



Orally bioavailable Cyclin A/B RxL macrocycle has antitumor effect in SCLC and NSCLC cell line derived (CDX) and patient derived xenograft (PDX) models

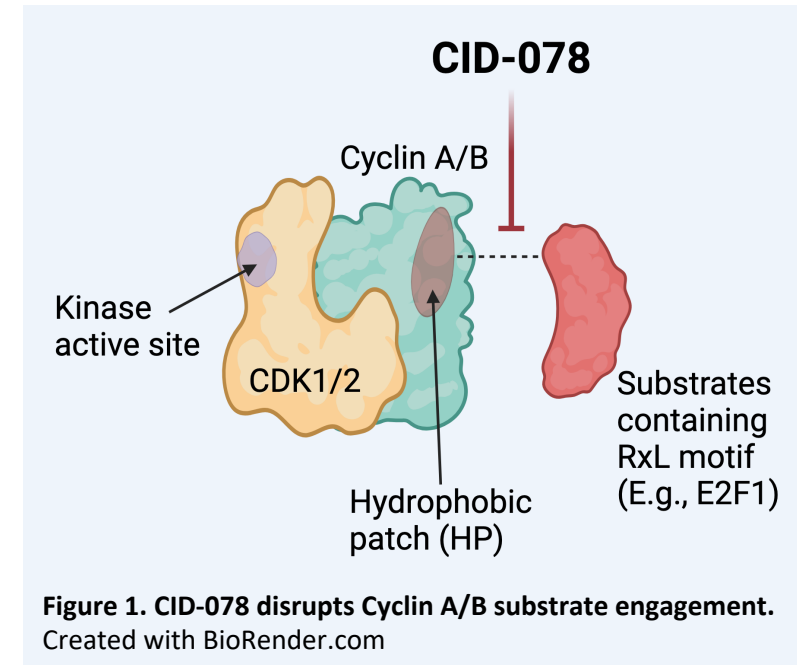
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Introduction and Background

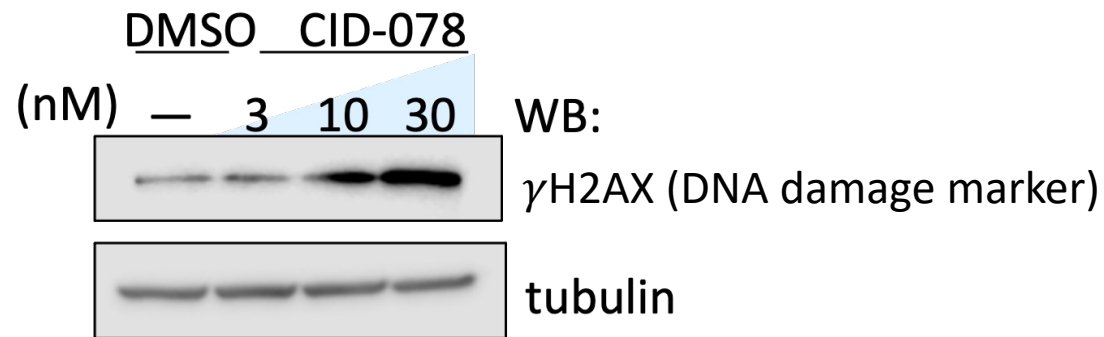
- CID-078 is an orally bioavailable, passively cell permeable, potent, and selective macrocycle that binds to the hydrophobic patch (HP) of cyclins A and B and blocks RxL-dependent interactions with proteins such as E2F1 with cyclin A-CDK2 and Myt1 with cyclin B-CDK1, providing a novel therapeutic strategy for SCLC and NSCLC patients (Figure 1).
- Previously, we demonstrated that disruption of these interactions selectively drives lethality in cancer cells containing oncogenic alterations that elevate expression of E2F1¹.
- We have further identified a pharmacodynamic (PD) biomarker, separase (ESPL1), a mitosis specific protein which is a direct substrate of cyclin B1-CDK1 and is inhibited by securin and phosphorylation-dependent binding to cyclin B1².
- Previously, we demonstrated that cyclin A/B RxL inhibition induces potent antiproliferative activity in SCLC cell line derived xenograft (CDX) and triple negative breast cancer (TNBC) patient derived xenograft (PDX) models^{1,3}.
- Here we demonstrate single agent efficacy of CID-078 in SCLC and NSCLC PDX models and show that elevated E2F targets and G2M checkpoint hallmark pathway scores and/or elevated levels of E2F1, cyclin B1 and ESPL1 (separase) are associated with response to cyclin A/B RxL inhibition in SCLC and NSCLC xenografts.



