

Supplemental Figures and Legends:

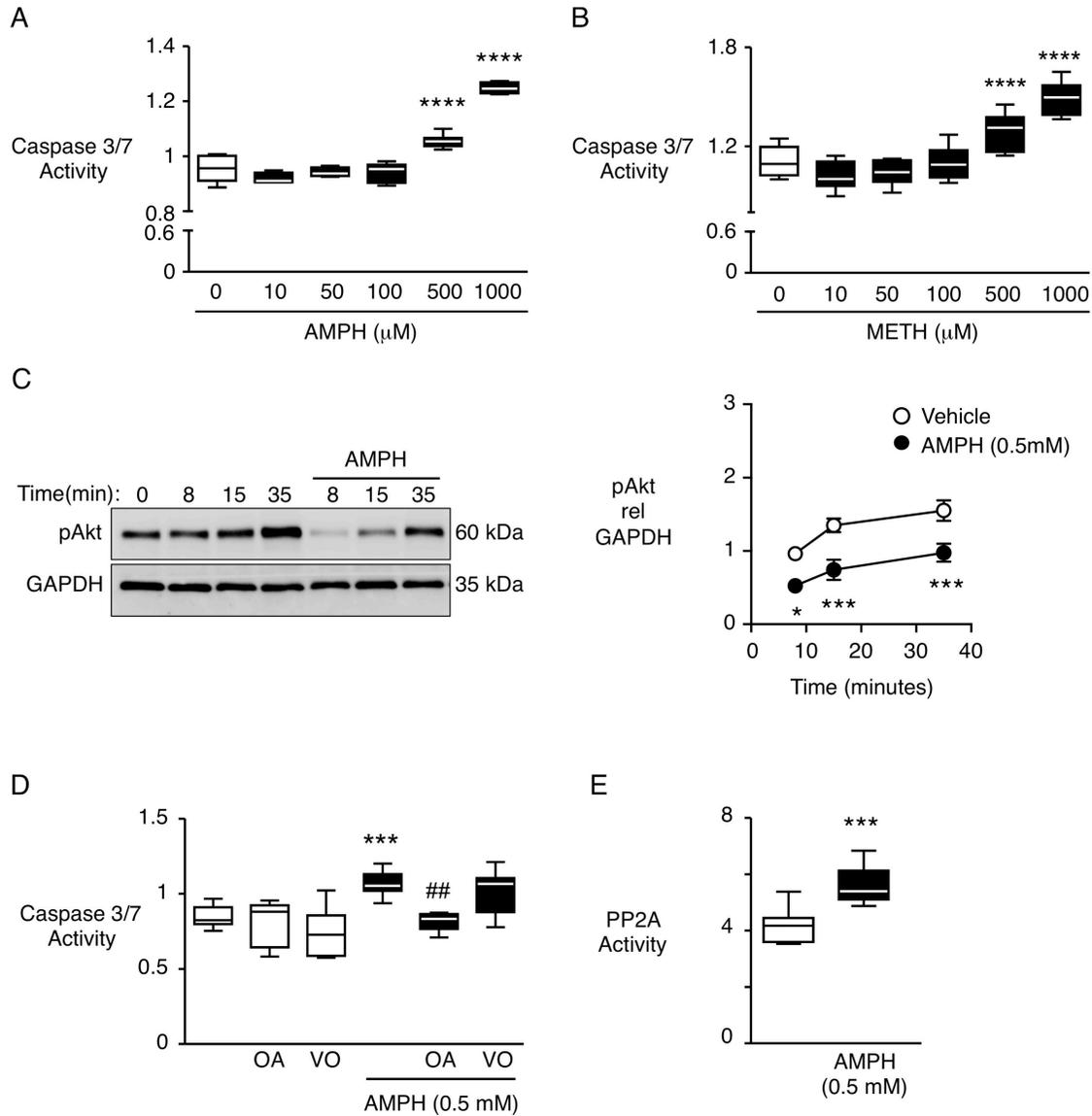


Figure S1: AMPH and METH induce caspase3/7 activation in a pAkt-dependent manner. (Related to data in Figure 2)

Commercially available human PAEC were treated in serum free medium for 24h with (A) AMPH or (B) METH in increasing doses, or with PBS (Veh) and then assayed for caspase3/7 activity. (C) PAEC starved in serum free medium overnight were stimulated with 0.5mM AMPH for the indicated times. Cell lysates were immunoblotted for pAkt, and GAPDH (loading control). (D) PAEC in serum free medium were treated with Veh, 0.5mM AMPH or AMPH together with okadaic acid (OA, 10μM) or VO-OHpic (VO, 5μM) for 24h and then assayed for caspase3/7 activity. (E) PAEC were treated with 0.5mM AMPH in serum free medium overnight, and PP2A activity measured using the DuoSet®-IC activity assay following the manufacturer's instructions.

(A, B, D and E) Box and whisker plots represent values within the interquartile range (boxes) and the minimum to maximum (whiskers). The line within the box shows the median. n=3 independent experiments with 3-4 replicates per experiment. (C) Line graph represent mean±SEM, n=3. * $p < 0.05$, *** $p < 0.0005$, **** $p < 0.0001$ vs. Veh; ## $p < 0.005$ vs. AMPH alone by 2-way ANOVA and Bonferroni's post-test. (E) *** $p < 0.0005$ by Student's t test.

