SUPPLEMENTAL MATERIAL

Upregulation of HERV-K is Linked to Immunity and Inflammation in Pulmonary Arterial Hypertension

Toshie Saito, Kazuya Miyagawa, Shih-Yu Chen, Rasa Tamosiuniene, Lingli Wang, Orr Sharpe, Erik Samayoa, Daisuke Harada, Jan-Renier A. J. Moonen, Aiqin Cao, Pin-I Chen, Jan K. Hennigs, Mingxia Gu, Caiyun G. Li, Ryan D. Leib, Dan Li, Christopher M. Adams, Patricia A. del Rosario, Matthew Bill, Francois Haddad, Jose G. Montoya, William H. Robinson, Wendy J. Fantl, Garry P. Nolan, Roham T. Zamanian, Mark R. Nicolls, Charles Y. Chiu, Maria E. Ariza, Marlene Rabinovitch

Expanded Methods:

Human samples (lung tissue and peripheral blood mononuclear cells, pulmonary artery endothelial cells (PAEC), pulmonary artery smooth muscle cells (PASMC), induced pluripotent stem cells (iPSC), iSPC differentiated into endothelial cells (iPSC-EC) from PAH patients and donor controls

Procurement of the tissues from human subjects was approved by the Administrative Panel on Human Subjects in Medical Research at the PHBI Transplant Procurement Centers (lung tissues) and at Stanford University (for blood) and at Vanderbilt University courtesy of Dr. Eric Austin for some of the fibroblasts used for induced pluripotent stem cells. Written informed consent was received from participants prior to inclusion in the study. Characteristics of PAH patients and healthy controls used in the various studies are provided in Supplemental Tables 1 and 2.

Lungs (PAH and donor controls): Lung tissues from heredity and idiopathic PAH patients and control subjects (unused donor lungs) were provided by the Pulmonary Hypertension Breakthrough Initiative (PHBI), which is funded by NIH/NHLBI and the Cardiovascular Medical Research and Education Fund (CMREF). The tissues were obtained from the PHBI Transplant Procurement Centers at Allegheny Hospital, Pittsburgh, PA, Baylor University, The Cleveland Clinic, Stanford University, University of Alabama at Birmingham, The University of California, San Diego, University of Michigan, and Vanderbilt University and de-identified patient data were obtained via the PHBI Data Coordinating Center at the University of Michigan. The lungs were

processed and stored as described previously[1]. In brief, lung tissues were kept in DMEM media supplemented with antibiotics for up to 24h during transportation from the transplant procurement centers. Small pieces of lung tissues were snap-frozen in liquid nitrogen and stored at -80^{o}C for biochemical experiments or fixed in 10% formaldehyde overnight for immunohistochemistry.

Pulmonary artery endothelial cells (PAEC), pulmonary artery smooth muscle cells (PASMC), induced pluripotent stem cells (iPSC) and induced pluripotent stem cells differentiated into endothelial cells (iPSC-EC): Most of the cells were obtained from PAH and control (unused donor) lungs provided by PHBI network. The cells were harvested using standard protocols previously published[2,3] Some of the fibroblasts used for induced pluripotent stem cells were obtained from Vanderbilt University as described before[2,4].

Peripheral blood mononuclear cells (PBMC): PBMC were isolated from blood of PAH patients, provided by the Stanford Human Immune Monitoring Center (HIMC) Biobank (Director: Dr. Gupta; in collaboration with Dr. Zamanian), and from blood of healthy donors, provided by Dr. Montoya. Additional PBMC for the HERV-K dUTPase experiments (experimental samples and assay controls) were isolated from blood obtained from the Stanford Blood Center.

Cell isolation and culture

PAH patient and control cells were obtained described above. Primary pulmonary arterial endothelial cells (PAEC) were also obtained from PromoCell (Heidelberg, Germany). PAEC were cultured in EC media supplemented with 5% fetal bovine serum (FBS), EC growth supplement and penicillin/streptomycin (ScienCell, Carlsbad, CA) and used at passage 3-6. PASMC were cultured in smooth muscle cell media supplemented with 5% fetal bovine serum (FBS), SMC growth supplement and gentamaicin/amphotericin-B (Lonza, Basel, Switzerland) and used at passage 4-10. iPSC and iPS-EC were cultured as described before[4]. PBMC were separated by Ficoll-Paque (GE Healthcare, Pittsburgh, PA) following centrifugation of whole blood at 400 g for 30 min. PBMC ($1X10^7$) were used for further preparation of enriched monocytes. Cells that attached to wells after two hours represented an enriched monocyte preparation. For detection of HERV-K dUTPase in monocytes by qPCR, we used the Pan Monocyte Isolation kit (Miltenyi Biotec, Auburn, CA), according to the manufacturer's protocol. PBMC were cultured with RPMI-1640 (ATCC, Manassas, VA), supplemented with 10% FBS and penicillin/streptomycin.

HERV-K dUTPase protein purification

Recombinant HERV-K dUTPase protein was provided by Dr. Ariza. The herv-k gene encoding the dUTPase was cloned into the pTrcHis Topo TA expression vector and the sequence verified by DNA sequencing analysis, as previously described[5]. The purity of the protein was assessed by SDS-PAGE and capillary-liquid chromatography nanospray tandem mass spectrometry (nano-LC/MSMS) performed at the Ohio State

University Mass Spectrometry and Proteomics Facility. Purified dUTPase preparations were routinely tested for contaminating DNA, RNA, lipopolysaccharide and peptidoglycan as previously described[5] to ensure high purity.

Treatment of cells with HERV-K dUTPase recombinant protein

PAEC ($1x10^6$), PBMCs ($1x10^6$), or enriched monocytes (isolated from $1x10^7$ PBMCs as described above) were used. Cells were incubated with 0.1, 1.0, or 10 μg/ml of recombinant HERV-K dUTPase for 24 hr. Supernatants were collected and used for cytokine measurement by ELISA, and cell lysates were used for western immunoblotting. PBMC were analyzed by single mass cytometry (CyTOF). PAEC were also assed for apoptosis judged by heightened caspase activity (Caspase-Glo 3/&) (Promega, Madison, WI) during serum withdrawal for 16hrs[2]. PASMC were assed for proliferation (MTT proliferation assay, ATCC, Manassas, VA)[6] in response to HERV-K dUTPase for 48 hr.

Caspase assay

Survival of PAEC was assessed by the Caspase 3/7 assay. In some experiments, IL-6 neutralizing antibody (InvioGen, San Diego, CA) vs. Isotype control (InvioGen, San Diego, CA) was used to assess whether PAEC apoptosis induced by HERV-K was IL-6 dependent.

Monoculture of PAEC: PAEC ($8X10^3$) were seeded in 96-well plate with 5% FBS and allowed to adhere overnight. Cells were incubated with 10 μg/ml of recombinant

HERV-K dUTPase in FBS free media for 16 hr. Cells were assessed with Caspase 3/7 Luciferase Reagent Mix (Promega, Madison, WI), according to the manufacturer's protocol.

