Supplementary Materials and Methods

Primer sequences

Genotyping primers:

Δ28-30-wtF: 5'-TGAGAGAGGACACCTACTCT-3';

Δ28-30-wtR: 5'-GCAGGGAGCAAACTGTCTCTT-3';

Δ28-30-mutR: 5'-GGTGTGACCTTTGTGCGAGA-3'.

RT-qPCR primers:

Ttr F: 5'-AAAGTCCTGGATGCTGTCCG-3'

Ttr R: 5'-TTCTCATCTGTGGTGAGCCC-3'

Aqp1 F: 5'-CAGTACCAGCTGCAGAGTGC-3'

Aqp1 R: 5'-CATCACCTCCTCCCTAGTCG-3'

Lmx1a F: 5'-TGAGTGTCCGTGTGGTTCAG-3'

Lmx1a R: 5'-CCCGCATTCCCACTACCATT-3'

Otx2 F: 5'-CTGACCTCCATTCTGCTGCT-3'

Otx2 R: 5'-GGAAGAGGTGGCACTGAAAA-3'

Gmnc F: 5'-GAGGCTCAGCTCTCATCTCA-3'

Gmnc R: 5'-ATGACAGCAACTTCTTGGCC-3'

Mcidas F: 5'-CCAGCTCTCACAACCATAGAC-3'

Mcidas R: 5'-GCATCTCTGAAATTCTGCAGG-3'

Msx2 F: 5'-GGAAAATTCCGAAGACGGAG-3'

Msx2 R: 5'-CTTCCGGTTGGTCTTGTGTT-3'

Grem1 F: 5'-CCTTTCTTTTTCCCCTCAGC-3'

Grem1 R: 5'-ACAGCGAAGAACCTGAGGAC-3'

Wnt1 F: 5'-AAATGGCAATTCCGAAACC-3'

Wnt1 R: 5'-GAGGTGATTGCGAAGATGAA-3'

Wnt3 F: 5'-CTTCTAATGGAGCCCCACCT-3'

Wnt3 R: 5'-GAGGCCAGAGATGTGTACTGC-3'

Gdf7 F: 5'-GGCTTCACAGACCAAGCAAC-3'

Gdf7 R: 5'-GCACTGTCCCTGTCTGGTTC-3'

I*gf2* F: 5'-GGTTTGCATACCCGCAGCA-3'

I*gf2* R: 5'-CACAAGGCGAAGGCCAAAGA-3'

Akap12 F: 5'-CCTGACAGAATCCTAAGACGTG-3'

Akap12 R: 5'-GGTTGAAATCATTGGACGGC

Foxj1 F: 5'-ACGGACAACTTCTGCTACTTC-3'

Foxj1 R: 5'-CTCCCGAGGCACTTTGATG-3'

β-actin F: 5'-CTGTCGAGTCGCGTCCACC-3'

β-actin R: 5'-TCGTCATCCATGGCGAACTG-3'

