

1 **MCPH1 inhibits condensin II during interphase by regulating its SMC2-kleisin**
2 **interface**

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24

25 **ABSTRACT:**

26

27 The dramatic change in morphology of chromosomal DNAs between interphase and
28 mitosis is one of the defining features of the eukaryotic cell cycle. Two types of
29 enzymes, namely cohesin and condensin confer the topology of chromosomal DNA
30 by extruding DNA loops. While condensin normally configures chromosomes
31 exclusively during mitosis, cohesin does so during interphase. The processivity of
32 cohesin's LE during interphase is limited by a regulatory factor called WAPL, which
33 induces cohesin to dissociate from chromosomes via a mechanism that requires
34 dissociation of its kleisin from the neck of SMC3. We show here that a related
35 mechanism may be responsible for blocking condensin II from acting during
36 interphase. Cells from patients carrying mutations in the *Mcp1* gene undergo
37 premature chromosome condensation but it has never been established for certain
38 whether MCPH1 regulates condensin II directly. We show that deletion of *Mcp1* in
39 mouse embryonic stem cells unleashes an activity of condensin II that triggers
40 formation of compact chromosomes in G1 and G2 phases, which is accompanied by
41 enhanced mixing of A and B chromatin compartments, and that this occurs even in
42 the absence of CDK1 activity. Crucially, inhibition of condensin II by MCPH1 depends
43 on the binding of a short linear motif within MCPH1 to condensin II's NCAPG2 subunit.
44 We show that the activities of both Cohesin and Condensin II may be restricted during
45 interphase by similar types of mechanisms as MCPH1's ability to block condensin II's
46 association with chromatin is abrogated by the fusion of SMC2 with NCAPH2.
47 Remarkably, in the absence of both WAPL and MCPH1, cohesin and condensin II
48 transform chromosomal DNAs of G2 cells into chromosomes with a solenoidal axis
49 showing that both SMC complexes must be tightly regulated to adjust both the
50 chromatid's structure and their segregation.

51

52

53 INTRODUCTION

54

55 The segregation of sister DNAs during mitosis requires that they are first disentangled
56 and organised into individual sister chromatids before being pulled in opposite
57 directions by the mitotic spindle upon the dissolution of sister chromatid cohesion by
58 separate. Chromatid formation during mitosis and sister chromatid cohesion
59 necessary for bi-orientation are mediated by highly related structural maintenance of
60 chromosomes (SMC)-kleisin complexes, namely condensin and cohesin respectively.
61 In both cases, the activity of their ring-like SMC-kleisin trimers is regulated by large
62 hook-shaped proteins composed of tandem HEAT repeats, known as HAWKs (HEAT
63 repeat proteins associated with kleisins) ¹.

64 The conundrum that complexes with such similar geometries appear to perform
65 such dissimilar functions has been resolved by the realisation that cohesin also
66 organises DNAs into chromatid-like structures, albeit during interphase and only upon
67 ablation of a regulatory protein called WAPL ² as well as naturally during meiotic
68 prophase ³ or during V(D)J recombination when WAPL is downregulated in pro-B cells
69 ⁴. There is now considerable evidence that cohesin and condensin organise
70 chromosomal DNAs during interphase and mitosis, respectively, by extruding DNA
71 loops in a processive manner. Indeed, both exhibit such loop extrusion (LE) activity *in*
72 *vitro* ^{5,6}. LE mediated by cohesin is halted or at least retarded at specific sequences
73 bound by CTCF, and the processivity of the complex is reduced by WAPL which
74 induces cohesin's dissociation from chromatin, albeit only infrequently every 10-20
75 min ⁷⁻⁹. No such site-specific DNA binding proteins are known to regulate condensin.
76 In both cases, chromatid formation is envisaged to arise from processive LE activity,

77 which organises DNA into a series of loops, each emanating from a central core
78 containing the SMC-kleisin loop extruders ¹.

79 Mammalian cells possess two types of condensin complexes: condensin I and
80 II. Both complexes share the same SMC proteins, SMC2 and SMC4, which both
81 contain 50 nm long anti-parallel coiled coils connecting a hinge domain at one end
82 with an ATPase domain, formed from the N- and C-terminal extremities, at the other.
83 Dimerisation via their hinges creates V-shaped SMC2/4 heterodimers whose ATPase
84 heads are inter-connected by their kleisin subunits, NCAPH1 for condensin I and
85 NCAPH2 for condensin II. These in turn recruit different HAWK regulatory subunits,
86 NCAPD2 or D3 and NCAPG or G2 for condensin I and II respectively ¹⁰. While
87 condensin II remains in the nucleus during interphase and starts to organise
88 chromosomal DNAs during prophase, condensin I only has access to the DNA after
89 nuclear envelope break down ¹¹⁻¹³. Despite this difference, both accumulate along the
90 longitudinal axes of metaphase chromosomes ^{13,14}. HiC data suggest that condensin
91 I extrudes shorter loops that are nested within longer ones previously created by
92 condensin II. Interactions between distant loops suggest that the loops created by
93 condensin II may be organised radially around a coiled or solenoidal axis ¹⁵. The ratio
94 between condensin I and condensin II adjusts the coiling of chromosome axes, partly
95 by altering the width of the central spiral and generating curled chromosomes ¹⁶.

96 An important factor in limiting the processivity of loop extrusion by cohesin is its
97 release from chromatin. This process that depends on WAPL involves engagement of
98 cohesin's ATPase heads ¹⁷ and dissociation of the N-terminal domain (NTD) of its
99 SCC1 kleisin subunit from the coiled coil (neck) that emerges from SMC3's ATPase
100 head domain ¹⁸. Because a co-translational fusion of SCC1's NTD to the C-terminal
101 domain of SMC3's ATPase blocks release, it has been proposed that it involves

102 passage of DNAs previously entrapped inside its SMC-kleisin ring through an exit gate
103 created by dissociation of SCC1 from SMC3^{19,20}. Indeed, the kleisin NTDs appear to
104 dissociate *in vitro* from engaged SMC2 and SMC4 heads^{21,22} as well as those of
105 SMC1 and SMC3^{19,23,24}. This observation raises the possibility that release via a
106 kleisin-SMC exit gate might be a feature of chromosomal condensin complexes as
107 well as cohesin. However, whether release of this nature occurs *in vivo* and whether
108 it has an important function is not known.

109 Condensin II's activity and functions within the nuclei of interphase cells are
110 poorly understood. While some studies have reported that it binds to chromatin during
111 interphase²⁵, its depletion from post mitotic mouse liver cells has little or no effect on
112 genome organisation or transcription²⁶. Likewise, the notion that condensin II is
113 activated as cells enter mitosis via kinase cascade involving CDK1 and PLK1²⁷ also
114 begs the question as to whether it has any significant role during interphase. However,
115 an important clue that condensin II can in principle function during this stage of the cell
116 cycle comes from the characterization of patients carrying a mutation of the *Mcp1*
117 gene, which causes a reduction in the size of the cerebral cortex known as primary
118 microcephaly (OMIM 608585)^{28,29}. Cells from such patients have an increased
119 number of prophase-like cells^{30,31}. The prophase-like organisation of chromosomal
120 DNA has since been shown to be mediated by condensin II^{32,33}, whose abnormal
121 activity triggers premature condensation in G2 and late de-condensation in G1^{30,31,34}.

122 MCPH1 is only found in metazoa. It contains three BRCT domains, one N-
123 terminal and two C-terminal, separated by a disordered region. The diverse
124 phenotypes of mutant cells have led to many different and often conflicting
125 suggestions for its roles. Binding of MCPH1's C-terminal BRCT domains to
126 phosphorylated histone H2AX³⁶ is thought to play a part in the DNA damage

127 response, while the premature chromosome condensation caused by mutations within
128 its N-terminal BRCT has been attributed to this domain's defective association with
129 the SWI/SNF nucleosome remodelling complex and SET1³⁷. MCPH1 is also thought
130 to bind DNA and has been proposed to function in telomere maintenance, centriole
131 organisation and CHK1 activation³⁸⁻⁴². In contradiction to the finding that MCPH1
132 binds directly to condensin II, it is widely believed that the N-terminal ~200 residues of
133 MCPH1 compete with condensin II for chromosomal binding³³, in other words,
134 MCPH1 has been proposed to occupy loci required for condensin II's chromosomal
135 activity.

136 Given the diversity and conflicting views of MCPH1's function, we have re-
137 addressed its role in regulating chromosome structure by analysing the consequences
138 of deleting its gene in mouse ES cells. We show that loss of MCPH1 induces a
139 prophase-like organisation of chromatin during G1 and G2 but not during S phase,
140 and that this depends on condensin II. Crucially, this phenotype, which is
141 accompanied by condensin II's stable association with chromosomes, is unaffected
142 by inhibiting CDK1, suggesting that MCPH1 does not inhibit chromosome
143 condensation merely by delaying the CDK1 activation normally necessary for
144 condensin II activity as previously suggested^{40,41,43}. We demonstrate that MCPH1
145 instead regulates the organisation of chromosomal DNA through the binding of
146 condensin II's NCAPG2 subunit by a conserved short linear motif (SLiM) situated
147 within its central domain. Such binding does not *per se* explain how MCPH1 regulates
148 condensin II and has little or no effect on its ATPase activity, at least *in vitro*.
149 Presumably, other domains, for example its N-terminal BRCT, which is frequently
150 mutated in human microcephaly patients, have an effector function, by interacting with
151 other sites within the condensin II pentamer or recruit other cellular factors.

152 A clue to the role of MCPH1 is the resemblance between the stable association of
153 condensin II with chromosomal DNA induced by MCPH1 ablation with the effect on
154 cohesin of mutating *Wapl*, which binds to the cohesin HAWK equivalent to NCAPG2,
155 namely STAG2, also using an FY SLiM. We therefore tested whether like WAPL,
156 MCPH1 prevents condensin II from associating stably with DNA by opening the
157 interface between NCAPH2's NTD with the neck of SMC2's ATPase domain.
158 Remarkably, a translational fusion between SMC2's C-terminus and NCAPH2's N-
159 terminus is not only functional in mouse oocytes but also resistant to the inhibition
160 mediated by an excess of MCPH1 induced by mRNA micro-injection. This raises the
161 possibility that like WAPL, MCPH1 acts by opening the interface between the kleisin's
162 NTD and the neck of the SMC ATPase domain. Finally, we investigated the
163 consequences of inducing the stable association with G2 chromosomes of both
164 cohesin and condensin and found that the chromosomal axes created by cohesin upon
165 mutating *Wapl* are turned into solenoids in cells deleted for both *Wapl* and *Mcp1*.

166

167 **RESULTS**

168

169 ***Mcp1* deletion induces chromosome condensation during interphase in** 170 **embryonic stem cells.**

171 Premature chromosome condensation is a key feature of cells isolated from patients
172 carrying *Mcp1* mutations. To study this in greater detail, we used CRISPR/Cas9 to
173 delete *Mcp1* in mouse E14 embryonic stem cells in which we had previously
174 introduced a Halo-tag at the C-terminal end of NCAPH2 (NCAPH2-Halo) at its
175 endogenous locus. Western blot using an anti-NCAPH2 antibody confirmed that all
176 the NCAPH2 protein present in the cell is shifted to a higher molecular weight that

177 matches the size of the band detected by in-gel Halo-TMR fluorescence. The protein
178 expression levels are identical to the untagged protein in control cells (Figure 1A).
179 Deleting the second exon of *Mcph1* induces a frameshift between exon 1 and 3 and
180 thereby complete inactivation of the gene. Western blot analysis of the targeted cells
181 confirmed the lack of any MCPH1 protein (Figure 1A).

182 Immunofluorescence microscopy revealed that *Mcph1* deletion leads to a
183 substantial increase in the fraction of cells with prophase like chromosomes (Figure
184 1B). FACS analysis of cells stained with propidium iodide to measure DNA content,
185 H3PS10 specific antibodies to detect G2/M cells, and pulse labelled with EdU to
186 identify S phase cells showed no overall change in cell cycle progression
187 (Supplementary figure 1). Thus, *Mcph1* deletion caused little or no prophase arrest.
188 Crucially, all EdU negative cells, whether they were in G1 (H3PS10 negative) or G2
189 (H3PS10 positive), contained prophase-like chromosomes (>100 cells counted) while
190 few if any of the EdU positive S phase cells did so (Figure 1C and 3D). In wild type G1
191 and G2 cells, different centromeres and peri-centric regions cluster in chromocenters.
192 This feature is abolished in the mutant cells, where every centromere occupies an
193 individual location (Supplementary figure 2) and each chromosome is likewise
194 individualised into prophase-like chromatids (Figure 1B). The localisation of NCAPH2
195 was analysed by labelling its Halo-tag with the fluorescent TMR ligand. In wild type,
196 NCAPH2 has a diffuse nuclear distribution throughout most of the interphase, with no
197 enrichment at any particular site. The protein starts to accumulate at centromeres
198 during G2 and subsequently along the length of chromosomes from prophase until the
199 end of telophase. Deletion of *Mcph1* caused NCAPH2 to associate with the prophase-
200 like chromosomes in all G1 and G2 cells and become enriched at their individualised
201 centromeres (Figure 1B).

202

203 **MCPH1 restricts condensin II activity during G1 and G2.**

204 The change in chromosome organisation caused by deletion of *McpH1* is
205 accompanied by a change in the localisation of condensin II. To address whether
206 condensin II is causing the observed phenotype, we used a Halo-PROTAC ligand to
207 specifically induce degradation of NCAPH2-Halo (Figure 1D). Because this completely
208 reversed the re-organisation of chromosomal DNA caused by *McpH1* deletion (Figure
209 1E), we conclude that altered regulation of condensin II is largely if not completely
210 responsible. Unlike the centromere dispersion, chromosome condensation, and
211 chromosome unpairing induced by loss of Slimb ubiquitin ligase or casein kinase 1 in
212 *Drosophila* cells, which is accompanied and caused by an increase in the level of
213 NCAPH2^{44,45}, deletion of *McpH1* in ES cells is accompanied by a modest but
214 nevertheless significant reduction in NCAPH2 levels (Figure 1 A & D). The implication
215 is that MCPH1 restricts the activity of individual condensin II complexes in G1 and G2
216 cells.

217

218 **MCPH1 prevents condensin II's stable association with interphase chromatin.**

219 Photobleaching studies with GFP tagged condensin II subunits has revealed that they
220 are highly mobile during interphase, suggesting that they are rarely or only transiently
221 associated with chromatin fibres⁸. FRAP of TMR labelled NCAPH2-Halo confirmed
222 this as photobleached spots of Halo-TMR fluorescence recovered 95% of their
223 fluorescence within 1 min (Figure 2). However, deletion of *McpH1* caused a dramatic
224 change in the dynamics, with fluorescence merely recovering to 28% of its starting
225 level within 1 minute. After that, no further change occurred during the next 10 min
226 (Figure 2), implying that *McpH1* deletion causes 72% of condensin II complexes to

227 associate stably with chromatin in G1 or G2 cells, thereby altering chromatin
228 compaction during interphase.

229

230 **The chromosome re-organisation induced by *McpH1* deletion does not depend**
231 **on CDK1.**

232 To check whether the premature formation of prophase-like chromatids when *McpH1*
233 deleted cells enter G2 might be caused by the precocious activation of CDK1, which
234 activates condensin II as wild type cells enter prophase, we compared inhibitory
235 phosphorylation of CDK1's Y15 residue and cyclin B1 localisation in wild type and
236 mutant cells. Deletion of *McpH1* neither reduced Y15 phosphorylation nor caused
237 cyclin B1 to enter nuclei prematurely (Figure 3A and B). To address this issue more
238 directly, namely whether CDK1 activity is required for condensin II's hyperactivity, we
239 asked whether inhibition of the kinase would suppress the chromosomal re-
240 organisation. Treatment of both wild type and *McpH1* deleted cultures with the CDK1
241 inhibitor RO-3306 for seven hours caused most cells to accumulate in G2 with a 4C
242 DNA content (Figure 3C). The DNA organisation of wild type cells resembled that of
243 normal G2 cells, namely no chromatid-like structures were formed and centromeres
244 were clustered in chromocenters (Figure 3D). In contrast, the chromosomal DNAs of
245 all mutant cells were organized into prophase-like chromatids with individualised
246 centromeres (Figure 3D). We conclude that the hyperactivity of condensin II in G2
247 *McpH1* deleted cells is independent of CDK1, suggesting that MCPH1 regulates
248 condensin II directly.

249

250 ***McpH1* deletion induces chromosomal compaction and alters inter-**
251 **chromosomal interactions**

252 *In situ* Hi-C libraries were generated for wild-type mouse E14 and *McpH1* deleted cells.
253 In wild-type cells, chromosomal p-termini have enhanced contact frequencies with one
254 another, as do q-termini. In contrast, p-termini are less likely to contact q-termini
255 (Figure 4A). This is consistent with the presence of chromocenters within which p-
256 termini co-localize with each other and likewise q-termini with each other. Because
257 mouse chromosomes are telocentric, with centromeres located at their p-termini, the
258 HiC maps confirm that centromeres co-localize with one another as do telomeres.

259 Strikingly, the enhanced spatial proximity between chromosomal p-termini
260 (resp., q-termini) is lost in the absence of MCPH1 (Figure 4B and C), consistent with
261 the disappearance of chromocenters as observed by microscopy. Deletion of *McpH1*
262 also results in an enhancement in the frequency of long-range, intra-chromosomal
263 contacts (Figure 4D, E and F) and enhances the frequency of inter-compartment (A to
264 B) contacts as compared to intra-compartment contacts (A to A and B to B)
265 (Supplementary figure 3A and B). This finding is consistent with the compaction of
266 individual chromosomes upon loss of MCPH1.

267 The HiC maps did not reveal loci moving from one compartment to the other
268 nor any major changes in loops or contact domains (Supplementary figure 3C).

269

270 **Recombinant MCPH1 forms a stable complex with condensin II.**

271 Our results suggest that MCPH1 directly represses condensin II activity, possibly via
272 a direct interaction. Previous *in vitro* work suggested that MCPH1 binds condensin II
273 via two interfaces. One interface between the N-terminal 195 residues of MCPH1 and
274 NCAPD3 and a second binding site between a highly conserved central domain of
275 MCPH1 (381-435) and NCAPG2 (Figure 5A and B)³³. To confirm a direct interaction
276 between MCPH1 and condensin II, full length human MCPH1 and condensin II were

277 expressed in insect cells and separately purified. While full-length MCPH1 was largely
278 insoluble, we purified sufficient strep-tagged full-length MCPH1 to confirm that it could
279 pull-down pentameric condensin II complex (Figure 5C). To localise the binding site,
280 we expressed and purified N-terminal His-MBP-tagged truncations of MCPH1 in *E.*
281 *coli*: MBP-MCPH1₁₋₄₃₅, MBP-MCPH1₁₋₁₉₅, MBP-MCPH1₁₉₆₋₄₃₅ and MBP-MCPH1₃₄₈₋₄₆₉
282 (Figure 5A). We found that strep-tagged condensin II could only pull-down MCPH1
283 constructs that included the central domain (Figure 5D). MCPH1 binding was specific
284 to condensin II, as condensin I-strep was unable to pull down any MCPH1 constructs
285 (Supplementary figure 4A).

286 We then tested whether tetrameric condensin II, lacking either NCAPD3 or
287 NCAPG2, could bind MCPH1₁₋₄₃₅ using pull-down assays. To exclude the MBP tag
288 interfering with the interaction at the N-terminus of MCPH1, we moved the tag to the
289 C-terminus. We found that removing NCAPG2 greatly reduced MCPH1₁₋₄₃₅ MBP pull-
290 down, while removing NCAPD3 had no effect (Figure 5E), suggesting that the binding
291 was mediated by the central domain of MCPH1 to NCAPG2. Further analysis with
292 analytical size exclusion chromatography demonstrated that MBP-MCPH1₁₉₆₋₄₃₅ and
293 condensin II co-eluted in one peak, separated from the void volume, suggesting they
294 form a stable, soluble complex (Supplementary figure 4B).

295

296 **MCPH1 binds condensin II via a short linear motif.**

297 To address which part of MCPH1's central domain is necessary for binding condensin
298 II, we analysed the sequence of MCPH1 using the ConSurf server^{46,47} to identify
299 conserved sequences. Between residues 381-435 of human MCPH1, the 410-424
300 interval stands out as a highly conserved patch within a region that is otherwise poorly
301 conserved (Figure 5B and Supplementary figure 4C). Despite its conservation, the

302 sequences are predicted to be disordered, suggesting that it could be a short linear
303 motif (SLiM) that binds to condensin II. To test this, we performed fluorescence
304 polarisation binding assays with a 5-FAM labelled MCPH1 peptide spanning residues
305 407-424 and found that it bound to condensin II, with a fit K_d of $0.64 \pm 0.12 \mu\text{M}$ (mean
306 \pm SEM) (Figure 5F). As expected, no binding was detected to a tetrameric version of
307 condensin II lacking NCAPG2.

