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Supplemental Information

EGFR Signaling Termination via Numb Trafficking in Ependymal Progenitors Controls Postnatal Neurogenic Niche Differentiation

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Supplemental Figures and Legends

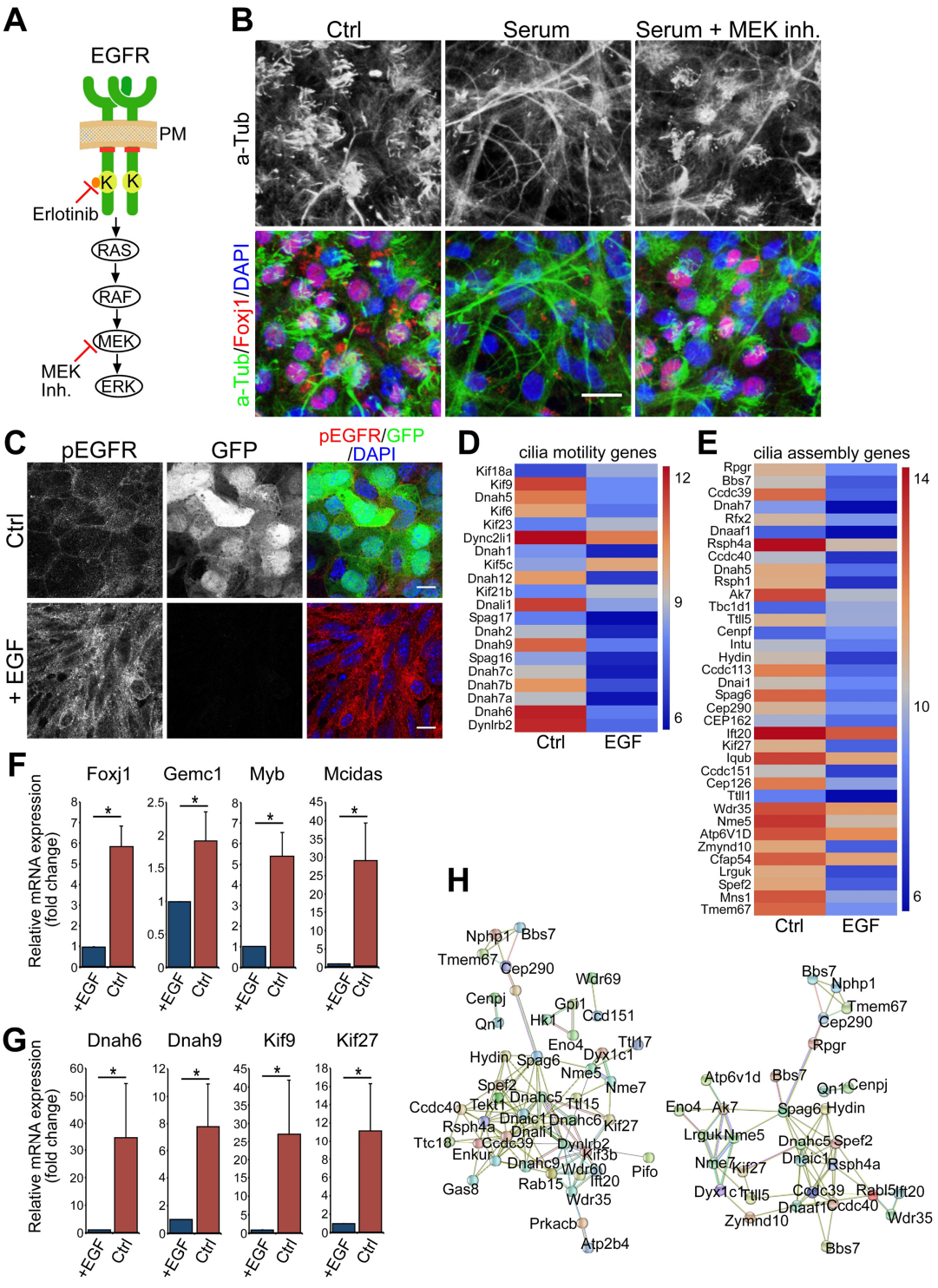

Figure S1. EGF inhibition of molecular pathways during postnatal ependymal niche development. Related to Figure 1. (**A**) Schematic showing EGFR signal transduction cascade: Erlotinib inhibits EGFR signaling at the level of receptor activation by blocking auto-phosphorylation; MEK inhibitor blocks MEK1/2 phosphorylated activation thereby inhibiting downstream signal transduction cascade. (**B**) Differentiating primary ECs in control (Ctrl), 10% serum (Serum), or 10% serum + MEK inhibitor (MEK inh.) culturing media, labeled with antibodies to Foxj1, acetylated-tubulin (a-Tub), and DAPI. Note the abundance of multiciliated ECs in Serum + MEK inh. but not in the Serum condition. Scale

bar: 20 μm. (**C**) IHC images of primary ependymal cultures grown in control differentiation media (Ctrl) or with EGF addition (+ EGF), stained with anti-pEGFR, GFP antibodies, and DAPI. Scale bar: 10 μm. (**D**) Heatmaps of genes found within the cilia motility GO term, differentially expressed between EGF-treated and untreated conditions. (**E**) Gene heatmaps within the cilia assembly GO term, differentially expressed between EGF-treated and untreated conditions. (**F, G**) Quantitative RT–PCR of mRNA expression levels of EC developmental genes Mcidas, Myb, Foxj1, GEMC1 (**F**), and multiciliary component genes DNAH6, DNAH9, KIF9, KIF27 (**G**), comparing EGF-treated (+EGF) and untreated (Ctrl) conditions. * $P < 0.05$, Wilcoxon 2-sample test, $n = 4$, mean ± SEM. (**H**) STRING analyses showing interactions/associations between differentially expressed genes identified in transcriptomic experiment, where line thickness corresponds to confidence level of gene interactions.

