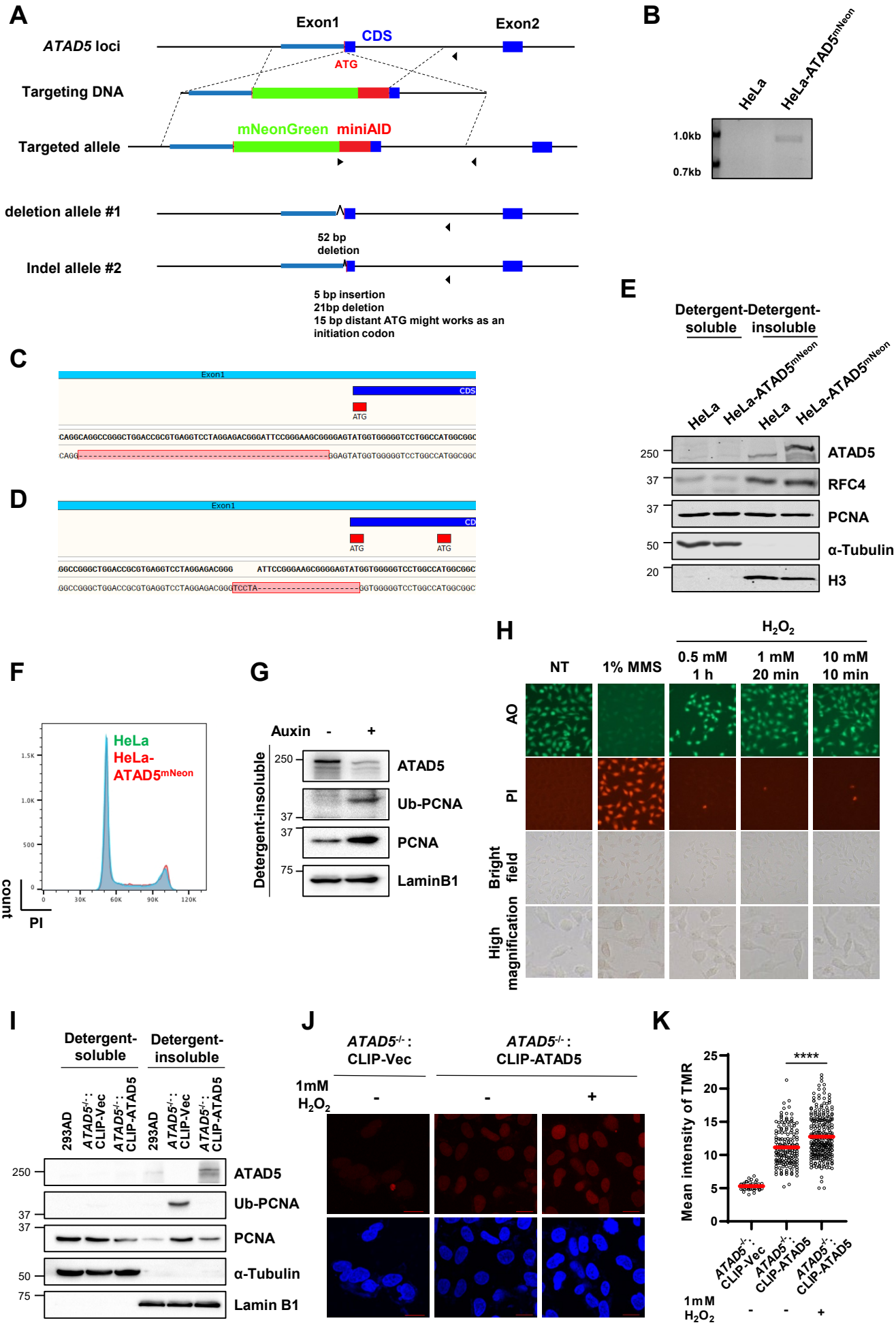
Supplementary Information

Timely termination of repair DNA synthesis by ATAD5 is important in oxidative DNA damage-induced single-strand break repair

Park et al.

