Figure S1

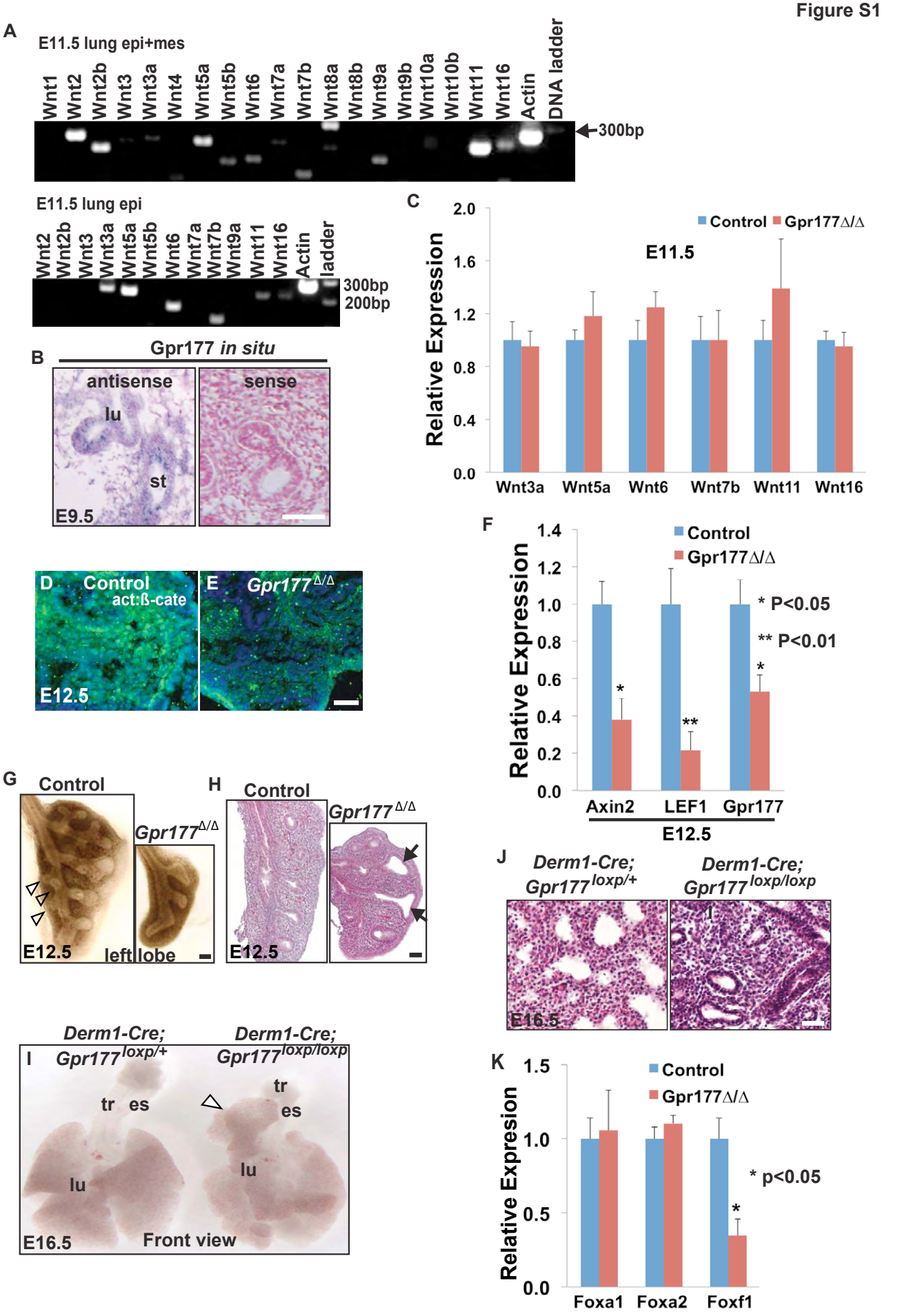
A
E11.5 lung epi+mes
Wnt1 Wnt2 Wnt2b Wnt3 Wnt3a Wnt4 Wnt5a Wnt5b Wnt6 Wnt7a Wnt7b Wnt8a Wnt8b Wnt9a Wnt9b Wnt10a Wnt10b Wnt11 Wnt16 Actin DNA ladder
300bp
E11.5 lung epi
Wnt2 Wnt2b Wnt3 Wnt3a Wnt5a Wnt5b Wnt6 Wnt7a Wnt7b Wnt9a Wnt11 Wnt16 Actin ladder
300bp
200bp
B
Gpr177 in situ
antisense
sense
lu
st
E9.5
C
Control
Gpr177Δ/Δ
E11.5
Relative Expression
2.0
1.6
1.2
0.8
0.4
0.0
Wnt3a Wnt5a Wnt6 Wnt7b Wnt11 Wnt16
D
Control
act:ß-cate
E12.5
E
Gpr177Δ/Δ
F
Control
Gpr177Δ/Δ
* P<0.05
** P<0.01
Relative Expression
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
Axin2 LEF1 Gpr177
E12.5
G
Control
Gpr177Δ/Δ
E12.5
left lobe
H
Control
Gpr177Δ/Δ
E12.5
J
Derm1-Cre; Gpr177loxp/+
Derm1-Cre; Gpr177loxp/loxp
E16.5
I
Derm1-Cre; Gpr177loxp/+
Derm1-Cre; Gpr177loxp/loxp
tr
es
lu
E16.5
Front view
K
Control
Gpr177Δ/Δ
* p<0.05
Relative Expresion
1.5
1.0
0.5
0.0
Foxa1 Foxa2 Foxf1

Fig. S1. Deletion of epithelial *Gpr177* leads to lung morphogenesis defects. (**A**) RT-PCR was used to examine the expression of Wnts in the E11.5 lung. Wnt2, Wnt2b, Wnt3a, Wnt5a, Wnt5b, Wnt6, Wnt7b, Wnt8a, Wnt9a, Wnt11 and Wnt16 are detected, and Wnt3a, Wnt5a, Wnt6, Wnt7b, Wnt11 and Wnt16 are enriched in the epithelium. (**B**) Gpr177 is expressed ubiquitously in the E9.5 lung, as shown by *in situ* hybridization. (**C**) The transcript levels of lung epithelial Wnts are not affected by the removal