

Figure S1. Colony formation assays. HuH7 and HLE cells were seeded in 6-well plates and incubated with AZD4573 at the indicated concentration for 10-14 days (*denotes statistical significance compared to treatment with vehicle only).

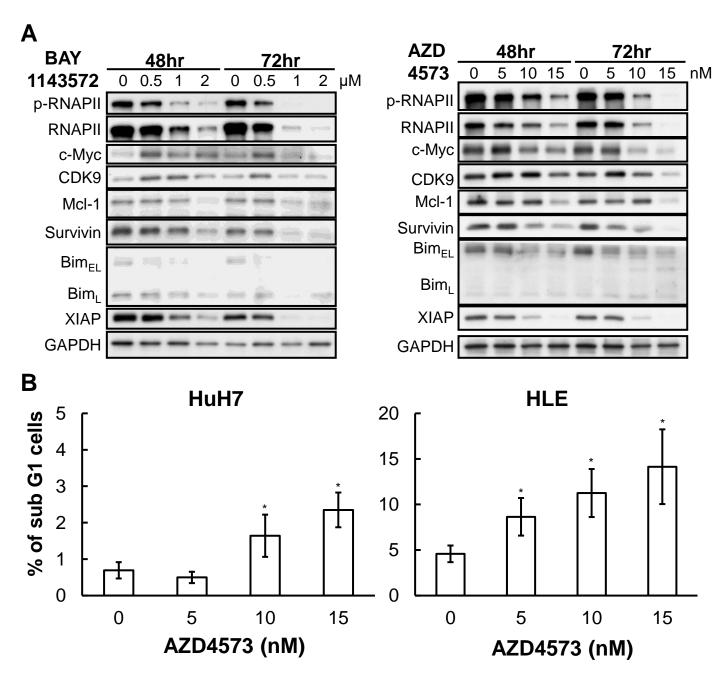


Figure S2. (A)Western blot results indicating the expression and phosphorylation of the downstream targets of CDK9 in HLE cells after treatment with BAY1143572 or AZD4573. The expression of antiapoptotic molecules after treatment with CDK9 inhibitors were also demonstrated. (B) Cell cycle assays indicating the sub-G1 fractions of HuH7 and HLE cells after treatment with AZD4573 for 48 hours. After the indicated treatment, cells were harvested, stained with PI, and then counted according their cell cycle phase using flow cytometry. (RNAPII = RNA polymerase II, XIAP = X-linked inhibitor of apoptosis protein (XIAP)

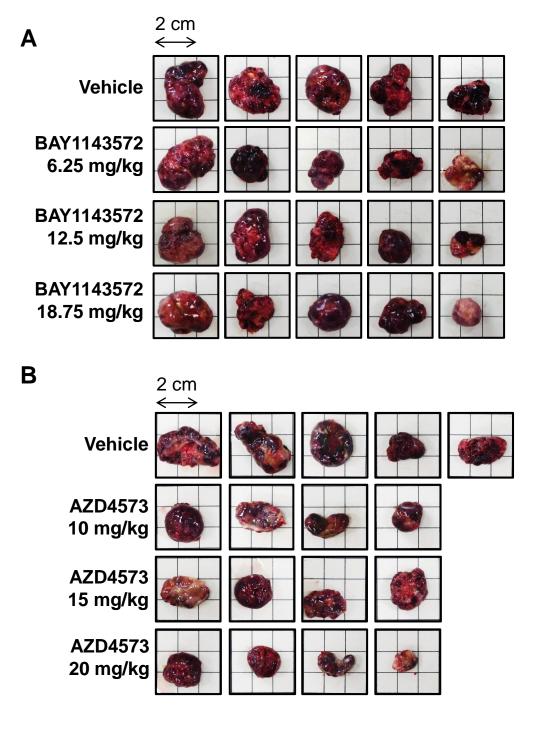


Figure S3. Xenograft studies. Mice were subcutaneously injected with HuH7 cells. (A) Treatment with vehicle (n = 5), BAY1143572 6.25 mg/Kg (n = 5), BAY1143572 12.5 mg/Kg (n = 5), or BAY1143572 18.75 mg/Kg (n = 5) was administered orally once per day, 5 days per week. (B) Treatment with vehicle (n = 5), AZD4573 10 mg/Kg (n = 4), AZD4573 15 mg/Kg (n = 4), or AZD4573 20 mg/Kg (n = 4) was administered via intraperitoneal injection twice per week. After sacrifice, the tumors were harvested and are shown here.

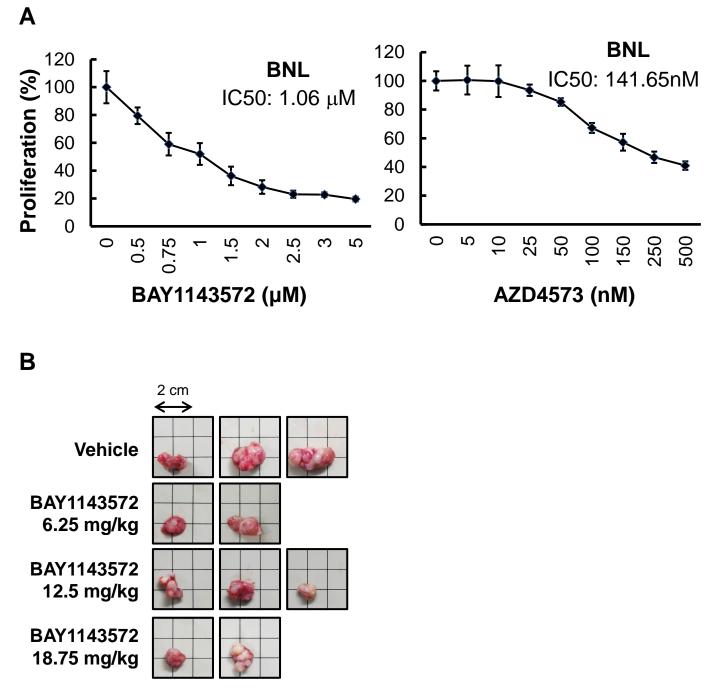


Figure S4. (A) MTT assays examining the viability of the BNL HCC cell line after treatment with BAY1143572 or AZD4573 at the indicated concentration for 72 hours. (B) An orthotopic mouse model. BNL cells were injected into the subcapsular area of the mouse livers. Treatment as indicated was administered orally once per day, 5 days per week. After sacrifice, the tumors were harvested and are shown here.