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Supplementary Materials for

A synthetic lethal dependency on casein kinase 2 in response to replication-perturbing therapeutics in RB1-deficient cancer cells

Daria Bulanova *et al.*

Corresponding author: Krister Wennerberg, krister.wennerberg@bric.ku.dk

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The PDF file includes:

Other Supplementary Material for this manuscript includes the following:

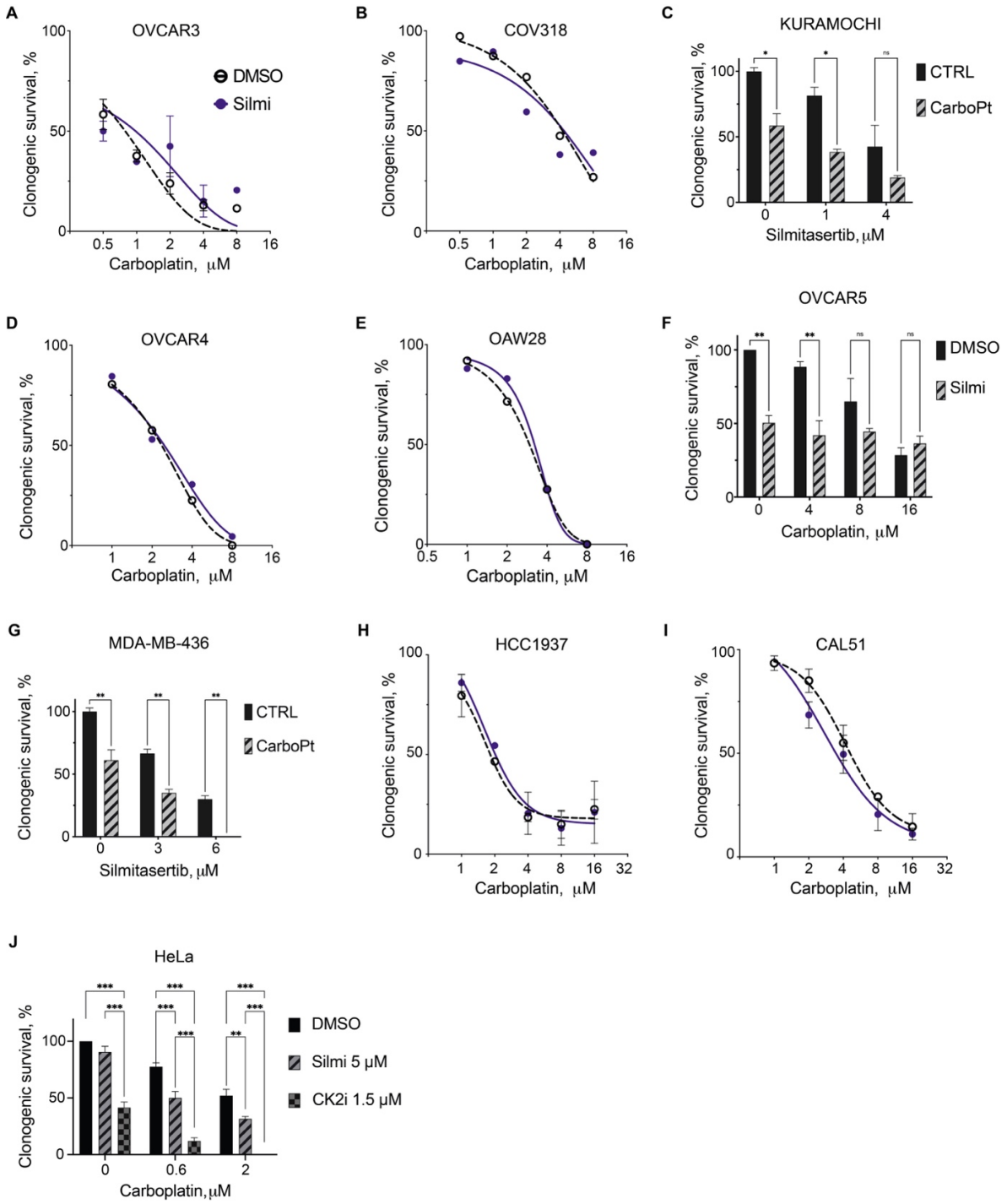

Fig. S1. Clonogenic survival of cancer cell lines treated with carboplatin in the presence or absence of CK2 inhibitor silmitasertib. A-J, Quantification of the clonogenic tests in indicated cell lines treated for 5 days with carboplatin in presence or absence of silmitasertib, followed by 3 days in medium with silmitasertib alone or vehicle alone, respectively, and by another 6 days in drug-free medium for all wells. Bars, mean+/-SD, n=2. *$0.01 < P \leq 0.05$, **$0.001 < P \leq 0.01$, ***$P \leq 0.001$, two-way ANOVA with Tukey post-test for multiple comparisons.

