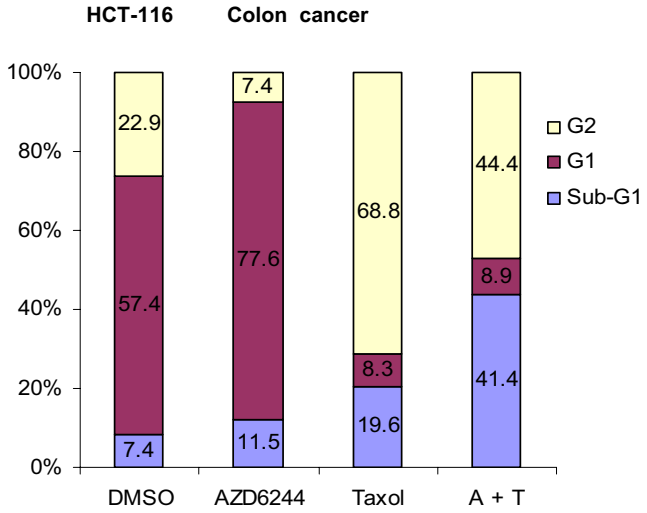
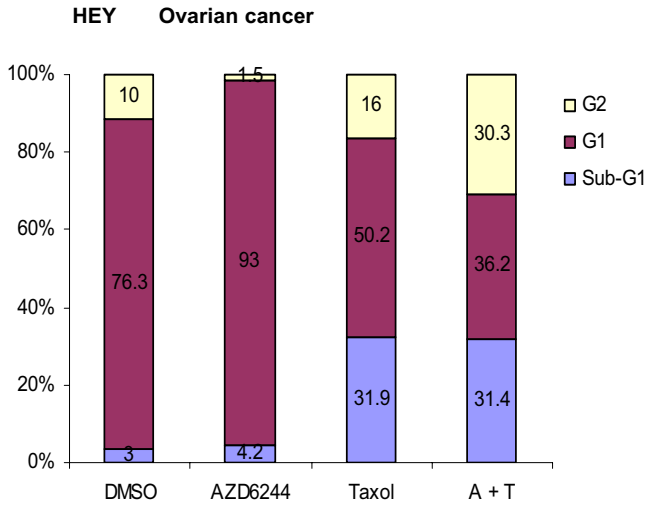
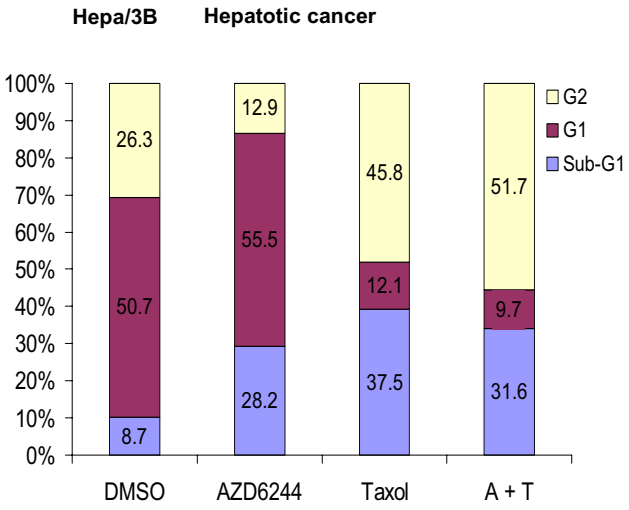
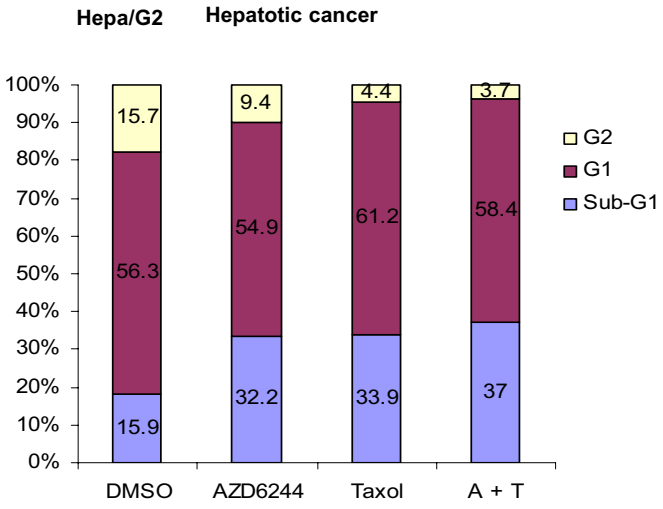
Supplemental Figure 1