of epithelial *Gpr177* in the E11.5 *Shh-Cre;Gpr177*$^{loxp/loxp}$ (*Gpr177* $^{\Delta/\Delta}$) mutants. Quantitative real-time PCR was used, and the value was normalized to GAPDH. (**D**,**E**) The levels of activated β-catenin are reduced in the E12.5 *Gpr177*$^{\Delta/\Delta}$ mutant lungs. (**F**) Reduced mRNA levels of Wnt downstream targets Axin2 and Lef1 in the E12.5 mutant lung. The levels of Gpr177 are also significantly reduced (P<0.05). The transcript levels were normalized against GAPDH mRNA. (**G**) Abnormal branching in the E12.5 mutant lung. Arrowheads indicate lateral secondary branching in the normal lung. The size of the mutant lung is reduced. (**H**) Dilated terminal airways in the mutant lung (arrows). (**I**) Deletion of *Gpr177* in the mesenchyme of the *Derm1-Cre;Gpr177*$^{loxp/loxp}$ mutants leads to abnormal lung branching morphogenesis. The arrowhead indicates the abnormal cranial lobe. (**J**) Hematoxylin and Eosin staining of the *Derm1-Cre;Gpr177*$^{loxp/loxp}$ mutant lung. Airways are enlarged and there is a lack of hemorrhage in the mutant lung. (**K**) The levels of Foxf1 transcript are significantly reduced in the E16.5 lungs of *Shh-Cre;Gpr177*$^{loxp/loxp}$ mutants (P<0.05), while the levels of Foxa1 and Foxa2 are not significantly changed. Real-time PCR was used to measure the transcript levels. lu, lung; st, stomach; tr, trachea; es, esophagus. Scale bar: 50 μm.

