

1 **Title:** Microneedle manipulation of the mammalian spindle reveals specialized, short-
2 lived reinforcement near chromosomes

3

4 Pooja Suresh^{1,3}, Alexandra F. Long^{2,3}, Sophie Dumont¹⁻³⁺

5 ¹ Biophysics Graduate Program, ² Tetrad Graduate Program, ³ Department of Cell and
6 Tissue Biology, University of California, San Francisco, ⁺ corresponding author

7

8 ⁺Corresponding author: sophie.dumont@ucsf.edu

9

10 **Abstract**

11

12 The spindle generates force to segregate chromosomes at cell division. In mammalian
13 cells, kinetochore-fibers connect chromosomes to the spindle. The dynamic spindle
14 anchors kinetochore-fibers in space and time to coordinate chromosome movement.
15 Yet, how it does so remains poorly understood as we lack tools to directly challenge this
16 anchorage. Here, we adapt microneedle manipulation to exert local forces on the
17 spindle with spatiotemporal control. Pulling on kinetochore-fibers reveals that the
18 spindle retains local architecture in its center on the seconds timescale. Upon pulling,
19 sister, but not neighbor, kinetochore-fibers remain tightly coupled, restricting
20 chromosome stretching. Further, pulled kinetochore-fibers freely pivot around poles but
21 not around chromosomes, retaining their orientation within 3 μm of chromosomes. This
22 local reinforcement has a 20 s lifetime, and requires the microtubule crosslinker PRC1.
23 Together, these observations indicate short-lived, specialized reinforcement of the

24 kinetochore-fiber in the spindle center. This could help the spindle protect local structure
25 near chromosomes from transient forces while allowing its remodeling over longer
26 timescales, thereby supporting robust chromosome attachments and movements.

27

28 **Introduction**

29

30 The spindle is the macromolecular machine that segregates chromosomes at cell
31 division. Mechanical force helps build the spindle, stabilize chromosome attachments
32 (Nicklas & Koch, 1969), and ultimately move chromosomes (Inoue & Salmon, 1995). To
33 perform its function, the mammalian spindle must generate and respond to force while
34 maintaining a mechanically robust structure that can persist for about an hour. Yet, to
35 remodel itself during mitosis, the spindle must also be dynamic, with its microtubules
36 turning over on the order of seconds and minutes (Gorbsky & Borisy, 1989; Saxton et
37 al., 1984; Zhai, Kronebusch & Borisy, 1995). How the spindle can be dynamic while also
38 being mechanically robust remains an open question. While we know most of the
39 molecules required for mammalian spindle function (Hutchins et al., 2010; Neumann et
40 al., 2010), the spindle's emergent mechanical properties and underlying physical
41 principles remain poorly understood. In large part, this is because of a lack of tools to
42 probe the mammalian spindle's physical properties.

43 A key structural element of the mammalian spindle is the kinetochore-fiber (k-
44 fiber), a bundle of 15-25 kinetochore-bound microtubules (kMTs) (McEwen, Ding &
45 Heagle, 1998) of which many reach the spindle pole (McDonald et al., 1992; Rieder,
46 1981). K-fibers generate force to move chromosomes (Grishchuk et al., 2005;

47 Koshland, Mitchison & Kirschner, 1988) and provide connections to opposite spindle
48 poles. To do so, k-fibers must be robustly anchored and correctly oriented within the
49 spindle. K-fibers make contacts along their length with a dense network of non-
50 kinetochore microtubules (non-kMTs) (Mastronarde et al., 1993; McDonald et al., 1992),
51 likely through both motor and non-motor microtubule binding-proteins (Elting et al.,
52 2017; Kajtez et al., 2016; Vladimirou et al., 2013). We know that the non-kMT network
53 bridges sister k-fibers together (Kajtez et al., 2016; Mastronarde et al., 1993; Witt, Ris &
54 Borisy, 1981), and that it can locally anchor k-fibers and bear load in the spindle's
55 longitudinal (pole-pole) axis (Elting et al., 2017). Yet, how the dynamic spindle
56 mechanically anchors k-fibers in space and time remains poorly mapped and
57 understood. Specifically, we do not know if k-fibers are anchored uniformly along their
58 length, to what structures they are anchored to, over what timescale this anchorage
59 persists before remodeling is allowed, or more broadly how local forces propagate
60 through the spindle's longitudinal and lateral axes. These questions are central to the
61 spindle's ability to robustly maintain its structure, respond to force and ultimately move
62 chromosomes.

63 We currently lack tools to apply forces with both spatial and temporal control to
64 mammalian spindles. For example, laser ablation, commonly used to alter forces in the
65 spindle, can locally perturb spindle structure, but lacks control over the duration and
66 direction of ensuing force changes. Further, mammalian spindles cannot yet be
67 reconstituted *in vitro*. To understand how the dynamic spindle robustly anchors k-fibers,
68 and to ultimately map mammalian spindle mechanics to function, we need approaches
69 to apply local and reproducible forces inside cells, with spatiotemporal control. Here, we

70 adapt microneedle manipulation of the metaphase spindle in mammalian cells for the
71 first time, combining it with live fluorescence imaging and molecular perturbations. We
72 base our manipulation efforts on pioneering work in insect spermatocyte cells (Nicklas &
73 Koch, 1969; Nicklas, Kubai & Hays, 1982), newt cells (Skibbens & Salmon, 1997) and
74 more recent work in *Xenopus* extract meiotic spindles (Gatlin et al., 2010; Shimamoto et
75 al., 2011; Takagi et al., 2019).

76 Using this approach, we find that the mammalian mitotic spindle prioritizes the
77 preservation of local structure in its center under seconds-long forces. We show that k-
78 fibers can freely pivot around spindle poles but resist movement near chromosomes
79 due to lateral and longitudinal reinforcement in the spindle center. We find that this
80 reinforcement is specialized, only present near chromosomes, and short-lived with a
81 lifetime of seconds. Finally, we show that this reinforcement is mediated by the
82 microtubule crosslinker PRC1. Our work suggests a model for k-fiber anchorage that is
83 local in both space and time: short-lived, local reinforcement isolates k-fibers from
84 transient but not sustained forces in the spindle center. Thus, the spindle center can
85 robustly maintain its connections to k-fibers and chromosomes, and yet remodel its
86 structure and move chromosomes over minutes. Together, this study provides a
87 framework for understanding how the spindle and other macromolecular machines can
88 be dynamic yet mechanically robust to perform their cellular functions.

89

90 **Results**

91

92 *Microneedle manipulation can exert local forces with spatiotemporal control on the*
93 *mammalian spindle*

94

95 To probe how the k-fiber is anchored in the mammalian spindle in space and time, we
96 sought to mechanically challenge its connections to the rest of the spindle. Specifically,
97 we looked for an approach to apply local forces on a k-fiber with the ability to control the
98 position, direction and duration of force, in a system compatible with live fluorescence
99 imaging to visualize spindle deformations. Based on work in insect meiotic spindles (Lin
100 et al., 2018; Nicklas & Koch, 1969; Nicklas et al., 1982), we adapted microneedle
101 manipulation to mammalian cells. We used PtK2 cells since they are molecularly
102 tractable (Udy et al., 2015), flat, strongly adherent, and have only 13 chromosomes
103 (Walen & Brown, 1962), helpful for pulling on individual k-fibers. We optimized several
104 parameters to make this approach reproducible and compatible with cell health (detailed
105 in Methods). We used a glass microneedle whose outer diameter was $1.1 \pm 0.1 \mu\text{m}$ in the
106 imaging plane, bent to contact the cell at a 90° angle, and fluorescently coated its tip to
107 visualize its position. We connected the microneedle to a stepper-motor
108 micromanipulator, and changed x-y-z position either manually or with computer control.
109 The latter ensured the smooth movements necessary to prevent cell membrane rupture,
110 and to achieve reproducible microneedle motions from cell to cell.

111 To assess whether manipulation locally or globally perturbed dividing PtK2 cells,
112 we imaged microtubules (GFP-tubulin), the microneedle (BSA-Alexa 555 or 647) and
113 the membrane (CellMask Orange). We found that the microneedle deformed the
114 membrane locally, rather than globally pressing the membrane against the whole

115 spindle (Figure 1A-B). Consistent with local deformation, the overall cell height did not
116 change upon manipulation (Figure 1C). The membrane appeared intact since the
117 membrane contoured the microneedle during manipulation (Figure 1B) and the cell
118 impermeable dye propidium iodide did not enter the cell during and after manipulation
119 (Figure 1- figure supplement 1) (Nicklas et al., 1982).

120 We used this approach to exert local, spatiotemporally controlled pulls on
121 individual outer k-fibers in PtK2 GFP-tubulin metaphase cells (Figure 1D), and were
122 able to deform their spindles. We pulled the k-fiber in the lateral direction (roughly
123 perpendicular to the pole-pole axis) away from the spindle by $1.8 \pm 0.4 \mu\text{m}$ for $11.9 \pm 2.1 \text{ s}$
124 ($n=7$ cells) and $2.5 \pm 0.2 \mu\text{m}$ for $60.5 \pm 8.8 \text{ s}$ ($n=23$ cells) (Figure 1E-F). We imaged the
125 spindle before, during and after the pull (Figure 1G) and found that the spindle returned
126 to its original structure upon microneedle removal (Figure 1H). The spindle typically
127 entered anaphase within 15 min of microneedle removal (Figure 1G), consistent with
128 cell health. These observations indicate that we now have a local and reproducible
129 approach to mechanically challenge the k-fiber's connections to the mammalian spindle
130 over space and time.

131

132 *Pulling on kinetochore-fibers reveals the spindle's ability to retain local architecture near*
133 *chromosomes under seconds-long forces*

134

135 To probe how k-fibers are crosslinked to the spindle microtubule network, we examined
136 the dataset where we manipulated the outer k-fiber over $11.9 \pm 2.1 \text{ s}$ (Figure 1G, red
137 traces, $n=7$ cells). This short timescale was chosen to probe the spindle's passive

138 connections before significant remodeling had occurred: it is shorter than the lifetime of
139 kinetochore-microtubules or detectable k-fiber growth or shrinkage (Gorbsky & Borisy,
140 1989; Zhai et al., 1995) (Figure 2- figure supplement 1), and on the order of the half-life
141 of non-kMTs (Saxton et al., 1984). We constructed strain maps (Figure 2A) to quantify
142 the extent of deformation across the spindle in response to the manipulation. In
143 principle, which structures move with the deformed k-fiber, and how much they move,
144 could reveal the position and strength of anchorage within the spindle.

145 Upon manipulating the k-fiber over 12 s (Figure 2B), we observed structural
146 changes in the spindle that were local (Figure 2C). The deformed k-fiber bent and
147 deformations in the same spindle-half were only detectable within the first 5 μm from the
148 microneedle (exponential decay constant = $0.54 \mu\text{m}^{-1}$ (Figure 2D, Figure 2- figure
149 supplement 2A)), suggesting weak mechanical coupling between neighboring k-fibers
150 (Elting et al., 2017; Vladimirov et al., 2013). As a control, increasing crosslinking with a
151 Kinesin-5 rigor drug (FCPT (Groen et al., 2008), n=4 cells) led to a more gradual spatial
152 decay of deformation (exponential decay constant = $0.25 \mu\text{m}^{-1}$ (Figure 2D, Figure 2-
153 figure supplement 2B)), with deformations propagating further through the spindle-half.
154 This is consistent with the idea that crosslinking strength tunes anchorage within the
155 spindle and thereby modulates its material properties (Shimamoto et al., 2011; Takagi
156 et al., 2019). Together, these findings suggest that force propagation is dampened
157 between neighboring k-fibers, which may effectively mechanically isolate them (Matos
158 et al., 2009) and promote their independent functions.

159 Surprisingly, pulling on the k-fiber over this short timescale did not lead to an
160 increase in inter-kinetochore distance (distance between sister k-fiber plus-ends, Figure

161 2E,F). Yet, we know that chromosomes relax and then stretch after k-fiber ablation near
162 plus-ends over a similar timescale (Elting et al., 2014; Sikirzhytski et al., 2014),
163 indicating that they are elastic on the timescale of these manipulations. Instead, the
164 spindle shortened by $0.5 \pm 0.1 \mu\text{m}$ in response to the manipulation (Figure 2E,G) (Gatlin
165 et al., 2010; Itabashi et al., 2009). This suggests that a structure other than the
166 chromosome couples sister k-fibers across spindle halves on the seconds timescale.
167 Consistent with this idea, the sister k-fiber, opposite the k-fiber being pulled, moved in
168 towards the pole-pole axis by $5.8 \pm 0.9^\circ$ upon pulling (Figure 2E,H), preserving the angle
169 between sister k-fibers (Figure 2- figure supplement 3). Together, this reveals that the
170 spindle maintains local architecture around chromosomes against transient forces,
171 instead adjusting its global architecture, and that sister k-fibers are tightly crosslinked to
172 each other on the seconds timescale at metaphase.

173

174 *The deformed kinetochore-fiber's shape indicates specialized, short-lived crosslinking to*
175 *the spindle near chromosomes*

176

177 To probe the basis of the tight coupling between sister k-fibers, we measured curvature
178 along the deformed k-fiber on the premise that it could inform on the spatial distribution
179 of the effective underlying crosslinking (Figure 3A). In the k-fibers manipulated over 12
180 s, we observed high positive curvature at the position of the microneedle and,
181 unexpectedly, a region of negative curvature near the chromosome ($n=4/7$ cells) (Figure
182 3- figure supplement 1A-B). This configuration, more energetically costly than a single
183 bent region, indicates that k-fibers are unable to freely pivot near their plus-ends, which

184 is well suited to promote their biorientation.

185 To define the location and lifetime of these underlying connections, key to
186 uncovering both mechanism and function, we repeated the above manipulation assay
187 varying the position and duration of microneedle pulls. This time, we deformed the k-
188 fiber by a larger magnitude of $2.5 \pm 0.2 \mu\text{m}$ and for longer ($60.5 \pm 8.7 \text{ s}$, Figure 1G, navy
189 traces, $n=23$ cells) to accentuate the curvature profile (Figure 3B). Similar to the 12 s
190 manipulations (Figure 2F,H), there was no increase in the inter-kinetochore distance
191 and the sister k-fiber's plus-end moved in towards the pole-pole axis (Figure 3- figure
192 supplement 2A-C). We observed negative curvature near the chromosome in 74%
193 ($n=17/23$) of the spindles, and near the pole in just 13% ($n=3/23$) of the spindles (Figure
194 3C-D). This indicates that k-fibers freely pivot around poles, as in insect cells (Begg &
195 Ellis, 1979), but cannot freely pivot around chromosomes. Often, manipulating the outer
196 k-fiber exposed non-kMTs in contact with the k-fiber (Figure 3B, Figure 3- figure
197 supplement 1); this revealed connections observed in electron microscopy (McDonald
198 et al., 1992; Nicklas et al., 1982), that are harder to see with light microscopy. These
199 non-kMT connections, observed close to the region of negative curvature (Figure 3-
200 figure supplement 3), may contribute to reinforcing k-fibers in the spindle center.

