Supplemental Materials

Molecular Biology of the Cell

Wang et al.
**SUPPLEMENTARY FIGURES**

**Movie S1. Cell-cycle-dependent localization of Hos3**
Wild type cells transformed with Hos3-GFP (CEN) and the nucleus reporter Rpb10-RFP (CEN) were grown to mid-log phase in synthetic minimal medium and prepared for fluorescence microscopy with the cover slip sealed by nail polish to prevent evaporation. Fluorescence images were taken at approximately 7 min intervals over the time period indicated. Hos3 is constantly at the bud neck and appears later as a focus in the daughter cell. Size bar: 5 µm.

**Figure S1. Sequence alignment of A. aeolicus AcuC1 and S. cerevisiae class I and II HDACs**
The crystal structure of a histone deacetylase homologue (AcuC1, also called HDLP) from the hyperthermophilic bacterium *A. aeolicus* reveals a catalytic core that establishes the lysine deacetylation mechanism for class I and II HDACs (Finnin et al., 1999). The amino acid sequences of Hos3, Rpd3, Hda1, Hos1, and Hos2 were aligned with that of AcuC1 (accession no. NP_213698) by ClustalW. Residues identical to AcuC1 are highlighted in black. Degrees of residue conservation are coded by color bars. The secondary structure and specific residues of AcuC1 are annotated as in (Finnin et al., 1999).

**Figure S2. Localization of truncated Hos3 alleles**
Wild type cells transformed with systematically truncated versions of Hos3-GFP (CEN) were analyzed by fluorescence microscopy. Hos3(1-566) and Hos3(1-487) are enhanced for an “around-the-nucleus” signal which significantly colocalizes with the nuclear pore complex (data not shown). Stars denote Hos3 at the mother bud neck. Arrowheads point to the daughter-SPB-associated Hos3. A second arrowhead (left) in Hos3(NΔ) points to its association with the mother SPB.

**Figure S3. Catalytic mechanism of Hos3**
(A) Identifying Hos3 equivalents of the key catalytic residues in AcuC1. The two charge-relay systems of AcuC1 required for catalysis are conserved in Hos3. The first D166-H131 pair (aligned to D231-H195 of Hos3) is involved in coordinating an activated water molecule while the second D173-H132 charge-relay pair (aligned to D238-H196 of Hos3) donates a proton to cleave the amide bond, yielding the acetate and the deacetylated
lysine products (Finnin et al., 1999). AcuC1 also requires three residues to stabilize a zinc ion for catalysis (Finnin et al., 1999). Two of these residues are clearly conserved (aligned to D233 and H235 of Hos3), but no Hos3 counterpart to D258 can be identified from the primary sequence alignment. The schematic representation of AcuC1-TSA interactions is adapted and modified from Finnin et al., 1999. TSA is in black and AcuC1 residues are labeled in red with their aligned counterparts in Hos3 in parentheses (see Fig. S1; conserved residues in black and non-conserved residues in gray). Thatched semi-circles indicate van der Waals contacts between hydrophobic protein residues and TSA. Hydrogen bonds are shown as green dashed lines.

(B and C) Hos3 catalyzes lysine deacetylation using residues analogous to the AcuC1 catalytic mechanism. Mutations of “H196E, D231N” (B) or “D233A” (C) abolish Hos3 lysine deacetylase activity. Cells transformed with vectors bearing the corresponding genes were assayed as in Fig. 2C.

(D) Loss of HDAC activity does not alter Hos3 level. hos3Δ cells transformed with CEN vectors bearing Hos3, Hos3-3XHA and Hos3H196E, D231N-3XHA were analyzed for Hos3 level by preparing the whole cell extracts for immunoblotting against HA. Tub1 was used as loading control.

(E) Loss of HDAC activity does not change Hos3 localization. Hos3H196E, D231N-GFP (CEN) was imaged in hos3Δ cells. An asterisk denotes Hos3 at the mother-bud neck, the daughter SPB-associated Hos3 is indicated by an arrow.

**Figure S4. The morphogenesis checkpoint components regulate Hos3 localization to the bud neck**

(A) Hos3 protein levels are unchanged in the mutant cells which mislocalize Hos3. The Hos3 levels in the mutants identified by screening are comparable to that in wild type cells. Hos3-3XHA (CEN) was transformed into hos3Δ, hsl1Δ hos3Δ, hsl7Δ hos3Δ, elm1Δ hos3Δ, and gin4Δ hos3Δ cells respectively. Hos3 level in these cells was analyzed by preparing the whole cell extracts for immunoblotting against HA tag. Tub1 was used as loading control.

