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Supporting information for article:

Structure and evolution of the spliceosomal peptidyl-prolyl *cis-trans* isomerase Cwc27

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Supporting Tables

Table S1. Thermostability assay with *H. sapiens* and *C. thermophilum* PPlase domains.

	<i>hsCwc27</i> PPlase	<i>ctCwc27</i> PPlase
Average T_m [°C] ^a	48.12 ± 0.52	51.32 ± 0.12
Temp. transition start [°C] ^b	35.24 ± 0.83	38.14 ± 1.56
Temp. transition end [°C] ^c	55.38 ± 1.60	55.71 ± 0.56
Slope [%/K] ^d	11.50 ± 1.01	18.23 ± 0.91

^a Average transition temperature of 21 independent measurements ± standard deviation.

^b Average temperature of transition start ± standard deviation.

^c Average temperature of transition end ± standard deviation.

^d Maximal slope of transition curve in percentage of transition per degree Kelvin ± standard deviation.

Table S2. Amino acid composition of PPlase domain parts of Cwc27 structures.

Amino acid	<i>hsCwc27</i> (6-173) aa% (#) ^a	<i>ctCwc27</i> (6-201) aa% (#) ^a
Non-polar		
Ala	5.4% (9)	5.1% (10)
Val	5.4% (9)	3.1% (6)
Leu	7.2% (12)	9.1% (18)
Ile	6.0% (10)	5.6% (11)
Pro	5.4% (9)	6.6% (13)
Met	1.2% (2)	2.0% (4)
Phe	7.2% (12)	6.6% (13)
Trp	0.6% (1)	1.0% (2)
Polar		
Gly	9.5% (16)	13.2% (26)
Ser	4.8% (8)	2.5% (5)
Thr	6.0% (10)	7.1% (14)
Cys	1.8% (3)	0.5% (1)
Tyr	2.4% (4)	2.5% (5)
Asn	6.6% (11)	6.6% (13)
Gln	2.4% (4)	4.1% (8)
Acidic		
Asp	8.3% (14)	6.1% (12)
Glu	6.0% (10)	6.1% (12)
Basic		
Lys	5.4% (9)	4.6% (9)
Arg	5.4% (9)	4.1% (8)
His	3.6% (6)	3.1% (6)
Total	100% (168)	100% (196)

^a Percentage of particular amino acid of total number of amino acids present in indicated PPlase domain parts of Cwc27 structures. In parentheses, number of particular amino acid in indicated PPlase domain parts of Cwc27 structures.

