SUPPLEMENTAL INFORMATION

Checkpoint activation by Spd1: a competition-based system relying on tandem disordered PCNA binding motifs

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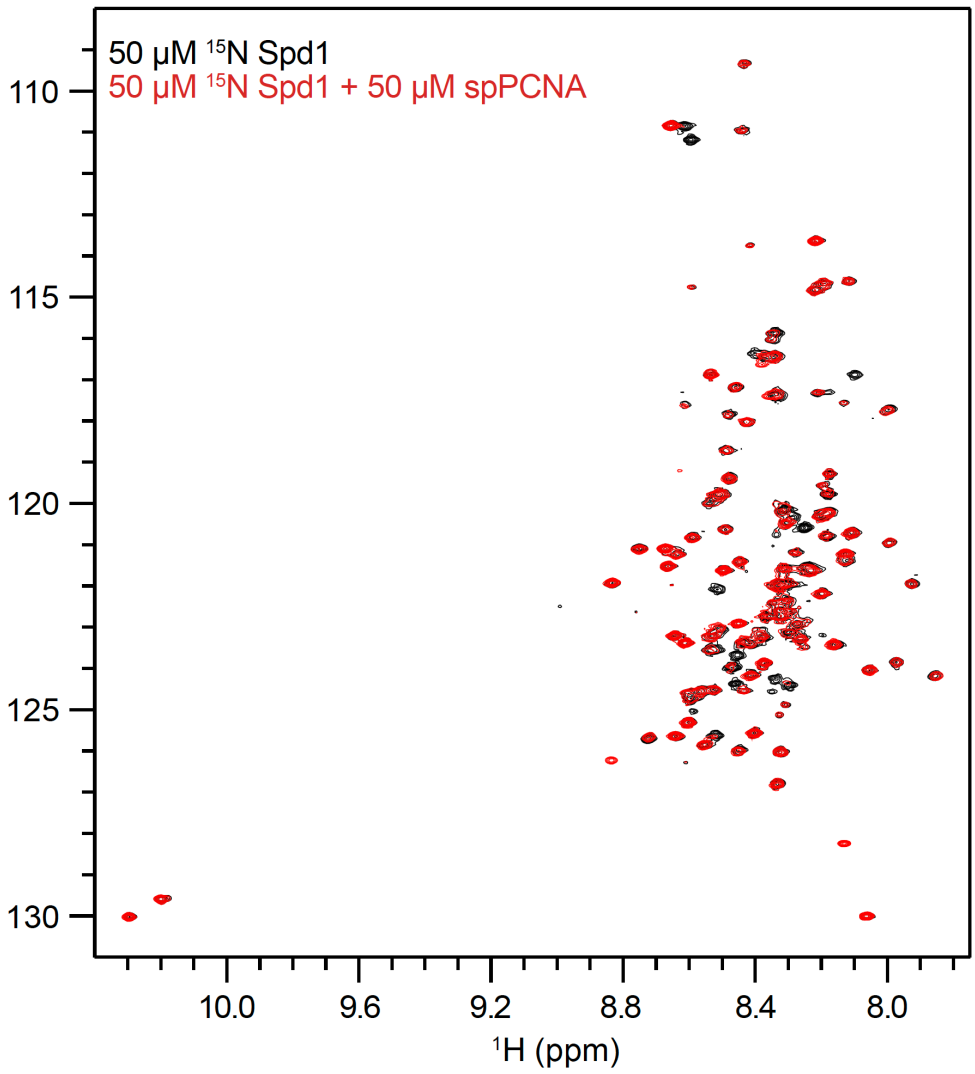
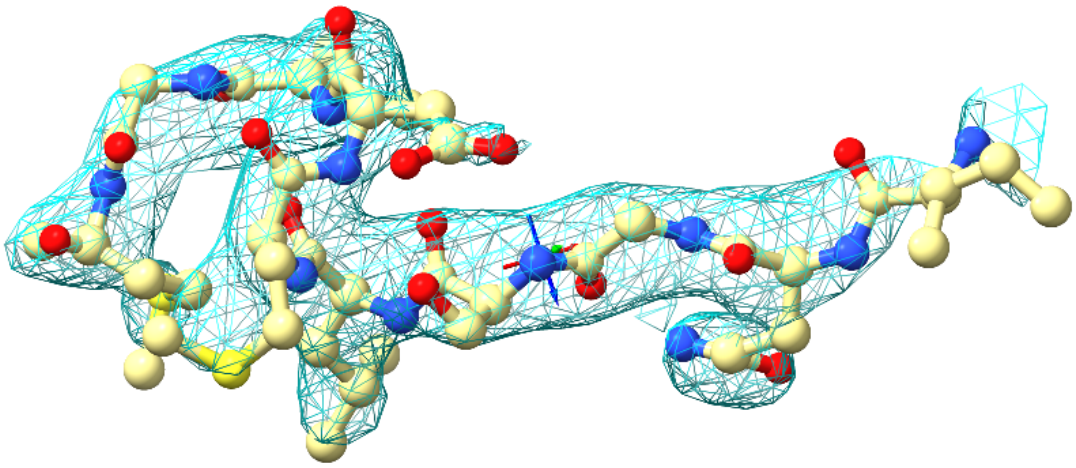
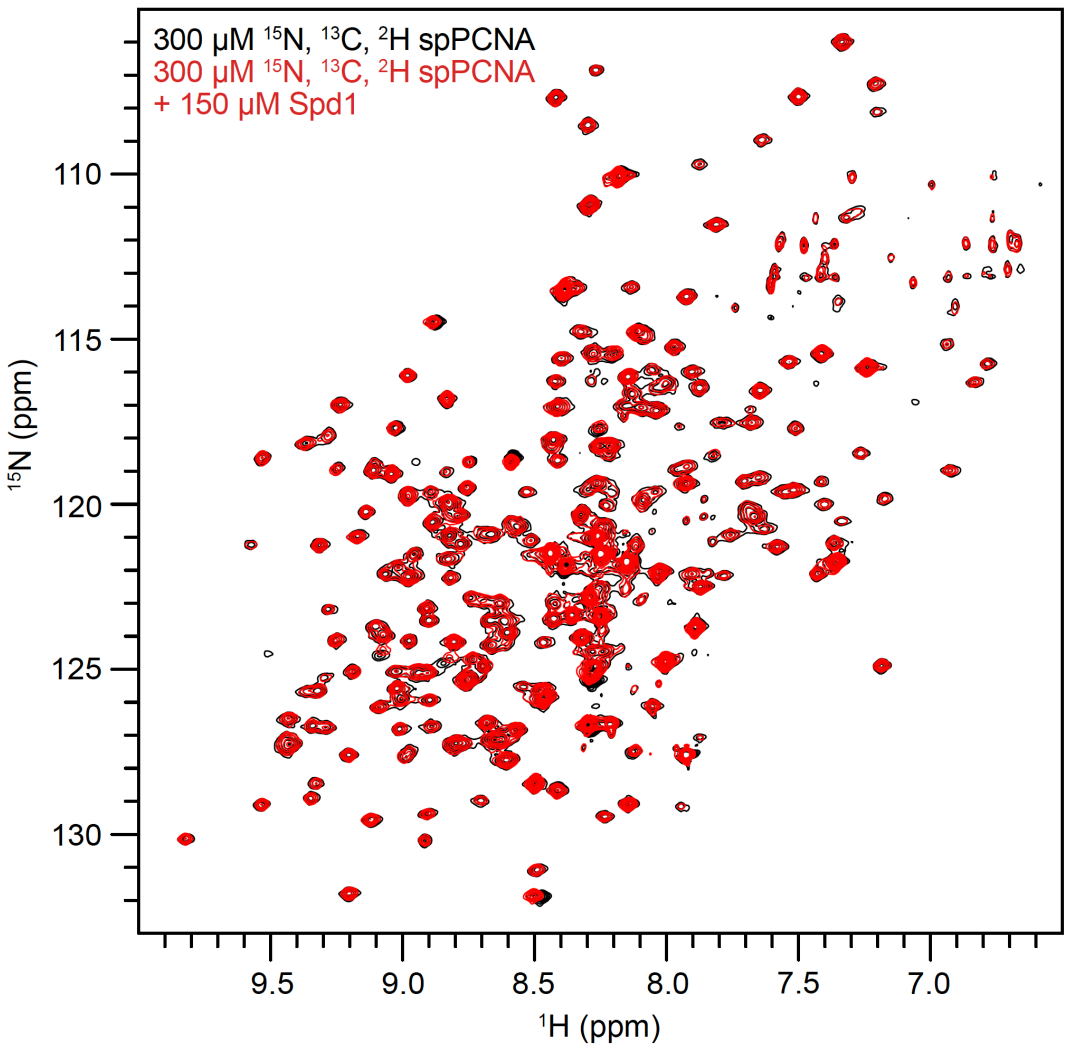