¹Gleason C, Garcia P, Odeh R, et al. Poster #1560 presented at AACR Annual Meeting, April 14-19, 2023, Orlando, FL; ²Haaß W, Stehle M, Nittka S, et al. *PLoS One* 7(8): e42863 (2012); ³Dias M, Liu L-F, Molina C, et al. Poster presented at EORTC-NCI-AACR International Conference on Molecular Targets and Cancer Therapeutics, October 11-15, 2023, Boston, MA.

Background: CID-078 induces DNA damage and G₂/M phase arrest in NCI-H446 SCLC cell line

A. Induction of DNA damage



B. Cell cycle profile

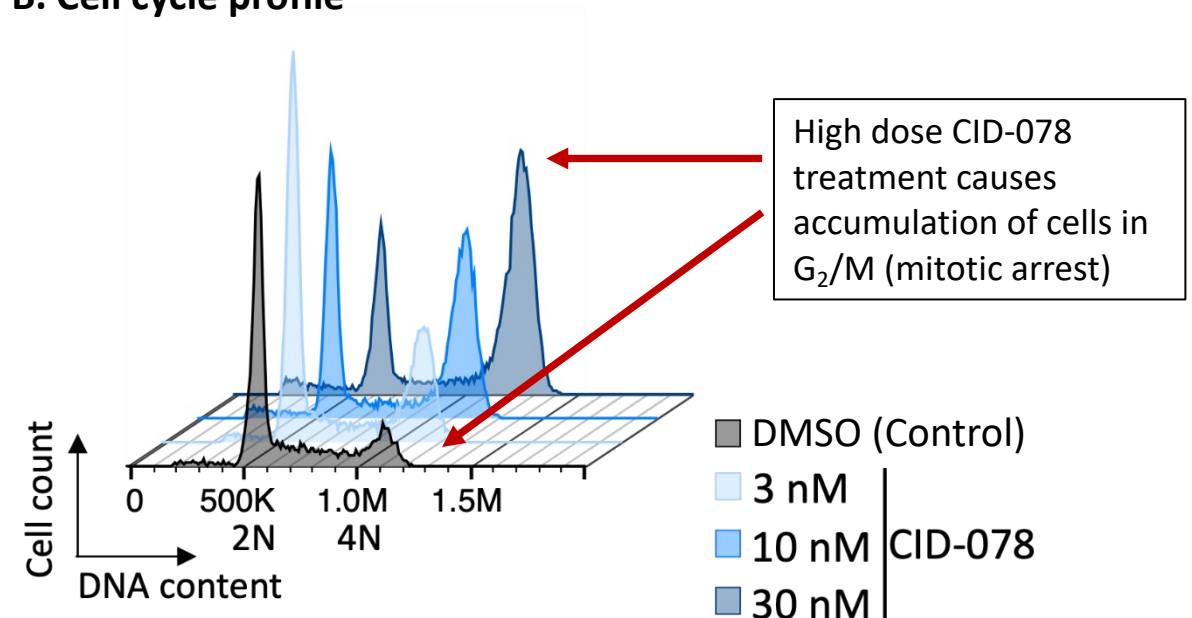


Figure 2. (A) CID-078 induces DNA damage. NCI-H446 cells were dosed with CID-078 at the indicated concentrations for 24h and processed for analysis of γ H2AX levels, a marker for activation of the DNA damage response pathway, by Western blot. **(B) Representative flow cytometry plots showing G₂/M phase arrest after treatment with CID-078.** NCI-H446 cells were dosed with DMSO or CID-078 at the indicated concentrations for 24h. DNA content was measured by FxCycle violet.

