A Dynamical Model of the Spindle Position Checkpoint

Supplementary Information

Ayse Koca Caydasi[1], Maiko Lohel[1], Gerd Grünert[2],
Peter Dittrich[2], Gislene Pereira[1], and Bashar Ibrahim[1,2]

[1] Molecular Biology of Centrosomes and Cilia, German Cancer Research Center,
DKFZ-ZMBH Alliance, Heidelberg, Germany

[2] Bio Systems Analysis Group, Jena center of Bioinformatics and
Friedrich Schiller University, Jena, Germany

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1 A reduced model of the intrinsic GTPase-cycle of Tem1

We modelled the intrinsic GTPase-cycle of Tem1 with only two states and two irreversible reactions interconverting between them. We obtain this minimal model through reduction of the more comprehensive reaction scheme below:

$$\mathrm{GTP} + \mathrm{Tem1} \underset{k_{\mathrm{off}}^{\mathrm{T}}}{\overset{\alpha}{\rightleftharpoons}} \mathrm{Tem1}^{\mathrm{GTP}} \xrightarrow{k_{\mathrm{cat}}} \mathrm{Tem1}^{\mathrm{GDP}} \underset{\alpha}{\overset{k_{\mathrm{off}}^{\mathrm{D}}}{\rightleftharpoons}} \mathrm{Tem1} + \mathrm{GDP}\,. \tag{1.1}$$

Depending on the context, Tem1, $\mathrm{Tem1}^{\mathrm{GTP}}$, and $\mathrm{Tem1}^{\mathrm{GDP}}$ represent either the molecular species or the concentration thereof.

Hydrolysis of GTP by Tem1 takes place with a rate $k_{\mathrm{cat}} = 0.002\ \mathrm{s}^{-1}$ (at 30° C) (Geymonat et al., 2002), which comprises cleavage and subsequent release of the γ-phosphate of GTP. Binding of free phosphate to $\mathrm{Ras}^{\mathrm{GDP}}$ in complex with a GTPase-activating protein (GAP) has been reported to occur with a low rate of about 108 $\mathrm{M}^{-1}\mathrm{s}^{-1}$(Phillips et al., 2003). The same complex releases the cleaved phosphate with a high rate of 7.8 s^{-1}, resulting in a very low affinity with a dissociation constant of 72 mM. We suppose that $\mathrm{Tem1}^{\mathrm{GDP}}$ has a similar affinity for free phosphate, thus we regard the GTP-hydrolysis step as practically irreversible.

Dissociation of GTP from Tem1 takes place with a rate coefficient of about $k_{\mathrm{off}}^{\mathrm{T}} = 0.0012\ \mathrm{s}^{-1}$(at 30° C) (Geymonat et al., 2002), the same coefficient as measured for its *S. pombe* homologue Spg1 (Furge et al., 1998). The rate coefficient for GDP release from Tem1 was found to be about 0.0033 s^{-1}at 14° C (Geymonat et al., 2002), 3.5 times slower than the GDP release rate coefficient for Spg1 measured at 30° C (Furge et al., 1998). GDP release from Tem1 was too fast to be measured at 30° C, therefore we assume that at 30° C the release of GDP from Tem1 is faster than release of GDP from Spg1 and set $k_{\mathrm{off}}^{\mathrm{D}} = 0.017\ \mathrm{s}^{-1}$, five times the rate coefficient measured at 14° C.

The intracellular concentrations of GTP and GDP in budding yeast in cultures during mid-exponential growth are about 200 μM and 50 μM, respectively (Rudoni et al., 2001). The concentrations of GTP and GDP vastly exceed the intracellular concentration of Tem1. Because we find Tem1 to be about 2.5 times as concentrated as Bfa1, which is present in about 1380 molecules per cell in average (Ghaemmaghami et al., 2003). With an cellular volume of about 100 fL, we estimate the Tem1 concentration to be around 0.06 μM. The total levels of GTP and GDP are presumably not substantially altered by the above reactions and we treat GTP and GDP as constant external metabolites. The ratio of GTP and GDP is sensitive to nutritional conditions (Rudoni et al., 2001), yet we will show that the above scheme is insensitive to a wide range of GTP to GDP ratios.

The affinity of Ras for GDP is $K_{\mathrm{D}} \approx 10^{-8}$ M, and its affinity for GTP is slightly higher (Tucker et al., 1986). We assume that the affinity of Tem1 for GDP is similar and that the increased affinity for GTP is due to the lower dissociation rate. We can then estimate the common association rate constant $\alpha = k_{\mathrm{off}}^{\mathrm{D}}/K_{\mathrm{D}} = 1.7 \times 10^{6}\ \mathrm{M}^{-1}\mathrm{s}^{-1}$. This leads to a dissociation constant for GTP binding of 7×10^{-9} M, which is 1.4-fold of the affinity for GDP, well in agreement with what was reported for Ras (Tucker et al., 1986).

We obtain pseudo first order rate constants for GTP and GDP binding by multiplying this association rate by the respective nucleotide concentrations, i.e., we get $k_{\mathrm{on}}^{\mathrm{T}} = 340\ \mathrm{s}^{-1}$and $k_{\mathrm{on}}^{\mathrm{D}} = 85\ \mathrm{s}^{-1}$. Note that these association rates constants are several orders of magnitude higher than the respective dissociation rate constants.

We can now simplify the reaction scheme (1.1) to

$$\mathrm{Tem1} \underset{k_{\mathrm{off}}^{\mathrm{T}}}{\overset{k_{\mathrm{on}}^{\mathrm{T}}}{\rightleftharpoons}} \mathrm{Tem1}^{\mathrm{GTP}} \xrightarrow{k_{\mathrm{cat}}} \mathrm{Tem1}^{\mathrm{GDP}} \underset{k_{\mathrm{on}}^{\mathrm{D}}}{\overset{k_{\mathrm{off}}^{\mathrm{D}}}{\rightleftharpoons}} \mathrm{Tem1}\,, \tag{1.2}$$

which features only first order processes. The respective reaction rate constants are summarized in supplementary table 1.

Table 1: Parameters for the simplified reaction scheme (1.2)

k_{on}^{T}	$k_{\text{off}}^{\text{T}}$	k_{on}^{D}	$k_{\text{off}}^{\text{D}}$	k_{cat}
340 s^{-1}	0.0012 s^{-1}	85 s^{-1}	0.017 s^{-1}	0.002 s^{-1}

1.1 Steady state ratio of Tem1$^{\text{GTP}}$ and Tem1$^{\text{GDP}}$

We can translate equation (1.2) into a system of ordinary differential equations:

$$\begin{aligned}
\frac{\mathrm{d}}{\mathrm{d}t}\text{Tem1}^{\text{GTP}} &= k_{\text{on}}^{\text{T}}\,\text{Tem1} - \left(k_{\text{off}}^{\text{T}} + k_{\text{cat}}\right)\text{Tem1}^{\text{GTP}} \\
\frac{\mathrm{d}}{\mathrm{d}t}\text{Tem1}^{\text{GDP}} &= k_{\text{on}}^{\text{D}}\,\text{Tem1} - k_{\text{off}}^{\text{D}}\,\text{Tem1}^{\text{GDP}} + k_{\text{cat}}\,\text{Tem1}^{\text{GTP}} \\
\frac{\mathrm{d}}{\mathrm{d}t}\text{Tem1} &= k_{\text{off}}^{\text{T}}\,\text{Tem1}^{\text{GTP}} + k_{\text{off}}^{\text{D}}\,\text{Tem1}^{\text{GDP}} - \left(k_{\text{on}}^{\text{T}} + k_{\text{on}}^{\text{D}}\right)\text{Tem1}\,.
\end{aligned} \tag{1.3}$$

Using the conservation law $\text{Tem1} + \text{Tem1}^{\text{GTP}} + \text{Tem1}^{\text{GDP}} = \text{Tem1}_{\text{T}} = const$ we can eliminate the third equation and determine the steady state concentrations

$$\begin{aligned}
\text{Tem1}_{\text{ss}}^{\text{GTP}} &= \frac{\text{Tem1}_{\text{T}}}{a}\,k_{\text{off}}^{\text{D}}\,k_{\text{on}}^{\text{T}} \\
\text{Tem1}_{\text{ss}}^{\text{GDP}} &= \frac{\text{Tem1}_{\text{T}}}{a}\left(k_{\text{on}}^{\text{T}}\,k_{\text{cat}} + k_{\text{on}}^{\text{D}}\,k_{\text{off}}^{\text{T}} + k_{\text{on}}^{\text{D}}\,k_{\text{cat}}\right) \\
\text{Tem1}_{\text{ss}} &= \frac{\text{Tem1}_{\text{T}}}{a}\left(k_{\text{off}}^{\text{D}}\,k_{\text{off}}^{\text{T}} + k_{\text{off}}^{\text{D}}\,k_{\text{cat}}\right)
\end{aligned} \tag{1.4}$$

with

$$a = k_{\text{on}}^{\text{T}}\,k_{\text{cat}} + k_{\text{on}}^{\text{D}}\,k_{\text{off}}^{\text{T}} + k_{\text{on}}^{\text{D}}\,k_{\text{cat}} + k_{\text{off}}^{\text{D}}\,k_{\text{on}}^{\text{T}} + k_{\text{off}}^{\text{D}}\,k_{\text{off}}^{\text{T}} + k_{\text{off}}^{\text{D}}\,k_{\text{cat}}\,. \tag{1.5}$$

Obviously is the steady state concentration of Tem1 significantly smaller than the steady state concentrations of Tem1$^{\text{GTP}}$ and Tem1$^{\text{GDP}}$ if the association rate constants k_{on}^{T} and k_{on}^{D} are large compared to the dissociation rate constants $k_{\text{off}}^{\text{T}}$, $k_{\text{off}}^{\text{D}}$, and the hydrolysis rate constant k_{cat}.

With our choice of rate constants we obtain steady state concentrations $\text{Tem1}_{\text{ss}}^{\text{GTP}} \approx 5.15 \times 10^{-8}$ M, $\text{Tem1}_{\text{ss}}^{\text{GDP}} \approx 0.85 \times 10^{-8}$ M, and $\text{Tem1}_{\text{ss}} < 5 \times 10^{-13}$ M ≈ 0 M. Thus, in steady state, Tem1$^{\text{GTP}}$ accounts for 86% of the total Tem1 population (0.06 μM).

1.2 Sensitivity of the steady state to the kinetic parameters

We analyse the local sensitivities of the steady state concentrations from equation (1.4), $\text{Tem1}_{\text{ss}}^{\text{GDP}}$ and $\text{Tem1}_{\text{ss}}^{\text{GTP}}$, with respect to the kinetic parameters. Normalized local sensitivities are defined as

$$\mathcal{S}(X, p) := \frac{p}{X} \cdot \frac{\partial X}{\partial p}$$

where X is a model output of interest and p is a parameter of the model. For the steady state concentrations, the sensitivities with respect to Tem1$^{\text{GDP}}$ read

$$\begin{aligned}
\mathcal{S}\left(\text{Tem1}_{\text{ss}}^{\text{GDP}}, k_{\text{on}}^{\text{T}}\right) &= \frac{1}{a\,b}\,k_{\text{off}}^{\text{D}}\,k_{\text{on}}^{\text{T}}\left(k_{\text{off}}^{\text{T}} + k_{\text{cat}}\right)\left(k_{\text{cat}} - k_{\text{on}}^{\text{D}}\right) \\
\mathcal{S}\left(\text{Tem1}_{\text{ss}}^{\text{GDP}}, k_{\text{off}}^{\text{T}}\right) &= -\frac{1}{a\,b}\,k_{\text{off}}^{\text{D}}\,k_{\text{on}}^{\text{T}}\,k_{\text{off}}^{\text{T}}\left(k_{\text{cat}} - k_{\text{on}}^{\text{D}}\right) \\
\mathcal{S}\left(\text{Tem1}_{\text{ss}}^{\text{GDP}}, k_{\text{on}}^{\text{D}}\right) &= \frac{1}{a\,b}\,k_{\text{on}}^{\text{D}}\,k_{\text{off}}^{\text{D}}\left(k_{\text{off}}^{\text{T}} + k_{\text{cat}}\right)\left(k_{\text{on}}^{\text{T}} + k_{\text{off}}^{\text{T}} + k_{\text{cat}}\right) \\
\mathcal{S}\left(\text{Tem1}_{\text{ss}}^{\text{GDP}}, k_{\text{off}}^{\text{D}}\right) &= -\frac{1}{a\,b}\,k_{\text{off}}^{\text{D}}\left(k_{\text{on}}^{\text{T}} + k_{\text{off}}^{\text{T}} + k_{\text{cat}}\right) \\
\mathcal{S}\left(\text{Tem1}_{\text{ss}}^{\text{GDP}}, k_{\text{cat}}\right) &= \frac{1}{a\,b}\,k_{\text{off}}^{\text{D}}\,k_{\text{on}}^{\text{T}}\,k_{\text{cat}}\left(k_{\text{on}}^{\text{T}} + k_{\text{off}}^{\text{T}} + k_{\text{on}}^{\text{D}}\right)
\end{aligned}$$

and similarly we get for Tem1^{GTP}

$$\begin{aligned}
\mathcal{S}\big(\text{Tem1}_{\text{ss}}^{\text{GTP}}, k_{\text{on}}^{\text{T}}\big) &= \frac{1}{a}\ \big(k_{\text{on}}^{\text{D}} + k_{\text{off}}^{\text{D}}\big)\ \big(k_{\text{off}}^{\text{T}} + k_{\text{cat}}\big) \\
\mathcal{S}\big(\text{Tem1}_{\text{ss}}^{\text{GTP}}, k_{\text{off}}^{\text{T}}\big) &= -\frac{1}{a}\ \big(k_{\text{on}}^{\text{D}} + k_{\text{off}}^{\text{D}}\big)\ k_{\text{off}}^{\text{T}} \\
\mathcal{S}\big(\text{Tem1}_{\text{ss}}^{\text{GTP}}, k_{\text{on}}^{\text{D}}\big) &= -\frac{1}{a}\ \big(k_{\text{off}}^{\text{T}} + k_{\text{cat}}\big)\ k_{\text{on}}^{\text{D}} \\
\mathcal{S}\big(\text{Tem1}_{\text{ss}}^{\text{GTP}}, k_{\text{off}}^{\text{D}}\big) &= \frac{b}{a} \\
\mathcal{S}\big(\text{Tem1}_{\text{ss}}^{\text{GTP}}, k_{\text{cat}}\big) &= -\frac{1}{a}\ \big(k_{\text{on}}^{\text{D}} + k_{\text{on}}^{\text{T}} + k_{\text{off}}^{\text{D}}\big)\ k_{\text{cat}}
\end{aligned}$$

with a as in equation (1.5) and

$$b = k_{\text{on}}^{\text{T}}\, k_{\text{cat}} + k_{\text{on}}^{\text{D}}\, k_{\text{off}}^{\text{T}} + k_{\text{on}}^{\text{D}}\, k_{\text{cat}} \tag{1.6}$$

The numerical sensitivities are given in supplementary table 1.2. It is evident that the steady state is most sensitive to the hydrolysis rate k_{cat} and the GDP-release rate $k_{\text{off}}^{\text{D}}$, while the the steady state appears to be only weakly dependent on the GTP-release rate.

