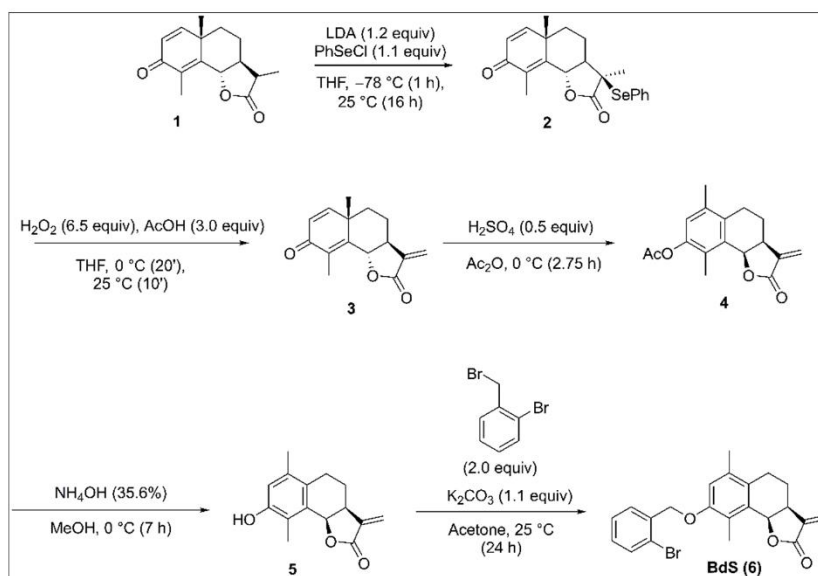
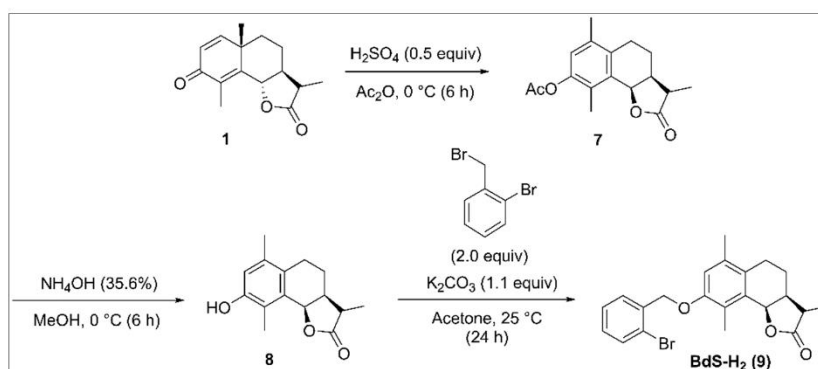


