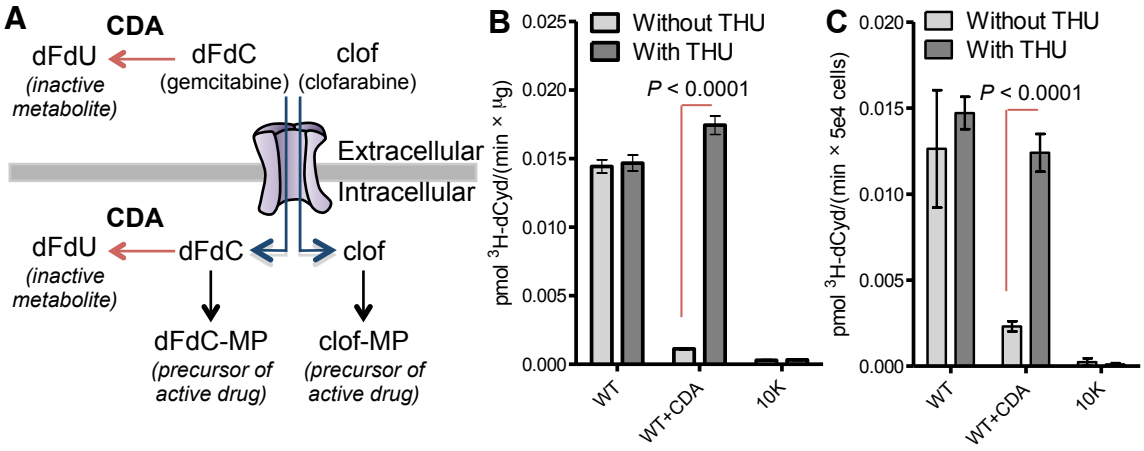
SUPPORTING METHODS

In vitro kinase and uptake assays using ^{3}H-dCyd, ^{3}H-FAC and ^{18}F-FAC

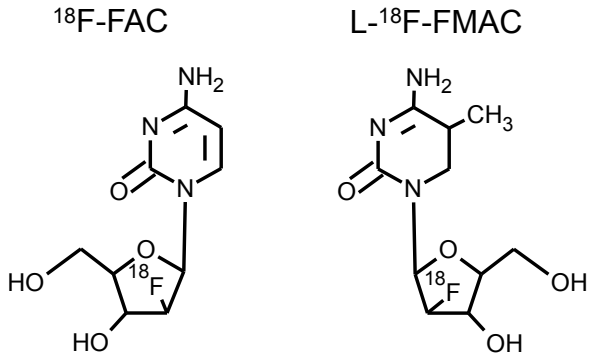
Kinase and cell-based uptake assays were performed as previously described in the main text using 0.9 μM ^{3}H-dCyd (2'-deoxycytidine, [5-^{3}H(N)], MT673, Moravek Biochemicals; specific activity, 0.833 TBq [22.5 Ci] /mmol), 1.5 μM ^{3}H-FAC (2'-deoxy-2'-fluoro-D-arbinofuranosylcytosine, [^{3}H]-, MT1858, Moravek Biochemicals; specific activity, 0.503 TBq [13.6 Ci] /mmol) or 185 kBq (5 μCi) ^{18}F-FAC. The radiochemical purities of ^{18}F-FAC were >99% and the specific activity was >1000 Ci/mmol. Briefly, 5 x 10^{6} cells growing in exponential phase were lysed by three rounds of freeze-thaw. Supernatant containing purified protein was incubated with the radiolabeled probe for 20 min at 37°C and spotted on positively-charged DE-61 Whatman filters which bind negatively-charged phosphorylated products. Filters were washed, allowed to dry and analyzed for radioactivity. In uptake assays, cells were plated for 4-5 hours in growth media followed by incubation with the radiolabeled probe. In uptake assays, cells were plated for 4-5 hours in growth media followed by incubation with the radiolabeled probe. Samples were measured for radioactivity using a Wallac Wizard 3" 1480 Automatic Gamma Counter (PerkinElmer) for ^{18}F and a Beckman Liquid Scintillation counter for ^{3}H. For uptake assays, 38.85 kBq (1.05 μCi) of ^{3}H-dCyd were added to L1210 cells (1 x 10^{5} cells per well in 96-well plates). After 1 h at 37°C, wells were washed 3 times with ice-cold phosphate-buffered saline using a vacuum filtration system (Millipore). Plates were oven-dried at 45−50°C for 30 min and 200 μL of scintillation fluid were added per well. Radioactivity was measured using a Trilux MicroBeta scintillation counter (Perkin Elmer). Tetrahydrouridine (THU, 584222, EMD Chemicals) was prepared in water.

Quantitative Real-Time PCR gene expression analysis (qPCR)

