Appendix - additional supporting results

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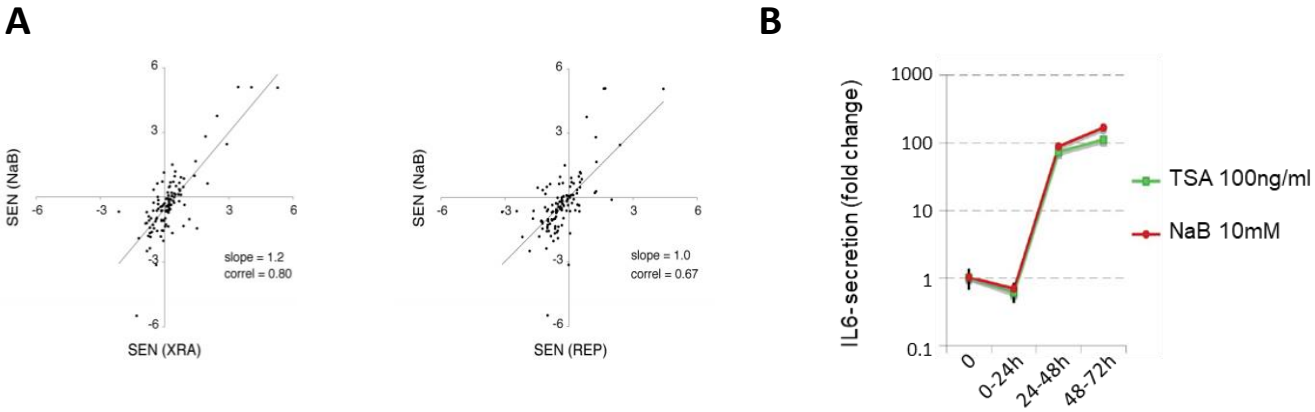
Appendix figure S1

