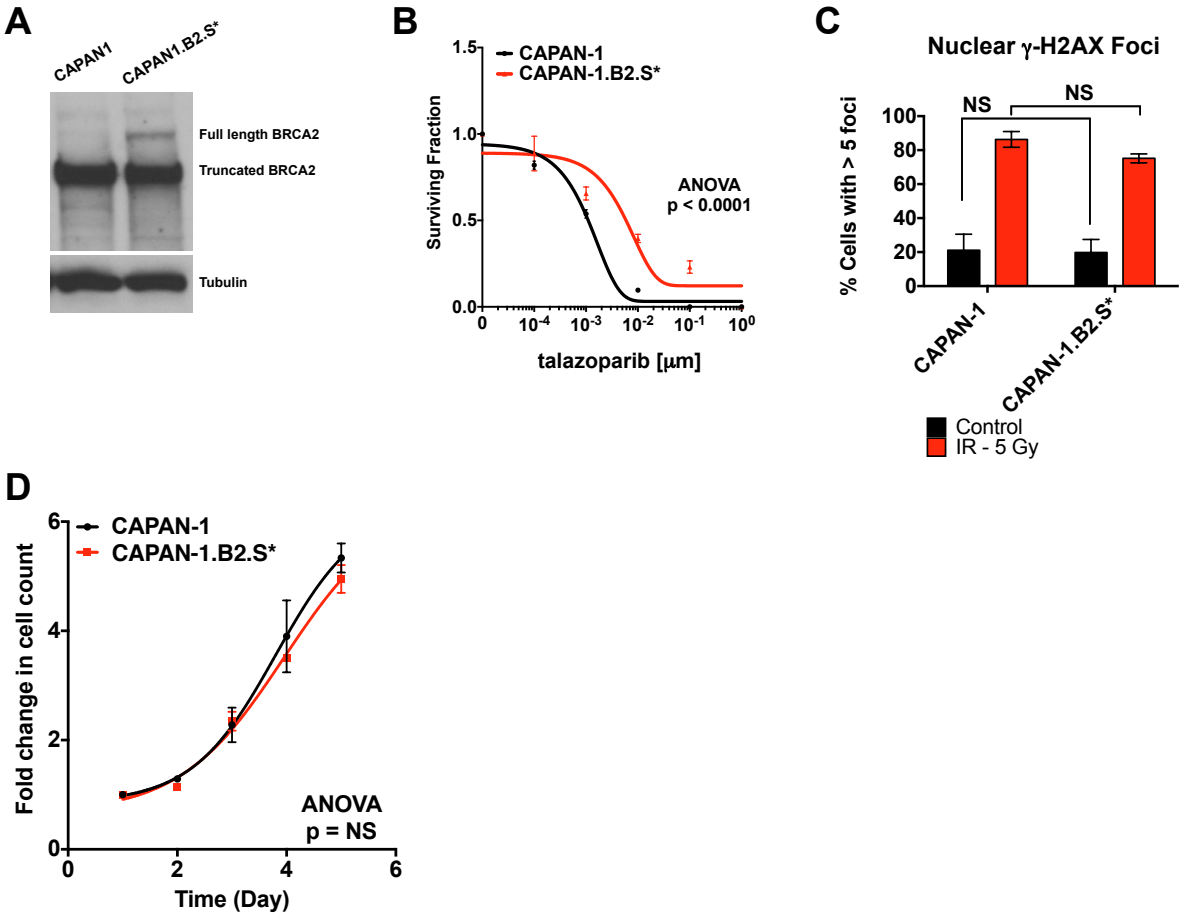
Supplementary Figure 1



Supplementary Figure 1.

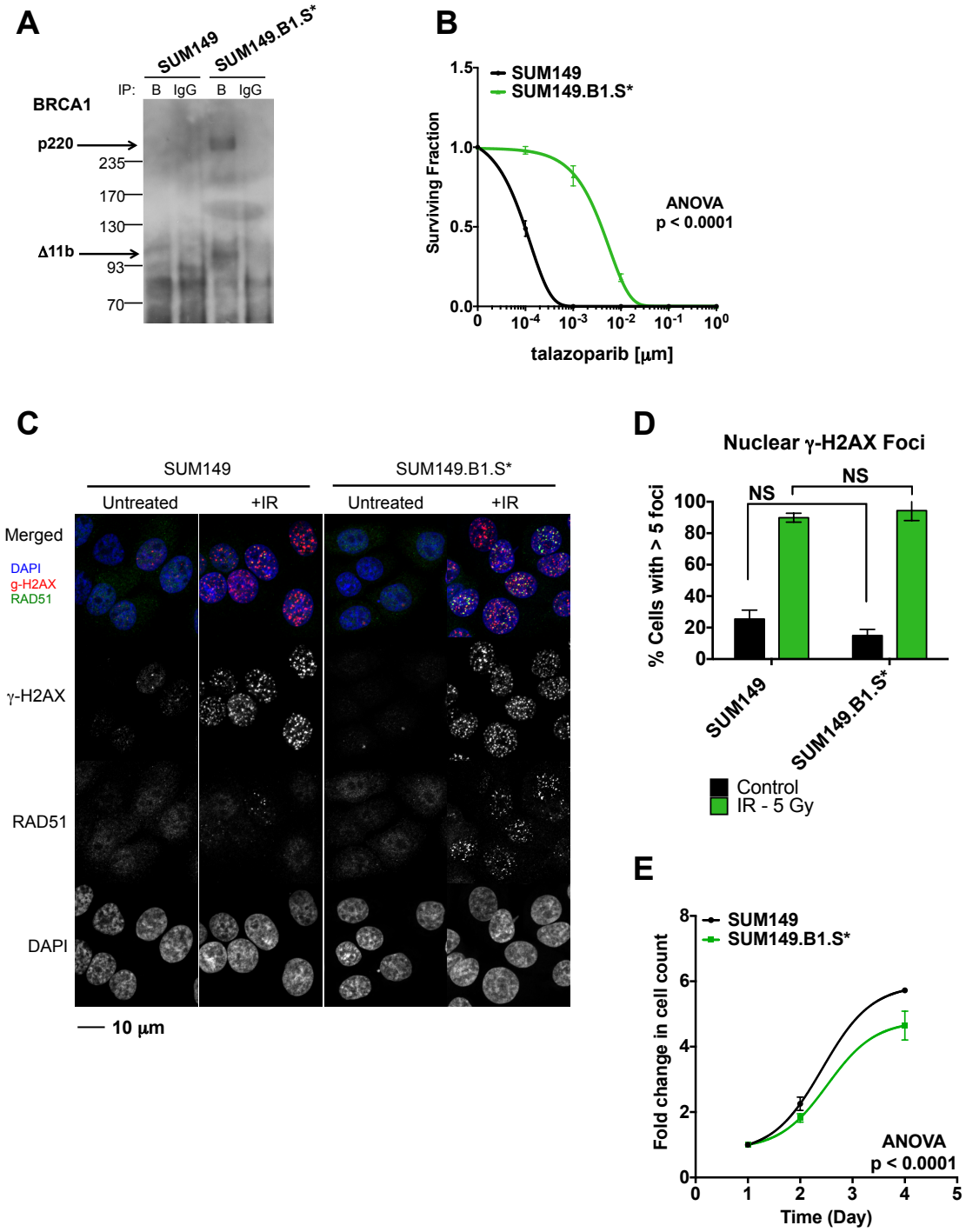
A. Western blot of lysates with anti-BRCA2 antibodies show almost full length BRCA2 is present in CAPAN1.B2.S*.

