

1 Computational modelling and 2 near-complete kinetochore tracking 3 reveal how chromosome dynamics 4 during cell division are co-ordinated 5 in space and time

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11

12 **Abstract** Mitotic chromosome segregation is a self-organising process that achieves high
13 fidelity separation of 46 duplicated chromosomes into two daughter cells. Chromosomes must
14 be captured by the microtubule-based spindle, aligned at the spindle equator where they
15 undergo oscillatory motion (metaphase) and then pulled to opposite spindle poles (anaphase).
16 These large and small-scale chromosome movements are driven by kinetochores, multi-protein
17 machines, that link chromosomes to microtubules and generate directional forces. Through
18 automated near-complete tracking of kinetochores at fine spatio-temporal resolution over long
19 timescales, we produce a detailed atlas of kinetochore dynamics throughout metaphase and
20 anaphase in human cells. We develop a hierarchical biophysical model of kinetochore dynamics
21 and fit this model to 4D lattice light sheet experimental data using Bayesian inference. We
22 demonstrate that location in the metaphase plate is the largest factor in the variation in
23 kinetochore dynamics, exceeding the variation between cells, whilst within the spindle there is
24 local spatio-temporal coordination between neighbouring kinetochores of directional switching
25 events, kinetochore-fibre (K-fibre) polymerization/depolymerization state and the segregation of
26 chromosomes. Thus, metaphase oscillations are robust to variation in the mechanical forces
27 throughout the spindle, whilst the spindle environment couples kinetochore dynamics across the
28 plate. Our methods provide a framework for detailed quantification of chromosome dynamics
29 during mitosis in human cells.

30

31 Introduction

32 The metaphase-anaphase transition is a critical, irreversible step during mitotic cell division, whereby
33 the 46 pairs of duplicated chromosomes (called sister chromatids) are segregated into two daugh-
34 ter cells. During metaphase, chromosomes undergo quasi-periodic saw-toothed oscillations across
35 the spindle equatorial plane (*Skibbens et al., 1993; Wan et al., 2012; Burroughs et al., 2015*). Once
36 the mitotic checkpoint is satisfied, the sister chromatids are pulled towards opposite poles during
37 anaphase (*Musacchio, 2011*). It is crucial that this segregation occurs with high fidelity since errors

38 can cause aneuploidy which is a hallmark of cancer and various developmental disorders (*Gregan*
39 *et al., 2011*).

40 The forces necessary for these exquisite chromosome movements are largely driven by inter-
41 actions between mitotic spindle microtubules and kinetochores - multi-protein machines that as-
42 semble on each sister chromatid (*Rago and Cheeseman, 2013*). Sister kinetochores are capable
43 of maintaining attachment to both growing and shrinking microtubule bundles (K-fibres) that gen-
44 erate pushing and pulling forces on the chromosomes respectively (*Armond et al., 2015*). More-
45 over, sister kinetochores are physically connected by centromeric chromatin which behaves as an
46 elastic spring (*Stephens et al., 2013; Harasymiw et al., 2019*) and enables mechanical cues to be
47 transmitted between kinetochores (*Burroughs et al., 2015; Wan et al., 2012*). Such cues control
48 directional switching, giving rise to the metaphase oscillatory dynamic (*Burroughs et al., 2015*). It
49 is this cohesive linkage between sisters that is severed at the onset of anaphase to allow segre-
50 gation of sisters (*Hauf et al., 2001*), although global phosphorylation states are also important in
51 driving anaphase dynamics (*Su et al., 2016; Vázquez-Novelle et al., 2014*). Sister kinetochores, do
52 not however, operate in isolation in driving chromosome movements; dynamic non-kinetochore
53 spindle microtubules exert forces on chromosome arms and together with arm-tethered molecu-
54 lar motors give rise to a polar ejection force (PEF, (*Ke et al., 2009*)), and microtubule bridging fibers
55 connecting sister K-fibers can slide to push the K-fibre poleward and generate tension between
56 sisters (*Polak et al., 2017; Kajtez et al., 2016*). Within the mitotic spindle, the cross-linked micro-
57 tubule network produces the highly viscoelastic environment in which chromosomes movements
58 need to be understood (*Shimamoto et al., 2011*).

59 Chromosomes in human mitotic cells are typically treated as 46 identical objects whilst conclu-
60 sions are often based on the analysis of (visible/trackable) subsets of sister kinetochores/chromosomes.
61 However, recent reports suggest that there are chromosome specific differences. For instance,
62 chromosomes 1 and 2 have a higher mis-segregation rate than would be expected if all chromo-
63 somes behaved identically (*Worrall et al., 2018*), while centromere differences between chromo-
64 somes (*Dumont et al., 2020*) and kinetochore size (*Drpic et al., 2018*) have both also been implicated
65 in biasing chromosome segregation errors. A further challenge is that mitotic events occur over
66 multiple time scales, with fast kinetochore-directional switching (on the timescale of seconds) (*Bur-*
67 *roughs et al., 2015*), and a slow self organisation dynamic with chromosome congression taking
68 approximately 10-20 mins (*Paul et al., 2009; Auckland et al., 2017*).

69 Understanding this complex multi-scale mechanical system requires development of quantita-
70 tive mathematical models that can capture crucial elements of the system's biophysics and regu-
71 latory properties, provide quantitative support for conceptual ideas, and generate testable predic-
72 tions. Efforts in this direction have been ongoing since the 1980's with previous work focusing on
73 microscopic models of kinetochore-microtubule attachment (*Hill, 1985; Joglekar and Hunt, 2002;*
74 *Civelekoglu-Scholey and Cimini, 2014*), on the role of bridging fibres and spindle geometry (*Kajtez*
75 *et al., 2016; Miles et al., 2021*), and on chromosome congression dynamics to the spindle equa-
76 tor (*Mogilner et al., 2006; Zaytsev and Grishchuk, 2015; Blackwell et al., 2017*). Careful calibration
77 of models to experimental data is crucial to ensure model validity. However, few studies have per-
78 formed inference of model parameters directly from experimental data. In previous work (*Armond*
79 *et al., 2015*), we proposed a biophysical model of metaphase oscillations, and fitted the model to
80 3D kinetochore tracking data from HeLa cells using Bayesian inference, a methodology that prop-
81 agates uncertainty so parameter confidence can also be determined. The fitted model provided
82 insight into the forces acting on kinetochores across directional switching events, and how sister
83 kinetochores coordinated directional switching (*Armond et al., 2015; Burroughs et al., 2015*).

84 However, a high degree of heterogeneity in oscillatory dynamics has also been revealed, *i.e.*
85 there was substantial variation of the biophysical properties. In particular, the position of the chro-
86 mosome within the 3D spindle influences mechanical forces with both the polar ejection forces
87 (*Armond et al., 2015; Civelekoglu-Scholey et al., 2013*) and kinetochore swivel (*Smith et al., 2016*)
88 increasing towards the periphery of the metaphase plate. Non-sister kinetochores can also influ-

89 ence each others' behaviour and exhibit motion correlated to that of their neighbours (*Vladimirov*
90 *et al., 2013*), hypothesised to be due to cross-linking connections between K-fibres (*Vladimirov*
91 *et al., 2013; Elting et al., 2017*). To further investigate spatial interactions within the cell and spatio-
92 temporal dynamics through to anaphase, a biophysical model is required that includes the transi-
93 tion from metaphase to anaphase.

94 In this work we present a metaphase-anaphase model of kinetochore dynamics for human reti-
95 nal pigment epithelial cells (RPE1); a karyotypically stable, non-transformed cell line. The model
96 incorporates metaphase oscillations, captures the transition to anaphase and the segregation of
97 chromosomes to respective poles. Chromosome movements are driven through force balance
98 between the 4 primary forces acting on a chromosome in mitosis: the K-fibre forces, the PEF, the
99 centromeric spring connecting sisters and drag. We use a stochastic differential equation formula-
100 tion for the mechanics and a discrete hidden Markov model to model the K-fibre dynamics of the
101 chromatid pair and the transition into anaphase. Using Bayesian inference, specifically a Markov
102 chain Monte Carlo (MCMC) algorithm, we parametrise our biophysical model, providing a power-
103 ful tool to interpret/annotate and analyse experimental trajectory data. By combining lattice light
104 sheet microscopy (LLSM (*Chen et al., 2014*)) and endogenous protein labelling, we achieve a high
105 signal-to-noise ratio and temporal resolution whilst ensuring minimal photobleaching and photo-
106 toxicity. We demonstrate near-complete 3D tracking of the 46 kinetochore pairs for up to 15 mins.
107 This allows fitting of the metaphase-anaphase model to kinetochore trajectories and character-
108 isation of the biophysical parameters describing chromosome dynamics over the population of
109 chromosomes in human cells.

110 Our analysis provides a comprehensive atlas of kinetochore dynamics in normal human cells
111 throughout metaphase and anaphase, revealing how spatial positioning defines the mechanical
112 behavior of sister kinetochores such that kinetochore behavior is less variable between cells than
113 previously thought. We show local spatial coordination of directional switches and the timing of
114 anaphase onset. We further demonstrate how dynamics mature as anaphase approaches, caused
115 by a stiffening of the kinetochore-microtubule attachment. Together, these results reveal how
116 interactions between kinetochore pairs can account for the coordination of directional switching
117 events and anaphase onset itself.

118 Results

119 Near-complete kinetochore tracking through the metaphase-anaphase transition

120 To obtain insight into chromosome dynamics at the anaphase-metaphase transition, we developed
121 a tracking algorithm that achieves near-complete tracking of all 46 fluorescently labelled kineto-
122 chores, using an endogenous label of a kinetochore protein (*Roscioli et al., 2020*). The tracking
123 pipeline is outlined in Supp. Fig. 1 and consists of: deconvolving the 4D movies; detecting candi-
124 date spots via an adaptive threshold technique; refining spot locations using a Gaussian mixture
125 model to provide subpixel resolution; fitting a plane to give the metaphase plate as a reference co-
126 ordinate system; linking detected particles between frames over time to form tracks; and grouping
127 kinetochore sister pairs based on metaphase dynamics. This provides subpixel resolution for the
128 positions of each kinetochore, and allows us to study dynamics of sister kinetochore pairs, rather
129 than simply individual kinetochores.

130 We performed live-cell imaging of untransformed human RPE1 cells using LLSM (Fig. 1) and gen-
131 erated tracks with our tracking pipeline (details in Methods). Data were collected at a high tempo-
132 ral resolution of 2.05s per z -stack over long timescales, typically tens of minutes, from metaphase
133 through to anaphase. A higher time resolution was required than in previous work (*Sen et al., 2021*)
134 (which used 4.7s per z -stack) to properly assess fast directional switching dynamics. We obtain par-
135 tial tracks (in the coordinate system indicated in Fig. 1B) for all 46 sister pairs of kinetochores for
136 the cell shown in Fig. 1A, with 22 of these extending throughout the duration of the movie from
137 metaphase through to anaphase. Across a population of $N = 58$ cells, we obtain an average of

138 72 [quartiles Q1-Q3:65-77] kinetochores (Fig. 1C) and 29 [24-34] sister kinetochore pairs (Fig. 1D)
 139 tracked through at least 50% of the movie.

140 As expected, kinetochores form a metaphase plate (Fig. 1E) and undergo saw-toothed oscillations
 141 perpendicular to the metaphase plate (Fig. 1F) before separating in anaphase when kineto-
 142 chores segregate towards their respective spindle poles (Fig. 1F). Visualising trajectories over time
 143 highlights spatial differences between the kinetochore pairs distributed throughout the metaphase
 144 plate (Fig. 1G). In particular, kinetochores at the centre of the metaphase plate have greater am-
 145 plitude of oscillation compared to the edge of the plate (Fig 1G), as expected from previous work
 146 in HeLa cells (*Armond et al., 2015*) and Ptk1 cells (*Civelekoglu-Scholey et al., 2013*). Quantitative
 147 analysis of variation of the dynamics across the metaphase plate requires a computational model
 148 to annotate the trajectories and estimate parameters.

149 **Metaphase-anaphase model of kinetochore dynamics in human cells**

In order to address hypotheses about how chromosome segregation is co-ordinated in space and
 time, we extended the metaphase dynamics model of *Armond et al. (2015)* to incorporate the
 metaphase-anaphase transition. Let X_t^k denote the (1D) position of kinetochore sister k at time t in
 the x direction perpendicular to the metaphase plate. Metaphase dynamics are described by the
 stochastic differential equations

$$\begin{aligned} dX_t^1 &= (-v_{\sigma_t^1} - \kappa (X_t^1 - X_t^2 - L \cos \theta_t) - \alpha X_t^1)dt + sdW_t^1, \\ dX_t^2 &= (v_{\sigma_t^2} - \kappa (X_t^2 - X_t^1 + L \cos \theta_t) - \alpha X_t^2)dt + sdW_t^2, \end{aligned} \quad (1)$$

150 where θ_t is the angle between the normal to the metaphase plate and the vector connecting a
 151 sister pair at time t (thereby projecting the spring force perpendicular to the metaphase plate), α
 152 is the polar ejection force parameter, κ and L are the spring constant and natural length of the
 153 centromeric chromatin spring connecting the kinetochore sisters, W_t^j are Weiner processes for
 154 sister j , s the magnitude of the diffusive noise, v_+, v_- the velocities associated with polymerising (+)
 155 and depolymerising (-) microtubule states respectively, and the microtubule states σ_t^1 and σ_t^2 are
 156 hidden states taking values in the set

$$(\sigma^1, \sigma^2) \in \{(+, +), (+, -), (-, +), (-, -)\}, \quad (2)$$

with +/− states denoting polymerising and depolymerising K-fibers respectively. The term $\kappa(X_t^1 - X_t^2 - L \cos \theta_t)$ is a Hookean spring force term due to the centromeric chromatin spring connecting a kinetochore pair. The αX_t^1 term corresponds to the polar ejection force (PEF) pushing the chromosomes towards the equator. We assume that the PEF is linear in the spring extension near the metaphase plate (*Ke et al., 2009*). The $v_{\sigma_t^j}$ term (taking values v_+ or v_-) represents the polymerisation/depolymerisation force from the K-fiber attached to sister j , (dependent on the current microtubule state σ_t^j). The schematic in Fig. 2A illustrates the forces acting on a kinetochore sister pair, and how these forces depend on the configuration of hidden states (Fig. 2B) at any given time. The stochastic differential equations (1) follow from force balance, dividing through by the unknown drag coefficient as in *Armond et al. (2015)* (see Appendix 2 for further details); the effect of this is that all terms in eq. (1) have dimensions of speed, and units of the force parameters α and κ are [s^{-1}]. We integrate over a time frame, Δt , approximating the system of equations in (1) as a discretised system of equations as follows:

$$\begin{aligned} (X_{t+\Delta t}^1 - X_t^1)/\Delta t &= -v_{\sigma_t^1} - \kappa (X_t^1 - X_t^2 - L \cos \theta_t) - \alpha X_t^1 + N(0, \tilde{s}^2), \\ (X_{t+\Delta t}^2 - X_t^2)/\Delta t &= +v_{\sigma_t^2} - \kappa (X_t^2 - X_t^1 + L \cos \theta_t) - \alpha X_t^2 + N(0, \tilde{s}^2), \end{aligned}$$

157 where $\tilde{s}^2 = s^2/\Delta t$.

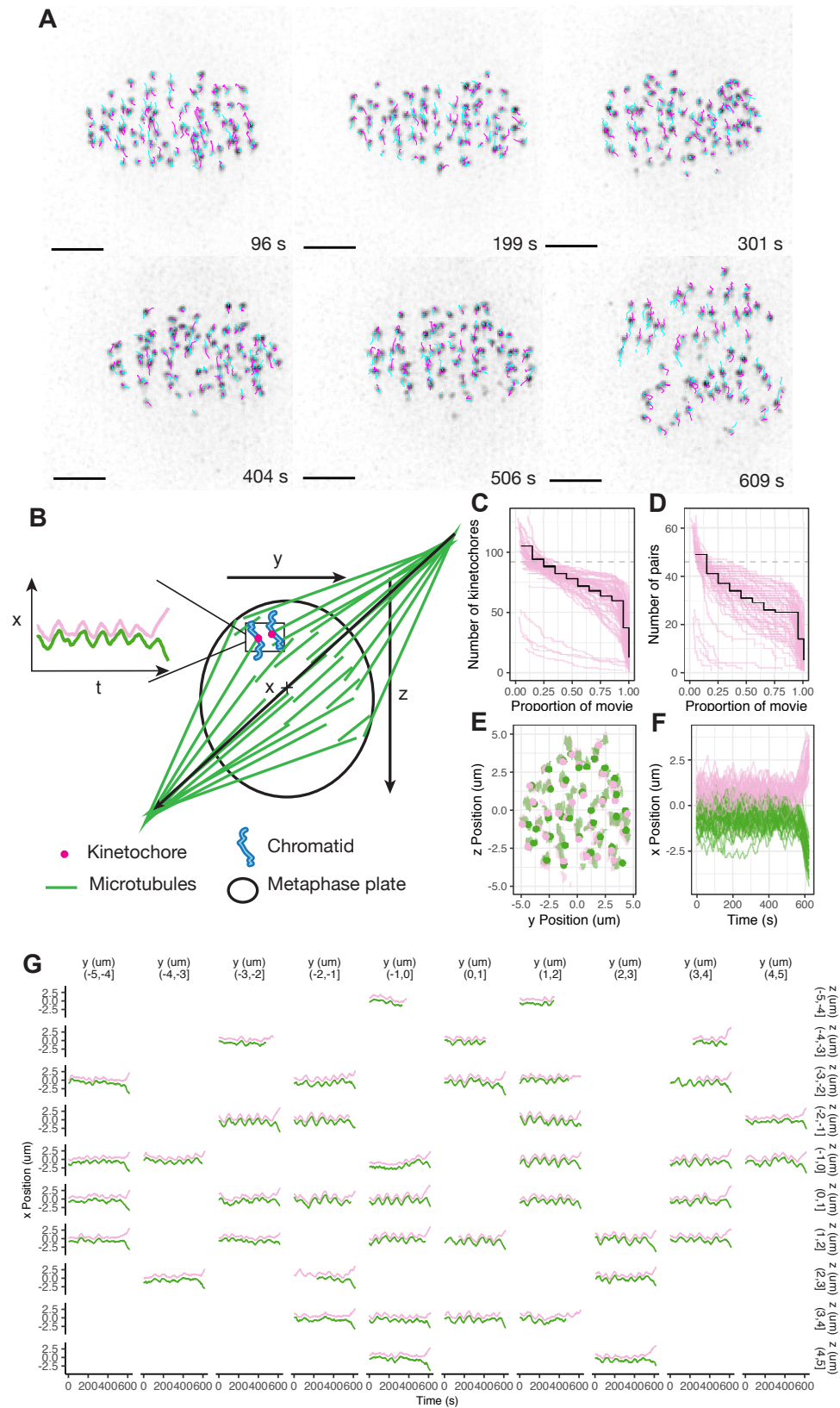


Figure 1. Near-complete tracking of kinetochores through metaphase and anaphase A in human RPE1 cells.

Figure 1. Near-complete tracking of kinetochores through metaphase and anaphase A in human RPE1 cells. (A) Sequence of z -projected images showing metaphase in the lead up to anaphase, and the metaphase-anaphase transition in a movie of 619s duration. Dragontails indicate tracked kinetochores in an example cell; cyan (magenta) lines show previous (next) 5 frames of trajectories. Scalebar shows 3 μ m. (B) Schematic showing the coordinate system and metaphase plate. The x axes is perpendicular to the metaphase plate, with y and z axes mutually perpendicular within the metaphase plate. (C) The number of kinetochores tracked through at least a given proportion of the duration of a movie. Magenta lines show individual cells and the black line shows the median over the population of $N = 58$ cells. Dashed grey line indicates 92 kinetochores. (D) The number of kinetochore pairs such that both kinetochores in a pair are tracked for at least a given proportion of the movie. Magenta lines show individual cells and the black line shows the median over the population of $N = 58$ cells. Dashed grey line indicates 46 kinetochore pairs. (E) Positions of kinetochores for the cell in (A) are shown within the metaphase plate during metaphase, with sister kinetochores coloured green and magenta and circles indicating the position at 492 s. Kinetochores have a set position within the metaphase plate with limited diffusion in the plate over time. (F) Tracks for all kinetochore pairs in the cell from (A) showing position relative to the metaphase plate over time perpendicular to the plate. (G) Trajectories of kinetochore pairs over time for the cell from (A) shown with subplots positioned corresponding to their average position within the metaphase plate.