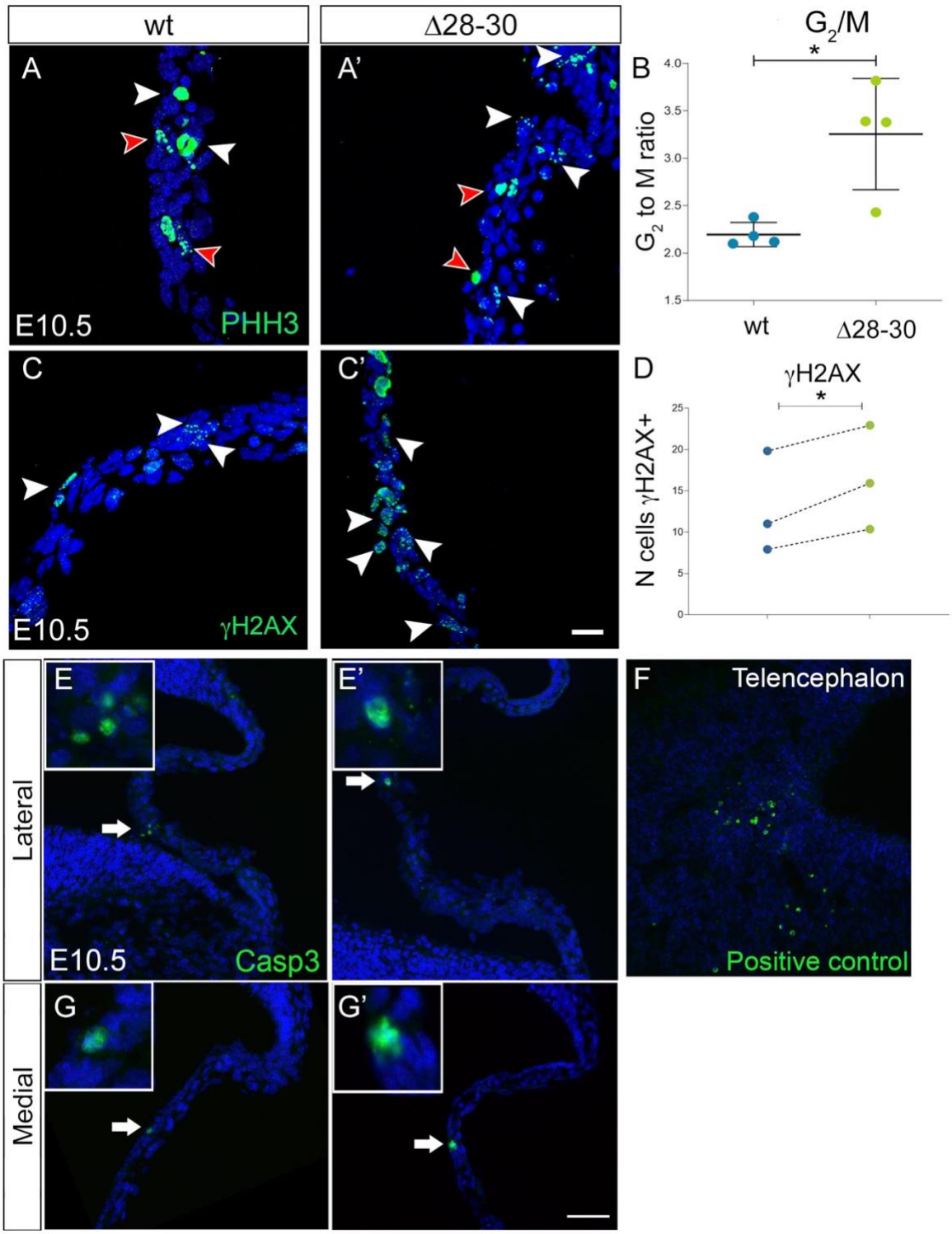


Fig S1 - Stall of cell cycle progression and increase of DNA damage markers in the Δ28.30 hChP. A-G'. Immunostaining of parasagittal cerebellar sections at E10.5. A-A'. PHH3 antibody detects cells in G_2 phase (dotted staining, red arrowheads) and in M phase (full staining, white arrowheads). In the mutant hChP progenitors the G_2/M ratio is increased indicating a delay in cell cycle progression from G_2 to M phase (A',B). B.

Unpaired t-test with Welch's correction was performed to determine statistically significant differences of the ratio G2/M between wt and mutant (*, $P < 0.05$). C,C'. In the mutant the DNA damage marker γH2AX is slightly but significantly increased (C',D). D. Paired t-test was performed to determine statistically significant differences between wt and mutant (*, $P < 0.05$). G,G'. No differences in cell death were observed in the mutant hChP compared to the wt (E',G'). A positive control of caspase3 immunostaining is shown in F. Size bar: 20μm in A-C', 50μm in E-G'.

