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Structure of mono-ubiquitinated PCNA: implications for DNA polymerase switching and Okazaki fragment maturation

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Supplementary Figure S1

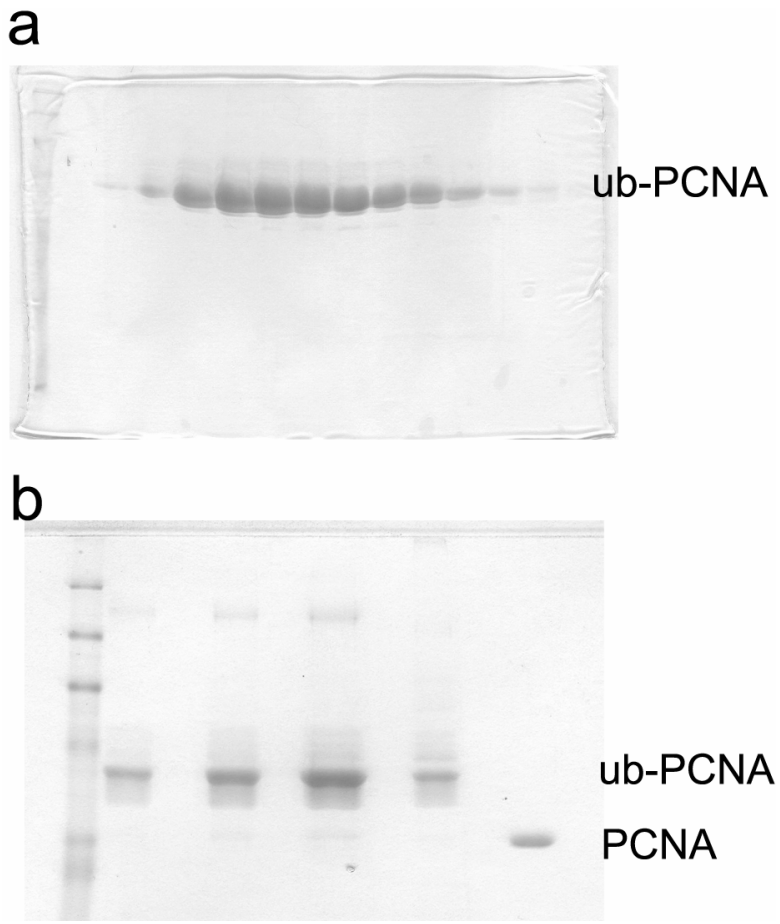

Figure S1. Quality of purified ub-PCNA. (**a**) Ub-PCNA was analyzed by SDS-PAGE after FPLC gel-filtration chromatography. The protein was visualized by coomassie-blue staining. (**b**) The dissolved crystals (first four protein lanes after the marker lane) were analyzed by SDS-PAGE. PCNA (last lane, 1 μg) was used as a control. The ub-PCNA bands from the dissolved crystals displayed some spreading due to high salt contents in the samples as ub-PCNA crystallizes in the presence of 1.1 M sodium citrate.