To describe anaphase, we introduce an additional hidden state, A , with dynamics (Fig. 2C) in this state given by

$$\begin{aligned} dX_t^1 &= v_A dt + sdW_t^1, \\ dX_t^2 &= -v_A dt + sdW_t^2, \end{aligned}$$

where v_A is the velocity in anaphase. We have removed the terms for the spring forces and the polar ejection force (as these are lost at anaphase onset), and allowed the velocity in anaphase to be different to the velocities associated with polymerising or depolymerising microtubule states. The transition to anaphase is controlled by a smooth switch whereby there is a transition probability at time t given by,

$$p_A(t) = \frac{1}{1 + \exp(-(t - t_A)/\beta)}.$$

158 Thus, the transition to anaphase occurs around time t_A with $\beta = \Delta t/2$ determining the range over
 159 which switching can occur. We assume the anaphase state A is accessible from each of the other
 160 states, but transitions back from anaphase to metaphase are not possible, state A is absorbing.
 161 Thus, once anaphase onset occurs the chromosomes segregate.

Switches between hidden states (see Fig. 2B) occur at each time step according to the time dependent transition matrix (state order as (2), with anaphase state A at the end),

$$\mathbf{P}(t) = \begin{bmatrix} p_{\text{icoh}}p_{\text{icoh}}q_A(t) & p_{\text{icoh}}q_{\text{icoh}}q_A(t) & p_{\text{icoh}}q_{\text{icoh}}q_A(t) & q_{\text{icoh}}q_{\text{icoh}}q_A & p_A(t) \\ p_{\text{coh}}q_{\text{coh}}q_A(t) & p_{\text{coh}}p_{\text{coh}}q_A(t) & q_{\text{coh}}q_{\text{coh}}q_A(t) & p_{\text{coh}}q_{\text{coh}}q_A & p_A(t) \\ p_{\text{coh}}q_{\text{coh}}q_A(t) & q_{\text{coh}}q_{\text{coh}}q_A(t) & p_{\text{coh}}p_{\text{coh}}q_A(t) & p_{\text{coh}}q_{\text{coh}}q_A & p_A(t) \\ q_{\text{icoh}}q_{\text{icoh}}q_A(t) & p_{\text{icoh}}q_{\text{icoh}}q_A(t) & p_{\text{icoh}}q_{\text{icoh}}q_A(t) & p_{\text{icoh}}p_{\text{icoh}}q_A & p_A(t) \\ 0 & 0 & 0 & 0 & 1 \end{bmatrix}$$

162 with $q_{\text{coh}} = 1 - p_{\text{coh}}$ and $q_{\text{icoh}} = 1 - p_{\text{icoh}}$ and $q_A(t) = 1 - p_A(t)$, where p_{coh} and p_{icoh} are the probabilities
 163 of a kinetochore remaining in the coherent (sisters move in the same direction), respectively inco-
 164 herent (sisters move in opposite direction) state over a time interval Δt , and p_A is the probability
 165 of transition to the anaphase state, A . Simulating from this biophysical model produces trajecto-
 166 ries with quasi-periodic oscillations qualitatively similar to observed data (Fig. 2 D). Saw-tooth like
 167 oscillations occur when the coherent mean lifetime is larger than the incoherent lifetime.

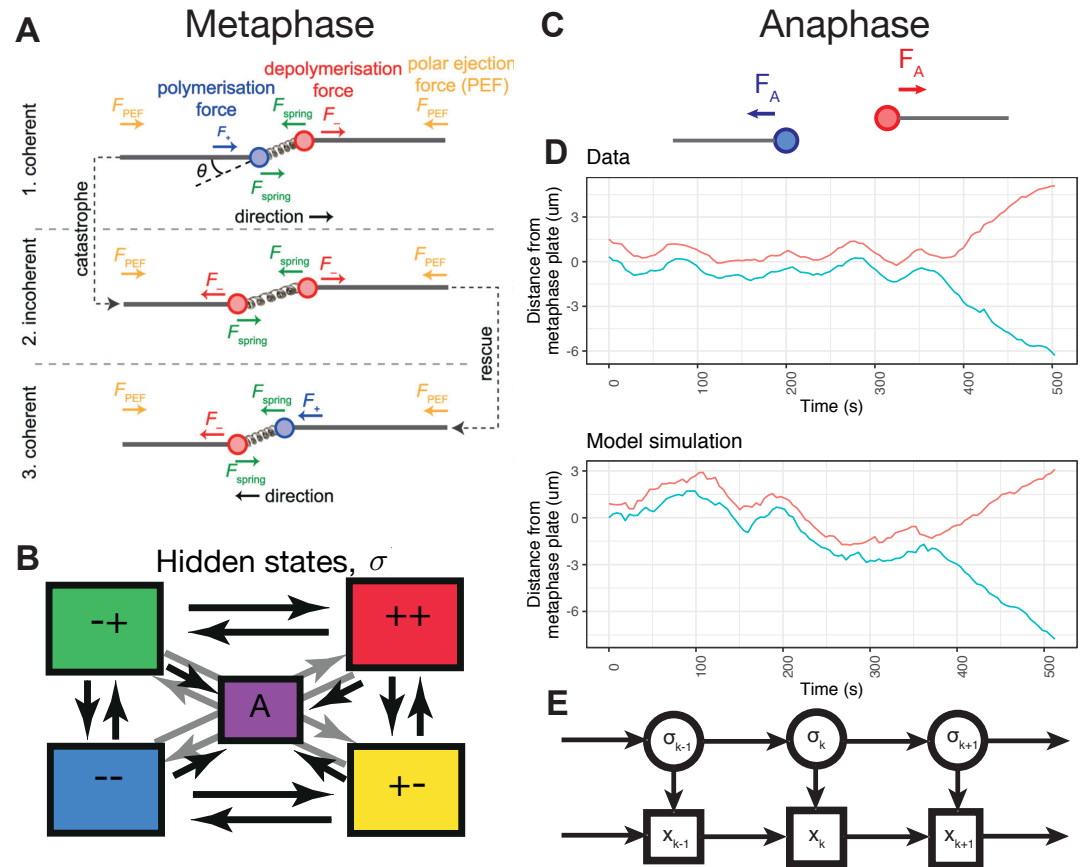


Figure 2. Biophysical model for metaphase-anaphase transition in mitosis in human cells. (A) Schematic of forces on a kinetochore pair in metaphase (inset comparison with zoomed in pair); adapted from *Armond et al. (2015)*. (B) Schematic of hidden states and transitions between hidden states. (c) Schematic of forces on a kinetochore pair in anaphase (inset comparison with zoomed in pair after anaphase onset). (D) Comparison of forward simulation from the model with experimental data for a single pair. (E) Graphical model structure as a state space model, similar to a Hidden Markov model. Here the K-fibre polymerisation states σ_i^k are unobserved hidden variables.

168 **Bayesian inference enables automated annotation of microtubule attachment hid-** 169 **den states and biophysical parameter estimation**

170 Automated annotation of kinetochore trajectories is a key tool in the analysis of metaphase dynam-
171 ics, the transition to anaphase, and inferring potential mechanisms. We take a Bayesian approach
172 to fit the model described in the previous section directly to experimental data. We use MCMC
173 techniques (*Gelman et al., 2014*) to obtain samples from the posterior distribution $P(\theta|x_{1:T})$ of the
174 model parameters $\theta = (\tau, \alpha, \kappa, v_-, v_+, L, p_{\text{coh}}, p_{\text{icoh}}, v_A, t_A)$ given the observed time series data where
175 $x_t = [X_t^1, X_t^2]$ is the observed position of the sisters at time t . Here $\tau = 1/\bar{s}^2$ is the precision. Further-
176 more, we can sample from the distribution of hidden microtubule states, $P(\sigma_t|x_{1:T})$ allowing us to
177 annotate trajectories.

178 The structure of the model considered here is that of a state space model, very similar to the
179 standard hidden Markov model (HMM) set up, except that we have additional dependencies on
180 the previous observation term, as shown in Fig. 2E. We derive a version of the forward-backward
181 algorithm (*Rabiner, 1989*) that accounts for these additional dependencies, thereby allowing sam-
182 pling from the distribution of the hidden states, see Methods. In this way, we are able to compute
183 $P(\sigma_k|x_{1:k})$ via the forward algorithm, and to sample backwards from $P(\sigma_k|x_{1:T})$ with the forward-
184 backward algorithm, for any $k = 1, \dots, T$. Using the formulation of the likelihood described in the
185 Methods, and automatic differentiation to provide derivatives, we sample from the posterior dis-
186 tribution using an Hamiltonian Monte Carlo algorithm (*Neal, 2011; Hoffman and Gelman, 2014*),
187 implemented in Stan (*Carpenter et al., 2017*). On synthetic data simulated from the anaphase
188 model of eq. (1), we are able to recover the true parameters used to simulate the data (Supp. Fig.
189 2), and infer the hidden microtubule attachment states and directional switches.

190 We demonstrate the automated annotation of hidden microtubule attachment states on a tra-
191 jectory of a kinetochore pair in Figures 3A,B,C. Estimates for the probability of occupying each
192 discrete hidden state at a given time point are shown in Fig. 3B, showing a clear transition to the
193 anaphase state, A , around 230s (black line, Fig. 3B). Based on the sampled hidden states, intermedi-
194 ate states during directional switches can be identified, *i.e.* directional switches (between coherent
195 states $+-$ to $-+$, or $-+$ to $+-$), can occur via the intermediate incoherent states, $--$ or $++$ (Fig. 3C).
196 Where intermediate states cannot be identified (eg. $+-$ followed by $-+$), we refer to the switch as
197 a joint switch. We infer all the biophysical model parameters jointly; the inferred marginal distribu-
198 tions for each of the biophysical model parameters are shown in Fig. 3D. Based on trace plots and
199 comparison between prior and posterior marginals, all model parameters satisfy practical identifi-
200 ability (*Hines et al., 2014; Browning et al., 2020*), except L for which an informative prior was used,
201 as (*Armond et al., 2015*), because of an identifiability issue, see Methods. The Bayesian framework
202 allows us to propagate forward uncertainty in the parameter estimates, and thus simulate from
203 the posterior predictive distribution, $p(x_t|x_{1:(t-1)}, \sigma_t)$, as shown in Fig. 3E. The true data points lie
204 within the predictive interval from the model, indicating that the model describes the observed
205 data.

206 **Incorporating anaphase directional reversals with a hierarchical model**

207 One aspect of chromosome dynamics not captured by the model of the previous Section are tran-
208 sient reversals of the usual poleward motion during anaphase (see Figures 3E and 4C). These re-
209 versals have been observed in previous studies (*Skibbens et al., 1993*), whilst metaphase-like chro-
210 mosome oscillations have been shown to persist into anaphase upon inhibition of protein dephos-
211 phorylation (*Su et al., 2016*). However, the cause of these reversals is not understood. Without
212 reversals in the model of chromosome dynamics, there is a mismatch between the model and
213 data due to misspecification of the model, resulting in MCMC convergence issues (Supp. Fig. 3) for
214 some trajectories where reversals occur.

To incorporate reversals into the mechanical model of the previous section, we add an addi-
tional discrete hidden state, R , only accessible from the anaphase state, A , as shown in Fig. 4A.
Since the forces acting on a kinetochore that cause, and act during, a reversal are not well under-

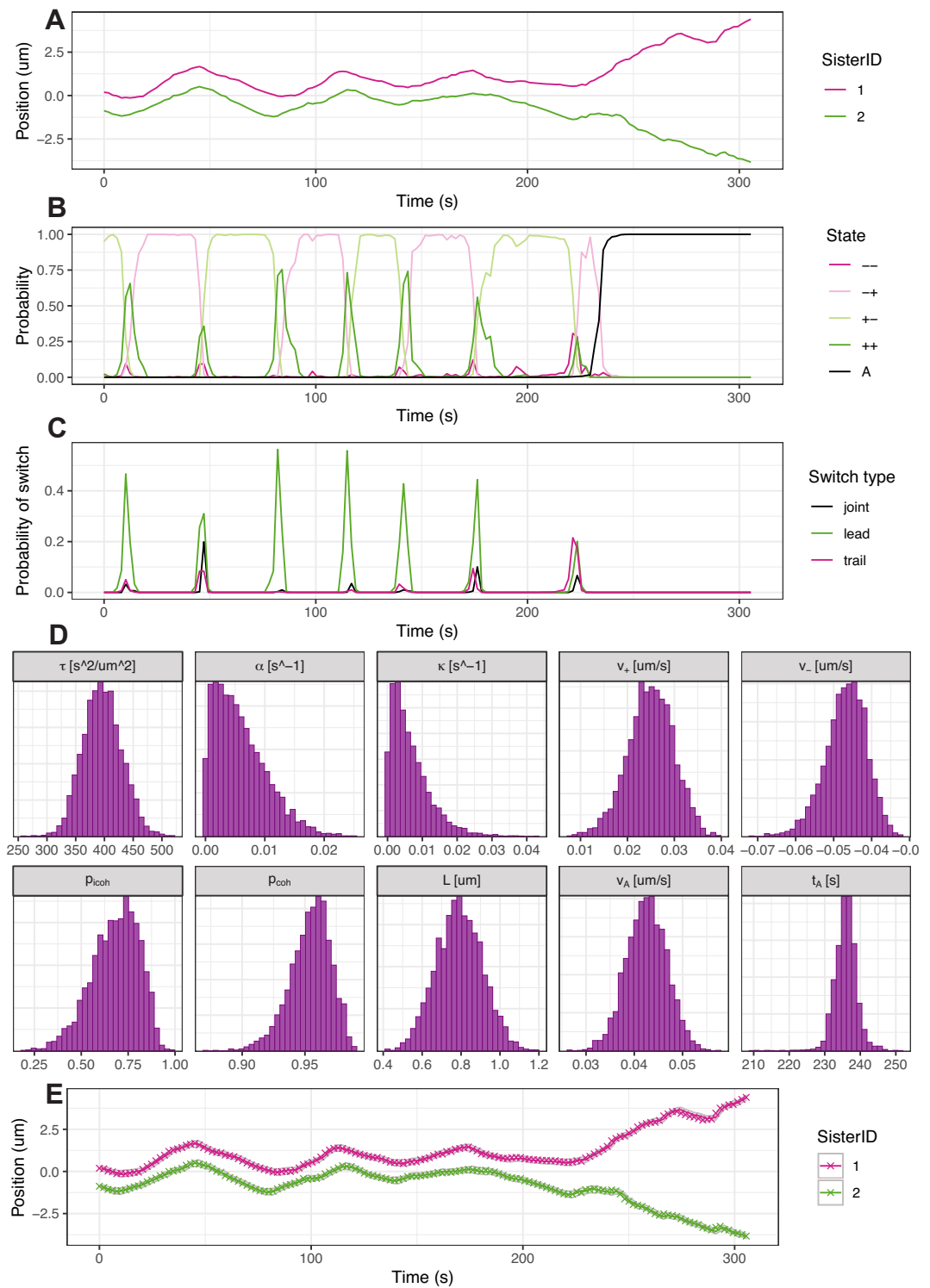


Figure 3. Bayesian inference enables automated annotation of microtubule attachment hidden states, and estimation of biophysical parameters (A) Observed track of kinetochore pair from experimental data. (B) Inferred probability of hidden states over time, $P(\sigma_t|x_{1:T};\theta)$ as sampled via the backward algorithm (see Methods). (C) Probability of a directional switch initiated by the leading kinetochore, trailing kinetochore, or a joint switch. Switching probability is assessed using the sampled hidden states and corresponds to a proportion of MCMC samples matching a particular pattern of states (eg. $[-+,-+,++,+-]$ or equivalent for a LIDS) corresponding to a given switch type. (D) Marginal posterior distribution of biophysical parameters for the trajectory data in (A). (E) Prediction from the filtering distribution $P(x_t|x_{1:(t-1)},\sigma_t)$. Coloured crosses indicate observed data.

stood, we model the reversal state dynamics as a pure diffusion:

$$\frac{X_{t+\Delta t}^1 - X_t^1}{\Delta t} = 2N(0, \tilde{s}^2),$$
$$\frac{X_{t+\Delta t}^1 - X_t^1}{\Delta t} = 2N(0, \tilde{s}^2),$$

215 using a higher diffusion coefficient (we found the factor of 2 worked on our data). This ensures
216 that a wide variety of reversal behaviours will be compatible with the model, and can be refined in
217 future as mechanisms are identified. Crucially, the additional reversal state avoids model misspec-
218 ification that would otherwise be present because of these unaccounted for reversal events, and
219 thus avoids convergence issues of the MCMC algorithm.

220 However, a key complication is that reversals are relatively rare events and do not occur in
221 every sister pair. In order to parametrise this model from experimental data, we need sufficient
222 reversal events to infer the rate of transition from state *A* to state *R*, and vice versa. If fitted to data
223 for a single pair with no reversals then there would be an identifiability problem. We therefore
224 developed a hierarchical model for joint inference of all the (tracked) kinetochore pairs in a cell,
225 with both shared parameters and individual kinetochore pair parameters. This enables estimation
226 of rare event transition rates (shared parameters), including between states *A* and *R*, based on
227 trajectories from all kinetochore sister pairs in the cell. A further advantage is that when pooling
228 all the directional switching events for all kinetochore pairs in a cell, we have a large sample of
229 switching events and thus obtain a tighter, more informative posterior distribution for the switch-
230 ing parameters compared to inference on individual sister pair trajectories.

231 The graphical structure of the hierarchical model with shared transition rates is shown in Fig.
232 4B. The transition probabilities between the hidden states are shared parameters relevant to all
233 kinetochore pairs in a cell, $\theta_{SP} = (p_{\text{icoh}}, p_{\text{coh}}, p_{AR}, p_{RA})$, where p_{AR} is the probability of remaining¹ in
234 the anaphase state, *A*, over a time step Δt (and similarly for p_{RA} the probability of remaining in the
235 reversal state). The remaining biophysical parameters $\theta_{BP} = (\tau, \kappa, \alpha, L, v_-, v_+, v_A, t_A)$ are assumed to
236 be unique to each kinetochore pair and are inferred independently for each pair. Several of these
237 biophysical parameters have been shown to vary based on position in the metaphase plate (*Ar-*
238 *mond et al., 2015*) which motivates keeping independent parameters for each sister kinetochore
239 pair.

240 We demonstrate inference for the hierarchical model based on data from all tracked pairs in
241 a single cell (Fig. 4C-E); inferred hidden states including the reversal state, *R* are shown for some
242 representative trajectories in Supp. Fig. 4. The marginal posterior distribution of the individual bio-
243 physical model parameters for each kinetochore sister pair are shown in Fig. 4D, with the posterior
244 distribution of the shared rate parameters shown in Fig. 4E to visualise marginal distributions and
245 pairwise relationships between rate parameters. We obtain tight estimates of all parameters; re-
246 call that the natural spring length parameter, *L*, has a strong informative prior as described in
247 Methods. In particular the switching rates for metaphase oscillations are tighter than for single tra-
248 jectory pairs (posterior standard deviation 4.1 and 3.5 times smaller for p_{coh} and p_{icoh} respectively
249 for the trajectory shown in Fig. 3A), reflecting the greater number of directional switching events
250 in a cell, whilst the reversal transition rate p_{AR} is also well inferred. The transition from *A* to *R* is
251 rare hence the probability of remaining in the *A* state per frame, p_{AR} is close to 1. This model is
252 consistent with observations with reversals being rare events; only 6% of kinetochore pairs spend
253 on average more than 10 frames in the reversal state (Supp. Fig. 5).

254 The hierarchical model's predictions are better than those from the model without reversals.
255 Specifically, we compared the posterior predictive distribution, $p(x_t | x_{1:(t-1)}, \sigma_t)$ for the cell based hier-
256 archical model with shared rate parameters and the previous kinetochore pair based metaphase-
257 anaphase model. For a trajectory with a reversal, we find the observed data (coloured crosses) lies

¹ $q_{AR} = 1 - p_{AR}$ is thus the probability of switching from the anaphase state, *A*, to the reversal state, *R*.

258 within the credible region for the hierarchical model (Fig. 4C), whereas the data lies outside the
 259 model's credible region for the simpler anaphase model during the reversal (Fig. 3E, and Fig. 4C).

260 We observe heterogeneity between different kinetochore sister pairs in cell (Fig. 4D). Ordering
 261 the kinetochore sister pairs by the relative time of anaphase onset, t_A , reveals correlations with
 262 parameters such as τ and v_A (Fig. 4D, Supp. Fig. 6). The velocity in anaphase, v_A and the preci-
 263 sion parameter, τ , both show an increase among pairs with later anaphase onset times; increased
 264 velocity in anaphase for late separating pairs is consistent with previous studies (*Armond et al.*,
 265 *2019*).

