Expanded View Figures

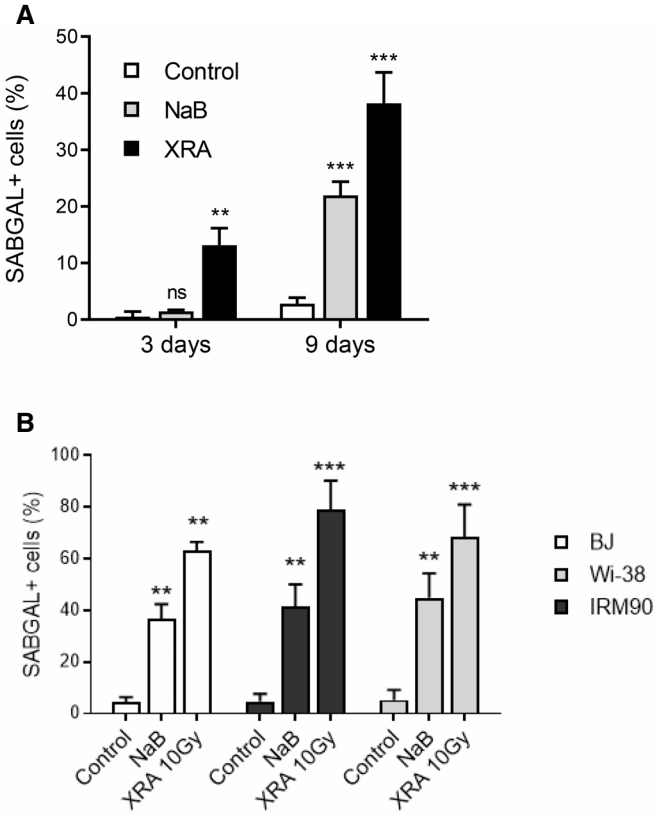
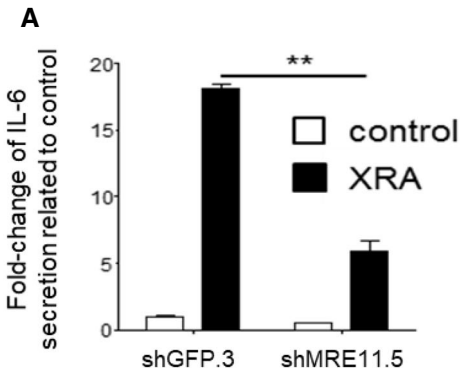


Figure EV1. Quantification of SA-β-galactosidase assays in NaB- or XRA-treated fibroblasts.

A HCA2-hTert fibroblasts were irradiated (XRA) with 10 Gy or treated with 2 mM of NaB. Three or 9 days later, cells were fixed and SA-ß-galactosidase assays were performed. Data are means ± SD of triplicates and are representative of two independent experiments.

B BJ, WI-38, or IMR90 fibroblasts were irradiated (XRA) with 10 Gy or treated with 5 mM of NaB. Nine days later, cells were fixed and SA-ß-galactosidase assays were performed. Data are means ± SD of triplicates and are representative of two independent experiments. Unpaired *t*-test: $^{**}P < 0.01$; $^{***}P < 0.001$, ns, non-significant.



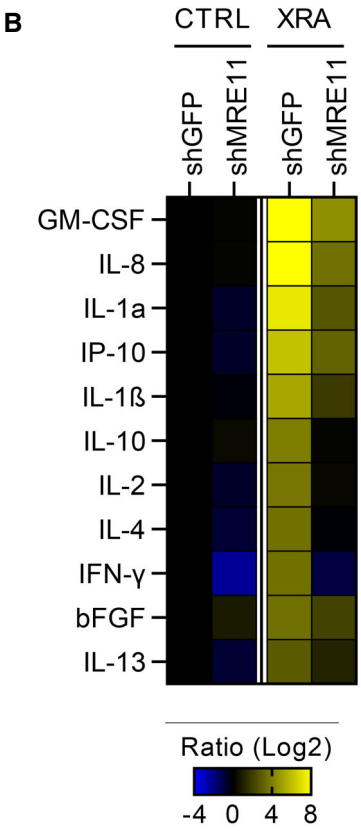


Figure EV2. Impact of MRE11 depletion on XRA-SASP.