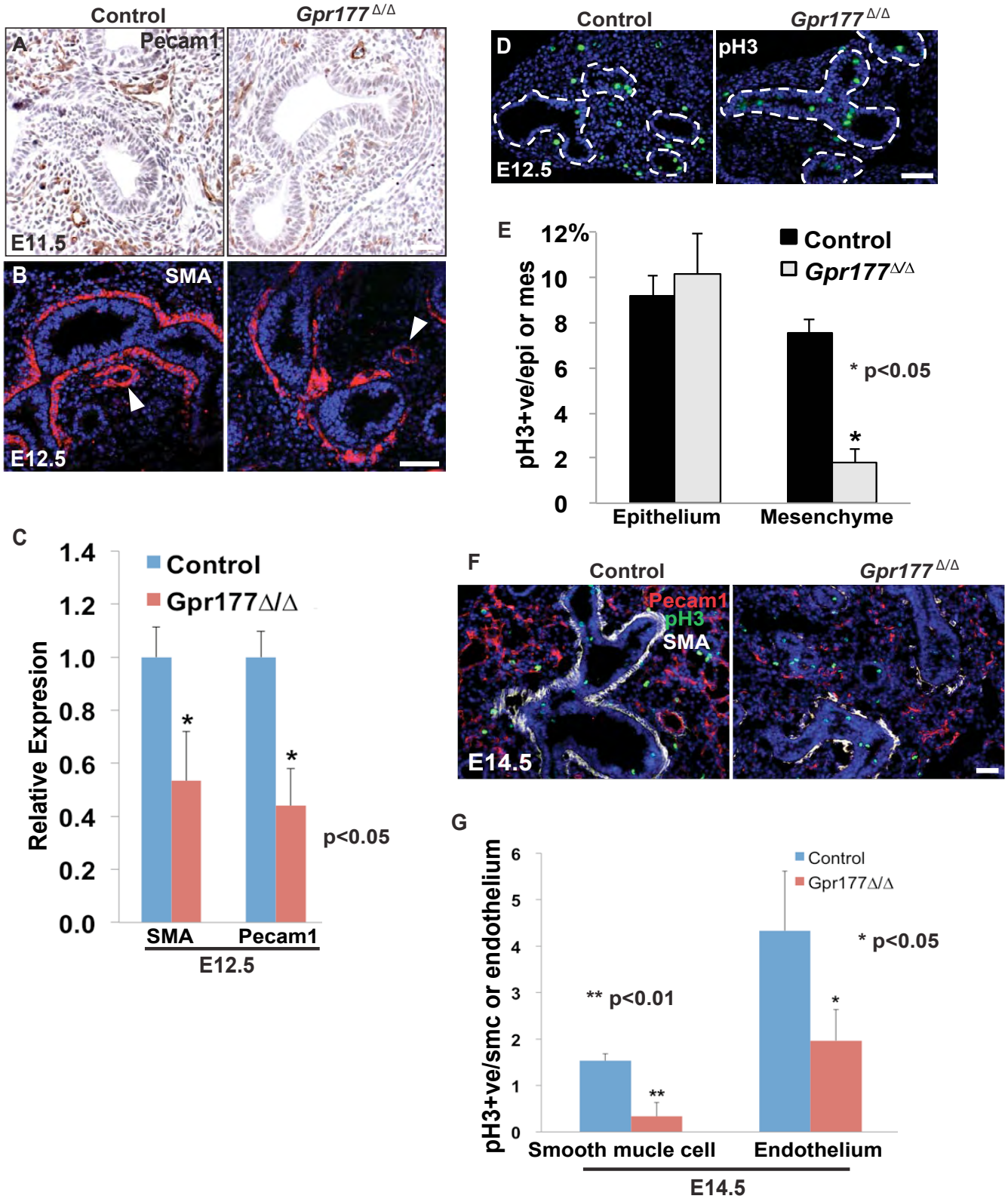

Fig. S2. Decreased proliferation in the mesenchymal compartment contributes to the vasculature defects in *Gpr177*$^{\Delta/\Delta}$ mutants. (**A**) Reduced number of blood vessels (Pecam1^{+}) in the E11.5 *Gpr177*$^{\Delta/\Delta}$ mutant lung. (**B**) The number of smooth muscle cells surrounding the blood vessels (arrowhead) and airways is decreased in the E12.5 mutant lung. (**C**) The transcript levels of smooth muscle actin and Pecam1 are significantly decreased in the *Gpr177*$^{\Delta/\Delta}$ mutant lung (P<0.05). Real-time PCR was used to measure the transcript levels. (**D**) Representative sections stained with anti-pH3 antibody to show decreased proliferation of mesenchyme but not epithelium in the E12.5 mutant lung. (**E**) Quantification of proliferating cells in the E12.5 mutant and wild-type lungs. The data are presented as mean±s.e.m. (**F**,**G**) Reduced proliferation of smooth muscle and endothelial cells in the E14.5 *Gpr177*$^{\Delta/\Delta}$ mutant lungs. The data are presented as mean±s.e.m. Scale bar: 50 μm.

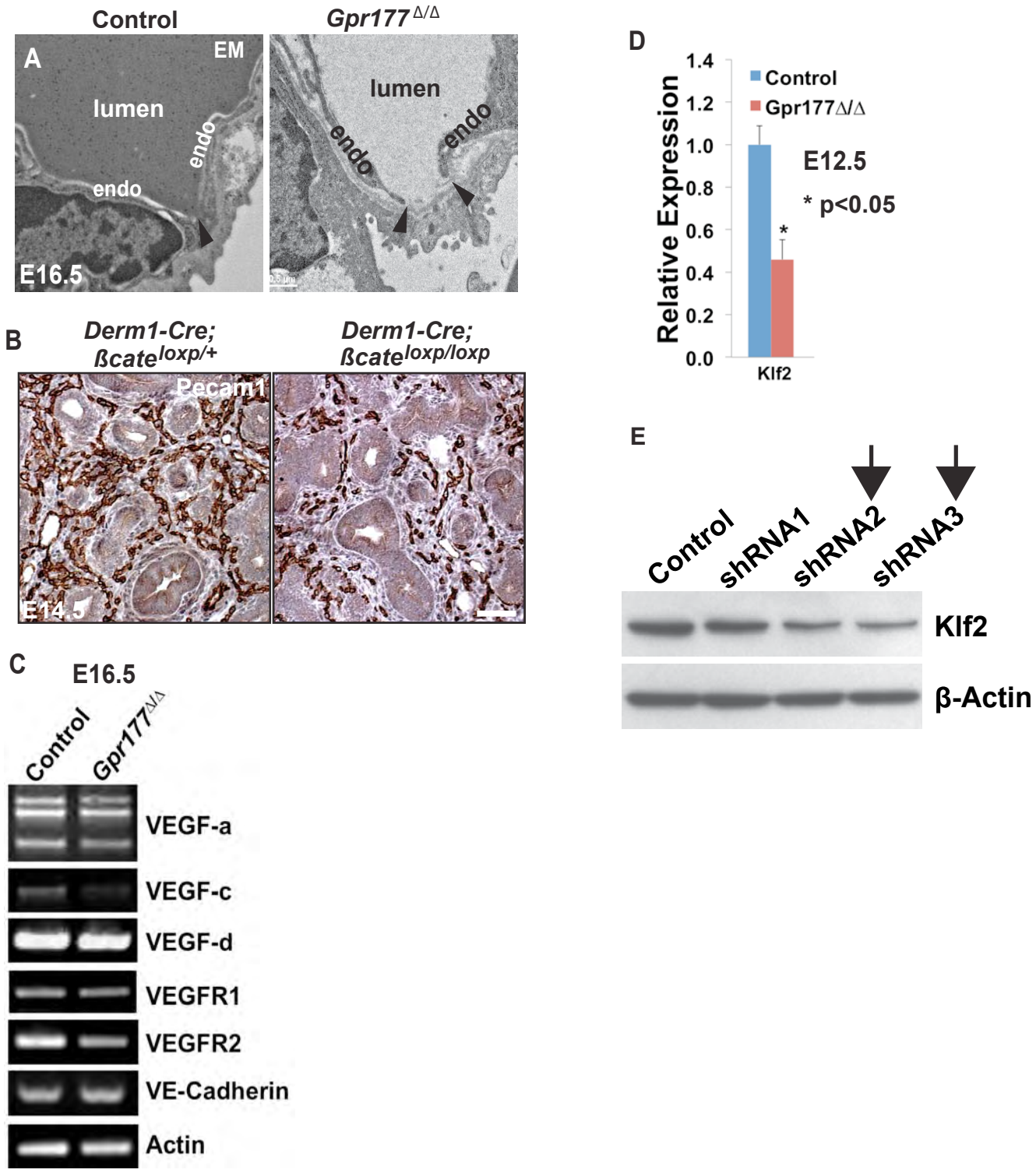