Higher E2F targets and G2M checkpoint hallmark pathways scores predict favorable response to CID-078 *in vitro*

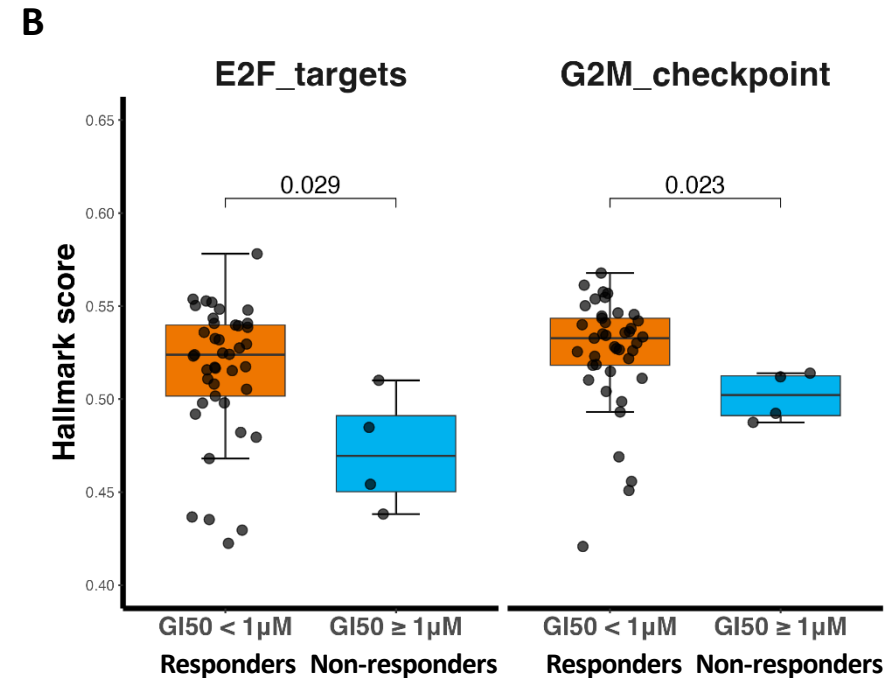
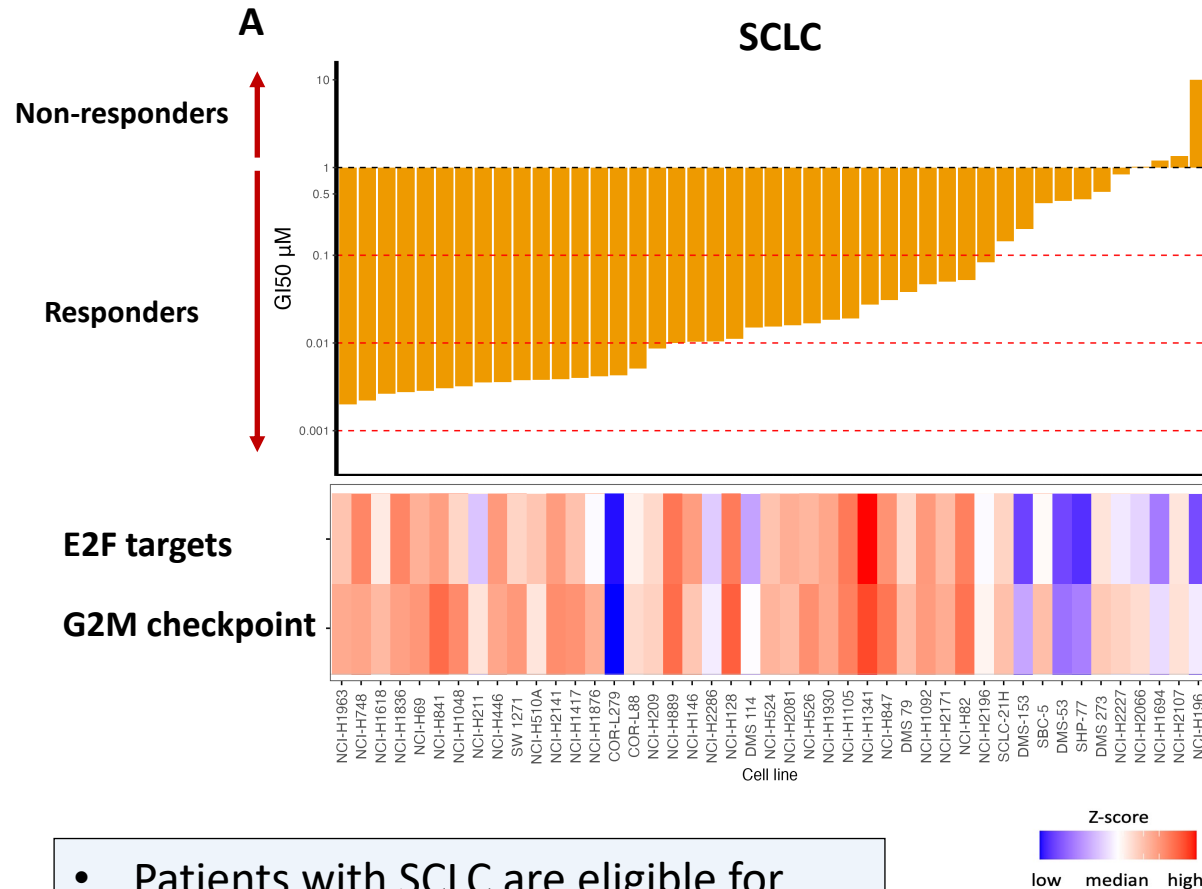


