Distel et al., http://www.jcb.org/cgi/content/full/jcb.201004154/DC1

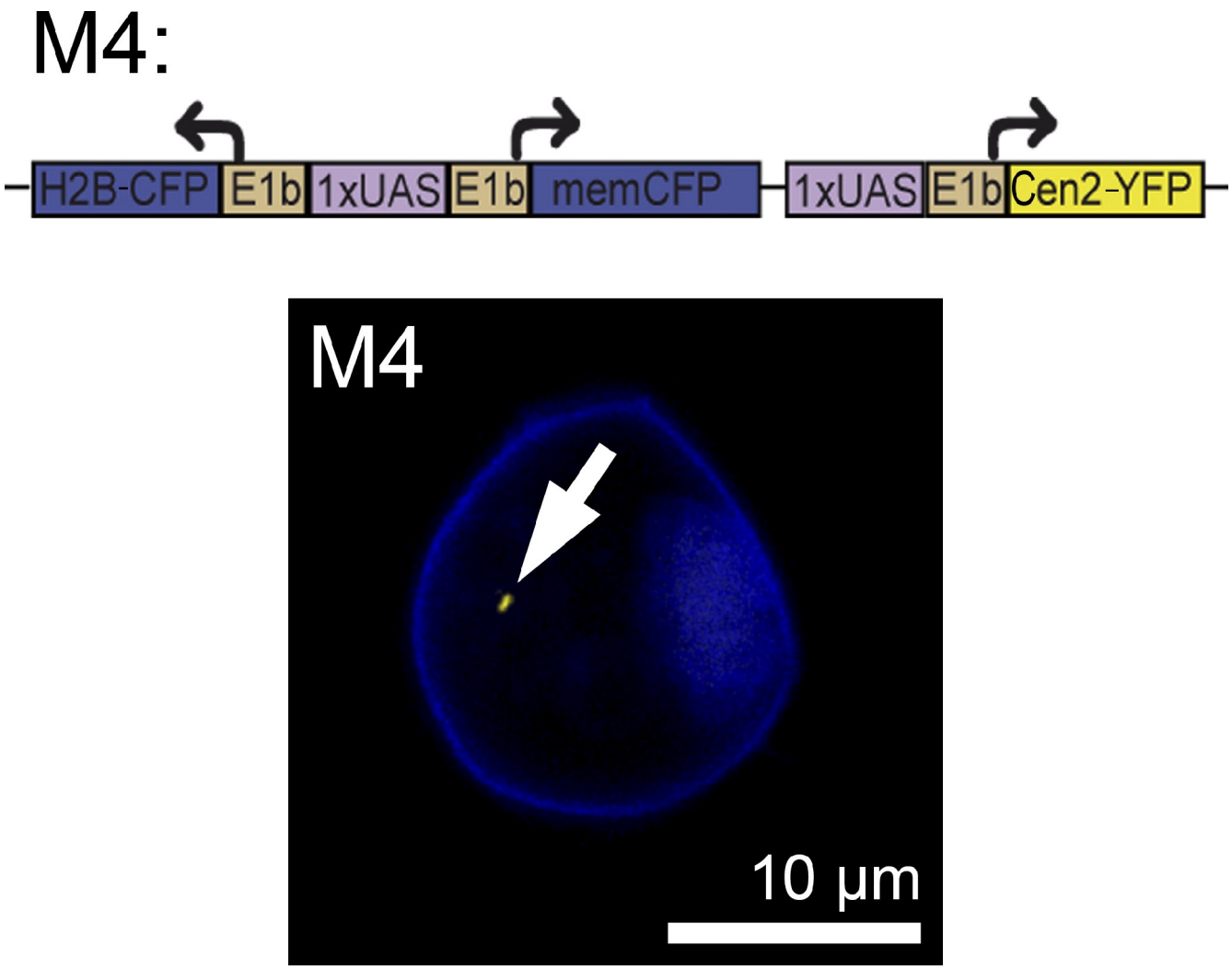


Figure S1. **Medusa vector expression obtained from a 1xUAS site.** Schematic representation of Medusa vector M4. Gal4 binding to a single UAS site activates expression of H2B-CFP to label the nucleus in blue, memCFP to label the membrane in blue, and Centrin2-YFP to label the centrioles of the centrosome in yellow fluorescence (white arrow). This shows that a single Gal4-binding site is sufficient for bidirectional activation of expression by Gal4-activator molecules. Images were obtained from living gastrulation stage zebrafish embryos coinjected with M4 and a vector coding for KalTA4. Images were recorded using a confocal microscope (LSM 510; Carl Zeiss, Inc.) and 40x water objective.

