

1 **JNK signaling controls branching, nucleokinesis, and positioning of**
2 **centrosomes and primary cilia in migrating cortical interneurons**

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12 **Running title:** Interneuron dynamics require JNK

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14 **Key Words:** GABAergic interneuron; development; forebrain; intracellular signaling; neuronal
15 migration; live imaging

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17 **Summary Statement:** Loss of JNK signaling reduces growth cone branching frequency, limits
18 interstitial side branch duration, alters rate and amplitude of nucleokinesis, and mislocalizes
19 centrosomes and primary cilia in migrating cortical interneurons.

34 **ABSTRACT**

35 Aberrant migration of inhibitory interneurons can alter the formation of cortical circuitry and lead
36 to severe neurological disorders including epilepsy, autism, and schizophrenia. However,
37 mechanisms involved in directing the migration of these cells remain incompletely understood.
38 In the current study, we used live-cell confocal microscopy to explore the mechanisms by which
39 the c-Jun NH₂-terminal kinase (JNK) pathway coordinates leading process branching and
40 nucleokinesis, two cell biological processes that are essential for the guided migration of cortical
41 interneurons. Pharmacological inhibition of JNK signaling disrupts the kinetics of leading
42 process branching, rate and amplitude of nucleokinesis, and leads to the rearward
43 mislocalization of the centrosome and primary cilium to the trailing process. Genetic loss of *Jnk*
44 from interneurons corroborates our pharmacological observations and suggests that important
45 mechanics of interneuron migration depend on the intrinsic activity of JNK. These findings
46 suggest that JNK signaling regulates leading process branching, nucleokinesis, and the
47 trafficking of centrosomes and cilia during interneuron migration, and further implicates JNK
48 signaling as an important mediator of cortical development.

49

50 **SYMBOLS AND ABBREVIATIONS**

51 MGE: Medial ganglionic eminence
52 CGE: Caudal ganglionic eminence
53 JNK: c-Jun NH₂-terminal kinase
54 Dcx: Doublecortin
55 Cetn2-mCherry: Centrin2-mCherry
56 *Dlx5/6-CIE: Dlx5/6-Cre-IRES-EGFP*
57 *cTKO*: conditional *Jnk* triple knockout
58 WT: Wild type
59 MAPK: mitogen-activated protein kinase
60 cHBSS: complete Hank's Balanced Salt Solution
61 Shh: Sonic hedgehog
62 E14.5: Embryonic day 14.5
63 μ: Micro
64 Cxcr4: C-X-C motif chemokine receptor 4
65 ErbB4: erb-b2 receptor tyrosine kinase 4
66 5-Htr6: Serotonin receptor 6
67 s.e.m.: standard error of the mean

68 INTRODUCTION

69 During embryonic development, cortical interneurons are born in the medial and caudal
70 ganglionic eminences (MGE and CGE) of the ventral forebrain and then migrate long distances
71 to reach the place of their terminal differentiation in the overlying cerebral cortex (Miyoshi et al.,
72 2010; Nery et al., 2002; Wichterle et al., 1999; Xu et al., 2004). While navigating their
73 environments, cortical interneurons must integrate extracellular guidance cues with intracellular
74 machinery in order to reach the cortex, assemble and travel in tangentially oriented streams,
75 and disembark from streams at the correct time and place to properly infiltrate the cortical plate.
76 Two cellular mechanisms that enable interneurons to make these complex migratory decisions
77 are leading process branching, where cortical interneurons dynamically remodel their leading
78 processes to sense and respond to extracellular guidance cues, and nucleokinesis, where
79 interneurons propel their cell bodies forward in the selected direction of migration (Ang et al.,
80 2003; Bellion et al., 2005; Moya and Valdeolmillos, 2004; Nadarajah et al., 2003; Polleux et al.,
81 2002). Moreover, proper positioning and signaling from two subcellular organelles, the
82 centrosome and primary cilium, have been implicated in the guided migration of cortical
83 interneurons (Higginbotham et al., 2012; Luccardini et al., 2013; Luccardini et al., 2015;
84 Yanagida et al., 2012). Failure to coordinate these cellular and subcellular events can alter
85 cortical interneuron migration and impair the development of cortical circuitry, which may
86 underlie severe neurological disorders such as autism spectrum disorder, schizophrenia, and
87 epilepsy (Hildebrandt et al., 2011; Kato and Dobyns, 2005; Meechan et al., 2012; Volk et al.,
88 2015). While progress has been made on elucidating the complex molecular mechanisms
89 underlying nucleokinesis and leading process branching (Baudoin et al., 2012; Godin et al.,
90 2012; Silva et al., 2018; Tsai and Gleeson, 2005), the intracellular signaling pathways that
91 regulate these cellular mechanisms remain largely unknown.

92 The c-Jun NH₂-terminal kinases (JNKs) are evolutionarily conserved members of the mitogen-
93 activated protein kinase (MAPK) super-family (Chang and Karin, 2001; Davis, 2000). The JNK
94 proteins are encoded by three genes, *Jnk1* (*Mapk8*), *Jnk2* (*Mapk9*), and *Jnk3* (*Mapk10*). JNKs
95 phosphorylate numerous substrates in response to extracellular stimuli to mediate physiological
96 processes including cellular proliferation, apoptosis, differentiation, and migration (Davis, 2000).
97 Disruption to JNK signaling has been linked to aberrant migration of excitatory cortical neurons
98 (Hirai et al., 2006; Wang et al., 2007; Westerlund et al., 2011; Yamasaki et al., 2011; Zhang et
99 al., 2016) as well as cognitive disorders in humans (Kunde et al., 2013; McGuire et al., 2017).
100 More recently, we found that JNK signaling controls the timing of interneuron entry into the

101 cerebral cortex, as well as the formation and maintenance of tangential streams of cortical
102 interneurons (Myers et al., 2020; Myers et al., 2014), but the role that JNK plays in the migratory
103 properties of individual cortical interneurons has not been examined.

104 In the current study, we use a combination of pharmacological and genetic manipulations in an
105 MGE explant cortical cell co-culture assay to demonstrate that interneurons have a requirement
106 for JNK-signaling in the regulation of leading process branching and nucleokinesis. JNK-
107 inhibited MGE interneurons dramatically slow their migration while displaying more variable
108 speeds, and exhibit decreased migratory displacement. Concomitantly, JNK-inhibited
109 interneurons display significant defects in leading process branching with decreased growth
110 cone splitting frequency and interstitial side branch duration, as well as disrupted nucleokinesis
111 and swelling dynamics. Similarly, genetic ablation of *Jnk* from MGE interneurons also results in
112 leading process branching and nucleokinesis defects, suggesting interneurons have a cell-
113 intrinsic requirement for JNK signaling during migration. In addition, we discovered a novel role
114 for JNK signaling in the dynamic localization of the centrosome and primary cilium in migrating
115 interneurons. Surprisingly, the centrosomes and the primary cilia of JNK-inhibited interneurons
116 aberrantly localized to the cell body or trailing process, regardless of whether the leading
117 process contained a swelling. These findings implicate the JNK pathway as a key intracellular
118 mediator of leading process branching, nucleokinesis, and organelle dynamics in migrating
119 MGE interneurons.

120 **RESULTS**

121 ***Pharmacological inhibition of JNK signaling disrupts MGE interneuron migration in vitro***

122 c-Jun NH₂-terminal kinase (JNK) signaling is required for the initial entry of cortical interneurons
123 into the cortical rudiment and the tangential progression of interneurons in migratory streams
124 (Myers et al., 2020; Myers et al., 2014). In the current study, we examined the role that JNK
125 plays in the migratory dynamics of individual interneurons. To study interneuron migration at
126 high spatial and temporal resolution, we performed live-cell confocal imaging of medial
127 ganglionic eminence (MGE) explant cortical cell co-cultures. MGE explants from embryonic day
128 14.5 (E14.5) *Dlx5/6-Cre-IRES-EGFP (Dlx5/6-CIE)* positive embryos were cultured on top of a
129 *Dlx5/6-CIE* negative (wild type, WT) monolayer of dissociated cortical cells for 24 hours (Fig.
130 1A). Cultures were treated with 20 μM SP600125, a pan JNK inhibitor (Bennett et al., 2001), or
131 vehicle control and immediately imaged live for 12 hours (Fig. 1A). At the beginning of imaging
132 (Time 0), the field of view was placed at the distal edge of interneuron outgrowth (Fig. 1B-C).

133 Many control interneurons migrated into the field of view by 12 hours of imaging (Fig. 1B; Movie
134 1), but SP600125-treated cells failed to progress through the frame and appeared to move
135 slower (Fig. 1C; Movie 2). To assess potential differences in their migratory dynamics, we
136 tracked individual cells in order to evaluate how JNK inhibition affects interneuron migration on a
137 single cell level (representative cell tracks in Fig. 1B, C). The migratory speeds of JNK-inhibited
138 interneurons were significantly slower than controls, including the maximum (values =
139 mean \pm s.e.m.; control: 132.28 \pm 4.25 μ m/hour; SP600125: 78.02 \pm 1.69 μ m/hour; $p=1.68\times 10^{-10}$),
140 mean (control: 54.62 \pm 2.54 μ m/hour; SP600125: 26.48 \pm 0.94 μ m/hour; $p=1.68\times 10^{-9}$), and minimum
141 (control: 6.64 \pm 0.91 μ m/hour; SP600125: 1.96 \pm 0.21 μ m/hour; $p=7.17\times 10^{-5}$) migratory speeds (Fig.
142 1D). While JNK-inhibited interneurons migrated slower, speed variation, which is the ratio of
143 track standard deviation to track mean speed was significantly increased in SP600125-treated
144 conditions (control: 0.62 \pm 0.02; SP600125 0.76 \pm 0.02; $p=0.00019$; Fig. 1E). Due to the decrease
145 in migratory speed, the normalized migratory displacement of SP600125-treated interneurons
146 was also significantly reduced compared to control interneurons (control: 156.93 \pm 10.37 μ m;
147 SP600125: 75.76 \pm 4.04 μ m; $p=4.73\times 10^{-7}$; Fig. 1F). Despite these changes in overall migratory
148 dynamics, JNK-inhibited interneurons displayed no change in their migratory straightness
149 (control: 0.71 \pm 0.03; SP600125: 0.68 \pm 0.02; $p=0.45$; Fig. 1G). Collectively, these data suggest
150 that JNK inhibition alters the migratory behavior of MGE interneurons by reducing their
151 migratory speed and the overall displacement of their migratory trajectories.

