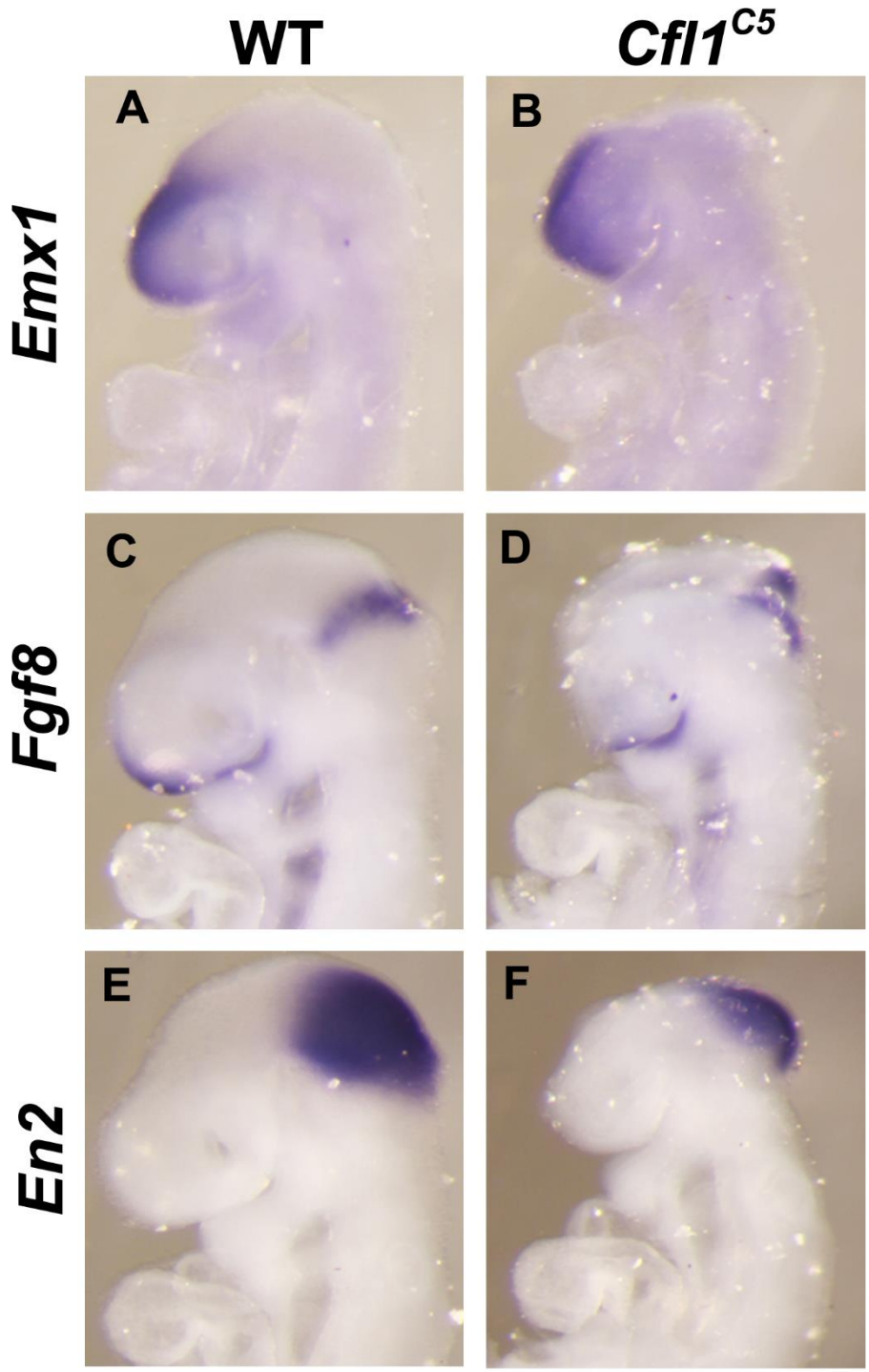


Figure S1. Normal anterior-posterior patterning in the developing *Cfl1*^{C5} brain.
In situ hybridization of E9.5 WT and *Cfl1*^{C5} embryos with **A, B)** the forebrain marker *emx1*, **C, D)** the midbrain-hindbrain junction marker *fgf8* and **E, F)** the hindbrain marker *en2*.

