

## Legends for supplemental figures

**Figure S1. Structural analysis of the interaction between the extra loop and the LRR motif.** (A) 2D plot of the interaction between the C-terminal loop (aa139-160) and the LRR motif of TbCFAP410-NTD. The plot was generated using DIMPLOT in the LigPlot plus suite. Residues involved in hydrogen bond formation are shown as ball-and-sticks, with oxygen, nitrogen and carbon atoms colored in red, blue, and gray, respectively. Dotted lines in cyan indicate hydrogen bonds. Non-bonded residues involved in hydrophobic interactions are shown as spoked arcs. (B) Sequence alignment of TbCFAP410 and orthologs in their NTDs. Residues involved in hydrogen bond hydrophobic interactions as shown in (A) are indicated by arrows and dots, respectively. (C) Two orthogonal views of the crystal structure of TbCFAP410-NTD, with the LRR motif (aa1-138) shown as semitransparent surface and the loop as green cartoon and sticks. Highly and relatively conserved residues on the LRR motif are shaded in blue and light cyan, respectively. (D) Open-book views of the interfaces on the LRR and the loop.  $2F_o - F_c$  electron density maps are shown (purple) for the entire loop at  $2\sigma$  level.

**Figure S2. Conservation analysis of the NTDs of various CFAP410 orthologs.** (A) Sequence alignment of orthologs of *T. brucei*, *H. sapiens*, and *C. reinhardtii* CFAP410 proteins. Trypanosomes, animals and algae/ciliates are colored in blue, orange and green, respectively. Conserved surface residues are marked with arrows, where shades of blue represent varying degrees of conservation: dark blue (>90%), ocean blue (80-90%), and light blue (70-80%). Diamonds denote residues linked to disease-causing mutations, numbered according to the *T. brucei* CFAP410 sequence. (B & C) Mapping the conserved

residues onto the crystal structure of TbCFAP410-NTD. The solvent-exposed flat surface on the  $\beta$  sheet contains many conserved residues (B), whereas the opposite side of the LRR motif with the extra loop is devoid of conserved residues (C).

**Figure S3. Homology modeling of HsCFAP410-NTD and comparison with TbCFAP410-NTD.** (A) NCBI-BLAST report for the NTDs of *H. sapiens* and *T. brucei* CFAP410. Residues associated with disease-causing mutations in humans are shaded in cyan. Accession codes: HsCFAP410: NP\_004919, TbCFAP410: XP\_011776929/Tb927.9.12170. (B) Ribbon diagram showing the crystal structure of *TbCFAP410*-NTD (blue), superimposed with homology models of *HsCFAP410*-NTD generated using SWISS-MODEL (green) and AlphaFold2 (pink). Five disease-related residues highlighted in (A) are displayed as sticks and labeled. (C) SWISS-MODEL report for the homology modeling of HsCFAP410-NTD. (D) Predicted alignment error (PAE) plots of AlphaFold2 homology models of HsCFAP410-NTD. (E) Predicted local distance difference test (pLDDT) plots of the AlphaFold2 homology models of HsCFAP410-NTD, showing per-residue confidence scores on a scale of 0–100 (higher values indicate higher confidence).

