

# Evaluation of New Targeted Therapies in 3D Culture Models for Pancreatic Cancer



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## Introduction

Pancreatic Ductal Adenocarcinoma (PDAC) is a deadly cancer that displays a highly heterogeneous tumor microenvironment. Novel therapeutic agents that target transcription regulatory networks known as super-enhancers have recently gained attention in the potential treatment of PDAC. To accurately study the effects of super-enhancer-inhibitors, cell culture models that mimic the tumor microenvironment are necessary. In this study, two PDAC patient-derived cell models (PDCs), B011 and B028, are grown in both 3D and 2D culture methods and the inhibitory concentrations of three super-enhancer-inhibitors are determined.

## Research Question

Do PDAC PDCs B011 and B028 grown in a 3D geometry exhibit more resistance to drug treatment with super-enhancer-inhibitors (triptolide, THZ1, and THZ2) than their 2D cell culture counterparts? In other words, is the half maximal inhibitory concentration ( $IC_{50}$ ) of triptolide, THZ1 and THZ2 higher for 3D PDAC cultures?

## Materials and Methods

### Cell culture maintenance and conditions

Low passage tumor cells were isolated from the tumor samples of two PDAC patients (B011 and B028). 5000 cells were added to 96-well flat bottom plates for 2D cultures or 96-well U-bottom plates with 1.5% Matrigel for 3D organoid cultures. 2D cultures were grown for 24 hours and 3D cultures were grown for 72 hours prior to drug treatment.

### Preparation of super-enhancer inhibitor agents

Serial dilutions of TPL, THZ1, and THZ2 were made to achieve 10x the final concentration and were transferred to the culture plates to achieve the desired dose. Triplicates were created for each condition.

### Cell viability assay

Following 72 hours of treatment, the CellTiter-Glo<sup>®</sup> Luminescent Cell Viability Assay was performed to determine the relative cell viability.

## Results

- The 2D B011 cultures demonstrated a lower  $IC_{50}$  compared to their 3D counterparts for all 3 super-enhancer-inhibitors; triptolide, THZ1, and THZ2.
- 2D B011 that were treated with THZ1 demonstrated an  $IC_{50}$  of 16.3 nM while the 3D B011 cells demonstrated an  $IC_{50}$  of 57.5 nM.
- 2D B011 that were treated with THZ2 demonstrated an  $IC_{50}$  of 61.2 nM while the 3D B011 cells demonstrated an  $IC_{50}$  of 182 nM.
- 2D B011 cultures treated with triptolide (TPL) exhibited less resistance to drugs compared to their 3D counterparts. The 2D B011 cultures treated with TPL had an  $IC_{50}$  of 11.9 nM while the 3D B011 cultures demonstrated an  $IC_{50}$  of 26.3 nM.
- The B028 cell line did not grow as well as the B011 cell line and the luminescence results demonstrated unviable cells. However, THZ1 was successfully tested in the B028 cell line and 2D B028 cultures demonstrated an  $IC_{50}$  of 9.5 nM while the 3D B028 cultures demonstrated an  $IC_{50}$  of 71.9 nM.