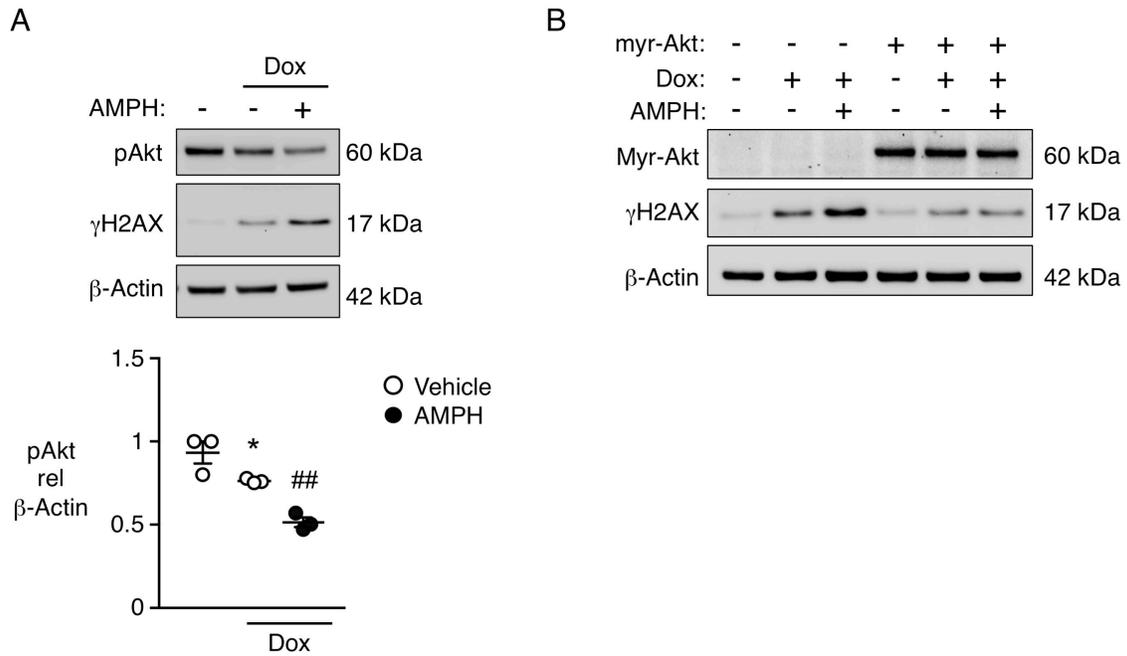


Figure S2: AMPH exacerbates pAkt inhibition under genotoxic stress (Related to data in Figure 2)

(A) PAEC or (B) PAEC transfected with myristoylated-Akt1 (myr-Akt) or with vector alone for 24h were treated with 1mM of AMPH daily for three days and then stimulated with Dox for 24h. Cells lysates were immunoblotted for pAkt, γ H2AX, β -actin (loading control), and in B, for HA, to verify the expression of myr-Akt. Dot plot represents mean \pm SEM, n=3 independent experiments. * p <0.05, **** p <0.0001 vs. Veh, ## p <0.005 vs. Dox by 2-way ANOVA and Bonferroni's post-test.

