

**Supplementary Data:**

**Senolytic-mediated elimination of head and neck tumor cells**

**induced into senescence by cisplatin**

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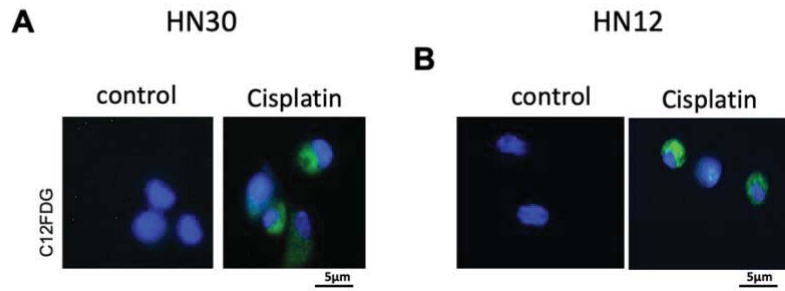
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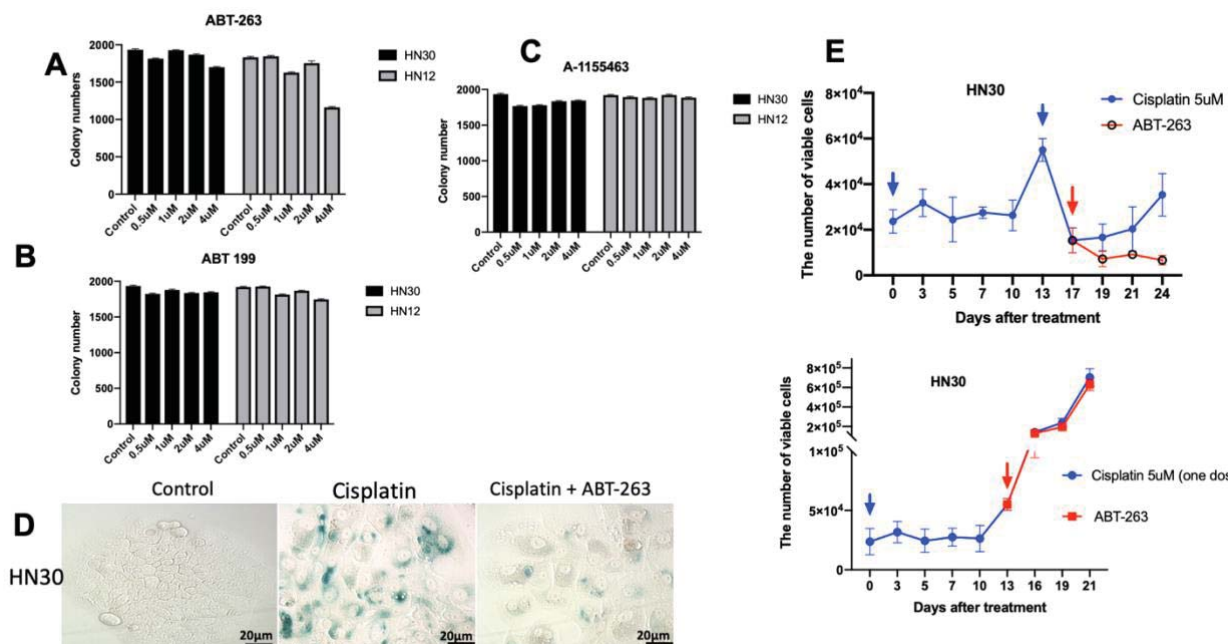
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**Figure S1**



