Science Advances
AAAS

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Supplementary Materials for

Inhibition of the translesion synthesis polymerase REV1 exploits replication gaps as a cancer vulnerability

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Published 10 June 2020, *Sci. Adv.* **6**, eaaz7808 (2020)
DOI: 10.1126/sciadv.aaz7808

This PDF file includes:

Supplemental Figures:

Figure S1. Both WT and pro-TLS mutant S990A correct defects in replication restart due to FANCJ deficiency.

(A) Cell survival assays with U2OS FANCJ K/O cells complemented with $FANCJ^{WT}$ (WT), empty vector (V) and the FANCJ-BRCA1 binding deficient, $FANCJ^{S990A}$ (S990A) treated with increasing concentrations of MMC. Data represent the mean percent ± SD of survival from three independent experiments.

(B) Western blot analysis with the indicated Abs of WCE from FANCJ null FA-J cells complemented with $FANCJ^{WT}$ (WT), empty vector (V) and the FANCJ-BRCA1 binding deficient, $FANCJ^{S990A}$ (S990A), the FANCJ helicase dead mutant (K52R) and the FANCJ-BRCA1 binding deficient and helicase dead (S990A+K52R) double mutant.

(C) Experimental schematic and quantification of CldU (red) tract length either after treatment with 20uM TLSi alone or under unchallenged conditions as observed in the U2OS FANCJ K/O or FA-J complemented cells.

(D) Experimental schematic and the quantification of CldU (red) tract length after co-incubation with 0.5mM HU or $2J/m^2$ of UV as observed in the FA-J complemented cells.

(E) Experimental schematic and quantification of CldU (red) tract length, percent CldU (red) tract connected with IdU (green), percent only IdU (green) and percent only CldU (red) tract as observed in the FA-J complemented cells.

(F) Experimental schematic and quantification of CldU (red) track length following 1h treatment with 0.5mM HU immediately followed by -/+ S1 nuclease treatment and as observed in the FA-J complemented cells.

(G) Experimental schematic and quantification of CldU/IdU (red/green) ratio as observed in the FA-J complemented cells.

(H) Cell survival assays with PEO1 cells expressing V5 tagged - empty vector (V), $FANCJ^{WT}$ (WT), and the FANCJ-BRCA1 binding deficient, $FANCJ^{S990A}$ (pro-TLS) under increasing concentrations of cisplatin. Data represent the mean percent ± SD of survival from three independent experiments. Experimental schematic and quantification of CldU (red) tract length after co-incubation with 0.5mM HU as observed in these cell lines.

For every DNA fiber assay analysis, each dot represents one individual fiber. Experiments were performed in biological triplicate with at least 100 fibers per replicate. Bars represent

the mean ± SD. Statistical analysis were performed according to two-tailed Mann-Whitney test. All p values are further described in the statistical methods.

Figure S2. TLS polymerases overcome replication stress associated ssDNA gaps.
(A-B) Representative images (63X) of eGFP-pol-eta (pol-η) or eGFP-Rev1 foci as observed in U2OS FANCJ K/O cells complemented with $FANCJ^{WT}$ (WT) and the FANCJ-BRCA1 binding deficient, $FANCJ^{S990A}$ (S990A) and U2OS cells expressing shRNA against NSC or p21 transiently transfected with eGFP-pol-η or eGFP-Rev1 and assessed either after no treatment (Unt), post 4h 15J/m^2 UV or post 48h 250nM MMC treatment. Around 300 cells were counted from multiple fields.
(C) Experimental schematic, representative images and quantification of the EdU (green) positive cells after varying doses of UV treatment as observed in U2OS FANCJ K/O cells complemented with $FANCJ^{WT}$ (WT - Control) and the FANCJ-BRCA1 binding deficient, $FANCJ^{S990A}$ (S990A – pro-TLS). EdU staining was performed as described in Figure 2
(D-G) Experimental schematic, representative images and quantification of EdU (green) and ssDNA (red) positive cells following initial labeling with CldU for 48h followed by EdU treatment with or without 0.5mM HU -/+ 20μM TLSi or -/+ 5μM ATRi. EdU and ssDNA staining was performed as described in the Figure 2.
Experiments were performed in biological triplicate. Bars represent the mean ± SD. Statistical analysis were performed according to two-tailed Mann-Whitney test. All p values are further described in the statistical methods.

Figure S3. TLS polymerases counteract oncogene induced replication stress response.
(A and C) Experimental schematic and representative images of EdU and ssDNA positive cells as previously described in Figure 3(B and E).
(B) Representative images and quantification of the colony formation assay as observed in $FANCJ^{WT}$ or $FANCJ^{S990A}$ complemented FANCJ K/O U2OS cell lines stably infected with only vector (V) and as described in the Figure 3(C).
Experiments were performed in biological triplicate. Bars represent the mean ± SD. Statistical analysis were performed according to two-tailed Mann-Whitney test. All p values are further described in the statistical methods.

Figure S4. TLS polymerase re-wiring of cancer cells promote unrestrained replication during stress.

(A) Experimental schematic and quantification of EdU (green) positive cells. Cells were either labeled for 30mins with EdU or for 2h with EdU and varying doses of HU. Cells were stained for EdU as previously described in Figure 2. Percent EdU positive cells were quantified from around 300 cells counted from multiple fields.

(B) Experimental schematic and quantification of EdU (green) and ssDNA (red) positive cells as previously described in Figure 4(A).

Experiments were performed in biological triplicate. Bars represent the mean ± SD. Statistical analysis were performed according to two-tailed Mann-Whitney test. All p values are further described in the statistical methods.

