

Supplementary Figures

Supplementary Figure Legends

Supplementary Figure 1. A, B. Bar charts showing effect of siATR SMARTpool and individual ATR siRNAs on cell survival in two SS cell lines. Error bars represent standard deviation from triplicate experiments. *p* value calculated by Student's *t* test. **C.** Western blot illustrating ATR silencing 48 hours after transfection with siRNAs targeting *ATR*. Effects of SMARTpool and the four different constituent siRNAs are shown. **D.** Dose-response curves showing resistance of non-tumour cell lines (C2C12, MCF10A and HFF1) to VX970 in five-day survival assays. Error bars represent SD from triplicate experiments. **E.** Scatter dot plots showing SF₅₀ values for SS tumour cell lines screened for sensitivity to VX970 in five-day survival assays compared to non-SS tumour cells ("other"), ATM defective, ARID1A defective or Ewing's sarcoma (EWS) tumour cell lines. *p*-values represent Mann-Whitney test. Error bars represent standard deviation. **F-H.** Dose-response curves showing sensitivity of SS tumour cell lines to AZD6738, AZ20 and VE821 ATR inhibitors in five-day survival assays. HCT116 cells were used as a negative control for resistance to ATR inhibitors. *p*-values represent 2-way ANOVA compared to HCT116 cells. Error bars represent SD from triplicate experiments.

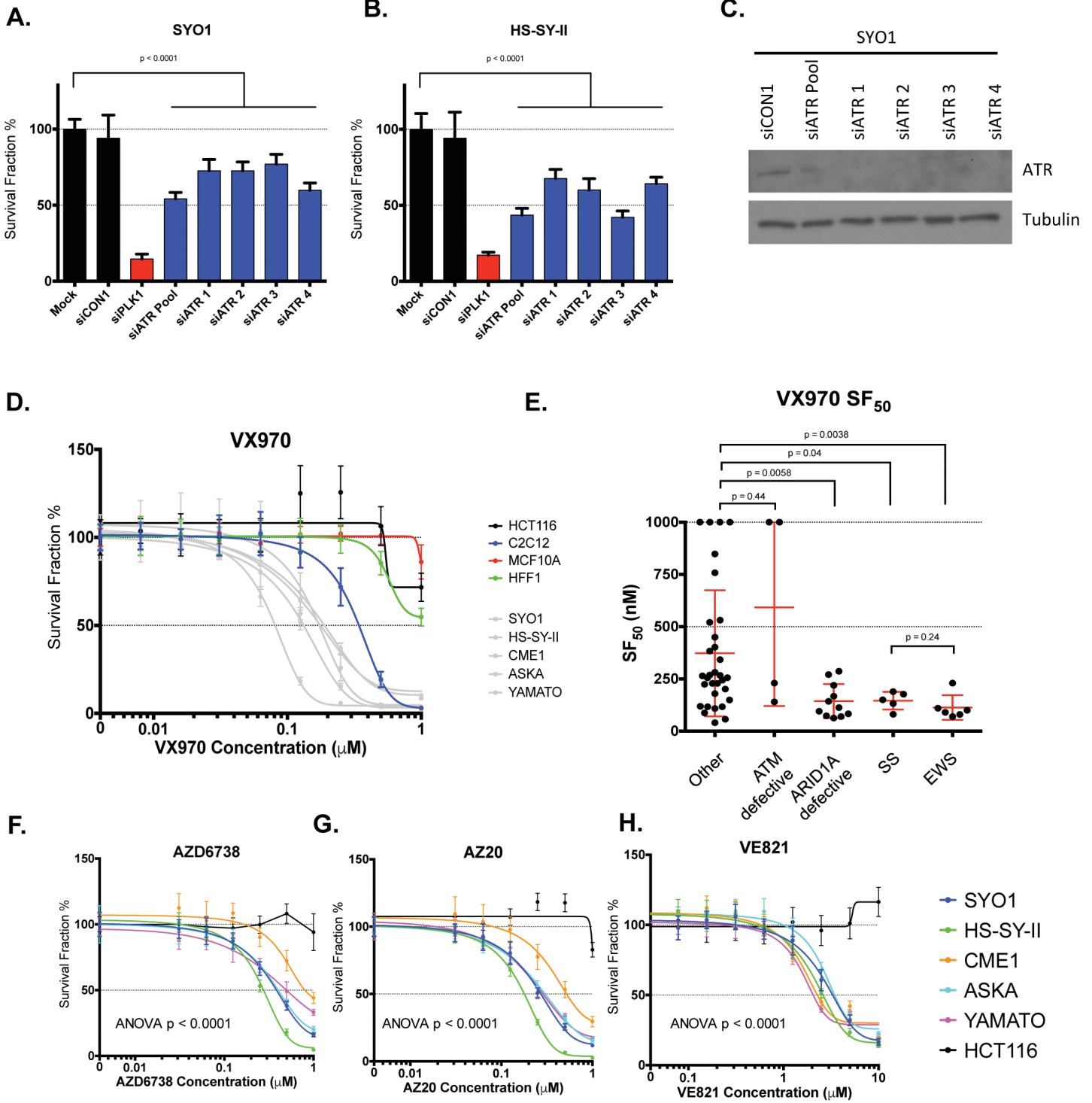
Supplementary Figure 2. A. Bar chart illustrating effect of SS18-SSX fusions on viability of HCT116 cells over six days. Error bars represent standard deviation from triplicate experiments. **B.** Bar chart illustrating effect of SS18-SSX on viability of HFF1 cells over six days. Error bars represent standard deviation. *p*-values calculated using Student's *t*-test. **C.** Western blot illustrating SS18-SSX expression and effect on SMARCB1 in HFF1 cells. **D.** Western blot illustrating SS18-SSX expression and effect on SMARCB1 in U2OS cells. **E.** Dose-response curves illustrating effect of SS18-SSX expression on sensitivity of U2OS cells to VX970. *p*-values calculated by 2-way ANOVA. Error bars represent SD from triplicate experiments. **F-G.** Gene silencing of *SMARCB1* causes ATR inhibitor sensitivity in HCT116 cells. HCT116 cells were reverse transfected with *ARID1A*, *SMARCB1* or non-targeting control siRNA. Twenty-four hours after transfection, cells were exposed to VX970 for five continuous days at which point cell viability was estimated by CellTiterGlo reagent. **F.** Dose response curves from three independent experiments. Error bars represent standard error of the mean. **G.** Western blots of lysates isolated from cells 24 hours after reverse transfection with siRNA.