The absolute values of the sensitivities of $\text{Tem1}_{\text{ss}}^{\text{GDP}}$are larger then those of $\text{Tem1}_{\text{ss}}^{\text{GTP}}$. This is the case because of the conservation law $\text{Tem1}^{\text{GDP}} = \text{Tem1}_{\text{T}} - \text{Tem1} - \text{Tem1}^{\text{GTP}}$ together with the vanishingly small concentration of Tem1_{ss}, every change in $\text{Tem1}_{\text{ss}}^{\text{GTP}}$causes a change with the same absolute magnitude in $\text{Tem1}_{\text{ss}}^{\text{GDP}}$; however, the relative change in $\text{Tem1}_{\text{ss}}^{\text{GDP}}$is larger due to the approximately $6:1$ steady state ratio of $\text{Tem1}_{\text{ss}}^{\text{GTP}}$ and $\text{Tem1}_{\text{ss}}^{\text{GDP}}$.

Because no data on the affinities of Tem1 for GTP and GDP are available, k_{on}^{T} and k_{on}^{D} are our parameters with the largest uncertainity. Because they also have moderate influence on the steady state (supplementary table 1.2), we decided to analyse the relative Tem1^{GTP} amount in steady state as when k_{on}^{T} and k_{on}^{D} are changed within one order of magnitude up or down.

Supplementary Figure 7 shows that with our choice for k_{on}^{T} and k_{on}^{D} a steady state lies in a region where the relative steady state level of $\text{Tem1}_{\text{ss}}^{\text{GTP}}$is high and no significant increase is possible without changing the parameters significantly. We can also observe that the steady state is insensitive to a proportional change of both rate constants. This holds true also for the sensitivities of the steady state with respect to all parameters (Supplementary Figure 8). Moderate changes of the association rate constants do not alter the sensitivity profile significantly. Considering that the association rates k_{on}^{T} and k_{on}^{D} are products of the "true" second order association rate and the nucleotide concentrations, our estimated affinities result in a rather robust system with respect to moderate changes in the nucleotide concentrations or the affinities.

Table 2: Sensitivities of the steady state concentrations $\text{Tem1}_{\text{ss}}^{\text{GDP}}$ and $\text{Tem1}_{\text{ss}}^{\text{GTP}}$

	k_{on}^{T}	$k_{\text{off}}^{\text{T}}$	k_{on}^{D}	$k_{\text{off}}^{\text{D}}$	k_{cat}
$\text{Tem1}_{\text{ss}}^{\text{GDP}}$	-0.2453	0.0920	0.2453	-0.8586	0.7666
$\text{Tem1}_{\text{ss}}^{\text{GTP}}$	0.0404	-0.0152	-0.0404	0.1414	-0.1263

1.3 Reduction of the GTPase-cycle to a minimal model

We can reduce the reaction scheme (1.2) by eliminating variable Tem1 for the the vanishingly small nucleotide free GTPase. Assuming quasi steady state for Tem1 in equations (1.3), one obtains

$$\frac{\mathrm{d}}{\mathrm{d}t}\text{Tem1} \approx 0$$

and so

$$\text{Tem1} = \frac{k_{\text{off}}^{\text{T}}\,\text{Tem1}^{\text{GTP}} + k_{\text{off}}^{\text{D}}\,\text{Tem1}^{\text{GDP}}}{k_{\text{on}}^{\text{T}} + k_{\text{on}}^{\text{D}}}\,. \tag{1.7}$$

Substitution of (1.7) into equation (1.3) yields

$$\frac{\mathrm{d}}{\mathrm{d}t}\mathrm{Tem1}^{\mathrm{GTP}} = -\left(k_{\mathrm{off}}^{\mathrm{T}} + k_{\mathrm{cat}}\right)\mathrm{Tem1}^{\mathrm{GTP}} + \frac{k_{\mathrm{on}}^{\mathrm{T}}}{k_{\mathrm{on}}^{\mathrm{T}} + k_{\mathrm{on}}^{\mathrm{D}}}\left(k_{\mathrm{off}}^{\mathrm{T}}\,\mathrm{Tem1}^{\mathrm{GTP}} + k_{\mathrm{off}}^{\mathrm{D}}\,\mathrm{Tem1}^{\mathrm{GDP}}\right)$$
$$\frac{\mathrm{d}}{\mathrm{d}t}\mathrm{Tem1}^{\mathrm{GDP}} = -k_{\mathrm{off}}^{\mathrm{D}}\,\mathrm{Tem1}^{\mathrm{GDP}} + k_{\mathrm{cat}}\,\mathrm{Tem1}^{\mathrm{GTP}} + \frac{k_{\mathrm{on}}^{\mathrm{D}}}{k_{\mathrm{on}}^{\mathrm{T}} + k_{\mathrm{on}}^{\mathrm{D}}}\left(k_{\mathrm{off}}^{\mathrm{T}}\,\mathrm{Tem1}^{\mathrm{GTP}} + k_{\mathrm{off}}^{\mathrm{D}}\,\mathrm{Tem1}^{\mathrm{GDP}}\right)$$

We define

$$p_{\mathrm{GTP}} = \frac{k_{\mathrm{on}}^{\mathrm{T}}}{k_{\mathrm{on}}^{\mathrm{T}} + k_{\mathrm{on}}^{\mathrm{D}}} \qquad \text{and} \qquad p_{\mathrm{GDP}} = \frac{k_{\mathrm{on}}^{\mathrm{D}}}{k_{\mathrm{on}}^{\mathrm{T}} + k_{\mathrm{on}}^{\mathrm{D}}}$$

which can be interpreted as the probabilities of the nucleotide free GTPase to bind either GTP or GDP. The identity $p_{\mathrm{GTP}} = 1 - p_{\mathrm{GDP}}$ leads to

$$\begin{aligned}\frac{\mathrm{d}}{\mathrm{d}t}\mathrm{Tem1}^{\mathrm{GTP}} &= -\left(k_{\mathrm{off}}^{\mathrm{T}} + k_{\mathrm{cat}}\right)\mathrm{Tem1}^{\mathrm{GTP}} + p_{\mathrm{GTP}}\left(k_{\mathrm{off}}^{\mathrm{T}}\,\mathrm{Tem1}^{\mathrm{GTP}} + k_{\mathrm{off}}^{\mathrm{D}}\,\mathrm{Tem1}^{\mathrm{GDP}}\right)\\ &= k_{\mathrm{off}}^{\mathrm{D}}\,\mathrm{Tem1}^{\mathrm{GDP}} - k_{\mathrm{cat}}\,\mathrm{Tem1}^{\mathrm{GTP}} - p_{\mathrm{GDP}}\left(k_{\mathrm{off}}^{\mathrm{T}}\,\mathrm{Tem1}^{\mathrm{GTP}} + k_{\mathrm{off}}^{\mathrm{D}}\,\mathrm{Tem1}^{\mathrm{GDP}}\right)\\ &= -\frac{\mathrm{d}}{\mathrm{d}t}\mathrm{Tem1}^{\mathrm{GDP}}.\end{aligned}$$

Hence, the system is fully described with only one ODE. Collecting the terms in $\mathrm{Tem1}^{\mathrm{GTP}}$ yields

$$\frac{\mathrm{d}}{\mathrm{d}t}\mathrm{Tem1}^{\mathrm{GTP}} = -\underbrace{\left(p_{\mathrm{GDP}}\,k_{\mathrm{off}}^{\mathrm{T}} + k_{\mathrm{cat}}\right)}_{k_{\mathrm{hyd}}}\mathrm{Tem1}^{\mathrm{GTP}} + \underbrace{p_{\mathrm{GTP}}\,k_{\mathrm{off}}^{\mathrm{D}}}_{k_{\mathrm{nex}}}\mathrm{Tem1}^{\mathrm{GDP}}. \tag{1.8}$$

We can interpret $k_{\mathrm{hyd}} = p_{\mathrm{GDP}}\,k_{\mathrm{off}}^{\mathrm{T}} + k_{\mathrm{cat}}$ and $k_{\mathrm{nex}} = p_{\mathrm{GTP}}\,k_{\mathrm{off}}^{\mathrm{D}}$ as effective hydrolysis and GDP-GTP-exchange rate coefficients, respectively. We can reduce the chemical reaction equation (1.2) to

$$\mathrm{Tem1}^{\mathrm{GTP}} \underset{k_{\mathrm{nex}}}{\overset{k_{\mathrm{hyd}}}{\rightleftharpoons}} \mathrm{Tem1}^{\mathrm{GDP}}. \tag{1.9}$$

Interaction of Tem1 with GTPase-activating-protein (GAP) complex Bfa1-Bub2 will likely prevent nucleotide dissociation (Geymonat et al., 2002) but in turn accelerate the catalytic step. We can integrate this by the definition of a second effective hydrolysis rate coefficient for Tem1 when in complex with its GAP:

$$k_{\mathrm{hyd}}^{\mathrm{GAP}} = \lambda k_{\mathrm{cat}} \tag{1.10}$$

where λ is the fold-acceleration of GTP-hydrolysis in the GAP-$\mathrm{Tem1}^{\mathrm{GTP}}$ complex.

With equation (1.8), the steady state concentration of $\mathrm{Tem1}^{\mathrm{GTP}}$ is given by

$$\mathrm{Tem1}_{\mathrm{ss}}^{\mathrm{GTP}} = \frac{\mathrm{Tem1}_{\mathrm{T}}}{b + k_{\mathrm{off}}^{\mathrm{D}}\,k_{\mathrm{on}}^{\mathrm{T}}}\,k_{\mathrm{off}}^{\mathrm{D}}\,k_{\mathrm{on}}^{\mathrm{T}} \tag{1.11}$$

with b from equation (1.6). The relative error of the steady state concentration achieved in the reduced model compared to the reaction scheme (1.2) is

$$\frac{\overline{\mathrm{Tem1}_{\mathrm{ss}}^{\mathrm{GTP}}} - \mathrm{Tem1}_{\mathrm{ss}}^{\mathrm{GTP}}}{\overline{\mathrm{Tem1}_{\mathrm{ss}}^{\mathrm{GTP}}}} = \frac{b + k_{\mathrm{off}}^{\mathrm{D}}\,k_{\mathrm{on}}^{\mathrm{T}}}{a} - 1$$

where $\overline{\mathrm{Tem1}_{\mathrm{ss}}^{\mathrm{GTP}}}$ is the steady state concentration of $\mathrm{Tem1}^{\mathrm{GTP}}$ according to equation (1.4). With the parameters given in supplementary table 1, the relative error is less than 10^{-5} and it remains below 10^{-3} even if both, $k_{\mathrm{on}}^{\mathrm{D}}$ and $k_{\mathrm{on}}^{\mathrm{T}}$, are varied in a range from 10^{-2} to 10^{2} around their nominal values. Furthermore, the dynamics of the minimal model are indistinguishable from the original model as long as $k_{\mathrm{on}}^{\mathrm{T}}$ and $k_{\mathrm{on}}^{\mathrm{D}}$ are large compared to the respective dissociation rates and the quasi steady state assumption is valid.

2 Reaction equations

In our model, we consider merely four different proteins, namely Bfa1, Tem1, and their respective binding sites at the SPB, S and T. Though, Bfa1 and Tem1 can occur in several states, and all relevant complexes of the four proteins have to be considered. We denote complexes by listing the subunits separated by a colon, B : Bfa1, for instance, denotes the complex consisting of unphosphorylated Bfa1 and its binding site at the SPB. The possible subunits are defined below:

Bfa1 'unphosphorylated' Bfa1, that is, Bfa1 which is neither phosphorylated by Kin4 nor hyperphosphorylated by Cdc5.

Bfa1$^{\mathbf{P4}}$ Bfa1 phosphorylated by Kin4.

Bfa1$^{\mathbf{P5}}$ Bfa1 hyperphosphorylated by Cdc5.

Tem1$^{\mathbf{GTP}}$ Tem1 in GTP-bound state.

Tem1$^{\mathbf{GDP}}$ Tem1 in GDP-bound state.

B The unknown binding site for Bfa1 at the SPB.

T The unknown GAP-independent binding site for Tem1 at the SPB.

All reaction rate coefficients (indicated above and, where apropriate, below the raction arrows) are defined in Table 4 in section 3.5.