Figure S5. FANCJ promotes TLS pathway by suppressing the negative regulator, p21

(A) Western blot analysis with the indicated Abs of WCE from HeLa CRISPR control and FANCJ CRISPR knockout (K/O) cells and HeLa FANCJ K/O cells expressing shRNA against NSC or p21. Experimental schematic and quantification of EdU (green) positive cells. Cells were either labeled for 30mins with EdU or 2h with EdU and 0.5mM HU. Cells were stained for EdU as previously described in Figure 2.

(B and D) Representative images and quantification of the colony formation assay as observed in HeLa CRISPR control and FANCJ CRISPR knockout (K/O) cells and HeLa FANCJ K/O cells expressing shRNA against NSC or p21. The HeLa FANCJ K/O shRNA p21 cells were either grown in absence or continuous presence of 20μM TLSi.

(C) Experimental schematic and quantification of EdU (green) and ssDNA (red) positive cells as previously described in Figure 4(A). EdU and ssDNA was staining performed as described in Figure 2.

Experiments were performed in biological triplicate. Bars represent the mean ± SD. Statistical analysis were performed according to two-tailed Mann-Whitney test. All p values are further described in the statistical method.

Figure S6. TLSi as a promising cancer therapy (A-D) Representative images and quantification of the colony formation assay after dose dependent treatment with ATRi (μM) and WEE1i (nM) alone or in combination with 20μM TLSi across different cell lines. Experiments were performed in biological triplicate. Bars represent the mean ± SD. Statistical analysis were performed according to two-tailed Mann-Whitney test. All p values are further described in the statistical methods.

Fig S1

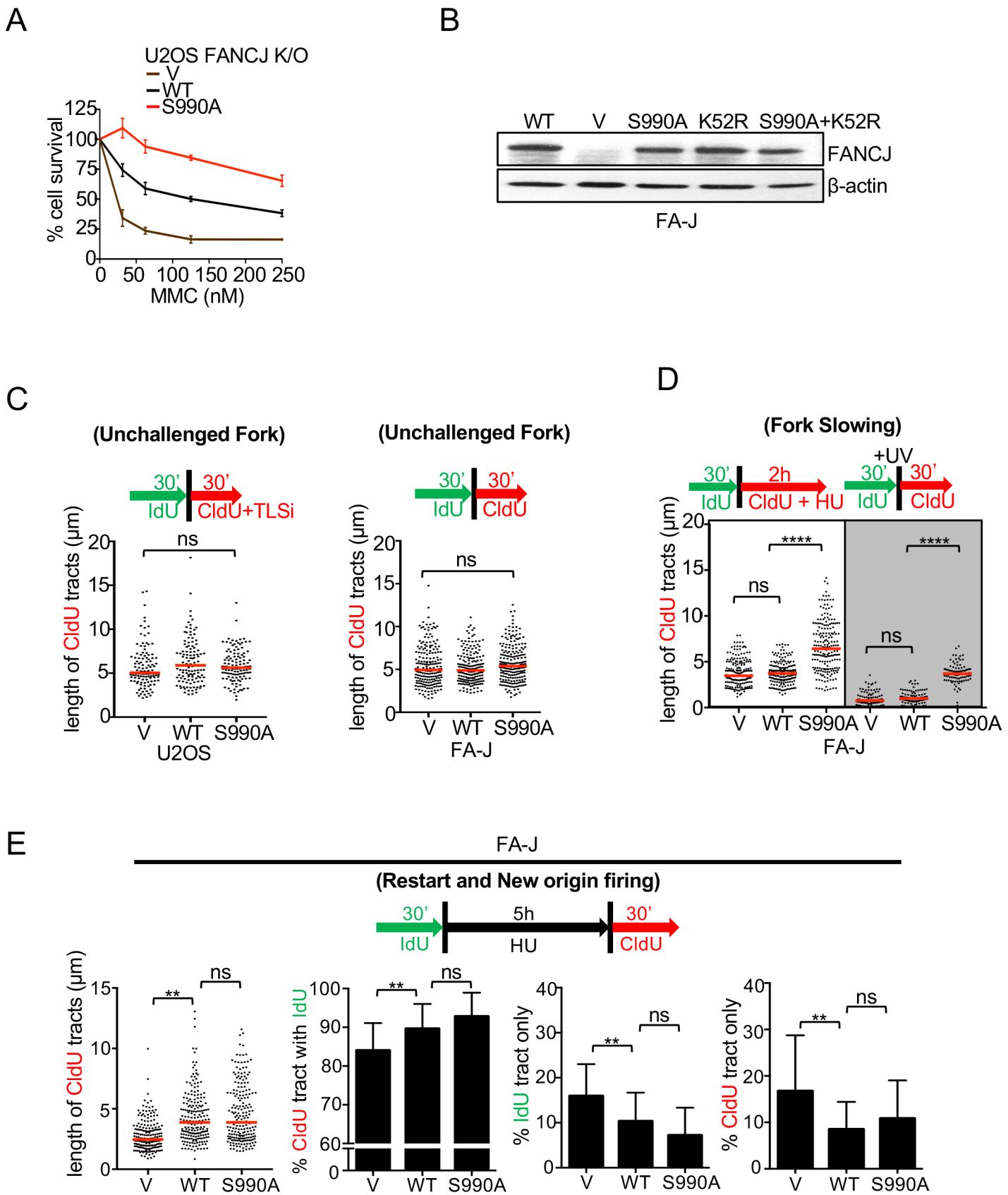
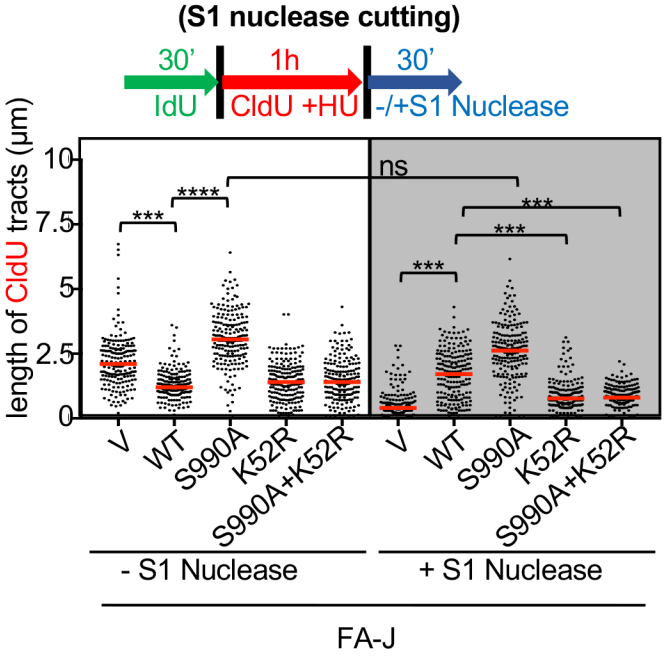
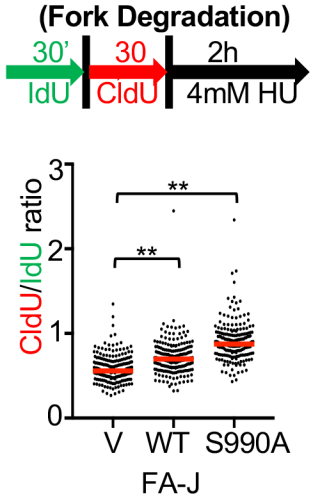

