

## SUPPLEMENTAL APPENDIX

### SCUBE1 Controls BMPR2-Relevant Pulmonary Endothelial Function: Implications for Diagnostic Marker Development in Pulmonary Arterial Hypertension

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**Table S1. PH, COPD and ALI patient demographics for plasma samples.**

<b>Cohort</b>	<b>Non-diseased Controls</b>	<b>Group 1 PAH</b>	<b>Group 2 PH</b>	<b>COPD</b>	<b>ALI</b>
n	56	62	16	39	39
Age (Mean±SD, years)	47.4±17.6	58.4±13.6	64.9±11.1	63.5±9.8	51.9±15.9
Gender (n and % female)	37(66.1%)	48(77.4%)	9(56.3%)	14(35.9%)	18(46.2%)
Race (n and % white)	38(67.9%)	56(90.3%)	14(87.5%)	39(100%)	38(97.4%)

PH: pulmonary hypertension; PAH: pulmonary arterial hypertension; COPD: chronic obstructive pulmonary disease; ALI: acute lung injury.

**Table S2. Patient hemodynamic parameters by PH classification.** Hemodynamic parameters are shown as median with 25th and 75th (Q1-Q3) interquartile range. P-values were calculated by Mann-Whitney nonparametric test.

<b>PH Group</b>	<b>Group 1 PAH</b>	<b>Group 2 PH</b>	<b>P-Value</b>
n	62	16	
mean PAP (mmHg)	42.0 (32.8-53.0)	42.5 (37.5-52.3)	0.521
PCWP (mmHg)	10.0 (9.0-12.0)	22.5 (17.0-27.0)	<0.001
CO (Fick, L/min)	5.3 (4.5-6.8)	5.8 (5.1-7.9)	0.193
PVR (WU)	5.2 (3.8-7.1)	3.1 (1.9-4.4)	<0.001

PH: pulmonary hypertension; PAH: pulmonary arterial hypertension; PAP: pulmonary artery pressure; PCWP: pulmonary capillary wedge pressure; CO: cardiac output; PVR: pulmonary vascular resistance; WU: Wood units.

**Table S3. Demographics and hemodynamic parameters of Group 1 PAH patients at time of blood draw.**

<b>Etiology</b>	<b>Age</b>	<b>Gender</b>	<b>Race</b>	<b>mean PAP (mmHg)</b>	<b>PVR (WU)</b>
Idiopathic	39	Female	White	45	3.8
Portal hypertension	63	Male	White	56	9.1
Idiopathic	59	Female	White	36	4
Scleroderma	70	Female	White	44	9.4
Congenital heart disease	40	Male	White	31	5.2
Scleroderma	72	Female	White	27	5.2
Scleroderma	73	Female	White	41	6.4
Scleroderma	65	Female	White	32	3.8
Idiopathic	57	Male	White	53	9.1
Idiopathic	26	Female	White	34	5.4
Scleroderma	73	Female	White	32	3.8
Scleroderma	37	Male	White	37	3.4
Dermatomyositis	68	Male	White	35	5.3
Idiopathic	43	Female	White	52	11.2
Idiopathic	36	Female	White	36	7
HIV	48	Male	White	72	16
Scleroderma	71	Female	White	33	4.8
Idiopathic	53	Male	White	27	4.7
Idiopathic	67	Male	White	54	6.6
Scleroderma	64	Female	White	46	6.6
Scleroderma	72	Female	White	26	2.5*
Idiopathic	75	Female	White	42	3.1
Scleroderma	43	Female	Black	27	2.7*
Scleroderma	75	Female	White	51	6.4
Scleroderma	73	Female	White	57	8.5
Idiopathic	66	Female	White	54	6.9
Scleroderma	76	Female	Black	39	3.2
Idiopathic	70	Female	White	29	4.3
Idiopathic	76	Female	White	42	11
Scleroderma	86	Female	White	42	8.4
Congenital heart disease	36	Female	White	58	7.5
Scleroderma	57	Female	White	27	2.5*
Systemic sclerosis	73	Female	White	38	5
Idiopathic	53	Female	White	56	13
Scleroderma	58	Female	White	53	6.8
Scleroderma	65	Female	White	47	6.9
Scleroderma	47	Female	White	32	2.2*

