Supporting Information

Thorpe et al. 10.1073/pnas.0811248106

SI Methods

Image Analysis. To reconstruct a 3-dimensional fluorescent image of the cells a stack of vertically-separated fluorescent images (z-stack) was deconvolved using Volocity deconvolution software (Improvision, Waltham, MA USA). The resulting volume was used to calculate the amount of fluorescence (in relative units) for each kinetochore or nucleus using Volocity software as previously described (3, 4). Briefly, a 3-dimensional volume within the image was selected, containing either the nucleus (for the histones) or the kinetochore (for kinetochore proteins) and quantified. To control for background, an extracellular region was also selected and measured. We subtract the background fluorescence levels from those of either the nucleus or the kinetochore to derive the relative units provided in the Dataset S1 (more information concerning the levels of cellular background is provided below). The fluorescence signal generated from fluorescently-tagged protein equates with their concentration, as shown by numerous studies (for example see ref. 5). However, we wished to perform a number of controls (in addition to those described in the printed text of the manuscript) to ensure that the fluorescence measurements made using our Zeiss microscope and the subsequent image analysis using Volocity deconvolution software are representative of the amount of fluorescence in the yeast cells.

Deconvolution. Although the deconvolution algorithm is a linear function, we were concerned that the process would distort the relative levels of fluorescence of 2 objects in an image. Since our analysis depends on this quantitation, we used fluorescent beads (Tetraspeck Fluorescent Microspheres, Molecular Probes) to determine the effects of iterative restorations using Volocity deconvolution software. We find that although Volocity deconvolution can affect the relative intensity of fluorescence, it does not affect the ratio of intensities between 2 fluorescent foci in the same image (two examples are shown in Fig. S5).

Cellular Background. To rule out the possibility that the asymmetry we observe is caused by the background fluorescence in the mother lineage, we checked the background fluorescence levels of the mother and the bud. This is particularly pertinent for the study of spores, since spore wall components, which are often retained after germination, are autofluorescent in the blue channel (CFP, 480 nm). We find that the background levels of fluorescence are equivalent in the mother versus bud in several of the strains used in this study. For spores derived from the diploid strain, W7247 (*MTW1-YFP/MTW1-CFP*), the mean cellular background m/b ratio at the first division for CFP is 1.1 (SD = 0.12) and for YFP 1.0 (SD = 0.03). For spores derived from 2 other diploid strains W7909 and W7908 (*CTF19-YFP/CTF19-CFP* and *ASK1-YFP/ASK1-CFP* respectively) the equivalent ratios are 1.2 and 1.2 for CFP respectively (SD = 0.01 and 0.13, respectively) and for YFP 1.0 and 1.0 respectively (SD = 0.04 and 0.03, respectively).

To obtain another independent confirmation that the background in the mother cell is not the cause of the asymmetry found in our analysis, we compared fluorescence intensity with m/b ratio. If high background fluorescence caused the asymmetry in the mother lineage, we would expect to find that kinetochore foci with a low intensity of fluorescence would show greater levels of asymmetry (since they would be more sensitive to the background levels). For experiments using Mtw1 and Ask1, we plotted the intensity of nonencoded fluorescence in the kinetochore foci versus the m/b ratio. There is no correlation (positive or negative) between the intensity of fluorescence and the m/b ratios for any of the experiments that we examined (Fig. S6).

Z Plane. It has been observed that the distance of the fluorescence signal from the coverslip affects the measured fluorescence intensity (5). Both of our cell preparation methods put the yeast cells adjacent to the coverslip and in all of our images the kinetochore pairs in the mother and bud were approximately equivalent distances from the coverslip with a variation of ≈ 2 μm. Using a variety of fluorescent proteins we have determined the effect of z position on fluorescence intensity. As published previously (5), we find that fluorescence intensity decreases with increasing distance from the coverslip. However, this effect is $<10\%$ at 5 μm with our system.

Dilution of Kinetochore Proteins. It is relatively straightforward to assay the dilution of the nonencoded histone Hta1 (histone H2A) and we find that it dilutes by approximately half at each division. Since, we are measuring the total fluorescence in the nucleus, this measurement likely includes the free and DNA-bound Hta1. In contrast, we do not measure the total amounts of Mtw1, Ctf19, Ask1 and Ndc10 in the nucleus—we only measure fluorescence in the kinetochore focus. Since kinetochore proteins can exchange between the free pools in the nucleus and the kinetochore structure, the dilution of nonencoded kinetochore proteins is more complex than that of the histone. Our study was not designed to address the stability of proteins in the kinetochore structure, nor do we measure the exchange of kinetochore proteins between the kinetochore structure and the nucleus as a whole.

Symmetric Division of TetR-mRFP. Although we find that Hta1 segregates symmetrically, we also measured a different specific focus within the nucleus as another negative control. We used the tetracycline repressor tagged with RFP, which binds specifically to an array of Tet operator sequences integrated into chromosome 3. We examined the intensity of the RFP foci in cells derived from the spore in the first postmeiotic division obtained from a homozygous diploid parent (W8368). We find that the m/b ratio is 1.2 (Dataset S1), indicative of symmetrical segregation of this protein.

In summary, there are a number of potential sources of error in the quantitation of fluorescence using microscopy. *In toto*, we measured (and compared) fluorescence levels in adjacent cells from the same images along with structure of interest, we conclude that the measured fluorescence intensities are indicative of the amount of tagged protein within the cells.

Rad52. We examined spores derived from diploid cells encoding Rad52-YFP to determine whether foci arise during the first cell division. We captured fluorescence, images as previously reported (3), every 10 min as the spores and their progeny underwent divisions. It should be noted that although a 10-min time interval reduces photobleaching, it likely prevents us from observing some short-lived Rad52-YFP foci. We found that Rad52-YFP foci form in dividing spores ($\approx 18\%$ of S phases) and also in their progeny, and this result was not detectably different from the frequency in haploid cells. Additionally, we derived spores from a diploid strain encoding Rad52-YFP/Rad52-CFP and followed these spores through 2 divisions. We found that m/b ratios for Rad52 protein in the nucleus are equivalent in the mother lineage versus other lineages (m/b ratios ≈ 1.4 in all lineages).

1. Thomas BJ, Rothstein R (1989) The genetic control of direct-repeat recombination in *Saccharomyces*: the effect of rad52 and rad1 on mitotic recombination at *GAL10*, a transcriptionally regulated gene. *Genetics* 123:725–738.
2. Zou H, Rothstein R (1997) Holliday junctions accumulate in replication mutants via a RecA homolog-independent mechanism. *Cell* 90:87–96.
3. Lisby M, Barlow JH, Burgess RC, Rothstein R (2004) Choreography of the DNA damage response: spatiotemporal relationships among checkpoint and repair proteins. *Cell* 118:699–713.
4. Thorpe PH, Marrero VA, Savitzky MH, Sunjevaric I, Rothstein R (2006) Cells expressing murine *RAD52* splice variants favor sister chromatid repair. *Mol Cell Biol* 26:3752–3763.
5. Joglekar AP, Bouck DC, Molk JN, Bloom KS, Salmon ED (2006) Molecular architecture of a kinetochore-microtubule attachment site. *Nat Cell Biol* 8:581–585.