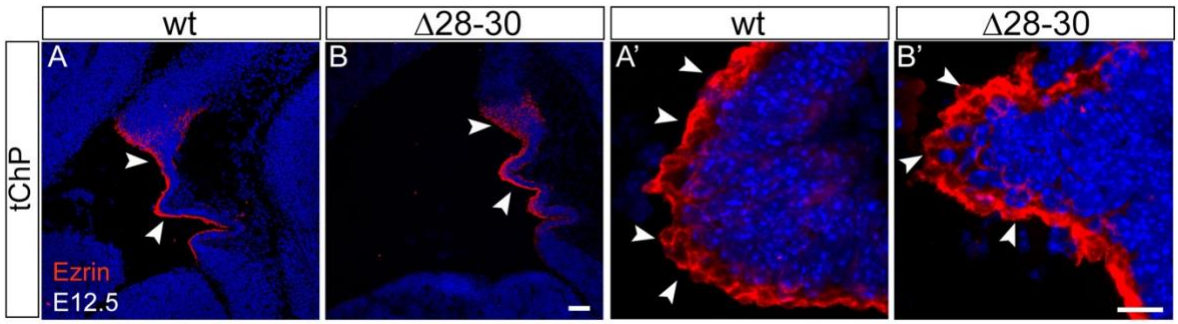


Fig S2 - The apical microvillus marker Ezrin is normally expressed in the mutant telencephalic ChP. A-B'. Parasagittal sections of the lateral ventricles at E12.5, stained fort Ezrin. Ezrin is expressed at high levels and apically located in wt and mutant epithelia alike in the developing tChP (white arrowheads). Size bar: 100μm in A, B, 10μm in A', B'.

