Table 1. Human TS interface residues (yellow background: interesting Ala Scan [9] results)**:**

Residue	Contacts with the other monomer (CSU [8])	Mutations	G [a] (dimer)	G (mono-mer)	Other things
Val45	Y202 (dc) V204 (hphob) N205 (dc)	V45A&D254N, V45I&T125I& A144S&K308R --> 5-FdUR resistant [7]	0.5/0.5[b] 0.3/0.5	0.6/0.1 0.7/0.5	binding mRNA? [10] peptide N22 [11]
Arg46	V204 (dc) N205 (H-bond, dc)		-[c]	1.0/0.4	binding mRNA? peptide N22
Lys47*[d]	P172 (vdW) D173 (H-bond, dc) D174 (H-bond) R175 (dc) Y202 (hphob, dc) V203 (dc) V204 (hphob)	K47E --> active but not resistant [12]; K47Q&D48E double mutant --> high level of resistance to 5-FdUR [13]	1.9/2.4 2.6/2.0	0.9/0.5 0.5/0.01	binding mRNA? peptide N22
Asp48	D173 (H-bond)	D48E/V --> moderate resistance to 5-FdUR; high-level of resistance in double mutant with K47Q; other resistant multimutants: D48E&T51S&G52, D48E&Y33W [7,13]	-0.6/-0.6 0.04/0.04	-0.2/-0.1 -0.9/-0.9	peptide N22
Asp49*	D173 (dc) R175 (H-bond, dc)	D49G --> resistance to 5-FdUR and Thymitaq, but not Tomudex or BW1843U89; D49N --> inactive [12] EcTS: D20G/A/P/F/LY/Q/ H/K/R/C/S/E --> inactive/active[22]	2.2/0.2 1.0/0.8	4.5/5.5 0.7/1.6	peptide N22

Residue	Contacts with the other monomer (CSU [8])	Mutations	G [a] (dimer)	G (mono-mer)	Other things
Arg50* [e]	N171 (dc) D173 (H-bond, dc) R175 (dc) R176 (dc)	R50C --> inactive [12] EcTS: R21G/P/S/H/A/F/L/C/Y/Q/K/E --> less active than wt/inactive [14, 22] LcTS: R23A/D/E/F/G/H/I/K/L/N/P/C/Q/S/V/Y -->activity lost/decreased; effect on co-factor binding and catalysis [21, 22]	-2.7/-2.7 0.1/0.1	0.7/0.6 -0.7/-0.7	peptide N22 active site core residue
Thr51*	R175 (dc)	T51S --> 5-FdUR resistant; T51A --> inactive; resistant multimutants: T51S&G52S, D48E&T51S&G52, T51S&K82Q& K99D&N171S --> 5-FdUR resistant [7,12,13] EcTS: T22A/G/F/L/C/H/R/E/P/S/Y/Q/K --> inactive/active [22]	0.2/-1.5	4.8/4.0	peptide N22
Thr55*	R175 (H-bond, dc)	T55I&V106A& K284I --> 5-FdUR resistant [7] EcTS: T26Y/K/R/A/G/P/F/L/C/S/Q/H/E --> activity lost/decreased/active [22]	0.1/0.1	1.0/0.7	peptide N22
Ser57*	Y202 (H-bond)	EcTS: S28P/L/Y/H/K/A/G/F/C/Q/R/E --> activity lost/decreased/active [22]	1.1/-0.5 1.2/0.2	0.6/0.7 1.1/0.6	binding mRNA? peptide N22
Val58*	R64 (dc) Y202 (vdW)	EcTS: I29A/G/P/K/R/E/F/L/C/S/Y/Q/H --> inactive/active [22]	0.1/0.1	1.9/1.7	binding mRNA? peptide N22