201 To determine whether this chromosome-proximal reinforcement is mediated by
202 uniform crosslinking all along the k-fiber length (Model 1) or specialized crosslinking
203 near chromosomes (Model 2), we pulled the k-fiber at different distances from
204 chromosomes (Figure 3E). Negative curvature was not correlated with microneedle
205 position (Figure 3F), and was always observed between 1 and 3 μm from the
206 chromosome regardless of where we pulled (Figure 3G). This strongly supports a model

207 whereby a specialized structure in the spindle center laterally reinforces k-fibers near
208 chromosomes (Model 2).

209 To define the lifetime of this specialized reinforcement, we measured k-fiber
210 curvature over time while we held the microneedle in place after manipulating for 60 s
211 (“manipulate-and-hold”, n=5 cells). The negative curvature near chromosomes lasted for
212 18.8 ± 2.6 s before it was no longer detectable, likely reflecting the lifetime of the
213 underlying connections (Figure 3H-I). Together, these findings indicate the presence of
214 short-lived, non-uniform reinforcement of the k-fiber near chromosomes that is stable
215 enough to preserve spindle structure over short timescales, but sufficiently dynamic to
216 allow spindle remodeling over long timescales.

217

218

219 *The microtubule crosslinker PRC1 mediates the specialized and short-lived*
220 *kinetochore-fiber reinforcement near chromosomes*

221

222 We next sought to determine the underlying molecular basis for this specialized, short-
223 lived reinforcement near chromosomes. We hypothesized that the microtubule
224 crosslinker PRC1 plays this role based on its localization in the spindle center during
225 metaphase (Mollinari et al., 2002) and its proposed role in linking sister k-fibers at
226 metaphase and anaphase (Jiang et al., 1998; Kajtez et al., 2016; Mollinari et al., 2002;
227 Polak et al., 2017). Using immunofluorescence, we first asked if PRC1 localization in
228 PtK2 cells correlated with the location of this specialized reinforcement region (Figure
229 4A). We found that PRC1 enrichment spanned a region of 6.9 ± 0.3 μm (n=6 cells) in the

230 spindle center (Figure 4B). This maps well to the expected location of a specialized
231 crosslinker, spanning the inter-kinetochore region ($\sim 2 \mu\text{m}$), and the region of
232 mechanical reinforcement near chromosomes ($1\text{-}3 \mu\text{m}$ along each sister k-fiber).

233 To assess whether PRC1 played a role in this specialized reinforcement, we
234 depleted it by RNAi in PtK2 cells (Figure 4C) (Udy et al., 2015). Using
235 immunofluorescence, we observed a decrease in the inter-kinetochore distance (from
236 $2.35 \pm 0.04 \mu\text{m}$ in WT cells to $1.86 \pm 0.09 \mu\text{m}$ in PRC1 RNAi cells) (Figure 4- figure
237 supplement 1A), similar to human cells (Polak et al., 2017). When subjecting PRC1
238 RNAi spindles to the same manipulation assay (deformed by $2.6 \pm 0.1 \mu\text{m}$ over $66 \pm 6 \text{ s}$
239 ($n=12$ cells)) as WT spindles (Figure 3), we found that 91% of the spindles lacked a
240 detectable k-fiber negative curvature near chromosomes upon pulling (Figure 4D-E). In
241 order to directly compare the WT and PRC1 RNAi datasets, we only looked at curvature
242 profiles in which the distribution of microneedle positions overlapped: 78% ($n=14/18$) of
243 deformed k-fibers in WT cells showed negative curvature near chromosomes compared
244 to only 8% ($n=1/12$) of deformed k-fibers in PRC1 RNAi cells (Figure 4F). Thus, PRC1
245 laterally reinforces k-fibers near chromosomes, and enables them to resist pivoting
246 under force.

247 In PRC1 RNAi spindles, the inter-kinetochore distance increased by $0.7 \pm 0.1 \mu\text{m}$
248 upon manipulation, compared to $0.1 \pm 0.1 \mu\text{m}$ in WT spindles (Figure 4G,H). This
249 suggests that PRC1-mediated crosslinking not only resists compressive forces (Kajtez
250 et al., 2016) but also extensile forces. Furthermore, upon pulling, the sister k-fiber
251 opposite the deformed k-fiber moved in towards the pole-pole axis by $4.5 \pm 1.4^\circ$, less
252 than in WT cells where it moved by $10.6 \pm 1.2^\circ$ (Figure 4G,I). Finally, the angle between

253 the sister k-fibers' chromosome-proximal regions was less well-preserved after
254 manipulation than in WT (Figure 4G,J). These findings suggest that PRC1 promotes
255 tight coupling between sister k-fibers, ensuring they behave as a single mechanical unit
256 and maintain biorientation. While we do not know if PRC1 acts directly or indirectly to
257 locally reinforce k-fibers, we find that microtubule intensity remains similar upon PRC1
258 depletion (Figure 4- figure supplement 1B), suggesting that it is unlikely to act simply by
259 changing microtubule density in the spindle. Together, our findings indicate that PRC1
260 provides the specialized, short-lived structural reinforcement in the spindle center near
261 chromosomes. They suggest a model whereby PRC1 can locally protect the spindle
262 center from transient lateral and longitudinal forces while allowing it to move
263 chromosomes and remodel over longer timescales (Figure 5).

264

265 **Discussion**

266

267 The spindle's ability to be dynamic and constantly generate and respond to force while
268 robustly maintaining its structure is central to chromosome segregation. Here, we
269 asked: what mechanisms allow the dynamic mammalian spindle to robustly hold on to
270 its k-fibers? Using microneedle manipulation to directly pull on k-fibers, we were able to
271 challenge the robustness of their anchorage over different locations and timescales
272 (Figure 1). We show that k-fibers' anchorage in the spindle is local in space and short-
273 lived in time (Figure 5). K-fibers are weakly coupled to their neighbors but strongly
274 coupled to their sisters (Figure 2) through specialized, short-lived reinforcement within
275 the first 3 μm of chromosomes (Figure 3), mediated by PRC1 (Figure 4). Such

276 mechanical reinforcement could help protect chromosome-to-spindle connections while
277 allowing them to remodel over the course of mitosis. Together, our work provides a
278 framework for understanding the molecular and physical mechanisms giving rise to the
279 dynamics and robust mechanics of the mammalian spindle.

280 Spindle mechanics emerge from both active (energy consuming) and passive
281 molecular force generators (Elting, Suresh & Dumont, 2018). Here, we find a spatially
282 and temporally well-defined role for PRC1, a passive force generator that preferentially
283 binds anti-parallel microtubules (Mollinari et al., 2002) and can maintain and reinforce
284 microtubule overlaps *in vitro* (Bieling, Telley & Surrey, 2010; Braun et al., 2011). In the
285 *longitudinal axis*, PRC1 can help promote chromosome stretch, which has been
286 proposed to maintain tension and biorientation of sister-kinetochore pairs (Polak et al.,
287 2017). We find that PRC1 can also help limit chromosome stretch (Figure 4H), thereby
288 mechanically buffering chromosomes from transient forces. Whether PRC1
289 mechanically reinforces the spindle center directly or indirectly remains unknown; for
290 example, it could do so by generating friction along microtubules (Forth et al., 2014),
291 thereby limiting the timescale of microtubule sliding and spindle reorganization. PRC1's
292 microtubule binding is phosphoregulated during mitosis (Mollinari et al., 2002; Zhu &
293 Jiang, 2005; Zhu et al., 2006), and as such these frictional forces may be regulated as
294 the spindle's mechanical functions change. In the *lateral axis*, we find that PRC1
295 restricts free pivoting of k-fibers around chromosomes (Figure 4C-D), even under
296 external force, thereby promoting biorientation between sister k-fibers. Whether PRC1
297 mediates this mechanical reinforcement by crosslinking k-fibers to a non-specific or
298 specific set of non-kMTs (such as bridge-fibers (Kajtez et al., 2016; Polak et al., 2017)),

299 and whether PRC1 plays the same mechanical role over different timescales or spindle
300 axes (Elting et al., 2017), remain open questions. Looking forward, defining the
301 mechanical roles of diverse crosslinkers such as NuMA (Elting et al., 2017) and Kinesin-
302 5 (Shimamoto, Forth & Kapoor, 2015; Takagi et al., 2019), combined with the use of
303 calibrated microneedles (Nicklas, 1983; Shimamoto & Kapoor, 2012; Shimamoto et al.,
304 2011; Takagi et al., 2019), will allow us to quantitatively link molecular-scale mechanics
305 to cellular-scale ones in the mammalian spindle.

306 Mapping mechanics over space, microneedle manipulation reveals that
307 anchorage along k-fibers is non-uniform and locally reinforced near chromosomes in
308 both the longitudinal and lateral axes (Figure 3). While the spindle was known to be able
309 to bear load locally in space (Elting et al., 2017; Milas & Tolić, 2016), whether
310 anchorage was uniform along the k-fiber's length, and along which axes it acted, were
311 not known. Our work suggests a strategy whereby anchoring forces are spatially
312 mapped to regions of active force generation at the kinetochore (Grishchuk, 2017; Inoue
313 & Salmon, 1995; Mitchison et al., 1986) and spindle poles (Elting et al., 2014;
314 Sikirzhytski et al., 2014), similar to patterns in *Xenopus* extract spindles (Takagi et al.,
315 2019) despite significant differences in spindle architecture (Crowder et al., 2015).
316 Probing mechanical heterogeneity in other regions in the spindle will further enable us
317 to map local mechanical properties to function. In principle, specialized reinforcement
318 near chromosomes could help protect kinetochore-microtubule attachments and
319 chromosomes from transient forces, and ensure that sister k-fibers point to opposite
320 poles (Figure 2E-H) – while allowing them to bend further away and focus into poles.

321 Mapping mechanics over time, our findings indicate that local anchorage at the
322 spindle center can robustly resist structural changes due to forces over seconds (Figure
323 2G, 3), and yet remodel over minutes. Our findings suggest that spindle structural and
324 functional robustness emerge in part from differentially responding to forces over
325 different timescales. While the molecular basis of the spindle center's remodeling
326 timescale is not known, it likely reflects the turnover of underlying connections, for
327 example of non-kMTs (Saxton et al., 1984) or of PRC1 (Pamula et al., 2019), just as
328 crosslinking dynamics contribute to the physical properties of *Xenopus* extract spindles
329 (Shimamoto et al., 2011). By tuning the lifetime of these connections that reinforce the
330 spindle center, the cell could in principle regulate its remodeling to allow kinetochores to
331 only sense forces of a given timescale. This could, for example, result in only sustained
332 forces being communicated to kinetochores, thereby ensuring that the error correction
333 machinery responds to the appropriate mechanical cues (Li & Nicklas, 1995;
334 Sarangapani & Asbury, 2014). Furthermore, regulating the timescale of remodeling can
335 enable spindle morphology to change only when needed, for instance at the
336 metaphase-to-anaphase transition (Zhu & Jiang, 2005; Zhu et al., 2006). Looking
337 forward, the ability to exert controlled forces on the mammalian spindle will be key to
338 understanding how its mechanics emerge (Brugues & Needleman, 2014; Oriola,
339 Needleman & Brugues, 2018) from the dynamics of its individual components (Roostalu
340 et al., 2018; Ross et al., 2019; Surrey et al., 2001).

341 Altogether, our work suggests that mechanical heterogeneity is a simple principle
342 for how the spindle and other macromolecular machines can be at once dynamic and
343 mechanically robust. Mechanical heterogeneity over space and time allows these

344 machines to be reinforced in specific regions and on short timescales for local functions,
345 while allowing remodeling elsewhere and on longer timescales – to ultimately perform
346 robust cellular-scale functions.

347

348

349 **Acknowledgements**

350 We thank Alexey Khodjakov for Ptk2 GFP- α -tubulin cells and Timothy Mitchison for
351 FCPT. We are grateful to Justin Biel, the Fred Chang Lab, Maya Anjur-Deitrich, Daniel
352 Fletcher, Wallace Marshall, Dan Needleman, Adair Oesterle, Le Paliulis, Iva Tolic and
353 Orion Weiner for helpful discussions, Deepak Krishnamurthy and Jason Town for image
354 analysis discussions, the Verkman Lab for the microforge, and Arthur Molines and
355 members of the Dumont Lab for discussions and critical reading of the manuscript. This
356 work was supported by NIH DP2GM119177, NIH 1R01GM134132, NSF CAREER
357 1554139, NSF 1548297 Center for Cellular Construction (S.D.), the Rita Allen
358 Foundation and Searle Scholars' Program (S.D.), NSF Graduate Research Fellowships
359 (P.S. and A.F.L.) and a UCSF Kozloff Fellowship (A.F.L.).

360

361 **Author Contributions**

362 Conceptualization, P.S. and S.D.; Methodology, P.S. and A.F.L.; Investigation, P.S.;
363 Data Curation, P.S. and A.F.L.; Software, P.S.; Writing – Original Draft, P.S.; Writing –
364 Review and Editing, P.S., A.F.L. and S.D.; Visualization, P.S.; Funding Acquisition, S.D.

