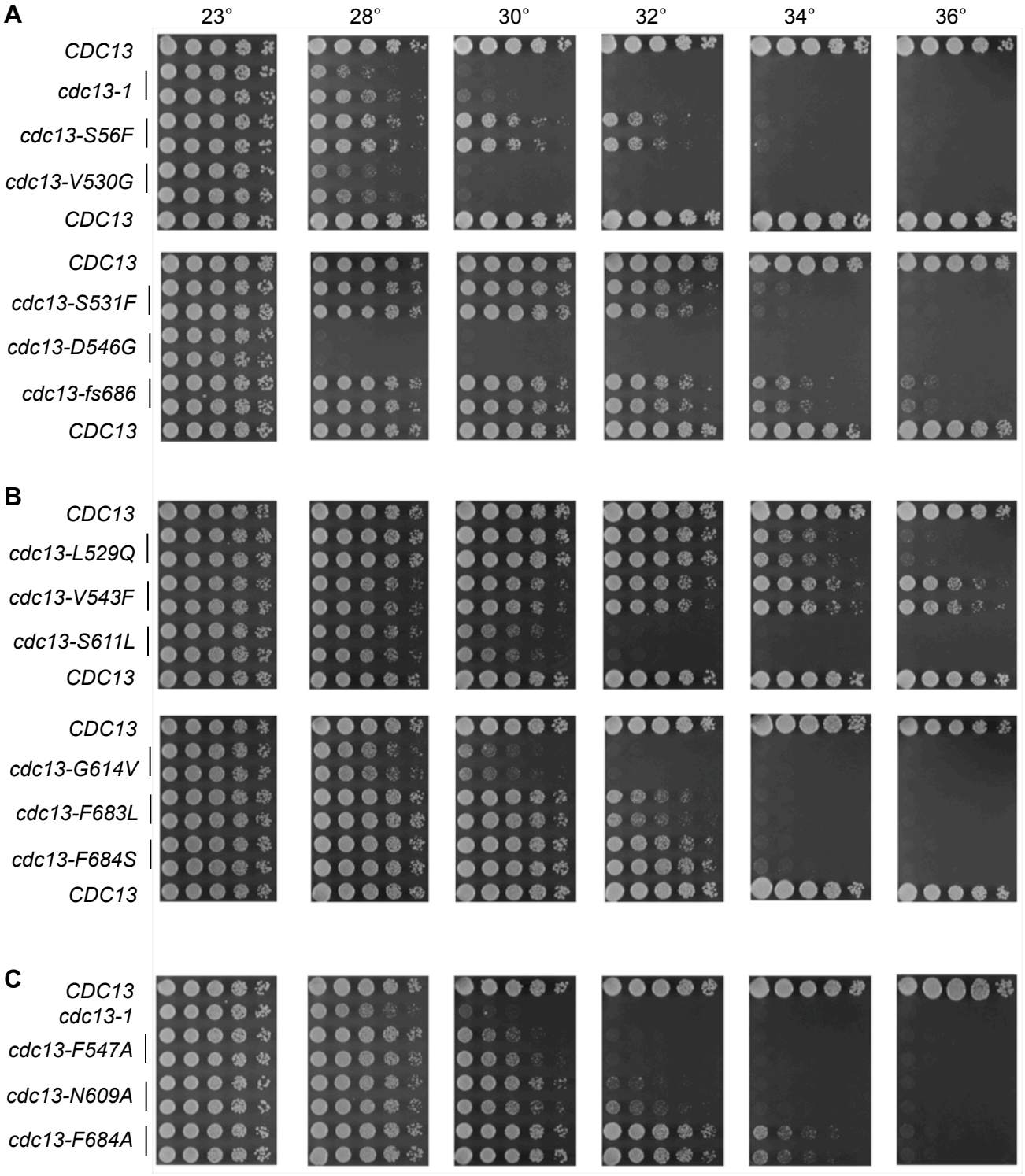
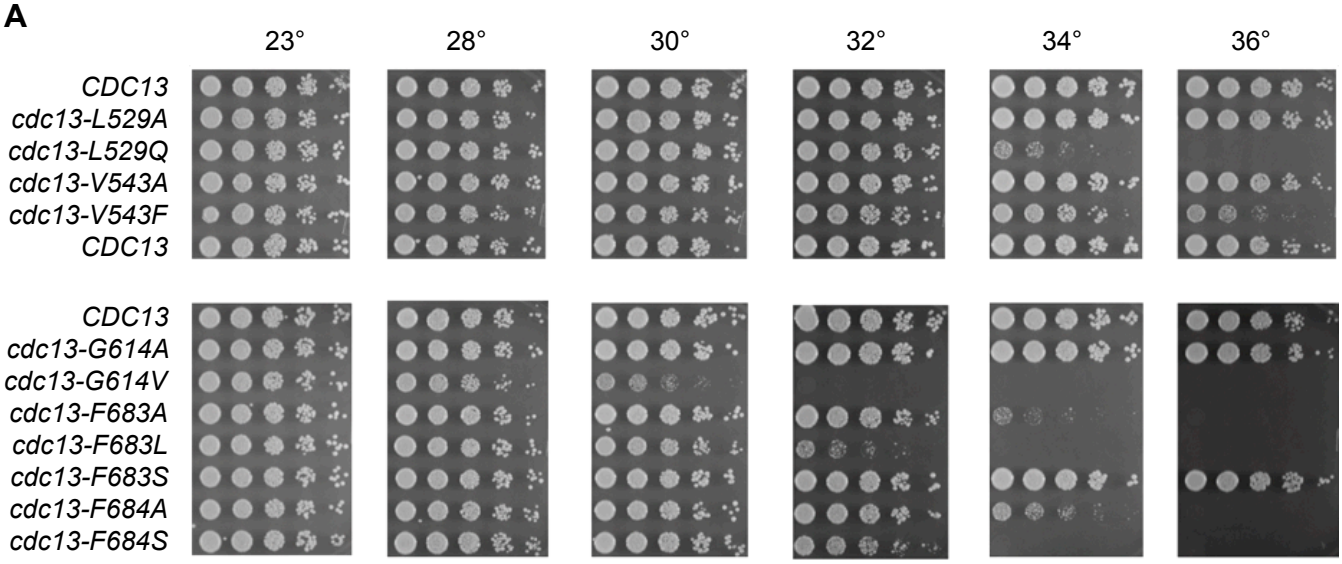
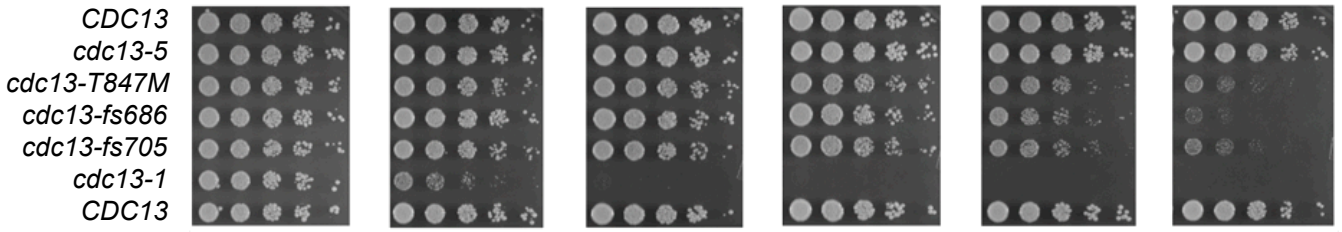


