**Supplementary Figure 1: *Pten* inactivation by *Dermo1-Cre.* (A,B)** PTEN staining in control (A) and mutant (B) lungs. Note PTEN absence in *Pten*$^{f/f;Dermo\text{-}Cre}$ mesenchyme. Also PTEN is both cytoplasmic and nuclear localized in control mesenchyme, while in mutant PTEN is in epithelium (n=3/genotype). **(C,D)** Increased p-AKT staining in mutant (D) compared to controls (n=3/genotype). **(E)** qRT-PCR showing 42% *Pten* decrease in *Pten*$^{f/f;Dermo\text{-}Cre}$ lungs (n=4/ experiment). **(F)** Western analysis for PTEN and p-AKT (n=4 mice). **(G)** PECAM staining in a β-gal background of adult *Dermo1*$^{Cre/+}$*/Rosa26R* WT lungs show Cre activity in lung mesenchyme not endothelial cells (PECAM-positive). Scale bars: 50μm.

**Supplementary Figure 2: Embryonic and perinatal mortality in *Pten*$^{f/f;Dermo\text{-}Cre}$ mice due to vascular defects.**

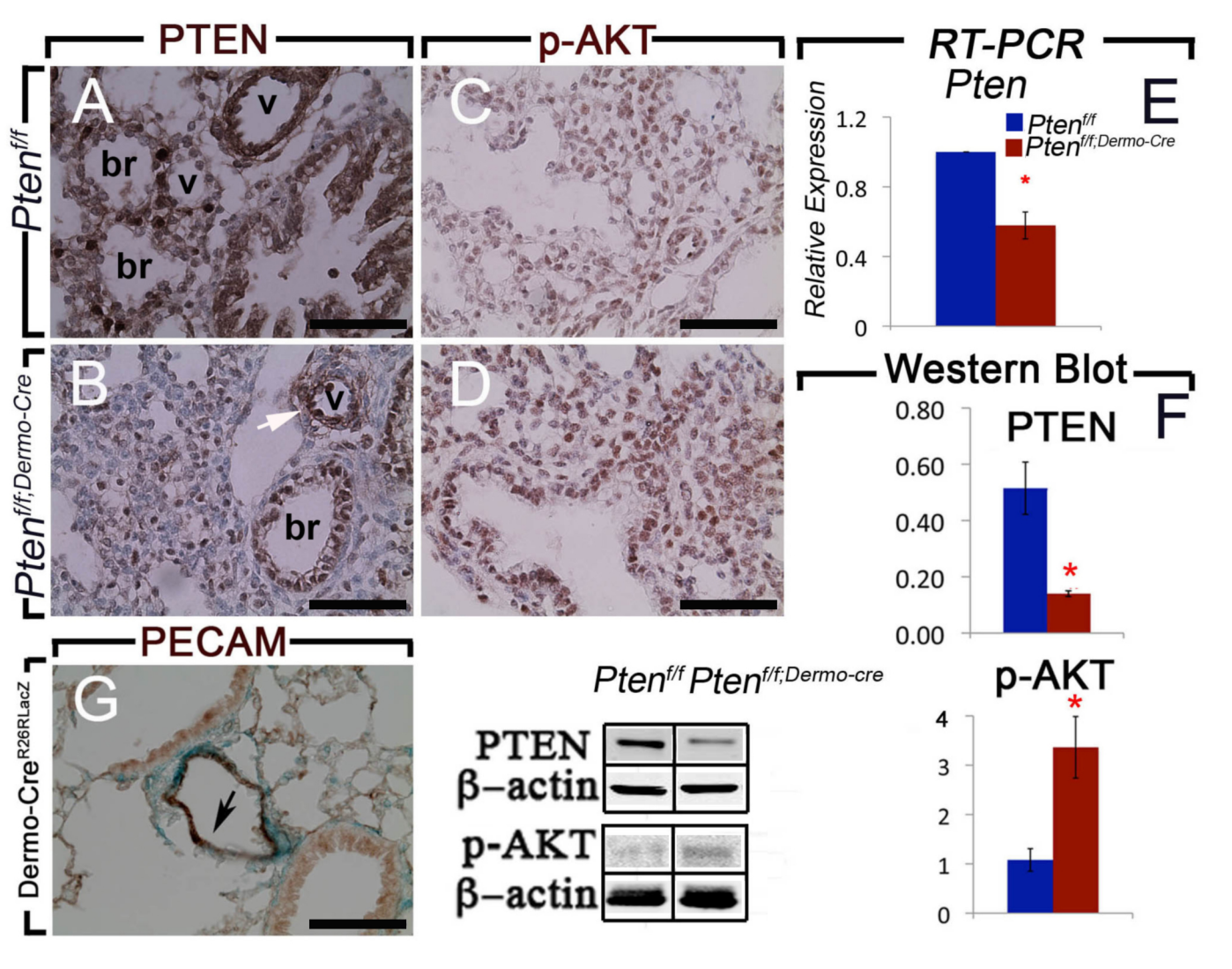
**(A-D)** Gross appearance of representative control (A,C) and mutant (B,D) E15.5 (A,B) and E18.5 (C,D) embryos. Note skin pallor in mutants caused vascular failure (B) with hemorrhages at E18.5 (D). **(E)** *Pten*$^{f/f;Dermo\text{-}Cre}$ mice (left) at birth with cyanosis.