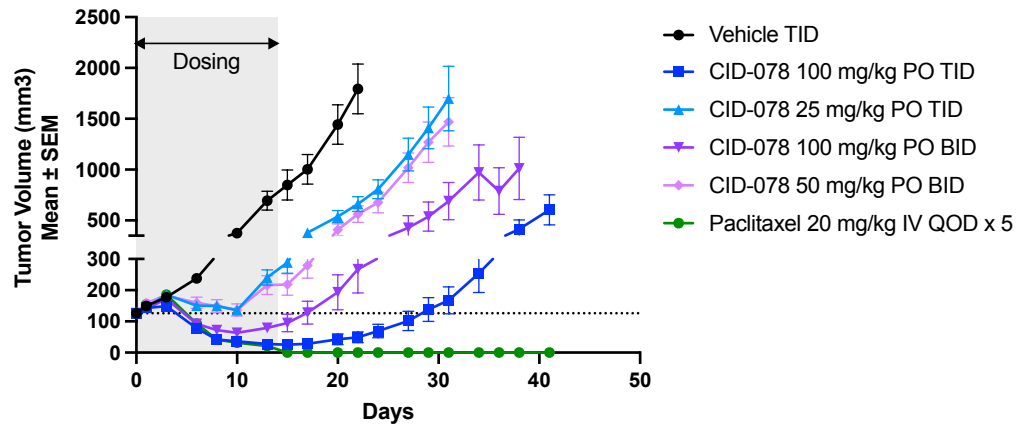
Figure 3. (A) Waterfall plots of sensitivity (as assessed by GI₅₀) and heatmaps of E2F targets and G2M checkpoint hallmark pathways Z-scores of CID-078 tested in 45 SCLC cell lines. **(B)** E2F targets and G2M checkpoint hallmark pathway scores were associated with sensitivity to CID-078 in SCLC cell lines.

P-values are shown and calculated by the Wilcoxon rank sum exact.

- Patients with SCLC are eligible for enrollment into CID-AB1-24001

CID-078 demonstrates monotherapy benefit in SCLC models with high E2F targets and G2M checkpoint hallmark pathways scores

A NCI-H446 (CDX)



B LUX083 (PDX)

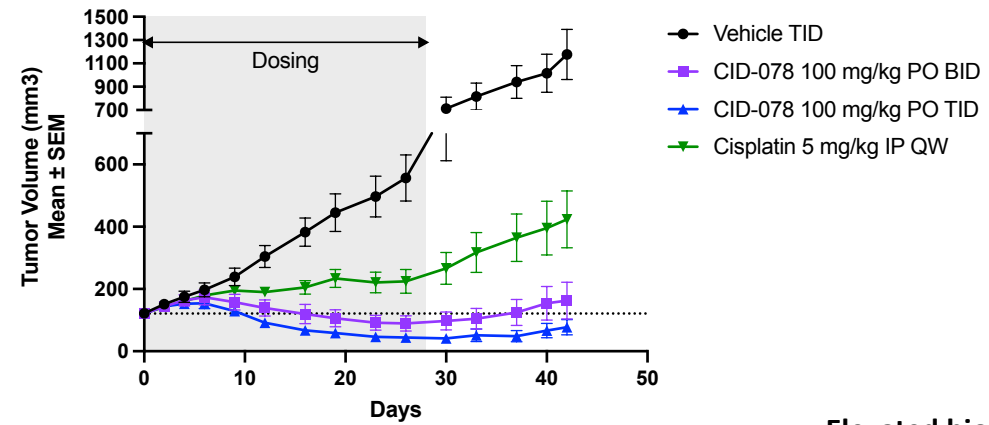
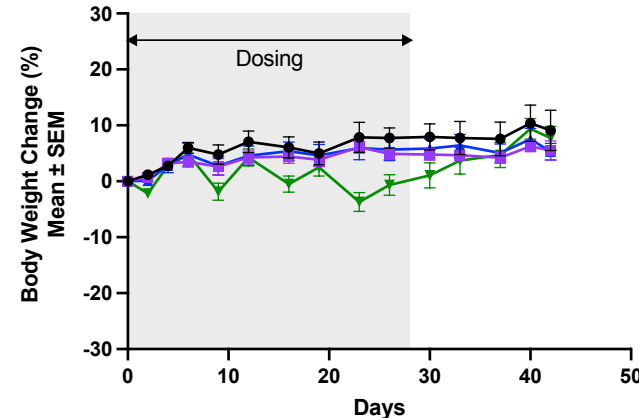


