Title: Using CRISPR to mimic a phosphorylated state of a MELT-like motif in *S. cerevisiae's* Stu1 protein

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The kinetochore (KT) drives chromosomal segregation during anaphase which is a highly regulated process that ensures daughter cells contain the right amount of genetic material. Errors in this process lead to incorrect amounts of genetic material, or aneuploidy, often resulting in cell death. KT proteins are involved in activating cell cycle checkpoints to prevent aneuploidy through phosphorylation. Physiologically relevant phosphosites on proteins play a role in communicating cell cycle checkpoints.

We sought to analyze the physiological relevance of a phosphosite on the KT protein Stu1p in yeast. Stu1p is known to control microtubule (MT) dynamics and retain MT network organization. Furthermore, MELT motifs are highly conserved sequences of amino acids that serve as phosphosites targeted by the Mps1 kinase. Stu1p has two variations of the MELT consensus known as expanded MELT motifs: IDLT716 and MEMT1031. We used the CRISPR-Cas9 system to mutagenize T719 in IDLT716 to aspartic acid, mimicking a phosphorylated state at this position. Any phenotype differences that arise from this phosphomimetic mutation may provide valuable insights into IDLT716's physiological relevance and a better understanding of Stu1p's role in cell division.

We transformed the CRISPR vector containing a small guide RNA (sgRNA) into yeast which allowed the Cas9 enzyme to target a locus near IDLT716. Using a designed homology-directed repair template, we confirmed mutagenesis of one isolate and initiated phenotype testing by heat stress. While our preliminary results suggest that IDLT716 is physiologically irrelevant, we are awaiting sequencing data of our remaining isolates before continuing phenotype testing.