Analysis of the "RadC"-containing ORF.
We examined this ORF in some detail because it is semi-conserved in SCC elements and thus may confer some advantage to its host. In both patterns of SCC elements, the DUF 950-960-1643 trio is often but not always followed by this ORF. In some cases this is interrupted by insertion of Tn554. The RadC conserved domain is also annotated as Mov34 and DUF2466, and contains a JAB1/MPN/Mov34 metalloenzyme (JAMM) motif.
The RadC found on SCC elements contains only this conserved domain, whereas a chromosomal RadC-containing gene in *S. aureus* has an N-terminal extension that the RaptorX [54] threading server predicts will form a helix-hairpin-helix fold (a motif that often used for nonspecific dsDNA binding). Despite the annotation, RadC does not function in DNA repair, but it may be involved in natural competence, at least in some bacteria [55]. The structure of RadC (2QLC) from *Chlorobium tepidum* has been determined, and is very closely related to those of zinc-dependent deubiquitinases [56], suggesting it may be involved in eukaryotic host -pathogen interactions [57]. Alternatively, this motif is also found in some bacteriophage tail assembly proteins, suggesting it may function as a bacterial cell wall hydrolase.

Coordinates and structure factors have been deposited in the RCSB database under number 5DGK, and raw data have been deposited with SBgrid[58], doi:10.15785/SBGRID/166.

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55. Attaiech, L., Granadel, C., Claverys, J.-P. & Martin, B. RadC, a misleading name? *J. Bacteriol.* **190,** 5729–5732 (2008).
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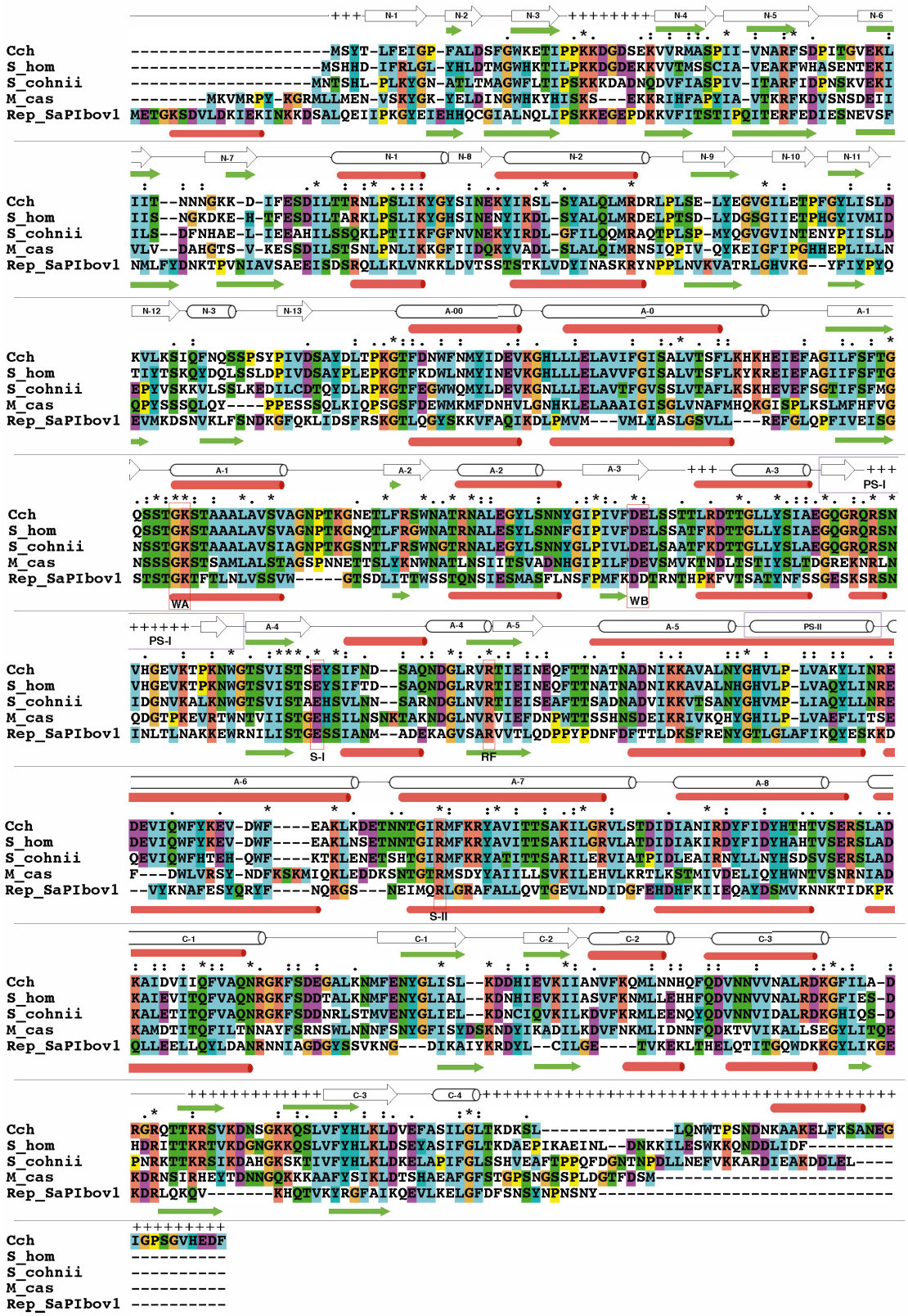

