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1 **Forebrain Eml1 depletion reveals early centrosomal** 2 **dysfunction causing subcortical heterotopia**

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26 **eTOC summary**

27 Mechanisms leading to abnormal distribution of neural progenitors during cortical
28 development in the context of subcortical heterotopia associated with EML1 mutations remain
29 unknown. Using a forebrain-specific mouse model and mutant human cells, this work
30 innovatively demonstrates that by restoring microtubule function, abnormal progenitor
31 distribution and heterotopic volume are significantly reduced.

33 **Abstract**

34 Subcortical heterotopia is a cortical malformation associated with epilepsy, intellectual
35 disability and an excessive number of cortical neurons in the white matter. EML1
36 (Echinoderm microtubule associated protein like 1) mutations lead to subcortical heterotopia,
37 associated with abnormal radial glia positioning in the cortical wall, prior to malformation
38 onset. This perturbed distribution of proliferative cells is likely to be a critical event for
39 heterotopia formation, however the underlying mechanisms remain unexplained. This study
40 aimed to decipher the early cellular alterations leading to abnormal radial glia. In a forebrain
41 conditional Eml1 mutant model and human patient cells, primary cilia and centrosomes are
42 altered. Microtubule dynamics and cell cycle kinetics are also abnormal in mouse mutant
43 radial glia. By rescuing microtubule formation in Eml1 mutant embryonic brains, abnormal
44 radial glia delamination and heterotopia volume were significantly reduced. Thus, our new
45 model of subcortical heterotopia reveals the causal link between Eml1's function in
46 microtubule regulation and cell position, both critical for correct cortical development.

47 **Keywords**

48 Cortical development; brain malformation; microtubule; primary cilia; centrosome; cell cycle

49 **Introduction**

50 Cortical development is finely regulated to result in the formation of a functional cerebral
51 cortex required for advanced cognitive functions such as perception and language. At the
52 onset of neurogenesis, neuroepithelial cells (NECs) give rise to apical progenitors named
53 radial glial cells (RG), that become the predominant neuronal progenitor cell type, able to
54 self-renew but also give birth to post-mitotic neurons directly or indirectly *via* basal
55 progenitors (intermediate progenitors or basal RG) (Götz & Barde, 2005; Noctor et al., 2001,
56 2004). They are highly polarized cells with a short apical process that faces the ventricle and
57 terminates in an enlarged apical domain where a primary cilium (PC), docked to a basal body
58 (modified centrosome), protrudes into the embryonic cerebrospinal fluid (eCSF) to act as a
59 signaling hub, sensing secreted molecules. RG also possess a long basal process that crosses
60 the entire cortical wall and acts as a support for neuronal migration, before terminating at the
61 pial surface (Rakic, 1972). RG somata are restricted to the ventricular zone (VZ) and exhibit
62 interkinetic nuclear migration (INM), a process that defines the cell cycle-dependent
63 movement of the nucleus within the VZ. More precisely, nuclei move in G1 to reach the more

64 basal part of the VZ to undergo S-phase and then re-descend apically in G2 to undergo
65 mitosis at the ventricular surface (Sauer & Walker, 1959). INM relies on the actin and
66 microtubule (MT) cytoskeletons, as well as motor proteins such as kinesin and dynein (Spear
67 & Erickson, 2012; Tsai et al., 2010). Genetic mutations can alter RG in different ways,
68 affecting cortical development and leading to cortical malformations (Romero et al., 2018;
69 Stouffer et al., 2016).

70 Mutations in microtubule (MT) associated proteins can cause cortical malformations (Romero
71 et al., 2018; Stouffer et al., 2016). We and others have identified *Echinoderm microtubule*
72 *associated protein like 1* (*EML1*, also known as *EMAPLI*) mutations associated with human
73 ribbon-like Subcortical heterotopia (SH) (Kielar et al., 2014; Shaheen et al., 2017). Patients
74 with compound heterozygous, or homozygous mutations in *EML1* exhibit large clusters of
75 abnormally positioned neurons within the white matter (Kielar et al., 2014; Shaheen et al.,
76 2017). Patients may also present hydrocephalus, polymicrogyria, megalencephaly and corpus
77 callosum pathology (Kielar et al., 2014; Markus et al., 2021; Oegema et al., 2019; Shaheen et
78 al., 2017). Defects are associated with severe epilepsy, intellectual disability, and/or
79 psychomotor developmental delay (Kielar et al., 2014; Oegema et al., 2019). *Eml1* mutations
80 were also originally identified in a spontaneously arisen *Heterotopic Cortex* (*HeCo*) mouse
81 model (Kielar et al., 2014). To our knowledge, *Eml1/EML1* is the only known gene found
82 mutated in both human patients and rodent models with an SH phenotype (Bizzotto &
83 Francist, 2015; Collins et al., 2019; Grosenbaugh et al., 2020; Kielar et al., 2014; Uzquiano et
84 al., 2019). The EML1 protein, a member of the EMAP family, binds to MTs and has an N-
85 terminal domain with a coiled coil motif and a C-terminal region with a tandem β -propeller
86 structure (Richards et al., 2015). However, its role during neurodevelopment has remained
87 elusive.

88 In the *HeCo* mouse, the spontaneous mutation in *Eml1* resulted from an insertion of a
89 retrotransposon in an intron towards the end of the gene, leading to the absence of full-length
90 transcripts and the presence of small quantities of chimeric transcripts (Kielar et al., 2014). In
91 this model, it was shown that RG distribution is abnormal during cortical development. Many
92 of these cells were found in basal locations in the cortex (e.g. the intermediate zone and
93 cortical plate), hence proliferating outside the VZ and unable to act as a proper support for
94 neuronal migration from the VZ (Kielar et al., 2014). RG detachment may hence be the
95 primary event leading to heterotopia formation. It is important to further understand why

96 *Eml1* mutant RG detach, especially since similar mechanisms may contribute to basal radial
97 glia (bRG) production in gyrencephalic brains (Borrell & Götz, 2014; Penisson et al., 2019).

98 Previously, apical extremity defects were revealed in *HeCo* RG, notably the PC did not form
99 correctly, shown in both mutant murine and human progenitor cells (Bizzotto et al. 2017;
100 Jabali et al. 2022; Markus et al. 2021; Uzquiano et al. 2019). We showed that *Eml1* full
101 knockout reproduces heterotopia in mice, as well as RG detachment, but some lethality was
102 observed, as well as hydrocephalus (Collins et al., 2019). In this study, we produced *Eml1*
103 conditional knockout (cKO) mice with a forebrain-specific inactivation of *Eml1*, also
104 revealing severe heterotopia but without hydrocephalus. In this new model, we focused on
105 subcellular and cellular RG alterations in early and mid-corticogenesis to understand
106 pathogenic mechanisms which may lead to heterotopia formation. Assessing centrosomes *in*
107 *vitro* and in brain sections, we determined that their structure is affected in both human and
108 mouse RG, impacting MT nucleation. This important phenotype may precede PC defects and
109 RG apical detachment in early corticogenesis. By artificially stabilizing MT in embryonic
110 brains at early stages of corticogenesis, we were able to partially inhibit RG delamination and
111 reduce heterotopia volume, demonstrating a causal relationship between MT physiology,
112 ectopic RG and heterotopia formation. With these data, we reveal new roles for *Eml1* in early
113 cortical development, whilst unveiling key cellular patho-mechanisms contributing to the
114 early onset of SH formation.

115 **Materials and Methods**

116 **Animals**

117 Research was carried out conforming to national and international directives (directive CE
118 2010/63 / EU, French national APAFIS n° 23424; 46509) with protocols followed and
119 approved by the local ethical committee (Charles Darwin, Paris, France). Mice were housed
120 with a light/dark cycle of 12 h (lights on at 07:00). Males and females were used in all
121 analyses.

122 **Generation of *Eml1* cKO animals**

123 The *Eml1* mutant mouse line was established at the PHENOMIN MCI/ICS (Mouse Clinical
124 Institute - Institut Clinique de la Souris, Illkirch, France; <http://www-mci.u-strasbg.fr>), in the
125 framework of funding from the French Rare Disease Foundation (Collins et al., 2019).
126 The *Eml1* mutant line was generated and analysed on the mouse genetic background

127 C57BL/6N (B6N). LoxP sites surrounding exon 8 allowed conditional knockout. *Eml1* cKO
128 mice were generated by crossing with *Emx1*-Cre knock-in animals (Gorski et al., 2002).
129 Homozygote *Eml1*-Flox mice (*Eml1* fl/fl) were crossed with homozygote *Eml1*-Flox mice
130 also heterozygote for *Emx1*-Cre (Gorski et al., 2002) (*Eml1* fl/fl x *Eml1* fl/fl, *Emx1*-Cre/+).
131 All mice were housed in the IFM institute animal facility or at the CDTA, Orléans, France.

132 **Crosses and genotyping**

133 *Eml1* flox/flox animals were crossed with *Eml1* flox/flox-*Emx1*Cre/+ animals. Females were
134 placed in the male cage and the following morning the presence of a vaginal plug was
135 observed and considered to be embryonic day 0.5 (E0.5). Embryonic brains were collected at
136 the indicated times.

137 Genotyping primers used to detect Cre were:

138 Cre 1: GAACCTGATGGACATGTTTCAGG

139 Cre 2: AGTGCGTTCGAACGCTAGAGCCTGT

140 Primers used to detect the floxed *Eml1* allele were:

141 Primer Lf: GAAAACGTGCTTTGCTGTGTACATAGG

142 Primer Er: CACCCACTGAAGAAATGACTGGCAG

143 **Antibodies**

144 Primary antibodies used were: mouse anti-BrdU (ThermoFisher Scientific Cat# B35141,
145 RRID:AB_2536441 MobU-1, 1:100), rabbit anti-Pax6 (Covance Cat# PRB-278P,
146 RRID:AB_291612, 1:300), rabbit anti-Tbr2 (Abcam Cat# ab23345, RRID:AB_778267,
147 1:300), mouse anti-Ki-67 (BD Biosciences Cat# 556003, RRID:AB_396287, 1:200), rabbit
148 anti-PCNA (Proteintech Cat# 10205-2-AP, RRID:AB_2160330), mouse anti-GM130 (BD
149 Biosciences Cat# 610822, RRID:AB_398141, 1:300), mouse anti-Cep170 (ThermoFisher
150 Cat# 41-3200, RRID:AB_2533502, 1: 500), mouse anti- γ -tubulin (Sigma-Aldrich Cat#
151 T6557, RRID:AB_477584, 1:400), mouse anti- α -tubulin (Sigma-Aldrich Cat# T9026,
152 RRID:AB_477593, 1:500), rabbit anti-Arl13b (Proteintech Cat# 17711-1-AP,
153 RRID:AB_2060867, 1:400), mouse anti-*Eml1* 3E8 (Santa Cruz Biotechnology Cat# sc-
154 100938, RRID:AB_1122523, 1:500), rabbit anti-*Eml1* C3 (GeneTex Cat# GTX100252,
155 RRID:AB_1240763, 1:500), goat anti-Sox2 (Santa Cruz Biotechnology Cat# sc-17320,
156 RRID:AB_2286684, 1:500) and rabbit anti-GFP (ThermoFisher Cat# A-6455,
157 RRID:AB_221570, 1:2000), rabbit anti-Pericentrin (Abcam Cat# ab4448,

158 RRID:AB_304461), rabbit anti-Satb2 (Abcam Cat# ab92446, RRID:AB_10563678, 1:1000).
159 For Neuro-2A cells: Streptavidin Alexa Fluor 488 conjugate (Life Technologies, S32354,
160 1:5000, RRID:AB_2315383), Myc-tag (Cell Signaling, 2278, 1:400, RRID:AB_490778), and
161 γ -tubulin (Sigma-Aldrich, T5326, 1:200, RRID:AB_532292) were used.

162 Secondary antibodies used for immunohistochemistry and immunocytochemistry were goat
163 anti-rabbit Alexa Fluor 555 (Cell Signaling Technology Cat# 4413, RRID:AB_10694110),
164 goat anti-mouse Alexa Fluor 633 (Thermo Fisher Scientific Cat# A-21052,
165 RRID:AB_2535719), Streptavidin conjugated Alexa Fluor 488 (Invitrogen, S32354), goat
166 anti-rabbit Alexa 488 (ThermoFisher Cat#A-11008; RRID: AB_143165), goat anti-mouse
167 Alexa 488 (ThermoFisher, Cat#A28175; RRID: AB_2536161), goat anti-rat Alexa 568
168 (ThermoFisher Cat# A-11006; RRID: AB_141373, 1:800), goat anti-rabbit Alexa 568
169 (ThermoFisher, Cat# A-11011; RRID: AB_143157; 1:800), goat anti-mouse Alexa 633
170 (ThermoFisher, Cat# A-21050; RRID: AB_2535718; 1:800), goat anti-rabbit Alexa 633
171 (ThermoFisher, Cat# A-21070, RRID: AB_2535731; 1:800). Secondaries used for Western
172 blot were Dylight anti-mouse 800 (ThermoFisher, Cat#SA5-35521; RRID: AB_2556774,
173 1:5000), anti-rabbit 680 (ThermoFisher, Cat# 35568, RRID: AB_614946, 1:5000), anti-rabbit
174 800 (ThermoFisher, Cat#SA5-35571; RRID: AB_2556775, 1:5000).

175 **Plasmids**

176 Plasmids used were BLBP-GFP (Kielar et al, 2014) for *in utero* electroporation, CMV-
177 3xFlag-EML1 (Uzquiano et al, 2019) and YFP-EML4 (Richards et al, 2015) for
178 immunoprecipitation. For BioID, full-length mouse Eml1 and Eml1*T243A cDNAs were
179 cloned into the pcDNA3.1 mycBioID (Addgene#35700) vector. Retention using selective
180 hooks (RUSH (Boncompain et al. 2012)) was performed with SSTR3-GFP (generated in
181 Franck Perez's lab) and PKD2-GFP (kindly provided by Dominic Norris' lab, London UK).

182 **Embryonic brain collection and sectioning**

183 Females were sacrificed by cervical dislocation and embryos were collected. Brains were
184 fixed overnight with paraformaldehyde (PFA) 4% and then rinsed and stored with phosphate
185 buffered saline 1X (PBS). For vibratome sectioning: brains were placed in an inclusion of
186 10% sucrose and 7.5% agarose in PBS 1X. Brains were cut in 70 μ m thick coronal sections
187 using a vibrating blade microtome (Leica VT1000 S). For cryostat sectioning, brains were

188 embedded in OCT (Eprelia, Ref 6502) and frozen. Brains were cut in 20 μ m thick coronal
189 sections using a cryostat (Cryostar NX70, HOMVDP, Microm).

190 **Cresyl-violet labeling**

191 Cresyl violet staining was performed on sections mounted on Superfrost slides
192 (ThermoFisher). Sections were treated 5 minutes with EtOH 100%, then 5 min with EtOH
193 95% and 2 min with EtOH 70%. Slices were rinsed in water and then incubated in Cresyl-
194 Violet solution (Sigma 46597MJ) for 8 minutes and then with water (1 min), EtOH 70% (2
195 min), 95% (1 min) and 100% (3 min). After 5 minutes in xylene, they were mounted with
196 Eukitt medium.

197 Sections were analyzed at room temperature with a brightfield microscope (Provis; Olympus)
198 using a charge-coupled device (CCD) camera (CoolSNAP CF; Photometrics) with 2 \times (NA =
199 0.08), 4 \times (NA = 0.13) and 10X (NA=0.30) objectives. Minimum contrasts were adjusted
200 using Photoshop software (Adobe).

201 **Protein lysates and dosage**

202 Embryonic cortices were collected, and lysis of each embryonic cortex was performed
203 individually, by resuspending the tissue continuously with lysis buffer for a period of 1h at
204 4°C. The lysate was then centrifuged (30 min, 15000 rcf, 4°C), the supernatant was collected,
205 and the protein concentration was measured using the BCA protein assay kit (ThermoFisher)
206 and the BertholdTech Mithras ELISA microplate reader.

