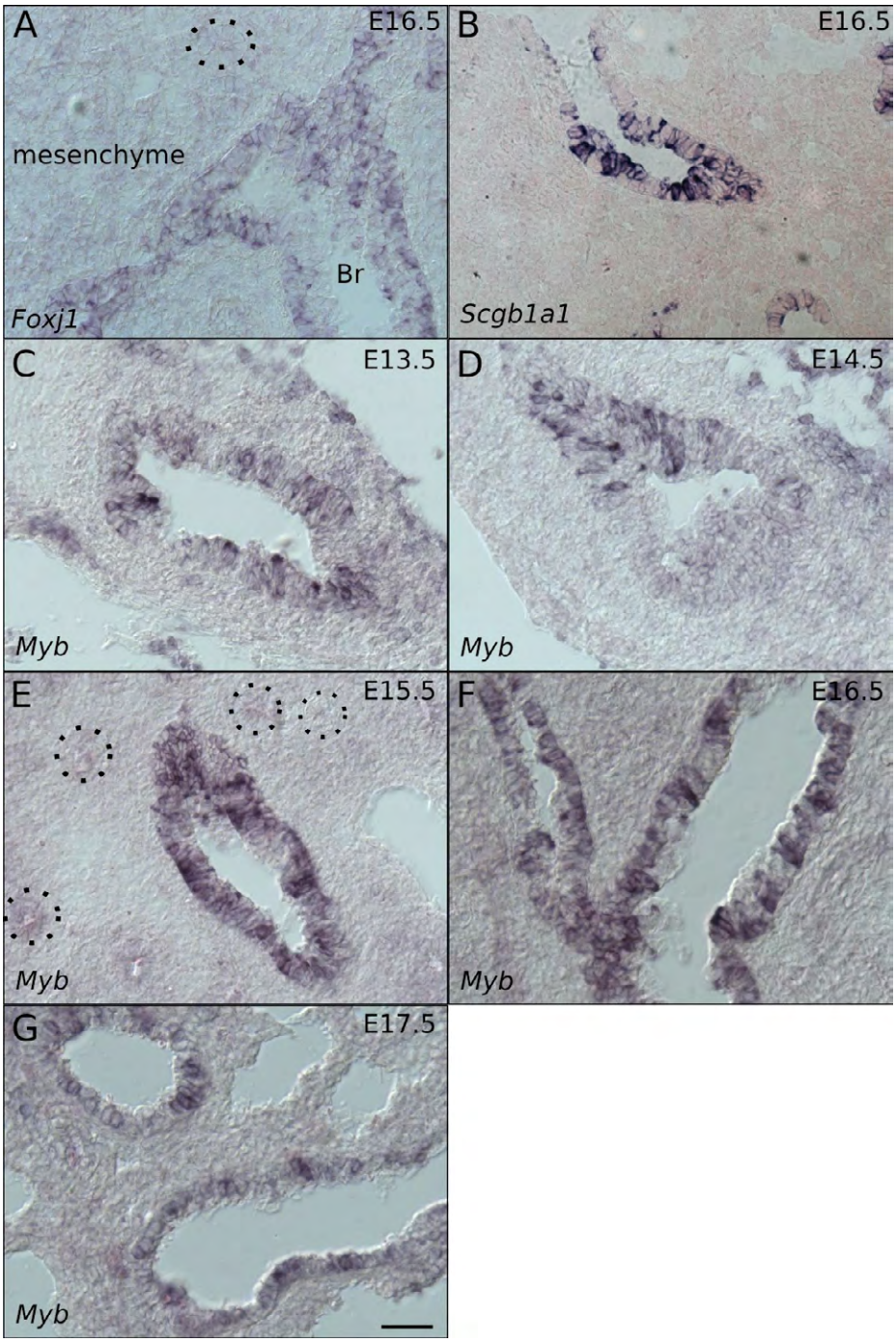


Figure S1. *Myb* is expressed in a pattern similar to *Foxj1* and *Scgb1a1* during embryonic lung development. (A) In situ hybridization for *Foxj1* mRNA in an E16.5 mouse lung. *Foxj1* is expressed in the large bore proximal airway (bronchus, Br) in a "salt and pepper" pattern but is not expressed in the small developing distal airways (dotted outline). (B) In situ hybridization for *Scgb1a1*, a marker for Clara cells, shows a similar "salt and pepper" pattern in large bore proximal airways. (C-G) In situ hybridization for *Myb* from E13.5 through E17.5. *Myb* is expressed in large bore proximal lumenized airways in a "salt and pepper" pattern at all time points shown. Note in E (reproduced from Figure 1) that *Myb* is absent from small diameter distal airways (dotted outlines). Bar, 50 μm (A-G).

