SUPPLEMENTARY FIGURES

TITLE: Nearly complete deletion of BubR1 causes microcephaly through shortened mitosis and massive cell death

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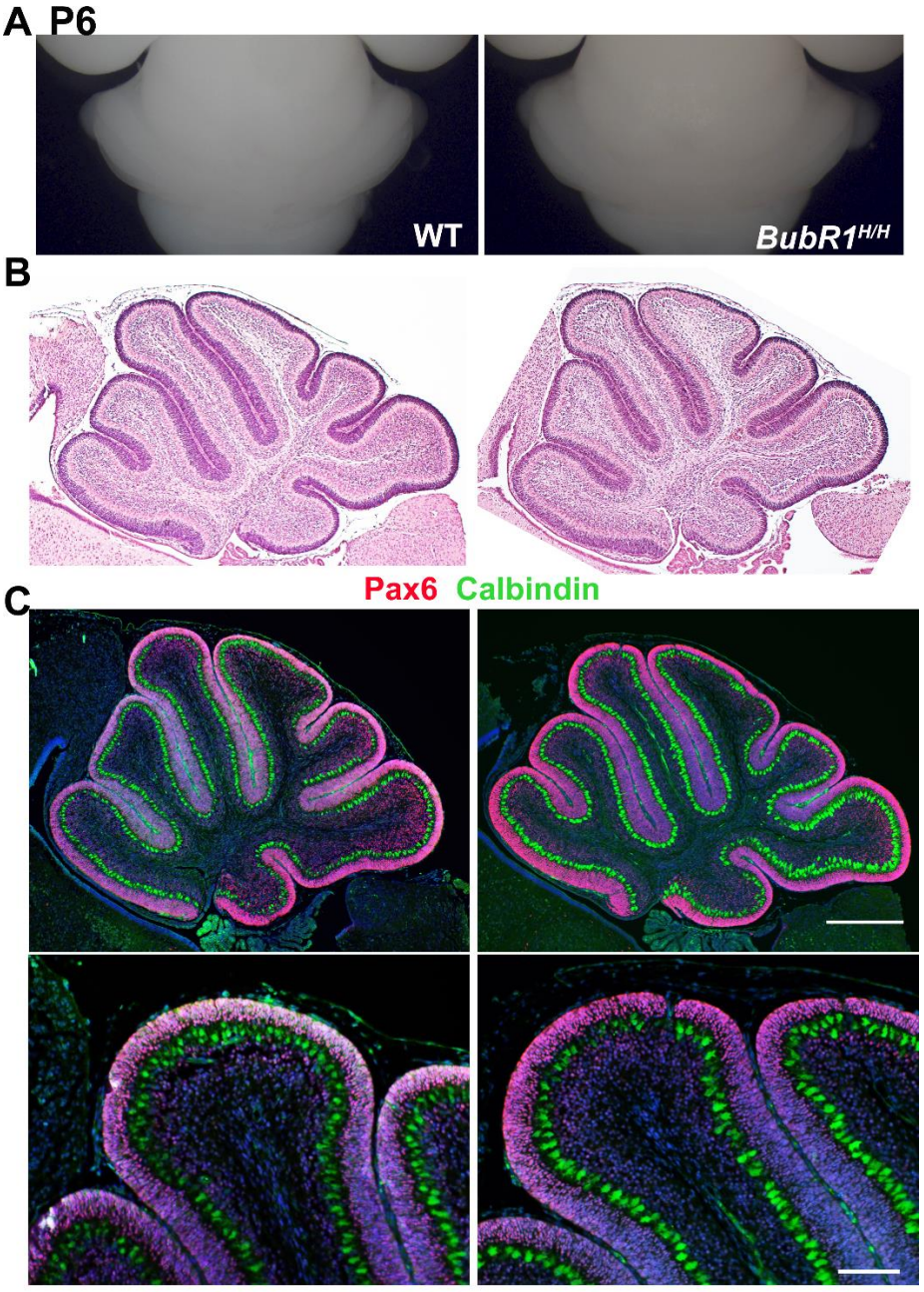

