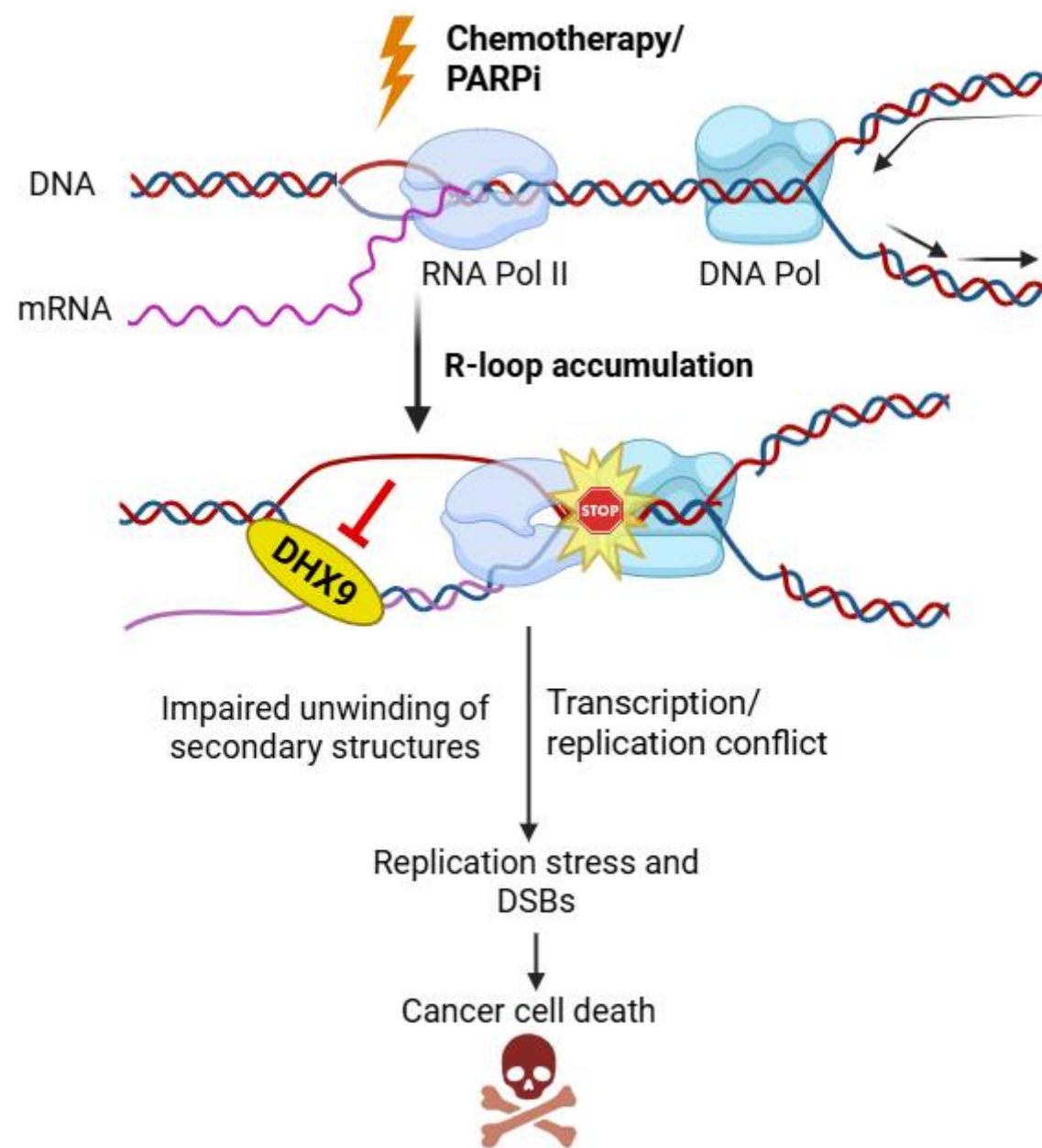


# DHX9 Inhibition is Synthetically Lethal With Homologous Recombination Deficient and dMMR/MSI-H Cancers and Synergizes With PARP1 Inhibition and Chemotherapy

