Supplemental Information

Supplemental Table S1: Details of the sequences of the synthetic peptides used is tabulated, the 'p' in each sequence indicates the residue phosphorylated.

Label	Peptide Sequence
Ser^{5}	H-Tyr-Ser-Pro-Thr-**p**Ser-Pro-Ser-Tyr-Ser-Pro-NH2
Tyr^{1}	H-Thr-Ser-Pro-Ser-**p**Tyr-Ser-Pro-Thr-Ser-NH2
Tyr^{1}-Ser^{5}	H-Tyr-Ser-Pro-Thr-**p**Ser-Pro-Ser-**p**Tyr-Ser-Pro-NH2
Thr^{4}-Ser^{5}	H-Ser-Pro-Ser-Tyr-Ser-Pro-Thr-Ser-Pro-Ser-Tyr-Ser-Pro-**p**Thr-**p**Ser-Pro-Ser-Tyr-Ser-OH
Ser^{2}	Ac-Ser-Pro-Ser-Tyr-**p**Ser-Pro-Thr-Ser-Pro-Ser-NH2
Ser^{2}-Ser^{5}	H-Ser-Pro-Ser-Tyr-**p**Ser-Pro-Thr-Ser-Pro-Ser-Tyr-Ser-Pro-Thr-**p**Ser-Pro-Ser-Tyr-Ser-OH
Tyr^{1}-Ser^{2}	Ac-Thr-Ser-Pro-Ser-**p**Tyr-**p**Ser-Pro-Thr-Ser-Pro-Ser-Tyr-Ser-Pro-NH2
Tyr^{1} 14-mer	Ac-Thr-Ser-Pro-Ser-**p**Tyr-Ser-Pro-Thr-Ser-Pro-Ser-Tyr-Ser-Pro-NH2
Ser^{5} 19-mer	H-Ser-Pro-Ser-Tyr-Ser-Pro-Thr-Ser-Pro-Ser-Tyr-Ser-Pro-Thr-**p**Ser-Pro-Ser-Tyr-Ser-OH
Ser^{5}-Ser^{7}	H-Ser-Pro-Ser-Tyr-Ser-Pro-Thr-Ser-Pro-Ser-Tyr-Ser-Pro-Thr-**p**Ser-Pro-**p**Ser-Tyr-Ser-OH

Supplementary Table S2: Crystallographic Data Collection and Refinement Statistics

	Ssu72 C13D/D144N + Symplekin Ser2/Ser5 peptide
Data collection	
Space group	P4
Cell dimensions	
a, *b*, *c* (Å)	127.70, 127.70, 105.98
α, β, γ (°)	90.00, 90.00, 90.00
Resolution (Å)	50.00-2.50 (2.54-2.50)*
R_{merge}/R_{pim}	0.108 (0.743)/ 0.053
I / σI	13.4 (1.5)
Completeness (%)	100.0 (100.0)
Redundancy	5.1 (5.1)
Refinement	
Resolution (Å)	48.95-2.49 (2.55-2.49)
No. reflections	59967 (4110)
R_{work}	0.1958 (0.2711)
R_{free}±	0.2370 (0.3423)
No. atoms	
Protein	8196
Ligand/ion	37
Water	210
***B*-factors** (Å^2)	
Protein	57.5
Ligand/ion	87.7
Water	45.4
R.m.s. deviations	
Bond lengths (Å)	0.003
Bond angles (°)	0.497
Ramachandran Favored£	96.3%

*Values within parenthesis represent corresponding values in the outermost shell.

±R_{free} is the R_{model} calculated for 3.36% of the reflections, a maximum of 2000 reflections were randomly selected and omitted from refinement.

£There are only two Ramachandran outliers which correspond to a proline residue (Pro 53 on Chain B) and glycine residue (Gly 58 on Chain A) which shows strong electron density.

Supplementary Fig. S1 - Amino acid sequences of GST-CTD constructs. Sequence of CTD portion of GST-CTD constructs used in the manuscript. Sequences presented N to C-terminal as individual heptads.