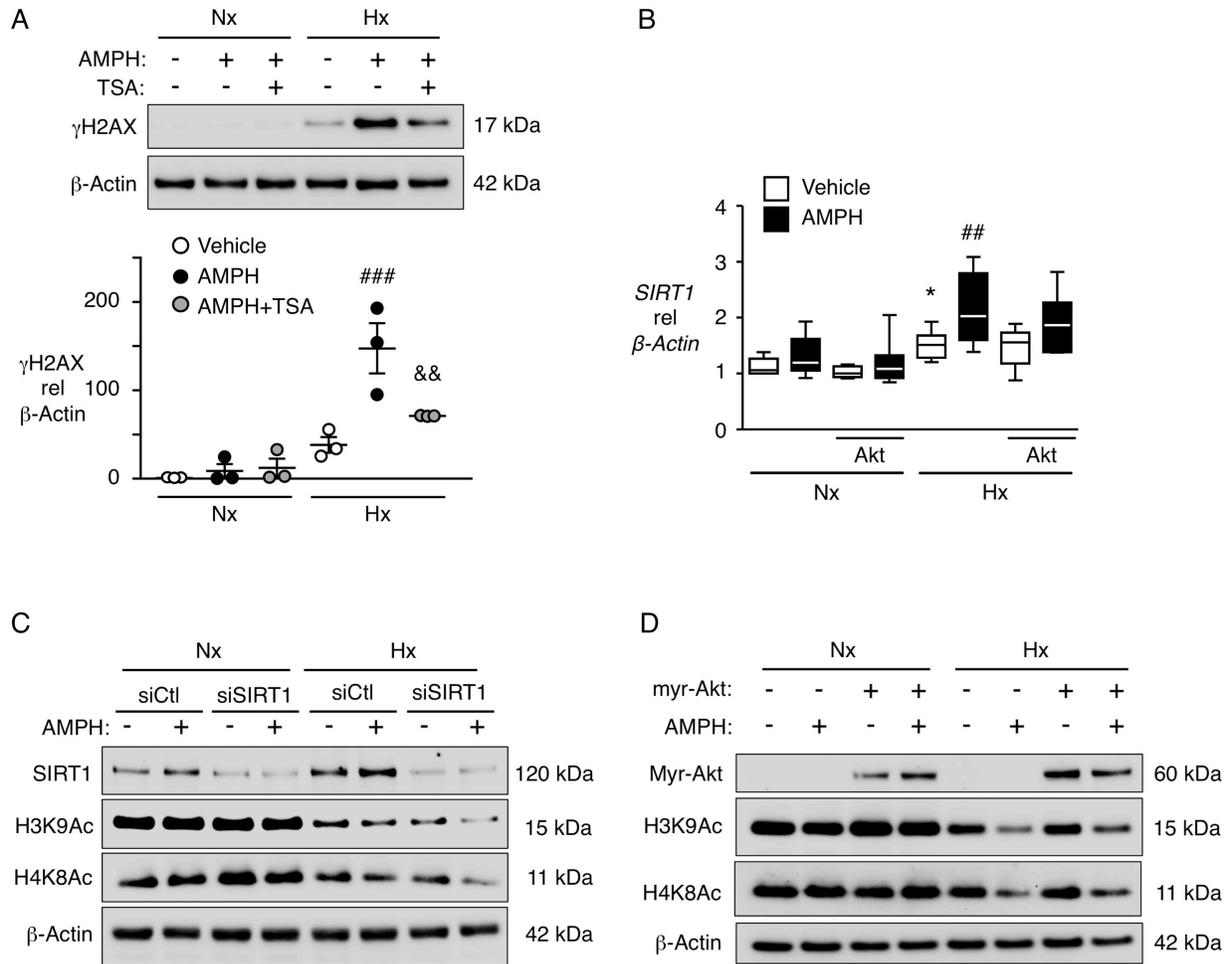


Figure S3: Pan-HDAC inhibitor TSA abrogates AMPH-exaggerated DNA damage (Related to data in Figure 3)

(A) PAEC were treated with PBS or 0.5mM AMPH with or without TSA (0.25 μ M) daily for three days and then cultured with the stimuli under Nx or Hx (0.5% O₂) for 48h. Cell lysates were immunoblotted for γ H2AX and β -actin (loading control). (B) PAEC transfected with vector or myr-Akt constructs for 24h were treated with AMPH daily for three days and then cultured with the stimuli under Nx or Hx for 8h. mRNA was extracted and subjected to SIRT1 qRT-PCR. (C) PAEC transfected with scrambled (siCtl) or SIRT1 (siSIRT1) siRNA, and (D), PAEC transfected with vector or myr-Akt constructs, were treated as in A. Cells lysates were immunoblotted for H3K9Ac, H4K9Ac, SIRT1, HA and β -actin (loading control).

(A) Dot plot represents mean \pm SEM, n=3 independent experiments. (B) Box and whisker plots represent values within the interquartile range (boxes) and the minimum to maximum (whiskers). The line within the box shows the median. n=3 independent experiments with 2-3 replicates per experiment. * p <0.05, vs. Nx+Veh, ### p <0.005, ### p <0.0005 vs. Hx+Veh, && p <0.005 vs. Hx+AMPH by 2-way ANOVA and Bonferroni's post-test.

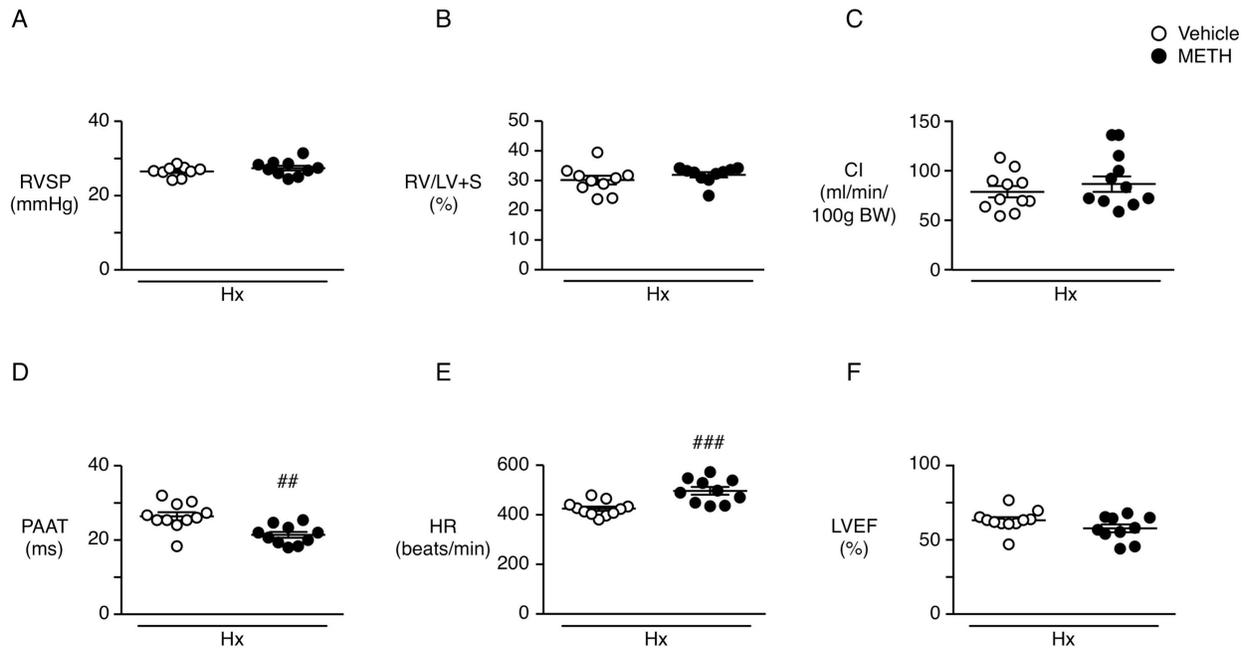


Figure S4: Mice exposed to METH and hypoxia exhibited irregular hemodynamics (Related to data in Figure 6)

Mice were injected with 10mg/kg of METH twice daily for three days, followed by 4-day hypoxia (10% O₂), and the sequence repeated for a total of four weeks. Hemodynamic measurements were conducted as described in “Methods”. **(A)** Right ventricle systolic pressure (RVSP), **(B)** The ratio of the right ventricle over the left ventricle+septum (RV/LV+S), **(C)** cardiac index (CI), **(D)** pulmonary artery acceleration time (PAAT), **(E)** heart rate (HR), and **(F)** left ventricle ejection fraction (LVEF). In (A), RVSP values at normoxia (Nx), were measured in a separate group treated with vehicle.