152 ***JNK signaling regulates branching dynamics of migrating MGE interneurons***

153 Migrating cortical interneurons repeatedly extend and retract leading process branches to sense
154 extracellular guidance cues and establish a forward direction of movement (Bellion et al., 2005;
155 Polleux et al., 2002; Yanagida et al., 2012). Leading process branching normally occurs through
156 two mechanisms: growth cone splitting at the distal end of the leading process, and formation of
157 interstitial side branches along the length of the leading process (Lysko et al., 2011; Martini et
158 al., 2009).

159 To determine if JNK inhibition effected leading process morphology, we first measured the
160 length of leading processes over time from live-imaged *Dlx5/6-CIE* positive MGE interneurons.
161 Maximum (control: 84.96 \pm 4.45 μ m; SP600125: 85.14 \pm 4.02 μ m/hour; $p=0.977$), mean (control:
162 60.39 \pm 2.88 μ m; SP600125: 60.14 \pm 2.56 μ m; $p=0.947$), or minimum lengths (control:
163 37.40 \pm 2.47 μ m; SP600125: 37.81 \pm 2.72 μ m; $p=0.912$) of leading processes of SP600125-treated
164 interneurons remained unchanged (Fig. 2C). However, when we analyzed the dynamic

165 behavior of leading processes, significant differences were found between interneurons in
166 control and SP600125-treated conditions (Fig. 2; Movies 3-4). In control conditions, migrating
167 MGE interneurons show frequent initiation of new branches from growth cone splitting at the tip
168 of their leading processes (Fig. 2A; Movie 3, Clip 1). In JNK-inhibited conditions, interneurons
169 still underwent growth cone splitting, but the frequency appeared to be reduced (Fig. 2B; Movie
170 4, Clip1). When we measured the rate of growth cone splitting, JNK-inhibited interneurons had a
171 statistically significant reduction compared to controls (control: 1.83 ± 0.19 splits/hour; SP600125
172 1.15 ± 0.20 splits/hour; $p=0.02$; Fig. 2D). In addition to branching from their growth cones, MGE
173 interneurons extend and retract interstitial side branches from their leading processes. To
174 determine whether JNK inhibition impacted the frequency and duration of interstitial branching,
175 we measured the rate in which new side branches formed and determined the amount of time
176 each newly generated branch was retained. Both control and SP600125-treated interneurons
177 extended side branches at similar frequencies (control: 1.33 ± 0.22 branches/hour;
178 SP600125: 1.37 ± 0.19 branches/hour; $p=0.91$; Fig. 2 E-G; Movies 3-4, Clip 2). However, the
179 duration of time in which *de novo* side branches persisted was significantly reduced in
180 interneurons treated with JNK inhibitor (control: 28.77 ± 2.53 min; SP600125: 21.19 ± 1.76 min;
181 $p=0.02$; Fig. 2H).

182 Here, we found that initiation of branching from growth cone splitting was significantly reduced
183 during JNK inhibition. JNK-inhibited interneurons also formed side branches at similar rates, but
184 these branches were shorter-lived than controls. Our data indicate that JNK influences
185 branching dynamics of migratory MGE interneurons by regulating the rate of growth cone
186 splitting, and by promoting the stability of newly formed side branches.

187 ***Acute loss of JNK signaling impairs nucleokinesis and cytoplasmic swelling dynamics of*** 188 ***migrating MGE interneurons***

189 Since pharmacological inhibition of JNK signaling disrupted the overall migratory properties and
190 leading process branching dynamics of MGE interneurons, we further examined the role for JNK
191 in nucleokinesis, an obligate cell biological process in neuronal migration (Bellion et al., 2005;
192 Yanagida et al., 2012). To closely examine the movement of interneuron cell bodies during
193 migration, we imaged cultures at higher spatial and temporal resolution and analyzed the effect
194 of JNK inhibition on nucleokinesis (Fig. 3). Time-lapse recordings show that under control
195 conditions, a single cycle of nucleokinesis starts with the extension of a cytoplasmic swelling
196 into the leading process and ends with the translocation of the cell body into the swelling (Fig.

197 3A; Movie 5, Clip 1). Although JNK-inhibited interneurons still engaged in nucleokinesis, the
198 distance and kinetics of individual nucleokinesis events were disrupted (Fig. 3B; Fig. 3H; Movie
199 5, Clip 2). When we measured the mean distance that cell bodies advanced over time, JNK-
200 inhibited interneurons translocated significantly shorter distances compared to control cells
201 (control: $14.87 \pm 0.32 \mu\text{m}$; SP600125: $8.50 \pm 0.39 \mu\text{m}$; $p = 2.36 \times 10^{-10}$; Fig. 3C). Thus, while cell
202 bodies of JNK-inhibited interneurons still translocated forward into the leading process, the
203 distance of their movement was reduced.

204 Since nucleokinesis is cyclical, with the cell extending a swelling, translocating its cell body,
205 then pausing before repeating the process, we measured the rate of nucleokinesis in control
206 and JNK-inhibited conditions. Upon treatment with SP600125, interneurons completed
207 significantly fewer translocation events per hour (control: 2.50 ± 0.06 events/hour; SP600125:
208 1.73 ± 0.06 events/hour; $p = 1.92 \times 10^{-8}$; Fig. 3D). Along with this, interneurons in JNK-inhibited
209 cultures displayed longer pauses between the initiation of nucleokinesis events (control:
210 31.21 ± 1.05 min; SP600125: 40.71 ± 0.58 min; $p = 1.45 \times 10^{-7}$; Fig. 3E). Because nuclear
211 translocation is preceded by swelling extension, we measured the average distance from the
212 soma to the swelling before translocation and found that SP600125-treated interneurons did not
213 extend cytoplasmic swellings as far as controls (control: $13.13 \pm 0.38 \mu\text{m}$; SP600125:
214 $11.34 \pm 0.30 \mu\text{m}$; $p = 0.002$; Fig. 3F). Since JNK-inhibited interneurons paused for longer periods of
215 time, we asked if this was strictly due to delayed nuclear propulsion towards the swelling, or if
216 the dynamics of swelling extension were also affected. Interneurons treated with SP600125
217 displayed significantly longer lasting cytoplasmic swellings (control: 11.27 ± 0.99 min; SP600125:
218 18.31 ± 1.33 min; $p = 0.0005$; Fig. 3G), indicating that swelling duration is concomitantly increased
219 with pause duration. Finally, the frequency and amplitude of nuclear translocations that exceed
220 a minimum distance of 5 microns was notably reduced when individual control and JNK-
221 inhibited cells were compared (Fig. 3H).

222 Together, these data point to a role for JNK signaling in regulating the distance and kinetics of
223 nucleokinesis in migrating MGE interneurons, which likely contributes to the decrease in
224 migratory speed and displacement that occurs during JNK inhibition.

225

226

227 **Complete genetic loss of JNK impairs nucleokinesis and leading process branching of**
228 **migrating MGE interneurons in vitro**

229 Since acute pharmacological inhibition of JNK activity altered the dynamic behavior of migratory
230 cortical interneurons, we next asked whether genetic removal of JNK function from MGE
231 interneurons also impaired their migration. In order to genetically ablate all three JNK genes
232 from interneurons, we used mice containing the *Dlx5/6-CIE* transgene to conditionally remove
233 *Jnk1* from *Jnk2;Jnk3* double knockout embryos (*Dlx5/6-CIE;Jnk1^{fl/m};Jnk2^{-/-};Jnk3^{-/-}*). Using this
234 conditional triple knockout (*cTKO*) model, we modified our assay to determine if MGE
235 interneurons have an intrinsic genetic requirement for JNK in their migration. MGE explants
236 from *Dlx5/6-CIE+* wild type (WT) and *cTKO* brains were cultured on a WT cortical feeder layer
237 and imaged live (Fig. 4A). We tracked individual interneurons over time to assess the overall
238 migratory properties of WT and *cTKO* interneurons (Fig. 4B,C). While there were no changes in
239 migratory speed (Fig. 4D), *cTKO* interneurons exhibited greater variations in migratory speed
240 compared to WT cells (WT: 0.54 ± 0.01 ; *cTKO*: 0.59 ± 0.02 ; $p=0.02$; Figure 4E). We also found
241 that *cTKO* interneurons have shorter migratory displacements than WT interneurons (WT:
242 195.06 ± 6.80 μm ; *cTKO*: 165.99 ± 12.49 μm ; $p=0.05$; Fig. 4F). Additionally, the track straightness
243 of *cTKO* interneurons was decreased (WT: 0.77 ± 0.02 ; *cTKO*: 0.71 ± 0.02 ; $p=0.03$; Figure 4G).
244 The combination of increased speed variability and decreased migratory straightness explain
245 why *cTKO* interneurons exhibited shorter migratory displacements. Together, these data
246 indicate that *cTKO* interneurons have subtle yet statistically significant deficits in their overall
247 migratory dynamics, similar to pharmacological inhibition of JNK .

248 To determine the genetic requirement for JNK signaling in branching, we analyzed leading
249 process branching dynamics of *cTKO* and WT interneurons (Movies 6-7). *cTKO* interneurons
250 displayed a significant reduction in the frequency of growth cone splitting compared to WT
251 interneurons (WT: 1.92 ± 0.18 splits/hour; *cTKO*: 1.30 ± 0.11 splits/hour; $p=0.04$; Fig. 4H; Movie 6-
252 7, Clip 1). In addition, genetic removal of JNK signaling from interneurons resulted in no change
253 in side branch initiation (WT: 1.43 ± 0.15 ; *cTKO*: 1.32 ± 0.20 branches/hour; $p=0.66$; Fig. 4I), but
254 significant decreases in the duration that side branches persisted (WT: 25.51 ± 3.39 min;
255 *cTKO*: 17.29 ± 1.71 min; $p=0.05$; Fig. 4J; Movie 6-7, Clip 2). These data corroborate the findings
256 from our pharmacological analyses and further suggest a key role for JNK signaling in
257 controlling leading process branching dynamics.