308 SLiMs are frequently regulated by post-translation modification ⁴⁸ and
309 proteomic analysis of mitotic cells previously found that MCPH1 can be
310 phosphorylated within the central motif at S417 ⁴⁹, with S417/P418 forming a potential
311 CDK consensus site ⁵⁰. We therefore used a fluorescence polarisation competition
312 assay to test the effect of phosphorylating S417. In these assays, the concentration of
313 condensin II and 5-FAM-MCPH1₄₀₇₋₄₂₄ is fixed and unlabelled peptides of either wild-
314 type or S417 phosphorylated MCPH1₄₀₇₋₄₂₄ are added at increasing concentrations.
315 While the wild-type MCPH1₄₀₇₋₄₂₄ readily competed with 5-FAM-MCPH1₄₀₇₋₄₂₄,
316 resulting in a fit competition K_D of $5.3 \pm 1.0 \mu\text{M}$, phosphorylation at S417 reduced the
317 affinity \sim 10 fold to $53 \pm 8 \mu\text{M}$ (Figure 5G). This suggests that CDK1 phosphorylation
318 of MCPH1 may reduce its interaction with condensin II, an effect that might have an
319 important role in initiating chromosome condensation during prophase.

320

321 **MCPH1 central domain is essential for its interaction with condensin II and its**
322 **regulation *in vivo*.**

323 To address whether this central motif is necessary for binding and regulating
324 condensin II *in vivo*, we created an E14 cell line in which both copies of the *Ncaph2*
325 gene is tagged at its C-terminus with GFP. Western blotting revealed MCPH1 in
326 immunoprecipitates generated using antibodies against GFP, and only in GFP-tagged

327 cells expressing wild-type MCPH1 (Figure 6A). Because the MCPH1-specific antibody
328 was raised against the central domain, we used a cell line in which both copies of
329 *Mcph1* and *Ncaph2* were tagged with GFP and Halo respectively to test the role of the
330 central domain motif and then created a variant (*Mcph1*^{ΔCenGFP/ΔCenGFP}) lacking 15
331 residues containing the motif (S₄₀₀SYEDYFSPDNLKER₄₁₄). Western blotting
332 confirmed that *Mcph1*^{GFP/GFP} cells and *Mcph1*^{ΔCenGFP/ΔCenGFP} were expressed at similar
333 levels (Figure 6C). The slightly increased mobility of *Mcph1*^{ΔCenGFP/ΔCenGFP} and its
334 failure to be detected by the MCPH1-specific antibody confirmed deletion of the central
335 domain motif (Figure 6B). Because TMR labelled NCAPH2-Halo was detected in GFP
336 immunoprecipitates from *Mcph1*^{GFP/GFP} but not *Mcph1*^{ΔCenGFP/ΔCenGFP} cells (Figure 6C),
337 we conclude that MCPH1's central domain is essential for its stable interaction with
338 condensin II *in vivo*.

339 Immunofluorescence revealed that MCPH1 and MCPH1^{ΔCen}-GFP proteins
340 show the same localisation within cells. They are both exclusively nuclear and
341 enriched in small clusters that colocalise with sites of DNA damage marked by γH2aX
342 DNA (Figure 6D), as previously reported⁵¹. The significance of this association is
343 unclear as *Mcph1* deletion has no effect on the level of γH2aX (Supplementary figure
344 1D). Crucially, the chromosomes of *Mcph1*^{ΔCenGFP/ΔCenGFP} but not *Mcph1*^{GFP/GFP} G1 and
345 G2 cells adopted the prophase-like appearance characteristic of *Mcph1* deleted cells
346 (Figure 6E). Therefore, we conclude that MCPH1's central domain SLiM is essential
347 for inhibiting condensin II during interphase and inhibiting premature condensin-
348 mediated chromatin condensation.

349

350 **MCPH1 does not alter condensin II ATPase activity or DNA binding *in vitro*.**

351 To address whether MCPH1 affects condensin II's activity *in vitro*, purified the
352 condensin II- MCPH1₁₋₄₃₅ complex using size exclusion chromatography and
353 measured its ATPase activity. The condensin II-MCPH1₁₋₄₃₅ complex possessed a
354 similar activity to that of condensin II alone but its stimulation by DNA was modestly
355 lower (Figure 7A). To ensure that the ATPase activity measured in these assays was
356 genuinely due to condensin II, we also purified a condensin II-MCPH1₁₋₄₃₅ complex
357 deficient in ATP binding (Q-loop mutation, SMC2 Q147L, SMC4 Q229L). As expected,
358 this mutation effectively eliminated ATPase hydrolysis (Figure 7A).

359 Previous work has suggested that full length MCPH1 binds to DNA and
360 chromatin^{33,42} so we tested whether MBP-MCPH1₁₋₄₃₅ and MBP-MCPH1₁₉₆₋₄₃₅ are
361 able to bind to a 50 bp sequence of dsDNA using electrophoretic mobility shift assay
362 (EMSA). Both MCPH1₁₋₄₃₅-MBP and MCPH1₁₉₆₋₄₃₅-MBP were able to induce a shift,
363 however higher concentrations of MCPH1₁₉₆₋₄₃₅ MBP were required for a complete
364 shift in the free DNA band, suggesting MCPH1₁₋₁₉₅ MBP could have a role in DNA
365 binding (Figure 7B). We then examined if MCPH1 affected condensin II DNA binding,
366 by performing condensin II EMSAs in the presence or absence of MCPH1₁₋₄₃₅-MBP.
367 The distinct MCPH1₁₋₄₃₅-MBP shifted band disappeared with increasing
368 concentrations of condensin II, and there was an upward shift in the condensin II
369 bands in the presence of MCPH1₁₋₄₃₅-MBP relative to the MBP condensin II control,
370 suggesting MCPH1 was binding with condensin II (Figure 7C). We then performed
371 condensin II EMSAs in the presence or absence of 1 μ M 5-FAM-MCPH1₄₀₇₋₄₂₄ peptide.
372 5-FAM signal was present with condensin II shifted DNA band demonstrating that the
373 MCPH1 peptide was sufficient to mediate comigration of MCPH1 with condensin II
374 (Figure 7D). Additionally, the presence of the 5-FAM-MCPH1₄₀₇₋₄₂₄ peptide did not
375 affect condensin II DNA binding. Collectively, this indicates that *in vitro*, using purified

376 proteins, condensin II can bind MCPH1 and DNA simultaneously. Finally, these results
377 suggest that the inhibitory effect of MCPH1 on condensin II loading observed *in vivo*
378 involves a feature absent from the *in vitro* DNA binding assay.

379

380 **MCPH1 overexpression inhibits the loading of condensin II on mouse meiotic**
381 **chromosomes.**

382 Our results show that MCPH1 plays a crucial role in chromosome organisation during
383 interphase by inhibiting condensin II's activity in mitotic cells. We next extended our
384 analysis of MCPH1 to meiotic cells by testing the effect of increased MCPH1
385 expression during the first meiotic division of mouse oocytes. To image the
386 chromosomes, oocytes were injected with *in vitro* transcribed mRNA coding for H2B-
387 mCherry to illuminate the chromosomes in magenta (Figure 8A). As previously
388 described⁵², after the germinal vesicle breakdown (GVBD), bivalent chromosomes
389 form a ball (3.3 h) before congressing to a metaphase plate (7.4 h). Cleavage of
390 cohesin by separase along chromosome arms then converts each bivalent into a pair
391 of dyads that segregate highly synchronously to opposite poles of the cells during
392 anaphase I (10 h), which is followed by extrusion of the first polar body (Figure 8B,
393 control).

394 Co-injection with *M. musculus* MCPH1 mRNAs (Figure 8B, +MCPH1) had little
395 effect on the chromosome congression until metaphase. However, it caused
396 chromosomes to unravel soon after the onset of anaphase, presumably because
397 chromosomes are not stiff enough to resist to the pulling forces of the spindle, and this
398 was accompanied by a catastrophic failure to disjoin the chromosome arms of dyads
399 to opposite poles (Figure 8B, +MCPH1, 16 h). The lack of chromosome rigidity and

400 the unravelling of the chromatin in response to traction by the spindle are reminiscent
401 of the phenotype caused by depletion of NCAPH2⁵².

402 To address whether MCPH1 overexpression inhibits the association of
403 condensin II with chromosomes, we rescued mouse oocytes deleted for *Ncaph2*
404 (*Ncaph2^{Lox/Lox}, ZP3^{TgCre}*) by injecting an mRNA coding for NCAPH2-GFP as previously
405 described⁵². These oocytes were also injected with mRNA encoding MAD2 to arrest
406 them in meiosis I and H2B-mCherry to image and quantify the amount of chromosomal
407 NCAPH2-GFP (Figure 8C). This revealed that the injection of MCPH1 mRNAs greatly
408 reduced association of condensin II with chromosomes during metaphase I (Figure 8D
409 and E). To see if MCPH1 can also release condensin II previously associated with
410 chromosomes, we injected MCPH1 mRNA into meiosis I arrested oocytes. However,
411 this induced a much milder reduction within the first two hours (not shown), suggesting
412 that MCPH1 prevents the initial loading of condensin II on chromosomes but has little
413 effect on complexes already stably associated with them.

414 The N-terminal BRCT domain of MCPH1 was previously shown to have an
415 essential role in regulating chromosome condensation both *in vitro* and *in vivo*³³.
416 Consistent with this, deletion of the N-terminal 200 amino acids of MCPH1 abolishes
417 its inhibitory effect in mouse oocytes (Figure 8D). It has also been claimed that the N-
418 terminal domain of MCPH1 can on its own inhibit condensin II by competing for its
419 binding sites on chromosomal DNA³³. However, we were unable to detect any impact
420 of over-expressing only the NTD of MCPH1 (not shown).

421

422 **Fusion of SMC2 to NCAPH2 is resistant to MCPH1 inhibitory effect.**

423 The effects of MCPH1 on condensin II resemble those of WAPL on cohesin, which is
424 thought to act by dissociating the NTD of its kleisin from the neck of SMC3's

425 ATPase domain^{18,19,53}. A key finding in this regard is that the fusion of the C-terminus
426 of SMC3 to the N-terminus of SCC1 causes cohesin to resist WAPL. To address
427 whether fusion of this nature has similar effect on condensin II, we created a cDNA
428 encoding a protein in which the C-terminus of SMC2 and the N-terminus of NCAPH2
429 are connected by a 57 amino acid linker containing three TEV protease cleavage sites.
430 A GFP tag was introduced at the C-terminal end of NCAPH2 to image the protein
431 (Figure 9A). Importantly, mRNAs encoding this fusion fully rescued the meiosis I
432 chromosome segregation defects of oocytes deleted for *Ncaph2*. Furthermore, GFP
433 fluorescence associated with the fusion protein was detected along the chromosome
434 axes of bivalent chromosomes, a distribution that is similar if not identical to that of
435 wild-type NCAPH2. Remarkably, co-injection of MCPH1 mRNAs had no adverse
436 effect on this activity, unlike controls in which MCPH1 mRNAs were co-injected with
437 NCAPH2-GFP mRNAs (Figure 9B and C). Likewise, MCPH1 prevented association of
438 NCAPH2-GFP with chromosomes but not that of the SMC2-NCAPH2-GFP fusion
439 (Figure 9C).

440 A caveat to this experiment is that the resistance to MCPH1 of the SMC2-
441 NCAPH2-GFP fusion could be due to the linker sequences associated with the N- and
442 C-termini of NCAPH2 and SMC2 respectively rather than their stable inter-connection
443 *per se*. If the latter were the case, cleavage of the linker using TEV protease should
444 restore sensitivity to MCPH1. We therefore repeated the rescue experiment but, in this
445 case co-injected mRNA encoding TEV protease (Figure 9D). Quantification of the
446 chromosomal GFP fluorescence showed that the fusion's resistance to MCPH1
447 activity is abolished by the TEV (Figure 9E and F), which is consistent with the notion
448 that resistance arises from connecting the interface between SMC2 and NCAPH2.

449

450 ***Mcph1* deletion induces the coiling of cohesin vermicelli.**

451 Our results suggest that condensin II's association with chromosomal DNA might be
452 regulated by MCPH1 through a mechanism that resembles that of cohesin by WAPL.
453 By inducing cohesin's dissociation from chromatin, albeit only rarely approximately
454 every 15 min., WAPL merely moderates the processivity of loop extrusion mediated
455 by cohesin. MCPH1 has a more drastic effect, preventing most condensin II
456 complexes from ever associating stably with chromatin. The entire architecture of
457 interphase chromatin therefore depends on these two key regulatory factors. Though
458 condensin II is presumed like cohesin to act as a DNA loop extruder, the deregulation
459 of cohesin and condensin II induces different chromosomal morphologies. *Wapl*
460 deletion enables cohesin to form thread-like structures and to accumulate along their
461 longitudinal axes, creating so called vermicelli. In contrast, *Mcph1* deletion enables
462 condensin II to produce soft spherical or "gumball" chromosomes and to associate
463 stably throughout chromosomal DNA, albeit at high levels at centromeres. Strangely,
464 condensin II does not form or accumulate along the sort of axes observed when wild
465 type cells enter mitosis. Thus, the activities of condensin II and cohesin unleashed by
466 *Mcph1* and *Wapl* deletion respectively produce DNA loops with very different
467 arrangements. We therefore set out to address two questions. First, is this difference
468 intrinsic to differences in the behaviour of cohesin and condensin II or merely due to
469 differences in the type of cell used, namely mouse fibroblasts and ES cells? Assuming
470 that it is in fact, the former, what happens when both factors are deregulated
471 simultaneously? To this end, we altered the *Mcph1* and *Wapl* genes in E14 cells in
472 which SCC1 is tagged with Halo and NCAPH2 with GFP. Because *Wapl* deletion is
473 lethal, we generated a tamoxifen-inducible deletion allele, which enabled us to
474 compare chromosomal DNA morphology as well as localisation of SCC1-Halo and

475 NCAPH2-GFP in four different conditions: Wild type, $\Delta Wapl$, $\Delta Mcph1$ and the double
476 mutation $\Delta Wapl$, $\Delta Mcph1$ (Supplementary figure 5).

477 In wild-type cells, SCC1-Halo accumulates throughout nuclei and their
478 genomes during interphase and apart from centromeres, is largely removed from
479 chromosomes through the action of WAPL in M phase. Condensin II's distribution
480 resembles that of cohesin throughout most of interphase (Figure 2 and ⁸). Condensin
481 II accumulates around centromeres during G2 and along the chromatid axes created
482 through its activity during prophase (Figure 10, WT).

483 As previously reported for fibroblasts, *Wapl* deletion in E14 cells causes
484 cohesin to create chromatid-like structures, especially during G2, and to accumulate
485 along their longitudinal axes ². Because the majority of SCC1 remains on
486 chromosomes during mitosis, most is cleaved by separase during anaphase and
487 daughter cells inherit considerably less cohesin than normal. As a consequence, axial
488 cohesin vermicelli are rarely if ever observed during G1, especially as this cell cycle
489 phase is very short in ES cells. Pronounced vermicelli are only observed in G2. As
490 expected, *Wapl* deletion neither alters condensin II's distribution during interphase nor
491 hinders its accumulation along chromatid axes during mitosis. Despite cohesin's
492 persistence on mitotic chromosomes and the participation of a large fraction in sister
493 chromatid cohesion, condensin II still manages to localise to and help create the axes
494 of individual chromatids, between which run inter-chromatid axes coated in cohesin
495 (Figure 10A and B, $\Delta Wapl$). There are presumably two pools of chromosomal cohesin
496 in post-replicative *Wapl* deleted cells, one involved in cohesion and a second engaged
497 in the loop extrusion responsible for the formation of vermicelli. The former clearly
498 persists and accumulates along the inter-chromatid axis when loop extrusion mediated
499 by condensin I and II individualise chromatids, but the fate of the latter is unclear.

500 Deletion of *Mcph1* had little effect on cohesin's distribution. Despite the
501 formation of gumball chromosomes during G1 and G2, cohesin remains uniformly
502 associated with chromatin and does not form vermicelli. As in wild type cells, most
503 dissociates from chromosome arms when cells enter mitosis and little can be detected
504 along the inter-chromatid axes connecting the two condensin II axes of individualised
505 chromatids (Figure 10, $\Delta Mcph1$).

506 Deletion of both *Wapl* and *Mcph1* had a dramatic effect. The cohesin vermicelli
507 caused by the lack of WAPL in G2 cells adopt a coiled configuration upon the
508 simultaneous deletion of *Mcph1*. This coiling increases during prophase. By the time
509 cells reach metaphase, the coiling leads to the formation of chromosomes that have
510 the shape of a spring (or solenoid), a configuration that is visible with SCC1-Halo,
511 NCAPH2-GFP and DNA staining (DAPI) (Figure 10, $\Delta Wapl$, $\Delta Mcph1$). Moreover, the
512 two distinct axes of condensin II associated with each chromatid remain intermingled
513 in the double mutant.

514 To analyse the axial organisation of these chromosomes in greater detail, we
515 used super-resolution three-dimensional structured illumination microscopy (3D-SIM)
516 to compare the distribution of SCC1-Halo in *Wapl* deleted and double mutant cells.
517 Unfortunately, fluorescence due to NCAPH2-GFP was insufficient to reveal reliable
518 images using this technique. Analysis of G2 cells revealed that a modest coiling of
519 cohesin axes surrounded by DNA loops in *Wapl* single mutants is greatly accentuated
520 in double-mutant cells, with a pronounced increase in the radii of coils (Figure 11 and
521 Supplementary video 1). In metaphase cells, the cohesin axes that seem to have a
522 spring-like appearance in confocal microscopy are revealed to have a much more
523 complicated organisation, being composed of twisted segments that regularly change
524 handedness. It is noticeable that the formation of chromosomes with this morphology

525 is not simply due to the combined activity/presence of cohesin and condensin II during
526 mitosis, which also occurs in *Wapl* single mutants. It only arises when both cohesin
527 and condensin were stably associated with chromatin during G2.

528 We conclude that combining the abnormal activity of condensin II unmasked by
529 deleting *Mcp1* with that of cohesin unmasked by deleting *Wapl* leads to a major
530 transformation of chromosome structure when cells enter G2, that is associated with
531 coiling of the entire axis of the chromosome. Interestingly, this coiling does not have a
532 handedness that persists throughout the chromosome in metaphase. Instead, the
533 chromosome appears divided into segments whose axes are coiled with alternating
534 handedness.

535

536 **DISCUSSION**

537 Despite accumulation within interphase nuclei, condensin II associates with chromatin
538 only fleetingly if at all and exerts little or no effect on chromosome topology during this
539 phase of the cell cycle. It normally only associates stably with chromosomal DNA and
540 organises it into a series of loops when cells enter M phase. The restriction of
541 condensin II's activity to M phase was previously attributed to its phosphorylation by
542 CDK1^{27,40,41,43}. Our finding that in the absence of MCPH1, condensin II is capable of
543 transforming the topology of chromosomal DNA in cells arrested in G2 using a CDK1
544 inhibitor implies that condensin II is capable of substantial activity in the absence of
545 CDK1-mediated phosphorylation normally associated with M phase. In other words, it
546 is MCPH1 that prevents condensin II's association with chromosomes during G2 not
547 the lack of CDK1 phosphorylation.

548 Our observation that phosphorylation of a CDK1 consensus sequence
549 abolishes association between a conserved and essential SLiM within MCPH1's

550 central domain and condensin II's NCAPG2 subunit raises the possibility that CDK1
551 exerts at least part of its effect by preventing MCPH1's association with condensin II.
552 Our conclusion that the SLiM within MCPH1's central domain is essential for its
553 inhibitory activity contradicts the claim that it is not necessary, an inconsistency that
554 we attribute to the fact that previous studies tested the function of over-expressed
555 MCPH1 alleles ^{33,54}.

556 Our findings as well as those of others ^{30,31,35} show that MCPH1 is responsible
557 for inhibiting condensin II during G1 as well as G2 phase. Thus, in the absence of
558 MCPH1, condensin II organises chromosomal DNAs into chromatid-like structures
559 during G1 and G2 but strikingly not during S phase when some other (MCPH1-
560 independent) mechanism prevents it from associating stably with chromatin.

561 MCPH1's inhibition of condensin II depends on its N-terminal BRCT domain in
562 addition to its central SLiM. Indeed, most of *McpH1* mutations identified in
563 microcephaly patients affect the BRCT domain, which possibly interacts with some
564 other part of condensin II once MCPH1 has been recruited via its SLiM. However, the
565 function of this domain remains mysterious.

566 How does MCPH1 inhibit condensin II? An important clue stemmed from the
567 numerous similarities between MCPH1 and WAPL, a protein that facilitates cohesin's
568 release from chromatin ¹. WAPL binds to STAG, the cohesin subunit equivalent to
569 NCAPG2, using a SLiM and its inactivation leads to cohesin's stable association with
570 chromatin ^{2,7,55}. Cohesin release mediated by WAPL involves dissociation of the NTD
571 of cohesin's SCC1 kleisin subunit from the coiled coil that emerges from SMC3's
572 ATPase head, known as its neck ^{18,19,53}. It is currently thought that kleisin-neck
573 dissociation takes place, albeit rarely, upon engagement of cohesin's SMC1 and
574 SMC3 ATPase heads in the presence of ATP when SMC3 is unacetylated and in the

575 absence of SCC2^{18,19,56,57}. Crucially, the fusion of SMC3's C-terminus to SCC1's N-
576 terminus completely blocks WAPL from triggering cohesin's release from chromatin
577^{19,53}. It could do so either by creating a barrier to the passage of DNA through an
578 opened kleisin-neck interface, i.e. by blocking the exit of DNAs previously entrapped
579 within cohesin rings, or merely by hindering kleisin-neck dissociation, which has some
580 other poorly understood function necessary for release.

