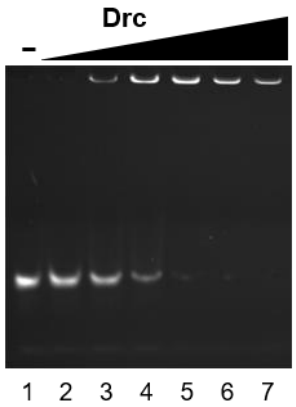
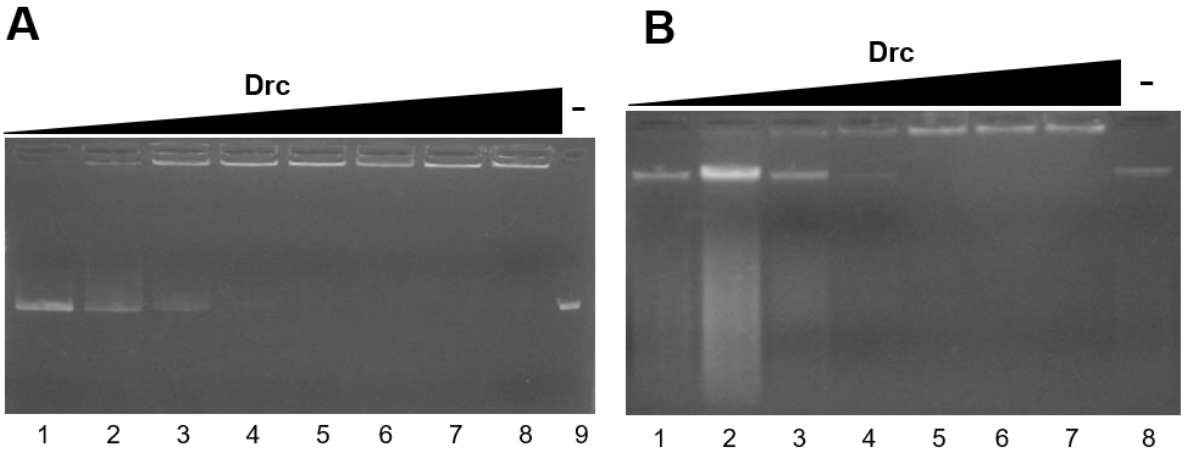
SUPPLEMENTARY MATERIALS



Supplementary figure S1. (A) Replica of EMSA on native acrylamide gel with 100 nM 5'-6-FAM-labeled ssDNA oligonucleotides combined with Drc (lane 1-7). Different concentrations of protein were used from left to right: 0, 0.5, 1, 1.5, 5, 20, 50 µM. In this replica, the shifted DNA is visible inside the wells.



Supplementary figure S2. (A) EMSA on agarose gel with 100 ng pUC19 DNA and increasing concentrations of Drc (from left to right: 3, 6, 9, 12, 15, 18, 21, 22.5 µM). Lane 9 contains a control in which Drc was replaced with protein buffer. (B) EMSA on agarose gel with 100 ng genomic LUZ7 DNA with increasing concentrations of Drc (from left to right: 1.5, 3, 6, 9, 12, 15, 18 µM). In lane 2 double the amount was loaded to enhance visibility of smaller DNA (most likely mechanically sheared DNA due to pipetting). This sheared DNA is also shifted at higher protein concentrations, further supporting that Drc binds non-sequence specific. Lane 8 contains a control in which Drc was replaced with protein buffer.

