Prolonged Mitosis of Neural Progenitors Alters Cell Fate in the Developing Brain

Highlights
- Mitotically delayed Magoh+/- radial glia directly produce altered progeny
- Pharmacological prolonging of mitosis recapitulates Magoh+/- progenitor phenotypes
- Prolonged progenitor mitosis causes increased neurogenic and apoptotic divisions
- Apoptosis and differentiation are mutually exclusive outcomes of mitotic delay

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In Brief
Pilaz et al. use live imaging and genetic and pharmacological approaches to demonstrate a causal relationship between prolonged mitosis of neural progenitors and fate specification in the developing brain. Their study has implications for understanding neurodevelopmental disorders such as microcephaly.
Prolonged Mitosis of Neural Progenitors Alters Cell Fate in the Developing Brain

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SUMMARY
Embryonic neocortical development depends on balanced production of progenitors and neurons. Genetic mutations disrupting progenitor mitosis frequently impair neurogenesis; however, the link between altered mitosis and cell fate remains poorly understood. Here we demonstrate that prolonged mitosis of radial glial progenitors directly alters neuronal fate specification and progeny viability. Live imaging of progenitors from a neurogenesis mutant, Magoh+/−, reveals that mitotic delay significantly correlates with preferential production of neurons instead of progenitors, as well as apoptotic progeny. Independently, two pharmacological approaches reveal a causal relationship between mitotic delay and progeny fate. As mitotic duration increases, progenitors produce substantially more apoptotic progeny or neurons. We show that apoptosis, but not differentiation, is p53 dependent, demonstrating that these are distinct outcomes of mitotic delay. Together our findings reveal that prolonged mitosis is sufficient to alter fates of radial glia progeny and define a new paradigm to understand how mitosis perturbations underlie brain size disorders such as microcephaly.

INTRODUCTION
During neurogenesis of the developing dorsal telencephalon, radial glial progenitors (RGCs) divide adjacent to the ventricle to generate new neurons and progenitors (Figure 1A) (Malatesta et al., 2000; Noctor et al., 2001). RGCs undergo symmetric proliferative divisions to self-renew and asymmetric neurogenic divisions to generate a new RGC and either a neuron or an intermediate progenitor (IP) (Franco and Müller, 2013). IPs undergo one to two self-renewal divisions before producing neurons (Kowalczyk et al., 2009). The precise balance of RGC divisions and resulting progeny ultimately influences the size and function of the mature neocortex. This is exemplified in human and mouse microcephaly models in which reduced brain size is associated with altered neurogenesis, including common phenotypes of depleted progenitors, precocious neurons, and apoptosis (Asami et al., 2011; Gruber et al., 2011; Lizarraga et al., 2010; Marthiens et al., 2013; McIntyre et al., 2012; Silver et al., 2010; Xie et al., 2013; Yingling et al., 2008). As most human microcephaly-associated genes identified to date encode mitotic regulators, aberrant progenitor division has been invoked in the etiology of this disease (Hu et al., 2014). Yet how altered progenitor mitosis impacts cell fate changes associated with microcephaly remains unclear.

Several aspects of progenitor mitosis have been implicated in neocortical fate specification. Spindle orientation is strongly linked to neuron production and survival, and generation of basally dividing cells (Asami et al., 2011; Konno et al., 2008; La-Monica et al., 2013; Lizarraga et al., 2010; Mora-Bermúdez et al., 2014; Xie et al., 2013; Yingling et al., 2008). Spindle-size asymmetry is associated with asymmetric divisions (Delaunay et al., 2014). Mitotic progression defects can trigger mitotic catastrophe, in which progenitors die prior to division (Chen et al., 2014; Novorol et al., 2013) or massive apoptosis in mice with altered centrosome number (Insolera et al., 2014; Marthiens et al., 2013). However, the specific impact of prolonged mitosis on fate specification is unclear. An important unanswered question is whether there is a direct causal relationship between prolonged progenitor mitosis and pathogenic production of neurons and apoptotic progeny in the developing brain (Figure 1B).

Here we address this critical issue by demonstrating that prolonged M phase of RGCs directly alters progenitor and neuronal cell fates in the developing brain. First we employ live imaging of Magoh+/− brain slices and primary progenitors. This mutant exhibits microcephaly and neurogenesis phenotypes, which are attributable in part to altered levels of the microtubule protein LIS1 (Silver et al., 2010). Compared with non-delayed Magoh+/− RGCs, we find that mitotically delayed Magoh+/− RGCs produce significantly fewer RGCs and more apoptotic progeny. Independently, we employ a reversible pharmacological paradigm using two distinct drugs to prolong mitosis both ex vivo and in vitro. We show that prolonged RGC mitosis alone recapitulates altered
differentiation and apoptotic fates, with only the latter occurring via a p53-dependent mechanism. Our results indicate prolonged mitosis of RGCs causes preferential generation of progeny with two mutually independent fates, differentiation and apoptosis.

RESULTS

In Vivo Cell-Cycle Analysis Reveals that Magoh−/− Radial Glia Progenitors Exhibit a Significant Mitotic Delay

We previously discovered that mice haploinsufficient for Magoh exhibit microcephaly and severe neurogenesis defects, including depleted IPs, ectopic neurons, and apoptosis (McMahon et al., 2014; Silver et al., 2010). Although Magoh is required for proper mitosis of cultured cells and melanoblasts (Silver et al., 2010, 2013), it remains unclear whether it regulates mitosis duration of neural progenitors and whether this is relevant for altered cell fate during corticogenesis. Moreover, G1- and S-phase durations are linked to RGC proliferative behavior (Arai et al., 2011; Lange et al., 2009; Pilaz et al., 2009), but the role of M phase in RGC function is poorly understood. We assessed population level cell cycle using a cumulative BrdU/EdU labeling paradigm at E13.5 (Quinn et al., 2007), when neurogenesis is markedly disrupted in Magoh+/- (Silver et al., 2010) (Figures 1C–1G). Relative mitotic index was calculated as the fraction of mitotic cells among Ki67+ cycling progenitors (Figures 1H–1J). Although total cell cycle (Tc) and S phase (Ts) were similar between control and Magoh+/- (Figures 1G), mitotic index was increased in Magoh+/- (Figure 1J, p < 0.05). These results suggest that within the normal Tc-Ts fraction, RGC mitosis is lengthened.

Using γ-TUBULIN and Hoechst staining to mark centrosomes, we quantified a significantly higher fraction of Magoh+/- in prometaphase (PM) and metaphase (M) (Figures 1K–1O). These findings were corroborated by fixed and live analyses of Magoh siRNA-treated HeLa cells, which displayed increased...
mitotic duration (Figures S1A–S1C; Movie S5). Overall, these data indicate Magoh+/− RGCs exhibit significant mitotic delay. In Magoh+/− brains, mitotic delay is concomitant with increased neuronal apoptosis (Silver et al., 2010). To examine whether apoptosis is due to a requirement of Magoh in post-mitotic neurons, we used a conditional MagohLox/Lox allele. Magoh depletion from neural progenitors using Emx1-Cre causes severe microcephaly and apoptosis (McMahon et al., 2014) (Figures S1D and S1E). In contrast, Magoh loss from post-mitotic neurons using CamKIIα-Cre caused no discernable apoptosis or altered neuron number, suggesting that Magoh is not required for post-mitotic neuron viability (Figures S1F–S1H). These experiments indicate Magoh deficiency in progenitors is a likely cause of apoptosis. Altogether, these data prompted us to further examine M phase to assess its relevance for the Magoh+/− phenotype.

**Live Imaging Reveals Magoh-Deficient Radial Glia Exhibit Prolonged Prometaphase**

We evaluated mitosis progression live using time-lapse imaging of dividing RGCs in organotypic brain slices prepared from E13.5 Tg-H2B-EGFP (control) and Tg-H2B-EGFP;Magoh+/− embryos (Figures 2A–2C; Movies S1 and S2) (Plaz and Silver, 2014). Control RGCs completed mitosis on average in 79.9 min (Figure S2A), similar to previous measurements of mitosis duration (Haydar et al., 2003). In contrast, Magoh+/− RGCs were markedly delayed in mitosis, averaging 94.7 min. RGC mitotic delay was also evident in Syto11-stained metaphase/spindles, confirming that they were mitotic (Figures S2C and S2D; Movie S4). Hence, both fixed and live analyses show that mitosis is prolonged in Magoh+/− RGCs.

Live imaging uncovered a significant delay in prometaphase/metaphase (PM+M). On average PM+M was 2.4-fold longer for Magoh+/− RGCs than control (compare 39.7 with 16.8 min) (Figure 2D). Magoh+/− RGCs exhibited a broad range of PM+M durations, with 66% dividing normally (<60 min) and 34% significantly delayed (>60 min, p < 0.001) (Figure 2E). Among those Magoh+/− delayed progenitors, 62% eventually divided (Figure S2E). During the 5-hr imaging session, we never observed mitotic catastrophe, suggesting that most delayed Magoh+/− RGCs eventually divide. Together these data indicate a significant fraction of Magoh+/− RGCs progress slower through PM+M.

We sought to further define the cellular mechanism by which Magoh levels affect mitotic progression. Defective centrosome number and maturation are associated with microcephaly and mitosis defects (Gruber et al., 2011; Insolera et al., 2014; Marthiens et al., 2013). However, Magoh-deficient RGCs showed normal centrosome distance and number as demonstrated by γ-TUBULIN staining (Figures 1K–1N and S3A). Magoh-depleted HeLa cells also showed normal centriole number and centrosome maturation as assessed with ODF2 staining (Figures S3K–S3Q). Analysis of Ndc80/Hec1, Mad1, and CENP-A staining in Magoh siRNA-treated HeLa cells indicates intact kinetochore function and microtubule attachments, respectively (Figures S3R–S3OQ). Together, these data reveal that Magoh does not impact centrosome maturation, duplication, or chromosome attachments.

