Supplemental Material to:

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Autophagy-dependent senescence in response to DNA damage and chronic apoptotic stress

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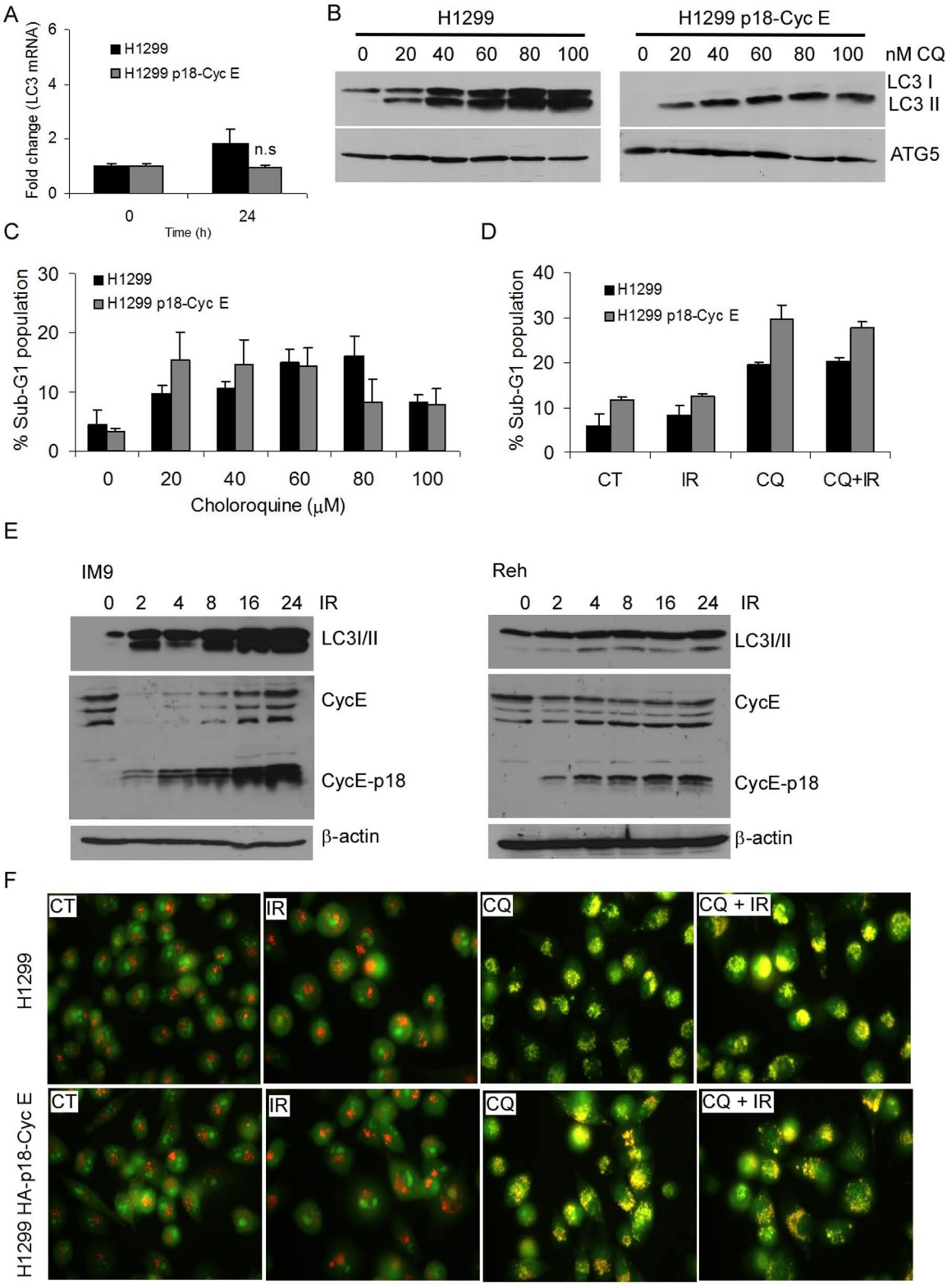

