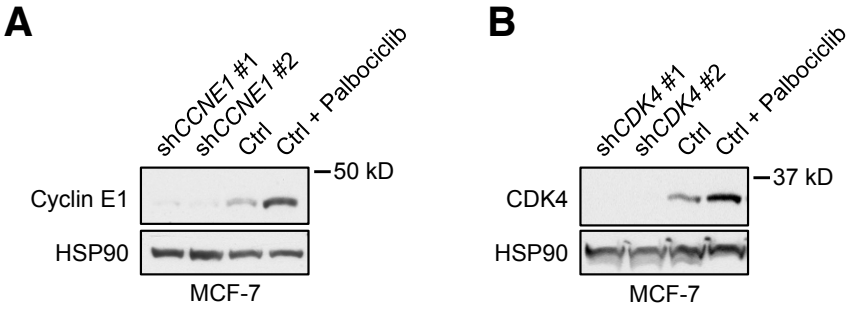
Supplemental Figures

eIF4A inhibitors suppress cell cycle feedback response and acquired resistance to CDK4/6 inhibition in cancer

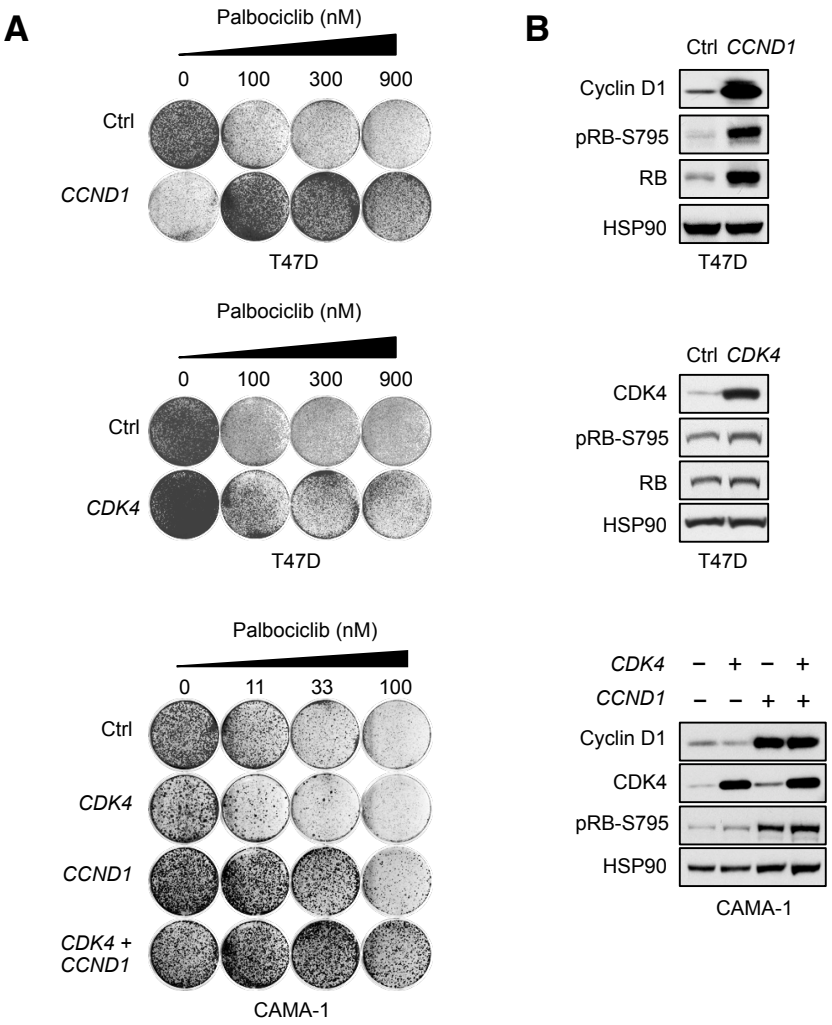
Kong et al

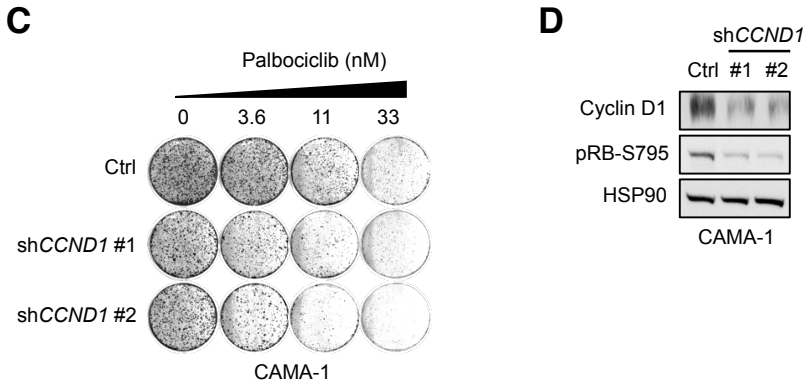


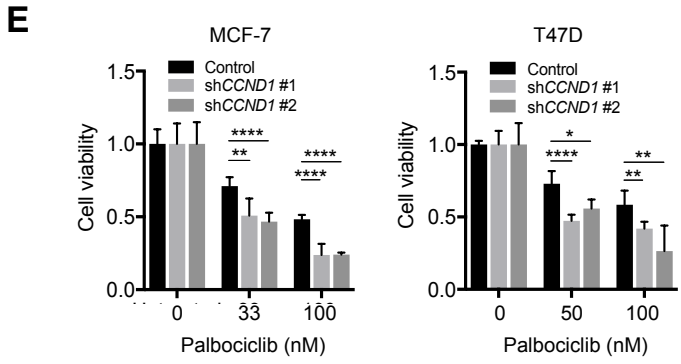
Supplemental Figure 1. Validation of cyclin E and CDK4 signals with RNAi

A) Immunoblot verifying a low-molecular-weight isoform of cyclin E1 (~ 45 kDa) upregulation after palbociclib treatment. Cyclin E1 is suppressed upon *CCNE1* knockdown using two independent shRNAs. Cells were treated with 300 nM palbociclib for 24 hours.