207

208 **Western blot**

209 Samples were denatured with 2X NuPAGE LDS Buffer (ThermoFisher) for 10 min at 70°C.
210 Denaturing electrophoresis was performed on a 4%–12% Bis-Tris Gel with MOPS SDS
211 running buffer for 2h at 110V. Proteins were transferred onto nitrocellulose membranes for 1h
212 at 110V and 4°C in a Tris-Glycine transfer buffer (Tris 25 mM, Glycine 192 mM, ethanol
213 10%). After protein transfer, the membranes were stained with Ponceau red to check the
214 transfer quality. The membranes were extensively washed with distilled water, followed by a
215 60 min incubation in 1X TBST (100 mM Tris pH 7.5, 150 mM NaCl, 10% ethanol, 0.05%
216 Tween) + 5% non-fat milk. Incubation for 45 min in the dark with the secondary antibody
217 (DyLight, see above) was performed to identify non-specific binding sites. The primary
218 antibody was incubated overnight (O/N) at 4°C, and abundantly washed with 1X TBST,

219 followed by incubation with the secondary antibody (see above). After extensive washes in
220 TBST then with 1X TBS, the membranes were scanned using an Odyssey (Li-Cor) infrared
221 scanner, with ImageStudio software.

222 **In utero electroporation (IUE)**

223 Timed-pregnant mice (E12.5 to E14.5) were anesthetized with isoflurane (4% during
224 induction and 2–2.5% during surgery) and embryos were revealed within the intact uterine
225 wall after sectioning the abdomen. Embryos were constantly hydrated with NaCl 0.9% (B.
226 Braun). A solution containing Blbp-GFP plasmid DNA (1 µg/µl, Blbp-promoter specific for
227 RG) and 20 % w/v fast green in sterile endo-free water was injected in the lateral ventricles of
228 the embryos. Forcep electrodes (System CUY650P5 NepaGene Co) were placed around the
229 embryo head at a 45° angle and plasmids electroporated by discharging a 4,000-µF capacitor
230 charged to 35 V (five electric pulses of 50 ms with 950 ms intervals) with a CUY21
231 NepaGene electroporator. The embryos were then placed back in the abdominal cavity for 24
232 h prior to subsequent analyses.

233 Embryonic heads were harvested and fixed overnight with 4% PFA at 4°C. Brains were
234 extracted, washed in PBS and cryoprotected overnight serially in 15% and 30% sucrose.
235 Brains were embedded in an embedding chamber using cryomedium Neg-50 (Epredia, Ref
236 6502), frozen under isopentane and dry ice, and cryo-sectioned at 20 µm with a Cryostar
237 NX70 (HOMVPD, Microm). Brain sections were fluorescently immunolabelled (see below)
238 with antibodies detecting Pax6 and GFP in electroporated progenitors. Images were acquired
239 at room temperature with a TCS Leica SP5-II confocal microscope, with analyses focused on
240 the future somatosensory cortex. 40X (NA = 1.25–0.75) and 100 X (NA=1.44) objectives
241 were used controlled by LAS-AF software for acquisition (Leica). ImageJ was used for cell
242 counting.

243 **EpoD treatment in mice**

244 Females were injected intraperitoneally with 20mg/kg Epothilone D (EpoD, Abcam) at E11.5,
245 E12.5 and sacrificed at E13.5 (for vibratome sectioning and immunohistochemistry) or E18.5
246 (for 3D imaging). EpoD was stored in PBS 1X and the exact needed volume was resuspended
247 in saline 37°C in a final volume of 200 µL. For control injections, 200 µL of saline 37°C was
248 injected at the indicated times.

249 **Immunohistochemistry**

250 Immunohistochemistry for all other experiments was performed on floating brains slices.
251 These were permeabilized with 1X PBST (0.1% Triton X-100) for 15 min. After washes,
252 blocking was performed for 1 h at room temperature (RT) with 1X PBS containing 10%
253 normal goat serum (NGS) and 0.1% Triton X-100 before incubation overnight (O/N) at 4°C
254 with the primary antibody. After extensive washes, sections were incubated with the
255 secondary antibodies for 2 h at RT protected from the light. This was followed by 10 min
256 incubation in Hoechst stain (ThermoFisher, 1:10000) prior to washing with 1X PBS. Brain
257 slices were mounted using Fluoromount G (Invitrogen). Images were acquired at room
258 temperature with a TCS Leica SP5-II confocal microscope, with analyses focused on the
259 future somatosensory cortex. Fluorochromes are as described above in the antibody section.
260 40X (NA = 1.25–0.75) and 100 X (NA=1.44) objectives were used controlled by LAS-AF
261 software (Leica). Minimum contrast adjustment was performed using ImageJ software.

262 For Tbr2, Ki67 and PCNA labelling, antigen retrieval was performed by incubating the
263 sections in sodium citrate 10 mM pH 6 at 95°C for 20 min and allowing them to cool down
264 before blocking.

265 For EdU and BrdU labelling after permeabilization, DNA was denatured with hydrochloric
266 acid (HCl) 2N treatment during 30 min at 37°C, followed by washes and blocking. EdU was
267 labelled using a click it EdU kit 647 following manufacturer's instructions (C10340,
268 ThermoFisher).

269 **3D visualization of the heterotopia**

270 E18.5 embryonic brains were dissected and fixed in 4% PFA overnight. They were then
271 incubated in PBS1X gelatin 0.2% Triton 0.5% (PBSGT) at RT for 24 hours. Anti-Satb2
272 antibody was diluted in PBSGT containing 0.1% saponin at 37°C for at least 5 days, after
273 which the brains were washed six times in PBSGT at RT. The next day, secondary antibodies
274 were diluted in PBSGT+saponin and incubated at 37°C for at least 2 days. All steps were
275 performed on a rocking platform or wheel. Immunolabeled brains were embedded in 1.5%
276 low-melting agarose (in 1% ultrapure Tris-acetate-EDTA solution). The embedded samples
277 were cleared according to a modified version of the original iDISCO+ protocol (Renier et al.,
278 2016). Samples were dehydrated by consecutive treatment with increasing concentrations of
279 methanol and finally incubated in 1 volume of methanol / 2 volumes of dichloromethane

280 (DCM, Sigma 270997-12X100ML). The samples were then washed twice in 100% DCM.
281 Finally, samples were incubated in di-benzyl ether (DBE, Sigma 108014-1KG) overnight. For
282 3D imaging, cleared samples were imaged on a light sheet microscope (LaVision Biotec)
283 equipped with a sCMOS camera (Andor Neo). Samples were imaged at room temperature in
284 DBE. Inspector microscope control software was used. Scans were taken at 1.25X zoom
285 magnification. Imaris (Bitplane, <http://www.bitplane.com/imaris/imaris>) was used for 3D
286 reconstruction, snapshots and movies.

287 **Mouse neuronal progenitor primary cell culture**

288 The neuronal progenitor cell cultures were adapted from (Sun et al., 2011) giving highly
289 enriched populations of Pax6(+) cells. For this, 6-well cell culture plates were coated with
290 Poly-D-lysine (PDL, P6407, Sigma Aldrich) 2 $\mu\text{g}/\text{cm}^2$ in sterile 1X PBS, O/N, at 37 °C and 5
291 % CO₂. The following day, the PDL was removed, and the plates were coated with 1 $\mu\text{g}/\text{cm}^2$
292 fibronectin (F1141, Sigma Aldrich) in sterile 1X PBS. E14.5 timed-pregnant mice were
293 sacrificed by cervical dislocation and the uterus was placed in ice cold basal medium
294 (DMEM/F12 Hams, 21041, ThermoFisher, 1% Pen-Strep (Gibco), 2.9 mg/ml glucose and 1.2
295 mg/ml sodium bicarbonate). The embryos were collected and the cortex from both
296 hemispheres was dissected and kept at 4°C in basal medium. The medium was removed and
297 substituted by pre-warmed sterile complete medium (basal medium complemented with 1X
298 B27 without vitamin A (12589-010, Gibco), 20 ng/ml of EGF (E9644, Sigma Aldrich) and 20
299 ng/ml of FGF (F0291, Sigma Aldrich). The tissue was dissociated, and each sample was
300 centrifuged (3 min, 1000 rcf). The medium was removed and substituted by fresh pre-warmed
301 complete medium followed by re-suspension of the cells. 1×10^5 cells were plated in coated 6-
302 well culture plates. The cells were split once at seven days in vitro (DIV) before performing
303 experiments. Half of the culture medium was changed by fresh complete medium every 2
304 days for one week. For splitting, cells were washed with pre-warmed Versene (Gibco),
305 followed by a 3 min incubation with pre-warmed StemPro Accutase (Gibco) at 37°C. Cells
306 were plated ($6-8 \times 10^5$) on coated 14 mm glass coverslips and cultured for 2 DIV for
307 immunocytochemistry experiments.

308 **Immunocytochemistry**

309 Cells were washed in 1X PBS prior to fixation with 4% w/v PFA in 0.1 M phosphate buffer,
310 pH 7.4, for 15 min at RT or fixed with methanol at -20°C. The cells were extensively washed

311 for 15 min in PBST (Triton X-100 0.1% in 1X PBS). Incubation with blocking solution (10%
312 NGS, ThermoFisher, 0.1% Triton X-100 in 1X PBS) was performed for 1 h at RT and
313 primary antibodies were applied for 2h at RT or O/N at 4°C (see above for antibodies). The
314 cells were extensively washed with blocking solution and secondary antibodies were
315 incubated for 2h at RT in the dark. After washes, Hoechst (1:10000, ThermoFisher) was
316 applied for 15 min at RT in the dark. The cells were extensively washed in PBS and the
317 coverslips mounted with Fluoromount G (Southern Biotechnology). Images were acquired at
318 room temperature with a TCS Leica SP5-II confocal microscope as above.

319 **RUSH trafficking experiments**

320 Retention using selective hooks (RUSH (Boncompain et al., 2012)) was performed with
321 plasmids SSTR3-GFP (lab) and PKD2-GFP. Plasmids were individually transfected in
322 neuronal progenitor Pax6+ cells in culture, using Lipofectamine 3000 (Invitrogen, L3000-
323 008) following manufacturer's instructions. An avidin concentration of 0.28 µg/mL was
324 added to block biotin already present in the DMEM F12 medium (Invitrogen, 21041025)
325 (Boncompain et al., 2021). After 24h, biotin at a concentration of 40µM in new medium was
326 added for a given time duration (0, 30, 60 and 90 minutes) and cells were fixed with 4% PFA
327 at RT for 15 min. Co-immunolabelling with GM130 and Arl13b antibodies as described
328 above was performed to assess the concentration of the RUSH protein respectively in the
329 Golgi Apparatus (GA) and primary cilia (PC) over time. Images were acquired at room
330 temperature with a TCS Leica SP5-II confocal microscope as above and analyzed using
331 Image J.

332 **Microtubule (MT) regrowth assay**

333 Primary cultures of mouse neuronal progenitor cells were plated on coated 14 mm glass
334 coverslips and placed directly on ice, then incubated for 40 min to allow disassembly of the
335 MT cytoskeleton. The cells were then placed at 37°C for the indicated times (1 min or 2 min)
336 and immediately fixed with methanol at -20°C. Immunolabelling for pericentrin and α-tubulin
337 were then performed as described above to analyze MT regrowth at the centrosomes. Images
338 were acquired at room temperature with a TCS Leica SP5-II confocal microscope as above
339 and analyzed using Image J.

340 **Human cells and electron microscopy (EM)**

341 Centrosomes were identified and analyzed in previously generated EM images of human
342 cortical progenitors (Uzquiano et al. 2019, Jabali et al. 2022). Control cells were Ctrl1: id-
343 number B7_028#4, female origin; Ctrl2: catalog ID GM08680, male origin. EML1
344 heterotopia patients were P135 (T243A compound heterozygous mutations) and P3489
345 (homozygous W225R mutations) (Kielar et al., 2014).

346 Progenitor cells were previously produced, fixed and imaged with an electron microscope
347 (Philips CM100), digitalized with a CDD camera (Gatan Orius). Images were analyzed using
348 ImageJ. In this study, centrosome aspect and numbers were assessed.

349 **Cell cycle analysis**

350 **EdU and BrdU injections**

351 BrdU (10 mg/ml) and EdU (5 mg/ml) were dissolved with 1X PBS. Intraperitoneal injections
352 of the appropriate volume of EdU and BrdU solutions were carried out for an injection of 50
353 mg/kg of EdU and 50 mg/kg of BrdU (one hour after the first injection). 1.5h after the first
354 injection, the pregnant mouse was euthanized by cervical dislocation and brains of embryos
355 were collected. After immunolabeling for EdU, BrdU and Pax6 on embryonic floating coronal
356 brain sections, RG were assessed according to their S-phase state (Harris et al., 2018;
357 Martynoga et al., 2005): The confocal SP5 microscope was used as already mentioned above.
358 Pax6 (+)Edu(+)BrdU(-) cells were considered to have left S-phase, Pax6(+)Edu(-)BrdU(+)
359 cells to have entered in S-phase and Pax6(+)Edu(+)BrdU(+) to be still in S-phase.

360 Considering that in a population of proliferating asynchronous cells, the fraction of cells in a
361 given phase of the cell cycle is directly proportional to the duration of that phase relative to
362 the total duration of the cell cycle (Nowakowski et al., 1989) we could thus calculate S-phase
363 length $T_s = (\text{EdU}^+; \text{BrdU}^+ \text{ cells} / \text{EdU}^+; \text{BrdU}^- \text{ cells})$. Also, because RG are cycling cells, we
364 could estimate their cell cycle duration using the formula $T_c = T_s / (\text{BrdU}^+ / \text{Pax6}^+ \text{ cells})$. In more
365 details: the ratio between the duration of one period of the cell cycle and that of another
366 period is equal to the ratio between the number of cells in the first period and the number of
367 cells in the second period (Nowakowski et al., 1989). The sequential exposure of proliferating
368 cells to EdU and BrdU allowed us to differentiate defined cell populations. Specifically,
369 progenitors are exposed to EdU in vivo at $T = 0\text{h}$ so that all cells in S phase at the start of the
370 experiment are labeled with EdU. At $T = 1\text{h}$, cells are exposed to BrdU to label all cells in S
371 phase at the end of the experiment. Cells still in S phase are also labelled with EdU. The

372 interval during which cells can incorporate EdU but not BrdU (T') is 1h. Cells from the initial
 373 EdU-labeled S-phase cohort will leave S-phase at a constant rate during the T' interval. This
 374 outgoing fraction will be labeled by EdU but not by BrdU. Consequently, the ratio between
 375 the number of EdU+BrdU- and EdU+BrdU+ cells is equal to the ratio between T' (which
 376 equals 1h) and Ts (Shibui et al., 1989). Thus, $T_s = \text{number of EdU+BrdU+} / \text{EdU+BrdU- cells}$
 377 and $T_c = T_s / (\text{BrdU+} / \text{Pax6+ cells})$.

378 **Dual labelling for Ki67 and PCNA**

379 The method was adapted from Fousse et al (Fousse et al., 2019). The confocal SP5
 380 microscope was used as already mentioned above. Cells with punctate PCNA in the nucleus
 381 and diffuse Ki67 were considered in S-phase. Cells with punctate Ki67 and diffuse PCNA
 382 were counted as cells in G1 phase. Nuclei with both Ki67 and PCNA in a diffuse pattern were
 383 considered as G2/M cells. Cells were counted using the plugin cell counter in ImageJ.

