iScience, Volume *27*

Supplemental information

Distinct effects of sacituzumab govitecan and berzosertib on DNA damage response in ovarian cancer

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Supplementary figure legends

Supplementary Figure S1. TROP2 expression in HGSOC cell lines, related to figure 1C, 1D and 1E.

(A) Flow cytometric analysis for TROP2 expression (grey) on HGSOC cells was performed after incubating cells with recombinant anti-TROP2 hRS7 antibody followed by goat anti-human $F(ab)_2$-AF488 secondary antibody as given in methods. In control tubes (white), cells were treated only with secondary antibody.

(B) Western blot analysis of the same cell lines for protein expression. Densitometric analysis of expression of TROP2 relative to GAPDH are shown below the respective images.

(C) Immunofluorescent microscopic analysis of HGSOC cells for surface expression of TROP2 (green), with matched isotype antibodies or those specific to TROP2. Slides were counterstained with nuclear dye DAPI (blue). Scale bar (white) represents a length of 20 µm.

Supplementary Figure S2. Comparative efficacy analysis of SN-38 versus SG for combination with ATR inhibitor (ATRi) ceralasertib or berzosertib, related to figures 1A and 2.

(A) XTT assays were performed over 72 hours for the given cell lines with SN-38 (0 – 40 nM) to estimate IC_{50} values for growth inhibition (GI). The mean $\pm$ SD (*n* = 3 biological replicates) for each treatment are plotted as line curves. The calculated IC_{50} values for SN-38 are given on the right and used as basis for subsequent experiments with SN-38 or with SG.

(B) Combination screening for synergy was conducted by treating cells plated in 96 well plates (3000 cells/well) with SN-38 (0-20 nM) with or without berzosertib (0 – 1 µM) or ceralasertib (0 – 10 µM). After 72 hours, plates were assayed with XTT reagent for growth inhibition and values were analyzed by Combenefit™ software[1] for synergy using the HSA scoring system[2]. Positive excessHSA scores (given within squares) of the combination indicates synergy as pictured by the color scale below.

(C) To assess whether equimolar SG would produce similar synergy with ATRi as SN-38, cells in 96-well plates (3000 cells/well) were treated with three different concentrations of SN-38, selected for synergy from (B) or equimolar concentrations of SG in combination with ATRi over 72 hours (*n* = 3 biological replicates per treatment). Synergy was measured as described in methods. Data is representative of 2 biologically independent experiments.

Supplementary Figure S3. Inhibitory effect of combination treatment of SN-38 or equimolar SG with ATRi on HGSOC cell growth, related to figures 2A and 2B.

XTT assays were performed to compare the inhibitory efficacy of combining SN-38 or equimolar SG with ceralasertib or berzosertib. Cells were plated in 96-well plates (3000 cells/well) 24 hours prior to addition of drugs. Cells were treated with IC_{50} concentration of SN-38 as determined from Figure S2A for OVCAR8 (3 nM), PEO1 (2 nM), PEO1-olaR (4 nM) and PEO1-olaJR (4 nM) or the equivalent molar concentrations for control ADC (cADC) or SG, followed by the addition of ceralasertib (0 – 10μM) (left) or berzosertib (0 – 2μM) (right) and plates were read with XTT reagent after 72 hours of incubation. IC_{50} values for the respective ATRi against combination with either SG, cADC or SN-38 are given on the right of each plot as a measure of potency. Data were analyzed using Student's *t*-test and the mean $\pm$ SD (*n* = 3 biological replicates per treatment) are plotted as line curves. Statistical difference between respective points on the curves for control versus SN-38 or SG are given as "*" while those between SG and cADC are shown as "#". *,# $p < 0.05$, **,## $p < 0.01$, and ***,### $p < 0.001$, ns, not significant. Experiment was repeated twice with similar results.

Supplementary Figure S4. Effect of SN-38 or equimolar SG on ATRi induced cell death in HGSOC, related to figure 2E.

Flow cytometric analysis for viability (AnnexinV/7AAD) of HGSOC cells treated with either SN-38 (4 nM) or equimolar concentration of SG (0.57 nM) with or without berzosertib (500 nM) or ceralasertib (2 μM) for 48 hours in 6-well plates. % Viable cells (negative for both AnnexinV and 7-AAD) were plotted as bar charts as shown on the right. Data is representative of 2 biologically independent experiments.

Supplementary Figure S5. Effect of SG and berzosertib combination treatment on DNA damage markers in HGSOC, related to figures 3C and 4D.

(A-B) Immunofluorescent confocal microscopic analysis of OVCAR8 and PEO1-olaR for RPA1 (green) and γH2AX-S139 (pink) of cells pretreated with SG (10 µg/ml, for 30 min at 37°C, washed 3 times with PBS and resuspended in media) and then exposed to berzosertib (ATRi) (1 µM) overnight. Graphs show mean ± SD of percent positive cells for either RPA1 or γH2AX foci relative to total cells from each sampled field (*n* = 3-5 sampled fields). Scale bar (white) represents a length of 20 µm. Significance was assessed using Student's *t*-test. * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$, ns, not significant.

(C) Microscopic analysis of cells pretreated with SG and exposed to a gradient of berzosertib (0, 0.25, 0.5 and 1 µM). At least 200-300 cells were examined for each treatment and cells positive for >15 γH2AX foci were plotted as bar charts. Graphs show mean ± SD of % $\gamma H2AX^{+ve}$ cells relative to total cells from each sampled field (*n* = 3-5 sampled fields). Significance was assessed using Student's *t*-test. * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$, ns, not significant. Data is representative of 2 biologically independent experiments.

(D) Representative images of cells pretreated with SG followed by overnight treatment with or without berzosertib (ATRi) prior to staining for RPA1 (green foci) and Cyclin B1 (CCNB1, red). The graph shows percent distribution of RPA1+ve cells across cell cycle phases as defined by staining pattern of nuclear CCNB1 from the sampled fields (*n* = 3-5 biological replicates per treatment). Representative of *n* = 2 biologically independent experiments. Scale bar (white) represents a length of 20 µm.

Supplementary Figure S6. Role of TLS in SG induced sensitization to olaparib, related to figure 5.

