Supplementary Data

Structural mechanism of the phosphorylation-dependent dimerization of the MDC1 forkhead-associated domain

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Supplementary Data includes Supplementary table 1 and Supplementary figures 1-5.

Supplementary figures

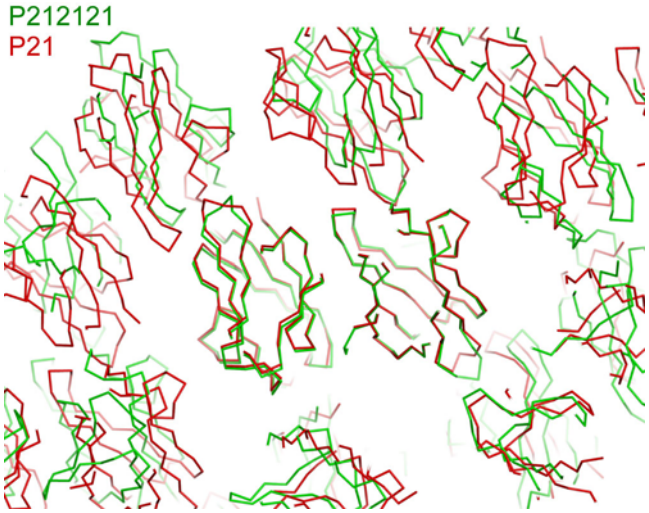

Figure S1. Crystal lattices of MDC1 FHA structure in the $P2_1$ (red) and $P2_12_12_1$ space groups (green). The two protomers in the asymmetric unit at the center of the figure are aligned.

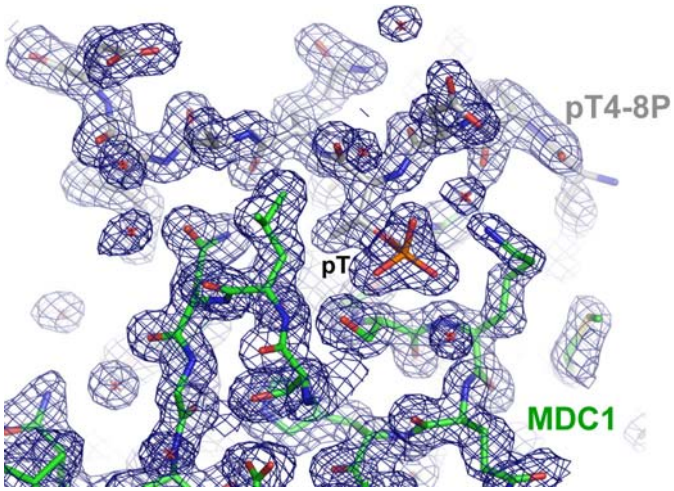

Figure S2. The 1.7 Å $2f_o$-f_c electron density map of the MDC1$^{27\text{-}138}$ and pT4-8P complex structure is contoured at the 1.0 σ level. The pT-binding pocket is shown.

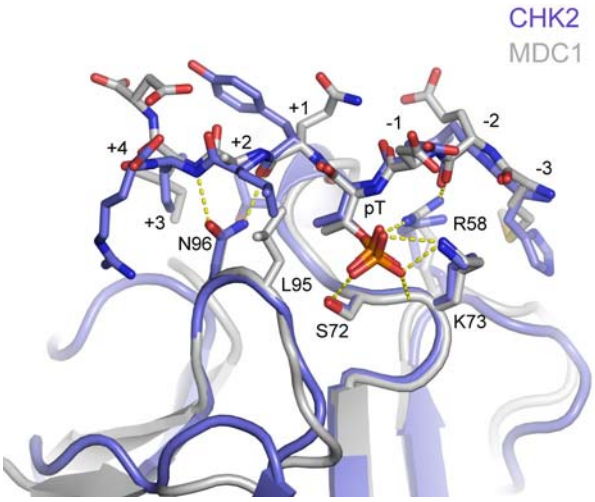

Figure S3. Superimposition of the structure of MDC1$^{27-138}$ bound with the phosphopeptide pT4-8P (grey) with the structure of CHK2 FHA domain bound with the phosphopeptide [RHFD(pT)YLIRR] (PDB: 1GXC, blue). The two FHA domains were aligned by their pT-binding pockets. The two phosphopeptides and the side chains of interacting residues in FHA domains are shown as sticks. The interacting residues of MDC1 are labeled. Residues of the two phosphopeptides are indicated by their relative position to pT.

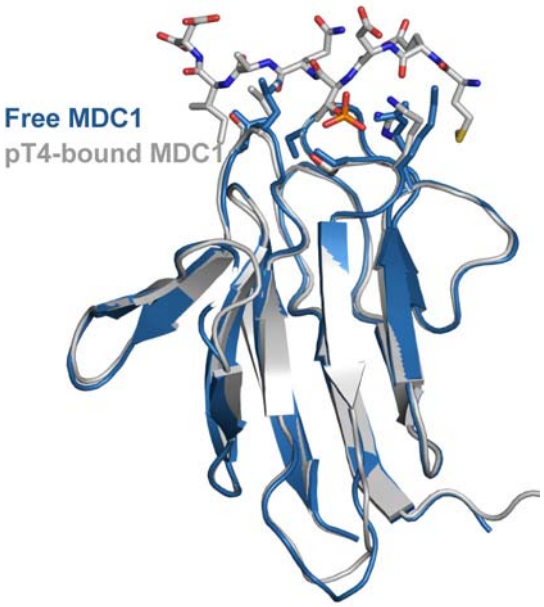

Figure S4. Structural alignment of MDC1 FHA structures in the free and pT4-bound states. The alignment yields an rmsd of 0.4 Å over 96 Cα pairs.

