Selective inhibition of ATM-dependent double-strand break repair and checkpoint control synergistically enhances the efficacy of ATR inhibitors

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Supplementary Figures

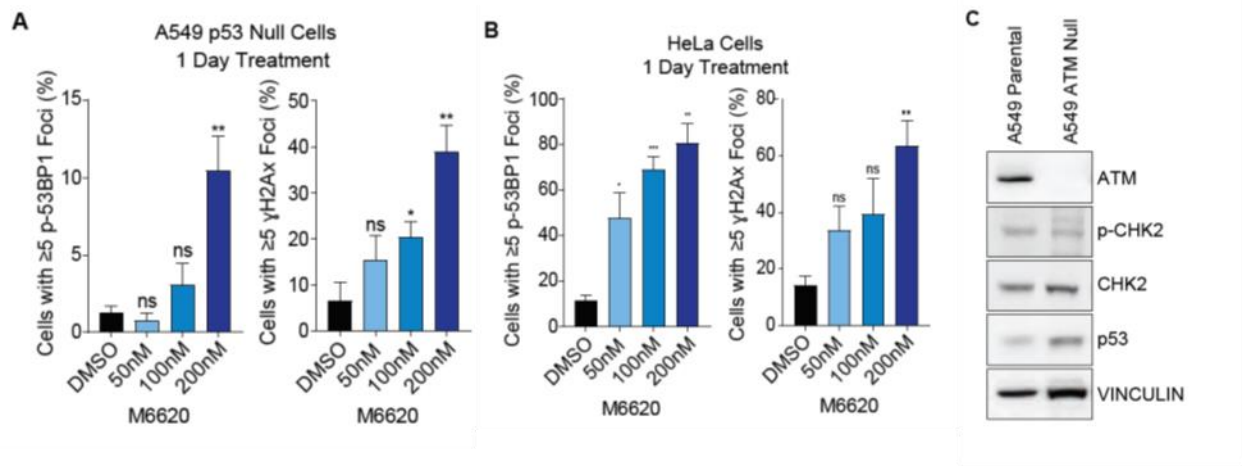

Figure S1. ATR inhibition by berzosertib (M6620) elevates DSB DNA damage in cancer cells.
A-B. Immunofluorescence analysis of p-53BP1 and γH2Ax foci in A549 p53-null and HeLa cells after 24 h exposure to DMSO and the indicated berzosertib concentrations. Error bars represent the SEM; ***P < 0.001, **P < 0.01, and *P < 0.05 by unpaired t-test. **C**. Western blot analysis of A549 parental and ATM-null cells to confirm loss of ATM protein and establish basal downstream pCHK2 and CHK2 protein levels.