384 **BioID (Proximity Dependent Biotin Identification)**

385 **Cloning**

386 Full-length mouse *Eml1* and *Eml1*^{T243A}* cDNAs were cloned into pcDNA3.1 mycBioID
 387 (Addgene#35700) vector using the following primers:

Primer Name	5' to 3'
Eml1_BioID_N_ F	CTGTGCTGGATATCTGCAGAATTCATGGAGGACGGCTTCTCC
Eml1_BioID_N_ R	TGATCAGCGGTTTAAACTTAAACTTAAGCTTCTAAATGACTCGCCAC TGC

388

389 **BioID (Proximity Dependent Biotin Identification) Analysis**

390 Mouse Neuro2A cells (ATCC CCL-131) were transiently transfected 40 µg BioID constructs
 391 with Polyethylenimine (PEI) (Polysciences Inc., Cat. #23966) and after 24 hours, cells were
 392 incubated overnight in the presence of 0.05 mM D-biotin (Life Technologies, Cat. #B1595),
 393 followed by cell lysis in a lysis buffer (150 mM NaCl, 0.5% SDS, 2% NP40, 1mM EDTA,
 394 10mM IAA, 10 mM Tris pH 7.6 and Protease Inhibitor Cocktail, Roche, Cat.#11836170001).
 395 The lysates were centrifuged at 14,000g for 10 min at 4°C, and the protein concentrations

396 were measured using a bicinchoninic acid (BCA) assay (BCA Protein Assay Kit (Thermo
397 Scientific, Cat. #23225). Equal amounts of protein lysates from different conditions were
398 incubated with streptavidin beads (Pierce® Streptavidin Plus UltraLink® Resin, Thermo
399 Scientific, Cat.#53117) overnight at 4°C. The bound proteins were washed with several wash
400 buffers respectively for 10 min, wash buffer 1 (2% SDS), wash buffer 2 (1% Triton X-100,
401 2% sodium deoxycholate, 1mM EDTA, 50 mM NaCl, 50 mM HEPES pH 7.5), wash buffer 3
402 (1% Triton X-100, 0.5% NP-40, 0.5% sodium deoxycholate, 1mM EDTA, 50 mM NaCl, 10
403 mM Tris pH 8.1) and wash buffer 4 (50 mM NaCl, 50 mM Tris pH 7.4).

404 To identify biotinylated proteins by mass spectrometry (MS), on bead digestion was
405 performed. Proteins on the beads were reduced with 100 mM DTT in 50 mM ammonium
406 bicarbonate buffer at 56°C for 45 min and alkylated with 100 mM iodoacetamide at RT in the
407 dark for 30 min. MS grade trypsin (Pierce) was added to the beads for overnight digestion at
408 37°C. The resulting peptides were purified using C18 StageTips. Finally, the eluted peptides
409 were analyzed by a Thermo Fisher Scientific Q-Exactive LC-MS/MS mass spectrometer. This
410 experiment was performed three independent times.

411 The raw data were processed using Proteom Discoverer 2.3 (Thermo Fisher Scientific) and
412 searched against the UniProt *Mus musculus* database (accessed August 2022) with Sequest
413 HT search engine with tryptic digest specificity, allowing maximum two missed cleavage,
414 precursor mass tolerance 15 ppm, and fragment mass tolerance 0.05 Da (Liu et al., 2020).
415 SAINTexpress analysis was performed using three biological replicates (Teo et al., 2014).
416 Biotinylated proteins of negative control, transfected without DNA, were analyzed to define
417 non-specific interactions. Saint probability score ≥ 0.6 was used to define the proximal
418 interactors of EML1 and EML1*T243A. The final protein lists were analyzed using the
419 STRING v11.5 database (Szklarczyk et al., 2021) and g:Profiler annotation server (Raudvere
420 et al., 2019). Selected protein interactions were visualized using Cytoscape 3.9.1 (Shannon et
421 al., 2003).

422 **Immunocytochemistry of Neuro2A cells**

423 For immunofluorescence staining, Neuro2A cells (ATCC CCL-131) were transfected with
424 Lipofectamine 3000 (Invitrogen). Two days after transfection, the cells were fixed with 4%
425 paraformaldehyde (PFA). The antibodies and their dilutions used were as follows:
426 Streptavidin Alexa Fluor 488 conjugate (Life Technologies, S32354, 1:5000,

427 RRID:AB_2315383), Myc-tag (Cell Signaling, 2278, 1:400, RRID:AB_490778), and
428 Gamma-Tubulin (Sigma-Aldrich, T5326, 1:200, RRID:AB_532292). Secondary antibodies
429 were DAPI (359 nm), Alexa Fluor 488 (Invitrogen, S32354), Alexa Fluor 555 (Cell
430 Signaling, 4413), Alexa Fluor 633 (Invitrogen, A21052). Vectashield mounting medium was
431 used (Vector Laboratories, H-1200-10)

432 After fixation, images were acquired at room temperature with a Leica DMI8 / SP8 TCS DLS
433 laser scanning confocal microscope: HyD detectors provide signal collection in channels, then
434 acquired using a DFC365 FX camera (version FCAM2 V1.0.10). A 63× Plan Apo 1.4 NA oil-
435 immersion objective was used controlled by LAS-X software (Leica).

436

437 **Co-immunoprecipitation experiments**

438 For co-immunoprecipitation (IP) experiments, Neuro2A cells were co-transfected with CMV-
439 3xFlag-EML1 (Uzquiano et al, 2019) and YFP-EML4 (Richards et al, 2015) plasmids, or
440 CMV-3xFlag vector only (Uzquiano et al, 2019) with YFP-EML4 (Richards et al, 2015).
441 After 24 h in culture, cells reaching 70%–80% confluence were transfected using a ratio of
442 1:3 DNA and PEI, respectively (10 µg of DNA and 30 µg of PEI, Polysciences). After 48 h,
443 cells were recovered and washed twice with 1 X PBS and lysed by rotation for 20 min at 4°C
444 in 150 µl of RIPA buffer (50 mM Tris-HCl, pH 8, 100 mM NaCl, 1 mM EDTA, 0.1% SDS,
445 1% Nonidet P-40, 0.5% sodium deoxycholate and Protease inhibitor cocktail). The samples
446 were centrifuged at 14000 rcf. for 15 min at 4°C. Protein G Sepharose beads (Sigma Aldrich)
447 were diluted 1/10 in TNE buffer (50 mM Tris-HCl pH 7.4, 100 mM NaCl, 5 mM EDTA) and
448 60 µl of diluted beads were used for each IP experiment. The beads were extensively washed
449 with 1 x RIPA buffer, followed by centrifugation (12000 rcf., 4°C, 30 s). A pre-clearing step
450 was performed by incubating the cell lysates with the already-washed beads on a rotating
451 wheel at 4°C for 1 h 30 min. The samples were then centrifuged (12000 rcf., 4°C, 30 s) and
452 the supernatant collected. The latter was incubated with the following primary antibodies (1
453 µg): rabbit anti-GFP (A6455, Invitrogen), mouse anti-Flag (F1804, Sigma Aldrich), (O/N, 4°,
454 rotating wheel). Afterward, these samples were incubated with 100 µl of diluted washed
455 Protein G Sepharose beads (5 h, 4°C, rotating wheel). The unbound fraction was recovered
456 after centrifugation (12000 rcf., 4°C, 30 s). 5x washes of the beads in 1 x RIPA buffer were
457 performed before elution with NuPAGE LDS Buffer (Thermo Fisher), 10 min at 70°C. The
458 bound fraction was recovered after centrifugation (12000 rcf., RT, 2 min). Western blots and
459 immunodetection were performed as mentioned above.

460 **Image acquisitions**

461 As mentioned in the above methods, acquisitions of immunolabeled brain sections and plated
462 cells were carried out using confocal microscopes Leica SP5 and SP8. For vibratome brain
463 imaging a total of $z=10\ \mu\text{m}$ was imaged and for cryostat a total of $z=20\ \mu\text{m}$ was imaged. For
464 cells a total of $z=7\ \mu\text{m}$ was imaged. Hoechst (DAPI, 359 nm), Alexa Fluor 488 (Invitrogen,
465 S32354), Alexa Fluor 555 (Cell Signaling, 4413), Alexa Fluor 633 (Invitrogen, A21052) were
466 used.

467 **Image analysis**

468 Cell and element counting (Fig 1, Fig 2, Fig 3, Fig 4, Fig 6, Fig 7, Fig 8, Supp Fig 2) was
469 performed using Image J. Fluorescence intensity (Fig 4, Fig 5, Fig 6, Supp Fig 4, Supp Fig 5)
470 was measured using ImageJ. Images were acquired the same manner, with the same
471 parameters. For sections, the analyzed region of interest (ROI) was 200 microns width and the
472 total cortical wall in height, except for Ki67/PCNA analyses where the ROI was 100 microns.
473 Imaris (Bitplane, <http://www.bitplane.com/imaris/imaris>) was used for 3D reconstruction,
474 snapshots and movies (Fig 8, Supp Fig 1).

475 **Statistical analysis**

476 The sample size selection for experiments was based on both published and previous pilot
477 studies considering the sensitivity of the applied approaches. When possible, data were
478 collected and analysed in a blind manner to the experimenter. One main experimenter
479 performed each experiment. Statistical tests were carried out using GraphPad Prism 9.
480 Normality and homogeneity of variances were tested using either a D'Agostino-Pearson
481 omnibus, Shapiro-Wilk or KS normality tests, or data distribution was assumed to be normal
482 but this was not formally tested, depending on the number of samples. Significance was
483 established with $p\text{-value} < 0.05$. For each experiment, the statistical test used (Mann Whitney
484 or Two-way Anova) is described in the figure legend, as well as the number of individuals
485 analyzed. Data were collected and processed randomly.

486

487 **Supplemental material**

488 This manuscript contains 5 Supplemental figures and 2 Supplemental videos.

489

490 **Results**

491 **Ectopic RG detachment and PC defects occur during early** 492 **embryonic cortical development in *Eml1* cKO mouse brains**

493 To study the role of *Eml1* in RG detachment linked to heterotopia formation, we generated a
494 forebrain-specific *Eml1* cKO mouse model (Supplementary Figure 1 A). By crossing
495 homozygote *Eml1*-Flox mice (*Eml1* fl/fl) with *Eml1*-Flox mice also heterozygote for *Emx1*-
496 Cre (Gorski et al., 2002), homozygote forebrain-specific *Eml1* cKO (*Eml1* fl/fl *Emx1*-Cre/+) animals were obtained. Controls were Cre-negative *Eml1* fl/fl animals. 100% of *Eml1* cKO
497 animals were obtained. Controls were Cre-negative *Eml1* fl/fl animals. 100% of *Eml1* cKO
498 mice exhibit SH, as shown with cresyl violet staining at 8 weeks (Supplementary Figure 1 B),
499 as well as *Satb2* staining at embryonic stage E18.5 and 3D visualization of the heterotopia
500 (Supplementary Figure 1 C, Video 1 and 2). *Eml1* protein was shown to be absent by Western
501 blot (WB, Supplementary Figure 1 D).

502 After confirming the presence of heterotopia in adult *Eml1* cKO animals, RG were
503 characterized during development. Performing immuno-labelling for Pax6, a transcription
504 factor marker of RG (Englund et al., 2005), on embryonic coronal brain sections at different
505 developmental stages, the distribution of cells was analyzed throughout the cortical regions.
506 At E12.5, no differences in the total count of Pax6 positive (+) cells, neither in the proportion
507 of detached RG (outside the VZ) were observed in *Eml1* cKO brains (Figure 1 A-C)
508 compared to controls. From E13.5, the proportion of detached Pax6+ cells was increased in
509 *Eml1* cKO embryonic brains, as well as the total number of Pax6+ cells (Figure 1 A-C). This
510 suggests that RG begin to detach from E12.5 to E13.5 in the absence of *Eml1*. Cortical wall
511 thickness (CW) was unchanged until E15.5 when it was thicker in the *Eml1* cKO condition
512 (Figure 1 D). VZ thickness compared to CW thickness was significantly reduced at E14.5 and
513 E15.5 suggesting that the progenitor pool in the VZ is depleted over time (Figure 1 D).

514 Previously PC defects were observed in *HeCo Eml1* mutants (Bizzotto et al., 2017; Uzquiano
515 et al., 2019), we hence assessed if they were defective at early- and mid-corticogenesis in
516 *Eml1* cKO brains. *Arl13b* (a marker of PC) was immunodetected, revealing puncta at the
517 ventricular surface. At E12.5, the total number of *Arl13b*+ puncta was not changed, but the
518 mean length of PC was reduced in mutants (Figure 1 E-G). At E15.5, PC mean size was not
519 significantly different, although a tendency for reduction was observed. However, the number
520 of *Arl13b*+ puncta was decreased (Figure 1 H-J). These data suggest that PC defects are

521 present before observation of detached RG and that they are consistently observed in all *Eml1*
522 mouse models.

523 Thus, these data suggest that Pax6⁺ cells start to detach from E12.5 to E13.5, when an overall
524 increased number of Pax6⁺ RG is observed. Detachment correlates with a depletion of the VZ
525 pool of RG during corticogenesis.

526 We further tested if RG detach in a stage-specific manner in *Eml1* mutant conditions. *In utero*
527 *electroporation* (IUE) was performed to introduce the BLBP-GFP plasmid into RG at the
528 ventricular surface at different stages. One day after IUE, embryos were sacrificed and
529 immuno-labelling for Pax6 was performed to assess the percentage of electroporated RG in
530 the VZ versus those outside the VZ (detached or ectopic progenitors). In the *Eml1* cKO an
531 increased percentage of BLBP-GFP⁺ Pax6⁺ cells was found outside the VZ compared to the
532 WT after IUE at E12.5 and sacrificed at E13.5 (Figure 2 A, B). There were correspondingly
533 fewer cells observed in the VZ. From E14.5 to E15.5, a proportion of BLBP-GFP⁺ Pax6⁺
534 cells outside the VZ was observed but this was not significantly different from WT (Figure 2
535 C, D). This suggests that in the *Eml1* cKO, more RG delaminate at early, relative to mid-
536 corticogenesis.

537 Based on the progenitor abnormalities detected, we decided to investigate if progenitors
538 exhibit cell cycle defects in the absence of *Eml1*.

539 ***Eml1* cKO induces stage-specific cell cycle alterations in VZ RG at** 540 **E12 and E15**

541 We first investigated cell cycle using a method of staggered injection of dual thymidine
542 analogs in pregnant females at E12.5 and E15.5 (adapted from Harris et al. (Harris et al.,
543 2018) and schematized in Figure 3 A). Briefly, EdU⁺, BrdU⁺, and Edu+BrdU⁺ cells (labeled
544 with red, green, and white fluorescence) were counted, allowing a calculation of S-phase and
545 cell cycle length for apical RG (Pax6⁺) (Martynoga et al., 2005; Nowakowski et al., 1989).

546 S-phase duration was increased in *Eml1* cKO RG at E12.5 compared to WT (4.98 h in WT; 9
547 h in cKO) (Figure 3 B, C, G). The cell cycle duration was also lengthened (8.716 h in WT; 15
548 h in *Eml1* cKO). G1+G2+M duration was not significantly different, although there was a
549 tendency for a longer duration in cKO cells (Figure 3 C). We also looked at the distribution of
550 the cells in S-phase by dividing the cortical wall into 6 identical bins. In the *Eml1* cKO

551 cortical wall, there was an increased proportion of cells entering in S-phase apically (e.g. bin
552 2) and an inversed tendency for those exiting (Figure 3 D).

553 At E15.5 the cell cycle duration was calculated for *Eml1* cKO RG in the VZ, *Eml1* cKO RG
554 outside the VZ (ectopic RG), compared to WT RG in the VZ. The total cell cycle duration
555 was shorter in *Eml1* cKO RG in the VZ (20.67 h in WT; 11.05 h in VZ cKO RG and 15.99 h
556 in basal ectopic cKO RG) associated with a shortening of G1+G2+M phase (Figure 3 E-G). S-
557 phase length was not altered significantly at this time point. *Eml1* cKO RG outside the VZ
558 exhibited an intermediate phenotype between WT and *Eml1* cKO RG in the VZ (Figure 3 E-
559 G). Cell cycle phase and length durations did not differ significantly from the WT, nor from
560 cKO RG in the VZ.

561 With the dual injection method, it was not possible to determine the duration for G1 and
562 G2/M separately, so we additionally performed co-immunolabelling of Ki67 and PCNA,
563 associating cells to a cell cycle phase based on their labelling pattern (protocol adapted from
564 Fousse et al. (Fousse et al., 2019), Supplementary Figure 2 A). At E12.5, the proportion of S
565 phase cells was greater in *Eml1* cKO RG, indicating that the relative duration of S-phase is
566 longer, as also shown with the dual injection method (Supplementary Figure 2 B, C). At
567 E15.5, in *Eml1* cKO cells in the VZ, the S-phase proportion of cells was unchanged compared
568 to WT, whereas G1 phase cells were reduced, and G2/M cells were increased, suggesting a
569 relative lengthening of G2/M and shortening of G1 duration. Ectopic RG showed no
570 significant differences compared to WT (Supplementary Figure 2 D, E). Thus, there is overlap
571 between the results of the two methods, revealing robust defects in S-phase at E12.5, and
572 pinpointing alterations in G1, G2/M in *Eml1* cKO RG in the VZ at E15.5. In the absence of
573 *Eml1*, it is clear that progenitors exhibit stage-specific cell cycle alterations. Based on these
574 and the PC results, we were interested in assessing the possible upstream subcellular and
575 molecular mechanisms that might help explain the defects.