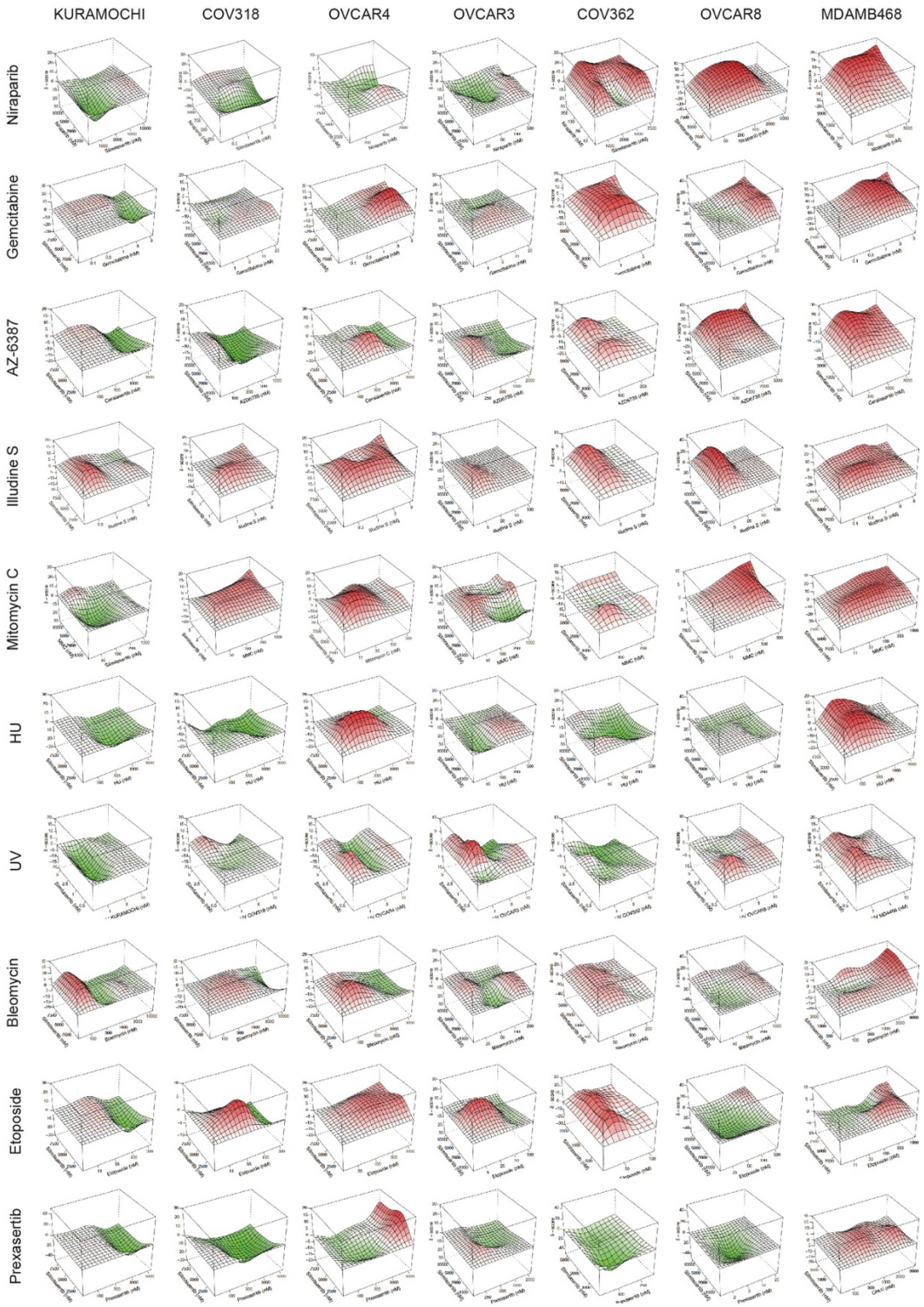

Fig. S2. Synergy score heat maps for drug combinations with silmitasertib.
Surface heat maps related to Figure 2C representing the synergy scores (Z axis) between silmitasertib (2.5, 5, 7.5 μM, Y axis) and the indicated drugs in the range of concentrations (X axis), n=3, N=2.

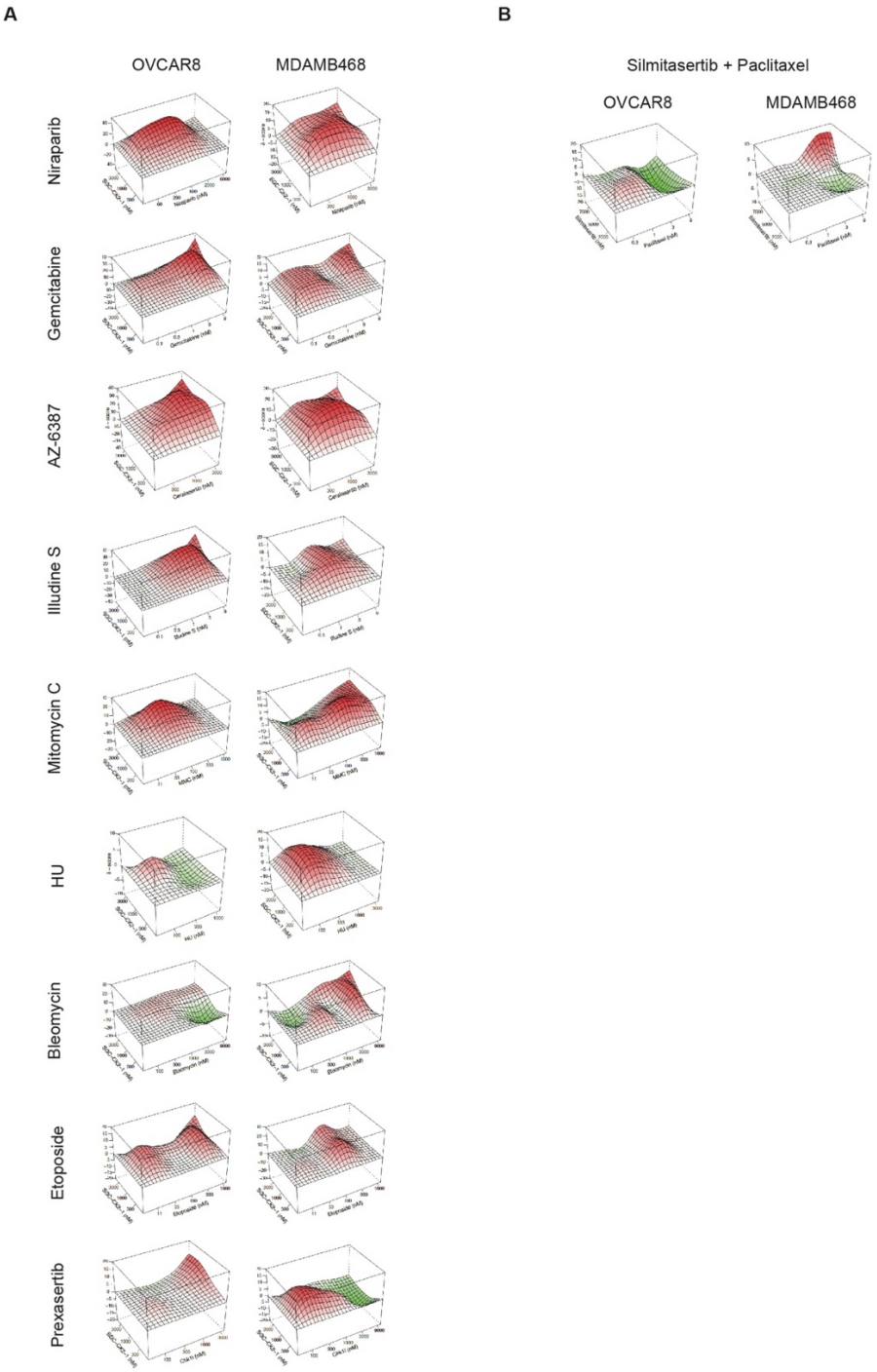

Fig. S3. Synergy score heat maps for drug combinations with SGC-CK2-1 or silmitasertib. A, Surface heat maps for the synergy scores (Z axis) between SGC-CK2-1 (0.3, 1, 3 μM, Y axis) and the indicated drugs in the range of concentrations (X axis). B, Surface heat maps for the synergy scores (Z axis) between silmitasertib (2.5, 5, 7.5 μM, Y axis) and paclitaxel in the range of concentrations (X axis), n=3, N=2.