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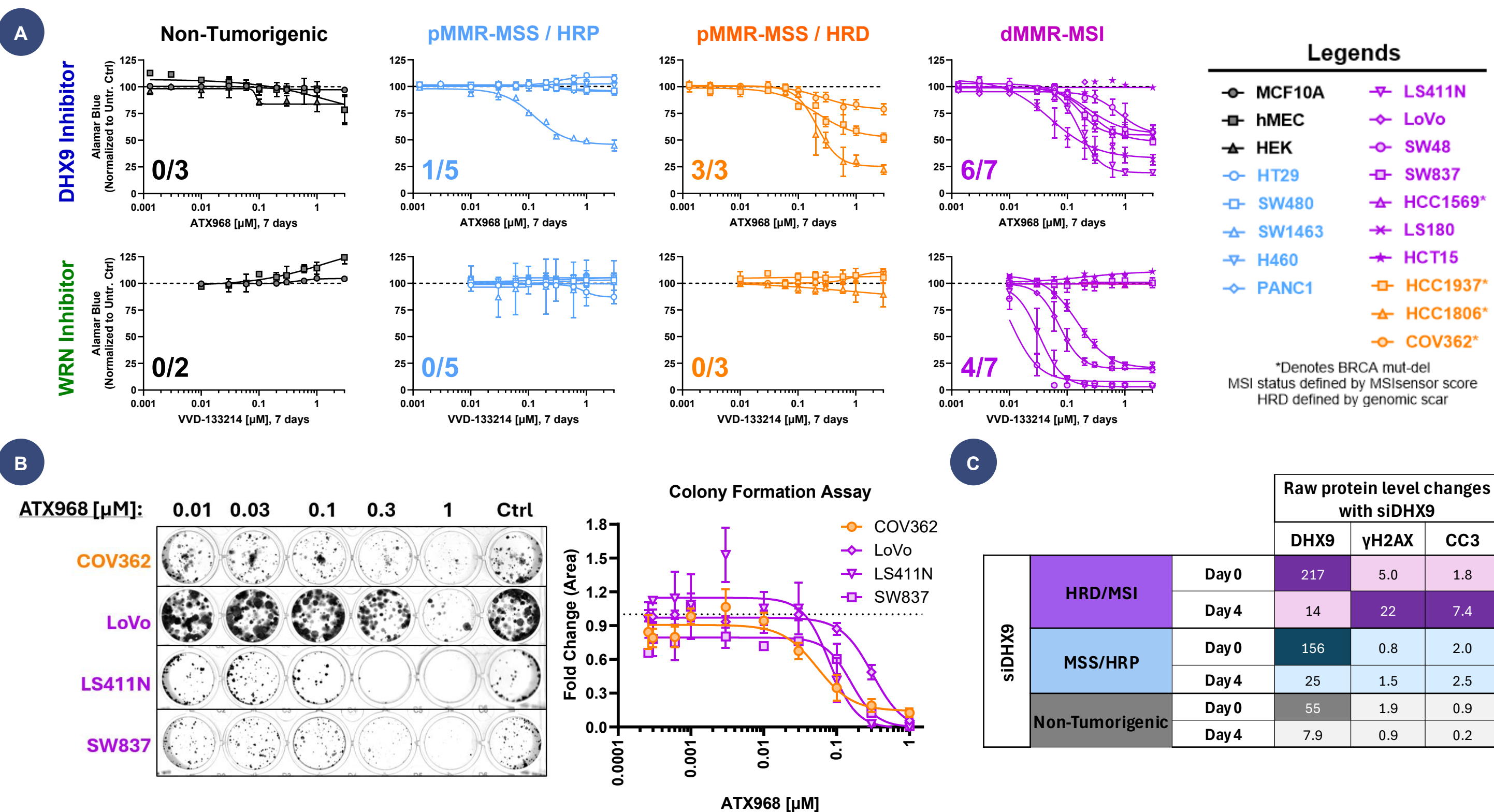
## Background