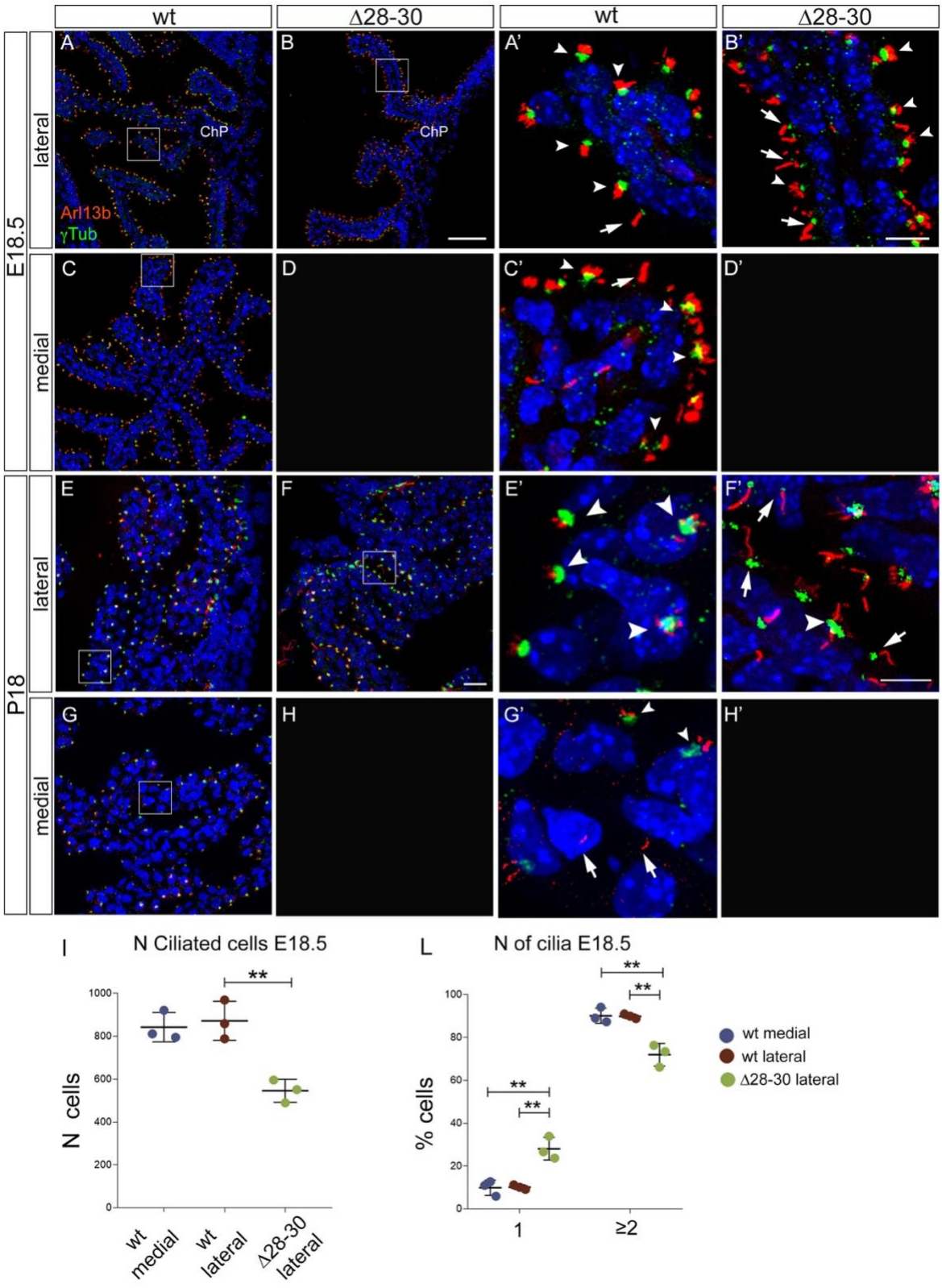


Fig S3 - Ciliary defects persist in the mutant hChP at birth and in adulthood. A-H'. Parasagittal cerebellar sections at late embryonic (E18.5) and postnatal (P18) stages, stained for Arl13b and γTubulin, to visualize cilia and basal bodies, respectively. At E18.5, most wt hChP epithelial cells are multiciliated, with multiple γTubulin+ basal bodies and Arl13b-positive cilia (A,A' white arrowheads), while few monociliated cells (A,A' white arrow) can be observed in lateral parasagittal sections of the hChP. Conversely, in the

mutant, the minute hChP segment confined to the lateral most aspect of the 4th ventricle, exhibits more monociliated (C', white arrows) and fewer multiciliated (C', white arrowheads) cells than the wt. At E18.5, the medial segment of the hChP is totally absent in mutant embryos (D). At P18, the wt hChP epithelial cells display mostly multiciliated cells with multiple γTubulin-positive basal bodies and Arl13b-positive cilia (E,E'' white arrowheads), in lateral sections of the hChP. Conversely the small lateral segment of the mutant hChP exhibits mostly monociliated (F,F', white arrows) and only a minority of multiciliated (F,F' white arrowheads) cells compared to the wt. Again, the bulk of the hChP is deleted in mutant embryos (H). I. The histogram shows the number of ciliated cells in the lateral and medial section of the wt hChP, and in the lateral section of the mutant hChP at E18.5. Data are plotted as mean ± SD. N=3/genotype. A two-tailed unpaired (Welch's) t-test was performed to determine statistically significant differences between lateral sections of the wt and mutant (**, $P < 0.005$). L. In the histogram the percentage of cells with 1 cilium or ≥2 cilia for genotype. A 2way ANOVA multiple comparison was performed to determine statistically significant differences between samples. Data are plotted as mean ± SD. Bonferroni post-hoc analysis was applied. (**, $P < 0.005$). Size bar: 20μm in A,B,C, 10μm in A',B',C', 20μm in E,F,G, 10μm in E',F',G'.