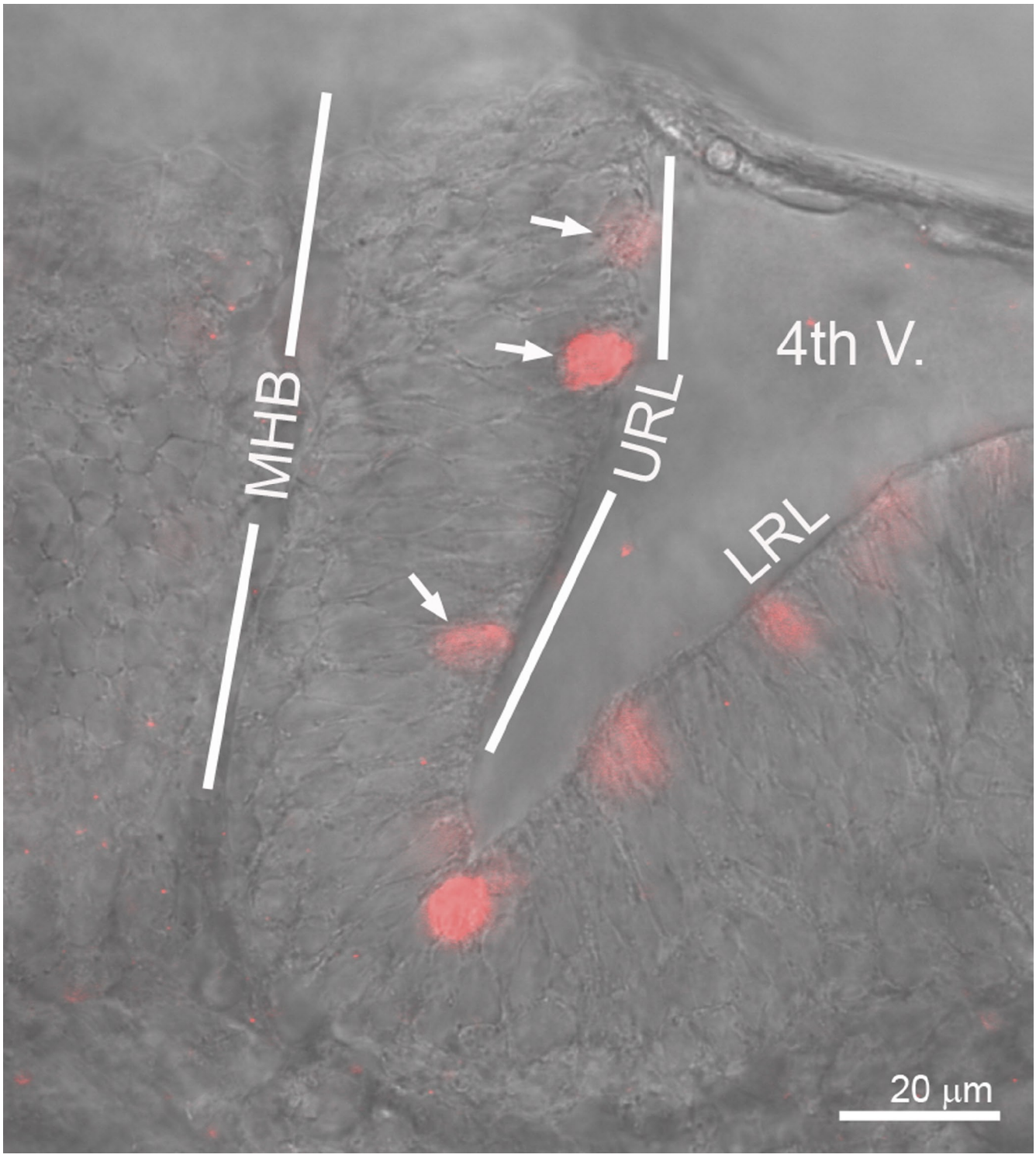


Figure S2. **Cell division in the cerebellum occurs at the ventricular surface.** Lateral view of the developing cerebellum of a 36-hpf wild-type zebrafish embryo processed for whole mount immunohistochemistry using anti-PH3 (red) to label dividing cells. This staining shows that proliferation of URL-derived progenitors occurs strictly along the apical rhombic lip, which is also observed in time-lapse recordings of THN progenitors (see Video 5). Image is a single confocal plane recorded with a confocal microscope (LSM 510; Carl Zeiss, Inc.) and 40x water objective. Note that the dividing cells are present at the apical side of the cerebellum, along the fourth ventricle. LRL, lower rhombic lip; MHB, midbrain–hindbrain boundary; URL, upper rhombic lip.

