FGF-Regulated Etv Transcription Factors Control FGF-SHH Feedback Loop in Lung Branching

Supplemental Figures and Legends:

Supplemental Figure 1 (related to Figure 1). *Etv* mutant lungs show branching defects leading to reduced overall size at birth.

(A-D) *Etv4* and *Etv5* expression as analyzed by wholemount (A,B) or section (C,D) RNA in situ hybridization. *Etv5* is restricted to the epithelial tips while *Etv4* expression is present in both the epithlium and mesenchyme. (E.F) Dorsal view of wholemount control and *Etv* mutant lungs taken from P0 pups. At birth *Etv4;5* mutant lungs are noticeably smaller than control lungs. (G,H) Still images of three-dimensional surface rendering of E12.5 lungs stained with anti-E-Cadherin antibody (green) and anti-ACTA2 (also termed smooth muscle actin) antibody (red). (I) Quantification indicates a relatively increased tip volume. (J,K) Representative EdU staining of E12.5 lung sections. (L,M) Relative percentage of EdU+ cells in the mutants compared to controls. No statistical significant difference was observed in either the epithelium or the mesenchyme.

Supplemental Figure 1
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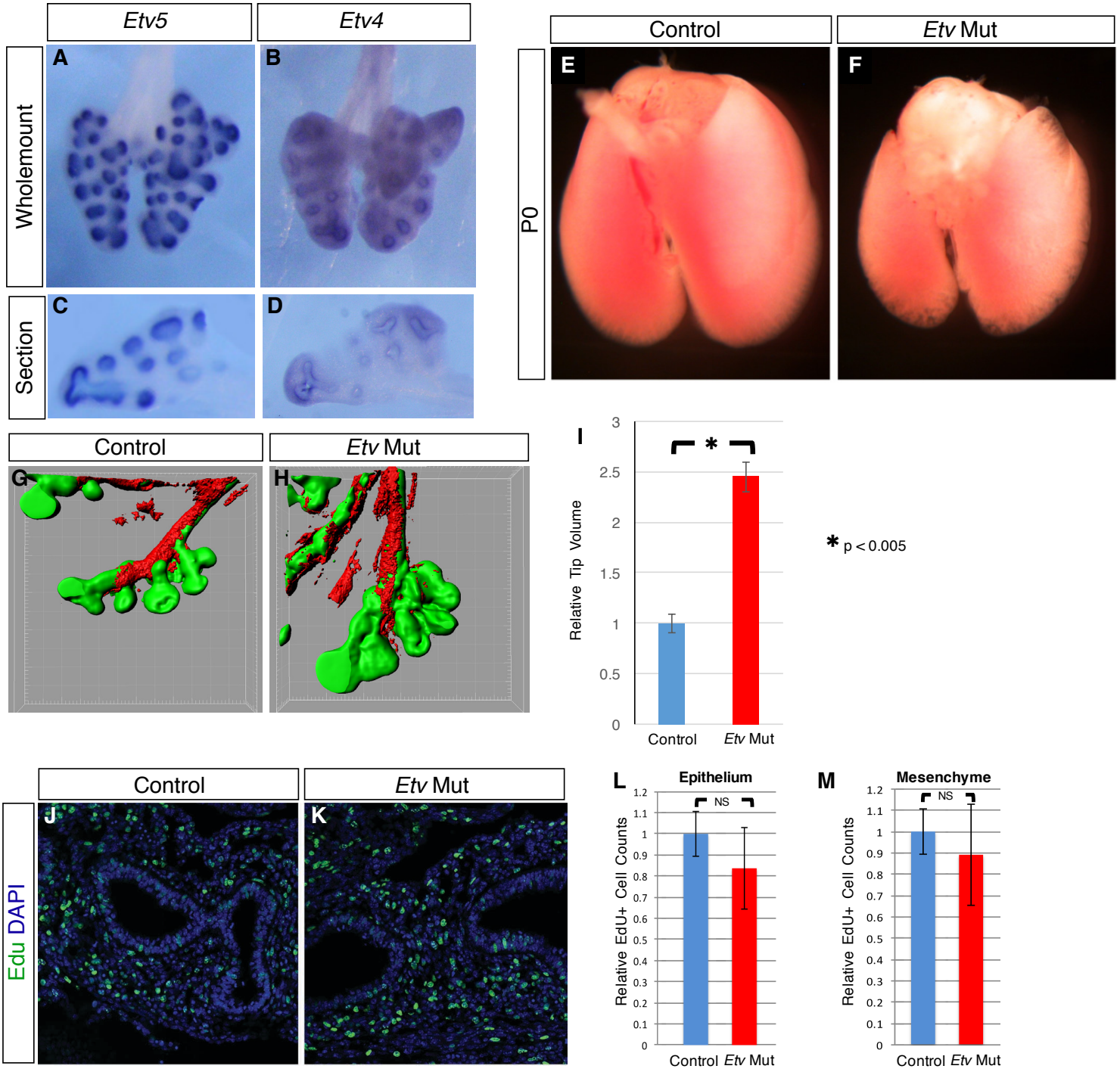