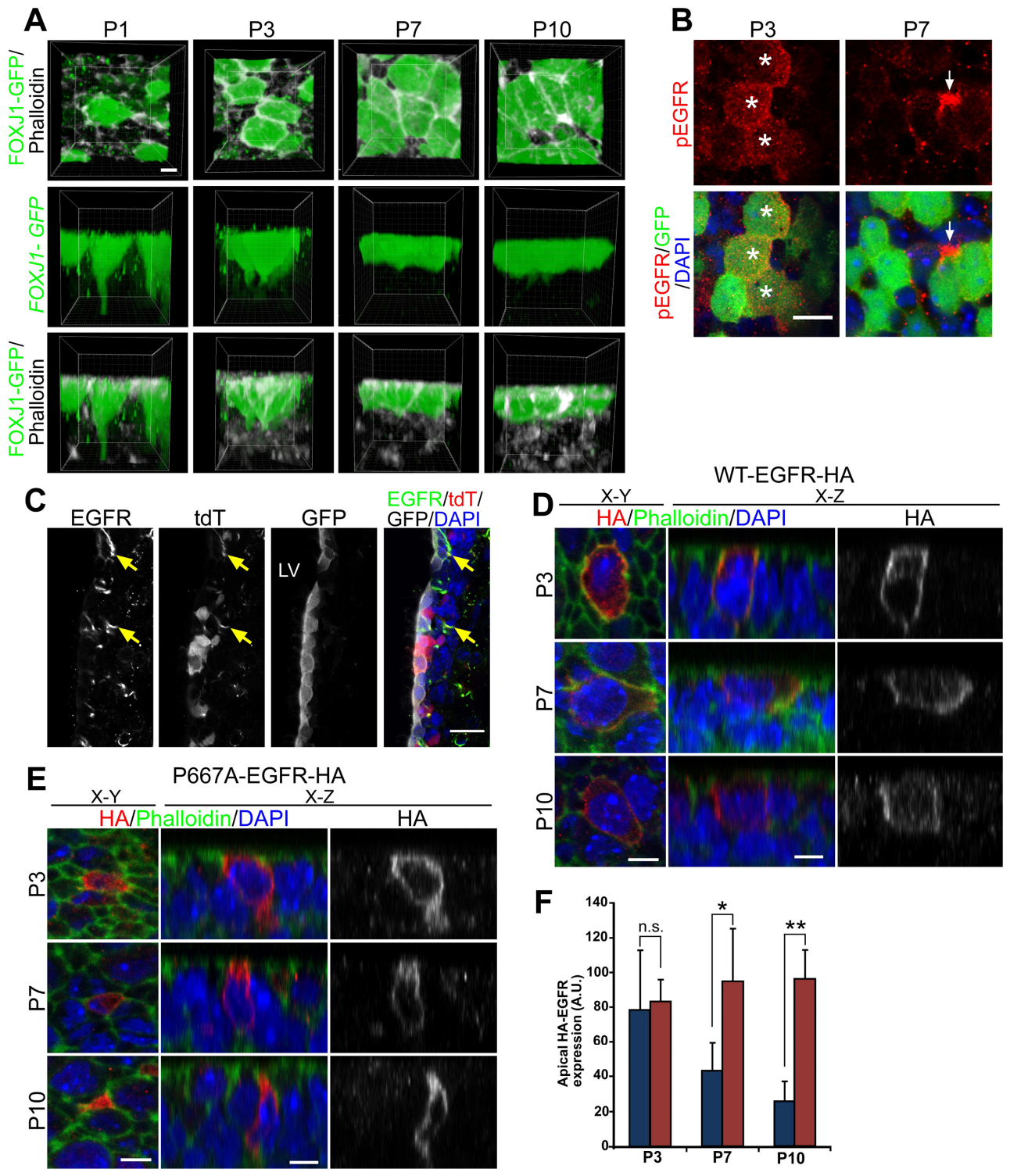

Figure S2. EGFR in postnatal ependymal niche development. Related to Figure 2. (**A**) Imaris 3D projections of lateral ventricular wholemounts from *FOXJ1-GFP*$^{+}$ animals of the indicated postnatal ages (X-Y, X-Z plane views), stained with phalloidin, with native GFP fluorescence signal, showing basal processes of undifferentiated GFP^{+} pRGPs at postnatal day 1 (P1), and their apical surface area expansions during differentiation (P3 – 10). Scale bar: 10 µm. (**B**) IHC images of P3, P7 ventricular wholemounts from *FOXJ1-GFP*$^{+}$ animals stained with pEGFR, GFP antibodies, and DAPI. Note that pEGFR is strongly expressed in GFP^{+} ependymal cells at P3 (*), but at P7 becomes restricted to lateral cellular domains in GFP^{+} cells, and small ventricular surface contacts of GFP-negative cells (arrows).

Scale bars: 10 µm. (**C**) IHC images of coronal lateral ventricular sections from 2 month old *nestin-CreER*^{tm4}*; R26R-tdTomato; FOXJ1-GFP* animal, P14 tamoxifen-induced, stained with EGFR, RFP, GFP (in white for clarity) antibodies, and DAPI, showing EGFR in NSCs (arrows) but not in the ECs. Scale Bar: 20 µm. (**D, E**) X-Y and X-Z view images of LV wholemounts from the indicated postnatal stages expressing WT-EGFR-HA (**D**) or P667A-EGFR-HA (**E**) and labeled with anti-HA antibody, phalloidin, and DAPI. Scale bar: 10 µm. Note the persistent apical expression of P667A-EGFR-HA in (**E**). (**F**) Apical expression of WT-EGFR-HA and P667A-EGFR-HA at the postnatal time points indicated. * $P < 0.001$, ** $P < 0.0001$, n.s. = non significant, Student's *t*-test, $n = 10$ cells for each group, mean ± SEM.