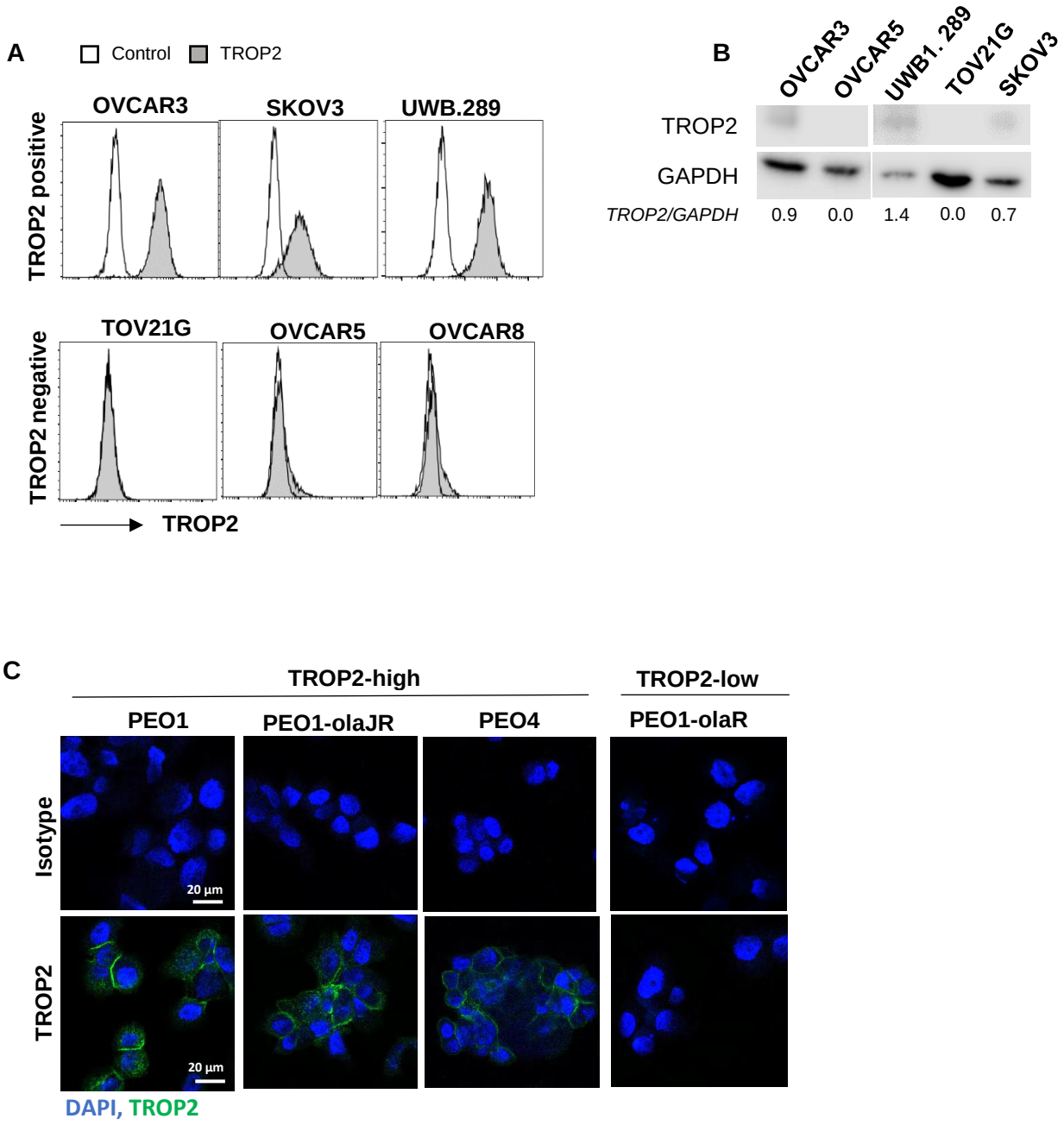
(A) Cell lines positive and negative for TROP2 were treated with JH-RE-06 (0 – 10 µM) on Day 0 and Day 3 of a 5-day experiment. Graph shows mean absorbance values at 490nm ± SD (*n* = 3 biological replicates). Zero drug values were replaced with 0.01 for the first datapoint (untreated), on each curve to enable plotting on a logarithmic scale.

(B) Western assays on the components and markers of TLS activity. Densitometric analysis of expression of each target protein was performed relative to GAPDH or total proteins are shown below the respective images.
(C) XTT assays of untreated PEO1-olaR cells or cells pretreated with SG (10 µg/ml for 30 min at 37°C, washed 3 times with PBS) were incubated for 5 days with different concentrations of olaparib (0-10 µM). Graph shows mean ± SD of absorbance at 490 nm (*n* = 3 biological replicates).
(D) Graph shows mean ± SD of cell growth values from XTT assays of PEO1-olaR cells with or without SG pretreatment and incubated with olaparib (10 µM) for 5 days against a gradient of specific TLS inhibitor JH-RE-06 (*n* = 3 biological replicates).
For (C) and (D), Student's *t*-test was used to assess significance. * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$, ns, not significant.

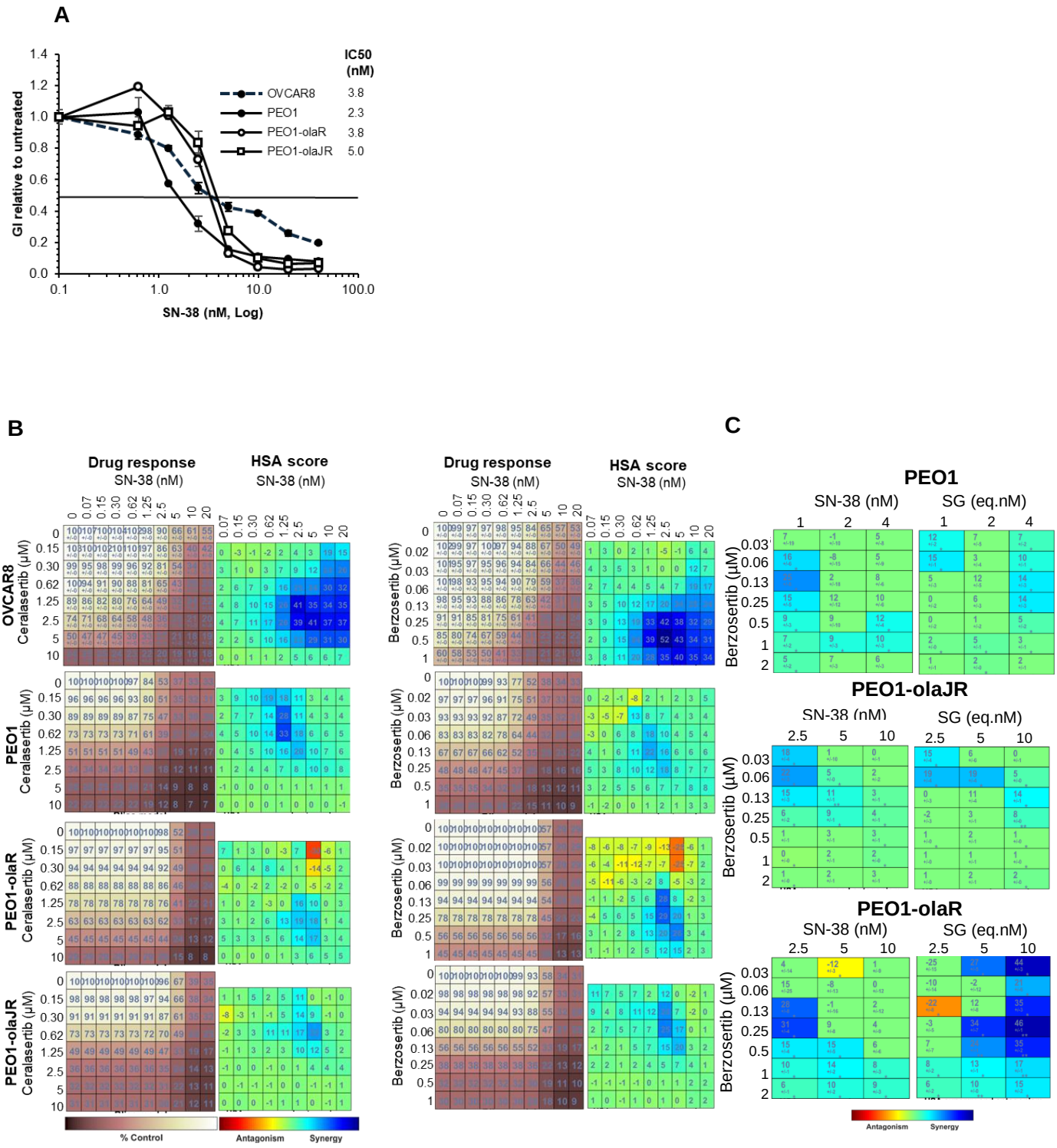
Supplementary Figure S7. Efficacy of SG and berzosertib combination treatment in HGSOC murine models, related to figure 7.