Figure 4. *In vivo* SCLC efficacy studies. (A) Mice were inoculated SC with 5×10^6 NCI-H446 CDX cells or (B) fragments of LUX083 PDX tumors. Treatment was initiated when tumors reached 100-200mm³. CID-078 was administered PO at the doses indicated. (C) All treatment regimens were tolerated as assessed by body weight measurements. (D) Summary table of biomarker characteristics for each model.

SC, subcutaneous; PO, orally; QD, once daily; BID, twice daily; TID, three times daily; QOD, once every two days; SEM, standard error of mean

C



D

Elevated biomarker characteristics are associated with response

	CDX-H446 SCLC	PDX-LUX083 SCLC
RB1	MUT	WT
E2F targets	High	NA
G2M checkpoint	High	NA
E2F1	High	High
ESPL1	High	High
CCNB1	High	High

Mitosis specific protein p-separase S1126 is a candidate PD biomarker

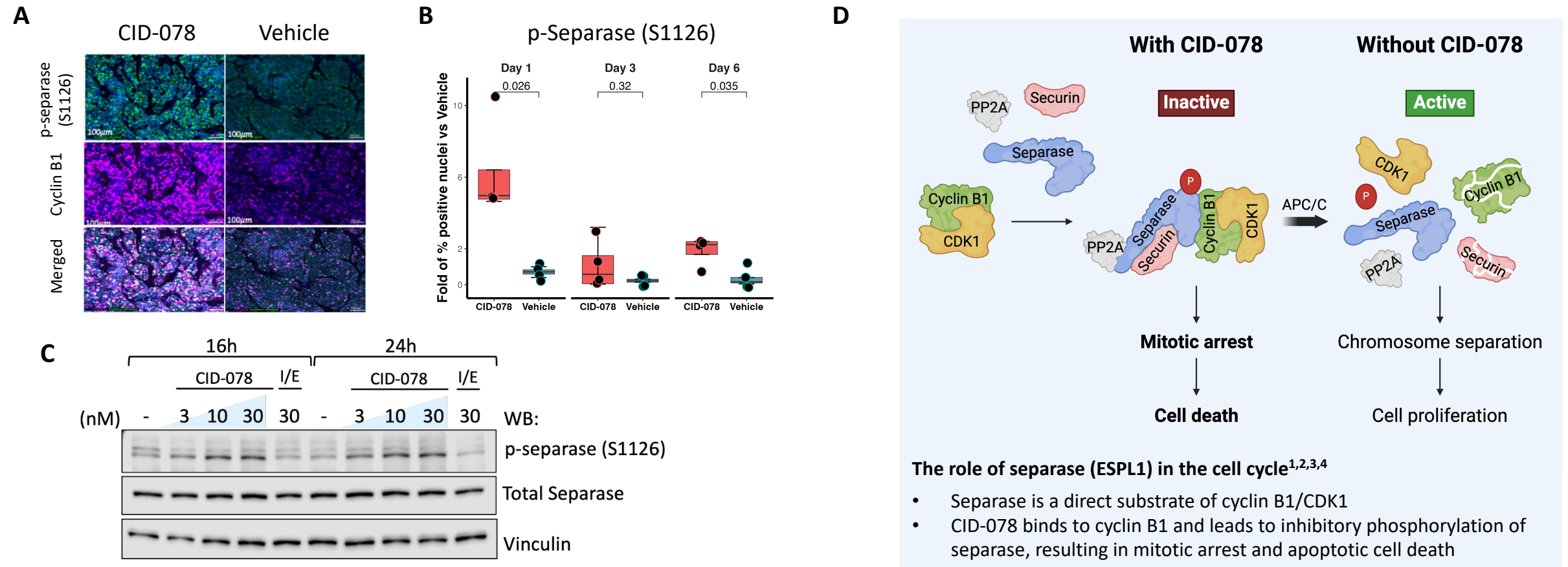


Figure 5. Time- and dose-dependent PD effect observed upon CID-078 treatment in the NCI-H446 SCLC model. (A) Representative immunohistochemistry (IHC) images and **(B)** quantification of IHC results of tumors treated with vehicle or 100 mg/kg CID-078 QD x 1 or TID x 3 and 6 show an increase in p-separase (S1126), and co-expression of p-separase (S1126) and cyclin B1 by multiplex IHC analysis. Samples were collected 4 h post final dose except for CID-078 QD x 1, collected 10 h post dose. **(C)** CID-078 treatment leads to a dose-dependent increase in p-separase (S1126). Western blot for p-separase (S1126) in cells dosed with CID-078 for 16 or 24 hours at the indicated concentrations. **(D)** Proposed model for pharmacodynamic effect of CID-078, created with BioRender.com.

I/E, inactive enantiomer of CID-078

¹Haaß W, Stehle M, Nittka S, et al. *PLoS One* 7(8): e42863 (2012); ²Pérez de Castro I, de Cárcer G, Malumbres M. *Carcinogenesis*, 28(5):899-912, (2007); ³Morgan DO. *The Cell Cycle: Principles of Control*. New Science Press (2007); ⁴Holland AJ, Taylor SS. *J Cell Sci*, 119(Pt 16):3325-36, (2006).

High E2F targets and G2M checkpoint hallmark pathways scores in large cell and squamous cell NSCLC predict favorable response to CID-078 *in vitro*

