

Appendix

Nrm1 is a bistable switch connecting cell cycle progression to transcriptional control

Guillem Murciano-Julià, Montserrat Vega, Esther Pazo, Àlex Pascual-Serra, Isabel Alves-Rodrigues, Oriol Bagudanch, Roger Anglada, Núria Bonet, Rosa Aligué, Sergio Moreno, Baldo Oliva, Elena Hidalgo and José Ayté

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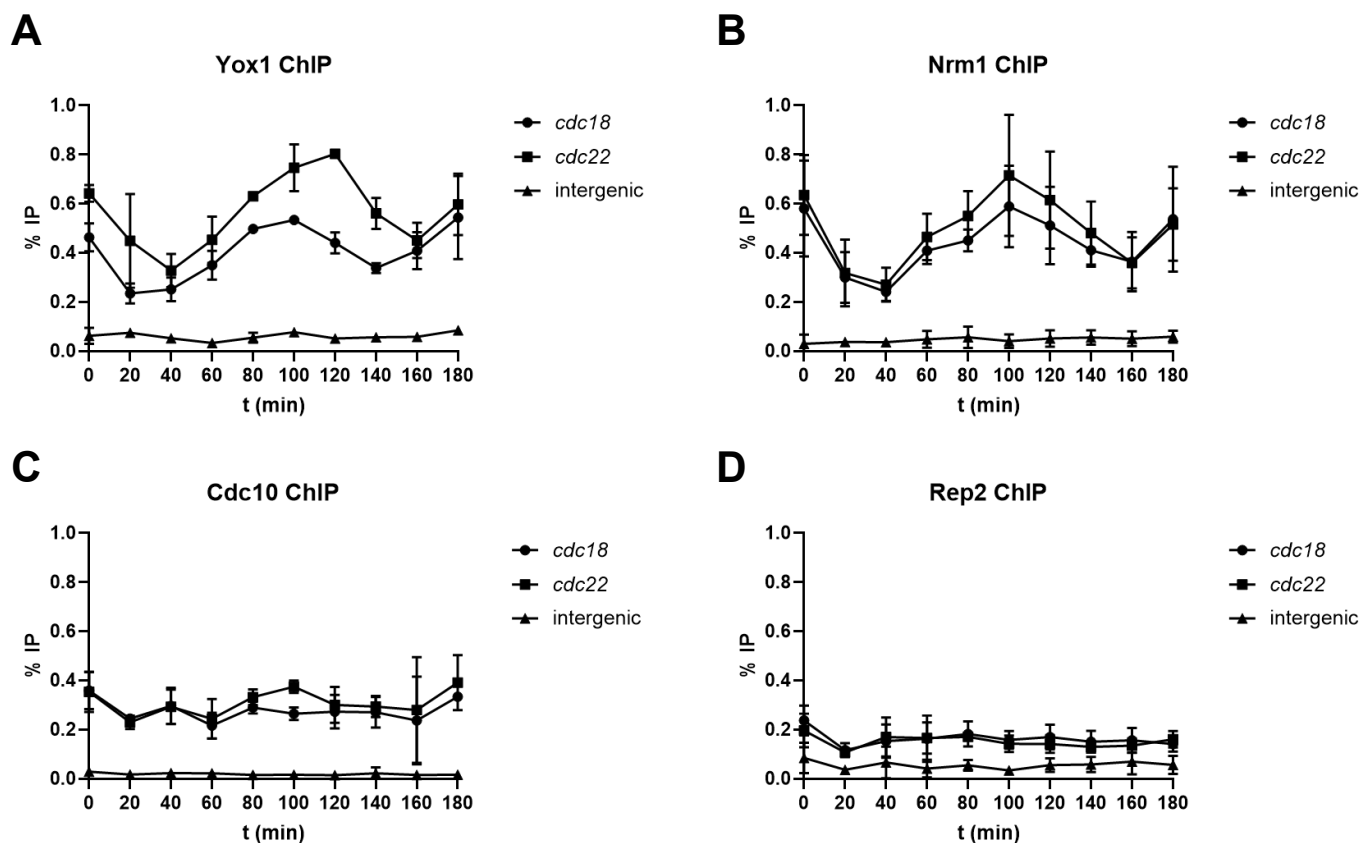
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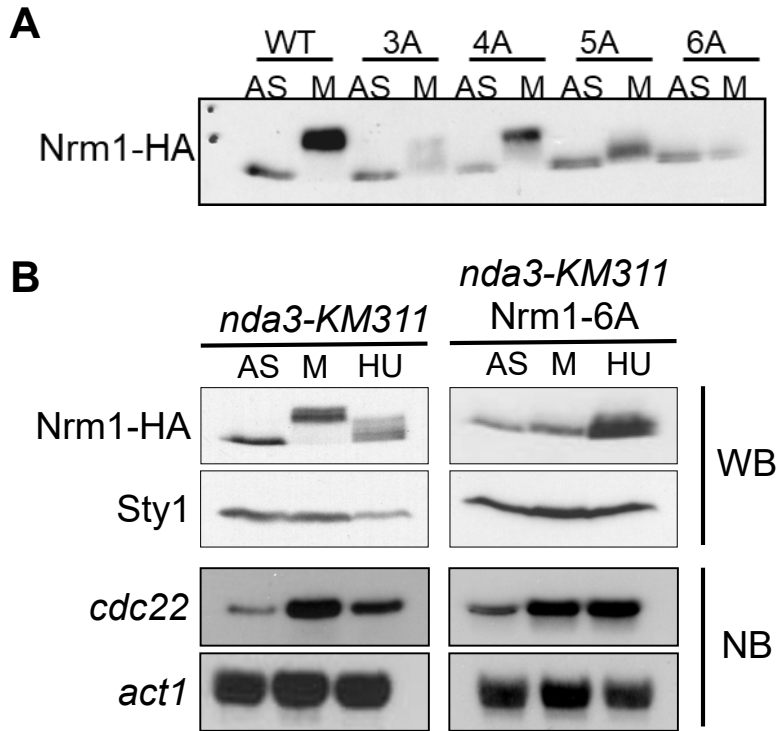
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Appendix Figure S1.