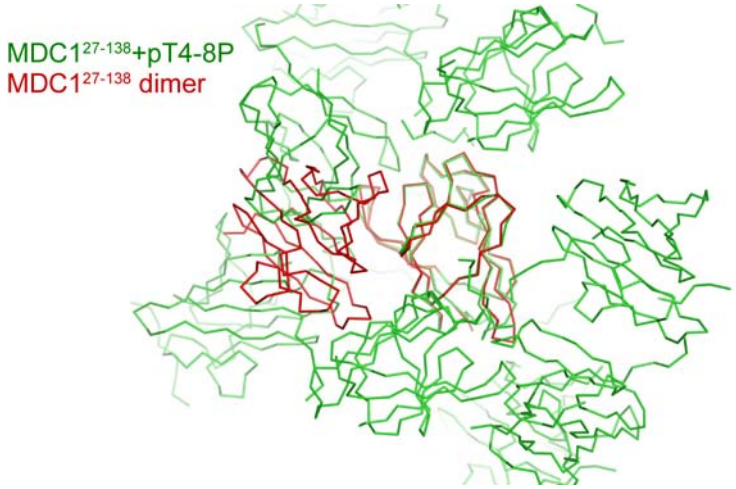

Figure S5. Crystal lattice of the monomeric structure of MDC1$^{27-138}$ in complex with phosphopeptide pT4-8P. One subunit of the unliganded MDC1$^{27-138}$ dimer (red) is aligned to a pT4-bound MDC1$^{27-138}$ molecule (green) at the center of the figure. The other subunit in the MDC1$^{27-138}$ dimer would be occluded by neighboring molecules in the pT4-bound MDC1$^{27-138}$ crystal.

Table S1. Data collection and refinement statistics

Crystal form	MDC1$^{27-138}$ +KAu(CN)2	MDC1$^{27-138}$	MDC1$^{27-138}$	MDC1$^{27-138}$ + pT4-8P
Data collection				
PDB code		3UMZ	3UNM	3UNN
Space group	$P2_12_12_1$	$P2_12_12_1$	$P2_1$	$P3_121$
Cell dimensions				
a, b, c (Å)	56.2, 58.7, 66.6	58.0, 58.9, 67.1	30.7, 56.1, 64.9	52.9, 52.9, 81.2
α, β, γ (º)	90, 90, 90	90, 90, 90	90, 97.53, 90	90, 90, 120
Wavelength (Å)	1.5418	0.9796	0.9796	0.96396
X-ray source	Cu-K	SSRF, BL17U	SSRF, BL17U	SSRF, BL17U
Resolution range (Å) [a]	25-2.70 (2.75-2.70)	50-1.65 (1.68-1.65)	20-1.80 (1.83-1.80)	45-1.7 (1.73-1.7)
Unique reflections	6357	28513	20009	14992
Redundancy	7.8 (4.3)	6.5 (4.8)	3.4 (2.3)	10.7 (10.7)
I/σ	11.0 (2.0)	25.3 (2.4)	22.5(2.3)	58.5(6.6)
Completeness (%)	99.4 (96.6)	98.8 (99.5)	97.7 (91.2)	99.0(100)
R_{merge} [b]	0.164 (0.525)	0.108 (0.586)	0.079 (0.448)	0.046(0.425)
Structure refinement				
Resolution range (Å)		20-1.65 (1.69-1.65)	20-1.80 (1.85-1.80)	20-1.7 (1.75-1.70)
No. reflections		26402 (1906)	18794 (1267)	14190 (994)
No. atoms		1814	1719	981
Average B factor (Å^2)		20.2	31.2	21.4
R_{work} [c]		0.236 (0.246)	0.236 (0.258)	0.208 (0.242)
R_{free} [d]		0.267 (0.288)	0.274 (0.310)	0.232 (0.268)
Rmsd bond length (Å)		0.009	0.009	0.007
Rmsd bond angles (º)		1.288	1.241	1.192
RAMPAGE analysis				
Favoured (%)		98.5	98.5	99.1
Allowed (%)		1.5	1.5	0.9
Outlier (%)		0	0	0

[a]The values for the data in the highest resolution shell are shown in parentheses.

[b]$R_{merge} = \sum|I_i - I_m|/\sum I_i$, where I_i is the intensity of the measured reflection and I_m is the mean intensity of all symmetry related reflections.

[c]$R_{work} = \sum_h|F_o - F_c|/\sum_h F_o$, where F_o and F_c are the observed and calculated structure factor amplitudes of reflection h.

[d]R_{free} is the same as R_{work}, but calculated on 5% reflections not used in refinement