

**Supplementary Table S1.**

Function	Residue	Mutation	Location on Protein	Organism	Hs Equivalent	Reference
Iron Binding	E92	E92A	$\alpha 1$	<i>Hs</i>	E92	[54]
	E96	E96A	$\alpha 1$	<i>Hs</i>	E96	[54]
	E100	E100A	$\alpha 1$	<i>Hs</i>	E100	[54]
	E101	E101A	$\alpha 1$	<i>Hs</i>	E101	[54]
	D104	D104A	$\alpha 1$	<i>Hs</i>	D104	[54]
	S105	*1	$\alpha 1$	<i>Hs</i>	S105	[16]
	H83	*1	$\alpha 1$	<i>Sc</i>	S105	[33]
	A107	*1	$\alpha 1$	<i>Hs</i>	A107	[16]
	D86	D86A	$\alpha 1$	<i>Sc</i>	E108	[33]
	E108	E108A	$\alpha 1$	<i>Hs</i>	E108	[16]
	F109-F110	*1	$\alpha 1$	<i>Hs</i>	F109-F110	[16]
	E111	E111A	$\alpha 1$	<i>Hs</i>	E111	[53]
	D112	D112A	$\alpha 1$	<i>Hs</i>	D112	[82]
	E90	E90A	$\alpha 1$	<i>Sc</i>	D112	[33]
	L91	*1	$\alpha 1$	<i>Sc</i>	L113	[34]
	L113-A114	*1	$\alpha 1$	<i>Hs</i>	L113-A114	[16]
	E93	E93A	$\alpha 1$	<i>Sc</i>	D115	[33]
	D115	D115A	$\alpha 1$ - $\beta 1$ Loop	<i>Hs</i>	D115	[82]
	A94	*1	$\alpha 1$	<i>Sc</i>	K116	[33]
	K116	*1	$\alpha 1$ - $\beta 1$ Loop	<i>Hs</i>	K116	[16]
	H95	*1	$\alpha 1$	<i>Sc</i>	P117	[33]
	E121	E121A	$\alpha 1$ - $\beta 1$ Loop	<i>Hs</i>	E121	[82]
	D122	D122A/Y	$\beta 1$	<i>Hs</i>	D122	[82] [54]
	Y123	*1	$\beta 1$	<i>Hs</i>	Y123	[16]
	D124	D124A	$\beta 1$	<i>Hs</i>	D124	[82]
	D101	D101A	$\beta 1$	<i>Sc</i>	D124	[33]
	V102	*1	$\beta 1$	<i>Sc</i>	V125	[18]
	V125-F127	*1	$\beta 1$	<i>Hs</i>	V125-F127	[16]
	E103	E103A	$\beta 1$	<i>Sc</i>	S126	[33]
	L104	*1	$\beta 1$	<i>Sc</i>	F127	[33]
	S105	*1	$\beta 1$	<i>Sc</i>	G128	[33]
	G130	G130V	$\beta 1$	<i>Hs</i>	G130	[54]
	I154	I154F	$\beta 4$	<i>Hs</i>	I154	[54]
	W155	W155R	$\beta 4$	<i>Hs</i>	W155	[54]
	N140	*1	$\beta 4$ - $\beta 5$ Loop	<i>Sc</i>	K164	[33]
NFS Binding	D104-S105	*1	$\alpha 1$	<i>Hs</i>	D104-S105	[16]
	E108-F109	*1	$\alpha 1$	<i>Hs</i>	E108-F109	[18]
	F110	*1	$\alpha 1$	<i>Hs</i>	F110	[16]
	E111	*1	$\alpha 1$	<i>Hs</i>	E111	[18]
	D112-A114	*1	$\alpha 1$	<i>Hs</i>	D112-A114	[16]
	D115-T119	*1	$\alpha 1$ - $\beta 1$ Loop	<i>Hs</i>	D115-T119	[16]
	E121-Y123	*2	$\alpha 1$ - $\beta 1$ Loop	<i>Hs</i>	E121-Y123	[18]
	D124	D124A/K	$\beta 1$	<i>Hs</i>	D124	[18,55]
	V125-F127	*1	$\beta 1$	<i>Hs</i>	V125-F127	[16]
	G128	*2	$\beta 1$	<i>Hs</i>	G128	[18]
	V131	*2	$\beta 2$	<i>Hs</i>	V131	[18]
	N146	N146K	$\beta 3$	<i>Hs</i>	N146	[18]
	K147	*4	$\beta 3$	<i>Hs</i>	K147	[86]
	N151	N151A	$\beta 3$ - $\beta 4$ Loop	<i>Hs</i>	N151	[18]
	K152	*4	$\beta 3$ - $\beta 4$ Loop	<i>Hs</i>	K152	[86]
	I154	I154F	$\beta 4$	<i>Hs</i>	I154	[18], [54]
	W155	W155R	$\beta 4$	<i>Hs</i>	W155	[18] [54]
	K171-N172	*1	$\beta 5$ - $\beta 6$ Loop	<i>Hs</i>	K171-N172	[16]
	W149	W149G	$\beta 6$	<i>Sc</i>	W173	[35]
	Y175	*2	$\beta 6$	<i>Hs</i>	Y175	[18]
	H177	*2	$\beta 6$ - $\alpha 2$ Loop	<i>Hs</i>	H177	[18]
	A204	*1	C-Termini	<i>Hs</i>	A204	[16]

