Supplemental Figure 1

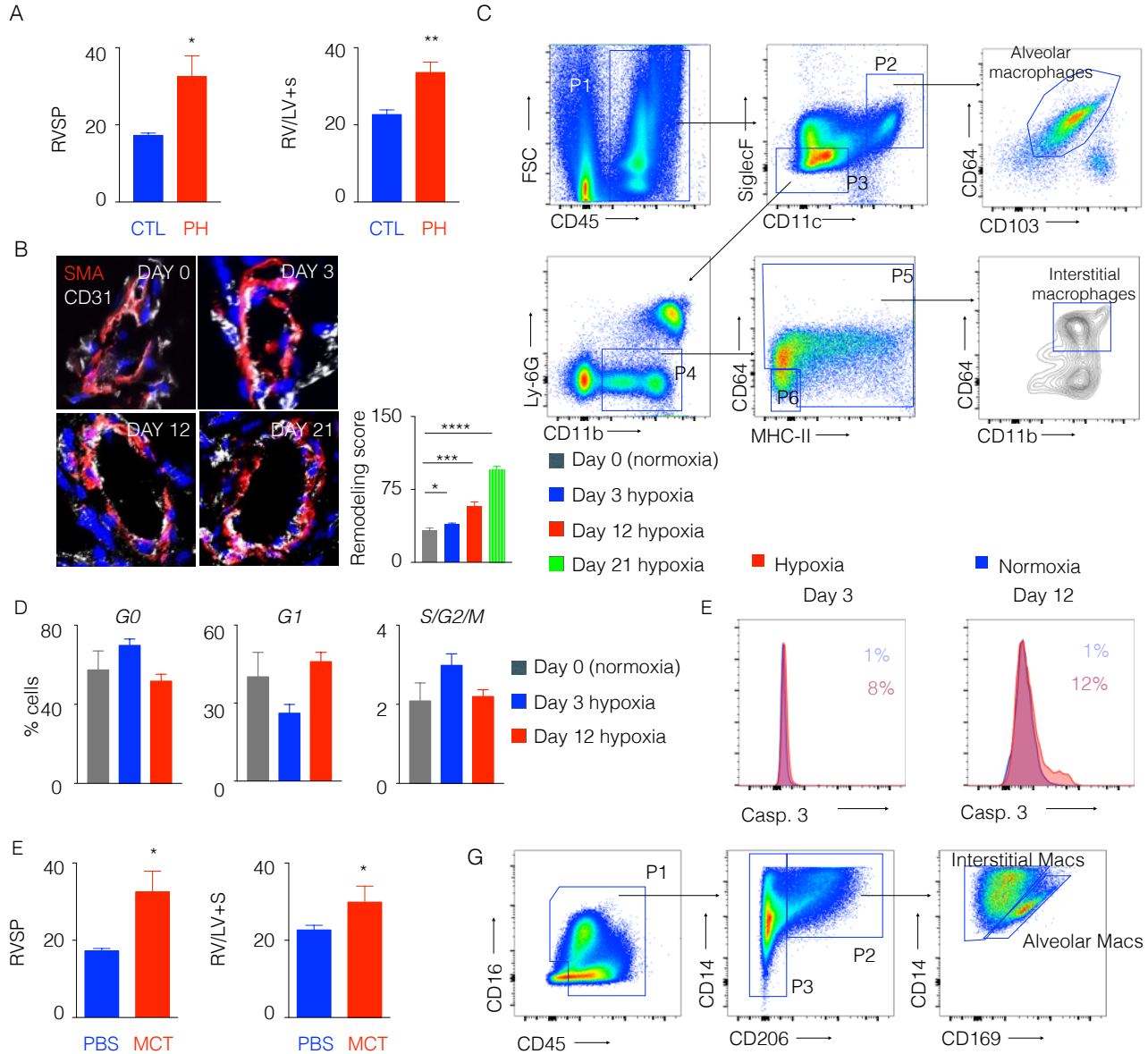


Figure S1. Flow cytometric gating strategy for mouse and human lung macrophages: A) Histograms showing right ventricular systolic pressure (RVSP) and right ventricular remodeling (Fulton index, RV/LV+S of hypoxic mice compared to normoxic mice (CTL). **B)** Representative confocal images showing vasculature remodeling of mice at different time points of hypoxia. Bar graphs showing the quantification of the remodeling. **C)** Flow cytometric plots showing the gating strategy for mouse alveolar (Siglec F^+, $CD11c^+/CD64^+/CD103^+$) and interstitial macrophages ($CD11c^-/CD64^+/CD11b^+$). **D)** The bar graphs show the frequency of alveolar macrophages at different stages of the cell cycle. **E)** Histograms showing the percentage of caspase 3^+ alveolar macrophages in normoxic compared to hypoxic mice. **F)** Bar graphs showing RVSP and RV/LV+S of rat injected with PBS (CTL) and monocrotaline (PH). **G)** Flow cytometric plots showing the gating strategy for human alveolar ($CD169^+/CD14^{int}$) and interstitial ($CD169^{int}/CD14^+$) macrophages. n=4, Mean ± s.e.m., * $P < 0.05$, ** $P < 0.01$.