A
U2OS FANCJ K/O
V
WT
S990A
% cell survival
MMC (nM)
B
WT
V
S990A
K52R
S990A+K52R
FANCJ
β-actin
FA-J
C
(Unchallenged Fork)
30'
IdU
CldU+TLSi
length of CldU tracts (μm)
ns
V
WT
S990A
U2OS
(Unchallenged Fork)
CldU
FA-J
D
(Fork Slowing)
+UV
2h
CldU + HU
****
E
FA-J
(Restart and New origin firing)
5h
HU
% CldU tract with IdU
% IdU tract only
% CldU tract only
**

Fig S1 Cont

F



G



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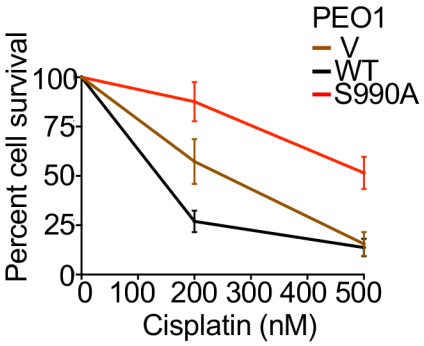


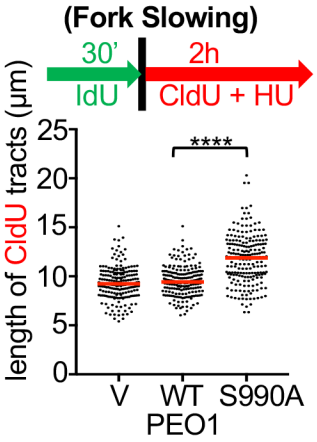


Fig S2

