**SUPPLEMENTAL Figure S1**

**Overexpressed Cse4 is stabilized in *hir1∆* strain.**

Cse4 protein stability was determined by Western blot analysis with wild type and *hir1∆* strains containing *GALCSE4HA* (pMB1458) induced in galactose medium for 3 hours. Samples were collected at indicated time after treatment of CHX (10 ug/ml) and shifted to glucose medium. Half-lives, determined as described in Materials and Methods, are the average of two biological replicates.

**SUPPLEMENTAL Figure S2**

Kinetochore protein Mtw1-GFP is not mislocalized in *hir2∆* strains. Wild type and *hir2∆* strains with *GALCSE4HA* (pMB1458) were transformed with plasmid pMB1059 expressing Mtw1-GFP and grown for 1 hour in SC-Ura with 2% raffinose. MTW1-GFP foci within the nucleus were counted in WT (n=98) and *hir2∆* (n=147) cells, respectively.

**SUPPLEMENTAL Figure S3**

Cse4 is mislocalized to non-centromeric regions in *hir2∆* strain. ChIP for Cse4was performed as described in Figure 6. Enrichment of Cse4to *CEN* (*CEN1* and *CEN3*), peri-*CEN* (R3, Chr 1: 151357-151436; and R4, Chr 14: 628862-629047), coding region (R9, Chr 10: 391376-391504), ARS (R8, Chr 5: 442614-442691), non-coding intergenic regions (R5, Chr 5: 559132-559242; R7, Chr 2: 419616-419768; and R11, Chr 4: 916200-916500), promoters (R6, Chr 2: 680382-680488; R10, Chr 10: 526735-526917; R12, Chr 5: 210000-210300; R13, Chr 11: 234400-234700; and R14, Chr 16: 502900-503200), and negative controls (R15, Chr 4: 896650-890950; and R16, Chr 16: 530600-530900) was determined by ChIP-qPCR and is reported as % input. Average from three independent experiments ± standard error is shown. \**p*-value <0.05, \*\**p*-value <0.01, ns = not significant, Student’s t test.

**SUPPLEMENTAL Figure S4**

Cse4 is mislocalized to non-centromeric regions in *hir2∆* *cac2∆* strain. ChIP was performed using chromatin prepared from wild type, *hir2∆*, *cac2∆,* and *hir2∆* *cac2∆* strains with *GALCSE4HA* (pMB1458) after growth for 6 hours in SC-Ura with galactose and raffinose (2% each). Immunoprecipitation was with α-HA agarose as described in materials and methods. Enrichment of Cse4to *CEN3* (*p*-value = 0.62), peri-*CEN* (R4, Chr 14: 628862-629047, *p*-value = 0.97), coding region (R9, Chr 10: 391376-391504, *p*-value = 0.17), promoters (R6, Chr 2: 680382-680488, *p*-value = 0.57; R10, Chr 10: 526735-526917, *p*-value = 0.82; R12, Chr 5: 210000-210300, *p*-value = 0.70; and R13, Chr 11: 234400-234700, *p*-value = 0.30), and negative control (R16, Chr 16: 530600-530900, *p*-value = 0.35) were determined by ChIP-qPCR and reported as % input. Average from three independent experiments ± standard error is shown. ns = not significant, Student’s t test.

**SUPPLEMENTAL Figure S5**

Overexpression of *UBI4* fails to suppress the lethality of *hir2∆ GALCSE4* or *hir2∆ psh1∆ GALCSE4.* Spot test by 5-fold serial dilution was performed at 30˚ C on glucose- or galactose-containing medium with wild type, *psh1∆*, *hir2∆,* and *hir2∆ psh1∆* strains, all containing *GALCSE4* (pMB1458) transformed with either MoBY 2-micron vector or *UBI4*. Image was photographed 4 days post plating.