Supplemental Figure 2

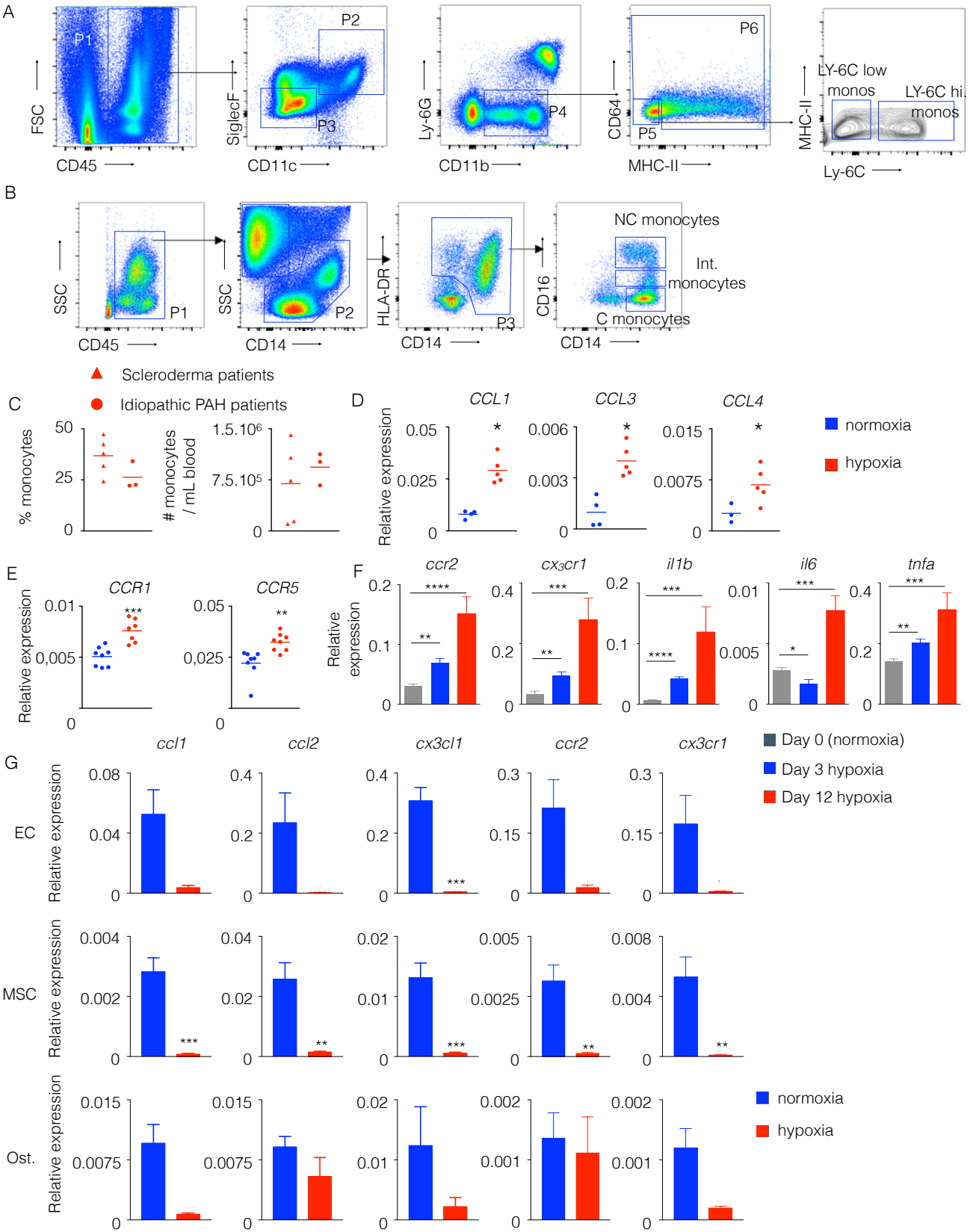