Co-culture of PAEC and Monocytes: PAEC ($1X10^5$) were seeded in 12-well plates with 5% FBS and allowed to adhere overnight. Pan Monocyte Isolation kit (Miltenyi Biotec, Auburn, CA) was used to isolated monocytes as described above. Then $5X10^5$ monocytes were pre-treated on a cell culture insert with 10 μg/ml of recombinant HERV-K dUTPase for 2 hr. Then the monocytes were added to the PAEC cultures for 8 hr. After co-culture, the cell culture insert (monocytes) was removed, media of PAEC were changed to serum free media and incubated for 16 hr. Cells were assessed with Caspase 3/7 Luciferase Reagent Mix (Promega, Madison, WI), according to the manufacturer's protocol.

MTT Assay

Proliferation of PASMC was assessed by MTT. PASMC ($8X10^3$) were seeded in 96-well plates with 5% FBS and allowed to adhere overnight. Cells were incubated with 10 μg/ml of recombinant HERV-K dUTPase in 5% FBS media for 48 hr. Cells were assessed with MTT (ATCC, Manassas, VA), according to the manufacturer's protocol.

Treatment with lipopolysaccharide (LPS)

Monocytes and PAEC cultured as described above were treated with 1 μg/ml LPS for 6 hr and cell lysates were used to asses HERV-K dUTPase mRNA by qPCR.

Immunohistochemistry

Lung tissue sections (approx. 1.2 cm x 1.2 cm) were fixed with 10% formaldehyde (Thermo Scientific, Waltham, MA) and embedded in paraffin (Leica Biosystem, Buffalo Grove, IL). After deparaffinization, sections were permeabilized with 0.2% Triton (Sigma Aldrich, St. Louis, MO). Antigen retrieval was done with either citrate buffer pH 6.0 (Sigma Aldrich, St. Louis, MO) or 1 mM EDTA pH 8.0 (Life Technologies, Grand Island, NY), depending on the primary antibodies. Sections were immersed in 0.3% hydrogen peroxide (Sigma Aldrich, St. Louis, MO) to prevent endogenous peroxidase activity, blocked with 5% goat serum (Jackson ImmunoResearch, West Grove, PA) and incubated with primary antibodies overnight at 4^{o}C, or for one hour at room temperature, depending on the primary antibodies. The following primary antibodies were used: SAMHD1 (1:500, Abcam), CD3 (1:50, Dako), CD19 (1:100, Dako), Plasma Cell (1:100 Dako), Follicular Dendritic cell (FDC) (1:50, Dako), IL6 (1:1000, Abcam). After incubation with secondary antibodies for one hour at RT and amplification with streptavidin-biotin (LSAB2 kit, DAKO, Carpinteria, CA), sections were stained with 3,3-diaminobenzidine (DAB) and (LSAB2 kit, DAKO, Carpinteria, CA) counterstained with hematoxylin (Vector Laboratories, Burlingame, CA). Images were acquired using a Leica DMLB microscope (Leica, Buffalo Grove, IL).

Quantification of SAMHD1 staining: Six PAs and associated perivascular regions were randomly chosen for each sample. SAMHD1-immunestained nuclei were evaluated by the ImmunoRatio[7], a publicly available automated web-based image analysis program. We used the program to calculate the percentage of DAB-stained

SAMHD1 positive nuclear area relative to the total nuclear area. The average values of six PAs for each sample are shown.

Quantification of tertiary lymphoid tissue: Tertiary lymphoid tissue was evaluated by H&E and T cell, B cell, plasma cell and follicular dendritic cell markers. The ratio of PAs with tertiary lymphoid tissue relative to the total number of PAs in each sample was calculated and shown as % of total for each patient and each control.

Immunofluorescent staining

Lung sections (approx. 1.2 cm X 1.2 cm) were fixed with 10% formaldehyde (Thermo Scientific, Waltham, MA) and embedded in paraffin (Leica Biosystem, Buffalo Grove, IL). After deparaffinization, sections were permeabilized with 0.2% Triton (Sigma Aldrich, St. Louis, MO). Antigen retrieval was done with either citrate buffer pH 6.0 (Sigma Aldrich, St. Louis, MO) or 1 mM EDTA pH 8.0 (Life Technologies, Grand Island, NY), depending on the primary antibodies. Sections were blocked with 5% goat serum (Jackson ImmunoResearch, West Grove, PA) and incubated with primary antibodies overnight at 4^{o}C, or for one hour at room temperature, depending on the primary antibodies. The following primary antibodies were used: SAMHD1 (1:500, Abcam), VVF (1:1000, Abcam), CD11c (1:100, Abcam), CD68 (1:100, Dako), CD3 (1:50, Dako), HERV-K env (1:1000, Austral), HERV-K dUTPase (1:2000, provided by Dr. Ariza), α-Actin (1:400, Sigma Aldrich), followed by incubation with fluorophore-conjugated secondary antibodies (Alexa Fluor 488 anti mouse and Alexa Fluor 594 anti rabbit) for one hour at RT. When double staining with the primary antibodies from same species

was done, Zenon kit (Life technologies, Grand Island, NY) was used for direct labeling of primary antibodies with fluorophores, according to the manufacturer's protocol. Nuclei were stained with DAPI (Vector Laboratories, Burlingame, CA). Images were acquired using a FlouView 1000 (Olympus, Center Valley, PA) or a Leica TCS SP8 (Leica, Buffalo Grove, IL) confocal microscope.

In situ SAMHD1 antibody production

To localize SAMHD1 antibody producing cells, frozen lung tissues were fixed with acetone (Sigma Aldrich, St. Louis, MO), blocked with 5% normal goat serum (Jackson ImmunoResearch, West Grove, PA) and incubated with GST-tagged SAMHD1 recombinant protein (Novus Biologicals, Littleton, CO) in PBS (20 μg/ml) overnight at 4˚C. Sections were washed with PBS and incubated with FITC-conjugated anti GST antibody (Abcam, 1:800) for one hour at RT. Nuclei were stained with DAPI (Vector Laboratories, Burlingame, CA). Images were acquired using a FlouView 1000 (Olympus, Center Valley, PA) confocal microscope.

Immune complex immunoprecipitation and mass spectrometry to identify target antigens

Immune complexes were captured using a Direct IP kit (Life Technologies, Grand Island, NY), according to the manufacturer's protocol. In brief, 10 μg of C1q (Sigma, St. Louis, MO) was coupled to AminoLink Plus Coupling Resin (Life Technologies, Grand Island, NY). Lung tissue (100mg) was lysed using a dounce homogenizer and 500 μl of lysis buffer (0.025 M Tris HCl pH 7.5, 0.15 M NaCl, 1 mM EDTA, 0.5% NP40,

5% glycerol) with proteinase/phosphatase inhibitors (Life Technologies, Grand Island, NY). After centrifugation at 20,000 g for 20 min at 4˚C, the supernatant was collected. The lysates were incubated with C1q coupled resin overnight at 4˚C. After the incubation, the beads were washed four times and eluted with 50 μl of IgG Elution Buffer (Life Technologies, Grand Island, NY) for 10 min using gentle vortex and neutralizes with 1 M Tris-HCl pH 9.0. The samples were then prepared using filter aided sample preparation (FASP)[8]. In brief, solubilized proteins in a 4% SDS, 0.1 M DTT, 0.1 M Tris-HCl pH 7.8 were exchanged into first an 8 M urea containing buffer, followed by a 50 mM ammonium bicarbonate buffer on an ultracentrifuge filter. Following a buffer exchange, digestion using trypsin was performed on the membrane filter overnight at 37˚C, where peptides were spun out, collected and further cleaned on C18 reverse phase material and analyzed by liquid chromatography (LC) and mass spectrometry (MSMS).