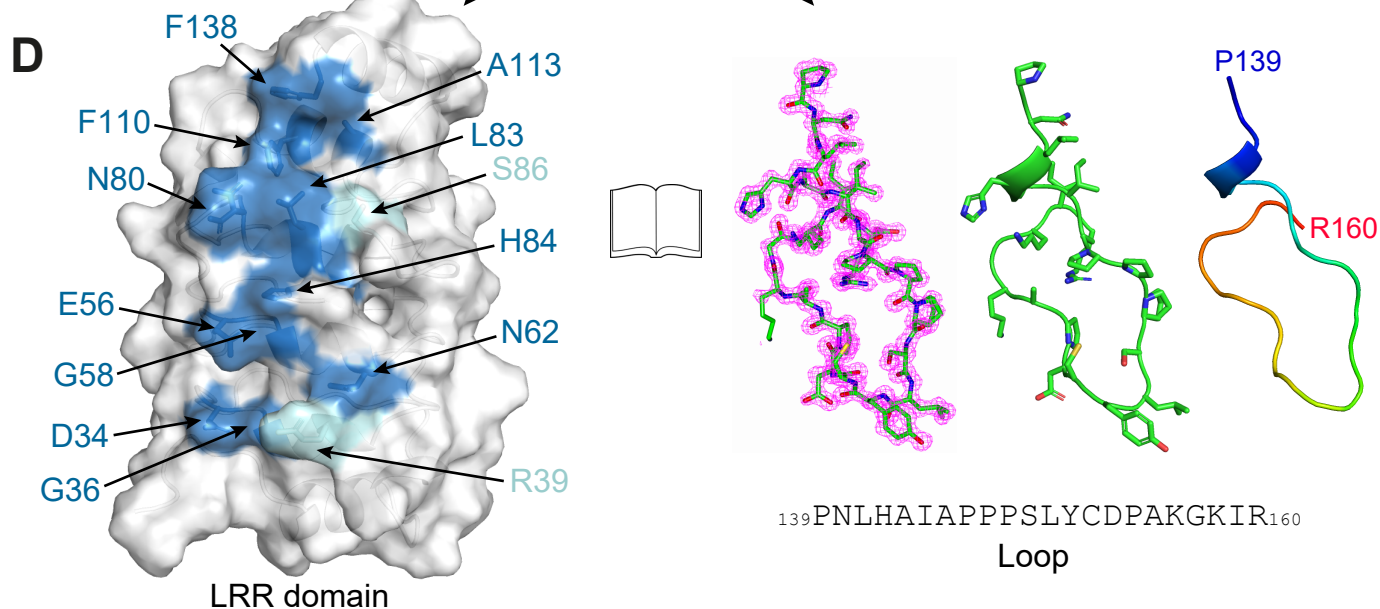
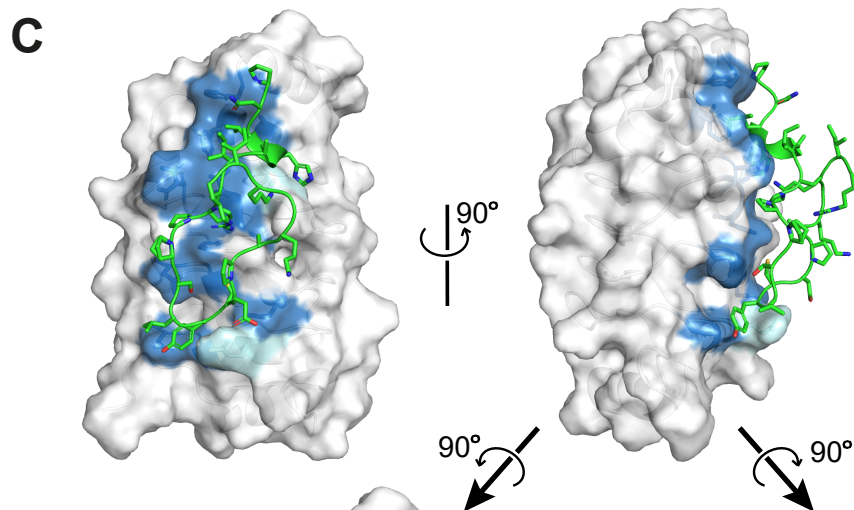
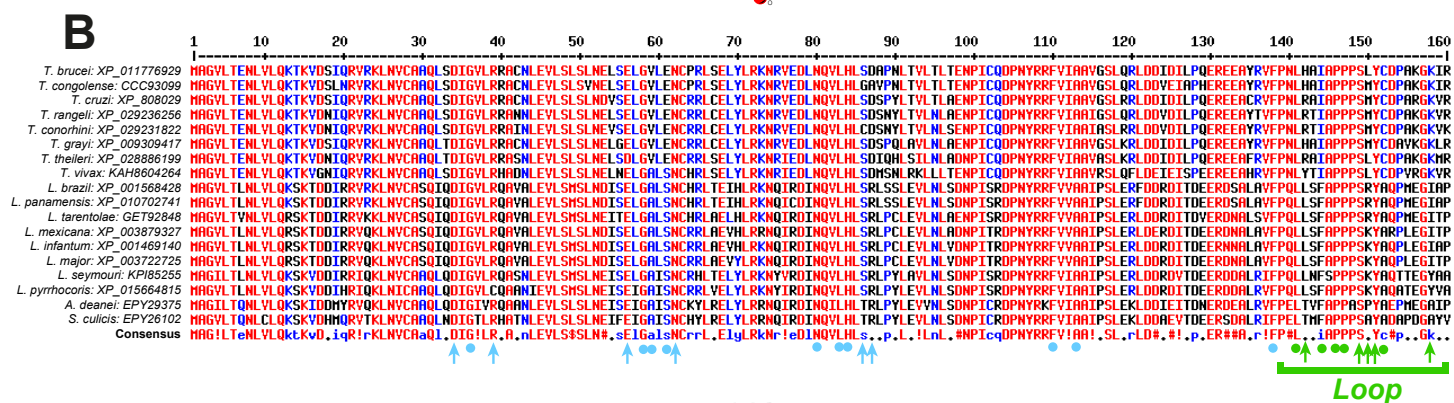
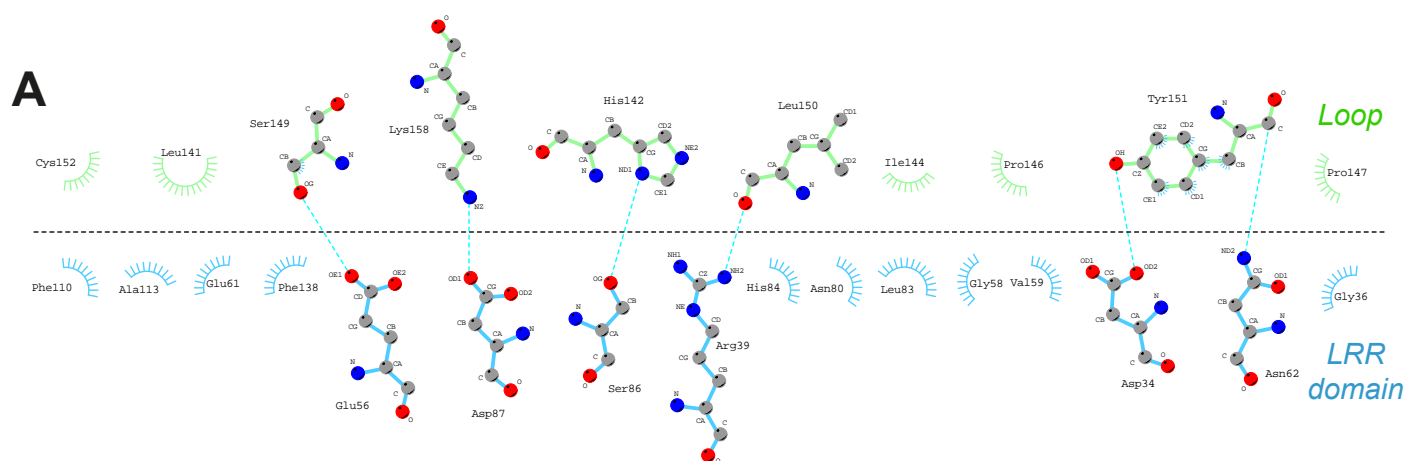
**Figure S4. Simulation and prediction analyses of wild-type and mutant TbCFAP410-NTD.** (A) Conformational analysis of the C63Y mutant simulations. *Left*: central configuration of the most populated cluster in the hNTD C63Y simulations (cluster occupancy 21%); *Right*: central configuration of the second most populated cluster in the C63Y simulations showing the large opening of the C-terminal cleft, which coincides with