(A) Flow cytometric analysis for TROP2 expression (grey) was performed after incubating cells with recombinant anti-TROP2 hRS7 antibody followed by Goat anti-human $F(ab)_2$-AF488 secondary antibody as given in methods. In control tubes (white), cells were treated only with secondary antibody. TROP2 negative A2780 cell line was used to verify non-specificity of antibodies. Mean fluorescence intensity (Mean FI) of TROP2 histograms relative to control for each cell line is plotted as bar charts on the right.
(B) Line plots from Figures 7B and 7C with error bars showing median ± 95% CI. Charts show % change in disease burden against time on the X-axis (days 0 – 77) measured at weekly intervals (*n* = 8-10 mice per group).
(C) Luciferase imaging performed on PEO1-olaJR bearing NSG mice over 80 days and plotted for each treatment for PEO1-olaJR. The weight charts for the same mice are shown on the right. This experiment was performed once.

Supplementary Figure S1.



Supplementary Figure S2.



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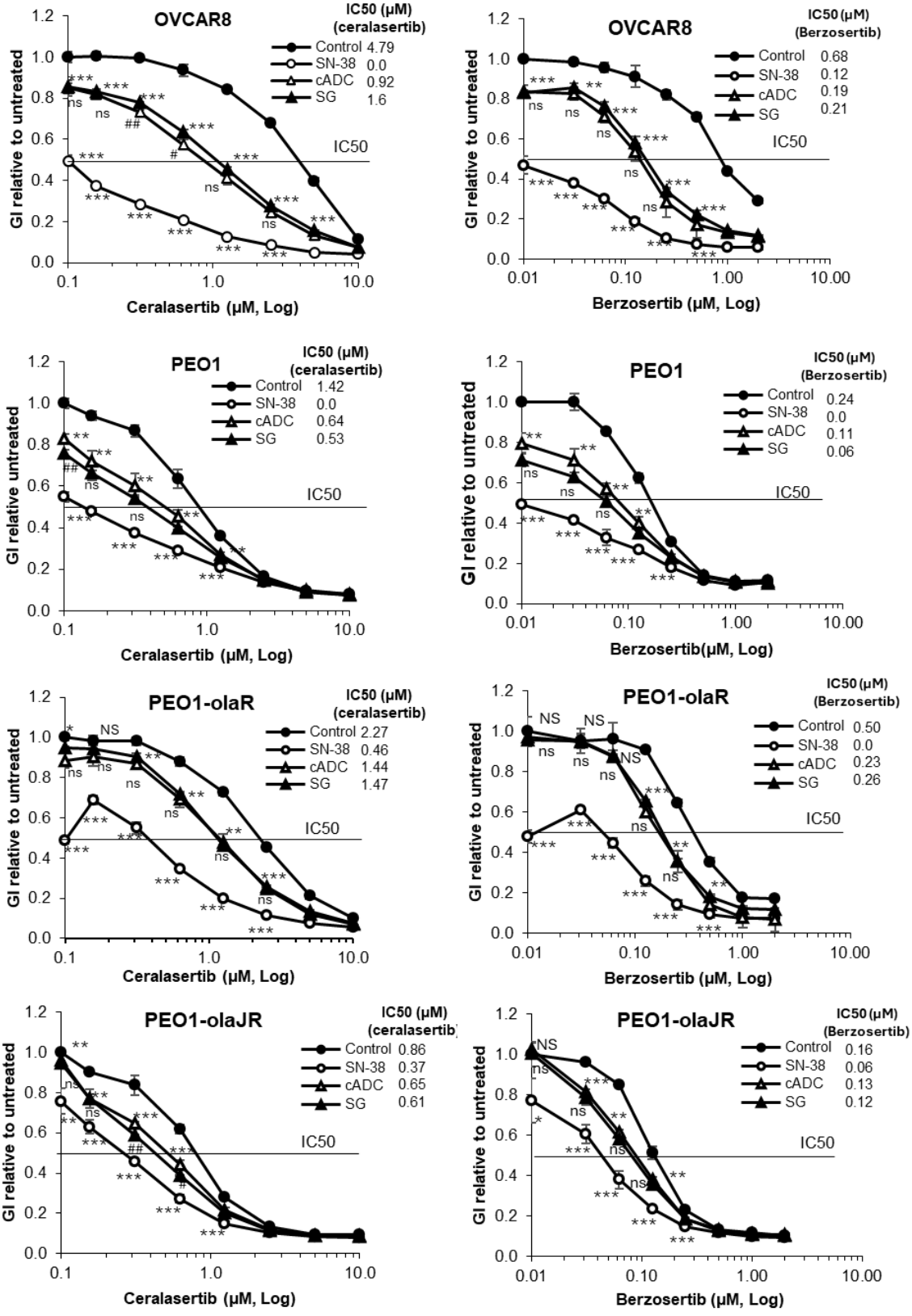