The LC was a NanoLC-2D (Eksigent) where mobile phase A was 99.9% water, 0.1% formic acid and mobile phase B was 99.9% acetonitrile, 0.1% formic acid run at a flow rate of 600 nL/min. The reversed phase C18 column was packed in-house using PEEKE C18 3 μM material to a length of 15 cm where the column inner diameter was 100 μM. The source was a Michrom-Bruker Advance Captive Spray with an electrospray potential of 1.7 kV. The mass spectrometer was a LTQ Orbitrap Velos set in data dependent acquisition (DDA) mode, to isolate and fragment the top 12 most intense multiply charged precursor ions. The raw data were converted to .mgf format and searched by Byonic (Protein Metrics) using typical search conditions and a

1% False Discovery Rate determined using the standard reverse decoy strategy. Byonic output files were further analyzed using custom scripts developed in Matlab to aid in data visualization. We excluded proteins where we did not observe at least 3 peptides in at least one sample. Common contaminants were also excluded. For all other proteins, control and PAH patient data were assed using significance analysis of microarray (SAM) to determine q values with a false discovery rate (FDR) cutoff of 5%[9].

Quantification of SAMHD1 specific Immune complexes

Lung tissue (100 mg) was lysed using a dounce homogenizer and 500 μl of lysis buffer (0.025 M Tris-HCl pH 7.5, 0.15 M NaCl, 1 mM EDTA, 0.5% NP40, 5% glycerol) with proteinase/phosphatase inhibitors (Life Technologies, Grand Island, NY). After centrifugation at 20,000 g for 20 min at 4˚C, total protein concentration of the supernatant was measured and diluted in PBS to 1 μg/μl total protein. ELISA plates were coated with 0.02 mg/mL SAMHD1 antibody (Abcam) in PBS overnight at 4˚C. The plates were blocked with 5% BSA in PBS for one hour. Lung lysate was applied and incubated for two hours. HRP-conjugated species-specific anti human IgG (GE Healthcare, 1:10000) added to the plates, and incubated for one hour at room temperature. Tetramethylbenzidine substrate (R&D, Minneapolis, MN) was added for 15 min, then the reaction was stopped with 2 N sulfuric acid (R&D, Minneapolis, MN), and OD values were determined at 450 nm.

Western immunoblotting

Lung lysates (50 mg) were prepared by homogenization with 500 μl of modified RIPA buffer (50 mM Tris-HCl pH 7.4, 150 mM NaCl, 1 mM EDTA, 1% Triton X-100, 0.1% SDS, 1% Sodium deoxycholate, 1 mM PMSF) containing protease and phosphatase inhibitors (Life Technologies, Grand Island, NY). After centrifugation at 20,000 g for 20 min at 4˚C, the supernatant was collected. Protein concentration was determined by BCA. Equal amounts of protein were loaded on a precast NuPage 4-12% Bis-Tris (LifeTechnologies) gel and subjected to electrophoresis under reducing conditions and electrotransferred onto polyvinylidene difluoride (PVDF) membranes. After blocking with 5% milk in 0.5% Tween-PBS, membranes were incubated with primary antibodies against SAMHD1 (1:500, Abcam), pSTAT3 (1:1000, Invitrogen), STAT3 (1:1000, Invitrogen), β-Actin (1:10000, Santa Cruz Biotechnology), α-Tubulin (1:2000, Sigma Aldrich). Anti mouse IgG secondary antibody (1:5000, Santa Cruz Biotechnology) was used. After incubation with HRP-conjugated secondary antibodies, signals were visualized with ECL or ECL prime (GE Healthcare, Pittsburgh, PA).

Enzyme-linked immunosorbent assay (ELISA)

Cytokine levels in human enriched monocytes, human PAEC, and rat lung lysates were measured using the Quantikine ELISA kit (R&D, Minneapolis, MN) for human TNFα, IL1β and IL6, and for rat IL6 (LSBio, Seattle, WA), according to the manufacturer's protocol.

Quantitative (q)PCR

Total RNA was extracted and purified from lung tissue or cells using spin column based kits (Qiagen, Valencia, CA, or Zymo Research, Irvine, CA). RT-PCR was performed according to the manufacture's protocol (Applied Biosystems, Grand Island, NY). qPCR was performed with a 7900HT Sequence Detection System (Applied Biosystems, Foster, CA) or a CFX384 Real Time System (BioRad, Hercules, CA). Primers used: TaqMan Gene Expression Assays, (β-actin (Hs01060665_g1), HERV-K(II) env (PN4441114, custom probe, Applied Biosystems) and Syber Green assays, HERV-K dUTPase (Forward, 5'-AAATGGGCAACCATTGTCGGGAAACGAGC-3'; Reverse, 5'-TAGTACAT AAATCTACTGCTGCACTGC-3), β-actin (Forward,5'-CATGCCATCCTGCGTCTGGA-3'; Reverse, 5'-CCGTGGCCATCTCTTGCTCG).

Unbiased pan-viral metagenomic next-generation sequencing

A 0.5 cm^3 piece of previously snap frozen lung tissue was placed in a 2 mL tube with RLT Buffer (Qiagen, Hilden, Germany) and 0.1 mm silica beads (MPBiomedicals, Solon OH). Next, the samples were homogenized using an Omni Bead Ruptor (Omni Intl, Kennesaw, GA), and cellular debris was pelleted. Total RNA was extracted using the EZ1 RNA Universal Tissue kit (Qiagen, Hilden, Germany). An on-instrument DNase treatment was performed to minimize any background genomic DNA, followed by enrichment for polyadenylated mRNA using OligoTex (Qiagen, Hilden, Germany). Nucleic acid samples were randomly amplified to generate a cDNA library as previously described[10], and the quality and size distributions of the libraries were examined by 2% agarose gel electrophoresis. Metagenomic sequencing libraries were then generated

using a modified Illumina TruSeq protocol[10]. The BioAnalyzer High-Sensitivity DNA kit (Agilent, Santa Clara, CA) was used to assess library size and the Kapa Universal qPCR kit (Kapa Biosystems, Woburn, MA) was used to quantitate the library concentration. After validation, samples were sequenced on an Illumina MiSeq instrument using 300/200 base pair (bp) paired-end sequencing. Approximately 18.3 million sequencing reads were analyzed using a modified version of SURPI (Sequence-Based Ultra-Rapid Pathogen Identification)[11], a computational pipeline for detection of microbes, including viruses, from next-generation sequencing data. Briefly, after detection of viral sequences aligning to human endogenous retroviruses (HERVs) with an initial run using SURPI, the pipeline was rerun with the following modifications: (1) raw sequencing reads were preprocessed by adapter trimming and low-quality / low-complexity filtering (generating ~1.3 to 3.5 million reads per sample), (2) the human computational subtraction step was skipped to retain human endogenous viral sequences, and (3) preprocessed reads were aligned to the viral portion of the NCBI (National Center for Biotechnology Information) NT database to detect HERV sequences. Viral reads were identified as HERVs using a stringent edit distance requirement of 0 (no mismatches) across 75 bp of sequence. HERV reads were also taxonomically classified to the appropriate rank (family, genus, species, or subspecies/strain) by use of an in-house developed classification algorithm using the SNAP nucleotide aligner (v0.15)[11]. Heat maps were generated using matrix2png[12].