Supporting Figures

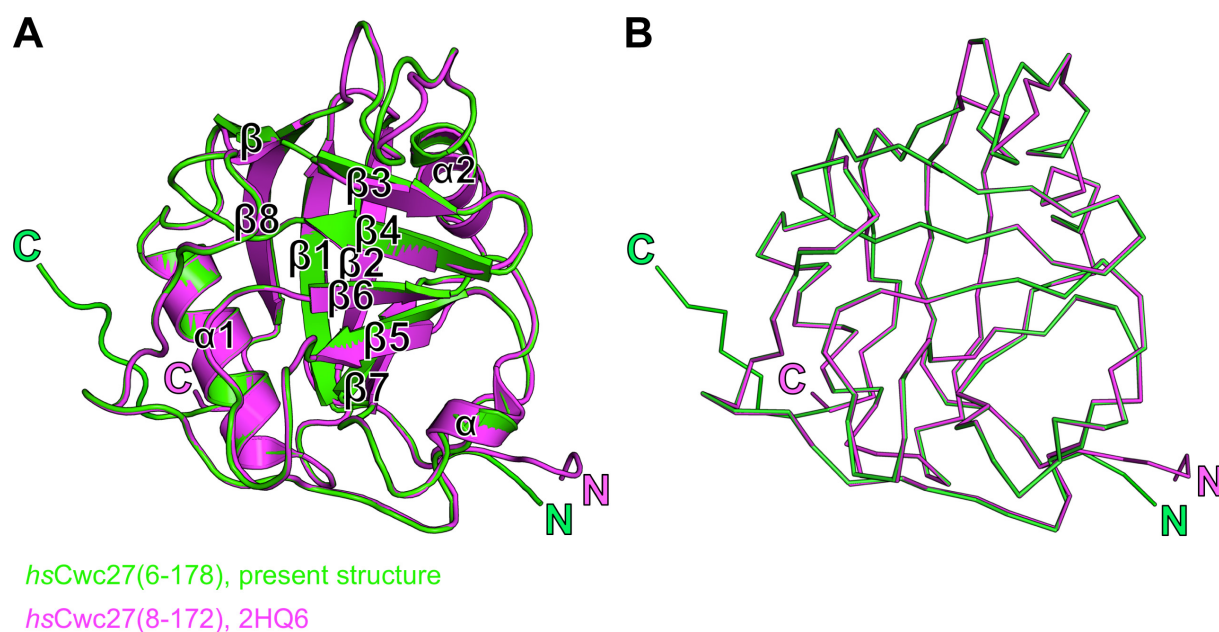


Figure S1. Superposition of *hsCwc27* structures. Superposition of *hsCwc27*⁶⁻¹⁷⁸ (green; present structure) and the previously published *hsCwc27*⁸⁻¹⁷² (pink; PDB ID 2HQ6; (Davis et al., 2010)) in cartoon representation (**A**) and as C α -traces (**B**). RMSD = 0.5 Å of 167 C α -atoms.

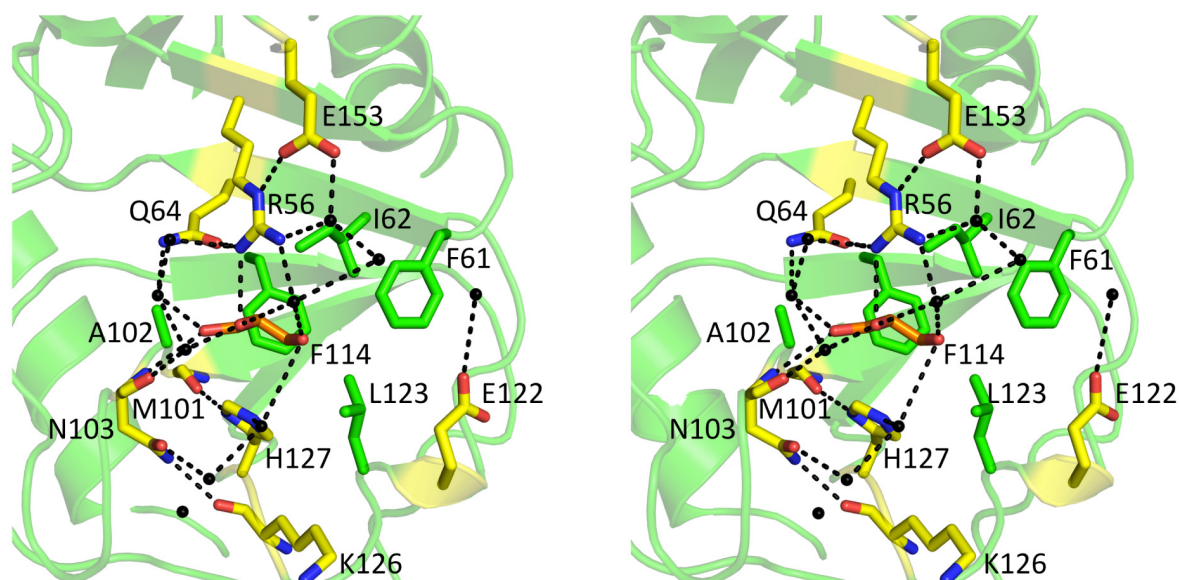


Figure S2. Stereo view of the hydrogen bond network within the proline-binding pocket of *hsCwc27*⁶⁻¹⁷⁸. Participating hydrophilic residues are presented as yellow sticks; hydrophobic residues building the hydrophobic pocket are presented as green sticks. A glycerol molecule located in the center of the pocket is depicted as orange sticks. Relevant water molecules are shown in black, hydrogen bonds as dashed black lines.



Figure S3. Multiple sequence alignment of Cwc27 proteins. Multiple sequence alignment of 22 Cwc27 proteins identified in our tree of life analysis. The alignment was built with Jalview (Waterhouse et al., 2009) and calculated with the Muscle algorithm (Edgar, 2004). Secondary structure annotations of β -strands 1-8 and of helices 1-2 are derived from our structures; annotations C-terminal of β 8 are derived from secondary structure predictions with PROFsec (PredictProtein, (Rost et al., 2004)) and are marked with an asterisk.