A BJ fibroblasts were infected with lentiviruses expressing shGFP.3 or shMRE11.5, selected with puromycin and allowed to recover for 7 days. BJ-shGFP.3 and BJ-shMRE11.5 cells were untreated (control) or irradiated with 10 Gy of XRA. After 9 days, serum-free conditioned medium (SF-CM) was collected over 24 h. IL-6 secretion in SF-CM was analyzed by ELISA. Data are the means ± SD of triplicates and representative of two independent experiments.

B Secreted soluble factors were evaluated using a multiplex immunoassay (40-Plex MSD®). Average secretion of untreated BJ-shGFP.3 cells was used as baseline. Heat map key indicates $\log_2$-fold changes from control. Unpaired *t*-test: $^{**}P < 0.01$.

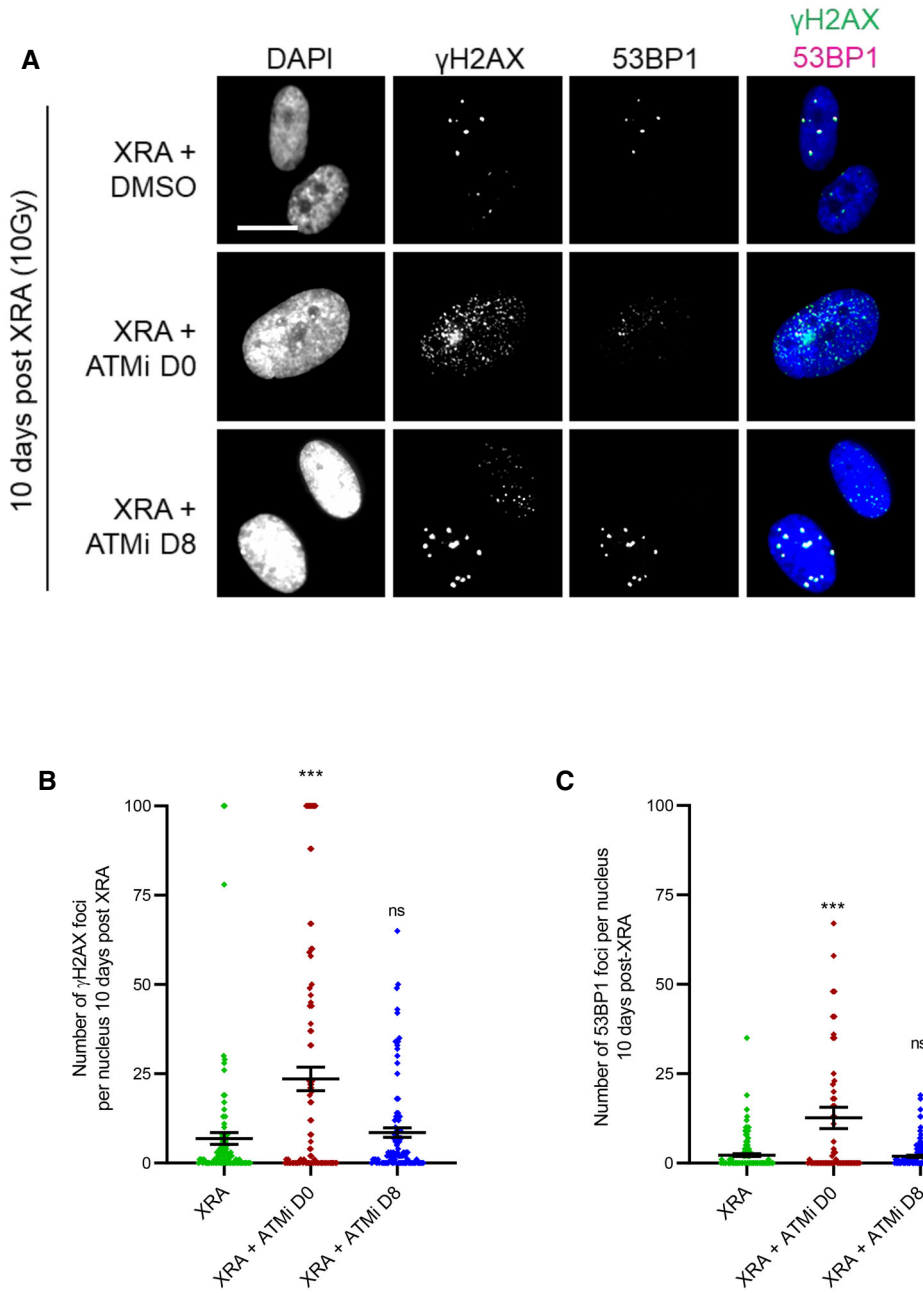


Figure EV3. Impact of ATM inhibitor on persistent DNA damage foci.

A–C HCA2-hT or BJ cells were irradiated with 10 Gy of XRA. Ku-55933 (ATMi, 5 μM) was added 1 h before XRA (ATMi D0) or 8 days post-XRA (ATMi D8). (A) BJ cells were fixed 10 days post-XRA and analyzed for γH2AX or 53BP1 immunofluorescence. Images of γH2AX (green) or 53BP1 (red) and DAPI staining. (B) Quantification of the number of γH2AX foci per nucleus (n = 150). (C) Quantification of the number of 53BP1 foci per nucleus (n = 150). Means ± SEM of foci per nucleus are representative of three independent experiments. Unpaired t-test: ***$P < 0.001$, ns: non-significant.

