

1 **Title**

2 A new centrosomal protein regulates neurogenesis by microtubule organization

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43

44 **Abstract**

45 The expansion of brain size in species with a large and gyrified cerebral cortex is triggered by
46 a relative enlargement of the subventricular zone (SVZ) during development. Here, we
47 uncover the key role of the novel interphase centrosome protein Akna in this process and
48 show that it localizes mainly at subdistal appendages of the mother centriole in subtypes of
49 neural stem and progenitor cells. Akna is necessary and sufficient to organize microtubules
50 (MT) at the centrosome and regulate their polymerization. These processes show an
51 unprecedented role of MT dynamics controlled by Akna in regulating entry to, and exit from,
52 the SVZ by controlling delamination from the neuroepithelial ventricular zone and retention

53 of cells in the SVZ. Importantly, Akna plays a similar role in mammary epithelial cells
54 undergoing epithelial-to-mesenchymal transition (EMT), generalizing the importance of this
55 new centrosomal protein in orchestrating MT polymerization to control cell delamination.

56

57 **Main Text**

58 Expansion of the SVZ is the developmental hallmark of enlarged and folded cerebral cortices,
59 underpinning the importance of understanding the mechanisms that govern its formation.
60 Epithelial-like neural stem cells (NSCs) divide in the ventricular zone (VZ), and mostly
61 generate a new NSC and a committed progenitor cell at midneurogenesis. The latter
62 delaminates and transforms into a basal progenitor (BP) which constitute the SVZ^{1,2}. Keeping
63 cells for a defined temporal window in the SVZ is essential to control further amplification
64 and fate determination^{3,4}. To identify novel regulators of these processes we compared the
65 transcriptome of murine NSC sub-types that generate BPs from those that do not⁵⁻⁷. We report
66 here a novel and unexpected regulator of BP generation and SVZ formation, called Akna. Our
67 work uncovers the function of this mis-annotated protein at the centrosome and reveals
68 interphase centrosomal microtubule organizing center (MTOC) activity as a novel mechanism
69 regulating EMT-like delamination of cells from the VZ to enter the SVZ and their retention
70 therein.

71

72 *Akna is an integral component of the interphase centrosome*

73 In murine cerebral cortex, Akna mRNA levels correlate with the time of SVZ generation (low
74 at embryonic day 11 (E11), high at E14, low at E18) and NSCs isolated at the peak of SVZ
75 generation have higher Akna levels when transitioning to BPs⁶ (Extended Data Fig. 1a,b). We
76 therefore chose Akna as a candidate regulator of SVZ and BP generation and generated
77 several rat and mouse monoclonal antibodies against Akna, validated by means of RNA
78 interference, to test this hypothesis further (Extended Data Fig. 1c-f, information about clones

79 used in Methods). Given the annotation of Akna as a transcription factor⁸, we were surprised
80 to find specific immunofluorescence (IF) signals at centrosomes (Fig. 1a and Extended Data
81 Fig. 1f, g) in different cell types (B cells, cell lines, NSCs, cerebral cortex cells) of murine,
82 ferret, primate and human origin (Extended data Figs. 1-4,7,9). This location was confirmed
83 using BAC-transgenic cell lines with a c-terminal EGFP-tag (Extended Data Fig. 1h). Indeed,
84 Akna lacks a nuclear localization signal and, in mice, the GRP core motif⁹ of the AT hook
85 domain required for RNA and DNA binding (see Extended Data Fig. 1i, j) and is not
86 detectable in the nuclear fraction (Extended Data Fig. 1k), but rather in centrosome enriched
87 fractions (Extended Data Fig. 1l).

88 Using immune electron microscopy (EM) to detect endogenous Akna in the mouse brain and
89 STED nanoscopy in cultured NSCs we found that Akna largely localizes at the distal part of
90 the subdistal appendages (SDAs) of the mother centriole (MC) in interphase, although a
91 minor signal is also detected in the proximal ends (PEs) of both centrioles, (Extended Data
92 Fig. 2a-c and Supplementary Movie 1). Importantly, the carboxyterminal part of Akna is
93 necessary for its localization at the centrosome (a region omitted in its first description⁸), with
94 all forms lacking these last 370 amino acids distributing within the cytoplasm (Extended Data
95 Fig. 2d-k). The centrosomal localization of Akna is not MT- or cargo-motor dependent, since
96 it remained at centrosomes upon treatment with: 1) the MT de-polymerization factor,
97 nocodazole; 2) the MT stabilization factor, Taxol (not shown); 3) destabilization of the
98 Dynein/Dynactin complex by p50/Dynamitin (Dcnt2) overexpression (Extended Data Fig. 2i-
99 k). Akna is an integral SDA component as it was lost from the centrosome remnants in the
100 cerebral cortex of Sas4/P53 double knock-out mice, that lack centrioles but retain
101 pericentriolar material (containing pericentrin (Pcnt), Extended Data Fig. 2l) and it also
102 disappeared from the centrosome upon deletion of the SDA protein Odf2 (Extended Data Fig.
103 2m, n).

104 During mitosis, Akna was no longer detectable at the centrosomes or spindle poles (Extended

105 Data Fig. 1h'', 3a), as is often the case for SDA-associated proteins.¹⁰⁻¹² Immunoblots of
106 synchronized cells and live imaging of Akna-Kusabira-Orange (mKO2) fusion protein
107 showed that Akna is not degraded during M-phase, but rather dissociates from the centrosome
108 and reassembles there in late telophase and G1 (Extended Data Fig. 3b and Supplementary
109 Movie 2). This process is regulated by phosphorylation as inhibition of phosphatases by
110 okadaic acid (OA) led to the delocalization of Akna from the centrosome in primary cerebral
111 cortex cells 3-4 hours after treatment (Extended Data Fig. 3c, d). Thus, Akna is a novel
112 interphase centrosome protein raising the question about its role in development.

113

114 *Akna regulates NSCs delamination and seeding of the SVZ*

115 To understand Akna's function in the developing murine cerebral cortex, we first determined
116 which cells have Akna positive (Akna+) centrosomes. Consistent with mRNA analysis, only a
117 few centrosomes were very weakly Akna+ before (E9) and at the end of (E18) neurogenesis
118 (Extended data Fig. 4a, b). Conversely, at E14, the peak of SVZ generation, Akna+
119 centrosomes were present and their number were highest in the SVZ (Fig. 1b, c; Extended
120 data Fig. 4c), while almost no signal was observed in the layer of differentiating neurons, the
121 cortical plate (CP). At the apical surface of the VZ, only a fraction of centrosomes (20% and
122 46% quantified by IF and EM respectively, Extended Data Fig. 4d, e) were Akna+. These
123 NSCs with Akna+ centrosomes were Pax6+/Tbr2+ differentiating NSCs¹³ as shown in both
124 primary cultures (4 hours post isolation) and FACS-sorted Prominin1+ NSCs, in full
125 agreement with Akna mRNA expression profile¹³ (Extended Data Fig. 4f, g). Thus, Akna
126 exhibits an unprecedented subtype-specificity for a centrosomal protein largely restricted to
127 differentiating NSCs and BPs.

128 To functionally analyze the role of Akna in corticogenesis, we used shRNA mediated knock-
129 down (KD) (see WB in Extended Data Fig. 1d) via *in utero* electroporation (IUE) at E13. In
130 control conditions, large proportions of GFP+ cells had left the VZ and SVZ of the cerebral

131 cortex and were migrating into the CP two days after IUE (Fig. 2a). In contrast, under Akna
132 KD conditions, GFP+ cells were mainly retained in the VZ and SVZ with very few migrating
133 towards the CP (Fig. 2c and Supplementary Video 3). Notably, this phenotype was observed
134 upon KD with 2 different shRNAs, and also occurred when p53-mediated cell death induced
135 by Akna KD was blocked (Fig. 2b, c, Extended data Fig. 5). According to the positional
136 changes upon Akna knock-down with more cells in the VZ, we also observed an increase in
137 Pax6+/Tbr2- NSCs and decrease in Pax6-/Tbr2+ BPs (Fig. 2d). Thus, Akna loss-of-function
138 impairs NSC delamination and subsequently blocks further lineage progression.

139 Conversely, Akna OE in E13 cortex induced delamination of NSCs from the VZ with most
140 electroporated GFP+ cells being in the SVZ already 24 hours after IUE (Fig. 2e-g).
141 Accordingly, Akna OE also induced higher percentages of Tbr2+ BPs and NeuN+ neurons,
142 with a concomitant decrease in Pax6+ and Ki67+ cells, as compared to controls (Fig. 2h, i).
143 Live imaging in cortical slices showed that this accelerated delamination occurred by
144 retraction of the apical processes without undergoing cell division (28%, n=33 compared to
145 3%; n=165, in controls, Supplementary Video 4). Thus, the delamination induced by Akna
146 OE does not require M-phase or the orientation of cell division when other centrosomal
147 proteins act, but rather occurs in interphase, when endogenous levels of Akna are also at their
148 peak. Akna gain-of-function thus induces precocious delamination and subsequently
149 premature neuronal differentiation.

150

151 *Akna affects microtubule organization in subtypes of neural progenitors*

152 To determine how Akna mediates the effects described above, we first examined whether
153 Akna OE also elicits cell fate changes *in vitro*. When Akna was overexpressed for 48 hours in
154 dissociated E14 cortex primary cells no changes in the proportion of Pax6+, Tbr2+ or Ki67+
155 cells were observed (Fig. 2j). This suggests that the increased neuronal differentiation

156 observed upon Akna OE *in vivo* likely occurs due to re-localization to the SVZ niche (Fig.
157 2k).

158 We next asked how Akna could mediate the delamination of cells from the VZ. So far only
159 mechanisms influencing the F-actin belt and the adherens junctions (AJs) between NSCs as
160 well as primary cilium positioning have been implicated in retaining NSCs in the VZ¹⁴⁻¹⁶.
161 Given the localization of Akna at the SDAs that anchor MTs, we suspected a different role of
162 Akna and used nocodazole wash-out assays to monitor MT regrowth (Extended Data Fig. 6a)
163 upon shRNA-mediated Akna knock-down (KD) in E14 primary cortical cells (Fig 3a).
164 Interestingly, the proportion of cells exhibiting centrosomal MT regrowth was significantly
165 reduced compared to control transfected cells with the remaining cells (which still showed
166 centrosome-based nucleation) having an overall reduction in length of the MT fibers (Fig. 3a-
167 c). Thus, lowering Akna protein levels dramatically reduces centrosomal interphase MTOC
168 activity *in vitro*.

169 To determine whether Akna is also sufficient to organize MTs we transfected E14 primary
170 cortical cells with Akna expressing plasmids and performed the nocodazole wash-out assay.
171 Strikingly, MT-asters emanated from ectopic Akna protein foci (Fig. 3d and Extended Data
172 Fig. 6b) that were also sufficient to recruit gamma-Tubulin Ring Complex (gTurC)
173 components gamma-Tubulin (Tubg) and Tubgcp4 (Extended Data Fig. 6c). Neither the MT-
174 minus end capping-protein - Camsap2 - nor the centrosomal protein - Pcnt - were enriched at
175 Akna foci, ruling out unspecific binding of antibodies to Akna foci (Extended Data Fig. 6d).
176 Thus, Akna is sufficient to recruit mainly gTuRC-capped MTs and proteins involved in MT
177 organization at SDAs such as Mapre1/EB1, Dctn1/p150Glued and Odf2¹⁷⁻²⁰ (Extended Data
178 Fig. 6e). Co-immunoprecipitation experiments proved association of Akna with EB1, Dctn1
179 and Odf2, but not with the gTuRC component Tubgcp2 (Extended Data Fig. 6f), suggesting
180 Akna organizes MTs together with the above-mentioned proteins at SDAs while its
181 interaction with gTuRC may be weaker or indirect. Thus, we identified here a novel regulator

182 of centrosomal MTOC activity selectively enriched in differentiating NSCs. This is intriguing
183 as centrosomal MTOC activity regulated by Akna may counteract the often non-centrosomal
184 MT anchoring in epithelial cells mediated by the Camsap-family of proteins²¹ and thereby
185 change the major mode of MT anchoring and polymerization in delaminating NSCs.

186 To directly observe such changes *in vivo*, we monitored growing MTs by live imaging in slice
187 preparations using EB3-GFP after Akna IUE. Consistent with previous data²², EB3-GFP
188 comets preferentially moved basally into the radial glial process in control cells with an
189 average angle of 78° relative to the apical surface (Fig. 3e, f). Upon Akna OE, we observed
190 comets moving more obliquely (average angle 55°, Fig. 3e, g; Supplementary Videos 5, 6),
191 indicating a change in the orientation of MT nucleation and repositioning of the MTOC to
192 non-apical positions. Moreover, the speed of the EB3-GFP comets was significantly higher
193 upon Akna OE, suggesting that MT polymerization speed is increased when Akna levels are
194 high (Fig. 3h). On the contrary, upon lowering Akna levels with shRNA constructs harboring
195 a EB3-neonGreen reporter, we observed a significant reduction in EB3 comet speed (Fig. 3i
196 and Supplementary Videos 7,8). Together this indicates, in agreement with the above
197 observations, that knocking down Akna decreases MT polymerization. Hence, Akna levels
198 regulate MT nucleation and organization in the cell and influence MT polymerization both *in*
199 *vitro* and *in vivo*. This suggests that Akna levels are critical to shift the balance of MTs
200 anchored at non-centrosomal positions, such as in the basal process and at AJs in NSCs with
201 epithelial hallmarks^{2,23-25}, to the centrosome which is also accompanied by faster MT
202 polymerization. This in turn might be necessary for destabilization of AJs mediating the
203 delamination observed *in vivo*.