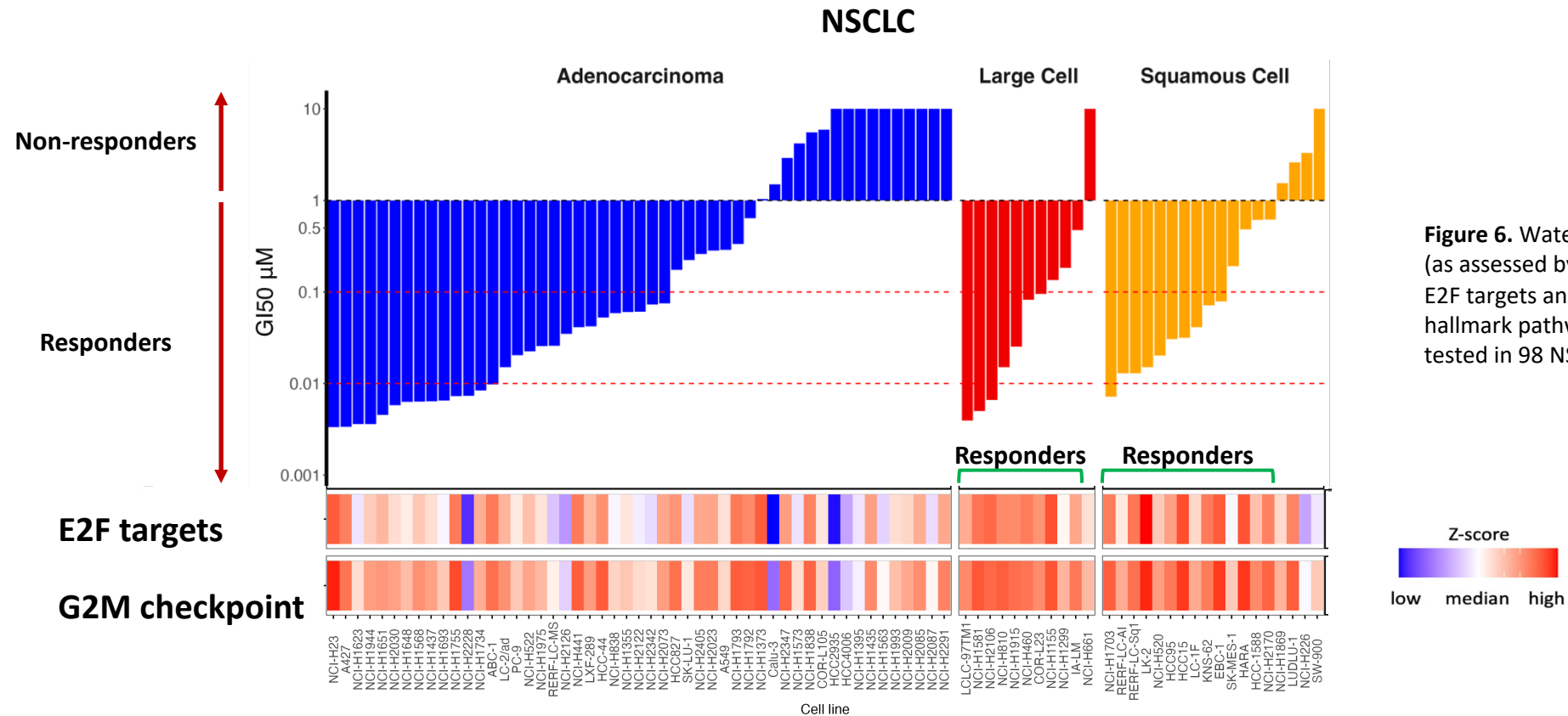


Figure 6. Waterfall plots of sensitivity (as assessed by GI₅₀) and heatmaps of E2F targets and G2M checkpoint hallmark pathways Z-scores of CID-078 tested in 98 NSCLC cell lines.

- Patients with NSCLC are eligible for enrollment into CID-AB1-24001

CID-078 demonstrates monotherapy benefit in NSCLC CDX models with high E2F targets and G2M checkpoint hallmark pathways scores

