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1 **A transient pool of nuclear F-actin at mitotic exit controls chromatin**
2 **organization.**

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21

22 **Reestablishment of nuclear structure and chromatin organization after cell division is integral**
23 **for genome regulation or development and is frequently altered during cancer progression.**
24 **The mechanisms underlying chromatin expansion in daughter cells remain largely unclear.**
25 **Here, we describe the transient formation of nuclear actin filaments (F-actin) during mitotic**
26 **exit. These nuclear F-actin structures assemble in daughter cell nuclei and undergo dynamic**
27 **reorganization to promote nuclear protrusions and volume expansion throughout early G₁ of**
28 **the cell cycle. Specific inhibition of this nuclear F-actin assembly impaired nuclear expansion**
29 **and chromatin decondensation after mitosis and during early mouse embryonic**
30 **development. Biochemical screening for mitotic nuclear F-actin interactors identified the**
31 **actin-disassembling factor Cofilin-1. Optogenetic regulation of Cofilin-1 revealed its critical**
32 **role for controlling timing, turnover and dynamics of F-actin assembly inside daughter cell**
33 **nuclei. Our findings identify a cell cycle-specific and spatiotemporally controlled form of**
34 **nuclear F-actin that reorganizes the mammalian nucleus after mitosis.**

35

36 **Introduction**

37 Cytoplasmic actin polymerization at plasma membranes is an essential and versatile process
38 that defines cellular shape, determines cell polarity, cell-cell and cell-matrix interactions, and
39 drives cytokinesis ¹. In addition, it is well established that actin resides in the nuclear
40 compartment of somatic cells ^{2,3}. For example, monomeric actin is stably assembled into

41 chromatin remodeling complexes ^{4,5}, while a dynamic pool of actin appears to constantly
42 shuttle between the nucleus and cytoplasm in an Importin 9- and Exportin 6-dependent
43 manner ⁶. Similarly, many actin-regulatory proteins have been described to enter the nuclear
44 compartment ². More recently, using nuclear-targeted probes, the presence of F-actin
45 structures was demonstrated in mammalian cell nuclei in response to serum, integrin signaling
46 or DNA damage ⁷⁻⁹. However, whether transient and dynamic nuclear actin filaments exert
47 fundamental structural functions in somatic cells to spatially reorganize nuclear architecture
48 has not been investigated ¹⁰.

49 Mitotic cell division relies on a complex cascade of mechanistic processes to precisely
50 ensure maintenance of genomic organization and integrity in the emerging daughter cells.
51 During mitotic exit, newly formed cells undergo a profound reorganization of their nuclear
52 content to reestablish an interphase nucleus, which is accompanied by a striking expansion in
53 nuclear size and volume ^{11,12}. Further key processes include the reformation of a nuclear
54 envelope and lamina, assembly of nuclear pore complexes, and decondensation as well as
55 reorganization of the highly condensed mitotic chromosomes ^{13,14}. Surprisingly, the cellular
56 mechanisms, which drive nuclear expansion while reversing mitotic chromosomes into an
57 interphase chromatin state, remain largely unexplored ^{11,15,16}.

58

59 **RESULTS**

60 **Transient nuclear F-actin assembly during mitotic exit**

61 We recently reported an approach to monitor endogenous nuclear F-actin dynamics without
62 detectable effects on nucleocytoplasmic shuttling or the polymerization state of actin⁸. Our
63 live-cell compatible approach relies on a transiently binding nanobody directed against actin
64 fused to a nuclear localization signal (anti-Actin-Chromobody-GFP-NLS), herein referred to as
65 nAC-GFP (nuclear Actin-Chromobody-GFP)⁸. While examining mouse fibroblasts stably
66 expressing nAC-GFP together with LifeAct-mCherry to co-visualize cytoplasmic actin, we
67 observed the striking and transient appearance of nuclear actin filament structures when
68 daughter cell nuclei formed (Fig. 1a and Supplementary Video 1). These actin filaments were
69 constantly and dynamically reorganized within the nuclear compartment (as visualized by a
70 nanobody against Lamin A/C), arguing for a spatiotemporal function during the final stages of
71 cell division (Fig. 1b and Supplementary Video 2). Of note, nuclear actin concentrations
72 appeared to be constant during exit from mitosis and were not affected by the presence of nAC
73 (Supplementary Fig. 1a, b).

74 Cell cycle-associated nuclear actin filaments could be detected and quantified using
75 nAC-GFP or a shuttling Actin-Chromobody (sAC) with comparable frequencies (Fig. 1c, 3a and
76 Supplementary Video 3). Transient nuclear actin polymerization persisted for 60-70 minutes
77 during early G₁ phase, followed by filament disassembly upon further progression into
78 interphase (Fig. 1d and Supplementary Video 1). Importantly, we confirmed our observations
79 by using the F-actin marker phalloidin in fixed, but otherwise native, untreated cells (Fig. 1e).
80 Nuclear actin polymerization at mitotic exit could also be observed in MCF10A breast epithelial,
81 RPE-1 retinal pigmented epithelial as well as HT1080 fibrosarcoma cells, arguing for a conserved
82 feature among mammalian cell types (Supplementary Fig. 1c-e).

83 Mitotic nuclear actin filaments were not affected by silencing of the nucleoskeletal
84 proteins Emerin or Lamin A/C (Supplementary Fig. 1f-h), or expression of a dominant-negative
85 KASH (Klarsicht/ANC-1/Syne-1 homology) domain (Supplementary Fig. 1i), shown to disrupt the
86 LINC (Linker of Nucleoskeleton and Cytoskeleton) complex¹⁷, suggesting that these filaments
87 are different in origin and function from those forming upon cell spreading and integrin-
88 dependent signaling⁸.

89

90 **Analysis of nuclear F-actin at mitotic exit using super-resolution microscopy**

91 Next, cells stably expressing nAC fused to the photoconvertible fluorescent protein Dendra2
92 were imaged by PALM (Fig. 2a and Supplementary Fig. 2a). Under these conditions, 96% of all
93 cells showed nuclear actin filaments at mitotic exit. Actin filaments were of several micrometer
94 lengths with an apparent width of about 50-90 nm, which can be explained by single actin fibers
95 stained with nAC-Dendra2 of about 10 nm width and thin bundles of about 2-5 fiber diameters
96 convolved with the achieved PALM resolution of about 40 nm (Fig. 2b and Supplementary Fig.
97 2a, b).

98 To further investigate native cells, phalloidin staining was performed to confirm these
99 findings using STORM imaging on cells fixed at defined time points during mitotic exit
100 (Supplementary Fig. 2c). This revealed nuclear actin fibers for up to 60 minutes after anaphase.
101 The reduced background and improved resolution of 30 nm allowed us to detect a population
102 of even thinner, 40 nm wide nuclear actin filaments in addition to thicker, more bundled
103 subsets of F-actin structures (Fig. 2c, d and Supplementary Fig. 2c).

104

105 **Postmitotic nuclear volume expansion requires nuclear actin polymerization**

106 To gain insight into the functional role of nuclear F-actin formation in early G₁, we visualized
107 nuclear actin filaments together with a fluorescently-labeled histone H2B to label the nuclear
108 compartment. This revealed a substantial expansion of nuclear volume during the period of
109 nuclear F-actin assembly as well as distinct nuclear protrusions associated with the emerging
110 dynamic nuclear F-actin structures (Fig. 3a and Supplementary Video 4).

111 To assess a potential role of nuclear F-actin formation in reshaping the newly forming
112 nuclei after cell division, we first tested for the effects of various pharmacological inhibitors of
113 the actin cytoskeleton that were directly added under the microscope to cells exiting mitosis.
114 The actin-depolymerizing agents Cytochalasin D or Latrunculin B robustly impaired nuclear
115 volume expansion, while the Arp2/3 inhibitor CK-666 had no effect (Supplementary Fig. 3a, b).

116 As pharmacological agents interfere globally with actin dynamics, we aimed to directly
117 assess the impact of nuclear actin by expression of its specific nuclear export factor Exportin 6
118 to enhance nuclear export of actin monomers (Supplementary Fig. 1b)¹⁵. This not only resulted
119 in a strongly reduced number of cells displaying nuclear F-actin during mitotic exit (Fig. 3b), but
120 also in a significantly impaired volume expansion of daughter nuclei (Fig. 3c, d).

121 To more specifically address the role of polymerized nuclear actin, we generated cell
122 lines stably expressing a doxycycline-inducible, nuclear-targeted version of the polymerization-
123 deficient actin mutant actin^{R62D} (NLS-BFP-actin^{R62D})^{8,18}, to dilute the amount of polymerization-
124 competent actin monomers within the nuclear compartment. Similar to expression of Exportin

125 6, this approach resulted in a strong reduction of nuclear actin filaments as well as impaired
126 nuclear expansion compared to control cells expressing BFP-NLS only (Fig. 3e-g). To control for
127 any effects potentially arising from an increased concentration of nuclear actin monomers, we
128 additionally compared the effects of actin^{R62D} to wild-type actin (actin^{wt}) instead of BFP. Given
129 the potential limitations of fluorophore-tagged actin¹⁹, we generated doxycycline-inducible
130 Flag-NLS-actin constructs fused to a self-cleavable SNAP-tag, which allowed us to indirectly
131 identify expression of these Flag-actin derivatives in live dividing cells (Supplementary Fig. 3c-f).
132 Importantly, this experimental setup confirmed our results (Fig. 3g); clearly demonstrating the
133 critical need of a polymerization-competent pool of nuclear actin required for nuclear volume
134 expansion.

135 Noteworthy, the effects of nuclear F-actin on nuclear volume expansion were specific
136 for cells at the mitotic exit, as no differences in nuclear volume could be detected among nuclei
137 of cells arrested in interphase during induction of actin^{R62D} expression (Supplementary Fig. 3g).
138 Furthermore, global transcriptional inhibition by the CDK inhibitory drug Flavopiridol during cell
139 division did not affect the rate of daughter nuclei expansion (Supplementary Fig. 3h, i),
140 excluding altered transcription as a cause of the observed phenotype.

141 Using atomic force microscopy (AFM) on isolated nuclei from synchronized live cells
142 enabled us to visualize the structure of the nuclear surface in early G₁ as well as interphase (Fig.
143 3h). This revealed a remarkably rough nuclear surface indicative of nuclear protrusions in
144 postmitotic control cells expressing Flag-NLS-actin^{wt}, which were absent in Flag-NLS-actin^{R62D}
145 expressing nuclei displaying an overall much flatter nuclear surface (Fig. 3h, i). Similar nuclear
146 protrusions were observed on postmitotic nuclei from untransfected cells. Of note, induction of

147 chromatin decompaction by Trichostatin A (TSA)-treatment of interphase nuclei ²⁰ did not result
148 in an increased nuclear surface roughness (Fig. 3h, j), arguing for a cell cycle-specific
149 phenomenon that critically depends on the involvement of nuclear F-actin formation in early
150 G₁.

151

152 **Nuclear actin assembly affects early G₁ chromatin reorganization**

153 While visualizing nuclear actin dynamics together with the histone H2B, we found nuclear F-
154 actin to reside within interchromatin spaces in the emerging daughter cell nuclei (Fig. 4a,
155 Supplementary Video 3). This observation as well as the time period of nuclear actin
156 polymerization during which chromatin decondensation takes place ¹², prompted us to further
157 investigate a potential role for F-actin in this process.

158 Quantifications of chromatin density assessed by 3D H2B-mCherry fluorescence
159 intensities revealed a significantly higher degree of chromatin compaction in postmitotic nuclei
160 impaired for the assembly of nuclear F-actin either by expression of Exportin 6 or nuclear-
161 targeted actin^{R62D} (Fig. 4b-e).

162 To more directly measure the degree of chromatin compaction, we established a
163 fluorescent lifetime imaging microscopy (FLIM) assay to determine fluorescence energy transfer
164 (FRET) between GFP- and mCherry-tagged histone H2B (Supplementary Fig. 4a, b) ²¹. Consistent
165 with a role of nuclear F-actin in postmitotic chromatin dynamics, we found that induced
166 expression of Flag-NLS-actin^{R62D} but not Flag-NLS-actin^{wt} (Fig. 4f, g), as well as expression of

167 Exportin 6 (Fig. 4h, i) resulted in a significantly reduced fluorescence lifetime of GFP-H2B in
168 synchronized early G₁ cells (Fig. 4f-i).

169 Given the conserved roles of increased histone H3 Ser-10 phosphorylation (H3S10ph)
170 and decreased histone H4 Lys-16 acetylation (H4K16ac) in mitotic chromatin condensation ²²,
171 we confirmed the effects of altered nuclear F-actin assembly on chromatin reorganization by
172 analyzing these histone modifications in cells synchronized by mitotic shake-off. While control
173 cells (BFP-NLS) reestablished lower levels of H3S10ph and higher levels of H4K16ac within 90
174 minutes, cells expressing NLS-BFP-actin^{R62D} sustained mitotic levels of these histone
175 modifications (Supplementary Fig. 4c). In addition, we found higher nuclear levels of Aurora B, a
176 kinase responsible for H3S10 phosphorylation ²³, as well as a lower levels of KAT5, known to
177 mediate H4K16 acetylation²⁴, in cells expressing Flag-NLS-actin^{R62D} (Supplementary Fig. 4d, e).
178 Consistently, chromatin obtained from cells exiting mitosis and expressing NLS-BFP-actin^{R62D}
179 appeared more resistant to MNase digestion (Supplementary Fig. 4f), arguing for reduced
180 chromatin accessibility.

181 Furthermore, electron microscopy of cryopreserved samples allowed us to directly
182 quantify the amount of condensed chromatin, which appears as an electron-dense structure in
183 unlabeled samples (Fig. 4j and Supplementary Fig. 4g). This approach confirmed a highly
184 significant increase in the proportion of condensed chromatin at mitotic exit in cells expressing
185 either nuclear-targeted actin^{R62D} (Fig. 4j, k) or Exportin 6 (Fig. 4l and Supplementary Fig. 4h)
186 compared to control. Altogether, these data support a requirement for polymerization-
187 competent nuclear actin to achieve proper chromatin organization after mitosis.

188

189 **Nuclear F-actin is formed and required for nuclear volume expansion in early mouse embryos**

190 To gain insight into the biological consequences of impaired mitotic nuclear actin assembly we
191 determined general transcriptional activity by quantifying nuclear 3D RNA polymerase II
192 phospho-Serine 5 (pS5) fluorescence. This revealed strongly reduced transcriptional activity in
193 cells expressing GFP-Exportin 6 (Fig. 5a and Supplementary Fig. 5a) as well as significantly lower
194 proliferation rates (Fig. 5b).

195 Next, we asked if similar functions of nuclear F-actin are exerted during development.
196 For this, we investigated fertilized mouse embryos, which rely on substantial chromatin
197 decondensation of sperm and oocyte nuclei. 150 ng mRNA of nAC-GFP was injected into mouse
198 fertilized embryos and analyzed at indicated time points post insemination (hpi) (Fig. 5c).
199 Notably, nuclear actin filaments were readily detected in pronuclei, as well as in the early G₁
200 phase after the first mitotic division (71.43%, 18 hpi) and disassembled upon further cell cycle
201 progression of the 2-cell embryos (25%, 24 hpi) (Fig. 5d). Of note, expression of actin^{R62D}-HA-
202 NLS or Exportin 6 inhibited nuclear volume expansion after the first mitotic division (Fig. 5e, f),
203 consistent with our findings in mammalian somatic cells. Furthermore, a significant
204 developmental delay was observed in Exportin 6 mRNA-injected embryos (Fig. 5g and
205 Supplementary Fig. 5b), providing further evidence for a physiological role of nuclear F-actin
206 assembly in the early steps of mouse embryo development.

207

208 **Cofilin-1 controls nuclear F-actin assembly, volume expansion and chromatin organization in**
209 **daughter cell nuclei**

210 As siRNA against several known actin assembly factors had no obvious effect on nuclear F-actin
211 formation (Supplementary Table 1) and to gain further mechanistic insight, we performed a
212 proteomic screen using biotin-phalloidin to identify potential nuclear F-actin binding proteins
213 from nuclear fractions of cells released from a mitotic nocodazole arrest (Fig. 6a, b). Hereby, we
214 identified the F-actin disassembly factor Cofilin-1 as a candidate interactor (Fig. 6c, d and
215 Supplementary Table 1). Since Cofilin-1 is inactivated by phosphorylation on Ser-3 (p-Cofilin)²⁵,
216 we analyzed nuclear p-Cofilin levels by measuring 3D nuclear fluorescence intensities in
217 synchronized NIH3T3 cells and observed a pronounced increase in nuclear p-Cofilin levels
218 during mitotic exit, revealing Cofilin-1 as a cell cycle-controlled nuclear actin regulator (Fig. 6e
219 and Supplementary Fig. 6a-d). Indeed, and consistent with the role of Cofilin-1 in F-actin
220 disassembly, siRNA against Cofilin-1 resulted in a pronounced stabilization of nuclear F-actin
221 during mitotic exit (Fig. 6f, g and Supplementary Video 5).

222 To specifically address the nuclear function of Cofilin-1 during this cell cycle phase, we
223 generated cells expressing either wildtype (WT-Cofilin) or cytoplasm-targeted Cofilin-1 (NES-
224 Cofilin; NES, nuclear export signal) resistant to siRNA targeting (Fig. 6h, i). Imaging mitotic cells
225 revealed that upon silencing of endogenous Cofilin-1, cells devoid of its nuclear localization
226 failed to prevent extensive nuclear F-actin stabilization during mitotic exit (Fig. 6j and
227 Supplementary Fig. 6e) similar to depletion of total Cofilin-1 (Fig. 6g). Consistent with
228 deregulated actin filament dynamics and turnover, this resulted in defective nuclear volume
229 expansion (Fig. 6k) and chromatin decompaction (Fig. 6l), underscoring the critical importance
230 of dynamic F-actin reorganization for the processes of nuclear volume expansion and chromatin
231 reorganization.

232

233 **Optogenetic control of Cofilin-1 establishes its role in reorganizing daughter cell nuclei after**
234 **mitosis**

235 To corroborate our findings that nuclear Cofilin-1 controls chromatin dynamics, we expressed a
236 nuclear-targeted version of Cofilin-1 (NLS-Cofilin) to inhibit nuclear F-actin formation in early G₁
237 (Supplementary Fig. 6f), and observed a striking defect in establishing open chromatin in cryo-
238 EM samples (Fig. 7a, b).