Supplementary Figure S2

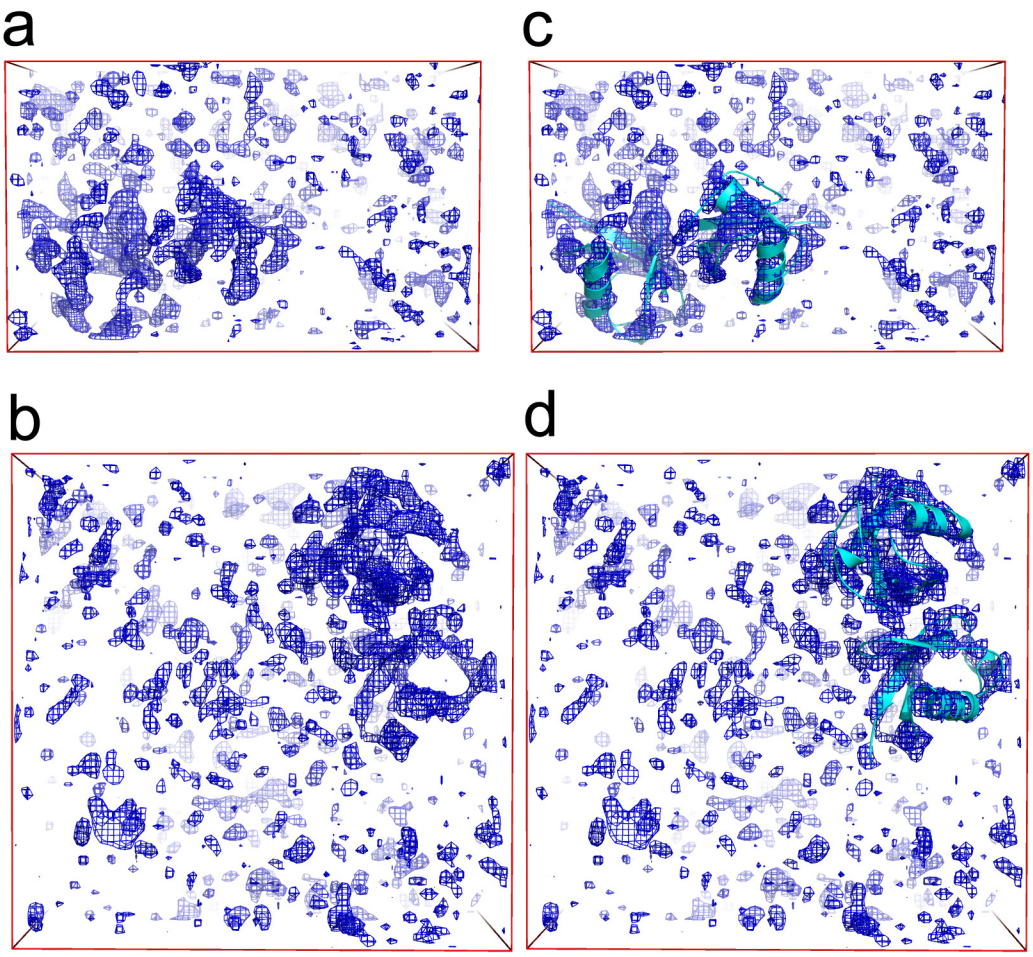

Figure S2. The initial electron density of ubiquitin molecules after maximum entropy refinement (BUSTER) with the PCNA trimer identified by molecular replacement. (a, b) The (fo-fc) electron density maps for two ubiquitin molecules contoured at 1 σ at two different angles (the red rectangles are the outlines of the unit cell). (**c, d**) The fit of the final ubiquitin molecules to the electron density.

