Appendix for

Two-step nuclear centring by competing microtubule- and actin-based mechanisms in 2-cell mouse embryos

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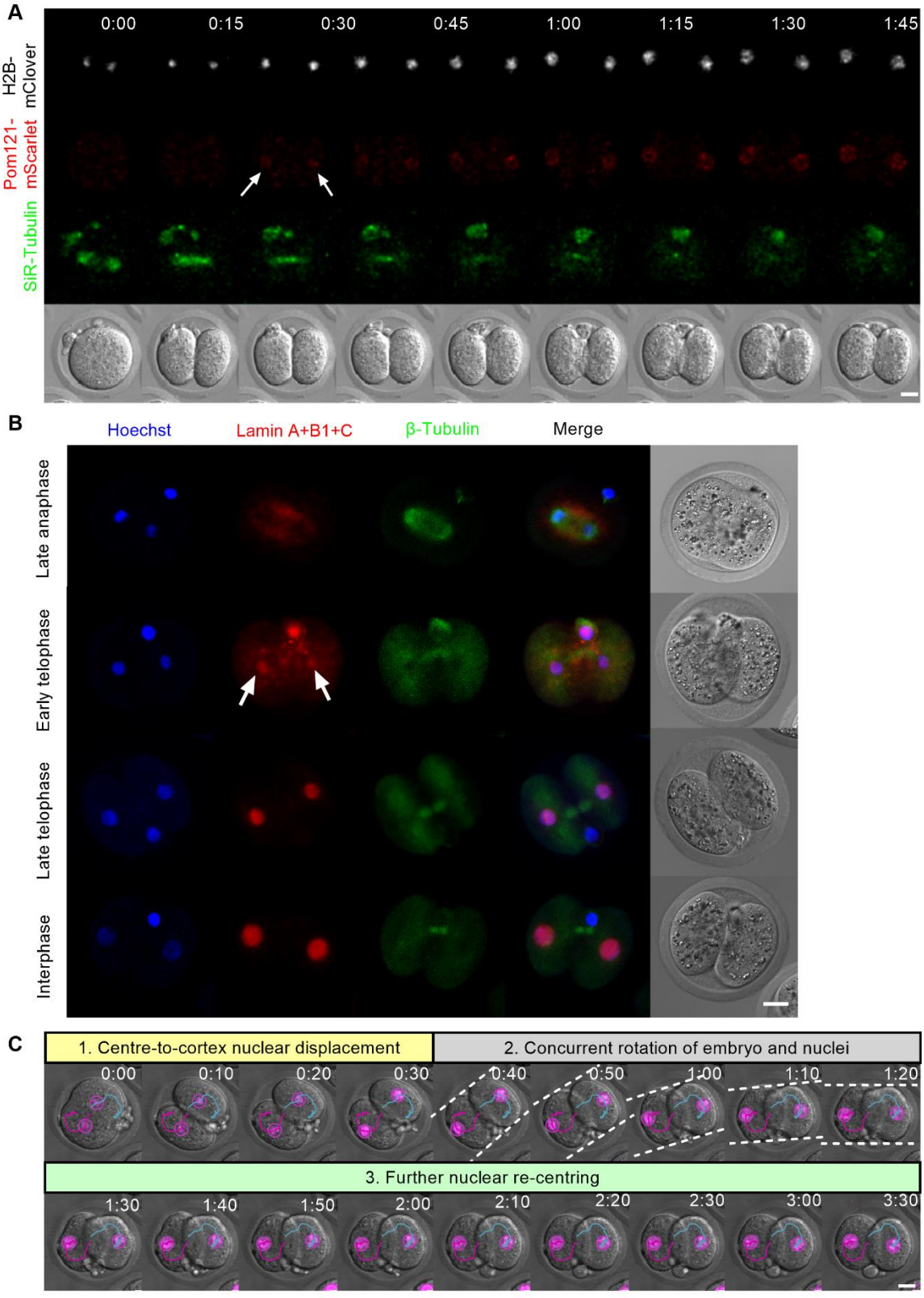

A
0:00
0:15
0:30
0:45
1:00
1:15
1:30
1:45
H2B-mClover
Pom121-mScarlet
SiR-Tubulin
B
Hoechst
Lamin A+B1+C
β-Tubulin
Merge
Late anaphase
Early telophase
Late telophase
Interphase
C
1. Centre-to-cortex nuclear displacement
2. Concurrent rotation of embryo and nuclei
0:00
0:10
0:20
0:30
0:40
0:50
1:00
1:10
1:20
3. Further nuclear re-centring
1:30
1:40
1:50
2:00
2:10
2:20
2:30
3:00
3:30

