Supplementary Materials

N-terminal segment of *Tv*CyP2 cyclophilin is involved in self-association, membrane interaction and subcellular localization

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Table S1. Comparison of interactions among N-terminal segment with *Tv*CyP2, Myb1$_{104-111}$ peptide with *Tv*CyP1 (PDB ID 5YBA) and CsA with *h*CyPA (PDB ID 1CWL) according to the X-ray structural data. Data were analyzed and interpreted using software tools *LigPlot, Discoveries studio* and *PyMOL*.

Types of Interaction	***Tv*CyP2_N-terminal segment**		***Tv*CyP1_Myb1 peptide**		***h*CyPA_Cyclosporine A**	
H-bonds	N-terminal segment	*Tv*CyP2 active site	Myb1 peptide	*Tv*CyP1 active site	Cyclosporine A	*h*CyPA active site
	R8 :O	R75:NH2	P107:O	R63:NH2	MeLeu3:O	R55:NH2
	I10:O	Q83:NH2	Y105:O	Q71:NH2	MeLeu3:O	R55:NH2
	S11:NH	N122:O	G106:NH	N110:O	MeBmt5:O	Q63:NH2
	S11:O	Q131:NH2	Y105:OH	T115:O	ABU6:NH	N102:O
	T7:O	W141:NH	K108:O	W129:NH	MeLeu2:O	W121:NH
Hydrophobic	A6	F80	W109	Q67	MeLeu2	F60
	T7	L142	K108	H133	MeVAL4	M61
	R8	H146	P107	F68	MeVAL4	F113
	V9	M81	P107	M69	MeVAL4	L122
	V9	A121	P107	A109	MeVAL4	H126
	V9	F133	P107	F121	MeBmt5	A103
	V9	H146	P107	L130	ABU6	G72
	V9	L142	P107	H134	ABU6	A101
	S11	G92	Y105	G80	ABU6	Q111
	S11	Q131	Y105	A111		
	P13	Y93	Y105	Q119		

