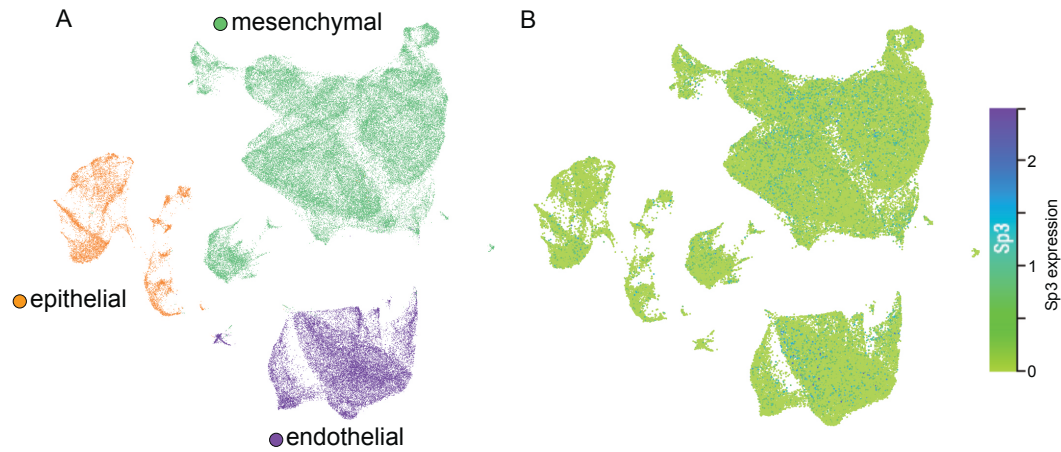
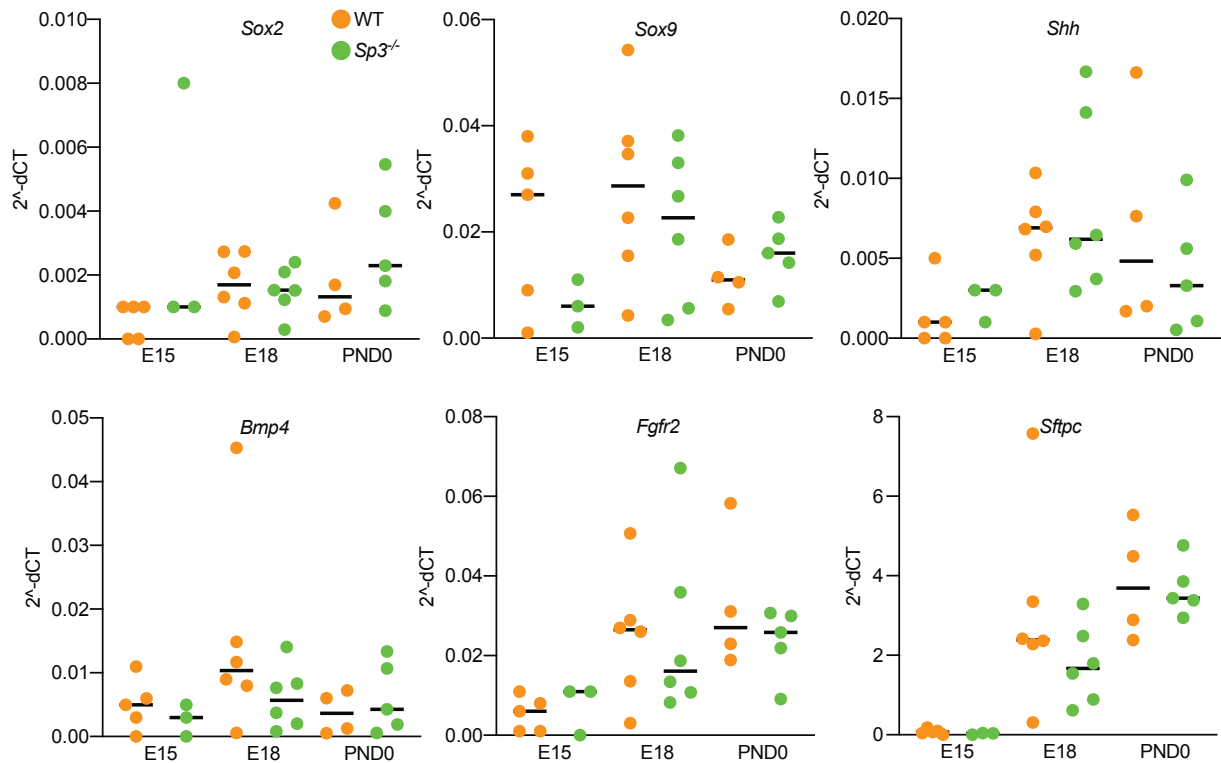
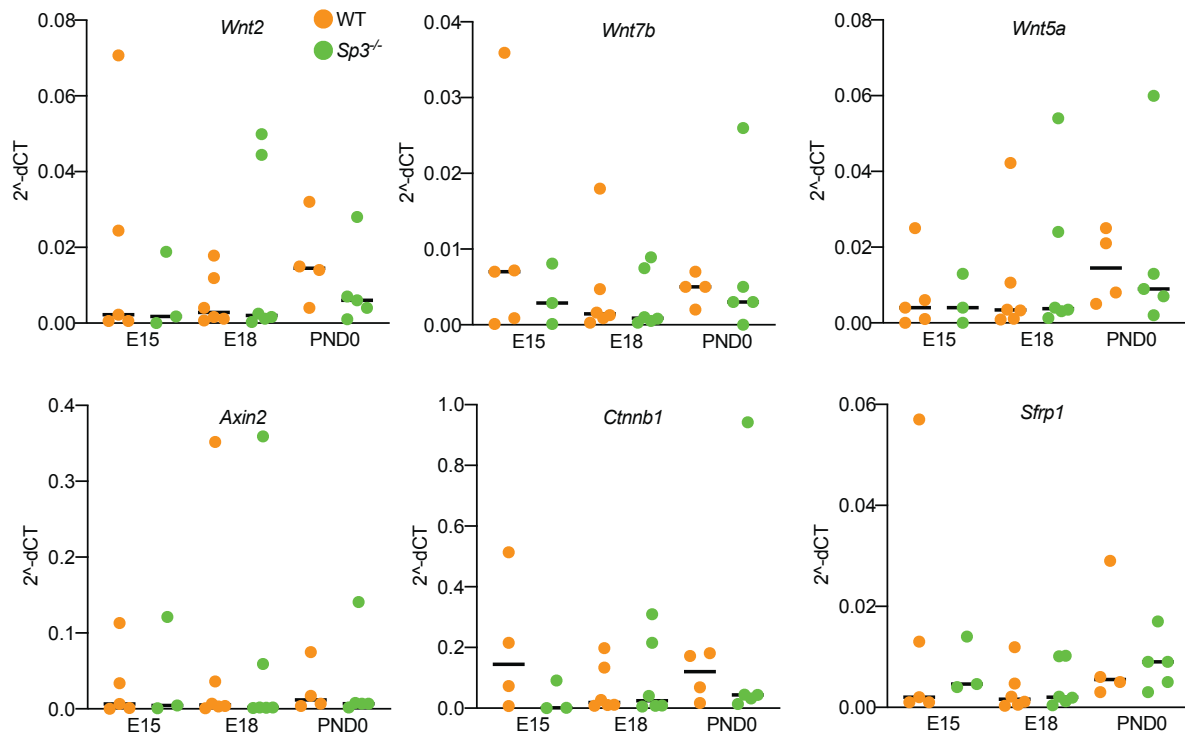




