Supplemental Material

Centrosome Duplication Defective Yeast Cells are More Prone to Membrane Damage

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Measurement of extracellular conductivity

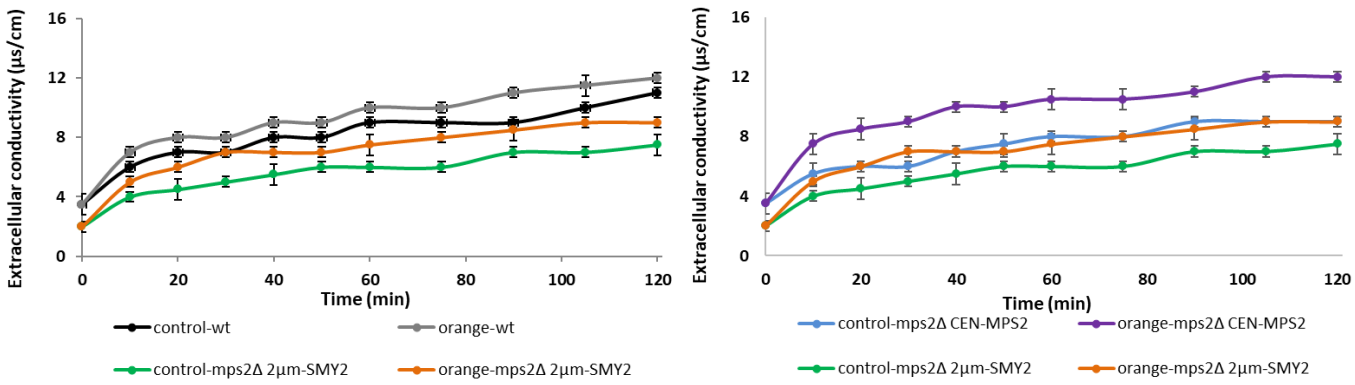

Figure S1. Measurement of extracellular conductivity of WT, mps2Δ CEN-MPS2, and mps2Δ 2µm-SMY2 cells after treatment with 0.1 µL/mL orange peel oil

Measurement of cellular material release

Saccharomyces cerevisiae strains (YPH499, *mps2*Δ CEN-*MPS2*, *mps2*Δ 2µm-*SMY2* and *mps2Δ pom34Δ*) were cultured overnight at 25˚C in YPD broth and was suspended in YPD to give a final density of 1 × 106 CFU/mL. The release of cellular material from cells treated with turpentine oil, oregano oil, thyme oil, benzyl alcohol and chlorpromazine (which absorbs light at 260 nm) were performed. Effects of hexanol with different concentrations were determined in another study (Gaskova et al., 2013). According to this study, we use 1% hexanol as positive control. Exponential cells were harvested, washed twice with double-distilled water, and resuspended in double-distilled water. The cell suspension was divided into the seven equal parts. First one is the oil or chemical free control that is called as negative control, second one is 1% hexanol as positive control, and the others are oils and chemicals. Cells were treated with 0.2 µL/mL of turpentine oil, 1 µL/mL of oregano oil, 1 µL/mL of thyme oil, 10 mM of benzyl alcohol and 1 mM of chlorpromazine for 60 minutes. After adding oils and chemicals into the cell suspension (at 0. minute), 3 mL aliquots were centrifuged, and 2 mL supernatants were used to measure absorbance at 260 nm. Cells were treated with essential oils and chemicals for 1 hour. The same procedure was applied to obtain absorbance at 260 nm at the end of the 60 minutes. All experiments were performed three times and the results are shown in Figure S2. Absorbance (260 nm) was measured at 0. minute in order to determine the background of the oils and chemicals.