Fig. S3. Downregulation of vascular factors in the *Gpr177* conditional mutant lungs. (**A**) Deletion of *Gpr177* leads to thinning of the basement membrane and breakdown of tight junctions between endothelial cells, as revealed by electron microscopy. The arrowheads indicate the ends of two neighboring endothelial cells. (**B**) β-catenin deletion in the mesenchyme leads to reduced number of blood vessels in E14.5 *Derm1-Cre;β-cate*$^{loxp/loxp}$ mutants. (**C**) RT-PCR analysis shows the levels of VEGF-c are reduced in the E16.5 *Gpr177*$^{\Delta/\Delta}$ mutant lungs. (**D**) The transcript levels of Klf2 are significantly reduced in the E12.5 *Gpr177*$^{\Delta/\Delta}$ mutant lungs, as shown by qPCR. (**E**) Western blot analysis shows that the two shRNAs (shRNA2 and shRNA3) targeting different regions of Klf2 mRNA efficiently reduce the protein levels of Klf2 in PAC1 cells. endo, endothelium. Scale bar: 0.5 μm in A; 50 μm in B.

Table S1. Primer sequences used to determine the transcripts expressed in the lungs of *Gpr177* conditional deletion mutants and controls

Gene	Primer sequence 5′→3′
Wnt 1 forward	5-ATGAACCTTCACAACAACGAG-3
Wnt 1 reverse	GGTTGCTGCCTCGGTTG
Wnt 2 forward	CTGGCTCTGGCTCCCTCTG
Wnt 2 reverse	GGAACTGGTGTTGGCACTCTG
Wnt 2b forward	CGTTCGTCTATGCTATCTCGTCAG