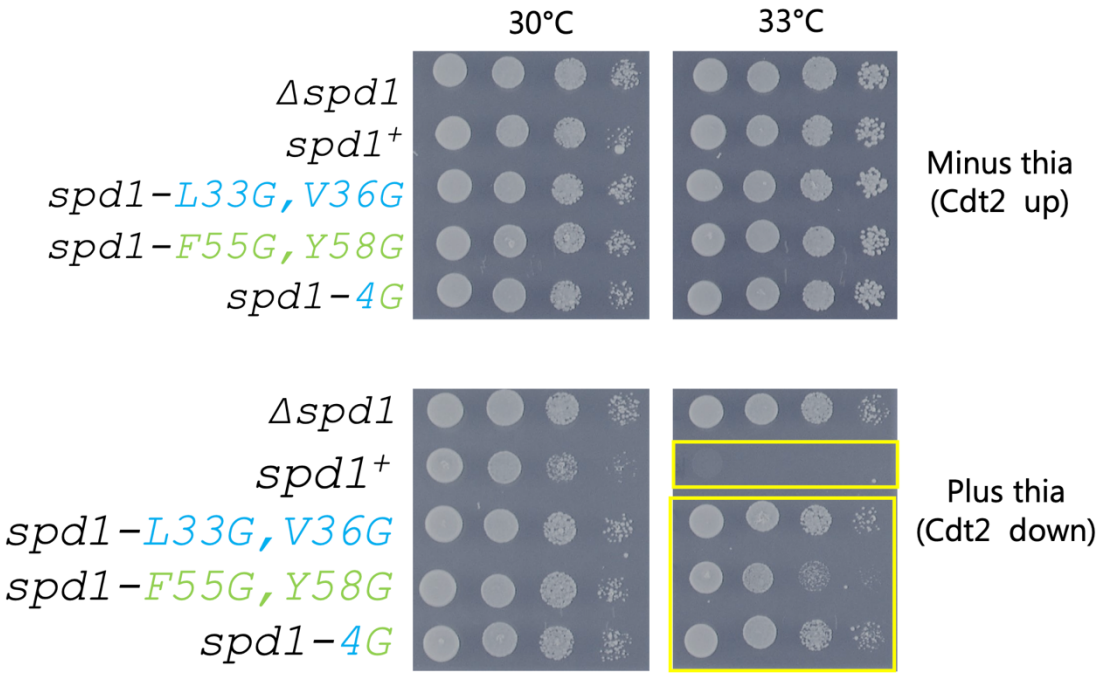
Supplementary Fig. S1: $^{1}H,^{15}N$-HSQC spectra of ^{15}N-Spd1 with and without addition of spPCNA



Supplementary Fig. S2: Electrons density of subunit D.



Supplementary Fig. S3: ^{1}H,^{15}N-HSQC spectra of ^{15}N,^{13}C,^{2}H-spPCNA with and without addition of Spd1



Supplementary Fig. S4: Serial dilutions of strains with the indicated genotypes spotted on plates either untreated or treated with thiamine and incubated at the indicated temperature for three days. All strains also harbour the *cdt2*-TR and the *rad3-TS* alleles.

Table S1. Data collection and refinement data for PCNA:Spd1. Values in parenthesis refer to data from the outer resolution shell (3.06-2.90 Å).