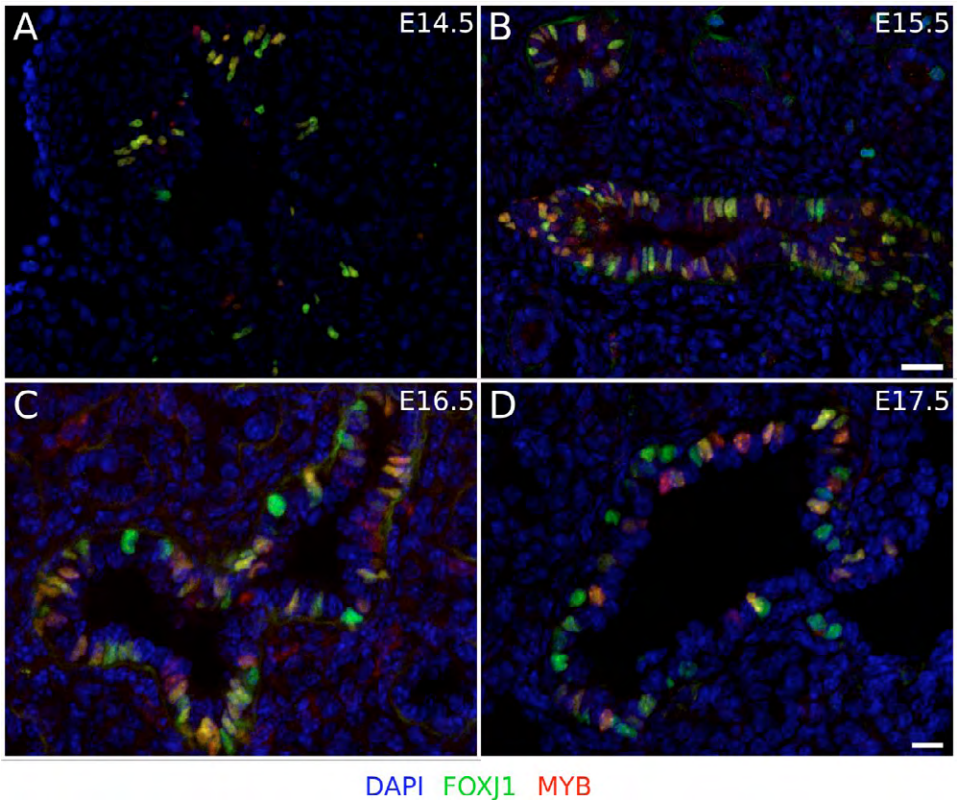


Figure S2. Overlap of MYB expression and FOXJ1 expression during lung development. (A-D) Sections of lung tissue from E14.5 through E17.5 immunostained for MYB and FOXJ1. Bar, 20 μm (A, B), 10 μm (C, D).

