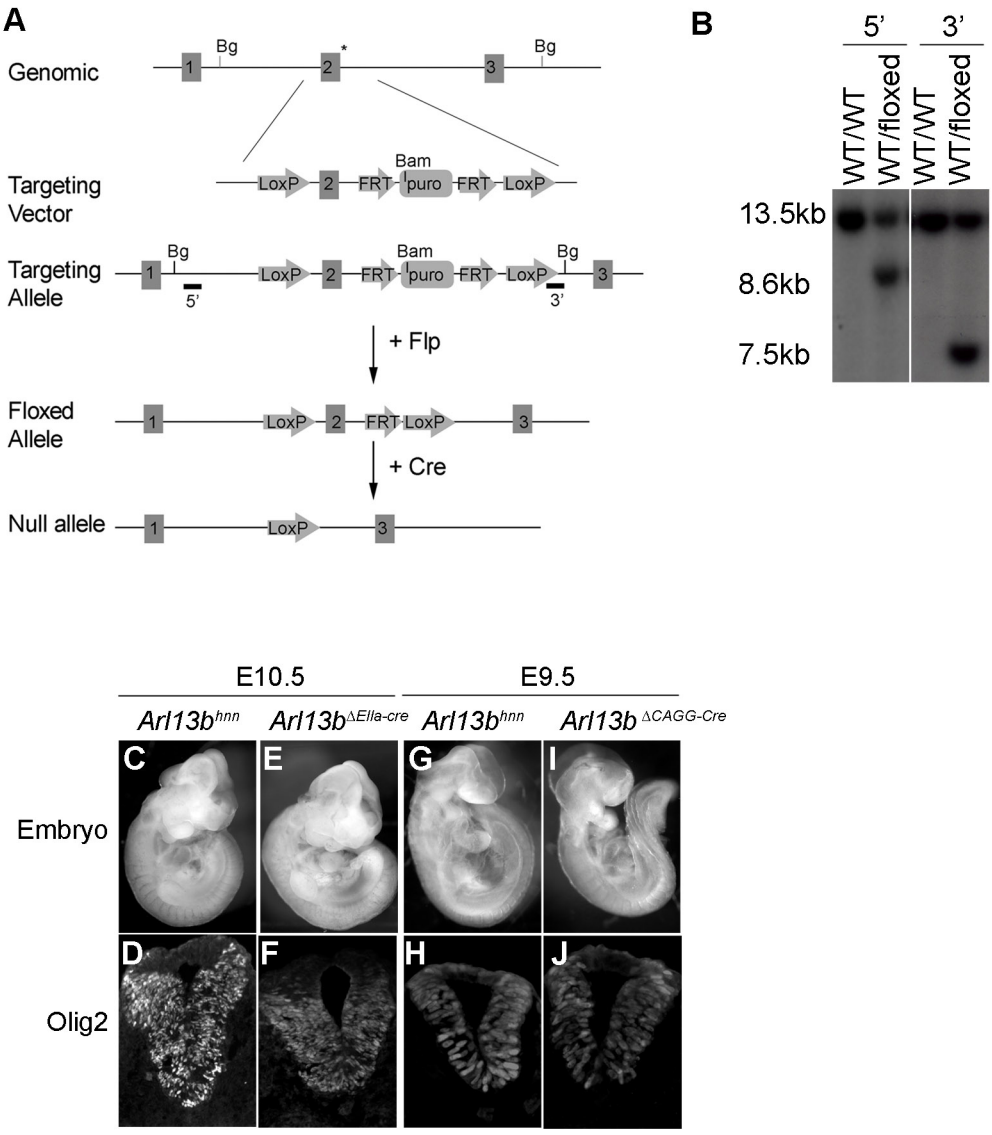

Fig. S1. The generation of a conditional *Arl13b* allele. (**A**) Schematic shows that exon 2 of the wild-type *Arl13b* locus is flanked by loxP sites with a puromycin-resistance cassette. The *hnn* mutation (*) disrupts the splice site of exon 2 of *Arl13b* in $Arl13b^{hnn}$. The puromycin-resistance cassette is removed by Flp recombinase, and exon 2 can be deleted upon Cre recombination. Genomic DNA is digested with *Bgl*II (Bg) and *Bam*HI (Bam), and probed with two unique external probes (black bars) to confirm homologous targeting. (**B**) The targeted locus creates a *Bam*HI site that reduces a 13.5 kb *Bgl*II fragment into one 8.6 kb and one 7.5 kb fragment that can be detected by unique 5′ and 3′ external probes, respectively. (**C-F**) When *Arl13b* is deleted by germline-expressed *EIIa-Cre*, the E10.5 $Arl13b^{\Delta EIIa\text{-}Cre/hnn}$ embryo shows exencephaly (E) and an expansion of Olig2 cells (F) that are identical to $Arl13b^{hnn}$ (C,D). (**G-J**) When a high dose of tamoxifen is injected at E6.5, $Arl13b^{\Delta CAGG\text{-}Cre}$ can recapitulate the null phenotype by embryo morphology (G,I) and the expansion of Olig2 cells (H,J) at E9.5.

