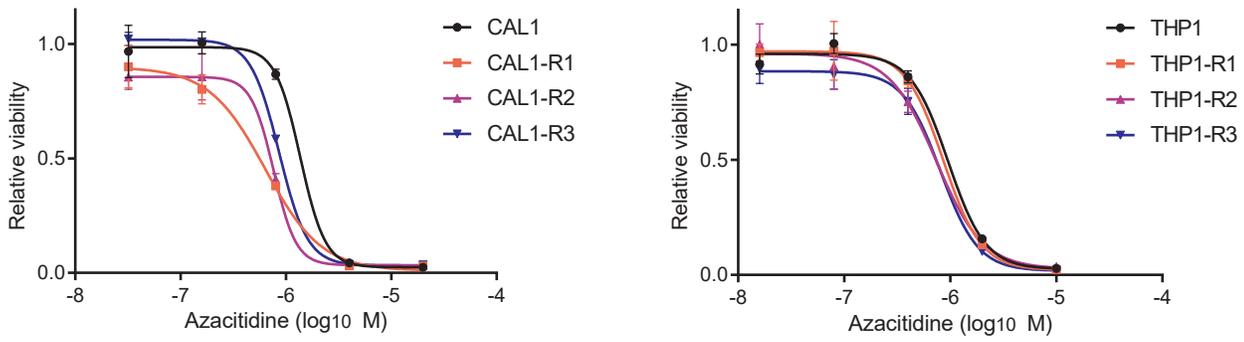
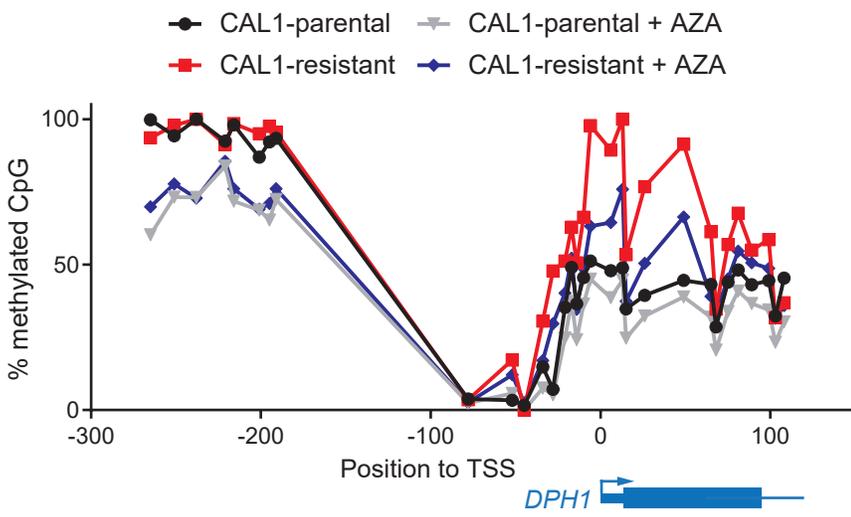