Supplemental Figure 2 (related to Figure 2). Patterning and cell differentiation are largely normal in *Etv* mutant lungs.

(A-D) *Sox2* and *Sox9* expression as assayed by wholemount RNA in situ hybridization show that no overt patterning defect is observed in the mutants. (E) qRT-PCR analysis indicates that there is a trending increase of *Sox2* expression (1.0 for controls, 1.38 for mutants, p=0.042), but no change in *Sox9* expression (1.0 for controls, 1.01 for mutants, p=0.972). (F,G) Immunofluorescence staining of Type I (red) and type II (green) cells show normal distribution in the mutant compared to control at P0. (H) Quantification of relative cell number indicates no difference (1.0 for controls, 0.90 for mutants, p=0.26). (I) qRT-PCR of SpC gene expression at E18.5 indicates no change (1.0 for control, 0.96 for mutant, p=0.83). (J-M) Immunofluoresence labeling of vasculature (J) and smooth muscle (M) at E18.5 indicate no changes. (N,O) Alcian blue staining of trachea cartilage at E18.5 indicates reduced and disorganized cartilage in the mutant.

Supplemental Figure 2
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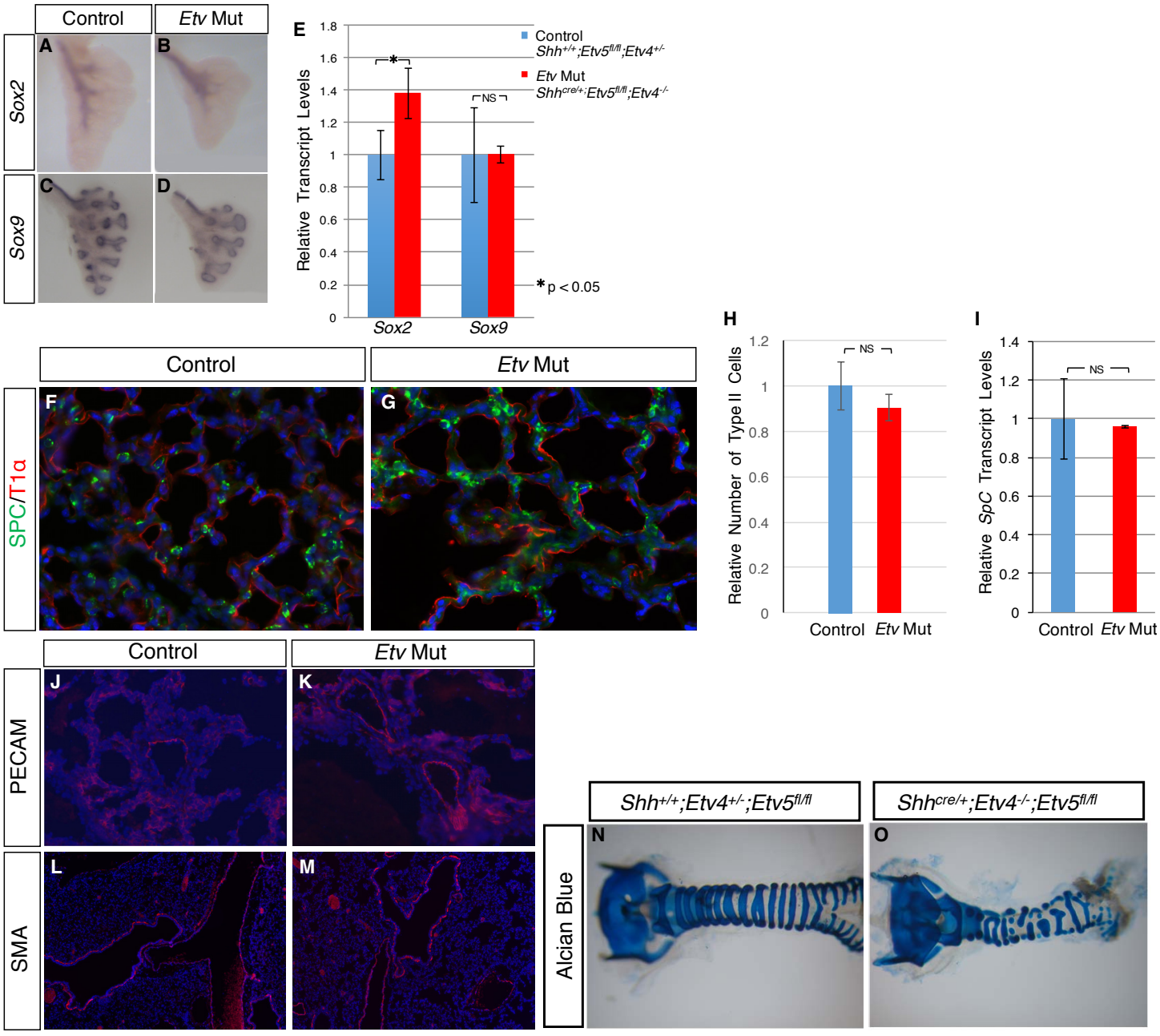

Supplemental Figure 3 (Related to Figure 3). *Etv* mutant lungs exhibit an increase in FGF signaling activity.

(A-F) Single slice confocal images of E12.5 lung epithelial tips stained with anti-E-Cadherin antibody to label the epithelium and anti-PERK antibody to report FGF activity. The anti-E-Cadherin staining is apically biased leading to high signal when the apical surfaces appose as in (D). The increase of pERK signal in the mutant is largely in the epithelium. (G-J) RNA in situ hybridization indicate increase expression of *Spry2* and *Bmp4* in the mutants. (K) qRT-PCR results indicate an increase in *Spry2* and *Bmp4* expression (for *Spry2*: 1.0 for controls, 2.90 for mutants, p=4.9x10-5; for *Bmp4*: 1.0 for controls, 1.96 for mutants, p=0.011).

Supplemental Figure 3
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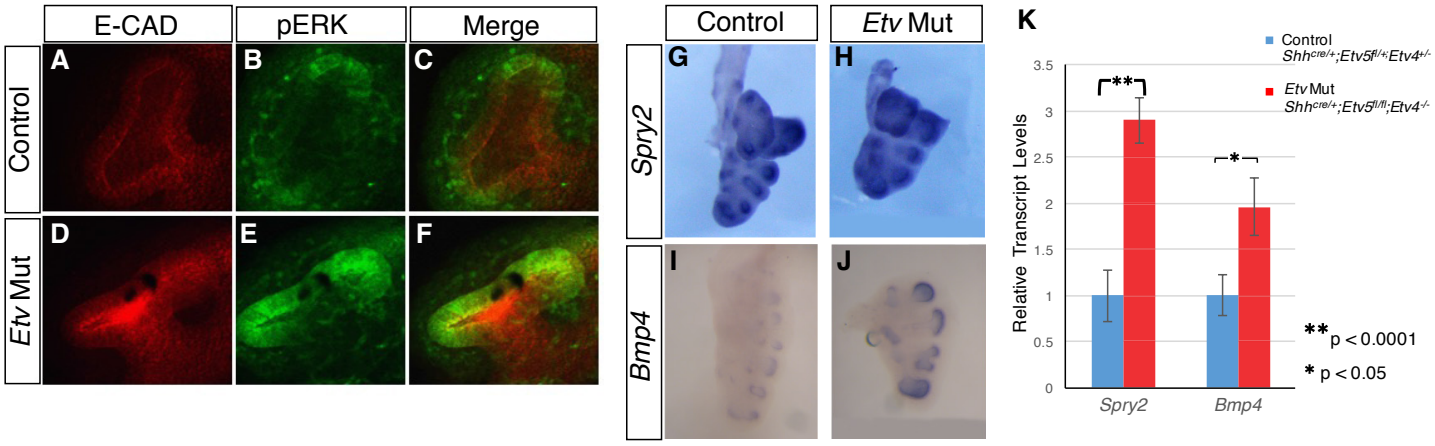