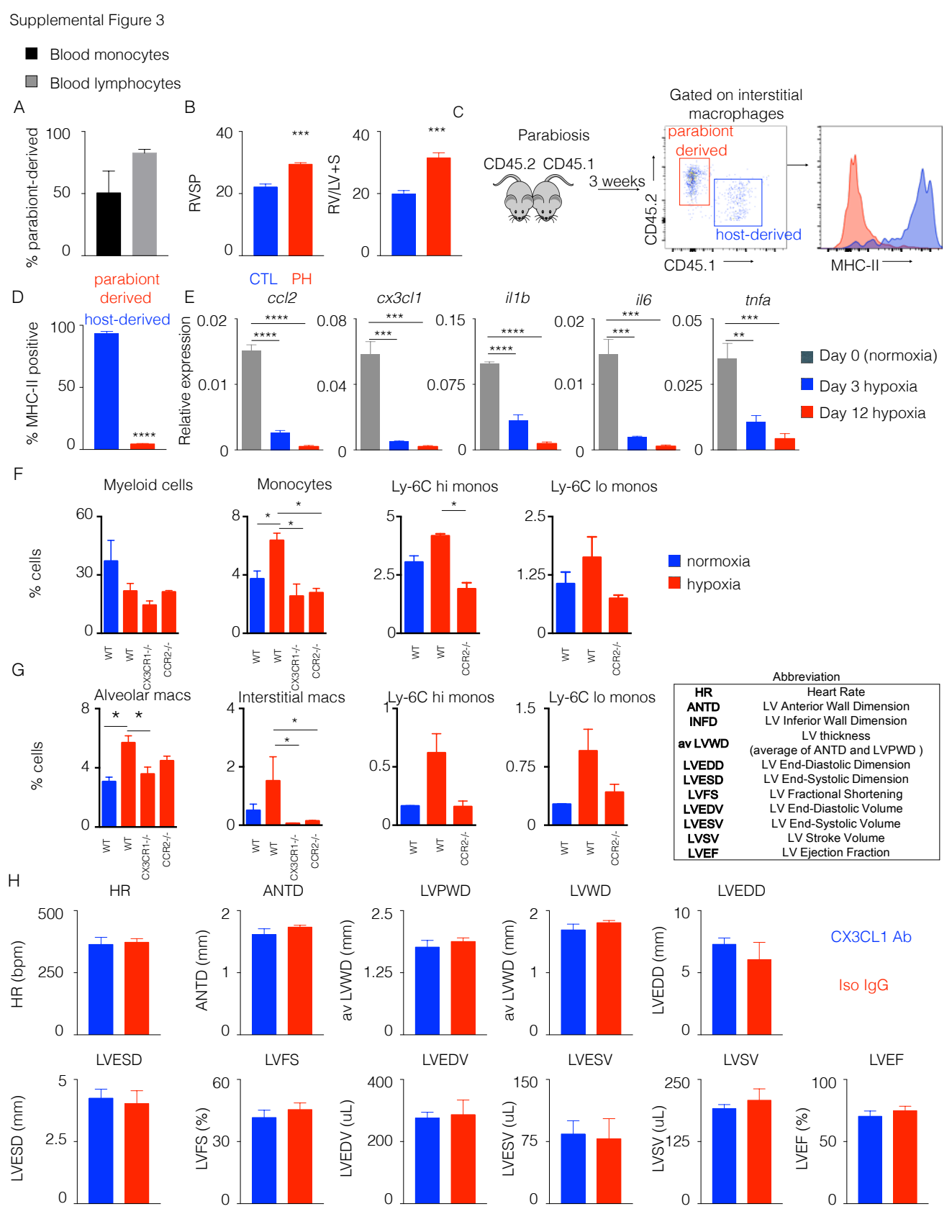