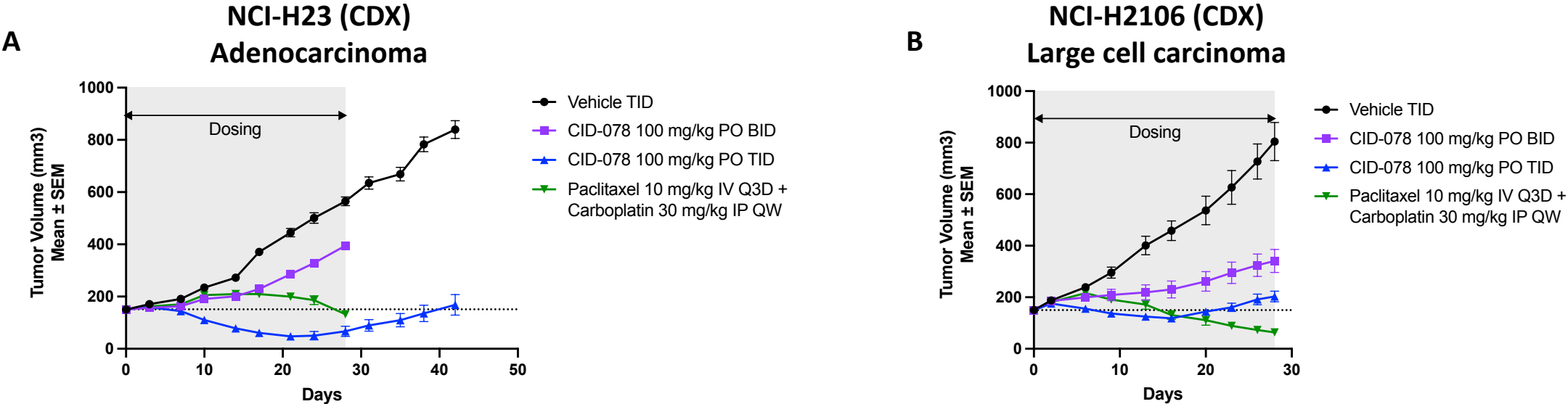


Figure 7. *In vivo* NSCLC CDX efficacy studies. (A) Mice were inoculated SC with 1.5×10^7 NCI-H23 or (B) 1×10^7 NCI-H2106 CDX cells. Treatment was initiated when tumors reached 100-200mm³. CID-078 was administered PO at the doses indicated for each model. All treatment regimens were tolerated as assessed by body weight measurements (not shown). (C) Summary table of biomarker characteristics for each model is shown.

