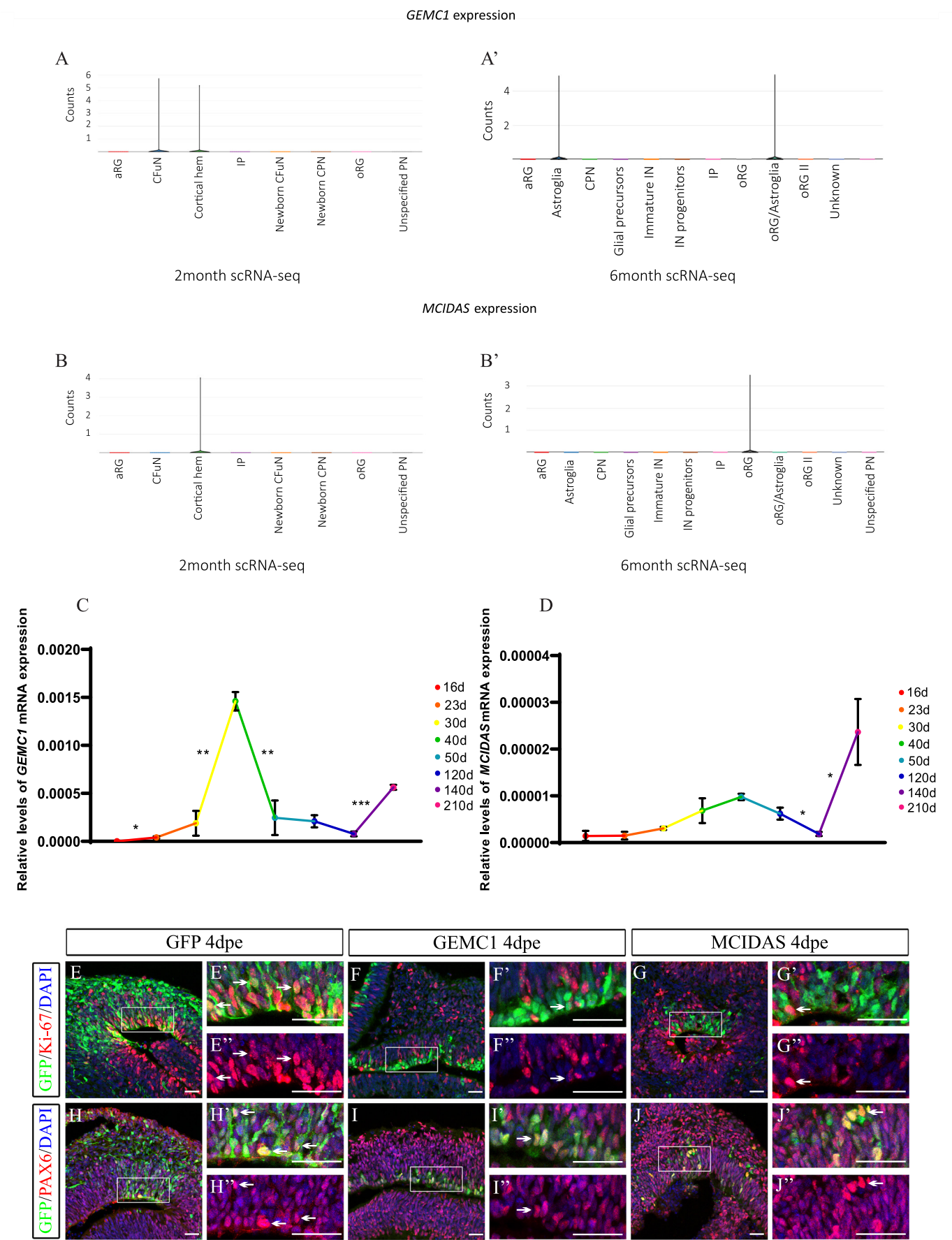


Expanded View Figures

Figure EV1. Cell- and time-specific expression patterns of GEMC1 and MCIDAS and their influence on aRGs number.