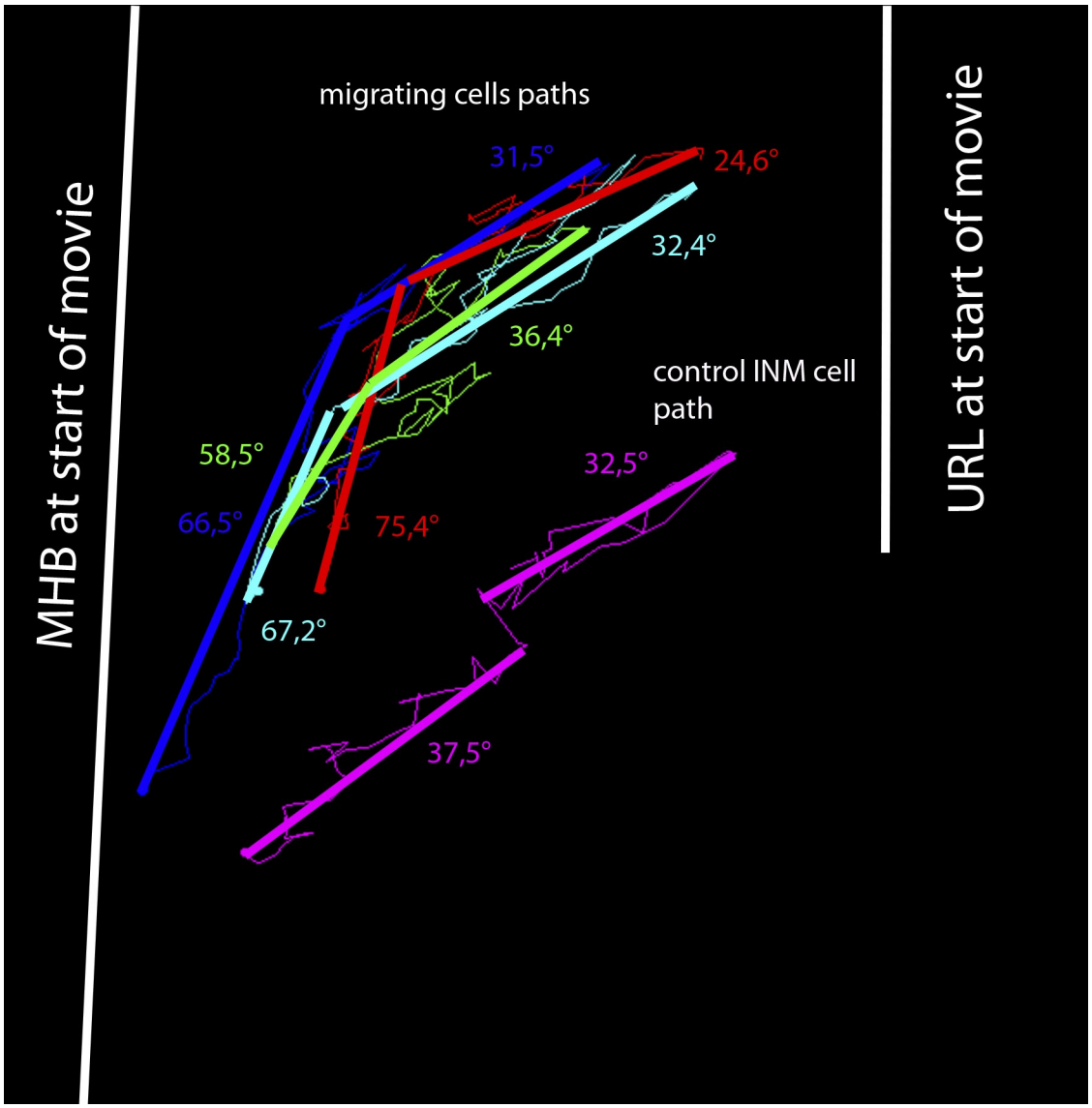


Figure S3. **THN progenitors exhibit a change in migratory angle before ventral migration.** Tracks of THN progenitors (shown in Video 6) during their final movement from the URL toward the MHB and at the start of ventral nucleokinetic migration (blue, green, red, and turquoise) along the MHB. The paths were generated by manually tracking the center of individual nuclei using the Manual Tracking tool in ImageJ. For