Residue	Contacts with the other monomer (CSU [8])	Mutations	G [a] (dimer)	G (mono-mer)	Other things
Phe59*	R64 (H-bond, dc) Q200 (hphob) Y202 (arom, hphob) S209 (dc) C210 (vdW) Q211 (hphob) I249 (hphob, dc)	F59L --> inactive [12] EcTS: F30A/G/P/C/S/Q/H/K/R/E/L/Y --> inactive/active [22]	3.8/3.9 4.3/4.3	0.4/0.8 0.1/0.02	binding mRNA? peptide N22
Gly60*	Q62 (dc) R64 (H-bond) Q211 (hphob) I249 (hphob, dc)	EcTS: G31P/F/L/Y/Q/H/K/R/E/A/C/S --> activity lost/decreased/active [22]	-[e]	0.5/0.4	binding mRNA? peptide N22
Met61*	Q62 (H-bond, dc)		0.1/0.2 -0.02/-0.02	2.1/2.4 1.7/1.7	binding mRNA? peptide N22
Gln62*	G60 (dc) M61 (H-bond, dc) Q62 (H-bond, hphob, dc) T251 (dc)	EcTS: Q33A/G/P/L/F/C/S/Y/H/R/K/E --> active [22]	-0.5/-0.8 4.5/4.8	1.2/1.1 0.7/0.6	binding mRNA? peptide N22
Arg64*	H39 (H-bond) V58 (dc) F59 (H-bond, dc) G60 (H-bond)	R64A --> mRNA binding lost [10] EcTS: R35A/G/P/L/F/C/S/Y/Q/H/K/E --> active/activity decreased [22]	2.2/0.8 2.6/1.5	1.8/1.8 0.9/1.0	binding mRNA? peptide N22
Phe142*	F142 (arom) W182 (vdW) N183 (vdW) P184 (hphob) R185 (hphob, dc)	F142S&F225I --> 5-FdUR resistant [7]	1.4/1.4 1.6/1.5	1.4/1.7 1.7/1.7	binding mRNA?
Gly143	R185 (H-bond)		-	1.2/0.7	binding mRNA?
Val158*	P184 (hphob, dc) R185 (hphob)		0.4/0.5 0.4/0.6	1.1/0.7 1.0/1.0	
Gln160*	W182 (H-bond) P184 (hphob, dc)		0.3/0.4 0.2/0.3	2.6/2.8 2.3/2.8	

Residue	Contacts with the other monomer (CSU [8])	Mutations	G [a] (dimer)	G (mono-mer)	Other things
Arg163	-		-	0.5/0.2	not binding mRNA? [10] at the interface in the inactive form
Asp173	K47 (H-bond, dc) D48 (") D49 (vdW) R50 (H-bond, dc)		-0.7/-0.9 -0.1/-0.1	-0.1/0.1 -0.9/-0.9	not binding mRNA? peptide M17 [11]
Asp174	K47 (H-bond)		- -0.1/-0.1	0.4/0.6 -0.7/0.01	not binding mRNA? peptide M17
Arg175*	K47 (dc) D49 (H-bond, dc) R50 (dc) T55 (H-bond, dc) R215 (") S216 (dc) D254 (hphob, dc) H256 (H-bond) Y258 (")	EcTS: R126E --> void of activity (especially the double mutant with C146W, cf. hTS C195) [15]; R126G/A/P/F/H/L/C/S/Y/Q/K--> activity lost/decreased/active [22] LcTS: R178F --> more labile tertiary&quaternary interactions; no activity [16, 22]; R178A/C/D/E/G/I/K/L/P/S/T/V/W --> activity lost/decreased; effect on dUMP binding [21, 22]	-1.3/0.2 3.4/5.1	0.4/0.2 -0.1/0.04	not binding mRNA? peptide M17 active site core residue
Arg176*	R50 (dc) W182 (hphob) L192 (dc) P193 (dc) R215 (dc)	EcTS: R127A/C/E/F/G/H/K/L/P/Q/S/Y --> active [22] LcTS: R179A/C/D/E/F/G/H/K/L/M/P/Q/S/T/V/W --> activity retained except for R179P; effect on dUMP binding [21]	-3.2/-2.9 0.7/0.8	0.4/0.4 -0.3/-0.2	binding mRNA? not binding mRNA? peptide M17 active site core residue

