Title: CDC20B is required for deuterosome-mediated centriole production in multiciliated cells

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Abstract: Multiciliated cells (MCCs) harbour dozens to hundreds of motile cilia, which beat in a synchronized and directional manner, thus generating hydrodynamic forces important in animal physiology. In vertebrates, MCC differentiation critically depends on the synthesis and release of numerous centrioles by specialized structures called deuterosomes. Little is known about the composition, organization and regulation of deuterosomes. Here, single-cell RNA sequencing reveals that human deuterosome-stage MCCs are characterized by the expression of many cell cycle-related genes. We further investigated the uncharacterized vertebrate-specific *cell division cycle 20B* (*CDC20B*) gene, the host gene of microRNA-449abc. We show that the CDC20B protein associates to deuterosomes and is required for the release of centrioles and the subsequent production of cilia in mouse and *Xenopus* MCCs. CDC20B interacts with PLK1, which has been shown to coordinate centriole disengagement with the protease Separase in mitotic cells. Strikingly, over-expression of Separase rescued centriole disengagement and cilia production in CDC20B-deficient MCCs. This work reveals the shaping of a new biological function, deuterosome-mediated centriole production in vertebrate MCCs, by adaptation of canonical and recently evolved cell cycle-related molecules.

Multiciliated cells (MCCs) are present throughout metazoan evolution and serve functions ranging from locomotion of marine larvae and flatworms, to brain homeostasis, mucociliary clearance of pathogens and transportation of oocytes in vertebrates[1-3]. The formation of MCCs requires the production of numerous motile cilia through a complex process called multiciliogenesis[2, 3]. The transcriptional control of multiciliogenesis has been decrypted to a large extent, through studies in *Xenopus* and mouse[2]. Seating at the top of the cascade, the Geminin-related factors GemC1[4-7] and Multicilin[8, 9] (MCIDAS in mammals) are both necessary and sufficient to initiate MCC differentiation. GemC1 and Multicilin in complex with E2F transcription factors have been reported to activate the expression of Myb, FoxJ1, Rfx2 and

45 Rfx3, which collectively regulate the expression of a large body of effectors required for the
46 formation of multiple motile cilia[4, 5, 8-11]. Recently, defective multiciliogenesis caused by
47 mutations in MCIDAS and Cyclin O (CCNO) has been associated with congenital respiratory
48 and fertility syndromes in human[12, 13].
49 Each cilium sits atop a modified centriole, called a basal body (BB). After they exit from the
50 cell cycle, maturing MCCs face the challenge of producing dozens to hundreds of centrioles in
51 a limited time window. In vertebrate MCCs, bulk centriole biogenesis is mostly achieved
52 through an acentriolar structure named the deuterosome, although canonical amplification from
53 parental centrioles also occurs[1-3]. The deuterosome was first described in early electron
54 microscopy studies of various multiciliated tissues including the mammalian lung[14] and
55 oviduct[15, 16], the avian trachea[17], and the *Xenopus* tadpole epidermis and trachea[18]. In
56 mammalian MCCs, the deuterosome was described as a spherical mass of fibers organized into
57 an inner dense region and an outer, more delicate, corona[16]. In *Xenopus*, deuterosomes were
58 initially named procentriole organizers and were reported as dense amorphous masses[18]. Recent
59 studies have revealed that deuterosome-mediated centriole synthesis mobilizes key components
60 of the centriole-dependent duplication pathway of the cell cycle, including CEP152, PLK4 and
61 SAS6[19-21]. However, the deuterosome itself differs from the centriole and may contain specific
62 components. The identification of one such component, called DEUP1 for Deuterosome
63 assembly protein 1, opened the possibility to investigate the deuterosome at the molecular
64 level[21]. In mouse tracheal ependymal cells, DEUP1 was detected in the core of the
65 deuterosome[21]. DEUP1, also known as CCDC67, is a conserved vertebrate paralogue of
66 CEP63, itself known for its importance in initiation of centriole duplication during the cell
67 cycle[21, 22]. Consistently, DEUP1 was shown to be essential for centriole multiplication in mouse
68 and *Xenopus* MCCs[21]. Both CEP63 and DEUP1 interact with CEP152, an event essential for
69 centriole duplication and multiplication in cycling cells and MCCs, respectively[21, 22]. Once

70 centriole multiplication is over, neo-synthesized centrioles must disengage from deuterosomes
71 and parental centrioles, convert into BBs and migrate apically to dock at the plasma membrane
72 to initiate cilium elongation.
73 In this study, we aimed at better understanding deuterosome biology. We found that the gene
74 *CDC20B* was specifically expressed in maturing MCCs during the phase of centriole
75 multiplication. We established the corresponding CDC20B protein as an essential regulator of
76 centriole-deuterosome disengagement. This work illustrates well the strong functional
77 relationships that exist between centriole release from deuterosomes and centriole
78 disengagement in mitotic cells. It also posits CDC20B as a new component of a "multiciliary
79 locus" that contains several gene products, either proteins, such as MCIDAS, CCNO or
80 CDC20B itself, or microRNAs, such as miR-449abc, which are all actively involved into
81 multiciliogenesis.
82
83 **Results**
84 To identify new regulators of centriole multiplication, we analyzed the transcriptome of human
85 airway epithelial cells (HAECs) at the differentiation stage corresponding to active centriole
86 multiplication[23] at the single-cell level (Fig. 1a). Gene expression data from 1663 cells was
87 projected on a 2D space by *t*-distributed Stochastic Neighbor Embedding (tSNE) (Fig. 1b). We
88 identified a small group of 37 cells corresponding to maturing MCCs engaged in deuterosome-
89 mediated centriole amplification, as revealed by the specific expression of *MCIDAS*[8], *MYB*[24],
90 and *DEUP1*[21] (Fig. 1c,d and Supplementary Fig. 1). This subpopulation was characterized by
91 the expression of known effectors of centriole synthesis, such as *PLK4*, *STIL*, *CEP152*, *SASS6*,
92 but also of cell cycle regulators, such as *CDK1*, *CCNB1*, *CDC20*, *SGOL2* and *NEK2* (Fig. 1d,
93 Supplementary Fig. 1 and Supplementary Table 1). We reasoned that uncharacterized cell
94 cycle-related genes that are specific to this subpopulation could encode new components of the

95 deuterosome-dependent centriole amplification pathway. A particularly interesting candidate
96 in this category was *CDC20B* (Fig. 1d), which is related to the cell cycle regulators *CDC20* and
97 *FZR1*[25] (Supplementary Fig. 2a). First, the *CDC20B* gene is present in the vertebrate genomic
98 locus that also contains the key MCC regulators *MCIDAS*[8] and *CCNO*[13]. Co-expression of
99 *CDC20B*, *MCIDAS* and *CCNO* throughout HAEC differentiation was indeed observed in an
100 independent RNA sequencing study, performed on a bulk population of HAECs
101 (Supplementary Fig. 2b). These results fit well with the observation that the promoter of human
102 *CDC20B* was strongly activated by the MCIDAS partners E2F1 and E2F4 (Supplementary Fig.
103 2c), as also shown in *Xenopus* by others[9] (Supplementary Fig. 2d). Second, the *CDC20B* gene
104 bears in its second intron the miR-449 microRNAs, which were shown to contribute to MCC
105 differentiation[23, 26-30]. Finally, in *Xenopus* epidermal MCCs, *cdc20b* transcripts were
106 specifically detected during the phase of centriole amplification (Supplementary Fig. 2e-m).
107 This first set of data pointed out the specific and conserved expression pattern of *CDC20B* in
108 immature MCCs. In the rest of this study, we analyzed the putative role of CDC20B in
109 deuterosome-mediated centriole multiplication,.
110 We first conducted a series of immunofluorescence analyses to gain a better understanding of
111 deuterosome organization in mouse ependymal and *Xenopus* epidermal MCCs as models. In
112 whole-mounts of mouse ependymal walls, mature deuterosomes revealed by DEUP1 staining
113 appeared as circular structures around a lumen (Fig. 2a). We noticed that DEUP1 also stained
114 fibers emanating from the core into the corona. Nascent centrioles revealed by the marker FOP
115 were organized around the DEUP1-positive core ring. STED super-resolution microscopy
116 helped to better appreciate the regular organization of individual FOP-positive procentrioles
117 (Fig. 2b). Proximity labeling assays have revealed that when ectopically expressed in
118 centrosomes CCDC67/DEUP1 is found close to Pericentrin (PCNT) and γ-tubulin, two main
119 components of the pericentriolar material (PCM)[31]. Interestingly, we found that PCNT was

120 present in the deuterosome corona (Fig. 2a), and STED microscopy further revealed that PCNT
121 formed fibers around growing procentrioles (Fig. 2b). γ-tubulin staining was detected in the
122 DEUP1-positive deuterosome core, as well as in the corona (Fig. 2a). STED microscopy
123 indicated that PCNT and γ-tubulin stained distinct interwoven fibers in the deuterosome corona.
124 Next, we stained immature *Xenopus* epidermal MCCs with γ-Tubulin and Centrin to reveal
125 centriole amplification platforms. These platforms displayed irregular shapes and sizes (Fig.
126 2c), in agreement with early electron microscopy studies[18]. Expression of low amounts of GFP-
127 Deup1 in MCCs induced by Multicilin confirmed that active deuterosomes are embedded in γ-
128 Tubulin-positive masses (Fig. 2d). Overall, this analysis is consistent with early ultrastructural
129 studies, as the deuterosome core and corona can be distinguished by the presence of DEUP1
130 and PCNT, respectively. Moreover, γ-tubulin is a conserved marker of centriole amplification
131 platforms in vertebrate MCCs. By analogy to the organization of the centrosome, we propose
132 to coin the term perideuterosomal material (PDM) to describe the corona, as this region may
133 prove important for deuterosome function.
134 We then analyzed the subcellular localization of CDC20B protein in deuterosome-stage mouse
135 and *Xenopus* MCCs. In immature mouse tracheal MCCs, double immunofluorescence revealed
136 the association of CDC20B to DEUP1-positive deuterosomes (Fig. 3a). We noticed that
137 CDC20B tended to associate primarily to large DEUP1 foci. As deuterosomes grow as they
138 mature[21], this suggests that CDC20B may penetrate into the deuterosomal environment at a late
139 stage of the centriole multiplication process. The same observation was made when comparing
140 CDC20B staining in the region of immature and mature deuterosomes of mouse ependymal
141 MCCs (Fig. 3b). As double DEUP1/CDC20B staining could not be performed on these cells,
142 we analyzed CDC20B distribution relative to FOP-positive procentrioles. In early
143 deuterosome-stage MCCs, CDC20B was expressed at low levels and FOP staining was mostly
144 concentrated in a large amorphous cloud (Fig. 3b). In such cells, no CDC20B staining was

145 detected in association to FOP-positive procentrioles growing around deuterosomes. In
146 contrast, in mature deuterosome-stage MCCs, CDC20B was enriched in the innermost part of
147 the PDM, probably very close to the deuterosome core (Fig. 3b). Further evidence was provided
148 with a custom-made polyclonal antibody (Supplementary Fig. 3b,c) used to analyze Cdc20b
149 protein distribution in *Xenopus* epidermal MCCs. Here also, Cdc20b was found associated to
150 Deup1-positive deuterosomes actively engaged in centriole synthesis (Fig. 3c). We finally
151 analyzed the distribution of CDC20B in mature MCCs. As previously reported, the CDC20B
152 protein was detected near BBs[23], but also in cilia of fully differentiated human airway MCCs
153 (Supplementary Fig. 4a-c). This was confirmed by proximity ligation assays that revealed a
154 tight association of CDC20B with Centrin2 and acetylated α-Tubulin, in BBs and cilia,
155 respectively (Supplementary Fig. 4d-f). Fluorescent immunostaining also revealed the presence
156 of Cdc20b in the vicinity of BBs in *Xenopus* epidermal MCCs (Supplementary Fig. 4g-i). In
157 contrast, no cilia staining was observed in these cells. We conclude that in three distinct types
158 of MCCs in two distant vertebrate species, CDC20B is tightly associated to mature
159 deuterosomes. We next investigated whether it may control their function.
160 For that purpose, *Cdc20b* was knocked down in mouse ependymal MCCs, through post-natal
161 brain electroporation of three distinct shRNAs. One of them, sh274, which targets the junction
162 between exons 3 and 4, and can therefore only interact with mature mRNA, was useful to rule
163 out possible interference with the production of miR-449 molecules from the *Cdc20b* pre-
164 mRNA (Supplementary Fig. 5a). Five days after electroporation, all three shRNAs significantly
165 reduced the expression of CDC20B in deuterosome-stage MCCs (Fig. 4c), but did not alter
166 MCC identity as revealed by FOXJ1 expression (Fig. 4a,b,d). Centriole production by
167 deuterosomes was analyzed by FOP/DEUP1 double staining nine days after electroporation. At
168 this stage, control MCCs had nearly all released their centrioles and disassembled their
169 deuterosomes (Fig. 4e,g). In sharp contrast, *Cdc20b* shRNAs caused a significant increase in

170 the number of defective MCCs that displayed centrioles still engaged on deuterosomes (Fig.
171 4f,g). Fifteen days after electroporation, a majority of CDC20B-deficient MCCs still showed a
172 severely reduced number of released centrioles, and consequently lacked cilia (Fig. 4h-k).
173 *Cdc20b* was also knocked down in *Xenopus* epidermal MCCs, through injection of two
174 independent morpholino antisense oligonucleotides targeting either the ATG (Mo ATG), or the
175 exon 1/intron 1 junction (Mo Spl) (Supplementary Fig. 5b). The efficiency of Mo ATG was
176 verified through fluorescence extinction of co-injected Cdc20b-Venus (Supplementary Fig. 5c).
177 RT-PCR confirmed that Mo Spl caused intron 1 retention (Supplementary Fig. 5d), which was
178 expected to introduce a premature stop codon, and to produce a Cdc20b protein lacking 96%
179 of its amino-acids, likely to undergo unfolded protein response-mediated degradation. Thus,
180 both morpholinos were expected to generate severe loss of Cdc20b function. Consistent with
181 this interpretation, both morpholinos strongly reduced Cdc20b immunostaining in
182 deuterosome-stage MCCs (Supplementary Fig. 5e). We verified that neither morpholinos
183 caused *p53* transcript up-regulation (Supplementary Fig. 5f), a non-specific response to
184 morpholinos that is sometimes detected in zebrafish embryos[32]. Importantly, whole-mount *in*
185 *situ* hybridization indicated that miR-449 expression was not perturbed in the presence of either
186 morpholino (Supplementary Fig. 5g). We found that *cdc20b* knockdown did not interfere with
187 acquisition of the MCC fate (Supplementary Fig. 6a-e), but severely impaired multiciliogenesis,
188 as revealed by immunofluorescence and electron microscopy (Fig. 5a-i). This defect stemmed
189 from a dramatic reduction in the number of centrioles, and poor docking at the plasma
190 membrane (Fig. 5g-o and Supplementary Fig. 6f-k). Importantly, centrioles and cilia were
191 rescued in Mo Spl MCCs by co-injection of *cdc20b*, *venus-cdc20b* or *cdc20b-venus* mRNAs
192 (Fig. 5j-o and Supplementary Fig. 6f-k). In normal condition, *Xenopus* epidermal MCCs arise
193 in the inner mesenchymal layer and intercalate into the outer epithelial layer, while the process
194 of centriole amplification is underway[33]. To rule out secondary defects due to poor radial

195 intercalation, we assessed the consequences of *cdc20b* knockdown in MCCs induced in the
196 outer layer by Multicilin overexpression[8]. Like in natural MCCs, Cdc20b proved to be essential
197 for the production of centrioles and cilia in response to Multicilin activity (Supplementary Fig.
198 7a-g). We also noted that the apical actin network that normally surrounds BBs was not
199 maintained in absence of Cdc20b, although this defect could be secondary to the absence of
200 centrioles (Supplementary Fig. 7d-g). Centrioles in Cdc20b morphant cells often formed
201 clusters, suggesting that disengagement from deuterosomes could have failed (Fig. 5l,m). To
202 better assess this process we injected GFP-Deup1 in Multicilin-induced MCCs and stained
203 centrioles with Centrin. In mature control MCCs, deuterosomes were disassembled, centrioles
204 were converted into BBs, had docked and initiated cilium growth (Fig. 5p,s). In contrast, both
205 morpholinos caused a dramatic increase in the number of defective MCCs, which were devoid
206 of cilia and displayed centrioles still engaged on deuterosomes (Fig. 5q-u). Altogether our
207 functional assays in mouse and *Xenopus* indicate that CDC20B is required for centriole
208 disengagement from deuterosomes and subsequent ciliogenesis in MCCs. We next investigated
209 the molecular mechanism of action of CDC20B underlying its role in centriole release.
210 In mitotic cells, centriole disengagement is necessary to license centriole duplication in the
211 following cell cycle[34]. This process is known to depend on the coordinated activities of the
212 mitotic kinase PLK1 and the protease Separase[35]. One proposed mechanism involves the
213 phosphorylation of PCNT by PLK1, which induces its cleavage by Separase, thereby allowing
214 centriole disengagement through disassembly of the PCM[36, 37] (Fig. 7h). Separase is known to
215 be activated by the degradation of its inhibitor Securin, which is triggered by the Anaphase
216 Promoting Complex (APC/C) upon binding to CDC20[25]. *PLK1*, *Separase* (*ESPL1*), *Securin*
217 (*PTTG1*), *CDC20* and *PCNT* were all found to be expressed in human deuterosome-stage
218 MCCs (Fig. 1d and Supplementary Fig. 1). We have shown above that PCNT is present in the
219 PDM and a recent study revealed the presence of CDC20 and the APC/C component APC3 in

220 mouse ependymal MCCs at the stage of centriole disengagement[38]. Based on this large body of
221 information, we hypothesized that centriole-deuterosome disengagement involves the
222 coordinated activities of PLK1 and Separase, and that CDC20B would be involved in this
223 scenario. *CDC20B* encodes a protein of about 519 amino-acids largely distributed across the
224 vertebrate phylum[23]. In its C-terminal half, CDC20B contains seven well conserved WD40
225 repeats, predicted to form a β-propeller, showing 49% and 37% identity to CDC20 and FZR1
226 repeats, respectively (Supplementary Fig. 2a). However, CDC20B lacks canonical APC/C
227 binding domains (Supplementary Fig. 2a). Using mass spectrometry on immunoprecipitated
228 protein complexes from transfected HEK cells, we could identify multiple APC/C components
229 interacting with CDC20 but not with CDC20B (Supplementary Table 2). We conclude that
230 CDC20B is probably incapable of activating APC/C. Interestingly, an unbiased interactome
231 study reported association of CDC20B with PLK1[39]. Using reciprocal co-immunoprecipitation
232 assays in HEK transfected cells, we confirmed that CDC20B and PLK1 could be found in the
233 same complex (Fig. 6a). This suggested that CDC20B could cooperate with PLK1 to trigger
234 centriole disengagement. Consistent with this hypothesis, we found that PLK1 was enriched in
235 the PDM of mature deuterosomes in mouse ependymal MCCs (Fig. 6d), in agreement with a
236 recent report[38]. Another interesting partner of CDC20B identified in a second unbiased
237 interactome study[40] was SPAG5 (Astrin), which was reported to control timely activation of
238 Separase during the cell cycle[41, 42]. Using the same strategy as above, we could detect CDC20B
239 and SPAG5 in the same complex (Fig. 6b). As SPAG5 was found associated to DEUP1 in a
240 proximity labeling assay[31], we assessed its localization in deuterosomes. Strikingly, SPAG5
241 was detectable in mature deuterosomes of mouse ependymal MCCs, with a clear enrichment in
242 the deuterosome core (Fig. 6d). Finally, reciprocal co-immunoprecipitations revealed that
243 CDC20B and DEUP1 were detected in the same complex when co-expressed in HEK cells (Fig.
244 6c). Consistent with this result, we observed that RFP-Cdc20b was recruited around spherical