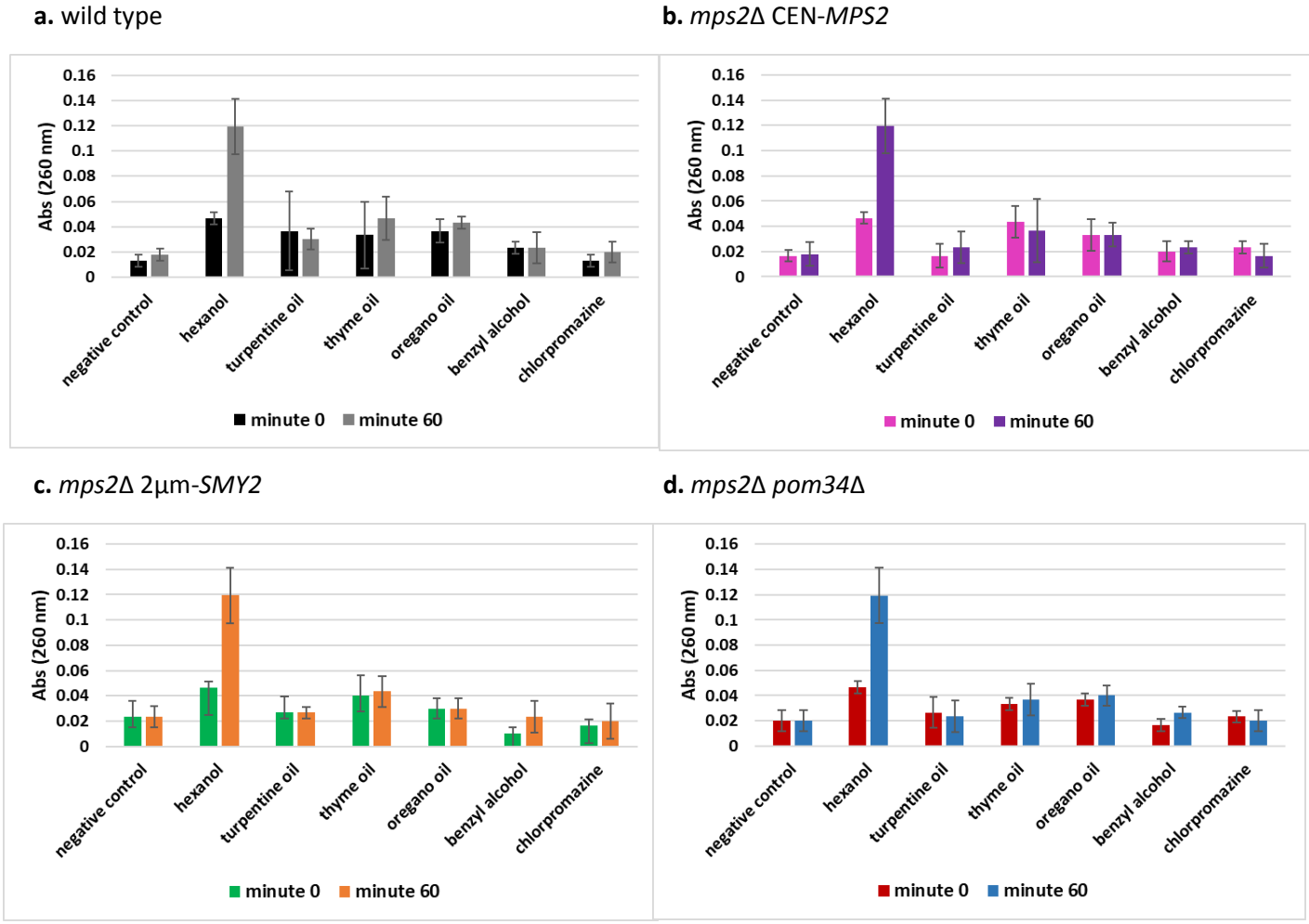

Figure S2. Exposure of a) wild type cells, b) *mps2*Δ CEN-*MPS2* cells, c) *mps2*Δ 2µm-*SMY2* cells and d) *mps2*Δ *pom34*Δ cells with 1% hexanol as a positive control, 0.2 µL/mL turpentine oil, 1 µL/mL thyme oil, 1 µL/mL oregano oil, 10 mM benzyl alcohol and 1 mM chlorpromazine in order to find out release of cellular material