Supplemental Figure 4 (Related to Figure 4). Reducing *Fgf10* gene dosage in the *Etv* mutant background led to attenuation of the tip dilation phenotype.

The *Etv;Fgf10* mutants exhibited a mild but consistent attenuation of the distal dilation defect starting at E11.5, which becomes more pronounced at later stages.

Supplemental Figure 4
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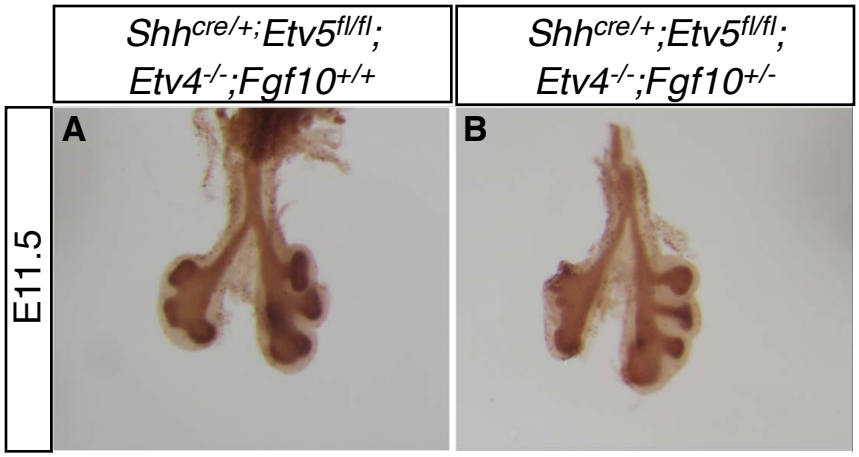

Supplemental Figure 5 (Related to Figure 6). SHH activity is increased following SAG treatment.

(A-B) *Ptch1* expression, as an indicator of SHH activity, was assayed by in situ hybridization in wild-type E11.5 lungs cultured for 24 hours in the presence or absence of SAG.