245 Deup1-GFP structures positive for γ-Tubulin and Centrin in *Xenopus* epidermal MCCs
246 (Supplementary Fig. 7h-m). This series of experiments suggested that CDC20B could
247 participate in the assembly of a protein complex in mature deuterosomes, required to coordinate
248 the activities of PLK1 and Separase for centriole disengagement. As Separase is the last effector
249 in this scenario, we tested whether over-expressing human Separase in *Xenopus cdc20b*
250 morphant MCCs could rescue centriole disengagement. In support to our hypothesis, over-
251 expression of wild-type, but not protease-dead Separase, efficiently rescued centriole
252 disengagement and cilia formation in *cdc20b* morphant MCCs (Fig. 7a-g and Supplementary
253 Fig. 7n-s). Separase could also rescue multiciliogenesis in Multicilin-induced MCCs injected
254 with *cdc20b* Mos (Supplementary Fig. 7t-z). We conclude that CDC20B is involved in
255 Separase-mediated release of mature centrioles from deuterosomes in vertebrate MCCs (Fig.
256 7h).
257
258 **Discussion**
259 In this study, we report for the first time the essential and conserved role of CDC20B in
260 vertebrate multiciliogenesis. Our data suggest that the presence of CDC20B in the
261 perideuterosomal region is necessary to allow Separase-dependent proteolysis leading to
262 centriole disengagement. Our findings are consistent with a recent report showing that centriole
263 disengagement in murine ependymal MCCs involves the activities of PLK1 and APC/C[38]. How
264 are CDC20B and Separase activities integrated? The simple scenario of a CDC20-like function
265 of CDC20B is very unlikely as it does not appear to bind APC/C (Supplementary Table 2).
266 CDC20 was detected in cultured murine ependymal MCCs during the phase of centriole
267 disengagement[38], and FZR1 genetic ablation was reported to cause reduced production of
268 centrioles and cilia in the same cells[43]. APC/C is therefore likely activated in maturing MCCs
269 by its classical activators, CDC20 and/or FZR1, leading to Separase activation through

270 degradation of its inhibitor Securin. In that context, we propose that additional factors linked
271 directly or indirectly to CDC20B may contribute to activation of Separase. It was shown that
272 SPAG5 inhibits or activates Separase depending on its status of phosphorylation[41, 42]. As the
273 phosphorylation status of SPAG5 was shown to be controlled by PLK1[44], our data suggest that
274 the CDC20B/PLK1/SPAG5 complex could control the timing of Separase activation locally in
275 deuterosomes. It is therefore possible that multiple modes of activation of Separase may act in
276 parallel to trigger the release of neo-synthesized centrioles in maturing MCCs. Alternatively,
277 different pathways may be used in distinct species, or in distinct types of MCCs. An important
278 question for future studies regards the identity of PLK1 and Separase substrates involved in
279 centriole disengagement. Work on mitotic cells[36, 37] and our own analysis suggest that PCNT
280 may represent a prime target. Another potentially relevant candidate could be DEUP1 itself as
281 it is clear that deuterosomes are disassembled after the release of centrioles. In that respect, it
282 is interesting to note the presence of multiple PLK1 consensus phosphorylation sites in human,
283 mouse and *Xenopus* DEUP1.
284 In this study, we have introduced the notion of perideuterosomal material, in analogy to the
285 pericentriolar material. It is striking that the two main components of the PCM, PCNT and γ-
286 Tubulin, are also present in the PDM, which begs the question whether additional PCM proteins
287 may be present in the PDM. The PDM may constitute a platform to sustain procentriole growth,
288 through the concentration and delivery of elementary parts. It could also play a mechanical role
289 to hold in place the growing procentrioles. Future work should evaluate deuterosome-mediated
290 centriole synthesis in absence of major PDM components.
291 We found that beyond its association to deuterosomes during the phase of centriole
292 amplification, CDC20B was also associated to BBs and cilia in fully differentiated mammalian
293 MCCs. This dual localization is consistent with failed ciliogenesis upon CDC20B knockdown
294 in mouse ependymal MCCs. However, while we could detect Cdc20b near BBs of mature

295 MCCs in *Xenopus*, we found no evidence of its presence in cilia. Furthermore, cilia were
296 rescued by Separase overexpression in Cdc20b morphant MCCs. This suggests that Cdc20b is
297 not required for ciliogenesis in this species, although it could potentially contribute to cilium
298 structure and/or function. Thus, refined temporal and spatial control of CDC20B inhibition will
299 be needed to study its function beyond centriole synthesis.
300 This and previous studies[23, 26-28] establish that the miR-449 cluster and its host gene *CDC20B*
301 are commonly involved in multiciliogenesis. Consistent with its early expression, it was
302 suggested that miR-449 controls cell cycle exit and entry into differentiation of MCCs[23, 27, 30].
303 This study reveals that CDC20B itself is involved in the production of centrioles, the first key
304 step of the multiciliogenesis process. From that perspective, the nested organization of miR-
305 449 and *CDC20B* in vertebrate genomes, which allows their coordinated expression, appears
306 crucial for successful multiciliogenesis.
307 It is also noteworthy to point out the location of this gene in a genomic locus where congenital
308 mutations in MCIDAS and CCNO were recently shown to cause a newly-recognized MCC-
309 specific disease, called Reduced Generation of Multiple motile Cilia (RGMC). RGMC is
310 characterized by severe chronic lung infections and increased risk of infertility[12, 13]. Its location
311 in the same genetic locus as MCIDAS and CCNO makes CDC20B a putative candidate for
312 RGMC. By extension, new deuterosome-stage specific genes uncovered by scRNA-seq in this
313 study also represent potential candidates for additional RGMC mutations.
314 Previous works have established the involvement of the centriole duplication machinery active
315 in S-phase of the cell cycle, during centriole multiplication of vertebrate post-mitotic MCCs[19-
316 21]. Our study further reveals a striking analogy between centriole disengagement from
317 deuterosomes in MCCs, and centriole disengagement that occurs during the M/G1 transition of
318 the cell cycle (Fig. 7g). Thus, it appears that centriole production in MCCs recapitulates the key
319 steps of the centriole duplication cycle[34]. However, the cell cycle machinery must adapt to the

320 acentriolar deuterosome to massively produce centrioles. Such adaptation appears to involve
321 physical and functional interactions between canonical cell cycle molecules, such as CEP152
322 and PLK1, and recently evolved cell cycle-related deuterosomal molecules, such as DEUP1[21]
323 and CDC20B. It remains to examine whether additional deuterosomal cell cycle-related
324 molecules have emerged in the vertebrate phylum to sustain massive centriole production.
325 In conclusion, this work illustrates how coordination between ancestral and recently evolved
326 cell cycle-related molecules can give rise to a new differentiation mechanism in vertebrates.
327
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452
453

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474 **Author contributions**

475 PB, BM and LK designed and supervised the study, and obtained funding. LEZ, SRG, OM
476 performed and analyzed human and mouse airway cells experiments. DRR and VT performed
477 and analyzed *Xenopus* experiments. CB performed and analyzed all experiments on mouse
478 ependymal MCCs and contributed to the description of *Xenopus* deuterosomes. OR
479 characterized CDC20B antibodies. MD and AP performed the bioinformatic analysis. NP
480 carried out scRNAseq experiments. ASG performed mass spectrometry analyses. GP designed
481 and performed CDC20B interaction studies. All authors were involved in data interpretation.

DRR, LEZ and CB designed the figures. LK drafted the original manuscript. DRR, LEZ, CB, BM, PB and LK edited the manuscript.

Competing financial interests

The authors declare no competing financial interests.

Figures and legends

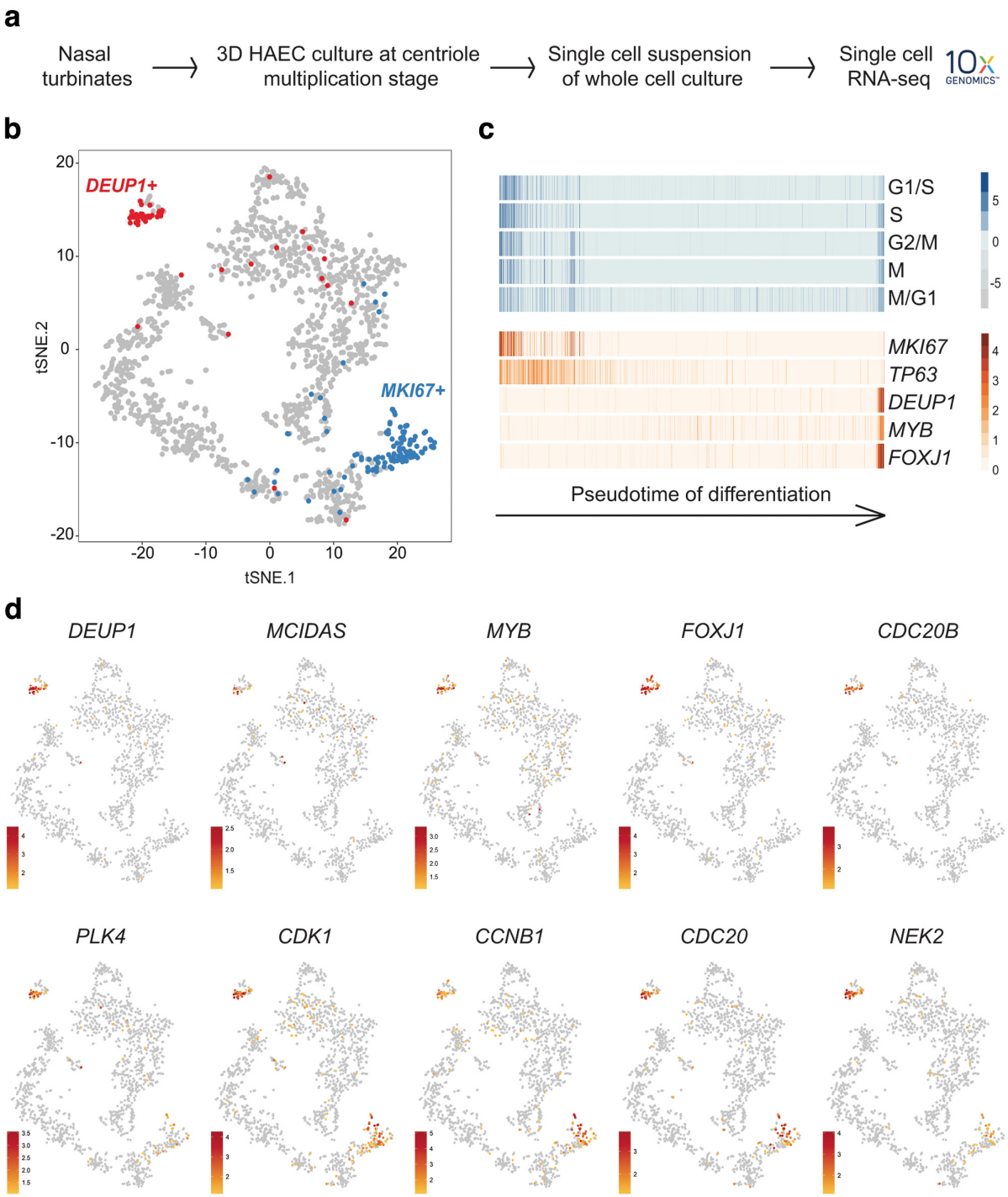
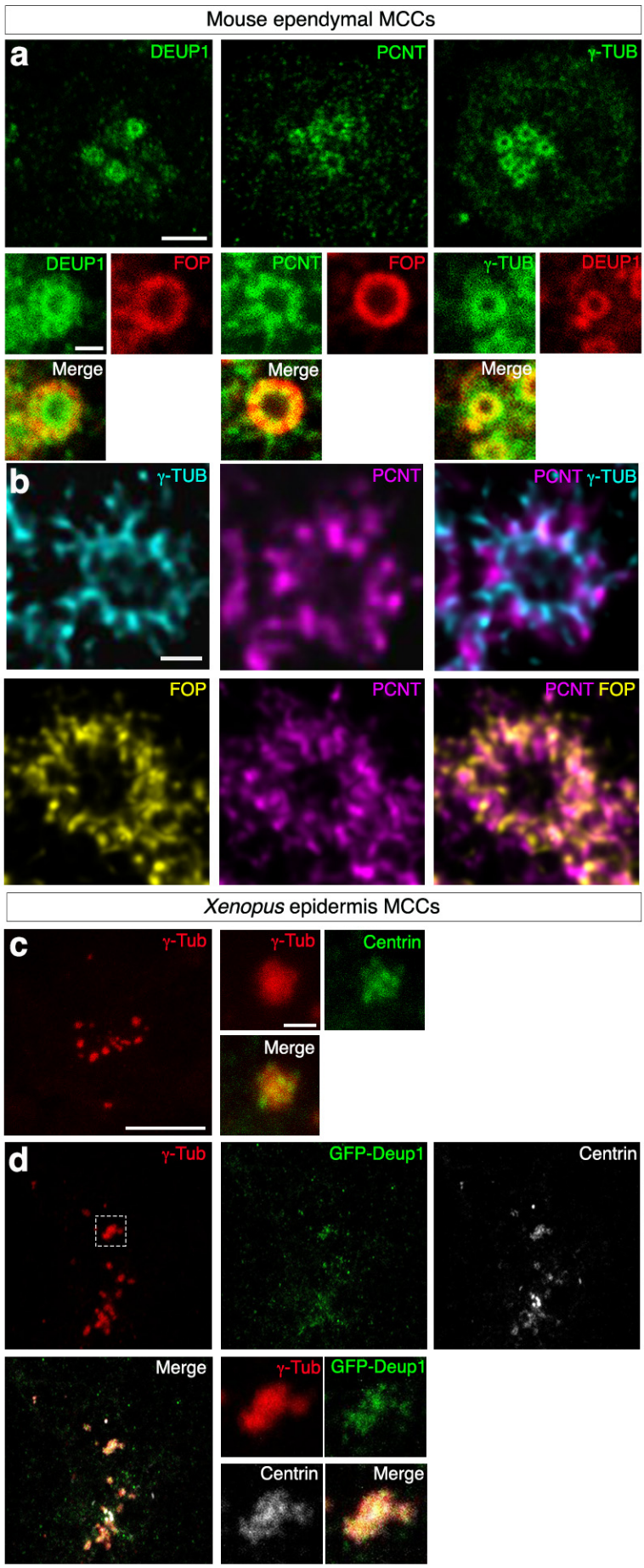


Figure 1: Single-cell RNA-seq analysis reveals *CDC20B* as a novel specific marker of deuterosomal stage MCCs, and enrichment of cell cycle-related genes in this cell population.

(**a**) Experimental design of the scRNA-seq experiment. (**b**) tSNE plot. Each point is a projection of a unique cell on a 2D space generated by the tSNE algorithm. Blue dots represent *MKI67*-

497 positive proliferating cells, and red dots represent *DEUP1*-positive cells corresponding to
498 maturing MCCs at deuterosome stage. (**c**) Cell cycle-related gene set expression in HAECs
499 measured by scRNA-seq. Cells were ordered along a pseudotime axis, defined with the
500 Monocle2 package. Phase specific scores are displayed in the top heatmap. Expression of
501 selected genes is displayed in the bottom heatmap. (**d**) tSNEs plots for a selection of genes
502 specifically enriched in deuterosome-stage cells. Note that *CDC20B* exhibits the most specific
503 expression among deuterosome marker genes.
504


Mouse ependymal MCCs
a
DEUP1
PCNT
γ-TUB
DEUP1
FOP
PCNT
FOP
γ-TUB
DEUP1
Merge
Merge
Merge
b
γ-TUB
PCNT
PCNT γ-TUB
FOP
PCNT
PCNT FOP
Xenopus epidermis MCCs
c
γ-Tub
γ-Tub
Centrin
Merge
d
γ-Tub
GFP-Deup1
Centrin
Merge
γ-Tub
GFP-Deup1
Centrin
Merge

505

506 **Figure 2: Composition and organization of vertebrate deuterosomes**

507 (**a-b**) Maturing mouse ependymal MCCs were immunostained as indicated, pictures were taken
508 with confocal (**a**) or STED (**b**) microscope. (**a**) DEUP1 stains the deuterosome core (ring) and
509 a close fibrous area that defines the perideuterosomal region. The centriolar marker FOP reveals
510 procentrioles arranged in a circle around the deuterosome. Pericentrin (PCNT) is enriched in
511 the perideuterosomal region. γ-Tubulin (γ-TUB) stains the core as well as the periphery of the
512 deuterosome. (**b**) STED pictures showing the organization of FOP, PCNT and γ-TUB in the
513 perideuterosomal region. (**c**) *Xenopus* embryos were immunostained for γ-Tubulin (γ-Tub) and
514 Centrin and high-magnification pictures of immature epidermal MCCs were taken. In these
515 cells, Centrin-positive procentrioles grow around γ-Tubulin positive structures. (**d**) *Xenopus*
516 embryos were injected with *Multicilin-hGR* and *GFP-Deup1* mRNAs, treated with
517 dexamethasone at gastrula st11 to induce Multicilin activity, and immunostained at neurula st18
518 for γ-Tubulin, GFP and Centrin. Scale bars: 5µm (**a**, top), 500nm (**a**, bottom), 500nm (**b**), 10µm
519 (**c**, **d**, large view), 1µm (**c**, **d**, high magnification).

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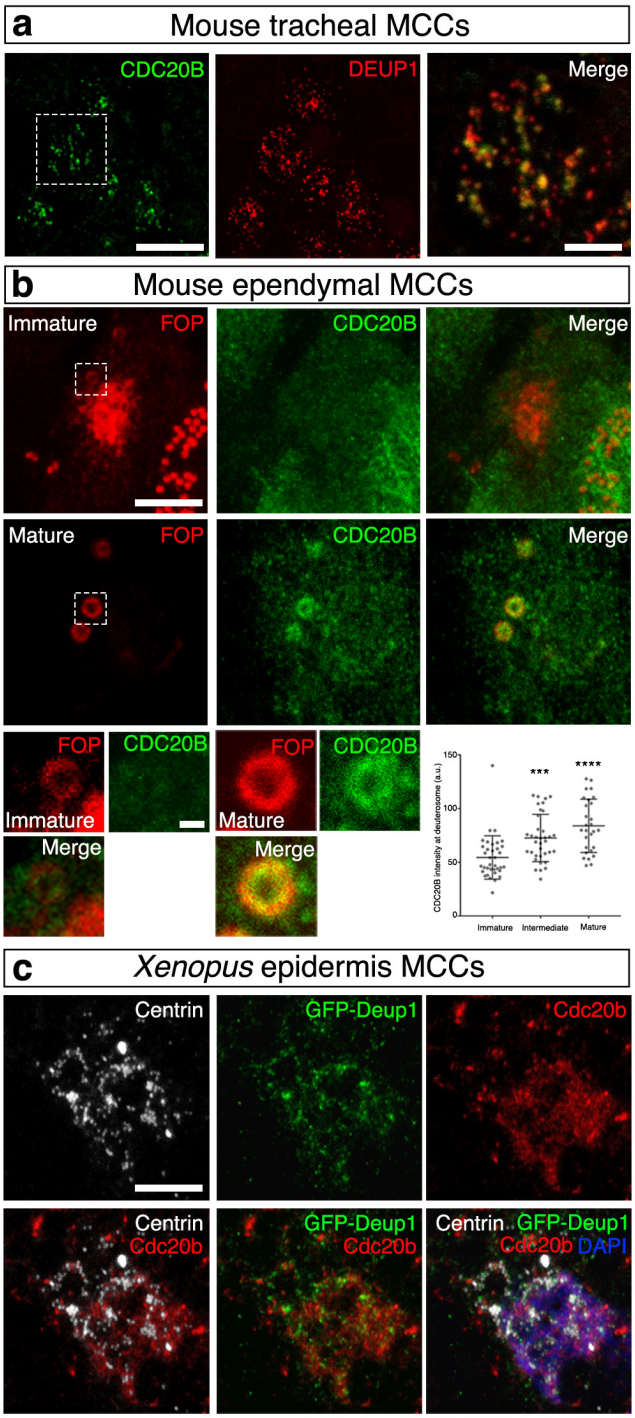
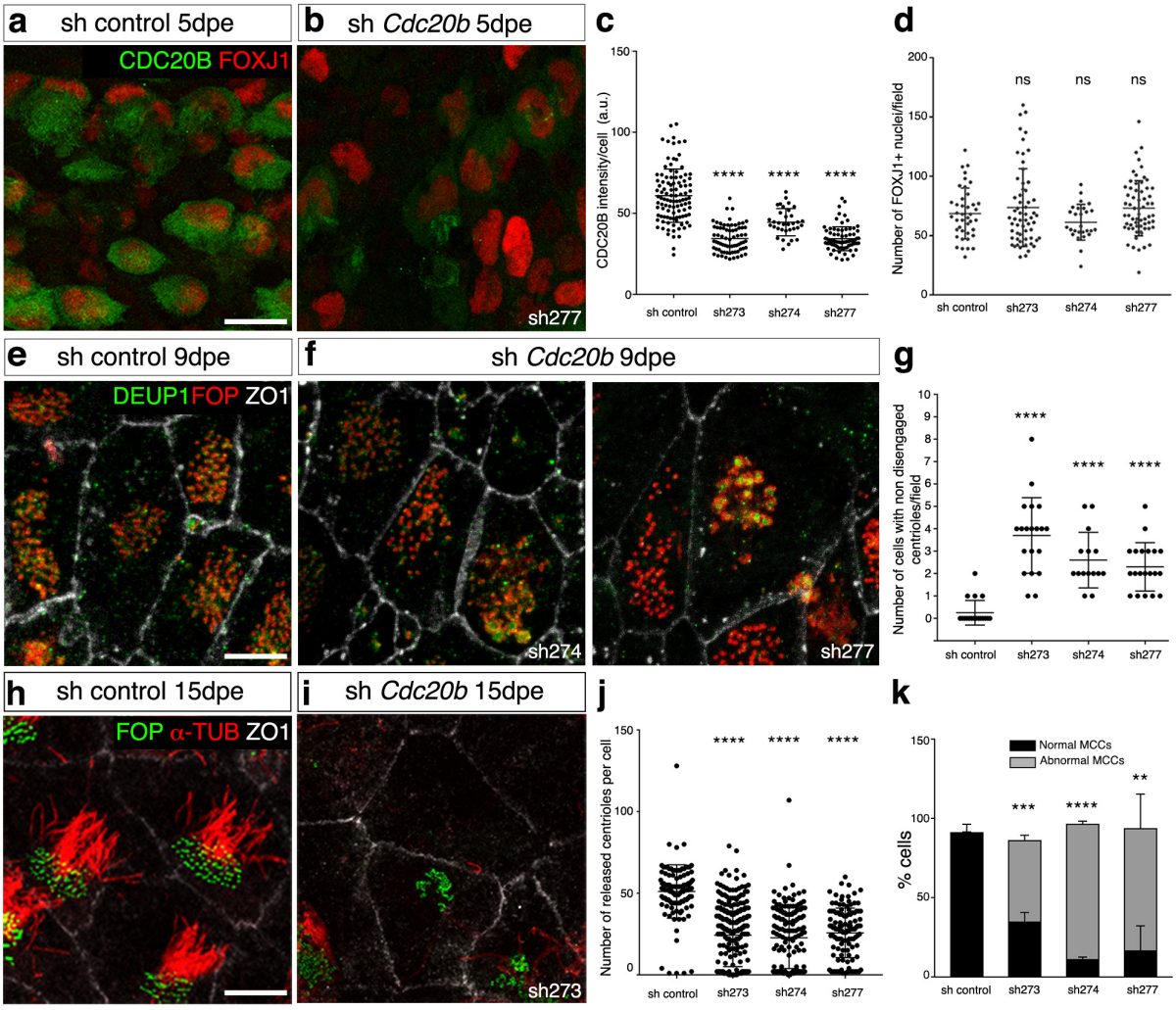


