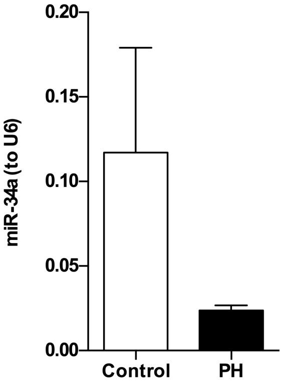
A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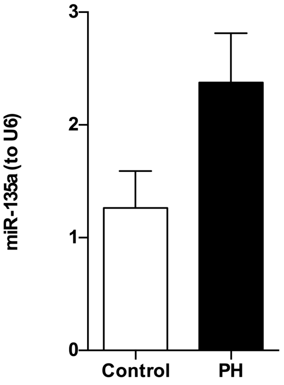
0.10
0.05
0.00
miR-34a (to U6)
Control
PH

B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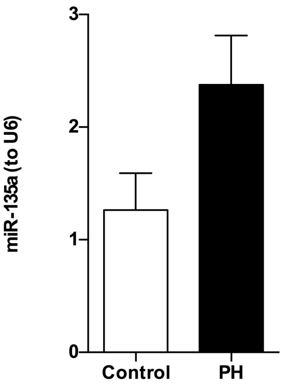
1
0
miR-135a (to U6)
Control
PH

C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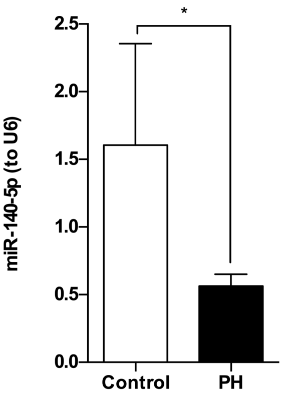
2
1
0
miR-135a (to U6)
Control
PH

D


*
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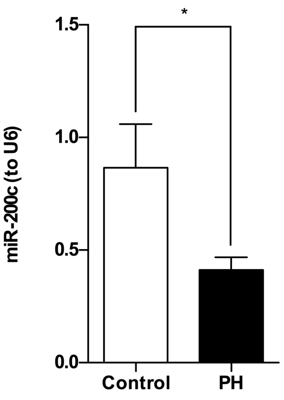
0.0
miR-140-5p (to U6)
Control
PH

E


*
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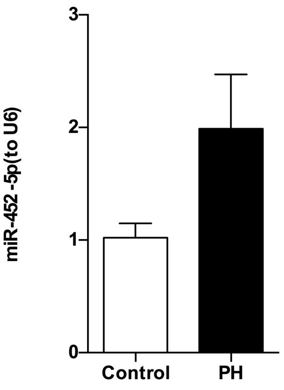
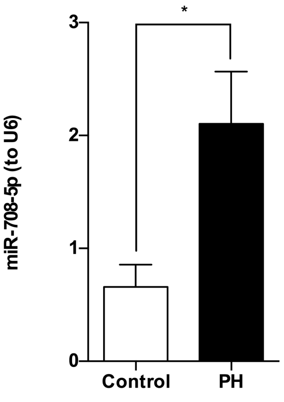
1.0
0.5
0.0
miR-200c (to U6)
Control
PH