Figure S1

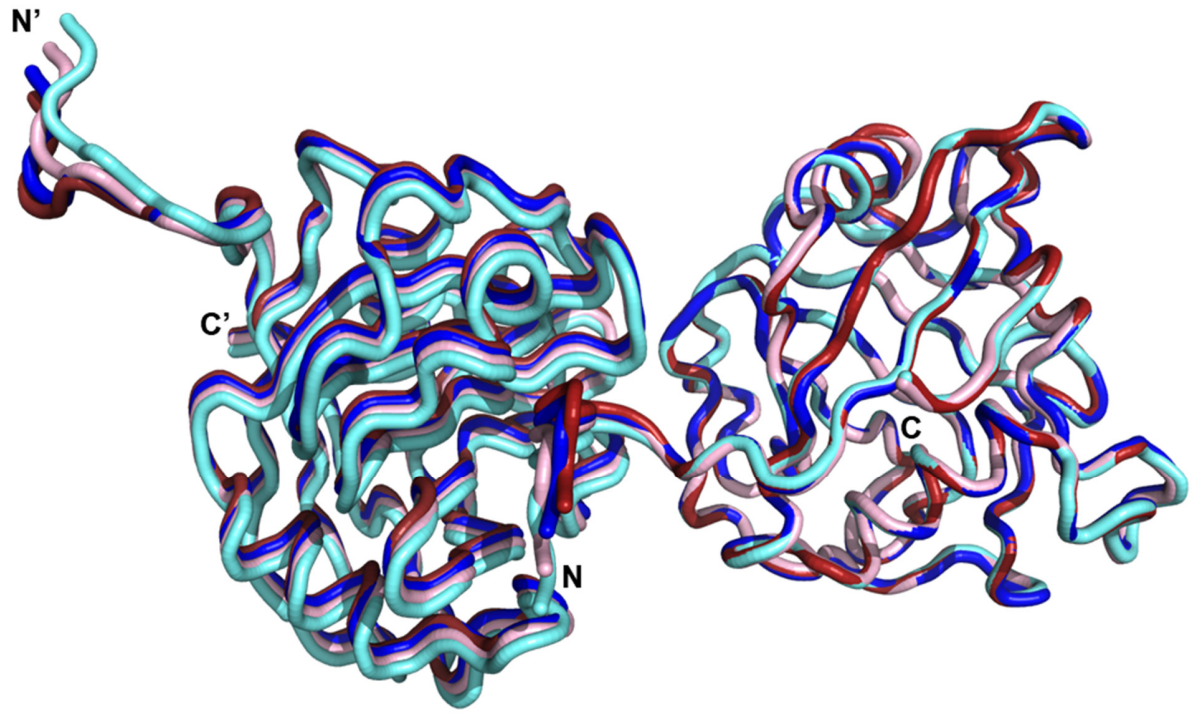

Figure S1. Structural superimposition of four X-ray structures, *Tv*CyP2_apo1 (cyan), *Tv*CyP2_apo2 (pink) *Tv*CyP2_apo3 (red) and *Tv*CyP2_apo4 (blue), determined under different conditions.