521

522 **Figure 3: CDC20B is a component of vertebrate deuterosomes**

523 (**a**) Double immunofluorescence was performed on mouse tracheal MCCs after 3 days of
524 culture in air-liquid interface. Low magnification confocal panels show coincident CDC20B
525 and DEUP1 staining in several individual MCCs. High magnification on a single MCC reveals
526 the prominent association of CDC20B to deuterosomes marked by DEUP1. (**b**) Mouse

527 ependymal MCCs were immunostained as indicated, and high magnification confocal pictures
528 of cells with immature and mature deuterosomal figures were taken. In these cells, centrioles
529 revealed by FOP form a ring around deuterosomes. CDC20B staining forms a ring inside the
530 ring of FOP-positive procentrioles indicating that CDC20B is tightly associated to
531 deuterosomes. Note that the CDC20B signal associated to deuterosome increased with their
532 maturation (high-magnification pictures quantified in the graph). (**c**) *Xenopus* embryos were
533 injected with *GFP-Deup1* mRNA and immunostained at neurula st18 as indicated. Scale bars:
534 5µm (**a**, **b**, large view), 1.5µm (**a**, high magnification), 500nm (**b**, high magnification), 10µm
535 (**c**).
536



537

538 **Figure 4: CDC20B knockdown impairs centriole disengagement from deuterosomes and**

539 **ciliogenesis in mouse ependymal MCCs**.

540 (**a**,**b**) Ependyma were stained for CDC20B (green) and FOXJ1 (nuclear MCC fate marker, red)

541 5 days post electroporation (5dpe) of control shRNA (**a**) or *Cdc20b* shRNA (**b**). sh277 is

542 exemplified here, but all three *Cdc20b* shRNAs produced similar effects. (**c**) Graph showing

543 the quantification of CDC20B protein levels in cells at the deuterosomal stage at 5dpe.

544 Horizontal lines are mean values and vertical lines are standard deviations. (**d**) Histogram

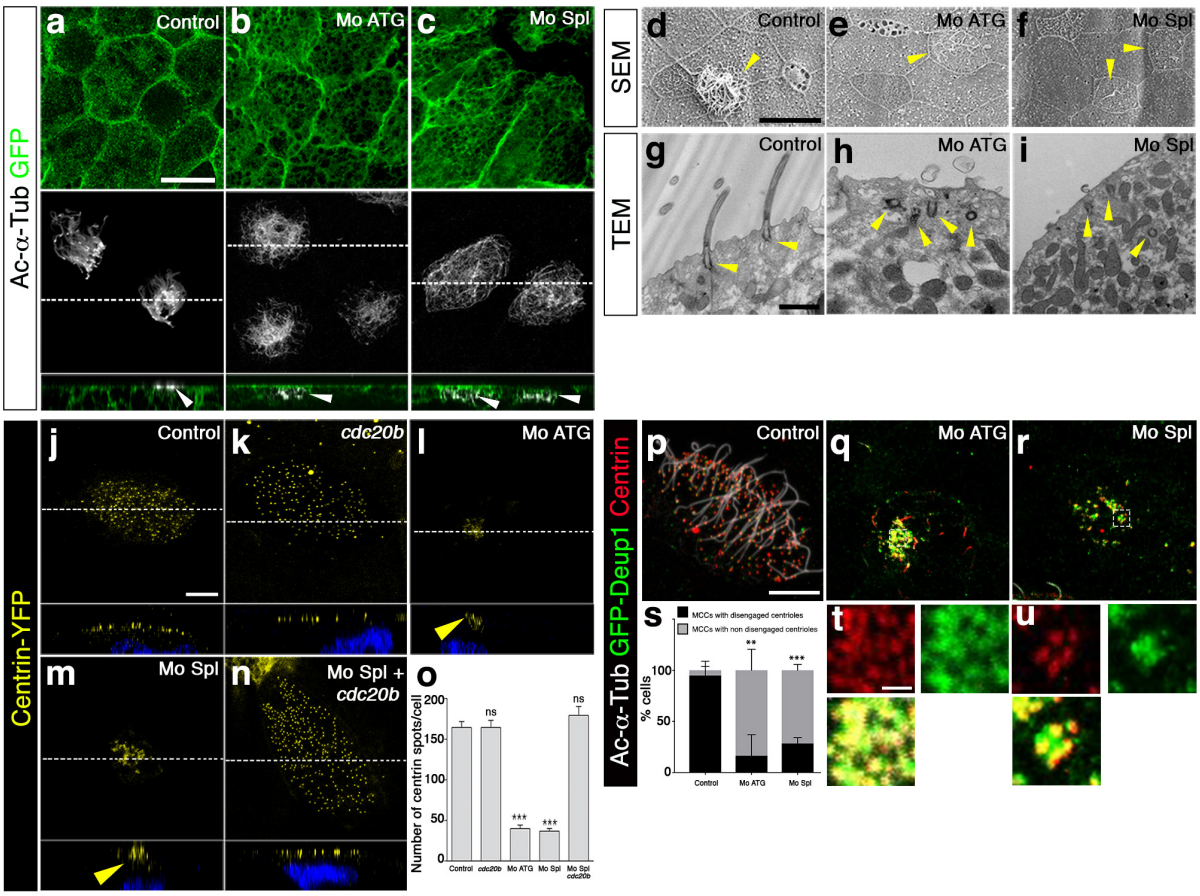
545 showing the number of FOXJ1-positive nuclei observed for each field (dot), with mean values

546 (horizontal lines) and standard deviations (vertical lines). No significant variations were

547 observed between conditions, indicating that MCC fate acquisition was not affected by *Cdc20b*

548 knockdown. (**e-f**) Confocal pictures of 9dpe ependyma electroporated with control shRNA (**e**)

549 or *Cdc20b* shRNAs (**f**) and stained for DEUP1 (deuterosome, green), FOP (centrioles, red) and
550 ZO1 (cell junction, white). DEUP1 positive deuterosomes with non-disengaged FOP positive
551 centrioles were observed much more frequently in MCCs electroporated with *Cdc20b* shRNAs
552 compared to control shRNA. (**g**) Histogram showing the number of cells with non-disengaged
553 centrioles per field (dots), with mean values (horizontal bars) and standard deviations (vertical
554 lines). (**h-i**) Confocal pictures of 15dpe ependyma stained for FOP (centrioles, green), α-
555 Tubulin (α-TUB, cilia, red) and ZO1 (cell junction, white) showing the morphology of normal
556 MCCs in shRNA control condition (**h**), and examples of defects observed in MCCs treated with
557 sh *Cdc20b* (**i**). (**j**) Histogram showing the number of released centrioles per cell (dots), with
558 mean values (horizontal bars) and standard deviations (vertical lines). (**k**) Bar graph showing
559 the percentage of normal and abnormal MCCs. MCCs were scored abnormal when they did not
560 display organized centriole patches associated to cilia. Scale bars: 20μm (**a**), 5μm (**e**, **i**).
561



562

563 **Figure 5: *cdc20b* knockdown impairs centriole disengagement, docking and ciliogenesis**

564 **in *Xenopus* epidermal MCCs.**

565 (**a-c**) 8-cell embryos were injected in presumptive epidermis with *GFP-CAAX* mRNA and

566 *cdc20b* morpholinos, as indicated. Embryos at tailbud st25 were processed for fluorescent

567 staining against GFP (injection tracer, green) and Acetylated-α-Tubulin (Ac-α-Tub, cilia,

568 white). White dotted lines indicate the position of orthogonal projections shown in bottom

569 panels. Note that *cdc20b* morphant MCCs display cytoplasmic filaments but do not grow cilia

570 (white arrowheads). (**d-f**) Scanning Electron Microscopy (SEM) of control (**d**) and *cdc20b*

571 morphant (**e**,**f**) embryos at tadpole st31. Yellow arrowheads point at normal (**d**) and defective

572 MCCs (**e**,**f**). (**g-i**) Transmission Electron Microscopy (TEM) of control (**g**) and *cdc20b*

573 morphant (**h**,**i**) embryos at tailbud st25. Yellow arrowheads point at normally docked basal

574 bodies supporting cilia (**g**) and undocked centrioles unable to support cilia (**h**,**i**). (**j-n**) 8-cell

575 embryos were injected in presumptive epidermis with *centrin-YFP* mRNA, *cdc20b*
576 morpholinos, and *cdc20b* mRNA, as indicated. Centrin-YFP fluorescence was observed
577 directly to reveal centrioles (yellow). Nuclei were revealed by DAPI staining in blue. White
578 dotted lines indicate the position of orthogonal projections shown in bottom panels. Yellow
579 arrowheads point at undocked centrioles. (**o**) Bar graph showing the number of BBs per MCC,
580 as counted by Centrin-YFP dots. *cdc20b* knockdown significantly reduced the number of BBs
581 per cell, and this defect could be corrected by *cdc20b* co-injection with Mo Spl. (**p-u**) Embryos
582 were injected with *Multicilin-hGR* and *GFP-Deup1* mRNAs, treated with dexamethasone at
583 gastrula st11 to induce Multicilin activity, and immunostained at neurula st23 against
584 Acetylated-α-tubulin (cilia, white), GFP (deuterosomes, green) and Centrin (centrioles, red).
585 (**p**) Control cells showed individual centrioles, many of which had initiated ciliogenesis. Note
586 that Deup1-positive deuterosomes were no longer visible at this stage. (**q**,**r**,**t**,**u**) *cdc20b*
587 morphant MCCs showed procentrioles still engaged on deuterosomes and lacked cilia. (**s**) bar
588 graph showing the percentage of cells that completed or not centriole disengagement. Scale
589 bars: 20µm (**a**, **d**), 1µm (**g**, **t**), 5µm (**j**, **p**).
590

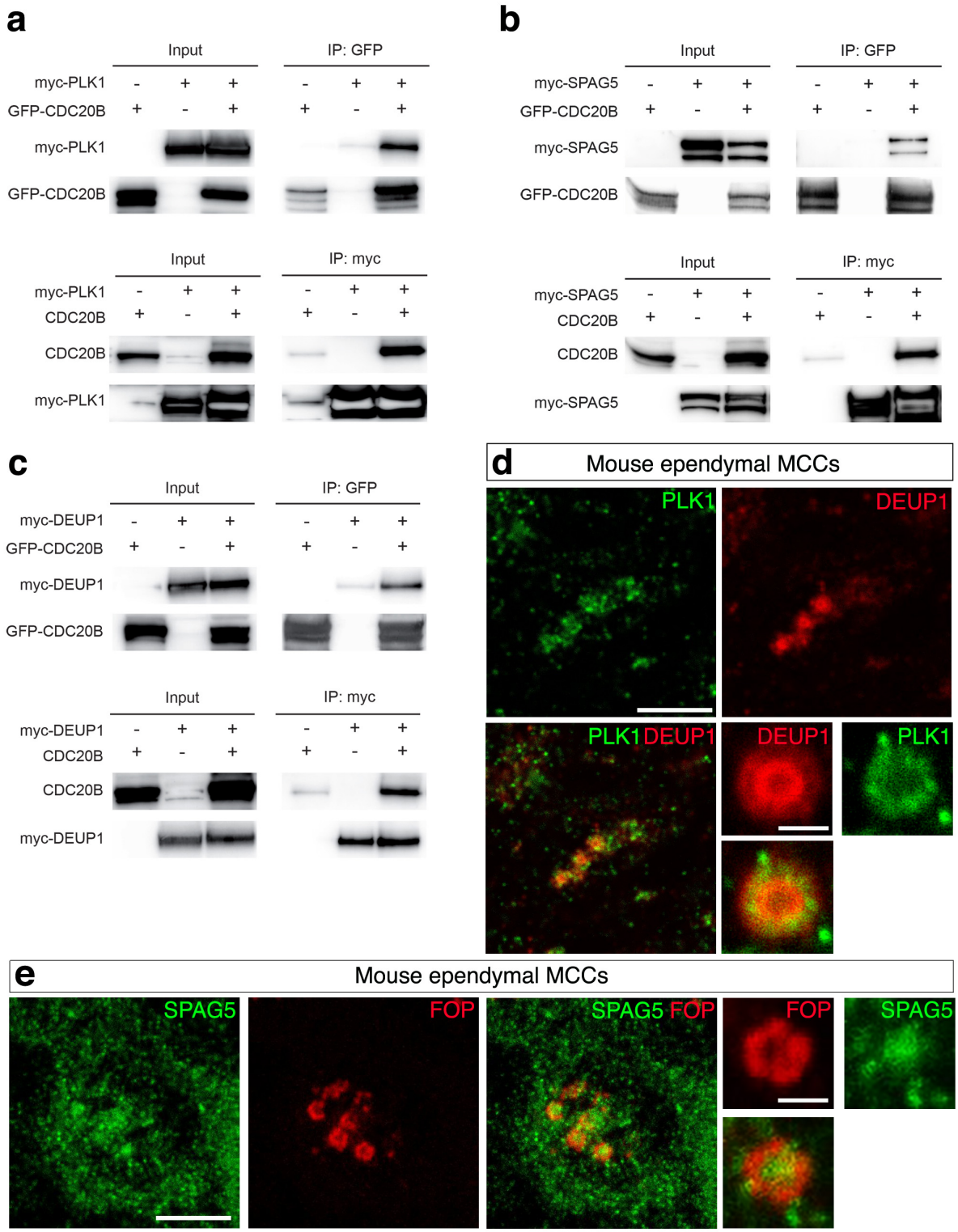
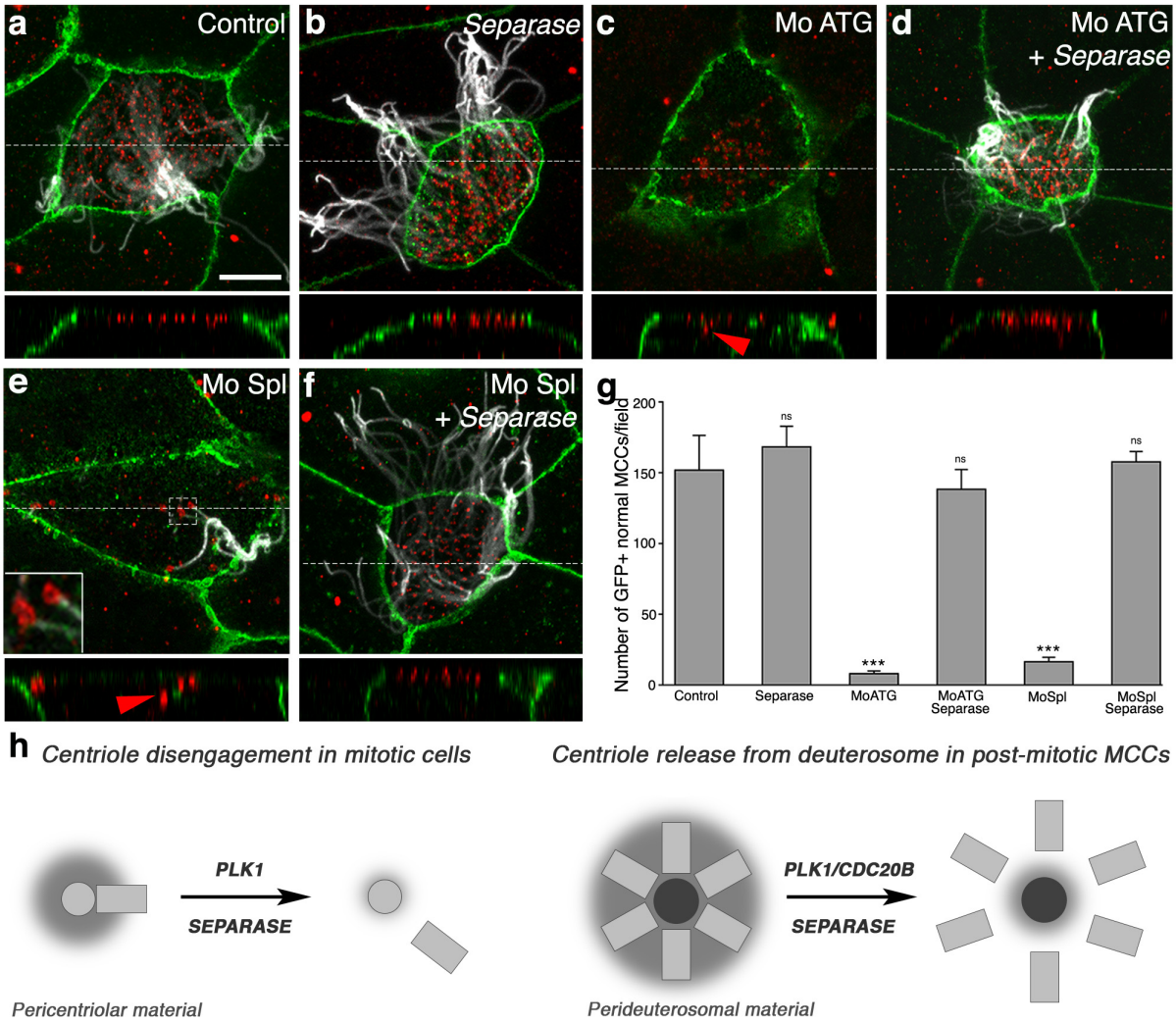


Figure 6: CDC20B interacts with PLK1, SPAG5 and DEUP1

(**a-c**) Co-immunoprecipitations of PLK1, SPAG5 and DEUP1 with CDC20B were tested after transfections of different constructs in HEK cells, indicated at the top of each panel. Proteins

595 (left legend) were revealed by immunoblotting. (**d**) Maturing mouse ependyma were
596 immunostained for the indicated proteins, and pictures were taken with a confocal microscope.
597 PLK1 and SPAG5 are expressed in maturing MCCs. High magnifications show that PLK1 is
598 enriched in the perideuterosomal region, while SPAG5 is enriched in the deuterosome core.
599 Scale bars: 5µm (**d**, **e**, large view), 1µm (**d**, **e**, high magnification).
600



601

602 **Figure 7: Separase overexpression rescues multiciliogenesis in absence of Cdc20b.**

603 (**a-f**) 8-cell *Xenopus* embryos were injected in the presumptive epidermis with *GFP-gpi* mRNA,