Supplementary Figure S3

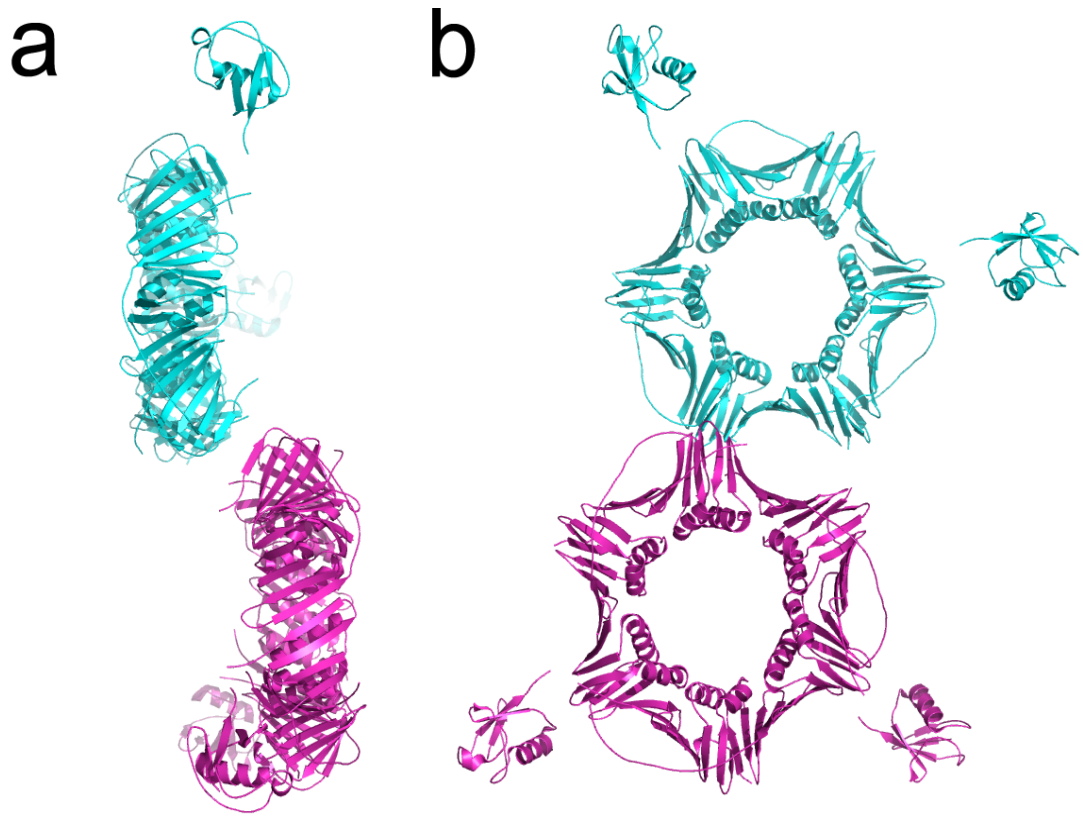

Figure S3. The position of the third ubiquitin differs from the two modeled ubiquitins. (a) Side-view of ub-PCNA with a symmetry molecule. (**b**) Front-view of the two symmetry related molecules rotated 90° from the view in "a". The position that would be occupied by the third ubiquitin in a corresponding manner to the two modeled ubiquitins is occupied by PCNA-PCNA contacts of the symmetry molecules in the crystal packing. Areas of electron densities indicate that the third ubiquitin is displaced to a location in the groove between the PCNA ridges.

