# Appendix

## The CDK Pho85 inhibits Whi7 Start repressor to promote cell cycle entry in budding yeast

Cristina Ros-Carrero *et al.*

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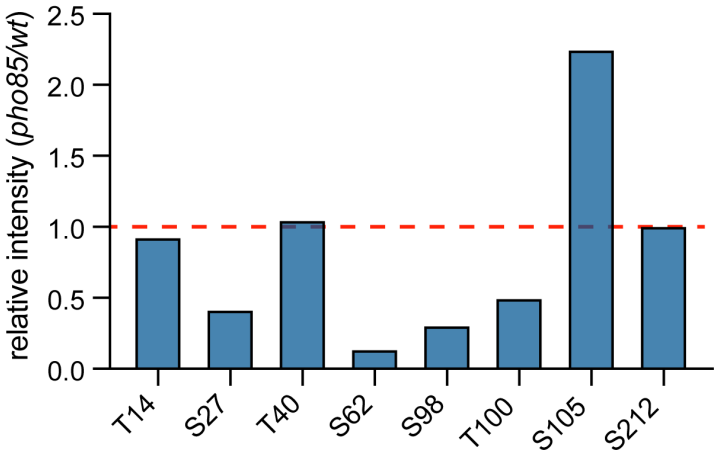
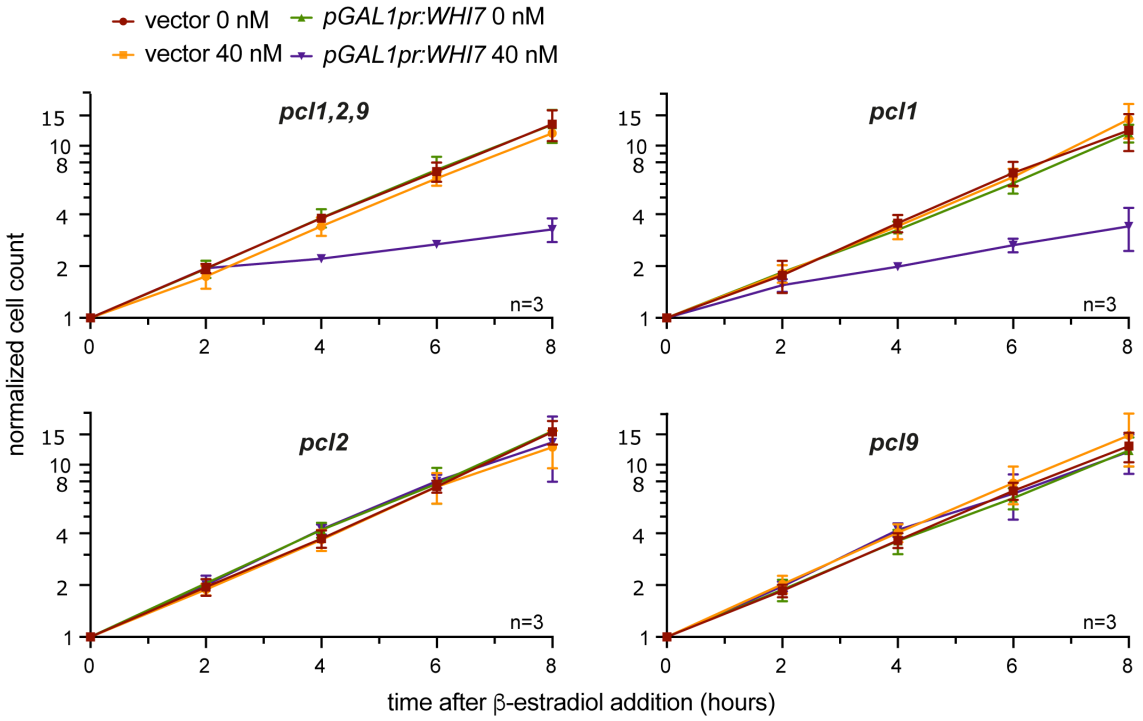



**Appendix Figure S1. Several CDK consensus sites show a reduced relative phosphorylation in the *pho85* mutant**. In an approximated comparison of the relative abundance of phosphorylated CDK sites in wild type and *pho85* mutant cells, we calculated in each sample the percentage of the MaxLFQ intensity for each phosphorylated CDK site relative to the total intensity of all the phosphorylated non-CDK sites in the sample. The ratio of the average of *pho85* against wild type strains for the common CDK sites is represented.