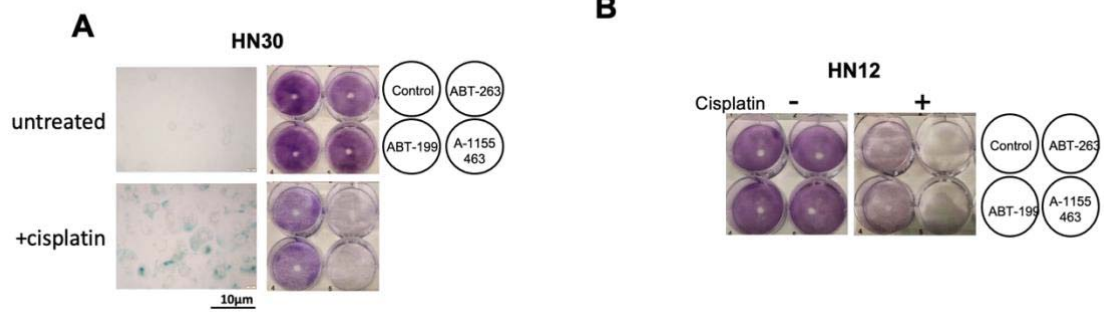
**Figure S1. Immunofluorescence imaging of C<sub>12</sub>FDG in A) HN30 and B) HN12 cells after senescence induction by 5 μM cisplatin. Blue fluorescence indicates nuclear staining with DAPI, and green fluorescence reflects C<sub>12</sub>FDG immunostaining.**

**Figure S2**



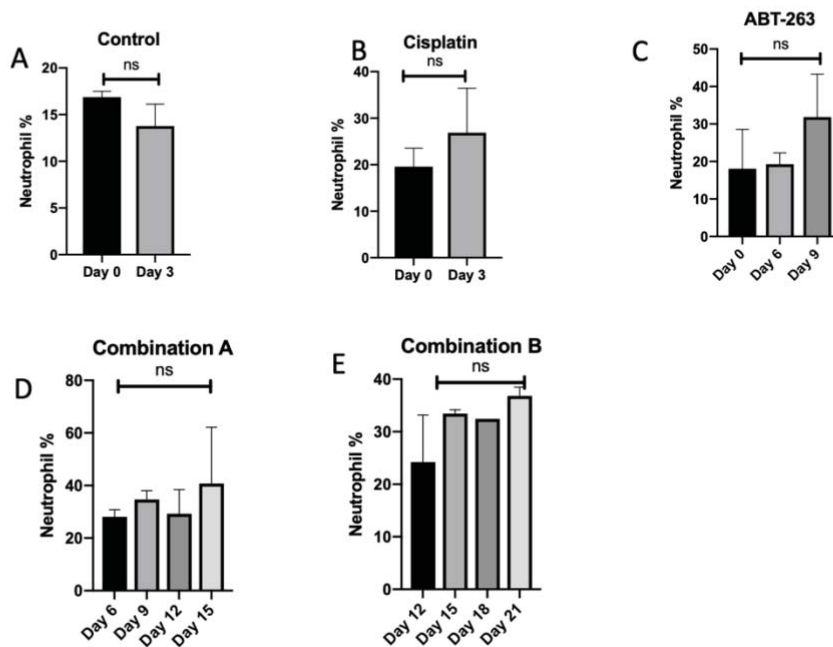
**Figure S2. ABT-263 has minimal cytotoxicity on non-senescent cells or proliferative recovering cells from senescence.** **A, B, and C)** Clonogenic survival assay performed on control cells treated with different concentrations of ABT-263 for 24 hours. The number of colonies were counted and analyzed. **D)** X-gal staining after sequential treatment of HN30 cells with cisplatin and ABT-263; decreased population of SA-β-gal positive cells show that ABT-263 treatment eliminates senescent cells. **E)** ABT-263 effectiveness diminishes over time when HN30 cells recover their proliferative capacity. Blue arrows indicate the cisplatin treatment timepoint. Red arrows are ABT-263 treatment timepoints. Note that HN30 cells undergo cell death only when they are in senescence state (top), but not in recovery stage (bottom). All quantitative graphs are mean ± SD from at least three independent experiments.