Supplementary Figure S4

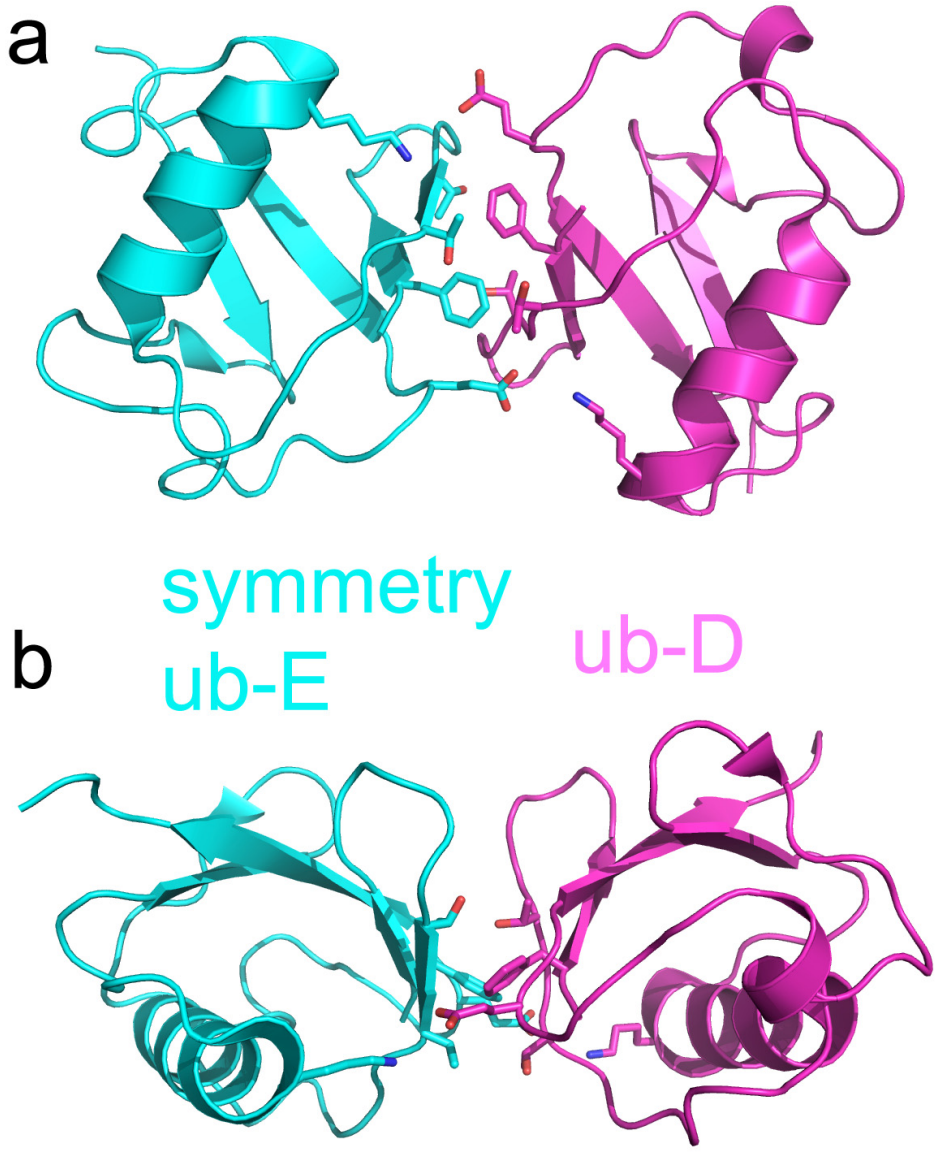

Figure S4. Ub-D forms a dimer with Ub-E from an adjacent symmetry molecule. The two ubiquitin molecules form a loosely associated dimer (view **a** and **b**) due to crystal packing with a buried surface area of 285 $Å^2$. However, ub-PCNA does not dimerize in solution as judged by size-exclusion chromatography.