365

366 **Competing Interests**

367 The authors declare no competing financial or non-financial interests.

368

369 **Materials and Methods**

370

371 *Cell culture and siRNA*

372

373 PtK2 GFP- α -tubulin cells were cultured in MEM (11095; Thermo Fisher, Waltham, MA)
374 supplemented with sodium pyruvate (11360; Thermo Fisher), nonessential amino acids
375 (11140; Thermo Fisher), penicillin/streptomycin, and 10% heat-inactivated fetal bovine
376 serum (10438; Thermo Fisher). Cells were maintained at 37°C and 5% CO₂. For
377 depletion of PRC1, cells were transfected with siRNA (5'-
378 GGACTGAGGUUGUCAAGAA-3') for PRC1 using Oligofectamine (Life Technologies,
379 Carlsbad, CA) as previously described (Udy et al., 2015). Cells were imaged 72 h after
380 siRNA treatment. For PRC1 RNAi, knockdown was previously validated in our hands by
381 western blot (Udy et al., 2015), and was verified here by immunofluorescence. We
382 quantified PRC1 immunofluorescence intensity in mock RNAi (Luciferase) versus PRC1
383 RNAi: the average per pixel PRC1 intensity in the spindle above that in the cytoplasm
384 was 581.1±53.2 (AU) (SEM, n=20) in control and 128.533±21.1 (AU) (SEM, n=17) in
385 PRC1 RNAi cells (78% knock-down). Cells used for quantification were selected based
386 on examining the DNA channel only (so as to be unbiased for the amount of PRC1
387 present when selecting cells, as a control for live experiments). We confirmed PRC1
388 knockdown in the particular coverslips used for live imaging by verifying at low
389 magnification the enrichment of binucleated cells (35% of cells, n=100), a previously

390 characterized consequence of PRC1 knockdown (Mollinari et al., 2002; Udy et al.,
391 2015).

392

393 *Drug/dye treatment*

394

395 To image the cell membrane, we added CellMask-Orange (Thermo Fisher) (1:1000
396 dilution) to the imaging dish 1 min before imaging (Figure 1A-D): we observed rapid
397 incorporation of dye into the cell membrane and imaged cells for 30-45 min, before too
398 much membrane dye became internalized.

399 To test whether the membrane was ruptured during microneedle manipulation, we
400 added propidium iodide (Thermofisher) (50 μ l of 1 mg/ml stock solution) to the cell
401 media 1 min before imaging (Figure 1- figure supplement 1): We observed rapid
402 chromosome labeling in dead cells and no labeling of chromosomes in cells that were
403 successfully manipulated.

404 To increase microtubule crosslinking by rigor binding Eg5, we treated with 200 μ M
405 FCPT (2-(1-(4-fluorophenyl)cyclopropyl)-4-(pyridin-4-yl)thiazole) (gift of T. Mitchison,
406 Harvard Medical School, Boston, MA) for 15-30 min (Groen et al., 2008) (Figure 2D).

407

408 *Immunofluorescence*

409

410 To quantify the region of PRC1 enrichment in the metaphase spindle in WT cells (Figure
411 4A-B) and confirm PRC1 depletion following RNAi (Figure 4C), cells were fixed with
412 95% methanol + 5 mM EGTA at -20°C for 1 min, washed with TBS-T (0.1% Triton-X-

413 100 in TBS), and blocked with 2% BSA in TBS-T for 1 h. Primary and secondary
414 antibodies were diluted in TBS-T+2% BSA and incubated with cells for 1 h (primary) and
415 for 25 min at room temperature (secondary). DNA was labeled with Hoescht 33342
416 (Sigma, St. Louis, MO) before cells were mounted in ProLongGold Antifade (P36934;
417 Thermo Fisher). Cells were imaged using the spinning disk confocal microscope
418 described above. Antibodies: rabbit anti-PRC1 (1:100, BioLegend, San Diego, CA),
419 mouse anti- α -tubulin DM1 α (1:1000, Sigma-Aldrich), anti-mouse secondary antibodies
420 (1:500) conjugated to Alexa Fluor 488 (A11001; Invitrogen), anti-rabbit secondary
421 antibodies (1:500) conjugated to Alexa Fluor 647 (A21244; Life Technologies).

422

423 *Imaging*

424

425 Ptk2 GFP- α -tubulin cells (stable line expressing human α -tubulin in pEGFP-C1; Takara
426 Bio Inc.; a gift from A. Khodjakov, Wadsworth Center, Albany, NY (Khodjakov et al.,
427 2003)) were plated on 35 mm #1.5 coverslip glass-bottom dishes coated with poly-D-
428 lysine (MatTek, Ashland, MA) and imaged in CO₂-independent MEM (Thermo Fisher).
429 The cells were maintained at 27-32°C in a stage top incubator (Tokai Hit, Fujinomiya-
430 shi, Japan), without a lid. Live imaging was performed on two similar CSU-X1 spinning-
431 disk confocal (Yokogawa, Tokyo, Japan) Eclipse Ti-E inverted microscopes (Nikon) with
432 a perfect focus system (Nikon, Tokyo, Japan). The 12 s manipulations (Figure 2) were
433 performed on a microscope with the following components: head dichroic Semrock
434 Di01-T405/488/561, 488 nm (150 mW) and 561 (100 mW) diode lasers (for tubulin and
435 microneedle respectively), emission filters ETGFP/mCherry dual bandpass 59022M

436 (Chroma Technology, Bellows Falls, VT), and Zyla camera (Andor Technology, Belfast,
437 United Kingdom). The 60 s manipulations (Figure 3,4) and immunofluorescence (Figure
438 4) were performed on a microscope with the following components: head dichroic
439 Semrock Di01-T405/488/568/647, 488 nm (120 mW) and 642 nm (100 mW) diode
440 lasers (for tubulin and microneedle respectively), emission filters ET 525/50M and
441 ET690/50M (Chroma Technology), and iXon3 camera (Andor Technology). Cells were
442 imaged via Metamorph (7.7.8.0, MDS Analytical Technologies) by fluorescence (50-70
443 ms exposures) with a 100X 1.45 Ph3 oil objective through a 1.5X lens yielding 105
444 nm/pixel at bin=1. For the 3D whole cell membrane imaging (Figure 1A-B), z-stacks
445 were taken through the entire cell with a z step-size of 400 nm. For the 12 s
446 manipulations (Figure 2), the camera was used in continuous streaming mode, where
447 single z-plane images were taken every 120 ms, which enabled us to build the strain
448 map more accurately. For the 60 s manipulations (Figure 3,4), cells were imaged by
449 taking either a single slice or 3 z-slices of 400 nm spacing every 5-7 s, helping us track
450 the deformed k-fiber over time despite z-height changes induced by microneedle
451 movement.

452

453 *Microneedle manipulation*

454

455 Microneedle manipulation was adapted to mammalian cells by optimizing the following
456 key parameters:

- 457 • Glass capillaries with an inner and outer diameter of 1 mm and 0.58 mm
458 respectively (1B100-4 or 1B100F-4, World Precision Instruments) were used to

459 create microneedles. A micropipette puller (P-87, Sutter Instruments, Novato,
460 CA) was used to create uniform glass microneedles. When pulled the tip of the
461 capillary was closed, as seen in the microneedle-labeled image in Figure 1B. For
462 a ramp value of 504 (specific to the type of glass capillary and micropipette
463 puller), we used the following settings: Heat = 509, Pull = 70, velocity = 45, delay
464 = 90, pressure = 200, prescribed to generate microneedles of 0.2 μm outer tip
465 diameter (Sutter Instruments pipette cookbook). In the plane of imaging,
466 microneedle diameter was measured to be $1.1 \pm 0.1 \mu\text{m}$. This variability comes
467 from the microneedle tip sometimes being in a slightly different z-plane than the
468 plane imaged. Microneedles with longer tapers and smaller tips than above
469 ruptured the cell membrane more frequently.

- 470 • Microneedles were bent ~ 1.5 mm away from their tip to a 45° angle using a
471 microforge (Narishige International, Amityville, NY), so as to have them approach
472 the coverslip at a 90° angle (the microneedle holder was 45° from the coverslip).
473 The angle of microneedle approach was critical towards improving cell health
474 during manipulations, likely because it minimizes the surface area of the
475 membrane and cortex deformed by the microneedle.
- 476 • Microneedles used for manipulation were coated with BSA Alexa Fluor 555
477 conjugate (BSA-Alexa-555; A-34786, Invitrogen) (Figure 2) or BSA Alexa Fluor
478 647 conjugates (BSA-Alexa-647; A-34785, Invitrogen) (Figure 1,3,4) by soaking
479 in the solution for 60 s before imaging (Sasaki, Matsuki & Ikegaya, 2012): BSA-
480 Alexa dye and Sodium Azide (Nacalai Tesque, Kyoto, Japan) were dissolved in
481 0.1 M phosphate-buffered saline (PBS) at the final concentration of 0.02% and 3

482 mM, respectively (Sasaki et al., 2012). Tip labeling was critical towards improving
483 cell health during manipulations because it allowed us to better visualize the
484 microneedle tip in fluorescence along with the spindle and prevented us from
485 going too deeply into the cell, thereby causing rupture.

- 486 • Mitotic cells for microneedle manipulation were chosen based on the following
487 criteria: spindles in metaphase, flat, bipolar shape with both poles in the same
488 focal plane. These criteria were important for pulling on single k-fibers close to
489 the top of the cell and simultaneously being able to image the whole spindle's
490 response to manipulation.
- 491 • Manipulations were performed in 3D using an x-y-z stepper-motor
492 micromanipulator (MP-225, Sutter Instruments, Novato, CA). A 3-axis-knob
493 (ROE-200) or joystick (Xenoworks BRJOY, Sutter Instruments) connected to the
494 manipulator via a controller box (MPC-200, Sutter Instruments) allowed fine
495 manual movements and was used to both find and position the microneedle tip in
496 the field of view and manipulate the spindle while imaging.
- 497 • Towards setting up the micromanipulator on our scope, we used a metal bracket
498 attached to the scope body such that it sits above the stage and is directly
499 coupled to the scope body. The micromanipulator was attached to this bracket.
- 500 • To find and position the microneedle, we first located and centered the
501 microneedle tip in the field of view using low magnification (10X or 20X 0.5 Ph1
502 air objectives). Critically, we brought the microneedle tip close to the coverslip,
503 placing it just above the cells, after which we switched to higher magnification
504 (100X 1.45 Ph3 oil objective) and refined the x-y-z position of the microneedle so

505 that it was right above the cell. When refining the microneedle position in higher
506 magnification, using the Ph1 phase ring helped see the microneedle more clearly
507 than with a Ph3 ring.

- 508 • When starting a manipulation experiment, we placed the microneedle $\sim 5 \mu\text{m}$
509 above the cell and acquired images every 5-7 sec. Once we identified an outer k-
510 fiber in a plane that is close to the top of the cell, we slowly brought the
511 microneedle down into the cell, using the fluorescent label of the microneedle tip
512 to help inform us on its position. If the microneedle's position were far away from
513 the k-fiber of interest, we slowly moved the microneedle out of the cell, adjusted
514 its x-y position and brought it back down into the cell. Through this iterative
515 process, we could correctly position the microneedle such that it was inside the
516 spindle, right next to the outer k-fiber.
- 517 • Once the microneedle was positioned next to an outer k-fiber near the top of the
518 cell, it was moved in a direction that was roughly perpendicular to the pole-pole
519 axis.
- 520 • All 12 s manipulations were done manually using the joystick, and most 60 s
521 manipulations were done with computer control.
- 522 • For manipulations done with computer control, we used a custom-written Python
523 script that took the following inputs: Angle of movement (based on the orientation
524 of the spindle in the cell), duration of movement and total distance. The script
525 generates a text file with a sequence of steps (with the smallest step-size being
526 $0.0625 \mu\text{m}$) in xyz and wait/delay times, which are the instructions for the
527 software (Multi-Link, Sutter Instruments) that makes the manipulator move.

528 Computer control ensured smooth and reproducible microneedle movements
529 over a longer period. Our manipulation programs generated microneedle
530 movements of the following speeds: $9.3 \pm 1.8 \mu\text{m}/\text{min}$ and $2.5 \pm 0.1 \mu\text{m}/\text{min}$.
531 Microneedle speeds that exceeded these killed cells more frequently. For the
532 manipulate-and-hold experiments (Figure 3H-I), the microneedle was left in the
533 same position at the end of its movement and only removed after 45-60 s. At the
534 end of the manipulation, the microneedle was manually removed from the cell
535 slowly ($< \sim 5 \mu\text{m}/\text{min}$) to avoid membrane rupture or cell detachment from the
536 coverslip.

- 537 • Cells were included in our datasets if they did not appear negatively affected by
538 micromanipulation. We excluded cells from the dataset if they met the following
539 criteria: cells that underwent sudden and continuous blebbing upon microneedle
540 contact, spindles that started to rapidly collapse during manipulation,
541 chromosomes decondensed, mitochondria become punctate. When we followed
542 the spindle post manipulation (n=10), 70% of cells entered anaphase within 15
543 min after manipulation.

544

545 *Quantification and statistical analyses*

546

- 547 • Building strain maps (Figure 2): First, we aligned all images during the
548 manipulation in order to correct for whole spindle rotation and translation, using
549 the Stackreg plugin on ImageJ (Thevenaz, Ruttimann & Unser, 1998). This
550 correction allowed us to only look at structural changes within the spindle and not

551 whole spindle translation or rotation. K-fibers were included in the data set
552 (Figure 2) only if their entire length stayed within the same z-plane over time. The
553 images taken in the continuous streaming mode (50 frames) helped ensure that
554 the same k-fiber could be followed over a long time period, being correctly
555 mapped from frame to frame. K-fibers were traced and tracked semi-
556 automatically using using GFP- α -tubulin images in home-written Python scripts at
557 frames 0 and 50, over their entire length. We stored 100 equally spaced
558 coordinates along each k-fiber in frames 0 and 50, which were then connected to
559 each other (coordinate 1 in frame 0 connects to coordinate 1 in the frame 50, and
560 so on). This approach provided a linear mapping between the undeformed
561 (purple) and deformed (green) spindle image to build a strain map (Figure 2C).
562 This linear mapping was possible because k-fiber lengths remained constant
563 during these 12 s manipulations (Figure 2- figure supplement 1).

- 564 • Tracking features of interest in live images (Figure 2,3,4): Inter-kinetochore
565 distance (Figure 2F, 4G, Figure 3- figure supplement 3B) was calculated as the
566 distance between sister k-fiber plus-ends. To make sure we measured this
567 distance between correctly identified sister pairs, we confirmed that there were
568 correlated movements between them before and during the manipulation. Angle
569 of sister k-fiber plus-end from the pole-pole axis was calculated by measuring the
570 angle between the position of the sister k-fiber's plus-end (connected to the same
571 chromosomes as the deformed one) to the pole-pole axis (Figure 2H, 4H, Figure
572 3- figure supplement 3D). For the control dataset (unmanipulated spindles), the
573 same measurements were made only on outer k-fibers, in order to be able to

574 compare k-fibers in a similar part of the spindle. Pole-pole distance was
575 calculated as the distance between centroids of the two spindle poles (Figure
576 2G). The number of measurements (n) represent a subset of the manipulation
577 data-set per figure, depending on which features were trackable in the
578 manipulation.

