Cell Reports, Volume *32*

Supplemental Information

Cell Cycle Checkpoints Cooperate to Suppress DNA- and RNA-Associated Molecular Pattern Recognition and Anti-Tumor Immune Responses

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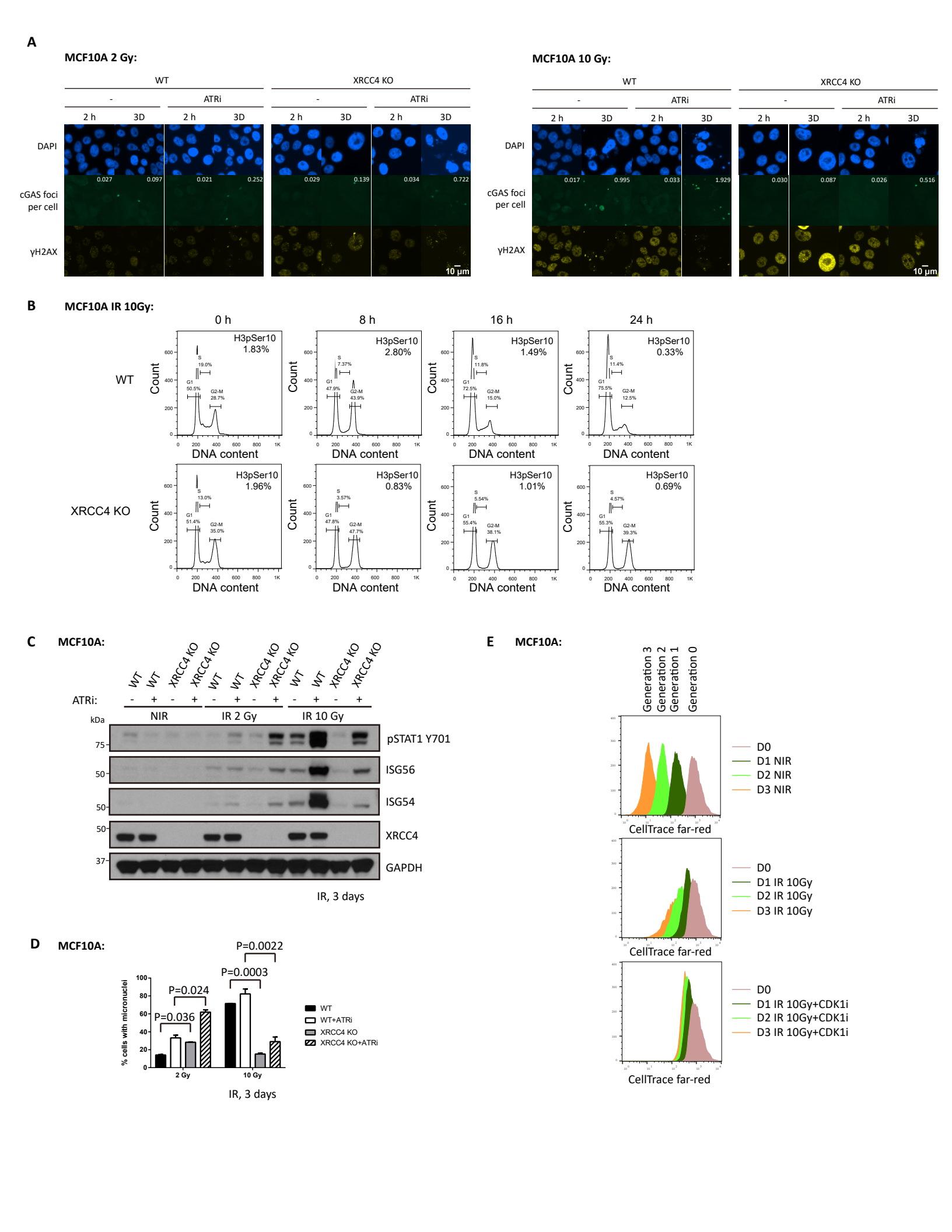