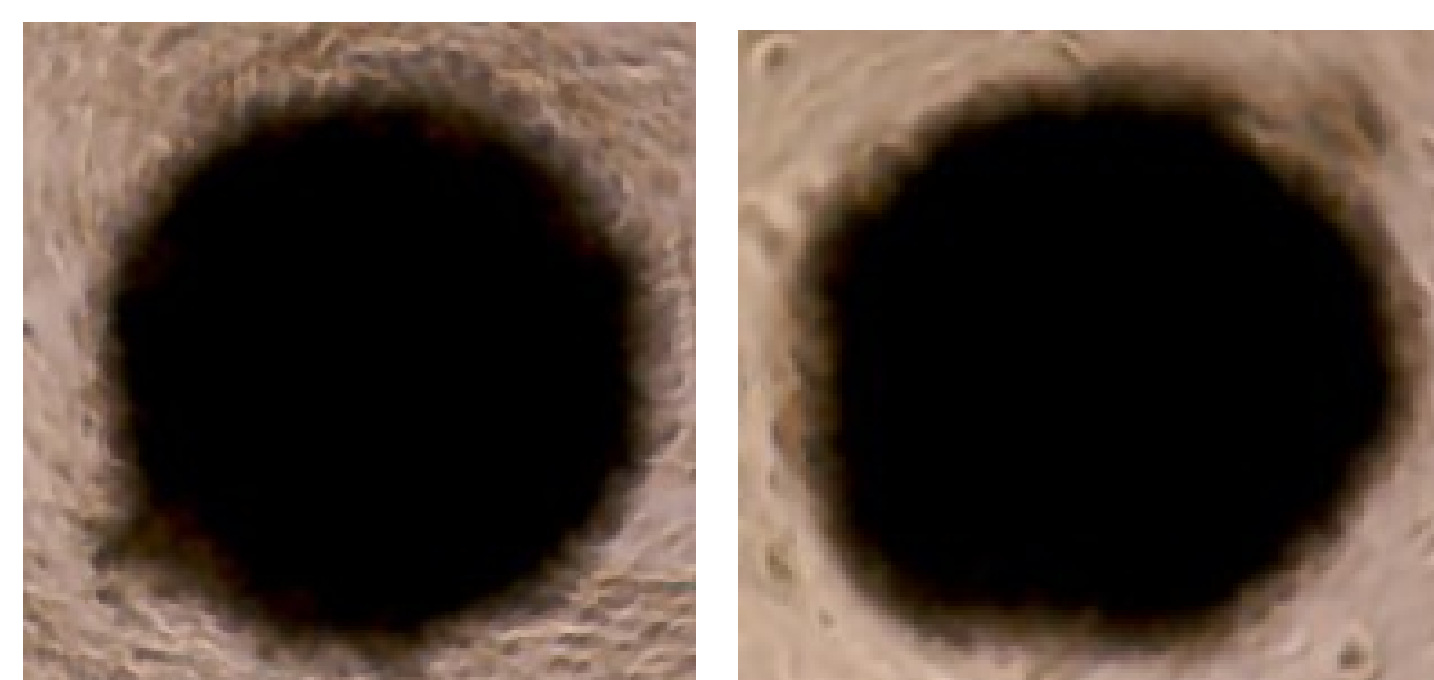


Figure 1: 3D B011 (left) and 3D B028 (right) visualized under a microscope prior to drug treatment.