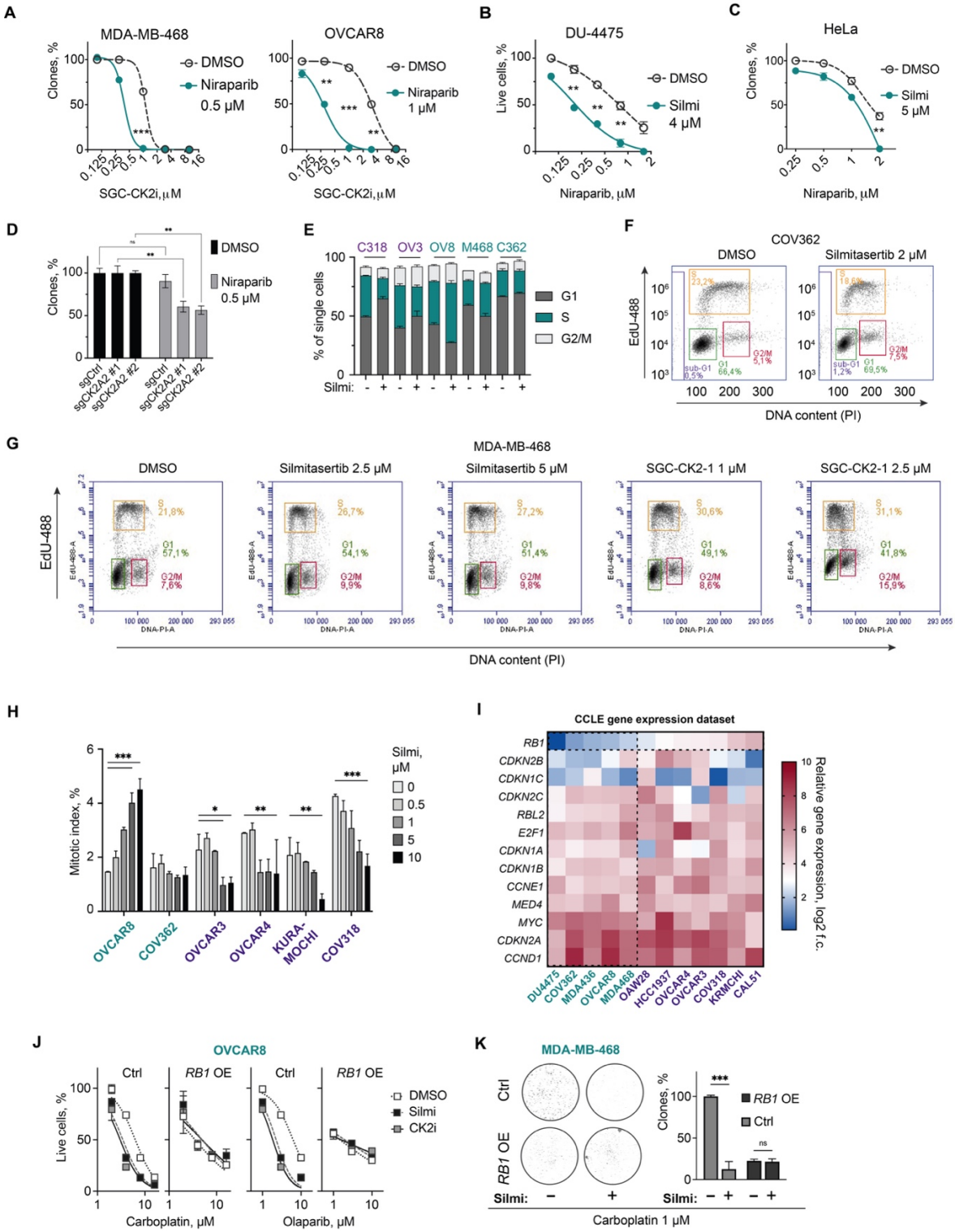

Fig. S4. The effect of CK2 inhibition on PARP inhibition response and cell division rate associates with the differential expression of RB1. A, Quantification of the clonogenic survival of OVCAR8 and MDA-MB-468 treated with niraparib and CK2 inhibitor SGC-CK2-1 for 5 days. Data points represent mean+/-SD, n=2, N=1 for each cell line. B, Quantification of the survival of DU-4475 TNBC cell line treated with the drugs for 5 days and allowed to grow for 9 more days in

drug-free medium. Viability is calculated by the fraction of live cells of DMSO-treated control (counted using Trypan Blue staining and Countess II automated cell counter (Thermofisher). Data points represent mean+/-SD for one experiment, n=2, N=2. C, Quantification of the clonogenic survival of HeLa treated with niraparib and silmitasertib for 5 days. Data points represent mean+/-SD, n=2, N=1. D, Quantification of the clonogenic survival of niraparib-treated OVCAR8 cells with CSNK2A2 CRISPR knock-out. Bars represent mean+/-SD for one experiment, n=2, N=1. E, Flow cytometry analysis of the EdU incorporation assay in MDA-MB-468 cells treated with silmitasertib and SGC-CK2-1 for 72 h. F, Quantification of the cell cycle phase distribution determined in EdU incorporation assay, related to Figure 3. G, Flow cytometry analysis of the EdU incorporation assay in COV362 cells treated with silmitasertib for 72 h. H, Quantification of the micronuclei formation in COV318 and OVCAR8 cells treated with carboplatin for 24 h and then released to the carboplatin-free medium in presence or absence of 5 μM silmitasertib for 6 h. Confocal imaging of 500-1000 cells per condition. H, Mitotic index quantification. Adherently growing cells were incubated with silmitasertib for 72 h, fixed, and stained with Hoechst. Linear classifier algorithm-based detection of mitotic figures was used to assess the % of mitotic nuclei out of the total number of nuclei on the confocal microscopy images. The bars represent mean+/-SD for one experiment, n=2, N=2. I, Heat-map of the expression of RB pathway genes, CCLE gene expression dataset. J, Carboplatin and olaparib dose response of OVCAR8 expressing RB1 in the presence of 5 μM silmitasertib or 1 μM SGC-CK2-1. The numbers of live and dead cells were determined after 7 days of incubation with the compounds using image cytometry and Hoechst / CellTox green staining; data points represent mean+/-SD (n=3). K, Clonogenic survival of RB1-expressing MDA-MB-468. The cells were treated with 1 μM carboplatin in presense or absence of 5 μM silmitasertib for 96 h and replated to 6-well plates for clonogenic growth for 7 days. The number of colonies in carboplatin-treated control transfectants is taken as 100%. The bars represent mean+/-SD, n=2, N=1. *P* values are calculated using a two-way ANOVA with Tukey post-test for multiple comparisons. *$0.01 < P \leq 0.05$, **$0.001 < P \leq 0.01$.

