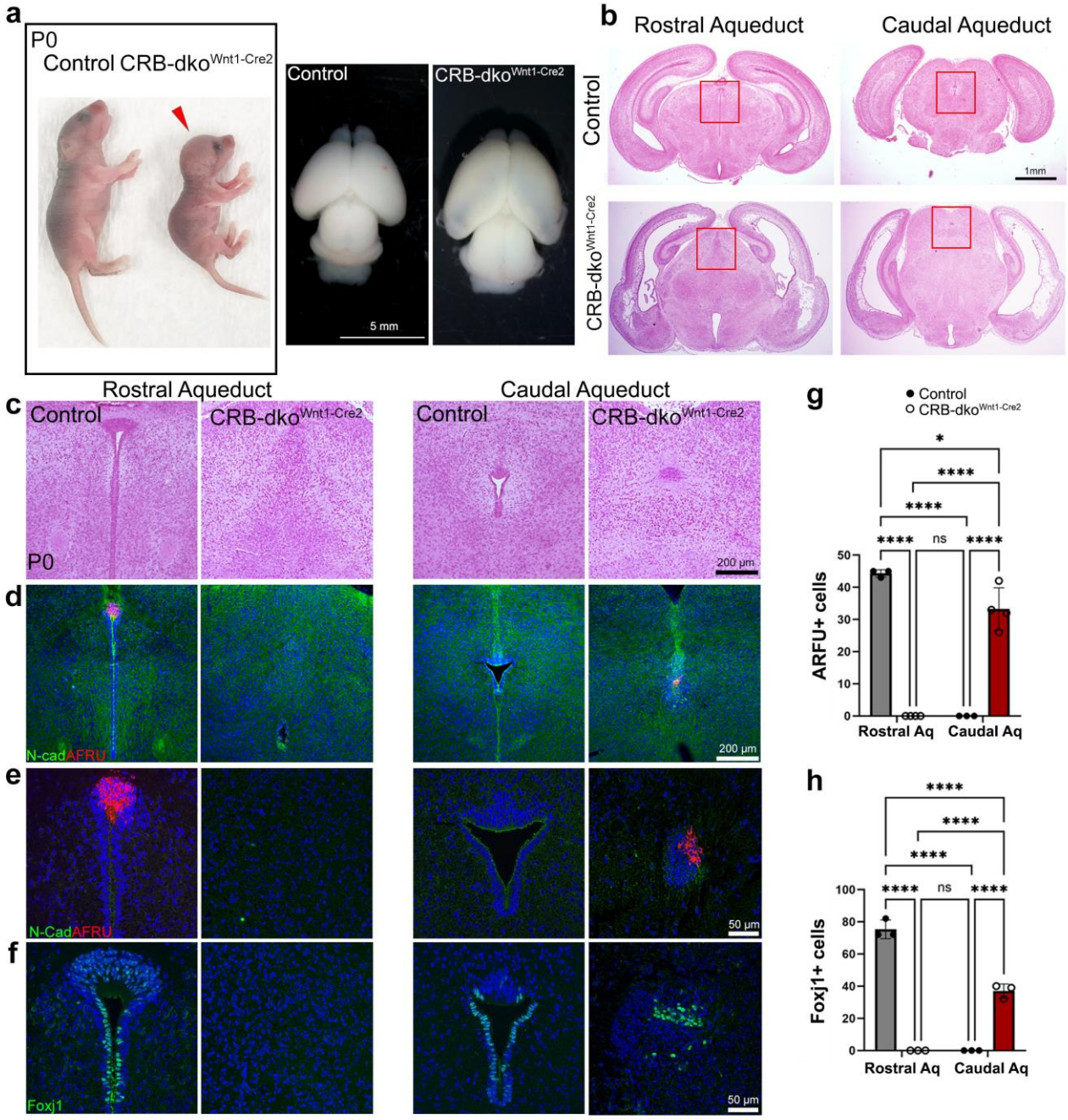

Supplementary Material 9. Fig. 9. Crb2 loss in midbrain disrupts aqueduct integrity but does not prevent generation of multiciliated ependymal cells (ECs) at P0. **(a)** Gross morphology and dorsal brain views show domed head and abnormal brain morphology in CRB-dko$^{Wnt1\text{-}Cre2}$ mice, consistent with hydrocephalus. **(b)** Representative coronal H&E-stained sections through the rostral and caudal aqueduct regions (Controls vs CRB-dko$^{Wnt1\text{-}Cre2}$). **(c)** Higher magnification of the boxed areas at (b). Representative coronal sections of the rostral and caudal aqueduct regions, stained for **(d)** N-cad (AJ labeling, green) and AFRU (SCO/Reissner fiber-associated cells marker). Control animals show the expected distribution, whereas mutants show altered localization, with signal reduced rostrally and detected ectopically/accumulated caudally. **(e)** Higher magnification of the N-cad/ARFU staining. **(f)** Representative coronal sections of the rostral and caudal aqueduct regions, stained for Foxj1^{+} ECs**. (g)** Quantification of ARFU^{+} cells per section in rostral and caudal aqueduct regions (rostral aqueduct: N=3 Controls, N=4 CRB-dko$^{Wnt1\text{-}Cre2}$, caudal aqueduct: N=3 Controls, N=4 CRB-dko$^{Wnt1\text{-}Cre2}$), P = 0.0118 (Rostral Controls vs. Caudal CRB-dko$^{Wnt1\text{-}Cre2}$), P > 0.9999 (ns). **(h)** Quantification at P0 of Foxj1^{+} cells per section, (rostral aqueduct: N=3 Controls, N=3 CRB-dko$^{Wnt1\text{-}Cre2}$, caudal aqueduct: N=3 Controls, N=3 CRB-dko$^{Wnt1\text{-}Cre2}$), P>0.9999 (ns). (g,h): Two-way ANOVA with post-hoc tests. In the plots significance is indicated as *P<0.05, **P<0.01,***P< 0.001, ****P < 0.0001, ns, not significant. Data are presented as mean ± SD.