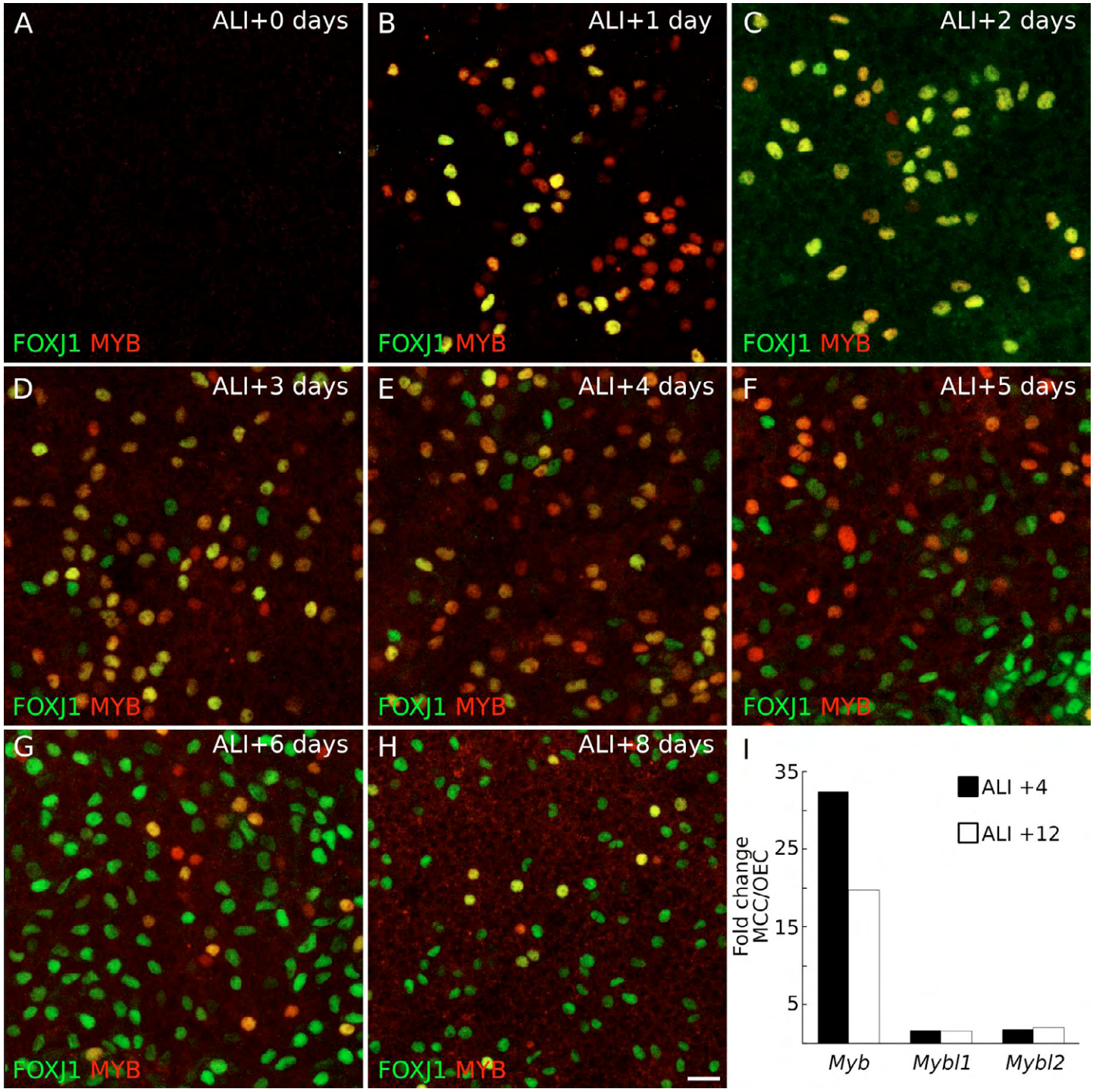


Figure S3. MYB and FOXJ1 expression during multiciliogenesis in mouse tracheal epithelial cell (MTEC) cultures. (A-H) MTECs were fixed at the indicated times after establishment of air liquid interface (ALI) and immunostained for MYB and FOXJ1. MYB expression largely overlaps with FOXJ1, similar to the results obtained *in vivo*. MYB expression was never detected before ALI creation. (I) Expression of *Myb* genes during multiciliogenesis in MTECs. FOXJ1-GFP-positive (multiciliated cells, MCCs) and FOXJ1-GFP-negative (other epithelial cells, OECs) cell populations at ALI+4 days and ALI+12 days were isolated by FACS from MTECs cultured from mice carrying GFP driven by the *Foxj1* promoter (Ostrowski et al., 2003), and RNA was isolated and analyzed on DNA microarrays (Hoh et al., 2012). Values shown are ratios of expression levels in FOXJ1-GFP-positive cells compared to FOXJ1-GFP-negative cells, each being first normalized to a common microarray reference standard. *Myb* expression was 33-fold higher in FOXJ1-GFP-positive cells as compared to the reference set than in FOXJ1-GFP-negative cells at ALI +4 days. Levels of *Myb* expression drop to 19-fold higher by ALI +12 days, when most MCCs are mature. Bar, 25 μm (A-H).