Idiopathic	27	Female	White	60	4.9
Congenital heart disease	39	Female	White	40	4.2
Connective tissue disease	83	Female	White	41	5
Idiopathic	58	Female	White	53	5.5
Idiopathic	47	Male	White	52	11.7
Idiopathic	53	Female	White	54	5.1
Connective tissue disease	57	Female	White	65	5.7
Idiopathic	73	Female	White	28	3.6
Idiopathic	41	Male	White	51	2.9*
Connective tissue disease	61	Female	White	25	4.1
Idiopathic	55	Female	Black	44	3.4
Connective tissue disease	58	Female	White	53	3.6
Idiopathic	58	Male	White	61	4.2
Connective tissue disease	58	Male	White	43	3
Idiopathic	55	Female	White	50	6.5
Scleroderma	62	Male	White	33	4.5
Scleroderma	61	Male	White	25	3.1
Scleroderma	56	Female	White	38	5
Scleroderma	51	Female	Black	39	9.2
Idiopathic	59	Female	White	56	13.1
Scleroderma	50	Female	White	23*	1.5*
Idiopathic	71	Female	White	45	7.7
Congenital heart disease	43	Female	White	53	5.6
Connective tissue disease	55	Female	White	52	7.5
Idiopathic	63	Female	White	20*	3.8

\*: The diagnosis was made based on prior invasive hemodynamics measurement, which fulfilled the criteria for Group 1 PAH.

PAP: pulmonary artery pressure; PVR: pulmonary vascular resistance; WU: Wood units.

**Table S4. Patient demographics for lung tissue donors.**

<b>Cohort</b>	<b>Non-diseased controls</b>	<b>PAH</b>	<b>COPD</b>
n	11	8	20
Age (Mean±SD, years)	46.6±12.8	52.8±16.7	62.1±9.6
Gender (n and % female)	5(45.5%)	7(87.5%)	8(40.0%)
Race (n and % white)	8(72.7%)	7(87.5%)	17(85.0%)

PAH: pulmonary arterial hypertension; COPD: chronic obstructive pulmonary disease.

**Table S5. CAD patient demographics for serum samples.**

<b>Cohort</b>	<b>Non-CAD controls</b>	<b>CAD</b>
n	22	21
Age (Mean±SD, years)	60.8±9.9	61.3±11.0
Gender (n and % female)	8 (26.7%)	6 (28.6%)
Race (n and % white)	19 (86.4%)	20 (95.2%)

CAD: coronary artery disease.

**Table S6. Patient demographics for heart tissue donors.**

<b>Cohort</b>	<b>Non-diseased controls</b>	<b>ICM</b>	<b>NICM</b>
n	12	12	12
Age (Mean±SD)	52.6±10.6	61.4±8.3	49.3±10.0
Gender (n and % female)	2(16.7%)	0(0.0%)	6(50.0%)
Race (n and % white)	9(75.0%)	11(91.7%)	8(66.7%)

ICM: ischemic cardiomyopathy; NICM: non-ischemic cardiomyopathy.



**Table S7. Summary of statistics for ROC analysis between PAH and a combined non-PAH cohort composed of control, COPD, and ALI subjects.**