Both Magoh+/− RGCs and Magoh-depleted HeLa cells showed evidence of altered mitotic microtubules. Staining for EB1, a microtubule plus-tip binding protein, revealed aberrant spindle microtubules in Magoh knockdown cells (Figures S3RR and S3SS). Acetylated TUBULIN staining of Magoh+/− RGCs also revealed altered mitotic spindle morphology (Figures S3C–S3I). Magoh+/− brains showed reduced protein levels of EG5 (Kinesins/Kif11) (Skoufias et al., 2006) and NUMA (Gaglio et al., 1995), both required for mitotic microtubules (Figures S3B–S3J). Of note, mutations in Kif11 cause human microcephaly (Ostergaard et al., 2012). Together with the previous finding that Magoh controls microtubule-assoicated LIS1 protein levels (Silver et al., 2010), these analyses indicate altered microtubule regulation, rather than centrosome dysfunction, is associated with Magoh+/− mitotic defects (Figure S3TT).

We next assessed whether prometaphase delay was associated with aneuploidy (Figures S4A–S4F). Control and Magoh+/− E13.5 RGCs showed no evidence of aneuploidy, as evaluated by DNA FISH (Figures S4A–S4D). This result was not due to death of aneuploid cells, as evidenced by normal ploidy in a p53+/− background, which prevents apoptosis (Figure S4E). Thus, in contrast to some microcephaly models (Marthiens et al., 2013), aneuploidy is not associated with Magoh+/−-delayed RGCs.

As spindle orientation is strongly implicated in neurogenesis and microcephaly phenotypes (Asami et al., 2011; Xie et al., 2013; Yingling et al., 2008), it is possible that mitotic delay causes spindle mis-orientation, or alternatively, that these could be independent phenotypes. To distinguish between these scenarios, we analyzed spindle orientation and rotation in our 3D time-lapse imaging of E13.5 mitotic RGCs (Figures 2B and 2C; Movies S1, S2, S3, and S4). Magoh+/− PM+M spindles underwent 1.6-fold more rotation than control (Figures S4G–S4J) (Haydar et al., 2003). In control anaphase progenitors, most cleavage planes were oriented between 60°–90°, as previously reported (Konno et al., 2008; Xie et al., 2013) (Figures S4K and S4L). In comparison, Magoh+/− anaphase progenitors exhibited a significantly broader range of cleavage plane orientations spanning from 0°–90° (Figures S4L and S4M). This corroborates previous analyses of fixed Magoh+/− E11.5 and E12.5 brains (Silver et al., 2010). Importantly, we observed no significant correlation between prometaphase duration and altered spindle rotation or anaphase cleavage plane orientation (Figures S4J and S4M). Thus, in E13.5 Magoh+/− brains, prolonged mitosis and altered spindle orientation can occur in individual progenitors.

**Live Imaging Clonal Analysis Reveals Magoh+/− Radial Glia Exhibit Mitotic Delay, Generate Apoptotic Progeny, and Undergo Increased Neurogenic Divisions**

Altogether, these data prompted us to examine whether Magoh+/− progenitors exhibiting prolonged mitosis produce cells with altered fates. We employed live imaging of dividing progenitors coupled with clonal analysis of direct progeny (Shen et al., 2002). Dissociated progenitors were generated from control and Magoh+/− brains expressing Dcx:DsRed as a live reporter for new neurons (Wang et al., 2007). Analysis was at E12.5, when Magoh+/− brains contain ectopic neurons and apoptosis (evidenced by cleaved caspase 3 [CC3] staining) (Silver et al., 2010) (Figures 3A and 3B). As observed in vivo,
Magoh+/– progenitors underwent 1.5-fold longer average mitoses than control (p < 0.05, data not shown). Compared with control, this same cohort of Magoh+/– progenitors generated significantly more apoptotic progeny (at least one apoptotic daughter cell) (Figure 3C; Movie S6). In vivo, Magoh+/– apoptosis is accompanied by altered numbers of neurons and progenitors, prompting us to assess the fates of viable divisions. We defined proliferative divisions as production of two progenitors (RGCs and/or IPs, both DsRed+/C0) and neurogenic divisions as generation of at least one neuron (DsRed+). In comparison to control, Magoh+/– E12.5 progenitors underwent significantly more neurogenic divisions (Figure 3D). Apoptosis and Dcx::DsRed expression were evident concurrently around 6 hr after division, making it difficult to disentangle these two fates. Altogether, these findings indicate that Magoh+/– progenitors exhibit prolonged mitosis, as well as altered proliferative, neurogenic, and apoptotic divisions.

We refined our live-imaging analysis to specifically examine RGCs. Mutant mice were bred onto a Tbr2-EGFP background (Arnold et al., 2009). At E12.5, RGCs were identified as EGFP– (93% of dividing cells), and IPs were identified as EGFP+. We performed live-imaging clonal analysis as before with two

Figure 2. Live Imaging of Embryonic Brain Slices Reveals Magoh+/– Radial Glia Progenitors Exhibiting Prolonged Mitosis
(A) Protocol utilized to visualize mitosis progression in live brain slices.
(B and C) Time-lapse images of dividing progenitors in E13.5 H2B-EGFP control (B) and Magoh+/– (C) brain slices. Dotted lines, ventricular border; arrows, progenitor and progeny; orange lines, metaphase plate orientation; blue lines, cleavage plane orientation. Time equals h:min, and mitotic stages are indicated beneath each panel. Time-lapse panels correspond to Movies S1 and S2.
(D and E) Duration of mitosis stages (D) and distribution of prometaphase (E) (PM)+metaphase (M) duration of E13.5 progenitors in indicated genotypes.
*p < 0.05, **p < 0.01, ### p < 0.001. *, t test; #, chi-square analysis. Scale bars represent (B and C) 10 μm. Error bars represent SD.

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exceptions: only RGC divisions were analyzed, and progeny were assessed by immunostaining for neurons (TUJ1⁺), RGCs (PAX6⁺), and Tbr2-EGFP for IPs (Figures 3E and 3F). Consistent with all prior experiments described above, Magoh haploinsufficient RGCs displayed 1.8-fold longer mitoses relative to control (Figure 3G). All Magoh⁺⁻ RGCs produced significantly more apoptotic progeny than control RGCs, generating either one or two apoptotic progeny to equivalent degrees (Figures S5A and S5B). Additionally, all Magoh⁺⁻ RGCs underwent more neurogenic divisions than control (Figure S5C). Thus, two independent reporters (Dcx::DsRed and TUJ1 staining) reveal that Magoh⁺⁻ progenitors/RGCs exhibit increased neuron-generating divisions.

**Generation of Aberrant Progeny in Mitotically Delayed but Not Normally Dividing Magoh⁺⁻ Radial Glia**

These findings established that total progenitor and RGC populations exhibit mitotic delay concomitant with altered progeny cell fates. We hypothesized that among Magoh⁺⁻ RGCs, those delayed in mitosis preferentially generate neurons and apoptotic progeny. To assess this, we examined progeny derived from either delayed or non-delayed RGCs. Virtually all control RGCs (98%) completed mitosis within 40 min (Figures 3H and S5D). Therefore, normal and delayed mitosis was classified as <40 or >40 min, respectively. In contrast, only 62% of Magoh⁺⁻ RGCs completed mitosis normally, and 38% were delayed (Figure 3H). This distribution of mitotic delay is remarkably similar to that observed in Magoh⁺⁻ slices (compare Figures 2E and 3H).

We then assessed production of apoptotic progeny in delayed versus non-delayed Magoh⁺⁻ RGCs. Both control and non-delayed Magoh⁺⁻ RGCs produced primarily viable progeny, with no significant difference between genotypes (Figure 3I). In sharp contrast, delayed Magoh⁺⁻ RGCs produced significantly more apoptotic progeny than non-delayed mutant RGCs (Figure 3I, p < 0.01). Strikingly, as mitotic duration increased up to 80 min or greater, there was a significant correlative increase in production of apoptotic cells (Figure 3J, p < 0.001). Consistent with this, apoptotic divisions were on average significantly longer than divisions generating viable progeny (Figure S5E). This suggests two important findings: that non-delayed Magoh⁺⁻ RGCs do not contribute significantly to apoptosis in Magoh⁺⁻ E12.5 brain and that the probability of Magoh⁺⁻ RGCs generating non-viable progeny increases proportionally with mitotic duration.

We used the same strategy to assess neuron and progenitor production from mitotically delayed Magoh⁺⁻ RGCs. Relative to control and non-delayed Magoh⁺⁻ RGCs, mitotically delayed Magoh⁺⁻ RGCs exhibited striking and significant increases in neuron generation and proportionate decreases in RGC production (Figures 3K and 3L, p < 0.001). Increased neurons were a result of more asymmetric neurogenic Magoh⁺⁻ divisions, and not increased symmetric neurogenic divisions or indirect (IP producing) divisions (Figures 3L and S5G–S5I). Importantly, as seen for apoptosis, as Magoh⁺⁻ RGC mitosis duration lengthened, the probability of producing neurons significantly increased (Figures 3M and S5F).

We also assessed the potential contribution of non-delayed Magoh⁺⁻ RGCs for neuron production. Distinguishing proliferative and neurogenic divisions into distinct subtypes, including RGC/IP generating divisions, revealed no significant differences between non-delayed control and Magoh⁺⁻ RGCs (Figures 3L and S5G–S5I). Together with the prior data, this indicates that non-delayed RGCs show no increased propensity for differentiation or apoptosis. As Magoh is a RNA binding protein, it is possible that delayed Magoh⁺⁻ RGCs carry other pro-neurogenic and pro-apoptotic influences or potentially accumulated damage. Regardless, live-imaging analyses clearly demonstrate that Magoh⁺⁻ RGCs show a strong and highly significant correlation between mitotic delay and altered cell fate. Therefore, we sought to independently extend our findings to challenge the idea of a causal relationship between mitotic duration and cell fate of progeny.