# Supplementary Figure S1

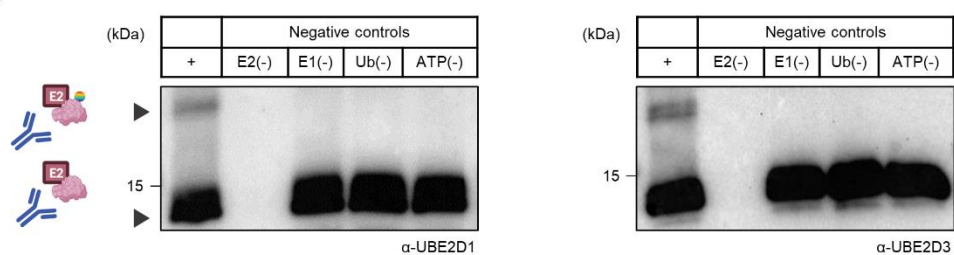
**A**



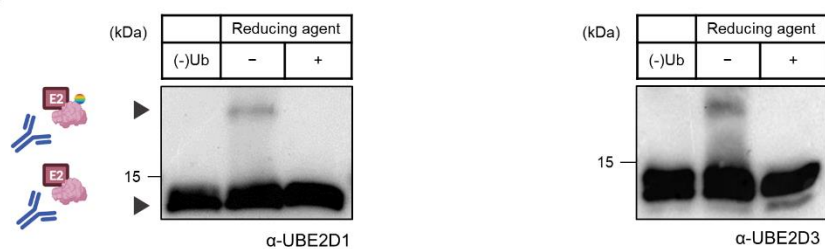
**B**



**C**

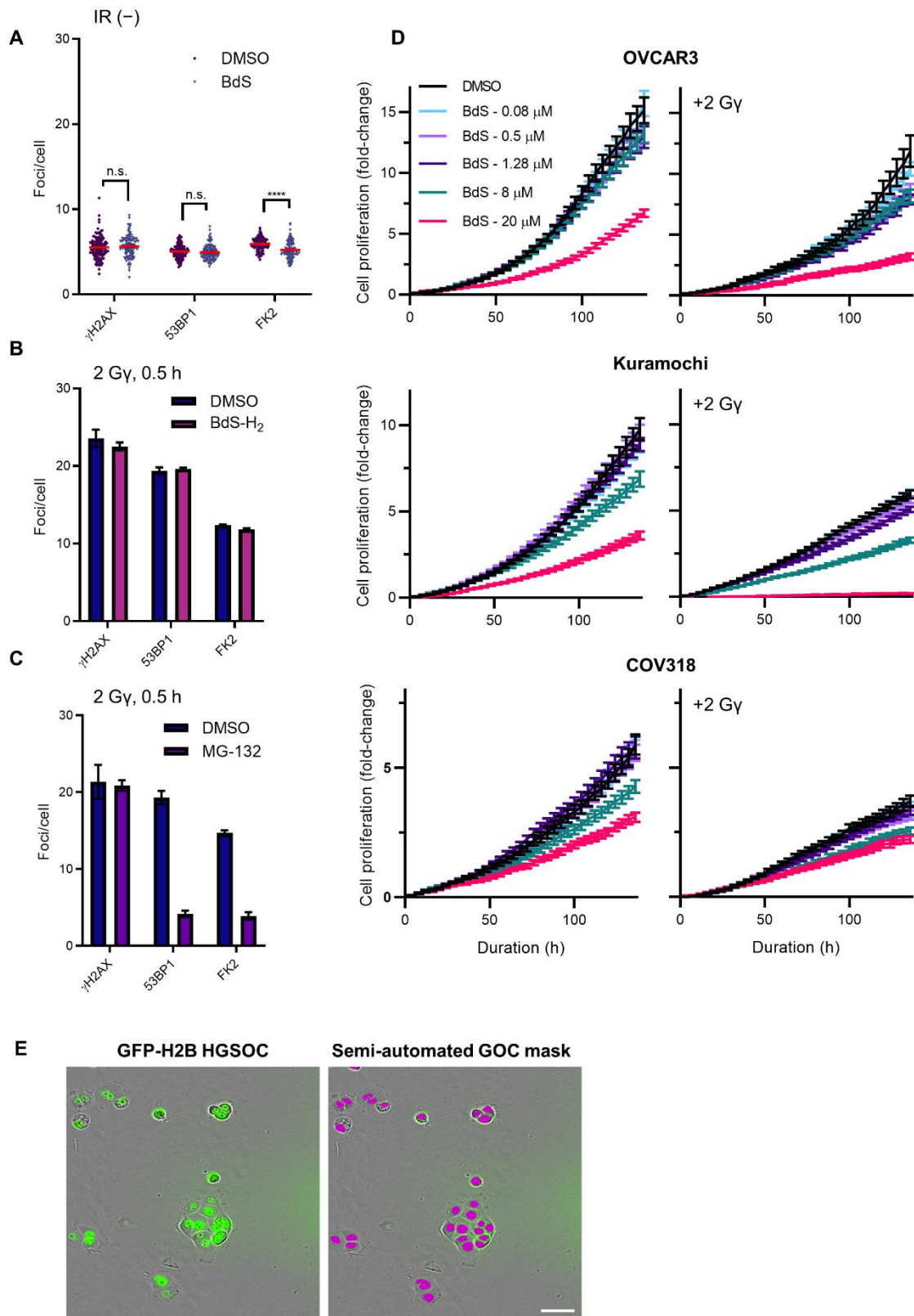


**D**



**Supplementary Figure S1. Synthesis of BdS and BdS-H<sub>2</sub>, and *in vitro* ubiquitylation assay workflow controls.** (A) Synthetic route towards sesquiterpene lactone, BdS, as previously described [21]. (B) Synthetic route towards negative control compound, BdS-H<sub>2</sub>. (C) Systematic negative controls for the *in vitro* ubiquitylation assays performed for UBE2D1 (left,  $n = 1$ ), and UBE2D3 (right,  $n = 1$ ), with all the components being required for ubiquitin-loading of the relevant E2 as indicated by the formation of the relevant band in the left-most lane (+). (D) Representative blots of a control experiment demonstrating that ubiquitin-loaded E2 formation is abrogated with the addition of reducing agent ( $\beta$ -mercaptoethanol) for both UBE2D1 ( $n = 2$  independent experiments) and UBE2D3 ( $n = 2$  independent experiments).

Supplementary Figure S2

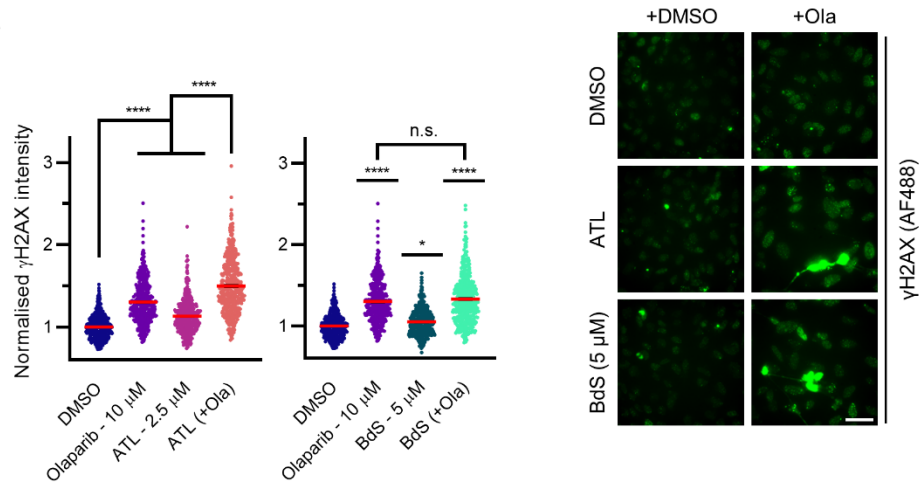


**Supplementary Figure S2. Establishing DNA repair and HGSOC cell growth workflows. (A)**

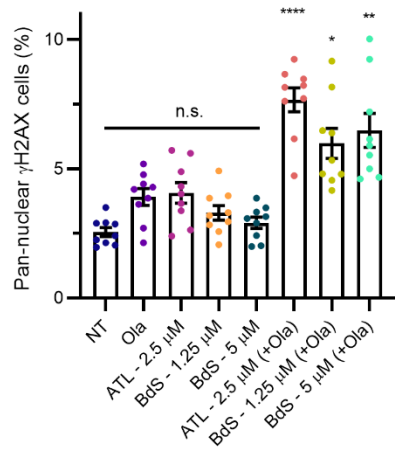