C Elevated biomarker characteristics are associated with response

	CDX-H23 Adenocarcinoma	CDX-H2106 Large cell carcinoma
RB1	WT	MUT
E2F targets	High	High
G2M Checkpoint	High	High
E2F1	High	High
ESPL1	High	High
CCNB1	High	Med

CID-078 demonstrates monotherapy benefit in a NSCLC PDX model with favorable biomarker characteristics

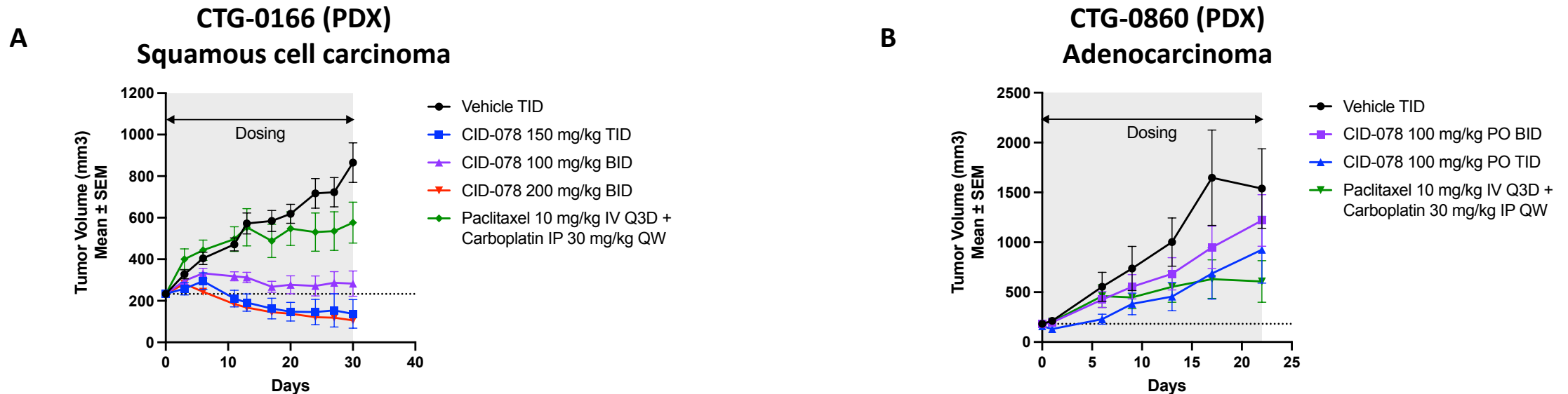


Figure 8. *In vivo* NSCLC PDX efficacy studies. Mice were inoculated SC with fragments of (A) CTG-0166 or (B) CTG-0860 PDX tumors. Treatment was initiated when tumors reached 150-250mm³. CID-078 was administered PO at the doses indicated for each model. All treatment regimens were tolerated as assessed by body weight measurements (not shown). (C) Summary table of best response and biomarker characteristics for each model is shown.

PR, partial response; PD, progressive disease

C Elevated biomarker characteristics are associated with response

	PDX-CTG-0166 Squamous cell carcinoma (PR)	PDX-CTG-0860 Adenocarcinoma (PD)
RB1	MUT	WT
E2F targets	High	Med
G2M Checkpoint	High	Med
E2F1	High	Low
ESPL1	High	Low
CCNB1	High	Med

Conclusions

- CID-078 induced antiproliferative activity in SCLC and NSCLC cell lines (Figures 3 and 6). In the SCLC cell line, NCI-H446, CID-078 induces significant DNA damage and G₂/M phase accumulation that correlates with increased separase S1126 inhibitory phosphorylation (Figure 5).
- Tumor models with high E2F targets and G2M checkpoint hallmark pathways scores and elevated levels of E2F1, cyclin B1 and ESPL1 demonstrate tumor growth inhibition and/or regression when treated with CID-078 dosed at 100 mg/kg BID and TID (Figures 4, 7, and 8).
- In the SCLC tumor model NCI-H446, we observed an increase in p-separase (S1126) and co-expression of p-separase and cyclin B1 following treatment with CID-078 (Figure 5) as well as p-ATM nuclear staining and E2F1 (not shown).
- CID-078 demonstrates potent anti-tumor activity in the SCLC and NSCLC preclinical models evaluated, correlating well with E2F1 and ESPL1 expression and consistent with proposed cyclin A/B RxL inhibition leading to DNA damage (γ -H2AX & pATM).
- CID-078 activity further correlates with an increase in a mitotic phase-specific protein (p-separase S1126) and leads to apoptotic tumor cell death.
- Based on these compelling results, CID-078 may provide a novel therapeutic option patients with SCLC and NSCLC. This hypothesis and the correlation of anti-cancer activity with the proposed biomarkers will be explored in the first-in-human clinical trial, CID-AB1-24001 (NCT06577987).