**Pharmacologically Induced Mitotic Delay Causes Generation of Fewer Progenitors, More Neurons, and Apoptotic Cells**

To test the hypothesis that prolonged mitosis alone can directly impact progeny fate, we established an experimental approach to reversibly delay wild-type progenitors in prometaphase. We used two traditional mitotic inhibitors, S-trityl-L-cysteine (STLC), which inhibits EG5-mediated centrosome separation, (Skoufias et al., 2006) and nocodazole, which depolymerizes microtubules (Bazzi and Anderson, 2014; Uetake and Sluder, 2010). The use of pharmacology in brain slices is well established for studying neural progenitor mitosis (Mora-Bermúdez et al., 2014; Ostrem et al., 2014; Tsai et al., 2007). E13.5 brain slices were cultured in media containing either STLC, nocodazole, or DMSO (control) for 3 hr and subsequently cultured in drug-free media for up to 14 hr (Figures 4A–4G). Compared with control, both STLC and nocodazole treatment significantly increased the number of mitotic cells (Figures 4A–F and 4H). Upon inhibitor washout, prometaphase-arrested progenitors proceeded to anaphase within 2–3 hr in STLC-treated slices and within 1 hr in nocodazole-treated slices (Figures 4A–4F and 4H). Neither drug significantly impacted anaphase cleavage plane orientation (Figures 4I and 4J). Moreover, there was no increased aneuploidy in STLC treated brain slices (Figures 4K–4N). Hence, both pharmacological treatments reversibly prolong prometa-phase of neural progenitors without perturbing ploidy or cleavage plane orientation.

We next coupled EdU pulse-chase with the pharmacological paradigm to assess progeny fate at a population level. We pulsed slices with EdU for 30 min to indelibly label a cohort of S-phase progenitors and subsequently applied either DMSO or a mitotic inhibitor for 3 additional hr (Figures 5A–5C, S6A, and S6B). Prior to drug treatment, the majority of EdU⁺ cells were PAX6⁺ RGCs, and 16% were TBR2⁺ IPs (Figure S6C). The 1-hr delay between EdU pulse and drug-treatment corresponds to the time required for EdU⁺ progenitors to enter mitosis (Takahashi et al., 1995). Over the 3-hr drug treatment, EdU⁺ progenitors continued to enter mitosis but were stalled at prometaphase (Figure 5C). Both drug treatments led to significantly more mitotically delayed progenitors within the EdU⁺ population compared with control (Figure 5B). At 6-hr post-washout, the localization of EdU⁺ cells in STLC treated slices was similar to control-treated slices 3 hr earlier, consistent with mitotic delay (Figure S6B).
Mitosis duration (min)

Control    Magoh +/-

![Diagram of mitosis duration](image)

Symmetric RGC divisions (%)

- Proliferative
- Neurogenic

![Graph of symmetric RGC divisions](image)

Division subtypes

- Proliferative
- Asymmetric neurogenic (direct)
- Symmetric neurogenic (direct)

![Graph of division subtypes](image)

Neuron precursor (RGC or IP) (Dcx::DsRed+)

![Diagram of neuron precursor](image)

Mitosis duration (min)

Control    Magoh +/-

![Graph of mitosis duration](image)

Neurogenic RGC divisions (%)

- Proliferative
- Asymmetric neurogenic (direct)
- Symmetric neurogenic (direct)

![Graph of neurogenic RGC divisions](image)

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14-hr post-washout, EdU+ cells were located in the basal VZ and SVZ/CP layers (Figures 5D–5O and S6B). Given cell cycle and migration kinetics, this population corresponds to progeny of RGCs and IPs (Takahashi et al., 1995). Based on published cell-cycle measurements of progenitors (Arai et al., 2011; Takahashi et al., 1995), we can infer that by 14-hr post-washout, new EdU+ progeny do not have time to complete a second round of mitosis. Thus, a significant proportion of EdU+ cells is direct progeny of mitotically delayed progenitors.

We next assessed apoptosis of EdU+ cells 14-hr post-washout following progenitor mitotic delay. STLC- and nocodazole-treated slices had 21-fold and 8-fold higher respective fractions of apoptotic (CC3+) EdU+ cells compared with control (Figures 5D–5P). Apoptotic cells were not detectable until 6-hr post-washout, well after the drug was absent from the media and after progenitors had exited mitosis, arguing against global cytotoxicity (Figure S6G). Consistent with the specificity of apoptosis, the majority of CC3+ cells were EdU+ in both STLC and nocodazole-treated slices (91% and 85%, respectively) (Figure S6E). Altogether these pulse-chase experiments demonstrate that prolonged prometaphase is associated with production of more apoptotic cells, primarily in progeny of mitotically delayed progenitors.

We next assessed neuronal and precursor fates following mitotic delay. We quantified the proportion of EdU-positive cells at 14-hr post-washout as neurons, RGCs, or IPs using TUJ1, Pax6, and Tbr2 staining, respectively (Figures 5D–5Q, 5S, 5S–5X, S6D, and S6F). Strikingly, compared with control, prometaphase delay resulted in 1.5-fold more neurons, evident with either STLC or nocodazole treatment (Figure 5Q). Concomitant with the increase of neurons, we observed a significant 25% reduction in IPs (EdU+Tbr2+) with either STLC or nocodazole (Figure 5W). RGCs (EdU+Pax6+Tbr2–) were significantly reduced in STLC, but not in nocodazole (Figure 5X). Compared with STLC, nocodazole induces shorter mitotic arrest, which could explain the reduced impact on RGCs. It is possible these overall population changes could be due to preferential progenitor apoptosis. However, apoptosis was evident in equivalent fractions of both EdU+ neurons and EdU+ progenitors, suggesting that it impacts both populations to a similar extent (Figure 5R). In sum, these data show that prometaphase delay produces imbalanced numbers of neurons and progenitors.

Prometaphase-Delayed Progenitors Generate Significantly More Neurons, Fewer Progenitors, and Apoptotic Progeny

With the observation that mitotic delay induces population level changes, we next used live imaging to directly follow individual progenitor divisions and fate of their progeny. A similar paradigm was used as described in Figure 3A, except E13.5 progenitors were treated with DMSO, STLC, or nocodazole for the first 3 hr of imaging (Figure 6A). Importantly, with this paradigm we captured co-cultured progenitors with different mitosis durations, depending on their cell-cycle state at the beginning of the drug treatment. We noted no significant apoptosis of interphase progenitors treated with either STLC or nocodazole (Figures S7A). This suggests that neither drug impacts overall cell viability, as seen in slice culture experiments. On average, control E13.5 progenitors remained in mitosis about 22 min (Figures S7B). In contrast, mitoses of nocodazole and STLC treated progenitors were on average 54 and 63 min, respectively (Figure S7B). 91% of control progenitors completed mitosis within 40 min, whereas only 45% of nocodazole and 52% of STLC-treated progenitors exhibited normal mitosis duration (Figures S6C and S7B). These data indicate that nocodazole and STLC treatments can be used to prolong mitosis and evaluate its direct impact on progeny cell fate.

With this paradigm, we examined apoptosis in progeny derived from delayed and non-delayed progenitors. Strikingly, we discovered causal relationships between mitotic delay and subsequent apoptosis for all three conditions: control, STLC-, and nocodazole-treated progenitors. The small fraction of control E13.5 progenitors displaying prolonged mitosis generated significantly more apoptotic progeny, an outcome also manifest by mitotically delayed E14.5 control progenitors (Figures 5D, S7H, and S7I). Analysis of the drug-treated progenitors showed a dramatic trend. Delayed progenitors treated with either inhibitor produced significantly more apoptotic progeny than non-delayed counterparts. Approximately 70%–80% of apoptotic divisions gave rise to two dying cells (Figures 5D and S7C). Most strikingly, as mitosis duration lengthened in drug-treated progenitors, the probability of producing non-viable progeny significantly increased (Figure 6E). For example, STLC-treated progenitors delayed for 60 min produced viable progeny in 80% of their divisions, whereas those delayed beyond 120 min underwent only 14% viable divisions. This relationship between
Figure 4. Pharmacological Inhibitors Reversibly Prolong Progenitor Prometaphase

(A–F) Hoechst-stained sections from slices treated with DMSO, STLC, or nocodazole (for 3 hr) fixed at +0 hr (A–C), or +3 hr washout (D–F). At 0 hr, STLC- and nocodazole-treated progenitors accumulate in prometaphase (brown arrows) and by 3-hr anaphase cells are evident (blue arrowheads).

(G) Timeline of slice culture experiment.

(H) Quantification of mitoses in different conditions. Stars indicate significant differences between DMSO and STLC (red) and DMSO and nocodazole (blue) conditions at each time point. Absence of a star indicates ns.

(I and J) Average spindle orientation (I) and distribution of angles (J) in DMSO-treated (gray), STLC-treated (black), and nocodazole-treated (blue) brain slices after 3 hr (STLC) and 1 hr washout (nocodazole). n = cells examined.

(K–N) DNA FISH analysis of STLC-treated brain slice (K) with higher magnification images (L and M). (N) Graph depicting lack of significant aneuploidy in STLC-treated slices.

ns, not significant; *p < 0.05, **p < 0.01, ***p < 0.001. Scale bars represent (A–F) 10 μm, (K) 20 μm, and (L and M) 5 μm. Error bars represent SD.
Figure 5. Prometaphase Delay of Progenitors Causes Ectopic Neurons, Reduced Progenitors, and Apoptosis in Embryonic Brain Slices
(A) Timeline of the slice culture experiment.
(B) Quantification of the fraction of EdU+ mitotic cells in different conditions. Stars indicate significant differences between DMSO and STLC (red) and DMSO and nocodazole (blue) conditions at each time point.
(C) Paradigm for EdU pulse-chase of E13.5 brain slices.
(D and J) Sections from E13.5 brain slices 14 hr after washout, using paradigm outlined in (A) and (C), and stained for EdU (red), CC3 (blue), and TUJ1 (green).
(E–H and K–N) High-magnification images of (D) and (J) stained for EdU (red), CC3 (white or blue), and TUJ1 (green).
(I and O) High-magnification images of (H) and (N) depicting more EdU+TUJ1+ cells (yellow) in the STLC-treated brain slices versus control.

