Supplementary material legends

Supplementary table1: Primers utilized for genotyping.

Supplementary table 2: List of antibodies utilized for these studies.

Supplementary Figure 1: *Wls* was efficiently deleted from tracheal tissue by Cre mediated recombinase activity. DNA from E11.5 embryonic tracheal tissue was isolated to confirm deletion of *Wls* by PCR. Primers P2-P4 were utilized to detect a 441 bp WT band and a 556 bp Wls^{fl} Neo deleted band, while primers P1-P4 detected a 1,625 bp WT band and a 410 bp- null band validating Cre mediated deletion of *Wls* in $Wls^{f/f};Shh^{Cre/+}$ tracheal tissue.

Supplementary figure 2: Wnt ligands are expressed in developing respiratory tract and body wall. In situ hybridization was performed to detect transcripts for *Wnt2, Wnt2b and Wnt11*. *Wnt2* and *Wnt2b* transcripts were detected in the periphery of the developing lung; *Wnt11* expression was detected in the mesenchyme of the body wall. Higher magnification of the areas in squares are shown. *Ppib* transcripts, a housekeeping control, were detected throughout the embryo while *Dapb* transcripts, a bacterial gene, were not detected in embryonic mouse samples.

Supplementary figure 3: Epithelial deletion of *Wls* causes tracheomalacia and severely impaired Sox9 expression. Cross sections of E18.5 embryos were stained with Sox9 and αSMA antibody. In control embryos tracheal mesenchyme Sox9 expression was observed in sites where cartilage was formed while αSMA staining was restricted to

the dorsal aspect labeling trachealis muscle. In contrast, Sox9 staining was virtually absent from the *Wls*$^{f/f}$;*Shh*$^{Cre/+}$ tracheal mesenchyme while αSMA was present across the tracheal mesenchyme (A). Higher magnifications of images depicting trachea and esophagus are shown in panel B. T=trachea, E=esophagus.

Supplementary Figure 4: Deletion of Sox9 from the tracheal mesenchymal impaired cartilage formation. Whole mount images of E18.5 embryos depict the anomalous and short forelimbs as well as micrognathia (arrows B, D) observed after mesenchymal deletion of *Sox9* (B, D). Alcian blue staining was near absent in E18.5 tracheas of *Sox9*$^{f/f}$;*Col2a1*$^{Cre/+}$ mice (F). Tracheal cartilaginous rings in *Sox9*$^{f/f}$;*Dermo1*$^{Cre/+}$ embryos, were abnormal and reduced in number resulting in a shorter trachea (H).

Supplementary Figure 5: **Mesenchymal Wls is not required for Sox9 expression or tracheal cartilage formation.** *Wls*$^{f/f}$;*Dermo1*$^{Cre/+}$ mice were bred to *Rosa mT/mG* mice. Longitudinal sections stained for Nkx2.1, Sox9 and GFP antibody showed specific recombination in tracheal mesenchyme driven by Cre recombinase in control and *Wls*$^{f/f}$;*Dermo1*$^{Cre/+}$ at E12.5 (A, B). Sox9 was expressed in control (C) and *Wls*$^{f/f}$;*Dermo1*$^{Cre/+}$ tracheal mesenchyme of E12.5 embryos (D). RT-PCR analysis showed similar levels of *Sox9* and *Col2a1* mRNA in tracheal tissue of *Wls*$^{f/f}$;*Dermo1*$^{Cre/+}$ and control embryos (E). Alcian blue staining of E18.5 tracheal tissue showed that cartilage was present in tracheal mesenchyme of *Wls*$^{f/f}$;*Col2a1*$^{Cre/+}$ embryos (F). T=trachea.

Supplementary material