Supplementary Information

Insights into docking in megasynthases from investigation of the toblerol *trans*-AT polyketide synthase: many α-helical means to an end†

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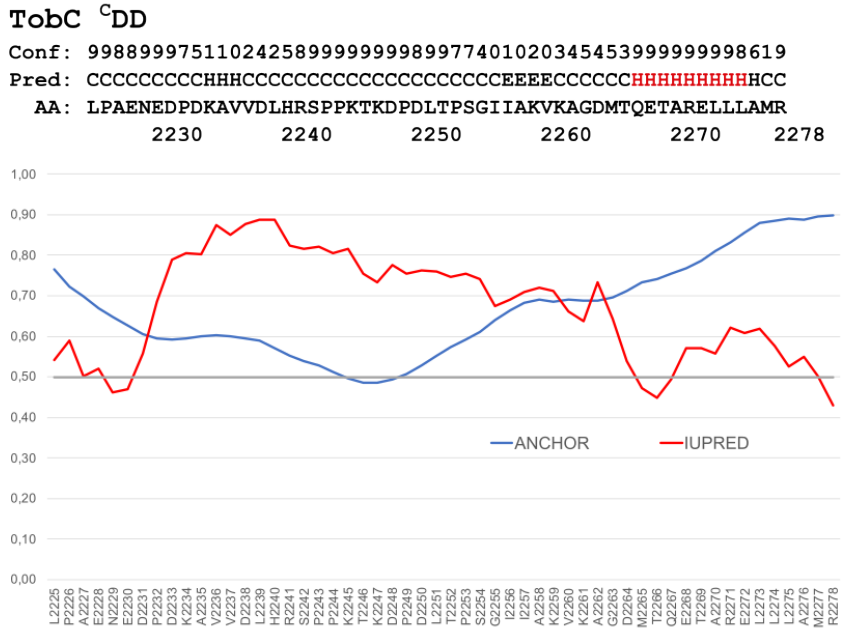
Supplementary Figures

a



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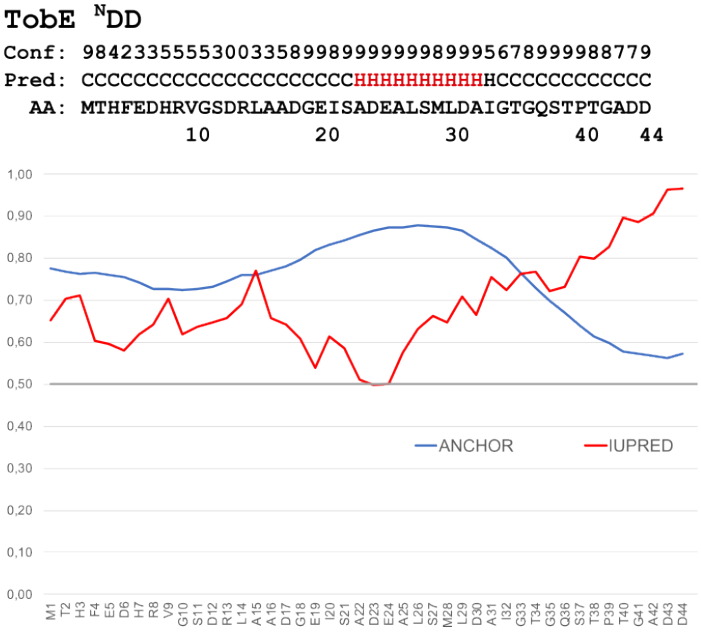


Fig. S1 Bioinformatics analysis of the TobC/TobE interface. The analyses of TobC CDD and TobE NDD are shown in panels (a) and (b), respectively. Secondary structure prediction was carried out using PSIPRED (top, with potential α-helices indicated in red),[1] and the intrinsic disorder/interaction propensity prediction was predicted using the IUPred2A interface[2] (bottom). IUPred2 indicates the disorder tendency of each residue in a given protein, with higher values corresponding to a higher probability of disorder. ANCHOR2 predicts the probability of each residue belonging to a region involved in binding. Key: Conf. is the confidence score of the prediction (from 0 (lowest) to 9 (highest)); Pred. is the type of secondary structure predicted (C = coil, H = helix and E = sheet); AA is the amino acid sequence.

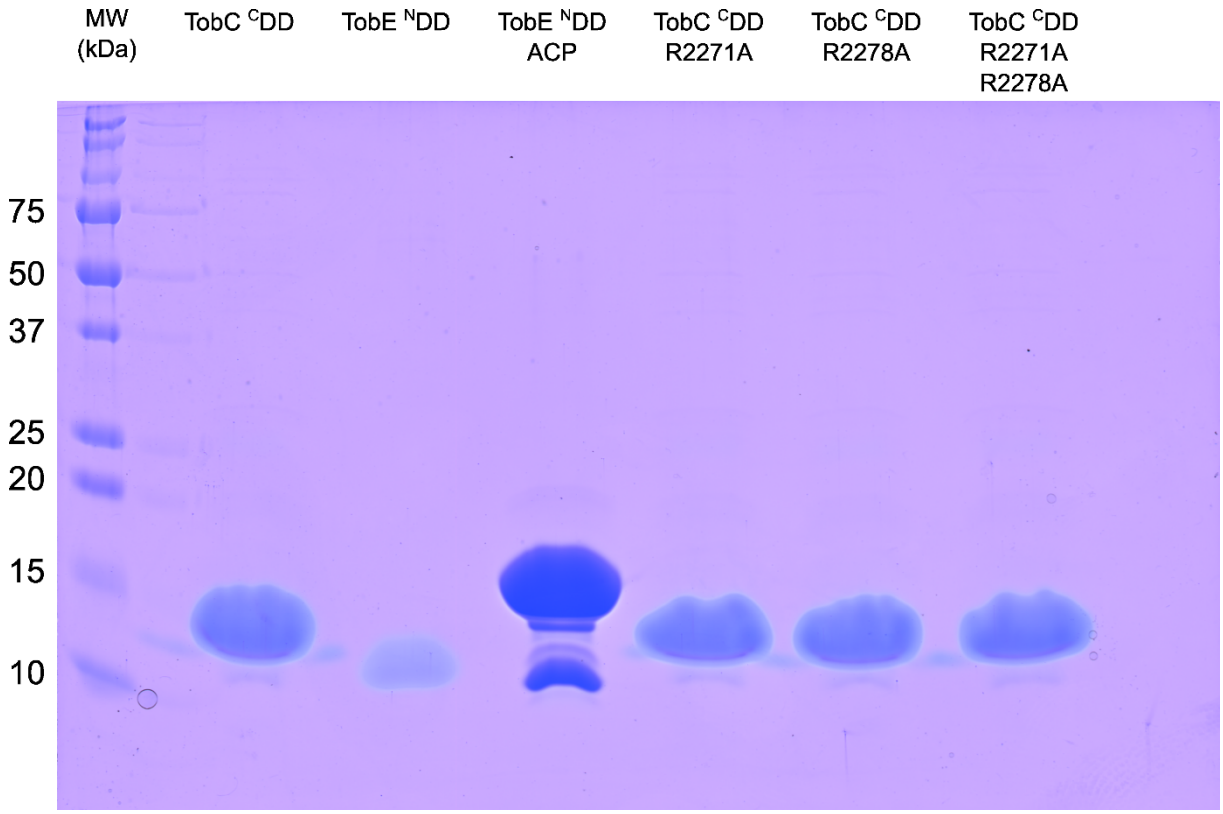


Fig. S2 SDS-PAGE analysis of the constructs used in this study. TobC ^{C}DD (calc'd: 6.64 kDa); TobE ^{N}DD (calc'd: 5.02 kDa); TobE ^{N}DD-ACP (calc'd: 14.71 kDa); TobC ^{C}DD R2271A (calc'd: 6.55 kDa); TobC ^{C}DD R2278A (calc'd: 6.55 kDa); TobC ^{C}DD R2271A/R2278A (calc'd: 6.47 kDa). The molecular weights of the markers are indicated.