(legend continued on next page)
mitosis duration and increased production of apoptotic progeny parallels results obtained with Magoh+/− delayed RGCs (compare with Figure 3J). For all conditions, non-delayed progenitor divisions produced almost 100% viable progeny, consistent with the notion that exposure to inhibitors alone is insufficient to induce cell death (Figures 6D and 6E).

We then assessed the causal relationship between prolonged mitosis and neuron and progenitor production. We quantified divisions as follows: proliferative P/P (both TUJ1− progenitors), neurogenic P/N (1 TUJ1+), and neurogenic N/N (both TUJ1+) (Figures 6F and S7D). A comparison of non-delayed DMSO or drug-treated progenitors showed no significant difference in differentiation or proliferation, indicating that pharmacological treatment alone in cell culture is insufficient to induce differentiation (Figure 6F). Strikingly, only those precursors with prolonged mitosis showed altered proliferative and neurogenic divisions, evident to a similar extent with either STLC or nocodazole (Figures 6F, 6G, and S7D). Moreover, as mitosis duration was prolonged using either drug, there was a concomitant increase in neurogenic divisions, as evident in Magoh+/−-delayed progenitors (Figure 6G, p < 0.001, both). Although control delayed E13.5 progenitors did not show altered neuron production, control E14.5 progenitors, which have higher neurogenic potency, did show a correlation between prolonged mitosis and increased neurogenic divisions (Figure S7J).

Finally, we queried whether these phenotypes were evident specifically in E13.5 RGCs (Figure 6H). The same causal relationship emerged as in the previous experiment. Prometaphase-delayed RGCs (DsRed-EGFP-) generated significantly more apoptotic progeny compared with control, and underwent significantly more direct neurogenic divisions (Figures 6I and 6J). We noted no notable increase in indirect IP-generating divisions (Figures 6J and S7E–S7G). Thus, both Magoh+/− and STLC-treated RGCs produce more neurons specifically via asymmetric direct neurogenic divisions. Taken together, these pharmacology results demonstrate that prolonged mitosis alters the balance of progenitor division subtypes, directly leading to increased neuron production, decreased RGC production, and increased apoptotic progeny.

**p53 Signaling Distinguishes Mitotic Mechanisms of Differentiation and Apoptosis**

These findings indicate that abnormally long mitosis induces both differentiation and cell death in progeny. However, it remains unclear whether these fates are caused by distinct molecular events and how mitotically delayed progenitors direct progeny to die or differentiate. To begin to address these questions, we examined STLC-delayed progenitors to identify candidate pathways linking prolonged mitosis to apoptosis and differentiation. In mitotically delayed immortalized cells, DNA damage response (DDR) can be increased (Garner and Pellman, 2012). Indeed, we noted significantly higher DDR in mitotically delayed but not non-delayed telophase progenitors, as assessed by γH2AX staining (Figures 7A–7E). Interestingly, depletion of Magoh either genetically or in immortalized cells also triggers increased DDR (Silver et al., 2010). p53 is induced following DDR and is also upregulated following PM-arrest (Bazzi and Anderson, 2014; Uetake and Sluder, 2010), suggesting that it could be an important pathway downstream of damage and mitotic delay. In addition, both p53 signaling and DDR are implicated in apoptosis and differentiation of various stem cell populations, including adult neural stem cells (Gil-Pe rrotin et al., 2006; Inomata et al., 2009; Wang et al., 2012).

We hypothesized that p53 induces both apoptosis and neuronal differentiation following mitotic delay. We first assessed accumulation of p53 in nuclei of STLC-treated slices, as a proxy for pathway activation (Inolsera et al., 2014). By 3 hr following pharmacological treatment, we observed a significant 50-fold increase in p53+EdU+ cells (Figures 7F–7J, S8A, and S8B). To assess the role of p53 signaling in apoptosis and differentiation, we repeated the EdU pulse-chase experiments described above in a p53−/− background, using STLC to reversibly delay progenitors in prometaphase (Figure 7K). At 14 hr after washout, we observed a complete rescue of apoptosis in STLC treated p53 null slices (Figures 7L–7P). If p53 were also required for differentiation, then we expected equivalent rescue of neuron, RGC, and IP number. However, this was not the case. Compared with DMSO-treated p53−/− slices, significantly more neurons (TUJ1+EdU+) were present in STLC-treated p53−/− slices (Figure 7Q). This indicates aberrant neuronal differentiation occurs in the absence of p53 following mitotic delay. We noted similar fold changes in the fraction of EdU+ neurons and RGCs in both the p53 WT and null backgrounds (Figure 7R). In contrast, IP depletion was rescued in the p53−/− background. This result could be due to either a role of p53 in IP apoptosis or IP generation. However, if p53 were required for IP generation, one would expect reciprocal rescue of PAX6 and TUJ1 populations, which was not evident. Along with the data in control slices (Figure 5R), these findings indicate the following outcomes of mitotic delay: RGCs are depleted due to both apoptosis and fewer RGC producing divisions; IPs are depleted primarily due to apoptosis; and neurons are increased due to more neurogenic divisions. These data are consistent with clonal analysis of drug-treated and Magoh+/− progenitors, in which mitotic delay did not dramatically impact IP generating divisions.

The above data indicate that following mitotic delay at the population level, p53 signaling is required for apoptosis, but not for neuronal differentiation. We sought to assess directly the requirement of p53 signaling in these progeny fates using clonal live imaging (Figure 8A). Similar to WT, p53-depleted progenitors exhibited mitotic delay following STLC treatment (compare Figures 6B and 6C with Figures 8B and 8C). Mitotically delayed p53−/− progenitors produced negligible apoptotic progeny, similar to slice experiments (Figures 6D, S8C, and S8D).

(P, Q, W, and X) Percentage of EdU+ cells in DMSO (gray), nocodazole (blue), and STLC (red) treated slices that are CC3+ (P), TUJ1+ (Q), TBR2+ (W), and PAX6+ (X) after 14-hr washout.

(R) Percentage of CC3+EdU+ cells in each condition that are TUJ1+ neurons (black) or TUJ1− (white).

(S–V) Section from E13.5 brain slices 14 hr after washout and stained for EdU (red), PAX6 (blue), and TBR2 (green).

ns, not significant, *p < 0.05, **p < 0.01, ***p < 0.001. Scale bars represent (D and J) 500 μm, (E–I and K–O) 20 μm, and (S–V) 10 μm. Error bars represent SD.
**A**

Control embryos

E13.5

Dissociation inhibitor

Fixation Tuj1 immuno.

Live imaging

0 +3 +18 time (h)

---

**B**

Mitosis duration (min)

0-40 40-60 60-80 80-100 100-120 >120

DMSO Noco STLC

---

**C**

Divisions (%)

0-40 40-60 60-80 80-100 100-120 >120

DMSO Noco STLC

Statistics (χ²)

DMSO vs Noco: ***

DMSO vs STLC: ***

Noco vs STLC: ***

---

**D**

Survive Apoptosis

---

**E**

Apoptosis in progeny (%)

0-40 40-60 60-80 80-100 100-120 >120

DMSO Noco STLC

---

**F**

Divisions (%)

0-40 40-60 60-80 80-100 100-120 >120

DMSO Noco STLC

---

**G**

Neurogenic divisions (%)

0-40 40-60 60-80 80-100 100-120 >120

DMSO Noco STLC

---

**H**

Division subtypes

Proliferative Direct neurogenic

Symmetric RGC

Asymmetric neurogenic

IP generating (indirect)

Symmetric neurogenic

RGC (Tbr2-EGFP; Dcx::DsRed+)

Intermediates progenitor (Tbr2-EGFP+)

Neuron (Dcx::DsRed+)

---

**I**

RGC progeny (%)

0-40 40-60 60-80 80-100 100-120 >120

DMSO STLC

---

**J**

RGC divisions (%)

0-40 40-60 60-80 80-100 100-120 >120

DMSO STLC

Symmetric RGC

Indirect IP generating

Direct asymmetric neurogenic

Direct symmetric neurogenic

---

(legend on next page)
Also reinforcing the slice findings, mitotic-delay induced differentiation was p53 independent. Compared with non-delayed p53−/− progenitors, delayed p53−/− progenitors underwent significantly more neurogenic but fewer proliferative divisions (Figure 8E). Notably, both p53 null and WT progenitors showed similar fractions of proliferative and neurogenic divisions (Figure 8E). Thus, in the absence of apoptosis, there is no significant shift in the balance of proliferative and neurogenic divisions. These data demonstrate that prolonged mitosis of progenitors induces apoptosis in a p53-dependent fashion. In contrast, p53 signaling is not involved in the induction of differentiation following prolonged mitosis (Figures 8F–8I). Altogether, these analyses reveal that apoptosis and differentiation are mutually exclusive outcomes of mitotic delay.

DISCUSSION

Control of cell-cycle progression has been linked to regulation of cortical development (Arai et al., 2011; Lange et al., 2009; Pilaz et al., 2009; Takahashi et al., 1995), yet the specific impact of prolonged M phase in specification of neural cell fates has remained enigmatic. Using genetic models and two independent pharmacological approaches, we demonstrate that prolonged mitosis directly alters balanced divisions of RGCs, generating more neurons and apoptotic progeny. Our findings indicate that prolonged mitosis in the Magoh+/− mutant contributes to aberrant neurogenesis and is likely a major contributor to microcephaly of these animals. Taken together, our study has implications for understanding the behavior of neural stem cell populations and for the etiology of microcephaly.