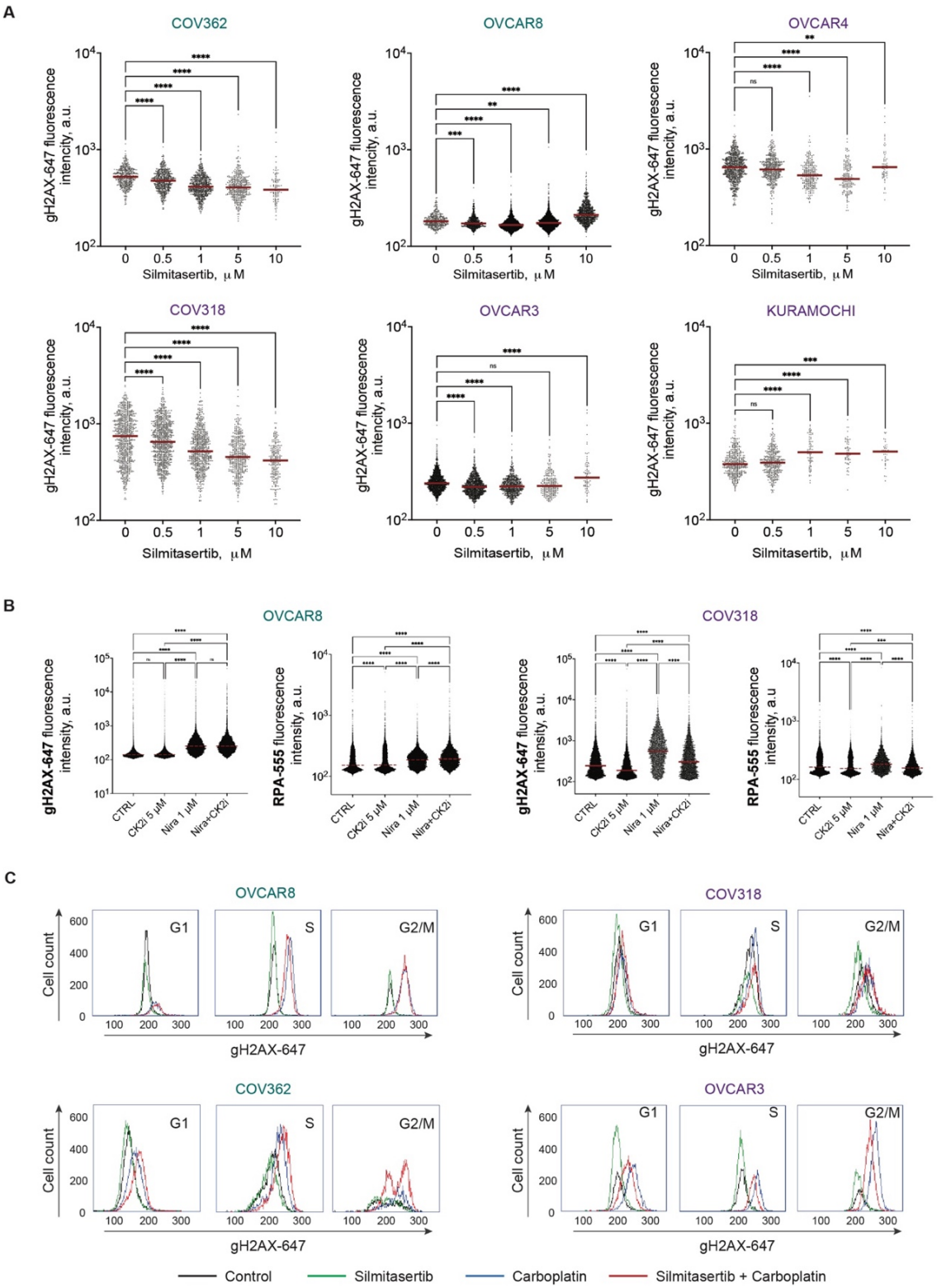

Fig. S5. Effect of CK2 inhibition on DNA damage markers gH2AX and RPA2 in cells treated with carboplatin or niraparib. A, Quantification of the confocal imaging of the nuclear fluorescence intensity of the γH2AX immunostaining for the indicated cell lines treated for 72 h with silmitasertib. Cell line names in cerulean correspond to the models where silmitasertib acted

synergistically with carboplatin or niraparib; cell line names in violet correspond to the models where no interaction between CK2 inhibitors and carboplatin or PARP inhibitors was observed in drug survival tests. Data points represent individual cells, and the red line represents the mean. B, Quantification of the nuclear fluorescence intensity of the γH2AX-Alexa647 or RPA-Alexa555 immunostaining for the indicated cell lines treated with 5 µM silmitasertib +/- 1 µM niraparib for 72 h. C, Flow cytometry-based analysis of the γH2AX immunostaining intensity in the different cell cycle phases after 72 h treatment with carboplatin +/- silmitasertib. The cell cycle phases were discriminated by EdU/propidium iodide staining as described for EdU incorporation assay in the Materials and methods. *P* values are calculated using a two-way ANOVA with Tukey post-test for multiple comparisons. *$0.01 < P \leq 0.05$, **$0.001 < P \leq 0.01$, ***$0.0001 < P \leq 0.001$ and ****$P \leq 0.0001$.