Measurement of glucose leakage

Saccharomyces cerevisiae strains (YPH499, *mps2*Δ CEN-*MPS2*, *mps2*Δ 2µm-*SMY2* and *mps2Δ pom34Δ*) were cultured overnight at 25˚C in YPD broth and was suspended in YPD to give a final density of 1 × 106 CFU/mL. Measurement of the glucose leakage from the cells treated with turpentine oil, oregano oil, thyme oil, benzyl alcohol and chlorpromazine were performed at 540 nm. Exponential cells were harvested, washed twice with double-distilled water, and resuspended in double-distilled water. The cell suspension was divided into the six equal parts. First one is the oil or chemical free control that is called as negative control, and the others are oils and chemicals. Cells were treated with 0.2 µL/mL of turpentine oil, 1 µL/mL of oregano oil, 1 µL/mL of thyme oil, 10 mM of benzyl alcohol and 1 mM of chlorpromazine for 60 minutes. After adding oils and chemicals into the cell suspension, glucose leakage was measured at 0. Minute and 60. Minute. For this processes, 1 mL of 3,5-dinitrosalicylic acid (1% of DNS) reagent to 1 mL of samples and all mixtures were heated at 90º C for 5 minutes to develop the red-brown color (Miller, 1959). After cooling to room temperature in a cold-water bath, absorbance with a spectrophotometer at 540 nm was recorded for each sample. All experiments were performed three times and the results are shown in Figure S3. Absorbance (540 nm) was measured at 0. minute in order to determine the background of the oils and chemicals. Additionally DNS method was performed on 1 mg/ml glucose solution as a positive control of the method, which gave Abs (540 nm)= 1.06.