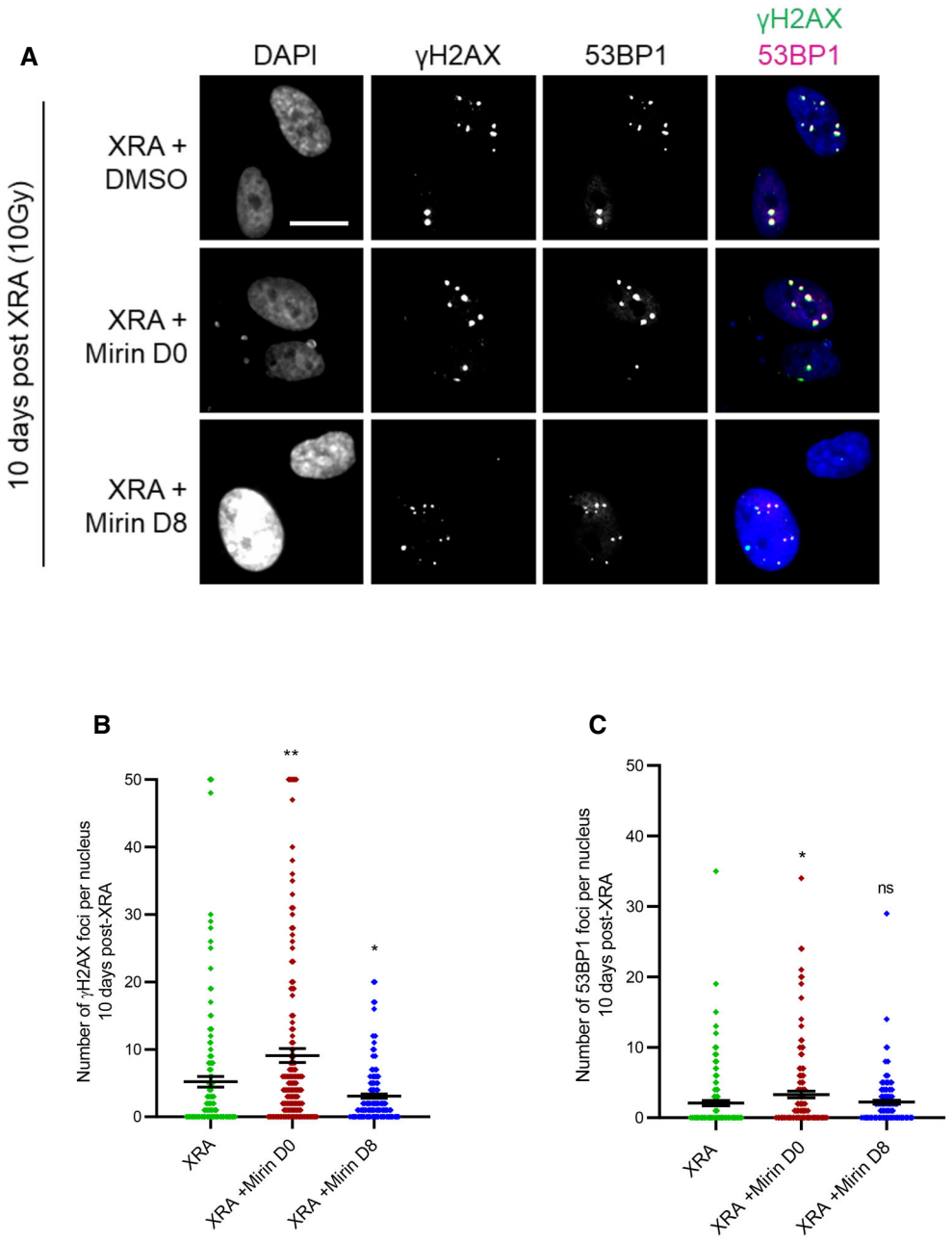


Figure EV4. Impact of MRE11 inhibitor on persistent DNA damage foci.

A–C HCA2-hT or BJ cells were irradiated with 10 Gy of XRA. Five μM of Mirin was added 1 h before XRA (Mirin D0) or 8 days post-XRA (Mirin D8). (A) BJ cells were fixed 10 days post-XRA and analyzed for γH2AX or 53BP1 immunofluorescence. Left panel: images of γH2AX (green) or 53BP1 (red) and DAPI staining. Middle panel: quantification of the number of γH2AX foci per nucleus (n = 150). Right panel: quantification of the number of 53BP1 foci per nucleus (n = 150). Means ± SEM of foci per nucleus are representative of three independent experiments. Unpaired t-test: *P < 0.05; **P < 0.01, ns: non-significant.

