope of HicB (white). The FoXS profile of the proposed scattering for the crystal structure (red) against the experimental raw scattering data (black) is underneath (χ^2= 8.94). (B) *Ab initio* modelling of the crystal structure of the dimer of dimers form of HicB into the shape envelope of HicAB (white), with the corresponding FoXS profile underneath (χ^2= 8.06).

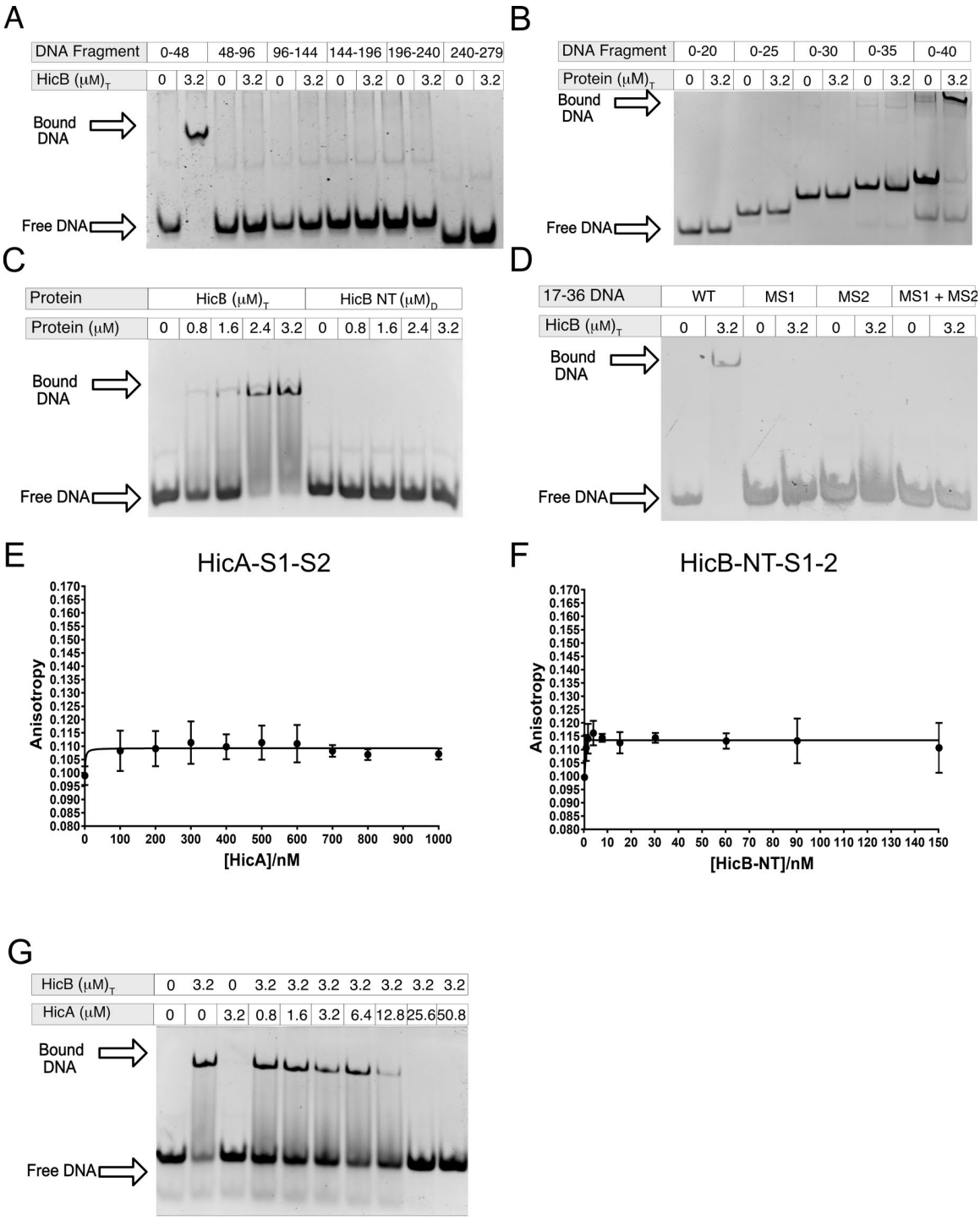
A
DNA Fragment
0-48
48-96
96-144
144-196
196-240
240-279
HicB (μM)T
0 3.2 0 3.2 0 3.2 0 3.2 0 3.2 0 3.2
Bound DNA
Free DNA
B
DNA Fragment
0-20
0-25
0-30
0-35
0-40
Protein (μM)T
0 3.2 0 3.2 0 3.2 0 3.2 0 3.2
Bound DNA
Free DNA
C
Protein
HicB (μM)T
HicB NT (μM)D
Protein (μM)
0 0.8 1.6 2.4 3.2 0 0.8 1.6 2.4 3.2
Bound DNA
Free DNA
D
17-36 DNA
WT
MS1
MS2
MS1 + MS2
HicB (μM)T
0 3.2 0 3.2 0 3.2 0 3.2
Bound DNA
Free DNA
E
HicA-S1-S2
Anisotropy
0.170 0.165 0.160 0.155 0.150 0.145 0.140 0.135 0.130 0.125 0.120 0.115 0.110 0.105 0.100 0.095 0.090 0.085 0.080
0 100 200 300 400 500 600 700 800 900 1000
[HicA]/nM
F
HicB-NT-S1-2
Anisotropy
0.170 0.165 0.160 0.155 0.150 0.145 0.140 0.135 0.130 0.125 0.120 0.115 0.110 0.105 0.100 0.095 0.090 0.085 0.080
0 10 20 30 40 50 60 70 80 90 100 110 120 130 140 150
[HicB-NT]/nM
G
HicB (μM)T
0 3.2 0 3.2 3.2 3.2 3.2 3.2 3.2 3.2
HicA (μM)
0 0 3.2 0.8 1.6 3.2 6.4 12.8 25.6 50.8
Bound DNA
Free DNA

