

Table S3. PCR and Sanger sequencing analysis of Ura⁺ transformants obtained in the temperature-sensitive (ts) mutation knock-in experiments.

sgRNA	Phenotype	Mutations
<i>tor2</i> -sgRNA1	ts	C6111A / G6129A / <u>T6143C</u> (3/5); G6129A / <u>T6143C</u> (2/5)
	Not ts	C6111A / G6129A (3/11); G6129A (8/11)
<i>tor2</i> -sgRNA2	ts	C6111A / G6129A / <u>T6143C</u> (6/6)
	Not ts	C6111A / G6129A (1/8); C6111A (6/8); WT (1/8)
<i>cdc25</i> -sgRNA1	ts	C1581T / C1590T / <u>G1595A</u> (8/8);
	Not ts	WT (8/8)
<i>cdc25</i> -sgRNA2	ts	C1581T / C1590T / <u>G1595A</u> (1/2); C1590T / <u>G1595A</u> (1/2)
	Not ts	WT (8/13); C1581T / C1590T (3/13); C1581T (2/13)

Intended temperature-sensitive (ts) mutations are indicated by underline. PAM-disrupting silent

mutations are indicated by bold letters.