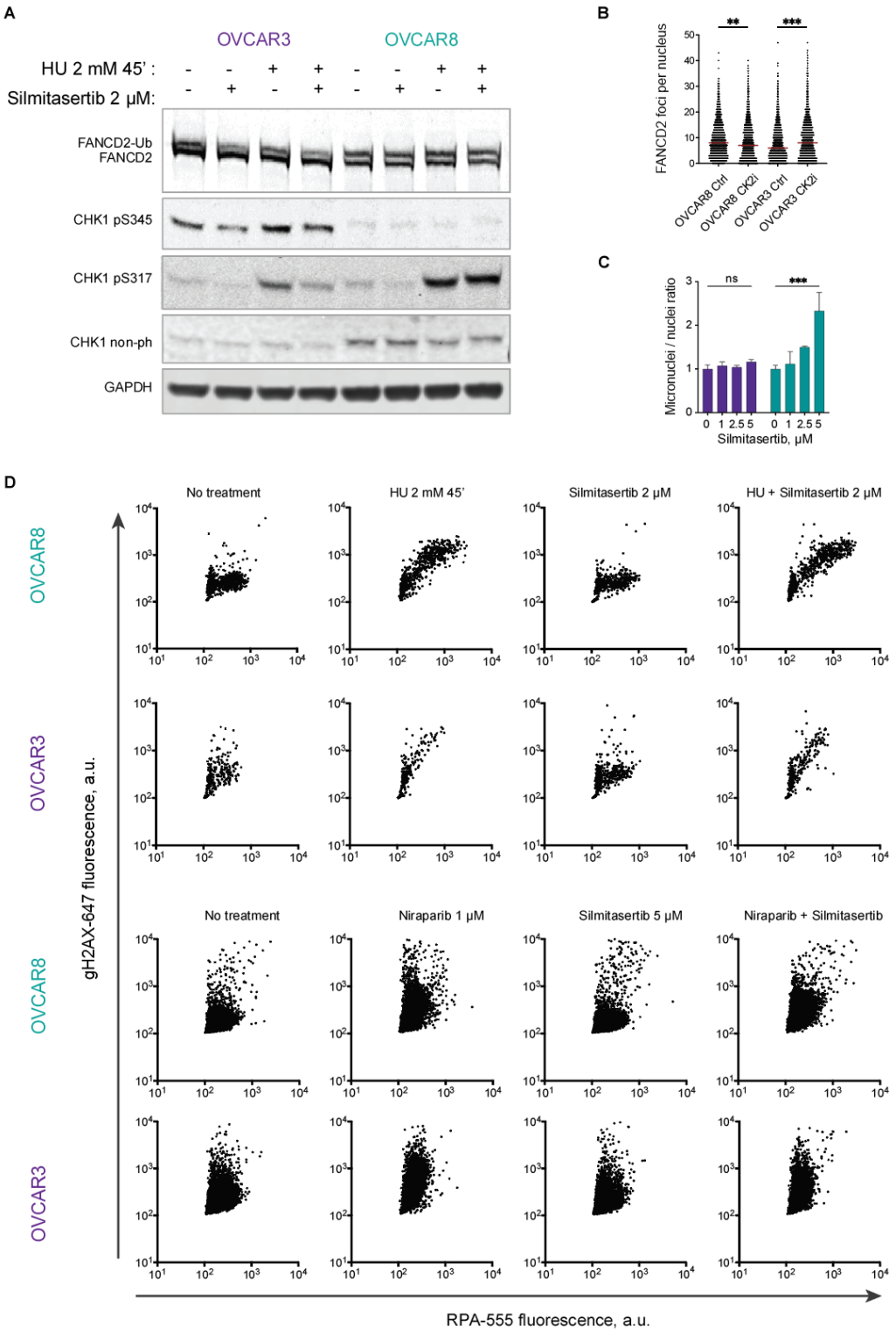

Fig. S6. Replicative DNA damage markers in silmitasertib-treated cells. A, Immunoblotting analysis of the replicative DNA damage signaling triggered by replication blocking nucleotide depletion by control replication stress inducer hydroxyurea in presence or absence of CK2 inhibitor. Cells were pre-treated for 24 with silmitasertib to achieve a full loss of CK2 activity and then were treated with an acute dose of HU for 45 min as described[36]. B, Quantification of the

imaging for immunostained inter-strand crosslink repair foci of FANCD2 in the pulse-labeled EdU-positive cells after 72 h of silmitasertib treatment. C, Quantification of the micronuclei formation in COV318 and OVCAR8 cells treated with carboplatin for 24 h and then released to the carboplatin-free medium in the presence or absence of 5 μM silmitasertib for 6 h. Confocal imaging of 500-1000 cells per condition. The bars represent mean+/-SD for one experiment, n=2, N=2. D, Quantification of the nuclear fluorescence intensity of the γH2AX-Alexa647 or RPA-Alexa555 immunostaining for the indicated cell lines treated with silmitasertib and HU as described in A, or with the combination of silmitasertib and niraparib for 72 h. Dots represent individual nuclei, 500-2000 nuclei analyzed by confocal imaging. Cell line names in cerulean correspond to the models where silmitasertib acted synergistically with carboplatin or niraparib; cell line names in violet correspond to the models where no interaction between CK2 inhibitors and carboplatin or PARP inhibitors was observed in drug survival tests. *P* values are calculated using a two-way ANOVA with Tukey post-test for multiple comparisons. *$0.01 < P \leq 0.05$, **$0.001 < P \leq 0.01$, ***$0.0001 < P \leq 0.001$.