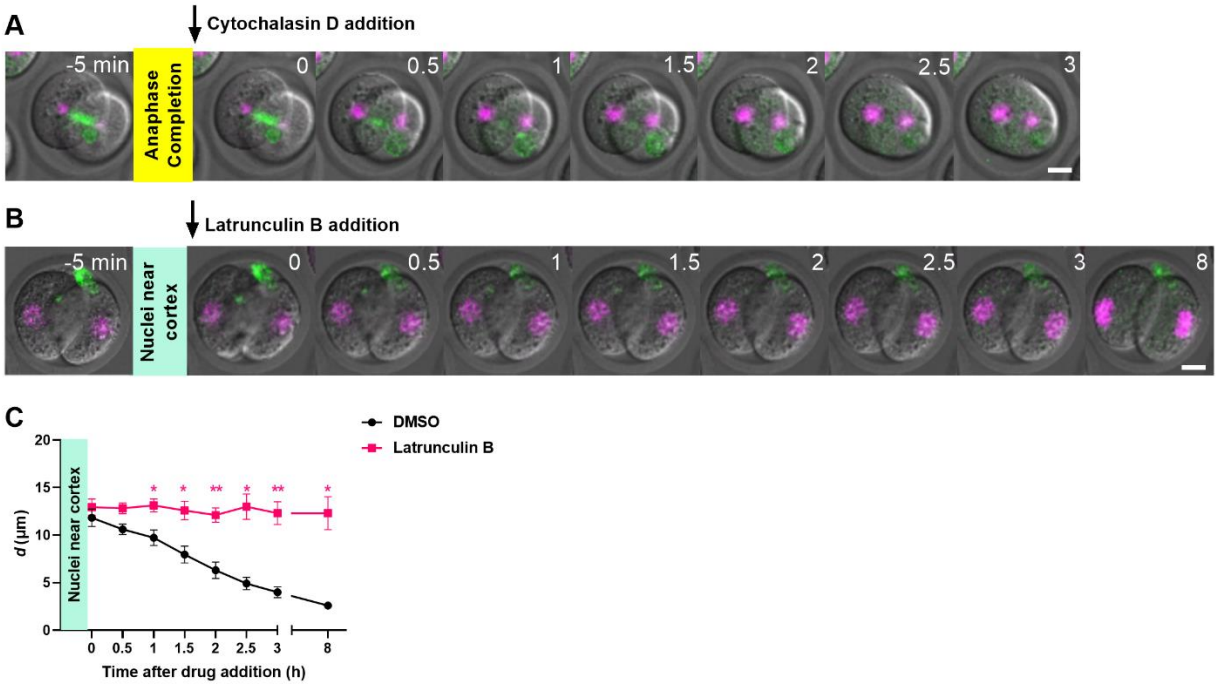
Appendix Figure S1.

A Time-lapse images of an embryo expressing a fluorescently labelled nuclear pore complex protein, Pom121-mScarlet, and H2B-mClover with tubulin labelled using SiR-tubulin. White arrows indicate when Pom121-mScarlet first shows clear co-localisation with DNA (n = 20 embryos). Time is shown as h:min post-anaphase onset.

B Shown are representative images of embryos immunostained for lamin A+B1+C, β-tubulin and Hoechst at the cell-cycle stages shown. White arrows indicate the first appearance of lamin signals co-localising with decondensed DNA (n > 3 embryos each time point).

C Example of concurrent rotation of embryo and nuclei during nuclear re-centring. Time is shown as h:min post-anaphase onset.

Data information: In (A – C), scale bar = 20 μm.