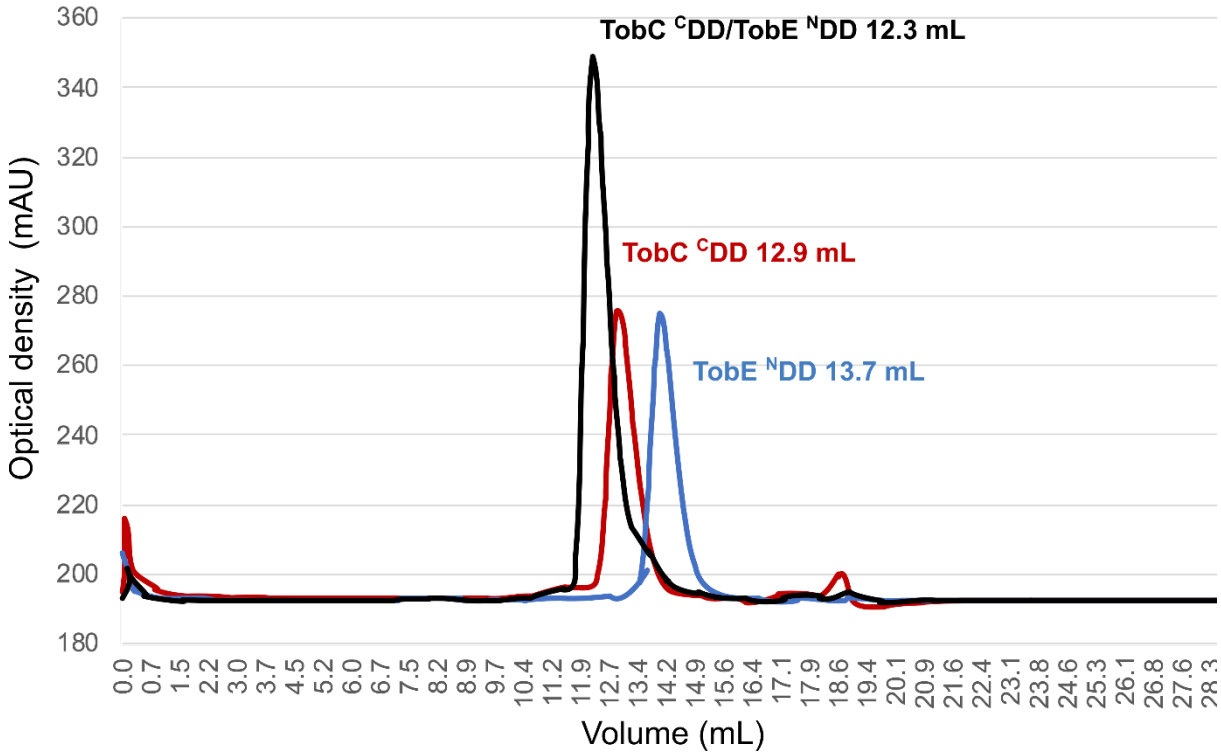


Fig. S3 Gel filtration analysis of the interaction between TobC ^{C}DD and TobE ^{N}DD (both at 400 µM). TobC ^{C}DD eluted at a volume of 12.9 mL and TobE ^{N}DD at 13.7 mL. When mixed (individual concentrations of 400 µM), a single peak was observed at a lower elution volume of 12.3 mL, consistent with formation of a complex.

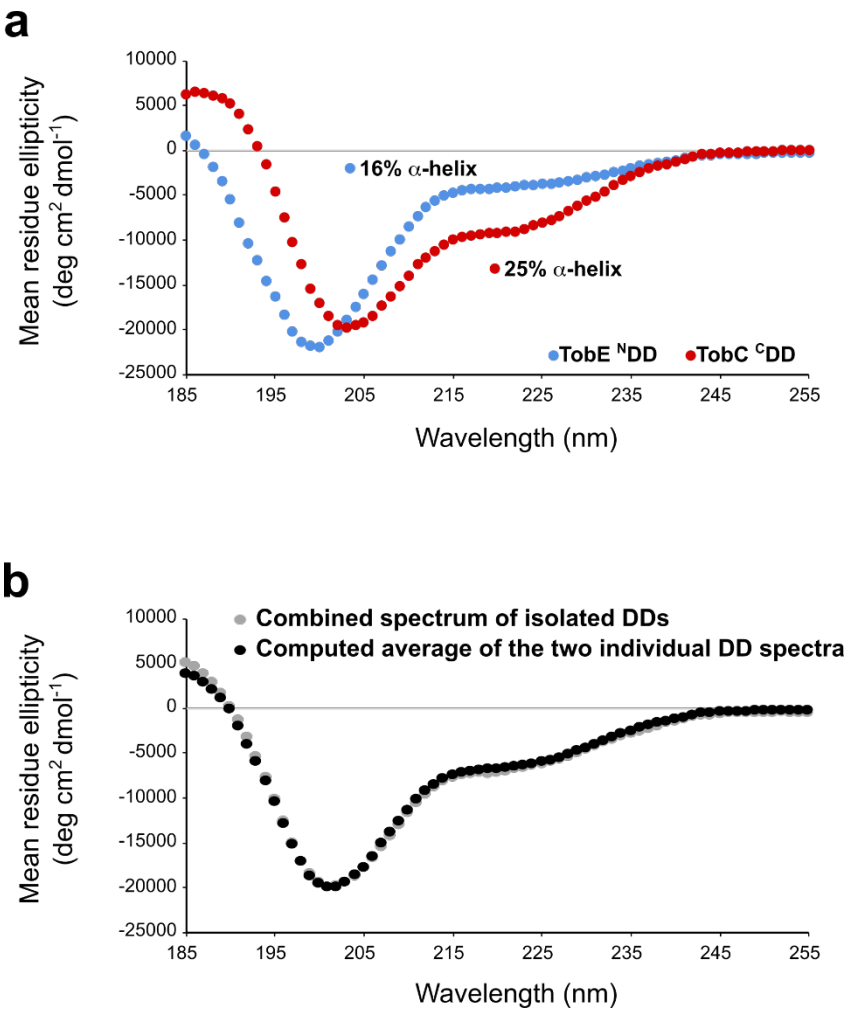


Fig. S4 Circular dichroism analysis of TobE ^{N}DD and TobC ^{C}DD. (a) Circular dichroism analysis of individual TobC ^{C}DD and TobE ^{N}DD (100 μM). The % α-helix calculated using the Pro-Data Viewer software (Applied Photophysics) under the two conditions is indicated. (b) CD analysis showing the superposition of the combined spectrum of the isolated DDs and the computed average of the two individual DD spectra.