604 *cdc20b* morpholinos, and human *Separase* mRNA, as indicated. Embryos were fixed at tailbud

605 st25 and immunostained against GFP (injection tracer, green), Acetylated-α-Tubulin (cilia,

606 white) and ɣ-Tubulin (BBs, red). White dotted lines indicate the position of orthogonal

607 projections shown in bottom panels. Red arrowheads point undocked BBs. Left inset in (**e**)

608 shows zoom on clustered centrioles. (**g**) Bar graph showing the number of properly ciliated

609 MCCs among injected cells, per field of observation. Counting was performed on pictures taken

610 at low magnification (20x), in order to score a large number of cells. Separase overexpression

611 fully rescued multiciliogenesis in *cdc20b* morphant MCCs. Scale bars: 5µm (**a**). (**h**) Model

612 illustrating the analogy between centriole disengagement in mitotic cells and centriole release
613 from deuterosomes in post-mitotic MCCs.
614
615 **Materials and Methods**
616 Subjects/human samples
617 Inferior turbinates were from patients who underwent surgical intervention for nasal obstruction
618 or septoplasty (provided by L. Castillo, Nice University Hospital, France). The use of human
619 tissues was authorized by the bioethical law 94-654 of the French Public Health Code and
620 written consent from the patients.
621
622 Single-cell RNA sequencing of HAECs
623 HAECs were cultured as previously described[23]. They were induced to differentiate at the air-
624 liquid interface for 14 days, which corresponds to the maximum centriole multiplication stage.
625 Cells were incubated on Transwell® (Corning®, NY 14831 USA) with 0.1% protease type
626 XIV from *Streptomyces griseus* (Sigma-Aldrich) in HBSS (Hanks' balanced salts) for 4 hours
627 at 4°C. Cells were gently detached from the Transwells by pipetting and then transferred to a
628 microtube. 50 units of DNase I (EN0523 Thermo Fisher Scientific) per 250µl, were directly
629 added and cells were further incubated at room temperature for 10 min. Cells were centrifuged
630 (150g for 5 min) and resuspended in 500 µL HBSS 10% Fetal Bovine Serum (Gibco),
631 centrifuged again (150g for 5 min) and resuspended in 500 µL HBSS before being mechanically
632 dissociated through a 26G syringe (4 times). Finally, cell suspensions were filtered through a
633 Scienceware® Flowmi™ Cell Strainer (40µm porosity), centrifuged (150g for 5 min) and
634 resuspended in 500 µL of cold HBSS. Cell concentration measurements were performed with
635 Scepter™ 2.0 Cell Counter (millipore) and Countess™ automated cell counter (ThermoFisher
636 Scientific). Cell viability was checked with Countess™ automated cell counter (ThermoFisher

637 Scientific). All steps except the DNAse I incubation were performed on ice. For the cell capture
638 by the 10X genomics device, the cell concentration was adjusted to 300 cells/µl in HBSS aiming
639 to capture 1500 cells. We then followed the manufacturer's protocol (Chromium™ Single Cell
640 3' Reagent Kit, v2 Chemistry) to obtain single cell 3' libraries for Illumina sequencing. Libraries
641 were sequenced with a NextSeq 500/550 High Output v2 kit (75 cycles) that allows up to
642 *91* cycles of paired-end sequencing: the forward read had a length of 26 basse that included the
643 cell barcode and the UMI; the reverse read had a length of 57 bases that contained the cDNA
644 insert. CellRanger Single-Cell Software Suite v1.3 was used to perform sample demultiplexing,
645 barcode processing and single-cell 3′ gene counting using standards default parameters and
646 human build hg19. Additional analyses were performed using R. Pseudotemporal ordering of
647 single cells was performed with the last release of the Monocle package[45]. Cell cycle scores
648 were calculated by summing the normalized intensities of genes belonging to phase-specific
649 gene sets then centered and scaled by phase. Gene sets for each phase were curated from
650 previously described sets of genes[46] (Supplementary Table 1). Data was submitted to the GEO
651 portal under series reference GSE103518. Data shown in Figure 1 is representative of 4
652 independent experiments performed on distinct primary cultures.
653
654 <u>RNA sequencing of HAECs</u>
655 For Figure S3A, three independent HAEC cultures (HAEC1, HAEC2, HAEC3) were triggered
656 to differentiate in air-liquid interface (ALI) cultures for 2 days (ALI day 2, undifferentiated),
657 ALI day 14 (first cilia) or ALI day 28 (well ciliated). RNA was extracted with the miRNeasy
658 mini kit (Qiagen) following manufacturer's instructions. mRNA-seq was performed from 2 µg
659 of RNA that was first subjected to mRNA selection with Dynabeads® mRNA Purification Kit
660 (Invitrogen). mRNA was fragmented 10 min at 95°C in RNAseIII buffer (Invitrogen) then
661 adapter-ligated, reverse transcribed and amplified (6 cycles) with the reagents from the

662 NEBNext Small RNA Library Prep Set for SOLiD. Small RNA-seq was performed from 500
663 ng RNA with the NEBNext Small RNA Library Prep Set for SOLiD (12 PCR cycles) according
664 to manufacturer's instructions. Both types of amplified libraries were purified on Purelink PCR
665 micro kit (Invitrogen), then subjected to additional PCR rounds (8 cycles for RNA-seq and 4
666 cycles for small RNA-seq) with primers from the 5500 W Conversion Primers Kit (Life
667 Technologies). After Agencourt® AMPure® XP beads purification (Beckman Coulter),
668 libraries were size-selected from 150 nt to 250 nt (for RNA-seq) and 105 nt to 130 nt (for small
669 RNA-seq) with the LabChip XT DNA 300 Assay Kit (Caliper Lifesciences), and finally
670 quantified with the Bioanalyzer High Sensitivity DNA Kit (Agilent). Libraries were sequenced
671 on SOLiD 5500XL (Life Technologies) with single-end 50b reads. SOLiD data were analyzed
672 with lifescope v2.5.1, using the small RNA pipeline for miRNA libraries and whole
673 transcriptome pipeline for RNA-seq libraries with default parameters. Annotation files used for
674 production of raw count tables correspond to Refseq Gene model v20130707 for mRNAs and
675 miRBase v18 for small RNAs. Data generated from RNA sequencing were then analyzed with
676 Bioconductor (http://www.bioconductor.org) package DESeq and size-factor normalization
677 was applied to the count tables. Heatmaps were generated with GenePattern using the
678 "Hierarchical Clustering" Module, applying median row centering and Euclidian distance.
679
680 Re-analysis of *Xenopus* E2F4 Chip-seq and RNA-seq
681 RNA-seq (samples GSM1434783 to GSM1434788) and ChIP-seq (samples GSM1434789 to
682 GSM1434792) data were downloaded from GSE59309. Reads from RNA-seq were aligned to
683 the *Xenopus laevis* genome release 7.1 using TopHat2[47] with default parameters. Quantification
684 of genes was then performed using HTSeq-count[48] release 0.6.1 with "-m intersection-
685 nonempty" option. Normalization and statistical analysis were performed using Bioconductor
686 package DESeq2[49]. Differential expression analysis was done between Multicilin-hGR alone

687 versus Multicilin-hGR in the presence of E2f4ΔCT. Reads from ChIP-seq were mapped to the
688 *Xenopus laevis* genome release 7.1 using Bowtie2[50]. Peaks were called and annotated according
689 to their positions on known exons with HOMER[51]. Peak enrichments of E2F4 binding site in
690 the promoters of centriole genes and cell cycle genes[9] were estimated in presence or absence of
691 Multicilin and a ratio of E2F4 binding (Multicilin vs no Multicilin) was calculated.
692
693 Promoter reporter studies
694 The human *CDC20B* promoter was cloned into the pGL3 Firefly Luciferase reporter vector
695 (Promega) with SacI and NheI cloning sites. The promoter sequenced ranged from -1073 to
696 +104 relative to the transcription start site. 37.5 ng of pGL3 plasmid were applied per well.
697 pCMV6-Neg, pCMV6-E2F1 (NM_005225) and pCMV6-E2F4 (NM_001950) constructs were
698 from Origene. 37.5 ng of each plasmid was applied per well. 25 ng per well of pRL-CMV
699 (Promega) was applied in the transfection mix for transfection normalization (Renilla
700 luciferase). HEK 293T cells were seeded at 20 000 cells per well on 96-well plates. The
701 following day, cells were transfected with the indicated plasmids (100 ng of total DNA) with
702 lipofectamine 3000 (Invitrogen). After 24 hours, cells were processed with the DualGlo kit
703 (Promega) and luciferase activity was recorded on a plate reader.
704
705 Proximity ligation Assays
706 Fully differentiated HAECs were dissociated by incubation with 0.1% protease type XIV from
707 *Streptomyces griseus* (Sigma-Aldrich) in HBSS (Hanks' balanced salts) for 4 hours at 4°C. Cells
708 were gently detached from the Transwells by pipetting and then transferred to a microtube.
709 Cells were then cytocentrifuged at 300 rpm for 8 min onto SuperFrostPlus slides using a
710 Shandon Cytospin 3 cytocentrifuge. Slides were fixed for 10 min in methanol at -20°C for
711 Centrin2 and ZO1 assays, and for 10 min in 4% paraformaldehyde at room temperature and

712 then permeabilized with 0.5% Triton X-100 in PBS for 10 min for Acetylated-α-tubulin assays.
713 Cells were blocked with 3% BSA in PBS for 30 min. The incubation with primary antibodies
714 was carried out at room temperature for 2 h. Then, mouse and rabbit secondary antibodies from
715 the Duolink® Red kit (Sigma-Aldrich) were applied and slides were processed according to
716 manufacturer's instructions. Images were acquired using the Olympus Fv10i confocal imaging
717 systems with 60X oil immersion objective and Alexa 647 detection parameters.
718
719 Animals
720 All experiments were performed following the Directive 2010/63/EU of the European
721 parliament and of the council of 22 September 2010 on the protection of animals used for
722 scientific purposes. Experiments on *Xenopus laevis* and mouse were approved by the 'Direction
723 départementale de la Protection des Populations, Pôle Alimentation, Santé Animale,
724 Environnement, des Bouches du Rhône' (agreement number F 13 055 21). Mouse experiments
725 were approved by the French ethical committee n°14 (permission number: 62-12112012).
726 Timed pregnant CD1 mice were used (Charles Rivers, Lyon, France).
727
728 Immunostaining on mouse ependyma
729 Immunostaining on ependyma preparations were performed as previously described [52]. Briefly,
730 dissected brains were subjected to 12 min fixation in 4% paraformaldehyde, 0.1% Triton X-
731 100, blocked 1 hour in PBS, 3% BSA, incubated overnight with primary antibodies diluted in
732 PBS, 3% BSA, and incubated 1 h with secondary antibodies at room temperature. Ependyma
733 were dissected further and mounted with Mowiol before imaging using an SP8 confocal
734 microscope (Leica microsystems) equipped with a 63x oil objective. The same protocol was
735 used to prepare samples for super-resolution acquisition. Pictures were acquired with a TCS
736 SP8 STED 3X microscope equipped with an HC PL APO 93X/1.30 GLYC motCORR™

737 objective (Leica microsystems). Pericentrin was revealed using Alexa 514 (detection 535-
738 564nm, depletion 660nm), γ-tubulin was revealed using Alexa 568 (detection 582-667nm,
739 depletion 775), and FOP was revealed using Alexa 488 (detection 498-531nm, depletion
740 592nm). Pictures were deconvoluted using Huygens software. Maximum intensity projection
741 of 3 deconvoluted pictures is presented in Figure 4G. Primary antibodies: rabbit anti-CDC20B
742 (1:500; Proteintech), mouse IgG anti-PLK1 (1:500; Thermo Fisher), rabbit anti-Pericentrin
743 (1:500, Abcam), mouse IgG2a anti-Securin (1:100; Abcam), rabbit anti-Separase (1:200;
744 Abcam), mouse IgG1 anti-FoxJ1 (1:1000; eBioscience), rabbit anti-Deup1 (1:1000; kindly
745 provided by Dr Xueliang Zhu), rabbit anti-Deup1 (1:250; Proteintech), mIgG1 anti-γ-Tubulin
746 (clone GTU88) (1:250; Abcam), rabbit anti-ZO1 (1:600; Thermo Fisher Scientific), rabbit anti-
747 Spag5 (1:500; Proteintech), mouse IgG1 anti-ZO1 (1:600; Invitrogen), mouse IgG2b anti-
748 FGFR1OP (FOP) (1:2000; Abnova), mouse IgG1 anti-α-tubulin (1:500; Sigma-Aldrich).
749 Secondary antibodies: Alexa Fluor 488 goat anti-rabbit (1:800; Thermo Fisher Scientific),
750 Alexa Fluor 647 goat anti-rabbit (1:800; Thermo Fisher Scientific), Alexa Fluor 514 goat anti-
751 rabbit (1:800; Thermo Fisher Scientific), Alexa Fluor 488 goat anti-mouse IgG2b (1:800;
752 Thermo Fisher Scientific), Alexa Fluor 568 goat anti-mouse IgG2b (1:800; Thermo Fisher
753 Scientific), Alexa Fluor 488 goat anti-mouse IgG2a (1:800; Thermo Fisher Scientific), Alexa
754 Fluor 568 goat anti-mouse IgG1 (1:800; Thermo Fisher Scientific), Alexa Fluor 647 goat anti-
755 mouse IgG1 (1:800; Thermo Fisher Scientific).
756
757 Mouse constructs
758 Expression constructs containing shRNA targeting specific sequences in the CDC20B coding
759 sequence under the control of the U6 promoter were obtained from Sigma-Aldrich (ref.
760 TRCN0000088273 (sh273), TRCN0000088274 (sh274), TRCN0000088277 (sh277)). PCX-
761 mcs2-GFP vector (Control GFP) kindly provided by Xavier Morin (ENS, Paris, France), and

762 U6 vector containing a validated shRNA targeting a specific sequence in the NeuroD1 coding
763 sequence[53](Control sh, ref. TRCN0000081777, Sigma-Aldrich) were used as controls for
764 electroporation experiments.
765
766 Postnatal mouse brain electroporation
767 Postnatal mouse brain electroporation was performed as described previously[54]. Briefly, P1
768 pups were anesthetized by hypothermia. A glass micropipette was inserted into the lateral
769 ventricle, and 2 µl of plasmid solution (concentration 3µg/µl) was injected by expiratory
770 pressure using an aspirator tube assembly (Drummond). Successfully injected animals were
771 subjected to five 95V electrical pulses (50 ms, separated by 950 ms intervals) using the CUY21
772 edit device (Nepagene, Chiba, Japan), and 10 mm tweezer electrodes (CUY650P10, Nepagene)
773 coated with conductive gel (Signagel, Parker laboratories). Electroporated animals were
774 reanimated in a 37°C incubator before returning to the mother.
775
776 Statistical analyses of mouse experiments
777 Analysis of CDC20B signal intensity in deuterosomes (graph in Fig. 3b). For each
778 category, >25 cells from two different animals were analyzed. Deuterosome regions were
779 delineated based on FOP staining and the intensity of CDC20B fluorescent immunostaining
780 was recorded using ImageJ software, and expressed as arbitrary units. Unpaired t test vs
781 immature: p=0,0005 (intermediate,***); p<0,0001 (Mature, ****).
782 Analysis of *Cdc20b* shRNAs efficiency (Fig. 4c): For each cell at the deuterosomal stage, the
783 intensity of CDC20B fluorescent immunostaining was recorded using ImageJ software and
784 expressed as arbitrary units. Data are mean ± sem. Two independent experiments were
785 analyzed. A minimum of 35 cells per condition was analyzed. n= 3, 4, 5 and 5 animals for sh

786 control, sh273, sh274 and sh277, respectively. Unpaired t test vs sh control: p<0.0001 (sh273,
787 sh274 and sh277 ****).
788 Analysis of the number of FOXJ1 positive cells at 5dpe (Fig. 4d): Unpaired t test vs sh control:
789 0.3961 (sh273, ns), 0.1265 (sh274, ns), 0.3250 (sh277, ns).
790 Analysis of the number of cells with non-disengaged centrioles at 9dpe (Fig. 4g): 15-20 fields
791 were analyzed per condition. n= 4, 4, 3, and 4 animals for sh control, sh273, sh274 and sh277,
792 respectively, from 2 independent experiments. Unpaired t test vs sh control: p<0.0001 (sh273,
793 sh274, sh277 ****).
794 Analysis of the number of centrioles per cell at 15dpe (Fig. 4j): >100 cells were analyzed per
795 condition. n= 3, 3, 3, and 3 animals for sh control, sh273, sh274 and sh277, respectively, from
796 2 independent experiments. Unpaired t test vs sh control: p<0.0001 (sh273, sh274, sh277 ****).
797 Analysis of ependymal cell categories at 15dpe (Fig. 4k): Data are mean ± sem from three
798 independent experiments. More than 500 cells were analyzed for each condition. n= 4, 4, 3, and
799 3 animals for sh control, sh273, sh274 and sh277, respectively. Unpaired t test vs sh control:
800 p= 0.0004 (sh273, ***), 0.0001 (sh274, ****), 0.0038 (sh277, **).
801
802 Mouse tracheal epithelial cells (MTECs)
803 MTECs cell cultures were established from the tracheas of 12 weeks old mice, according to the
804 procedure previously published[55], with the following modification: in differentiation medium,
805 NuSerum™ was replaced with Ultroser-G™ (Pall Corporation) and 10 µM DAPT (N-[N-(3,5-
806 difluorophenacetyl)-L- alanyl]-S-phenylglycine t-butyl ester) (Sigma) was added one day after
807 setting-up the air-liquid interface.
808
809 Immunostaining on HAECs and MTECs

810 Three days after setting-up the air-liquid interface, MTECs on Transwell membranes were pre-
811 extracted with 0.5% Triton X-100 in PBS for 3 min, and then fixed with 4% paraformaldehyde
812 in PBS for 15 min at room temperature. HAECs were treated 21 days after setting-up the air-
813 liquid interface. They were fixed directly on Transwells with 100% cold methanol for 10 min
814 at -20°C (for CDC20B and Centrin2 co-staining, Supplementary Fig. 4a,b) or with 4%
815 paraformaldehyde in PBS for 15 min at room temperature (for CDC20B single staining,
816 Supplementary Fig. 4c). All cells were then permeabilized with 0.5% Triton X-100 in PBS for
817 5 min and blocked with 3% BSA in PBS for 30 min. The incubation with primary and secondary
818 antibodies was carried out at room temperature for 2 h and 1 h, respectively. Nuclei were stained
819 with 4,6-diamidino-2-phenylindole (DAPI). Transwell membranes were cut with a razor blade
820 and mounted with ProLong Gold medium (Thermofisher). Primary antibodies: rabbit anti-
821 CDC20B (1:500; Proteintech), rabbit anti-DEUP1 (1:500), anti-Centrin2 (Clone 20H5, 1:500;
822 Millipore). Secondary antibodies: Alexa Fluor 488 goat anti-rabbit (1:1000; Thermo Fisher
823 Scientific), Alexa Fluor 647 goat anti-mouse (1:1000; Thermo Fisher Scientific). For co-
824 staining of CDC20B and DEUP1, CDC20B was directly coupled to CF™ 633 with the Mix-n-
825 Stain™ kit (Sigma-Aldrich) according to the manufacturer's instruction. Coupled primary
826 antibody was applied after secondary antibodies had been extensively washed and after a 30
827 min blocking stage in 3% normal rabbit serum in PBS.
828
829 Western blot and immunofluorescence analysis of transfected cells
830 Cos1 or Hela cells cells were grown in DMEM supplemented with 10% heat inactivated FCS
831 and transfected with Fugene HD (Roche Applied Science) according to manufacturer's
832 protocol. Transfected or control cells were washed in PBS and lysed in 50 mM Tris-HCl pH
833 7.5, 150 mM NaCl, 1mM EDTA, containing 1% NP-40 and 0.25% sodium deoxycholate
834 (modified RIPA) plus a Complete Protease Inhibitor Cocktail (Roche Applied Science) on ice.

