SUPPLEMENTALY DATA

Conditional Degradation of SDE2 by the Arg/N-End Rule Pathway Regulates Stress Response at Replication Forks

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SUPPLEMENTARY MATERIALS AND METHODS

EdU staining and flow cytometry

G1/S-arrested cells were incubated with 10 μM EdU (5-ethynyl-2´-deoxyuridine) (ThermoFisher) for 30 min before release into fresh medium. Harvested cells were fixed with 4 % paraformaldehyde at RT for 15 min, permeabilized by saponin-based permeabilization buffer (ThermoFisher) for 15 min, and subjected to EdU-click reaction using Alexa Fluor 488 picolyl azide and click-iT Plus EdU flow cytometry assay kit (ThermoFisher) according to the manufacturer's protocol. Cells were washed once and resuspended with 200 μg/mL PureLink™ RNase A and eBioscience™ 7-AAD viability staining solution (ThermoFisher). After 30 min incubation at 37 °C, cells were analyzed with the Attune NxT acoustic focusing cytometer and the Attune NxT software v2.7 (ThermoFisher).

Fluorescence microscopy for GFP-Pol η foci detection

Cells were reverse-transfected onto coverslips with GFP-Pol η, and after 24 h, UVC irradiated with 40 J/m^2. Where indicated, cells were pre-incubated with 10 μM EdU for 30 min to mark S-phase cells. After 2 h recovery time, coverslips were fixed for 10 min at 4 °C using 4% paraformaldehyde. Cells were then permeabilized using PBS/0.3% Triton X-100 for 3 min at 4 °C. After quickly blocking coverslips with 1% BSA, EdU-click reaction was performed with Alexa Fluor 647 picolyl azide using Click-iT Plus EdU Imaging kits (ThermoFisher) according to the manufacturer's protocol. After another 1 % BSA blocking step, cells were incubated with 1:300 anti-GFP (B-2) for 2 h at RT and, after washing with PBS, with 1:1000 Alexa Fluor 488 goat anti-mouse IgG for 45 min in 1 % BSA. After washing again with PBS, coverslips were mounted using DAPI-containing mounting medium (Vector Lab) and analyzed with either the Leica TCS SP8 X confocal microscope or the Nikon Eclipse Ts2R inverted microscope. For GFP epifluorescence, MSCV-transduced cells were fixed with 4% paraformaldehyde for 10 min

at 4 °C, washed three times with PBS and mounted with DAPI-containing mounting medium (Vector Lab).

RT-qPCR

RNA was isolated using TRIzol (Invitrogen) and chloroform (Sigma). cDNA synthesis was performed using a high-capacity cDNA reverse transcription kit (Applied Biosystems) according to the manufacturer's protocols. Real-time quantitative PCR was performed using Fast SYBR Green Master Mix (Applied Biosystems) and a StepOnePlus Real-Time PCR system (Applied Biosystems). GAPDH mRNA levels were used as a control for normalization. The following primers were used for cDNA amplification: ETAA1 forward 5′-TCCTTGTACTCCCAGTGTAG-3′; ETAA1 reverse 5′-TTGAATCACATCTAGCTCTTCC-3′.

Chromatin extraction

Cells were lysed using lysis (S) buffer (10 mM HEPES pH 7.4, 10 mM KCl, 0.05% NP-40) for 20 min on ice. After centrifugation at 14,000 rpm for 10 min, nuclei were lysed with low salt buffer (10 mM Tris-HCl pH 7.5, 0.2 mM $MgCl_2$ + 1% Triton X-100) for 15 min on ice. After centrifugation at 14,000 rpm for 10 min, the pellet containing chromatin were resuspended with chromatin buffer 0.2 N HCl and incubated for 20 min on ice. The supernatant was neutralized with 1 M Tris-HCl pH 8.0.

CRISPR/Cas9 gene editing

A pair of oligos containing the human *UBR1* sgRNA targeting sequence were designed from crispr.mit.edu. The forward oligo sequence is 5′-CACCGTGAGAGGATGGAAATCAGCG-3′ and the reverse oligo sequence is 5′-AAACCGCTGATTTCCATCCTCTCAC-3′. Annealed oligos were cloned into pSpCas9(BB)-2A-Puro (pX459; a gift from Feng Zhang, Addgene plasmid #48139 (Ran et al., 2013)) and transfected into U2OS cells using GeneJuice (Millipore). Control

cells were transfected with a pX459 empty vector. Twenty-four hours after transfection, cells were selected with 2 μg/mL puromycin. After recovery from selection, cells were seeded onto 96-well plates, in medium without puromycin, for clonal selection. Selected clones were subsequently analyzed by Western blotting, using anti-UBR1 antibody, to confirm successful knockout.

3D modeling

All molecular modeling was done in UCSF Chimera, production version 1.12 (build 41623) (Pettersen et al., 2004). The receptor protein was generated by removing the ligand from PDB 3NY3, a structure of the UBR-box of UBR2 interacting with the N-degron Arg-Ile-Phe-Ser, solved by X-ray diffraction. For docking, a .pdb file of the receptor protein was uploaded to the online docking server ClusPro 2.0 and run in Peptide Mode. The peptide sequence KGGFG (searched using motif KXG[FY]G) was docked using default settings, resulting in 129 results. From those, all models that did not have hydrogen bonds between the ligand and either D150 or D153 of the receptor were eliminated, reducing the number of potential models to 12. These residues were determined to be important based on the hydrogen bonds made between them and the native ligand in the source file (3NY3). Each model was then manually checked for positional similarity to the native ligand of 3NY3, with only 4 models that reasonably aligned to the position of the native ligand. Next, solvent-excluded molecular surface maps of the receptor and the ligands were generated within Chimera using the MSMS package, and model 001.048 was chosen as the final model based on best fit into the receptor pocket. This model was from the pool of results generated using the algorithm for biasing toward van der Waals and electrostatic interactions. Additionally, model 001.048 had the best hydrogen-bonding network, making two bonds from the lysine to D153 and one to D150, while the other models only had one hydrogen bond to each residue or fewer. For the final model, the receptor without any ligands was charged and energy-minimized and the chosen ligand then added. Hydrogen bonds

were calculated through Chimera using the default settings. To calculate the electrostatic surface potential of the receptor protein, the PDB2PQR and energy minimization tools within Chimera were used with default settings to add hydrogens and assign charges and atomic radii, using the forcefields AMBER ff14SB with the AM1-BCC option for non-standard residues. APBS was used from within Chimera with default settings to generate a .dx potential file. This file was used to color the MSMS surface map according to electrostatic potential.