Supplementary Figure 3. A. Western blots illustrating phosphorylation of CHK1 following exposure to 2 mM hydroxyurea (HU) for 4 hours. **B.** Western blots illustrating phosphorylation of CHK1 and H2AX following exposure to 5 μ M cisplatin for 24 hours. **C.** Western blot illustrating phosphorylation of ATM and CHK2 4 hours after exposure to 5 Gy ionising radiation (IR). **D.** Bar chart illustrating percentage of SYO-1 cells with >5 nuclear foci RAD51 foci in the presence of 500 nM VX970, 2 mM hydroxyurea (HU), or 4 hours after exposure to 10 Gy IR. 100 cells were counted and scored for >5 foci. All *p*-values were calculated using student's *t*-tests. **E.** Western blots illustrating successful fractionation of the chromatin enriched fraction from HCT116 cells. Tubulin was used as a cytoplasmic control and histone H3 as a chromatin-bound control. G = GIPZ transfected cells, E = empty vector transfected cells, SS1 = SS18-SSX1 cDNA transfected cells, SS2 = SS18-SSX2 cDNA transfected cells. **F.** Western blot illustrating effect of SS18-SSX expression on TOP2A levels in the chromatin fraction of HCT116 cells. **G.** Scatter dot plots showing SF₅₀ values for cell lines screened for BMN673 (talazoparib) sensitivity in five-day cell survival assays. *p*-values calculated using Mann-Whitney test. Error bars represent standard deviation. BRCA = Breast Cancer; EWS = Ewing's sarcoma. **H.** Dose-response curves showing sensitivity of SS tumour cell lines to BMN673 (talazoparib) in five-day survival assays. SUM149 (BRCA1 mutant) and SUM149 R2.5 (BRCA1 revertant mutation) breast cancer cell lines were included as PARP inhibitor-sensitive and PARP inhibitor-resistant controls, respectively. Error bars represent standard error of the mean (SEM) from triplicate experiments. **I-J.** Dose-response curves illustrating effect of SS18-SSX1, SS18-SSX2 and D71-78 fusion expression on sensitivity of HCT116 cells to talazoparib (BMN673) (I) or olaparib (J). Error bars represent standard error of the mean (SEM) from triplicate experiments SYO-1 SS tumour cell line was included as a PARP inhibitor-sensitive SS cell line.