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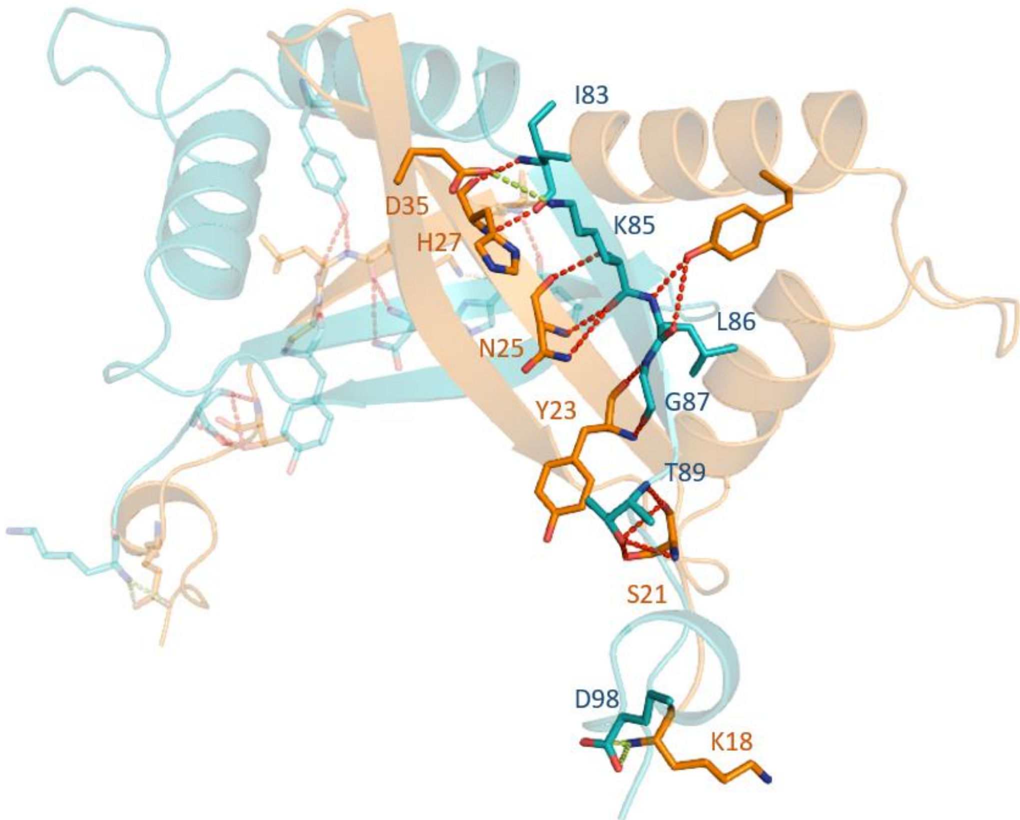
>Drc
MALVKKNQARNTQATDNKGASAYLNFHFPTRDGKDVRLVSLGLRADDALHMQLQEFLTVDDKGKPLSETAYA
ERCKKLVSRLIIKLGVTRSEEERALDL
>Drc_disorder
DDDDDDDDDDDDDDDDDDDDOOOOOOOOOOOOOOOOOOOOOOOOOOOOOOOOOOOOOOOOOOOOOOOOOOOO
OOOOOOOOOOOOOOOOOODDDDDDDDD
>Drc_disorder_confidence_label
666555555555443322218999999999999999999999999999999999999999999999999999
999999999999999998122233432
```

Supplementary figure S3. Protein disorder prediction by ESpritz [v 1.3] (X-ray prediction type and 5% false positive rate). For each amino acid under '>Drc' the disorder is predicted and represented in '>Drc_disorder'. Disordered amino acids are represented by 'D', ordered amino acids by 'O'. The confidence of the prediction for each amino acid is given in '>Drc_disorder_confidence_label', with numbers ranging from 1 (lowest confidence) to 9 (highest confidence). Disordered amino acids and associated values are colored red.



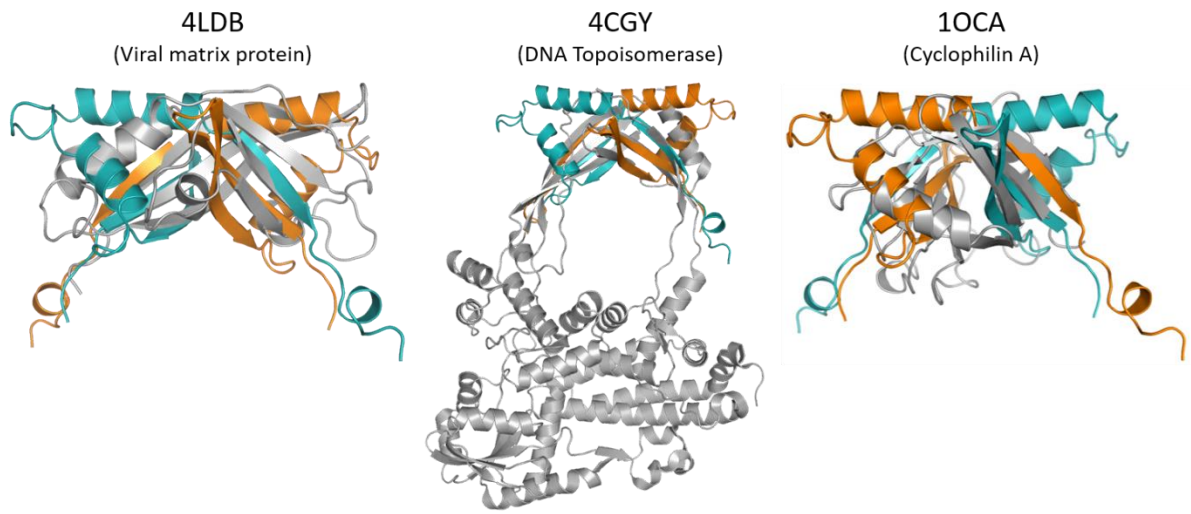
Supplementary figure S4. Overview of the different hydrogen and salt bridges present between both chains in the Drc dimer. Interacting residues on one side of the dimer are highlighted together with their hydrogen bonds (red dashed lines) or salt bridges (yellow dashes).