Figure S6. Overview of DNA binding with HicB

(A) Gel-shift analysis investigating HicB binding of individual fragments of the intergenic region of DNA upstream of the *hicAB* operon. Concentrations quoted at $(\mu M)_T$ correspond to the concentration of the HicB tetramer. (B) Gel-shift assay probing the HicB DNA target site within the 0-48 bp region. (C) Gel shift assay of HicB and HicB-NT (Concentrations quoted at $[\mu M]_T$ or $[\mu M]_D$ correspond to the concentration of the HicB tetramer or HicB-NT dimer respectively) binding to 2 μM S1-2. (D) Mutations within S1-2 (2 μM) at either S1 or S2 abolishes binding to HicB (3.2 μM). (E) Quantification of HicA binding to HEX-S1-2 (n=1). Three independent repeats were fit to equation (2). (F) Quantification of HicB-NT binding to HEX-S1-2. The proportion of HEX-S1-2 bound by varying concentrations of HicB-NT was followed (n=1). Again 3 independent repeats were fit to equation (2). For each experiment the mean value is plotted with error bars representing the S.E.M. Standard errors of K_d values were calculated in GraphPad Prism. (G) HicA acts as a de-repressor. EMSA gel of varying HicA ratios (0.8 μM – 50.8 μM) titrated into HicB (3.2 μM) resulting in de-repression of HicB-S1-2 binding upon an excess of HicA.

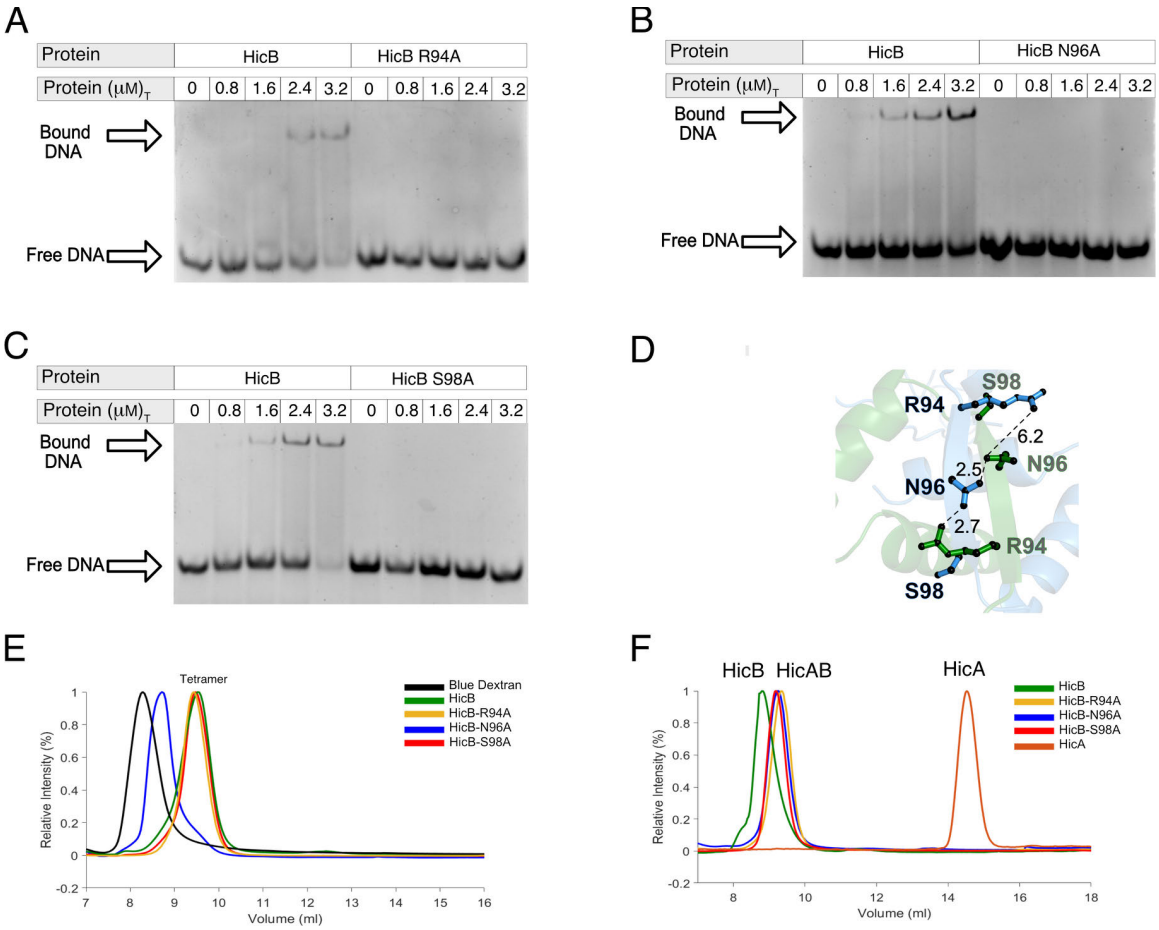

Figure S7. Gel shift assays of DNA binding with HicB alanine mutants and gel filtration.