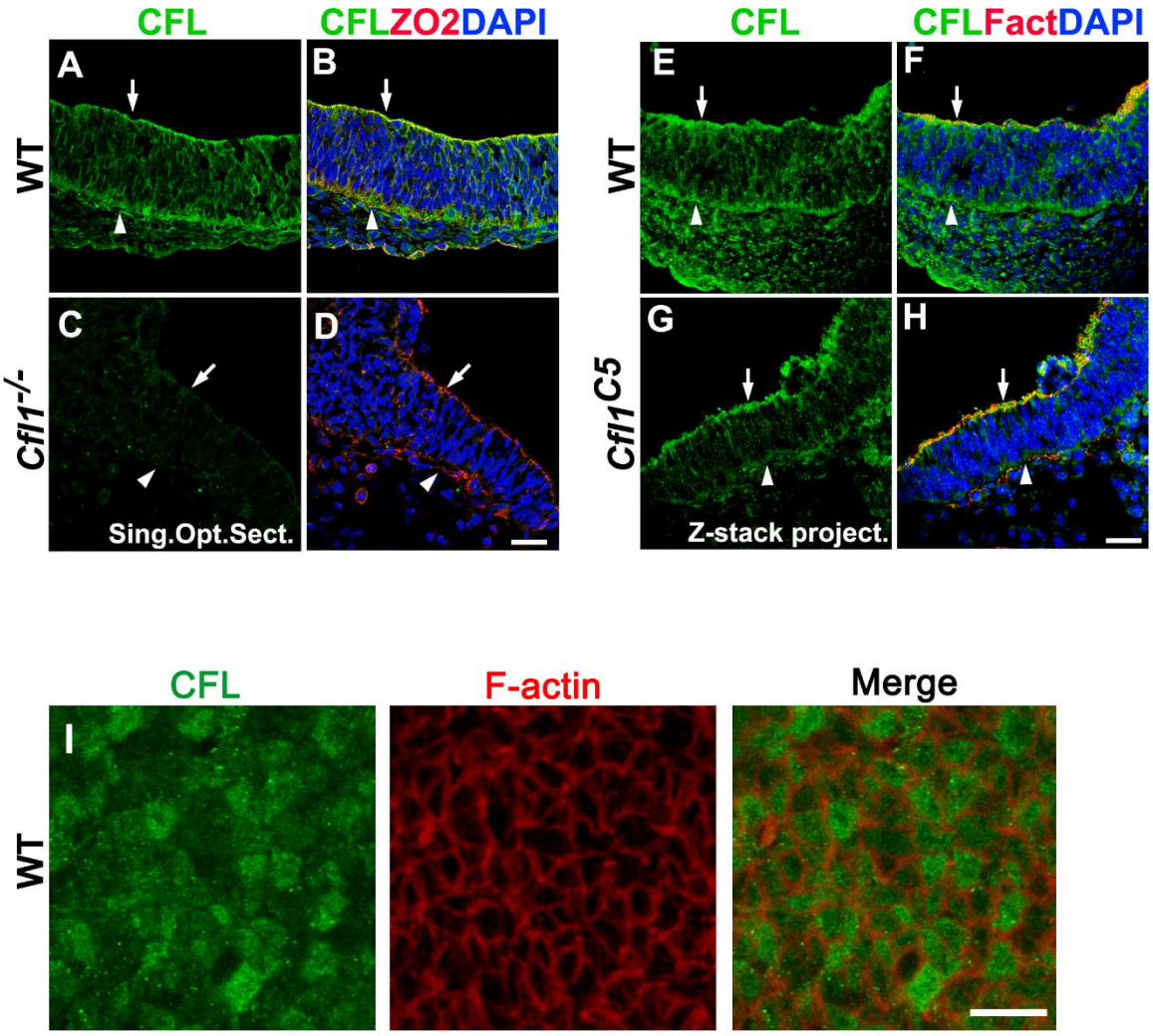


Figure S2. CFL expression in WT and *Cfl1*^{tm1Wit} E9.5 neural plate.
Immunodetection of CFL and ZO2 in transverse sections of WT (**A, B**) and the *Cfl1*^{tm1Wit} **null** allele at E9.5 (**C, D**) shows the specificity of CFL staining. Z-stack projection of 3 optical sections taken every 1μm, showing double staining of CFL and F-actin in WT (**E, F**) and *Cfl1*^{C5} (**G, H**) transverse sections. **(I)** *en face* images of CFL (green) and F-actin (red) expression of the apical domain of wild-type E9.5 cephalic neural tube. Arrows indicate apical, arrowheads indicate basal. Scale bar in A-D is 30 μm; in E is 10 μm.