258 Since we found alterations to overall migratory properties and branching dynamics, we next
259 analyzed migrating *cTKO* interneurons for defects in nucleokinesis. Although *cTKO* interneurons
260 engaged in nucleokinesis, the kinetics of nucleokinesis were significantly altered compared to
261 WT interneurons (Fig. 5). The average distance *cTKO* cells traveled forward during
262 nucleokinesis was significantly shorter compared to that of the WT cells (WT: $15.08 \pm 0.28 \mu\text{m}$;
263 *cTKO*: $14.16 \pm 0.26 \mu\text{m}$; $p=0.03$; Fig. 5A-C). However, unlike during acute pharmacological
264 inhibition of JNK signaling, *cTKO* interneurons displayed increased rates of nucleokinesis (Fig
265 5A, B; Movie 8). Genetic ablation of JNK signaling in migrating MGE interneurons resulted in
266 increased frequency of translocation events (WT: 2.76 ± 0.05 events/hour; *cTKO*:
267 3.22 ± 0.11 events/hour; $p=0.002$; Fig. 5D). While both WT and *cTKO* cells paused after the
268 completion of a nucleokinesis event (after the cell body moves into the swelling), *cTKO* cells
269 spent significantly less time pausing before they extended a new swelling (WT: 32.10 ± 0.62 min;
270 *cTKO* 27.01 ± 1.02 min; $p=0.0005$; Fig. 5E). When we measured the duration of time that
271 cytoplasmic swellings persisted, the swellings in *cTKO* interneurons were significantly shorter-
272 lived (WT: 10.47 ± 0.62 min; *cTKO*: 7.86 ± 0.19 min; $p=0.003$; Fig 5F). These data likely explain
273 why we did not observe an overall change in migratory speeds between *cTKO* and WT
274 interneurons. While *cTKO* interneurons are not migrating as far during each translocation event
275 they are initiating nucleokinesis at a faster rate, thus moving at similar speeds compared to
276 controls.

277 Collectively, our data suggest that genetic removal of *Jnk* alters the migratory behavior of MGE
278 interneurons. While the phenotypes observed with conditional removal of *Jnk* from migrating
279 interneurons was not identical to pharmacological inhibition of JNK signaling, our results
280 indicate that interneurons require *Jnk* for correct leading process branching dynamics and
281 nucleokinesis.

282 ***Subcellular localization and dynamic behavior of the centrosome and primary cilia in*** 283 ***migrating MGE interneurons depend on intact JNK-signaling***

284 The cytoplasmic swelling emerges from the cell body during nucleokinesis and contains multiple
285 subcellular organelles involved in the forward movement of cortical interneurons (Bellion et al.,
286 2005; Martini and Valdeolmillos, 2010; Yanagida et al., 2012). One organelle involved in
287 nucleokinesis is the centrosome, which translocates from the cell body into the swelling during
288 nucleokinesis. The centrosome is tethered to the nucleus through a perinuclear cage of
289 microtubules and acts to generate a forward pulling force on the nucleus during nucleokinesis

290 (Bellion et al., 2005; Umeshima et al., 2007). Disruptions in centrosome motility and positioning
291 are thought to underly nucleokinesis defects seen in other studies of neuronal migration
292 (Luccardini et al., 2013; Luccardini et al., 2015; Silva et al., 2018; Solecki et al., 2009). Since we
293 found significant defects in nucleokinesis in migrating MGE interneurons, we sought to
294 determine if centrosome dynamics were also disrupted during JNK inhibition.

295 To visualize the centrosome and study the role of JNK signaling in centrosome dynamics in
296 migrating MGE interneurons, we live-imaged *Dlx5/6-CIE+* cells expressing a red-fluorescent
297 centrosome marker, Cctn2-mCherry (Fig. 6A). In control cells, the centrosome moved correctly
298 into the cytoplasmic swelling (Fig 6B; Movie 9, Clip 1), with centrioles occasionally splitting
299 between the soma and swelling preceding nucleokinesis (Fig. 6B, frames 0:00-0:10 minutes), as
300 reported elsewhere (Bellion et al., 2005; Umeshima et al., 2007). Upon JNK-inhibition, the
301 centrosome often maintained a position near the soma regardless of the presence of a swelling
302 (Fig. 6C; Movie 9, Clip 2-3). Moreover, in many JNK-inhibited cells, the centrosome moved
303 backwards into the trailing process, even when the cell body translocated forward (Fig. 6C;
304 Movie 9, Clip 2-3). When we tracked the positioning of the centrosome over time, the
305 centrosome of JNK-inhibited cells spent significantly more time in the trailing process and less
306 time in the leading process ($P=0.0001$; Fig. 6D). Additionally, when a swelling was formed in
307 front of the soma, the centrosome of JNK-inhibited cells spent significantly less time inside of
308 the swelling than controls (control: $66.64\pm 5.99\%$; SP600125: $16.08\pm 5.52\%$ of time; $P=0.0001$;
309 Fig. 6E). When we measured the average maximal distance that the centrosome was displaced
310 from the somal front, the centrosome of JNK-inhibited interneurons maintained a significantly
311 closer position to the leading pole of the soma compared to controls (control: $9.93\pm 0.99\mu\text{m}$;
312 SP600125: $6.73\pm 0.88\mu\text{m}$; $p=0.03$; Fig. 6F). This was not surprising since the soma-to-swelling
313 distance in JNK-inhibited interneurons was decreased (Fig. 3E). However, when we compared
314 the average maximal rearward distance between the centrosome and somal front, the
315 centrosome of JNK-inhibited interneurons was significantly further behind that of
316 controls (control: $9.40\pm 0.77\mu\text{m}$; SP600125: $19.75\pm 1.94\mu\text{m}$; $p=1.48\times 10^{-5}$; Fig. 6F).

317 Since we found defects in centrosome dynamics, we wanted to determine whether primary cilia,
318 which normally extend from the mother centriole and house receptors important for the guided
319 migration of cortical interneurons (Baudoin et al., 2012; Higginbotham et al., 2012), were also
320 perturbed in interneurons following JNK-inhibition. In order to study the localization of cilia in
321 migrating interneurons, we performed live-cell confocal imaging on *Dlx5/6-CIE+* MGE cells
322 expressing Arl13b-tdTomato, a red-fluorescent cilia marker.

323 Almost identical to that of our centrosome analyses, we found significant alterations in the
324 dynamic positioning of primary cilia in migrating MGE interneurons (Fig. 7). In control cells, the
325 primary cilium moved into the cytoplasmic swelling before nuclear translocation (Fig. 7A; Movie
326 10, Clip 1). However, upon JNK inhibition, the cilium was frequently positioned in the soma and
327 often moved into the trailing process as the cell body translocated forward (Fig. 7B; Movie 10,
328 Clip 2-3). Overall, the cilia spent significantly more time in the cell soma and behind the cell in
329 the trailing process, and significantly less time in the leading process of JNK-inhibited cells
330 ($P=0.0001$; Fig. 7C). Additionally, the primary cilia in JNK-inhibited interneurons failed to spend
331 as much time in formed cytoplasmic swellings as controls (control: $73.73\pm 7.81\%$ of time;
332 SP600125: $33.85\pm 8.20\%$ of time; $P=0.0001$; Fig. 7D). When we measured the maximal distance
333 behind the somal front, the cilia of JNK-inhibited interneurons were also positioned further
334 behind the cell body than controls, matching our centrosome findings (control: $9.71\pm 1.16\mu\text{m}$;
335 SP600125: $16.09\pm 2.10\mu\text{m}$; $p=0.02$; Fig. 7E). Taken together, these data highlight a novel role
336 for JNK signaling in the dynamic movement and positioning of the centrosome and primary
337 cilium in migrating MGE interneurons.

338 DISCUSSION

339 In the present study, we demonstrated that migrating MGE interneurons rely on the JNK
340 signaling pathway to properly undergo leading process branching and nucleokinesis.
341 Pharmacological inhibition of JNK signaling in an *in vitro* assay resulted in reduced migratory
342 speed and displacement with an increase in speed variation of migrating interneurons.
343 Concomitant with these alterations in migratory properties, JNK-inhibited interneurons displayed
344 decreased initiation of branches arising from growth cone tips, decreased persistence of
345 interstitial side branches, as well as shorter, less frequent nucleokinesis events. Using a
346 conditional triple knockout (*cTKO*) mouse line to completely remove *Jnk* from MGE
347 interneurons, *cTKO* interneurons had decreased migratory displacement without reductions in
348 overall migratory speed, apparently resulting from migratory trajectories that had more variable
349 speeds and reduced track straightness compared to controls. Moreover, *cTKO* interneurons
350 displayed significant defects in leading process branching and nucleokinesis. Similar to
351 pharmacological manipulation, *cTKO* cells displayed shorter nuclear translocations, but unlike
352 JNK-inhibited interneurons, *cTKO* interneurons completed nucleokinesis at faster rates relative
353 to controls, which further explained why the overall migratory speed of *cTKO* interneurons was
354 not impaired. These results indicate that MGE interneurons have a cell-intrinsic requirement in
355 the coordination of leading process branching and nucleokinesis. Finally, we found a novel role

356 of JNK signaling in regulating the dynamic positioning of two organelles involved in
357 nucleokinesis: the centrosome and primary cilium. Centrosomes and primary cilia failed to
358 properly translocate into a leading process swelling and spent significantly more time
359 mislocalized to the trailing process of JNK-inhibited interneurons. Together, these results
360 suggest that JNK signaling is required to maintain the cellular kinetics underlying MGE
361 interneuron migration.

