

# **SUPPLEMENTAL MATERIAL**

**Table S1. Detailed characteristics of human pulmonary arteries used in this study.**

#	Category	Origin	Diagnosis	Age	Sex	WHO class	mPAP	CO	PVR
1	CTRL	Autopsy	Sudden death	43	Female	NA	NA	NA	NA
2	CTRL	Autopsy	Sudden death	49	Female	NA	NA	NA	NA
3	CTRL	Biopsy	Adenocarcinoma	65	Male	NA	NA	NA	NA
4	CTRL	Biopsy	Adenocarcinoma	79	Female	NA	NA	NA	NA
5	CTRL	Biopsy	Squamous cell carcinoma	65	Female	NA	NA	NA	NA
6	CTRL	Biopsy	Adenocarcinoma + Solitary fibrous tumor	54	Female	NA	NA	NA	NA
7	CTRL	Biopsy	Adenocarcinoma	77	Male	NA	NA	NA	NA
8	CTRL	Biopsy	Squamous cell carcinoma	76	Male	NA	NA	NA	NA
9	CTRL	Autopsy	Sudden death	52	Male	NA	NA	NA	NA
10	CTRL	Autopsy	Sudden death	29	Male	NA	NA	NA	NA
11	CTRL	Autopsy	Sudden death	65	Male	NA	NA	NA	NA
12	CTRL	Autopsy	Sudden death	31	Male	NA	NA	NA	NA
13	CTRL	Biopsy	Adenocarcinoma	74	Female	NA	NA	NA	NA
14	CTRL	Autopsy	Coronary artery disease	78	Male	NA	NA	NA	NA
15	CTRL	Autopsy	Sudden death	48	Female	NA	NA	NA	NA
16	PAH	Biopsy	PAH-SSc	36	Female	III	50	4,4	799
17	PAH	Autopsy	PAH-SSc	54	Female	IV	48	3,7	735
18	PAH	Biopsy	iPAH	52	Male	IV	51	5,7	575
19	PAH	Autopsy	PAH-SSc	77	Male	III	47	5,4	563
20	PAH	Autopsy	PAH-SSc	47	Female	III	46	3,9	NA
21	PAH	Autopsy	iPAH	74	Female	IV	35	4,1	NA
22	PAH	Autopsy	PAH-SSc	72	Female	IV	44	3	826
23	PAH	Autopsy	PoPH	46	Female	IV	NA	NA	NA
24	PAH	Autopsy	PAH-SSc	53	Female	IV	68	5	864
25	PAH	Autopsy	Heritable PAH	61	Female	III	66	4,5	1012
26	PAH	Biopsy	iPAH	59	Male	IV	66	6,47	630
27	PAH	Autopsy	iPAH	65	Male	III	76	4,9	1125
28	PAH	Biopsy	PAH-SSc and PVODPAH	45	Female	IV	43	7,25	375
29	PAH	Autopsy	iPAH	52	Male	IV	51	2,97	NA
30	PAH	Autopsy	PVOD-PAH	72	Female	IV	61	3,36	630

CTRL: control; mPAP, mean pulmonary artery pressure; CO: cardiac output; WHO class: World Health Organisation functional class assessed at the time of last clinic visit; iPAH: idiopathic PAH; SScPAH: PAH associated with scleroderma; PVR: pulmonary vascular resistance; PVOD: pulmonary veno-occlusive disease.