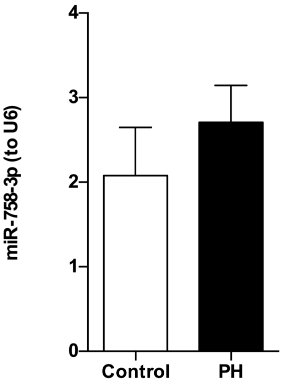
F


3
2
1
0
miR-452-5p (to U6)
Control
PH

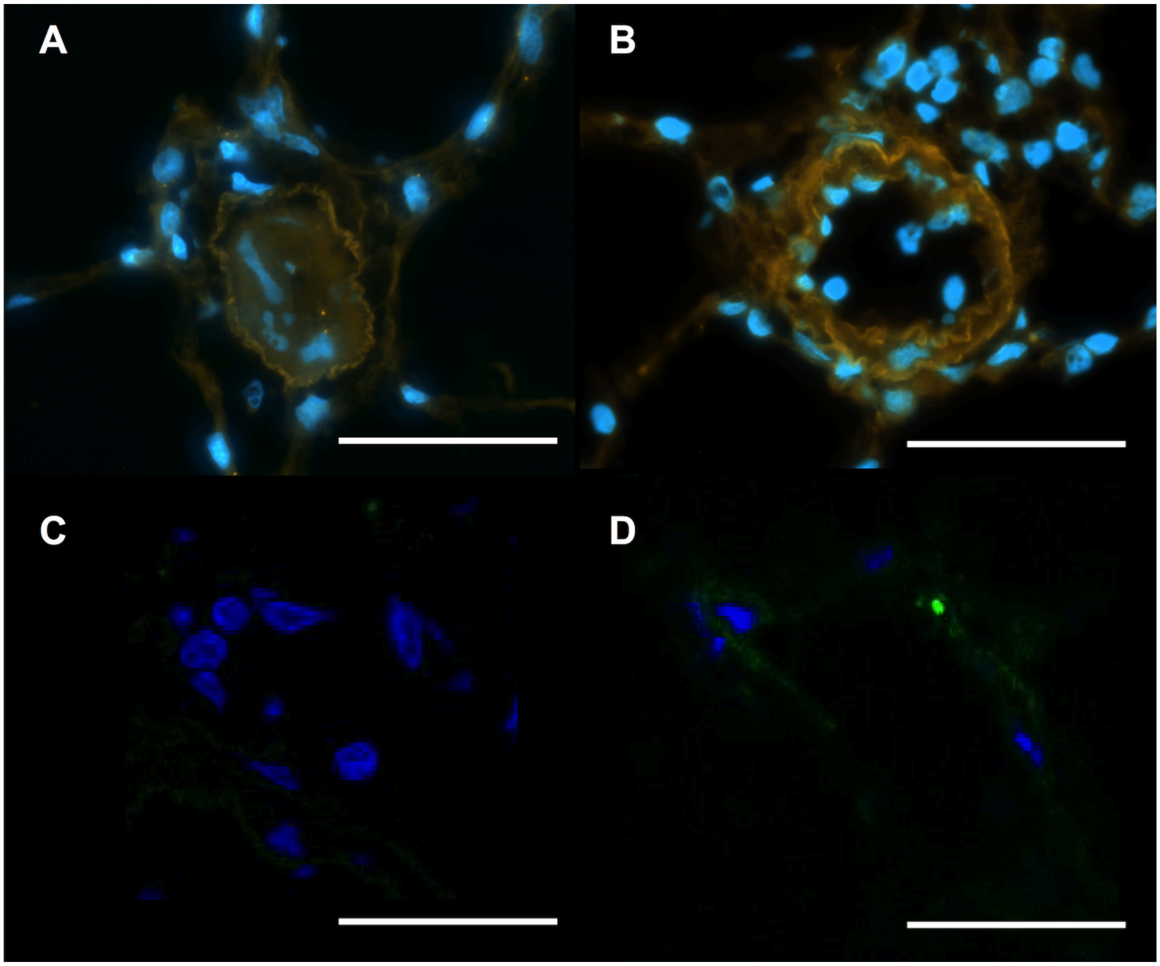
G


*
3
2
1
0
miR-708-5p (to U6)
Control
PH

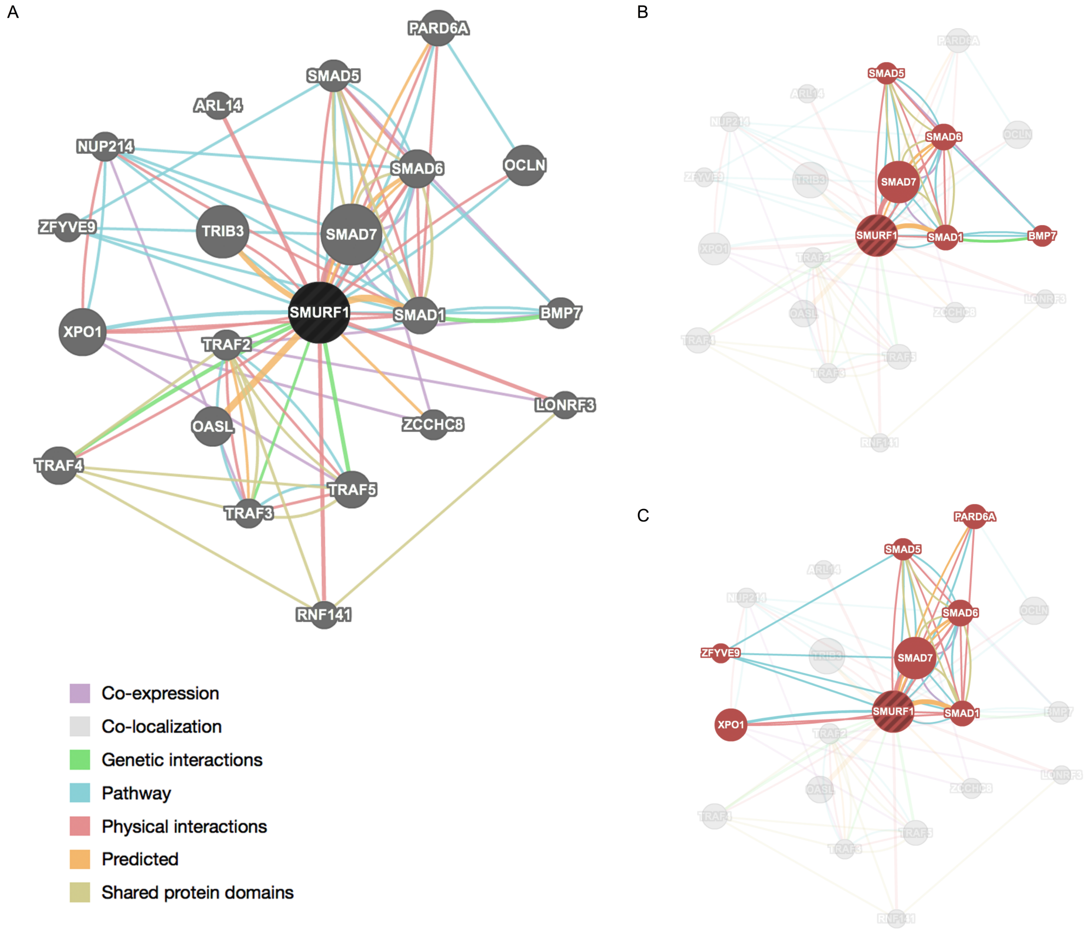
H


4
3
2
1
0
miR-758-3p (to U6)
Control
PH

Supplementary Figure 1: Validation of altered expression of miR in individual patient whole blood samples. A-H) Expression of miR was quantified by single sample assay from individual whole blood samples of patients included in array studies. Patients with idiopathic PAH, connective tissue disease associated PAH and chronic thromboembolic PH were grouped as pulmonary hypertension (PH). Healthy volunteers and patients with connective tissue disease referred for investigation for PH, but found to have normal pulmonary haemodynamics were grouped as control (n = 12 PH and n = 8 control, * $P < 0.05$, two-tailed Student's t-test, mean +/- S.E.M.)



Supplementary Figure 2: Delivery of miR to pulmonary vasculature in the SuHx rat model of PAH. A-B) Florescence microscopy of lung cryosections following two weekly nebulized doses of scramble control (A) or Cy3 labeled miR (B) (Cy3 – orange) (A-B: scale bar 50 µm, photomicrographs representative of n = 4 per group, x200). C-D) Confocal microscopy demonstrating delivery of Cy3 labeled miR to the pulmonary vasculature. C) Scramble control. D) Cy3 labeled miR (Cy3 – green, DAPI –blue) (C-D: scale bar 50 µm, photomicrographs representative of n = 4 per group, x200).



Supplementary Figure 3: Network analysis of SMURF1 interactions. A) SMURF1 interaction network. B) BMP signaling related SMURF1 interactions. C) TGFβ signaling related SMURF1 interactions. Genes are represented by nodes and interaction by edges. Networks constructed using Gene-Ontology and Biological Process based weighting in GeneMania (http://www.genemania.org).