Supplemental Figure 1

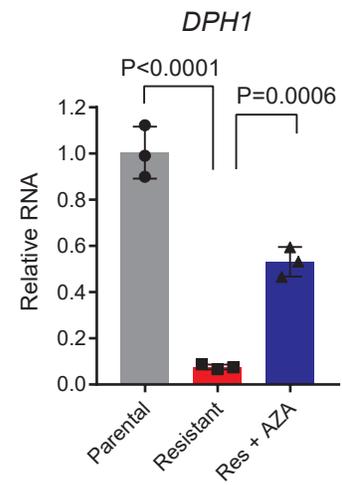
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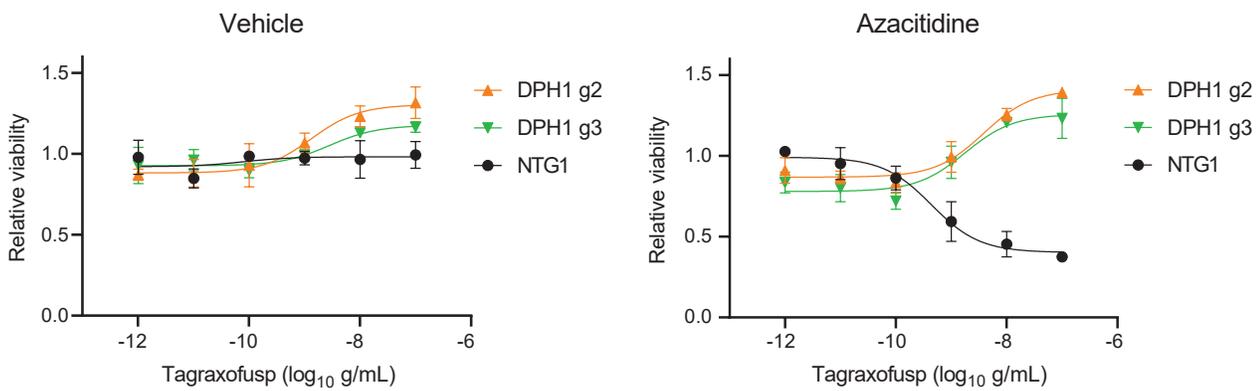
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C



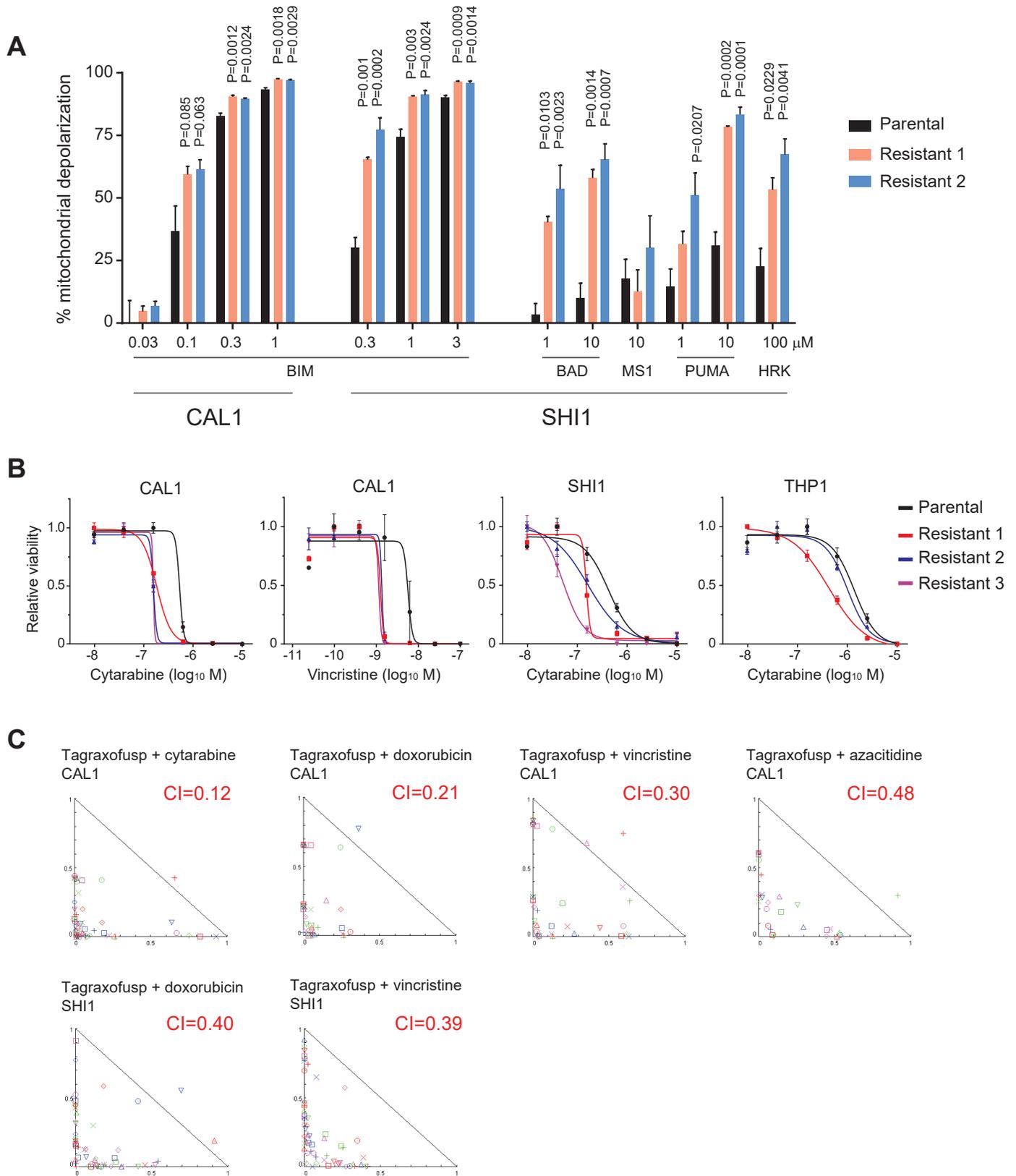
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Supplemental Figure 1. Azacitidine reverses *DPH1* locus CpG methylation and expression, and restoration of tagraxofusp cytotoxicity is dependent on intact *DPH1* in BPDCN cells.