B. Dose-response survival curves for talazoparib for CAPAN1.B2.S* (red) compared to the parental cell line (P <0.0001, ANOVA). Error bars represent SEM from triplicate experiments.

C. Bar chart illustrating quantitation of nuclear g-H2AX foci. Cells containing more than five foci were counted as positive. Mean ± SEM (standard error of the mean) for three independent experiments are shown. p values were calculated using Student's t test.

D. Fold change in cell count plotted against time in CAPAN1 and CAPAN1.B2.S* show isogenic cell lines have similar growth rates. Error bars represent SEM from triplicate experiments.

Supplementary Figure 2



Supplementary Figure 2.

A. IP followed by western blotting of lysates with anti-BRCA1 antibodies showing re-expression of near full-length BRCA1 in SUM149.B1.S*. B, BRCA1 IP; IgG, control IP.

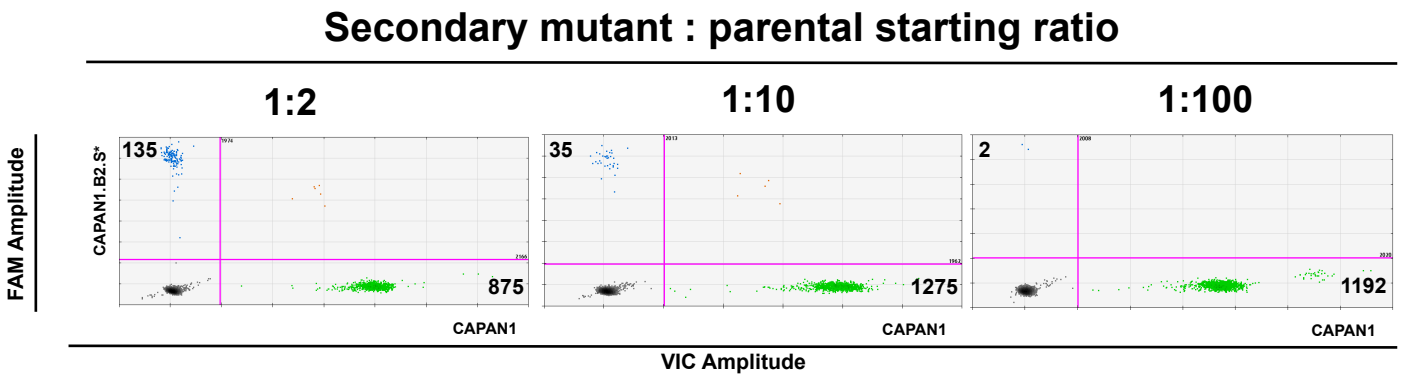
B. Dose-response survival curves for talazoparib for SUM149.B1.S* (green) compared to the parental cell line (P <0.0001, ANOVA). Error bars represent SEM from triplicate experiments.

C. Representative images for nuclear RAD51 foci formation in SUM149 and SUM149.B1.S* cells following IR exposure. Scale bar = 10 mm.

D. Bar chart illustrating quantitation of nuclear g-H2AX foci. Cells containing more than five foci were counted as positive. Mean ± SEM (standard error of the mean) for three independent experiments are shown. p values were calculated using Student's t test.

E. Fold change in cell count plotted against time in SUM149 and SUM149.B1.S* show isogenic cell lines growth rates. Error bars represent SEM from triplicate experiments.