Supplementary Table 1

Derivation cohort – clinical characteristics

Diagnosis	Gender	Age (years)	mPAP (mmHg)	RV CO (L/min)
No PH	Female	74	18	6.8
No PH	Female	60	17	2.5
No PH	Female	74	20	4.1
No PH	Female	67	21	4.4
HV	Female	58	NA	NA
HV	Female	53	NA	NA
HV	Female	45	NA	NA
HV	Female	26	NA	NA
CTD PH	Female	55	44	5.1
CTD PH	Female	61	44	5.3
CTD PH	Female	60	65	5.2
CTD PH	Female	63	66	3.3
CTEPH	Male	61	54	4.0
CTEPH	Female	72	55	3.2
CTEPH	Female	49	44	6.2
CTEPH	Female	53	70	5.5
IPAH	Female	73	53	3.9
IPAH	Female	34	50	7.8
IPAH	Female	71	53	2.7
IPAH	Female	53	62	3.2

Supplementary Table 2

Validation cohort: clinical characteristics

Diagnosis	Gender	Age (Years)	mPAP (mmHg)	RV CO (L/min)
IPAH	Female	48	73	6.1
IPAH	Female	44	64	5.1
IPAH	Female	34	49	7.8
IPAH	Male	68	48	3.3
IPAH	Male	64	59	5.5
IPAH	Female	64	42	7.2
IPAH	Female	34	53	3.3
IPAH	Female	65	53	4.7
IPAH	Female	65	46	3.2
IPAH	Female	27	58	2.5
IPAH	Female	75	60	5.3
IPAH	Female	56	72	7.0
IPAH	Male	52	62	NA
IPAH	Male	66	56	4.9
IPAH	Male	68	47	5.8
IPAH	Male	54	44	5.1
IPAH	Female	52	75	3.3
IPAH	Male	37	71	4.6
IPAH	Male	68	41	5.7
IPAH	Male	34	65	6.1
HV	Female	34	NA	NA
HV	Female	35	NA	NA
HV	Male	29	NA	NA
HV	Female	47	NA	NA
HV	Male	46	NA	NA
HV	Female	32	NA	NA
HV	Male	45	NA	NA
HV	Male	57	NA	NA
HV	Male	74	NA	NA
HV	Male	57	NA	NA
HV	Female	31	NA	NA
HV	Female	48	NA	NA
HV	Female	62	NA	NA
HV	Female	73	NA	NA
HV	Male	60	NA	NA
HV	Female	43	NA	NA

Supplementary Table 3

Monocrotaline Prevention Study

Phenotype parameter	Scramble miR	miR-140-5p mimic	P Value
Heart rate (BPM)	346 (15)	311 (13)	NS
Cardiac Output (µl/min)	60.8 (4.6)	79.8 (3.6)	<0.01
RVSP (mm Hg)	62 (4)	35 (3)	<0.001
RVEDP (mm Hg)	4 (1)	4 (1)	NS
RV dP/dt$_{max}$ (mm Hg/s)	3038 (307)	2790 (512)	NS
RV dP/dt$_{min}$ (mm Hg/s)	-3112 (15)	- 2078 (209)	<0.001
LVESP (mm Hg)	102 (5)	98 (4)	NS
LVEDP (mm Hg)	6 (1)	7 (1)	NS
LV dP/dt$_{max}$ (mm Hg/s)	6946 (503)	6037 (475)	NS
LV dP/dt$_{min}$ (mm Hg/s)	-6272 (780)	-5361 (598)	NS
Mean Aortic pressure (mm Hg)	90 (4)	82 (4)	NS
ePVRi (mm Hg/ml/min/g)	270 (40)	143 (11)	<0.01
PAAT (ms)	18.6 (1.4)	22.7 (1.2)	<0.05
RV/LV & Septum	0.37 (0.02)	0.34 (0.01)	NS

Two-tailed Student's t-test, mean +/- S.E.M., RV/ LV & septum, right ventricle/left ventricle including septum.