Supplementary Table S1. siRNA oligo information used in this study

Gene	Sequence	Manufacturer	Catalog #
Control	CAGGGTATCGACGATTACAAA	Qiagen	SI03650318
ETAA1	GCAUGUCAUCAAUUAGAUAtt	Ambion	s29018
NPL4	AACAGCCtCCTCCAACAAATC	Qiagen	Custom
p97-1	AAGATGGATCtCATTGACCTA	Qiagen	SI03019681
p97-2	AACAGCCATTCtCAAACAGAA	Qiagen	SI03019730
p97-3	AAACAGAUCCUAGCCCUUA	Dharmacon	D-008727-06-0005
SDE2-1	AAACGGCAATGGCCTACTAAA	Qiagen	SI03121503
TOPBP1	GGAUAUAUCUUUGCGGUUUtt	Ambion	s21823
UBR1-1	AAGCAGGAGGAAAGTGTACAA	Qiagen	Custom
UBR1-2	ATGGAAATCAGCGCGGAGTTA	Qiagen	Custom

Supplementary Table S2. Antibody information used in this study

Antigen	Manufacturer	Catalog Number
BrdU (BU1/75 ICR1) for CldU	Abcam	Cat# ab6326
BrdU (B44) for IdU	BD	Cat# 347580
C1orf55 (SDE2)	Sigma Atlas	Cat# HPA031255
CDT1	Cell Signaling	Cat# 3386
CHK1 (G-4)	Santa Cruz	Cat# sc-8408
pCHK1 (S317)	Cell Signaling	Cat# 2344
pCHK1 (S345)	Cell Signaling	Cat# 2341
Flag M2	Sigma	Cat# F1804
GFP (B-2)	Santa Cruz	Cat# sc-9996
GFP (JL-8)	Clontech	Cat# 632381
GFP	Abcam	Cat# Ab6556
HA (6E2)	Cell Signaling	Cat# 2367
Histone H4	Millipore	Cat# 07-108
HSC70	Santa Cruz	Cat# sc-7298
MCM6 (H-8)	Santa Cruz	Cat# sc-393618
NPL4	Bethyl	Cat# A304-102A
p97	Cell Signaling	Cat# 2648
PCNA (PC-10)	Santa Cruz	Cat# sc-56
RAD18 (3H7)	Abcam	Cat# ab57447
RPA32	Millipore	Cat# NA19L
pRPA32 (Ser33)	Bethyl	Cat# A300-246A
pSDE2 (T319)	Custom from Genscript	Antigen: TETEE(pT)QEKKAC
TOPBP1	Bethyl	Cat# A300-111A
Tubulin	Bethyl	Cat# A302-631A
Ubiquitin (P4D1)	Cell Signaling	Cat# 3936
UBR1	Bethyl	Cat# A302-988A
UBR2	Bethyl	Cat# A305-416A
UBXN7	Bethyl	Cat# A303-865A
UFD1	Bethyl	Cat# A301-875A

Supplementary Table S3. Chemical information used in this study

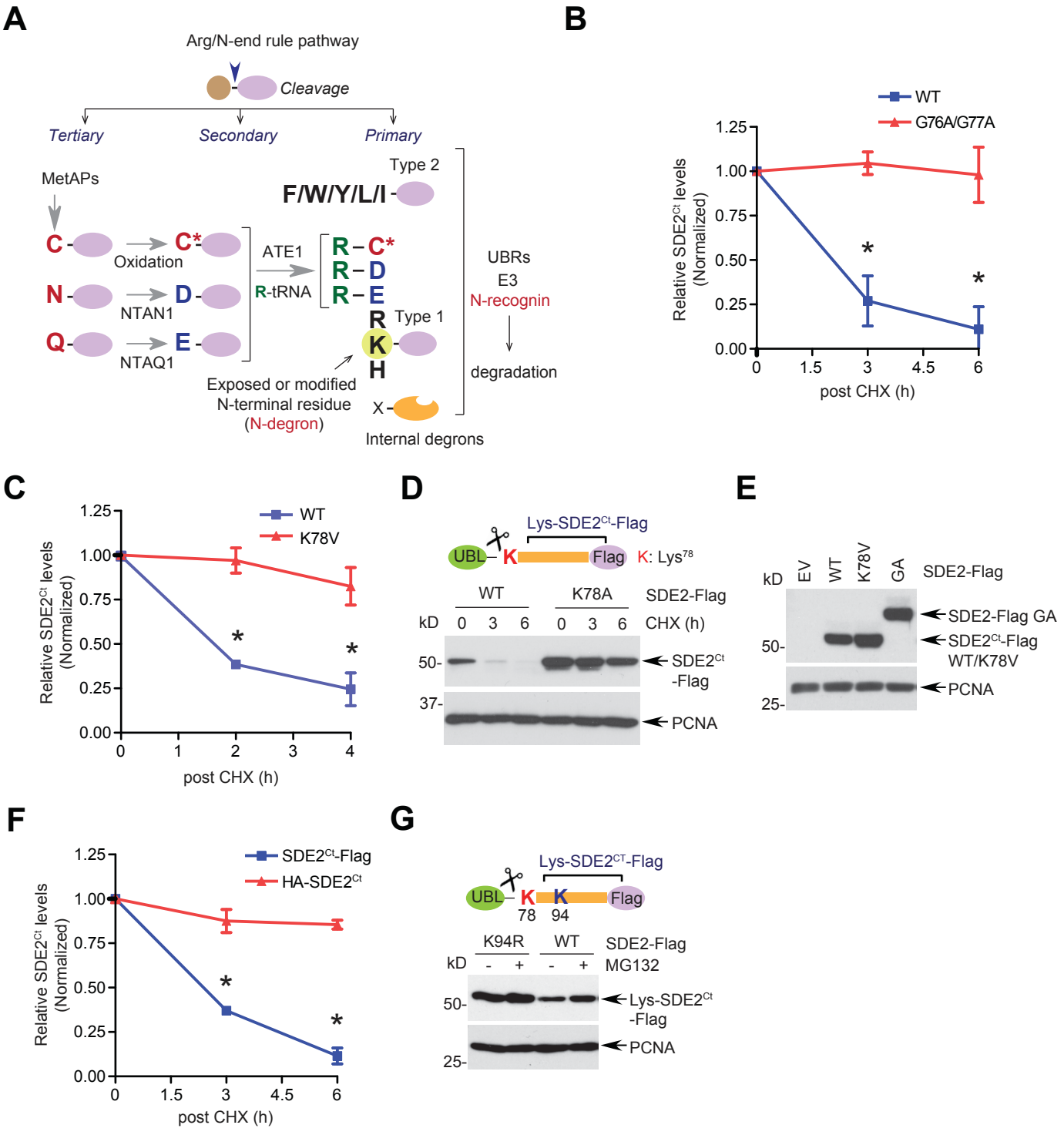
Name	Manufacturer	Catalog Number
BrdU	Sigma	Cat# B5002
Camptothecin (CPT)	ThermoFisher	Cat# A10044
CldU	Sigma	Cat# C9911
Cycloheximide	Sigma	Cat# C6891
Doxycycline	Sigma	Cat# C4859
EdU	Sigma	Cat# D9891
Hydroxyurea Crystallin (HU)	Sigma	Cat# H8627
IdU	Sigma	Cat# I7125
MG132	Sigma	Cat# C2211
Mitomycin C	Sigma	Cat# M0503
NMS-873	Selleckchem	Cat# S7285
Puromycin	Sigma	Cat# P8833
Thymidine	Sigma	Cat# T1895
VE-821	Selleckchem	Cat# S8007

References

Pettersen, E.F., Goddard, T.D., Huang, C.C., Couch, G.S., Greenblatt, D.M., Meng, E.C., and Ferrin, T.E. (2004). UCSF Chimera--a visualization system for exploratory research and analysis. Journal of computational chemistry *25*, 1605-1612.

