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The inhibition of triosephosphate isomerase by phosphoenolpyruvate in the feedback-regulation of glycolysis

Supplementary Figures

S1

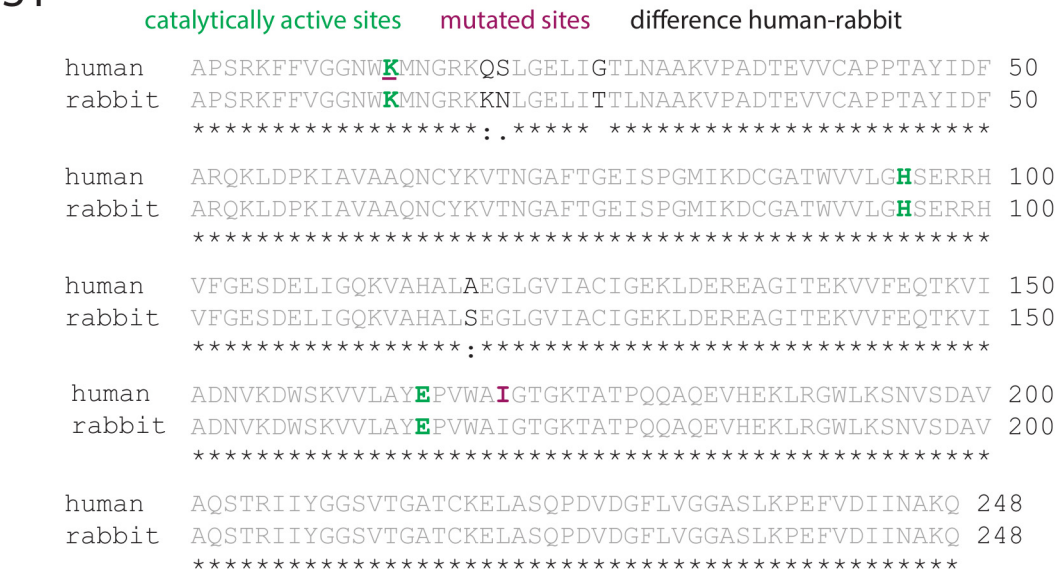

Fig S1: Alignment of human and rabbit TPI, as well as overview of catalytic and mutant residues

The alignment of human TPI (Swiss-Prot: P60174) and rabbit TPI (Swiss-Prot: P00939) amino acid sequences was generated with an EMBL-EBI clustalW. The catalytically active residues are highlighted in green and the residues that were experimentally exchanged are indicated in purple. Human and Rabbit TPI are virtually identical, and differ in four non-catalytic residues.

S2

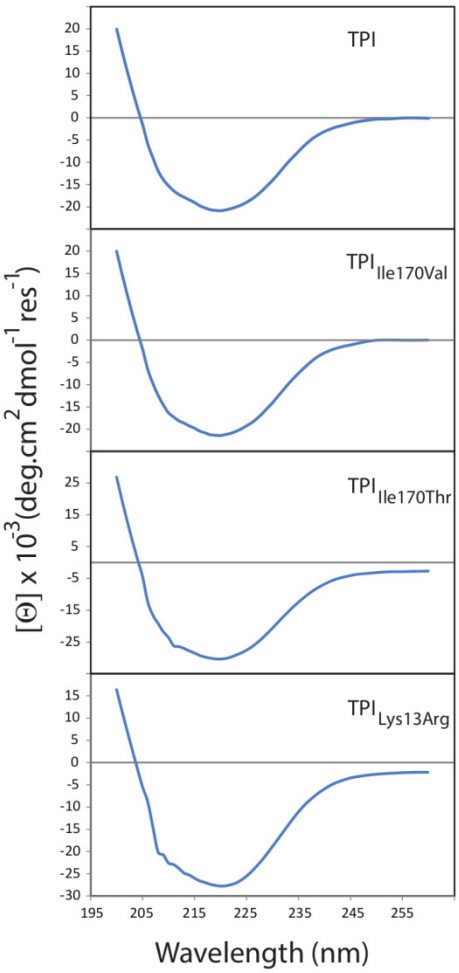

Fig S2: Far UV-Circular Dichroism spectra for purified TPI, $TPI_{Ile170Val}$, $TPI_{Ile170Thr}$ and $TPI_{Lys13Arg}$

The secondary structures of the four purified proteins were measured in 20 mM HEPES buffer, pH 7.5. The CD spectra indicate similar secondary structure composition of TPI enzymes, indicating correct folding of the TPI mutants.

