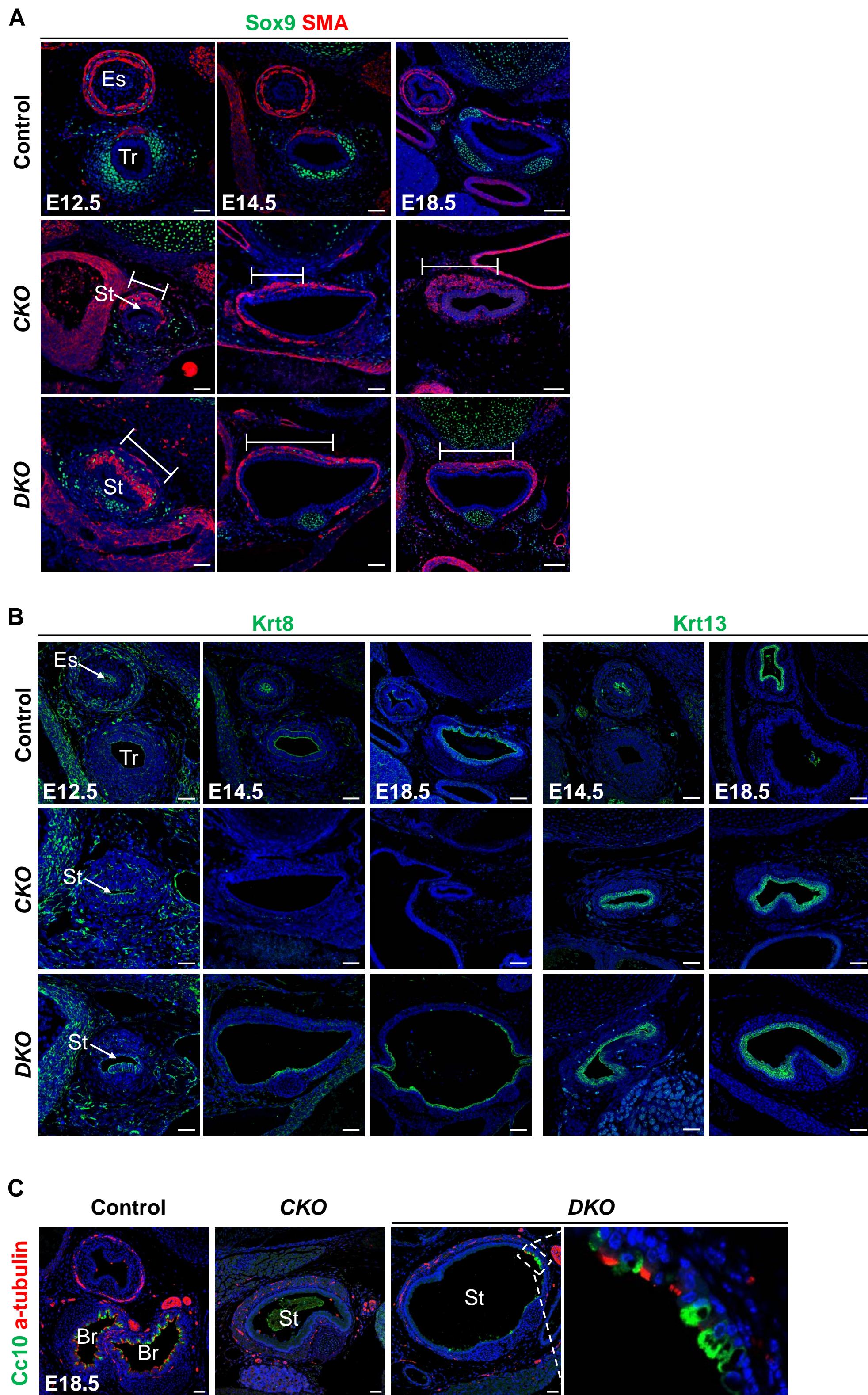
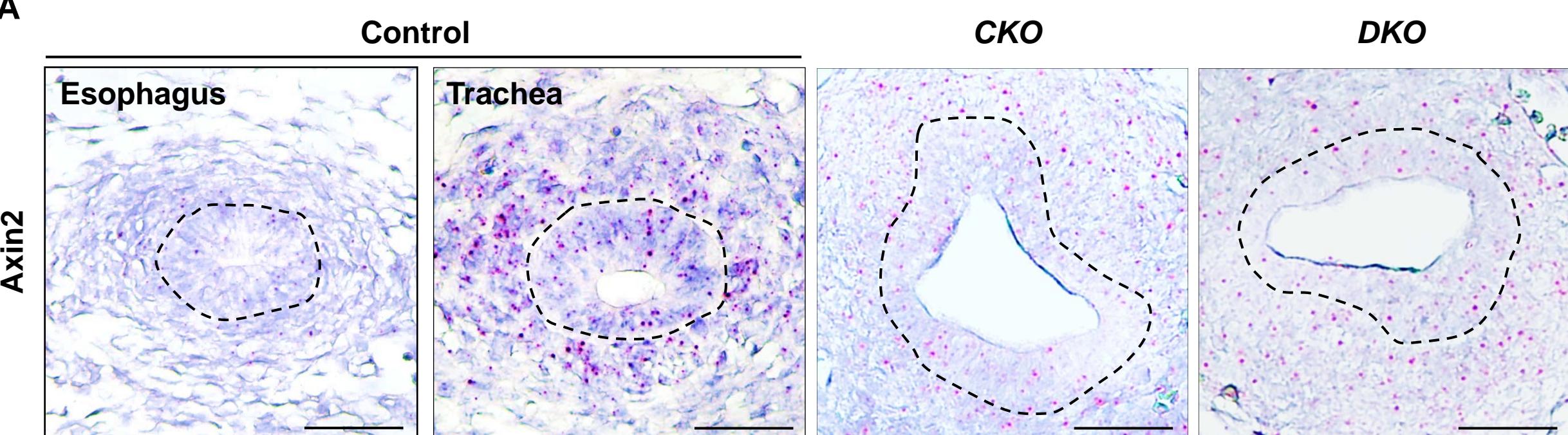
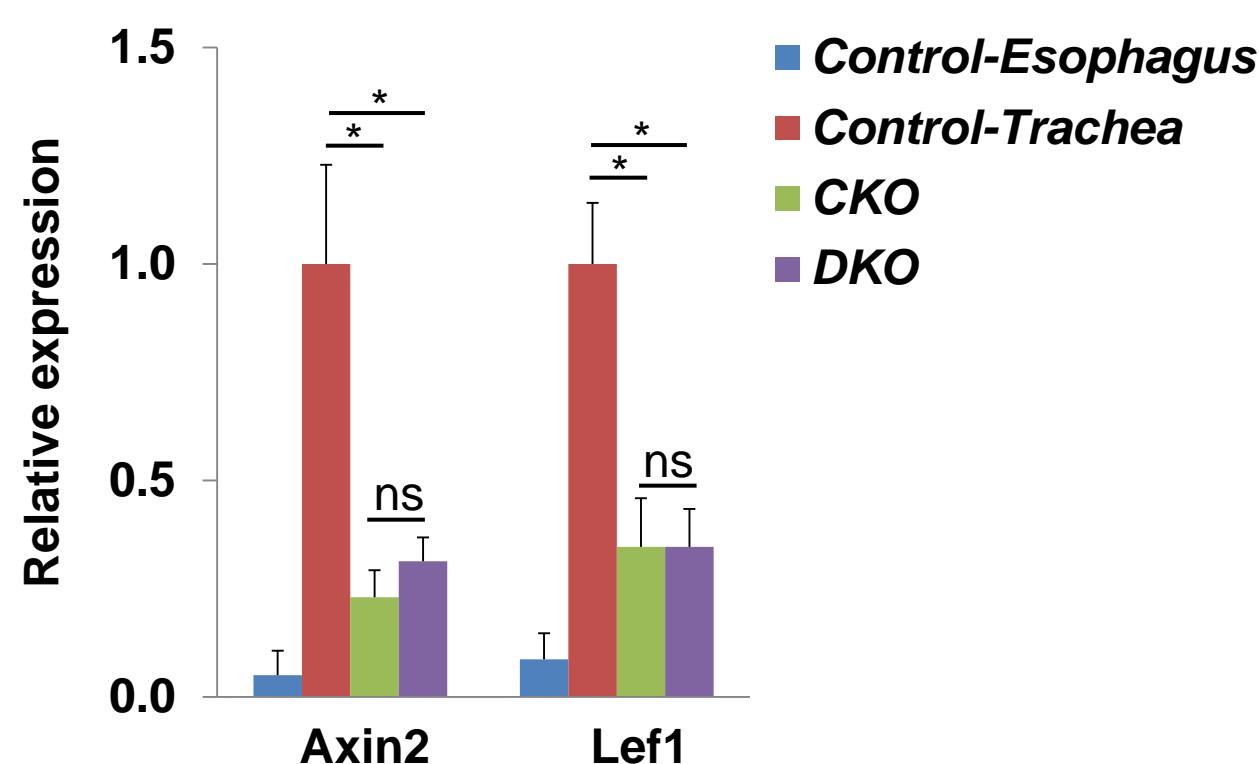


**Fig. S1.  $\beta$ -catenin protein is retained in the epithelial junction of  $Ctnnb1^{DKO}$  but not  $Ctnnb1^{CKO}$  mutants.** Abbreviation: Es: esophagus; Tr: trachea; St: single lumen tube; CKO:  $Shh\text{-}Cre};Ctnnb1^{loxp/loxp}$ ; DKO:  $Shh\text{-}Cre};Ctnnb1^{DM/loxp}$ . Scale bar: 50  $\mu\text{m}$ .



**Fig. S2. Presence of residual respiratory epithelium in the unseparated foregut tube in *Ctnnb1*<sup>DKO</sup> but not *Ctnnb1*<sup>CKO</sup> mutants.