CyTOF (single cell mass cytometry)

Details of the CyTOF method were described previously[13]. In brief, PBMC were stained with metal-conjugated antibodies (listed below) and analyzed with a mass cytometer. A subset of the antibodies was obtained pre-labeled by Fluidigm (San Francisco, CA) and others were conjugated with metals in-house, using MaxPAR antibody conjugation kit (Fluidigm, San Francisco, CA). $1X10^6$ cells were used for each sample. After staining with cisplatin for viability evaluation[14], cells were treated with 002% Saponin (Sigma, St. Louis, MO) in cell staining media (CSM, low barium PBS with 0.5% BSA, 0.02% NaN3), then stained with barcoding dye for 15 min for identification, to enable pooling for processing and measurement as a single multiplexed sample[13]. Cells were stained with surface markers for one hr at room temperature. After permeabilization with methanol for 10 min at 4^oC, cells were stained with intracellular markers for one hr at room temperature, then with DNA intercalator (Fluidigm, South San Francisco, CA) overnight at 4^oC. Cells were washed with CSM twice between individual steps and washed three times with MilliQ water before the samples were analyzed with mass cytometer (CyTOF2, Fluidigm, South San Francisco, CA). Stained cells were analyzed on mass cytometer at event rate of 400-500 cells per second. Data files were concatenated. The data were normalized using NormalizerR2013b and de-barcoded using the Matlab DebarcoderR2013b. Gating was performed in http://nolanlab.cytobank.org[15]. The data were transformed to arcsinh values by taking the inverse hyperbolic sine of the raw data. The arcsinh ratio is the difference between the median arcsinh values of the two samples. For Figure 2D, all the data from PAH and controls are shown as arcsinh ratio

over assay control. For Figure 4F, the data shows HERV-K dUTPase-treated over untreated (control) PBMCs (Stanford Blood Bank).

CyTOF antibodies:

(i) PAH vs. control samples

Isotope	Antigen	Clone	Supplier
Y 89	CD45	HI30	Fluidigm, South San Francisco, CA
Er 170	CD3	UCHT1	Fluidigm, South San Francisco, CA
Nd 142	CD19	HIB19	Fluidigm, South San Francisco, CA
Yb 176	CD7	M-T701	BD Biosciences, San Jose, CA
Nd 143	CD11c	Bu15	Biolegend, San Diego, CA
Gd 160	CD14	M5E2	Biolegend, San Diego, CA
Bi 209	CD16	3G8	Fluidigm, South San Francisco, CA
In 115	HLADR	L243	Trace Sciences, Richmond Hill, Canada
Pr 141	cPARP	F21-852	BD Biosciences, San Jose, CA

(ii) HERV-K dUTPase treatment of PBMC

Isotope	Antigen	Clone	Phosphorylation site	Supplier
In 115	CD45	HI30	N/A	Biolegend, San Diego, CA
Er 170	CD3	UCHT1	N/A	Fluidigm, South San Francisco, CA
Nd 142	CD19	HIB19	N/A	Fluidigm, South San Francisco, CA
Yb 174	HLADR	L243	N/A	Fluidigm, South San Francisco, CA
Dy 162	CD69	FN50	N/A	Fluidigm, South San Francisco, CA
Pr 141	Caspase3	C92-605	N/A	BD Biosciences, San Jose, CA

Isotope	Antigen	Clone	Phosphorylation site	Supplier
Eu 153	pSTAT1	58D6	pY701	Fluidigm, South San Francisco, CA
Gd 156	pSTAT3	4	pY705	BD Biosciences, San Jose, CA
Dy 164	pSTAT5	47	pY694	BD Biosciences, San Jose, CA
Gd 158	pMAPKAPK2	27B7	pT334	Cell Signaling Technology, Danvers, MA
Sm 149	pNFkB	K10-895.12.50	pS529	BD Biosciences, San Jose, CA
Sm 152	pAKT	D9E	pS473	Fluidigm, South San Francisco, CA
Er 167	pERK	D13.14.4E	pT202/Y204	Fluidigm, South San Francisco, CA

N/A, not applicable

Rat model for the induction of pulmonary hypertension by HERV-K dUTPase

The experimental protocol used in this study was approved by the Animal Care Committee at Stanford University following the published guidelines of the National Institutes of Health and the American Physiological Society. Adult male Sprague-Dawley rats (7 wks, 180-200 g) were randomly assigned to a control or treatment group. Rats were either untreated or given a single subcutaneous dose of SU5416 (20 mg/kg body weight) one day prior to the first of three weekly intravenous injections of HERV-K dUTPase (0.2 mg/kg body weight). Rats in the control group were treated with saline vehicle. Twenty-one days after the first HERV-K dUTPase injection, cardiac function, right ventricular systolic pressure and right ventricular hypertrophy were assessed as previously described[1]. In brief, cardiac function was measured by Vivid 7 ultrasound machine (GE Healthcare, Pittsburgh, PA) and 13-MHz linear array transducer. RVSP

was measured by inserting 1.4F Millar catheter (Millar Instruments, Houston, TX) via right jugular vein. Pressure measurements were repeated three times. Data were collected by Power Lab Data Acquisition system (AD Instruments, Colorado Springs, CO) and analyzed by LabChart software (AD Instruments, Colorado Springs, CO). Right ventricular hypertrophy was assessed by the weight ratio of the RV to LV plus septum. Isoflurane anesthesia (1.5%, 1 liter/min oxygen) was used during these procedures. After hemodynamic measurements, the lungs were flushed with saline. Right lungs were snap-frozen in liquid nitrogen and stored at -80^{o}C for ELISA. Left lungs were fixed with 10% formalin for histology. For histology, staining methods are described above. Images were acquired using Leica DMLB microscope (Leica, Buffalo Grove, IL). Quantification of muscularization and arterial number relative to alveoli was conducted in a blinded manner as described below. For IL6 measurement by ELISA, 20 μg of tissues was homogenized per the manufacturer's protocol. After centrifugation, total protein concentration of supernatant was measured and prepared in PBS at 0.5 μg/μL total protein.

Quantification of Immunohistochemistry

Evaluation of muscularized arteries: To evaluate the distal pulmonary arteries (DPAs) at alveolar duct and alveolar wall level, we counted DPAs in six randomly chosen low magnification (200X) fields per rat, in the SMA and vWF double stained immunofluorescent images. The artery was defined as muscularized if a thick double line of SMA staining was observed in multiple areas. The ratio of muscularized DPAs over total DPAs was calculated.