comparison, the tracks of cells still undergoing INM were also calculated (pink). Changes in the migration angle along the pathway were measured using Adobe Photoshop CS3. This analysis shows that THN progenitors display a turn of ~30 degrees before initiating ventral migration (see Materials and methods for details of analysis).

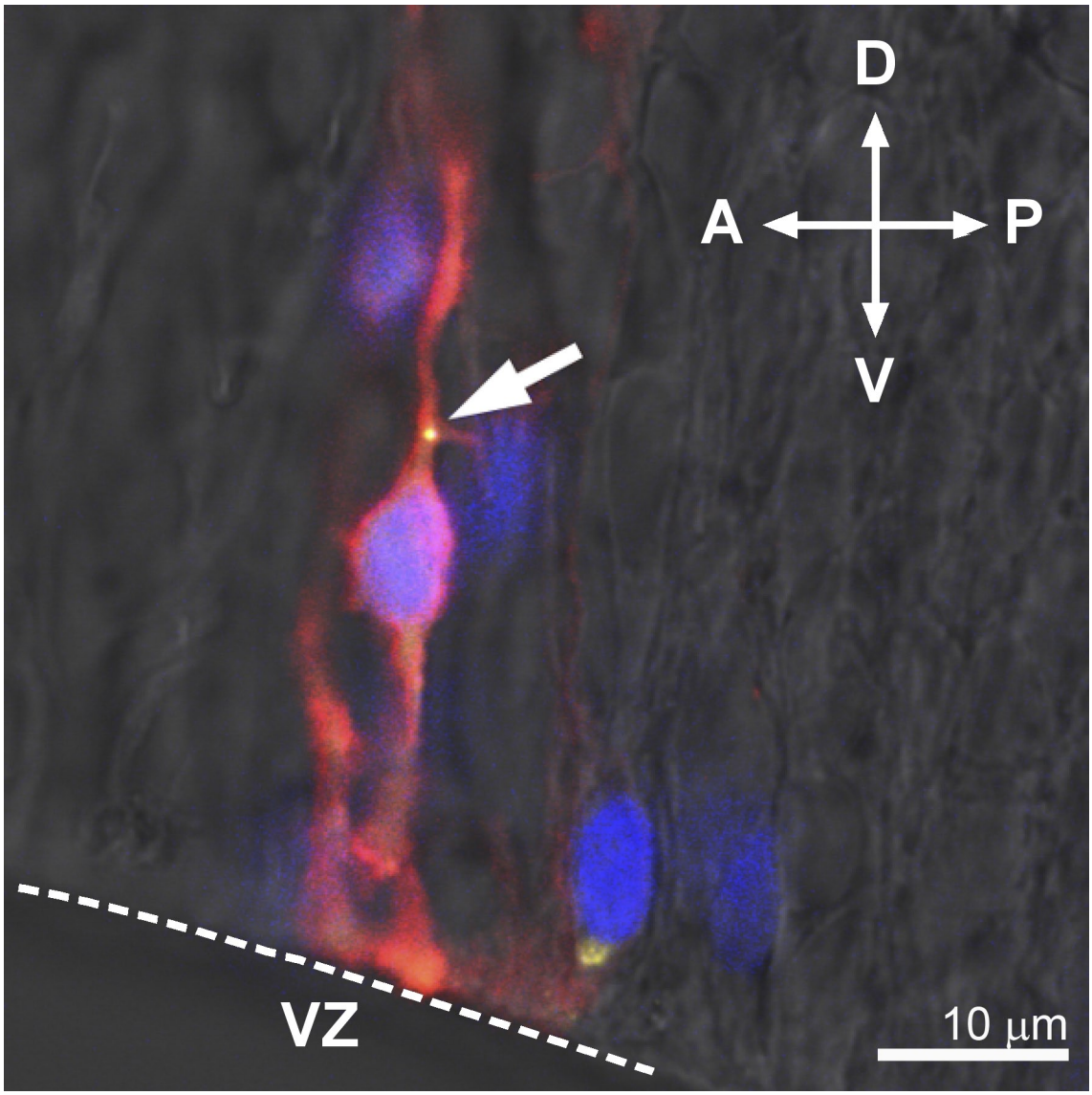
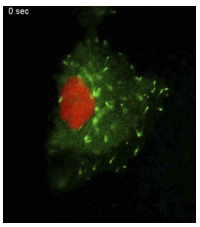
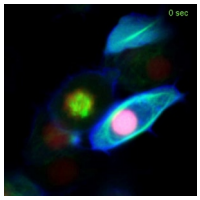