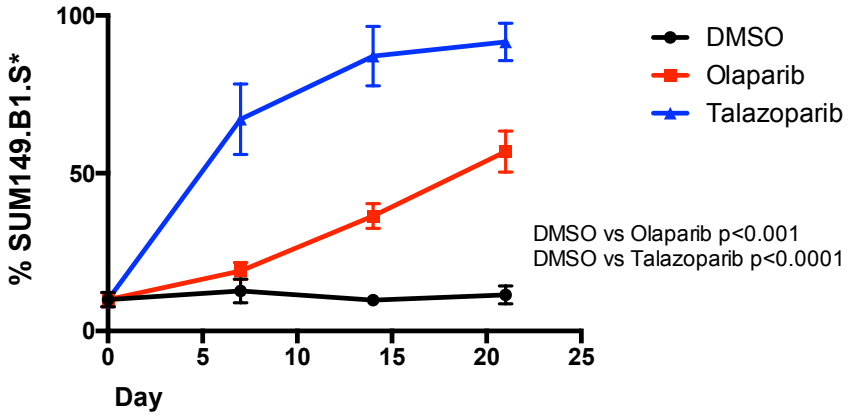
Supplementary Figure 3

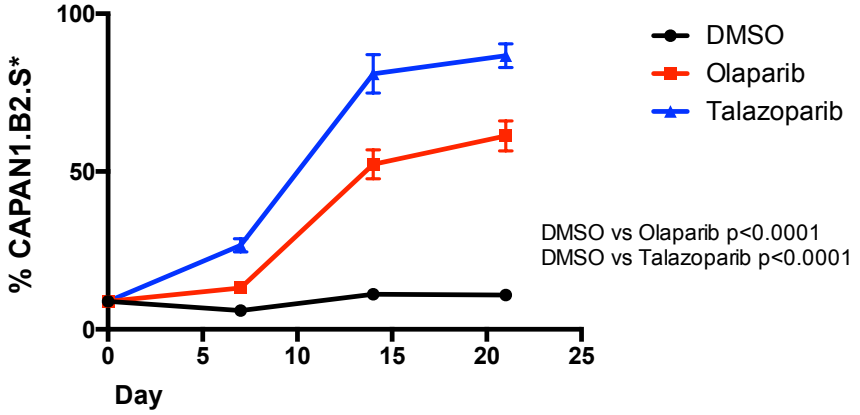


Supplementary Figure 3. The ddPCR assay can detect up to 1:100 secondary mutant to parental tumour cell events.
ddPCR plots showing droplet populations observed for 1:2, 1:10, or 1:100 starting ratios of secondary mutant:parental tumour cells with either CAPAN1.B2.S* (blue, FAM amplitude) or CAPAN1 (green, VIC amplitude). Key: Black drops- empty droplets, blue- CAPAN1.B2.S* DNA FAM positive droplets, green- CAPAN1 DNA VIC positive droplets.

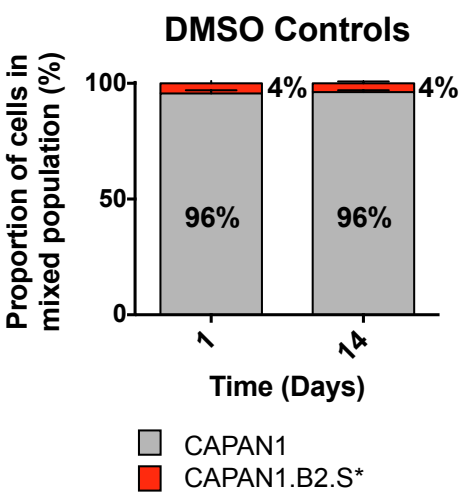
Supplementary Figure 4

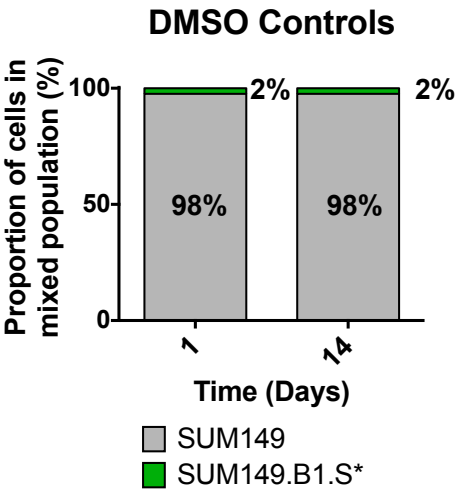
A





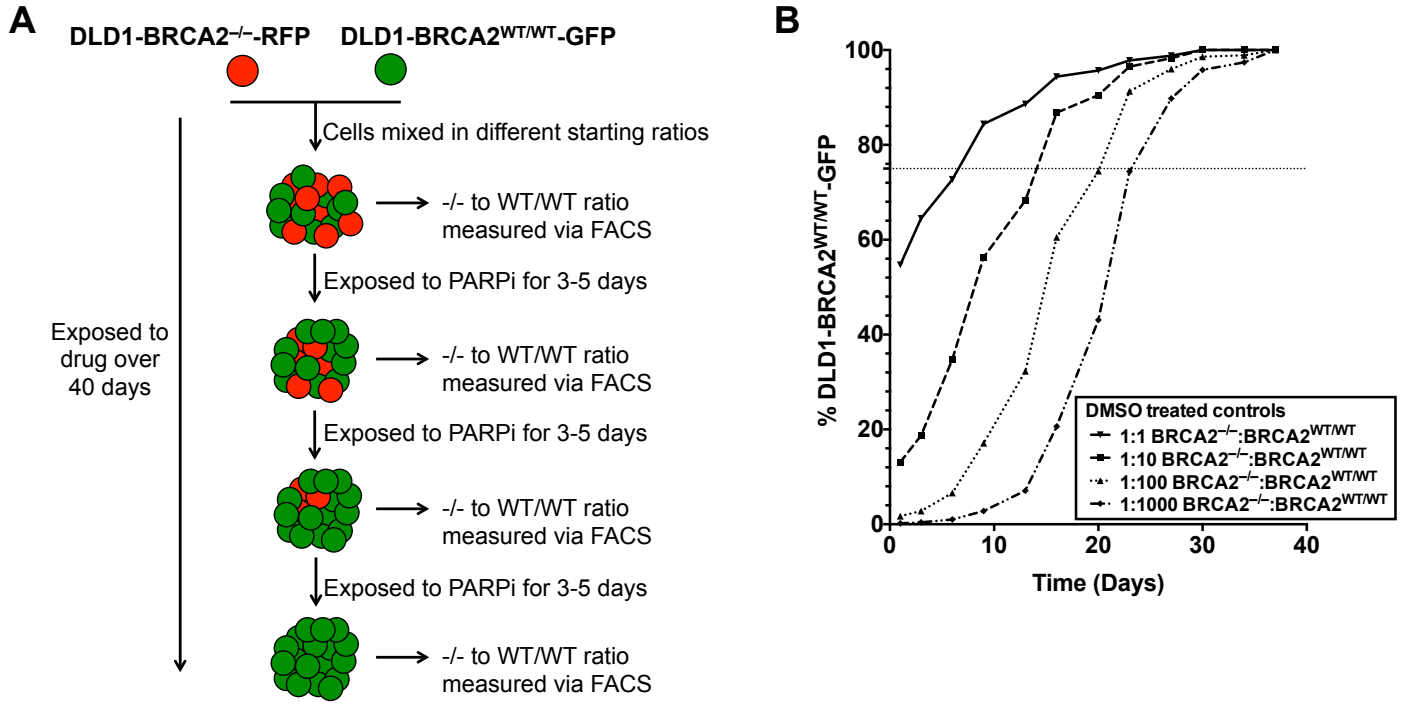
B





Supplementary Figure 4. Olaparib and talazoparib select for secondary mutant tumour cells.
A. CAPAN1/ CAPAN1.B2.S* or SUM149/ SUM149.B1.S* mixed cultures (1:10 secondary mutant:parental cell ratio) were exposed to either either 1 μM olaparib or 0.1 μM talazoparib for 21 days. The frequency of secondary mutant cells was monitored by ddPCR. Graphs show the frequency of secondary mutant tumour cells in the population over time.
B. No fitness discrepancy between parental and secondary mutant clone observed for mixed CAPAN-1 or SUM149 co-cultures. Bar graphs showing day 1 and day 14 DMSO exposed controls for both CAPAN1 and SUM149 mixed secondary mutant:parental tumour cell populations.