362 ***Cytoskeletal regulation during leading process branching and nucleokinesis of migrating*** 363 ***interneurons***

364 Leading process branching and nucleokinesis—the two main features of guided interneuron
365 migration—rely on the coordination of actomyosin and microtubule-based cytoskeletal networks.
366 Leading process branches initially form through membrane protrusions containing a F-actin
367 meshwork, which are then stabilized by microtubules to allow for the emergence of the nascent
368 branch (Lysko et al., 2014; Martini et al., 2009; Peyre et al., 2015; Spillane et al., 2011).
369 Nucleokinesis is thought to be mediated through the combination of the forward pulling forces
370 from microtubules at the front of the cell and pushing forces from actomyosin contraction at the
371 rear (Bellion et al., 2005; Martini and Valdeolmillos, 2010; Martini et al., 2009). While
372 mechanisms underlying these processes are still under investigation, several molecular
373 mediators of microtubule and actin dynamics in migrating interneurons have emerged, and
374 interestingly, have been linked to JNK signaling in other cells.

375 For instance, p27^{kip1}, a microtubule associated protein, coordinates both actomyosin contraction
376 and microtubule organization to control leading process branching and nucleokinesis in
377 migrating interneurons (Godin et al., 2012). Conditional deletion of p27^{kip1} from post-mitotic
378 interneurons resulted in slower migratory speed, increased frequency of nucleokinesis, and
379 shorter distance of translocations. Similarly, *cTKO* interneurons had shorter translocation
380 distances and increased rates of nucleokinesis. In addition, p27^{kip1} knockout interneurons
381 displayed shorter-lived side branches, similar to our findings with both pharmacological and
382 genetic loss of JNK. JNK signaling was reported to regulate p27^{kip1} phosphorylation during
383 cancer cell migration (Kim et al., 2012), suggesting a possible link between JNK signaling and
384 this molecular mediator of cellular migration.

385 Another important regulator of nucleokinesis and leading process branching is the microtubule
386 associated protein Doublecortin (Dcx; Friocourt et al., 2007; Kappeler et al., 2006), which is a
387 downstream target of JNK signaling in neurons (Gdalyahu et al., 2004; Jin et al., 2010). Cortical

388 interneurons lacking Dcx show a decreased duration of interstitial side branches, and
389 significantly shorter nuclear translocation distances with no overall changes in migratory speed
390 (Kappeler et al., 2006), similar to what we found in *cTKO* interneurons. Thus, it is possible that
391 JNK signaling fine-tunes leading process branching and nucleokinesis in cortical interneurons
392 by phosphorylating Dcx.

393 Recently, the role of the Elongator complex, specifically the enzymatic core Elp3, was found to
394 control both leading process branching and nucleokinesis through the regulation of actomyosin
395 activity (Tielens et al., 2016). MGE interneurons devoid of Elp3 displayed nucleokinesis and
396 leading process branching defects strikingly similar to our pharmacological results, including
397 decreased migratory speed, translocation frequency, nucleokinesis amplitude, and frequency of
398 growth cone splitting (Tielens et al., 2016). Moreover, the Elongator complex was found to
399 potentiate JNK activity during cellular stress in HeLa and HEK293 cells (Holmberg et al., 2002;
400 Kojic and Wainwright, 2016). This suggests that the Elongator complex may potentiate the
401 activity of JNK to phosphorylate effector proteins required for proper migration of interneurons.
402 While the exact mechanisms underlying how cytoskeletal modulators interact to control the
403 guided migration of cortical interneurons remain to be determined, JNK may be a key signaling
404 node required to coordinate these cellular behaviors.

405 ***Position and function of the centrosome and primary cilium during cortical interneuron*** 406 ***migration***

407 During the migration cycle of cortical interneurons, a cytoplasmic swelling containing two
408 interconnected organelles, the centrosome and primary cilium, extends ahead of the soma into
409 the leading process (Bellion et al., 2005; Tsai and Gleeson, 2005). Disruptions to the
410 movement, positioning, and function of these organelles are often found in interneurons with
411 migratory deficits (Baudoin et al., 2012; Higginbotham et al., 2012; Luccardini et al., 2013;
412 Matsumoto et al., 2019; Nakamuta et al., 2017).

413 Migratory olfactory bulb interneurons require DOCK7, a member of the DOCK180 family of
414 atypical Rac/Cdc42 guanine nucleotide exchange factors, for migration (Nakamuta et al., 2017).
415 Knockdown of DOCK7 led to unstable movement of the centrosome from the swelling back into
416 the cell body (Nakamuta et al., 2017), which was attributed to slower migration of olfactory bulb
417 interneurons devoid of DOCK7. We observed similar migratory deficits and disrupted
418 centrosome positioning in MGE interneurons treated with JNK inhibitor. Interestingly,

419 knockdown of DOCK7 was previously shown to reduce JNK phosphorylation during Schwann
420 cell development and migration (Yamauchi et al., 2008; Yamauchi et al., 2011).

421 Furthermore, inactivation of the cell adhesion molecule N-cadherin from MGE interneurons
422 leads to mislocalization of the centrosome to the rear of the cell body (Luccardini et al., 2013).
423 JNK-inhibition not only impeded the forward progression of centrosomes into the swelling, but
424 also led to their unobstructed movement into the trailing process. Interestingly, JNK-inhibition
425 has been reported to decrease N-cadherin levels and cellular migration of myofibroblasts (De
426 Wever et al., 2004), which suggests a potential role for JNK signaling in the regulation of N-
427 cadherin during migration. While mechanisms that control the positioning of the centrosome in
428 migrating neurons remain to be explored, JNK signaling may help synchronize the activity of cell
429 adhesion molecules, cytoskeletal proteins, and cytoplasmic machinery that are critically involved
430 in centrosome motility.

431 Finally, disruptions to ciliary proteins including Arl13b, Kif3a, and IFT88 or to the sonic
432 hedgehog (Shh) signal transduction pathway all result in cortical interneuron migratory deficits
433 (Baudoin et al., 2012; Higginbotham et al., 2012). Conditional deletion of Arl13b disrupts the
434 formation of the primary cilium from the centrosome and the localization/transport of key
435 receptors known to be critical for interneuron migration, including C-X-C motif chemokine
436 receptor 4 (Cxcr4), neuregulin-1 receptor (ErbB4), and the Serotonin Receptor 6 (5-Htr6)
437 (Higginbotham et al., 2012; Riccio et al., 2009; Wang et al., 2011). Dominant negative
438 knockdown of Kif3a, a molecular motor required for cilium-specific Shh signal transduction,
439 results in rearward movement of the centrosome of migrating olfactory bulb interneurons
440 (Matsumoto et al., 2019), suggesting that functional primary cilia are necessary for the proper
441 localization of the centrosome-cilium complex. Thus, cortical interneurons may require the
442 function of signal transduction machinery inside the primary cilium for the centrosome-cilium
443 complex to localize correctly, and to sense and respond to environmental guidance cues that
444 promote directed migration of interneurons. Additionally, cortical interneurons lacking Arl13b
445 exhibited leading process branching defects, suggesting that the primary cilium may have
446 cytoskeletal functions along with its role in transduction of guidance signals (Higginbotham et
447 al., 2012). Here, we provided evidence that JNK signaling is required for the proper positioning
448 of the primary cilium during MGE interneuron migration. Future studies are needed to determine
449 whether inhibition of JNK signaling impairs the localization of centrosome and cilia by disrupting
450 the function of ciliary proteins such as Kif3a, and whether mislocalized cilia can compromise the
451 guided migration of cortical interneurons *in vivo*.

452 ***Cellular influences of JNK signaling during cortical interneuron migration***

453 Our work here has shown that the proper cellular mechanics of MGE interneuron migration
454 depend on the JNK signaling pathway. Loss of JNK function disrupted leading process
455 branching and nucleokinesis of MGE interneurons and led to significant alterations of their
456 migratory properties. The requirement of JNK in interneuron migration could be multifactorial,
457 however, and regulate interneuron migration through intrinsic mechanisms, extrinsic
458 mechanisms, or both. Since SP600125 treatment inhibits JNK function in all cells of the MGE
459 explant cortical cell co-culture assay, we cannot exclude the possibility that JNK inhibition
460 disrupts cell-cell interactions between interneurons and the cortical feeder cells on which they
461 are grown. To determine whether migrating MGE interneurons have a cell-autonomous
462 requirement for JNK signaling, we genetically removed *Jnk* from interneurons and cultured them
463 on WT cortical cells. Although we found migratory deficits in *cTKO* interneurons that were
464 indicative of an intrinsic function for JNK, the deficits we uncovered were somewhat distinct from
465 pharmacological experiments, suggesting that there may be additive effects when JNK is
466 simultaneously removed from both populations of cells. Both pharmacological inhibition and
467 genetic removal of *Jnk* resulted in consistent leading process branching phenotypes with
468 decreased growth cone splitting and short-lived interstitial side branches. However, when we
469 analyzed nucleokinesis, the kinetics of movement were opposite: JNK-inhibited cells completed
470 nucleokinesis at slower rates, whereas *cTKO* cells completed at faster rates. These data imply
471 that cortical interneuron migration is dependent on both intrinsic and extrinsic requirements for
472 JNK signaling, as suggested from recent *in vivo* and *ex vivo* experiments (Myers et al., 2020).
473 While the exact mechanisms that cortical interneurons utilize to navigate their environment
474 remain to be fully elucidated, we have found that JNK signaling exerts fine-tune control over cell
475 biological processes required for proper interneuron migration.

476 ***Conclusions***

477 Using a combination of pharmacological and genetic approaches, we found a novel requirement
478 for JNK signaling in MGE interneuron leading process branching and nucleokinesis. Our
479 findings are also the first to implicate the JNK signaling pathway as a key intracellular regulator
480 of the dynamic positioning of multiple subcellular organelles involved in interneuron migration.
481 The exact molecular mechanisms controlling JNK signaling in interneuron migration remain to
482 be determined. Therefore, identifying the upstream activators and downstream targets of JNK

483 signaling will provide further insight into the role of JNK signaling in cortical development and
484 disease.