- 579 • Measuring curvature along k-fiber (Figure 3,4): We used a custom-written Python
580 script to calculate local curvature along k-fiber length. We calculated the radius of
581 a circle that was fit to three points along the k-fiber. These three points were
582 chosen to be spaced apart by 1 μm in order to calculate curvature on the relevant
583 length scale. This radius (radius of curvature, units = μm) was used to calculate
584 curvature (units = $1/\mu\text{m}$) by taking its inverse, which we then mapped on to the
585 traced k-fiber using a color spectrum from blue (negative curvature) to red
586 (positive curvature).
- 587 • Immunofluorescence quantification: In order to quantify the length of PRC1
588 enrichment in WT spindles (Figure 4A,B), we calculated the ratio of PRC1 to
589 tubulin intensity inside a region of the spindle (whole spindle excluding spindle
590 poles) (Figure 4A, bottom panel). In order to quantify the percentage of PRC1
591 knocked down, we calculated the per pixel intensity of PRC1 in PRC1 RNAi
592 spindles relative to the background levels and compared it to that in WT spindles.
593 A similar analysis was done to quantify microtubule intensity in WT and PRC1
594 RNAi spindles inside two regions (whole spindle excluding spindle poles and just
595 the spindle center) (Figure 4- figure supplement 4A). Inter-kinetochore distance
596 in WT and PRC1 RNAi spindles (Figure 4- figure supplement 4A) was calculated

597 as the distance between sister k-fiber plus-ends. We equally sampled outer and
598 middle sister k-fiber pairs.

599 • Statistical tests: We used the non-parametric two-sided Mann-Whitney U test
600 when comparing two independent datasets and the Wilcoxon signed rank test
601 when comparing two paired datasets. In the text, whenever we state a significant
602 change or difference, the p-value for those comparisons were less than 0.05. In
603 the figures, we display the exact p-value from every statistical comparison made,
604 and in the legends we state what test was conducted. Quoted n's are described
605 in more detail where mentioned in the text or figure legend, but in general refer to
606 the number of independent individual measurements (e.g., individual k-fibers,
607 sister pairs, spindles, manipulations, etc.).

608

609 *Script Packages*

610

611 All scripts were written in Python. Pandas was used for all data organization and
612 compilations, Scipy for statistical analyses, Matplotlib and Seaborn for plotting and data
613 visualization as well as Numpy for general use. FIJI was used for movie formatting,
614 immunofluorescence quantification and tracking manipulations (Schindelin et al., 2012).
615 In FIJI, StackReg for spindle rigid body motion correction was necessary for building
616 strain maps and MtrackJ was used to track the microneedle over time (Meijering,
617 Dzyubachyk & Smal, 2012; Thevenaz et al., 1998).

618

619 *Video preparation*

620

621 Videos show a single spinning disk confocal z-slice imaged over time (Video 2) or a
622 maximum intensity projection (Video 1,3,4,5) and were formatted for publication using
623 ImageJ and set to play at 5 frames per second (Video 1-5).

624

625 **References:**

626

- 627 Begg, D. A., & Ellis, G. W. (1979). Micromanipulation studies of chromosome
628 movement. I. Chromosome-spindle attachment and the mechanical properties of
629 chromosomal spindle fibers. *J Cell Biol*, 82(2), 528-541. doi:10.1083/jcb.82.2.528
630 Bieling, P., Telley, I. A., & Surrey, T. (2010). A minimal midzone protein module controls
631 formation and length of antiparallel microtubule overlaps. *Cell*, 142(3), 420-432.
632 doi:10.1016/j.cell.2010.06.033
633 Braun, M., Lansky, Z., Fink, G., Ruhnnow, F., Diez, S., & Janson, M. E. (2011). Adaptive
634 braking by Ase1 prevents overlapping microtubules from sliding completely apart.
635 *Nat Cell Biol*, 13(10), 1259-1264. doi:10.1038/ncb2323
636 Brugues, J., & Needleman, D. (2014). Physical basis of spindle self-organization. *Proc*
637 *Natl Acad Sci U S A*, 111(52), 18496-18500. doi:10.1073/pnas.1409404111
638 Crowder, M. E., Strzelecka, M., Wilbur, J. D., Good, M. C., von Dassow, G., & Heald, R.
639 (2015). A comparative analysis of spindle morphometrics across metazoans.
640 *Curr Biol*, 25(11), 1542-1550. doi:10.1016/j.cub.2015.04.036
641 Elting, M. W., Hueschen, C. L., Udy, D. B., & Dumont, S. (2014). Force on spindle
642 microtubule minus ends moves chromosomes. *J Cell Biol*, 206(2), 245-256.
643 doi:10.1083/jcb.201401091
644 Elting, M. W., Prakash, M., Udy, D. B., & Dumont, S. (2017). Mapping Load-Bearing in
645 the Mammalian Spindle Reveals Local Kinetochore Fiber Anchorage that
646 Provides Mechanical Isolation and Redundancy. *Curr Biol*, 27(14), 2112-
647 2122.E2115. doi:10.1016/j.cub.2017.06.018
648 Elting, M. W., Suresh, P., & Dumont, S. (2018). The Spindle: Integrating Architecture
649 and Mechanics across Scales. *Trends Cell Biol*, 28(11), 896-910.
650 doi:10.1016/j.tcb.2018.07.003
651 Forth, S., Hsia, K. C., Shimamoto, Y., & Kapoor, T. M. (2014). Asymmetric friction of
652 nonmotor MAPs can lead to their directional motion in active microtubule
653 networks. *Cell*, 157(2), 420-432. doi:10.1016/j.cell.2014.02.018
654 Gatlin, J. C., Matov, A., Danuser, G., Mitchison, T. J., & Salmon, E. D. (2010). Directly
655 probing the mechanical properties of the spindle and its matrix. *J Cell Biol*,
656 188(4), 481-489. doi:10.1083/jcb.200907110
657 Gorbisky, G. J., & Borisy, G. G. (1989). Microtubules of the kinetochore fiber turn over in
658 metaphase but not in anaphase. *J Cell Biol*, 109(2), 653-662.
659 doi:10.1083/jcb.109.2.653

- 660 Grishchuk, E. L. (2017). Biophysics of Microtubule End Coupling at the Kinetochore. In
661 B. E. Black (Ed.), *Centromeres and Kinetochores: Discovering the Molecular*
662 *Mechanisms Underlying Chromosome Inheritance* (pp. 397-428). Cham:
663 Springer International Publishing.
- 664 Grishchuk, E. L., Molodtsov, M. I., Ataulakhanov, F. I., & McIntosh, J. R. (2005). Force
665 production by disassembling microtubules. *Nature*, *438*(7066), 384-388.
666 doi:10.1038/nature04132
- 667 Groen, A. C., Needleman, D., Brangwynne, C., Gradinaru, C., Fowler, B., Mazitschek,
668 R., et al. (2008). A novel small-molecule inhibitor reveals a possible role of
669 kinesin-5 in anastral spindle-pole assembly. *J Cell Sci*, *121*(Pt 14), 2293-2300.
670 doi:10.1242/jcs.024018
- 671 Hutchins, J. R., Toyoda, Y., Hegemann, B., Poser, I., Heriche, J. K., Sykora, M. M., et
672 al. (2010). Systematic analysis of human protein complexes identifies
673 chromosome segregation proteins. *Science*, *328*(5978), 593-599.
674 doi:10.1126/science.1181348
- 675 Inoue, S., & Salmon, E. D. (1995). Force generation by microtubule
676 assembly/disassembly in mitosis and related movements. *Mol Biol Cell*, *6*(12),
677 1619-1640. doi:10.1091/mbc.6.12.1619
- 678 Itabashi, T., Takagi, J., Shimamoto, Y., Onoe, H., Kuwana, K., Shimoyama, I., et al.
679 (2009). Probing the mechanical architecture of the vertebrate meiotic spindle. *Nat*
680 *Methods*, *6*(2), 167-172. doi:10.1038/nmeth.1297
- 681 Jiang, W., Jimenez, G., Wells, N. J., Hope, T. J., Wahl, G. M., Hunter, T., et al. (1998).
682 PRC1: a human mitotic spindle-associated CDK substrate protein required for
683 cytokinesis. *Mol Cell*, *2*(6), 877-885. doi:10.1016/s1097-2765(00)80302-0
- 684 Kajtez, J., Solomatina, A., Novak, M., Polak, B., Vukusic, K., Rudiger, J., et al. (2016).
685 Overlap microtubules link sister k-fibres and balance the forces on bi-oriented
686 kinetochores. *Nat Commun*, *7*, 10298. doi:10.1038/ncomms10298
- 687 Khodjakov, A., Copenagle, L., Gordon, M. B., Compton, D. A., & Kapoor, T. M. (2003).
688 Minus-end capture of preformed kinetochore fibers contributes to spindle
689 morphogenesis. *J Cell Biol*, *160*(5), 671-683. doi:10.1083/jcb.200208143
- 690 Koshland, D. E., Mitchison, T. J., & Kirschner, M. W. (1988). Polewards chromosome
691 movement driven by microtubule depolymerization in vitro. *Nature*, *331*(6156),
692 499-504. doi:10.1038/331499a0
- 693 Li, X., & Nicklas, R. B. (1995). Mitotic forces control a cell-cycle checkpoint. *Nature*,
694 *373*(6515), 630-632. doi:10.1038/373630a0
- 695 Lin, N. K. H., Nance, R., Szybist, J., Cheville, A., & Paliulis, L. V. (2018).
696 Micromanipulation of Chromosomes in Insect Spermatocytes. *J Vis Exp*(140).
697 doi:10.3791/57359
- 698 Mastronarde, D. N., McDonald, K. L., Ding, R., & McIntosh, J. R. (1993). Interpolar
699 spindle microtubules in PTK cells. *J Cell Biol*, *123*(6 Pt 1), 1475-1489.
700 doi:10.1083/jcb.123.6.1475
- 701 Matos, I., Pereira, A. J., Lince-Faria, M., Cameron, L. A., Salmon, E. D., & Maiato, H.
702 (2009). Synchronizing chromosome segregation by flux-dependent force
703 equalization at kinetochores. *J Cell Biol*, *186*(1), 11-26.
704 doi:10.1083/jcb.200904153

- 705 McDonald, K. L., O'Toole, E. T., Mastronarde, D. N., & McIntosh, J. R. (1992).
706 Kinetochore microtubules in PTK cells. *J Cell Biol*, *118*(2), 369-383.
707 doi:10.1083/jcb.118.2.369
- 708 McEwen, B. F., Ding, Y., & Heagle, A. B. (1998). Relevance of kinetochore size and
709 microtubule-binding capacity for stable chromosome attachment during mitosis in
710 PtK1 cells. *Chromosome Res*, *6*(2), 123-132.
- 711 Meijering, E., Dzyubachyk, O., & Smal, I. (2012). Methods for cell and particle tracking.
712 *Methods Enzymol*, *504*, 183-200. doi:10.1016/B978-0-12-391857-4.00009-4
- 713 Milas, A., & Tolić, I. (2016). Relaxation of interkinetochore tension after severing of a k-
714 fiber depends on the length of the k-fiber stub. *Matters Select*.
715 doi:10.19185/matters.201603000025
- 716 Mitchison, T., Evans, L., Schulze, E., & Kirschner, M. (1986). Sites of microtubule
717 assembly and disassembly in the mitotic spindle. *Cell*, *45*(4), 515-527.
718 doi:10.1016/0092-8674(86)90283-7
- 719 Mollinari, C., Kleman, J. P., Jiang, W., Schoehn, G., Hunter, T., & Margolis, R. L.
720 (2002). PRC1 is a microtubule binding and bundling protein essential to maintain
721 the mitotic spindle midzone. *J Cell Biol*, *157*(7), 1175-1186.
722 doi:10.1083/jcb.200111052
- 723 Neumann, B., Walter, T., Heriche, J. K., Bulkescher, J., Erfle, H., Conrad, C., et al.
724 (2010). Phenotypic profiling of the human genome by time-lapse microscopy
725 reveals cell division genes. *Nature*, *464*(7289), 721-727.
726 doi:10.1038/nature08869
- 727 Nicklas, R. B. (1983). Measurements of the force produced by the mitotic spindle in
728 anaphase. *J Cell Biol*, *97*(2), 542-548. doi:10.1083/jcb.97.2.542
- 729 Nicklas, R. B., & Koch, C. A. (1969). Chromosome micromanipulation. 3. Spindle fiber
730 tension and the reorientation of mal-oriented chromosomes. *J Cell Biol*, *43*(1),
731 40-50. doi:10.1083/jcb.43.1.40
- 732 Nicklas, R. B., Kubai, D. F., & Hays, T. S. (1982). Spindle microtubules and their
733 mechanical associations after micromanipulation in anaphase. *J Cell Biol*, *95*(1),
734 91-104. doi:10.1083/jcb.95.1.91
- 735 Oriola, D., Needleman, D. J., & Bruges, J. (2018). The Physics of the Metaphase
736 Spindle. *Annu Rev Biophys*, *47*, 655-673. doi:10.1146/annurev-biophys-060414-
737 034107
- 738 Pamula, M. C., Carlini, L., Forth, S., Verma, P., Suresh, S., Legant, W. R., et al. (2019).
739 High-resolution imaging reveals how the spindle midzone impacts chromosome
740 movement. *J Cell Biol*, *218*(8), 2529-2544. doi:10.1083/jcb.201904169
- 741 Polak, B., Risteski, P., Lesjak, S., & Tolic, I. M. (2017). PRC1-labeled microtubule
742 bundles and kinetochore pairs show one-to-one association in metaphase.
743 *EMBO Rep*, *18*(2), 217-230. doi:10.15252/embr.201642650
- 744 Rieder, C. L. (1981). The structure of the cold-stable kinetochore fiber in metaphase
745 PtK1 cells. *Chromosoma*, *84*(1), 145-158. doi:10.1007/bf00293368
- 746 Roostalu, J., Rickman, J., Thomas, C., Nedelec, F., & Surrey, T. (2018). Determinants
747 of Polar versus Nematic Organization in Networks of Dynamic Microtubules and
748 Mitotic Motors. *Cell*, *175*(3), 796-808 e714. doi:10.1016/j.cell.2018.09.029