(A–B') Analyses of published single-cell RNA sequencing data in cells isolated from hBOs in different developmental stages (Data ref: Uzquiano et al, 2022). Violin plots showing the GEMC1 and MCIDAS normalized counts at different cell types in 2 m (A, B) and 6 m hBOs (A', B'). (C, D) GEMC1 and MCIDAS quantitative mRNA expression analyses in hBOs in different developmental stages. Data normalized against GAPDH. Three independent replicates were analyzed, each bearing the homogenate of three individual brain organoids. (E–J'') GFP, *GEMC1* or *MCIDAS* OE BOs at 4 dpe stained using antibodies against GFP and Ki-67 (E–G'') or PAX6 (H–J''). Data are represented as the mean $\pm$ s.e.m. Statistical analysis was performed using the unpaired *t* test (**P* < 0.05, ****P* < 0.001, *****P* < 0.0001). White boxes indicate the area zoomed-in in the corresponding pictures. Arrows indicate Ki-67+GFP+ or PAX6+GFP+ cells. Scale bars: 30 μ m.



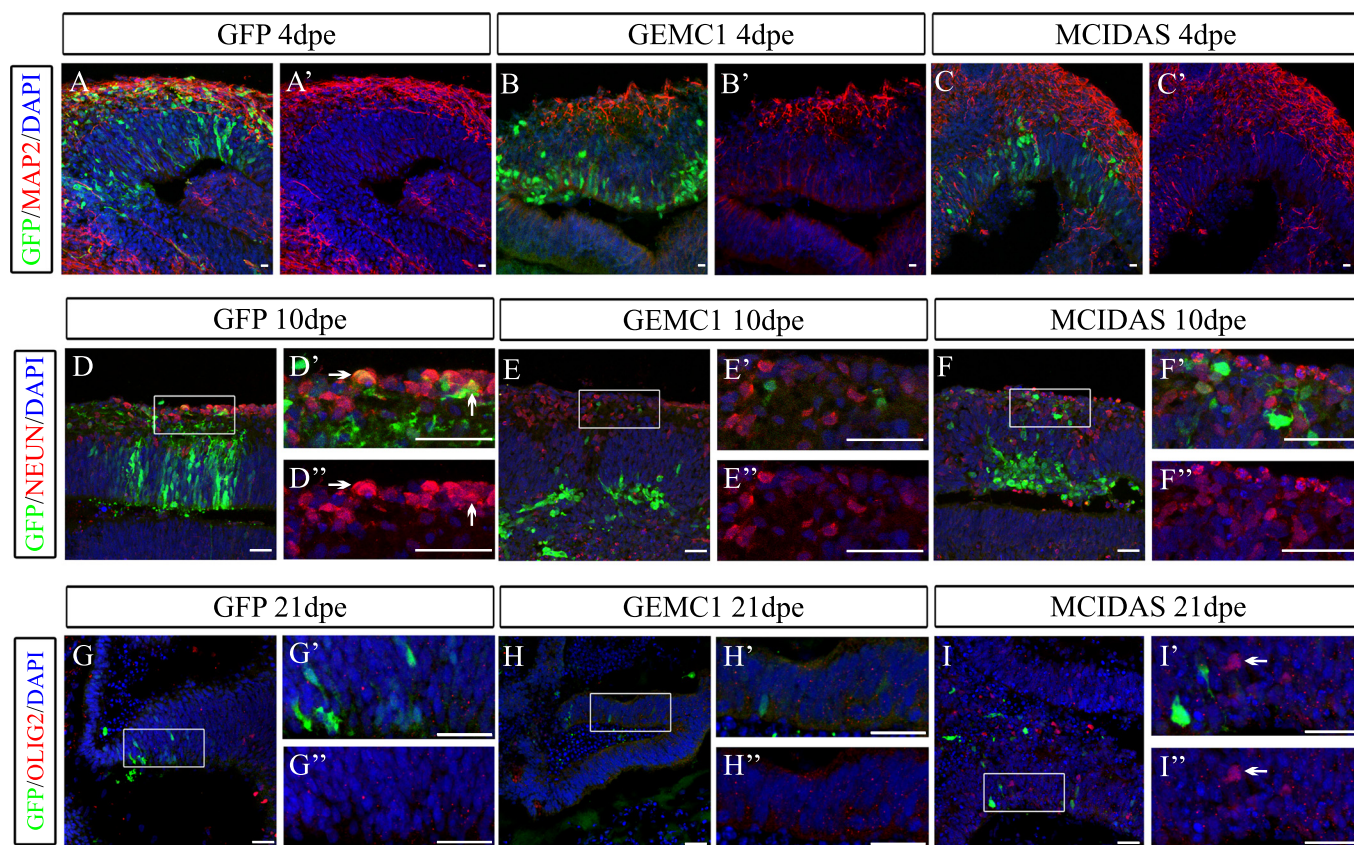


Figure EV2. GEMC1 and MCIDAS OE cells fail to differentiate into neurons.

(A–C') GFP, *GEMC1* or *MCIDAS* OE BOs at 4 dpe stained using antibodies against GFP and MAP2. (D–F') GFP, *GEMC1* or *MCIDAS* OE BOs at 10 dpe stained using antibodies against GFP and NEUN. (G–I') GFP, *GEMC1* or *MCIDAS* OE BOs at 21 dpe stained using antibodies against GFP and OLIG2. Biological replicates: MAP2: GFP: $v = 9$, *GEMC1*: $v = 4$, *MCIDAS*: $v = 4$. NEUN: $v = 3$ for all conditions. OLIG2: GFP: $v = 1$, *GEMC1*: $v = 2$, *MCIDAS*: $v = 2$. White and red boxes indicate the area zoomed-in in the corresponding pictures. Arrows indicate NEUN+GFP+ or OLIG2+ cells. Scale bars: 30 μm .