Figure S2

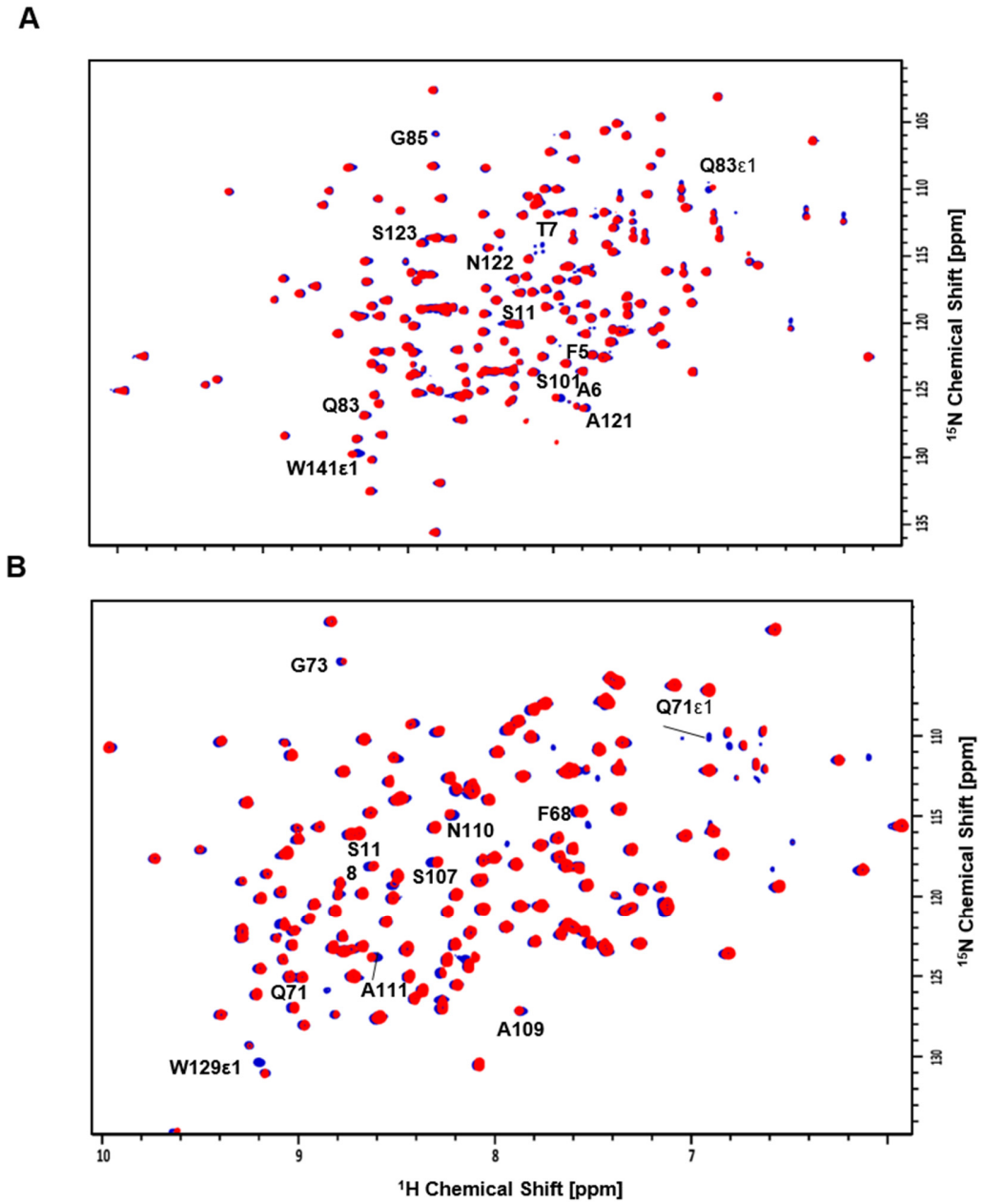

Figure S2. (A) 2D ^{1}H-^{15}N HSQC spectra for *Tv*Cyp2 in blue overlapped with that for *Tv*Cyp2 titrated with unlabeled *Tv*CyP2$_{3-18}$ (1:5 molar ratio) shown in red. (B) 2D ^{1}H-^{15}N HSQC spectra for *Tv*Cyp1 in blue overlapped with that for *Tv*Cyp1 titrated with unlabeled *Tv*CyP2$_{3-18}$ (1:5 molar ratio) shown in red. Cross peaks that showed chemical

shift perturbation or line width broadening upon adding the *Tv*CyP2$_{3-18}$ peptide are labelled.

Figure S3

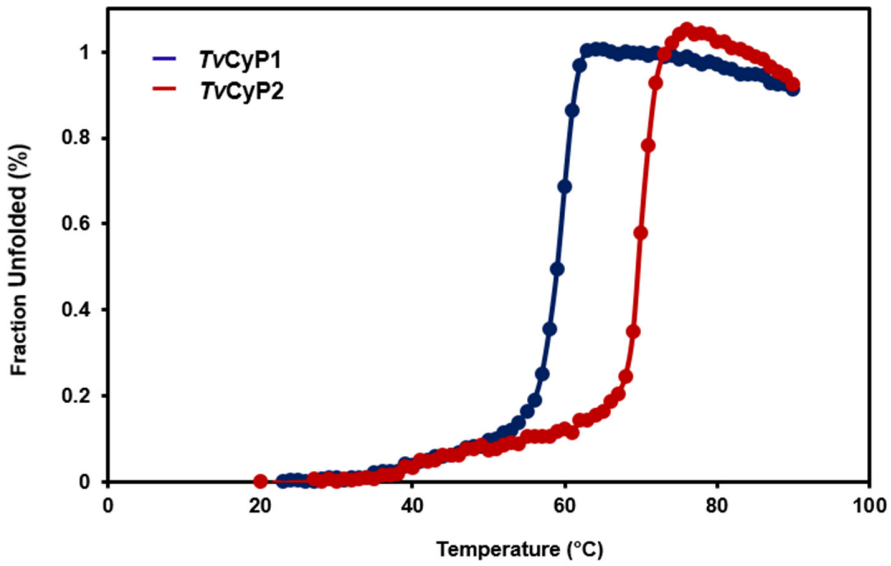

Figure S3. Thermal unfolding CD spectra of *Tv*CyP2 and *Tv*CyP1 shown in red and blue, respectively, from 20 to 90 °C at wavelength 222 nm. *Tv*CyP2 with T_m 70°C was more stable than *Tv*CyP1 with T_m 60°C.

