Supplementary Figures

Figure Legends

Supplementary Figure S1. Left panel shows western blots performed on HeLa cells 48 h after transient expression of PNKP-mGFP/mRFP (top), XRCC1-mGFP/mRFP (middle), and EGFP-FL LIG3/EGFP-ΔZnF LIG3 (bottom). Right panel shows a schematic outline for all fluorescent proteins used in this study

Supplementary Figure S2. Cells co-expressing mGFP L360D (see Figure 2C) and WT XRCC1 mRFP were tested in different laser micro-irradiation systems (405-nm laser diode and multi-photon 750-nm). WT XRCC1 mRFP shows robust recruitment in response to both systems.

Supplementary Figure S3. Western blot showing PARP1 levels in PARP1 WT (F20) and null (A1) MEFs, where A549 lysate was used as a positive control.

Supplementary Figure S4. (A) Immunofluorescence staining, showing reduced formation of PAR following laser micro-irradiation in cells pretreated with AG14361. γH2AX was used as a marker for DNA damage at laser tracks. (B) To ensure that the γH2AX seen in the laser micro-irradiated tracks did not arise from stalled replication (i.e. conversion of SSB into DSB), non-synchronized HeLa cells transiently expressing mRFP-PCNA (marker for S-phase) were subjected to multi-photon 750-nm laser micro-irradiation as described in Materials and Methods (approximately 40 cells were analyzed). Subsequently (5-10 minutes post laser micro-irradiation) cells were fixed and stained for γH2AX. A robust signal for γH2AX was observed in all cells analyzed. A higher magnification of individual cells reveals PCNA distribution in S-phase (upper panel showing punctate PCNA distribution) and in non S-phase cells (lower panel showing a more homogeneous distribution of PCNA outside of the nucleoli). γH2AX is detected in laser micro-IR tracks in S-phase and non S-phase cells. (C) Confirmation of the PARP inhibition results shown in Figure 5, using different PARP inhibitor, PJ-34. For recruitment curve, error bars represent S.E.M; *n= 24*.

Supplementary Figure S5. Western blot showing the knock down of LIG3 by the Hush plasmid at different time points post transfection. Actin levels represent loading controls.

Supplementary Figure S6. Endogenous LIG3 shows robust accumulation at sites of DNA damage created by either (A) laser micro-irradiation or (B) by 10 mM H_20_2 even upon pre-treatment of cells with 1 and 2 μM AG14361. (C and D) LIG3 did not co-localize with γH2AX (universal DSB marker) or 53BP1 (NHEJ DSB marker). For AG14361 treatments, cells were incubated with either 1 or 2 μM drug at 37ºC for 1-2 hours, then cells were either subjected to laser micro-irradiation (see Materials and Methods), or treated with 10 mM H_20_2 for 10 minutes, then fixed and stained for immunofluorescence. We need to say something about the zoomed images in B and C

Supplementary Figure S7. FRAP analysis showing differences in binding kinetics in response to H_2O_2 damage between FL-LIG3 and ΔZnF-LIG3. Error bars represent S.E.M; *n=24.*

Supplementary Figure S8. FRAP analysis showing the differences in binding kinetics of PARP1 in the presence and absence of DNA damage introduced by H_2O_2. Error bars represent S.E.M; *n=24.*