Figure S1. Stable p18-CycE-expression induces autophagy while transient p18-CycE-expression leads to apoptosis. (A) Quantitative RT-PCR analysis for LC3 in parental and p18-CycE-expressing cells at 24 h following irradiation (n.s). (B) Cells were lysed at 24 h following chloroquine treatment at the indicated doses and immunoblotted for LC3 I/II, with ATG5 used as a loading control. (C and D) Cell death is shown as percentage of cells with sub-G1 DNA content at 24 h following irradiation and/or chloroquine (100 μM) treatment, as indicated. (E) Protein gel blot analysis for endogenous LC3 I/II and p18-CycE in IM-9 and Reh cells at the indicated time following IR. (F) Micrograph using the supravital cell-stain acridine orange in parental and HA-p18-CycE expressing cells following irradiation and/or chloroquine (100 μM) treatment for 24 h.

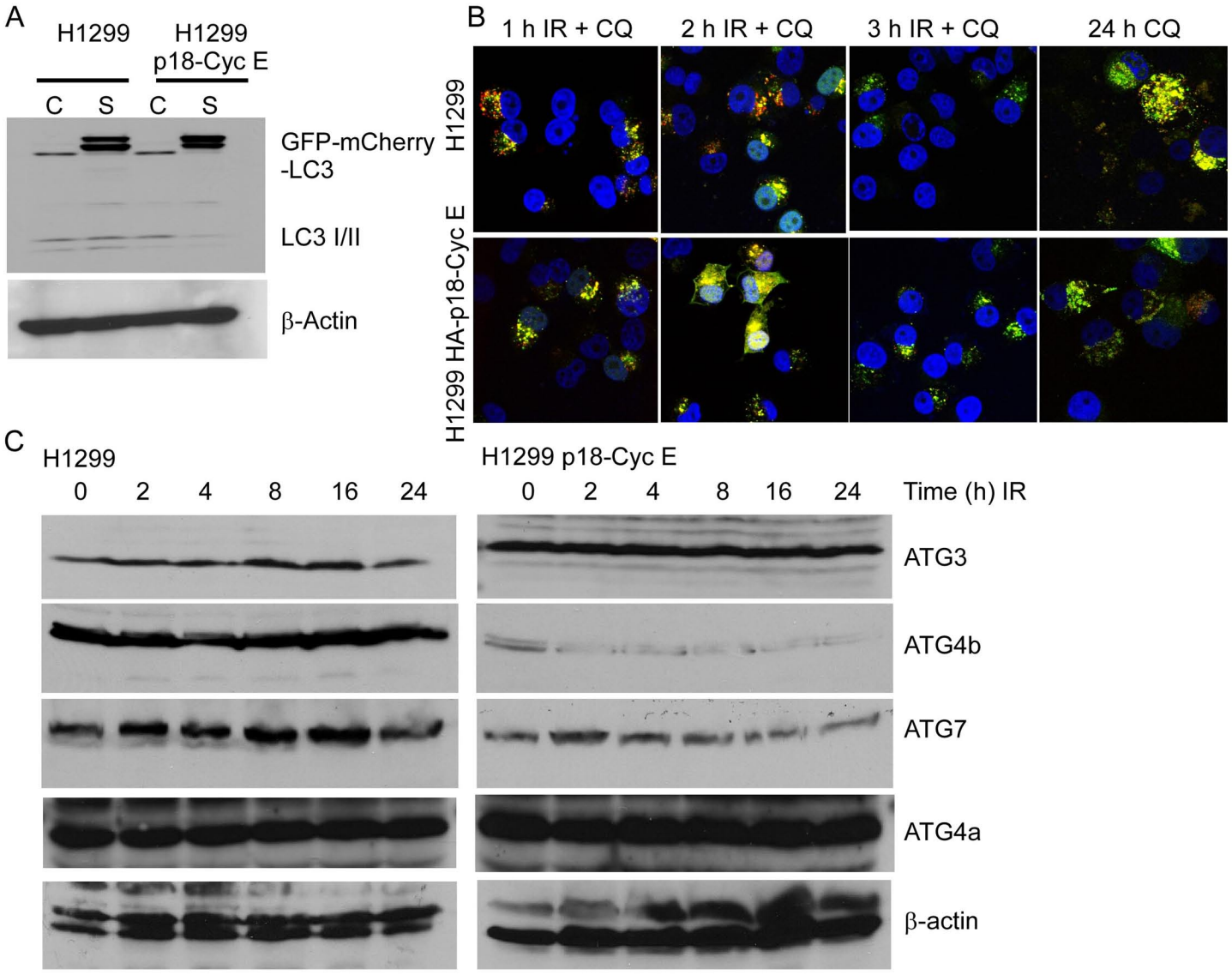

