**SUPPLEMENTARY INFORMATION**

# The CDK regulators Cdh1 and Sic1 promote efficient usage of DNA replication origins to prevent chromosomal rearrangements at a chromosome arm

Pilar Ayuda-Durán [1], Fernando Devesa [1], Fábia Gomes [1], Joana Sequeira-Mendes [2], Carmelo Ávila-Zarza [3], María Gómez [2], and Arturo Calzada [1,*]

[1] Department of Microbial Biotechnology, Centro Nacional de Biotecnología CNB-CSIC, Darwin 3, Madrid, 28049, Spain
[2] Centro de Biología Molecular Severo Ochoa CBMSO-CSIC/UAM, Nicolás Cabrera 1, Madrid, 28049, Spain
[3] Department of Statistics, Universidad de Salamanca, Salamanca, 37007, Spain

Supplementary Tables 1 and 2; Supplementary Figure legends and Supplementary Figures 1-5.

Supplementary Table 1. Yeast strains

| Strain | Genotype | Source |
|---|---|---|
| YAC36 | W303-1a *MATa ade2-1 ura3-1 his3-11,15 trp1-1 leu2-3,112 can1-100* | K. Labib |
| YAC37 | W303-1b *MATα ade2-1 ura3-1 his3-11,15 trp1-1 leu2-3,112 can1-100* | K. Labib |
| RDKY3615 | S288C *MATa ura3-52, leu2Δ1 trp1Δ63 his3Δ200 lys2ΔBgl hom3-10 ade2Δ 1 ade8 YEL069::URA3* | Chen and Kolodner, 99 |
| YAC177 | S288C *yjl193w::hphNT* | This study |
| YAC188 | W303-1a *yjl193w::hphNT* | This study |
| YAC198 | W303-1a *sic1::(TRP1)GAL1,10p-SIC1* | This study |
| YAC217 | S288C *sic1::(TRP1)GAL1,10p-SIC1* | This study |
| YAC272 | W303-1a *bar1Δ::URA3* | This study |
| YAC276 | W303-1a *bar1Δ::URA3 sic1::(TRP1)GAL1,10p-SIC1* | This study |
| YAC300 | S288C *cdh1Δ::HIS3* | This study |
| YAC304 | S288C *sic1::(TRP1)GAL1,10p-SIC1 cdh1Δ::HIS3* | This study |
| YAC308 | W303-1a *cdh1Δ::HIS3* | This study |
| YAC314 | W303-1a *cdh1Δ::HIS3 sic1::(TRP1)GAL1,10p-SIC1* | This study |
| YAC571 | S288C *sic1Δ::HIS3* | This study |
| YAC768 | W303-1a *ura3Δ::KanMX4 ars507Δ::ARS305* | This study |
| YAC796 | W303-1a *leu2::pRS305SIC1p-SIC1* | This study |
| YAC797 | W303-1a *sic1::(TRP1)GAL1,10p-SIC1 leu2::pRS305SIC1p-SIC1* | This study |
| YAC799 | W303-1a *cdh1Δ::HIS3 leu2::pRS305SIC1p-SIC1* | This study |
| YAC800 | S288C *leu2::pRS305SIC1p-SIC1* | This study |
| YAC801 | S288C *sic1::(TRP1)GAL1,10p-SIC1 leu2::pRS305SIC1p-SIC1* | This study |
| YAC803 | S288C *cdh1Δ::HIS3 leu2::pRS305SIC1p-SIC1* | This study |
| YAC810 | W303-1a *ura3Δ::KanMX4 cdh1Δ::HIS3 ars507Δ::ARS305* | This study |
| YAC828 | W303-1a *ura3Δ::KanMX4 sic1::(TRP1)GAL1,10p-SIC1 ars507Δ::ARS305* | This study |
| YAC1158 | W303-1a *sic1Δ::HIS3* | This study |

Supplementary Table 2. Maintenance of origin efficiency in the absence of Cdh1 or Sic1 is independent on normal origin efficiency, firing timing, or chromosomal location.