Supplemental Figure 5
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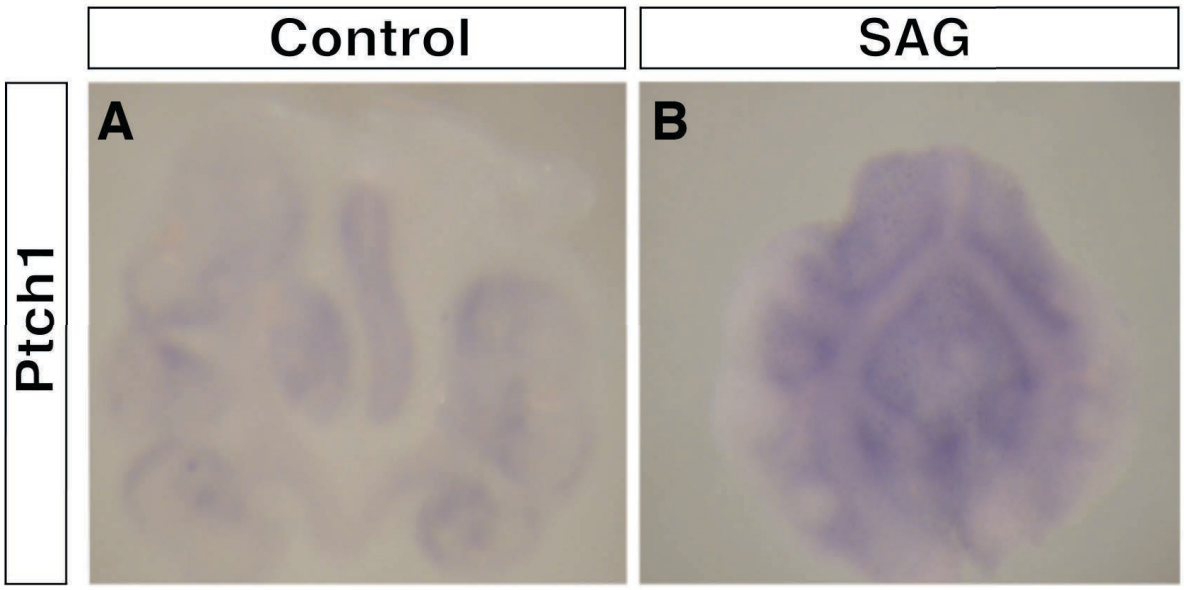

Supplemental Figure 6 (related to Figure 7). ETV controls *Shh* expression through putative binding sites in a long-range enhancer.

(A) Normalized luciferase activity from assays in MLE12 cells, using wild-type MACS1 *Shh* enhancer, mutant MACS1 *Shh* enhancer where all three putative ETV sites were mutated (mut MACS1) or just one site was mutated (mut1, 2, or 3). Either blank, ETV5 and/or NKX2-1 full-length expression construct was used as indicated. Relative p values are indicated, n=3 for each group. (B) β-gal staining of transgenic lungs carrying lacZ reporter constructs driven by either wild-type (wt) or mutant (mut, all three sites mutanted) MACS1 *Shh* enhancer. All lungs that showed βgal staining specific in the epithelium were arbitrarily separated into groups with strong (s), medium (m) and weak (w) expression, and are shown. No lungs carrying the mutant enhancer-lacZ construct showed strong straining.

Supplemental Figure 6
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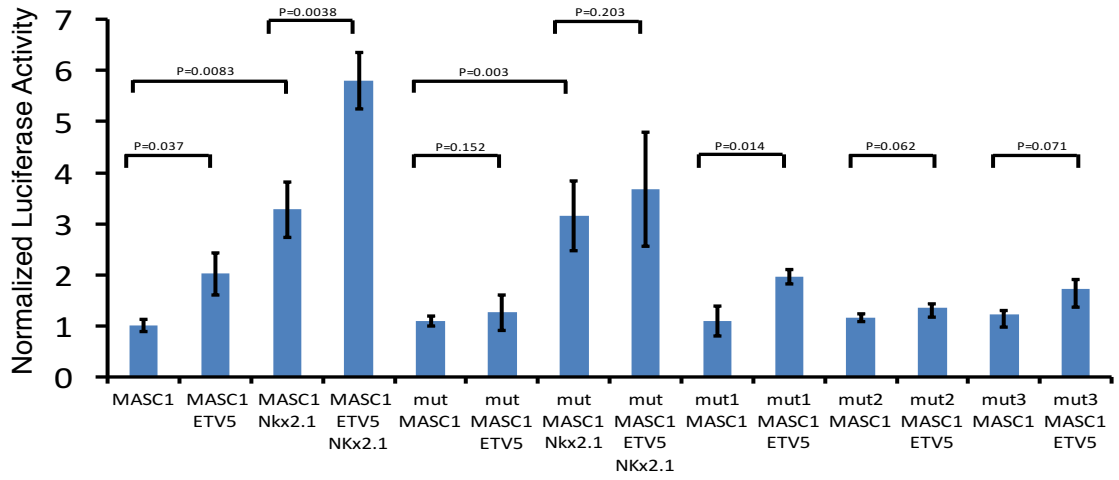
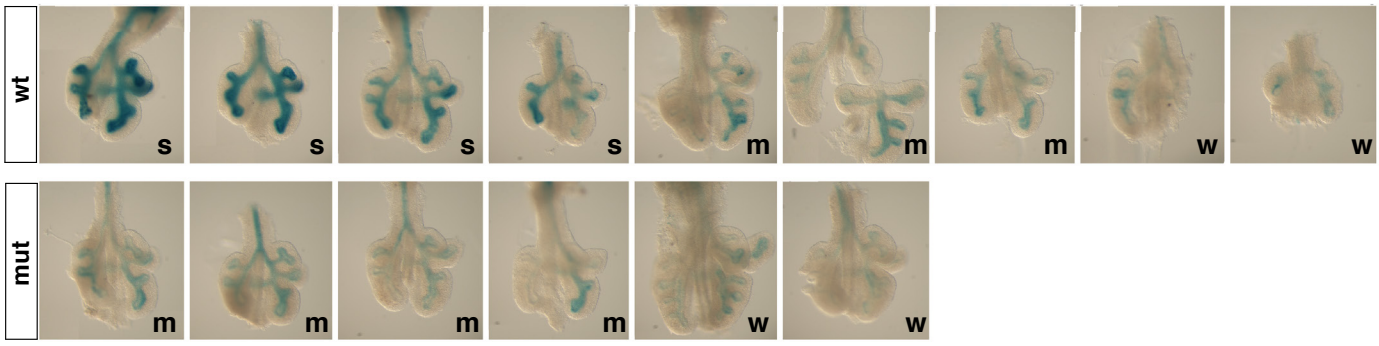
A