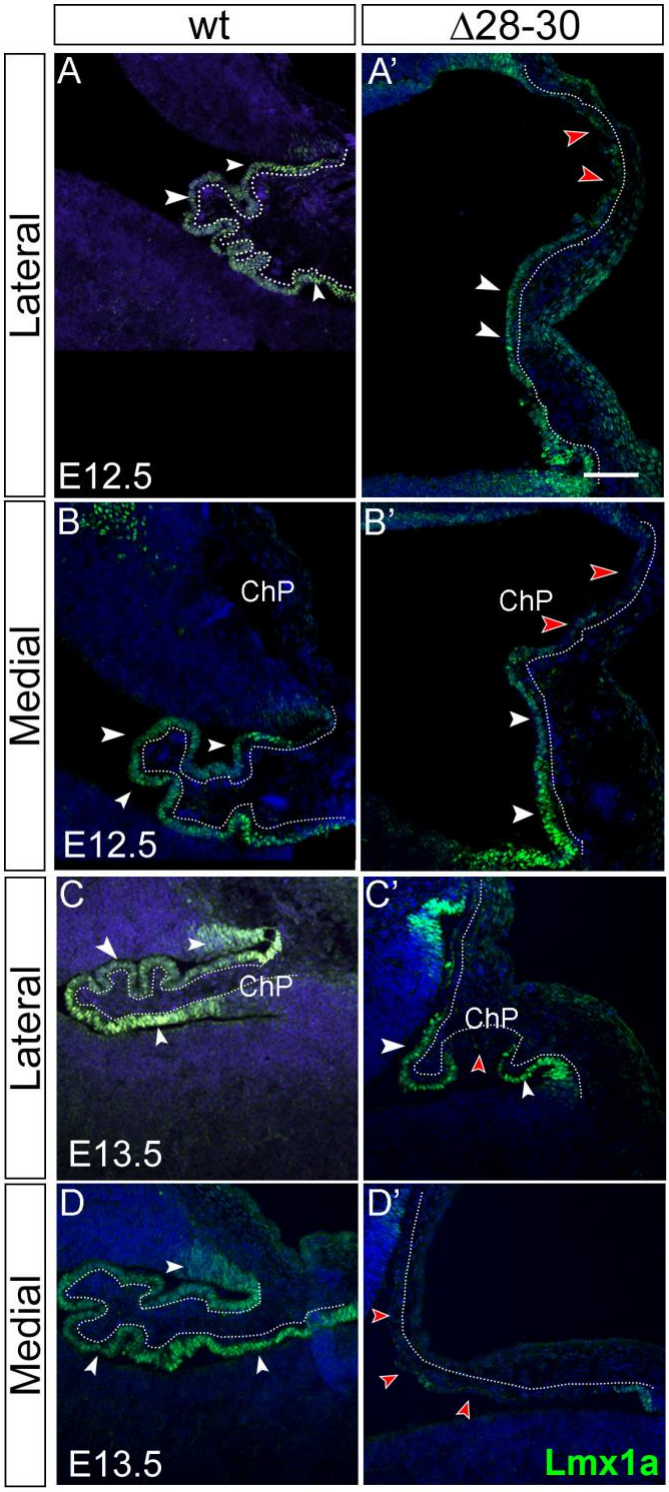


Fig S4 - Loss of Lmx1a protein expression in the *Zfp423* mutant epithelium. A-D'. Parasagittal cerebellar sections at different stages of development, stained for Lmx1a to visualize its distribution in the developing hChP. A-B'. Lateral and medial parasagittal cerebellar sections at E12.5 show a robust expression of the protein in the wt columnar epithelium (A and B, white arrowheads and dotted lines), while the expression in mutant epithelium is discontinuous (A' and B', red arrowheads and dotted lines). C,D', lateral and medial parasagittal cerebellar sections at E13.5 show robust expression of the protein in the wt columnar epithelium (white arrowheads and dotted lines in C and D), while the expression in mutant epithelium is discontinuous in the lateral section (C' red arrowheads) and absent in the medial section (D' red arrowheads). Size bar: 100μm in A-D'.