Figure S1 Identification of new *cdc13-ts* mutations, part I. Mutant alleles recovered from (A) forward mutagenesis of the full length *CDC13* gene, (B) forward mutagenesis directed at the DBD of *CDC13* or (C) reverse mutagenesis of the DBD of *CDC13* were transformed into a *cdc13-Δ/* p*CEN CDC13 URA3* shuffle strain and subsequently streaked on 5-FOA media at 23°, to recover isolates which had lost the wild type *CDC13* plasmid. The resulting strains were grown overnight in culture and viability was assessed by plating serial dilutions on pre-warmed rich media plates and photographing after 2.5 days (for ≥ 28° incubations) or 4 days (for 23° and 25°).

A



B



C

Allele	Mutations present (**cluster 1**, **cluster 2**, **cluster 3**)
isolate 22	**V543F**
isolate 23	N609D
isolate 26	N609D, Y626F
isolate 19	L599P, S650P
isolate 16	**M525T**, I633V
isolate 30	T507A, **L529Q**
isolate 21	**S533L**, **V616A**
isolate 15	M463I, **S611L**
isolate 31	P506S, **G614V**
isolate 28	**K618E**, **F683S**
isolate 20	N631D, **F684S**
isolate 17	**D535G**, P651L, **F683L**
isolate 24	V481E, A489T, L562Q
isolate 25	T496S, E519V, I578T
isolate 27	F575L, N627S, Y680C, **I686V**
isolate 29	M498V, **F544L**,T586M, K629E
isolate 18	**S531T**, I563V, E608V, K629E, L693P
isolate 14	N455D, T473A, D492G, I552T, F574S

Figure S2 Identification of new *cdc13-ts* mutations, Part II. (A) Additional mutant alleles, as discussed in the text, were analyzed as described in Figure S1. (B) Missense mutations identified in a panel of *cdc13-ts* alleles, following mutagenesis of the DNA binding domain with error-prone PCR, remissense; clusters of residues discussed in the text are indicated.

