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Supporting information for article:

Structure of *Thermococcus litoralis* Δ^1-pyrroline-2-carboxylate reductase in complex with NADH and L-proline

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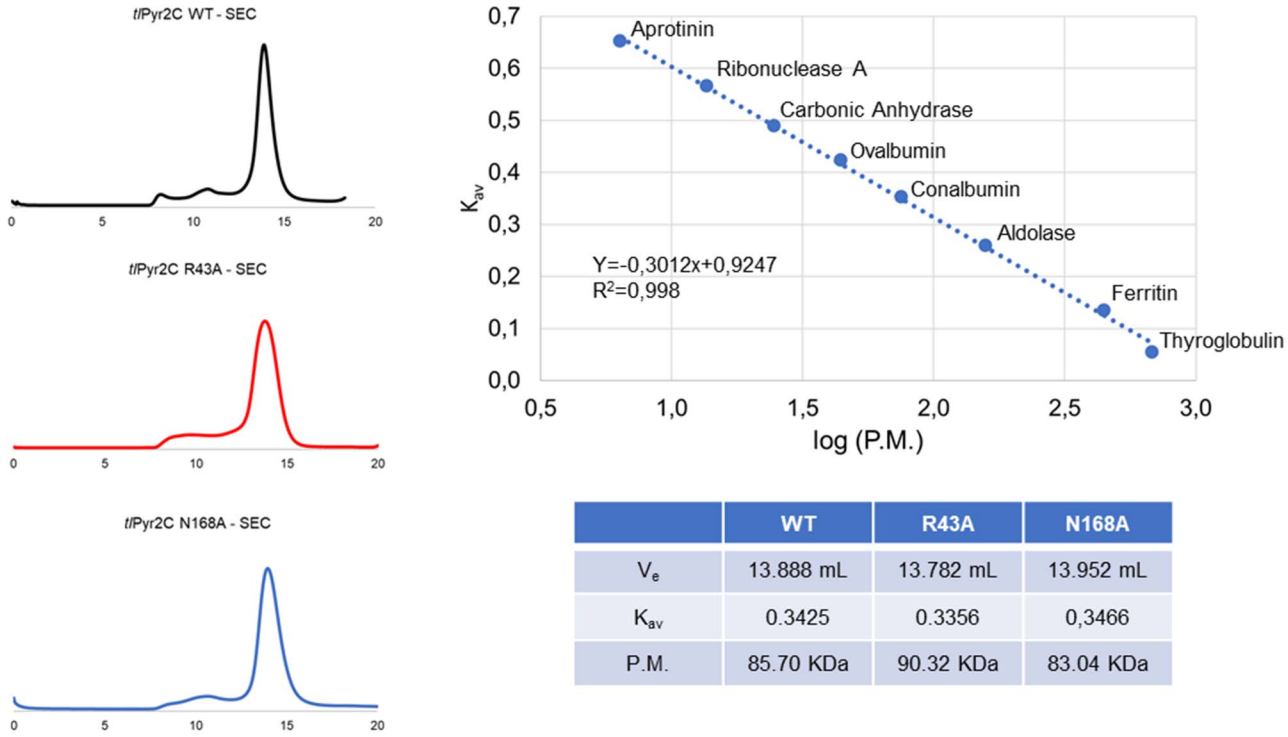

	WT	R43A	N168A
V_e	13.888 mL	13.782 mL	13.952 mL
K_{av}	0.3425	0.3356	0,3466
P.M.	85.70 KDa	90.32 KDa	83.04 KDa

Figure S1 Left: size-exclusion (SEC) chromatography reporting the absorbance at 280 nm of eluted *tl*Pyr2C (black line), *tl*Pyr2C R43A mutant (red line) and *tl*Pyr2C N168A (blue line). On the top right: calibration curve used to estimate molecular weights for *tl*Pyr2C and mutants. The calibration curve was plotted using the gel-phase distribution coefficient (K_{av}) versus the logarithm of the molecular weight (Log Mw). $K_{av} = (V_e - V_o)/(V_c - V_o)$ where V_e = elution volume, V_o = column void volume. Straight line is the calibration curve calculated from the data for molecular weight standards ($R^2 = 0.998$). The equation Y=-0.3012x + 0.9247 from the calibration curve was used to calculate the experimental molecular weights reported in the bottom-right table.