- 749 Ross, T. D., Lee, H. J., Qu, Z., Banks, R. A., Phillips, R., & Thomson, M. (2019).
750 Controlling organization and forces in active matter through optically defined
751 boundaries. *Nature*, *572*(7768), 224-229. doi:10.1038/s41586-019-1447-1
752 Sarangapani, K. K., & Asbury, C. L. (2014). Catch and release: how do kinetochores
753 hook the right microtubules during mitosis? *Trends in Genetics*, *30*(4), 150-159.
754 doi:10.1016/j.tig.2014.02.004
755 Sasaki, T., Matsuki, N., & Ikegaya, Y. (2012). Targeted axon-attached recording with
756 fluorescent patch-clamp pipettes in brain slices. *Nat Protoc*, *7*(6), 1228-1234.
757 doi:10.1038/nprot.2012.061
758 Saxton, W. M., Stemple, D. L., Leslie, R. J., Salmon, E. D., Zavortink, M., & McIntosh, J.
759 R. (1984). Tubulin dynamics in cultured mammalian cells. *J Cell Biol*, *99*(6),
760 2175-2186. doi:10.1083/jcb.99.6.2175
761 Schindelin, J., Arganda-Carreras, I., Frise, E., Kaynig, V., Longair, M., Pietzsch, T., et
762 al. (2012). Fiji: an open-source platform for biological-image analysis. *Nat*
763 *Methods*, *9*(7), 676-682. doi:10.1038/nmeth.2019
764 Shimamoto, Y., Forth, S., & Kapoor, T. M. (2015). Measuring Pushing and Braking
765 Forces Generated by Ensembles of Kinesin-5 Crosslinking Two Microtubules.
766 *Dev Cell*, *34*(6), 669-681. doi:10.1016/j.devcel.2015.08.017
767 Shimamoto, Y., & Kapoor, T. M. (2012). Microneedle-based analysis of the
768 micromechanics of the metaphase spindle assembled in *Xenopus laevis* egg
769 extracts. *Nat Protoc*, *7*(5), 959-969. doi:10.1038/nprot.2012.033
770 Shimamoto, Y., Maeda, Y. T., Ishiwata, S., Libchaber, A. J., & Kapoor, T. M. (2011).
771 Insights into the micromechanical properties of the metaphase spindle. *Cell*,
772 *145*(7), 1062-1074. doi:10.1016/j.cell.2011.05.038
773 Sikirzhyski, V., Magidson, V., Steinman, J. B., He, J., Le Berre, M., Tikhonenko, I., et
774 al. (2014). Direct kinetochore-spindle pole connections are not required for
775 chromosome segregation. *J Cell Biol*, *206*(2), 231-243.
776 doi:10.1083/jcb.201401090
777 Skibbens, R. V., & Salmon, E. D. (1997). Micromanipulation of chromosomes in mitotic
778 vertebrate tissue cells: tension controls the state of kinetochore movement. *Exp*
779 *Cell Res*, *235*(2), 314-324. doi:10.1006/excr.1997.3691
780 Surrey, T., Nedelec, F., Leibler, S., & Karsenti, E. (2001). Physical properties
781 determining self-organization of motors and microtubules. *Science*, *292*(5519),
782 1167-1171. doi:10.1126/science.1059758
783 Takagi, J., Sakamoto, R., Shiratsuchi, G., Maeda, Y. T., & Shimamoto, Y. (2019).
784 Mechanically Distinct Microtubule Arrays Determine the Length and Force
785 Response of the Meiotic Spindle. *Dev Cell*, *49*(2), 267-278 e265.
786 doi:10.1016/j.devcel.2019.03.014
787 Thevenaz, P., Ruttimann, U. E., & Unser, M. (1998). A pyramid approach to subpixel
788 registration based on intensity. *IEEE Trans Image Process*, *7*(1), 27-41.
789 doi:10.1109/83.650848
790 Udy, D. B., Voorhies, M., Chan, P. P., Lowe, T. M., & Dumont, S. (2015). Draft De Novo
791 Transcriptome of the Rat Kangaroo Potorous tridactylus as a Tool for Cell
792 Biology. *PLoS One*, *10*(8), e0134738. doi:10.1371/journal.pone.0134738

- 793 Vladimirou, E., McHedlishvili, N., Gasic, I., Armond, J. W., Samora, C. P., Meraldi, P., et
794 al. (2013). Nonautonomous movement of chromosomes in mitosis. *Dev Cell*,
795 27(1), 60-71. doi:10.1016/j.devcel.2013.08.004
796 Walen, K. H., & Brown, S. W. (1962). Chromosomes in a marsupial (*Potorous*
797 *tridactylis*) tissue culture. *Nature*, 194, 406. doi:10.1038/194406a0
798 Witt, P. L., Ris, H., & Borisy, G. G. (1981). Structure of kinetochore fibers: Microtubule
799 continuity and inter-microtubule bridges. *Chromosoma*, 83(4), 523-540.
800 doi:10.1007/bf00328277
801 Zhai, Y., Kronebusch, P. J., & Borisy, G. G. (1995). Kinetochore microtubule dynamics
802 and the metaphase-anaphase transition. *J Cell Biol*, 131(3), 721-734.
803 doi:10.1083/jcb.131.3.721
804 Zhu, C., & Jiang, W. (2005). Cell cycle-dependent translocation of PRC1 on the spindle
805 by Kif4 is essential for midzone formation and cytokinesis. *Proc Natl Acad Sci U*
806 *S A*, 102(2), 343-348. doi:10.1073/pnas.0408438102
807 Zhu, C., Lau, E., Schwarzenbacher, R., Bossy-Wetzel, E., & Jiang, W. (2006).
808 Spatiotemporal control of spindle midzone formation by PRC1 in human cells.
809 *Proc Natl Acad Sci U S A*, 103(16), 6196-6201. doi:10.1073/pnas.0506926103
810
811
812

813 **Main figure legends:**

814

815 **Figure 1: Microneedle manipulation can exert local forces with spatiotemporal**
816 **control on the mammalian spindle.** See also Figure 1-figure supplement 1 and Video
817 1.

818 **(A-B)** Representative PtK2 cell (GFP-tubulin, yellow) and membrane label (CellMask
819 Orange, magenta) **(A)** before (undeformed cell) and **(B)** during (deformed cell)
820 microneedle (Alexa-647, blue) manipulation. x-y and y-z views displayed (left and right
821 panels). y-z view taken along the white dashed line shown in the left panels.
822 **(C)** Left: Overlay of the y-z view of the membrane labeled images before (undeformed,
823 magenta) and during (deformed, green) microneedle manipulation, in order to compare
824 membrane shape and cell height (white line) adjacent to the microneedle due to
825 manipulation. Right: Cell height adjacent to the microneedle, measured using the

826 membrane label, in its undeformed versus deformed state (n=7 cells, Spearman R
827 coefficient=0.93, p=0.003). Dashed line represents no change in cell height. Solid grey
828 line is the linear regression fit to the data ($r^2=0.88$).

829 **(D)** Schematic showing a very local deformation of the cell by the microneedle during
830 manipulation, based on **(A-C)**.

831 **(E)** Schematic of the microneedle (black circle) manipulation assay used throughout this
832 study, pulling (arrow) on a spindle's outer k-fiber for two different magnitudes and
833 durations.

834 **(F)** Microneedle displacement over time for two different manipulation datasets: 12 s
835 (red, n=7 cells) and 60 s (navy, n=23 cells) pulls. Plot shows mean \pm SEM.

836 **(G)** Timelapse images of the representative response of a metaphase spindle in a PtK2
837 cell (GFP-tubulin, yellow), when its outer k-fiber is deformed by the microneedle (Alexa-
838 647, blue, white circle) by 2.5 μm over 60 s. The spindle enters anaphase about 20 min
839 after manipulation. Microneedle begins moving at 00:00 (first frame). Scale bar = 5 μm .
840 Time in min:sec.

841 **(H)** Overlay of the tubulin labeled images of the spindle **(G)** pre-manipulation
842 (undeformed, magenta) and post-manipulation and microneedle removal (relaxed,
843 cyan). The spindle's structure is similar pre- and post-manipulation, after correcting for
844 spindle movement.

845

846 **Figure 2: Pulling on kinetochore-fibers reveals the spindle's ability to retain local**
847 **architecture near chromosomes under seconds-long forces.** See also Figure 2-
848 figure supplement 1-3 and Video 2.

849 **(A)** Schematic of the assay to measure spindle deformation under local force:
850 manipulation of the outer k-fiber for 12 s (perturbation) and generation of strain maps
851 between undeformed (magenta) and deformed (green) spindles (measurement).
852 **(B)** Timelapse images of a representative PtK2 metaphase spindle (GFP-tubulin, grey)
853 during a 12 s manipulation, with microneedle position (white circle) displayed on
854 images. Scale bar = 5 μm . Time in min:sec.
855 **(C)** Strain map showing structural changes between undeformed (00:00, magenta
856 circles) and deformed (00:11, green stars) spindles shown in **(B)**, after correcting for
857 spindle movement. Strain corresponds to the distance (black line) between magenta
858 circles (undeformed spindle) and green stars (deformed spindle).
859 **(D)** Magnitude of deformation in the structure (mean \pm SEM) versus distance from the
860 microneedle in unmanipulated WT (control, grey, n=4 cells), manipulated WT (black,
861 n=7 cells) and manipulated FCPT-treated (positive control, red, n=4 cells) spindles.
862 **(E)** Schematic of the three measurements made in **(F,G,H)**: Inter-kinetochore distance
863 (measured between the manipulated k-fiber's and its sister's plus-ends), pole-pole
864 distance, and angle between the sister k-fiber plus-end (opposite the manipulated k-
865 fiber) and the pole-pole axis.
866 **(F)** Change in inter-kinetochore distance in WT unmanipulated (control, n=8 kinetochore
867 pairs from 4 cells) and WT manipulated (between undeformed and deformed, n=7
868 kinetochore pairs from 7 cells) spindles, measured over 12 s. There is no significant
869 difference in the inter-kinetochore distance upon manipulation (p=0.28, Mann-Whitney U
870 test).

871 **(G)** Change in pole-pole distance in WT unmanipulated (control, n=4 cells) and WT
872 manipulated (between undeformed and deformed, n=7 cells) spindles, measured over
873 12 s. Pole-pole distance decreases in manipulated spindles (p=0.008, Mann-Whitney U
874 test). Plot shows mean±SEM.

875 **(H)** Change in angle of sister k-fiber plus-end with respect to the pole-pole axis, in WT
876 unmanipulated (control, n=8 k-fibers from 4 cells) and WT manipulated (between
877 undeformed and deformed, n=7 k-fibers from 7 cells) spindles, measured over 12 s. The
878 sister k-fiber moves in towards the pole-pole axis in manipulated spindles (p=0.001,
879 Mann-Whitney U test). Plot shows mean±SEM.

880

881 **Figure 3: The deformed kinetochore-fiber's shape indicates specialized, short-**
882 **lived crosslinking to the spindle near chromosomes.** See also Figure 3-figure
883 supplement 1-3 and Videos 3-4.

884 **(A)** Schematic of the assay to probe the physical basis of k-fiber anchorage in the
885 spindle: manipulation of the outer k-fiber for 60 s and quantification of local curvature
886 along its length. The absence of k-fiber negative curvature (1) would suggest free
887 pivoting at poles and chromosomes. K-fiber negative curvature at poles (2) or
888 chromosomes (3) or at both (4) would suggest it is laterally anchored there, and
889 prevented from freely pivoting.

890 **(B)** Top: Timelapse images of a representative PtK2 metaphase spindle (GFP-tubulin,
891 grey) during a 60 s manipulation, with microneedle position (white circle) and traced
892 manipulated k-fiber (white) displayed on the images. Scale bar = 5 μ m. Time in min:sec.
893 Bottom: Curvature mapped along highlighted k-fiber for each time point in the top panel

894 (blue, negative curvature; red, positive curvature). This manipulation can expose
895 contact points (asterisk) between the k-fiber and non-kMTs.

896 **(C)** Local curvature of deformed k-fibers for normalized positions along the k-fiber (n=23
897 cells). Most k-fibers exhibit negative curvature near the chromosome (orange), and a
898 few show no negative curvature (grey) near the chromosome. Few k-fibers also show
899 negative curvature near poles. Scatter plot of microneedle positions shown above
900 (inset).

901 **(D)** Percentage of k-fiber curvature profiles with negative curvature less than $-0.1 \text{ 1}/\mu\text{m}$,
902 proximal to chromosomes (n=17/23 cells) and the pole (n=3/23 cells).

903 **(E)** Schematic of two possible outcomes of manipulating the outer k-fiber at different
904 locations along its length: either the negative curvature position (orange star) remains
905 fixed relative to the microneedle (black circle) position (uniform anchorage along the k-
906 fiber, Model 1) or remains fixed relative to the chromosome (specialized, non-uniform
907 anchorage near chromosome, Model 2).

908 **(F-G)** Position of the curvature maxima (microneedle, white circle) and curvature
909 minima (negative curvature, orange star) **(F)** measured from the microneedle position
910 (n=23 cells), and **(G)** measured from the chromosome (n=23 cells). Dashed lines
911 connect the maxima (microneedle) and minima (negative curvature) for a given
912 manipulation. The negative curvature position is tightly distributed near chromosomes,
913 regardless of the microneedle's position, supporting a specialized crosslinking model
914 (Model 2, Figure 3E). Plot also shows microneedle positions of manipulations that do
915 not result in negative curvature (grey circles).

916 **(H)** Top: Timelapse images of a PtK2 metaphase spindle (GFP-tubulin, grey)
917 manipulate-and-hold experiment to probe the timescale of k-fiber reinforcement in the
918 spindle center, performing a 60 s manipulation and then holding the microneedle (white
919 circle) in place to measure when the negative curvature in the manipulated k-fiber (white
920 trace) disappears (1:39, 21 s after 1:18 hold started). Scale bar = 5 μm . Time in
921 min:sec. Bottom: Curvature mapped along highlighted k-fiber for each point in the top
922 panel (blue, negative curvature; red, positive curvature). Negative curvature (black
923 arrow) disappears over 21 s of holding time.