239 To directly and reversibly control Cofilin-1 function within the nucleus in real time, we
240 generated an optogenetic Cofilin-1 (opto-Cofilin) (Fig. 7c) based on a previously described light-
241 inducible nuclear export system²⁶. Under control conditions, opto-Cofilin exhibited a
242 subcellular distribution similar to WT-cofilin, but allowed for rapid and efficient light-induced
243 nuclear export within 200 seconds, which could be reverted within 500 seconds after
244 illumination was switched off (Fig. 7d and Supplementary Video 6).

245 In cells silenced for endogenous Cofilin-1 (Supplementary Fig. 6g), light-induced nuclear
246 export of opto-Cofilin resulted in extensive stabilization of nuclear F-actin during mitotic exit
247 (Fig. 7e and Supplementary Videos 7, 8), while its timely controlled nuclear re-accumulation, by
248 switching off illumination, triggered rapid re-organization and successive disassembly of nuclear
249 F-actin (Fig. 7e and Supplementary Video 8). Notably, light-controlled export of opto-Cofilin
250 during mitotic exit also resulted in arrested growth of daughter cell nuclei, while nuclear
251 reimport of opto-Cofilin promoted their further volume expansion (Fig. 7f).

252 Together, these results uncover a critical nuclear-specific function of Cofilin-1 in
253 spatiotemporally controlling actin dynamics for nuclear reorganization in the early phases after
254 mitotic cell division.

255

256 **DISCUSSION**

257 Here, we discovered dynamic and transient F-actin assembly in the growing nuclei of daughter
258 cells exiting mitosis. The mechanisms and cellular factors that determine nuclear volume
259 regulation are poorly understood ¹¹. Our data reveal a key function for nuclear actin filaments
260 in nuclear volume and chromatin expansion during mitotic exit as well as a critical nuclear
261 function of Cofilin-1 in tightly controlling the spatiotemporal turnover of these actin filaments.
262 As such, nuclear reorganization during mitotic exit is impaired upon loss of polymerization-
263 competent nuclear actin as well as excessive nuclear F-actin formation, illustrating that the
264 dynamic interplay between polymerization and depolymerization of nuclear F-actin appears to
265 be critical during this cell cycle-specific process. Accordingly, it is tempting to speculate that
266 additional actin-regulatory factors as well as actin bundling proteins are involved in nuclear
267 actin assembly during early G₁.

268 Since cytoskeletal actin dynamics are well known to exert contractile and mechanical
269 forces in order to shape or move a variety of cellular components ²⁷ and in light of our
270 observation of F-actin-dependent nuclear protrusions, one may envisage similar functions for
271 nuclear F-actin during mitotic exit in rearranging the chromatin and nuclear content of
272 mammalian cells. Thus, future work will be directed to dissect whether the role for nuclear

273 actin in promoting efficient reorganization of chromatin is primarily exerted through direct
274 effects of F-actin on chromatin, or more indirectly through expanding and reshaping the nuclear
275 compartment, or both.

276 Our findings thus open a perspective to gain a better understanding of nuclear actin
277 filament dynamics and its role in regulating spatiotemporal chromatin organization and
278 maintenance of a defined nuclear architecture, all of which have profound implications for
279 genome stability and regulation in health and disease.

280

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341

342 **Supplementary Information** is linked to the online version of the paper at
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355

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357 and analysed most of the experiments with help from R.G. and A.K.; R.H. assisted in electron
358 microscopy, D.A. in FLIM/FRET; K.Mo., S.M. and K.Mi. performed experiments on fertilized
359 mouse embryos; E.M.K, D.V. and U.E. performed PALM/STORM, and S.B. mass spectrometry.
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364 (robert.grosse@staff.uni-marburg.de).

365 **Figure Legends**

366 **Figure 1 | Spatiotemporal features and dynamics of nuclear F-actin formation at mitotic exit.**

367 **(a)** Time-lapse imaging of NIH3T3 cells stably expressing nAC-GFP (nuclear Actin-Chromobody-
368 GFP, anti-Actin-Chromobody-GFP-NLS, green, grey in magnifications) together with LifeAct-
369 mCherry (red) during anaphase and mitotic exit. The asterisk indicates a nucleus shown
370 magnified in the lower panel. Images show maximum intensity projections of confocal z-stacks.
371 See also Supplementary Video 1. Scale bar, 10 μm ; time stamp, h:min:s. **(b)** Live cell imaging of
372 NIH3T3 cells stably expressing nAC-GFP (green) together with Lamin-Cb-SNAP (Lamin-
373 Chromobody-SNAP, labelled by SiR-647, magenta) during mitotic exit. The nucleus indicated by
374 an asterisk is shown magnified for individual time points. See also Supplementary Video 2. Scale
375 bar, 10 μm (overview) and 1 μm (magnifications). **(c)** Quantification of incidence and **(d)**
376 duration of nuclear actin filament formation at mitotic exit using indicated nuclear actin probes.
377 Data are shown as mean + SEM from at least n=3 independent experiments and n=60 (nAC-
378 GFP), n=30 (sAC-GFP, shuttling Actin-Chromobody, anti-Actin-Chromobody-GFP-NLS-NES), n=60
379 (phalloidin, formaldehyde fixation 70 min after mitotic shake-off) mitotic events. **(e)** NIH3T3
380 cells at mitotic exit were fixed using glutaraldehyde and stained for actin filaments using
381 phalloidin-Atto 488. The area indicated by a dashed rectangle is shown magnified for individual
382 confocal slices in z-direction with a step size of 0.37 μm . Scale bar, 10 μm (overview) and 1 μm
383 (magnifications).

384

385 **Figure 2 | Super-resolution microscopy reveals structural features of nuclear F-actin at mitotic**
386 **exit.**

387 **(a)** Photoactivated localization microscopy (PALM) of nuclear actin filaments in fixed NIH3T3
388 cells stably expressing nAC-Dendra2 (anti-Actin-Chromobody-Dendra2-NLS, orange) at mitotic
389 exit. DIC (differential interference contrast) images were acquired over time to monitor mitotic
390 progression and to allow for time-defined fixation 20 min after anaphase. Experimental
391 resolution after drift correction is 33 ± 1 nm. Scale bars, 5 μ m. **(b)** Filament widths of nAC-
392 Dendra2-labeled nuclear actin filaments (n = 81, data were collected from at least n=3
393 independent experiments) as histograms (up) with a bin size of 10.65 nm (x-axis) plotted
394 against frequency (y-axis). Full data is represented underneath the histograms as box + scatter
395 plots with the same x-axis. The box marks the first and third quartiles and the indent represents
396 the median. Whiskers mark minimal and maximal values. **(c)** Stochastic Optical Reconstruction
397 Microscopy (STORM) of phalloidin-stained nuclear actin filaments in native NIH3T3 cells at
398 mitotic exit. The overview image focuses on a nucleus 45 min after anaphase (see also
399 Supplementary Fig. 2c). The magnifications show representative thin (1) and thick (2) filaments
400 with different labelling densities. Scale bars, 5 μ m (overview) and 200 nm (magnifications). **(d)**
401 Filament widths of phalloidin-labelled nuclear actin filaments (n=53, data were collected from
402 n=1 experiment) as histograms (up) with a bin size of 14.3 nm (x-axis) plotted against frequency
403 (y-axis). Full data is represented underneath the histograms as box + scatter plots with the
404 same x-axis. The box marks the first and third quartiles and the indent represents the median.
405 Whiskers mark minimal and maximal values.

406

407 **Figure 3 | Nuclear F-actin reshapes nuclei, and promotes nuclear volume expansion after**
408 **mitotic cell division.**

409 **(a)** NIH3T3 cells stably expressing nAC-GFP (green) together with H2B-mCherry (red) during
410 mitotic exit. Asterisk indicates the nucleus shown for additional time points. Dashed rectangles
411 indicate areas of dynamic nuclear protrusions, shown magnified over time. Arrows indicate
412 direction of protrusions. See also Supplementary Video 4. Scale bar, 10 μm (overviews) and 1
413 μm (magnifications); time stamp, min:s. **(b)** NIH3T3 cells expressing nAC-GFP (green) together
414 with mCherry or mCherry-Exportin 6 (gray, insets) after anaphase. Figures give proportion of
415 cells showing nuclear F-actin in the presence (n=15) or absence (n=17 mitotic events) of GFP-
416 Exportin 6, visualized by nAC-mCherry. Scale bar, 10 μm . **(c)** Nuclear expansion after anaphase
417 (00:00) was visualized by H2B-mCherry in NIH3T3 cells expressing GFP or GFP-Exportin 6.
418 Images show representative coloured 3D surface reconstructions of daughter nuclei. Time
419 stamp, h:min. **(d)** Quantification of nuclear volume during mitotic exit in NIH3T3 cells
420 expressing either GFP or GFP-Exportin 6. Data shows mean \pm SEM from n=4 independent
421 experiments and n=30 nuclei per condition. **(e)** Images of NIH3T3 cells stably expressing nAC-
422 GFP (green) together with doxycycline-induced BFP-NLS or NLS-BFP-actin^{R62D} (gray, insets) at
423 mitotic exit. Figures give proportion of cells showing nuclear F-actin (BFP-NLS, n=22; NLS-BFP-
424 actin^{R62D}, n=18 mitotic events). Scale bar, 10 μm . **(f)** Visualization of nuclear expansion in
425 NIH3T3 cells expressing doxycycline-induced BFP-NLS or NLS-BFP-actin^{R62D} similar to **c**. **(g)**
426 Quantification of nuclear volume during mitotic exit in NIH3T3 cells expressing doxycycline-
427 induced BFP-NLS or actin derivatives, as indicated. Data shows mean \pm SEM from n=3
428 independent experiments and n=60 (BFP-NLS, NLS-BFP-actin^{R62D}), n=50 nuclei (Flag-NLS-actin^{wt},
429 Flag-NLS-actin^{R62D}). **(h)** Representative AFM images show nuclear surface morphology of
430 isolated NIH3T3 nuclei. Early G₁ nuclei were obtained from cells expressing Flag-NLS-actin^{wt} or

431 Flag-NLS-actin^{R62D}, whereas interphase nuclei were treated with or without TSA (1 μ M, 5 h) to
432 induce chromatin decondensation. Scale bar, 1 μ m. **(i)** Quantification of surface roughness of
433 early G₁ nuclei expressing Flag-NLS-actin^{wt} or Flag-NLS-actin^{R62D}. Data shows mean \pm SEM from
434 n=2 independent experiments and n=9 nuclei per condition; P values were calculated by *t*-test.
435 **(j)** Quantification of surface roughness of control or TSA-treated interphase nuclei. Data shows
436 mean \pm SEM from n=2 independent experiments and n=7 nuclei per condition. P values were
437 calculated by *t*-test.

438

439 **Figure 4 | Nuclear actin dynamics promote chromatin decondensation at mitotic exit.**

440 **(a)** NIH3T3 cells stably expressing a shuttling Actin Chromobody (sAC-GFP, green) together with
441 H2B-mCherry. Asterisk indicates the nucleus shown magnified for additional time points to
442 visualize nuclear F-actin within interchromatin spaces. See also Supplementary Video 3. Scale
443 bars, 10 μ m; time stamp, min:s. **(b)** Maximum intensity projections of confocal z-stacks
444 illustrate H2B-mCherry fluorescence densities (grey) during mitotic exit in NIH3T3 cells co-
445 expressing BFP-NLS or NLS-BFP-actin^{R62D}. Scale bar, 10 μ m; time stamp, h:min. **(c-e)** Chromatin
446 densities of NIH3T3 cells were assessed in G₀ or 90 min after anaphase (mitotic exit) and
447 compared between **(c)** expression of BFP-NLS and NLS-BFP-actin^{R62D} (each n=60 nuclei), **(d)**
448 expression of Flag-NLS actin^{wt} and Flag-NLS-actin^{R62D} (each n=50 nuclei) or **(e)** expression of GFP
449 and GFP-Exportin 6 (each n=30 nuclei). In each panel data is shown as mean + SEM from n=3
450 independent experiments. P values were calculated by two-way ANOVA. **(f-i)** Fluorescence
451 lifetime imaging microscopy (FLIM) of fluorescence resonance energy transfer (FRET) between
452 GFP- and H2B-mCherry NIH3T3 cells at mitotic exit. **f** and **h** provide image examples for the

453 tested conditions. The colour code indicates fluorescence lifetime of GFP-H2B. Scale bar, 10
454 μm . **(g, i)** Quantifications of GFP-H2B fluorescence lifetime (Tau) in early G₁ NIH3T3 cells
455 expressing either Flag-NLS-actin^{wt} or Flag-NLS-actin^{R62D} **(g)** or Flag-Exportin 6 **(i)**. Data is shown
456 as boxplot of n \geq 20 **(g)** or n \geq 30 **(i)** cells per condition from n=3 independent experiments. P
457 values were calculated by *t*-test. See also Supplementary Fig. 4a, b. **(j)** Representative electron
458 microscopy images of cryo-preserved, synchronized early G₁ NIH3T3 cells induced to express
459 BFP-NLS or NLS-BFP-actin^{R62D}. Areas indicated by a dashed rectangle are shown magnified. Scale
460 bar, 2 μm . **(k, l)** Quantifications of condensed chromatin based on cryo-EM images. Chromatin
461 condensation was compared between control conditions and expression of either NLS-BFP-
462 actin^{R62D} (n \geq 28 nuclei) **(k)** or expression of GFP-Exportin 6 (n \geq 14 nuclei) **(l)**. In each panel data is
463 shown as mean + SEM from n=2 independent experiments. P values were calculated by *t*-test.

464

465 **Figure 5 | Inhibition of nuclear F-actin formation impairs transcription, proliferation and early**
466 **embryonic development.**

467 **(a)** 3D quantification of nuclear RNA Pol II pS5 fluorescence intensities in NIH3T3 cells
468 expressing either GFP or GFP-Exportin 6 at indicated time points after mitotic shake off. See
469 also Supplementary Fig. 5a. Data are shown as mean + SD from n=2 independent experiments
470 and n \geq 10 nuclei per condition. P values were calculated by two-way ANOVA. **(b)** Proliferation of
471 NIH3T3 cells expressing either GFP or GFP-Exportin 6 was measured using WST-1. Data were
472 normalized to values of control cells at time 0 h and are shown as mean + SEM from n=3
473 independent experiments. P values were calculated by two-way ANOVA. **(c)** Experimental
474 scheme for studying nuclear F-actin in fertilized mouse embryos. At 2 hpi (hours post

475 insemination) mRNA encoding for the indicated proteins was injected into fertilized embryos to
476 either visualize or manipulate nuclear actin dynamics. **(d)** Image examples showing early
477 embryo development at 8 hpi (pronuclei), 18 hpi and 24 hpi (2-cell). DIC, Differential
478 interference contrast. Nuclear F-actin corresponding to dashed rectangles is shown magnified
479 below (nAC-GFP, green). Scale bars, 20 μm (overview) and 10 μm (magnifications). **(e, f)** Image
480 examples and quantitative analysis of nuclear volume in 2-cell embryos expressing either
481 actin^{R62D}-HA-NLS or actin^{wt}-HA-NLS **(e)** or mCherry-Exportin 6 **(f)**. Scale bar, 20 μm . Quantitative
482 data is shown as mean + SD from n=3 independent experiments and n \geq 30 nuclei per condition.
483 P values were calculated by *t*-test. **(g)** Preimplantation development of embryos after a single
484 injection of mCherry-Exportin 6 mRNA. Note the significant developmental delay at 48 hpi, 60
485 hpi, 72 hpi and 96 hpi compared to injection of mRNA encoding myc-tagged GFP. Data is shown
486 as mean + SEM from n=3 independent experiments. P values were calculated by chi-squared
487 test. See also Supplementary Fig. 5b.