A
MCF10A 2 Gy:
WT
XRCC4 KO
-
ATRi
2 h
3D
DAPI
cGAS foci per cell
γH2AX
0.027
0.097
0.021
0.252
0.029
0.139
0.034
0.722
10 µm
MCF10A 10 Gy:
0.017
0.995
0.033
1.929
0.030
0.087
0.026
0.516
B
MCF10A IR 10Gy:
0 h
8 h
16 h
24 h
WT
XRCC4 KO
H3pSer10
1.83%
2.80%
1.49%
0.33%
1.96%
0.83%
1.01%
0.69%
Count
DNA content
C
MCF10A:
ATRi:
NIR
IR 2 Gy
IR 10 Gy
kDa
75
50
pSTAT1 Y701
ISG56
ISG54
XRCC4
GAPDH
IR, 3 days
D
MCF10A:
P=0.0022
P=0.0003
P=0.024
P=0.036
% cells with micronuclei
2 Gy
10 Gy
WT
WT+ATRi
XRCC4 KO
XRCC4 KO+ATRi
IR, 3 days
E
MCF10A:
Generation 3
Generation 2
Generation 1
Generation 0
D0
D1 NIR
D2 NIR
D3 NIR
CellTrace far-red
D1 IR 10Gy
D2 IR 10Gy
D3 IR 10Gy
D1 IR 10Gy+CDK1i
D2 IR 10Gy+CDK1i
D3 IR 10Gy+CDK1i

Figure S1. Low dose irradiation allows micronuclei formation and inflammatory signaling in c-NHEJ deficient MCF10A cells, Related to Figure 1.

(A) WT cells or XRCC4 KO cells were irradiated with 2 Gy or 10 Gy, and then cultured with or without ATRi for indicated time before fixation for immunofluorescence staining.

(B) WT cells or XRCC4 KO cells were irradiated with 10 Gy followed by time course collection for cell cycle and mitotic population analysis using flow cytometry.

(C and D) WT cells or XRCC4 KO cells were treated with 2 Gy or 10 Gy IR, and then maintained for 3 days with or without ATRi before western blot analysis (C) or qualification of cells with micronuclei (D). Mean values and S.E.M (n=3).

(E) MCF10A cells were resuspended in medium containing 1 uM CellTrace far-red dye (Thermo) for 20 mins and subsequently washed and seeded in dye-free medium. The next day, cells were either left untreated (NIR, top panel) or treated with 10 Gy (middle panel) or 10 Gy+CDK1i (bottom panel), followed by collection for CellTrace far red signal analysis using flowcytometry. Reductions in dye content over time indicate cell division due to dye dilution. Non-irradiated (NIR) cells undergo three divisions whereas irradiated controls perform 1-2 divisions. CDK1i prevents cell division.