Supplementary table S1. Output from the DALI protein structure comparison server. The Drc dimer was used as input in the form of a single chain and run against the full PDB database. For each chain hit, the Z-score, Root Mean Square Distance (rmsd, Å), number of structurally aligned residues (lali), number of amino acids in the hit protein (nres) and percentage of identical amino acids (%id) is given together with a description of the protein function.

Chain	Z-score	rmsd	lali	nres	%id	Description
4ldb-A	3.6	3.5	86	266	10	Matrix protein VP40
4cgy-A	3.5	6.9	100	619	5	DNA topoisomerase 3-alpha
3f1j-A	3.2	5.0	89	140	9	Matrix protein
4rul-A	3.1	7.1	90	821	8	DNA topoisomerase 1
6bk6-A	3.0	3.9	82	280	17	Hendra virus matrix protein
5b0v-A	3.0	4.2	89	258	6	Matrix protein VP40
3kop-F	2.9	3.0	83	169	12	Protein with cyclophilin-like fold
5ex1-C	2.7	5.0	94	272	7	Peptidyl-prolyl cis-trans isomerase cyclophilin T
3x27-D	2.7	3.2	82	315	6	Cucumopine synthase
2k49-A	2.7	4.0	80	118	8	UPF0339 protein SO_3888
5gvc-A	2.7	6.6	97	612	11	DNA topoisomerase 3-beta-1
2ykd-A	2.6	3.6	83	255	8	Matrix protein
3tcq-A	2.6	3.5	80	239	6	Matrix protein VP40
4ddt-A	2.5	7.4	83	1102	10	Reverse gyrase
2ose-A	2.3	4.3	94	195	7	Cyclophilin
5uwb-A	2.2	4.3	56	187	2	Toluene tolerance protein
3d9r-B	2.2	8.8	55	134	9	Ketosteroid isomerase-like protein
5e27-A	2.1	11.5	51	226	0	Resuscitation-promoting factor RPFB
4ldb-C	2.0	4.6	81	237	7	Matrix protein VP40

Supplementary table S2. Output from the PDBeFOLD protein structure comparison server. The Drc dimer was used as input in the form of a single chain and run against the full PDB database at default parameters except for the similarity cut-off level, which was decreased to 60 %. For each chain hit, the Z-score, Root Mean Square Distance (rmsd, Å), number of structurally aligned residues (lali), number of amino acids in the hit protein (nres) and percentage of identical amino acids (%id) is given together with a description of the protein function.

Chain	Z-score	rmsd	lali	nres	%id	Description
1oca-A	2.7	3.6	84	165	5	Cyclophilin A
4yui-A	2.6	3.8	84	163	6	Cyclophilin A
3yuj-A	2.7	4.2	86	163	5	Cyclophilin A



Supplementary figure S5. Overlay of the Drc dimer (orange, cyan) on the full A chain of three structures that are representative for the top scoring DALI and PDBeFOLD hits (grey) from table S1. Part of the 4LDB C-terminus is hidden for clarity.

