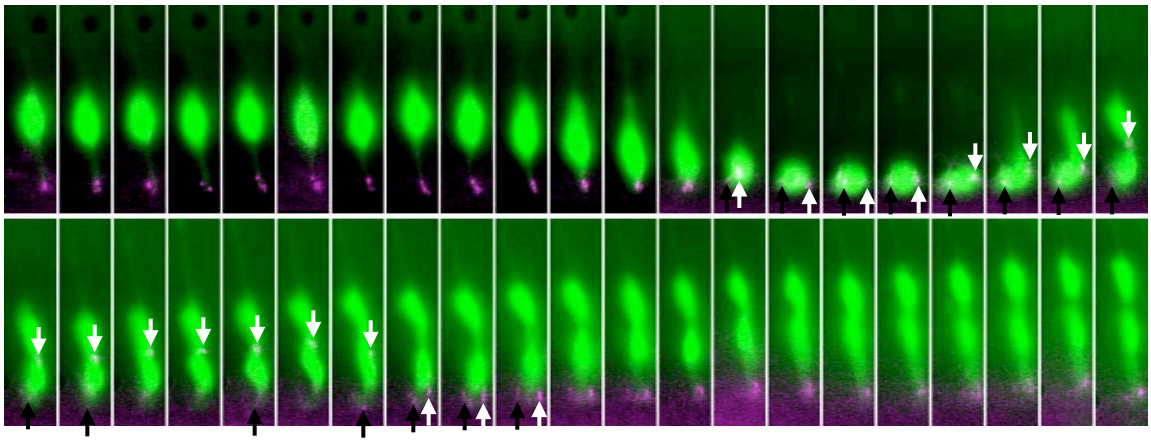
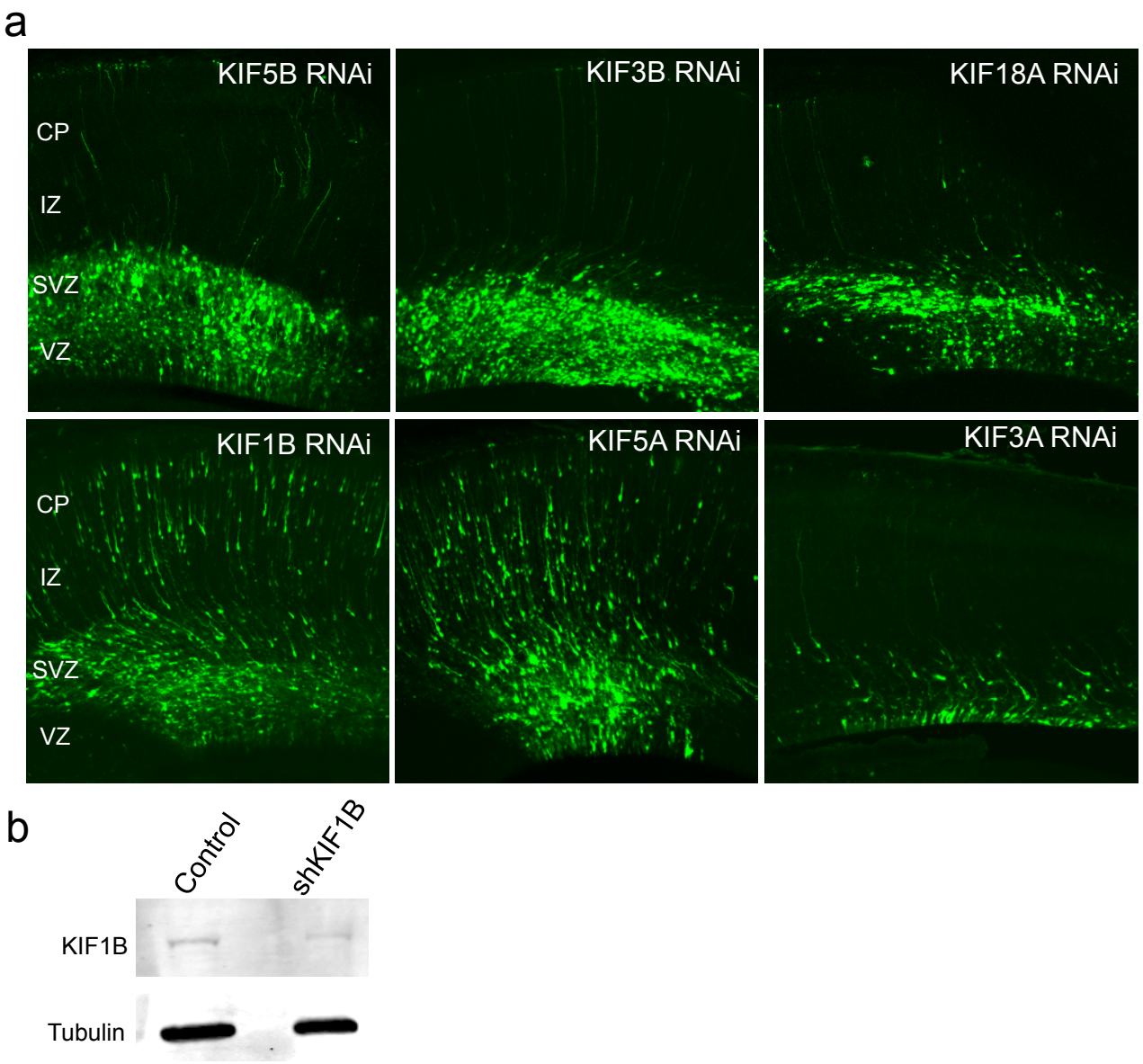
Supplementary Figure 1