Figure S4

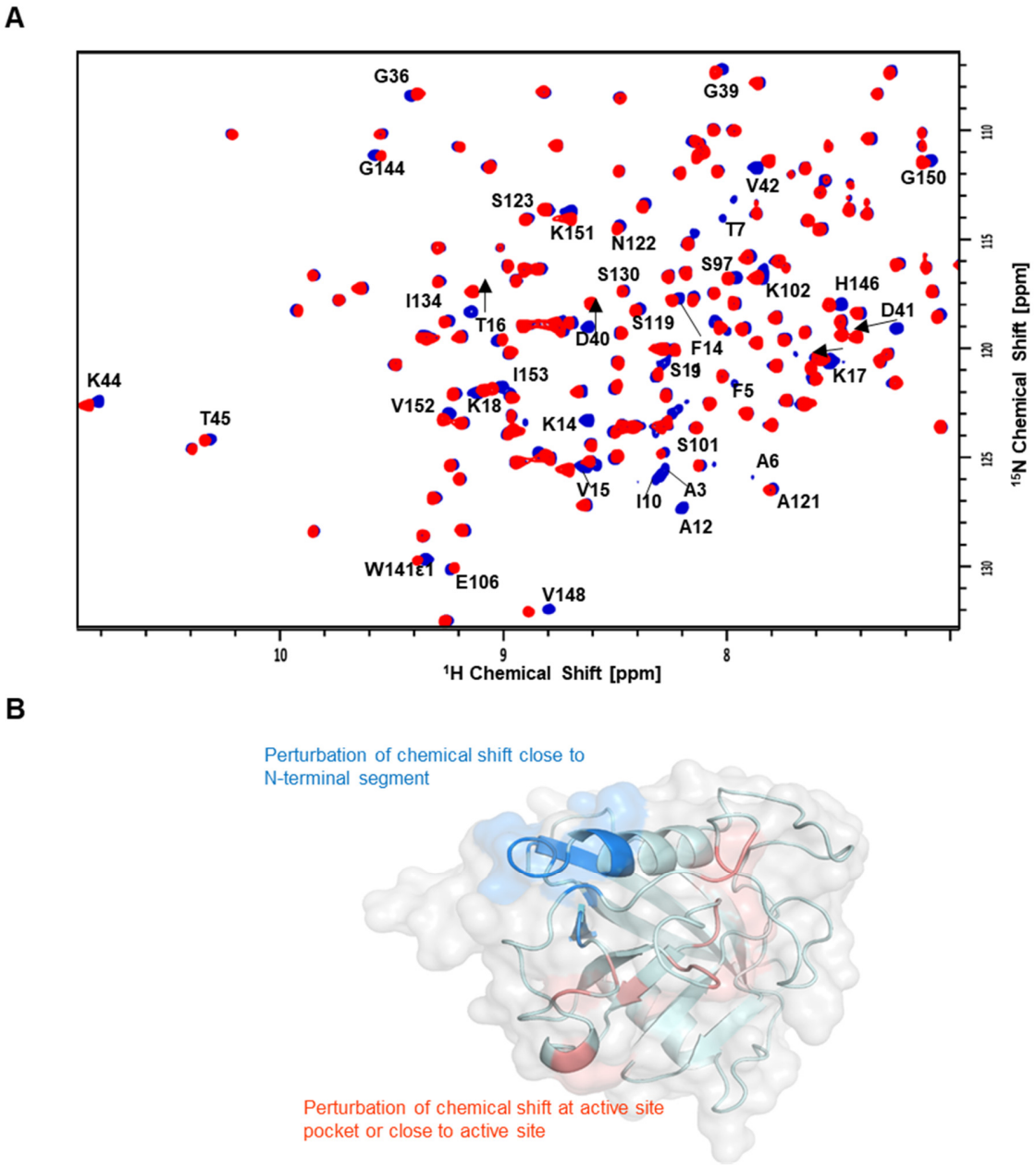

Figure S4. (A) The superimposed 2D ^{1}H-^{15}N HSQC spectra between *Tv*Cyp2 in blue and *Tv*Cyp2-ΔN in red. Residues showing chemical shift perturbation between the two spectra and disappeared in *Tv*Cyp2-ΔN due to the lack of N-terminal segment are annotated. (B) Residues with shift perturbations are mapped onto the structure,

showing they are close to the N-terminal segment (blue) or in the active-site pocket and its nearby region (red).