581 Our finding that an analogous fusion, between the C-terminus of SMC2 and the
582 N-terminus of NCAPH2, prevents the release of condensin II from meiosis I
583 chromosomes in oocytes upon over-expression of MCPH1 suggests that MCPH1
584 prevents condensin II's association with chromatin by a mechanism that is similar to
585 cohesin's release by WAPL. It has been reported that engagement of SMC2 and
586 SMC4 heads triggered by ATP binding induces the release of the NTD of the kleisin
587 from SMC2's neck^{21,58}, raising the possibility that MCPH1 blocks condensin II's stable
588 association with chromatin by facilitating such a process. Irrespective of the actual
589 mechanics, which remains poorly understood, our observations emphasise that
590 MCPH1 blocks condensin II's association with chromosomes using a mechanism
591 similar to that that employed by WAPL to cause cohesin release.

592 Despite these striking similarities, the mode of action of these two proteins may
593 differ in an important respect. Though WAPL alters cohesin's residence time on
594 chromatin exclusively by facilitating release, our finding that over-expression of
595 MCPH1 in oocytes prevents *de novo* association of condensin II but does not remove
596 complexes previously associated with chromosomes raises the possibility that MCPH1
597 acts predominantly by aborting *de novo* loading.

598 Recent advances have shown that both human condensin and cohesin
599 complexes extrude DNA loops^{5,59-61}, and cryo-electron microscopy structures of yeast

600 condensin, and yeast and human cohesin are starting to provide insight into how these
601 complexes engage with DNA ^{58,62–64}. In the case of cohesin, it has been suggested
602 that an early step is the clamping of DNA on top of SMC1 and SMC3 ATPase domains
603 that have engaged with each other in the presence of ATP. Clamping in this manner
604 requires cohesin's SCC2 HAWK protein, which is equivalent to condensin II's
605 NCAPD3. SCC2 is necessary to prevent cohesin's release from chromatin and may
606 perform this function by preventing dissociation of its kleisin subunit from SMC3's neck
607 ^{57,62}. If NCAPD3 had a similar role, then MCPH1 could conceivably block condensin
608 II's association with chromatin by interfering with NCAPD3's ability to block the release
609 of NCAPH2 from SMC2.

610 One of the earliest insights into the cell division cycle was that one of the main
611 constituents of the nucleus undergoes a dramatic morphological transformation shortly
612 before division, namely the transformation of an apparently amorphous mass of
613 chromatin into thread-like structures now known as chromosomes, each composed of
614 a pair of chromatids joined together. It is now recognised that this transformation is
615 brought about by condensins I and II which act by extruding DNA loops. It is also
616 recognised that interphase chromosomal DNA is not in fact amorphous but instead
617 characterised by a complex and dynamic network of interactions known as
618 topologically associated domains or TADs, which are created by cohesin that like
619 condensin is a DNA loop extruder, albeit one that is active during interphase and
620 blocked by the site specific DNA binding protein CTCF ^{55,65}.

621 There are two reasons why DNAs are not organised into thread-like chromatids
622 during interphase. By causing cohesin release, WAPL prevents loop extrusion by
623 cohesin going to completion while MCPH1 prevents condensin II associating with DNA
624 stably and thereby extruding loops. Crucially, inactivation of either protein leads to the

625 formation of chromatid-like structures during interphase, albeit by different loop
626 extruders and with different actual morphologies. Interestingly, depletion of both
627 proteins simultaneously leads to a major transformation of chromosome structure as
628 cells enter mitosis, which is associated with coiling of the entire axis of the
629 chromosome. Understanding how and why this comes about through the unregulated
630 activities of cohesin and condensin II may help reveal further insight into how loop
631 extrusion creates chromosomes.

632

633 **Glossary:**

634 HAWK, HEAT repeat protein associated with the kleisin

635 LE, loop extrusion

636 PLC, prophase like cells

637 SLiM, short linear motif

638 TADs, topologically associating domains

639 Cryo EM, cryo electron microscopy

640 AFM, atomic force microscopy

641 GVBD, germinal vesicle break down

642

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655

656 **Author contribution**

657 MH design, performed the experiments, wrote the manuscript and coordinated the
658 project. EC designed, performed all the *in vitro* biochemistry experiments and wrote
659 the manuscript. JG provided advice for the manipulation of oocytes. LS acquired and
660 analysed the SIM data. APA performed the Hi-C experiments. ELA supervised HiC
661 experiments and wrote the corresponding section. MSS and DW processed and
662 analyzed the Hi-C data. AV supervised the project and wrote the manuscript. KN
663 supervised the project and wrote the manuscript.

664

665 **Competing financial interests**

666 The authors declare no competing financial interests.

667

668

669

670 **FIGURE LEGENDS**

671 **Figure 1.** *The deletion of Mcph1 in E14 cells induces condensin II-dependent*
672 *chromosome condensation in both G1 and G2 phases of the cell cycle. (A)* In gel TMR-
673 Halo detection and Western blot analysis of E14 cells wild type, *Ncaph2^{Halo/Halo}* and
674 *Ncaph2^{Halo/Halo} Mcph1^{ΔΔ}*. TMR signal detects NCAPH2-Halo tagged. The anti-
675 NCAPH2 antibody shows that all the NCAPH2 protein expressed is fused to the Halo-
676 tag and that the expression levels are similar to wild type but reduced after *Mcph1*
677 deletion. The anti-SMC2 detection shows similar levels of condensin in the three cell
678 lines. **(B)** Immunofluorescence analysis of Histone H3 phosphorylated on serine 10
679 (green) combined with TMR detection of NCAPH2-Halo (Red) in *Mcph1^{wt/wt}* and
680 *Mcph1^{ΔΔ}* cells. The DNA organization was analysed using Hoechst. **(C)** EdU
681 incorporation in *Mcph1^{wt/wt}* or *Mcph1^{ΔΔ}* cells. **(D)** Western blot analysis of Halo-
682 PROTAC induced NCAPH2-Halo degradation in wild-type, *Ncaph2^{Halo/Halo} Mcph1^{wt/wt}*
683 and *Ncaph2^{Halo/Halo} Mcph1^{ΔΔ}* cells using an anti-NCAPH2 antibody. Anti-SCC1 was
684 used as a loading control. **(E)** Immunofluorescence analysis of the chromosome
685 decompaction induced by 16 h treatment of *Mcph1^{ΔΔ}* cells with Halo-PROTAC.
686 Immunofluorescence analysis of Histone H3 phosphorylated on serine 10 (green) was
687 used to compare similar cell cycle stages. Scale bar, 5 μm.

688

689 **Figure 2.** *Mcph1 deletion induces the stable binding of condensin II to the condensed*
690 *chromosomes in interphase.* FRAP analysis of NCAPH2-Halo turn-over on chromatin
691 in *Mcph1^{wt/wt}* **(A)** and *Mcph1^{ΔΔ}* cells **(B)**. Top row: NCAPH2-Halo signal pre-bleach,
692 post bleach and after 70 sec recovery. The region bleached correspond to the white
693 circle. Scale bar, 5 μm. **(C)** Quantification of the fluorescence recovery after
694 photobleaching over a 10 min post-bleach period. (Average of three experiments, total

695 number of cells analysed: WT 28 cells, *Mcp1*^{ΔΔ} 30 cells, standard deviation is
696 represented for every time point)

697

698 **Figure 3.** *CDK1 activity is not required for the condensation phenotype induced by*
699 *Mcp1 deletion.* **(A)** Western blot analysis of CDK1 phosphorylation on tyrosine 15 in
700 wild type cells compared to *Mcp1* deleted cells. Wild-type protein extracts were
701 treated by λ phosphatase as a control of antibody specificity. An anti-CDK1 protein
702 was used as a loading control. **(B)** Immunolocalisation of Cyclin B in *Mcp1* deleted
703 cells. **(C)** CDK1 activity was inhibited by incubating wild-type or *Mcp1* deleted cells
704 to RO-3306 for 7 h. The cell cycle profile was analysed by FACS for both wild-type
705 and *Mcp1* deleted cells without treatment or after 7 h incubation with 9 μM RO-3306.
706 **(D)** All the G2 cells in *Mcp1* deleted cells present the condensed phenotype after
707 CDK1 inhibition. Scale bar, 5 μm.

708

709 **Figure 4.** *Mcp1 deletion causes chromosome compaction and loss of*
710 *chromocenters.* **(A)** Representative subset of interactions between chromosomes 1-7
711 for wild-type and *Mcp1* deletion maps (wild-type below diagonal) shows loss of
712 chromocenters in the *Mcp1* deletion maps. **(B)** Log fold change for *Mcp1* deletion
713 over wild-type for chromosomes 1-7. **(C)** Log fold enrichment of the *Mcp1* deletion
714 map over wild-type map for the aggregated inter-chromosomal matrix. **(D)** Balanced
715 KR-normalized Hi-C Maps for wild-type and *Mcp1* deletion maps for the
716 intrachromosomal region of chromosome 1 (wild-type below diagonal). Increased
717 interactions between distant loci in the intrachromosomal *Mcp1* deletion maps is
718 seen. Color scale threshold is at the average value of each respective Hi-C map. **(E)**
719 Intrachromosomal contact probability for all chromosomes shows increased long-

720 range interactions and diminished contact drop-off for *McpH1* deletion. **(F)** Average
721 intrachromosomal contact frequency for all chromosomes shows increased long-
722 range interactions with *McpH1* deletion.

723

724 **Figure 5. Human condensin II interaction with MCPH1. (A)** Domain structure of
725 MCPH1 and MBP fusion constructs that were expressed in *E. coli* and used in binding
726 assays. BRCT domains are indicated in green and the central domain in yellow. **(B)**
727 Conservation analysis of the MCPH1 central domain with the ConSurf server. **(C)**
728 Strep tag pull-down indicating full length MCPH1 binds condensin II. Full length
729 MCPH1 and condensin II were expressed in insect cells and separately purified,
730 before being mixed on strep-tactin sepharose. Samples of input and resin after run on
731 SDS page and visualised with silver stain. **(D)** Strep-tag pull-down assay indicating
732 strep tagged condensin II pulls down MBP-MCPH1 constructs that contain the central
733 domain, but not MBP-MCPH1₁₋₁₉₅ or MBP alone. SDS page gel visualised with
734 Coomassie stain. **(E)** Strep pull-down assay showing strep-tagged pentameric
735 condensin II or tetrameric condensin II lacking NCAPD3 can pull down MBP-MCPH1₁₋
736 ₄₃₅, while tetrameric condensin lacking NCAPG2 does not pull down MCPH1. The
737 lower panel shows a western blot performed using strep-resin samples, blotted using
738 an anti-NCAPD3 antibody. **(F)** Fluorescence polarization binding assay using 5-FAM
739 labelled MCPH1₄₀₇₋₄₂₄ peptide and increasing concentration of either pentameric
740 condensin or tetrameric condensin II lacking MCPH1 binding subunit NCAPG2
741 (CIIΔG2). **(G)** Peptide competition assay using a fixed concentration of 5-FAM labelled
742 MCPH1₄₀₇₋₄₂₄ and condensin II with an increasing amount of MCPH1₄₀₇₋₄₂₄ wild-type
743 or phosphorylated at serine 417. All error bars indicate standard deviation from three
744 replicates.

745

746 **Figure 6.** *MCPH1* interaction with condensin II is essential to prevent interphasic

747 chromosome condensation. **(A)** Co-immunoprecipitation of MCPH1 with NCAPH2-

748 GFP. Nuclear extracts were prepared from wild type, *Ncaph2*^{GFP/GFP} and

749 *Ncaph2*^{GFP/GFP} *Mcph1*^{Δ/Δ} cells. Immunoprecipitation was performed using GFP-trap

750 agarose beads and analysed by western blot using an anti-MCPH1 antibody (IP-GFP).

751 5% of the lysate used for IP was loaded as INPUT control. Anti-Lamin B1 antibody

752 was used as a loading control. **(B)** Deletion of the central domain of MCPH1. To

753 address if the central domain of MCPH1 is necessary to mediate the interaction with

754 condensin II, we first introduced a GFP-tag at the C-terminal end of MCPH1 in

755 *Ncaph2*^{Halo/Halo} cells as the antibody against the protein was raised against the central

756 domain. Then a second targeting was done to delete the central domain. As a result,

757 the western blot represented in panel B using anti-MCPH1 antibody detects the wild-

758 type protein or the GFP-tagged protein, homozygous *Mcph1*^{GFP/GFP} but does not detect

759 anything after deletion of the central domain. Using anti-GFP antibody reveals that the

760 protein deleted for the central domain is present in the cell at similar levels as the wild-

761 type GFP-tagged protein. A slight decrease in size is observed due to the deletion of

762 the central domain. Anti-Lamin B1 antibody was used as a loading control. **(C)** Co-

763 immunoprecipitation of NCAPH2-Halo with MCPH1-GFP. Nuclear extracts were

764 prepared from *Ncaph2*^{Halo/Halo}, *Ncaph2*^{Halo/Halo}*Mcph1*^{GFP/GFP} and

765 *Ncaph2*^{Halo/Halo}*Mcph1*^{ΔcenGFP/ΔcenGFP} cells. Immunoprecipitation was performed using

766 GFP-trap agarose beads and analysed by in-gel detection of NCAPH2-Halo using the

767 Halo-ligand TMR or by western blot using an anti-NCAPD3 antibody (IP-GFP). 5% of

768 the lysate used for IP was loaded as input control. Anti-Lamin B1 antibody was used

769 as a loading control. **(D,E)** Immunofluorescence analysis of the chromatin organisation

770 in *Ncaph2^{Halo/Halo}Mcph1^{GFP/GFP}* and *Ncaph2^{Halo/Halo}Mcph1^{ΔcenGFP/ΔcenGFP}* cells. MCPH1-
771 GFP is only detected in the cell nucleus enriched in dots colocalising with some γ H2AX
772 foci (D). The deletion of its central domain induces a similar condensation of
773 interphasic chromosomes as the one observed after the complete loss of function of
774 *Mcph1* (E).

775

776 **Figure 7.** *MCPH1 has little effect on condensin II ATP hydrolysis and DNA binding.*

777 **(A)** ATPase rate of condensin II complex in the presence of MCPH1. Q refers to
778 condensin II with an ATPase deficient mutation in the Q-loop. Below is an SDS page
779 gel of the completed reaction. Error bars indicate standard deviation from three
780 repeats. **(B)** EMSA assay of MBP, MCPH1₁₋₄₃₅-MBP and -MCPH1₁₉₅₋₄₃₅-MBP using
781 50 bp of Cy5 labelled dsDNA. **(C)** EMSA assay of condensin II in the presence of MBP
782 or MCPH1₁₋₄₃₅-MBP. **(D)** EMSA assay of condensin II in the presence or absence of
783 5FAM-MCPH1 peptide. Top image detecting Cy5 and bottom imaged detecting 5FAM.
784

785 **Figure 8.** *MCPH1 prevents the association of condensin II with chromosomes. (A)*

786 Cartoon summarizing the experimental procedure corresponding to panel B. **(B)** Wild-
787 type mouse oocytes were injected at the GV stage with *in vitro* transcribed mRNA
788 coding for H2B-mCherry alone to mark the chromosomes in magenta (Control) or in
789 combination with MCPH1 (+MCPH1). Meiosis I progression was followed by live cell
790 confocal imaging. The segregation defects observed in the presence of MCPH1 are
791 indicated by a yellow arrowhead. Maximum intensity z projection images of the main
792 time points are shown between 3.3 h post GVBD onwards (number of oocytes
793 analysed in three independent experiments: control :12; +MCPH1: 19). **(C)** Cartoon
794 summarizing the experimental procedure corresponding to panel D. **(D)** Oocytes from

795 *Ncaph2^{ff} Tg(ZP3Cre)* females were injected at the GV stage with mRNA coding for
796 H2B-mCherry, MAD2 and NCAPH2-GFP only (control) or in combination with MCPH1
797 (+MCPH1) or MCPH1 deleted of the first N-terminal 200 amino acids (+MCPH1 Δ 200).
798 Oocytes were arrested 16 h after GVBD in metaphase I owing to MAD2
799 overexpression, and maximum-intensity z projection images of chromosomes were
800 acquired by live cell confocal imaging (Total number of oocytes analysed in three
801 experiments: control: 37, +MCPH1: 58, +MCPH1 Δ 200: 12). **(E)** Quantification of
802 NCAPH2-GFP signal on the chromosomes. (Number of oocytes analysed in two
803 independent experiments: control: 11; +MCPH1: 14). Scale bar, 5 μ m

804

805 **Figure 9.** *The closure of the SMC2-NCAPH2 interface prevents MCPH1 inhibitory*
806 *effect. (A)* Schematic representation of the protein fusion between SMC2 C-terminus
807 and the N-terminus of NCAPH2 using a linker comprising three TEV protease
808 cleavage sites. **(B)** Cartoon summarizing the experimental procedure corresponding
809 to panel C. **(C)** Oocytes from *Ncaph2^{ff} Tg(ZP3Cre)* females were injected at the GV
810 stage with mRNA coding for H2B-mCherry and MCPH1 in combination with NCAPH2-
811 GFP (NCAPH2-GFP+MCPH1) or with the fusion (Fusion-GFP+MCPH1). Meiosis I
812 progression was followed by live cell confocal imaging. Maximum intensity z-
813 projections images of the time points corresponding to anaphase I when segregation
814 defects are observed (total number of oocytes analysed in three experiments:
815 NCAPH2-GFP+MCPH1: 17, Fusion-GFP+MCPH1: 18). **(D)** Cartoon summarizing the
816 experimental procedure corresponding to panel E. **(E)** Oocytes from *Ncaph2^{ff}*
817 *Tg(ZP3Cre)* females were injected at the GV stage with mRNA coding for H2B-
818 mCherry, MAD2 and Fusion-GFP only or in combination with TEV protease. Oocytes
819 were arrested 16 h after GVBD in metaphase I owing to MAD2 over-expression and

820 maximum-intensity z-projection images of chromosomes were acquired by live cell
821 confocal imaging. **(F)** Quantification of Fusion-GFP signal on the chromosomes (Total
822 number of oocytes analysed in three experiments: Fusion: 9; Fusion + MCPH1: 14,
823 Fusion + TEV: 13, Fusion + TEV + MCPH1: 18). Scale bar, 5 μm .

824

825 **Figure 10.** *Mcph1* deletion induces the coiling of the vermicelli. **(A)**
826 Immunofluorescence analysis of the chromatin organisation in the four conditions:
827 wild-type, $\Delta Wapl$, $\Delta Mcph1$ and $\Delta Wapl \Delta Mcph1$. In order to compare cells that are in
828 G2, prophase or metaphase, Histone H3-serine 10 (cyan) was used as a cell cycle
829 marker. The localization of SCC1-Halo was analysed using Halo-JFX554, NCAPH2-
830 GFP using nanobodies and DNA was detected using DAPI. **(B)** Magnified view of cells
831 marked with a white star in panel A. Scale bar, 5 μm .

832

833 **Figure 11.** *Super-resolution 3D-SIM analysis of G2 and metaphase cells deleted for*
834 *Wapl or both Mcph1 and Wapl.* **(A)** Cells deleted for $\Delta Wapl$ or $\Delta Wapl + \Delta Mcph1$ in G2
835 or metaphase (M) were analysed by 3D-SIM. Maximum intensity projections of 16
836 consecutive mid sections covering 2 μm in depth. The left panel shows DNA coloured in
837 magenta and SCC1-Halo in green. The right panel shows the SCC1 signal with z-depth
838 colour-coded. Scale bar, 5 μm (inset, 1 μm) **(B)** Representative SCC1-Halo solenoid
839 structure corresponding to one chromosome from a $\Delta Wapl + \Delta Mcph1$ cell in G2 was
840 segmented (green) and overlaid to the DNA (magenta). Scale bar, 2 μm . **(C)** 3D
841 surface rendering of the segmented and isolated solenoid from panel B. View from the
842 top, right, left and bottom of one segmented solenoid. Scale bar, 1 μm .

843

844

845 **SUPPLEMENTARY FIGURE LEGENDS**

846

847 **Supplementary figure 1.** *The cell cycle parameters are unchanged in Mcph1 deleted*
848 *cells.* FACS analysis of the cell cycle parameters of *Mcph1* deleted cells compared to
849 wild type: **(A)** Analysis of the DNA content using propidium iodide (repeated twice).
850 **(B)** EdU incorporation (repeated twice). **(C)** H3 Phosphorylation on serine 10
851 (repeated twice). **(D)** Western blot analysis of the amount of γ H2AX and H3
852 phosphorylation on serine 10 in wild-type cells compared to *Mcph1* deleted cells. Scale
853 bar, 5 μ m.