485 **MATERIALS AND METHODS**

486 ***Animals***

487 Animals were housed and cared for by the Office of Laboratory Animal Resources at West
488 Virginia University (Morgantown, WV, USA). Timed-pregnant dams (day of vaginal plug =
489 embryonic day 0.5) were euthanized by rapid cervical dislocation at embryonic day 14.5 (E14.5)
490 and mouse embryos were immediately harvested for tissue culture. CF-1 (Charles River;
491 Wilmington, MA, US) or C57BL/6J dams (Stock # 000664; The Jackson Laboratory; Bar
492 Harbour, ME, USA) were crossed to hemizygous *Dlx5/6-Cre-IRES-EGFP* (*Dlx5/6-CIE*; Stenman
493 et al., 2003) males maintained on a C57BL/6J background to achieve timed pregnancies at
494 E14.5. To generate JNK triple knockout embryos at E14.5, *Jnk1^{fl/fl}*; *Jnk2^{-/-}*; *Jnk3^{-/-}* dams were
495 crossed to *Dlx5/6-CIE*; *Jnk1^{fl/+}*; *Jnk2^{-/-}*; *Jnk3^{+/-}* males maintained on a C57BL/6J background. All
496 animal procedures were performed in accordance to protocols approved by the Institutional
497 Animal Care and Use Committee at West Virginia University.

498 ***MGE explant cortical cell co-culture***

499 8-well chamber coverslip slides (Thermo Fisher 155411) were coated with a solution of poly-L-
500 lysine (Sigma P5899) and laminin (Sigma L2020) diluted in sterile water (Polleux and Ghosh,
501 2002), incubated overnight at 37°C with 5% CO₂, and rinsed with sterile water prior to cell
502 plating. E14.5 *Dlx5/6-CIE+* and *Dlx5/6-CIE-* embryos were sorted by GFP fluorescence and
503 dissected in ice-cold complete Hank's Balanced Salt Solution (cHBSS; Tucker et al., 2006).
504 Cortices were dissected from the negative brains and pooled together for dissociation (Polleux
505 and Ghosh, 2002). After dissociation, 250µL of cell suspension diluted to 1680cells/µL was
506 added to each well and allowed to settle for 2 hours. MGE explants were dissected from GFP+
507 brains and plated on top of cortical cells. Cultures were grown for 24 hours before treatments
508 and live imaging. Two E14.5 timed-pregnant dams were used for each genetic experiment.
509 *Dlx5/6-CIE+* and *Dlx5/6-CIE-* embryos were obtained from a *Dlx5/6-Cre-IRES-EGFP* x
510 C57BL/6J cross, while *cTKO* embryos were obtained by crossing a *Dlx5/6-CIE*; *Jnk1^{fl/+}*;
511 *Jnk2^{-/-}*; *Jnk3^{+/-}* male to a *Jnk1^{fl/fl}*; *Jnk2^{-/-}*; *Jnk3^{-/-}* dam. MGE explants from *Dlx5/6-CIE+* WT and
512 *cTKO* embryos were dissected and plated into separate wells containing a monolayer of *Dlx5/6-*
513 *CIE-* WT cortical cells. Cultures were grown 24 hours prior to live imaging.

514 ***Electroporations***

515 Intact ventral forebrains were microdissected from *Dlx5/6-CIE+* embryos and placed on thin
516 slices of 3% low-melting point agarose (Fisher BP165-25) in cHBSS. Agar slices containing
517 ventral forebrain tissue were placed onto a positive genepaddles electrode (5x7mm; Harvard
518 Apparatus Inc #45-0123; Holliston, MA, USA) from a BTX ECM 830 squarewave electroporation
519 system under a stereo microscope. Endotoxin-free plasmid DNA (1-3 mg/ml) for *Cetn2-mCherry*
520 and *Arl13b-tdTomato* (gift from Dr. Eva Anton) was injected into the MGE with a picospritzer
521 (6ms/spritz; General Valve Picospritzer II), a negative genepaddles electrode (5x7mm; Harvard
522 Apparatus Inc #45-0123) containing a droplet of cHBSS was lowered to the tissue, and
523 electroporated (5 x 60mV/5ms pulse length/200ms interval pulses). Electroporated MGE
524 explants were then dissected, plated as above, and grown for 48 hours before imaging.

525 ***Live Imaging Experiments***

526 Cultures were treated with pre-warmed 37°C serum-free media containing a 1:1000 dilution of
527 DMSO for vehicle control or 20 µM SP600125 pan-JNK inhibitor (Enzo Life Sciences BML-
528 EI305-0010; Farmingdale, NY, USA) and immediately transferred to a Zeiss 710 Confocal
529 Microscope with stable environmental controls maintained at 37°C with 5% humidified CO₂.
530 Multi-position time-lapse z-series were acquired at 10-minute intervals over a 12-hour period
531 with a 20X Plan-Apo objective (Zeiss; Oberkochen, Germany) for overall migration analysis,
532 nucleokinesis distance, and swelling distance measurements. For measurements requiring
533 higher temporal and spatial resolution, such as swelling duration, branch dynamics, and
534 visualization of subcellular structures in electroporated cells, cultures were imaged using multi-
535 position time-lapse z-series at 2-2.5 minute intervals over a 4-10 hour period with a 40X C-
536 apochromat 1.2W M27 objective (Zeiss; Oberkochen, Germany).

537 ***Analysis of Live Imaging***

538 4D live imaging movies were analyzed using Imaris 9.5.1 (Bitplane; Zürich, Switzerland)
539 software. Movies collected at 20X were evaluated in the first 12 h of each recording. Individual
540 interneurons were tracked for a minimum of 4 h. Tracks were discontinued if a cell remained
541 stationary for 60 contiguous minutes, or if the tracked cell could no longer be unambiguously
542 identified. All tracks from each movie were averaged together for dynamic analyses. Cortical
543 interneurons were tracked using the Spots feature of Imaris to capture migratory speed,
544 distance, displacement, and track straightness data. Displacement was normalized to the

545 minimum track length of 4 h. Data sets were acquired from a minimum of four experimental
546 days with genetic experiments containing 5 conditional triple knockout (*cTKO*) embryos.
547 Pharmacological swelling duration data was obtained from movies collected over 4 experimental
548 days. Genetic swelling duration was obtained from 3 experimental days with 3 *cTKO* embryos.
549 The minimum criteria for an interstitial side branch to be included in our analysis was as follows:
550 the cell had to remain in frame for a minimum of 3 hours, an interstitial side branch had to
551 persist for a minimum of 10 minutes, and the branch could not become the new leading
552 process. Two-tailed unpaired Student's *t* tests were used to determine statistical differences
553 between groups.

554 For electroporation experiments, cultures were imaged at 40X and cells were selected for
555 centrosome and cilia analyses under the following criteria: the cell remained in frame for a
556 minimum of 1 hour, the cell displayed low to moderate expression levels of the construct
557 (without additional expression of aggregated fluorescent protein), and the cell was discernable
558 from surrounding cells. Centrosome and ciliary distance from the front of the cell body, and
559 localization were manually tracked and recorded using Imaris software. Two-way Anova
560 followed by Fisher's LSD post-hoc analyses were performed to determine statistical differences
561 for organelle distribution analyses (Prism Version 8 using GraphPad Software; San Diego, CA,
562 USA). Statistical significances were determined by χ^2 test for the presence of absence of
563 organelles to a formed swelling over time (Prism Version 8 using GraphPad Software; San
564 Diego, CA, USA). Two-tailed unpaired Student's *t* tests were used to determine statistical
565 differences between groups for distance measurements. Confocal micrographs were uniformly
566 adjusted for levels, brightness, and contrast in Imaris for movie preparation, and Adobe
567 Photoshop for figure images.

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574 **COMPETING INTERESTS**

575 No competing interests declared.

576 **AUTHOR CONTRIBUTIONS**

577 Conceptualization: S.E.S. and E.S.T.; Methodology: S.E.S. and E.S.T.; Formal analysis: S.E.S.,
578 and N.K.C.; Investigation: S.E.S.; Writing: S.E.S., and E.S.T.; Visualization: S.E.S.; Supervision:
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800 **FIGURE LEGENDS**

801 **Figure 1. JNK signaling regulates the dynamic migratory properties of MGE interneurons.**

802 A. Schematic diagram of MGE explant cortical cell co-culture assay with pharmacological
803 inhibition of JNK signaling. B-C. Individual cell tracks (pseudo-colored by time) from four
804 interneurons in control (B) or 20 μ M SP600125 (C) treated cultures imaged live for 12 hours. D-
805 G. Quantification of interneuron migratory properties revealed significant disruptions in migration
806 speed (D), speed variation (E), and displacement (F), but not straightness (G) during JNK
807 inhibition. For each condition, 10 cells were tracked from $n = 11$ movies (110 cells/condition)
808 obtained over 4 experimental days. Data are mean \pm s.e.m. **** $p < 0.0001$, *** $p < 0.001$, Student's
809 t -test. Time in hours. Scale bar: 50 μ m.

810 **Figure 2. Migrating MGE interneurons require intact JNK signaling for proper leading**

811 **process branching.** A-B. Time series depicting growth cone (GC) splitting from control (A) or
812 JNK-inhibited (B) MGE interneurons. Closed arrowhead = GC, open arrowhead = new GC
813 branch. C. Quantification of leading process length. $n=10$ cells were measured from 8
814 movies/condition obtained over 4 experimental days. D. Quantification of GC splitting frequency.
815 $n=17$ control cells from 8 movies and $n=19$ SP600125 cells from 10 movies were measured. E-
816 F. Interstitial side branching from control (E) or JNK-inhibited (F) interneurons. Closed
817 arrowhead = new side branch. G. Quantification of interstitial side branch frequency of control
818 and SP600125 treated interneurons. $n=17$ control cells from 8 movies; $n=19$ SP600125 cells
819 from 10 movies. H. Quantification of interstitial side branch duration in control and JNK-inhibited
820 conditions. $n=52$ branches from 14 control cells and 18 SP600125 cells were measured from 10
821 movies/condition. All branching data were from movies obtained over 5 experimental days. Data
822 are mean \pm s.e.m. * $p < 0.05$, Student's t -test. Time in minutes. Scale bar: 15 μ m.