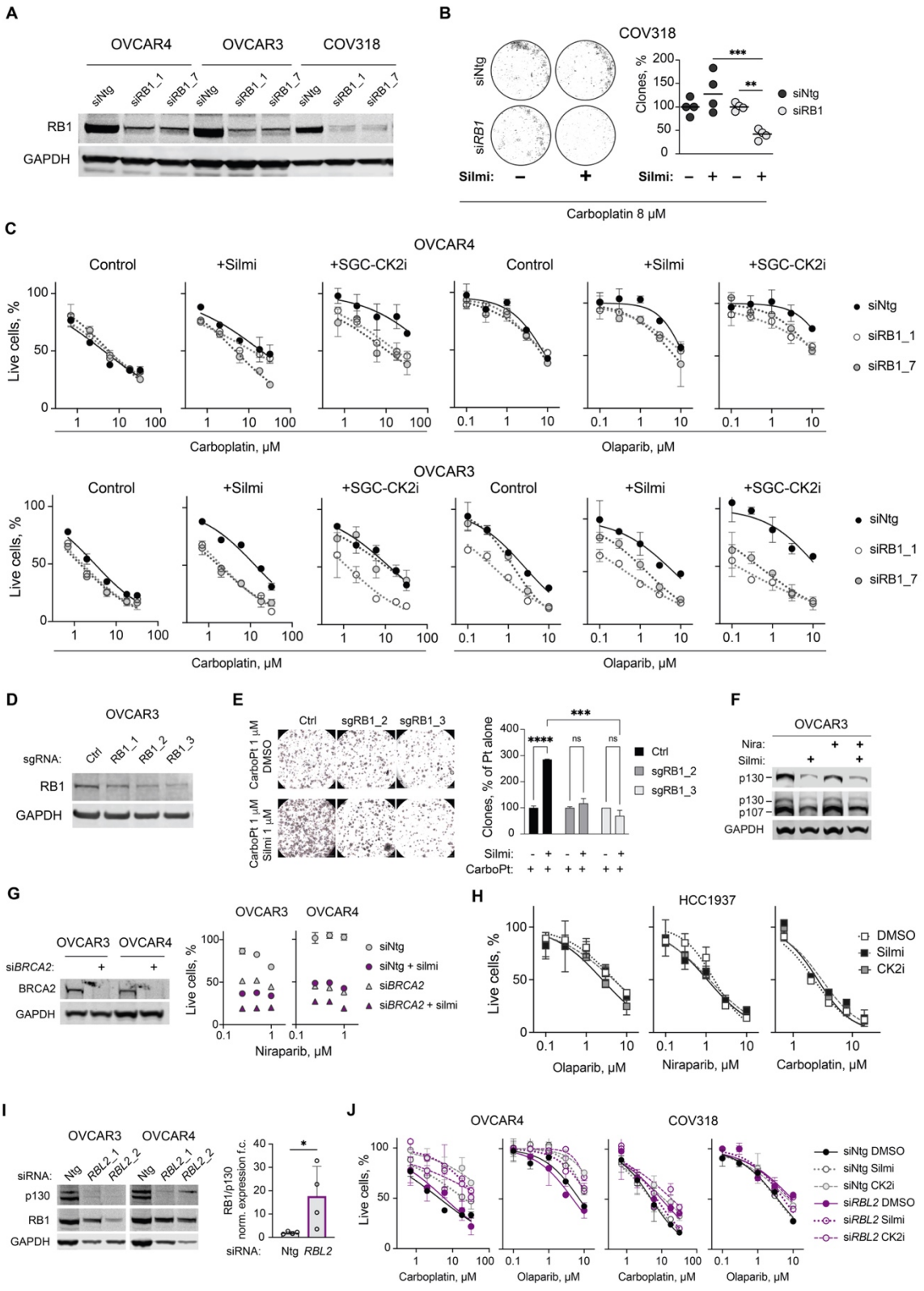

Fig. S7. Modulation of RB1 expression affects the response to the combination of CK2 inhibition with carboplatin or PARP inhibitors. A, Immunoblotting analysis of RB1 protein

expression upon transfection of the cell lines with 30 nM of non-targeting or RB1-targeting siRNAs. B, Clonogenic survival of RB1-depleted COV318 cells. The cells were treated with 8 μM carboplatin in the presence or absence of 5 μM silmitasertib for 96 h and replated to 6-well plates for clonogenic growth for 7 days. The number of colonies in carboplatin-treated control transfectants is taken as 100%. The bars represent mean+/-SD, n=2, N=1. C, Carboplatin and olaparib dose response in RB1-depleted OVCAR3 and OVCAR4 in the presence of 5 μM silmitasertib or 1 μM SGC-CK2-1. The numbers of live and dead cells were determined after 7 days of incubation with the compounds using image cytometry and Hoechst / CellTox green staining; n=3, N=1, data points represent mean+/-SD. D, Immunoblotting for RB1 expression in OVCAR3-Cas9 cells infected with control guide RNA or sgRB1. E, Representative images of the clonogenic survival of sgRB1-transduced and control OVCAR3 cells treated with niraparib in the presence or absence of silmitasertib and quantification of the clonogenic assay. F, Immunoblotting for p107 and p130 protein abundance in OVCAR3 cell line treated with 1 μM niraparib alone or combined with 1 μM silmitasertib for 96 h. G, Immunoblotting for BRCA2 in *BRCA2*-depleted and control OVCAR3 and OVCAR4 and their niraparib dose response assessed as in C. H, Carboplatin or PARPi dose responses in HCC1937 in the presence of 5 μM silmitasertib or 1 μM SGC-CK2-1 assessed as in C. I, Immunoblotting analysis of p130 protein expression upon transfection of the cell lines with 30 nM of non-targeting or *RBL2*-targeting siRNAs and quantification of the ratio of the GAPDH-normalized abundance of RB1/p130. J, Carboplatin or PARPi dose responses in *RBL2*-depleted cell lines in the presence of 5 μM silmitasertib or 1 μM SGC-CK2-1 assessed as in C. *$P < 0.05$; **$P < 0.01$, ***$P < 0.001$, two-way ANOVA with Tukey post-test. For I, *$P < 0.05$, Mann-Whitney test. For the Western blot images, image-wide non-linear adjustments of the brightness of the western blot membrane scan were applied for presentation clarity. Quantification has been done from the raw scan images.