B) Immunoblot verifying CDK4 upregulation after palbociclib treatment. CDK4 is suppressed upon *CDK4* knockdown using two independent shRNAs. Cells were treated with 300 nM palbociclib for 24 hours.







Supplemental Figure 2

Supplemental Figure 2. Cyclin D1 dictates response to palbociclib and is limiting in the formation of CDK4/6- Cyclin D1 complexes in ER-positive breast cancer.

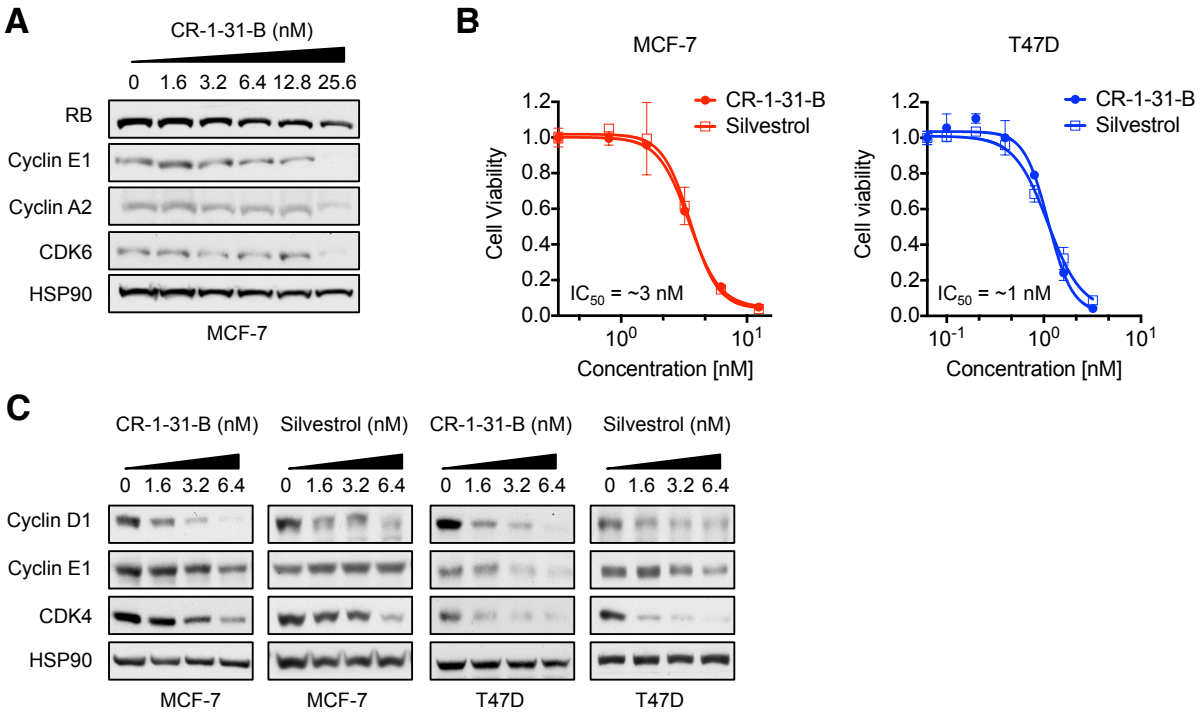
A) Long-term colony formation assays showing resistance to palbociclib after overexpression of *CCND1* (top panel) or *CDK4* (middle panel) in T47D cells or overexpression of *CDK4, CCND1,* and their combination (bottom panel) in CAMA-1 cells. Cells were seeded and grown in the presence of palbociclib for 8-14 days. Palbociclib was refreshed every 3 days.