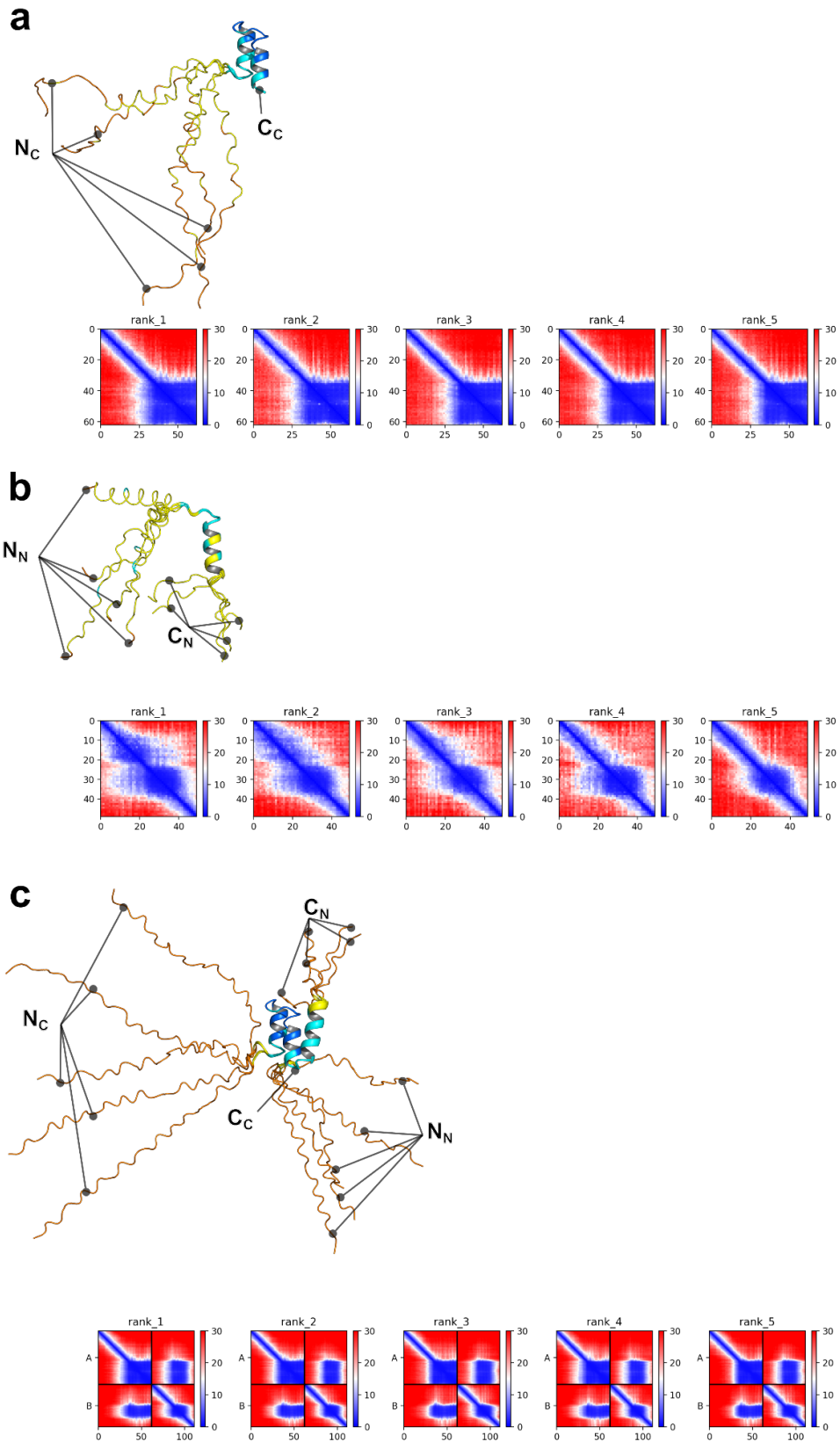


Fig. S5 AlphaFold2[3] structure predictions (top) and associated PAE Matrix Score (bottom). (a) Prediction for TobC ^{C}DD. (b) Prediction for TobE ^{N}DD. (c) Prediction for the TobE ^{N}DD/TobC ^{C}DD complex. All models have been relaxed using Amber. The five predicted models are superimposed and the residues are colored according to a per-residue confidence metric called pLDDT:[3] very high (pLDDT > 90) (blue), confident (90 > pLDDT > 70) (cyan), low (70 > pLDDT > 50) (yellow) and very low (pLDDT < 50) (orange). The N- and C- termini of the models are labeled (N_C and C_C for the ^{C}DD and N_N and C_N for the ^{N}DD).

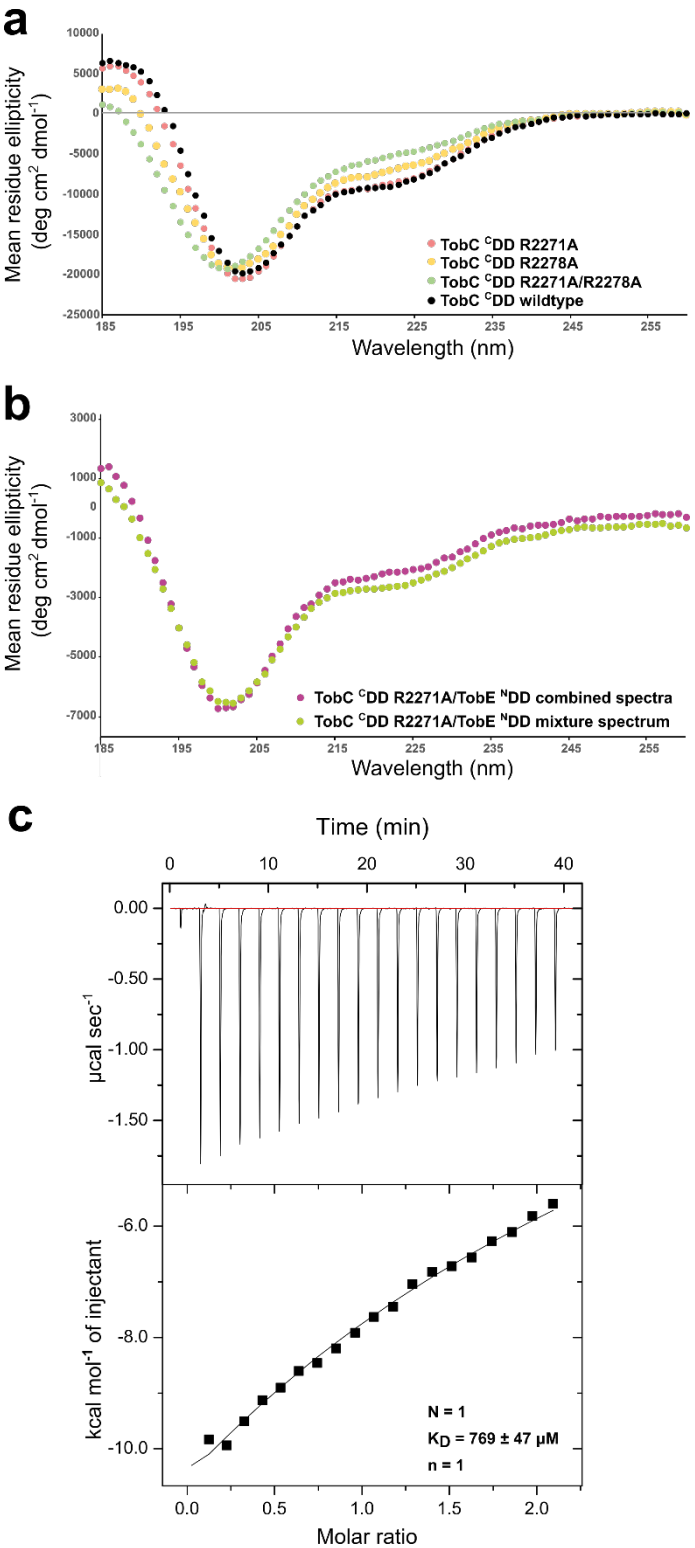
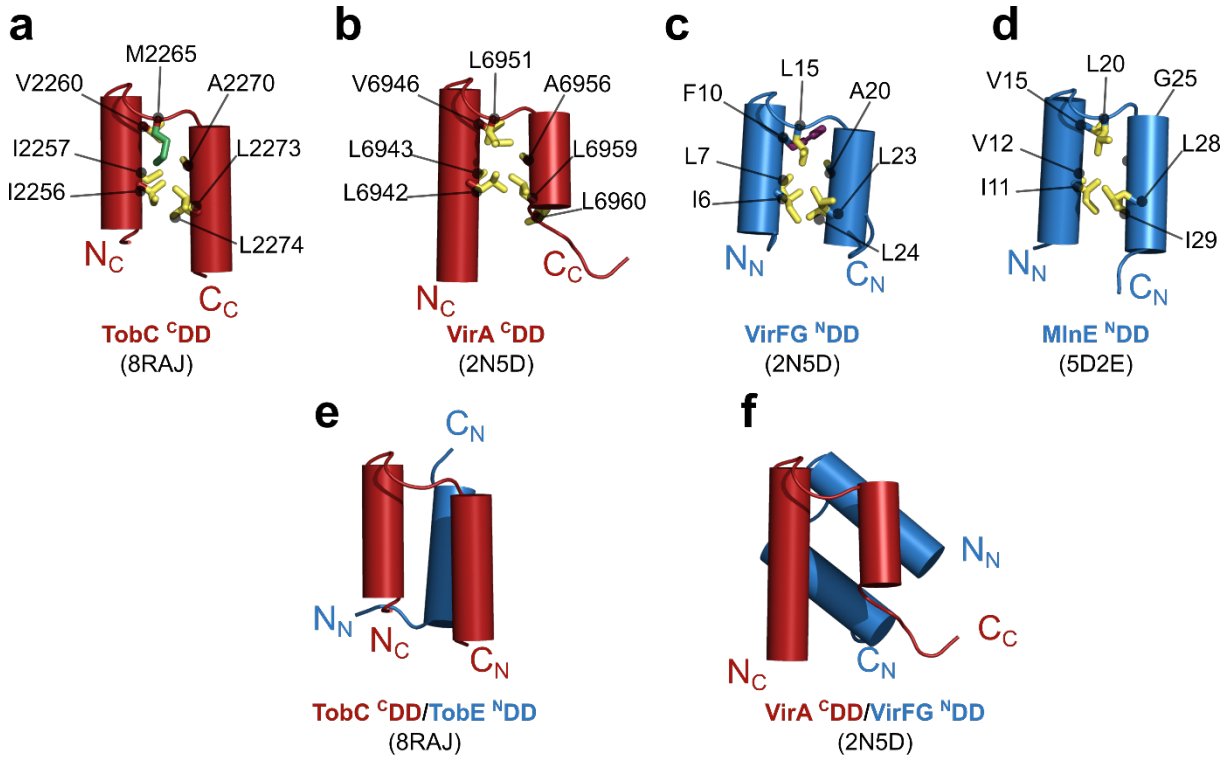