Supplementary Figures

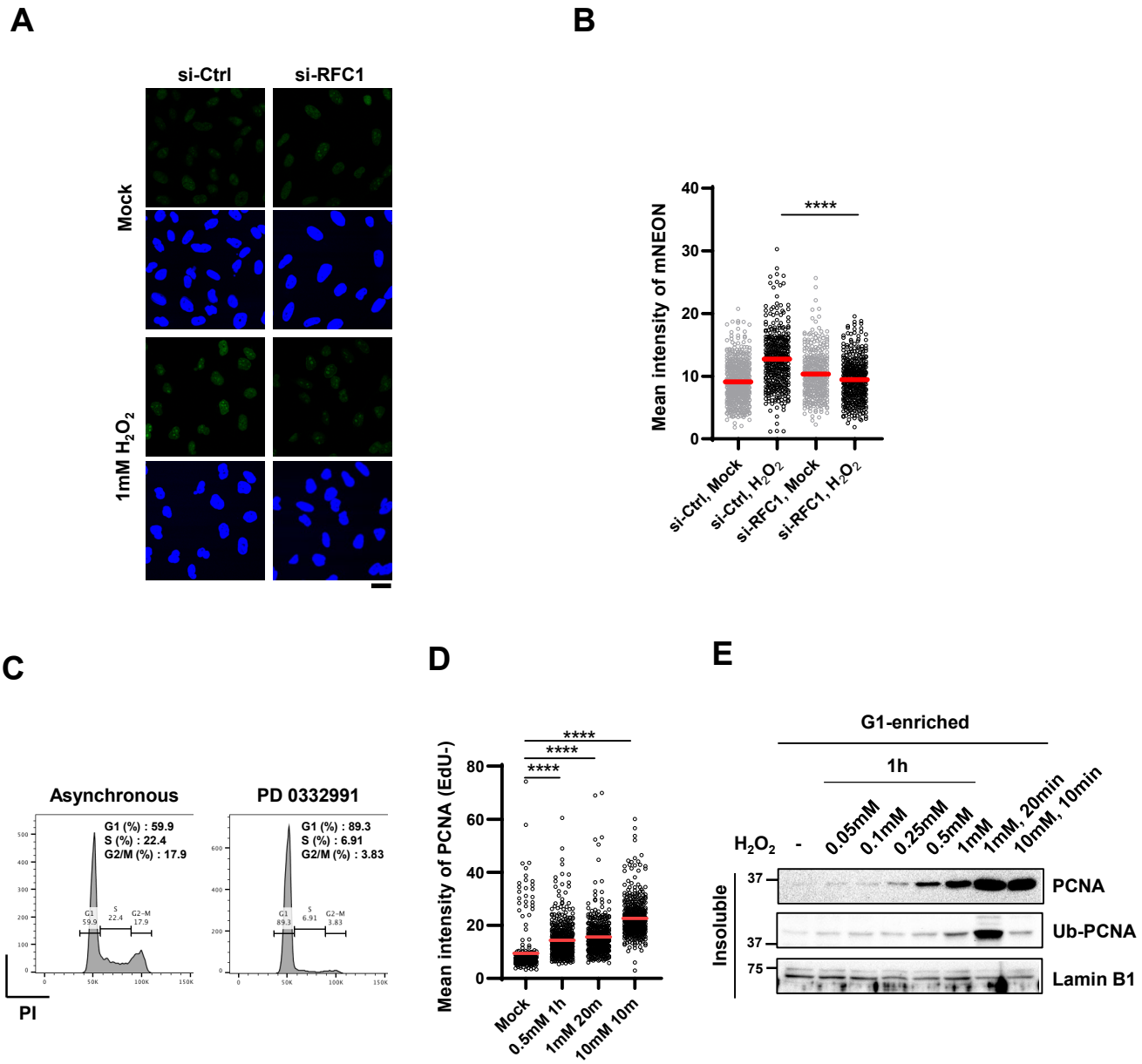
Supplementary Figure 1



Supplementary Figure 1. ATAD5 accumulates on chromatin upon oxidative DNA damage.

