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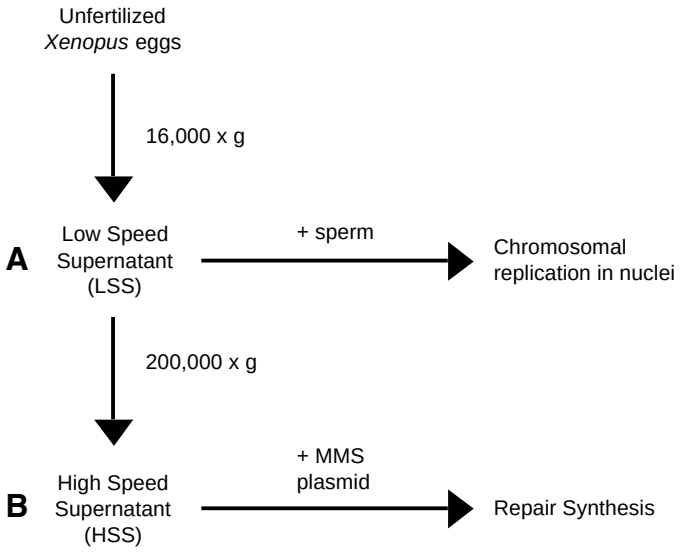

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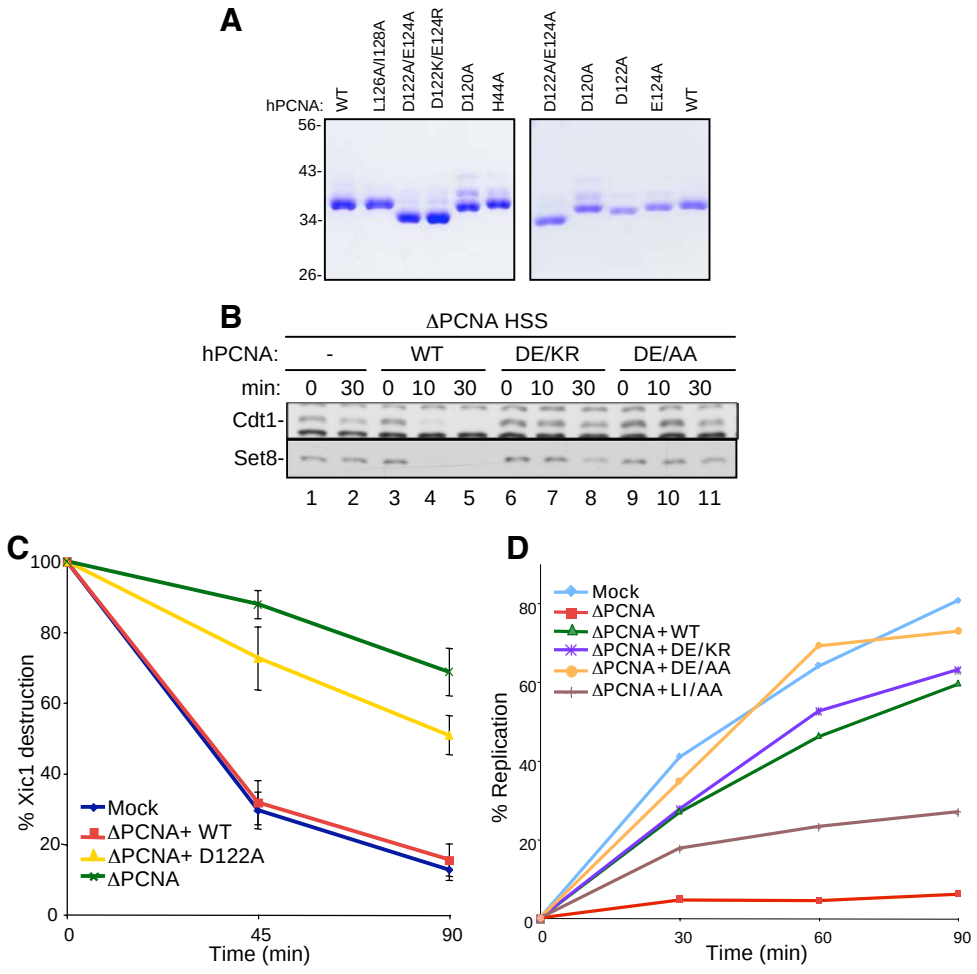
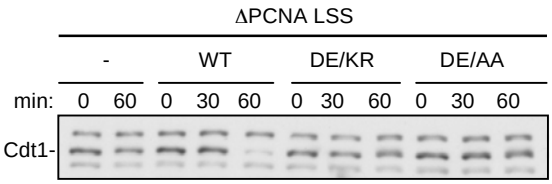