Figure S2. Stable but not transient p18-CycE-expression increases autophagic flux. (A) Expression of GFP-mCherry-LC3 in untransfected and (c) stably transfected (s) cells, with β-actin serving as loading control. (B) Confocal microscopic images of parental and HA-p18-CycE expressing cells stably expressing GFP-mCherry-LC3 at the indicated times following irradiation combined with chloroquine (100 μM). (C) Parental and p18-CycE-expressing cells were lysed at the indicated times following irradiation and immunoblotted for ATG3, ATG4b, ATG7, ATG4a, with β-actin serving as loading control.

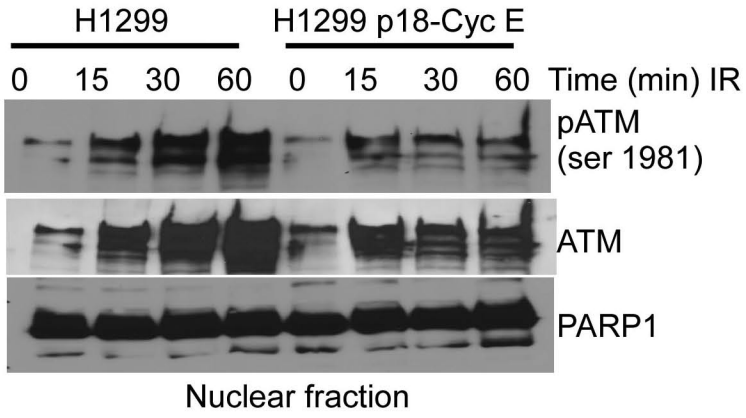

Figure S3. Deregulated nuclear ATM signaling in p18-CycE-expressing cells following irradiation with shATG7 enhancing cell death. Nuclear fractions of cells, at the indicated time following irradiation, immunoblotted for pATM phosphorylaiton on ser1981, ATM, and PARP1 as loading control.