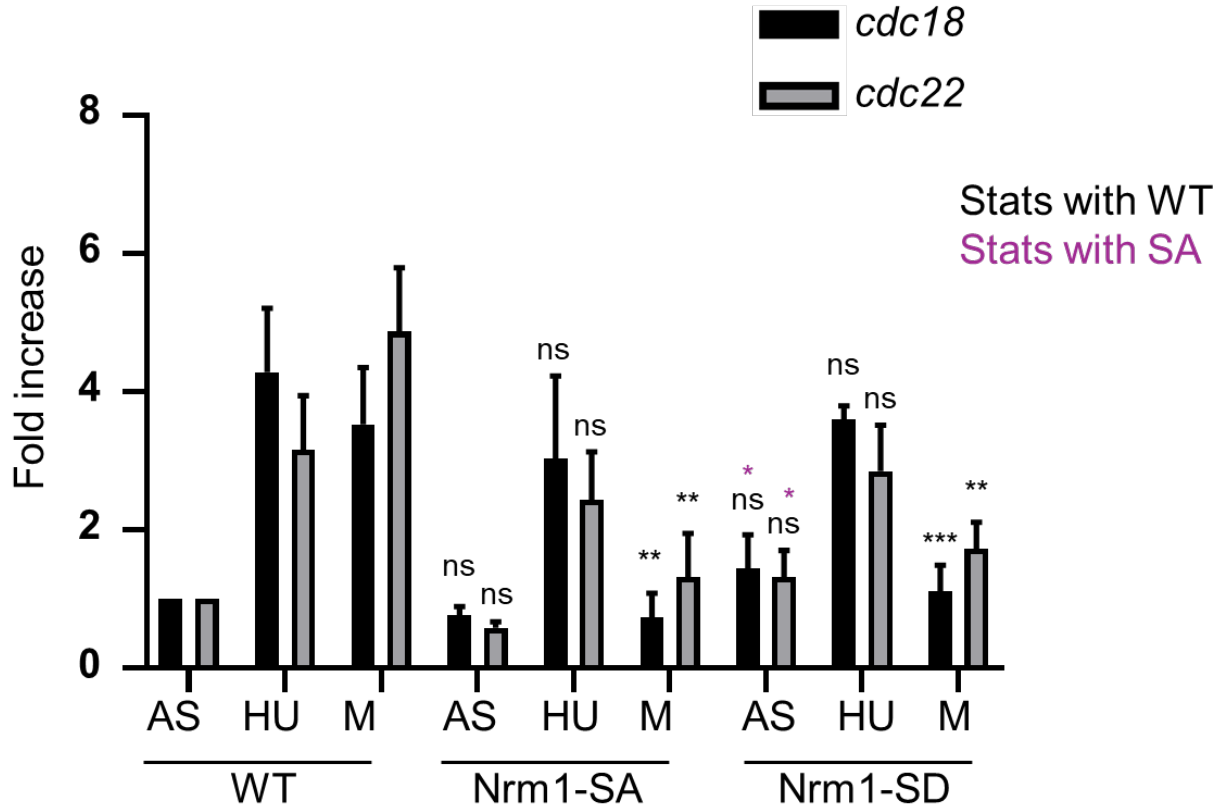
ChIP experiments from Fig. 2A showing each immunoprecipitated protein in individual graphs, with interaction to *cdc18* and *cdc22* promoters and an intergenic region as control. **(A)** Yox1 ChIP; **(B)** Nrm1-HA ChIP; **(C)** Cdc10 ChIP; **(D)** Rep2-TAP ChIP.