(A) Gel-shift analysis of HicB-R94A against S1-2 (17-36 bp). (B) Gel-shift analysis of HicB-N96A against S1-2. (C) Gel-shift analysis of HicB-S98A against S1-2. (D) Structure of the RHH domain, with key DNA binding residues highlighted. (E) Analytical SEC traces of HicB variants R94A, N96A and S98A and Blue Dextran. (F) Analytical SEC of HicB variants R94A, N96A and S98A complexed with HicA.

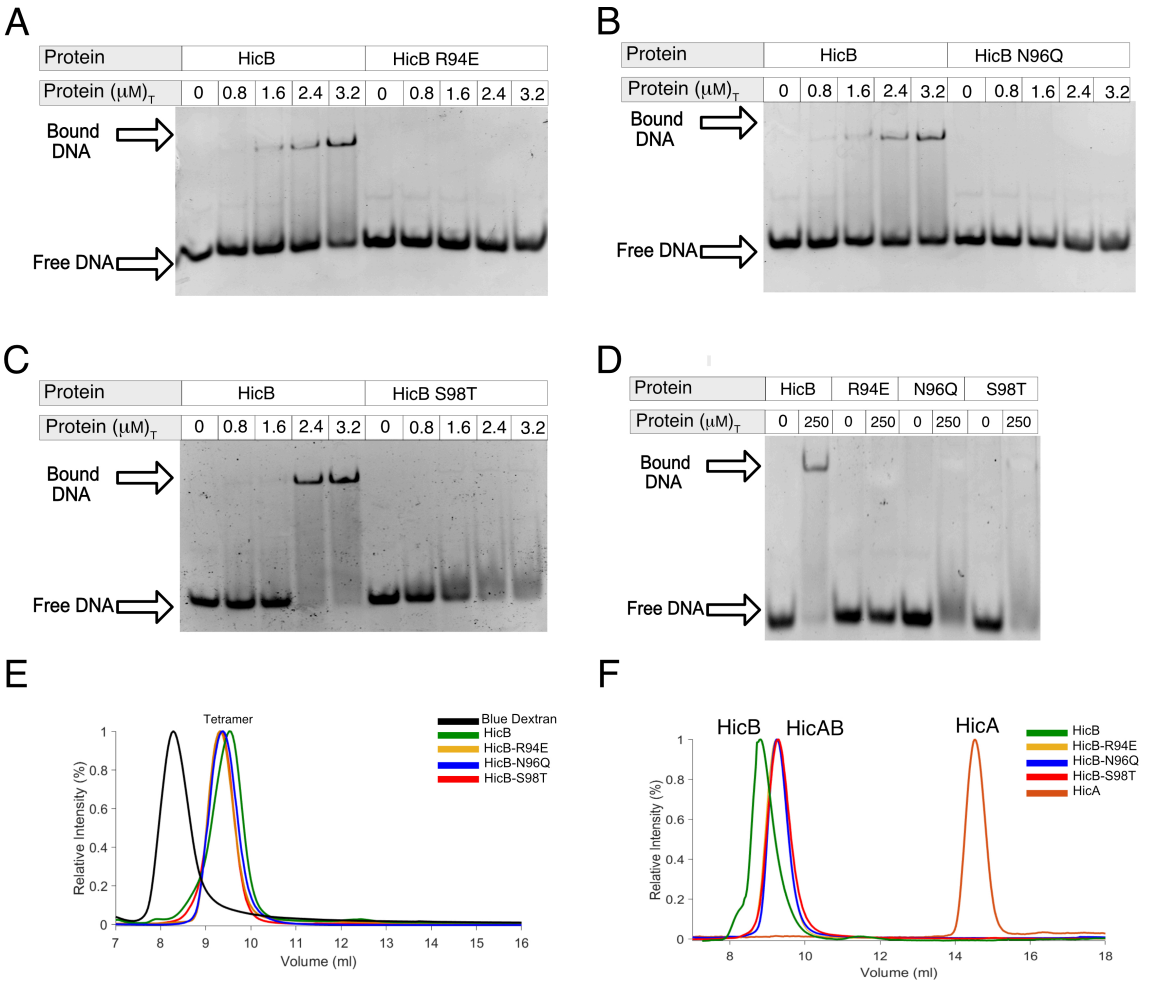

Figure S8. Gel shift assays of DNA binding of HicB semi-conservative mutants and gel filtration.

(A) Gel-shift assay of HicB-R94E binding S1-2. (B) Gel-shift assay of HicB-N96Q binding S1-2. (C) Gel-shift assay of HicB-S98T binding S1-2. (D) Gel shift assay of HicB variants, R94E, N96Q and S98T at 1000 μM binding to 2 μM S1-2. (E) Analytical SEC traces of all HicB semi conservative mutants with Blue Dextran standard. (F) Analytical SEC of HicB semi-conservative mutants complexed with HicA.