488

489 **Figure 6 | Nuclear Cofilin-1 regulates nuclear F-actin during mitotic exit.**

490 **(a)** Cartoon illustrating nuclear F-actin pulldown at mitotic exit. **(b)** Immunoblot detecting α -
491 Tubulin (cytoplasm) and Histone 3 (H3, nucleus) confirms successful subcellular fractionation.
492 **(c)** Immunoblot detecting β -actin and Cofilin-1 validates efficient pulldown of nuclear F-actin
493 and co-precipitation of Cofilin-1. **(d)** Table listing nuclear F-actin-binding proteins as identified
494 by mass spectrometry (cov., coverage). **(e)** 3D quantitative immunofluorescence analysis of
495 nuclear p-Cofilin at indicated times after mitotic shake-off. Data are shown as mean + SD from
496 n=30 mitotic events for each time point. See also Supplementary Fig. 6a, b. **(f)** Time-lapse

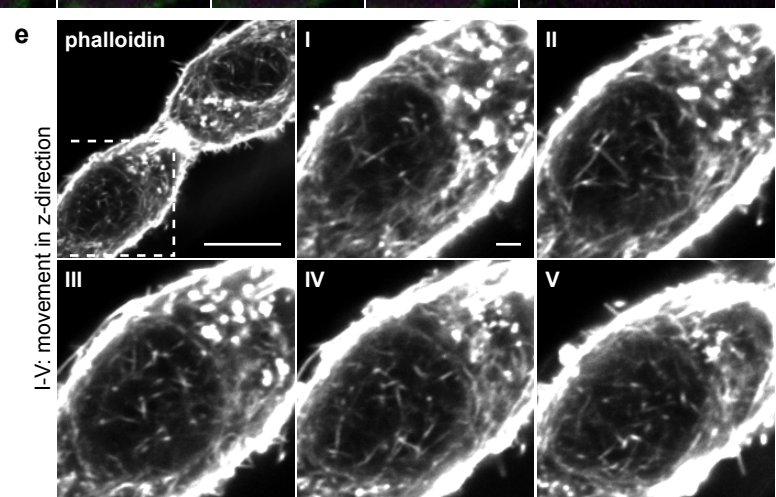
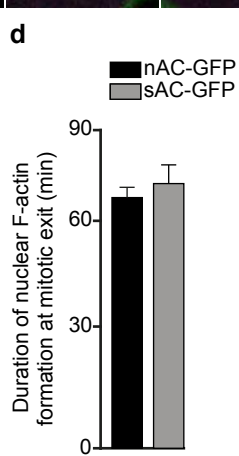
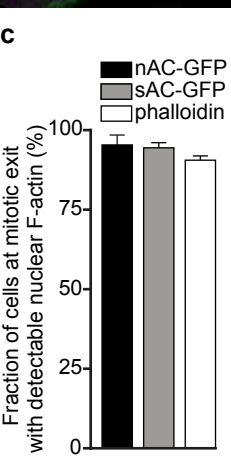
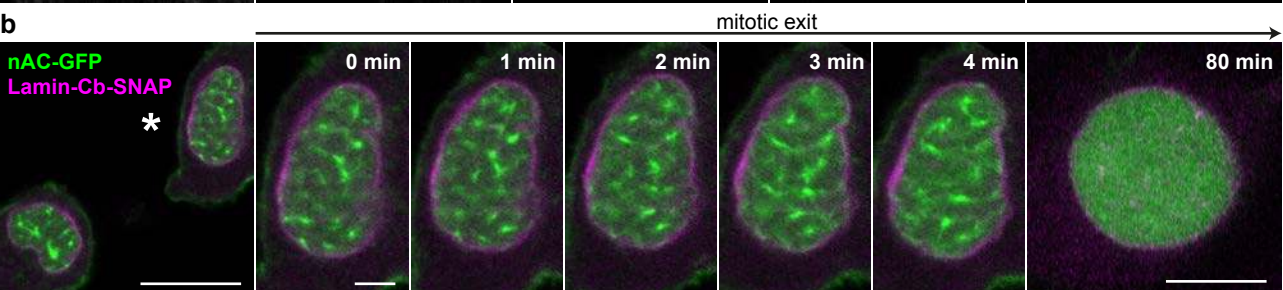
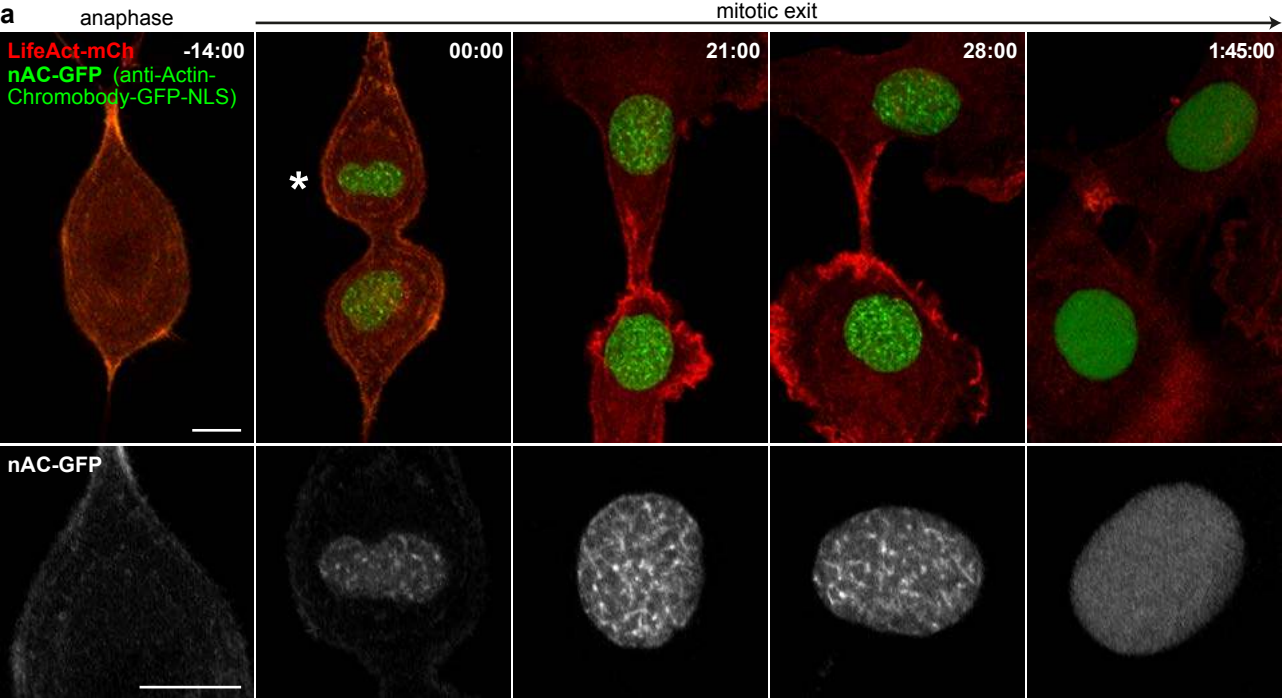
497 imaging shows nAC-GFP expressing NIH3T3 cells transfected with si-control or si-Cofilin at
498 indicated times after anaphase. See also Supplementary Video 5. Scale bar, 5 μm ; time stamp,
499 h:min. **(g)** Quantifications of stabilized nuclear F-actin (present for ≥ 2 h after anaphase) in
500 NIH3T3 cells treated with indicated siRNAs. Data are shown as mean + SD from n=3
501 independent experiments and n=49 (si-control), n=58 (si-Cofilin), n=59 (si-Cofilin (3'-UTR))
502 mitotic events. Immunoblot confirms efficient silencing of Cofilin-1. **(h)** Confocal images of fixed
503 NIH3T3 cells stably expressing WT- or NES-mCherry-Cofilin (red). Scale bar, 10 μm . **(i)**
504 Immunoblot of NIH3T3 cells stably expressing WT- or NES-mCherry-Cofilin confirms siRNA (si-
505 Cofilin (3' UTR))-resistant expression of mCherry-Cofilin derivatives. **(j)** Quantifications of
506 stabilized nuclear F-actin (present for ≥ 2.5 h after anaphase) in NIH3T3 cells treated with si-
507 Cofilin (3'-UTR) in the presence of either WT- or NES-mCherry-Cofilin. Data are shown as mean
508 + SD from n=3 independent experiments and n=30 (WT), n=38 (NES) mitotic events. **(k)**
509 Quantifications of nuclear volume during mitotic exit in NIH3T3 cells treated with si-control or
510 si-Cofilin (3'-UTR) in the presence of either WT- or NES-mCherry-Cofilin. Data are shown as
511 mean + SD from n=3 independent experiments and n=50 nuclei per condition. **(l)** NIH3T3 cells
512 expressing WT- or NES-mCherry-Cofilin were treated with si-control or si-Cofilin (3'-UTR) and
513 chromatin densities were assessed 90 min after anaphase. Data are shown as mean + SEM from
514 n=3 independent experiments and n=50 nuclei per condition. Unprocessed original scans of
515 blots are shown in Supplementary Fig. 7.

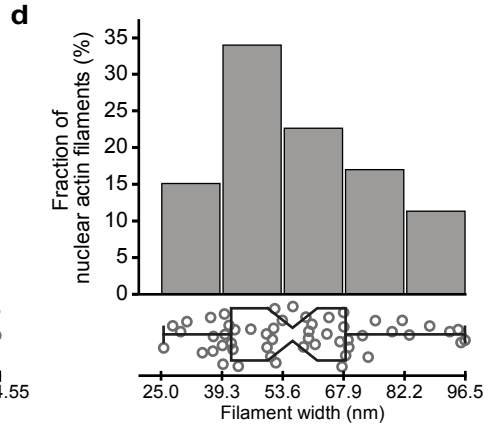
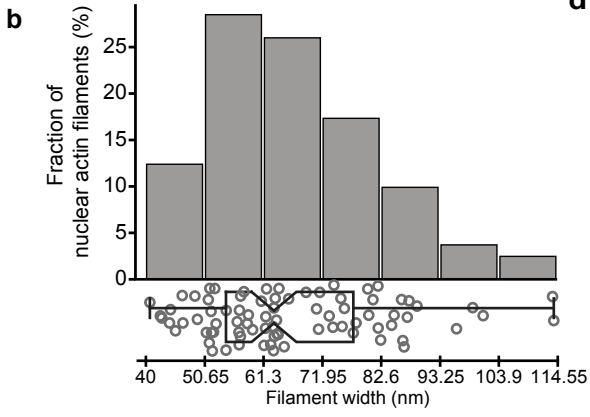
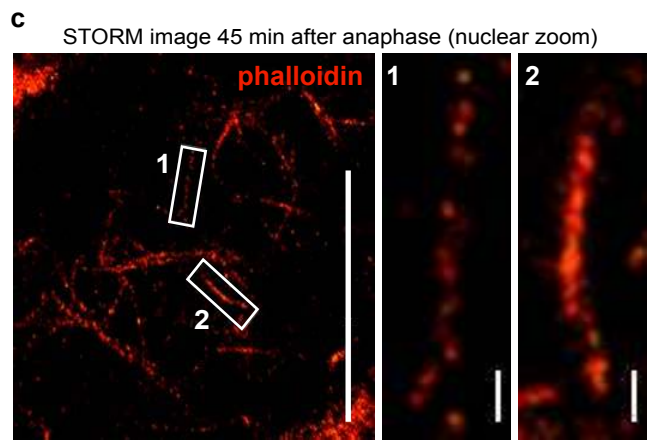
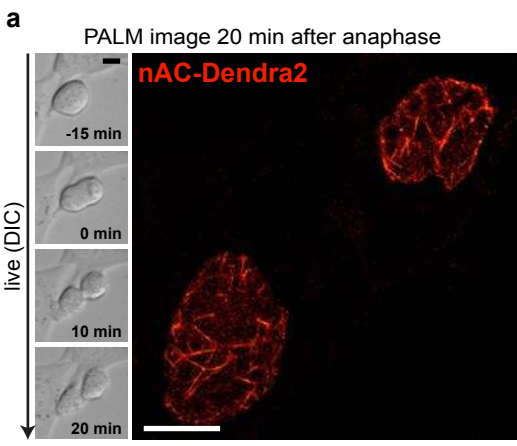
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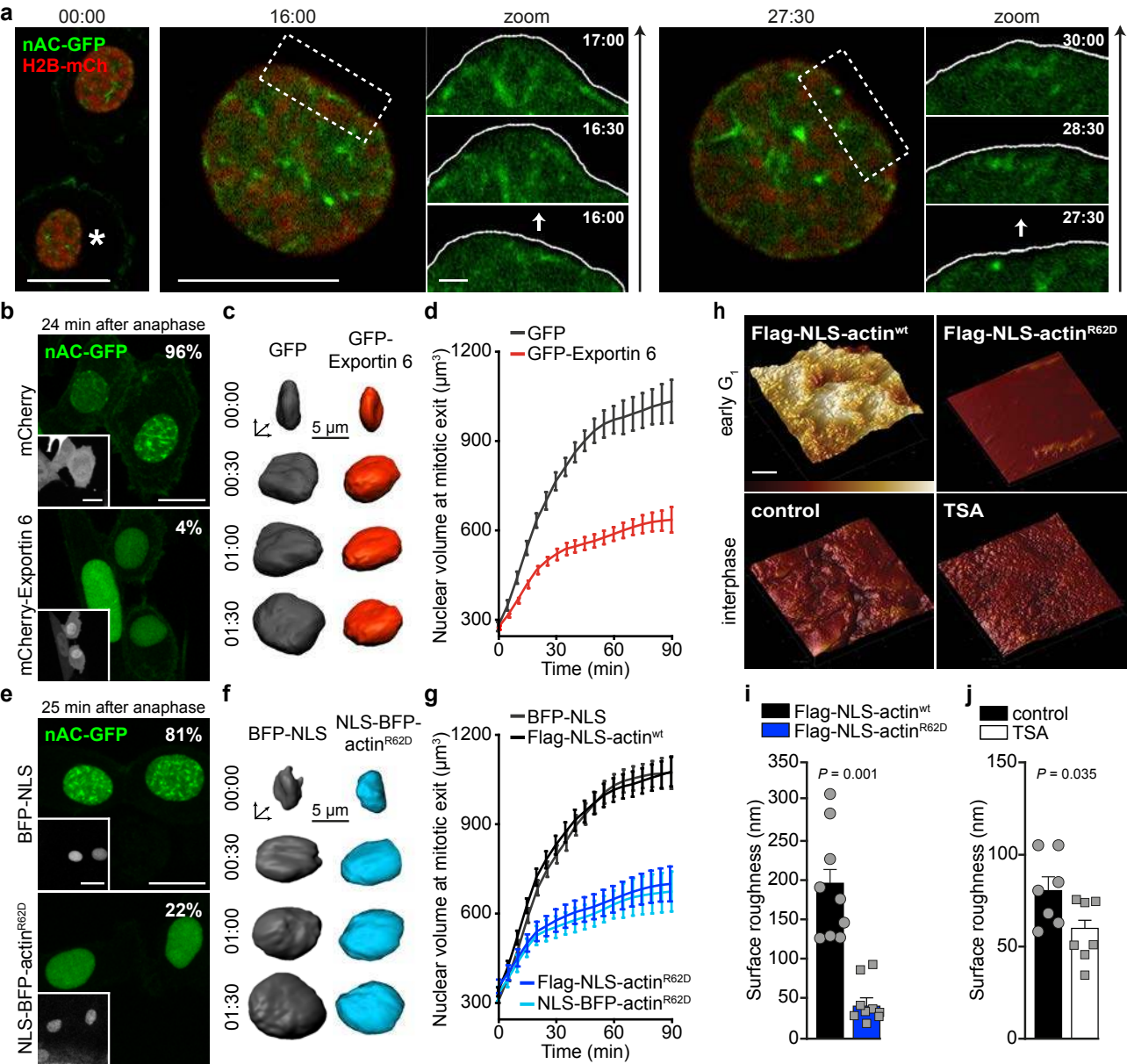
517 **Figure 7 | Nuclear Cofilin-1 affects chromatin organization and its optogenetic control reveals**
518 **critical functions in nuclear F-actin disassembly at mitotic exit.**

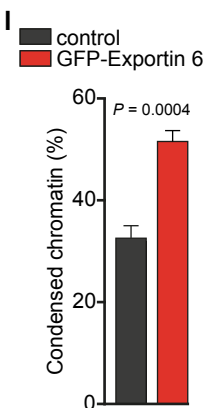
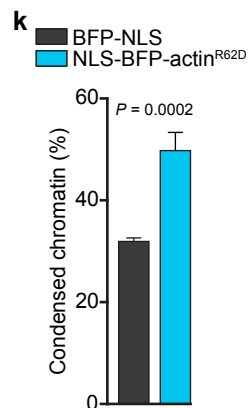
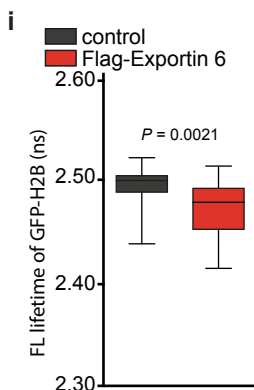
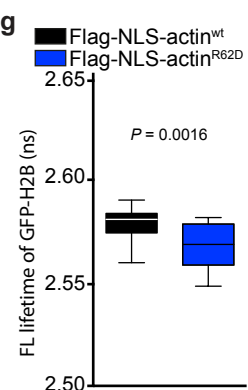
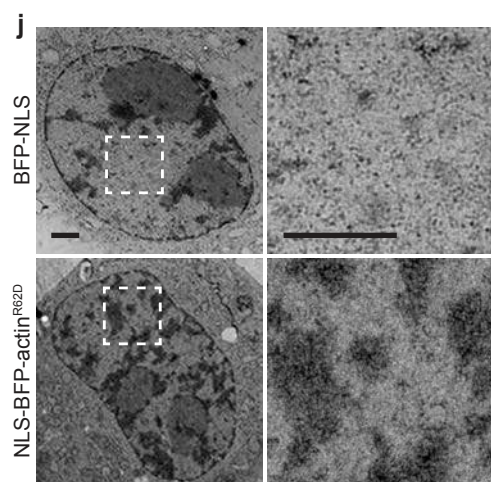
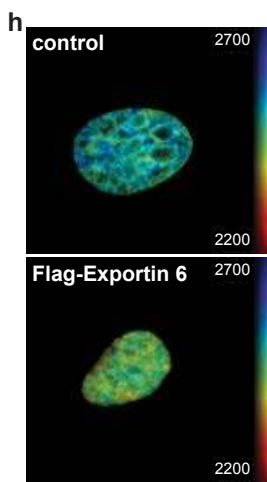
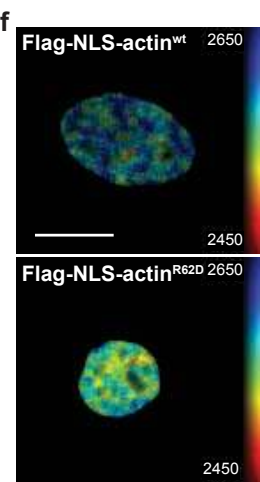
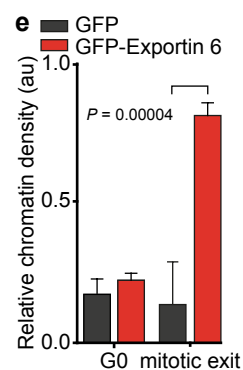
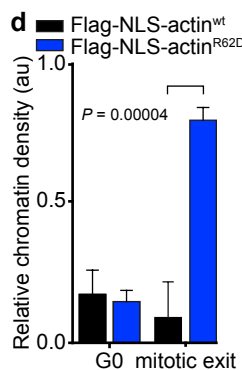
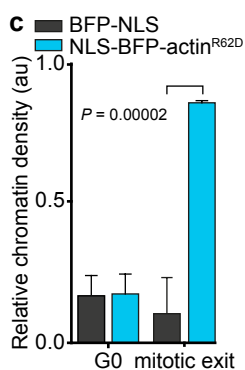
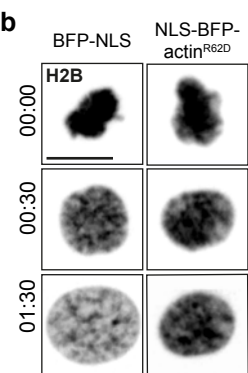
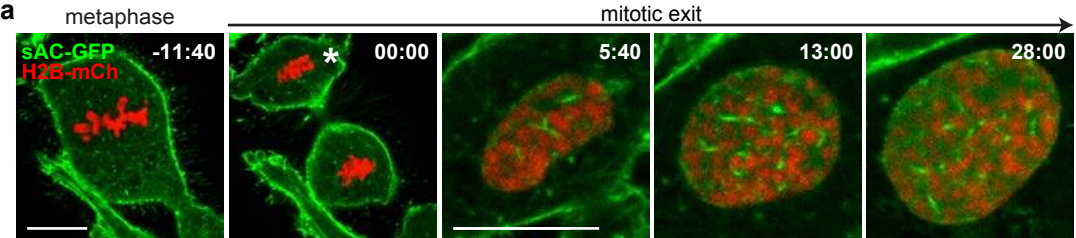
519 **(a)** Representative electron microscopy images of cryo-preserved NIH3T3 cells at mitotic exit in
520 the absence or presence of NLS-mCherry-Cofilin. Scale bar, 2 μm . **(b)** Quantifications of
521 condensed chromatin based on cyro-EM images. Data are shown as mean + SEM from n=2
522 independent experiments and n \geq 15 nuclei per condition. P values were calculated by t-test. **(c)**
523 Cartoon illustrating design and photo-convertibility of opto-Cofilin. Blue light induces exposure
524 of a photocaged NES (nuclear export sequence), thereby promoting its nuclear export. **(d)** Time-
525 lapse imaging of NIH3T3 cells demonstrates light-regulated control of opto-Cofilin subcellular
526 localization. NIH3T3 cells stably expressing opto-Cofilin (grey) were imaged at 10 second
527 intervals either with (+ light) or without (- light) additional irradiation by blue laser light (488
528 nm). See also Supplementary Video 6. Scale bar, 10 μm . **(e)** NIH3T3 cells stably expressing nAC-
529 SNAP (labelled by SiR-647, grey) and opto-Cofilin (red) were treated with si-Cofilin (3'-UTR) and
530 imaged during and after mitosis. To promote nuclear export of opto-Cofilin, cells were exposed
531 to blue laser light (488 nm) at 2.5 min intervals (+ light). Note the excessive formation of
532 stabilized nuclear F-actin in the absence of nuclear Cofilin as well as the onset of filament
533 disassembly upon controlled nuclear re-import of opto-Cofilin (- light). See also Supplementary
534 Video 8. Scale bar, 10 μm . **(f)** Nuclear fluorescence intensities of opto-Cofilin (red line) and
535 relative nuclear area (black line) were quantified before and during light-regulated re-import of
536 opto-Cofilin. Nuclear re-import of opto-Cofilin is accompanied by nuclear shape changes and
537 overall nuclear expansion. Data are shown as mean from n=5 nuclei.