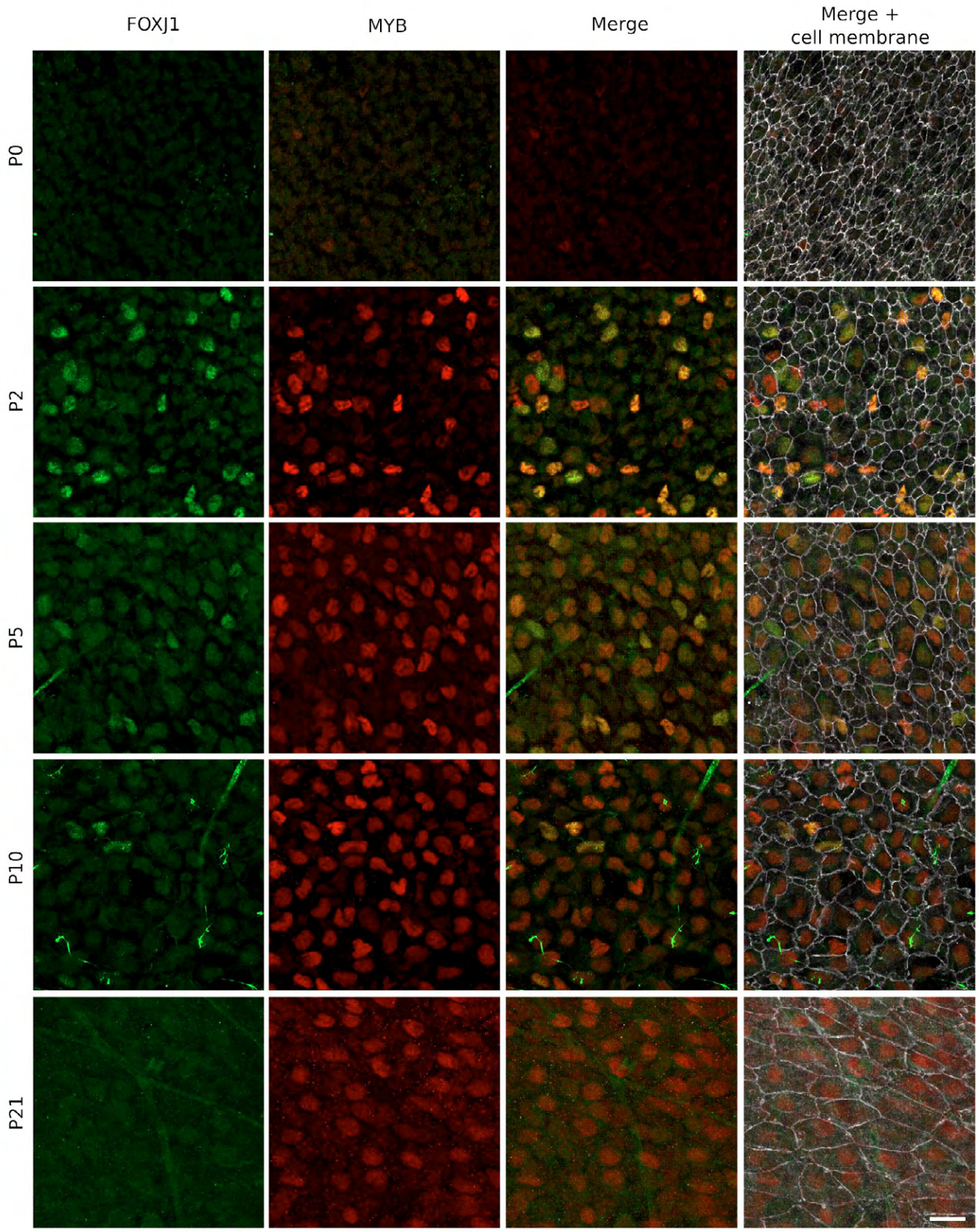


Figure S4. Expression of MYB and FOXJ1 during mouse ependymal multiciliogenesis. Wholemount *en face* ventricle preparations of brains from animals of postnatal ages indicated, including P0 through P10, which encompasses the period of multiciliogenesis, were stained for MYB, FOXJ1 and catenin beta 1 (β-catenin) to outline apical cell membrane. The anterior-dorsal region of the lateral ventricular wall is shown (Mirzadeh et al., 2008). MYB expression overlapped with FOXJ1 expression during early multiciliogenesis, but was not expressed at later time points and when ependymal cells are mature. Note that panel P2 is also presented in Figure 2. Bar, 15 μm.