Figure S2. Gating strategy for mouse and human monocytes, and chemokine and chemokine receptor expression in control and PH patients: A) Flow cytometric plots showing the gating strategy for mouse Ly-6C^{low} and Ly-6C^{high} monocytes. **B**) Flow cytometric plots showing the gating strategy for human classical (CD14^{high}/CD16^{-}), intermediate (CD14^{high}/CD16^{int}) and non-classical monocytes (CD14^{int}/CD16^{high}). **C**) Scatter plots showing the difference in monocyte frequency and number in scleroderma compared to idiopathic PAH patients. **D**) Quantitation of CCL1, CCL3 and CCL4 expression in the lungs of PAH patients and healthy controls. **E)** Quantitation of the expression of chemokine receptors such as CCR1 and CCR5 in monocytes. The monocytes were sorted from the blood of PAH patients and healthy controls. n=9. **F)** Quantitation of the expression of the chemokine receptors and pro-inflammatory cytokines in isolated blood monocytes in normoxic and hypoxic mice. n=5 per group. **G)** Quantitation of the expression of various chemokines and chemokine receptors in endothelial cells (EC), mesenchymal stem cells (MSC) and osteoblasts (Ost.) isolated from the bone marrow of normoxic and hypoxic mice. n=5 per group, Mean ± s.e.m., * $P < 0.05$, ** $P < 0.01$, *** $P< 0.001$.

Supplemental Figure 3



Abbreviation	
HR	Heart Rate
ANTD	LV Anterior Wall Dimension
INFD	LV Inferior Wall Dimension
av LVWD	LV thickness (average of ANTD and LVPWD)
LVEDD	LV End-Diastolic Dimension
LVESD	LV End-Systolic Dimension
LVFS	LV Fractional Shortening
LVEDV	LV End-Diastolic Volume
LVESV	LV End-Systolic Volume
LVSV	LV Stroke Volume
LVEF	LV Ejection Fraction

Figure S3. Chimera levels at steady state and frequency of cells in the blood and lungs of CCR2 KO and CX_3CR1 KO hypoxic mice:

