SUPPLEMENTARY INFORMATION

Supplementary Figure S1. TIP does not interact with mtPCNA monomers. Proteins (100 μg each) were separated on a Superdex-200 gel filtration column as described in "Materials and Methods". Aliquots (80 μl) from the indicated fractions were separated on 15% SDS-PAGE and stained with Coomassie brilliant blue (R-250). A. mtPCNA; B. mtPCNAm; C. TIP; D. mtPCNA and TIP; E, mtPCNAm and TIP.

Supplementary Figure S2. TIP affects the interactions of PCNA1 and PCNA2 with PolB. Proteins (100 μg each) were separated on a Superdex-200 gel filtration column as described in "Materials and Methods". Aliquots (80 μl) from the indicated fractions were separated on 15% SDS-PAGE and followed by Coomassie brilliant blue (R-250) staining. A. PCNA1; B. PCNA2; C. TIP; D, PolB; E, PCNA1 and PolB; F, PCNA1, PolB and TIP; G, PCNA2 and PolB; H, PCNA2, PolB and TIP; I, PolB and TIP.

Supplementary Figure S3. Total ion chromatogram of peptides obtained from PCNA1 (top) and PCNA2 (bottom) through on-line digestion platform. PCNA1 showed poor digestion efficiency and mainly yielded the undigested protein.

Supplementary Figure S4. Peptic peptides of native PCNA2 and TIP. An on-line digestion device provides 98% sequence coverage of PCNA2 (A) and 99% sequence coverage of TIP (B) (53).

Supplementary Figure S5. Differential HDX map of wild-type PCNA2 in which the regions having negative values of deuterium uptake difference (PCNA2 vs. TIP-PCNA2 complex) suggest that the protein conformation changes toward a more protected conformation upon the binding of TIP.

Supplementary Figure S6. Alignment of the residues on PCNA involved in TIP binding. Residues 187-204 in PCNA2 are aligned with the region in PCNA1 and mtPCNA proteins. Amino acids which are identical in two out of the three proteins are highlighted

in red.

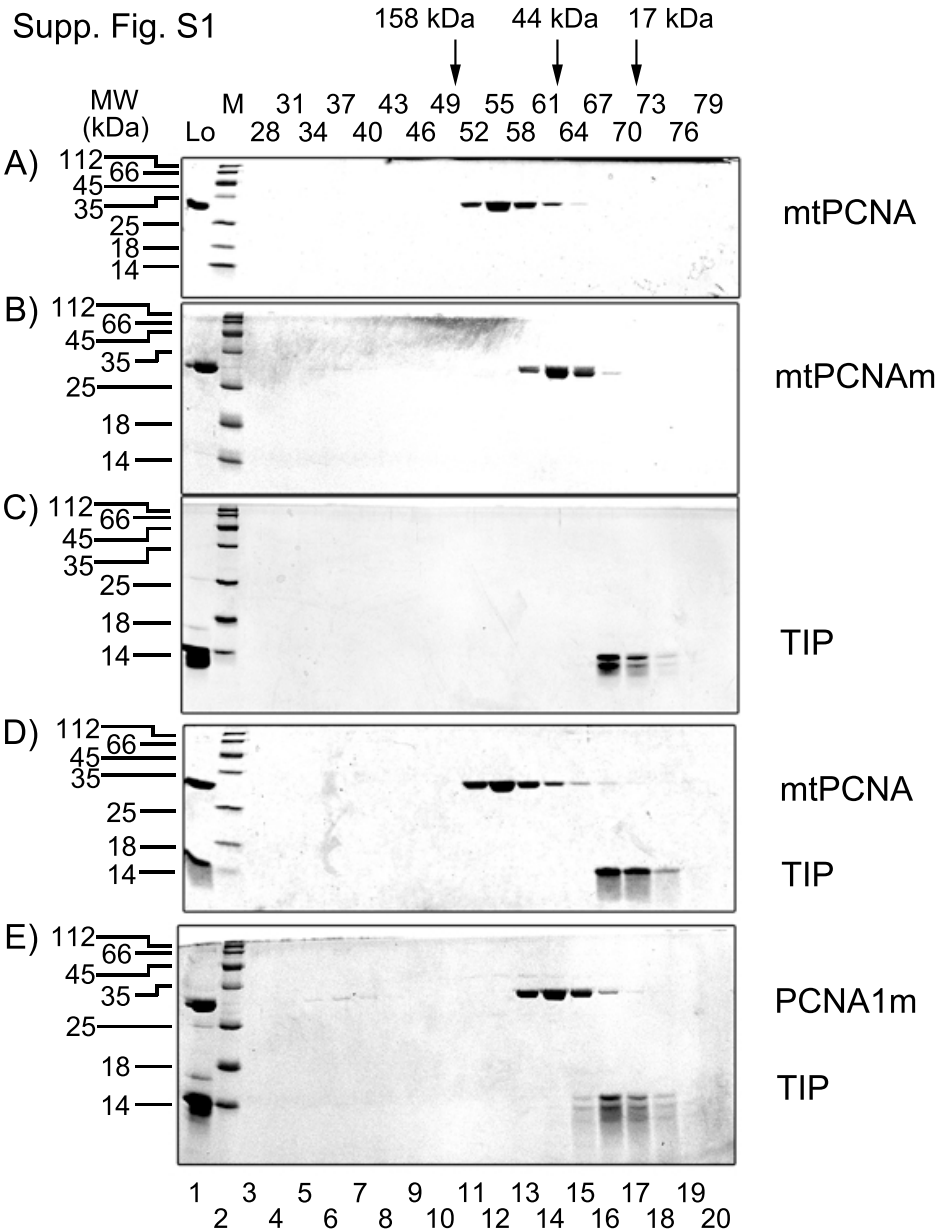
Supplementary Figure S7. TIP does not interact with PCNA2-A and PCNA2-E. Proteins (100 μg each) were separated on a Superdex-200 gel filtration column as described in "Materials and Methods". Aliquots (80 μl) from the indicated fractions were separated on 15% SDS-PAGE and stained with Coomassie brilliant blue (R-250). A. PCNA2-E; B. PCNA2-E and TIP; C. PCNA2-A; D. PCNA2-A and TIP.