823 **Figure 3. Pharmacological inhibition of JNK signaling impairs nucleokinesis in migrating**

824 **MGE interneurons.** A-B. Time series of a control (A) and SP600125-treated (B) interneuron
825 undergoing a single cycle of nucleokinesis. Closed arrowhead = leading process swelling, $n =$
826 nucleus. C-E. Cortical interneurons treated with JNK inhibitor have significantly shorter somal
827 translocation distances (C), decreased frequency of nucleokinesis (D), and increased pause
828 duration (E) compared to controls. C. Cartoon showing how the distance (d) that an interneuron
829 cell body translocates over time was measured. In each condition, 50 cells were measured from
830 $n=10$ movies obtained over 4 experimental days. F. Cartoon showing how the distance (d) that
831 a swelling extends from a cell body was measured. JNK-inhibited cells display significantly

832 decreased distance of swelling extension. G. Swelling duration is significantly increased in JNK-
833 inhibited interneurons. 43 control cells were measured from n=10 control movies and 53 treated
834 cells were measured from n=6 SP600125 movies, each obtained over 4 experimental days. H.
835 Histogram showing nuclear translocation over time for a single cell in each condition. Distance
836 traveled between two points is plotted and every movement above 5 μm (grey dashed line) is
837 considered to be a nucleokinesis event. Data are mean \pm s.e.m. ****p<0.0001, ***p<0.001,
838 **p<0.01, Student's *t*-test. Time in minutes. Scale bar: 15 μm .

839 **Figure 4. Genetic removal of JNK signaling impairs migratory properties and leading**
840 **process dynamics of MGE interneurons.** A. Diagram of MGE explant assay with *Dlx5/6-CIE+*
841 wild-type (WT) or JNK conditional triple knockout (*cTKO*) explants cultured on WT cortical
842 feeder-cells. B-C. Four individual cell tracks (pseudo-colored by time) from WT or *cTKO*
843 interneurons imaged live for 12 hours. D-G. Quantification of migratory properties reveals
844 significant disruptions in migratory speed, speed variation, displacement, and straightness
845 between control and *cTKO* interneurons. 120 WT cells were measured from n=12 control
846 movies and 130 *cTKO* cells were measured from n=13 *cTKO* movies, each obtained over 4
847 experimental days. H-I. *cTKO* interneurons have significantly decreased growth cone split
848 frequency (H) without changes in interstitial side branch frequency (I). n=11 WT cells and n=12
849 *cTKO* cells measured from 6 movies/condition collected over 4 experimental days. J. Side
850 branches from *cTKO* interneurons are significantly shorter-lived than controls. n=34 branches
851 were measured from 10 WT cells and n=28 branches were measured from 10 *cTKO* cells
852 recorded from 6 movies/condition obtained over 4 experimental days. Data are mean \pm s.e.m.
853 *p<0.05, Student's *t*-test. Time in hours. Scale bar: 50 μm .

854 **Figure 5. Genetic removal of *Jnk* disrupts nucleokinesis in migrating MGE interneurons.**
855 A. WT cortical interneuron undergoing a single nucleokinesis event. B. *cTKO* cortical
856 interneuron completing two nucleokinesis events over the same interval of time. Closed
857 arrowhead = leading process swelling, n = nucleus. C-E. *cTKO* interneurons have significantly
858 decreased translocation distance (C), increased translocation frequency (D), and decreased
859 pause duration (E) compared to WT interneurons. In each condition, 50 cells were measured
860 from n=10 movies obtained over 4 experimental days. F. *cTKO* interneurons have decreased
861 swelling duration compared to WT interneurons. 37 WT cells were measured from n=6 WT
862 movies and 38 *cTKO* cells were measured from n=6 *cTKO* movies, each obtained over 4
863 experimental days. Data are mean \pm s.e.m. ***p<0.001, **p<0.01, *p<0.05, Student's *t*-test. Time
864 in minutes. Scale bar: 15 μm .

865 **Figure 6. The subcellular localization of the centrosome is disrupted during JNK**
866 **inhibition.** A. Diagram depicting *ex vivo* electroporation of MGE tissue and subsequent culture
867 of MGE explants on cortical feeder cells. B. An interneuron expressing a fluorescently tagged
868 centrosome protein (Centrin2; Cetn2-mCherry) shows translocation of the centrosome into the
869 cytoplasmic swelling prior to nucleokinesis in control conditions. C. A Cetn2-mCherry
870 expressing interneuron treated with SP600125 shows aberrant rearward movement of the
871 centrosome into the trailing process. Arrowhead = Cetn2-mCherry. D. Quantification of
872 centrosome distribution over time (Two-way ANOVA: $F_{(2,114)} = 13.82$; $P < 0.0001$). Error bars
873 represent mean \pm s.e.m., post-hoc by Fisher's LSD *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$. E.
874 Quantification of centrosome presence in a formed swelling over time (χ^2 test; **** $P < 0.0001$). F.
875 Average maximum distance the centrosome traveled from the soma front (Student's *t*-test;
876 **** $p < 0.0001$, *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$). In each condition, $n = 20$ cells were measured
877 from 11 movies obtained over 5 experimental days. Data are mean \pm s.e.m. Time in minutes.
878 Scale bar: 15 μ m.

879 **Figure 7. Primary cilium localization is disrupted during JNK inhibition.** A. An interneuron
880 expressing a fluorescently tagged primary ciliary marker (Arl13b-tdTomato) shows translocation
881 of the primary cilium into the cytoplasmic swelling prior to nucleokinesis in control conditions. B.
882 An interneuron expressing Arl13b-tdTomato shows aberrant rearward movement of the primary
883 cilium into the trailing process when treated with SP600125. Arrowhead = Arl13b-tdTomato. C.
884 Quantification of primary cilium distribution over time (Two-way ANOVA: $F_{(2,114)} = 12.13$;
885 $P < 0.0001$). Error bars represent mean \pm s.e.m., post-hoc by Fisher's LSD *** $p < 0.001$, ** $p < 0.01$,
886 * $p < 0.05$. D. Quantification of primary cilium presence in a formed swelling over time (χ^2 test;
887 **** $P < 0.0001$). E. Average maximum distance the primary cilium traveled from the soma front
888 (Student's *t*-test; ** $p < 0.01$). In each condition, $n = 20$ cells were measured from 15 movies
889 obtained over 6 experimental days. Data are mean \pm s.e.m. Time in minutes. Scale bar: 15 μ m.