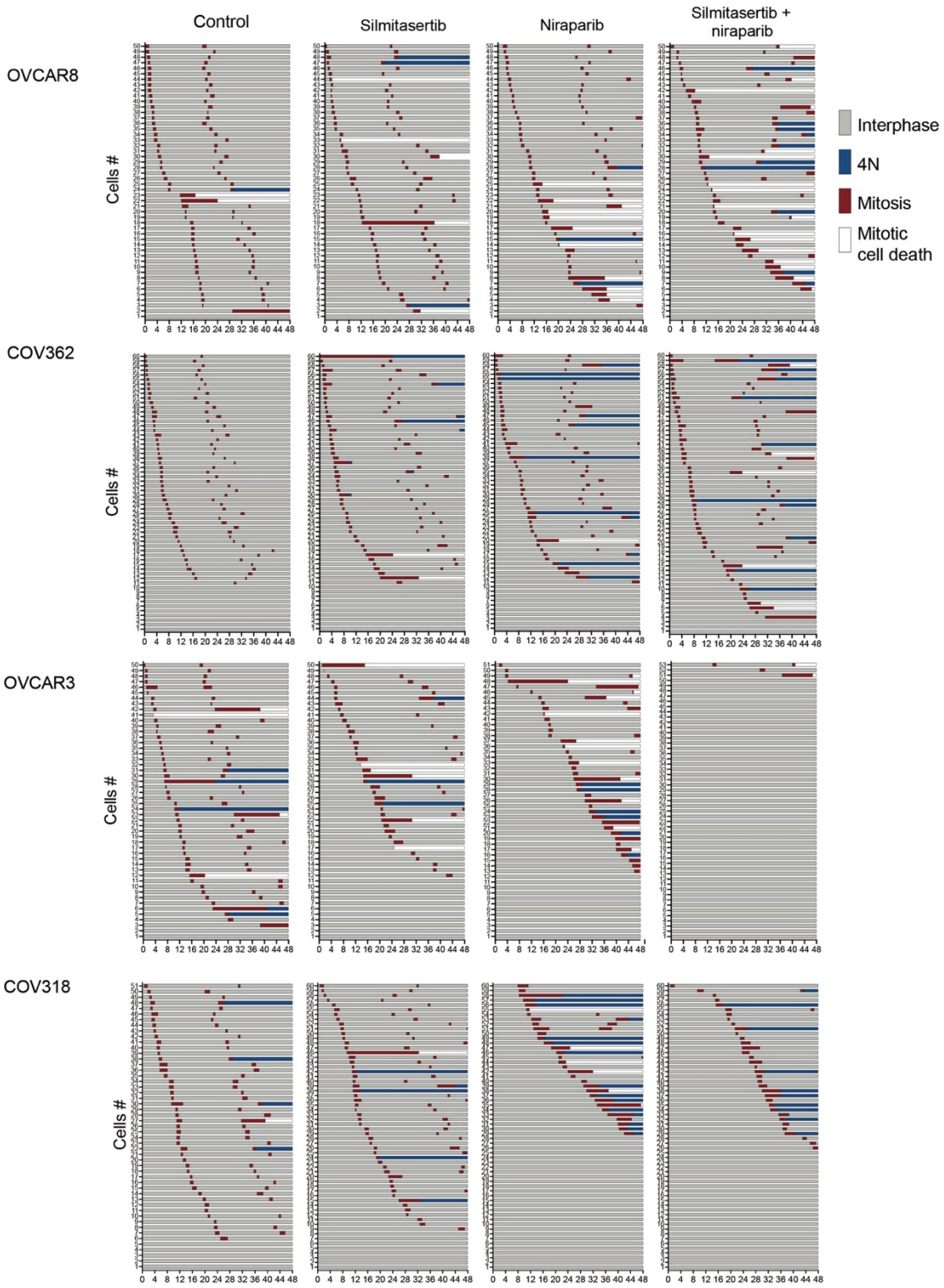

Fig. S8. Cell division timing in the cells treated with niraparib and silmitasertib.
Duration of interphase and mitosis in individual cells quantified from the time-lapse videos SM1-16 taken at IncuCyte Zoom microscope using 10x objective for 96 h. Cells were exposed to niraparib for 48 h, followed by additional 48 h in presence or absence of silmitasertib. Rounding of the cells in prometaphase was considered the start of mitosis, while cell spreading after

cytokinesis was considered as the end of the cell division. Irreversible detachment and signs of apoptosis (membrane blebbing) in mitosis were assigned as mitotic cell death. Exit from mitosis without division to 2 daughter cells was assigned as aneuploidy (4N). 50-60 cell divisions were followed up for each treatment. OVCAR8 and COV362 are RB1-deficient, and COV318 and OVCAR3 are RB1-proficient cell lines.

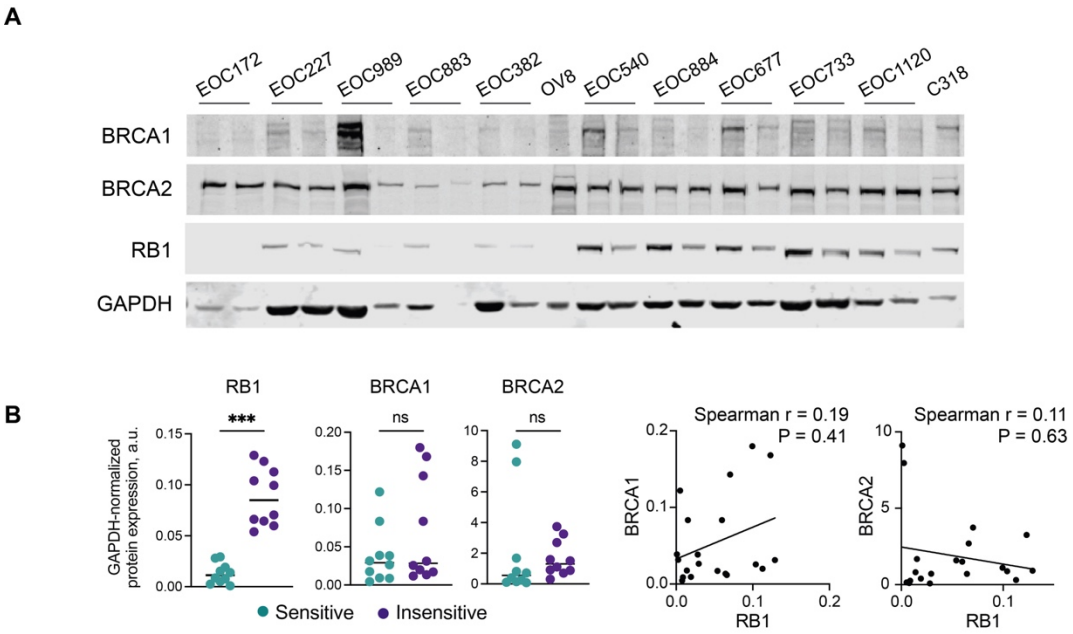

Fig. S9. Expression of RB1 and BRCA proteins in the HGSC organoid cultures (related to main Fig.5). A, Immunoblotting for BRCA1, BRCA2, and RB1 in the protein samples collected from asynchronously growing organoid cultures collected at 2 different passages (between passages 7-21). B, Quantitative analysis of the RB1, BRCA1, and BRCA2 band intensities normalized to the corresponding GAPDH band intensity. *P* values are calculated Mann-Whitney non-parametric test. ***$0.0001 < P \leq 0.001$. C, Spearman correlation analysis of the GAPDH-normalized RB1 protein abundance with the abundance of BRCA1 or BRCA2 in the analysis in A. Image-wide non-linear adjustments of the brightness of the western blot membrane scan were applied for presentation clarity. Quantification has been done from the raw scan images.