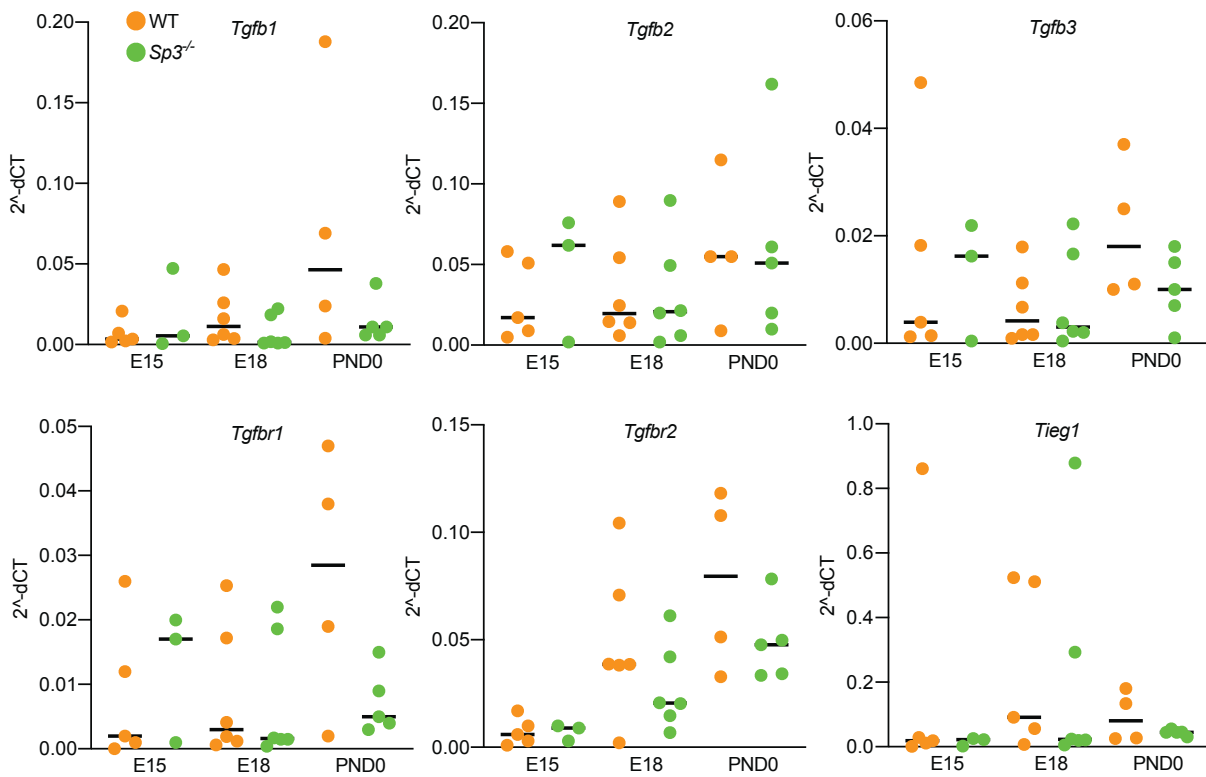
**Fig. S1.** **A.** UMAP plot of fetal mouse lung single cell RNAseq data originally reported (Negretti et al., 2021) and now publicly available through GEO. **B.** Sp3 expression throughout all lung cell populations. <https://lungcells.app.vumc.org/>