Figure S1. Cerebellar development is unaffected in *BubR1*$^{H/H}$ mice. (A-B) Cerebellum size and lobule structure appear normal in *BubR1*$^{H/H}$ mice and are indistinguishable from WT. (C) The distribution of granule neuron precursors (Pax6+) and Purkinje cells (Calbindin+) is unaltered in *BubR1*$^{H/H}$ mice compared to WT. Scale bar: 500 μm, 100 μm (Enlarged)

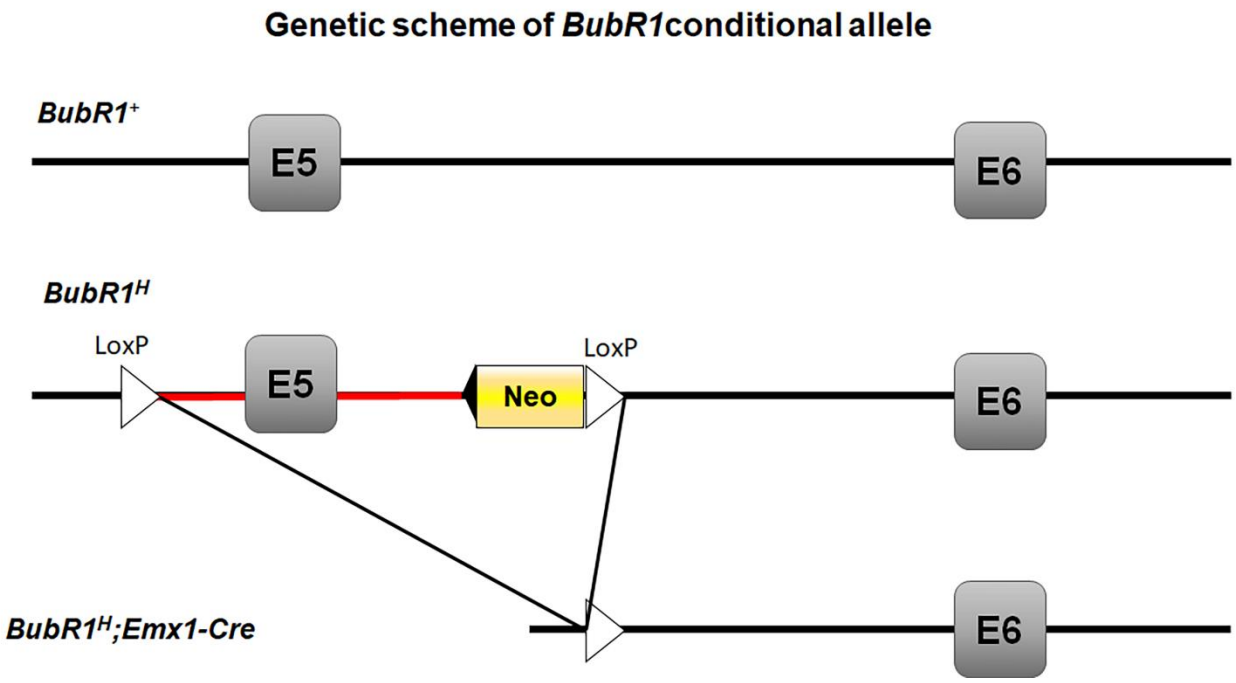

Figure S2. Schematic representation of *BubR1* CKO generation. The *BubR1*$^{H/H}$ allele contains a neo cassette in the intron between exons 5 and 6 resulting in decreased BubR1 expression; it was generated with LoxP sites flanking exon 5[1]. Emx1-Cre expression excises exon 5 resulting in frame shift and early termination in cortical progenitors.