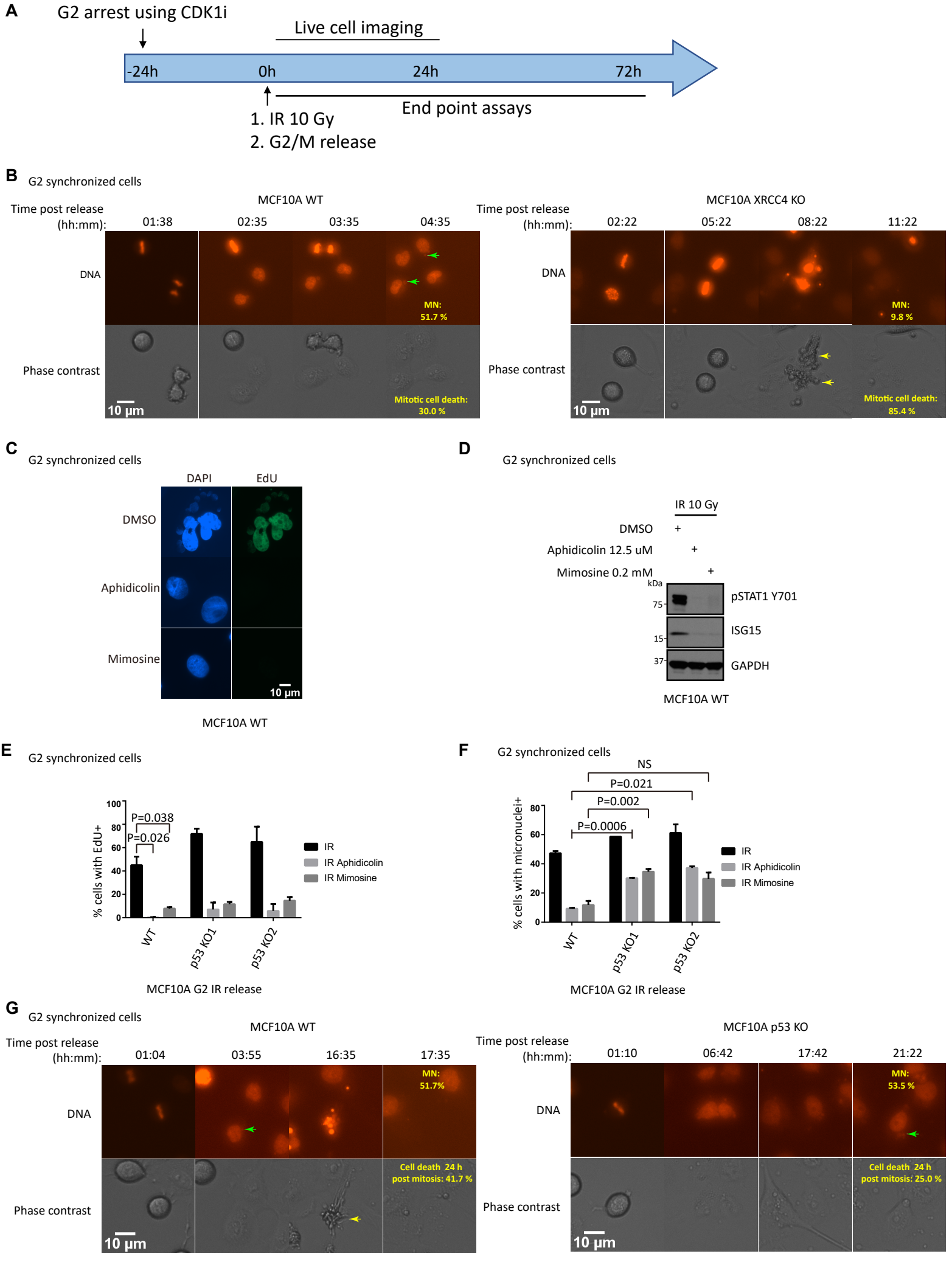

A
G2 arrest using CDK1i
Live cell imaging
-24h
0h
24h
72h
End point assays
1. IR 10 Gy
2. G2/M release
B
G2 synchronized cells
MCF10A WT
Time post release (hh:mm):
01:38
02:35
03:35
04:35
DNA
Phase contrast
MN: 51.7 %
Mitotic cell death: 30.0 %
10 µm
MCF10A XRCC4 KO
Time post release (hh:mm):
02:22
05:22
08:22
11:22
MN: 9.8 %
Mitotic cell death: 85.4 %
C
G2 synchronized cells
DAPI
EdU
DMSO
Aphidicolin
Mimosine
10 µm
MCF10A WT
D
G2 synchronized cells
IR 10 Gy
DMSO +
Aphidicolin 12.5 uM +
Mimosine 0.2 mM +
kDa
75
15
37
pSTAT1 Y701
ISG15
GAPDH
MCF10A WT
E
G2 synchronized cells
% cells with EdU+
P=0.038
P=0.026
IR
IR Aphidicolin
IR Mimosine
WT
p53 KO1
p53 KO2
MCF10A G2 IR release
F
G2 synchronized cells
NS
P=0.021
P=0.002
P=0.0006
% cells with micronuclei+
IR
IR Aphidicolin
IR Mimosine
WT
p53 KO1
p53 KO2
MCF10A G2 IR release
G
G2 synchronized cells
MCF10A WT
Time post release (hh:mm):
01:04
03:55
16:35
17:35
DNA
Phase contrast
MN: 51.7%
Cell death 24 h post mitosis: 41.7 %
10 µm
MCF10A p53 KO
Time post release (hh:mm):
01:10
06:42
17:42
21:22
MN: 53.5 %
Cell death 24 h post mitosis: 25.0 %