924 **(I)** Curvature minima near chromosome as a function of time the microneedle has been
925 held in place (n=5 cells). Negative curvature disappears after holding for 20 s. Plot
926 shows mean \pm SEM (orange).

927

928 **Figure 4: The microtubule crosslinker PRC1 mediates the specialized and short-**
929 **lived kinetochore-fiber reinforcement near chromosomes.** See also Figure 4-figure
930 supplement 1 and Video 5.

931 **(A)** Immunofluorescence images of a representative PtK2 WT metaphase spindle
932 showing where PRC1 is localized in the spindle (tubulin, yellow; PRC1, magenta). White
933 box (bottom panel) shows the region in which intensity **(B)** was quantified. Scale bar = 5
934 μm .

935 **(B)** Fluorescence intensity ratio of PRC1 to tubulin along the pole-pole axis (n=6 cells),
936 showing PRC1 localization in the spindle center. Plot shows mean \pm SEM.

937 **(C)** Immunofluorescence images of a representative PtK2 PRC1 RNAi metaphase
938 spindle (tubulin, yellow; PRC1, magenta), showing PRC1 depletion. Scale bar = 5 μm .

939 **(D)** Top: Timelapse images of a representative PtK2 metaphase PRC1 RNAi spindle
940 (GFP-tubulin, grey) during a 60 s manipulation, showing microneedle position (white
941 circle) and traced manipulated k-fiber (white). Scale bar = 5 μ m. Time in min:sec.
942 Bottom: Curvature mapped along traced k-fiber for each point in the top panel (blue,
943 negative curvature; red, positive curvature), showing the absence of negative curvature
944 near chromosomes without PRC1.

945 **(E)** Local curvature of deformed k-fibers for normalized positions along the k-fiber (n=12
946 k-fibers in 12 cells). Most k-fibers exhibit no negative curvature (grey) and one shows
947 negative curvature similar to WT k-fibers (orange). Scatter plot of microneedle positions
948 shown above (inset).

949 **(F)** Left: Distribution of microneedle positions along the k-fiber in WT (n=18 cells) and
950 PRC1 RNAi (n=12 cells) spindles, after datasets are minimally downsampled to
951 maximize microneedle position overlap between them. There is no significant difference
952 in microneedle position in the two conditions ($p=0.19$, Mann-Whitney U test). Plot shows
953 mean \pm SEM. Right: Percentage of deformed k-fiber profiles showing negative curvature
954 near chromosomes in WT and PRC1 RNAi manipulated spindles, showing loss of
955 negative curvature without PRC1.

956 **(G)** Schematic of the three measurements made in **(H,I,J)**: Inter-kinetochore distance
957 between the manipulated k-fiber and its sister, angle between the sister k-fiber plus-end
958 (opposite the manipulated k-fiber) and the pole-pole axis, and the angle between sister
959 k-fiber plus-end regions.

960 **(H)** Change in inter-kinetochore distance in WT unmanipulated (control, n=13
961 kinetochore pairs from 6 cells), WT manipulated (n=8 kinetochore pairs from 8 cells)

962 and PRC1 RNAi manipulated (n=8 kinetochore pairs from 8 cells) spindles, measured
963 over 60 s. Inter-kinetochore distance after manipulation is significantly higher in spindles
964 with PRC1 RNAi than WT ($p=0.003$, Mann-Whitney U test). Plot shows mean \pm SEM.

965 **(I)** Change in angle of sister k-fiber plus-end with respect to the pole-pole axis in WT
966 unmanipulated (control, n=12 k-fibers from 6 cells) and WT manipulated (n=11 k-fibers
967 from 11 cells) and PRC1 RNAi manipulated (n=9 k-fibers from 9 cells) spindles,
968 measured over 60 s. The sister k-fiber moves less (smaller angle) towards the pole-pole
969 axis after manipulation in PRC1 RNAi spindles compared to WT ($p=0.004$, Mann-
970 Whitney U test). Plot shows mean \pm SEM.

971 **(J)** Distribution of the angle between sister k-fiber plus-end regions at the end of
972 manipulation in WT (n=19 cells) and PRC1 RNAi (n=10 cells) spindles, measured
973 between the chromosome-proximal regions of the k-fibers.

974

975 **Figure 5: Model for specialized, short-lived reinforcement near chromosomes**
976 **in the mammalian spindle**

977 **(A)** K-fiber reinforcement in space: Microneedle (black circle) manipulation of the
978 mammalian spindle reveals that k-fibers (light grey) are weakly coupled to their
979 neighbors (thin dashed vertical line), strongly coupled to their sisters (thick horizontal
980 line), and freely pivot around the pole (black arrow) but not around chromosomes: they
981 are locally reinforced (dashed box, 3 μ m) near chromosomes (spring) through
982 specialized, non-uniform mechanisms requiring the microtubule crosslinker PRC1 (light
983 blue squares). Other crosslinkers in the spindle are shown in dark grey.

984 **(B)** K-fiber reinforcement in time: Local reinforcement near chromosomes is preserved

985 over seconds (with a lifetime of 20 s, black star) yet remodels over minutes as
986 molecules turn over in the spindle center. This allows the local architecture in the
987 spindle center to persist under transient force fluctuations, and yet respond to sustained
988 forces. Such short-lived reinforcement could help protect chromosome-to-spindle
989 connections while allowing them to remodel (green arrow) as mitosis progresses. For
990 simplicity, we only depict PRC1 turning over as time evolves (from light blue to dark
991 blue PRC1 molecules), though microtubules and other crosslinkers also turn over.

992

993 **Supplemental figure legends:**

994

995 **Figure 1 - figure supplement 1: Propidium iodide remains outside cells during**
996 **microneedle manipulation**

997 **(A)** Representative images of two PtK2 cells (GFP-tubulin, yellow) with compromised
998 membranes in which cell impermeable propidium iodide binds and labels DNA
999 (magenta). Scale bar = 5 μm .

1000 **(B)** Timelapse images of a PtK2 spindle (GFP-tubulin, yellow) during a 60 s
1001 manipulation in which propidium iodide (magenta) in the media does not enter the cell,
1002 suggesting that the membrane is sealed and does not rupture due to the microneedle
1003 (Alexa-647, blue, white circle) during this process. Scale bar = 5 μm . Time in min:sec

1004

1005 **Figure 2 - figure supplement 1: Kinetochore-fiber length does not change over 12**
1006 **s manipulations**

1007 Magnitude of change in k-fiber length in WT unmanipulated (control, n=8 k-fibers from 4
1008 cells) and WT manipulated spindles (between undeformed and deformed, n=7 k-fibers
1009 from 7 cells), measured over 12 s. Mean±SEM displayed over points. There is no
1010 significant difference in k-fiber length during manipulation over this timescale (p=0.69,
1011 Mann-Whitney U test).

1012

1013 **Figure 2 - figure supplement 2: Estimating the exponential decay rate of spindle**
1014 **deformations over space**

1015 Magnitude of deformation in the structure versus distance from the microneedle in **(A)**
1016 WT manipulated (n=7 cells) and **(B)** FCPT-treated manipulated (n=4 cells) spindles, due
1017 to 12 s manipulations. Thin grey lines are individual traces, thick lines (WT, black;
1018 FCPT, red) are exponential decay functions fit to the data. The equation that produced
1019 the best fits to the data is displayed.

1020

1021 **Figure 2 - figure supplement 3: The angle between sister kinetochore-fibers is**
1022 **preserved in 12 s manipulations**

1023 Change in angle between sister k-fiber plus-end regions in the outer pair, in WT
1024 unmanipulated (control, n=6 k-fiber pairs from 4 cells) and WT manipulated (n=6 k-fiber
1025 pairs from 6 cells) spindles, measured over 12 s. There is no significant change in angle
1026 between sister k-fibers in WT spindles after manipulation (p=0.22, Mann-Whitney U
1027 test).

1028

1029 **Figure 3 - figure supplement 1: Deformed kinetochore-fibers exhibit negative**

1030 **curvature in 12 s manipulations**

1031 **(A)** Top: Timelapse images of a representative spindle (GFP-tubulin, grey) during a 12 s
1032 manipulation. Microneedle position (white circle) and traced manipulated k-fiber (white)
1033 displayed on the images. Scale bar = 5 μm . Time in min:sec. Bottom: Curvature
1034 mapped along highlighted k-fiber for each point in the top panel (blue, negative
1035 curvature; red, positive curvature). This manipulation can expose contact points
1036 between the k-fiber and non-kMTs (asterisk).

1037 **(B)** Local curvature of deformed k-fibers for normalized positions along the k-fiber (n=7
1038 cells). Some k-fibers exhibit negative curvature near the chromosome (orange), and
1039 others do not (grey).

1040

1041 **Figure 3 - figure supplement 2: Tight coupling between sister kinetochore-fibers**
1042 **in 60 s manipulations**

1043 **(A)** Schematic of the two measurements made in **(B-C)**: Inter-kinetochore distance
1044 measured between sister k-fiber plus-ends of the manipulated k-fiber, and angle
1045 measured between the sister k-fiber plus-end (opposite the manipulated k-fiber) and the
1046 pole-pole axis, measured over 60 s.

1047 **(B)** Change in inter-kinetochore distance in WT unmanipulated (control, n=13
1048 kinetochore pairs from 6 cells) and WT manipulated (n=8 kinetochore pairs from 8 cells)
1049 spindles, measured over 60 s. There was no significant difference in inter-kinetochore
1050 distance after manipulation ($p=0.06$, Mann-Whitney U test). Plot shows mean \pm SEM.

1051 **(C)** Change in angle of sister k-fiber plus-end with respect to the pole-pole axis, in WT
1052 unmanipulated (control, n=12 k-fibers from 6 cells) and WT manipulated (n=11 k-fibers

1053 from 11 cells) spindles, measured over 60 s. The sister k-fiber in manipulated spindles
1054 moves in towards the pole-pole axis ($p=0.0005$, Mann-Whitney U test). Plot shows
1055 mean \pm SEM.

1056

1057 **Figure 3 - figure supplement 3: Non-kinetochore microtubule contacts distributed**
1058 **close to observed negative curvature**

1059 Frequency distribution of the distance from a contact point between the k-fiber and non-
1060 kMTs (non-kMT contact) to the curvature minima (negative curvature) position. The
1061 average distance between a non-kMT contact and the negative curvature near
1062 chromosomes is 1 ± 0.1 μm (mean \pm SEM, $n=14$ cells).

1063

1064 **Figure 4 - figure supplement 1: Immunofluorescence quantifications of inter-**
1065 **kinetochore distance and tubulin intensity between PRC1 RNAi and WT spindles**

1066 **(A)** Inter-kinetochore distance of WT ($n=22$ kinetochore pairs from 12 cells) and PRC1
1067 RNAi spindles ($n=44$ kinetochore pairs from 24 cells) measured from
1068 immunofluorescence images. Plot shows mean \pm SEM. The inter-kinetochore distance in
1069 PRC1 RNAi spindles is smaller than that of WT spindles ($p=0.0006$, Mann-Whitney U
1070 test).

1071 **(B)** Average fluorescence intensity of tubulin above cytoplasmic background levels in
1072 WT ($n=12$ cells) and PRC1 RNAi ($n=24$ cells) spindles. Regions of interest (dashed-line
1073 box) include the whole spindle excluding poles (similar to Figure 4A) and the equator
1074 region near chromosomes. Plot shows mean \pm SEM. Tubulin intensity remains
1075 unchanged upon PRC1 RNAi in the whole spindle ($p=0.43$, Mann-Whitney U test), but

1076 slightly lower in the equator region though not significant ($p=0.08$, Mann-Whitney U
1077 test).

1078

1079 **Video Legends:**

1080

1081 **Video 1: Microneedle manipulation of a mammalian mitotic spindle at metaphase**

1082 **showing spindle relaxation and anaphase entry post-manipulation.** Related to

1083 Figure 1.

1084

1085 Microneedle manipulation of a metaphase spindle in a PtK2 cell. The microneedle

1086 (Alexa-647, blue) pulls (time 00:00) on the spindle's outer k-fiber (GFP-tubulin, yellow)

1087 over 60 s and deforms the spindle. Upon needle removal (time 00:51), the spindle

1088 typically returns to its original structure. About 20 min after manipulation, the spindle has

1089 progressed to anaphase (time 25:10), consistent with cell health post manipulation.

1090 Scale bar = 5 μm . Time in min:sec. Video was collected using a spinning disk confocal

1091 microscope, at a rate of 1 frame every 5 s before and during manipulation. Video has

1092 been set to play back at constant rate of 5 frames per second. Movie corresponds to still

1093 images from Figure 1G.

1094

1095 **Video 2: The spindle locally deforms under seconds-long forces.** Related to Figure

1096 2.

1097

1098 Microneedle manipulation of a metaphase spindle in a PtK2 cell. The microneedle
1099 (Alexa-647, white circle) pulls (time 00:00) on the spindle's outer k-fiber (GFP-tubulin,
1100 grey) over 12 s and deforms the spindle. The k-fiber bends around the microneedle and
1101 the rest of the spindle structure appears unaffected by the force exerted, indicating a
1102 local structural response. Scale bar = 5 μm . Time in min:sec. Video was collected using
1103 a spinning disk confocal microscope, at a rate of 4 frames per second during
1104 manipulation. Video has been set to play back at constant rate of 5 frames per second.
1105 Movie corresponds to still images from Figure 2B.

1106

1107 **Video 3: Microneedle manipulation of a kinetochore-fiber reveals free pivoting**
1108 **around poles and local reinforcement near chromosomes.** Related to Figure 3.

1109

1110 Microneedle manipulation of a metaphase spindle in a PtK2 cell. The microneedle
1111 (Alexa-647, white circle) pulls (time 00:00) on the spindle's outer k-fiber (GFP-tubulin,
1112 grey) over 60 s and deforms the spindle. The k-fiber bends around the needle, freely
1113 pivots around the pole but does not pivot around chromosomes, instead remaining
1114 straight in the spindle center. This indicates the presence of a region of k-fiber
1115 reinforcement in the spindle center. Scale bar = 5 μm . Time in min:sec. Video was
1116 collected using a spinning disk confocal microscope, at a rate of 1 frame every 4 s
1117 before and during manipulation. Video has been set to play back at constant rate of 5
1118 frames per second. Movie corresponds to still images from Figure 3B.

1119

1120 **Video 4: Manipulate-and-hold assay reveals that local reinforcement near**

1121 **chromosome has a 20 s lifetime.** Related to Figure 3.