Ran, F.A., Hsu, P.D., Wright, J., Agarwala, V., Scott, D.A., and Zhang, F. (2013). Genome engineering using the CRISPR-Cas9 system. Nature protocols *8*, 2281-2308.

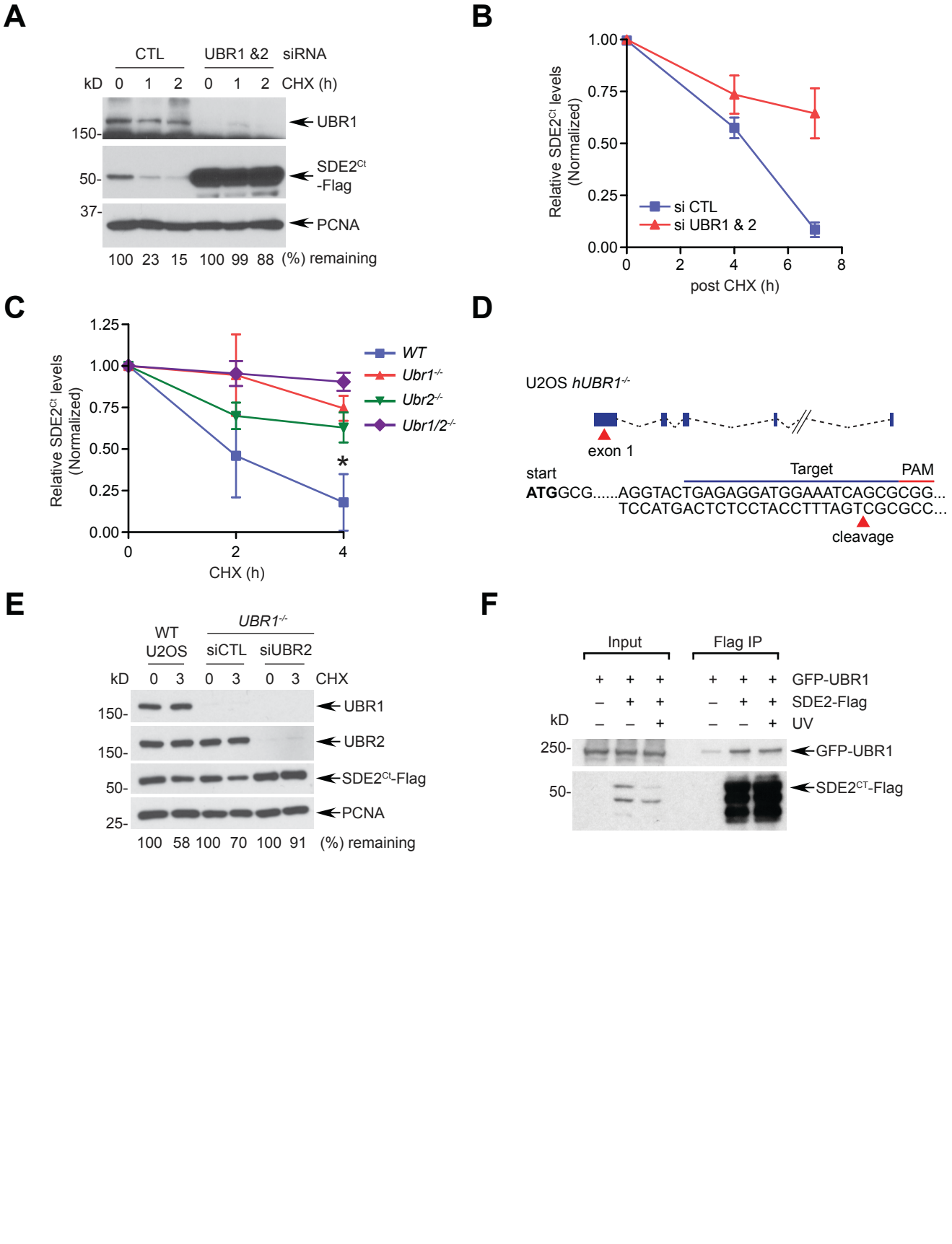
Figure S1


A
Arg/N-end rule pathway
Cleavage
Tertiary
Secondary
Primary
MetAPs
Type 2
F/W/Y/L/I
C
C*
Oxidation
N
D
NTAN1
Q
E
NTAQ1
ATE1
R-tRNA
R – C*
R – D
R – E
R
Type 1
K
H
X
UBRs
E3
N-recognin
degradation
Exposed or modified
N-terminal residue
(N-degron)
Internal degrons
B
WT
G76A/G77A
Relative SDE2Ct levels
(Normalized)
post CHX (h)
C
WT
K78V
Relative SDE2Ct levels
(Normalized)
post CHX (h)
D
Lys-SDE2Ct-Flag
UBL
K
Flag
K: Lys78
WT
K78A
SDE2-Flag
kD
0 3 6 0 3 6
CHX (h)
50-
37-
SDE2Ct
-Flag
PCNA
E
kD
EV
WT
K78V
GA
SDE2-Flag
50-
25-
SDE2-Flag GA
SDE2Ct-Flag
WT/K78V
PCNA
F
SDE2Ct-Flag
HA-SDE2Ct
Relative SDE2Ct levels
(Normalized)
post CHX (h)
G
Lys-SDE2CT-Flag
UBL
K
K
Flag
78
94
K94R
WT
SDE2-Flag
kD
- + - +
MG132
50-
25-
Lys-SDE2Ct
-Flag
PCNA

Supplementary Figure S1. Characterization of SDE2^{Ct} as an Arg/N-end rule substrate (Related to Figure 1)