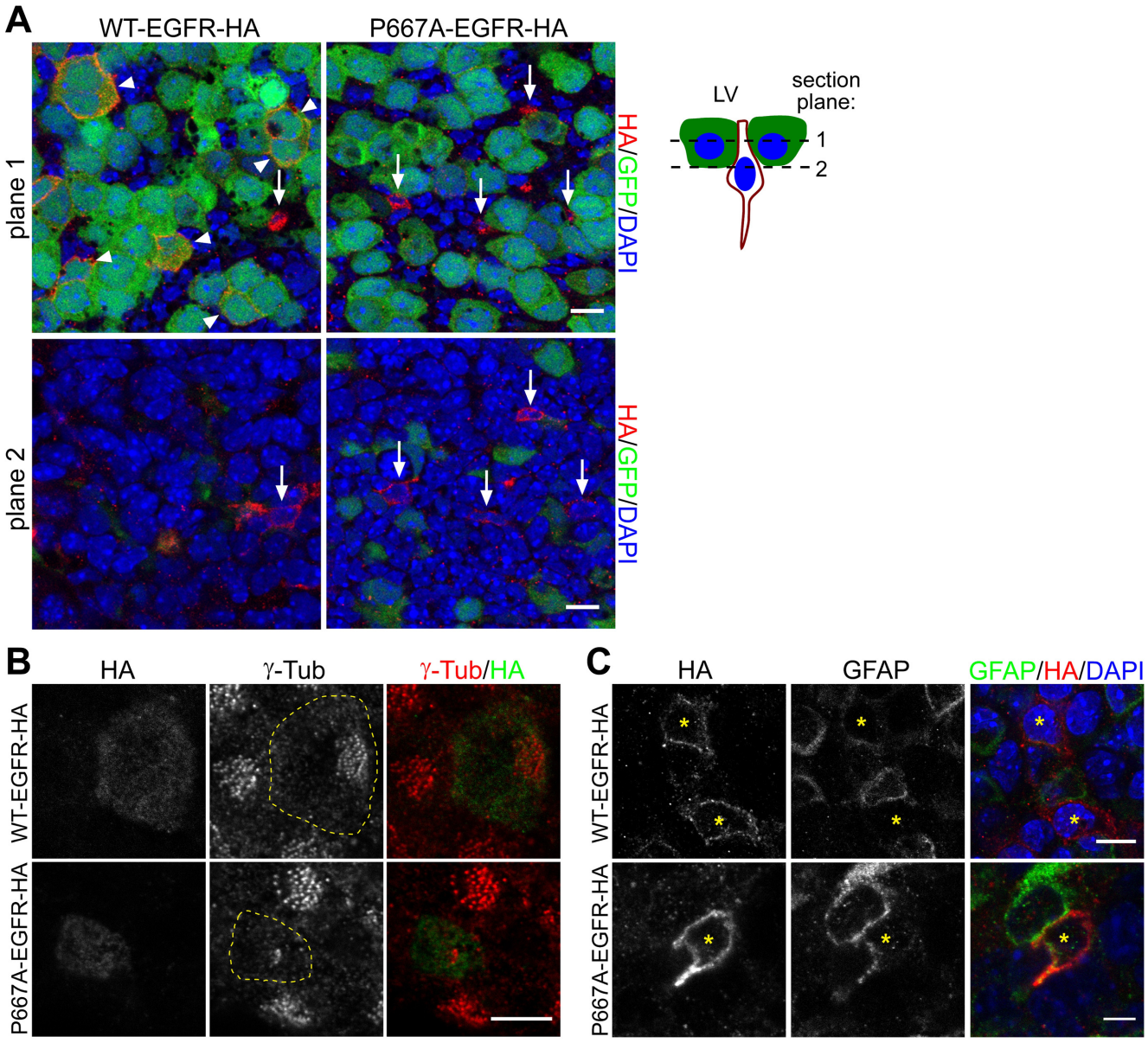

Figure S3. Lentiviral EGFR construct expression in vivo. Related to Figure 2. (A) Larger field views of LV wholemounts from *FOXJ1-GFP* animals, labeled with HA antibody, DAPI, and native GFP fluorescence, showing 2 sectional plane views (see schematic diagram). Note that with WT-EGFR-HA, many GFP^+ cells showed punctate HA^+ co-localization (arrowheads), while HA^+/GFP^- cells showed glial-like features located between ECs (arrows). With P667A-EGFR-HA mutant, HA^+ cells were mostly GFP^-, showing small apical surface area contacts between ECs (arrows). Scale bar: 20 µm. (**B, C**) IHC images of LV wholemounts with cells expressing WT-EGFR-HA or P667A-EGFR-HA (dashed lines in **B,** * in **C**), stained with anti-HA antibody, DAPI, and either anti-γ-tubulin (γ-Tub, **B**) or anti-GFAP (**C**) antibodies. Scale bars: 5 µm (**B**), 10 µm (**C**).