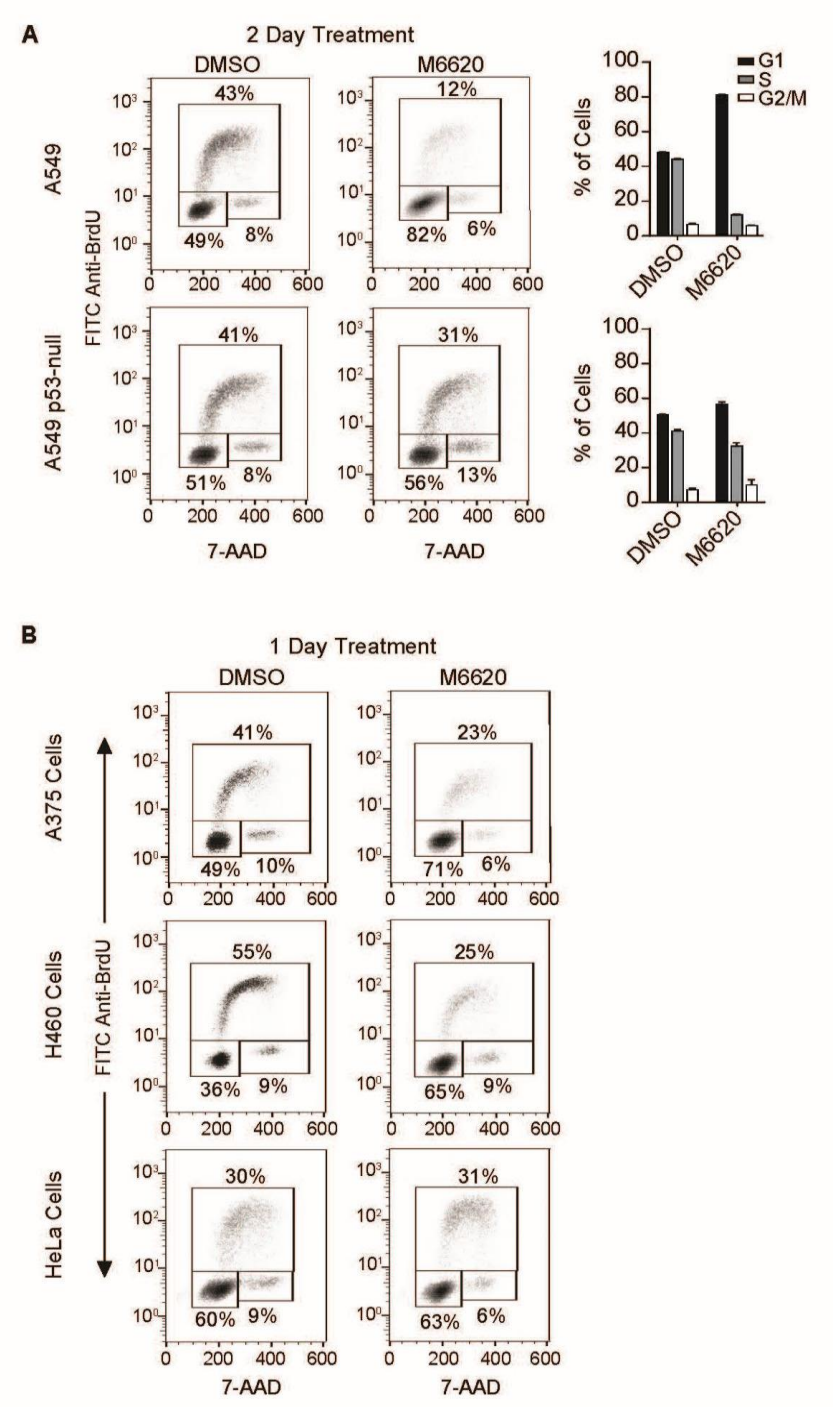

Figure S2. Inhibition of ATR by berzosertib (M6620) activates the ATM/p53 G1 checkpoint. **A.** BrdU/7-AAD cell cycle analysis in A549 parental and A549 p53-null cells after 48 h exposure to DMSO or 200 nM berzosertib (representative flow cytometry dot plots and quantification across replicates). Error bars represent the SEM. **B.** BrdU/7-AAD cell cycle analysis of proliferating A375, H460, and HeLa cells after 24 h exposure to DMSO or 200 nM berzosertib.