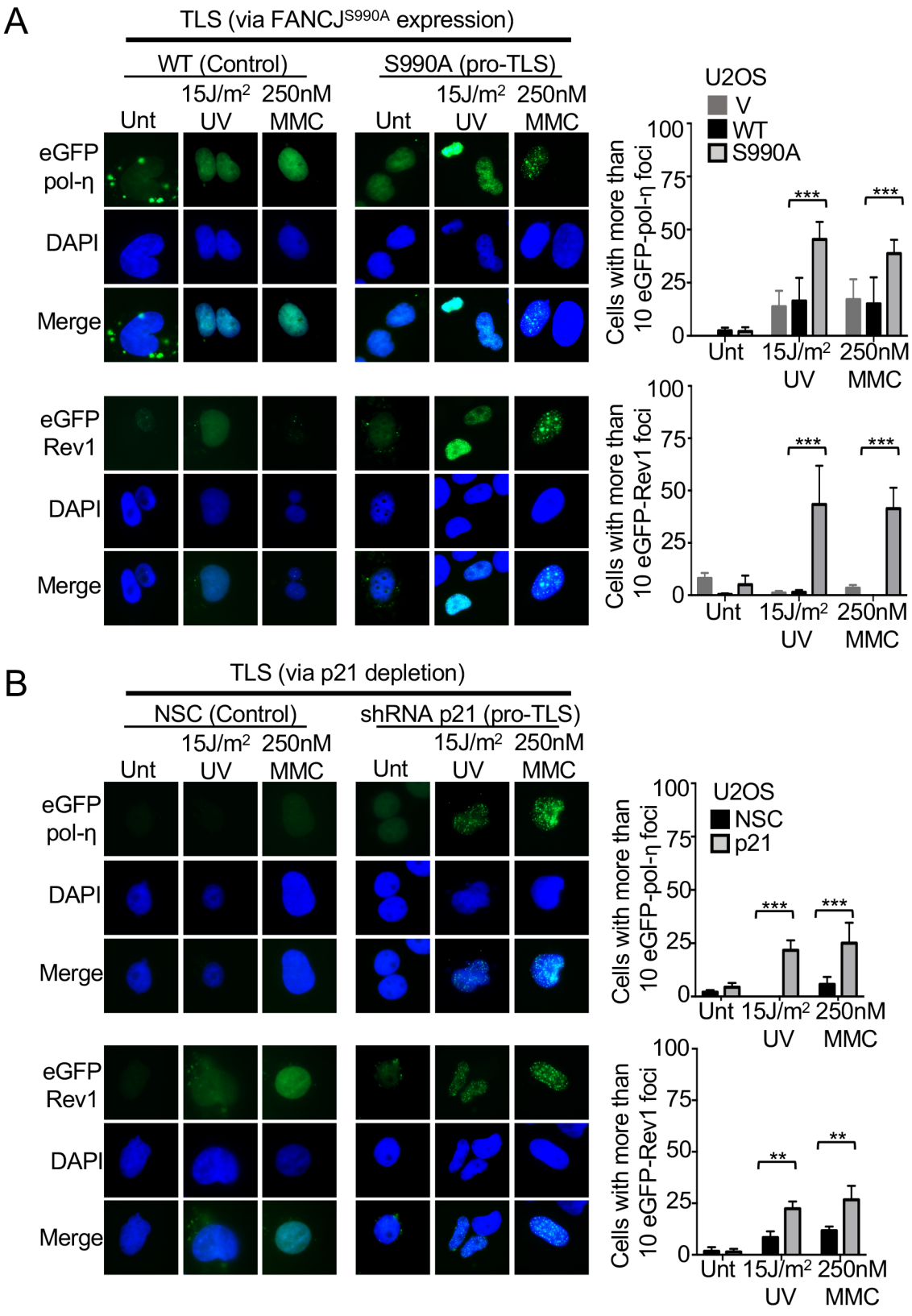
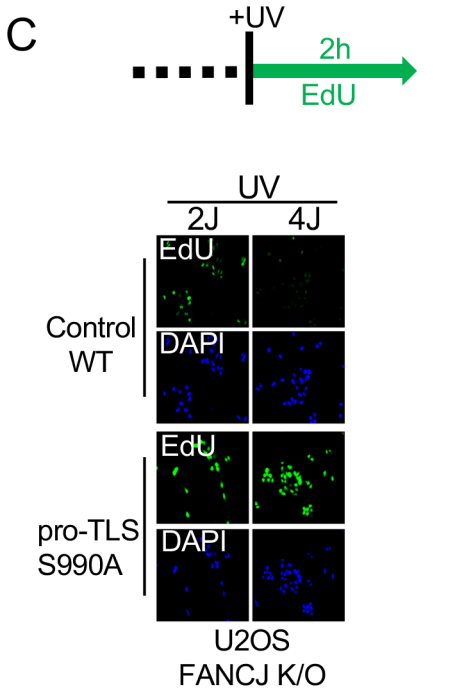
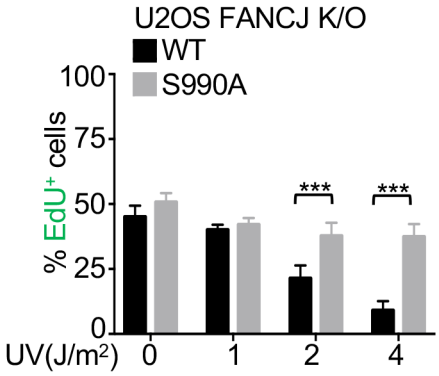

A
TLS (via FANCJ^{S990A} expression)
WT (Control)
S990A (pro-TLS)
Unt
15J/m^2 UV
250nM MMC
eGFP pol-η
DAPI
Merge
eGFP Rev1
U2OS
V
WT
S990A
Cells with more than 10 eGFP-pol-η foci
Cells with more than 10 eGFP-Rev1 foci
***
B
TLS (via p21 depletion)
NSC (Control)
shRNA p21 (pro-TLS)
NSC
p21
**

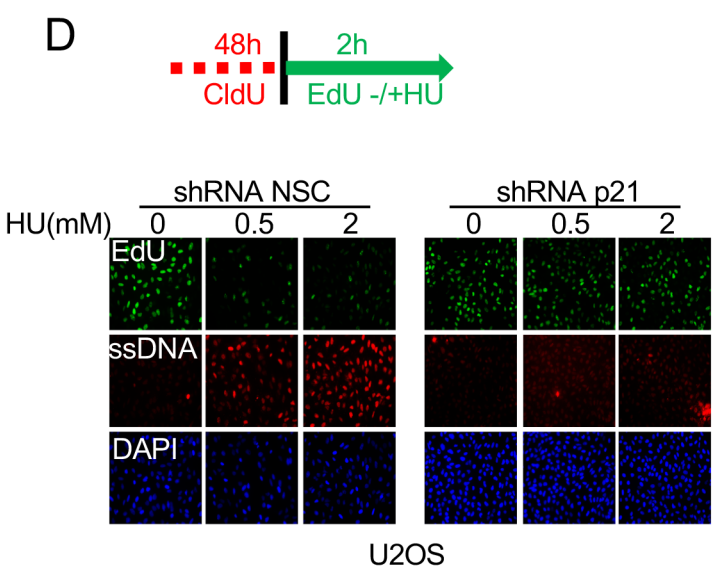
Fig S2 Cont

TLS (via FANCJ^{S990A} expression)





TLS (via p21 depletion)