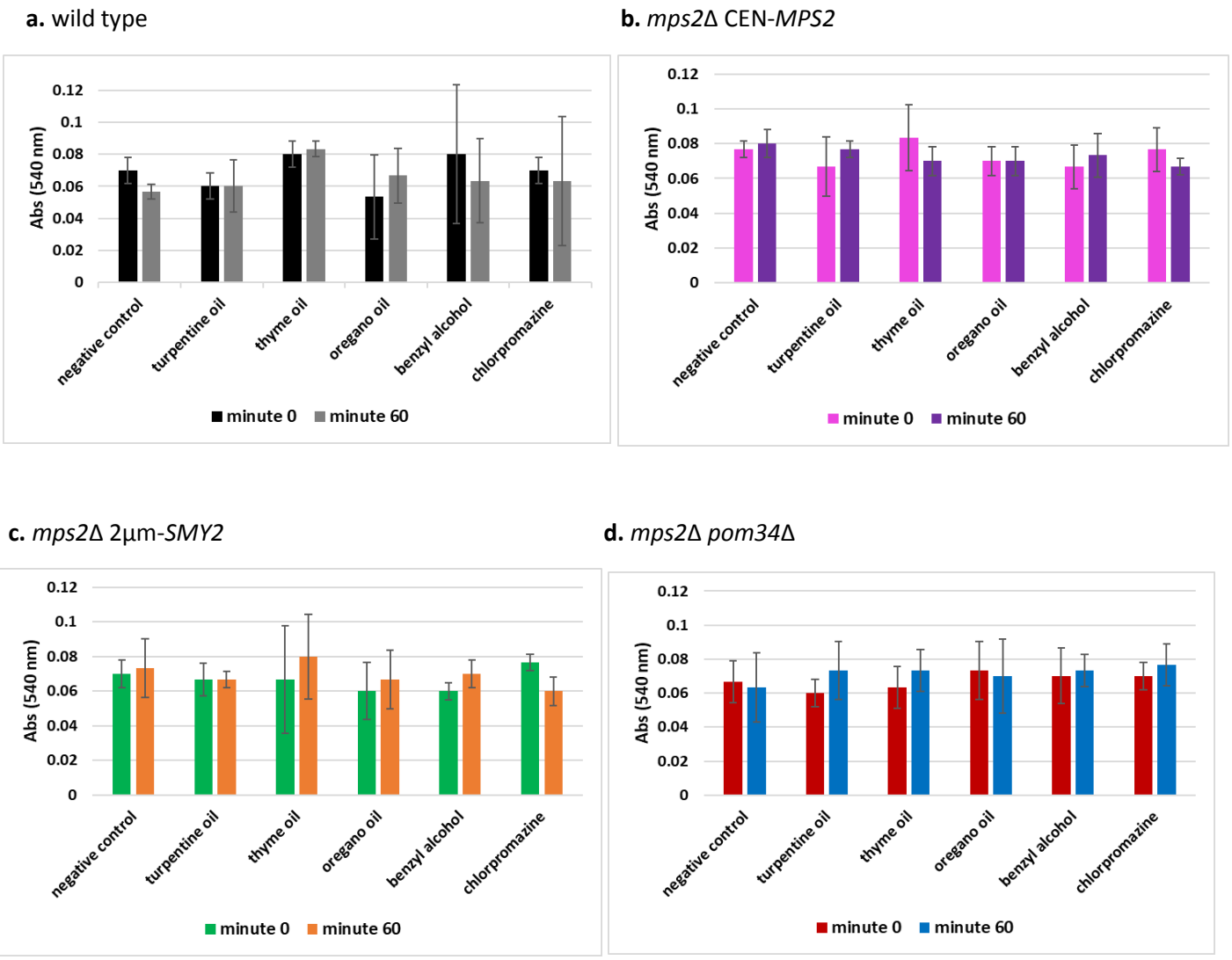

Figure S3. Exposure of a) wild type cells, b) *mps2*Δ CEN-*MPS2* cells, c) *mps2*Δ 2µm-*SMY2* cells and d) *mps2*Δ *pom34*Δ cells with 0.2 µL/mL turpentine oil, 1 µL/mL thyme oil, 1 µL/mL oregano oil, 10 mM benzyl alcohol and 1 mM chlorpromazine in order to find out glucose leakage from the cells

Measurement of extracellular pH

Extracellular pH was determined by the same method mentioned in the article. Figure S4 demonstrates the extracellular pH measurement of wild type cells treated with different concentration of benzyl alcohol (1 mM, 5 mM and 10 mM) and chlorpromazine (0.1 mM, 0.5 mM and 1 mM) from 0 to 60 minutes. Benzyl alcohol causes membrane damage into the yeast cells based on the concentration and we select the 10 mM of benzyl alcohol to perform the other experiments. On the other hand, chlorpromazine has no effect on the membrane of the yeast cells.

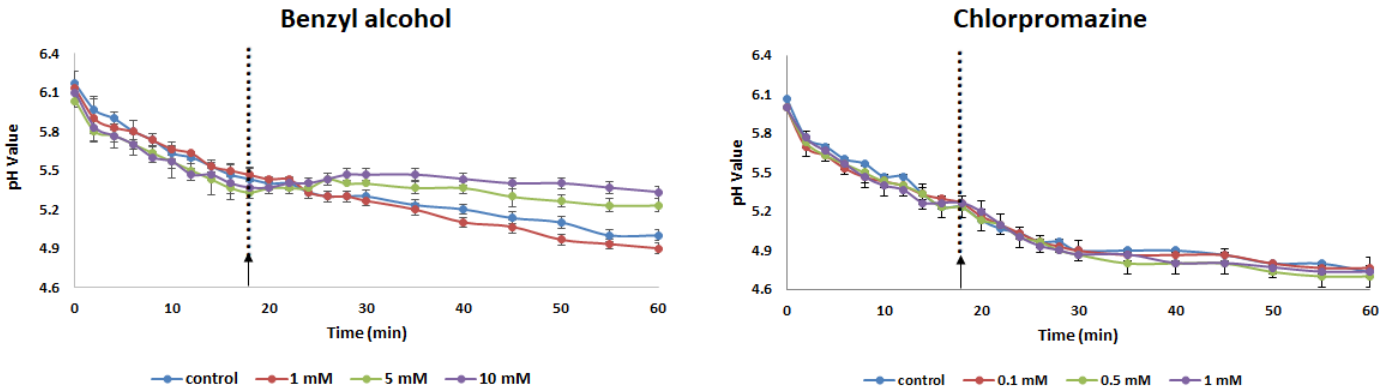

Figure S4. Effects of benzyl alcohol and chlorpromazine on the extracellular pH of *S. cerevisiae* wild type cells. Concentration dependent effects of essential oils on yeast cells in glucose-induced medium are shown. The arrows indicate the time of addition of a) benzyl alcohol: 1, 5 and 10 mM, b) chlorpromazine: 0.1, 0.5 and 1 mM.