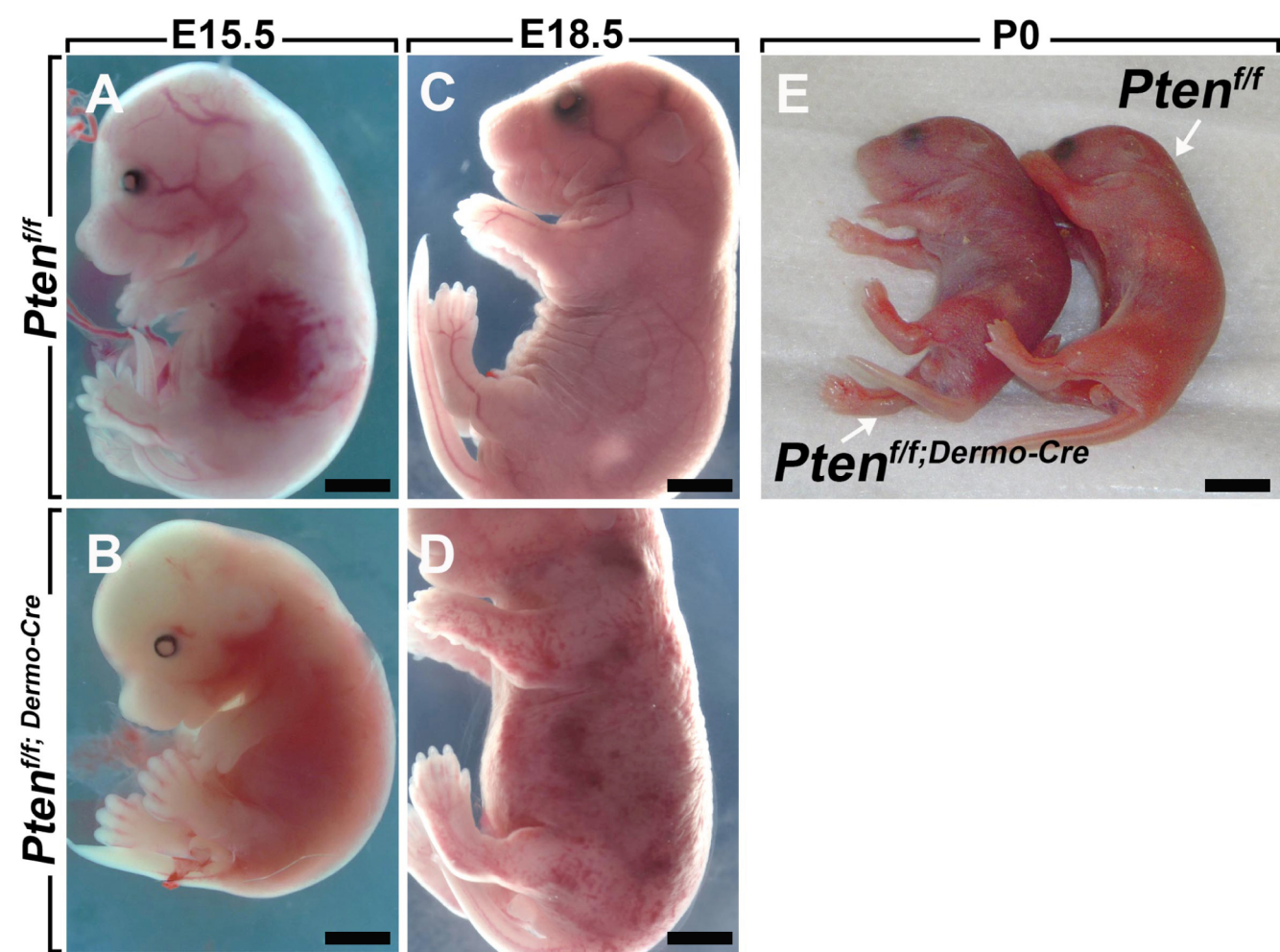
**Supplementary Figure 3: Generalized lack of vascularization in *Pten*$^{f/f;Dermo\text{-}Cre}$ mice.**