Loss of distal vessels: We counted DPAs as described above and alveoli in six randomly chosen low magnification (200X) fields per rat in Movat stained sections, and calculated the ratio of total counted DPAs over total alveoli.

Statistical Analysis

Data were analyzed using Prism 6.0 (GraphPad Software, La Jolla, CA). Statistical significance was determined by one-way ANOVA followed by Dunnett's test or Tukey's test of multiple comparisons when more than two groups were being compared. When only two groups were compared, we used Student's t-test. For some experiments, as indicated in the figure legends, we applied the Welch or Mann Whitney test depending on the data distribution, i.e., when the distribution was not normal we used the Mann-Whitney and when the variance was unequal by F-test, we used the Welch. A P-value of <0.05 was considered significant. Data are shown as mean $\pm$ SEM or median with interquartile range depending on the test applied. For target identification of immune complexes by mass spectrometry, significance analysis of microarray (SAM) was applied with false discovery rate (FDR) cutoff of 5% [9]. For signaling data by CyTOF, Bonferroni-adjusted P-value ($P=7.14X10^{-3}$) was applied to signaling response with arcsinh ratio $>|0.2|$[13].

Accession Numbers

Next-generation sequencing data with human sequences removed, using BLASTn to the human genome at a low-stringency cutoff of 10^{-5} have been publicly deposited in the NIH Sequence Read Archive (SRA accession number SRP056561).

Supplemental Tables:

Supplemental Table 1: Characteristics of PAH patients

Patient	PAH Diagnosis[a]	Age - Gender	Race	PAP[b] mean (mmHg)	PVR[c] (Wood Units)	6 Min[d] Walk (m)	PAH Medications[e]	Study
PAH-01	HPAH (BMPR2 mutation)	33-F	AA	48	15.57	326	epoprostenol, bosentan, sildenafil, treprostinil	tLTs, IHC, qPCR (lung, PAEC, iPSC, iPSC-EC)
PAH-02	HPAH	47-M	White	62	11.95	282	bosentan, sildenafil, epoprostenol	SAMHD1 IC
PAH-03	IPAH	39-F	Asian	47	N/A	161	epoprostenol, bosentan, ambrisentan, sildenafil	SAMHD1 IC, WB, viral screen
PAH-04	IPAH	58-F	White	50	N/A	206	bosentan, tresprostinil, sildenafil, epoprostenol	tLT, SAMHD1 IC
PAH-05	IPAH	28-F	White	37	6.38	434	sildenafil, epoprostenol, bosentan	tLT, SAMHD1 IC
PAH-06	IPAH	27-F	AA	55	N/A	316	sildenafil, treprostinil (inhaled)	tLT, SAMHD1 IC
PAH-07	IPAH	16-F	White	80	N/A	348	bosentan, ambrisentan, epoprostenol, sildenafil, tresprostinil	tLT
PAH-08	IPAH	41-F	Unknown	43	N/A	335	tresprostinil, bosentan, sildenafil	tLT
PAH-09	IPAH	40-F	White	47	N/A	294	ambrisentan, sildenafil, iloprost, epoprostenol	tLT
PAH-10	IPAH	29-F	AA	41	N/A	339	epoprostenol, ambrisentan, sildenafil	SAMHD1 IC
PAH-11	IPAH	24-M	Asian	48	9.56	356	sildenafil, ambrisentan, epoprostenol, bosentan	SAMHD1 IC
PAH-12	HPAH	56-F	White	75	N/A	372	epoprostenol, bosentan, ambrisentan, sildenafil	SAMHD1 IC, MS

Patient	PAH Diagnosis[a]	Age - Gender	Race	PAP[b] mean (mmHg)	PVR[c] (Wood Units)	6 Min[d] Walk (m)	PAH Medications[e]	Study
PAH-13	IPAH	49-F	White	75	16.76	326	ambrisentan, sildenafil, epoprostenol	tLT, SAMHD1 IC, MS, WB, IHC, viral screen, qPCR (lung)
PAH-14	HPAH (BMPR2 mutation)	27-F	White	69	12.11	360	sildenafil, tresprostinil bosentan iloprost	qPCR (lung, PAEC, PASMC)
PAH-15	IPAH	45-M	White	48	11.08	293	treprostinil bosentan sildenafil epoprostenol	SAMHD1 IC.
PAH-16	HPAH (BMPR2 mutation)	33-F	White	48	9.74	288	bosentan treprostinil sildenafil epoprostenol	SAMHD1 ICs, qPCR (lung, PASMC)
PAH-17	IPAH	15-F	White	102	25.24	387	sildenafil epoprostenol	tLT, qPCR (lung)
PAH-18	HPAH (SMAD9 mutation) +APAH (CHD)	16-F	Asian	92	16.15	512	sildenafil treprostinil bosentan epoprostenol	tLT, SAMHD1 IC, WB, IHC, viral screen, qPCR (lung)
PAH-19	IPAH	25-F	Asian	87	20.96	201	epoprostenol sildenafil bosentan	tLTs, qPCR (lung)
PAH-20	IPAH	55-F	AA	53	12.29	273	sildenafil bosentan epoprostenol	tLTs, SAMHD1 IC, MS, IHC, qPCR (lung)
PAH-21	IPAH	25-M	White	36	N/A	511	epoprostenol sildenafil treprostinil	SAMHD1 IC, WB, qPCR
PAH-22	IPAH	56-F	White	57	11.41	137	sildenafil ambrisentan treprostinil	SAMHD1 IC, qPCR (PAEC, iPSC, iPSC-EC)
PAH-23	IPAH	41-F	White	55	9.84	472	sildenafil bosentan epoprostenol	tLTs, SAMHD1 IC, WB, viral screen, qPCR (lung, PAEC)
PAH-24	IPAH	58-F	White	47	12.66	642	bosentan sildenafil	CyTOF
PAH-25	IPAH	27-F	White	27	5.26	707	tadalafil	CyTOF
PAH-26	IPAH	47-F	AA	47	5.5	344	treprostinil (inhaled) tadalafil	CyTOF