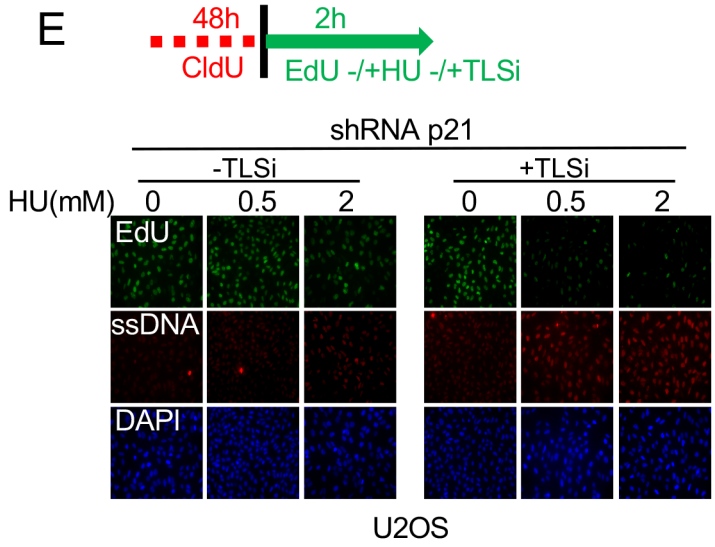


Fig S2 Cont

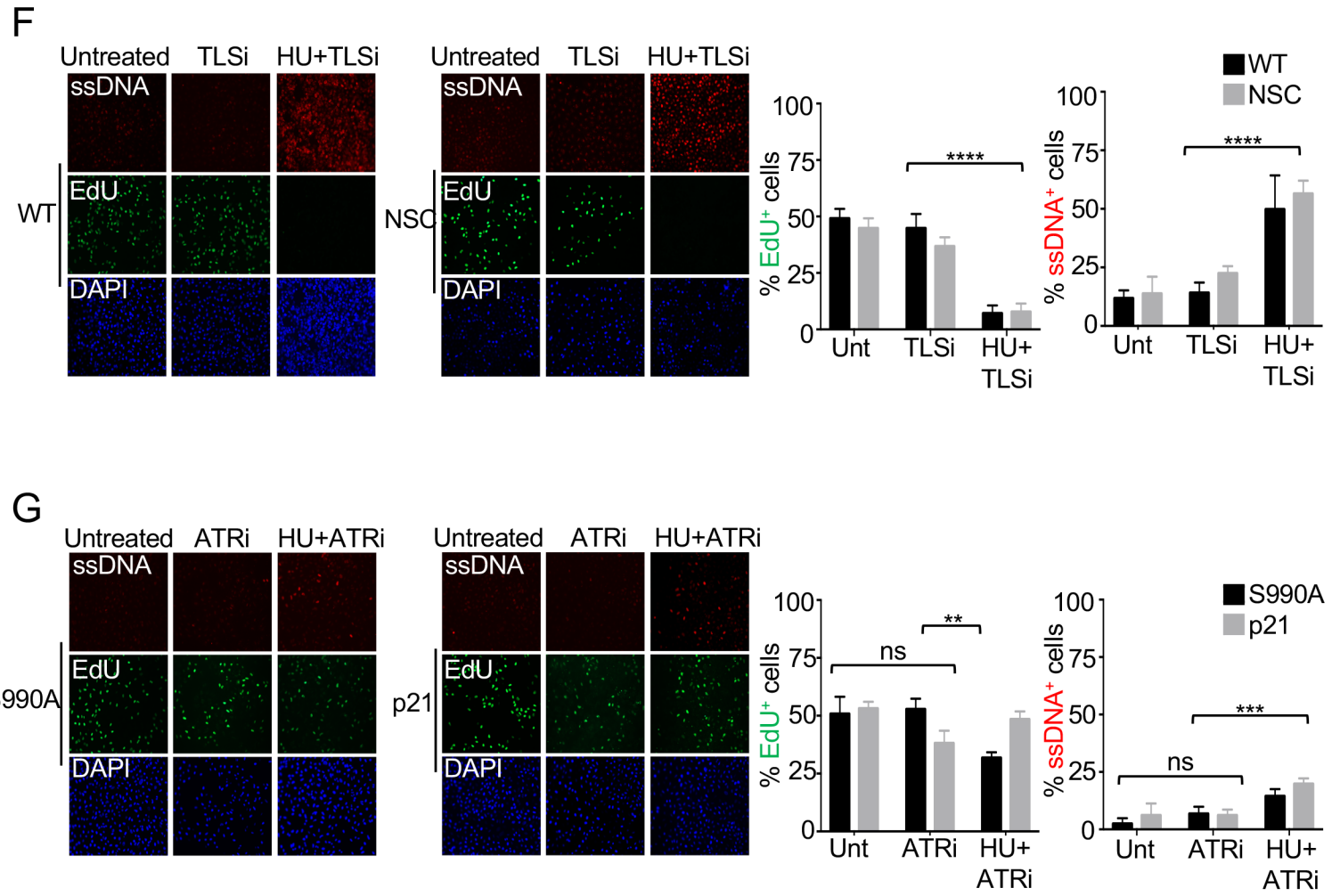