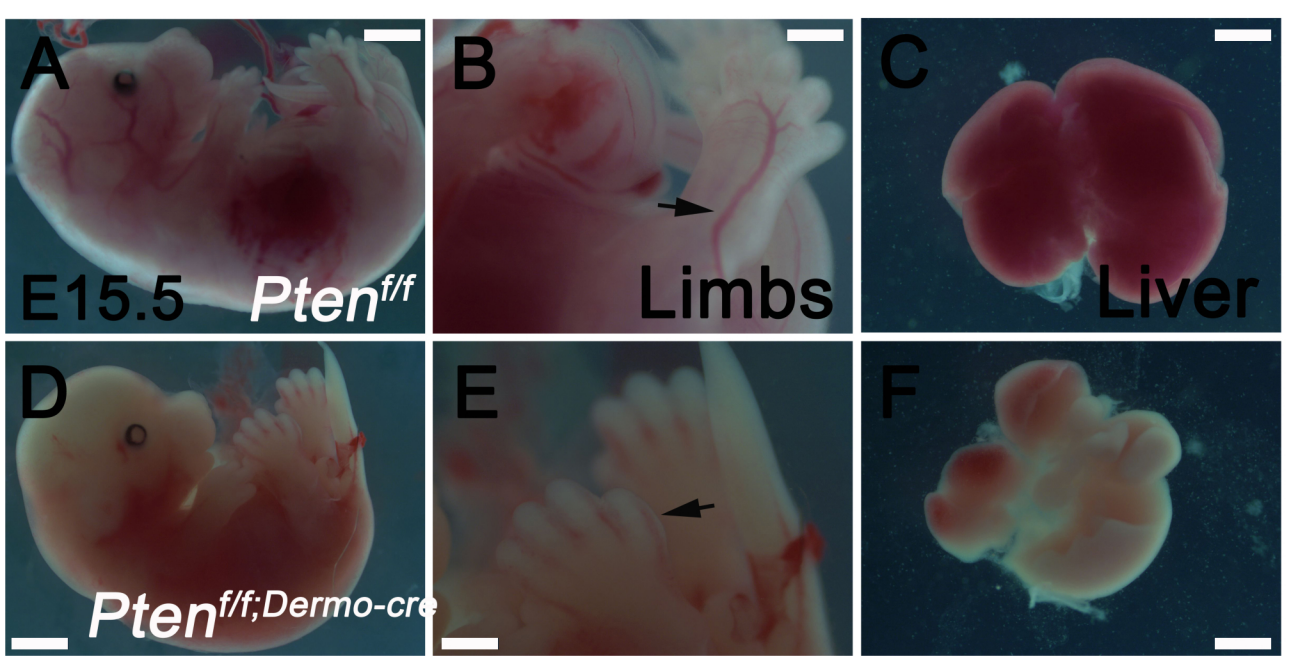
E15.5 control and mutant embryos (A,D), whole body; (B,E) limbs, and (C,F) liver.