Prometaphase Delay Produces Common Microcephaly Phenotypes

Mitosis defects are proposed to underlie microcephaly and associated neurogenesis phenotypes. Several specific mitotic mechanisms have been invoked to explain this. Altered centrosome number and spindle orientation cause apoptosis, premature differentiation, and mislocalized progenitors (Insolera et al., 2014; Matthijs et al., 2013). Progenitor depletion and apoptosis can also arise from mitotic catastrophe in which progenitors die prior to division (Chen et al., 2014; Novorol et al., 2013). Our study highlights prolonged prometaphase as an additional mechanism for influencing cell fate, which can occur independent of aneuploidy, altered cleavage plane orientation, and centrosome integrity. Many mouse microcephaly mutants are reported to increase mitotic index, but few studies have directly analyzed mitotic duration using live imaging. Thus, it will be of interest to examine whether other microcephaly-associated genes also operate via prolonged mitosis. Moreover, we propose prolonged metaphase duration is a valuable parameter to examine in human organoid models of microcephaly (Lancaster et al., 2013).

Exposing Mechanisms by which Magoh Influences Neurogenesis

We previously demonstrated Magoh haploinsufficient mice exhibit microcephaly due to apoptosis, precocious neurons and reduced IPs; however, the cellular cause of these phenotypes was unclear (Silver et al., 2010). We can now pinpoint altered balance of neurogenic, proliferative, and viable RGC divisions as a major contributing mechanism. At E12.5, delayed Magoh+/− RGCs directly produce fewer RGCs, more neurons, and more apoptotic progeny. As mitosis duration of Magoh deficient RGCs lengthens, so does the probability of producing fewer progenitors and non-viable cells. The net result is a severe depletion of the progenitor pool, which is predicted to ultimately reduce neurons, as is evident in E18.5 Magoh−/− brains (Silver et al., 2010). Interestingly, RGCs are not depleted at the population level in microcephalic Magoh−/− cortical columns (Silver et al., 2010), suggesting that Magoh regulation of RGCs is especially relevant in the tangential dimension. Magoh is part of the exon junction RNA binding complex, components of which are associated with human neurodevelopmental phenotypes, including microcephaly (Mao et al., 2015; Pilaz and Silver, 2015). Our findings may have broad relevance for understanding roles of this complex in human brain development.

It is striking that only mitotically delayed Magoh+/− RGCs produce altered progeny. However, Magoh+/− delayed RGCs are likely to carry additional factors that influence cell fate. In support of this, for identical mitotic durations of 60–80 min, Magoh−/− E12.5 progenitors produce substantially more apoptotic progeny than STLC delayed E13.5 progenitors. What could these contributing factors be? Magoh−/− brains have increased DNA damage (Silver et al., 2010), and it is plausible that mitotic delay is a consequence of earlier damage, which could also influence progeny fate. It is also possible that mitotically delayed Magoh+/− progenitors express a slightly different repertoire of Magoh-dependent pro-differentiation determinants, such as Ngn2, Tbr2, and LIS1 protein (Silver et al., 2010). In considering these possibilities, it will be of interest to understand why only a
A subpopulation of RGCs are affected by Magoh haploinsufficiency. One potential explanation is stochastic Magoh levels but another intriguing idea is that delayed progenitors are fundamentally distinct. Indeed, heterogeneous populations of RGCs have been reported (Franco and Müller, 2013; Tyler et al., 2015). Defining transcriptomic and proteomic differences in Magoh+/- progenitors with normal and prolonged mitoses will help to address these fascinating questions.

Figure 7. p53 Signaling Distinguishes Apoptosis and Differentiation Fates of Mitotic Progenitors in Embryonic Brain Slices

(A–D) γH2AX staining of non-delayed and STLC delayed progenitors (dotted lines).

(F–I) Sections from E13.5 brain slices treated with either DMSO (F and G) or STLC (H and I) after 0-hr (F and H) or 3-hr washout (G and I) and stained for P53 (green).

(J) Quantification of nuclei with high P53 signal.

(K) Timeline of the slice culture experiment.

(L–O) Sections from E13.5 control (L and N) and p53−/− (M and O) brain slices 14 hr after washout from either DMSO (L and M) or STLC treatment (N and O) and stained for CC3 (green) and Hoechst (blue).

(P and Q) Fraction of EdU+ cells in DMSO (gray) and STLC (red) treated E13.5 p53−/− slices that are CC3+ (P) or TUJ1+ (Q) after 14-hr washout.

(R) Fold changes in the fraction of neurons (TUJ1+), IPs (Tbr2+), and RGCs (PAX6+ TBR2−) in STLC/DMSO-treated slices from WT (gray) versus p53−/− (black) embryonic brains.

ns, not significant, *p < 0.05, **p < 0.01, ***p < 0.001. Scale bars represent (A–D) 10 µm, (F–I) 50 µm, and (L–O) 75 µm. Error bars represent SD.
How does Magoh influence mitosis? The most striking defect in Magoh-depleted mitotic cells was in microtubule organization, leading us to posit Magoh regulates mitotic progression by controlling spindle dynamics and anaphase onset. Consistent with this notion, mago nashi, the Magoh ortholog, is required for microtubule dynamics in Drosophila oocytes (Micklem et al., 1997). Our data indicate that Magoh can influence microtubules indirectly, by modulating expression of key microtubule/mitosis regulatory proteins. However, Magoh may also directly influence microtubule organization; it localizes to the mitotic spindle (Ishigaki et al., 2014), was identified in proteomic studies as a mitotic spindle component (Bonner et al., 2011; Sauer et al., 2005), and physically associates with the microtubule-associated protein MAP1B (Tretyakova et al., 2005). It will be interesting to assess whether Magoh’s RNA binding function is necessary for microtubule regulation and if so to identify its RNA cargos specifically at M phase. Future studies aimed at understanding how Magoh influences microtubules will help illuminate the root causes of the mitosis delay.

Figure 8. Mitotically Delayed Progenitors Directly Produce p53-Dependent Apoptotic Progeny and p53-Independent Neuronal Progeny
(A) Overview of experiment for live imaging progenitors and visualizing cell fate.
(B) Average mitosis duration and distribution of mitosis durations for DMSO (white) and STLC (red) treated p53−/− progenitors.
(D) Absence of apoptosis (black) in both DMSO- and STLC-treated p53−/− progenitors.
(E) Proportion of P/P (white), P/N (gray), and N/N (black) divisions of delayed and non-delayed progenitors from indicated treatments.
(F–I) Models depicting how prolonged neural progenitor mitosis influences the survival and fate of direct progeny. RGCs with prolonged mitosis directly produce neurons at the expense of new RGCs in a p53-independent manner (F and H). With excessively longer mitosis, RGCs are more likely to generate apoptotic progeny via p53-dependent mechanisms (F and I).

ns, not significant, *p < 0.05, ***p < 0.001.
Potential Mechanisms Linking Prometaphase to Altered Cell Fate

We show mitotically delayed progenitors have increased propensity for generating apoptotic progeny and differentiated neurons. Our data indicate that as mitosis lengthens, the probability for producing apoptotic progeny increases. In Magoh\textsuperscript{loxP/loxP}, STLC and nocodazole treated progenitors, extremely long mitoses generate primarily apoptotic fates, whereas increased neurogenic divisions are first evident in shorter delays. We speculate that neural progenitors have an internal clock, which measures mitosis duration. This model has been invoked to explain why mitotically delayed immortalized cells produce G1-arrested progeny (Uetake and Sluder, 2010). Progenitors with longer mitoses may be deemed damaged or problematic, forcing their progeny to die or exit the cell cycle. In support of this model, our genetic and pharmacology experiments indicate that delayed cells generate aberrant neurons via direct neurogenic rather than indirect IP-generating divisions. Thus, mitotic delay causes progenitors to preferentially produce post-mitotic or apoptotic progeny rather than generating new progenitors.

How does longer mitosis induce cell fate changes in progeny? In this study, we examined several potential mechanisms. In drug-treated progenitors, neither increased aneuploidy nor misoriented spindles explained observed cell fate changes. In contrast, mitotically delayed progenitors showed increased γH2AX staining prior to completion of mitosis. Subsequent progeny exhibited P53 activation, which induced apoptosis. As p53 loss rescued apoptosis but not differentiation, it is intriguing to consider if other DDR-dependent signaling pathways promote differentiation. Notably, DDR can trigger differentiation of stem cell populations, including adult neural stem cells, making it an interesting avenue for future studies (Gil-Perotin et al., 2006; Inomata et al., 2009; Sherman et al., 2011; Wang et al., 2012). Identifying the molecular mechanisms driving delay-induced differentiation is a high priority for future studies.

Mitotic Regulation and the Developing Brain

Our study indicates prolonged mitosis can alter cell fates in a pathogenic state, but it is interesting to consider its potential role during normal development. Haydar et al. (2003) showed that over the course of corticogenesis as more neurons are being produced, neural progenitor metaphase duration increases. We observed that prolonged mitosis could impact normal development as evidenced by significantly more apoptotic progeny from delayed control progenitors at E13.5 and E14.5. E14.5 control progenitors delayed in mitosis also underwent more neurogenic divisions. These outcomes were not evident at E12.5, perhaps because there are fewer delayed progenitors at this age. Thus, the relatively subtle relationships between duration and cell fate may be more pronounced at older stages when progenitor divisions are longer and more neurogenic and/or when measured with less pro-proliferative culture conditions. Studies of older stages are also particularly relevant for assessing the role of mitotic duration in different progenitor populations and in the generation of distinct neuronal and glial subtypes. Our findings provide rationale for future detailed analysis of the role of mitosis duration in influencing normal brain development.

Beyond normal mouse development, cell-cycle differences are one of the most distinguishing traits differentiating the brains of mice, non-human primates, and humans (Geschwind and Rakic, 2013). Such differences are postulated to influence brain size, shape, and neuronal number. Thus, in future studies, we should consider not only the potential role that prolonged mitotic duration plays in the etiology of microcephaly, but also in defining species-specific differences in brain development.