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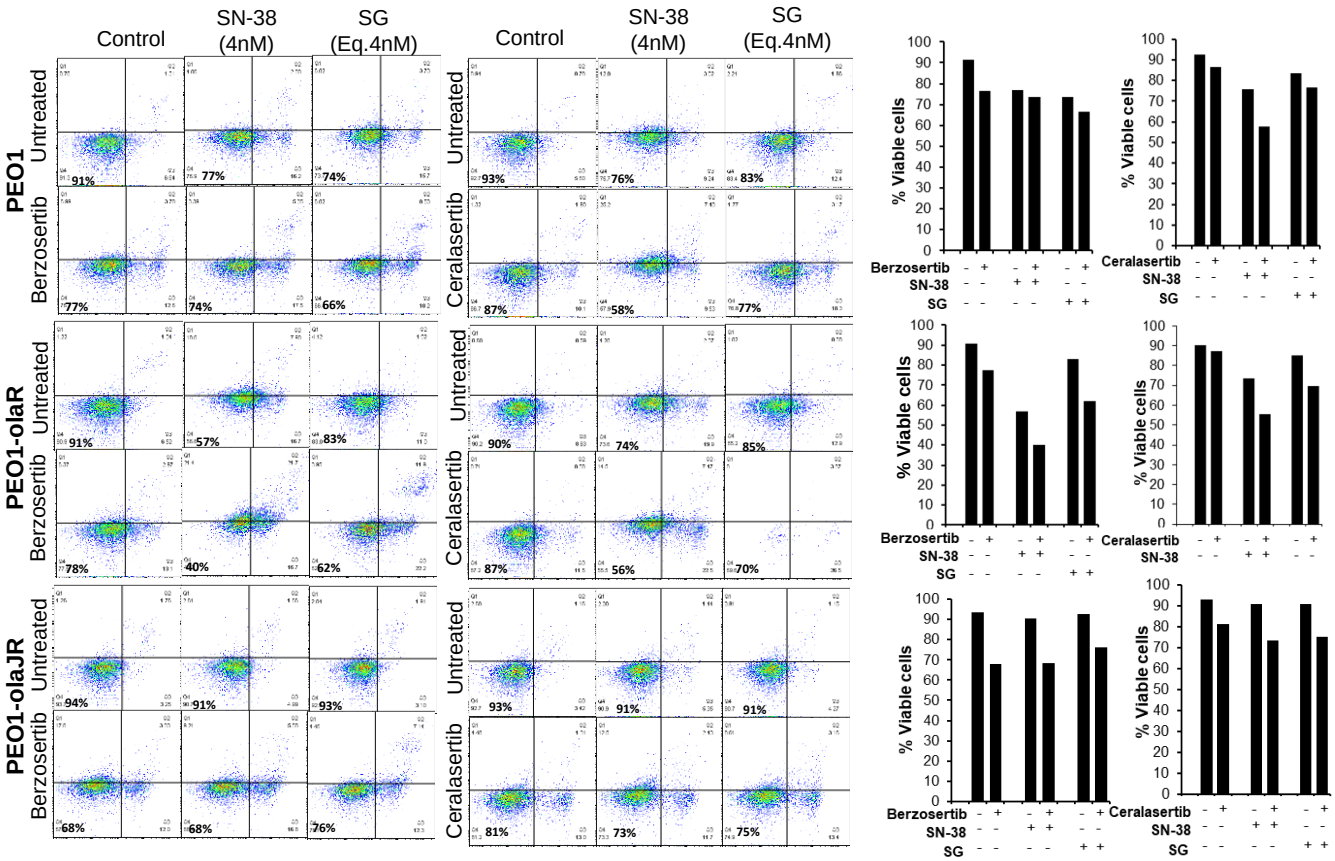
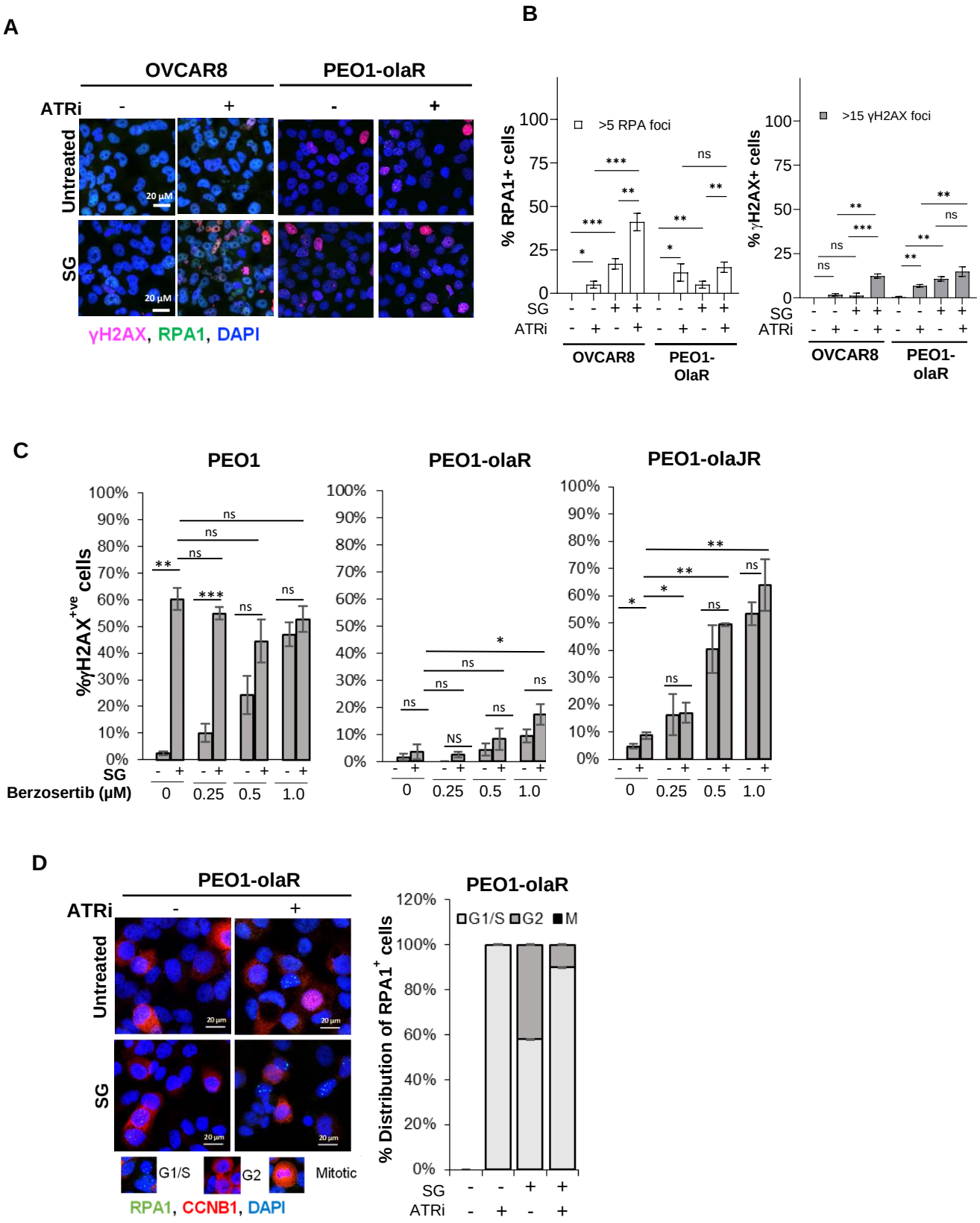