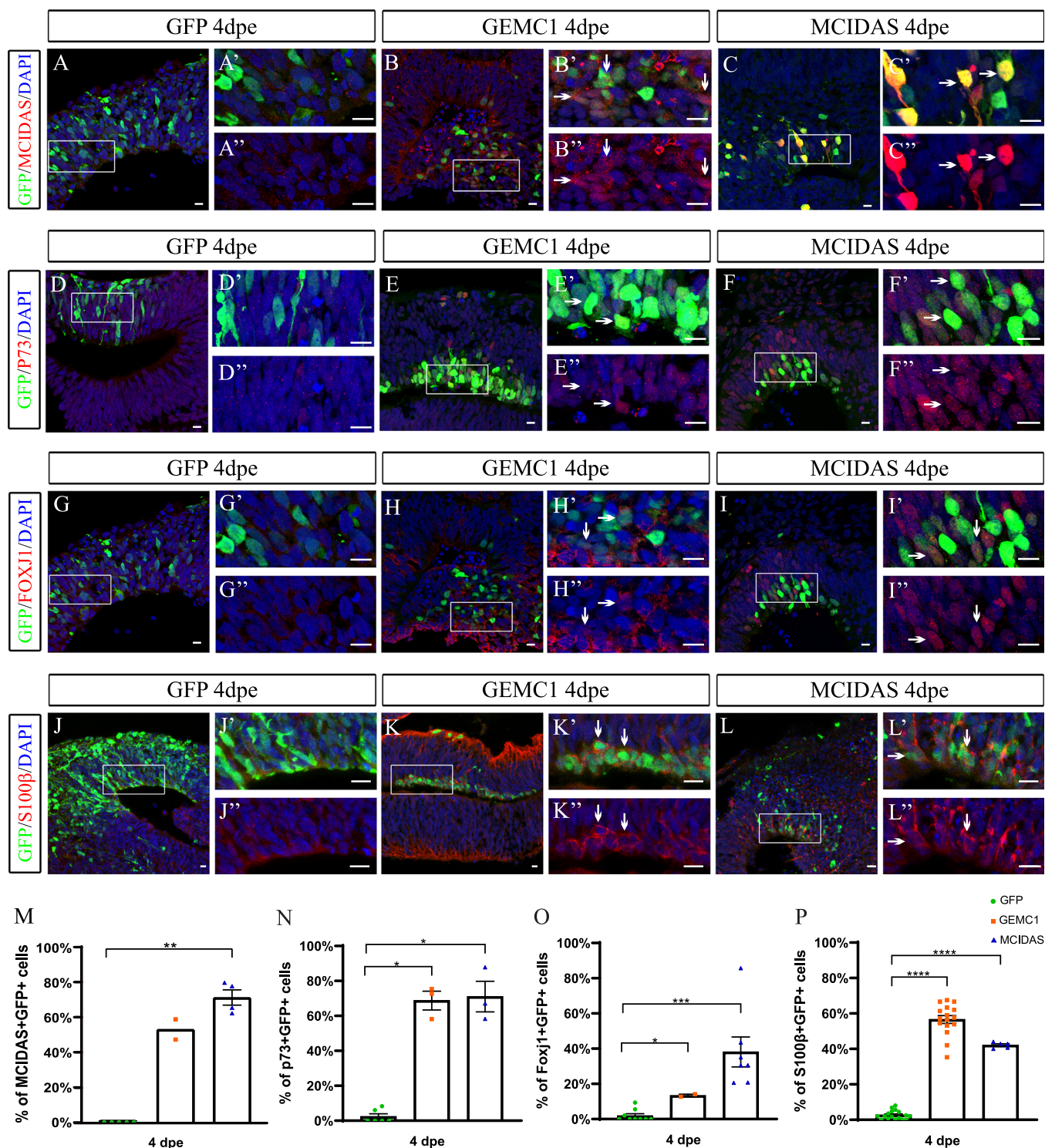


Figure EV3. GEMC1 and MCIDAS OE cells express ECs markers.

(A–L'') GFP, *GEMC1* or *MCIDAS* OE BOs at 4 dpe stained using antibodies against GFP and *MCIDAS* (A–C''), *P73* (D–F''), *FOXJ1* (G–I'') or *S100β* (J–L''). DAPI and GFP channels in images (A–A'') are reused in (G–G''), DAPI and GFP channels in images (B–B'') are reused in (H–H'') and DAPI and GFP channels in images (F–F'') are reused in (I–I''). (M–P) Quantification of the percentage of *MCIDAS*+GFP+ (M), *P73*+GFP+ (N), *FOXJ1*+GFP+ (O) and *S100β*+GFP+ cells (P). Biological replicates: *MCIDAS*: GFP: $v = 5$, *GEMC1*: $v = 2$, *MCIDAS*: $v = 4$ (P value: 0.0079). *P73*: GFP: $v = 6$, *GEMC1*: $v = 3$, *MCIDAS*: $v = 3$ (P value: 0.0119 and 0.0119, respectively). *FOXJ1*: GFP: $v = 9$, *GEMC1*: $v = 2$, *MCIDAS*: $v = 7$ (P value: 0.0182 and 0.0002, respectively). *S100β*: GFP: $v = 17$, *GEMC1*: $v = 16$, *MCIDAS*: $v = 5$ (P value: <0.0001 and <0.0001, respectively). Data are represented as the mean $\pm$ s.e.m. Statistical analysis was performed using the nonparametric two-tailed Mann-Whitney test (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$). White boxes indicate the area zoomed-in in the corresponding pictures. Arrows indicate *MCIDAS*+GFP+, *P73*+GFP+ cells, *FOXJ1*+GFP+ cells or *S100β*+GFP+ cells. Scale bars: 10 μ m.