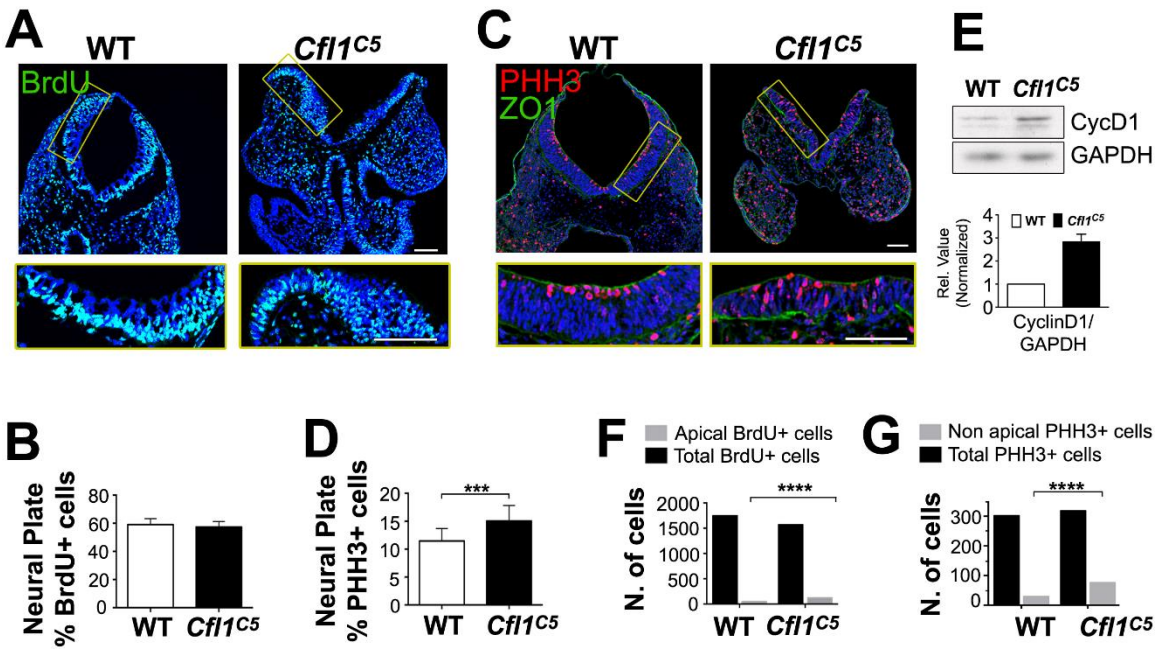
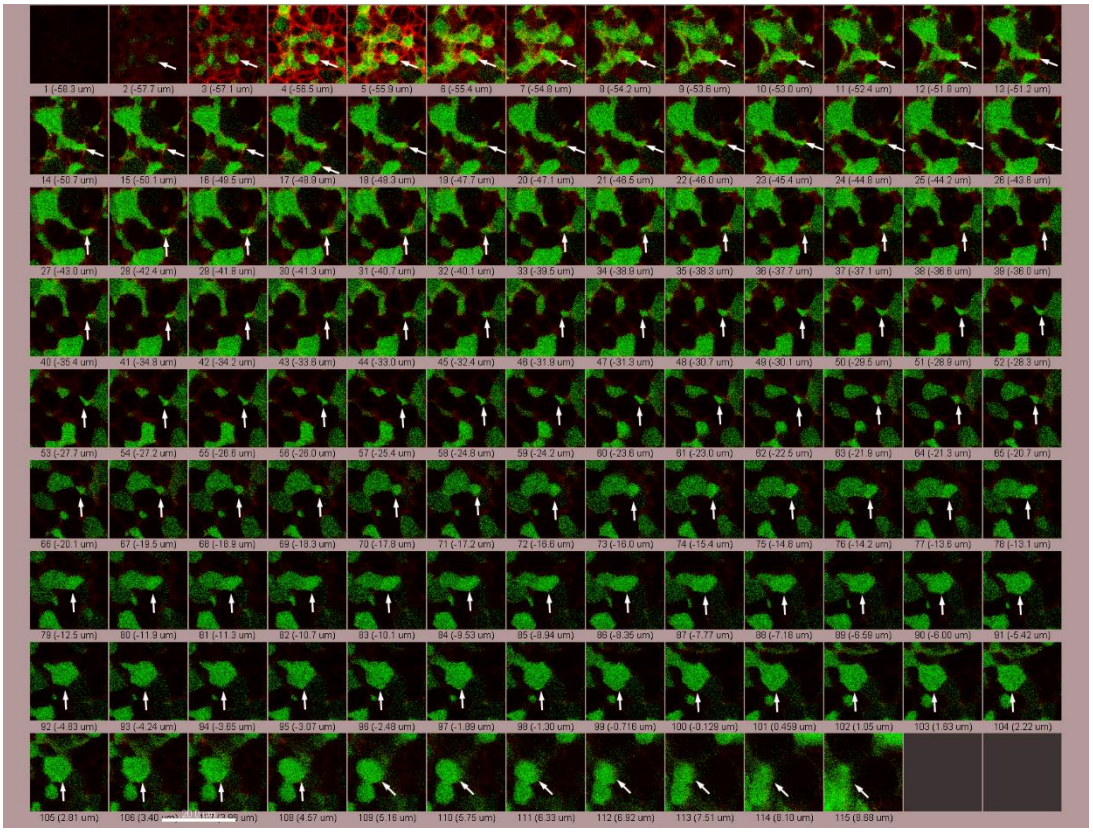