| Origin | Genomic location (1) | Analysed by 2D gels (1) | Replication Index | | Origin efficiency in this study (4) |
|---|---|---|---|---|---|
| | | | Heavy:Light (2) | Copy Number (3) | |
| ARS306 | III-75 | Yes | 0 | 0.045 | Reduced |
| ARS305 | III-39 | Yes | 0.033 | 0.119 | Unaffected |
| ARS432 | IV-1159 | This study | 0.034 | 0.008 | Reduced |
| ARS607 | VI-199 | Yes | 0.036 | 0.151 | Reduced |
| ARS508 | V-94 | This study | 0.064 | 0.114 | Unaffected |
| ARS1014 | X-417 | This study | 0.108 | 0.096 | Unaffected |
| ARS507 | V-59 | This study | 0.165 | 0.14 | Reduced |
| ARS603.5 | VI-119 | Yes | 0.197 | 0.214 | Reduced ** |
| ARS1-ARS416 | IV-463 | Yes | 0.231 | 0.186 | Reduced |
| ARS501-ARS522 | V-550 | Yes | 0.399 | - | Unaffected |
| ARS608 | VI-217 | Yes | - | - | No firing |
| ARS302 | III-15 | Yes | - | - | No firing |
| ARS303 | III-15 | Yes | - | - | No firing |
| ARS320 | III-16 | Yes | - | - | No firing |
| ARS503 | V-7 | This study | - | - | No firing |
| ARS504 | V-9 | This study | - | - | No firing |
| ARS504.2 | V-12 | This study | - | - | Increased*/Reduced** |
| proARS506# | V-18 | This study | - | - | No firing |
| ars507Δ::ARS305 | V-59 | This study | n.d. | n.d. | Unaffected |

(1) See www.OriDB.com
(2) Raghuraman et al. (62).
(3) Yabuki et al. (86).
(4) As detected in 2D blots in either *Δcdh1* or Sic1-depleted cells, *versus* control cells.
# Likely ARS
* in *Δcdh1* cells
** in *sic1* cells
n.d. Not determined

62. Raghuraman, M.K., Winzeler, E.A., Collingwood, D., Hunt, S., Wodicka, L., Conway, A., Lockhart, D.J., Davis, R.W., Brewer, B.J. and Fangman, W.L. (2001) Replication dynamics of the yeast genome. Science, 294, 115-121.
86. Yabuki, N., Terashima, H. and Kitada, K. (2002) Mapping of early firing origins on a replication profile of budding yeast. Genes Cells, 7, 781-789.

**SUPPLEMENTARY FIGURE LEGENDS**

Supplementary Figure 1. Cell growth and cell cycle distribution analysis of cells lacking Cdh1 and Sic1. (a) Dilution spotting of control, single, and double mutants in the indicated conditions in W303-1a (i) and S288C (ii) cells. (b) FACS analysis of cells of the indicated strains of W303-1a (i) and S288C (ii) backgrounds in logarithmic growth. *sic1* cells are shown in expressing (RaffGal, so that Sic1 is overexpressed) and repressing conditions (after switching to glucose for 4 hours, and Sic1 is depleted). Cultures in glucose correspond to cells employed for 2D gel analysis. Note S288C cells show milder phenotypes for cells lacking Cdh1 or Sic1.

