Cell Reports, Volume *29*

Supplemental Information

The Ciliary Protein Arl13b Functions Outside of the Primary Cilium in Shh-Mediated Axon Guidance

Julien Ferent, Sandii Constable, Eduardo D. Gigante, Patricia T. Yam, Laura E. Mariani, Emilie Legué, Karel F. Liem Jr., Tamara Caspary, and Frédéric Charron

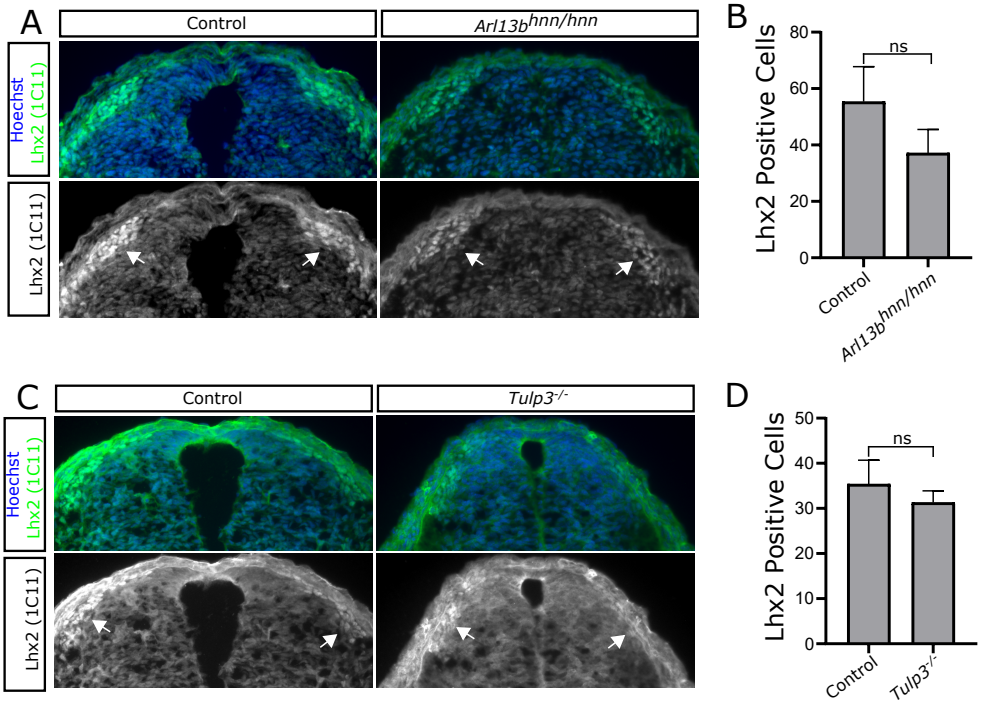

Figure S1. Lhx2+ cell number is not affected in *Arl13b*$^{hnn/hnn}$ and *Tulp3*$^{-/-}$ embryos. Related to Figures 1 and 2.

(A) Lhx2 immunostaining in the neural tube of control and *Arl13b*$^{hnn/hnn}$ embryos. Forelimb-level E11.5 neural tube sections were stained for Lhx2 positive cells (1:100; DHSB PCRP-LHX2-1C11). (B) The number of Lhx2 positive cells in 3-4 neural tube sections per embryo was quantified. Each section was separated by ~100-200 μm along the anterior-posterior axis representing different segments of the forelimb neural tube region. Cell counting was performed in Image J software (NIH; Bethesda, MD). Data are presented as average number of Lhx2-positive cells (±SD) and analyzed by Student's t test. Number of embryos examined per genotype n = 3-4. (C) Lhx2 immunostaining in the neural tube of control and *Tulp3*$^{-/-}$ embryos. (D) Quantification of the number of Lhx2 positive cells in 3-4 neural tube sections per embryo. Number of embryos examined per genotype n = 3-4.