EXPERIMENTAL PROCEDURES

Statistical Analyses and Quantitation

See Table S1 for details regarding statistical analysis. All graphs depict average and SD values, and error bars denote biological variation. When possible, all analyses were performed by more than one person, blind to genotype. For this manuscript, RGCs were denoted either by their division at the apical surface, expression of PAX6, or absence of TBR2 expression at E12.5.

Live Analyses

For clonal analysis, progenitors were cultured as previously described (Shen et al., 2003). For brain-slice imaging, analysis was performed as previously described (Pilaz and Silver, 2014). Because pharmacology can potentially have off-target effects, in our experimental paradigm, we rule out impacts of nocodazole and STLC on interphase viability, cleavage plane orientation, and exclude aneuploidy as an outcome of STLC.

Mouse Genetics

Magoh\textsuperscript{loxP/loxP} (Magoh\textsuperscript{-/-}) (Silver et al., 2010), Magoh\textsuperscript{flox} (McMahon et al., 2014), and Tbr2-EGFP (Arnold et al., 2009) mice were previously described. The following mouse strains were obtained from Jackson Labs: B6.129S2-Emx1<tm1(cre)Ky/J, B6.Cg-Tg(Camk2a-cre)T29-1St/J, B6.129S2-Trp53tm1Ify/J, B6.Cg-Tg(Hist1H2BB/EGFP)1Pa/J, and C57BL/6J-Tg(Dcx-DsRed)14Qlu/J. All experiments were approved by the Institutional Animal Care and Use Committee of Duke University.

SUPPLEMENTAL INFORMATION

Supplemental Information includes Supplemental Experimental Procedures, eight figures, one table, and seven movies can be found with this article online at http://dx.doi.org/10.1016/j.neuron.2015.12.007.

AUTHOR CONTRIBUTIONS

L.-J.P., J.J.M., and D.L.S. designed the study, analyzed all data, and wrote the manuscript. L.-J.P. performed Figures 1, 2, 4, 5, 7, S2, S3, S4, and S6. J.J.M. performed Figures 3, 6, 7, S1, S5, S7, and S8. A.S., E.E.M., and E.E.M. contributed to Figures S1 and S3. A.L.L. contributed to Figure S4.

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Supplemental Information

Prolonged Mitosis of Neural Progenitors Alters Cell Fate in the Developing Brain

Louis-Jan Pilaz, John J. McMahon, Emily E. Miller, Ashley L. Lennox, Aussie Suzuki, Edward Salmon, and Debra L. Silver
Scramble siRNA HeLa H2B-EGFP

Magoh siRNA HeLa H2B-EGFP

C

Scramble siRNA HeLa H2B-EGFP

Magoh siRNA HeLa H2B-EGFP

Mitotic index

PM index

Control

Emx1-Cre; Magoh^Lox/Lox

CC3- Hoechst

NeuN- Hoechst

NeuN+Cells / 225µm column

CamKIIα-Cre; Magoh^Lox/Lox

E11

P30

F

G

H

ns

NeuN- Cells / 225µm column

Control

CamKIIα-Cre; Magoh^Lox/Lox

ns
Figure S1. Mitosis defects in siRNA-treated HeLa cells and absence of neuron loss in a postnatal conditional Magoh knockout, related to Figure 1. (A) Mitotic index of Scramble (n=609 cells) or Magoh siRNA (n=651 cells) treated cells 2 days after transfection. (B) Proportions of scramble (n=182 cells) or Magoh siRNA (n=168 cells) treated mitotic HeLa cells in the different phases of mitosis. (C) Time-lapse images of dividing histone H2B-EGFP HeLa cells treated with scrambled siRNA (n=10 cells), or Magoh siRNA (n=33 cells) with indicated stages. In Magoh-siRNA treated cells, 31 of 33 mitotic cells arrested at metaphase and exhibited donut shaped metaphase chromosomes. Time-lapse panels correspond to Movies S5. (D,E) E11.0 brain sections of Emx1-Cre (control) (D) and Emx1-Cre;Magohlox/lox mice (E) stained for CC3 (apoptosis, green) and Hoechst (blue). (F,G) Sections form P30 brains of CamKII-Cre (control) (F) and CamKII-Cre;Magohlox/lox mice (G) stained for NEUN (neurons, green). (H) Quantification of NEUN-positive cells in indicated genotypes. ns, not significant. Scale bar, C: 5µm, D,E: 50 µm F,G: 100 µm. Error bars, S.D.
**A**

H2B-EGFP

Duration (min)

- Control
- Magoh**+/−**

***

^*

**B**

Syto 11

Mitotic Cells (%)

- Control
- Magoh**+/−**

PM+M duration (min)

15-29
30-44
45-60
>60

**C**

Syto11 + electroporated mCherry-α-Tubulin in Magoh**+/−** brain slice

**D**

Electroporated mCherry-α-Tubulin in Magoh**+/−** brain slice + Syto11

mitotic spindle

condensed DNA

**E**

- Divide
- Don’t divide

62%

38%
Figure S2: Live imaging reveals a subset of Magoh-deficient RGCs with prolonged prometaphase, related to Figure 2. (A) Graph depicting the average mitosis duration in H2B-EGFP; Magoh\textsuperscript{+/−} (black) and H2B-EGFP control (grey) E13.5 brain slices. (B) Graph showing the proportion of mitotic cells with different metaphase durations in Syto11-stained control (grey) and Magoh\textsuperscript{+/−} (black) E13.5 brain slices as exemplified in Movie S3. (C) Time lapse images from Syto11-stained Magoh\textsuperscript{+/−} brain slice electroporated with mCherry-\(\alpha\)-TUBULIN (red). The dotted box indicates the image shown at high magnification in (D). The time-lapse panel corresponds to Movie S4. (E) Pie chart depicting proportion of mitotically delayed E13.5 Magoh\textsuperscript{+/−} progenitors that were observed to divide (black) or not divide (grey) during the 5 hour live-imaging session. *, \(P<0.05\), ***, \(P<0.001\). Scale bar, C, D: 5\(\mu\)m. Error bars, S.D.
Figure S3. Analysis of mitosis phenotypes in embryonic cortices and siRNA treated HeLa cells, related to Figure 2. (A) Graph depicting centrosome distance in E13.5 metaphase RGCs in control (grey) and Magoh^{+/−} (black) cortices. (B,C) Western blot analysis of EG5 protein levels in E12.5 control and Magoh^{+/−} littermate cortices. (D-I) Representative images of mitotic RGCs labeled with Hoechst (blue) and antibodies targeting NUMA (green, D,E) and acetylated TUBULIN (white, F, G, red H, I). (J) Graph depicting quantitative NUMA signal intensity in metaphase spindles of RGCs from E13.5 control and Magoh^{+/−} littermates. (K-P) Transfected HeLa cells stained with anti-γ-TUBULIN (green) and anti-ODF2 (red) labeling both centrioles and mother centriole, respectively. Although Magoh-depleted HeLa cells exhibit centrosome separation defects, this is likely due to a stronger mitotic checkpoint and greater Magoh depletion (70%) (Silver et al., 2010). (Q) Quantification of ODF2-immunofluorescence signal intensity in the daughter centriole relative to the mother centriole, comparing Scramble (n=46 cells) and Magoh (n=42 cells) siRNA transfected cells. (R-MM) Analysis of kinetochore integrity and spindle assembly checkpoint in transfected HeLa cells using NDC80/HEC1 (R-V) (Scramble: n=177 cells, Magoh: n=202 cells) and Mad1 targeted immunofluorescence (W-MM) (Nocodazole: n=168 cells, Prometaphase: n=215 cells, Monastrol: n=168, Magoh: n=165 cells). (NN-QQ) Visualization of mitotic microtubule-centromere attachments in HeLa cells using anti-CENP-A and anti-α-TUBULIN. (RR,SS) Visualization of mitotic microtubules in HeLa cells using anti-EB1, with cell outline indicated by dotted line. (TT) Cartoon representation of the cellular defects observed in Magoh-deficient mitotic cells. Altogether, our data show Magoh deficiency leads to altered mitotic microtubule organization associated with aberrant mitotic spindles and delay in progression to anaphase. Altered microtubule dynamics may originate from a direct role of MAGOH in that process, or by establishing the levels of key microtubule
regulatory proteins such as LIS1, EG5 and NUMA, which are critical for helping pull microtubules apart and for mitosis. ns, not significant, *, $P<0.05$, **, $P<0.01$. Scale bars: D-I: 2µm, K-P, NN-SS: 5 µm. Error bars, S.D.
**Figure S4.** Prolonged mitosis of *Magoh* deficient neural progenitors does not cause aneuploidy or spindle mis-orientation, related to Figure 2. (A-C) DNA FISH (targeting Chromosome 11) staining of E13.5 *Magoh*<sup>+/−</sup> brain section, and (B,C) higher magnification view of region boxed in left image. (D) Graph depicting lack of significant aneuploidy in E13.5 *Magoh*<sup>+/−</sup> cortices. (E) Graph depicting lack of significant aneuploidy in E13.5 *Magoh*<sup>+/−</sup>; *p53<sup>−/−</sup>* cortices (black) compared with control *p53<sup>−/−</sup>* cortices (grey). (F) Graph showing aneuploidy in passage-3 *Magoh*<sup>+/−</sup> MEFS (black) compared with control MEFS (grey). This result confirms previous findings and serves as a positive control for detecting aneuploidy events (Silver et al., 2010). (G) Cartoon representation of rotation angle (α) relative to the ventricle (vent.). (H) Example of a 3D reconstruction of a metaphase progenitor, with spindle angle (red line). (I) Average metaphase spindle rotation angle/time point in control (grey) and *Magoh*<sup>+/−</sup> (black). Control PM+M spindles rotated extensively, as previously reported (Haydar et al., 2003). (J) Spindle rotation angle relative to metaphase length (colored dots) in *Magoh*<sup>+/−</sup>. No significant difference was observed for spindle rotation between each category of metaphase duration. (K) Examples of anaphase progenitors with 3 cleavage plane angles used for quantitation in L, M. (L) Average anaphase cleavage plane orientation angle in E13.5 control (grey) and *Magoh*<sup>+/−</sup> (black) H2B-EGFP slices. (M) Anaphase cleavage plane orientation angle relative to PM+M duration in *Magoh*<sup>+/−</sup>. Note the lack of a correlation between mitotically delayed progenitors and any specific cleavage plane angle. **, *P*<0.01, ***, *P*<0.001, ns, not significant. Scale bars: A: 15µm, B,C: 3µm, H,K: 5µm. Error bars, S.D.
**E12.5 Division subtypes**