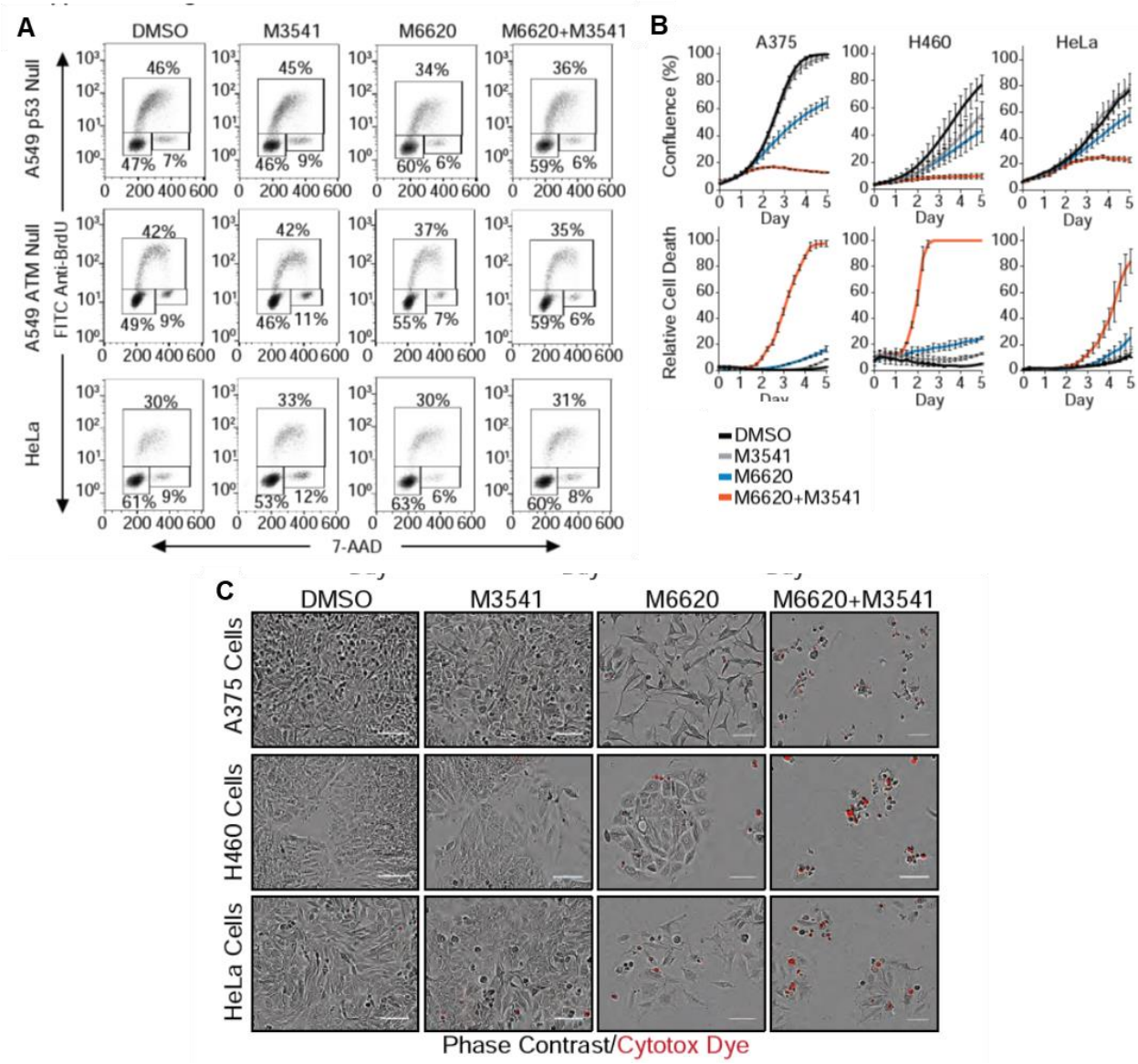

Figure S3. Dual inhibition of ATM by M3541 and ATR by berzosertib (M6620) leads to aberrant cell cycle progression in ATM-p53-proficient cells and enhances cytotoxicity. A. BrdU/7-AAD cell cycle analysis in A549 p53-null, A549 ATM-null, and HeLa cells after 24 h exposure to DMSO, M3541 (1 μM), berzosertib (200 nM), or their combination. **B.** Confluence (top row) and relative cell death (bottom row) from IncuCyte live imaging in A375, H460, and HeLa cells exposed to DMSO, M3541 (1 μM), berzosertib (100 nM) or the combination. Relative cell death was calculated from the number of cells positively stained with CytoTox dye normalized to confluence. Error bars represent the SEM. **C.** Representative images from IncuCyte live imaging (10x objective) of A375, H460, and HeLa cells after 6-day exposure to DMSO, M3541 (1 μM), berzosertib (100 nM) or their combination. Scale bars = 100μm.

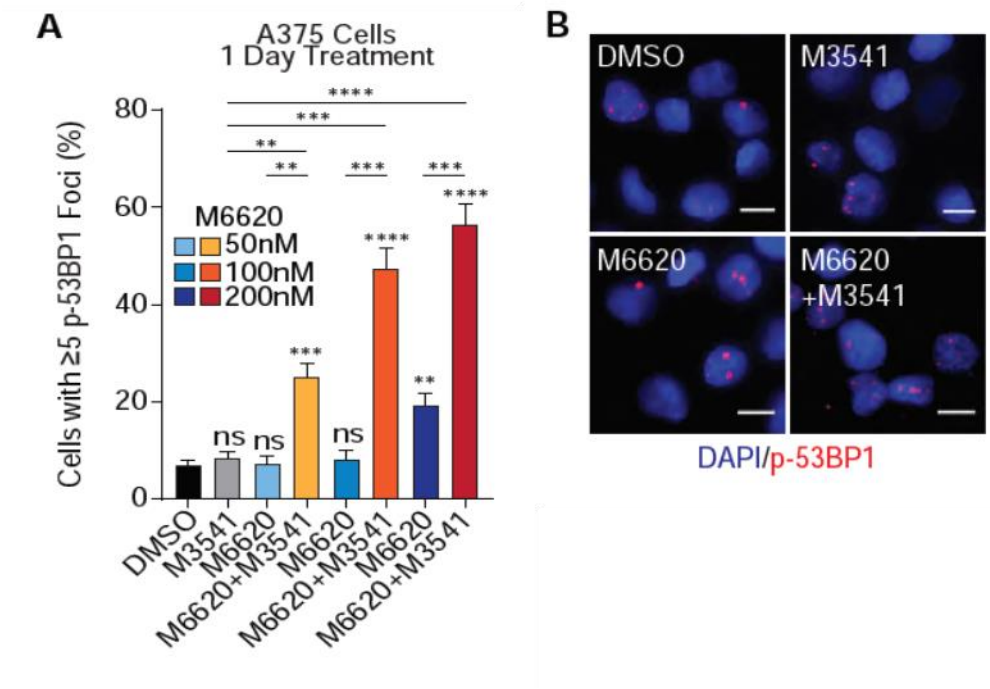

Figure S4. Dual inhibition of ATM and ATR by M3541 and berzosertib (M6620) leads to increased DNA damage in A375 cells. **A.** Immunofluorescence analysis of phospho-53BP1 foci in A375 cells after 24 h exposure to DMSO, M3541 (1 μM), berzosertib (50, 100, 200 nM), or their combination. Error bars represent the SEM; ****P < 0.0001, ***P < 0.001, and **P < 0.01 by unpaired t-test. **B.** Representative immunofluorescence images of phospho-53BP1 foci in A375 cells after 24 h exposure to DMSO, M3541 (1 μM), berzosertib (50 nM), or their combination. Scale bars = 10 μm.