B) Immunoblot analysis of overexpression of *CDK4*, *CCND1*, or their combination in T47D or CAMA-1 cells.

C) Long-term colony formation assays showing CAMA-1 sensitivity to palbociclib after knockdown of *CCND1* utilizing two independent shRNAs. CAMA-1 cells were grown for 14-18 in the presence of palbociclib. Palbociclib was refreshed every 3 days.

D) Immunoblot showing cyclin D1 knockdown using two independent shRNAs in CAMA-1 cells.

E) Cell viability assays showing increased sensitivity to palbociclib after *CCND1* knockdown in MCF-7 and T47D cells. *CCND1* was perturbed using two independent shRNAs and treated with two doses of palbociclib. Cell viability was assessed with CellTiter-Blue. Error bars represent mean ± standard deviation. * $p < 0.05$; ** $p < 0.01$; **** $p < 0.0001$; by Student's t-test, unpaired, two-sided.

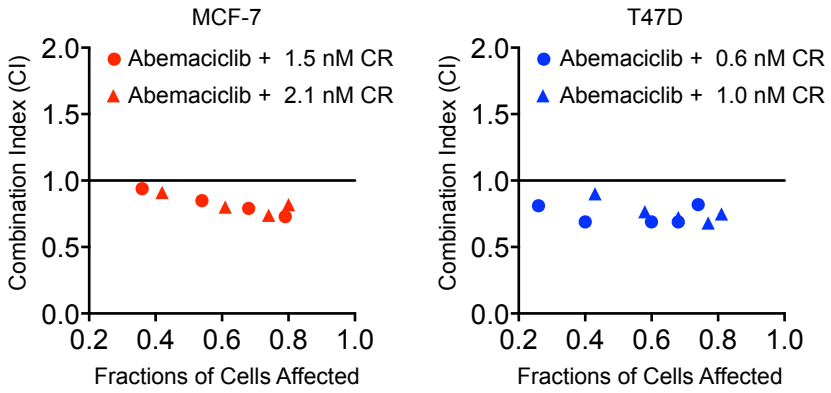


Supplemental Figure 3. Rocaglates preferentially suppresses protein expression of cyclin D1, CDK4, and cyclin E1.

A) Immunoblot analysis showing cyclin E1 and other effectors in MCF-7 cells can be inhibited at higher concentrations of CR-1-31-B. Cells were treated for 24 hours at the indicated drug concentrations.

