SUPPORTING INFORMATION

Functional Impact of a Cancer-Related Variant in Human Δ^1-Pyrroline-5-Carboxylate Reductase 1

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Table S1. PYCR1 variants identified in cancer patients

Mutation[1]	Cancer type	Conservation	PolyPhen score	gnomAD
Val126Ile(Ala)	Uterus	Human, Bovine, Mouse	0.081	Found
Arg293Trp	Skin, lung	Human, Bovine, Mouse	0.881	Found
Thr198Met	Skin, lung	Human, Bovine, Mouse, Yeast, Drosophila	0.988	**Not Found**
Gly324Glu(Arg)	Lung, Sarcoma	Human, Bovine, Mouse	0.001	Found

[1]The residue numbers in TCGA reference sequence UniProt P32322-3, which is an isoform of the UniProt canonical PYCR1 sequence P32322-1. P32322-3 is generated by alternative splicing and includes the 27-residue mitochondrion signaling peptide. In this study, we use the UniProt P32322-1 and refer to the Thr198Met variant as Thr171Met.

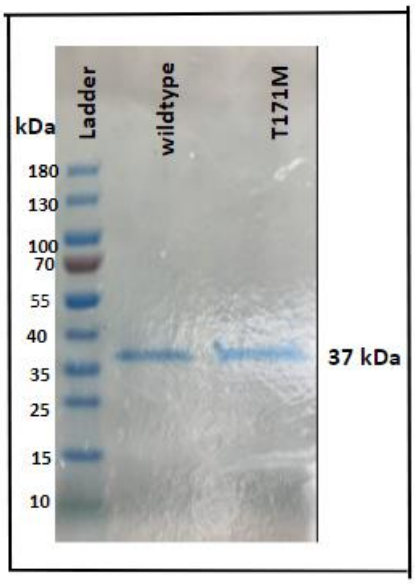

Figure S1. SDS-PAGE analysis of purified PYCR1 wild-type and T171M variant (Coomassie staining).

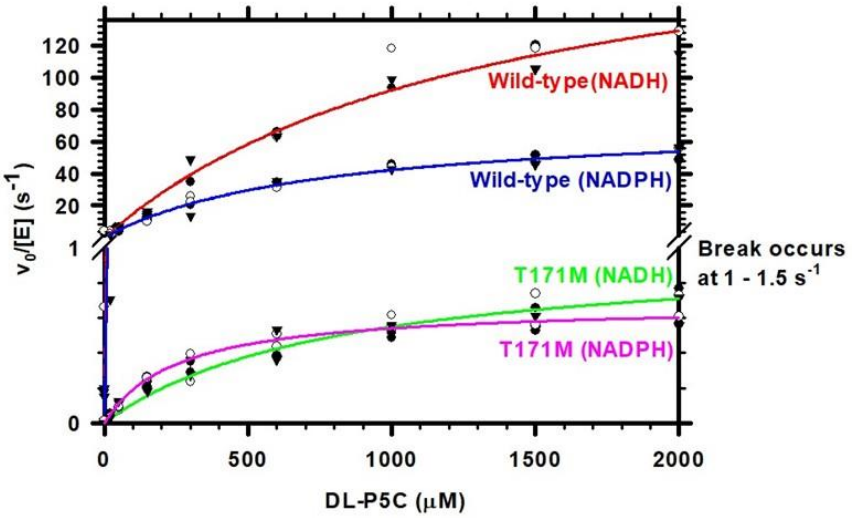

Figure S2. Michaelis-Menten plots of PYCR1. PYCR1 wild-type (0.05 μM or 0.39 U) and T171M variant (0.5 μM or 0.018 U) were assayed with DL-P5C as the variable substrate. Shown are the fits for assays of PYCR1 wild-type with NADH (red line) and NADPH (blue line) fixed at 0.5 mM and the PYCR1 T171M variant with NADH (green line) and NADPH (magenta line) fixed at 0.5 mM. Note the break in the vertical axis. Each curve is a global fit of three replicates to the Michaelis-Menten equation using SigmaPlot 12.5.