Figure S1

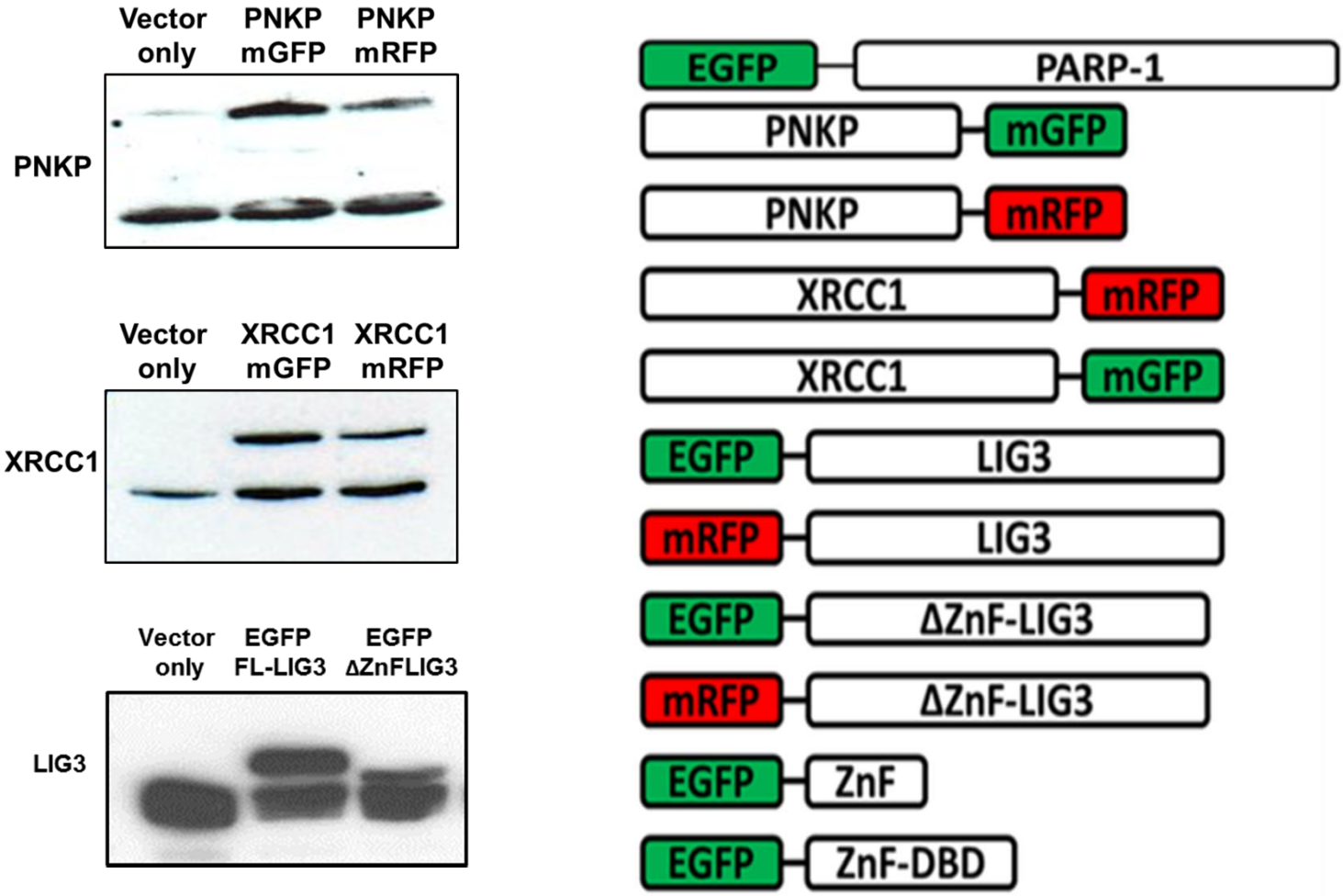

Vector only
PNKP mGFP
PNKP mRFP
PNKP
Vector only
XRCC1 mGFP
XRCC1 mRFP
XRCC1
Vector only
EGFP FL-LIG3
EGFP ΔZnFLIG3
LIG3
EGFP
PARP-1
PNKP
mGFP
PNKP
mRFP
XRCC1
mRFP
XRCC1
mGFP
EGFP
LIG3
mRFP
LIG3
EGFP
ΔZnF-LIG3
mRFP
ΔZnF-LIG3
EGFP
ZnF
EGFP
ZnF-DBD