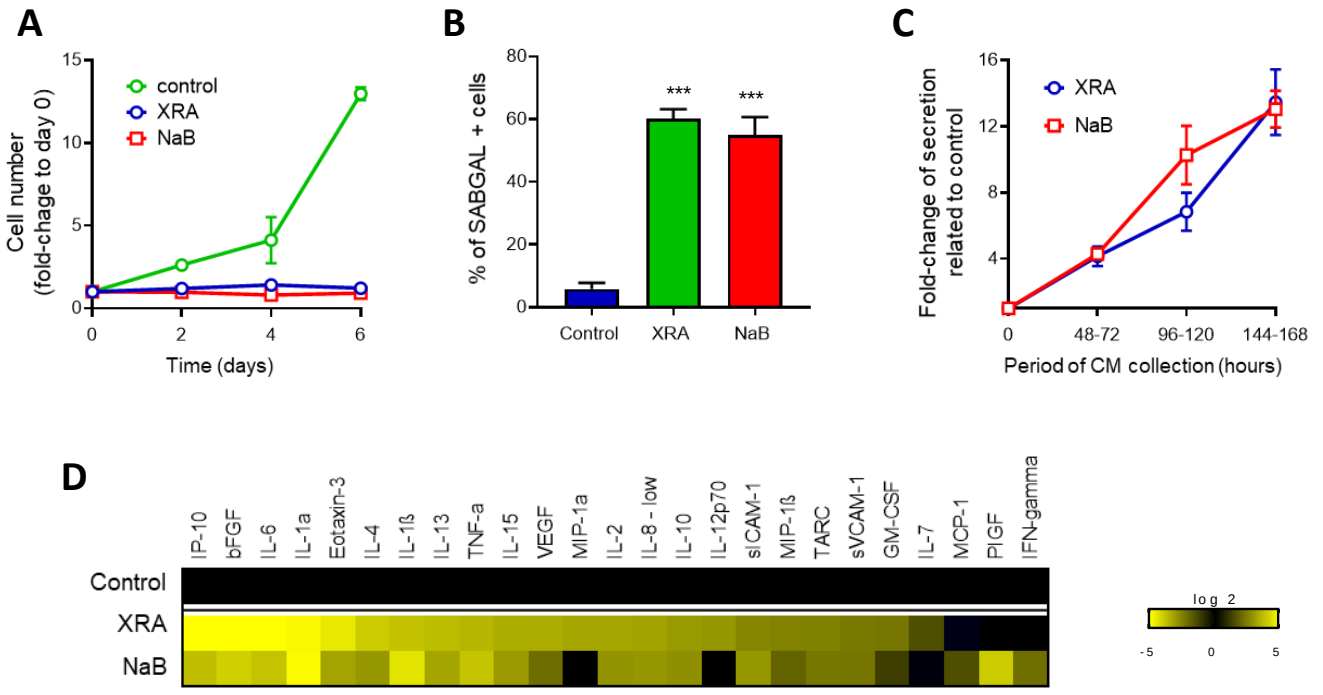


Appendix Figure S1. Complementary data for figure 1

(A) Correlation between NaB and other senescence-induced secretory profiles analyzed in Figure 1D. Correlations between NaB vs. XRA or NaB vs. REP were done using control as a baseline and are depicted as log2-fold changes.

(B) HCA2-hT cells were untreated or treated 1 or 2 days with NaB (10 mM) or Trichostatin A (TSA; 100 ng/ml) and replaced by serum-free medium over the next 24 hours. IL-6 secretion was analyzed by ELISA. Data are reported as fold increase relative to untreated control cells.

Appendix figure S2



Appendix Figure S2. XRA and NaB trigger SA phenotype and SASP in PC-3

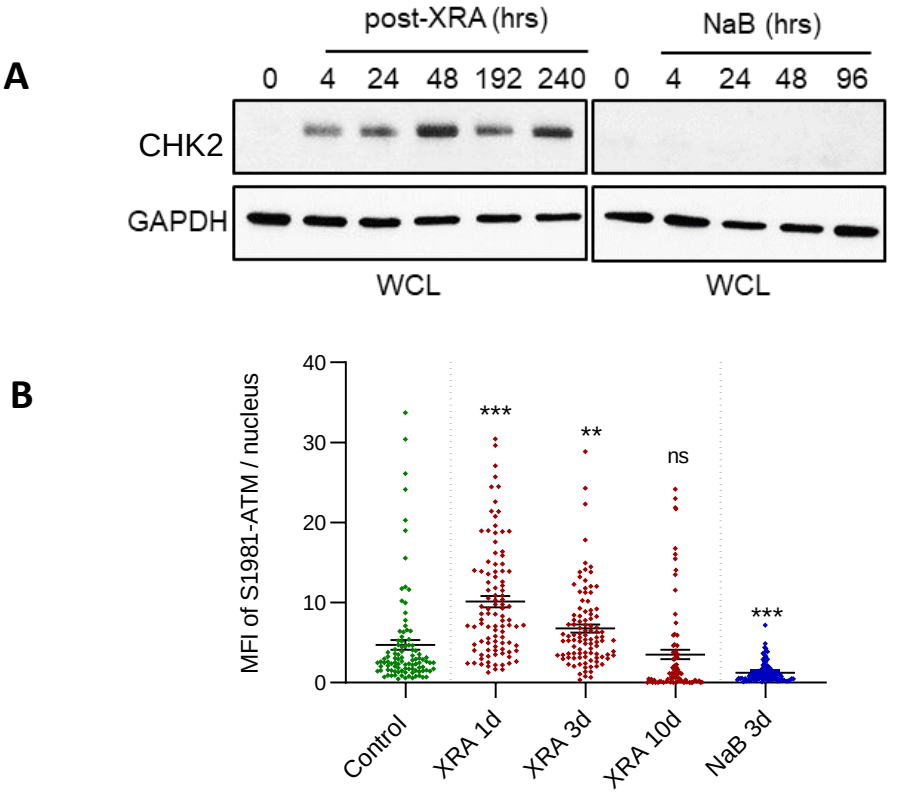
(A) PC-3 prostate cancer cells were untreated (control), exposed to 2 mM of NaB or XRA (4 Gy). Cells were fixed 2, 4 and 6 days after treatment and stained with the fluorescent DNA dye DRAQ5. Cell growth was evaluated using the fluorescence intensity.

(B) SA-ß-Galactosidase assay performed 5 days post-treatment on PC-3. Unpaired T-test: *** $p < 0.001$

(C) Il-6 secreted by PC-3 cells untreated, irradiated (XRA, 4Gy) or treated with NaB (2mM) XRA (4Gy) or PC-3 treated with NaB (2mM) were assessed by ELISA. CM were collected in serum-free condition during the indicated times. Data are means ± S.D. of triplicates nd are representative of two independent experiments.