(B) Localization of Hos3-GFP (CEN) in cla4Δ and kcc4Δ cells.
Figure S5. Targeting Hos3 to the neck is upstream of CDK regulation and downstream of the four regulator proteins

(A) Hos3 neck defect in the septin or hit mutants is not caused by Swe1-dependent cell cycle delay. Hos3-GFP (CEN) was imaged in shs1Δ swe1Δ, hsl1Δ swe1Δ, hsl7Δ swe1Δ, elm1Δ swe1Δ, and gin4Δ swe1Δ cells respectively.

(B) Localization of Hos3S47A, T201A, S533A, T671A-GFP, or Hos3(4A)-GFP (CEN) in wild type cells. S47, T201, S533, and T671 are the four Cdc28 consensus sites within Hos3.

(C) An extra copy of Hsl1 rescues Hos3 to the neck in elm1Δ gin4Δ double mutant cells. Hos3-GFP (CEN) was imaged in elm1Δ gin4Δ cells transformed with an empty vector or a CEN vector bearing Hsl1.

(D) Hsl1 kinase activity is essential for the rescue effect. Hos3-GFP (CEN) was imaged in elm1Δ, gin4Δ, and elm1Δ gin4Δ cells transformed with a CEN vector bearing kinase-dead Hsl1K110R.

Figure S6. Reciprocal localization profile of Hos3 and its regulators

(A-F) Hos3-GFP (CEN) was co-imaged with Cdc10-RFP (CEN), Hsl1-RFP (CEN), Hsl7-RFP (CEN), Elm1-RFP (CEN), and Gin4-RFP (CEN) respectively in wild type (A), shs1Δ (B), hsl1Δ (C), hsl7Δ (D), elm1Δ (E), and gin4Δ (F) cells. Images are rotated with the bud neck parallel to the horizontal axis. XZ and YZ dimensions are shown to reveal the neck localization pattern.

Figure S7. Investigating the function of neck-localized Hos3

(A) Hos3 is dispensable for assembling septins and hit proteins at the bud neck. Cdc10-RFP (CEN), Hsl1-RFP (CEN), Hsl7-RFP (CEN), Elm1-RFP (CEN), and Gin4-RFP (CEN) were respectively imaged in hos3Δ cells. Images are rotated with the bud neck parallel to the horizontal axis. XZ and YZ dimensions are shown to reveal the neck localization pattern.

(B) Wild type, hsl1Δ, hsl7Δ, and hos3Δ cells were grown to mid-log phase in YPD medium, stained with propidium iodide, and analyzed for DNA content by flow cytometry. Cells with 2N content of DNA reveal the G2/M subpopulation. Data from three independent experiments were analyzed for statistics. Error bars represent SEM. For statistical significance by t-test, ** denotes “p value < 0.001”; n.s. denotes “not significant (p value > 0.05)”. 
(C and D) Wild type, *hsl7Δ*, and *hos3Δ* cells of W303 background were grown to mid-log phase in YPD medium and analyzed by DIC microscopy (C) or by 10-serial dilution onto YPD plates for incubation at 30°C and 37°C (D).

(E) Neck-localization is dispensable for Hos3 association with the daughter SPB. Hos3-GFP (*CEN*) and Tub4-RFP (*CEN*) were co-imaged in *hsl7Δ* cells. Arrowhead points to the daughter-SPB-associated Hos3.

(F) Neck-localization is not involved with the genetic interaction between Hos3 and the *apc5CA* allele. Wild type, *apc5CA*, *hos3Δ*, *hsl7Δ*, *apc5CA hos3Δ*, and *apc5CA hsl7Δ* cells were grown to mid-log phase in YPD medium and analyzed by 10-serial dilution onto YPD plates for incubation at 30°C and 38°C.
Table S1. List of genes screened for defective targeting of Hos3 to the mother-bud neck.

