Figure S3  Viability assays of stn1 missense mutations. (A) Plasmids carrying the indicated Stn1 alleles were transformed into a stn1-Δ/pCEN 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.