Figure S2

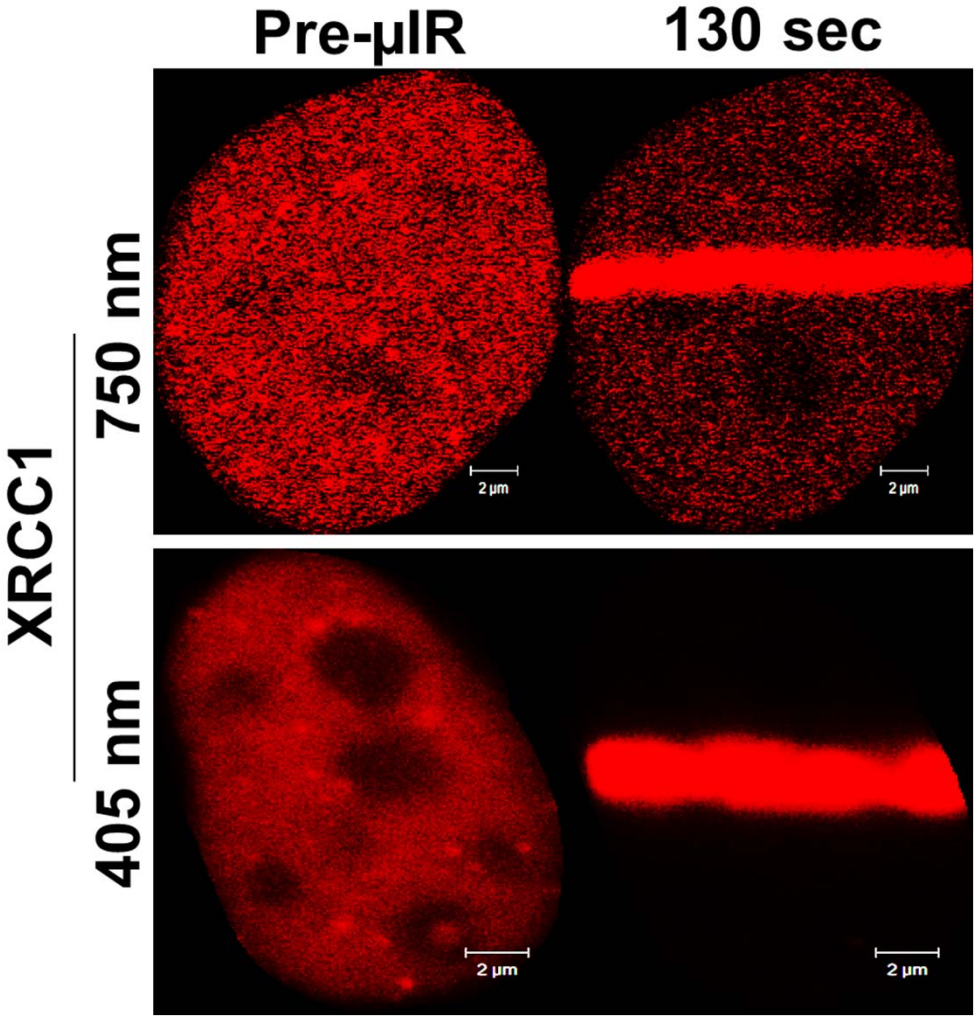


Figure S3

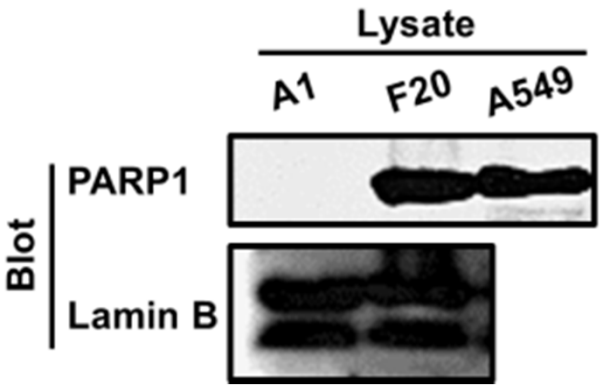
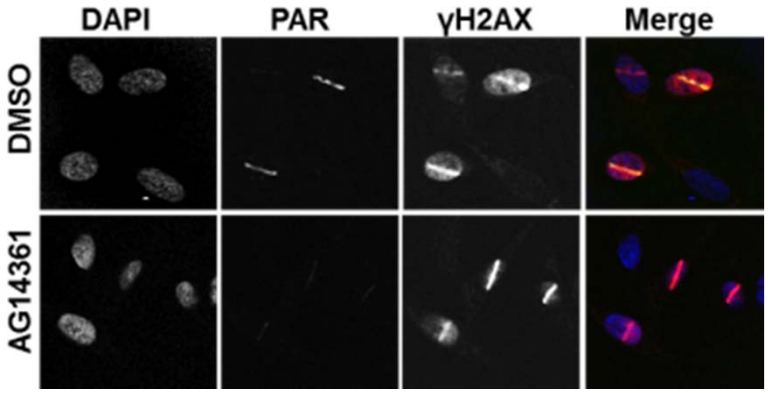
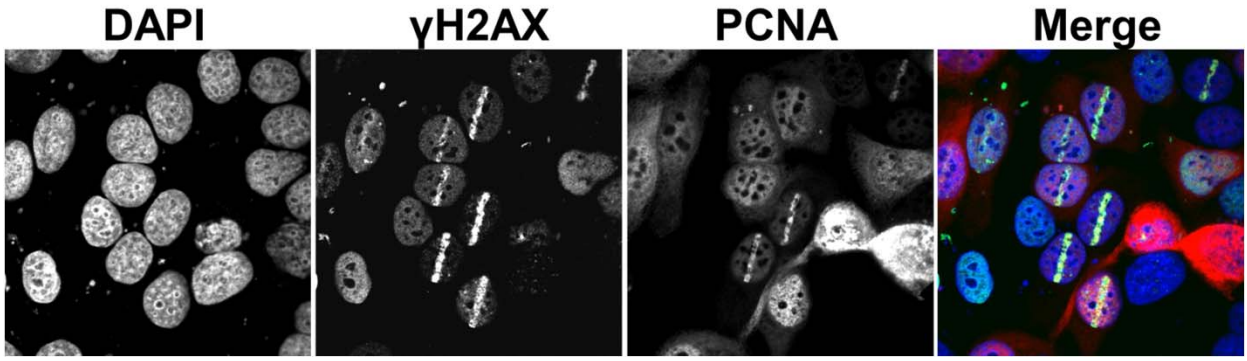


