# Supporting Information

Thayer et al. 10.1073/pnas.1416079111

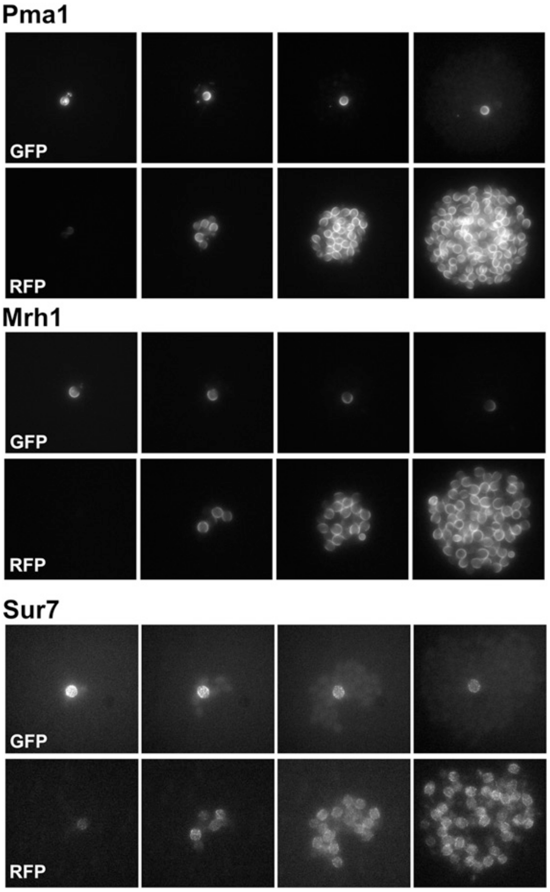


**Fig. S1.** PMA1, MRH1, and SUR7 recombination-induced tag exchange (RITE)-tag time-lapse images with separated channels. Images from Fig. 2*B* presented with GFP and RFP channels as separate grayscale images. PMA1-RITE (*Top*), MRH1-RITE (*Middle*), and SUR7-RITE (*Bottom*). (Magnification: Pma1, 200×; Mrh1 and Sur7, 250×.)

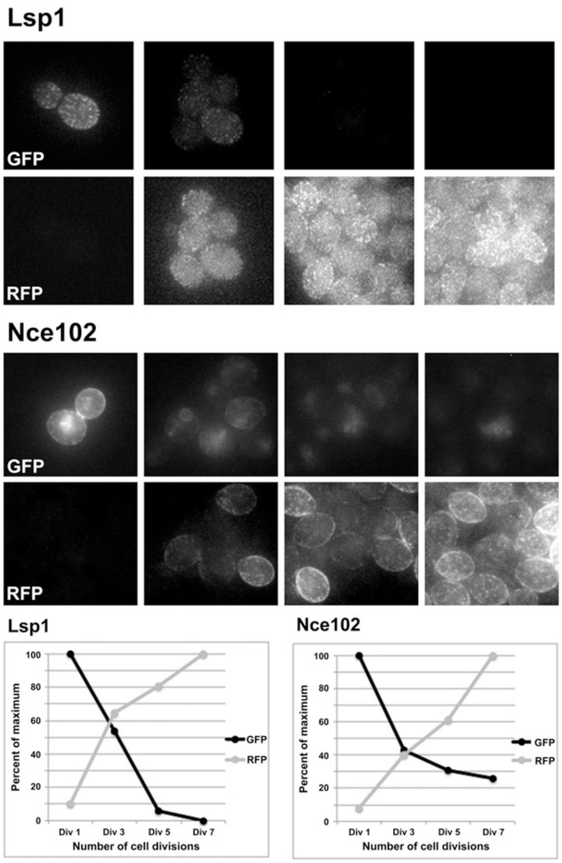


**Fig. S2.** LSP1 and Nce102 RITE-tag time-lapse images with separated channels. Images from Fig. 4 *B* and *C* presented with GFP and RFP channels as separate grayscale images. LSP1-RITE (*Top*) and Nce102 (*Middle*). (Magnification: 700×.) Quantification of fluorescence at the plasma membrane is graphically displayed (*Bottom*).