(A) The mammalian Arg/N-end rule pathway. The N-end rule determines the *in vivo* half-life of a protein based on the identity of its N-terminal residue (N-degron), identified as a degradation signal, and mostly exposed following endolytic cleavage. Modification of Asn or Gln via the N-terminal amidases NTAN1 and NTAQ1, generating Asp and Glu, respectively, followed by arginylation mediated by the arginyltransferase 1 (ATE1) constitutes the tertiary, secondary, and primary degrons, respectively. Their primary degrons, together with other positively charged type 1 residues, such as Lys (marked in a light green circle in the case of SDE2^{Ct}) are recognized by the UBR box-containing ubiquitin E3 ligase UBRs (also called N-recognins) for polyubiquitination and proteasome delivery. C* denotes oxidized N-terminal Cys caused by nitric oxide (NO) and oxygen. **(B)** Quantification of the immunoblot intensity of SDE2^{Ct} WT or GA **(C)** Quantification of the immunoblot intensity of SDE2^{Ct} WT or K78V **(D)** U2OS cells expressing wild-type (WT) or K78A full-length SDE2-Flag were treated with 50 μg/mL cycloheximide (CHX) or DMSO (vehicle) for the indicated times and analyzed by Western blotting (WB). **(E)** WB of U2OS cells transiently expressing WT, K78V, or G76A/G77A SDE2-Flag. EV: empty vector **(F)** Quantification of the immunoblot intensity of SDE2^{Ct}-Flag or HA-SDE2^{Ct} **(G)** U2OS cells expressing the WT or K94R SDE2-Flag were treated with DMSO or 10 μM of the proteasome inhibitor MG132 for 6 h and subsequently analyzed by WB. For (B), (C), and (F), data are mean ± SD from two independent experiments., * $p < 0.05$, Student's t-test. Immunoblot intensities were normalized by loading controls.

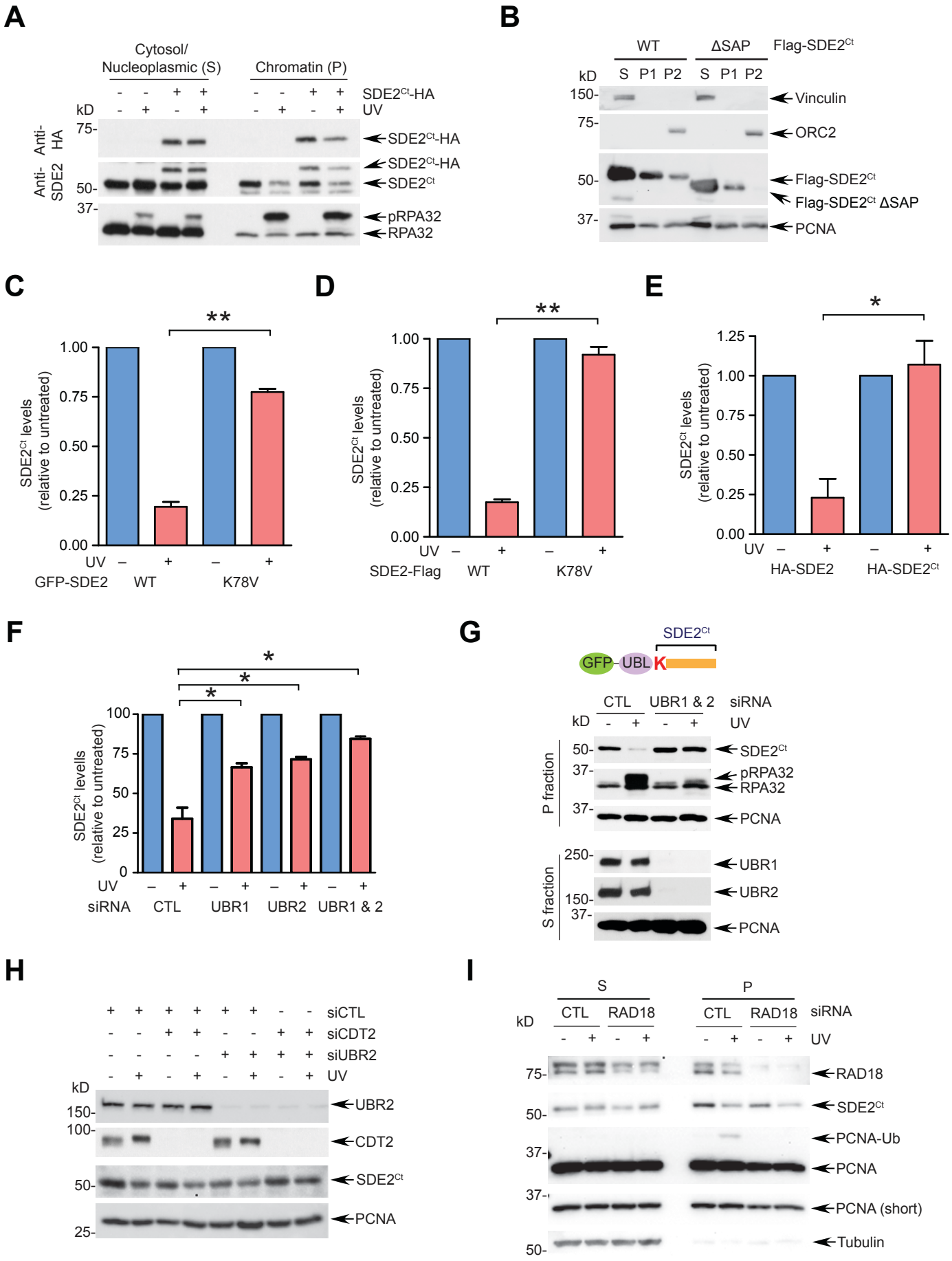
Figure S2


A
CTL
UBR1 &2
siRNA
kD 0 1 2 0 1 2 CHX (h)
150-
UBR1
50-
SDE2Ct -Flag
37-
PCNA
100 23 15 100 99 88 (%) remaining
B
Relative SDE2Ct levels (Normalized)
1.00
0.75
0.50
0.25
0.00
si CTL
si UBR1 & 2
0 2 4 6 8
post CHX (h)
C
Relative SDE2Ct levels (Normalized)
1.25
1.00
0.75
0.50
0.25
0.00
WT
Ubr1-/-
Ubr2-/-
Ubr1/2-/-
*
0 2 4
CHX (h)
D
U2OS hUBR1-/-
exon 1
start
Target
PAM
ATGGCG.....AGGTACTGAGAGGATGGAAATCAGCGCGG...
TCCATGACTCTCCTACCTTTAGTCGCGCC...
cleavage
E
WT U2OS
UBR1-/-
siCTL
siUBR2
kD 0 3 0 3 0 3 CHX
150-
UBR1
150-
UBR2
50-
SDE2Ct-Flag
25-
PCNA
100 58 100 70 100 91 (%) remaining
F
Input
Flag IP
+ + + + + + GFP-UBR1
– + + – + + SDE2-Flag
kD – – + – – + UV
250-
GFP-UBR1
50-
SDE2CT-Flag