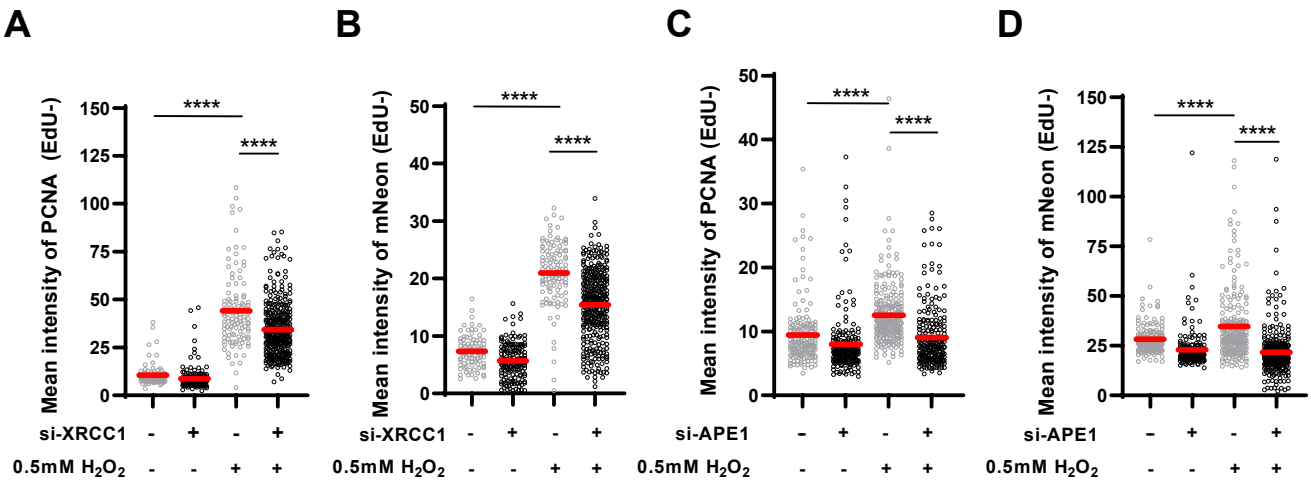
(A) Schematic diagram showing the generation of a HeLa cell line expressing an endogenously mNeonGreen and auxin-inducible-degron (AID)-tagged ATAD5 (HeLa-ATAD5$^{mNeonGreen-AID}$). The targeted allele and the other two alleles with deletions and indels, respectively, were shown. The filled triangles represent the PCR primers used for genomic PCR. (B) Genomic DNA extracted from HeLa or HeLa-ATAD5$^{mNeonGreen-AID}$ cells were subjected to PCR assay using the primers indicated in (A). (C, D) The sequence information of the deletion allele #1 (C) and the indel allele #2 (D) was displayed. (E) Detergent-soluble and detergent-insoluble proteins were fractionated from HeLa or HeLa-ATAD5$^{mNeonGreen-AID}$ cells and subjected to immunoblotting. (F) HeLa or HeLa-ATAD5$^{mNeonGreen-AID}$ cells were fixed and cellular DNA content was stained with propidium iodide (PI) and measured by flow cytometry. (G) HeLa-ATAD5$^{mNeonGreen-AID}$ cells were treated with auxin for 10 h, and then detergent-insoluble proteins were fractionated and subjected to immunoblotting. (H) HeLa-ATAD5$^{mNeonGreen-AID}$ cells were treated with drugs as indicated and stained with acridine orange (AO) for live cells and propidium iodide (PI) for dead cells. (I) HEK293AD, HEK293AD-*ATAD5*$^{-/-}$: CLIP vector (Vec), and HEK293AD-*ATAD5*$^{-/-}$: CLIP-ATAD5 cells were fractionated and subjected to immunoblotting. (J, K) HEK293AD-*ATAD5*$^{-/-}$: CLIP vector (Vec) and HEK293AD-*ATAD5*$^{-/-}$: CLIP-ATAD5 cells were incubated with benzylcytosine-tetramethylrhodamine (TMR) for 1 h, treated with 1mM H_2O_2 for 20 min, and fixed after pre-extraction with CSK buffer for microscope analysis. (J) Representative images are shown. Scale bar 20 μm. (K) Quantification of TMR signals. Three independent experiments were performed and one representative result is displayed. Red bar indicates mean value. Statistical analysis: two-tailed unpaired Student's *t*-test; ****P < 0.001.