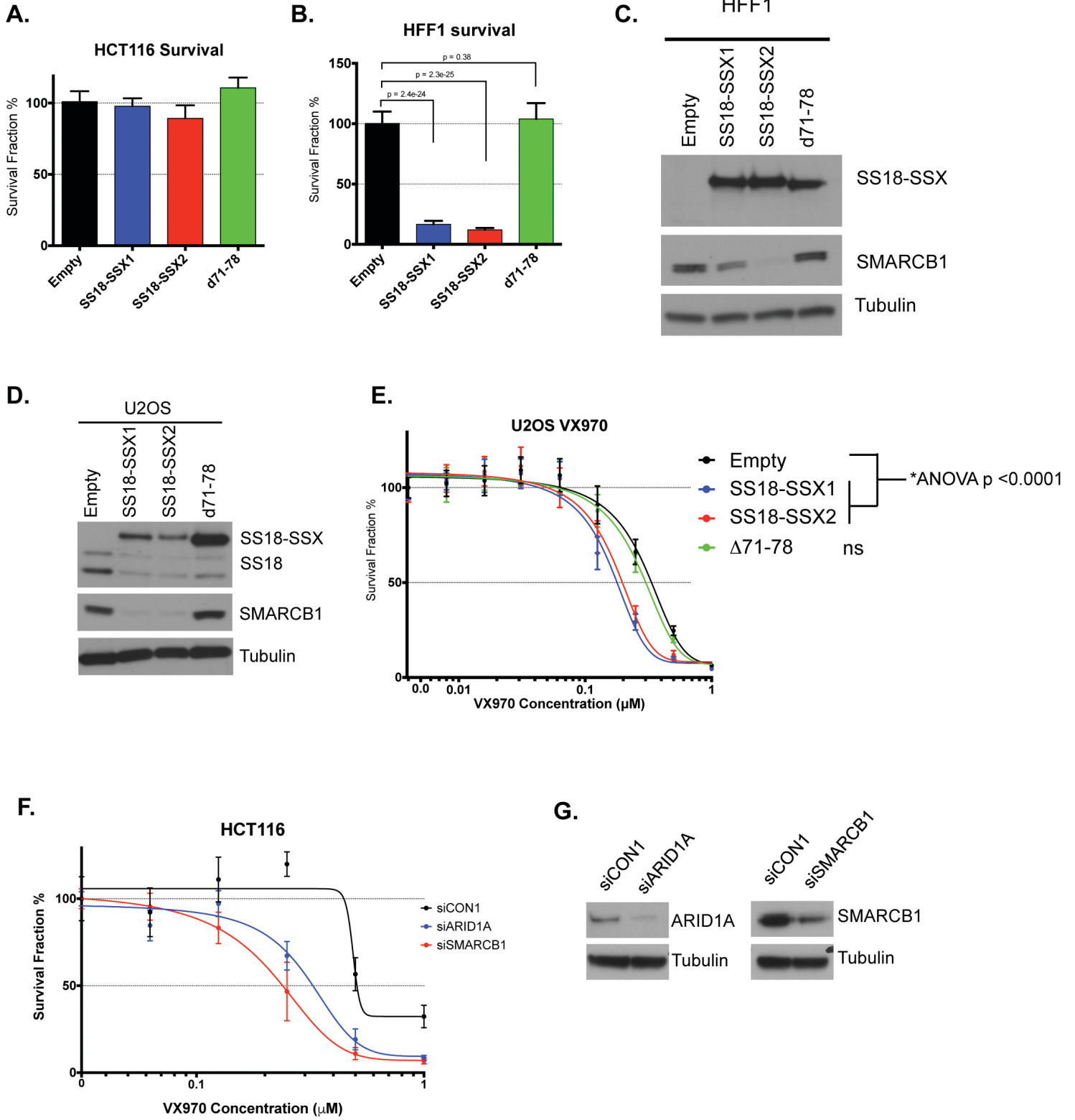
Supplementary Figure 4. A. Fork rates measured in DNA fibres prepared from SYO-1 cells exposed to DMSO for 2 hours, then IdU (30 mins) followed by CldU (30 mins). Example image is shown in lower panel. **B.** Fork rates measured in DNA fibres prepared from SYO-1 cells exposed to 500 nM VX970 for 2 hours, then IdU (30 mins) followed by CldU (30 mins). Example image is shown in lower panel. **C.** Fork rates measured in DNA fibres prepared from HCT116 cells expressing SS18-SSX1 or empty constructs exposed to DMSO or 500 nM VX970 for 2 hours, then IdU (30 mins) followed by CldU (30 mins). At least 100 tracks were measured for each condition. **D.** Quantification of number of SYO-1 cells in S-phase presented in Figure 3J. **E.** Western blot illustrating that ectopic expression of SS18-SSX expression does not alter levels of Cyclin E protein expression. **4F,G.** Cell cycle profiles of SYO-1 SS tumour cells transfected with siAllstar (control, F) or siCCNE1 (G) for 72h. DAPI was used to estimate DNA content and EdU incorporation was used to estimate the S phase fraction. Numbers indicate fraction of cells present in each cell cycle phase.