| Genes          |
|----------------|
| Abp1, Ace2, Acf2, Acm1, Aip1, Arp1, Axl1, Axl2, Bem1, Bem2, Bem3, Bem4, Bfy1, Bfa1, Bik1, Bim1, Bni1, Bni4, Bnr1, Boi1, Boi2, Bud2, Bud3, Bud4, Bud6, Bud8, Cap1, Cap2, Cdc24, Cdc26, Cdc42, Cdh1, Chs3, Chs4, Chs5, Cin8, Cka1, Cka2, Ckb1, Cla4, Cnm67, Cyk3, Db2b, Db2d, Dfg5, Dsk2, Dss4, Dyn1, Dyn2, Dyn3, Elm1, Fkh1, Fkh2, Gic1, Gic2, Gin4, Hof1, Hog1, Hsl1, Hsl7, Jnm1, Kar3, Kar9, Kcc4, Kin3, Kin4, Kip1, Kip2, Kip3, Lte1, Mih1, Mkk1, Mpc54, Msb1, Msb2, Msb3, Msb4, Mss4, Nap1, Ndc1, Nfi1, Nip100, Nis1, Num1, Pac1, Pac11, Pea2, Rad23, Rdi1, Rga1, Rga2, Rho2, Rho4, Rom1, Rom2, Rsr1, Rvs167, Sac6, Sac7, Sic1, Skg6, Skm1, Sla1, Slt2, Spa2, Spo21, Srv2, Sso1, Sso2, Ste20, Stt4, Swe1, Swi5, Syp1, Tos2, Tpm1, Tus1 |

(For nonessential genes, a null mutant was used; for essential genes, a heat-sensitive conditional allele was assayed after 2h shift to non-permissive temperature.)