Supplementary Figure S2. Characterization of UBR1 and UBR2 as ubiquitin E3 ligases involved in SDE2^{Ct} degradation (Related to Figure 2)

(A) U2OS cells sequentially transfected with the indicated siRNA oligos (vs. control: CTL) and full-length SDE2-Flag were treated with 50 μg/mL CHX for the indicated times, and SDE2^{Ct}-Flag levels were analyzed by WB. SDE2^{Ct} levels were normalized by loading control and quantitated by ImageJ (0 h set as 100 %). **(B)** Quantification of endogenous SDE2^{Ct} levels of Fig. 2C from two independent experiments. * $p < 0.05$, Student's t-test.**(C)** Quantification of the SDE2^{Ct} immunoblot intensity of Fig. 2D from two independent experiments. * $p < 0.05$, WT vs. *Ubr1/2*$^{-/-}$, unpaired two-tailed t-test. **(D)** Schematic of the *UBR1* knockout strategy in U2OS cells using CRISPR/Cas9. The 20-nucleotide sgRNA target locus on exon 1 is delineated with a blue line, along with the PAM sequence delineated as a red line. The cleavage site for the Cas9 nuclease is indicated by a red triangle. **(E)** U2OS control (WT) or *UBR1*$^{-/-}$ clones were first transfected with either control or UBR2 siRNA oligos, then SDE2-Flag expression plasmid. Three hours after treatment with 50 μg/mL (CHX) or DMSO, SDE2^{Ct}-Flag levels were analyzed by WB. **(F)** Anti-Flag co-immunoprecipitation (IP) of 293T cells co-expressing GFP-UBR1 and SDE2-Flag after 40 J/m^2 UVC or mock treatment. (-) indicates empty vector transfection.

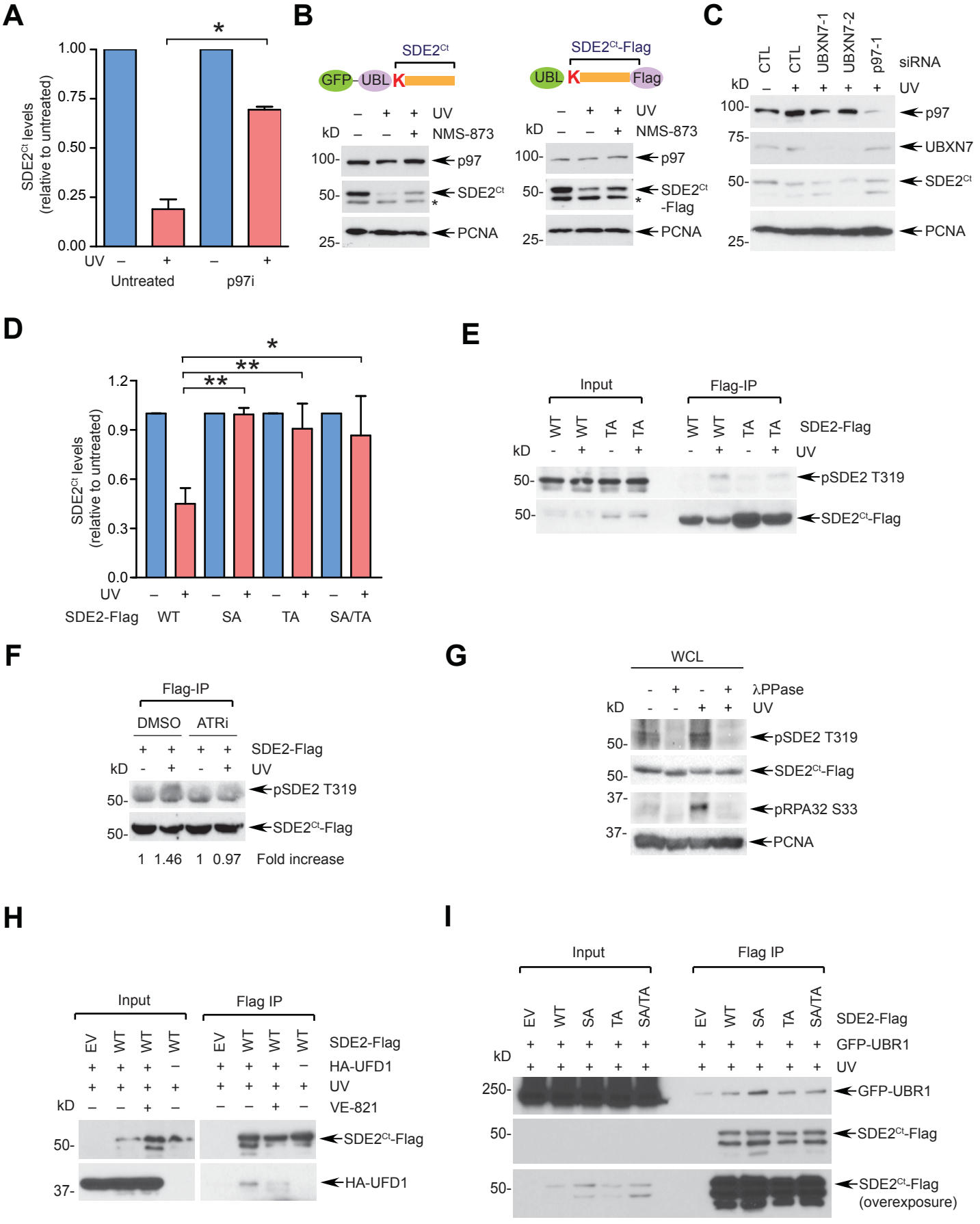
Figure S3


A
Cytosol/ Nucleoplasmic (S)
Chromatin (P)
SDE2Ct-HA
UV
kD
Anti-HA
Anti-SDE2
75-
50-
37-
SDE2Ct-HA
SDE2Ct-HA
SDE2Ct
pRPA32
RPA32
B
WT
ΔSAP
Flag-SDE2Ct
kD
S P1 P2 S P1 P2
150-
75-
50-
37-
Vinculin
ORC2
Flag-SDE2Ct
Flag-SDE2Ct ΔSAP
PCNA
C
SDE2Ct levels (relative to untreated)
1.00
0.75
0.50
0.25
0.00
**
UV – + – +
GFP-SDE2 WT K78V
D
SDE2Ct levels (relative to untreated)
**
UV – + – +
SDE2-Flag WT K78V
E
SDE2Ct levels (relative to untreated)
1.25
1.00
0.75
0.50
0.25
0.00
*
UV – + – +
HA-SDE2 HA-SDE2Ct
F
SDE2Ct levels (relative to untreated)
100
75
50
25
0
*
*
*
UV – + – + – + – +
siRNA CTL UBR1 UBR2 UBR1 & 2
G
SDE2Ct
GFP
UBL
K
CTL
UBR1 & 2
siRNA
kD
UV
P fraction
50-
37-
37-
SDE2Ct
pRPA32
RPA32
PCNA
S fraction
250-
150-
37-
UBR1
UBR2
PCNA
H
siCTL
siCDT2
siUBR2
UV
kD
150-
100-
50-
25-
UBR2
CDT2
SDE2Ct
PCNA
I
S
P
CTL
RAD18
CTL
RAD18
siRNA
kD
UV
75-
50-
37-
37-
50-
RAD18
SDE2Ct
PCNA-Ub
PCNA
PCNA (short)
Tubulin