**Table S2. Clinical information of human right ventricular samples used in this study.**

#	Category	Origin	Diagnosis	Age	Sex	WHO/NYHA class	LVEF (%)	RVSP (mmHg)	TAPSE (mm)	CI (L/min/m <sup>2</sup> )	Interval between last CI recorded and tissue sampling (days)
1	CTRL	Autopsy	Sudden death	43	Female	NA	NA	NA	NA	NA	NA
2	CTRL	Autopsy	Sudden death	52	Male	NA	NA	NA	NA	NA	NA
3	CTRL	Autopsy	Sudden death	29	Male	NA	NA	NA	NA	NA	NA
4	CTRL	Autopsy	Sudden death	65	Male	NA	NA	NA	NA	NA	NA
5	CTRL	Autopsy	Sudden death	48	Female	NA	NA	NA	NA	NA	NA
6	CTRL	Autopsy	Coronary artery disease	56	Male	NA	NA	NA	NA	NA	NA
7	CTRL	Biopsy	Aortic valve stenosis	56	Male	NA	60	NA	25	2,77	56
8	CTRL	Autopsy	Sudden death	28	Male	NA	NA	NA	NA	NA	NA
9	CTRL	Biopsy	Aortic valve stenosis	46	Female	NA	60	NA	26	3,09	72
10	CTRL	Biopsy	Aortic valve stenosis	52	Female	NA	55	15	NA	2,53	204
11	CTRL	Biopsy	Aortic valve stenosis	53	Male	NA	NA	NA	NA	NA	NA
12	CTRL	Biopsy	Aortic valve stenosis	29	Male	II	60	25	NA	2,79	37
13	CTRL	Biopsy	Aortic valve stenosis	47	Male	NA	60	29	23	NA	25
14	CTRL	Biopsy	Aortic valve stenosis	59	Male	NA	60	20	28	2,52	63
15	CTRL	Biopsy	Aortic valve stenosis	50	Male	NA	60	30	NA	3,36	63
16	CTRL	Biopsy	Aortic valve stenosis	22	Male	NA	65	20	NA	2,52	15
17	CTRL	Biopsy	Aortic valve stenosis	58	Male	NA	55	NA	NA	3,67	84
18	CTRL	Biopsy	Aortic valve stenosis	46	Male	NA	60	2	23	2,61	107
19	PAH	Autopsy	SSc-PAH	54	Female	IV	60	60	17	1,51	233
20	PAH	Autopsy	SSc-PAH	77	Male	III	50	44	13	1,7	363
21	PAH	Autopsy	SSc-PAH	47	Female	III	60	80	12	0,7	17
22	PAH	Autopsy	iPAH	74	Female	IV	55	84	NA	2,03	667
23	PAH	Autopsy	CHD-PAH	57	Female	III	60	53	15	1,74	174
24	PAH	Autopsy	PoPH	46	Female	IV	80	115	NA	NA	2
25	PAH	Autopsy	SSc-PAH	53	Female	iV	55	110	NA	1,96	124
26	PAH	Autopsy	Heritable PAH	61	Female	III	45	100	16	1,73	7
27	PAH	Autopsy	IPAH	65	Male	III	60	100	NA	1,73	162
28	PAH	Autopsy	PVOD-PAH	72	Female	IV	65	108	13	2,55	1142
29	PAH	Autopsy	SSc-PAH	62	Male	III-IV	60	62	9	NA	262
30	PAH	Autopsy	SSc-PAH	77	Female	III	50	NA	NA	1,34	16
31	PAH	Autopsy	PVOD-PAH	76	Female	IV	60	66	16	2,25	96

CTRL: control; CI: cardiac index; iPAH: idiopathic PAH; LVEF: left ventricular ejection fraction; PVOD: pulmonary veno-occlusive disease; TAPSE: tricuspid annular plane systolic excursion; RVSP, right ventricular systolic pressure; SSc-PAH: PAH associated with scleroderma; WHO class: World Health Organisation functional class assessed at the time of last clinic visit.