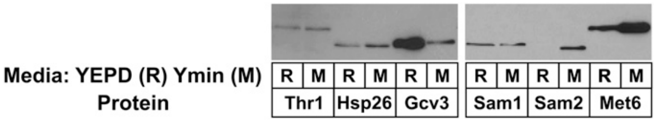


**Fig. S3.** Comparison of putative long-lived asymmetrically retained proteins (LARP) levels under stable isotope labeling by amino acid conditions. RITE-tagged strains were grown overnight in either YEPD (R) or Ymin+his+leu (M) to mid-log phase cell density. Equivalent amounts of total whole-cell protein were probed with anti–GFP antibody on Western blots. All strains were heterozygous *MET15/met15*Δ. Met6 and Sam2 were more highly expressed in minimal media, and Hsp26 only slightly more expressed. Sam1 and Thr1 appeared to have no difference in expression, and Gcv3 had a decrease in expression in minimal media.

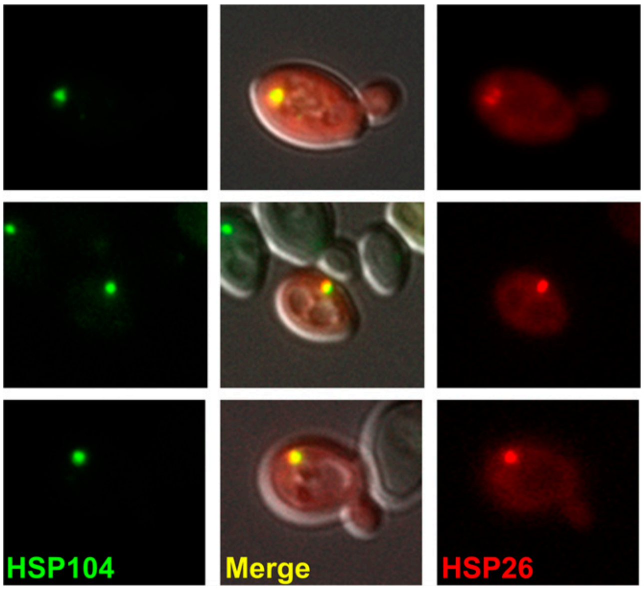


**Fig. S4.** Hsp26 foci are coincident with Hsp104 foci. Cells from a strain containing Hsp104-GFP and Hsp26-mCherry are shown. Hsp26-mCherry foci were visible in ∼10% of cells, whereas Hsp104-GFP foci were visible in >50% of cells. However, all Hsp26-mCherry foci overlapped with an Hsp104-GFP focus. (Magnification: 2,750×.)