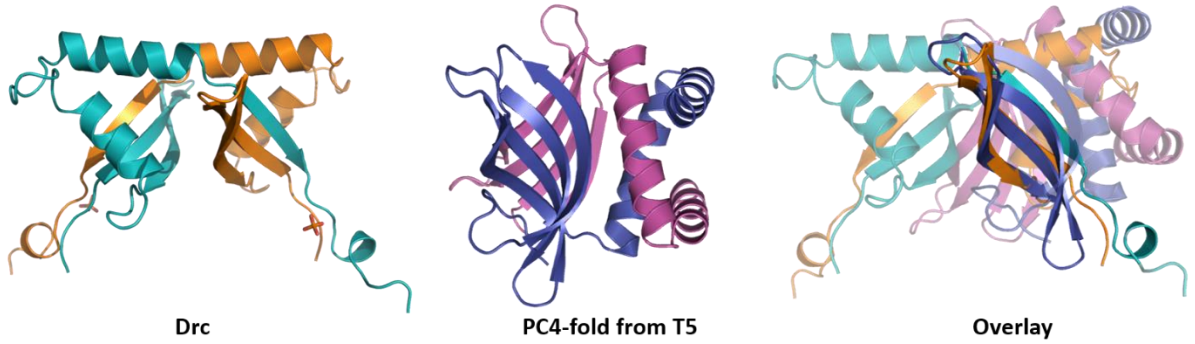
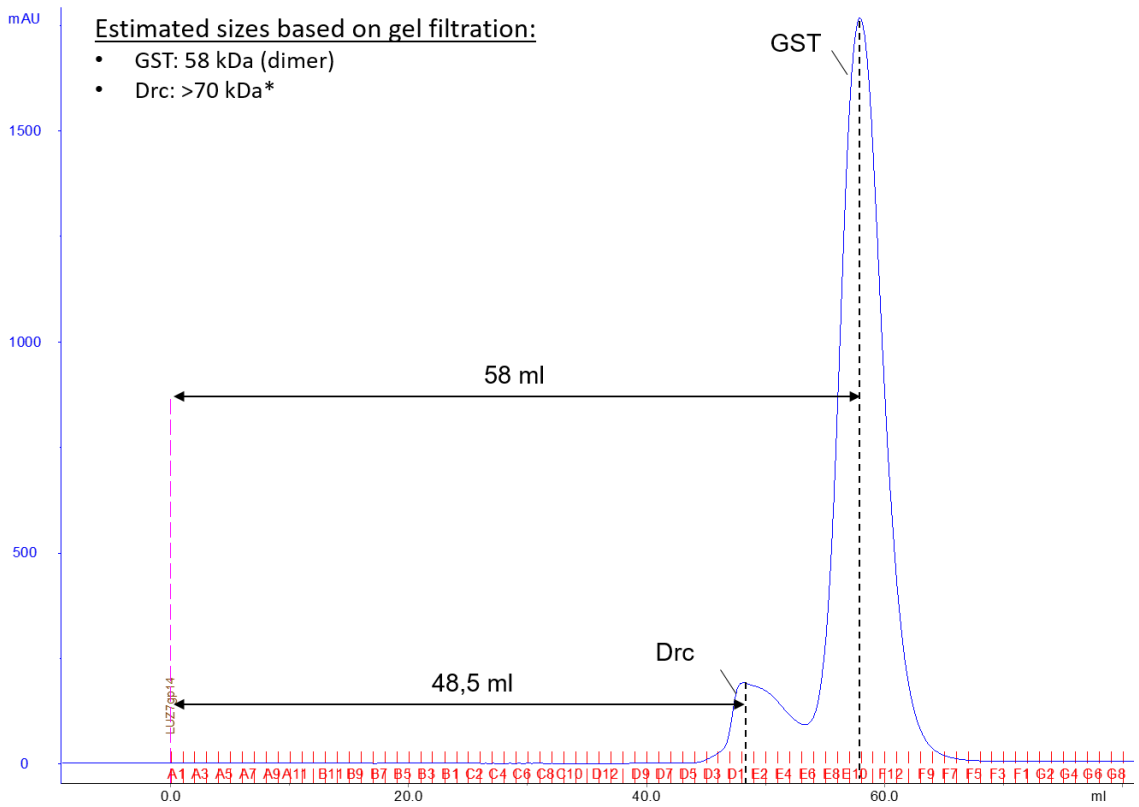
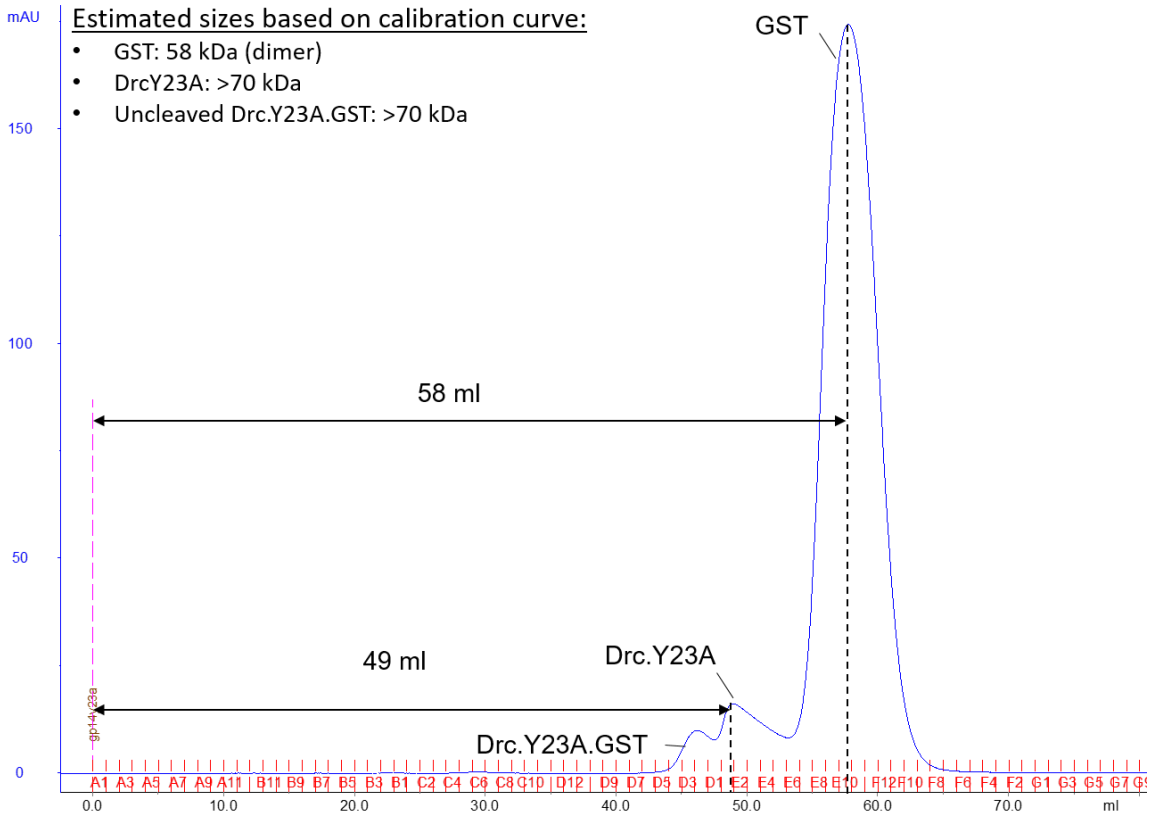


Figure S6. Comparison of the Drc dimer (left; colored cyan and orange) with the PC4-fold protein encoded by ORF115 of phage T5 (middle; PDB ID: 4BG7). The chains of the ORF115 dimer are colored purple and blue. Right shows the overlay of both proteins when the β-sheets are aligned. A single sheet can be overlaid well, while the other parts of both proteins (transparent) do not overlap.

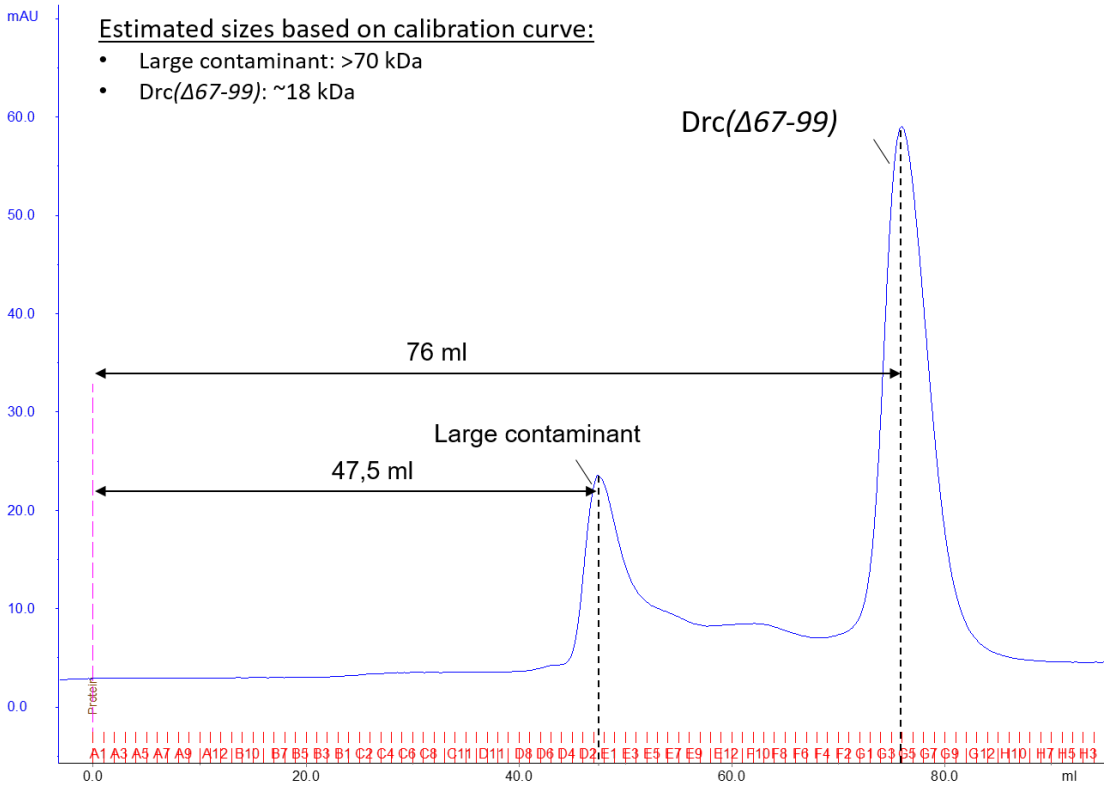
A


Estimated sizes based on gel filtration:
• GST: 58 kDa (dimer)
• Drc: >70 kDa*
mAU
1500
1000
500
0
GST
58 ml
Drc
48,5 ml
0.0
20.0
40.0
60.0
ml

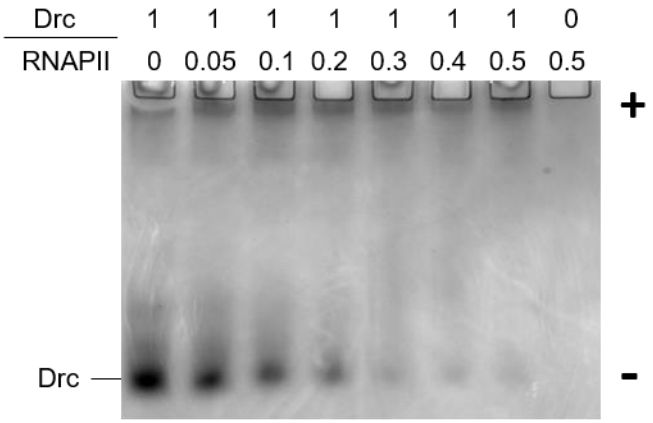
B


Estimated sizes based on calibration curve:
• GST: 58 kDa (dimer)