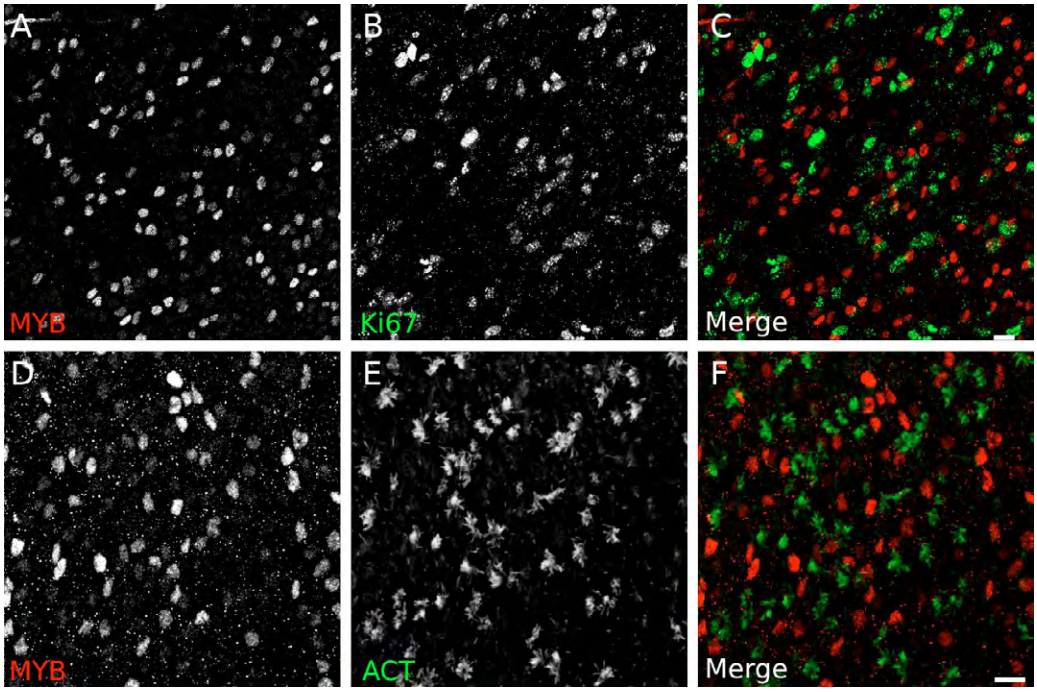


Figure S5. Timing of MYB expression during ependymal multiciliogenesis. *En face* view of brain ventricles from P2 animals were immunostained for MYB and Ki67 or acetylated tubulin (ACT) as indicated. (A-C) MYB is not co-expressed with Ki67, a marker of cycling cells. (Panel C is reproduced from Figure 2.) (D-F) MYB is not co-expressed with ACT, a marker of mature multiciliated ependymal cells. Bar, 10 μm (A-F).

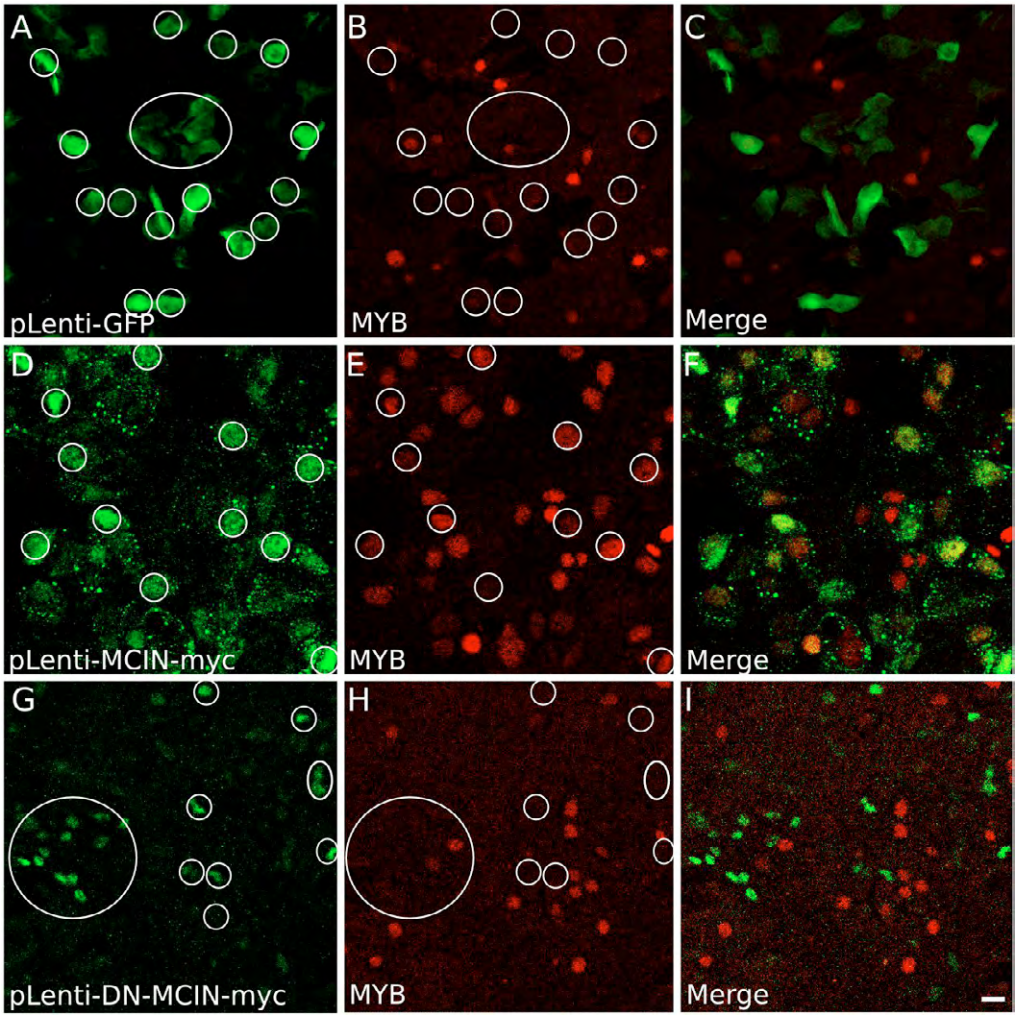


Figure S6. Effect of MCIN on MYB expression in MTECs. MTECs were infected with a control lentivirus expressing GFP (A-C), myc-tagged MCIN, which induces motile ciliogenesis in infected cells (D-F), or a myc-tagged dominant negative form of MCIN, which blocks motile ciliogenesis in infected cells (Stubbs et al., 2012) (G-I), harvested at ALI+4 days and stained for MYB and myc. Circles indicate examples of infected cells. Bar, 10 μm (A-I).