854

855 **Supplementary figure 2.** *Chromocenters disruption in Mcph1 deleted cells.*
856 Immunofluorescence analysis of centromere clustering using CREST antibody
857 showing that in *Mcph1* deleted cells, the centromeres are scattered in the nucleus
858 even in replicating, EdU-positive cells.

859

860 **Supplementary figure 3.** *Mcph1 deletion decreases intra-compartment strength and*
861 *does not affect looping.* **(A)** Pearson correlation map at 250 kB for wildtype and *Mcph1*
862 deletion maps for the intrachromosomal region of chromosome 11 (wildtype below
863 diagonal). **(B)** Pearson correlation map at 250 kB for wildtype and *Mcph1* deletion
864 maps for the intrachromosomal region of chromosome 11 sorted by values of the
865 principal eigenvector (wildtype below diagonal). Intra-compartmental interactions (A-
866 A and B-B) vs inter-compartment interactions (A-B) show a relative decrease in the
867 *Mcph1* deletion maps. **(C)** Aggregate peak analysis using the loop lists for both
868 wildtype and *mcph1* deletion on both Hi-C maps shows no significant global change
869 to chromatin looping, which is also verified by direct observation of the maps.

870

871 **Supplementary figure 4.** *Condensin I does not interact with MCPH1.* **(A)** Strep-tag
872 pull-down assay indicating strep tagged condensin I does not pull-down any MBP-
873 MCPH1 construct. SDS page visualised with Coomassie stain for input samples and
874 silver stain for resin samples. **(B)** Condensin II and MBP-MCPH1₁₉₆₋₄₃₅ form a stable
875 complex and co-elute in size exclusion chromatography. Elution profiles of condensin
876 II + MBP-MCPH1₁₉₆₋₄₃₅, condensin II alone and MBP-MCPH1₁₉₆₋₄₃₅ alone are shown
877 in green, blue and red respectively. Fractions from void, condensin II and MCPH1
878 peak were run on an SDS page and stained with Coomassie. **(C)** Sequence
879 conservation map of MCPH1₁₋₄₃₅ generated using the ConSurf server^{46,47}, with the
880 central motif indicated. Colours range from non-conserved (green) to conserved
881 (purple), 'b' indicates residues predicted to be buried and 'e' indicates residues
882 predicted to be solvent-exposed.

883

884 **Supplementary figure 5.** *Western blot analysis of the four conditions analysed in*
885 *Figures 10 and 11.* The first cell line is: *Ncaph2*^{GFP/GFP} *Sccl*^{Halo/Halo} *Wapl*^{TevLox/Δ}
886 *McpH1*^{wt/wt}. The second cell line is: *Ncaph2*^{GFP/GFP} *Sccl*^{Halo/Halo} *Wapl*^{TevLox/Δ} *McpH1*^{Δ/Δ}.
887 *Wapl* can be deleted in both cell lines after Tamoxifen treatment giving four
888 experimental conditions: Wild type, Δ*Wapl*, Δ*McpH1* and Δ*Wapl* Δ*McpH1*.

889

890 MATERIAL AND METHODS

891 Mouse strain, *in vitro* culture and oocytes micro-injection.

892 *Ncaph2*^{tm1a(EUCOMM)Wtsi} (MBCH;EPD0070-2-G090) were obtained from the Wellcome
893 Trust Sanger Institute. The corresponding *lox* allele was obtained as previously
894 described⁵². The fusion was obtained by cloning in frame *Smc2* cDNA, a linker
895 containing three TEV protease cleavage sites
896 (GGGGSGGGSGGGGTGSENYFYQGPRENYFYQGGSENYFYQGTRGGGGSGGG
897 GSGGGG), *Ncaph2* cDNA (Origene, MC200537) and the eGFP ORF in the pUC19
898 vector.

899 Fully grown prophase-arrested GV oocytes were isolated and injected with mRNAs
900 (5–10 pl) diluted in RNase-free water at the following concentrations: H2B–mCherry:
901 150 ng μl^{-1} , Mad2: 200 ng μl^{-1} , NCAPH2–eGFP: 50 ng μl^{-1} , Fusion-EGFP (50 ng μl^{-1}),
902 MCPH1 (200 ng μl^{-1}), TEV protease: 250 ng μl^{-1} . All experimental procedures were
903 approved by the University of Oxford ethical review committee and licensed by the
904 Home Office under the Animal (Scientific procedures) Act 1986. No statistical method
905 was used to predetermine sample size. The experiments were not randomised and
906 the investigators were not blinded to allocation during experiments and outcome
907 assessment.

908 Live cell confocal imaging.

909 Oocyte live cell imaging was done in 4-well Labtek chambers (ref. 155383) in 4 μl
910 drops of M16 medium covered with mineral oil (Sigma) in a 5% CO₂ environmental
911 microscope incubator at 37 °C (Pecon). Images were acquired using an LSM-780
912 confocal microscope (Zeiss) using the ZEN 2011 software. Between 7 and 12 slices
913 (between 1 and 4 μm) were acquired every 5 to 15 min for each stage position using

914 the autofocus tracking macro developed in J. Ellenberg's laboratory at EMBL. For
915 detection of eGFP and mCherry, 488-nm and 561-nm excitation wavelengths and
916 MBS 488/561 filters were used. Images were further analysed using Volocity software.
917 For high-resolution videos, the lens used was a C-Apochromat ×63/1.20 W Corr UV–
918 VIS–IR. The fluorescence was quantified using Fiji.

919 **E14 mouse embryonic stem cells culture**

920 E14 mouse embryonic stem cells were grown in Dulbecco's modified Eagle's medium
921 (DMEM; Life Technologies) supplemented with 10% foetal calf serum (Seralab),
922 2 mM L-glutamine (Life Technologies), 1x non-essential amino acids (Life
923 Technologies), 50 µM β-mercaptoethanol (Life Technologies), 1X penicillin-
924 streptomycin solution (Life Technologies) and leukaemia inhibitory factor (LIF) made
925 in-house. All E14 cells were grown in feeder-free conditions on gelatinised plates at
926 37 °C in a humid atmosphere with 5% CO₂.

927 For conditional deletion of *Wapl*, Cre recombinase was induced by treating the cells
928 with 800 nM 4-hydroxytamoxifen (OHT) for the indicated time. The degradation of
929 NCAPH2-HALO was triggered by adding HaloTag® PROTAC Ligand (Promega) at
930 1µM for 16 hours.

931 The Halo Ligands (Halo-TMR Ligand, Promega, Ref G8251) were added in the culture
932 medium for 20 min at 100 nM. Cells were washed and left in the incubator with fresh
933 medium for an extra 30 min to remove the unbound ligand before being analysed by
934 immunofluorescence or western blot.

935

936 **Genomic engineering using CRISPR Homology-directed repair**

937 The sgRNAs were designed using the CRISPOR online tool
938 (<http://crispor.tefor.net/crispor.py>) and cloned in the pSptCas9(BB)-2A-Puro(PX459)-
939 V2.0 vector (Addgene #62988). The sgRNA cloning was done according to the
940 protocol from ⁶⁶.

941 To generate the targeting constructs, 1 kb homology arms were amplified by PCR (Q5-
942 NEB) from E14 cells genomic DNA and cloned in pUC19 vector using Gibson
943 Assembly Master Mix kit (New England Biolabs). The targeting construct was
944 designed such that the guide RNA sequence used for the specific targeting was
945 interrupted by the tag or contained silent mutations.

946 For each targeting, a 6 cm dish of E14 cells 50% confluent was transfected using 2 μ g
947 of pX459-Cas9-sgRNA and 5 μ g of targeting construct using Lipofectamine 2000
948 (ThermoFisher) according to manufacturer's guidelines. The next day, cells were
949 trypsinised and plated at three different densities in 20 cm dishes in medium
950 supplemented with puromycin (1 μ g/ml). The selection medium was removed 48 h
951 later and cells were grown for approximately ten days. 96 Individual clones were then
952 picked in 96 well plates, grown for 48 h and split into two 96 well plates. The next day,
953 genomic DNA was prepared using 50 μ l of Lysis buffer (10 mM Tris HCl pH8, 1 mM
954 EDTA, 25 mM NaCl, 200 μ g/ml Proteinase K), incubated at 65 °C for 1 h, 95 °C for 10
955 min to inactivate Proteinase K. The clones were then screened by PCR (Q5-NEB) and
956 amplified to be further analysed by western blot.

957

958 **Conditional Wapl deletion**

959 To generate the TEV protease conditional cells, a series of four tandem STOP
960 cassette (Addgene, pBS.DAT-LoxStop, Jacks Lab) flanked by two LoxP sites was
961 cloned between the pCAG promoter and the PK tagged TEV protease cDNA. The

962 CRE-ERT2 cDNA was cloned upstream under the transcriptional control of the
963 Rosa26 Splice acceptor. This construct was then flanked by 1 kb homology arms to
964 target the construct at the Rosa26 locus using CRISPR-HDR. After selecting and
965 amplifying of the targeted E14 clones, the TEV protease could be detected by western
966 blot 8 h post hydroxytamoxifen induction. Immunofluorescence analysis revealed a
967 homogeneous expression of TEV protease in all the cells.

968 In the selected clones, three TEV protease cleavage sites were targeted in *Wapl*
969 coding sequence after Proline 499 using CRISPR-HDR. After selecting of the targeted
970 clones, it appeared that TEV cleavage of the *Wapl* protein led to a new steady-state
971 in the cells in which a small amount of full-length protein was present preventing the
972 formation of vermicelli. To avoid this compensation effect, we targeted loxP sites in
973 the *Wapl* gene on both sides of exon 4 to induce the deletion of the gene
974 simultaneously as the TEV cleavage of the protein already present in the cell. This
975 combined strategy induced a complete loss of function of *Wapl* within 8 hours and the
976 formation of vermicelli.

977

978 **sgRNA used for CRISPR-HDR**

<i>Targeted Gene</i>	sgRNA
NCAPH2-GFP	GGTGGAAAGTAGTATATAACC
NCAPH2-Halo	GGTGGAAAGTAGTATATAACC
MCPH1 deletion Sg5'	GGTGTGCAATTCCTAGTGTG
MCPH1 deletion Sg3'	AGCTGTTCTTAGAACACGA
MCPH1-GFP	ACAGTGAGACATCTACAATG
STOP-TEV	CATGGATTTCTCCGGTGAAT
WAPL-Lox-TEV Sg5'	AATGGGTGCTTATAATTAGC

WAPL-Lox-TEV Sg3'	ACAATGTCACAATGGCTCAT
SCC1-Halo	ATAATATGGAACCGTGGTCC
DELCEN-MCPH1	CGTTGAGGCTTCTTCCTATG
TEV sites in Wapl Two Guide RNA used in combination	AATGGGTGCTTATAATTAGC ACAATGTCACAATGGCTCAT

979

980 **Immunofluorescence detection**

981 E14 cells were plated on glass coverslips (Marienfeld, High precision, 22x22, N°1.5H,
982 Ref 0107052) in 6-well plates. 48 h later, cells were labelled with the Halo Ligand
983 (Halo-JFX554, Janelia 100 nM 30 min)⁶⁷ or EdU (5 min at 10 μ M), washed PBS and
984 then fixed in 3.8% Formaldehyde (Sigma-F8775) in PBS for 15 min. After three PBS
985 washes, cells were permeabilised in 0.5% Triton X-100 in PBS for 10 min and then
986 washed three times in PBS. After 20 min in blocking buffer: PBS 3% BSA (Sigma,
987 A4503). The coverslips were transferred in a wet chamber and covered with 100 μ l of
988 antibody solution in blocking buffer. After 1 h at room temperature, the coverslips were
989 washed three times in blocking solution and incubated with the secondary antibody for
990 1 h (AlexaFluor 594 or 488, 1/500, Life Technology). After three washes in PBS, DNA
991 was labelled using Hoechst (Sigma, 33342, 5 μ g/ml) for 15 min. The coverslips were
992 then washed in PBS, mounted in Vectashield (H1000) and sealed using nail varnish.
993 Imaging was done using an LSM 780 confocal microscope (Zeiss) using the ZEN 2011
994 software. EdU was detected according to the manufacturer's instruction (Click-iT EdU
995 Imaging kit, Invitrogen). CDK1 inhibition was performed by incubating the cells in E14
996 medium supplemented with 9 μ M RO-3306 (SIGMA SML0569) for 7 h before fixation.

997

998 **Immunoprecipitation and western blot**

999 The cells were collected using Trypsin and washed in PBS twice. The cell pellet was
1000 resuspended in 10 vol. of buffer A (10 mM HEPES pH7.9, 1.5 mM MgCl₂, 10 mM KCl,
1001 1 mM DTT, 1 mM PMSF, 1x complete protease inhibitor (Roche, 04693132001) and
1002 incubated 15 min on ice. After centrifugation at 4°C at 2000 rpm for 5 min to remove
1003 the supernatant, the pellet was resuspended in 3 vol. of buffer A + NP40 (0.1%) and
1004 incubated for 15 min on ice followed by another centrifugation at 4 °C 4000 rpm for
1005 5min to remove the supernatant and resuspended in lysis buffer: (20 mM Tris pH7.5,
1006 200 mM NaCl, 1% Triton X-100, 0.1% sodium deoxycholate, 1 mM DTT, 1 mM PMSF,
1007 1x complete protease inhibitor (Roche, 04693132001), 1x super nuclease). The
1008 suspension was passed through a 26GA needle 10 times, left on ice for 1 h and
1009 centrifuged at 20000g for 30min. Proteins were quantified by Bradford (KitBiorad
1010 5000006, spectrophotometer BIOCHROM). Each IP was performed using 1 mg of
1011 protein in 1 ml in lysis buffer. GFP-Trap agarose beads (GTA-10, Chromotek) were
1012 washed in lysis buffer and incubated according to the supplier's instructions with the
1013 protein extract overnight at 4 °C. Washed five times in Lysis buffer and separated by
1014 SDS-PAGE, imaged using Fujifilm FLA-7000 imager if Halo ligand was used and then
1015 transferred overnight on Nitrocellulose membrane. After Ponceau red evaluation of
1016 the transfer quality, the membrane was blocked in PBS-0,1%Tween 20 + 5% non-fatty
1017 milk for an hour and incubated with the antibody overnight in PBS-0,1%Tween 20 +
1018 5% non-fatty milk. Western blots were analysed using LI-COR Odyssey Fc imager.

1019

1020 **Antibodies list**

1021 NCAPH2: Rabbit polyclonal produced on demand by Eurogentec (WB:1/1000).

1022 SCC1: Millipore, 53A303, mouse monoclonal antibody (WB:1/1000).

- 1023 SMC2: Cell Signaling Technology, D23C5, rabbit monoclonal Antibody (WB:1/500).
- 1024 Lamin B1: Abcam Ab133741, rabbit monoclonal (WB:1/1000).
- 1025 MCPH1: Cell Signaling Technology, D38G5, rabbit monoclonal Antibody (WB:1/1000).
- 1026 CREST: Immunovision HCT0-100, human autoantibody (IF: 1/500)
- 1027 H3PS10: Millipore, clone 3H10, mouse monoclonal (WB:, IF:1/2000).
- 1028 γ H2aX: Millipore, clone JBW301, mouse monoclonal (WB:, IF:1/500).
- 1029 CDK1: Cell Signaling Technology, cdc2, 77055, rabbit polyclonal (WB:1/1000).
- 1030 Phospho-CDK1: Cell Signaling Technology, phospho-cdc2 (Tyr15) antibody, 9111,
- 1031 rabbit polyclonal (WB:1/1000).
- 1032 WAPL: provided by J.M. Peters's Lab (WB:1/1000).
- 1033 GFP: Abcam, ab290, rabbit polyclonal (WB: 1/1000, IF:1/500).
- 1034 PK-Tag: Biorad, MCA 1360G, mouse monoclonal (WB:1/1000).
- 1035 Cyclin B1: Cell Signaling Technology, 4138T, rabbit monoclonal (IF:1/200).
- 1036 NCAPD3: Bethyl Laboratory, A300-604A-M, rabbit polyclonal (WB:1/1000).

1037

1038 **Halo in gel imaging**

1039 Cells were incubated with Halo-TMR ligand (100 nM) for 30 min then washed in ligand-

1040 free medium and analysed by immunofluorescence or for protein purification. After

1041 SDS-PAGE, the fluorescence was measured in the gel using an Imager Fujifilm FLA-

1042 7000.

1043

1044 **FRAP**

1045 Live-cell imaging was performed in a spinning disk confocal system (PerkinElmer

1046 UltraVIEW) with an EMCCD (Hamamatsu) mounted on an Olympus IX8 microscope

1047 with Olympus 60x 1.4 N.A. and 100x 1.35 N.A. objectives. Image acquisition and

1048 quantitation were performed using Volocity software. During imaging, cells were
1049 maintained at 37°C and 5% CO₂ in a humidified chamber. FRAP was carried out with
1050 a 488 nm laser beam, 100% power, 15–30 ms. The fluorescence intensity
1051 measurement was performed by using ImageJ. All signals were subjected to
1052 background correction. The fluorescence intensity of unbleached and bleached areas
1053 was normalized to that of initial pre-bleaching images using the EasyFRAP website.

1054

1055 **In-situ Hi-C**

1056 Hi-C libraries were generated as described in ⁶⁸, analysed using the Juicer pipeline ⁶⁹
1057 and visualised with Juicebox ⁷⁰. We sequenced 785,454,085 Hi-C read pairs in wild-
1058 type mouse ES cells, yielding 509,278,039 Hi-C contacts; we also sequenced
1059 1,814,815,287 Hi-C read pairs in *Mcp1* deleted cells, yielding 1,284,169,272 Hi-C
1060 contacts. Loci were assigned to A and B compartments at 100 kB resolution. Loops
1061 were called with HiCCUPS at 25 kB, 10 kB, and 5 kB resolution. Contact domains
1062 were called at 10 kB resolution. Contact frequency analysis was performed as
1063 described in ⁷¹. All code used for these analyses is publicly available at
1064 (github.com/aidenlab), with a help forum for questions at (aidenlab.org/forum.html).

1065

1066 **Structured illumination microscopy**

1067 Super-resolution 3D-SIM was performed on a DeltaVision OMX SR system (GE
1068 Healthcare) equipped with sCMOS cameras (PCO) and 405, 488 and 568 nm lasers,
1069 using a 60x NA 1.42 PlanApo oil immersion objective (Olympus). To minimise
1070 artefacts due to spherical aberration

1071 Raw data sets were acquired with a z-distance of 125 nm and 15 raw images
1072 per plane (5 phases, 3 angles). Reconstructions were performed with SoftWoRx 6.2
1073 (GE Healthcare) using channel-specifically measured optical transfer functions (OTFs)

1074 generated from 100 nm diameter green and red FluoSphere beads (ThermoFisher),
1075 respectively, and Wiener filter set to 0.0030. For DAPI acquisitions, the sample was
1076 excited with the 405 nm laser and the emission detected in the green channel and
1077 reconstructed with a green OTF. This is enabled by the broad emission spectrum of
1078 DAPI and empirically resulted in better reconstructions than reconstructing blue
1079 emission with a 'blue' OTF obtained typically less intense blue FluoSphere beads.

1080 All data underwent quality assessment via SIMcheck ⁷² to determine image
1081 quality via analysis of modulation contrast to noise ratio (MCNR), spherical aberration
1082 mismatch, reconstructed Fourier plot and reconstructed intensity histogram values.
1083 Reconstructed 32-bit 3D-SIM datasets were thresholded to the stack modal intensity
1084 value and converted to 16-bit composite z-stacks to discard negative intensity values
1085 using SIMcheck's "threshold and 16-bit conversion" utility and MCNR maps were
1086 generated using the "raw data modulation contrast" tool of SIMcheck. To eliminate
1087 false positive signals from reconstructed noise, we applied SIMcheck's 'modulation
1088 contrast filter' utility. Briefly, this filter sets masks out all pixels, where the underlying
1089 MCNR value in the raw data fall below an empirically chosen threshold MCNR value
1090 of 6.0, followed by a Gaussian filter with 0.8 pixel radius (xy) to smoothen hard edges
1091 ⁷³.

1092 Colour channels were registered in 3D with the open-source software
1093 Chromagnon 0.85 ⁷⁴ determining alignment parameter (x,y,z-translation, x,y,z-
1094 magnification, and z-rotation) from a 3D-SIM dataset acquired on the date of image
1095 acquisition of multicolour-detected 5-ethenyl-2'-deoxyuridine (EdU) pulse replication
1096 labelled C127 mouse cells serving as biological 3D alignment calibration sample ⁷⁵.

1097

1098 **FACS**

1099 Cells were incubated with EdU (10 μ M) for 5 min, collected using trypsin, washed with
1100 PBS and fixed in 3.8% Formaldehyde (Sigma-F8775) in PBS for 15 min. After three
1101 PBS washes, cells were permeabilised in 0.5% Triton X-100 in PBS for 10 min and
1102 then washed three times in PBS. Cells were then incubated for 20 min in blocking
1103 buffer: PBS 3% BSA (Sigma, A4503), incubated for 1 h with H3PS10 antibody
1104 (1/1000), washed three times in PBS and then incubated with fluorescent secondary
1105 antibody (Alexa Fluor 488, 1/500, Life Technology) incubated with propidium iodide
1106 (Sigma, 30 μ g/ml) and then analysed by FACS. 15,000 cells were analysed for each
1107 data point. EdU detection was done following the manufacturer's instruction (Click-iT
1108 EdU Imaging kit, Invitrogen).