**Supplemental Video 1: Video of Tridimensional reconstruction of E18.5 lungs**

Lung vascular system is revealed using FITC-leptin showing deceased and uncoupled capillary network in mutant lungs (right video).


PTEN
p-AKT
RT-PCR
Pten
E
Pten f/f
Pten f/f;Dermo-Cre
Relative Expression
1.2
0.8
0.4
0
*
A
v
br
v
br
C
B
v
br
D
Western Blot
F
PTEN
0.80
0.60
0.40
0.20
0.00
*
Pten f/f
Pten f/f;Dermo-cre
PTEN
β–actin
p-AKT
β–actin
p-AKT
4
3
2
1
0
*
PECAM
G
Dermo-Cre R26RLacZ


E15.5
E18.5
P0
A
C
E
Pten f/f
B
D
Pten f/f; Dermo-Cre
Pten f/f
Pten f/f;Dermo-Cre


A
E15.5
Pten f/f
B
Limbs
C
Liver
D
Pten f/f;Dermo-cre
E
F

**Supplementary Methods:**

**Immunohistochemistry:**

Immunohistochemistry protocols used have been previously described in detail (reference) . IHC for all antibodies (except $\alpha$-SMA) was performed using antigen retrieval (reference). Antibodies were, Anti-PTEN (1:100, Cell Signaling), anti-p-AKT (Cell Signaling, 1:50), anti-PECAM (1:50, Labvision) or anti-ADRP (Santa Cruz, 1:200). The slides were visualized using Dako EnVision+ Dual Link System-HRP (DAB+), according to manufacturer and Photographed.

For immunofluorescence (IF), the following antibodies were used: anti-E-CAD (1:200, BD), anti-VEGF (Thermo Scientific, 1:200), anti-PH3 (Cell Signaling, 1:200), anti-β-CAT Ser552 (1:200, kindly provided by Dr. Linheng Li, Stowers Institute Medical Research, Kansas City, Missoury, USA), anti-BEK (1:100, Santa Cruz), anti-ID2 (1:50, Cell Bioreagents), anti-CC10 (1:100, Santa Cruz), anti-T1α (Developmental Studies Hybridoma Bank, 1:200) and anti-SPC (1:500, Seven Hills). Secondary antibodies were obtained from Jackson Immunoresearch. Mounting medium was Vectashield with DAPI. IF for a-SMA was performed with a conjugated primary antibody (Sigma, 1:200).

**RNA Extraction and Quantitative PCR:**

Total RNA was isolated from the lungs of transgenic mice and wild-type littermate controls using a Qiagen RNAeasy kit, according to the manufacturer's specifications. A Nano Drop ND-1000 determined the concentration of the isolated RNA.

Total RNA (1 µg) was reverse-transcribed using the Superscript-III first strand super mix (Invitrogen) in accordance with the manufacturer's directions. 2 ng of cDNA was used for each of the quantitative PCR (qPCR) reactions using the primers and probes designed by the online Roche software: Probe finder version 2.20, https://www.roche-applied%1Escience.com/sis/rtpcr/upl/adc.jsp. The used primers are described in supplementary table 1. All qPCR reactions were performed with Roche FastStart TaqMan® Probe Master kits, according to the manufacturer's instructions in a Roche Light Cycler 1.5 qPCR machine. *$\beta$-actin* and *Gapdh* RNA were used as internal controls for all analyses.

**Western Blot:**

E18.5 control and mutant (n=4 for each group) lung tissues were individually homogenized in Tissue Protein Extraction Reagent (T-PER) buffer (Thermo Scientific Cat #: 78510) containing protease and phosphatase inhibitors (Roche Cat #: 04 693 124 001). Equal amounts of total

lung proteins (13 mg) from each assayed sample were separated on a 4-12% Bis-Tris Gel (Invitrogen Cat #: NP0322BOX) and then transferred to a nitrocellulose membrane (Biorad Cat #: 162-0094). ®-ACTIN, p-AKT, PTEN, proSP-C and TTF-1 proteins were detected with the following antibodies: anti-β-ACTIN (1:5000), anti-p-AKT (Cell Signaling, 1:1000), anti-PTEN (Cell Signaling, 1:1000), anti-proSP-C (Seven Hills Bioreagents, 1:10000) and anti-TTF-1 (Seven Hills Bioreagents, 1:5000) as suggested by the manufacturer. Membrane bands were detected using the Odyssey Infrared Imaging System (LI-COR Lot# 9201-01) and quantitatively analyzed using the accompanying Odyssey Software (LI-COR Lot# 9201-500).