Supplementary Figure 1. Alignment of Cch and SaPIbovI Rep primary sequences and secondary structures.

Cch sequences from *S. aureus* USA300 strain (SAUSA300_0039), *S. cohnii* (ADM43452), *S. hominis* (BAB83484), *M. caseolyticus* (BAI83364) and Rep from SaPIBov1 (CAI80039) were aligned using ClustalW [59]. Asterisks indicate positions that have a fully conserved residue, colon indicates strong conservation between groups of strongly similar properties and period indicates partial conservation. Secondary structure predictions (using the jpred server [60]) of Cch_USA300 and Rep_SaPIbov1 are displayed above and below their sequences respectively. Predicted alpha helices and beta strands are

shown as orange cylinders and green arrows respectively. Real secondary structure, based on our crystal structure, is displayed above the Cch_USA300 sequence as open cylinders and open arrows. Plus signs indicate residues that couldn't be modeled due to disorder. Helices and arrows have been numbered accordingly to their domain. N-terminal domain: N1, N2, N3…ATPase domain: A1, A2, A3… C-terminal domain: C1, C2, C3…Note that secondary structure elements in the ATPase domain have been labeled according to a standard system (article reference 27). Catalytic residues are highlighted in red rectangles: WA, Walker A; WB, Walker B; S-I, sensor-I; RF, arginine finger; S-II, sensor-II. Black rectangles within the top line mark the Pre-sensor I (PS-I) and Pre-sensor II insertions. Color code of the residues is according to ClustalX default values.