Figure S5

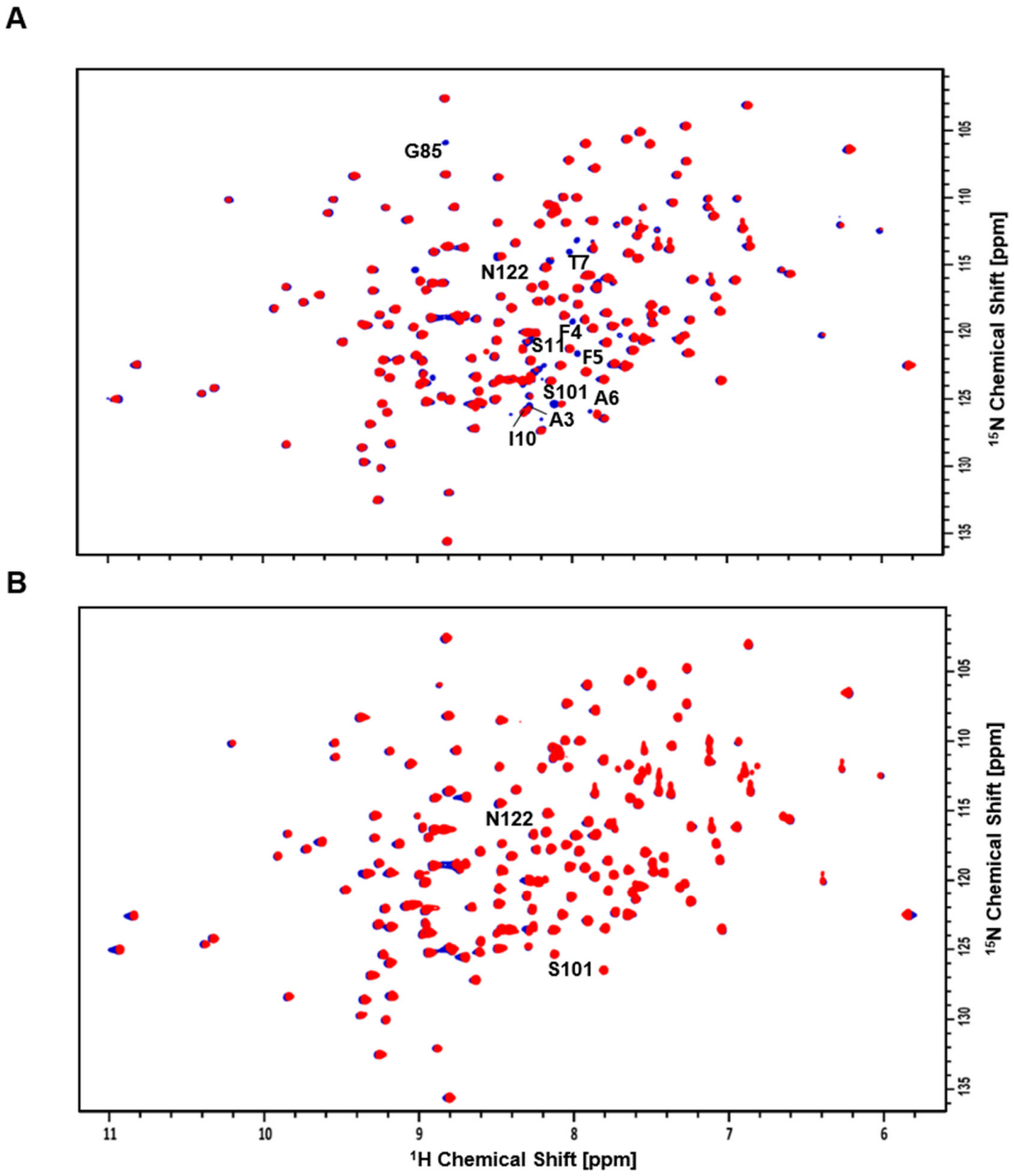

Figure S5. (A) The overlapped 2D ^{1}H-^{15}N HSQC spectra of *Tv*CyP2 in aqueous solution (blue) and in the presence of DPC micelles (red). Cross peaks with shift perturbation, line width changing, and disappeared are labelled. (B) The overlapped

2D ^{1}H-^{15}N HSQC spectra of *Tv*CyP2-◎N in aqueous solution (blue) and in the presence of DPC micelles (red). Nearly all cross peaks are identical, which indicates no interaction between *Tv*CyP2-◎N and DPC micelles.