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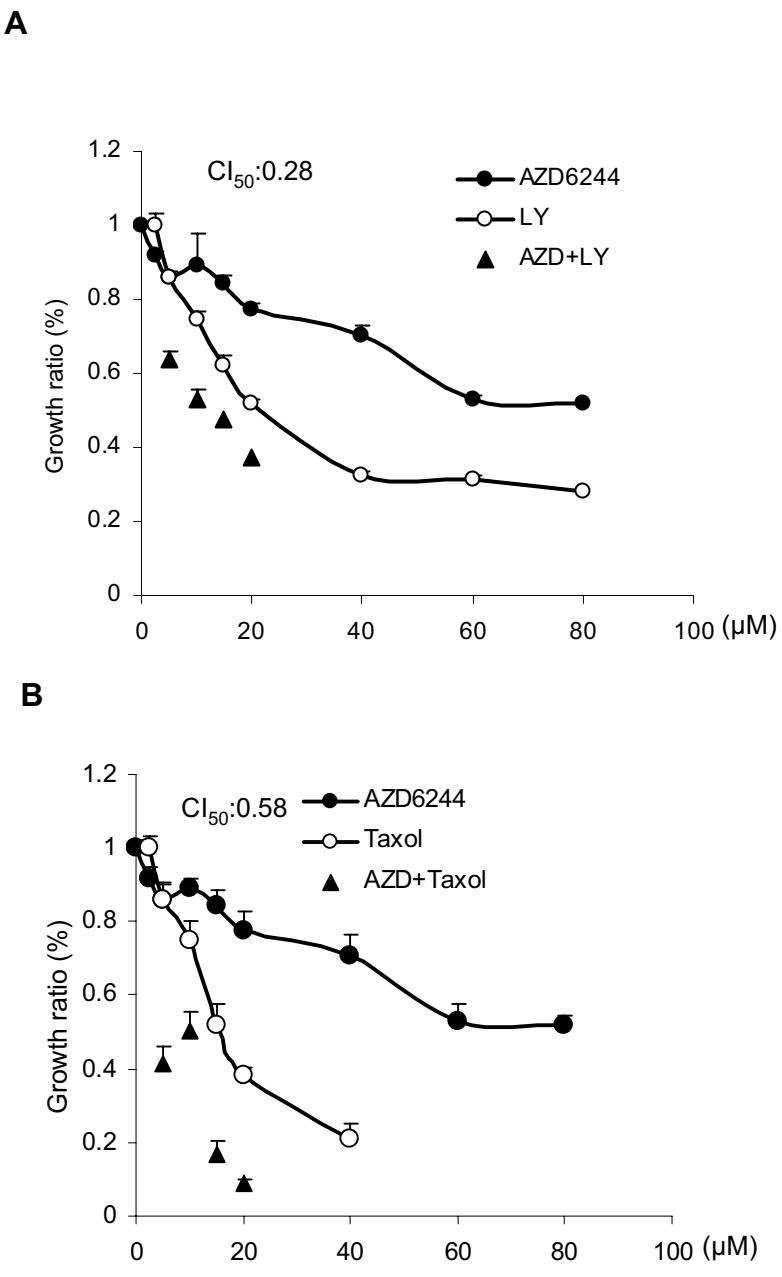