Appendix Figure S2.

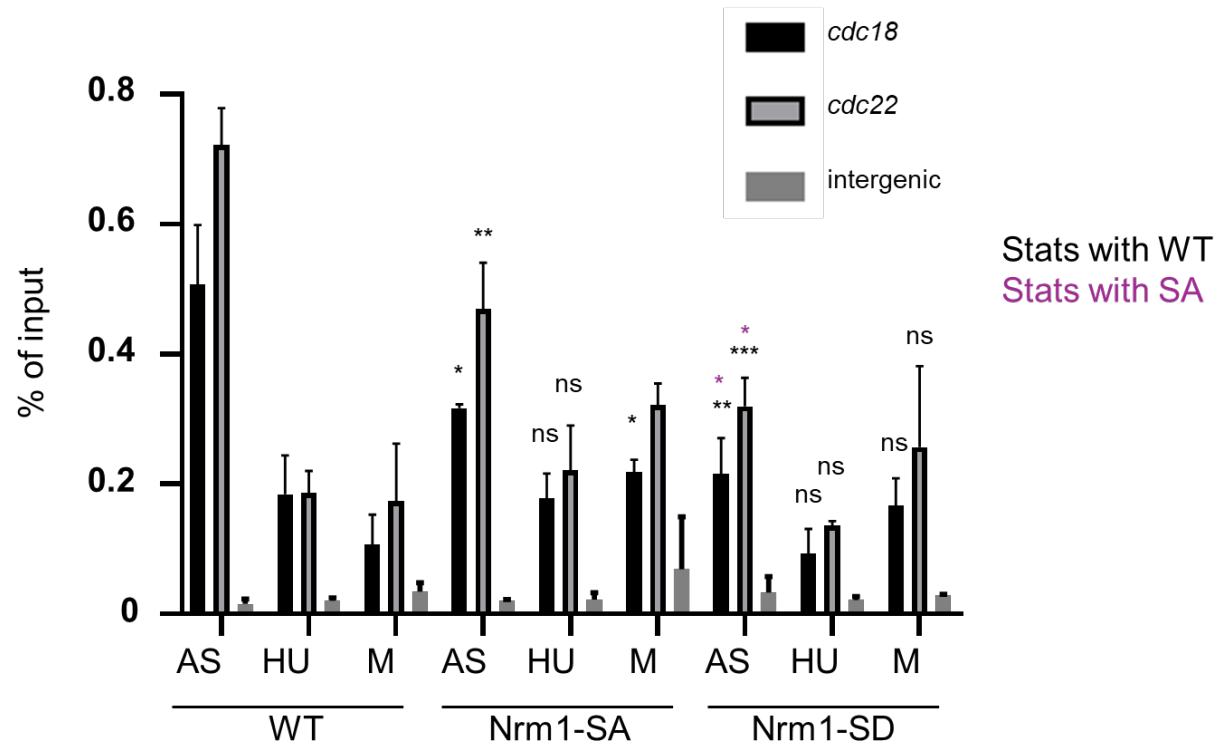
(A) Western blot analysis of asynchronous (AS) or metaphase arrested (M) *nda3-KM311* cells with wild type Nrm1 (WT) or different phosphorylation mutants: 3A (T9A, S57A, T287A), 4A (T9A, S57A, T116A, T287A); 5A (T9A, S57A, S237A, T241A, T287A); 6A (T9A, S57A, T116A, S237A, T241A, T287A). Cells were grown in YE5S at 30°C and shifted at 18°C for 5h for metaphase arrest prior to TCA sample collection.

(B) Western Blot (WB) and Northern Blot from *nda3-KM311* strain expressing Nrm1-HA (left) or Nrm1-6A-HA (right); asynchronous culture (AS), cells arrested in metaphase for 5 hours at 18°C (M), or cells treated with 10 mM Hydroxyurea (HU) for 3 hours at 30°C. Sty1 is shown as loading control in the Western Blot and *act1* as a control in the Northern blot. Representative experiments of at least three different experiments are shown.