Supplementary Figure S3. Damage-dependent SDE2^{Ct} degradation after UVC-induced replication stress (Related to Figure 3)

(A) U2OS cells expressing empty vector or SDE2-HA were irradiated or not with 40 J/m^2 UVC, and 4 h later, cell lysates were fractionated, and endogenous SDE2^{Ct} and exogenous SDE2^{Ct}-HA levels were analyzed by WB. **(B)** U2OS cells transfected with N-terminally Flag-tagged SDE2^{Ct} (i.e. Flag-SDE2 ΔUBL) wild-type or ΔSAP were fractionated into cytoplasmic (S), nucleoplasmic (P1), and chromatin (P2) fractions, and each fraction was analyzed by WB. **(C-F)** Quantification of the SDE2^{Ct} immunoblot intensities in the P fractions of Fig. 3A (S3C), Fig. 3B (S3D), Fig. 3C (S3E), and Fig. 3D (S3F) from two independent experiments. * $p < 0.05$, ** $p < 0.01$, unpaired two-tailed t-test. **(G)** U2OS cells expressing N-terminally GFP-tagged SDE2 were transfected with both UBR1 and UBR2 siRNA oligos (vs. CTL), irradiated with 40 J/m^2, and 4 h later, cell lysates were fractionated and SDE2^{Ct} levels were analyzed by WB. **(H)** U2OS cells were transfected with indicated combination of siRNA oligos and GFP-SDE2, irradiated with 40 J/m^2 UVC, and P fractions were analyzed by WB (UBR2 blot from S fraction). **(I)** U2OS cells transfected with RAD18 siRNA (vs. CTL) were irradiated with 40 J/m^2 UVC, fractionated into S and P fractions, and analyzed by WB.

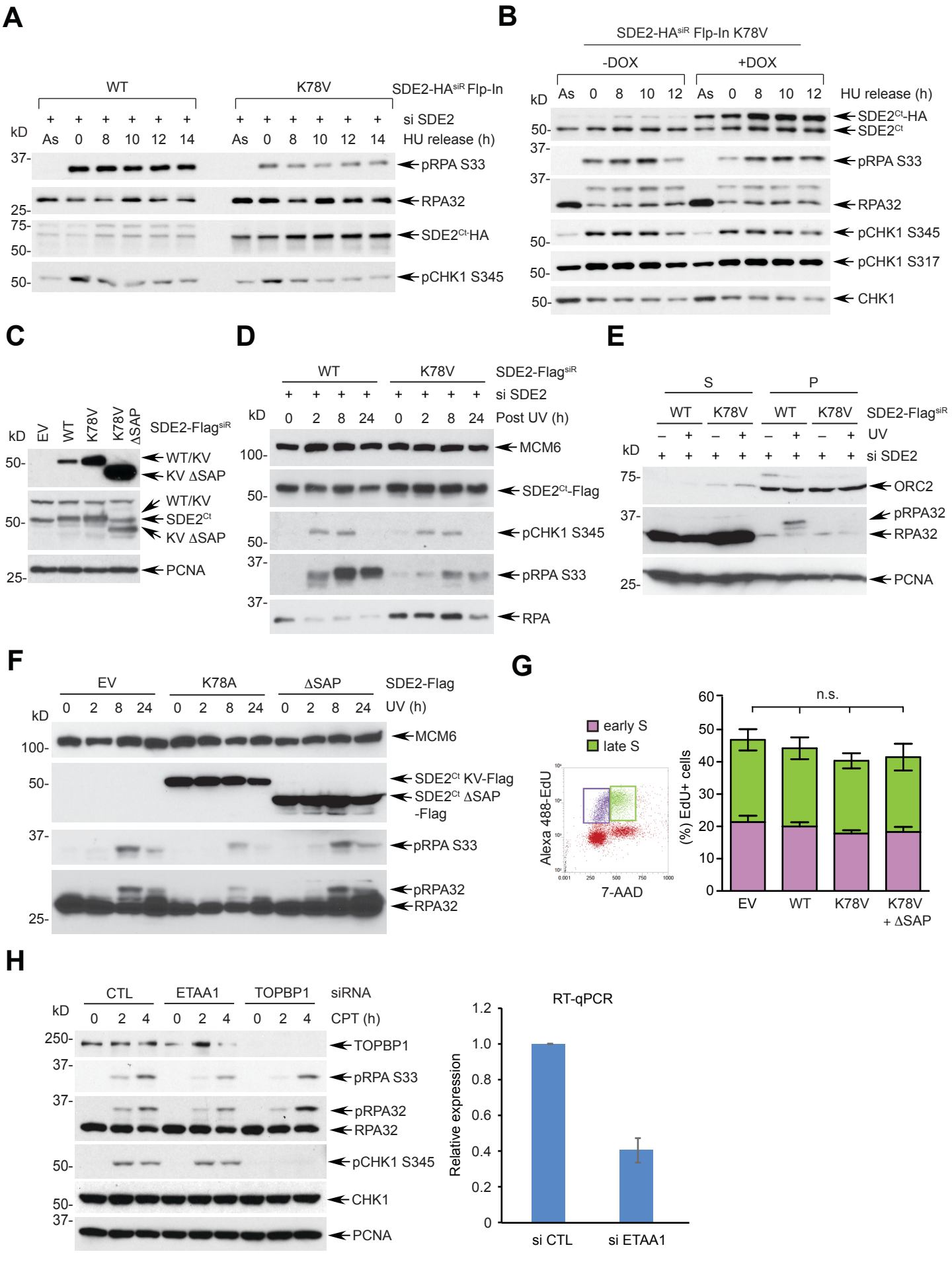
Figure S4


A
B
C
D
E
F
G
H
I
SDE2Ct levels (relative to untreated)
Untreated
p97i
SDE2Ct
SDE2Ct-Flag
GFP
UBL
K
Flag
NMS-873
p97
SDE2Ct
PCNA
siRNA
CTL
UBXN7-1
UBXN7-2
p97-1
UBXN7
SDE2-Flag
WT
SA
TA
SA/TA
Input
Flag-IP
pSDE2 T319
Fold increase
DMSO
ATRi
WCL
λPPase
pRPA32 S33
Flag IP
EV
HA-UFD1
VE-821
GFP-UBR1
(overexposure)

Supplementary Figure S4. Characterization of the p97 adapters required for SDE2^{Ct} degradation (Related to Figure 4).