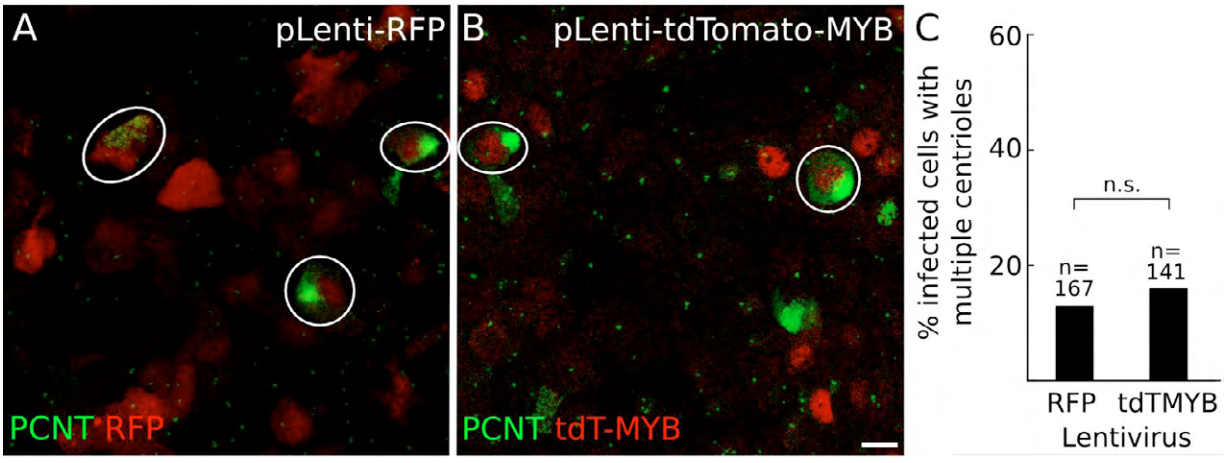


Figure S7. Effect of MYB on centriole amplification in MTECs. MTECs infected with a lentivirus expressing either RFP alone (A) or a tdTomato-MYB fusion protein (B) were harvested at ALI+6 days and stained for PCNT, a centriole marker, to assess centriole amplification. (C) Quantification of the percent of infected cells that display multiple centrioles. There was no significant difference (n.s.) in the number of infected cells with amplified centrioles between control and tdTomato-MYB infected filters by chi-square test. Ovals, examples of cells with amplified centrioles. Bar, 10 μm (A, B).

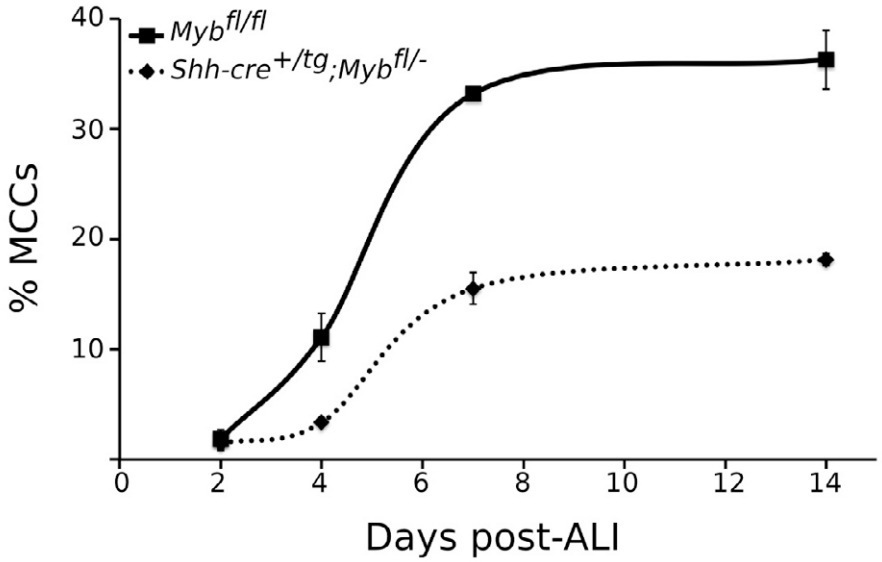


Figure S8. Incomplete recovery of multiciliogenesis in *Myb* CKO MTECs. MTECs were cultured from *Myb* CKO (dashed line) and control animals (solid line). The number of MCCs (cells expressing ACT) was counted at the indicated times after ALI. Even at ALI +14 days, *Myb* CKO MTECs produce less MCCs than controls, similar to what is observed in vivo. Bars, s.e.m.