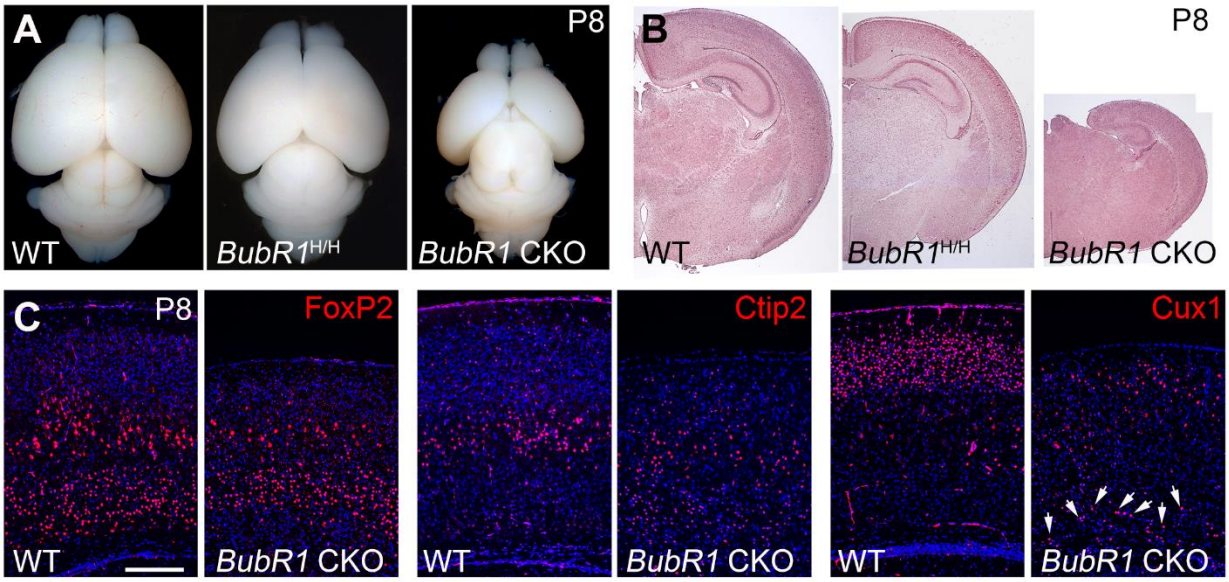

Figure S3. Microcephaly is evident in early postnatal stages of *BubR1* CKO. (A, B) Cortex of *BubR1* CKO, but not of *BubR1*$^{H/H}$, is strikingly reduced at P8, as at P21. (C) Early-born neurons (FoxP2+, Ctip2) are maintained, but late-born neurons (Cux1+) are dramatically reduced. Cux1+ late-born neurons are ectopic in the deep cortical layers (arrows). Scale bar; 100 μm