Supplementary Figure 5

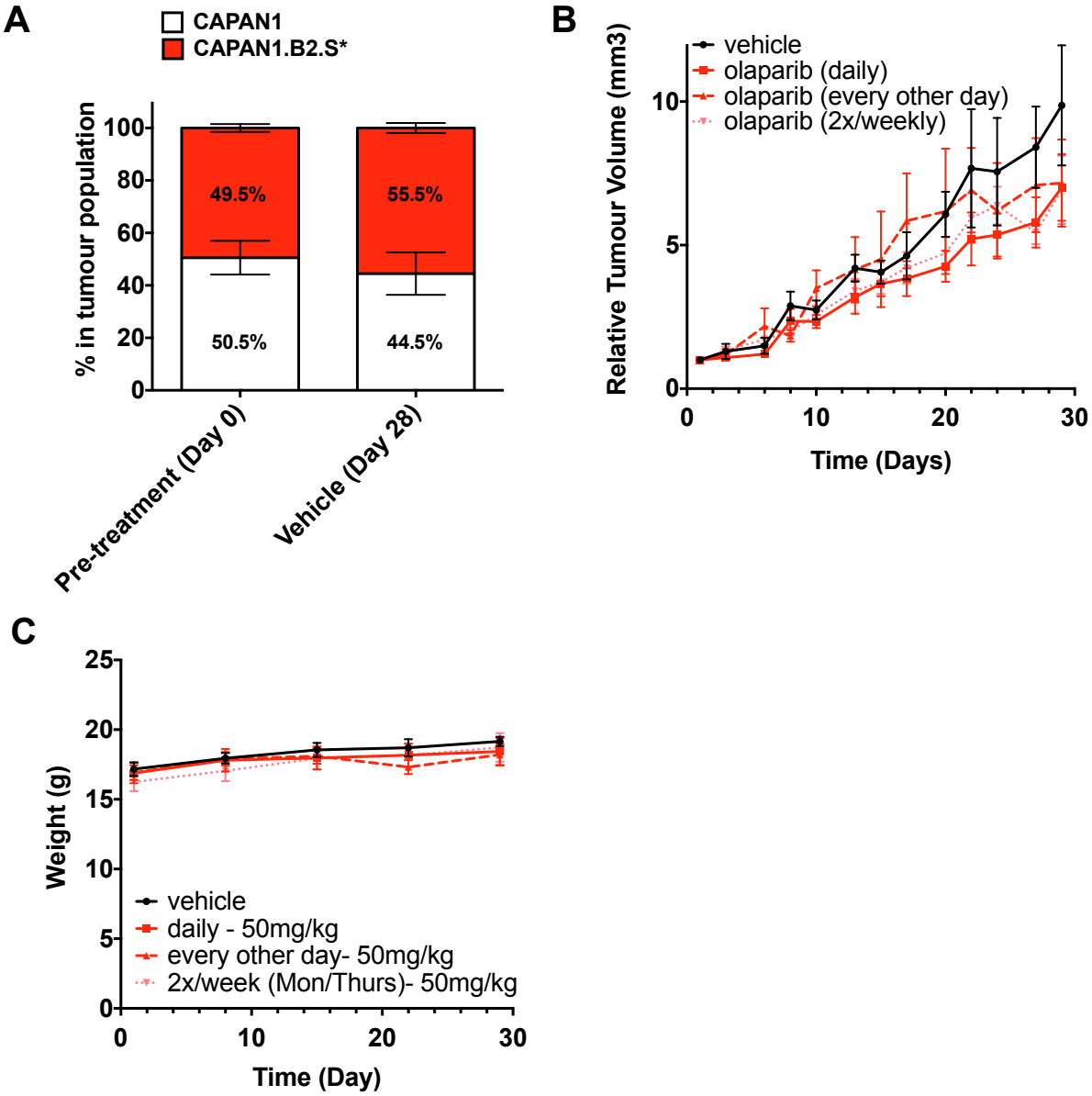


Supplementary Figure 5. DLD1.BRCA2$^{WT/WT}$ tumour cells have a fitness advantage over DLD1.BRCA2$^{-/-}$ cells *in vitro*.

A. Experimental schematic for evaluating mixed cell populations consisting of DLD1.*BRCA2*$^{WT/WT}$-GFP and DLD1.*BRCA2*$^{-/-}$-RFP cells at different starting ratios (1:1, 1:10, 1:100, 1:1000) over 40 days. Cells were exposed to either olaparib or talazoparib at a low and high dose and periodically monitored by FACS analysis.

B. Graph showing temporal evaluation of DLD1.*BRCA2*$^{WT/WT}$ tumour cell population within mixed DLD1.*BRCA2*$^{WT/WT}$-GFP:DLD1.*BRCA2*$^{-/-}$-RFP co-cultures over 40 days.