2.1 Association of Bfa1 and Bfa1 : Tem1 complexes with their SPB-binding site B

$$\mathrm{B} + \mathrm{Bfa1} \underset{k^{\mathrm{B}}_{\mathrm{off}}}{\overset{k^{\mathrm{B}}_{\mathrm{on}}}{\rightleftharpoons}} \mathrm{B:Bfa1} \tag{2.1}$$

$$\mathrm{B} + \mathrm{Bfa1^{P4}} \underset{k^{\mathrm{B4}}_{\mathrm{off}}}{\overset{k^{\mathrm{B4}}_{\mathrm{on}}}{\rightleftharpoons}} \mathrm{B:Bfa1^{P4}} \tag{2.2}$$

$$\mathrm{B} + \mathrm{Bfa1^{P5}} \underset{k^{\mathrm{B}}_{\mathrm{off}}}{\overset{k^{\mathrm{B}}_{\mathrm{on}}}{\rightleftharpoons}} \mathrm{B:Bfa1^{P5}} \tag{2.3}$$

$$\mathrm{B} + \mathrm{Bfa1:Tem1^{GTP}} \underset{k^{\mathrm{B}}_{\mathrm{off}}}{\overset{k^{\mathrm{B}}_{\mathrm{on}}}{\rightleftharpoons}} \mathrm{B:Bfa1:Tem1^{GTP}} \tag{2.4}$$

$$\mathrm{B} + \mathrm{Bfa1^{P4}:Tem1^{GTP}} \underset{k^{\mathrm{B4}}_{\mathrm{off}}}{\overset{k^{\mathrm{B4}}_{\mathrm{on}}}{\rightleftharpoons}} \mathrm{B:Bfa1^{P4}:Tem1^{GTP}} \tag{2.5}$$

$$\mathrm{B} + \mathrm{Bfa1^{P5}:Tem1^{GTP}} \underset{k^{\mathrm{B}}_{\mathrm{off}}}{\overset{k^{\mathrm{B}}_{\mathrm{on}}}{\rightleftharpoons}} \mathrm{B:Bfa1^{P5}:Tem1^{GTP}} \tag{2.6}$$

$$\mathrm{B} + \mathrm{Bfa1:Tem1^{GDP}} \underset{k^{\mathrm{B}}_{\mathrm{off}}}{\overset{k^{\mathrm{B}}_{\mathrm{on}}}{\rightleftharpoons}} \mathrm{B:Bfa1:Tem1^{GDP}} \tag{2.7}$$

$$\mathrm{B} + \mathrm{Bfa1^{P4}:Tem1^{GDP}} \underset{k^{\mathrm{B4}}_{\mathrm{off}}}{\overset{k^{\mathrm{B4}}_{\mathrm{on}}}{\rightleftharpoons}} \mathrm{B:Bfa1^{P4}:Tem1^{GDP}} \tag{2.8}$$

$$\mathrm{B} + \mathrm{Bfa1^{P5}:Tem1^{GDP}} \underset{k^{\mathrm{B}}_{\mathrm{off}}}{\overset{k^{\mathrm{B}}_{\mathrm{on}}}{\rightleftharpoons}} \mathrm{B:Bfa1^{P5}:Tem1^{GDP}} \tag{2.9}$$

2.2 Association of Tem1 with SPB-bound Bfa1

$$\mathrm{B:Bfa1} + \mathrm{Tem1^{GTP}} \underset{k_{\mathrm{off}}^{\mathrm{BT}}}{\overset{k_{\mathrm{on}}^{\mathrm{BT}}}{\rightleftharpoons}} \mathrm{B:Bfa1:Tem1^{GTP}} \tag{2.10}$$

$$\mathrm{B:Bfa1^{P4}} + \mathrm{Tem1^{GTP}} \underset{k_{\mathrm{off}}^{\mathrm{BT}}}{\overset{k_{\mathrm{on}}^{\mathrm{B4T}}}{\rightleftharpoons}} \mathrm{B:Bfa1^{P4}:Tem1^{GTP}} \tag{2.11}$$

$$\mathrm{B:Bfa1^{P5}} + \mathrm{Tem1^{GTP}} \underset{k_{\mathrm{off}}^{\mathrm{BT}}}{\overset{k_{\mathrm{on}}^{\mathrm{B5T}}}{\rightleftharpoons}} \mathrm{B:Bfa1^{P5}:Tem1^{GTP}} \tag{2.12}$$

$$\mathrm{B:Bfa1} + \mathrm{Tem1^{GDP}} \underset{k_{\mathrm{off}}^{\mathrm{BT}}}{\overset{k_{\mathrm{on}}^{\mathrm{BT}}}{\rightleftharpoons}} \mathrm{B:Bfa1:Tem1^{GDP}} \tag{2.13}$$

$$\mathrm{B:Bfa1^{P4}} + \mathrm{Tem1^{GDP}} \underset{k_{\mathrm{off}}^{\mathrm{BT}}}{\overset{k_{\mathrm{on}}^{\mathrm{B4T}}}{\rightleftharpoons}} \mathrm{B:Bfa1^{P4}:Tem1^{GDP}} \tag{2.14}$$

$$\mathrm{B:Bfa1^{P5}} + \mathrm{Tem1^{GDP}} \underset{k_{\mathrm{off}}^{\mathrm{BT}}}{\overset{k_{\mathrm{on}}^{\mathrm{B5T}}}{\rightleftharpoons}} \mathrm{B:Bfa1^{P5}:Tem1^{GDP}} \tag{2.15}$$

2.3 Association of Tem1 with cytosolic Bfa1

$$\mathrm{Bfa1} + \mathrm{Tem1^{GTP}} \underset{k_{\mathrm{off}}^{\mathrm{BT}}}{\overset{k_{\mathrm{on}}^{\mathrm{BT}}}{\rightleftharpoons}} \mathrm{Bfa1:Tem1^{GTP}} \tag{2.16}$$

$$\mathrm{Bfa1^{P4}} + \mathrm{Tem1^{GTP}} \underset{k_{\mathrm{off}}^{\mathrm{BT}}}{\overset{k_{\mathrm{on}}^{\mathrm{B4T}}}{\rightleftharpoons}} \mathrm{Bfa1^{P4}:Tem1^{GTP}} \tag{2.17}$$

$$\mathrm{Bfa1^{P5}} + \mathrm{Tem1^{GTP}} \underset{k_{\mathrm{off}}^{\mathrm{BT}}}{\overset{k_{\mathrm{on}}^{\mathrm{B5T}}}{\rightleftharpoons}} \mathrm{Bfa1^{P5}:Tem1^{GTP}} \tag{2.18}$$

$$\mathrm{Bfa1} + \mathrm{Tem1^{GDP}} \underset{k_{\mathrm{off}}^{\mathrm{BT}}}{\overset{k_{\mathrm{on}}^{\mathrm{BT}}}{\rightleftharpoons}} \mathrm{Bfa1:Tem1^{GDP}} \tag{2.19}$$

$$\mathrm{Bfa1^{P4}} + \mathrm{Tem1^{GDP}} \underset{k_{\mathrm{off}}^{\mathrm{BT}}}{\overset{k_{\mathrm{on}}^{\mathrm{B4T}}}{\rightleftharpoons}} \mathrm{Bfa1^{P4}:Tem1^{GDP}} \tag{2.20}$$

$$\mathrm{Bfa1^{P5}} + \mathrm{Tem1^{GDP}} \underset{k_{\mathrm{off}}^{\mathrm{BT}}}{\overset{k_{\mathrm{on}}^{\mathrm{B5T}}}{\rightleftharpoons}} \mathrm{Bfa1^{P5}:Tem1^{GDP}} \tag{2.21}$$

2.4 Association of Tem1 with its SPB-binding site T

$$\mathrm{T} + \mathrm{Tem1^{GTP}} \underset{k_{\mathrm{off}}^{\mathrm{T}}}{\overset{k_{\mathrm{on}}^{\mathrm{T}}}{\rightleftharpoons}} \mathrm{T:Tem1^{GTP}} \tag{2.22}$$

$$\mathrm{T} + \mathrm{Tem1^{GDP}} \underset{k_{\mathrm{off}}^{\mathrm{T}}}{\overset{k_{\mathrm{on}}^{\mathrm{T}}}{\rightleftharpoons}} \mathrm{T:Tem1^{GDP}} \tag{2.23}$$

2.5 Regulation of Bfa1 at the SPB

$$\mathrm{B:Bfa1} \xrightarrow{k_{+}^{\mathrm{Kin4}}} \mathrm{B:Bfa1^{P4}} \tag{2.24}$$

$$\mathrm{B:Bfa1:Tem1^{GTP}} \xrightarrow{k_{+}^{\mathrm{Kin4}}} \mathrm{B:Bfa1^{P4}:Tem1^{GTP}} \tag{2.25}$$

$$\mathrm{B:Bfa1:Tem1^{GDP}} \xrightarrow{k_{+}^{\mathrm{Kin4}}} \mathrm{B:Bfa1^{P4}:Tem1^{GDP}} \tag{2.26}$$

$$\mathrm{B:Bfa1} \xrightarrow{k_+^{\mathrm{Cdc5}}} \mathrm{B:Bfa1^{P5}} \tag{2.27}$$

$$\mathrm{B:Bfa1:Tem1^{GTP}} \xrightarrow{k_+^{\mathrm{Cdc5}}} \mathrm{B:Bfa1^{P5}:Tem1^{GTP}} \tag{2.28}$$

$$\mathrm{B:Bfa1:Tem1^{GDP}} \xrightarrow{k_+^{\mathrm{Cdc5}}} \mathrm{B:Bfa1^{P5}:Tem1^{GDP}} \tag{2.29}$$

2.6 Regulation of Bfa1 in the cytosol

$$\mathrm{Bfa1} \xrightarrow{k_{+,\mathrm{cyto}}^{\mathrm{Kin4}}} \mathrm{Bfa1^{P4}} \tag{2.30}$$

$$\mathrm{Bfa1:Tem1^{GTP}} \xrightarrow{k_{+,\mathrm{cyto}}^{\mathrm{Kin4}}} \mathrm{Bfa1^{P4}:Tem1^{GTP}} \tag{2.31}$$

$$\mathrm{Bfa1:Tem1^{GDP}} \xrightarrow{k_{+,\mathrm{cyto}}^{\mathrm{Kin4}}} \mathrm{Bfa1^{P4}:Tem1^{GDP}} \tag{2.32}$$

$$\mathrm{Bfa1^{P4}} \xrightarrow{k_-^{\mathrm{Kin4}}} \mathrm{Bfa1} \tag{2.33}$$

$$\mathrm{Bfa1^{P4}:Tem1^{GTP}} \xrightarrow{k_-^{\mathrm{Kin4}}} \mathrm{Bfa1:Tem1^{GTP}} \tag{2.34}$$

$$\mathrm{Bfa1^{P4}:Tem1^{GDP}} \xrightarrow{k_-^{\mathrm{Kin4}}} \mathrm{Bfa1:Tem1^{GDP}} \tag{2.35}$$

$$\mathrm{Bfa1^{P5}} \xrightarrow{k_-^{\mathrm{Cdc5}}} \mathrm{Bfa1} \tag{2.36}$$

$$\mathrm{Bfa1^{P5}:Tem1^{GTP}} \xrightarrow{k_-^{\mathrm{Cdc5}}} \mathrm{Bfa1:Tem1^{GTP}} \tag{2.37}$$

$$\mathrm{Bfa1^{P5}:Tem1^{GDP}} \xrightarrow{k_-^{\mathrm{Cdc5}}} \mathrm{Bfa1:Tem1^{GDP}} \tag{2.38}$$

2.7 Tem1-GTPase-cycle

$$\mathrm{Tem1^{GTP}} \xrightarrow{k_{\mathrm{hyd}}} \mathrm{Tem1^{GDP}} \tag{2.39}$$

$$\mathrm{Tem1^{GDP}} \xrightarrow{k_{\mathrm{nex}}} \mathrm{Tem1^{GTP}} \tag{2.40}$$

$$\mathrm{T:Tem1^{GTP}} \xrightarrow{k_{\mathrm{hyd}}} \mathrm{T:Tem1^{GDP}} \tag{2.41}$$

$$\mathrm{T:Tem1^{GDP}} \xrightarrow{k_{\mathrm{nex}}} \mathrm{T:Tem1^{GTP}} \tag{2.42}$$

$$\mathrm{Bfa1:Tem1^{GTP}} \xrightarrow{k_{\mathrm{hyd}}^{\mathrm{BT}}} \mathrm{Bfa1:Tem1^{GDP}} \tag{2.43}$$

$$\mathrm{Bfa1^{P4}:Tem1^{GTP}} \xrightarrow{k_{\mathrm{hyd}}^{\mathrm{B4T}}} \mathrm{Bfa1^{P4}:Tem1^{GDP}} \tag{2.44}$$

$$\mathrm{Bfa1^{P5}:Tem1^{GTP}} \xrightarrow{k_{\mathrm{hyd}}} \mathrm{Bfa1^{P5}:Tem1^{GDP}} \tag{2.45}$$

$$\mathrm{B:Bfa1:Tem1^{GTP}} \xrightarrow{k_{\mathrm{hyd}}^{\mathrm{BT}}} \mathrm{B:Bfa1:Tem1^{GDP}} \tag{2.46}$$

$$\mathrm{B:Bfa1^{P4}:Tem1^{GTP}} \xrightarrow{k_{\mathrm{hyd}}^{\mathrm{B4T}}} \mathrm{B:Bfa1^{P4}:Tem1^{GDP}} \tag{2.47}$$

$$\mathrm{B:Bfa1^{P5}:Tem1^{GTP}} \xrightarrow{k_{\mathrm{hyd}}} \mathrm{B:Bfa1^{P5}:Tem1^{GDP}} \tag{2.48}$$

3 Ordinary differential equations of the model

We translated the reaction equations (2.1)–(2.48) into the set of coupled nonlinear ordinary differential equations given below. Conventionally we enclose the species name as it appears in the reaction equations in brackets to refer to their respective molar concentrations. Association of cytosolic Bfa1 and Tem1 with their respective binding sites at the SPB involves a transition from a large (cytosol, V_C) to a small (SPB, V_S)

reference volume . The fluxes of reactions (2.1)–(2.15), (2.22), and (2.23) must therefore be scaled with $V_\mathrm{S}/V_\mathrm{C}$ when they contribute to the differential equations of the respective cytosolic species. The fluxes v_1 to v_{48} correspond to reactions (2.1)–(2.48) and are given in section 3.2, initial conditions and parameters are listed in tables 3 (section 3.4) and 4 (section 3.5), respectively.