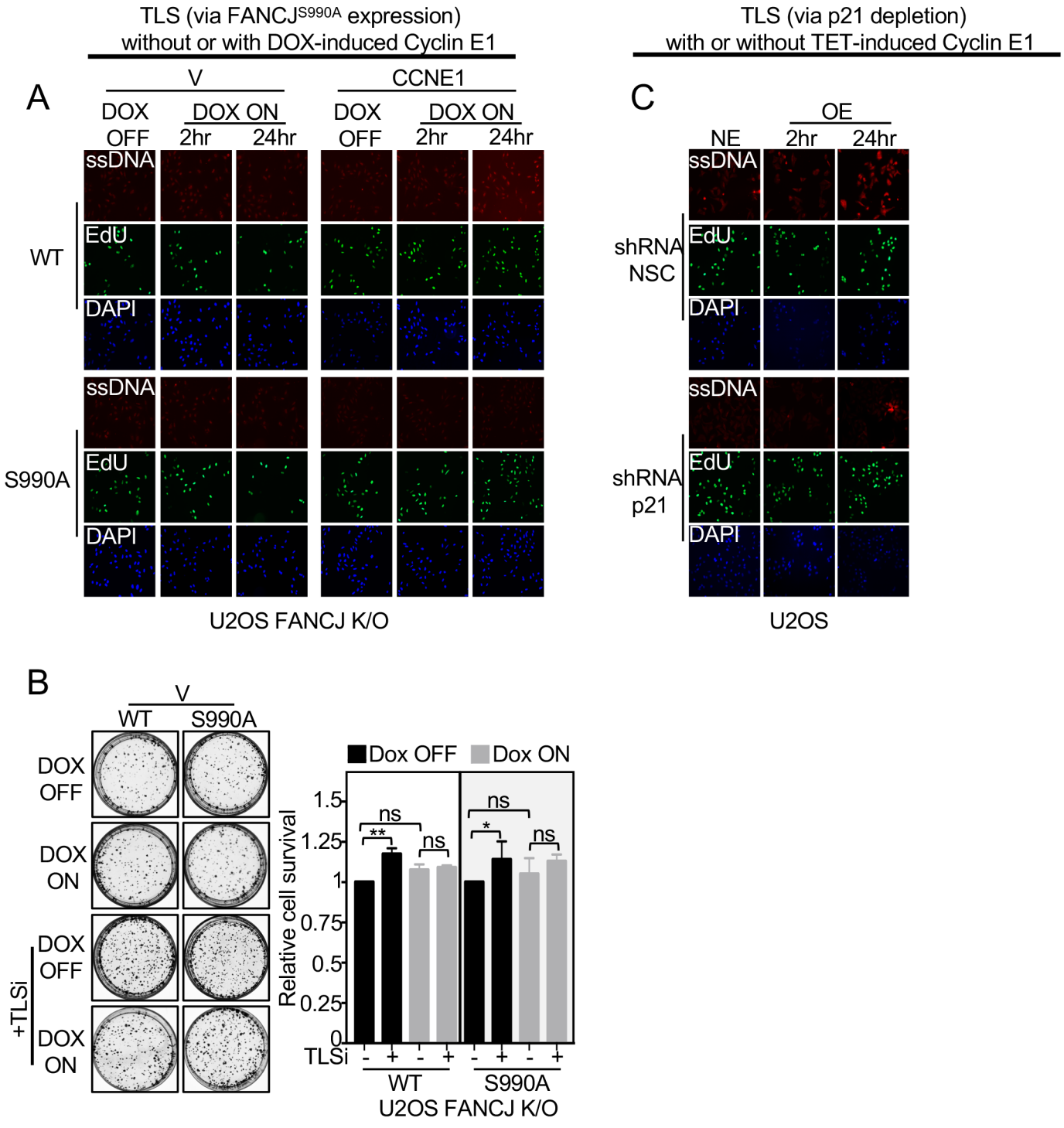
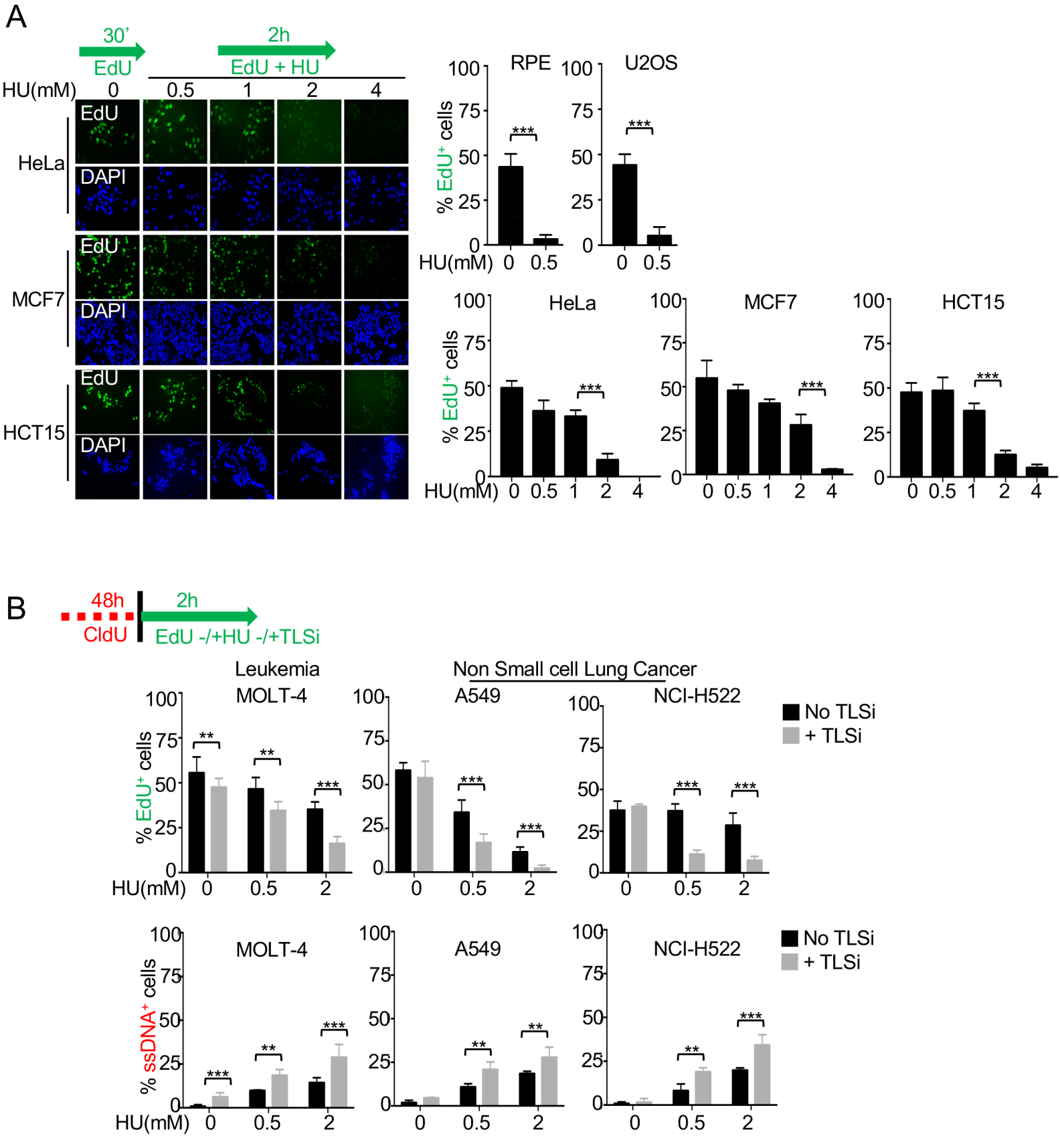
