1 **ONLINE DATA SUPPLEMENT: METHODS**

2 **Hemodynamic and Morphometric Studies on Mice**

3 All the experimental protocols used in this study were approved by the Animal Care
4 Committee at Stanford University and adhered to the published guidelines of the
5 National Institutes of Health and the American Physiological Society.

6 C57Bl6J and C57 $Apc^{Min/+}$ mice were purchased from the Jackson Laboratory (Jackson
7 Laboratories, Bar Harbor, ME). All animals used in our experiments were obtained by
8 crossing male C57 $Apc^{Min/+}$ mice and a female C57Bl6J for at least five generations. Tail
9 based genotyping of the progeny was used to identify littermate controls and $Apc^{Min/+}$
10 mice. For hypoxia studies, mice were placed in a hypoxia chamber where they were
11 exposed to 10% inspired O_2 with access to food and water ad libitum for 3 weeks. To
12 establish the hypoxic environment, the chamber was flushed with a mixture of room air
13 and nitrogen, and the gas was re-circulated continuously. The chamber environment
14 was monitored using an oxygen analyzer (Servomex, Sugar Land, TX). Carbon dioxide
15 was removed by soda lime granules and chamber temperature was maintained at 22–
16 24°C. The chamber was inspected daily to document temperature, O_2+CO_2 atmospheric
17 concentration and animal welfare, and was opened every third day for 1 hour to clean
18 the cages and replenish food and water supplies. RVSP, RV and heart rate were
19 measured under isoflurane anesthesia (1.5–2.5% in 2 L O_2/min) in unventilated mice
20 using a closed chest technique as previously described in detail[1]. A 1.4-F Millar catheter
21 (Millar Instruments, Houston, TX) was inserted into the jugular vein and directing to the
22 right ventricle. Systemic blood pressure was determined in conscious animals by a
23 noninvasive computerized tail-cuff method. Pulmonary artery acceleration time (PAAT)
24 was determined from Doppler echocardiograms acquired with a GE Vivid 7 ultrasound
25 system (GE Healthcare, Milwaukee, WI) equipped with 13-MHz transducer. The Doppler
26 sample volume was centrally positioned within the main PA, just distal from the
27 pulmonary valve with the beam oriented parallel to the flow as described[2]. The sweep
28 speed for flow recordings was 200 mm/s. PAAT was defined as the interval between the
29 onset of systolic pulmonary arterial flow and peak flow velocity[3]. LVEDP measurements
30 were performed under isoflurane anesthesia (1.5% in 1L/min oxygen flow).   A 1.4-F
31 Millar catheter was inserted into the right carotid artery and advanced into the left
32 ventricle. Intraventricular pressure signals from Millar transducer were analyzed using a
33 PowerLab/4sp data acquisition system (AD Instruments Ltd, Castle Hill, Australia)
34 connected to a ThinkPad computer. Systolic and diastolic left ventricular pressure were
35 measured directly from the pressure waveforms. Left ventricular shortening fraction and
36 cardiac output were evaluated by echocardiography (Acuson Sequoia 256, Garnerville,
37 NY). The mice were euthanized by exsanguination. The heart and lungs were removed
38 en bloc, and right ventricular hypertrophy was evaluated by the Fulton index, i.e., weight
39 of right ventricle/left ventricle plus septum (RV/LV+S). The pulmonary circulation was
40 flushed with 3ml of PBS at 37°C, and the lungs were prepared for morphometric
41 analyses by barium gelatin injection of the pulmonary arterial vasculature and formalin
42 inflation-fixation of the lung. Morphometric analyses were performed on paraffin-
43 embedded lung sections stained using elastic van Giessen or Movat pentachrome
44 stains. The total number of peripheral arteries was calculated as a ratio of the number of
45 arteries per 100 alveoli in each of 5–6 different microscopic fields (200x magnification)
46 per section from each lung. Muscularization was assessed in 15 higher magnification
47 fields/per mouse (400x magnification) by calculating the proportion of fully and partially
48 muscularized peripheral (alveolar duct and wall) PAs to total peripheral PAs.   All
49 morphometric analyses were performed by one observer, blinded as to genotype and
50 condition, i.e., room air, hypoxia, and recovery.

1

Isolation of Mouse and Human Microvascular Pulmonary Endothelial Cells

Mouse mvPAEC were isolated by digesting whole lung tissue with collagenase IA (0.5mg/ml) for 45 minutes at 37°C. The cell suspension was filtered through 70μm cell strainers, and then centrifuged at 250G for five minutes. The cell pellet was then washed three times with PBS and the cell suspension was incubated with sheep anti-rat IgG magnetic beads (Invitrogen, Carlsbad, CA) coated with rat anti-mouse CD-31 antibody (BD Biosciences, San Diego, CA) to select out mvPAEC for culture. Characterization of the culture after isolation was performed by labeling with Dil-conjugated Ac-LDL (Dil-Ac-LDL) and CD31 staining and confirmed as 95% pure ECs. Human mvPAEC were isolated from fresh lungs of control (unused donor) and IPAH patients obtained through the PHBI Network (PHBI). The tissues were procured at the Transplant Procurement Centers at Stanford University, Cleveland Clinic and Allegheny General Hospital and de-identified patient data were obtained via the Data Coordinating Center at the University of Michigan. Lung tissue was digested with collagenase IA (1.0mg/ml) (Sigma-Aldrich, St Louis, MO) for 1h and followed the mouse mvPAEC protocol. Anti-human CD31-coated beads were used for EC purification (Cat: 111.55D, Invitrogen). To ensure the purity of the culture we re-purified these cultures with CD31 beads after first passage. Staining using Dil-conjugated Ac-LDL (Dil-Ac-LDL, Invitrogen) and CD31 show over 95% purity for ECs. The expression analyses were done at passage 2. All cells were used between passages 3 and 6.