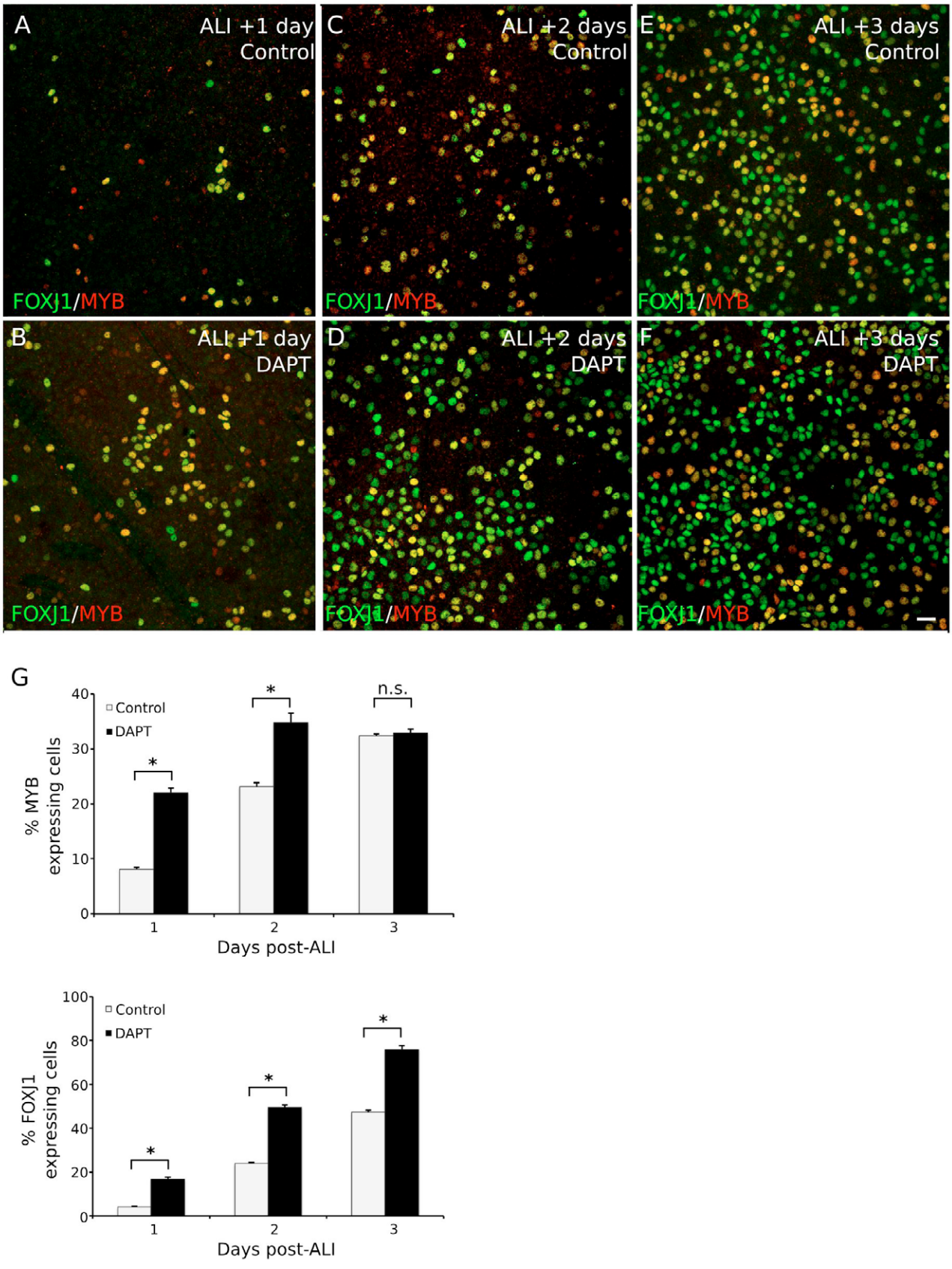
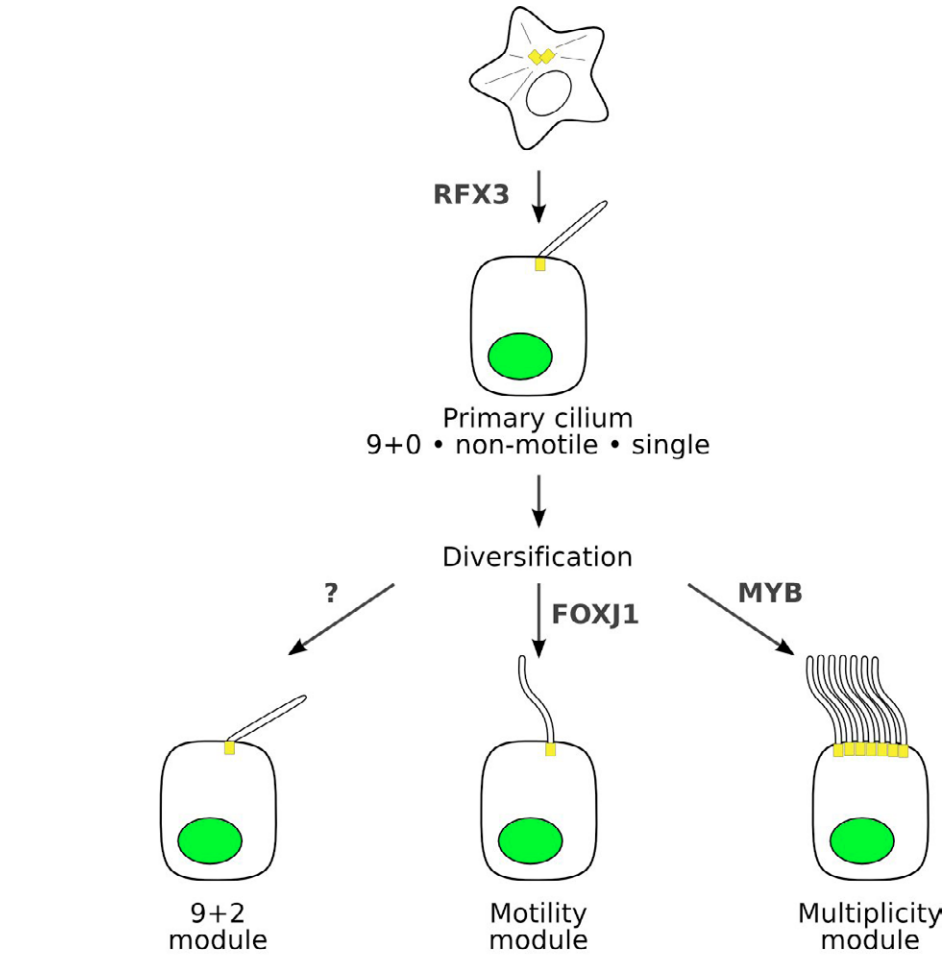