(D) Soluble factors secreted by untreated, XRA (5 days post-4Gy) or PC-3 treated with NaB (5 days; 2mM) were analyzed with multiplex immunoassay (40-VPlex MSD). Secretion of control cells was a reference for baseline. Signals higher than baseline are yellow; signals below baseline are blue; unchanged fold variations are black (heat map key indicates log2-fold changes from the control).

Appendix figure S3

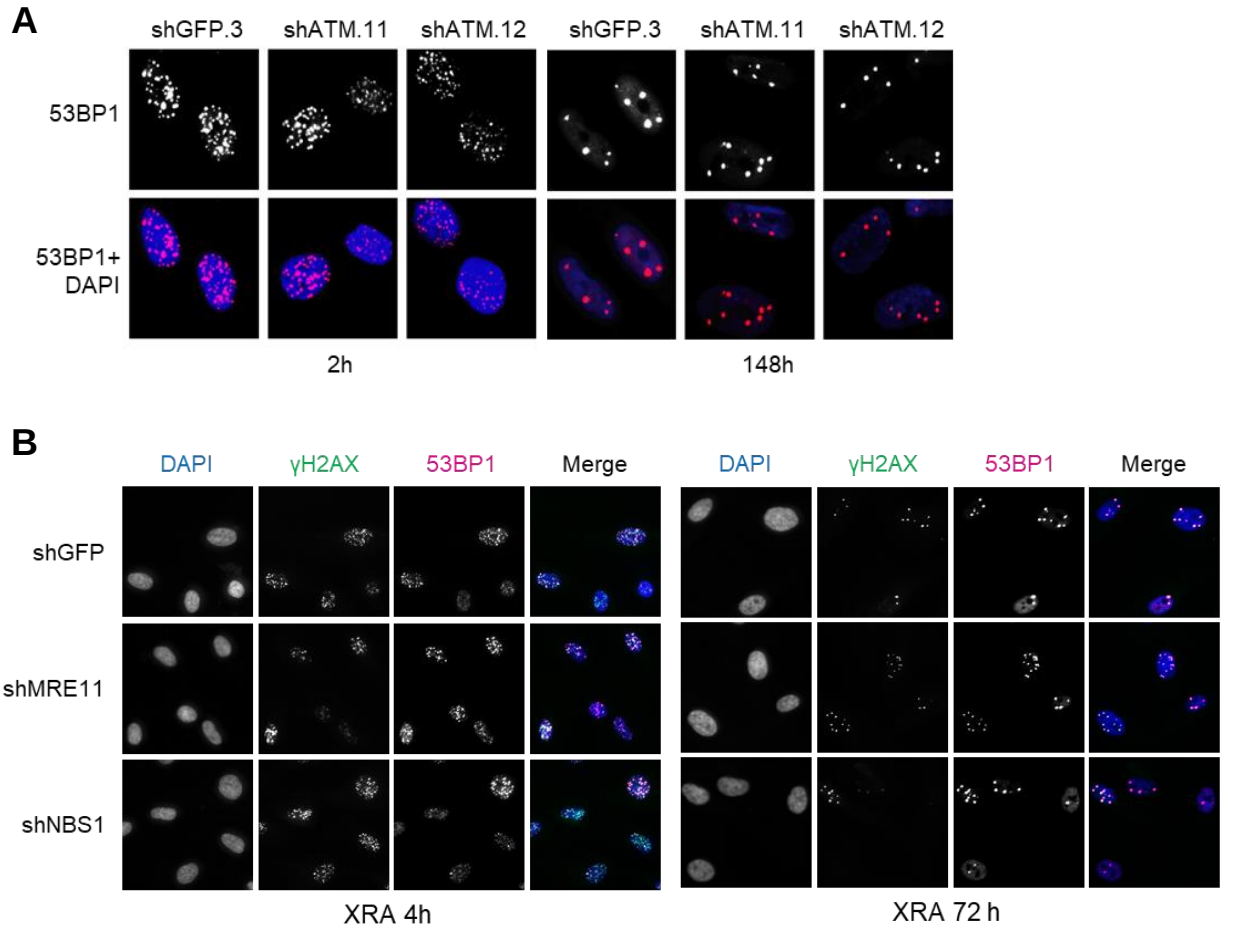


Appendix Figure S3. NaB do not induced canonical DDR activation

(A) HCA2-hT cells were irradiated with 10 Gy of XRA or treated with 5 mM of NaB. Total cell lysates were collected at the indicated times. Expression of phosphorylated CHK2 (T68CHK2) was analyzed by western blot. GAPDH was used as loading control. Data are representative of two independent experiments.