576 ***Eml1* interactome indicates cell cycle and microtubule proteins**

577 To gain insight into potential perturbations of protein interactions that occur when EML1 is
578 mutated (SH mutation EML1*T243A (Kielar et al., 2014)), BioID proximity labeling was
579 performed. BioID uses an abortive *E.coli* biotin ligase (BirA) fused to a target protein to
580 biotinylate its nearby interactors (Roux et al., 2012). Here, BirA was fused to the N-terminus
581 of EML1 and EML1*T243A and expressed in Neuro2A cells. We carefully checked for
582 centrosomal defects in transfected cells (in interphase and during mitosis), without identifying

583 any obvious abnormalities (Supplementary Figure 3 A). Cell size and multi-nucleation defects
584 were also not observed.

585 After isolation of biotinylated proteins and tryptic digestion (Figure 4 A), peptides were
586 analyzed by LC-MS/MS. A SAINT (Significance Analysis of INTeractome) probability (SP)
587 score (Teo et al., 2014) and fold changes were generated. Using an SP score ≥ 0.6 resulted in
588 49 and 30 proximal interactor proteins for EML1 and EML1*T243A respectively (Figure 4 B,
589 C). Thus, the EML1*T243A interactome comprised fewer proteins compared with the WT.
590 Also, when 26 common interactors for EML1 and EML1*T243A were plotted according to
591 their SP scores (not necessarily falling below $SP < 0.6$), these were found reduced in the
592 mutant (Figure 4 D). Gene ontology (GO) analyses were performed based on molecular
593 function, cellular compartment and biological process (Figure 4 E and Supplementary Figure
594 3 B, C). This showed that the most enriched biological processes are organelle organization,
595 translation, peptide biosynthetic process, peptide metabolic process, cell cycle and MT-based
596 processes (Figure 4 E). The GO analyses related to the SH-associated mutant form of EML1
597 reveal correspondingly fewer of these biological processes. Cross-referencing the BioID hits
598 showed that a number were also uncovered previously (e.g. (Cep170, Eml4, Dnmt1, Dhx15,
599 Ddx3x, Nat10, Ddx6, Ruvb1, Eif2s3x, Rpl10a, Tjp1, Dnajc7 and Rpl10) (Bizzotto et al.,
600 2017).

601 To further investigate the BioID hits of EML1, cellular components related to the
602 cytoskeleton, spindle and organelles were highlighted (Figure 4 F). Validating the BioID
603 screen, we were able to co-immunoprecipitate EML1 with EML4 (Supplementary Figure 3
604 D). Also, Vcpip1 is a previously established WT Eml1 partner, for which the interaction is
605 lost with the missense mutation (Uzquiano et al., 2019). A further interacting protein that was
606 specifically identified with WT EML1 is Cep170. This was not identified when EML1 carried
607 the patient mutation (T243A). Cep170 is known to be essential for centrosome maturation and
608 is a marker of the mature centriole. We checked for Cep170 intensity at the centrosomes
609 located at the ventricular surface of E12.5 and E15.5 WT and cKO embryonic brains and
610 observed that its intensity was significantly decreased in the *Eml1* cKO at E12.5, with a
611 possible tendency for reduction at E15.5 (Figure 4 G-J, Supplementary Figure 3 E). Eml1
612 interaction with Cep170 may thus be important for the localization of the latter to
613 centrosomes. Eml1 could be essential for centrosome integrity during early cortical
614 development, influencing cell cycle and PC.

615 **Human and mouse centrosomes are defective upon *EML1/Eml1*** 616 **mutation and this is rescued by EpoD treatment**

617 Along with centrosomal and PC proteins identified by BioID (e.g. Cep170, Ckap2, Cct2,
618 Cct28), several proteins related to the Golgi apparatus were also identified (e.g. Copg2 and
619 Vcpip1). General secretory trafficking from the Golgi apparatus was indeed shown to be
620 delayed in *Eml1* mutant RG ((Uzquiano et al., 2019) and data not shown). Cargo trafficking
621 and Golgi-derived protein transport are key to PC formation, we therefore decided to
622 investigate PC protein trafficking. Using primary cultures of Pax6+ cells (Sun et al., 2011),
623 we first tested the Retention Using Selective Hook (RUSH) approach (Boncompain et al.,
624 2012). Proteins are released from the endoplasmic reticulum (ER)/Golgi after addition of
625 biotin, and can be monitored in fixed cells at different time points (0, 30, 60 and 90 min, as
626 schematized in Supplementary Figure 4 A). By performing immuno-labelling of GM130 to
627 visualize the Golgi (Nakamura et al., 1995) and Arl13b to label the PC, we assessed the
628 concentration of SSTR3 and PKD2 PC proteins in each organelle over time. In control cells,
629 we observed that SSTR3-GFP increased in the Golgi at 30 min and in the PC at 60 min
630 (Supplementary Figure 4 B, C). In *Eml1* cKO progenitors compared to WT, SSTR3-GFP
631 levels were lower both in the Golgi at 30 min and in the PC at 60 min (Supplementary Figure
632 4 B, C). For PKD2-GFP, under control conditions, we observed a small increase at the Golgi
633 at 30 min and in the PC at 60 min, but in *Eml1* cKO a significant increase of the protein was
634 still observed at the Golgi at 60 min with a tendency for decrease at the PC (Supplementary
635 Figure 4 D). These abnormal profiles suggest that the anterograde trafficking of PC proteins is
636 impaired.

637 The centrosome serves as the base of the PC and plays a role in assembly, maintenance and
638 signaling pathways. Dysfunction of the centrosome can contribute to defects in PC formation
639 and function, resulting in a variety of diseases, including ciliopathies. Understanding the
640 relationship between the centrosome and PC is thus important for uncovering the molecular
641 mechanisms underlying cilia-related dysfunctions. Centrosome structure was hence assessed
642 using electron microscopy (EM) in human cells from 2 EML1 patients (patient 1, compound
643 heterozygous, R138X, T243A; patient 2, homozygous, W225R (Jabali et al., 2022)). In cortical
644 progenitors derived from induced pluripotent stem cells (IPSCs (Jabali et al., 2022)), all
645 patient cells examined exhibited abnormal centrosomes with poorly formed MT triplets
646 (Figure 5 A, B). In addition, 83.33% showed MT aggregates near the centrosomes, suggesting

647 centriole assembly defects in patient cells (Figure 5 A, B). Treatment with Etoposide
648 (EpoD, a microtubule polymerizing and stabilizing agent) was shown previously to rescue PC
649 length defects in *EML1* patient cells (Jabali et al. 2022). We therefore asked whether this
650 treatment could also rescue centrosome defects. After EpoD treatment, 86% of EML1 patient
651 cells showed rescued centrosomal structure and fewer cells (37.93%) showed MT aggregates
652 in the vicinity of the centrosomes (Figure 5 A, B). These results may indicate that centrosome
653 defects contribute to PC formation defects in mutant cells.

654 Given these results, key centrosome proteins were assessed in primary cultures of Pax6+ cells
655 from *Eml1* cKO mouse brain. Labeling for γ -tubulin (a marker of centrosomes) showed that
656 the number of γ -tubulin puncta per cell was not altered significantly in mutant cells however
657 the intensity of γ -tubulin was decreased (Figure 5 C, D). Also, pericentrin (pcnt), a marker of
658 pericentriolar material (PCM), a complex of proteins surrounding centrioles, was reduced in
659 intensity (Figure 5 C, E). The relative dispersion of the PCM, measured from the pcnt-labelled
660 area, may also be decreased (Figure 5 E). Thus, *EML1/ Eml1* seems to contribute to
661 centrosome integrity in human and in mouse progenitors.

662 ***Eml1* is essential for the recruitment of key proteins at the** 663 **centrosome**

664 To detect centrosomes *in vivo* during early and mid-corticogenesis, γ -tubulin and pcnt were
665 examined in coronal brain sections. The distribution of centrosomes along the cortical wall at
666 E12.5 and E15.5 was revealed by labelling with γ -tubulin. At E12.5, mutant brain
667 centrosomes were abnormally distributed with fewer γ -tubulin (+) puncta in the first 10 μ m
668 above the ventricular surface and more within the 10 - 20 μ m range compared to WT (Figure
669 6 A, B). A similar trend was observed at E15.5 (Figure 6 D, E). This suggests that centrosome
670 distribution may be altered prior to the observation of detached RG. Overall numbers of
671 puncta at each stage are unchanged (Supplementary Figure 5 A). We also checked for γ -
672 tubulin intensity at the ventricular surface and observed at E12.5 a tendency for decreased
673 fluorescence intensity in *Eml1* cKO conditions, with fewer obvious changes at E15.5 (Figure
674 6 C, F; Supplementary Figure 5 B). Likewise, labelling for pcnt revealed that PCM dispersion
675 was reduced in the cKO condition specifically at E12.5, but not at E15.5 (Figure 6 G, H;
676 Supplementary Figure 5 C). These results suggest that at E12.5, γ -tubulin and pcnt are not
677 well recruited to the centrosomes in the absence of *Eml1*.

678 **Microtubule growth is reduced in the absence of Eml1**

679 The centrioles and the PCM contain proteins that nucleate and stabilize MTs. Indeed,
680 centrioles act as templates for the formation of MTs that grow outward from the centrosome,
681 providing the cell with a polarized cytoskeleton. Since we observed PC and centrosomal
682 defects in Eml1 deficient cortical cells, we also tested how possible reduced protein
683 complexes at the MTOC might alter MT nucleation activity. In Pax6⁺ cells in culture, MT
684 regrowth was studied at 1 and 2 minutes after MT depolymerization, showing robust growth
685 in WT around pericentrin⁺ puncta (Figure 7 A, B). In Eml1 mutant cells, less puncta were
686 associated with MT regrowth (Figure 7 A, B). Among MTs surrounding centrosomes, their
687 average length appeared unaffected (Figure 7 C).

688 We next assessed MTs in brain tissue with α -tubulin labeling in coronal sections of E12.5
689 embryonic brains. In the VZ, assessing the distribution of fluorescence intensity from the
690 ventricular surface to a height of 100- μ m in the WT VZ, an enrichment of α -tubulin was
691 observed at the apical surface, however this was greatly reduced in the Eml1 cKO
692 (Supplementary Figure 5 D, E). There was also a tendency for decreased α -tubulin
693 fluorescence intensity in the absence of Eml1 (Supplementary Figure 5 D, F).

694 These combined results suggest that centrosome phenotypes, including in the VZ, may
695 influence efficient MT formation, potentially impacting RG behavior.

696 **RG detachment is partially rescued with EpoD treatment in vivo**

697 Because EpoD treatment rescues centrosome and PC defects, we tested if this treatment could
698 also rescue RG detachment *in vivo*. To do so, we injected EpoD intraperitoneally into
699 pregnant females consecutively at E11.5 and E12.5 and sacrificed them at E13.5. Pax6
700 immunolabelling was then performed in embryonic brain slices, and results were compared
701 with saline-injected embryos. Cortical wall and VZ thickness were compared and no
702 significant differences were found for these parameters (Figure 8 A, B), nor for overall cell
703 number in the VZ, although there was a possible tendency for reduction in the cKO (upon
704 EpoD treatment 114.08 ± 3.02 Hoechst labeled cells per ROI in control versus 104.42 ± 3.86 in
705 Eml1 cKO). The proportion of detached Pax6⁺ cells in the Eml1 cKO in both saline and
706 EpoD conditions was still increased, however with the EpoD treatment, detachment was
707 significantly less than in saline conditions (Figure 8 C, right). Of note, the number of Pax6(+)
708 cells in the Eml1 cKO is increased under saline conditions compared to the WT but this is no

709 longer the case after EpoD treatment (Figure 8 C, left), suggesting that the increased number
710 of Pax6+ cells may be linked to increased detached RG. Further verifying the cells outside the
711 VZ, shows in the cKO that 60 % of cells are Pax6+ve in the cKO at this age under saline
712 conditions and is reduced to 32% upon EpoD treatment. Verifying the number of Pax6
713 negative cells outside the VZ, although the results were not significant between the
714 conditions, a tendency for reduction in the cKO was observed compared to control under
715 saline conditions and the trend remains the same upon EpoD treatment (133.33±8.99 per ROI
716 in control vs 101.5±7.11 in *Eml1* cKO under saline conditions and 122.92±13.88 in control vs
717 103.33±13.33 in *Eml1* cKO under EpoD treatment). We hence do not rule out that
718 neurogenesis is slightly reduced at this timepoint in the cKO.

719 To further analyze the effects of EpoD on heterotopia formation subsequent to RG
720 detachment, we allowed the embryos to develop until E18.5 after being treated either by
721 saline or EpoD consecutively at E11.5 and E12.5. Whole embryonic brains were stained for
722 SatB2 and subsequently cleared for 3D imaging. As *Satb2*+ neurons form a compact layer of
723 cells covering the whole surface of the heterotopia, we used this staining to delineate the
724 shape of the malformation (Figure 8 D). The volume of the heterotopia was thus quantified
725 and normalized to the corresponding homotopic cortex. Our results suggest that EpoD
726 treatment in early corticogenesis partially reduces the volume of the heterotopia at E18.5
727 (Figure 8 E). *Satb2* mean fluorescent intensities did not appear to change between conditions
728 (Figure 8 F). Thus, we pinpoint early MT-dependent defects in the *Eml1* cKO that influence
729 both apical RG attachment and heterotopia formation.

730

731 **Discussion**

732 With this study we pinpoint early roles for *Eml1* in cortical development by analyzing the
733 phenotypes occurring before the heterotopia arises in a dorsal telencephalon-specific cKO.
734 Specifically, we identified that 1) RG start to delaminate after E12.5; 2) there are cell cycle
735 modifications which differ across the developmental window starting from this time point; 3)
736 *Eml1* is central for the centrosome function and 4) transient microtubule stabilization at early
737 stages partially rescues the *Eml1* cKO defects. Altogether these novel results raise several
738 intriguing questions.

739 RG detachment preferentially occurs at early but not mid-corticogenesis suggesting that there
740 may be a critical time window in the *Eml1* cKO mouse to produce ectopic RG. Thus, the early

741 defects we detected are likely to be correlated with detachment. We checked if *Eml1*
742 expression from E12.5 to E15.5 varies, using the humous.org database (Klingler et al., 2021),
743 and observed that in mouse, the expression of *Eml1* does not vary, but in Human it is enriched
744 at early corticogenesis compared to mid-corticogenesis in RG. To further understand mouse
745 developmental stage progenitor differences, and focusing on the specific alteration of γ -
746 tubulin, *pcnt* and their defects at the centrosomes, we analyzed expression in WT apical
747 progenitors using RNA sequencing data from Telley and colleagues (Telley et al., 2019).
748 Expression of *pcnt*, and γ -tubulin are reduced from E12.5 to E15.5 in mouse, suggesting that
749 they could be more important in early than late stages. Cortical progenitors were shown to
750 have different features at specific stages (Telley et al., 2019) so it is expected that they change
751 over time. Centrosomal protein composition also varies along corticogenesis and
752 differentiation (O'Neill et al., 2022). Our work hence further underlines that centrosome
753 function could be critical at certain stages. These combined data may help explain specific
754 defects occurring in particular time-windows, compatible with the deregulated processes we
755 identify. It is also of interest to note that basal RG (bRG) formation in gyrencephalic species
756 also may occur in a defined period. In ferret for example, it has been shown that there is a
757 peak of bRG production between E30 to E34 (Martínez-Martínez et al., 2016). This process
758 may share common features with *Eml1* mutant results.