Figure S3. Proliferation analysis in *Cfl1*^{C5} mutant embryos.

A) BrdU^{+} cells (green) after a 25 min pulse in E9.5 WT and *Cfl1*^{C5} transverse cephalic sections. **B)** Quantification S-phase neural plate cells. WT: 1692 BrdU^{+} cells out of 3024 total, n=3 embryos; 56 ± 5%. *Cfl1*^{C5}: 1578 BrdU^{+} cells out of 2871 cells, n=3 embryos; 55 ± 4%; p = 0.0932; **C)** Phospho-histone H3 (PHH3) (red) and ZO1 (green) staining in E9.5 WT and *Cfl1*^{C5} transverse cephalic sections. **D)** Mitotic index of cephalic neural plate: WT: 277 PHH3^{+} cells out of 2321 total; 12 ± 2%; *Cfl1*^{C5}: 224 PHH3^{+} cells out of 1361; 15 ± 3%; ***p = 0.0001. **E**) Western blot of Cyclin D1 and GAPDH, and quantification normalized to WT. **F-G)** Analysis of interkinetic nuclear migration. **F**) Quantification of apical BrdU^{+} cells. WT: 48 apical BrdU^{+} out of 1747 cells, n=3 embryos; 3 ± 1%. *Cfl1*^{C5}: 123 apical BrdU^{+} out of 1569 cells, n=3 embryos; 8 ± 2%; ****p < 0.0001. **G)** Quantification of non-apical mitotic cells: WT: 29 out of 302; 10 ± 2%; *Cfl1*^{C5}: 76 out of 318; 24 ± 11%; ****p < 0.0001. Scale bars: A and D 80 μm. Statistical analysis was performed using Chi square (Fisher's exact) test. Error bars indicate standard deviation.