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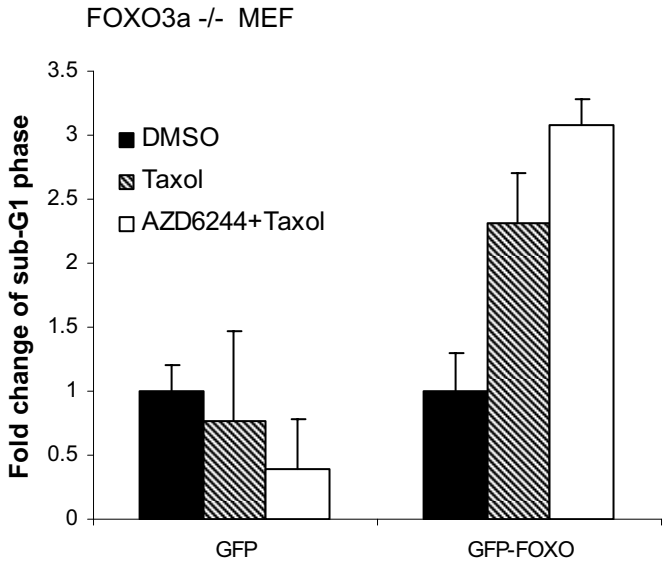

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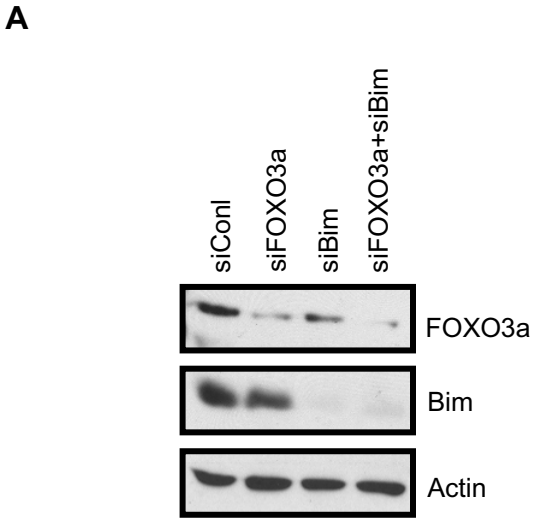