Dot plots represent individual animals (mean±SEM, Nx+Veh group n=4, Hx+Veh group n=10-11, Hx-METH group n=9-10). ** $p < 0.005$ vs. Nx, # $p < 0.05$, Hx-Veh vs. METH-Veh by un-paired t test.

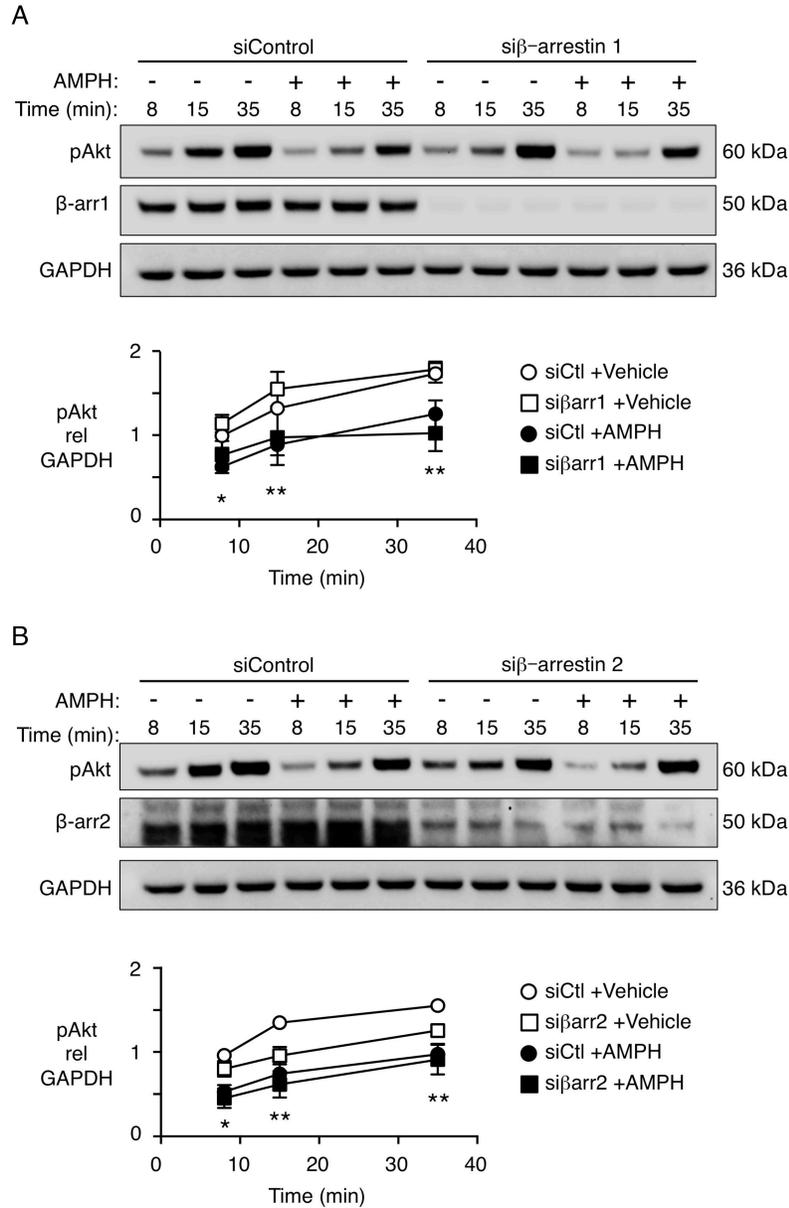


Figure S5: Knock-down of β -arrestin 1 or 2 did not reverse AMPH-suppressed pAkt (Related to Discussion)

PAEC transfected with 20nM of siRNA against β -arrestin1 or 2 (si β -arrestin1 or 2) or scrambled control siRNA (siCtl) for 24 hrs were starved overnight and then stimulated with 0.5mM AMPH for the indicated times. Cell lysates were immunoblotted for pAkt, β -arrestin1 (β -arr1, in **A**) or β -arrestin2 (β -arr2, in **B**), and GAPDH (for normalization). Representative immunoblots above and densitometric quantification of pAkt in the line graphs below, showing the mean \pm SEM, n=3. * p <0.05, ** p <0.005 vs. siCtl+Veh by 2-way ANOVA, Bonferroni's post-test.