Quantification of foci number per cell for DNA damage response (DDR) factors ( $\gamma$ H2AX, 53BP1, FK2), omitting irradiation for U2OS cells treated with BdS (20  $\mu$ M, 2 h). Each dataset represents a minimum of 3,000 cells (3 replicates;  $n = 126$ ; mean  $\pm$  SEM). Data points correspond to each recorded field. **(B)** Quantification of foci number per cell for DDR factors ( $\gamma$ H2AX, 53BP1, FK2), following irradiation (2 Gy, 0.5 h recovery) and treatment with BdS-H<sub>2</sub> (200  $\mu$ M, 2 h). Each dataset represents a minimum of 1,500 U2OS cells (2 replicates;  $n = 2$ ; mean  $\pm$  SEM). **(C)** Quantification of foci number per cell for DDR factors ( $\gamma$ H2AX, 53BP1, FK2), following irradiation (2 Gy, 0.5 h recovery) and treatment with proteasome inhibitor, MG132 (10  $\mu$ M, 2 h). Each dataset represents a minimum of 1,500 U2OS cells (2 replicates;  $n = 2$ ; mean  $\pm$  SEM). **(D)** Representative fold-change growth curves ( $n = 27$  for each data point) for high-grade serous ovarian carcinoma (HGSOC) cell lines in the presence of BdS over 136 h – top to bottom: OVCAR3, Kuramochi, COV318. Left to right: non-irradiated, irradiated (2 Gy) for each cell line. **(E)** Representative images of the green object count (GOC) mask applied to GFP-H2B HGSOC live cell imaging in order to track proliferation. Scale – 50  $\mu$ M. Statistical significance indicated as follows: n.s.— $p > 0.05$ , and \*\*\*\*— $p < 0.0001$ .