Supplementary Figure S8. Differential HDX map of the PCNA2-A mutant in which the regions having negative values of deuterium uptake difference (PCNA2-A mutant vs. TIP-PCNA2-A mutant complex) suggest that the protein conformation changes toward a more protected conformation upon the binding of TIP.

Supplementary Figure S9. Differential HDX map of the PCNA2-E mutant in which the regions having negative values of deuterium uptake difference (PCNA2-E mutant vs. TIP-PCNA2-E mutant complex) suggest that the protein conformation changes toward a more protected conformation upon the binding of TIP.

Supplementary Figure S10. Differential HDX map of TIP in which the regions having negative values of deuterium uptake difference (TIP vs. TIP-PCNA2 complex) suggest that the protein conformation changes toward a more protected conformation upon the binding of native PCNA2 (A), PCNA2-A mutant (B), and PCNA2-E mutant (C).

Supp. Fig. S1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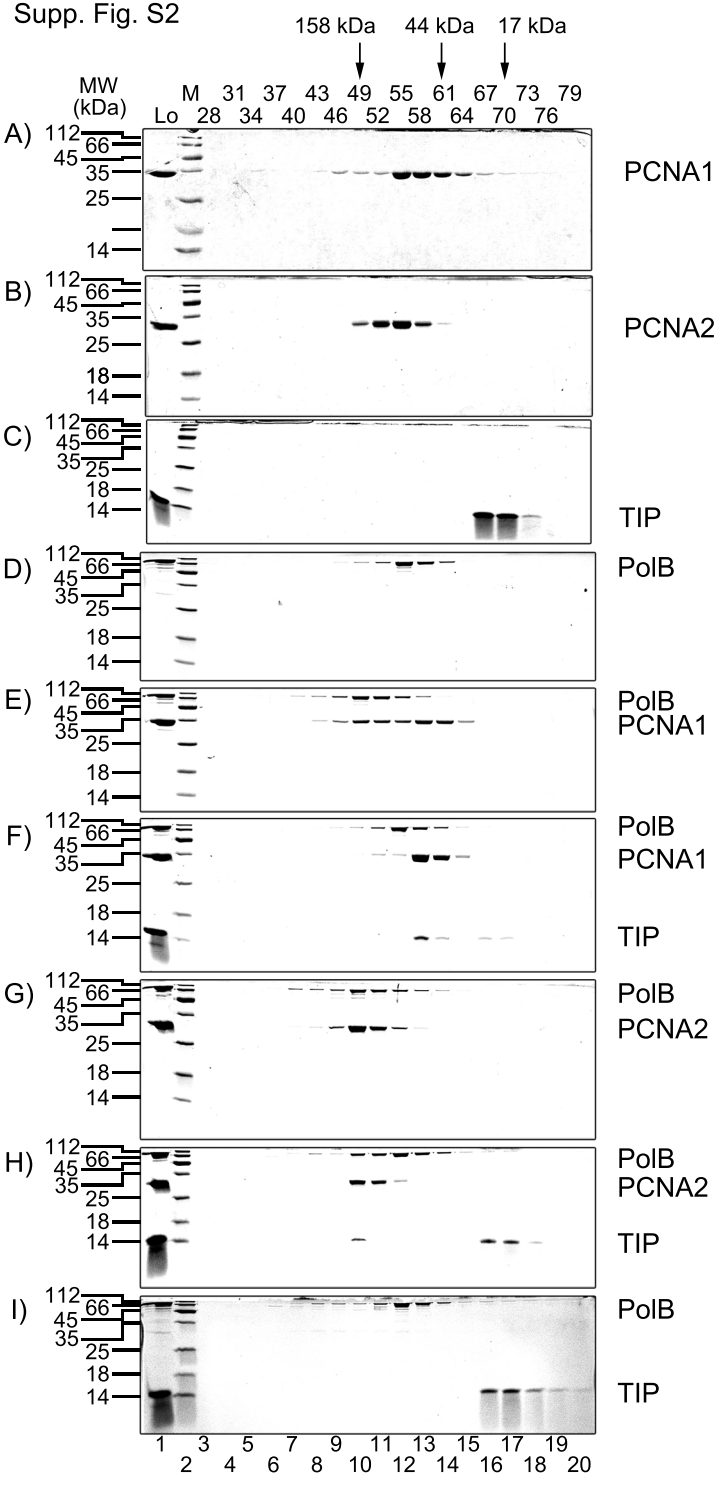
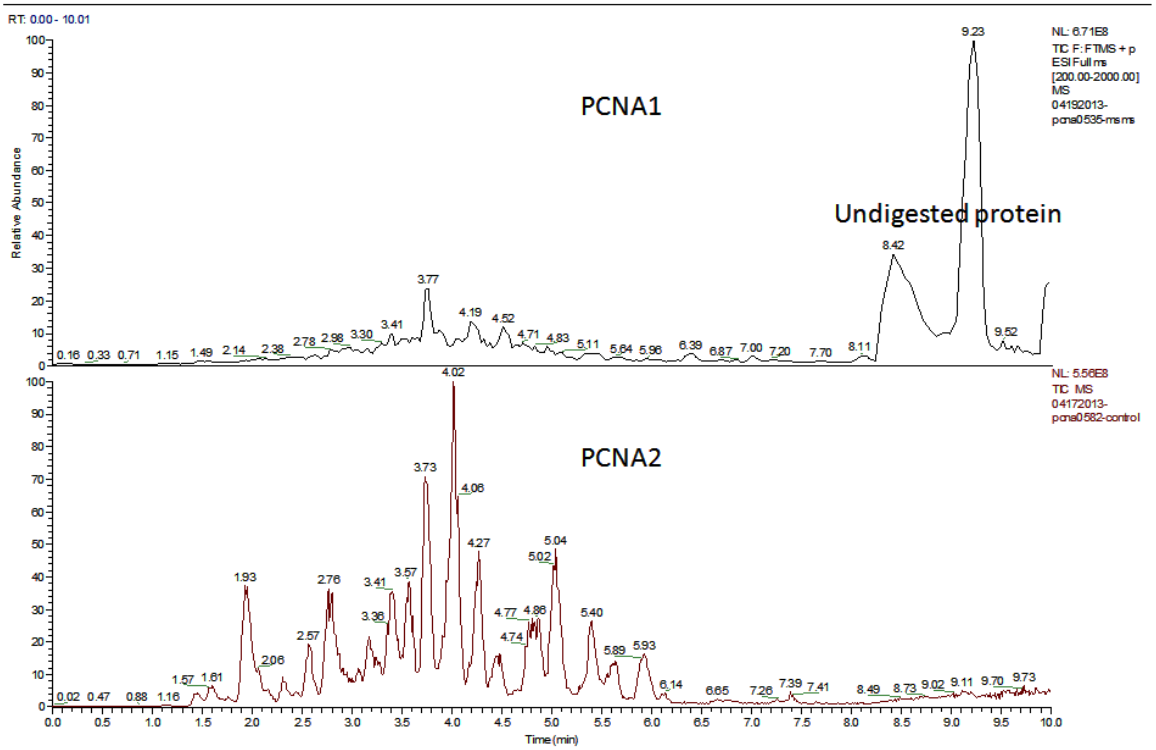
44 kDa
17 kDa
MW (kDa)
M
Lo
31 37 43 49 55 61 67 73 79
28 34 40 46 52 58 64 70 76
A)
B)
C)
D)
E)
112
66
45
35
25
18
14
mtPCNA
mtPCNAm
TIP
mtPCNA
TIP
PCNA1m
TIP
1 3 5 7 9 11 13 15 17 19
2 4 6 8 10 12 14 16 18 20

Supp. Fig. S2


158 kDa
44 kDa
17 kDa
MW (kDa)
M 31 37 43 49 55 61 67 73 79
Lo 28 34 40 46 52 58 64 70 76
A)
B)
C)
D)
E)
F)
G)
H)
I)
112
66
45
35
25
18
14
PCNA1
PCNA2
TIP
PolB
PolB
PCNA1
PolB
PCNA1
TIP
PolB
PCNA2
PolB
PCNA2
TIP
PolB
TIP
1 3 5 7 9 11 13 15 17 19
2 4 6 8 10 12 14 16 18 20