Supplementary Figure 5. A. MacSynergy plots for SYO-1 and HS-SY-II cells exposed to escalating concentrations of VX970 combined with doxorubicin. 3D synergy plots represent synergy volumes in mM²; volumes > 120 mM² were considered as synergistic (details in Methods). **B.** MacSynergy plots for SYO-1 and HS-SY-II cells exposed to escalating concentrations of VX970 combined with 4-HC (pre-activated cyclophosphamide). **C.** MacSynergy plots for SYO-1 and HS-SY-II cells exposed to escalating doses of VX970 combined with the multi-kinase PDGFR/VEGFR-inhibitor Pazopanib. **D.** Dose-response curves for SYO-1 and HS-SY-II cells exposed to escalating doses of VX970 combined with doxorubicin. Error bars represent standard deviation. ***p* < 0.01; *****p* < 0.0001. **E.** Dose-response curves for SYO-1 and HS-SY-II cells exposed to escalating doses of VX970 combined with the pre-activated derivative of cyclophosphamide, 4-HC. Error bars represent standard deviation. ***p* < 0.01; ****p* < 0.001. **F.** Dose-response curves for SYO-1 and HS-SY-II cells treated with escalating doses of VX970 (0 – 1 mM) combined with the multi-kinase PDGFR/VEGFR-inhibitor Pazopanib (0 – 10 mM). Error bars represent standard deviation.

Supplementary Figure 1.

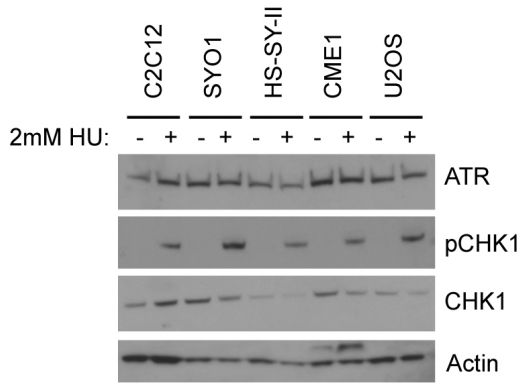


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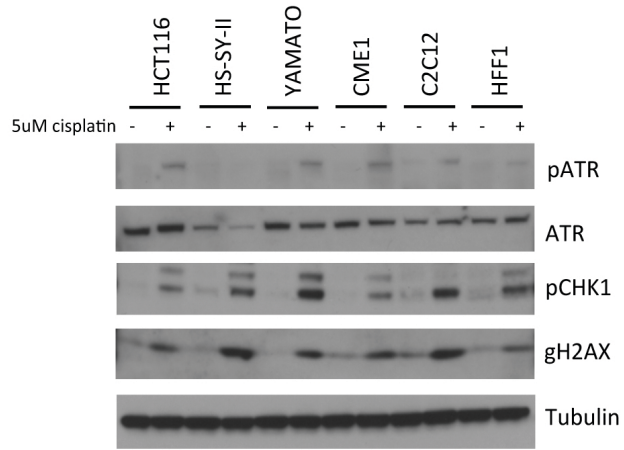