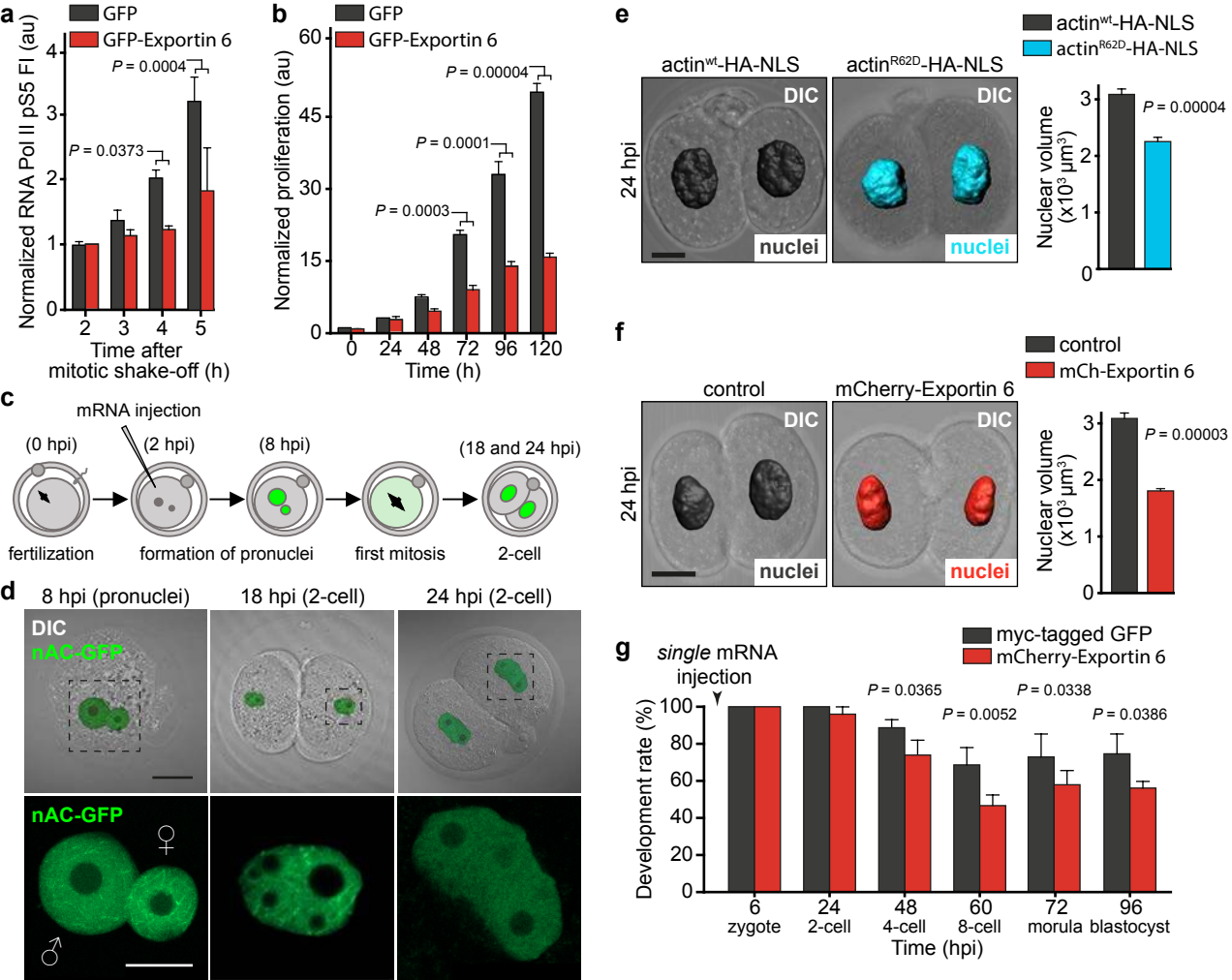
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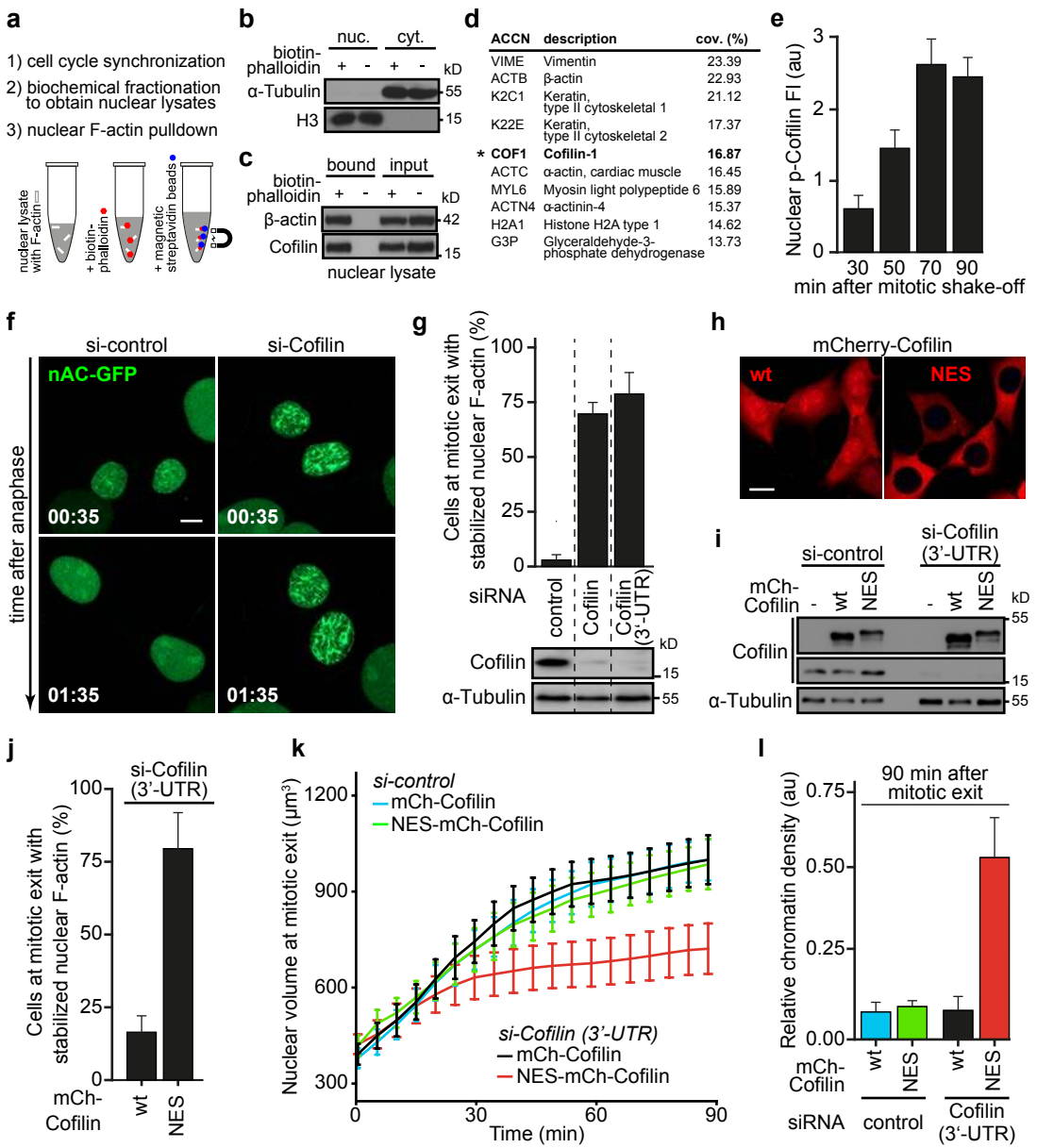


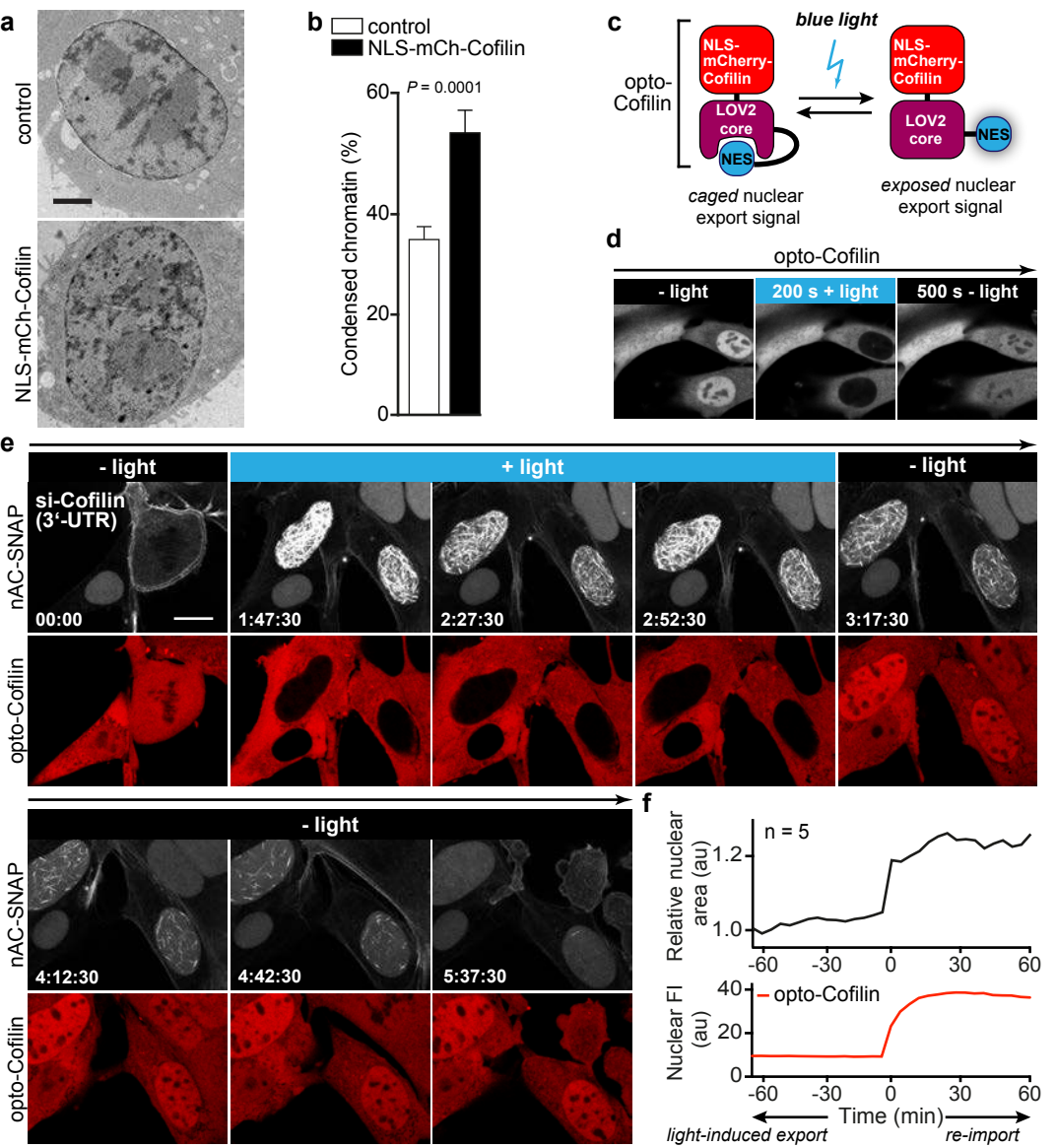












1 **Methods**

2 **Antibodies and reagents**

3 Cell culture and transfection reagents (Lipofectamine 2000 and RNAiMax) were obtained
4 from Invitrogen.

5 Rhodamine-phalloidin, biotin-phalloidin, phalloidin-AF647 and phalloidin-Atto 488
6 were purchased from Life Technologies. SNAP-Cell 647-SiR was obtained from NEB. SiR-DNA
7 was from Spirochrome. CK-666, Cytochalasin D, DMSO, doxycycline, Latrunculin B, and
8 nocodazole were from Sigma-Aldrich and used at indicated concentrations. Flavopiridol was
9 obtained from Santa Cruz and used at a final concentration of 1 μ M.

10 Information on antibodies used is listed in Table S3.

11

12 **Plasmids and constructs**

13 The generation of nuclear Actin-Chromobody-GFP (nAC-GFP, anti-actin-Chromobody-GFP-
14 NLS) and sAC was described previously ⁸. The nuclear Actin-Chromobody is a genetically
15 encoded, NLS-tagged nanobody against the actin protein. Due to its NLS-fusion, the nuclear
16 Actin-Chromobody is enriched in the nuclei of interphasic cells. Nuclear envelope
17 breakdown in prophase is accompanied by a temporary loss of its specific nuclear
18 localization, which reestablishes during mitotic exit and daughter nuclei assembly.

19 nAC-Dendra2, nAC-mCherry and nAC-SNAP were generated by replacing the GFP of
20 pWPXL-nAC-GFP by either pDendra2 (Clontech Laboratories, Inc.), mCherry or the SNAP-tag
21 (New England Biolabs).

22 To obtain a Lamin-nanobody fused to the SNAP-tag, the tagRFP2 of pLC-TagRFP2
23 (ChromoTek) was replaced by a cDNA encoding the SNAP-tag. For stable expression of

24 Lamin-nanobody-SNAP, the corresponding cDNA was subcloned into pWPXL using the
25 MluI/SpeI restriction sites.

26 For stable expression of H2B-mCherry, the GFP of pWPXL was replaced by mCherry,
27 before insertion of a cDNA encoding human H2B via the BamHI/MluI restriction sites.

28 For generation of tagRFP-KASH, the cDNA of murine Nesprin-1 α was amplified as described
29 ¹⁷. KASH was expressed from the EFpLink plasmid carrying a N-terminal tagRFP.

30 The cDNA of human Exportin 6 was obtained by reverse transcription of total mRNA
31 obtained from HeLa cells. Exportin 6 was expressed from the EFpLink plasmid carrying a N-
32 terminal Flag-, Flag-GFP- or mCherry-tag.

33 To obtain mCherry-Cofilin plasmids for lentiviral transductions, the cDNA of mouse
34 Cofilin-1 (kindly provided by M. Rust, University of Marburg) was N-terminally fused to
35 mCherry and cloned into pWPXL via MluI/SpeI. For NES-mCherry-Cofilin the NES (nuclear
36 export signal) of HIV1-Rev (LPPLERLTL) was fused to the N-terminus of mCherry. Opto-
37 Cofilin was generated by addition of the cMyc^{P1A} NLS (AAAKRVKLD) to the N-terminus of
38 mCherry-Cofilin and a C-terminal fusion to the LEXY module ²⁶. Opto-Cofilin was inserted
39 into pWPXL via MluI/SpeI to allow for production of lentiviral particles.

40 To obtain NLS-BFP-actin^{R62D}, the SV40 large T antigen NLS (PPKKRKRKV) was N-
41 terminally fused to tagBFP2 (separated by one linking glycine), which was further fused to
42 the N-terminus of actin^{R62D} ¹⁸, separated by a SGLRSRA linker. For BFP-NLS, the cDNA
43 encoding tagBFP2 was C-terminally fused to the SV40 large T antigen NLS, separated by a
44 GDPPVAT linker. To obtain Flag-NLS-actin-T2A-SNAP derivatives, a cDNA encoding human β -
45 actin (either wild-type or containing the point mutation R62D) was N-terminally fused to a
46 Flag-tag and the SV40 large T antigen NLS (separated by a BamHI restriction site) and C-
47 terminally linked to a SNAP-tag by a self-cleaving T2A peptide (GSGEGRGSLTTCGDVEENPGP).

48 To allow for stable doxycycline-inducible expression of NLS-BFP-actin^{R62D}, BFP-NLS or Flag-
49 NLS-actin-T2A derivatives, the corresponding cDNAs were inserted into the pInducer20
50 plasmid²⁸ by homologous recombination using the Gateway technology (Invitrogen).
51 LifeAct-mCherry lentiviral particles were a gift from O. Fackler (University of Heidelberg).

52

53 **Cell culture, viral transductions, transfections, and treatments**

54 NIH3T3, HT1080, RPE-1 cells and all their derivatives were grown in DMEM supplemented
55 with 10% FCS (fetal calf serum), 100 U/ml penicillin and 100 mg/ml streptomycin at 37 °C in
56 a 5% CO₂ atmosphere. MCF10A cells and derivatives were cultured as described previously
57²⁹.