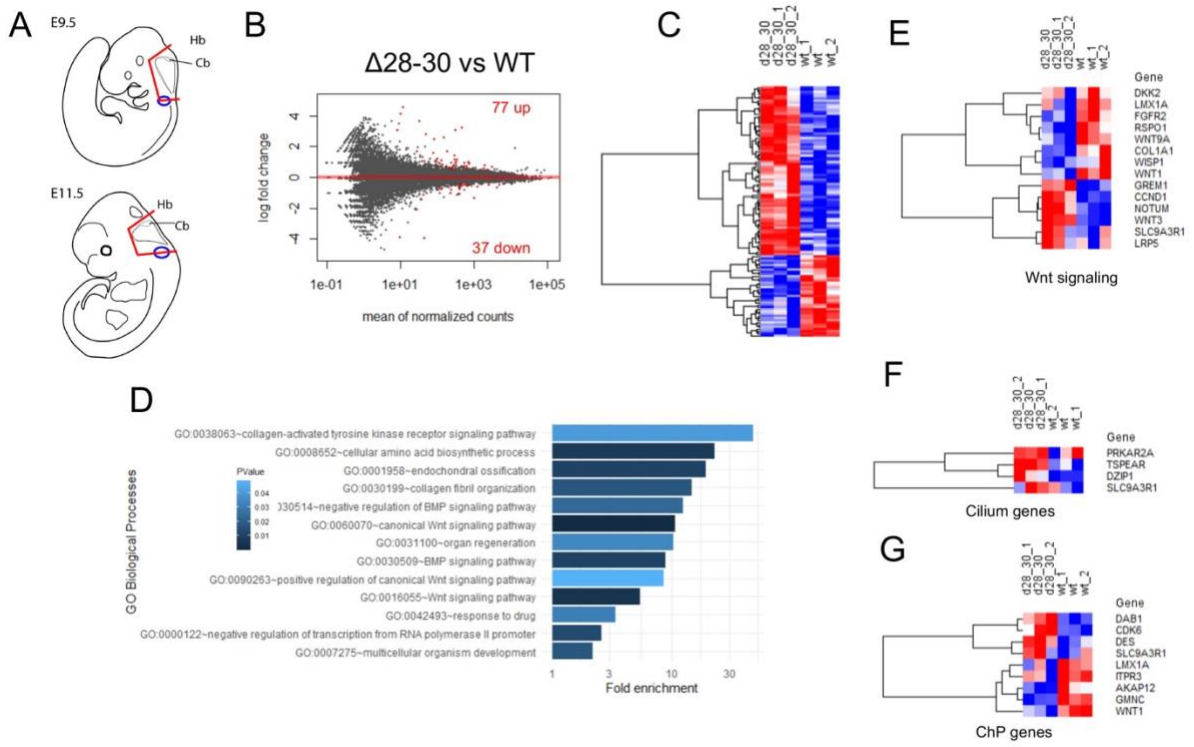


Fig S5 - Differential RNA sequencing analysis of wt vs Δ28-30 hindbrain at 9.5 days of embryonic development. A. Schematic representation of the sectioning carried out on E9.5 and E11.5 embryos. Red lines indicate the boundaries of the tissue specimen analyzed by RNA-seq (E9.5) and by RT-qPCR (E9.5, E11.5 and E13.5). The blue circle indicates the otic vesicle used as a caudal reference point. B. MA plot showing log2 fold changes as a function of average gene expression. Differentially expressed genes (DEGs) with false discovery rate (FDR)<0.1 are highlighted in red. C. Heatmap of the DEGs with FDR<0.1. D. Functional enrichment analysis of gene ontology biological processes. E-G. Heatmaps showing DEGs related to Wnt signaling pathway (E), Cilium-associated (F), and ChP-associated (G) DEGs.

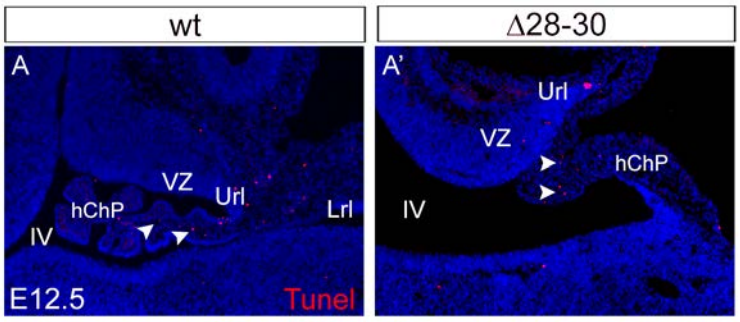


Fig S6 – No differences in cell death were observed in the mutant hChP compared to the wt. A-A' E12.5 parasagittal cerebellar sections processed for Tunel staining. Only few cells were positive in wt and mutant sections alike (white arrowheads). hChP: hindbrain choroid plexus; IV: 4th ventricle, Url: upper rhombic lip; Lrl: lower rhombic lip; VZ: ventricular zone. Size bar: 50μm.

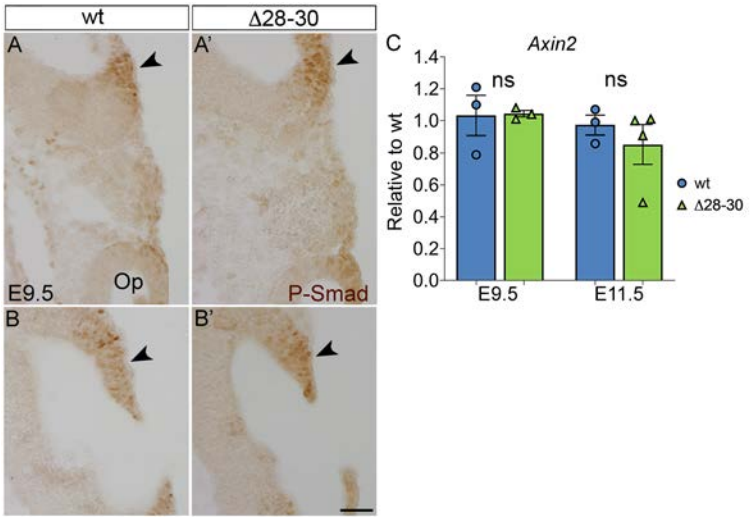


Fig S7 – No substantial alterations of BMP signalling and canonical Wnt signaling are observed in the mutant. A-B'. E9.5 parasagittal sections stained for P-Smad. The P-Smad signal is unchanged in the mutant hindbrain compared to the wt (arrowheads). E. RTqPCR experiments reveal unaltered expression of *Axin2* in the mutant at E9.5 and E11.5. ns = not significantly different. n= 3/genotype at E9.5; n= 3/wt and n= 4/ 28-30 at E11.5. Results are plotted as mean ± s.e.ms; Welch's unequal variances t-test. Op: otic pit. Size bar: 50μm.