835 Cell extracts separated on polyacrylamide gels were transfered onto Optitran membrane
836 (Whatman) followed by incubation with rabbit anti-mouse CDC20B (1:500, Proteintech) or
837 homemade rabbit anti-*Xenopus* Cdc20b (1:300) antibody and horseradish peroxidase
838 conjugated secondary antibody (Jackson Immunoresearch Laboratories). Signal obtained from
839 enhanced chemiluminescence (Western Lightning ECL Pro, Perkin Elmer) was detected with
840 MyECL Imager (Thermo Fisher Scientific).
841 For immunofluorescence staining, transfected cells were grown on glass coverslips and fixed
842 for 6 min in methanol at -20°C. Cells were washed in PBS, blocked in PBS, 3% BSA and
843 stained with rabbit anti-*Xenopus* Cdc20b (1:300) or rabbit anti-CFTR (1:200, Santa-Cruz
844 Biotechnology) as a negative control, in blocking buffer. After washings in PBS 0.1% Tween-
845 20, cells were incubated with Alexa fluor 488 donkey anti-rabbit antibody (Thermo Fisher
846 Scientific), washed, and DNA was stained with 250 ng/ml DAPI. Coverslip were then rinsed
847 and mounted in Prolong Gold antifade reagent (ThermoFisher Scientific) and confocal images
848 were acquired by capturing Z-series with 0.3 µm step size on a Zeiss LSM 510 laser scanning
849 confocal microscope.
850
851 Co-immunoprecipitation studies
852 Asynchronous HEK cells were rinsed on ice with chilled Ca++ and Mg++ free Dulbecco's PBS
853 (DPBS, Invitrogen), harvested using acell scraper and lysed on ice for 5 min in lysis buffer
854 (0.025M Tris, 0.15M NaCl, 0.001M EDTA, 1% NP-40, 5% glycerol; pH 7.4) supplemented
855 with EDTA and Halt™ Protease and Phosphatase Inhibitor Cocktail (Pierce, Thermofisher).
856 Lysates were clarified (12000g, 4°C, 10 min) and the protein concentrations were determined
857 using the Bradford assay (Bio-Rad). Immunoprecipitations were performed with the Pierce co-
858 immunoprecipitation kit (Pierce, Thermofisher) according to the manufacturer's instructions.
859 For each immunoprecipitation, 1-1.5 mg of total lysate was precleared on a control column,

860 then incubated on columns coupled with 20 µg of anti-GFP or anti-c-myc-antibody (clone
861 9E10). Incubation was performed overnight at 4°C. Columns were washed and eluted with 50
862 µL elution buffer. Samples were denatured at 70°C for 10 min with Bolt™ LDS Sample Buffer
863 and Bolt reducing agent, then separated on 4-12% gradient Bolt precast gels (Thermofisher),
864 transferred onto nitrocellulose (Millipore), and subjected to immunoblot analysis using either
865 anti-CDC20B (ProteinTech) or anti-c-myc antibody (clone 9E10). In Figure 6, note that the
866 high level of expression of myc-PLK1 (**a**) and myc-SPAG5 (**b**) drained out locally the ECL
867 reagent at the peak of the protein. The resulting double bands correspond in fact to unique ones.
868 Human SPAG5, subcloned into pCMV6-MT, was from OriGene. Human DEUP1 and PLK1
869 were cloned into pCS2-MT vector (Addgene). Human CDC20B was cloned into pEGFP-C1,
870 pEGFP-N1 (Clontech) for the GFP fusion protein and pIRES-EYFP (Addgene) for the
871 untagged protein.
872
873 <u>In-Gel digestion, NanoHPLC, and Q-exactive*plus* analysis</u>
874 For mass spectrometry analysis, protein spots were manually excised from the gel and destained
875 with 100 µL of H2O/ACN (1/1). After 10 min vortexing, liquid was discarded and the
876 procedure was repeated 2 times. They were rinsed with acetonitrile and dried under vacuum.
877 Extracts were reduced with 50 µL of 10 mM dithiothreitol for 30 min at 56 °C, then alkylated
878 with 15 µL of 55 mM iodoacetamide for 15 min at room temperature in the dark. They were
879 washed successively by: i) 100 µL of H2O/ACN (1/1) (2 times) and ii) 100 µL of acetonitrile.
880 Gel pieces were rehydrated in 60 µL of 50 mM NH_4HCO_3 containing 10 ng/µL of trypsin
881 (modified porcine trypsin, sequence grade, Promega) incubated for one hour at 4°C. After the
882 removal of trypsin, samples were incubated overnight at 37°C. Tryptic peptides were extracted
883 with: i) 60 µL of 1% FA (formic acid) in water (10 min at RT), ii) 60 µL acetonitrile (10 min

884 at RT). Extracts were pooled, concentrated under vacuum, resuspended in 15 µL of aqueous
885 0.1% formic acid for NanoHPLC separation.
886 Separation was carried out using a nanoHPLC (Ultimate *3000,* Thermo Fisher Scientific). After
887 concentration on a µ-Precolumn Cartridge Acclaim PepMap 100 C_{18} (i.d. 5 mm, 5 µm, 100 Å,
888 Thermo Fisher Scientific) at a flow rate of 10 µL/min, using a solution of H_2O/ACN/FA
889 98%/2%/0.1%, a second peptide separation was performed on a 75 µm i.d. x 250 mm (3 µm,
890 100 Å) Acclaim PepMap 100 C_{18} column (Thermo Fisher Scientific) at a flow rate of 300
891 nL/min. Solvent systems were: (A) 100% water, 0.1% FA, (B) 100% acetonitrile, 0.08% FA.
892 The following gradient was used t = 0min 6% B; t = 3 min 6%B; t = 119min, 45% B; t = 120
893 min, 90% B; t = 130 min 90% B (temperature at 35°C).
894 NanoHPLC was coupled via a nanoelectrospray ionization source to the Hybrid Quadrupole-
895 Orbitrap High Resolution Mass Spectrometer (Thermo Fisher Scientific). MS spectra were
896 acquired at a resolution of 70 000 (200 m/z) in a mass range of 300−2000 m/z with an AGC
897 target 3e6 value of and a maximum injection time of 100ms. The 10 most intense precursor
898 ions were selected and isolated with a window of 2m/z and fragmented by HCD (Higher energy
899 C-Trap Dissociation) with normalized collision energy (NCE) of 27. MS/MS spectra were
900 acquired in the ion trap with an AGC target 2e5 value, the resolution was set at 17 500 at 200
901 m/z combined with an injection time of 100 ms.
902 Data were reprocessed using Proteome Discoverer 2.1 equipped with Sequest HT. Files were
903 searched against the Swissprot Homo sapiens FASTA database (update of February 2016). A
904 mass accuracy of ± 10 ppm was used to precursor ions and 0.02 Da for product ions. Enzyme
905 specificity was fixed to trypsin, allowing at most two miscleavages. Because of the previous
906 chemical modifications, carbamidomethylation of cysteines was set as a fixed modification and
907 only oxydation of methionine was considered as a dynamic modification. Reverse decoy

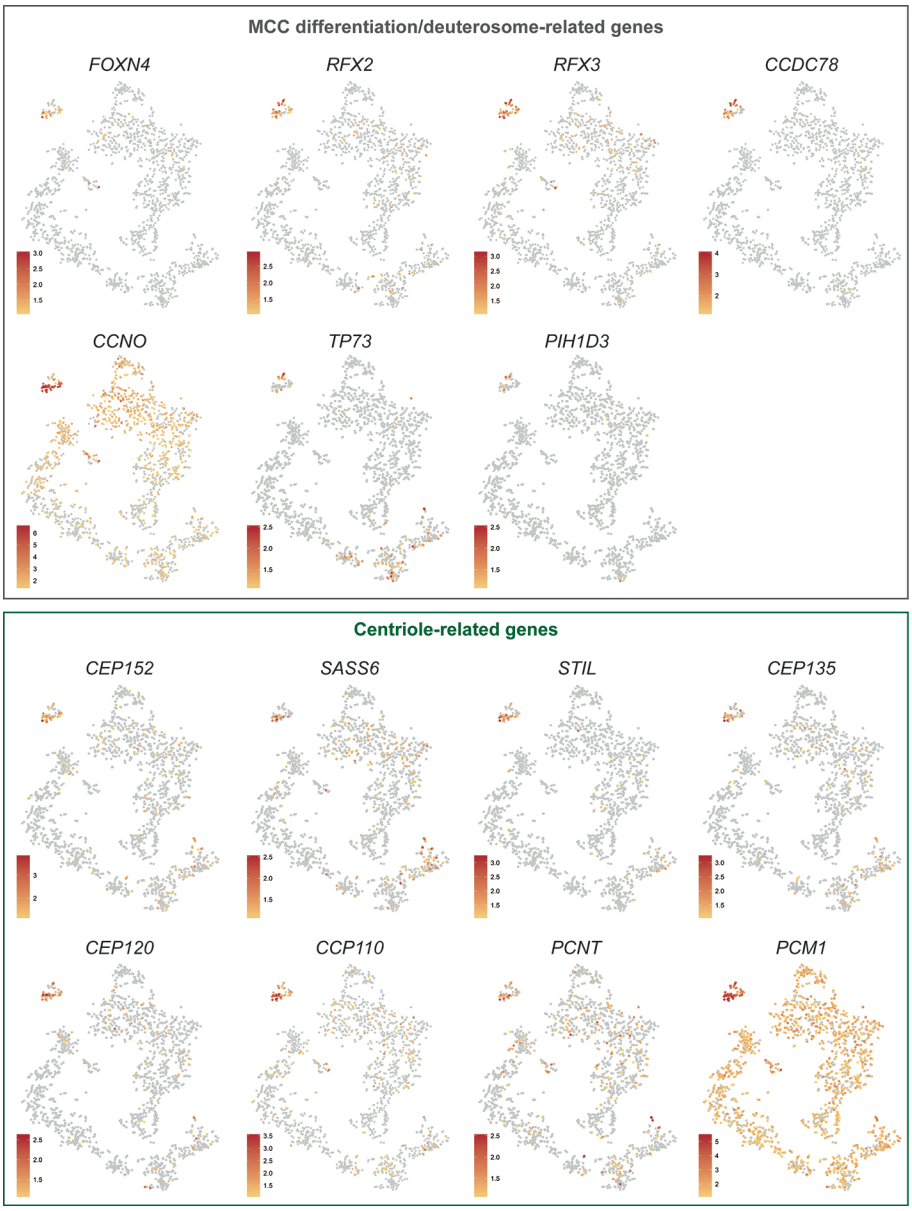
908 databases were included for all searches to estimate false discovery rates, and filtered using the
909 Percolator algorithm at a 1% FDR.
910
911 *Xenopus* embryo injections, plasmids, RNAs, and mopholinos
912 Eggs obtained from NASCO females were fertilized *in vitro*, dejellied and cultured as described
913 previously[56]. All injections were done at the 8-cell stage in one animal-ventral blastomere
914 (presumptive epidermis), except for electron microscopy analysis for which both sides of the
915 embryo was injected, and for RT-PCR analysis for which 2-cell embryos were injected.
916 *cdc20b* riboprobe was generated from *Xenopus laevis* cDNA. Full-length sequence was
917 subcloned in pGEM™-T Easy Vector Systems (Promega). For sense probe it was linearized by
918 SpeI and transcribed by T7. For antisense probe it was linearized by ApaI and transcribed by
919 Sp6 RNA polymerase. Synthetic capped mRNAs were produced with the Ambion
920 mMESSAGE mMACHINE Kit. pCS105/GFP-CAAX was linearized with AseI and mRNA
921 was synthesized with Sp6 polymerase. pCS2-mRFP and pCS2-GFP-gpi were linearized with
922 NotI and mRNA was synthesized with Sp6 polymerase. pCS-Centrin4-YFP (a gift from
923 Reinhard Köster, Technische Universität Braunschweig, Germany) was linearized with NotI
924 and mRNA was synthesized with Sp6 polymerase. pCS2-GFP-Deup1 and pCS2-
925 Multicilin(MCI)-hGR were kindly provided by Chris Kintner and the mRNAs were obtained
926 as described previously[8]. Embryos injected with *MCI-hGR* mRNA were cultured in
927 Dexamethasone 20μM in MBS 0,1X from st11 until fixation. pCS2-Separase wild-type and
928 phosphomutant 2/4 (protease dead, PD) were provided by Marc Kirchner and Olaf Stemann,
929 respectively; plasmids were linearized with NotI and mRNAs were synthesized with Sp6
930 polymerase. *Venus-cdc20b*, *cdc20b-Venus* and *cdc20b* were generated by GATEWAY™
931 Cloning Technology (GIBCO BRL) from *Xenopus laevis cdc20b* cDNA. *cdc20b* was also
932 subcloned in pCS2-RFP to make *RFP-cdc20b* and *cdc20b-RFP* fusions. All *cdc20b* constructs

933 were linearized with NotI and mRNAs were synthesized with Sp6 polymerase. Quantities of
934 mRNA injected: 500pg for *GFP-CAAX*, *RFP*, *GFP-gpi, Separase* and *Separase(PD);* 25 to
935 500pg for *GFP-Deup1*; 40 to 500pg for *MCI-hGR*; 1ng for *Venus-cdc20b*, *cdc20b-Venus*,
936 *cdc20b*, and *cdc20b-RFP*; 500pg to 1ng for *RFP-cdc20b.*
937 Two independent morpholino antisense oligonucleotides were designed against *cdc20b*
938 (GeneTools, LLC). *cdc20b* ATG Mo: 5'-aaatcttctctaacttccagtccat-3', *cdc20b* Spl Mo 5'-
939 acacatggcacaacgtacccacatc-3'. 20ng of MOs was injected per blastomere or 10ng of each Mo
940 for co-injection.
941
942 PCR and Quantitative RT-qPCR
943 *Xenopus* embryos were snap frozen at different stages and stored at -80°C. Total RNAs were
944 purified with a Qiagen RNeasy kit (Qiagen). Primers were designed using Primer-BLAST
945 Software. PCR reactions were carried out using GoTaq® G2 Flexi DNA Polymerase
946 (Promega). RT reactions were carried out using iScript™ Reverse Transcription Supermix for
947 RT-qPCR (BIO-RAD). qPCR reactions were carried out using SYBRGreen on a CFX Biorad
948 qPCR cycler. To check *cdc20b* temporal expression by qPCR we directed primers to exons 9/10
949 junction (Forward: 5'-ggctatgaattggtgcccg-3') and exons 10/11 junction (Reverse: 5'-
950 gcagggagcagatctggg-3') to avoid amplification from genomic DNA. The relative expression of
951 *cdc20b* was normalized to the expression of the housekeeping gene *ornithine decarboxylase*
952 (*ODC*) for which primers were as follows: forward: 5'-gccattgtgaagactctctccattc-3': reverse: 5'-
953 ttcgggtgattccttgccac-3'.
954 To check the efficiency of Mo SPL, expected to cause retention of intron1 in the mature mRNA
955 of *cdc20b* we directed forward (5'-cctcccgagagttagagga-3') and reverse (5'-
956 gcatgttgtactttctgctcca-3') primers in exon1 and exon2, respectively.

957 To check the expression of *p53* in morphants by qPCR, primers were as follows: forward: 5'-
958 cgcagccgctatgagatgatt-3'; reverse: 5'-cacttgcggcacttaatggt-3'. The relative expression of p53
959 was normalized to Histone4 expression (H4) for which primers were as follows: forward: 5'-
960 ggtgatgccctggatgttgt-3'; reverse: 5'-ggcaaaggaggaaaaggactg-3'.
961
962 Immunostainining on *Xenopus* embryos
963 Embryos were fixed in 4% paraformaldehyde (PFA) overnight at 4°C and stored in 100%
964 methanol at -20°C. Embryos were rehydrated in PBT and washed in MABX (Maleic Acid
965 Buffer + Triton X100 0,1% v/v). Next, embryos were incubated in Blocking Reagent (Roche)
966 2% BR + 15% Serum + MABX with respective primary and secondary antibodies. The anti-
967 *Xenopus laevis* CDC20B antibody was obtained by rabbit immunization with the peptide
968 SPDQRRIFSAAANGT (amino acids 495–509) conjugated to keyhole limpet hemocyanin,
969 followed by affinity purification (Eurogentec). For immunofluorescence, embryos were fixed
970 at RT in PFA 4% in PBS, and incubated in the CDC20B antibody diluted 1/150 in BSA 3% in
971 PBS. For all experiments secondary antibodies conjugated with Alexa were used. GFP-CAAX
972 in Fig. 5g was revealed using a rabbit anti-GFP antibody together with a secondary antibody
973 coupled to HRP, which was revealed as described previously[23]. To mark cortical actin in MCCs,
974 embryos were fixed in 4% paraformaldehyde (PFA) in PBT (PBS + 0,1% Tween v/v) for 1h at
975 room temperature (RT), washed 3x10 min in PBT at RT, then stained with phalloidin-Alexa
976 Fluor 555 (Invitrogen, 1:40 in PBT) for 4 h at RT, and washed 3x10 min in PBT at RT. Primary
977 antibodies: mouse anti-Acetylated–α-Tubulin (Clone 6-11B-1, Sigma-Aldrich, 1:1000), rabbit
978 anti-γ-Tubulin (Abcam, 1:500), mouse anti-γ-Tubulin (Clone GTU88, Abcam, 1:500), Chicken
979 anti-GFP (2B scientific, 1:1000), rabbit anti-GFP (Torrey Pines Biolabs, 1:500), mouse anti-
980 Centrin (Clone 20H5, EMD Millipore; 1:500). Secondary antibodies: Alexa Fluor 647 goat

981 anti-mouse IgG2a (1:500; Thermo Fisher Scientific), Alexa Fluor 488 goat anti-chicken (1:500;
982 Thermo Fisher Scientific), Alexa Fluor 568 goat anti-rabbit (1:500; Thermo Fisher Scientific).
983
984 *In situ* hybridization on *Xenopus* embryos
985 Whole-mount chromogenic *in situ* hybridization was performed as described previously[56].
986 Whole-mount fluorescent *in situ* hybridization (FISH) was performed as described
987 previously[57]. For single staining, all RNA probes were labeled with digoxigenin. For FISH on
988 section, embryos were fixed in 4% paraformaldehyde (PFA), stored in methanol for at least 4
989 h at −20°C, then rehydrated in PBT (PBS + Tween 0.1% v/v), treated with triethanolamine and
990 acetic anhydride, incubated in increasing sucrose concentrations and finally embedded with
991 OCT (VWR Chemicals). 12µm-thick cryosections were made. Double FISH on sections was
992 an adaptation of the whole-mount FISH method. 80ng of *cdc20b* digoxigenin-labeled sense and
993 antisense riboprobes and 40ng of antisense *α-tubulin* fluorescein-labeled riboprobe[58] were used
994 for hybridization. All probes were generated from linearized plasmids using RNA-labeling mix
995 (Roche). FISH was carried out using Tyramide Signal Amplification – TSA TM Plus Cyanine
996 3/Fluorescein System (PerkinElmer). Antibodies: Anti-rabbit-HRP (Interchim, 1:5000), Anti-
997 DigAP (Roche, 1:5000), Anti-DigPOD (Roche, 1:500), Anti-FluoPOD (Roche, 1:500).
998
999 Microscopy
1000 Confocal: Flat-mounted epidermal explants were examined with a Zeiss LSM 780 confocal
1001 microscope. Four-colors confocal z-series images were acquired using sequential laser
1002 excitation, converted into single plane projection and analyzed using ImageJ software.
1003 Scanning Electron Microscopy (SEM): skin epidermis of *Xenopus* embryos from stage 37 was
1004 observed and analyzed into a digital imaging microscope (FEI TENEO). Embryos were
1005 processed as described previously[57]. Transmission Electron Microscopy (TEM): St25 embryos

1006 were fixed overnight at 4°C in 2.5% glutaraldehyde, 2% paraformaldehyde, 0.1% tannic acid
1007 in a sodium cacodylate buffer 0.05 M pH7.3. Next, embryos were washed 3x15 min in
1008 cacodylate 0.05 M at 4°C. Post-fixation was done in 1% osmium buffer for 2 h. Next, embryos
1009 were washed in buffer for 15 min. Then, embryos were washed in water and dehydrated
1010 conventionally with alcohol, followed by a step in 70% alcohol containing 2% uranyl during 1
1011 to 2 h at RT, or overnight at 4°C. After 3 times in 100% alcohol, completed with 3 washes of
1012 acetone. Next, embryos were included in classical epon resin, which was polymerized in oven
1013 at 60°C for 48 h. Sections of 80 nm were made and analyzed into an FMI TECNAI microscope
1014 with acceleration of 200kV.
1015
1016 Statistical analysis of *Xenopus* experiments
1017 To quantify the effect of our different experiments, we applied One-way ANOVA analysis and
1018 Bonferroni's multiple comparisons test (t test). ***P<0.05; ns = not significant. Statistical
1019 analyses were done using GraphPad Prism 6.
1020 Fig. 5o and Fig. S6k: 10 cells per condition were analyzed and the total number of Centrin-YFP
1021 or γ-tubulin positive spots per injected cell was counted.
1022 Fig. 7g: 5 fields (20x zoom) per condition were analyzed, and the total number of properly
1023 ciliated MCCs based on acetylated α-tubulin staining among GFP positive cells per field was
1024 counted. Each field corresponded to a different embryo.
1025 Fig. 5s : 160-200 cells per condition were analyzed. n= 6, 8, and 10 embryos from 3
1026 independent experiments for control, Mo ATG and Mo Spl, respectively. Unpaired t test vs
1027 control: p=0,0037 (Mo ATG **) and 0,0004 (Mo Spl ***).
1028
1029 **Supplementary figures and legends**
1030