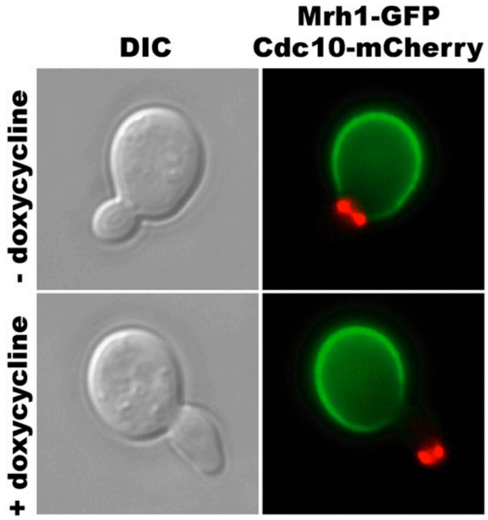


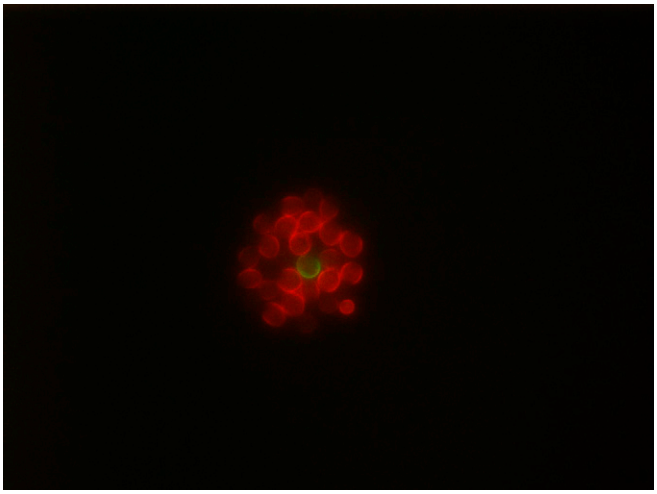
**Fig. S5.** Plasma membrane LARP asymmetry is not mediated by the septin ring. Mrh1-GFP asymmetry between mother cells and buds was evaluated upon septin ring disruption by repressing *TetO$_7$-CDC12* (1) transcription by treating cells with 20 μg/mL of doxycycline for 5 h. Cells also expressed Cdc10-mCherry to follow septin morphology. Bud elongation and Cdc10-mCherry mislocalization to the bud tip occurred upon *CDC12* repression and indicate septin ring disruption (2, 3). (Magnification: 2,500×.)

1. Mnaimneh S, et al. (2004) Exploration of essential gene functions via titratable promoter alleles. *Cell* 118(1):31–44.
2. Bouquin N, et al. (2000) Regulation of cytokinesis by the Elm1 protein kinase in *Saccharomyces cerevisiae*. *J Cell Sci* 113(Pt 8):1435–1445.
3. Hartwell LH (1971) Genetic control of the cell division cycle in yeast. IV. Genes controlling bud emergence and cytokinesis. *Exp Cell Res* 69(2):265–276.