759 We also show distinct cell cycle alterations at early and mid-corticogenesis. S-phase
760 lengthening was observed in E12.5 *Eml1* cKO RG, which may be correlated with a more
761 proliferative behavior of these cells. Indeed, it has been shown that RG with a longer S-phase
762 are prone to be more proliferative (Arai et al., 2011). Coherent with this, we found more
763 Pax6⁺ cells in total at E13.5 but not at E12.5, suggesting an amplification of the Pax6⁺ cell
764 pool. Of note, we also observe that, while Pax6⁺ cells increase significantly starting from
765 E13.5, the VZ thickness does not decrease until E14.5/E15.5. It is possible that Pax6⁺ cells
766 outside the VZ are the product of already detached and proliferating Pax6⁺ cells, and not
767 simply those directly detached from the VZ. However, in *EML1*-mutant organoids, it was
768 shown that Pax6⁺ cells in the VZ-like niche exhibited increased nuclear YAP1, a downstream
769 effector of HIPPO signaling linked with proliferation and S-phase entry (Jabali et al., 2022).
770 Pharmacological inhibition of YAP1 significantly reduced the numbers of ectopic neural
771 rosettes in *EML1*-mutant organoids (Jabali et al., 2022). Also, positive regulators of
772 proliferation are increased at the transcriptional level in *EML1* mutant organoids. Other works
773 show that, especially during S phase, centrosomes can have an MT-independent role. For

774 instance, targeting of polo kinase 1 (Plk1) to the centrosomes or γ -tubulin subcellular
775 localization are both essential for S-phase progression (Shen et al., 2013). Thus, there may be
776 several reasons for S-phase lengthening.

777 At later stages, we observed cell cycle duration shortening in the VZ, a decreased number of
778 cells in G1 phase and increased numbers in G2/M. At E15.5, there are less cells in the VZ
779 leading to its thinning. We can imagine that INM distance is shortened, and thus perhaps cells
780 can advance faster through the cell cycle. It has also already been observed that recombinant
781 Eml1 can localize to the mitotic spindle (Kielar et al., 2014) which may help explain in its
782 absence, why there might be a longer G2/M in mutant cells, although it is unclear why this
783 might specifically affect later stages. Moreover, at E15.5 we observe that ectopic versus VZ
784 RG are not equally affected in terms of cell cycle. It is indeed known that human bRG are
785 subject to a different environment compared to RG in the VZ, and they locally produce
786 growth factors such as PDGFD (Lui et al., 2014), and require a modified extracellular matrix
787 (ECM), in terms of components and receptors, that play crucial role in neuronal progenitor
788 proliferation and behaviour (Ferent et al., 2020). Furthermore, bRG have less contact with the
789 embryonic cerebrospinal fluid (eCSF) and hence less exposure to its secreted factors with an
790 impact on signaling. These elements might help explain the differences between the two
791 populations. Concerning the neuronal output of altered VZ progenitors, these increased cells
792 might collectively increase the number of neurons at a later timepoint; on the other hand, VZ
793 cells at E15.5 may also individually produce less neurons, as they have a shorter G1
794 (Takahashi et al., 1995). VZ cell composition may differ in the mutant, potentially containing
795 a higher proportion of Pax6-negative cells than in controls, although this remains to be
796 examined.

797 Because of the central role of the centrosome and PC in proliferation, we investigated protein
798 trafficking to these organelles. Regarding the diversity of possible routes taken by ciliary
799 proteins to reach the PC (Long & Huang, 2020), we do not yet know which of those may be
800 exclusively or partially altered in *Eml1* mutant conditions. Recently, it was shown in RG, that
801 post-Golgi apical trafficking occurs in the direction of the MT minus ends, via the Rab6-
802 dynein-Lis1 complex, which is required for apical localization of Crumbs3 (Brault et al.,
803 2022), a major apical domain determinant (Bulgakova & Knust, 2009). Therefore, genetic
804 inactivation of Rab6A/B or Lis1 induces a retraction of the RG apical process, leading to
805 delamination and ectopic division (Brault et al., 2022). Thus, known apical trafficking defects
806 to regions of the cell containing the centrosome and PC strongly resemble the *Eml1* cKO

807 phenotype. For example, Eml1 interacts with the GA protein Vcpip1, and its interaction is
808 reduced when Eml1 has the T234A patient mutation ((Uzquiano et al., 2019) and our BioID
809 data). Also, GA morphological defects were found in human and mouse cells in *EML1/Eml1*
810 mutant conditions (Uzquiano et al., 2019), suggesting that ciliary routes from this organelle
811 could be impaired. Indeed, increased accumulation of PKD2 in the GA in RUSH experiments
812 suggest a GA trafficking defect. SSTR3, for which we found decreased protein in the GA as
813 well as the PC, may exhibit an ER-GA protein transport alteration in the mutant. Recombinant
814 EML1 exhibits a vesicular pattern on MTs, altered with T243A mutation (Kielar et al., 2014).
815 We can hypothesize that through its ability to bind MTs, Eml1 can influence MT integrity and
816 the trafficking of cargos including PC proteins. Eml1 can also influence centrosome MT
817 integrity.

818 It is known that mutations of genes coding for tubulin proteins are associated with cortical
819 malformations (Poirier et al., 2013). Here, we showed that during early cortical development,
820 MTs are defective at the apical surface, and MT re-growth is impaired in Pax6+ cells in
821 culture. It was recently shown that EML1 is involved in MT nucleation as well as associating
822 strongly with growing MTs, with a unique pattern of accumulation towards their ends
823 (Jijumon et al., 2022). Patient mutations alter this co-localization with MTs, quite possibly
824 impacting MT dynamics and architecture. MT stabilization with EpoD rescues centrosomal
825 defects in human patient cells. EpoD treatment also partially rescued RG detachment in the
826 *Eml1* cKO, underlining an involvement of MT-dependent mechanisms in pathogenic
827 detachment. EpoD's effect may be by stabilization of centrosomes and nucleated MTs, even
828 though the latter may be fewer in number in the cKO. We showed that EpoD injections in
829 early corticogenesis not only partially rescues RG detachment at E13.5, but remarkably, can
830 also reduce the volume of heterotopia several days later at E18.5. It would be interesting to
831 assess if EpoD treatment rescues cell cycle parameters as well, in order to determine their link
832 to MT defects, and the extent to which cell cycle alterations are involved in RG detachment.
833 Further in-depth studies will address this, as our transient, early EpoD treatment clearly
834 targets an important time window for RG manipulation. These promising results in a
835 preclinical mouse model of heterotopia may help pinpoint future pertinent interventions for
836 this severe disorder.

837 **Data availability**

838 The mass spectrometry proteomics data (BioID) have been deposited to the ProteomeXchange
839 Consortium via the PRIDE partner repository (<https://www.ebi.ac.uk/pride/>) with the dataset

840 identifier PXD045846. The main body of data (including images and analyses) are available
841 in the article or online supplemental material. Source data are provided with this paper for
842 Western blots. All other source data are available from the authors upon reasonable request.

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865 **Conflicts of interest**

866 The authors declare no competing financial interests.

867 **Contributions**

868 Donia Zaidi: conceptualization, investigation, formal analysis, validation, visualization,
869 methodology, supervision for animal and in vitro experiments, as well as writing (original

870 draft as well as review and editing). Kaviya Chinnappa: investigation, formal analysis,
871 validation, methodology for animal experiments, especially in utero electroporation, as well
872 as writing (original draft as well as review and editing). Valeria Viola: investigation, formal
873 analysis, validation, for animal experiments, as well as writing (original draft as well as
874 review and editing). Berfu Nur-Yigit: investigation, formal analysis, visualization and
875 validation for BioID experiments, as well as writing (original draft as well as review and
876 editing). Nurhan Ozlu: conceptualization, project administration, funding acquisition,
877 methodology, investigation, formal analysis, supervision, visualization and validation for
878 BioID experiments, as well as writing (original draft as well as review and editing). Carmen
879 Cifuentes-Diaz: investigation, formal analysis, validation, visualization for performing EM
880 experiments., and writing (original draft as well as review and editing). Julien Ferent: formal
881 analysis, funding acquisition, investigation, methodology, supervision, validation and
882 visualization for model characterization and 3D heterotopia data, as well as writing (original
883 draft as well as review and editing). Ana Uzquiano: investigation, formal analysis,
884 visualization, methodology, supervision for animal and in vitro experiments, and writing
885 (original draft as well as review and editing). Ammar Jabali: investigation, formal analysis,
886 validation, methodology, for human in vitro experiments, and writing of the original draft.
887 Julia Ladewig: formal analysis, funding acquisition, investigation, methodology, supervision,
888 validation and visualization for human in vitro work, as well as writing (original draft as well
889 as review and editing). Emilie Lemesre: methodology and resources generating and supplying
890 RUSH constructs. Franck Perez: methodology and supervision for generating and supplying
891 RUSH constructs, validation and visualization. Fiona Francis: project administration, funding
892 acquisition, conceptualization, investigation, formal analysis, validation, visualization,
893 methodology and supervision, as well as writing (original draft as well as review and editing).

894 **Supplementary material**

895

896 Supplementary material is available at *JCB* online.

897

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1044

1045 **Figure legends**

1046 **Figure 1. Pax6⁺ cells start to detach during early corticogenesis with PC defects**
1047 **observed earlier in the *Em11* cKO mouse model. A. Representative images of**

1048 immunolabelling for Pax6 in WT and *Eml1* cKO embryonic brain coronal sections from
1049 E12.5 to E15.5. **B.** Quantifications of Pax6+ cell counts per region of interest (ROI of 200 μ m
1050 width) and **C.** proportion of detached Pax6+ cells above the VZ, expressed as mean +/- SEM.
1051 **D.** Quantifications of cortical wall thickness (CW), ventricular zone thickness (VZ) and the
1052 percentage of VZ/CW, represented as mean +/- SEM. Analyses performed at least on 3
1053 individuals from 2 different litters and 2 sections per individual for each genotype and age. **E.**
1054 Representative images of immunolabelling for Arl13b at the ventricular surface at E12.5 for
1055 WT and *Eml1* cKO. **F, G.** Quantification of cilia mean size and number at E12.5, expressed as
1056 mean +/- SEM. **H, I, J.** Similar analyses performed at E15.5 with 5 individuals per genotype
1057 and age, analyzed from 3 different litters. Test and significance: Two-way Anova, Sidak's
1058 multiple comparison (Pax6 analyses, CW and VZ thickness (data distribution was assumed to
1059 be normal but this was not formally tested)), Mann-Whitney (Arl13b analyses). *P-value* <0.05
1060 *, <0.01 **, <0.001 ***, <0.0001 ****. Scale bars (equivalent for WT and cKO): 30 μ m in A,
1061 20 μ m in E and 10 μ m in H.

1062

1063 **Figure 2. Abnormal detachment of RG occurs at early but not mid-corticogenesis. A.**
1064 Representative images and, **B.** Quantification of the distribution of GFP+Pax6+ cells in the
1065 VZ or outside the VZ between WT and *Eml1* cKO brains one day post-IUE, at E13.5. **C.**
1066 Representative images and, **D.** Quantification of the distribution of GFP+Pax6+ cells in the
1067 VZ or outside the VZ between WT and *Eml1* cKO brains one day post-IUE, at E15.5.
1068 Histograms show mean +/- SEM. Boxes with dotted lines indicate the areas of higher
1069 magnification images displayed. Arrows indicate GFP+Pax6+ double positive cells. N=5 for
1070 each condition from 3-4 litters. Test and significance: Two-way Anova, Sidak's multiple
1071 comparison test. Data passed normality test. *P-value* <0.0001 ****. Scale bars (A,C,
1072 equivalent for WT and cKO): 50 μ m (for main and insets).

1073

1074 **Figure 3. Cell cycle analyses of Pax6 (+) cells in E12.5 and E15.5 WT and *Eml1* cKO**
1075 **brains. A.** Schematic representation of EdU and BrdU injections performed for cell cycle
1076 analyses and related formulae to calculate duration of S-phase and the cell cycle. **B.**
1077 Representative images of EdU/BrdU/Pax6 labelling of E12.5 coronal brain slices from WT
1078 and *Eml1* cKO. **C.** Quantifications of S-phase length (Ts), cell cycle length (Tc), G1+G2+M
1079 length in hours at E12.5, expressed as mean +/- SEM. **D.** Distribution of cells in the E12.5

1080 cortical wall divided into 6 identical bins. Distributions are shown for cells entering in S-
1081 phase, maintained in S-phase and for those that exit S-phase, expressed as mean +/- SEM. **E,**
1082 **F.** Similar analyses performed at E15.5, comparing mutant cells in the VZ and outside. **G.**
1083 Schematic representation of cell cycle phase lengths for results obtained at E12.5 and E15.5 in
1084 WT and *Eml1* cKO embryos. Analysis was performed on 5 individuals from 3 litters per
1085 genotype and 2 ROI were analyzed per individual. Test and significance: Mann-Whitney,
1086 Two-way Anova, Dunn's post test (distribution analyses: data passed normality test). *P-value*
1087 <0.05 *, <0.01 **. Scale bars (equivalent for WT and cKO): 15 μ m at E12.5 and 30 μ m at
1088 E15.5.

1089

1090 **Figure 4. Eml1 interacting partner analyses reveal centrosomal protein Cep170 as an**
1091 **interactor, and reduced presence at the centrosome in *Eml1* mutant cells *in vivo*.** **A.**
1092 BioID workflow to identify proximal interactors of EML1 and EML1*T243A. **B.** For EML1
1093 and EML1*T243A BioID analysis, each hit is represented on the scatter plot displays by its
1094 Saint Probability (SP) score versus its fold change in the spectral count over the control. **C.**
1095 Venn diagram displaying overlapping hits for EML1 and EML1*T243A with an $SP \geq 0.6$. **D.**
1096 Heat map showing the SP scores of EML1 and EML1*T243A proximal interactors. **E.** Gene
1097 ontology (GO) annotation grouped into biological process of EML1 and EML1*T243A
1098 proximal interactors. **F.** Proximal interactors of EML1 related to microtubule cytoskeleton,
1099 spindle, and organelle cellular components (underlined proteins lose interaction significance
1100 in EML1*T243A $SP < 0.6$). **G.** Representative images of Cep170 labelling at the ventricular
1101 surface in E12.5 WT and *Eml1* cKO brains **H.** Quantifications of Cep170 fluorescence
1102 intensity at the centrosomes, also normalized to γ -tubulin intensity, expressed as mean +/-
1103 SEM (the *P-value* is indicated). **I, J.** Similar analyses performed at E15.5. For BioID
1104 experiments each condition has three replicates stemming from three independent
1105 experiments. Cep170 fluorescence intensity analyzes were performed from at least 4
1106 individuals per genotype from 3 different litters and 2 ROI analyzed per individual. Test and
1107 significance: Mann-Whitney. *P-value* <0.05 *. Scale bars (equivalent for WT and cKO): 10
1108 μ m.

1109

1110 **Figure 5. Centrosomal alterations in human patient and mouse mutant cells.** **A.**
1111 Representative electron microscopy (EM) images of control and EML1 patient cortical

1112 progenitors. Cells are untreated or treated with Epothilone D (EpoD). **B.** Quantifications (cells
1113 with defective centrosomes, black arrowhead in A, and MT aggregates, circled in A)
1114 performed on treated or non-treated human cells, values expressed as mean +/- SEM. **C.**
1115 Representative images of pericentrin and γ -tubulin labelling on Pax6+ cells cultured from WT
1116 and *Eml1* cKO embryonic brains. Enlargement of pericentrin is shown in the right panel and
1117 the red contours show the pericentrin areas in WT and *Eml1* cKO cells. **D.** Quantification of
1118 the total number of centrosome puncta per cell, and γ -tubulin fluorescence intensity per
1119 centrosome represented as mean +/- SEM. **E.** Quantification of pericentrin fluorescence
1120 intensity and pericentrin area represented as mean +/- SEM. γ -tubulin and pericentrin intensity
1121 were analyzed in 90 WT and 89 cKO cells from 3 independent cultures, indicated by different
1122 colors. Pericentrin area measurement was performed on 72 WT cells and 73 cKO cells. Test
1123 and significance: Mann-Whitney. *P-value* <0.0001 *****. Scale bars: for A 0.2 μ m (equivalent
1124 for all images); for C (equivalent for WT and cKO): 5 μ m (for main and insets).