Statistical Analyses

All experiments were repeated at least three times to decrease the experimental errors. In extracellular pH measurement method, we statistically compared the increase in pH for wild type and *mps2*Δ 2µm-*SMY2* cells by using the last 6 measurement points (from 35. minute to 60. minutes). Calculations were illustrated in the Figure S5.

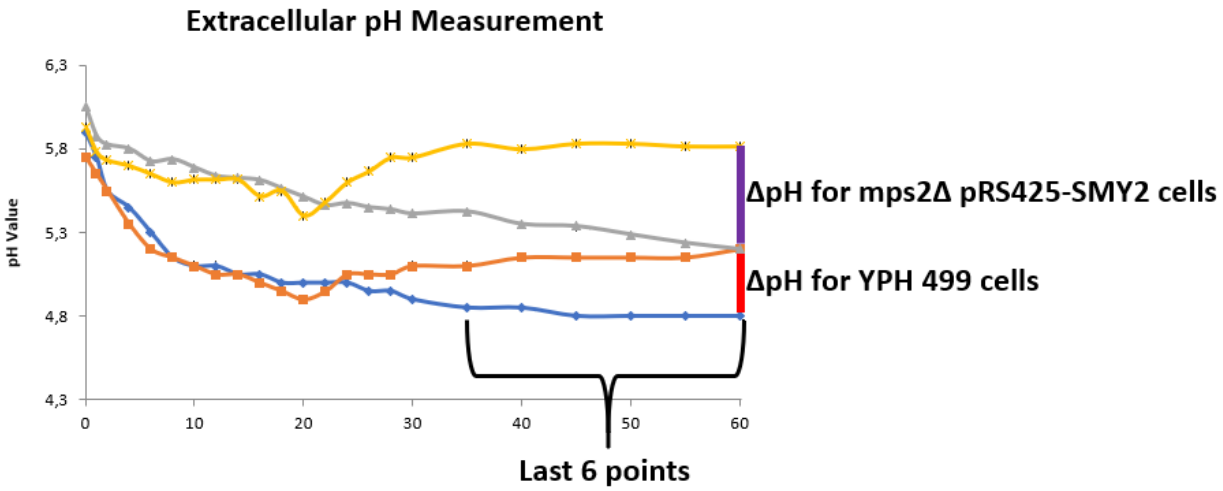

Figure S5. Illustration of the calculation of ΔpH values for YPH 499 and mps2Δ pRS425-SMY2 cells to compare our results for two data sets.

After calculation of ΔpH values for wild type and *mps2*Δ 2µm-*SMY2* cells, t-test was performed in order to define statistically significant differences between two cell types in Microsoft Excel Data Analysis Add-in. According to our analyses (Table S1), orange peel, oregano, turpentine and thyme oils cause statistically significant pH increase of *mps2*Δ 2µm-*SMY2* cells compared to wild type yeast cells. The same statistic analyses were performed for the conductivity measurements. Oregano, turpentine and thyme oils cause statistically significant increase in extracellular conductivity of *mps2*Δ 2µm-*SMY2* cells compared to wild type cells (Table S2).

Table S1 ΔpH values for YPH499 and mps2Δ pRS425-SMY2 cells

essential oil	YPH499	mps2Δ 2µm-SMY2
orange peel	0.22 ± 0.04	0.31 ± 0.03*
turpentine	0.14 ± 0.03	0.68 ± 0.04*
oregano	0.33 ± 0.04	0.51 ± 0.07*
thyme	0.23 ± 0.04	0.40 ± 0.04*

*Extracellular pH change in the *mps2*Δ 2µm-*SMY2* cells is statistically significantly different than YPH499 cells, $p<0.05$.