**Table S1. Plasmids and strains used**

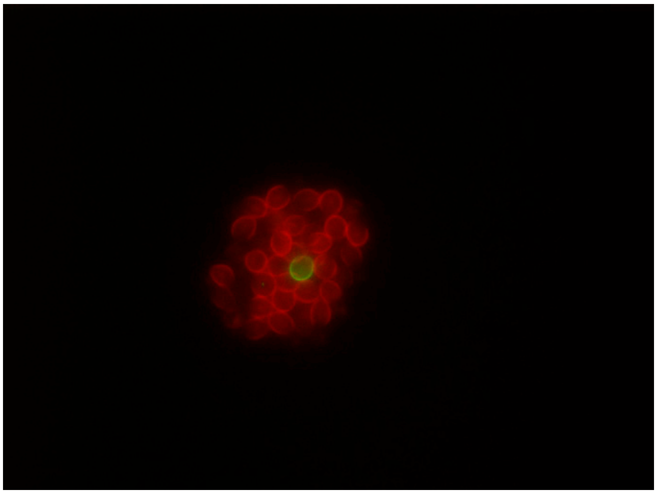
| Plasmid or strain | Genotype or descriptions | Source |
|---|---|---|
| Plasmids | | |
| pKV015 | V5-loxP-HA-GFP-HphMX-loxP-T7-mRFP used to fuse RITE tag to gene of interest | (1) |
| pSS146 | pINT-URA3-$P_{GPD/TDH3}$-cre-EBD78, MluI linearized fragment used to integrate cre-EBD78 into CYC1$_{term}$ locus | (1) |
| pKT127 | pFA6a-link-yEGFP-KanMX used to fuse GFP to gene of interest | (2) |
| pKT128 | pFA6a-link-yEGFP-SpHIS5 used to fuse GFP to gene of interest | (2) |
| pKTmCherry | mCherry-KanMX used to fuse mCherry to gene of interest | W. Shou laboratory, Fred Hutchinson Cancer Research Center, Seattle |
| Yeast strains | | |
| BY4741 | MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 | (3) |
| BY4742 | MATα his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0 | (3) |
| UCC4044 | MATa/α his3Δ1/his3Δ1 leu2Δ0/leu2Δ0 ura3Δ0/ura3Δ0 met15Δ0/+ lys2Δ0/+ LAP4-V5-loxP-HA-GFP-HphMX-loxP-T7-mRFP/LAP4 CYC1$_{term}$:URA3-$P_{GPD/TDH3}$-cre-EBD78:CYC1$_{term}$/+ | Present study |
| UCC4181 | MATa/α his3Δ1/his3Δ1 leu2Δ0/leu2Δ0 ura3Δ0/ura3Δ0 met15Δ0/+ lys2Δ0/+ MRH1-V5-loxP-HA-GFP-HphMX-loxP-T7-mRFP/MRH1 CYC1$_{term}$:URA3-$P_{GPD/TDH3}$-cre-EBD78:CYC1$_{term}$/+ | Present study |
| UCC4190 | MATa/α his3Δ1/his3Δ1 leu2Δ0/leu2Δ0 ura3Δ0/ura3Δ0 met15Δ0/+ lys2Δ0/+ w/ PMA1-V5-loxP-HA-GFP-HphMX-loxP-T7-mRFP/PMA1 CYC1$_{term}$:URA3-$P_{GPD/TDH3}$-cre-EBD78:CYC1$_{term}$/+ | Present study |
| UCC4243 | MATa/α his3Δ1/his3Δ1 leu2Δ0/leu2Δ0 ura3Δ0/ura3Δ0 met15Δ0/+ lys2Δ0/+ MET6-V5-loxP-HA-GFP-HphMX-loxP-T7-mRFP/MET6 CYC1$_{term}$:URA3-$P_{GPD/TDH3}$-cre-EBD78:CYC1$_{term}$/+ | Present study |
| UCC4277 | MATa/α his3Δ1/his3Δ1 leu2Δ0/leu2Δ0 ura3Δ0/ura3Δ0 met15Δ0/+ lys2Δ0/+ YNL134C-V5-loxP-HA-GFP-HphMX-loxP-T7-mRFP/YNL134C CYC1$_{term}$:URA3-$P_{GPD/TDH3}$-cre-EBD78:CYC1$_{term}$/+ | Present study |
| UCC4395 | MATa/α his3Δ1/hisΔ1 leu2Δ0/leu2Δ0 ura3Δ0/ura3Δ0 lys2Δ0/+ trp1Δ63/+ hoΔ:: $P_{SCW11}$-cre-EBD78-NatMX/hoΔ:: $P_{SCW11}$-cre-EBD78-NatMX loxP-CDC20-Intron-loxP-HphMX/loxP-CDC20-Intron-loxP-HphMX loxP-UBC9-loxP-LEU2/loxP-UBC9-loxP-LEU2 LSP1-mCherry-KanMX/LSP1-mCherry-KanMX SUR7-GFP-SpHIS5/SUR7-GFP-SpHIS5 | Present study |
| UCC4925 | MATa/α his3Δ1/his3Δ1 leu2Δ0/leu2Δ0 ura3Δ0/ura3Δ0 lys2Δ0/+ trp1Δ63/+ hoΔ::SCW11pr-cre-EBD78-NatMX/hoΔ:: $P_{SCW11}$-cre-EBD78-NatMX loxP-CDC20-Intron-loxP-HphMX/loxP-CDC20-Intron-loxP-HphMX loxP-UBC9-loxP-LEU2/loxP-UBC9-loxP-LEU2 | (4) |
| UCC5406 | MATa/α ade2Δ::hisG/ade2Δ::hisG his3Δ1/his3Δ1 leu2Δ0/leu2Δ0 met15Δ::ADE2/+ ura3Δ0/ura3Δ0 trp1Δ63/trpΔD63 hoΔ::$P_{SCW11}$-cre-EBD78-NatMX/hoΔ:: $P_{SCW11}$-cre-EBD78-NatMX loxP-UBC9-loxP-LEU2/loxP-UBC9-loxP-LEU2 loxP-CDC20-Intron-loxP-HphMX/loxP-CDC20-Intron-loxP-HphMX arg4Δ0::KanMX/arg4Δ0::KanMX lys1Δ0::KanMX/lys1Δ0::KanMX | Present study |