**Appendix Figure S2. *WHI7* overexpression causes growth defects in the absence of Pcl1 cyclin.** Exponentially growing cells of *pcl1,2,9* (JCY2626), *pcl1* (JCY2831), *pcl2* (JCY2832), or *pcl9* (JCY2833) transformed with an empty vector or the p*GAL1pr:WHI7-HA* plasmid were incubated in the presence of 0 nM or 40 nM β-estradiol (to induce overexpression of *WHI7*) and the increase in cell number over time was analyzed. Cell number was normalized to 1.

**Appendix Table S1.** Whi7 phosphorylation sites identified in each biological replicate.

| | **wild type** | | | ***pho85*** | | |
|---|---|---|---|---|---|---|
| | replicate 1 | replicate 2 | replicate 3 | replicate 1 | replicate 2 | replicate 3 |
| **T5** | | X | X | | | |
| **T14** | X | X | X | X | X | X |
| **S17** | | X | | | | |
| **S27** | X | X | X | X | | X |
| **T32** | X | X | X | | | |
| **S34** | X | X | | | | X |
| **S36** | X | X | X | X | | X |
| **T40** | X | X | X | X | X | X |
| **S56** | X | X | | X | | |
| **S62** | X | X | X | X | X | X |
| **T70** | X | | | | X | |
| **Y71** | | X | X | | | |
| **Y73** | X | X | | X | | X |
| **T84** | X | X | X | X | | |
| **S90** | X | | | | | |
| **S98** | X | X | X | X | X | X |
| **T100** | X | X | X | X | X | X |
| **S105** | X | X | X | X | | X |
| **T107** | | X | | | | |
| **T151** | X | | X | X | X | |
| **S158** | X | | X | | X | X |
| **S172** | | X | X | X | X | X |
| **S176** | | | X | | | |
| **S186** | | X | X | | | |
| **S187** | | X | | X | | |
| **S203** | | X | | | | |
| **S212** | X | X | X | X | X | X |
| **T222** | X | X | X | X | X | X |
| **S224** | X | X | X | X | X | X |
| **T225** | X | X | X | X | X | |
| **S229** | X | X | X | X | X | |

**Appendix Table S2.** *Saccharomyces cerevisiae* strains used in this work

| Name | Strain | Genotype | Source |
|---|---|---|---|
| W303-1a | *wild type (WT)* | *MATa ade2-1 trp1-1 leu2-3,112 his3-11,15 ura3-52 can1-100* | |
| JCY1539 | *grr1::LEU2* | *MATa ade2-1 trp1-1 leu2-3,112 his3-11,15 ura3-52 can1-100 grr1::LEU2* | Gomar-Alba et al., 2017 |
| JCY1728 | *WHI7-HA* | *MATa ade2-1 trp1-1 leu2-3,112 his3-11,15 ura3-52 can1-100 WHI7-HA-TRP1* | Gomar-Alba et al., 2017 |
| JCY1746 | *WHI7-GFP* | *MATa ade2-1 trp1-1 leu2-3,112 his3-11,15 ura3-52 can1-100 WHI7-GFP-kanMX6* | Gomar-Alba et al., 2017 |
| JCY1819 | *whi7* | *MATa ade2-1 trp1-1 leu2-3,112 his3-11,15 ura3-52 can1-100 whi7::kanMX6* | Gomar-Alba et al., 2017 |
| JCY1874 | *whi5* | *MATa ade2-1 trp1-1 leu2-3,112 his3-11,15 ura3-52 can1-100 whi5::LEU2* | Gomar-Alba et al., 2017 |
| JCY1976 | *SWI4-myc* | *MATa ade2-1 trp1-1 leu2-3,112 his3-11,15 ura3-52 can1-100 SWI4-myc-HIS3* | Gomar-Alba et al., 2017 |
| JCY2036 | *WHI5-HA* | *MATa ade2-1 trp1-1 leu2-3,112 his3-11,15 ura3-52 can1-100 WHI5-HA-HIS3* | Gomar-Alba et al., 2017 |
| JCY2221 | *WHI7-HA pho85* | *MATa ade2-1 trp1-1 leu2-3,112 his3-11,15 ura3-52 can1-100 WHI7-HA-TRP1 pho85::kanMX6* | This study |
| JCY2241 | *pho85* | *MATa ade2-1 trp1-1 leu2-3,112 his3-11,15 ura3-52 can1-100 pho85::kanMX6* | This study |
| JCY2256 | *WHI7-HA pcl1 pcl2 pcl9* | *MATa ade2-1 trp1-1 leu2-3,112 his3-11,15 ura3-52 can1-100 WHI7-HA-TRP1 pcl1::kanMX6 pcl2::HIS3 pcl9::NAT* | This study |
| JCY2282 | *WHI5-HA pho85* | *MATa ade2-1 trp1-1 leu2-3,112 his3-11,15 ura3-52 can1-100 WHI5-HA-HIS3 pho85::kanMX6* | This study |
| JCY2315 | *pcl1 pcl2 pcl9* | *MATa ade2-1 trp1-1 leu2-3,112 his3-11,15 ura3-52 can1-100 pcl1::kanMX6 pcl2::HYG pcl9::NAT* | This study |
| JCY2333 | *pho80* | *MATa ade2-1 trp1-1 leu2-3,112 his3-11,15 ura3-52 can1 pho80::kanMX6* | This study |
| JCY2335 | *WHI7-HA pho80* | *MATa ade2-1 trp1-1 leu2-3,112 his3-11,15 ura3-52 can1-100 WHI7-HA-TRP1 pho80::kanMX6* | This study |
| JCY2337 | *WHI7-HA pcl1 pcl2 pcl9 pho80* | *MATa ade2-1 trp1-1 leu2-3,112 his3-11,15 ura3-52 can1-100 WHI7-HA-TRP1 pcl1::kanMX6 pcl2::HIS3 pcl9::NAT pho80::HYG* | This study |