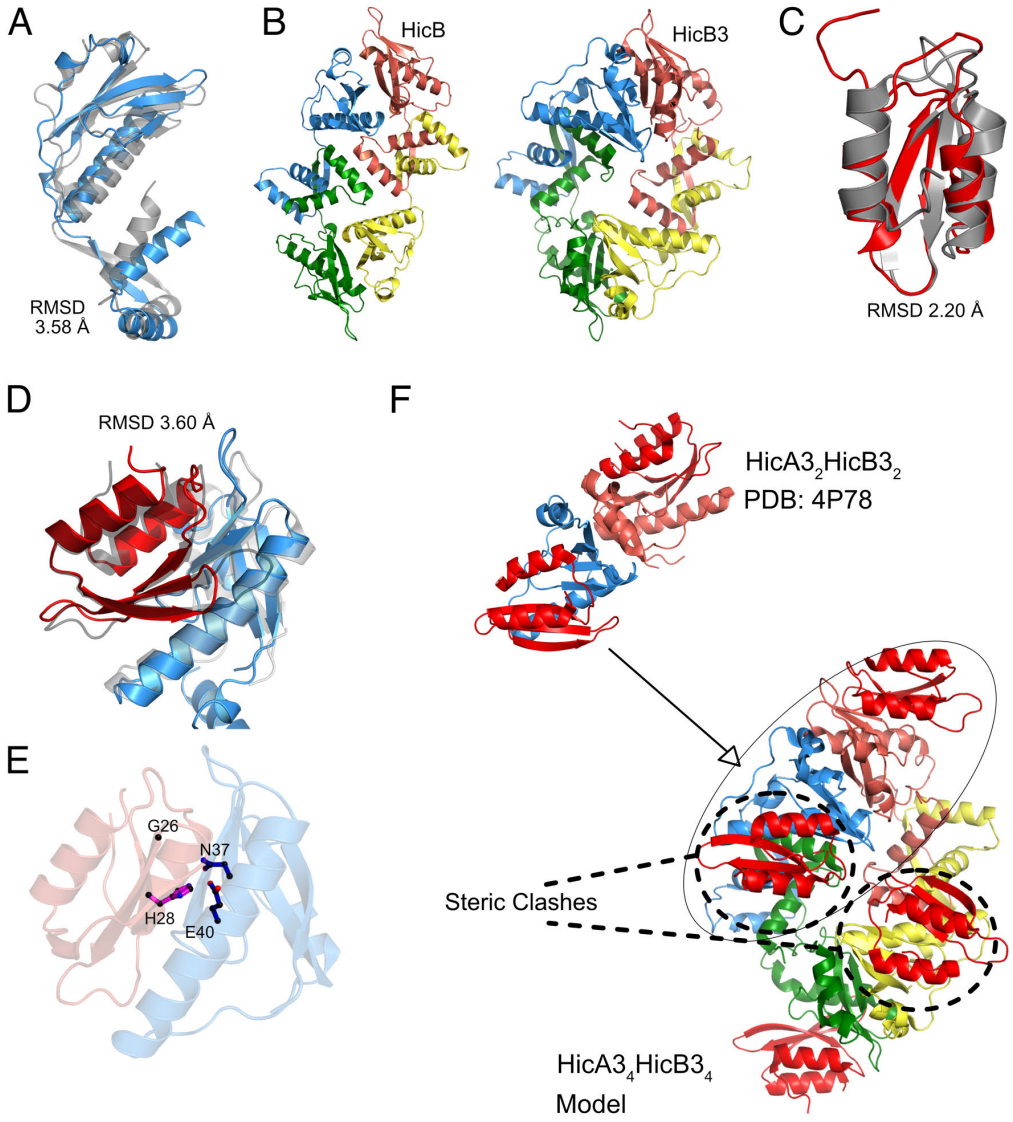

Figure S9. Comparison of HicB and HicAB crystal structures to HicB3 and HicA3B3.

(A) Superimposition of HicB (Blue) to HicB3 (Light grey), RMSD backbone atoms: 3.58 Å. (B) Comparison of the tetrameric organisation of HicB (Left) and HicB3, PDB:4P7D (Right). Individual subunits are highlighted in blue, red, green and yellow. (C) Superimposition of HicA (red) to HicA3 (Dark grey) (PDB:4P78), RMSD backbone atoms: 2.20 Å (D) The superimposition of HicA$_1$(red)HicB$_1$ (blue) to HicA3$_1$ (Dark grey) HicB3-NT (Light grey) (PDB:4P78), RMSD backbone atoms: 3.60 Å. (E) Sequestration of G26 and H28 in HicA3HicB3-NT model. (F) The HicA3$_2$HicB3$_2$ dimer (PDB: 4P78) that was reported (top) and extension to the hypothesized HicA3$_4$HicB3$_4$ structure with C-domains modelled. This revealed steric clashes between HicA3 monomers and the C-domains of subunit 1 and 4 of HicB3 as the N-terminal domains of complexed HicA3B3 are not correctly rearranged as seen in HicAB.