3.1 Ordinary differential equation system

$$
\begin{aligned}
\mathrm{d}[\mathrm{T}]/\mathrm{d}t &= -v_{22} - v_{23} \\
\mathrm{d}[\mathrm{B}]/\mathrm{d}t &= -\textstyle\sum_{i=1}^{9} v_i \\
\mathrm{d}[\mathrm{Bfa1}]/\mathrm{d}t &= -\left(V_\mathrm{S}/V_\mathrm{C}\right) v_1 - v_{16} - v_{19} - v_{30} + v_{33} + v_{36} \\
\mathrm{d}[\mathrm{Bfa1}^{\mathrm{P4}}]/\mathrm{d}t &= -\left(V_\mathrm{S}/V_\mathrm{C}\right) v_2 - v_{17} - v_{20} + v_{30} - v_{33} \\
\mathrm{d}[\mathrm{Bfa1}^{\mathrm{P5}}]/\mathrm{d}t &= -\left(V_\mathrm{S}/V_\mathrm{C}\right) v_3 - v_{18} - v_{21} - v_{36} \\
\mathrm{d}[\mathrm{Bfa1}:\mathrm{Tem1}^{\mathrm{GTP}}]/\mathrm{d}t &= -\left(V_\mathrm{S}/V_\mathrm{C}\right) v_4 + v_{16} - v_{31} + v_{34} + v_{37} - v_{43} \\
\mathrm{d}[\mathrm{Bfa1}^{\mathrm{P4}}:\mathrm{Tem1}^{\mathrm{GTP}}]/\mathrm{d}t &= -\left(V_\mathrm{S}/V_\mathrm{C}\right) v_5 + v_{17} + v_{31} - v_{34} - v_{44} \\
\mathrm{d}[\mathrm{Bfa1}^{\mathrm{P5}}:\mathrm{Tem1}^{\mathrm{GTP}}]/\mathrm{d}t &= -\left(V_\mathrm{S}/V_\mathrm{C}\right) v_6 + v_{18} - v_{37} - v_{45} \\
\mathrm{d}[\mathrm{Bfa1}:\mathrm{Tem1}^{\mathrm{GDP}}]/\mathrm{d}t &= -\left(V_\mathrm{S}/V_\mathrm{C}\right) v_7 + v_{19} - v_{32} + v_{35} + v_{38} + v_{43} \\
\mathrm{d}[\mathrm{Bfa1}^{\mathrm{P4}}:\mathrm{Tem1}^{\mathrm{GDP}}]/\mathrm{d}t &= -\left(V_\mathrm{S}/V_\mathrm{C}\right) v_8 + v_{20} + v_{32} - v_{35} + v_{44} \\
\mathrm{d}[\mathrm{Bfa1}^{\mathrm{P5}}:\mathrm{Tem1}^{\mathrm{GDP}}]/\mathrm{d}t &= -\left(V_\mathrm{S}/V_\mathrm{C}\right) v_9 + v_{21} - v_{38} + v_{45} \\
\mathrm{d}[\mathrm{B}:\mathrm{Bfa1}]/\mathrm{d}t &= v_1 - v_{10} - v_{13} - v_{24} - v_{27} \\
\mathrm{d}[\mathrm{B}:\mathrm{Bfa1}^{\mathrm{P4}}]/\mathrm{d}t &= v_2 - v_{11} - v_{14} + v_{24} \\
\mathrm{d}[\mathrm{B}:\mathrm{Bfa1}^{\mathrm{P5}}]/\mathrm{d}t &= v_3 - v_{12} - v_{15} + v_{27} \\
\mathrm{d}[\mathrm{B}:\mathrm{Bfa1}:\mathrm{Tem1}^{\mathrm{GTP}}]/\mathrm{d}t &= v_4 + v_{10} - v_{25} - v_{28} - v_{46} \\
\mathrm{d}[\mathrm{B}:\mathrm{Bfa1}^{\mathrm{P4}}:\mathrm{Tem1}^{\mathrm{GTP}}]/\mathrm{d}t &= v_5 + v_{11} + v_{25} - v_{47} \\
\mathrm{d}[\mathrm{B}:\mathrm{Bfa1}^{\mathrm{P5}}:\mathrm{Tem1}^{\mathrm{GTP}}]/\mathrm{d}t &= v_6 + v_{12} + v_{28} - v_{48} \\
\mathrm{d}[\mathrm{B}:\mathrm{Bfa1}:\mathrm{Tem1}^{\mathrm{GDP}}]/\mathrm{d}t &= v_7 + v_{13} - v_{26} - v_{29} + v_{46} \\
\mathrm{d}[\mathrm{B}:\mathrm{Bfa1}^{\mathrm{P4}}:\mathrm{Tem1}^{\mathrm{GDP}}]/\mathrm{d}t &= v_8 + v_{14} + v_{26} + v_{47} \\
\mathrm{d}[\mathrm{B}:\mathrm{Bfa1}^{\mathrm{P5}}:\mathrm{Tem1}^{\mathrm{GDP}}]/\mathrm{d}t &= v_9 + v_{15} + v_{29} + v_{48} \\
\mathrm{d}[\mathrm{Tem1}^{\mathrm{GTP}}]/\mathrm{d}t &= -\left(V_\mathrm{S}/V_\mathrm{C}\right)\left(\textstyle\sum_{i=10}^{12} v_i + v_{22}\right) - \textstyle\sum_{i=16}^{18} v_i - v_{39} + v_{40} \\
\mathrm{d}[\mathrm{Tem1}^{\mathrm{GDP}}]/\mathrm{d}t &= -\left(V_\mathrm{S}/V_\mathrm{C}\right)\left(\textstyle\sum_{i=13}^{15} v_i + v_{23}\right) - \textstyle\sum_{i=19}^{21} v_i + v_{39} - v_{40} \\
\mathrm{d}[\mathrm{T}:\mathrm{Tem1}^{\mathrm{GTP}}]/\mathrm{d}t &= v_{22} - v_{41} + v_{42} \\
\mathrm{d}[\mathrm{T}:\mathrm{Tem1}^{\mathrm{GDP}}]/\mathrm{d}t &= v_{23} + v_{41} - v_{42}
\end{aligned}
$$

3.2 Reaction fluxes

$$
\begin{aligned}
v_1 &= k_{\mathrm{on}}^{\mathrm{B}}\,[\mathrm{B}]\,[\mathrm{Bfa1}] - k_{\mathrm{off}}^{\mathrm{B}}\,[\mathrm{B}:\mathrm{Bfa1}] \\
v_2 &= k_{\mathrm{on}}^{\mathrm{B4}}\,[\mathrm{B}]\,[\mathrm{Bfa1}^{\mathrm{P4}}] - k_{\mathrm{off}}^{\mathrm{B4}}\,[\mathrm{B}:\mathrm{Bfa1}^{\mathrm{P4}}] \\
v_3 &= k_{\mathrm{on}}^{\mathrm{B}}\,[\mathrm{B}]\,[\mathrm{Bfa1}] - k_{\mathrm{off}}^{\mathrm{B}}\,[\mathrm{B}:\mathrm{Bfa1}^{\mathrm{P5}}] \\
v_4 &= k_{\mathrm{on}}^{\mathrm{B}}\,[\mathrm{B}]\,[\mathrm{Bfa1}:\mathrm{Tem1}^{\mathrm{GTP}}] - k_{\mathrm{off}}^{\mathrm{B}}\,[\mathrm{B}:\mathrm{Bfa1}:\mathrm{Tem1}^{\mathrm{GTP}}] \\
v_5 &= k_{\mathrm{on}}^{\mathrm{B4}}\,[\mathrm{B}]\,[\mathrm{Bfa1}^{\mathrm{P4}}:\mathrm{Tem1}^{\mathrm{GTP}}] - k_{\mathrm{off}}^{\mathrm{B4}}\,[\mathrm{B}:\mathrm{Bfa1}^{\mathrm{P4}}:\mathrm{Tem1}^{\mathrm{GTP}}] \\
v_6 &= k_{\mathrm{on}}^{\mathrm{B}}\,[\mathrm{B}]\,[\mathrm{Bfa1}^{\mathrm{P5}}:\mathrm{Tem1}^{\mathrm{GTP}}] - k_{\mathrm{off}}^{\mathrm{B}}\,[\mathrm{B}:\mathrm{Bfa1}^{\mathrm{P5}}:\mathrm{Tem1}^{\mathrm{GTP}}]
\end{aligned}
$$

$$v_7 = k_{\text{on}}^{\text{B}}\,[\text{B}]\,[\text{Bfa1}:\text{Tem1}^{\text{GDP}}] - k_{\text{off}}^{\text{B}}\,[\text{B}:\text{Bfa1}:\text{Tem1}^{\text{GDP}}]$$
$$v_8 = k_{\text{on}}^{\text{B4}}\,[\text{B}]\,[\text{Bfa1}^{\text{P4}}:\text{Tem1}^{\text{GDP}}] - k_{\text{off}}^{\text{B4}}\,[\text{B}:\text{Bfa1}^{\text{P4}}:\text{Tem1}^{\text{GDP}}]$$
$$v_9 = k_{\text{on}}^{\text{B}}\,[\text{B}]\,[\text{Bfa1}^{\text{P5}}:\text{Tem1}^{\text{GDP}}] - k_{\text{off}}^{\text{B}}\,[\text{B}:\text{Bfa1}^{\text{P5}}:\text{Tem1}^{\text{GDP}}]$$
$$v_{10} = k_{\text{on}}^{\text{BT}}\,[\text{B}:\text{Bfa1}]\,[\text{Tem1}^{\text{GTP}}] - k_{\text{off}}^{\text{BT}}\,[\text{B}:\text{Bfa1}:\text{Tem1}^{\text{GTP}}]$$
$$v_{11} = k_{\text{on}}^{\text{B4T}}\,[\text{B}:\text{Bfa1}^{\text{P4}}]\,[\text{Tem1}^{\text{GTP}}] - k_{\text{off}}^{\text{BT}}\,[\text{B}:\text{Bfa1}^{\text{P4}}:\text{Tem1}^{\text{GTP}}]$$
$$v_{12} = k_{\text{on}}^{\text{B5T}}\,[\text{B}:\text{Bfa1}^{\text{P5}}]\,[\text{Tem1}^{\text{GTP}}] - k_{\text{off}}^{\text{BT}}\,[\text{B}:\text{Bfa1}^{\text{P5}}:\text{Tem1}^{\text{GTP}}]$$
$$v_{13} = k_{\text{on}}^{\text{BT}}\,[\text{B}:\text{Bfa1}]\,[\text{Tem1}^{\text{GDP}}] - k_{\text{off}}^{\text{BT}}\,[\text{B}:\text{Bfa1}:\text{Tem1}^{\text{GDP}}]$$
$$v_{14} = k_{\text{on}}^{\text{B4T}}\,[\text{B}:\text{Bfa1}^{\text{P4}}]\,[\text{Tem1}^{\text{GDP}}] - k_{\text{off}}^{\text{BT}}\,[\text{B}:\text{Bfa1}^{\text{P4}}:\text{Tem1}^{\text{GDP}}]$$
$$v_{15} = k_{\text{on}}^{\text{B5T}}\,[\text{B}:\text{Bfa1}^{\text{P5}}]\,[\text{Tem1}^{\text{GDP}}] - k_{\text{off}}^{\text{BT}}\,[\text{B}:\text{Bfa1}^{\text{P5}}:\text{Tem1}^{\text{GDP}}]$$
$$v_{16} = \alpha k_{\text{on}}^{\text{BT}}\,[\text{Bfa1}]\,[\text{Tem1}^{\text{GTP}}] - k_{\text{off}}^{\text{BT}}\,[\text{Bfa1}:\text{Tem1}^{\text{GTP}}]$$
$$v_{17} = \alpha k_{\text{on}}^{\text{B4T}}\,[\text{Bfa1}^{\text{P4}}]\,[\text{Tem1}^{\text{GTP}}] - k_{\text{off}}^{\text{BT}}\,[\text{Bfa1}^{\text{P4}}:\text{Tem1}^{\text{GTP}}]$$
$$v_{18} = \alpha k_{\text{on}}^{\text{B5T}}\,[\text{Bfa1}^{\text{P5}}]\,[\text{Tem1}^{\text{GTP}}] - k_{\text{off}}^{\text{BT}}\,[\text{Bfa1}^{\text{P5}}:\text{Tem1}^{\text{GTP}}]$$
$$v_{19} = \alpha k_{\text{on}}^{\text{BT}}\,[\text{Bfa1}]\,[\text{Tem1}^{\text{GDP}}] - k_{\text{off}}^{\text{BT}}\,[\text{Bfa1}:\text{Tem1}^{\text{GDP}}]$$
$$v_{20} = \alpha k_{\text{on}}^{\text{B4T}}\,[\text{Bfa1}^{\text{P4}}]\,[\text{Tem1}^{\text{GDP}}] - k_{\text{off}}^{\text{BT}}\,[\text{Bfa1}^{\text{P4}}:\text{Tem1}^{\text{GDP}}]$$
$$v_{21} = \alpha k_{\text{on}}^{\text{B5T}}\,[\text{Bfa1}^{\text{P5}}]\,[\text{Tem1}^{\text{GDP}}] - k_{\text{off}}^{\text{BT}}\,[\text{Bfa1}^{\text{P5}}:\text{Tem1}^{\text{GDP}}]$$
$$v_{22} = k_{\text{on}}^{\text{T}}\,[\text{T}]\,[\text{Tem1}^{\text{GTP}}] - k_{\text{off}}^{\text{T}}\,[\text{T}:\text{Tem1}^{\text{GTP}}]$$
$$v_{23} = k_{\text{on}}^{\text{T}}\,[\text{T}]\,[\text{Tem1}^{\text{GDP}}] - k_{\text{off}}^{\text{T}}\,[\text{T}:\text{Tem1}^{\text{GDP}}]$$
$$v_{24} = u k_{+}^{\text{Kin4}}\,[\text{B}:\text{Bfa1}]$$
$$v_{25} = u k_{+}^{\text{Kin4}}\,[\text{B}:\text{Bfa1}:\text{Tem1}^{\text{GTP}}]$$
$$v_{26} = u k_{+}^{\text{Kin4}}\,[\text{B}:\text{Bfa1}:\text{Tem1}^{\text{GDP}}]$$
$$v_{27} = k_{+}^{\text{Cdc5}}\,[\text{B}:\text{Bfa1}]$$
$$v_{28} = k_{+}^{\text{Cdc5}}\,[\text{B}:\text{Bfa1}:\text{Tem1}^{\text{GTP}}]$$
$$v_{29} = k_{+}^{\text{Cdc5}}\,[\text{B}:\text{Bfa1}:\text{Tem1}^{\text{GDP}}]$$
$$v_{30} = u k_{+,\text{cyto}}^{\text{Kin4}}\,[\text{Bfa1}]$$
$$v_{31} = u k_{+,\text{cyto}}^{\text{Kin4}}\,[\text{Bfa1}:\text{Tem1}^{\text{GTP}}]$$
$$v_{32} = u k_{+,\text{cyto}}^{\text{Kin4}}\,[\text{Bfa1}:\text{Tem1}^{\text{GDP}}]$$
$$v_{33} = k_{-}^{\text{Kin4}}\,[\text{Bfa1}^{\text{P4}}]$$
$$v_{34} = k_{-}^{\text{Kin4}}\,[\text{Bfa1}^{\text{P4}}:\text{Tem1}^{\text{GTP}}]$$
$$v_{35} = k_{-}^{\text{Kin4}}\,[\text{Bfa1}^{\text{P4}}:\text{Tem1}^{\text{GDP}}]$$
$$v_{36} = u k_{-}^{\text{Cdc5}}\,[\text{Bfa1}^{\text{P5}}]$$
$$v_{37} = u k_{-}^{\text{Cdc5}}\,[\text{Bfa1}^{\text{P5}}:\text{Tem1}^{\text{GTP}}]$$
$$v_{38} = u k_{-}^{\text{Cdc5}}\,[\text{Bfa1}^{\text{P5}}:\text{Tem1}^{\text{GDP}}]$$
$$v_{39} = k_{\text{hyd}}\,[\text{Tem1}^{\text{GTP}}]$$
$$v_{40} = k_{\text{nex}}\,[\text{Tem1}^{\text{GDP}}]$$
$$v_{41} = k_{\text{hyd}}\,[\text{T}:\text{Tem1}^{\text{GTP}}]$$
$$v_{42} = k_{\text{nex}}\,[\text{T}:\text{Tem1}^{\text{GDP}}]$$
$$v_{43} = k_{\text{hyd}}^{\text{BT}}\,[\text{Bfa1}:\text{Tem1}^{\text{GTP}}]$$
$$v_{44} = k_{\text{hyd}}^{\text{B4T}}\,[\text{Bfa1}^{\text{P4}}:\text{Tem1}^{\text{GTP}}]$$