Patient	PAH Diagnosis[a]	Age - Gender	Race	PAP[b] mean (mmHg)	PVR[c] (Wood Units)	6 Min[d] Walk (m)	PAH Medications[e]	Study
PAH-27	IPAH	36-M	Asian	70	12.7	488	treprostinil (inhaled) ambrisentan sildenafil	CyTOF
PAH-28	IPAH	27-F	White	40	7.82	548	treprostinil (inhaled) sildenafil	CyTOF
PAH-29	IPAH	36-M	Other	44	7.17	481	tadalafil	CyTOF
PAH-30	IPAH	29-F	White	53	8.95	590	treprostinil (subcutaneous) tadalafil	CyTOF
PAH-31	IPAH	50-F	White	40	5.99	550	ambrisentan sildenafil	CyTOF
PAH-32	IPAH	46-F	AA	52	10.25	572	None	CyTOF
PAH-33	IPAH	36-F	Asian	33	3.83	594	treprostinil (inhaled) tadalafil	CyTOF
PAH-34	IPAH	46-F	Asian	57	13.69	518	epoprostenol sildenafil	qPCR (monocytes)
PAH-35	IPAH	35-F	White	61	26.96	366	None	qPCR (monocytes)
PAH-36	IPAH	53-F	White	50	12.6	238	None	qPCR (monocytes)
PAH-37	IPAH	20-F	White	33	6.84	675	ambrisentan tadalafil	qPCR (monocytes)
PAH-38	IPAH	46-F	White	58	11.11	232	treprostinil (inhaled) sildenafil	qPCR (monocytes)
PAH-39	IPAH	40-M	White	64	73	420	sildenafil, ambrisentan, treprostinil	qPCR (PAEC, iPSC, iPSC-EC)
PAH-40	IPAH	11-F	White	95	N/A	244	sildenafil, ambrisentan, epoprostenol, treprostinil	qPCR (PASMC)
PAH-41	IPAH	39-F	Unknown	69	14.97	262	sildenafil, bosentan iloprost, epoprostenol	qPCR (PAEC, iPSC, iPSC-EC)
PAH-42	HPAH	37-M	White	77	14.22	309	sildenafil, sitaxsentan, ambrisentan, epoprostenol, Imatinib(investigational medication), treprostinil	qPCR (PASMC)
PAH-43	HPAH (BMPR2 Mutation)	N/A -M	White	N/A	N/A	N/A	N/A	qPCR (iPSC)

Patient	PAH Diagnosis[a]	Age - Gender	Race	PAP[b] mean (mmHg)	PVR[c] (Wood Units)	6 Min[d] Walk (m)	PAH Medications[e]	Study
PAH-44	HPAH (BMPR2 Mutation)	N/A -F	White	N/A	N/A	N/A	N/A	qPCR (iPSC)
PAH-45	HPAH (BMPR2 Mutation)	N/A -F	White	N/A	N/A	N/A	N/A	qPCR (iPSC)

[a] Diagnosis: Pulmonary arterial hypertension (PAH) classification: IPAH, Idiopathic PAH; HPAH, hereditary PAH, known mutation as stated; APAH, Associated PAH; CHD, congenital heart disease

[b] PAP, Mean pulmonary arterial pressure. Data were obtained from catheterization study performed closest to transplantation or blood draw.

[c] PVR, Pulmonary vascular resistance. Data were obtained from catheterization study performed closest to transplantation or blood draw.

[d] 6 Min Walk = distance walked in six minutes. Data were obtained from study performed closest to transplantation or blood draw.

[e] PAH medications: For samples provided by the PAH biobank (CyTOF experiments) we list current medications at the time of blood draw. All others, medications are listed according to total drug exposure during treatment period up to transplantation, not necessarily in combination.

AA, African American

IHC, immunohistochemistry of SAMHD1

MS, complement 1q (C1q) mass spectroscopy

NA, data not available

qPCR (lung), evaluation of HERV-K(II) envelope and HERV-K dUTPase in the lung, by qPCR

qPCR (monocytes, PAEC, PASMC, iPSC, iPSC-EC), evaluation of HERV-K dUTPase in this cell type, by qPCR

SAMHD1 ICs, immunohistochemistry of SAMHD1 in ICs

tLTs, immunohistochemistry to detect tertiary lymphoid tissues

Viral screen, screen for HERV-K viruses

WB, expression of SAMHD1 assayed by western immunoblot

CyTOF, single cell mass cytometry

Supplemental Table 2: Characteristics of Controls (unused donor lungs or healthy volunteers)

For definitions, see Supplemental Table 1.

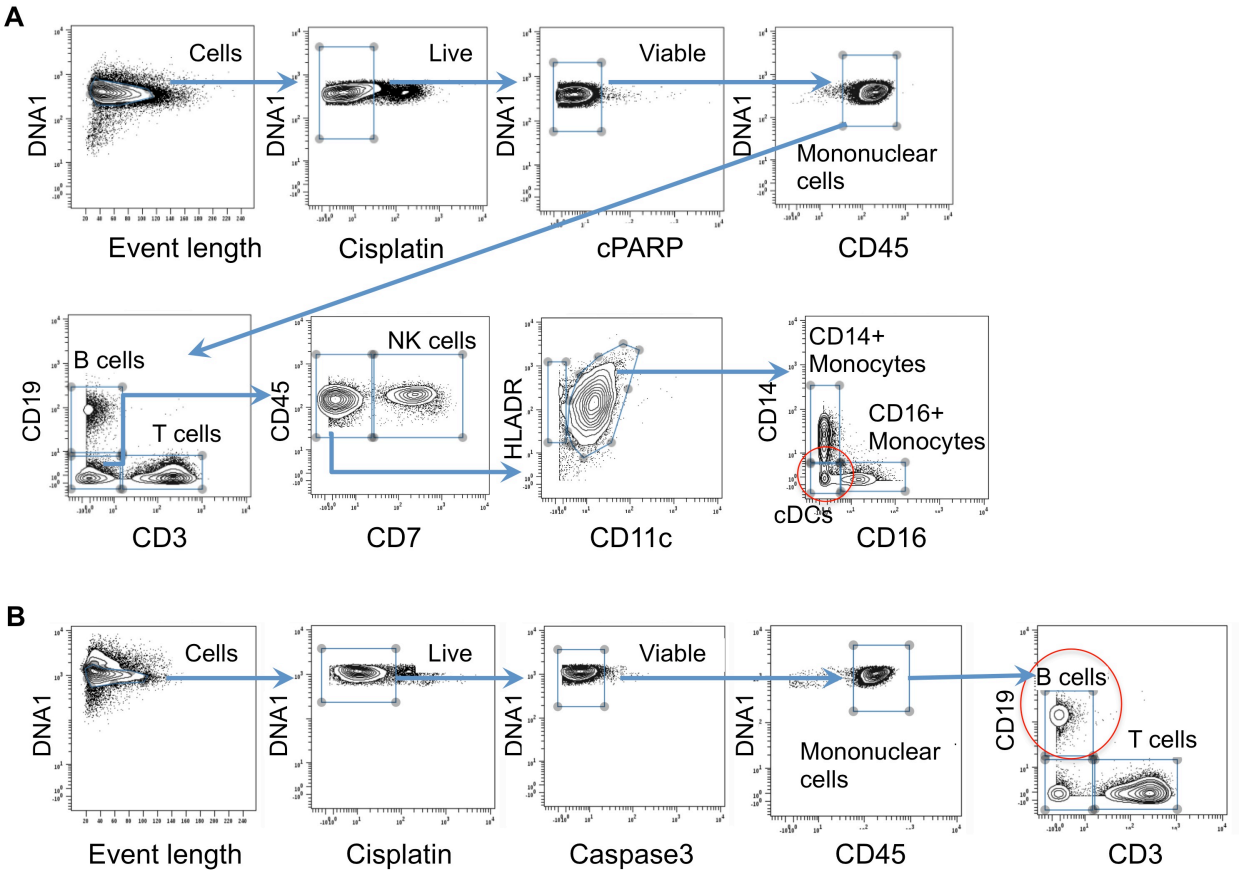
Patient	Age - Gender	Race	Cause of Death	Study
CON-01	18-M	White	Gunshot wound to the head	SAMHD1 IC
CON-02	26-M	White	Cerebrovascular accident/Stroke	tLT, SAMHD1 IC
CON-03	14-M	White	Gun shot wound to head	tLT
CON-04	55-M	White	Anoxia/cardiovascular/ natural causes	SAMHD1 IC, WB
CON-05	14-M	White	Cardiac arrest secondary to diabetic ketoacidosis	tLT
CON-06	54-F	White	Cerebrovascular accident /stroke	qPCR (lung)
CON-07	28-F	White	Anoxia following motor vehicle accident	tLT, SAMHD1 IC, WB, IHC, viral screen, qPCR (lung)
CON-08	62-M	White	Cerebrovascular/ intercranial hemorrhage/stroke	SAMHD1 IC
CON-09	47-M	White	Motor vehicle accident	qPCR (lung, PAEC, iPSC-EC, iPSC)
CON-10	56-F	White	Cerebrovascular accident	tLT, IHC, qPCR (lung)
CON-11	49-F	White	Intracranial hemorrhage	qPCR
CON-12	30-M	White	Head trauma due to motor vehicle accident	tLT, IHC, qPCR (lung)
CON-13	55-F	Unknown	Intracranial stroke/hemorrhage	tLT, qPCR (lung, PAEC)
CON-14	12-M	AA	Brain death/cerebral edema/diabetic ketoacidosis/diabetes-type1	tLT
CON-15	40-F	White	Extensive intracranial injury/subarachnoid hemorrhage secondary to motor vehicle accident	Viral screen
CON-16	45-F	White	Cerebrovascular accident/ subarachnoid hemorrhage	SAMHD1 IC
CON-17	25-M	White	Cerebrovascular/ intracranial hemorrhage/stroke	tLT, IHC, qPCR (lung)