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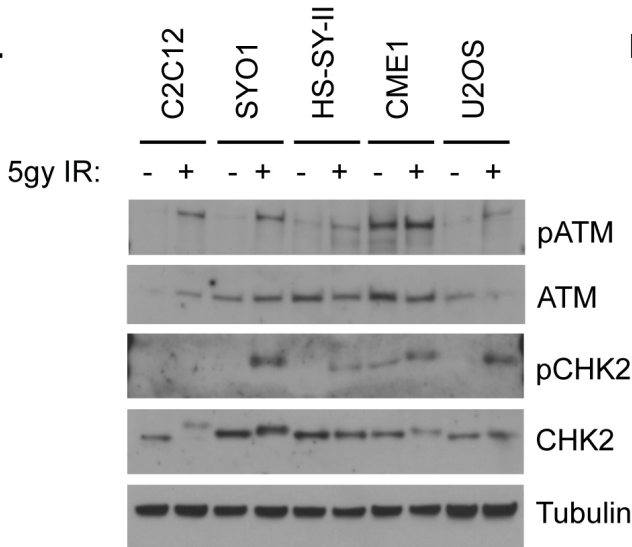
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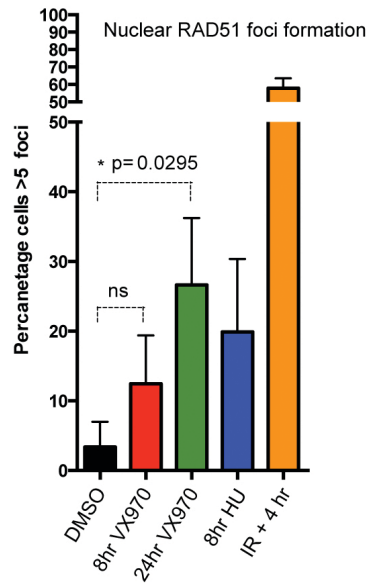
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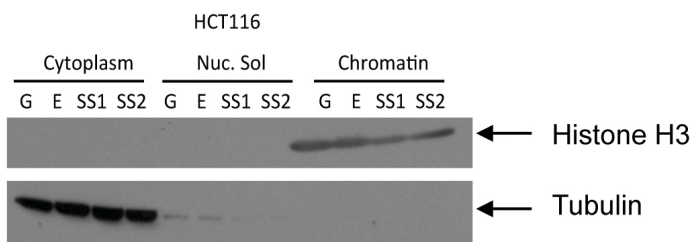
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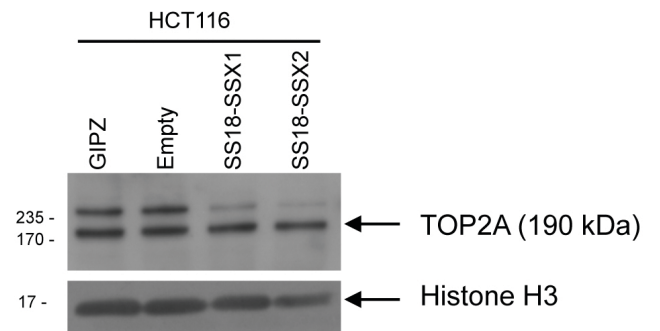
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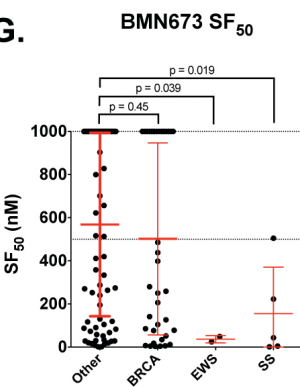
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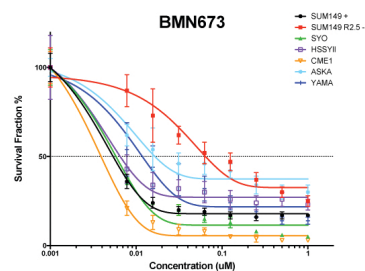
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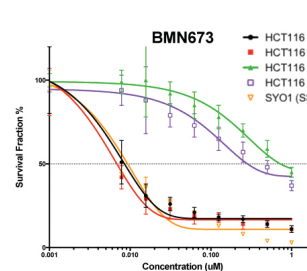
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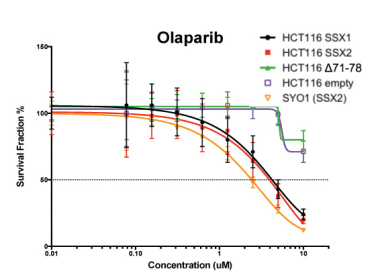
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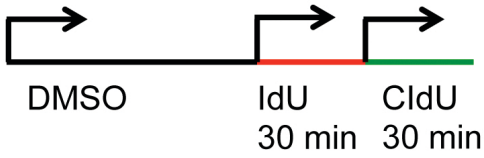