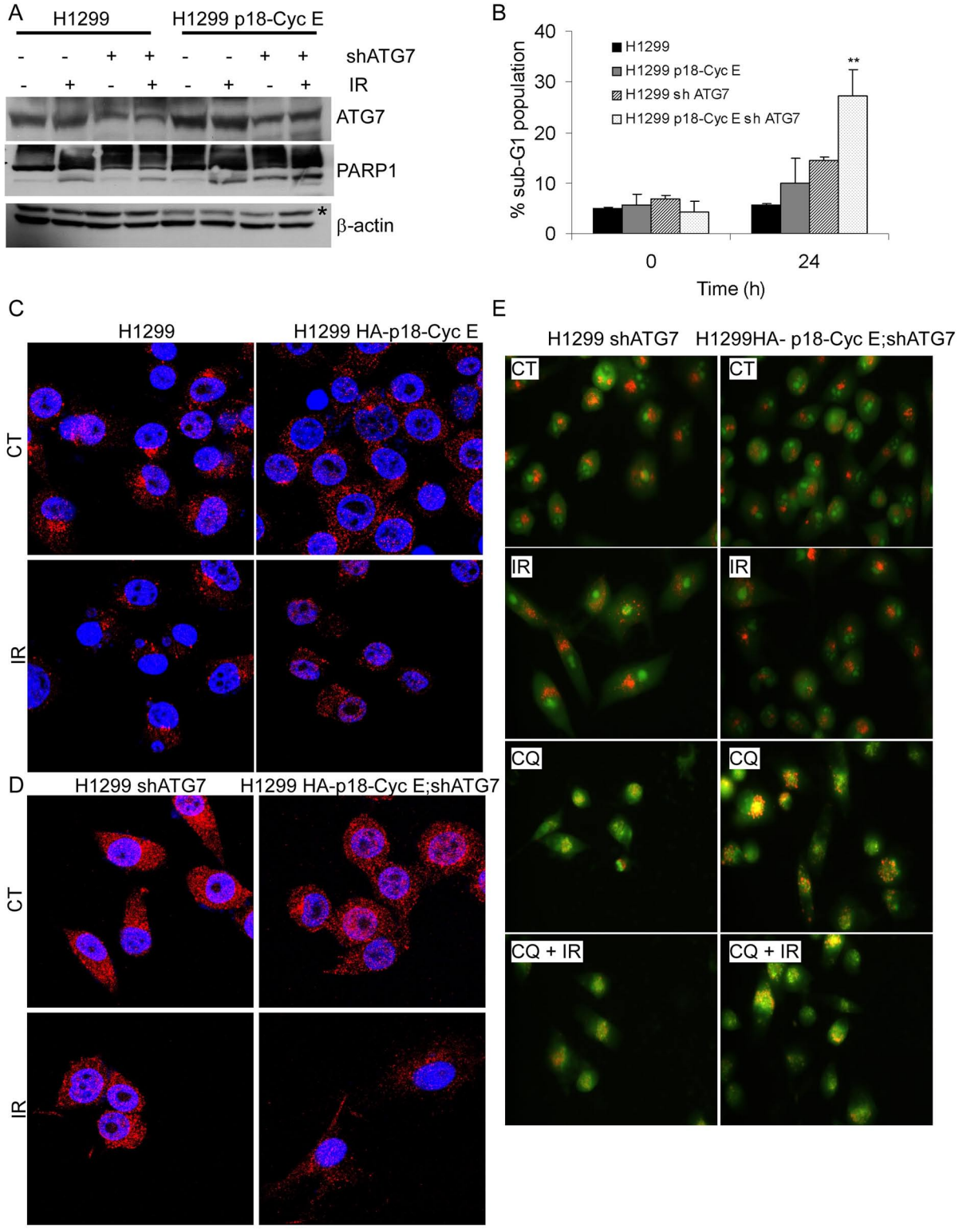

Figure S4. Autophagy inhibition by shATG7. (A) p18-CycE-expressing cells without or with shATG7 were lysed at the indicated time following irradiation and immunoblotted for ATG7, PARP1, with β-actin as loading control. "*" represent non-specific proteins. (B) Cell death is shown as percentage of cells with sub-G1 DNA content that stably express p18-CycE in the absence or presence of shATG7 (Clone 2) at 24 h following irradiation (*p = 0.036). (C and D) Visualization of endogenous LC3 I/II at 24 h following irradiation in parental and HA-p18-CycE-expressing cells with or without shATG7 expression. Nuclei were stained with DAPI. (E) Micrograph using the supravital cell-stain acridine orange in parental and HA-p18-CycE expressing cells with or without shATG7 expression following irradiation and/or chloroquine (100 μM) treatment for 24 h.