Figure S9. Effect of DAPT treatment on MYB expression in MTECs. (A-F) MTECs were mock-treated (control) or treated with the Notch inhibitor DAPT, which induces motile ciliogenesis in MTECs, from ALI -1 day to ALI +3 days and then harvested at the times indicated and stained for MYB and FOXJ1. Bar, 20 μm. (G) Quantification of percentage of total cells that are MYB-expressing (top) or FOXJ1-expressing (bottom) in cultures as in A-F. * = $p<0.0002$, n.s. = not significant by Student's T test. Bars, s.e.m.



	9+2 module	Motility module	Multiplicity module
Kinocilia	+	−	−
Nodal cilia	−	+	−
Flagella	+	+	−
MCC cilia	+	+	+

Figure S10. Transcriptional control of cilium generation and diversity. Rfx transcription factors control primary cilium formation (9+0 axoneme, non-motile, single) and *Myb*, *Foxj1* and other, as yet unidentified transcription factors are proposed to control different structural (9+2 axoneme, multiplicity) and functional (motility) modules that are deployed in different combinations by various cell types to generate ciliary diversity. The modules used (+) to form four different types of cilia are shown below the diagram. MCC, multiciliated cell.

Table S1. PCR primer sequences

Primers for *X. laevis* animal cap qRT-PCR	
Primer name	Primer sequence
cmyb for1	GCCCCAAAAGGTGGTCAGT
cmyb rev1	CCTCTCTCTGCATTGTTTGCCTAT
foxj1 QF4	TGAGCTAACGGGCAGCAAAT
foxj1 QR4	TTCTTTTCATTGTGGCCATGTC
odc QF2	CTGCCGCCTCAGTGTGAA
odc QR2	TGCCCGCTCCAGAAGC
Primers for cloning *X. laevis Myb* for microinjections	
Primer name	Primer sequence
Stu-XLcMybForward	CGAGGCCTATGGACAGGAGACCAAGCC
Xba1-XLcMybReverse	CGTCTAGATTATCATATCACTAGTGTAG

Table S2. Quantification of lung epithelial cells co-expressing MYB and Ki67

Age	Cells scored	Expressing MYB	Expressing MYB and Ki67	$MYB^+/Ki67^+$
E14.5	249	245	4	2%
E15.5	286	282	4	1%
E16.5	215	215	0	0%
E17.5	98	98	0	0%