Supplementary Figure S5



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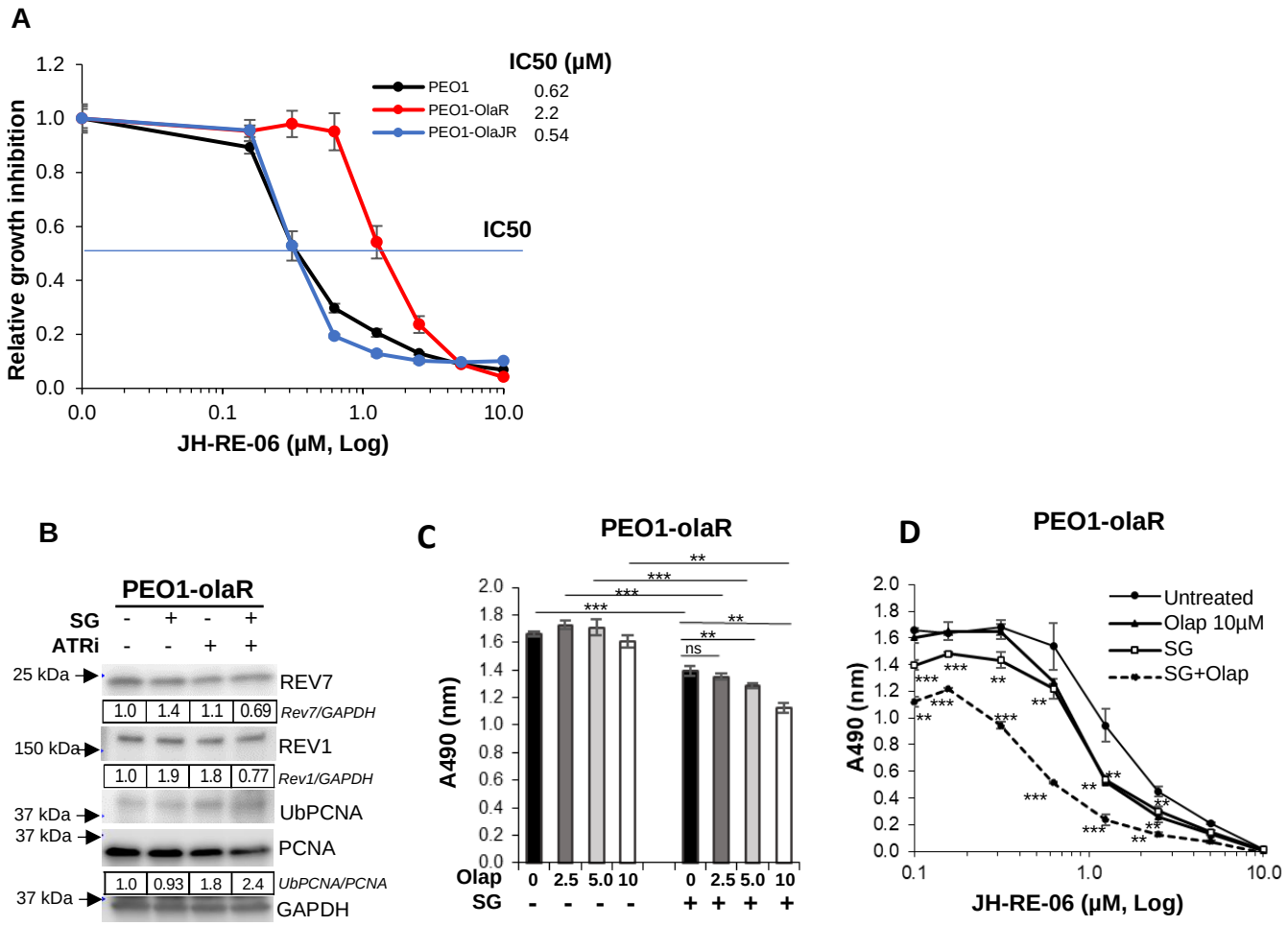
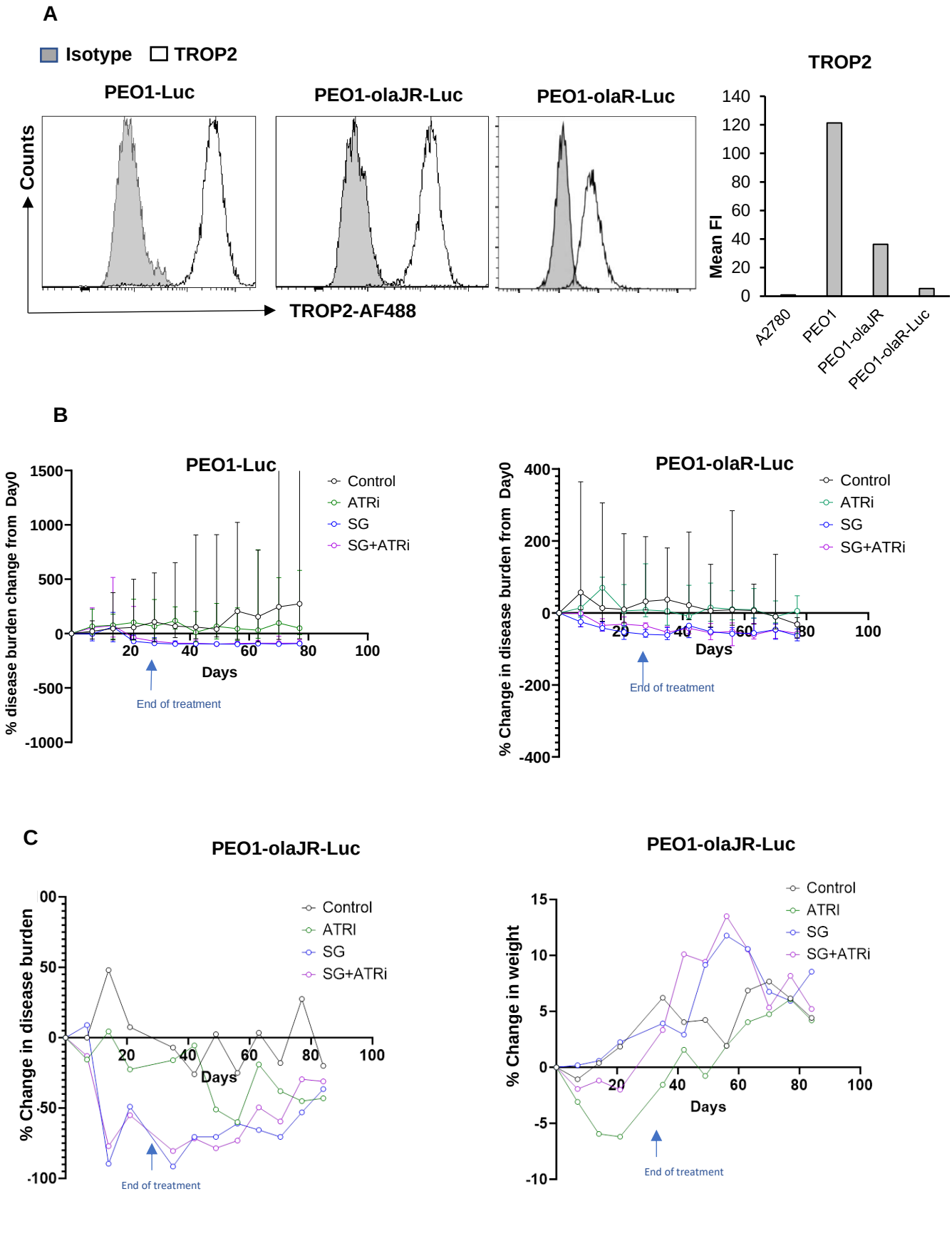