1109

1110 **Protein purification**

1111 Human condensin II pentameric and tetrameric complexes were purified as previously
1112 described⁵⁹. Full length MCPH1 was cloned into the pLIB vector and viral bacmids
1113 were generated using Tn7 transposition in DH10EMBacY cells (Geneva biotech),
1114 transfected into Sf9 cells using Cellfectin II (GIBCO) and resultant virus harvested after
1115 3 days. Virus was further amplified in Sf9 cells before being used to infect High Five
1116 cells for protein expression. High Five cells were harvested by centrifugation 3 days
1117 after infection. Cell pellets were resuspended in purification buffer (20 mM HEPES pH
1118 8, 300 mM KCl, 5 mM MgCl₂, 1 mM DTT, 10% glycerol) supplemented with 1 Pierce
1119 protease inhibitor EDTA-free tablet (Thermo Scientific) per 50 mL and 25 U/ml of
1120 Benzonase (Sigma) and lysed with a Dounce homogenizer followed by brief
1121 sonication. The lysate was cleared with centrifugation, loaded onto a StrepTrap HP
1122 (GE), washed with purification buffer and eluted with purification buffer supplemented
1123 with 5 mM desthiobiotin (Sigma). Size exclusion chromatography was performed

1124 using purification buffer on a Superdex 200 16/60 column (GE), and protein containing
1125 fractions separated from the void volume were pooled and concentrated.
1126 MCPH1 residue 1-435, 1-195 and 196-435 were cloned into a pET vector with an N-
1127 terminal 6xHis-MBP fusion tag or with an N-terminal 6xHis and C-terminal MBP tag,
1128 and expressed in *E. coli* BL21 (DE3) pLysS cells (Novagen). Cells were grown at 37
1129 °C, induced with 1 mM IPTG for 4 h, before being harvested by centrifugation and
1130 flash frozen. The cell pellet was resuspended in MCPH1 purification buffer (20 mM
1131 Tris pH 7.5, 150 mM NaCl, 10% glycerol, 1 mM DTT, Pierce protease inhibitor EDTA-
1132 free tablet), lysed with sonication on ice, treated with Benzonase (Sigma-Aldrich) (10
1133 µL per 100 mL, with 1 mM MgCl₂) and cleared via centrifugation. Cleared cell lysate
1134 was filtered with a 5 µm filter, imidazole was added to a final concentration of 10 mM
1135 and incubated with pre-equilibrated His-Pure NTA resin. The resin was washed with
1136 MCPH1 purification buffer, then washed with wash buffer 20 mM Tris pH 7.5, 500 mM
1137 NaCl, 20 mM imidazole, before elution with 20 mM Tris pH 8, 300mM NaCl, 500 mM
1138 Imimidazole, 10% glycerol. Protein was diluted 2-fold with buffer TA (20 mM Tris pH 8,
1139 5% glycerol, 1 mM DTT), and loaded onto a HiTrap Q Fast Flow column or loaded on
1140 to HiTrap Heparin HP column (GE) and eluted with a gradient of buffer TB (20 mM
1141 Tris pH 8, 2 M NaCl, 5% glycerol, 1 mM DTT). For protein used in ATPase assays,
1142 final size exclusion chromatography was performed using purification buffer and a
1143 Superdex 200 10/300 or 16/60 column (GE).

1144

1145 **Pull-downs**

1146 Condensin complexes (0.1 µM) were mixed with at least a 10-fold molecular excess
1147 of MBP MCPH1 constructs or MBP protein in 200 µL and incubated with 40 µL of
1148 Strep-tactin sepharose resin (IBA) in MCPH1 purification buffer. The resin was

1149 washed 5 times, before being eluted by boiling in 1x NuPAGE LDS sample buffer with
1150 50 mM DTT. Samples of 10% input and resin elution were run on 4-12% NuPAGE Bis-
1151 Tris gels against Color Prestained Protein Standard, Broad Range (NEB) and stained
1152 with Instant Blue (Expedeon) or silver stain (Life Technology).

1153 The absence of the CAP-D3 subunit was confirmed by western blot analysis of the
1154 output protein samples. For this, the tetrameric condensin II pull-down was run on an
1155 SDS page gel and transferred to a nitrocellulose membrane (Amersham). The
1156 membrane was then blocked with 5% milk-powder in TBS-T, before being probed with
1157 a mouse anti-CAP-D3 antibody (Santa Cruz, Sc-81597, used at 1/1000), then a goat
1158 DyLight 800 florescent anti-mouse secondary antibodies (Cell Signaling Technology,
1159 5257, used at 1/5000). The membrane was imaged using a LI-COR imager.

1160

1161 **Fluorescence polarisation assays**

1162 Peptides used in fluorescence polarization assays were synthesised by Genscript and
1163 are shown in Table 1. The concentration of 5FAM wild-type MCPH1₄₀₇₋₄₂₂ was
1164 determined using the 5-FAM extinction coefficient of 83,000 (cmM)⁻¹ at 493 nm. Non-
1165 labelled peptides had TFA removed to less than 1% and were accurately quantified
1166 using Genscript's amino acid analysis service. All peptides were solubilised in DMSO
1167 and diluted to a working concentration in FP assay buffer (20 mM Tris pH 7.5, 200 mM
1168 NaCl, 1 mM DTT). Peptides in competition experiments were diluted in a two-fold
1169 series with FP assay buffer supplemented with DMSO, such that DMSO concentration
1170 was constant for all peptide concentrations.

1171

1172 Table 1: Peptides used in FP experiments

Name	Sequence
------	----------

5FAM-MCPH1 ₄₀₇₋₄₂₂	5FAM- CGESSYDDYFSPDNLKER
MCPH1 ₄₀₇₋₄₂₂	CGESSYDDYFSPDNLKER
MCPH1 ₄₀₇₋₄₂₂ pS417	CGESSYDDYF{pSER}PDNLKER

1173

1174 FP binding assays were performed with 0.3 μ M of 5-FAM labelled wild-type MCPH1₄₀₇₋
1175 ₄₂₂ and 0.625, 1.25, 1.75, 2.5, 3.5 and 5 μ M of pentameric condensin II or tetrameric
1176 condensin II lacking the CAPG2 subunit, in a total volume of 40 μ l in half-area black
1177 plates (Constar). The plate was incubated at room temperature for 20 min, before
1178 being read with an Omega plate reader (BMG Labtech) at 5 min intervals and
1179 monitored to ensure binding had reached equilibrium. Each plate was read three
1180 times, and three replicates were performed at each protein concentration.

1181 FP competition titrations were performed as above, but with 0.63 μ M of condensin II,
1182 0.3 μ M of 5FAM-MCPH1₄₀₇₋₄₂₂ and indicated amount of competing peptide. Data was
1183 normalised by subtracting the FP signal for 5FAM-MCPH1₄₀₇₋₄₂₂ in the absence of
1184 condensin II at each peptide concentration and divided by the background-subtracted
1185 signal of sample with 0.63 μ M of condensin II and 0.3 μ M of 5FAM-MCPH1₄₀₇₋₄₂₂
1186 without any competing peptide. Each plate was read 4 times and each experiment was
1187 performed a total of 3 times.

1188 Fluorescence polarisation data was fit using equations for direct binding and directly
1189 competitive binding, as presented by ⁷⁶.

1190

1191 **ATPase assays**

1192 Complexes of wild type or ATPase hydrolysis deficient Q-loop mutants condensin II
1193 with MCPH1₁₋₄₃₅MBP were purified with gel filtration on a Superose 6 10/300 column,
1194 along with wild-type only control in ATPase assay buffer (20 mM Tris pH 7.5, 150 mM

1195 NaCl, 1 mM DTT). Prior to gel-filtration all samples were treated with Tev protease to
1196 remove N-terminal 6x His-tag on MCPH1₁₋₄₃₅MBP. ATPase assays were performed
1197 using the EnzChek™ Phosphate Assay Kit (Invitrogen) modified for a 96 well plate
1198 format ⁷⁷. 50 bp double stranded DNA sequence with the same as that used in the
1199 EMSA (without a fluorescent label). Reactions contained 50 nM protein with or without
1200 800 nM DNA. Final conditions included 1 mM ATP and a total salt concentration of 50
1201 mM. Protein/DNA was preincubated in reaction mix without ATP for 15 minutes at
1202 room temperature before the reaction was started by addition of ATP immediately
1203 before putting it in the plate reader to track phosphate release. A standard curve
1204 ATPase rate was determined from a linear fit of data from the first 60 min.

1205

1206 **Electromobility shift assays**

1207 EMSAs were performed using 50 nM of 50 bp double stranded DNA labelled with Cy5
1208 at the 5' with the sequence:

1209 CTGTCACACCCTGTCACACCCTGTCACACCCTGTCACACCCTGTCACACC

1210 For MCPH1 EMSAs, MCPH1-MBP constructs were diluted in 20 mM Tris pH 7.5, 150
1211 mM NaCl, 10% glycerol, 1 mM DTT and mixed 1 in 2 with DNA in 20 mM HEPES pH
1212 8, 300 mM KCl, 5 mM MgCl₂, 10% glycerol and 1 mM DTT.

1213 For condensin II/MCPH1 EMSAs, condensin II diluted in 20 mM HEPES pH 8, 300
1214 mM KCl, 5 mM MgCl₂, 10% glycerol and 1 mM DTT and mixed with MCPH1₁₋₄₃₅-MBP,
1215 MBP or 5-FAM-MCPH1₄₀₇₋₄₂₄ diluted in in 20 mM Tris pH 7.5, 150mM NaCl, 10%
1216 glycerol, 1 mM DTT. DNA was then added to a final concentration of 50 nM. Protein
1217 and DNA were incubated on ice for 15 min, before being loaded in a 2% agarose gel
1218 and run in 0.5x TBE buffer for 30 min. Gel was imaged using a Typhoon.

1219 **REFERENCES**

- 1220 1. Yatskevich, S., Rhodes, J. & Nasmyth, K. Organization of Chromosomal DNA
1221 by SMC Complexes. *Annu. Rev. Genet.* **53**, 445–482 (2019).
- 1222 2. Tedeschi, A. *et al.* Wapl is an essential regulator of chromatin structure and
1223 chromosome segregation. *Nature* **501**, 564–568 (2013).
- 1224 3. Challa, K. *et al.* Meiosis-specific prophase-like pathway controls cleavage-
1225 independent release of cohesin by Wapl phosphorylation. *bioRxiv* 1–27 (2018)
1226 doi:10.1101/250589.
- 1227 4. Hill, L. *et al.* Wapl repression by Pax5 promotes V gene recombination by Igh
1228 loop extrusion. *Nature* **584**, 142–147 (2020).
- 1229 5. Davidson, I. F. *et al.* DNA loop extrusion by human cohesin. *Science (80-.)*.
1230 **366**, 1338–1345 (2019).
- 1231 6. Ganji, M. *et al.* Real-time imaging of DNA loop extrusion by condensin.
1232 *Science (80-.)*. **360**, 102–105 (2018).
- 1233 7. Wutz, G. *et al.* ESCO1 and CTCF enable formation of long chromatin loops by
1234 protecting cohesin¹ from WAPL. *Elife* **9**, 1–33 (2020).
- 1235 8. Gerlich, D., Hirota, T., Koch, B., Peters, J. M. & Ellenberg, J. Condensin I
1236 stabilizes chromosomes mechanically through a dynamic interaction in live
1237 cells. *Curr. Biol.* **16**, 333–344 (2006).
- 1238 9. Hansen, A. S., Pustova, I., Cattoglio, C., Tjian, R. & Darzacq, X. CTCF and
1239 cohesin regulate chromatin loop stability with distinct dynamics. *Elife* **6**, 1–33
1240 (2017).
- 1241 10. Cutts, E. E. & Vannini, A. Condensin complexes: understanding loop extrusion
1242 one conformational change at a time. *Biochem. Soc. Trans.* **48**, 2089–2100
1243 (2020).

- 1244 11. Hirota, T., Gerlich, D., Koch, B., Ellenberg, J. & Peters, J. M. Distinct functions
1245 of condensin I and II in mitotic chromosome assembly. *J. Cell Sci.* **117**, 6435–
1246 6445 (2004).
- 1247 12. Ono, T. *et al.* Differential contributions of condensin I and condensin II to
1248 mitotic chromosome architecture in vertebrate cells. *Cell* **115**, 109–121 (2003).
- 1249 13. Ono, T., Fang, Y., Spector, D. L. & Hirano, T. Spatial and Temporal Regulation
1250 of Condensins I and II in Mitotic Chromosome Assembly in Human Cells. *Mol.*
1251 *Biol. Cell* **15**, 3296–3308 (2004).
- 1252 14. Walther, N. *et al.* A quantitative map of human Condensins provides new
1253 insights into mitotic chromosome architecture. *J. Cell Biol.* **217**, 2309–2328
1254 (2018).
- 1255 15. Gibcus, J. H. *et al.* A pathway for mitotic chromosome formation. *Science* (80-
1256). **359**, (2018).
- 1257 16. Baxter, J. & Aragón, L. A model for chromosome condensation based on the
1258 interplay between condensin and topoisomerase II. *Trends Genet.* **28**, 110–
1259 117 (2012).
- 1260 17. Elbatsh, A. M. O. *et al.* Cohesin Releases DNA through Asymmetric ATPase-
1261 Driven Ring Opening. *Mol. Cell* **61**, 575–588 (2016).
- 1262 18. Beckouët, F. *et al.* Releasing Activity Disengages Cohesin's Smc3/Scc1
1263 Interface in a Process Blocked by Acetylation. *Mol. Cell* **61**, 563–574 (2016).
- 1264 19. Chan, K. L. *et al.* Cohesin's DNA exit gate is distinct from its entrance gate and
1265 is regulated by acetylation. *Cell* **150**, 961–974 (2012).
- 1266 20. Gligoris, T. G. *et al.* Closing the cohesin ring: Structure and function of its
1267 Smc3-kleisin interface. *Science* (80-). **346**, 963–967 (2014).
- 1268 21. Hassler, M. *et al.* Structural Basis of an Asymmetric Condensin ATPase Cycle.

- 1269 *Mol. Cell* **74**, 1175-1188.e9 (2019).
- 1270 22. Lee, B. G. *et al.* Cryo-EM structures of holo condensin reveal a subunit flip-flop
1271 mechanism. *Nat. Struct. Mol. Biol.* **27**, 743–751 (2020).
- 1272 23. Buheitel, J. & Stemmann, O. Prophase pathway-dependent removal of cohesin
1273 from human chromosomes requires opening of the Smc3-Scc1 gate. *EMBO J.*
1274 **32**, 666–676 (2013).
- 1275 24. Huis In't Veld, P. J. *et al.* Characterization of a DNA exit gate in the human
1276 cohesin ring. *Science (80-.)*. **346**, 968–972 (2014).
- 1277 25. Ono, T., Yamashita, D. & Hirano, T. Condensin II initiates sister chromatid
1278 resolution during S phase. *J. Cell Biol.* **200**, 429–441 (2013).
- 1279 26. Schwarzer, W. *et al.* Two independent modes of chromatin organization
1280 revealed by cohesin removal. *Nature* **551**, 51–56 (2017).
- 1281 27. Abe, S. *et al.* The initial phase of chromosome condensation requires Cdk1-
1282 mediated phosphorylation of the CAP-D3 subunit of condensin II. *Genes Dev.*
1283 **25**, 863–874 (2011).
- 1284 28. Woods, C. G., Bond, J. & Enard, W. Autosomal recessive primary
1285 microcephaly (MCPH): A review of clinical, molecular, and evolutionary
1286 findings. *Am. J. Hum. Genet.* **76**, 717–728 (2005).
- 1287 29. Thornton, G. K. & Woods, C. G. Primary microcephaly: do all roads lead to
1288 Rome? *Trends Genet.* **25**, 501–510 (2009).
- 1289 30. Neitzel, H. *et al.* Premature chromosome condensation in humans associated
1290 with microcephaly and mental retardation: A novel autosomal recessive
1291 condition. *Am. J. Hum. Genet.* **70**, 1015–1022 (2002).
- 1292 31. Trimborn, M. *et al.* Mutations in microcephalin cause aberrant regulation of
1293 chromosome condensation. *Am. J. Hum. Genet.* **75**, 261–266 (2004).

- 1294 32. Trimborn, M., Schindler, D., Neitzel, H. & Hirano, T. Misregulated chromosome
1295 condensation in MCPH1 primary microcephaly is mediated by condensin II.
1296 *Cell Cycle* **5**, 322–326 (2006).
- 1297 33. Yamashita, D. *et al.* MCPH1 regulates chromosome condensation and shaping
1298 as a composite modulator of condensin II. *J. Cell Biol.* **194**, 841–854 (2011).
- 1299 34. Arroyo, M. *et al.* MCPH1 is essential for cellular adaptation to the G2-phase
1300 decatenation checkpoint. *FASEB J.* **33**, 8363–8374 (2019).
- 1301 35. Arroyo, M. *et al.* MCPH1, mutated in primary microcephaly, is required for
1302 efficient chromosome alignment during mitosis. *Sci. Rep.* **7**, 1–12 (2017).
- 1303 36. Venkatesh, T. & Suresh, P. S. Emerging roles of MCPH1: Expedition from
1304 primary microcephaly to cancer. *Eur. J. Cell Biol.* **93**, 98–105 (2014).
- 1305 37. Leung, J. W. *et al.* SET nuclear oncogene associates with
1306 microcephalin/MCPH1 and regulates chromosome condensation. *J. Biol.*
1307 *Chem.* **286**, 21393–21400 (2011).
- 1308 38. Cicconi, A. *et al.* Microcephalin 1/BRIT1-TRF2 interaction promotes telomere
1309 replication and repair, linking telomere dysfunction to primary microcephaly.
1310 *Nat. Commun.* **11**, (2020).
- 1311 39. Lin, S. Y. & Elledge, S. J. Multiple tumor suppressor pathways negatively
1312 regulate telomerase. *Cell* **113**, 881–889 (2003).
- 1313 40. Alderton, G. K. *et al.* Regulation of mitotic entry by microcephalin and its
1314 overlap with ATR signalling. *Nat. Cell Biol.* **8**, 725–733 (2006).
- 1315 41. Gruber, R. *et al.* MCPH1 regulates the neuroprogenitor division mode by
1316 coupling the centrosomal cycle with mitotic entry through the Chk1-Cdc25
1317 pathway. *Nat. Cell Biol.* **13**, 1325–1334 (2011).
- 1318 42. Chang, H. Y. *et al.* Microcephaly family protein MCPH1 stabilizes RAD51

- 1319 filaments. *Nucleic Acids Res.* **48**, 9135–9146 (2020).
- 1320 43. Tibelius, A. *et al.* Microcephalin and pericentrin regulate mitotic entry via
1321 centrosome-associated Chk1. *J. Cell Biol.* **185**, 1149–1157 (2009).
- 1322 44. Buster, D. W. *et al.* SCFSlimb ubiquitin ligase suppresses condensin II-
1323 mediated nuclear reorganization by degrading Cap-H2. *J. Cell Biol.* **201**, 49–63
1324 (2013).
- 1325 45. Nguyen, H. Q. *et al.* Drosophila Casein Kinase I Alpha Regulates Homolog
1326 Pairing and Genome Organization by Modulating Condensin II Subunit Cap-H2
1327 Levels. *PLoS Genet.* **11**, 1–32 (2015).
- 1328 46. Berezin, C. *et al.* ConSeq: The identification of functionally and structurally
1329 important residues in protein sequences. *Bioinformatics* **20**, 1322–1324
1330 (2004).
- 1331 47. Ashkenazy, H. *et al.* ConSurf 2016: an improved methodology to estimate and
1332 visualize evolutionary conservation in macromolecules. *Nucleic Acids Res.* **44**,
1333 W344–W350 (2016).
- 1334 48. Van Roey, K. *et al.* Short linear motifs: Ubiquitous and functionally diverse
1335 protein interaction modules directing cell regulation. *Chem. Rev.* **114**, 6733–
1336 6778 (2014).
- 1337 49. Oppermann, F. S. *et al.* Combination of chemical genetics and
1338 phosphoproteomics for kinase signaling analysis enables confident
1339 identification of cellular downstream targets. *Mol. Cell. Proteomics* **11**, 1–12
1340 (2012).
- 1341 50. Errico, A., Deshmukh, K., Tanaka, Y., Pozniakovsky, A. & Hunt, T.
1342 Identification of substrates for cyclin dependent kinases. *Adv. Enzyme Regul.*
1343 **50**, 375–399 (2010).