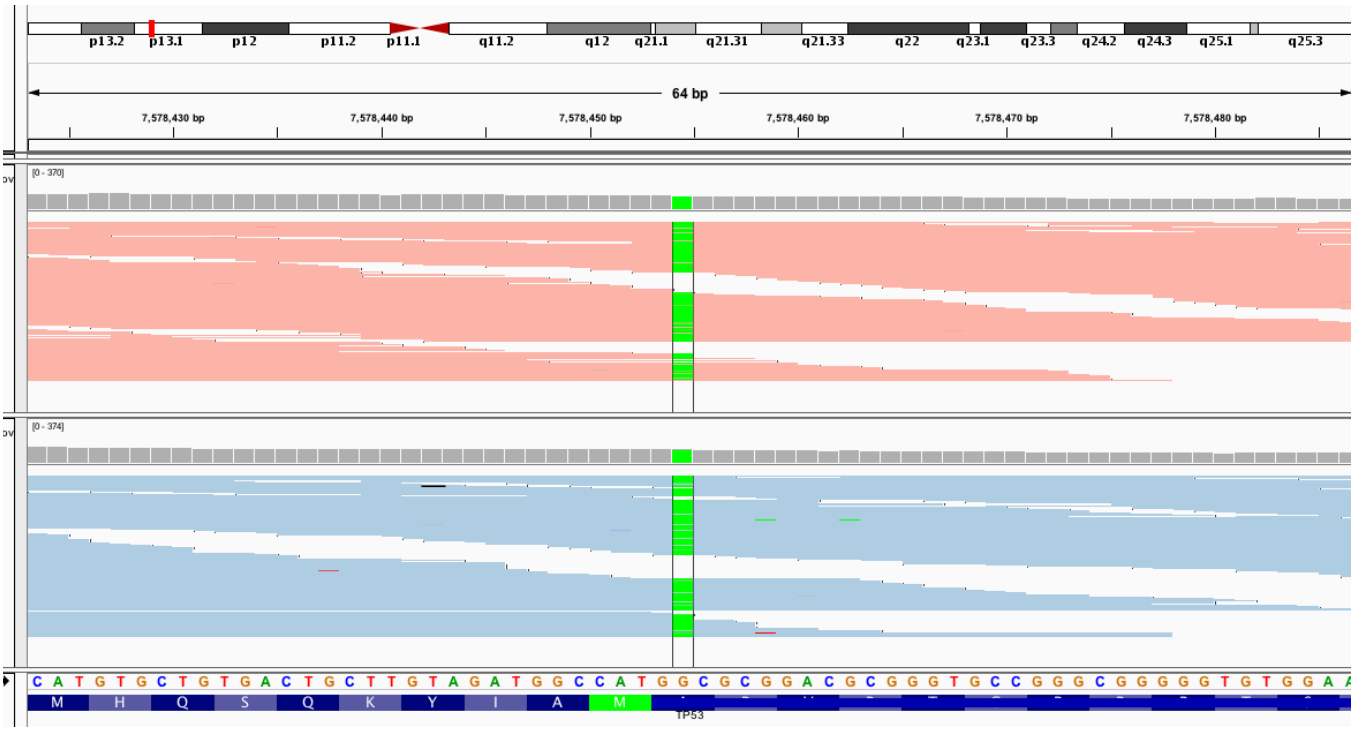
Supplementary Figure 6



Supplementary Figure 6. Olaparib has little efficacy in mixed CAPAN-1 xenografts.
A. Bar chart illustrating CAPAN1 (white) to CAPAN1.B2.S* (red) ratio in day 0 (pretreatment control) xenografts (n=6, mean ± SEM) and after 28 days exposure to vehicle.
B. Tumour response in mixed CAPAN1:CAPAN1.B2.S* xenografts treated for 28 days with 1) vehicle, 2) olaparib – 50 mg/kg (daily), 3) olaparib – 50 mg/kg (every-other-day), and 4) olaparib – 50 mg/kg (2x/weekly) (n=6, mean ± SEM).
C. Tolerability of olaparib treatment *in vivo* over 28 day exposure (n=6, mean ± SEM).