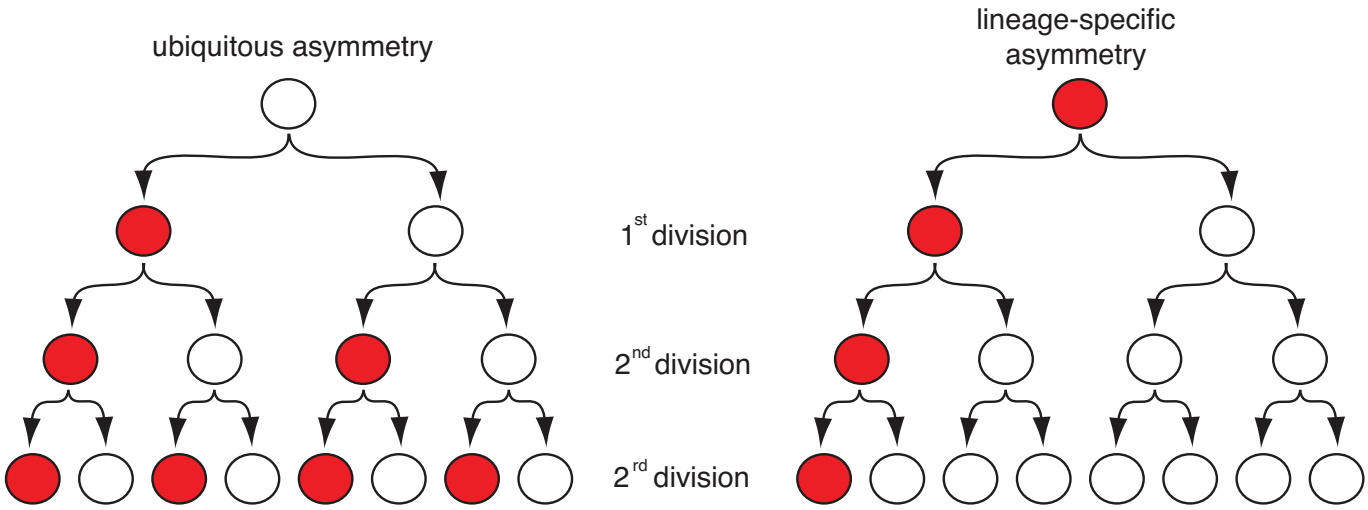

Fig. S1. Models of ubiquitous asymmetry (*Left*) and lineage-specific asymmetry (*Right*). (*Left*) An example of ubiquitous asymmetry is shown; the cells shown in red are capable of switching mating type—these are the mother cells from each and every division. Mating-type switching is inhibited in the bud lineage due to asymmetric segregation of RNA encoding Ash1, an inhibitor of the HO endonuclease. (*Right*) A lineage-specific pattern of asymmetry is shown, which differs from ubiquitous asymmetry in that only a single lineage of cells (shaded in red) shows the asymmetric phenotype.