266 Distribution of biophysical parameters across the population of kinetochores

267 To assess heterogeneity of kinetochore dynamics in a population of non-transformed human RPE1
 268 cells, we consider the distribution of posterior median parameter estimates for the population
 269 of kinetochore pairs tracked in $N = 25$ cells. This distribution is shown in Fig. 5 for both the
 270 individual parameters unique to each kinetochore pair (Fig. 5A, summarised in Table 1), and the
 271 rate parameters shared across cells (Fig. 5B). We exclude cells and kinetochore pairs for which
 272 diagnostics of the MCMC chains indicate that convergence has failed (see Methods and Supp. Fig.
 273 7).

Table 1. Estimates of biophysical parameters in RPE1 cells, and correlation with radial position in metaphase plate based on $n = 684$ kinetochore pairs from $N = 25$ cells

Parameter	Population median	Population standard deviation	Within-cell standard deviation	Between-cell standard deviation	Ratio between/within variance	p value assessing variance ratio	Correlation with radius ρ	p value assessing correlation with radius
α (s^{-1})	0.011	0.009	0.0084	0.0031	0.14	<0.001	0.08	0.041
κ (s^{-1})	0.007	0.007	0.0055	0.0038	0.48	0.015	-0.11	0.0026
L (nm)	797	8.0	5.9	2.0	0.11	<0.001	0.08	0.0332
t_A (s)	0.000	14.1	13.4	NA	NA	NA	-0.36	<0.001
τ ($s^2/\mu m^2$)	450	139	92	119	1.69	0.978	-0.37	<0.001
v_A (nm/s)	41	13	8.7	10.6	1.48	0.935	-0.20	<0.001
v_- (nm/s)	-40	18	12.8	13.8	1.16	0.730	0.34	<0.001
v_+ (nm/s)	7	8	5.2	4.9	0.89	0.380	-0.23	<0.001
p_{icoh}	0.809	0.077	NA	0.0774	NA	NA	NA	NA
p_{coh}	0.956	0.009	NA	0.0093	NA	NA	NA	NA
p_{AR}	0.985	0.030	NA	0.0303	NA	NA	NA	NA
p_{RA}	0.832	0.121	NA	0.1210	NA	NA	NA	NA
Proportion	0.623	0.204	0.175	0.107	0.38	0.002	-0.521	<0.001
LIDS Proportion	0.281	0.188	0.163	0.092	0.32	<0.001	0.505	<0.001
TIDS								

274 The main difference to highlight compared to previous estimates based on HeLa cells (*Armond*
 275 *et al.*, *2015*) is the distribution of the spring constant, κ , which has shifted to smaller values for
 276 RPE1 cells ($0.009 \pm 0.007 s^{-1}$; mean \pm population standard deviation) compared to the previous esti-
 277 mates for HeLa cells ($0.03 \pm 0.01 s^{-1}$). This therefore indicates that RPE1 cells have a more compliant
 278 (weaker) centromeric chromatin spring than for HeLa cells. Other notable differences compared to
 279 previous estimates in HeLa cells relate to the microtubule speed parameters, v_- and v_+ . The magni-
 280 tude of these parameters have a greater difference ($|v_-| - v_+$) in RPE1 cells compared to HeLa cells;
 281 v_- is estimated as -40 ± 18 nm/s in RPE1 cells versus -35 ± 15 nm/s in HeLa, similarly v_+ is estimated
 282 as 7 ± 8 nm/s in RPE1 cells versus 13 ± 16 nm/s in HeLa, where mean values \pm standard deviation of
 283 the population are given. It should be noted however that the HeLa cell analysis in *Armond et al.*
 284 (*2015*) is based on far lower coverage of kinetochores than the current analysis.

285 To assess whether there are any links between biophysical parameters and segregation errors,
 286 we considered laziness of kinetochores, as defined in *Sen et al.* (*2021*). Lazy kinetochores show
 287 impaired segregation compared to other kinetochores that segregate to the same daughter cell.

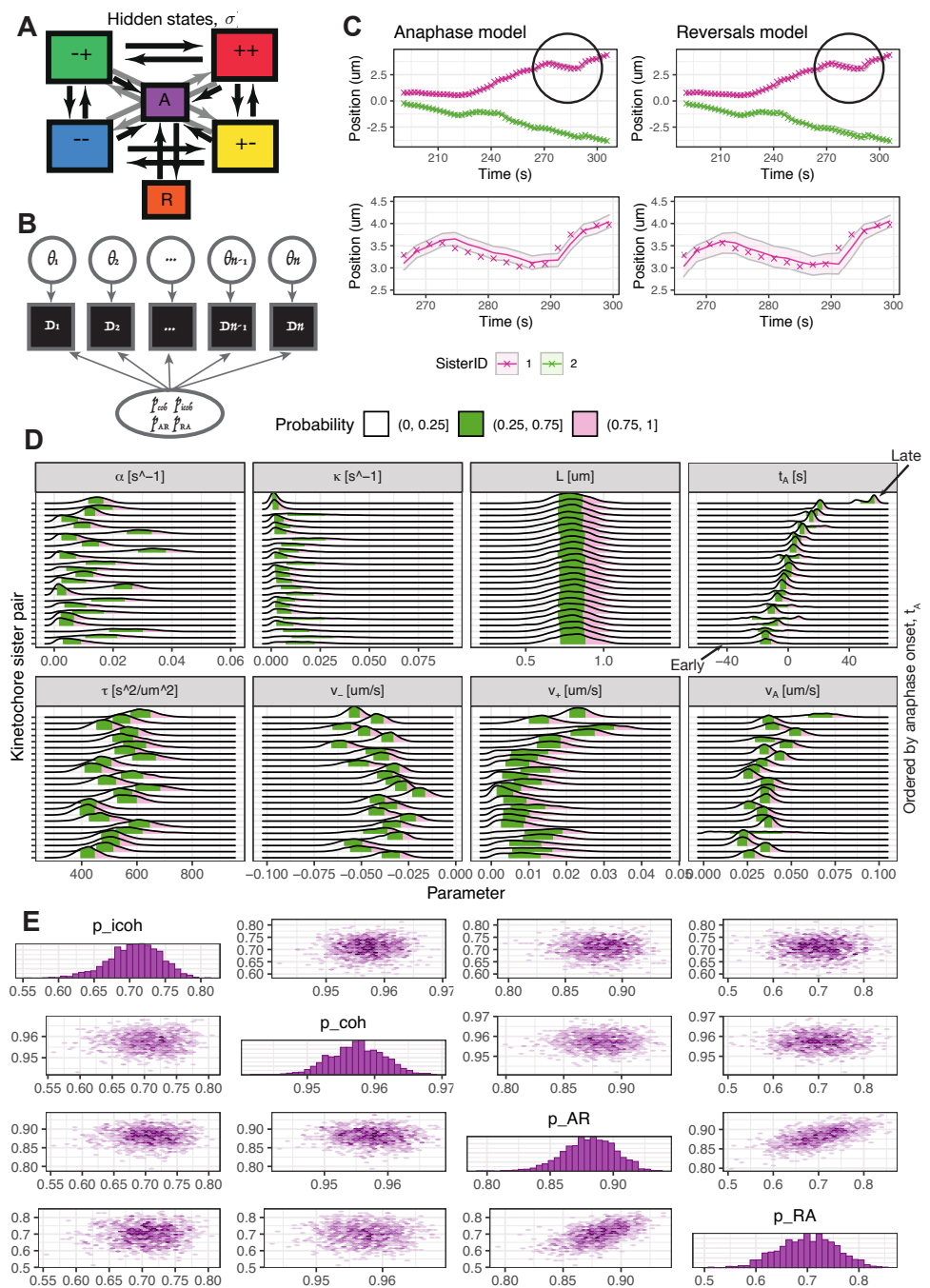


Figure 4. Hierarchical Bayesian framework for inference of rare reversals in anaphase. (A) Graphical structure of transitions between hidden states. All metaphase states, $\{++, +-, -+, --\}$, are accessible from each other by either a single sister switching, or both sisters switching within a time step. The anaphase state, A , is accessible from all the metaphase states. From the anaphase state, A , transitions to and from the reversal state, R , are possible. (B) Schematic showing the hierarchical structure of shared rate parameters, $\theta_{SP} = (p_{\text{icoh}}, p_{\text{coh}}, p_{AR}, p_{RA})$, and individual biophysical parameters, $\theta_{BP} = (\tau, \alpha, \kappa, v_-, v_+, L)$, unique to each kinetochore sister pair. (C) Prediction from the filtering distribution $P(x_t | x_{1:t-1}, \sigma_t; \theta)$ on a trajectory with reversals during anaphase. The reversal in anaphase is circled and the observed data (coloured crosses) lies outside the shaded region, the 95% credible region for the model predictions, between 270s and 290s for the simple biophysical anaphase model, but not for the hierarchical model. (D) Biophysical parameter marginal posterior distributions for individual kinetochore sister pairs. Pairs are ordered by the posterior mean time of anaphase onset, t_A , which are shown relative to the median time of anaphase onset for the cell. The green shaded region highlights the interquartile range, while the grey and pink shaded regions show the lower and upper tails respectively. (E) Density plot of the pairwise posterior distribution for shared rate parameters, $p_{\text{icoh}}, p_{\text{coh}}, p_{AR}, p_{RA}$ for a single cell, showing correlations between parameters. Histograms of the marginal posterior distributions are shown on the diagonal.

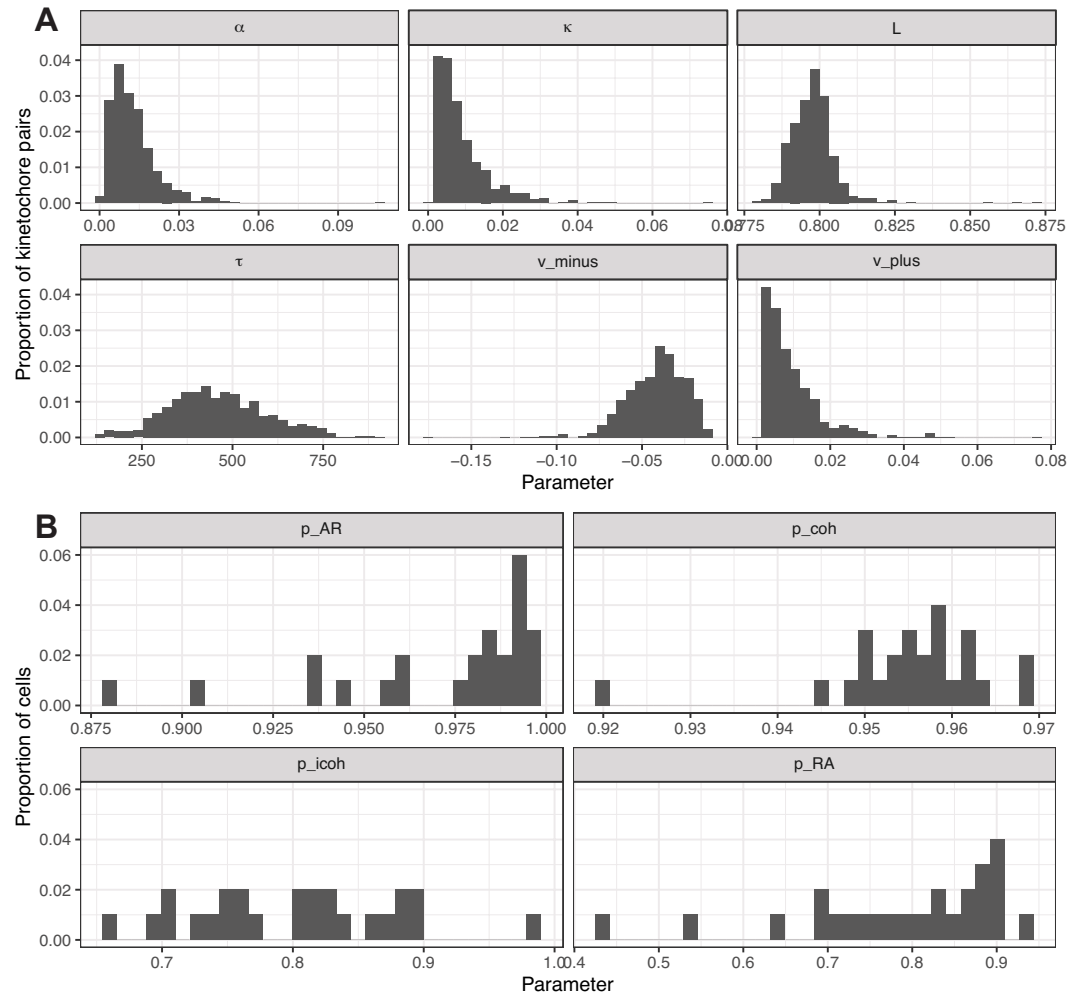


Figure 5. Distribution of model parameters for a population of $n = 684$ kinetochore pairs from $N = 25$ cells. (A) Histograms of the median posterior estimate for biophysical parameters $\alpha, \kappa, L, \tau, v_{-}, v_{+}$ across a population of trajectories from multiple cells. (B) Histograms of the median posterior estimate for each of the shared rate parameters, $\theta_{SP} = (p_{\text{icoh}}, p_{\text{coh}}, p_{\text{AR}}, p_{\text{RA}})$, across the population of cells.

288 We found that kinetochores with high laziness have significantly higher values for v_+ ($p = 0.02$,
289 see Supp. Fig. 8) compared to the remaining population of kinetochores. No other biophysical
290 variables were significant.

291 The parameter distributions over the cell population demonstrate heterogeneity between cells,
292 Fig. 5. Since heterogeneity is observed within cells, for instance, trends with metaphase plate lo-
293 cation (see Table 1 and Supp. Fig. 9), it is unclear how much of population heterogeneity derives
294 from variation within cells. To determine whether variation between cells or within cells contributes
295 more to the variation, for each parameter we consider between-cell variation: the standard devia-
296 tion across the population of the median parameter estimates per cell (median over kinetochore
297 pairs in a cell); versus samples of the within-cell variation: the parameter standard deviation of each
298 cell's kinetochore pairs (Fig. 6A). As indicated in Fig. 6B and Table 1, we find greater within-cell varia-
299 tion compared to between-cell variation, particularly for the polar ejection force parameter, α , and
300 the spring parameters, L and κ . This is evident because the cell-to-cell variation (red dot) lies be-
301 low the within-cell variability (black dots) for these parameters. Assessing the ratio of between-cell
302 variance to within-cell variance and whether this differs from 1 with an F -test indicates a signifi-
303 cant difference for the polar ejection force parameter, α , ($p < 0.001$), the natural spring length, L ,
304 ($p < 0.001$), and spring constant, κ , ($p = 0.015$). Although this result may be expected for the natural
305 spring length, L , which is subject to a strong informative prior distribution and thus has near zero
306 variation between cells, it is perhaps surprising that the PEF parameter, α , and spring constant, κ ,
307 have a prescribed variation over the metaphase plate that is similar in different cells.

308 Since spatial variation of the PEF was previously demonstrated by *Armond et al. (2015)*, we
309 therefore examined the extent to which this heterogeneity can be explained by subpopulations
310 of kinetochores located in particular spatial locations of the metaphase plate. We compared the
311 same heterogeneity statistics for kinetochores located, on average, 0 to 2 μm , 2 to 4 μm , and 4
312 to 6 μm from the centre of the metaphase plate (Fig. 6C). For parameter α , between-cell variation
313 (red dot) was below the within-cell variability (black dots) when considering all kinetochore pairs,
314 but on the radial kinetochore subsets the red dot lies within the cell variation distribution (black
315 dots) suggesting that heterogeneity within this population and between cells are similar for these
316 subsets. In contrast, after accounting for radius, the red dot lies above the black points for the
317 precision parameter, τ , suggesting that, once radius is accounted for, the noise varies much more
318 between cells than within cells. These results indicate that radial position in the metaphase plate
319 dictates kinetochore dynamic behaviour, making a notable contribution to variability in biophysical
320 properties.

321 **Directional switching events vary spatially across the metaphase plate**

322 Given the spatial variation in the biophysical parameters (see Table 1), we reasoned that there may
323 also be effects on directional switching of chromosomes. Metaphase quasi-periodic oscillations re-
324 quire that both sister kinetochores change direction at a directional switch. A key distinction is
325 between which sister switches first - specifically switching events initiated by the leading kineto-
326 chore sister (lead induced directional switch, or LIDS, events), and those initiated by the trailing
327 kinetochore sister (trail induced directional switch, or TIDS, events) are observed (*Armond et al.,*
328 *2015; Burroughs et al., 2015*). From the annotation of kinetochore trajectories, we can identify
329 directional switching events and determine which sister initiated that switch, and subsequently
330 correlate LIDS/TIDS events with our trajectory summary statistics, Fig. 7.

331 By fitting the hierarchical model of metaphase-anaphase dynamics to data from $N = 25$ cells
332 at a time resolution of $\Delta t = 2.05\text{s}$ per image stack, and using forward filtering backward sampling
333 (as described in Methods), we are able to sample from the distribution of hidden states through
334 a trajectory. Given a sequence of states, a directional switch is defined as a pattern of states that
335 changes between consecutive coherent runs moving in the opposite directions (+- to -+ or vice
336 versa), which can have intermediate incoherent states ++ or --, the sister kinetochores then mov-
337 ing in opposite directions. For example the sequence [+-, +-, +-, +-, --, -+, -+, -+, -+] would

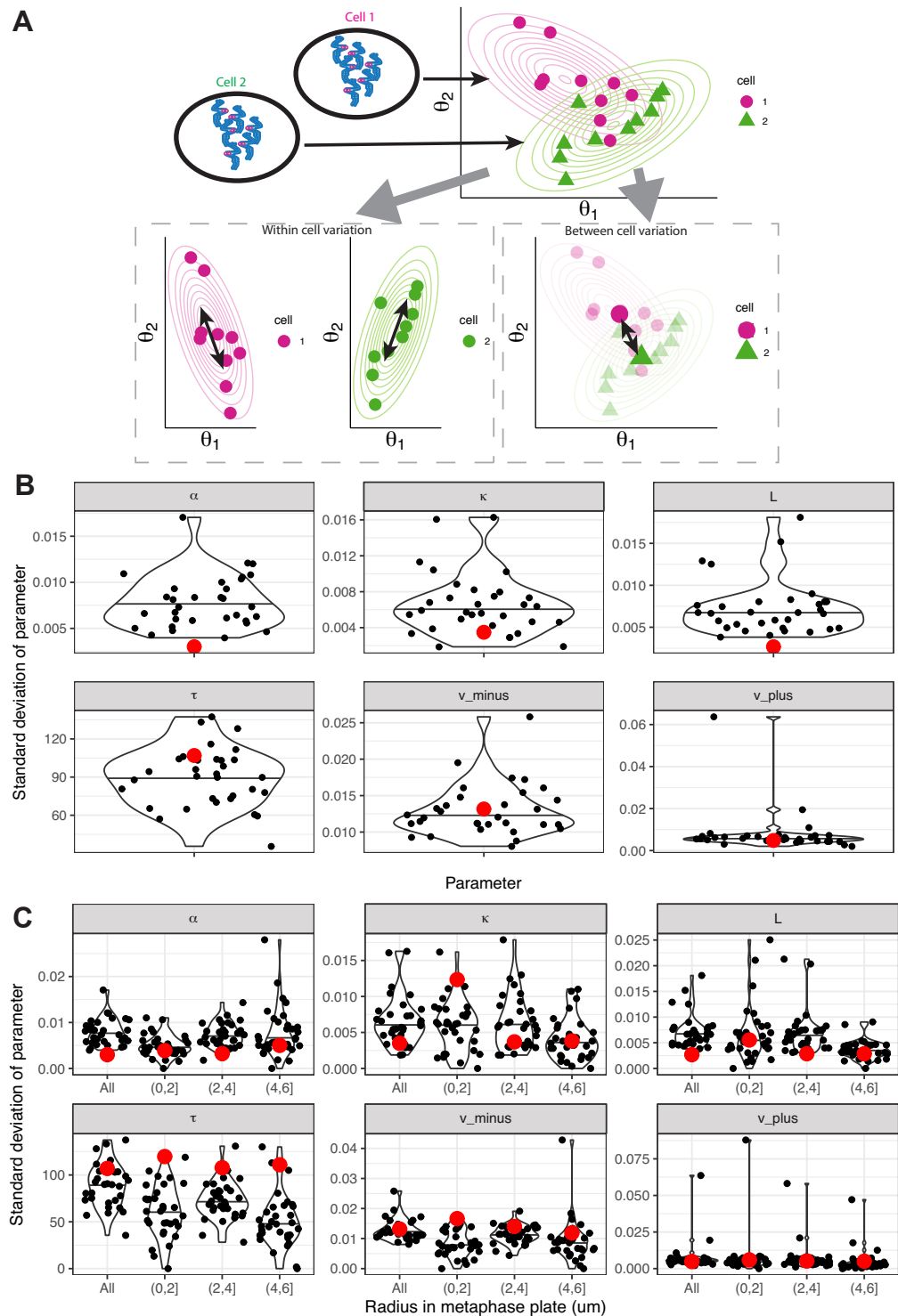


Figure 6. Comparison of heterogeneity in biophysical parameters between and within cells. (A) Schematic diagram defining within cell variation and between cell variation. For two cells (green and magenta), their estimated parameter values for θ_1 and θ_2 are shown by magenta circles and green triangles respectively. The standard deviation of θ_i for the magenta and green points respectively quantifies the variation within a cell (left), whereas taking a cell average and considering the standard deviation between cells quantifies the variation between cells. (B) Violin plots of the standard deviation of the variation between kinetochore pairs within each cell for biophysical parameters α , κ , L , τ , v_- , v_+ . Each black dot corresponds to the variation within a cell. Marked red dots are the standard deviation of the median parameter estimates for each cell showing the level of between-cell heterogeneity. (C) Violin plots as in (B) showing the full data (all kinetochores) and subpopulations of kinetochores in the indicated ranges for the radial position in the metaphase plate. Assuming a normal distribution for within-cell variation and evaluating the percentile of the between-cell variation, for α this gives $p = 0.044$ for all kinetochores, but is no longer significant for subpopulations of kinetochores at particular radial locations. Results based on $n = 684$ kinetochore pairs from $N = 25$ cells.