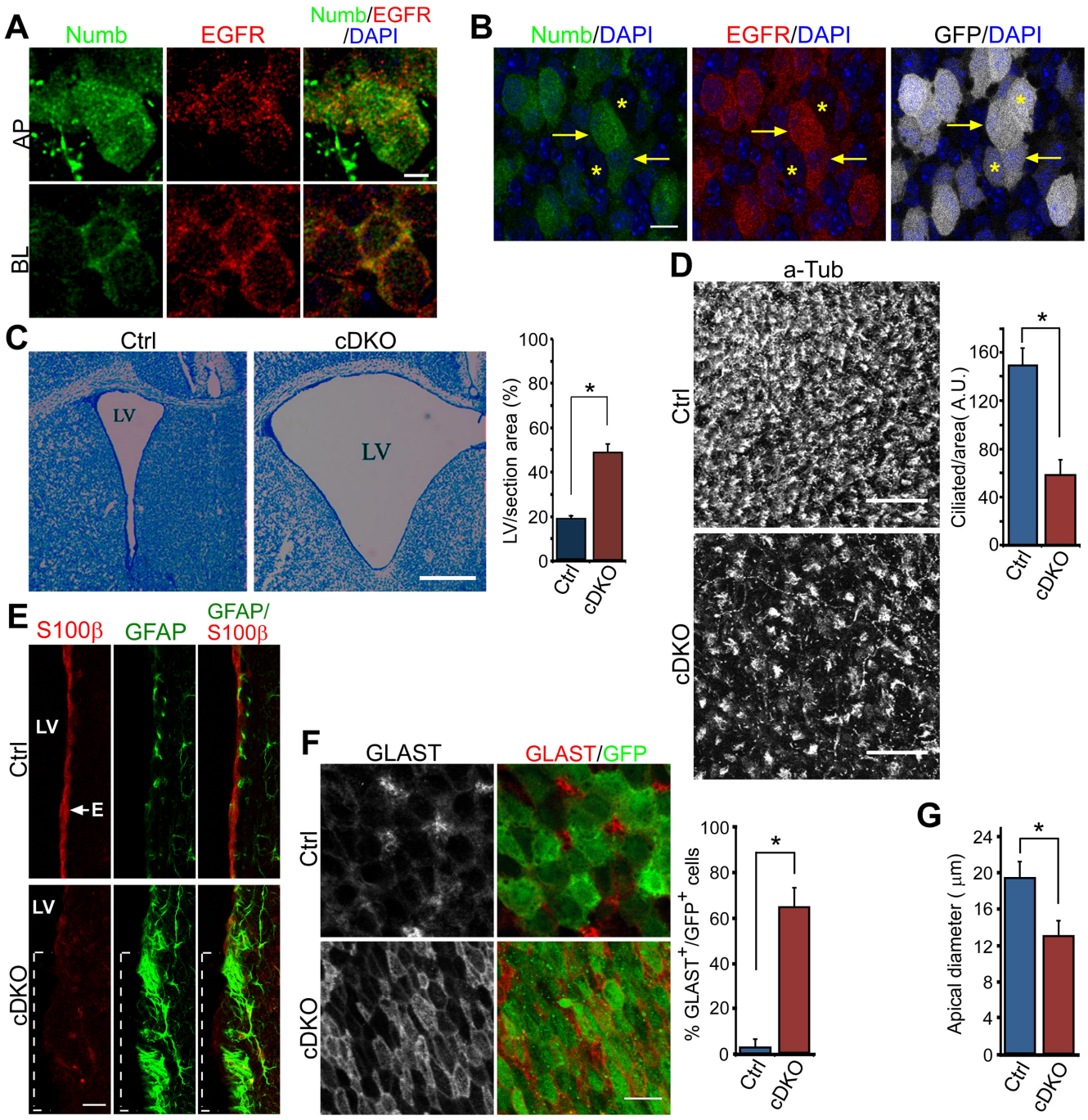

Figure S4. Ependymal defects in Numb/Numblike conditional mutants. Related to Figure 3. (**A**) STED super-resolution microscopy images of lateral ventricular wholemounts from P7 *FOXJ1-GFP*$^{+}$ animals, labeled with antibodies to Numb, EGFR, and DAPI. AP = apical domain; BL = basolateral domain. X-Z view = optical section projection from longest X-Y axis. Scale bar: 5 µm. (**B**) IHC images stained with anti-EGFR, Numb antibodies, and DAPI. Note FOXJ1-GFP^{+} cells expressing both Numb and EGFR (arrows), and FOXJ1-GFP^{+} cells negative for Numb and EGFR (*). Scale bar: 10 µm. (**C**) Nissl staining of coronal brain sections from P21 control (Ctrl) and *FOXJ1-Cre; Nb*$^{flox/flox}$*; Nbl*$^{KO/KO}$ (cDKO) mutant animals, showing ventricular enlargement in cDKO animals. Scale bar: 1 mm. Ventricle volume as % of total section size in Ctrl and cDKO animals. * $P < 0.008$, Wilcoxon 2-sample test, $n = 5$ for each group, mean ± SEM. (**D**) IHC images of LV wholemounts from P7 control or Numb cDKO animals stained for anti-a-Tub antibody. Scale bar: 60 µm. Number of ciliated cells per area in control