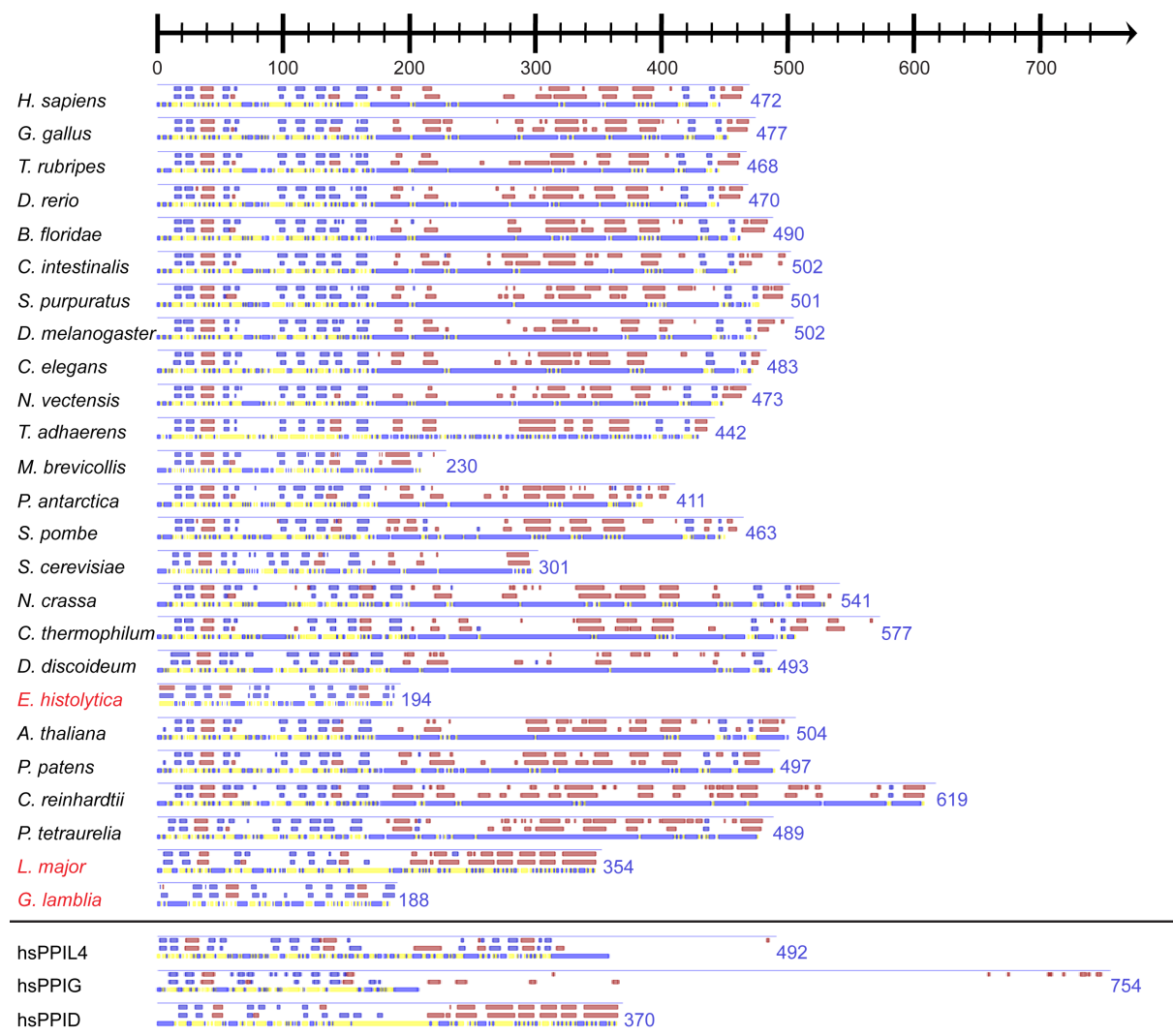


Figure S4. Secondary structure prediction of Cwc27 proteins. Proteins from species in black contain a Cwc27-characteristic secondary structure signature; proteins from species in red lack a Cwc27-characteristic secondary structure signature. Depicted are secondary structure predictions with REPROFSec (blue = β -strand, red = helix; top row) or PROFsec (blue = β -strand, red = helix; middle row) and prediction of solvent accessibility with PROFAcc (blue = solvent exposed, yellow = buried; bottom row (PredictProtein; (Rost et al., 2004))). For comparison, secondary structure predictions for *hsPPIL4*, *hsPPIG* and *hsPPID* are displayed below the black line.