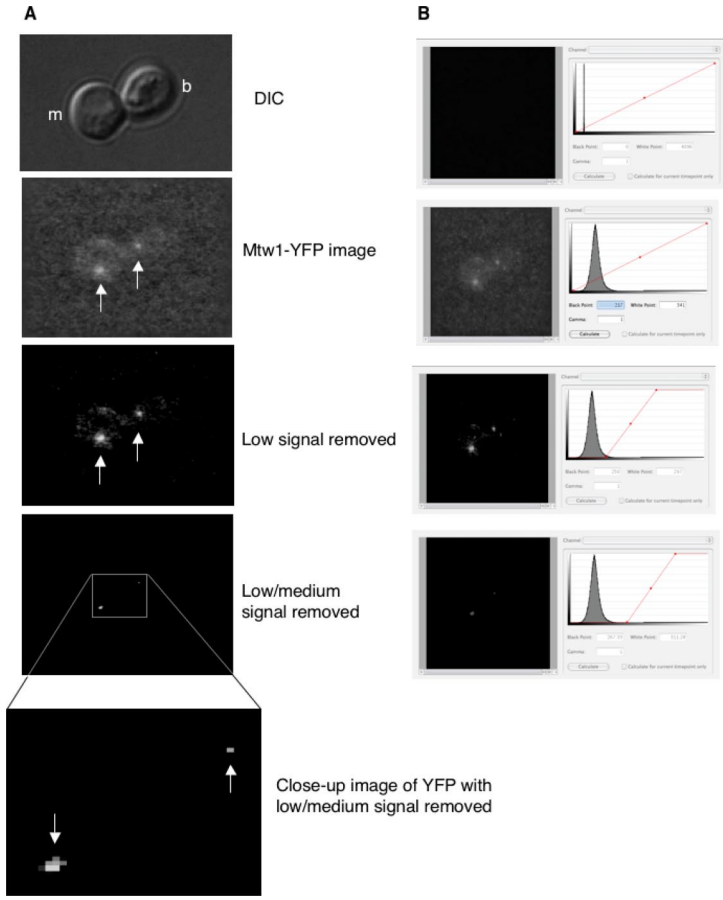

Fig. S2. (*A*) An example of a time-lapse image of the first division of a spore containing the *MTW1* gene tagged with YFP (derived from a YFP/CFP diploid). The *Top* DIC image shows the mother cell (*m, Left*) and bud cell (*b, Right*). Below, in the deconvolved YFP image, the asymmetry between the mother and bud is not clearly apparent, the 2 kinetochores are indicated with white arrows. Quantitation of the kinetochore fluorescence (minus background) indicates a mother to bud ratio of 2.42 (mother kinetochore = 67.2 relative units and bud kinetochore = 27.8 relative units, see Dataset S1, Mtw1 spores #MTW1-Z). (*Lower*) The same YFP image with the low signal and then the low to medium signal removed (see below for an explanation of the method). *Lower* is a magnification of the boxed region of the enhanced YFP image containing the 2 kinetochores to illustrate the asymmetry. (*B*) Examples are shown of the contrast enhancements performed on the deconvolved YFP images in Fig. S2*A*. Screen captures of the Volocity software contrast enhancement feature are shown. Each pixel in the fluorescence images contains 4096 levels of fluorescence. The ''black point'' is the level (of signal) for an image pixel to be represented as black and the ''white point'' is the level (of signal) for an image pixel to be represented as white. (*Upper*) No signal is visible, with a black point set to 0 and a white point set to 4096, the full range measured in our experiments. The next image shows the black point increased to 241 and the white point reduced to 341. By progressively increasing the black point and reducing the white point it is possible to visualize only the pixels with the highest levels of fluorescence in an image, shown in *Lower*. Importantly, this enhancement is linear and is applied uniformly across an image. (*C* and *D*) An example of a time-lapse sequence of images of the first 3 divisions of a spore containing the *MTW1* gene tagged with CFP (derived from a YFP/CFP diploid). In the series of images at *Left*, the *Upper* DIC image shows the mother cell (*m, Lower*) and bud cell (*b, Upper*). Below, in the CFP image, the 2 kinetochores are indicated with white arrows. (*Lower*) The same CFP image, with the low signal and then the low to medium signal removed. *Lower* is a close-up image of the 2 kinetochores to illustrate the asymmetry. (*Right*) The same format but for the subsequent second cell division. The kinetochore CFP quantitation of these images, produces values as follows, m = 333.2, b = 174, mm = 235, mb = 202, bm = 202 and bb = 168 relative units, full details are provided in Dataset S1 (Mtw1 spores, MTW1-AK). (*D*) The same colony as for Fig. 5*C*, at the third division, to clarify the position of cells, a dotted line indicates the position of the cells in the DIC and fluorescence images. The enhanced CFP image is divided since the mother lineage cell and its progeny (mmm and mmb) are in a different focal plane to the other cells. The kinetochore CFP quantitation of these images, produces values as follows, mmm = 847, mmb = 141, mbm = 162, mbb = 182, bmm = 319, bmb = 154, bbm = 151 and bbb = 142 relative units, full details are provided in Dataset S1 (Mtw1 spores, MTW1-AK). (*E*) An example of a time-lapse image of the first division of a spore containing the *ASK1* gene tagged with YFP (derived from a YFP/CFP diploid). The *Top* DIC image shows the mother cell (*m, Upper*) and bud cell (*b, Lower*). Below, in the YFP image, the asymmetry between the mother and bud is not clearly apparent in the deconvolved image, the 2 kinetochores are indicated with white arrows. Quantitation of the kinetochore fluorescence (minus background) indicates a mother to bud ratio of 2.9 (mother kinetochore = 51.1 relative units and bud kinetochore = 17.4 relative units, see Dataset S1, Ask1 spores, #ASK1-B1). (*Lower*) The same YFP image, with the low signal and then the low to medium signal removed. *Lower* is a close-up image of the 2 kinetochore cluster signals to illustrate the asymmetry. (*F*) An example of a time-lapse image of the first division of a spore containing the *CTF19* gene tagged with YFP (derived from a YFP/CFP diploid). (*Upper*) DIC image showing the mother cell (*m, Left*) and bud cell (*b, Right*). Quantitation of the kinetochore fluorescence (minus background) indicates a mother to bud ratio of 1.85 (mother kinetochore = 51 relative units and bud kinetochore = 27.5 relative units, see Dataset S1, Ctf19 spores #CTF19-C3). (*Lower*) The same YFP image, with the low signal and then the low to medium signal removed. *Lower* is a close-up image of the 2 kinetochores to illustrate the asymmetry. (*G*) An example of a time-lapse image of the first division of a spore containing the *NDC10* gene tagged with YFP (derived from a YFP/CFP diploid). (*Upper*) DIC image showing the mother cell (*m, Right*) and bud cell (*b, Left*). Below, in the YFP image, the 2 kinetochores are indicated with white arrows. Quantitation of the kinetochore fluorescence (minus background) indicates a mother to bud ratio of 1.97 (mother kinetochore = 129.3 relative units and bud kinetochore = 65.6 relative units, see Dataset S1, Ndc10 spores, #NDC10-C9). (*Lower*) The same YFP image, with the low signal and then the low to medium signal removed. *Lower* is a close-up image of the 2 kinetochores to illustrate the asymmetry.

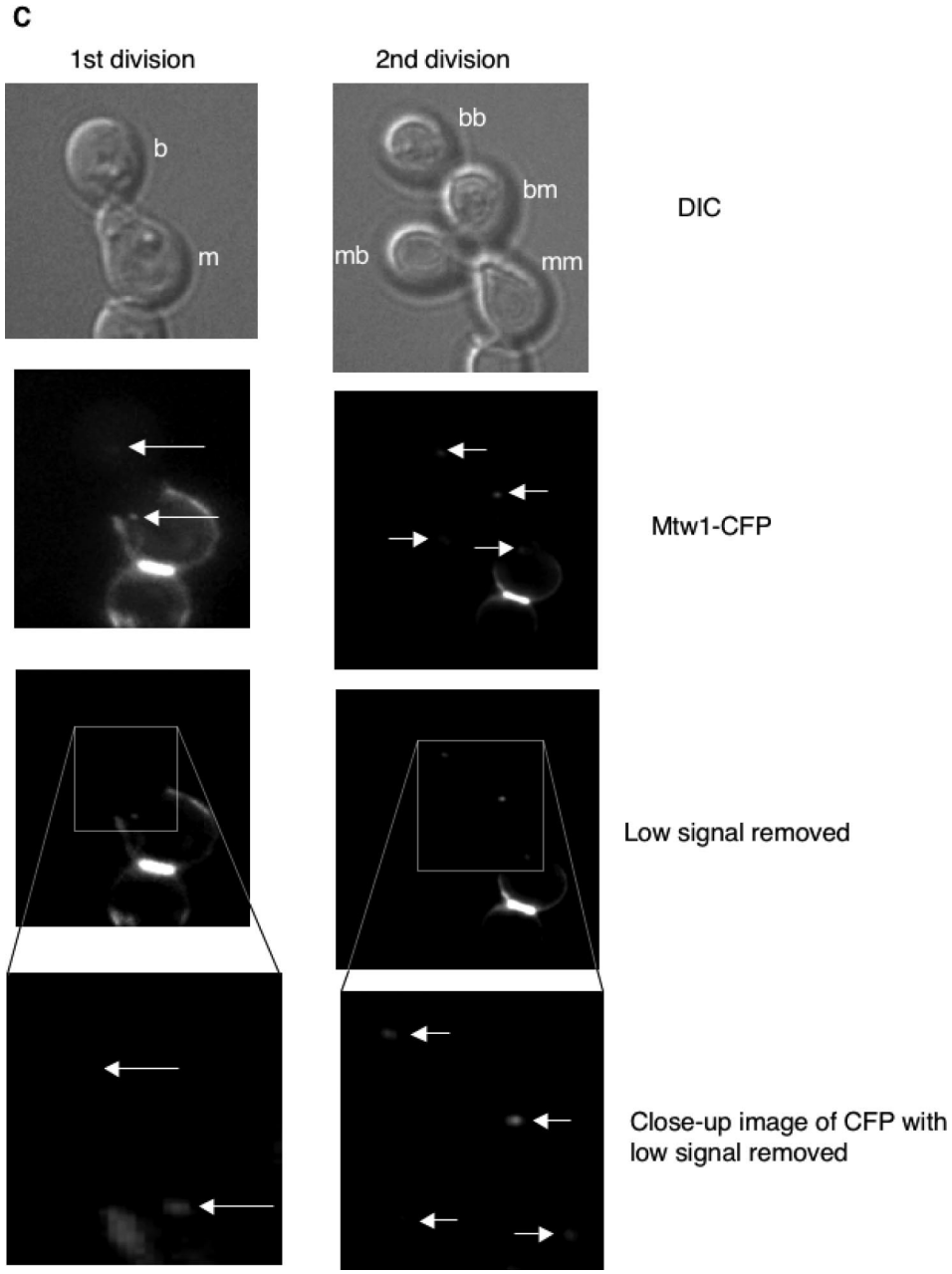
C
1st division
2nd division
b
m
bb
bm
mb
mm
DIC
Mtw1-CFP
Low signal removed
Close-up image of CFP with
low signal removed