Figure S6

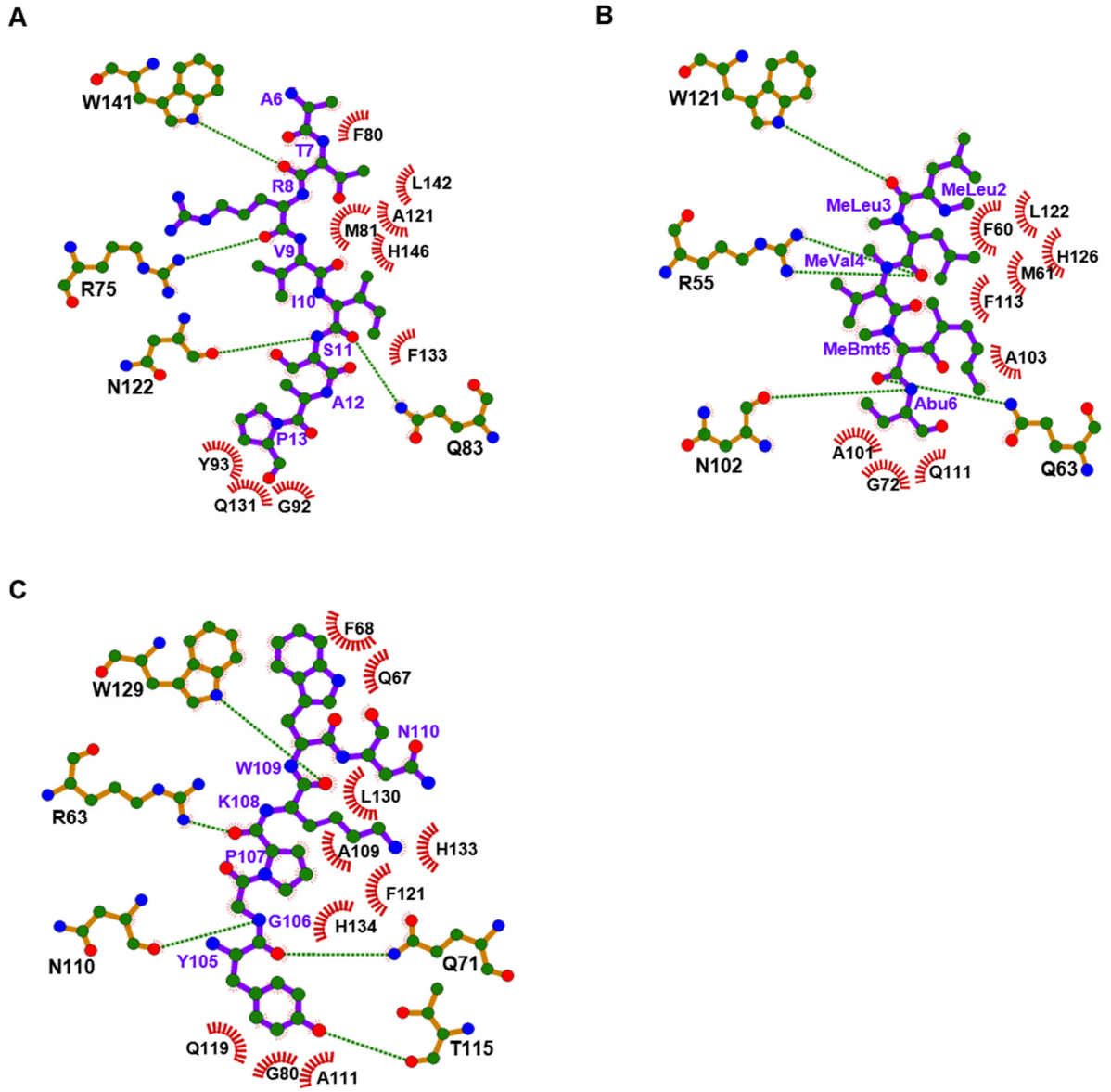

Figure S6. (A) H-bond and hydrophobic interactions observed between N-terminal segment and a neighboring *Tv*CyP2. (B) H-bond and hydrophobic interactions observed in *h*CyPA–CsA complex. (C) H-bond and hydrophobic interactions

observed in *Tv*CyP1–Myb1$_{105\text{-}110}$ complex. All figures were generated by LigPlot+, with H-bonds indicated by green dashed lines and hydrophobic interactions by red spokes. Carbon atom is shown in green, oxygen in red and nitrogen in blue.