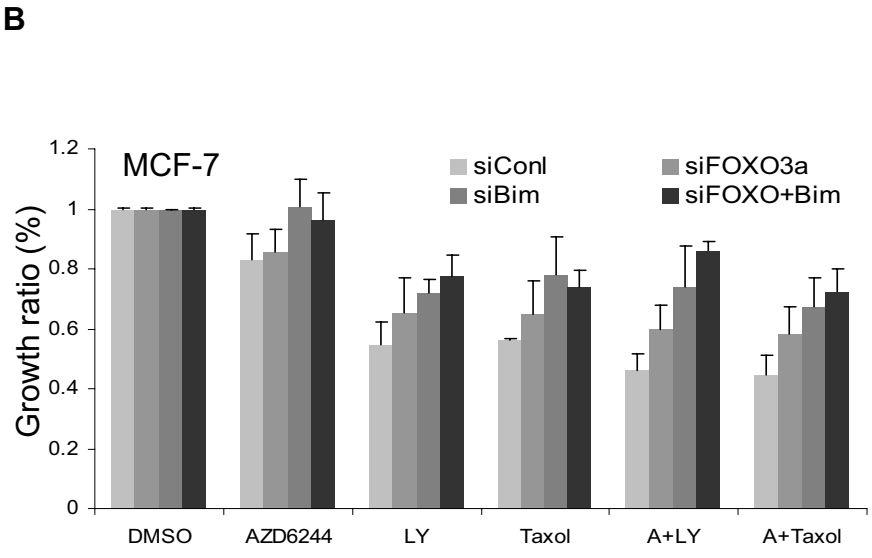

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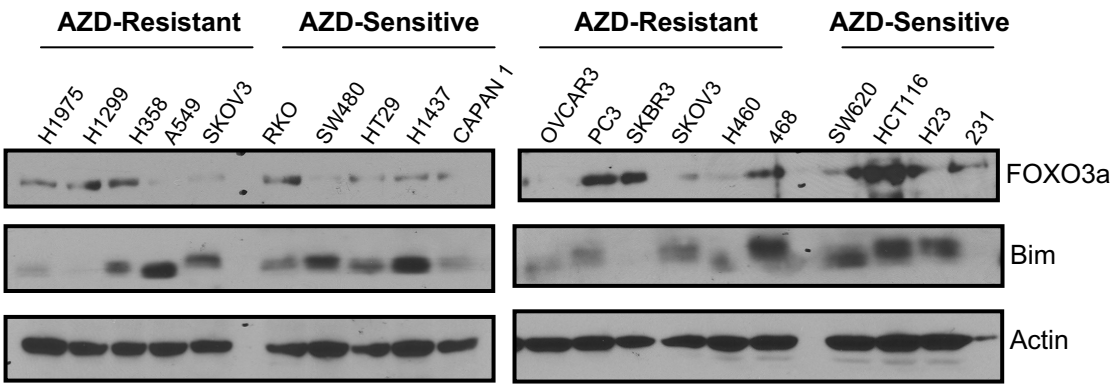

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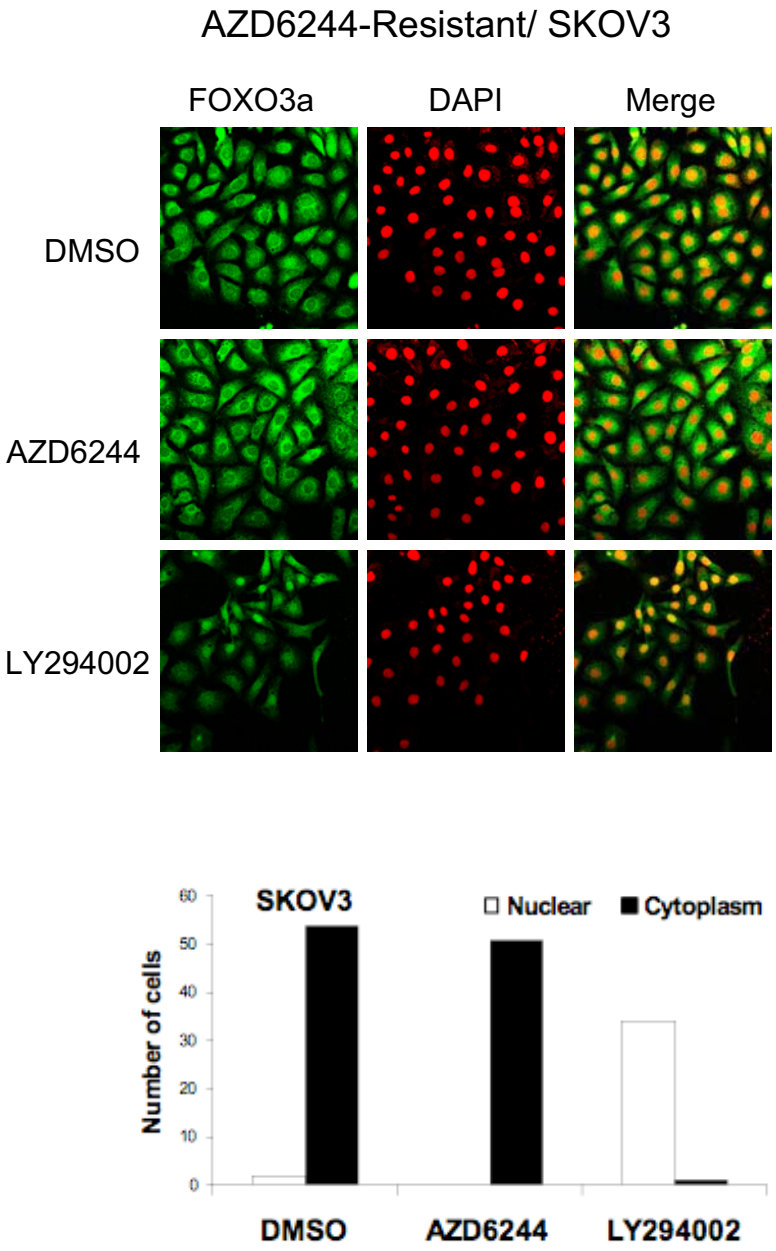