Supplementary Figure S7



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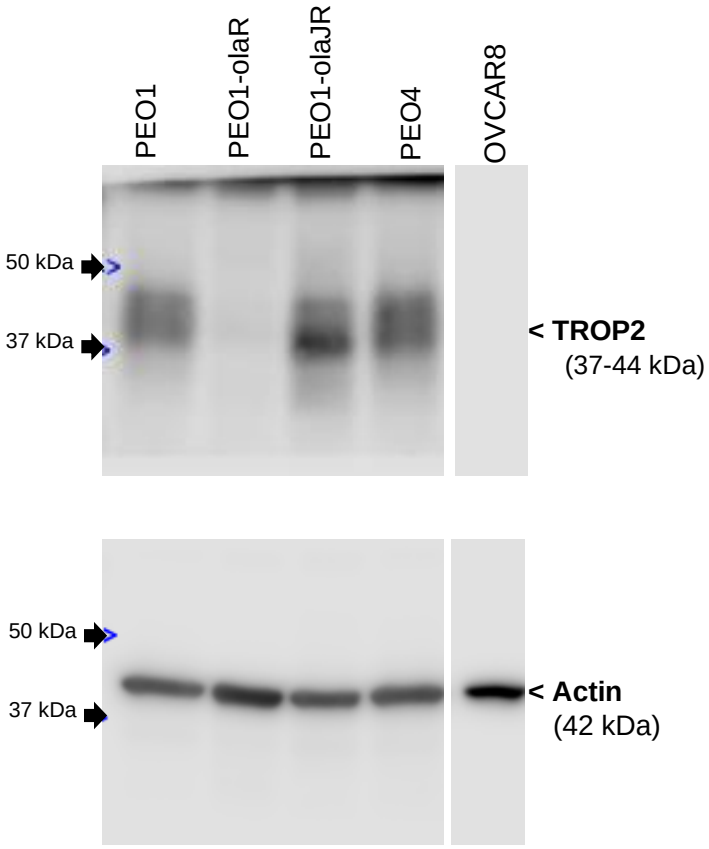
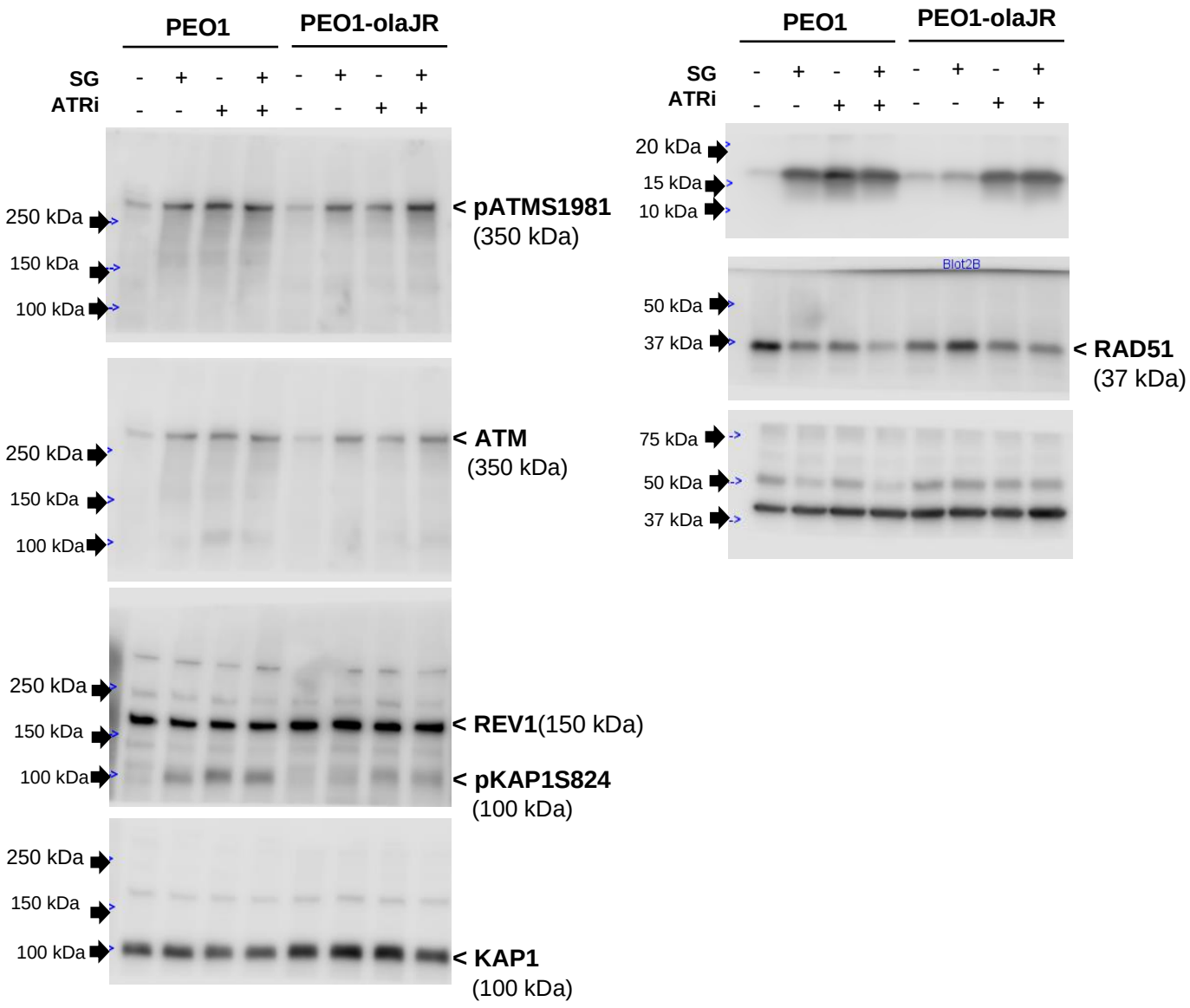
Supplementary Table S2. The list of drugs showing synergistic cytotoxic effects with an ATR inhibitor ceralasertib in all cell lines (10x10 screen), Related to Figure 1A.

		ExcessHSA				
Drug name	**Target**	**PEO1**	**PEO1-olaR**	**PEO1-olaJR**	**PEO4**	**average**
Gemcitabine	Ribonucleotide reductase inhibitor	-1675.0	-887.0	-1299.3	-1525.3	-1346.7
BMN-673	PARP inhibitor	-2534.1	-562.3	-657.7	-1265.4	-1254.9
Exatecan	**DNA topoisomerase I inhibitor**	**-2223.1**	**-235.8**	**-238.0**	**-1035.7**	**-933.2**
SN-38	**DNA topoisomerase I inhibitor**	**-2021.1**	**-259.1**	**-440.1**	**-751.1**	**-867.9**
Birinapant	IAP antagonist	-746.7	-598.8	-650.4	-1116.1	-778.0
Sirolimus	mTOR inhibitor	-458.2	-862.6	-728.0	-667.5	-679.1
Triciribine	AKT inhibitor	-421.6	-748.3	-665.0	-866.3	-675.3
Ridaforolimus	mTOR inhibitor	-527.4	-521.7	-404.7	-610.2	-516.0
Prexasertib	CHK1 inhibitor	-316.2	-507.6	-564.8	-504.7	-473.3
AZD8055	mTORC1/2 inhibitor	-517.8	-502.6	-327.3	-428.1	-443.9
MK-1775	Wee1 kinase inhibitor	-930.3	-238.0	-209.2	-393.2	-442.6
Paclitaxel	Tubulin depolymerization inhibitor	-458.2	-532.8	-344.9	-409.6	-436.4
Bimiralisib	PI3K inhibitor	-103.1	-669.9	-348.6	-473.0	-398.7
AZD5363	AKT inhibitor	-338.4	-363.9	-166.6	-156.8	-256.5
BEZ-235	mTOR inhibitor	-479.1	-180.4	-60.2	-248.6	-242.1
SAR-260301	PI3Kbeta inhibitor	-96.9	-395.8	-146.7	-279.0	-229.6
Acalisib	PI3Kdelta inhibitor	-183.2	-151.0	-68.0	-402.0	-201.1
Vistusertib	mTORC1/2 inhibitor	-205.4	-212.2	-185.9	-125.1	-182.2
CUDC-907	PI3K inhibitor	-285.7	-119.0	-71.4	-246.1	-180.6
Afuresertib	AKT inhibitor	-384.3	-93.3	-52.9	-65.9	-149.1
GSK-2126458	PI3K$\alpha/\beta/\delta/\gamma$ inhibitor	-131.7	-192.5	-19.2	-174.5	-129.5