Havens_SFig3

A



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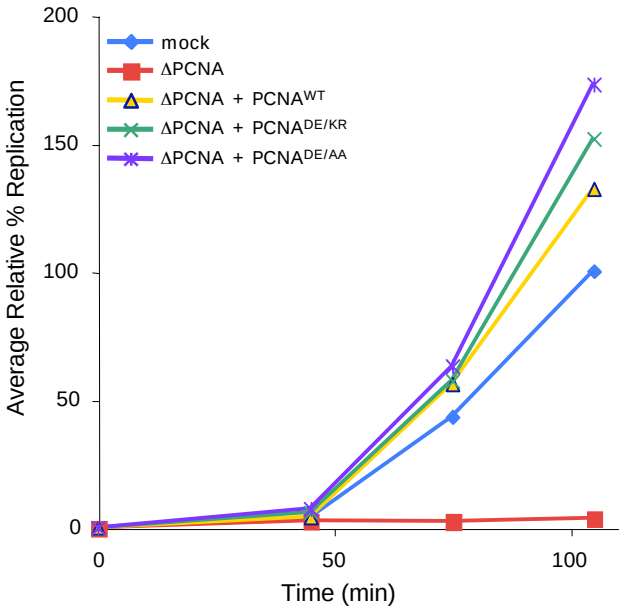

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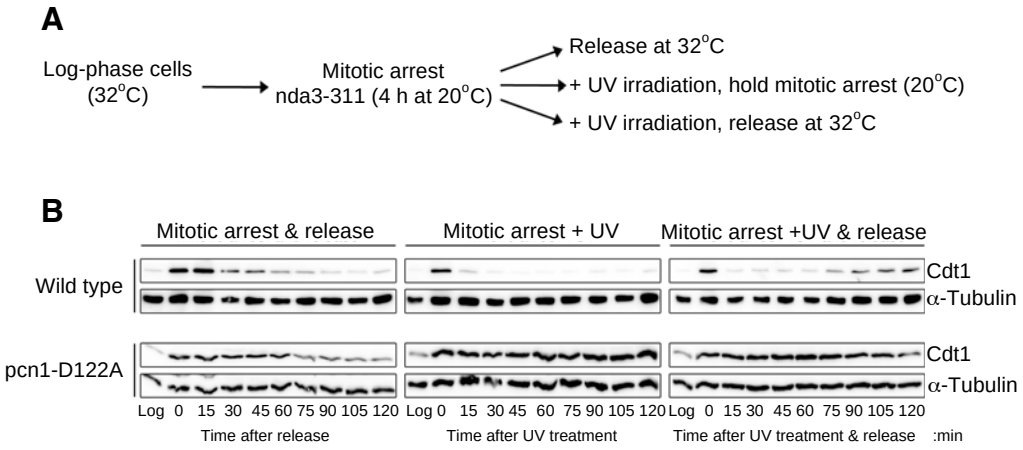

Supplemental Figure Legends:

Figure S1. Two experimental systems to induce CRL4^{Cdt2}-mediated destruction in Xenopus egg extracts.

A) For replication-dependent destruction, Low Speed Supernatant (LSS) is supplemented with sperm chromatin.