**Table S3. Baseline clinical characteristics between the PAH groups.**

	<b>IPAH (n=39)</b>	<b>CTD-PAH (n=21)</b>	<b>P value</b>
Female, n (%)	23 (59)	16 (76)	0.182
Age, years	63±16	69±7	0.031
HPAH, n (%)	2 (5)		
WHO functional class, n (%)			0.524
I	0 (0)	0 (0)	
II	7 (18)	3 (14)	
III	27 (69)	14 (67)	
IV	5 (13)	4 (19)	
Hemodynamics			
Mean PAP, mmHg	48±9	43±7	0.043
Mean RAP, mmHg	8±4	8±6	0.943
Cardiac index (L/min/m <sup>2</sup> )	2.4±0.7	2.5±0.8	0.365
PVR (dynes·s·cm <sup>-5</sup> )	842±403	812±339	0.775
Heart rate (bpm)	81±12	81±8	0.944
	63±10	62±7	0.571
NT-	1364 (3653598)	1558 (3054409)	0.812
SvO <sub>2</sub> (%)	70±22	66±21	0.539
proBNP (pg/mL)*			
eGFR (MDRD)(mL/min/1.73 m <sup>2</sup> )			
6-minute walking distance, m			
PAH medication categories, n (%)			
	324±109	279±136	0.201
None	25 (64)	12 (57)	0.028
Mono therapy	8 (21)	7 (33)	0.019
Double therapy	6 (15)	1 (5)	0.643
Triple therapy	0 (0)	1 (5)	0.459
Specific PAH therapy at enrollment, n (%)			
ERA	6 (15)	4 (19)	0.298
PDE5 inhibitor	12 (31)	7 (33)	0.051
Epoprostenol	2 (5)	1 (5)	0.545

Data are expressed by mean±SEM, median (interquartile range)\*, or n (%). PAH, pulmonary arterial hypertension; IPAH, idiopathic pulmonary arterial hypertension; HPAH, heritable PAH; CTD, connective tissue disease; WHO, world health organization; PAP, pulmonary arterial pressure; RAP, right atrium pressure; PVR, pulmonary vascular resistance; SvO<sub>2</sub>, mixed venous oxygen blood saturation; NT-proBNP, N-terminal pro b-type natriuretic peptide; eGFR, estimated glomerular

filtration rate; MDRD, Modification of Diet in Renal Disease; ERA, endothelin receptor antagonist; PDE5, phosphodiesterase type 5.

**Table S4. Univariate Cox proportional hazard analyses for death or lung transplantation in PAH patients.**

	Hazard ratio	95% confidence interval	P value
CRIM1	4.619	2.144–9.951	<0.001
HGF	2.943	1.508–5.744	0.002
MATN2	2.475	0.925–6.618	0.071
EGLN1	1.081	0.802–1.457	0.610
ANGPTL4	2.401	1.285–4.485	0.006
FST	2.312	1.145–4.668	0.019
DNPH1	0.960	0.518–1.779	0.896
AGER	1.456	0.926–2.289	0.103
DFFA	1.036	0.581–1.847	0.906
RABGAP1L	0.654	0.337–1.270	0.210
TGFA	3.852	1.608–9.232	0.002
CLEC4D	1.396	0.837–2.330	0.201
CLSTN2	2.528	1.485–4.304	0.001
PKLR	0.540	0.273–1.068	0.077
NBN	1.272	0.662–2.443	0.470
NTF3	1.427	1.004–2.026	0.047
ENPP7	1.291	0.959–1.739	0.092
DAG1	2.411	1.055–5.512	0.037
FSTL3	3.635	2.061–6.413	<0.001
TGFB1	2.144	0.886–5.186	0.091
TPP1	1.631	0.733–3.626	0.231
CCL28	1.228	0.594–2.540	0.579
OSM	1.021	0.713–1.461	0.910
AGRN	1.761	1.001–3.099	0.050
PSIP1	1.860	0.980–3.530	0.058
TNFRSF13B	2.369	1.410–3.979	0.001
PAPPA	2.156	1.176–3.953	0.013
CXCL10	1.017	0.711–1.455	0.926
SPON1	4.602	2.215–9.560	<0.001
VEGFD	1.697	0.929–3.100	0.085
CXCL17	2.285	1.267–4.119	0.006
NCF2	1.269	0.811–1.984	0.297
CCN2	0.816	0.426–1.563	0.540
HEXIM1	1.153	0.792–1.678	0.457
IRAK1	1.048	0.650–1.689	0.848
ESM1	2.202	1.289–3.762	0.004
GZMB	0.770	0.498–1.190	0.240
CCL21	0.969	0.586–1.601	0.901
PIK3AP1	0.810	0.464–1.415	0.460
ENAH	2.455	1.299–4.640	0.006
LAP3	0.769	0.414–1.428	0.405
GMPR	0.959	0.578–1.592	0.872
PLAUR	5.902	2.672–13.039	<0.001
AGRP	1.652	0.977–2.795	0.061
NPPC	1.781	1.056–3.003	0.030
LHPP	0.689	0.392–1.211	0.196
ICAM4	1.518	0.954–2.416	0.078
VEGFA	0.825	0.458–1.488	0.523
MZB1	1.668	0.999–2.787	0.051
MGMT	1.033	0.803–1.330	0.799