** (A) Presence of cartilage nodules in *Ctnnb1*<sup>DKO</sup> but not *Ctnnb1*<sup>CKO</sup> mutants. Note expanded smooth muscle cells in the foregut of both *Ctnnb1*<sup>CKO</sup> and *Ctnnb1*<sup>DKO</sup> mutants. (B) The residual respiratory epithelial cells in *Ctnnb1*<sup>DKO</sup> mutants express the columnar cell marker Krt8, but not the squamous cell protein Krt13 which is enriched throughout the esophagus-like tube in *Ctnnb1*<sup>CKO</sup> mutants. (C) Presence of club cells (Cc10<sup>+</sup>) and ciliated cells ( $\alpha$ -tubulin<sup>+</sup>) in the ventral side of the unseparated foregut of *Ctnnb1*<sup>DKO</sup> mutants at E18.5. Abbreviation: Es, esophagus; Tr, trachea; Br, bronchus; St, single lumen tube; CKO: *Shh-Cre; Ctnnb1*<sup>loxP/loxP</sup>; DKO: *Shh-Cre; Ctnnb1*<sup>DM/loxP</sup>. Scale bar: 50  $\mu$ m.

**A****B**

**Fig. S3. Deletion of  $\beta$ -catenin reduces the mRNA expression of Wnt signaling target genes.** (A) In situ hybridization of *Axin2* indicates reduction of mRNA expression in both *CKO* and *DKO* mutants. Note that the expression of *Axin2* mRNA is low in the esophagus. (B) The mRNA expression of *Axin2* and *Lef1* is reduced in both *CKO* and *DKO* mutants (\* $p < 0.05$ ,  $n = 3$  for each). Abbreviation: *CKO*: *Shh-Cre*; *Ctnnb1*<sup>loxP/loxP</sup>; *DKO*: *Shh-Cre*; *Ctnnb1*<sup>DM/loxP</sup>. Scale bar: 50  $\mu$ m.