Fig. S2C.

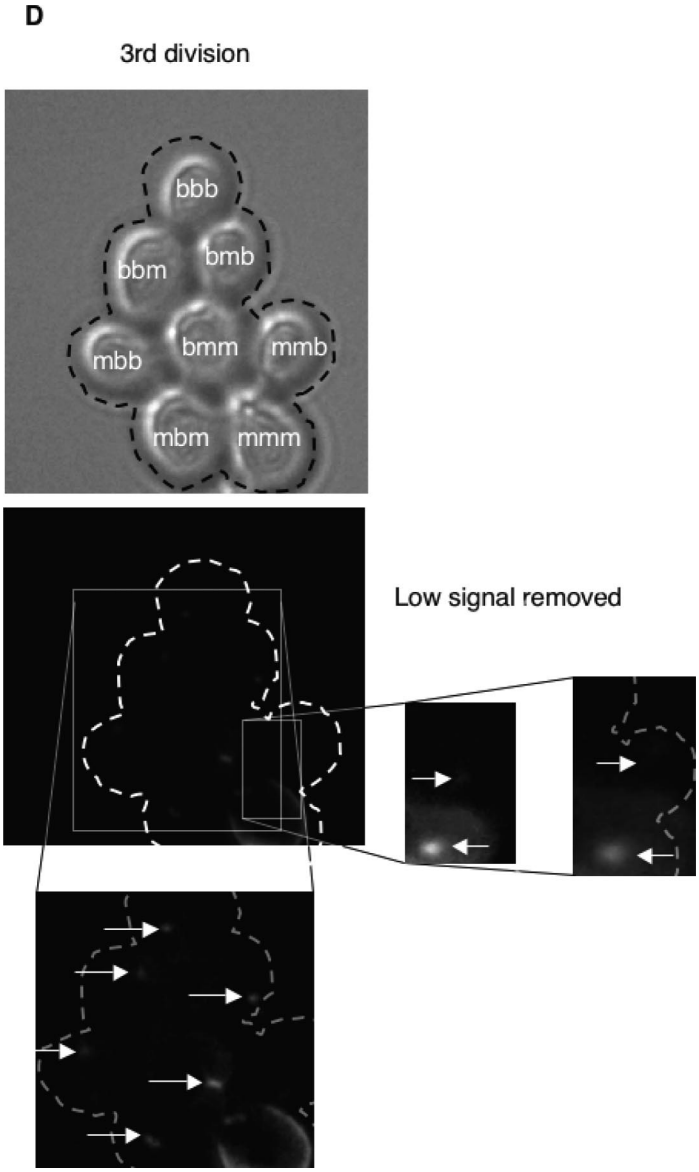
D
3rd division
bbb
bmb
bbm
bmm
mmb
mbb
mbm
mmm
Low signal removed

Fig. S2*D*.

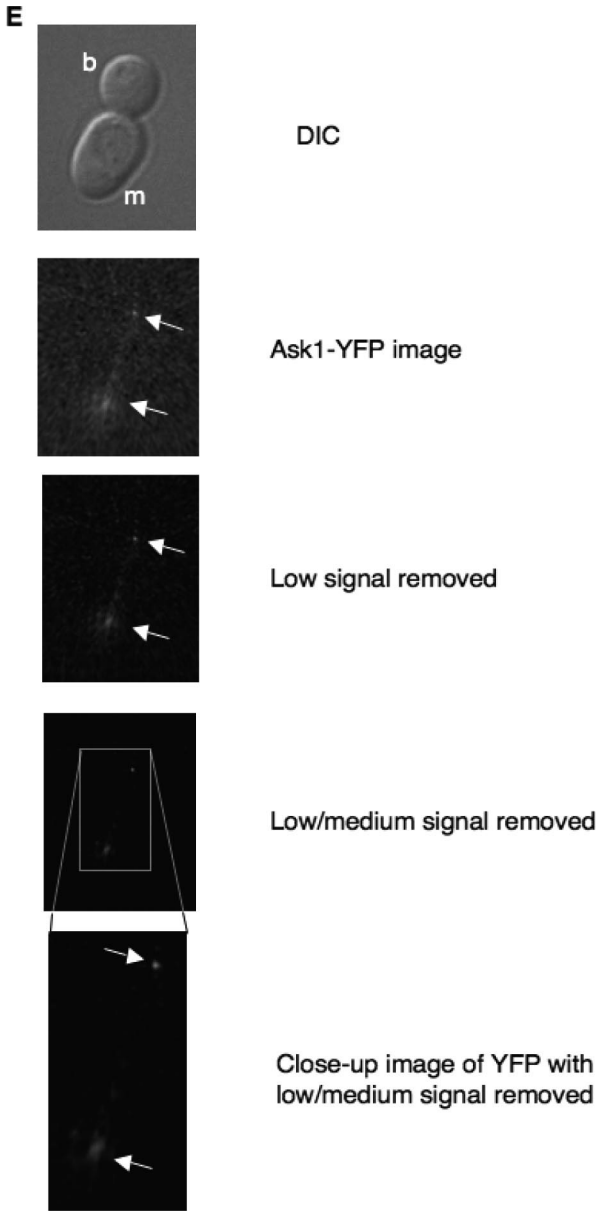
E
b
m
DIC
Ask1-YFP image
Low signal removed
Low/medium signal removed
Close-up image of YFP with
low/medium signal removed

Fig. S2*E*.