(A) CAL1 BPDCN and THP1 AML parental (black) and tagraxofusp-resistant (orange, purple, blue) cultures were tested for cytotoxicity after exposure to five-fold decreasing concentrations of azacitidine. Each point was assessed in triplicate and plotted relative to cells growing in vehicle alone. (B) Percentage of methylated CpGs in the *DPH1* locus are shown for the indicated genomic positions in parental CAL1 cells and a tagraxofusp-resistant subculture, before and after 2 weeks of pulsatile treatment with non-cytotoxic doses of azacitidine. (C) Quantitative RT-PCR for *DPH1* expression in parental CAL1 cells and a tagraxofusp-resistant subclone treated with vehicle or azacitidine (n=3 replicates each). Dots represent relative expression, bars +/-SD. Conditions compared by one-way ANOVA with Dunnett's multiple comparisons correction, adjusted P value shown. Data are representative of two independent resistant subclones with similar results. (D) Tagraxofusp cytotoxicity assays in BPDCN (CAL1) cells harboring *DPH1*-targeting (*DPH1* g2, g3) or control non-targeting (NTG1) sgRNAs made resistant to tagraxofusp and then treated for 2 weeks with pulsatile non-cytotoxic doses of azacitidine or vehicle. Each point was assessed in triplicate and plotted relative to cells growing in vehicle alone.

Supplemental Figure 2

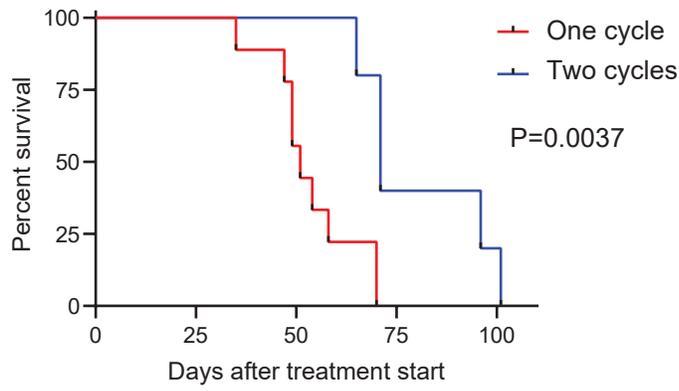


Supplemental Figure 2. Tagraxofusp resistance is associated with increased apoptotic priming and sensitivity to chemotherapy.