Cell Culture

Primary human mvPAECs and EC growth medium were obtained from Sciencell (Sciencell, Carlsbad, CA). Cells were grown in EC growth medium (2% FBS, 1 μg/ml hydrocortisone, 10ng/ml human epidermal growth factor, 3ng/ml basic fibroblast growth factor, 10μg/ml heparin, and gentamycin/amphotericin); subcultured at a 1:4 ratio in 100mm dishes (Corning, Lowell, MA) and used between passages 4-8. Cells were starved in EC starvation medium (0.2% FBS and gentamycin/amphotericin) for 24 hours prior to the experiment. SW480 cells were obtained from ATCC (ATCC, Manassas, VA) and maintained in Leibovitz F12 media (ATCC). For hypoxia studies, cells were placed in an incubator containing a hypoxia chamber (Biospherix, New York, NY) that provided 1% O_2 concentration for 24 hours.

For affinity purification (see "Affinity Purification" below), 293T cells were used and maintained in DMEM high glucose/10% FCS plus Penicillin/Streptamycin.

Adhesion Assay

A 96-well plate was coated with fibronectin (purified from human plasma and purchased from Sigma-Aldrich, St. Louis MO), collagen IV (purified from human fibroblasts and purchased from Sigma-Aldrich, St. Louis, MO) and laminin (derived from Engelbreth-Holm-Swarm murine sarcoma basement membrane and purchased from Sigma-Aldrich, St. Louis, MO) overnight at 4°C using concentrations of 1.0, 5.0 and 10.0 μg/ml for each ECM component per well, respectively. Human mvPAECs between passages three and five were seeded (20,000 cells/well) and incubated at 37°C for 30 minutes. At the end of the experiment, the cells were washed in PBS and fixed in 4% PFA at room temperature for 10 minutes. Subsequently, cells were stained with crystal violet for 10 minutes

1 followed by a water wash for 10 minutes. The average number of cells was calculated by
2 counting the total number of cells in six random fields per well (200x magnification). All
3 assays were done in triplicate, and three separate cell harvests were assessed.
4
5 **Tube Formation Assay**
6 Human and murine mvPAECs (5×10^3 per well) were cultured in a 96-well plate (Corning,
7 Lowell, MA) coated with 50µl Matrigel Basement Membrane Matrix or Growth Factor
8 Reduced Matrigel (Trevigen, Gaithersburg, MD). Tube length was quantified after 6
9 hours by measuring the cumulative tube length in three random microscopic fields using
10 a Leica computer-assisted microscope with the program KS300 3.0 (Zeiss).
11
12 **Integrin Blockade Assay**
13 A 96-well plate was coated with LN overnight at 4^oC using a concentration of 5.0µg/ml.
14 Prior to seeding in the cell culture wells, mvPAECs between passages three and five
15 were incubated with different integrin blocking antibodies (α1, α2, α3, α4, α5, αV, α6, β1
16 and β4, Millipore, Billerica, MA) at the following concentrations: 0.1, 0.5, 1.0 and 5 µg/ml,
17 for one hour at 4^oC. Following integrin blockade, the cells were seeded in the coated 96
18 well plates and adhesion studies performed as described above.
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20 **Proliferation and Survival assays**
21 To measure proliferation, cells were seeded at 25,000 cells per well on 24-well plates in
22 EC growth medium and allowed to adhere overnight. The next day, cells were washed
23 3x with PBS and incubated in EC starvation medium for 24 hours. Cells were then
24 trypsinized and counted in a hemocytometer as previously described[4]. All assays were
25 done in triplicate, and three separate cell harvests were assessed. For Caspase 3/7
26 assays, cells were seeded in a 96-well plate (4 wells per condition and 5,000 cells per
27 well) and allowed to attach overnight, then incubated for 24 hours in serum-free media to
28 induce apoptosis. Then cells were incubated for 1 hour in 100µl of Caspase 3/7
29 Luciferase Reagent Mix (Promega, Madison, WI) and total luminescence measured in a
30 Turner 20/20 luminometer (Turner Biosystems, Sunnyvale, CA).
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32 **RNA Interference**
33 The siRNA duplexes (Dharmacon, Lafayette, CO) specific for β–catenin (Dharmacon on-
34 target plus; accession number NM_001012329, NM_020248), and APC (Ambion
35 Validated siRNA, Ambion, Grand Island, NY), were transfected into human mvPAECs
36 using nucleofection as described previously [4]. Knockdown efficiency was evaluated 48
37 hours later by measuring protein levels in lysates using a western immunoblot as
38 described below.
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40 **Plasmids and Transfection Methods**
41 A pCMV-Neo-Bam plasmid containing the H. sapiens WT APC sequence was a kind gift
42 from Dr. Bert Vogelstein (Johns Hopkins University). A constitutively active (CA) Akt
43 expression construct (pcDNA3 T7 Akt1 K179M T308A S473A) was a kind gift from Dr.
44 William Sellers (Novartis). Plasmids containing inserts with the integrin α3 and α4
45 sequences in a pBluescript II KS phagemid were obtained from ATCC. Cells were
46 transfected in a Nucleofector II (Program T-032, Lonza) using the basic endothelial

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1   Nucleofection Kit (Lonza, Basel, Switzerland).