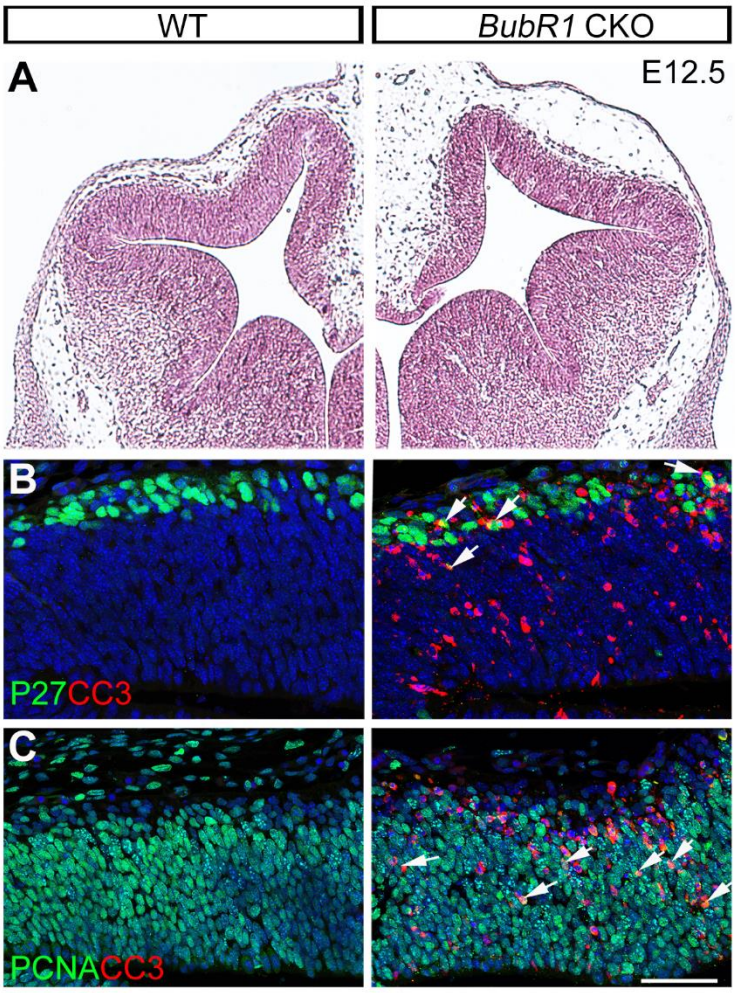

Figure S4. In the absence of BubR1, cell death is profound during early cortical development.

(A) At E12.5, cortical thickness in *BubR1* CKO is similar to WT. (B) Post-mitotic (P27+) cells (arrows) are clearly among the cells undergoing apoptosis (CC3+). (C) Many progenitors (PCNA+) are also undergoing apoptotic cell death (arrows). Scale bar; 100 μm

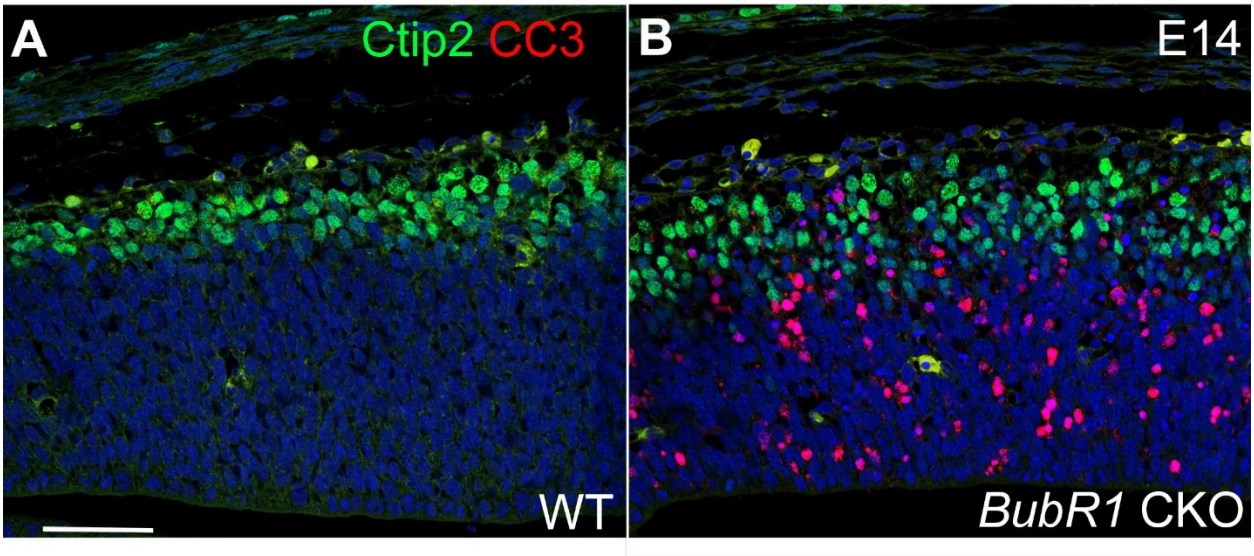

Figure S5. Differentiated neurons may not undergo cell death (A, B) At E14.5 Ctip2+ neurons in the cortical plate rarely overlap with CC3 in the BubR1 CKO although many CC3+ cells are present; few CC3 + cells are found in the WT.

Scale bar; 50μm.