$$
\begin{aligned}
v_{45} &= k_{\text{hyd}}\,[\text{Bfa1}^{\text{P5}}:\text{Tem1}^{\text{GTP}}] \\
v_{46} &= k_{\text{hyd}}^{\text{BT}}\,[\text{B}:\text{Bfa1}:\text{Tem1}^{\text{GTP}}] \\
v_{47} &= k_{\text{hyd}}^{\text{B4T}}\,[\text{B}:\text{Bfa1}^{\text{P4}}:\text{Tem1}^{\text{GTP}}] \\
v_{48} &= k_{\text{hyd}}\,[\text{B}:\text{Bfa1}^{\text{P5}}:\text{Tem1}^{\text{GTP}}]
\end{aligned}
$$

3.3 Observables

We were particularly interested in the time evolution of active and inactive Bfa1 and Tem1, respectively. However, these observables we demand are not directly represented as model variables because the attributed activity of Bfa1 or Tem1 must be tracked even within their respective complexes. We therefore have to sum up the respective variables appropriately. We consider GTP-bound Tem1 to be active, regardless of whether it is bound to Bfa1 or not, thus

$$
\begin{aligned}
[\text{Tem1}^{\text{active}}_{\text{SPB}}] &= [\text{T}:\text{Tem1}^{\text{GTP}}] + [\text{B}:\text{Bfa1}:\text{Tem1}^{\text{GTP}}] \\
&\quad + [\text{B}:\text{Bfa1}^{\text{P4}}:\text{Tem1}^{\text{GTP}}] + [\text{B}:\text{Bfa1}^{\text{P5}}:\text{Tem1}^{\text{GTP}}] \\
[\text{Tem1}^{\text{active}}_{\text{Cyto}}] &= [\text{Tem1}^{\text{GTP}}] + [\text{Bfa1}:\text{Tem1}^{\text{GTP}}] \\
&\quad + [\text{Bfa1}^{\text{P4}}:\text{Tem1}^{\text{GTP}}] + [\text{Bfa1}^{\text{P5}}:\text{Tem1}^{\text{GTP}}]
\end{aligned}
$$

for active Tem1 at the SPB or in the cytosol, respectively. Similarly, Tem1 bound to GDP is always accounted inactive, therefore we define

$$
\begin{aligned}
[\text{Tem1}^{\text{inactive}}_{\text{SPB}}] &= [\text{T}:\text{Tem1}^{\text{GDP}}] + [\text{B}:\text{Bfa1}:\text{Tem1}^{\text{GDP}}] \\
&\quad + [\text{B}:\text{Bfa1}^{\text{P4}}:\text{Tem1}^{\text{GDP}}] + [\text{B}:\text{Bfa1}^{\text{P5}}:\text{Tem1}^{\text{GDP}}] \\
[\text{Tem1}^{\text{inactive}}_{\text{Cyto}}] &= [\text{Tem1}^{\text{GDP}}] + [\text{Bfa1}:\text{Tem1}^{\text{GDP}}] \\
&\quad + [\text{Bfa1}^{\text{P4}}:\text{Tem1}^{\text{GDP}}] + [\text{Bfa1}^{\text{P5}}:\text{Tem1}^{\text{GDP}}]
\end{aligned}
$$

for inactive Tem1 at the SPB or in the cytosol. The activity of Bfa1 is regulated by its phosphorylation status, irrespectively of being in complex with Tem1. However, dependent upon the model variant, we consider unphosphorylated Bfa1 active (ubiquitous-association and hot-spot-association models) while we consider it inactive in the ubiquitous-inactive variant. The appropriate sums for active Bfa1 read

$$
\begin{aligned}
[\text{Bfa1}^{\text{active}}_{\text{SPB}}] &= q\left\{[\text{B}:\text{Bfa1}] + [\text{B}:\text{Bfa1}:\text{Tem1}^{\text{GTP}}] + [\text{B}:\text{Bfa1}:\text{Tem1}^{\text{GDP}}]\right\} \\
&\quad + [\text{B}:\text{Bfa1}^{\text{P4}}] + [\text{B}:\text{Bfa1}^{\text{P4}}:\text{Tem1}^{\text{GTP}}] + [\text{B}:\text{Bfa1}^{\text{P4}}:\text{Tem1}^{\text{GDP}}] \\
[\text{Bfa1}^{\text{active}}_{\text{Cyto}}] &= q\left\{[\text{Bfa1}] + [\text{Bfa1}:\text{Tem1}^{\text{GTP}}] + [\text{Bfa1}:\text{Tem1}^{\text{GDP}}]\right\} \\
&\quad + [\text{Bfa1}^{\text{P4}}] + [\text{Bfa1}^{\text{P4}}:\text{Tem1}^{\text{GTP}}] + [\text{Bfa1}^{\text{P4}}:\text{Tem1}^{\text{GDP}}]\,,
\end{aligned}
$$

where $q = 1$ for ubiquitous-association and hot-spot association variant, and $q = 0$ for the ubiquitous-inactive model. Accordingly, for inactive Bfa1

$$
\begin{aligned}
[\text{Bfa1}^{\text{inactive}}_{\text{SPB}}] &= (1-q)\left\{[\text{B}:\text{Bfa1}] + [\text{B}:\text{Bfa1}:\text{Tem1}^{\text{GTP}}] + [\text{B}:\text{Bfa1}:\text{Tem1}^{\text{GDP}}]\right\} \\
&\quad + [\text{B}:\text{Bfa1}^{\text{P5}}] + [\text{B}:\text{Bfa1}^{\text{P5}}:\text{Tem1}^{\text{GTP}}] + [\text{B}:\text{Bfa1}^{\text{P5}}:\text{Tem1}^{\text{GDP}}] \\
[\text{Bfa1}^{\text{inactive}}_{\text{Cyto}}] &= (1-q)\left\{[\text{Bfa1}] + [\text{Bfa1}:\text{Tem1}^{\text{GTP}}] + [\text{Bfa1}:\text{Tem1}^{\text{GDP}}]\right\} \\
&\quad + [\text{Bfa1}^{\text{P5}}] + [\text{Bfa1}^{\text{P5}}:\text{Tem1}^{\text{GTP}}] + [\text{Bfa1}^{\text{P5}}:\text{Tem1}^{\text{GDP}}]\,.
\end{aligned}
$$

3.4 Initial conditions

Table 3: Initial conditions of the model variables. All concentrations are given in molar.

Variable	Conc.	Molecules	Notes	References
B	8.3×10^{-5}	150	Unidentified Bfa1-binding site at the SPB. Adds up to 300 binding sites in total together with B : $Bfa1^{P5}$.	This study
$B:Bfa1^{P5}$	8.3×10^{-5}	150		This study
Bfa1	2.03×10^{-8}	1224	Adds up to 1374 together with B : $Bfa1^{P5}$.	This study
$Tem1^{GTP}$	4.91×10^{-8}	2954	Adds up to 3435 together with $Tem1^{GDP}$(2.5-fold of total Bfa1). Ratio of $Tem1^{GTP}$ and $Tem1^{GDP}$ according to section 1.	This study
$Tem1^{GDP}$	7.99×10^{-9}	481	Adds up to 3435 together with $Tem1^{GTP}$(2.5-fold of total Bfa1). Ratio of $Tem1^{GTP}$ and $Tem1^{GDP}$ according to section 1.	This study
T	1.66×10^{-4}	300	Unidentified Tem1-binding site at the SPB.	This study
all others	0			

3.5 Parameters

Table 4: Parameters used for the simulations of the models.

Parameter	Value			Unit	Notes	Reactions	References
	Ubiquitous-association	*Hot-spot-association*	*Ubiquitous-inactive*				
k_{on}^{B}	1.25×10^6	1.25×10^6	1.25×10^6	$\text{M}^{-1}\text{s}^{-1}$	Association of Bfa1 with the SPB.[a]	1, 3, 4, 6, 7, 9	This study
$k_{\text{off}}^{\text{B}}$	1.2×10^{-3}	1.2×10^{-3}	1.2×10^{-3}	s^{-1}	Dissociation of Bfa1 from the SPB. Corresponding to a half-life of $t_{1/2} = 600$ s.[b,c]	1, 3, 4, 6, 7, 9	(Caydasi and Pereira, 2009)
$k_{\text{on}}^{\text{B4}}$	2×10^4	2×10^4	2×10^4	$\text{M}^{-1}\text{s}^{-1}$	Association of Bfa1 with the SPB, low affinity after phosphorylation by Kin4.[a]	2, 5, 8	This study
$k_{\text{off}}^{\text{B4}}$	3.65×10^{-2}	3.65×10^{-2}	3.65×10^{-2}	s^{-1}	Dissociation of Bfa1 from the SPB, low affinity after phosphorylation by Kin4. Corresponding to a half-life of $t_{1/2} \approx 19$ s in cells with misaligned spindle.[b]	2, 5, 8	(Caydasi and Pereira, 2009)
$k_{\text{on}}^{\text{BT}}$	3.65×10^7	3.65×10^7	3.65×10^7	$\text{M}^{-1}\text{s}^{-1}$	Association of Tem1 and Bfa1.[a]	10, 13, 16, 19	This study
$k_{\text{on}}^{\text{B4T}}$	3.65×10^7	3.65×10^7	3.65×10^7	$\text{M}^{-1}\text{s}^{-1}$	Association of Tem1 and Bfa1^{P4}.[a]	11, 14, 17, 20	This study
$k_{\text{on}}^{\text{B5T}}$	7×10^6	5.5×10^6	7×10^6	$\text{M}^{-1}\text{s}^{-1}$	Association of Tem1 and Bfa1^{P5}.[a]	12, 15, 18, 21	This study
$k_{\text{off}}^{\text{BT}}$	1.83×10^{-1}	1.83×10^{-1}	1.83×10^{-1}	s^{-1}	Dissociation of Bfa1:Tem1 complex. Estimated from Tem1 half-life at the SPB of $t_{1/2} \approx 3.8$ s in WT cells with normal anaphase spindle, where Bfa1 is the primary binding site.[b]	10 – 21	(Caydasi and Pereira, 2009)
k_{on}^{T}	1.9×10^6	1.25×10^6	1.9×10^6	$\text{M}^{-1}\text{s}^{-1}$	Association of Tem1 with the GAP-independent binding site.[a]	22, 23	This study
$k_{\text{off}}^{\text{T}}$	1.83×10^{-1}	1.83×10^{-1}	1.83×10^{-1}	s^{-1}	Dissociation of Tem1 from the GAP-independent binding site. Estimated from Tem1 at the SPB of $t_{1/2} \approx 3.8$ s in cells with misapligned spindle.[b]	22, 23	(Caydasi and Pereira, 2009)
k_{+}^{Kin4}	10^3	10^3	10^3	s^{-1}	Pseudo first-order rate coefficient for the phosphorylation of Bfa1 by Kin4 at the SPB.	24 – 26	This study.
$k_{+,\text{cyto}}^{\text{Kin4}}$	0.09	0.09	0.09	s^{-1}	Pseudo first-order rate coefficient for the phosphorylation of Bfa1 by Kin4.	30 – 32	This study.
k_{-}^{Kin4}	2.51×10^{-2}	2.51×10^{-2}	2.51×10^{-2}	s^{-1}	Pseudo first-order rate coefficient for the dephosphorylation of Bfa1 by the unknown phosphatase counteracting Kin4.	33 – 35	This study
k_{+}^{Cdc5}	1	1	1	s^{-1}	Pseudo first-order rate coefficient for the phosphorylation of Bfa1 by Cdc5.	27 – 29	This study
k_{-}^{Cdc5}	10^{-2}	10^{-2}	10^{-2}	s^{-1}	Pseudo first-order rate coefficient for the dephosphorylation of Bfa1 by the unknown phosphatase counteracting Cdc5.	36 – 38	This study
k_{hyd}	2.24×10^{-3}	2.24×10^{-3}	2.24×10^{-3}	s^{-1}	Effective intrinsic GTP-hydrolysis by Tem1. See section 1.3 and equation (1.8) and table 1 therein.	39, 41, 45, 48	Section 1
k_{nex}	1.36×10^{-2}	1.36×10^{-2}	1.36×10^{-2}	s^{-1}	Effective intrinsic GDP-GTP-exchange rate by Tem1. See section 1.3 and equation (1.8) and table 1 therein.	40, 42	Section 1