(A) Quantification of the SDE2^{Ct} immunoblot intensity of Fig. 4A from two independent experiments. * $p < 0.05$, unpaired two-tailed t-test. (**B**) U2OS cells expressing Nt GFP-tagged SDE2 or Ct Flag-tagged SDE2 were UVC irradiated, let recover for 4 h, and P fractions were analyzed by WB. Where indicated, cells were pretreated with 10 μM NMS-873 for 1 h. * nonspecific. **(C)** U2OS cells transfected with indicated with siRNA oligos were irradiated or not with 40 J/m^2 UVC, and 4 h later, cell lysates were fractionated, and P fractions were analyzed by WB. **(D)** Quantification of SDE2^{Ct}-Flag levels of Fig. 4D from three independent experiments. * $p < 0.05$, ** $p < 0.01$, unpaired two-tailed t-test **(E)** U2OS cells expressing SDE2-Flag WT or T319A were irradiated with 40 J/m^2 UVC, and recovered for 2 h. Chromatin fractions were subjected to anti-Flag IP and WB with anti-pSDE2 T319 and Flag antibodies. **(F)** U2OS cells expressing SDE2-Flag was pretreated with 10 μM ATR inhibitor (VE-821) for 1 h, irradiated with 50 J/m^2 UVC, and after 2 h, P fractions were subjected to anti-Flag IP and WB. Fold increase average was quantitated from two independent experiments. **(G)** 293T cells expressing SDE2-Flag were irradiated with 50 J/m^2 UVC (2 h recovery with 20 μM MG132), and whole cell lysates were treated with lambda phosphatase at 30 °C for 30 min. pRPA32 serves as a control for phosphatase treatment. **(H)** 293T cells transfected with indicated plasmids were pretreated with 10 μM VE-821 (ATR inhibitor) for 1 h, UVC irradiated and let recover for 2 h in the presence of the inhibitor. Cell lysates were analyzed by anti-Flag IP and WB. **(I)** 293T cells transfected with indicated plasmids were subjected to anti-Flag IP and WB.

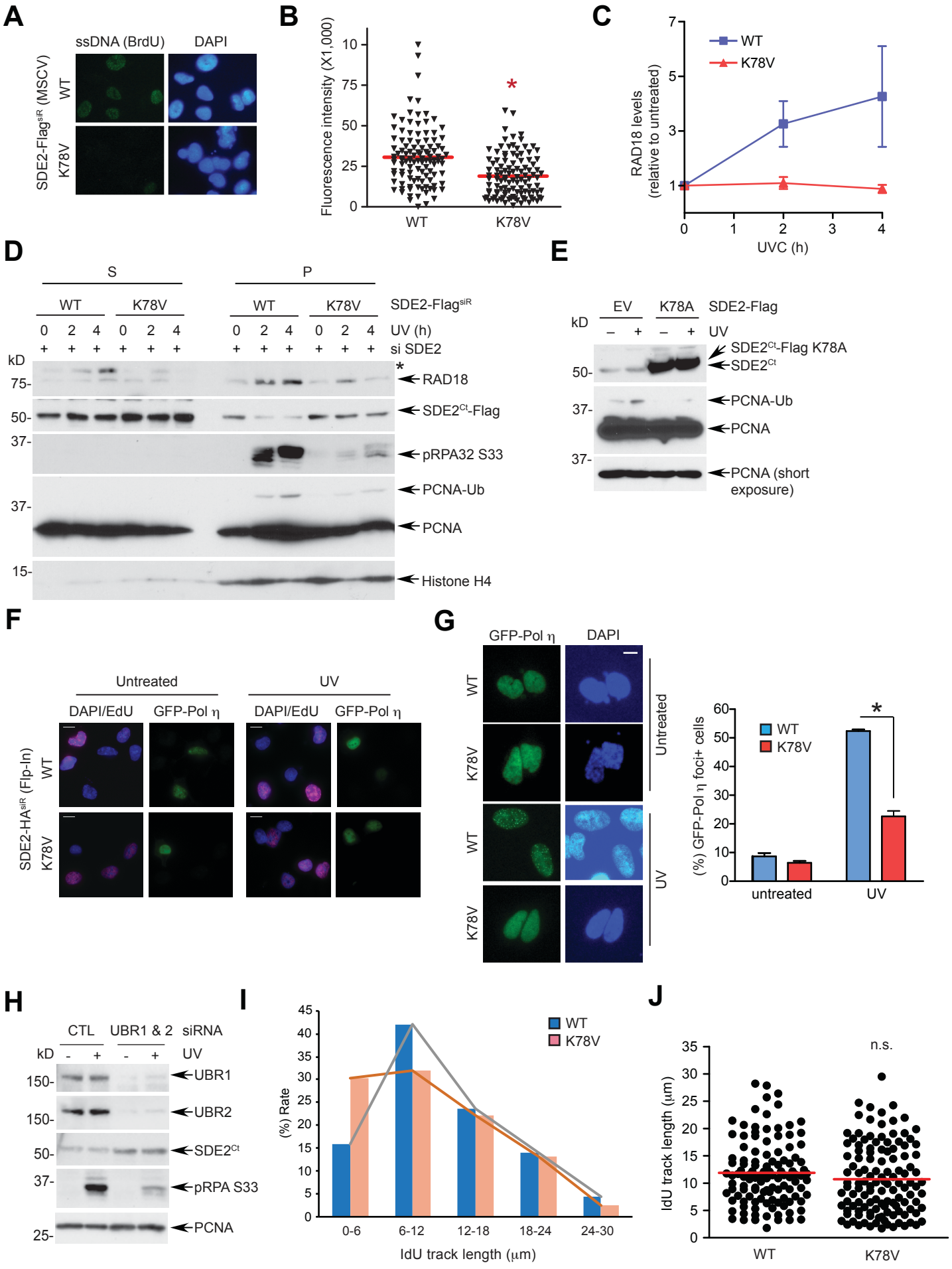
Figure S5


A
WT
K78V
SDE2-HA^siR Flp-In
si SDE2
kD
As 0 8 10 12 14
HU release (h)
37-
pRPA S33
RPA32
25-
75-
50-
SDE2^Ct-HA
pCHK1 S345
B
SDE2-HA^siR Flp-In K78V
-DOX
+DOX
SDE2^Ct-HA
SDE2^Ct
pRPA S33
RPA32
pCHK1 S345
pCHK1 S317
CHK1
C
EV
WT
K78V
K78V ΔSAP
SDE2-Flag^siR
WT/KV
KV ΔSAP
SDE2^Ct
PCNA
D
SDE2-Flag^siR
Post UV (h)
MCM6
SDE2^Ct-Flag
pCHK1 S345
pRPA S33
RPA
E
S
P
UV
ORC2
pRPA32
RPA32
PCNA
F
EV
K78A
ΔSAP
UV (h)
MCM6
SDE2^Ct KV-Flag
SDE2^Ct ΔSAP -Flag
pRPA S33
pRPA32
RPA32
G
early S
late S
Alexa 488-EdU
7-AAD
n.s.
(%) EdU+ cells
EV
WT
K78V
K78V + ΔSAP
H
CTL
ETAA1
TOPBP1
siRNA
CPT (h)
TOPBP1
pRPA S33
pRPA32
RPA32
pCHK1 S345
CHK1
PCNA
RT-qPCR
Relative expression
si CTL
si ETAA1