1125

1126 **Figure 6. *Eml1* is essential for the recruitment of key proteins at the centrosomes in**
1127 **early corticogenesis. A.** Representative images of immunofluorescence labelling of γ -tubulin
1128 at the ventricular surface of embryonic coronal sections at E12.5 from WT and *Eml1* cKO
1129 individuals. **B.** Quantifications of γ -tubulin (+) puncta distribution from the 0 to 70 μ m from
1130 the apical surface at E12.5 in WT and *Eml1* cKO embryonic brain sections, expressed as
1131 mean +/- SEM. **C.** Quantification of γ -tubulin fluorescence intensity at E12.5 (the *P-value* is
1132 indicated), expressed as mean +/- SEM. **D, E, F.** Similar analyses performed at E15.5. **G.**
1133 Representative images of pericentrin labelling at the ventricular surface at E12.5 in WT and
1134 *Eml1* cKO brains, and quantification of pericentrin dispersion at E12.5, expressed as mean +/-
1135 SEM. **H.** Similar analyses performed at E15.5 (the *P-value* is indicated). For centrosome
1136 analyses *n*=5 individuals from 3 litters were analyzed per genotype and age. 2 ROI were
1137 analyzed per individual. For pericentrin area analyses: at least 4 individuals were analyzed
1138 from 3 litters per genotype and age. Test and significance: Two-way Anova, Bonferroni post
1139 tests (distribution analyses: data passed normality test), Mann-Whitney. *P-value* <0.05 *.
1140 Scale bars (equivalent for WT and cKO): 30 μ m (for A and D); 10 μ m in G, H (main), 5 μ m
1141 (for G,H insets).

1142

1143 **Figure 7. Microtubule regrowth at centrosomes is impaired in *Eml1* mutant conditions.**
1144 **A.** Representative images for pericentrin+ puncta ('centrosomes') with α -tubulin labeling
1145 ('MTs') on WT and *Eml1* cKO cells after ice recovery MT assays (1 and 2 minutes). **B.**
1146 Quantifications of percentage (%) of centrosomes exhibiting MT regrowth after 1 or 2
1147 minutes per analyzed ROI, represented as mean +/- SEM **C.** Quantifications for mean length
1148 of MTs per centrosome 2 minutes after ice recovery, represented as mean +/- SEM ($n=49$
1149 centrosomes for *Eml1* cKO and 53 for WT). 16 ROI analyzed from 2 different cultures after 2
1150 min and 12 ROI analyzed from 2 different cultures after 1 min. Different cultures are
1151 indicated by dots of different colors. Test and significance: Mann-Whitney. *P*-value <0.01 **,
1152 <0.001 ***. Scale bars (equivalent for WT and cKO, and for 1 min, 2 min): 10 μ m.

1153

1154 **Figure 8. Abnormal detachment and subsequent heterotopia formation is partially**
1155 **rescued with EpoD treatment.** **A.** Representative images of Pax6 labelling for WT and *Eml1*
1156 cKO in saline or EpoD conditions at E13.5. **B.** Quantification of the cortical wall and
1157 ventricular zone (VZ) thickness and **C.** total count for Pax6+ cells and distribution outside of
1158 VZ in WT and *Eml1* cKO from saline and EpoD conditions represented as mean +/- SEM
1159 ($n=6$ individuals from 2 litters at least, indicated by dots of different colors). **D.**
1160 Representative images of the heterotopia volume in 3D visualized by SatB2
1161 immunofluorescence. The homotopic cortex is depicted in transparency (purple) and the
1162 heterotopia is shown with a solid rendering (red). *Eml1* cKO embryos received saline or EpoD
1163 at E11.5 and E12.5 and were analyzed at E18.5. Three different angles are shown. **E.**
1164 Quantification of the ratio between heterotopia volume and that of the homotopic cortex in
1165 *Eml1* cKO with Saline or EpoD, represented as mean +/- SD ($n = 7$ embryos from 2 litters).
1166 Two independent litters are color-coded. **F.** Quantification of Satb2 mean fluorescence
1167 intensity in the homotopic cortex in *Eml1* cKO with Saline or EpoD, expressed as mean +/-
1168 SD. Tests and significance: Two-way Anova, Sidak multiple comparison (Pax6 analyses, CW
1169 and VZ thickness. data passed normality test), Mann Whitney test (heterotopia volume and
1170 Satb2 analyses). $n=7$ samples from 2 litters. *P*-value <0.05*, <0.01 **, <0.001 ***, <0.0001
1171 ****. Scale bars: 50 μ m in A (equivalent for WT and cKO, all conditions) and 500 μ m in D.

1172

1173 **Supplementary Figure 1. Forebrain inactivation of *Eml1* leads to subcortical heterotopia**
1174 **in mouse.** **A.** Schematic representation of the alleles used to generate *Eml1* cKO animals. **B.**

1175 Cresyl violet staining of individual brains at 8 weeks showing SH (red outline) in homozygote
1176 mutant animals. **C.** Representative images of SatB2 immunofluorescence in 3D visualized by
1177 light sheet microscopy in a control E18.5 embryo compared to an *Eml1* cKO. No heterotopia
1178 can be detected in the control. The homotopic cortex is depicted in transparency (purple) and
1179 the heterotopia is shown with a solid rendering (red). Three different angles are shown. **D.**
1180 Western blot analyses showing that Eml1 expression is lost in P7 cortices. Specific anti-Eml1
1181 antibodies 3E8 are shown in upper panels, and C3GTX in lower panels. Scale bars (equivalent
1182 for WT and cKO): 250 μm in B (main); 100 μm in B inset; 500 μm in C.

1183

1184 **Supplementary Figure 2. Altered proportion of cells in cell cycle phases in *Eml1* mutant**
1185 **conditions. A.** Examples of PCNA and Ki67 labelling patterns (cropped nuclei) for different
1186 cell cycle phases, punctate Ki67 or PCNA are indicated with a red arrow. **B.** Representative
1187 images for PCNA and Ki67 labelling at E12.5 in WT and *Eml1* cKO cells in the VZ. **C.**
1188 Quantification for percentage of proliferating cells in S, G1 or G2/M phases of the cell cycle
1189 expressed as mean \pm SEM. $n=6$ individuals per genotype at E12.5. **D, E.** Similar analyses
1190 were performed at E15.5, with $n=5$ per individuals per genotype. Test and significance: Two-
1191 way Anova, Sidak's multiple comparison (E12.5), Tukey's post-test at (E15.5). Data passed
1192 normality test. *P-value* <0.05 *, <0.001 *** Scale bar (equivalent for WT and cKO): 30 μm .

1193

1194 **Supplementary Figure 3. BioID and gene ontology analyses of WT and T243A Eml1**
1195 **interacting partners, Cep170 cell analyses. A.** Centrosomes were carefully checked in
1196 transfected Neuro2A cells, in interphase and during mitosis. No obvious abnormalities were
1197 identified. **B, C.** Gene ontology (GO) annotation grouped into molecular function (B) and
1198 cellular component (C) of EML1 and EML1*T243A proximal interactors. **D.** Representative
1199 immunoblots of co-immunoprecipitation of EML1 and EML4. Mutant EML1 was not
1200 assessed. **E.** Analyses of individual puncta of Cep170 fluorescence intensity at the
1201 centrosomes, also normalized to γ -tubulin intensity, expressed as mean \pm SEM ($n = 4$
1202 embryos from 2 litters, 2 ROI analyzed per embryo). Test and significance: Mann-Whitney.
1203 *P-value* <0.0001 ****. Scale bar (equivalent all images): 10 μm .

1204

1205 **Supplementary Figure 4. Trafficking to the PC is altered in *Eml1* cKO RG. A.**
1206 Schematized method of the Retention Using Selective Hook (RUSH) approach, used here for

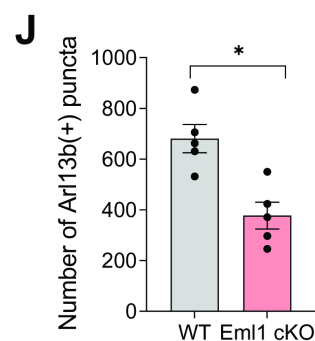
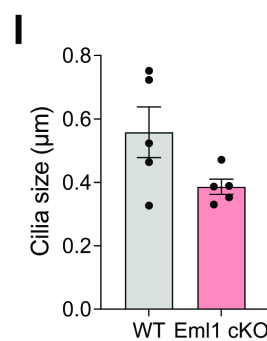
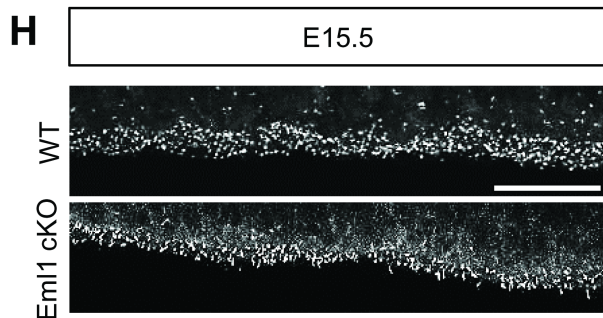
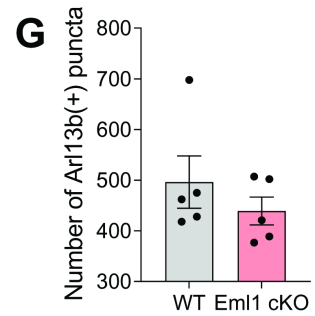
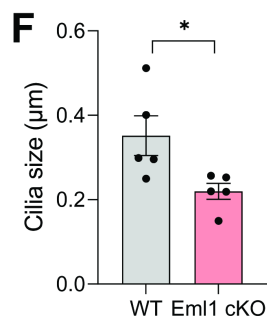
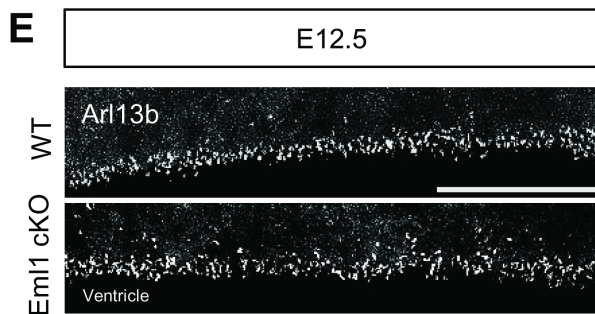
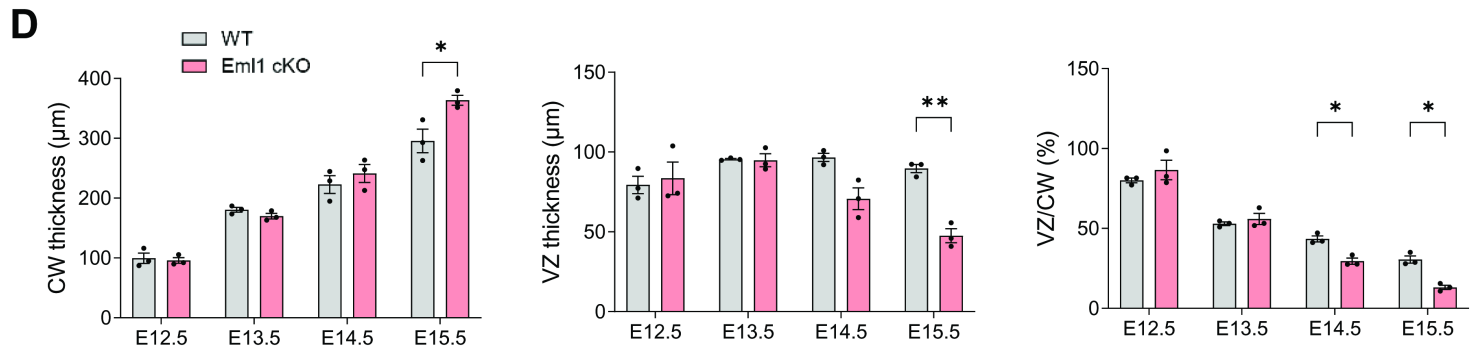
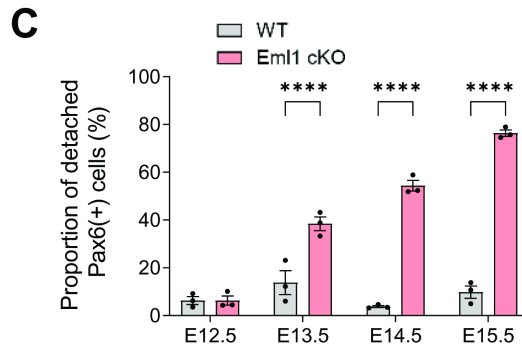
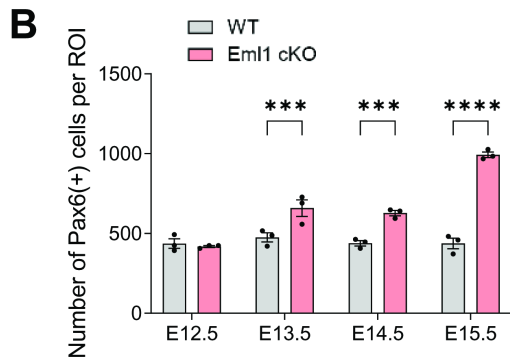
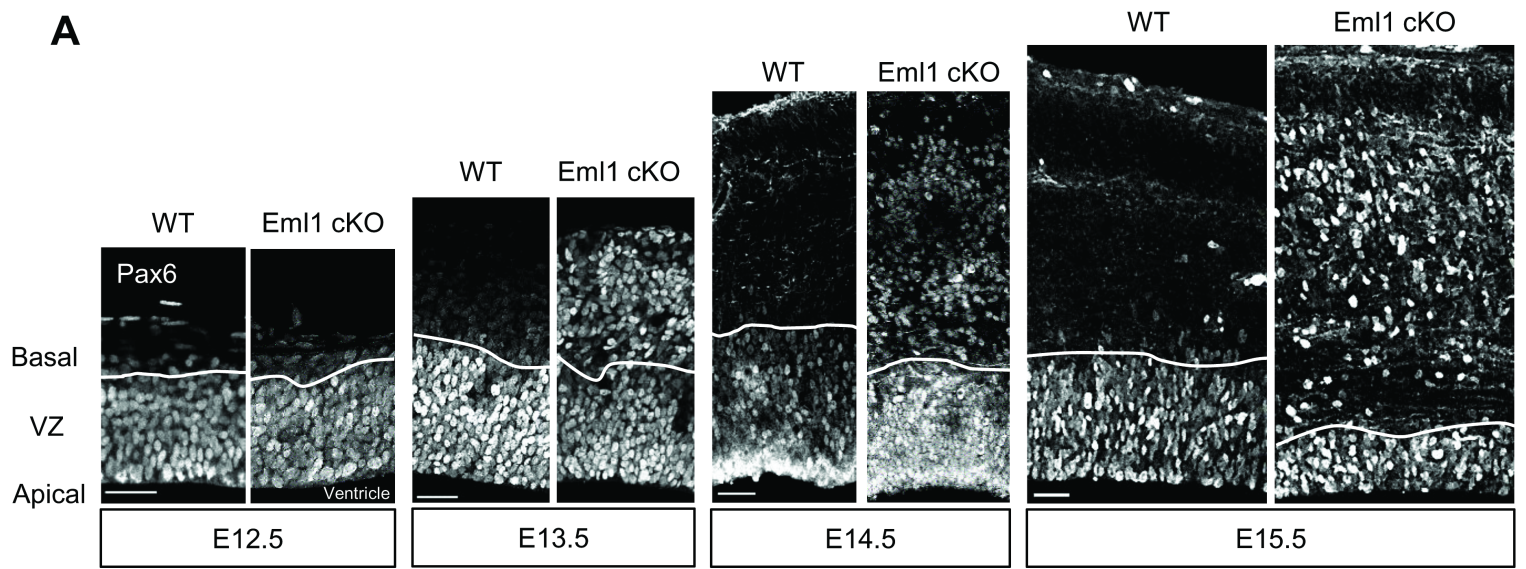
1207 PC protein trafficking analyses in primary cultures of Pax6+ cells. **B.** Representative images
1208 of GM130, Arl13b labelling and SSTR3-GFP signal on WT and *Eml1* cKO cells in culture at
1209 the different time points analyzed. Quantifications for SSTR3 (**C**), PKD2 (**D**) RUSH construct
1210 concentration in the Golgi and in the PC over time (0, 30, 60 and 90 min) in WT and *Eml1*
1211 cKO cells in culture, values represent mean +/- SEM. Quantifications were performed on at
1212 least 15 cells from two independent cultures for each genotype and analyzed protein. Test and
1213 significance: Two-way Anova. Sidak's multiple comparison. Data distribution was assumed
1214 to be normal. *P-value* <0.05 *, <0.01 **, <0.001 ***. Scale bar (equivalent for WT and cKO,
1215 all timepoints): 5 μ m.