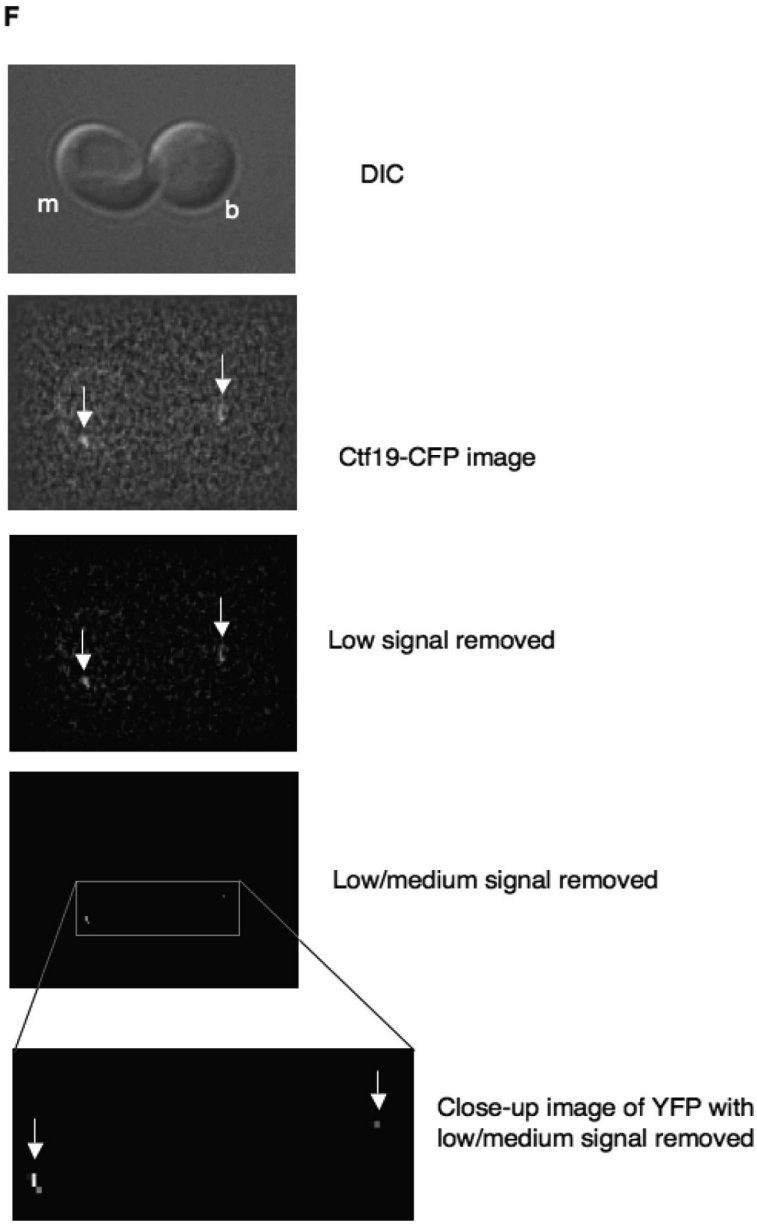
F
m
b
DIC
Ctf19-CFP image
Low signal removed
Low/medium signal removed
Close-up image of YFP with
low/medium signal removed

Fig. S2*F*.

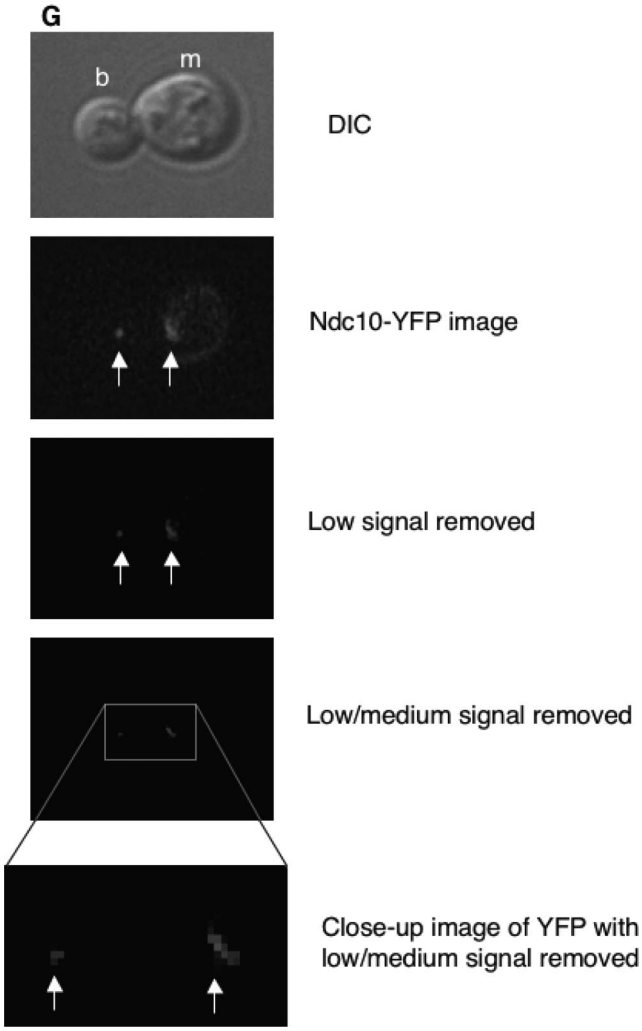
G
b
m
DIC
Ndc10-YFP image
Low signal removed
Low/medium signal removed
Close-up image of YFP with
low/medium signal removed

Fig. S2*G*.