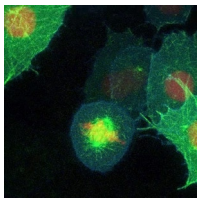
Figure S4. **Medusa vector expression in chick.** Histological section (lateral view, anterior is to the left) of an embryonic chick brain electroporated with vectors M1 and pCS-KalTA4. Neuronal cells in the midbrain tegmentum (ventricular zone is marked by white dashed line) show the expression of subcellular fluorescent fusion proteins encoded by Medusa vector M1 correctly localizing to the membrane (red), nucleus (blue), and centrosome (yellow, white arrow). These data suggest that Janus and Medusa vectors work in a cross-species manner and may be of use for cell biological in vivo studies in avian and mammals. Images were recorded using a 40x water objective and a confocal microscope (LSM 510; Carl Zeiss, Inc.).



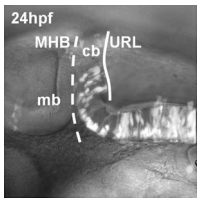
Video 1. **Microtubule dynamics imaged in vivo.** Time-lapse video of microtubule dynamics in keratinocytes of a zebrafish embryo coinjected with M3 and pCS-KalTA4 (each 25 ng/μl) at the one-cell stage. Images are single planes recorded every 2 s using a 63x oil objective and a confocal microscope (LSM 510; Carl Zeiss, Inc.). Images were rendered using Adobe Photoshop CS2 and ImageJ. The video (6 frames/sec) was animated using QuickTime Pro. The video demonstrates that Medusa vectors are capable of reliably visualizing subcellular structures like microtubule plus-ends for in vivo time-lapse microscopy.



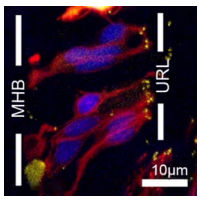
Video 2. **Microtubule dynamics during mitosis.** Time-lapse video of mitotic cells of a gastrulation-stage zebrafish embryo that had been coinjected with M2 (nucleus in red, membrane in blue, and microtubules in green) and KalTA4 at the one-cell stage. Images are single planes recorded every 20 s using a 63x oil objective and a confocal microscope (LSM 510; Carl Zeiss, Inc.). Images were rendered using Adobe Photoshop CS2 and ImageJ. The video was animated at 6 frames/sec using QuickTime Pro. This video demonstrates that the dynamics of cell components during mitosis can be monitored in live zebrafish using Medusa-mediated subcellular labeling.