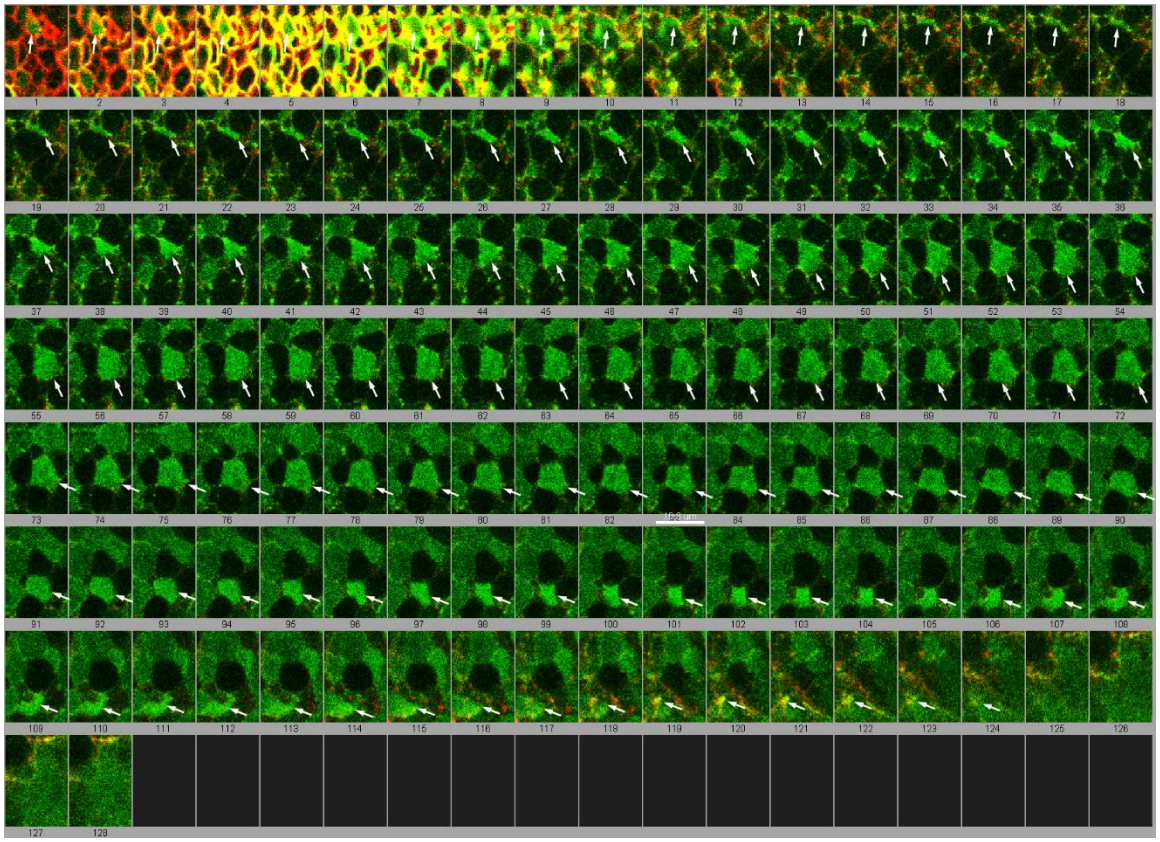


Figure S4 and S5. Single optical sections along the Z axis of WT (Fig. S4) and *Cfl1*^{C5} (Fig. S5) neuroepithelium.

Single planes used to make the 3D rendering shown in Fig. 3D. Red is F-actin: Green is X-linked GFP. Arrows point a single cell along the apicobasal axis.