Fig. S6 ITC and CD analysis of single point mutations. (a) CD analysis of TobC ^{C}DD mutants at 100 µM. TobC ^{C}DD wildtype is shown in black. (b) CD analysis for structuration upon mixing TobC ^{C}DD R2271A mutant and TobE ^{N}DD. The combined spectrum was obtained by simultaneously analyzing the two isolated docking domains (both at 100 µM). Comparison with the spectrum of a mixture of the two DDs (again at an overall concentration of 100 µM), demonstrates a lack of increased structuration upon mixing. (c) Analysis by ITC of binding between the mutant TobC ^{C}DD R2271A in the syringe and TobE ^{N}DD in the cell. To ensure data fitting, the stoichiometry was set to 1.



g

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TobC   (8RAJ)      -----2250------2260------2270-----
                   KTKDPDLTPSGIIAKVKAGDMTQETARELLLAMR
VirA   (2N5D)      6931---6940-------6950-------6960-
                   AGEPSQADLDALLSAVRDNRLSIEQAVTLLTPRR
VirFG  (2N5D)            1--------10--------20--------30
                         MDAKEILTRFKDGGLDRAAAQALLAGRTPAAA
MlnE   (5D2E)       1--------10--------20--------30------
                    MNEKTMQIEQIVKKVKECSLTPEEGLELIKSLGKTHL
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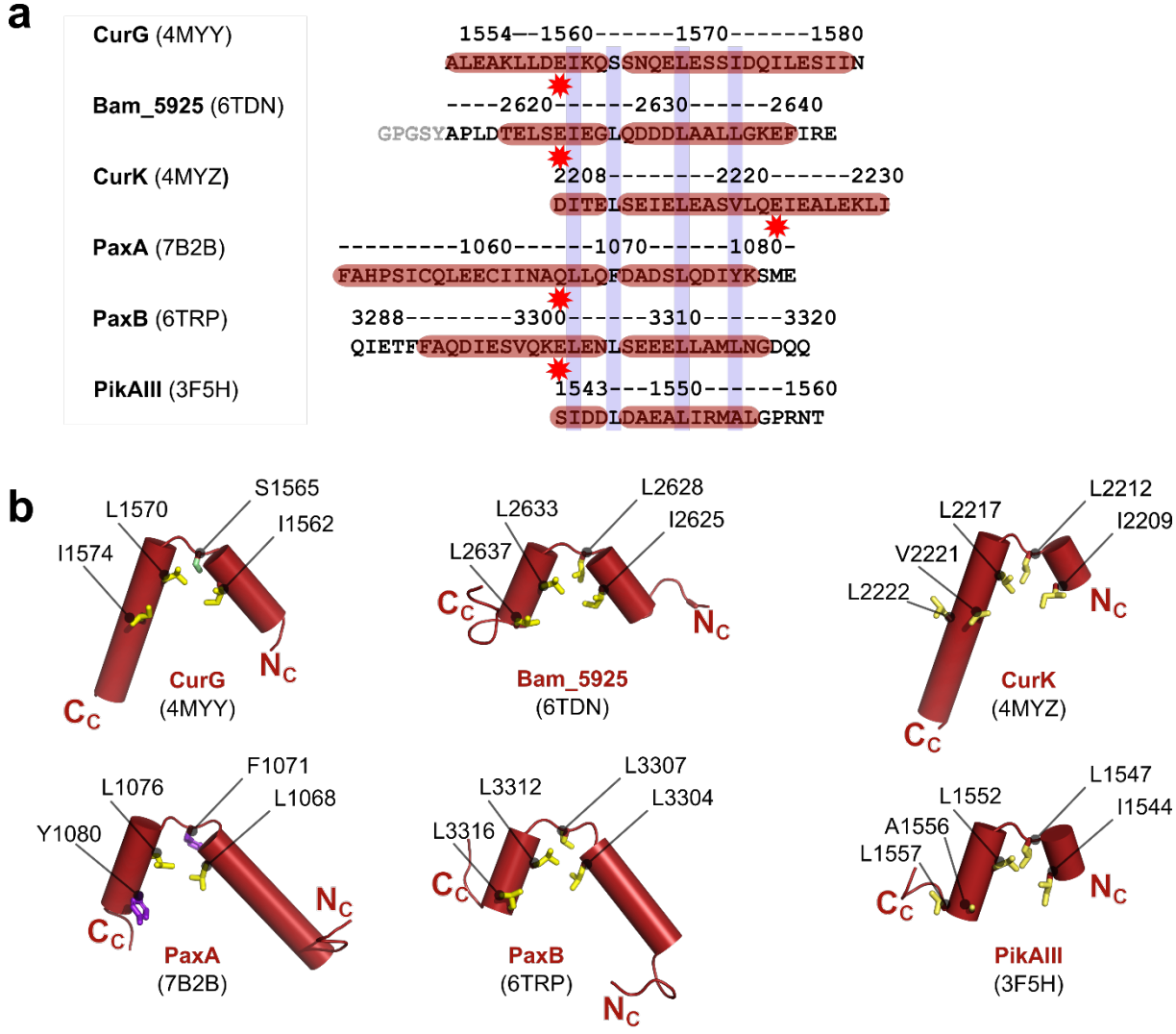
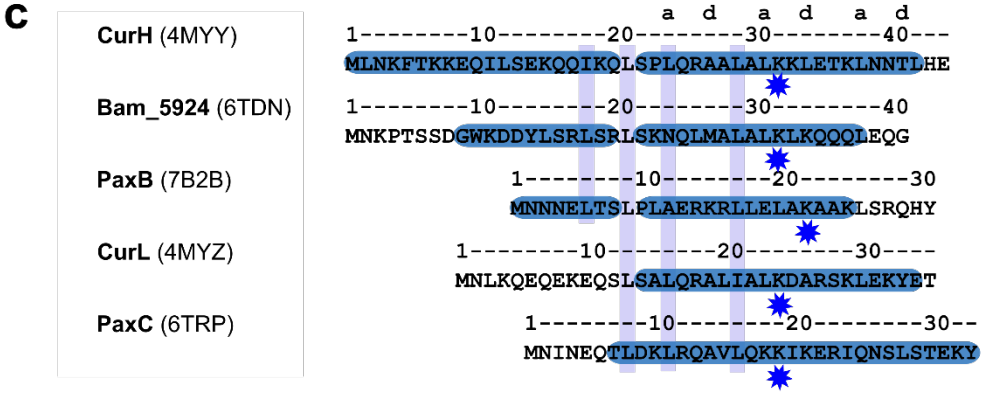
Fig. S7 Comparison between 4 α-helix bundle (4HB) docking domains and the TobC ^{C}DD/TobE ^{N}DD complex fold. (a) TobC ^{C}DD. (b) VirA ^{C}DD (PDB ID: 2N5D[4]). (c) VirFG ^{N}DD (PDB ID: 2N5D[4]). (d) MlnE ^{N}DD (PDB ID: 5D2E[5]). (e)–(f) Structures and comparison between Vir and Tob docking complexes. (e) Complex between TobC ^{C}DD (red) and TobE ^{N}DD (blue). (f) Complex between VirA ^{C}DD (grey) and VirFG ^{N}DD (purple). (g) Sequence alignment between TobC ^{C}DD and 4HB docking domains. The ^{C}DD and ^{N}DD α-helices are colored red and blue, respectively.