58 Lentiviral transductions were performed as previously described⁸.

59 Transient transfections of Exportin 6 or tagRFP-KASH were carried out using
60 Lipofectamine LTX&PLUS Reagent according to the manufacturer's instructions.
61 Transfection of siRNAs and sequence for si-Emerin and si-Lamin A/C have been described
62 previously⁸. In addition, the following siRNAs were obtained from Qiagen:
63 CCGCTGCACCCTGGCAGAGAA (si-Cofilin), TGCCAACTTCTAACCACAATA (si-Cofilin (3'-UTR)),
64 and TTGGAATATCTGACAAGTAAA (si-Exportin 6). Sequences of siRNAs used to obtain data
65 presented in Table S1 are shown within the table.

66 SNAP-Cell 647-SiR and SiR-DNA were used according to the manufacturer's
67 instructions.

68 Induction of BFP-NLS, NLS-BFP-actin^{R62D} or Flag-NLS-actin-T2A-SNAP derivatives was
69 achieved by addition of 0.5 µg/ml doxycycline for 16 hours to the cell culture medium.
70 NIH3T3 cells were arrested in G₀ phase by serum starvation (growth medium without FCS)
71 for at least 16 hours.

72

73 **Immunofluorescence and phalloidin staining**

74 For immunofluorescence stainings, cells were grown on cover slips, before fixation using 4%
75 formaldehyde (15 min at RT). Immunolabeling was performed as described previously²⁹.

76 For phalloidin staining in Fig. 1e, cells were fixed using glutaraldehyde according to⁷.
77 Phalloidin staining for the quantification shown in Fig. 1c and for super-resolution
78 microscopy (Fig. 2c,d and Supplementary Fig. 2c) was performed on formaldehyde fixed
79 samples (4% for 15 min at RT). After washing with PBS, samples were incubated with
80 phalloidin-AF647 or phalloidin-Atto 488 at 4°C for 96 hours. For super-resolution
81 microscopy, samples were post-fixed using 1% formaldehyde for 10 min at RT.

82

83 **Image acquisition and live cell imaging**

84 All confocal image acquisitions were performed on a LSM 700 or LSM 800 confocal laser
85 scanning microscope (Zeiss) equipped with a 63X/1.4 NA oil objective. For live cell imaging,
86 cells were cultured in μ -slides (ibidi) at 37 °C in a 5% CO₂ atmosphere using a humidified
87 incubation chamber (Pecon).

88 Experiments with opto-Cofilin were carried out using the LSM 800 microscope. To
89 induce nuclear export of opto-Cofilin the excitation of mCherry (555 nm) was combined
90 with 488 nm irradiation at 1% laser power and a pixel dwell of 2.06 μ sec.

91

92 **Image processing and quantification of nuclear volumes, chromatin densities and 3D**
93 **fluorescence intensities**

94 Image processing was performed with IMARIS (Bitplane), FIJI (NIH) and Photoshop CS6
95 (Adobe).

96 For quantification of nuclear volume in living NIH3T3 cells, images were acquired
97 every 2.5 or 5 min over an interval of 90 min. Complete z-stacks of the signal obtained by
98 either H2B-mCherry (Fig. 3c, d, f, g) or SiR-DNA (Fig. 6k; Supp. Fig. 3a, b) were analyzed with
99 IMARIS. 3D surfaces based on the nuclear-specific signal were generated, and their
100 respective volumes were measured over time. Chromatin density was calculated by dividing
101 the sum of H2B or SiR-DNA fluorescence intensities by total nuclear volume.

102 For measuring nuclear fluorescence intensities in 3D, a nuclear staining (i.e. DAPI)
103 was used to threshold a nuclear region of interest (with FIJI for 2D data, and IMARIS for 3D
104 data), from which the respective pixel intensities were calculated.

105

106 **PALM and measurement of nuclear actin filament width**

107 For super-resolution imaging of nAC-Dendra2, NIH3T3 cells were washed and fixed in 3.7 %
108 formaldehyde (FA), 20 min at room temperature. For all super-resolution imaging, a 1:5000
109 dilution of fluorescent beads (TetraSpeck™ Microspheres, 0.1 μm, Life Sciences T7279 or
110 FluoSpheres 715/755, Life Sciences F8799) was sonicated to break up clumps of beads. ~5
111 μL of the beads were added to the sample and allowed to settle and adhere for 15 minutes,
112 to serve as fiducial markers for drift correction. Super-resolution imaging was performed as
113 described in detail elsewhere³⁰. Briefly, a customized and automated Nikon Eclipse Ti
114 microscope was equipped with 405 nm, 488 nm, 561 and 640 nm lasers (all OBIS, Coherent
115 Inc.). Laser intensities were controlled by an acousto-optical tunable filter (Acal BFi Germany
116 GmbH) to illuminate the sample using the quad color dichroic zt405/488/561/640rpc. The
117 illumination could be switched from epifluorescence to total internal reflection fluorescence
118 (TIRF) mode by a motorized TIRF mirror (Thorlabs, Germany). Fluorescence signals were
119 collected by a quad line laser rejection filter ZET405/488/561/640 and the bandpass filters

120 ET 525/50, ET 610/75 or HC 689/23 dependent on the imaging channel (all filters AHF,
121 Germany). The detection of the single fluorophore emissions was performed using an
122 electron multiplying charged coupled device (emCCD iXon 888, Andor).

123 For PALM imaging of Dendra2, the sample was irradiated by about 1-2 kW/cm² (561
124 nm) and < 0.5 kW/cm² (405 nm) and imaged at an acquisition frame rate of 60 ms. Cells
125 were imaged until all Dendra2 fluorophores were read-out. For STORM imaging of
126 Phalloidin-Alexa 647, the sample was imaged in 100 mM Methyl diethanolamine (MEA) with
127 a glucose oxidase oxygen scavenger system³¹ illuminated with about 2-4 kW/cm² (640 nm)
128 and recorded at an acquisition frame rate of 70 ms.

129 Super-resolution movies were analyzed by the RapidSTORM software³² and post-
130 processed by customized scripts written in Python programming language (Python Software
131 Foundation, <https://www.python.org/>) to correct for sample drift during the image
132 acquisition. From the post-processed data, the experimental Nearest Neighbor Approach
133 (NeNA) resolution³³ was calculated and super-resolution images were reconstructed
134 according to their individual resolution.

135 Filament widths were analyzed by a self-written, customized script for the FIJI
136 software³⁴. Briefly, the filaments were selected by a segmented line profile covering the
137 filament width and length. To minimize the selection and pixelation error, selections were
138 shifted by 0.5 pixels (5 nm) in all directions to obtain five measures in total for each
139 filament. These selected ROIs were straightened to remove the individual curvatures of the
140 filaments and projected along their long axis. The obtained profiles were fitted by a
141 Gaussian which yields the filament widths by its FWHM-value. The optimal histogram bin
142 size was calculated using the Freedman – Diaconis rule³⁵.

143

144 **Fluorescent lifetime imaging microscopy (FLIM) of fluorescence resonance energy transfer**
145 **(FRET)**

146 For FLIM/FRET, NIH3T3 cells were transduced with lentiviruses encoding PGK-H2B-mCherry
147 and PGK-GFP-H2B, and sorted for homogeneous expression. For experiments involving
148 expression of Exportin 6, cells were seeded and synchronized by a single thymidine block, 24
149 hours after transfection of Flag-Exportin 6. After this, cells were washed 3x in PBS and
150 cultured in normal media for 10 hrs. Cells were then fixed (2% PFA in PBS, 10 min),
151 permeabilised (0.1% Triton in PBS, 10 min), and blocked (2% BSA in PBS, 30 min), before
152 incubation with anti-Flag antibodies for 2 hours. Cells were washed 3x in PBS, and incubated
153 with secondary Alexa Fluor 405-conjugated antibodies for 45 minutes. Cells were then
154 washed again in PBS. Post-mitotic, Flag-Exportin 6-overexpressing cells were identified using
155 405 nm excitation.

156 In the case of Flag-WT/R62D mutant actin, transfected cells expressing Flag-
157 WT/R62D-NLS-actin were sorted through SNAP-mediated tagging. Following this, cells were
158 transduced to express GFP-H2B and mCherry-H2B, and sorted by FACS. For these
159 experiments, cells were synchronised at G1/S transition using thymidine (2 mM for 20
160 hours). Cells were then washed three times in PBS, and returned to normal media
161 containing 500 ng/ml Doxycycline to induce expression of Flag-actin derivatives. After 4
162 hours, 1 μ M of CDK1i (RO-3306, Sigma) was added to the medium for a subsequent period
163 of 4 hours. Cells were washed three times in PBS, and then returned to normal media
164 containing 500 ng/ml Doxycycline. 30 min later, mitotic cells were isolated by mitotic shake
165 off, centrifuged at 1500 x g for 5 min and plated onto PLL-coated 35 mm dishes. After one
166 hour cells were pre-extracted with CSK buffer for 5 minutes, washed three times in PBS and
167 fixed with 2% PFA.

168 Lifetime measurements were taken on a Leica TCS SP8 system, using a white light
169 laser with a repetition rate of 20 MHz and an excitation wavelength of 488 nm. GFP-H2B
170 emission was detected over an emission range of 495 – 530 nm. Data was fitted using
171 FLIMfit software³⁶. Temporal binning of the fluorescence decays was performed prior to
172 fitting, resulting in 256 time bins per decay. Tail-fitting of the fluorescence images was
173 performed pixel-wise with a single exponential model on all pixels above an intensity
174 threshold of 175 photons, allowing spatial variations in fluorescence lifetime to be
175 visualized.

176

177 **Nuclear F-actin pulldown at mitotic exit**

178 For nuclear F-actin pulldowns, RPE-1 cells were mitotically blocked by nocodazole (100 nM
179 for 24 hours), before washout with growth medium. 4 hours after washout, cells were lysed
180 and subjected to subcellular fractionation as described previously⁸. Purity of subcellular
181 fractionations was controlled by immunoblotting for α -Tubulin and histone H3. The
182 obtained nuclear lysates were incubated with 5 μ g biotin-phalloidin at 4 °C and constant
183 rotation for 2 hours. Then, pre-washed magnetic streptavidin dynabeads (Thermo Fisher)
184 were added, following incubation at 4 °C and constant rotation for 2 hours. After washing,
185 the magnetic beads were collected and boiled in 2x Laemmli buffer for 10 min. The
186 supernatant containing lysed nuclear F-actin and associated proteins was used for further
187 analyses.

188

189 **Mass spectrometry-based protein identification**

190

191

192

193 Samples were loaded on an SDS gel and immediately after they had entered the separation
194 gel, electrophoresis was stopped and the protein bands were excised and subjected to in-gel
195 digest using trypsin³⁷.

196 For mass spectrometric analysis an Orbitrap Velos Pro mass spectrometer
197 (ThermoScientific) was used which was connected online with an Ultimate nanoRSLC-HPLC
198 system (Dionex), equipped with a nano C18 RP column. 10 µL of the tryptic digest were
199 usually injected onto a C18 pre-concentration column and automated trapping and
200 desalting of the sample was performed at a flowrate of 6 µL/min using water/0.05% formic
201 acid as solvent.

202 Tryptic peptides were separated with water/0.045% formic acid (solvent A) and 80%
203 acetonitrile/0.05% formic acid (solvent B) at a flow rate of 300 nL/min: holding 4% B for five
204 minutes, followed by a linear gradient to 45% B within 30 minutes and linear increase to
205 95% solvent B for 5 minutes. The column was connected to a stainless steel nanoemitter
206 (Proxeon, Denmark) and the eluent sprayed directly towards the heated capillary of the
207 mass spectrometer using a potential of 2300 V. A survey scan with a resolution of 60000
208 within the Orbitrap mass analyzer was combined with at least three data-dependent MS/MS
209 scans with dynamic exclusion for 30 s either using CID with the linear ion-trap or using HCD
210 and Orbitrap detection at a resolution of 7500.

211 Data analysis was performed using Proteome Discoverer (v4.0; ThermoScientific)
212 with SEQUEST and MASCOT (v2.4; Matrix science) search engines using either SwissProt or
213 NCBI databases.

214

215 **Mitotic shake-off**

216 For indicated immunoblot analyses, immunofluorescence staining and MNase assays, cells
217 were seeded at 40% confluency and allowed to adhere for 8 hours. Cells were then serum
218 starved for 24 hours, followed by addition of growth media, containing 0.33 µg/ml
219 doxycycline for experiments involving dox-inducible protein expression. After 16 hours,
220 nocodazole (100 nM) was added for 3 additional hours. Mitotic cells were collected by
221 mitotic shake-off and washed three times in growth media. These mitotic cells were then
222 reseeded and further processed for subsequent analyses.

223

224 **Micrococcal nuclease (MNase) digestion assay**

225 One million cells were harvested, and washed once with 1 ml of 1x RSB buffer (10 mM Tris,
226 pH 7.6, 15 mM NaCl, and 1.5 mM MgCl₂). After centrifugation (3,000 x g), the cell pellet was
227 resuspended in 1 ml of 1x RSB buffer with 1% Triton-X 100 and homogenized. Nuclei were
228 collected by centrifugation (13,000 x g) and washed twice with 1 ml of buffer A (15 mM Tris,
229 pH 7.5, 15 mM NaCl, 60 mM KCl, 0.34 M sucrose, and 0.1% β-mercaptoethanol, EDTA-free
230 protease inhibitor cocktail). Nuclei were resuspended in 500 µl MNase reaction buffer (from
231 NEB, 50 mM Tris-HCl, 5 mM CaCl₂ pH 7.9) and aliquoted into 100 µl aliquots. MNase
232 digestion was performed in 100 µl reactions by addition of 50 Kunitz units of MNase (NEB)
233 at 37 °C for 5 minutes. Reactions were terminated by adding 25 mM EDTA. DNA was purified
234 using a PCR purification kit and 1000 ng of DNA was analyzed on a 1.5% agarose gel.

235

236 **Animals**

237 ICR mice were obtained from Kiwa Experimental Animals (Wakayama, Japan). This study
238 conformed to the Guide for the Care and Use of Laboratory Animals. All animal experiments

239 were approved and performed under the guidelines of the Animal Research Committee of
240 Kindai University.

241

242 ***In vitro* fertilization of mouse oocytes and mRNA injection**

243 Female ICR mice (or ICR x ICR), aged 8-13 weeks, were superovulated with pregnant mare
244 serum gonadotropin (PMSG; Novartis Animal Health, Japan), followed 48 hours later with
245 human chorionic gonadotropin (hCG; ASKA Pharmaceutical). Cumulus–oocyte complexes
246 were collected from the oviducts in HTF medium. The sperm suspension was added to the
247 oocyte cultures, and morphologically normal fertilized oocytes were collected 1-1.5 hours
248 after insemination at 37°C under 5% CO₂ in air. Fertilized oocytes were transferred to
249 HEPES-CZB medium. mRNAs for nAC-GFP, actin^{R62D}-HA-NLS, actin^{wt}-HA-NLS, mCherry-
250 Exportin 6, myc-GFP were injected using a piezo manipulator (Prime Tech). mRNAs were
251 prepared from pCS2³⁸ or pcDNA3.1 vectors³⁹. In the case of pCS2 vectors, mRNAs produced
252 from the SP6 promoter were subjected to the addition of polyA tails while pcDNA3.1 vectors
253 were transcribed from the T7 promoter. Since the translation efficiency is different between
254 mRNAs produced from pCS2 vectors and those from pcDNA3.1 vectors, different
255 concentrations of mRNA were injected; nAC-GFP (150 ng/μl), HA-NLS-actin^{R62D} (650 ng/μl),
256 HA-NLS-actin^{wt} (650 ng/μl), mCherry-Exportin 6 (1,000 ng/μl) and myc-GFP (1,000 ng/μl),
257 histone H2B-mCherry (5 ng/μl). After mRNA injections, the fertilized embryos were cultured
258 in KSOM medium at 37°C in a 5% CO₂ atmosphere. mCherry-Exportin 6 mRNA or control
259 myc-tagged GFP mRNA was injected into oocytes denuded by 0.1% hyaluronidase before
260 subsequent *in vitro* fertilization.

261

262 **Confocal microscopy of oocytes**

263 mRNA-injected embryos were fixed in 4% formaldehyde for 10 min and washed three times
264 in PBS containing 0.01% PVA. Then, fixed embryos were incubated in PBS-BSA with 0.2%
265 Triton-X for 60 min, followed by three time washes with PBS-BSA. Washed embryos were
266 stained with 5 µg/ml DAPI for 15 min, followed by three times washing using PBS-BSA.
267 Embryos were mounted and observed under a confocal microscope (LSM800, ZEISS). Images
268 were analyzed using the ZEN software (ZEISS).

269

270 **Electron microscopy (EM)**

271 For EM-based analysis of chromatin compaction at mitotic exit, NIH3T3 cells (either stably
272 expressing BFP-NLS, NLS-BFP-actin^{R62D} or transfected with GFP-Exportin 6 or NLS-mCherry-
273 Cofilin) were synchronised at G1/S transition using thymidine (2 mM for 20 h). Expression of
274 GFP-Exportin 6 and NLS-mCherry-Cofilin was ensured by FACS-based cell sorting prior to
275 sample preparation. Cells were washed three times in PBS, and then returned to normal
276 media containing 500 ng/ml Doxycycline. After 4 hours, analysis of flow cytometry data,
277 using the Watson (Pragmatic) model, determined that 40% of cells had completed S phase.
278 This time point was therefore chosen to add 1 µM of CDK1i (RO-3306, Sigma), for a period
279 of 4 hours. Cells were washed three times in PBS, and then returned to normal media
280 containing 500 ng/ml Doxycycline. 30 min later, mitotic cells were isolated by mitotic shake
281 off. Cells were centrifuged at 1500 x g for 5 min, and plated onto PLL-coated 35 mm dishes.

282 After 1 hour, cells were trypsinised and centrifuged at 1500 x g for 5 min. Pellets
283 were re-suspended in complete media containing 10% BSA, and centrifuged at 1500 x g for
284 5 min. 1 µl of this cell pellet was then put into a 0.1 mm gold membrane carrier and high
285 pressure frozen (Leica EM PACT2). Samples were then freeze-substituted in a freeze-
286 substitution acetone mix, containing 0.1% uranyl acetate and 1% osmium tetroxide. During

287 this procedure, samples were first held at -90°C, then brought to 0°C, over a period of 18
288 hrs. These samples were then embedded in EPON, and baked at 60°C for 48 hrs. 70 nm
289 sections were cut using an ultratome, which were stained with uranyl acetate and lead
290 citrate and images were taken at 2900x magnification on a FEI Tecnai 12 TEM, operated at
291 120kV.