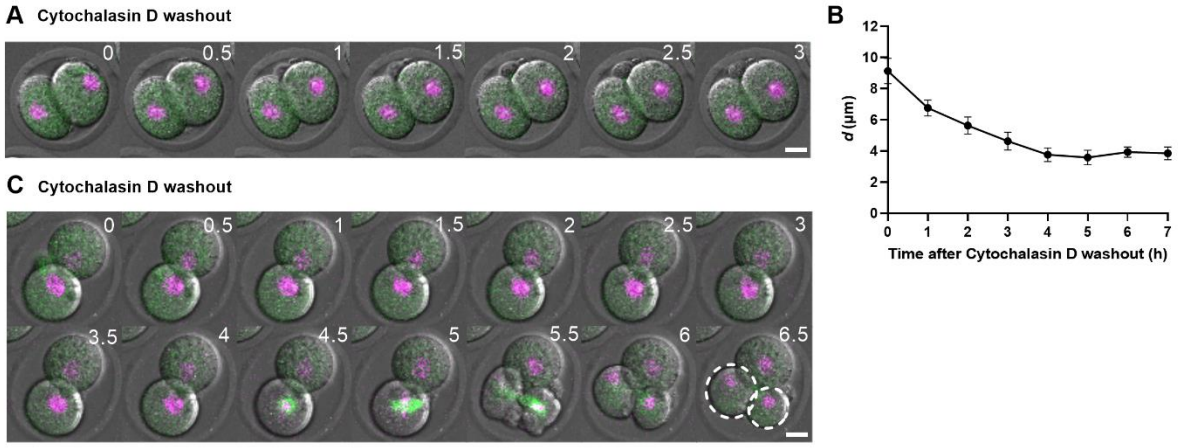


Appendix Figure S2.

A Representative time-lapse images of embryos expressing H2B-RFP and labelled with SiR-tubulin following cytochalasin D addition after cytokinesis completion (n = 11 embryos).

B, C Representative time-lapse images (B) and quantification (C) of embryos following treatment with Latrunculin B after nuclei reached the cortex (n = 5 blastomeres, mean ± SEM).

Data information: Scale bar = 20 µm. In (A, B), time is shown as h post-drug addition. Statistical significance was assessed by Mann-Whitney test, (*P < 0.05, **P < 0.01, unremarked datapoints = not significant).