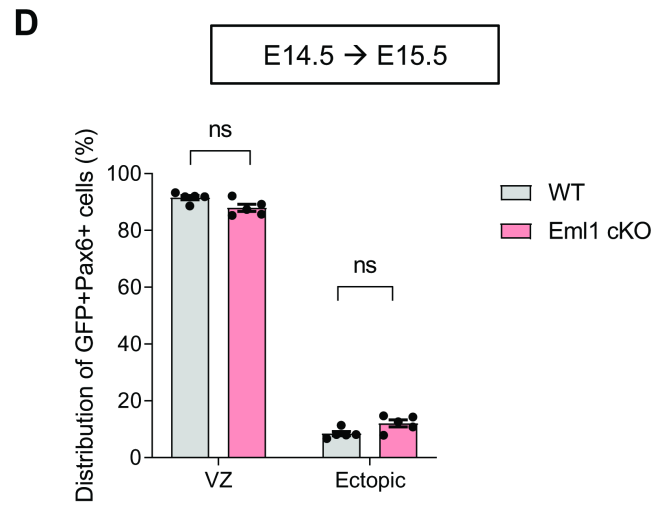
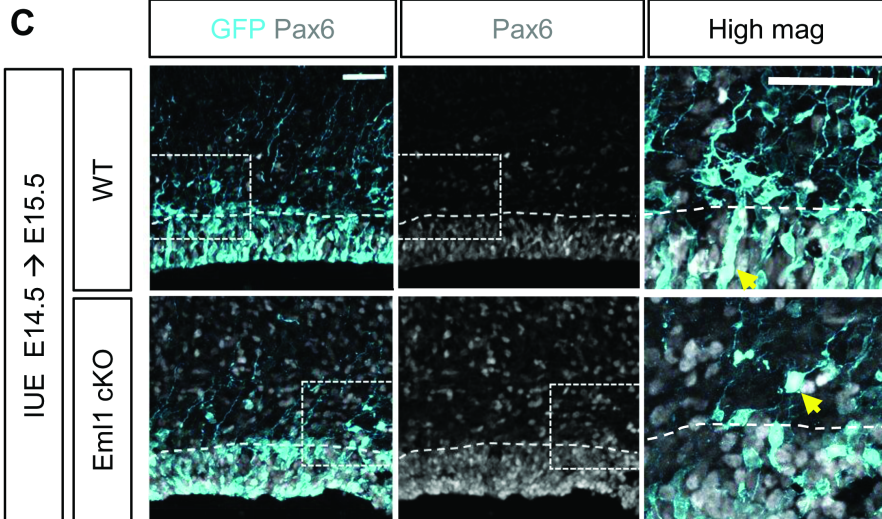
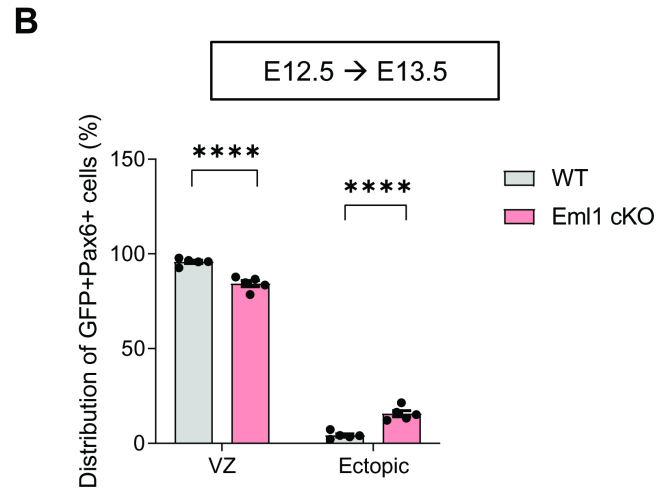
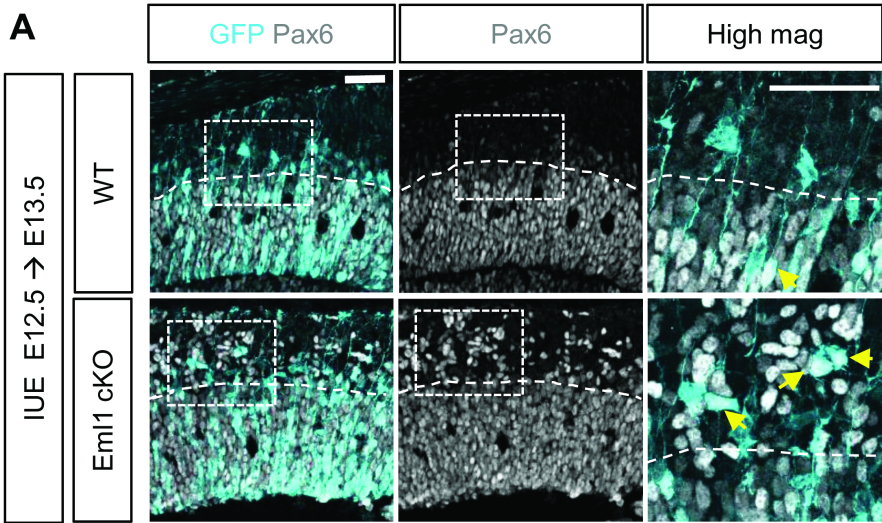
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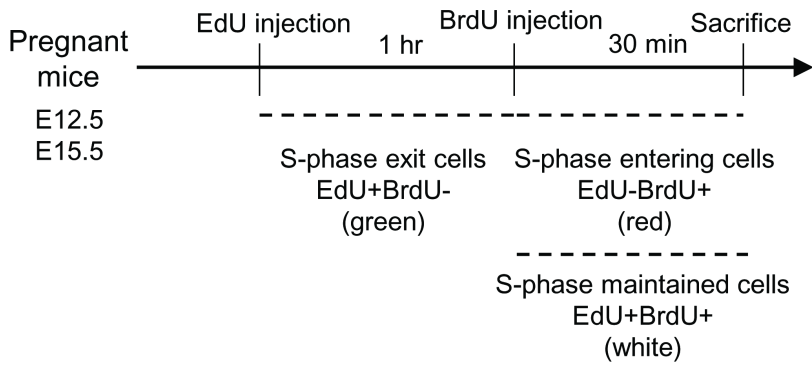
1217 **Supplementary Figure 5. Centrosome and α -tubulin modifications at E12.5 and/or E15.5**
1218 **in WT and *Eml1* cKO brains.** **A.** Analyses of overall numbers of γ -tubulin puncta at each
1219 stage. **B, C.** γ -tubulin intensity (B) and pcnt dispersion (C) analyses at E12.5 and E15.5 by
1220 individual puncta, expressed as mean +/- SEM. For centrosome analyses *n*=5 individuals from
1221 3 litters were analyzed per genotype and age. 2 ROI were analyzed per individual. For
1222 pericentrin area analyses: at least 4 individuals were analyzed from 3 litters per genotype and
1223 age. **D.** Immunolabelling of α -tubulin on embryonic brain slices at E12.5 in WT and *Eml1*
1224 cKO. **E, F.** Quantifications from the ventricular surface to 100 μ m height show a reduction in
1225 intensity, especially close to the ventricular surface. Quantification of α -tubulin mean
1226 intensity per ROI (47% decrease was observed in the VZ). Values represent mean +/- SEM (*n*
1227 = 4 embryos for each case). Test and significance: Mann-Whitney (γ -tubulin and pcnt
1228 analyses), Two-way Anova (α -tubulin analyses, data distribution was assumed to be normal
1229 but this was not formally tested). *P-value* <.0.001 ***, <0.0001 *****. Scale bar (equivalent
1230 for WT and cKO): 30 μ m.

1231 **Supplemental Video 1:** 3D visualization of Satb2 staining (in green) of a cortex from a wild
1232 type E18 embryo, with corresponding surfacing.

1233 **Supplemental Video 2:** 3D visualization of Satb2 staining of a cortex from an *Eml1* cKO
1234 E18 embryo, with corresponding surfacing. The homotopic cortex is depicted in green
1235 whereas the heterotopia is shown in pink.



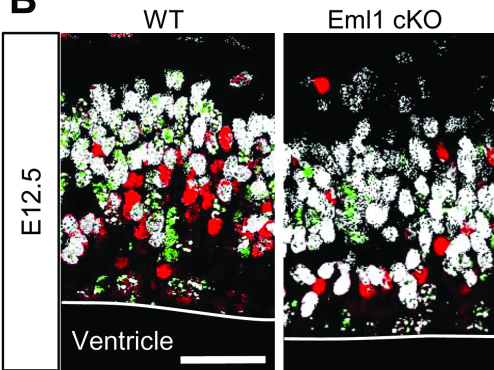
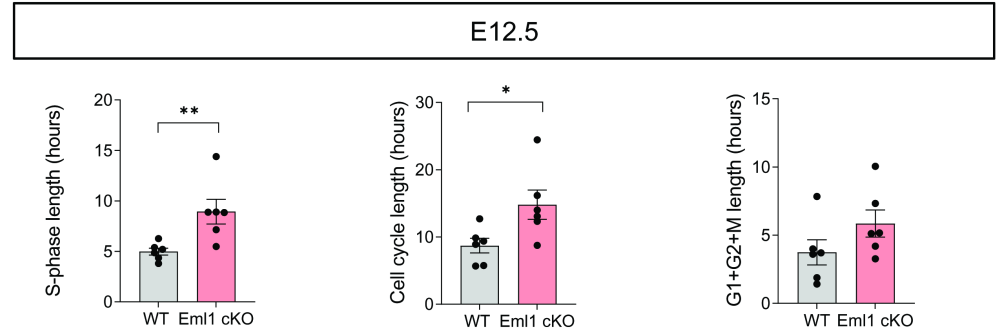
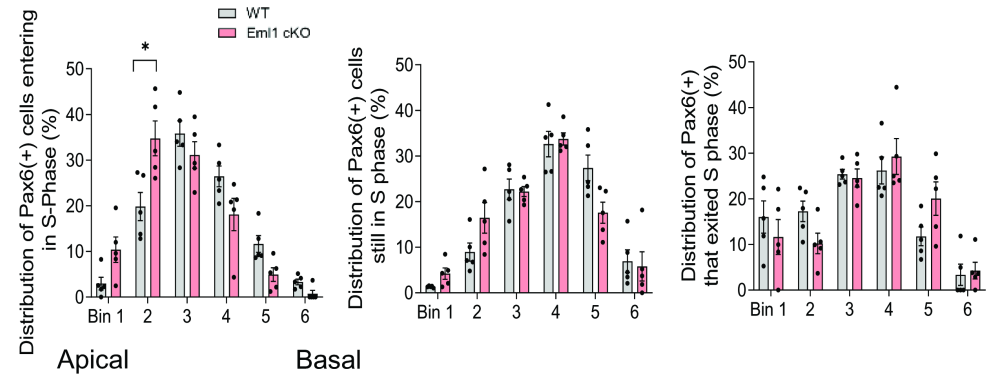
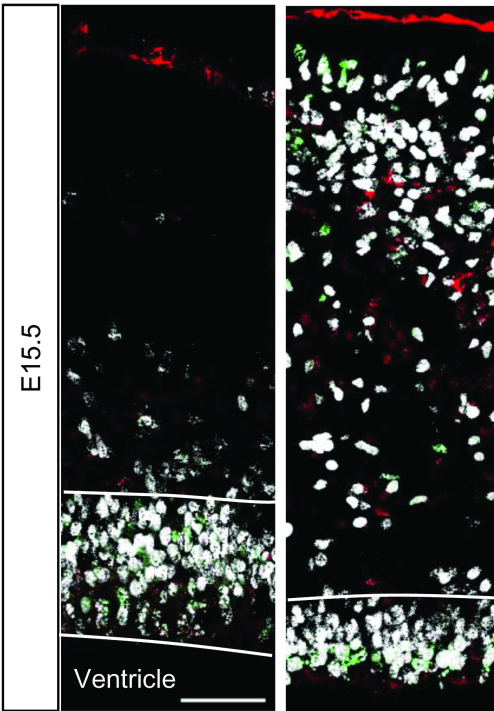
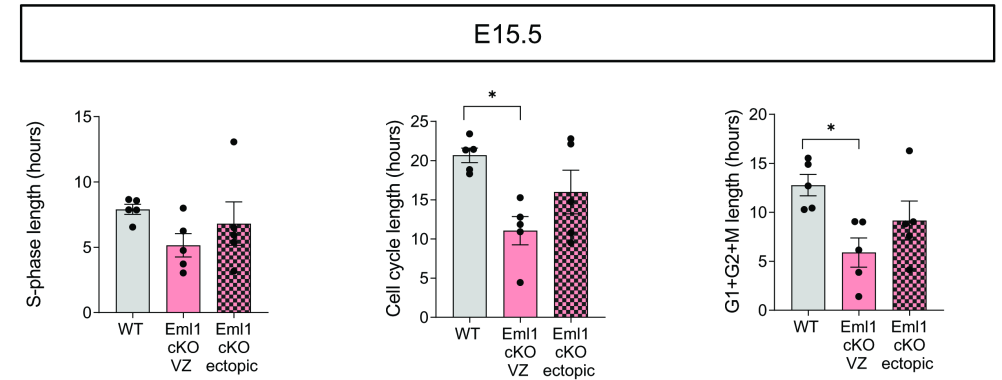
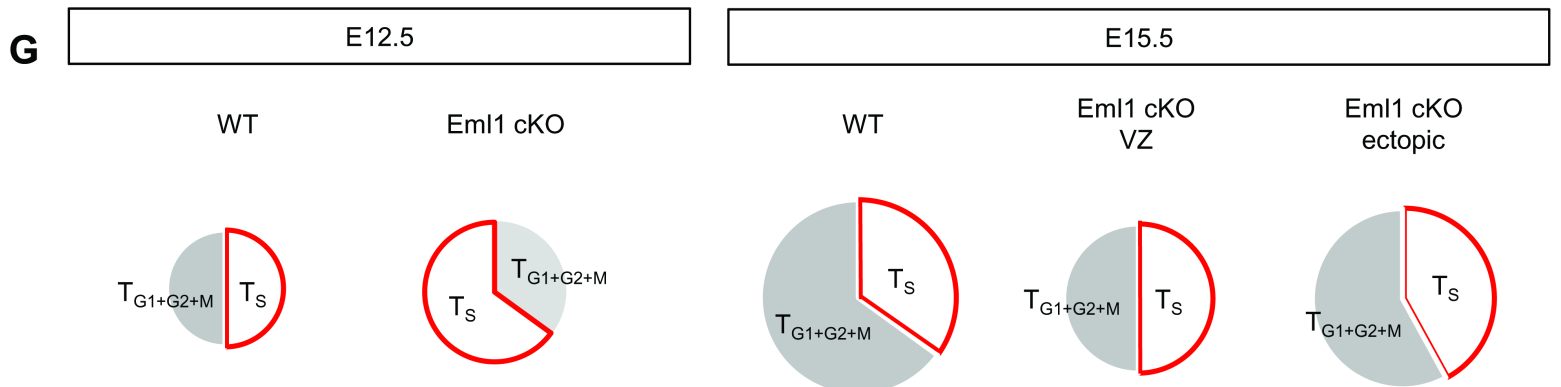


