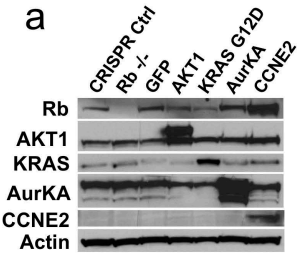
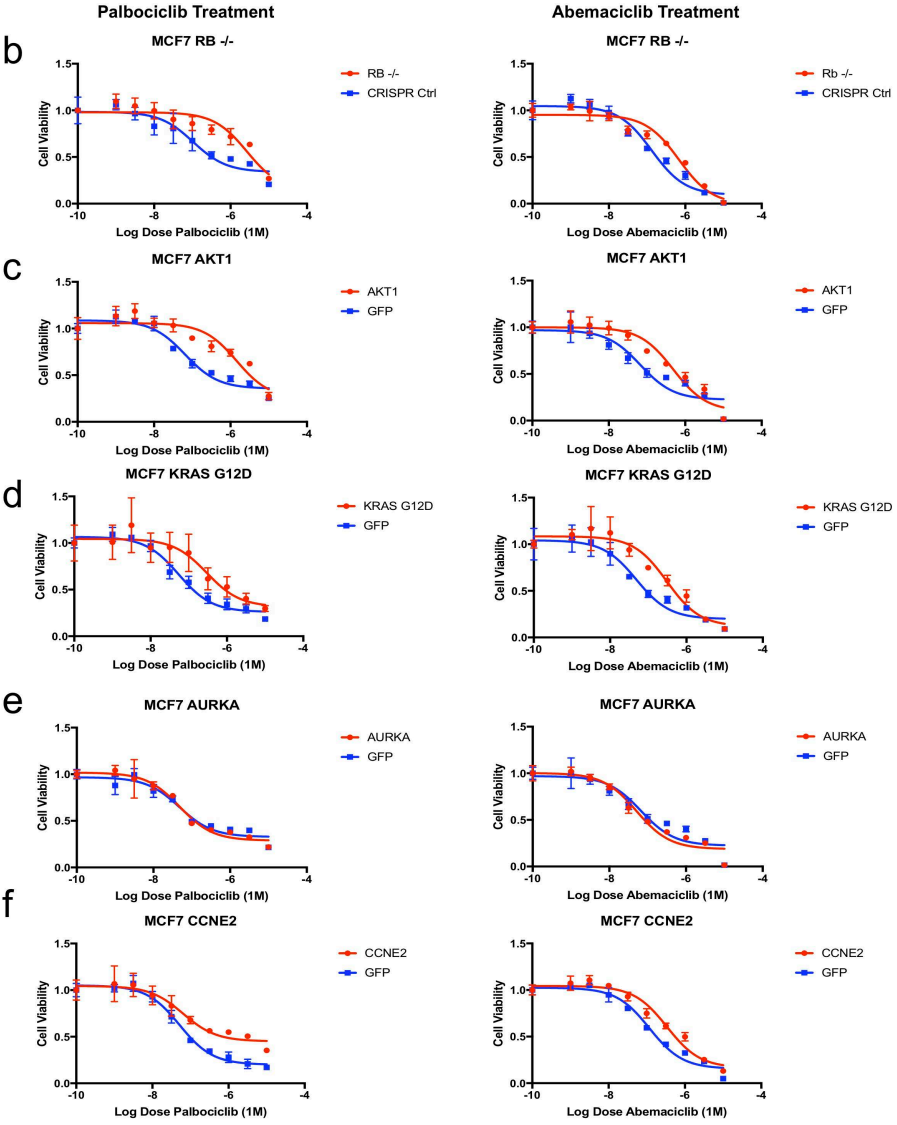
Figure S5


a
CRISPR Ctrl
Rb -/-
GFP
AKT1
KRAS G12D
AurKA
CCNE2
Rb
AKT1
KRAS
AurKA
CCNE2
Actin


Palbociclib Treatment
Abemaciclib Treatment
b
MCF7 RB -/-
RB -/-
CRISPR Ctrl
Cell Viability
Log Dose Palbociclib (1M)
Log Dose Abemaciclib (1M)
c
MCF7 AKT1
AKT1
GFP
d
MCF7 KRAS G12D
KRAS G12D
GFP
e
MCF7 AURKA
AURKA
GFP
f
MCF7 CCNE2
CCNE2
GFP

Supplemental Figure 5. Candidate alterations provoke CDK4/6i resistance *in vitro* (MCF7).

(a) MCF7 cells were modified via CRISPR-mediated downregulation (RB1) or lentiviral overexpression (AKT1, KRAS G12D, AURKA, CCNE2) to interrogate potential resistance mediators identified in patient biopsy samples. Western blotting with the indicated antibodies is included. (b-f) Modified MCF7 cells were exposed to escalating doses of CDK4/6i (palbociclib – left, abemaciclib – right) and viability was estimated via cell-titer-glo (CTG) assay. Control (CRISPR non-targeting guide, GFP) cells are plotted along with the resistance driver of interest (RB1 – b, AKT1 – c, KRAS G12D – d, AURKA – e, CCNE2 – f). Parental and variant cell lines are normalized to vehicle control and viability is plotted as a function of increasing CDK4/6i (graphed as triplicate average +/- standard deviation). RB1, AKT1, and CCNE2 provoke CDK4/6i resistance (to both palbociclib and abemaciclib) *in vitro* in MCF7 cells. Corresponding IC50 values are included in Supplemental Table 7.