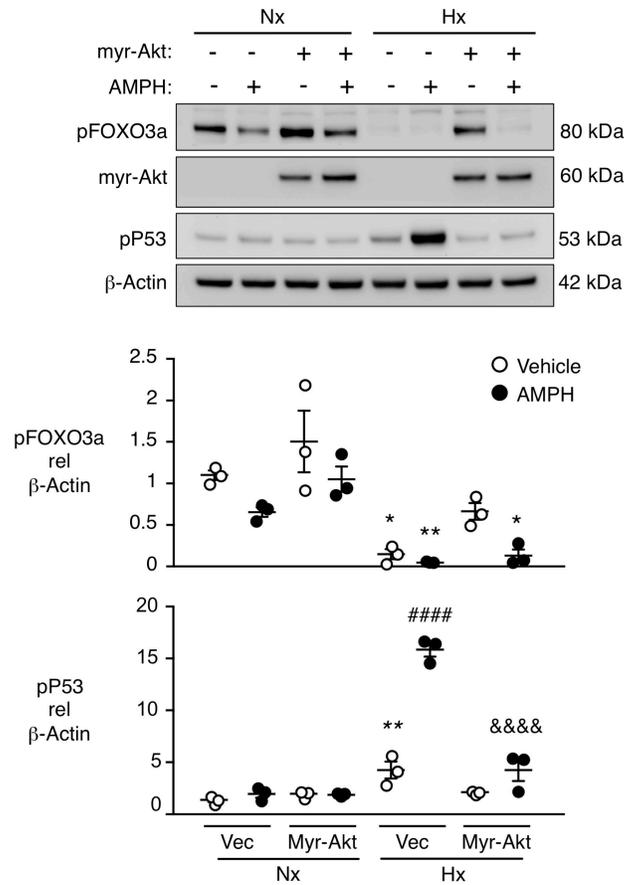


Figure S6: Constitutively-active Akt reversed AMPH-exaggerated pP53, but not the reduction of pFOXO3a (Related to Discussion)

PAEC transfected with vector (Vec) or myr-Akt for 24h were treated with PBS (Veh), or 0.5mM AMPH daily for three days and then cultured with the stimuli under Nx or Hx for 48h. Cell lysates were immunoblotted for pFOXO3a, pP53, β -actin as loading control and HA to verify the expression of myr-Akt. Representative immunoblots are above with densitometric quantification of pFOXO3a and pP53 normalized for β -actin in the dot plots (mean \pm SEM, n=3). * p <0.05, ** p <0.005 vs. Nx+Veh, # p <0.05, #### p <0.0001 vs. Hx+Veh, &&&& p <0.0001 vs. Hx+AMPH by 2-way ANOVA, Bonferroni's post-test.

Supplemental Experimental Procedures

Human Pulmonary Artery EC (PAEC)

Primary human PAEC were commercially obtained from PromoCell GmbH (Heidelberg, Germany). Additional primary PAEC were obtained from lungs removed at transplantation from D&T-PAH patients, and from unused donor lungs as controls (patient and donor characteristics in Table S1). The data and tissue samples were provided by PHBI under the Pulmonary Hypertension Breakthrough Initiative (PHBI). Funding for the PHBI is provided under an NHLBI R24 grant, #R24HL123767, and by the Cardiovascular Medical Research and Education Fund (CMREF). Lung tissues were processed as previously described (S1). PAEC were grown in complete EC media (EC basal medium, ECGS and 5% FBS) (ScienCell, Carlsbad, CA) and used between passages 4 and 7. In the studies examining AMPH-exaggerated DNA damage, human PAEC were treated daily with AMPH in full medium for three days and then exposed to genotoxic stress (Doxorubicin) for the indicated times.

Mouse Model

C57BL/6 (6-8 weeks old) mice were obtained from Charles River (San Diego, CA), maintained in the animal facility at Stanford University School of Medicine and fed food and water ad libitum. Methamphetamine hydrochloride (METH) purchased from United States Pharmacopeial Convention (Rockville, MD) via Stanford University Environmental Health and Safety (EH&S) Department was dissolved in sterile saline. METH at a dose of 10 mg/kg was intraperitoneally administered twice daily for 3 days, followed by 4-day hypoxia (10% O₂). This regimen was repeated for a total of 4 weeks. Control animals were injected with an equal volume of sterile saline. At the end of the regimen, RVSP and RVH, cardiac function and output, and pulmonary artery acceleration time (PAAT) were measured. RVSP was measured in unventilated mice under isoflurane anaesthesia (1.5–2.5%, 2l O₂/min) using a closed chest technique, by introducing a 1.4-F Millar catheter (Millar Instruments, Houston, TX) into the jugular vein and directing it to the right ventricle as previously described (S2). Cardiac output, left ventricular function (fractional shortening) as well as PAAT were evaluated by echocardiography under isoflurane anaesthesia (1.5–2.5%, 2l O₂/min) before RVSP measurements using a Vivid 7 ultrasound machine (GE Healthcare, Fairfield, CT) and a 13-MHz linear array transducer. Following the hemodynamic measurements, the heart and lungs were perfused with PBS, the left lung fixed, and sections embedded in paraffin for immunohistochemistry and morphometry. The right lung was snap-frozen in liquid N₂ and kept at –80°C for protein extraction. The number of mice per experiment is indicated in the figure legends.

Reagents and Cellular Assays

Reagents: D-Amphetamine hemisulfate salt (AMPH, research grade) was purchased from Sigma Aldrich (St. Louis, MO) via Stanford University EH&S under the control II substance regulation. The reagent is dissolved in PBS as 500mM stock for treating cells, zVAD-FMK were from R&D system (Minneapolis, MN). VO-OHpic trihydrate (PTEN inhibitor), Trichostatin A (TSA) and Antimycin A (AMA) were from Sigma Aldrich (St. Louis, MO). Okadaic acid was purchased from Cell Signaling Technology (Danvers, MA). Sirtinol was purchased from Cayman Chemical (Ann Arbor, Michigan). All reagents were dissolved in solvents following instructions in the data sheets and stored accordingly.