| UCC6884 | MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 CYC1$_{term}$:URA3-$P_{GPD/TDH3}$-cre-EBD78:CYC1$_{term}$ | Present study |
| UCC6886 | MATα his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0 CYC1$_{term}$:URA3-$P_{GPD/TDH3}$-cre-EBD78:CYC1$_{term}$ | Present study |
| UCC8773 | MATa his3Δ1 leu2Δ0 ura3Δ0 lys2Δ0 hoΔ:: $P_{SCW11}$-cre-EBD78-NatMX loxP-CDC20-Intron-loxP-HphMX loxP-UBC9-loxP-LEU2 | (4) |
| UCC8774 | MATα his3D1 leu2D0 ura3D0 trp1D63 hoD:: $P_{SCW11}$-cre-EBD78-NatMX loxP-CDC20-Intron-loxP-HphMX loxP-UBC9-loxP-LEU2 | (4) |
| UCC10141 | MATa/α his3Δ1/his3Δ1 leu2Δ0/leu2Δ0 ura3Δ0/ura3Δ0 lys2Δ0/+ trp1Δ63/+ hoΔ:: $P_{SCW11}$-cre-EBD78-NatMX/hoΔ:: $P_{SCW11}$-cre-EBD78-NatMX loxP-CDC20-Intron-loxP-HphMX/loxP-CDC20-Intron-loxP-HphMX loxP-UBC9-loxP-LEU2/loxP-UBC9-loxP-LEU2 MRH1-GFP-KanMX/MRH1-GFP-KanMX | Present study |
| UCC11298 | MATa/α his3Δ1/his3Δ1 leu2Δ0/leu2Δ0 ura3Δ0/ura3Δ0 lys2Δ0/+ trp1Δ63/+ hoΔ:: $P_{SCW11}$-cre-EBD78-NatMX/hoΔ:: $P_{SCW11}$-cre-EBD78-NatMX loxP-CDC20-Intron-loxP-HphMX/loxP-CDC20-Intron-loxP-HphMX loxP-UBC9-loxP-LEU2/loxP-UBC9-loxP-LEU2 SUR7-GFP-SpHIS5/SUR7 | Present study |
| UCC12510 | MATa/α his3Δ1/his3Δ1 leu2Δ0/leu2Δ0 ura3Δ0/ura3Δ0 met15Δ0/+ lys2Δ0/+ HSP26-V5-loxP-HA-GFP-HphMX-loxP-T7-mRFP/HSP26 CYC1$_{term}$:URA3-$P_{GPD/TDH3}$-cre-EBD78:CYC1$_{term}$/+ | Present study |
| UCC12520 | MATa/α his3Δ1/his3Δ1 leu2Δ0/leu2Δ0 ura3Δ0/ura3Δ0 met15Δ0/+ lys2Δ0/+ THR1-V5-loxP-HA-GFP-HphMX-loxP-T7-mRFP/THR1 CYC1$_{term}$:URA3-$P_{GPD/TDH3}$-cre-EBD78:CYC1$_{term}$/+ | Present study |
| UCC12526 | MATa/α his3Δ1/his3Δ1 leu2Δ0/leu2Δ0 ura3Δ0/ura3Δ0 met15Δ0/+ lys2Δ0/+ SAM2-V5-loxP-HA-GFP-HphMX-loxP-T7-mRFP/SAM2 CYC1$_{term}$:URA3-$P_{GPD/TDH3}$-cre-EBD78:CYC1$_{term}$/+ | Present study |
| UCC12543 | MATa/α his3Δ1/his3Δ1 leu2Δ0/leu2Δ0 ura3Δ0/ura3Δ0 met15Δ0/+ lys2Δ0/+ NCE102-V5-loxP-HA-GFP-HphMX-loxP-T7-mRFP/NCE102 CYC1$_{term}$:URA3-$P_{GPD/TDH3}$-cre-EBD78:CYC1$_{term}$/+ | Present study |
| UCC12561 | MATa/α his3Δ1/his3Δ1 leu2Δ0/leu2Δ0 ura3Δ0/ura3Δ0 met15Δ0/+ lys2Δ0/+ GCV3-V5-loxP-HA-GFP-HphMX-loxP-T7-mRFP/GCV3 CYC1$_{term}$:URA3-$P_{GPD/TDH3}$-cre-EBD78:CYC1$_{term}$/+ | Present study |
| UCC12592 | MATa/α his3Δ1/hisΔ1 leu2Δ0/leu2Δ0 ura3Δ0/ura3Δ0 lys2Δ0/+ trp1Δ63/+ hoΔ:: $P_{SCW11}$-cre-EBD78-NatMX/hoΔ:: $P_{SCW11}$-cre-EBD78-NatMX loxP-CDC20-Intron-loxP-HphMX/loxP-CDC20-Intron-loxP-HphMX loxP-UBC9-loxP-LEU2/loxP-UBC9-loxP-LEU2 HSP26-mCherry-KanMX/HSP26 HSP104-GFP-SpHIS5/HSP104 | Present study |