the formation of the E61-K156 salt bridge. **(B)** Analysis of hNTD wild-type (WT) and mutant simulations with the number of conformational clusters with cluster occupancy  $P > 1\%$ ; cluster occupancy of the most populated cluster ( $P_{MPC}$ ); all-atom RMSD between the central structures of the most populated clusters in mutant and wildtype simulations; change in conformational entropy upon mutation ( $DS_{conf} = S_{mutant} - S_{wt}$ ) whereby  $S_{mutant}$  was rescaled by the relative number of degrees of freedom; change in the total solvent-accessible surface area ( $DSASA_T$ ) and its hydrophilic ( $DSASA_{Hphi}$ ) and hydrophobic components ( $DSASA_{Hpho}$ ) upon mutation ( $DSASA = SASA_{mutant} - SASA_{wt}$ ). **(C)** Superposition of TbCFAP410-NTD crystal structure with five models of the mutant C63Y generated by AlphaFold. The bulky aromatic sidechain of tyrosine in the mutant causes the flip of the helix outwards (insert) that subsequently destabilizes the interaction with the loop.

**Figure S5. Comparison of TbCFAP410 with its paralog.** **(A)** Sequence alignment of TbCFAP410 and its paralog from *T. brucei*. Accession codes: TbCFAP410: XP\_011776929/Tb927.9.12170; TbCFAP410-paralog: XP\_011777799/Tb927.10.5130. Residues in the conserved surface patch on TbCFAP410-NTD are indicated by arrows (green, conserved in paralog; orange, different in paralog). **(B)** Superposition of the AlphaFold model of the paralog on the crystal structure of TbCFAP410-NTD. **(C)** The conserved surface patch on TbCFAP410-NTD. **(D)** Conservation analysis of the surface patch on TbCFAP410-NTD versus its paralog.

**Figure S6. Sequence comparison of all HsCFAP410 isoforms.** (A) Sequence alignment of all 11 isoforms (i.e. 1-4 and X1-X7) of HsCFAP410. The ranges of both NTD and CTD are indicated. The alignment was carried out using the option of “Muscle with defaults” in Jalview. Residues are shaded by the degree of conservation. Accession codes: HsCFAP410\_isoform-1: NP\_004919; isoform-2: NP\_001258369; isoform-3: NP\_001258370; isoform-4: NP\_001258371; isoform-X1: XP\_047296936; isoform-X2: XP\_047296937; isoform-X3: XP\_047296938; isoform-X4: XP\_006724114; isoform-X5: XP\_047296939; isoform-X6: XP\_047296940; isoform-X7: XP\_047296943. (B) Two orthogonal views of the three-dimensional structure of a tetrameric model of HsCFAP410 generated by AlphaFold2. The N- and C-terminal regions are colored orange and teal, respectively.