Patient	Age - Gender	Race	Cause of Death	Study
CON-18	60-M	White	Type A aortic dissection with brain death	SAMHD1 IC, WB
CON-19	54-M	White	Choking with anoxic brain injury	SAMHD1 IC
CON-20	41-F	White	Grade 4 subarachnoid hemorrhage, ruptured anterior cerebral artery aneurysm	tLT, SAMHD1 IC, MS, WB, viral screen, qPCR (lung)
CON-21	17-M	White	Head injury progression to brain death secondary to motor vehicle accident	tLT
CON-22	49-M	White	Intracranial hemorrhage	SAMHD1 IC, MS, WB
CON-23	43-M	White	Fatal gun shot to head	tLT, SAMHD1 IC
CON-24	19-M	White	Anoxic brain injury	tLT, MS, viral screen, qPCR (lung)
CON-25	19-M	AA	Anoxia of brain	tLT, SAMHD1 IC
CON-26	52-F	White	Hypoxic brain death	tLT
CON-27	59-F	White	N/A – Healthy volunteer	CyTOF
CON-28	27-F	White	N/A – Healthy volunteer	CyTOF
CON-29	47-F	White	N/A – Healthy volunteer	CyTOF
CON-30	36-M	Asian	N/A – Healthy volunteer	CyTOF
CON-31	28-F	White	N/A – Healthy volunteer	CyTOF
CON-32	36-M	White	N/A – Healthy volunteer	CyTOF
CON-33	46-F	White	N/A – Healthy volunteer	CyTOF
CON-34	51-F	AA & White	N/A – Healthy volunteer	CyTOF
CON-35	30-F	White	N/A – Healthy volunteer	qPCR (monocytes)
CON-36	35-F	Asian	N/A – Healthy volunteer	qPCR (monocytes)
CON-37	52-F	White	N/A – Healthy volunteer	qPCR (monocytes)

Patient	Age - Gender	Race	Cause of Death	Study
CON-38	46-F	White	N/A – Healthy volunteer	qPCR (monocytes)
CON-39	57M	White	N/A – Healthy volunteer	qPCR (monocytes)
CON-40	45-M	White	Anoxia	qPCR (PAEC, iPSC-EC, iPSC)
CON-41	43-F	White	Cerebrovascular/Stroke	qPCR (PAEC, iPSC-EC, iPSC, PASMC)
CON-42	33-F	White	Head trauma. Blunt injury	qPCR (PAEC, iPSC-EC, iPSC)
CON-43	1-M	White	Anoxia/Drowning	MTT (PASMC)
CON-44	46-M	Asian	Cerebrovascular/Stroke	qPCR (PASMC)
CON-45	36-F	White	Subarachnoid hemorrhage	qPCR (PASMC)
CON-46	51-M	White	Cerebrovascular accident	qPCR (PAEC)
CON-47	N/A - M	White	N/A	qPCR (iPSC)
CON-48	N/A – F	White	N/A	qPCR (iPSC)
CON-49	N/A - F	White	N/A	qPCR (iPSC)

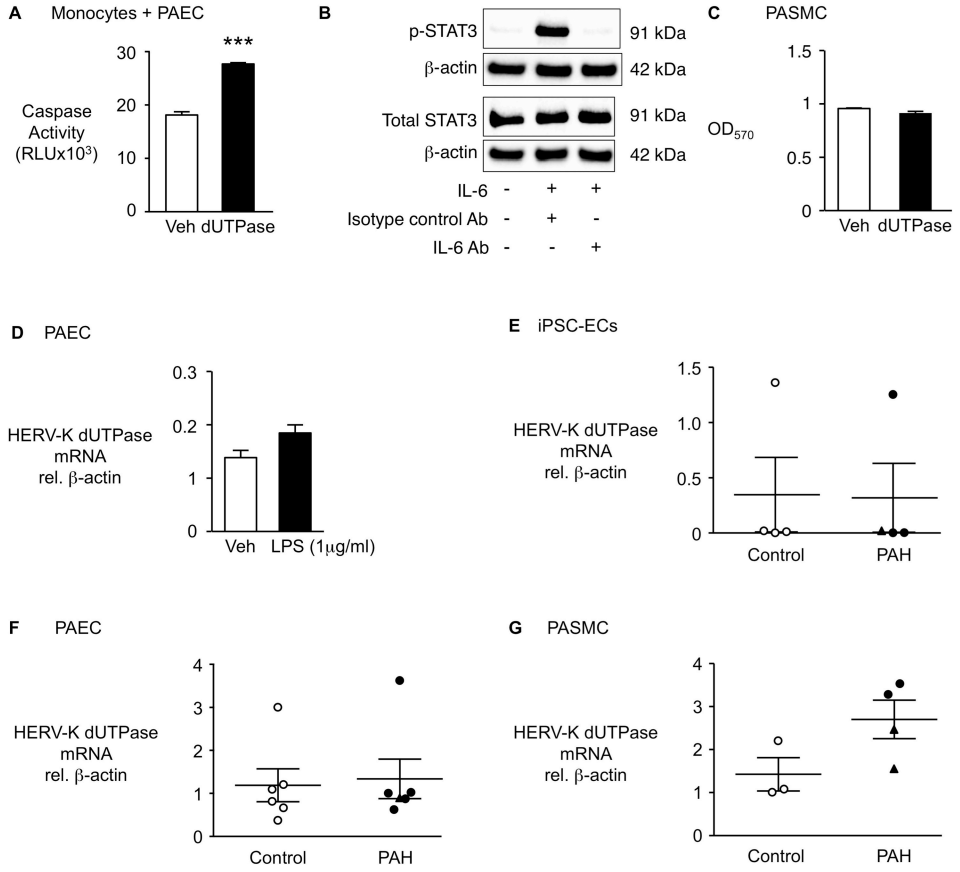
Supplemental Figures and Legends:

Supplemental Figure 1: Gating strategy for classical dendritic cells (cDC) or B-cells of PAH patients and controls, used in the experiments shown in Figures 2D, 4E and 4F.