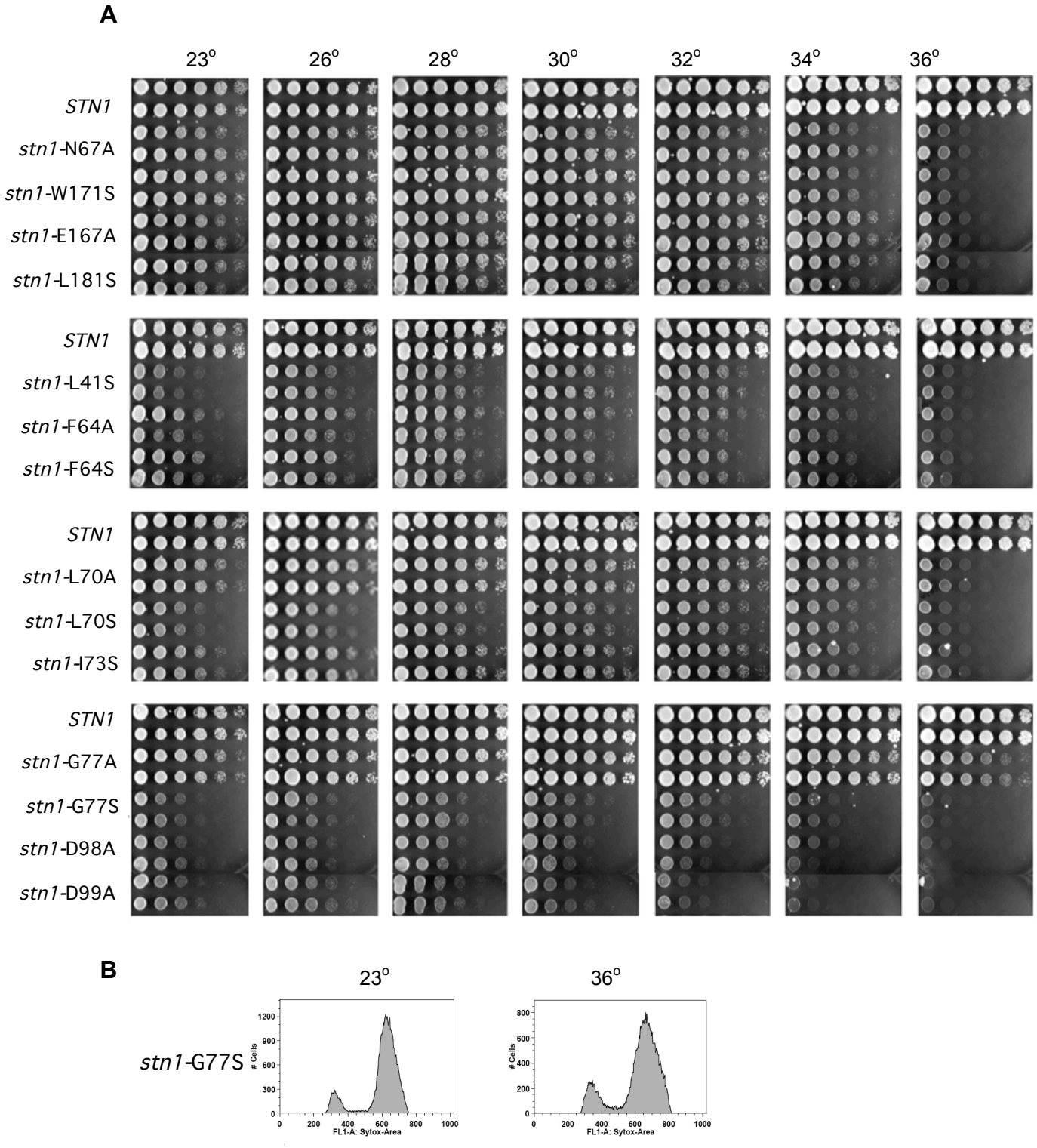


Figure S3 Viability assays of *stn1*¯ missense mutations. (A) Plasmids carrying the indicated *Stn1* alleles were transformed into a *stn1-Δ/* p*CEN STN1 URA3* shuffle strain and subsequently streaked on 5-FOA media at 23°, to recover isolates which had lost the wild type *STN1* plasmid. The resulting strains were grown overnight in culture and viability was assessed by plating serial dilutions of two isolates for each genotype on pre-warmed rich media plates and photographing after 2.5 days (for ≥ 28° incubations) or 4 days (for 23° and 25°). (B) Flow cytometry profile of log-phase cultures of a *stn1-Δ*/p *stn1-G77S* strain grown at 23° and 36° for 3.5h.