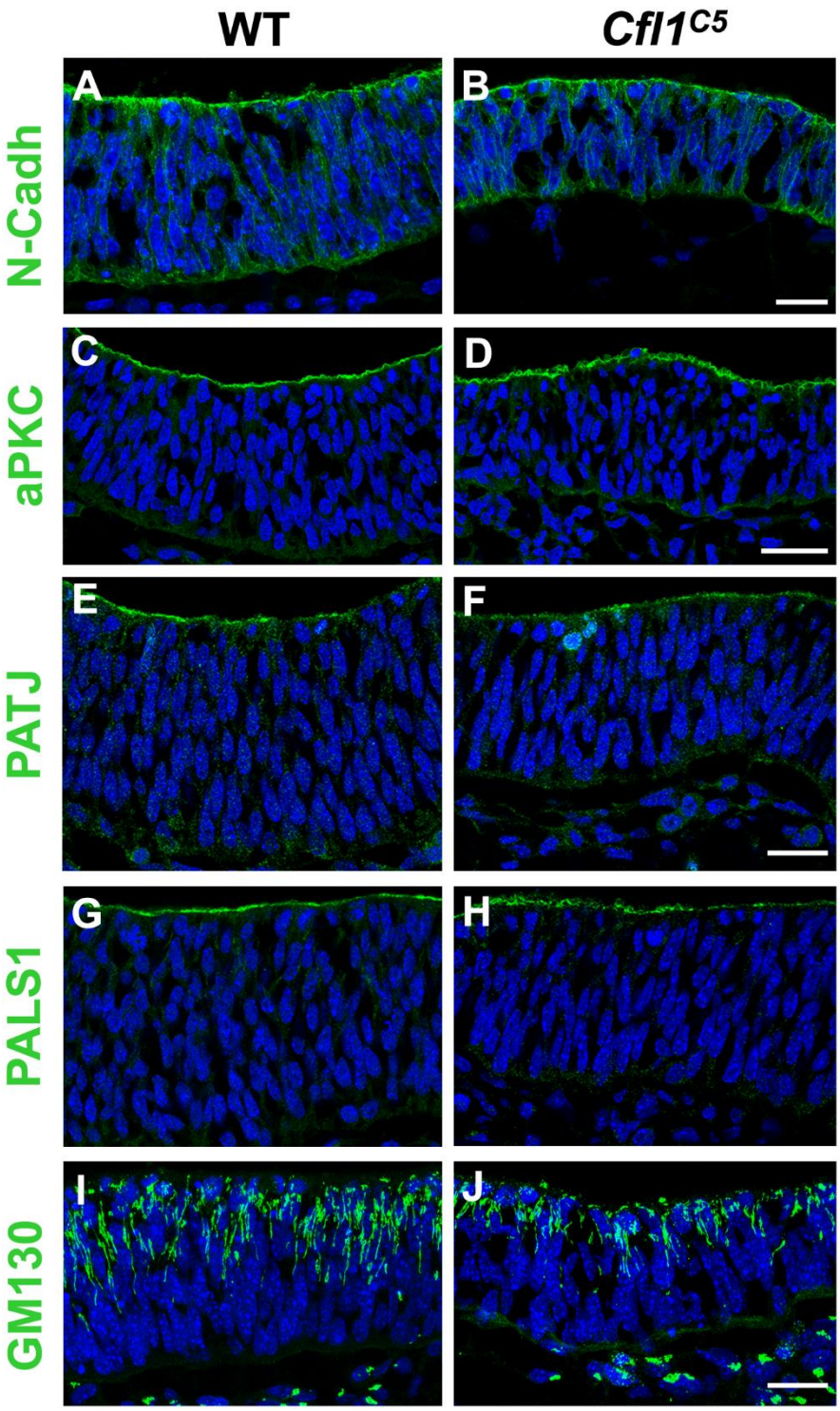


Figure S6. Normal apical-basal polarity marker expression in the *Cfl1*^{C5} neural plate. Transverse sections of E9.5 WT and *Cfl1*^{C5} cephalic neural plates stained for **A, B)** N-Cadherin, **C, D)** aPKC, **E, F)** PATJ, **G, H)** PALS1, **I, J)** GM130. Blue is DAPI. **A**, **B**, **I** and **J** are Z-stack projections of 3 single optical sections taken every 1μm. Scale bar is 30μm.

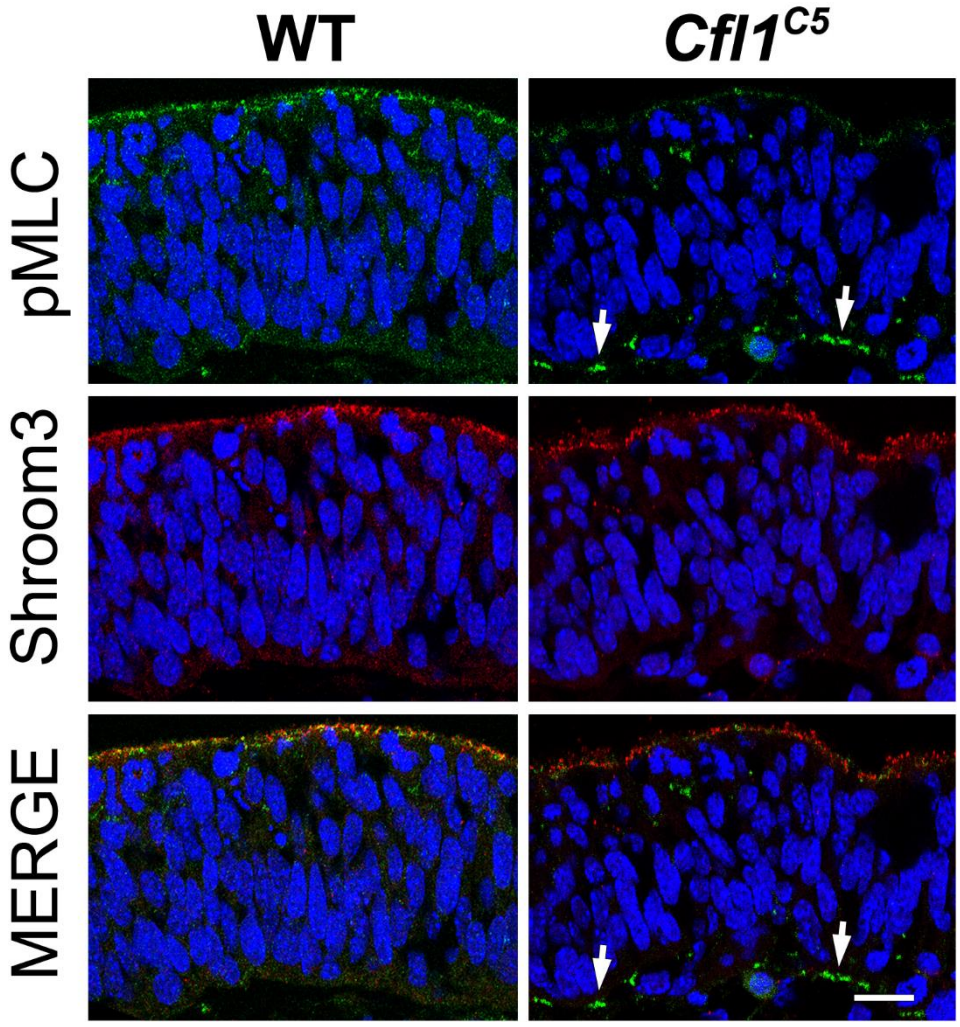


Figure S7. Double labeling of pMLC and SHROOM3 in WT and *Cfl1*^{C5}embryos at E9.5.