MCC differentiation/deuterosome-related genes
FOXN4
RFX2
RFX3
CCDC78
CCNO
TP73
PIH1D3
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CEP120
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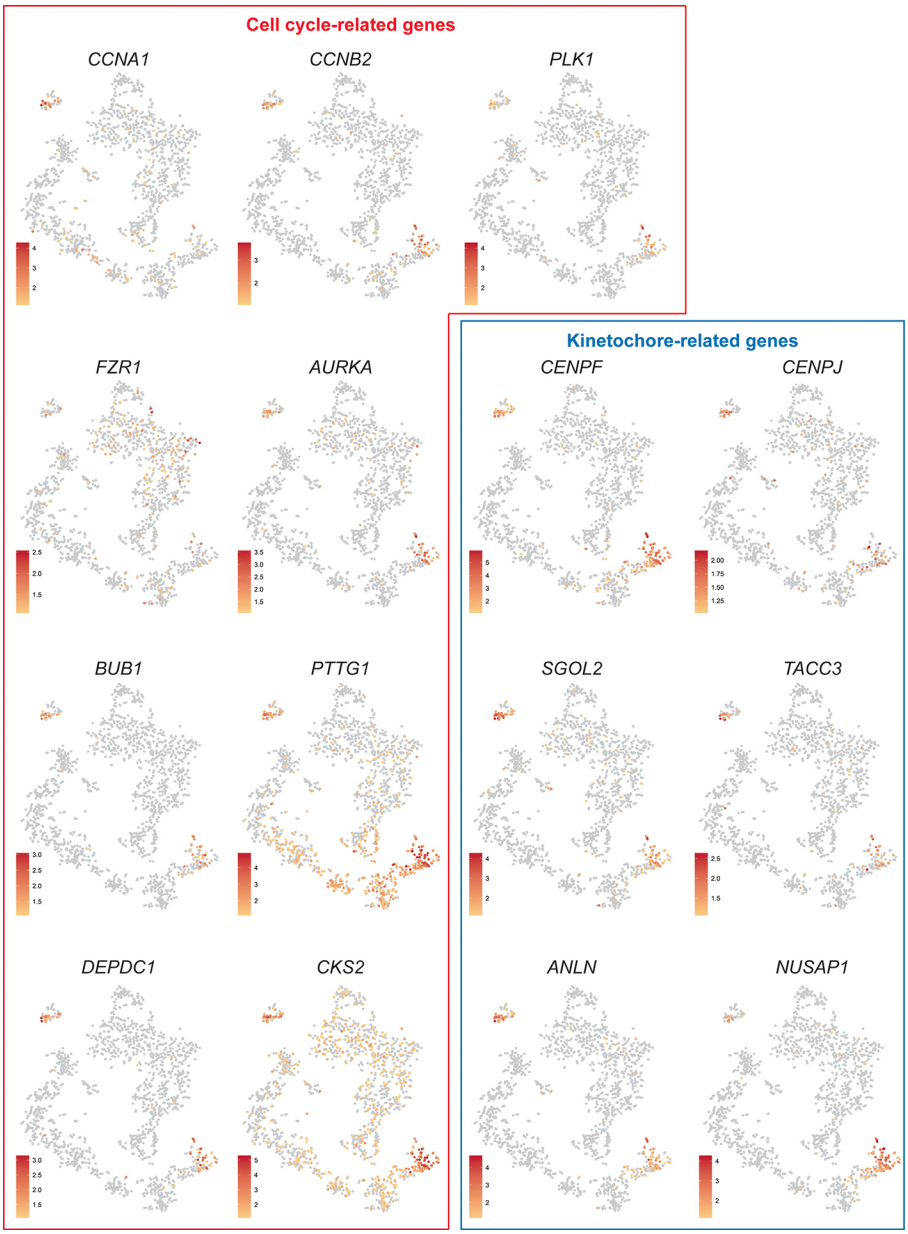
PCNT
PCM1

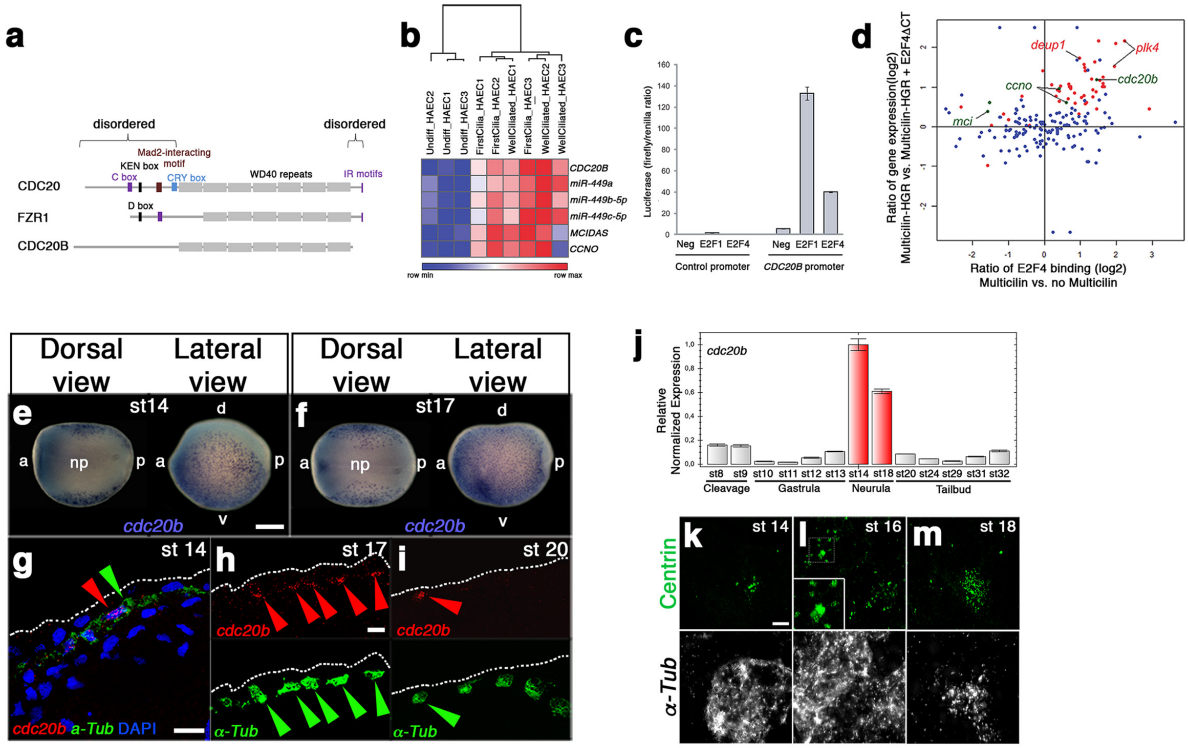
1031

1032



Supplementary Figure 1: Single cell RNA-seq analysis of HAECs.

tSNE plots for a selection of genes expressed at the single-cell level, in deuterosomal-stage differentiating HAECs. Genes were grouped into functional categories.



1038

1039 **Supplementary Figure 2: Structure, regulation and sub-cellular localization of CDC20B.**

1040 **Domain composition of CDC20 family members. (a)** The C box and IR motifs in CDC20

1041 and FZR1 serve as APC/C binding domains. The KEN box and the Cry box in CDC20, and the

1042 D box in FZR1 are involved in their regulation by degradation. The Mad2-interacting motif in

1043 CDC20 is important for its regulation by the spindle assembly checkpoint. WD40 repeats are

1044 involved in substrate recognition. Note that CDC20B lacks degradation motifs and the APC/C

1045 binding domains present in CDC20 and FZR1. ***CDC20B* is induced during multiciliogenesis**

1046 **under the control of E2Fs/MCIDAS. (b)** Heatmap of gene expression measured by RNA-seq

1047 or small RNA-seq on 3 independent HAEC differentiation time courses (HAEC1 to HAEC3).

1048 Normalized read counts were Log2-transformed and median-centered by gene. Hierarchical

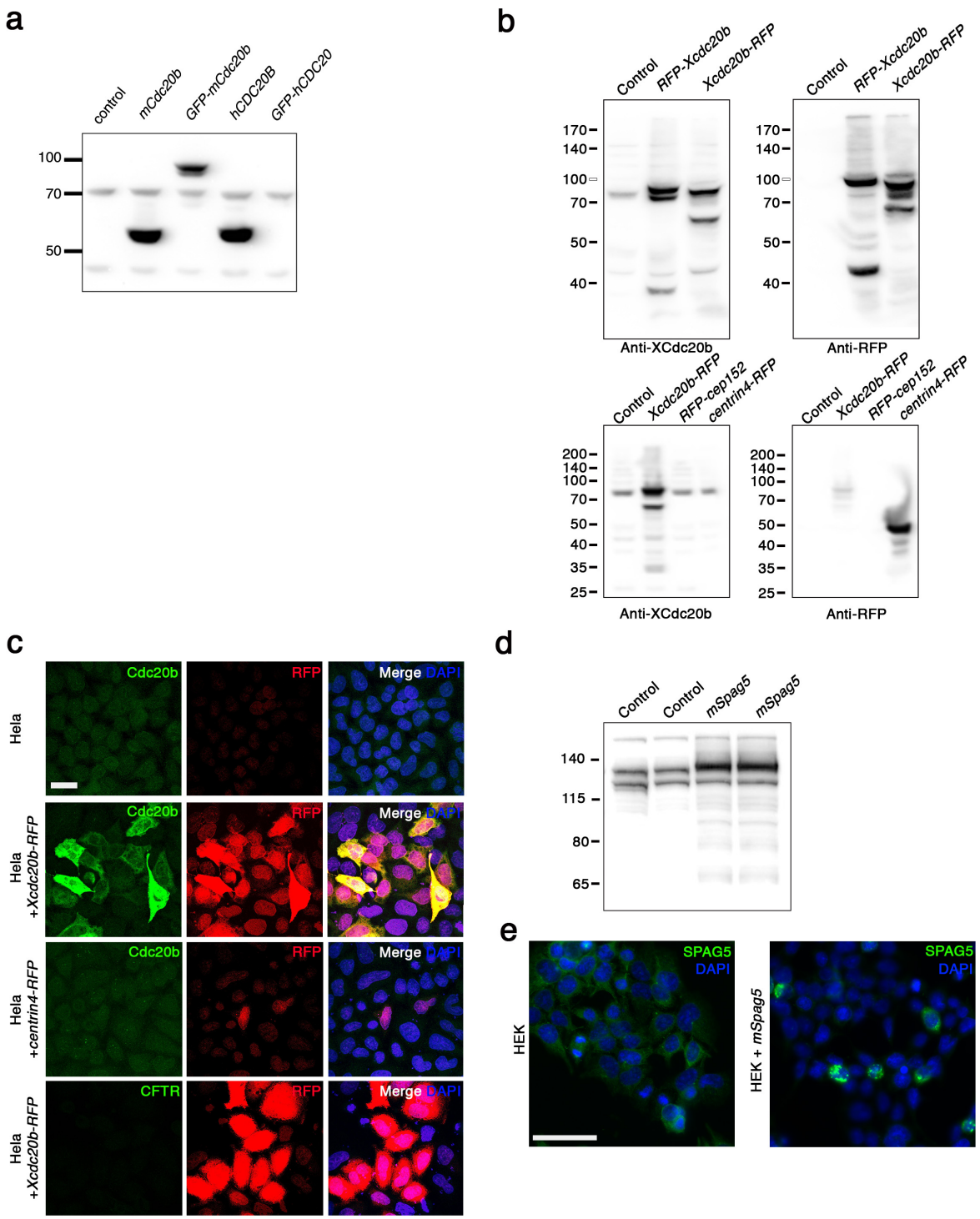
1049 clustering (Euclidian distance) was performed on samples. The scale color bar indicates the

1050 minimum and maximum values per row. (**c**) Promoter luciferase reporter assay. Promoter-

1051 associated firefly luciferase was normalized by constitutive renilla luciferase. Control and

1052 *CDC20B* promoter were co-expressed with a plasmid expressing E2F1 or E2F4, or a negative

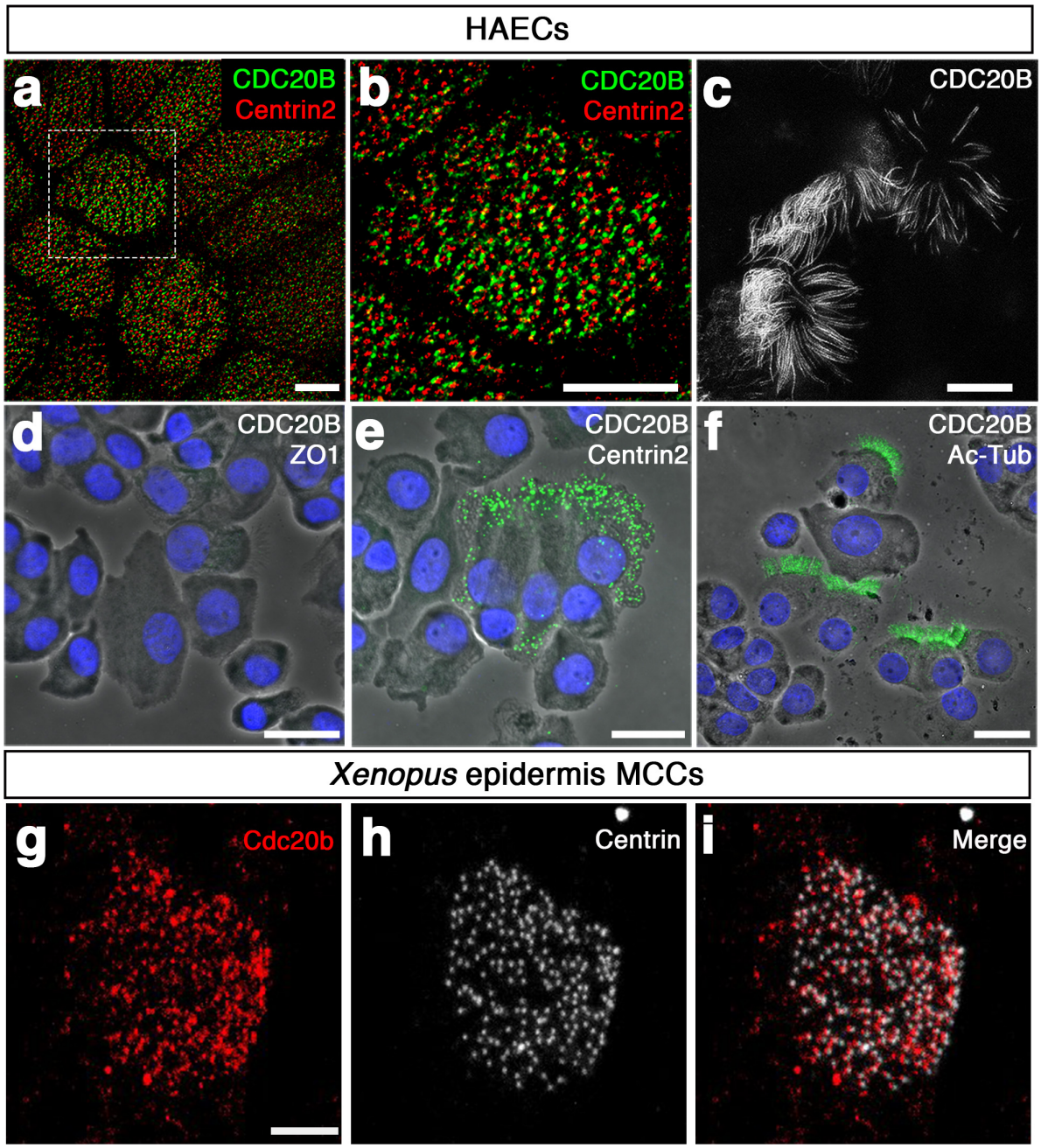
1053 control. Bars represent the average of 3 independent experiments. Error bars represent the
1054 standard deviation. (**d**) Ratio of gene expression (Multicilin-HGR vs. Multicilin-HGR
1055 +E2F4ΔCT) vs. ratio of E2F4 binding (Multicilin vs. no Multicilin). E2F4ΔCT prevents the
1056 formation of transcriptionally active Multicilin/E2F complexes. Centriole-related genes are
1057 highlighted in red. Genes from the multiciliary locus are highlighted in green. The graph was
1058 built by mapping and quantifying previously published raw data[9]. **CDC20B expression in**
1059 ***Xenopus* epidermis MCCs.** (**e**,**f**) *cdc20b* whole-mount *in situ* hybridization in early neurula
1060 st14 and st17, respectively. *cdc20b* mRNA is expressed in epidermal cells but not in the neural
1061 plate (np), as revealed on dorsal views. a: anterior, p: posterior, d: dorsal, v: ventral. (**g-i**) *cdc20b*
1062 (red) and *α-Tubulin* (α-*Tub,* green) double fluorescent *in situ* hybridization (FISH) on sectioned
1063 embryos at st14 (**g**), st17 (**h**) and st20 (**i**). Red and green arrows point immature MCCs co-
1064 expressing *cdc20b* and *α-Tub*. Nuclei are revealed by DAPI staining in blue. White dotted lines
1065 indicate the surface of the epidermis. Note that the majority of MCCs become negative for
1066 *cdc20b* expression at st20. (**j**) RT-qPCR showing the relative expression of *cdc20b* from st8
1067 (mid-blastula transition) until tadpole st32 normalized with *ODC* expression. Red bars indicate
1068 the peak of *cdc20b* transcript accumulation between st14 and st18, when centriole amplification
1069 occurs. (**k-m**) To reveal the dynamics of centriole multiplication, MCCs were stained by α-*Tub*
1070 FISH and by immunostaining against Centrin. Multiple Centrin-positive foci were detected at
1071 st14, marking the onset of centriologenesis. Procentriole aggregates, presumably organized
1072 around deuterosomes were clearly visualized at st16 (inset). Dispersed multiple centrioles were
1073 detected at st18. Scale bars: 250µm (**e**), 20µm (**g**,**h**), 5µm (**k**).
1074



Supplementary Figure 3: Antibody validations

(a) COS1 cells were transfected with vectors coding for the indicated proteins and immunoblot was performed using Proteintech rabbit antibody raised against human CDC20B. This antibody recognized human and mouse CDC20B but did not cross-react with human CDC20. (**b**) Hela cells were transfected with vectors coding for the indicated proteins and immunoblot was

1081 performed using a custom-made rabbit antibody raised against *Xenopus* CDC20B. (**c**) Hela cells
1082 were transfected with vectors coding for the indicated proteins and immunostainings were
1083 performed using the antibodies indicated on the photographs. Note that the antibody directed
1084 against *Xenopus* CDC20B did not cross-react with the centriole marker Centrin4. (**d**) HEK cells
1085 were transfected in duplicate with pCMV6-mSpag5, lysed 24 hours later and western blot was
1086 performed using proteintech rabbit polyclonal antibody raised against human SPAG5. (**e**) HEK
1087 cells were transfected with pCMV6-mSpag5, fixed with methanol 24 hours later and
1088 immunostained using proteintech rabbit polyclonal antibody raised against human SPAG5.
1089 This antibody cross-reacted with mouse SPAG5. Scale bars: 20µm (**c**), 50µm (**e**).
1090



1091

1092 **Supplementary Figure 4: CDC20B localization in mature MCCs.**

1093 **(a-f) CDC20B sub-cellular localization in human mature MCCs. (a**,**b**) ALI day 21 HAECs