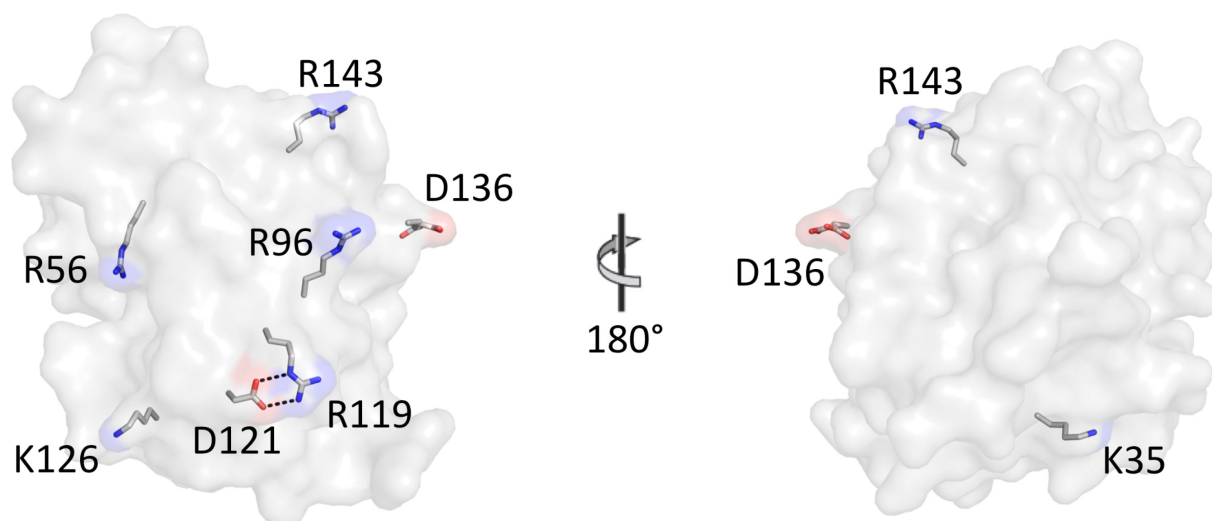


Figure S5. Charge-flipping between *H. sapiens* and *S. cerevisiae* Cwc27 PPlase domains. Surface representation of *hsCwc27*⁶⁻¹⁷⁸. The left image is rotated by -60 °, the right image is rotated 120 ° along the vertical axis compared to compared to the view in Figure 3A. *HsCwc27* residues with a different charge compared to corresponding residues in *scCwc27* are depicted as grey sticks. Nitrogen atoms - blue, oxygen atoms - red.