Plasmid transfection: pcDNA3 Myr HA Akt1 was purchased from Addgene (Cambridge, MA) and expanded. pcDNA3 Myr HA Akt1 and pcDNA3 vector control were transfected into PAEC by the 4D Nucleofector™ Core unit (Lonza, Walkersville, MD) using the p5 Primary Cell Solution (Lonza) and re-seeded in cell culture plates. Treatments as indicated in the figure legends were initiated 24 h post-transfection.

siRNA transfection: siRNA specific for SIRT1, β -arrestin1 and β -arrestin2 were purchased from Dharmacon (ON-TARGETplus SMARTpool, Lafayette, CO) and Ambion (Grand Island, NY) targeting different sequences of the mRNA. siRNAs were transfected into PAEC by the 4D Nucleofector™ Core unit (Lonza, Walkersville, MD) using the p5 Primary Cell Solution (Lonza) and re-seeded in cell culture plates. The knockdown efficiency was determined by immunoblotting or qPCR. ON-TARGETplus siControl (Dharmacon) or *Silencer*® Select Negative Control (Ambion) was used as control. Treatments as indicated in the figure legends were initiated 24 h post-transfection. The siRNA against SIRT1 targeting different sequences of SIRT1 were tested with similar results.

Caspase Assay: For Caspase-3/7 assays, cells were seeded in a 96-well plate at 8,000-10,000 cells per well, in full EC medium. 12-24h after seeding, cells were treated with the indicated stimuli in serum free medium as described in the figure legends for 24h, and then incubated for 1h in 40 μ l of Caspase 3/7 Luciferase Reagent Mix (Promega, Madison, WI). Total luminescence was measured in a plate reader (BioTek Synergy H1 Hybrid Reader).

PP2A assay: Promocell PAEC were treated with 1mM AMPH overnight in full medium. Cells were rinsed twice with TBS and then solubilized for 15min in Lysis Buffer (50mM HEPES, 0.1mM EGTA, 0.1mM EDTA, 120mM NaCl, 0.5% NP-40 Alternative (pH 7.5), 25 μ g/mL Leupeptin, 25 μ g/mL Pepstatin, 2 μ g/mL Aprotinin, and 1mM PMSF; Protease inhibitors were added just before use and placed on ice). Cell lysates were cleared with centrifugation and then subjected to active PP2A DuoSet® IC assay (Promega, Madison, WI) following the manufacturer's instructions. In brief, PP2A antibodies were coated in a 96-well plate overnight, and then blocked with blocking buffer. Cell lysates were added to the coated plates and then incubated for 3h at 2-8°C. After removing the cell lysates, plates were rinsed with appropriate diluents and then Serine/Threonine Phosphatase substrate was added to react with bound PP2A in the wells. Phosphate released from PP2A dephosphorylation is then detected with sensitive dye-binding assay using malachite green and molybdic acid. The activity of PP2A is determined by calculating the rate of phosphate release.

Comet assay: DNA breaks were monitored using the Comet Assay Reagent Kit for Single Cell Gel Electrophoresis Assay (Trevigen, Gaithersburg, MD) according to the manufacturer's protocol. In brief, PAEC were trypsinized, rinsed and resuspended with cold PBS to have 3x10⁵ cells/mL. 50 μ l of cells were mixed with 500 μ l of low-melting agarose, and 50 μ l of the cell-agarose mixture was spread on each comet slides. Cells in the agarose were cooled and then lysed in Trevigen Lysis Solution over night. Comet slides were subjected to alkaline unwinding followed by electrophoresis. Slides were rinsed and then dried on heat block. DNA was stained with SYBR Green (Invitrogen) and then imaged with fluorescent microscope. The comet tail lengths (from the center of DNA head to the end of the DNA tail) were measured in 100-150 cells per slide per condition for each sample per condition and analyzed with ImageJ.

Signal Reporter Assay: PAEC were transfected with HIF responsive luciferase constructs (Qiagen, Hilden, Germany) that encode the firefly luciferase reporter gene under the control of a promoter containing the HIF response elements, by 4D Nucleofector™ Core unit (Lonza, Walkersville, MD) using the P5 Primary Cell Solution (Lonza). A Renilla construct encoding the Renilla luciferase reporter gene under the control of a CMV promoter was used as an internal control for normalizing transfection efficiencies and monitoring cell viability. The luciferase reporter was co-transfected with siRNA against SIRT1, or control siRNA. A firefly luciferase reporter construct without any transcriptional response elements served as the negative control. A firefly luciferase reporter construct driven by a CMV promoter served as a positive control. Luciferase production was measured in a luminometer (BioTek Synergy H1 Hybrid Reader, BioTek, Winooski, VT) using the Dual Luciferase kit (Promega) according to the manufacturer's protocol.

Mitochondrial Membrane Potential Assay: The mitochondrial membrane potential was determined using the MitoPT JC-1 (5',6,6'-tetrachloro-1,1',3,3'-tetraethylbenzimidazolylcarbocyanine iodide) assay kit (Immunochemistry Technologies, Bloomington, MN). In brief, PAEC were trypsinized, rinsed, re-suspended and incubated in staining buffer containing JC-1 dye for 15-20 minutes at 37°C. The rinsed pellet was re-suspended again in the assay buffer at 50,000-100,000 cells/100 μ l. 100 μ l of cell suspension per well, in a 96-well plate, were used to measure the dye emission in a fluorescence plate reader (BioTek Synergy H1 Hybrid Reader). Potential-dependent accumulation of the dye in the mitochondria is indicated by a fluorescence emission shift from green (~527nm) to red (~600nm).