Z-stack projections of 3 optical sections taken every 1 μm. from a cephalic transverse section. Arrows indicates ectopic localization. Scale bar is 30 μm.

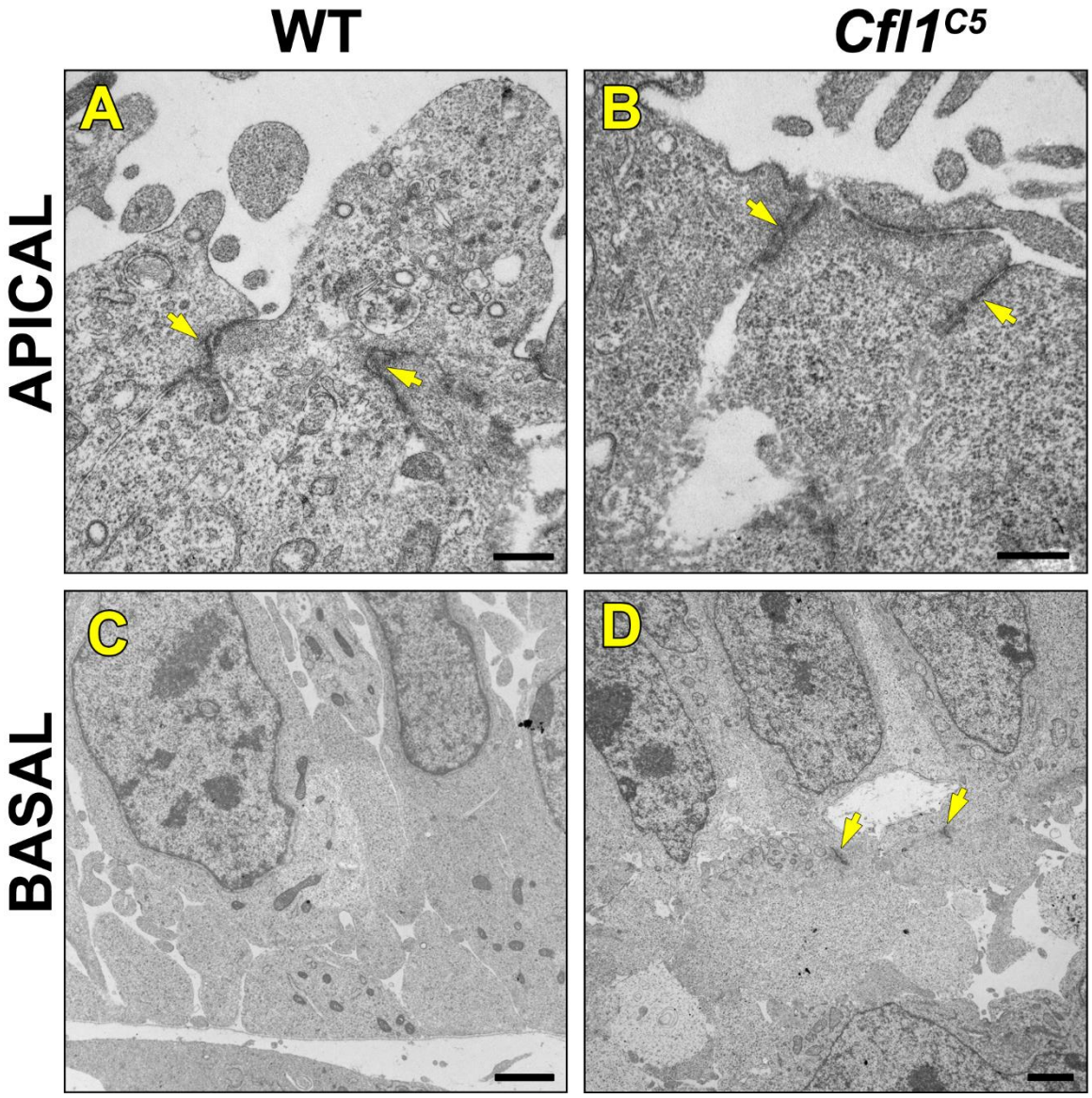


Figure S8. Ultrastructure of apical and basal domains of cephalic neural plate.
TEM analysis of E9.5 WT and *Cfl1*^{C5} cephalic neural plates. **A, B)** Apical domain: **B, C)** Basal domain. Arrows indicate electron-dense particles. Scale bar in A and B is 500nm; in C and D is 2μm.