Continued on the next page

Table 4: continued

Parameter	Value			Unit	Notes	Reactions	References
	Ubiquitous-association	*Hot-spot-association*	*Ubiquitous-inactive*				
$k_{\text{hyd}}^{\text{BT}}$	2	2	2.24×10^{-3}	s^{-1}	GAP-accelerated GTP-hydrolysis by Tem1. See section 1.3 and equation (1.10) and table 1 therein. Fold-acceleration $\lambda = 1000$. Unphosphorylated Bfa1 considered inactive in the Bfa1-control model, thus value according to equation (1.10).	43, 46	Section 1
$k_{\text{hyd}}^{\text{B4T}}$	2	2	2	s^{-1}	GAP-accelerated GTP-hydrolysis by Tem1. See section 1.3 and equation (1.10) and table 1 therein. Fold-acceleration $\lambda = 1000$.	44, 47	Section 1
α	1	0	1		Structural parameter preventing association of Bfa1 and Tem1 in the cytosol if 0.		
u	0 or 1	0 or 1	0 or 1		Input signal modeling correct spindle alignment $u = 0$ or spindle misalignment $u = 1$. Our simulations start with $u = 1$ at $t = 0$ min and switch to $u = 0$ at $t = 30$ min.		
V_{S}	3×10^{-18}	3×10^{-18}	3×10^{-18}	l	Volume of the SPB compartment by overestimating the SPB as a cylindrical volume element approximately 100 nm high and 200 nm in diameter		This study, (Jaspersen and M, 2004)
V_{S}	1×10^{-13}	1×10^{-13}	1×10^{-13}	l	Volume of the cytosol compartment considered similar to the whole cell volume		(Bryan et al., 2010)

[a] Association rate was manually adjusted to match the molecule numbers at the SPB, given the respective dissociation rate. [b] Dissociation rate coefficient estimated according to $k_{\text{off}} = \ln(2)/t_{1/2}$ where $t_{1/2}$ is the average half-life at the SPB. [c] Bfa1 is tightly bound to SPBs of correctly aligned anaphase spindles (Caydasi and Pereira, 2009). However, in order to allow Cdc5 to deactivate also the cytosolic Bfa1 pool, we assume a more rapid half-life of 600s.

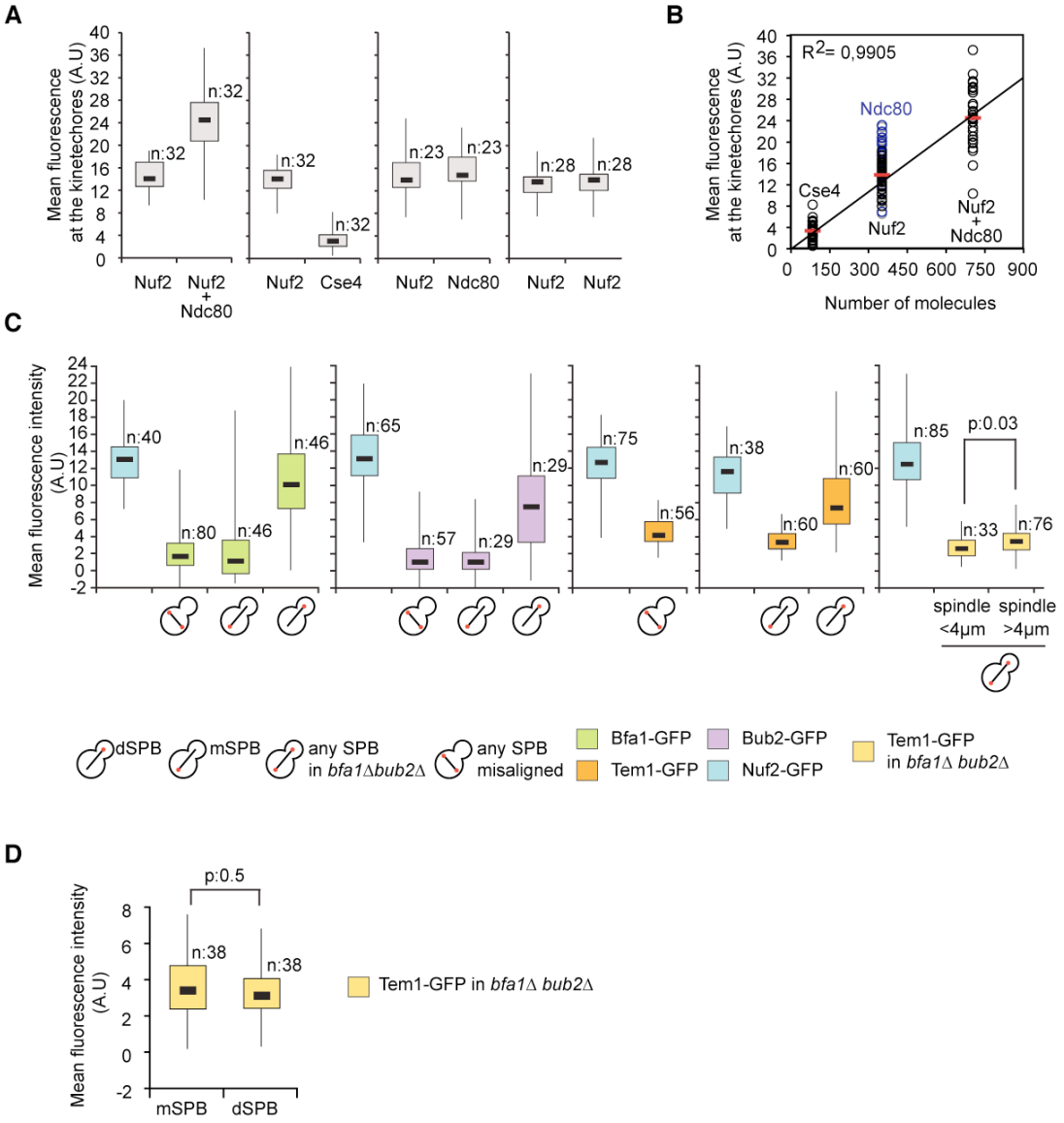
4 Supplementary Tables

4.1 Supplementary Table 5. Table of Strains

	Genotype	Source/ Reference
Yeast strains		
AKY01	*MATa ura3-52 leu2Δ1 his3Δ200 trp1Δ63 BUB2-GFP-kanMX6 SPC42-eqFP-hphNT1 kar9Δ::klTRP1*	Caydasi & Pereira 2008
AKY02	*MATa ura3-52 leu2Δ1 his3Δ200 trp1Δ63 TEM1-GFP-kanMX6 SPC42-eqFP-hphNT1 kar9Δ::klTRP1*	Caydasi & Pereira 2008
AKY03	*MATa ura3-52 leu2Δ1 his3Δ200 trp1Δ63 BFA1-GFP-kanMX6 SPC42-eqFP-hphNT1 kar9Δ::klTRP1*	Caydasi & Pereira 2008
AKY39	*MATa ura3-52 leu2Δ1 his3Δ200 trp1Δ63 TEM1-GFP-kanMX6 SPC42-eqFP-hphNT1 bfa1Δ::klTRP bub2Δ::his3MX6*	Caydasi & Pereira 2008
GPY1054	*MATa ura3-52 leu2Δ1 his3Δ200 trp1Δ63 MOB1-GFP-kanMX6 SPC42-eqFP-hphNT1*	This Study
AKY278	*MATa ura3-52 leu2Δ1 his3Δ200 trp1Δ63 SPC42- eqFP -kanMX6 NUF2-GFP-klTRP1*	This Study
AKY279	*MATa ura3-52 leu2Δ1 his3Δ200 trp1Δ63 SPC42- eqFP -kanMX6 NDC80-GFP-klTRP1*	This Study
AKY285	*MATa ura3-52 leu2Δ1 his3Δ200 trp1Δ63 NUF2-GFP-kanMX6*	This Study
AKY289	*MATa ura3-52 leu2Δ1 his3Δ200 trp1Δ63 NUF2-GFP-kanMX6 NDC80-GFP-klTRP1*	This Study
AKY395	*MATa ura3-52 lys2-801 trp1Δ63 his3Δ200 leu2Δ1 kar9Δ::klTRP1 bfa1Δ::his ade2-101::GFP-TUB1-ADE2*	This Study
AKY773	*MATa ura3-52 leu2Δ1 his3Δ200 trp1Δ63 TEM1-GFP-kanMX6 BFA1-3mCherry-hphNT1*	This Study
AKY836	*MATa ura3-52 leu2Δ1 his3Δ200 trp1Δ63 MOB1-GFP-kanMX6 SPC42-eqFP-hphNT1 bfa1Δ::his3MX6 bub2Δ::URA3*	This Study
AKY871	*MATa ura3-52 leu2Δ1 his3Δ200 trp1Δ63 TEM1-GFP-kanMX6 SPC42-eqFP-hphNT1* Gal1-*clb2ΔDB-URA3 p425*-Gal1	This Study
AKY872	*MATa ura3-52 leu2Δ1 his3Δ200 trp1Δ63 TEM1-GFP-kanMX6 SPC42-eqFP-hphNT1* Gal1-*clb2ΔDB-URA3 p425*-Gal1-*BFA1*	This Study
AKY1126	*MATa ura3-52 leu2Δ1 his3Δ200 trp1Δ63 BUB2-GFP-kanMX6 BFA1-3mCherry-hphNT1*	This Study
CJY492	*MATa ura3-52 leu2Δ1 his3Δ200 trp1Δ63 CSE4-GFP-kanMX6*	Gift from Elmar Schiebel
ESM356-1	*MATa ura3-52 leu2Δ1 his3Δ200 trp1Δ63*	Pereira et al., 2001