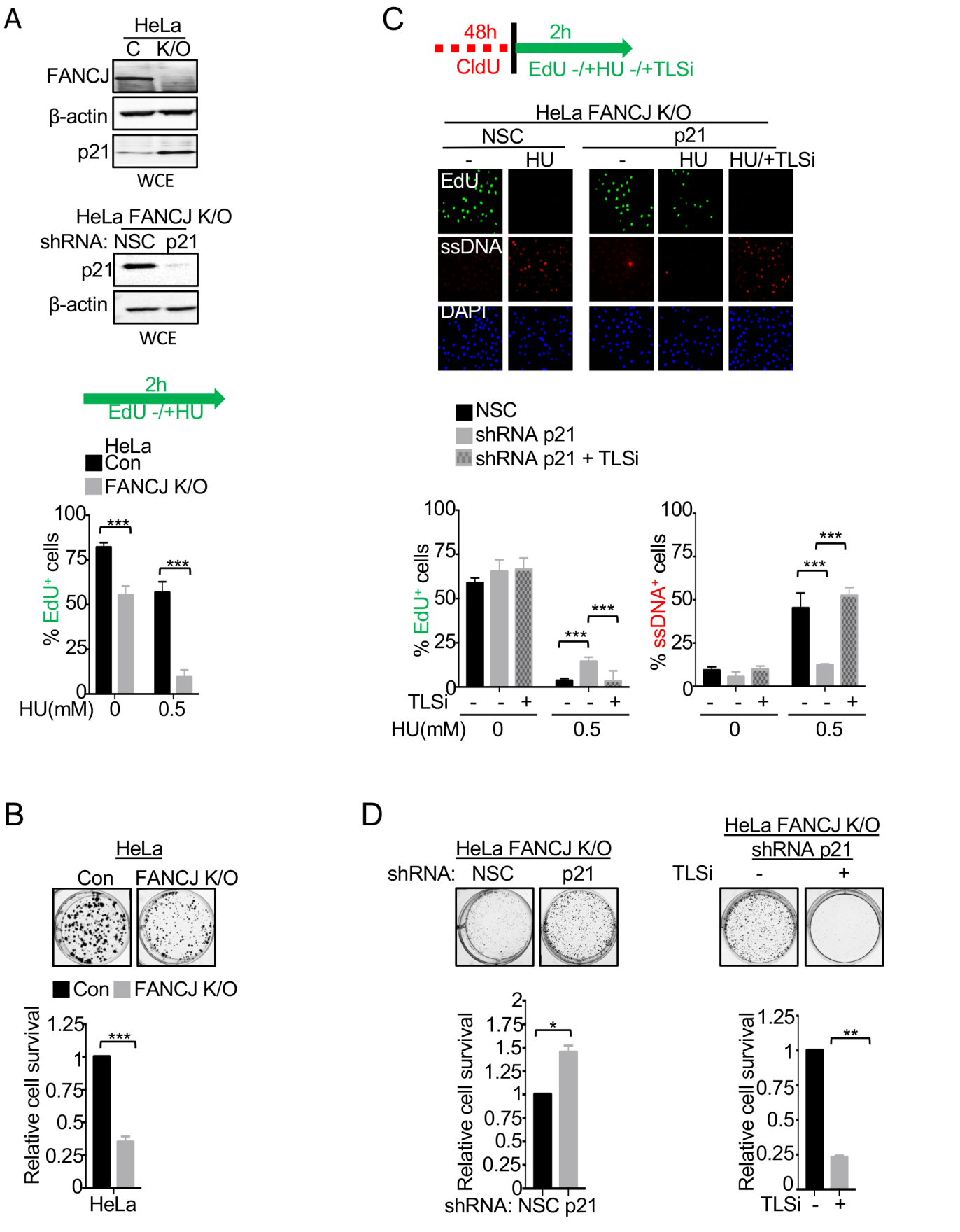
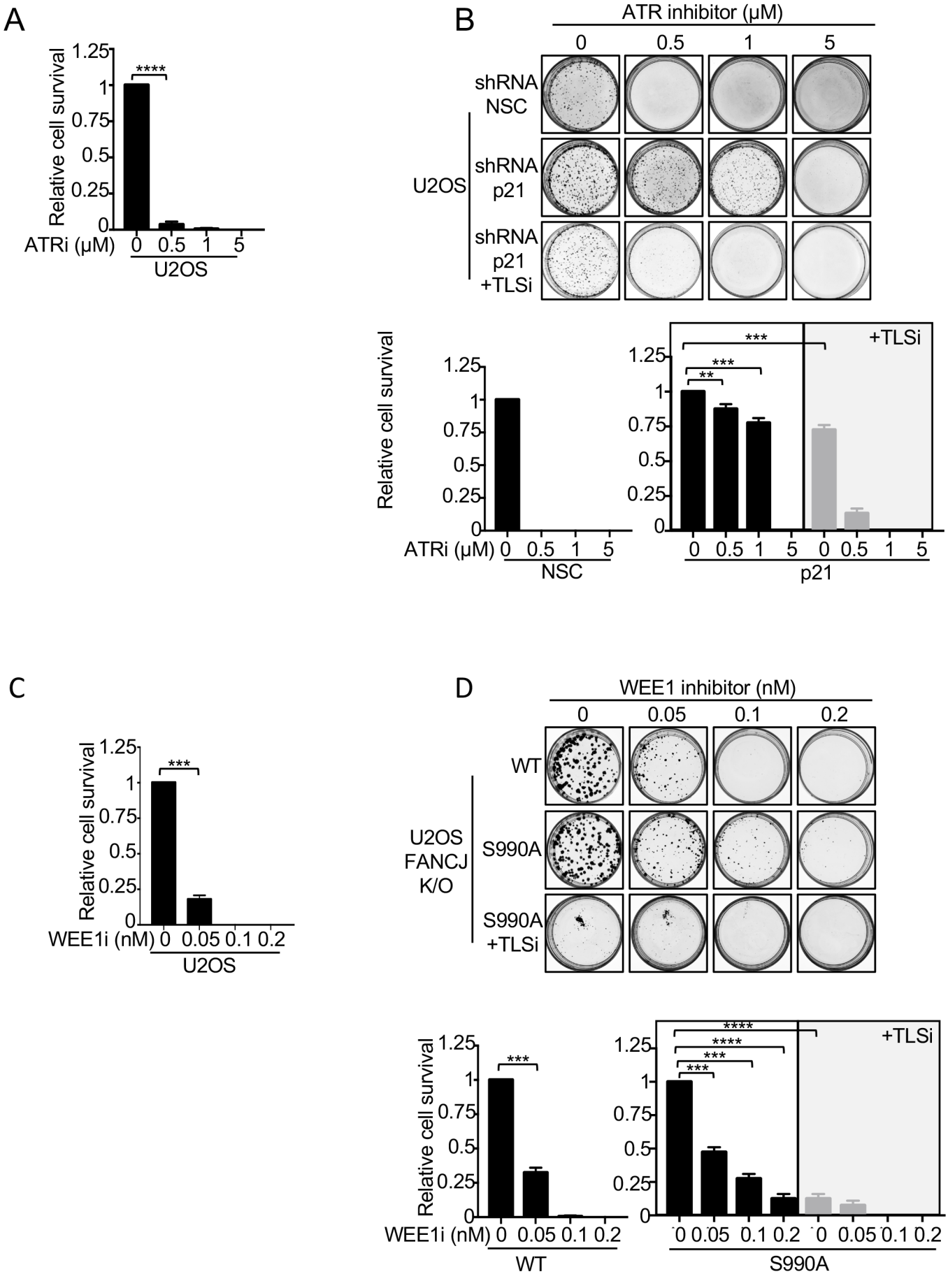

