

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

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

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