Supp. Fig. S3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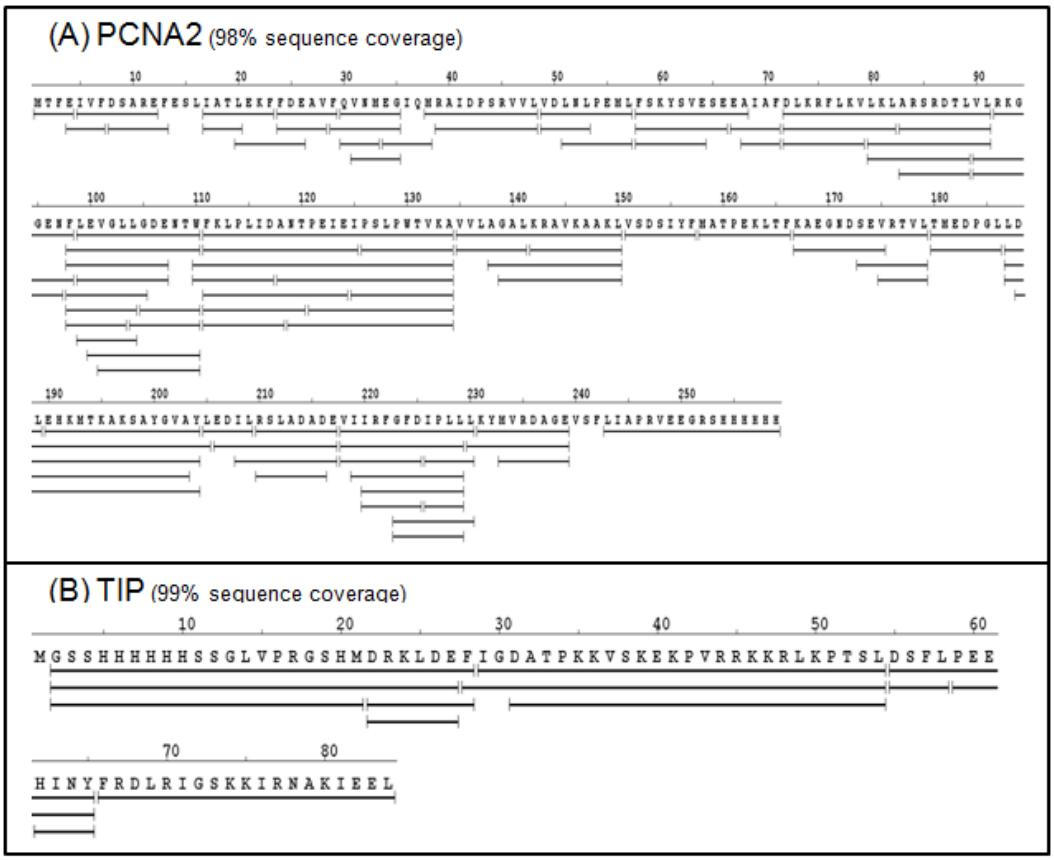
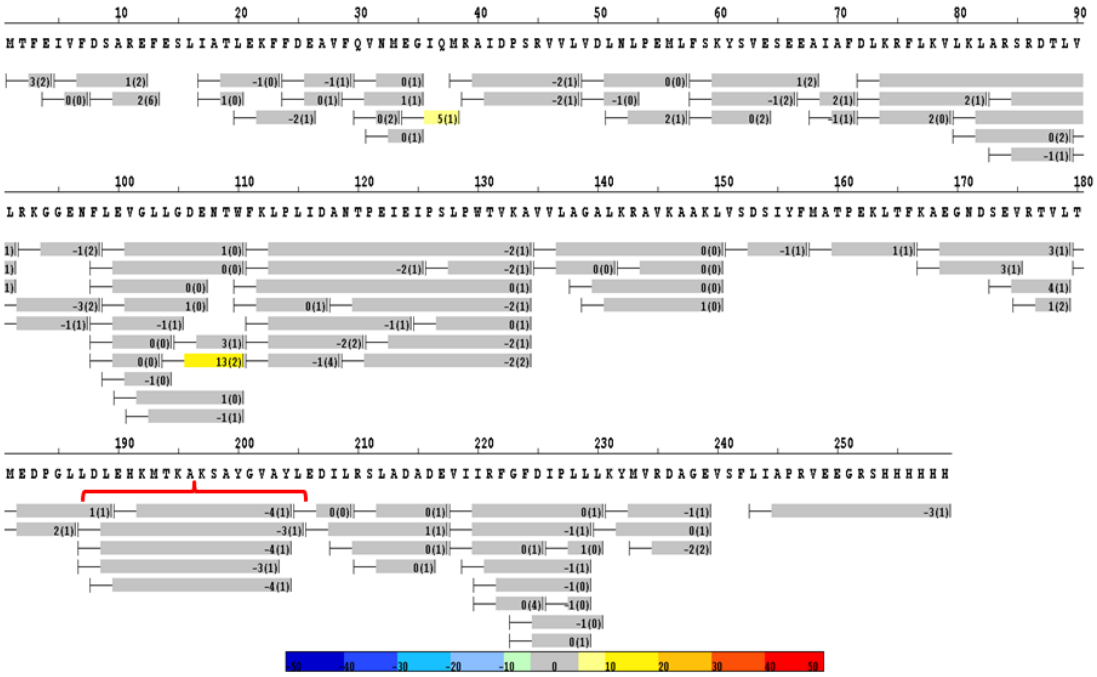
PCNA1
Undigested protein
NL: 6.71E8
TIC F: FTMS + p ESI Full ms [200.00-2000.00] MS 04192013-pcna0535-msms
Relative Abundance
PCNA2
NL: 5.56E8
TIC MS 04172013-pcna0582-control
Time (min)

Supp. Fig. S4


(A) PCNA2 (98% sequence coverage)
(B) TIP (99% sequence coverage)
MGSSHHHHHHSSGLVPRGSHMDRKLDEFIGDATPKKVSKEKPVRRKKRLKPTSLDSFLPEE
HINYFRDLRIGSKKIRNAKIEEL

Supp. Fig. S5

Native PCNA2_HDX Protection (Ligand: TIP)