## Figure S1



## Figure S2

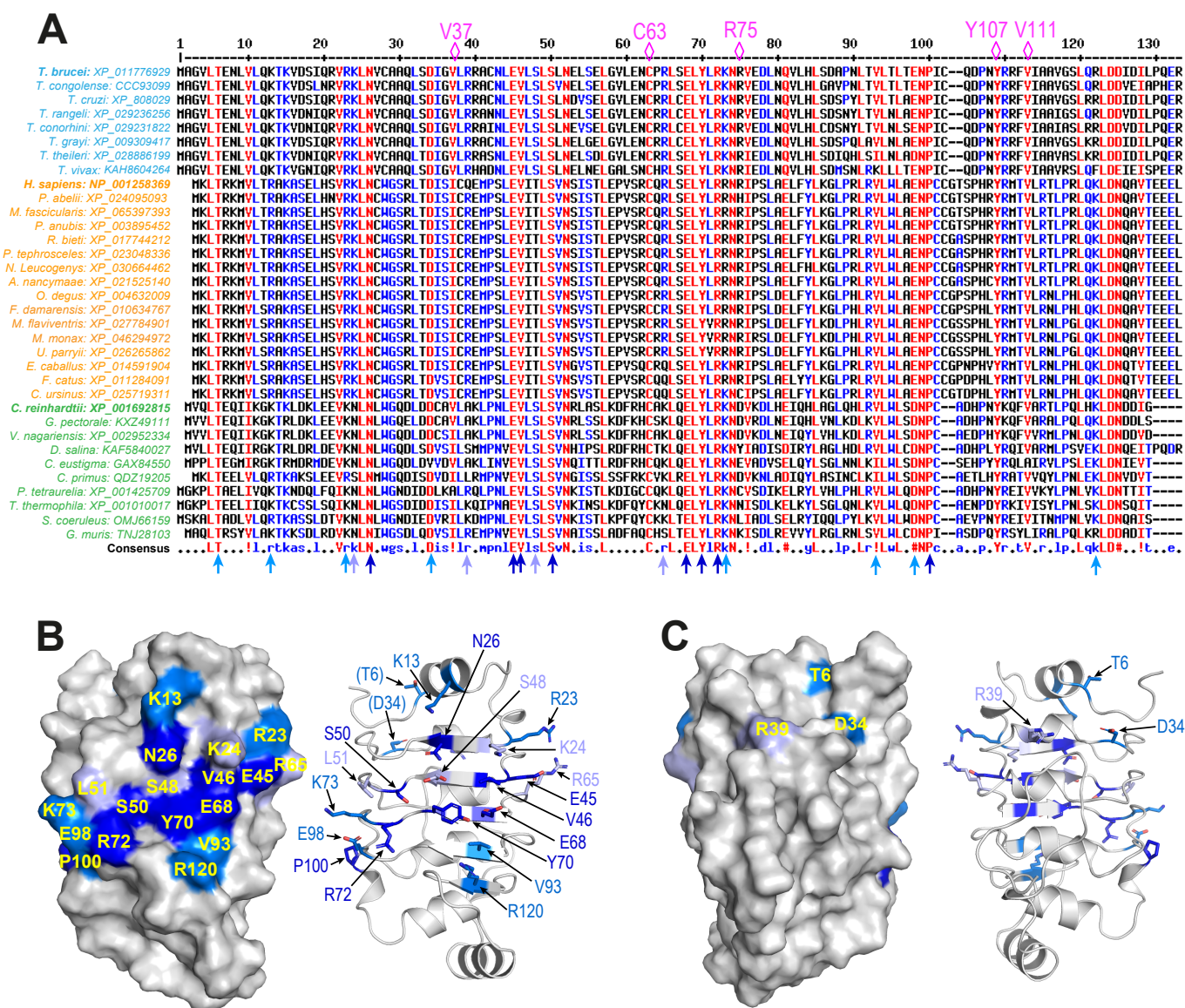




Figure S3