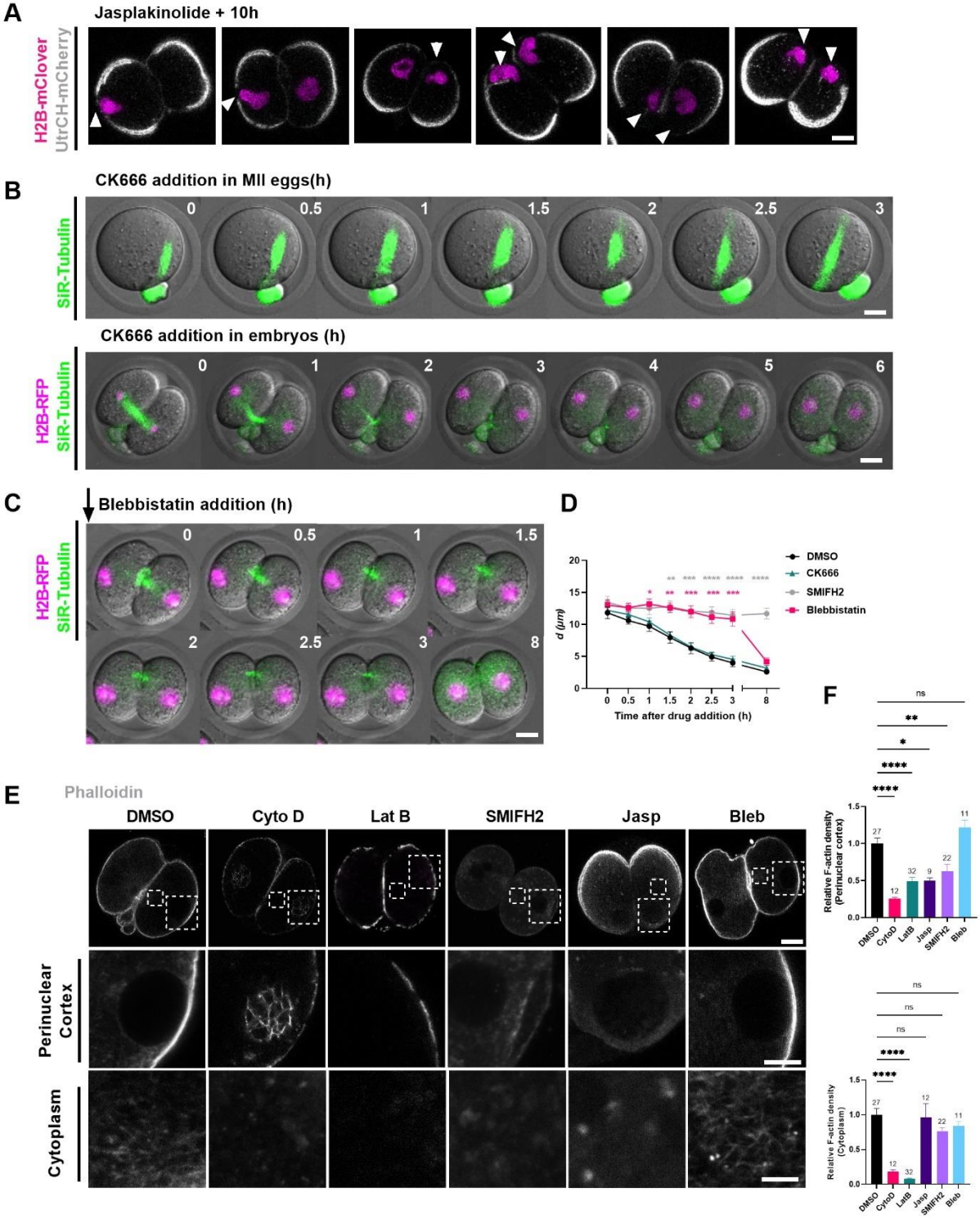
Appendix Figure S3

A Representative time-lapse images of embryos whose nuclei had been trapped at the cortex by cytochalasin D following drug washout.

B Changes in nucleus-to-cell centre distances (*d*) over time following cytochalasin D washout (n = 22 blastomeres).

C An example of asymmetric division that occurred before nuclei were centred following cytochalasin D washout. Time is shown as h post-drug washout.

Data information: In (A, C), time is shown as h post-drug washout and scale bar = 20 µm. In (B), data are shown as mean ± SEM.


A
Jasplakinolide + 10h
H2B-mClover
UtrCH-mCherry
B
CK666 addition in MII eggs(h)
SiR-Tubulin
0
0.5
1
1.5
2
2.5
3
CK666 addition in embryos (h)
H2B-RFP
SiR-Tubulin
0
1
2
3
4
5
6
C
Blebbistatin addition (h)
H2B-RFP
SiR-Tubulin
0
0.5
1
1.5
2
2.5
3
8
D
DMSO
CK666
SMIFH2
Blebbistatin
d (μm)
Time after drug addition (h)
E
Phalloidin
DMSO
Cyto D
Lat B
SMIFH2
Jasp
Bleb
Perinuclear Cortex
Cytoplasm
F
Relative F-actin density (Perinuclear cortex)
Relative F-actin density (Cytoplasm)
DMSO
CytoD
LatB
Jasp
SMIFH2
Bleb

Appendix Figure S4

A Effect of Jasplakinolide on actin and nuclei 10h post-drug addition. Arrowheads indicate F-actin-deprived actin cortex near displaced nuclei.

B Representative time-lapse images of meiosis II-arrested oocytes (top, n = 7 oocytes) and 2-cell embryos (bottom, n = 16 blastomeres) labelled with SiR-tubulin in the presence of CK666.

C Representative time-lapse images of 2-cell embryos expressing H2B-RFP and labelled with SiR-Tubulin following treatment with blebbistatin when the nuclei are near the cortex.

D Changes in nucleus-to-cell centre distances (*d*) over time relative to time of drug addition in DMSO- (n = 20 blastomeres), SMIFH2 (n = 9 blastomeres), and CK666- (n = 16 blastomeres), and blebbistatin (n = 12 blastomeres) treated 2-cell blastomeres. Time is shown as h post-drug addition.

E Shown are representative images of embryos immunostained for phalloidin. DMSO-treated embryos were cultured in DMSO from pronucleus-stage and fixed when nuclei were near the cortex. In the treatment groups, drugs were added when nuclei reached the cortex and embryos were fixed 3-10 h later. Enlarged images of large and small dashed boxes are shown as “Perinuclear cortex” and “Cytoplasm” panels, respectively.

F Bar charts of relative F-actin densities in cytoplasmic and perinuclear cortex regions normalised to respective mean intensities measured from DMSO-control embryos. The number of blastomeres measured is shown above individual bars.

Data information: In (A - C), scale bar = 20 µm. In (E), scale bar = 20 µm (top panel), 10 µm (middle panel), 5 µm (lower panel). In (B – C), time is shown as h post-drug addition. In (D, F), data are shown as mean ± SEM. Statistical significance was assessed by an ordinary two-way ANOVA with Šidák’s multiple comparisons test (D) or Statis Kruskal-Wallis test (F) (*P < 0.05, **P < 0.01, ****P < 0.0001, unremarked datapoints = not significant).