Supplementary Figure 2



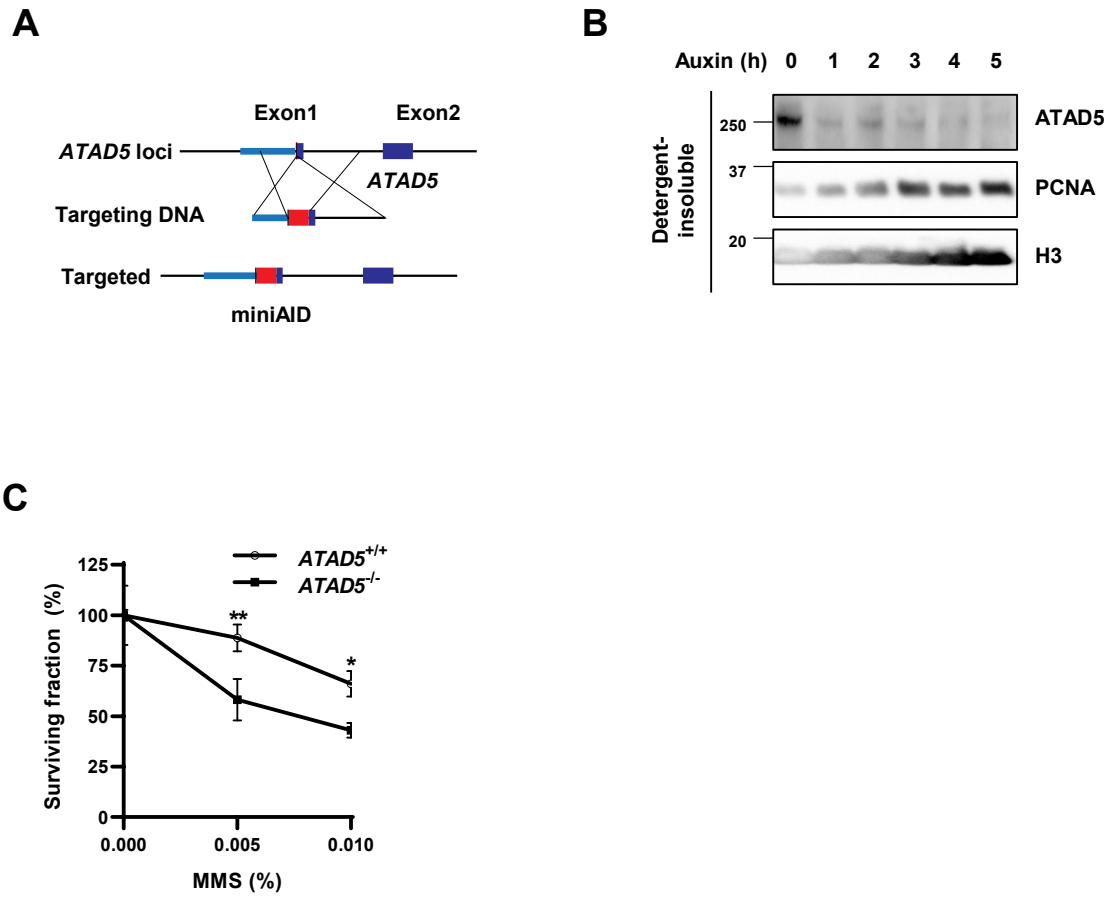
Supplementary Figure 2. PCNA accumulates on chromatin upon oxidative DNA damage.

(A, B) HeLa-ATAD5$^{mNeonGreen\text{-}AID}$ cells were transfected with small interfering RNAs (siRNAs) as indicated for 48 h. Cells were treated with 1 mM H_2O_2 for 20 min, detergent-pre-extracted and then fixed for detection of mNeonGreen signal. (A) Representative images are shown. Scale bar 20 μm. (B) Quantification of mNeonGreen signals. Three independent experiments were performed and one representative result is displayed. Red bar indicates mean value. Statistical analysis: two-tailed unpaired Student's *t*-test; ****P < 0.001. (C) U2OS-ATAD5^{AID} cells were treated with PD 0332991 for 24 h. After that, cellular DNA content was stained with propidium iodide (PI) and measured by flow cytometry. (D) HeLa-ATAD5$^{mNeonGreen\text{-}AID}$ cells were incubated with EdU for 1 h before detergent-pre-extraction. Cells were treated with H_2O_2 as indicated, detergent-pre-extracted, and fixed for EdU-click reactions. PCNA signals were quantified in strong EdU-signal-negative cells. Red bar indicates mean value. Statistical analysis: two-tailed unpaired Student's *t*-test; ****P < 0.001. (E) U2OS-ATAD5^{AID} cells enriched at G1 by treatment with PD 0332991 and PHA-767491 were treated with H_2O_2 as indicated, and detergent-insoluble proteins were fractionated for immunoblotting.



