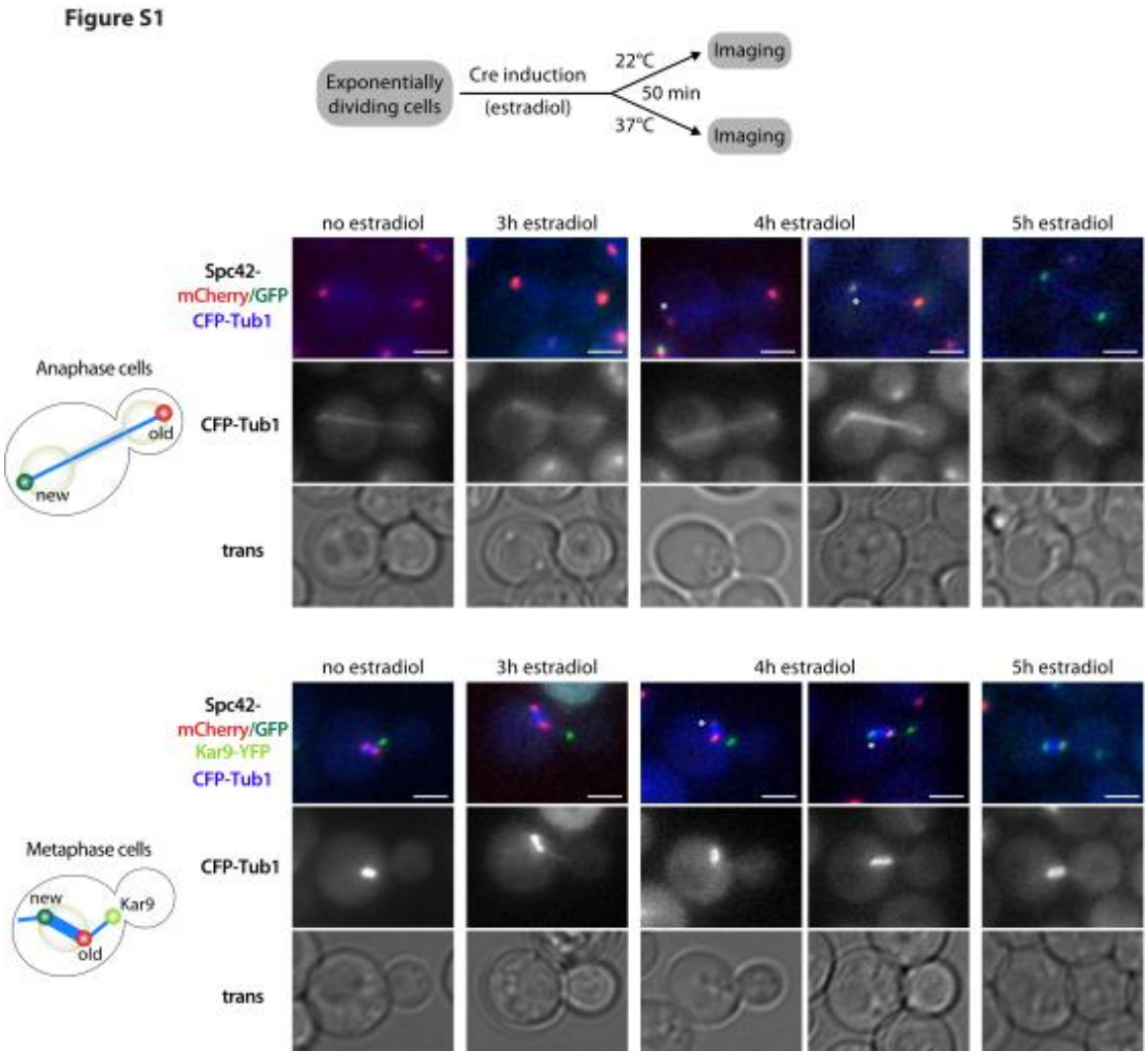

Figure S1, related to Figure 1, 6 and 7.