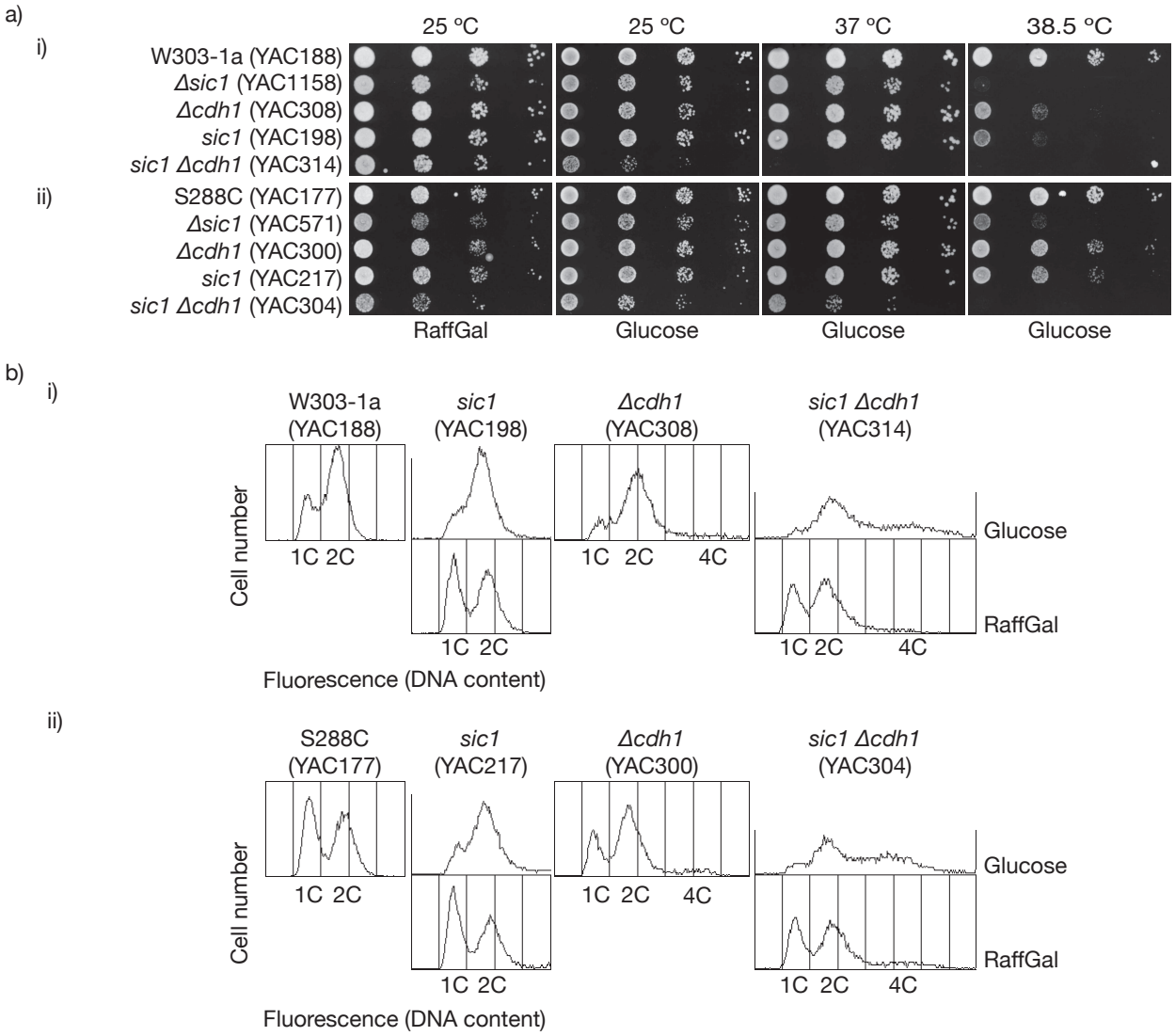
Supplementary Figure 2. Depletion of Sic1 upon addition of glucose to *GAL1,10p-SIC1* cells. (a) Sic1 levels in control and *GAL1,10p-SIC1* W303-1a, and (b) S288C cells, in raffinose-galactose or glucose as indicated. As, asynchronous cells; Noc, nocodazole; $\alpha$-F, alpha-factor. Pgk1 levels are shown as a loading control. Note a less efficient Sic1-depletion in S288C cells than in W303-1a cells. *sic1* cells are shown in expressing (RaffGal) and repressing conditions (Glucose).