1. Verzijlbergen KF, et al. (2010) Recombination-induced tag exchange to track old and new proteins. *Proc Natl Acad Sci USA* 107(1):64–68.
2. Sheff MA, Thorn KS (2004) Optimized cassettes for fluorescent protein tagging in *Saccharomyces cerevisiae*. *Yeast* 21(8):661–670.
3. Brachmann CB, et al. (1998) Designer deletion strains derived from *Saccharomyces cerevisiae* S288C: A useful set of strains and plasmids for PCR-mediated gene disruption and other applications. *Yeast* 14(2):115–132.
4. Hughes AL, Gottschling DE (2012) An early age increase in vacuolar pH limits mitochondrial function and lifespan in yeast. *Nature* 492(7428):261–265.



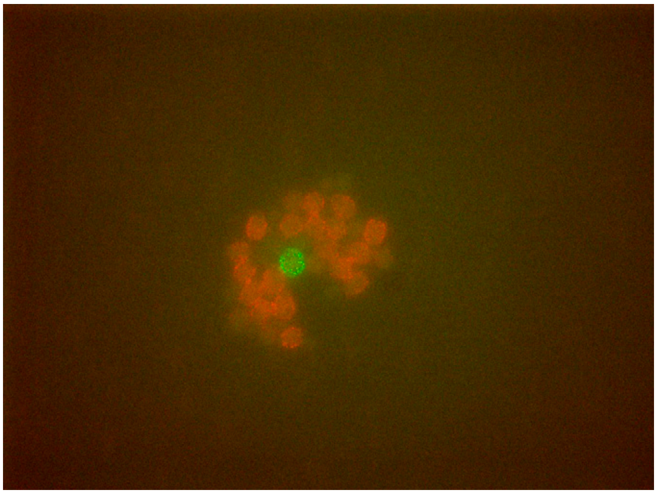
**Movie S1.** Mrh1-RITE time-lapse. Mrh1 is retained in mother cells at the plasma membrane. (Magnification: 500×.)

Movie S1



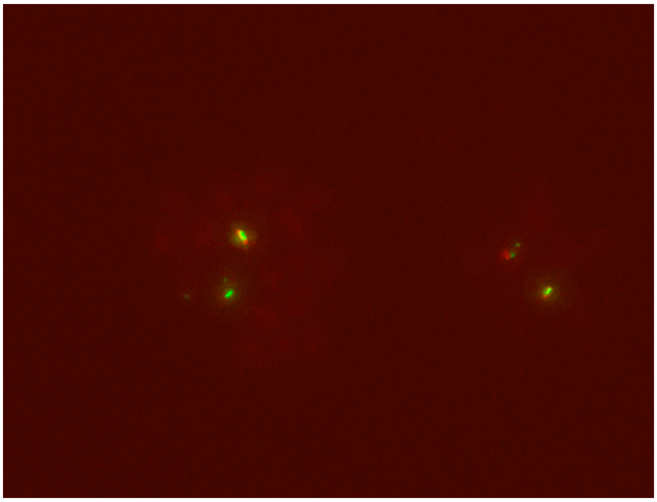
**Movie S2.** Pma1-RITE time-lapse. Pma1 is retained in mother cells at the plasma membrane. (Magnification: 500×.)