Mitochondrial Reactive Oxygen Species (ROS) assay: PAEC seeded onto live-cell imaging glass bottom dishes (Ibidi Labware, Madison, WI) were treated with vehicle (veh), 0.5mM AMPH, or AMPH with 10 μ M sirtinol daily for three days and placed in Hx for 24h with the stimuli. Following treatment, cells were loaded with 100nM MitoTracker Green FM (Molecular Probes, Eugene, OR) to stain mitochondria, and 5 μ M MitoSOX Red (Molecular Probes) to detect mitochondrial superoxide, for 20min at 37°C in HBSS in the dark, and then washed three times with pre-warmed HBSS (37°C). Live image acquisition was performed on an Olympus FluoView FV1000 equipped with a PrecisionControl WeatherStation (Olympus, Japan). Fluorescence intensities (MitoTracker: Excitation 488nm, emission 500–545nm; MitoSOX: Excitation 559nm, emission 570-670nm) were quantified using Fiji software (NIH). ROS production, normalized to cell number and mitochondria content, is given by the ratio of MitoSOX to MitoTracker fluorescence ($F_{\text{MitoSOX/MitoTracker}}$).

Mitochondrial Function Assays: Baseline mitochondrial function and mitochondrial stress response were measured by oxygen consumption rate (OCR) using the Cell Mito Stress Kit with an XF24 extracellular flux analyzer (SeaHorse Biosciences, North Billerica, Massachusetts) following manufacturer's instructions. Glycolytic capacity was measured using the XF Glycolysis Stress Test Kit (SeaHorse Bioscience) on the same device. Briefly, 30,000 cells per well were seeded overnight and then treated as indicated in the figure legends. Following treatment,

for OCR measurements, cells were washed with XF assay medium (SeaHorse Bioscience) containing 10mM Glucose, 1mM Sodium-Pyruvate and 2mM L-Glutamine set to pH 7.40. OCR was measured over time at baseline and following consecutive injections of 1 μ M Oligomycin, 1 μ M FCCP and a mix of 1 μ M Rotenone + 1 μ M Antimycin A. Maximal mitochondrial respiration is defined as the OCR following FCCP injection, spare respiratory capacity is defined as $\Delta\text{OCR}_{\text{FCCP-baseline}}$ and mitochondrial ATP production as $\Delta\text{OCR}_{\text{Baseline-Oligomycin}}$. For extracellular acidification rate (ECAR) measurements, cells were washed in glucose-free XF base medium (SeaHorse Bioscience) containing 2mM L-Glutamine at pH 7.35. ECAR was determined after serial injection with 10mM D-Glucose, 1 μ M Oligomycin and 100 mM 2-Deoxyglucose. Aerobic glycolysis is defined as ECAR in response to Oligomycin after glucose injection. Glycolytic reserve is defined as $\Delta\text{ECAR}_{\text{Oligomycin-Glucose}}$. Non-glycolytic acidification is ECAR after 2-Deoxyglucose injection.

Western immunoblotting: Western immunoblotting was performed as previously described (S3). The antibody dilutions in 5% BSA are listed in the table below. For normalization, the membrane was re-probed with an antibody against a housekeeping protein, β -Actin or GAPDH. For technical reasons, in a few of the experiments (indicated in the figure legend), the samples were normalized to β -Actin or GAPDH measured from the same samples that were run on separate gels. HRP-anti-rabbit and anti-mouse secondary antibodies were used as 1:10,000 (Santa Cruz Biotechnology, Santa Cruz, CA).

Primary antibodies used for immunoblotting:

Antibody	Dilution	Company/Catalog #
<i>SIRT1</i>	1:1000	Cell Signaling Technology (Danvers, MA) / 8469
<i>p-SIRT1(Ser⁴⁷)</i>	1:1000	Cell Signaling Technology (Danvers, MA) / 2314
<i>p-Akt (Ser⁴⁷³)</i>	1:1000	Cell Signaling Technology (Danvers, MA) / 4060
<i>cleaved caspase 3</i>	1:1000	Cell Signaling Technology (Danvers, MA) / 9664
<i>PDK1</i>	1:1000	Cell Signaling Technology (Danvers, MA) / 3820
<i>Acetyl-lysine</i>	1-2 μ g for IP	Cell Signaling Technology (Danvers, MA) / 9441
<i>H3K9Ac</i>	1:1000	Cell Signaling Technology (Danvers, MA) / 9649
<i>H4K8Ac</i>	1:1000	Cell Signaling Technology (Danvers, MA)
<i>p-FOXO3a</i>	1:1000	Cell Signaling Technology (Danvers, MA) / 9465
<i>p-p53(Ser¹⁵)</i>	1:1000	Cell Signaling Technology (Danvers, MA) / 9284
<i>p-JNK</i>	1:500	Cell Signaling Technology (Danvers, MA) /
<i>αSMA</i>	1:1000	Santa Cruz Biotechnology Dallas, TX
<i>COX4II</i>	1:1000	Thermo Scientific (Rockford, IL) / MA5-15686
<i>β-actin</i>	1:2000	Santa Cruz Biotechnology (Santa Cruz, CA) /
<i>HIF1α</i>	1:2000	BD Biosciences (San Jose, CA) / 610958
<i>γH2AX</i>	1:1000	EMD millipore (Billerica, MA) / 05-636
<i>GAPDH</i>	1:2000	EMD millipore (Billerica, MA)

qRT-PCR: Total RNA was extracted and purified from cells using the Quick-RNA MiniPrep Kit (Zymo Research, Irvine, CA). The quantity and quality of RNA was determined by using a spectrophotometer, and then the RNA was reverse transcribed using High Capacity RNA to cDNA Kit (Applied Biosystems, Foster City, CA) according to the manufacturer's instructions. RT-PCR was performed using 1 μ l of 5 μ M mixed primers, 5 μ l Power SYBR green PCR Master Mix (Applied Biosystems), 2 μ l dH₂O and 2 μ l cDNA sample (10ng cDNA). Each measurement was carried

out in duplicate or triplicate using a CFX384 real Time System (Biorad) in a 10 μ l reaction. The PCR conditions were: 95°C for 10min, followed by 45 cycles of 95°C for 15sec, and 56°C for 60sec. Primer sequences were designed using NCBI's Primer-BLAST function or PrimerBank (<http://pga.mgh.harvard.edu>) and are as listed in the table below. Expression levels of selected genes were normalized to β -actin. The housekeeping gene that was chosen was the most consistent throughout the experimental protocol.

