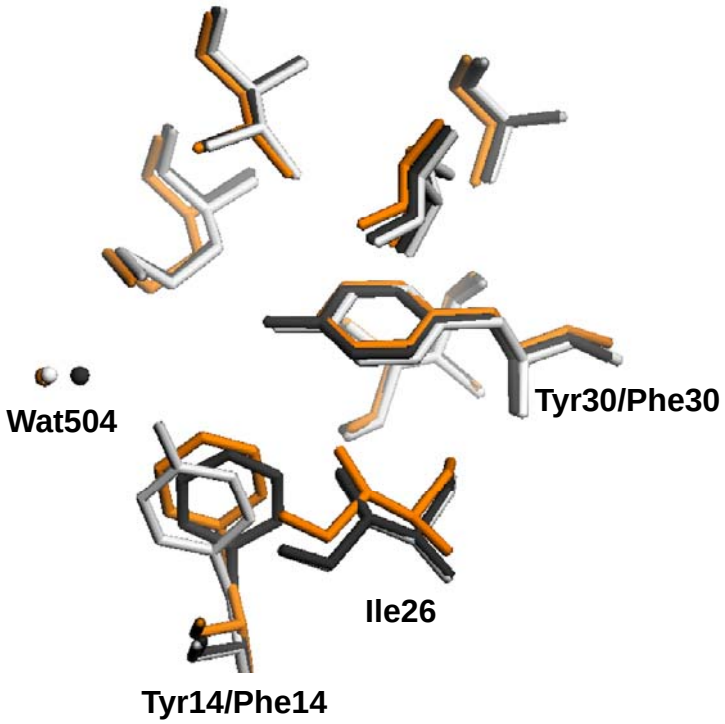

Supplementary Fig. 1 The conformational changes near residue 30. WT (light gray; PDB code 1OPY), Y14F (dark gray; PDB code 1EA2) and Y14F/Y30F (yellow; PDB code 4K1U). The figure was prepared using PyMOL.

Supplementary Table 1. Thermodynamic parameters from urea denaturation of WT and its mutant KSIs [a]

Enzyme	$\Delta G_U^{H_2O}$ [b] (kcal/mol)	*m*-value [c] (kcal/mol M)	$urea_{50\%}$ [d] (M)	$\Delta\Delta G_U^{H_2O}$ (kcal/mol)
WT	24.5 ± 0.89	3.37 ± 0.19	5.31 ± 0.06	
Y30F	24.4 ± 0.55	3.18 ± 0.06	5.61 ± 0.07	0.1
Y14F	20.1 ± 0.53	3.07 ± 0.10	4.40 ± 0.03	4.4
Y14F/Y30F	23.3 ± 0.05	3.24 ± 0.06	5.15 ± 0.08	1.2
Y55F	21.0 ± 0.21	2.98 ± 0.02	4.79 ± 0.08	3.6
Y30F/Y55F	24.3 ± 0.28	2.99 ± 0.08	5.91 ± 0.08	0.3
Y14F/Y55F	21.8 ± 0.33	3.68 ± 0.07	4.14 ± 0.10	2.7
Y14F/Y30F/Y55F	26.1 ± 0.48	3.46 ± 0.07	5.63 ± 0.03	-1.6

[a] All data collected at 25 °C in 20mM potassium phosphate, pH 7.0, 1mM DTT. [b] Free energy difference between the native and denatured states in units of kilocalories per mole. [c] The *m*-value is the change in free energy with respect to the change in [urea]. [d] Midpoint concentration, $urea_{50\%}$, is the [urea] at which half of the protein is denatured in units of molar.

Supplementary Table 2. Thermodynamic parameters from thermal denaturation of WT and its mutant KSIs

Enzyme	T_m (°C)
WT	65.6 ± 0.3
Y30F	66.4 ± 0.4
Y14F	59.4 ± 0.5
Y14F/Y30F	65.8 ± 0.1
Y55F	61.7 ± 0.4
Y30F/Y55F	66.8 ± 0.3
Y14F/Y55F	58.5 ± 0.7
Y14F/Y30F/Y55F	68.5 ± 0.2

Supplementary Table 3. Contacts between the aromatic moiety of residue 30 and its surrounding residues in WT and mutant KSIs

Enzyme	Number of contacts [a]
WT	17
Y30F	17
Y14F	18
Y14F/Y30F	21
Y55F	14
Y30F/Y55F	19
Y14F/Y55F	19
Y14F/Y30F/Y55F	21

[a] All the interatomic contacts were calculated using 4.0 Å cut-off