Supplementary Figure S5. SDE2^{Ct} degradation upon replication stress promotes RPA phosphorylation (Related to Figure 5)

(A) SDE2 WT or K78V flipped cells by siRNA and doxycycline (dox) induction were incubated with 2 mM HU for 18 h and released for the indicated times, followed by WB. As: asynchronous. **(B)** U2OS K78V SDE2-HA Flp-In cells were treated or not with 10 ng/mL dox for 48 h to induce the expression of the K78V mutant (on endogenous SDE2 background) prior to treatment with 2 mM hydroxyurea (HU) for 20 h. Cells were then released into fresh medium for the indicated times before WB analysis. **(C)** U2OS cells transduced with the indicated siRNA-resistant (siR) WT or mutant SDE2-Flag using the Murine Stem Cell Virus (MSCV) retroviral vectors were analyzed by WB following puromycin selection. **(D)** U2OS cells stably expressing WT or K78V SDE2-Flag (MSCV) were transfected with SDE2 siRNA oligos, then irradiated or not (0 h) with 10 J/m^2 UVC, and harvested at the indicated times for WB analysis. **(E)** SDE2 siRNA-transfected U2OS cells stably expressing WT or K78V SDE2-Flag (MSCV) were irradiated or not with 10 J/m^2 UVC, and 4 h later, cell lysates were fractionated and analyzed by WB for the localization of phosphorylated RPA. **(F)** U2OS cells transiently expressing K78V or ΔSAP SDE2-Flag (vs. empty vector) were irradiated or not (0 h) with 10 J/m^2 UVC, and harvested at the indicated times to examine the suppression of RPA phosphorylation by the SDE2 mutants. **(G)** Analysis of cycling cells (MSCV) at S phase by EdU/7-ADD staining and flow cytometry.Mean ± SD (n=3 independent experiments), n.s. not significant. **(H)** (Left) siRNA-transfected U2OS cells were treated with 100 nM CPT, and cell lysates were analyzed by WB. (Right) confirmation of ETAA1 knockdown by RT-qPCR. Experiments were performed in triplicates, and error bar indicates mean ± SD from three independent experiments.

Figure S6


A
ssDNA (BrdU)
DAPI
SDE2-Flag^siR (MSCV)
WT
K78V
B
Fluorescence intensity (X1,000)
WT
K78V
C
RAD18 levels (relative to untreated)
UVC (h)
WT
K78V
D
S
P
WT
K78V
SDE2-Flag^siR
UV (h)
si SDE2
kD
RAD18
SDE2^Ct-Flag
pRPA32 S33
PCNA-Ub
PCNA
Histone H4
E
EV
K78A
SDE2-Flag
UV
SDE2^Ct-Flag K78A
SDE2^Ct
PCNA-Ub
PCNA
PCNA (short exposure)
F
Untreated
UV
DAPI/EdU
GFP-Pol η
SDE2-HA^siR (Flp-In)
WT
K78V
G
GFP-Pol η
DAPI
Untreated
UV
(%) GFP-Pol η foci+ cells
untreated
UV
H
CTL
UBR1 & 2 siRNA
UV
UBR1
UBR2
SDE2^Ct
pRPA S33
PCNA
I
(%) Rate
IdU track length (μm)
0-6
6-12
12-18
18-24
24-30
J
IdU track length (μm)
n.s.
WT
K78V

Supplementary Figure S6. SDE2^{Ct} degradation upon UVC-induced replication stress is required for DNA damage bypass signaling (Related to Figure 6)

(A) U2OS cells stably expressing WT or K78V SDE2-Flag WT or K78V (MSCV) were transfected with SDE2 siRNA oligos, incubated with 10 μM BrdU for 48 h, and irradiated with 40 J/m^2 UVC. Two hours later, coverslips were fixed and processed for anti-BrdU immunofluorescence in non-denaturing conditions. Representative images demonstrate that ssDNA formation, as revealed by BrdU immunofluorescence, is reduced in the K78V-expressing cells. Scale bar, 10 μm. **(B)** Quantification of BrdU fluorescence intensity from (A) using Image J. A representative result from two independent experiments is shown. *p < 0.0001, two-tailed unpaired Student's t-test. **(C)** Quantification of the RAD18 immunoblot intensity of Fig. 6C normalized by PCNA from two independent experiments. **(D)** SDE2 siRNA-transfected U2OS cells stably expressing WT or K78V SDE2-Flag (MSCV) were irradiated with 40 J/m^2 UVC, fractionated, and cell lysates were analyzed by WB for pRPA, RAD18 recruitment, and PCNA monoubiquitination. **(E)** U2OS cells transiently expressing K78V SDE2-Flag were left untreated or irradiated with 10 J/m^2 UVC, and 6 h later, cell lysates were fractionated and analyzed by WB for PCNA monoubiquitination. **(F)** Representative images GFP-Pol η from EdU- cells in Figure 6D. Scale bar, 10 μm. **(G)** (Left) siRNA-transfected U2OS cells stably expressing WT or K78V SDE2-Flag (MSCV) were transiently expressed with GFP-pol η, irradiated with 40 J/m^2 UVC, and 4 h later, cell were fixed and GFP-Pol η focus formation was examined by GFP epifluorescence. Representative images are shown. Scale bar, 5 μm. (Right) quantification of GFP-Pol η foci using ImageJ(foci+ indicates more than 20 defined GFP-Pol η foci). Mean ± SEM (n=2). * p = 0.0043, unpaired Student's t-test. **(H)** siRNA-transfected U2OS cells were irradiated with 40 J/m^2 UVC, and 4 h later, cell lysates were analyzed by WB. **(I)** Distribution of IdU track length from ongoing forks of WT or K78V Flp-In cells (dox-induced and knocked-down) from Figure6G. A representative graph from three independent experiments is shown. **(J)** Plotting of IdU track length from (I). n.s. not significant, Mann-Whitney test.