Table S2. Yeast strains used in this study

| Strain | Genotype | Reference/Source |
|--------|----------|-----------------|
| W303-1A | MATα ura3Δ leu2-3,112 his3Δ11,15 trp1Δ1 can1Δ100 ade2Δ1 | This Study |
| W303-1B | MATα ura3Δ leu2-3,112 his3Δ11,15 trp1Δ1 can1Δ100 ade2Δ1 | This Study |
| BY4742 | MATα ura3Δ0 leu2Δ0 hisΔ3Δ1 lysΔ0 | Research Genetics, Inc. |
| RCY239 | MATα ura3Δ5-2 leu2-3,112 | M-12 from Mark Longtine |
| RCY263 | MATα ade6 | Research Genetics, Inc. |
| RCY3915 | BY4742 hos3Δ::KanMX4 | Research Genetics, Inc. |
| RCY4042 | MATα ura3 leu2 cdc3-3 | Research Genetics, Inc. |
| RCY4043 | MATα ura3 leu2 met1 cdc11-6 | Research Genetics, Inc. |
| RCY4564 | BY4742 shs1Δ::KanMX4 | Research Genetics, Inc. |
| RCY4565 | BY4742 cla1Δ::KanMX4 | Research Genetics, Inc. |
| RCY4568 | BY4742 elm1Δ::KanMX4 | Research Genetics, Inc. |
| RCY4570 | BY4742 gin4Δ::KanMX4 | Research Genetics, Inc. |
| RCY4582 | MATα ura3Δ0 leu2Δ0 hisΔ3Δ1 lysΔ2Δ0 Hos3::3GFP-Ura3 | Research Genetics, Inc. |
| RCY4607 | BY4742 kcc4Δ::KanMX4 | Research Genetics, Inc. |
| RCY4609 | MATα ura3Δ0 leu2Δ0 hisΔ3Δ1 lysΔ2Δ0 met15Δ0 hos3Δ::HIS3 | Research Genetics, Inc. |
| RCY4624 | BY4742 his1Δ::KanMX4 | Research Genetics, Inc. |
| RCY4656 | MATα ura3Δ0 leu2Δ0 hisΔ3Δ1 lysΔ2Δ0 elm1Δ::KanMX4 hos3Δ::HIS3 | Research Genetics, Inc. |
| RCY4658 | MATα ura3Δ0 leu2Δ0 hisΔ3Δ1 lysΔ2Δ0 met15Δ0 gin4Δ::KanMX4 hos3Δ::HIS3 | Research Genetics, Inc. |
| RCY4660 | MATα ura3Δ0 leu2Δ0 hisΔ3Δ1 lysΔ2Δ0 met15Δ0 his1Δ::KanMX4 hos3Δ::HIS3 | Research Genetics, Inc. |
| RCY4669 | MATα ura3Δ0 leu2Δ0 hisΔ3Δ1 lysΔ2Δ0 met15Δ0 elm1Δ::KanMX4 gin4Δ::KanMX4 | Research Genetics, Inc. |
| RCY4693 | MATα ura3Δ0 leu2Δ0 hisΔ3Δ1 lysΔ2Δ0 trp1Δ1 Hos3::3HA-HIS3 | Research Genetics, Inc. |
| RCY4696 | BY4742 swe1Δ::KanMX4 | Research Genetics, Inc. |
| RCY4702 | MATα ura3Δ0 leu2Δ0 hisΔ3Δ1 lysΔ2Δ0 his7Δ::KanMX4 | Research Genetics, Inc. |
| RCY4716 | BY4742 mh1Δ::KanMX4 | Research Genetics, Inc. |
| RCY4721 | MATα ura3Δ0 leu2Δ0 hisΔ3Δ1 lysΔ2Δ0 his7Δ::KanMX4 hos3Δ::HIS3 | Research Genetics, Inc. |
| RCY4728 | MATα ura3Δ0 leu2Δ0 hisΔ3Δ1 lysΔ2Δ0 his7Δ::KanMX4 swe1Δ::KanMX4 | Research Genetics, Inc. |
| RCY4730 | MATα ura3Δ0 leu2Δ0 hisΔ3Δ1 lysΔ2Δ0 his1Δ::KanMX4 swe1Δ::KanMX4 | Research Genetics, Inc. |
| RCY4731 | MATα ura3Δ0 leu2Δ0 hisΔ3Δ1 lysΔ2Δ0 met15Δ0 swe1Δ::KanMX4 swe1Δ::KanMX4 | Research Genetics, Inc. |
| RCY4732 | MATα ura3Δ0 leu2Δ0 hisΔ3Δ1 lysΔ2Δ0 met15Δ0 gin4Δ::KanMX4 swe1Δ::KanMX4 | Research Genetics, Inc. |
| RCY4733 | MATα ura3Δ0 leu2Δ0 hisΔ3Δ1 lysΔ2Δ0 gin4Δ::KanMX4 swe1Δ::KanMX4 | Research Genetics, Inc. |
| RCY4743 | W303-1B adh4::URA3::CI-3Δ4 stv2Δ::KanMX | CCC1 in Chou et al., 2008 |
| RCY4744 | W303-1B adh4::URA3::CI-3Δ4 stv2Δ::KanMX | CCC12 in Chou et al., 2008 |
| Code   | Constructs                                                                 | Source                  |
|--------|-----------------------------------------------------------------------------|-------------------------|
| RCY4749| MATa ura3Δ0 leu2Δ0 his3Δ1 lys2Δ0 bar1Δ::HIS3 Hos3::3HA-HIS3                 | This Study              |
| RCY4875| RCY239 rho<sup>6</sup>                                                      | This Study              |
| RCY4889| MATa ura3 leu2 his3 lys2 ade2                                              | YTH1636 in Turner et al., 2010. |
| RCY4890| MATa ura3 leu2 his3 lys2 ade2 apc5<sup>C4</sup>-PA::His5                   | YTH1637 in Turner et al., 2010. |
| RCY4891| MATa ura3 leu2 his3 lys2 ade2 hos3::KanMX6                                 | YTH2804 in Turner et al., 2010. |
| RCY4892| MATa ura3 leu2 his3 lys2 ade2 apc5<sup>C4</sup>-PA::His5 hos3::KanMX6       | YTH2806 in Turner et al., 2010. |
| RCY4896| W303-1A hos3Δ::HIS3                                                        | This Study              |
| RCY4897| W303-1A hsl7Δ::HIS3                                                        | This Study              |
| RCY4904| BY4742 dyn1Δ::KanMX4                                                       | Research Genetics, Inc. |
| RCY4920| MATa ura3Δ0 leu2Δ0 his3Δ1 met15Δ0 dyn1Δ::KanMX4 hos3Δ::HIS3                 | This Study              |
| RCY4922| BY4742 kin4Δ::KanMX4                                                       | Research Genetics, Inc. |
| RCY4932| MATa ura3Δ0 leu2Δ0 his3Δ1 lys2Δ0 dyn1Δ::KanMX4 kin4Δ::KanMX4               | This Study              |
| RCY4937| MATa ura3Δ0 leu2Δ0 his3Δ1 lys2Δ0 dyn1Δ::KanMX4 hsl7Δ::KanMX4               | This Study              |
| RCY4939| BY4742 nip100Δ::KanMX4                                                    | Research Genetics, Inc. |
| RCY4940| BY4742 bub2Δ::KanMX4                                                       | Research Genetics, Inc. |
| RCY4941| BY4742 hfa1Δ::KanMX4                                                       | Research Genetics, Inc. |
| RCY4943| MATa ura3 leu2 his3 lys2 ade2 apc5<sup>C4</sup>-PA::His5 hsl7Δ::KanMX4     | This Study              |
| RCY4947| MATa ura3 leu2 his3 lys2 ade2 hsl7Δ::KanMX4                                | This Study              |
| RCY4954| BY4742 num1Δ::KanMX4                                                       | Research Genetics, Inc. |
| RCY4955| BY4742 arp1Δ::KanMX4                                                       | Research Genetics, Inc. |
| RCY4956| BY4742 jnm1Δ::KanMX4                                                       | Research Genetics, Inc. |
| RCY4957| MATa ura3Δ0 leu2Δ0 his3Δ1 dyn1Δ::KanMX4 bub2Δ::KanMX4                     | This Study              |
| RCY4958| MATa ura3Δ0 leu2Δ0 his3Δ1 lys2Δ0 dyn1Δ::KanMX4 hfa1Δ::KanMX4               | This Study              |
| RCY4942| MATa ura3Δ leu2Δ his3Δ met15Δ0 bar1Δ::HIS3                                 | This Study              |
Table S3. Plasmids used in this study