qRT-PCR primers

Gene	Forward primer	Reverse Primer
<i>HDAC1</i>	ATCTATCGCCCTCACAAAGC	AATCTCTGCATCTGCTTGCT
<i>HDAC2</i>	TGCTACTACTACGACGGTGA	AGTGGCTTTATGGGGCCTA
<i>HDAC3</i>	GAGAGTCAGCCCCACCAATA	GTTGTTTCAGCTGGGTTGCTC
<i>HDAC4</i>	CAAGAACAAGGAGAAGGGCAAAG	GGACTCTGGTCAAGGGAACTG
<i>HDAC6</i>	ATCTGGCGGAGTGGAAAGAA	AAGTGACACTGGAGTCCTGA
<i>HDAC7A</i>	CTCACTGTCAGCCCCAGAG	CTGGTGCTTCAGCATGACC
<i>HDAC8</i>	AAACGGGCCAGTATGGTG	CTGACCTTCTGGAGATGCTG
<i>HDAC9</i>	CAACAAAACCCTAGCAGCCT	GCCCACAGGAACTTCTGACT
<i>HDAC10</i>	CACTAGCGAGGGCGTTTG	GGGTCGTCCCAGAGCA
<i>HDAC11</i>	GGATGCTACACACAACCCA	CCCATTTCCGGCATCAAAG
<i>SIRT1</i>	GCGATTGGGTACCGAGATAA	TTGCATGTGAGGCTCTATCC
<i>SIRT2</i>	GACCCCTCTACCCTCTG	CAGGAAGTCCATGTCTGCTT
<i>SIRT3</i>	TCCAGTTTCTTCTTTTCGAGTA	GAAAGCTTCCCCTTGCTACT
<i>SIRT4</i>	GACAGGGTCTGTGCTTG	GCTCCTCTGAGAGAAAGACG
<i>SIRT5</i>	GGGGCCCAAGTAAATGGAAA	TGAAGATGGTTGTCTCCACG
<i>SIRT6</i>	TCCCGGAGATCTTCGACC	CCAGAGGCAGTGCTGATG
<i>β-Actin</i>	ACCCGCCACCAGTTCGCCAT	CTTGACATGCCGGAGCCGT
<i>PDK1</i>	CTGTGATACGGATCAGAAACCG	TCCACCAAACAATAAAGAGTGCT
<i>COX4II</i>	CAGGGTATTTAGCCTAGTTGGC	GCCGATCCATATAAGCTGGGA

Immunofluorescence:

Tissue sections: Sections from formaldehyde-fixed and paraffin-embedded mouse lung tissues were de-paraffinized and rehydrated. Antigen retrieval was performed by boiling the sections in 0.25 mM EDTA. Sections were incubated with 3% hydrogen peroxide to block endogenous peroxidase, washed, and blocked with 5% BSA. The sections were then incubated with the primary antibodies against Von Willebrand factor (vWF) (1:1000; Abcam, Cambridge, CA), α -smooth muscle actin (α -SMA) (1:500; Sigma) or γ H2AX (1:100, Millipore, Billerica, MA), overnight at 4°C. The next day, sections were washed three times with PBS and then incubated with the secondary antibody (Alexa Fluor 488-labeled anti-rabbit 1:800, Alexa Fluor 594-labeled anti-mouse 1:800, Invitrogen) for 1h at RT. Sections were washed three times with PBS, mounted with Vectashield containing DAPI (Vector Laboratories, Burlingame, CA, USA) and stored at 4°C until analysis. Confocal analysis was performed on a confocal laser-scanning microscope (FV1000, Olympus, Center Valley, PA).

Cell culture: Cells treated per the experimental protocol were fixed in 4% paraformaldehyde, and then permeabilized with 0.1% TritonX-100. Following blocking by 5% BSA. To show DNA damage foci, cells were

immunostained overnight with antibodies against γ H2AX (1:300, Millipore, Billerica, MA) or phospho-DNA-PK (1:300, Abcam, Cambridge, MA). The next day, slides were washed three times with PBS and then incubated with the secondary antibody, washed, mounted and analyzed as above.

Supplemental References:

- S1. Sawada H, Saito T, Nickel NP, Alastalo TP, Glotzbach JP, Chan R, et al. Reduced BMP2 expression induces GM-CSF translation and macrophage recruitment in humans and mice to exacerbate pulmonary hypertension. *J Exp Med*. 2014;211(2):263-80.
- S2. Diebold I, Hennigs JK, Miyagawa K, Li CG, Nickel NP, Kaschwich M, et al. BMP2 preserves mitochondrial function and DNA during reoxygenation to promote endothelial cell survival and reverse pulmonary hypertension. *Cell Metab*. 2015;21(4):596-608.
- S3. Hopper RK, Moonen JR, Diebold I, Cao A, Rhodes CJ, Tojais NF, et al. In pulmonary arterial hypertension, reduced BMP2 promotes endothelial-to-mesenchymal transition via HMGA1 and its target Slug. *Circulation*. 2016;133(18):1783-94.