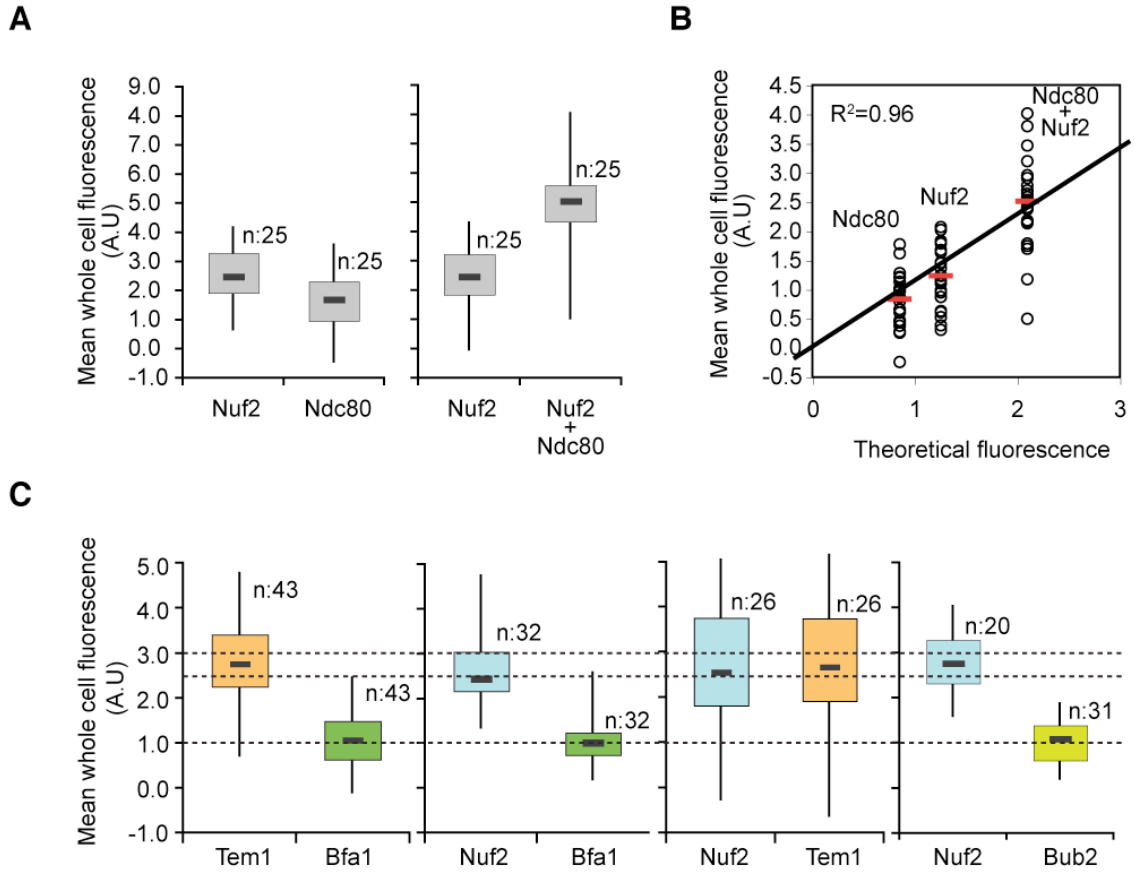
5 Figures

5.1 Supplementary Figure 1



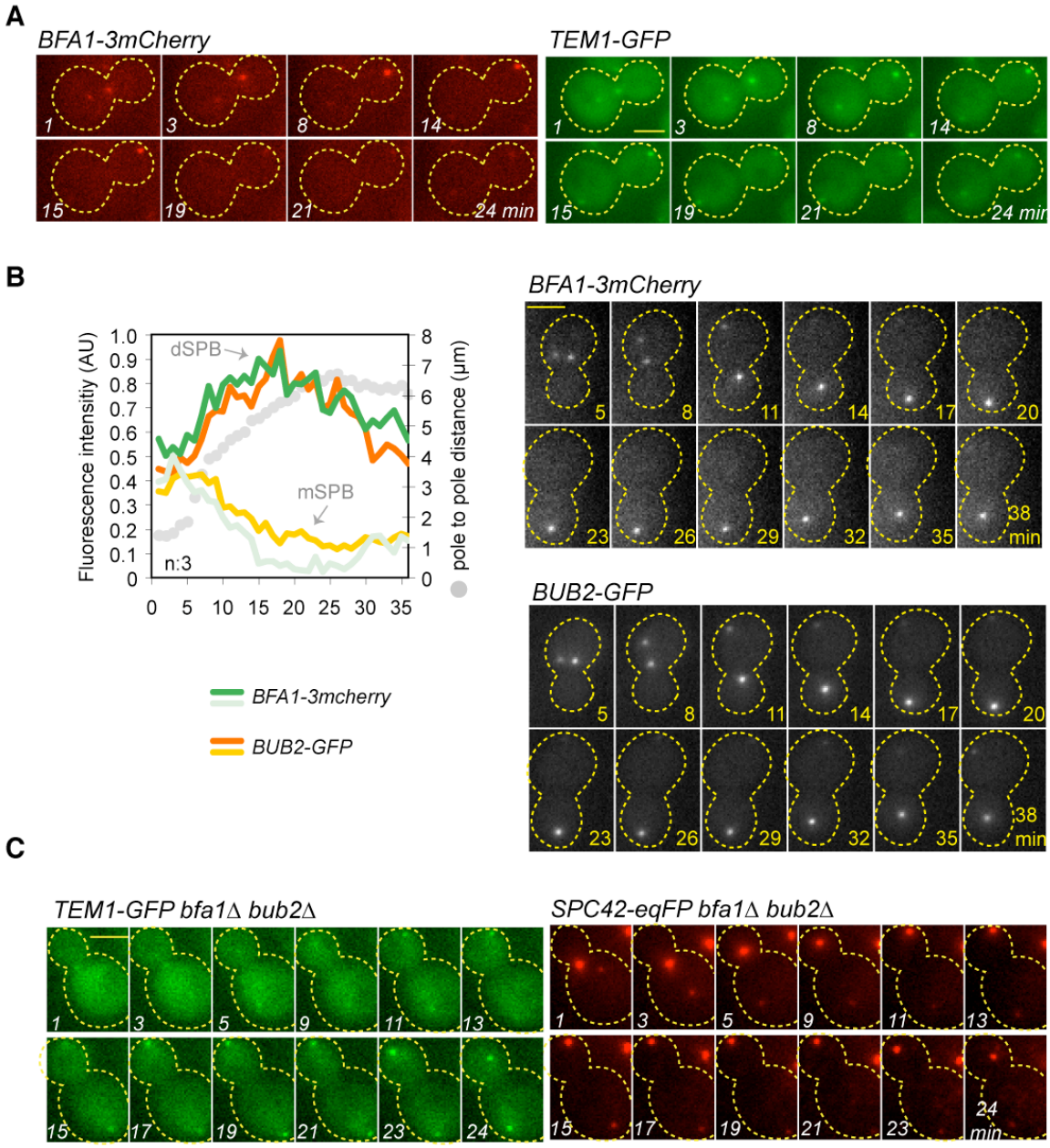
Supplementary Figure 1. SPB bound fluorescence intensities of Bfa1-GFP Bub2-GFP and Tem1-GFP in comparison to Nuf2-GFP. **(A-B)** Method verification. **(A)** Box and whisker plots representing the kinetechore signals of the cells expressing *CSE4-GFP*, *NUF2-GFP*, *NDC80-GFP* and *NDC80-GFP NUF2-GFP* in comparison to cells expressing *NUF2-GFP*. **(B)** Dot plot of the individual signals from A versus the known number of molecules of corresponding proteins at the kinetochore clusters. Red short lines mark the median value of the samples from which the depicted best fit line obtained. **(C)** Box and whisker plots of SPB bound mean fluorescence intensities of Bfa1-GFP, Bub2-GFP, Tem1-GFP in *kar9*Δ cells and Tem1-GFP in *bfa1*Δ *bub2*Δ cells in comparison to Nuf2-GFP signal at the kinetechore clusters. **(D)** Box and whisker plots comparing the amount of Tem1-GFP at the mSPB and the dSPB of *bfa1*Δ *bub2*Δ cells in late anaphase. Boxes are the first and the third quartiles; the line inside the box is the median while the whiskers are the minimum and the maximum values. "n" shows the sample size. p-values are obtained by two tailed students t-test.

5.2 Supplementary Figure 2



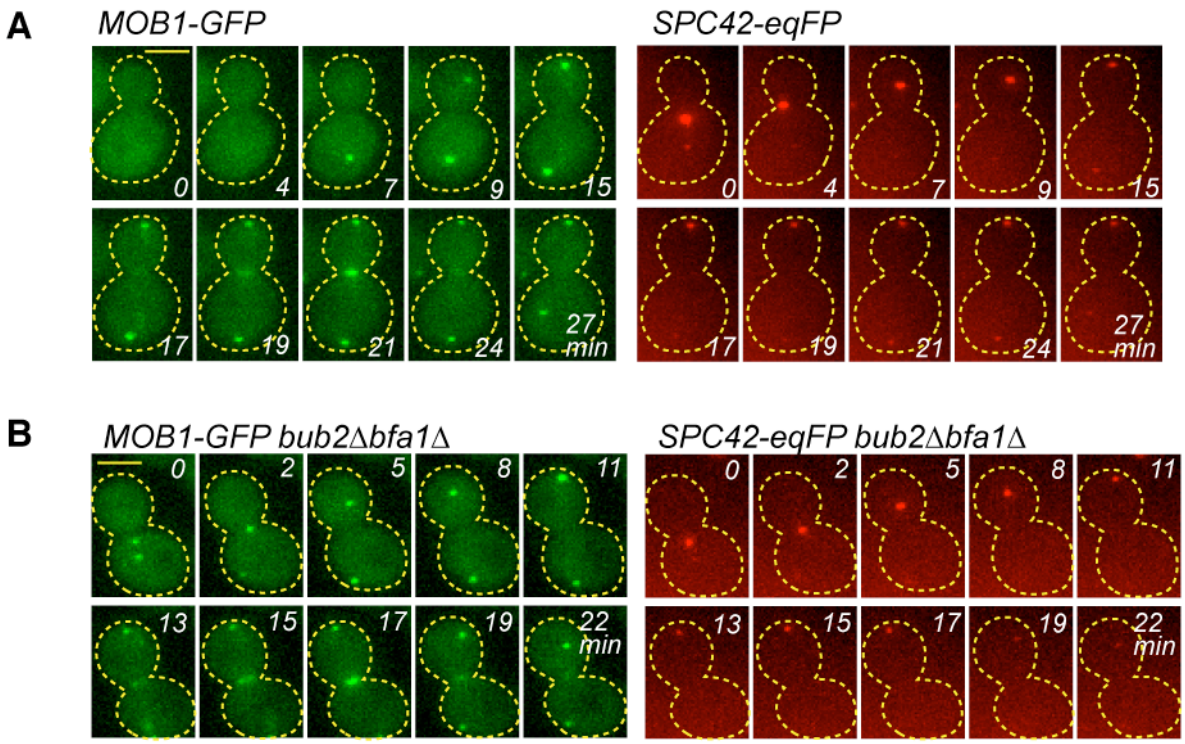
Supplementary Figure 2. Comparison of total cellular levels of Bfa1, Bub2 and Tem1. **(A-B)** Method verification. **(A)** Mean whole-cell fluorescence intensities of cells expressing *NDC80-GFP* and cells that simultaneously express *NDC80-GFP* and *NUF2-GFP* in comparison to *NUF2-GFP* cells. **(B)** Dot plots of the measurements from A. Theoretical values are calculated considering that Ndc80+Nuf2 whole cell fluorescence is equal to the sum of median values of Ndc80-GFP and Nuf2-GFP mean whole cell fluorescence measurements. Best-fit line of the short red lines (median values) is indicated. **(C)** Box and whisker plots of Tem1-GFP, Bfa1-GFP, Bub2-GFP and Nuf2-GFP obtained from the whole cell mean fluorescence measurements. Boxes indicate the first and the third quartiles; the line inside the box marks the median while the whiskers represent the minimum and the maximum values. "n" shows sample size.

5.3 Supplementary Figure 3



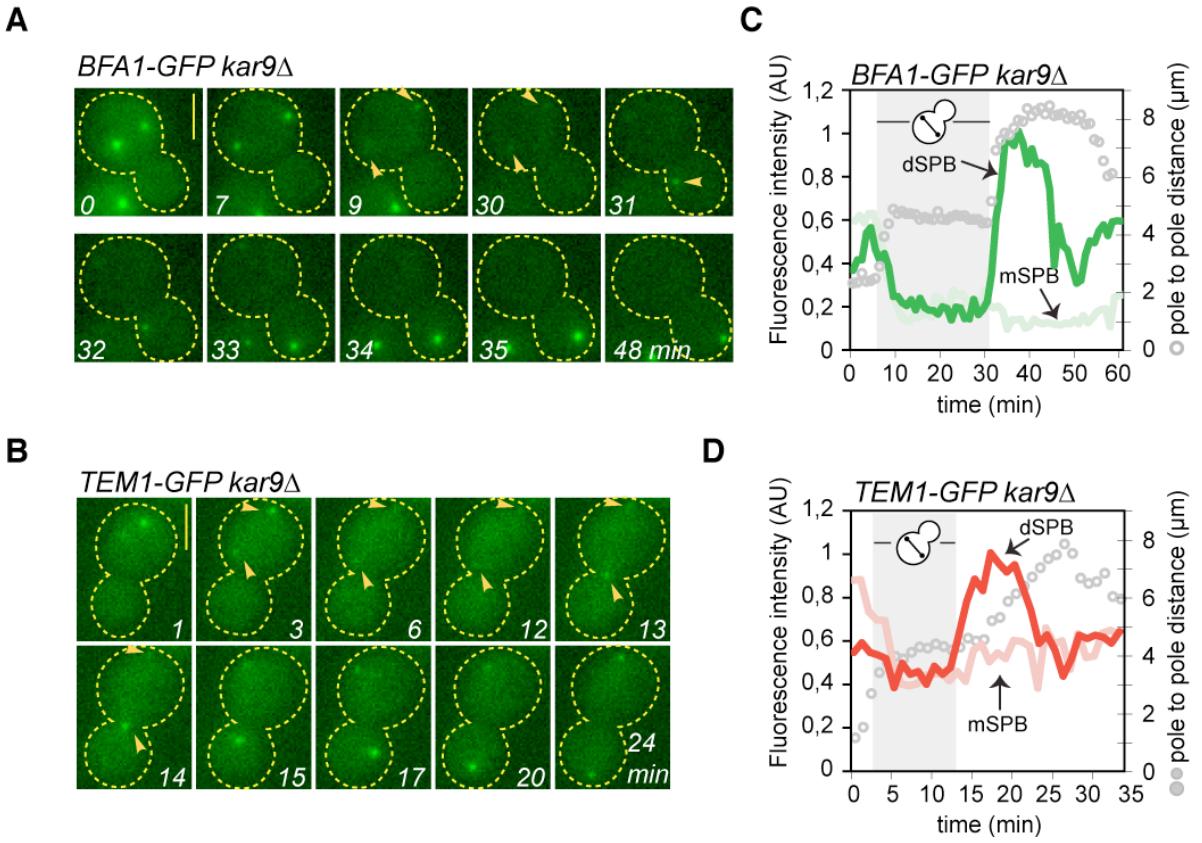
Supplementary Figure 3. Tem1-GFP and Bub2-GFP localization with respect to Bfa1-3mCherry during mitosis. **(A)** Representative still images of selected time points from the time-lapse series of *BFA1-3mCherry TEM1-GFP*. **(B)** Mean fluorescence intensities of Bfa1-3mCherry and Bub2-GFP at the SPBs during mitosis and the representative still images of the selected time points. Mean fluorescence intensity line graph is the average of n:3 cells monitored with 1 min time resolution. In each cell, maximum signal of each protein is normalized to 1. Pole-to-pole distances are plotted as a reference for cell cycle progression. **(C)** Representative still images of selected time points from the time-lapse series of *TEM1-GFP SPC42-eqFP bfa1Δ bub2Δ* cells Scale bars: 3 µm.