Figure S2. Increased micronuclei formation in MCF10A p53 KO cells after IR, Related to Figure 4.
(A) Scheme showing working flow of live cell imaging (B and G) and end point assays (C, D, E and F).
(B) G2 arrested MCF10A WT cells and MCF10A XRCC4 KO cells were irradiated with 10 Gy and then released from G2 phase followed by live cell imaging to monitor micronuclei formation. siR-DNA was used to visualize DNA in live cells. Micronuclei were recognized as positive siR-DNA staining outside of primary nuclei. Mitotic cell death was identified as anaphase cells broke into small pieces, detached from dish, and floated in medium under both phase contrast and siR-DNA staining channels.
(C and D) G2 arrested MCF10A cells were irradiated and then released but blocked before next S phase to probe micronuclei formation in the presence of EdU (C) and inflammatory signaling (D).
(E and F) MCF10A WT cells and MCF10A p53 KO cells were treated as in (C) for quantification of cells with EdU incorporation (E) and cells with micronuclei (F). Mean values and S.E.M (n=3).
(G) MCF10A WT cells and MCF10A p53 KO cells were treated as in (B) to monitor cell death after mitosis in the following 24 hours. Cell death post mitosis was qualified when cells broke into small pieces and detached from the slide following mitotic progression with micronuclei under both phase contrast and siR-DNA staining channels.