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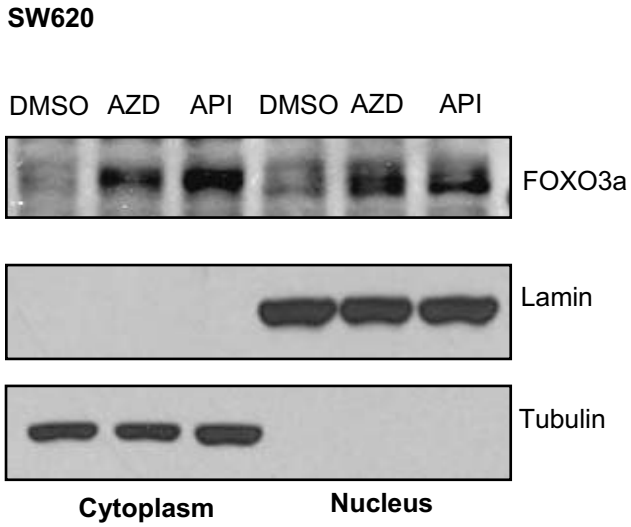
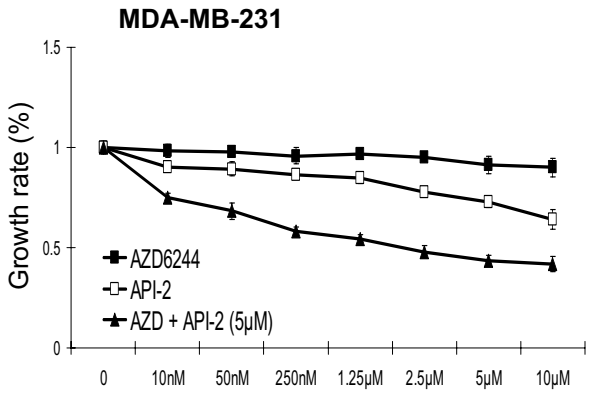


Supplemental Figure 8

A



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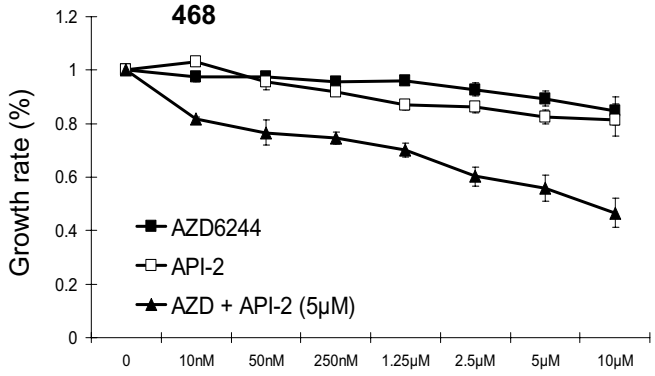

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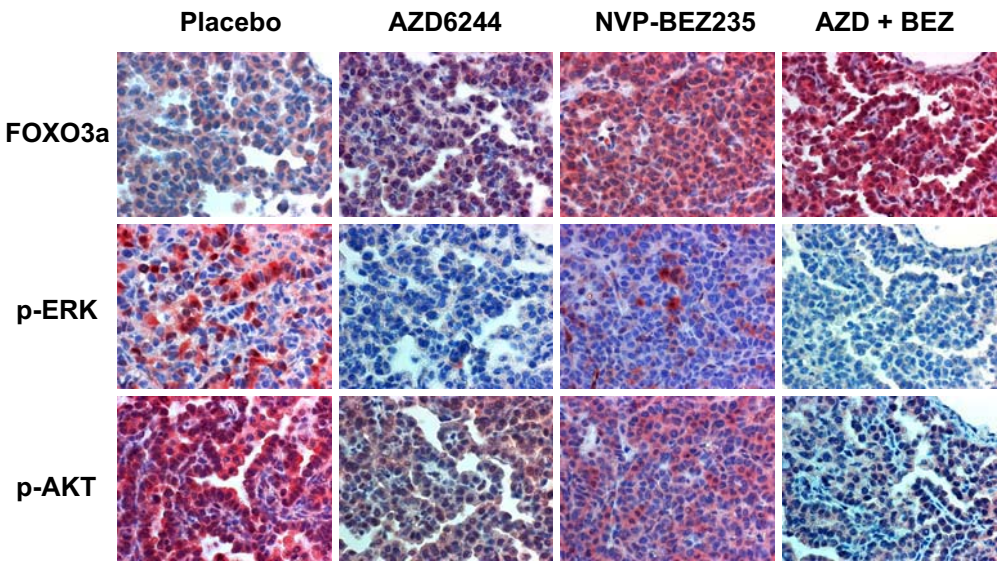