GST-tag

MHHHHHHSSMSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKKFELGLEFPNLPYYIDGDVK
LTQSMAIIRYIADKHNMLGGCPKERAEISMLEGAVLDIRYGVSRIAYSKDFETLKVDFLSKLPEMLKMFEDRLC
HKTYLNGDHVTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPQIDKYLKSSKYIAWPLQGWQATF
GGGDHPPKSSSLEVLFQ/GPGSGM

yCTD

Y_1	S_2	P_3	T_4	S_5	P_6	S_7
			G			
F	G	V	S	S	P	G
F	S	P	T	S	P	T
Y	S	P	T	S	P	A
Y	S	P	T	S	P	S
Y	S	P	T	S	P	S
Y	S	P	T	S	P	S
Y	S	P	T	S	P	S
Y	S	P	T	S	P	S
Y	S	P	T	S	P	S
Y	S	P	T	S	P	S
Y	S	P	T	S	P	S
Y	S	P	T	S	P	S
Y	S	P	T	S	P	S
Y	S	P	T	S	P	S
Y	S	P	T	S	P	A
Y	S	P	T	S	P	S
Y	S	P	T	S	P	S
Y	S	P	T	S	P	S
Y	S	P	T	S	P	S
Y	S	P	T	S	P	N
Y	S	P	T	S	P	S
Y	S	P	T	S	P	G
Y	S	P	G	S	P	A
Y	S	P	K	Q	D	E

Q K H N E N E N S R

5CTD and YtoH 5CTD

Y_1	S_2	P_3	T_4	S_5	P_6	S_7
Y/H	S	P	T	S	P	S
Y/H	S	P	T	S	P	S
Y/H	S	P	T	S	P	S
Y/H	S	P	T	S	P	S
Y/H	S	P	T	S	P	S

3CTD and YtoT 3CTD

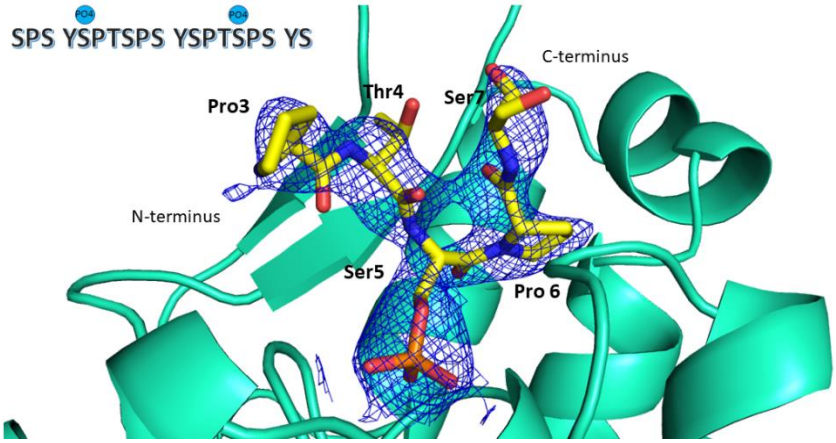
Y_1	S_2	P_3	T_4	S_5	P_6	S_7
Y/T	S	P	T	S	P	S
Y/T	S	P	T	S	P	S
Y/T	S	P	T	S	P	S

YTSPTSPS,3CTD

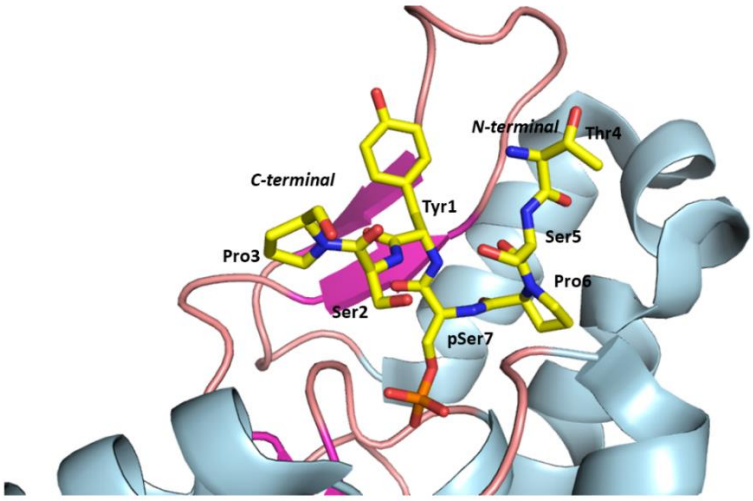
Y_1	T_2	S_3	P_4	T_5	S_6	P_7	S_8
Y	T	S	P	T	S	P	S
Y	T	S	P	T	S	P	S
Y	T	S	P	T	S	P	S
Y							

Supplemental Fig. S2: The structure of Ssu72 binding to CTD peptides. (A) The 2Fo-FC map (contoured at 1σ) shown as a blue mesh for the Ser2/Ser5 peptide soaked in Ssu72+Symplekin C13D/D144N complex. (B) Ssu72+Symplekin C13D/D144N complex with Ser7 peptide (shown as yellow sticks) as obtained from the previously solved structure (pdb code: 4H3H).