Table S2. Δconductivity values for YPH499 and mps2Δ pRS425-SMY2 cells

essential oil	YPH499	mps2Δ pRS425-SMY2
orange peel	1.25 ± 0.38	1.60 ± 0.34
turpentine	1.44 ± 0.15	5.44 ± 0.85*
oregano	2.33 ± 0.47	6.41 ± 0.44*
thyme	2.33 ± 0.68	7.91 ± 0.93*

*Extracellular conductivity change in the *mps2*Δ 2µm-*SMY2* cells is statistically significantly different than YPH499 cells, $p<0.05$.

Effect of Essential Oils on SPB Insertion

We previously showed that SPBs are inserted into the nuclear envelope in *mps2*Δ 2µm-*SMY2* cells at 23°C. However, the duplication plaque is not inserted into the nuclear envelope in *mps2*Δ 2µm-*SMY2* cells at 37°C (Sezen et al., 2009). In order to determine the effect of essential oils on SPB insertion, we repeated the analyses at 30°C. At this temperature uninserted SPB is a rare event (<3 %). Treating cells with essential oils, however, increases the number of uninserted SPBs (Figure S6).

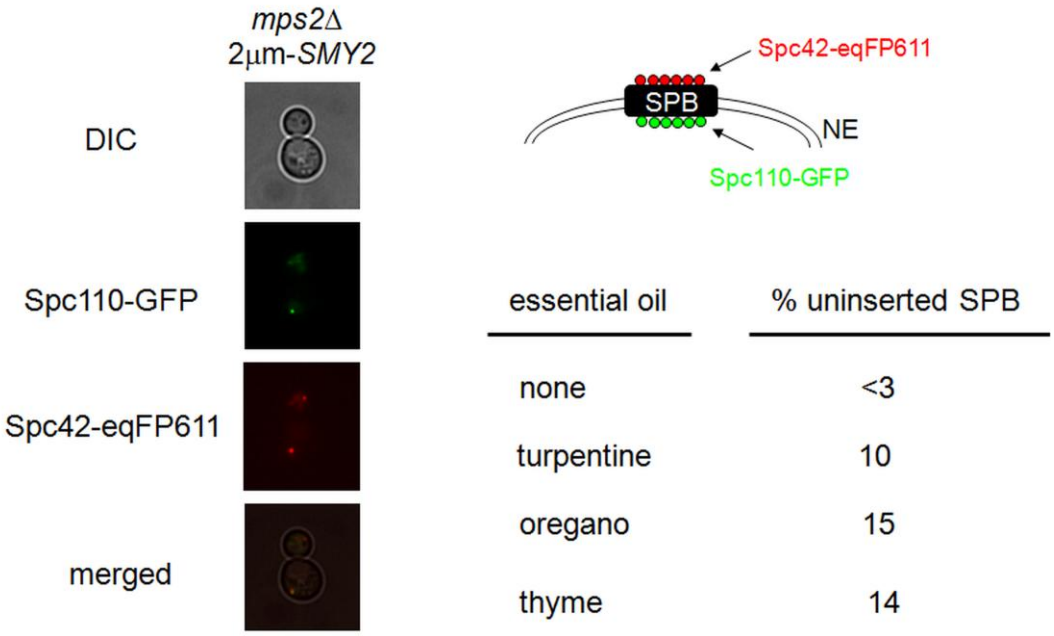

Figure S6. Analysis of *mps2*Δ 2µm-*SMY2* cells with SPC110-GFP SPC42-eqFP611 by fluorescence and phase contrast (DIC) microscopy at 30°C (2 h). Note that in uninserted SPB bearing cells, Spc110-GFP is only associated with one of the two Spc42-eqFP611-marked SPBs. This is the typical phenotype of cells with a defect in duplication plaque insertion (Schramm et al. 2000; Jaspersen and Winey 2004).

References

Gaskova, D., Plasek, J., Zahumensky, J., Benesova, I., Buriankova, L., Sigler, K., Alcohols are inhibitors of Saccharomyces cerevisiae multidrug-resistance pumps Pdr5p and Snq2p, FEMS Yeast Res 13: 782–795, 2013.

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Schramm C, Elliott S, Shevchenko A, Shevchenko A, Schiebel E. 2000. The Bbp1p–Mps2p complex connects the SPB to the nuclear envelope and is essential for SPB duplication. EMBO J 19: 421–433.

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