- 1344 51. Rai, R. *et al.* BRIT1 regulates early DNA damage response, chromosomal
1345 integrity, and cancer. *Cancer Cell* **10**, 145–157 (2006).
- 1346 52. Houlard, M. *et al.* Condensin confers the longitudinal rigidity of chromosomes.
1347 *Nat. Cell Biol.* **17**, 771–781 (2015).
- 1348 53. Eichinger, C. S., Kurze, A., Oliveira, R. A. & Nasmyth, K. Disengaging the
1349 Smc3/kleisin interface releases cohesin from Drosophila chromosomes during
1350 interphase and mitosis. *EMBO J.* **32**, 656–665 (2013).
- 1351 54. Wood, J. L., Liang, Y., Li, K. & Chen, J. Microcephalin/MCPH1 associates with
1352 the condensin II complex to function in homologous recombination repair. *J.*
1353 *Biol. Chem.* **283**, 29586–29592 (2008).
- 1354 55. Li, Y. *et al.* The structural basis for cohesin–CTCF-anchored loops. *Nature*
1355 **578**, 472–476 (2020).
- 1356 56. Chan, K. L. *et al.* Pds5 promotes and protects cohesin acetylation. *Proc. Natl.*
1357 *Acad. Sci. U. S. A.* **110**, 13020–13025 (2013).
- 1358 57. Srinivasan, M. *et al.* Scc2 counteracts a wapl-independent mechanism that
1359 releases cohesin from chromosomes during G1. *Elife* **8**, 1–34 (2019).
- 1360 58. Lee, B. G. *et al.* Cryo-EM structures of holo condensin reveal a subunit flip-flop
1361 mechanism. *Nat. Struct. Mol. Biol.* **27**, 743–751 (2020).
- 1362 59. Kong, M. *et al.* Human Condensin I and II Drive Extensive ATP-Dependent
1363 Compaction of Nucleosome-Bound DNA. *Mol. Cell* **79**, 99-114.e9 (2020).
- 1364 60. Golfier, S., Quail, T., Kimura, H. & Brugués, J. Cohesin and condensin extrude
1365 DNA loops in a cell-cycle dependent manner. *Elife* **9**, 1–34 (2020).
- 1366 61. Kim, Y., Shi, Z., Zhang, H., Finkelstein, I. J. & Yu, H. Human cohesin compacts
1367 DNA by loop extrusion. *Science (80-.).* **366**, 1345–1349 (2019).
- 1368 62. Collier, J. E. *et al.* Transport of DNA within cohesin involves clamping on top of

- 1369 engaged heads by SCC2 and entrapment within the ring by SCC3. *Elife* **9**, 1–
1370 36 (2020).
- 1371 63. Higashi, T. L. *et al.* A Structure-Based Mechanism for DNA Entry into the
1372 Cohesin Ring. *Mol. Cell* **79**, 917-933.e9 (2020).
- 1373 64. Shi, Z., Gao, H., Bai, X. & Yu, H. Cryo-EM structure of the human cohesin-
1374 NIPBL-DNA complex. **1459**, 1454–1459 (2020).
- 1375 65. Rao, S. S. P. *et al.* Cohesin Loss Eliminates All Loop Domains. *Cell* **171**, 305-
1376 320.e24 (2017).
- 1377 66. Ran, F. A. *et al.* Genome engineering using the CRISPR-Cas9 system. *Nat.*
1378 *Protoc.* **8**, 2281–2308 (2013).
- 1379 67. Grimm, J. B. *et al.* A General Method to Improve Fluorophores Using
1380 Deuterated Auxochromes. *JACS Au* **1**, 690–696 (2021).
- 1381 68. Rao, S. S. P. *et al.* A 3D map of the human genome at kilobase resolution
1382 reveals principles of chromatin looping. *Cell* **159**, 1665–1680 (2014).
- 1383 69. Durand, N. C. *et al.* Juicer Provides a One-Click System for Analyzing Loop-
1384 Resolution Hi-C Experiments. *Cell Syst.* **3**, 95–98 (2016).
- 1385 70. Durand, N. C. *et al.* Juicebox Provides a Visualization System for Hi-C Contact
1386 Maps with Unlimited Zoom. *Cell Syst.* **3**, 99–101 (2016).
- 1387 71. Sanborn, A. L. *et al.* Chromatin extrusion explains key features of loop and
1388 domain formation in wild-type and engineered genomes. *Proc. Natl. Acad. Sci.*
1389 *U. S. A.* **112**, E6456–E6465 (2015).
- 1390 72. Ball, G. *et al.* SIMcheck: A toolbox for successful super-resolution structured
1391 illumination microscopy. *Sci. Rep.* **5**, 1–12 (2015).
- 1392 73. Rodermund, L. *et al.* Time-resolved structured illumination microscopy reveals
1393 key principles of Xist RNA spreading. *Science (80-.).* **372**, eabe7500 (2021).

- 1394 74. Matsuda, A., Schermelleh, L., Hirano, Y., Haraguchi, T. & Hiraoka, Y. Accurate
1395 and fiducial-marker-free correction for three-dimensional chromatic shift in
1396 biological fluorescence microscopy. *Sci. Rep.* **8**, 1–3 (2018).
- 1397 75. Kraus, F. *et al.* Quantitative 3D structured illumination microscopy of nuclear
1398 structures. *Nat. Protoc.* **12**, 1011–1028 (2017).
- 1399 76. Roehrl, M. H. A., Wang, J. Y. & Wagner, G. A general framework for
1400 development and data analysis of competitive high-throughput screens for
1401 small-molecule inhibitors of protein-protein interactions by fluorescence
1402 polarization. *Biochemistry* **43**, 16056–16066 (2004).
- 1403 77. Voulgaris, M. & Gligoris, T. G. A protocol for assaying the ATPase activity of
1404 recombinant cohesin holocomplexes. *Methods Mol. Biol.* **2004**, 197–208
1405 (2019).
- 1406

