SUPPLEMENTARY FIGURE LEGENDS

Supplementary Figure S1. Prexasertib exhibits monotherapy activity in olaparib-resistant HGSOC PDX models. (A) NSG mice bearing luciferized PDXs were treated with vehicle, olaparib (100 mg/kg, daily) or prexasertib (8 mg/kg, BIDx3, rest 4 days) for 3 weeks. Tumor growth was monitored by weekly bioluminescence imaging for approximately 150 days. Tumor growth in mice bearing PDXs is shown. All tumor measurements are from n=1 mouse/group. (B) CCNE1 mRNA expression in 14 HGSOC PDX models.

Supplementary Figure S2. Body weights of HGSOC tumor-bearing mice treated with prexasertib as a monotherapy or in combination with olaparib. (A) NSG mice bearing luciferized PDXs (n=5 mice/group) were treated with vehicle, olaparib (100 mg/kg, daily) or prexasertib (8 mg/kg, BIDx3, rest 4 days) as monotherapy for 3 weeks. (B) NSG mice bearing luciferized PDXs, namely DF59 (n=10 mice/group) or DF83 (n=9 mice/ group) were treated with vehicle, olaparib (100 mg/kg, daily), prexasertib (8 mg/kg, BIDx3, rest 4 days) as monotherapy or olaparib (100 mg/kg, daily) + prexasertib (8 mg/kg, BIDx3, rest 4 days) combination for 3 weeks. Body weights of the mice in panels "A" and "B" were measured twice weekly.

Supplementary Figure S3. RAD51 and γ-H2AX staining in response to olaparib in PDX models. (A) RAD51 and (B) γ-H2AX immunohistochemical (IHC) staining in tumor cells from DF59 PDX model. NSG mice bearing PDX model DF59 were treated with vehicle, olaparib (100 mg/kg), prexasertib (8 mg/kg, BID) or olaparib (100 mg/kg) + prexasertib (8 mg/kg, BID). Tumors were harvested at 52 hrs after dosing and FFPE tissue sections of PDX models were stained using an anti-RAD51 antibody or anti-γH2AX antibody. Representative images (60X)

are shown. Lower panel in "A" shows the quantitation of RAD51 foci in tumor cells of vehicle-treated or olaparib-treated mice.

Supplementary Figure S4. Cyclin E expression in ovarian cancer cell lines. Western blots of lysates from prexasertib-sensitive and prexasertib-resistant ovarian cancer cells.

Supplementary Figure S5. Prexasertib is synergistic with olaparib in multiple ovarian cancer cell lines. Cells were grown for 24 hrs before exposure to graded concentrations of prexasertib and olaparib. Viability was assessed at 6 days after treatment using CellTiter- Glo. Synergy or antagonism between the drugs was determined using Combenefit software. Bliss synergy or antagonism levels on the experimental combination dose response surface are shown. Bliss scores greater than zero (green/blue shading) indicate synergy between prexasertib and olaparib. The experiments were done at least two times for each cell line, and data from a representative experiment are shown. Cell lines characteristics are as reported in Domcke et al. Nat Commun 2013;4:2126.

Supplementary Figure S6. (A–D) BRCA1-deficient RPE cells with acquired resistance to olaparib are sensitive to prexasertib. (A) Western blots of the lysates from p53-deficient (RPE *P53-/-*), as well as BRCA1-deficient RPE cells (B40 *P53-/-;BRCA1-/-*) and olaparib-resistant *B40 P53-/-;BRCA1-/-* cells (NA). (B, C) Survival curves of RPE *P53-/-* cells, B40 *P53-/-; BRCA1-/-* cells and NA cells after exposure to olaparib (n=6) or prexasertib (n=3). NA cells show resistance to olaparib but sensitivity to prexasertib. **** P <0.0001; * P = 0.0214, using the one-way Anova. (D) Increased RAD51 foci in olaparib-resistant NA cells compared to parental B40 *P53-/-;BRCA1-/-* cells. The cells demonstrate formation of RAD51 foci 5 hours

after IR (5Gy), indicating restoration of homologous recombination (HR). ** $P = 0.006$ for RPE *P53-/-* vs. B40 *P53-/-;BRCA1-/-* cells; ** $P = 0.003$ for B40 *P53-/-;BRCA1-/-* vs. NA cells using Kruskal-Walls test. **(E) Pre-treatment with prexasertib or olaparib does not alter the synergistic cytotoxicity between prexasertib and PARP inhibition in TOV112D cells.** Cells were grown for 24 hrs in triplicate before exposure to graded concentrations of prexasertib and olaparib. In left panels, cells were exposed to both the drugs concurrently. In middle panels, the cells were first exposed to olaparib for 24 hrs, followed by concurrent treatment with both drugs. In right panels, the cells were first exposed prexasertib for 24 hrs, followed by concurrent treatment with both drugs. Viability was assessed at 6 days using CellTiter- Glo. Synergy/antagonism between the drugs was determined using Combenefit software. (*Top panels*) Survival plots. (*Bottom panels*) Bliss synergy/antagonism levels in a matrix format.

Supplementary Figure S7. Prexasertib has activity in BRCA1-deficient ovarian cancer cells that have restored fork stability and acquired PARP inhibitor resistance. (A) UWB1.289 SYR12 and UWB1.289 SYR14 ovarian cancer cells with acquired PARP inhibitor resistance are sensitive to prexasertib. IC_{50} plots of cells exposed to prexasertib for 72 hrs are shown. The experiment was performed with quadruplicate replicates. (B) Synergy between prexasertib and olaparib in UWB1.289 SYR12 and UWB1.289 SYR14 PARP inhibitor-resistant ovarian cancer cells. Representative colony formation assays of BRCA1-deficient UWB1.289 cells and the SYR12 and SY14 derivatives treated with olaparib and/or prexasertib are shown. For colony formation assays, cells were exposed to olaparib and/or prexasertib in 6-well plates (n=3) for 12 days and stained with crystal violet. **** $P < 0.0001$; ** $P = 0.0017$; * $P = 0.038$ using the one-way Anova.