292 For analysis, nuclei and nucleoli were manually segmented in 2D slice images across
293 the cell using the freehand selection tool of ImageJ/Fiji to generate a binary mask of the
294 nucleoplasm. Condensed chromatin was then semi-automatically segmented across the
295 nucleoplasmic region using the WEKA Trainable Segmentation plugin for ImageJ/Fiji ⁴⁰.
296 Classification was based on the Gaussian blur, Sobel filter, Hessian, Difference of Gaussians
297 and membrane projections metrics using the built-in fast random forest algorithm. Due to
298 the variability in chromatin staining, it was necessary to train a new classification model for
299 each image. Condensed chromatin distribution was subsequently analysed in the
300 segmented images using a custom ImageJ/Fiji macro, which measured the total condensed
301 chromatin area and perimeter, as well as the area fraction of condensed chromatin, as a
302 proportion of the total nucleoplasmic area.

303

304 **Atomic force microscopy**

305 To obtain early G1 or interphase NIH3T3 nuclei, cells were collected 60 min or 7 hours after
306 mitotic shake-off, respectively. Nuclei were isolated as described previously ⁴¹. Atomic force
307 microscopy measurements were conducted in aqueous solution utilizing a Multi-mode VIII
308 microscope with Nanoscope V controller and a PeakForce feedback control mechanism with
309 an enclosed liquid cell. Isolated live nuclei were bound to a poly-L-lysine coated glass
310 coverslip and remained hydrated in this buffer (20 mM HEPES at pH 7.8, 25 mM KCl, 5 mM

311 MgCl₂, 0.25 M sucrose and 1 mM ATP) to increase the longevity of the nuclei for
312 investigation. The surface morphologies of nuclei were observed to remain unchanged
313 under these conditions, allowing multiple nuclei to be tested in each sample and an average
314 surface roughness and associated error to be calculated for each nucleus type. Using
315 SCANASYST-FLUID cantilevers [Bruker, CA, USA] of nominal spring constant 0.7 N/m and
316 nominal tip radius 2 nm, the force applied to plane of the sample by the AFM tip was kept
317 below 1 nN, thus imaging stability was maintained whilst avoiding tip-induced deformation
318 of the sample. Images were collected at a scan rate of 0.404 Hz with at 500 x 500 pixels
319 giving a digital resolution of ~10 nm/pixel. Nuclear height and roughness (R_q) were
320 calculated and quantified for the corresponding nuclei. Surface roughness (R_q) was defined
321 as root mean square average of height deviations.

322

323 **Statistics and Reproducibility**

324 For each experiment, sample sizes were chosen based on initial pilot experiments. Similar
325 experiments reported in previous publications were further used to direct sample sizes. No
326 data were excluded from the analysis. No blinding or randomization was used in the course
327 of the experiments. All attempts of replication were successful. Statistical analyses were
328 performed with Prism 7 (GraphPad). Data are presented as stated in the respective figure
329 legends. All *t*-tests were performed as unpaired, two-sided *t*-tests.

330

331 **Data availability**

332 Mass spectrometry data have been deposited in ProteomeXchange with the primary
333 accession code PXD213854. Datasets generated and analysed during the current study are
334 available from the corresponding author on reasonable request.

335

336

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- 370

1 **Supplementary Information**

2 **1. Supplementary Figures**

3 **Supplementary Figure 1 | Nuclear actin levels during mitotic exit are not affected by**
4 **expression of nAC-GFP. Nuclear actin filaments are a conserved feature among different**
5 **mammalian cell lines and form independent of Emerin, Lamin A/C, and the LINC complex.**
6 **(a)** 3D nuclear fluorescence intensities (FI) of stably expressed mCherry-actin were
7 measured at indicated times after cell division (0 min corresponds to anaphase) to compare
8 its nuclear distribution in the absence or presence of co-expressed nAC-GFP. Nuclei were
9 counterstained with SiR-DNA. Data are shown as mean \pm SD (n=13 mitotic events per
10 condition, pooled from several independent experiments). **(b)** NIH3T3 cells stably
11 expressing mCherry-actin were transfected with GFP-Exportin 6 and followed during the
12 time-course of mitotic cell division. Images show single confocal slices to illustrate nuclear
13 distribution of mCherry-actin in postmitotic cells in either the presence (indicated by
14 asterisks) or absence (arrowheads) of GFP-Exportin 6. Note the nuclear fluorescence signal
15 produced by mCherry-actin in control daughter nuclei (indicated by arrowheads). The
16 experiment was performed once. Time stamp, hours:min; scale bar, 10 μ m. **(c)** Time-lapse
17 imaging of stably nAC-GFP (green) expressing MCF10A, HT1080, and RPE-1 during cell
18 division reveals nuclear F-actin formation at mitotic exit. Images show maximum intensity
19 projections. Scale bar, 10 μ m; time stamp, min:sec. **(d, e)** Quantification of incidence **(d)** and
20 duration **(e)** of nuclear F-actin formation during mitotic exit in MCF10A, HT1080, and RPE-1
21 cells stably expressing nAC-GFP. Data are shown as mean + SEM (n=40 (MCF10A, HT1080),
22 n=30 (RPE-1) mitotic events, pooled from several independent experiments). **(f, g)**
23 Quantification of the incidence **(f)** and duration **(g)** of nuclear F-actin formation during

24 mitotic exit in nAC-GFP expressing NIH3T3 cells, treated with indicated siRNAs. Data are
25 shown as mean + SD (n=30 cells per condition, pooled from 2 independent experiments). **(h)**
26 Immunoblot validating efficient siRNA-mediated knockdown of Lamin A/C and Emerin. The
27 experiment was performed once. **(i)** Time-lapse imaging of NIH3T3 cells expressing nAC-GFP
28 (grey) together with tagRFP-KASH (red, insets) to disrupt the LINC complex during and after
29 mitotic division. The experiment was performed once. Scale bar, 10 μm ; time stamp,
30 min:sec. Unprocessed original scans of blots are shown in Supplementary Fig. 7.

31

32

33 **Supplementary Figure 2 | Super-resolution imaging of nuclear F-actin at mitotic exit.**

34 **(a)** PALM images of NIH3T3 cells stably expressing nAC-Dendra2 (orange) at mitotic exit
35 used to calculate filament widths in Fig. 2b. Experimental resolutions after drift correction
36 (see Methods) are $38 \pm 1 \text{ nm}$ (1, 2, 4), $39 \pm 1 \text{ nm}$ (3), $33 \pm 1 \text{ nm}$ (5, 6), $35 \pm 1 \text{ nm}$ (7,8). Cells 5
37 and 6 are shown in Fig. 2a. Scale bars, 5 μm . **(b)** Filaments were analysed by a self-written,
38 customized script for FIJI. First, ROIs were drawn along filaments (I). To minimize the
39 selection and pixelation error, selections were shifted by 0.5 pixels (5 nm) in all directions to
40 obtain five measures in total for each filament by straightening all selections (II). To
41 determine the filament width, lengthwise intensity profiles of the five filament selections
42 were fitted with Gaussians (III). The average full width at half maximum (FWHM) of the five
43 selections yields the average filament width. Scale bar, 0.5 μm . **(c)** Mitotic cell division of
44 native NIH3T3 cells was monitored over time using DIC (Differential interference contrast)
45 to allow for formaldehyde fixation at defined time points after anaphase, as indicated.
46 Stochastic Optical Reconstruction Microscopy (STORM) images focus on the corresponding
47 nuclei labelled by phalloidin (see Methods for details). The dashed rectangle is shown

48 magnified in Fig. 2c. Experimental resolutions after drift correction are 40 ± 1 nm (15 min,
49 45 min-cell 1), 30 ± 1 nm (45 min-cell 2) and 34 ± 1 nm (60 min). Scale bars, 5 μ m.

50

51 **Supplementary Figure 3 | Actin dynamics but not transcriptional inhibition affect early G1**
52 **nuclear expansion. Detection of Flag-actin derivatives by doxycycline-inducible T2A-SNAP**
53 **fusions.**

54 **(a)** 3D surface reconstructions of NIH3T3 nuclei (visualized by SiR-DNA) at indicated times
55 after drug treatment at mitotic exit. Scale bar, 10 μ m; time stamp, hours:min. **(b)** Nuclear
56 volume quantifications in cells treated similar to **a**. Data is shown as mean \pm SD (n=50 nuclei
57 per condition, pooled from at least 3 independent experiments). **(c)** Design of Flag-tagged
58 nuclear actin derivatives linked to the SNAP-tag by a T2A peptide. Upon translation, the T2A
59 peptide is cleaved resulting in equimolar expression of Flag-NLS-actin and the SNAP-tag.
60 Accordingly, labelling of the SNAP-tag allows for indirect detection of Flag-NLS-actin in living
61 cells. **(d)** Immunoblot confirms doxycycline-induced expression of Flag-NLS-actin-T2A-SNAP
62 derivatives. A single band indicates efficient cleavage of Flag-NLS-actin-T2A-SNAP. **(e)**
63 Confocal images of fixed NIH3T3 cells expressing nAC-GFP transfected with Flag-NLS-actin-
64 T2A-SNAP derivatives. In contrast to Flag-NLS-actin the SNAP-tag (labelled by SiR-647)
65 displays pan-cellular distribution. Scale bar, 10 μ m. **(f)** Confocal images of fixed NIH3T3 cells
66 at mitotic exit show expression of Flag-NLS-actin-T2A-SNAP derivatives, as indicated.
67 Magnifications correspond to dashed rectangles and highlight Flag-actin. Scale bar, 10 μ m.
68 **(g)** Nuclear volume quantifications in live NIH3T3 cells stably expressing H2B-mCherry and
69 doxycycline-induced BFP-NLS, NLS-BFP-actin^{R62D} or Flag-NLS-actin-T2A-SNAP derivatives.
70 Expression of the indicated constructs was induced during G₀. Data are mean + SD from
71 n=30 nuclei per condition. **(h)** RT-qPCR analysis of *FOS* expression in serum-starved NIH3T3

72 cells, pre-treated with Flavopiridol (1 μ M for indicated times) before stimulation with serum
73 (20% FCS, fetal calf serum) for 30 minutes. Note that 15 min pre-treatment with Flavopiridol
74 efficiently inhibits serum-induced transcriptional upregulation of *FOS* mRNA. Data are
75 shown as mean from n=2 independent experiments. **(i)** Nuclear volume analysis in NIH3T3
76 cells stably expressing H2B-mCherry in the presence of Flavopiridol (1 μ M) or DMSO (0.1%).
77 Flavopiridol was added at metaphase prior to imaging the subsequent expansion of
78 daughter nuclei. Data are shown as mean \pm SD (n>14 nuclei [precise n?] per condition,
79 pooled from 3 independent experiments). Unprocessed original scans of blots are shown in
80 Supplementary Fig. 7.

81

82 **Supplementary Figure 4 | Inhibition of nuclear F-actin formation impairs chromatin**
83 **decompaction at mitotic exit**

84 **(a)** Quantification of GFP-H2B fluorescence lifetime in cells expressing GFP-H2B alone, or in
85 combination with mCherry-H2B and indicated treatments: trichostatin A (TSA), or sodium
86 azide (NaN₃) together with 2-deoxyglucose (2-DG). ***P* < 0.01, ****P* < 0.001 calculated by
87 one-way ANOVA. **(b)** Quantification of fluorescence lifetime reveals no significant difference
88 in GFP fluorescence lifetime upon expression of and staining for Flag-Exportin 6 using a
89 Alexa Fluor 405-conjugated antibodies. ns, non-significant in one-way ANOVA. **(c)**
90 Comparative immunoblot analysis of histone modifications (H3S10ph, H4K16ac) in NIH3T3
91 cells induced to express BFP-NLS or NLS-BFP-actin^{R62D} and undergoing either asynchronous
92 (asyn.) or synchronized (mitotic shake off) culture, as indicated. **(d, e)** Images and
93 quantitative immunofluorescence analysis of nuclear Aurora B **(d)** and KAT5 **(e)** (both green;
94 nuclei are stained with DAPI (magenta)) in NIH3T3 cells at mitotic exit expressing Flag-NLS-
95 actin-T2A-SNAP derivatives, as indicated. Data are shown as mean \pm SD (n=20 nuclei per

96 condition, pooled from three independent experiments). Scale bar, 10 μm . **** $P < 0.0001$
97 calculated by *t*-test. **(f)** Analysis of chromatin compaction by an MNase accessibility assay 45
98 min after mitotic shake-off in NIH3T3 cells expressing either doxycycline-induced Flag-NLS-
99 actin^{wt} or -actin^{R62D}. Graph shows quantified pixel intensities corresponding to band
100 intensities. **(g)** Example images illustrating the pipeline used for the quantification of
101 condensed chromatin in cryo-electron microscopy images (for details see Methods). Based
102 on raw images (I) nuclei and nucleoli were manually segmented (II). Condensed chromatin
103 was semi-automatically segmented across the nucleoplasmic region and classified (III)
104 allowing for an assessment of its distribution using a custom ImageJ/Fiji macro (IV). **(h)**
105 Representative electron microscopy images of cryo-preserved, synchronized early G₁
106 NIH3T3 cells expressing GFP-Exportin 6 corresponding to Figure 4l. Scale bar, 2 μm .
107 Unprocessed original scans of blots are shown in Supplementary Fig. 7. Immunoblot in c and
108 MNase accessibility assay in f represent 1 out of 2 independent experiments.

109

110

111 **Supplementary Figure 5 | Inhibition of nuclear F-actin formation impairs Pol II-dependent**
112 **transcription and preimplantation development of mouse embryos.**

113 **(a)** Images corresponding to Figure 5a showing RNA Pol II pS5 stainings (grey) in NIH3T3 cells
114 expressing either GFP or GFP-Exportin 6 at indicated times after mitotic shake-off. Scale bar,
115 5 μm . **(b)** Images corresponding to Figure 5g showing preimplantation development of
116 mouse embryos expressing either mCherry-Exportin 6 or myc-tagged GFP as a control.
117 Similar amounts of mRNA were injected into oocytes at the metaphase II stage, followed by
118 *in vitro* fertilization. Scale bar, 100 μm .

119

120 **Supplementary Figure 6 | P-Cofilin levels change during mitotic exit and nuclear Cofilin-1**
121 **is essential for filament disassembly during mitotic exit.**

122 **(a)** Representative immunostaining of p-Cofilin (grey, DAPI (blue)) in NIH3T3 cells treated
123 with si-Control or si-Cofilin to validate specificity of the obtained fluorescence signals.
124 Asterisks indicate presumably non-silenced cells. Scale bar, 10 μm . The experiment was
125 performed once. **(b)** Images corresponding to quantifications shown in Figure 6e. Confocal
126 images show single slices at indicated time points after mitotic shake-off. Scale bar, 5 μm .
127 **(c)** Immunoblot detecting p-Cofilin and Cofilin in RPE-1 cells after washout of nocodazole.
128 Decreasing H3S10ph levels proof for successful release from the nocodazole-induced mitotic
129 block. **(d)** P-Cofilin/Cofilin ratio was calculated by densitometric quantification of
130 immunoblot intensities. Data are shown as mean + SD from n=3 independent experiments.
131 **(e)** Time-lapse imaging of NIH3T3 cells during mitotic exit corresponding to Figure 6j. Cells
132 stably express nAC-GFP (green) together with either WT- or NES-mCherry-Cofilin (red) and
133 were treated with siRNA against the 3'-UTR of endogenous Cofilin-1. Scale bar, 10 μm . **(f)**
134 Stably nAC-GFP expressing NIH3T3 cells were transfected with NLS-mCherry-Cofilin and
135 followed during mitotic exit. Images show maximum intensity projections of confocal z-
136 stacks and illustrate the absence of nuclear F-actin formation which was observed in 10 of
137 12 mitotic events. Time, hours:min; scale bar, 10 μm . **(g)** Immunoblot validating expression
138 of opto-Cofilin in cells treated with either control siRNA or siRNA directed against the 3'-UTR
139 of endogenous Cofilin-1. Unprocessed original scans of blots are shown in Supplementary
140 Fig. 7.

141

142 **Supplementary Figure 7 | Unprocessed original scans of Western blot analysis**

143

144 **2. Supplementary Tables**

145 **Supplementary Table 1**

146 Table illustrating the incidence and duration of nuclear F-actin formation at mitotic exit
147 upon siRNA-mediated knockdown of actin nucleators or regulators. All siRNA sequences
148 used showed greater than 50% knockdown efficiency on mRNA level for the intended
149 target, as determined by RT-qPCR (compared to control siRNA and normalized to expression
150 of TBP). Statistical analysis did not show a significant difference for any condition compared
151 to control cells, as determined by one-way ANOVA. n.d., not determined. Data are shown as
152 mean +/- SEM, pooled from at least 2 independent experiments. Sample sizes (number of
153 mitotic events) for each condition are shown within the table.

154

155 **Supplementary Table 2**

156 Table summarizing the results of nuclear F-actin pulldown, as well as a control pulldown
157 (without biotin-phalloidin), performed at mitotic exit and analysed by mass spectrometry
158 (see Methods for details).

159

160 **Supplementary Table 3**

161 Table listing the information of antibodies used in this study.

162

163 **3. Supplementary Videos**

164 **Supplementary Video 1 | Transient nuclear F-actin formation can be detected during**
165 **mitotic exit.**

166 Video corresponding to Fig. 1a shows transient formation of nuclear F-actin during and after
167 cell division in NIH3T3 cells as visualized by nAC-GFP (green). In addition, cells express
168 LifeAct-mCherry (red). Scale bar, 10 μm .

169

170 **Supplementary Video 2 | Nuclear F-actin shows dynamic turnover in cells at mitotic exit.**

171 Video corresponding to Fig. 1b shows dynamic reorganization of actin filaments after
172 mitotic division in NIH3T3 cells as visualized by nAC-GFP (green). In addition, cells express
173 Lamin-nanobody-SNAP, labelled by a SiR-647 dye (LaminCB-SNAP|SiR-647, magenta). Scale
174 bar, 10 μm .

175

176 **Supplementary Video 3 | Nuclear F-actin forms within interchromatin spaces.**

177 Video corresponding to Fig. 4a shows dynamic reorganization of actin filaments after mitotic
178 division in NIH3T3 cells as visualized by sAC-GFP (green). In addition, cells express H2B-
179 mCherry (red) to visualize chromatin content. Scale bar, 10 μm ; time stamp, h:min:s.