Figure 1

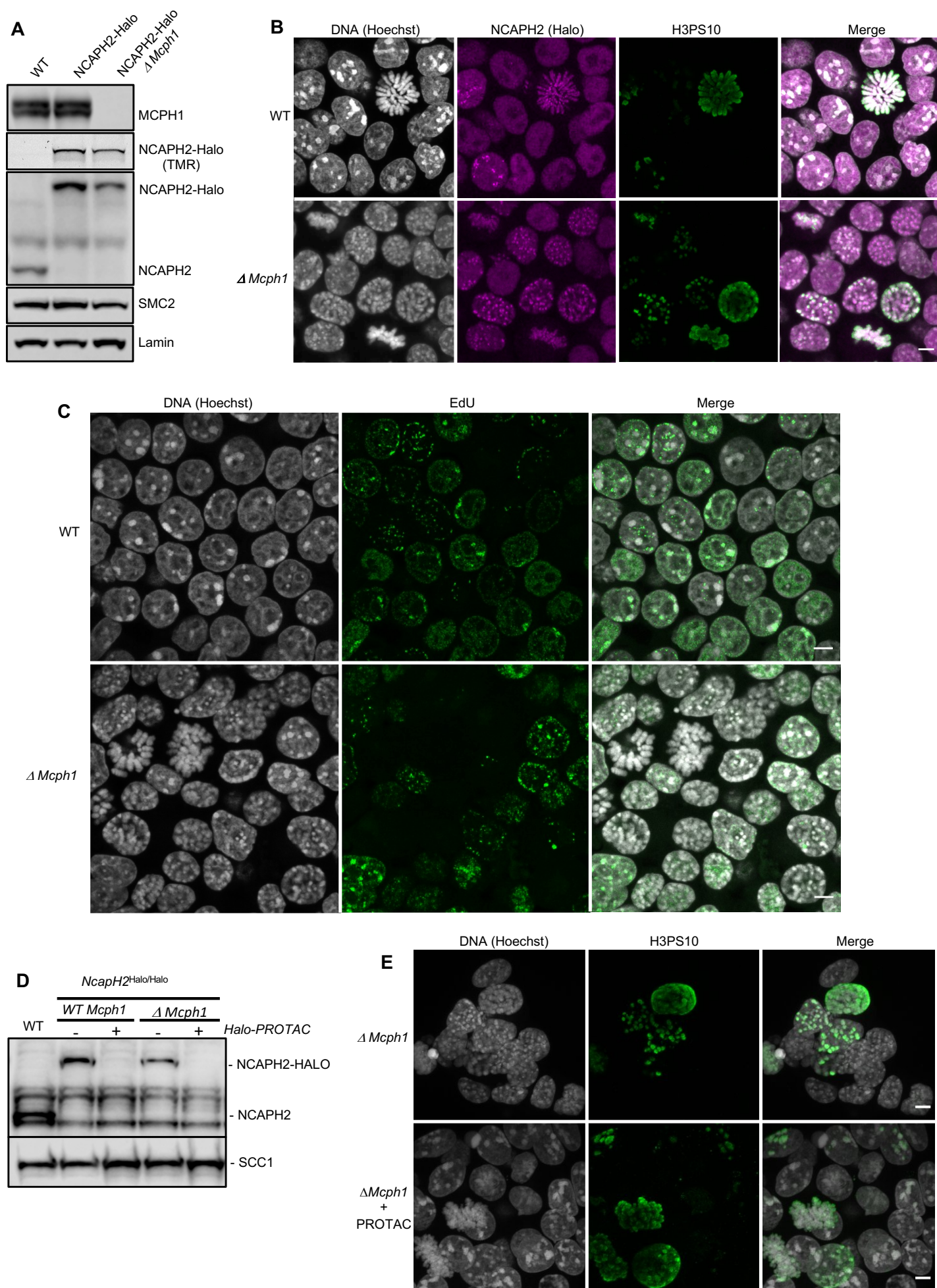


Figure 2

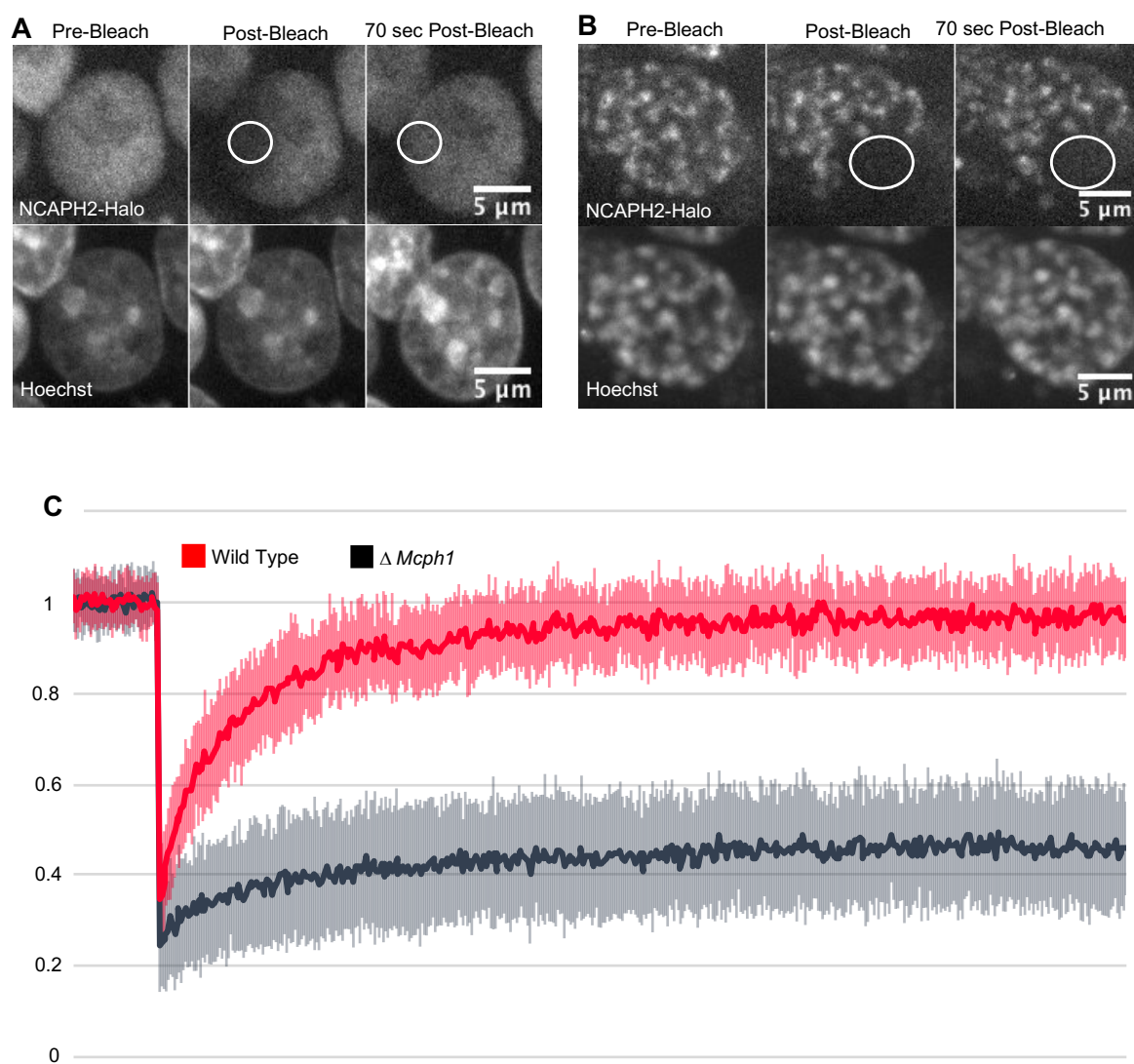


Figure 3

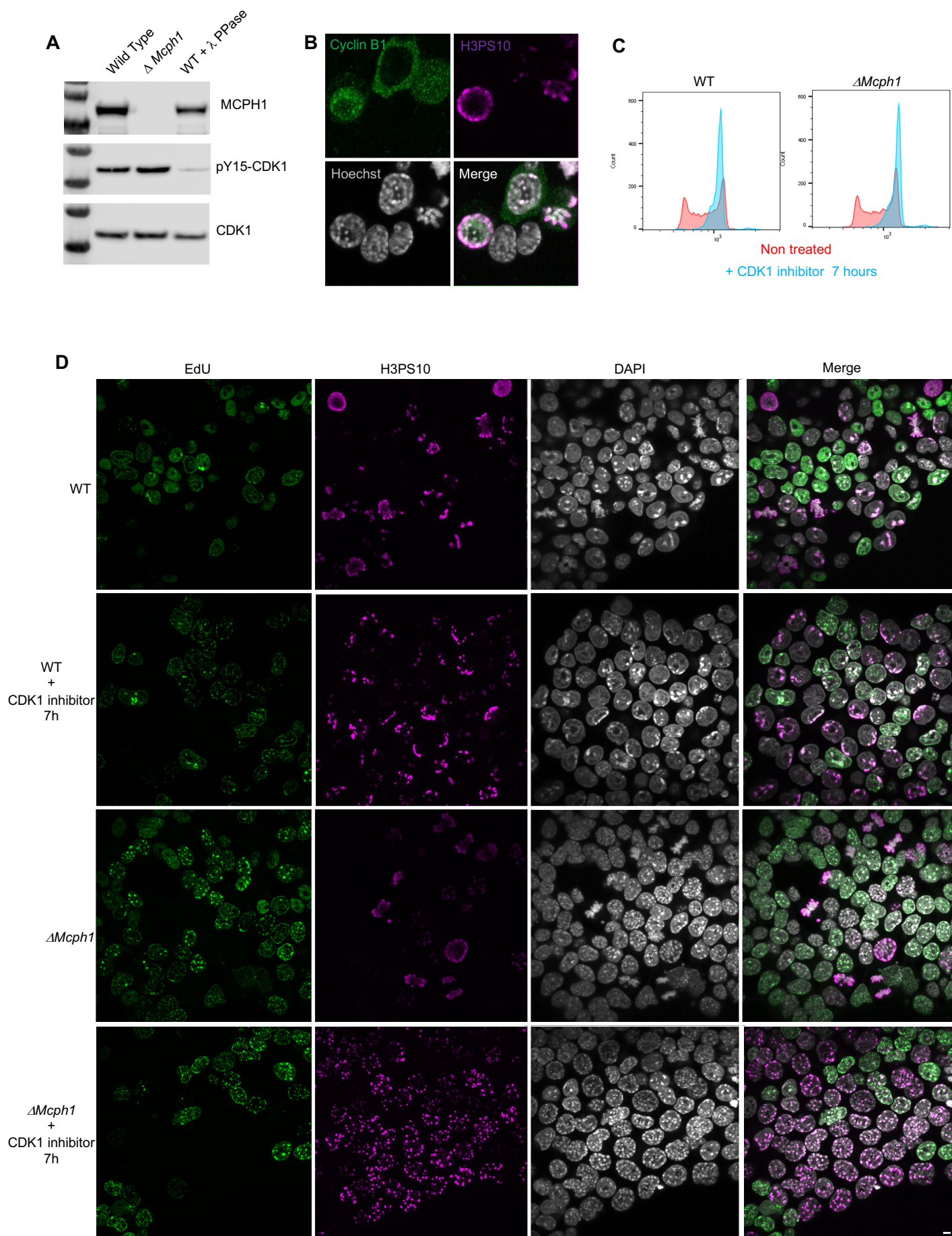


Figure 4

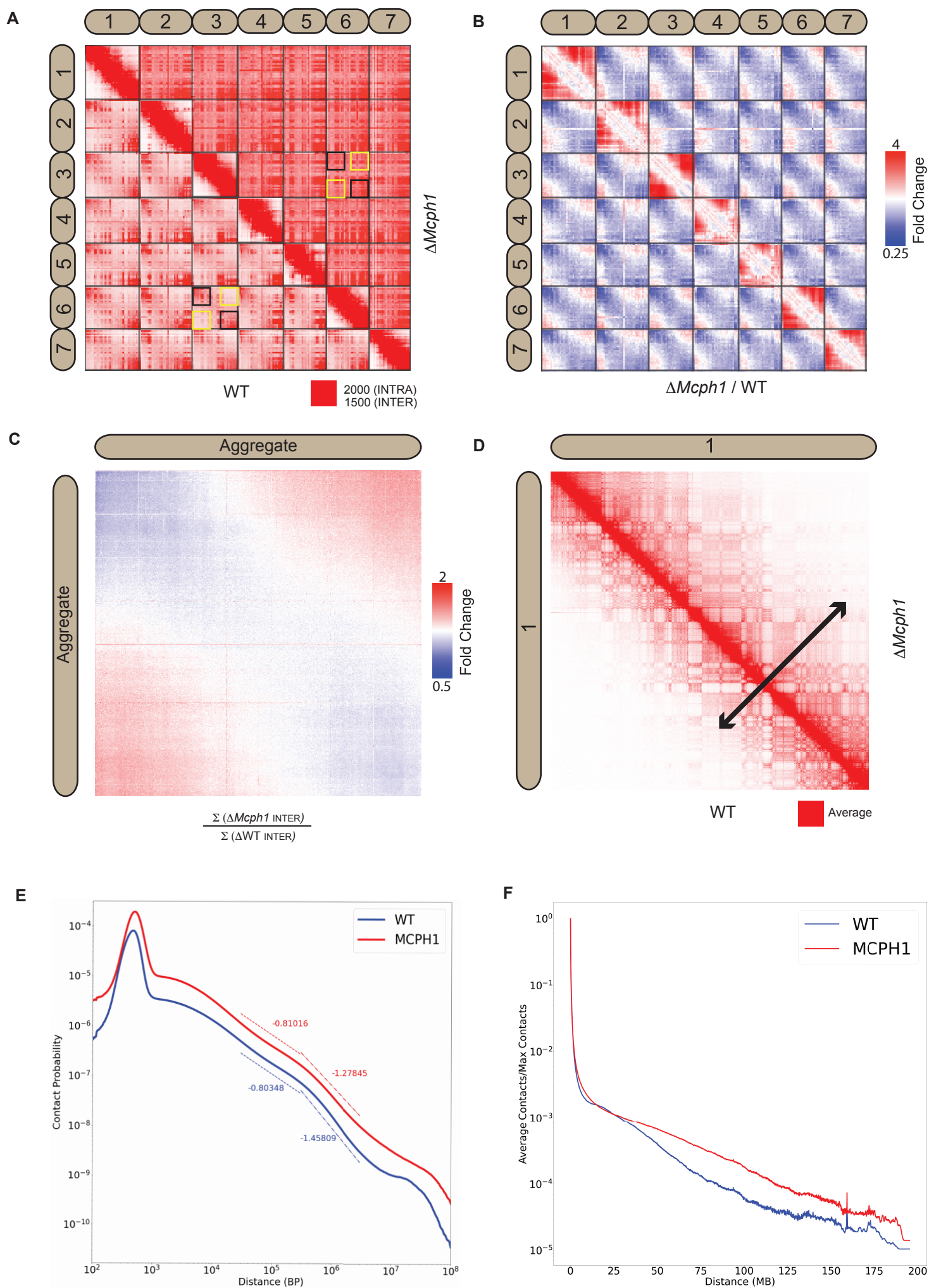


Figure 5

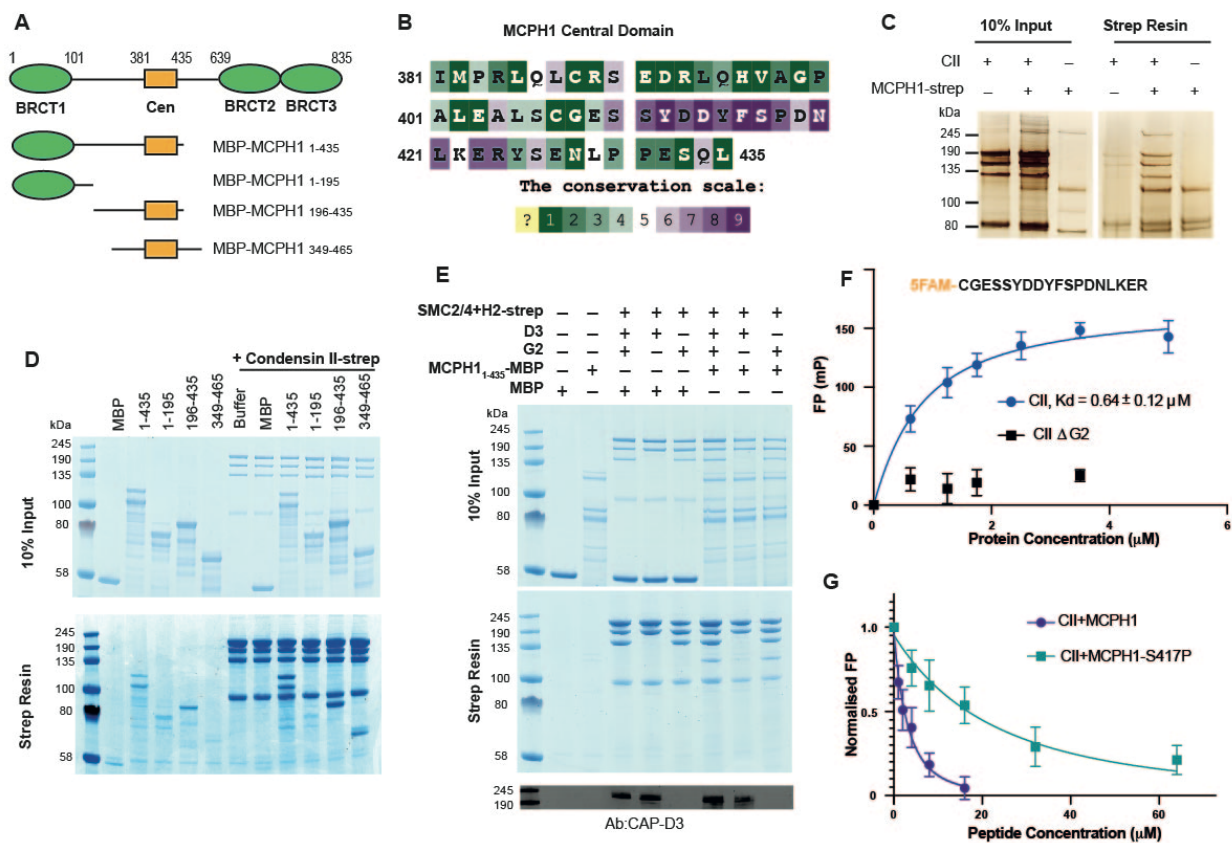


Figure 6

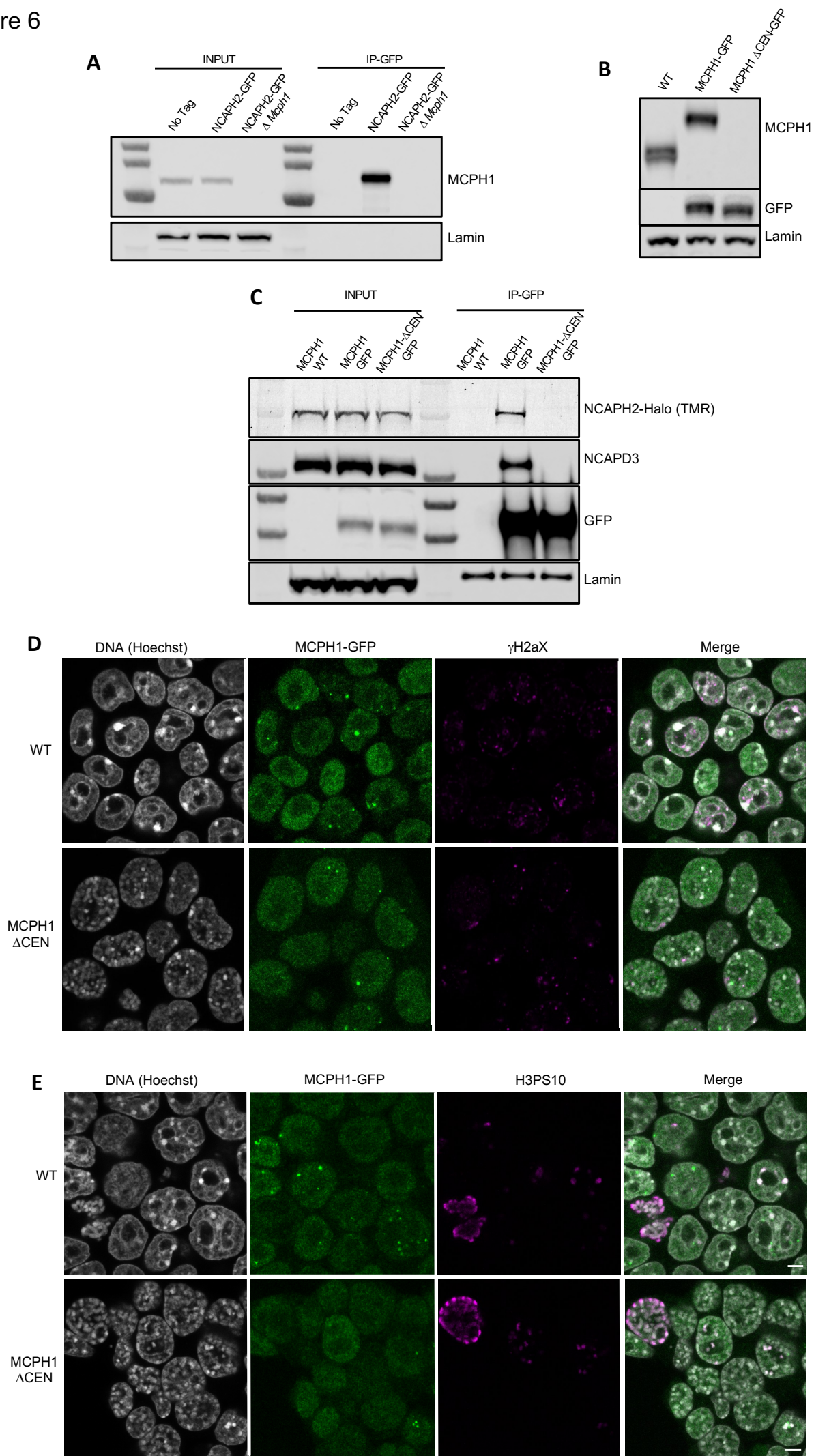


Figure 7

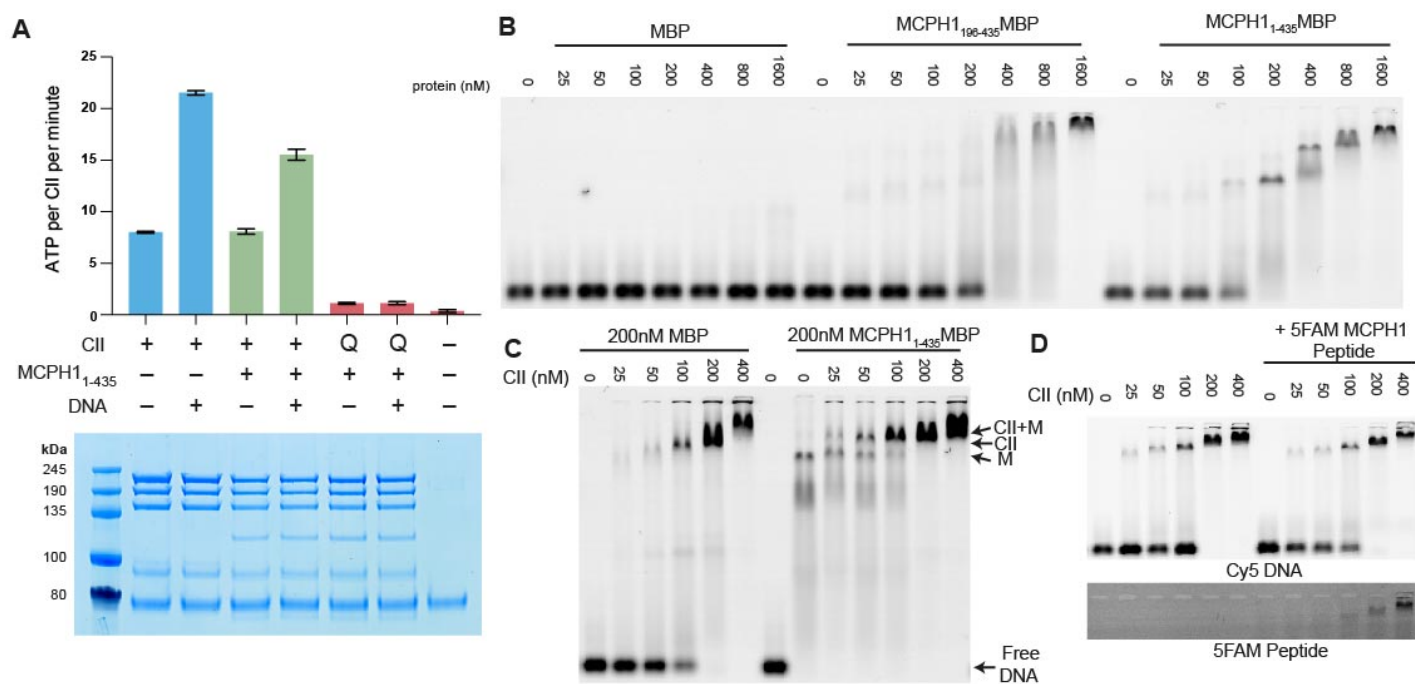


Figure 8

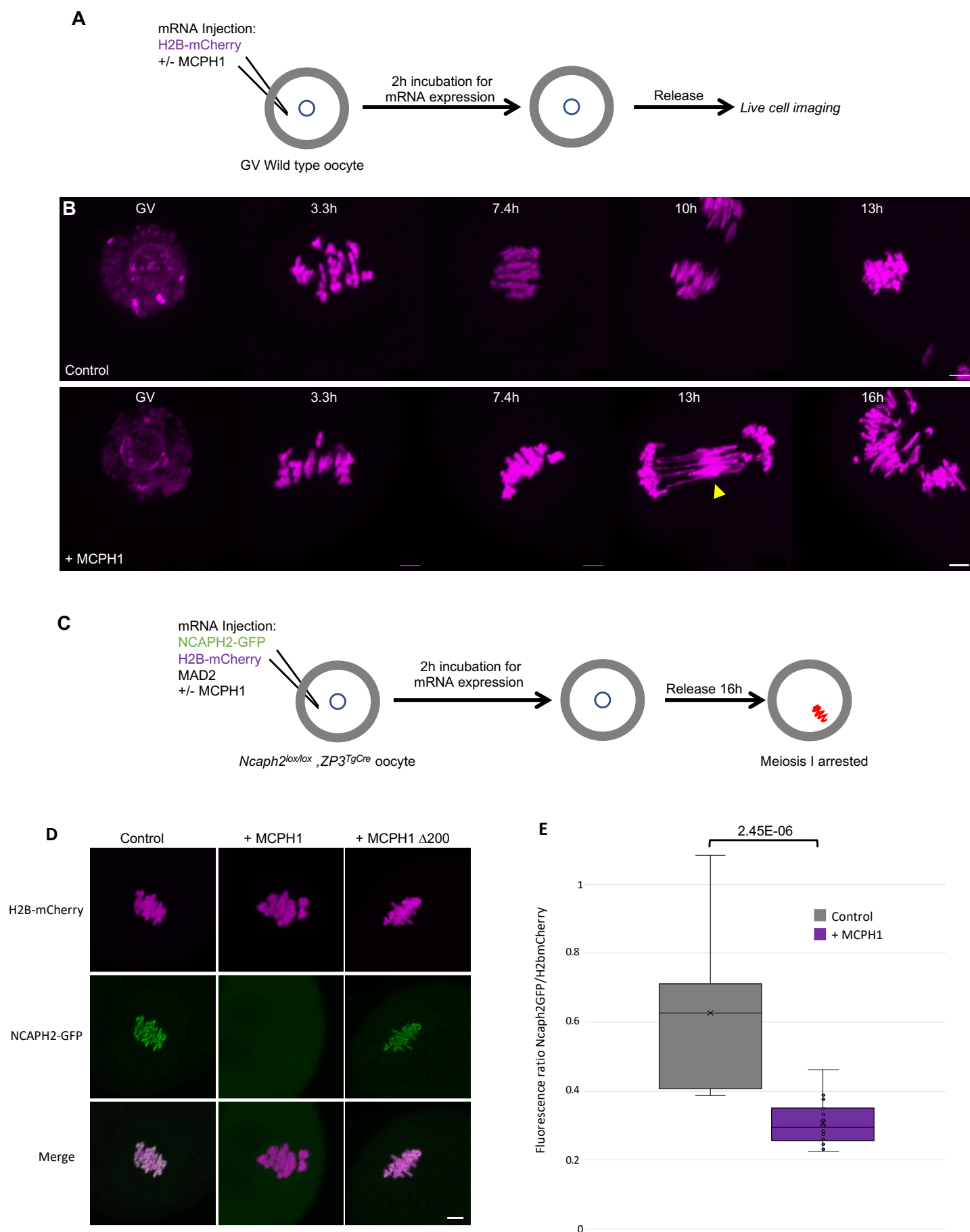


Figure 9

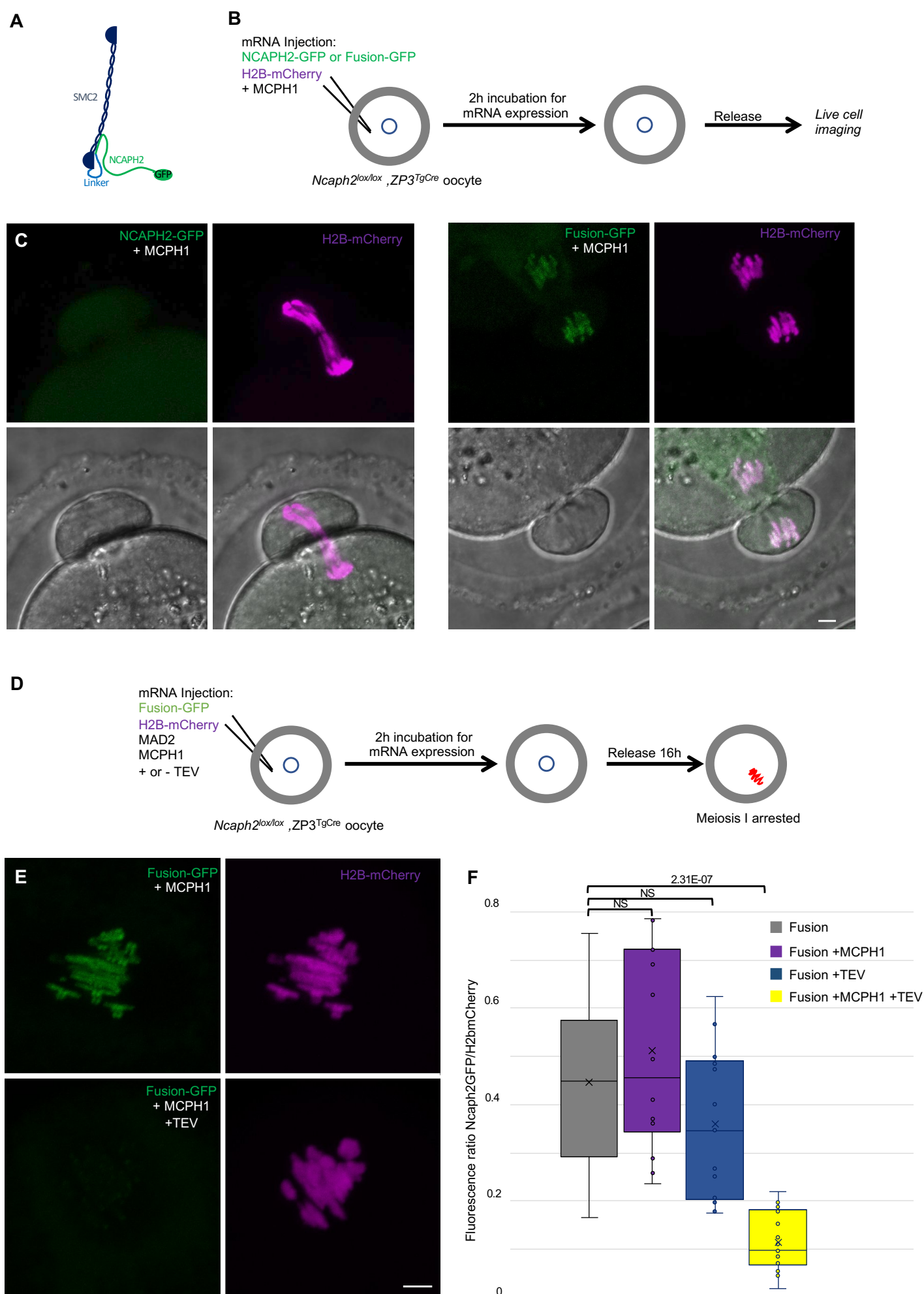