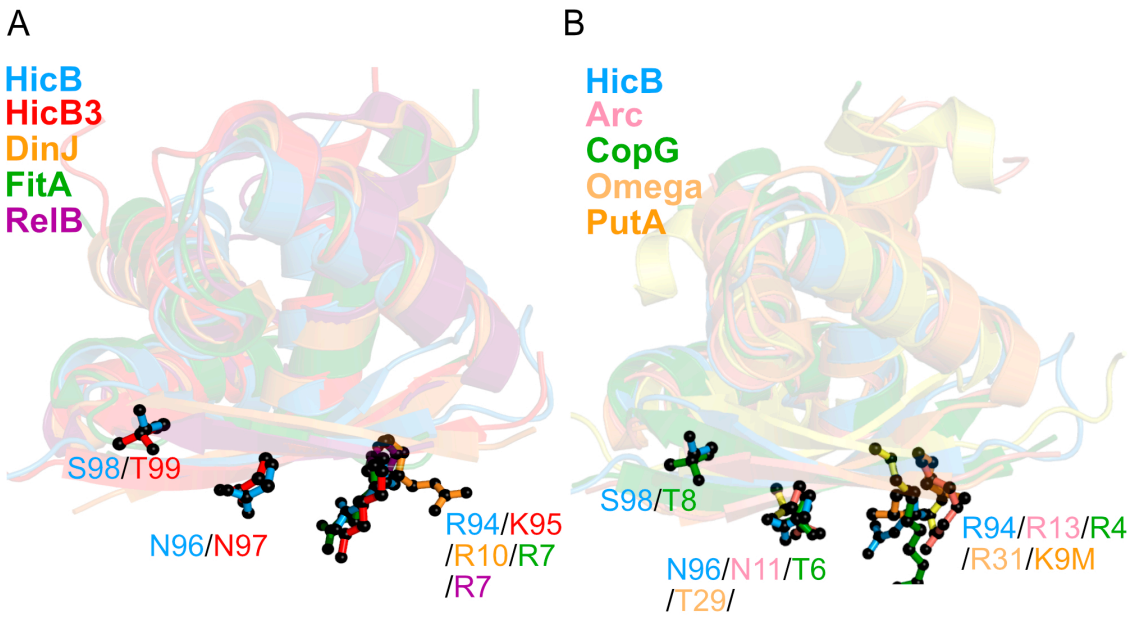

Figure S10. Comparisons of the RHH of HicB to other RHH containing proteins.

(A) Superimposition of HicB (blue) to HicB3 (PDB: 4P7D, red: RMSD: 1.43 Å), DinJ (PDB: 4Q2U, orange: RMSD: 2.66 Å), FitA (PDB: 2H10, green: RMS: 2.05 Å) and RelB (PDB: 4FXE, purple: RMSD: 2.71 Å). Direct conservation of the proximal basic residue is seen. (B) Overlay of Arc (PDB: 1BAZ, red), CopG (PDB: 2CPG, green), ORF Omega (PDB: 2BNZ, light orange) and PutA (PDB: 2AY0, orange) with HicB highlighting conservation of the proximal basic residue and the polar amino acid that flanks this in canonical DNA binding proteins. Individual superimpositions of HicB (Blue) with these protein domains are as follows: Arc (RMSD: 1.3 Å), CopG (RMSD: 1.3 Å), ORF Omega (RMSD: 1.6 Å) and PutA (RMSD: 1.8 Å).