(B) Quantification of the mean fluorescence intensity (MFI) of S1981-ATM staining per nucleus from the immunofluorescence in figure 2F using ImageJ.

Appendix figure S4

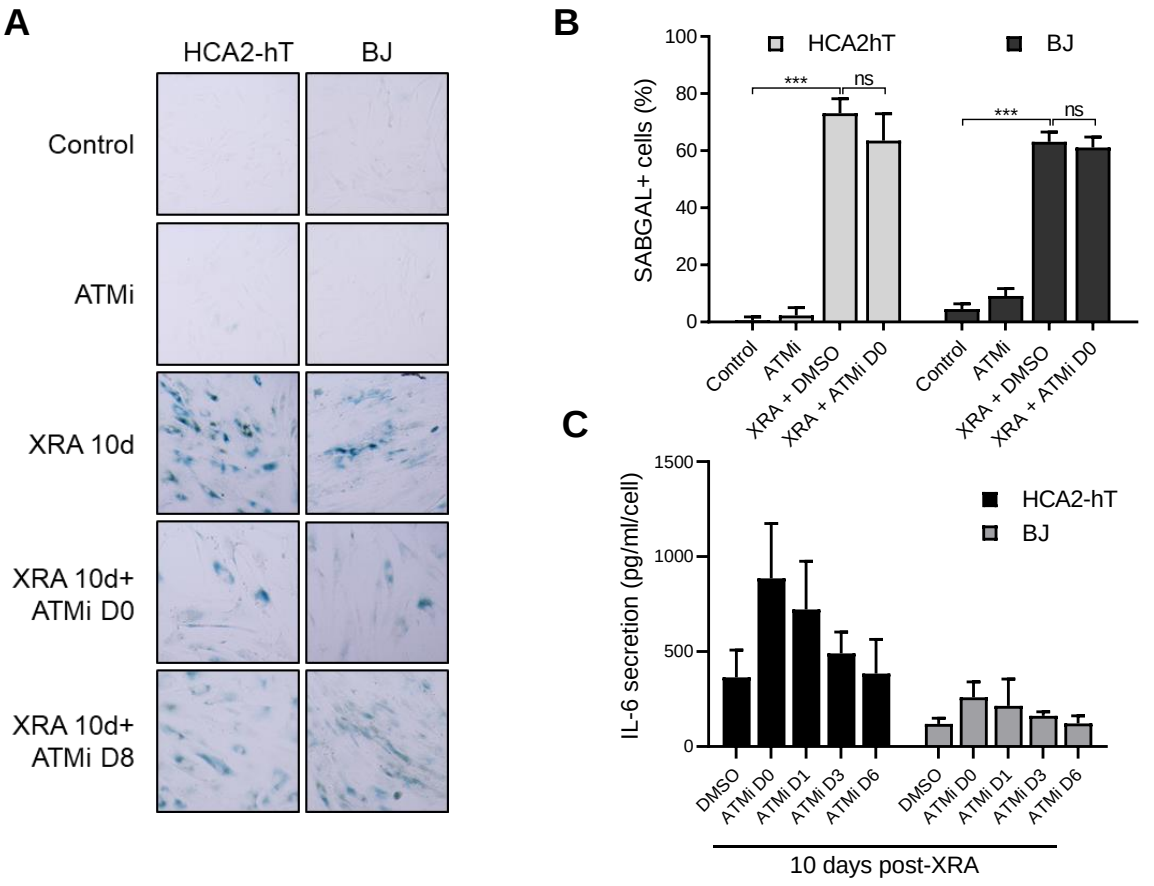


Appendix Figure S4. Impact of shATM, MRE11 or NBS1 on DDF

(A) BJ cells infected with lentiviruses expressing shGFP or shATM were irradiated with 10 Gy of XRA. Cells were fixed 2 hours or 6 days later. The number of 53BP1 foci (red) was measured by immunofluorescence (IF). Nuclei were counterstained using DAPI (blue).