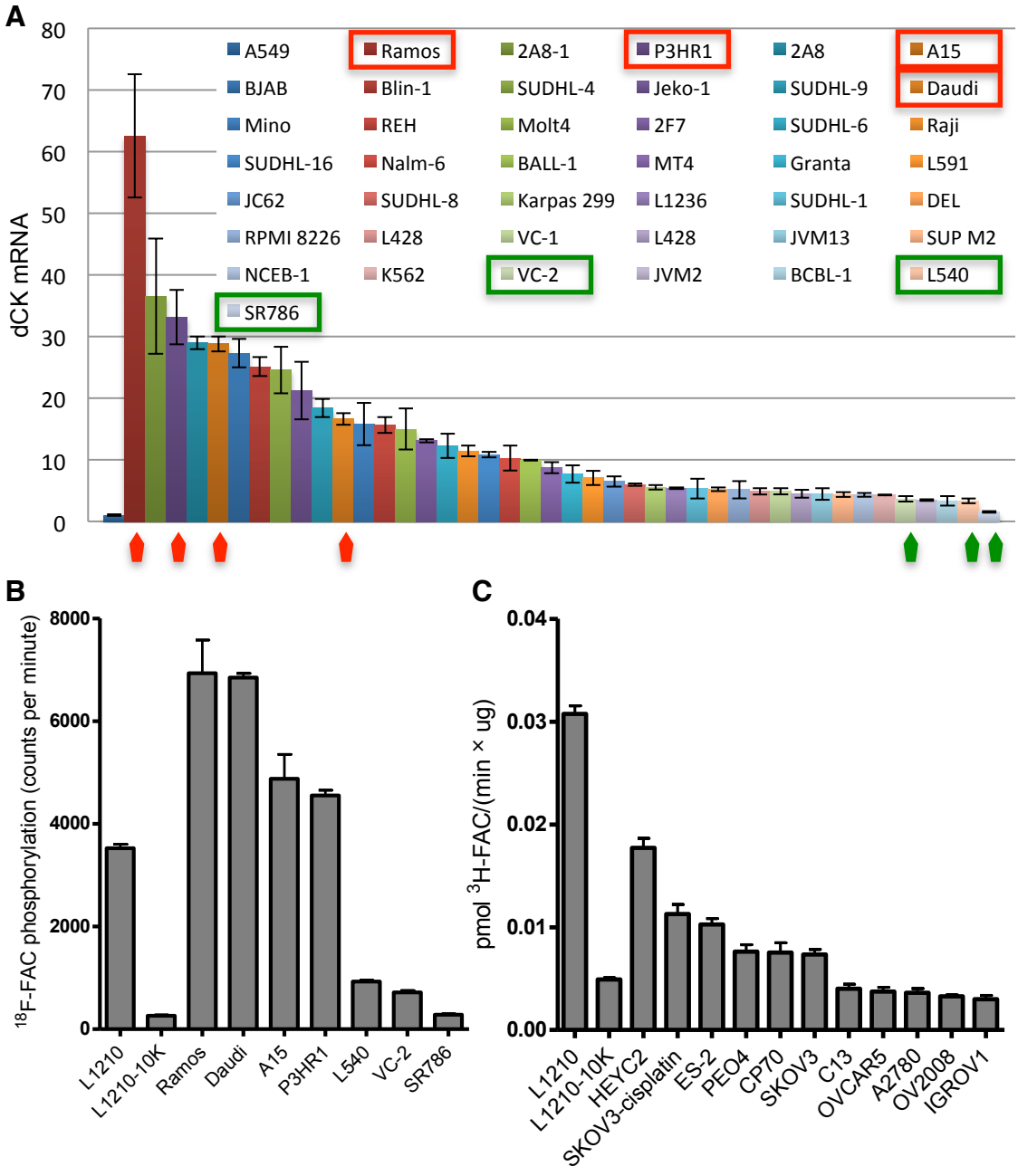
Total RNA was purified from tissues using the Qiagen RNeasy Mini kit. 1.5 μg of RNA was then used to synthesize cDNA using the TaqMan Reverse Transcription Reagents (Applied Biosystems). Pre-designed TaqMan Gene Expression assays for dCK (Assay ID: Hs01040726_m1) and GAPDH (Applied Biosystems, Part: Hs02758991_g1) as an endogenous control for quantification were used for qPCR analysis. The samples were run on a 48-well StepOne Real-Time PCR System (Applied Biosystems) and were analyzed with the StepOne Software v2.0 (Applied Biosystems) using the comparative C_t method ($\Delta\Delta C_t$). The qPCR mixture (50 μL) contained 15 ng cDNA, TaqMan Universal PCR Master mix and the appropriate TaqMan assay. Each assay included cDNA template in triplicates.



SUPPLEMENTAL FIGURE 1. Roles of dCK and CDA in gemcitabine and clofarabine metabolism. (A) Gemcitabine and clofarabine are phosphorylated and activated by dCK while only gemcitabine is deaminated and inactivated by CDA. *In vitro* ^{3}H-dCyd (B) enzymatic and (C) cell-based uptake assays using the L1210 cell lines, with or without the CDA inhibitor, tetrahydrouridine (THU, 100 µM). Results are normalized to protein concentration (B) or cell number (C), and representative of three independent experiments. dFdC – gemcitabine, clof – clofarabine, dCK – deoxycytidine kinase, CDA – cytidine deaminase, dFdU – deaminated gemcitabine, MP – monophosphate, THU – tetrahydrouridine.



SUPPLEMENTAL FIGURE 2. Structures of ^{18}F-FAC and L-^{18}F-FMAC, two PET probes that are phosphorylated and trapped by dCK. ^{18}F-FAC is also a substrate for CDA while L-^{18}F-FMAC is resistant to deamination.



SUPPLEMENTAL FIGURE 3. Variation in dCK levels in panels of human lymphoma and ovarian cancer cell lines. (A) Quantative real-time PCR assay of dCK mRNA levels relative to A549 and (B) ^{18}F-FAC enzymatic assay of dCK activity in a panel of human lymphoma cell lines. Arrows indicate cell lines common to both assays. (C) ^{3}H-FAC enzymatic assay of dCK activity in a panel of human ovarian cancer cell lines. Results are normalized to protein concentration.