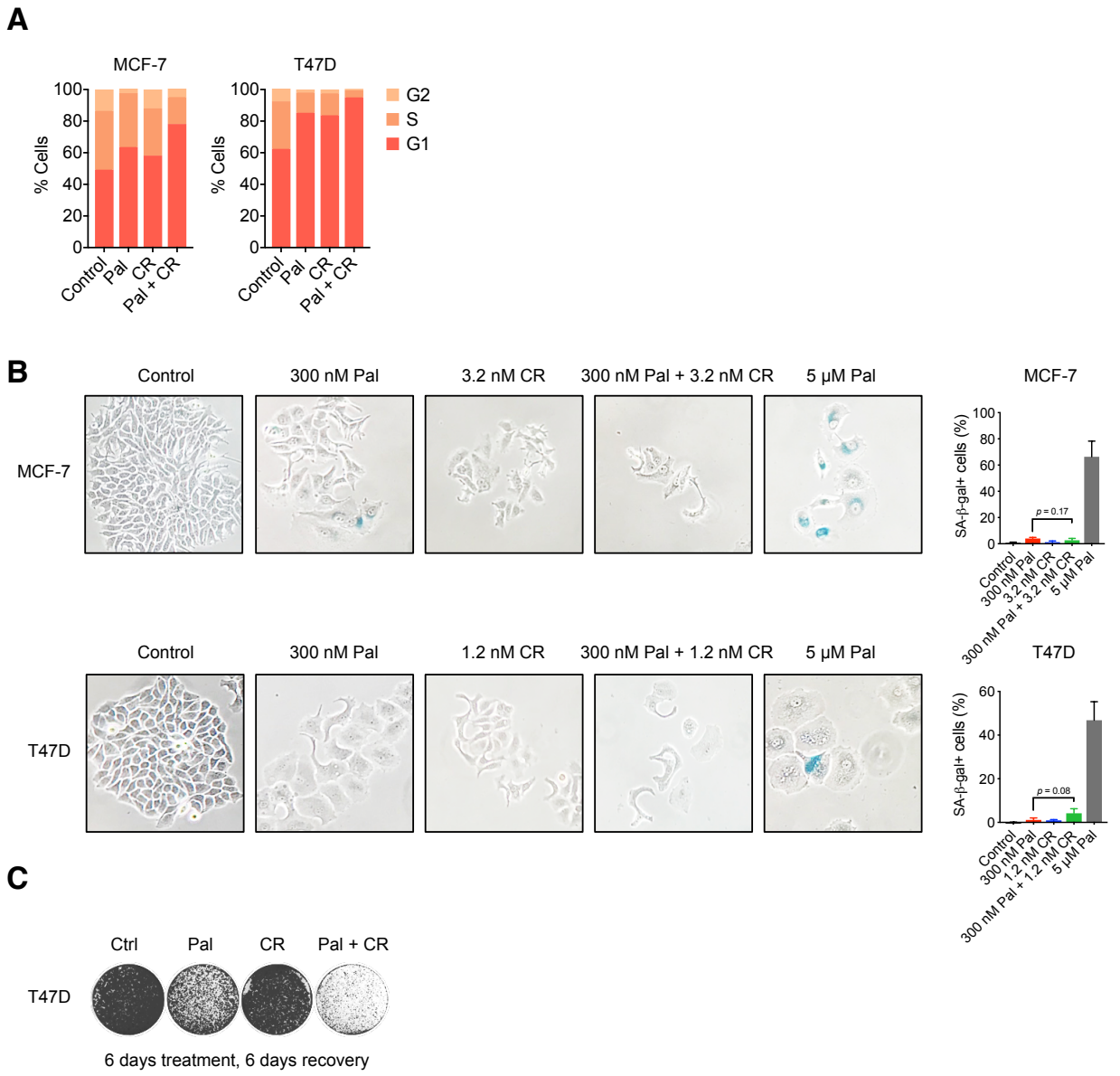
B) Cell viability assays of MCF-7 and T47D cells treated with increasing concentrations of CR-1-31-B or silvestrol. Cells were treated for 7-8 days, and cell viability was determined using CellTiter-Blue. Error bars represent mean ± standard deviation.

C) Western blot showing similar degree of protein suppression of cell cycle regulators by CR-1-31-B and silvestrol. MCF-7 and T47D cells were treated with CR-1-31-B or silvestrol for 24 hours at the indicated drug concentrations.