B) For DNA damage-induced destruction, High Speed Supernatant (HSS) is supplemented with DNA that has been damaged with methyl methane sulfonate.

Figure S2. Acidic residues in the PCNA interdomain connector loop are important for CRL4^{Cdt2}-mediated destruction, but dispensable for DNA synthesis.

A) Coomassie stained gels of purified human PCNA mutants. Note that mutation of the D122 and E124 to alanine causes the protein to run faster, likely due to the change in charge.

B) HSS was supplemented with MMS plasmid, human Set8^{WT} protein and either buffer, PCNA^{WT}, PCNA$^{DE/KR}$, or PCNA$^{DE/AA}$.

C) A graph showing the percentage of Xic1 protein remaining after MMS plasmid, in vitro translated ^{35}S labeled Xic1, and either buffer, PCNA^{WT} or PCNA^{D122A} were added to PCNA-depleted HSS for the indicated times. The amount of Xic1 remaining was measured by autoradiography.

D) HSS was mock-depleted or PCNA-depleted and supplemented with either PCNA^{WT}, PCNA$^{DE/KR}$ (PCNA$^{D122K/E124R}$), PCNA$^{DE/AA}$ (PCNA$^{D122A/E124A}$), or PCNA$^{LI/AA}$ (PCNA$^{L126A/I128A}$). 10 ng/μl single stranded M13 DNA and [α-^{32}P]dATP were added and

leading strand DNA synthesis was measured. Data from one representative experiment is shown.

Figure S3. Identification of PCNA residues that are essential for $CRL4^{Cdt2}$-mediated destruction, but not for chromosomal DNA replication.

A) PCNA-depleted LSS was supplemented with sperm chromatin, and either buffer, $PCNA^{WT}$, $PCNA^{DE/KR}$, or $PCNA^{DE/AA}$. Reactions were stopped at the indicated times and blotted for Cdt1.

B) PCNA-depleted LSS was supplemented with sperm chromatin, [α-^{32}P]dATP and either buffer, $PCNA^{WT}$, $PCNA^{DE/KR}$, or $PCNA^{DE/AA}$. Chromosomal DNA replication was measured and graphed.

Figure S4. The D122 residue of PCNA is required for $CRL4^{Cdt2}$-mediated destruction of Cdt1 in fission yeast cells released from a mitotic block.

A) Scheme of experiment.

B) Cdt1-TAP levels in cell extracts from wild type (2069) and *pcn1-D122A* mutant (2640) following the experimental procedure shown in *A*.

Supplementary table 1: List of yeast strains used in this work.

Yeast strains used		
1540	*h- cdt1-TAP::KanR*	Lab stock
2069	*h+ ade6-216 leu1-32 ura4-D18 cdt1-TAP::KanR nda3-311*	Lab stock
2640	*h+ ade6-216 leu1-32 ura4-D18 cdt1-TAP::KanR nda3-311 pcn1-D122A::HygR*	This study
2660	*h- cdt1-TAP::KanR pcn1-D122A::HygR*	This study

Supplementary table 2: List of primers used in this work for yeast strain construction.

Primer	***Sequence 5'-3'***	***Use***
974	AAAATGCATTCATTCTTATTTTCATAC	Forward primer to construct *NsiI-fragment* for *pcn1-D122A* mutation
975	CAAGTGTTCTTGGGCAATG	Reverse primer to construct *NsiI-fragment* for *pcn1-D122A* mutation
976	CATTGCCCAAGAACACTTG	Forward primer to construct *BamHI-fragment* for *pcn1-D122A* mutation
977	AAAGGATCCTCCATTTTATACTCC	Reverse primer to construct *BamHI-fragment* for *pcn1-D122A* mutation
978	ATGCTTGAAGCTAGATTTCAGCAG	DNA sequencing
619	CCGGCTCGTATGTTGTGTGGAATTGTG	Forward primer to check yeast integration
979	TTTGGCGCGCCGATTCCGTAATCTACTCCTCATCCTCCT	Reverse primer to check yeast integration