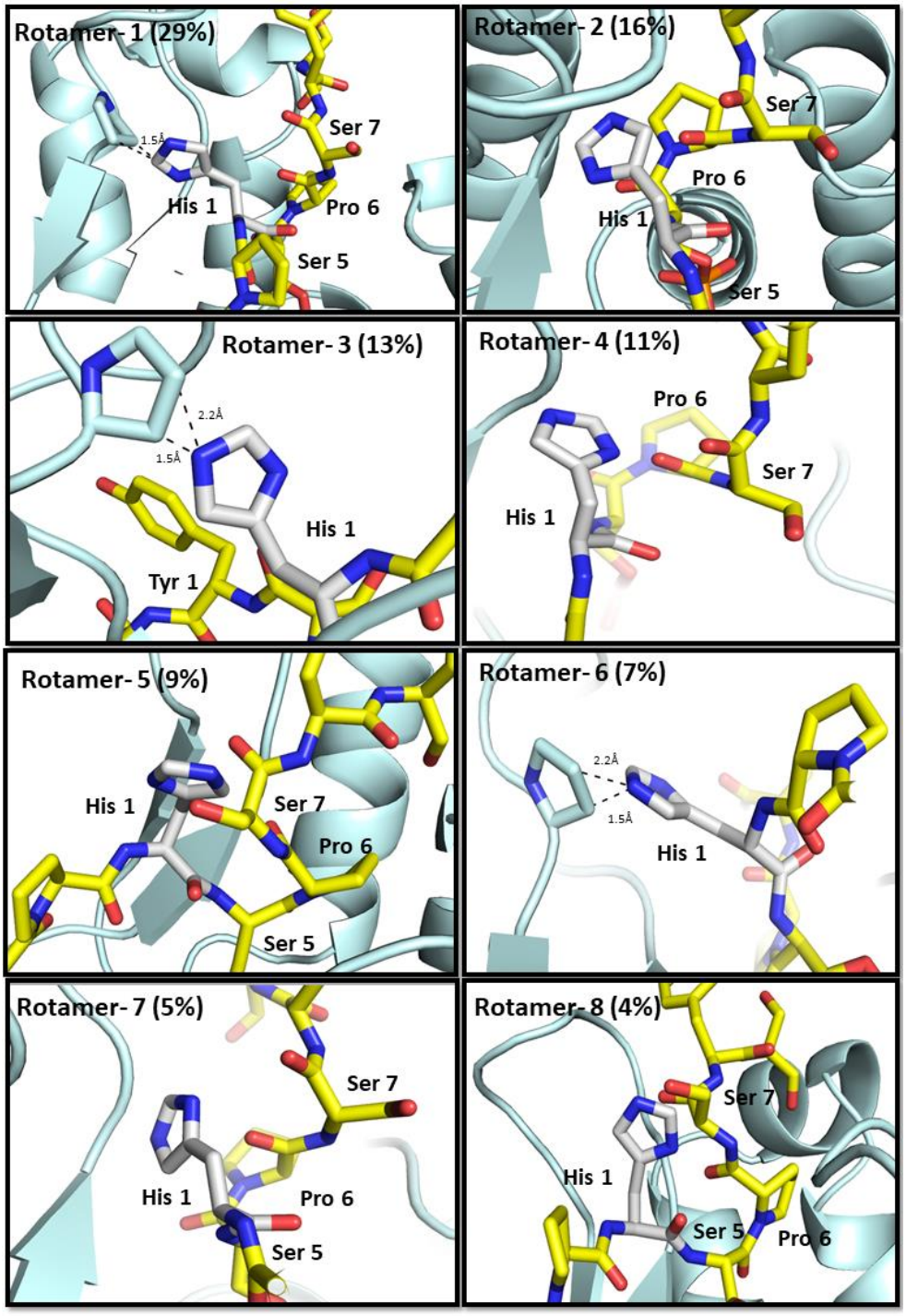
A.



B



Supplemental Fig. S3: Eight different rotamers of the histidine (shown in grey sticks) modelled in at the Thr4 position in the Ssu72 complex with Ser2/Ser5 structure (represented Fig 2A). Pro 53 which seems close to the histidine modelled in is shown as blue sticks and the unfavorable Van der Waals interactions have been indicated in terms of the distance between closely positioned atoms.



Supplemental Fig. S4: Specificity of PTP1B. Mass spectrometry analysis using MALDI-TOF for peptides (sequence of respective synthetic peptides mentioned in the top left hand corner of each graph, phosphorylation of a residue depicted as ‘p’ before the residue) before treatment with PTP1B, shown in black and after treatment with PTP1B shown in green.