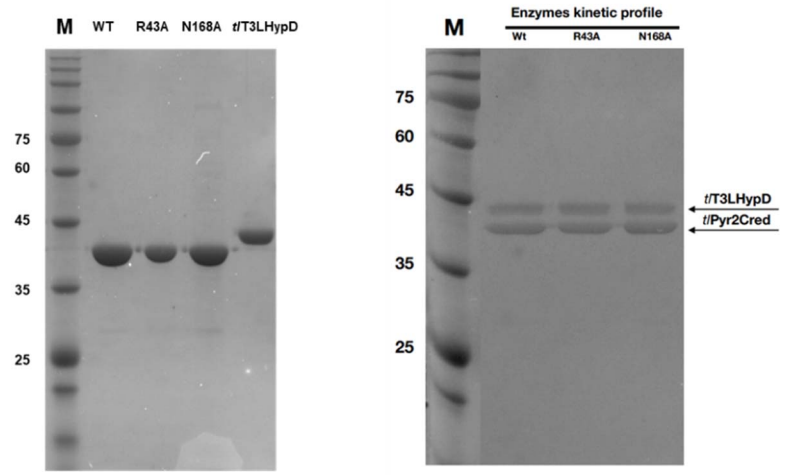

Figure S2 Left: SDS-PAGE of purified *tl*Pyr2C. Right: SDS-PAGE of representative *tl*T3LHypD and *tl*Pyr2C (wild type and mutants) coupled reactions.

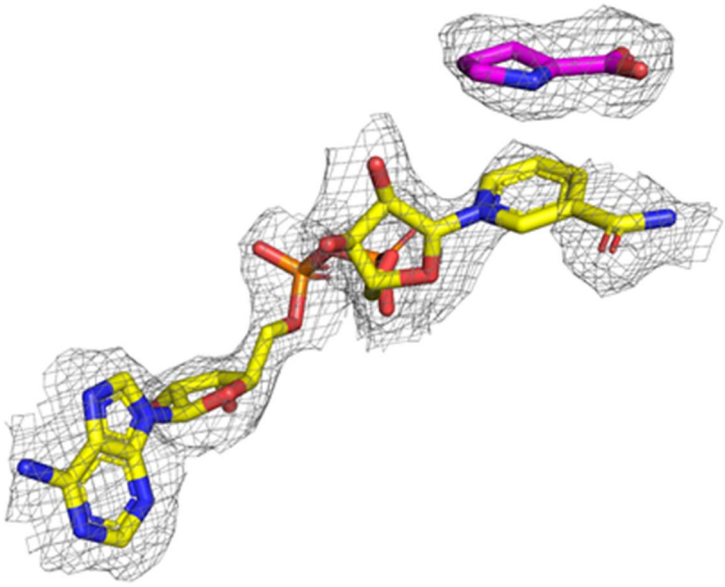

Figure S3 Unrefined, unbiased Fo-Fc map showing residual electron density (1.5σ) corresponding to NADH (in yellow) and L-Pro (in magenta).

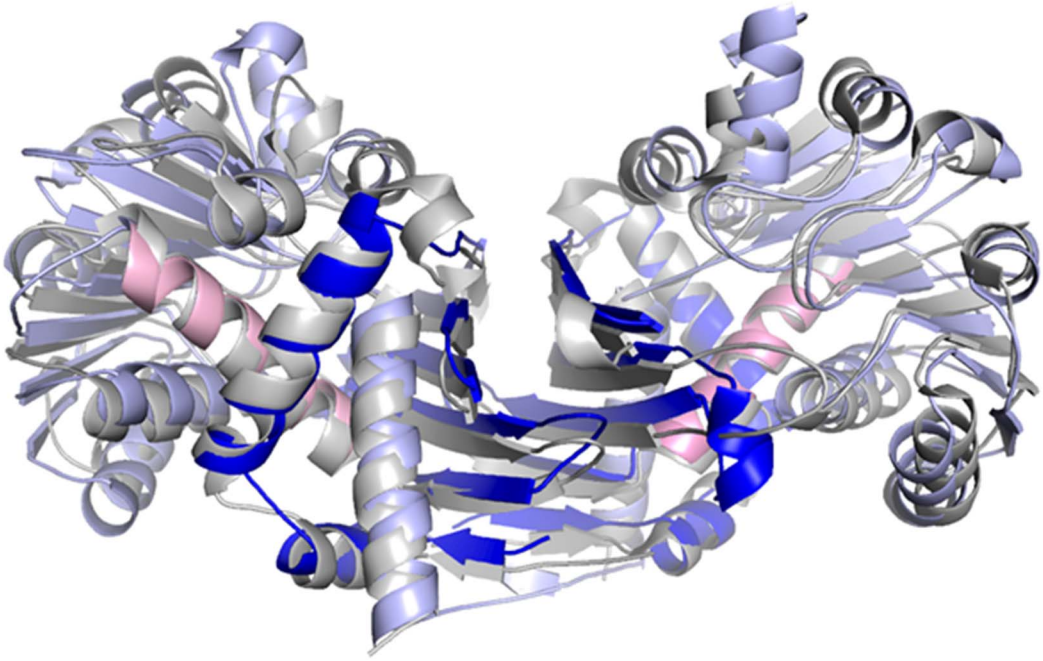

Figure S4 Structure overlay of *tl*Pyr2C and human μ-crystallin (RMSD=1.82 Å; PDB code: 2I99). In light grey: human μ-crystallin.