204

205 *Akna is required during EMT*

206 As the delamination from the VZ is regulated by transcription factors also involved in
207 EMT^{26,27}, we examined if the cell biological mechanisms of delamination mediated by Akna

208 may also be relevant in true epithelial cells undergoing EMT. Towards this aim, we monitored
209 normal murine mammary gland epithelial cells (NMuMG) during EMT induced by
210 Transforming growth factor beta-1 (TGF-beta-1)^{28,29}. While epithelial NMuMG cells have
211 low levels of Akna, it is up-regulated during EMT and localizes to the centrosome (Extended
212 Data Fig. 7a-c). Since epithelial cells show largely non-centrosomal MT polymerization prior
213 to EMT²¹, we hypothesized that, in analogy to NSCs, Akna might redirect MTOC activity to
214 the centrosome during EMT and thereby critically control EMT progression. To determine the
215 role of Akna in EMT, we used siRNAs to reduce its levels (Extended Data Fig. 7d). While
216 TGF-beta-1 mediated EMT induction in Akna knockdown conditions leads to a similar
217 upregulation of core EMT transcription factors like Twist and Zeb1 and the mesenchymal
218 target Fibronectin (data not shown), we observed the retention of the tight junction component
219 ZO1 (Fig. 4a-c) and increased levels of ZO1 and the cadherin interactor p120 (Extended Data
220 Fig. 7e, f). Moreover, the rearrangement of the actin cytoskeleton from the AJs to stress
221 fibers, visualized by Phalloidin stainings was attenuated upon knock-down of Akna and still
222 visible at many cell-cell contacts (Fig. 4a''-c'') and cells remained closer together (Extended
223 data Fig. 7g-i). Thus, Akna plays a key role in disassembling junctional coupling during
224 EMT. Taken together, these and the above data suggest that Akna up-regulation leads to the
225 re-distribution of MTs anchored through p120 at the junctional complex²³ to a centrosome-
226 based array, thereby facilitating the dissolution of cell-cell contacts that ultimately allows
227 mesenchymal cell scattering. To determine whether Akna also contributes to dissolving
228 junctional coupling in the developing cerebral cortex, we examined brains shortly after Akna
229 OE (18h after IUE; Fig. 4d, e) prior to delamination of most cells (Fig. 2f, g). In agreement
230 with the results obtained in mammary epithelial cells, Akna OE also reduced E-cadherin
231 levels within electroporated areas in the cortical VZ (Fig. 4e). Thus, Akna controls previously
232 unanticipated changes at the centrosome required for EMT and NSC to BP progression. This

233 provides new insights into how changing MT dynamics may contribute to remodeling of the
234 junctional complexes allowing cells to delaminate from their epithelial neighbors.

235

236 *Akna regulates retention of cells within the SVZ*

237 Given Akna's function in regulating MTOC activity and MT polymerization, we next
238 examined young neurons in the CP that lose Akna at the centrosomes (only 10% of
239 centrosomes are Akna+; Extended data Fig. 4e). As expected, neurons isolated by FACS
240 (PSA-NCAM+ cells) from E14 cerebral cortices showed much more non-centrosomal MT
241 polymerization in nocodazole-based MT regrowth assays (Extended Data Fig. 8a, b; compare
242 to NSCs), consistent with the decline of Akna in neurons and its role in mediating the
243 centrosomal MTOC activity. To determine the function of Akna when cells progress from a
244 multipolar state in the SVZ to a bipolar migratory phase entering the CP *in vivo*, we first
245 expressed Akna cDNA under the late BP/neuron-specific Doublecortin (Dcx)-promoter
246 (Extended Data Fig. 8c) at E13. When control cells were analyzed 5 days later most had
247 progressed into the CP, while approx. 50% of the GFP+ Akna OE cells were retained below
248 the CP (Fig. 5a-d). These were still able to differentiate into neurons and extend callosal
249 projections (Extended Data Fig. 8d-f). Conversely, downregulating Akna in late BPs and
250 young neurons by Dcx-promoter driven Akna miRNAs did not affect cells leaving the SVZ
251 and reaching normal positions within the CP similar to controls (Extended Data Fig. 8g, h).
252 Thus, increasing Akna levels prevents cells from moving into the CP indicating that
253 physiologically occurring Akna downregulation (Fig. 1b; Extended Data Fig. 1a, 4a-f) is
254 crucial in this transition.

255 To determine at which step Akna levels are critical in this transition from multipolar SVZ
256 cells to bipolar neurons migrating into the CP³⁰, we performed live imaging in slices upon
257 Akna OE or knock-down. Control and KD cells had similar migration speed (Fig. 5e), but KD
258 cells transited even faster from the multipolar SVZ morphology to a bipolar migrating

259 neuronal morphology (Fig. 5f, g). In contrast, many Akna OE cells retained a multipolar
260 morphology and migrated less (Fig. 5h,i and Supplementary Videos 9 and 10), demonstrating
261 the key role of Akna not only in bringing cells into the SVZ, but also in retaining them there.

262

263 *Akna levels affect SVZ formation in species with gyrified cortex*

264 Retaining cells in the SVZ is particularly important in species with an expanded outer SVZ
265 (oSVZ), such as ferrets and primates³¹. Indeed, Akna mRNA in ferret VZ peaks at the onset of
266 oSVZ formation when most cells forming the oSVZ delaminate (Extended Data Fig. 9a) and
267 differs in abundance at centrosomes in the oSVZ of future sulcus and gyrus regions (Extended
268 Data Fig. 9b-f). Indeed, Akna is also enriched at centrosomes in primate oSVZ, but not
269 detectable in the CP (Extended Data Fig. 9g, h). A feature that is fully consistent with its role
270 in mediating entry and exit to and from the SVZ in mice. To corroborate this at the functional
271 level in human cells, we first showed that AKNA is sufficient to organize MTs also in human
272 induced pluripotent stem cell (iPSC)-derived NSCs (Extended Data Fig. 9i, i'). We next used
273 AKNA OE (2 days) and KD (5 days) to test its function in human cerebral organoids grown
274 for 7-8 weeks following the Lancaster protocols^{32,33}. Importantly, AKNA OE also mediated
275 delamination from the VZ in human cortex organoids (Extended data Fig. 9l-n) and its KD
276 lead to a significant increase in retention of cells in the VZ (Extended data Fig. 9o). Thus, the
277 conserved function of Akna in recruiting and retaining cells in the SVZ may allow dynamical
278 change of SVZ size during evolution.

279

280 *Discussion*

281 Our work identified and characterized the function of a new component of the centrosome, the
282 former mis-annotated AT-hook transcription factor Akna, and reveals hitherto unknown cell
283 biological aspects controlling neurogenesis. We show that Akna confers centrosomal MTOC

284 activity during interphase specifically in subtypes of NSCs and progenitor cells. Akna is
285 downregulated during neuronal differentiation concomitant with the change in MT
286 organization in neurons, i.e. from primarily centrosome- to more non-centrosome-based
287 organization. In neurons, MTs originate preferentially from other subcellular compartments
288 such as the Golgi apparatus, the cell cortex or local pools of MTs³⁴, allowing the generation of
289 axons and dendrites while still moving into and within the CP^{35,36}. Indeed, reduction of Akna
290 levels is a requirement for neurons to proceed into the CP as its OE under Dcx-promoter
291 blocks this process. The molecular mechanisms of centrosome inactivation are not well
292 understood. Previously, re-localization of some nucleating and anchoring factors such as
293 Tubg, Cdk5rap2 (i.e. gTuRCs) and Ninein, as well as the down-regulation of Neddl^{35,37} have
294 been implicated. Here, we propose that Akna is an important regulator of the loss of
295 centrosomal MTOC activity in neurons³⁸. Importantly, we demonstrate that this switch occurs
296 at early stages of neuronal differentiation and is critical in regulating the exit from the SVZ.

297 Most strikingly, Akna also promotes entry into the SVZ as demonstrated by cells remaining in
298 the VZ upon knock-down and their fast delamination in interphase upon overexpression in the
299 VZ. *In vivo* live imaging upon Akna manipulation clearly demonstrated that it not only
300 regulates centrosomal MTOC activity, but also affects MT polymerization. Thus, Akna
301 affects at least two aspects of the delamination process. Firstly, the increase in MT
302 polymerization as mediated by Akna in differentiating NSCs could weaken cellular junctions
303 and hence promote the release of cells towards the SVZ. Indeed, MT polymerization per se
304 decreases p120-mediated stability of cadherins in cell lines³⁹. Moreover, members of the
305 Camsap/Patronin protein family promote MT nucleation at cellular junctions or, together with
306 Katanin, release centrosomal MTs⁴⁰ and tether them to the AJs²¹. This contributes, through
307 interaction with p120, to AJ stability.^{23,41} Importantly, if Camsaps are downregulated,
308 centrosomal MT nucleation increases⁴⁰ and both cell adhesion and junctional organization is

309 distorted.⁴² Our data thus supports a model in which Akna-mediated recruitment of MTs to
310 the centrosome reduces MTs at the junctional complex, thereby destabilizing it. This is
311 supported by the effects of Akna KD in mammary epithelial cells undergoing EMT impairing
312 the complete detachment of cells and retention of junctional components. Secondly,
313 positioning of the centrosome is affected by changes in MT organization upon Akna
314 manipulation. This may help to polarize organelles towards the SVZ⁴³ when cells become
315 multipolar and leave the VZ. Likewise, Akna levels need to be down-regulated when
316 multipolar SVZ cells become bipolar again when moving to the CP³⁰. Indeed, MT dynamics
317 regulate cell polarity by influencing centrosome and Golgi apparatus repositioning in cell
318 lines^{44,45}. Moreover, EMT in many developing organs is accompanied by changing polarity
319 and centrosome positioning⁴⁴. As Akna loss and gain of function experiments show changes
320 in the angle of MT polymerization after Akna OE and polarity defects in newborn neurons,
321 we propose that Akna plays a key role in mediating the changes in polarization when bipolar
322 RGCs transit to multipolar BPs (requiring high Akna levels) and when multipolar cells in the
323 SVZ progress towards bipolar migrating neurons (requiring low Akna levels).

324 Thus, Akna is a novel centrosomal protein crucially involved in the regulation of cell
325 delamination in the developing brain and other epithelial cells undergoing EMT, a wide-
326 spread process in many developing tissues⁴⁴. As neither Akna loss- nor gain-of-function have
327 any apparent effect on cilia growth, maintenance or position in cortical progenitors neither *in*
328 *vivo* nor *in vitro* (Extended Data Fig. 10), we conclude that Akna does not exert its effects via
329 cilia-mediated mechanisms. Importantly, the Akna phenotype and functions outlined here
330 differ from previously reported centrosome associated proteins in the developing cerebral
331 cortex often regulating spindle formation and orientation⁴⁶⁻⁴⁸. Interestingly, knockdown of the
332 SDA protein Ninein in NSCs has the opposite effect than Akna KD, namely triggering
333 delamination⁴⁹, suggesting that Akna may counteract Ninein in a subtype-specific manner.

334 Most importantly, Akna is the first and only known centrosomal protein regulating entry to
335 and exit from the SVZ thereby not only coordinating both of these processes, but also
336 highlighting the importance in the balance of centrosomal versus acentrosomal MT
337 recruitment in this crucial event during ontogeny and phylogeny of the brain.

