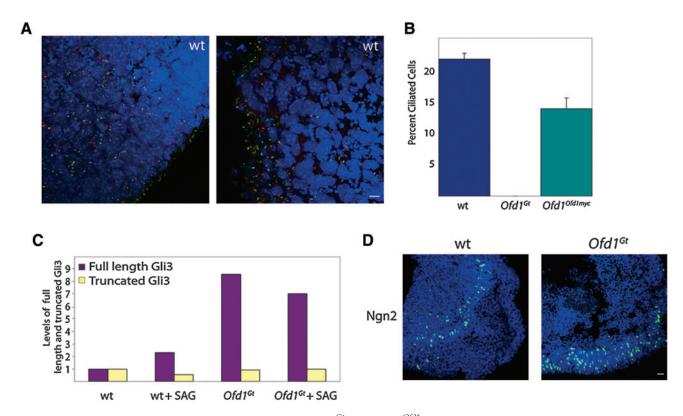
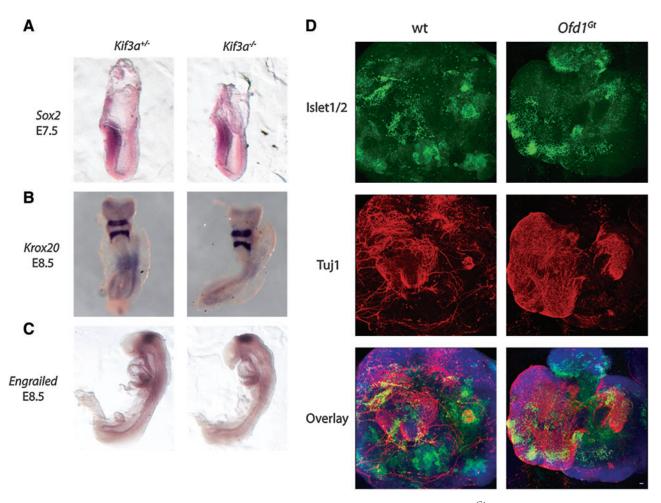
## **Supplementary Data**



**SUPPLEMENTARY FIG. S1.** Ciliation in wild-type,  $Ofd1^{Gt}$ , and  $Ofd1^{Ofd1myc}$  EBs. (A) Wild-type EBs stained for Arl13b (green) marking cilia and γ-tubulin (red) marking basal bodies. Scale bar  $10\,\mu\text{m}$ . (B) Percent ciliated cells in synchronized wild-type,  $Ofd1^{Gt}$ , and  $Ofd1^{Ofd1myc}$  embryonic stem cells. (C) Quantification of full-length and truncated Gli3 by immunoblot in wild-type and  $Ofd1^{Gt}$  control and SAG treated EBs. (D) Sections of wild-type and  $Ofd1^{Gt}$  EBs stained with Ngn2 (green) after 15 days of differentiation. Scale bar  $20\,\mu\text{m}$ . wt, wild type; Ofd1, Oral-Facial-Digital 1;  $Ofd1^{Gt}$ , gene trap insertion into the gene Ofd1; EB, embryoid body.



**SUPPLEMENTARY FIG. S2.** Neural specification and patterning in wild-type and  $Ofd1^{Gt}$  EBs and Kif3a mutant embryos. **(A–C)** Wholemount in situs of wild-type and Kif3a mutant embryos at E7.5 or E8.5 of development probed for ectoderm (Sox2), hindbrain (Krox20), and midbrain (Engrailed) markers. **(D)** Wild-type and  $Ofd1^{Gt}$  EBs stained for motor neurons (Islet1/2, green), postmitotic neurons (Tuj1, red), and nuclei (4',6-diamidino-2-phenylindole, blue). Scale bar 20  $\mu$ m.