Data collection	
Property	Value
Space group	P 2_1
Cell constants (Å) (a, b, c, α, β, γ)	80.9, 71.7, 84.0, 90.0°, 115.8°, 90.0°
Matthews coefficient, % solvent	2.3, 47
Protein molecules pr. asymmetric unit	3 PCNA subunits, 1 Spd1$^{27-46}$ ligand
Resolution (Å)	75.7-2.9 (2.975-2.900)
Number of reflections observed	79022
Number of unique reflections	19183
Completeness (%)	99.1 (99.2)
Multiplicity	4.1 (4.3)
Wavelength (Å)	0.8729
Rp.i.m.	0.10 (0.55)
CC1/2	0.99 (0.10)
<I/σI>	1.38
R/Rfree	0.23 (0.26)/0.26
Wilson B-factor (Å^2)	59.9
Total # atoms	5797
Structure building and refinement	
Software	CCP4i, Refmac5, Coot
Resolution (Å)	75.7-2.9
Number of protein atoms in model	5794
Number of water molecules	0
<B> all atoms (Å^2)	81.5
RMS deviation, bond lengths	0.009
RMS deviation, bond angles	1.9

Table S2. Fission yeast strains

Strain:	Genotype:
Eg3439	*h⁺ leu1+::nmt41-cdt2 cdt2::ura4+ ura4-D18 Δspd1::hygro rad3-ts*
Eg3490	*h⁺ leu1+::nmt41-cdt2 cdt2::ura4+ ura4-D18 rad3-ts*
Eg4191	*h⁺ leu1+::nmt41-cdt2 cdt2::ura4+ ura4-D18 rad3-TS spd1-L33G V36G*
Eg4193	*h⁺ leu1+::nmt41-cdt2 cdt2::ura4+ ura4-D18 rad3-TS spd1-F55G Y58G*
Eg4549	*h⁺ leu1+::nmt41-cdt2 cdt2::ura4+ ura4-D18 spd1-V36F G37F rad3-ts*
Eg4553	*h⁺ leu1+::nmt41-cdt2 cdt2::ura4+ ura4-D18 spd1-F55L N59F rad3-ts*
Eg4035	*h⁻ GFP-suc22*
Eg4031	*h⁻ GFP-suc22 Δspd1::hygro*
Eg4892	*h⁺ spd1-L33G V36G GFP-suc22*
Eg4894	*h⁺ spd1-F55G Y58G GFP-suc22*
Eg4900	*h⁺ spd1-V36F G37F GFP-suc22*
Eg4904	*h⁺ spd1-F55L N59F GFP-suc22*
Eg3947	*h⁺ leu1+::nmt41-cdt2 Δcdt2::ura4+ ura4-D18 VN173-pcn1::kmx spd1-VC155::nat*
Eg4926	*h⁺ leu1+::nmt41-cdt2 cdt2::ura4+ ura4-D18 VN173-pcn1::kmx spd1-L33G V36G-VC155::nat*
Eg4927	*h⁺ leu1+::nmt41-cdt2 cdt2::ura4+ ura4-D18 VN173-pcn1::kmx spd1-F55G Y58G-VC155::nat*
Eg4928	*h⁺ leu1+::nmt41-cdt2 cdt2::ura4+ ura4-D18 VN173-pcn1::kmx spd1-L33G V36G-F55G Y58G-VC155::nat*
Eg4929	*h⁺ leu1+::nmt41-cdt2 cdt2::ura4+ ura4-D18 VN173-pcn1::kmx spd1-V36F G37F-VC155::nat*
Eg4930	*h⁺ leu1+::nmt41-cdt2 cdt2::ura4+ ura4-D18 VN173-pcn1::kmx spd1-F55L N59F-VC155::nat*
Eg4514/ Eg4515	*h⁺ leu1+::nmt41-cdt2 cdt2::ura4+ ura4-D18 VN173-pcn1::kmx spd1-W109G F113G-VC155::nat*
Eg5074	*h⁺ spd1-VC155::nat*
Eg5075	*h⁺ spd1-L33G V36G-VC155::nat*
Eg5076	*h⁺ spd1-F55G Y58G-VC155::nat*
Eg5077	*h⁺ spd1-L33G V36G-F55G Y58G-VC155::nat*

Eg5078	*h⁺ spd1-V36F G37F-VC155::nat*
Eg5079	*h⁺ spd1-F55L N59F-VC155::nat*