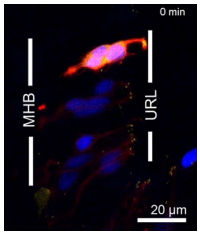
Video 3. **Microtubule plus-end dynamics in vivo.** Time-lapse video of mitotic cells of a gastrulation-stage zebrafish embryo that had been coinjected with M3 (nucleus in red, membrane in blue, and microtubule plus-ends in green) and KalTA4 at the one-cell stage. Images are maximum projections of z-stacks recorded every 2.96 s using a 63x glycerine objective and a confocal microscope (SP5; Leica). Images were rendered using Adobe Photoshop CS2. The video was animated at 10 frames/sec using QuickTime Pro. This video demonstrates that cellular processes like mitosis can be dissected with high precision in time and place using Medusa-mediated subcellular labeling in conjunction with high-speed confocal microscopy.



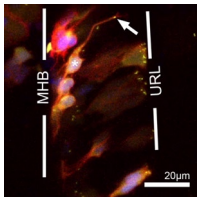
Video 4. **THN progenitor dynamics.** Time-lapse video of THN progenitor dynamics starting at ~24 hpf. Lateral view on the cerebellar anlage of a *Tg(atoh1a:Gal4TA4)*^{hzm2} x *Tg(shhb:Gal4TA4,5xUAS:mRFP)*^{hzm4} transgenic zebrafish embryo. Images are maximum projections of z-stacks (spacing = 2.5 µm; 38 planes) recorded every 12 min using a 40x water objective and a confocal microscope (LSM 510; Carl Zeiss, Inc.). Images were rendered using Adobe Photoshop CS2 and ImageJ. The video (6 frames/sec) was animated using QuickTime Pro. The video shows THN progenitors proliferating at the apical rhombic lip of the cerebellar primordium and the subsequent antero-ventral THN neuron migration. Retraction of long trailing processes is visible as well as formation of axons projecting into the midbrain once the THN neurons approach the MHB.



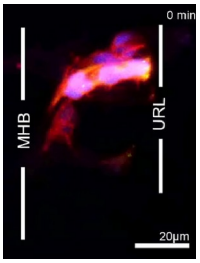
Video 5. **Subcellular dynamics during interkinetic nuclear movements and mitotic cleavages.** Time-lapse video of interkinetic nuclear movements and mitotic cleavages of THN progenitors starting at ~30 hpf. Lateral view of the cerebellar anlage of a *Tg(atoh1a:Gal4TA4)*^{hzm2} transgenic zebrafish embryo injected with vector M1 at the one-cell stage. Images are maximum projections of z-stacks (spacing = 1.8 µm; 26 planes) recorded every 404.2 s using a 40x water objective and a confocal microscope (LSM 510; Carl Zeiss, Inc.). Images were rendered using Adobe Photoshop CS2 and ImageJ. The video (2 frames/sec) was animated using QuickTime Pro. This video shows that the centrosome strictly localizes to the apical site in THN progenitors undergoing INM. Centrosome duplication in mitotic cells can be observed as well as centrosome relocation to the apical site after mitosis.



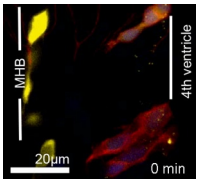
Video 6. **Trailing membrane retraction and centrosome translocation I.** Time-lapse video of trailing membrane retraction and centrosome translocation of THN progenitors starting at ~30 hpf. Lateral view on the cerebellar anlage of a *Tg(atoh1a:Gal4TA4)*^{hzm2} transgenic zebrafish embryo injected with vector M1 at the one-cell stage. Images are maximum projections of z-stacks (spacing = 1.8 µm; 26 planes) recorded every 404.2 s using a 40x water objective and a confocal microscope (LSM 510; Carl Zeiss, Inc.). Images were rendered using Adobe Photoshop CS2 and ImageJ. The video (6 frames/sec) was animated using QuickTime Pro. This video reveals that during anterior migration toward the MHB, THN progenitors are still connected to the apical rhombic lip by trailing processes, which contain the centrosome at their most apical end. This indicates that this phase rather depicts the end of INM rather than neuronal migration. However, as cells are changing positions and are not connected to the basal membrane, this phase also differs from INM.