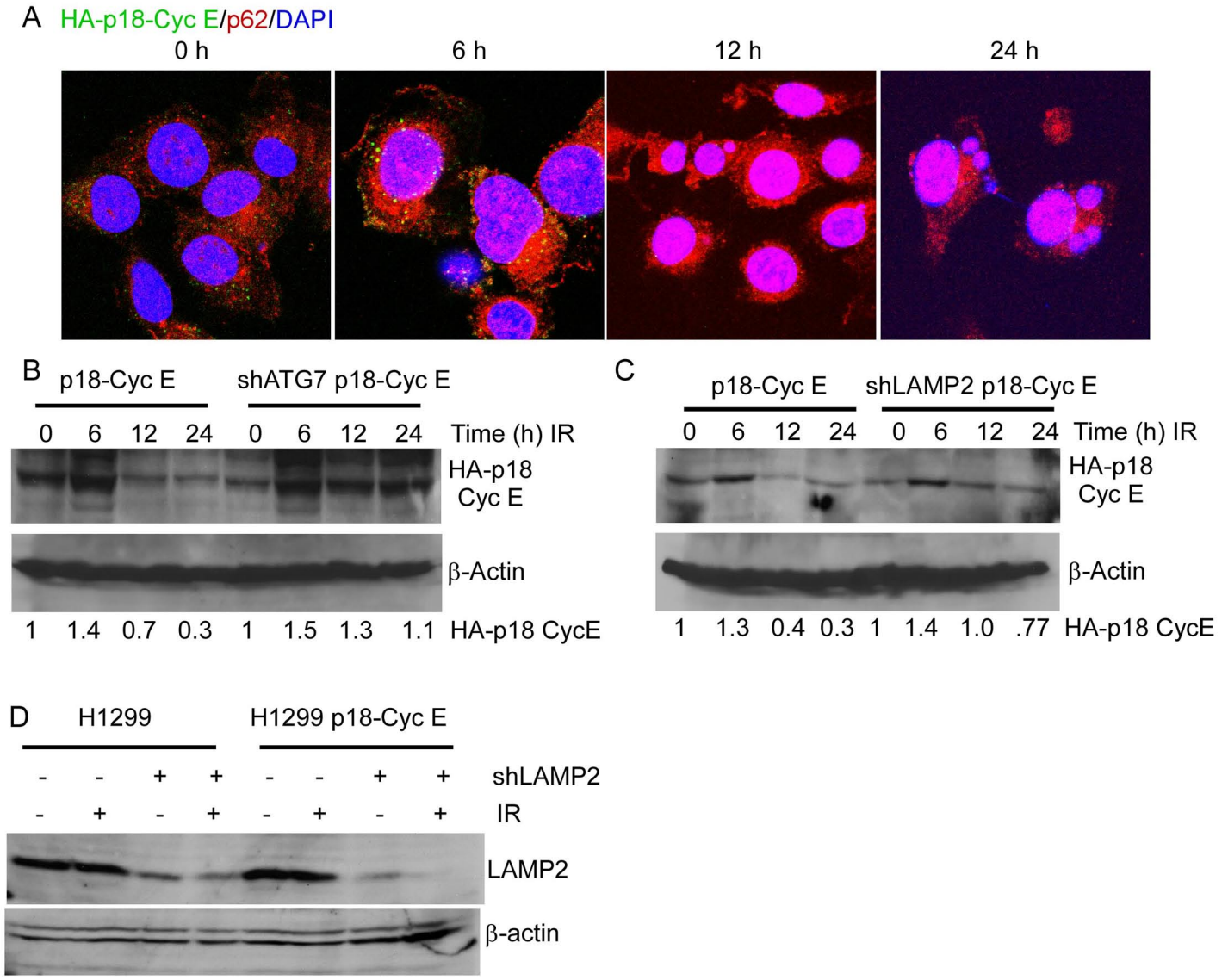

Figure S5. p18-CycE expression is regulated by autophagy. (A) Confocal co-immunostaining images for HA-p18-CycE and p62 in cells expressing stable HA-p18-CycE at the indicated times following irradiation. Nuclei were stained with DAPI. (B,C) Cells expressing p18-CycE and shATG7 or shLAMP2, respectively, were lysed at the indicated times following irradiation and immunoblotted for HA-p18-CycE with β-actin as loading control. (D) Parental and p18-CycE-expressing cells with or without shLAMP2 were lysed at 24 h following irradiation and immunoblotted for LAMP2, with β-actin as loading control.