Table S1. Sequences of the HicB constructs used in this study. Residues in bold indicate the additional His_6-tag or cloning artefacts.

```
          1         10        20        30        40        50        60        70
HicB      MMEFPIAVHKDDGSVYGVTVPDIPGVHSWGETIDDAIKNTREAIVGHVETLIELGEDVEFTCSTVEELVAKPEYAGAV
            80        90       100       110       120       130 134
          WALVSVDLSQLDSKPERINVSIPRFVLHKIDAYVASRHETRSGFLARAALEALNEGKKHHHHHH

          1         10        20        30        40        50        60        70
HicB_FL   MMEFPIAVHKDDGSVYGVTVPDIPGVHSWGETIDDAIKNTREAIVGHVETLIELGEDVEFTCSTVEELVAKPEYAGAV
            80        90       100       110       120       130  138
          WALVSVDLSQLDSKPERINVSIPRFVLHKIDAYVASRHETRSGFLARAALEALNEGKVRHAKHHHHHH

          1         10        20        30        40        50        60        70
HicB_NT   MEFPIAVHKDDGSVYGVTVPDIPGVHSWGETIDDAIKNTREAIVGHVETLIELGEDVEFTCSTVEELVAKPEYAGAV
            80   85
          WALVSVDLKHHHHHH

          1         10        20        30        40        50        60        70
HicB_DM   MMEFPIAVHKDDGSVYGVTVPDIPGVHSWGETIDDAIKNTREAIVGHVETLMELGEDVEFTCSTVEELVAKPEYAGAV
            80        90       100       110       120       130 134
          WALVSVDLSQLDSKPERINVSMPRFVLHKIDAYVASRHETRSGFLARAALEALNEGKKHHHHHH
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Table S2. Crystallisation conditions for each protein in this study.

Proteins at appropriate concentrations (25 mM Tris-HCl, pH 7.5, 150 mM NaCl) were obtained from the hanging drop vapour diffusion method at 16 °C. Crystals were flash frozen in liquid nitrogen prior to diffraction analysis.

Protein	Conditions	Cryoprotectant
HicB-NT	0.1 M NaOAc, pH 4.6, 8% (w/v) PEG 4000	10% (v/v) glycerol
HicB_DM	0.1 M NaOAc, pH 4.6, 2.0 M HCOONa, 11% (v/v) glycerol	N/A
HicB	0.1 M NaOAc, pH 4.2, 0.02 M $CaCl_2.dH_2O$, 15% (v/v) MPD	30% (v/v) glycerol
HicAB	0.1 M MES pH 6.5, 0.2 M NH_4SO_4, 16% (w/v) PEG 5000 MME	25% (v/v) glycerol

Table S3. Crystallography table with statistics

Project	HicB-NT	HicB	HicB SeMet	HicAB
Wavelength (Å)	0.9763	0.9763	0.9790	0.9795
Resolution range (Å)	54.30 - 1.56 (1.62-1.56)	39.42 - 1.85 (1.92 - 1.85)	59.85-2.73 (2.86-2.73)	34.02 – 2.49 (2.58-2.49)
Space group	$P2_12_12_1$	$P\ 4_1$	$P2_12_12_1$	$P2_1$
Unit cell ()	63.5 76.7 76.9Å 90 90 90°	62.6 62.6 173.5Å 90 90 90°	58.7 59.9 172.8Å 90 90 90°	85.1 72.2 85.3Å 90 90.1 90°
Multiplicity	13.2 (13.0)	4.7 (4.7)	12.7 (13.1)	6.8 (6.8)
Completeness (%)	100.0 (100.0)	99.0 (99.0)	99.3 (99.3)	99.9 (99.8)
Mean I/σ(I)	29.9 (2.1)	9.84 (0.40)	16.0 (1.5)	14.1 (1.4)
Wilson B-factor (Å²)	36.69	54.74	71.3	66.6
R-meas	0.04 (1.26)	0.07 (3.96)	0.11 (2.09)	0.12 (1.23)
CC1/2	1.000 (0.805)	0.998 (0.129)	0.998 (0.621)	0.998 (0.692)
DelAnom correlation between half-sets	-	-	0.493 (-0.03)	-
R/Rfree for partial model	-	-	0.327/0.390	-
Reflections used in refinement	53900	55828	-	35696
R-work	0.2001 (0.3972)	0.2016 (0.4480)	-	0.1814 (0.330)
R-free	0.2337 (0.4530)	0.2355 (0.4517)	-	0.2204 (0.375)
Number of protein atoms	2502	4327	-	6272
RMS (bonds)	0.013	0.008	-	0.034
RMS (angles	1.14	0.86	-	1.71
Average B-factors			-	
Protein	41.97	67.95	-	56.73
Solvent	47.56	53.65	-	52.17

Table S4. List of interactions between HicA and HicB.

Residues that have direct equivalents within the HicA3B3 interaction site are underlined.

Interaction	HicA-HicB
Hydrophobic	
	V18-L53
	A24-A42
	L35-L50
	L35-L53
	P39-W28
	P41-W28
	L45-V14
	L45-W28
	P46-V14
Hydrogen bonds	
	S23-W28
	T37-H46
Electrostatic	
	R19-E48
	R19-E52
	K28-E55
	K43-E30

Table S5. SAXS analysis for HicB and HicAB

Data-collection parameters		
Instrument	SEC-SAXS at B21 Diamond Light Source	
SEC column	Supedex 200 (GE Healthcare)	
Temperature	25 °C	
q range ($Å^{-1}$)	0.006-0.40	
Structural parameters	HicB	HicAB
I(0) (cm^{-1}) [from P(r)]	0.00378 ± 0.0000089	0.00349 ± 0.0000375
Rg (Å) [from P(r)]	30.12 ± 0.24	31.31 ± 0.21
I(0) (cm^{-1}) (from Guinier)	0.00382 ± 0.0000089	0.00363 ± 0.0000092
Dmax (Å)	97	100
Rg (Å) (from Guinier)	30.67 ± 0.37	31.90 ± 0.47
Porod volume estimate ($Å^{-3}$)	100,000	117,000
χ^2	0.07	0.75
Molecular-mass determination		
Partial specific volume ($cm^3\ g^{-1}$)	0.738384	0.738384
Contrast ($\Delta\rho$ x 10^{30} cm^{-2})	3.047	3.047