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Figure 1

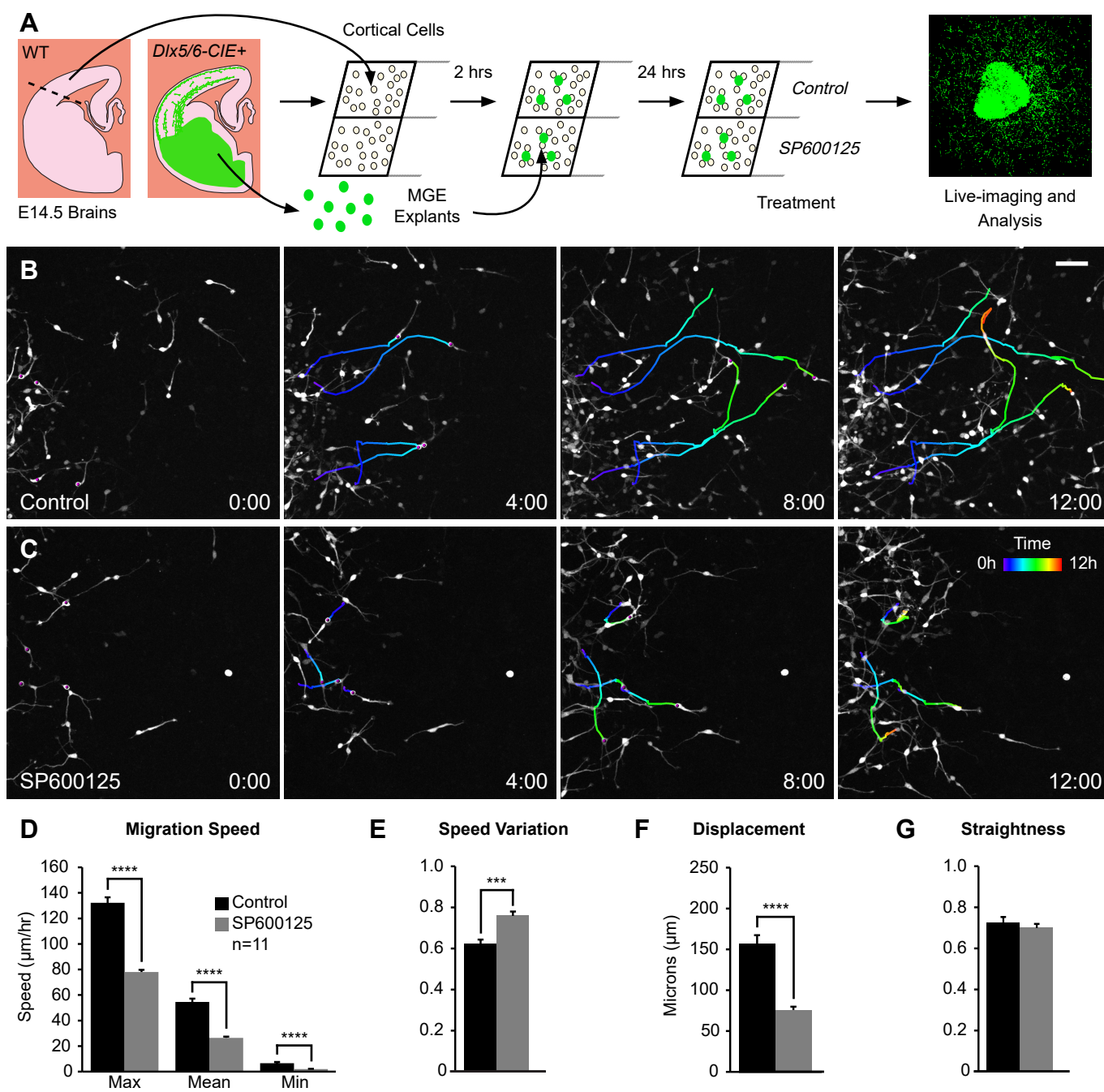


Figure 2

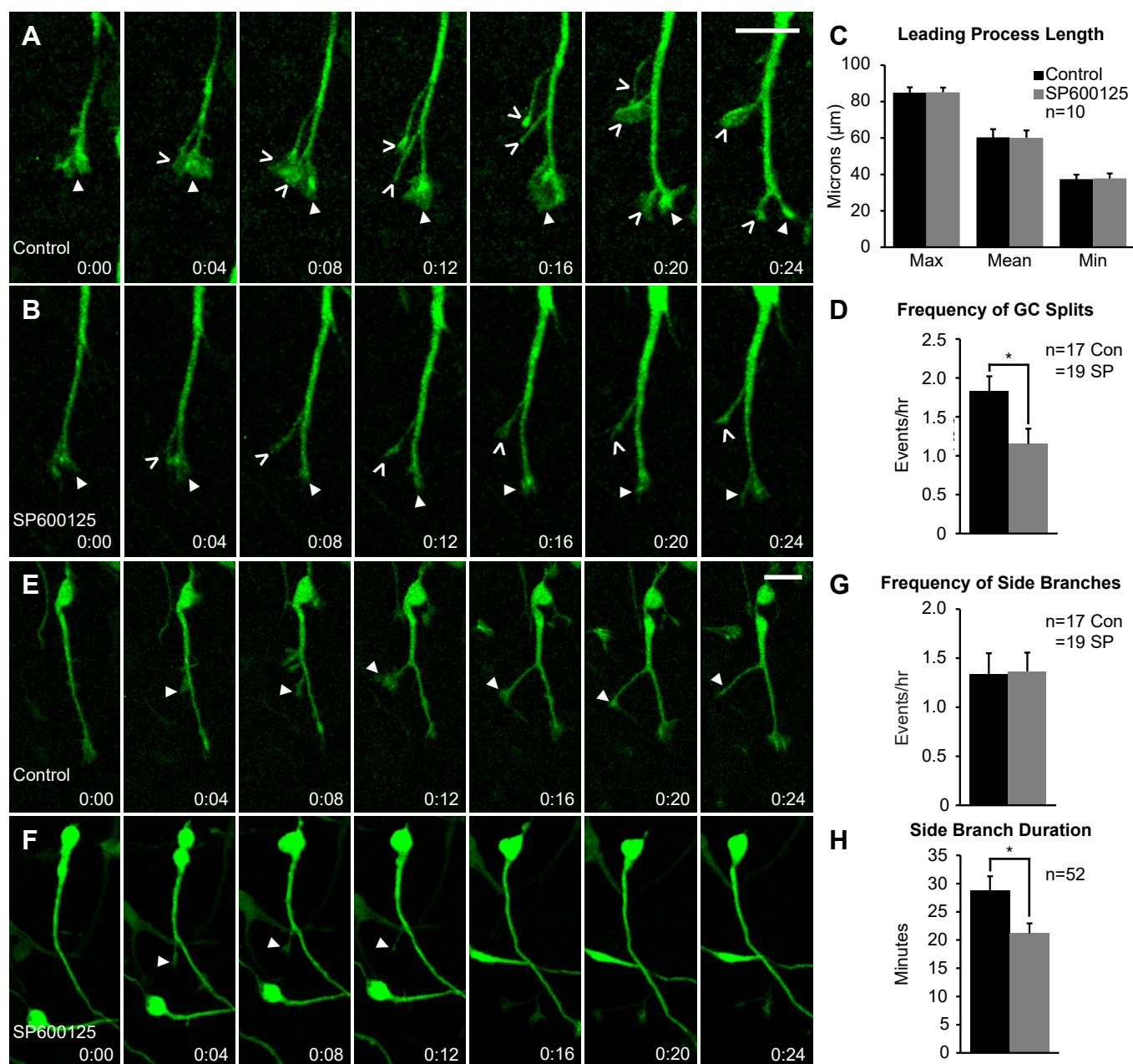


Figure 3