Supplementary Figure 3. Origin firing activity in $\Delta$*sic1* cells. 2D gels of the indicated origins in control and $\Delta$*sic1* cells performed in parallel. Origin efficiencies are indicated in the upper right corner. When firing efficiency loses are detected in $\Delta$*sic1* cells relative to wt cells, white arrowheads denote the active, bubble-arcs, and black arrowheads the passive Y-arcs,

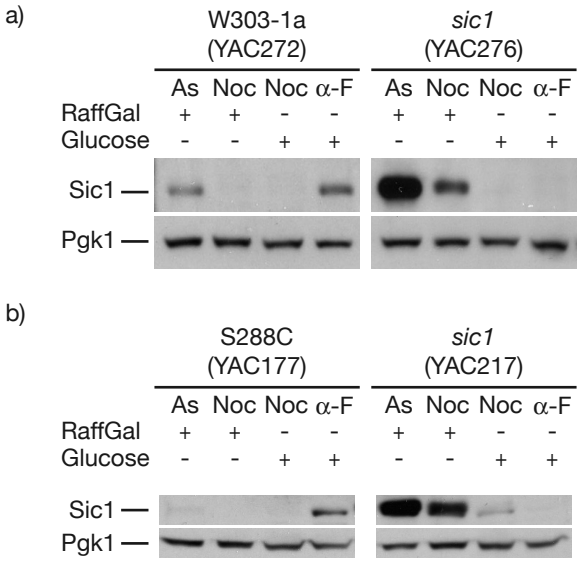
Supplementary Figure 4. (a) Repetition of some 2D gels for the indicated origins in control, single and double mutants, performed in parallel to evaluate reproducibility. (b) Repetition of some 2D gels from Figure 1B in double mutant cells of the S288C background. Origin efficiencies are indicated in the upper right corner. Open arrowheads show loss of efficiency relative to control cells.

Supplementary Figure 5. Analysis of fork direction at a region adjacent to ARS305 as in Figure 1C. The restriction fragment analysed -HindIII-BamHI (3.04 kb, chr. III, coordinates 41817-44862 '*downstream ARS305*')-, the EcoRI in-gel digestion (chr. III, 42444), the probe *downstream ARS305* (44213-44681 chr. III), and signal quantifications were performed as indicated (see Material and methods).