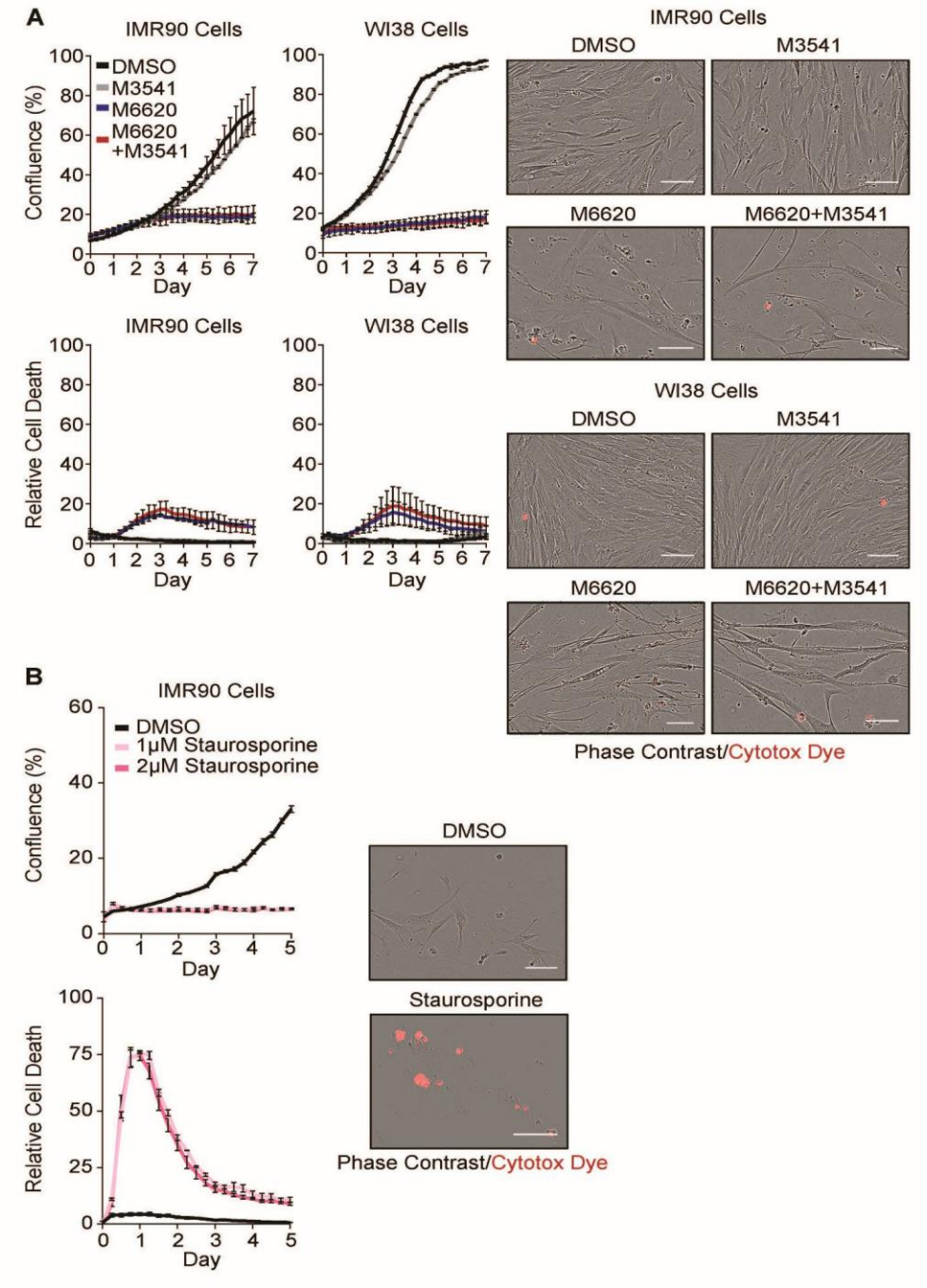

Figure S5. Effect of dual ATM/ATR inhibition by M3541 and berzosertib (M6620) in human fibroblasts. **A.** Confluence (top row) and relative cell death (bottom row) from IncuCyte live imaging in IMR90 and WI38 cells exposed to DMSO, M3541 (1 μM), berzosertib (200 nM) or the combination of berzosertib+M3541. Relative cell death was calculated from the number of cells positively stained with CytoTox dye normalized to confluence. Error bars represent the SEM. Representative images from IncuCyte live imaging (10x objective) after 6-day treatment. Scale bars = 100 μm. **B.** Confluence (top row) and relative cell death (bottom row) from IncuCyte live imaging in IMR90 cells exposed to DMSO or staurosporine (1 μM, 2 μM). Relative cell death was calculated from the number of cells positively stained with CytoTox dye normalized to confluence. Error bars represent the SEM. Representative images from IncuCyte live imaging (10x objective) after 1-day treatment. Scale bars = 100 μm.