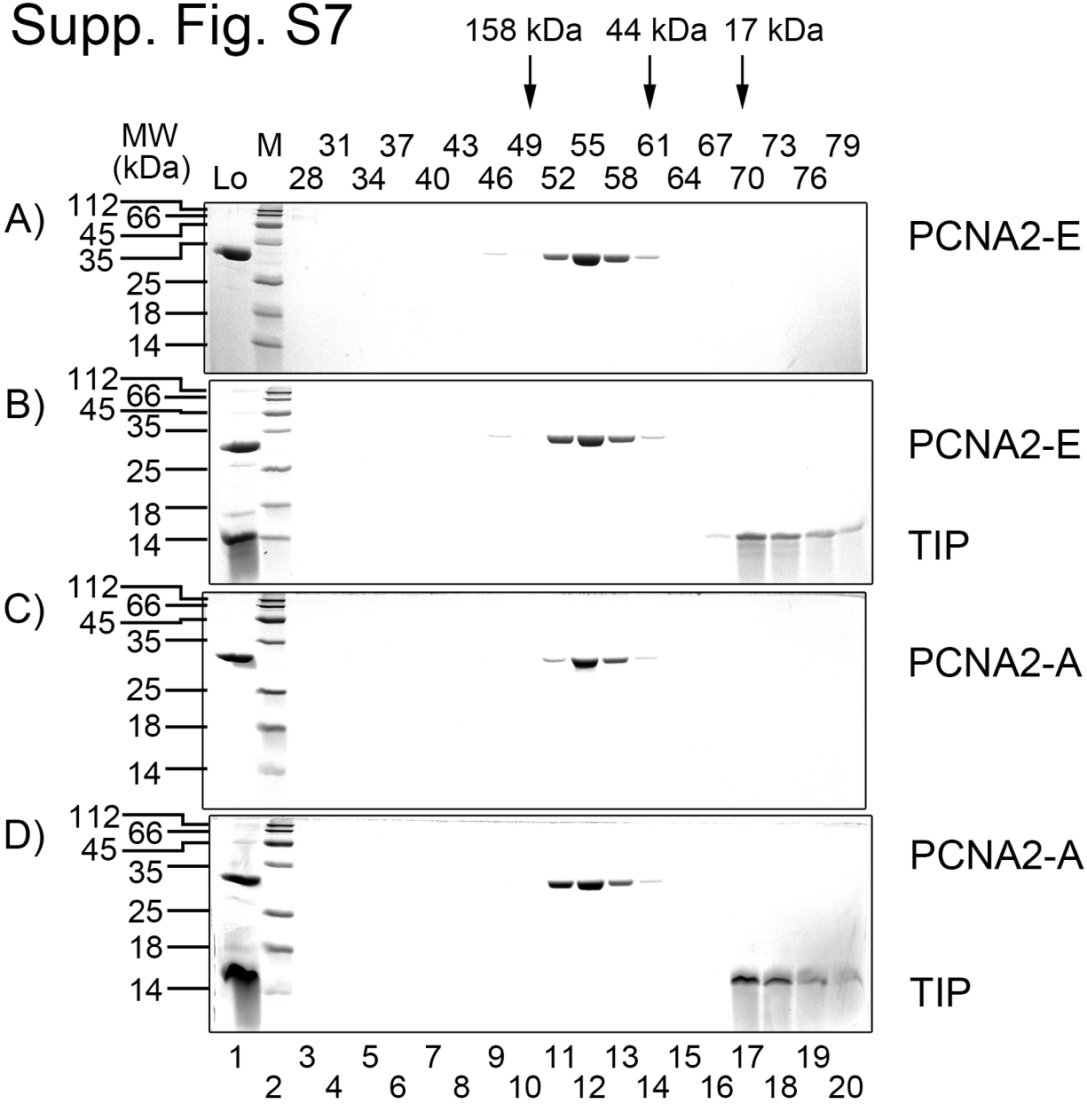


Deuterium uptake difference (%)