- ❖ DHX9 is an RNA/DNA helicase that resolves secondary structures, including R-loops and G-quadruplexes. Failure to clear these structures leads to replication stress and increased transcription–replication conflicts<sup>1</sup>
- ❖ HRD and dMMR/MSI-H tumors accumulate higher levels of RNA/DNA secondary structures and are therefore more dependent on DHX9 for maintaining genome stability<sup>2,3</sup>
- ❖ PARP inhibitors are standard treatments for BRCA-mutant and HRD cancers, but have limited depth and durability of response
- ❖ Topoisomerase1 (TOP1) inhibitors are frequently used in dMMR/MSI-H cancers after immunotherapy and in chemotherapy-refractory HRD tumors
- ❖ Both PARP1 and TOP1 participate in pathways that prevent or resolve R-loops, linking their mechanisms to DHX9 biology<sup>4</sup>
- ❖ Inhibiting DHX9 in combination with PARP or TOP1 inhibition will yield deeper, more durable antitumor responses in HRD and dMMR/MSI-H tumors by increasing unresolved RNA/DNA structures and replication stress

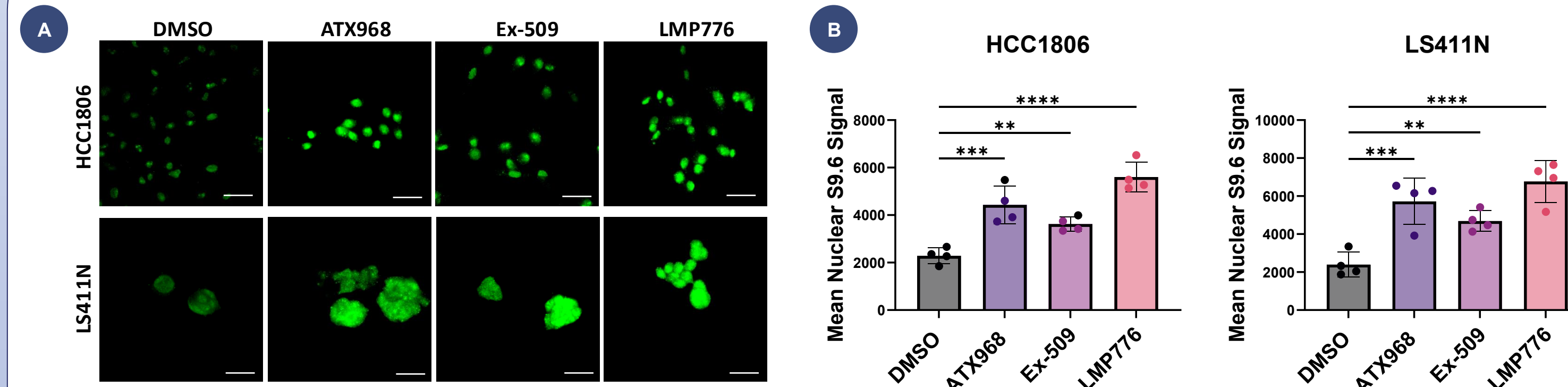
**Figure 1.** DHX9 inhibition combined with chemotherapy or PARP inhibition will increase replication stress and cancer cell death