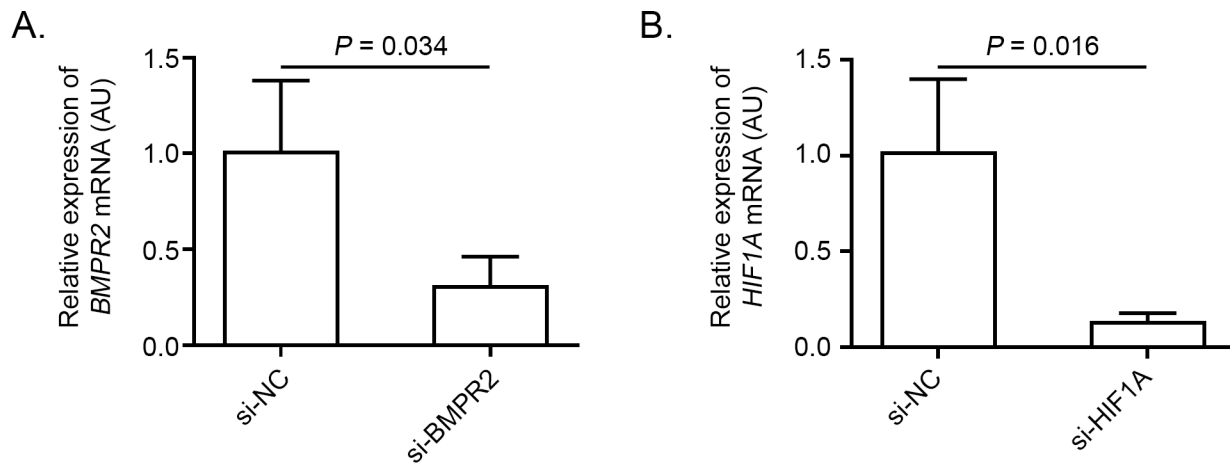
AUC area (C-statistics)	0.75
Standard error	0.035
95% confidence interval	0.681-0.817
P value of AUC area	<0.001
Sensitivity of optimal SCUBE1 cut point 5.46 ng/mL	0.53
95% confidence interval	0.45-0.61
Sensitivity of optimal SCUBE1 cut point 5.46 ng/mL	0.87
95% confidence interval	0.77-0.93
Likelihood ratio	4.11

**Table S8. Summary of statistics for ROC analysis between WSPH Group 1 PAH and Group 2 PH cohorts.**

AUC area (C-statistics)	0.68
Standard error	0.085
95% confidence interval	0.515-0.846
P value of AUC area	0.027
Sensitivity of optimal SCUBE1 cut point 5.01 ng/mL	0.50
95% confidence interval	0.28-0.72
Sensitivity of optimal SCUBE1 cut point 5.01 ng/mL	0.82
95% confidence interval	0.71-0.90
Likelihood ratio	2.82

