Cell Counting

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Plate cells

- 1 Cells were plated at 100,000 cells/well in 2 mL of media in 6-well plates. Three wells were used as technical replicates for each condition.
- 2 Three identical plates were prepared for each time point (24, 48, and 72 hours).

Perform drug treatments

- 3 After cells adhered, DMSO (0.05% final), idasanutlin (MedChemExpress, Monmouth Junction, NJ # HY-15676, 10 mM stock) (1 μ M final), or ATSP-7041 (5 μ M final), was added to the media.

Harvest and count cells

- 4 After cells grew for the specified amount of time, cells were washed with 1 mL of 1X PBS, harvested with 400 μ L of Trypsin-EDTA 0.25% (Thermo Fisher Scientific # 25200114), and quenched with 2 mL of base media (varies by cell line).
- 5 600 μ L of the cell solutions were counted using a Vi-Cell XR Cell Viability Analyzer (Beckman Coulter Life Sciences, Indianapolis, IN).
- 6 Counts (in viable cells/mL) were multiplied by 2.4 to obtain the total number of cells per condition. The three technical replicates were averaged, normalized to the initial plated number (100,000 cells), and transformed to a $\log_2$ scale.

Generate curves

- 7 The mean and SD of three independent biological replicates for each time point was then taken and plotted.
- 8 Significance between treated and DMSO groups was calculated by two-way ANOVA with Holm-Sidak's multiple comparisons correction using GraphPad Prism.