**In situ Hybridization:** Whole-mount in situ hybridization was performed on E12.5 embryonic lungs as previously described (S1). Briefly, embryonic lungs were fixed in 4% PFA with rocking at 4°C for 2 h, washed twice in PBS at 4°C, dehydrated with several washes with 70% ethanol (EtOH), and stored in absolute EtOH at −20 °C. Samples were then transferred to hybridization buffer (Formamide 50%, SSC 5×, tRNA 0.05 mg/ml, sodium dodecyl sulfate 1%, heparin 0.05 mg/ml) for 1 h at 50 °C and then hybridized with the probe at 55°C overnight. The next day specimens were washed with PBS several times at 50°C. After the washes the samples were added to blocking solution made with sheep serum for 1 h and then incubated overnight at 4°C with anti-DIG antibody. The color reaction was performed with BM Purple solution after washes with TBST and NTMT (NaCl 100 mM, Tris pH9.5 100 mM, Tween-20 0.1%). The following mouse cDNAs were used to generate antisense riboprobes: 584 bp *Fgf10* (18).

**FACS:**

Single lung cells were prepared from control and mutant lungs (n=14) as described previously (9). Cells were suspended at a concentration of $1x10^6$ /ml in DMEM, plus 10% FBS. Cells were stained with Hoechst dye (5 µg/ml) for 90 min at 37°C in the presence or absence of verapamil (50 mM; Sigma Cat# V105-5MG). After washing with 1 ml of HBSS+0.1%DNAse, cells were resuspended at $10X10^6$/500 µl, blocked for 10 min at 4ºC with 10 µl of FC block (BD Pharmingen Cat# 553141), washed in HBSS+0.1% DNAse and incubated for 15 min at 4ºC with 20 ∫l CD45PE (BD Pharmingen Cat# 553081) and CD31FITC ( BD Pharmingen Cat# 558738) antibodies. After two washes with HBSS+0.1%DNAse they were resuspended in HBSS. Analysis was performed in a FACSAria Cytometer (BD Bioscience). Analysis was by FACSDiva software.

**Sircol Collagen Quantification:**

The Sircol Soluble Collagen Assay (Biocolor life science assays) was performed according to the manufacturer's specifications from E18.5 control and mutant embryonic lungs (n=5 for each group). Absorbance was measured at 540 nm using Biorad Microplate Reader Model 680.

**Sirius Red Staining:**

Lung sections were deparaffinized with successive ethanol concentrations, left in Sirius red solution (0.1g of fast red green FCF) dissolved in 0.1% saturated picric acid for 60 min in room temperature, washed for 2 minutes in 0.01N hydrochloric acid, rinsed in 70% alcohol for 45 seconds and, after left in room temperature overnight, they were mounted.

**Detection of β-gal Expression:**

Lungs were dissected at E18.5 and fixed in 4% paraformaldehyde, PFA (for LacZ staining glutaraldehyde) for 10 min, washed twice for 10 min in PBS, transferred into freshly prepared X-gal solution, and stained at 37°C until a clear precipitate formed. After rinsing with PBS, tissues were post-fixed in 4% PFA in PBS. For microtome sections lungs were fixed in 4% PFA washed in PBS, dehydrated, and embedded with paraffin. Sections were counterstained with eosin for 5 min.

**Electron Microscopy:**

E18.5 lungs were dissected from embryos and fixed in 2% glutaraldehyde and post-fixed in 1% osmium tetroxide. Following dehydration in successive ethanol concentrations, the lungs were infiltrated with epoxy resin mixture (eponate 12 resin), embedded into a labeled beam capsule filled with epoxy mixture and polymerised overnight. The following day, the lungs were cut in ultrathin sections that were collected on copper grids. The grids were stained using 10% uranyl acetate in 50% methanol and sato lead stain. Lung samples were visualized with a Morgagni electron microscope.