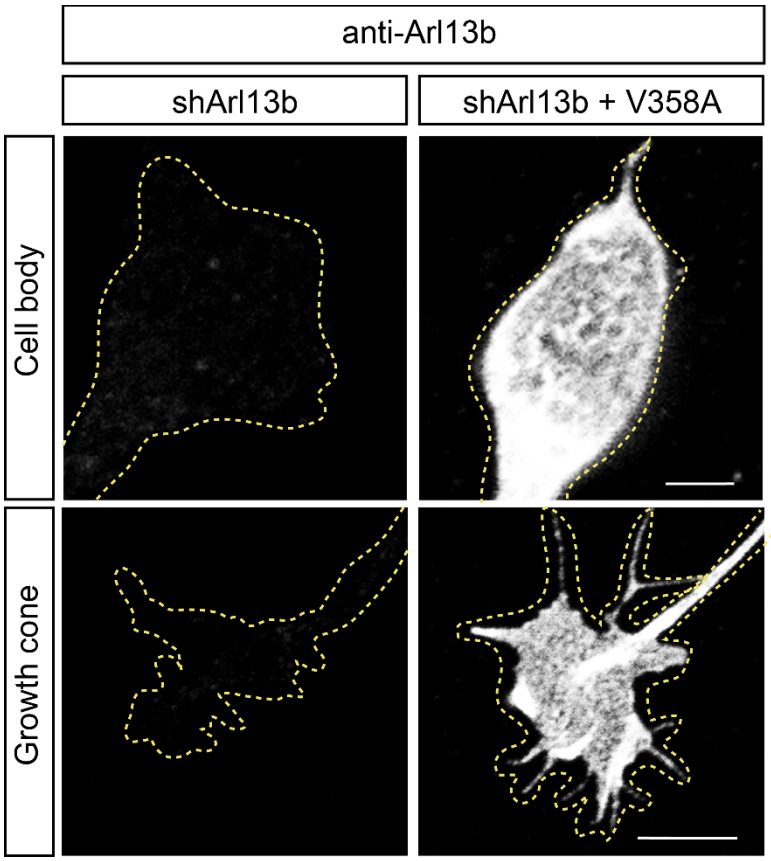

Figure S2. The Arl13b antibody can recognize the V358A mutant form. Related to Figure 4. Arl13b immunostaining on commissural neurons 2 days after electroporation with either shArl13b alone or in combination with the Arl13b^{V358A}-GFP expression vector. The antibody detects a strong signal when Arl13b^{V358A}-GFP is expressed, showing that this variant can still be detected by the anti-Arl13b antibody at the cell body and growth cone levels.

Scale bar, top row: 5 µm, bottom row: 10 µm.

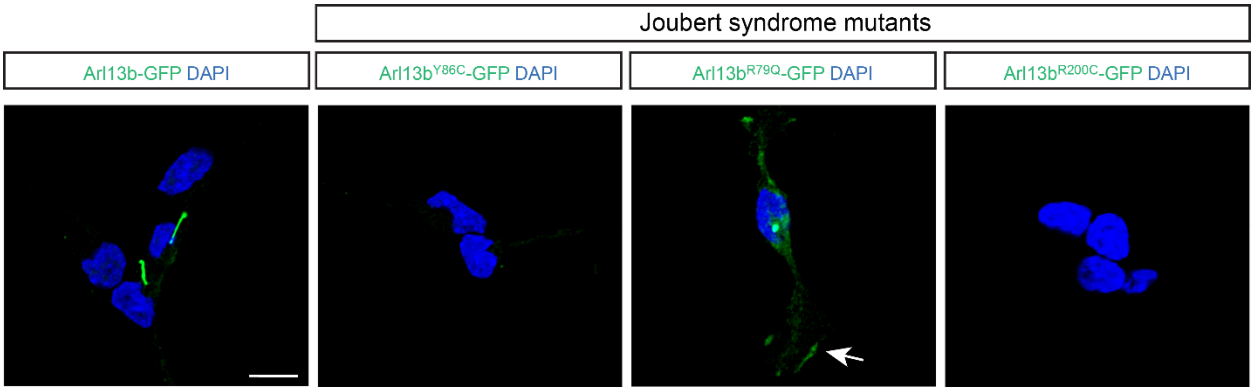

Figure S3. Expression and localization of Arl13b Joubert syndrome mutants in commissural neurons. Related to Figure 4.

GFP signal in commissural neurons 2 days after electroporation with Arl13b-GFP, Arl13b^{Y86C}-GFP, Arl13b^{R79Q}-GFP or Arl13b^{R200C}-GFP expression vector. Wild type Arl13b-GFP shows a clear signal within the primary cilia. Amongst the Joubert syndrome mutants, Arl13b^{R79Q}-GFP shows a signal present in the primary cilia, the cell body, the processes and growth cone (arrow). Arl13b^{Y86C}-GFP and Arl13b^{R200C}-GFP are not expressed in commissural neurons. The reason(s) for this remains elusive but could be due to a rapid degradation of the mutant protein, for example. Scale bar, 10 μm.