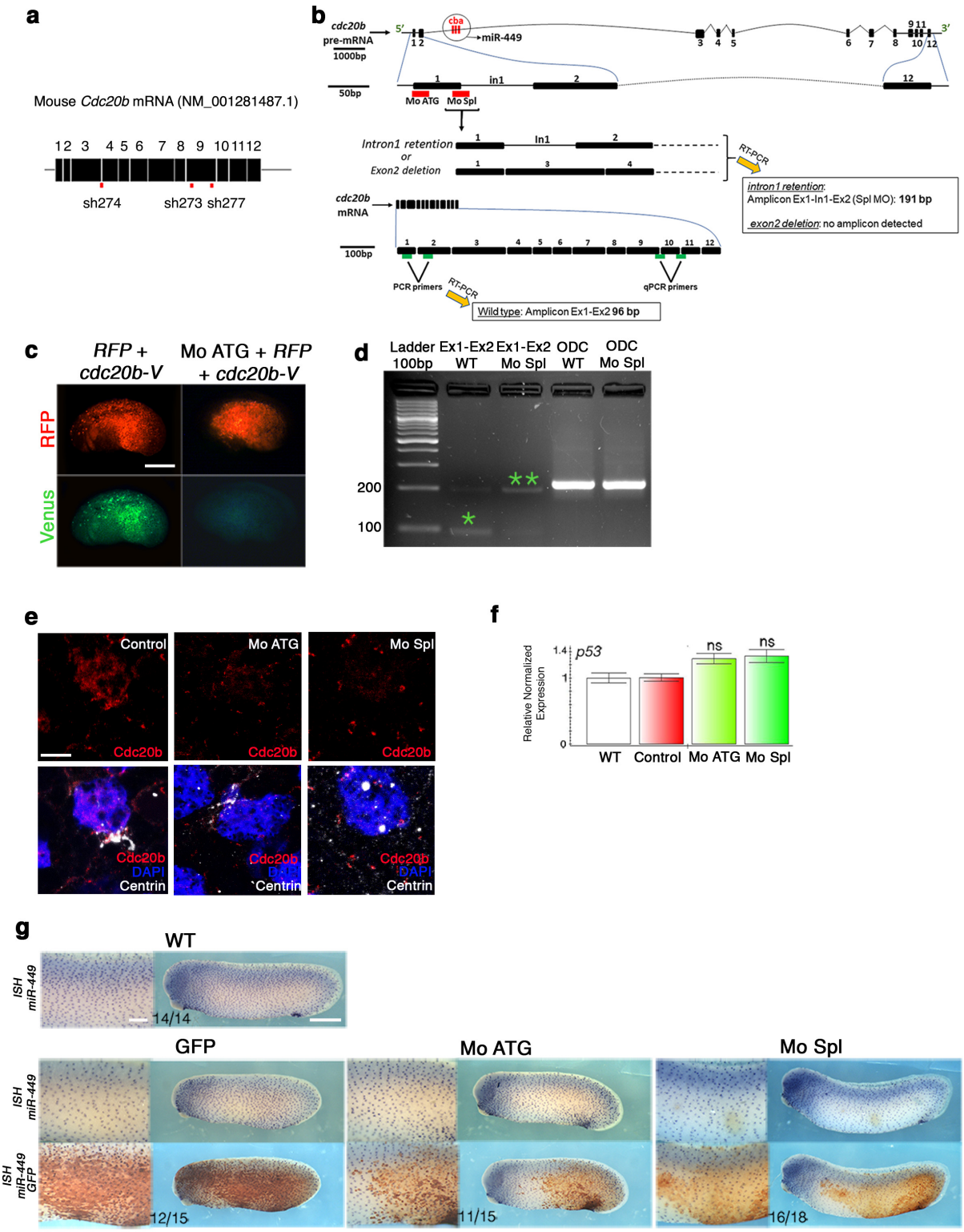
1094 were fixed in methanol, and immunostained against CDC20B and Centrin2. STED super-

1095 resolution microscopy revealed the association of CDC20B to BBs. (**c**) ALI day 21 HAECs

1096 were fixed in paraformaldehyde, and immunostained against CDC20B. STED super-resolution

1097 microscopy revealed the association of CDC20B with cilia. (**d-f**) DuoLink Assays on fully

1098 differentiated HAECs after cytospin. (**d**) Assay with CDC20B and ZO-1 antibodies was used
1099 as negative control. (**e**) Assay with CDC20B and Centrin2 (BBs) antibodies. (**f**) Assay with
1100 CDC20B and Acetylated-α-Tubulin (cilia) antibodies. Interaction between antibodies separated
1101 by less than 40nm generated green fluorescent signal. Nuclei are stained in blue. (**g-i**) **Cdc20b**
1102 **sub-cellular localization in *Xenopus* mature MCCs.** 4-cell *Xenopus* embryos were injected
1103 with *Multicilin-hGR* mRNA, induced with dexamethasone at stage 10.5 to activate Multicilin
1104 and immunostained for CDC20B (**g**) and Centrin (**h**) at stage 23. Scale bars: 5μm (**a-c**), 20μm
1105 (**d-f**), 5μm (**g**).
1106



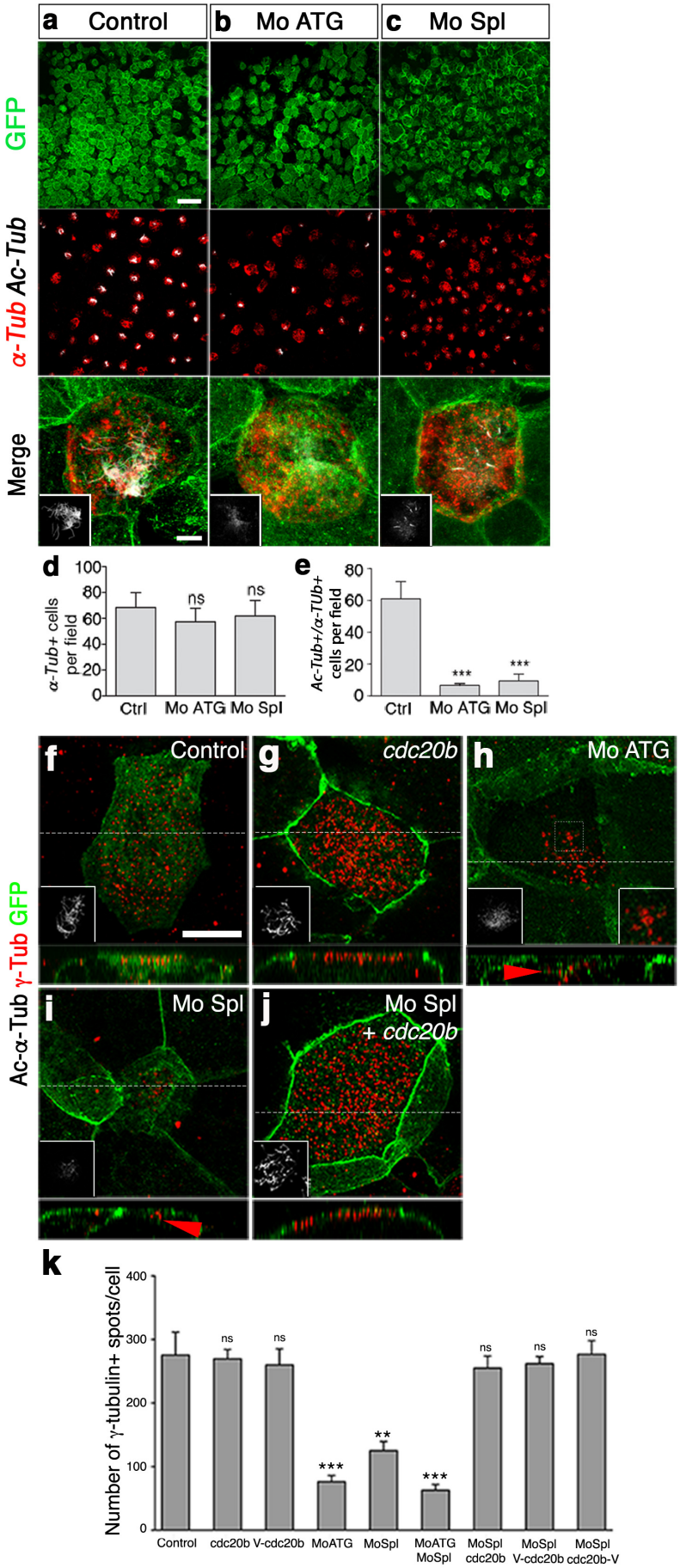
1107

1108 **Supplementary Figure 5: *cdc20b* knockdown in mouse and *Xenopus*.**

1109 (**a**) Schematic representation of mouse *Cdc20b* mRNA and position of shRNAs used in this

1110 study. Note that sh274 targeted the junction between exons 3 and 4, ruling out possible

1111 interference with the production of miR-449 molecules from the *Cdc20b* pre-mRNA. (**b**)
1112 Schematic representation of *Xenopus cdc20b* pre-mRNA with introns, exons and miR-449abc
1113 relative position and size. Red horizontal bars below exon1 show the position of *cdc20b* Mo
1114 ATG and Mo Spl. On the bottom, green horizontal bars indicate RT-PCR and qPCR primer
1115 positions. (**c**) The efficiency of Mo ATG was verified through fluorescence extinction of co-
1116 injected *cdc20b-Venus*. (**d**) RT-PCR confirmed that Mo Spl caused intron1 retention
1117 (amplicon=191bp; double green stars), which is expected to introduce a premature stop codon
1118 and to produce a Cdc20b protein lacking 96% of its amino-acids, likely to undergo unfolded
1119 protein response-mediated degradation. (**e**) Immunostaining with the anti-*Xenopus* CDC20B
1120 antibody confirmed that both Mo ATG and Mo Spl severely down-regulated CDC20B protein
1121 expression in st18 MCCs. (**f**) RTqPCR revealed that neither *cdc20b* mopholinos caused
1122 significant *p53* transcript up-regulation, a non-specific response sometimes detected in
1123 zebrafish embryos subjected to morpholinos. Four independent experiments were carried out
1124 to check *p53* expression in morphant conditions. (**g**) miR-449 expression revealed by whole-
1125 mount *in situ* hybridization with LNA probes was not perturbed in the presence of either
1126 mopholinos. Embryos were photographed before (top) and after (bottom) staining against co-
1127 injected GFP-CAAX to be able to detect miR-449 staining. The number of embryos showing
1128 normal miR-449 expression over the total number of embryos analyzed is indicated on the
1129 photographs. Scale bars: 500µm (**c**), 5µm (**e**), 500µm (**g**, whole embryo), 80µm (**g**, zoom).
1130

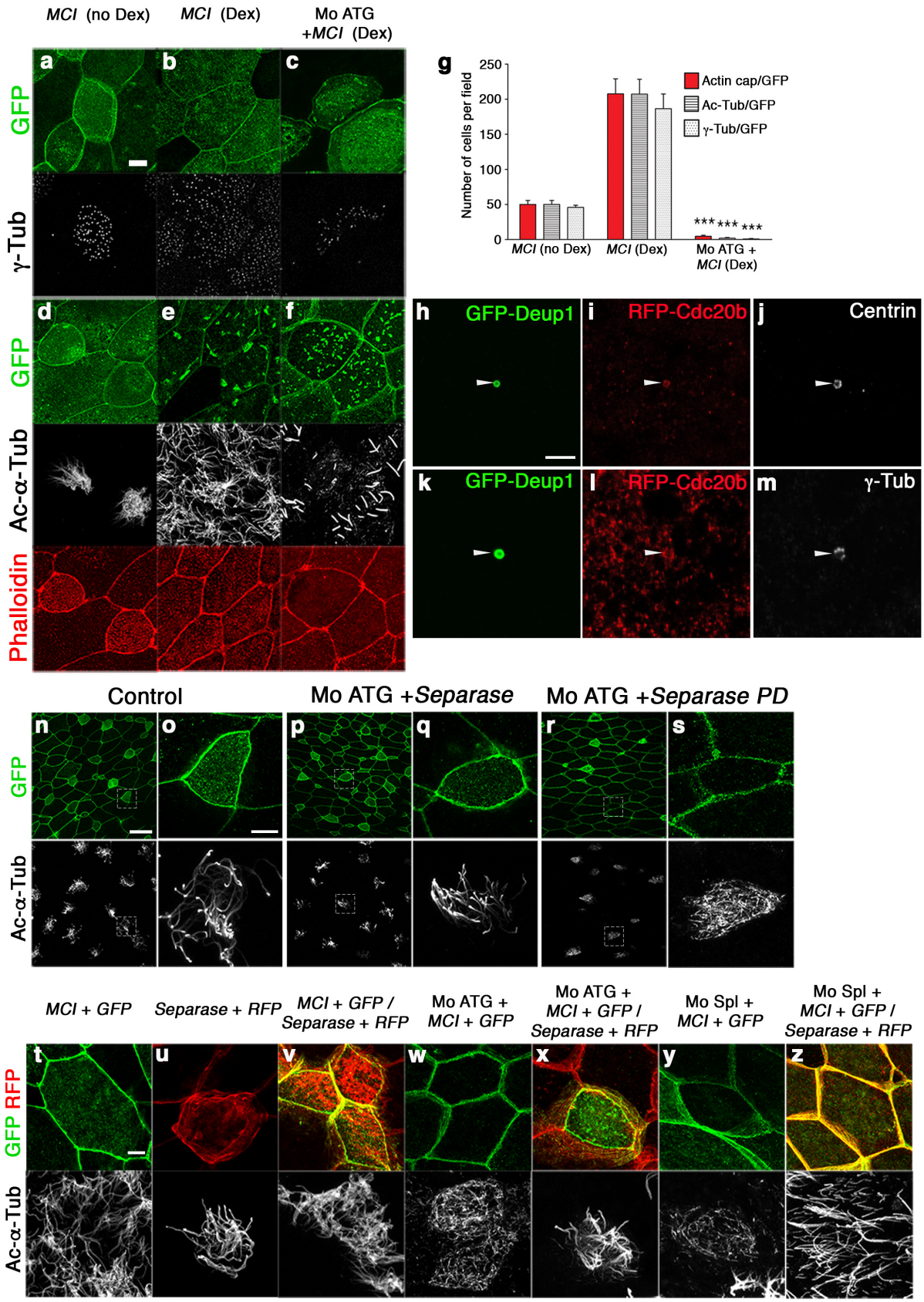

a Control
b Mo ATG
c Mo Spl
GFP
α-Tub Ac-Tub
Merge
d
α-Tub+ cells per field
ns
ns
Ctrl
Mo ATG
Mo Spl
e
Ac-Tub+/α-TUb+ cells per field
***
***
Ctrl
Mo ATG
Mo Spl
f Control
g cdc20b
h Mo ATG
Ac-α-Tub γ-Tub GFP
i Mo Spl
j Mo Spl + cdc20b
k
Number of γ-tubulin+ spots/cell
ns
ns
***
**
***
ns
ns
ns
Control
cdc20b
V-cdc20b
MoATG
MoSpl
MoATG MoSpl
MoSpl cdc20b
MoSpl V-cdc20b
MoSpl cdc20b-V

1131

1132 **Supplementary Figure 6: *cdc20b* knockdown impairs multiciliogenesis in *Xenopus*.**

1133 (**a**-**e**) 8-cell embryos were injected in presumptive epidermis with *cdc20b* morpholinos and
1134 *GFP-CAAX* mRNA (injection tracer) as indicated. Control was provided by *GPF-CAAX*
1135 injection alone. Embryos at tailbud st25 were processed for fluorescent staining against GFP
1136 (green), Acetylated α-Tubulin (cilia, white) and *α-Tub* mRNA (MCC marker, red). Insets on
1137 merged panels show cilia staining. Note that *cdc20b* morphant MCCs maintain expression of
1138 fate marker *α-Tub* but poorly grow cilia. (**d**) Bar graph showing quantification of *α-Tub*/GFP
1139 double positive cells per field of observation. (**e**) Bar graph showing quantification of *α-*
1140 *Tub*/Ac-Tub/GFP triple positive cells per field of observation. 10 fields corresponding to 10
1141 different embryos were analyzed for each condition. (**f**-**k**) 8-cell embryos were injected in
1142 presumptive epidermis with *cdc20b* morpholinos, *GFP-CAAX* and *cdc20b* mRNAs as
1143 indicated, and immunostained at tailbud st25 against GFP (injection tracer, green), γ-tubulin
1144 (BBs, red) and Acetylated α-Tubulin (cilia, white, left insets). Right inset in **h**: zoom on a
1145 stalled deuterosomal figure. z-projections made along white dotted lines are shown in bottom
1146 panels. Arrowheads point undocked BBs. (**k**) Bar graph showing the quantification of γ-tubulin
1147 spots per MCC. As that two individual γ-tubulin spots are detected around each basal body,
1148 twice as many spots are usually counted as compared to Centrin (Fig. 5j-o). Note that BB
1149 numbers were restored to normal levels in *cdc20b* Spl morphants injected with tagged and
1150 untagged versions of *cdc20b*. Scale bars: 50µm (**a**, top), 5µm (**a**, bottom), 5µm (**f**).

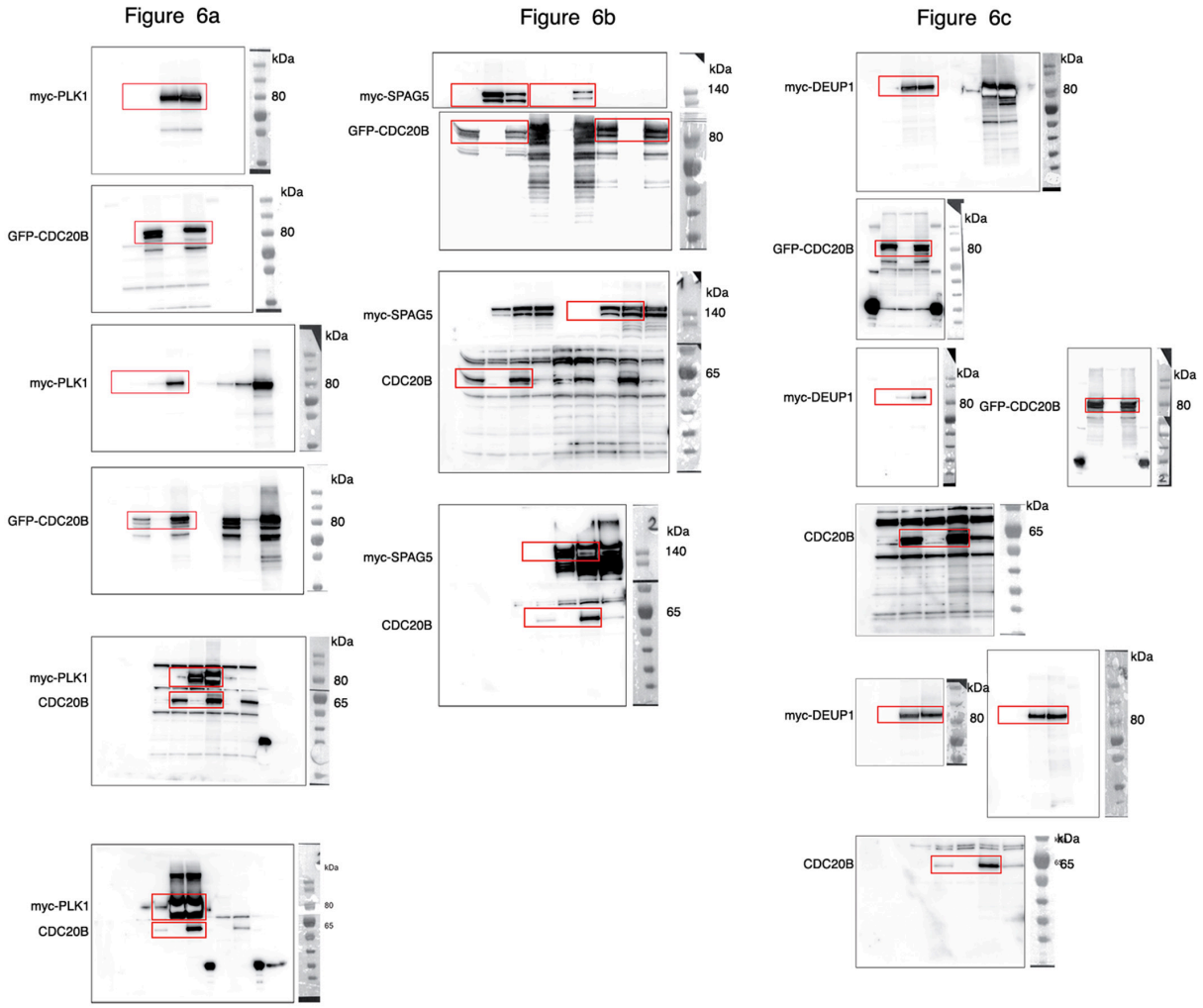
1151



Supplementary Figure 7: *cdc20b* knockdown prevents multiciliogenesis induced by Multicilin, and is counteracted by Separase overexpression.