(B) BJ cells infected with lentiviruses expressing shGFP.3, shMRE11.5 or shNBS1.6 were irradiated with 10 Gy of XRA. Cells were fixed 4 hours or 3 days later. The formation and persistence of DDF were evaluated by the co-localization of γH2AX (green) and 53BP1 (red) foci in IF. Nuclei were counterstained using DAPI (blue).

Appendix figure S5



Appendix Figure S5. Impact of ATM inhibitor on SA phenotypes

(A) Representative images of SA-ß-galactosidase assays performed on HCA2-hT or BJ cells 10 days post-XRA +/- ATMi, or treated 2 days with DMSO (Control) or ATMi.

(B) Quantification of SA-ß-galactosidase positive cells. Data are means ± S.D. of triplicates and representative of two independent experiments.

(C) HCA2-hT or BJ cells were irradiated with 10 Gy of XRA. Ku-55933 (5 µM, ATMi) was added 1 hour before XRA (ATMi D0) or 1 (ATMi D1), 3 (ATMi D3) or 6 days (ATMi D6) post-XRA. Ten days post-XRA, conditioned media (CM) of the final 16 hours was collected and assessed for IL-6 secretion by ELISA. Data are means ± S.D. of triplicates and representative of two independent experiments. Unpaired T-test: *** $p < 0.001$, ns = non-significant.

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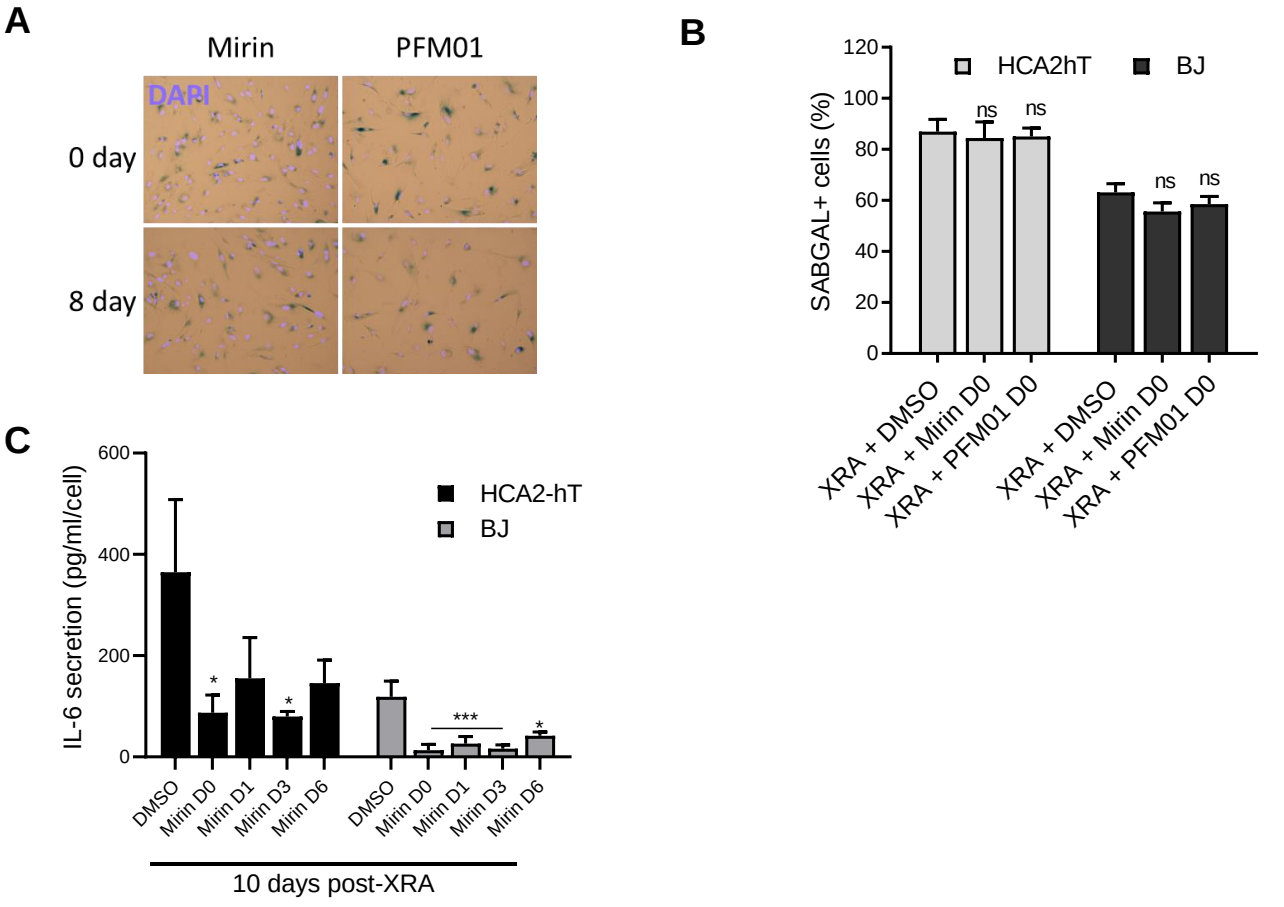
Appendix figure S6