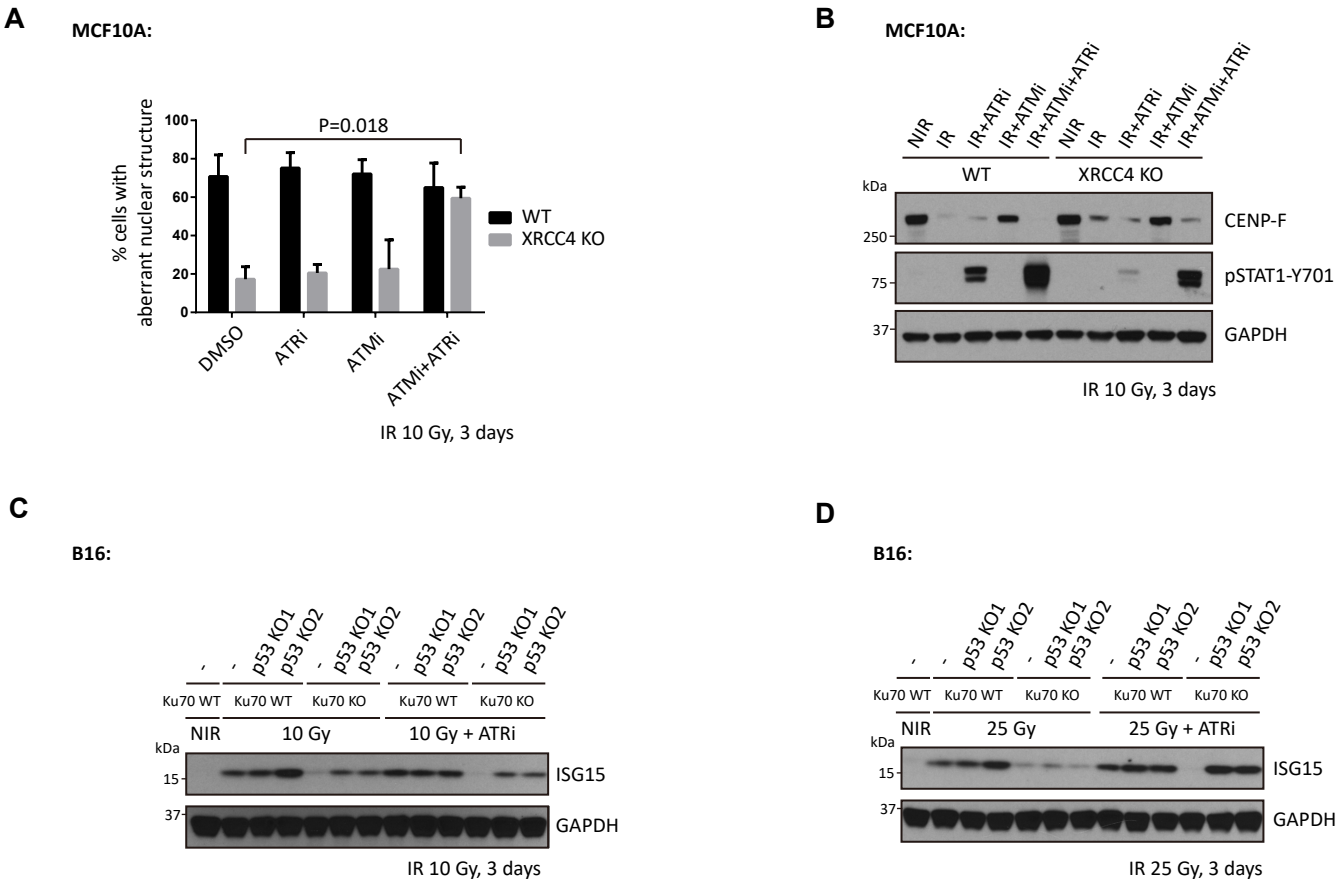
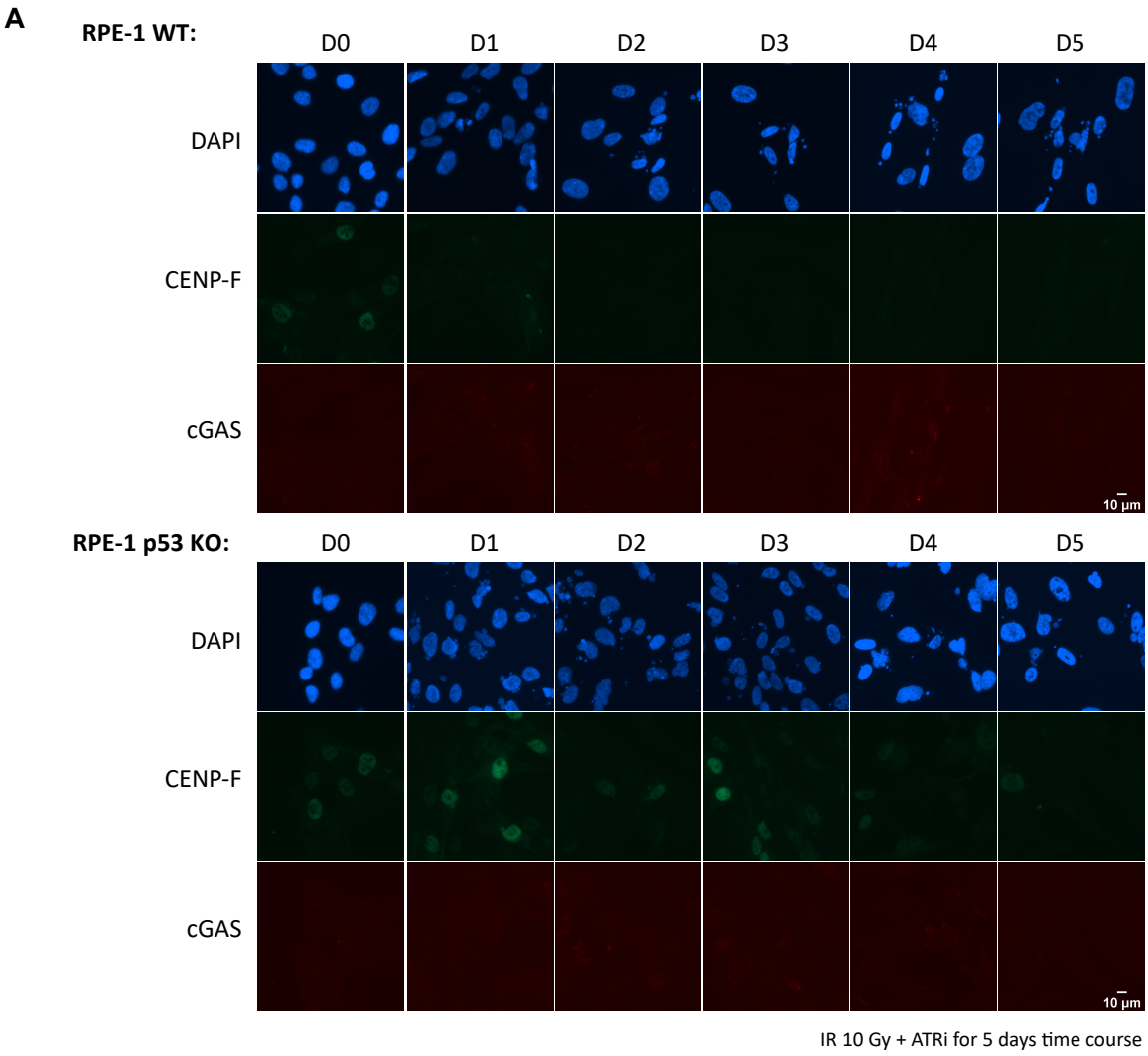


