**Supplemental Methods:**

**Dye-exclusion assay**

Cells were seeded at 3.5 x 105 cells per plate and treated with roscovitine for 24 hours, doxorubicin for 48 hours or sequential combination treatment at the IC50 concentrations (Supplemental Table 1B). Following the completion of treatments, cells were harvested and resuspended in PBS, and propidium iodide was added at a final concentration of 2 μg/mL prior to flow cytometric analysis using an LSR II flow cytometer equipped with the CellQuest Pro software program (BD Bioscience).

**Drugs**

Roscovitine, generously provided by Dr. Laurent Meijer (National Center for Scientific Research, Paris France), was diluted to 10 mM in dimethyl sulfoxide (DMSO). Staurosporine (Sigma-Aldrich) was diluted to 2.14 mM in DMSO. Doxorubicin-HCl (Bedford Laboratories, Bedford, OH) was reconstituted in a 0.9% sterile saline solution at 2 mg/mL and shielded from light. Etoposide (Sigma-Aldrich) was diluted in DMSO to 10 mM and used at 5 μM. Staurosporine (Sigma-Aldrich) was diluted to 2.14 mM in DMSO. Taxol (Sigma Aldrich) was diluted to 1mM in DMSO.

**Flow cytometry**

Flow cytometry was performed as previously described (1, 2) with the following modifications. Briefly, cells were seeded 10 cm dishes at 3.5 x 105 cells per plate to examine cell-cycle phases in response to drug treatment. After 24 hours, cells were treated with DMSO, roscovitine at 20 μM or its half-maximalinhibitory concentration (IC50) for 24 hours or doxorubicin at its IC50 for 48 hours as indicated (Supplemental Table 1B). To measure the effect of concomitant treatment on the cell cycle, cells were treated with roscovitine and doxorubicin at IC50 concentrations simultaneously for 72 hours (Supplemental Table 1B). Following treatment, cells were fixed and stained to measure their DNA content as described previously (3). Briefly, cells were resuspended in 1.5 mL of cold phosphate-buffered saline (PBS) and 3.5 mL of cold ethanol overnight. Cells were then washed with PBS, resuspended in PBS containing 10 μg/mL propidium iodide, RNase A, Tween 20, and bovine serum albumin and incubated at 4°C overnight. Prior to measuring their DNA content, cell samples were filtered and incubated for 1 hour at 37°C. Samples were analyzed at The University of Texas MD Anderson Cancer Center Flow Cytometry and Cellular Imaging Facility using Beckman Coulter Gallios Flow Cytometer (Indianapolis, IN) equipped with the Kaluza software program (Beckman Coulter)

**High-throughput survival assay**

 To assess the effect of combination drug treatment on HMEC, TNBC and HCT116 cell lines, cells were subjected to a high-throughput survival assay (HTSA) as described previously (2, 4) with the following modifications. Briefly, for all combinations cells were seeded in 96-well plates (Supplemental Table 1B). Roscovitine and doxorubicin combination drug treatment were administered simultaneously and sequentially, in both directions. For simultaneous drug administration, cells were treated for 72 hours with roscovitine and doxorubicin simultaneously (Supplemental Figure 1B, Supplemental Table 1B). For sequential drug administration, cells were treated with drug A at the 10% inhibitory concentration (IC10), IC25, and IC50 for 24 hours. Following drug A treatment, the medium was removed, and cells were treated with drug B at the IC10 to the IC50 for 48 hours, equaling 72 hours of drug treatment (Supplemental Figure 1C, Supplemental Table 1B). Following the completion of 72 hours of drug treatment, the medium was replaced with fresh-drug free medium every 48 hours for 9 days. Each 96-well plate also contained cells that remained untreated or treated with single drugs (A or B) and used as controls. Nine days following the removal of drug B, the plates were subjected to (3-(4, 5-[di](http://en.wikipedia.org/wiki/Di-)methyl[thiazol](http://en.wikipedia.org/wiki/Thiazole)-2-yl)-2, 5-di[phenyl](http://en.wikipedia.org/wiki/Phenyl)tetrazolium bromide (MTT) assay (2.5 mg/mL; Sigma-Aldrich), incubated for 4 hours at 37°C, and solubilized (0.04 N HCl and 1% sodium dodecyl sulfate [SDS], in isopropyl alcohol). Absorbance was read at 590 nM using Epoch microplate spectrophotometer with the Gen5 software program (BioTek, Winooski, VT). Isobolograms and combinational indices were generated using the CalcuSyn software program (Biosoft, Cambridge, UK).