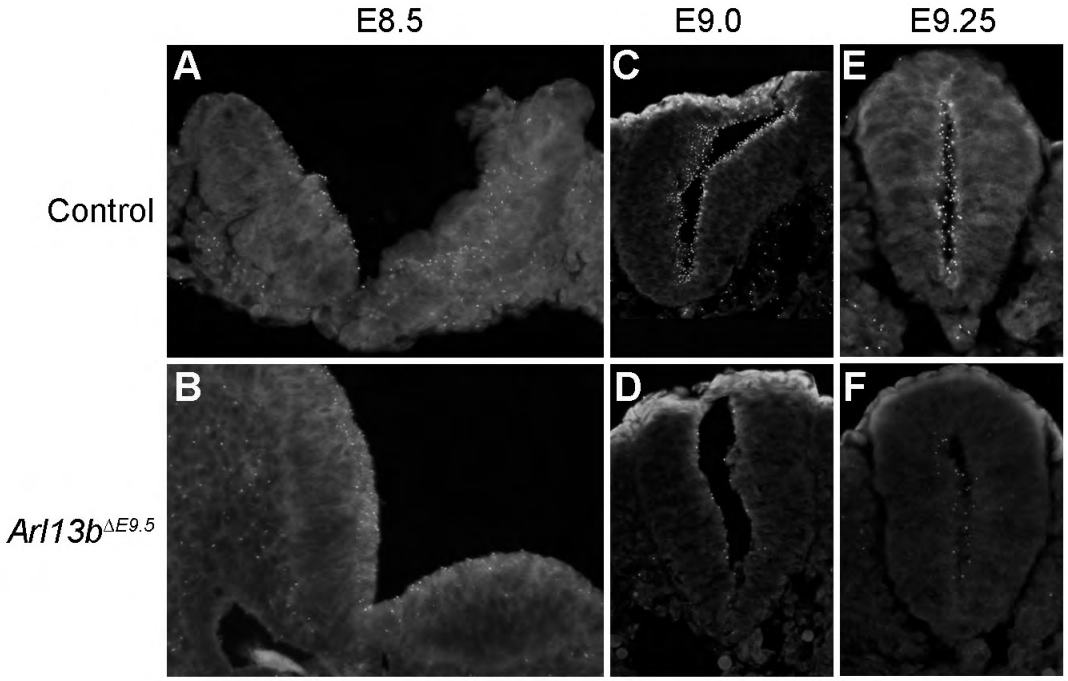

Fig. S2. The rate of Arl13b protein turnover in vivo. (**A**,**B**) Arl13b can be detected in all cilia at E8.5 in control (A), whereas some cells do not have Arl13b in *Arl13b*$^{\Delta E9.5}$ (B). (**C-F**) The expression of Arl13b can be observed along the ventricular zone in control embryos at E9.0 (C) and E9.25 (E). However, there is a dramatic decrease of Arl13b expression in *Arl13b*$^{\Delta E9.5}$ at both stages (D,F).