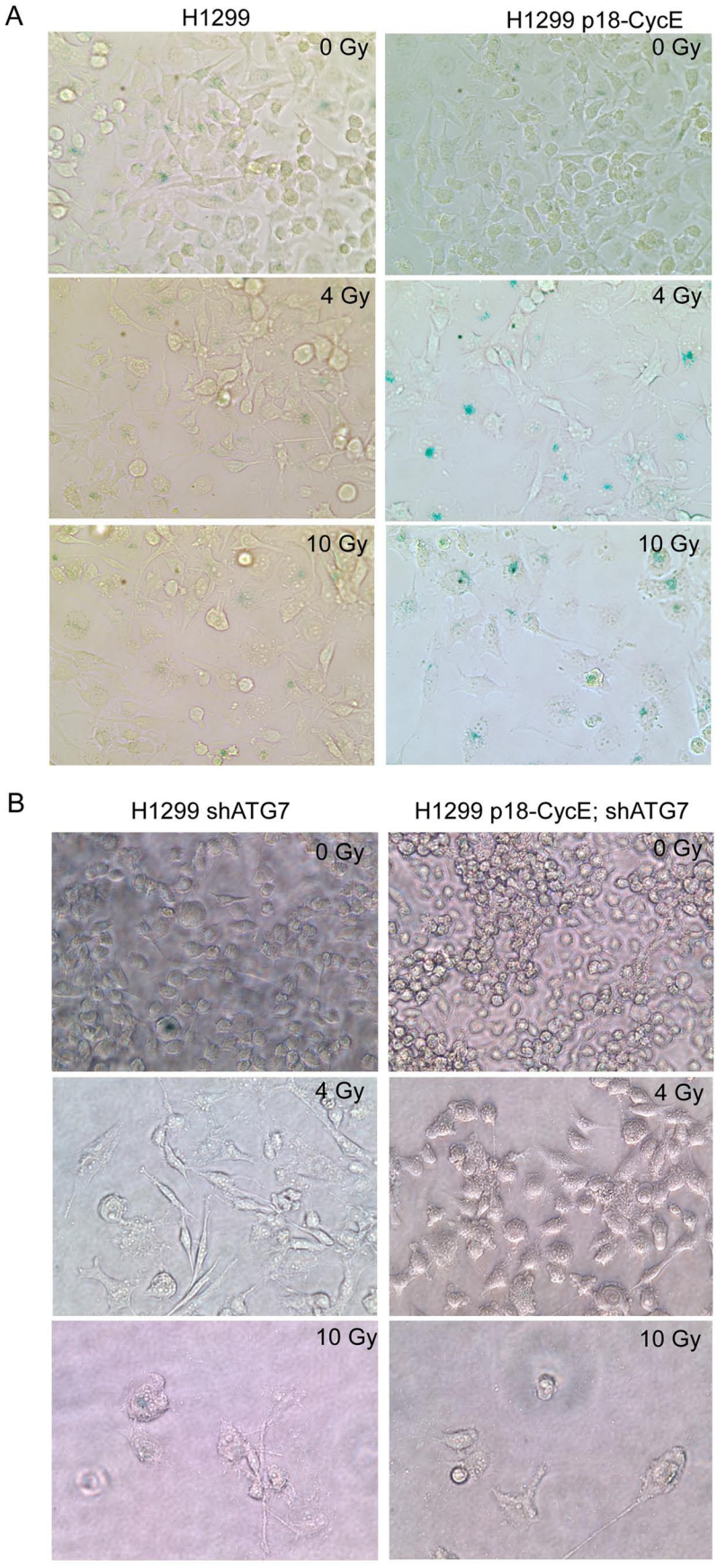

Figure S6 DNA Damage induces autophagy-dependent senescence in p18-CycE-expressing cells. (A) Representative images for SA-β-Galactosidase staining in p18-CycE-expressing and parental cells at 6 d post irradiation. (B) Representative images for SA-β-Galactosidase staining in p18-CycE-expressing and parental cells in the absence or presence of shATG7 at 6 d post irradiation.