**Western blot analysis of cell and tumor tissue lysates**

Western blot analysis was performed as previously described (1, 2) with the following modifications. Briefly, for cell lines, ollowing the indicated treatments for each western blot analysis, cells were washed with cold PBS, trypsinized (0.25%), and centrifuged. Next, cell pellets were washed with cold PBS and centrifuged again. Cell pellets were lysed with RIPA buffer (150 mM NaCl, 10 mM Tris, pH 7.3, 0.1% SDS, 1% Triton X-100, 1% deoxycholate, and 5 mM ethylenediaminetetraacetic acid) containing protease inhibitors, with occasional vortexing. Cell pellets were centrifuged at 16,000 rpm for 60 minutes at 4 deg. C, with supernatant containing protein lysates. Western blot analysis was performed to examine protein expression HMEC and TNBC cells as described previously (5). Blots were blocked with BLOTTO milk for 1 hour at room temperature and incubated with primary antibody overnight at 4°C. Antibodies against PARP-1, CDK1, total Rb and phosphor-Rb (Ser807/811) (Cell Signaling Technology, Danvers, MA), CDK2 (Santa Cruz, Dallas, TX) p27 (BD Biosciences, San Jose, CA), p53, p21, and actin (EMD Millipore, Billerica, MA), phosphor-H2AX (EMD Millipore), total H2AX (Cell Signaling Technology) and Rad51 (Santa Cruz) were used to probe for protein expression. Blots were incubated with goat anti-rabbit or goat anti-mouse immunoglobulin-horseradish peroxidase-conjugates (Pierce, Rockford, IL) at a dilution of 1:5000 in BLOTTO for 1 hour. Blots were then washed and developed using a Renaissance chemiluminescence system (Perkin Elmer Life Sciences, Inc., Boston, MA) as directed by the manufacturer.

For tumor tissues, following surgical resection, tumors were snap frozen in liquid nitrogen. Protein lysates were extracted via sonication in a protease inhibitor solutions as previously described (6). Homogenates were centrifuged 45,000 g for 45 minutes. Supernatants were subjected to Western blot analysis. Blots were probed with anti-PARP1 antibody (Cell Signaling Technology).

**Transfection of siRNA**

siRNA transfections were performed as previously described (1, 2), with the following modifications. Briefly, generate transient knockdown cells, HMEC, ER positive and TNBC cells were seeded for 96-well plates for survival analysis or 6-well plates for flow cytometry or Western blot analysis according to manufacture protocols (Thermo Scientific transfection Dhramafect siRNA transfection protocol). Thermo Scientific siGENOME smart pool siRNA, including siControl pool #1, siCDK1 and siCDK2, were resuspended according to manufacture protocol at 100 µm. Cells were transfected siRNA targeting CDK1, CDK1 or both using the transfection reagent Dharmafect formulation 1 according to manufacture protocol (Thermo Scientific). Non-coding siControl pool #1 was used as a negative control. Cells were harvested 48 hours post transfection or as indicated for Western blot analysis and flow cytometry. Cells were subjected to MTT for survival analysis 9 days after removal of doxorubicin.

**Generation of stable knockdown cells**

Stable knockdowns were performed as previously described (1, 2, 7, 8) with the following modifications. To generate 76NF2V cells with stable knockdown of Rb, HEK-293T cells were transfected lentiviral packaging vectors pMDG.2 and pCMV deltaR8.2 (produced by Didier Trono laboratory and made available through the Addgene repository) and lentiviral vector containing shRNA against Rb or scramble sequence (University of Texas MD Anderson Cancer Center ShRNA and ORFeome Core Facility) using LipoD293 transfection reagent (SignaGen, Rockville, MD) according to manufacturer’s protocols. Virus-containing medium was filtered through .45 μM filters and directly added to target cells in the presence of 8 μg/ml of polybrene (Millipore). 76NF2V cells were selected 2 μg/ml puromycin (InvivoGen, San Diego, CA) and maintained at half selection concentration. Knockdown or Rb was confirmed via Western blot analysis.

**Dose escalation study** (Supplemental Figure 6).

Non-tumor bearing mice were treated with vehicle, roscovitine (50 mg/kg), doxorubicin (5 mg/kg or 10 mg/kg) or sequential combination of roscovitine followed by doxorubicin (either 5 or 10 mg/kg) for four cycles. Mice that were treated with 10 mg/kg of doxorubicin were only received 3 rounds of treatment due to toxicities. Weight was measured once a week. Upon completion of treatment, mice were euthanized and blood was collected through a cardiac puncture through the left ventricle. A range of 0.1 to 1 ml of blood was collected. A blood count, including white blood cells, platelets and red blood cells, was performed using the Siemens Adiva 120 Hematology System (Erlangen, Germany).

**Histology of tumor tissues**

Upon sacrifice, tumors tissue was resected and fixed at least overnight in formalin. Tumor tissue preparation, H&E staining and assessment of proliferation using 5-bromo-2’doeyuridine (BRDU) was performed as previously described (9).