2

3   **Western Immunoblotting**

4   Cells were washed three times with ice-cold PBS and lysates prepared by adding boiling
5   lysis buffer (10mM Tris HCl, 1% SDS, 0.2mM PMSF) containing 1X protease and
6   phosphatase inhibitors (Sigma-Aldrich, St. Louis, MO), scraping into a 1.5ml
7   microcentrifuge tube and boiling for 10 minutes prior to centrifugation.   Protein
8   concentration was determined by the Lowry assay (Bio-Rad, Hercules, CA).   Equal
9   amounts of protein were loaded onto each lane of a 4-12% Bis-Tris gel and subjected to
10   electrophoresis under reducing conditions.   After blotting, PVDF membranes were
11   blocked for one hour in blocking buffer (nonfat milk powder 5% in TBS/Tween 0.1%) and
12   incubated with primary antibodies overnight at 4°C.   Binding of secondary HRP-
13   antibodies was visualized by ECL or ECL plus (Amersham Biosciences, Piscataway,
14   NJ).   A loading control was evaluated by re-probing the membrane with a mouse
15   monoclonal antibody to α-tubulin (Sigma-Aldrich). The following antibodies were used to
16   probe the membranes: β-catenin (BD Biosciences), APC and ILK-1 (Santa Cruz
17   Biotechnology, Santa Cruz, CA), α1, α3 and α5 integrins (Millipore), phospho and total
18   Akt (Cell Signaling Technologies, Boston, MA).

19

20   **Affinity purification (AP) and mass spectrometry to validate specificity of APC**
21   **antibody**
22   For AP, 293T cells were seeded to 20% subconfluence in 15 cm plates. When cells were
23   attached, cells were transfected with 20 μg pCMV-APC or pCMV empty vector using
24   calcium phosphate. 48 h after transfection, cells were detached and washed with PBS.
25   Cells were lysed in 1 ml cold lysis buffer (50 mM Tris pH 7.5, 150 mM NaCl, 1 mM
26   EDTA, 0.5% Nonidet P40, 2 mM sodium orthovanodate, 2 mM PMSF and HALT
27   protease inhibitor (Pierce)). Cells were dounced 20x on ice, and spun at 10,000 rpm for
28   30 min. The supernatant was pre-cleared with 20 μl Dynabeads Protein G (Invitrogen)
29   for 1 hr. The pre-cleared lysate was incubated with 4 μl of APC antibody (Santa Cruz)
30   overnight. The next day the antibody was chased with 40 μl of Dynabeads for 3 hr. The
31   beads were washed 4 times with lysis buffer but containing 0.05% Nonidet P40. Proteins
32   were eluted with 50 μl of IgG Elution Buffer (Pierce) for 10 min using gentle vortex. The
33   IP solution elutes were neutralized with 5 μl of 1M Tris pH 9.0. Half of the elutes were
34   analyzed by 4% SDS-PAGE (Invitrogen) followed by silver staining (Pierce). Protein
35   bands were excised and sent to the Stanford University Mass Spectrometry core facility
36   (Stanford, CA) for analysis. Excised gels were digested using Promega MS grade trypsin
37   overnight as previously reported (1), with the addition of the acid labile surfactant
38   protease max (Promega). Prior to digestion the gel slices were cut into approximately
39   1mm x 1mm cubes, reduced with 5 mM DTT and alkylated with acrylamide. Peptides
40   were extracted and dried down using a speed-vac prior to reconstitution and analysis.
41   The remaining IP solution elutes were sent to the SUMS facility. Elutes were digested
42   using the previously reported FASP (2) protocol using 30 μM filters from Pall.   Nano
43   reversed phase HPLC was done using an Eksigent 2D nanoLC (Eksigent, Dublin, CA)
44   with buffer A consisting of 0.1 % formic acid in water and buffer B 0.1 % formic acid in
45   acetonitrile. A fused silica column self packed with duragel C18 (Peeke, Redwood City,
46   CA) matrix was used with a linear gradient from 2 % B to 40 % B at a flow rate of 600
47   nL/minute. The nanoHPLC was interfaced with a Bruker/Michrom Advance Captive
48   spray source for nanoESI into the mass spectrometer. The mass spectrometer was a

1 LTQ Orbitrap Velos (Thermo Fischer Scientific) which was set in data dependent
2 acquisition mode to perform MS/MS on the top twelve most intense multiply charged
3 cations. The .RAW data were searched using Sequest on a Sorcerer platform against
4 the Uniprot database. Data was validated and visualized using Scaffold software.
5

Confocal Microscopy

7 Cells were plated in four-chamber polystyrene glass slides (15,000 cells per chamber).
8 For stimulation studies, cells were starved for 48 hours and then stimulated with BMP-2
9 as specified. Next, cells were fixed for ten minutes in 4% paraformaldehyde followed by
10 three washes with PBS. Cells were permeabilized for 30 minutes on room temperature
11 with 0.1% triton X-100 and 1% BSA in ice-cold PBS prior to overnight incubation with
12 primary antibody. The next day, samples were washed in PBS three times and
13 incubated with Alexa 488 or 555 tagged secondary antibody (Molecular Probes,
14 Carlsbad, CA) for one hour at room temperature. For actin labeling studies, slides were
15 treated with Alexa 488 labeled phalloidin for 20 minutes to stain for actin followed by
16 addition of Gold Antifade solution containing DAPI (Molecular Probes) and stored at 4°C
17 until analysis.

18 Confocal analysis was performed using a Leica SP2 AOBS confocal laser scanning
19 microscope using HCX PL APO 63X oil objective (N.A. 1.32-0.60) to locate areas of
20 interest on the slides. Image acquisition was performed using the Leica Confocal, v 2.5,
21 build 1347 software. Images were processed and saved in TIFF format using Adobe
22 Photoshop Creative Suite 2 (Adobe Systems, San Jose, CA).