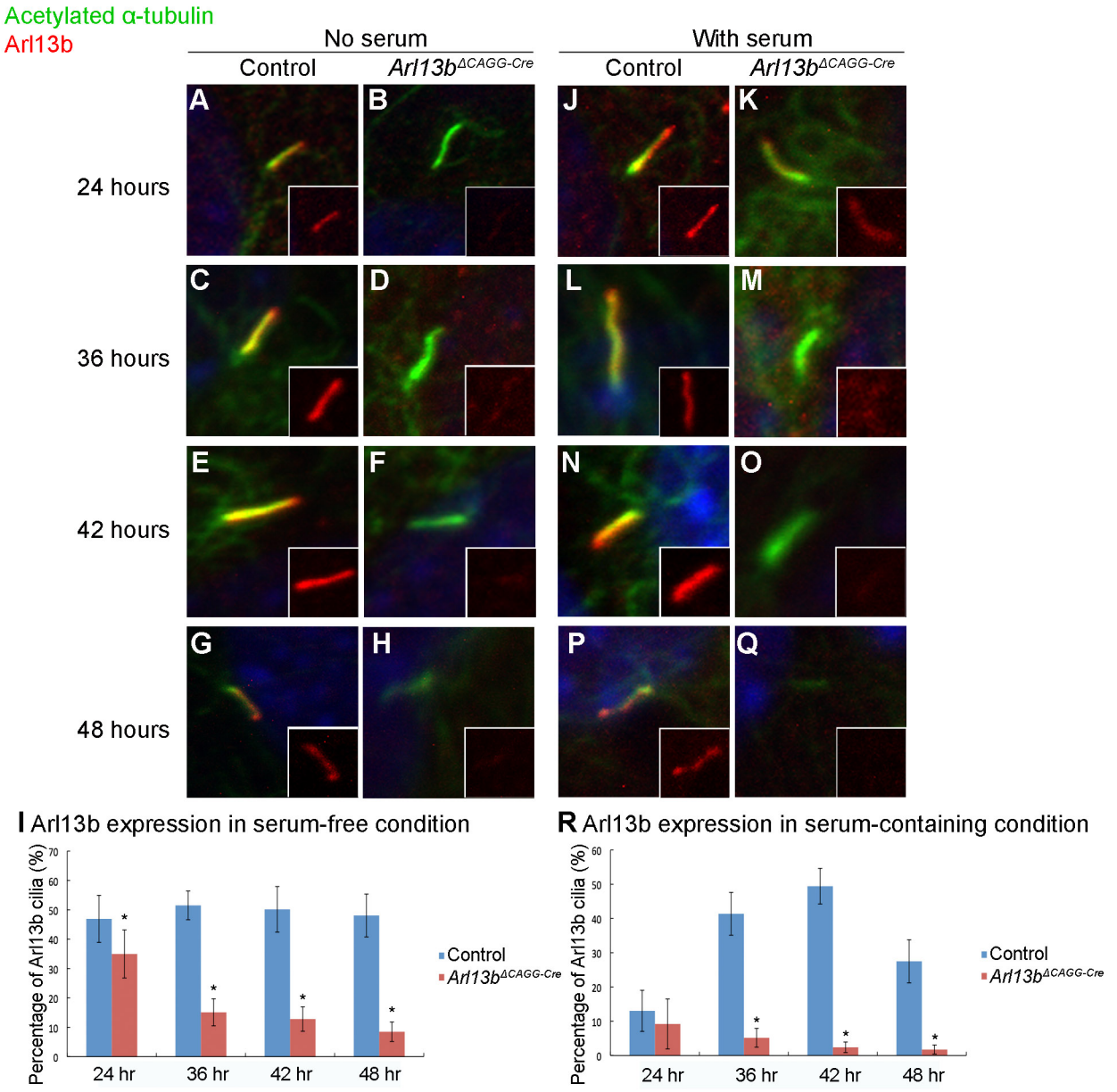

Fig. S3. The kinetics of Arl13b deletion in conditional Arl13b knockout MEFs is similar to in vivo. (**A-I**) Arl13b (red) is localized in the cilium (stained by acetylated α-tubulin, green) in control MEFs under serum-free conditions (A,C,E,G). Arl13b expression in *Arl13b*$^{\Delta CAGG\text{-}Cre}$ MEFs is barely detected after tamoxifen treatment for 24 (B), 36 (D), 42 (F) or 48 (H) hours under serum-free conditions. The insets show Arl13b staining alone. Quantification of Arl13b-expressing cells in control (blue) and *Arl13b*$^{\Delta CAGG\text{-}Cre}$ (red) (I). (**J-R**) Arl13b (red) is colocalized with acetylated α-tubulin (green) in control MEFs under serum-containing conditions (J,L,N,P). The decrease in Arl13b expression is more dramatic in *Arl13b*$^{\Delta CAGG\text{-}Cre}$ MEFs that are cultured in serum-containing media for 24-48 hours after adding tamoxifen (K,M,O,Q). Quantification of Arl13b expression in control (blue) and *Arl13b*$^{\Delta CAGG\text{-}Cre}$ (red) MEFs (R). *P<0.05.