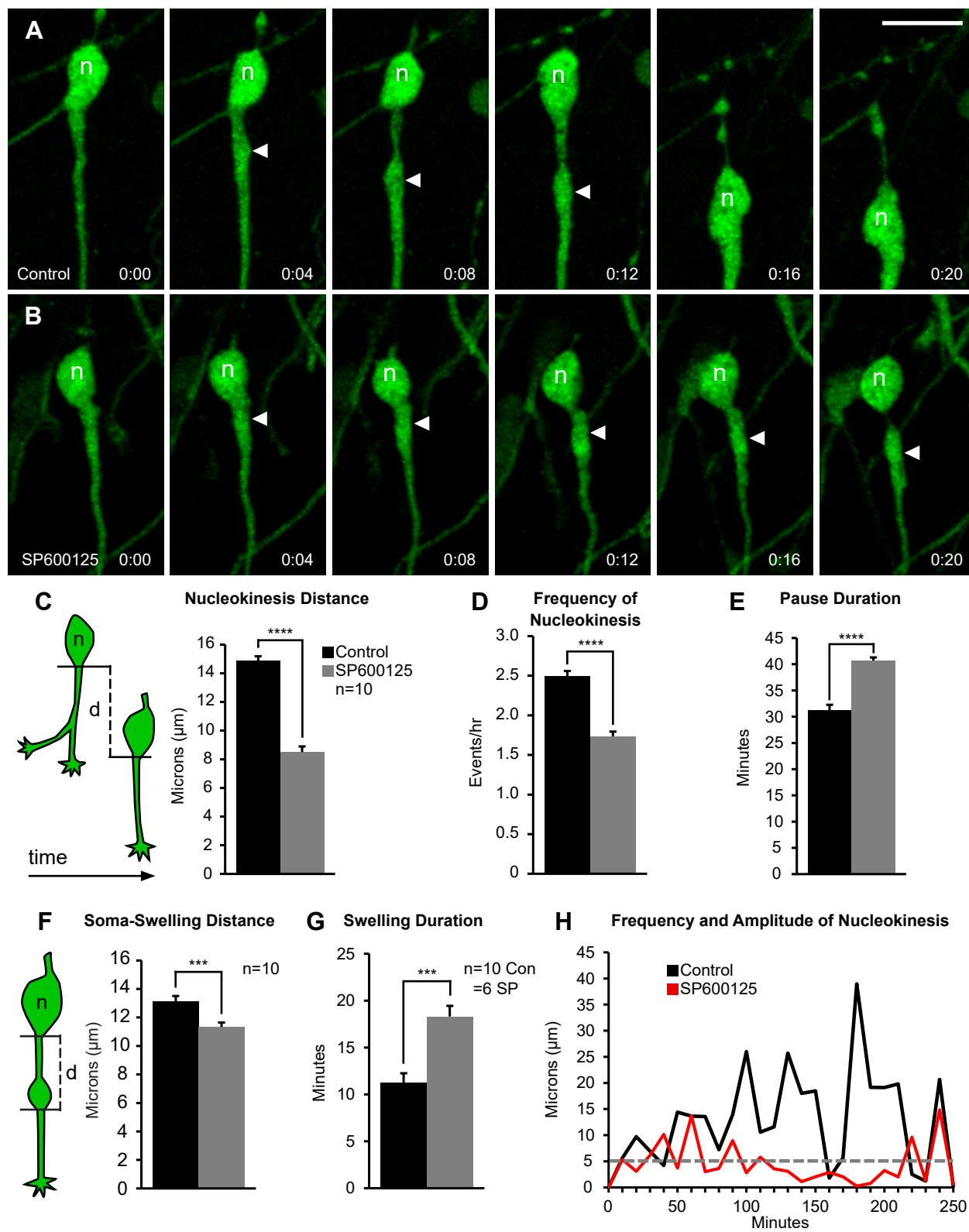


Figure 4

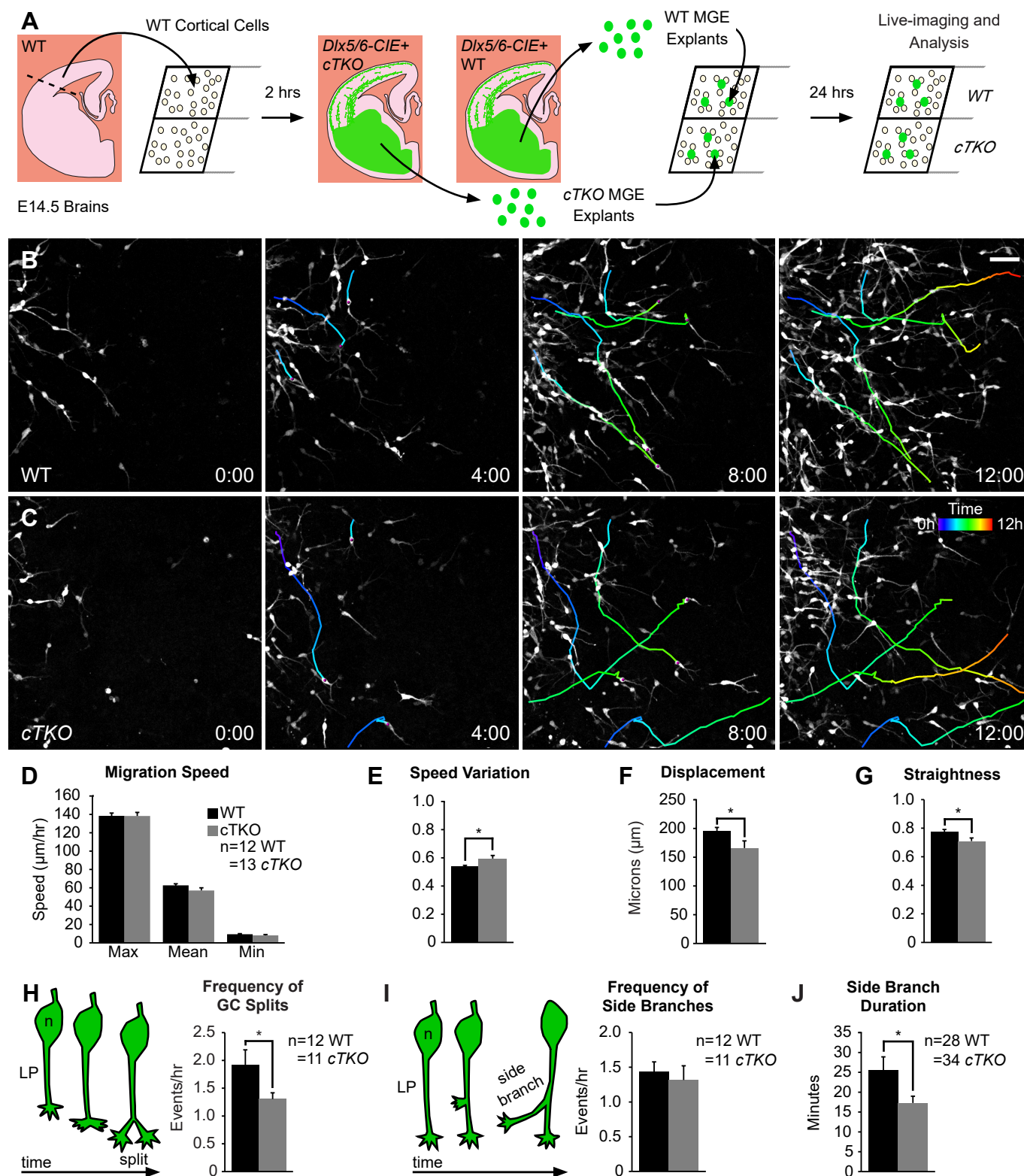


Figure 5

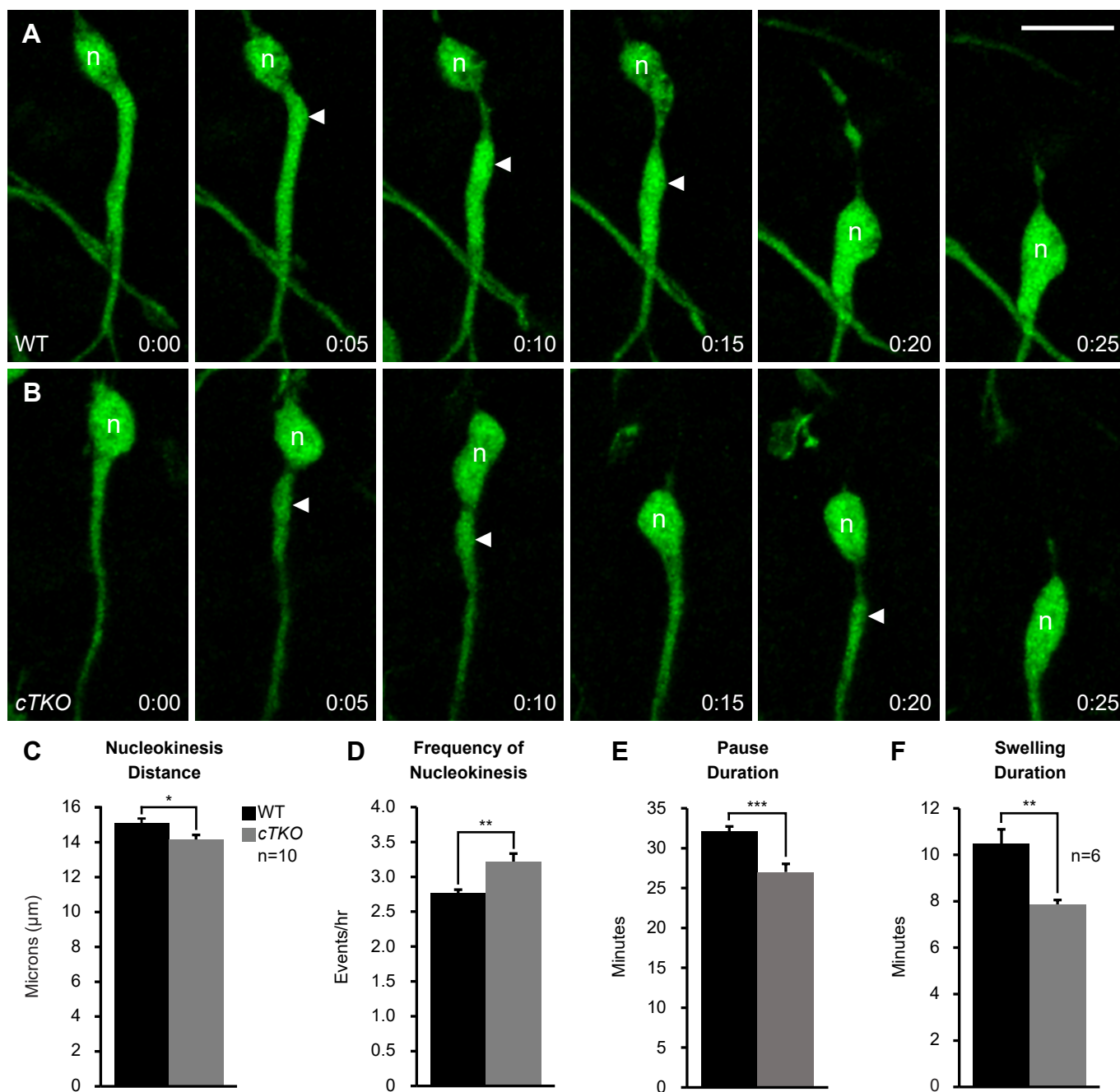


Figure 6

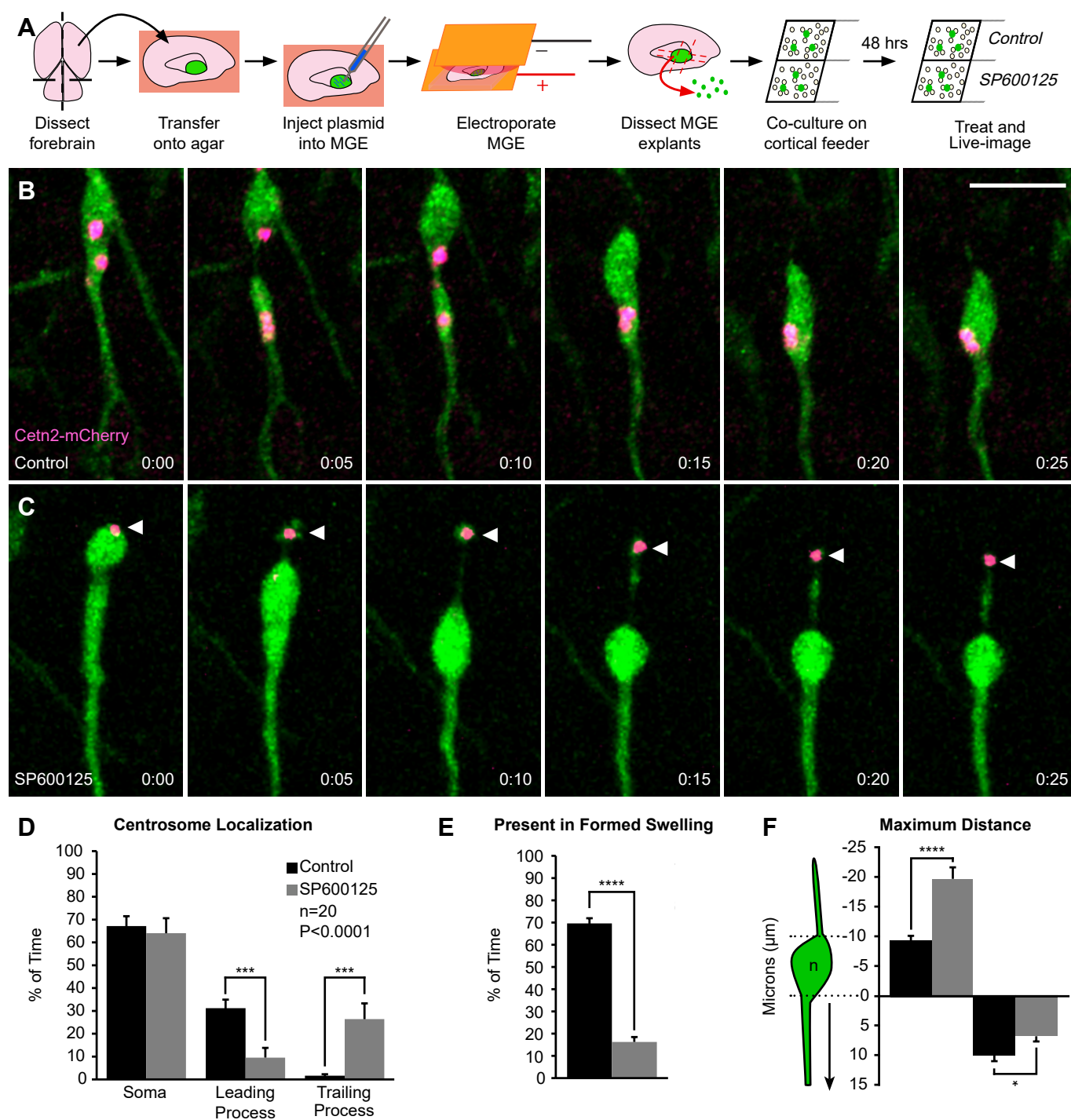


Figure 7