Supplementary Table 4

MCT rat therapeutic study

Phenotype parameter	Scramble miR	miR-140-5p mimic	P Value
Heart rate (BPM)	281 (17)	262 (18)	NS
Cardiac Output (µl/min)	58.47 (5.8)	76.18 (4.4)	<0.05
RVSP (mm Hg)	63 (6)	46 (7)	<0.05
RVEDP (mm Hg)	6 (1)	5 (1)	NS
RV dP/dt$_{max}$ (mm Hg/s)	2441 (159)	1941 (163)	<0.05
RV dP/dt$_{min}$ (mm Hg/s)	-2511 (192)	-2028 (241)	NS
LVESP (mm Hg)	77 (4)	83 (5)	NS
LVEDP (mm Hg)	7 (1)	7 (1)	NS
LV dP/dt$_{max}$ (mm Hg/s)	4372 (669)	4146 (580)	NS
LV dP/dt$_{min}$ (mm Hg/s)	-3204 (1337)	-4109 (59)	NS
Mean Aortic (mm Hg)	58 (6)	68 (6)	NS
ePVRi (mm Hg/ml/min/g)	349 (126)	204 (34)	<0.05
PAAT (ms)	17.9 (1.0)	22.0 (1.5)	<0.05
RV/LV & Septum	0.59 (0.02)	0.50 (0.04)	<0.05

Two-tailed Student's t-test, mean +/- S.E.M., RV/ LV & septum, right ventricle/left ventricle including septum.

Supplementary Table 5

SuHx Therapeutic Study

Phenotype parameter	**SCR**	**Mimic**	P value
Heart rate (BPM)	315 (17)	386 (21)	<0.05
Cardiac Output (µl/min)	68.9 (7.9)	90.1 (6.8)	NS
RVSP (mm Hg)	120 (6)	80 (9)	<0.05
RVEDP (mm Hg)	10 (1)	7 (1)	NS
RV dP/dt_{max} (mm Hg/s)	4792 (407)	4750 (425)	NS
RV dP/dt_{min} (mm Hg/s)	-4242 (404)	-5221 (452)	NS
LVESP (mm Hg)	93 (6)	118 (17)	NS
LVEDP (mm Hg)	7 (1)	6 (1)	NS
LV dP/dt_{max} (mm Hg/s)	5729 (464)	8047 (1667)	NS
LV dP/dt_{min} (mm Hg/s)	-4339 (352)	-7321 (703)	<0.05
Mean Aortic (mm Hg)	77 (4)	96 (12)	NS
ePVRi (mm Hg/ml/min/g)	364 (36)	243 (60)	NS
PAAT (ms)	15.81 (1.1)	20.61 (1.2)	<0.05
RV/LV & Septum	0.55 (0.03)	0.32 (0.05)	<0.01

Two-tailed Student's t-test, mean +/- S.E.M., RV/ LV & septum, right ventricle/left ventricle including septum.

Supplementary Table 6

Pathway analysis of miR-140-5p targets

Term	Count	P Value	Fold Enrichment
hsa04720:Long-term potentiation	7	0.003292391	4.71581876
hsa04144:Endocytosis	11	0.006120279	2.738689777
hsa04810:Regulation of actin cytoskeleton	11	0.017252393	2.343808925
hsa04330:Notch signaling pathway	5	0.018108894	4.873490512
hsa05200:Pathways in cancer	14	0.023791303	1.955339486
hsa04520:Adherens junction	6	0.024905094	3.56967357
hsa04010:MAPK signaling pathway	12	0.028597032	2.058912845
hsa04350:TGF-beta signaling pathway	6	0.039369185	3.159366263

Pathway analysis of computationally predicted miR-140-5p related targets performed in DAVID *(1)*.