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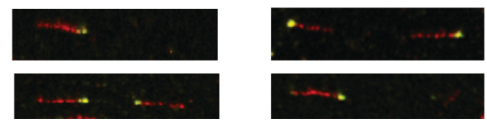
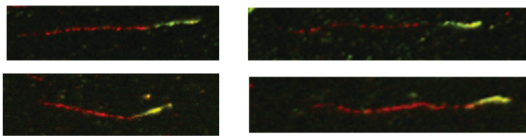
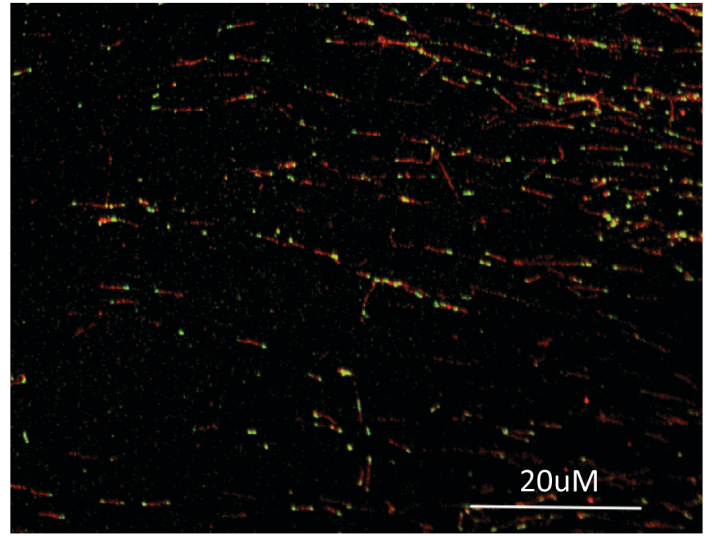
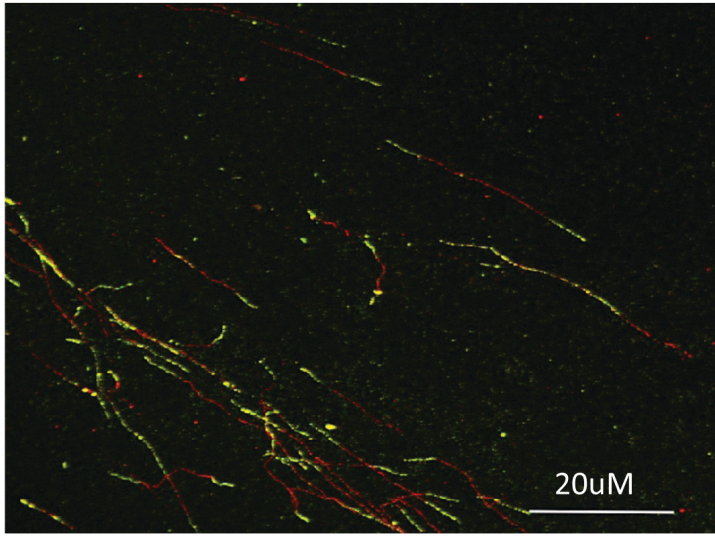
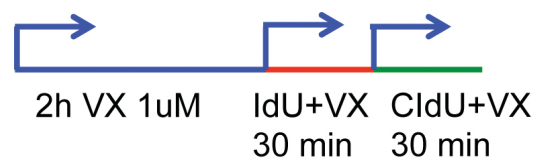


Supplementary Figure 4.