180

181 **Supplementary Video 4 | Nuclear actin filaments reshape newly assembled nuclei.**

182 Video corresponding to Fig. 3a shows NIH3T3 cells during mitotic exit, stably expressing
183 nAC-GFP (green) and H2B-mCherry (red). Scale bar, 10 μm ; time stamp, min:s.

184

185

186 **Supplementary Video 5 | Knockdown of Cofilin affects nuclear actin dynamics during**
187 **mitotic exit.**

188 Video corresponding to Fig. 6f, g. Time-lapse imaging of NIH3T3 cells stably expressing nAC-
189 GFP (green), treated with si-control or si-Cofilin during mitotic exit. Video shows three
190 representative examples for each condition. Note the appearance of excessive and stable
191 nuclear actin filaments in si-Cofilin-treated cells. Scale bar, 10 μm .

192

193 **Supplementary Video 6 | Light-regulated control of opto-Cofilin subcellular localization.**

194 Video corresponding to Fig. 7d shows NIH3T3 cells stably expressing opto-Cofilin (grey).
195 Single confocal slices were acquired at 10 sec intervals, and cells were temporarily
196 illuminated by additional blue laser light (488 nm, indicated by a green bar) to promote
197 reversible nuclear export of opto-Cofilin.

198

199 **Supplementary Video 7 | Formation of excessive, stable nuclear F-actin upon light-**
200 **regulated nuclear exclusion of opto-Cofilin.**

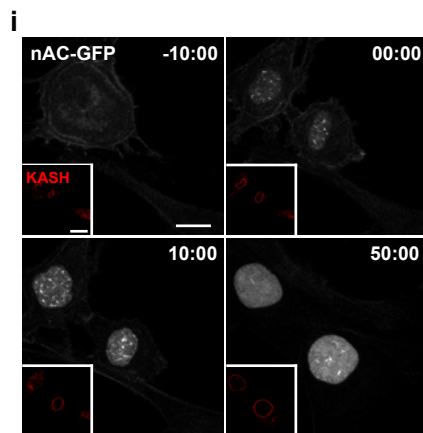
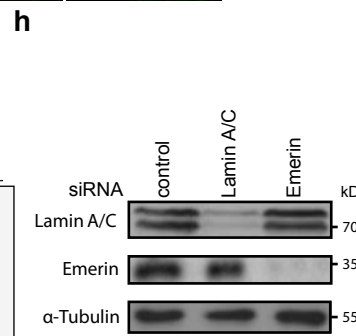
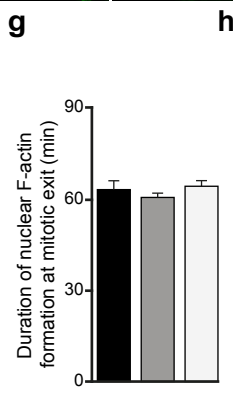
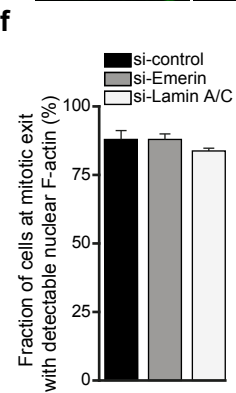
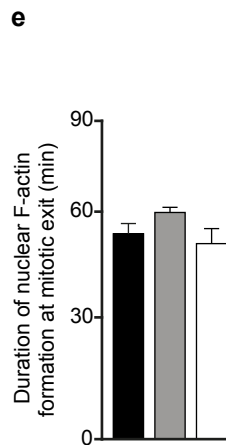
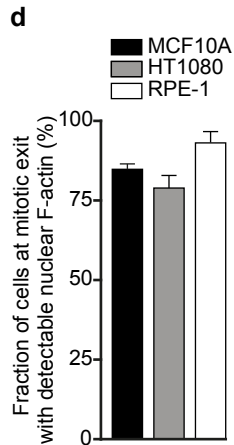
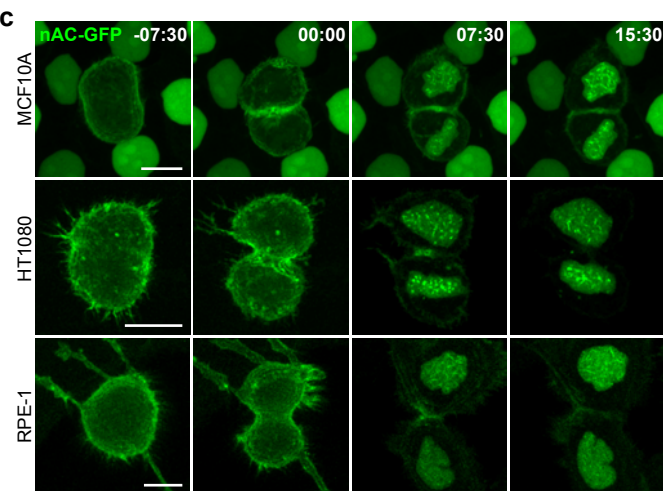
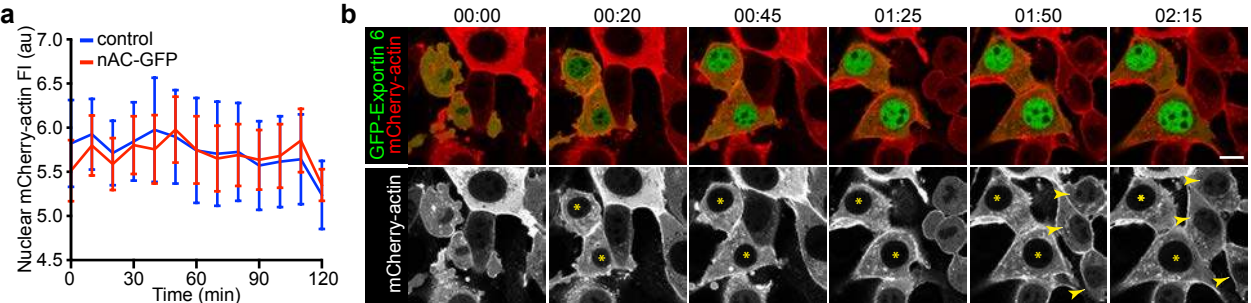
201 NIH3T3 cells stably expressing nAC-SNAP (labelled by SiR-647, grey) and opto-Cofilin (red)
202 were treated with si-Cofilin (3'-UTR) and imaged during and after mitosis. Cells were imaged
203 either with (+ light, lower panel) or without (- light, upper panel) additional blue laser light
204 (488 nm) to promote sustained nuclear export of opto-Cofilin.

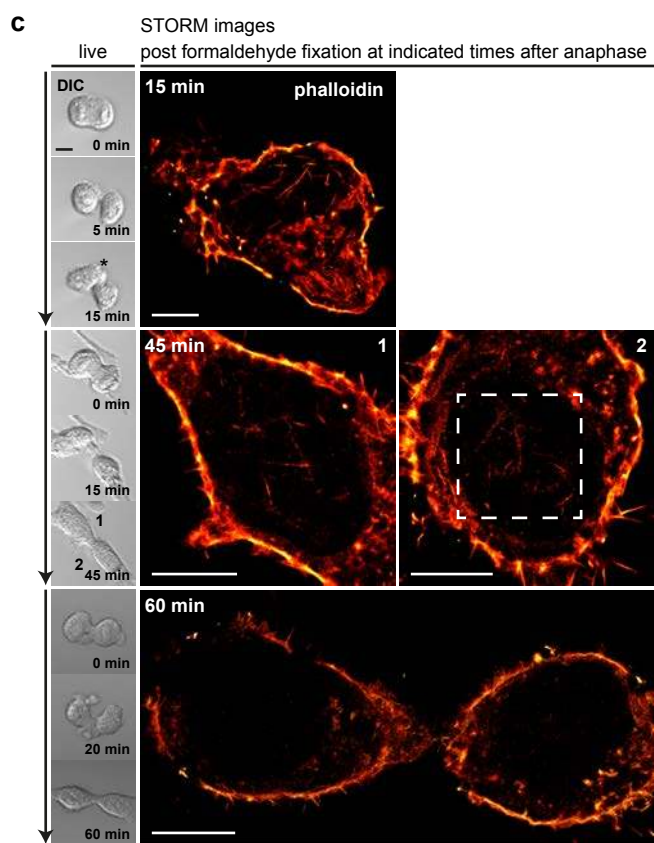
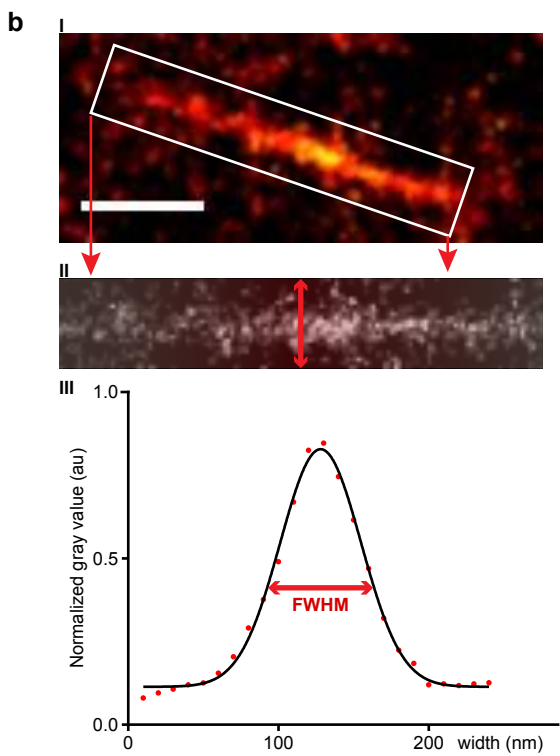
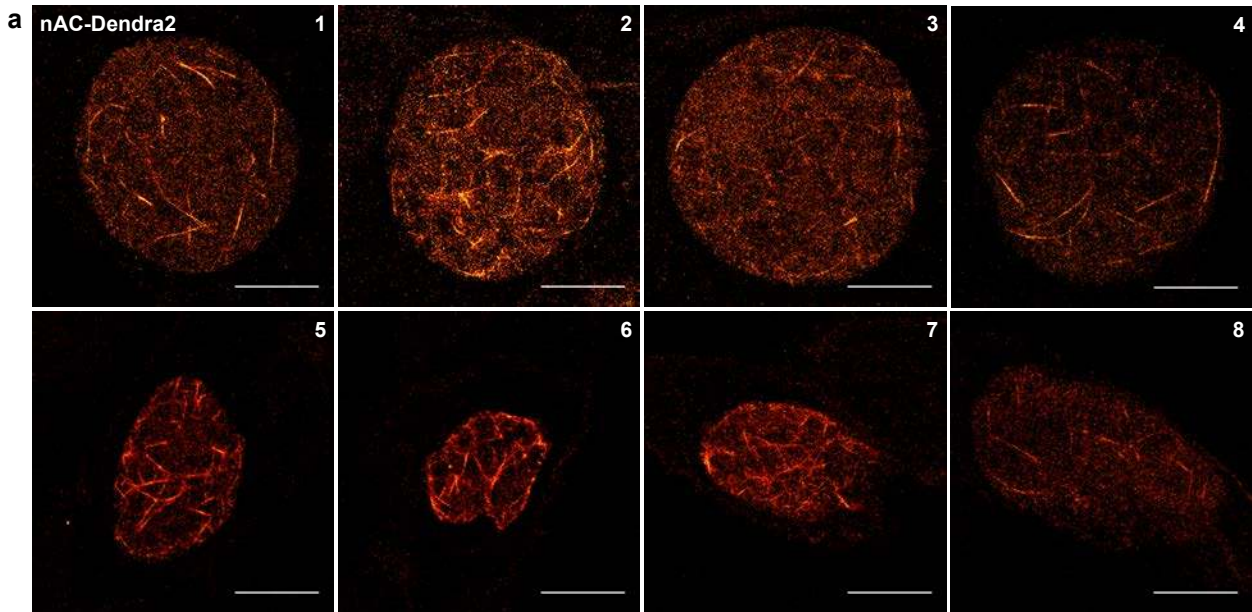
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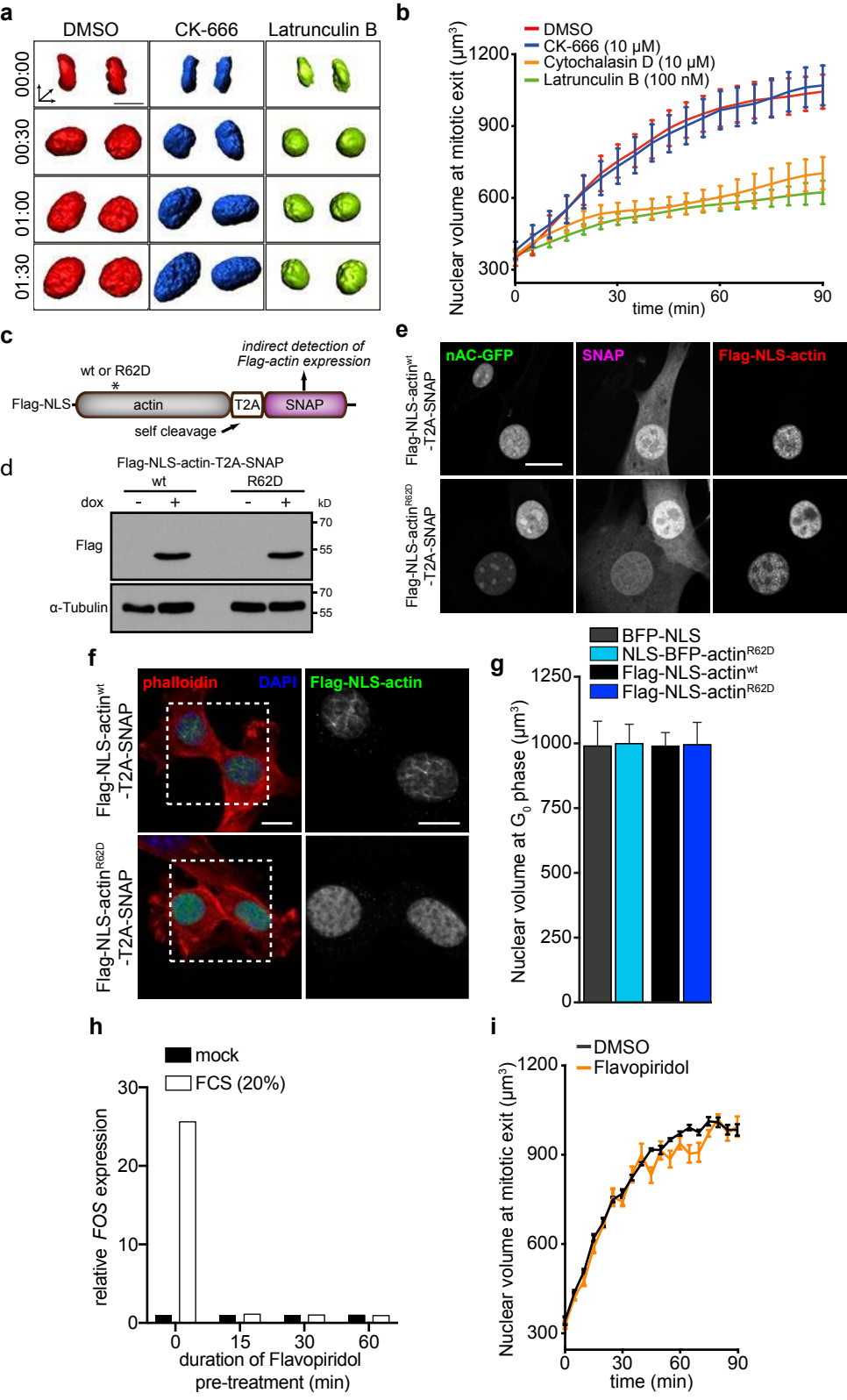
206 **Supplementary Video 8 | Reversible formation of excessive, stable nuclear F-actin by light-**
207 **controlled subcellular shuttling of opto-Cofilin.**

