G3 Genes | Genomes | Genetics

The C-terminal Residues of *Saccharomyces cerevisiae* Mec1 are required for its Localization, Stability and Function

Lance F. DaSilva[*], Samantha Pillon[*], Julie Genereaux[*], Megan J. Davey[*], Gregory B. Gloor[*], Jim Karagiannis[§] and Christopher J. Brandl*

[*]Department of Biochemistry, Schulich School of Medicine & Dentistry, The University of Western Ontario, London Canada N6A5C1

[§] Department of Biology, The University of Western Ontario,
London Canada N6A 5B7

DOI: 10.1534/g3.113.006841

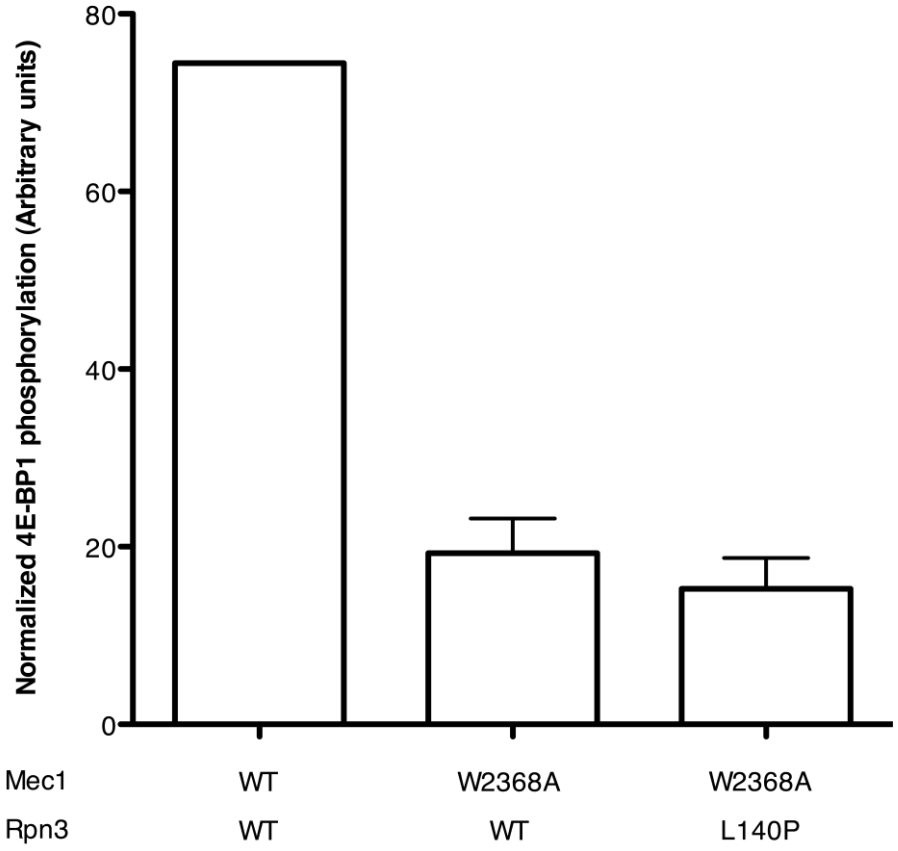

Figure S1 *rpn3-L140P* does not increase Mec1-W2368A kinase activity. Protein extracts were prepared by bead lysis in buffer containing protease inhibitors from strains CY6194 (*Flag^5-MEC1 RPN3*), CY6175 (*Flag^5-mec1-W2368A RPN3*) and CY6391 (*Flag^5-mec1-W2368A rpn3-L140P*) grown at 30°. Two milligrams of protein was immunoprecipitated with anti-Flag M2 magnetic beads. Two-thirds of the immunoprecipitate was suspended in 60 µl of kinase buffer. Aliquots of four microliters were used in kinase assays performed at 30° with 4E-BP1 as substrate. Shown is the amount of 4E-BP1 phosphorylation normalized to Mec1 protein levels. Error bars represent the mean ± SD of three replicates.