## DHX9 Inhibition Confers Synthetic Lethality in MSI and HRD Cell Lines



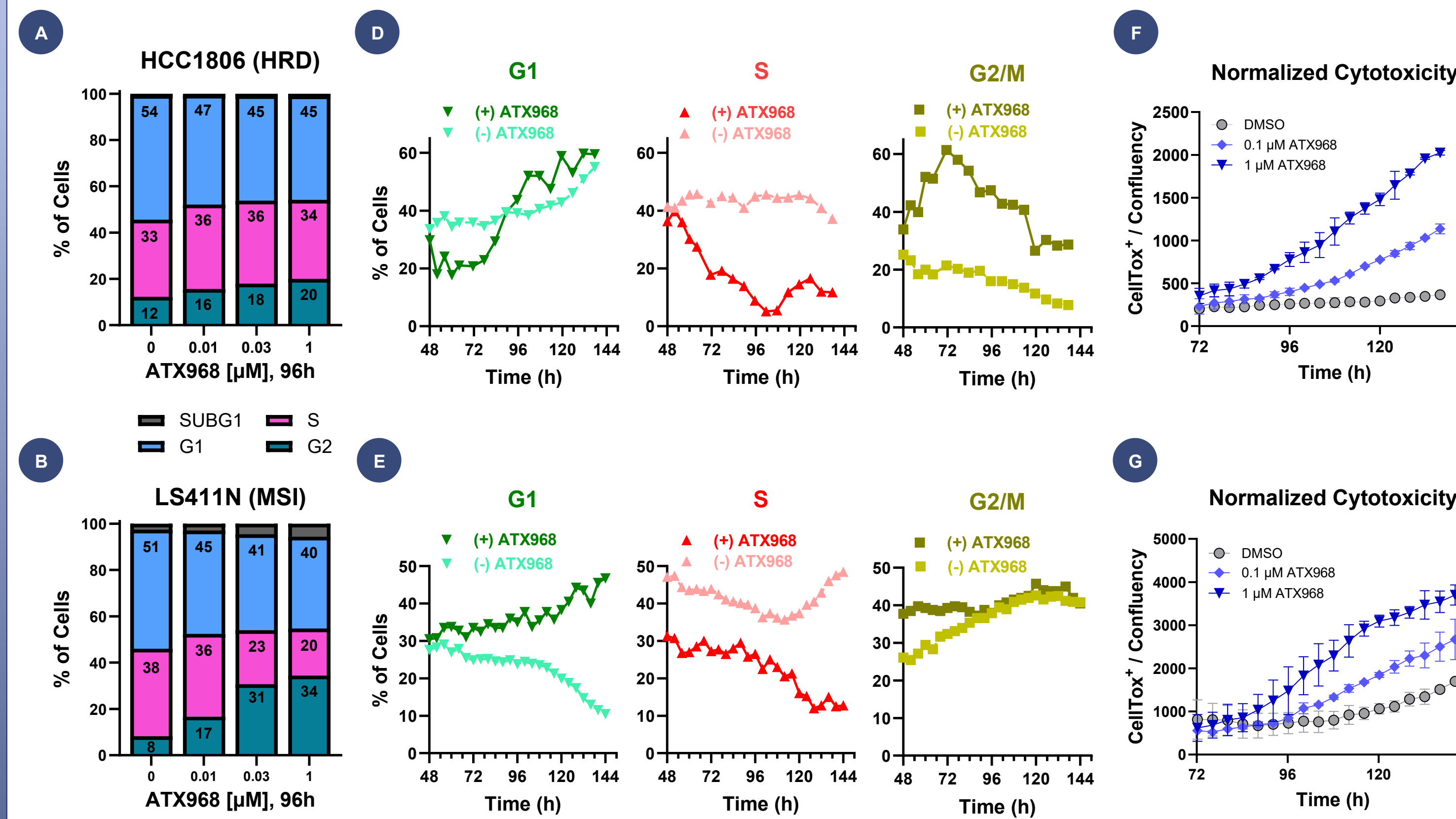
**Figure 2.** DHX9 inhibitors selectively kill MSI and HRD cells sparing MSS/HRP, and non-tumorigenic cell lines  
A. DHX9 inhibition with ATX968 reduces viability in both MSI and HRD cells, whereas WRN inhibition with R0759 targets cells only with high MSI scores and does not impact HRD cells. Similar results were observed with Ex-509 (DHX9i) and HRO761 (WRNi) (data not shown). All inhibitors spare non-tumorigenic and MSS/HRP cells. Cell viability was measured using AlamarBlue after 7 days of treatment. B. Prolonged DHX9 inhibition (14 days) results in complete cell death of HRD and MSI cells in colony-forming assays. C. Knockdown of DHX9 induces accumulation of yH2AX and cleaved caspase-3 (CC3) specifically in HRD/MSI cells, with no impact on HRP and non tumorigenic cells. DHX9, yH2AX and CC3 protein levels were assessed by Western blot following DHX9 knockdown.