| | | | |
|---|---|---|---|
| JCY2443 | *ADGEV* | *MATa ade2-1 trp1-1 leu2-3,112 his3-11,15 ura3-52 can1-100 URA3::ADGEV-URA3* | This study |
| JCY2446 | *ADGEV GAL1:WHI7-GFP* | *MATa ade2-1 trp1-1 leu2-3,112 his3-11,15 ura3-52 can1-100 URA3::ADGEV-URA3 HIS3-GAL1:WHI7-GFP-kanMX6* | This study |
| JCY2448 | *ADGEV GAL1:WHI5-GFP* | *MATa ade2-1 trp1-1 leu2-3,112 his3-11,15 ura3-52 can1-100 URA3::ADGEV-URA3 HIS3-GAL1:WHI5-GFP-kanMX6* | This study |
| JCY2475 | *pho85 whi7* | *MATa ade2-1 trp1-1 leu2-3,112 his3-11,15 ura3-52 can1-100 pho85::kanMX6 whi7::HIS3* | This study |
| JCY2486 | *ADGEV pho85* | *MATa ade2-1 trp1-1 leu2-3,112 his3-11,15 ura3-52 can1-100 pho85::kanMX6 URA3::ADGEV-URA3* | This study |
| JCY2495 | *WHI5-mNeonGreen* | *MATa ade2-1 trp1-1 leu2-3,112 his3-11,15 ura3-52 can1-100 WHI5-mNeonGreen-URA3* | This study |
| JCY2497 | *WHI7-mNeonGreen* | *MATa ade2-1 trp1-1 leu2-3,112 his3-11,15 ura3-52 can1-100 WHI7-mNeonGreen-URA3* | This study |
| JCY2499 | *WHI7-mNeonGreen pho85* | *MATa ade2-1 trp1-1 leu2-3,112 his3-11,15 ura3-52 can1-100 pho85::kanMX6 WHI7-mNeonGreen-URA3* | This study |
| JCY2501 | *WHI5-mNeonGreen pho85* | *MATa ade2-1 trp1-1 leu2-3,112 his3-11,15 ura3-52 can1-100 pho85::kanMX6 WHI5-mNeonGreen-URA3* | This study |
| JCY2503 | *PHO4-GFP-HIS3* | *MATa ade2-1 trp1-1 leu2-3,112 his3-11,15 ura3-52 can1-100 PHO4-GFP-HIS3* | This study |
| JCY2505 | *PHO4-GFP-HIS3 pho85* | *MATa ade2-1 trp1-1 leu2-3,112 his3-11,15 ura3-52 can1-100 PHO4-GFP-HIS3 pho85::kanMX6* | This study |
| JCY2538 | *pho85 whi5* | *MATa ade2-1 trp1-1 leu2-3,112 his3-11,15 ura3-52 can1-100 pho85::kanMX6 whi5::LEU2* | This study |
| JCY2540 | *pho85 whi5 whi7* | *MATa ade2-1 trp1-1 leu2-3,112 his3-11,15 ura3-52 can1-100 pho85::kanMX6 whi5::LEU2 whi7::kanMX6* | This study |
| JCY2553 | *WHI5-GFP WHI7-myc* | *MATa ade2-1 trp1-1 leu2-3,112 his3-11,15 ura3-52 can1-100 WHI5-GFP-kanMX6 WHI7-7myc-HIS3* | This study |
| JCY2556 | *WHI5-GFP WHI7-myc pho85* | *MATa ade2-1 trp1-1 leu2-3,112 his3-11,15 ura3-52 can1-100 WHI5-GFP-kanMX6 WHI7-7myc-HIS3 pho85::kanMX6* | This study |
| JCY2559 | *ADGEV GAL1:WHI5-GFP pho85* | *MATa ade2-1 trp1-1 leu2-3,112 his3-11,15 ura3-52 can1-100 URA3::ADGEV-URA3 HIS3-GAL1:WHI5-GFP-kanMX6 pho85::HYG* | This study |
| JCY2561 | *ADGEV GAL1:WHI7-GFP pho85* | *MATa ade2-1 trp1-1 leu2-3,112 his3-11,15 ura3-52 can1-100 URA3::ADGEV-URA3 HIS3-GAL1:WHI7-GFP-kanMX6 pho85::HYG* | This study |