Supp. Fig. S6

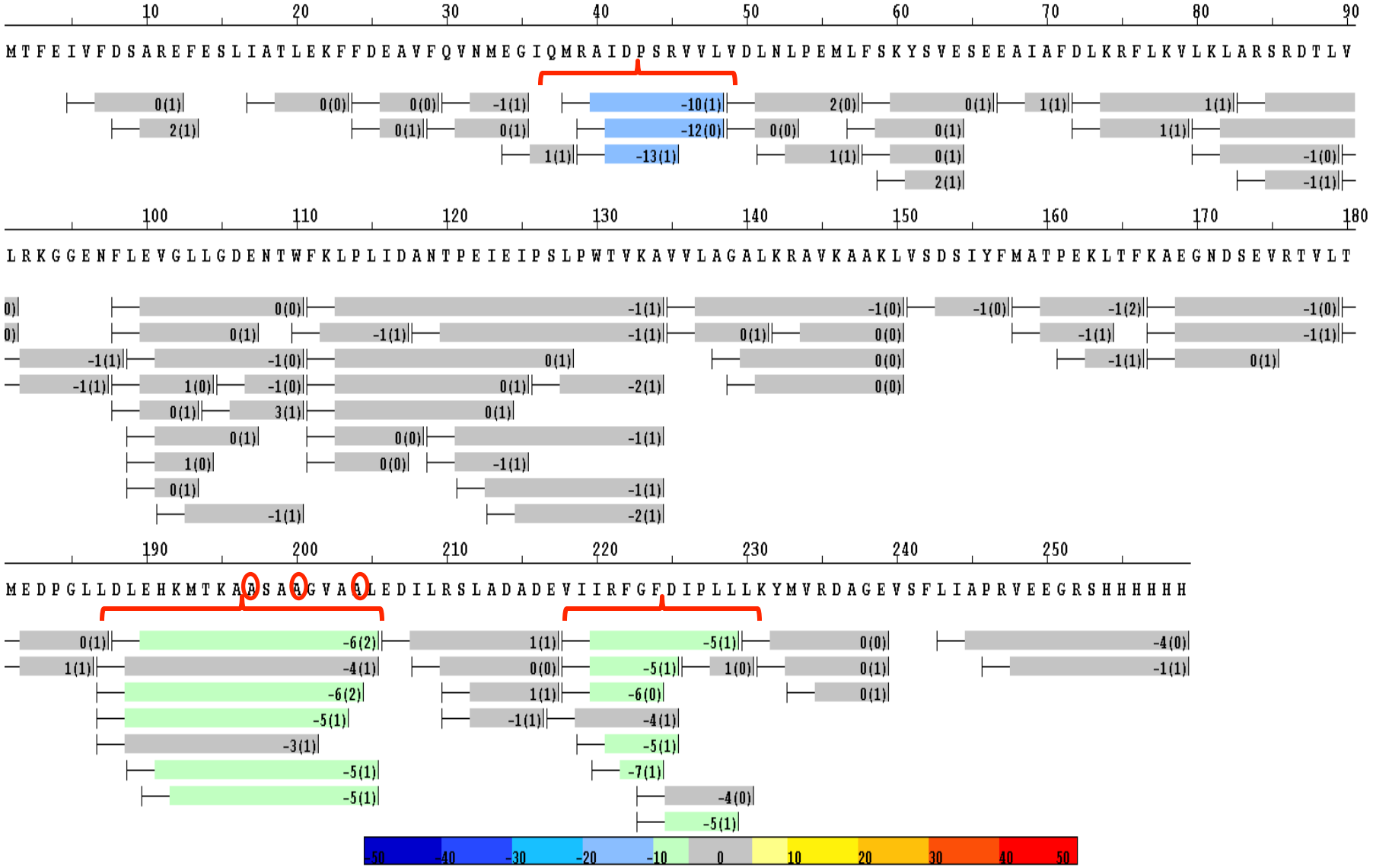
PCNA1	**187**	**LDLEVE-EETKSAYGIRY**	**203**
PCNA2	**187**	**LDLEHKMTKAKSAYGVAY**	**204**
mtPCNA	**178**	**LHGERIDKPARSIYSLDK**	**195**

Supp. Fig. S7


158 kDa
44 kDa
17 kDa
MW (kDa)
M
Lo
31 37 43 49 55 61 67 73 79
28 34 40 46 52 58 64 70 76
A)
B)
C)
D)
112
66
45
35
25
18
14
PCNA2-E
PCNA2-E
TIP
PCNA2-A
PCNA2-A
TIP
1 3 5 7 9 11 13 15 17 19
2 4 6 8 10 12 14 16 18 20

PCNA2-A_HDX Protection (Ligand: TIP)

