Supplementary Materials for

Nutrient limitation inactivates Mrc1-to-Cds1 checkpoint signalling in *Schizosaccharomyces pombe*

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Supplementary Figure 1. Glucose starvation transiently activates Cds1 and removes Mrc1. (a) Cds1-HA$_2$His$_6$, Δcds1, Δrad3 and Δtel1 cells were grown in 0.3% glucose rich medium at 30°C for the indicated times. Samples were withdrawn, fixed in methanol and scored for septated cells after staining the DNA with DAPI and the new septum with calcofluor (septation index = cells in G1/S). (b) Cds1-HA$_2$His$_6$ (56kDa) protein was immunoprecipitated at the indicated times and used to phosphorylate myelin basic protein in vitro after a shift from 3% to 0.3% glucose in rich medium at 30°C. (c) Total protein extracts prepared at the indicated times after a down-shift to 0.3% or 0.1% glucose in rich
medium at 30°C. Probed for Mrc1, Sty1-T171P+Y173P and Cdc2. (d) Total protein extracts prepared at 0h and 24h after the down-shift to 0.1% glucose in rich medium at 30°C. Probed for Mrc1 and Cdc2 in wild type cells (WT) and cells deleted for the indicated genes.
Supplementary Figure 2. Histone 2AX is phosphorylated towards the end of S phase at serine 129 and M159 is a cryptic translational start codon in the \textit{cds1} mRNA.

(a) \textit{Dfp1-His}_6\textit{nda3.KM311} cells were synchronised in mitosis at 20°C for 8h in rich medium with 3% glucose and then quickly released into rich medium with 3% glucose at 30°C. The DNA content was measured using a CUBE 8 (Sysmex) instrument and the histograms were produced using the free Flowing Software (http://flowingsoftware.btk.fi/). Cells completed S phase between 40 min and 60 min post-release. (b) Total protein extracts were probed for Dfp1-His\textit{6HA}_3 with an anti-HA antibody and for Mrc1, H2AX-S129-P and Rad4 with the corresponding antibodies. Dfp1 is hyper-modified as indicated by the
upper shift band in S phase (arrow). H2AX phosphorylation by Rad3 peaks towards the end of S phase when both, Dfp1 modification and Mrc1 are down-regulated. (c + d)

Location of the cryptic translational initiation codon AUG-159 (methionine 159) between the N-terminal FHA and C-terminal kinase domain of Cds1. The model of Cds1 is based on human Chk2 kinase (PDB ID: 3I6U) The model was produced with the Swiss Modelling tool (https://swissmodel.expasy.org/). (e) Total protein extracts of Cds1-HA<sub>2</sub>His<sub>6</sub> and Cds1-M159A-HA<sub>2</sub>His<sub>6</sub>, cells were prepared from growing (G) and non-growing (NG) (24h growth in 3% glucose rich medium at 30°C) cells. Cells were incubated with 12mM HU for 2h (+HU) under both conditions. Cds1 is only hyper-phosphorylated in growing cells, while its N-terminally truncated band is limited to non-growing, starved cells. The latter band is absent when M159 is replaced by an alanine codon (M159A). The second band above the shorter form may be a phosphorylated variant of the latter (arrow). PT = phostag SDS page.
Supplementary Figure 3. Cds1 is not required for the induction of the glucose transporter Ght4 and Ght5. (a) Ght4-GFP wild type (WT) cells or Ght4-GFP strains deleted for the indicated genes were shifted to 0.3% glucose and total protein extracts were prepared at the indicated times. The molecular weight is ~65kDa. Highly modified forms are detectable after induction. Up-regulation requires the MAP kinase Sty1/Spc1. (b) Induction of Ght5-GFP in wild type cells. The molecular weight is ~65kDa. A 25kDa breakdown product is detectable after 20h in 0.3% glucose medium. (c) Same experiment in a cds1 deletion background.