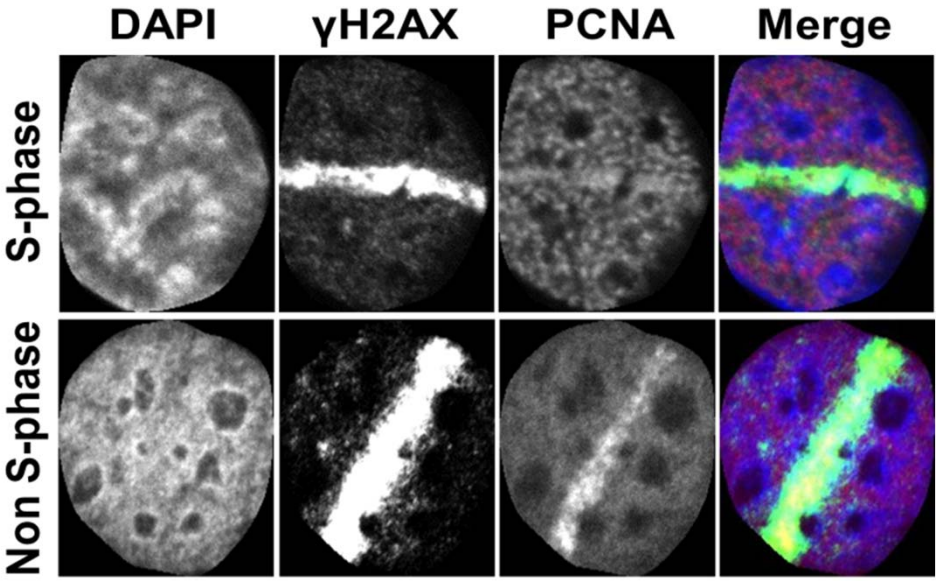
Figure S4

A)


DAPI
PAR
γH2AX
Merge
DMSO
AG14361

B)


DAPI
γH2AX
PCNA
Merge


DAPI
γH2AX
PCNA
Merge
S-phase
Non S-phase

C)