5.4 Supplementary Figure 4



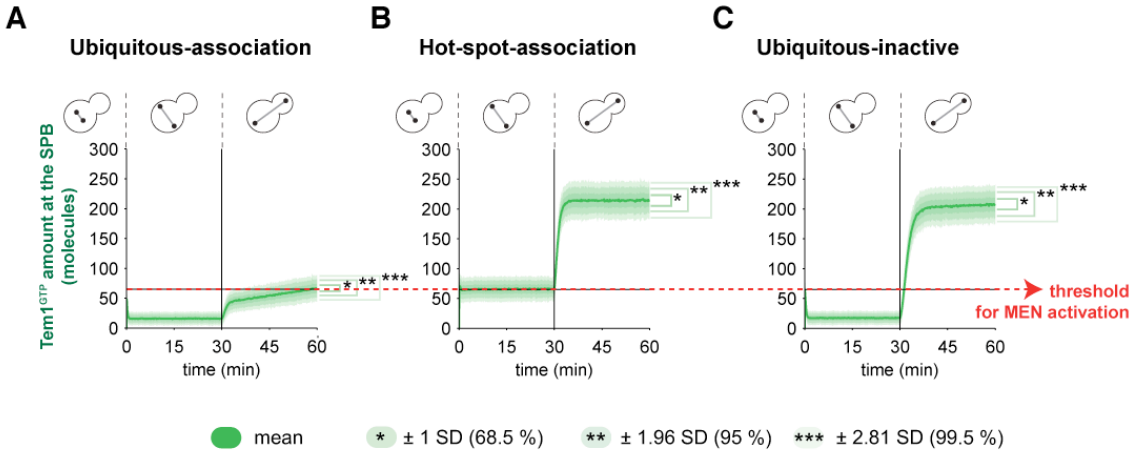
Supplementary Figure 4. Mob1-GFP localization during mitosis. Still images from the time-lapse series of *MOB1-GFP* in wild type **(A)** and *bfa1*Δ *bub2*Δ **(B)** cells. Spc42-eqFP is included as an SPB marker. Scale bars: 3 µm.

5.5 Supplementary Figure 5



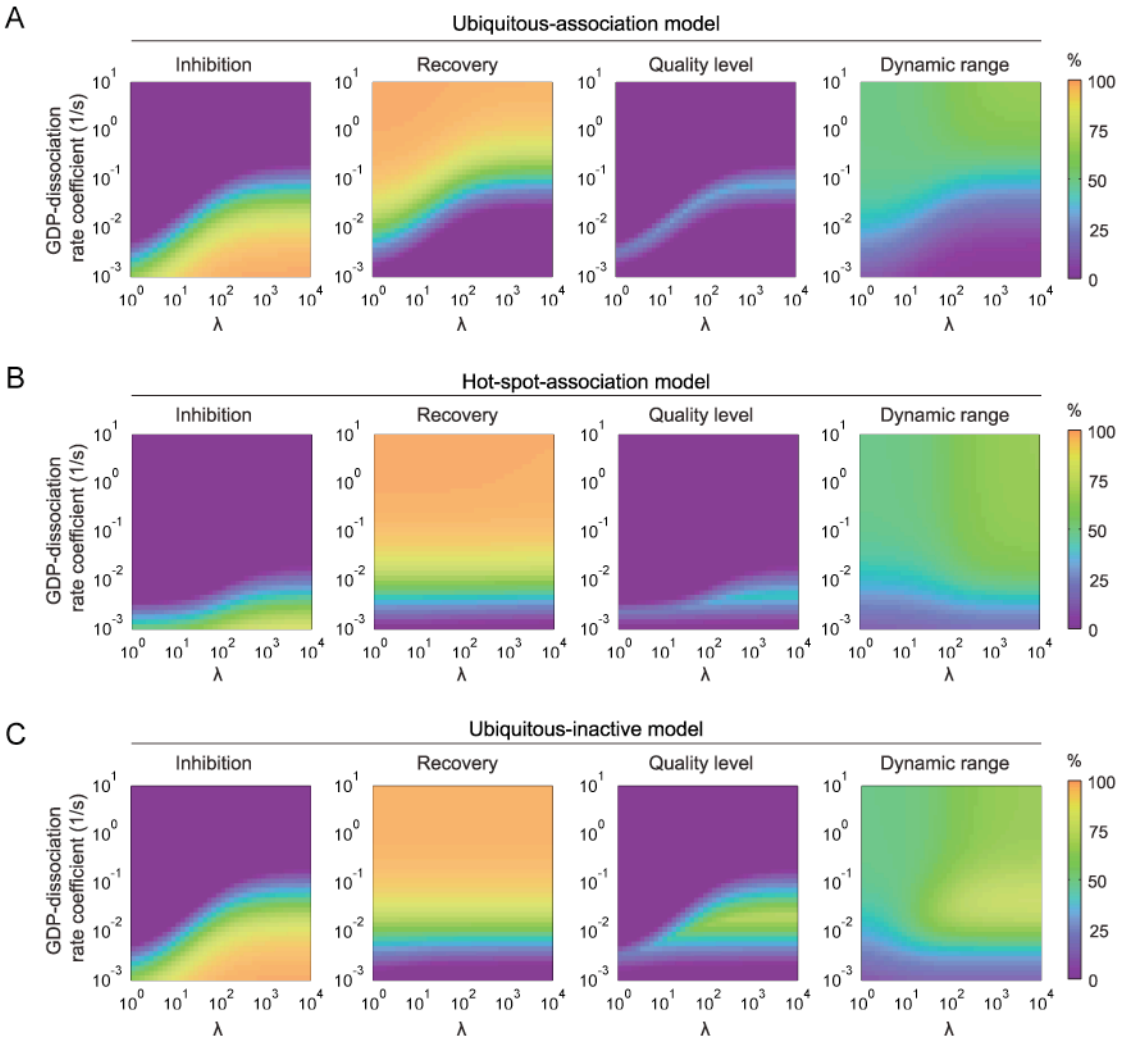
Supplementary Figure 5. Bfa1-GFP and Tem1-GFP localization during spindle misalignment. Still images of the selected time points from the time-lapse series of Bfa1-GFP **(A)** and Tem1-GFP **(B). (C-D)** Quantification of the mean fluorescence intensities at the SPBs of the cells in (A) and (B) respectively. Scale bars: 3 µm.

5.6 Supplementary Figure 6



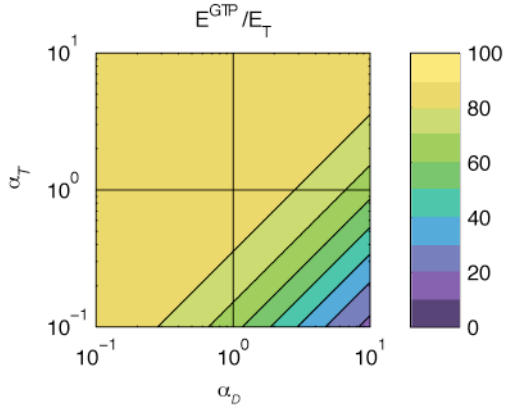
Supplementary Figure 6. Tem1GTP at the SPB from stochastic simulations of the ubiquitous-association **(A)**, hot-spot-association **(B),** and ubiquitous-inactive **(C)** models with the same parameters as in Figure 5A. Mean and 1 (*), 1.96 (**), and 2.81 (***) standard deviations (corresponding to 68.5%, 95%, and 99.5% confidence intervals) of ensembles of 200 individual stochastic simulations were indicated. Horizontal line indicates the number of active Tem1 molecules at the SPB sufficient for MEN activation.

5.7 Supplementary Figure 7



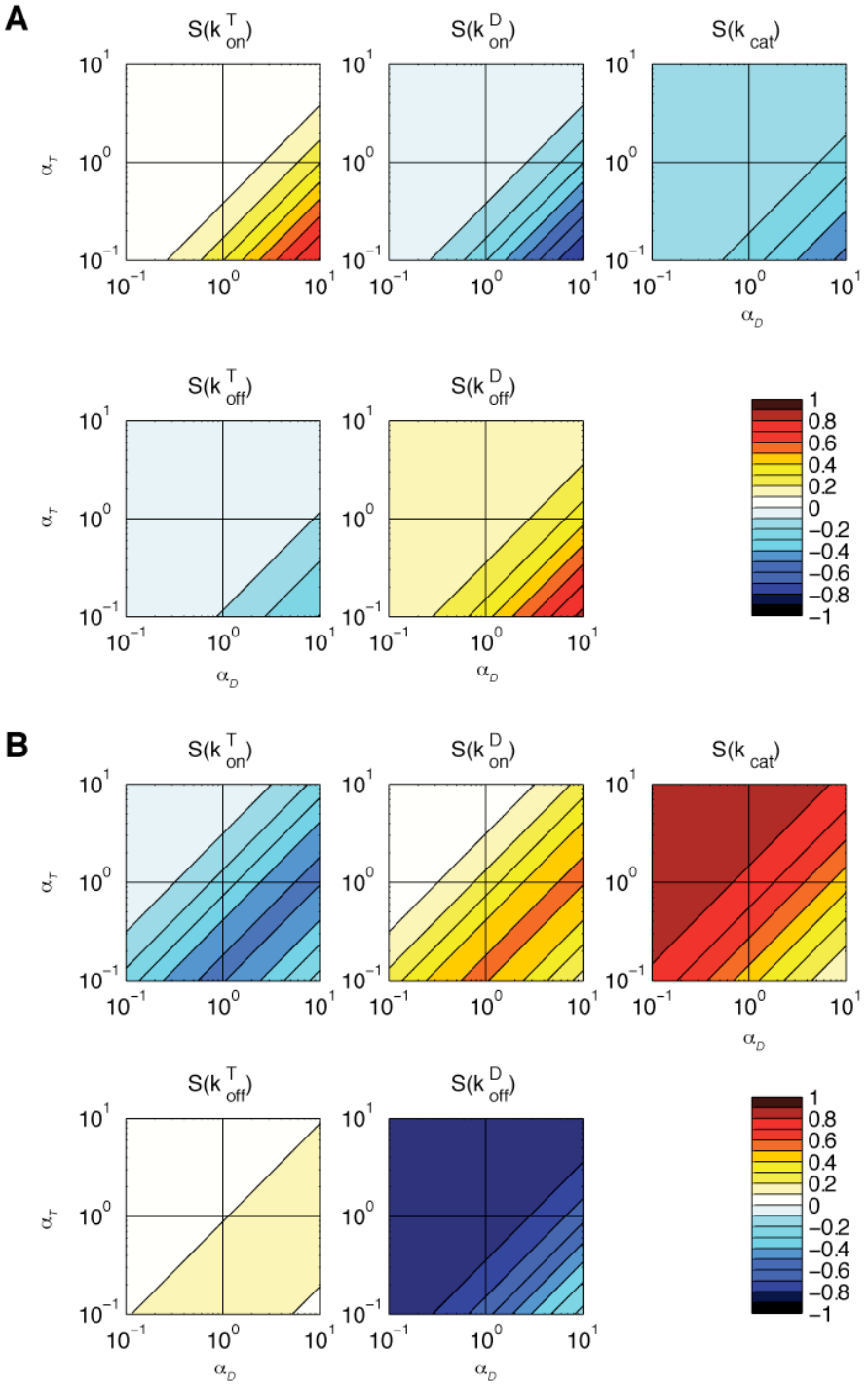
Supplementary Figure 7. Simultaneous parameter scans of GDP-dissociation rate coefficient and fold-acceleration of hydrolysis by the GAP complex ë . Columns from left to right show relative inhibition of Tem1 when SPOC is active (t = 30 min), relative recovery of active Tem1 10 minutes after SPOC deactivation (t = 40 min), and the corresponding quality level and dynamic range in ubiquitous-association model **(A),** hot-spot-association model **(B)** and ubiquitous-inactive model **(C)**.

5.8 Supplementary Figure 8



Supplementary Figure 8 Relative steady state level of Tem1-GTP as a function of fold-change of the GDP- and GTP association rate coefficients $k_{\mathrm{on}}^{\mathrm{D}}(\alpha_D)$ and $k_{\mathrm{on}}^{\mathrm{T}}(\alpha_T)$. Both parameters were varied in a range from 0.1 to 10 times of their respective nominal value (vertical and horizontal line, respectively). For the nominal values of the parameters, the steady state level of Tem1-GTP is near-maximal. Within the scanned parameter range, the steady state level depends only on the ratio of $k_{\mathrm{on}}^{\mathrm{D}}$ and $k_{\mathrm{on}}^{\mathrm{T}}$ rather than their absolute values. Higher steady state levels of Tem1-GTP can hardly be reached for any combination of the parameters. However, Tem1-GDP becomes the dominant form if the GDP-association rate $k_{\mathrm{on}}^{\mathrm{D}}$ is increased while the GTP-association rate $k_{\mathrm{on}}^{\mathrm{T}}$ is decreased significantly.

5.9 Supplementary Figure 9



Supplementary Figure 9. Sensitivities of the steady state concentrations of nucleotide-bound Tem1 as a function of fold-change of the GDP- and GTP association rate coefficients $k_{\mathrm{on}}^{\mathrm{D}}(\alpha_D)$ and $k_{\mathrm{on}}^{\mathrm{T}}(\alpha_D)$. Both parameters were varied in a range from 0.1 to 10 times their respective nominal value (vertical and horizontal line, respectively). Similar to the Tem1-GTP steady state level, sensitivities depend on the ratio of $k_{\mathrm{on}}^{\mathrm{D}}$ and $k_{\mathrm{on}}^{\mathrm{T}}$ instead of their absolute values within the scanned parameter range. **(A)** Sensitivities of the steady state concentration of Tem1-GTP. **(B)** Sensitivities of the steady state concentration of Tem1-GDP.

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