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464

465 **Figure Legends:**

466

467 **Figure 1. Akna is a centrosome component restricted to specific subtypes in the**
468 **developing cerebral cortex. a**, Immuno-staining of primary E14 cerebral cortex cells
469 showing Akna at the mother centriole together with the distal-subdistal appendage maker
470 Odf2 and the cilia and centriole marker GT335. **b**, Micrograph of E14 cerebral cortex
471 sections showing Akna+ centrosomes at the apical, ventricular surface of the ventricular zone
472 (VZ) and in the subventricular zone (SVZ). Very few Akna+ centrosomes are detectable in
473 the cortical plate (CP). Pericentrin (Pcnt) labels all centrosomes throughout the cortex. Blue
474 arrows show Pcnt+ Akna+ centrosomes, red arrows Pcnt+ Akna- centrosomes. Notice that
475 only subsets of centrosomes show Akna labelling at the apical surface of the VZ. V:
476 Ventricle. **c**, Quantification of the distribution of Akna+ centrosomes in the E14 CTX (n=3).
477 Data are presented as mean \pm standard error of the mean. Scale bars: 2.5 μ m (**a**), 20 μ m (**b**).
478

478

479 **Figure 2. Akna regulates NSC delamination and seeding of SVZ. a**, Confocal micrographs
480 illustrating binning and the distribution of control electroporated GFP+ cells 2 days after IUE.
481 **b**, Confocal micrograph showing the distribution of GFP+ cells after knockdown of Akna
482 using sh1. **c**, Line graph illustrating the distribution of GFP+ cells after IUE for control
483 (green), sh1- (orange) and sh2-mediated knockdown (KD) of Akna (magenta). The respective
484 standard error of the mean (SEM) is indicated as transparent band in the same colour (n=5
485 each condition). **d**, Boxplot showing the increase in Pax6+ NSCs and decrease of Tbr2+ BPs
486 after Akna KD (n=5 each condition). **e, f**, Confocal micrographs illustrating binning and the
487 distribution of GFP+ cells 1 day after IUE with control (**e**) or Akna overexpressing (OE)
488 plasmids (**f**). **g**, Line graph illustrating the distribution of GFP+ cells after control (green) and
489 Akna OE (blue) IUE with SEM as transparent band (n=5 each condition). **h, i**, Boxplots
490 showing the decrease of Pax6+ NSCs (**h**), proliferating (Ki67+) cells (**i**) and increase in
491 NeuN+ neurons (**h**) after Akna OE (n=5(**h**), n=4(**i**) each condition). **j**, Boxplot depicting the
492 identity of E14 primary cortical cells transfected *in vitro*. Note that Akna OE does not elicit
493 fate changes *in vitro* (n=3 each condition). **k**, Model describing the expression and functional
494 role of Akna in delamination and seeding of the SVZ. Scale bars: 50 μ m (**a,b,e,f**). (Mann-
495 Whitney U test; *P < 0.05, **P < 0.01, ***P < 0.001)

496

497 **Figure 3. Akna regulates microtubule organization in neural progenitors. a**, Example
498 images of control or Akna shRNA2 transfected primary E14 cortical cells in nocodazole

499 based MT re-polymerization assays. The yellow dashed line indicates a cell in which MTs did
500 not grow upon Akna knockdown. Boxplot showing reduced number of cells regrowing MTs
501 at the centrosome (**b**) and reduced length of MTs (**c**) (n=4) after Akna KD (co: 110 MT
502 endpoints, sh2: 92 MT endpoints). **d**, Ectopic Akna foci induced by Akna OE organize MT
503 independent of centrosomes (see also Extended Data Fig. 6b). **e-h**, Akna OE in the cerebral
504 cortex *in vivo* influences both the orientation (**e-g**, co: 117 EB3-comets, Akna OE: 122 EB3-
505 comets in 3 experiments) and speed (**h**, co: 178 EB3-comets, Akna OE: 113 EB3-comets) of
506 MT polymerization monitored by live imaging of EB3-GFP in cortical slices 1 day after IUE.
507 **i**, Speed of MT polymerization is reduced in shRNA mediated knockdown in vivo (sh (co): 56
508 EB3-comets, sh1(Akna): 96 EB3-comets). (Mann-Whitney U test; * P < 0.05, ** P < 0.01,
509 *** P < 0.001). Scale bars: 5 μ m.

510

511 **Figure 4. EMT progression requires Akna to dissolve junctional coupling.** Micrographs
512 of NMuMG cells immunostained in untreated (**a**) and TGF-beta-1-treated EMT inducing
513 conditions with control (**b**) or Akna siRNA (**c**). Staining for ZO1 (**a'**, **b'**, **c'**) shows that
514 junctional coupling dissolves only in control TGF-beta-1 induced EMT, but not when treated
515 with Akna siRNA. Phalloidin (**a''**, **b''**, **c''**) stainings reveal that the redistribution of Actin
516 filaments from junctions to cytoplasm during EMT is affected upon Akna KD. **d,e**, Confocal
517 micrographs of electroporated cortices 18h after IUE depict examples of GFP+ cells with
518 reduced levels of E-Cadherin upon Akna OE. Scale bars: 10 μ m (**a-c**), 20 μ m (**d,e**).

519

520 **Figure 5. Akna regulates retention of cells within the SVZ.** **a,b**, Confocal micrographs
521 illustrating binning and the distribution of electroporated cells expressing GFP+ (**a**) or Akna
522 and GFP (**b**) under the control of the Dcx promoter 5 days after IUE **c**, Line graph illustrating
523 the distribution of GFP+ cells after IUE for control (green), and Akna OE (blue) with SEM
524 indicated as transparent band (Dcx-GFP: n=5; Dcx-Akna n=4). **d**, Boxplot showing the
525 fraction of GFP+ cells retained below the CP labelled by Tbr1 (Dcx-GFP: n=5; Dcx-Akna
526 n=3). **e**, Dotplot overlaid with a Violin plot showing the distribution of migration speed of
527 control and Akna KD neurons assessed during live imaging in slices 2 days after IUE. The
528 mean \pm SEM is indicated in bold (Dcx-miR(neg): n=34; Dcx-miR(Akna): = 26). **f-i**,
529 Boxplots illustrating the morphology transitions of control and Akna KD cells (**f,g**) of after
530 Akna OE (**h,i**) (Dcx-miR(neg) n=2; Dcx-miRNA(Akna) n=3; Dcx-GFP n=3 and Dcx-Akna
531 n=3). Scale bars: 50 μ m (**a,b**). (**c,d,e**: Mann-Whitney U test; *P < 0.05; **f-i**: Students t-Test*P
532 < 0.05, **P < 0.01)

533 **Extended Data Figure 1. Akna expression, centrosome localization and antibody**
534 **verification. a**, RT-qPCR data show higher levels of Akna mRNA in E14 than E11 or E18
535 cerebral cortex (n=3). **b**, Microarray data depicting higher Akna expression in NSCs that
536 generate neurons via BPs (CD133+/GFPhigh)⁶ (n=3). **c**, WB of Akna in E14 cerebral cortex
537 lysate running at higher than predicted (153 kD) molecular weight due to phosphorylation
538 (data not shown, see also Extended Data Fig. 3d). **d,e**, WB of Akna in Neuro2a cell lysates
539 after transfection with Akna sh1 and sh2 or control (**d**) or 3 different miRNAs and control (**e**)
540 using the antibody clone 14D7. **f**, Akna IF using clone 25F1 in primary E14 cortical cells
541 showing Tbr2+ BPs lacking Akna IF signal upon transfection with Akna siRNAs but not in
542 control, showing specificity of the immunostaining. **g**, IF of Akna and Tubg1 in primary E14
543 cortical cells showing Akna signal surrounding a single Tubg+ centriole. **h**, A20 BAC
544 transgenic cell line showing GFP-tagged Akna at centrosomes marked by Tubg in interphase
545 (**h'** and **h''**), but not during mitosis (**h'''**). **i**, Predicted domains of murine Akna protein. **j**,
546 Amino acid sequence of Akna's AT-hook containing transcription factor domain and the AT-
547 hook-like domain. Notice that neither has the GRP-core sequence surrounded by several K/R
548 amino acids, which is required for DNA or RNA-binding AT-hook domains, such as the AT-
549 hook domains of Hmgal. **k**, WB of Akna in nuclear and cytoplasmic cell fractions of A20
550 cells showing Akna signal in the cytoplasm only. **l**, Mass-spectrometric analysis of sucrose
551 gradient-based isolated cellular sub-fractions of A20 cells. Akna is enriched in fractions 3 and
552 4 containing the centrosomal components listed on the right side. **m-o**, Immunostaining of
553 dissociated hiPSC-derived cerebral organoid cells showing AKNA localization at
554 centrosomes with three different monoclonal antibodies. Clone 9G1 and 4F5 recognize
555 epitopes at the N- and C-terminal part of the protein, respectively, suggesting that different
556 splice variants^{8,50} still localize at centrosomes. Also notice the enrichment at one (mother)
557 centriole. * P < 0.05, *** P < 0.001. Scale bars: 5 μm (**f, h, m, n, o**), 2.5 μm (**g**).
558

559 **Extended Data Figure 2. Mechanisms localizing Akna at the subdistal appendages. a, b**,
560 EM micrographs showing Akna immunogold-labeling at SDAs in the SVZ (**a**) and VZ (**b**) of
561 E13 cerebral cortex sections with (**b'**) as magnification of the boxed area showing SDA in
562 (**b**). **c**, STED nanoscopy picture showing Akna IF signal surrounding Odf2 IF signal showing
563 more distal localization of Akna at the SDA, while Odf2 localizes proximal in respect to the
564 centriole in E14 brain NSCs as summarized in the schematic drawing below (Akna indicated
565 in green, Odf2 in magenta). **d**, Schematic drawing of different truncated Akna variants used to
566 analyze the sub-cellular localization as indicated to the right. **e-h**, Micrographs showing the
567 localization of the truncated Akna forms expressed in primary E14 cortical cells. The
568 constructs containing the last 370 amino acids (**f, h**) localize to centrosomes. Otherwise IF
569 signal is observed in the cytoplasm (**e, g**) and in the nucleus (**e**). Notably, the clone used in
570 Siddiq et al.,⁸ lacked the c-terminal centrosome targeting part. **i-k**, E14 primary cortical cells

571 treated with DMSO (**i**), nocodazole (**j**) or Dctn2 overexpression (**k**) show Akna IF remaining
572 at centrosomes. **l**, Micrographs showing Akna IF signal at centrosomes at the apical,
573 ventricular surface in sections of E15 WT, but not *Sas4*^{-/-} *p53*^{-/-} mice lacking centrioles⁴⁷.
574 Note that Pcnt⁺ pericentriolar material remains present in the absence of centrioles *Sas4*^{-/-}
575 *p53*^{-/-} mice. **m**, **n**, Akna IF of WT (**m**) or Crispr-Cas9 generated *Odf2*-KO (**n**) mammary
576 epithelial cells showing that Akna is lost from centrioles lacking SDAs. Altogether, the data
577 demonstrates that Akna is an internal component of SDAs and is not recruited to centrosomes
578 by MT or Dynein/Dynactin motors. Scale bars: 0.1 μm (**a**, **b**, **c**), 10 μm (**e**, **g**, **h**, **l**, **m**, **n**), 5 μm
579 (**f**,**i**,**j**,**k**).

580

581 **Extended Data Figure 3. Akna dissociates from the centrosome in mitosis depending and**
582 **due to increased phosphorylation.** **a**, IF of Akna in primary E14 cortical cells at different
583 phases of the cell cycle showing lack of Akna IF at the centrosome during mitosis. **b**, WB of
584 Akna in synchronized A20 cells showing that Akna protein is not degraded during mitosis
585 indicated by the presence of phospho-Histone H3. **c**, Representative micrographs of Akna and
586 Pcnt IF in E14 primary cortical cells. Akna IF is observed at centrosomes at 0 hours, but it
587 is undetectable 3 hours after treatment with protein phosphatase inhibitor okadaic acid (OA).
588 This shows that centrosomal localization is phosphorylation-dependent. **d**, WB of lysates of
589 OA treated cells showing that phosphorylation, here caused by protein phosphatase inhibition,
590 delays Akna protein migration on SDS-PAGE and subsequently leads to protein degradation
591 as observed in lysates of cells 5 hours after OA washout. Scale bars: 5 μm (**a**), 10 μm (**c**).

592

593 **Extended Data Figure 4. Temporal and sub-type specific regulation of Akna in the**
594 **developing telencephalon.** **a-c**, Micrographs showing that Pcnt⁺ centrosomes lack Akna in
595 the cerebral cortex at E9 (**a**) and E18 (**b**) while Akna is enriched in the VZ and, specifically,
596 the SVZ at E14 in the ganglionic eminence (**c**) and the cerebral cortex (Fig. 1). **d-f**,
597 Histograms showing the percentage of Akna⁺ centrosomes in E14 cerebral cortex regions
598 (**d,e**, n=3) as indicated and in dissociated primary E14 cortical cells (**f**) revealing that cells
599 with Akna⁺ centrosomes are mostly differentiating NSCs (Pax6-Tbr2) and Tbr2⁺ BPs (n=3).
600 **g**, Micrographs of cells isolated from E14 cerebral cortex by FACS using prominin1 and
601 stained for Pax6 (red arrows) and Tbr2 (blue arrows) showing that double positive
602 (differentiating) NSCs have Akna⁺ centrosomes, while Pax6⁺/Tbr2-negative (proliferating)
603 NSCs do not. Scale bars: 10 μm (**a,c,g**), 20 μm (**b**).

604

605 **Extended Data Figure 5. Akna knock-down elicits cell death and delamination defects**
606 **persists upon cell death rescue by p53 reduction. a-c**, Micrographs showing TUNEL
607 staining in E15 cerebral cortex indicating cell death 2 days after IUE with Akna shRNA1 (b)
608 or 2 (c), but not with control plasmids (a). **d, e**, Micrographs showing the distribution of
609 GFP+ cells in E15 cerebral cortex 2 days after IUE with control shRNA (d) or Akna shRNA2
610 plus p53-miRNA plasmids (e). Note that p53-downregulation rescues the apoptotic effect of
611 Akna knock-down. **f**, Line graph illustrating the distribution of GFP+ cells in the cerebral
612 cortex after control (green, n=6) and Akna sh2/p53 miR (purple, n=8) IUE with SEM as
613 transparent band showing still a delamination defect upon Akna KD when cell death is
614 blocked (sh2 + p53-miR). **g**, Boxplot showing the decrease of GFP+ Tbr2+ cells after IUE of
615 Akna shRNA + p53-miR compared to control, showing that defects in delamination are
616 accompanied by retaining NSC fate. (Mann-Whitney U test; **P < 0.01). Scale bars: 50 μ m
617 (a-e).