a
CurG (4MYY)
Bam_5925 (6TDN)
CurK (4MYZ)
PaxA (7B2B)
PaxB (6TRP)
PikAIII (3F5H)
1554--1560------1570------1580
ALEAKLLDEIKQSSNQELESSIDQILESIIN
----2620------2630------2640
GPGSYAPLDTELSEIEGLQDDDLAALLGKEFIRE
2208--------2220------2230
DITELSEIELEASVLQEIEALEKLI
---------1060------1070------1080-
FAHPSICQLEECIINAQLLQFDADSLQDIYKSME
3288--------3300------3310------3320
QIETFFAQDIESVQKELENLSEEELLAMLNGDQQ
1543---1550------1560
SIDDLDAEALIRMALGPRNT
b
L1570
S1565
I1562
I1574
CurG
(4MYY)
L2633
L2628
I2625
L2637
Bam_5925
(6TDN)
L2217
L2212
I2209
V2221
L2222
CurK
(4MYZ)
L1076
F1071
L1068
Y1080
PaxA
(7B2B)
L3312
L3307
L3304
L3316
PaxB
(6TRP)
L1552
L1547
I1544
A1556
L1557
PikAIII
(3F5H)


c
CurH (4MYY)
Bam_5924 (6TDN)
PaxB (7B2B)
CurL (4MYZ)
PaxC (6TRP)
a d a d a d
1--------10--------20--------30--------40---
MLNKFTKKEQILSEKQQIKQLSPLQRAALALKKLETKLNNTLHE
1--------10--------20--------30--------40
MNKPTSSDGWKDDYLSRLSRLSKNQLMALALKLKQQQLEQG
1--------10--------20--------30
MNNNELTSLPLAERKRLLELAKAAKLSRQHY
1--------10--------20--------30----
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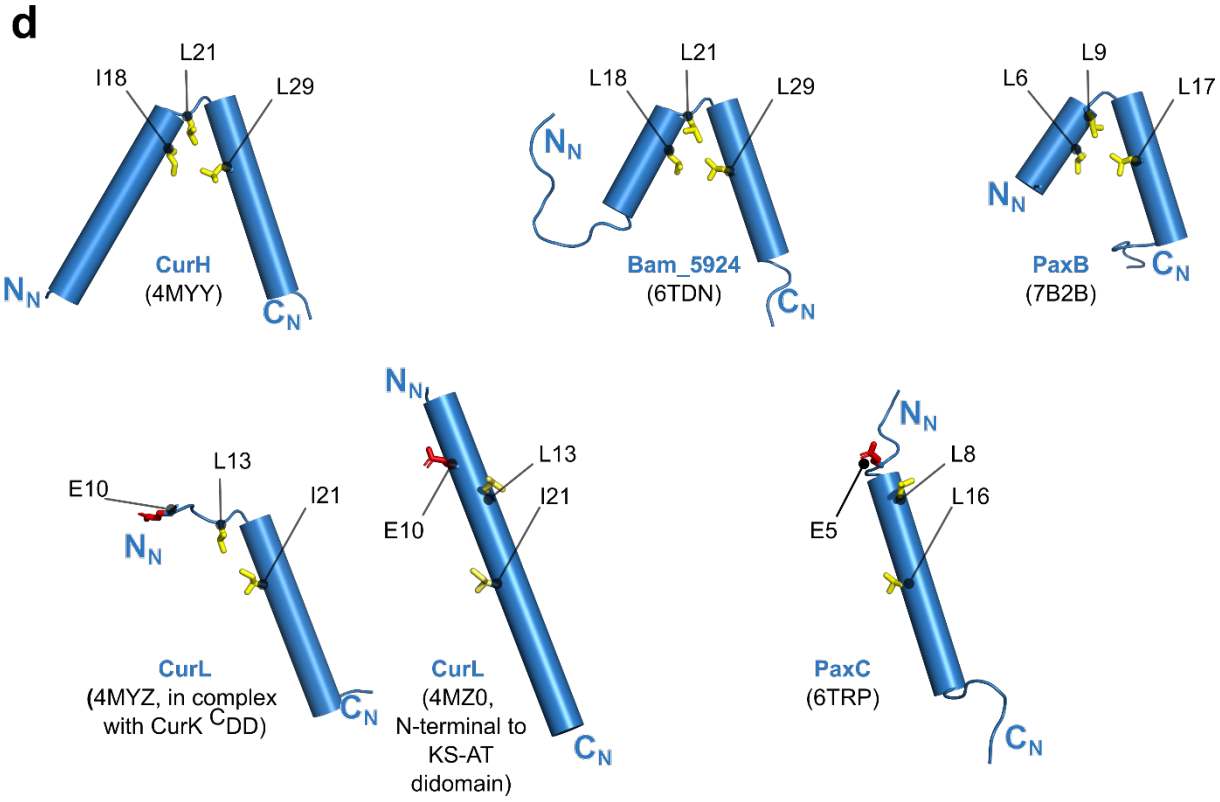
1--------10--------20--------30--
MNINEQTLDKLRQAVLQKKIKERIQNSLSTEKY


d
I18
L21
L29
NN
CN
CurH
(4MYY)
L18
L21
L29
NN
CN
Bam_5924
(6TDN)
L6
L9
L17
NN
CN
PaxB
(7B2B)
E10
L13
I21
NN
CN
CurL
(4MYZ, in complex
with CurK CDD)
NN
L13
E10
I21
CN
CurL
(4MZ0,
N-terminal to
KS-AT
didomain)
NN
L8
E5
L16
CN
PaxC
(6TRP)

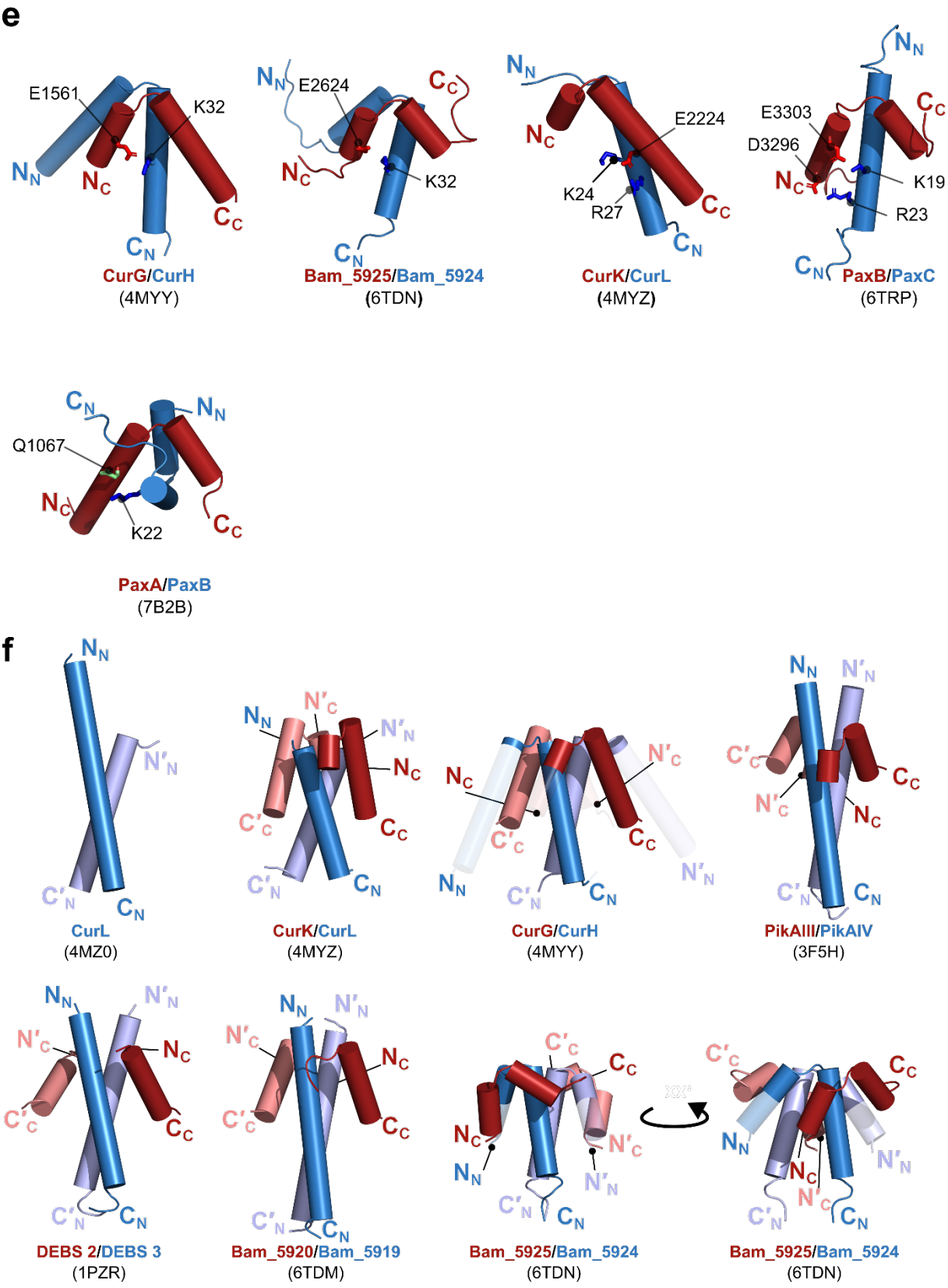


Fig. S8 Sequence and structure comparison of individual and complexed DDs. (a) Sequence alignment of type 2(-related) ^{C}DDs whose structures have been determined. The strips highlight the conserved aliphatic residues. Conserved residues mediating electrostatic interactions are highlighted by a star. (b) Structure comparison of type 2(-related) ^{C}DDs (aliphatic residues are colored in yellow and aromatic in purple) and the α-helices are represented as cylinders. The ^{C}DD names and PDB codes of the structures are indicated. (c) Sequence alignment of type 2(-related) ^{N}DDs whose structures have been determined. Conserved aliphatic residues are highlighted by a blue strip. The letters a and d indicate the first and fourth residues of the heptad repeats. (d) Structure comparison of type 2(-