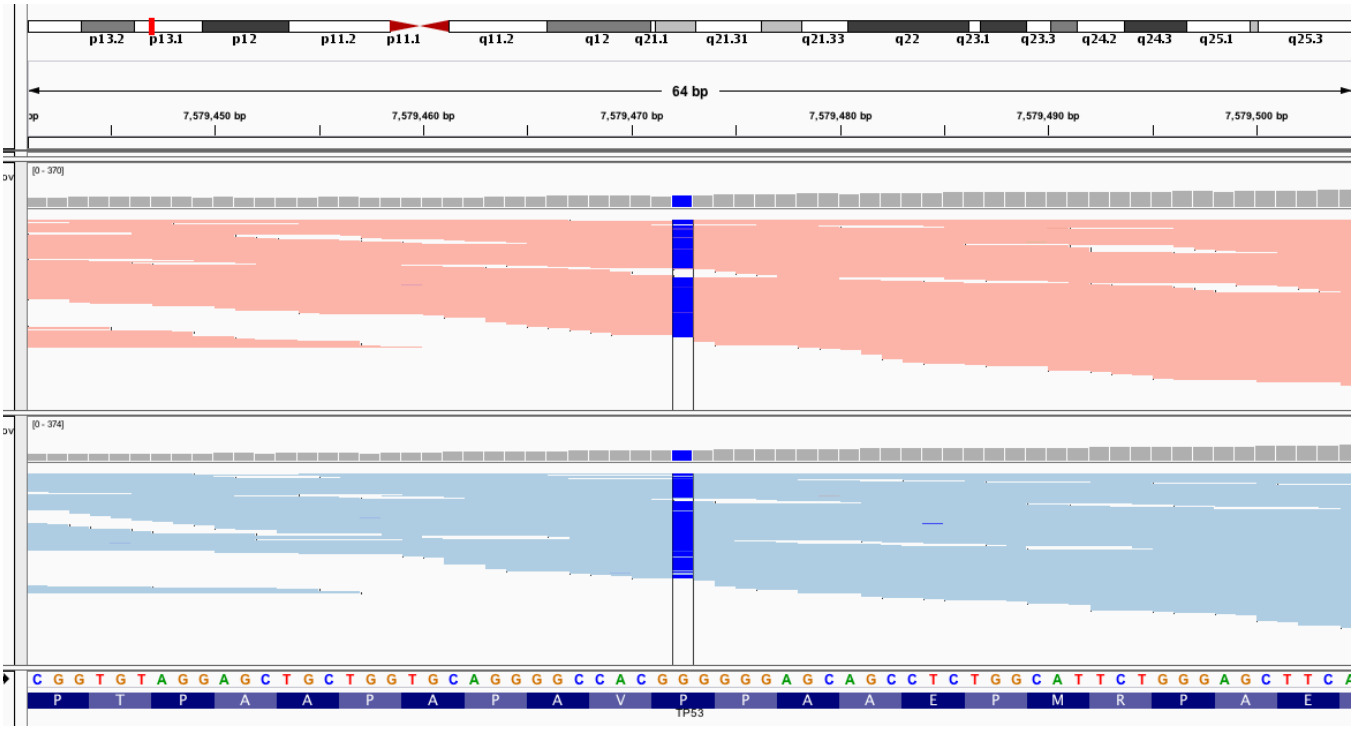
Supplementary Figure 7

A



17:7578454 G>A p.Ala159Val

B

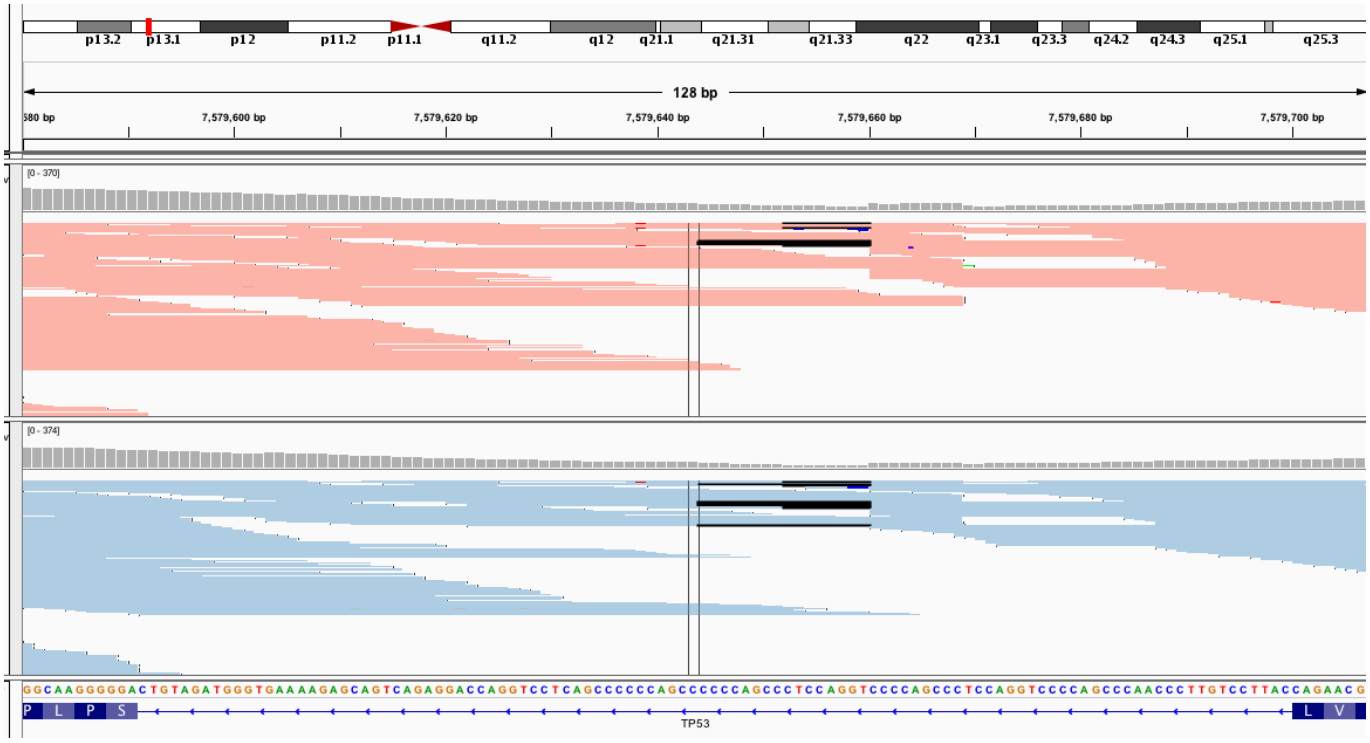


17:7579472 G>C p.Pro72Arg (dbSNP:rs1042522)

CAPAN-1 (parental)
CAPAN-1.B2.S*

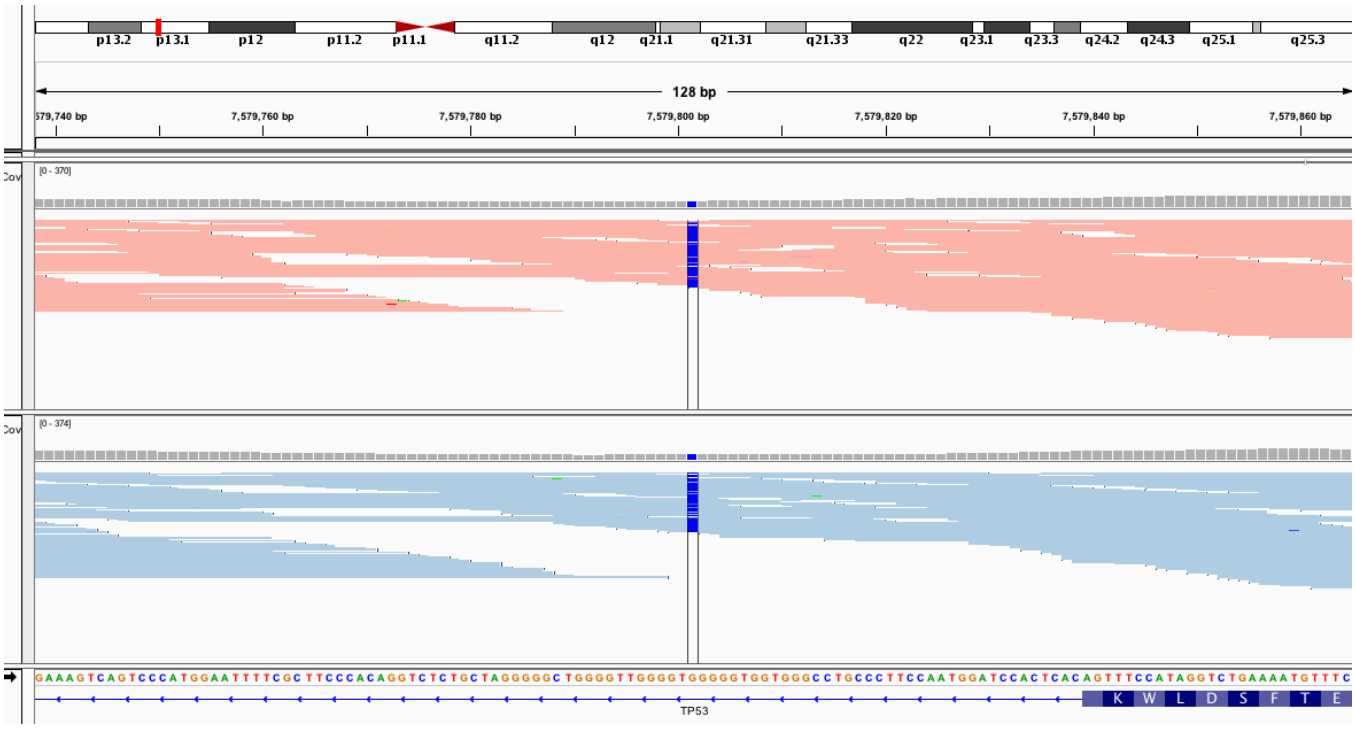
Supplementary Figure 7 con't.