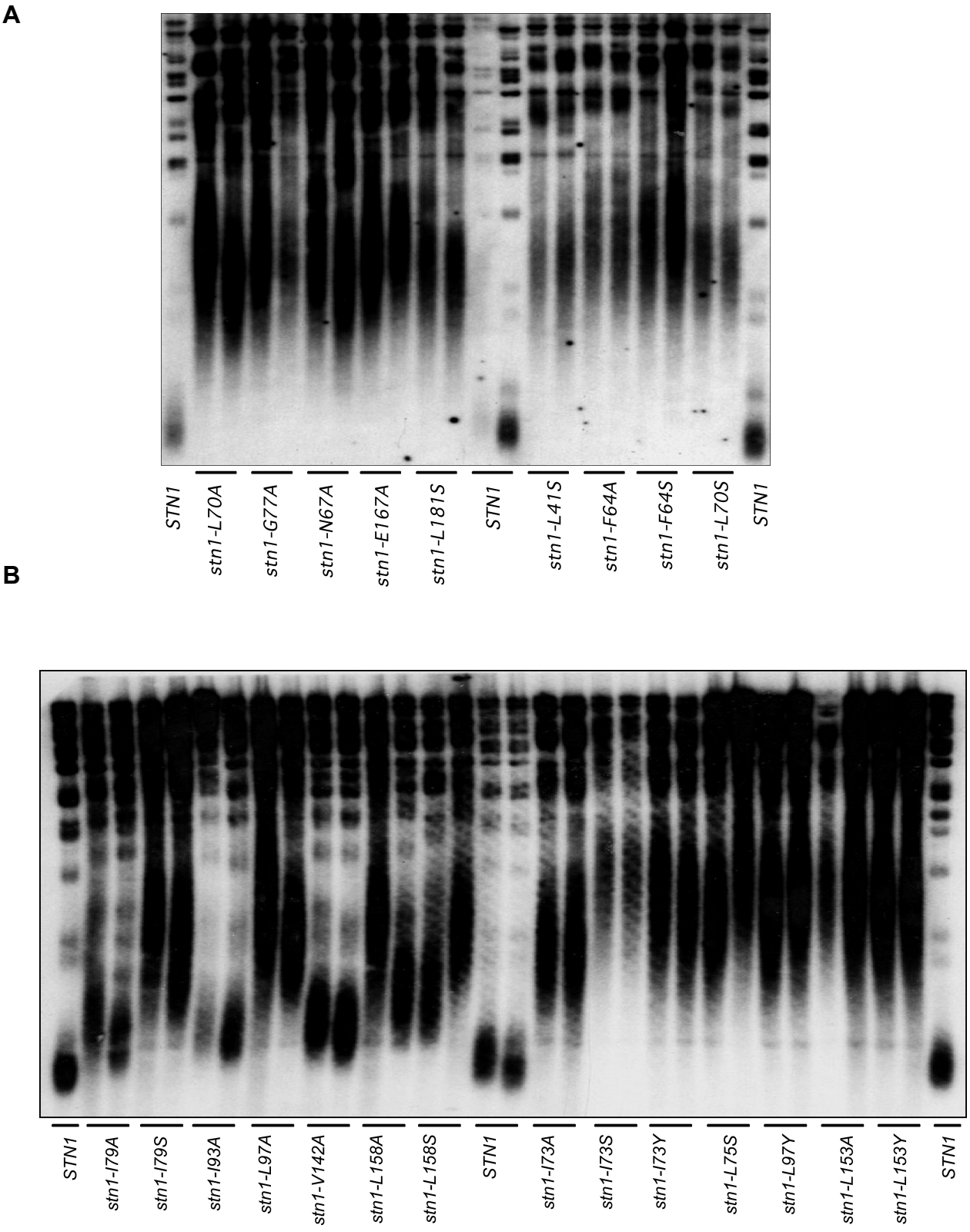


Figure S4 Telomere length of strains expressing *stn1*$^{-}$ missense mutations in residues selected for reverse mutagenesis based on either (A) Evolutionary Trace or (B) position of the side-chain relative to the interior of the β-barrel of the essential N-terminal OB-fold domain of Stn1; *stn1-Δ* strains with *CEN* plasmids expressing the indicated mutations were grown at 23° prior to preparing genomic DNA for telomere length analysis.

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

Figure S5 Summary of viability and telomere length of a panel of *stn1*⁻ 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.

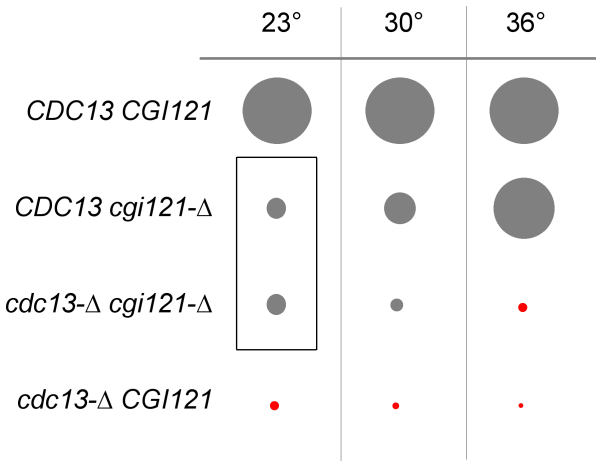


Figure S6 Schematic of relative colony size following dissection of a *CDC13/cdc13-Δ RAD24/rad24-Δ CGI121/cgi121-Δ* diploid; colony size is roughly to scale, with red dots indicating colonies that can only visulized with magnification. All indicated genoytpes are also *rad24-Δ.* Data for representative colonies indicated by the box are shown in Figure 8C.