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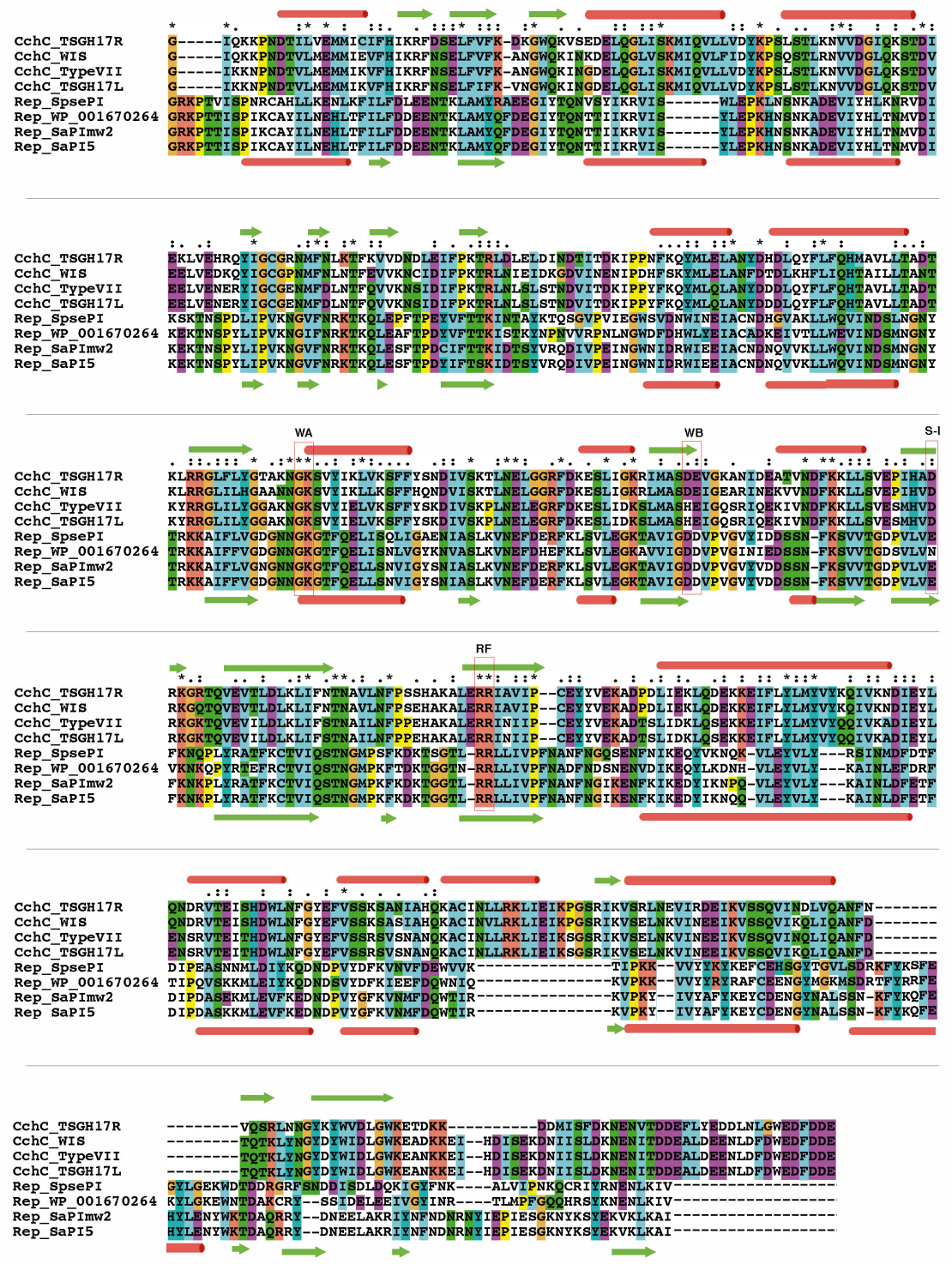
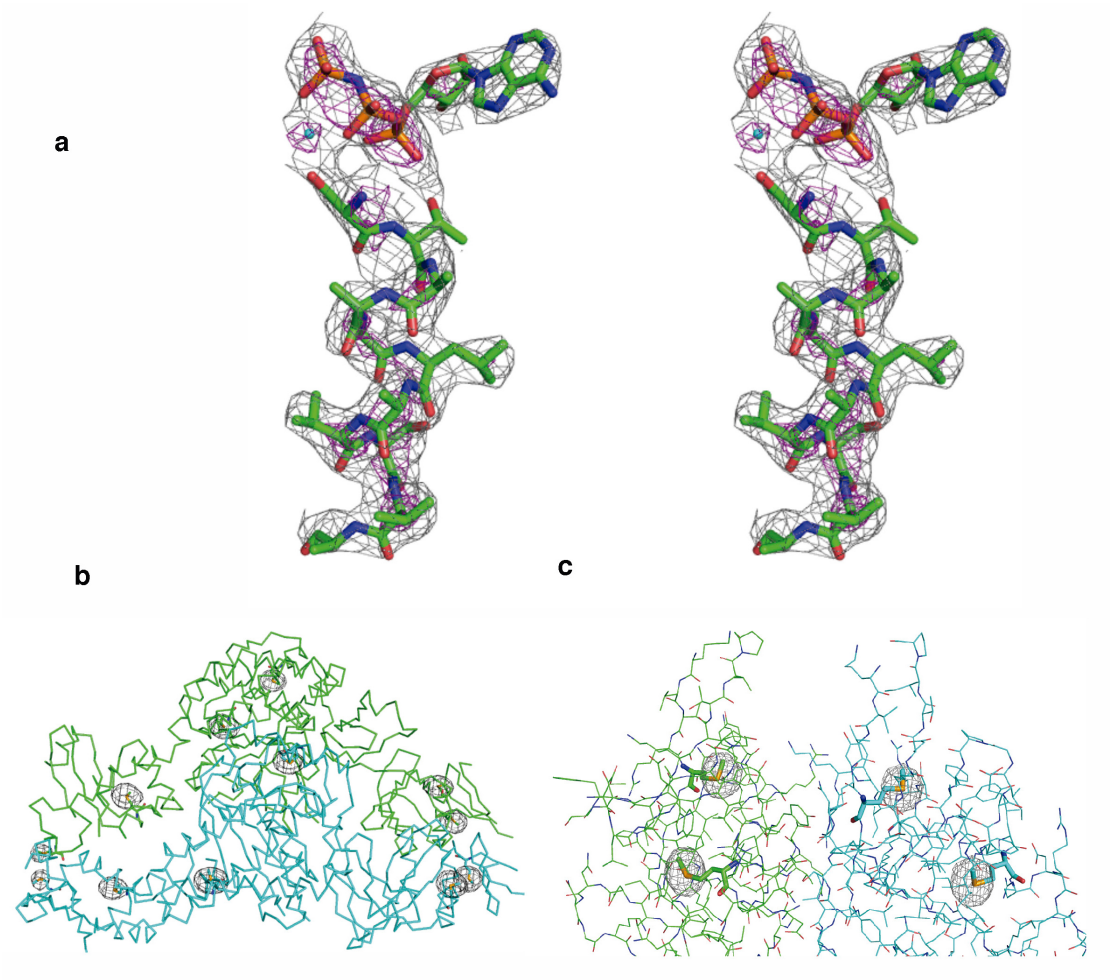