Figure 10

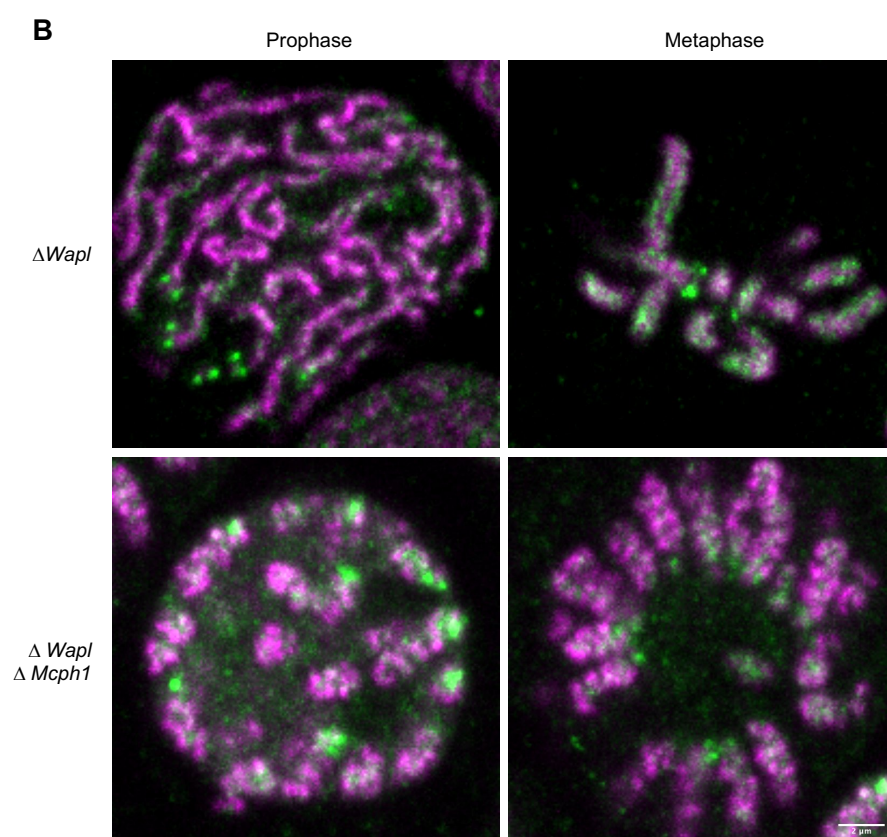
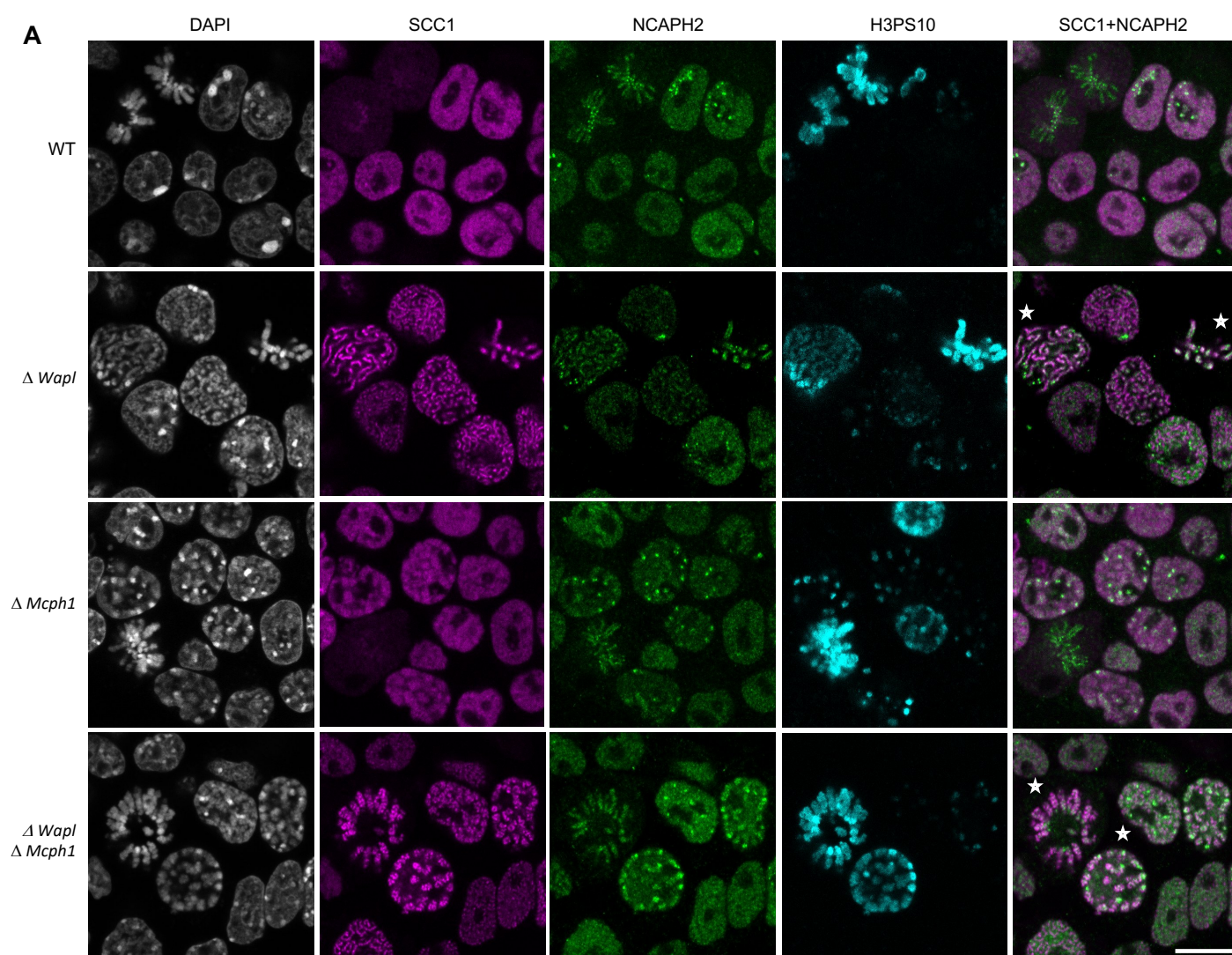
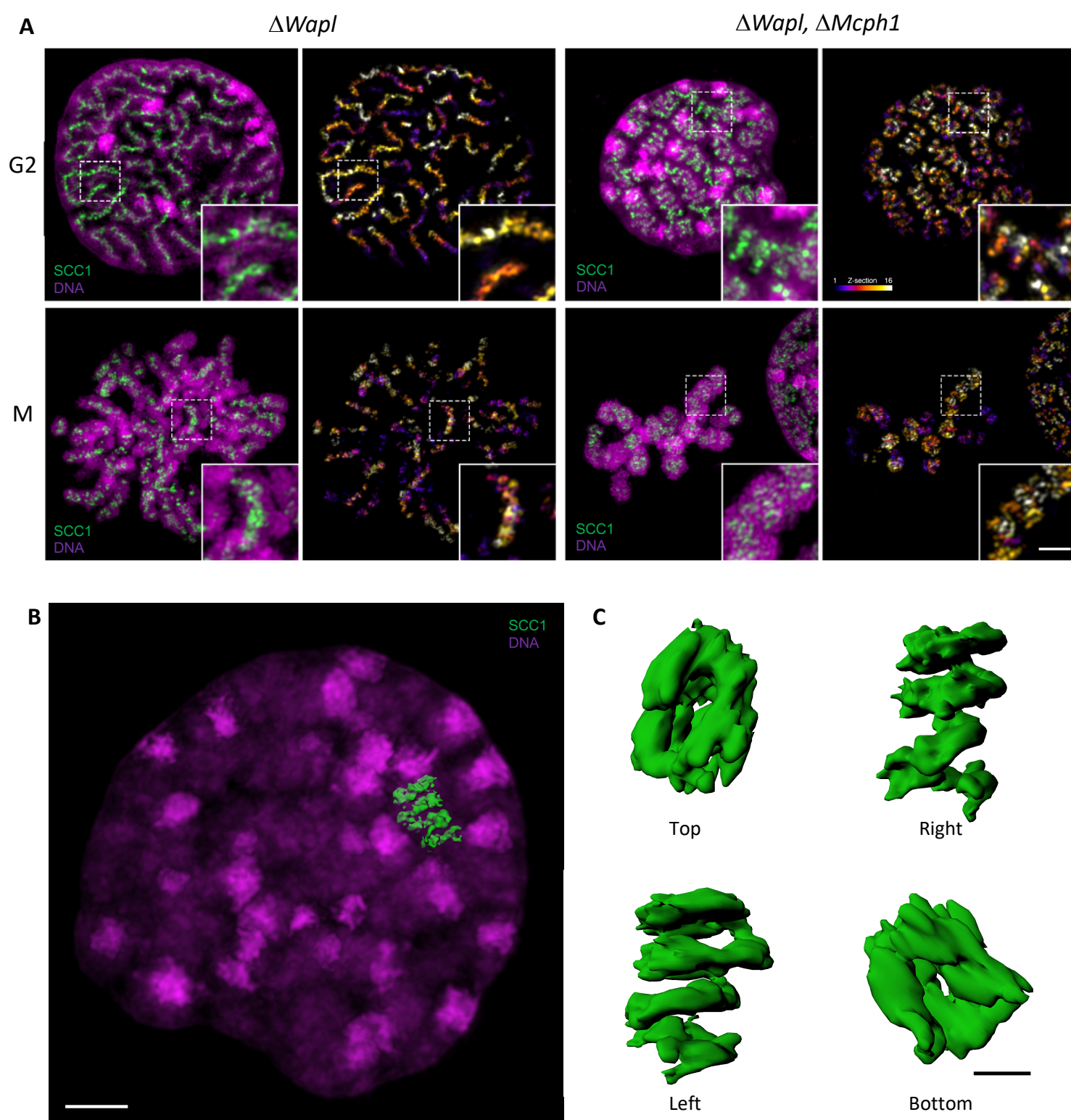
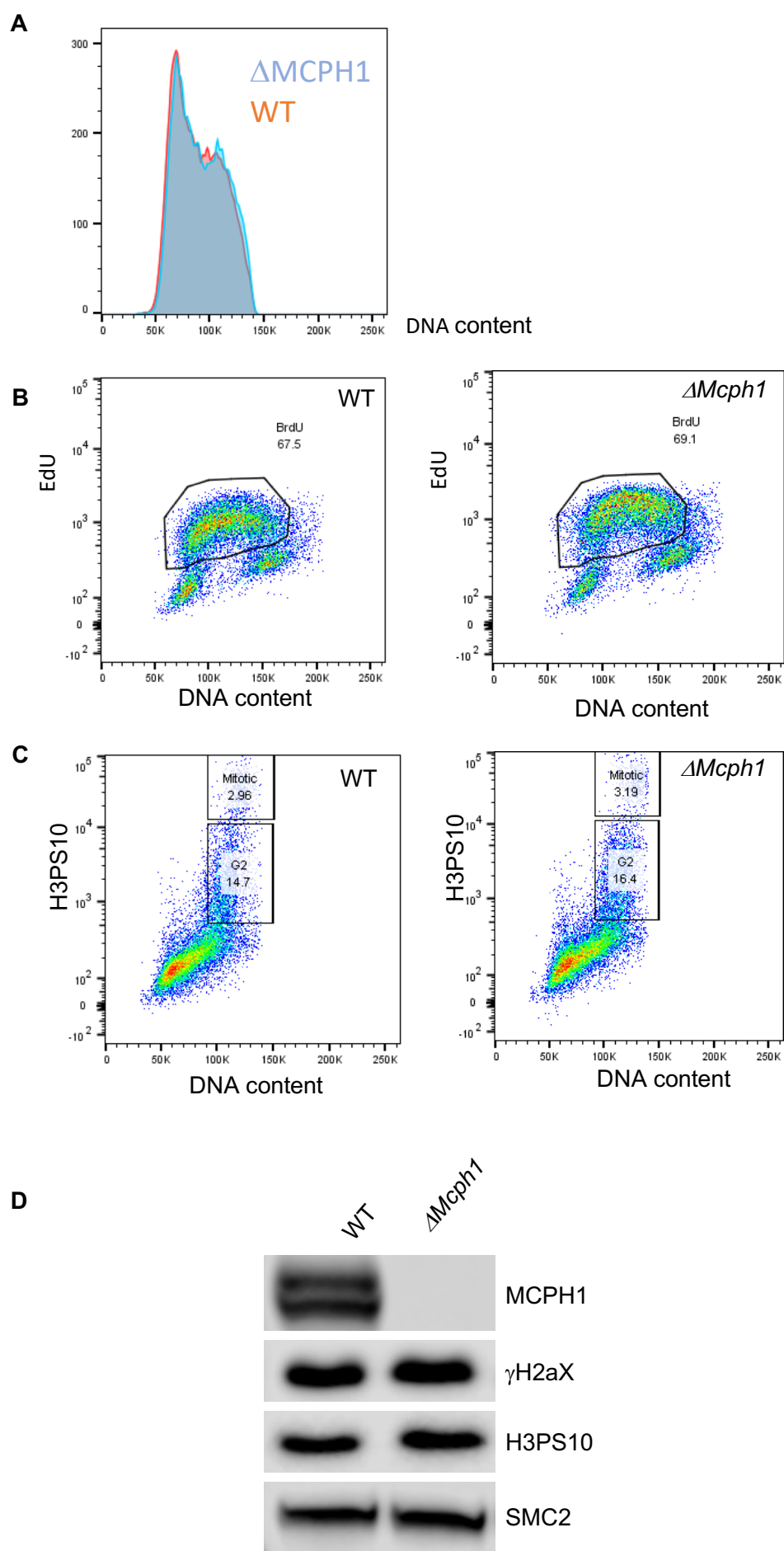


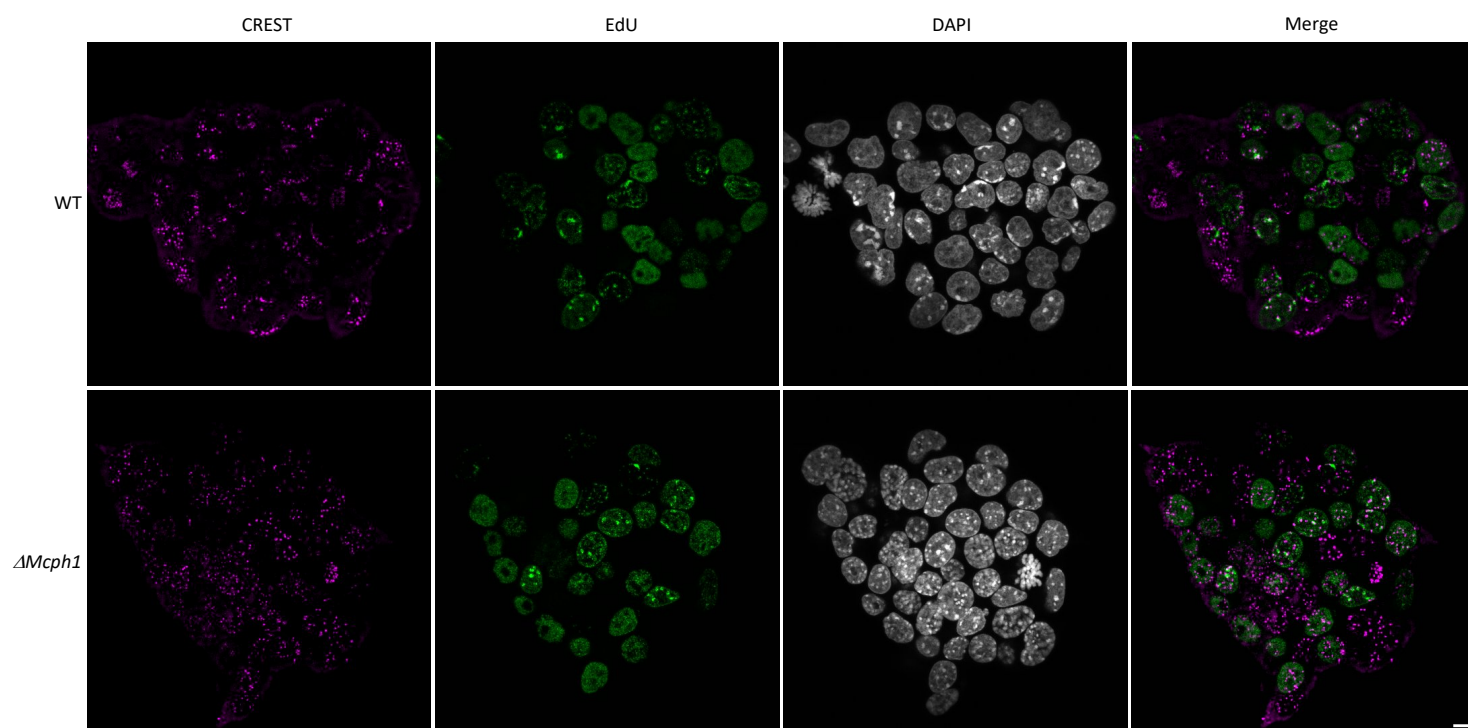
Figure 11



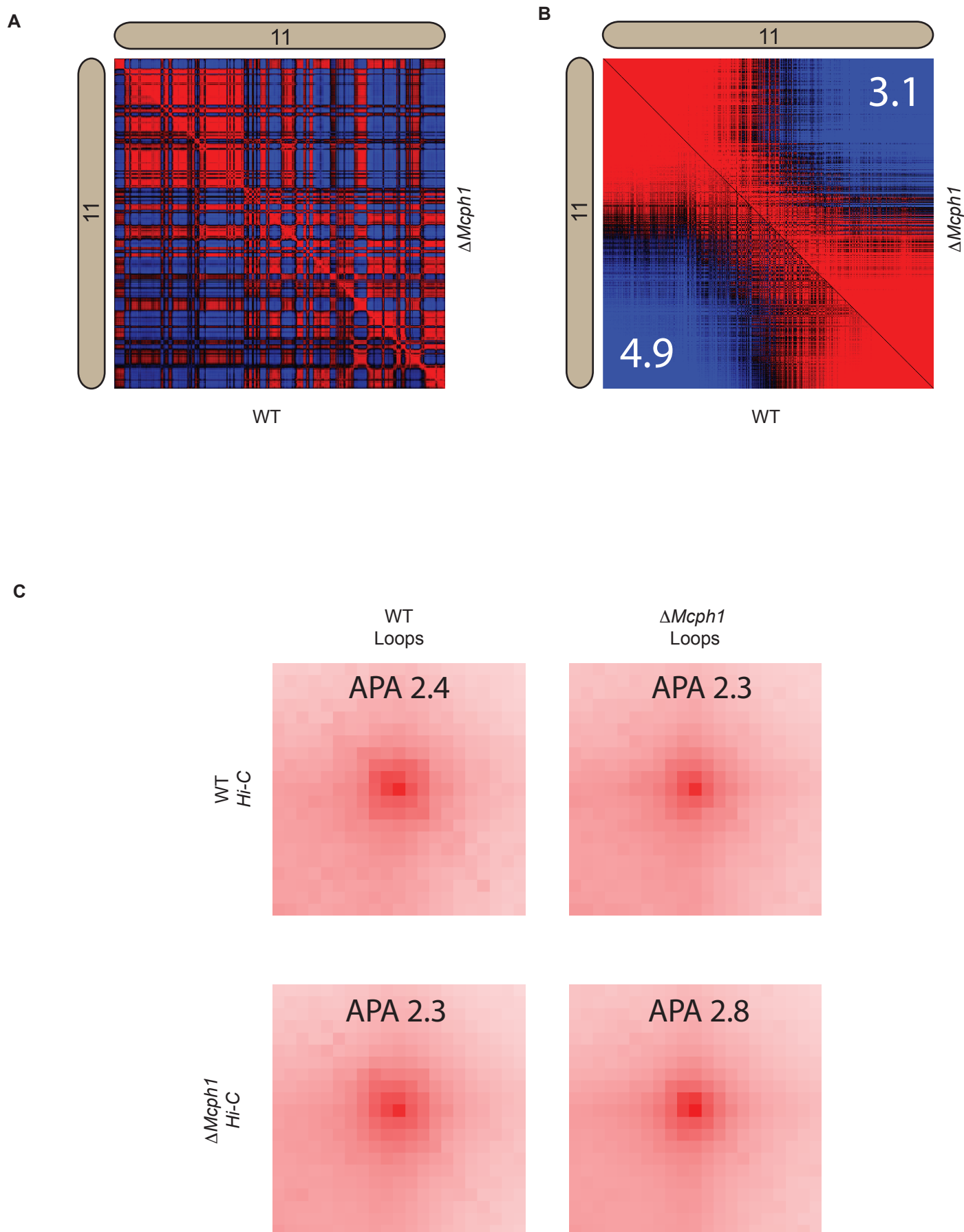
Supplementary Figure 1



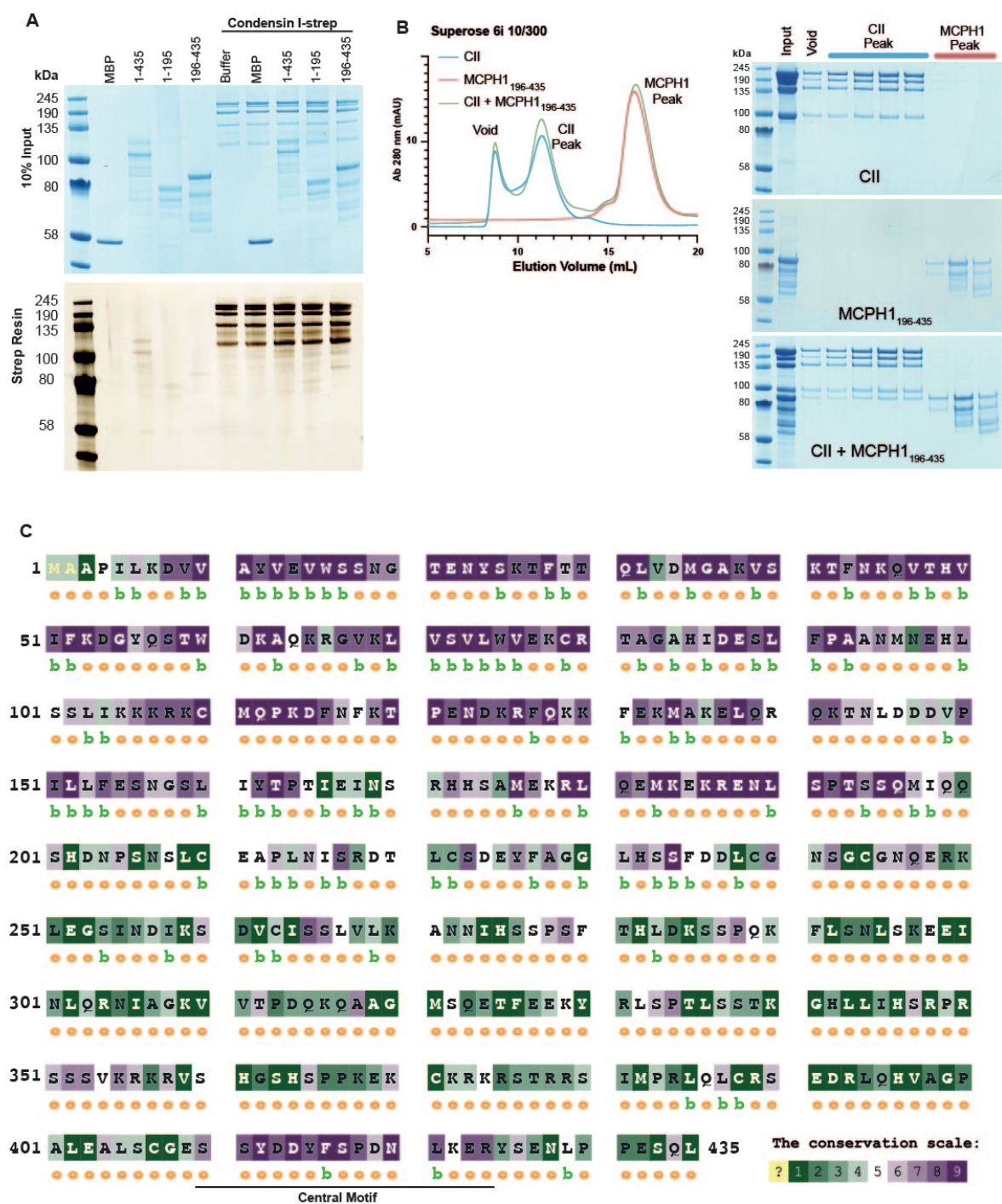
Supplementary Figure 2



Supplementary Figure 3



Supplementary Figure 4



Supplementary Figure 5