C



17:7579643 CCCCCAGCCCTCCAGGT>C Retained intron (dbSNP)

D



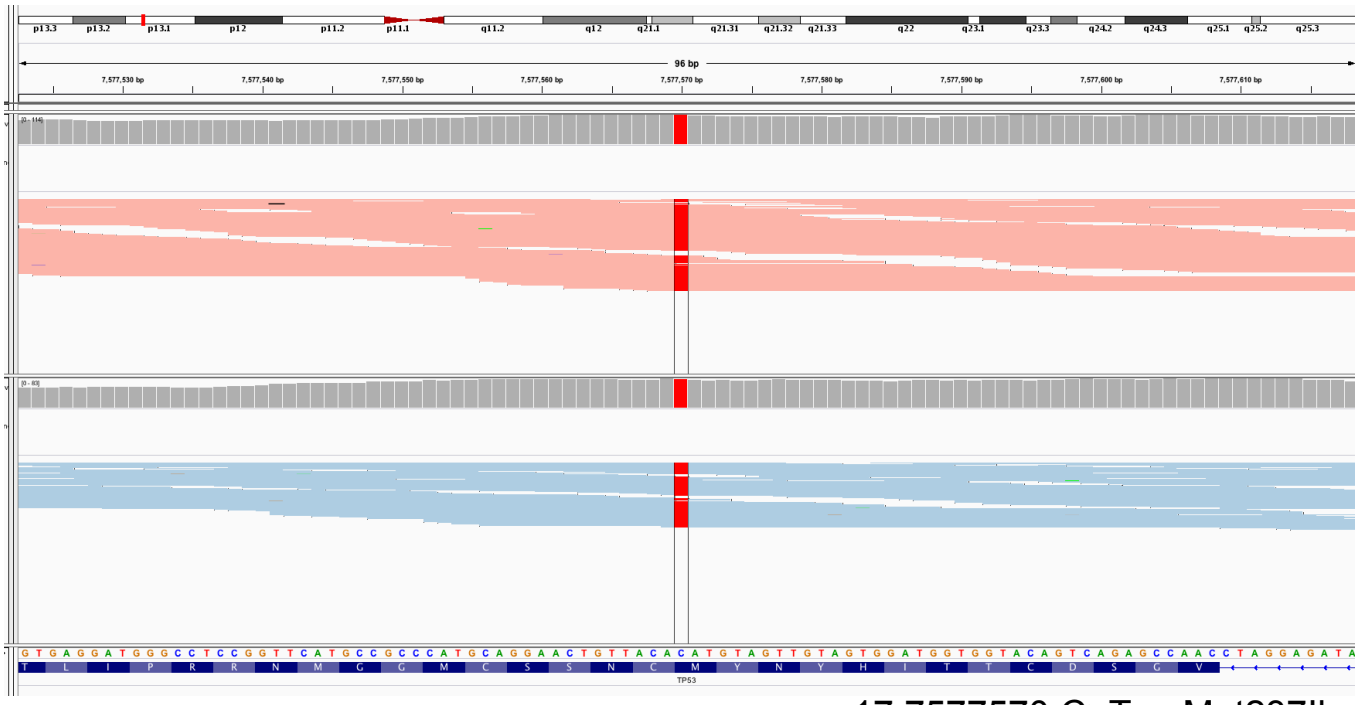
CAPAN-1 (parental)
CAPAN-1.B2.S*

17:7579801 G>C Retained intron (dbSNP:rs1642785)

Supplementary Figure 7. Exome sequencing of CAPAN-1.B2.S* show retention of TP53 mutations.
A-D. Exome sequencing confirms CAPAN1.B2.S* cells retained same *TP53* mutation **(A)** and variants **(B-D)** as observed in the CAPAN1 parental cell line.