618

619 **Extended Data Figure 6. Akna is sufficient to mediate MT polymerization and recruit g-**
620 **Tubulin and SDA components. a-e**, Micrographs of E14 primary cortex cells treated and
621 immunostained as indicated. **a**, Time-series depicting regrowth of the microtubule
622 cytoskeleton after nocodazole mediated de-polymerization. **b**, Regrowth of the microtubules
623 60 seconds after nocodazole mediated de-polymerization in Akna OE (GFP+) cells. Red
624 arrows indicate centrosomes. Note that MTs polymerize also from ectopic Akna+ sites. **c**,
625 Confocal images showing co-labeling of Akna foci with gTurC components Tubg1 and Gcp4.
626 **d**, Akna foci fail to recruit MT minus end capping-proteins Camsap2 and the centrosomal
627 protein Pcnt. **e**, Confocal micrographs depicting colocalization of ectopic Akna foci with the
628 Odf2, Dctn1, and Mapre1. **f**, Co-immunoprecipitation experiments with lysates from E14
629 cerebral cortex, IP with Akna antibody and WB with Akna, Odf2, Dctn1, Mapre1 and Gcp2,
630 showing that these proteins are in the same complex, except Gcp2 (i.e. the gTuRC). Scale
631 bars: 5 μ m (a-e).

632

633 **Extended Data Figure 7. Akna regulation and function during EMT in mammary**
634 **epithelial cells. a, b**, Akna IF in untreated (a) and 1 day with TGF-beta-1 treated (b)
635 NMuMG cells. Red arrows indicate Akna-negative centrosomes (Pcnt+) in untreated cells and
636 blue arrows Akna+ centrosomes in TGF-beta-1 treated cells. **c**, WB showing Akna protein
637 increase during the first days after EMT induction and subsequent decrease to levels in
638 untreated cells. Coomassie-stained gel showing equal loading is shown on the right side. **d**,

639 WB of Akna in untreated, control and Akna siRNA transfected cells at 4 days TGF-beta-1
640 treatment. Note the efficient knock-down of Akna by siRNA treatment. **e**, WB of ZO1 in
641 untreated, control and Akna siRNA transfected cells at 4 days TGF-beta-1 treatment. The
642 middle panel is a longer exposure. Note the increased ZO1 protein levels upon Akna KD in
643 equal loading as indicated in the lower panel. **f**, WB of p120 and phospho-p120 in untreated,
644 control and Akna siRNA transfected cells at 4 days TGF-beta-1 treatment. (**e**) and (**f**)
645 demonstrate that degradation of junctional proteins during EMT is blocked by Akna
646 knockdown. In accordance, cells are less scattered in Akna knockdown conditions compared
647 to control (**g-i**). Scale bars: 5 μ m (**a**), 30 μ m (**f, g, h**).

648

649 **Extended Data Figure 8. Lower Akna levels in differentiating neurons mediates**
650 **acentrosomal MT polymerization and allow migration into the cortical plate. a, b,**
651 Primary E14 cortical cells were sorted for Prominin1 (CD133) to isolate NSCs or PSA-
652 NCAM to isolate neurons as indicated in the panels. Prom1+ cells express the NSC marker
653 Nestin, while PSA-NCAM+ cells express the neuronal marker Tubb3 showing the specificity of
654 the FACS sort (**a**). Microtubule regrowth assay in purified NSCs shows centrosomal
655 microtubule polymerization (**b**, upper panels), purified neurons show largely non-centrosomal
656 microtubule polymerization patterns (**b**, lower panels). **c**, Micrographs showing co-
657 electroporation of a CAG-dsRED and Dcx-GFP at E13 and analysis at E15. Note the onset of
658 Dcx-GFP expression (**c''**) only in the BP/neuronal compartment of the developing cortex,
659 while DsRed+ cells are also found in the VZ (**c, c'**). **d**, Micrographs showing electroporated
660 cells (GFP+) in control (Dcx-GFP) and **e**, Akna overexpressing (Dcx-Akna) conditions. Note
661 that many Akna OE cells accumulate in the SVZ and are unable to migrate into the CP. **f**,
662 Micrographs of Akna OE cells retained in the SVZ co-stained for Tbr1, Ctip2, and Cux1
663 labelling neurons of different layer identity. Note that the neurons accumulating below the CP
664 upon Dcx-driven Akna contain neurons positive for each of these neuronal identities. **g**,
665 Micrographs showing that electroporated cells (GFP+) after control and Dcx-miR driven
666 downregulation of Akna enter the CP equally well. **h**, Line graph illustrating the distribution
667 of GFP+ cells after IUE for control (green), and Dcx-Akna overexpression (purple). In the
668 same color transparently underlying the line graph the respective standard error is indicated
669 (Dcx-miR (neg): n=4; Dcx-miR4 (Akna) n=3). Scale bars: 5 μ m (**a, b**); 50 μ m (**c-g**).

670

671

672 **Extended Data Figure 9. AKNA localization in ferret and macaque cerebral cortex and**
673 **function in human cerebral cortex organoids. a**, qPCR for AKNA showing the transient
674 but strong upregulation of AKNA mRNA in ferret VZ tissue at E34, the time when cells
675 forming the oSVZ leave the VZ³. **b**, Microarray data showing higher AKNA mRNA levels in
676 the lateral sulcus (LC) compared to splenial gyrus (SP) in oSVZ tissue of ferret brain at P1. **c**,
677 WB showing higher levels of AKNA protein in LS compared to SG from ferret oSVZ tissue
678 at P1. **d**, Micrograph showing AKNA and TUBG co-localization at centrosomes in ferret
679 brain cells at (P1). **e, f**, Comparison of AKNA IF in ferret VZ and oSVZ tissue in SP versus
680 LS. Notice more abundant IF signal in oSVZ of LS, corresponding with mRNA and protein
681 levels as determined by WB. Given that oSVZ of gyrus contains more basal radial glia with
682 bipolar morphology than sulcus, we propose that AKNA levels regulate the multipolar to
683 bipolar transition in ferret as in mouse SVZ with higher levels retaining more cells in a
684 multipolar state (see Fig. 5f-i). **g, h**, AKNA IF in macaque germinal zone (GZ) and cortical
685 plate (CP) at E64. Arrows indicate AKNA+ centrosomes and denote abundant IF signal. The
686 square in (**g**) shows a representative example of a TUBG+ AKNA+ centrosome in the GZ,
687 while the one in (**h**) depicts an AKNA-negative centrosome in the CP. **i**, Micrographs of
688 human NPCs derived from hiPS cells overexpressing Akna showing multiple foci of MT
689 polymerization. **j, k**, In situ hybridization (ISH) in hiPSC derived cerebral organoids showing
690 enrichment of AKNA mRNA in non-apical SVZ-like areas. **l, m**, Micrographs showing
691 sections of human brain organoids stained for GFP+ cells electroporated with a control
692 plasmid (**l**) or a plasmid overexpressing the human form of Akna (**m**). **n**, Line graph
693 illustrating the distribution of GFP+ electroporated cells after IUE for control (green) and
694 Akna OE (blue) in 3 different culture batches (n=3). **o**, Line graph illustrating the distribution
695 of GFP+ electroporated cells after IUE for control (green) and Akna knock down (orange)
696 (n=3). Data are shown as mean \pm SEM (**a,b**; Students t-Test *P < 0.05, ***p < 0.001; **n,o**:
697 Mann-Whitney U test; *P < 0.05). Scale bars: 5 μ m (**d**); 50 μ m (**e, f, i**); 10 μ m (**g, h**); 50 μ m
698 (**j, k**).

699
700 **Extended Data Figure 10. Akna does not regulate cilia formation or localization. a, b**,
701 Histograms depicting the percentages of ciliated cells (Arl13+) (**a**, n=3) and short vs. long
702 cilia (**b**, n=3) in control or Akna shRNA transfected E14 primary cortical cells. **c-d**, iCLEM
703 micrographs of in utero electroporated cells. The images compare two neighboring NSCs; one
704 electroporated (blue) and one non-electroporated (orange). Yellow and pink arrows show anti-
705 GFP and anti-Tuba immunogold-signal in the cytoplasm and cilium of the electroporated cell.

706 Notice that Akna electroporation does not notably affect cilia formation *in vivo*. Scale bars: 30
707 μm (**c-IF**); 1 μm (**c-EM**); 0.1 μm (**d**).