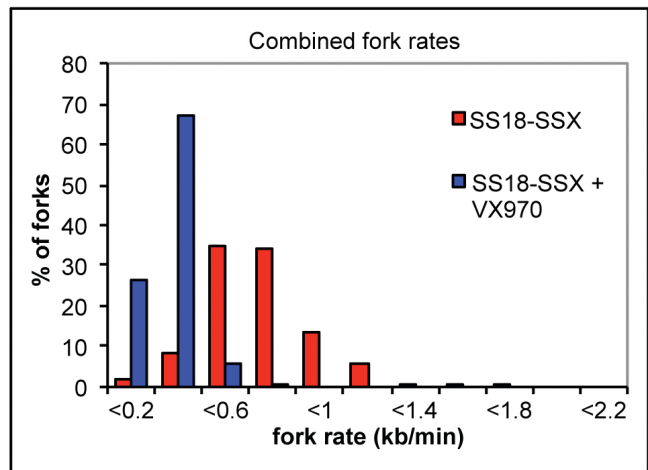
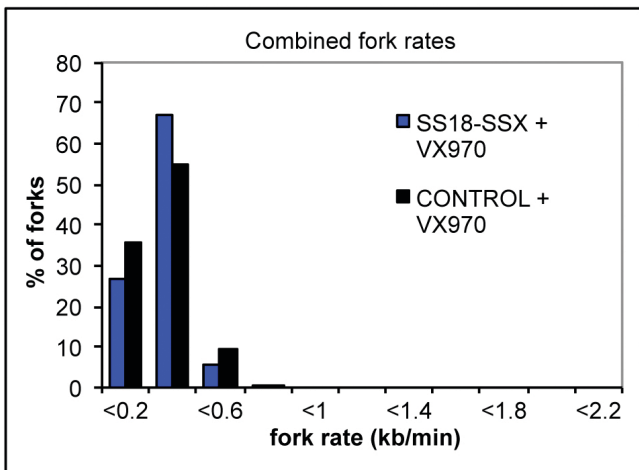
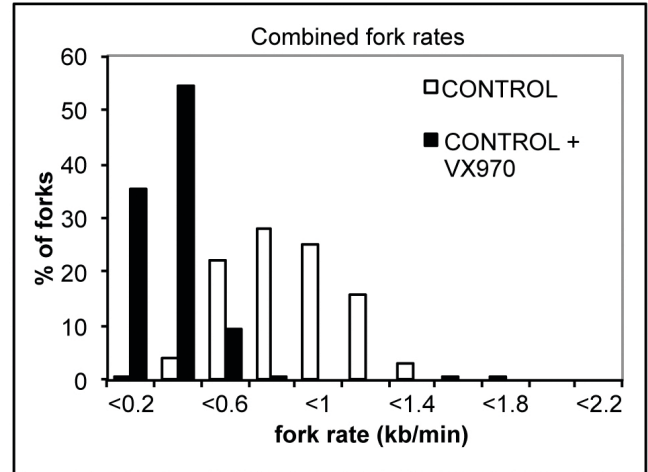
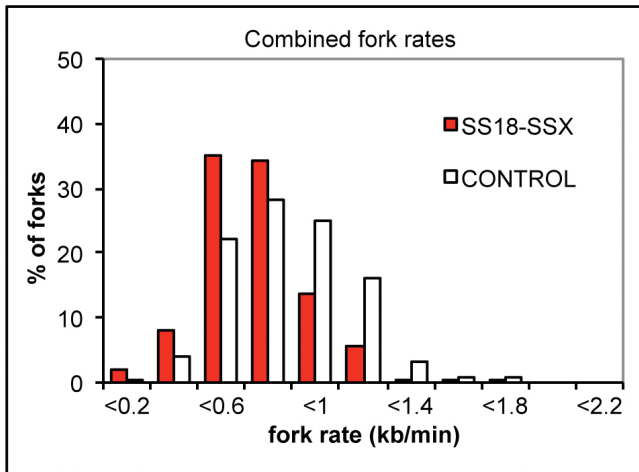
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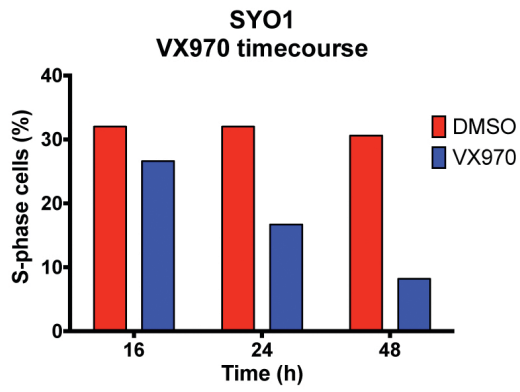


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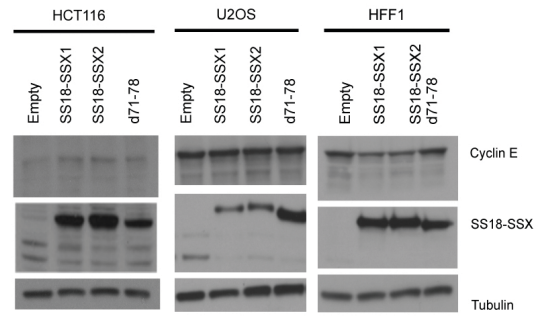