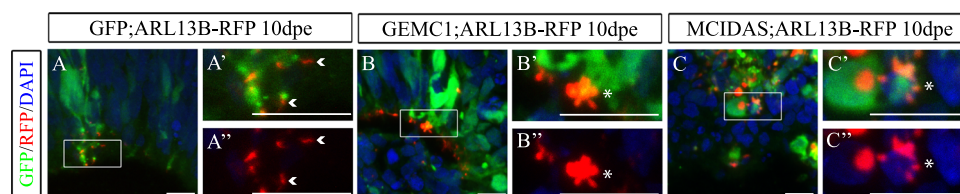


Figure EV4. GEMC1 and MCIDAS OE cells showcase multiple cilia.

(A–C'') BOs co-transfected with a GFP, GEMC1 or MCIDAS plasmid and an ARL13B-RFP plasmid at 10 dpe were stained using antibodies against GFP and RFP. Biological replicates: $v = 2$ for all conditions. White boxes indicate the area zoomed-in in the corresponding pictures. Arrowheads indicate cells that have one cilium and asterisks indicate electroporated cells that exhibit accumulation of ARL13B. Scale bars: 10 μm .

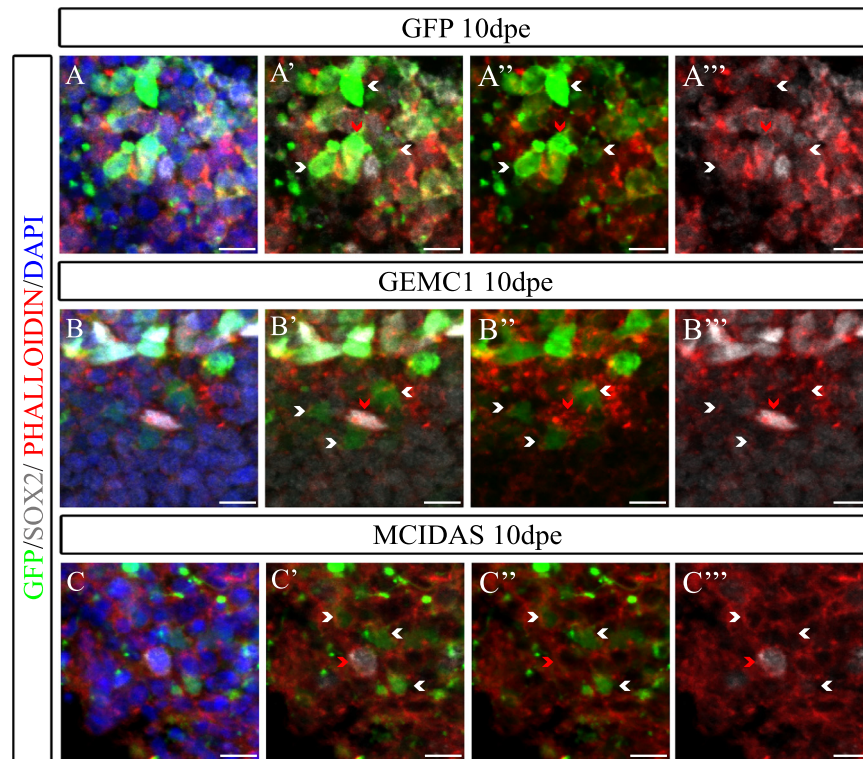


Figure EV5. *GEMC1* and *MCIDAS* OE leads to the formation of neural rosettes.

(A–C'') GFP, *GEMC1* or *MCIDAS* OE BOs at 10 dpe were stained using antibodies against GFP, SOX2 and PHALLOIDIN. Biological replicates: GFP: $v = 3$, *GEMC1*: $v = 1$, *MCIDAS*: $v = 3$. White arrowheads indicate GFP+ cells and red arrowheads indicate SOX2+ cells. Scale bars: 10 μm .