Supplementary Table 7

Curated PH-related gene list

TGF-β (Superfamily) Signaling	Bone Morphogenetic Protein Receptor, type II (2-4)	BMPR2
	Transforming Growth Factor-β, type 1 (2-4)	TGFB1
	Transforming Growth Factor-β Receptor type 1 (2-4)	TGFBR1
	Transforming Growth Factor-β Receptor type 2 (3)	TGFBR2
	Transforming Growth Factor-β, induced (5)	TGFBI
	SMAD 1 (6)	SMAD1
	SMAD 2 (6)	SMAD2
	SMAD 3 (6)	SMAD3
	SMAD 4 (6)	SMAD4
	SMAD 5 (6)	SMAD5
	SMAD 6 (6)	SMAD6
	SMAD 7 (6)	SMAD7
	SMAD 8 (6)	SMAD8
	SMAD 9 (6)	SMAD9
	Bone Morphogenetic Protein-2 (7, 8)	BMP2
	Bone Morphogenetic Protein-4 (7, 8)	BMP4
	Bone Morphogenetic Protein-6 (8)	BMP6
	Bone Morphogenetic Protein-7 (8)	BMP7
	Bone Morphogenetic Protein-9 (8)	BMP9
	Bone Morphogenetic Protein-10 (8)	BMP10
	LIM motif containing Protein Kinase 1 (9)	LIMK1
	Cofilin-1 (non-muscle) (9)	CFL1
	Cofilin-2 (muscle) (9)	CFL2
	Activin A Receptor type II-like 1 (5)	ACVRL1
	Bone Morphogenetic Protein Receptor, type IA (5)	BMPR1A
	Bone Morphogenetic Protein Receptor, type 1B (5, 10)	BMPR1B
	Endoglin (10, 11)	ENG
	X-linked Inhibitor of Apoptosis (12)	XIAP
	Guanine Nucleotide Binding Protein, beta Polypeptide 2-Like 1 (RACK1) (13)	GNB2L1
	Dynein, Light Chain, Tctex-type 1 (TCTEX) (14)	DYNLT1
	Gremlin(15)	GREM1
	Tumor necrosis factor (ligand) superfamily, member 10(16)	TNFSF10
	Tumor necrosis factor receptor superfamily, member 11B(17)	TNFRSF11B
	Tumor necrosis factor receptor superfamily, member 11A(17)	TNFRSF11A
	Tumor necrosis factor superfamily, member (17)	TNFSF11
	SMAD specific E3 ubiquitin protein ligase 1(18)	SMURF1
Serotonin Signaling	Serotonin Transporter 4 (19)	SLC6A4
	Serotonin Receptor 1B (20)	HTR1B
	Serotonin Receptor 2B (21)	HTR2B
Angiopoietin Signaling	Angiopoietin-1 (22)	ANGPT1
	Angiopoietin-2 (23)	ANGPT2
	Angiopoietin-3	ANGPT3
	Angiopoietin-4	ANGPT4
	Endothelium-Specific Receptor Tyrosine Kinase, type 2 (24)	TEK/TIE2
Endothelin Signaling	Endothelin-1 (25)	EDN1
	Endothelin-2	EDN2
	Endothelin-3	EDN3
	Endothelin Receptor A (26)	EDNRA
	Endothelin Receptor B (26)	ENDRB
Nitric Oxide Signaling	Nitric Oxide Synthase 1	NOS1
	Nitric Oxide Synthase 2 (27)	NOS2
	Nitric Oxide Synthase 3 (28)	NOS3
	Phosphodiesterase 1A (29)	PDE1A
	Phosphodiesterase 1C (29)	PDE1C
	Phosphodiesterase 3A (30)	PDE3A
	Phosphodiesterase 5A (30)	PDE5A
	Guanosine triphosphate cyclohydrolase 1 (31)	GCH1
	Soluble Guanylate Cyclase 1, beta 3 subunit (32)	GUCY1B3
	Soluble Guanylate Cyclase 1, beta 2 subunit (32)	GUCY1B2
	Soluble Guanylate Cyclase 1, alpha 3 subunit	GUCY1A3
	Soluble Guanylate Cyclase 1, alpha 2 subunit	GUCY1A2
Vascular Endothelial Growth Factor (VEGF) Signaling	Vascular Endothelial Growth Factor A (32, 33)	VEGFA
	Kinase Insert Domain Receptor (34)	KDR
	Cyclin-Dependent Kinase Inhibitor-1B (35)	CDKN1B