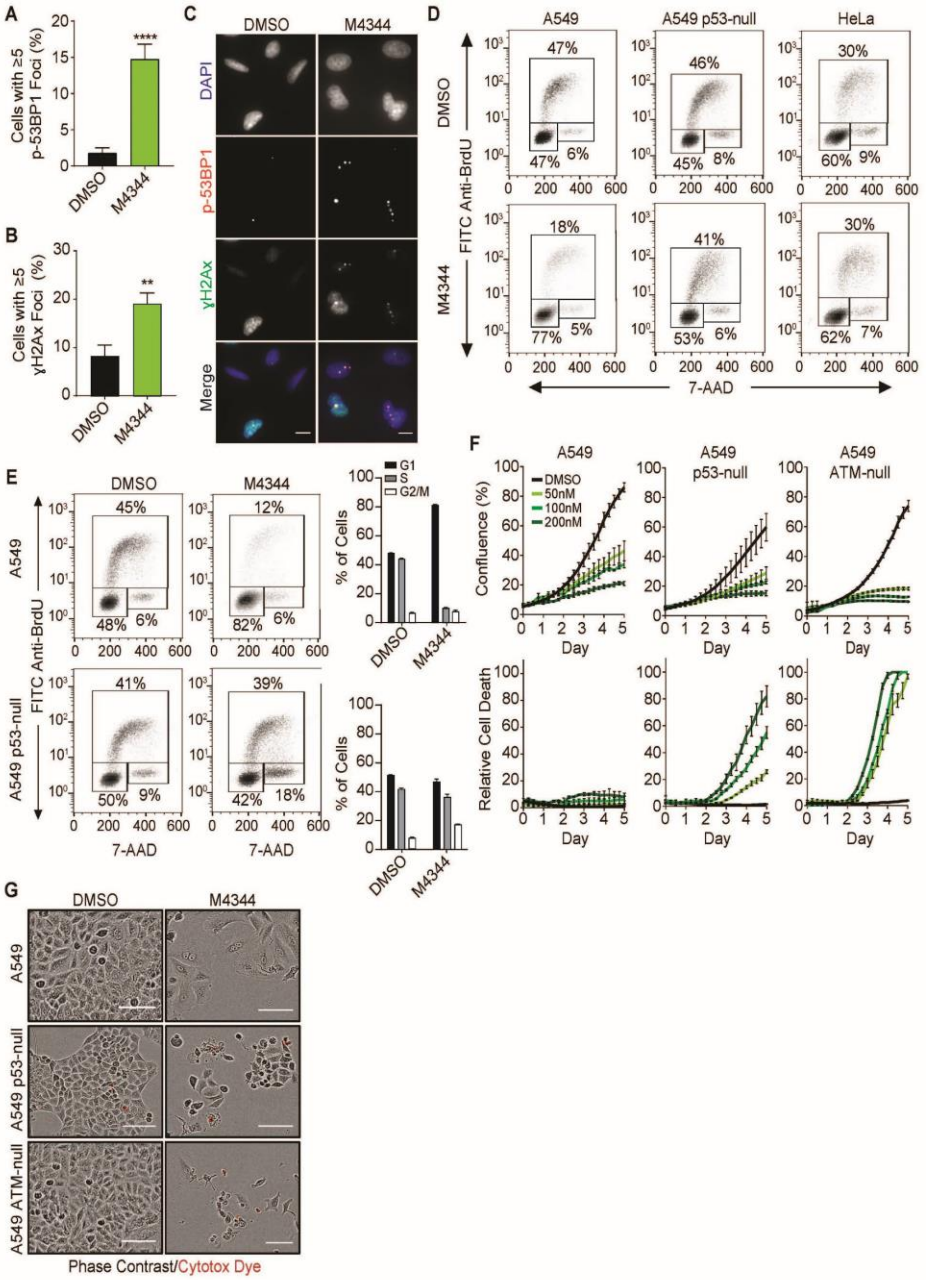

Figure S6. **ATR inhibition by gartisertib (M4344) increases DSB damage and induces G1 checkpoint in cancer cells. A-B.** Immunofluorescence analysis of phospho-53BP1 and γH2Ax foci in A549 cells after 24 h exposure to DMSO or 50 nM gartisertib. **C.** Representative immunofluorescence images of γH2Ax and phospho-53BP1 foci in A549 cells after 24 h exposure as in (A). Scale bars = 10 μm. **D.** BrdU/7-AAD cell cycle analysis in A549 parental, A549 p53-null, and HeLa cells after 24 h exposure to DMSO or 200 nM gartisertib. **E.** BrdU/7-AAD cell cycle analysis in A549 parental and A549 p53-null cells after 48 h exposure to DMSO or 200nM gartisertib. Quantification across replicates of G1, S, and G2 populations as determined by BrdU/7-AAD cell cycle analysis in A549 parental and A549 p53-null cells after treatment as above. Error bars represent the SEM. **F.** Confluence (top row) and relative cell death (bottom row) from IncuCyte live imaging in A549 parental, p53-null and ATM-null cells exposed to DMSO or gartisertib (50, 100, 200 nM). Relative cell death was calculated from the number of cells positively stained with CytoTox dye normalized to confluence. Error bars represent the SEM. **E.** Representative images from IncuCyte live imaging (10x objective) of A549 parental, p53-null and ATM-null cells after 6-day growth in media with 50 nM gartisertib. Scale bars = 100 μm.