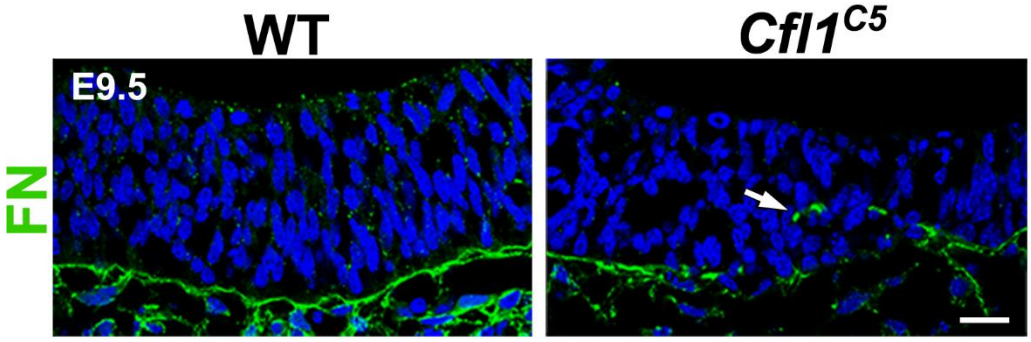
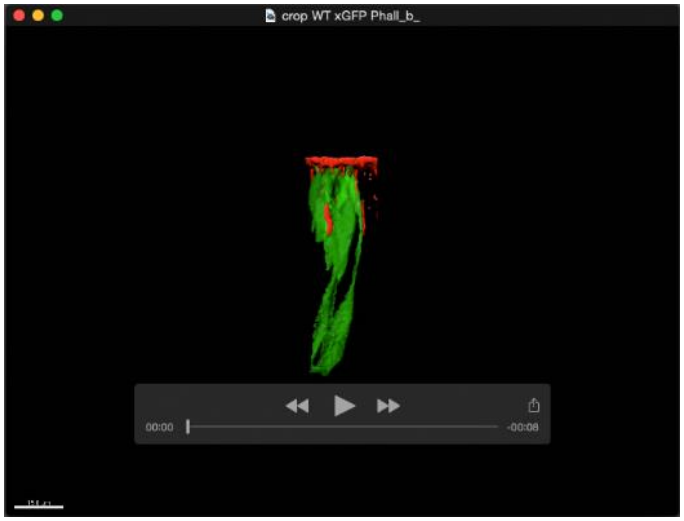
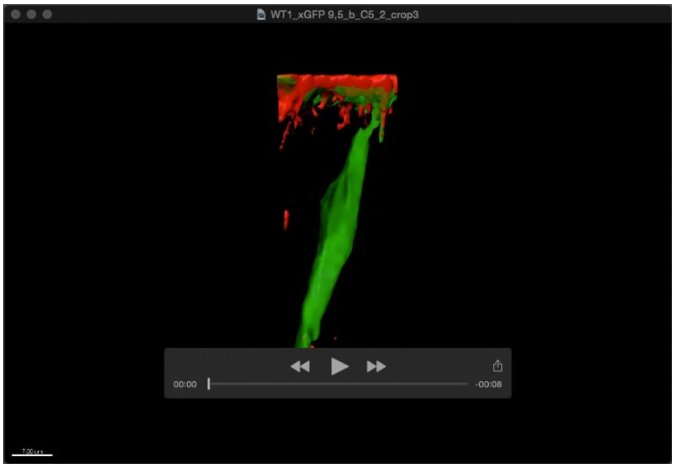


Figure S9. Ectopic localization of fibronectin in *Cfl1*^{C5} and ectopic cell clusters in the *Cfl1*^{C5} neural plate A) Fibronectin expression in E9.5 cephalic neural plate. Arrows indicate ectopic expression. Blue is DAPI. Scale bar 30 μm.



Movie 1. 3D rendering of a E9.5 WT X-linked GFP^{+} cephalic neuroepithelial cell. F-actin (red) is apical; no overlapping signal can be detected at the basal domain.



Movie 2. 3D rendering of a E9.5 *Cfl1*^{C5} X-linked GFP^{+} cephalic neuroepithelial cell. F-actin (red) is apical and basal, overlapping with endogenous GFP.