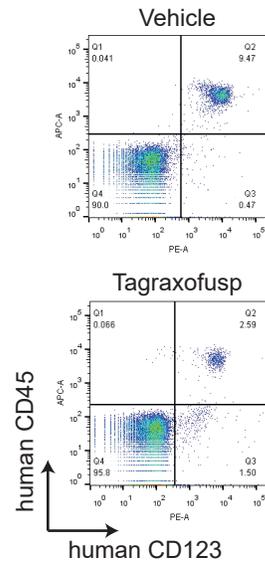
(A) BH3 profiling of CAL1 and SHI1 parental and tagraxofusp-resistant (R1-2) subcultures measuring overall mitochondrial apoptotic priming by mitochondrial depolarization in resistant cells after stimulation over increasing doses of a broadly proapoptotic BIM BH3-only peptide or with peptides that selectively bind to specific BCL-2 family members, as indicated. Numbers on the x-axis represent the dose of the indicated peptide in μM . Bars represent technical triplicates of biologically-independent resistant subcultures, each compared to technical triplicates of parental. Samples compared by one-way ANOVA with Dunnett's multiple comparisons correction, adjusted P value shown. (B) CAL1, SHI1, and THP1 parental and tagraxofusp-resistant cells tested in MTT viability assays after exposure to increasing doses of cytarabine (AraC) or vincristine (VCR). Each point was assessed in triplicate and plotted relative to cells growing in vehicle alone. (C) BPDCN (CAL1) and AML (SHI1) parental cell lines tested for synergistic cell death with combinations of tagraxofusp and cytarabine, doxorubicin, vincristine, or azacitidine, each varied over five-fold dilution series with data plotted in an isobologram, where points on the diagonal line indicate additivity, to the right of the line indicate antagonism, and to the left of the line indicate synergy. Combination indices (CI) are also shown for each drug pair, calculated using the method of Chou-Tallalay, where $\text{CI} < 1$ indicates synergy.