ATP5IF1	1.040	0.801–1.351	0.767
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AGER: advanced glycosylation end-product specific receptor; AGRN: agrin; AGRP: agouti related neuropeptide; ANGPTL4: angiopoietin Like 4; ATP5IF1: ATP synthase inhibitory factor subunit 1; CCL21: C-C motif chemokine ligand 21; CCL28: C-C motif chemokine ligand 28; CCN2: cellular communication network factor 2; CLEC4D: C-type lectin domain family 4 member D; CLSTN2: calstentenin 2; CRIM1: cysteine rich transmembrane BMP regulator 1; CXCL10: C-X-C motif chemokine ligand 10; CXCL17: C-X-C motif chemokine ligand 17; DAG1: dystroglycan 1; DFFA: DNA fragmentation factor subunit alpha; DNPH1: 2'-Deoxynucleoside 5'Phosphate N-Hydrolase 1; EGLN1: Egl-9 family hypoxia inducible factor 1; ENAH: ENAH actin regulator; ENPP7: ectonucleotide pyrophosphatase/phosphodiesterase 7; ESM1: endothelial cell specific molecule 1; FST: follistatin; FSTL3: follistatin-like 3; GMPR: guanosine monophosphate reductase; GZMB: granzyme B; HEXIM1: HEXIM P-TEFb complex subunit 1; HGF: hepatocyte growth factor; ICAM4: intercellular adhesion molecule 4; IRAK1: interleukin 1 receptor associated kinase 1; LAP3: leucine aminopeptidase 3; LHPP: phospholysine phosphohistidine inorganic pyrophosphate phosphatase; MATN2: matrilin-2; MGMT: O-6-methylguanine-DNA methyltransferase; MZB1: marginal zone B and B1 cell specific protein; NBN: nibrin; NCF2: neutrophil cytosolic factor 2; NPPC: natriuretic peptide C; NTF3: neurotrophin 3; OSM: oncostatin M; PAPP: pappalysin 1; PIK3AP1: phosphoinositide-3-kinase adaptor protein 1; PKLR: pyruvate kinase L/R; PLAUR: plasminogen activator, urokinase receptor; PSIP1: PC4 and SRSF1 interacting protein 1; RABGAP1L: RAB GTPase activating protein 1 like; SPON1: spondin 1; TGFA: transforming growth factor alpha; TGFB1: transforming growth factor beta 1; TNFRSF13B: TNF receptor superfamily member 13B; TPP1: tripeptidyl peptidase 1; VEGFA: vascular endothelial growth factor A; VEGFD: vascular endothelial growth factor D.



**Table S5. The area under the curves from time-dependent receiver operating characteristic curves for death or lung transplantation in PAH patients.**

Variables	Area under the curve of 1 year	P value	Area under the curve of 3 years	P value
CRIM1	0.808 (0.667–0.938)	<0.001	0.677 (0.519–0.832)	0.032
HGF	0.647 (0.438–0.857)	0.179	0.646 (0.475–0.792)	0.091
CLSTN2	0.766 (0.589–0.923)	0.006	0.713 (0.562–0.856)	0.008
FSTL3	0.872 (0.707–1.000)	0.003	0.743 (0.592–0.884)	0.007
SPON1	0.761 (0.578–0.920)	0.007	0.693 (0.545–0.826)	0.017
PLAUR	0.802 (0.625–0.966)	0.003	0.769 (0.629–0.903)	<0.001