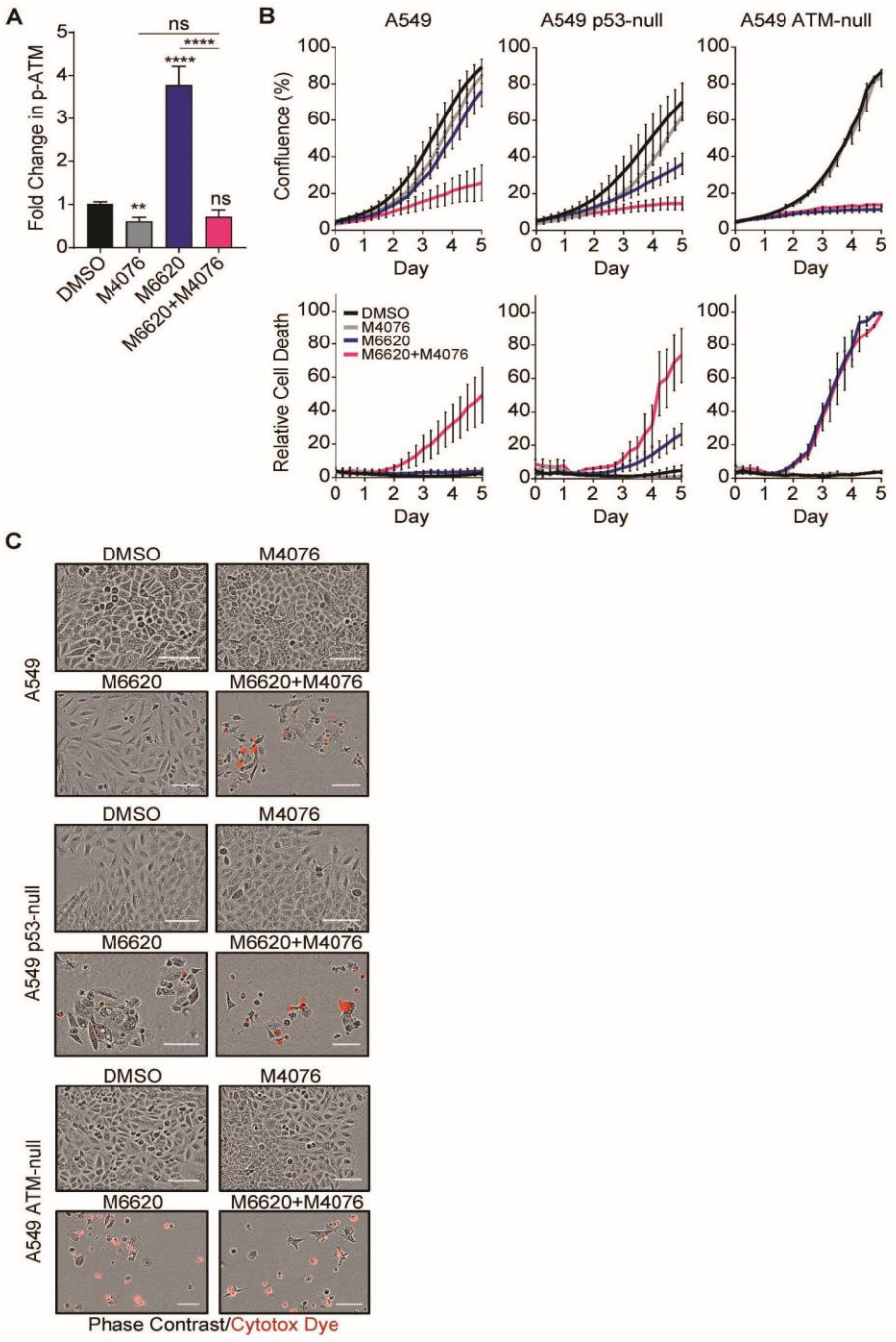

Figure S7. Dual inhibition of ATM by M4076 and ATR by berzosertib (M6620) bypasses ATM-p53 checkpoint signaling and enhances cytotoxicity. A. Quantification of ATM autophosphorylation (p-ATM^{Ser1981}/total ATM) in A549 cells by MSD assay after 24 h exposure to DMSO, M4076 (1 μM), berzosertib (200 nM) or their combination. Error bars represent the SEM; ****P < 0.0001 and **P < 0.01 by unpaired t-test. **B.** Confluence (top row) and relative cell death (bottom row) from IncuCyte live imaging in A549 parental, p53-null and ATM-null cells exposed to DMSO, M4076 (1 μM), berzosertib (200 nM) or their combination. Relative cell death was calculated from the number of cells positively stained with CytoTox dye normalized to confluence. Error bars represent the SEM. **C.** Representative images from IncuCyte live imaging (10x objective) after 6-day treatment. Scale bars = 100 μm.