Supplemental Figure 3

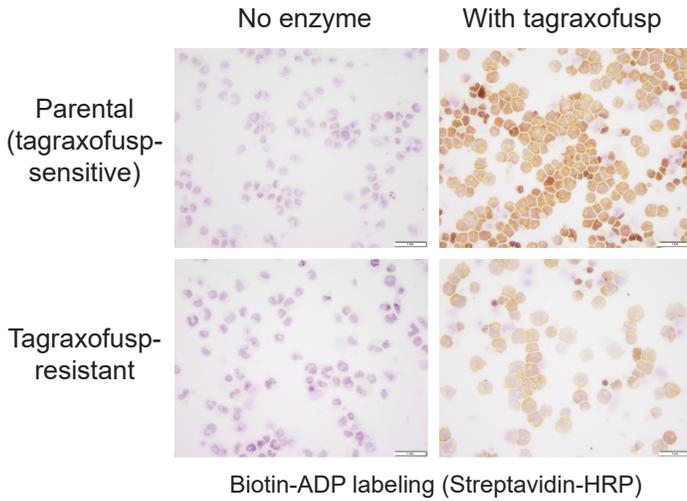
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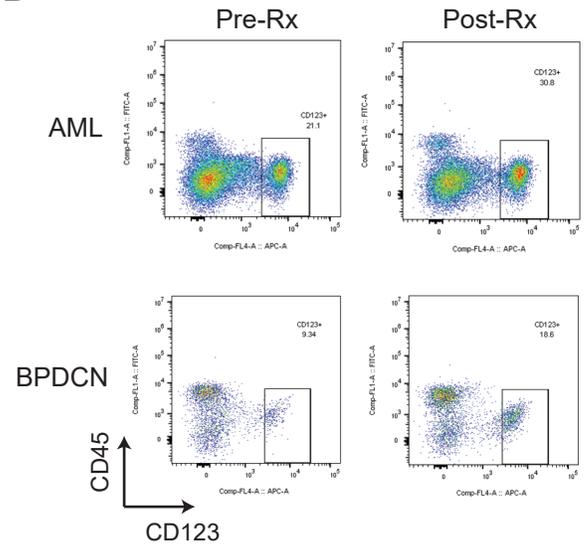
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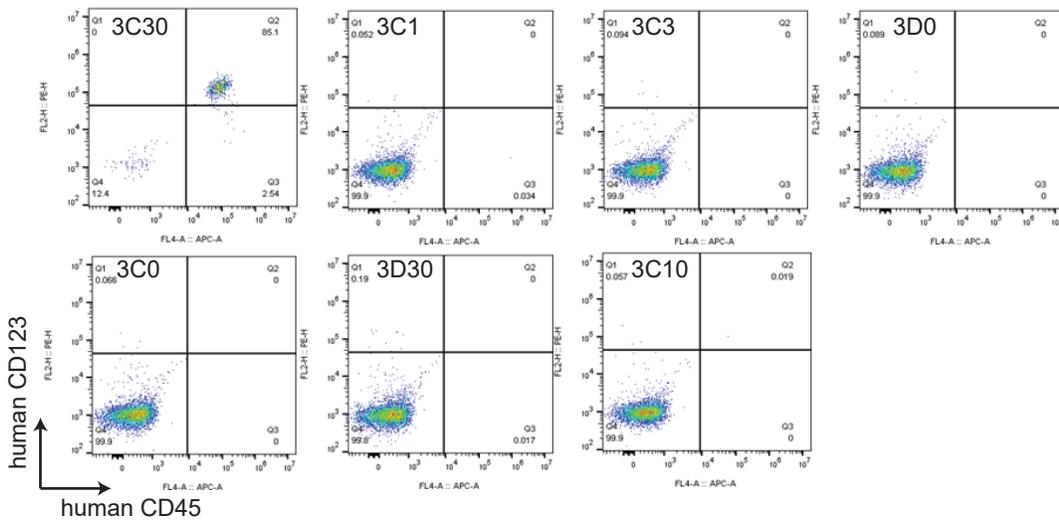
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E



Supplemental Figure 3. In vivo tagraxofusp resistance is associated with decreased ADP-ribosylation activity of tagraxofusp in permeabilized single-cell assays.

(A) Kaplan-Meier overall survival curves from the time of first treatment for animals harboring PDX101 and PDX102 that received one (n=9) or two (n=5) cycles of tagraxofusp. Curves compared pairwise by log-rank test. (B) Representative flow cytometry from animals bearing BPDCN PDXs that progressed after vehicle or tagraxofusp showing, like in patients, no significant decrease in the level of CD123 expression on the tumor cells. Data are representative of >10 PDXs assessed in each arm at the time of progression and all were similar. (C) Slide-based in vitro enzymatic labeling assay for tagraxofusp-induced ADP-ribosylation activity is shown in parental and tagraxofusp-resistant CAL1 cells, with or without inclusion of tagraxofusp in the reaction solution, showing decreased labeling with biotin-NAD⁺ in the setting of tagraxofusp-resistance. Staining was detected with streptavidin-HRP histochemistry. (D) Flow cytometry and gating for CD45 and CD123 in the flow cytometry-based tagraxofusp-induced ADP-ribosylation activity assay are shown for bone marrow cells from example patients with AML or BPDCN before (Pre-Rx) and after (Post-Rx) treatment with tagraxofusp. (E) Flow cytometry for human CD45 and CD123 in bone marrow harvested from BPDCN PDX-bearing animals that received combination therapy with tagraxofusp and azacitidine and were sacrificed at 200 days after treatment start, showing no measurable residual disease in 6 of 7 animals with analysis of 10,000 events (threshold for detection ~0.1% involvement).