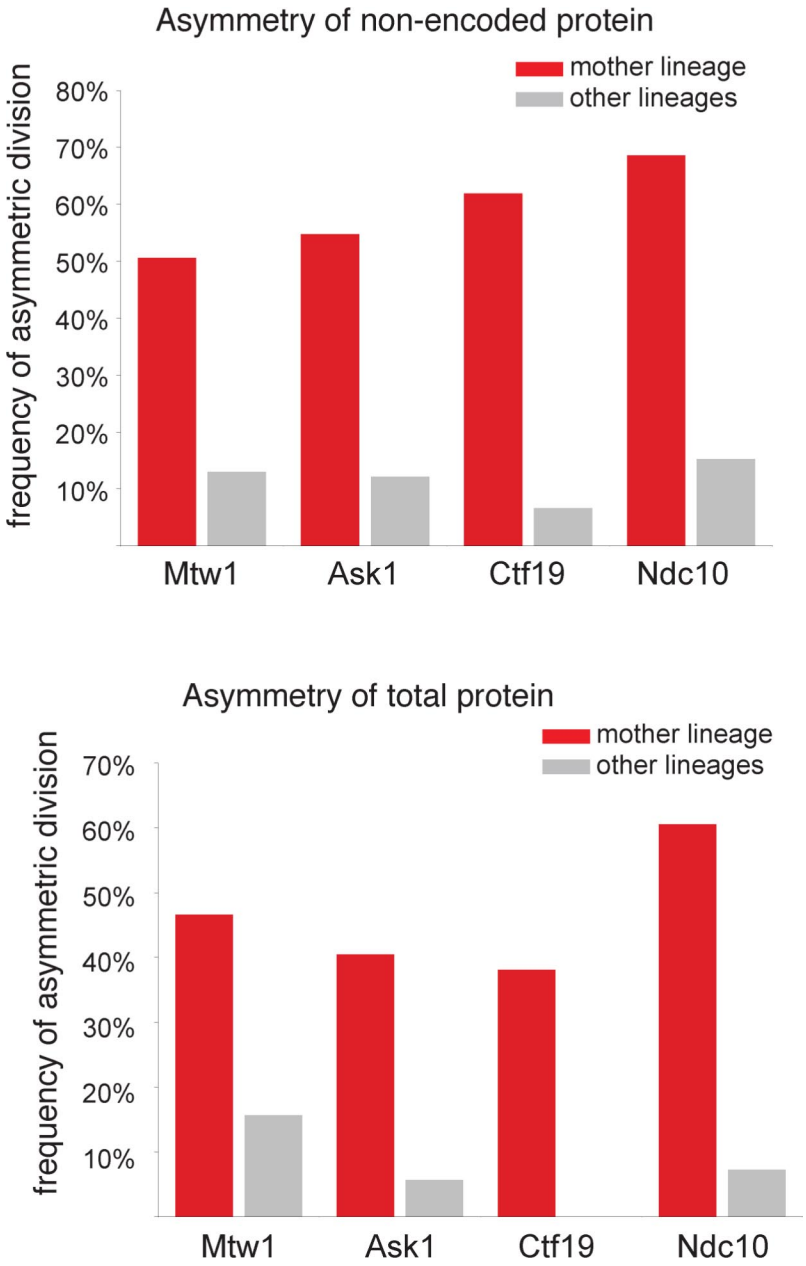

Fig. S3. The frequency of asymmetric divisions in both the mother lineage and the non-mother lineages for Mtw1, Ask1, Ctf19 and Ndc10. The bar graph indicates the % of divisions that are asymmetric, as defined by a mother to bud ratio of 1.6 or greater. The proportions of asymmetric divisions in the mother lineage are shaded red and the proportions in the other lineages are shaded gray. (*Upper* chart) Only the nonencoded protein is used to calculate the asymmetry frequencies. The frequencies of asymmetry in the mother lineage were compared with the non-mother lineages using Fischer's exact test (95% confidence). The resulting *P* values for Mtw1, Ask1, Ctf19 and Ndc10 are 1.2×10^{-5}, 1.1×10^{-4}, 8.3×10^{-4} and 1×10^{-6} respectively indicating that the frequency of asymmetry is significantly higher in the mother lineage than the non-mother lineages for each of these proteins. (*Lower* chart) The total kinetochore protein (encoded plus nonencoded) was used to calculate the asymmetry frequencies. The Fischer's exact test p values for Mtw1, Ask1, Ctf19 and Ndc10 are 1.6×10^{-4}, 3.2×10^{-4}, 5.8×10^{-6}, and 2.8×10^{-8}, respectively, again indicating that the frequency of asymmetry is significantly higher in the mother lineage compared to the other lineages.