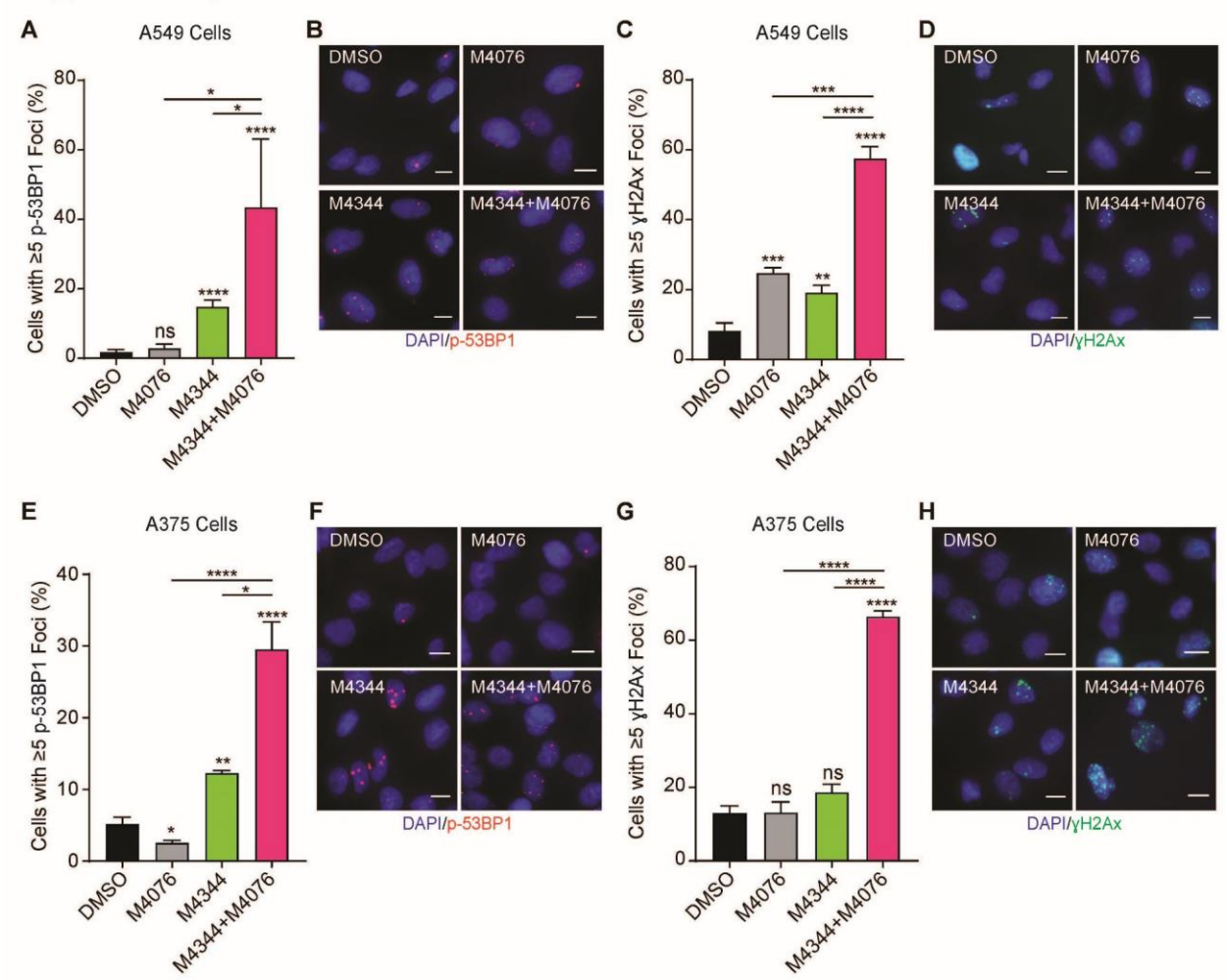

Figure S8. Dual inhibition of ATM by M4076 and ATR by gartisertib (M4344) leads to increased DNA damage in two human cancer cell lines. Immunofluorescence analysis **(A)** and representative images **(B)** of phospho-53BP1 foci in A549 cells after 24 h exposure to DMSO, M4076 (1 μM), gartisertib (50 nM) or the combination of gartisertib and M4076. Immunofluorescence analysis **(C)** and representative images **(D)** of γH2Ax foci in A549 cells after 24 h exposure to DMSO, M4076 (1 μM), gartisertib (50 nM) or their combination. Immunofluorescence analysis **(E)** and representative images **(F)** of phospho-53BP1 foci in A375 cells after 24 h exposure to DMSO, M4076 (1 μM), gartisertib (50 nM) or their combination. Immunofluorescence analysis **(G)** and representative images **(H)** of γH2Ax foci in A375 cells after 24 h exposure to DMSO, M4076 (1 μM), gartisertib (50 nM) or the combination of gartisertib+M4076. Error bars represent the SEM; ****P < 0.0001, ***P < 0.001, **P < 0.01, and *P < 0.05 by unpaired t-test. Scale bars on images = 10 μm.