## DHX9 Inhibition Induces Rapid Accumulation of R-Loops



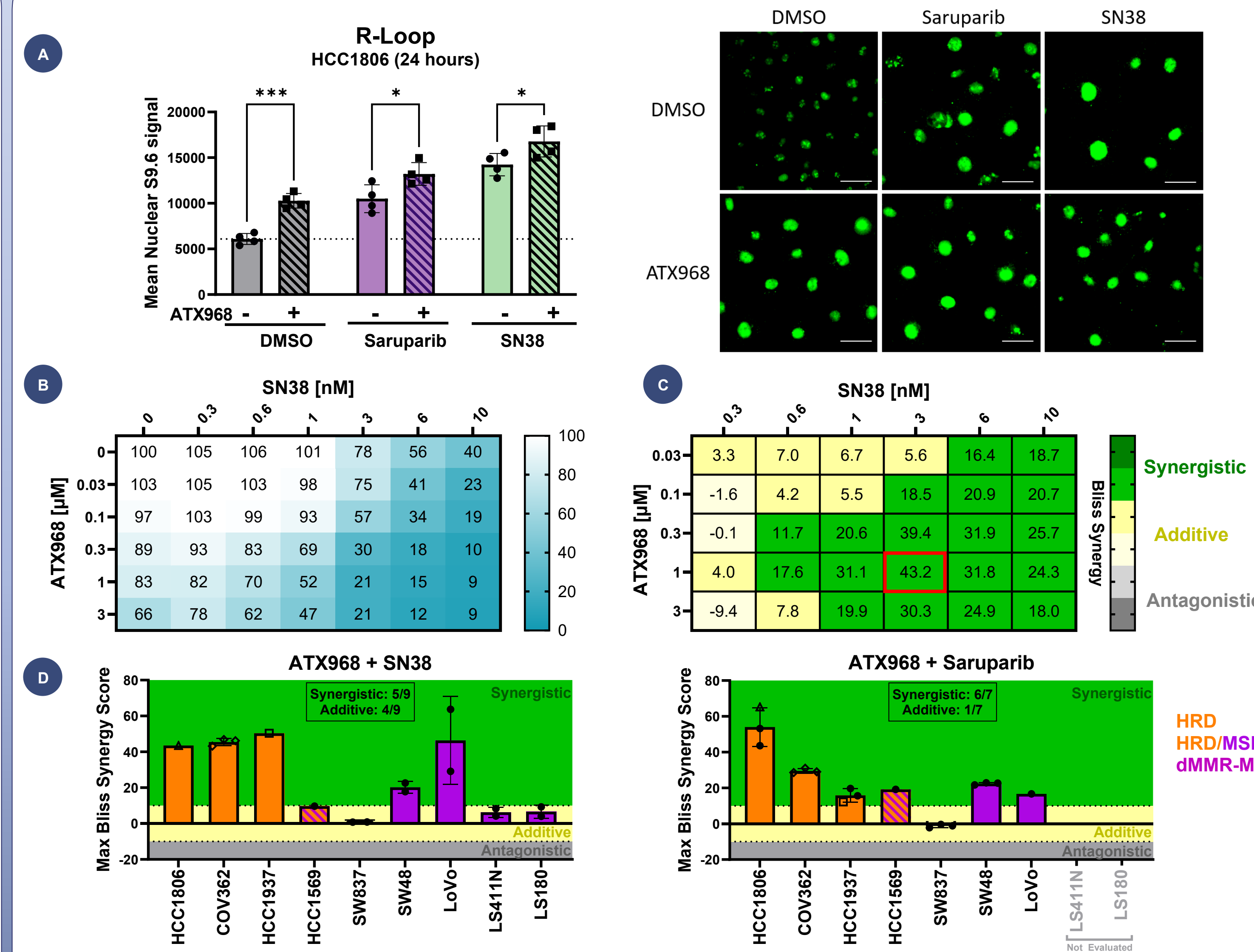
**Figure 3.** DHX9 inhibition with ATX968 or Ex-509 increases R-Loop accumulation in HCC1806 (HRD) and LS411N (MSI) cells  
A. R-loop staining with S9.6 in HCC1806 and LS411N cells after 24 h of DHX9 inhibitor treatment. LMP776 (TOP1 inhibitor) served as a positive control. Nuclei were identified using DAPI, which is not displayed to improve visualization of differences in S9.6 intensity between cells. B. Nuclear S9.6 signal was quantified based on DAPI-defined nuclear masks, excluding cytoplasmic signal. Scale bar: 50 μm.