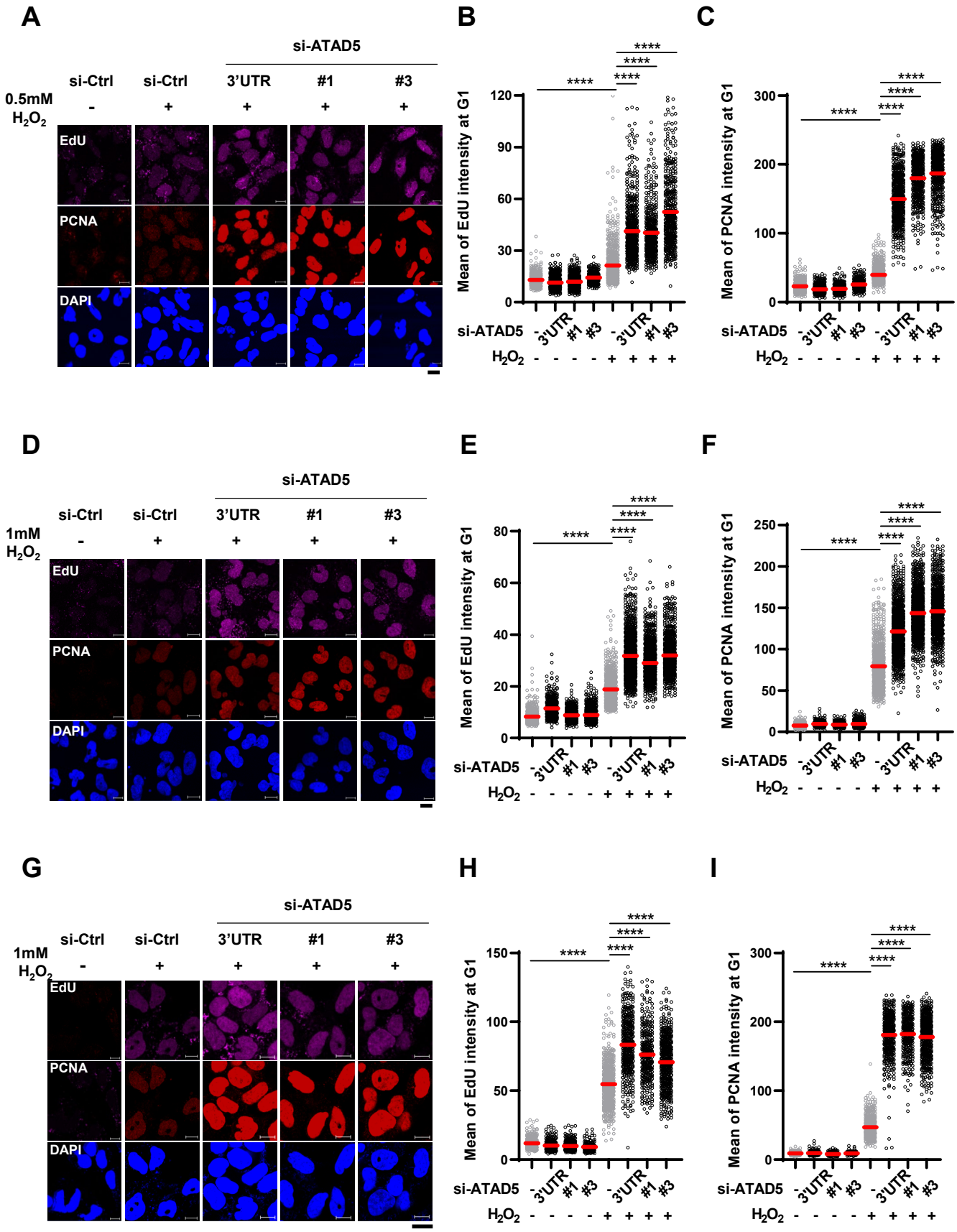
Supplementary Figure 3. Accumulation of PCNA and ATAD5 upon oxidative DNA damage depends on 3′-terminal-processing enzymes.