• DrcY23A: >70 kDa
• Uncleaved Drc.Y23A.GST: >70 kDa
mAU
150
100
50
0
GST
58 ml
49 ml
Drc.Y23A
Drc.Y23A.GST
0.0
10.0
20.0
30.0
40.0
50.0
60.0
70.0
ml

C



Supplementary figure S7. (A) Gel filtration chromatogram for Drc with cleaved GST tag (absorbance (mAU) in function of ml). Peaks were assigned to their protein based on SDS-PAGE analysis of the collected 1 ml fractions (red). Drc eluted at 48.5 ml, while GST eluted at 58 ml. Note that although this conforms with a >70 kDa protein (and thus higher than a Drc dimer), this size estimation is likely not correct due to the significant deviation from globularity by the terminal tails of Drc. (B) Gel filtration profile of Drc.Y23A, eluting at nearly identical volumes to wild type Drc. Some residual uncleaved protein was also observed (Drc.Y23A.GST). (C) Gel filtration chromatogram for Drc*(Δ67-99)* after His-tag purification (mAU in function of ml). A first peak containing contaminating proteins eluted at 47.5 ml while Drc*(Δ67-99)* eluted at 76 ml. This last one corresponds to a size of ~18 kDa, corresponding to a dimer. Note that with the removal of the tails in this protein, the protein now elutes at expected size.



Supplementary figure S8. MSA with Drc and the RNAPII complex on a native 10% acrylamide gel with reversal of the polarity (charge of the poles indicated by + and – symbols). At the top of each lane the ratio of Drc:RNAPII in each condition is indicated (with 1 being 50 µM and 0 being a buffer control). Drc was kept constant at 50 µM, while the concentration of RNAPII was increased from left to right to the indicated ratio. The gel was run for 3 hours on ice at 150V.