related) ^{N}DDs. Conserved residues are indicated on the structures in stick form (aliphatic residues are colored in yellow and acidic in red), and the α-helices are represented as cylinders. The ^{N}DD names and PDB codes of the structures are indicated. (e) Structure comparison of type 2(-related) ^{C}DD/^{N}DD complexes. The names of the DDs are indicated, with ^{C}DDs colored red and ^{N}DDs colored blue. Conserved residues participating in electrostatic interactions are represented as sticks and are labeled and numbered, with basic residues colored blue, acidic red and polar green. α-Helices are represented as cylinders, and the N- and C-termini are labeled. (f) Structure comparisons of type 2(-related) and type 1(-related) ^{C}DD/^{N}DD complexes (alongside the coiled-coil formed by extended CurL ^{N}DD). The names of the DDs are indicated with ^{C}DDs colored red and ^{N}DDs colored blue. α-Helices are represented as cylinders, and the N- and C-termini are labeled.

Supplementary Tables

Table S1 Sequences of constructs used in this study, and calculated molecular weights

Construct	Sequence[a]	Molecular weight
TobE ^{N}DD	GPGSYMTHFEDHRVGSDRLAADGEISADEALSMLDAIG TGQSTPTGADD	5023.32 Da
TobC ^{C}DD	GPGSPNSYLPAENEDPDKAVVDLHRSPPKTKDPDLTPS GIIAKVKAGDMTQETARELLLAMR	6640.50 Da
TobE ^{N}DD-ACP	GPGSMTHFEDHRVGSDRLAADGEISADEALSMLDAIGT GQSTPTGADDLKARIVQDLGLLIASVLKLQLEDLSPGRT WMDYGLDSIASTELATLFTEKYGLSLPPTIFFEFQNIDSF ADYLLSSHRVQLEKKYSV	14712.50 Da
TobC ^{C}DD R2271A	GPGSPNSYLPAENEDPDKAVVDLHRSPPKTKDPDLTPS GIIAKVKAGDMTQETAAELLLAMR	6555.39 Da
TobC ^{C}DD R2278A	GPGSPNSYLPAENEDPDKAVVDLHRSPPKTKDPDLTPS GIIAKVKAGDMTQETARELLLAMA	6555.39 Da
TobC ^{C}DD R2271A-R2278A	GPGSPNSYLPAENEDPDKAVVDLHRSPPKTKDPDLTPS GIIAKVKAGDMTQETAAELLLAMA	6470.29 Da

[a]Red: sequence introduced from the cloning procedure; green: added Tyr residue; yellow: introduced mutation.

Table S2 Primers used in this study

Construct	Primer Type	Oligonucleotide (5′-3′)[a]
Cloning		
TobE ^{N}DD	f	TTTCCGGGATCC**TAT**ATGACGCACTTCGAGGAC
	r	TTTCCGGCTAGC**TCA**ATCGTCCGCGCCGG
TobC ^{C}DD	f	TTTCCGGAATTCg**TAT**CTACCTGCAGAGAACGAGGATCC
	r	TTTCCGAAGCTTTCAGCGCATGGCAAGCAG
TobE ^{N}DD-ACP	f	TTTCCGGGATCCATGACGCACTTCGAGGAC
	r	TTTCCGGCTAGC**TCA**GACCGAATACTTTTTTTCCAATTGG
Mutagenesis		
TobC ^{C}DD R2271A	f	CAGGAGACCGCCGCCGAGCTGCTGCTT
	r	AAGCAGCAGCTCGGCGGCGGTCTCCTG
TobC ^{C}DD R2278A	f	CTGCTGCTTGCCATGGCCTGAAAGCTTGCGGCC
	r	GGCCGCAAGCTTTCAGGCCATGGCAAGCAGCAG

[a]Introduced restriction sites are underlined and the base pairs coding for an additional Tyr or stop codon are indicated in bold. In the case of TobC ^{C}DD, an additional base (in lower case) was introduced to conserve the reading frame.

Table S3 Structural statistics specified by the NMR Validation Task Force

Restraints used for structure calculation			
Distance restraints		TobC ^{C}DD	TobE ^{N}DD
Total		784	394
Intraresidue		256	134
Sequential		259	165
Medium range		168	79
Long range		101	16
Intermolecular	171		
Dihedral angle restraints		46	22

Constraint violations, mean ± SD[6]	
Distance violations	
0.1 < d ≤ 0.2 Å	0.15 ± 0.37
d > 0.2 Å	0
Average maximum distance violations (Å)	0.08 ± 0.02
Torsion angle violations	
θ < = 5.0 °	0
θ > 5.0°	0
Average maximum torsion angle violations (°)	0.0

Amber energies, mean ± SD (kcal mol^{-1})[6]	
Distance restraint	1.0 ± 0.2
Torsion restraint	0.001 ± 0.005
Van der Waals	−581 ± 13
Total molecular	−4469 ± 14

Precision, RMSD from the mean (Å)		
	TobC ^{C}DD P2249−M2277 and TobE ^{N}DD I20−A31 (defined as ordered by PSVS[7])	TobC ^{C}DD L2251−R2278 and TobE ^{N}DD E19−G33
Backbone	0.76 ± 0.25	0.59 ± 0.13
All heavy atoms	1.22 ± 0.21	1.11 ± 0.12

Ramachandran statistics (%)[8]		
	Whole construct	P2249−M2277, I20−A31 calculated with PSVS 1.5[7]
Most favored	82.4	98.1
Additionally allowed	15.1	1.9
Generously allowed	1.8	0.0
Disallowed	0.7	0.0

MolProbity scores (percentiles) for the lowest-restraint energy structure[9]		
MolProbity score	1.05	100th percentile

Clash score all-atom		0	100th percentile

Global quality scores (raw score/Z-score) calculated with PSVS 1.5[7]		
Verify3D		0.16 ± 0.02/−4.82
ProsaII		0.68 ± 0.06/0.12
Procheck G-factor (phi-psi)	P2249−M2277, I20−A31	0.41/1.93
Procheck G-factor (all)	P2249−M2277, I20−A31	0.36/2.13
Molprobity clashscore		0.16 ± 0.45/1.50

Deviations from ideal geometry[6]	
RMS deviation for bond angles	2.25° ± 0.02
RMS deviation for bond lengths	0.011 Å ± 0.001

Table S4 Summary of docking domain inter α-helix angles

Protein	Type of complex formed	Helix 1	Helix 2	Angle
TobC ^{C}DD	3HBb	P2253−A2262	Q2267−R2278	176 ± 2°
VirFG ^{N}DD	4HB	D2−D12	D16−G26	172 ± 2°
MlnE ^{N}DD	4HB	T5−E17	T21−S31	166°
VirA ^{C}DD	4HB	S6935−D6948	S6952−L6959	162 ± 4°
CurG ^{C}DD	2	E1555−Q1564	S1566−N1583	116 ± 1°
CurH ^{N}DD	2	M1−Q20	S22−L42	141 ± 9°
CurK ^{C}DD	2	D2208−L2212	S2213−S2232	125 ± 2°
Bam_5925 ^{C}DD	2-related	T2620−E2626	Q2629−G2638	125 ± 6°
Bam_5924 ^{N}DD	2-related	G9−L21	S22−L28	135 ± 3°
PaxA_T1 ^{C}DD	*	C1062−F1071	A1073−K1081	118 ± 3°
PaxB ^{N}DD	*	N2−L9	P10−K25	131 ± 3°
PaxB ^{C}DD	3HBa	F3293−E3305	S3308−G3318	119 ± 3°
PikAIII ^{C}DD	1b	S1543−D1546	A1549−A1556	132 ± 5°

*: In this case, the individual DDs resemble type 2(-related) domains, but the overall complex has not yet been classified.