and Numb cDKO animals. * $P < 0.001$, Student's *t*-test, $n = 10$ areas for each group, mean ± SEM. (**E**) IHC images of LV wholemounts from P26 control or Numb cDKO animals labeled with anti-GFAP, S100β antibodies. Dashed line bracket = glial scar in Numb cDKO animal, lacking $S100\beta^+$ ependymal layer seen in Ctrl (E, arrow). Scale bar: 30 μm. (**F**) IHC images of LV wholemounts from P10 control and Numb cDKO animals stained with anti-GLAST, GFP antibodies. Scale bar: 20 μm. % of cells positive for GFP and GLAST in control and Numb cDKO animals. * $P < 0.0001$, Student's *t*-test, $n = 10$ areas for each group, mean ± SEM. (**G**) Apical diameter in GFP^+ cells from control and Numb cDKO animals. * $P < 0.0001$, Student's *t*-test, $n = 20$ cells for each group, mean ± SEM.

Supplemental Table

Table S1. List of qPCR primers used in this study. Related to Key Resources Table in STAR Methods.

Primer Name RT-qPCR	Sequence
Dnah9-Fwd	5'-AGAGCACTATAGGCCAGCAG-3'
Dnah9-Rev	5'-GAAGGCCTTGAGGGAGAACT-3'
Dnah6-Fwd	5'-CGCAAGGAAGATGACACAGA-3'
Dnah6-Rev	5'-TTAGAGACCCAGCCATGACC-3'
Mcidas-Fwd	5'-AACCGAAGCGTCTCCTAGTG-3'
Mcidas-Rev	5'-GGTCATCCATTGCATCTCTG-3'
Myb-Fwd	5'-AGATGAAGACAATGTCCTCAAAGCC-3'
Myb-Rev	5'-CATGACCAGAGTTCGAGCTGAGAA-3'
Foxj1-Fwd	5'-GGCCACCAAGATCACTCTGT-3'
Foxj1-Rev	5'-TGTTCAAGGACAGGTTGTGG-3'
GemC1-Fwd	5'-TGGTCTCCTGGACAACACTG-3'
GemC1-Rev	5'-TAACTCAGAGGGCGATTCCA-3'
Kif9-Fwd	5'-AAGACTCCTTAGGGGGAAACTG-3'
Kif9-Rev	5'-GTCTTTGAGATCCCCATCTTTG-3'
Kif27-Fwd	5-GCGAGAAACGGAACGTAAAC-3
Kif27-Rev	5-CTTTTGCTGGAGGGTCAGTC-3