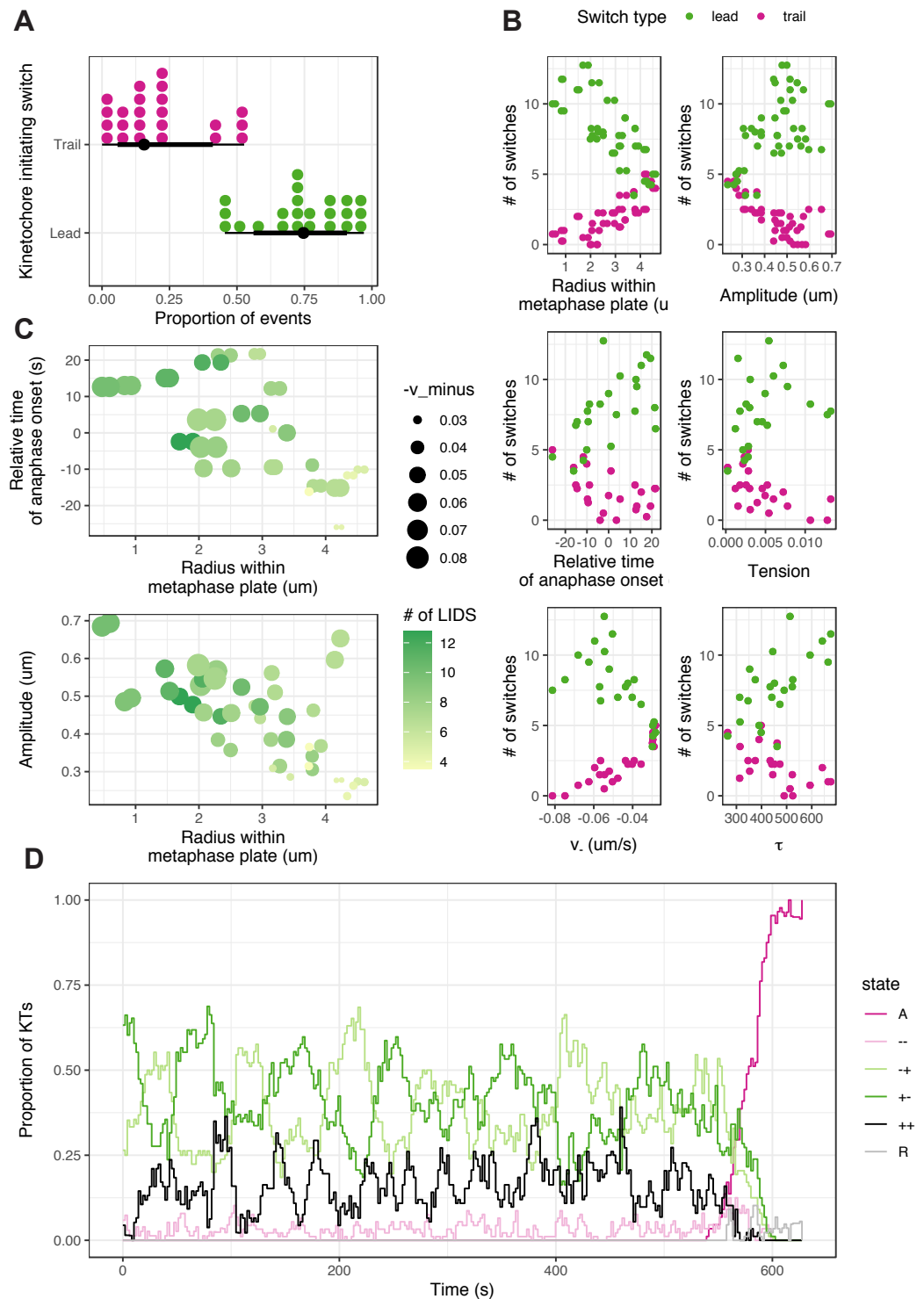


Figure 7. Directional switches of oscillating chromosomes vary across the metaphase plate. (A) Fraction of LIDS (green) and TIDS (pink) events as a proportion of the total number of switching events including joint switches. Each kinetochore pair gives rise to a LIDS and TIDS dot. (B) Relationship between the number of directional switches initiated by the leading (green) or trailing (pink) kinetochore sister, and other summary statistics describing the oscillatory dynamics. (C) Relationship between the number of LIDS events and other summary statistics indicating that many of these variables change together based on spatial position of kinetochore pairs within the metaphase plate. (D) Proportion of kinetochore sister pairs in a given hidden state at each time point. All data shown in this figure relate to a single cell; another cell is shown in Supp. Fig. 10 and population summaries are given in Table 1.

338 be assessed as containing a TIDS, as would a sequence with a longer incoherent sequence such as
339 [+−, +−, +−, +−, −−, −−, −−, −−, −−, −−, −−, −−, −−, −−, −−, −−]. We averaged over MCMC samples and obtained the
340 posterior number of LIDS and TIDS events in a sister kinetochore pair trajectory. This allows the
341 proportion of LIDS and TIDS events to be computed, relative to the total number of events (Fig. 7A,
342 Table 1).

343 Switch events in RPE1 cells are approximately two times more likely to be initiated by the lead-
344 ing kinetochore sister than the trailing kinetochore sister (Fig. 7A), consistent with previous results
345 in HeLa cells (Armond *et al.*, 2015; Burroughs *et al.*, 2015). Furthermore, we find that kinetochore
346 pairs close to the centre of the metaphase plate have more LIDS events and fewer TIDS events,
347 as shown in Fig. 7B and Table 1. Many other summary statistics and model parameters including
348 oscillation amplitude, the relative time of chromatid separation, tension, microtubule speed in the
349 (leading) depolymerization state v_- , and the noise parameter, τ , all show strong correlations with
350 the number of LIDS events (Fig. 7B). However, many of these covariates vary together spatially
351 within the metaphase plate (Fig. 7C) so determining causality of the factors responsible for these
352 effects on switching dynamics is unclear. Moreover, as for the biophysical parameters in the pre-
353 vious section, we observe significantly higher variation in the proportion of LIDS and TIDS events
354 within cells than between cells (Table 1) (F -test; LIDS: $p = 0.002$, TIDS: $p < 0.001$).

355 When considering the proportion of kinetochore pairs in a given state over time (averaged over
356 a cell's kinetochore population), we find oscillatory behaviour (Fig. 7D). These oscillations occur on
357 a similar timescale to the oscillations for single kinetochore pairs, perhaps suggesting coordination
358 across the population in the kinetochore oscillations, similar to the correlation between non-sister
359 kinetochore trajectories observed in Vladimirov *et al.* (2013). Intuitively we would expect that if
360 kinetochore pair oscillations were independent, the proportion of kinetochore pairs in each state
361 would be constant over time. Simulations from a 4 state Markov model with spontaneous switching
362 between states (see Methods) also exhibit fluctuations in the proportion of kinetochore pairs in
363 each state (Supp. Fig. 11), although lack the regular periodicity observed in the experimental data.
364 Thus, the oscillations seen in the average state proportion, Fig. 7D, are likely a result of averaging
365 over a finite number of oscillating kinetochore pairs (46 in human cells, and 46 in Supp. Fig. 11); in
366 the limit of infinite kinetochore pairs these fluctuations would disappear.

367 Finally, examination of the K-fibre polymerisation hidden states around anaphase in Fig. 7D
368 shows that no particular state dominates - thus anaphase onset is not coordinated with a particular
369 metaphase oscillation phase. There is only a small increased proportion of the (−−) states in the
370 lead up to anaphase (Fig. 7D, light pink line). Whether this is mechanistically relevant is unclear, as
371 (−−) is similar to the anaphase state in that both sisters are depolymerising.

372 **Coordination of directional switching events in space and time**

To further assess the extent of the coordination of directional switching across the metaphase
plate, we considered the influence of directional switching events on the switching of neighbour-
ing kinetochore pairs. Taking a dataset of directional switching events in a cell, as determined by
the metaphase-anaphase model, we use a self-exciting point process model known as a Hawkes
process (Hawkes, 1971; Reinhart, 2018) (Fig. 8A) to determine how contagious directional switching
events are. Label the switch events by i , then a switch event is given by (j_i, t_i, s_i) , the event occurring
in pair j_i at time t_i , kinetochore position s_i . The Hawkes process intensity for a switch event at time
 t , position s , kinetochore sister pair j is conditioned on the history of all previous switches, $t_i < t$,
 $i = 1, 2, \dots$, and given by

$$\lambda(s, t, j) = \mu(s, t) + \theta \sum_{i: t_i < t} k_t(t, t_i) k_s(s, s_i) k_j(j, j_i)$$

373 where $k_t(t, t_i)$ is the (exponential) kernel in time, $k_s(s, s_i)$ is the (Gaussian) kernel in space, and $k_j(j, j_i)$
374 is a kernel that removes interactions between sister kinetochores. We use an exponential decay
375 kernel for influence of events in the past, and a Gaussian kernel to discount for the 3D distance
376 of that event - the strengths of both of these kernels are inferred from the data. A point process

377 without self-exciting interactions would have only the $\mu(s, t)$ term, while the sum term represents
378 the influence of previous switching events.

379 Fitting the Hawkes process in a Bayesian framework (details in Methods) allows us to assess
380 whether one directional switch is likely to be coordinated with other directional switches locally
381 in space and time. This analysis revealed interactions between switching events over a timescale
382 of 36s [18.9,84.1] and a lengthscale of $1.3\mu\text{m}$ [1.02,1.66], where square brackets indicate the 95%
383 credible interval, as shown in Fig. 8B and C. The parameter θ represents the average number of
384 switching events triggered by a single switching event, and this was estimated as 0.72 [0.43,1.05];
385 since this is less than 1 the event structure in space and time comprises small clusters that are
386 excited from a spontaneous event, the cluster size being stochastic. From 100 switching events,
387 we would thus expect 72 switching events to be triggered by these, which will subsequently trigger
388 further switches. To visualise this, we classify events triggered by other switching events (and thus
389 a result of excitatory behaviour) as those with the highest estimated conditional intensity. This
390 allows us, in Fig. 8D, to highlight the switching events influenced by other switching events by
391 excitatory behaviour (filled circles) versus switching events that occur spontaneously due to the
392 background rate of switching (open squares). Thus, directional switching events of neighbouring
393 kinetochore pairs are spatio-temporally coordinated.

394 **Spatial coordination of K-fibre polymerisation and depolymerisation states**

395 Although the Hawkes process demonstrates an influence of switching events of other kinetochore
396 pairs on the switching rate of an individual kinetochore, it does not allow for the nature of the switch
397 or directionality of the kinetochores. To further investigate local coordination of kinetochore state,
398 and related movements, we define a measure of alignment between the hidden states of a kine-
399 tochore pair (polymerising/depolymerising for each K-fibre) and that of neighbouring kinetochore
400 pairs. Specifically, the alignment between kinetochore pair i and pair j at time t is defined as

$$\phi_{ij}^t = \sum_k p_i^t(k)p_j^t(k) \quad (3)$$

where $p_i^t(k)$ is the probability that pair i is in state k at time t . Based on average positions of kine-
tochore pairs within the metaphase plate, we identify k nearest neighbours of a kinetochore pair
(Fig. 9A), and average the alignment of neighbouring kinetochore pairs as follows

$$\phi^t = \frac{1}{N} \sum_i \frac{1}{|\Omega_i|} \sum_{(i,j) \in \Omega_i} \phi_{ij}^t$$

401 where Ω_i is the set of k nearest neighbours for a kinetochore pair i and N is the total number
402 of pairs. Full alignment between all kinetochore pairs would give $\phi^t = 1$. Using this measure of
403 alignment, we find that neighbouring kinetochore pairs have greater average state alignment in
404 the data than can be accounted for based on simulations from a model without any interactions
405 (Fig. 9C,D). This suggests that interactions between kinetochore pairs are required to account for
406 the alignment that we observe.

407 The alignment, ϕ^t , of all pairs in a cell oscillates over time, as shown in Fig. 9B. Taking the auto-
408 correlation in time of the average alignment of a kinetochore with its neighbours shows that these
409 oscillations have a frequency similar to that of kinetochore pair breathing ($\approx 20\text{s}$). This indicates
410 that neighbouring kinetochores interact, influencing each other so that there is local coordination
411 of direction of movement, potentially via interactions between their respective K-fibres (Tolić, 2018;
412 Simunić and Tolić, 2016).

413 **Spatial coordination of anaphase onset**

414 To determine if this local coordination of metaphase dynamics extends to local coordination of
415 chromosome segregation, we analysed whether there is evidence of spatial coordination in anaphase
416 onset. Taking the anaphase onset times inferred from the hierarchical metaphase-anaphase model,

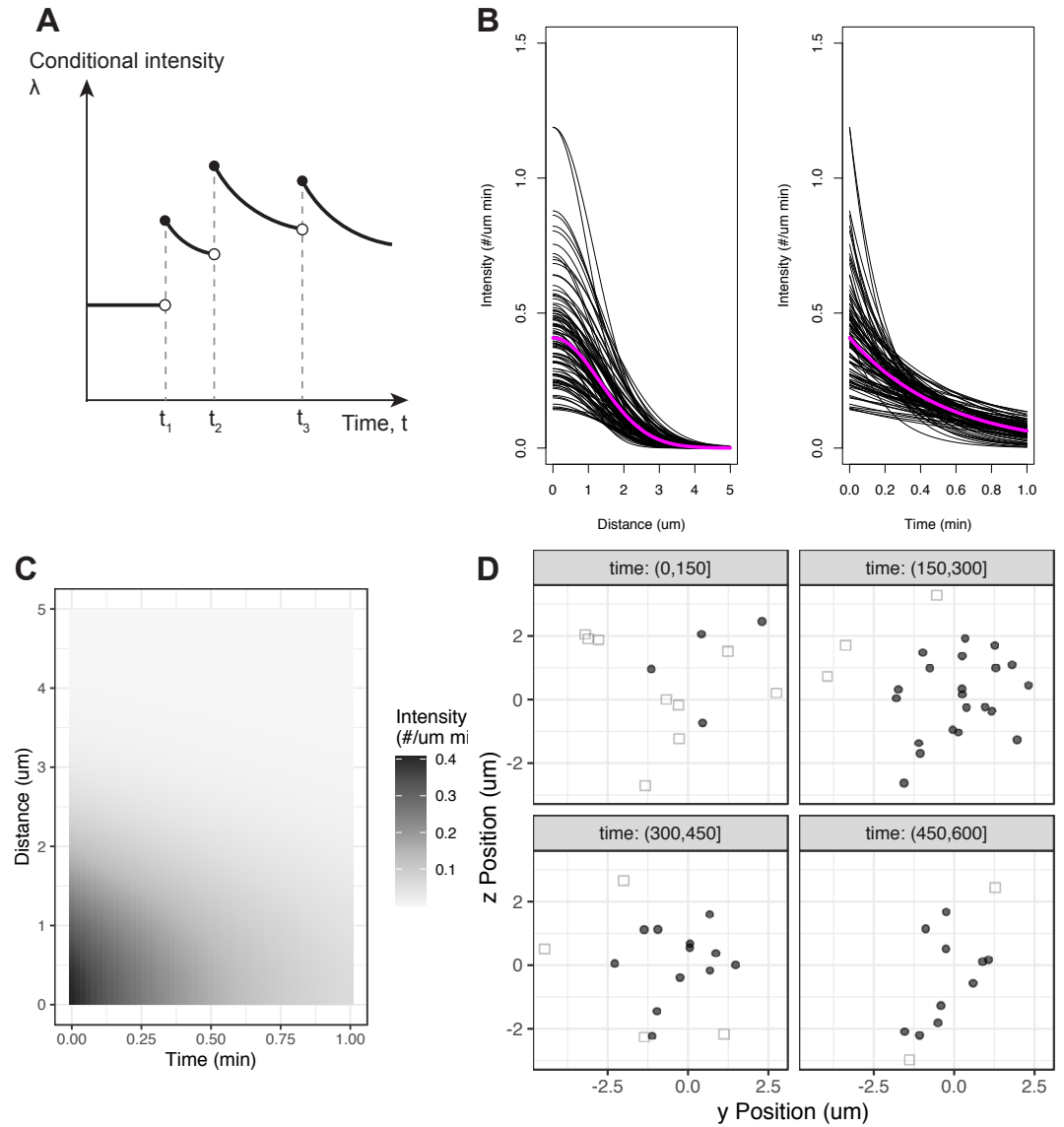


Figure 8. Directional switching events are coordinated in space and time, as revealed by a Hawkes process model. (A) Schematic of conditional intensity with a Hawkes process model (assuming only self-excitatory behaviour in time, and no spatial kernel) showing increase in the event rate after an event, the effect decaying with time from the event. (B) The self-excitatory component of the Hawkes process showing decay in interactions between switching events in space and time. The spatial kernel is visualised at $t = 0$, while the temporal kernel is visualised at a distance of 0. Each black line corresponds to a sample from the posterior, while the magenta line corresponds to the posterior mean. (C) Self-excitatory component of the Hawkes process visualised in space and time. (D) Example cell with switching events classified as excitatory (filled circles) or as background (open squares). The y and z positions shown correspond to positions within the metaphase plate. Subplots divide metaphase into sections of 150s. Data relates to a single cell.

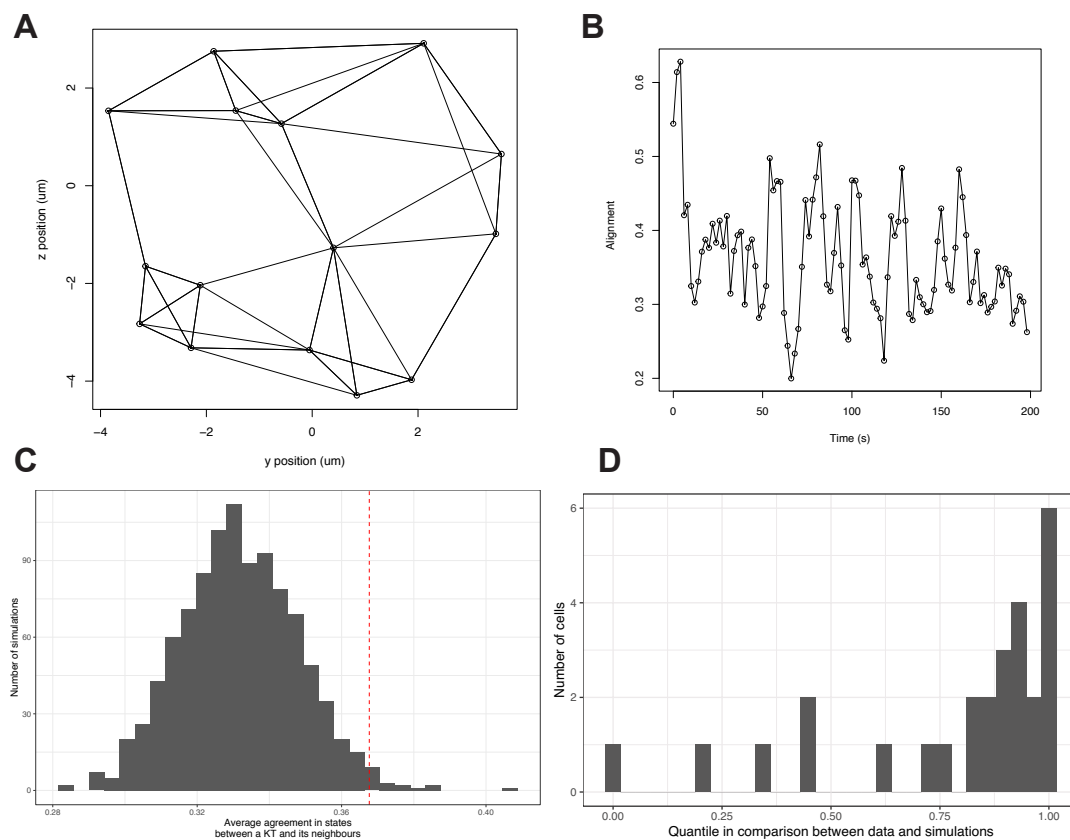


Figure 9. Local coordination of K-fibre polymerisation and depolymerisation states. (A) Network of $k = 4$ nearest neighbours of kinetochore pairs based on average positions within the metaphase plate. (B) Average alignment of states, ϕ^i , between neighbouring kinetochore pairs oscillates over time. (C) Comparison of average alignment from experimental data with simulations from a 4 state Markov process. Parameters for the Markov process are $p_{\text{coh}} = 0.96$, $p_{\text{icoh}} = 0.83$ are obtained from the posterior median estimate from fitting to this cell. The grey histogram shows average alignment computed for 1000 model simulations, while the dashed red line shows average alignment evaluated for experimental data. (D) Histogram of the percentile obtained from comparison between simulation and observed data for the average alignment for each cell in a population of $N = 25$ cells. A large proportion of percentiles are close to 1 indicating higher agreement of states in the observed data than expected from simulations of a null model. Plots (A-C) refer to the same example cell; data in (D) is based on $N = 25$ cells.