Table S5 Summary of RMSDs calculated between proteins or complexes in this study

Protein 1 (residues) (*DD type*[a])	Protein 2 (residues) (*DD type*[a])	RMSD
TobC ^{C}DD (G2255−L2274) (*3HBb*)	VirA ^{C}DD (A6941−L6960) (*4HB*)	0.56 ± 0.07 Å
	VirFG ^{N}DD (E5−L24) (*4HB*)	0.56 ± 0.05 Å
	MlnE ^{N}DD (Q10−I29) (*4HB*)	0.74 ± 0.06 Å
MlnE ^{N}DD (Q10−I29) (*4HB*)	VirFG ^{N}DD (E5−L24) (*4HB*)	0.90 ± 0.06 Å
	VirA ^{C}DD (A6941−L6960) (*4HB*)	0.88 ± 0.06 Å
VirA ^{C}DD (A6941−L6960) (*4HB*)	VirFG ^{N}DD (E5−L24) (*4HB*)	0.68 ± 0.08 Å
PaxB ^{N}DD (N4−A21) (*)	CurH ^{N}DD (Q16−K33) (*2*)	0.64 ± 0.05 Å
	Bam_5924 ^{N}DD (S16−L33) (*2-related*)	0.39 ± 0.09 Å
PaxC ^{N}DD (L11−Q25) (*3HBa*)	CurL ^{N}DD (L16−L30) (PDB ID: 4MYZ) (*2*)	0.59 ± 0.09 Å
PaxB ^{C}DD (V3300−L3316) (*3HBa*)	CurG ^{C}DD (L1558−I1574) (*2*)	0.71 ± 0.13 Å
	Bam_5925 ^{C}DD (E2621−L2637) (*2-related*)	0.87 ± 0.06 Å
	PaxA_T1 ^{C}DD (I1064−Y1080) (*)	1.40 ± 0.10 Å
PaxA_T1 ^{C}DD (I1064−Y1080) (*)	Bam_5925 ^{C}DD (E2621−L2637) (*2-related*)	1.41 ± 0.09 Å
	CurG ^{C}DD (L1558−I1574) (*2*)	1.30 ± 0.10 Å
CurK ^{C}DD (D2208−V2221) (*2*)	Bam_5925 ^{C}DD (E2624−L2637) (*2-related*)	0.95 ± 0.14 Å
	CurG ^{C}DD (E1561−I1574) (*2*)	0.69 ± 0.13 Å
	PaxB ^{C}DD (E3303−L3316) (*3HBa*)	0.92 ± 0.11 Å
	PaxA_T1 ^{C}DD (Q1067−Y1080) (*)	1.29 ± 0.16 Å
PikAIII ^{C}DD (S1543−A1556) (*1b*)	CurK ^{C}DD (D2208−V2221) (*2*)	0.58 ± 0.15 Å
	CurG ^{C}DD (E1561−I1574) (*2*)	0.54 ± 0.04 Å
	Bam_5925 ^{C}DD (E2624−L2637) (*2-related*)	0.69 ± 0.08 Å
	PaxB ^{C}DD (E3303−L3316) (*3HBa*)	0.74 ± 0.05 Å
	PaxA_T1 ^{C}DD (Q1067−Y1080) (*)	1.38 ± 0.05 Å
Complex 1 (residues) (*DD type*)	**Complex 2 (residues) (*DD type*)**	**RMSD**
PaxB ^{C}DD/PaxC ^{N}DD (V3300−L3316/D9−I20) (*3HBa*)	CurG ^{C}DD/CurH ^{N}DD (L1558−I1574/S22−K33) (*2*)	1.13 ± 0.07 Å
	Bam_5925 ^{C}DD/Bam_5924 ^{N}DD (E2621−L2637/S22−L33) (*2-related*)	1.18 ± 0.07 Å
CurK ^{C}DD/CurL ^{N}DD[b] (D2208−K2230/S12−K32) (*2*)	CurG ^{C}DD/CurH ^{N}DD (E1561−N1583/ Q20−N40) (*2*)	1.96 Å
CurL ^{N}DD/CurL ^{N}DD[b] (PDB ID: 4MYZ) (S12−T35) (*2*)	CurL ^{N}DD CurL ^{N}DD (PDB ID: 4MZ0) (S12−T35) (*2*)	1.66 Å
CurK ^{C}DD/CurL ^{N}DD (I2215−E2226/S14−K32) (*2*)	DEBS 2 ^{C}DD/ DEBS 3 ^{N}DD (D3551−G3562/R14−E32) (*1a*)	2.51 ± 0.20 Å
CurK ^{C}DD/CurL ^{N}DD (D2208−L2222/S14−E31) (*2*)	PikAIII ^{C}DD/ PikAIV ^{N}DD (S1543−L1557/D10−R27) (*1b*)	2.05 Å
CurK ^{C}DD/CurL ^{N}DD (I2209−E2226/S14−E31) (*2*)	Bam_5920 ^{C}DD/5919 ^{N}DD (D2956−G2973/Q6−Q23) (*1-related*)	1.77 ± 0.12 Å
CurK ^{C}DD/CurL ^{N}DD (D2208−V2221/S14−K29) (*2*)	Bam_5925 ^{C}DD/5924 ^{N}DD (E2624−L2637/S22−Q37) (*2-related*)	5.72 ± 0.11 Å

PikAIII ^{C}DD/ PikAIV ^{N}DD (L1547−L1557/S3−R29) (*1b*)	DEBS 2 ^{C}DD/ DEBS 3 ^{N}DD (A3548−D3558/M7−V33) (*1a*)	2.54 ± 0.10 Å
PikAIII ^{C}DD/ PikAIV ^{N}DD (S1543−G1558/D10−D31) (*1b*)	CurG ^{C}DD/CurH ^{N}DD (E1561−Q1576/S22−H43) (*2*)	2.03 Å
PikAIII ^{C}DD/ PikAIV ^{N}DD (I1544−G1558/N5−Q34) (*1b*)	Bam_5920 ^{C}DD/5919 ^{N}DD (D2956−Q2970/M1−R30) (*1-related*)	2.46 ± 0.15 Å
PikAIII ^{C}DD/ PikAIV ^{N}DD (S1543−A1556/A11−S26) (*1b*)	Bam_5925 ^{C}DD/5924 ^{N}DD (E2624−L2637/S22−Q37) (*2-related*)	5.79 ± 0.11 Å
DEBS 2 ^{C}DD/ DEBS 3 ^{N}DD (A3548−G3562/R14−H35) (*1a*)	CurG ^{C}DD/CurH ^{N}DD (S1565−E1579/S22−H43) (*2*)	2.06 ± 0.08 Å
DEBS 2 ^{C}DD/ DEBS 3 ^{N}DD (A3548−G3563/L12−R36) (*1a*)	Bam_5920 ^{C}DD/5919 ^{N}DD (L2959−D2974/I4−A28) (*1-related*)	2.48 ± 0.17 Å
DEBS 2 ^{C}DD/ DEBS 3 ^{N}DD (S3549−L3557/Y15−L30) (*1a*)	Bam_5925 ^{C}DD/5924 ^{N}DD (Q2629−L2637/S22−Q37) (*2-related*)	4.63 ± 0.11 Å
CurG ^{C}DD/CurH ^{N}DD (I1562−E1579/ S22−H43) (*2*)	Bam_5920 ^{C}DD/5919 ^{N}DD (D2956−G2973/Q6−Q27) (*1-related*)	1.71 ± 0.11 Å
CurG ^{C}DD/CurH ^{N}DD (E1561−I1574/ Q17−K37) (*2*)	Bam_5925 ^{C}DD/5924 ^{N}DD (E2624−L2637/R17−Q37) (*2-related*)	5.64 ± 0.11 Å
Bam_5920 ^{C}DD/5919 ^{N}DD (D2956−L2968/L7−N22) (*1-related*)	Bam_5925 ^{C}DD/5924 ^{N}DD (I2625−L2637/S22−Q37) (*2-related*)	5.45 ± 0.17 Å

[a]The 'type' designations refer to the initial classification of the DDs based on their structures within the docked complexes. *: In this case, the individual DDs resemble type 2(-related) domains, but the overall complex has not yet been classified.