Supplemental Figure 4. Synergism between a second CDK4/6 inhibitor, Abemaciclib, and CR-1-31-B

Isobologram synergy analysis showing abemaciclib synergizes with CR-1-31-B at multiple dose combinations. CR= CR-1-31-B.

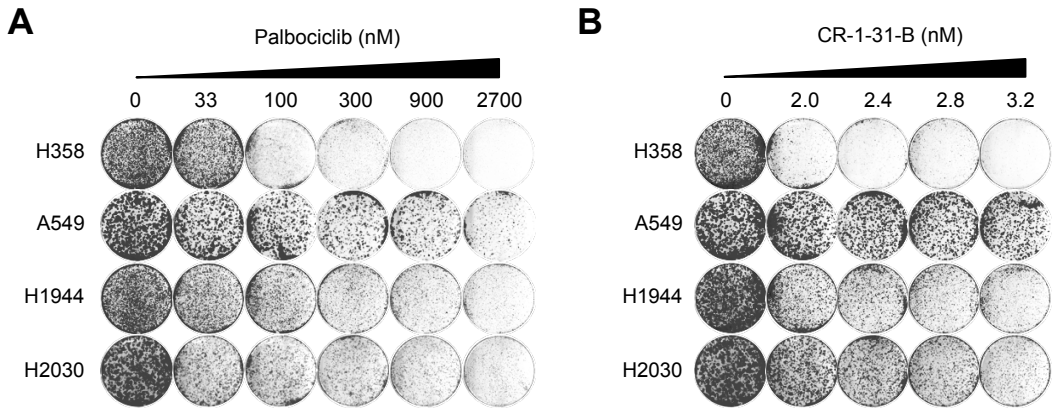


Supplemental Figure 5. Functional cell cycle effects of palbociclib and/or CR-1-31-B treatment in ER-positive breast cancer

A) Cell cycle analysis of MCF-7 and T47D cells. Cells were treated with 300 nM palbociclib, 3.2 nM CR-1-31-B, or combination for 72 hours. Percentage of cells in G1, S, and G2 cell cycle phases are as denoted.

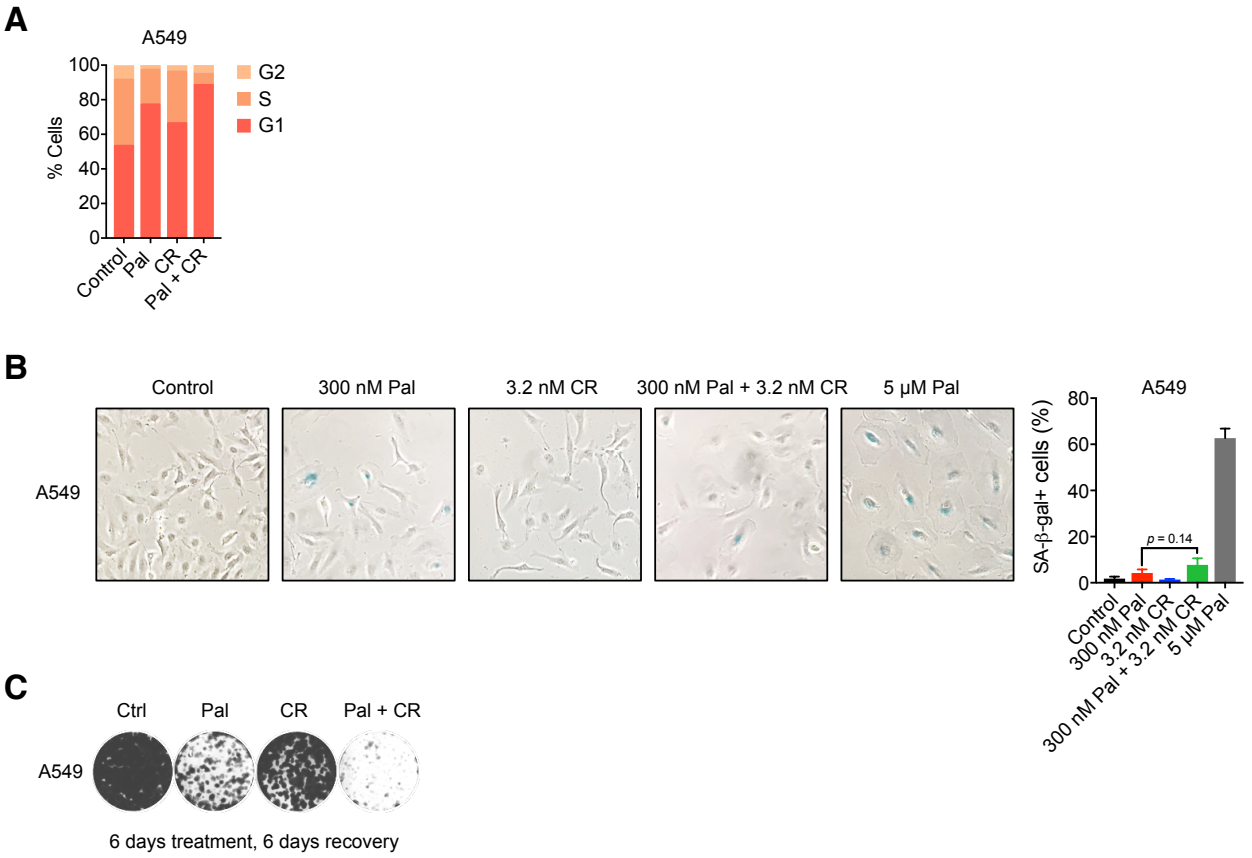
B) Senescence-associated β-galactosidase staining of MCF-7 and T47D cells treated with indicated doses of palbociclib and/or CR-1-3-1B for 7 days. Right panel: quantification of senescence-associated β-galactosidase stained positive cells.