Molecular mass M_r [from I(0)]	67,000	74,000
Calculated M_r from sequence (kDa)	62,952	91,164
From Porod Volume [Vporod/1.7] (Da)	58,823	73,000
Software employed		
Primary data reduction	GDA (Diamond Light Source)	
Data processing	Scatter	
Ab initio analysis	DAMMIN	
Validation and averaging	DAMAVER	
Rigid-body modelling	N/A	
Computation of model intensities	FoXS	
Three-dimensional graphics representations	Pymol	

Table S6 PISA analysis of HicB tetramer observed. Monomers were investigated for their total number of atoms (N_{at}), residues (N_{res}), total number of surface atoms (S_{at}) and residues (S_{res}). The total surface area (Area) and solvation energy of folding (ΔG) were calculated. Assemblies (Crystal splits) were investigated with the Accessible Solvent Area (ASA), Buried Surface Area (BSA), Standard Free Energy of dissociation into nearest stable assemblies (ΔG_diss), Standard Free Energy of dissociation into monomeric units (ΔG0) and Composition of assembly reported. It was reported that the tetramer was stable in solution. Chains A,B, C and D refer to subunits 2, 3, 1 and 4 in figure 1.

Monomer	N_{at}	N_{res}	S_{at}	S_{res}	Area (Å^2)	ΔG (kcal/mol)
A	1019	132	724	128	9294.5	-105.8
B	1019	132	719	128	9332.4	-106.9
C	1041	135	729	130	9737.4	-108.8
D	1041	135	725	129	9675.3	-107.1

Stable crystal assemblies							
Split No	Size	Type	ASA (Å^2)	BSA (Å^2)	ΔG_diss (kcal/mol)	ΔG0 (kcal/mol)	Composition
1	4	1	27034.1	11005.5	12.4	14.0	ABCD
2	2	2	15352.6	3717.2	22.0	11.0	BC
	2	2	15276.3	3693.5	21.7	10.9	AD
Metastable crystal assemblies							
3	2	3	17179.5	1630.8	0.6	0.6	AC
	2	3	17047.7	1624.6	-0.2	-0.2	BD

Table S7: Structural conservation of the HicB interfaces within the PDB. The PISA server reports the PDB entry, interface number, mmSize (number of macromolecular monomers within the assembly), Q score (relates from 0 to 1 for unrelated-identical proteins), Sequence identity (Seq ID), interface area, ΔG and Complexation significance score (CSS, relates from 0 to 1 as the interface relevance increases).

Entry	Interface Number	mmSize	Q score	Seq Id	Interface area, Å^2	ΔG kcal/mol	CSS
4P7D	1	4	0.395	0.273	2089.2	-30	0.844
4P78	3	4	0.369	0.268	951.5	-21.7	0.931
5YRZ	3	4	0.284	0.194	604.6	-7.7	0.268
3KWR	2	4	0.269	0.096	523.7	-9.4	0.815

Table S8: DALI. The DALI server search result against a single subunit of HicB. The PDB chain, Z score, RMSD, sequence identity and description are highlighted. The Z score relates to the structural similarity between proteins. Proteins with a Z score below 3 were not included in this table.

Chain	Z score	Rmsd	Identity	Description
4P7D-A	11.8	10.9	25	Antitoxin HicB3 from *Yersinia pestis*
5YRZ-A	9.9	2.6	23	Antitoxin HicB from *Streptococcus pneumoniae*
3KWR-B	10	3.7	15	Putative RNA binding protein
2DSY-C	6.1	2.2	21	Hypothetical protein TTHA0281
3K6Q-A	5.0	2.3	14	Putative anti-toxin from *Syntrophomonas Wolfei*
1ZBT-A	4.5	10	13	Peptide chain release factor
6CI7-F	3.9	14.3	8	YCAO
5CFF-F	3.7	2.2	10	Miranda/Staufen dsRBD5 complex
3VYY-A	3.6	3.5	10	ATP-Dependent RNA helicase A
4WYQ-B	3.5	2.6	8	Endoribonuclease DICER
2MDR-A	3.5	2.2	4	Double-stranded RNA-specific adenosine deaminase
2LTR-A	3.4	2.6	7	Protein RDE-4
3P1X-B	3.4	2.0	8	Interleukin enhancer binding factor 3
3ADL-A	3.3	2.4	14	RISC-loading complex subunit TARBP2
1WHQ-A	3.1	2.9	11	RNA helicase A

Table S9 PISA analysis of HicAB hetero-octamer. Monomers were investigated for their total number of atoms (N_{at}), residues (N_{res}), total number of surface atoms (S_{at}) and residues (S_{res}). The total surface area (Area) and solvation energy of folding (ΔG) were calculated. Assemblies (Crystal splits) were investigated with the Accessible Solvent Area (ASA), Buried Surface Area (BSA), Standard Free Energy of dissociation into nearest stable assemblies (ΔG_diss), Standard Free Energy of dissociation into monomeric units (ΔG0) and Composition of assembly reported. It was reported that the tetramer was stable in solution. Chains A,B,C,D refer to Subunit 4, 1, 3 and 2 of HicB, while chains F, E, G and H refer to HicA moieties bound to each respective subunit.

Monomer	N_{at}	N_{res}	S_{at}	S_{res}	Area (Å^2)	ΔG (kcal/mol)
A	1019	132	724	128	9294.5	-105.8
B	1019	132	719	128	9332.4	-106.9
C	1041	135	729	130	9737.4	-108.8