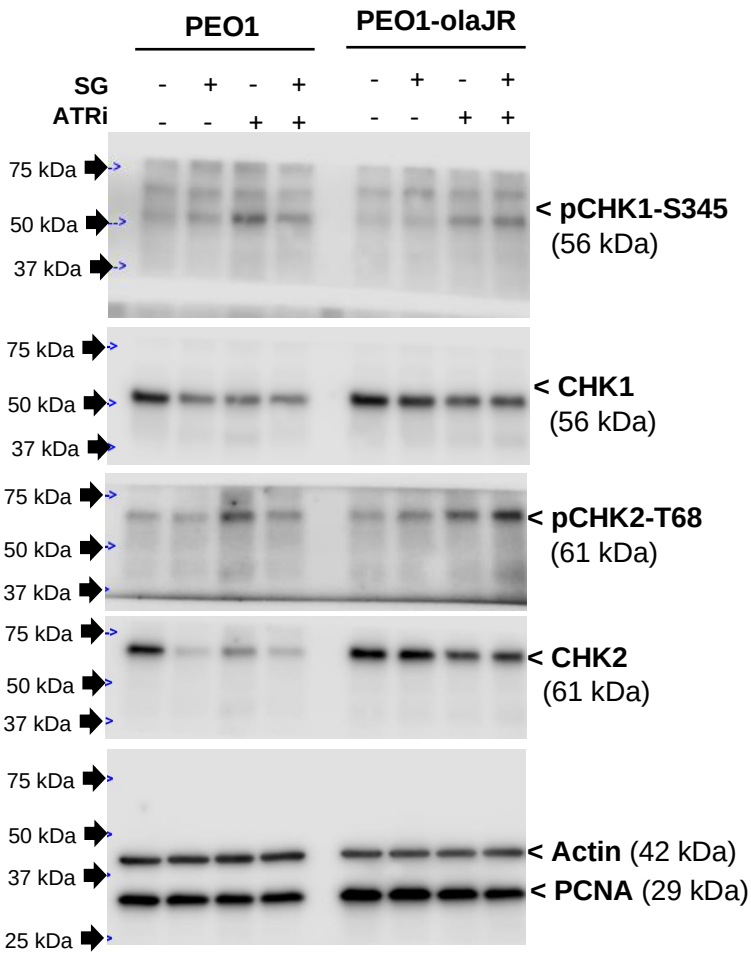
Data S1. Uncropped western blots, related to Figures 1D, 4A, 4B, 5A, 6F, 6G, S1B and S6B, related to figure 1D.



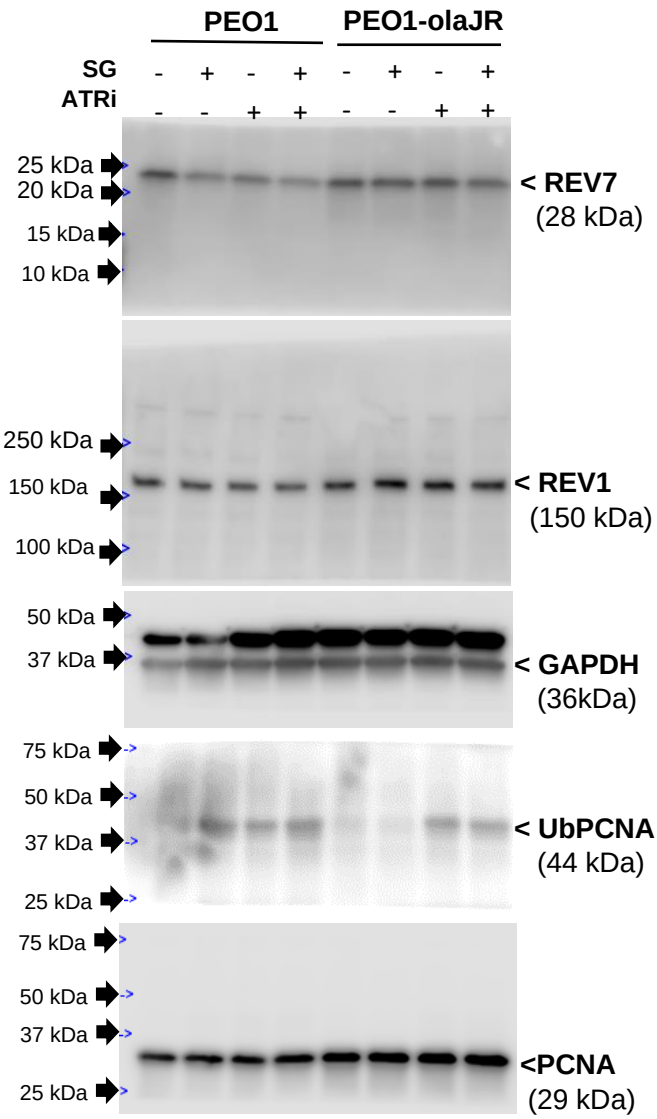
Data S1. Uncropped western blots, related to Figures 1D, 4A, 4B, 5A, 6F, 6G, S1B and S6B, related to figure 4A.



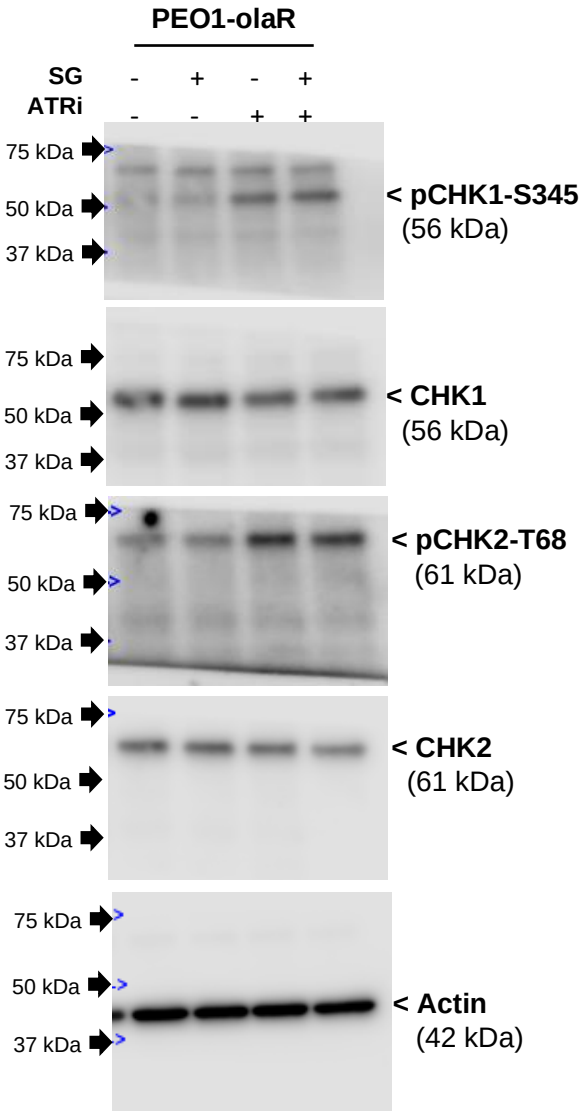
Data S1. Uncropped western blots, related to Figures 1D, 4A, 4B, 5A, 6F, 6G, S1B and S6B, related to Figure 4B.