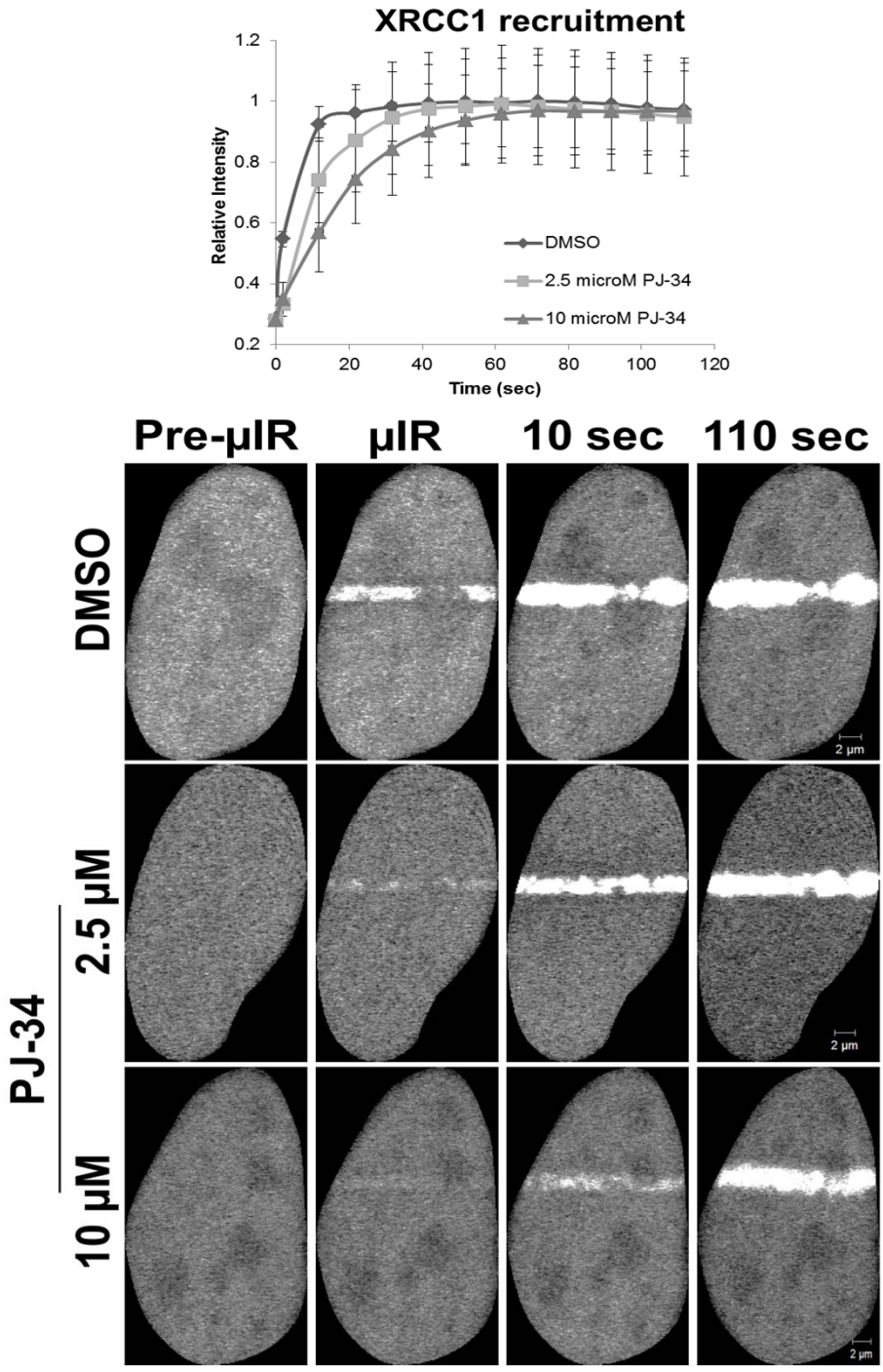

XRCC1 recruitment
Relative Intensity
1.2
1
0.8
0.6
0.4
0.2
0
20
40
60
80
100
120
Time (sec)
DMSO
2.5 microM PJ-34
10 microM PJ-34
Pre-μIR
μIR
10 sec
110 sec
DMSO
PJ-34
2.5 μM
10 μM
2 μm