Supplementary table S3. Homologs of N4 gp2 and LUZ7 gp14 (Drc). A psi-BLAST search was performed with the protein sequences of N4 gp2 (not the refseq sequence but the shorter sequence reported by Carter *et al.* (2003)) and Drc (YP_003358296.1) against the non-redundant NCBI database. Up to three iterations were performed with each iteration including all significant hits. Each phage strain containing a homolog is given, together with the NCBI accession nr. of the protein homolog, its e-value and % of identity with the query.

Organisms with N4 gp2 homolog	Accession nr.	E-value	Identity
Escherichia phage OLB145	AYR04185.1	1,00E-90	100%
Escherichia virus N4	YP_950480.1	3,00E-90	100%
Escherichia phage PMBT57	AUV59078.1	5,00E-90	99%
Klebsiella phage KP8	AVJ48917.1	5,00E-65	74%
Escherichia phage Bp4	YP_009113236.1	2,00E-62	72%
Escherichia phage PD38	AXY81283.1	2,00E-62	72%
Escherichia phage ECBP1	YP_006908773.1	3,00E-62	72%
Escherichia phage EC1-UPM	AGC31513.1	4,00E-62	71%
Escherichia phage IME11	YP_006990680.1	1,00E-61	73%
Escherichia phage St11Ph5	ATS92468.1	2,00E-61	70%
Escherichia phage PGN829.1	AXY82571.1	3,00E-61	71%
Escherichia phage vB_EcoP_G7C	YP_004782128.1	2,00E-60	71%
Escherichia phage vB_EcoP_PhAPEC5	YP_009055508.1	2,00E-58	70%
Escherichia phage vB_EcoP_PhAPEC7	YP_009056132.1	3,00E-58	70%
Pseudomonas phage inbricus	ATW58139.1	6,00E-41	58%
Erwinia phage vB_EamP_Frozen	YP_009286131.1	1,00E-30	45%
Erwinia phage vB_EamP_Rexella	ANJ65231.1	1,00E-30	45%
Erwinia phage vB_EamP_Gutmeister	ANJ65319.1	1,00E-30	45%
Erwinia phage Ea9-2	YP_009007375.1	3,00E-30	45%
Achromobacter phage phiAxp-3	YP_009208654.1	8,00E-28	43%
Xanthomonas phage RiverRider	AVO23096.1	2,00E-26	41%
Achromobacter phage JWAlpha	YP_009004703.1	4,00E-25	40%
Erwinia phage phiEaP-8	AWN06265.1	5,00E-25	40%
Achromobacter phage JWDelta	AHC56518.1	2,00E-24	41%
Podoviridae sp.	AXH72057.1	4,00E-23	41%
Shigella phage pSb-1	YP_009008442.1	9,00E-22	75%
Mesorhizobium sp. M4B.F.Ca.ET.058.02.1.1	WP_126055530.1	1,00E-19	39%
Mesorhizobium sp. LSJC255A00	WP_023705286.1	2,00E-16	40%
Delftia phage RG-2014	YP_009148364.1	9,00E-16	33%
Ochrobactrum anthropi	WP_125335540.1	7,00E-14	36%
Pantoea eucrina	WP_084798739.1	4,00E-12	40%
Escherichia phage Pollock	YP_009152107.1	1,00E-11	36%
Klebsiella phage Pylas	AYP69263.1	2,00E-10	34%
Pseudomonas phage ZC03	AMD43426.1	7,00E-09	36%
Pseudomonas phage ZC08	AMD43518.1	7,00E-09	36%
Pantoea brenneri	WP_069729479.1	6,00E-08	39%
Salmonella phage FSL SP-076	YP_008240229.1	4,00E-07	31%
Salmonella phage FSL SP-058	YP_008239413.1	6,00E-07	33%

Lucilia cuprina	KNC30488.1	3,00E-04	40%
Erwinia phage vB_EamP-S6	YP_007005741.1	0.001	34%
Shigella phage pSb-1	YP_009008441.1	0.003	50%
New hits in second iteration psi-BLAST*			
Ruegeria phage vB_RpoP-V12	AWY08791.1	3,00E-14	25%
Ruegeria phage vB_RpoP-V21	AWY08962.1	3,00E-14	25%
Ruegeria phage vB_RpoP-V17	AWY09523.1	3,00E-14	25%
Ruegeria phage vB_RpoP-V14	AXF42123.1	3,00E-14	25%
Dinoroseobacter phage DS-1410Ws-06	ANJ20665.1	6,00E-14	24%