- **Proliferative**
  - Symmetric RGC
  - IP generating (indirect)
  - Intermediate progenitor (Tbr2-EGFP)
  - Neuron (Tuj1+)

- **Direct neurogenic**
  - asymmetric neurogenic

- **Apoptosis**
  - RGC-RGC
  - RGC-N
  - Apoptosis

**H**

- 0-40 min WT
  - 63%
  - 19%
  - 12%
  - 1%
- 0-40 min Magoh+/−
  - 51%
  - 25%
  - 9%
  - 2%
- >40 min Magoh+/−
  - 4%
  - 31%
  - 9%
  - 2%

**I**

- < RGC-RGC
  - ns
  - ns
  - +++
- > RGC-N
  - < ***
  - > ***
- > Apoptosis
  - > ***
Figure S5. Live imaging clonal analysis of *Magoh* deficient progenitors, related to Figure 3. (A) Proportion of RGC progeny producing viable or apoptotic progeny from RGCs of indicated genotypes. (B) Proportion of RGC divisions generating either 2 apoptotic progeny (black) or one apoptotic and one viable cell (white). (C) Proportion of RGCs undergoing proliferative or neurogenic divisions in indicated genotypes. (D) Distribution of mitosis duration for control RGCs. (E) Graph depicting average mitosis duration for RGCs generating progeny that survive or undergo apoptosis. (F) Graph depicting average mitosis duration for different divisions for control and *Magoh* mutant. Note that in control RGCs, the average duration of mitosis is higher for IP generating divisions compared to RGC generating divisions. (G) Representation of different types of progenitor divisions analyzed. (H) Pie charts of normal and delayed control and *Magoh* +/- RGCs showing relative proportions of viable divisions generating proliferative and neurogenic progeny. (I) Proportion of normal and delayed control and *Magoh* +/- RGCs undergoing division types shown in (G). ns, not significant, *, P<0.05, **, P<0.01, ***, P<0.001. Error bars, S.D.
A) WT embryos

B) EdU +3h +6h +14h EdU - Hoechst

C) EdU CC3 Tuj1 Merge

D) EdU+ CC3+ Tuj1-

E) EdU+ cells within the CC3+ population

F) Pax6- Tbr2- Tbr2+ Pax6+ Tbr2-

G) CC3 immuno-labeling in DMSO and STLC treated slices
Figure S6. EdU pulse-chase analysis in embryonic brain slices, related to Figure 5. (A) Timeline of the slice culture experiment. (B) Images of sections from E13.5 brain slices following paradigm outlined in (A), prior to inhibitor treatment (-4 h), no washout (0 h), 3 hour washout, 6 hour washout and 14-hour washout. Sections were stained for EdU+ (red). (C) Fraction of EdU+ cells which were PAX6+ TBR2- (white) and TBR2+ (grey) following the 30-min EdU incorporation but prior to inhibitor treatment. (D) Examples of cells co-labeled for different markers, used for quantitation in Figure 5. D1-5 are high magnification images of regions highlighted in D respectively. (E) Fraction of CC3+ cells that were EdU+ in STLC and nocodazole treated slices after 14-hour washout. (F) Fraction of EdU+ cells in DMSO- (n=7 slices, 1872 cells) and STLC-treated E13.5 Control slices (n=7 slices, 840 EdU+ cells) that are PAX6+TBR2- (white), TBR2+ (grey) or PAX6-TBR2- (black) after 14-hour washout. (G) Sections from DMSO or STLC treated slices, after washout for 0, 3, 6, and 14 hours, stained for apoptosis, CC3. Ventricular zone, VZ, sub-ventricular zone, SVZ, cortical plate, CP. Scale bars, B, D: 20µm, D1-D5: 2µm.
E13.5 Division subtypes

- **Proliferative**
  - Symmetric RGC
  - IP generating (indirect)
  - Intermediate progenitor
  - Symmetric neurogenic

- **Direct neurogenic**
  - Symmetric neurogenic (Tbr2- Tuj1-)
  - Asymmetric neurogenic (Tbr2-EGFP)

- **Apoptosis**
  - RGC (Tbr2 - Tuj1-)
  - Intermediate progenitor (Tbr2-EGFP)
  - Neuron (Tuj1+)

**Mitosis duration (min)**

- **E14.5**

**Survive**

- Divisions (%)

**Apoptosis**

- Divisions (%)

**Division subtype**

- N/N
- P/N
- P/P
Figure S7. Live imaging clonal analysis, related to Figure 6. (A) Proportion of interphase cells treated under indicated conditions that survive or undergo apoptosis. (B) Distribution of mitosis duration for control progenitors. (C) Proportion of divisions giving rise to 2 viable (white) or 1 viable and 1 apoptotic (black) progeny. (D) Pie charts depicting proportion of progenitor divisions for each indicated condition. (E) Representation of different types of progenitor divisions analyzed. (F) Pie charts depicting proportion of total progenitor divisions for each indicated condition. (G) Proportion of DMSO and STLC-treated progenitors undergoing division types shown in E. (H-J) Analysis of the correlation between mitosis duration and cell fate in control E14.5 EGFP+ dissociated progenitors which were electroporated by in utero electroporation at E13.5. (H) Average mitosis duration (I) Proportion of divisions giving rise to 2 viable (white) or at least 1 apoptotic (black) progeny. (J) Bar graphs depicting proportion of progenitor divisions for each indicated condition. *, P<0.05, **: P<0.01, ns, not significant; error bars, SD.
> 40 min control

> 40 min p53−

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**STLC**

> 40 min

**DMSO**

**P53**

***

**+6h**
Figure S8. P53 STLC slice and clonal analysis, related to Figures 7, 8. (A,B) Images depicting P53 staining of DMSO (A) or STLC (B) treated slices 6h after the end of the treatment. (C) Proportion of indicated divisions for STLC-delayed progenitors from control or p53 null brains. (D) Pie charts depicting proportion of progenitor divisions for each indicated condition. ***, \( P<0.001 \). Scale bar, A,B: 50 µm.
Supplemental tables

**Table S1.** Table S1 lists all statistical tests, sample sizes used for data analysis, and actual $P$ values calculated.

Supplemental movies legends

**Movie S1.** Time-lapse imaging of dividing progenitors in an E13.5 H2B-EGFP control brain slice, related to Figure 2. Dotted line marks the ventricular border. See Methods for the criterion that was used to delineate the ventricular border. Time indicated in hh:mm.

**Movie S2.** Time-lapse imaging of dividing progenitors in an E13.5 H2B-EGFP *Magoh* $^{+/−}$ brain slice, related to Figure 2. Dotted line marks the ventricular border. White and yellow arrows point to dividing cells with or without a metaphase delay, respectively. Note that the appearance of two arrows highlights the separation of chromosomes at anaphase and prophase. Time indicated in hh:mm. In the first two movies, the cells highlighted with a white arrow divide during the live-imaging session, however in the third movie, the cell does not divide within the session.

**Movie S3.** Time-lapse imaging of dividing progenitors in E13.5 control and *Magoh* $^{+/−}$ brain slices, related to Figure 2. Brain slices were stained with Syto11. Dotted line marks the ventricular border. Time indicated in hh:mm.

**Movie S4.** Time-lapse imaging of $\alpha$-Tubulin labeled dividing progenitors in an E14.5 *Magoh* $^{+/−}$ brain slice, related to Figure 2. Slices were stained with Syto11 (in green) and cells were transfected with a plasmid expressing the fusion protein $\alpha$-Tubulin-mCherry by in utero electroporation at E13.5. The mitotic spindle highlighted by $\alpha$-Tubulin-mCherry (in red) confirms that *Magoh* $^{+/−}$ progenitors are in metaphase. Time indicated in hh:mm.

**Movie S5.** Time-lapse imaging of H2B-EGFP HeLa cells, related to Figure 2. Cells were transfected with a scrambled siRNA or *Magoh* siRNA 36h prior to live imaging. Time indicated in hh:mm.
**Movie S6.** Live imaging clonal assay showing progenitor divisions, related to Figures 3 and 6. A collection of movies depicting different division types as indicated in Figure S5G. Time indicated in hh:mm.

**Movie S7.** Live imaging clonal assay of STLC treated progenitors, related to Figures 6 and 8. A collection of movies depicting different division types as indicated in Figure S5G. Time indicated in hh:mm.
Supplemental Experimental Procedures

Immunofluorescence and fixed image analysis

Immunohistochemistry was performed on dorsal neocortices as previously described (Silver et al., 2010). Embryonic brains were harvested and fixed in 4% PFA overnight, then cryoprotected in 30% sucrose. 20µm-thick sections were collected on microscope slides with a cryostat. We used the following antibodies: rabbit: anti-Ki67 (1:200, Abcam SP6), anti-CC3 (1:200, Cell Signaling), anti-TBR2 (1:500, Abcam), PH3 (1:200, Millipore), CENP-A (1:800, Gift from Beth Sullivan), ODF2 (1:200, Abcam), Mad1 (1:20, kind gift of Dr. A. Mussachio), NUMA (Abcam, 1:100), γ-TUBULIN (1:500, Sigma), anti-P53 (1:250, Leica), mouse: anti-TUJ1 (1:1000, Covance), anti-γH2AX (1:100, Millipore), anti-EB1 (1:200, BD Biosciences), anti-phospho-VIMENTIN (1:500, MBL), anti-PAX6 (1:500, DSHB), NDC80/HEC1 (Abcam), acetylated TB11;OM (Sigma, 1:1000); rat: anti-BrdU (1:500, Abcam). Revelation of EdU was performed using Click-it technology according to the manufacturer’s protocol (Life Technologies). Images were acquired with an epifluorescence microscope equipped with the apotome technology (Zeiss). Image analysis and cell counting was performed in ImageJ. For the analysis of the percentage of mitotic cells within the Ki67+ population, mitotic cells were identified based on Ki67 staining pattern and DNA condensation observed with the Hoechst counterstaining.