| | | | |
|---|---|---|---|
| JCY2626 | *ADGEV pcl1 pcl2 pcl9* | *MATa ade2-1 trp1-1 leu2-3,112 his3-11,15 ura3-52 can1-100 URA3::ADGEV-URA3 pcl1::kanMX6 pcl2::HYG pcl9::NAT* | This study |
| JCY2627 | *ADGEV pho80* | *MATa ade2-1 trp1-1 leu2-3,112 his3-11,15 ura3-52 can1-100 URA3::ADGEV-URA3 pho80::kanMX6* | This study |
| JCY2652 | *PHO85-TAP* | *MATa ade2-1 trp1-1 leu2-3,112 his3-11,15 ura3-52 can1-100 PHO85-TAP-TRP1* | This study |
| JCY2831 | *ADGEV pcl1* | *MATa ade2-1 trp1-1 leu2-3,112 his3-11,15 ura3-52 can1-100 URA3::ADGEV-URA3 pcl1::kanMX6* | This study |
| JCY2832 | *ADGEV pcl2* | *MATa ade2-1 trp1-1 leu2-3,112 his3-11,15 ura3-52 can1-100 URA3::ADGEV-URA3 pcl2::kanMX6* | This study |
| JCY2833 | *ADGEV pcl9* | *MATa ade2-1 trp1-1 leu2-3,112 his3-11,15 ura3-52 can1-100 URA3::ADGEV-URA3 pcl9::kanMX6* | This study |
| JCY2941 | *GAL1:WHI7-GFP ADGEV HTB2-mCherry* | *MATa ade2-1 trp1-1 leu2-3,112 his3-11,15 ura3-52 can1-100 URA3::ADGEV-URA3 HIS3-GAL1:WHI7-GFP-kanMX6 HTB2-mCherry-HYG* | This study |
| JCY2951 | *GAL1:WHI7-GFP ADGEV HTB2-mCherry pho85* | *MATa ade2-1 trp1-1 leu2-3,112 his3-11,15 ura3-52 can1-100 URA3::ADGEV-URA3 HIS3-GAL1:WHI7-GFP-kanMX6 HTB2-mCherry-HYG pho85::NAT* | This study |
| JCY2978 | *GAL1:WHI5-GFP ADGEV HTB2-mCherry pho85* | *MATa ade2-1 trp1-1 leu2-3,112 his3-11,15 ura3-52 can1-100 URA3::ADGEV-URA3 HIS3-GAL1:WHI5-GFP-kanMX6 HTB2-mCherry-HYG pho85::NAT* | This study |
| JCY2981 | *HTB2-mCherry whi7* | *MATa ade2-1 trp1-1 leu2-3,112 his3-11,15 ura3-52 can1-100 HTB2-mCherry-HYG whi7::kanMX6* | This study |