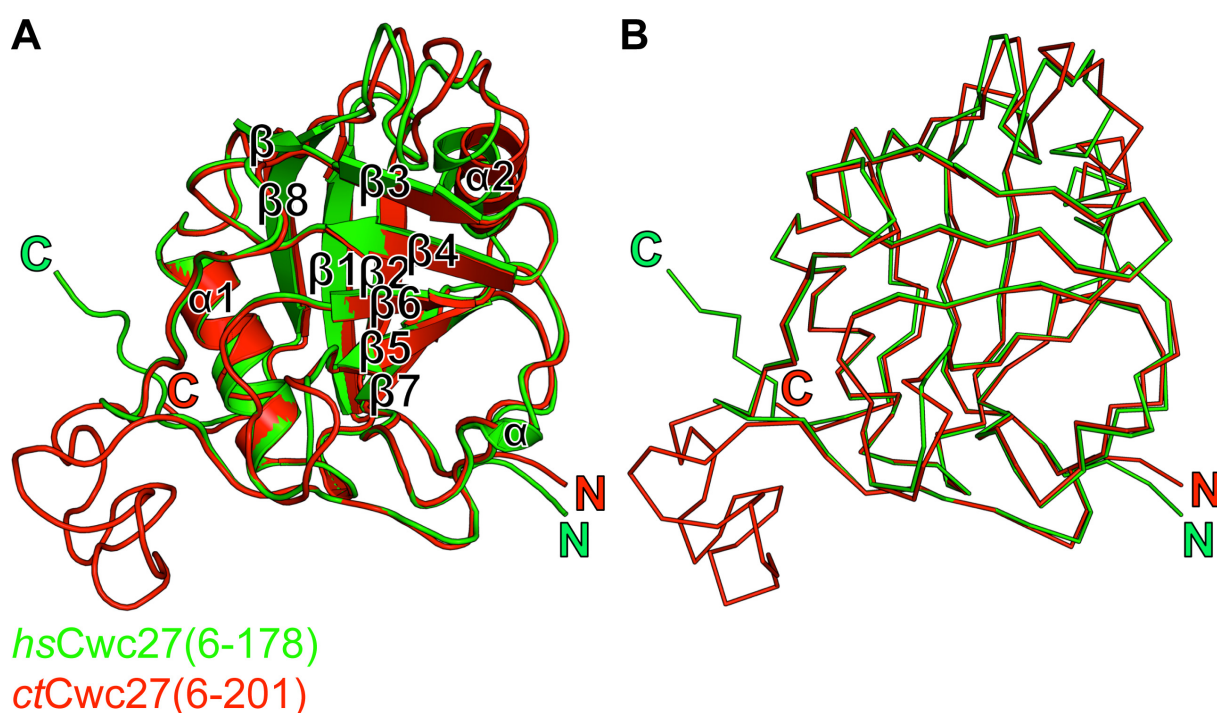


Figure S6. Superposition of *hsCwc27* and *ctCwc27* PPlase domains. Superposition of *hsCwc27*⁶⁻¹⁷⁸ (green) and *ctCwc27*⁶⁻²⁰¹ (red) in cartoon representation (A) and as Ca-traces (B). RMSD = 0.9 Å for 168 Ca-atoms.

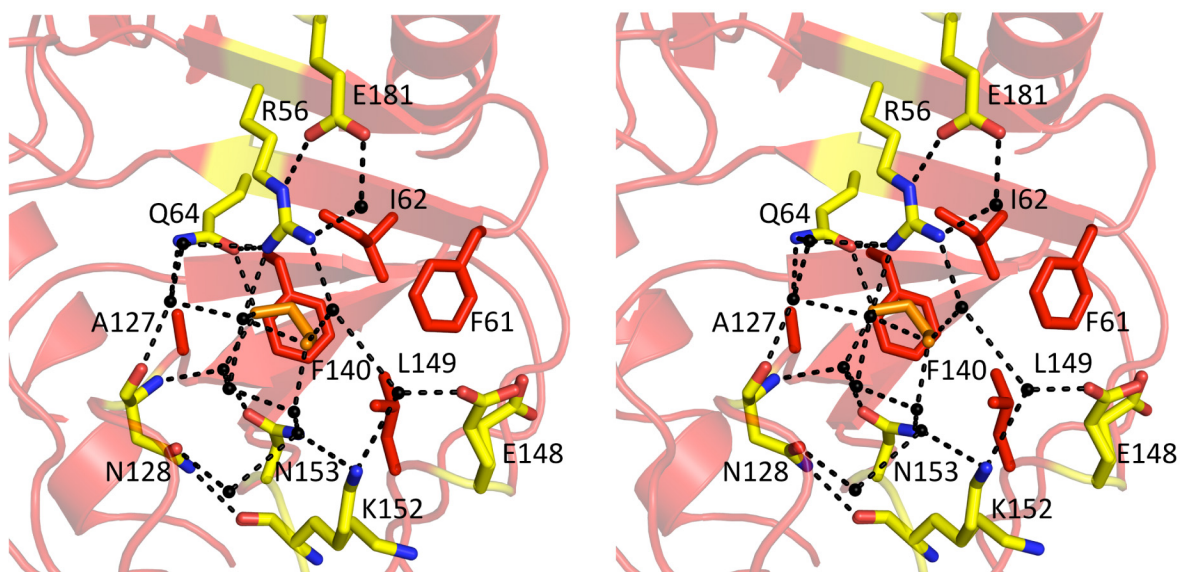


Figure S7. Stereo view of the hydrogen bond network within the proline-binding pocket of *ctCwc27*. Participating hydrophilic residues are presented as yellow sticks; hydrophobic residues building the hydrophobic pocket are presented as red sticks. An ethylene glycol molecule located in the pocket is depicted as orange sticks. Relevant water molecules are shown in black, hydrogen bonds as dashed black lines.