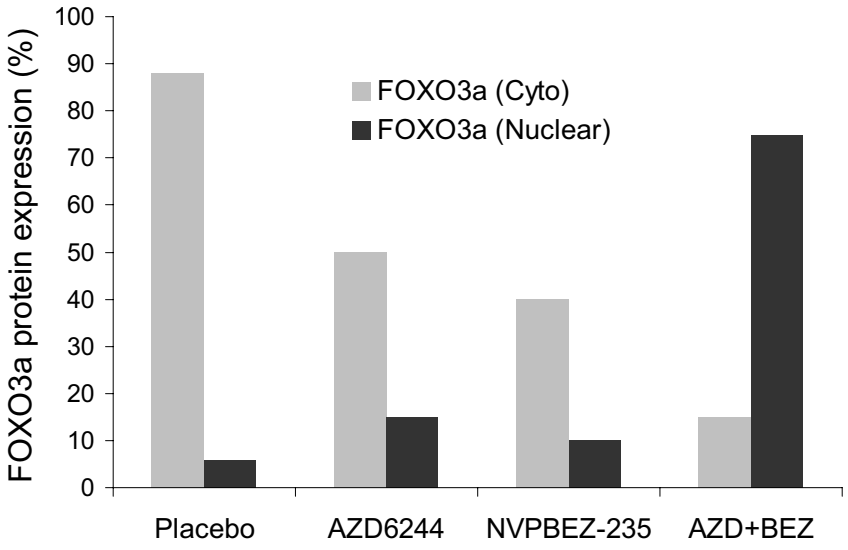

Supplemental Figure 10

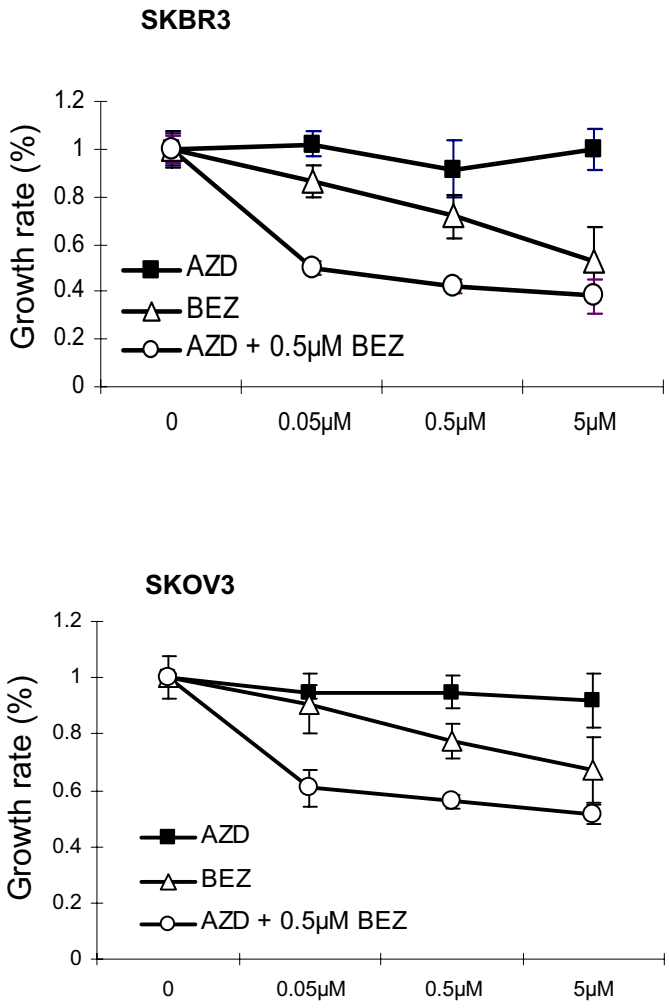

Figure S1. HCT-116, HET, Hep/3B and Hep/G2 cells were treated with AZD6244 for 24 hrs with or without Taxol, and then subjected to cell cycle analysis. Bar Graphs show the percentages of cells in the indicated cell phase.