Residue	Contacts with the other monomer (CSU [8])	Mutations	G [a] (dimer)	G (mono-mer)	Other things
Ile178*	W182 (H-bond, hphob, dc) A197 (hphob) Y213 (hphob) R215 (hphob)		2.2/2.2 1.6/1.6	1.8/2.2 1.7/1.8	binding mRNA? peptide M17
Cys180*	C180 (vdW) W182 (hphob) P184 (hphob) L198 (vdW)	C180A --> completely devoid of RNA binding activity; enzymatic activity >80% of wt [17]	0.2/02 -0.1/-0.1	0.5/1.6 5.7/5.9	binding mRNA? peptide M17
Trp182*	F142 (vdW) R176 (hphob) I178 (H-bond, hphob, dc) C180 (hphob) W182 (dc) L198 (hphob)		2.3/2.1 2.6/2.4	3.1/2.9 2.1/2.4	binding mRNA? peptide M17 active site loop
Asn183*	R185 (H-bond, dc)		1.9/0.1 0.5/0.1	1.4/1.0 4.2/4.3	binding mRNA? peptide M17 active site loop
Pro184*	F142 (hphob) V158 (hphob, dc) Q160 (") C180 (hphob)		1.1/0.8	2.0/2.1	binding mRNA? peptide M17 active site loop
Arg185	F142 (hphob) G143 (vdW) V158 (hphob) R185 (dc)		-1.2/ -0.02 0.2/0.8	2.0/-0.2 3.2/-0.03	binding mRNA? peptide M17 active site loop
Asp186	-		0.6/-	1.6/0.6	binding mRNA? peptide M17 active site loop
Leu187*	R163 (?)		-0.3/-0.3 0.1/0.1	3.0/3.0 2.6/2.6	binding mRNA? peptide M17 active site loop

Residue	Contacts with the other monomer (CSU [8])	Mutations	G [a] (dimer)	G (mono-mer)	Other things
Leu189	-		-	0.8/0.7	binding mRNA? peptide M17 active site loop at the interface in the inactive form
Met190*	-		-	2.8/3.0	binding mRNA? peptide M17 active site loop at the interface in the inactive form
Ala191*	R176 (H-bond)		-	0	binding mRNA? peptide M17 active site loop at the interface in the inactive form
Leu192*	R176 (dc)		-0.2/-0.3 0.1/-0.01	0.8/0.1 1.1/-0.4	binding mRNA? peptide M17 active site loop
Pro193*	R176	LcTS: P196G/F/V/I/T/N/C/S/Y/H/K/R/D/A/P/L/E --> active/activity decreased [22, 23]	0.1/0.1	2.4/2.4	peptide M17 active site loop
Pro194*	-	P194Q --> 5-FdUR resistant [7] LcTS: P197A/F/L/I/M/W/T/C/S/Y/Q/H/R --> active/activity decreased/lost [22, 23]	-	3.1/3.4	active site loop at the interface in the inactive form
Ala197	I178 (hphob) L198 (hphob)	A197V/M/L --> drug resistant; almost all aromatic residues --> inactive [18]; A197F, A197V&L198I& C199F --> 5-FdUR resistant [19]	0	0	active site loop

Residue	Contacts with the other monomer (CSU [8])	Mutations	G [a] (dimer)	G (mono-mer)	Other things
Leu198*	W182 (hphob) A197 (hphob, dc) L198 (hphob) Y213 (hphob)	L198I/T/F --> drug resistant (mostly hydrophobic); charged mutants -->inactive [18]; A197V&L198I& C199F --> 5-FdUR resistant [19]	2.1/2.1 1.6/1.5	1.4/1.7 1.0/1.5	peptide C20 [11]
Gln200*	F59 (hphob) Y213 (H-bond, hphob, dc) R215 (H-bond, dc) G253 (vdW) D254 (H-bond, dc)	Q200H&V204M --> 5-FdUR resistant [19] EcTS: Q203A/G/P/F/L/C/S/Y/K/R/H/E --> inactive/active [22]	1.1/-0.02 2.9/3.2	0.7/0.6. 0.2/0.5	peptide C20
Tyr202*	V45 (dc) K47 (hphob, dc) S57 (H-bond) V58 (H-bond) F59 (H-bond, arom, hphob) D254 (hphob)	C199L&Y202F& V204L&S206N --> 5-FdUR resistant [19]	2.5/3.1 2.4/3.4	0.7/1.2 0.3/0.6	binding mRNA? peptide C20
Val203*	K47 (dc)		0.6/-	2.1/2.6	binding mRNA? peptide C20
Val204	V45 (hphob) R46 (dc) K47 (hphob)	V204Y/S/T/R/Q/N/D/A/L --> drug resistant (mostly polar&charged); many 5-FdUR resistant multi-mutants [18,19]	1.7/1.3 0.8/0.8	-0.5/0.02 0.1/0.2	binding mRNA? peptide C20
Asn205	V45 (dc)	N205&S206R --> 5-FdUR resistant [19]	0.6/-0.4 0.2/0.1	-0.1/0.6 0.2/0.4	binding mRNA? peptide C20
Ser209*	F59 (dc)	C199L&V204L& N205Q&S209Y --> 5-FdUR resistant (see below the 5x-mutant) [19]	-0.02/-0.1 -0.1/-0.1	0.4/0.4 0.5/0.5	binding mRNA? peptide C20