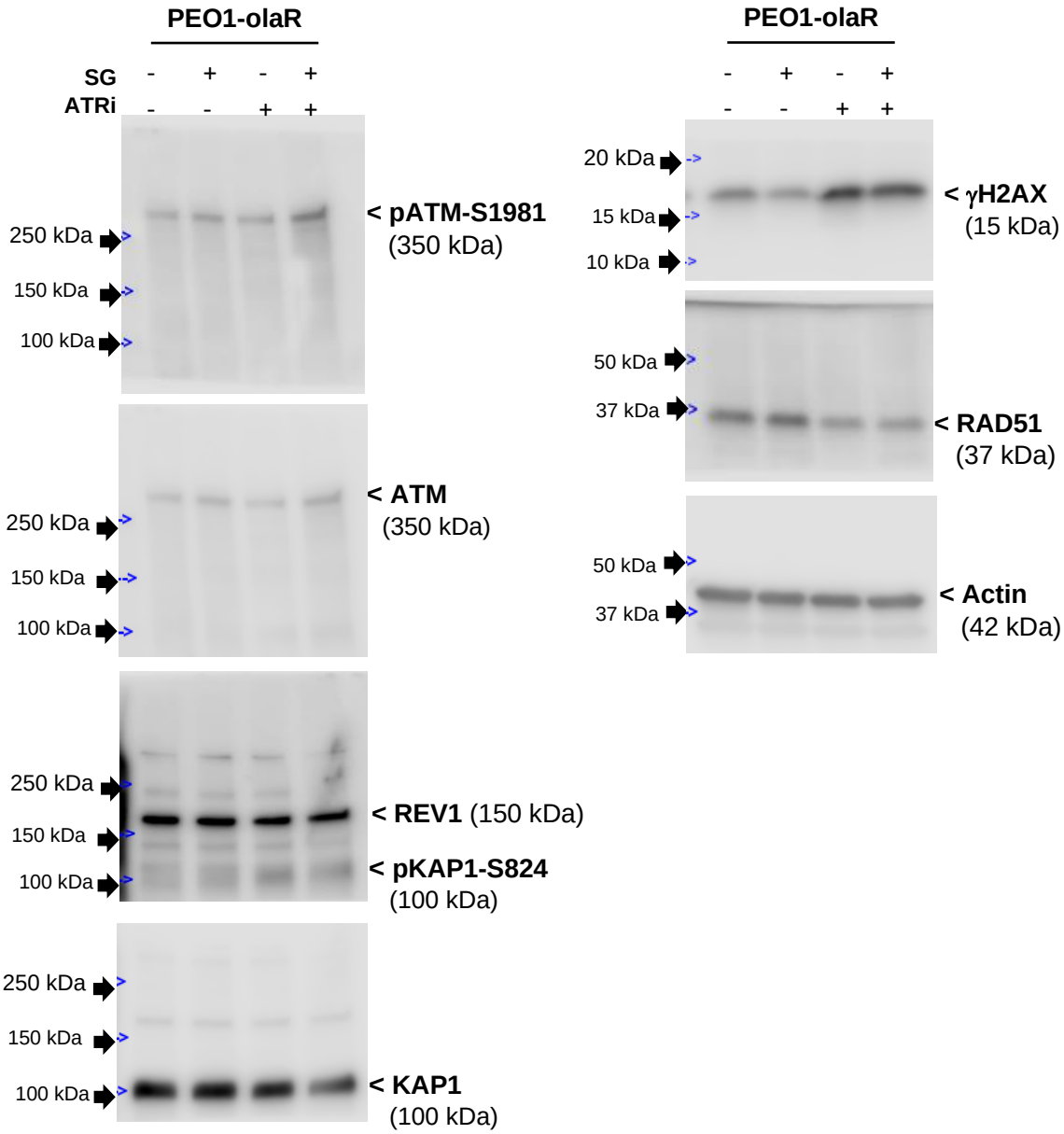
Data S1. Uncropped western blots, related to Figures 1D, 4A, 4B, 5A, 6F, 6G, S1B and S6B, related to Figure 5A.



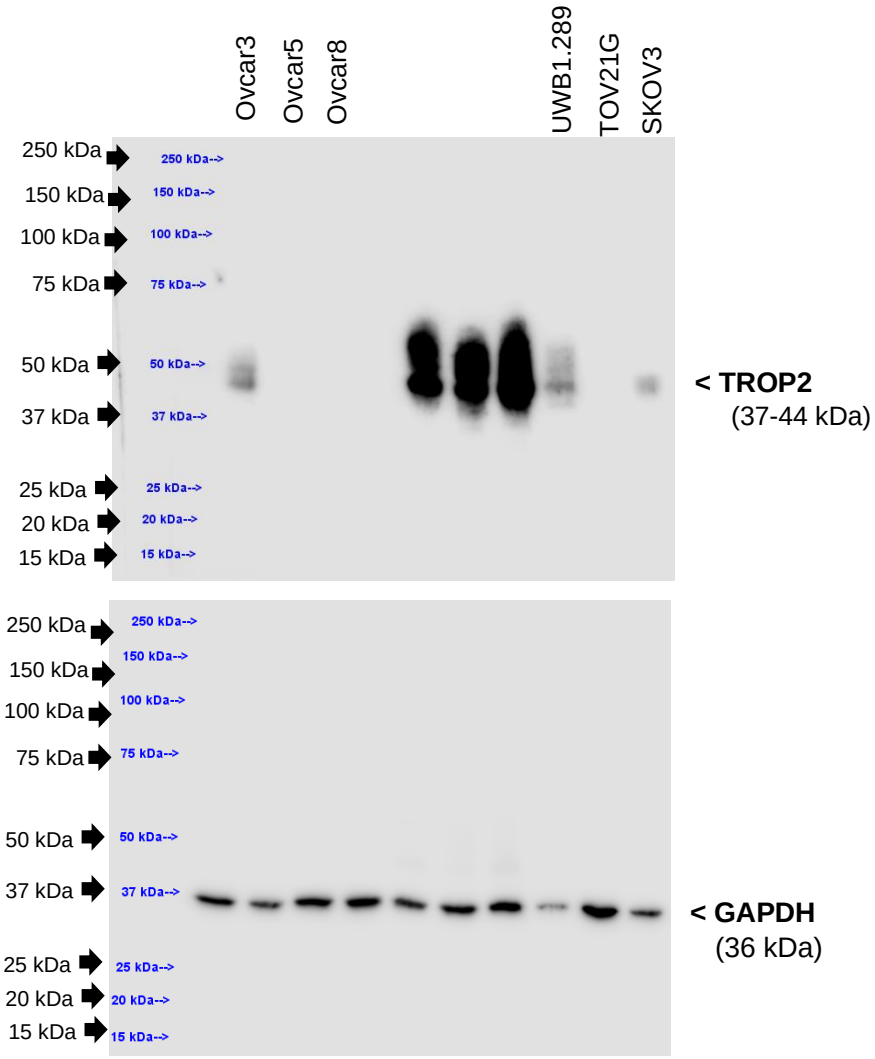
Data S1. Uncropped western blots, related to Figures 1D, 4A, 4B, 5A, 6F, 6G, S1B and S6B, related to Figure 6F.



Data S1. Uncropped western blots, related to Figures 1D, 4A, 4B, 5A, 6F, 6G, S1B and S6B, related to Figure 6G.



Data S1. Uncropped western blots, related to Figures 1D, 4A, 4B, 5A, 6F, 6G, S1B and S6B, related to supplementary Figure 1B.



Un-labelled lanes are unpublished data and is not part of this study.

Data S1. Uncropped western blots, related to Figures 1D, 4A, 4B, 5A, 6F, 6G, S1B and S6B, related to supplementary Figure 6B.