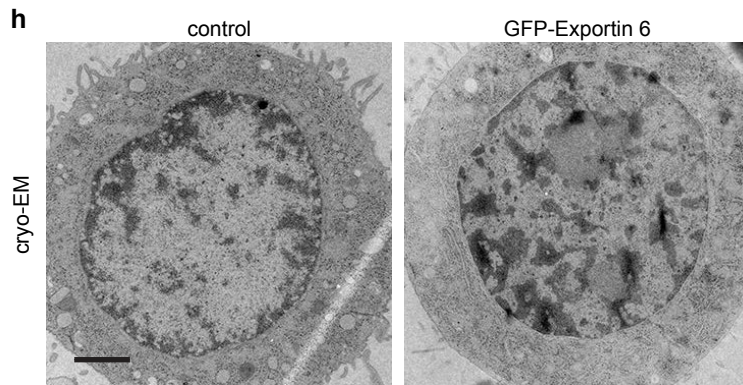
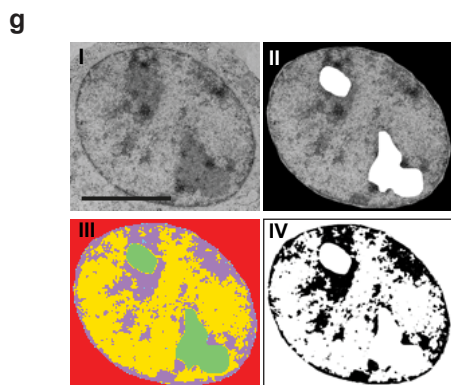
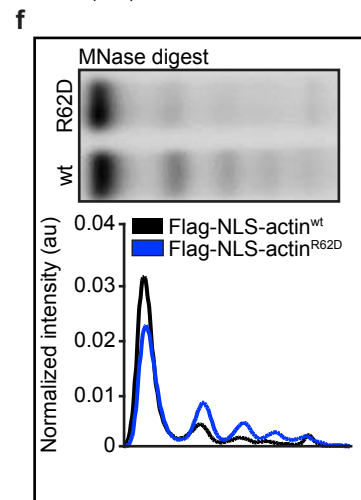
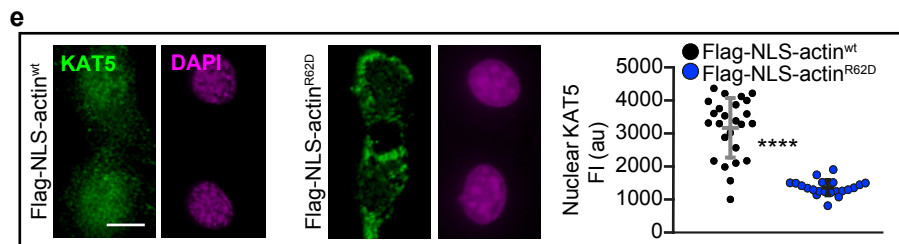
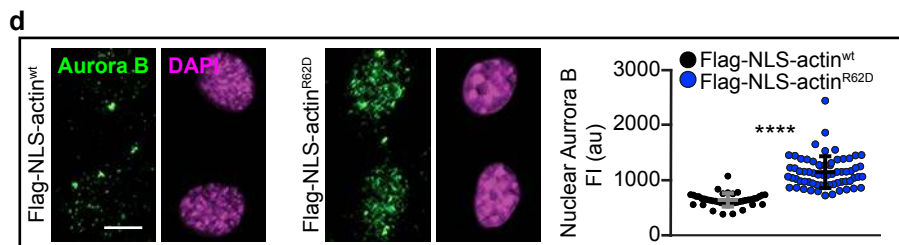
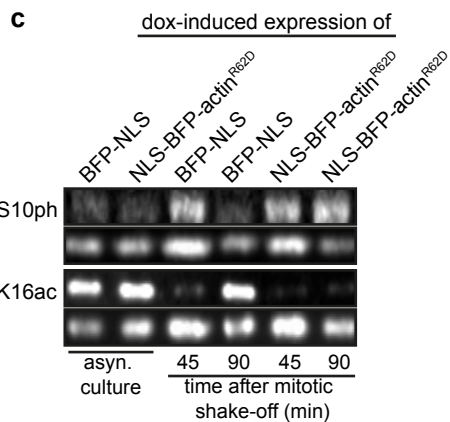
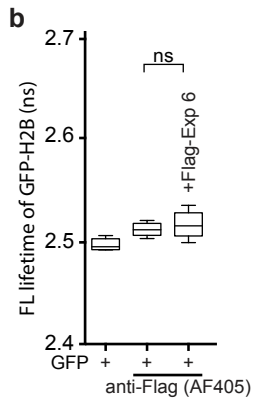
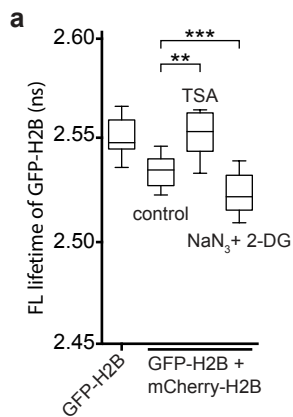
208 Video corresponding to Fig. 7f shows NIH3T3 cells stably expressing nAC-SNAP (labelled by
209 SiR-647, grey) and opto-Cofilin (red) during and after mitosis. Cells were treated with si-

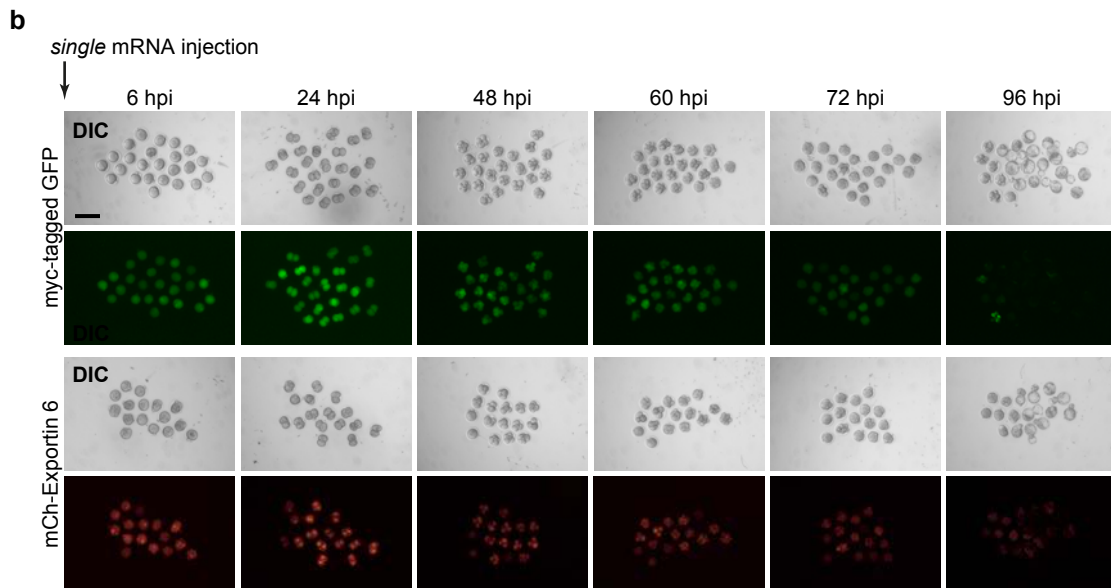
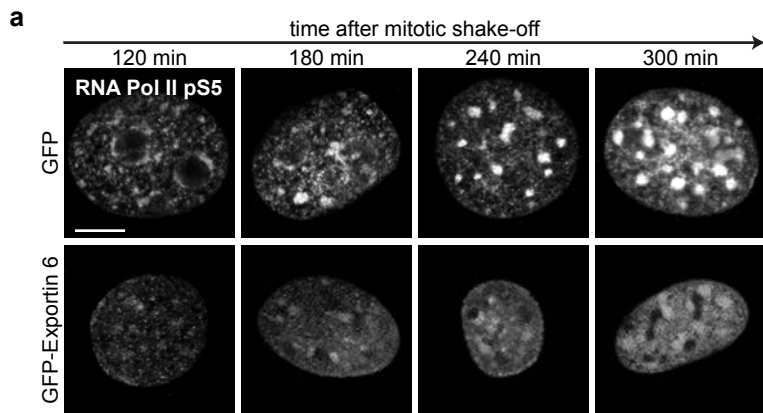
210 Cofilin (3'-UTR) and temporarily illuminated by blue laser light (488 nm) to promote nuclear
211 export of opto-Cofilin for a defined period of time (indicated by a green bar).
212











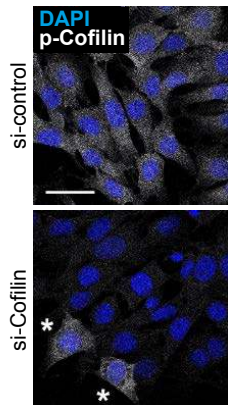
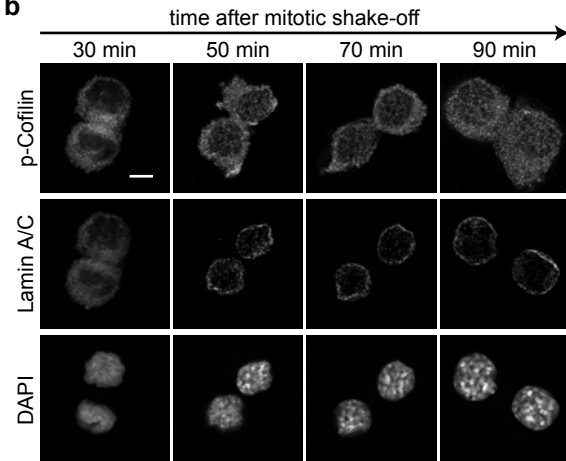
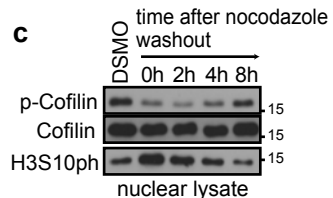
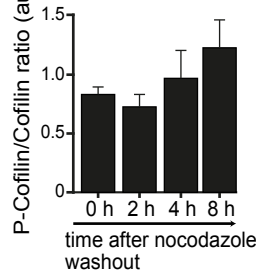
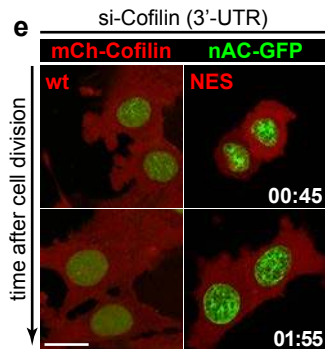
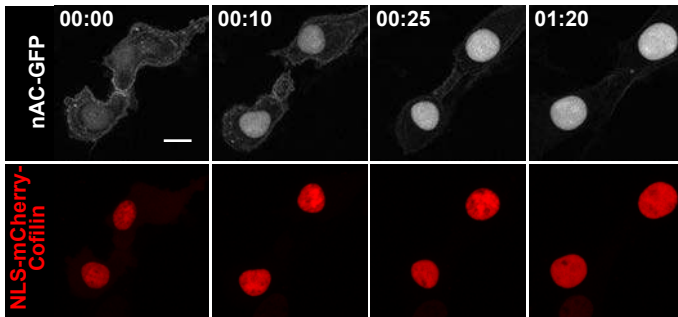
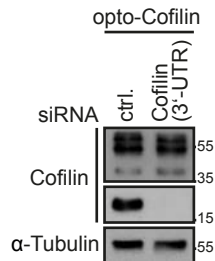
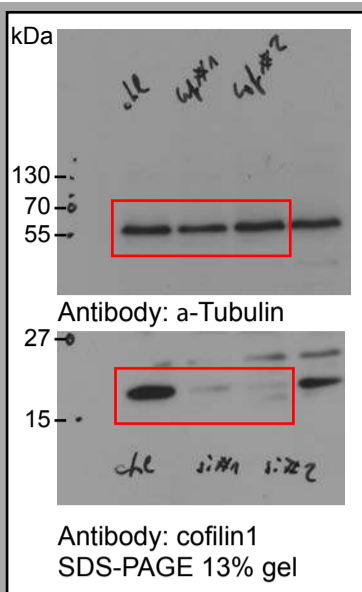
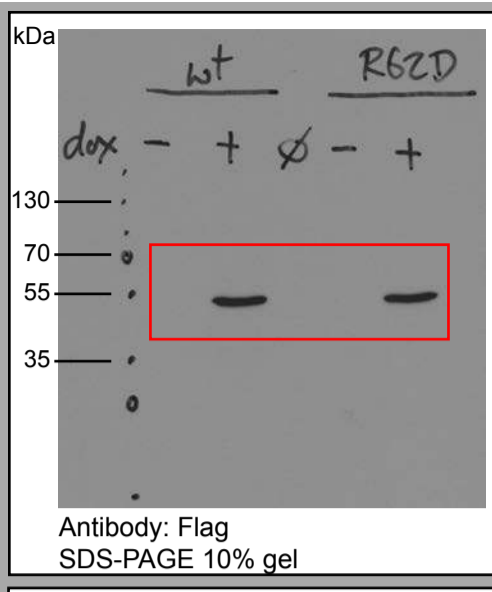
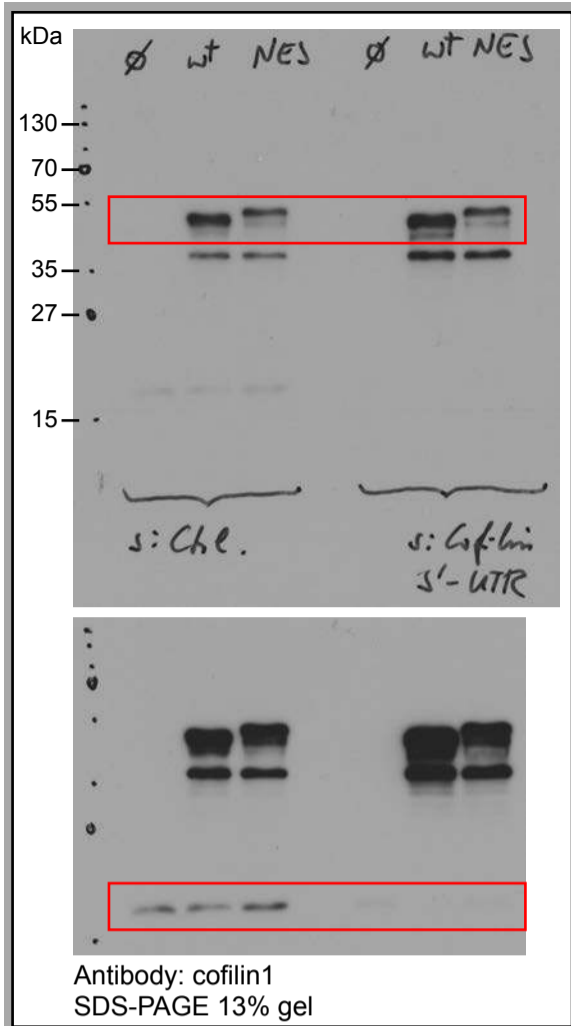
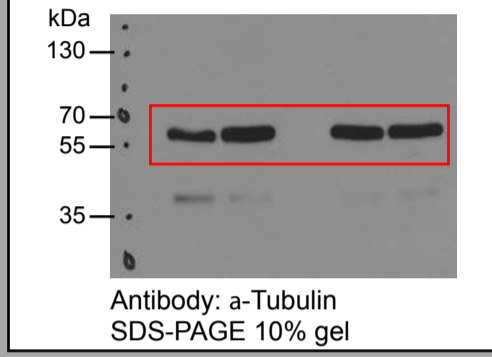
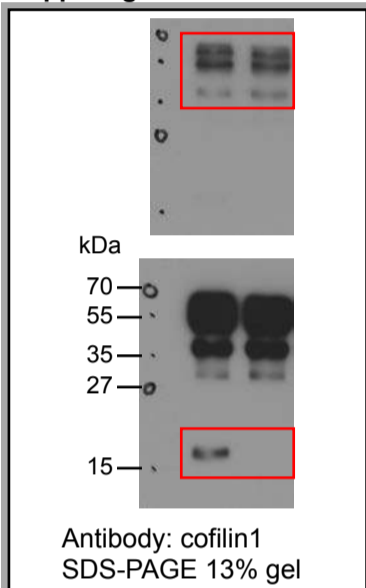
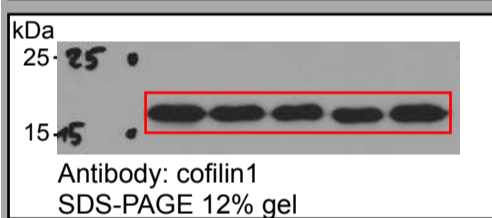
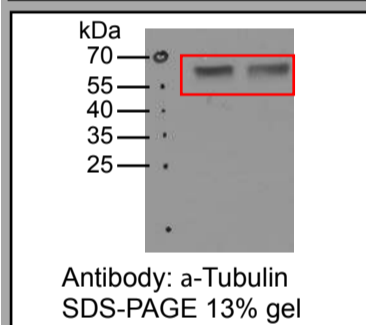
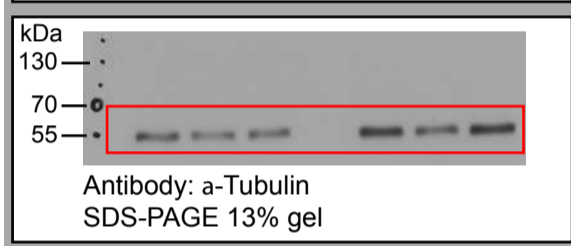
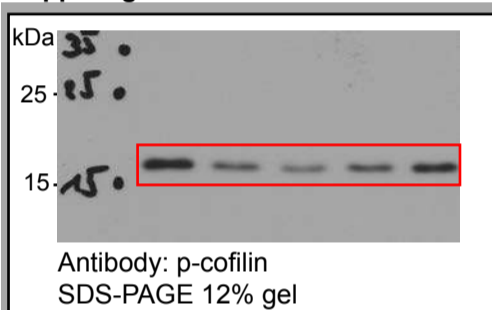
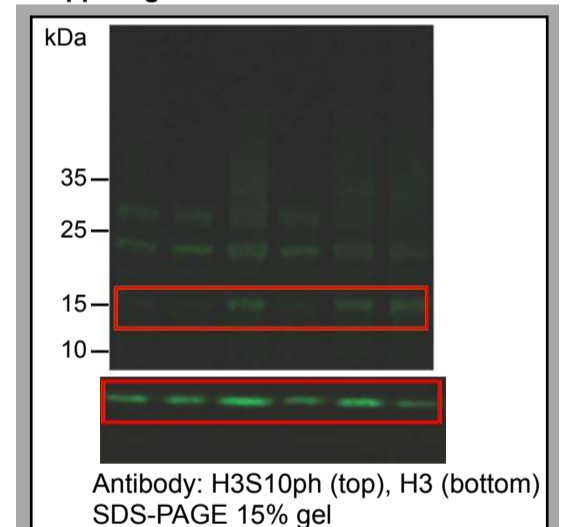
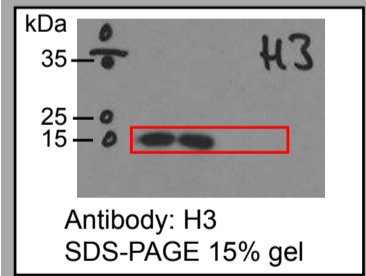
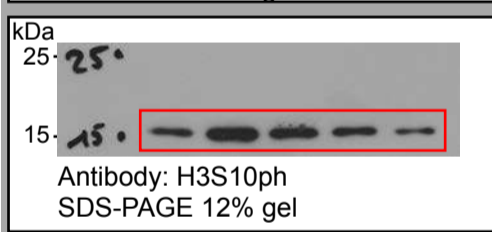
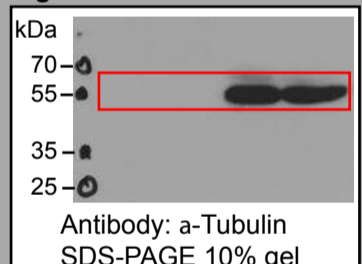
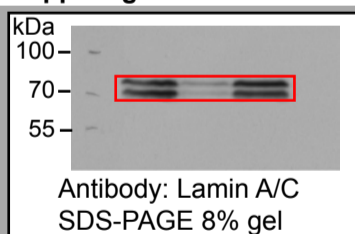
a**b****c****d****e****f****g**

Figure 6g**Suppl. Figure 3d****Figure 6i****Suppl. Figure 6h****Suppl. Figure 6h****Suppl. Figure 4c****Figure 6b****Suppl. Figure 1h****Figure 6c**