(A-D) HeLa-ATAD5$^{mNeonGreen\text{-}AID}$ cells were transfected with siRNAs as indicated and incubated for 48 h before 0.5 mM H_2O_2 treatment for 1 h. Cells were incubated with EdU for 30 min before detergent-pre-extraction. After H_2O_2 treatment, cells were detergent-pre-extracted and fixed for EdU-click reactions and PCNA immunostaining. PCNA (A, C) and mNeonGreen (B, D) signal intensity was quantified. Quantification was performed in strong-EdU-signal-negative cells. Four (A, B) or two (C, D) independent experiments were performed and one representative result is displayed. Red bar indicates mean value. Statistical analysis: two-tailed unpaired Student's *t*-test; ****P < 0.001.



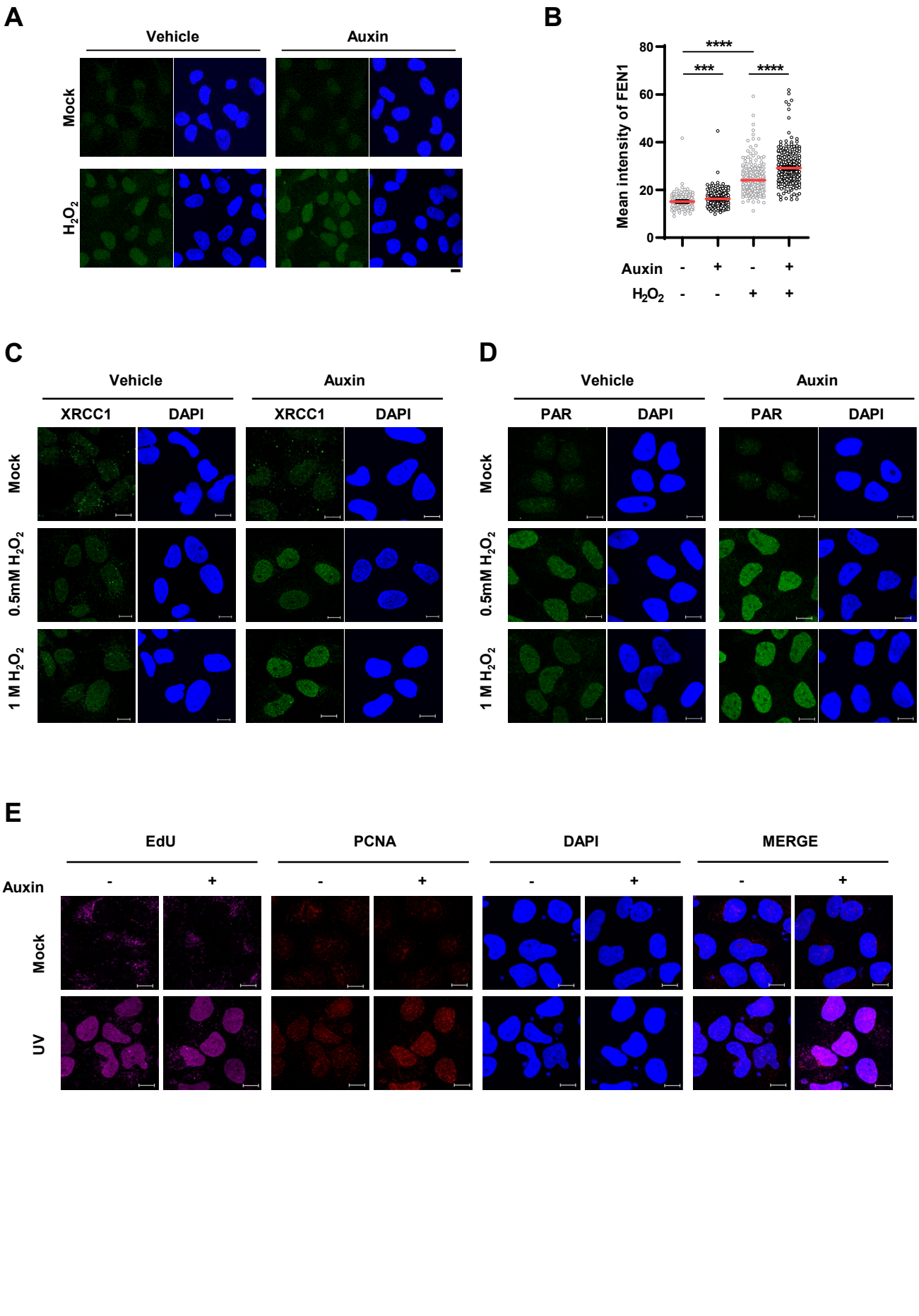
Supplementary Figure 4. ATAD5-depleted cells are sensitive to MMS in a clonogenic survival assay