| Plasmid   | Description                        | Reference/Source                  |
|-----------|------------------------------------|-----------------------------------|
| pRC374    | pRS314                             | Sikorski and Hieter, 1989.        |
| pRC380    | pRS317                             | Sikorski and Boeke, 1991.         |
| pRC540    | pRS315                             | Sikorski and Hieter, 1989.        |
| pRC1291   | pRS426                             | Christianson et al., 1992.        |
| pRC2601   | pRS315 Rpb10-RFP                   | This Study                        |
| pRC3664   | pRS316 Hos3-GFP                    | This Study                        |
| pRC3665   | pRS316 Rpd3-GFP                    | This Study                        |
| pRC4163   | pRS316 Hst3-GFP                    | This Study                        |
| pRC4164   | pRS316 Hst2-GFP                    | This Study                        |
| pRC4175   | pRS316 Hos2-GFP                    | This Study                        |
| pRC4176   | pRS316 Hst1-GFP                    | This Study                        |
| pRC4177   | pRS316 Hst4-GFP                    | This Study                        |
| pRC4189   | pRS316 Hda1-GFP                    | This Study                        |
| pRC4190   | pRS316 Sir2-GFP                    | This Study                        |
| pRC4191   | pRS316 Hos1-GFP                    | This Study                        |
| pRC4267   | pRS315 Cdc10-RFP                   | This Study                        |
| pRC4270   | pRS426 Hos3-GFP                    | This Study                        |
| pRC4333   | pRS315 Bni4-RFP                    | This Study                        |
| pRC4334   | pRS315 Kcc4-RFP                    | This Study                        |
| pRC4418   | pRS316 Hos3(1-443)-GFP             | This Study                        |
| pRC4421   | pRS316 Hos3(1-635)-GFP             | This Study                        |
| pRC4447   | pRS316 Hos3(1-487)-GFP             | This Study                        |
| pRC4448   | pRS316 Hos3(1-566)-GFP             | This Study                        |
| pRC4450   | pRS316 Hos3(40-697)-GFP            | This Study                        |
| pRC4489   | pRS426 P<sub>GAL</sub>10-Hos3     | This Study                        |
| pRC4511   | pRS315 Elm1-Hos3                   | This Study                        |
| pRC4512   | pRS315 Ssh1                       | This Study                        |
| pRC4515   | pRS315 Gin4                       | This Study                        |
| pRC4533   | pRS316 GFP-Hos3                    | This Study                        |
| pRC4548   | pRS316 Sir3-Hos3(2-549)-GFP       | This Study                        |
| pRC4549   | pRS316 Sir3-Hos3(2-697)-GFP       | This Study                        |
| pRC4552   | pRS315 Sir3-RFP                   | This Study                        |
| pRC4578   | pRS317 Hsl1                       | This Study                        |
| pRC4579   | pRS317 Hsl1<sup>K110R</sup>       | This Study                        |
| pRC4599   | pRS316 Hos3<sup>HMS6, D215S</sup>-GFP | This Study                       |
| pRC4620   | pRS315 Elm1-RFP                   | This Study                        |
| pRC4621   | pRS315 Gin4-RFP                   | This Study                        |
| pRC4623   | pRS315 Hsl1-RFP                   | This Study                        |
| pRC4624   | pRS315 Hsl7-RFP                   | This Study                        |
| pRC4676   | pRS316 Hos3<sup>S47A, T203A, S533A, T671A</sup>-GFP | This Study                       |
| pRC4678   | pRS315 Hsl7                       | This Study                        |
| pRC4682   | pRS317 Hsl7                       | This Study                        |
| pRC4738   | pRS314 Sir3-Hos3(2-697)            | This Study                        |
| pRC4739   | pRS314 Sir3-Hos3(2-697)-GFP       | This Study                        |
| pRC4741   | pRS316 Hos3(40-443)-GFP            | This Study                        |
| pRC4747   | pRS315 Hos3-3HA                    | This Study                        |
| pRC4750   | pRS316 Hos3(1-39 + 444-697)-GFP   | This Study                        |
| pRC4751   | pRS315 Gin4-Hsl7-RFP              | This Study                        |
| pRC4752   | pRS314 Sir2                       | This Study                        |
| pRC4753 | pRS314 Sir3 | This Study |
|---------|-------------|------------|
| pRC4754 | pRS314 Sir3-Hos3(2-549) | This Study |
| pRC4755 | pRS314 Sir3-Hos3(2-549)-GFP | This Study |
| pRC4762 | pRS315 Sec2-Hsl7-RFP | This Study |
| pRC4815 | pRS315 Hos3<sup>3196E, D231N</sup>-3HA | This Study |
| pRC4823 | pRS315 Hos3 | This Study |
| pRC4837 | pRS314 Sir3-Hos3(2-549)<sup>3196E, D231N</sup> | This Study |
| pRC4839 | pRS315 Sec2-RFP | This Study |
| pRC5060 | pRS315 Tub4-RFP | This Study |
| pRC5071 | pRS315 Hsl7<sup>254E</sup>-RFP | This Study |
| pRC5159 | pRS314 Sir3-Hos3(2-549)<sup>223A</sup> | This Study |
| pRC5163 | pRS426<sup>P<sub>GAL1</sub></sup><sub>Kin</sub>4 | This Study |
| pRC5165 | pRS426<sup>P<sub>GAL1</sub></sup><sub>Hos3<sup>3196E, D231N</sup></sub> | This Study |
| pRC5178 | pRS426<sup>P<sub>GAL1</sub></sup><sub>Bub2</sub> | This Study |
| pRC5179 | pRS426<sup>P<sub>GAL1</sup></sub><sub>Bfa1</sub> | This Study |