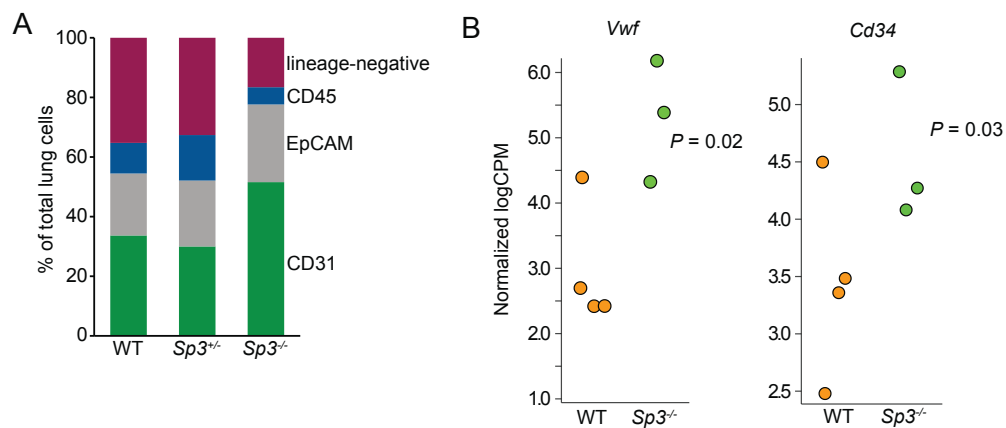
**Fig. S2.** Real time PCR data comparing epithelial marker gene expression in WT and *Sp3*<sup>-/-</sup> fetal (E15 and E18) and newborn (PND0) mouse lung tissue. Total RNA was isolated from lung homogenates and gene expression measured using commercially validated primer sets. Data for each independent biological replicate shown. Gene expression levels plotted as  $2^{-\Delta CT}$  after normalization of  $C_T$  values to *Gapdh* for each replicate.