Roseobacter phage RD-1410Ws-07	ANJ20816.1	6,00E-14	24%
Sulfitobacter phage phiCB2047-B	YP_007675849.1	1,00E-13	24%
Silicibacter phage DSS3phi2	YP_002899013.1	1,00E-13	26%
Sulfitobacter phage EE36phi1	YP_002898935.1	4,00E-13	26%
Roseovarius sp. 217 phage 1	CBW46996.1	5,00E-13	28%
Roseovarius Plymouth podovirus 1	CBX87933.1	5,00E-13	28%
Ruegeria phage vB_RpoP-V13	AWY09361.1	1,00E-11	26%
Dinoroseobacter phage DFL12phi1	YP_009043758.1	1,00E-06	24%
Dinoroseobacter phage vBDshPR2C	AID16822.1	1,00E-06	24%
New hits in third iteration psi-BLAST*			
Roseobacter phage RD-1410W1-01	ANJ20746.1	1E-12	21%

Organisms with Drc homolog	**Accession nr.**	**E-value**	**Identity**
Pseudomonas phage LUZ7	YP_003358296.1	2,00E-66	100%
Pseudomonas phage KPP21	YP_009219005.1	2,00E-65	99%
Pseudomonas phage Littlefix	AUV61861.1	1,00E-07	36%
Pseudomonas phage 98PfluR60PP	AWH15510.1	7,00E-07	36%
Pseudomonas phage PA26	AFO70512.1	4,00E-06	39%
Pseudomonas phage vB_PaeP_C2-10_Ab09	YP_009031789.1	5,00E-06	39%
Pseudomonas phage DL64	YP_009206282.1	5,00E-06	39%
Pseudomonas phage PEV2	YP_009286234.1	5,00E-06	39%
Pseudomonas phage vB_PaeP_MAG4	YP_009290547.1	5,00E-06	39%
Pseudomonas phage vB_PaeP_C2-10_Ab09	CDN96825.1	5,00E-06	39%
Pseudomonas phage RWG	AIZ94763.1	5,00E-06	39%
Pseudomonas phage vB_Pae575P-3	ANT44290.1	5,00E-06	39%
Pseudomonas phage vB_Pae1396P-5	ANT44382.	5,00E-06	39%
Pseudomonas phage vB_PaeP_PYO2	ASZ72062.1	5,00E-06	39%
Pseudomonas phage vB_PaeP_DEV	ASZ72220.1	5,00E-06	39%
Pseudomonas phage P3P1	SBT96773.2	6,00E-06	38%
Pseudomonas phage Pa2	YP_009148193.1	8,00E-06	38%
Pseudomonas phage phi176	AIZ94946.1	8,00E-06	38%
Pseudomonas phage YH6	YP_009152515.1	8,00E-06	39%
Pseudomonas phage YH30	YP_009226100.1	8,00E-06	39%
Pseudomonas phage LP14	AWY02785.1	2,00E-05	38%
Pseudomonas phage LIT1	YP_003358410.1	3,00E-05	37%
New hits in second iteration psi-BLAST*			

Pseudomonas phage phCDa	AXC36480.1	2,00E-05	25%
Marinobacterium litorale	WP_027854432.1	0.003	30%
New hits found with Drc homolog query: YH6 (YP_009152515.1)**			
Pectobacterium phage vB_PatP_CB1	ARB11747.1	0.001	30%
Pectobacterium phage Nepra	AWD92598.1	0.001	30%
Pectobacterium phage vB_PatP_CB4	AQT27863.1	0.004	29%
Pectobacterium phage vB_PatP_CB3	ARB11845.1	0.004	29%
Phage hit below threshold in second iteration psi-BLAST with YH6 Drc homolog***			
Acinetobacter phage Presley	YP_009007581.1	1.4	26%

* E-values obtained from consecutive psi-BLAST searches are the values for that specific iteration and are not comparable to values from other iterations.
** For Drc homologs, no new hits were observed in the third iteration. Instead a new search with a more distant Drc homolog did retrieve new hits within the evolutionary more distant CB1-like phages.
*** Although not within the threshold of significance, the hit is given for completeness as phage Presley is expected to have a Drc homolog based on its phylogenetics. Given the large evolutionary distance, it might be a Drc homolog which is too diverged to reach significance.