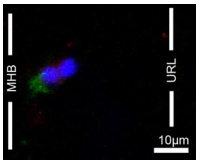
Video 7. **Trailing membrane retraction and centrosome translocation II.** Time-lapse video of the trailing process retraction and centrosome translocation of THN progenitors, starting at ~36 hpf. Lateral view of the cerebellar anlage of a *Tg(atoh1a:Gal4TA4)*^{hzm2} transgenic zebrafish embryo injected with vector M1 at the one-cell stage. Images are maximum projections of z-stacks (spacing = 1.8 µm; 18 planes) recorded every 300 s using a 40x water objective and a confocal microscope (LSM 510; Carl Zeiss, Inc.). Images were rendered using Adobe Photoshop CS2 and ImageJ. The video (6 frames/sec) was animated using QuickTime Pro. This video shows a THN neuron that is already migrating ventrally although the centrosome is localized in the trailing process, indicating that the centrosome is not required to permanently lead the nucleus during ventral migration.



Video 8. **Nucleokinetic ventral migration of THN neurons.** Time-lapse video of ventral migration of THN neurons starting at ~36 hpf. Lateral view of the cerebellar anlage of a *Tg(atoh1a:Gal4TA4)^{hzm2}* transgenic zebrafish embryo previously injected with vector M1. Images are maximum projections of z-stacks (spacing = 1.8 µm; 6 planes) recorded every 522.6 s using a 40x water objective and a confocal microscope (LSM 510; Carl Zeiss, Inc.). Images were rendered using Adobe Photoshop CS2 and ImageJ. The video (6 frames/sec) was animated using QuickTime Pro. This video reveals that the centrosome is not permanently leading the nucleus during ventral migration steps, but is instead overtaken by the nucleus during saltatory movements of the nucleus.



Video 9. **Axonogenesis.** Time-lapse video of axonogenesis in THN neurons starting at ~36 hpf. Lateral view of the cerebellar anlage of a *Tg(atoh1a:Gal4TA4)^{hzm2}* transgenic zebrafish embryo injected with vector M1 at the one-cell stage. Images are maximum projections of z-stacks (spacing = 1.78 µm; 23 planes) recorded every 360 s using a 40x water objective and a confocal microscope (LSM 510; Carl Zeiss, Inc.). Images were rendered using Adobe Photoshop CS2 and ImageJ. The video (6 frames/sec) was animated using QuickTime Pro. The video shows that the centrosome is not in proximity to the site of outgrowth of the presumptive axon in THN neurons.



Video 10. **In vivo visualization of the emerging axon using Kif5c-YFP.** Time-lapse video of axonogenesis in THN neurons starting at ~36 hpf. Lateral view of the cerebellar anlage of a *Tg(atoh1a:Gal4TA4)^{hzm2}* transgenic zebrafish embryo injected with vectors J8 and U14 at the one-cell stage. Images are maximum projections of z-stacks (spacing = 3 µm; 11 planes) recorded every 360 s using a 40x water objective and a confocal microscope (LSM 510; Carl Zeiss, Inc.). Images were rendered using Adobe Photoshop CS2 and ImageJ. The video (6 frames/sec) was animated using QuickTime Pro. This video demonstrates that axons (identified by Kif5c-YFP localization) are formed from the leading edge of THN neurons in the absence of a proximal centrosome.