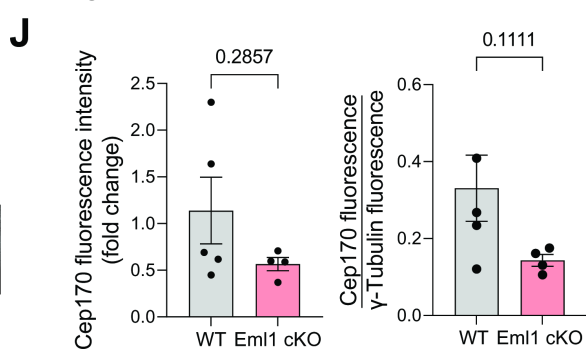
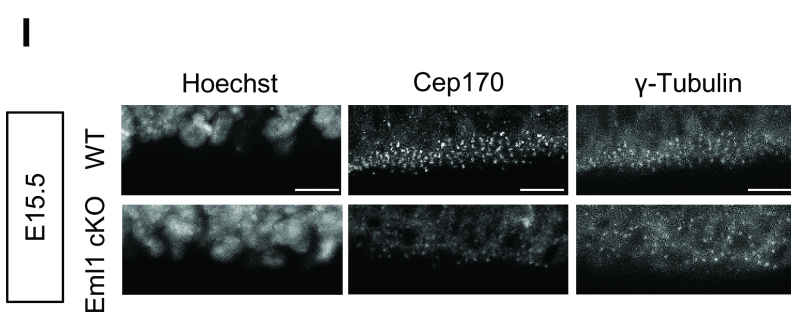
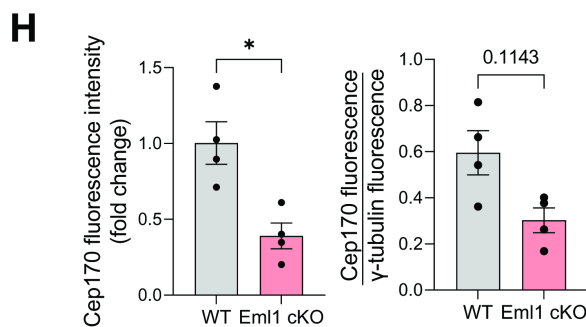
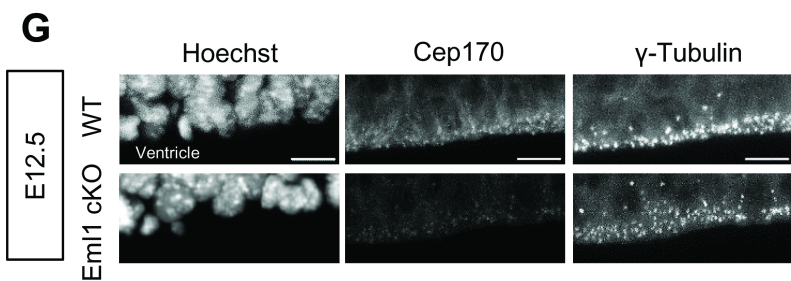
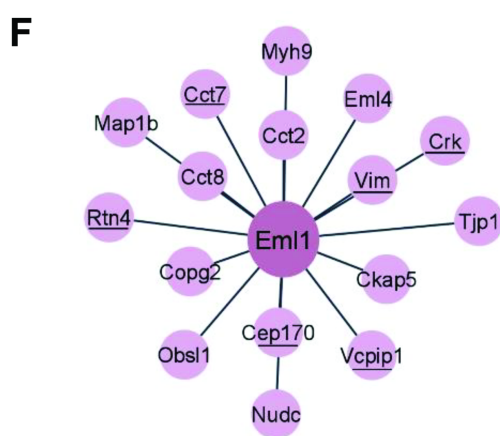
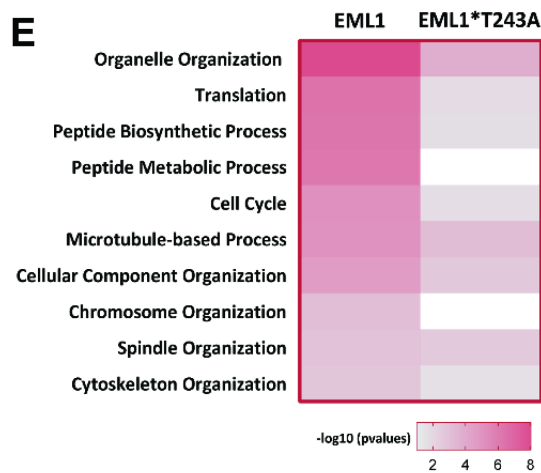
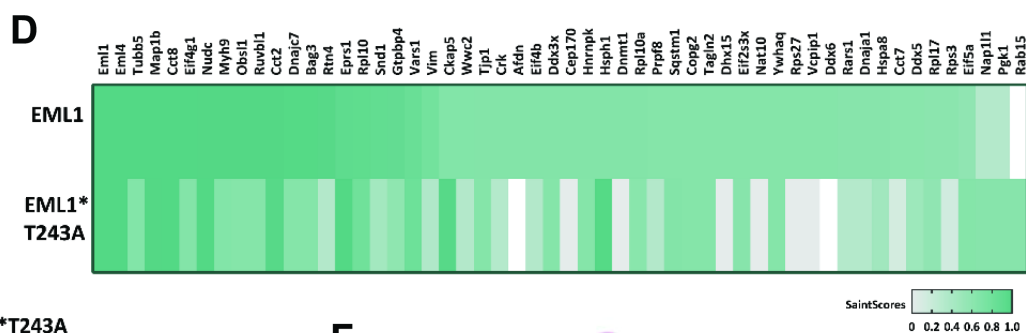
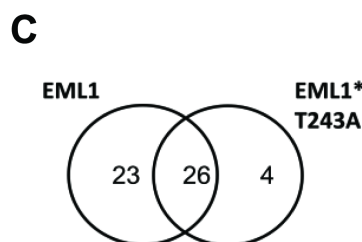
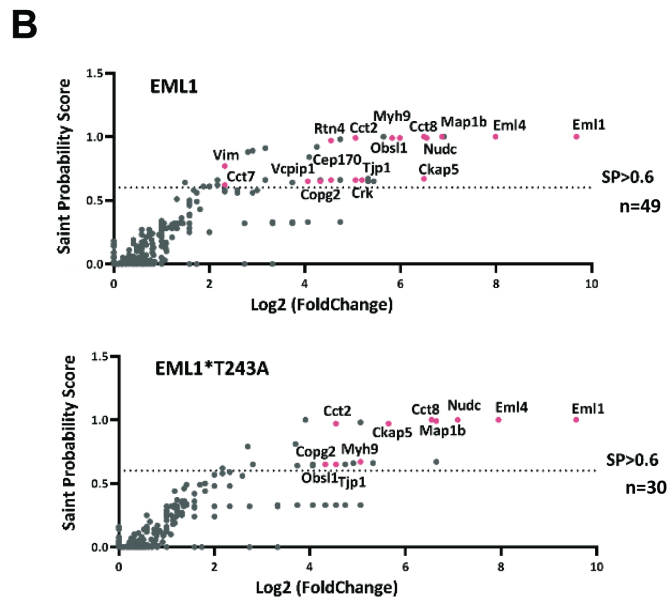
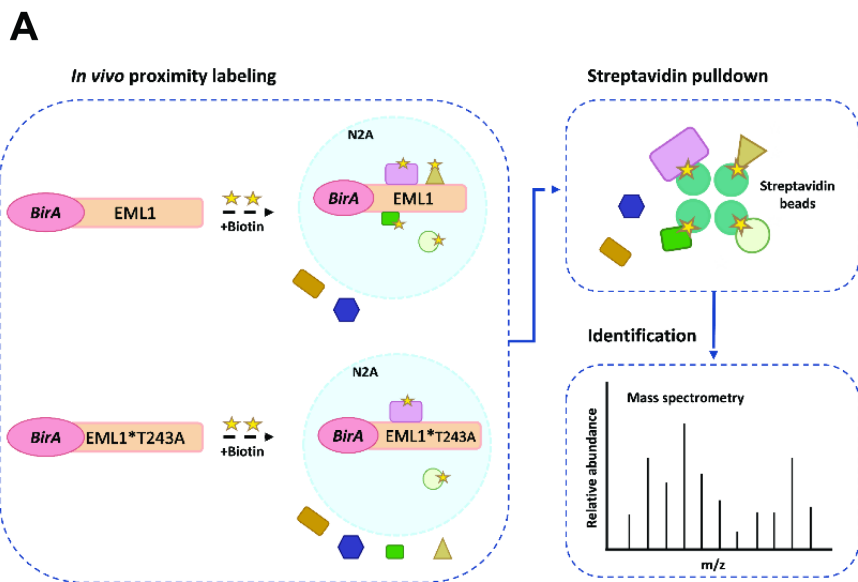
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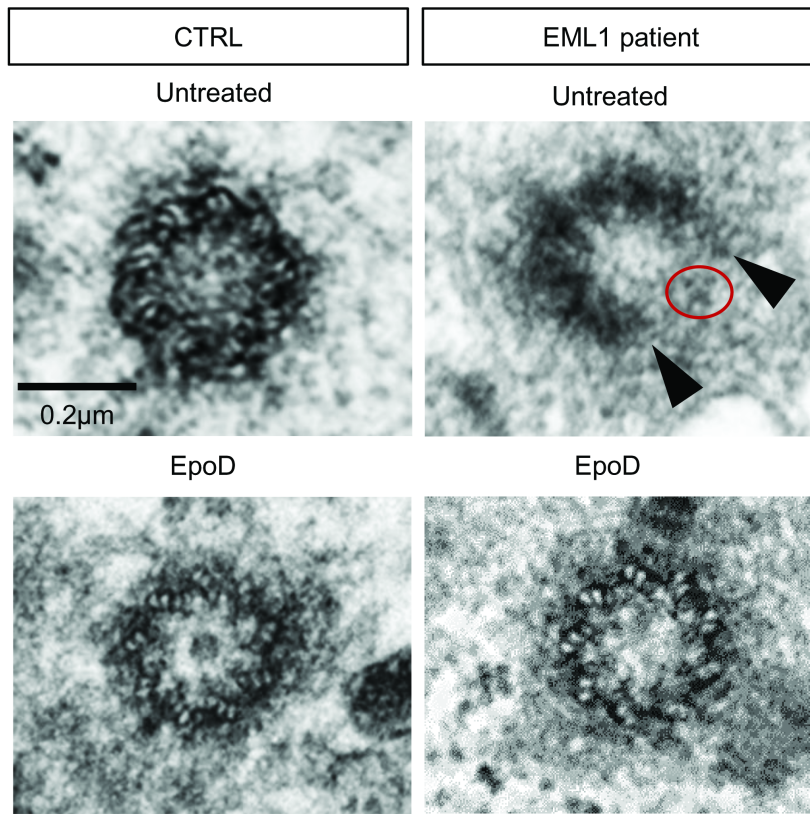
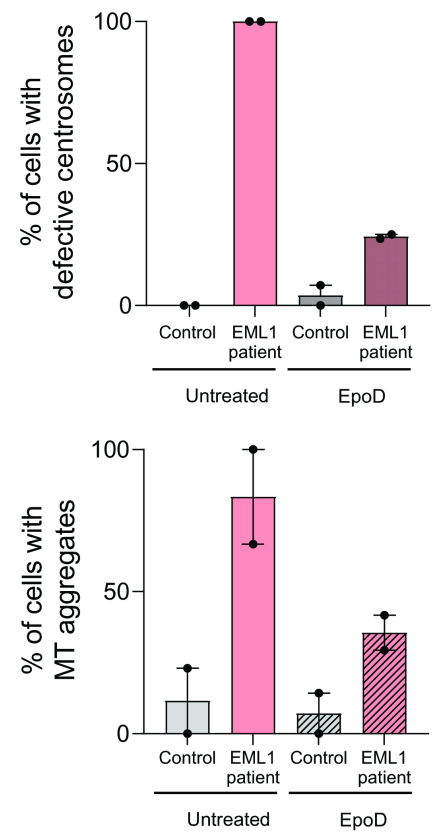
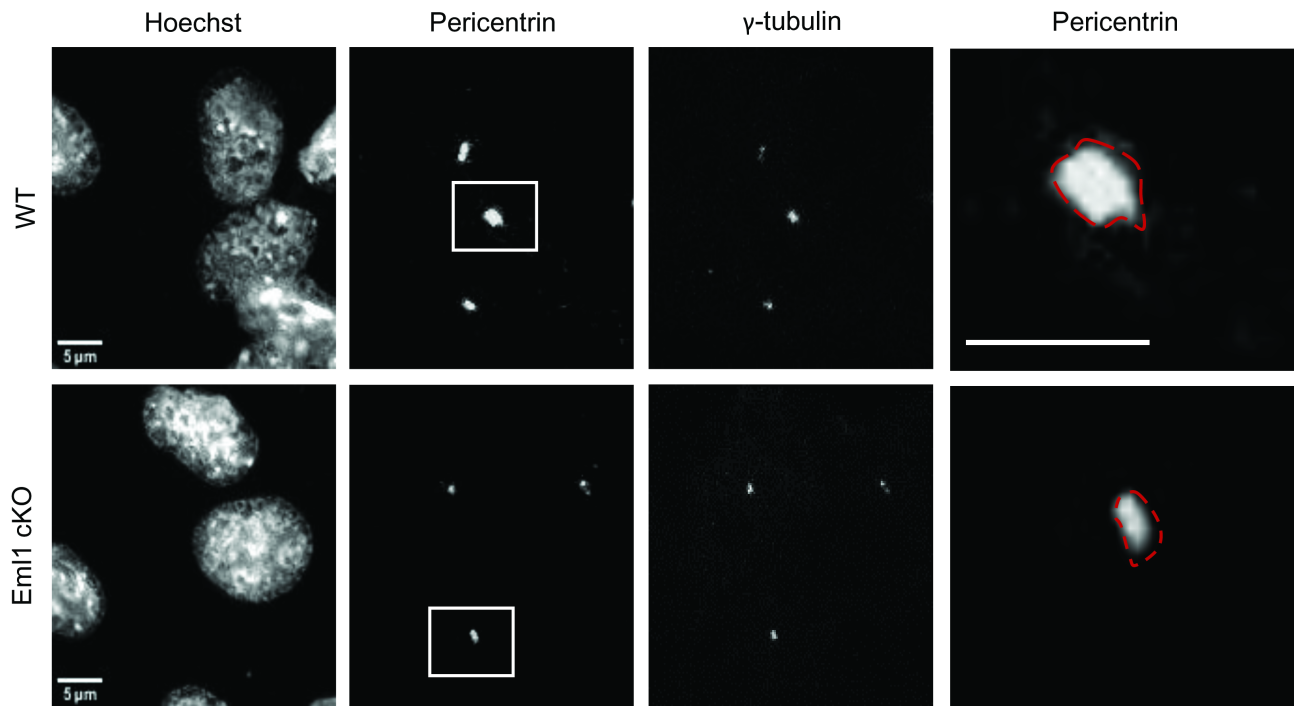
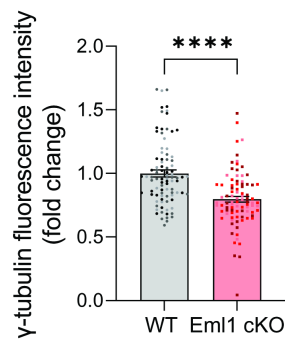
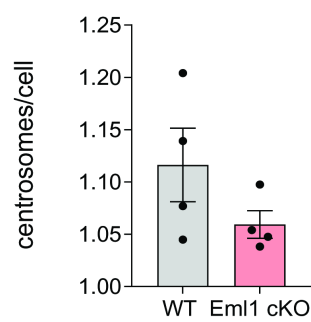
$$S\text{-phase length } (T_s) = \frac{\text{EdU+BrdU+}}{\text{Edu+BrdU-}}$$

$$\text{Cell cycle length } (T_c) = \frac{T_s}{[(\text{Edu+BrdU+})+(\text{Edu-BrdU+})]/\text{Pax6+}}$$

$$G1+G2+M \text{ length } (T_{G1+G2+M}) = T_c - T_s$$

B**C****D****E****F****G**



A Human**B****C Mouse****D****E**