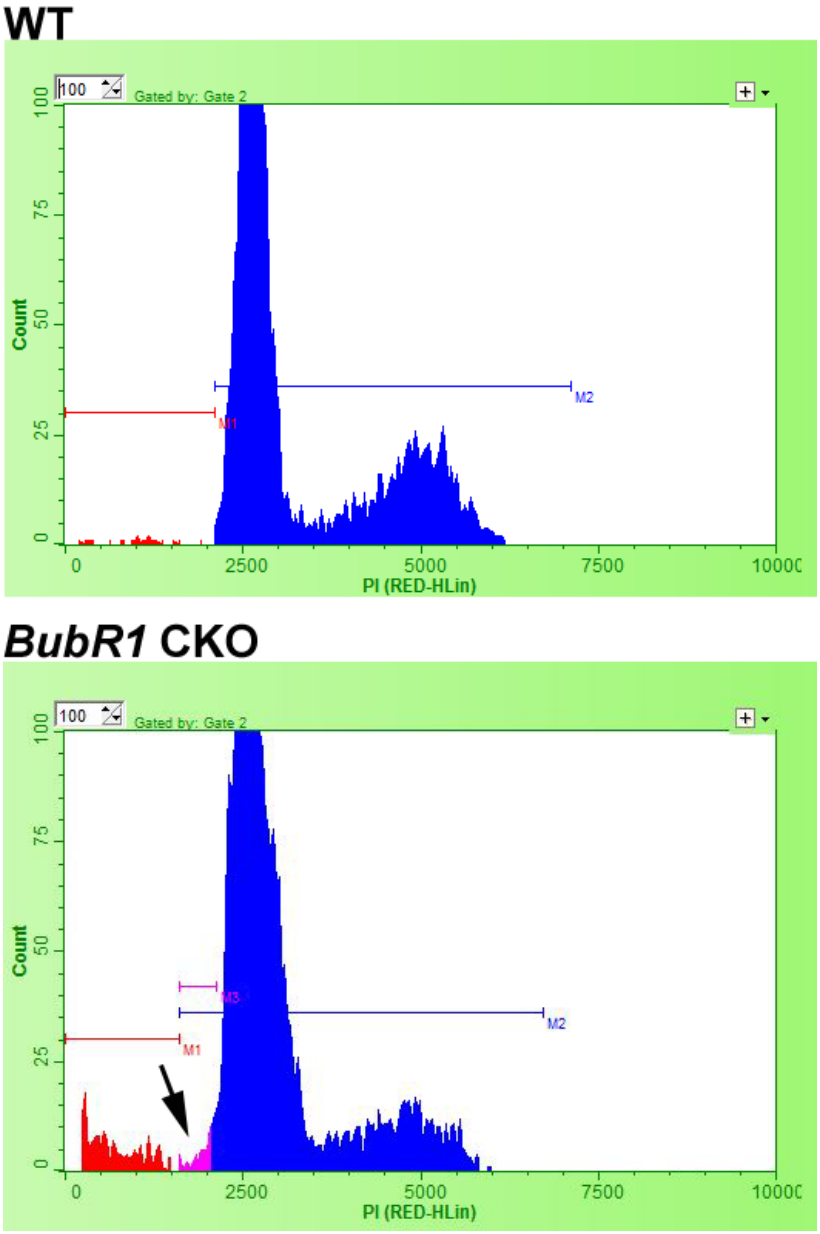

Figure S6. Large subG cell population in *BubR1* CKO cortex indicates profound cell death. Flow cytometry reveals a prominent subpopulation of cell debris (pink, black arrow) among the cortical cells analyzed, categorizing them as subG. Cells in both the blue and pink size thresholds were used for further grouping of cell cycle phase.