Movie S2



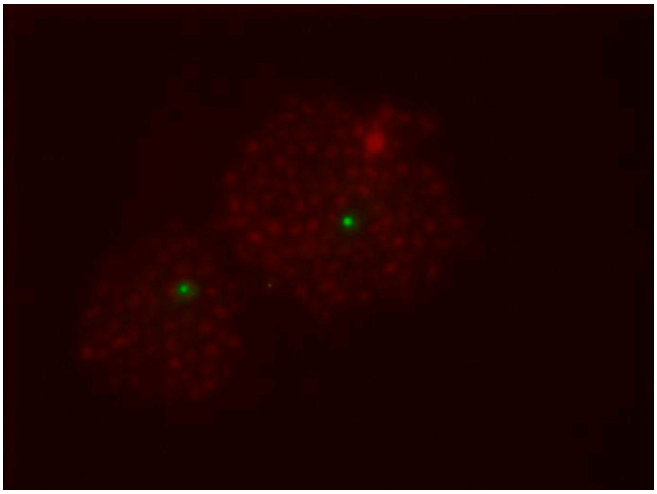
**Movie S3.** Sur7-RITE time-lapse. Sur7 is retained in mother cells at the plasma membrane. (Magnification: 500×.)

Movie S3



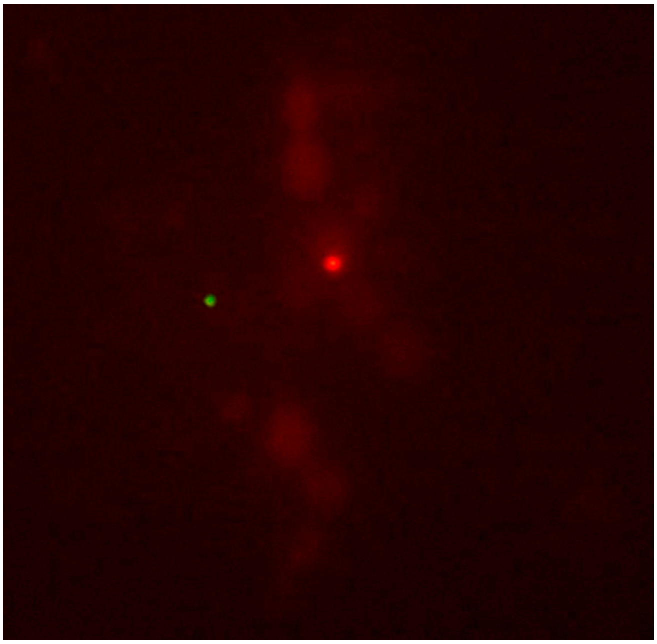
**Movie S4.** Thr1-RITE time-lapse. Thr1 foci have a propensity to remain in mother cells. (Magnification: 1,000×.)

Movie S4



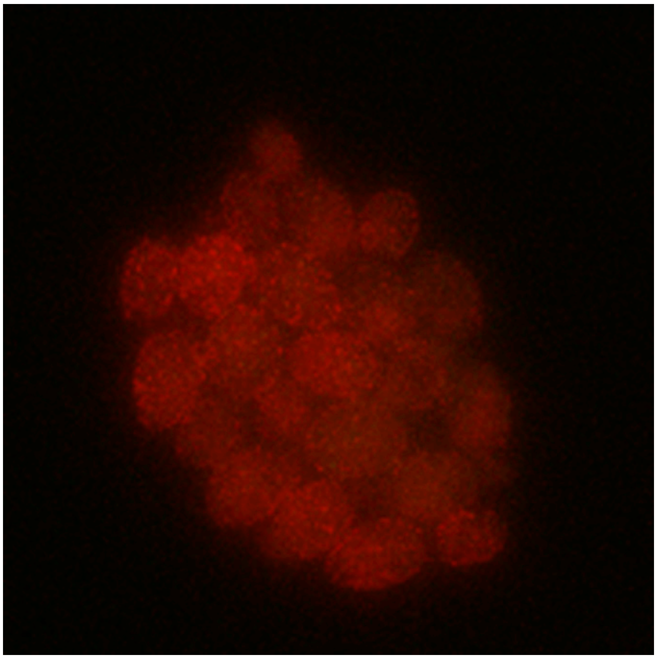
**Movie S5.** Hsp26-RITE time-lapse. Hsp26 foci have a propensity to remain in mother cells. (Magnification: 600×.)