Figure S5

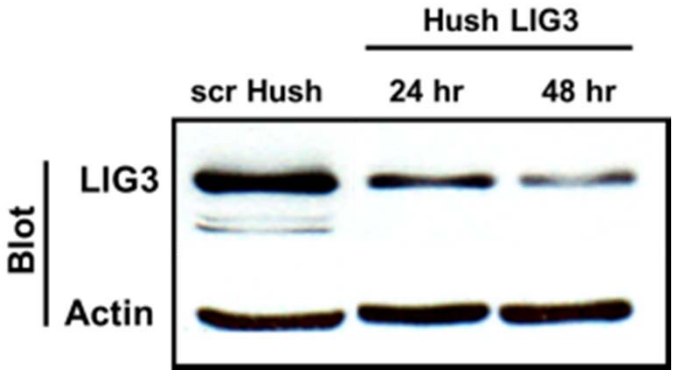


Figure S6

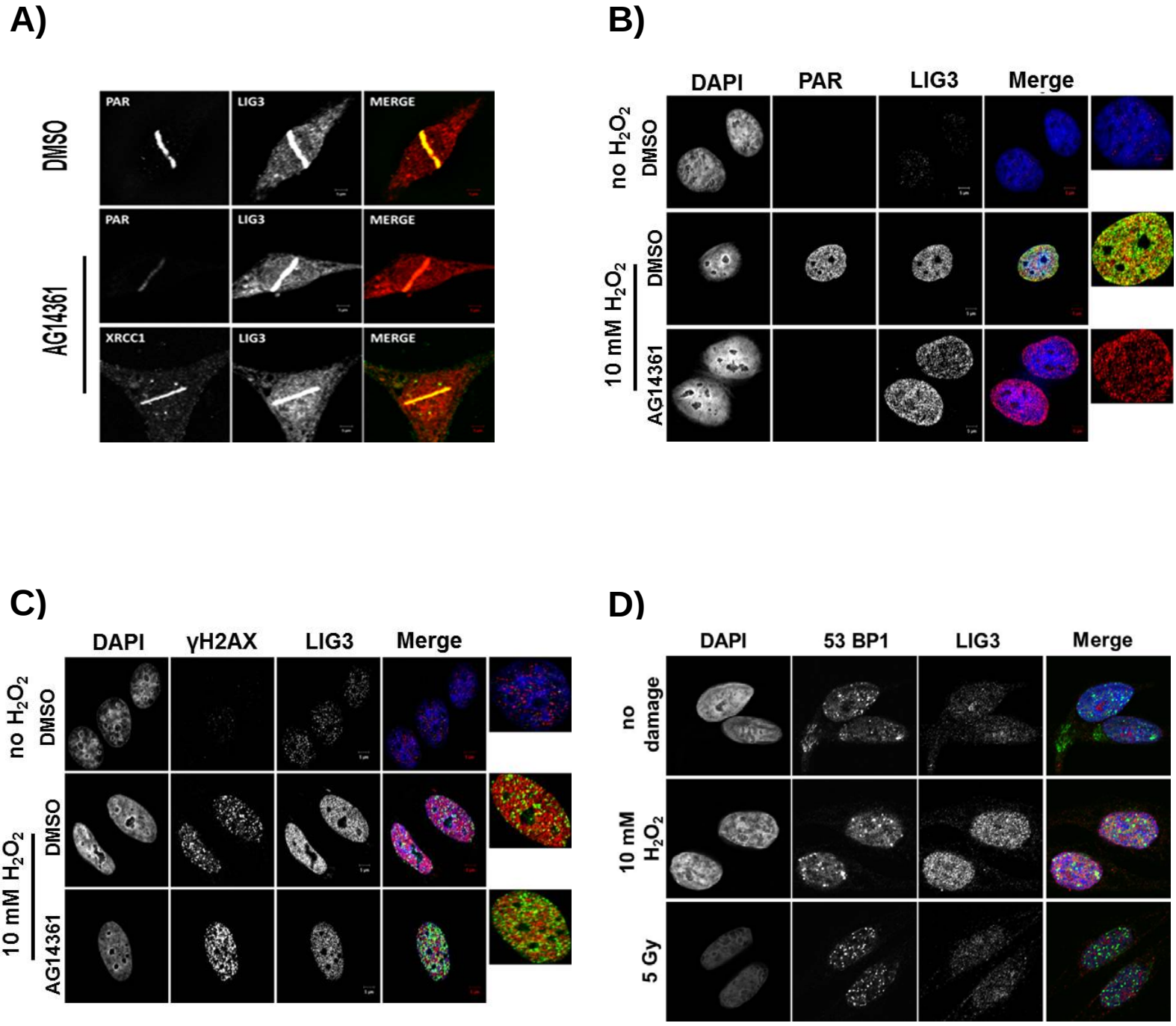

A)
DMSO
AG14361
PAR
LIG3
MERGE
XRCC1
B)
DAPI
PAR
LIG3
Merge
no H2O2
DMSO
10 mM H2O2
DMSO
AG14361
C)
DAPI
γH2AX
LIG3
Merge
no H2O2
DMSO
10 mM H2O2
DMSO
AG14361
D)
DAPI
53 BP1
LIG3
Merge
no damage
10 mM H2O2
5 Gy