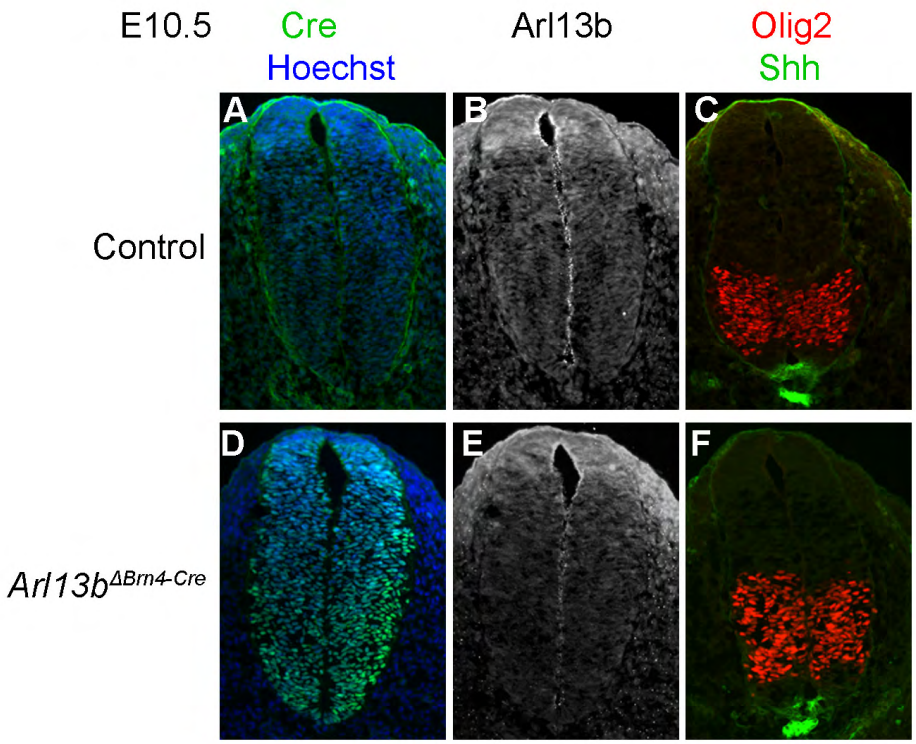

Fig. S4. Arl13b is still present at E10.5 when *Cre* expresses at E9.5. (**A**,**B**,**D**,**E**) Brn4-Cre (green) is expressed in the whole neural tube at E10.5 in *Arl13b*$^{\Delta Brn4\text{-}Cre}$ (D), but Arl13b can still be detected (E), even though *Brn4-Cre* begins to be expressed at E9.5. Control embryo does not contain Cre, but does express Arl13b in cilia (A,B). Hoechst (blue) stains nuclei. (**C**,**F**) E10.5 control (C) and *Arl13b*$^{\Delta Brn4\text{-}Cre}$ (F) caudal neural tubes show identical Olig2 (red) and Shh (green) expression.

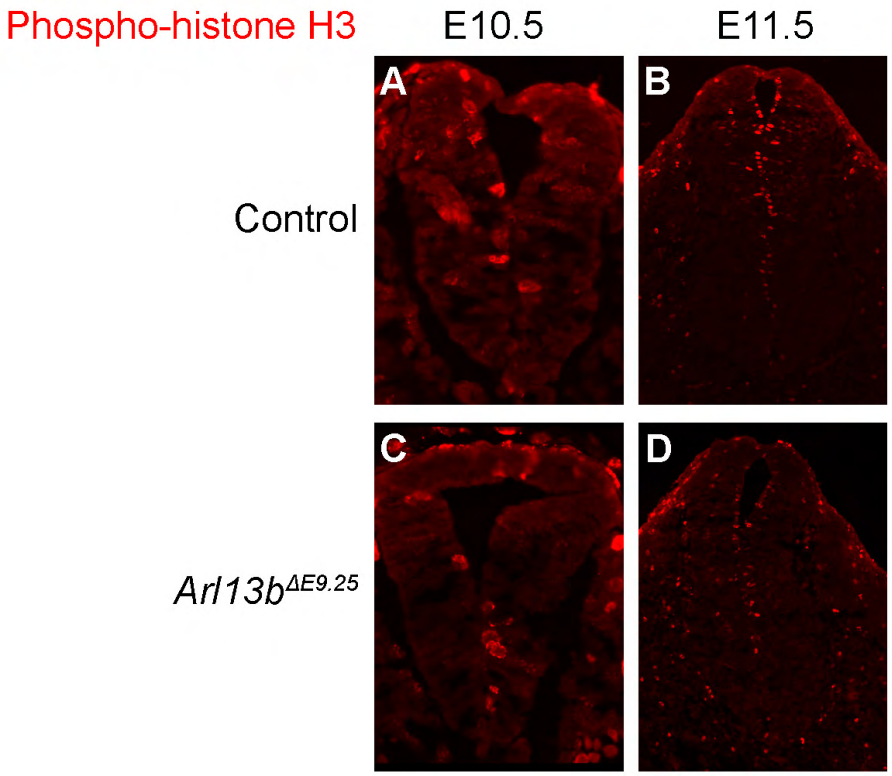

Fig. S5. Proliferation is normal in the absence of Arl13b. (**A-D**) Phospho-histone H3 (red) is localized along the ventricular zone of neural tubes at E10.5 (A,C) and E11.5 (B,D), and there is no significant difference between Cre-negative *Arl13b*$^{floxed/floxed}$ or Cre-negative *Arl13b*$^{floxed/hnn}$ (A,B) and *Arl13b*$^{\Delta E9.25}$ (C,D).