417 Fig. 4, we ordered the kinetochore pairs by increasing anaphase onset time. With this order-
418 ing, we visualised the spatial positions of kinetochore pairs over the sequence of anaphase on-
419 set times in a cell (Fig. 10A) and observed that kinetochore pairs close together in space tend
420 to enter anaphase close together in time. To quantify this, we computed the 2D Euclidean dis-
421 tance within the metaphase plate between kinetochore pairs, and the difference between their
422 separation times, t_A (Fig. 10B). We assessed whether there are a disproportionate number of inter-
423 actions between kinetochore pairs that are close in space and time, (within $2\mu\text{m}$ in space and $2s$,
424 or one frame, in time), compared to what would be expected from randomly permuting the times
425 of anaphase onset of pairs, (Fig. 10C). This is quantified via the empirical cumulative distribution
426 function (eCDF), indicating the percentile at which the experimental data lies in comparison with
427 random permutations. Based on a population of cells, a much larger proportion of cells have a
428 percentile close to 1 than would be expected ($p = 0.007$, KS test comparing to uniform distribution)
429 as shown in Fig. 10D. These results indicate that there are local interactions between kinetochore
430 pairs at anaphase onset, or that several pairs in similar spatial locations are all influenced by the
431 same external factors (eg. separate activity).

432 **Kinetochore pairs at the edge of the metaphase plate enter anaphase earlier than** 433 **pairs at the centre**

434 The onset of anaphase, when kinetochore pairs begin to separate and segregate towards their
435 respective spindle poles, is tightly controlled temporally (*Holt et al., 2008*) and appears entirely
436 synchronous at low time resolution. An asynchrony in anaphase onset times has previously been
437 observed with a difference between first and last separation times of 60-90s in RPE1 cells (*Armond*
438 *et al., 2019; Sen et al., 2021*). Using our high time resolution imaging and inferred anaphase onset
439 times t_A , we confirm that kinetochore pairs in different parts of the metaphase plate separate at
440 different times, with pairs at the edge of the plate separating on average over 20s earlier than pairs
441 at the centre (Fig. 11A).

442 Furthermore, estimated anaphase speeds, v_A , are lower for kinetochore pairs at the edge of the
443 plate (Fig. 11B). However, the same relationship is not seen for the framewise anaphase speed in
444 3D (Fig. 11C). By considering (a simple approximation of) the geometry of the mitotic spindle (see
445 Fig. 11D), we can account for this. We assume that the spindle at anaphase onset can be approxi-
446 mated by two cones joined at their flat faces; these faces being the metaphase plate. This assump-
447 tion ignores the curvature and chirality of the spindle (*Novak et al., 2018; Pavin and Tolić, 2020*),
448 but captures the key part of the spindle geometry relevant here. Consider the triangle between a
449 K-fibre to a kinetochore at the centre of the metaphase plate, and the K-fibre to a kinetochore at
450 the edge of the plate. The K-fibre at the edge is a distance R from the centre, and the K-fibre at the
451 centre is a distance S from the pole, equal to the half spindle length.

If we assume that both the kinetochore at the centre, and the kinetochore at the edge begin to
segregate at the same time and move at the same speed, then the kinetochore at the edge takes
approximately

$$\frac{\sqrt{R^2 + S^2}}{S}$$

452 times longer to reach the pole. For realistic values of $R = 4\mu\text{m}$ and $S = 9\mu\text{m}$ (see Fig. 11E), then
453 we find that the transit time for the kinetochore at the edge is 9% longer. Over the timescale of
454 anaphase A of around 5 mins (*Su et al., 2016*), this gives a similar timescale to the difference in seg-
455 regation times observed between kinetochore pairs at the edge and the centre of the metaphase
456 plate. This suggests that anaphase onset triggers earlier at the periphery to coordinate chromo-
457 some separation.

458 **Mechanical forces at anaphase onset and force maturation in metaphase**

459 From our annotated sister pair trajectories we are able to unlock a number of dynamic and me-
460 chanical questions. Using inferred parameters and K-fibre polymerisation states sampled from the

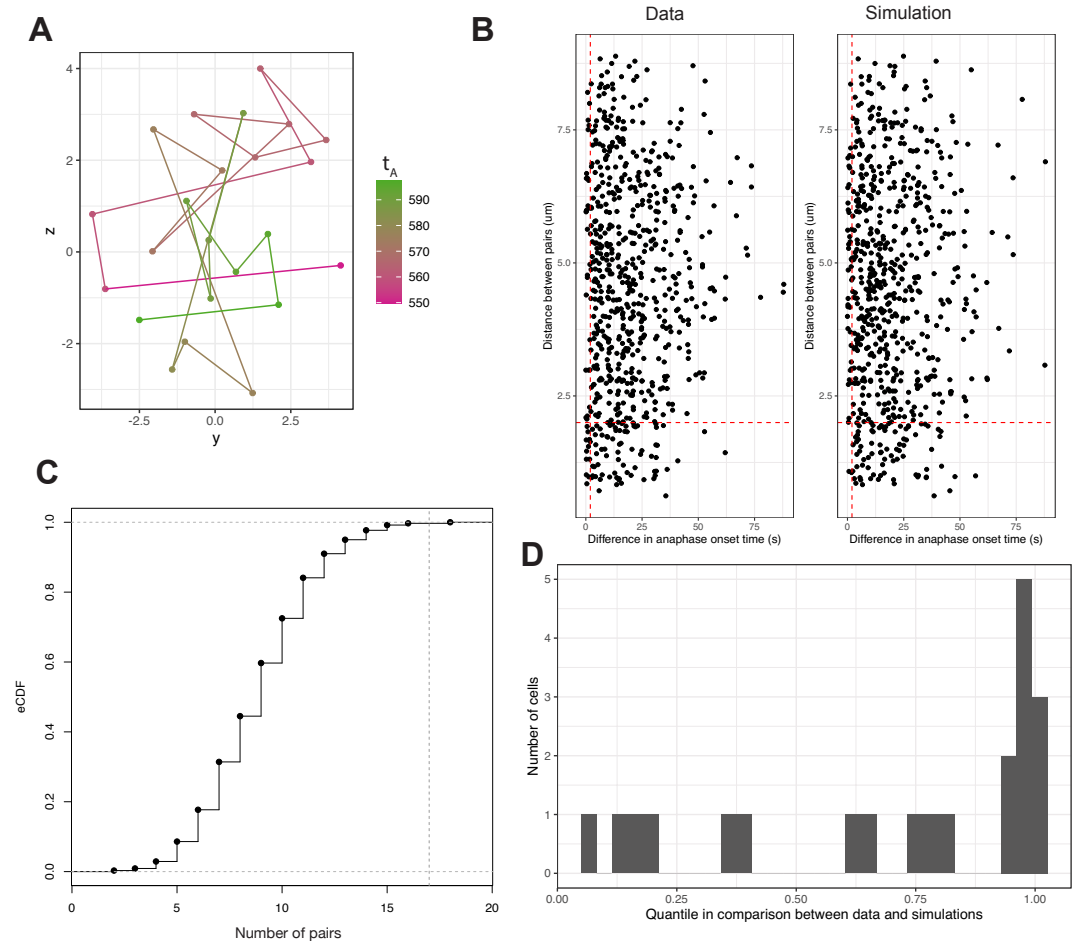


Figure 10. Anaphase onset times of kinetochore pairs exhibit local coordination. (A) Metaphase plate positions of kinetochore pairs in a cell at median anaphase onset time and coloured by time of anaphase onset t_A for each pair. Successive pairs in the anaphase onset time ordering are connected by lines. (B) Scatterplot of distances between all kinetochore pairs plotted against differences in their time of anaphase onset, t_A , in observed data (left) and simulated data (right) via randomly permuting the observed distances. Red dashed lines indicate a region of interest at the left bottom corner of the plot representing pairs close in space and time (within 2s and 2um). (C) Empirical cumulative density function (eCDF) of number of pairs close in both space and time (bottom left corner in (B)) in random permutations of the times of anaphase onset. Vertical dashed line indicates where the observed data lies in comparison. (D) Histogram of (empirical) percentiles (as in (C)) associated with comparison between the number of pairs close in both space and time (bottom left corner in (B)) in observed data versus simulations (obtained by randomly permuting times of anaphase onset for all pairs in a cell). Plots shown in (A), (B) and (C) are from a single cell, while (D) shows data from $N = 25$ cells.

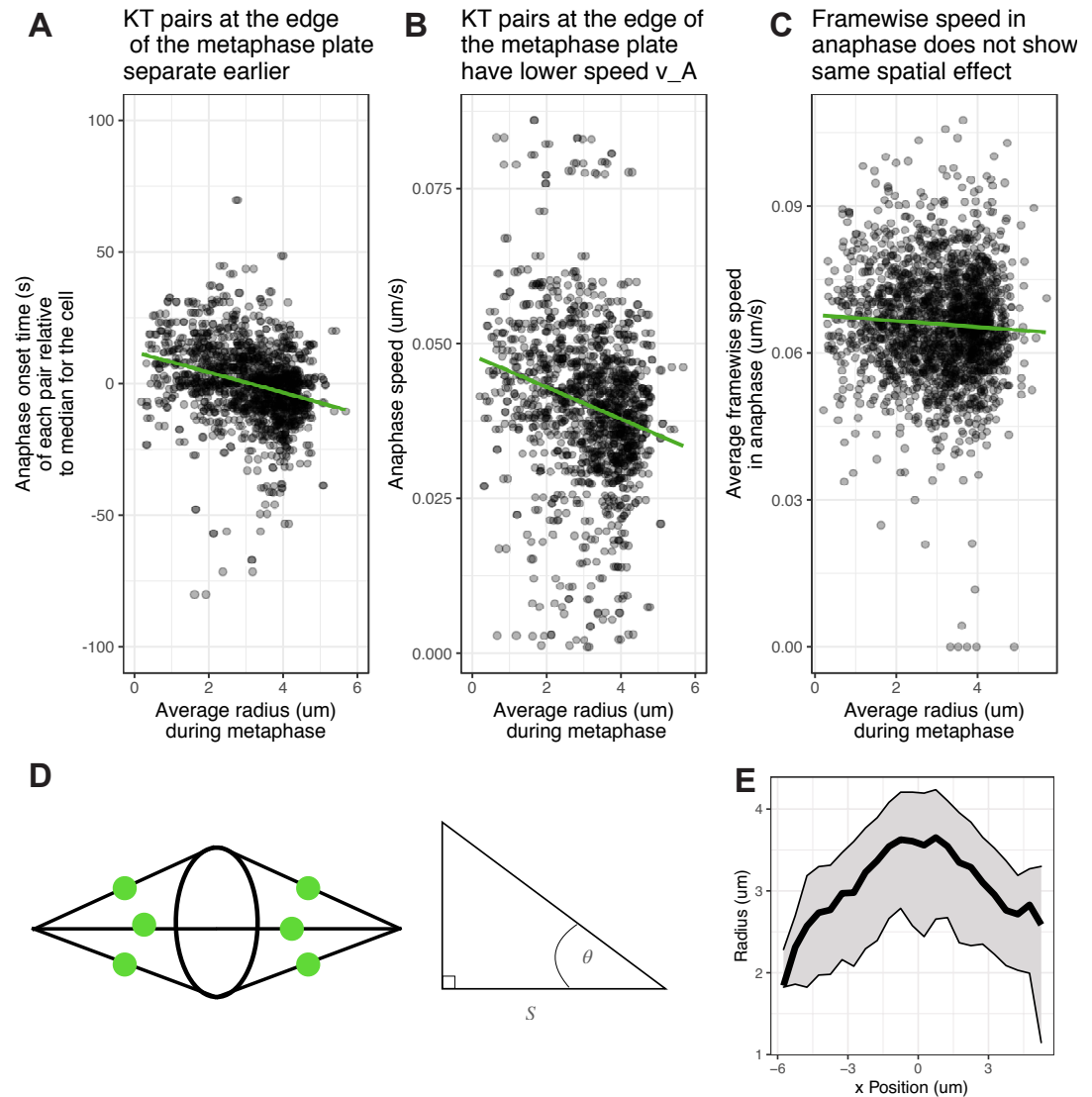


Figure 11. Anaphase onset occurs earlier at the edge of the metaphase plate. (A) Scatter plot of anaphase onset times relative to the median anaphase onset time in that cell versus the average radius of the kinetochore pair within the metaphase plate. (B) Scatter plot of anaphase speed, v_A , versus the average radial position of the kinetochore pair within the metaphase plate. (C) Scatter plot of the speed of kinetochore pairs in anaphase calculated framewise in 3D versus the average radial position of the kinetochore pair within the metaphase plate. For (A), (B) and (C), green lines indicate a linear fit to the data. (D) Schematic diagram indicating the metaphase plate and mitotic spindle (black lines) approximated via two cones, along with segregating kinetochores (green). The triangle depicted corresponds to the triangle between a kinetochore with average position at the centre of the metaphase plate, a kinetochore with average position at the edge of the plate, and one of the spindle poles. (E) Average radial location of kinetochores during anaphase for a given position on the x axis perpendicular to the metaphase plate. Solid black line shows median, with grey region showing 25% and 75% percentiles.

461 posterior distribution, we computed the expected forces acting on a kinetochore throughout time
 462 (recall forces are quantified as speeds by dividing through by the unknown drag force). Averaging
 463 over kinetochore pairs reveals a force profile around anaphase onset as shown in Fig. 12A. The
 464 largest force in metaphase is the force due to polymerisation/depolymerisation of the K-fibre, with
 465 a smaller contribution from the centromeric chromatin spring and polar ejection force, a similar
 466 pattern to that seen in Hela cells (*Armond et al., 2015*). The net force (Fig. 12B) increases immedi-
 467 ately after anaphase onset; during metaphase forces are in opposing directions.

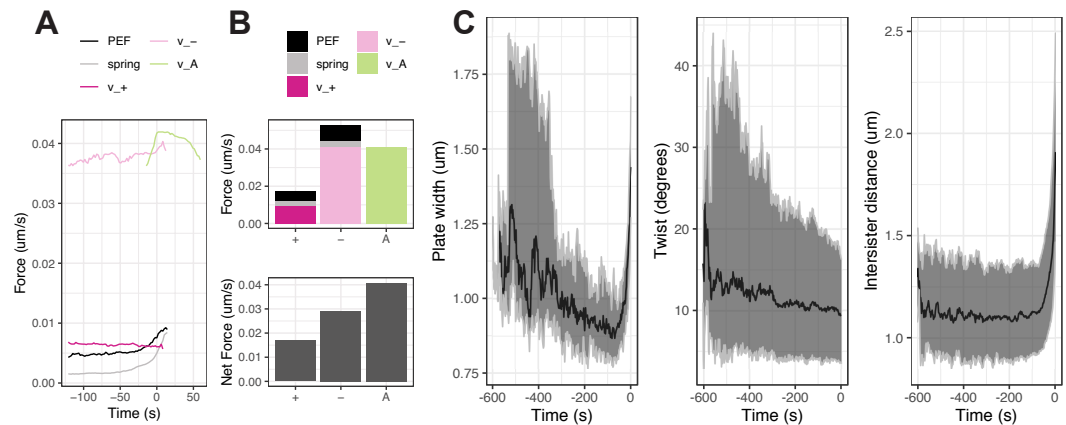


Figure 12. Force profiles at anaphase onset and kinetochore maturation characterisation during metaphase (A) Average force profile over time around anaphase onset across the population of kinetochores. Units of force terms are given as [um/s] as explained in Appendix 2. (B) Relative contributions of forces averaged over time shown as a bar plot (top) for K-fibres in polymerizing (+), depolymerizing (-) or anaphase (A) states, and total net force contributions in each of these states. (C) Changes over time during metaphase of the width of the metaphase plate (left), twist angle between the normal to the metaphase plate and the sister-sister vector (middle), and 3D intersister distance (right). Black line shows the median and grey region shows the 2.5% and 97.5% percentiles; data from $n = 684$ kinetochore pairs from $N = 25$ cells. The units of the inferred forces are [um/s] rather than [$g \text{ um/s}^2$] due to rescaling by the unknown drag coefficient.

468 Over the course of metaphase chromosome dynamics change, specifically there is an increase
 469 in centromere stiffness (*Harasymiw et al., 2019*) and a reduction of oscillation amplitudes (*Jaq-
 470 man et al., 2010*). By aligning trajectories of kinetochores to the median anaphase onset time of a
 471 cell, we can quantify these changes over time in higher resolution relative to a fixed reference point
 472 (anaphase onset). The metaphase plate becomes thinner over time (Fig. 12C, left) which is predom-
 473 inantly due to a decrease in the oscillation amplitude. The twist angle between the sister-sister axis
 474 and the normal to the metaphase plate reduces over time (Fig. 12C, middle) such that sisters be-
 475 come more aligned to the metaphase plate normal (which is likely to be aligned to the spindle
 476 axis (*McIntosh and Landis, 1971*)), suggesting a mechanical stiffening of the K-fibre-kinetochore
 477 attachment making it more rigid to torque (increased resistance to twist). The intersister distance
 478 between a kinetochore pair reduces slightly in metaphase until it begins to increase sharply as
 479 some kinetochore pairs separate (Fig. 12C, right).

480 Discussion

481 In this work, we provide an in depth quantitative analysis of kinetochore dynamics in metaphase
 482 and anaphase A in untransformed human cells (RPE1). This required development of computa-
 483 tional tools to fully utilise the high spatio-temporal resolution and quality achievable with LLSM.
 484 This included improvements in tracking software (KiT), development of a new metaphase-anaphase
 485 model and associated Bayesian inference algorithms, both for inference on data for single sister
 486 pairs and all the (tracked) sister pairs in a cell. We achieved near-complete kinetochore tracking,

487 performed a detailed state and event annotation of kinetochore trajectories, analysed the hetero-
488 geneity of sister dynamics between and within cells and demonstrated that kinetochore dynamics
489 are coordinated in time and space over the metaphase plate. We reveal that there is high variability
490 of kinetochore behaviour within a cell, with position in the metaphase plate being a major deter-
491 minant of behaviour, and that interactions between kinetochore pairs are important in governing
492 metaphase dynamics and, potentially, the transition to anaphase. This implies that it is essential to
493 consider chromosome dynamics in the context of the cell, accounting for the behaviour of neigh-
494 bouring chromosomes and variations in mechanical properties throughout the spindle, rather than
495 analysis of single kinetochore pairs in isolation.

496 We have demonstrated near-complete kinetochore tracking at high temporal resolution over
497 long timescales from prometaphase to late anaphase. Two key factors enabled this. Firstly, we
498 utilised LLSM (*Chen et al., 2014*) that uses an ultra thin sheet of light to limit effects of phototoxicity
499 and photobleaching, thus allowing imaging over long timescales at high temporal resolution (*Sen*
500 *et al., 2021*). Secondly, we made algorithmic improvements to our previous tracking algorithm (*Ar-*
501 *mond et al., 2016*), foremost among these being a change to the objective function in the adaptive
502 detection step to include biological information about the expected number of kinetochores in an
503 image.