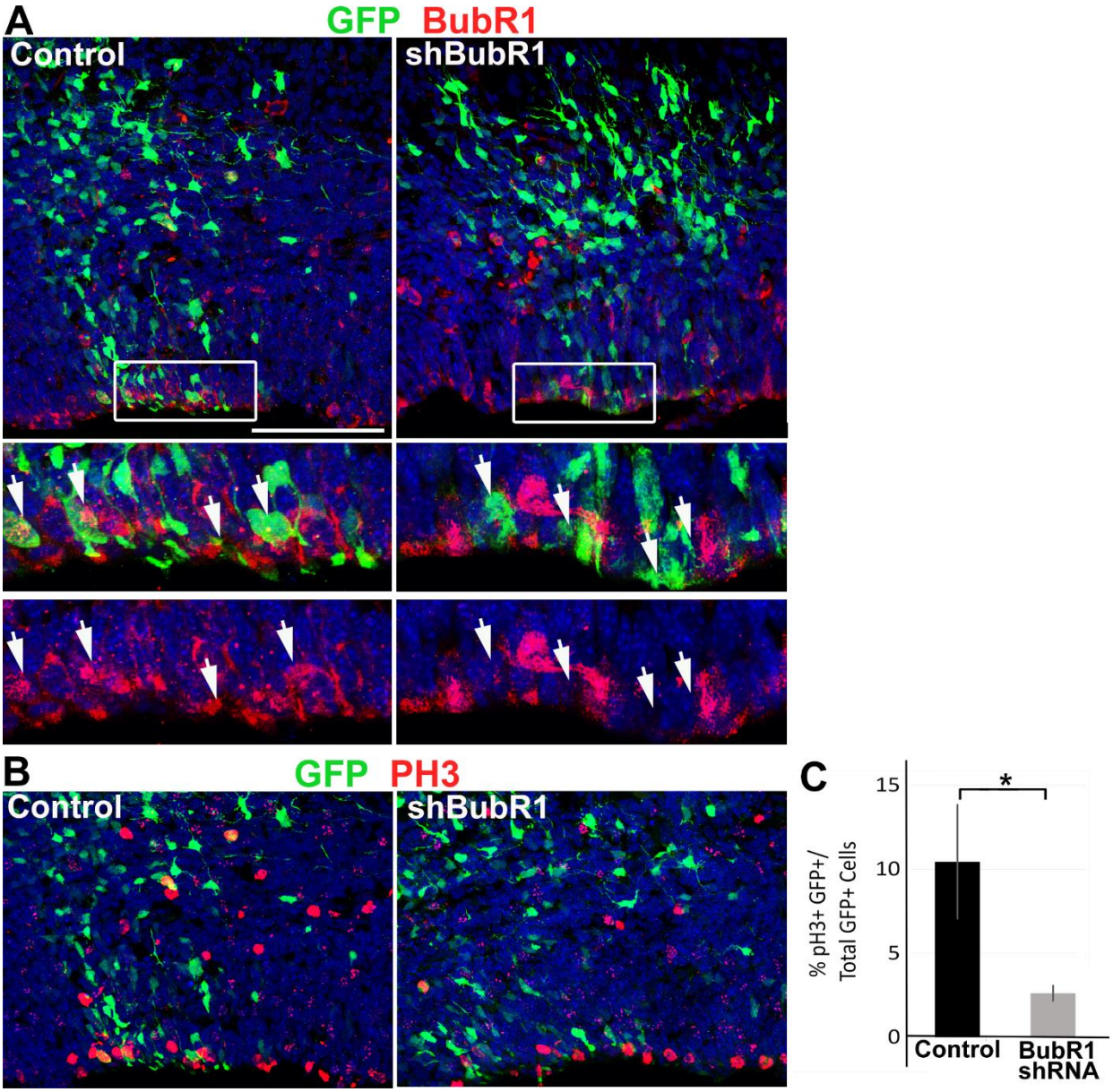

Figure S7. Cell-autonomous function of BubR1 is required to maintain cells in mitosis. (A) BubR1 shRNA[2] and pCAG-GFP were electroporated *in utero* into E13.5 cortices, which were harvested 2 days later. Cells electroporated with BubR1 shRNA and GFP (arrows) show reduction of BubR1 expression compared to control electroporated cells (arrows), confirming knockdown (GFP+, BubR1+, white arrows). (B and C) Knockdown of BubR1 decreases the cells in M-phase (pH3+, GFP+) among total electroporated cells (GFP+) compared to control shRNA electroporated cells. Scale bar; 100 μm