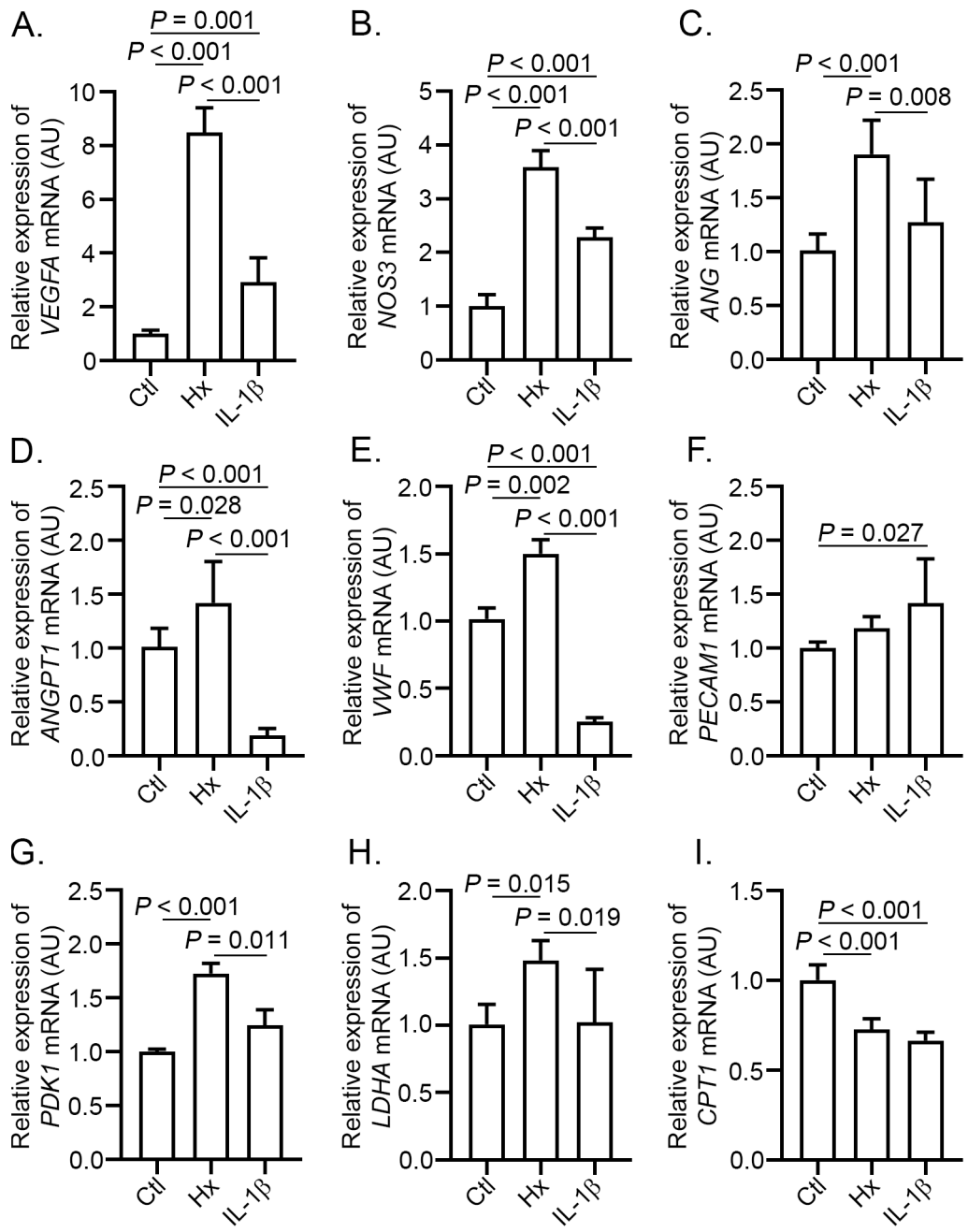
Supplemental Figure S1: Efficacy of siRNA knockdown for BMPR2 and HIF1A genes.

Cultured PAECs were incubated with siRNA targeting BMPR2 or HIF1A, and control scrambled RNAs were used as controls. (A,B) BMPR2 (A) or HIF1A (B) mRNA expression levels were determined by RT-qPCR. The data were derived from 3 independent experiments. Data are presented as mean  $\pm$  SD. The p values were calculated by unpaired 2-sided *t*-test. AU: arbitrary units.



Supplemental Figure S2: Expression profile of major endothelial function regulating genes in PAECs treated with hypoxia or IL-1 $\beta$  exposure.

Cultured PAECs were treated with hypoxia or IL-1 $\beta$  for 48 hours and mRNA (N = 6 samples per group) were quantified by RT-qPCR. The relative change in mRNA expression of angiogenesis, proliferation and apoptosis-related genes *VEGF*, *NOS3*, *ANG*, *ANGPT1* (A–D), adhesion molecule genes *vWF* and *VECAMI* (E-F), and endothelial metabolism-related genes *PDK1*, *LDHA*, *CPT1* (G–I) were profiled. Data are presented as mean  $\pm$  SD. The p values were calculated by 1-way ANOVA with post hoc Bonferroni test. The comparisons with  $p > 0.05$  were not explicitly stated in the panels.



Supplemental Figure S3: Correlation analysis of plasma SCUBE1 with left and right heart filling pressure and cardiac output in WSPH Group 1 PAH patients.

(A,B) Plasma was collected from patients with WSPH Group 1 at the time of right heart catheterization (RHC). Plasma SCUBE1 levels were measured by ELISA. From 62 Group 1 PAH patients where these specific catheterization indices were available, no significant correlation was found between plasma SCUBE1 levels with pulmonary capillary wedge pressure (PWP; A) and with cardiac index (B). The p values were calculated from Spearman correlation (rho: correlation coefficient).