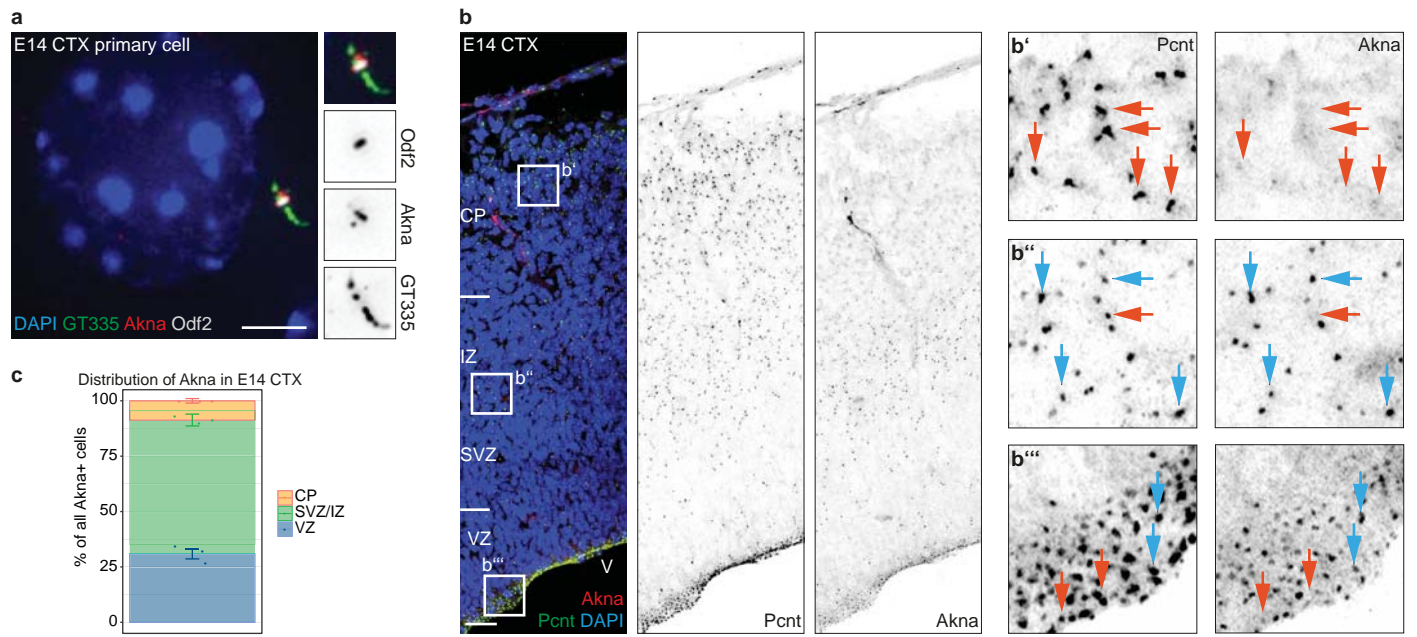


Figure 1, Camargo Ortega et al.,

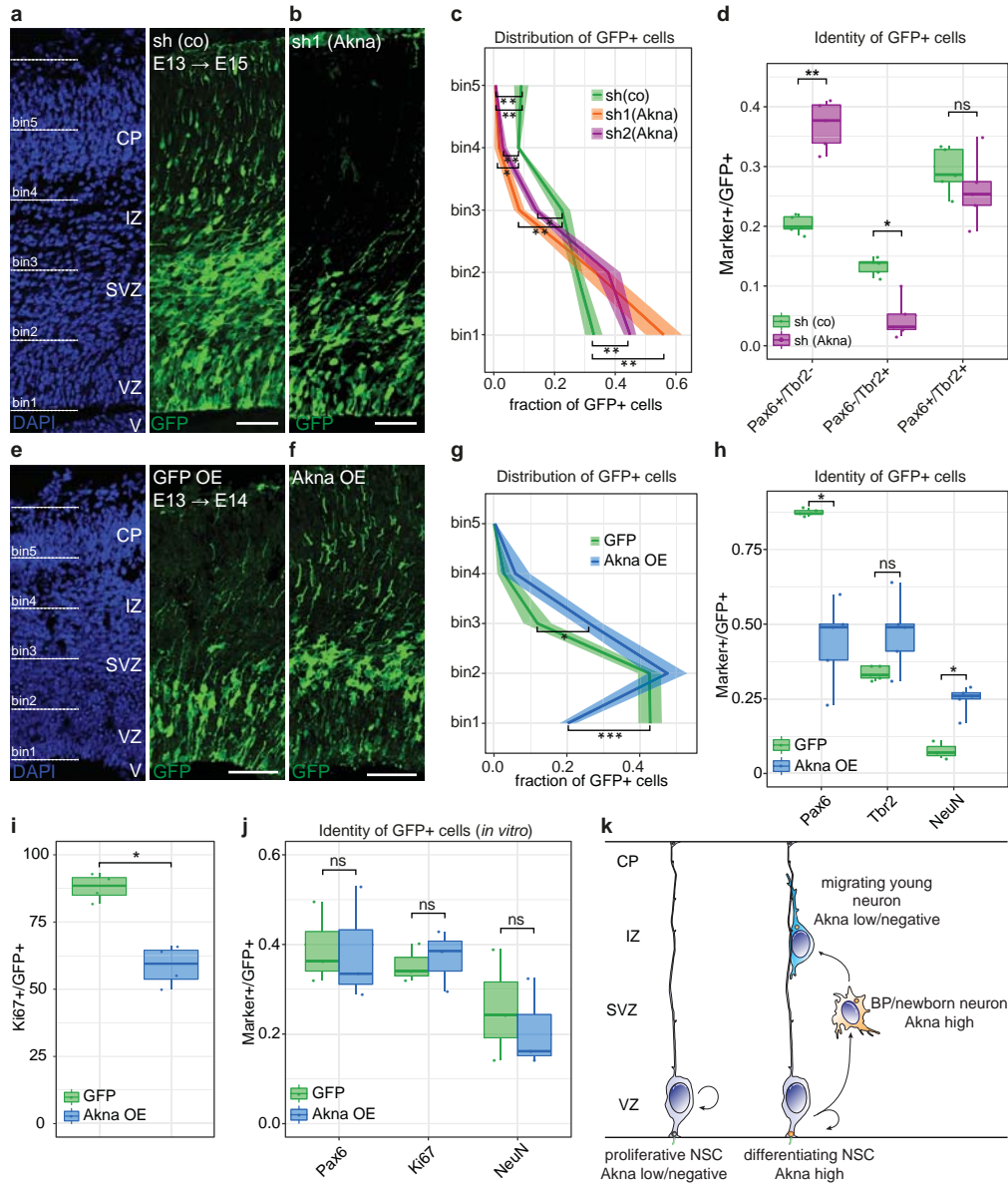


Figure 2, Camargo Ortega et al.,

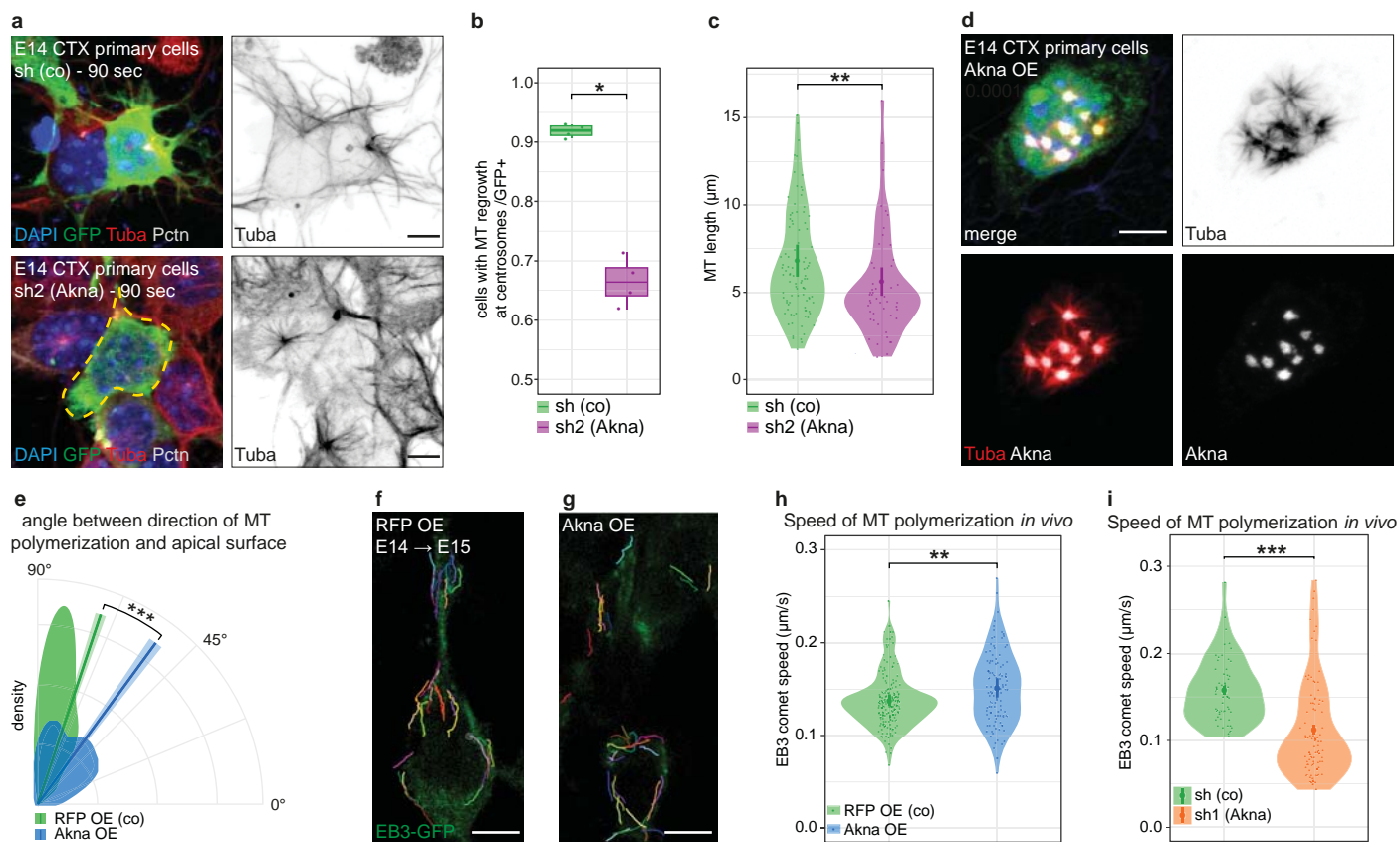


Figure 3, Camargo Ortega et al.

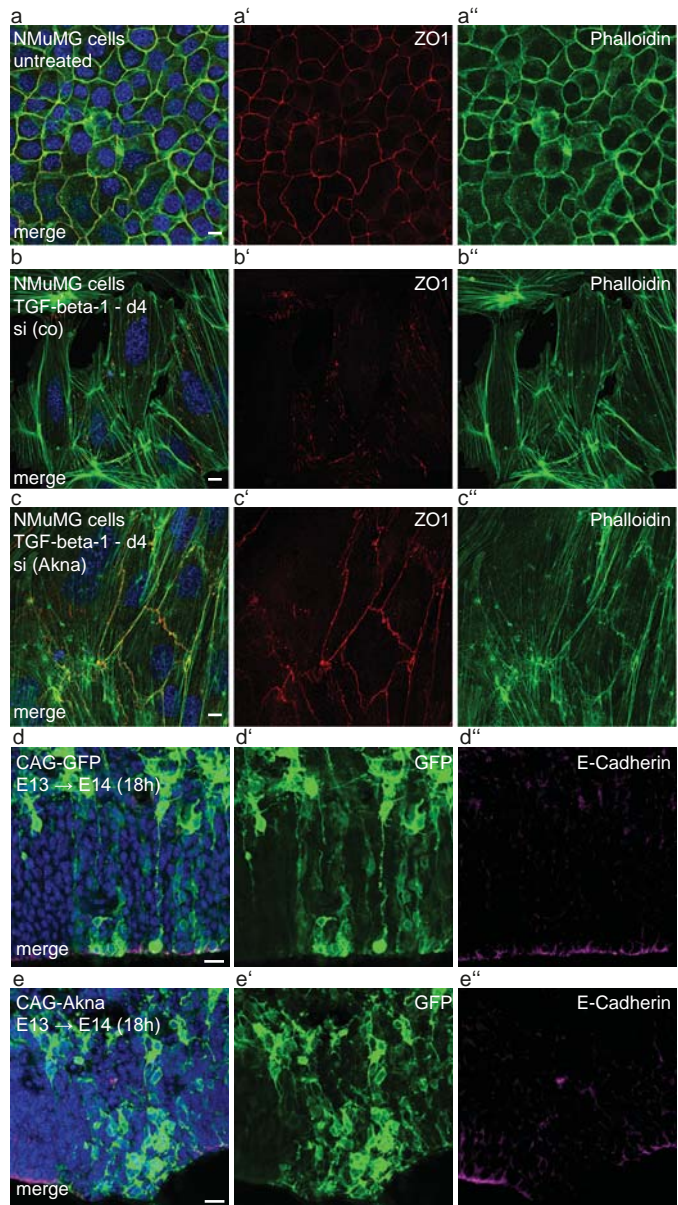


Figure 4, Camargo Ortega et al.