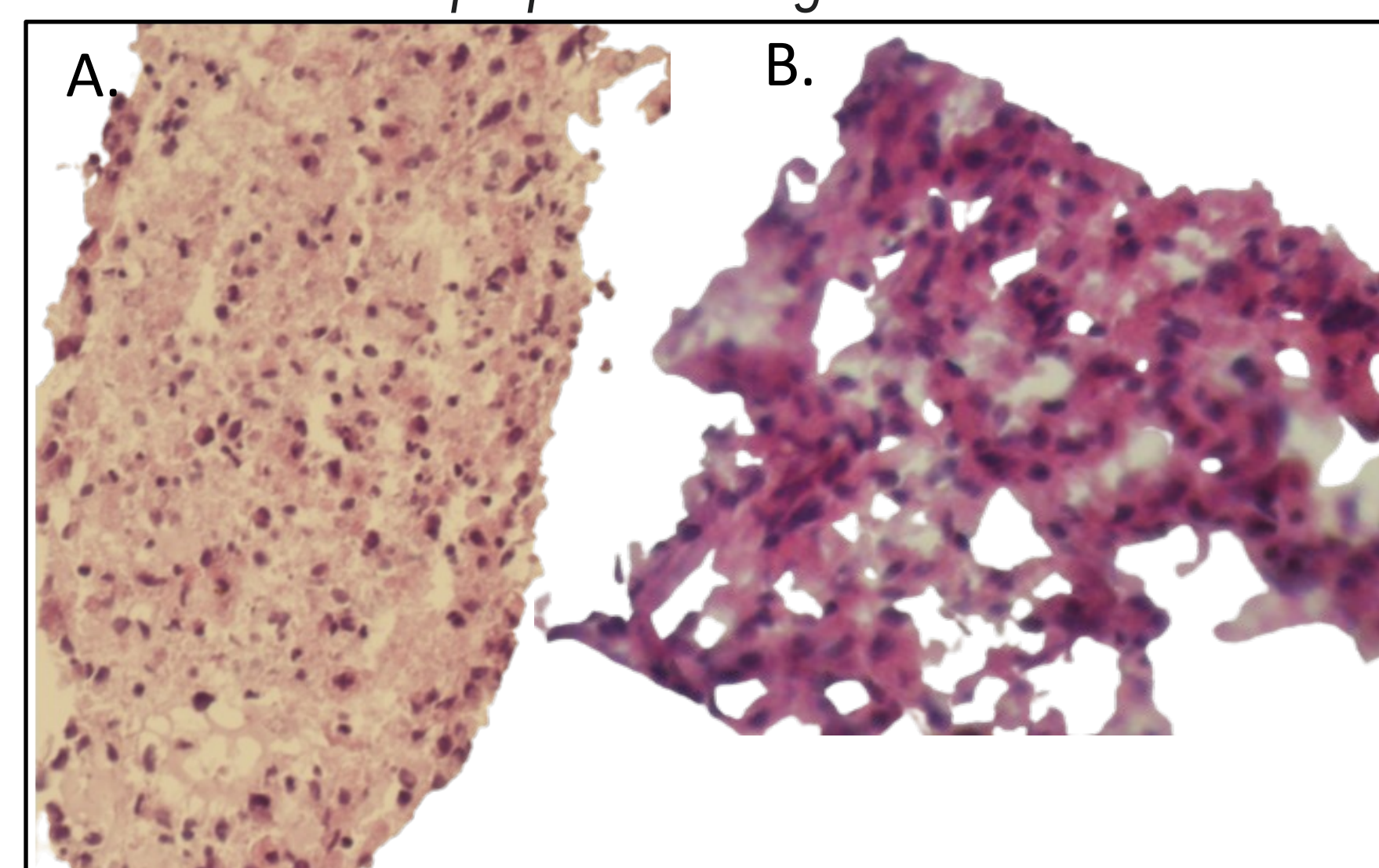


Figure 2: Hematoxylin and eosin (H&E) stains of 3D B028 (A) and 3D B011 (B).