C) Drug washout assay of T47D cells. T47D cells were treated with 100 nM palbociclib, 1.6 nM CR-1-31-B or combination for 6 days, after which cells were washed and refreshed with regular media for 6 days recovery.



Supplemental Figure 6. Sensitivity of *KRAS*-mutant non-small cell lung cancers to palbociclib and CR-1-31-B

Colony formation assays of *KRAS*-mutant non-small cell lung cancers after treatment with palbociclib (A) or CR-1-31-B (B). Cells were seeded for colony formation and treated with palbociclib or CR-1-31-B for 8-14 days. Drugs were refreshed every 3 days.

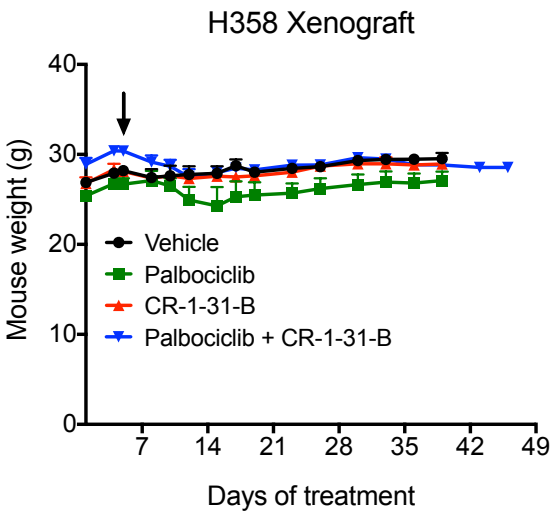


Supplemental Figure 7. Functional cell cycle effects of palbociclib and/or CR-1-31-B treatment in NSCLC