B



Movie Legends:

Movie 1 (Related to Figure 1). Three-dimensional surface rendering of E12.5 control lung stained with anti-E-Cadherin antibody (green) and anti-ACTA2 (also termed smooth muscle actin) antibody (red).

Movie 2 (Related to Figure 1). Three-dimensional surface rendering of E12.5 control lung stained with anti-E-Cadherin antibody (green) and anti-ACTA2 (also termed smooth muscle actin) antibody (red).

Supplemental Experimental Procedures:

β-actin	CGGCCAGGTCATCACTATTGGCAAC
	GCCACAGGATTCCATACCCAAGAAG
Etv5	GGTATTTCTCCAGCAGCCATGAAGG
	TCTCGGGTACCACGCAAGTATCATC
Fgf10	GATTGAGAAGAACGGCAAGGTCAG
	TTGACGGCAACAACTCCGATTTCC
Gli1	AGCTGCACTGAAGGATCTCTCT
	CATCAGAAAGGGGCGAGATG
Ptch1	CAGCATACCTCCTAGGTAAGCCTC
	CCAGCTGTCCACTTGGTAGTTTAT
Shh	GGCTGATGACTCAGAGGTGCAAAG
	GCTCGACCCTCATAGTGTAGAGAC
ShhEnhLucF	CGTTAGAGCTCTTGTACTGGTGAGTGTCAGGAT
ShhEnhLucR	CTGAAGGTACCATAACCAGGAGTCCAGGTACTT
ShhEnhMut1	CCTGAAGTTTGTTCATCCCTTTGACTTGGTTTATCACATAGAGATGGC
ShhEnhMut2	CTTGAAGTGGCTCCAAGCTGCAAGCCTGGAAGGATATTAAACCAG
ShhEnhMut3	CATTGAGATGCTATAAATTGGAGGCTTTTCATTTTGCTTTTGAGGG
ShhEnhHsp68F	TCGACCTCGAGGCGGCCGCAATAAAATATC
ShhEnhHsp68R	TCTAGCTGCAGCTAGCACGCGTAAGAGCTCGAT
CHIP-MACS1	TGCTAACTTGAAGTGGCTCC
	GCAAATTGCTTACTAATGTGTTGA
CHIP-MACS1 control	GCACTTGGGAGCTTTGTCCT
	ACTTTCCAAGGAAAAGAAGGGG
Mouse Etv5 cDNA	CGACGCGTATGGATGGGTTTTGTGATCAG
	GCGTCGACCTTAGTAAGCGAAGCCTTCG