Supplementary Figure 8

A

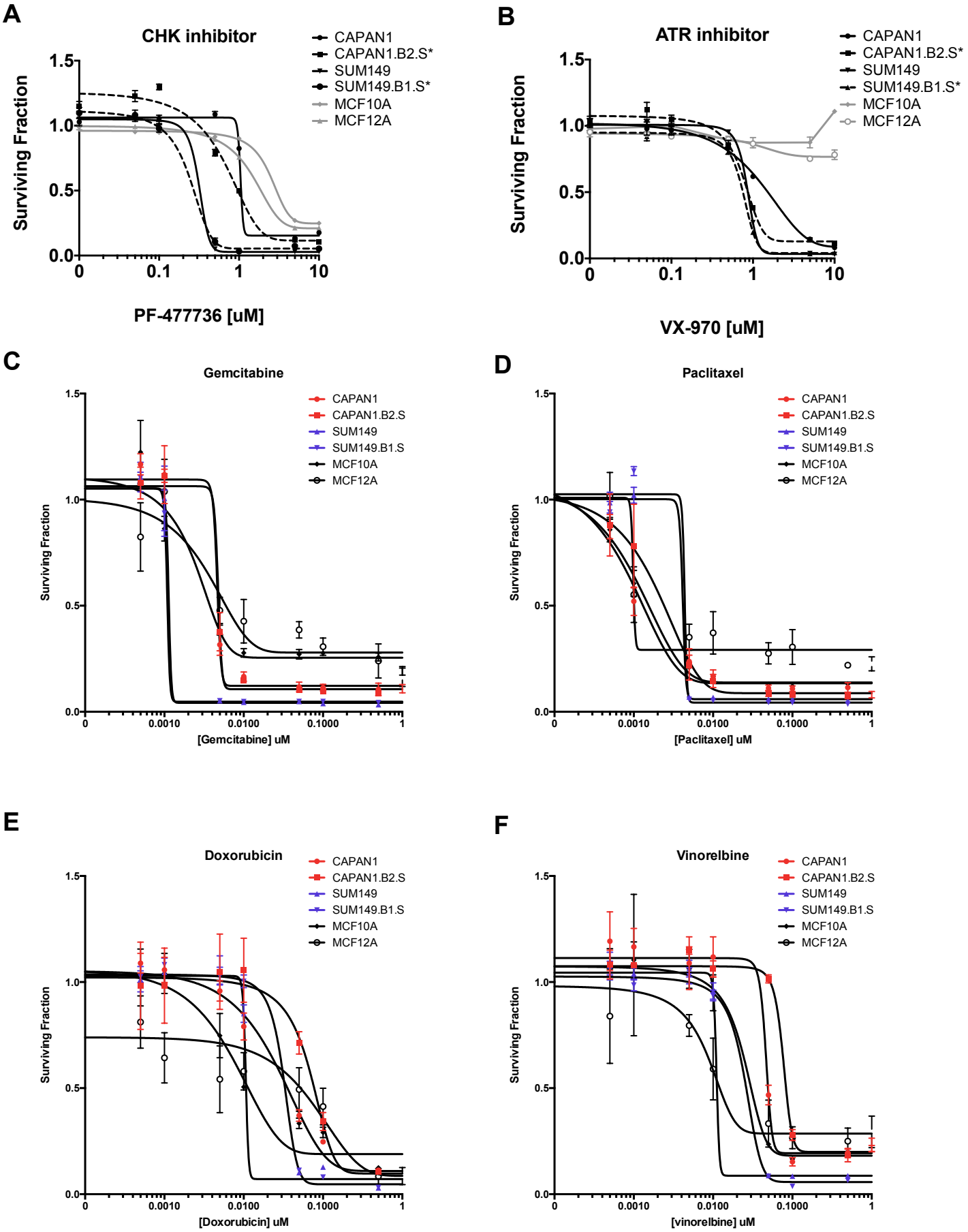


17:7577570 C>T p.Met237Ile

SUM149 (parental)
SUM149.B1.S*

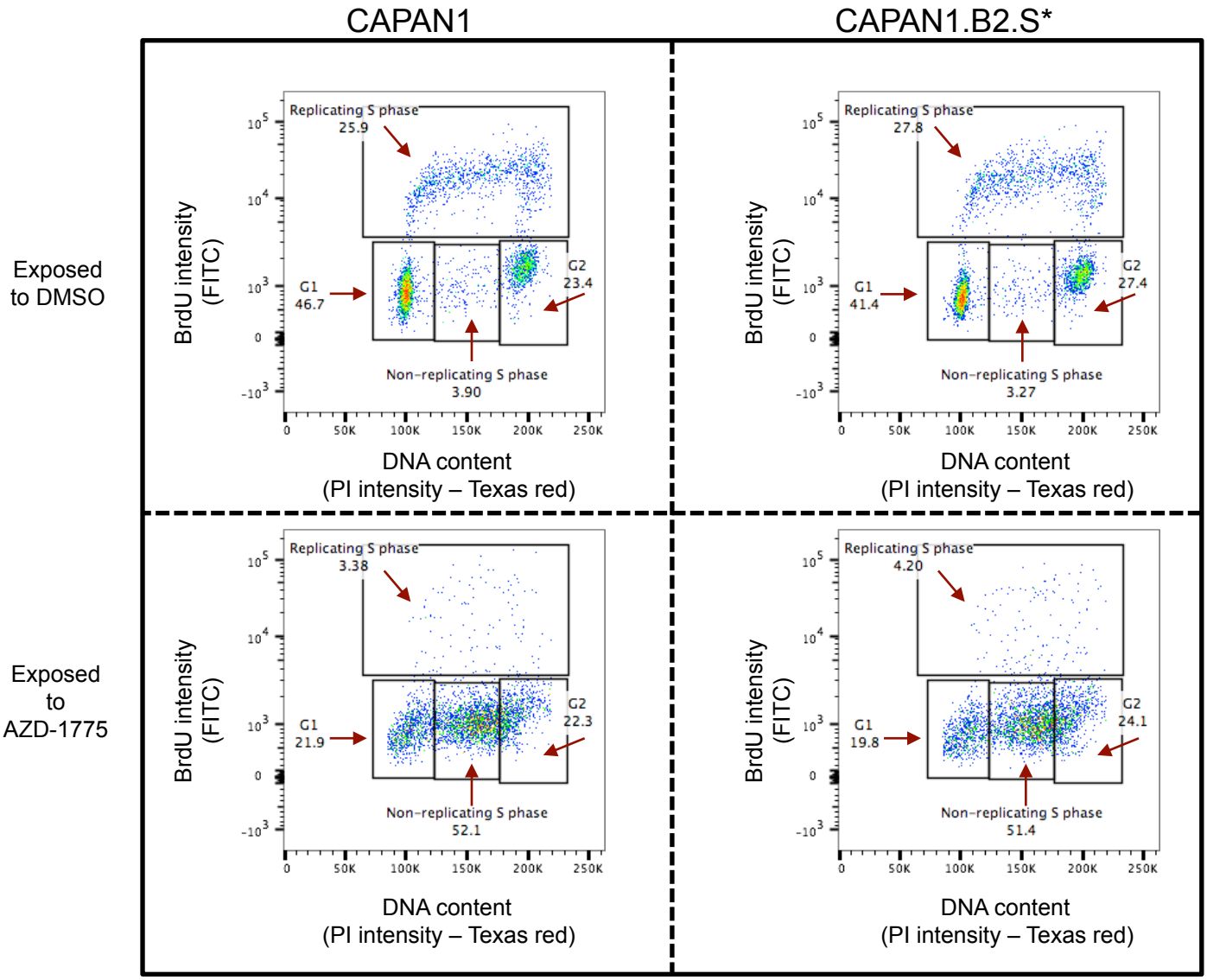
Supplementary Figure 8. Exome sequencing of SUM149.B1.S* show retention of TP53 mutation.
A. Exome sequencing confirms SUM149.B1.S* cells retained the same *TP53* mutation as observed in the SUM149 parental cell line.

Supplementary Figure 9



Supplementary Figure 9. BRCA-proficient and -deficient cells exhibit sensitivity to additional DNA damaging agents.
A-B. Five day dose-response survival curves for **(A)** PF-477736, **(B)** VX-970, **(C)** Gemcitabine, **(D)** Paclitaxel, **(E)** Doxorubicin and **(F)** Vinorelbine for CAPAN1, CAPAN1.B2.S*, SUM149 or SUM149.B1.S* compared to MCF-10A and MCF-12A cell lines. Error bars represent SEM from triplicate experiments.

Supplementary Figure 10



Supplementary Figure 10. AZD-1775 causes an active S phase reduction in both CAPAN1 and CAPAN-1.B2.S* cells.
BrdU and propidium iodide (PI) FACS profiling plots are shown with the fraction of cells in each cell cycle phase indicated. CAPAN1 and CAPAN1.B2.S* cells were exposed to 1 μM AZD-1775, or DMSO for 72 hours. Following this, the cell cycle distribution of the cells was assayed by BrdU/PI FACS analysis as shown.