504 We developed a mechanical switching model of chromosome dynamics in metaphase and
505 anaphase, including dynamics at the metaphase-anaphase transition. To capture rare reversal
506 events in anaphase, we extended this model from individual kinetochore pairs to a model of
507 the kinetochore population of a cell, fitted to experimental data using a hierarchical framework
508 whereby switching rate parameters are shared between kinetochore pairs in the same cell. Our
509 model captures key forces and events, although it ignores the complex dynamics of K-fibres and the
510 spindle. This simplicity ensures that all parameters of this model can be inferred from experimen-
511 tal trajectory data from a single cell (except for the natural spring length, see Methods). This would
512 not be possible with a highly detailed molecular model with large numbers of parameters, since tra-
513 jectory data is not informative on detailed molecular aspects governing the dynamics. Our model
514 thus ignores some biological phenomena. Further, model parameters are assumed constant in
515 time over the course of metaphase; however, mechanical maturation is observed during this time
516 period, see Fig. 12 and *Harasymiw et al. (2019)*. Future extensions of the model could address
517 such factors, along with directly modelling interactions between kinetochore pairs. In the hierar-
518 chical reversals model, both kinetochore sisters are assumed to be in the same anaphase/reversal
519 state; a model with independent reversals for each kinetochore sister could also be considered.

520 We demonstrated significant heterogeneity in kinetochore dynamics within a cell; in fact the
521 variation between kinetochore pairs in a cell is greater than variation between different cells (un-
522 der the same experimental conditions). Previous work on HeLa and PtK1 cells demonstrated that
523 biophysical parameters for the PEF (*Civelekoglu-Scholey et al., 2013*), α , the K-fibre forces, v_- , v_+ ,
524 and diffusive noise τ vary spatially across the metaphase plate (*Armond et al., 2015*), with larger
525 amplitude oscillations and lower noise kinetochores typically closer to the centre of the metaphase
526 plate and noisier, lower amplitude oscillations at the edge of the metaphase plate (*Armond et al.,*
527 *2015; Civelekoglu-Scholey et al., 2013*). This is also true for RPE1 cells (Table 1), whilst we have
528 further demonstrated that spatial variation is present in the switching events, Fig. 7. This sug-
529 gested that location in the spindle strongly affects kinetochore behaviour, which may explain the
530 higher variation within cells than between cells for certain parameters. This turned out to be the
531 case; once radial position is accounted for variation between cells is comparable to variation within
532 cells for the PEF parameter, α , (Fig. 6). In addition to metaphase plate position, there may be ad-
533 ditional factors contributing to this variation between kinetochore pairs in a cell. For instance,
534 dynamics may depend on specific chromosome properties, including size. This is suggested by the
535 mis-segregation bias for certain chromosomes (*Worrall et al., 2018*), and for large kinetochores (*Dr-*
536 *pic et al., 2018*). Our analysis thus suggests that metaphase dynamics are robust to changes in the
537 spindle environment, for instance robust to variation in K-fibre length and composition, our data in

538 fact indicating substantial spindle variation with transverse distance from the spindle axis. Despite
539 these variations, qualitatively similar dynamics occurs throughout the plate.

540 This robustness of metaphase oscillations is also evident in comparison between cell types. We
541 found that the centromeric chromatin spring is more compliant in RPE1 cells than in HeLa cells (*Ar-*
542 *mond et al., 2015*), corresponding to lower spring constant, κ , which is consistent with reduced
543 breathing between sister kinetochores in HeLa cells compared to that in RPE1 cells. Whether stiffer
544 centromeric chromatin springs is typical for cancer cells is unknown, but could explain disrupted
545 centromere mechanical maturation (*Harasymiw et al., 2019*) in aneuploid cell lines, and recent ob-
546 servations of attenuated chromosome oscillations in cancer cell lines *Iemura et al. (2021)*. Similarly,
547 the K-fibres forces v_- , v_+ have a larger difference in magnitude in RPE1 cells than in HeLa cells, with
548 possible relevance to the increased kinetochore-microtubule dynamics reported in RPE1 cells re-
549 lative to cancer cell lines (*Bakhoun et al., 2009*). This difference in K-fibre forces may be related to
550 misattachment errors such as merotelic; of note is that lazy kinetochores (*Sen et al., 2021*) in RPE1
551 cells have a higher value of v_+ than timely kinetochores, which adds further to the metaphase
552 signature (including reduced intersister distance and impaired oscillation regulatory) of lazy kine-
553 tochores reported in *Sen et al. (2021)*. In common with HeLa cells, we observed directional switching
554 is typically, but not exclusively, initiated by the leading sister kinetochore.

555 We showed that kinetochores at the edge of the metaphase plate enter anaphase earlier, and
556 have more directional switching events induced by the trailing kinetochore (and fewer induced by
557 the leading kinetochore). One interpretation of the earlier onset of anaphase at the edge of the
558 metaphase plate could be that in order to arrive near the spindle poles at the end of anaphase A in a
559 synchronous manner, kinetochore pairs at the edge of the metaphase plate must segregate earlier
560 because they have further to travel. This is consistent with calculations based on the simplified
561 mitotic spindle geometry shown in Fig. 11E, and may potentially be due to separase (part of a
562 positive feedback loop that increases the abruptness of anaphase (*Holt et al., 2008*)) penetrating
563 to the cohesins of peripheral chromosomes earlier.

564 An analysis of the coordination between kinetochore behaviour within a cell demonstrated
565 spatiotemporal interactions, including local coordination of directional switching events, kine-
566 tochore state and anaphase onset. Such local coordination is consistent with previous results re-
567 porting distance-dependent coupling of movements of neighbouring kinetochores in HeLa and
568 RPE1 cells (*Vladimirou et al., 2013*), and load distribution of forces anchoring a kinetochore within
569 $\sim 2\mu\text{m}$ laterally (*Elting et al., 2017*) in PtK2 cells. Interestingly, HeLa and RPE1 cells have similar size
570 spindles but very different numbers of kinetochores which is likely to affect the strength of the lo-
571 cal coupling; *Vladimirou et al. (2013)* found stronger correlated movements in HeLa than in RPE1
572 cells.

573 It is known that even weak physical coupling between oscillators leads to synchronisation, an
574 observation that goes back to Huygens in 1665 (*Willms et al., 2017*). Thus, interactions, or coupling
575 between non-sister chromosomes would lead to oscillation synchronisation across the plate, akin
576 to a beating drum, although microtubule dynamics are intrinsically noisy which would cause deteri-
577 oration in the synchronisation. The spindle is surrounded by the endoplasmic reticulum (*Ferrandiz*
578 *et al., 2021*) which likely increases the frictional and drag forces on the peripheral chromosomes,
579 thus reducing the amplitude of those oscillations towards the periphery. Inter-chromosome in-
580 teractions could arise through many mechanisms. The spindle is a visco-elastic anisotropic ma-
581 terial (*Shimamoto et al., 2011*) with chromosomes moving within the spindle primarily along the
582 spindle axis. Coupling between neighbouring chromosomes through this material would thus be
583 expected. In fact, viscosity alone is known to induce hydrodynamic forces between moving objects
584 through fluid movements. Thus, interactions that generate the space-time coordination observed
585 in our data could be a result of spindle material properties (visco-elastic or hydrodynamic), or arise
586 from direct interactions between kinetochore pairs (such as cross linking of K-fibres as proposed in
587 *Vladimirou et al. (2013)*), or between the chromosome arms, Ki67 acting as a surfactant to reduce
588 adherence in mitosis (*Cuylen et al., 2016*). The simplest hypothesis is that inherent variation in local

589 spindle architecture modulates mechanics locally at each point in metaphase plate, changes in spin-
590 dle geometry resulting in variation of the PEF with metaphase plate position (*Armond et al., 2015*;
591 *Civelekoglu-Scholey et al., 2013*) and inducing kinetochore swivel (*Smith et al., 2016*), whilst mater-
592 ial interactions (eg. drag) within the spindle environment and between chromosomes (*Cuylen*
593 *et al., 2016*) couple neighbouring kinetochores, which generates coordination between neighbour-
594 ing kinetochore movements. Finally, anaphase onset may be locally coordinated because of the
595 above mechanisms, or potentially through spatial variation in separase activity across the plate.
596 Thus we highlight how local interactions between kinetochores and feedback between spindle ar-
597 chitecture and mechanics (*Elting et al., 2018*) can give rise to emergent properties of mitotic cells
598 and ensure robustness of chromosome dynamics.

599 This study provides unprecedented detail and analysis of the behaviour of the complement of
600 chromosomes across the metaphase-anaphase transition in non-transformed human RPE1 cells.
601 This comprehensive characterisation should prove invaluable when comparing cell phenotypes (*Par-*
602 *gett and Umulis, 2013*) and assessing changes to chromosome dynamics upon perturbations, be it
603 genetic or through application of drugs. Thus, our methodology will allow mechanistic hypotheses
604 to be evaluated with high precision. This work sets the stage for future work to quantitatively anal-
605 yse the whole of mitosis at the cell level, from nuclear envelope breakdown through to segregation
606 of chromosomes.

607 **Methods and Materials**

608 **Code**

609 Code used to produce the results reported in this work is available at [www.github.com/shug3502/](http://www.github.com/shug3502/MitosisModels/)
610 [MitosisModels/](http://www.github.com/shug3502/MitosisModels/).

611 **Cell culture and generation of cell lines**

612 Immortalized (hTERT) diploid human retinal pigment epithelial (RPE1) cell line (MC191), express-
613 ing endogenously tagged Ndc80-eGFP, was generated by CRISPR-Cas9 gene editing *Roscioli et al.*
614 (*2020*). hTERT-RPE1 cells were grown in DMEM/F-12 medium containing 10% fetal bovine serum
615 (FBS), 2 mM L-glutamine, 100 U/ml penicillin and 100 mg/ml streptomycin (full growth medium);
616 and were maintained at 37°C with 5% CO₂ in a humidified incubator.

617 **Live cell imaging by lattice light sheet microscope**

618 The lattice light sheet microscope (LLSM) *Chen et al. (2014)* used in this study was manufactured by
619 3i (<https://www.intelligent-imaging.com>). Cells were seeded on 5 mm radius glass coverslips one
620 day before imaging. On the imaging day, each coverslip was transferred to the LLSM bath filled with
621 CO₂-independent L15 medium, where live imaging takes place. All imaged cells entered anaphase,
622 which is a suitable proxy for a lack of phototoxicity effects *Jaqaman et al. (2010)*. The LLSM light
623 path was aligned at the beginning of every imaging session by performing beam alignment, dye
624 alignment and bead alignment, followed by the acquisition of a bead image (at 488 nm channel)
625 for measuring the experimental point spread function (PSF). This PSF image is later used for the
626 deconvolution of images. 3D time-lapse images (movies) of Ndc80-eGFP were acquired at 488nm
627 channel using 1% laser power, 20 ms exposure time/z-plane, 75 z-planes, 307 nm z-step and 0.5
628 s laser off time, which results in 2 s/z-stack time/frame. Acquired movies were de-skewed and
629 cropped in XYZ and time, using Slidebook software in order to reduce the file size. Cropped movies
630 were then saved as OME-TIFF files in ImageJ.

631 **Tracking**

632 Kinetochore tracking is performed using the software package KiT (*Armond et al., 2016*) v2.4.0. The
633 tracking algorithm proceeds by detecting candidate spots via an adaptive threshold method to set
634 a threshold on the image histogram. Candidate spot locations are refined by fitting a Gaussian

635 mixture model. Spot locations are linked between frames by solving a linear assignment problem,
 636 with motion propagation via a Kalman filter. Tracked kinetochores are paired by solving another
 637 linear assignment problem.

638 Code to perform kinetochore tracking is available from <https://github.com/cmcb-warwick/KiT>,
 639 and this software includes a graphical user interface (GUI) for ease of use.

640 Likelihood calculation

To compute the likelihood for this model we apply the forward algorithm and marginalize out the discrete hidden states. Let $\xi_{\sigma_t,t} = P(\sigma_t|x_{1:t};\theta)$ be the probability of being in state σ_t at time t given all the data up to that time. Suppose that we define the likelihood contribution of an observation, x_t , as $\eta_{\sigma_t,t} = P(x_t|\sigma_t, x_{1:t-1};\theta)$. Notably, for the SDE model considered here, the likelihood contribution of an observation, x_t , depends only on the previous observation and not the entire history:

$$\begin{aligned}\eta_{\sigma_t,t} &= P(x_t|\sigma_t; x_{1:t-1}; \theta) \\ &= P(x_t|\sigma_t; x_{t-1}; \theta) \\ &= N(x_t|x_{t-1} + \Delta t M x_{t-1} + \Delta t \tilde{M} \sigma_t + \Delta t \mu),\end{aligned}$$

where $N(\cdot|\cdot)$ is the normal density function, and M , \tilde{M} , and μ are derived from the linear SDE in Eq. (1), and given by

$$M = \begin{bmatrix} -\kappa - \alpha & \kappa \\ \kappa & -\kappa - \alpha \end{bmatrix}$$

and

$$\tilde{M} = \begin{bmatrix} -v_+ & -v_+ & -v_- & -v_- \\ v_+ & v_- & v_+ & v_- \end{bmatrix}$$

and

$$\mu = [\kappa L \cos(\theta_t), \kappa L \cos(\theta_t)]^T.$$

641 In this notation, the state variable $x_t = [X_t^1, X_t^2]^T$ represents the positions of both sisters in a pair at
 642 time t and the state $\sigma_t \in \{[1, 0, 0, 0]^T, [0, 1, 0, 0]^T, [0, 0, 1, 0]^T, [0, 0, 0, 1]^T\}$ corresponding to the states
 643 $\{++, +-, -+, --\}$ for the sister kinetochore pair at time t .

Replacing these in the expressions above gives

$$\log L(x_{1:T}|\theta) = \sum_{t=1}^T \log(P(x_t|x_{1:t-1};\theta)),$$

where

$$P(x_t|x_{1:t-1};\theta) = \sum_{\sigma_{t-1}} \sum_{\sigma_t} P(\sigma_t|\sigma_{t-1};\theta) \xi_{\sigma_{t-1},t-1} \eta_{\sigma_t,t},$$

and hidden state probabilities are determined iteratively via:

$$\xi_{\sigma_t,t} = \frac{\sum_{\sigma_{t-1}} P(\sigma_t|\sigma_{t-1};\theta) \xi_{\sigma_{t-1},t-1} \eta_{\sigma_t,t}}{P(x_t|x_{1:t-1};\theta)}.$$

644 Sampling from the distribution of the hidden states

The expression required from the forward-backward algorithm is achieved by combining the results of the forward and backward algorithms as follows:

$$\begin{aligned}p(\sigma_k|x_{1:T}) &\propto p(\sigma_k, x_{1:T}) \\ &= p(x_{(k+1):T}|\sigma_k, x_{1:k})p(\sigma_k, x_{1:k}) \\ &= p(x_{(k+1):T}|\sigma_k, x_k)p(\sigma_k, x_{1:k})\end{aligned}$$

645 due to the graphical structure of the model (as in Fig. ??). Including the normalizing term, we have

$$\log p(\sigma_k|x_{1:T}) = \log p(x_{(k+1):T}|\sigma_k, x_k) + \log p(\sigma_k, x_{1:k}) - \log p(x_{1:T}). \quad (4)$$

646 Forward algorithm

The following derivation proceeds by summing over all possible values of the hidden state variable σ_{k-1} , using the definition of conditional probability, and the graphical structure of the model.

$$\begin{aligned}
 p(\sigma_k, x_{1:k}) &= \sum_{\sigma_{k-1}=1}^M p(\sigma_k, \sigma_{k-1}, x_{1:k}) \\
 &= \sum_{\sigma_{k-1}=1}^M p(x_k | \sigma_k, \sigma_{k-1}, x_{1:(k-1)}) p(\sigma_k | \sigma_{k-1}, x_{1:(k-1)}) p(\sigma_{k-1}, x_{1:(k-1)}) \\
 &= \sum_{\sigma_{k-1}=1}^M p(x_k | \sigma_k, x_{(k-1)}) p(\sigma_k | \sigma_{k-1}) p(\sigma_{k-1}, x_{1:(k-1)})
 \end{aligned} \tag{5}$$

647 where $p(x_k | \sigma_k, x_{(k-1)})$ is available via the SDE, $p(\sigma_k | \sigma_{k-1})$ is given via the transition matrix, and $p(\sigma_{k-1}, x_{1:(k-1)})$
 648 is equivalent to the term on the left hand side (LHS) for a different value of k . Applying this result
 649 iteratively forward in time from an initial condition allows us to solve for $p(\sigma_k, x_{1:k})$.

650 Backward algorithm

Similar steps are applied to derive a recursion for the backward algorithm.

$$\begin{aligned}
 p(x_{(k+1):T} | \sigma_k, x_k) &= \sum_{\sigma_{k+1}=1}^M p(x_{(k+1):T}, \sigma_{k+1} | \sigma_k, x_k) \\
 &= \sum_{\sigma_{k+1}=1}^M p(x_{(k+2):T} | \sigma_{k+1}, \sigma_k, x_{k+1}, x_k) p(x_{k+1} | \sigma_{k+1}, \sigma_k, x_k) p(\sigma_{k+1} | \sigma_k, x_k) \\
 &= \sum_{\sigma_{k+1}=1}^M p(x_{(k+2):T} | \sigma_{k+1}, x_{k+1}) p(x_{k+1} | \sigma_{k+1}, x_k) p(\sigma_{k+1} | \sigma_k)
 \end{aligned} \tag{6}$$

651 where $p(x_{(k+2):T} | \sigma_{k+1}, x_{k+1})$ is an iterated version of the LHS, $p(x_{k+1} | \sigma_{k+1}, x_k)$ is available via the SDE,
 652 and $p(\sigma_{k+1} | \sigma_k)$ is given via the transition matrix. We apply this iteratively from $k = T - 1, \dots, 1$.

653 Backward sampling

654 In order to make statements about switching events, we need to consider sequences of states
 655 forming a pattern corresponding to coherent switches from one coherent state to another via an
 656 intermediate state. To address this, we sample from the full hidden state sequence given all the
 657 data, and assess this for switches.

To enable the sampling, we observe that the pairwise marginal distribution can be expressed as

$$\begin{aligned}
 p(\sigma_k, \sigma_{k+1} | x_{1:T}) &\propto p(x_{1:k}, x_{k+1}, x_{k+2:T}, \sigma_k, \sigma_{k+1}) \\
 &= p(x_{k+2:T} | x_{k+1}, x_{1:k}, \sigma_k, \sigma_{k+1}) p(x_{k+1}, x_{1:k}, \sigma_k, \sigma_{k+1}) \\
 &= p(x_{k+2:T} | x_{k+1}, \sigma_{k+1}) p(x_{k+1} | x_{1:k}, \sigma_k, \sigma_{k+1}) p(x_{1:k}, \sigma_k, \sigma_{k+1}) \\
 &= p(x_{k+2:T} | x_{k+1}, \sigma_{k+1}) p(x_{k+1} | x_k, \sigma_{k+1}) p(\sigma_{k+1} | x_{1:k}, \sigma_k) p(\sigma_k, x_{1:k}) \\
 &\propto p(x_{k+2:T} | x_{k+1}, \sigma_{k+1}) p(x_{k+1} | x_k, \sigma_{k+1}) p(\sigma_{k+1} | \sigma_k) p(\sigma_k | x_{1:k}).
 \end{aligned}$$

This expression contains known terms and terms that can be computed via the forward and backward algorithms. Furthermore, when σ_{k+1} is known and we are considering how to sample back in time through the hidden states, we have that

$$\begin{aligned}
 p(\sigma_k | \sigma_{k+1}, x_{1:T}) &\propto p(\sigma_k, \sigma_{k+1} | x_{1:T}) \\
 &\propto p(\sigma_{k+1} | \sigma_k) p(\sigma_k | x_{1:k})
 \end{aligned} \tag{7}$$

658 since the other terms in the expression above do not depend on σ_k .

659 The strategy for the backward sampling algorithm is therefore to sample initially from $p(\sigma_T | x_{1:T})$,
 660 which is available from the forward algorithm, and subsequently to simulate backward in time from
 661 T to 1 via the conditional distribution given in Eq (7).

662 **Prior distributions**

663 We impose broad prior distributions on the parameters of the biophysical model, as shown in
 664 Supp. Table 1. For the natural length of the spring, L , we impose an informative prior based on an
 665 additional nocodazole washout experiment (nocodazole interferes with polymerization of micro-
 666 tubules). This avoids an unidentifiability in the model as in *Armond et al. (2015)*. Additionally, we
 667 use an informative prior for the time of anaphase, t_A , based on first fitting a changepoint model
 668 (with a uniform prior on t_A) to get an initial estimate for anaphase onset to guide the biophys-
 669 ical model and avoid exploring parameter space corresponding to pathological behaviour such as
 670 anaphase at the start or end of movies.

671 **Convergence diagnostics**

672 Convergence and mixing of MCMC chains is assessed via the Gelman-Rubin \hat{R} statistic *Gelman and*
 673 *Rubin (1992)*; *Vehtari et al. (2021)* using only results where $\hat{R} < 1.05$ for all parameters.