**Supplementary References**

Finnin, M.S., Donigian, J.R., Cohen, A., Richon, V.M., Rifkind, R.A., Marks, P.A., Breslow, R., and Pavletich, N.P. (1999). Structures of a histone deacetylase homologue bound to the TSA and SAHA inhibitors. Nature 401, 188-193.
Figure S2

|   | Hos3(residues)-GFP | Tub4-RFP | merge | DIC |
|---|-------------------|----------|-------|-----|
| (i) | 1-635            |          |       |     |
| (ii) | 1-566            |          |       |     |
| (iii) | 1-487             |          |       |     |
| (iv) | 1-443 "CΔ"       |          |       |     |
| (v) | 40-697 "NΔ"      |          |       |     |
| (vi) | 40-443 "NΔ+CΔ"   |          |       |     |
| (vii) | 1-39 + 444-697 "HDACΔ" |       |       |     |
## Figure S4

### A

**hos3Δ + Hos3-3XHA (CEN)**

|      | hsl1Δ | hsl7Δ | elm1Δ | gin4Δ |
|------|-------|-------|-------|-------|
| kDa  | HA    | HA    | HA    | HA    |
| 100  |       |       |       |       |
| 55   |       |       |       |       |

### B

**Hos3-GFP**

- **cla4Δ**
- **kcc4Δ**

**DIC**

- **cla4Δ**
- **kcc4Δ**