Wnt 2b reverse	ACACCGTAATGGATGTTGTCACTAC
Wnt 3 forward	CAAGCACAACAATGAAGCAGGC
Wnt 3 reverse	TCGGGACTCACGGTGTTTCTC
Wnt 3a forward	CACCACCGTCAGCAACAGCC
Wnt 3a reverse	AGGAGCGTGTCACTGCGAAAG
Wnt 4 forward	GAGAAGTGTGGCTGTGACCGG
Wnt 4 reverse	ATGTTGTCCGAGCATCCTGACC
Wnt 5a forward	CTCCTTCGCCCAGGTTGTTATAG
Wnt 5a reverse	TGTCTTCGCACCTTCTCCAATG
Wnt 5b forward	ATGCCCGAGAGCGTGAGAAG
Wnt 5b reverse	ACATTTGCAGGCGACATCAGC
Wnt 6 forward	TGCCCGAGGCGCAAGACTG
Wnt 6 reverse	ATTGCAAACACGAAAGCTGTCTCTC
Wnt 7a forward	CGACTGTGGCTGCGACAAG
Wnt 7a reverse	CTTCATGTTCTCCTCCAGGATCTTC
Wnt 7b forward	TCTCTGCTTTGGCGTCCTCTAC
Wnt 7b reverse	GCCAGGCCAGGAATCTTGTTG
Wnt 8a forward	ACGGTGGAATTGTCCTGAGCATG
Wnt 8a reverse	GATGGCAGCAGAGCGGATGG
Wnt 8b forward	TTGGGACCGTTGGAATTGCC
Wnt 8b reverse	AGTCATCACAGCCACAGTTGTC
Wnt 9a forward	GCAGCAAGTTTGTCAAGGAGTTCC
Wnt 9a reverse	GCAGGAGCCAGACACACCATG
Wnt 9b forward	AAGTACAGCACCAAGTTCCTCAGC
Wnt 9b reverse	GAACAGCACAGGAGCCTGACAC
Wnt 10a forward	CCTGTTCTTCCTACTGCTGCTGG
Wnt 10a reverse	CGATCTGGATGCCCTGGATAGC
Wnt 10b forward	TTCTCTCGGGATTTCTTGGATTC
Wnt 10b reverse	TGCACTTCCGCTTCAGGTTTTC
Wnt 11 forward	CTGAATCAGACGCAACACTGTAAAC
Wnt 11 reverse	CTCTCTCCAGGTCAAGCAGGTAG
Wnt 16 forward	AGTAGCGGCACCAAGGAGAC