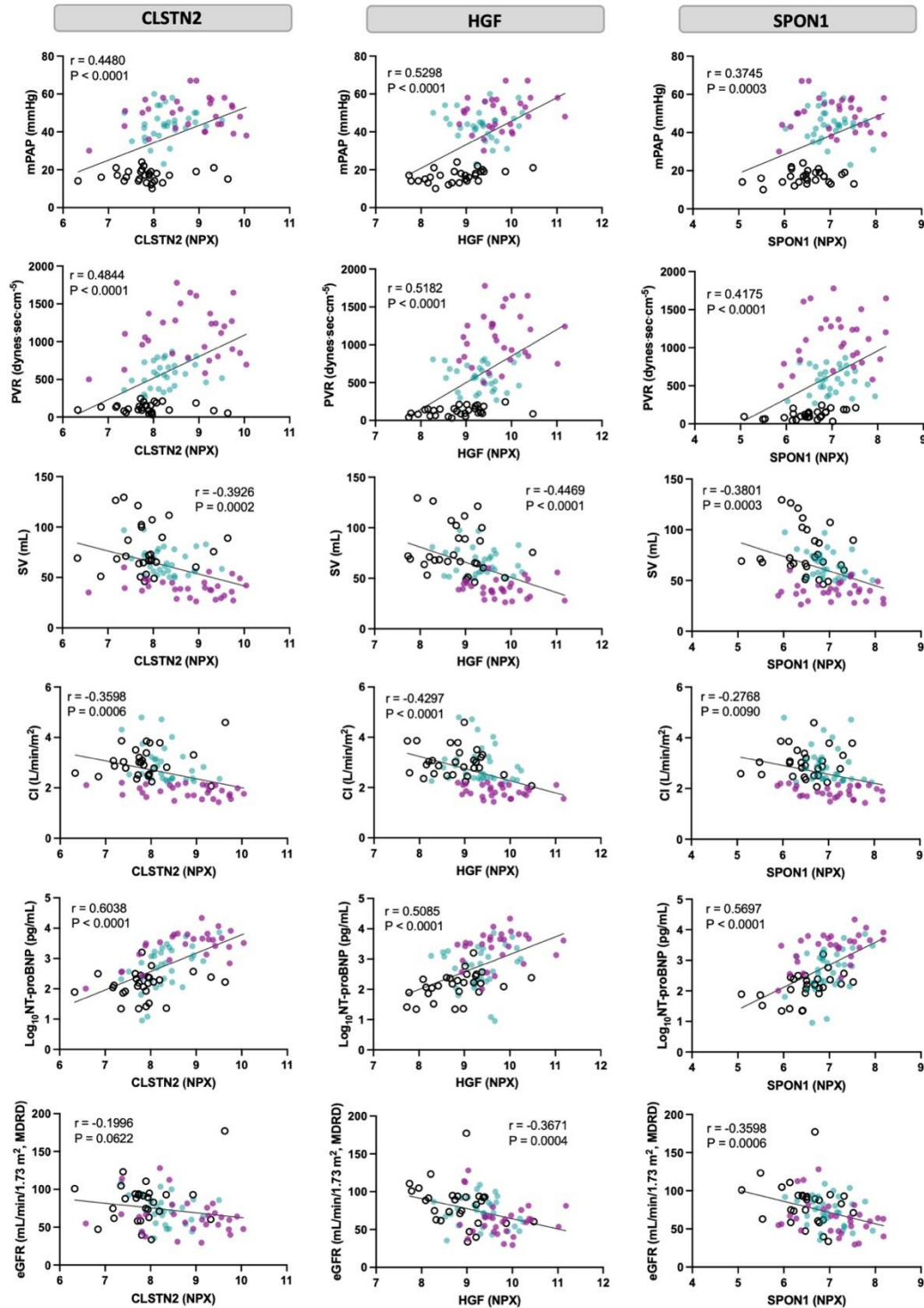
Data are shown in the area under the curves and 95% confidence intervals. CLSTN2: calstentenin 2; CRIM1: cysteine rich transmembrane BMP regulator 1; FSTL3: follistatin-like 3; HGF: hepatocyte growth factor; PLAUR: plasminogen activator, urokinase receptor; SPON1: spondin 1.