Movie S5



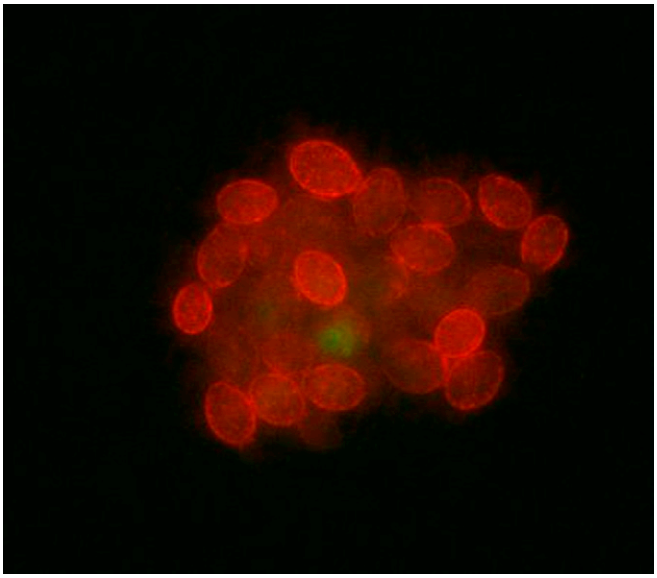
**Movie S6.** Hsp26-RITE time-lapse. Hsp26 foci are sometimes transferred to daughter cells. (Magnification: 1,000×.)

Movie S6



**Movie S7.** Lsp1-RITE time-lapse. Lsp1 is not retained in mother cells. (Magnification: 2,750×.)

Movie S7



**Movie S8.** Nce102-RITE time-lapse. Nce102 is not retained in mother cells. (Magnification: 2,500×.)

Movie S8

### Dataset S1. Peptides observed with calculated ratio columns

Dataset S1

(*i*) Systematic gene name. (*ii*) Common gene name. (*iii*) Approximate molecular weight of source fragment (gel slice). (*iv*) Peptide sequence. (*v*) Median $^{13}C/^{12}C$ ratio for all observed events of this peptide from the gel slice. (*vi*) Species of all peptides specifically identified, heavy or light. (*vii*) SD of the medians calculated from 1,000 bootstrapped samples of events for this peptide.

### Dataset S2. Plots of the $^{13}C/^{12}C$ ratio for peptides in given gel slices that correspond to all proteins that met threshold of having high quality data with ratio >0.1

Dataset S2

Each page represents a single predicted yeast ORF with its standard and systematic *S. cerevisiae* name in the title. The *x* axis is approximate molecular weight (MW) of peptide (estimated from gel slice); *y* axis is median ratio $^{13}C/^{12}C$ for all peptides mapped to that protein in a gel slice; size of dots reflect relative abundance of peptides in that slice; error bars are SD of the medians calculated from 1,000 bootstrapped samples. The expected MW of the full-length unmodified protein is plotted as a vertical dashed red line.

### Dataset S3. Plots of the $^{13}C/^{12}C$ ratio for peptides in given gel slices that correspond to all proteins observed

Dataset S3

Plots, as described for Dataset S2, for all observed proteins, irrespective of passing filtering criteria described in *Materials and Methods*. Some contaminating human proteins were left as light-labeled controls in the dataset; they are named by their International Protein Index accession number.