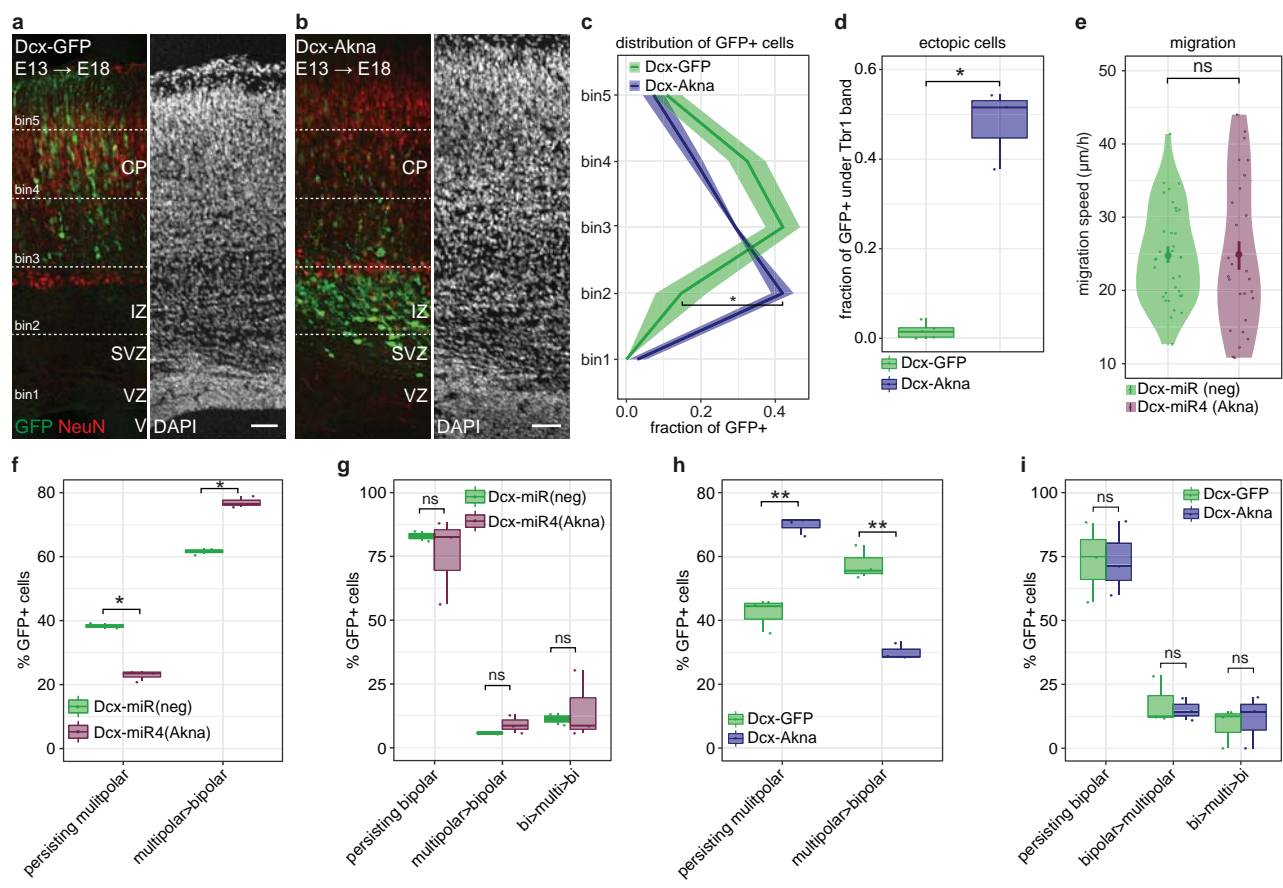
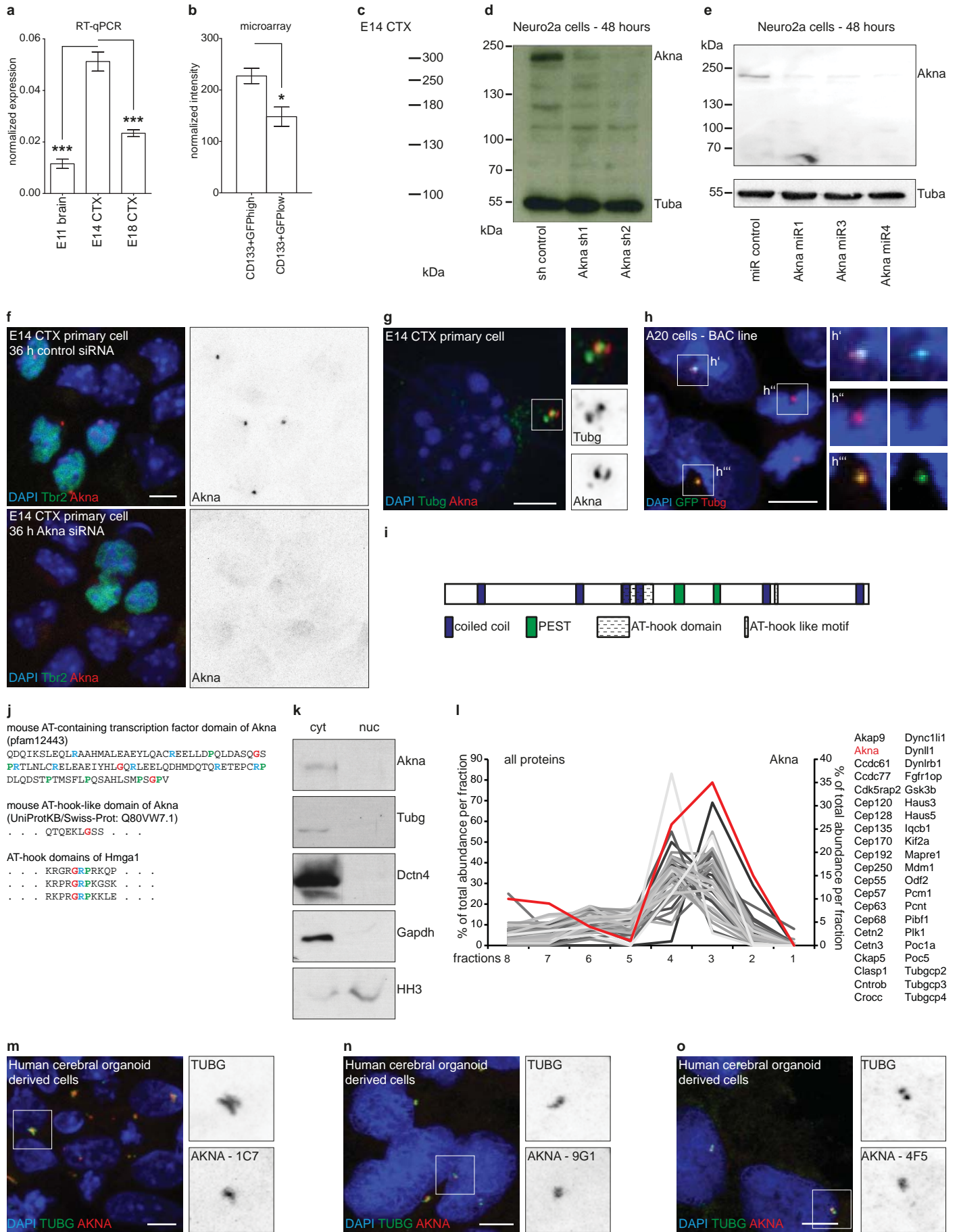
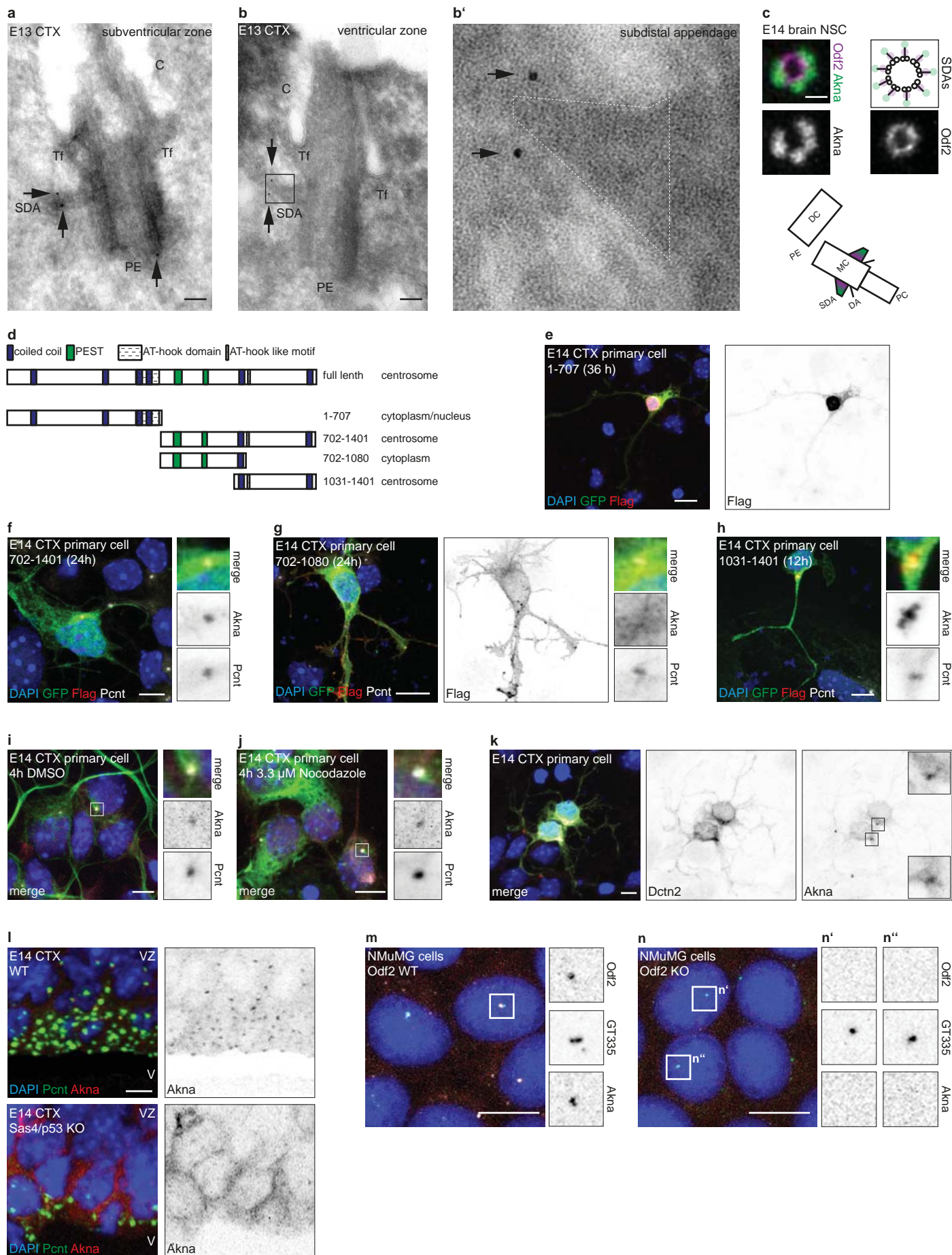


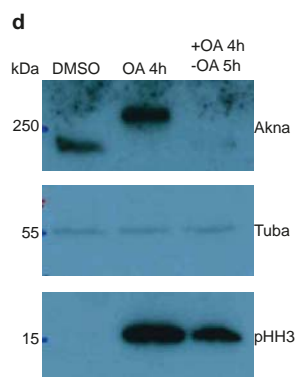
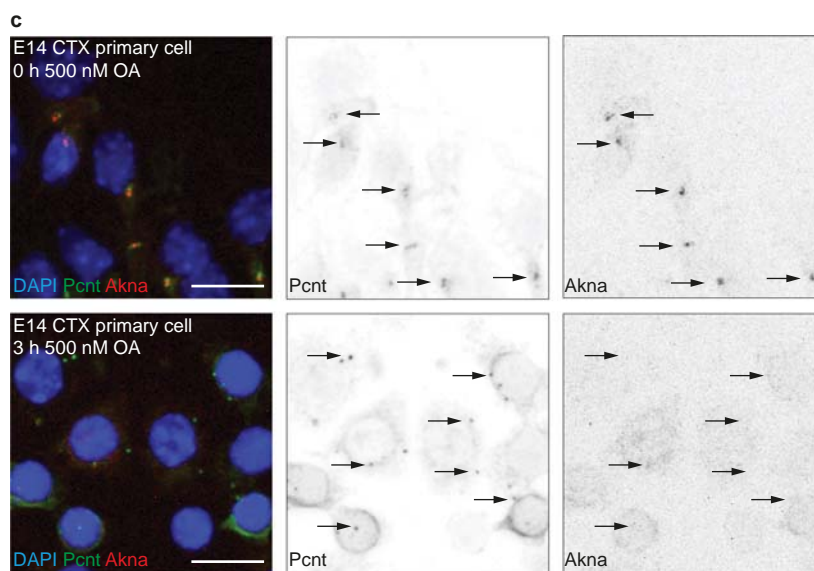
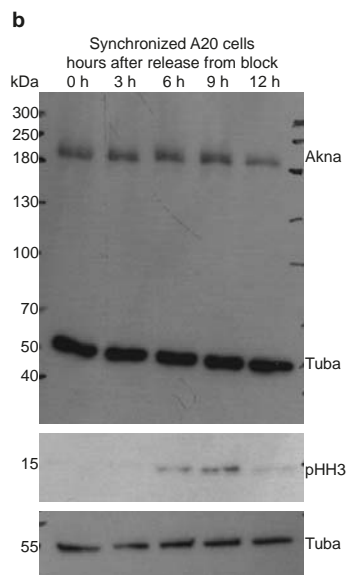
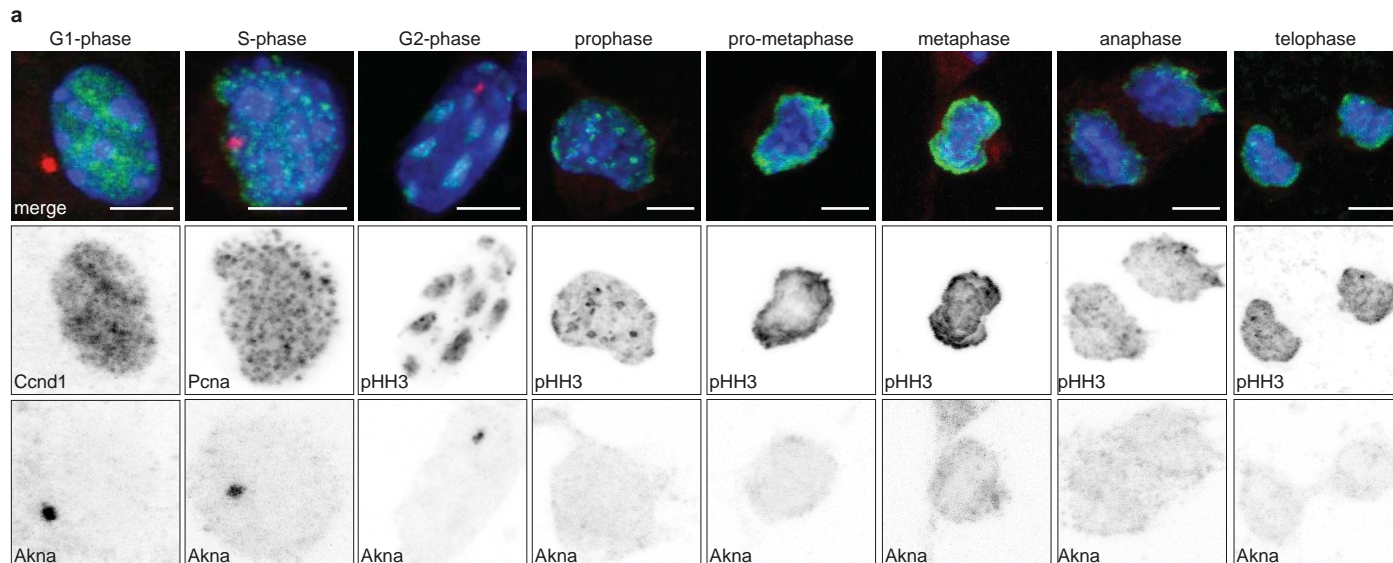
Figure 5, Camargo Ortega et al.