Supplementary Figure S5

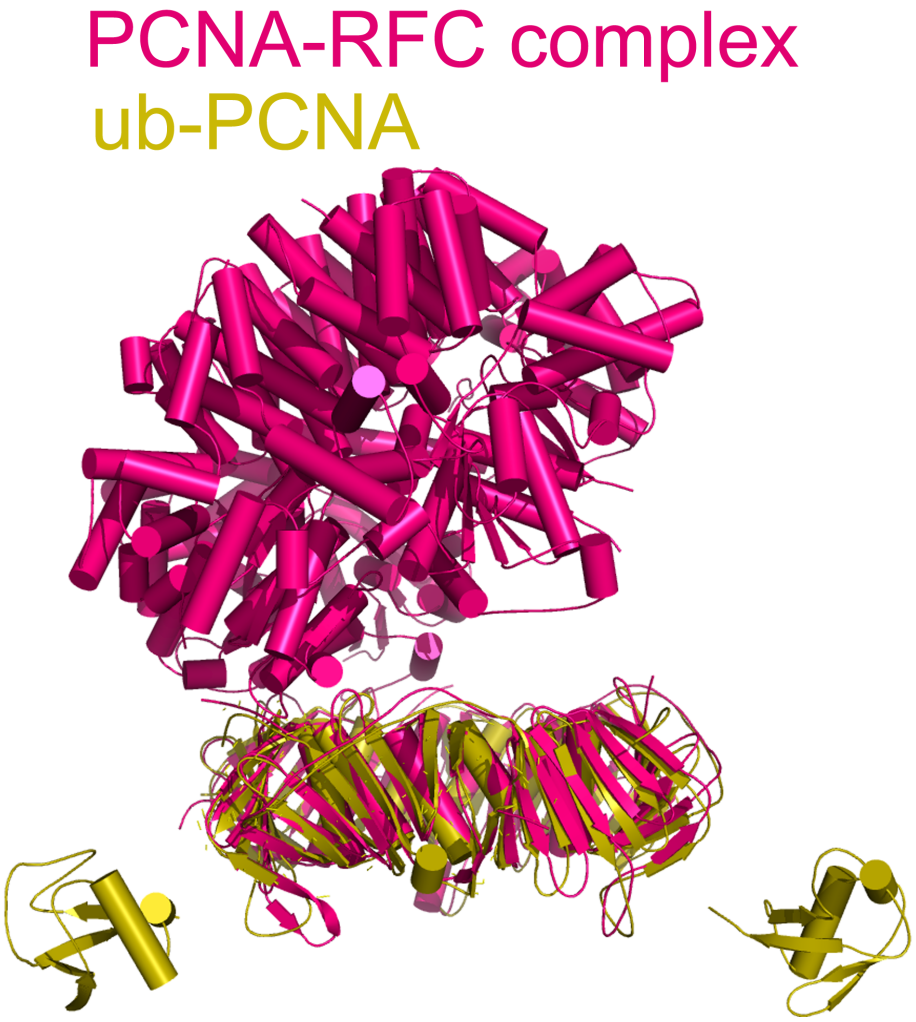

Figure S5. Structural alignment of ub-PCNA with the RFC-PCNA complex. Ubiquitin molecules in ub-PCNA do not interfere with the binding of replication factor C (RFC, 1SXJ, red) to PCNA when the PCNA portions of ub-PCNA and PCNA-RFC complex are aligned.