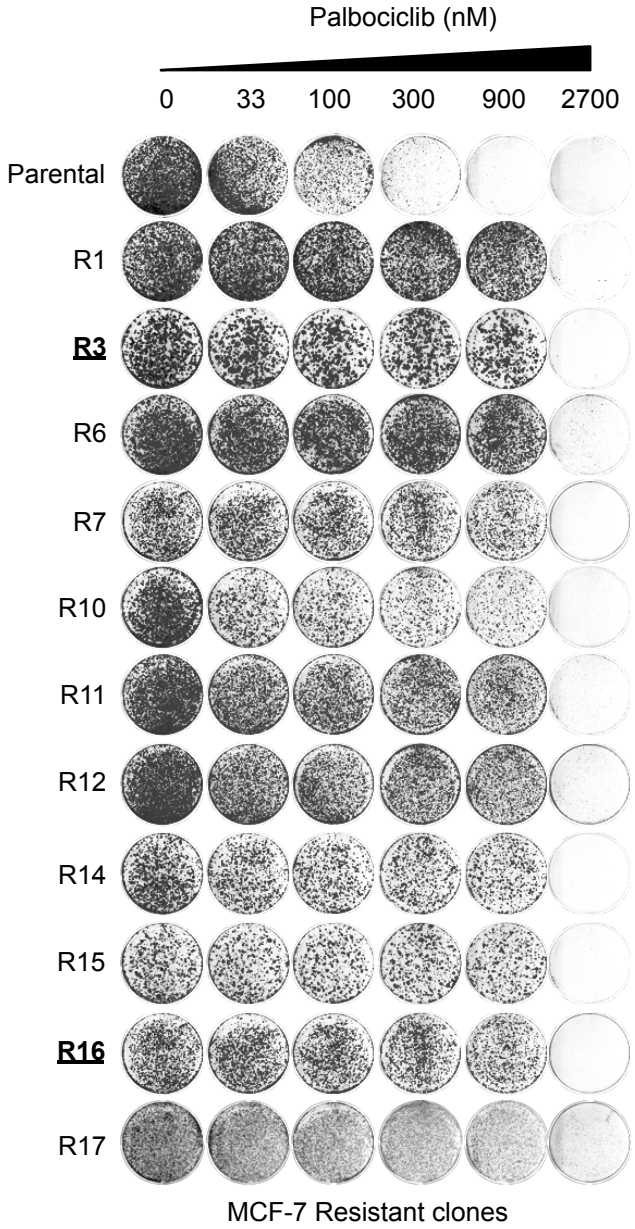
A) Cell cycle analysis of A549 cells treated with 300 nM palbociclib, 3.2 nM CR-1-31-B, or combination for 72 hours. Percentage of cells in G1, S, and G2 cell cycle phases are as denoted.

B) Senescence-associated β-galactosidase staining of A549 cells treated with indicated doses of palbociclib and/or CR-1-3-1B for 7 days. Right panel: quantification of senescence-associated β-galactosidase stained positive cells.

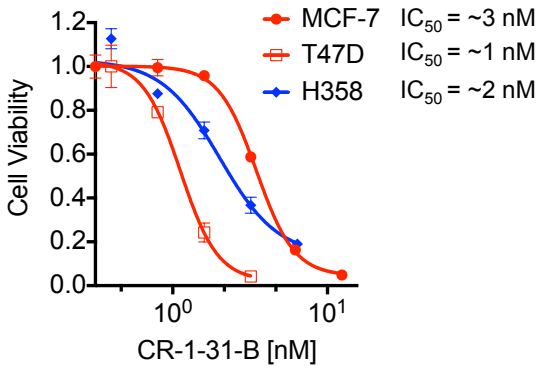
C) Drug washout assay of A549 cells. A549 cells were treated with 100 nM palbociclib, 3.2 nM CR-1-31-B or combination for 6 days, after which cells were washed and refreshed with regular media for 6 days recovery.



Supplemental Figure 8. Weights of subcutaneous xenografted NSG mice treated with palbociclib, CR-1-31-B, or their combination.
NSG mice were weighed twice a week to monitor changes in body weight in response to drug treatment over the xenograft period. The downward arrow indicates the start of drug treatment.



Supplemental Figure 9. Colony formation panel of palbociclib-resistant MCF-7 clones
11 palbociclib-resistant MCF-7 clones and their response to increasing doses of palbociclib. Cells were seeded and treated with palbociclib for 10-14 days at the indicated concentrations. Palbociclib was refreshed every 3 days.



Supplemental Figure 10. Cell viability assay of CR-1-31-B response.
Comparison of cell viability and CR-1-31-B IC_{50} of H358 cells to ER^+ breast cancer cell lines MCF-7 and T47D. Cells were treated with CR-1-31-B for 7-8 days, and cell viability was determined using CellTiter-Blue. Error bars represent mean ± standard deviation.