F
Untreated
TLSi
HU+TLSi
ssDNA
EdU
DAPI
WT
NSC
% EdU+ cells
% ssDNA+ cells
Unt
TLSi
HU+ TLSi
****
WT
NSC
G
Untreated
ATRi
HU+ATRi
S990A
p21
ns
**
***
S990A
p21
Unt
ATRi
HU+ ATRi

Fig S3


TLS (via FANCJ^{S990A} expression)
without or with DOX-induced Cyclin E1
A
V
CCNE1
DOX OFF
DOX ON 2hr
DOX ON 24hr
ssDNA
EdU
DAPI
WT
S990A
U2OS FANCJ K/O
TLS (via p21 depletion)
with or without TET-induced Cyclin E1
C
NE
OE 2hr
OE 24hr
shRNA NSC
shRNA p21
U2OS
B
V
WT
S990A
DOX OFF
DOX ON
+TLSi
Dox OFF
Dox ON
Relative cell survival
1.5
1.25
1
0.75
0.5
0.25
0
ns
**
*
TLSi
WT
S990A
U2OS FANCJ K/O


A
30'
EdU
2h
EdU + HU
HU(mM) 0 0.5 1 2 4
HeLa
MCF7
HCT15
EdU
DAPI
RPE
U2OS
% EdU+ cells
HeLa
MCF7
HCT15
B
48h
CldU
2h
EdU -/+HU -/+TLSi
Leukemia
MOLT-4
Non Small cell Lung Cancer
A549
NCI-H522
No TLSi
+ TLSi
% EdU+ cells
% ssDNA+ cells


A
HeLa
C K/O
FANCJ
β-actin
p21
WCE
HeLa FANCJ K/O
shRNA: NSC p21
p21
β-actin
WCE
2h
EdU -/+HU
HeLa
Con
FANCJ K/O
% EdU+ cells
HU(mM) 0 0.5
B
HeLa
Con FANCJ K/O
Con FANCJ K/O
Relative cell survival
HeLa
C
48h
CldU
2h
EdU -/+HU -/+TLSi
HeLa FANCJ K/O
NSC p21
- HU - HU HU/+TLSi
EdU
ssDNA
DAPI
NSC
shRNA p21
shRNA p21 + TLSi
% EdU+ cells
% ssDNA+ cells
TLSi
HU(mM) 0 0.5
D
HeLa FANCJ K/O
shRNA: NSC p21
HeLa FANCJ K/O
shRNA p21
TLSi - +
Relative cell survival
shRNA: NSC p21
TLSi - +


A
Relative cell survival
1.25
1
0.75
0.5
0.25
0
****
ATRi (μM) 0 0.5 1 5
U2OS
B
ATR inhibitor (μM)
0
0.5
1
5
shRNA NSC
U2OS
shRNA p21
shRNA p21 +TLSi
Relative cell survival
1.25
1
0.75
0.5
0.25
0
ATRi (μM) 0 0.5 1 5
NSC
***
**
***
+TLSi
0 0.5 1 5 0 0.5 1 5
p21
C
Relative cell survival
1.25
1
0.75
0.5
0.25
0
***
WEE1i (nM) 0 0.05 0.1 0.2
U2OS
D
WEE1 inhibitor (nM)
0
0.05
0.1
0.2
WT
U2OS FANCJ K/O
S990A
S990A +TLSi
Relative cell survival
1.25
1
0.75
0.5
0.25
0
***
WEE1i (nM) 0 0.05 0.1 0.2
WT
****
****
***
***
+TLSi
0 0.05 0.1 0.2 0 0.05 0.1 0.2
S990A