23

ILK-1 Kinase Assay

25 Cells were starved for 48 hours prior to the experiment. Cells were washed in PBS and
26 lysed with ice-cold RIPA buffer (500ml per T75 flask). Equivalent amounts (250 μg) of
27 lysates were incubated overnight at 4°C with 5 μl of goat polyclonal anti-ILK1 antibody
28 (Santa Cruz Biotechnology). The next day, the immune complexes were precipitated
29 with protein G sepharose 4 fast flow beads (Amersham) and washed three times with
30 RIPA lysis buffer and three times with kinase buffer (50mM HEPES [pH=7], 2mM MgCl$_2$,
31 2mM MnCl$_2$, 20mM Na$_3$VO$_4$, protease inhibitors). The kinase assay was performed
32 using 2µg of GSK3β fusion protein (Cell Signaling Technologies, Boston, MA) as a
33 substrate. This fusion protein is a low molecular weight (~28kDa) peptide that contains
34 the two serine residues (Ser21/9) which undergo phosphorylation in the presence of ILK-
35 1. GSK3β fusion protein is then incubated with 200µM ATP in the reaction buffer (50mM
36 HEPES [pH=7], 2mM MgCl$_2$, 2mM MnCl$_2$, 20mM Na$_3$VO$_4$, 20mM NaF) for 30 minutes at
37 30°C. Next, Laemmli buffer was added and samples were boiled for 10 minutes prior to
38 loading onto a 4-12% Bis-Tris SDS-PAGE gel. Phophorylation of the substrate was
39 detected by western immunoblot with anti-GSK3β serine 21/9 antibodies (Cell Signaling
40 Technologies).

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Co-Immunoprecipitation (Co-IP)

44 Analysis of protein-protein interaction was carried out using a previously published
45 protocol[5]. Briefly, after stimulation, cells were washed in ice-cold PBS followed by cell
46 lysis with 300μl of Co-IP buffer (50mM HEPES, pH=7.8, 300mM NaCl, 1% NP-40,
47 1.2mM EDTA pH=8.0, 5mM MgCl$_2$ with protease inhibitors). To the lysate, a total of 3-5

1   ml of the IP antibody was added followed by overnight incubation at 4°C. The next day,
2   the cell lysate was incubated with 20ml of equilibrated protein G beads for 2-3 hours at
3   4°C. The bead mixture was centrifuged and washed with Co-IP washing buffer (same as
4   Co-IP buffer but with 0.1% NP-40). The beads were then resuspended in Laemmli
5   Buffer and boiled for five minutes, followed by western immunoblot analysis of the
6   beads. Loading was confirmed by western immunoblot for α-tubulin in a sample of the
7   whole cell lysate obtained prior to addition of antibodies.
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9   **Generation of the ΔITGA3 and Integrin Chimeric Mutants**

10 For generation of the integrin chimeras, the cytoplasmic tail of α3 (3039G-3136A) and
11 α4 (3129C-3225C) were excised from their native sequence and swapped followed by
12 subcloning into a pcDNA 3.1 vector. For the generation of the ITGA3 mutant ($\Delta\alpha$3), we
13 substituted the serines found in the QPSXXE motif with alanines as illustrated below:
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15 AGCCCAGCCGTCAGAGACAGA (Native Sequence)
16 AGaCCAGCCGgCAGAGACcGA (Mutant Sequence)
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18 Site-directed mutagenesis and Chimera generation were performed at Mutagenex Labs
19 (Mutagenex, Somerset, NJ).
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21 **Statistical analysis**

22 The number of samples or animals studied per experiment is indicated in the Figure
23 Legends. Values from multiple experiments are expressed as mean±SEM. Statistical
24 significance was determined using unpaired t-test or one-way ANOVA followed by
25 Dunnett's or Bonferroni's multiple comparison tests unless stated otherwise. A value of
26 $P<0.05$ was considered significant.
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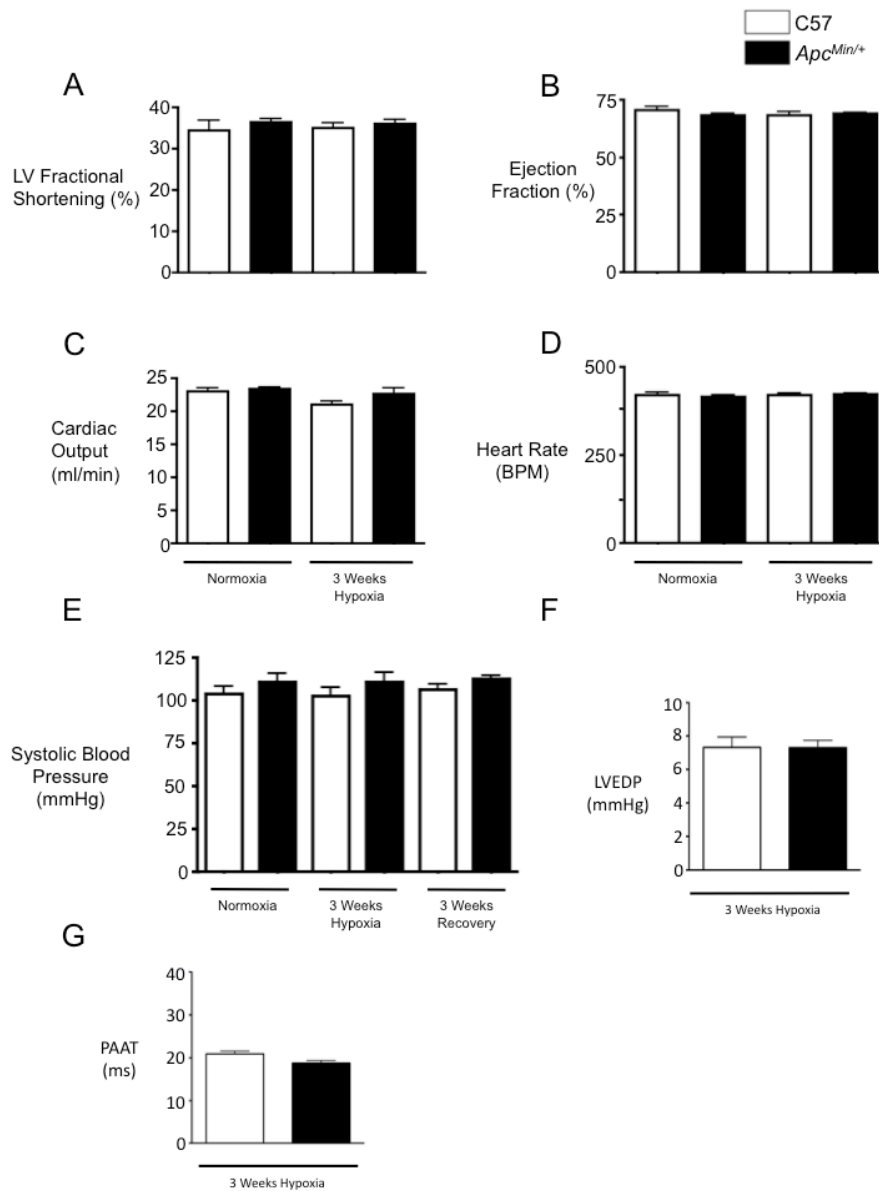
Supplement Figure I. *Apc^Min/+* **mice demonstrate no differences in echocardiographic parameters compared to C57 controls.**