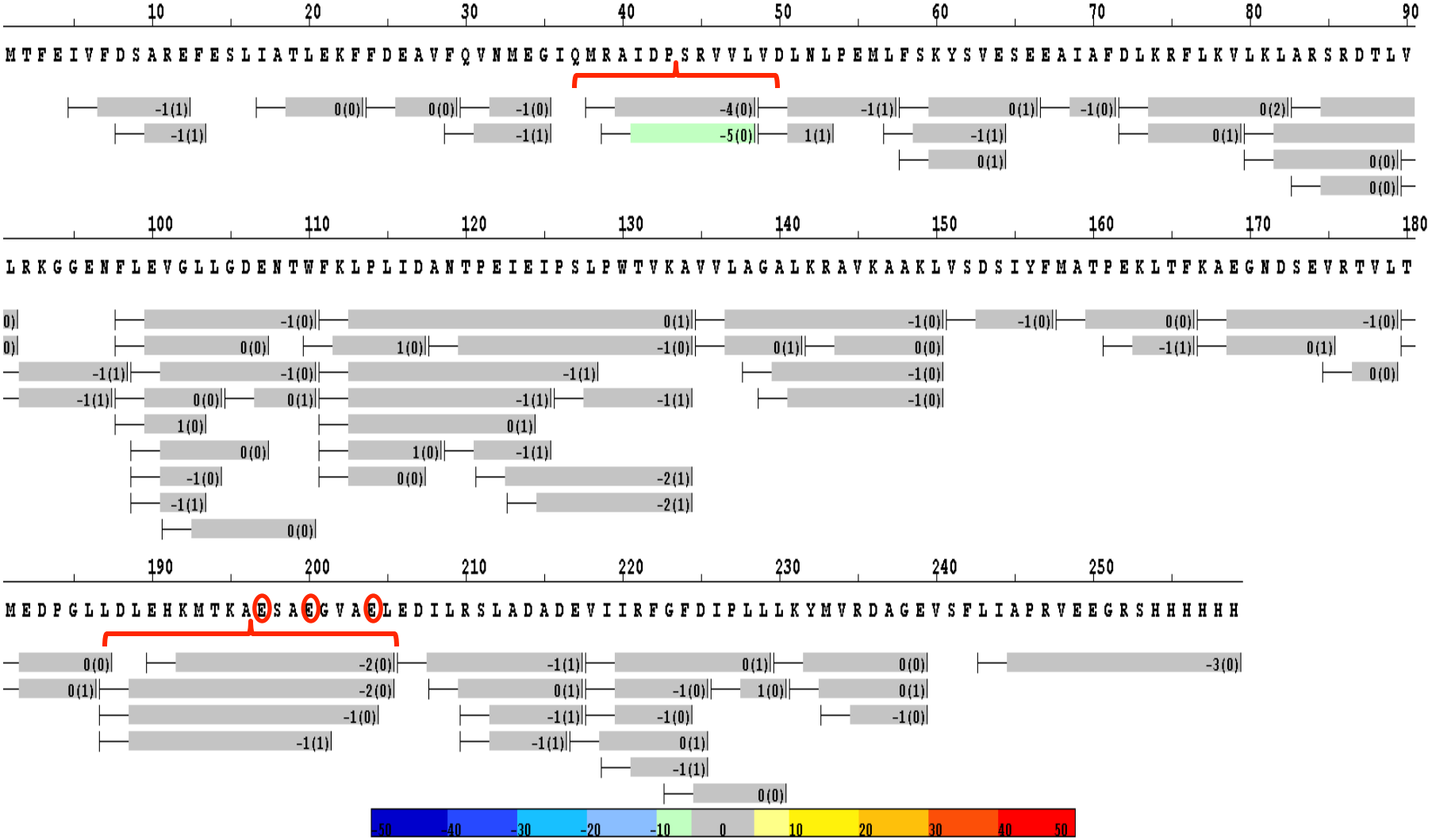
Supp. Fig. S8



Deuterium uptake difference (%)

PCNA2-E_HDX Protection (Ligand: TIP)

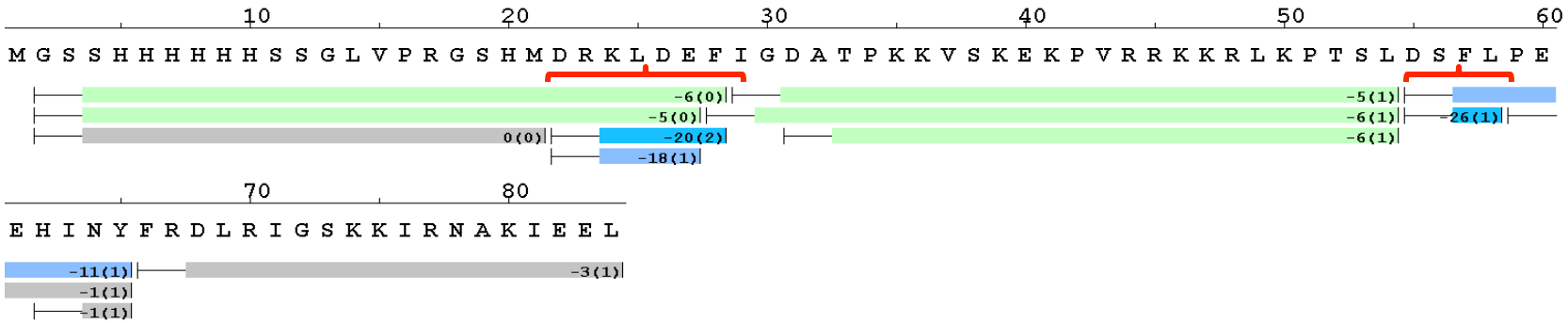
Supp. Fig. S9



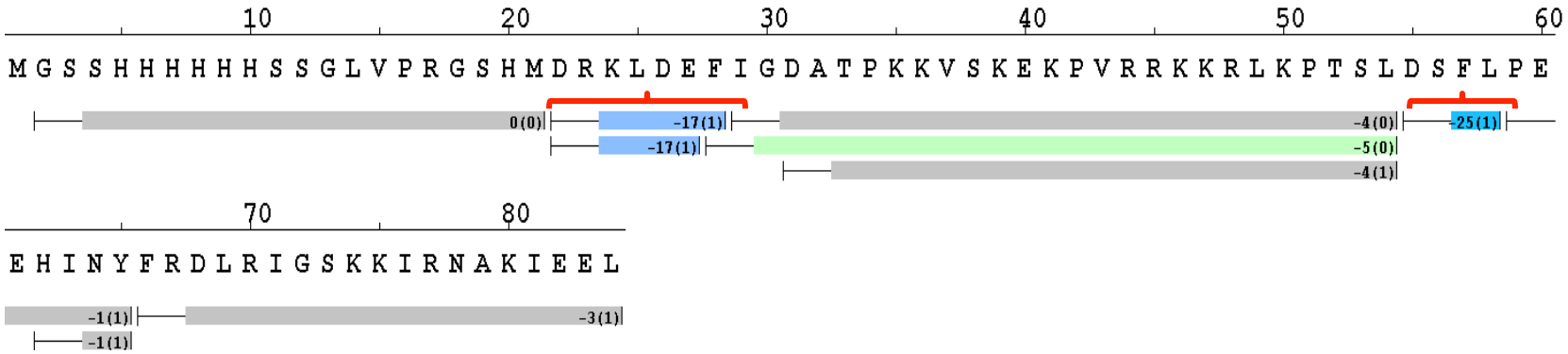
Deuterium uptake difference (%)