Figure S3. Restored inflammatory signaling after disruption of G1/S and G2/M checkpoints in c-NHEJ deficient cells, Related to Figure 4.

(A and B) MCF10A WT cells or XRCC4 KO cells were irradiated with 10 Gy and then maintained in medium with indicated inhibitors for 3 days before qualification for cells with micronuclei (A) or western blot analysis (B). Mean values and S.E.M (n=3).

(C and D) B16 WT cells and B16 cells with p53 KO or/and Ku70 KO were subjected to 10 Gy IR (C) or 25 Gy IR (D) treatment. Cells were then maintained in medium with or without ATR inhibitor for 3 days before western blot analysis.



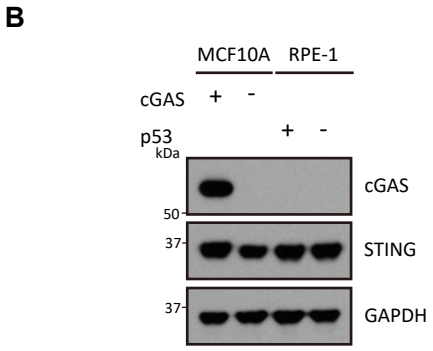


Figure S4. Undetectable cGAS expression in RPE-1 cells, Related to Figure 5.
(A) RPE-1 WT cells and RPE-1 p53 KO cells were irradiated with 10 Gy and subjected to culture in medium with ATRi. Cells were then fixed at indicated time for immunofluorescence staining.
(B) MCF10A cells and RPE-1 cells were collected to probe cGAS expression using western blot analysis.