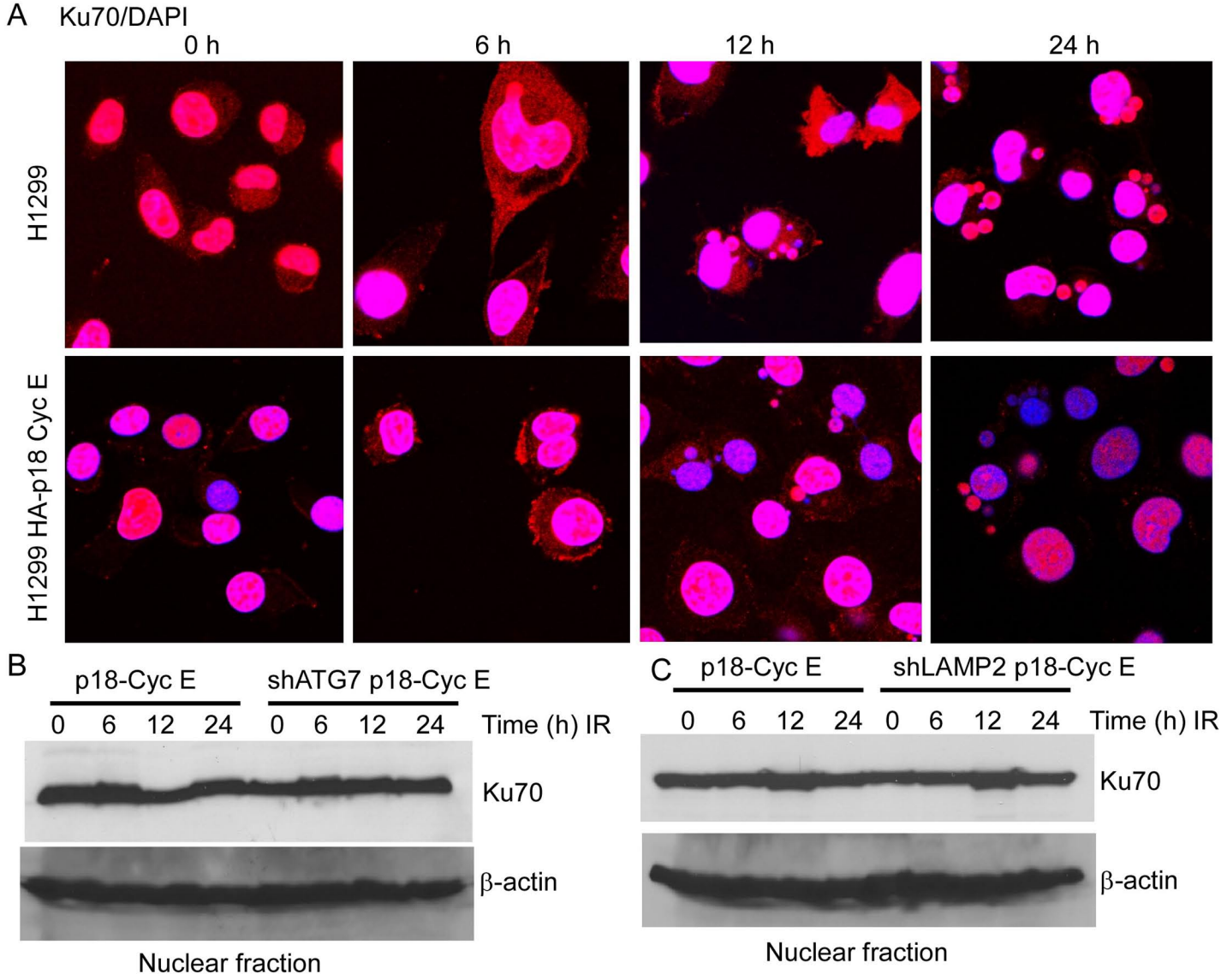

Figure S7. Cytoplasmic Ku70 degradation is prevented upon autophagy inhibition following irradiation. (A) Confocal co-immunostaining images for Ku70 in parental and HA-p18-CycE-expressing cells at the indicated times following irradiation. Nuclei were stained with DAPI. (B and C) Nuclear lysates of p18-CycE-expressing cells with or without shATG7 or shLAMP2 expression at the indicated times following irradiation were immunoblotted for Ku 70 and β-actin, as loading control.