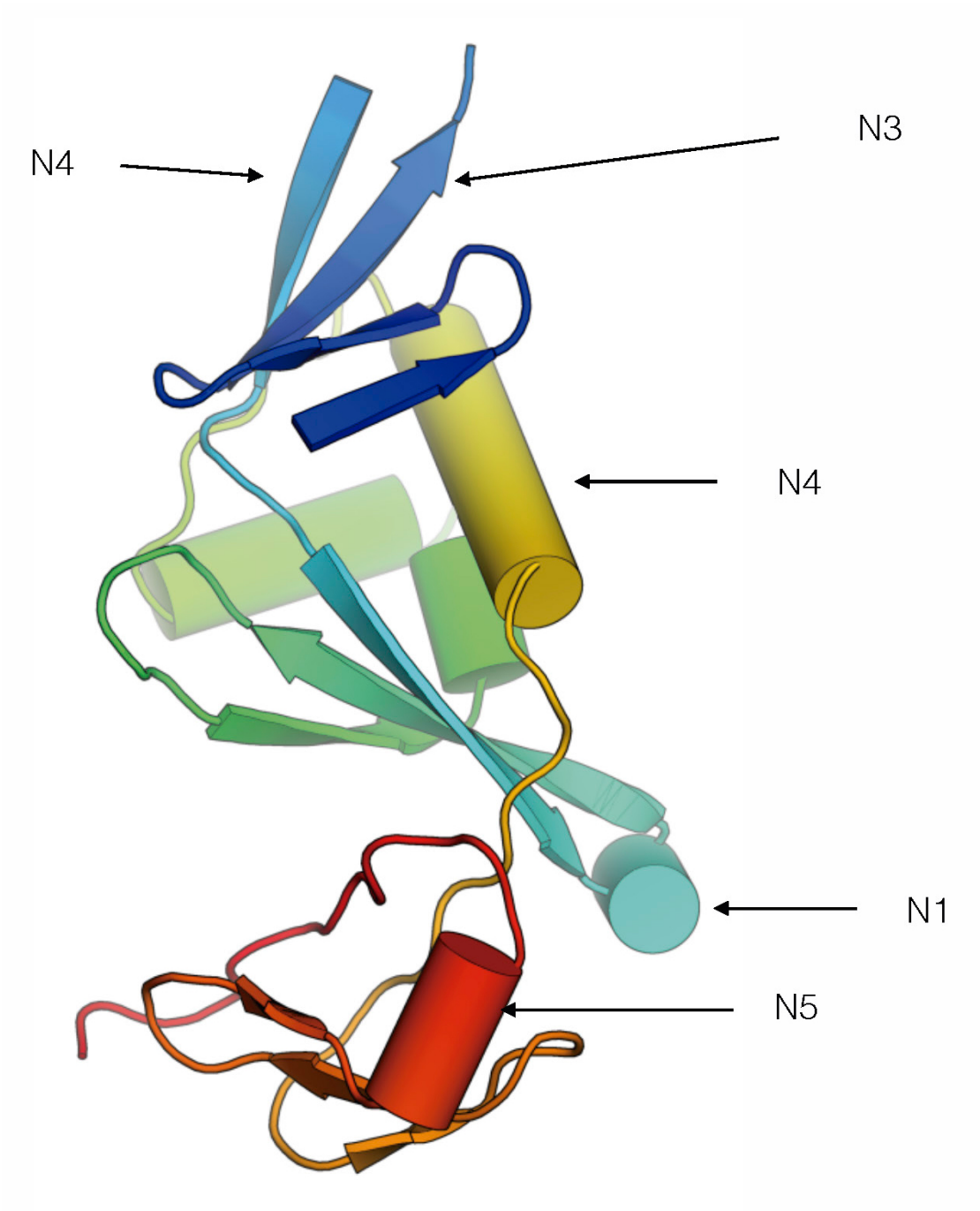
Supplementary Figure 2. Alignment of Cch2 and SaPI5-type Rep protein sequences and secondary structure