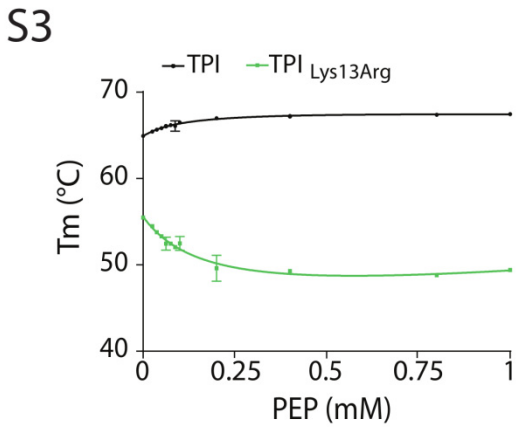

Fig S3: TPI $_{Lys13Arg}$ is thermally unstable.

Thermal melt assays upon adding increased concentrations of PEP to wild-type TPI and TPI $_{Lys13Arg}$. TPI $_{Lys13Arg}$ was thermally unstable, and PEP further destabilized the mutant enzyme.

S4

	G6P		F6P		F1,6BP		DHAP	
	AVR	STD	AVR	STD	AVR	STD	AVR	STD
TPI	106.75	5.30	75.38	25.55	223.63	21.97	33.57	29.41
TPI Ile170Val	84.64	6.42	40.90	2.83	260.59	25.80	107.15	31.22
TPI Ile170Thr	78.05	5.81	72.58	20.67	252.67	16.49	76.15	34.93

	G3P		2,3-PG		PEP		Pyr	
	AVR	STD	AVR	STD	AVR	STD	AVR	STD
TPI	7.15	1.27	20.03	1.85	2.13	0.30	90.93	15.01
TPI Ile170Val	4.31	0.76	17.38	4.61	1.60	0.29	83.40	5.58
TPI Ile170Thr	7.74	0.66	19.41	2.41	1.44	0.40	87.80	12.52

	6PG		R5P		Rl5P + X5P		S7P	
	AVR	STD	AVR	STD	AVR	STD	AVR	STD
TPI	5.82	0.80	1.31	0.25	3.23	1.08	76.98	10.45
TPI Ile170Val	7.30	2.89	1.95	0.31	4.96	1.34	82.48	20.36
TPI Ile170Thr	9.94	1.27	1.64	0.59	5.14	1.69	100.73	13.13

G6P, glucose 6-phosphate, **F6P,** fructose 6-phosphate, **F1,6BP,** fructose 1,6-phosphate, **DHAP,** Dihydroxyacetone phosphate, **G3P,** glyceraldehyde 3-phosphate, 2,3-PG, 2-phosphoglycerate + 3-phosphoglycerate, **PEP,** phosphoenolpyruvate, **Pyr,** pyruvate, **6PG,** 6-phosphogluconate, **R5P,** ribose 5-phosphate, **Rl5P + X5P,** ribulose 5-phosphate + xylulose 5-phosphate, **S7P,** seduheptulose 7-phosphate, **AVR,** average, **STD,** standard deviation. Concentrations are given in μM in a 100 μl cell extract.

Fig S4: Absolute concentrations

Glycolytic and PPP intermediate concentrations as determined by LC-SRM in yeast extracts expressing human wild-type TPI, TPI $_{Ile170Val}$ or TPI $_{Ile170Thr}$ in micromol / ml*OD_{600}; n=3