ISCU Binding	G107	*1	$\beta 1$ - $\beta 2$ Loop	<i>Sc</i>	G130	[18]
	T110	*1	$\beta 2$	<i>Sc</i>	V134	[18]
	L111	*1	$\beta 2$	<i>Sc</i>	K135	[18]
	T142	*2	$\beta 3$	<i>Hs</i>	T142	[18]
	Y119	*1	$\beta 3$	<i>Sc</i>	Y143	[18]
	V120	*1	$\beta 3$	<i>Sc</i>	V144	[38]
	V144	*2	$\beta 3$	<i>Hs</i>	V144	[18]
	N122	N122A/K	$\beta 3$	<i>Sc</i>	N146	[42]
	I145-K147	*1	$\beta 3$	<i>Hs</i>	I145-K147	[16]
	K123	K123T	$\beta 3$	<i>Sc</i>	K147	[42]
	Q124	Q124A	$\beta 3$	<i>Sc</i>	Q148	[42]
	T149	*1	$\beta 3$ - $\beta 4$ Loop	<i>Hs</i>	T149	[16]
	P150	*2	$\beta 3$ - $\beta 4$ Loop	<i>Hs</i>	P150	[18]
	N151	N151A	$\beta 3$ - $\beta 4$ Loop	<i>Hs</i>	N151	[18]
	N127	*1	$\beta 3$ - $\beta 4$ Loop	<i>Sc</i>	N151	[34]
	K152	*1	$\beta 3$ - $\beta 4$ Loop	<i>Hs</i>	K152	[16]
	Q153	*1	$\beta 4$	<i>Hs</i>	Q153	[16]
	Q129	Q129A	$\beta 3$ - $\beta 4$ Loop	<i>Sc</i>	Q153	[32]
	I154	I154F	$\beta 4$	<i>Hs</i>	I154	[54]
	W155	W155R/A/F	$\beta 4$	<i>Hs</i>	W155	[54,77]
	W131	W131A/F	$\beta 4$	<i>Sc</i>	W155	[32]
	L156-S158	*1	$\beta 4$	<i>Hs</i>	L156-S158	[16]
	P163	P163G	$\beta 5$	<i>Hs</i>	P163	[18]
	K164	*1	$\beta 5$	<i>Hs</i>	K164	[16]
	R165	R165C	$\beta 5$	<i>Hs</i>	R165	[18]
	R141	R141A	$\beta 5$	<i>Sc</i>	R165	[32]
	W168	*1	$\beta 5$	<i>Hs</i>	W168	[16]
	N154	*1	$\beta 6$ - $\beta 7$ Loop	<i>Sc</i>	D178	[34]
	V166	*1	$\alpha 2$	<i>Sc</i>	L190	[34]
	I170	*1	$\alpha 2$	<i>Sc</i>	L194	[34]
Stability	L106	L106S	$\alpha 1$	<i>Hs</i>	L106	[60]
	D86	D86A	$\alpha 1$	<i>Sc</i>	E108	[33]
	F109	F109L	$\alpha 1$	<i>Hs</i>	F109	[61]
	E93	E93A	$\alpha 1$	<i>Sc</i>	D115	[33]
	D122	D122Y	B1	<i>Hs</i>	D122	[54]
	Y123	Y123S	$\alpha 1$ - $\beta 1$ Loop	<i>Hs</i>	Y123	[61]
	D101	I01A	$\beta 1$	<i>Sc</i>	D124	[33]
	E103	I03A	$\beta 1$	<i>Sc</i>	S126	[33]
	G130	G130Y	$\beta 1$	<i>Hs</i>	G130	[54]
	T110A	T110A	$\beta 2$	<i>Sc</i>	V134	[32]
	G137	G137V	$\beta 2$ - $\beta 3$ Loop	<i>Hs</i>	G137	[84]
	T118A	T118A	$\beta 3$	<i>Sc</i>	T142	[32]
	V120	V120A	$\beta 3$	<i>Sc</i>	V144	[32]
	Q129A	Q129A	$\beta 3$ - $\beta 4$ Loop	<i>Sc</i>	Q153	[32]
	I130	I130A	$\beta 4$	<i>Sc</i>	I154	[32]
	I154	I154F	$\beta 4$	<i>Hs</i>	I154	[54]
	W155	W155R	$\beta 4$	<i>Hs</i>	W155	[54]
	W131	W131A/F	$\beta 4$	<i>Sc</i>	W155	[32]
	L132	L132A	$\beta 4$	<i>Sc</i>	L156	[32]
	S161	S161I	$\beta 4$ - $\beta 5$ Loop	<i>Hs</i>	S161	[61]
	W173	W173G	$\beta 6$	<i>Hs</i>	W173	[85]
	S181	S181F	$\alpha 2$	<i>Hs</i>	S181	[61]
	L182	L182F	$\alpha 2$	<i>Hs</i>	L182	[56]
	L185-L186	*3	$\alpha 2$	<i>Hs</i>	L185-L186	[56]
	L190	*3	$\alpha 2$	<i>Hs</i>	L190	[56]
	L194	*3	$\alpha 2$	<i>Hs</i>	L194	[56]
	T196-K197	Truncation	C-Termini	<i>Hs</i>	T196-K197	[56]
	L198	L198R/A/C	C-Termini	<i>Hs</i>	L198	[57]
	D199	Truncation	C-Termini	<i>Hs</i>	D199	[56]
	L200	L200C	C-Termini	<i>Hs</i>	L200	[57]

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\*1 Chemical shifts observed by NMR

\*2 Cryo-EM confirmed predictions from crosslinking, SAXS and NMR

\*3 Modeling

\*4 LC-MS/MS Analysis