(A) Left ventricular fractional shortening, (B) ejection fraction, (C) cardiac output and (D) heart rate of C57 littermate and *Apc^Min/+* mice in normoxia and chronic hypoxia. (E) Systolic blood pressure was measured using a tail cuff method. (F) Left ventricular end diastolic pressure (LVEDP) and (G) pulmonary artery aceleration times (PAAT) measurements were carried out as described in the Methods.
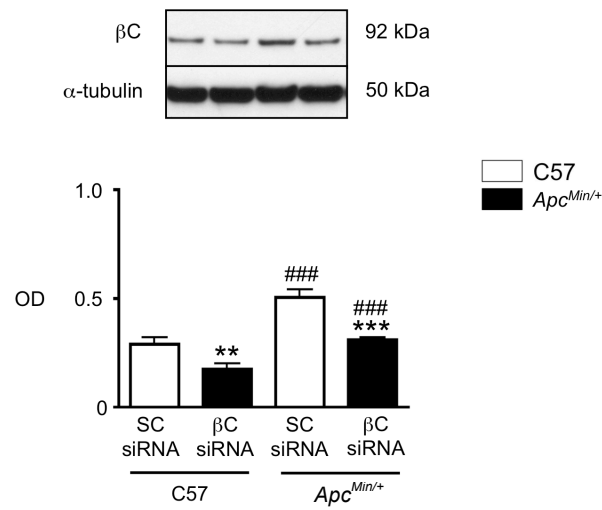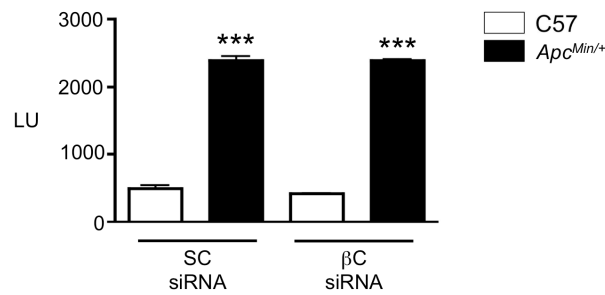
Supplement Figure II. βC knockdown does not promote survival in APC deficient human mvPAECs.

(A) siRNA mediated βC knockdown and (B) caspase 3/7 activation assay in scrambled and βC siRNA treated C57 littermate and *Apc*$^{Min/+}$ mvPAECs was performed as described in the Methods. Bars represent mean ±SEM from N=3 experiments. **P<0.001, ***P<0.0001 vs. C57, one way ANOVA with Bonferroni's post-test.
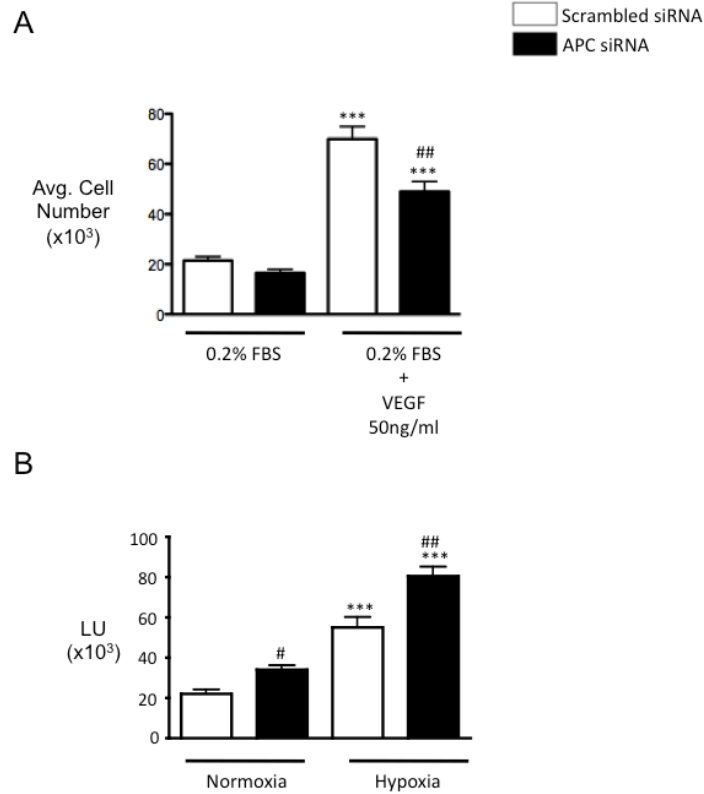
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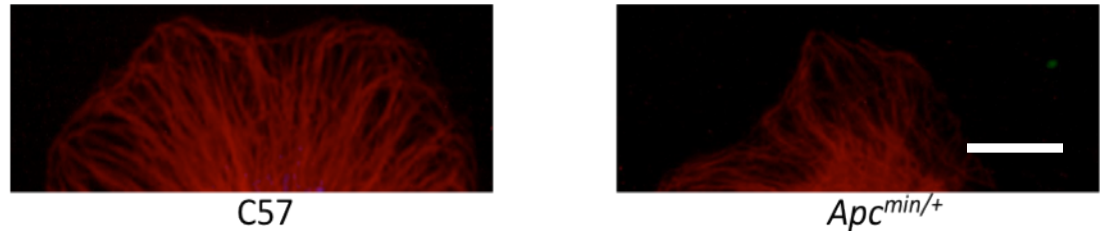
19 **Supplement Figure III. *APC^{Min/+}* mvPAECs demonstrate reduced clustering**
20 **of microtubule networks.**
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22 Representative IF images of C57 (left panel) and *APC^{Min/+}* (right panel)
23 mvPAECs probed for α-tubulin (red). Scale bar=50μm.
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**Supplement Figure IV. APC siRNA treated human mvPAECs demonstrate preserved proliferation response and reduced survival in hypoxia.**