Technique used to determine SPB inheritance and the orientation of Kar9 asymmetry with respect to SPB age. Cells expressing Spc42 fused to a switchable mCherry/GFP-tag (see also Figure 1A) co-expressed Kar9-YFP and CFP-Tub1. 4h after Cre recombinase induction by estradiol, cells were further incubated at 22°C or 37°C for 50 min and then either anaphase or metaphase cells were imaged.

Figure S2

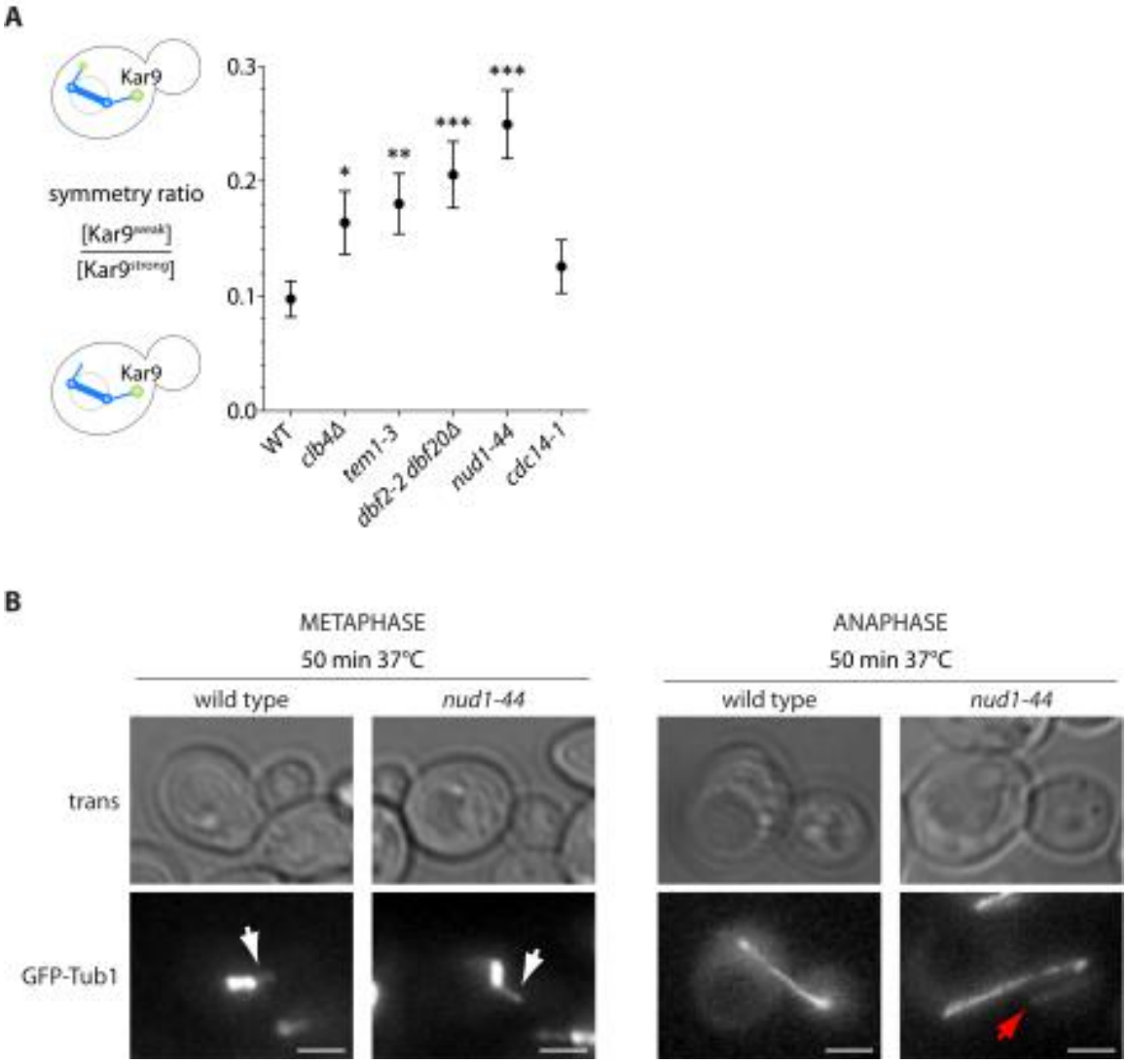

Figure S2, related to Figure 3.