# Supplementary Figure S3

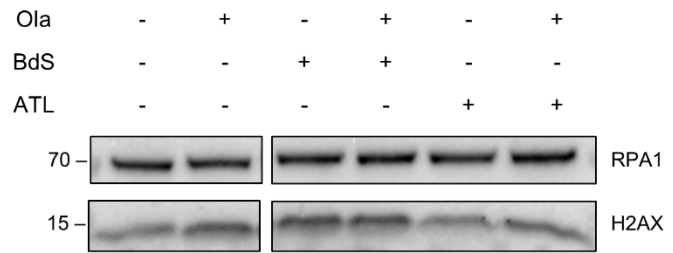
**A**



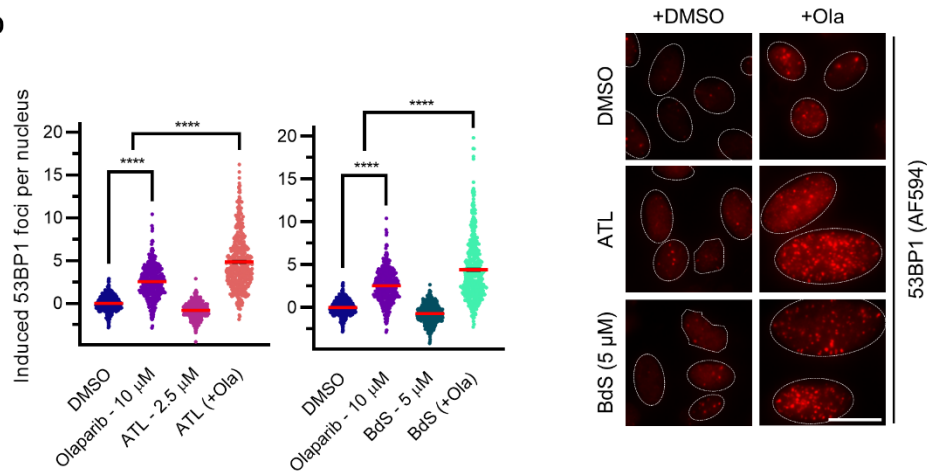
**B**



**C**



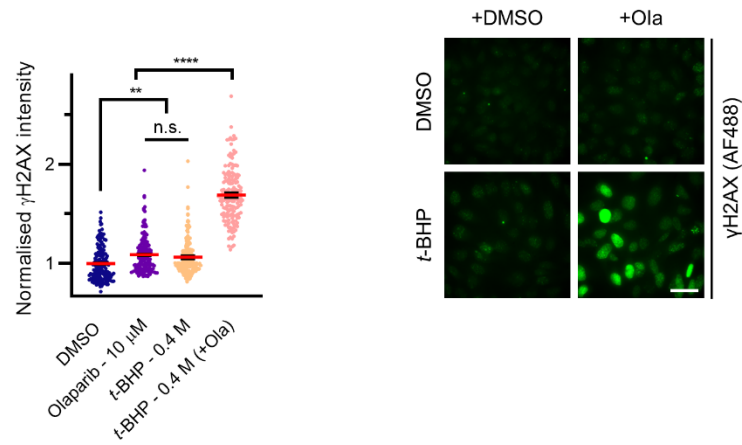
**D**



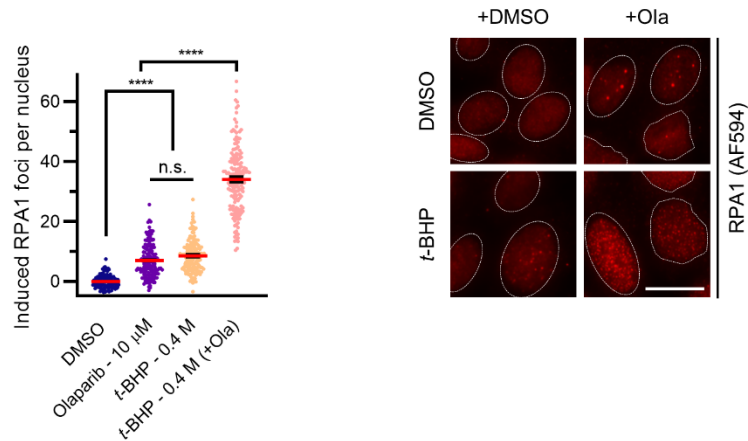
**Supplementary Figure S3. DNA damage markers characterise cellular response to combination treatments over longer durations.** (A) Quantification (left) and representative images (right, Alexa Fluor 488; AF488) of normalised nuclear  $\gamma$ H2AX immunofluorescence intensity (to vehicle-only – DMSO-treated cells) after 72 h treatment with the specified compounds alone or in combination. Each dataset represents a minimum of 15,000 U2OS cells (3 independent experiments;  $n = 504$ ; mean  $\pm$  SEM), while data points correspond to each recorded field. Scale bar – 50  $\mu$ m. (B) Quantification of pan-nuclear  $\gamma$ H2AX immunofluorescence at 72 h. Each dataset represents a minimum of 4,500 U2OS cells (3 independent experiments;  $n = 9$ ; mean  $\pm$  SEM). (C) Immunoblot showing whole cell extract levels of RPA1, and H2AX following treatment of U2OS cells with olaparib (10  $\mu$ M) alone, and in combination with alantolactone (ATL; 10  $\mu$ M), and BdS (10  $\mu$ M) for 72 h ( $n = 1$ ). Images taken from the same membrane for RPA1 and H2AX, respectively. (D) Quantification (left) and representative images (right Alexa Fluor 594; AF594) of induced 53BP1 foci (compared to vehicle-only – DMSO-treated cells) after 72 h treatment with the specified compounds alone or in combination as indicated. Each dataset represents a minimum of 15,000 U2OS cells (3 independent experiments;  $n = 504$ ; mean  $\pm$  SEM), while data points correspond to each recorded field. Nuclei are outlined in white using DAPI as a reference. Scale bar – 20  $\mu$ m. Statistical significance indicated as follows: n.s.— $p > 0.05$ , \*— $p < 0.05$ , \*\*— $p < 0.01$ , and \*\*\*\*— $p < 0.0001$

## Supplementary Figure S4

**A**



**B**



**Supplementary Figure S4. A combination of ROS inducer, *tert*-butyl hydroperoxide (*t*-BHP), and olaparib treatments phenocopies the effects seen with olaparib and ATL/BdS combination treatments. (A)** Quantification (left) and representative images (right, Alexa Fluor 488; AF488) of normalised nuclear  $\gamma$ H2AX immunofluorescence intensity (to vehicle-only – DMSO-treated cells) after 24 h treatment with olaparib, *t*-BHP, or in combination as specified. Each dataset represents a minimum of 7,500 U2OS cells (3 replicates;  $n = 168$ ; mean  $\pm$  SEM), while data points correspond to each recorded field. Scale bar – 50  $\mu$ m. **(B)** Quantification (left) and representative images (right) of induced RPA1 foci per cell (Alexa Fluor 594; AF594), after 24 h treatment with olaparib, *t*-BHP, or in combination as specified compared to vehicle only (DMSO). Each dataset represents a minimum of 7,500 U2OS cells (3 replicates;  $n = 168$ ; mean  $\pm$  SEM). Data points correspond to each recorded field (mean  $\pm$  SEM). Nuclei are outlined in white using DAPI as a reference. Scale bar – 20  $\mu$ m. Statistical significance indicated as follows: n.s.— $p > 0.05$ , \*\*— $p < 0.01$ , and \*\*\*\*— $p < 0.0001$ .