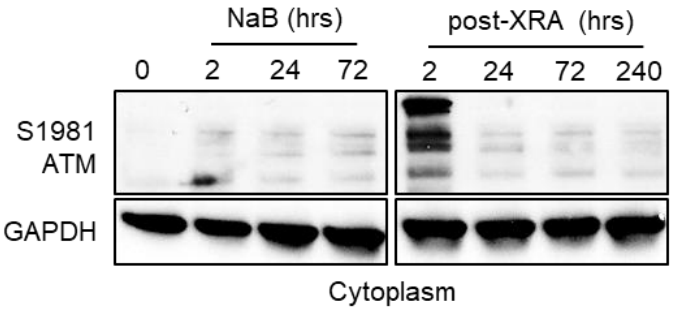
Appendix Figure S6. Impact of Mirin and PFM01 on SA phenotypes

(A) Representative images of SA-ß-galactosidase assays performed on HCA2-hT or BJ cells 10 days post-XRA +/- Mirin or PFM01 or treated 2 days with DMSO or Mirin or PFM01. Cells nuclei were counterstained using DAPI

(B) Quantification of SA-ß-galactosidase cells. Data are means ± S.D. of triplicates and representative of two independent experiments.

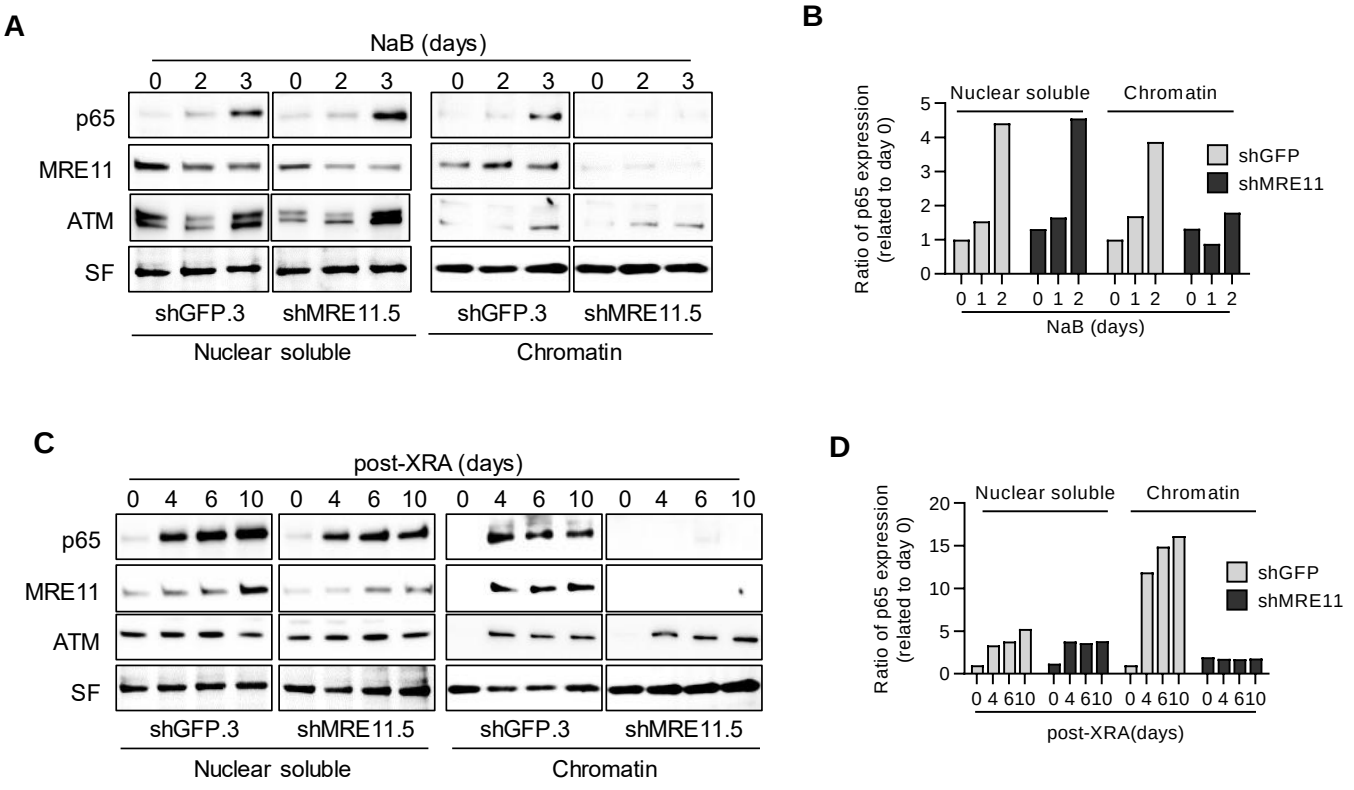
(C) HCA2-hT or BJ cells were irradiated with 10 Gy of XRA. Mirin was added 1 hour before XRA (Mirin D0) or 1 (Mirin D1), 3 (Mirin D3) or 6 days (Mirin