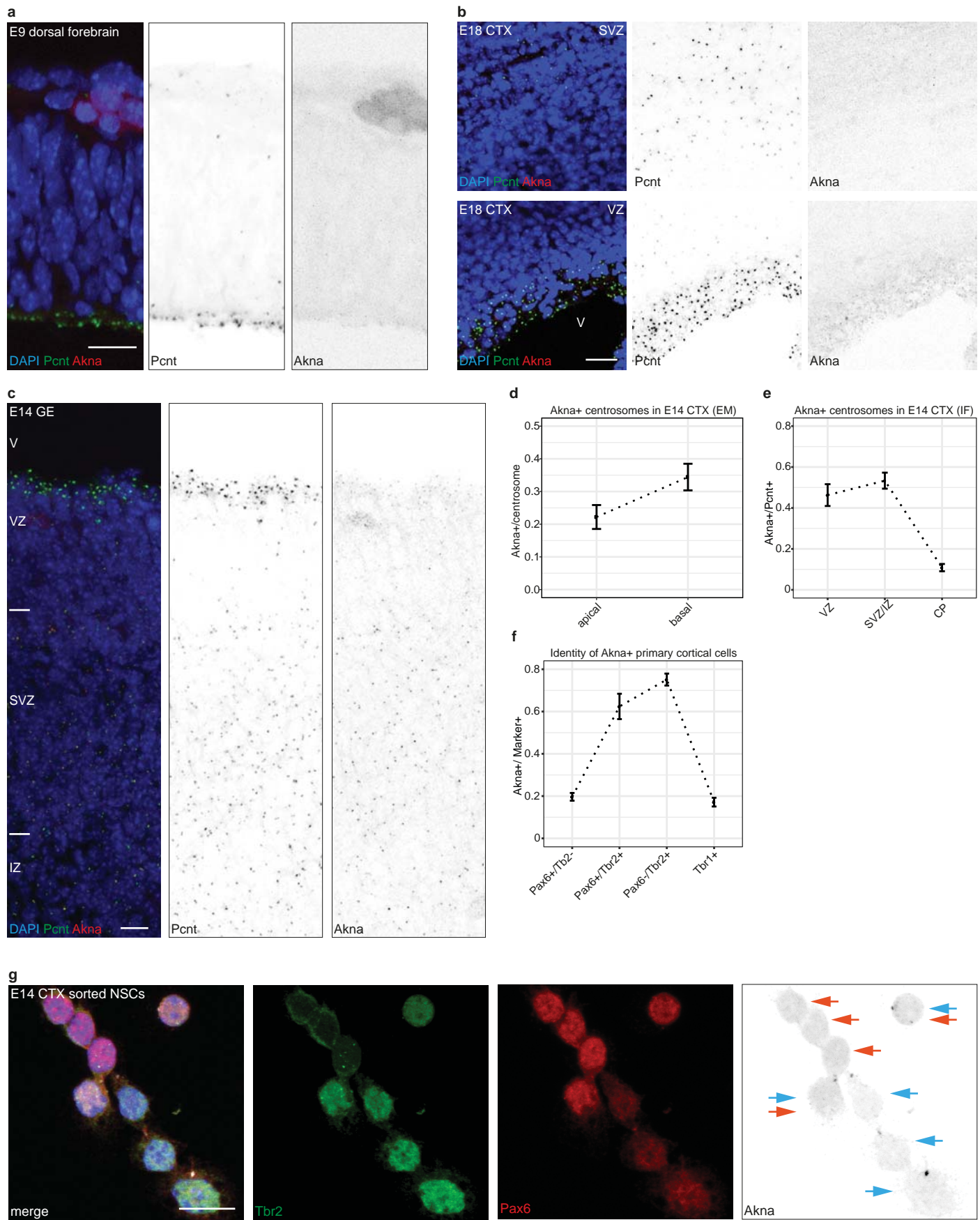
Extended Data Figure 1, Camargo Ortega et al.



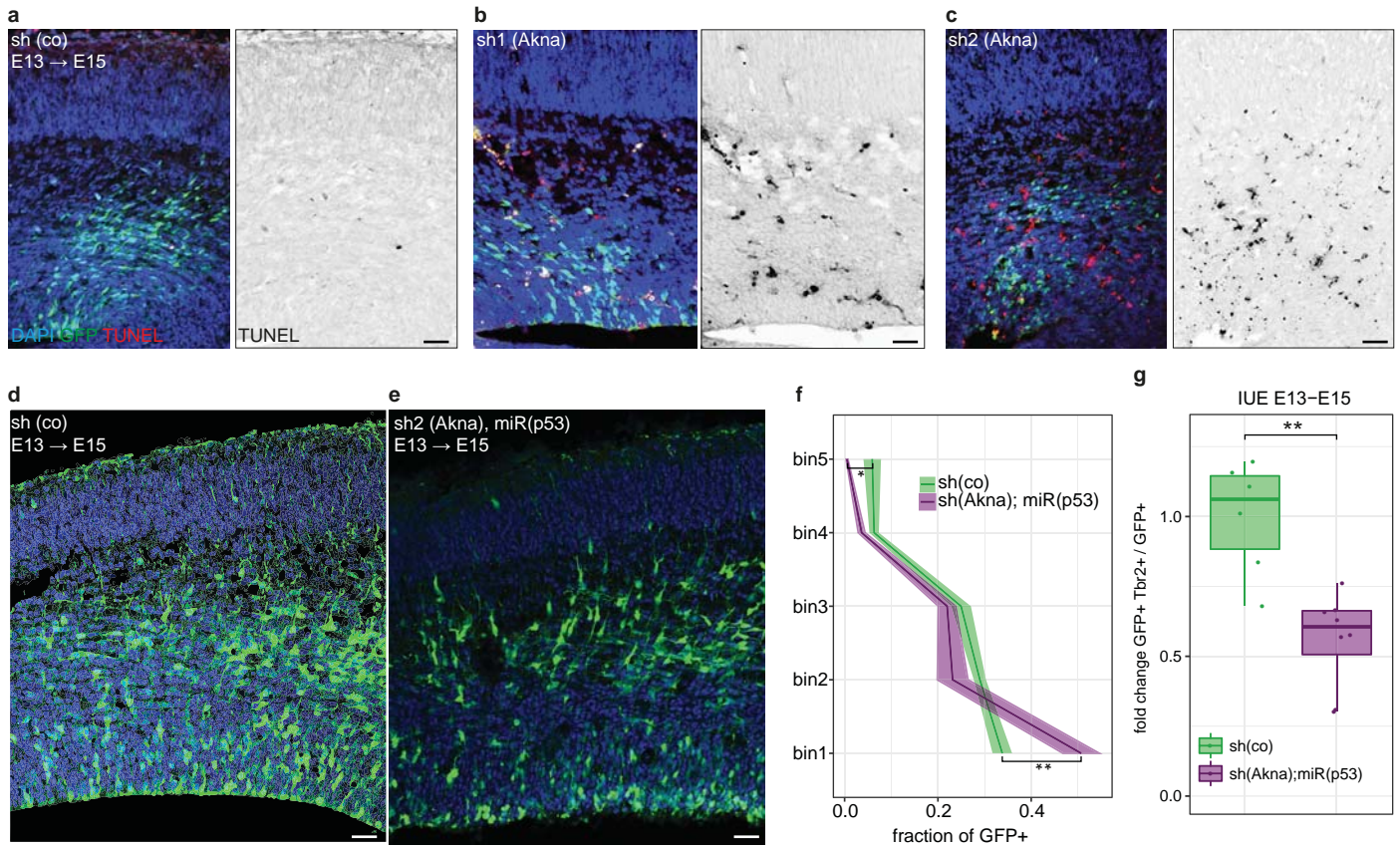
Extended Data Figure 2, Camargo Ortega et al.



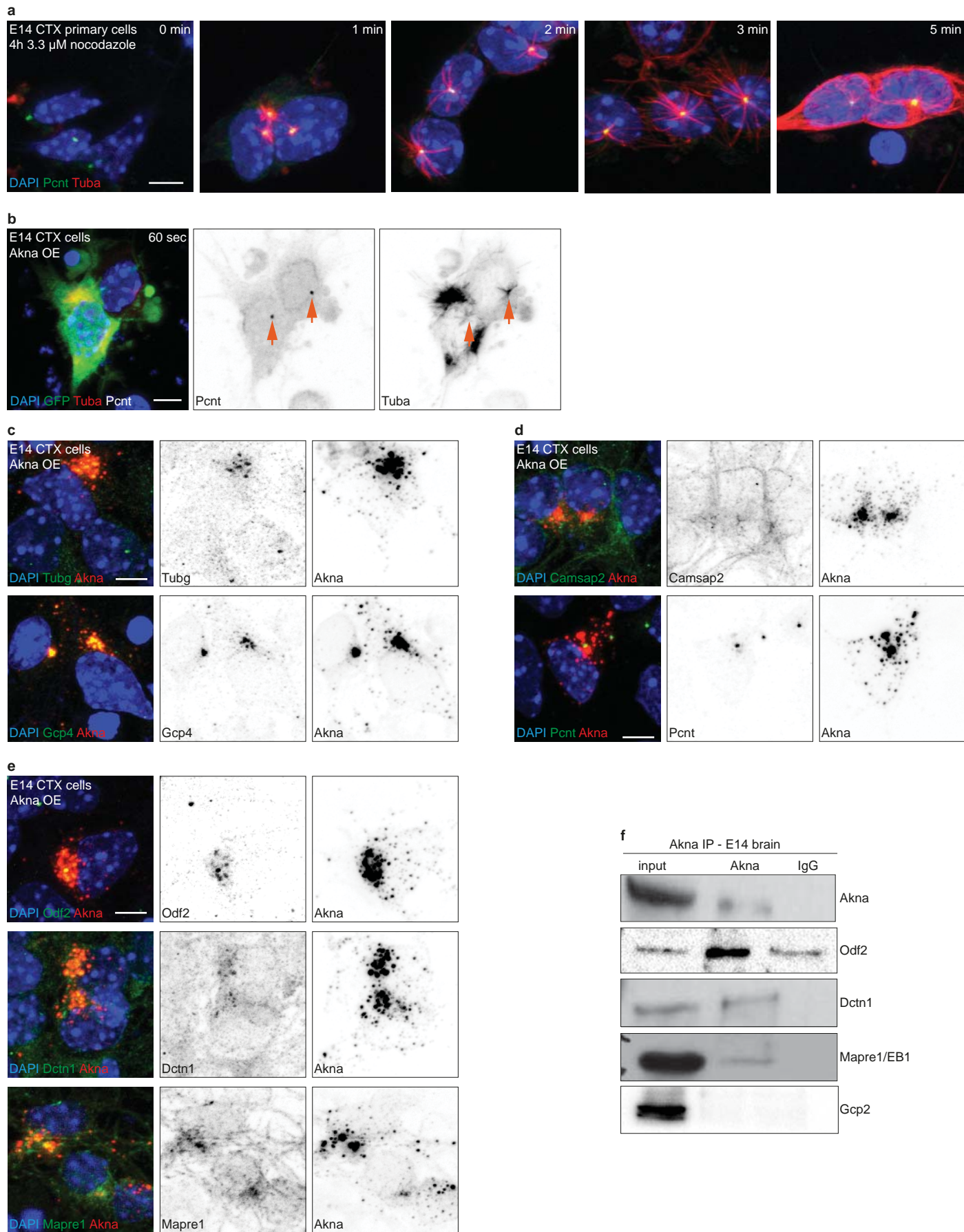
Extended Data Figure 3, Camargo Ortega et al.



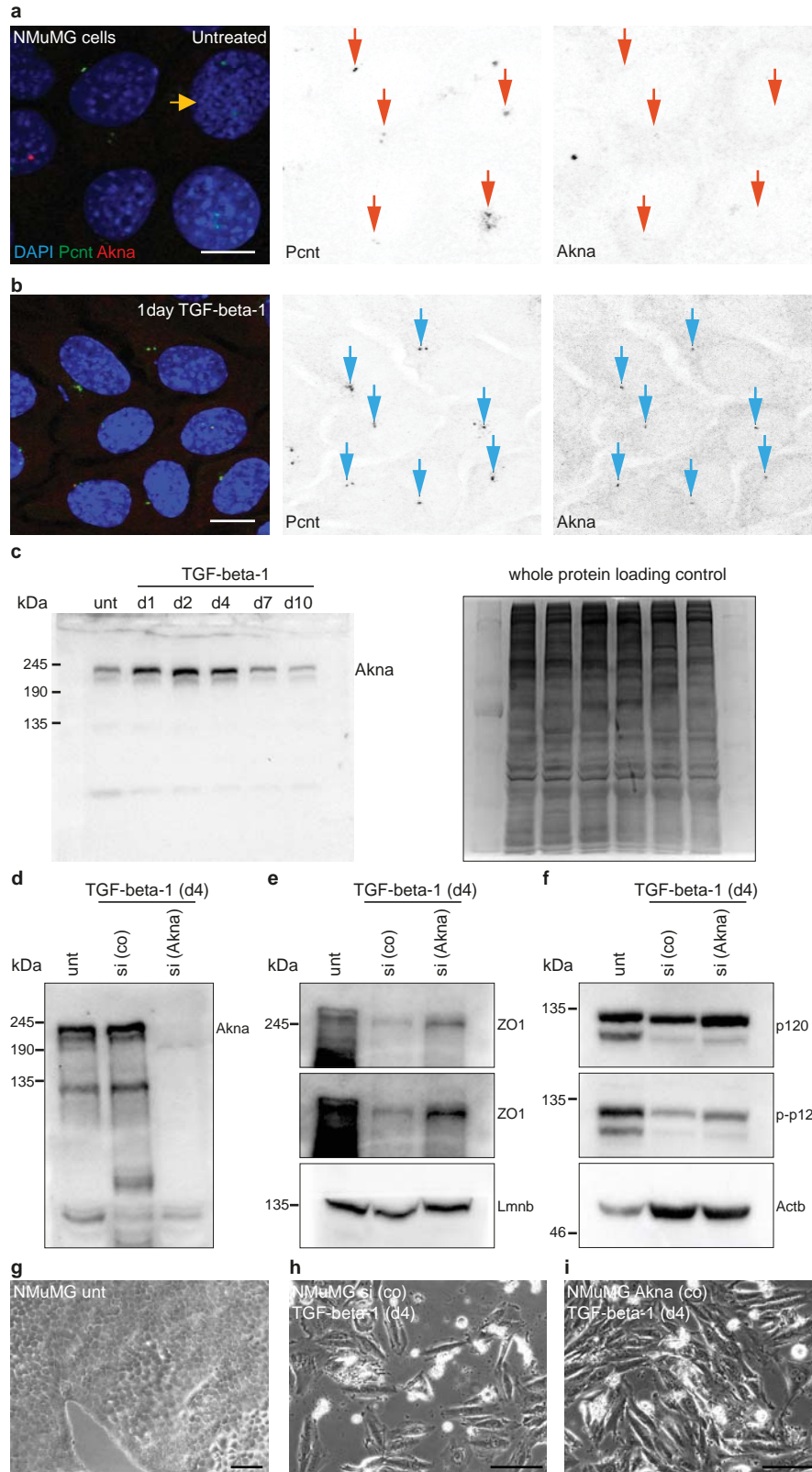
Extended Data Figure 4, Camargo Ortega et al.