Residue	Contacts with the other monomer (CSU [8])	Mutations	G [a] (dimer)	G (mono-mer)	Other things
Cys210*	F59 (vdW)	C210A --> enzymatic activity and RNA binding retained [17]; C210S in 5-FdUR resistant multi-mutants with C199L&V204L& S206G&S209F or A197C&C199L& V204G&S206G [19]	-0.1/-0.1	0.7/0.3	binding mRNA? peptide C20
Gln211*	F59 (hphob) G60 (H-bond) Y213 (H-bond, hphob, dc) T251 (dc) L252 (H-bond, dc) G253 (vdW)	Q211L, S6N&D69Q& Q211L --> 5-FdUR resistant [7,19]	-0.2/-0.4 2.5/2.9	1.6/1.4 1.0/0.8	binding mRNA? peptide C20
Tyr213*	I178 (hphob) L198 (hphob) Q200 (H-bond, phob, dc) Q211 (") Y213 (arom, hphob)		3.2/3.1 5.5/4.5	1.9/1.9 1.4/1.5	binding mRNA? peptide C20
Arg215*	R175 (H-bond, dc) R176 (dc) I178 (hphob) Q200 (H-bond, dc)	R215A --> mRNA binding lost [10] EcTS: R166G/A/P/F/L/C/S /Y/Q/H/K/E --> inactive [22] LcTS: R218K --> more labile tertiary&quaternary interactions [16]; R218A/E/F/G/H/K/ L/Q/S/T/V/Y/W --> all inactive except for R178K; essential for the structure of the catalytic site [21, 22]	-0.4/0.2 1.6/1.6	2.2/2.0 2.8/2.7	binding mRNA? peptide C20 active site core residue

Residue	Contacts with the other monomer (CSU [8])	Mutations	G [a] (dimer)	G (mono-mer)	Other things
Ser216*	R175 (vdW)	S216T --> as wt; S216C/R/L/M/D/A/H/Y/N/V/P --> inactive or much less active than wt [20]	-0.6/-0.7 -0.1/-0.04	-0.9/-0.8 -0.5/-0.4	binding mRNA? peptide C20 active site core residue
Asp247	-		0.4/0.4	1.0/-0.03	
Ile249*	F59 (hphob, dc) G60 (vdW)		0.8/0.6 1.0/1.0	2.9/2.9 2.3/2.3	
Thr251*	Q62 (dc) Q211 (dc) Y213 (vdW) T251 (hphob, dc)		0.3/0.3 4.1/4.0	2.6/2.4 2.0/1.7	
Gly253*	Q200 (vdW) Q211 (vdW)	EcTS: G204P/F/L/C/Y/Q/H/K/R/E/A/S --> inactive/active [22]	-	0.4/0.4	
Asp254*	R175 (H-bond, hphob, dc) Q200 (dc) Y202 (hphob)	D254N/E/A --> 5-FdUR resistant; also in multi-mutants [7]	1.7/0.3 0.8/0.3	3.4/3.4 4.5/3.0	
His256*	R175 (H-bond)	H256Q/L --> catalytic activity decreased [20]	-1.5/-1.1 0.5/0.5	1.2/2.6 4.2/3.8	active site core residue
Tyr258*	R175 (H-bond)	Y258F, T53S&Y258F --> 5-FdUR resistant [7] LcTS: Y261A/G/P/L/V/W/T/N/S/Q/H/R/E/D/M --> inactive/active [22]; Y261F/A/W/M --> activity decreased [24] EcTS: Y209W --> activity decreased [24]	1.5/0.9 3.6/2.0	3.4/3.5 2.0/2.1	active site core residue

[a]kcal/mol, change in free energy upon residue mutation to alanine, calculated by FoldX [9] and Robetta [25]; [b]A chain/B chain; [c]FoldX did not regard this residue as an interface residue; [d]* = conserved residue; [e]highly conserved residues in bold font

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