**Fig. S3.** Real time PCR data comparing Wnt signaling gene expression in WT and *Sp3*<sup>-/-</sup> fetal (E15 and E18) and newborn (PND0) mouse lung tissue. Total RNA was isolated from lung homogenates and gene expression measured using commercially validated primer sets. Data for each independent biological replicate shown. Gene expression levels plotted as  $2^{-\Delta CT}$  after normalization of  $C_T$  values to *Gapdh* for each replicate.



**Fig. S4.** Real time PCR data comparing TGF $\beta$  signaling gene expression in WT and *Sp3*<sup>-/-</sup> fetal (E15 and E18) and newborn (PND0) mouse lung tissue. Total RNA was isolated from lung homogenates and gene expression measured using commercially validated primer sets. Data for each independent biological replicate shown. Gene expression levels plotted as  $2^{-\Delta CT}$  after normalization of  $C_T$  values to *Gapdh* for each replicate.



**Fig. S5. A.** FACS of total lung cell populations from PND0 mouse lungs. Single cell suspensions from WT, *Sp3*<sup>+/-</sup>, and *Sp3*<sup>-/-</sup> lungs were labeled with antibodies against CD45 (immune cells), EpCAM (epithelial cells), and CD31 (endothelial cells). Lineage negative cells contained various mesenchymal cells. **B.** RNAseq data from fetal lung mesenchymal cells showing expression of the endothelial markers *Vwf* and *Cd34* in WT and *Sp3*<sup>-/-</sup> mesenchymal cells.

**Table S1.** qPCR primers

| Gene          | Company | Catalog #      |
|---------------|---------|----------------|
| <i>Axin2</i>  | IDT     | 421924103      |
| <i>Bmp4</i>   | IDT     | 421924082      |
| <i>Ctnnb1</i> | IDT     | 421924106      |
| <i>Etv4</i>   | IDT     | 421924076      |
| <i>Etv5</i>   | IDT     | 421924070      |
| <i>Fgf10</i>  | IDT     | 421924073      |
| <i>Fgfr2</i>  | BioRad  | qMmuCED0047697 |
| <i>Sfrp1</i>  | IDT     | 421924109      |
| <i>Sftpc</i>  | IDT     | 421924091      |
| <i>Shh</i>    | IDT     | 421924079      |
| <i>Sox2</i>   | IDT     | 421924085      |
| <i>Sox9</i>   | IDT     | 421924088      |
| <i>Tbx4</i>   | IDT     | 421924112      |
| <i>Tgfb1</i>  | IDT     | 421924115      |
| <i>Tgfb2</i>  | IDT     | 421924118      |
| <i>Tgfb3</i>  | IDT     | 421924121      |
| <i>Tgfbr1</i> | IDT     | 421924124      |
| <i>Tgfbr2</i> | IDT     | 421924127      |
| <i>Wnt2</i>   | IDT     | 421924094      |
| <i>Wnt5a</i>  | IDT     | 421924097      |
| <i>Wnt7b</i>  | IDT     | 421924100      |

**Supplemental Material References**

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