

Abstract

Pancreatic ductal adenocarcinoma (PDAC) is a leading cause of cancer-related mortality and comprises molecular subtypes characterized by MYC amplification (14%) or MYC overexpression (40%), both of which drive tumor proliferation, metabolic reprogramming, and cellular plasticity. MYC dysregulation is associated with poor clinical outcomes and resistance, but its direct targeting remains a challenge. Here, we investigate the response of a patient-derived cancer organoid (PCO) model of PDAC as a function of MYC amplification to evaluate the synthetic lethality of dual transcriptional inhibition through BET inhibition (ZEN3694) and CDK9 inhibition (SLS009).

Mechanism

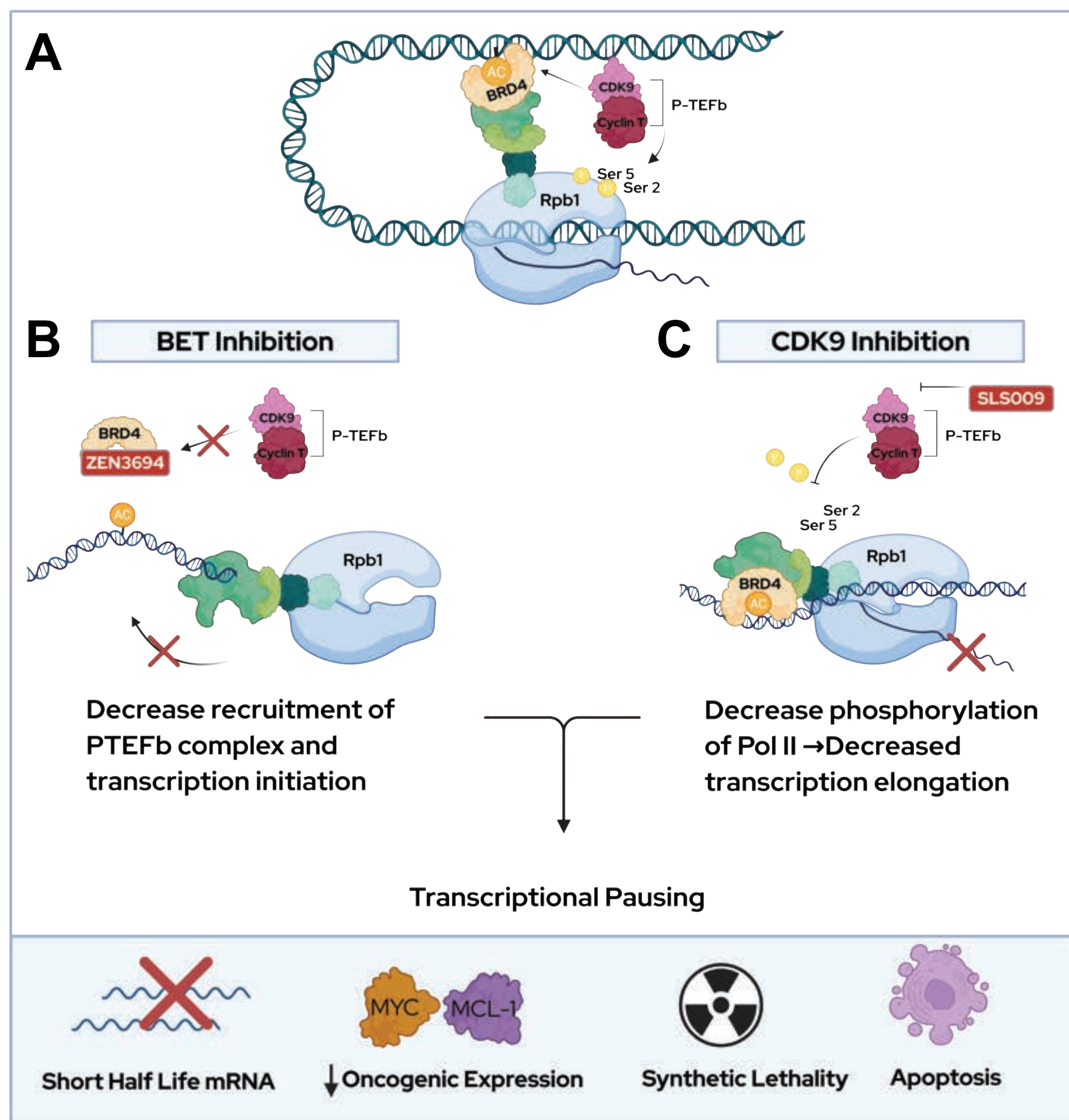


Figure 1. BRD4 and CDK9 roles in transcription. Mechanism of Transcription (A). BET inhibition disrupts BRD4 recruitment and P-TEFb activity, reducing BRD4-dependent transcription (B). CDK9 inhibition decreases RNA Pol II phosphorylation and inhibits transcriptional elongation (C). Dual inhibition synergistically reduces short half-life RNAs and oncogenic protein expression, promoting transcriptional pausing and apoptosis.