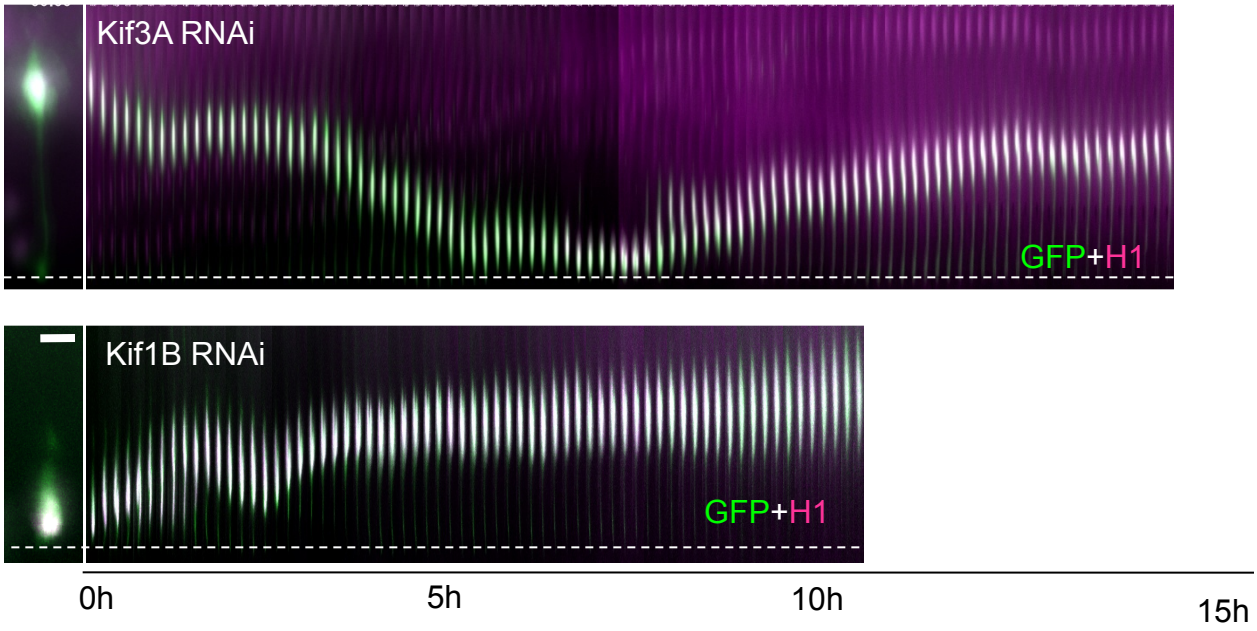
Supplementary Figure 1. Centrosomal dynamics in radial glial progenitors throughout cell cycle. In this example, both centrosomes left the ventricular surface and form spindles during mitosis, and, again, subsequently return to this site (Movie S3). Bar = 5 mm. Time = hh:mm.