CchC sequences (named Cch2 in Figure1) from S. aureus strain WIS (AB121219), SCCmec type VII (AB373032), strain TSGH17 (AB512767) and Pri-Rep sequences from SaPImw2 (BA000033), SaPI5 (NC_007793), strain WP_001670264, SaPI from S. pseudointermedius (WP_015728773) were used for alignment comparison using ClustalW 59. Note that the primase domain of the Pri-Rep fused proteins and the extreme N-terminus of the Cch proteins were excluded for the alignment (aligned portions began with Gly73 for CchC type V and Gly342 for SaPI5 Rep). The secondary structure prediction (from the jpred server 60) of CchC type VII and SaPI5 Pri-Rep proteins is displayed above and below the sequences respectively. Alpha helices, orange cylinders; beta-sheets, green arrows. Predicted catalytic residues have been highlighted as in Supplementary Fig. 1. Color code of the residues is according to ClustalX default values. See references 59 and 60 in Supplementary legend Fig. 1.



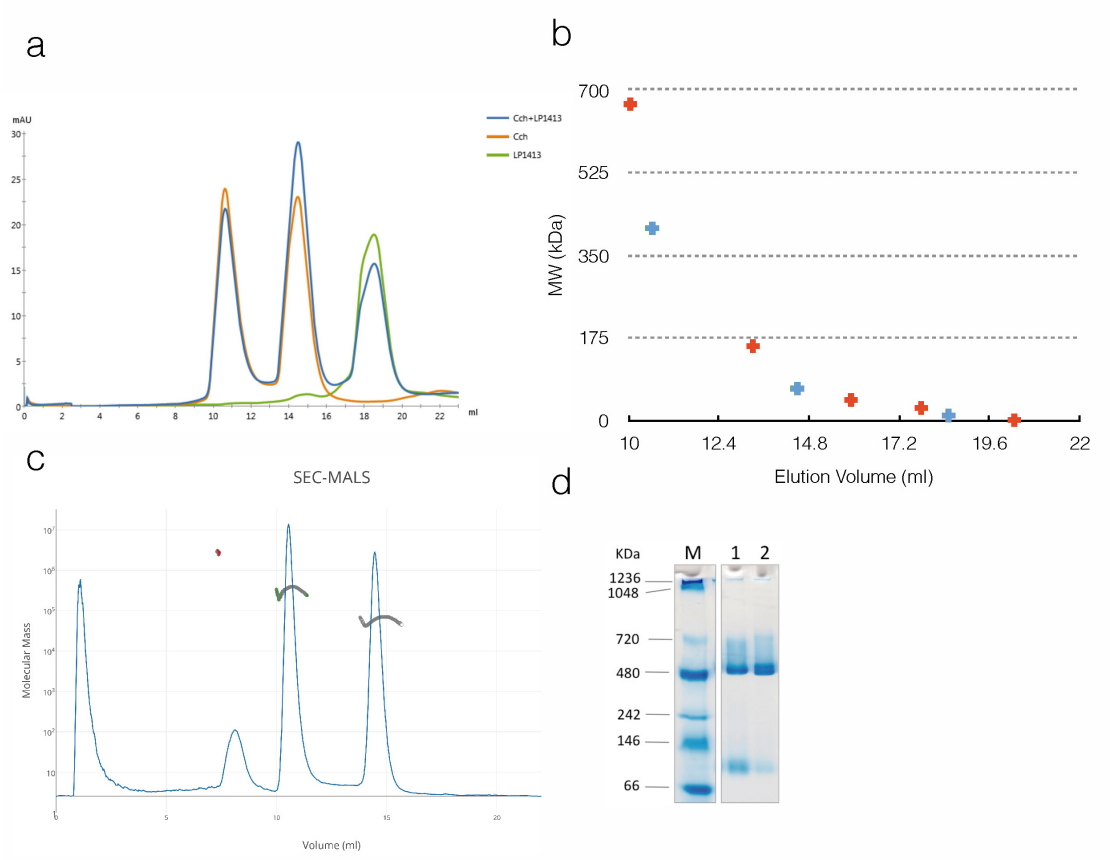
Supplementary Figure 3. Structure determination.