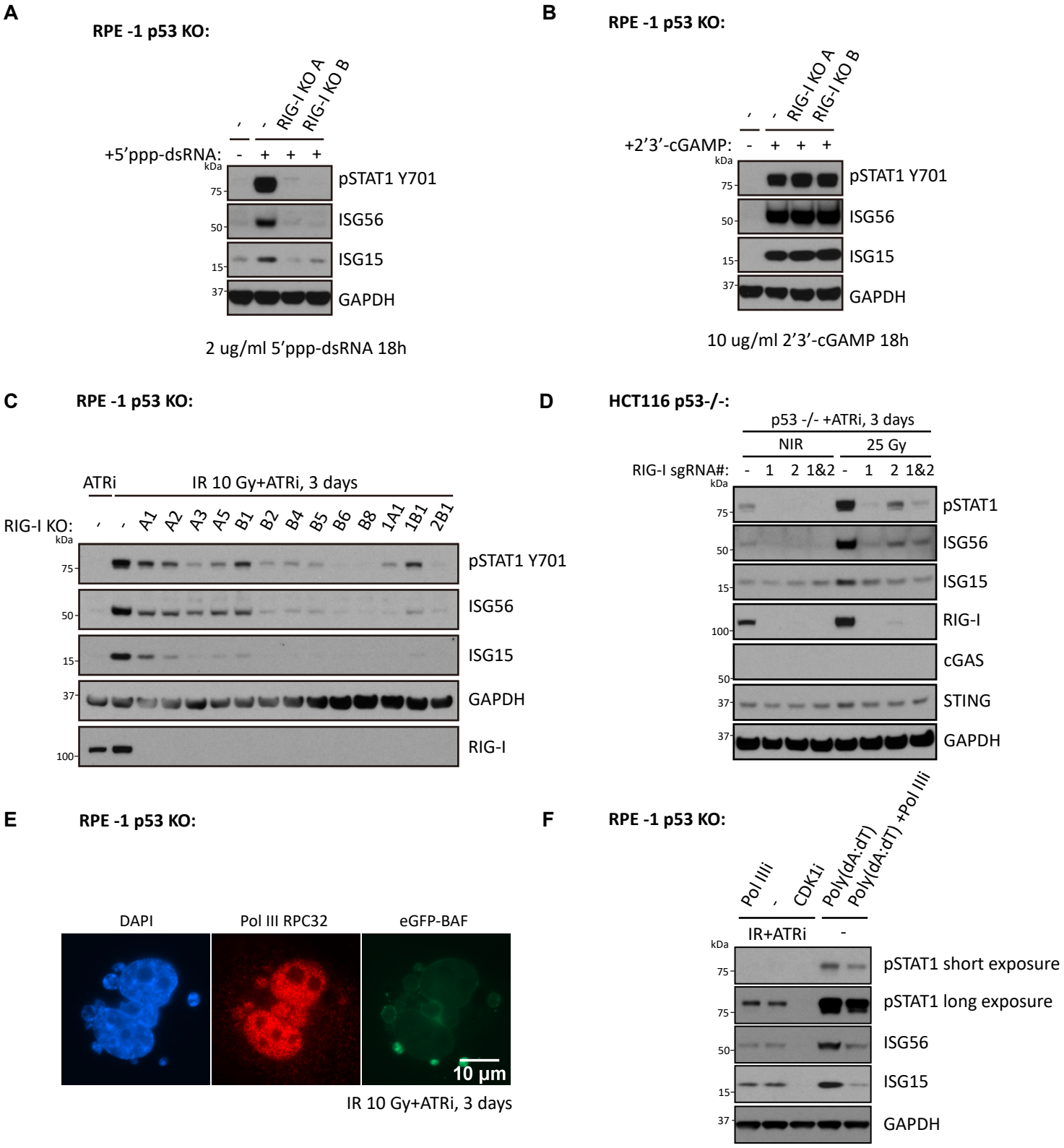


Figure S5. Pol III independent RIG-I activation in RPE-1 p53 KO cells following IR plus ATRi treatments, Related to Figure 6.

(A and B) RPE-1 p53 KO cells and RPE-1 p53 RIG-I DKO cells were transfected with RIG-I agonist (A) or STING agonist (B) and then collected 18 hours later for western blot analysis.

(C) RPE-1 p53 KO cells and RPE-1 p53 RIG-I DKO single clone cells were irradiated with 10 Gy and subjected to culture in medium with ATRi for 3 days before collection for western blot analysis.

(D) RIG-I depleted HCT116 p53-/- pool cells were achieved by sequential lentiviral infections of Cas9 and sgRNAs targeting RIG-I (#1, #2, or #1&2). Parental HCT116 p53-/- cells and HCT116 p53-/- cells with RIG-I depletion were left untreated (NIR) or treated with 25 Gy followed by culture in medium with ATRi for 3 days before collection for western blot analysis.

(E) RPE-1 p53 KO cells expressing ectopic eGFP tagged BAF were irradiated with 10 Gy and incubated in medium containing ATRi for 3 days before fixation for immunofluorescence staining.

(F) 10 Gy irradiated RPE-1 p53 KO cells were maintained in medium with 2.5 µM ATRi, 2.5 µM ATRi+ 40 µM Pol IIIi or 2.5 µM ATRi+ 9 µM CDK1i for 3 days before collection for western blot analysis. Cells transfected with poly(dA:dT) at a final concentration of 0.5 µg/ml were cultured in the absence or presence of 40 µM Pol IIIi for 1 day before western blot analysis.