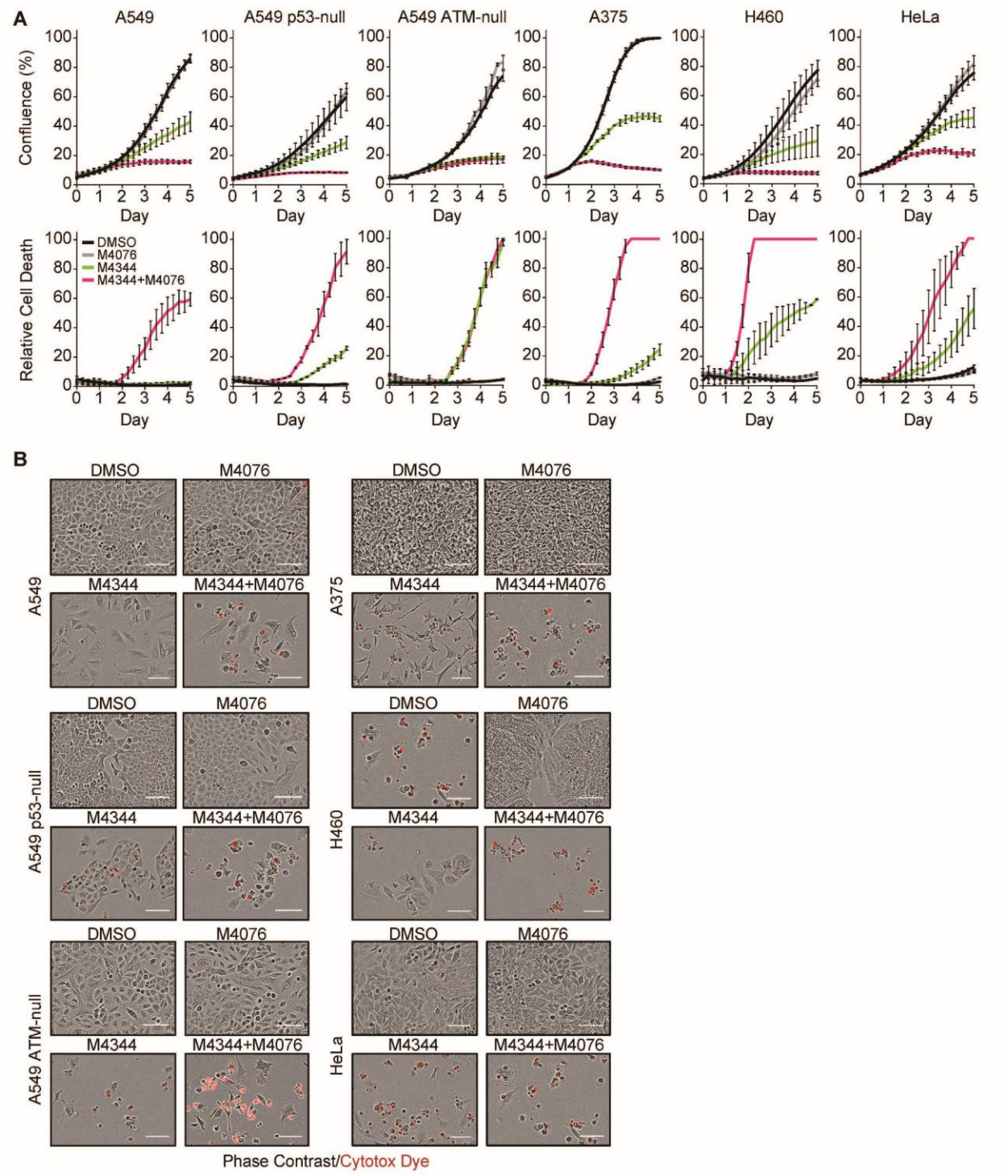

Figure S9. Dual inhibition of ATM by M4076 and ATR by gartisertib (M4344) leads to cytotoxicity in cancer cell lines. A. Confluence (top row) and relative cell death (bottom row) from IncuCyte live imaging in A549 parental, A549 p53-null, A549 ATM-null, A375, H460 and HeLa cells exposed to DMSO, M4076 (1 μM), gartisertib (50 nM) or their combination. Relative cell death was calculated from the number of cells positively stained with CytoTox dye normalized to confluence. Error bars represent the SEM. **b.** Representative images from IncuCyte live imaging (10x objective) after 6-day treatment. Scale bars = 100 μm.