D6) post-XRA. Ten days post-XRA, conditioned media (CM) of the final 16 hours were collected and assessed for IL-6 secretion by ELISA. Data are means ± S.D. of triplicates and representative of two independent experiments.

Appendix figure S7



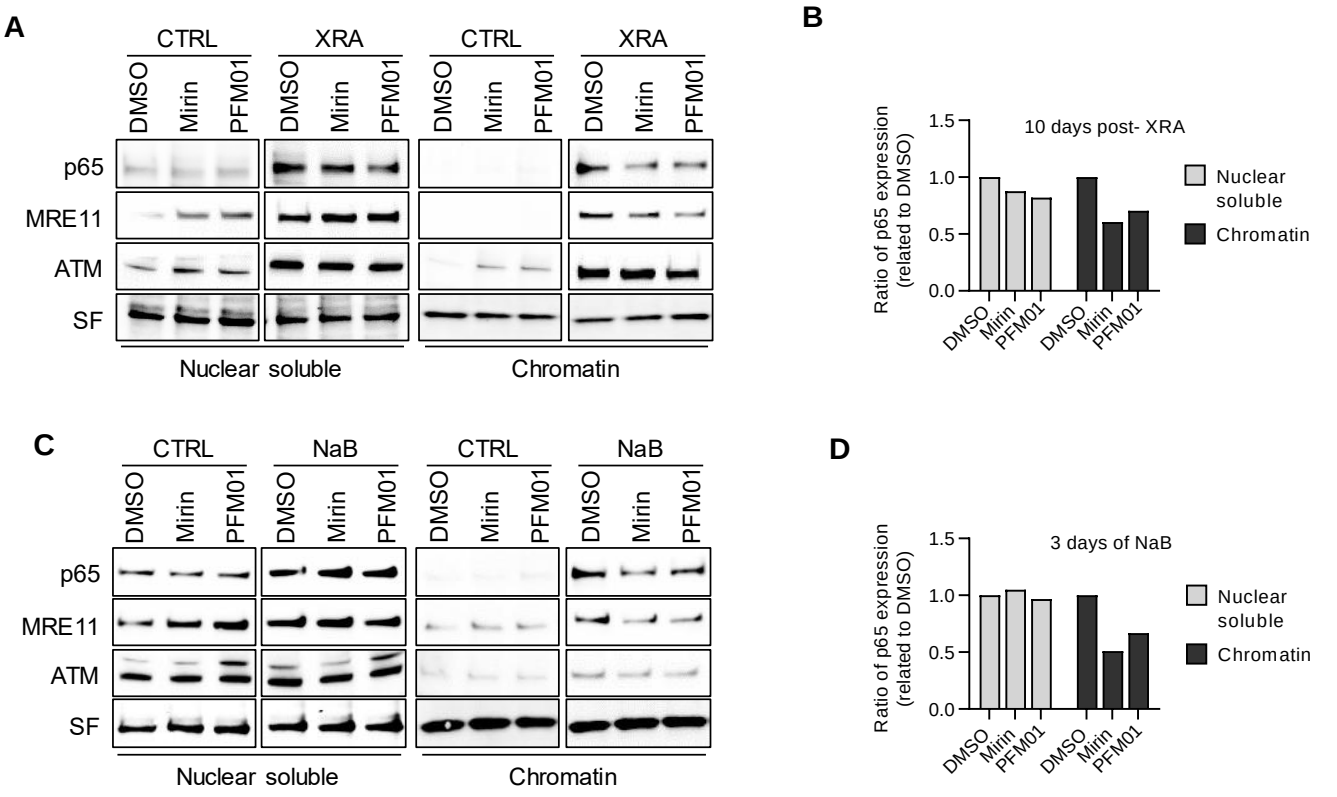
Appendix Figure S7. Expression of S1981-ATM in cytoplasmic fraction
HCA2-hTert cells were untreated (time 0), irradiated with 10 Gy of XRA or treated with 5 mM of NaB. Cells were collected at the indicated times for protein fractionation. Cytoplasm fractions were collected, and the expression of S1981-ATM was analyzed by western blot. GAPDH was used for loading control**.**