**Tridimensional Reconstruction of the Lungs:**

Fluorescein isothio-cyanate-dextran (FITC dextran: Sigma FD20005) was diluted in sterile water at a concentration of 2 mg/ml and injected intracardially into E18.5 embryos. The lungs were dissected and fixed overnight in 4% PFA and then dehydrated with different sucrose concentrations until embedding in OCT. Frozen sections were cut at 50 μm. Specimens were imaged with a Zeiss 700 confocal system mounted on an Axio Observer Z1 inverted microscope equipped with a 40x/1.3 EC Plan-Neofluar oil immersion objective lens (Thronwood, NY). FITC

was excited with a 488 nm laser and DAPI with a 405 nm laser. Transmitted 488 nm laser light was imaged simultaneously with FITC fluorescence.

## Tables

**Table 1: *Pten*$^{f/f;Dermo\text{-}Cre}$ Mice Display Embryonic and Neonatal Mortality.**

Genotyping of *Pten*$^{f/f;Dermo\text{-}Cre}$ mice at different gestational ages. At E12.5 and E15.5, the mutants accounted for respectively 29% (19 out of 65) and 25% (16 out of 63) of the total embryos, while at E18.5 their number was 21% (67 out of 311) indicating embryonic lethality between E15.5 and E18.5. At P21 no mutant mice was detected (total 121). *Pten*$^{f/f:Dermo\text{-}Cre}$ mice came from female *Pten*$^{f/f}$ and male *Pten*$^{f/+;Dermo\text{-}Cre}$ breeding.

**Supplementary Table 1**

| | | |
|---|---|---|
| *Fgf10* | atgactgttgacatcagactcctt | cactgttcagccttttgagga (probe 63) |
| *Pten* | aggcacaagaggccctagat | ctgactgggaattgtgactcc (probe 60) |
| *Flk1* | ggggtatggagacgagctg | gcacagatctgactaaattgctg (probe 80) |
| *Flt1* | ggcccgggatatttataagaac | ccatccattttaggggaagtc (probe 55) |
| *Spry2* | gagaggggttggtgcaaag | ctccatcaggtcttggcagt (probe 3) |
| *Pecam* | cggtgttcagcgagatcc | cgacaggatggaaatcacaa (probe45) |
| *Bmp4* | gaggagtttccatcacgaaga | gctctgccgaggagatca (probe 89) |
| *Vegfa* | aaaaacgaaagcgcaagaaa | tttctccgctctgaacaagg (probe 1) |
| *Adrp* | cctcagctctcctgttaggc | cactactgctgctgccattt (probe 79) |
| $\alpha$–*Sma* | cccacccagagtggagaa | acatagctggagcagcgtct (probe 20) |
| *Fgfr2b* | gcacaagctgaccaaacgta | ctggactcagccgaaactgt (probe 94) |
| *Pch1* | ggaaggggcaaagctacagt | tccaccgtaaaggaggctta (probe: 56) |
| *Shh* | accccgacatcatatttaagga | ttaacttgtctttgcacctctga ( probe 32) |
| *Gli1* | tggaggtctgcgtggtaga | ttgaacatggcgtctcagg (probe 85) |
| *Etv4* | cagagtccccgcacagac | ggggagtcataggcactgg (probe 4) |
| *Etv5* | gcagtttgtcccagattttca | gcagctcccgtttgatctt (probe 10) |
| *Fgf9* | tgcaggactggatttcatttag | ccaggcccactgctatactg (probe 60) |
| *Fgf7* | tggctgacaccatgactagc | ggctacaggctgtcgttttt (probe 42) |
| *Foxf1* | agcatctccacgcactcc | tgtgagtgataccgagggatg (probe 46) |
| *Foxc2* | cggctaggactggacaactc | ctgacagctcgcattgctc (probe 95) |
| $\beta$-*actin* | ctaaggccaaccgtgaaaag | accagaggcatacagggaca (probe 64) |

SUPPLEMENTAL REFERENCES

S1. Winnier G, Blessing M, Labosky PA, Hogan BL. Bone morphogenetic protein-4 is required for mesoderm formation and patterning in the mouse. *Genes Dev*. 1995;9(17):2105–2116.