Cell cycle analysis

Analysis of cell-cycle kinetics was performed as described previously (Martynoga et al., 2005; Quinn et al., 2007). Briefly, BrdU (50μg/g of Body weight) and EdU (10μg/g of body weight) were injected intraperitoneally to the pregnant dam 2.5 hours prior to sacrifice, respectively. DNA denaturation was performed by boiling the sections in citrate
buffer (Tang et al., 2007). Samples were prepared as described above. EdU revelation was performed according to the manufacturer’s instructions (Life Technologies, Click-iT 647). Subsequently, immunohistochemistry targeting BrdU (alexa 405), Pax6 (alexa 488) and Tbr2 (alexa 555) was performed as previously described (Silver et al., 2010). Images were acquired as described above. The total number of PAX6+ TBR2- cells (Cycling fraction), the number of PAX6+ TBR2- cells in S-phase (S fraction, PAX6+ TBR2- BrdU+ EdU+) and the number of PAX6+ TBR2- in the “leaving fraction” (L fraction, PAX6+ TBR2- BrdU+ EdU-) were assessed in ImageJ using the “Cell-counter” plugin. Ts and Tc were subsequently calculated using these equations:

\[
\frac{T_s}{T_L} = \frac{S_{\text{fraction}}}{L_{\text{fraction}}}
\]

\[
T_s = \frac{S_{\text{fraction}}}{L_{\text{fraction}}} \times T_L
\]

\[
T_s = \frac{S_{\text{fraction}}}{L_{\text{fraction}}} \times 1.5
\]

and Tc was calculated using these equations:

\[
\frac{T_c}{T_s} = \frac{\text{Cycling fraction}}{S_{\text{fraction}}}
\]

\[
T_c = \frac{\text{Cycling fraction}}{S_{\text{fraction}}} \times T_s
\]

**Quantification of Numa immunofluorescence at the mitotic spindle**

After immunofluorescent labeling of NUMA and acetylated TUBULIN, high-resolution z-stack images of metaphase cells were acquired at the ventricular border of E13.5 cortices using a 63x objective mounted on a Zeiss 780 confocal microscope. Using acetylated tubulin labeling, mitotic spindles were delineated and stored as regions of interest in ImageJ. The software subsequently calculated the average intensity of NUMA immunofluorescence signal inside those regions of interest. Of note the sections of all the embryos for each litter were processed for immunofluorescence in the same
experiment, and confocal acquisitions were acquired in the same session without changing acquisition parameters.

**Western blot analysis of Eg5 protein levels in Magoh**<sup>+/−</sup> **cortices**

SDS-PAGE and Western analysis was run using Mini-PROTEAN TGX gels (BioRad) and run at 150V. Blotting was done using the TransBlot Turbo Transfer System (BioRad). Blots were all blocked in 5% milk and the following primary antibodies were used: mouse anti-α-TUBULIN; 1:10,000 (Sigma), mouse anti-MAGO positional; 1:50 (Santa Cruz), rabbit anti-EG5; 1:200 (Novus). Secondary antibodies used were HRP goat anti-mouse or goat anti-rabbit (Amersham) both at 1:10,000. Western imaging was done using ECL Western blotting substrate (Pierce) and the Syngene G:Box acquisition system.

**Live Imaging of HeLa cells.**

Live imaging of HeLa cells was performed using the DeltaVision Elite microscope equipped with an Olympus IX-71 microscope, Coolsnap HQ2 high resolution CCD camera, and a 100x/1.40 oil UPLSAP0100X0 1-U2B836 WD 120 micron DIC ∞/0.17/FN26.5, UIS2 objective. For H2B-GFP analysis, images were taken every 10 minutes with Autofocus every 5 minutes and UltimateFocus selection. Images were deconvolved and a maximum image projection generated. siRNA knockdown was performed as previously described (Silver et al., 2010).

**Live Imaging of brain slices and quantification of mitosis**

Mitotic cells were assessed by morphology using Hoechst or by phospho-VIMENTIN or γ-TUBULIN staining. Mitotic index was calculated over the number of cycling cells (assessed by Ki67 staining). The procedure to generate time-lapse videomicroscopy
data from embryonic brain slices was performed as previously described (Pilaz and Silver, 2014). Of note, within the 5-hour imaging session, some Magoh<sup>+/−</sup> cells were already in prometaphase at the onset of imaging, and thus the “>60 minutes” category includes some cells which may have been delayed prior to imaging. The ventricular border was delineated based upon the abrupt change of H2B-EGFP or Syto11 signal intensity (the ventricle appears dark) that persists throughout the z-stack. Cellular debris observed in some Magoh<sup>+/−</sup> brain slices were not used to define the ventricular border. As reported by others (Adams, 1996), it is difficult to discriminate prometaphase from metaphase in embryonic brain progenitors because individual chromosomes are not easily resolved.

**DNA FISH analysis**

DNA FISH probes labeled with PlatinumBright550 and targeting the Tlk locus on chromosome 11 were purchased from Kreatech. Briefly, slides harboring frozen sections were washed three times in PBS + 0.5% triton for 10 minutes. Next, sections were equilibrated in 2X SSC for ten minutes. The probe solution was applied on top of the specimen. A cover slip was used to cover the samples. After sealing with Rubber Cement, probes and sample DNA were co-denatured on a hot plate at 80˚C for 15 minutes. Next, samples were incubated overnight at 37˚C. The following day, the seal was removed and Hoechst labeling of the sections was performed prior to mounting with Vectashield. Samples were analyzed with a 63x objective on an epifluorescence microscope equipped with the Apotome technology. Z-stacks covering the full depth of the section were acquired and the number of PlatinumBright550-positive foci within individual nuclei was counted using the “cell-counter” plugin in ImageJ. A similar approach was utilized to perform this assay in cultured MEFs (See Silver et al., 2010 for the preparation of MEFs from control and Magoh<sup>+/−</sup> embryos). However, in this case
permeabilization was performed using an ethanol gradient, and the probe solution was diluted 1:50 in hybridization buffer (15% dextran, 30% formamide).

**Mitosis inhibitor slice culture experiments**

225-250 µm thick organotypic brain slices were generated as described (Pilaz and Silver, 2014) and cultured as follows. 1. Slices were cultured for 1 hour on porous cell culture inserts (Millipore) in 6-well dishes containing 1000 µl of slice culture medium. 2. Inserts were transferred to new wells containing 20 µM EdU (Life Technologies) in medium for 30 minutes. 3. Inserts were transferred to new wells containing fresh medium for 1 hour. 4. 500µl of the medium was replaced with medium containing either DMSO or 10 µM of the Eg5 inhibitor STLC (Sigma) or 0.2 µM nocodazole (for a final concentration of 5 µM STLC and 0.1µM nocodazole). 5. After 3 hours slices were washed twice for 5 minutes by transferring the inserts into new wells containing fresh equilibrated medium. Slices were subsequently fixed in 2% PFA in PBS and processed for cryo-sectioning.

**Live Imaging Clonal Assay**

E12.5 dorsal cortices were isolated from either control or Magoh⁺⁻ embryos also expressing Dcx::DsRed and in some cases H2B-EGFP, as previously described (Silver et al., 2010). Cortices were prepared in single cell suspension and plated in neural progenitor media (DMEM with glucose, sodium pyruvate, B-27, N2, N-acetyl cystein and bFGF) as previously described (Shen et al., 2002). Cells were plated in 6 or 12 well glass bottom dishes (MatTek) and allowed 1-2 hours to settle at 37°C in 5% CO₂ prior to imaging. Images were captured every 10 or 20 minutes for 18 hours using a 20x magnification on a Zeiss Axio Observer Z.1 equipped with a XL multi S1 incubation chamber, CO₂ module S, temperature module S, and humidity control. Mitotic cells were
identified by morphology (rounding of cells and condensation of chromatin) and by pattern of H2B-EGFP expression. For fate tracking, only progeny that survived a minimum of 10 hours were quantified, excluding apoptotic fate. For STLC and nocodazole experiments neural progenitors were isolated from either B6, Dcx-DsRed or Dcx::DsRed;Tbr2-EGFP mice at E13.5. STLC and Nocodazole were applied in neural progenitor media at 2.5 µM and 0.1 µM for 3 hours respectively, and DMSO was used as a control. During which time DIC images were captured every 10 minutes to verify mitotic arrest. Cells were then rinsed in media 3 times and fluorescent images were acquired over 24 hours. For live analysis of RGC divisions Dcx::DsRed;Tbr2-EGFP mice were used and cells expressing Tbr2-EGFP prior to dividing (demarcating them as IPs), were excluded from analysis. For clonal assays of DMSO, STLC and Nocodazole in B6 mice, fate determination was performed post imaging by immunostaining for Pax6 and Tuj1. Apoptotic cells were evaluated by DIC morphology. For experiments using Dcx::DsRed for fate determination (in both STLC and Magoh<sup>+/−</sup>), apoptosis and Dcx::DsRed expression occurred on average around 6 hours after division, preventing us from distinguishing if apoptosis occurred preferentially in progenitors or neurons. For experiments quantifying H2AX intensity after DMSO, Nocodazole, and STLC expression cells were fixed in 4% PFA 90 minutes after removal of drugs (when many cells were still in mitosis). Mitotic cells were outlined using DIC imaging and mean fluorescence intensity was measured using Zen software.
Supplemental References