1155 (**a-g**) ***cdc20b* knockdown prevents multiciliogenesis induced by Multicilin.** 8-cell embryos
1156 were injected in presumptive epidermis with *Multicilin-hGR* mRNA (*MCI*) and *cdc20b* Mo
1157 ATG, as indicated. *GFP-GPI* mRNA was co-injected as a tracer. MCI-hGR-injected embryos
1158 were induced with dexamethasone at st11. To check the efficiency of MCI induction some
1159 embryos were not treated with dexamethasone and served as controls (no DEX). Embryos were
1160 fixed at tailbud st25, and were stained against GFP (green) and γ-Tubulin (basal bodies,
1161 white)(**a-c**), or against GFP (green), phalloidin (apical actin, red), and Acetylated-α-Tubulin
1162 (cilia, white)(**d-f**). Note that *cdc20b* morphant MCI-induced MCCs failed to amplify centrioles,
1163 to maintain a proper actin cap, and to grow cilia. (**g**) Bar graph showing the quantification of
1164 GFP-positive cells that displayed normal actin, basal body and cilium staining. 5 fields (40x)
1165 per condition were analyzed. (**h-m**) **Deup1 recruits CDC20B in centriole amplification**
1166 **platforms.** 8-cell embryos were injected in presumptive epidermis with *Multicilin-hGR*, *RFP-*
1167 *CDC20B*, and *GFP-Deup1*mRNAs. Multicilin activity was induced with dexamethasone at
1168 st11, embryos were fixed at st18 and stained for GFP, RFP, Centrin (centrioles) or γ-Tubulin
1169 (deuterosome). White arrowheads point at active deuterosome-like structures formed around
1170 overexpressed GFP-Deup1, which incorporate RFP-CDC20B, consistent with their capacity to
1171 form a complex (Fig. 6c). (**n-z**) **Wild-type but not protease-dead Separase rescues**
1172 **multiciliogenesis in MCCs deficient for Cdc20b.** (**n-s**) 8-cell embryos were injected in
1173 presumptive epidermis with *GFP-GPI* mRNA, human *Separase* mRNA, and *cdc20b* Mo ATG,
1174 as indicated. Immunofluorescence against GFP (injection tracer, green), and Acetylated-α-
1175 Tubulin (cilia, white) was performed at tailbud st25. Cells in dotted squares were blown up for
1176 better visualization. Note that multiciliogenesis was rescued in *cdc20b* morphant MCCs by
1177 wild-type (**p**,**q**) but not protease-dead Separase (**r**,**s**). (**t-z**) 4-cell embryos were injected in one
1178 ventral blastomere (presumptive epidermis) with *MCI-hGR* and *GFP-GPI* mRNAs, in the
1179 presence or not of *cdc20b* morpholinos, as indicated. Next, at 16-cell stage, half of those

1180 embryos were injected with human *Separase* and RFP mRNAs in one ventral-animal
1181 blastomere. This setup was designed to avoid co-injection of *cdc20b* morpholinos with
1182 *Separase* mRNA, ruling out non-specific interference *in vitro* between these reagents. MCI-
1183 hGR-injected embryos were induced with dexamethasone at st11. All embryos were fixed at
1184 tailbud st25 and stained for GFP (*cdc20b* Mo tracer, green), RFP (*Separase* tracer, red) and
1185 Acetylated-α-Tubulin (cilia, white). Note that multiciliogenesis failed in MCI-induced *cdc20b*
1186 morphant MCCs (**w**,**y).** The presence of Separase rescued multiciliogenesis in MCI-induced
1187 *cdc20b* morphant MCCs (**x**,**z**). Scale bars: 5µm (**a**, **h**, **o**, **t**), 20µm (**n**).
1188



1189

1190 **Supplementary Figure 8: Uncropped Western blots.**

1191 Red boxes mark the parts of the Western blot images that are shown in the indicated parts of

1192 Figure 6.

1193

G1/S	
Symbol	Ensembl
ORC1	ENSG00000085840
ZNF367	ENSG00000165244
ADAMTS1	ENSG00000154734
CCNE2	ENSG00000175305
CDC25A	ENSG00000164045
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DTL	ENSG00000143476
CDC6	ENSG00000094804
CCNE1	ENSG00000105173
MCM2	ENSG00000073111
GINS3	ENSG00000181938
CHAF1B	ENSG00000159259
WDR76	ENSG00000092470
MCM6	ENSG00000076003
CLSPN	ENSG00000092853
CDCA7	ENSG00000144354
OSBPL6	ENSG00000079156
RAB23	ENSG00000112210
PLCXD1	ENSG00000182378
SKP2	ENSG00000145604
MDM1	ENSG00000111554
GINS2	ENSG00000131153
E2F1	ENSG00000101412
MCM5	ENSG00000100297
SNHG10	ENSG00000247092
HSF2	ENSG00000025156
UBR7	ENSG00000012963
RMI2	ENSG00000175643
NUP43	ENSG00000120253
ACD	ENSG00000102977
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MSH2	ENSG00000095002
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PMS1	ENSG00000064933
UNG	ENSG00000076248
KIAA1147	ENSG00000257093
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BARD1	ENSG00000138376
INTS8	ENSG00000164941
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CAPN7	ENSG00000131375
TIPIN	ENSG00000075131
C14orf142	ENSG00000170270
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S	
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CDC7	ENSG00000097046
DNA2	ENSG00000138346
EXO1	ENSG00000174371
KDELC1	ENSG00000134901
ANKRD18A	ENSG00000180071
BRIP1	ENSG00000136492
PKMYT1	ENSG00000127564
CDC45	ENSG00000093009
C11orf82	ENSG00000165490
BLM	ENSG00000197299
RAD51	ENSG00000051180
CCDC150	ENSG00000144395
CDCA5	ENSG00000146670
CPNE8	ENSG00000139117
MCM8	ENSG00000125885
ESCO2	ENSG00000171320
GOLGA8B	ENSG00000215252
ASF1B	ENSG00000105011
FANCA	ENSG00000187741
INTS7	ENSG00000143493
POLA1	ENSG00000101868
FANCI	ENSG00000140525
RRM2	ENSG00000171848
TTLL7	ENSG00000137941
RAD51AP1	ENSG00000111247
KAT2B	ENSG00000114166
CHML	ENSG00000203668
BRCA1	ENSG00000012048
ABHD10	ENSG00000144827
TYMS	ENSG00000176890
PRIM1	ENSG00000198056
TTC31	ENSG00000115282
E2F8	ENSG00000129173
CENPQ	ENSG00000031691
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RRM1	ENSG00000167325
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G2/M	
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CDC25C	ENSG00000158402
NEIL3	ENSG00000109674
PIF1	ENSG00000140451
KIFC1	ENSG00000237649
HJURP	ENSG00000123485
NCAPH	ENSG00000121152
KIF23	ENSG00000137807
SKA3	ENSG00000165480
KIAA1524	ENSG00000163507
NDC80	ENSG00000080986
CCNF	ENSG00000162063
CDCA8	ENSG00000134690
PSRC1	ENSG00000134222
FANCD2	ENSG00000144554
ESPL1	ENSG00000135476
CDR2	ENSG00000140743
AURKB	ENSG00000178999
BORA	ENSG00000136122
LMNB1	ENSG00000113368
TRIM59	ENSG00000213186
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CKAP2L	ENSG00000169607
STIL	ENSG00000123473
POLQ	ENSG00000051341
MELK	ENSG00000165304
CENPL	ENSG00000120334
LIX1L	ENSG00000152022
KIF11	ENSG00000138160
C14orf80	ENSG00000185347
UBE2C	ENSG00000175063
NCAPD3	ENSG00000151503
HAUS8	ENSG00000131351
FAM83D	ENSG00000101447
CDK1	ENSG00000170312
MAD2L1	ENSG00000164109
GABPB1	ENSG00000104064
SAP30	ENSG00000164105
CFD	ENSG00000197766
TTF2	ENSG00000116830
MID1	ENSG00000101871
GAS1	ENSG00000180447
TUBA1A	ENSG00000167552
ZNF587	ENSG00000198466
TUBD1	ENSG00000108423
FAN1	ENSG00000198690
CDKN2C	ENSG00000123080
TUBB2A	ENSG00000137267
TNPO2	ENSG00000105576
ZNHIT2	ENSG00000174276
TRMT2A	ENSG00000099899
PKNOX1	ENSG00000160199
ENTPD5	ENSG00000187097
KDM4A	ENSG00000066135
STK17B	ENSG00000081320
KLF6	ENSG00000067082
KATNA1	ENSG00000186625
H2AFX	ENSG00000188486
BRD8	ENSG00000112983
RCCD1	ENSG00000166965
CDKN1B	ENSG00000111276
UACA	ENSG00000137831
KCTD9	ENSG00000104756
ATL2	ENSG00000119787
KPNA2	ENSG00000182481
HRSP12	ENSG00000132541
VTA1	ENSG00000009844
HMGB2	ENSG00000164104
C2orf69	ENSG00000178074
FADD	ENSG00000168040
HIPK2	ENSG00000064393
KIF22	ENSG00000079616
MGAT2	ENSG00000168282
NR3C1	ENSG00000113580
DHX8	ENSG00000067596
NMB	ENSG00000197696
TFAP2A	ENSG00000137203
HINT3	ENSG00000111911
CDC16	ENSG00000130177
NUMA1	ENSG00000137497
ARMC1	ENSG00000104442
STAT1	ENSG00000115415
CCDC107	ENSG00000159884
TMPO	ENSG00000120802

M	
Symbol	Ensembl
CKS1B	ENSG00000268942
DEPDC1B	ENSG00000035499
SHCBP1	ENSG00000171241
FAM64A	ENSG00000129195
FYN	ENSG00000010810
KIF2C	ENSG00000142945
SPAG5	ENSG00000076382
CIT	ENSG00000122966
CENPA	ENSG00000115163
DIAPH3	ENSG00000139734
CADM1	ENSG00000182985
KIF14	ENSG00000118193
PLK1	ENSG00000166851
MDC1	ENSG00000137337
DEPDC1	ENSG00000024526
BUB1	ENSG00000169679
DLGAP5	ENSG00000126787
NUF2	ENSG00000143228
CEP55	ENSG00000138180
GTSE1	ENSG00000075218
HMMR	ENSG00000072571
FOXM1	ENSG00000111206
E2F5	ENSG00000133740
PRR11	ENSG00000068489
NEK2	ENSG00000117650
TACC3	ENSG00000013810
CENPE	ENSG00000138778
CCNB2	ENSG00000157456
CDC20	ENSG00000117399
BIRC5	ENSG00000089685
CCDC88A	ENSG00000115355
POC1A	ENSG00000164087
MKI67	ENSG00000148773
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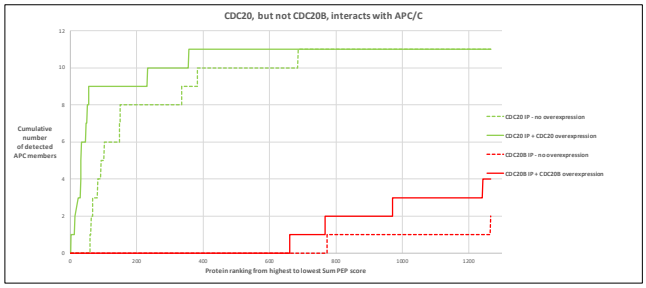
HSPA13	ENSG00000155304
CDC25B	ENSG00000101224
TPX2	ENSG00000088325
AURKA	ENSG00000087586
ANKRD40	ENSG00000154945
CENPF	ENSG00000117724
CNTROB	ENSG00000170037
NCAPD2	ENSG00000010292
SGOL2	ENSG00000163535
SRF	ENSG00000112658
DZIP3	ENSG00000198919
ECT2	ENSG00000114346
ORAOV1	ENSG00000149716
NUP35	ENSG00000163002
PTPN9	ENSG00000169410
HS2ST1	ENSG00000153936
RCAN1	ENSG00000159200
SS18	ENSG00000141380
HCFC1	ENSG00000172534
NUP98	ENSG00000110713
POM121	ENSG00000196313
TOMM34	ENSG00000025772
CKAP5	ENSG00000175216
GRK6	ENSG00000198055
SEPHS1	ENSG00000086475
QRICH1	ENSG00000198218
AHI1	ENSG00000135541
CNOT10	ENSG00000182973
KLF9	ENSG00000119138
SETD8	ENSG00000183955
ATF7IP	ENSG00000171681
RAD51C	ENSG00000108384
CDC42EP1	ENSG00000128283
HPS4	ENSG00000100099
GOT1	ENSG00000120053
MZT1	ENSG00000204899
RRP1	ENSG00000160214
AKIRIN2	ENSG00000135334
CDC27	ENSG00000004897
SMARCD1	ENSG00000066117
BIRC2	ENSG00000110330

M/G1	
Symbol	Ensembl
TROAP	ENSG00000135451
CDKN3	ENSG00000100526
PRC1	ENSG00000198901
HSD17B11	ENSG00000198189
BTBD3	ENSG00000132640
SLC39A10	ENSG00000196950
GTF3C4	ENSG00000125484
WWC1	ENSG00000113645
ELP3	ENSG00000134014
FOXK2	ENSG00000141568
OPN3	ENSG00000054277
KIAA0586	ENSG00000100578
ANTXR1	ENSG00000169604
CEP70	ENSG00000114107
HMGCR	ENSG00000113161
TULP4	ENSG00000130338
ZNF281	ENSG00000162702
CDK7	ENSG00000134058
LYAR	ENSG00000145220
PPP6R3	ENSG00000110075
DCP1A	ENSG00000162290
FAM189B	ENSG00000160767
AGPAT3	ENSG00000160216
PSEN1	ENSG00000080815
NUP37	ENSG00000075188
MSL1	ENSG00000188895
AGFG1	ENSG00000173744
SNUPN	ENSG00000169371
STAG1	ENSG00000118007
LRIF1	ENSG00000121931
PAK1IP1	ENSG00000111845
NCOA3	ENSG00000124151
PTTG1	ENSG00000164611
CTR9	ENSG00000198730
DKC1	ENSG00000130826
FOPNL	ENSG00000133393
VCL	ENSG00000035403
MRPS2	ENSG00000122140
WIPF2	ENSG00000171475

Supplementary Table 1: Gene sets used to assess enrichment of cell cycle phases related transcripts in individual cells analyzed by scRNA-seq.



Protein ranking from highest to lowest Sum PEP score	Cumulative number of detected APC/C members			
	CDC20 IP - no overexpression	CDC20 IP + CDC20 overexpression	CDC20B IP - no overexpression	CDC20B IP + CDC20B overexpression
1	0	0	0	0
2	0	1	0	0
6	0	1	0	0
7	0	1	0	0
12	0	1	0	0
13	0	2	0	0
24	0	3	0	0
27	0	3	0	0
28	0	3	0	0
30	0	3	0	0
31	0	4	0	0
32	0	5	0	0
33	0	6	0	0
46	0	6	0	0
47	0	7	0	0
50	0	7	0	0
51	0	8	0	0
55	0	8	0	0
56	0	9	0	0
59	0	9	0	0
60	1	9	0	0
62	1	9	0	0
63	2	9	0	0
67	2	9	0	0
68	3	9	0	0
81	3	9	0	0
82	4	9	0	0
91	4	9	0	0
92	5	9	0	0
101	5	9	0	0
102	6	9	0	0
147	6	9	0	0
148	7	9	0	0
149	7	9	0	0
150	8	9	0	0
231	8	9	0	0
232	8	10	0	0
335	8	10	0	0
336	9	10	0	0
356	9	10	0	0
357	9	11	0	0
382	9	11	0	0
383	10	11	0	0
660	10	11	0	0
661	10	11	0	1
685	10	11	0	1
686	11	11	0	1
767	11	11	0	1
768	11	11	0	2
773	11	11	0	2
774	11	11	1	2
970	11	11	1	2
971	11	11	1	3
1241	11	11	1	3
1242	11	11	1	4
1265	11	11	1	4
1266	11	11	2	4

IP CDC20 - no overexpression

Protein FDR Confidence	Accession	Symbol	Description	Sum PEP Score	Coverage	# Peptides	# PSMs	# Unique Peptides	MW [kDa]	emPAI	Score Sequest HT	global rank	APC rank
High	Q9UJX2	CDC23	Cell division c	109,78	47,74	27	48	27	68,79	6,80	88,85	60	1,00
High	**Q12834**	**CDC20**	**Cell division c**	**104,51**	**49,70**	**16**	**56**	**16**	**54,69**	**10,94**	**158,30**	63	2,00
High	Q9H1A4	ANAPC1	Anaphase-pro	101,92	23,66	32	42	32	216,36	1,30	36,79	68	3,00
High	Q9UJX4	ANAPC5	Anaphase-pro	91,89	42,38	23	50	23	85,02	3,19	83,00	82	4,00
High	Q9UJX3;A0A024RBJ3	ANAPC7	Anaphase-pro	85,18	46,58	21	38	21	66,81	6,13	61,05	92	5,00
High	A0A024RD22;Q13042	CDC16	CDC16 cell di	79,32	43,55	21	31	21	71,61	4,37	48,14	102	6,00
High	P30260	CDC27	Cell division c	63,63	26,33	16	31	1	91,81	2,00	41,46	148	7,00
High	G5EA36	CDC27	Cell division c	63,28	26,25	16	31	1	91,68	2,00	41,49	150	8,00
High	Q9UJX6	ANAPC2	Anaphase-pro	37,09	22,75	13	15	13	93,77	0,93	15,24	336	9,00
High	Q9UJX5	ANAPC4	Anaphase-pro	31,69	16,34	13	13	13	92,06	0,89	7,67	383	10,00
High	Q9UM13	ANAPC10	Anaphase-pro	16,01	31,89	5	9	5	21,24	1,85	11,56	686	11,00

IP CDC20 + CDC20 overexpression

Protein FDR Confidence	Accession	Symbol	Description	Sum PEP Score	Coverage	# Peptides	# PSMs	# Unique Peptides	MW [kDa]	emPAI	Score Sequest HT	global rank	APC rank
High	Q9H1A4	ANAPC1	Anaphase-pro	710,46	59,57	81	319	81	216,36	28,94	640,59	2	1
High	Q9UJX4	ANAPC5	Anaphase-pro	463,89	66,23	50	288	46	85,02	142,07	635,28	13	2
High	Q9UJX5	ANAPC4	Anaphase-pro	371,59	59,53	43	198	43	92,06	34,74	384,67	24	3
High	**Q12834**	**CDC20**	**Cell division c**	**332,93**	**69,54**	**28**	**791**	**28**	**54,69**	**202,09**	**1712,61**	28	3
High	P30260	CDC27	Cell division c	306,64	60,56	34	215	1	91,81	20,92	435,97	31	4
High	G5EA36	CDC27	Cell division c	306,44	60,51	34	212	1	91,68	20,92	426,79	32	5
High	Q9UJX6	ANAPC2	Anaphase-pro	301,10	54,50	45	165	45	93,77	27,12	297,57	33	6
High	A0A024RD22;Q13042	CDC16	CDC16 cell di	253,91	55,00	29	108	29	71,61	22,90	226,42	47	7
High	Q9UJX2	CDC23	Cell division c	244,49	54,77	33	121	33	68,79	27,80	262,31	51	8
High	Q9UJX3;A0A024RBJ3	ANAPC7	Anaphase-pro	236,24	64,61	32	117	32	66,81	37,75	239,65	56	9
High	Q9UM13	ANAPC10	Anaphase-pro	86,65	76,22	11	43	11	21,24	27,48	98,76	232	10
High	I3L3H6	CDC27	Cell division c	63,60	63,09	7	32	1	25,91	5,81	75,14	357	11

IP CDC20 B-no overexpression

Protein FDR Confidence	Accession	Symbol	Description	Sum PEP Score	Coverage	# Peptides	# PSMs	# Unique Peptides	MW [kDa]	emPAI	Score Sequest HT	global rank	APC rank
High	Q9UJX5	ANAPC4	Anaphase-pro	7,09	3,47	1	2	1	92,06	0,05	2,13	774	1
High	A0A024RD22;Q13042	CDC16	CDC16 cell di	2,31	4,68	1	1	1	71,61	0,06	0,00	1266	2

IP CDC20 B + CDC20B overexpression

Protein FDR Confidence	Accession	Symbol	Description	Sum PEP Score	Coverage	# Peptides	# PSMs	# Unique Peptides	MW [kDa]	emPAI	Score Sequest HT	global rank	APC rank
High	**Q86Y33**	**CDC20B**	**Cell division c**	**215,12**	**60,89**	**26**	**271**	**26**	**57,30**	**41,99**	**546,93**	7	0
High	Q9UJX2	CDC23	Cell division c	11,98	12,90	5	5	5	68,79	0,37	0,00	661	1
High	A0A024RD22;Q13042	CDC16	CDC16 cell di	9,77	5,65	3	3	3	71,61	0,21	3,53	768	2
High	P30260	CDC27	Cell division c	6,89	3,64	3	3	3	91,81	0,17	3,63	971	3
High	Q9UJX3;A0A024RBJ3	ANAPC7	Anaphase-pro	4,21	6,18	2	2	2	66,81	0,15	0,00	1242	4

Supplementary Table 2: CDC20, but not CDC20B, interacts with APC/C.