Methods

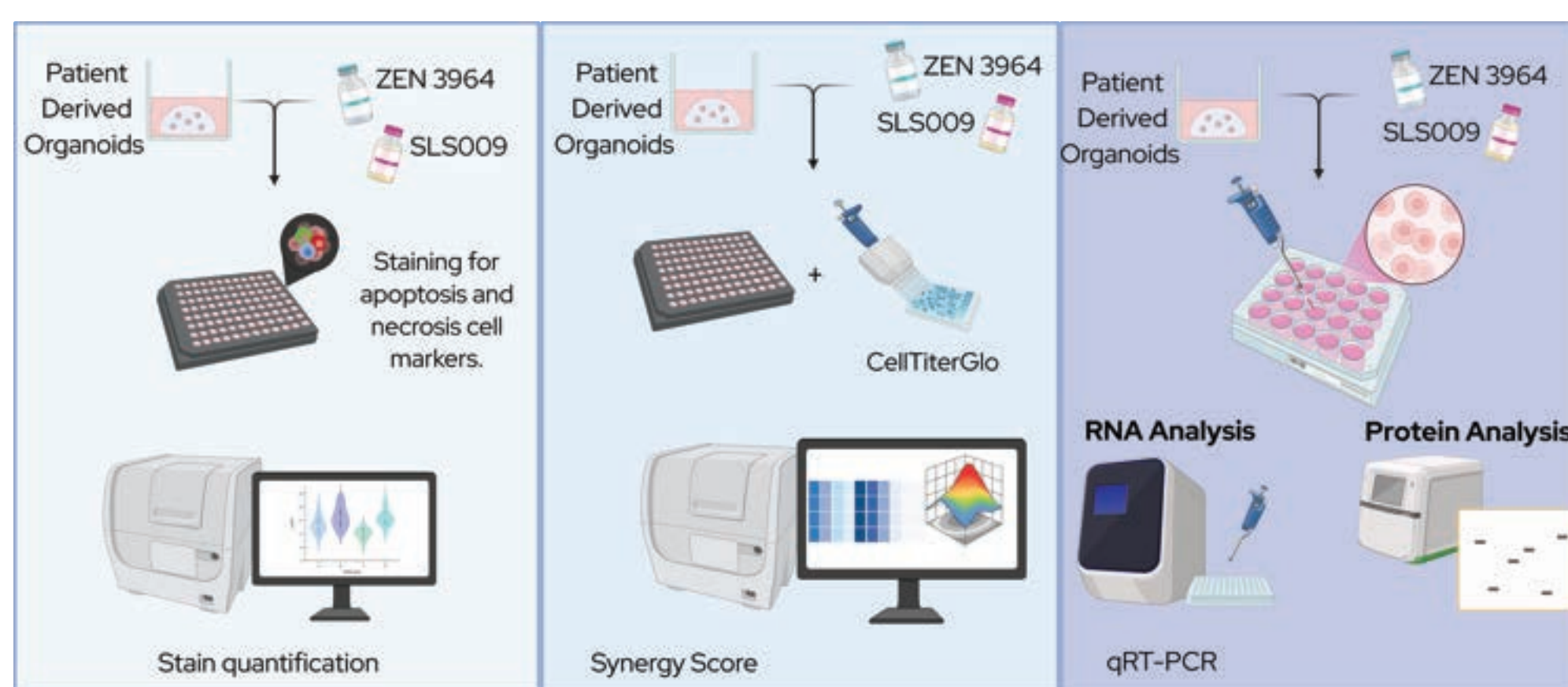
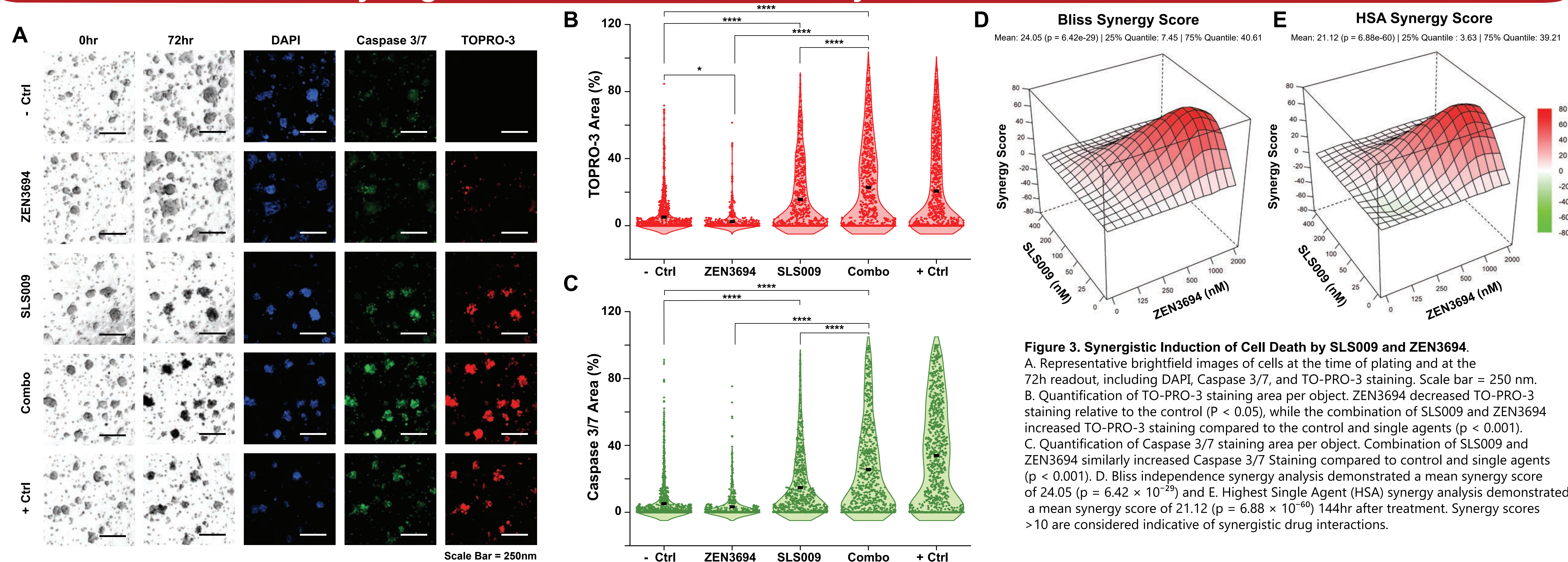
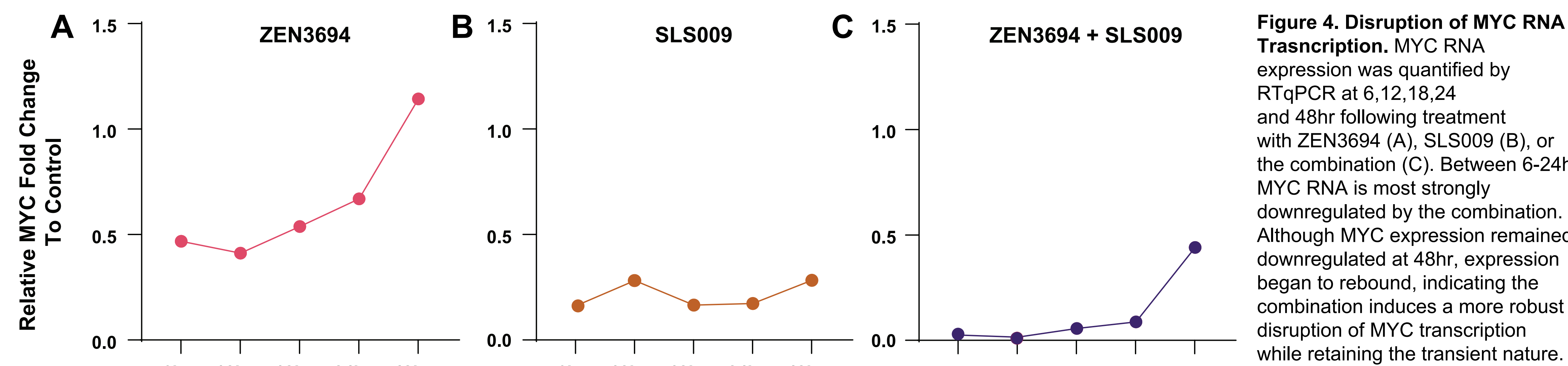


Figure 2. Schematic representation of 3D cell culture assays used. A. MYC-amplified PCO (copy number 35) was treated with ZEN3694 (500 nM), SLS009 (200 nM), or the combination. Apoptosis and necrosis were assessed at 72 h using caspase-3/7 and TO-PRO staining. Dose-response combination matrices were generated, and viability was measured at 144 h using 3D CellTiter-Glo (CTG, 50% v/v). RNA was isolated over a time course to assess MYC expression by qPCR. Protein was isolated at 24 h to evaluate MYC and MCL-1 expression, as well as RNA Pol II phosphorylation.