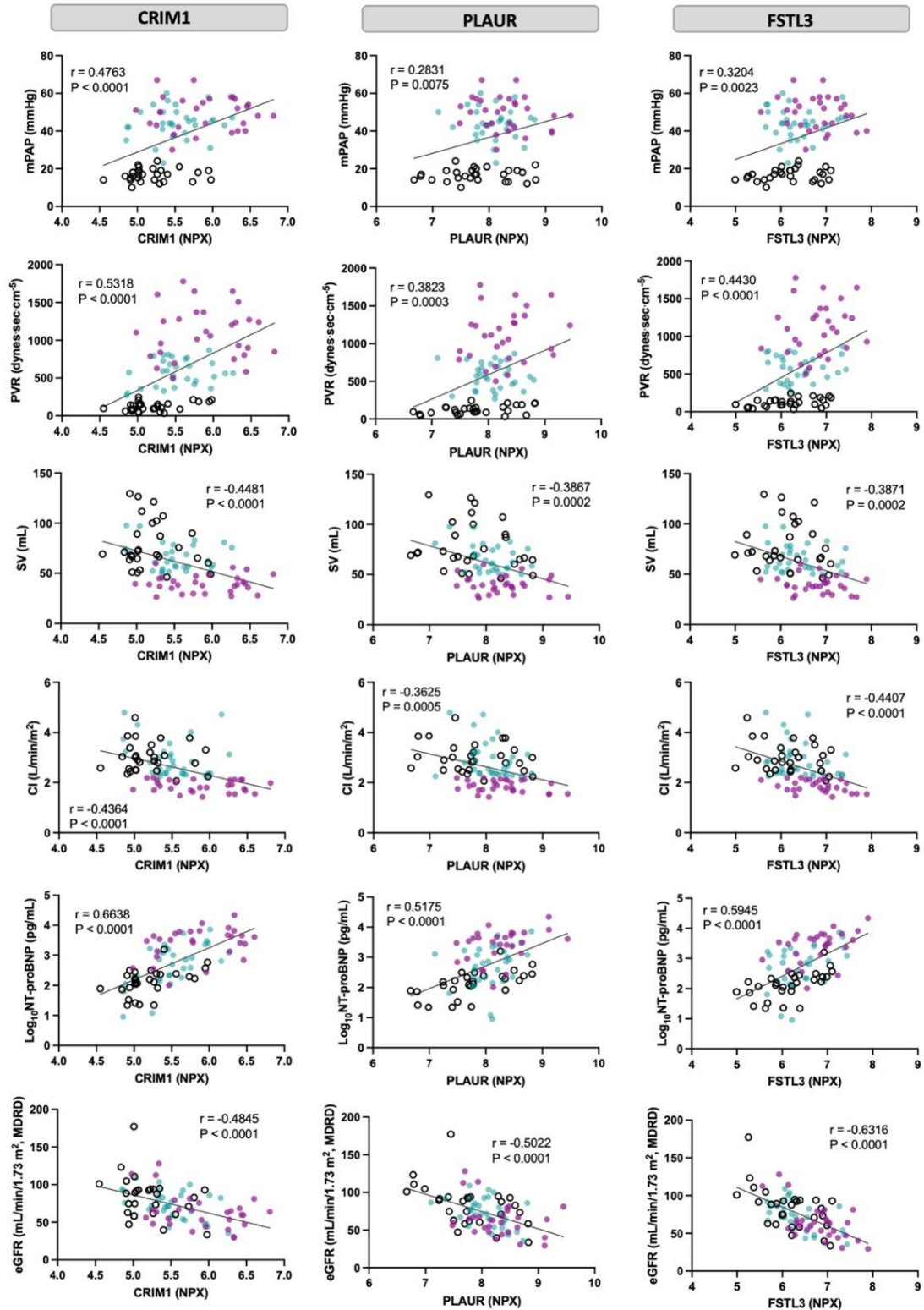
**Table S6. C-statistics of biomarkers with 2015 ESC/ERS risk score, REVEAL 2.0 risk score, and refined 4-strata risk assessment in PAH patients for death or lung transplantation.**