Table S1. **Collection of expression vectors for labeling subcellular structures in zebrafish**

Subcellular vectors (no. and name)	Labeled structure
S1: #879 pCSCentrin-tdTomato	centrioles
S2: #848 pCSCentrin2-YFP	centrioles
S3: #783 pEGFP-centrin2	centrioles
S4: #571 pCSH2B-mRFP	nucleus
S5: #895 pCSH2B-CFP	nucleus
S6: #938 pCSmemCFP	membrane
S7: #625 pCSmemmRFP	membrane
S8: #894 pCSDCX-tdTomato	microtubules
S9: #1406 pCSTau-GFP	microtubules
S10: #771 pCSEB3-GFP	microtubule plus-ends
S11: #765 pEB1-GFP	microtubule plus-ends
S12: #772 pEGFP-β3Tubulin	microtubules
S13: #88 pCSGFP-Tubulin	microtubules
S14: #801 pCSGFP-DCX	microtubules
S15: #1593 pCSYFP-DCX	microtubules
S16: #1424 pCSCitrine-Actin	actin cytoskeleton
S17: #926 pmito-DsRedT4-N1	mitochondria
S18: #1497 pDsRed2-ER	endoplasmic reticulum
S19: #1341 pCSGolgi-Citrine	Golgi apparatus
S20: #2328 pCSKif5c-YFP	axon

UAS vectors (no. and name)	Labeled structure
U1: #859 pSK5xUAS:memmRFP	membrane
U2: #860 pSKmemmRFP:5xUAS	membrane
U3: #922 pSK1xUAS:Centrin2-tdtomato	centrosome
U4: #925 pSK5xUAS:H2B-CFP	nucleus
U5: #996 pSK5xUAS:Centrin2-YFP	centrosome
U6: #997 pSK5xUAS:memCFP	membrane
U7: #1274 pSK1xUAS:H2B-CFPGI	nucleus
U8: #1275 pSK1xUAS:memCFPGI	membrane
U9: #1429 pSK1xUAS:Centrin2-YFPGI	centrosome
U10: #1404 pSK1xUAS:Citrine-DCXGI	microtubules
U11: #1597 pSKH2B-CFP:3xUAS	nucleus
U12: #1768 pSK4xUAS:GFPGI	not localized
U13: #1769 pSK14xUAS:GFPGI	not localized
U14: #2339 pSK5xUAS:Kif5c-YFP	axon
U15: #699 pSK14xUAS:H2B-mRFP	nucleus
U16: #709 pSKH2B-mRFP:5xUAS	nucleus
U17: #627 pSK14xUAS:H2B-GFP	nucleus
U18: #665 pSKH2B-GFP:5xUAS	nucleus
U19: #2622 pSK5xUAS:Tau-GFP	microtubules

Janus vectors (no. and name)	Labeled structure 1	Labeled structure 2
J1: #828 pBH2B-RFP:5xUAS:GFP-DCX	nucleus	microtubules
J2: #939 pSKmemmRFP:5xUAS:H2B-CFP	membrane	nucleus
J3: #827 pBH2B-RFP:5xUAS:GFP-Centrin2	nucleus	centrosome
J4: #780 pBH2B-mRFP:5xUAS:EB3-GFP	nucleus	microtubule plus-ends
J5: #1336 pSKH2B CFPGI:1xUAS:memCFPGI	nucleus	membrane
J6: #1525 pSKCentrin2-YFPGI:1xUAS:memCFPGI	centrosome	membrane
J7: #1773 pSKGFP:5xUAS:mRFP	cytosol	cytosol
J8: #2146 pSKH2B-CFP:5xUAS:Centrin2-tdtomato	nucleus	centrosome
J9: #1773 pSKGFP:5xUAS:mRFP	not localized	not localized
J10: pSKmRFP:5xUAS:GFP	not localized	not localized

Medusa vectors (no. and name)	Labeled structure 1	Labeled structure 2	Labeled structure 3
M1: #1595 pSKH2B-CFP:5xUAS:memmRFP-5xUAS:Centrin2-YFP	nucleus	membrane	centrosome
M2: #998 pSKH2B-mRFP:5xUAS:GFP-DCX-5xUAS:memCFP	nucleus	microtubules	membrane
M3: #999 pSKH2B-mRFP:5xUAS:EB3-GFP-5xUAS:memCFP	nucleus	microtubule plus-ends	membrane
M4: #1495 pSKH2B-CFPGI:1xUAS:memCFPGI-1xUAS:Centrin2-YFPGI	nucleus	membrane	centrosome