674 Of $N = 58$ cells tracked, MCMC chains were run successfully for $N = 32$ cells. Where MCMC
 675 chains failed to run this was due either to poor tracking results in that cell (insufficient tracked
 676 kinetochore pairs) or long time series such that the MCMC chains progressed extremely slowly
 677 (failed to find the typical set). Of the $n = 885$ kinetochore pairs across 32 cells where MCMC chains
 678 ran successfully, MCMC chains from $n = 201$ kinetochore pairs failed to converge as assessed by
 679 the \hat{R} statistic, leaving estimates from $n = 684$ kinetochore pairs. Of the 32 cells with MCMC results,
 680 chains failed to converge on any kinetochore pairs for 7 cells as assessed by the \hat{R} statistic. Thus
 681 results are reported for $n = 684$ kinetochore pairs from $N = 25$ cells.

682 **Changepoint model**

A simple changepoint model (*Armond et al., 2019*) to assess the time of anaphase assumes that the
 intersister distance between a kinetochore pair is constant in metaphase, and increases linearly in
 time during anaphase. If d_i is the 1D intersister distance (in the x direction perpendicular to the
 metaphase plate) between kinetochore sisters, then at time point t_i

$$d_{t_i} = \begin{cases} a_1 + \varepsilon_i & \text{for } t_i < t_A, \\ a_2 + b_2 t_i + \varepsilon_i & \text{for } t_i \geq t_A, \end{cases}$$

683 with the condition that $a_1 = a_2 + b_2 t_A$. Since this model is simpler than the biophysical model, a
 684 uniform prior can be used for t_A and weakly informative priors for other parameters.

685 **Hawkes process model**

The Hawkes process is a point process similar to an inhomogeneous Poisson process, with the key
 difference that the intensity depends on past events. The conditional intensity given the history of
 the process up to time t with switching events at times t_1, \dots, t_n is

$$\lambda(\mathbf{s}, t, j) = \mu(\mathbf{s}, t) + \theta \sum_{i: t_i < t} k_t(t, t_i) k_s(\mathbf{s}, \mathbf{s}_i) k_j(j, j_i)$$

where we have kernels in time, $k_t(t, t_i)$, in space, $k_s(\mathbf{s}, \mathbf{s}_i)$, and between kinetochore pairs, $k_j(j, j_i)$.
 We take an exponential kernel in time

$$k_t(t, t_i) = \omega \exp(-\omega(t - t_i)),$$

with timescale $1/\omega$, along with a Gaussian kernel in space

$$k_s(\mathbf{s}, \mathbf{s}_i) = 1/(2\pi l^2) \exp(-d(\mathbf{s}, \mathbf{s}_i)^2/2l^2),$$

where l is the length scale and $d(\mathbf{s}, \mathbf{s}_i)$ is the Euclidean distance between \mathbf{s} and \mathbf{s}_i . The kernel between kinetochore pairs ensures that we consider only interactions between switches of different pairs

$$k_J(j, j_i) = \begin{cases} 0 & \text{if } j = j_i, \\ 1 & \text{otherwise.} \end{cases}$$

686 We assume that the background rate is constant in time and space such that $\mu(\mathbf{s}, t) = \mu_0$.

To compute the likelihood for the Hawkes process model, we follow methods used in [Loeffler and Flaxman \(2018\)](#). The likelihood of the Hawkes process model is as for an inhomogeneous Poisson process, and is given by

$$\mathcal{L} = \prod_i \lambda(\mathbf{s}_i, t_i, j_i) \exp\left(-\sum_j \int_S \int_0^T \lambda(\mathbf{s}, t, j) ds dt\right).$$

The log likelihood can be written as

$$\begin{aligned} \log \mathcal{L} = & \sum_r \mu_0 + \theta \sum_{i:t_i < t_r} k_i(t_r, t_i) k_s(\mathbf{s}_r, \mathbf{s}_i) k_J(j_r, j_i) \\ & - \int_S \int_0^T \left(\mu_0 + \theta \sum_{i:t_i < t} k_i(t, t_i) k_s(\mathbf{s}, \mathbf{s}_i) k_J(j, j_i) \right) ds dt \end{aligned} \quad (8)$$

To simplify this expression, note that the final part of the integral term equates to the total number of events in the time interval considered

$$\int_S \int_0^T \left(\theta \sum_{i:t_i < t} k_i(t, t_i) k_s(\mathbf{s}, \mathbf{s}_i) k_J(j, j_i) \right) ds dt = n$$

687 assuming the kernels used are normalized; this term is therefore constant with respect to the pa-
688 rameters of the Hawkes process which simplifies the likelihood computation.

689 We implement this model via the log likelihood computation above in Stan ([Carpenter et al.,](#)
690 [2017](#)) and run 4 MCMC for 1000 iterations allowing us to sample from the posterior distribution
691 via Hamiltonian Monte Carlo.

692 Simulation from 4 state Markov model

693 To consider fluctuations in the proportion of kinetochore pairs in each state, we consider forward
694 simulations from the Markov model for the hidden states in the anaphase model (as in Fig. 2C),
695 but only including the metaphase states, $(++, +-, -+, -)$. The transition matrix thus depends on p_{coh}
696 and p_{icoh} , the framewise probability of switching out of a given state for an individual kinetochore
697 sister. Independent simulations are performed for $N = 46$ kinetochore pairs (corresponding to the
698 number of kinetochore pairs in untransformed human cells) and averaged to give the proportion
699 of pairs in a given state.

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706 Additional Information

707 Author contributions

708 Jonathan U Harrison, Conceptualization, Methodology, Software, Formal Analysis, Writing - Original
709 Draft, Writing - Review and Editing. Onur Sen, Conceptualization, Investigation, Design of Experi-
710 ments and Data Collection, Writing - Review and Editing. Andrew D McAinsh, Conceptualization,

711 Supervision, Project Administration, Funding Acquisition, Writing - Review and Editing. Nigel J Bur-
712 roughs, Conceptualization, Methodology, Supervision, Project Administration, Funding Acquisition,
713 Writing - Review and Editing.

714 References

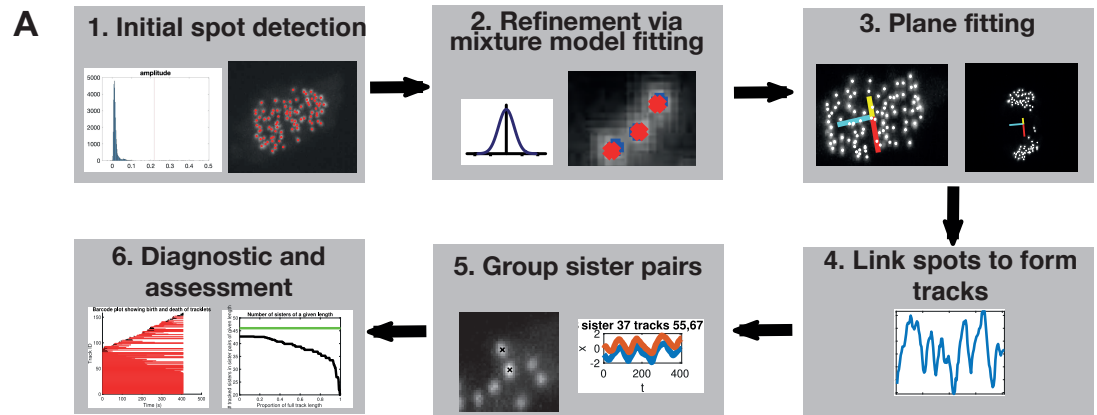
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852 **Appendix 1**

853 **Supplementary Figures**



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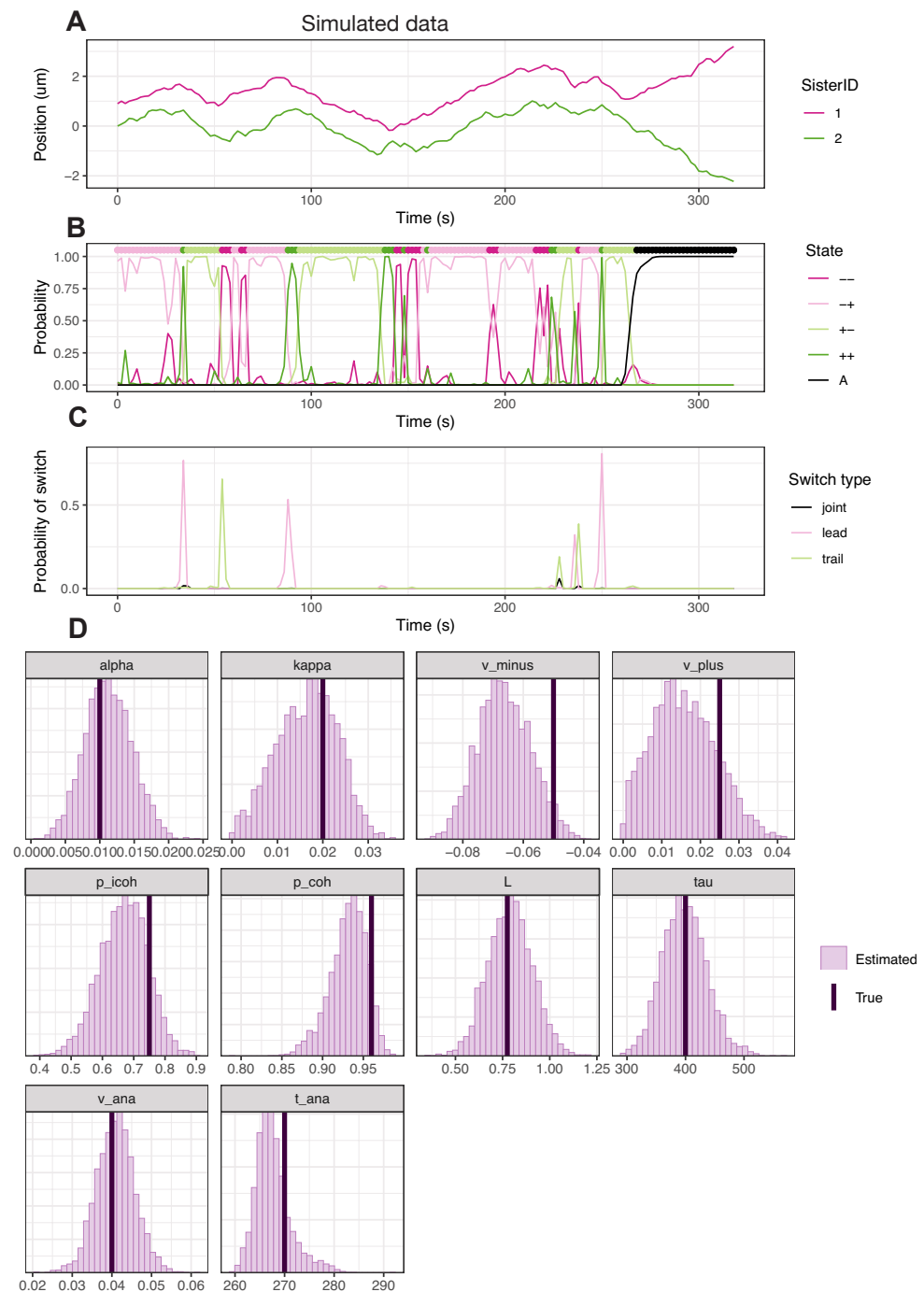
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Appendix 1 Figure 1. Supplementary Figure 1 Tracking pipeline for near-complete tracking of kinetochores. Candidate spots are detected via an adaptive threshold technique (step 1). Spot locations are refined using a Gaussian mixture model (step 2). A plane is fitted to orientate a reference coordinate system with respect to the metaphase plate (step 3). Detected particles are linked between frames over time to form tracks (step 4). Kinetochose sister pairs are grouped based on metaphase dynamics (step 5). Tracks of kinetochose sister pairs can be assessed via diagnostic tools (step 6).



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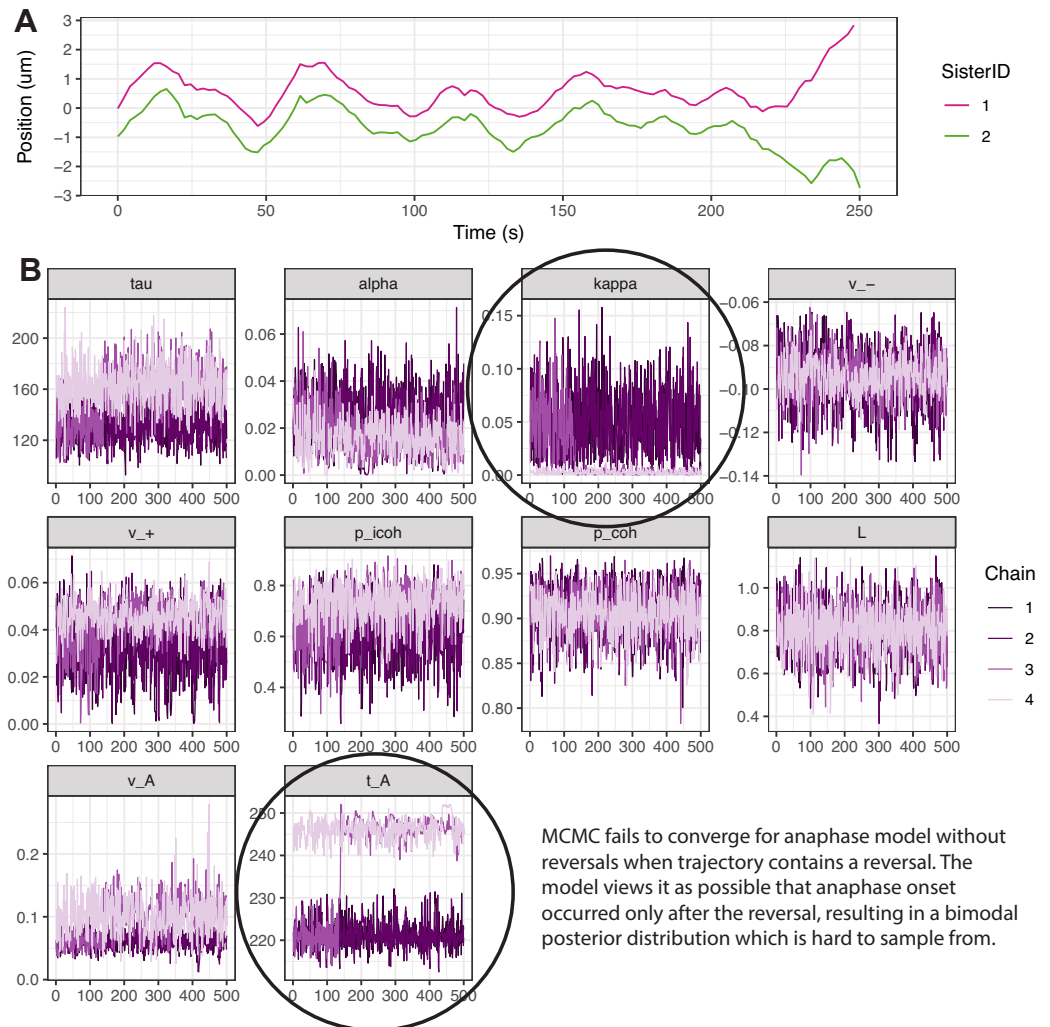
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Appendix 1 Figure 2. Supplementary Figure 2 Estimation of parameters of the biophysical mode and annotation of hidden states on simulated data. (A) Simulated data from the anaphase model until a final time of 320 s (160 frames) with parameters: $\tau = 400$, $\alpha = 0.01 \text{ s}^{-1}$, $\kappa = 0.02 \text{ s}^{-1}$, $v_- = -0.05 \text{ }\mu\text{m/s}$, $v_+ = 0.025 \text{ }\mu\text{m/s}$, $p_{\text{icoh}} = 0.75$, $p_{\text{coh}} = 0.96$, $L = 0.775 \text{ }\mu\text{m}$, $v_A = 0.04 \text{ }\mu\text{m/s}$, $t_A = 270 \text{ s}$, $\Delta t = 2 \text{ s}$. (B) Hidden microtubule attachment states in the simulated data and probabilities of each state as sampled from the posterior. Coloured points at the top of the plot indicate the true simulated hidden state, while the lines show the estimated probability of each state. (C) Switching probability based on patterns of hidden states sampled from the posterior distribution. Note that only switches from one coherent state (+- or --) to the opposite coherent state, via an intermediate incoherent state (++) or (--) are considered. (D) Marginal posterior histograms for each of the model parameters. Vertical lines indicate the true parameters used to simulate the data. These lines lie within the posterior density indicating that the parameters used to simulate the data can be recovered.



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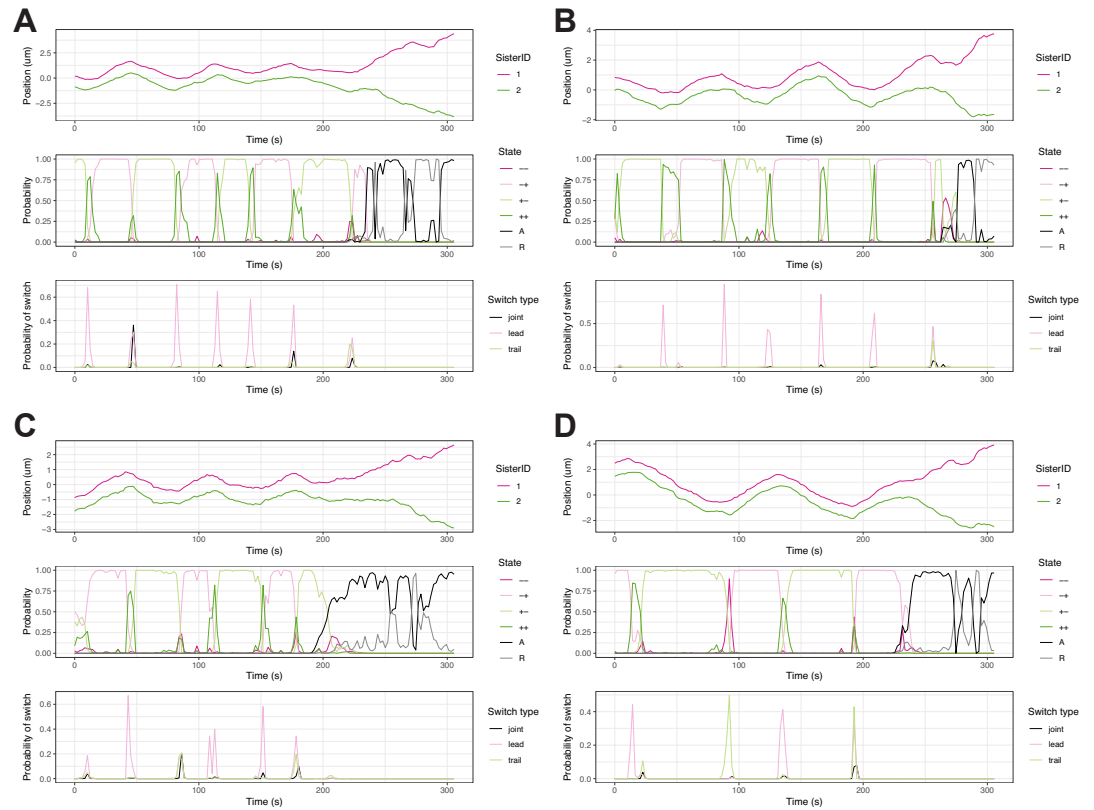
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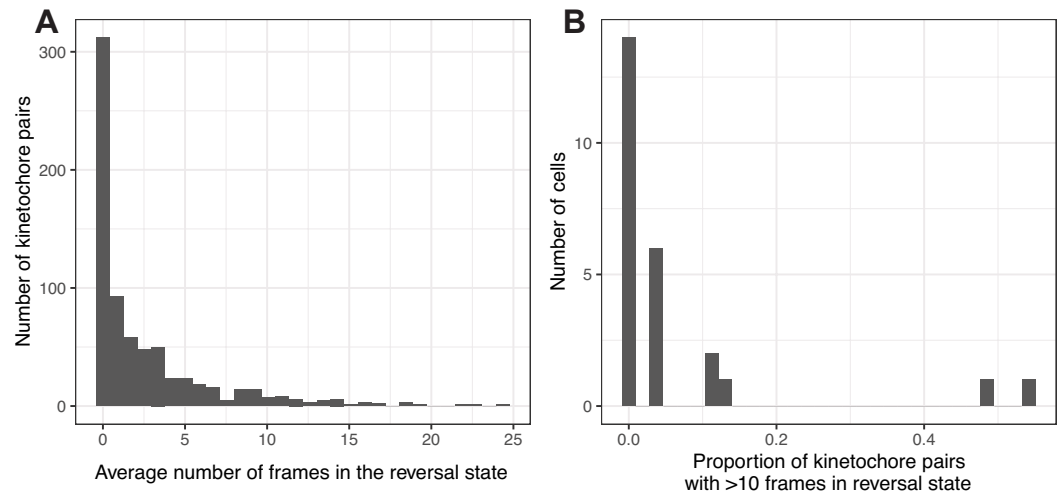
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Appendix 1 Figure 3. Supplementary Figure 3 MCMC convergence issues for anaphase model when reversals are present in the data. (A) Kinetochores tracking data for a single kinetochores pair exhibiting a reversal away from the pole in anaphase (Sister 2 around 230s), before returning to poleward motion. (B) MCMC traceplots for 4 chains and all model parameters of the anaphase model. For parameters κ (spring constant) and t_A (time of anaphase onset) the chains have not mixed properly due to a bimodal posterior distribution. The posterior has one model for a normal or early anaphase onset when the kinetochores pair separates at around 220s, and another mode for a late anaphase onset at around 245s after the reversal.



