

	Viable?	Telomere length	23 °	36 °
I73A I73S I73Y	Neatly inviable	Very long	+	+/-
		Extremely long	+/-	—
		Very long	+	+/- to (+)
L75A L75S L75Y	inviabie	Med long	+	(+) to +
		Very long	(+) to +	+/- to (+)
		n/a		
I79A I79S I79Y	inviabie	Med long	++	+
		Very long	+ to ++	(+)
		n/a		
I93A I93S I93Y		Med long	++	+
		Very long	+	+/- to (+)
		Slightly long	++	+
L97A L97S L97Y	inviabie	Very long	(+)	+/-
		n/a		
		Very long	(+) to +	+/-
L106A L106S L106Y		Wild type	++	++
		Wild type	++	++
		Wild type	++	++
L140A L140S L140Y		Wild type	++	++
		Wild type	++	++
		Wild type	++	++
V142A V142S V142Y	Neatly inviable inviabie	Slightly long	+ to ++	+
		n.t.	+/-	—
		n/a		
L153A L153S L153Y	inviabie	Very long	(+)	+/-
		n/a		
		Very long	(+)	+/-
V155A V155S V155Y	Neatly inviable inviabie	Very long	+	(+)
		n.t.	+/-	—
		n/a		
L158A L158S L158Y		Very long	+	+
		Very long	+	+
		n.t.	(+)	+/-
G77A G77S G77Y	Neatly inviable	Very long	(+) to +	+/- to +
		n.t.	(+)	+/-
		n.t.	+/-	—
G137A G137S G137Y	+	Very long	+ (+)	(+)
		Long	++	+
		n/a		

**Figure S5** Summary of viability and telomere length of a panel of *stn1*<sup>-</sup> missense mutations introduced into 11 hydrophobic residues with side-chains located in the interior of the β-barrel of the essential N-terminal OB-fold domain of Stn1. Telomere length of selected mutant isolates is shown in Figure S4. The results for mutagenesis of two highly conserved glycine residues are also included.