1122

1123 Manipulate-and-hold experiment in a metaphase spindle in a PtK2 cell. The
1124 microneedle (Alexa-647, white circle) pulls (time 00:00) on the spindle's outer k-fiber
1125 (GFP-tubulin, grey) over 60 s and is then held in place for 30 s. During the course of the
1126 microneedle hold, the negative curvature on the deformed k-fiber disappears in 20 s,
1127 indicating that this local, specialized reinforcement is short-lived. Scale bar = 5 μm . Time
1128 in min:sec. Video was collected using a spinning disk confocal microscope, with a
1129 variable frame rate of 1 frame every 10 s before manipulation and 1 frame per every 7 s
1130 during manipulation. Video has been set to play back at 7 frames per second despite
1131 the variable acquisition rate. Movie corresponds to still images from Figure 3H.

1132

1133 **Video 5: The microtubule crosslinker PRC1 mediates the specialized and short-**
1134 **lived kinetochore-fiber reinforcement near chromosomes.** Related to Figure 4.

1135

1136 Microneedle manipulation of a metaphase spindle in a PtK2 cell depleted of PRC1 by
1137 RNAi. The microneedle (Alexa-647, white circle) pulls (time 00:00) on the spindle's
1138 outer k-fiber (GFP-tubulin, grey) over 60 s and deforms the spindle. The k-fiber bends
1139 around the needle, similar to WT, however shows no negative curvature near
1140 chromosomes. This suggests that PRC1 is needed for the specialized, short-lived k-
1141 fiber reinforcement near chromosomes. Scale bar = 5 μm . Time in min:sec. Video was
1142 collected using a spinning disk confocal microscope, at a rate of 1 frame every 7 s

1143 during manipulation. Video has been set to play back at constant rate of 5 frames per
1144 second. Movie corresponds to still images from Figure 4D.

1145

1146

Figure 1: Microneedle manipulation can exert local forces with spatiotemporal control on the mammalian spindle

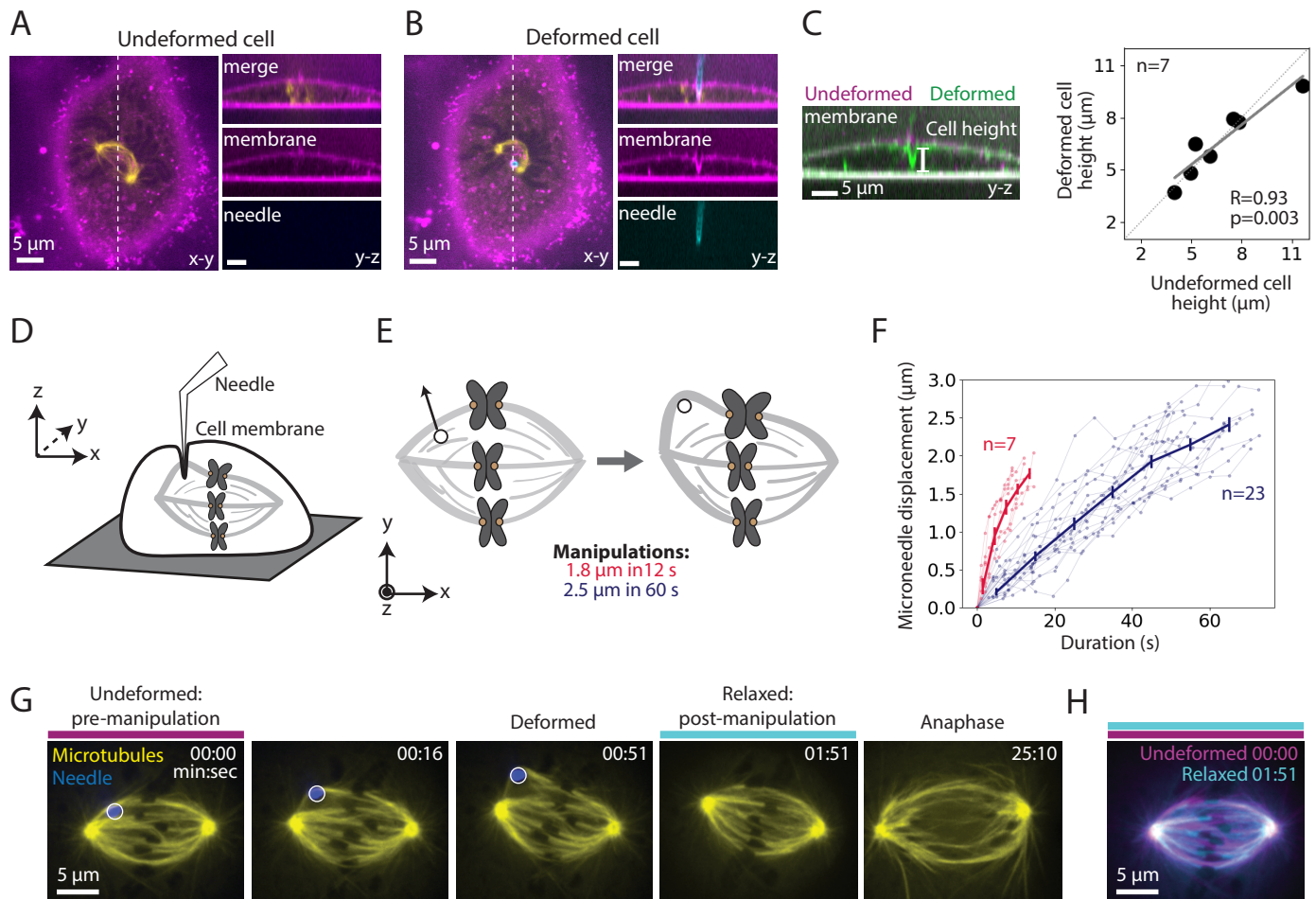


Figure 2: Pulling on kinetochore-fibers reveals the spindle's ability to retain local architecture near chromosomes under seconds-long forces

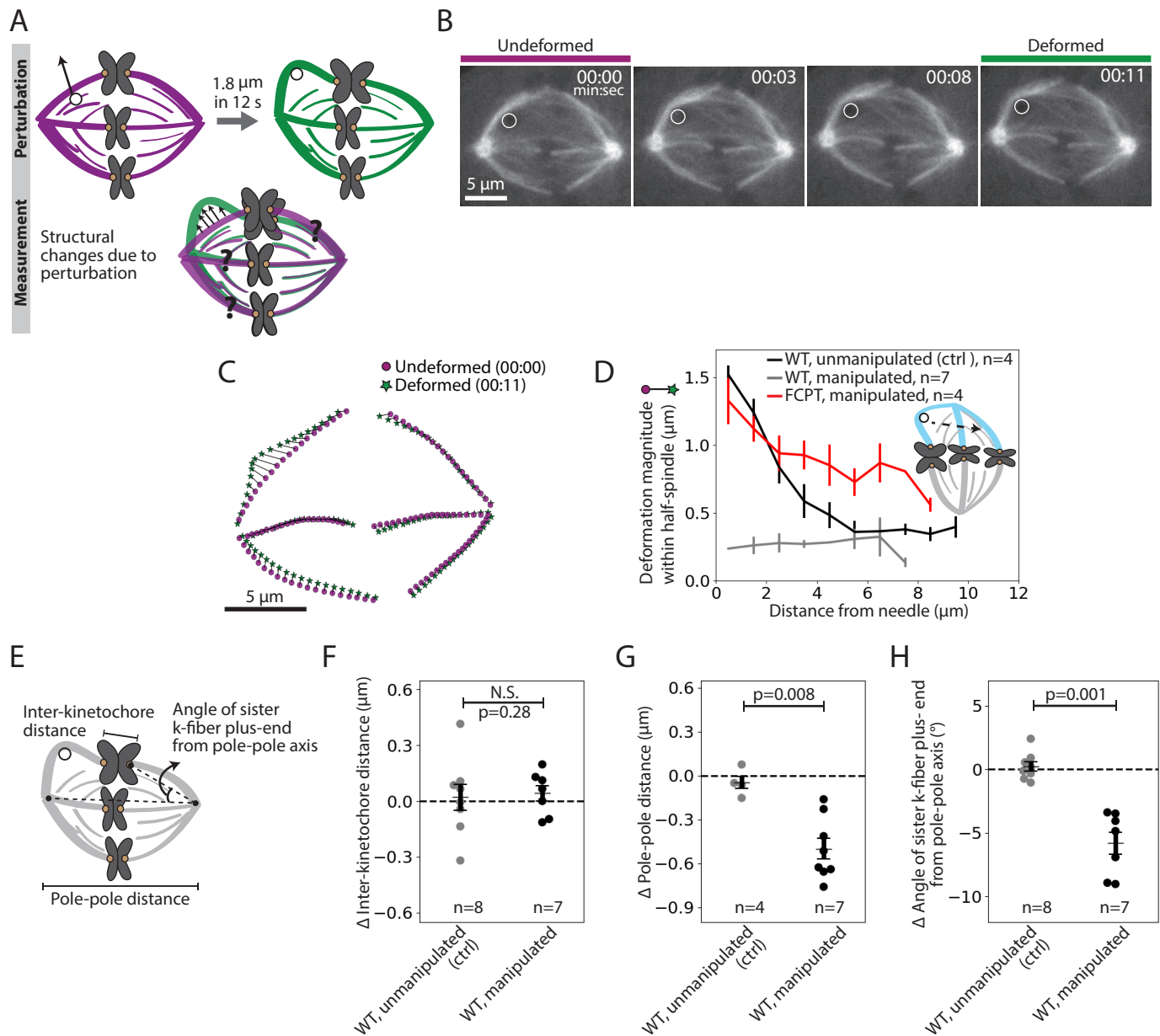


Figure 3: The deformed kinetochore-fiber's shape indicates specialized, short-lived crosslinking to the spindle near chromosomes

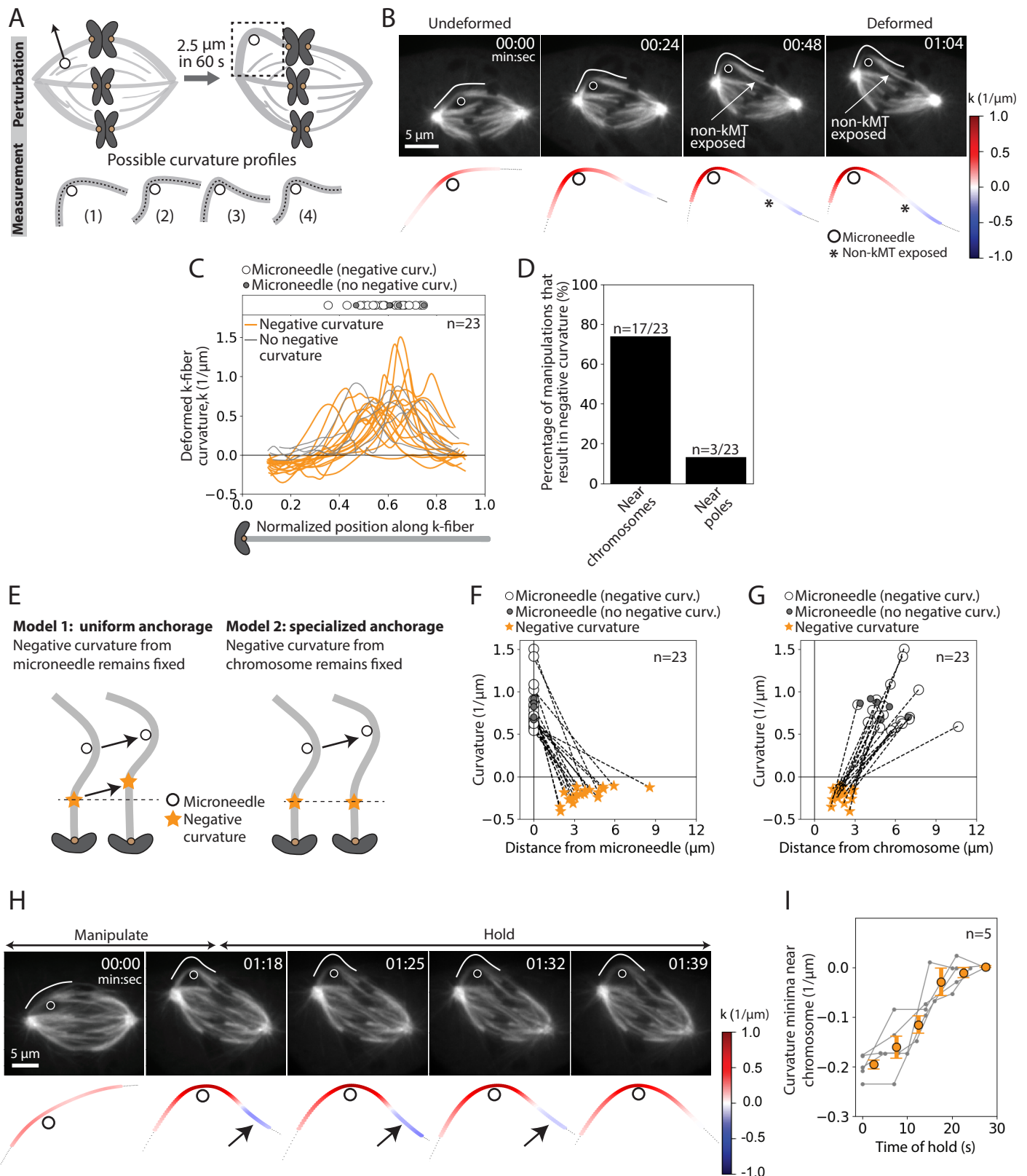


Figure 4: The microtubule crosslinker PRC1 mediates the specialized and short-lived kinetochore-fiber reinforcement near chromosomes