Appendix figure S8



Appendix Figure S8. Impact of shMRE11 on nuclear and chromatin localization of p65
BJ cells infected with lentiviruses expressing shGFP.3 or shMRE11.5 were (A-B) treated with NaB (5 mM) or (C-D) irradiated with 10 Gy of XRA. At the indicated times, cells were collected and protein from the nuclear soluble and chromatin fractions were isolated by subcellular fractionation. (A and C) p65, ATM and MRE11 protein expression on nuclear and chromatin fractions were analyzed western blot. (B and D) Expression of p65 protein in nuclear and chromatin fractions were quantified using Image lab software (Biorad). Data were normalized using the total protein quantification from the SF imaging. Protein expression at time 0 or in control was used as baseline.

Appendix figure S9



Appendix Figure S9. Impact of shMRE11 on nuclear and chromatin localization of p65
(A-B) HCA2-hT cells were irradiated with 10 Gy and 8 days later, DMSO, Mirin (5 µM) or PFM01 (5 µM) were added for 2 days. (C-D) HCA2-hT cells were treated with NaB (5 mM) +/- Mirin or PFM01 added 24 hours after NaB. Alternatively, non-irradiated HCA2-hT cells were treated with DMSO, Mirin or PFM01 for 2 days. Ten days post-XRA or after 3 days of NaB treatment, cells were collected and protein from the nuclear soluble and chromatin fractions were isolated by subcellular fractionation. (A and C) p65, ATM and MRE11 protein expression on nuclear and chromatin fractions were analyzed western blot. (B and D) Expression of p65 protein in nuclear and chromatin fractions were quantified using Image lab software (Biorad). Data were normalized using the total protein quantification from the SF imaging. Protein expression at time 0 or in control was used as baseline.

Appendix Table S1

Antibodies	Compagny	Clone/Cat #	WB dilution	IF dilution
53BP1	Novus	NB100-305	1:2000	1:2000
γH2AX	Millipore	JBW301	1:2000	1:1000
ATM	AbCam	2C11A1/Ab78	1:2000	-
Phospho-ATM (S1981)	Cell signaling	10H11.E12/4526	1:2000	1:500
NBS1	Cell signaling	D6J5I	1:1000	-
MRE-11	Cell signaling	31H4	1:2000	-
Rad51	AbCam	Ab213	-	1:1000
p65	Cell signaling	D14E12	1:1000	-
Phospho CHK2 (T68)	Santa Cruz	SC-16297-R	1:500	-
GAPDH	Cell signaling	14C10	1:2000	-

Appendix table S1. Antibodies used for western blot or immunofluorescence experiments