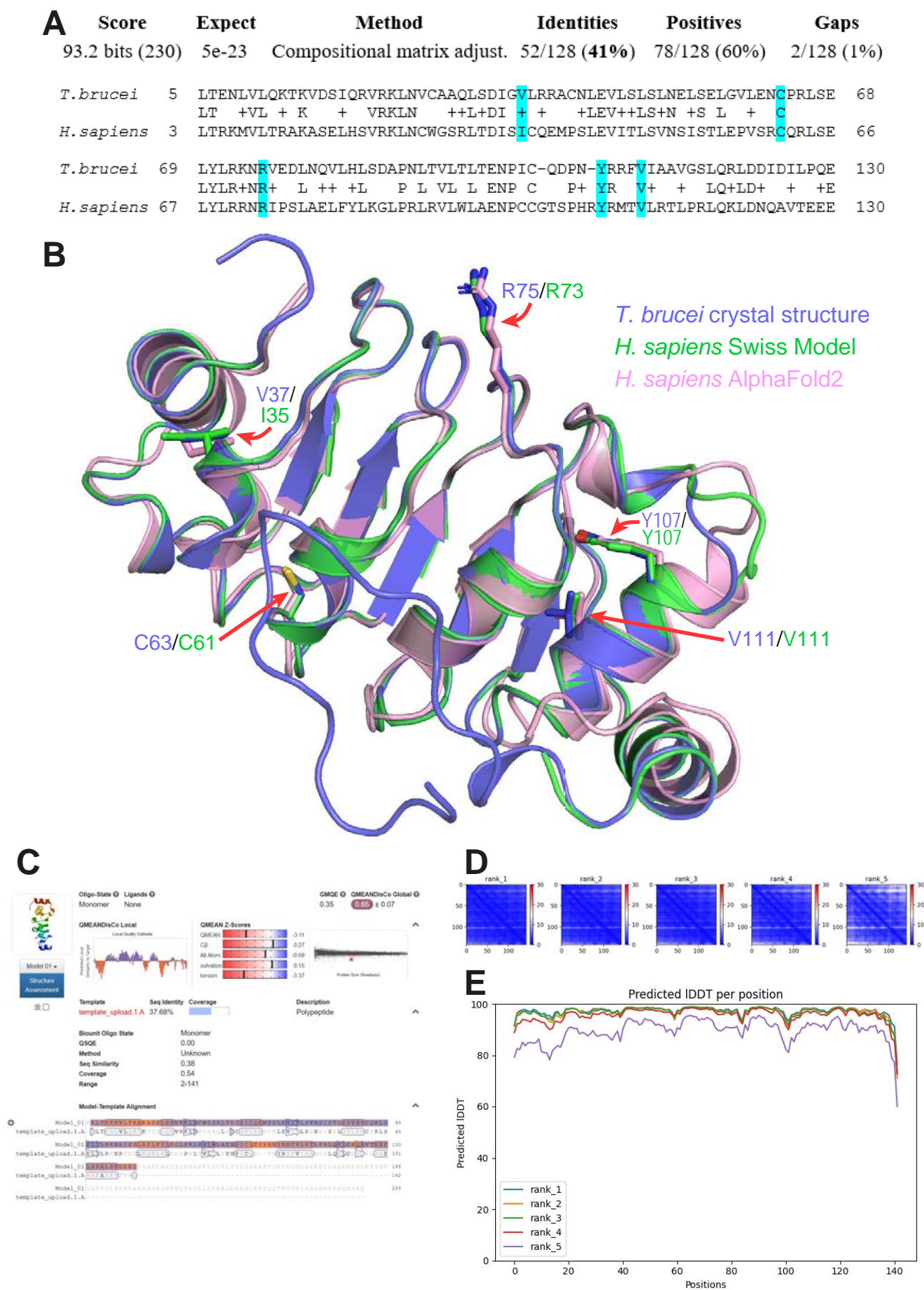
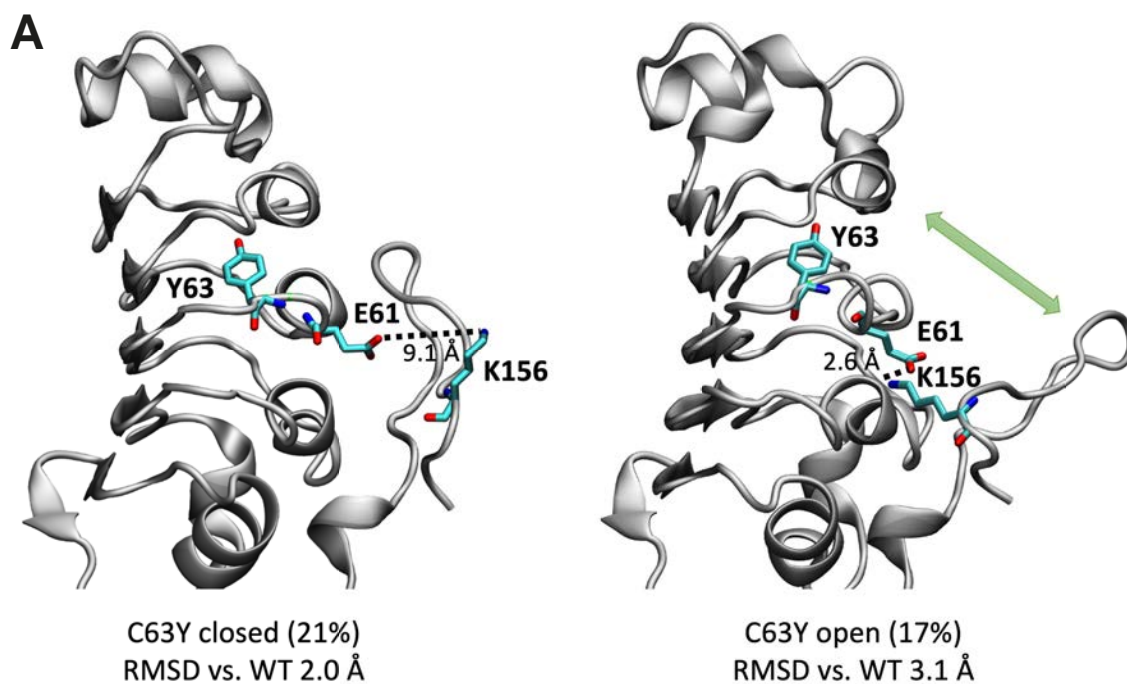