Figure S2. MCF-7 cells were treated with DMSO, AZD6244 (5μM) or AZD6244 (5μM) in combination with increasing concentrations of LY290024 (1μM, 2μM, 4μM and 10 μM) (A), or AZD6244 (5μM) in alternative combinations with increasing concentrations of Taxol (0.6ng/μl, 1.2ng/μl, 2.4ng/μl and 6ng/μl) (B), for 24 hrs and then subjected to MTT assays. Graphs show the mean values of the representative results from two experiments conducted in triplicates for each. Combination Index (24) value was calculated when 50% of the cells were killed. The combination index (24) calculated by the classic isobologram equation, represents synergism with CI<1, addition with CI=1, and antagonism with CI>1 as described in (27). The CI curves were plotted against ratios of cell survival inhibition when 25% (CI_{25}) and 50% (CI_{50}) of the MCF7 cells treated with (A) AZD6244/LY or (B) AZD6244/Taxol were killed. The calculation is briefly described as follows: CI_{50} = Dc1/D1 + Dc2/D2 (D1: IC_{50} of single agent D1, D2: IC_{50} of single agent D2, Dc1: IC_{50} of D1 when combined with D2, Dc2: IC_{50} of D2 when combined with D1).

Fig. S3. FOXO3a-/- cells transfected with GFP vector or GFP-FOXO3a were treated with DMSO or AZD6244 (5 μM) with or without Taxol (6ng/μl), then

subjected to FACS assays. Bar graphs show the mean values of the representative results from two experiments conducted in triplicates for each.

Figure S4. (A) After 48hr transfection with FOXO3a siRNA, Bim siRNA, or both were subjected to western blot with indicating antibodies. (B) MCF-7 cells from A were treated with DMSO or AZD6244 (5 μM) with or without LY290024 (10μM) and Taxol for 24 hrs and then subjected to MTT assays.

Figure S5. Lysates from nighteen AZD6244 sensitive cell lines and AZD6244 resistant cell lines were subjected to immunoblotting with FOXO3a and Bim antibodies.

Figure S6. (A) AZD6244-resistant SKOV3 cells were treated with DMSO, AZD6244 (10μM), LY290024 (10μM) for 24 hrs and then subjected to immunoflourescence analysis. (B) Bar Graphs show the percentages of cells with nuclear or cytoplasmic FOXO3a expression from immunoflourescence experiments in (A).

Figure S7. AZD6244-sensitive cells SW620 were treated with DMSO, AZD6244 (1μM), API-2 (10μM) for 24 hrs, subjected to nuclear-cytoplasmic fractionation, and then immunoblotting analysis.

Figure S8. API-2 synergizes with AZD6244 suppressing cell proliferation in AZD6244-resistant cancer cells. AZD6244-resistant cells (A) MDA-MB-231 (B) MDA-MB-468 were treated with DMSO, AZD6244, API-2 or AZD6244 along with API-2 for 48 hrs and then subjected to MTT assays.

Figure S9. The tumor sections of DMSO, AZD6244, NVP-BEZ235 or AZD6244 combined with NVP-BEZ235 treated-murine Kras-mutant lung tumors were subjected to immunohistochemistry with FOXO3a, pERK and pAKT antibody.

Figure S10. AZD6244-resistant cells (A) SKBR3, (B) SKOV3 treated with DMSO, AZD6244, NVP-BEZ235 or AZD6244 along with NVP-BEZ235 for 48 hrs were then subjected to MTT assays. Graphs show the mean values of the representative results from two experiments conducted in triplicates for each.