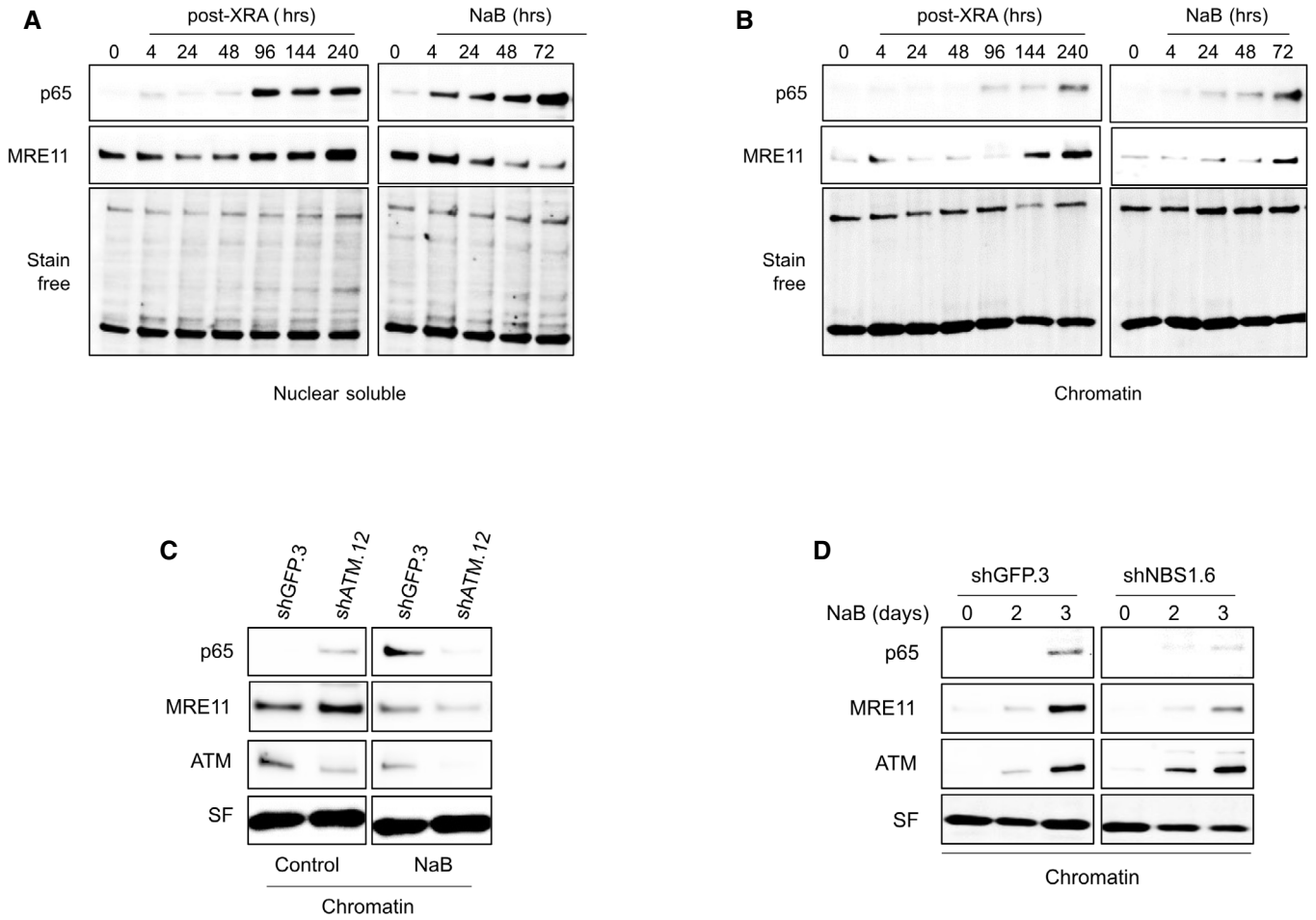


Figure EV5. Chromatin localization of p65 and MRE11 in senescent BJ cells.

A, B Cells were untreated (time 0), irradiated with 10 Gy of XRA, or treated with 5 mM of NaB. At the indicated time, BJ cells were collected and proteins from the (A) nuclear soluble or (B) chromatin fractions were isolated by subcellular fractionation. Expression of MRE11 or p65 was assessed by Western blot. Stain-free (SF) imaging of the membrane was used as loading control.

C BJ cells infected with lentiviruses expressing shGFP.3 or shATM.12 were treated with 5 mM of NaB for 3 days. Cells were collected and protein from chromatin fraction was isolated by subcellular fractionation. Expression of p65, MRE11, and ATM proteins in chromatin fractions was analyzed by Western blot. Stain-free (SF) imaging of the membrane was used as loading control.

D BJ cells infected with lentiviruses expressing shGFP.3 or shNBS1.6 were treated with 5 mM of NaB. At the indicated times, cells were collected protein from chromatin fraction were isolated by subcellular fractionation. Expression of p65, MRE11, and ATM proteins in chromatin fractions was analyzed by Western blot. Stain-free (SF) imaging of the membrane was used as loading control.

Source data are available online for this figure.