**Supplemental Figure Legends**

**Supplemental Figure 1. Changes in G2/M cell cycle phase following treatment as a function of receptor status A**, panel of HMEC, estrogen receptor (ER) positive, and triple-negative breast cancer (TNBC) cell lines were treated with roscovitine (R) for 24 hours at 20 μM. Percent of cells in G2/M cell cycle phase in untreated control (C) and R treated samples given **B**, A schematic depicting the method of concomitant combination drug treatment in an HTSA. **C**, A schematic depicting the method of sequential combination drug treatment in an HTSA.

**Supplemental Figure 2.** **Downreguation of CDK1, CDK2 or both in ER positive cells**. **A**, Two TNBC cell lines (MDA MB 468 and MDA MB 157) are treated with 72 hours of roscovitine treatment concomitantly with 48 hours of doxorubicin in an HTSA. **B**, Two TNBC cell lines (MDA MB 157 and MDA MB 468) were treated with sequential combination drug treatment of roscovitine (R) (24hours) followed by taxol (T) (48 hours) in an HTSA. Drug concentrations found in Supplemental Table 1B. Isobolograms and average combination index values generated using CalcuSyn. **C**, ER positive cell line ZR75-1 was transiently transfected with siRNA against CDK1, CDK2 or both CDK1 and CDK2. Cells were transfected with non-targeting siRNA control for a negative control. Western blot analysis used to confirm knockdown. Percent viability with and without doxorubicin treatment determined using MTT analysis. **D**, Percent viability of MCF10A, ZR75-1 and MDA MB 468 cells upon knockdown and treatment with doxorubicin at IC50 concentrations.  **E**, Images captured of untreated MCF10A cells at 3, 5, 7 and 9 days after seeding. Corresponding lysates collected at each point and Western blot analysis used to detect PARP expression.

**Supplemental Figure 3. Cell cycle analysis following single or combination treatments. A**, Cell cycle analysis was performed on HMEC and TNBC cells treated with roscovitine (R) for 24 hours, doxorubicin (D) for 48 hours or sequential roscovitine-doxorubicin (R🡪D) at IC50 concentrations. Cells were also harvested 24 hours post treatment (+24). Untreated cells served as a control (C). **B**, MCF10A and MDA MB 231 cells were concomitantly treated with roscovitine and doxorubicin (R+D) for 72 hours followed by fixation and cell cycle analysis. Sequential treatment (R🡪D) shown for comparison **C**, Stably expressing shScramble or shRb 76NF2V cells were treated with R, D or R🡪D followed by cell cycle analysis. C served as a control.

**Supplemental Figure 4. Sequential combination treatment of roscovitine and doxorubicin causes more DNA double strand breaks in p53 mutant in colon cancer cells. A and B**, The DNA repair foci γ-H2AX and Rad51 were detected HCT116 p53 wildtype and p53 knockout cells via immunofluorescence. Dapi was used to detect nuclei. Representative mages were captured at 100X magnification using an Eclipse 90i microscope equipped with the NIS-Elements Br 3.10 software program. **C,** Percent of γ-H2AX positive cells was quantified in response to single and combination drug treatment. Only cells with ≥ 5 foci were considered as positive**. D,** Percent of γ-H2AX positive cells with Rad51 foci recruitment were quantified. Cells with ≥ 1 Rad51 foci were considered positive. Experiments were repeated three times, and error barsare 95% confidence intervals. The student t-test (two-tailed, equal variance) was employed to derive the p-values.

**Supplemental Figure 5. Sequential roscovitine-doxorubicin combination treatment is effective and well tolerated in vivo**.  **A,** Images of representative tumors from each treatment arm were captured at the end of treatment (day 26). Statistical analysis based on tumor volume. **B,** Spider plots graphing individual tumor volumes per treatment group. Each line represents the tumor volume measured over time. **C,** Fold change in tumor volume for mice treated with vehicle, roscovitine (50 mg/kg), doxorubicin (2mg/kg), concomitant (R+D) and sequential (R🡪D) shown. **D**, H&E staining was performed on tumors resected at the end of treatment on day 26. **E,** Percent change in mouse weight compared to weight on first day of treatment.

**Supplemental Figure 6** **Dose escalation of doxorubicin in vivo**. **A,** Percent change in weight loss was used to examine toxicity during dose escalation of doxorubicin. Non-tumor bearing mice were treated with vehicle, roscovitine (50 mg/kg) for four days, doxorubicin (5 and 10 mg/kg) for 1 day or sequential (R🡪D) combination treatment. All mice received 4 rounds of treatment except for mice that received 10mg/kg of doxorubicin received 3 rounds due to toxicities. **B-D,** Following completion of drug treatment, blood was collected from vehicle, roscovitine, doxorubicin (10 mg/kg) and sequentially treated mice. Blood count of white blood cells (WBC), platelets and red blood cell count (RBC) analyzed.

**Supplemental References:**

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