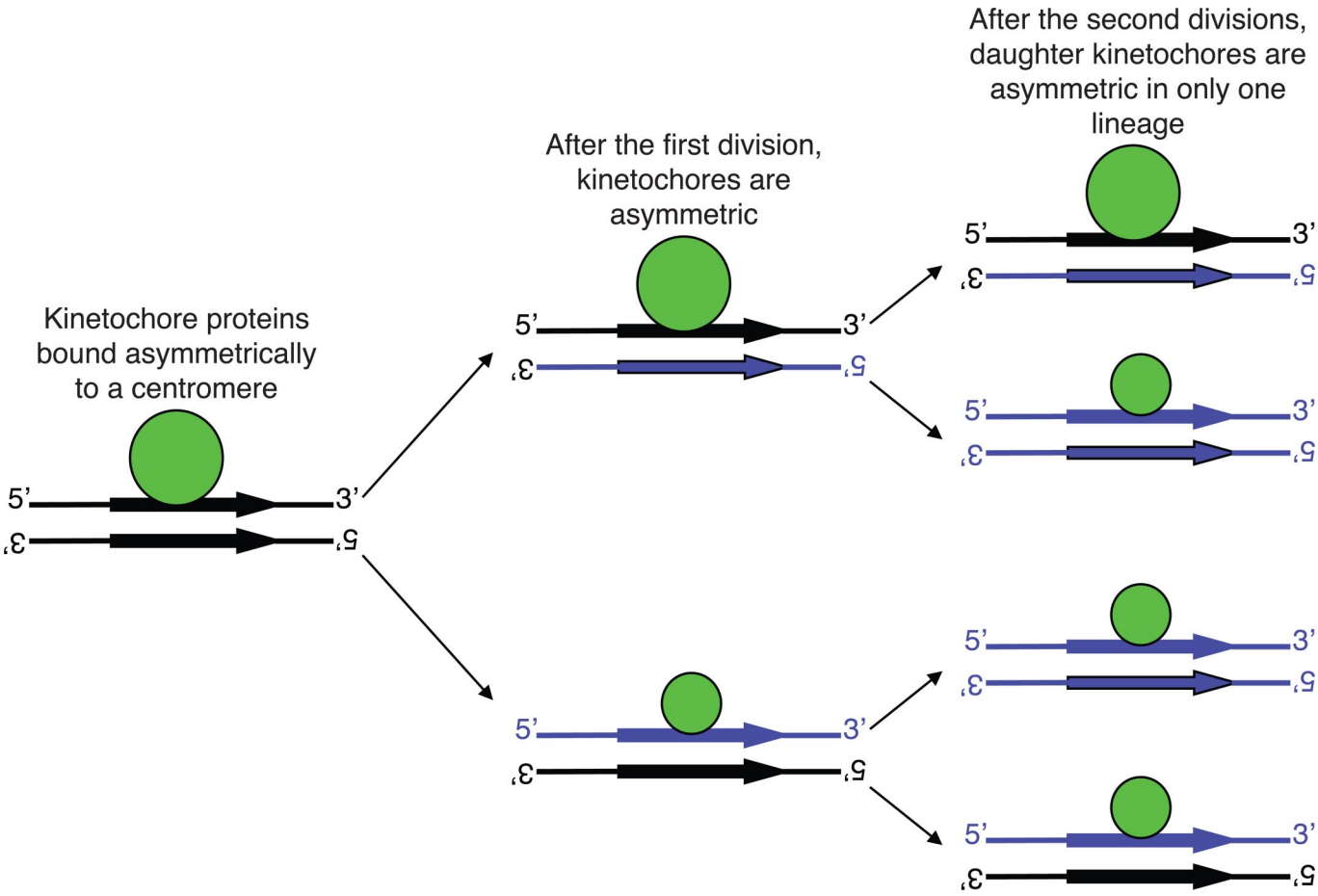

Fig. S4. Asymmetric binding of kinetochore components to a centromere sequence. The yeast centromere is unidirectional containing 3 conserved elements (CDEI, II and III); hence this sequence is illustrated as an arrow. If kinetochore proteins bind to the centromere unidirectionally, then asymmetry could be established in the original cell. After the first round of DNA replication (newly synthesized DNA is shown in blue), the 2 original DNA strands are not equivalent since the original kinetochore proteins are loaded asymmetrically. This pattern continues into the next division at which point only 1 of the 2 pairs of kinetochores is asymmetric. Such a model requires that either kinetochore proteins remain associated with their centromere sequences during replication or that there is mechanism to reestablish asymmetry after replication.

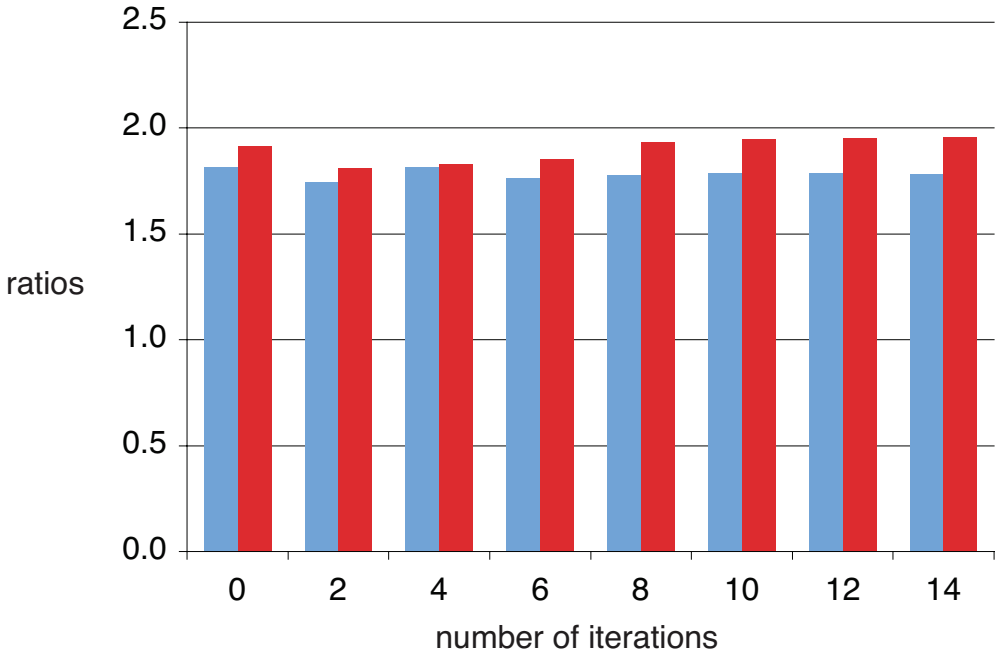

Fig. S5. Two examples of the effect of deconvolution on the ratio of intensity of 2 fluorescent foci. The chart shows the ratio of fluorescence between 2 foci, for 2 different samples (blue and red). The ratio of intensity is not dramatically altered by deconvolution. Zero iterations represents the image before deconvolution. All of our fluorescent images for quantitation were deconvolved for 5–9 iterations.