Supplementary Figure 2



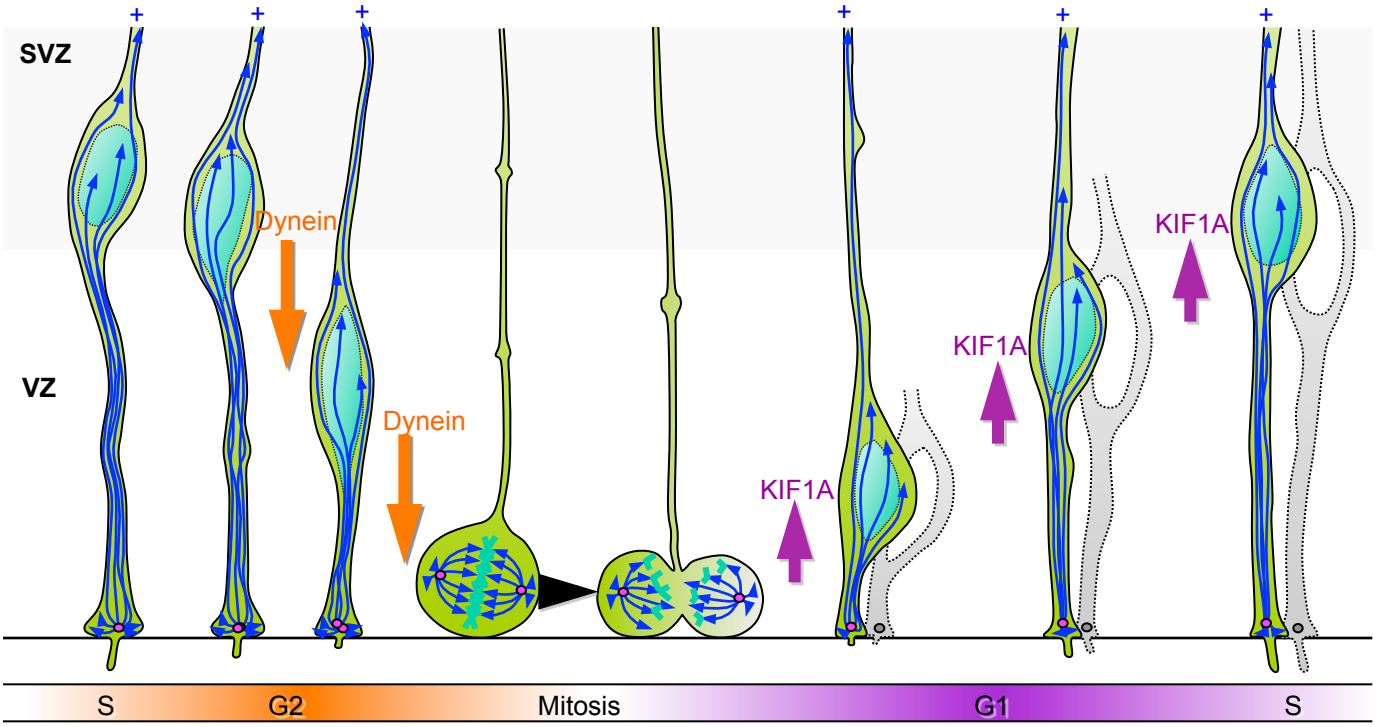
Supplementary Figure 2. Multiple kinesins involved in brain development. (a) shRNAs corresponding to sequences within KIF1B, KIF3A, KIF3B, KIF5A, KIF5B, and KIF18A were introduced into E16 rat brain by *in utero* electroporation. GFP fluorescence from the transfected cells was examined in fixed neocortical sections at E20. Strong inhibition of neuronal migration pathway was observed for KIF3A, KIF3B, KIF5B, and KIF18A as indicated by near absence of cells in intermediate zone (IZ) and cortical plate (CP) and accumulation in VZ and SVZ. (Compare with control scrambled E20 rat brain in Fig. 5B.) KIF1B and KIF5A RNAi produced more limited effect on neuronal migration pathway as indicated by loss of concentration of cells at CP. Bar = 50μm. (b) Immunoblot showing ~30% reduction of KIF1B in culture cells 3 days post tranfection.

Supplementary Figure 3



Supplementary Figure 3. Minimal effects of KIF1B and KIF3A RNAi observed by live brain slice imaging. E20 rat brain was subjected to *in utero* electroporation with cDNAs encoding KIF3A or KIF1B RNAi at E16. Basally directed nuclear movement in is normal in radial glial cells in each case, though we have noted somewhat faster average apically directed nuclear movement in KIF3A RNAi cells. (White dashed lines indicate ventricular surface; Movie S25, S26) Bar = 5µm.

Supplementary Figure 4



Supplementary Figure 4. Proposed mechanism for INM. Centrosomes remain at the ventricular surface throughout the cell cycle. Microtubules (dark blue) radiate from this region and are seen throughout the length of the cell with plus-ends towards the pial surface of the brain. Cytoplasmic dynein transports the nucleus along the microtubules apically toward the ventricular surface, possibly by a similar mechanism and in a similar saltatory manner to that observed in migrating neurons[4]. The basal process of the radial glial cell persists during mitosis, but depleted of microtubules. Microtubules re-enter the basal process by G1 phase, at which stage the plus-end directed microtubule motor KIF1A acts to moves the nucleus slowly and uniformly toward the pial surface of the brain until the cell enters S-phase.