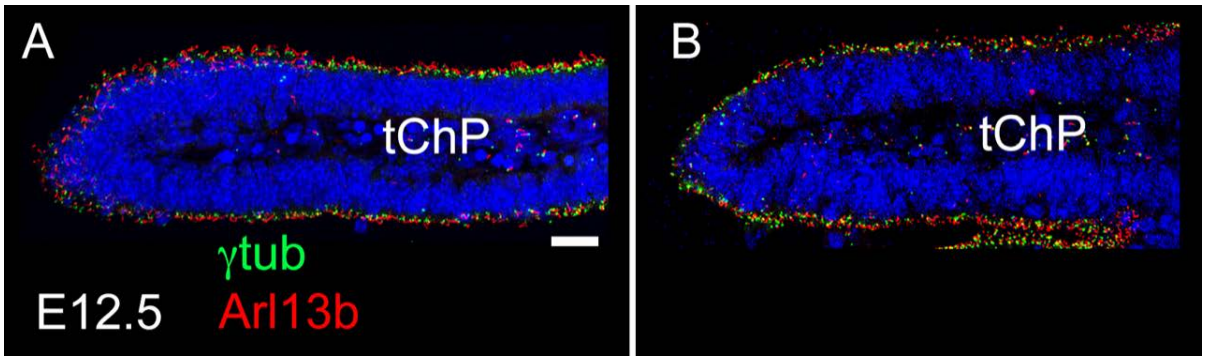
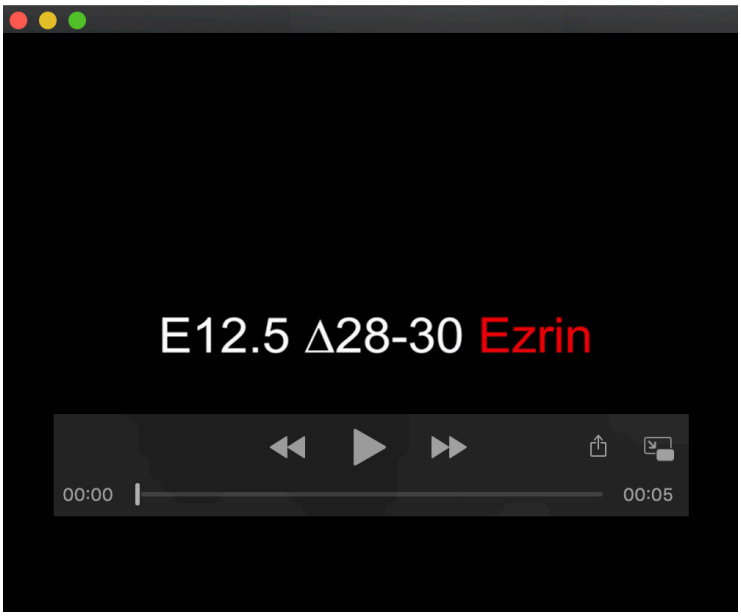


Fig. S8. Zfp423 does not affect tChP development. A,B. In the lateral ventricles wt (A) and mutant (B) tChP display a columnar and multiciliated epithelium positive for Arl13b+ and γ-tubulin. Scale bar: 20 μm.

Movie 1. Ezrin is strongly expressed on the apical margin of the wt hChP epithelium. E12.5 cerebellar section immunostained for the apical microvilli marker Ezrin shows a strong expression on the apical margin of the wt hChP epithelium (white arrowheads)



Movie 2. Ezrin is poorly polarized in the mutant hChP epithelium. E12.5 cerebellar sections immunostained for the apical microvilli marker Ezrin shows a weaker, patchy and poorly polarized signal in the mutant epithelium, extending to the basal domain of some hChP cells (red arrowheads)