(A) Cell count and (B) caspase 3/7 luciferase assay were performed as described in the Methods. Bars represent mean ±SEM from N=3 experiments. ***$P<0.0001$ vs. scrambled siRNA control at baseline and #$P<0.01$, ##$P<0.001$ vs. corresponding scrambled siRNA mvPAECs, one way ANOVA with Bonferroni post-test.
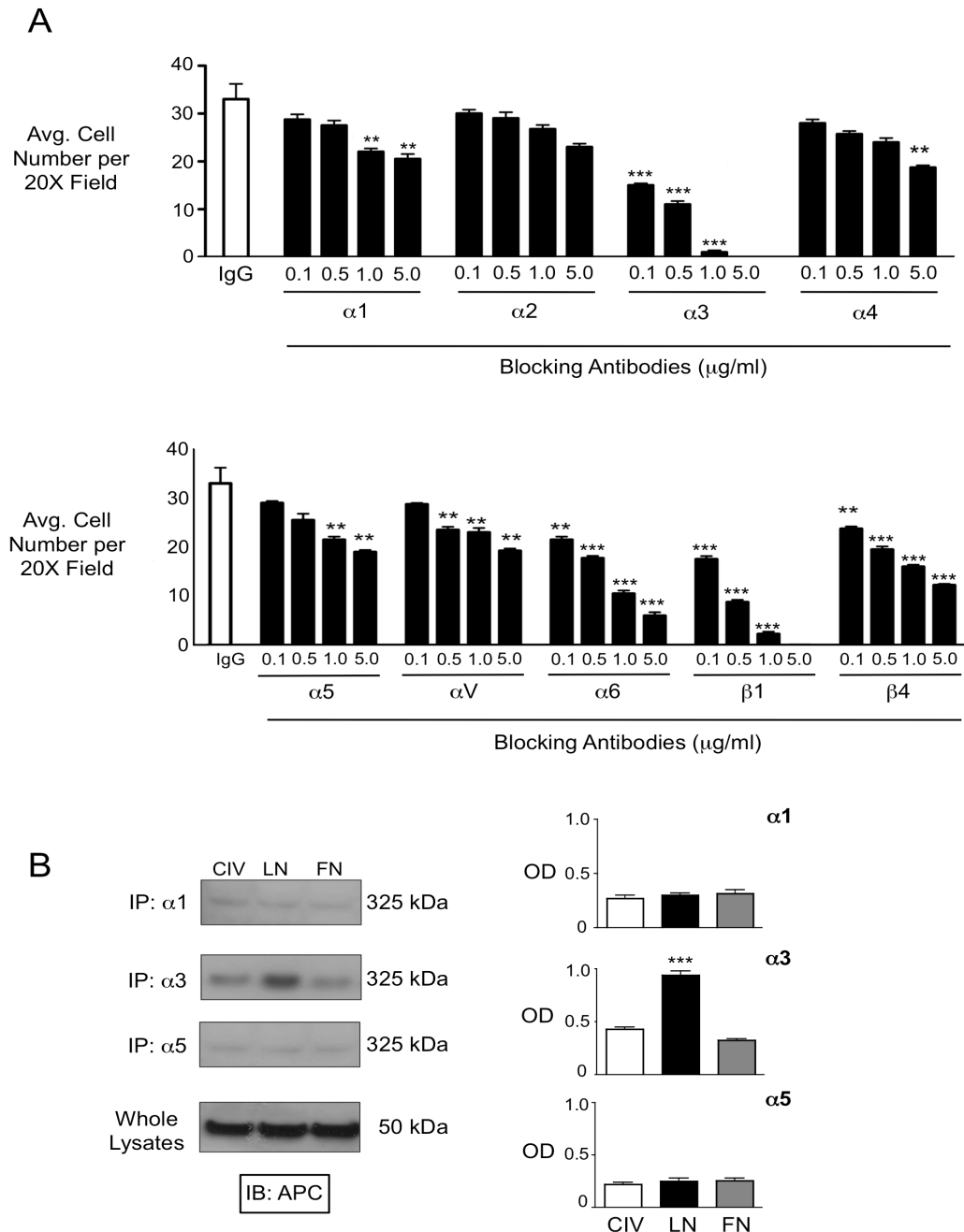
Supplement Figure V. Human mvPAECs bind to laminin via $\alpha3\beta1$ integrins resulting in the formation of an $\alpha3$/APC protein complex.