D	1041	135	725	129	9675.3	-107.1
E	479	61	297	56	4061.0	-55.1
F	466	60	296	56	3944.6	-53.1
G	472	60	294	56	4002.5	-54.7
H	461	59	305	57	4185.2	-49.4

Split No	Size	Type	ASA (Å^2)	BSA (Å^2)	ΔG_diss (kcal/mol)	ΔG0 (kcal/mol)	Composition
Stable crystal assemblies							
1	8	1	36213.4	17619.7	8.8	74.1	ABCDEFGH
2	4	2	19521.3	7473.8	9.2	32.6	ADFH
	4	2	192087	7629.2	8.8	32.6	BCEG
3	2	3	11537.3	1876.5	5.2	5.2	CG
	2	3	11710.0	1845.3	4.9	4.9	DH
	2	3	11611.9	1827.9	4.9	4.9	AF
	2	3	11567.2	1856.8	4.2	4.2	BE
4	4	4	27426.7	10213.1	9.6	56.9	ABCD
Metastable crystal assemblies							
5	4	5	22004.7	4974.7	-0.9	-0.9	BDEH
	4	5	21905.1	4948.5	-1.3	-1.3	ACFG
Marginally stable Assemblies							
6	4	4	27426.7	10213.1	9.6	56.9	ABCD
7	2	6	17460.6	1272.6	-0.2	-0.2	BD
	2	6	17662.5	1244.1	-0.6	-0.6	AC

Table S10: Structural conservation of the HicAB interface within the PDB The PISA server reports the PDB entry, interface number, mmSize (number of macromolecular monomers within the assembly), Q score (relates from 0 to 1 for unrelated-identical proteins), Sequence identity (Seq ID), interface area, ΔG and Complexation significance score (CSS, relates from 0 to 1 as the interface relevance increases).

Entry	Interface Number	mmSize	Q score	Seq Id	Interface area, Å^2	ΔG kcal/mol	CSS
5YRZ	6	4	0.782	0.246	1114.5	-7.9	0.468
4P78	2	4	0.710	0.328	1179.3	-7.5	0.608
3KWR	1	4	0.241	0.091	674.4	-12.5	0.436

Table S11. Oligonucleotides used in the project

Oligonucleotides		
0-48 Primer 1	IDT	gatcgtgattggatgtgtataattacacacaagacattcgggggagct
0-48 Primer 2	IDT	agctcccccgaatgtcttgtgtgtaattatacacatccaatcacgatc
48-96 Primer 1	IDT	ataggggcgaaacaatgtgaaaatacgcacggctacacaaaacttgag
48-96 Primer 2	IDT	ctcaagttttgtgtagccgtgcgtattttcacattgtttcgcccctat
96-144 Primer 1	IDT	tcgaggccgcgcgcgatgcttcagtcttgcccagcggacgggataaaa
96-144 Primer 2	IDT	ttttatcccgtccgctgggcaagactgaagcatcgcgcgcggcctcga
144-196 Primer 1	IDT	gccgcccgctggcggcagattacgacgagtggctacacacgaggaatg
144-196 Primer 2	IDT	cattcctcgtgtgtagccactcgtcgtaatctgccgccagcgggcggc
192-240 Primer 1	IDT	gtgccgaataagggtaactatcctgtcaatgttgacgggcaagaggtg
192-240 Primer 2	IDT	cacctcttgcccgtcaacattgacaggatagttacccttattcggcac
240-279 Primer 1	IDT	gccgaatcctcagtggaaaatccgcgaggactttagcac
240-279 Primer 2	IDT	gtgctaaagtcctcgcggattttccactgaggattcggc
0-20 Primer 1	IDT	acaagacattcgggggagct
0-20 Primer 2	IDT	agctcccccgaatgtcttgt
0-25 Primer 1	IDT	tacacacaagacattcgggggagct
0-25 Primer 2	IDT	agctcccccgaatgtcttgtgtgta
0-30 Primer 1	IDT	ataattacacacaagacattcgggggagct
0-30 Primer 2	IDT	agctcccccgaatgtcttgtgtgtaattat
0-35 Primer 1	IDT	tgtgtataattacacacaagacattcgggggagct
0-35 Primer 2	IDT	agctcccccgaatgtcttgtgtgtaattatacaca
0-40 Primer 1	IDT	ttggatgtgtataattacacacaagacattcggggga
0-40 Primer 2	IDT	agctcccccgaatgtcttgtgtgtaattatacacatc
17-36 Primer 1	IDT	atgtgtataattacacacaa
17-36 Primer 2	IDT	ttgtgtgtaattatacacat
MS1 Primer 1	IDT	atgtgtataattagggacaa
MS1 Primer 2	IDT	ttgtccctaattatacacat
MS2 Primer 1	IDT	atccctataattacacacaa
MS2 Primer 2	IDT	ttgtgtgtaattatagggat
MS1MS2 Primer 1	IDT	atccctataattagggacaa
MS1MS2 Primer 2	IDT	ttgtccctaattatagggat
HicB_FL Primer 1	IDT	aggagatataccatgatggaatttcccatcgcagt
HicB_FL Primer 2	IDT	gtgatggtgatgttttgcgtgcctaactttgccttc
HicB Primer 1	IDT	aggagatataccatgatggaatttcccatcgca
HicB Primer 2	IDT	gtgatggtgatgtttttgccttcattaagtgcctc
HicB-NT Primer 1	IDT	aggagatataccatggaatttcccatcgcagtg
HicB-NT Primer 2	IDT	gtgatggtgatgtttaagatcaacgctgacgag
I51M Primer 1	IDT	catgtagagacattgatggagcttggagaagat
I51M Primer 2	IDT	atcttctccaagctccatcaatgtctctacatg
I99M Primer 1	IDT	cggatcaatgtgagtatgcctcgcttcgtgctg
I99M Primer 2	IDT	cagcacgaagcgaggcatactcacattgatccg
Mutant Primer 1	IDT	aggagatataccatgatggaatttccgattgccgtgcat
Mutant Primer 2	IDT	gcactggaagcactgaatgaaggtaaaaaacatcaccatcac
R94E Primer 1	IDT	aaaccggaagaaattaatgttagc
R94E Primer 2	IDT	gctaacattaatttcttccggttt
S98T Primer 1	IDT	cgtattaatgttaccattccgcgtttt
S98T Primer 2	IDT	aaaacgcggaatggtaacattaatacg

Table S12. Samples analysed by SEC.

Elution volumes were used to calculate partition coefficient to indirectly determine the molecular weight based off the calibration curve.

Sample	Mw (Da)	Calculated Mw (Da)	$Log_{10}Mw$	V_e (ml)	K_{av}
HicA	7,053	8,240	0.92	14.5	0.44
HicB	62,952	65,610	1.82	9.03	0.11
HicAB	91,164	71,870	1.86	8.79	0.10