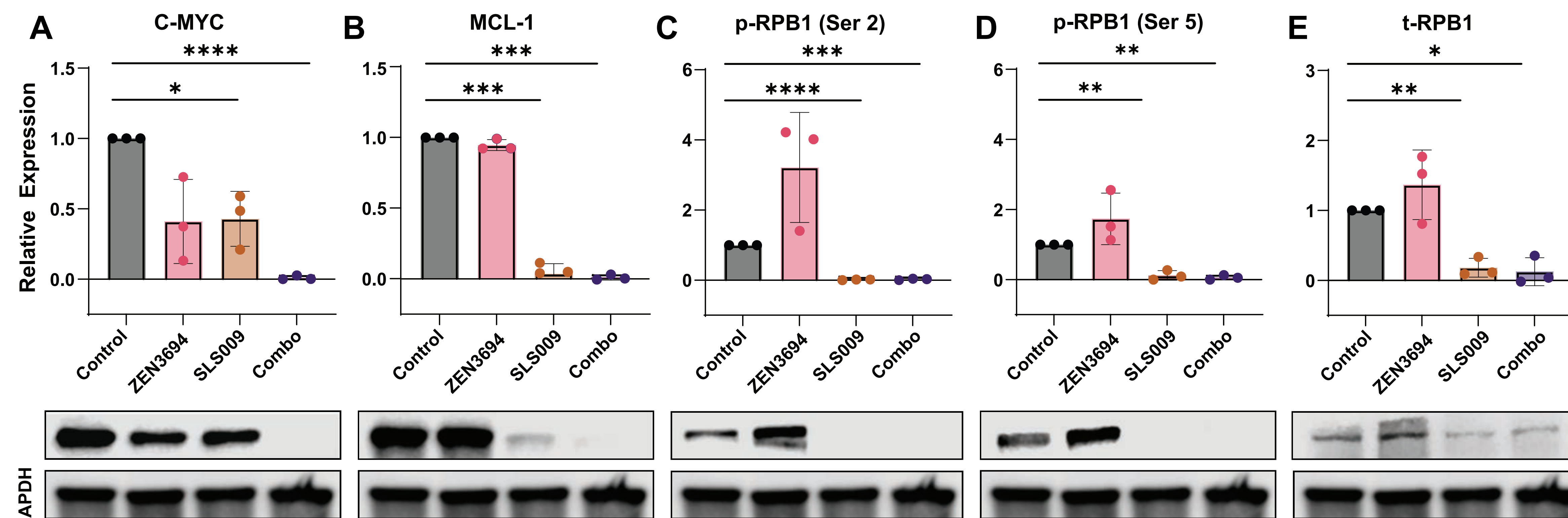
Synergistic Induction of Cell Death By BET and CDK9 Inhibition



Timecourse Analysis of MYC RNA Expression



Oncogene Expression and CDK9 Activity



Conclusions

This study demonstrates that dual BET and CDK9 inhibition with ZEN3694 and SLS009 induces greater apoptosis and necrosis than either agent alone, with synergistic activity. The combination sustained suppression of MYC RNA compared to single agents, suggesting prolonged transcriptional disruption. Protein analysis further showed decreased expression of the short-lived proteins MYC and MCL-1, consistent with transcriptional disruption. The combination also demonstrated on-target activity through decreased RNA Pol II phosphorylation. Notably, these effects were achieved using ZEN3694 at a concentration substantially below reported physiological levels, suggesting that CDK9i allows more tolerable BET inhibitor doses while enhancing antitumor activity.

Future Directions

Ongoing studies are evaluating this combination in additional PCO models with diverse mutational and treatment backgrounds to determine whether MYC non-amplified models may derive greater benefit from dual BET and CDK9 inhibition. Given the moderate sensitivity of the MYC-amplified model to each single agent, these studies will investigate whether reduced sensitivity to individual agents in MYC non-amplified models results in greater dependence on the combination for antitumor activity. Ongoing studies will validate these findings in vivo and confirm the mechanism of the combination. Additionally, in vivo studies will assess therapeutic efficacy and toxicity.

Acknowledgements & References

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