A) Histogram showing the percentage of parabiont-derived cells in blood lymphocytes and monocytes 3 weeks after parabiosis surgery in normoxic mice. n=4 Mean ± s.e.m. **B)** Histograms showing RVSP and RV/LV of normoxic (CTL) or hypoxic (PH) parabionts. n=6 per group. **C)** Flowchart depicting the parabiosis experiment **D)** Histogram showing the percentage of parabiont-derived and host-derived interstitial macrophages that are MHC-II^+. **E**) mRNA quantitation of gene expression of chemokines and pro-inflammatory cytokines in resident interstitial macrophages sorted from normoxic and hypoxic mice. n=5 per group. **F**) Histograms showing the frequency of myeloid cells, monocytes, and Ly-$6C^{high}$ and Ly-$6C^{low}$ monocytes in the blood of WT, $CX_3CR1^{-/-}$ and $CCR2^{-/-}$ mice in hypoxia and normoxia. **G)** Histograms showing the frequency of alveolar macrophages, interstitial macrophages, and Ly-$6C^{high}$ and Ly-$6C^{low}$ monocytes in the lungs of WT, $CX_3CR1^{-/-}$ and $CCR2^{-/-}$ mice in hypoxia and normoxia. n=6 per group. **H)** Echo data of monocrotaline injected rats treated with Cx3cl1 neutralizing antibody and isotype control antibody: Histograms showing heart rate (HR), LV anterior wall dimension (ANTD), LV inferior wall dimension (INFD), LV thickness (average of ANTD and LVPWD, avLVWD), LV end-diastolic dimension (LVEDD), LV end-systolic dimension (LVESD), LV fractional shortening (LVFS), LV end-diastolic volume (LVEDV), LV end-systolic volume (LVESV), LV stroke volume (LVSV) and LV ejection fraction (LVEF), n=5 per group. Mean ± s.e.m., * $P < 0.05$, ** $P < 0.01$, *** $P< 0.001$

Supplemental Table 1

A

Patient (Lung)	Age (years)	Gender (M/F)	PAH	mPAP (mm Hg)	PCWP (mm Hg)	PVR (WU)
PH1	41	M	Scleroderma	29	3	6.24
PH2	20	M	Idiopathic	69	12	19.52
PH3	50	F	Scleroderma	43	5	5.06
PH4	16	M	Idiopathic	62	<15	>3
PH5	19	M	Idiopathic	48	<15	>3
PH6	20	F	Idiopathic	55	<15	>3
PH7	53	F	Scleroderma	44	5	5.14
PH8	60	F	Scleroderma	45	14-16	5.5
PH9	44	F	Scleroderma	27	4	3.1
PH10	72	F	Idiopathic	42	6	12
PH11	23	F	Idiopathic	44	5	8
PH12	35	F	Congenital heart disease	41	7	5
PH13	78	F	Scleroderma	41	14	4.4
PH14	52	F	Scleroderma	42	10	7.9
PH15	40	F	Idiopathic	52	11	8.3

B

Patient	Age (years)	Gender (M/F)	Race	Smoking
Healthy control 1	32	M	White	Quit
Healthy control 2	31	F	White	<1/2 pack/day
Healthy control 3	34	M	Asian/Pacific Islander	1-5 cigarettes/week
Healthy control 4	53	F	White	Quit
Healthy control 5	36	M	Asian/Pacific Islander	Never
Healthy control 6	44	M	Asian/Pacific Islander	Never
Healthy control 7	32	F	African American	Never
Healthy control 8	20	M	Asian/Pacific Islander	Never
Healthy control 9	52	F	Asian/Pacific Islander	Never

Table S1. A. Demographics of PAH patients whose lungs and blood were used for study. Lung tissue analysis: Three PAH patients, 2 males and 1 female (PH1-3), were prospectively enrolled at the time of death or transplant for flow cytometric analysis of lung tissue. Paraffin-embedded lung tissue from those patients and 3 other PAH subjects (PH4-6) was further analyzed by *in situ* staining. **Blood analysis:** Peripheral venous blood from nine additional PAH patients (PH7-15) prospectively enrolled in this study and **B.** Peripheral venous blood from nine healthy donors was collected (Healthy donors: Mean age 46.4 $\pm$ 6.01 SEM, 86% female. PH patients: Mean age 47.7 $\pm$ 6.1 SEM). Hemodynamic parameters are listed: Pulmonary capillary wedge pressure (PCW), mean pulmonary arterial pressure (mPAP), and pulmonary vascular resistance (PVR) are displayed.