	Src (33)	SRC
Rho/Rho kinase Signaling (36)	Ras Homologue Gene Family member A	RHOA
	Ras Homologue Gene Family member B	RHOB
	Rho-associated, Coiled-Coil containing Protein Kinase 1	ROCK1
	Rho-associated, Coiled-Coil containing Protein Kinase 2	ROCK2
	Rho Guanine Nucleotide Exchange Factor 12	ARHGEF12
Cation Channel Activity (8)	Kv Channel Interacting Protein 2	KCNIP2
	K+ Voltage-Gated Channel, Shaker-related Subfamily, member 2 (Kv1.5) (37)	KCNA5
	CACNA1C calcium channel, voltage-dependent, L type, alpha 1C subunit (38)	CACNA1c
	Transient Receptor Potential Cation Channel, Subfamily C, member 1 (39)	TRPC1
	Transient Receptor Potential Cation Channel, Subfamily C, member 3 (39)	TRPC3
	Transient Receptor Potential Cation Channel, Subfamily C, member 6 (40)	TRPC6
	Potassium Channel, Subfamily K, Member 3(41)	KCNK3
Heme Oxygenase Activity	Heme Oxygenase (decycling) 1 (42)	HMOX1
Mitochondrial Metabolism (8)	Pyruvate Dehydrogenate Kinase 1	PDK1
	Pyruvate Dehydrogenate Kinase 2	PDK2
	Pyruvate Dehydrogenate Kinase 3	PDK3
	Pyruvate Dehydrogenate Kinase 4	PDK4
	Superoxide Dismutase 2	SOD2
	B-cell Lymphoma 2	BCL2
Hypoxia Inducible Factor (HIF) Activity	HIF-1alpha (43)	HIF1A
	HIF-2alpha (44)	EPAS1
	Von Hippel Lindau (45)	VHL
NFAT Signaling	Nuclear Factor of Activated T-cells, Cytoplasmic, Calcineurin-Dependent 2 (37)	NFATC2
	Nuclear Factor of Activated T-cells, Cytoplasmic, Calcineurin-Dependent 3 (46)	NFATc3
Phosphatase and Tensin Homolog Activity	Phosphatase and Tensin Homolog (47)	PTEN
Hypoxia Inducible Mitogenic Factor (HIMF) Activity	Resistin-like beta (48)	RETNLB
Thromboxane A2 Signaling	Thromboxane A2 Receptor (49)	TBXA2R
Prostacyclin Signaling	Prostacyclin Synthase (49)	PTGIS
	Prostacyclin Receptor (49)	PRGIR
5-lipoxygenase (5-LO) Activity	Arachidonate 5-lipoxygenase (50)	ALOX5
PPAR-γ Signaling (51)	Peroxisome Proliferator-activated Receptor-γ (52)	PPARG
	PTGIR Low Density Lipoprotein Receptor-related Protein 1	LRP1
	Apolipoprotein E	APOE
	3-Hydroxy-3- Methylglutaryl-Coa Reductase (53)	HMGCR
	Apelin(54, 55)	APLN
	Apelin Receptor(54, 55)	APLNR
Vascular Inflammation (56)	Chemokine (C-C motif) Ligand 3	CCL3
	Interleukin-1β (57)	IL1B
	Interleukin-1a (58)	IL1A
	Interleukin-6 (59)	IL6
	Interleukin-13 (60)	IL13
	Interleukin-18(61)	IL18
	Selectin P (62)	SELP
	Chemokine (C-X3-C motif) Ligand 1 (63)	CX3CL1
	Chemokine (C-X3-C motif) Receptor 1 (63)	CX3CR1
	Chemokine (C-C motif) Ligand 5 (RANTES) (64)	CCL5
	Chemokine (C-C motif) Ligand 2 (65)	CCL2
	Nuclear Factor of k Light Polypeptide Gene Enhancer in B-cells 1 (66)	NFKB1
	Nuclear Factor of k Light Polypeptide Gene Enhancer in B-cells 2 (66)	NFKB2
	Vascular Cell Adhesion Molecule 1 (67)	VCAM1
	Intracellular Adhesion Molecule 1 (67)	ICAM1
	Stromal Cell Derived Factor -1 (47)	CXCL12
	Osteopontin (68)	OPN
PDGF Signaling (69)	Platelet-derived Growth Factor alpha	PDGFA
	Platelet-derived Growth Factor beta	PDGFB
	Platelet-derived Growth Factor Receptor, alpha	PDGFRA
	Platelet-derived Growth Factor Receptor, beta	PDGFRB
EGF Signaling (70)	Epidermal Growth Factor	EGF
	Epidermal Growth Factor Receptor	EGFR
Virus-Associated Pathways (71)	Hepatitis C Virus*	HCV
	Human Herpes Virus-8*	HHV8
	Trans-activator of Transcription (HIV) *	Tat

	Negative Regulatory Factor (HIV) * Signal Transducer and Activator of Transcription	Nef STAT1
Scleroderma-Associated Signaling (72)	Major Histocompatibility Complex, class I, B Major Histocompatibility Complex, class II, DRβ5	HLA-B HLA-DRB5
Vasoactive Intestinal Peptide Activity (73)	Vasoactive Intestinal Peptide Vasoactive Intestinal Peptide Receptor 1 Vasoactive Intestinal Peptide Receptor 2	VIP VPAC1 VPAC2
Caveolae Activity (42)	Caveolin 1	CAV1
Modulation of Vascular Elastase and Extracellular Matrix (74)	Tenascin-C (75) Matrix Metallopeptidase 1 Matrix Metallopeptidase 2 Matrix Metallopeptidase 3 Matrix Metallopeptidase 9 Integrin alphaVbeta3 (Vitronectin Receptor)	TNC MMP1 MMP2 MMP3 MMP9 VTNR

Updated curated PH-related gene list (76).