Wnt 16 reverse	GAAACTTTCTGCTGAACCACATGC
Axin2 forward	CAGCCCTTGTGGTTCAAGCT
Axin2 reverse	GGTAGATTCCTGATGGCCGTAGT
LEF1 forward	GCAGCTATCAACCAGATCC
LEF1 reverse	GATGTAGGCAGCTGTCATTC
Gpr177 forward	TGTTGGAGGGATTCTTCTGG
Gpr177 reverse	ATTGCCGTGTAGGGTACTGC
Foxa1 forward	CCCTTTCTCCCTTTCACTCC
Foxa1 reverse	AGGGCTCCAATGTGCATAAC
Foxa2 forward	CCATCCAGCAGAGCCCCAACA
Foxa2 reverse	GTCTGGGTGCAGGGTCCAGAA
Foxf1 forward	GGCCTCCTACATCAAGCAAC
Foxf1 reverse	TAAGATCCTCCGCCTGTTGT
SMA forward	GGTCGTGGAGTTGGTGGAAA
SMA reverse	CTGCCATGTCCTCCACCTTAG
Pecam1 forward	TCCCTGGGAGGTCGTCCAT
Pecam1 reverse	GAACAAGGCAGCGGGGTTTA
Klf2 forward	GCCTGTGGGTTCGCTATAAA
Klf2 reverse	TTTCCCACTTGGGATACAGG
VEGF-a forward	GATCATGCGGATCAAACCTCACC
VEGF-a reverse	CCTCCGGACCCAAAGTGCTC
VEGF-c forward	AACGTGTCCAAGAAATCAGCC
VEGF-c reverse	AGTCCTCTCCCGCAGTAATCC
VEGF-d forward	GGTCCATGTTGGAACGATCT
VEGF-d reverse	ATGCTGAGCGTGAGTCCATA
VEGFR1 forward	CAACGTACAAAGAGATAGGACTGCT
VEGFR1 reverse	TTTGGACATCTAGGATTGTATTGGT
VEGFR2 forward	CGAAATTACTTTTTAGCCGAGGT
VEGFR2 reverse	TTAACATAAGCACACAGGCAGAA
VE-Cadherin forward	GGATGCAGAGGCTCACAGAGCTGG
VE-Cadherin reverse	CTTAGCATTCTGGCGGTTCACGTT
Actin forward	GACGGCCAGGTCATCACTAT
Actin reverse	GTACTTGCGCTCAGGAGGAG