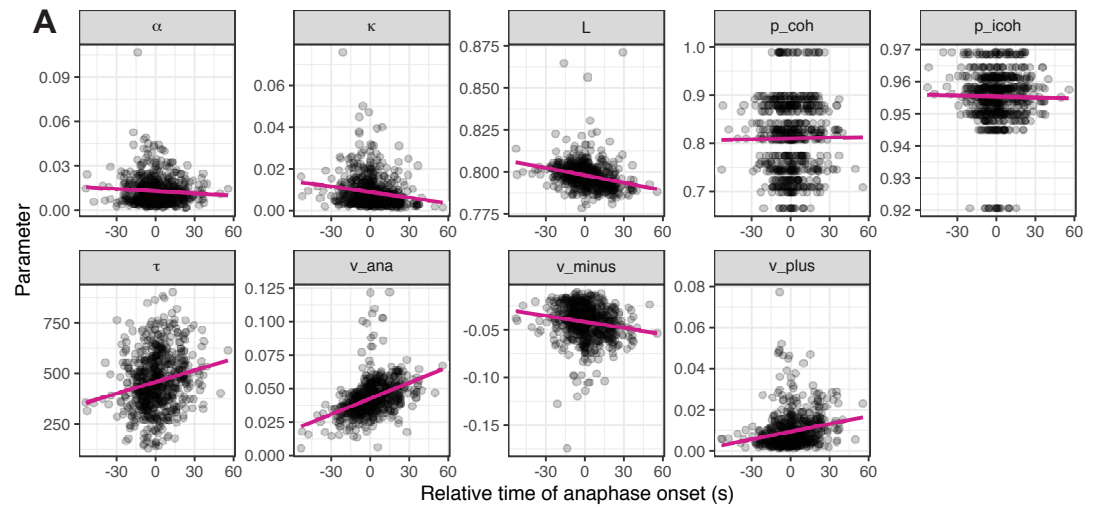
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Appendix 1 Figure 4. Supplementary Figure 4 Example trajectories and inferred hidden states (and probability of a switch based on these states) using the hierarchical anaphase model with reversals for 4 different kinetochore pairs shown in (A), (B), (C), and (D). All four pairs are from the same cell shown in Figure 4.



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Appendix 1 Figure 5. Supplementary Figure 5 Reversals are rare events for most cells. (A) Histogram of the average number of frames spent in the reversal state over the population of kinetochore pairs. (B) Histogram of the proportion of kinetochore pairs in a cell that spent on average more than 10 frames in the reversal state, *R*. Results from $N = 25$ cells and $n = 676$ kinetochore pairs.



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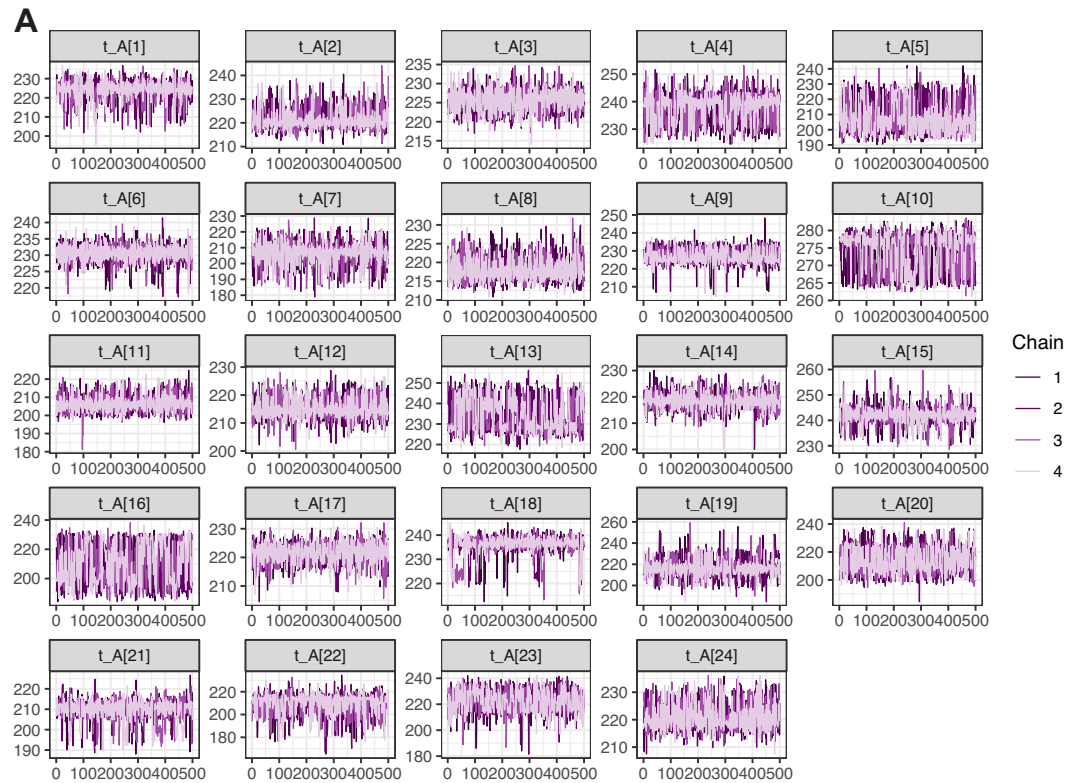
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Appendix 1 Figure 6. Supplementary Figure 6

Correlations between relative time of anaphase onset and model parameters, based on median of estimated posterior distributions. Each point corresponds to a kinetochore pair, with $n = 684$ kinetochore pairs from $N = 25$ cells. Solid magenta line shows linear fit between time of anaphase onset (relative to the median over pairs in a cell) and the estimated median model parameters.



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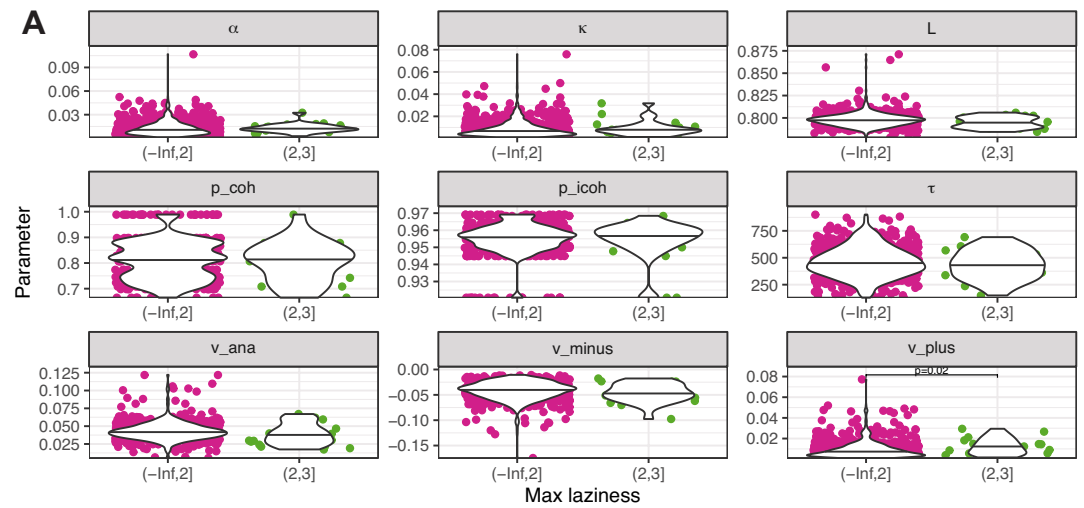
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Appendix 1 Figure 7. Supplementary Figure 7

MCMC traceplots based on the anaphase model with reversals for the time of anaphase onset, t_A , for each of the tracked kinetochore pairs in a cell. Four MCMC chains are run (see Methods) and are shown in different colours. The time of anaphase onset can have a bimodal distribution for some kinetochore pairs (eg. pairs 10 and 13 here) and thus checking traceplots for this parameter in particular can highlight problems with converged or mixing of MCMC chains. All pairs shown have $\hat{R} < 1.05$ and including the reversal state, R , has helped avoid convergence and mixing issues present without this state.



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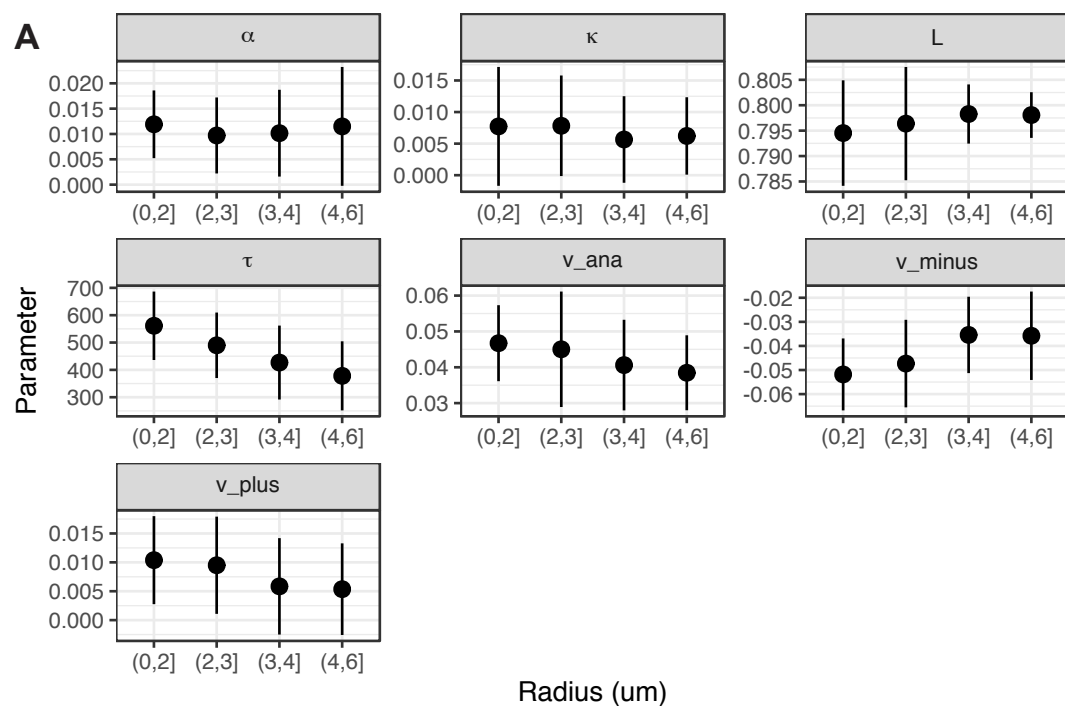
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Appendix 1 Figure 8. Supplementary Figure 8

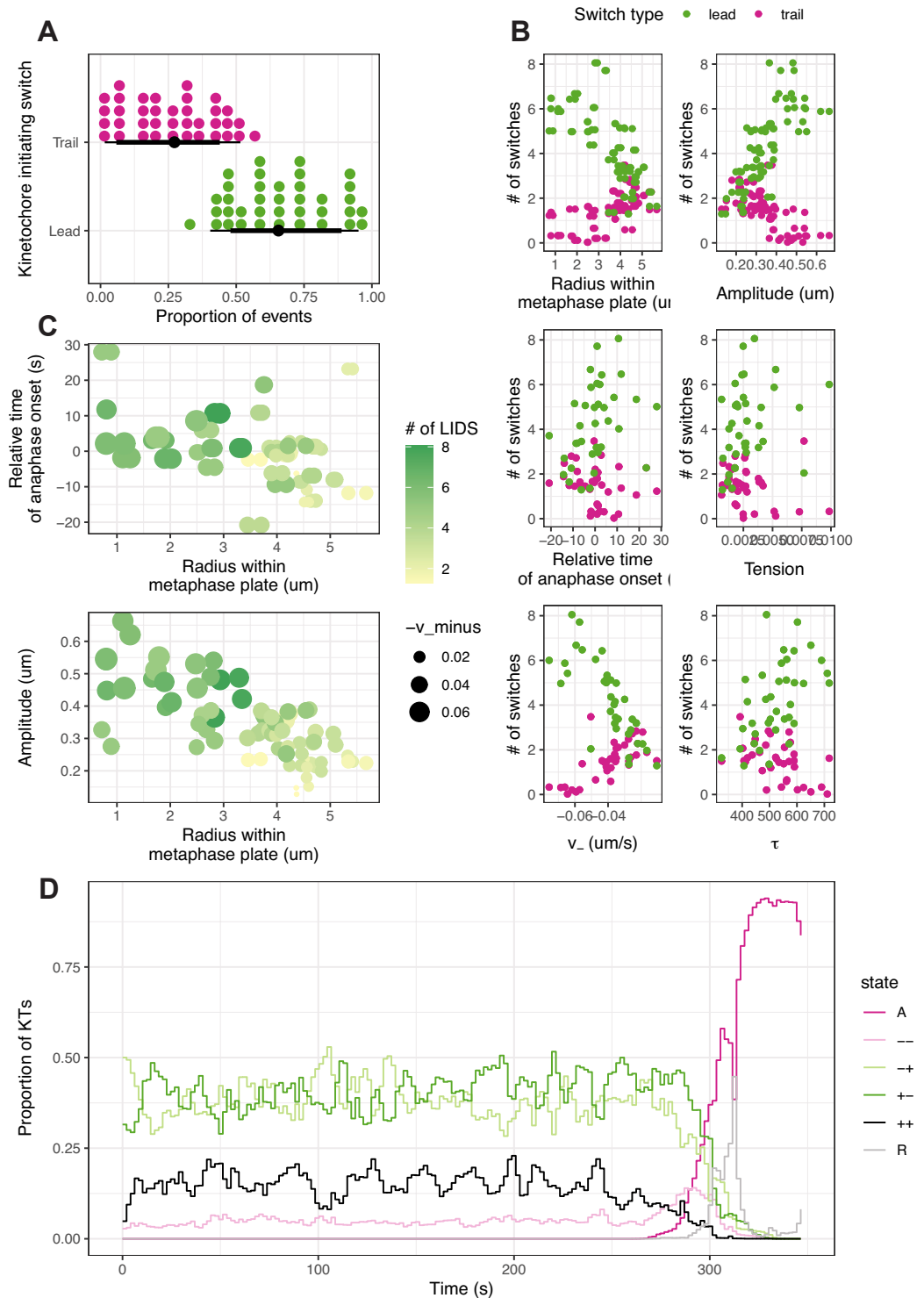
Distribution of inferred values for the biophysical parameters across kinetochore pairs with high laziness (>2) versus low laziness (<2). For definition of laziness, see *Sen et al. (2021)*. Results from $N = 25$ cells and $n = 684$ kinetochore pairs.



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Appendix 1 Figure 9. Supplementary Figure 9

Dependence of biophysical parameters on radial position within the metaphase plate. Points indicate medians across $n = 684$ kinetochores from $N = 25$ cells at a given radial location. Lines indicate median \pm one standard deviation across the population (at a given radial location). Radial position is divided into (unequal sized) bins.



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Appendix 1 Figure 10. Supplementary Figure 10

Directional switches of oscillating chromosomes vary across the metaphase plate. As for Figure 7, but for a different cell to indicate variability between cells. (A) Fraction of LIDS (green) and TIDS (pink) events as a proportion of the total number of switching events including joint switches. Each kinetochore pair gives rise to a LIDS and TIDS dot. (B) Relationship between the number of directional switches initiated by the leading (green) or trailing (pink) kinetochore sister, and other summary statistics describing the oscillatory dynamics. (C) Relationship between the number of

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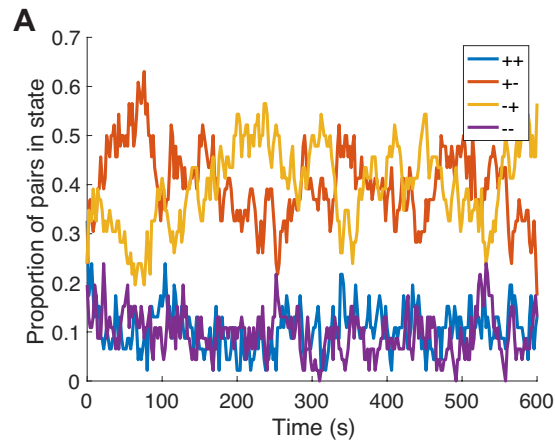
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LIDS events and other summary statistics indicating that many of these variables change together based on spatial position of kinetochore pairs within the metaphase plate. (D) Proportion of kinetochore sister pairs in a given hidden state at each time point.



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Appendix 1 Figure 11. Supplementary Figure 11

Simulating from a 4 state Markov model with spontaneous switching between states (see Methods) for $N = 46$ kinetochore pairs exhibits fluctuations in the proportion of kinetochore pairs in each state. Parameters used in simulation $(p_{\text{coh}}, p_{\text{icoh}}) = (0.96, 0.83)$.

Parameter	Prior	Support
α	$N(0.01, 0.1)$	$[0, \infty)$
κ	$N(0.05, 0.1)$	$[0, \infty)$
v_-	$N(-0.03, 0.1)$	$(-\infty, 0]$
v_+	$N(0.03, 0.1)$	$[0, \infty)$
p_{icoh}	$Beta(2, 1)$	$(0, 1)$
p_{coh}	$Beta(2.5, 1)$	$(0, 1)$
L	$N(0.79, 0.119)$	$[0, \infty)$
τ	$N(t^*, 14)$	$[0, \infty)$

⁹⁴⁵ **Supplementary Table 1: Priors for the anaphase model and anaphase rever-**
⁹⁴⁶ **sals model**

⁹⁴⁷ where t^* is the median posterior marginal estimate for t_A using the changepoint model to provide
⁹⁴⁸ an informative prior for t_A .

949 Appendix 2

950 Derivation of metaphase dynamics equations

951 To derive the metaphase dynamics in eq. (1), we proceed as follows. For kinetochore sister
952 j , we apply force balance, ignoring inertial forces since the system is in a high viscous limit:

$$953 F_{\text{drag}}^j + F_{\text{K-fibre}}^j + F_{\text{spring}}^j + F_{\text{PEF}}^j + F_{\text{noise}}^j = 0.$$

954 The drag force is taken as proportional to kinetochore velocity,
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$$956 F_{\text{drag}}^j = -\gamma \frac{dX_t^j}{dt}$$

957 where γ is the effective drag coefficient. The K-fibre force, $F_{\sigma_t^j}$, is dependent on the hidden
958 microtubule polymerization state, and other forces are as described in the main text. Thus
959 we find

$$960 \gamma dX_t^1 = (-F_{\sigma_t^1} - \kappa_0 (X_t^1 - X_t^2 - L \cos \theta_t) - \alpha_0 X_t^1) dt + s_0 dW_t^1, \quad (9)$$

$$961 \gamma dX_t^2 = (F_{\sigma_t^2} - \kappa_0 (X_t^2 - X_t^1 + L \cos \theta_t) - \alpha_0 X_t^2) dt + s_0 dW_t^2.$$

962 Dividing by the drag coefficient, γ , and redefining rescaled force parameters for the PEF,
963 $\alpha = \alpha_0/\gamma$, spring constant, $\kappa = \kappa_0/\gamma$, noise magnitude, $s = s_0/\gamma$ and microtubule force param-
964 eters $v_+ = F_+/\gamma$, $v_- = F_-/\gamma$, we obtain

$$965 dX_t^1 = (-v_{\sigma_t^1} - \kappa (X_t^1 - X_t^2 - L \cos \theta_t) - \alpha X_t^1) dt + s dW_t^1, \quad (10)$$

$$966 dX_t^2 = (v_{\sigma_t^2} - \kappa (X_t^2 - X_t^1 + L \cos \theta_t) - \alpha X_t^2) dt + s dW_t^2.$$

967 Due to this rescaling by the drag coefficient, which is hard to estimate, the K-fibre force
968 parameters (v_+ , v_-) that we infer in this work have units of speed [$\mu\text{m/s}$], while the PEF
969 parameter, α , and spring constant, κ , have units of [s^{-1}].
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