Supplementary Figure 4.
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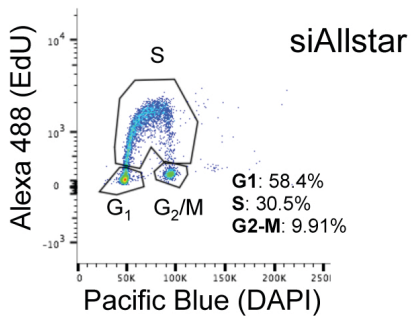
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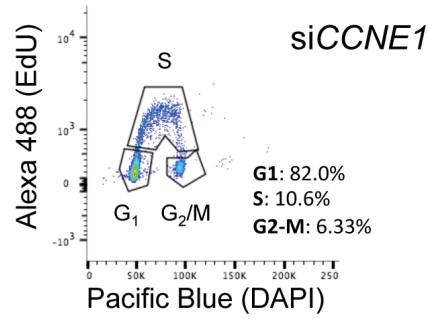
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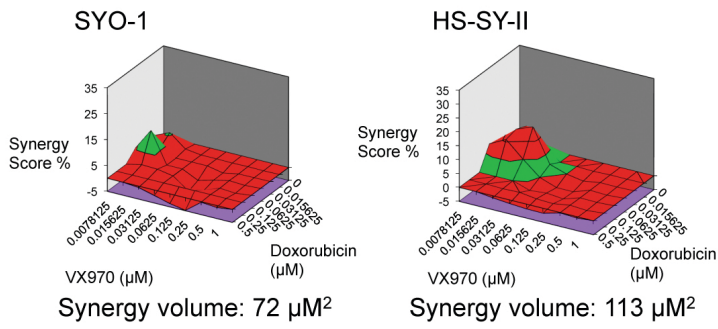


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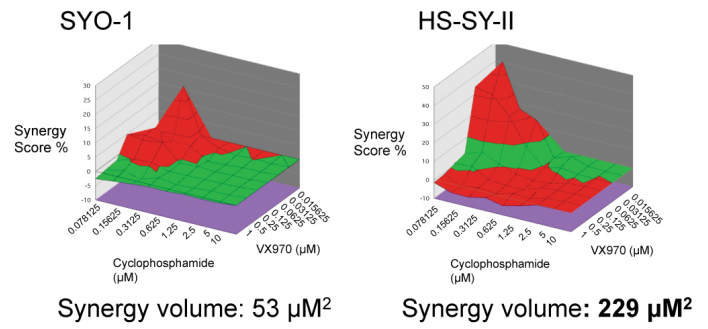


Supplementary Figure 5

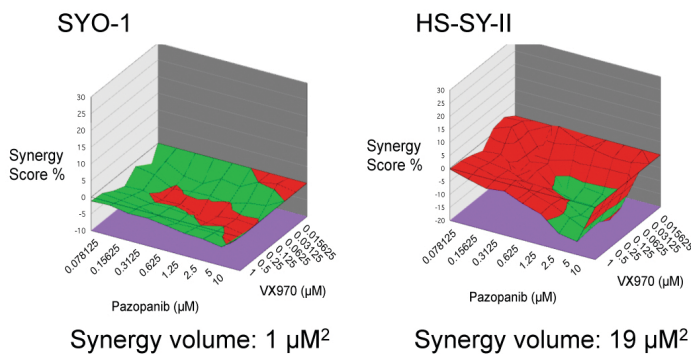
A. VX970 + Doxorubicin



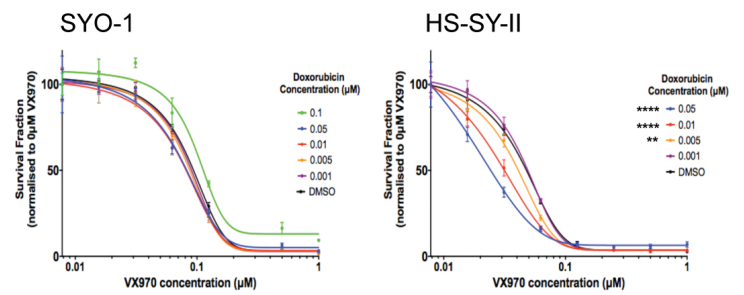
B. VX970 + 4HC



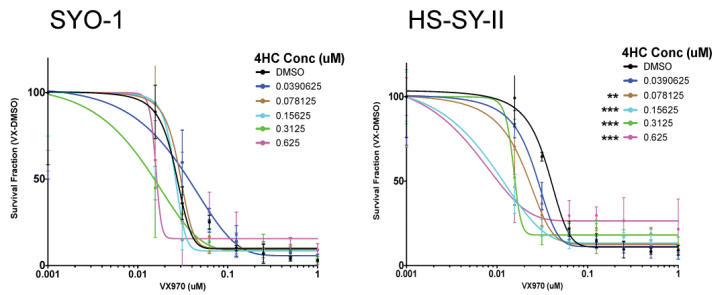
C. VX970 + Pazopanib



D. VX970 + Doxorubicin



E. VX970 + 4HC



F. VX970 + Pazopanib