Supp. Fig. S10

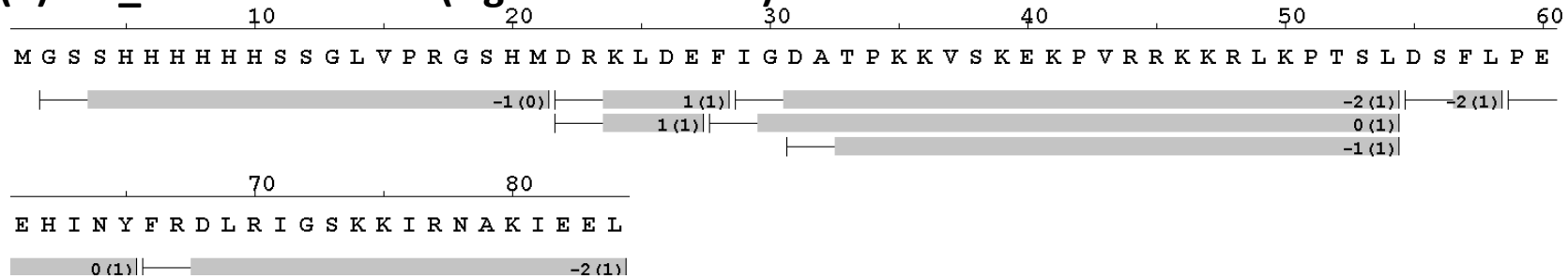
(A) TIP_HDX Protection (Ligand: Native PCNA2)

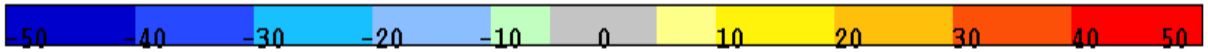


(B) TIP_HDX Protection (Ligand: PCNA2-A)



(C) TIP_HDX Protection (Ligand: PCNA2-E)





Deuterium uptake difference (%)

Supplementary Table S1. Oligonucleotides used for cloning and mutagenesis

Name	Used to generate	Sequence
Zhuo_0498	PCNA2-A	5'-AGATGACAAAGGCGGCCAGTGCGGCCGGGGTTGCAGCCCTC-3'
Zhuo_0499	PCNA2-A	5'-GAGGGCTGCAACCCCGGCCGCACTGGCCGCCTTTGTCATCT-3'
Zhuo_0500	PCNA2-E	5'-AGATGACAAAGGCGGAAAGTGCGGAAGGGGTTGCAGAACTCGAGGACATC-3'
Zhuo_0501	PCNA2-E	5'-GATGTCCTCGAGTTCTGCAACCCCTTCCGCACTTTCCGCCTTTGTCATCT-3'
Zhuo_0395	Fen1	5'-ATGCCATATGGGAGTCCAGATAGGTGAGC-3'
Zhuo_0396	Fen1	5'-AATTGTCGACCTATCGACCGAACCAGCTCTC-3'
Zhuo_0277	TIP	5'-ATGCCATATGGACAGGAAGCTCGACGAG-3'
Zhuo_0278	TIP	5'-GTACGTCGACTTATAACTCCTCGATTTTCGCG-3'
Zhuo_0502	TK0535 E143K	5'-CCTCGGTGAGGTTCTCAAGAAGGGCATAAAGGACGCTT-3'
Zhuo_0503	TK0535 E143K	5'-AAGCGTCCTTTATGCCCTTCTTGAGAACCTCACCGAGG-3'
Zhuo_0504	TK0535 V175D-I177K	5'-GCGAGACCAACGAGGACGAGAAGAGGCTTACCCTTGAGGA-3'
Zhuo_0505	TK0535 V175D-I177K	5'-TCCTCAAGGGTAAGCCTCTTCTCGTCCTCGTTGGTCTCGC-3'

Supplementary Table S2. P values obtained from paired t-test of five HDX time points of PCNA2 vs. PCNA2+TIP.

PCNA2 vs. PCNA2+TIP

PCNA2

Peptide	34-38	39-48	104-110	187-204	218-119
P value	<0.01	0.04	<0.01	0.01	0.05

PCNA2-A

Peptide	34-38	39-48	104-110	187-204	218-119
P value	0.19	<0.01	<0.01	<0.01	<0.01

PCNA2-E

Peptide	30-35	39-48	101-110	187-204	218-119
P value	0.06	<0.01	0.97	0.06	0.07