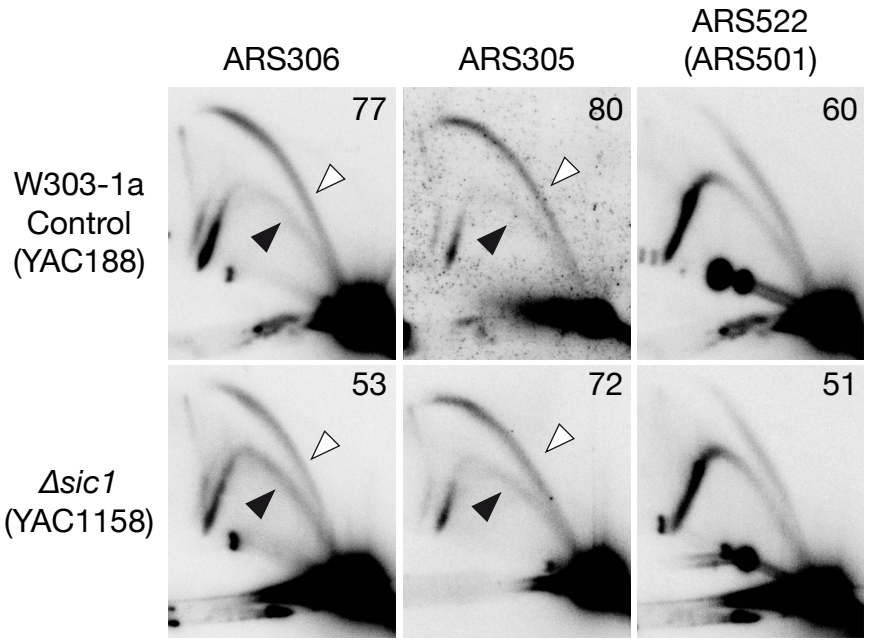
Supplementary Figure 1



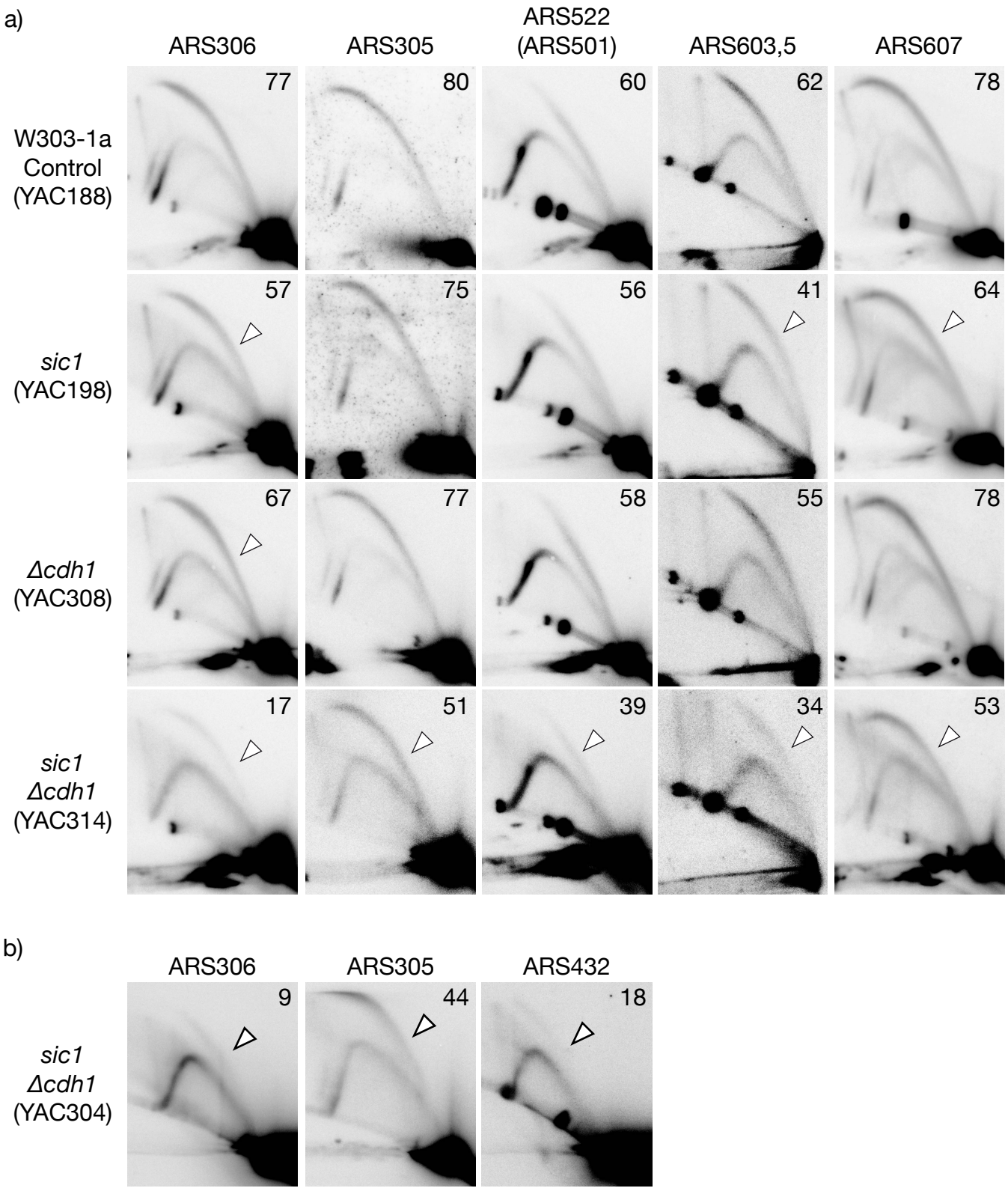
Supplementary Figure 2



Supplementary Figure 3



Supplementary Figure 4



Supplementary Figure 5