a. Experimental electron density map in stereo view. ATPase domain residues S217 to A227, AMP-PNP and magnesium (cyan sphere) are shown with the experimental density map at contour levels 1.5 σ (grey) and 4 σ (purple), carve=1.6 Å. The map was subjected to automated solvent modification and twofold averaging in Phenix Autosol, but contains no information from the model.
b. An anomalous difference Fourier map is shown contoured at 4 σ and carved at 4 Å. The two monomers in the asymmetric unit are displayed as green and cyan ribbons and SeMet residues are displayed as sticks.
c. Close-up of the N-terminal N-domains (displayed as lines) showing the agreement of the SeMet residues (shown as sticks) with the anomalous difference Fourier map contoured at 10 σ with carve set at 10 Å.



Supplementary Figure 4. The N-terminal DUF927-containing domain of Cch

The N-terminal DUF927-containing domain of Cch shaded from blue (L5) to red (L154). Three Helices (N1, N4 and N5) and two strands (N3 and N4) have been labeled according to Supplementary Fig. 1.



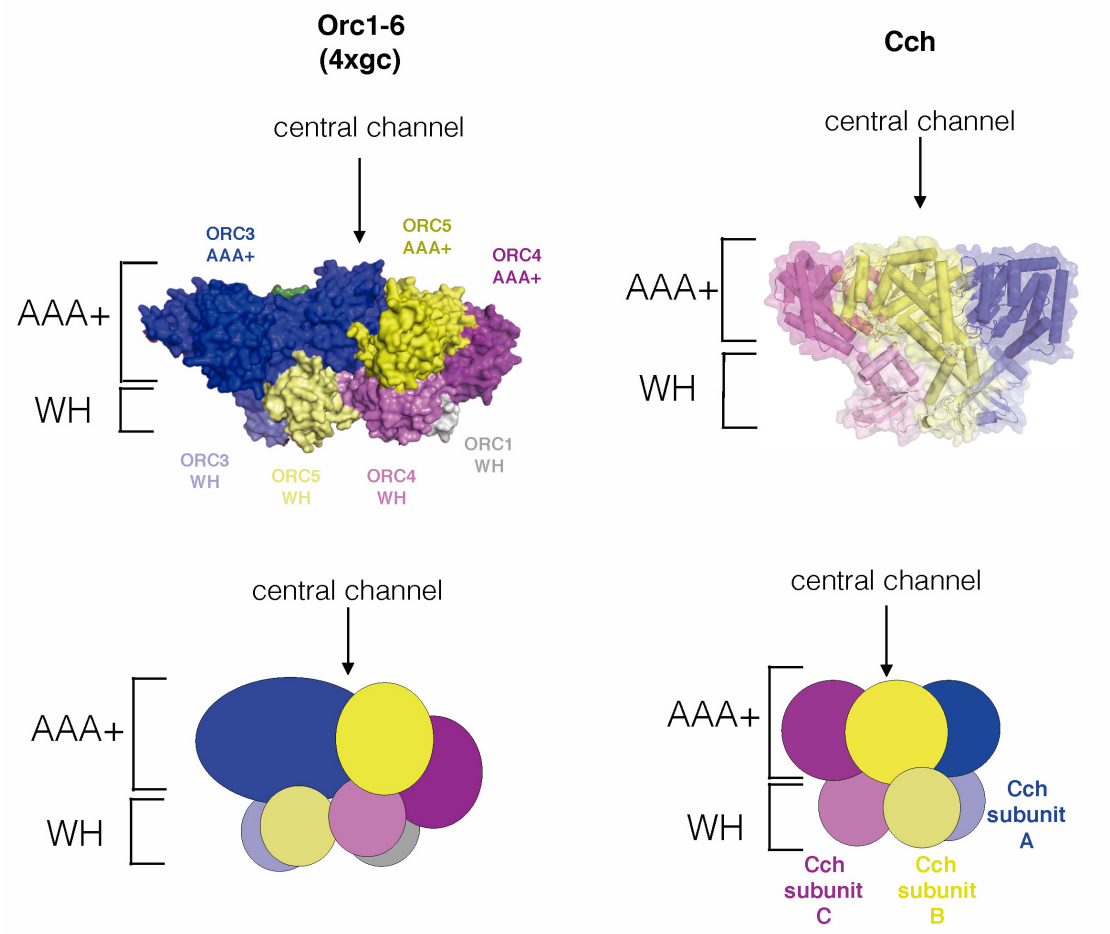
Supplementary Figure 5. Oligomerization state of Cch.