Figure S4

**B**

system	# clusters (P > 1%)	P <sub>MPC</sub>	RMSD	$\Delta S_{\text{conf}}$	$\Delta \text{SASA}_T$	$\Delta \text{SASA}_{\text{Hphi}}$	$\Delta \text{SASA}_{\text{Hpho}}$
WT	6	59.1	-	-	-	-	-
V37F	1	99.9	0.9	-34.1	-1.0	0.0	-1.0
C63Y	10	20.5	2.0	15.4	2.0	0.5	1.5
R75P	5	59.2	1.1	8.1	-1.0	-1.5	0.5
Y107C	8	31.3	1.1	25.7	0.5	0.3	0.2
Y107H	7	76.0	1.1	3.2	0.5	0.5	0.0
V111M	2	94.3	0.9	2.9	0.0	0.0	0.0
		%	Å	J/mol K	nm <sup>2</sup>		

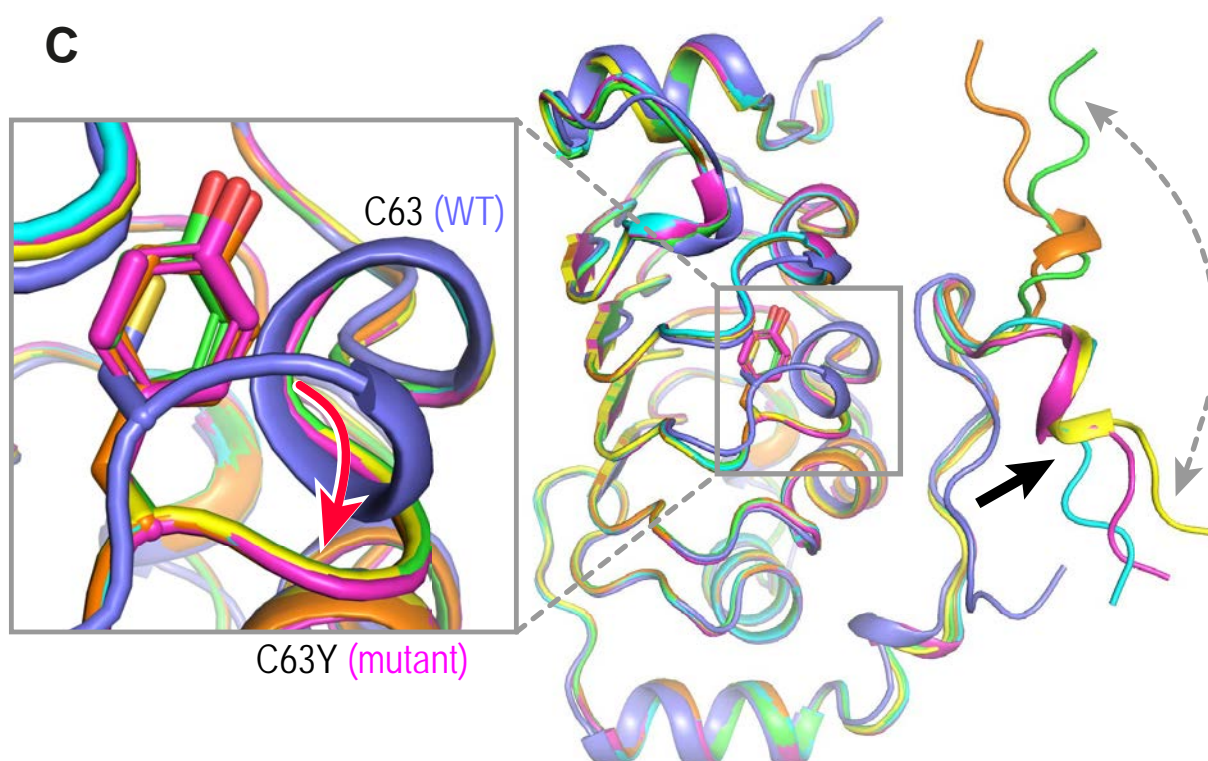
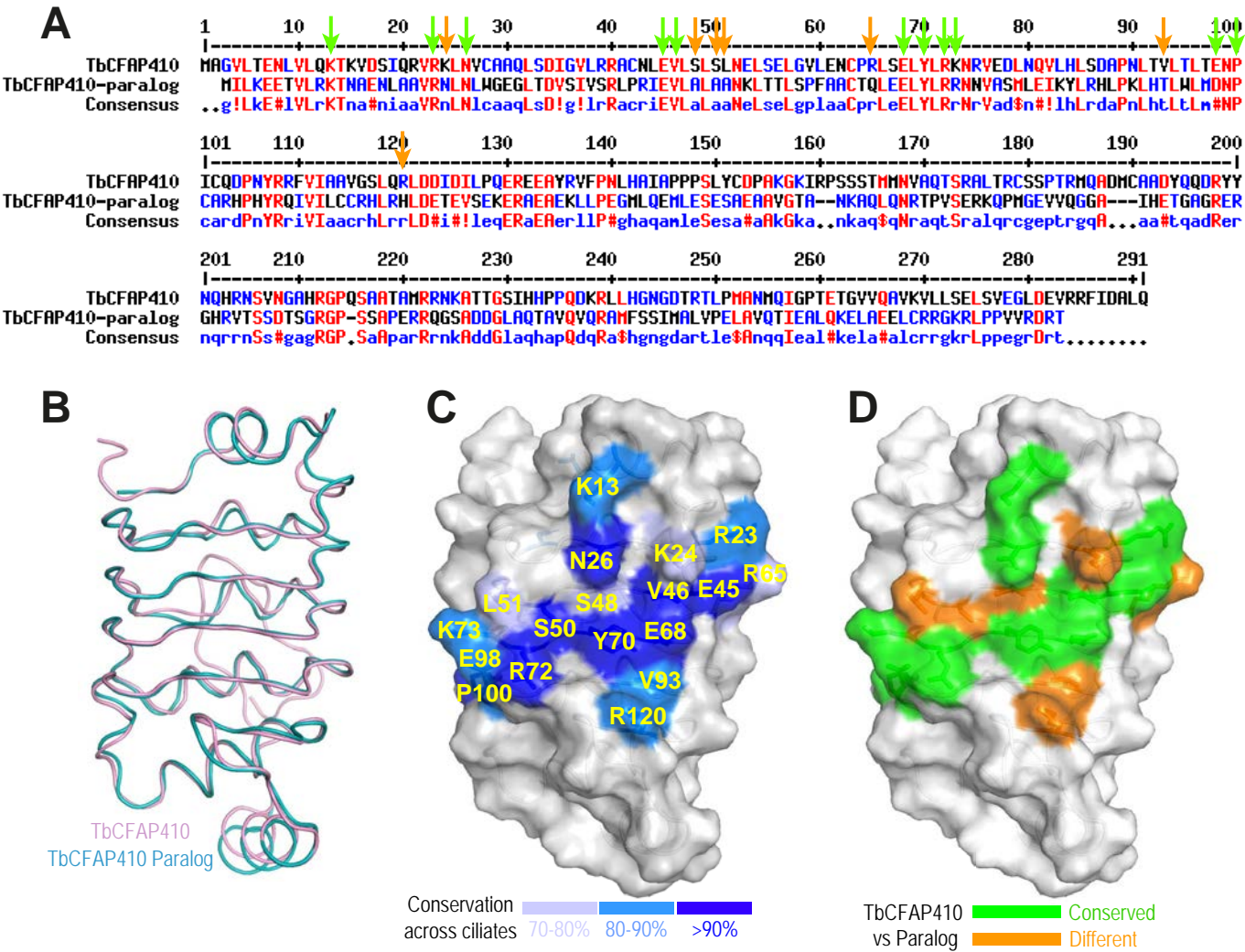
**C**



Figure S5



## Figure S6