Supplementary Figure S6

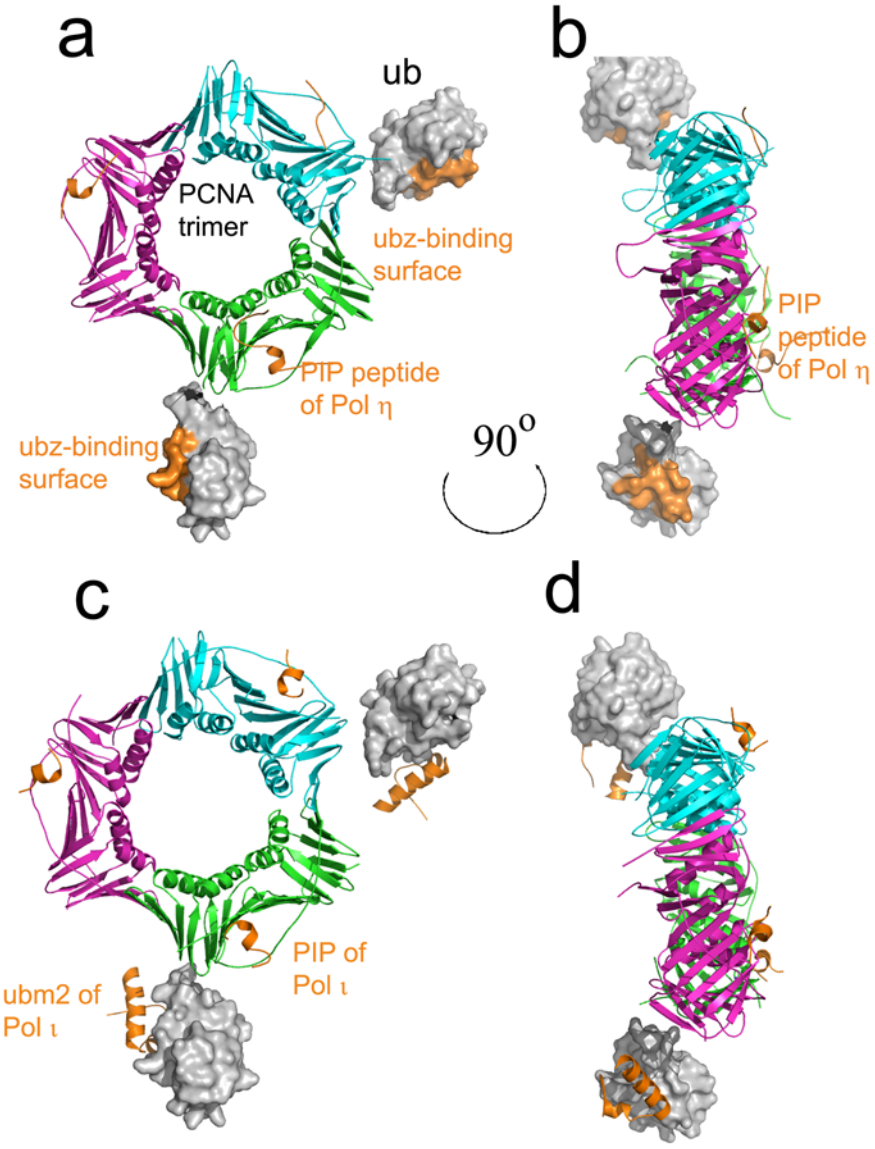

Figure S6. Relative orientation of the UBZ-binding surface (Pol η) and UBM domain (Pol ι) to the PCNA trimer ring. (**a**) the UBZ-binding surface on ubiquitin is almost perpendicular to the plane of the PCNA trimeric ring and resides opposite to the PIP peptide binding site across a ridge on PCNA. (**b**) view from a 90^0 angle to figure **a**. (**c**) The relative orientation of UBM-binding domain to the PIP motif binding site (as shown by the binding of the PIP peptide of Pol ι). (**d**) view from a 90^0 angle to **c.**

Supplementary Figure S7

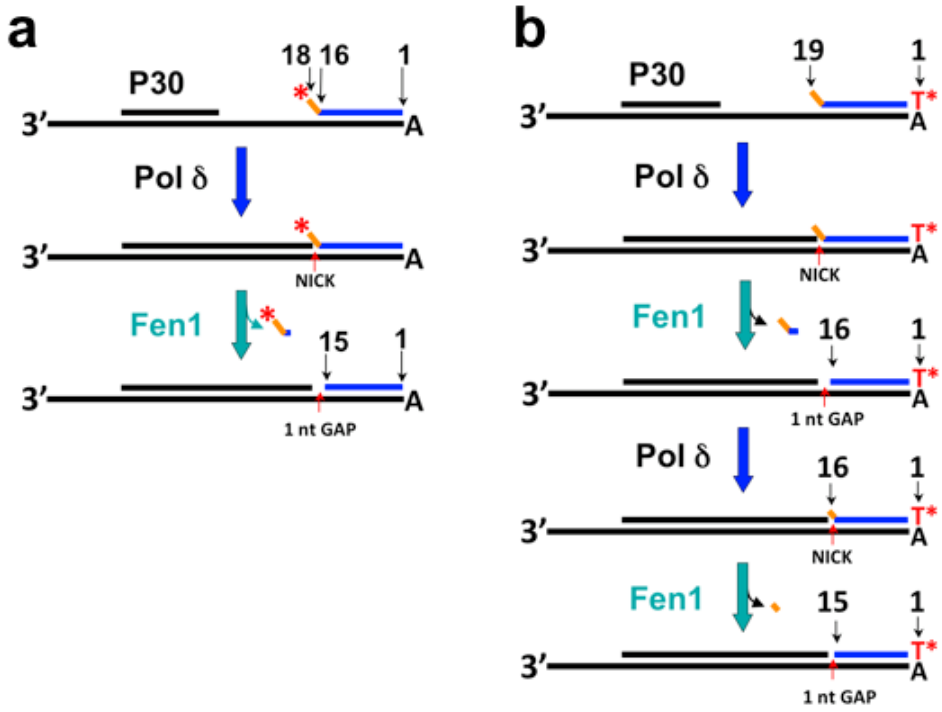

Figure S7. Substrates used for the assay of the combined actions of Fen1, Pol δ and PCNA. (**a**) 5'-end-labeled blocking 18nt oligonucleotide with a 2nt flap formed by two non-complementary nucleotides (orange). The reaction proceeds by gap filling by Pol δ until the blocking oligonucleotide is reached. Fen1 then cleaves off the flap at the Flap+1 position. (**b**) 3'-end labeled blocking 19nt oligonucleotide with a 2nt flap formed by two non-complementary nucleotides (orange). The reaction proceeds by gap filling by Pol δ until the blocking oligonucleotide is reached. Fen1 then cleaves off the flap at the Flap +1 position as in "**a**", to generate a 16 nt blocking oligonucleotide. Pol δ then fills the gap, and performs a limited strand displacement, creating a short flap (1nt or longer) which is then cleaved by Fen1. The combined reaction where a single nt flap is generated and cleaved is essentially a nick translation reaction.