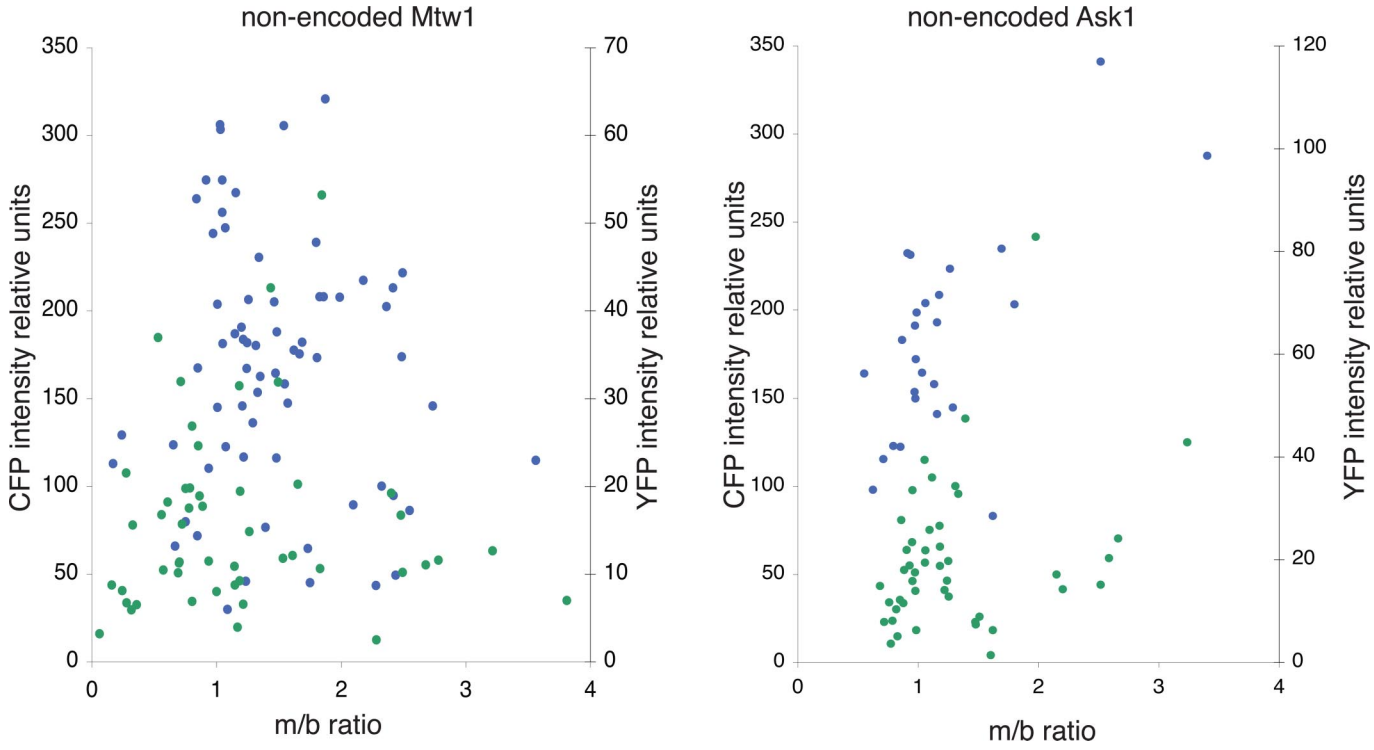

Fig. S6. Examples of the m/b ratios for different kinetochore divisions are plotted against fluorescence intensity. If the asymmetry that we observe is due to cellular background in the mother cell, then those kinetochores with the lowest intensity would show the highest m/b ratios and visa versa. No such correlation is found for 2 examples that we studied, Mtw1 and Ask1. The blue points indicate the ratios calculated using CFP and the green points indicate the ratios calculated using YFP. The CFP intensity is plotted on the left *y* axis and the YFP intensity is plotted on the right *y* axis. It is important to note that although only CFP shows autofluorescence of the spore wall, both YFP and CFP show a similar distibution indicating that background autofluoresence does not influence the m/b ratio.

Table S1. Summary statistics

Strain–lineage	Mean m/b ratio	Number of divisions, *n*	SD	SEM	*t* test, 2-tailed, unequal variance
Nonencoded protein					
Mtw1 spores–mother lineage	2.23	85	1.98	0.22	2.2×10^{-6}
Mtw1 spores–other lineages	1.10	46	0.45	0.07	
Ask1 spores–mother lineage	2.21	42	2.09	0.32	2.0×10^{-3}
Ask1 spores–other lineages	1.09	33	0.71	0.12	
Ndc10 spores–mother lineage	2.58	35	1.82	0.31	1.8×10^{-4}
Ndc10 spores–other lineages	1.25	46	0.70	0.10	
Ctf19 spores–mother lineage	2.94	21	2.77	0.60	5.3×10^{-3}
Ctf19 spores–other lineages	1.05	15	0.38	0.10	
Hta1 spores–mother lineage	0.90	12	0.18	0.05	0.73
Hta1 spores–other lineages	0.88	16	0.15	0.04	
Total protein (nonencoded plus encoded)					
Mtw1 haploid–all cells	1.20	94	0.69	0.07	
Mtw1 diploid–all cells	1.18	169	0.56	0.04	
Mtw1 spores–mother lineage	1.88	88	1.20	0.13	8.8×10^{-6}
Mtw1 spores–other lineages	1.22	51	0.41	0.06	
Ask1 haploid–all cells	1.00	101	0.36	0.04	
Ask1 diploid–all cells	1.05	73	0.60	0.07	
Ask1 spores–mother lineage	1.75	42	1.20	0.19	3.6×10^{-6}
Ask1 spores–other lineages	1.02	35	0.25	0.04	
Ndc10 haploid–all cells	1.08	131	0.42	0.04	
Ndc10 diploid–all cells	1.16	26	0.37	0.07	
Ndc10 spores–mother lineage	1.92	38	1.04	0.17	1.8×10^{-4}
Ndc10 spores–other lineages	1.19	55	0.46	0.06	
Ctf19 haploid–all cells	0.92	73	0.28	0.03	
Ctf19 diploid–all cells	1.03	13	0.24	0.07	
Ctf19 spores–mother lineage	2.48	21	1.64	0.36	9.7×10^{-4}
Ctf19 spores–other lineages	1.09	16	0.19	0.05	
Hta1 spores–mother lineage	0.87	12	0.14	0.04	0.98
Hta1 spores–other lineages	0.87	16	0.12	0.03	

Table S1 shows the mean m/b ratios for each of the alleles studied either in the mother lineage or other lineages (as illustrated in Figs. 1 and 2). The number of divisions studied (*n*), the standard deviation and standard error of the mean are included. A two tailed *t* test, with unequal variance, comparing the m/b ratios in the mother lineage with those of the other lineages are also shown. The upper part of the table is restricted to the nonencoded protein (i.e. the protein that was inherited from the diploid parent). The lower part of the table includes the data for total protein and consequently includes the data using both haploid and diploid cells. We use an alpha value of 0.05 as a threshold for statistical significance.

Table S2. Strains used in this study

Strain	Genotype
W7247	*MATa/MATα MTW1-YFP/MTW1-CFP [TetR-mRFP(iYGL119W) iYCL063W224xtetO::URA3]*
W7216–5C	*MATα MTW1-YFP TetR-mRFP(iYGL119W) iYCL063W224xtetO::URA3*
W7181–20D	*MATa MTW1-CFP TetR-mRFP(iYGL119W) iYCL063W224xtetO::URA3*
W7912	*MATa MATα HTA1-YFP HTA1-CFP*
W7908	*MATa/MATα ASK1-YFP/ASK1-CFP*
J1501	*MATa ASK1-YFP*
J1502	*MATα ASK1-CFP*
W7910	*MATa/MATα NDC10-YFP::HIS3/NDC10-CFP::KAN [BAR1]*
W7871–2A	*MATa NDC10-YFP::HIS3 BAR1*
W7872–1D	*MATα NDC10-CFP::KAN BAR1*
W7909	*MATa/MATα CTF19-YFP::HIS3/CTF19-CFP::KAN*
W7873–3D	*MATa CTF19-YFP-HIS3*
W7874–4D	*MATα CTF19-CFP-KAN*
W8368	*MATa/MATα [TetR-mRFP(iYGL119W) iYCL063W224xtetO::URA3]*

All strains used in this study are *ADE2*$^{+}$ *RAD5*$^{+}$ derivatives of W303, *can1–100 his3–11,15 leu2–3,112 ura3–1* (1,2) Genotypes enclosed in parenthesis square brackets indicate a homozygous diploid. In addition to the genotypes listed above all strains are *bar1::LEU2* unless otherwise indicated. Strains containing *MTW1–CFP/MTW1–YFP* and *ASK1–CFP/ASK1–YFP* were based upon 3′ gene-fusion alleles provided by Rebecca Burgess (Columbia University, New York), those containing *HTA1-CFP/HTA1-YFP* were based upon 3′ gene-fusion alleles provided by J. Barlow (Columbia University, New York). Strains containing the tet-operator sequences and tet-repressor tagged with red fluorescent protein are based upon those made by Michael Lisby (University of Copenhagen, Copenhagen) (3), which were derived from strains provided by Kim Nasmyth (University of Oxford, Oxford). Strains containing *CTF19-CFP/CTF19-YFP* and *NDC10-CFP/NDC10-YFP* are derived from 3′ gene-fusion alleles provided by Phil Heiter (University of British Columbia, Vancouver).

Other Supporting Information Files

Dataset S1