## Dose-Response Curves of Super-Enhancer-Inhibitors In B011 and B028 2D and 3D Cell Culture Models

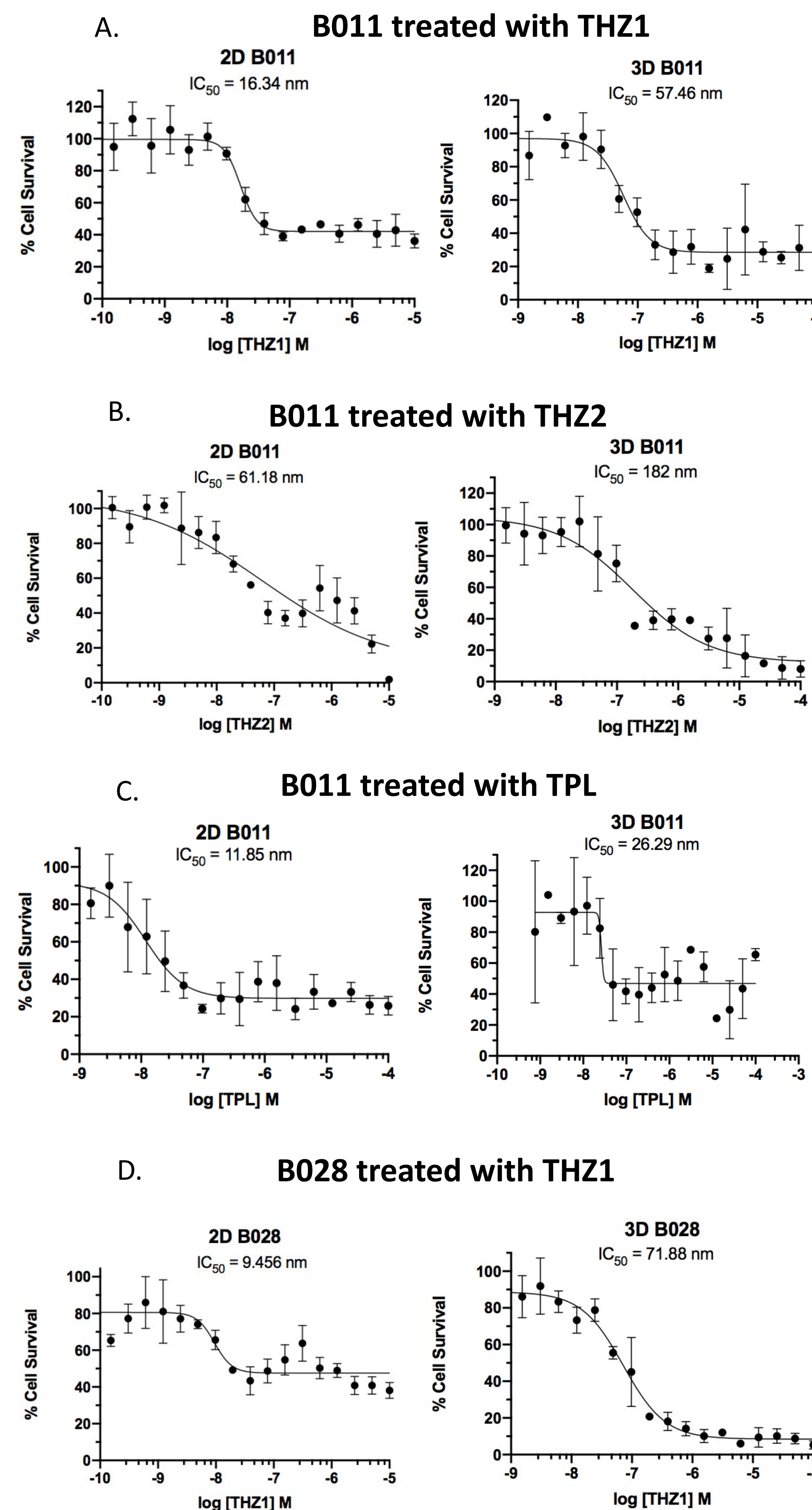


Figure 3: Dose response curves and  $IC_{50}$  values of 3D and 2D cultures of B011 treated with THZ1 (A), B011 treated with THZ2 (B), B011 treated with TPL (C), and B028 treated with THZ1 (D). A total of 5000 cells were seeded for each condition. The x-axis represents the log of drug concentration and the Y-axis represents percent of cell survivability. Error bars are demonstrated for each triplicate drug concentration.

## $IC_{50}$ Values of THZ1, THZ2, and TPL

B011	2D	3D
THZ1 $IC_{50}$ (nM)	16.3	57.5
THZ2 $IC_{50}$ (nM)	61.2	182.0
TPL $IC_{50}$ (nM)	11.9	26.3
B028	2D	3D
THZ1 $IC_{50}$ (nM)	9.5	71.9

Table 1: Summary of  $IC_{50}$  values for super-enhancer-inhibitors THZ1, THZ2 and TPL for 2D and 3D B011 cell cultures and THZ1 values for 3D and 2D B028 cultures.

## Conclusion

The 3D B011 cell cultures demonstrated larger  $IC_{50}$  values, indicating a greater resistance to treatment with all three super-enhancer inhibitors when compared to their 2D counterparts for each experiment.

The 3D B028 cell cultures demonstrated greater resistance to treatment with THZ1 but did not produce viable results when investigating THZ2 and triptolide.

## Summary

- 3D PDAC cell culture models are more resistant to treatment with super-enhancer-inhibitors (THZ1, THZ2, and TPL) when compared to their 2D counterparts.
- The implications of this study are clinically significant as it demonstrates that the same PDAC cell line, grown in different arrangements, demonstrates different inhibitory concentrations.

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