a. Size exclusion chromatography (SEC). Orange line: 400microliters of 32micromolar Cch was loaded onto a Superdex 200 increase 10/300 column that was both pre-equilibrated and eluted with buffer containing 20mM tris pH 7.8, 0.5mM EDTA, 5% glycerol and 0.5M NaCl. Blue line: Same, except that a 1.5 molar excess of LP1413 was added. Green line: LP1413 alone.

b. Elution volumes for standards and samples plotted as a function of molecular weight. Standards (red symbols) were (name, MW in Da, volume in ml): Thyroglobulin 670,000 10.043; γ-globulin 158,000 13.311; ovalbumin 44,000 15.921; Myoglobin 17,000 17.788; VitaminB12 1350 20.265. Elution volumes for the 3 Cch + LP peaks (blue symbols) were 10.64, 14.50 and 18.52 ml; for Cch alone (orange symbols, occluded by blue) were 10.62 and 14.48; for LP1413 alone the major peak eluted at 18.51 ml (green symbol, occluded by blue). The molecular weights plotted for the experimental samples were those of hexamers (407.4 kDa) and monomers (67.9 kDa) for Cch and of monomers for LP1413 (10.9 kDa). The shoulder on the LP1413 elution peak may represent a higher-order species, but under these conditions, it did not appear to interact with Cch.

c. SEC-MALS (SEC followed by multi angle light scattering) was carried out with150 microliters of 30 micromolar Cch (in the same buffer than a) on a Wyatt SEC-MALS GE AKTAPurifier UPC-10 with a DAWN HELEOS II detector. The molecular weights derived from the two peaks in this experiment were 380KDa for Cch hexamer and 64KDa for Cch monomer.

d. Blue Native Polyacrylamide Gel Electrophoresis shows that Cch (MW 67.9kDa) forms an oligomer consistent with a hexamer in solution. M, marker; 1, Cch (~3μM); 2, Cch and single stranded DNA. See methods section for details.



Supplementary Figure 6. Similarities and differences between ORC and Cch architectures.

In both cases the C-terminal WH domains make protein-protein contacts to one another and to a neighboring subunit's ATPase domain (article reference 26).

Supplementary Table 1

SCC type or microorganism	SCC Accession Number	Cch/CchC protein_id	Comments
Type I	AB033763	BAA86647.1	
Type II	D86934	BAA94656.1	
Type III	AB037671	BAB47592.1	
Type IV	FPR3757	WP_000122620	
Type V	AB512767	BAK57483.1	
Type VI	AF411935	unassigned	From nucleotide 5866 to 7648 in AF411935
Type VII	AB373032	BAG71444.1	
Type VIII	FJ670542	unassigned	C-terminal (truncated protein) From nucleotide 5709 to 6325 in FJ670542
Type IX	AB505628	BAK53060.1	
Type X	AB505630	BAK53159.1	
Type XI	FR821779	CCC86808.1	
SCC*mer*	AB037671	BAB47673.1	
S. sciuri	HG515014	CDH98047.1	
M. caseolyticus	AB498756	BAI83364.1	
S. hominis	AB063171	BAB83484.1	
Other SCC-like elements	**Accession Number**	**initiator of replication protein_id**	**Comments**
E. faecium	AAAK03000003	EAN10858.1	duf960 and duf1643 genes have been identified 4.1 kilobases away upstream the initiator gene.
B. cereus	JH792265	EJQ01250	
G. vulcani	JPOI01000001	WP_031406462.1	
P. difficile	AZSH01000015	WP_021390356	duf960 has been identified 1.6 kb upstream the initiator gene.