(A) Adhesion to laminin (LN) in human mvPAECs pretreated with increasing concentrations of α and β integrin blocking antibodies was evaluated 30 minutes after seeding the cells. Cells incubated with nonspecific IgG served as controls. (B) Immunoprecipitation of whole cell lysates of mvPAECs recovered following adhesion to collagen IV (CIV), LN and fibronectin (FN) using $\alpha1$, $\alpha3$ and $\alpha5$ integrin antibodies and immunoblotting using APC antibody. Bars represent mean ±SEM from N=3 experiments. *P<0.01, **P<0.001, ***P<0.0001 versus IgG, one way ANOVA with Bonferroni's post-test.

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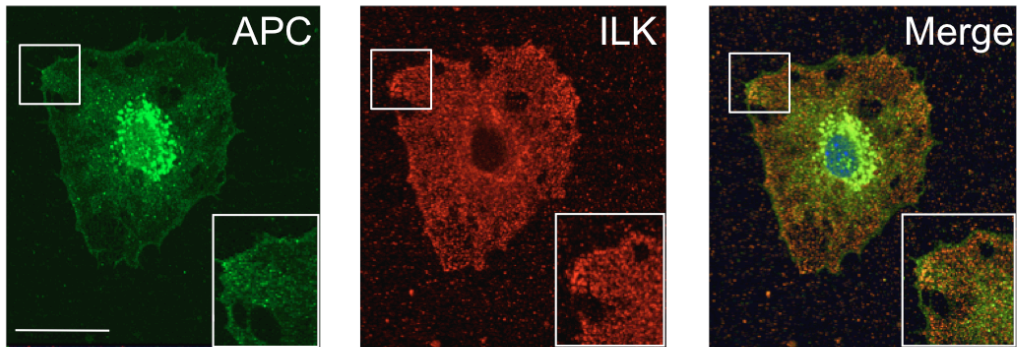
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6 **Supplement Figure VI. APC and ILK-1 colocalize in the periphery of**
7 **laminin adhered mvPAECs.**
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9 Representative confocal images of C57 mvPAECs probed for APC (green) and
10 ILK-1 (red). Merged images are shown in the right panels. Nuclei were stained
11 blue with DAPI. Scale bar=50μm.
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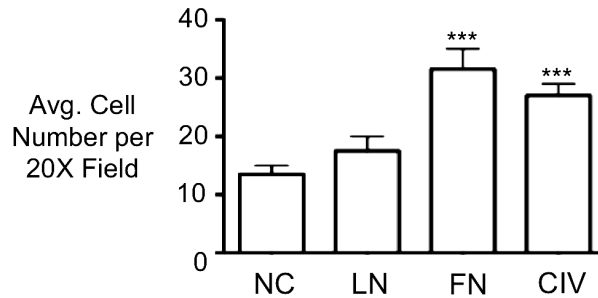
Supplement Figure VII. SW480 cells demonstrate reduced adhesion to laminin.
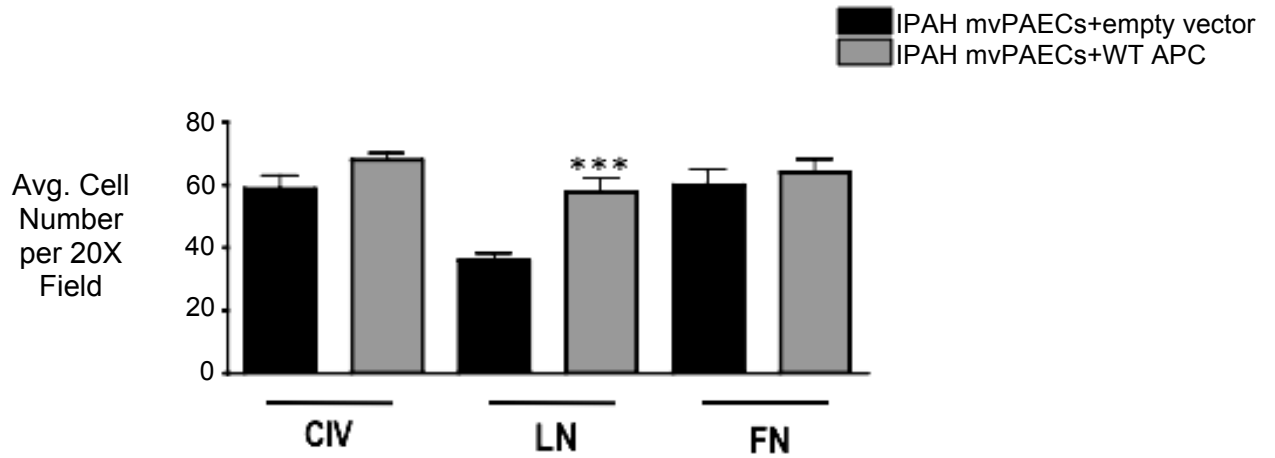
Adhesion assay of SW480 cells seeded in collagen IV (CIV), laminin (LN) and fibronectin (FN). Bars represent mean ±SEM from N=3 experiments.*P<0.01, ***P<0.0001, one way ANOVA with Bonferroni's post-test versus noncoated (NC).