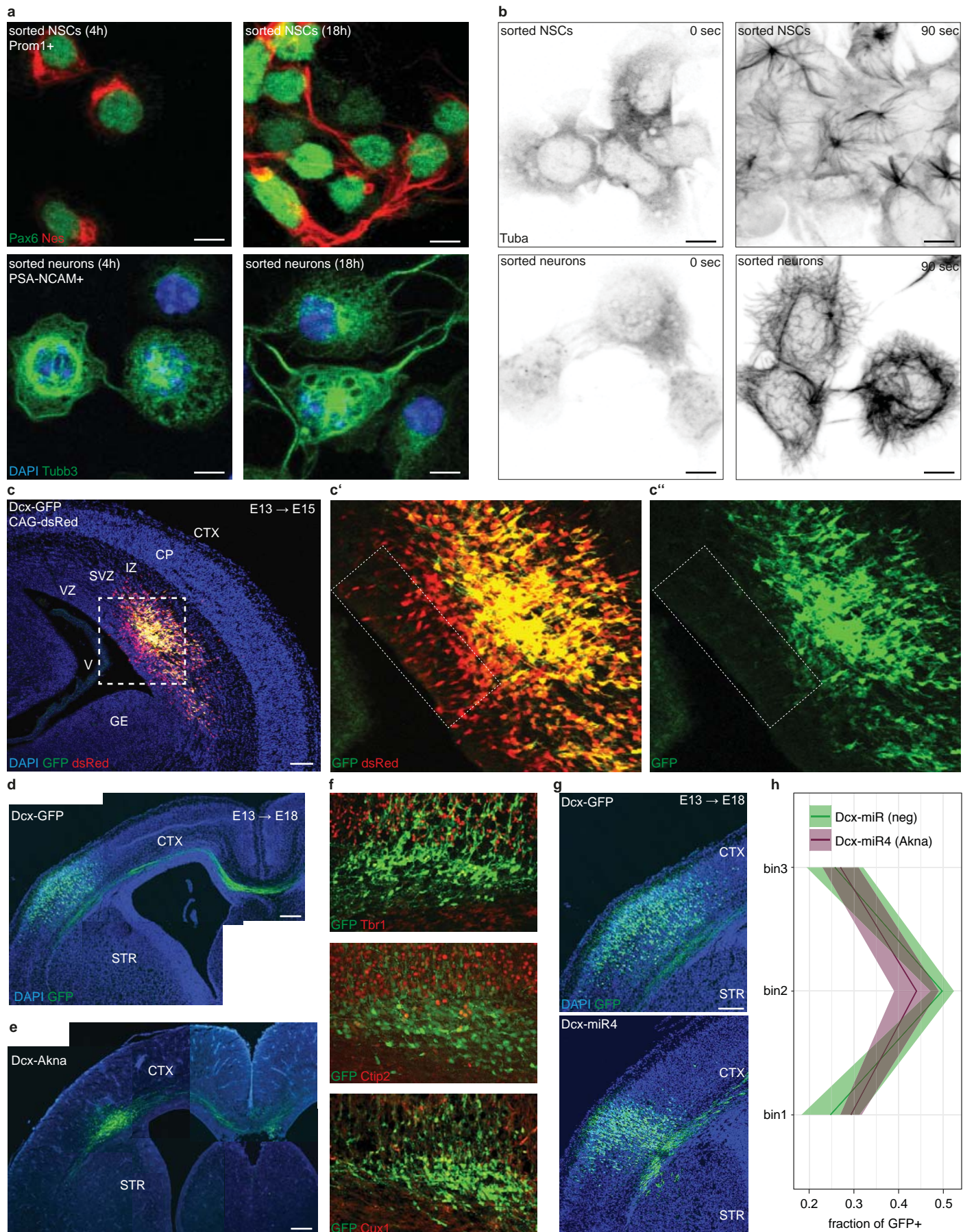
Extended Data Figure 5, Camargo Ortega et al.



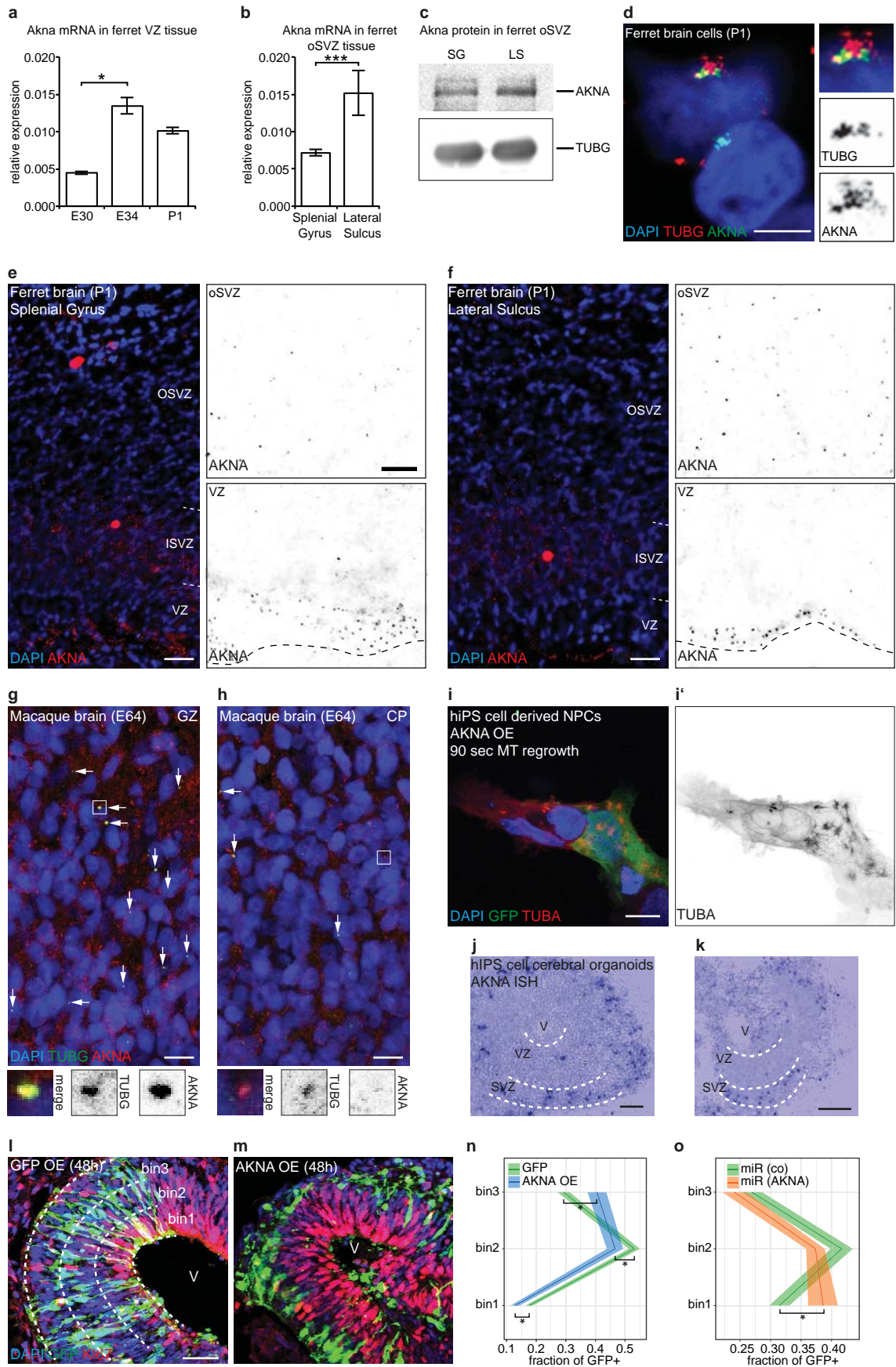
Extended Data Figure 6, Camargo Ortega et al.



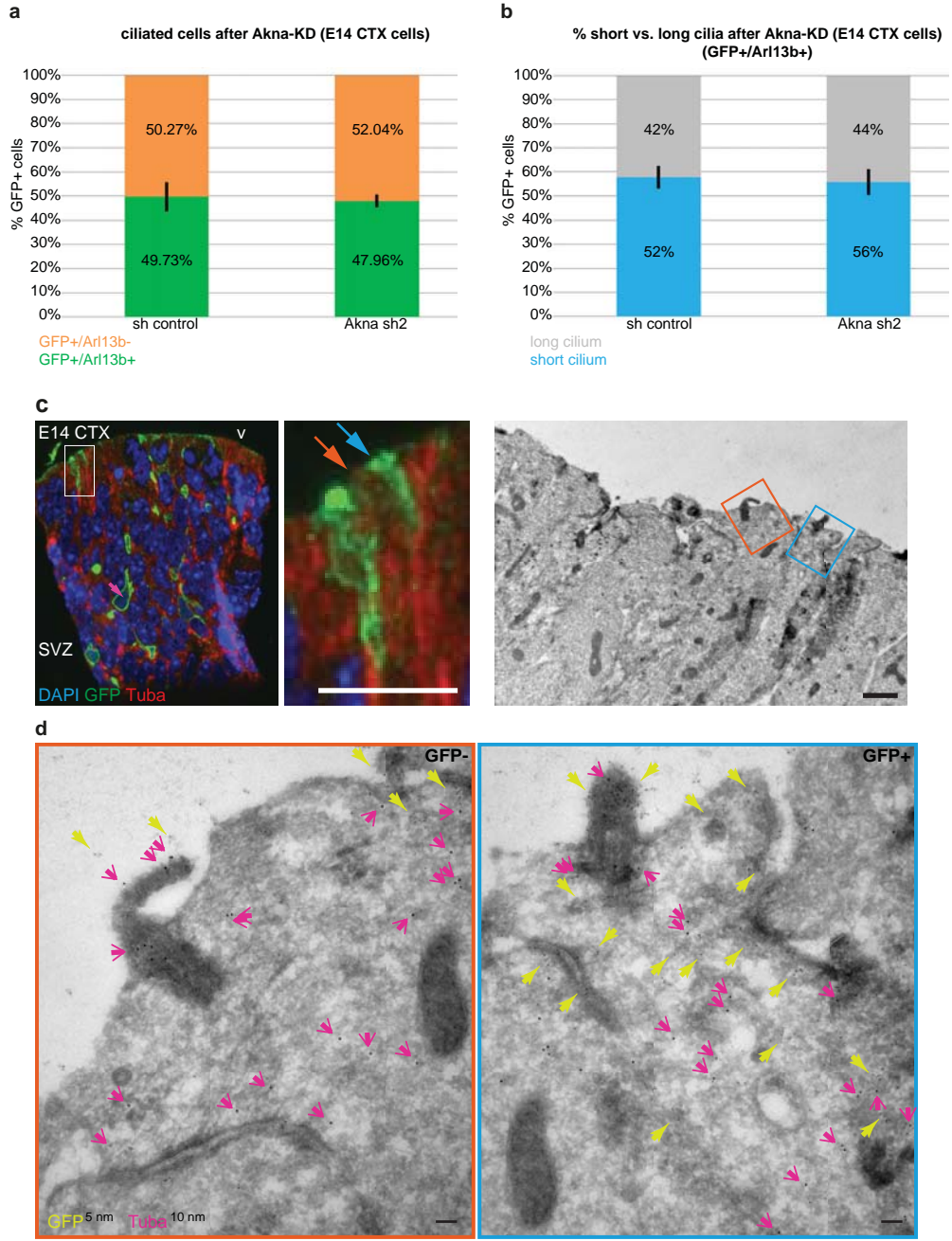
Extended Data Figure 7, Camargo Ortega et al.



Extended Data Figure 8, Camargo Ortega et al.



Extended Data Figure 9, Camargo Ortega et al.,



Extended Data Figure 10 Camargo Ortega et al.