(A) Kar9 fluorescence was measured in MEN and Nud1 mutants, as well as *clb4Δ* mutant cells. The intensity of the weaker Kar9 accumulation on one pole was divided by the stronger Kar9 accumulation on the other pole. Average and SEM of >86 individual cells are shown for each strain. Stars indicate p-values obtained from t-test comparing to WT.

(B) Metaphase and anaphase cells expressing GFP-Tub1 and either *NUD1* or *nud1-44*. Images were taken after 50 min at 37°C. White arrows indicate astral microtubules, red arrow indicates MT organization defect. Scale bar is 2µm.

Supplementary Materials and Methods

Construction of the switchable mCherry/GFP-cassette

First, the pFA6a-GFP-Trp1 vector was digested with *AscI-SpeI*, blunted and then religated. The first loxP site as well as an *XhoI* and *SacI* site were introduced by PCR. Then, the mCherry-Kan sequence was amplified from pYB967 to introduce *XhoI*-loxP sequence at the 5' end of the gene. The PCR product was cloned into the *XhoI-SacI* site from the previous reaction. Finally, the GFP sequence was reamplified from pFA6a-GFP-HisMX6 and cloned into the vector digested with *SacI-SacII*. The resulting plasmid contains the loxP-mCherry-KanMx4-loxP-GFP cassette.

Two-Dimensional SDS-PAGE

For sample preparation, cells were pelleted at 5000 g for 5 min, washed in 500 µl of PGSK buffer (50 mM $Na_2HPO_4{\cdot}2H_2O$, 4 mM $NaH_2PO_4{\cdot}H_2O$, 50 mM NaCl, 5 mM KCl, 60 mM glucose, 4 % CHAPs, also containing Protein inhibitor cocktail (PIC), 25 mM NaF, 20 mM BGP, 25 mM DTT, 10 mM o-vanadate, and PMSF). Cell pellets were resuspended in PGSK buffer and silica beads were added. Extracts were vortexed for 15 min, spun at 90000 g for 10 min and the supernatant was precipitated overnight with 9 Vs of ice-cold Acetone at -80°C. Pellets were air-dried for 10 min at room temperature, resuspended in 60-100 µl of rehydration buffer (7M Urea, 2M Thiourea, 4 % w/v CHAPS) and assayed for protein concentrations. For SDS-PAGE, IEF strips (7 cm pH 7-10 linear IEF strips, Bio-Rad) were rehydrated for 6-8 hours at room temperature in 125 µl of rehydration buffer, containing 1.25 µl of ampholytes (range pH7-10 Bio-Rad), 1.25 µl of DeStreak (GE Healthcare) and 0.002 % w/v Bromophenol Blue (BPB). 30 or 50 µg/strip of protein in 80 µl rehydration buffer was loaded near the cathode end of the strips by cup loading method. Samples were then focused at 50 mA/strip for a total of 20000 volt-hours. Strips were then either subjected to standard equilibration prior to second dimensional separation, or frozen in -80°C for storage. Second dimensional SDS-PAGE and immunoblotting was performed as previously described (Vogel et al., 2001). Anti-TAP (Open Biosystems) was used at 1:5000 in TBS-0.20% tween-20 (TBS/T-20). Anti-rabbit HRP-conjugated secondary antibody was used at 1:20000 in TBS/T-20 (GE Healthcare). Protein-antibody bindings were detected using ECL^+ and chemiluminescence films (GE Healthcare). For λ-Phosphatase treatment, samples prepared as before, but pellets were washed in 500 µl of PGSK buffer containing only PIC, 20 mM BGP and 25 mM DTT. To the final extract without PPase inhibitors and to one extract containing PPase

inhibitors, 50 units of λ-PPase (NEB) were added. All extracts were incubated at 37°C for an additional 30 min. 9 Vs of ice-cold Acetone were added to extracts and precipitated overnight at -80°C. Protein samples were then prepared for 2D-SDS PAGE analysis as described above.