Supplementary Table 8

Pathway analysis of miR-140-5p targets within PH-related gene list.

Term	Count	P Value	Fold Enrichment
hsa04350:TGF-beta signaling pathway	6	3.08E-07	31.88087774
hsa05200:Pathways in cancer	7	1.16E-05	9.865576497
hsa04520:Adherens junction	4	3.71E-04	24.01416765
hsa04510:Focal adhesion	5	4.13E-04	11.49932157
hsa05210:Colorectal cancer	4	4.80E-04	22.01298701
hsa04060:Cytokine-cytokine receptor interaction	5	0.001130238	8.821998612
hsa04144:Endocytosis	4	0.00463467	10.04940711
hsa05214:Glioma	3	0.006377122	22.01298701
hsa05218:Melanoma	3	0.008046337	19.53265045
hsa05212:Pancreatic cancer	3	0.00826754	19.26136364
hsa05215:Prostate cancer	3	0.012442265	15.58222676
hsa04010:MAPK signaling pathway	4	0.013042113	6.925434116
hsa04270:Vascular smooth muscle contraction	3	0.019278299	12.38230519
hsa04360:Axon guidance	3	0.025151621	10.75052854

Pathway analysis of computationally predicted PH-related miR-140-5p related targets performed in DAVID *(1)*.

Supplementary Table 9

miR-140-5p PH-related network node connections.

Node	Edge
SMURF1	9
MAPK1	7
IGF1R	6
SMAD3	6
TGFBR1	6
BMPR1B	5
PDGFRA	5
SP1	5
VEGFA	5
BMP2	4
FGF2	4
PARD3	3
SNX2	3
NCOA3	2
CACNA1C	1
CXCL12	1
PDK1	1
TRPC3	1

Supplementary Table 10

miR-140-5p PH-related network conserved binding sites.

Target gene	Gene name	Conserved sites				Poorly conserved sites				Aggregate P_{CT}
		total	8mer	7mer-m8	7mer-1A	total	8mer	7mer-m8	7mer-1A	
SMURF1	SMAD specific E3 ubiquitin protein ligase 1	1	0	1	0	0	0	0	0	0.71
CACNA1C	calcium channel, voltage-dependent, L type, alpha 1C subunit	1	0	1	0	0	0	0	0	0.7
PDGFRA	platelet-derived growth factor receptor, alpha polypeptide	1	0	1	0	0	0	0	0	0.68
BMP2	bone morphogenetic protein 2	1	0	1	0	0	0	0	0	0.62
SNX2	sorting nexin 2	1	1	0	0	0	0	0	0	0.62
TGFBR1	transforming growth factor, beta receptor 1	1	1	0	0	0	0	0	0	0.6
VEGFA	vascular endothelial growth factor A	1	1	0	0	0	0	0	0	0.6
IGF1R	insulin-like growth factor 1 receptor	1	0	1	0	1	0	1	0	0.6
PDK1	pyruvate dehydrogenase kinase, isozyme 1	0	0	0	0	4	3	1	0	0.27
FGF2	fibroblast growth factor 2	0	0	0	0	2	0	0	2	0.16
MAPK1	mitogen-activated protein kinase 1	1	1	0	0	0	0	0	0	0.14
PARD3	par-3 family cell polarity regulator beta	0	0	0	0	1	0	1	0	< 0.1
BMPR1B	bone morphogenetic protein receptor, type IB	0	0	0	0	1	0	0	1	< 0.1
NCOA3	nuclear receptor coactivator 3	0	0	0	0	1	0	1	0	< 0.1
TRPC3	transient receptor potential cation channel, subfamily C, member 3	0	0	0	0	1	0	1	0	< 0.1

Adapted from http://www.targetscan.org

Reference:

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