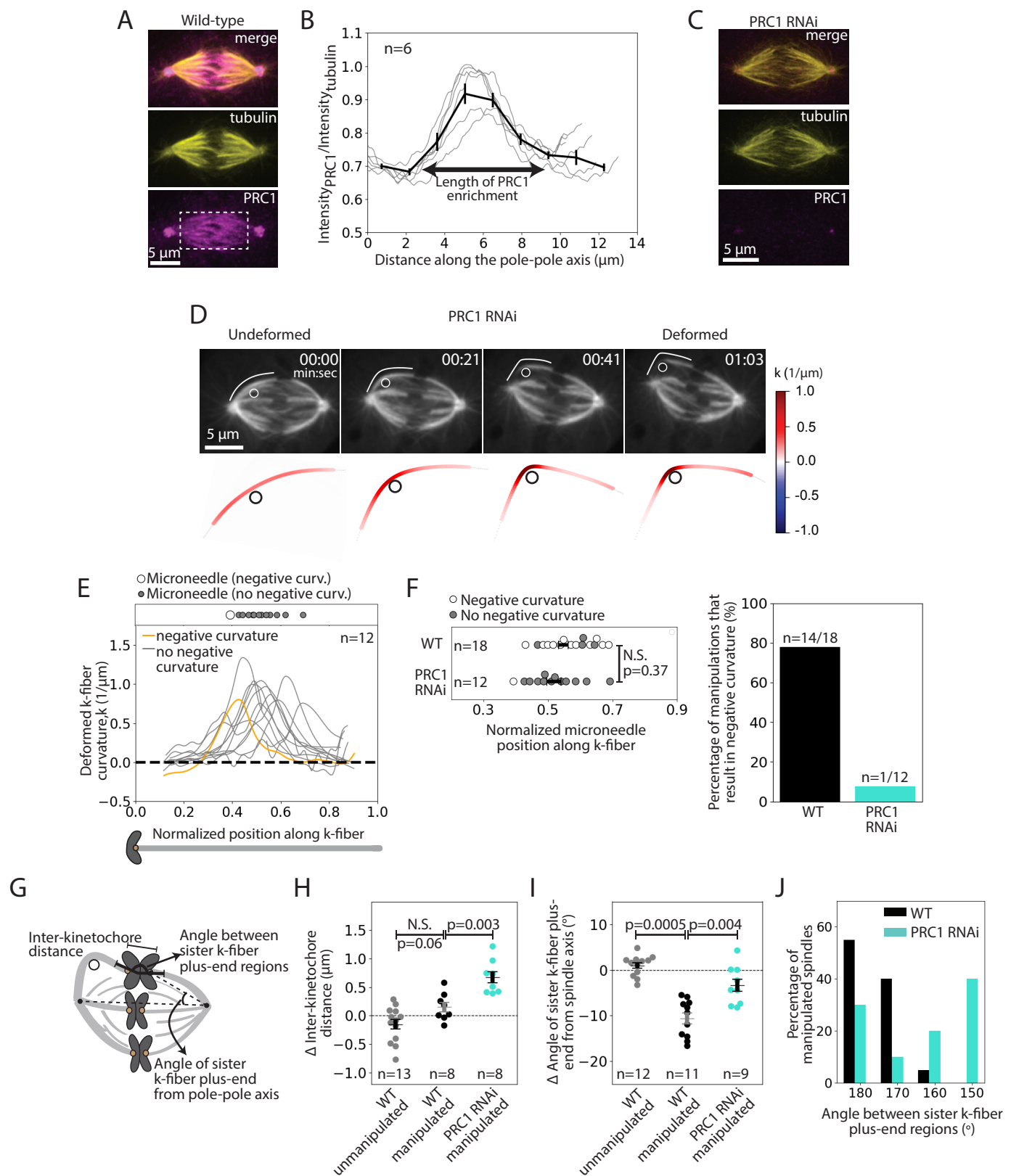


Figure 5: Model for specialized, short-lived reinforcement near chromosomes in the mammalian spindle

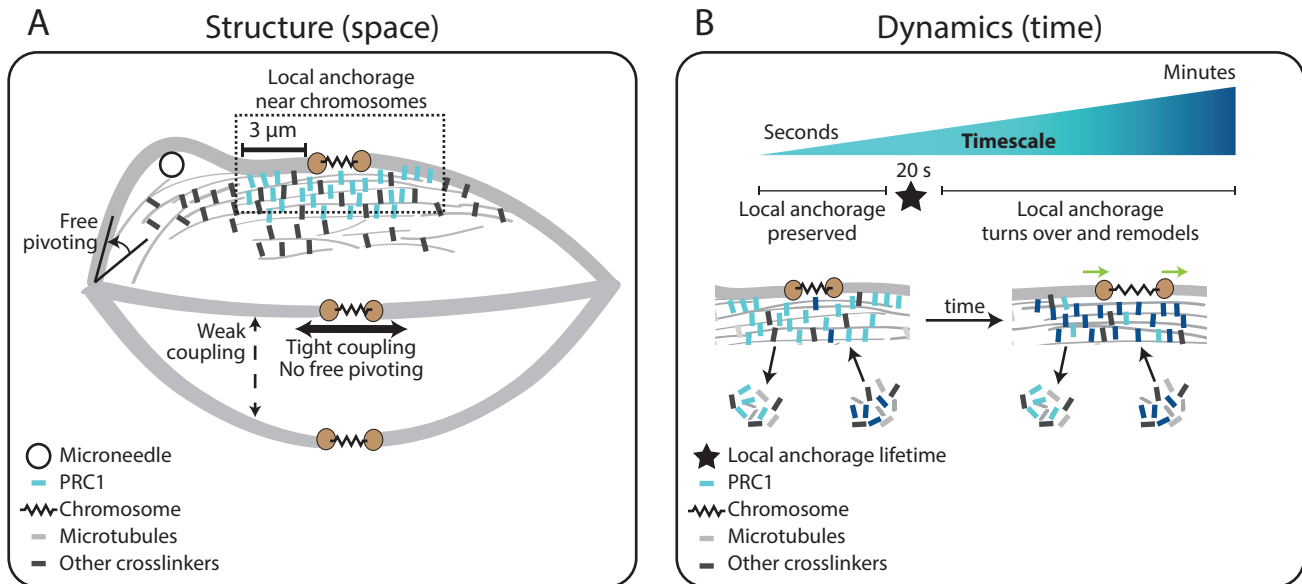
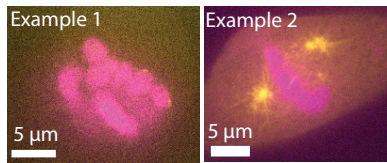


Figure 1- figure supplement 1:
Propidium iodide remains outside cells during microneedle manipulation

A

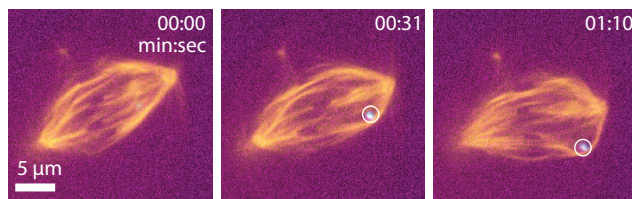
Cells with compromised membranes:
Propidium iodide bound to DNA



Microtubules
Propidium iodide

B

During manipulation: Propidium iodide excluded from cell



Microneedle
Microtubules
Propidium iodide

Figure 2- figure supplement 1:
Kinetochores-fiber length does not change over 12 s manipulations

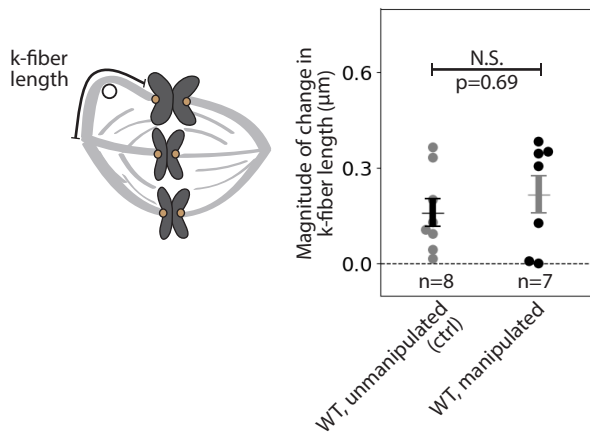


Figure 2- figure supplement 2:
Estimating the exponential decay rate of spindle deformations over space

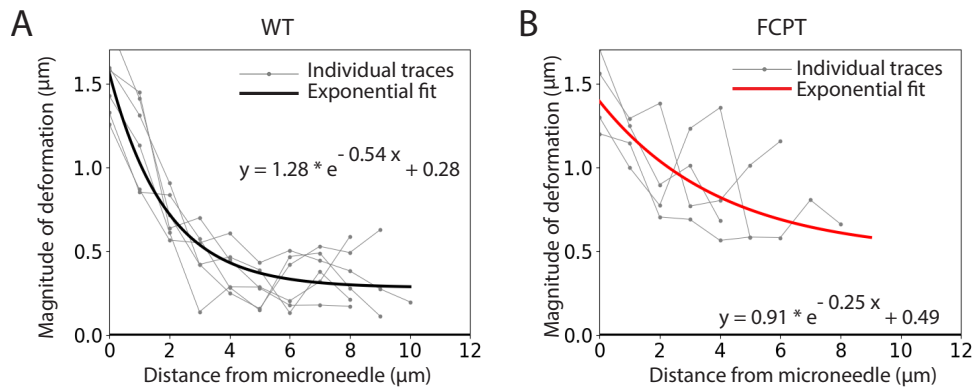


Figure 2- figure supplement 3:

The angle between sister kinetochore-fibers is preserved in 12 s manipulations

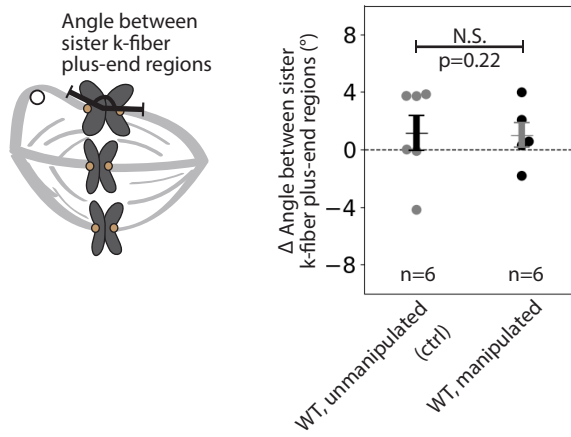


Figure 3- figure supplement 1:

Deformed kinetochore-fibers exhibit negative curvature in 12 s manipulations

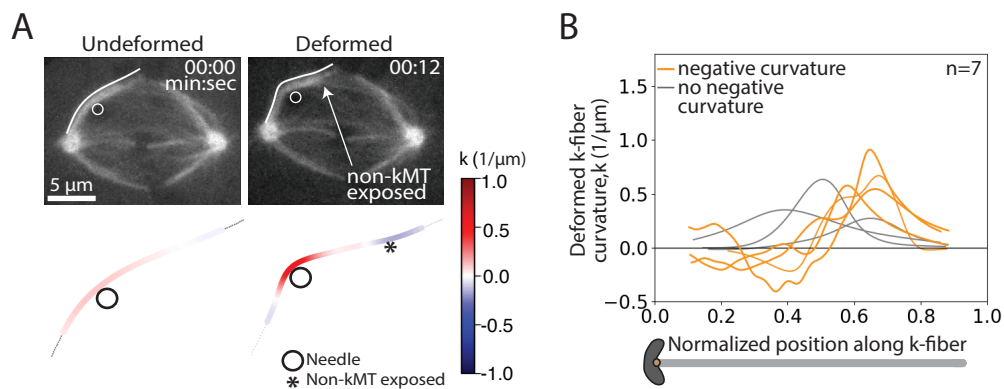


Figure 3- figure supplement 2:

Tight coupling between sister kinetochores-fibers in 60 s manipulations

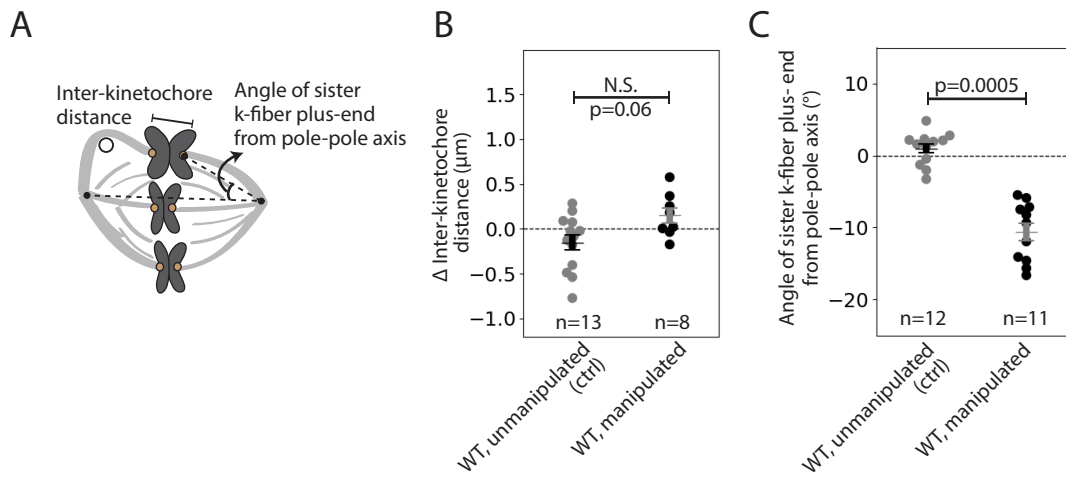


Figure 3- figure supplement 3:

Non-kinetochore microtubule contacts distributed close to observed negative curvature

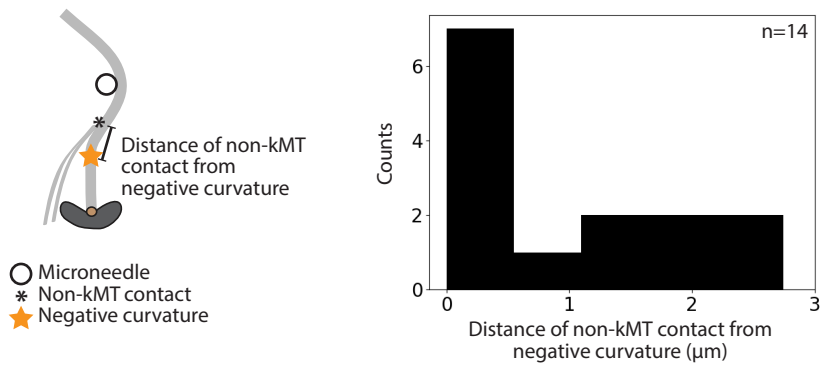


Figure 4- figure supplement 1:
Immunofluorescence quantifications of inter-kinetochore distance and tubulin intensity between PRC1 RNAi and WT spindles