Supplemental Table 1: Velocity of apical and basally-directed nuclear movement in radial glial cells.

Experiment	Animal used (*n*)	Cell recorded (*n*)	Age†	Velocity (μm/min)	
				Apically directed average#	Basally directed average#
DHC RNAi (5-day)	4	7	E16/E21	0.011±0.004***	ND
DHC RNAi (3-day)	6	20	E16/19	0.034±0.021***	0.064±0.016
Myosin 2B RNAi	5	16	E16/19	0.142±0.043	0.071±0.025
Blebbistatin	5	18	E16/19	0.122±0.036	0.085±0.029
KIF1A RNAi	6	22	E16/18	0.15±0.055	0.025±0.032***
KIF1A Scramble	4	25	E16/18	0.137±0.041	0.078±0.018
Control	7	23	E16/18	0.127±0.032	0.069±0.021
Rescue (DsRed KIF1A)	4	11	E16/18	0.132±0.041	0.074±0.026
Rescue (DsRed)	4	12	E16/18	0.125±0.038	0.034±0.044***

Data are compiled from live imaging of radial glial nuclei in Fig. 4, 5, 6, 7.
†: date of electroporation/date of experiment
#: average ±SEM
ND: non-detectable
***: $p<0.001$

Supplemental Table 2

Family	Gene	Neuronal distribution phenotype	Phenotypic Construct	RNAi sequence
Kinesin-1	KIF5A	+	Y	GGTGCTGAATGGACTGATGAA
			Y	GCACCGGAGGAAAGGCAGAAA
			-	CGTCACGACTCGAGTCAAGAA
	KIF5B	++	Y	AGGCTCATTTGTTCAGAACAA
			Y	ATCAGTAGTTTACGAGATGAA
			Y	TGACAAGGATGAAGAGATTAA
	KIF5C	+++	Y	CCAGCCGAATGCAGCATCAAA
			Y	TTGCTTATCTCGCAGCATGAA
			Y	GGCTGTCAATTACGACCAGAA
Kinesin-2	KIF3A	+++	Y	ATAGGAGAATGGCAGCTGAAA
			-	CGCACGCCATCTTCACAATTA
			Y	TTGCTCACATATTTGGTCATA
	KIF3B	+++	-	AGCTAAAGCTCAAGCATCTTA
			Y	ACGTATACCTCATTGCAGCAA
			Y	CAGCTTGGTTGCAGAGGAGAA
	KIF3C	++	Y	GGTGCCAGCTGGAGTGAATAA
			Y	GAGAGACCTTCCACGTCTAAA
			-	ATGGAGAATTACCTGCAGGAA
Kinesin-3	KIF1A	+++	Y	TCCAGAACTTGACTCCAAGAA
			Y	TTGGCGATATCACTGACATGA
			-	CTGGAGAAACTGAGCCTCTTA
	KIF1B	+	-	AGACATGAATGACTGGTTATA
			-	GCGCCAAAGGAACTCGATTAA
			Y	GAAGATCAATGACAACTGTAA
	KIF1C	+++	Y	AGGACCTCTTCTCTCGAGTTA
			Y	CTCATCTCCTCCTCCACATAA
			Y	GAGAAGCATTGCTGGCTGAGA
Kinesin-8	KIF18A	+++	-	GGTGCCAGCTGGAGTGAATAA
			Y	GAGAGACCTTCCACGTCTAAA
			-	CTATAATGATAGCTGCTGTTA
Kinesin-11	KIF26A	+++	Y	TGAAGGTTATGCTGCGAATAT
			-	CAGTCAGTATCATCAGTAGTA
			-	CATGATGGTCACCTGCTTTGA

Supplemental Table 3: RNAi effect on relative cell distribution in brain developmental layers

Experiment	Animal used (*n*)	Cell counted (*n*)	Age†	Cell distribution# (%)		
				VZ/SVZ	IZ	CP
Scramble RNAi	6	3815	E16/20	32.6±4.7	28.8±2.5	47.6±4.6
KIF1A RNAi	8	4721	E16/20	88.6±3.8	12.2±1.3	0.2±0.06
Rescue DsRed-KIF1A	6	4011	E16/20	45.7±2.8	33.8±4.1	20.5±2.2
Rescue Myc-Kif1A	3	1877	E16/20	40.6±5.3	38.2±6.9	21.2±3.4
Rescue-DsRed	5	3165	E16/20	82.6±5.5	18.1±4.2	0.3±0.07

†: date of electroporation/date of experiment
#: average ±SEM