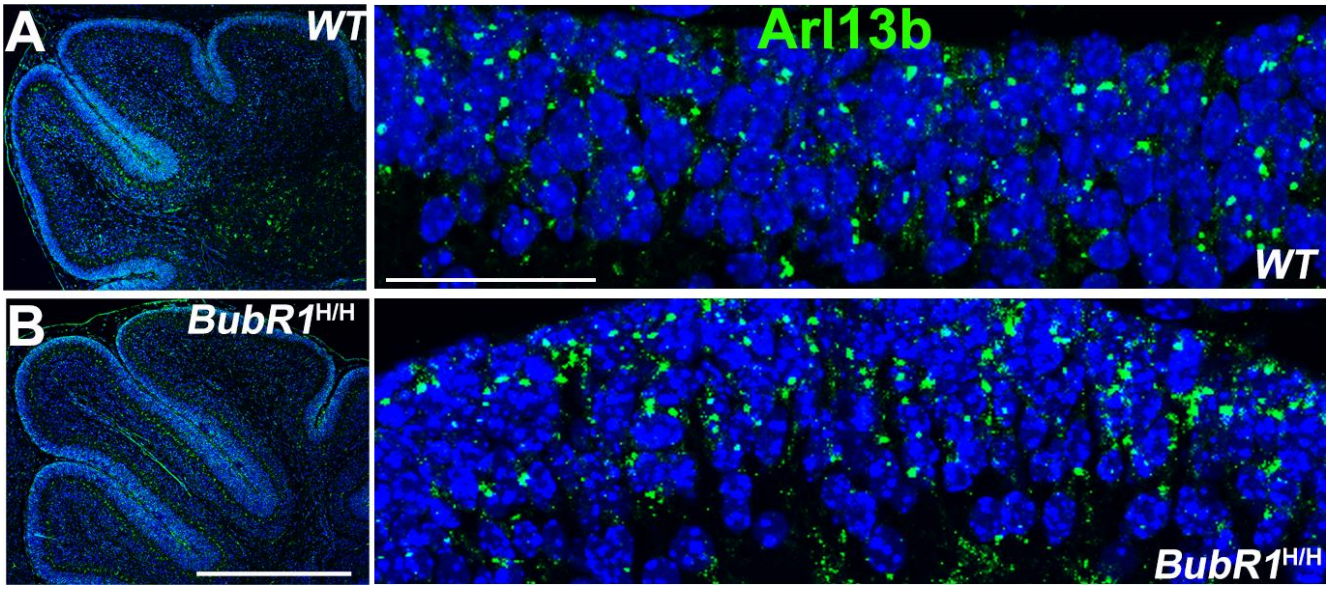

Figure S8. BubR1 reduction does not obviously disrupt primary cilia of cerebellar granule cell precursors. (A and B) At P8, primary cilia in cerebellar granule cell precursors in the external granular layer of *BubR1*$^{H/H}$ mice show no obvious alteration in presence or shape (B). Scale bar; 250 μm, 25 μm (enlarged)

REFERENCES

1. Baker, D.J., K.B. Jeganathan, J.D. Cameron, M. Thompson, S. Juneja, A. Kopecka, R. Kumar, R.B. Jenkins, P.C. de Groen, P. Roche, et al., *BubR1 insufficiency causes early onset of aging-associated phenotypes and infertility in mice.* Nat Genet, 2004. **36**(7): p. 744-9.
2. Yang, Z., H. Jun, C.I. Choi, K.H. Yoo, C.H. Cho, S.M.Q. Hussaini, A.J. Simmons, S. Kim, J.M. van Deursen, D.J. Baker, et al., *Age-related decline in BubR1 impairs adult hippocampal neurogenesis.* Aging Cell, 2017. **16**(3): p. 598-601.