**Figure S3**



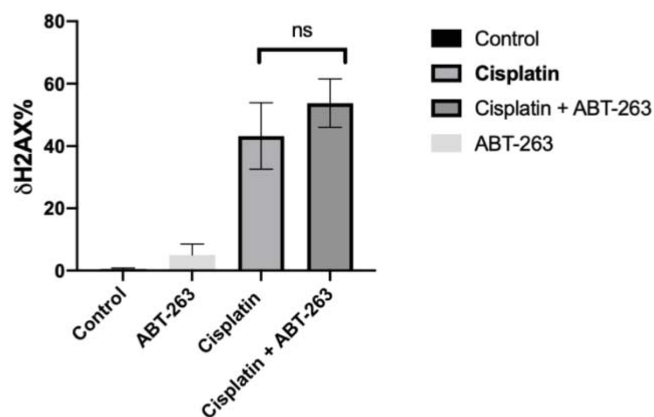
**Figure S3. BCL-X<sub>L</sub> is the primary target for ABT-263-induced senolysis.** Clonogenic survival assay performed on **A) HN30 and B) HN12** cells treated with vehicle or cisplatin followed by ABT-263, ABT-199, and A-1155463 (1µM for 24 hours).

**Figure S4**



**Figure S4.** Cisplatin, ABT-263 treatment alone or in combination did not result in significant Neutropenia. Blood samples were analyzed for neutrophil percentage at different time points in different groups of **A)** control, **B)** Cisplatin alone, **C)** ABT-263, **D)** and **E)** cisplatin in combination with ABT-263. Control vs ABT, cisplatin, Combination A or B:  $p > 0.05$  All quantitative graphs are mean  $\pm$  SD from at least three independent experiments. \*  $p \leq 0.05$ , \*\*  $p \leq 0.01$ , \*\*\*  $p \leq 0.001$ , \*\*\*\*  $p \leq 0.0001$  0001 indicate statistical significance of each condition compared to indicated condition as determined using two - way ANOVA with Sidak's post hoc test.

**Figure S5**



**Figure S5.** Cisplatin, ABT-263 treatment alone or in combination did not result in significant levels of DNA double strand breaks measured by  $\gamma$ -H2AX levels.  $p > 0.05$  All quantitative graphs are mean  $\pm$  SD from at least three independent experiments. \*  $p \leq 0.05$ , \*\*  $p \leq 0.01$ , \*\*\*  $p \leq 0.001$ , \*\*\*\*  $p \leq 0.0001$  0001 indicate statistical significance of each condition compared to indicated condition as determined using two - way ANOVA with Sidak's post hoc test.

Figure S6

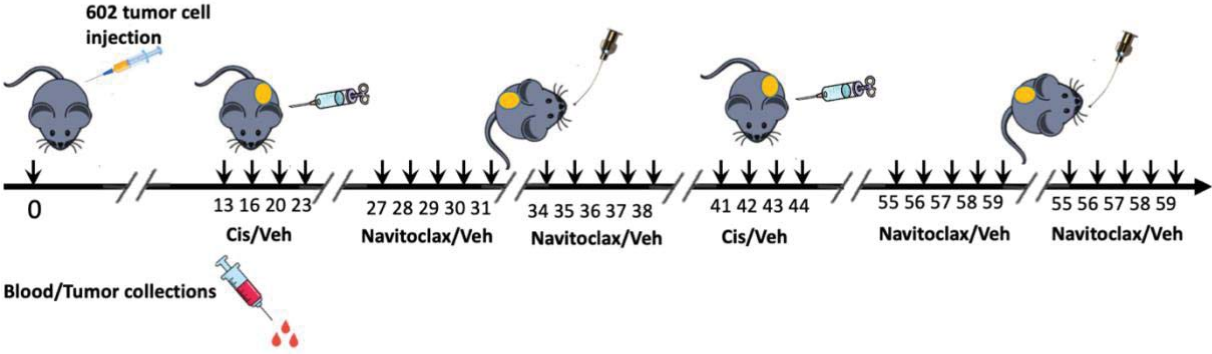


Figure S6. Animal experiments diagram.