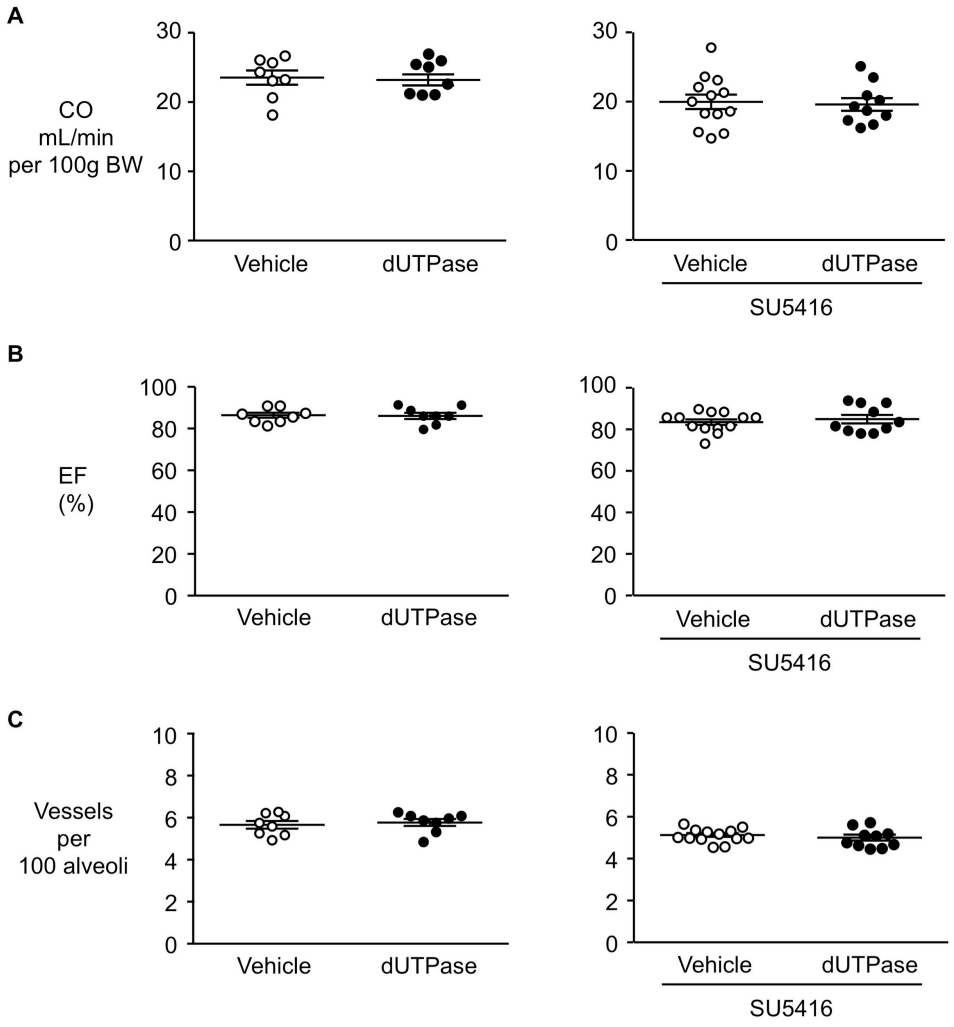
A schematic representation of the gating strategies used to define viable cells and immune cells types. **(A)** Gating for classical dendritic cells (cDC), used in rhe experiment shown in Figure 2D. **(B)** Gating for B-cells, used in the experiments shown in Figure 4E, F. Data are from representative samples. Gates and plots were created using cytobank.org. Live cells were gated by Cisplatin then viable cells were further gated by cPARP (A) or by Caspase 3 (B). NK cells: natural killer cells, cDC: classical dendritic cells, cPARP: cleaved PARP.

Supplemental Figure 2: HERV-K dUTPase and apoptosis in pulmonary arterial endothelial cell (PAEC) and monocyte co-cultures, IL6 antibody efficacy, HERV-K dUTPase and PASMC proliferation, lipopolysaccharide (LPS) and HERV-K dUTPase mRNA in PAEC, and in PAH vs. control iPSC-EC, PAEC and PASMC.



(A) Monocytes were treated with 10 μg/mL HERV-K dUTPase on a cell culture insert and then co-cultured with PAEC subjected to serum withdrawal media overnight (0% FBS). Apoptosis was assessed by Caspase-Glo 3/7 assay (n=3). **(B)** Corresponding to Figure 5B, the efficiency of neutralizing IL6 antibody was confirmed by blocking pSTAT3, assessed by immunoblot. **(C)** PASMC were treated with 10 μg/mL HERV-K dUTPase and proliferation was assessed by MTT assay (n=4) at 48 hr. **(D)** HERV-K dUTPase mRNA by qPCR in 1 μg/mL LPS-treated PAEC (n=3). **(E)** HERV-K dUTPase mRNA by qPCR in iPSC-EC from PAH patients (n=4) and controls (n=4). **(F)** HERV-K dUTPase mRNA by qPCR in PAEC from PAH patients (n=6) and controls (n=6). **(G)** HERV-K dUTPase mRNA by qPCR in PASMC from PAH patients (n=4) and controls (n=3). Ranges represent mean ± SEM (A, C-G). ***P<0.001 by Student's t-test. Closed symbols (PAH), open symbols (Controls), closed triangles hereditary PAH (HPAH).

Supplemental Figure 3: HERV-K dUTPase does not affect left ventricular function or the number of pulmonary vessels in a rat model of pulmonary hypertension.



Rats were pre-treated with or without the VEGF receptor blocker SU5416 then subsequently given three weekly injections of HERV-K dUTPase or vehicle (saline). Saline (n=8), HERV-K dUTPase (n=8), SU5416+Saline (n=13) or SU5416+HERV-K dUTPase (n=10) as described for Figure 6. **(A)** Cardiac output (CO) and **(B)** ejection fraction (EF) were evaluated by echocardiogram. **(C)** Vessels and alveoli were counted in Movat-stained lung sections from the rats. Ranges represent mean ± SEM. We found no significant difference between the groups by Student's t-test.

Supplemental Material References

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