## DHX9 Inhibition Alters Cell Cycle Progression and Induces Cell Death



**Figure 4.** DHX9 inhibition with ATX968 disrupts cell cycle progression and induces cell death in HRD and MSI cell lines  
A. ATX968 increases the G2 and reduces the G1 population in HCC1806 cells. Cells in S-phase were identified by EdU incorporation; DNA content was used to define cells in G1 and G2/M phases. B. In LS411N cells, ATX968 increases G2/M and decreases both the S-phase and G1 populations. Sub-G1 cells increase with ATX968 concentration dependently. C. Illustration of PIP-FUCCI reporter system<sup>5</sup>. D. PIP-FUCCI expressing cells demonstrate time-dependent shifts in cell-cycle distribution following DHX9 inhibition in HCC1806 cells E. Similar data is observed with LS411N cells. F. ATX968 induces cell death in a concentration- and time-dependent manner in HCC1806 and G. LS411N cells. Percent cell death was calculated by normalizing CellTox-positive cells to confluency.

## DHX9 Inhibition Synergizes With SN38 and Saruparib (PARP1i)



**Figure 5.** DHX9 inhibition in combination with Saruparib or SN38 enhances R-Loop accumulation and demonstrates synergy  
A. Single-agent treatment with ATX968, Saruparib, or SN38 increases R-loop accumulation, which is further enhanced by combining Saruparib or SN38 with ATX968 in HCC1806 cells. Right panel shows representative images of R-Loop staining with S9.6. Scale bar: 50 μm. B. Viability and C. Bliss Synergy Scores demonstrate that combined inhibition of DHX9 with SN38 is synergistic in COV362 cells. Red squares denote the maximum bliss synergy score. D. Inhibition of DHX9 in combination with SN38 or Saruparib were evaluated in multiple cell lines, and the maximum Bliss Scores were plotted. Combinations in all cell lines were either synergistic or additive.

## Conclusions

- ❖ DHX9 inhibition selectively kills MSI and HRD cells while sparing MSS/HRP and non-tumorigenic cells
  - WRN inhibition targets only cells with high MSI scores
- ❖ DHX9 knockdown increases yH2AX and CC3 in MSI/HRD cells, but not in MSS/HRP or non-tumorigenic cells
- ❖ R-loops rapidly accumulate following inhibition of DHX9, inducing replication stress
- ❖ Replication stress mediates cell cycle changes leading to increased cytotoxicity in MSI/HRD cells
- ❖ DHX9 inhibition is synergistic with SN38 and PARP1 inhibition

## References

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