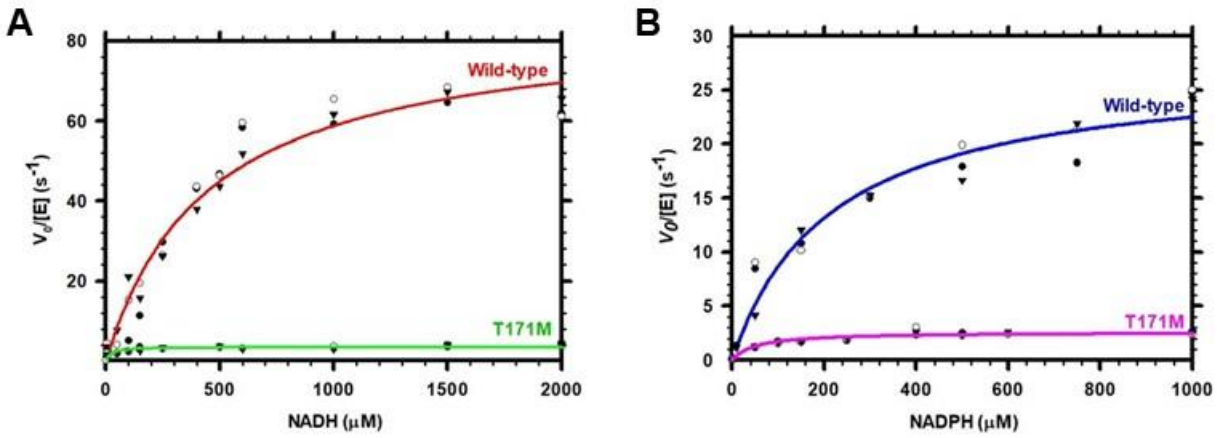

Figure S3. Michaelis-Menten analysis of PYCR1 wild-type (0.05 μM or 0.39 U) and T171M variant (0.5 μM or 0.018 U) with varying NADH and NADPH. (A) Initial rate data for PYCR wild-type (red line) and T171M variant (green line) as a function of NADH concentration with fixed DL-P5C (5 mM). (B) Initial rate data for PYCR wild-type (blue line) and T171M variant (magenta line) as a function of NADPH concentration with fixed DL-P5C (5 mM). In both panels, each curve is a global fit of three replicates to the Michaelis-Menten equation using SigmaPlot 12.5.

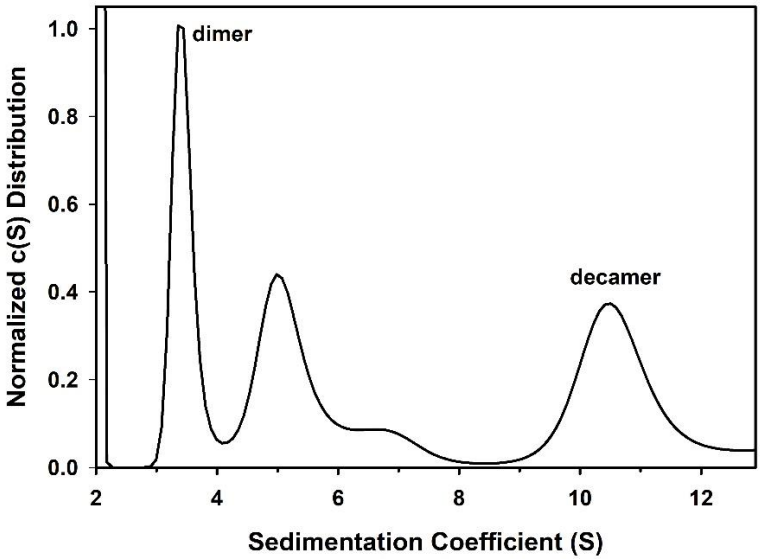

Figure S4. Sedimentation velocity analysis of the PYCR T171M variant. Sedimentation coefficient distribution for the PYCR T171M variant (4.3 mg/mL). Major peaks were observed at 3.4S, 5S, and 10.5S. The 3.4S peak is assigned to a dimer (~70 kDa) and the 10.5S peak is assigned to a decamer (~380 kDa), i.e., pentamer of dimers.