Supplementary Figure S8

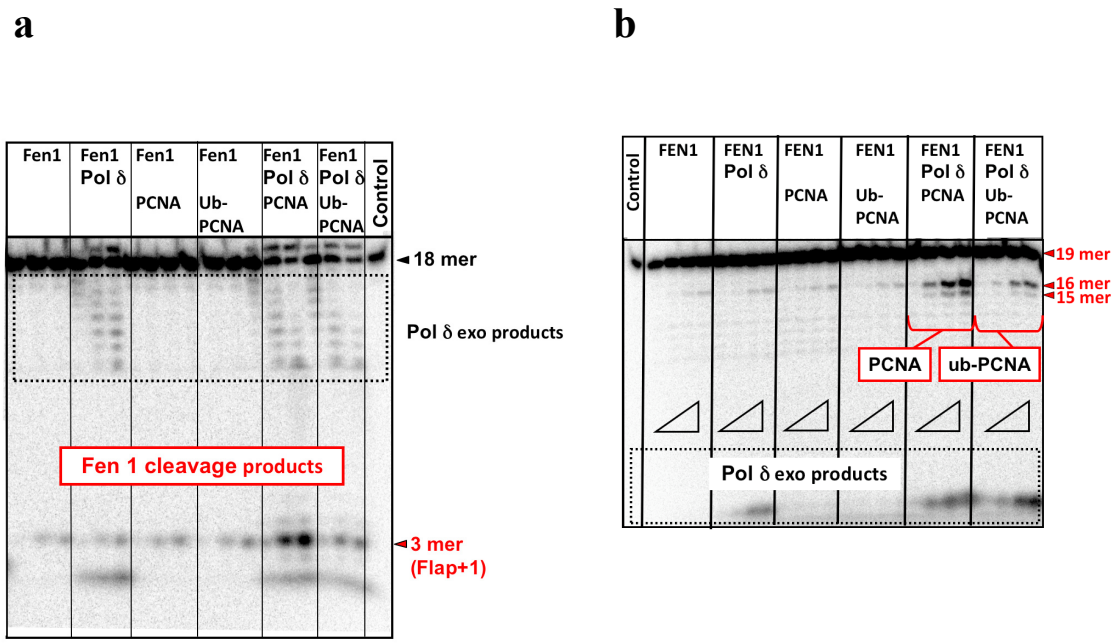

Figure S8. Gels for analysis of products of 3' and 5' end labels substrates used in Fig. 4. (**a**) Autoradiogram of gel of Fig. 4b, where a 5' end labeled blocking oligonucleotide was used (Figure S7a). Note that in addition to the Fen1 cleavage products, some exonucleolytic breakdown of the blocking oligonucleotide by the 3' to 5' exonuclease of Pol δ was also observed. Also seen is the addition of a single nt to the blocking oligonucleotide to form a small amount of 19 mer by Pol δ because the blocking oligonucleotide stopped 1nt short of the end of the template. **(b)** Autoradiogram of gel of Fig. 4e, where a 3' end labeled blocking oligonucleotide was used (Figure S7b). Also shown are the small amount of single nucleotide products (dTMP) coming of removal of the labeled end of the blocking oligonucleotide by the 3' to 5' exonuclease of Pol δ.