(A) Schematic diagram showing the generation of a HeLa cell line expressing an endogenously auxin-inducible-degron (AID)-tagged ATAD5 (HeLa-ATAD5^{AID}). The *AID* gene was inserted between the ATG start codon and the second codon of the endogenous *ATAD5* gene. (B) HeLa-ATAD5^{AID} cells were treated for the indicated times with auxin, and detergent-insoluble proteins were subjected to immunoblotting. (C) The wild-type or *ATAD5* knockout U2OS cells were treated with methyl methanesulfonate (MMS) for 1 h and subjected to clonogenic survival assay.


A
si-ATAD5
si-Ctrl
si-Ctrl
3'UTR
#1
#3
0.5mM H2O2
-
+
+
+
+
EdU
PCNA
DAPI
B
Mean of EdU intensity at G1
si-ATAD5
H2O2
C
Mean of PCNA intensity at G1
si-ATAD5
H2O2
D
si-ATAD5
si-Ctrl
si-Ctrl
3'UTR
#1
#3
1mM H2O2
EdU
PCNA
DAPI
E
Mean of EdU intensity at G1
si-ATAD5
H2O2
F
Mean of PCNA intensity at G1
si-ATAD5
H2O2
G
si-ATAD5
si-Ctrl
si-Ctrl
3'UTR
#1
#3
1mM H2O2
EdU
PCNA
DAPI
H
Mean of EdU intensity at G1
si-ATAD5
H2O2
I
Mean of PCNA intensity at G1
si-ATAD5
H2O2

Supplementary Figure 5. Unscheduled DNA synthesis is increased in ATAD5-depleted cells.

(A-I) U2OS cells (A-F) or HeLa cells (G-I) transfected with *ATAD5* siRNA were enriched at the G1 phase by releasing cells from nocodazole arrest; cells were treated with 0.5 mM H_2O_2 for 1 h (A-C) or 1 mM H_2O_2 for 20 min (D-I) with EdU incorporation, detergent-pre-extracted, and fixed for EdU-click reaction and immunostaining. UDS was measured based on EdU signal incorporation. (A, D, G) Representative images of immunostained cells are shown. Scale bar 10 μm. (B, C, E, F, H, I) The mean signal intensity of EdU (B, E, H) and PCNA (C, F, I) was quantified. Three independent experiments were performed and one representative result is displayed. Red bar indicates mean value. Statistical analysis: two-tailed unpaired Student's *t*-test; ****P < 0.001.


A
Vehicle
Auxin
Mock
H2O2
B
Mean intensity of FEN1
80
60
40
20
0
****
***
****
Auxin - + - +
H2O2 - - + +
C
Vehicle
Auxin
XRCC1
DAPI
XRCC1
DAPI
Mock
0.5mM H2O2
1 M H2O2
D
Vehicle
Auxin
PAR
DAPI
PAR
DAPI
Mock
0.5mM H2O2
1 M H2O2
E
EdU
PCNA
DAPI
MERGE
Auxin
- + - + - + - +
Mock
UV

Supplementary Figure 6. Unscheduled DNA synthesis is not increased upon UV irradiation in ATAD5-depleted cells.

(A, B) U2OS-ATAD5^{AID} cells G1-enriched by treatment with PD 0332991 and PHA-767491 were treated with 0.5 mM H_2O_2 for 1 h, pre-extracted, and fixed for immunostaining with an anti-FEN1 antibody. (A) Representative images of FEN1. (B) Quantification of FEN1 signal. Red bar indicates mean value. Statistical analysis: two-tailed unpaired Student's *t*-test; ****P < 0.001, ***P < 0.005. (C) Representative images of Figure 6I. (D) Representative images of Figure 6J. (E) Representative images of Figure 6K and L. (A, C, D, E) Scale bar 10 μm.