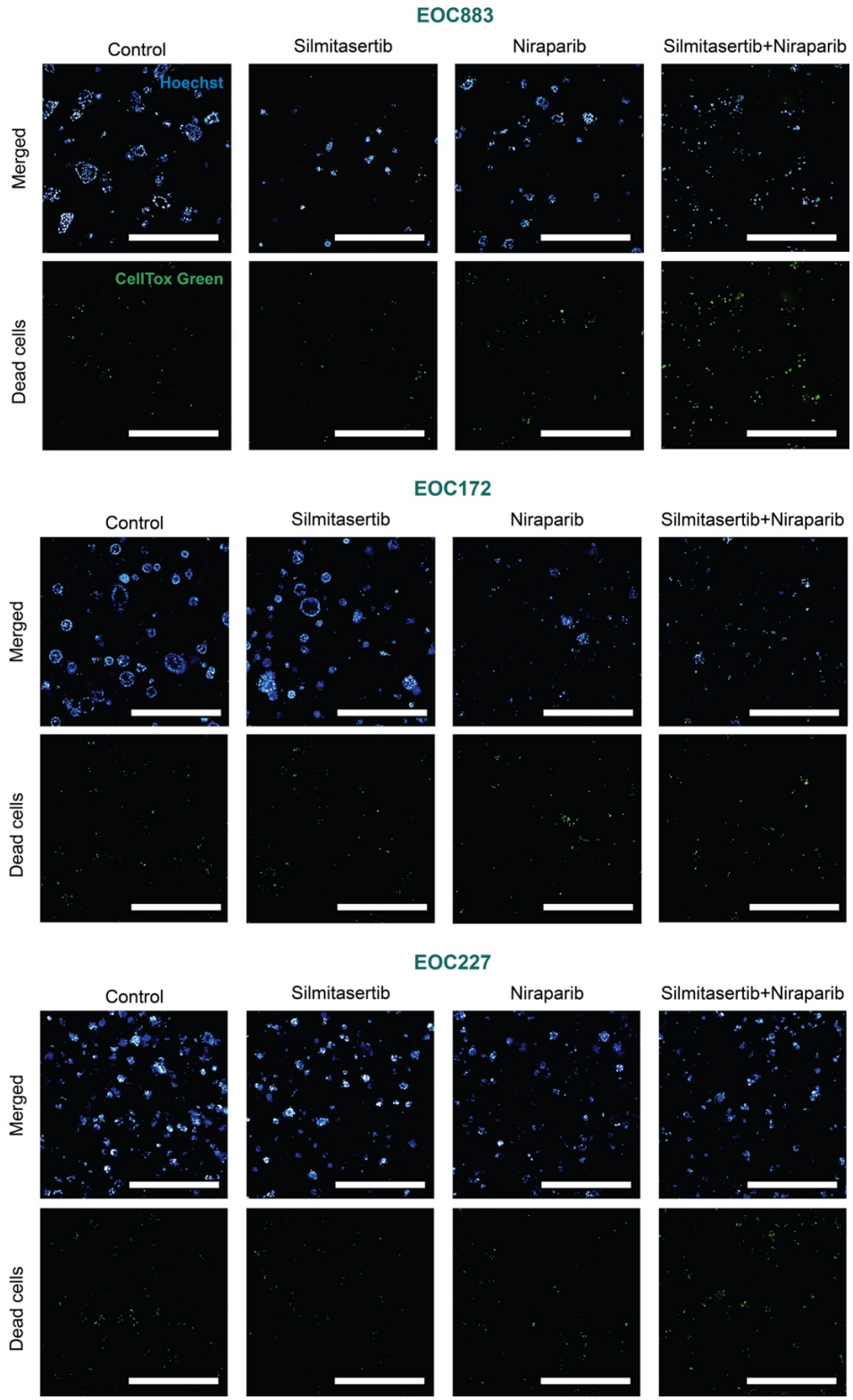

Fig. S10. Cytotoxicity of concurrent inhibition of PARP and CK2 in HGSC organoids. A-C, Confocal live imaging of RB1-deficient EOC172, EOC227, and EOC883p organoids treated with niraparib and silmitasertib for 7 days and stained with Hoechst (blue) and CellTox Green staining for 6 h at 37°C. Scale bar, 1 mm.

Supplementary Movies

Movie S1. Live imaging of OVCAR8 cells. Treatment: vehicle (DMSO).
Live microscopy using IncuCyte Zoom microscope (Sartorius), 10x objective, 48 h.

Movie S2. Live imaging of OVCAR8 cells. Treatment: silmitasertib, 5 µM.
Time-lapse microscopy using IncuCyte Zoom microscope (Sartorius), 10x objective, for 48 h.

Movie S3. Live imaging of OVCAR8 cells. Treatment: niraparib, 1 µM.
Time-lapse microscopy using IncuCyte Zoom microscope (Sartorius), 10x objective, for 48 h.

Movie S4. Live imaging of OVCAR8 cells. Treatment silmitasertib, 5 µM + niraparib, 1 µM.
Time-lapse microscopy using IncuCyte Zoom microscope (Sartorius), 10x objective, for 48 h.

Movie S5. Live imaging of OVCAR3 cells. Treatment: vehicle (DMSO).
Time-lapse microscopy using IncuCyte Zoom microscope (Sartorius), 10x objective, for 48 h.

Movie S6. Live imaging of OVCAR3 cells. Treatment: silmitasertib, 1 µM.
Time-lapse microscopy using IncuCyte Zoom microscope (Sartorius), 10x objective, for 48 h.

Movie S7. Live imaging of OVCAR3 cells. Treatment: niraparib, 1 µM.
Time-lapse microscopy using IncuCyte Zoom microscope (Sartorius), 10x objective, for 48 h.

Movie S8. Live imaging of OVCAR3 cells. Treatment: silmitasertib, 1 µM + niraparib, 1 µM.
Time-lapse microscopy using IncuCyte Zoom microscope (Sartorius), 10x objective, for 48 h.

Movie S9. Live imaging of COV362 cells. Treatment: vehicle (DMSO).
Time-lapse microscopy using IncuCyte Zoom microscope (Sartorius), 10x objective, for 48 h.

Movie S10. Live imaging of COV362 cells. Treatment: silmitasertib, 5 µM.
Time-lapse microscopy using IncuCyte Zoom microscope (Sartorius), 10x objective, for 48 h.

Movie S11. Live imaging of COV362 cells. Treatment: niraparib, 0.5 µM.
Time-lapse microscopy using IncuCyte Zoom microscope (Sartorius), 10x objective, for 48 h.

Movie S12. Live imaging of COV362 cells. Treatment silmitasertib, 5 µM + niraparib, 0.5 µM.
Time-lapse microscopy using IncuCyte Zoom microscope (Sartorius), 10x objective, for 48 h.

Movie S13. Live imaging of COV318 cells. Treatment: vehicle (DMSO).
Time-lapse microscopy using IncuCyte Zoom microscope (Sartorius), 10x objective, for 48 h.

Movie S14. Live imaging of COV318 cells. Treatment: silmitasertib, 5 µM.
Time-lapse microscopy using IncuCyte Zoom microscope (Sartorius), 10x objective, for 48 h.

Movie S15. Live imaging of COV318 cells. Treatment: niraparib, 1 µM.
Time-lapse microscopy using IncuCyte Zoom microscope (Sartorius), 10x objective, for 48 h.

Movie S16. Live imaging of COV318 cells. Treatment: silmitasertib, 5 µM + niraparib, 1 µM.
Time-lapse microscopy using IncuCyte Zoom microscope (Sartorius), 10x objective, for 48 h.

Data S1. (separate file)
Supplementary Tables ST1-ST7

Supplementary table ST1. Gene essentiality after OVCAR8 treatment with carboplatin. RRA is a P value-based aggregated gene essentiality score.

Supplementary table ST2. Gene set enrichment for the hit genes essential for long-term survival after carboplatin treatment in OVCAR8 cells.

Supplementary table ST3. Compounds used in the combinatorial drug screening related to Figure 2.

Supplementary table ST4. Cell lines used in the study.

Supplementary Table ST5. Primers and cycling conditions for sgRNA library amplification.

Supplementary Table ST6. List of sgRNAs, their sequences, and oligonucleotides for Golden Gate cloning.

Supplementary table ST7. Antibodies used in the study.