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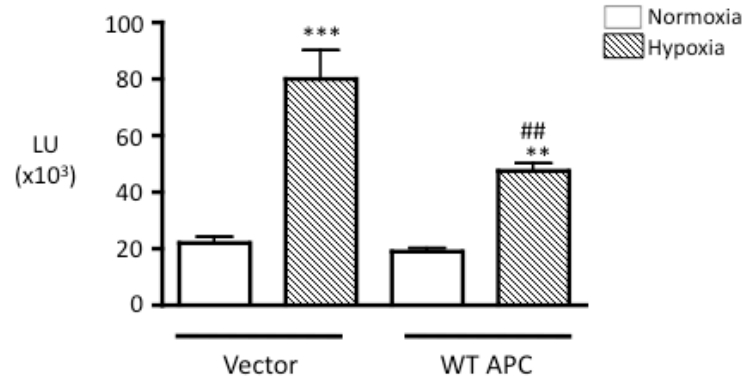
13 **Supplement Figure VIII. WT APC transfected human mvPAECs**
14 **demonstrate increased survival in hypoxia.**
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16 Caspase 3/7 luciferase assay were performed as described in the Methods. Bars
17 represent mean ±SEM from N=3 experiments. ***$P < 0.0001$ vs. normoxia control
18 at baseline and #$P < 0.01$, ##$P < 0.001$ vs. corresponding normoxia mvPAECs, one
19 way ANOVA with Bonferroni post-test.
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Supplement Figure IX. IPAH mvPAECs recover adhesion to laminin after transfection with WT APC expression construct.

Adhesion assay of IPAH mvPAECs cells seeded in collagen IV (CIV), laminin (LN) and fibronectin (FN). Bars represent mean ±SEM from N=3 experiments.***P<0.0001 versus empty vector, one way ANOVA with Bonferroni's post-test.

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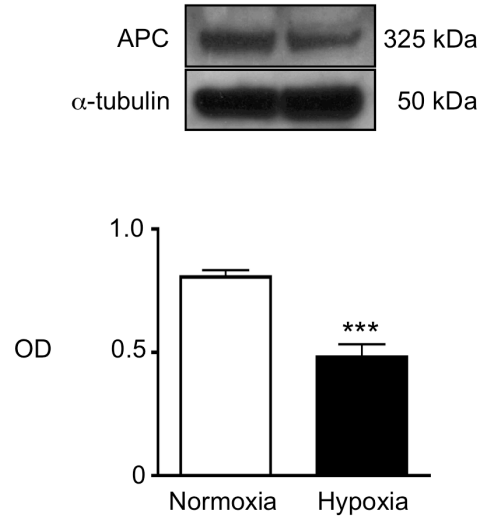
30 **Supplement Figure X. APC expression is reduced in human mvPAECs**
31 **incubated in hypoxia.**
32
33 Western immunoblot for APC in whole cell lysates of human mvPAECs exposed
34 to normoxia (FiO$_2$: 20%) and hypoxia (FiO$_2$: 1%) for 24 hours. Densitometry
35 values are shown relative α-tubulin in whole cell lysates. ***P<0.0001, unpaired
36 t-test.
37

38
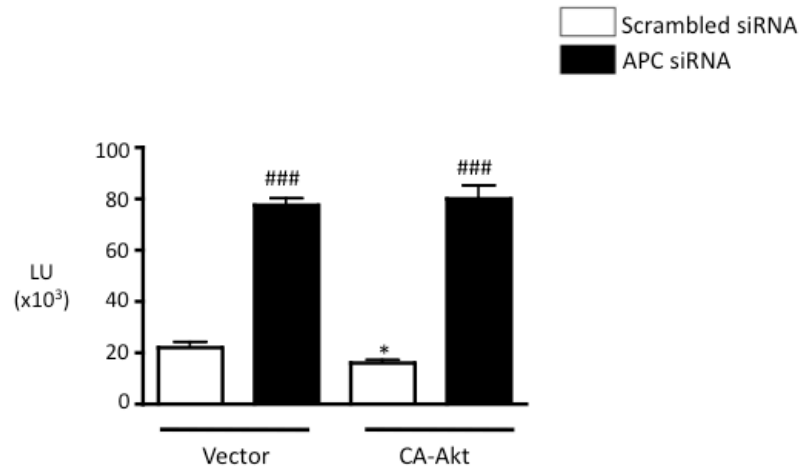
Supplement Figure XI. Transfection of a constitutively active (CA) Akt construct fails to protect APC siRNA treated mvPAECs incubated under serum free consitions.

Survival was measured using the Caspase 3/7 luciferase assay and results were compared against scrambled or APC siRNA treated mvPAECs transfected with an empty vector. ###$P<0.0001$ vs. corresponding scrambled siRNA and *$P<0.01$ vs. scrambled siRNA+empty vector, one way ANOVA with Bonferroni's post-test.

Unused Donor Control

Patient	Age	Gender	Cause of Death
1	25	Male	Head Gunshot
2	41	Male	Subarachnoid Hemorrhage
3	17	Male	Head Injury
4	28	Female	Car Accident
5	31	Male	Car Accident

IPAH Patients

Patient	Age	Gender	PAP (s/d/m)	PVR	6MW	Meds
1	43	Male	55/35/40	756	272	Bosentan sildenafil epoprostenol
2	24	Male	113/70/88	1356	182	Bosentan sildenafil epoprostenol
3	38	Female	89/26/50	1170	288	Bosentan sildenafil
4	41	Female	110/67/73	1124	299	Epoprostenol sildenafil
5	36	Male	108/67/72	1244	312	Sildenafil Ambrisentan

PAP, pulmonary artery pressures (mmHg), s: systolic, d: diastolic, m: mean.
PVR, pulmonary vascular resistance (dyne.sec.cm^{-5}.m^2), 6MW: distance (m) walked in 6 minutes.

1

2 **Supplement Table I.** Clinical characteristics of the patients who served as the
3 source for unused donor and IPAH mvPAECs.
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