Variables	2015 ESC/ERS risk score	P value vs. Reference	REVEAL 2.0 risk score	P value vs. Reference	Refined 4strata risk assessment	P value vs. Reference
Risk score alone	0.561 (0.456–0.667) (Reference)	-	0.663 (0.523–0.803) (Reference)	-	0.590 (0.453–0.727) (Reference)	-
+ CRIM1	0.798 (0.683–0.913)	<0.001	0.799 (0.684–0.915)	0.036	0.797 (0.681–0.913)	0.008
+ HGF	0.685 (0.548–0.823)	0.043	0.697 (0.560–0.834)	0.515	0.679 (0.540–0.818)	0.186
+ CLSTN2	0.661 (0.523–0.799)	0.143	0.688 (0.552–0.824)	0.571	0.657 (0.518–0.796)	0.331
+ FSTL3	0.770 (0.651–0.889)	0.003	0.768 (0.648–0.888)	0.116	0.767 (0.646–0.887)	0.024
+ SPON1	0.834 (0.730–0.938)	<0.001	0.832 (0.728–0.936)	0.017	0.831 (0.726–0.935)	0.002
+ PLAUR	0.753 (0.626–0.881)	0.008	0.742 (0.613–0.871)	0.183	0.748 (0.619–0.876)	0.036

CLSTN2: calyntenin 2; CRIM1: cysteine rich transmembrane BMP regulator 1; ERS, European Respiratory Society; ESC, European Society of Cardiology; FSTL3: follistatin-like 3; HGF: hepatocyte growth factor; IDI, integrated discrimination improvement; NRI, net reclassification improvement; PLAUR: plasminogen activator, urokinase receptor; SPON1: spondin 1.

**Figure S1. CLSTN2, HGF, SPON1, CRIM1, PLAUR and FSTL3 and hemodynamic/biochemical parameters of PAH patients.**

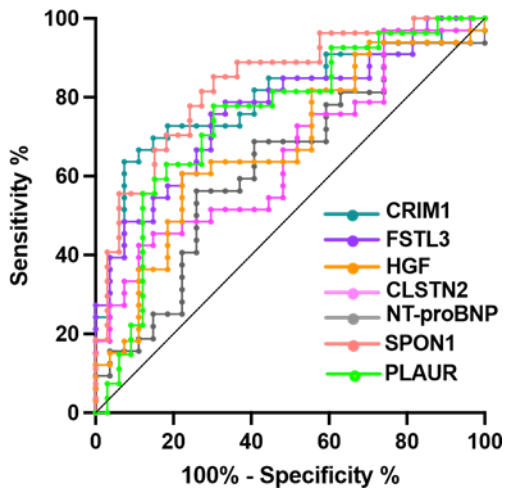




Pearson correlation coefficient with associated  $P$  value is shown in each graph. Open circles represent controls. Blue and purple circle represent cRV and dRV PAH patients, respectively. CI,

cardiac index; CLSTN2: calyntenin 2; CRIM1: cysteine rich transmembrane BMP regulator 1; eGFR, estimating glomerular filtration rate; FSTL3: follistatinlike 3; HGF: hepatocyte growth factor; mPAP, mean pulmonary artery pressure; NT-proBNP: Nterminal pro b-type natriuretic peptide; PLAUR: plasminogen activator, urokinase receptor; PVR, pulmonary vascular resistance; SPON1: spondin 1; SV, stroke volume.

Figure S2. Comparison of areas under the ROC curves between identified proteins and NTproBNP for the prediction of survival in PAH patients.



CLSTN2: calyntenin 2; CRIM1: cysteine rich transmembrane BMP regulator 1; FSTL3: follistatin-like 3; HGF: hepatocyte growth factor; NT-proBNP: N-terminal pro b-type natriuretic peptide; PLAUR: plasminogen activator, urokinase receptor; SPON1: spondin 1.