Figure S7

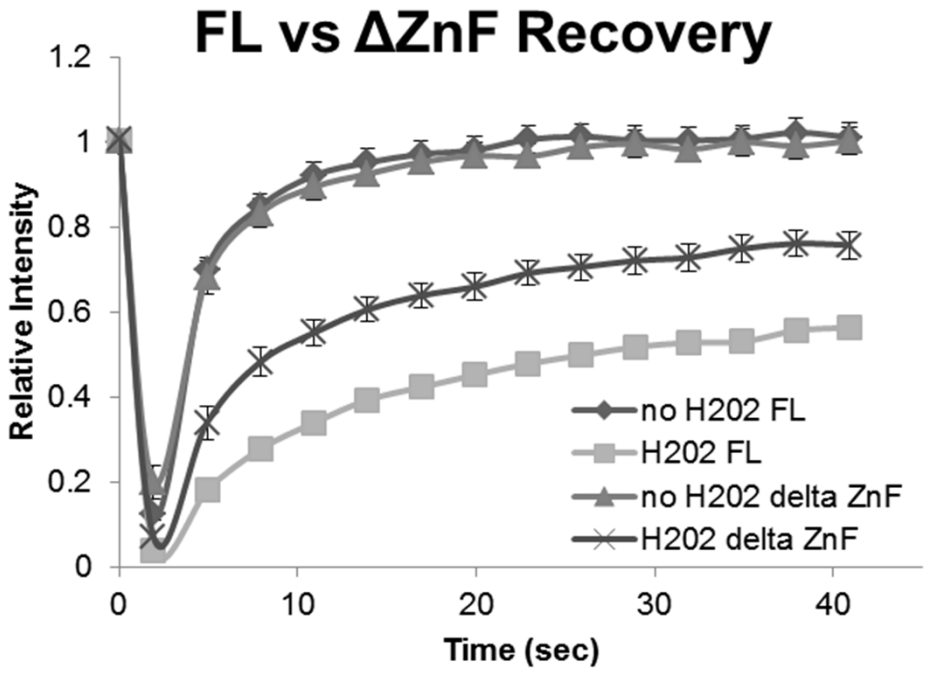
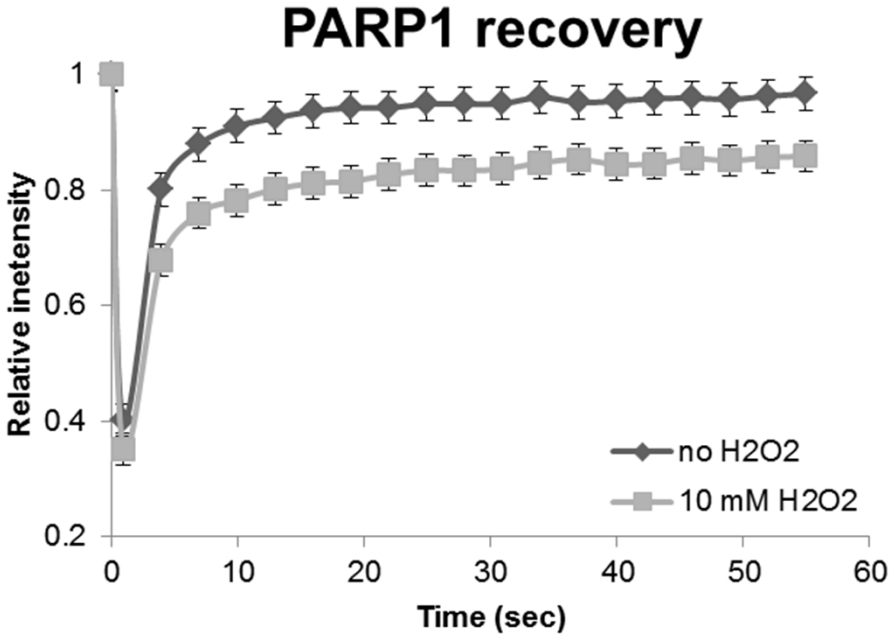

FL vs ΔZnF Recovery
Relative Intensity
Time (sec)
1.2
1
0.8
0.6
0.4
0.2
0
0
10
20
30
40
no H202 FL
H202 FL
no H202 delta ZnF
H202 delta ZnF

Figure S8


PARP1 recovery
Relative inetensity
1
0.8
0.6
0.4
0.2
0
10
20
30
40
50
60
Time (sec)
no H2O2
10 mM H2O2