Table S6 Summary of TM scores calculated between proteins or complexes in this study

Protein 1 (*DD type*[a])	Protein 2 (*DD type*[a])	TM score (min/max)
TobC ^{C}DD (T2252–L2275) (*3HBb*)	VirFG ^{N}DD (D2–A25) (*4HB*)	0.66 ± 0.04 (0.55/0.77)
	VirA ^{C}DD (D6938–T6961) (*4HB*)	0.62 ± 0.04 (0.49/0.71)
	MlnE ^{N}DD (Q7–K30) (*4HB*)	0.57 ± 0.03 (0.53/0.63)
MlnE ^{N}DD (Q7–K30) (*4HB*)	VirA ^{C}DD (D6938–T6961) (*4HB*)	0.49 ± 0.01 (0.47/0.52)
	VirFG ^{N}DD (D6938–T6961) (*4HB*)	0.51 ± 0.02 (0.47/0.54)
VirA ^{C}DD (D6938–T6961) (*4HB*)	VirFG ^{N}DD (D6938–T6961) (*4HB*)	0.64 ± 0.04 (0.54/0.72)
PaxB ^{N}DD (N3–K25) (*)	Bam_5924 ^{N}DD (L15–Q37) (2-related)	0.47 ± 0.03 (0.39/0.59)
	CurH ^{N}DD§ (K15–K37) (*2*)	0.51 ± 0.02 (0.47/0.55)
	CurH ^{N}DD¶ (K15–K37) (*2*)	0.48 ± 0.03 (0.42/0.51)
	CurH ^{N}DD overall (*2*)	0.49 ± 0.03 (0.42/0.55)
PaxC ^{N}DD (E5–Q25) (*3HBa*)	CurL ^{N}DD (K9–K29) 4MYZ (*2*)	0.40 ± 0.03 (0.33/0.49)
	CurL ^{N}DD (K9–K29) 4MZ0 (*2*)	0.45 ± 0.03 (0.39/0.49)
PaxB ^{C}DD (A3296–Q3320) (*3HBa*)	CurG ^{C}DD§ (L1554–L1578) (*2*)	0.51 ± 0.03 (0.44/0.56)
	CurG ^{C}DD¶ (L1554–L1578) (*2*)	0.39 ± 0.02 (0.35/0.43)
	CurG ^{C}DD overall (*2*)	0.45 ± 0.07 (0.35/0.56)
	Bam_5925 ^{C}DD (P2617–F2641) (*2-related*)	0.39 ± 0.03 (0.31/0.47)
	PaxA T1-^{C}DD (E1060–E1084) (*)	0.41 ± 0.04 (0.33/0.53)
PaxA T1-^{C}DD (E1060–E1084) (*)	CurG ^{C}DD§ (L1554–L1578) (*2*)	0.39 ± 0.02 (0.36/0.42)
	CurG ^{C}DD¶ (L1554–L1578) (*2*)	0.38 ± 0.03 (0.34/0.42)
	CurG ^{C}DD overall (*2*)	0.39 ± 0.02 (0.34/0.42)
	Bam_5925 ^{C}DD (P2617–F2641) (*2-related*)	0.31 ± 0.03 (0.25/0.43)
PikAIII ^{C}DD (S1543–L1557) (*1b*)	CurK ^{C}DD (D2208–Q2223) (*2*)	0.58 ± 0.03 (0.53/0.63)
	CurG ^{C}DD§ (L1554–L1578) (*2*)	0.67 ± 0.00 (0.67/0.67)
	CurG ^{C}DD¶ (L1554–L1578) (*2*)	0.54 ± 0.07 (0.50/0.59)
	CurG ^{C}DD overall (*2*)	0.61 ± 0.08 (0.50/0.67)
	Bam_5925 ^{C}DD (P2617–F2641) (*2-related*)	0.49 ± 0.03 (0.43/0.58)
	PaxB ^{C}DD (D3296–Q3320) (*3HBa*)	0.49 ± 0.03 (0.42/0.54)
	PaxA T1-^{C}DD (E1060–E1084) (*)	0.46 ± 0.03 (0.40/0.54)
Di-domain ^{C}DD/^{N}DD 1	Di-domain ^{C}DD/^{N}DD 2	TM score (min/max)
PaxB ^{C}DD/PaxC ^{N}DD (Q3288–Q3321/M1–Y33) (*3HBa*)	CurG ^{C}DD/CurH ^{N}DD§ (N1551–N1583/M1–E44) (*2*)	0.50 ± 0.01 (0.48/0.52)
	CurG ^{C}DD/CurH ^{N}DD¶ (N1551–N1583/M1–E44) (*2*)	0.51 ± 0.04 (0.37/0.54)
	CurG ^{C}DD/CurH ^{N}DD overall (*2*)	0.51 ± 0.04 (0.37/0.54)
	Bam_5925 ^{C}DD/Bam_5924 ^{N}DD (A2616–E2644/M1–G41) (*2-related*)	0.49 ± 0.01 (0.45/0.54)
^{N}DD/^{N}DD (1)[a]	^{N}DD/^{N}DD (2)	TM score (min/max)
CurL (S14–E34)/CurL (PDB ID: 4MZ0) (S13–T35) (*2*)	CurL(S14–E34)/CurL (PDB ID: 4MYZ) (S14–T35) (*2*)	0.70

^{C}DD/^{N}DD complex 1	^{C}DD/^{N}DD complex 2	TM score (min/max)
CurK ^{C}DD/CurL ^{N}DD (D2208–A2227/S14–E34) (*2*)	CurG ^{C}DD/Cur^{N}DD H (E1561–N1583/Q20–H43) (*2*)	0.68
	PikAIII ^{C}DD/ PikAIV ^{N}DD (S1543–G1558/S3–R33) (*1b*)	0.58
	DEBS 2 ^{C}DD/ DEBS 3 ^{N}DD (A3548–G3563/M7–A37) (*1a*)	0.56 ± 0.02 (0.54/0.59)
	Bam_5920 ^{C}DD 5919 ^{N}DD (D2956–G2973/M1–R30) (*1-related*)	0.66 ± 0.01 (0.64/0.68)
	Bam_5925 ^{C}DD 5924 ^{N}DD (E2624–E2644/R17–G41) (*2-related*)	0.42 ± 0.01 (0.40/0.45)
PikAIII ^{C}DD/ PikAIV ^{N}DD (S1543–G1558/S3–R33) (*1b*)	DEBS 2 ^{C}DD/ DEBS 3 ^{N}DD (A3548–G3563/M7–A37) (*1a*)	0.60 ± 0.01 (0.61/0.64)
	CurG ^{C}DD/Cur^{N}DD H (E1561–N1583/Q20–H43) (*2*)	0.67
	Bam_5920 ^{C}DD 5919 ^{N}DD (D2956–G2973/M1–R30) (*1-related*)	0.74 ± 0.02 (0.70/0.77)
	Bam_5925 ^{C}DD 5924 ^{N}DD (E2624–E2644/R17–G41) (*2-related*)	0.48 ± 0.01 (0.46/0.50)
DEBS 2 ^{C}DD/ DEBS 3 ^{N}DD (A3548–G3563/M7–A37) (*1a*)	CurG ^{C}DD/Cur^{N}DD H (E1561–N1583/Q20–H43) (*2*)	0.62 ± 0.01 (0.61/0.64)
	Bam_5920 ^{C}DD 5919 ^{N}DD (D2956–G2973/M1–R30) (*1-related*)	0.65 ± 0.02 (0.59/0.69)
	Bam_5925 ^{C}DD 5924 ^{N}DD (E2624–E2644/R17–G41) (*2-related*)	0.39 ± 0.02 (0.34/0.43)
CurG ^{C}DD/Cur^{N}DD H (E1561–N1583/Q20–H43) (*2*)	Bam_5920 ^{C}DD 5919 ^{N}DD (D2956–G2973/M1–R30) (*1-related*)	0.72 ± 0.01 (0.70/0.73)
	Bam_5925 ^{C}DD 5924 ^{N}DD (E2624–E2644/R17–G41) (*2-related*)	0.48 ± 0.01 (0.46/0.50)
Bam_5920 ^{C}DD 5919 ^{N}DD (D2956–G2973/M1–R30) (*1-related*)	Bam_5925 ^{C}DD 5924 ^{N}DD (E2624–E2644/R17–G41) (*2-related*)	0.45 ± 0.01 (0.42/0.48)

[a]The 'type' designations refer to the initial classification of the DDs based on their structures within the docked complexes. The symbols § and ¶ refer to distinct conformers observed in the crystal structures. *: In this case, the individual DDs resemble type 2(-related) domains, but the overall complex has not yet been classified.

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