Supplementary Figure S9

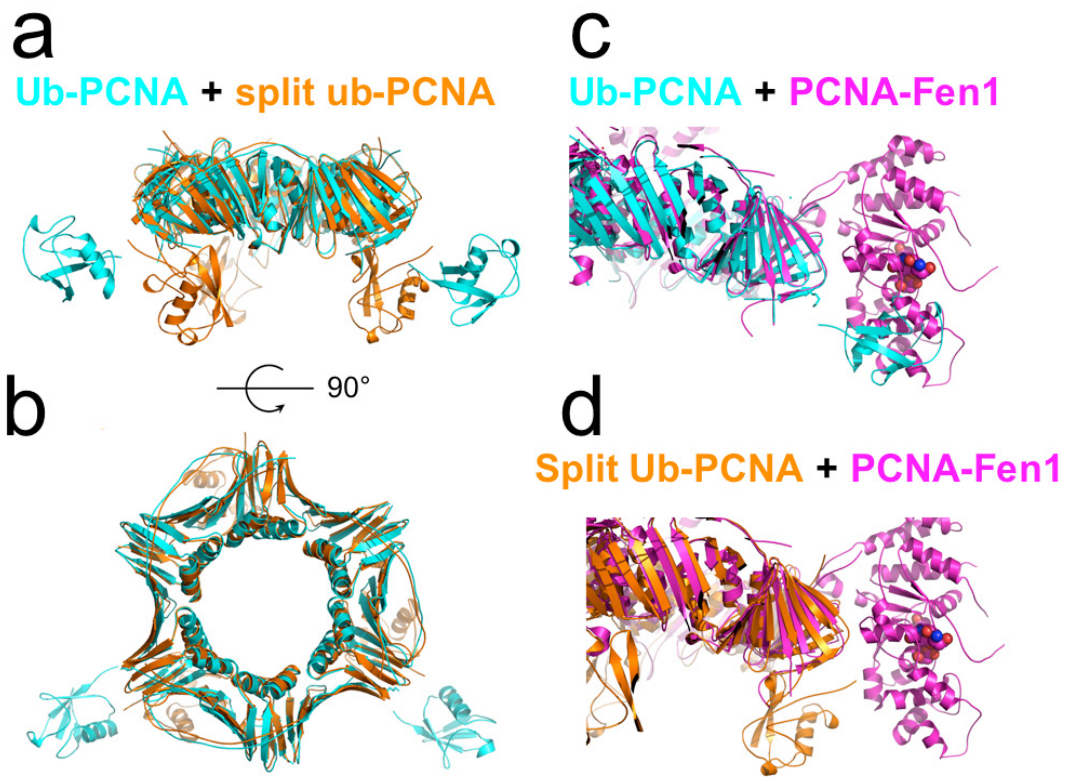

Figure S9. Alignments of the structures of ub-PCNA (cyan) and split ub-PCNA (3L10, orange) with the Fen1-PCNA complex. (**a, b)** View from two different angles of the difference between the spatial orientations of ubiquitin in ub-PCNA (cyan) and split ub-PCNA (orange). In contrast to the radial extension of ubiquitin in ub-PCNA, ubiquitin in split ub-PCNA is "tucked in" against the PCNA ring, burying its UBZ binding surface. This places the ubiquitin molecules directly under the plane of the PCNA trimer. (**c**) alignment of the ub-PCNA structure (cyan) with the PCNA-Fen1 structure (magenta). The ubiquitin molecule in ub-PCNA interferes with the conformational changes of Fen1 bound to PCNA. (**d**) alignment of the split ub-PCNA structure (orange) with the PCNA-Fen1 structure (magenta). Ubiquitin in split ub-PCNA does not sterically hinder potential conformational changes of Fen1.

Supplementary Table S1

Summary of crystallographic data and refinement statistics

Data collection	
Space group	$P4_32_12$
Cell dimensions	
a, *b*, *c* (Å)	161.05, 161.05, 97.36
α, β, γ (°)	90, 90, 90
Resolution (Å)	2.9 (2.95-2.90)
R_{sym} or R_{merge}	9% (76%)
$I / \sigma I$	48 (3.4)
Completeness (%)	99.8(100)
Redundancy	18 (16)
Refinement	
Resolution (Å)	40.6-2.9
No. reflections	28695
R_{work} / R_{free}	22.2%/29.2%
No. atoms	
Protein	6952
B-factors	
Protein	127
R.m.s. deviations	
Bond lengths (Å)	0.004
Bond angles (°)	1.033
Ramachandran (%)	
favored	91.7
allowed	8.3
outlier	0

Animation of an interpolation between the different conformations of Fen1 in the PCNA-Fen1 complex. The coordinate interpolation was performed by the Molecular Movement Database morph server at Yale University, and the movie was rendered with PyMol.