Effects of cingulin and SPTBN1 protein knockout on pancreatic ductal adenocarcinoma tumor cell polarity and subtype

This project seeks to discover how knocking out the cell polarity proteins cingulin (CGN) and spectrin beta, non erythrocytic 1 (SPTBN1) affects pancreatic ductal adenocarcinoma (PDAC) tumor cell polarity and growth. Inducible exogenous CGN and SPTBN1 constructs will be transfected into PDAC organoids, and afterwards CGN and SPTBN1 will be knocked out with CRISPR. The tumor organoids will then be viewed with immunofluorescence microscopy to assess cell polarity and subjected to a CellTiter-Glo growth assay to assess cell viability. To study the effect of CGN and SPTBN1 on cell viability, a Bravo Pipetting protocol was developed to plate efficient organoid growth assays. The CGN exogenous construct was also created and validated, SPTBN1 was sequence validated and initial immunofluorescence staining of CGN and SPTBN1 in a classical organoid line before knockout was negative. It is possible that the inability to stain for the proteins is due to non-specific primary antibodies, requiring optimization for staining organoids or a need for better control lines.