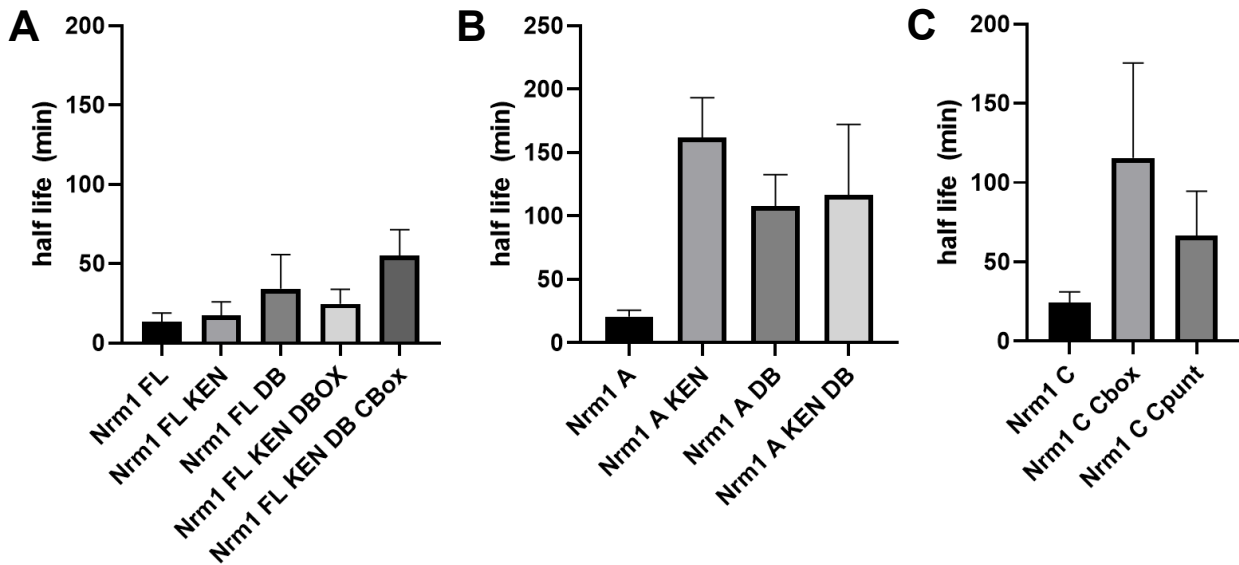
Appendix Figure S3.

qPCR transcription analysis of *cdc18* and *cdc22* expression in the Nrm1-HA, Nrm1-SA-HA and Nrm1-SD-HA in the *nda3-KM311* background. Cells were grown and treated as in Fig. 2B. AS, asynchronous culture; HU, cells treated with 10 mM Hydroxyurea for 3 hours at 30°C; M; cells arrested in metaphase for 5 hours at 18°C. *tfb2* was used as control gene. Plot represents mean $\pm$ SD of at least n=3 experiments. Statistics show significance from a student's T-test. In black relative to the same treatment with WT and in purple to the same treatment with SA. *: $p < 0.05$; **: $p < 0.01$; ***: $p < 0.001$



Appendix Figure S4.

ChIP analysis of Nrm1-HA, Nrm1-SA-HA and Nrm1-SD-HA in *nda3-KM311* strain. ChIP was done as described with α -HA antibodies. The isolated DNA was used to amplify the promoter region of *cdc18* or *cdc22*. Cells were grown and treated as in Fig. 2B. AS, asynchronous culture; HU, cells treated with 10 mM Hydroxyurea for 3 hours at 30°C; M, cells arrested in metaphase for 5 hours at 18°C. Plot represents mean $\pm$ SD of at least n=3 experiments. In black relative to the same treatment with WT and in purple to the same treatment with SA. Statistics show significance from a student's T-test. *: p<0.05; **: p<0.01; ***: p<0.001



Appendix Figure S5.

(A) Half-life in minutes of Nrm1 full length (FL), Nrm1 FL Δ KEN, Nrm1 FL Δ DB, Nrm1 FL Δ KEN Δ DB and Nrm1 FL Δ KEN Δ DB Δ CBox after thiamine and cycloheximide (CHX) addition. TCA samples were collected at 0, 10, 30 and 60 minutes after CHX addition and analyzed in Western blots. Sample loading was normalized with α -Sty1. Half-life was calculated using GraphPad Prism. Plot represents mean $\pm$ SD of at least $n = 3$ experiments.

(B) Half-life in minutes of Nrm1 A, Nrm1 A Δ KEN, Nrm1 A Δ DB and Nrm1 A Δ KEN Δ DB after thiamine and cycloheximide (CHX) addition. TCA samples were collected at 0, 10, 30 and 60 minutes after CHX addition and analyzed in Western blots. Sample loading was normalized with α -Sty1. Half-life was calculated using GraphPad Prism. Plot represents mean $\pm$ SD of at least $n = 3$ experiments.

(C) Half-life in minutes of Nrm1 C, Nrm1 C Δ CBox and Nrm1 C Δ Cpunt after thiamine and cycloheximide (CHX) addition. TCA samples were collected at 0, 10, 30 and 60 minutes after CHX addition and analyzed in Western blots. Sample loading was normalized with α -Sty1. Half-life was calculated using GraphPad Prism. Plot represents mean $\pm$ SD of at least $n = 3$ experiments.