Supplementary Data

Cellular pharmacodynamics of a novel pyrrolo[3,2-*d*]pyrimidine inhibitor targeting mitochondrial and cytosolic one-carbon metabolism

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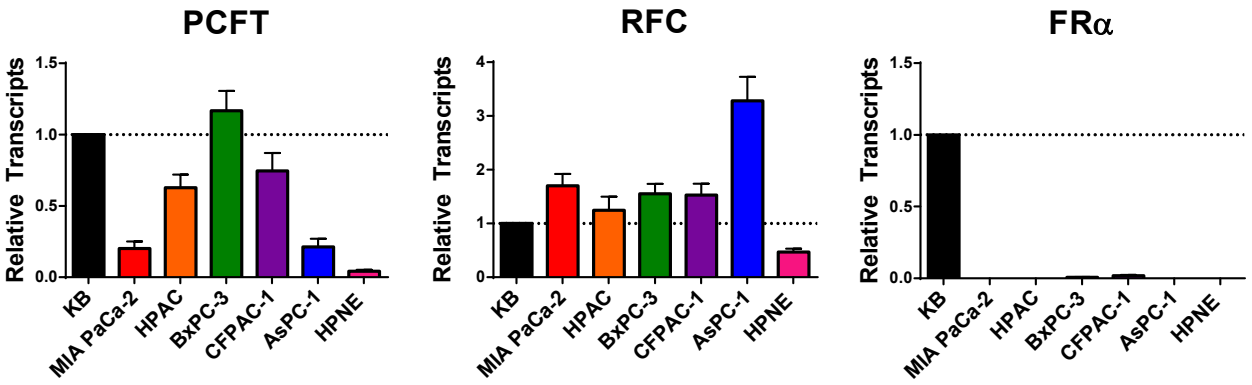

Figure S1. Relative transcripts of the proton-coupled folate transporter (PCFT), reduced folate carrier (RFC), and folate receptor α (FRα) were measured by RT-PCR in PaC cell lines and HPNE (human pancreatic normal epithelial) cells and compared to those in KB cells, which were assigned a value of 1. Results reflect mean values ± standard deviations of 3 biological replicates with transcript levels for target genes normalized to those of glyceraldehyde-3-phosphate dehydrogenase (GAPDH).

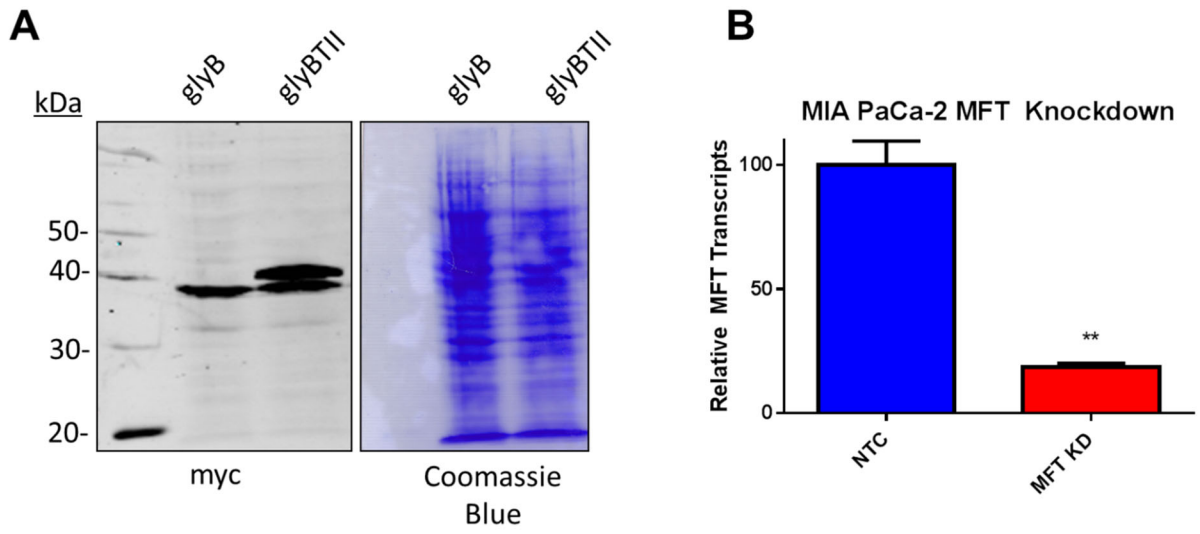

Figure S2. Generation of glyBTII and MIA PaCa-2 MFT KD cell lines. (A) Myc-tagged human MFT cDNA was transfected into glyB cells to generate glyBTII cells. Western Blot of mitochondrial fraction (see **Materials and Methods**) of glyB and glyBTII cells (left) with Coomassie Blue stain of the membrane used as a loading control (right). (B) Relative transcripts of MFT in MIA PaCa-2 cells transduced with non-targeted control (NTC) and MFT-targeted shRNA. Results (mean values ± standard deviations) represent three biological replicates. A pairwise statistical comparison was performed by a two-sided unpaired t-test against NTC. **, $p < 0.01$.

Table S1: Purity of Subcellular Fractions		
MIA PaCa-2 NTC	**Cytosol**	**Mitochondria**
LDH Activity	96.92 (1.69)	3.08 (1.69)
SDH Activity	77.53 (6.06)	22.47 (6.06)
MIA PaCa-2 MFT KD	**Cytosol**	**Mitochondria**
LDH Activity	97.22 (0.49)	2.78 (0.49)
SDH Activity	83.78 (3.63)	16.22 (3.63)
GlyB	**Cytosol**	**Mitochondria**
LDH Activity	99.62 (0.06)	0.38 (0.06)
SDH Activity	76.94 (10.93)	23.07 (10.93)
GlyBTII	**Cytosol**	**Mitochondria**
LDH Activity	98.48 (2.15)	1.52 (2.15)
SDH Activity	78.04 (2.64)	21.96 (2.64)
HPAC	**Cytosol**	**Mitochondria**
LDH Activity	94.27 (1.51)	5.73 (1.51)
SDH Activity	84.46 (2.07)	15.54 (2.07)

Subcellular fractions were generated as described in the **Materials and Methods**. Lactate dehydrogenase (LDH) and succinate dehydrogenase (SDH) were assayed spectrophotometrically as markers for cytosol and mitochondria respectively. Results reflect three biological replicates and the percentage of total enzyme activity in each fraction (mean ± standard deviation) is provided.