Disconnect between fibrotic response and right ventricular dysfunction

Slaven Crnkovic, PhD; Bakytbek Egemnazarov, MD, PhD; Rachel Damico, MD, PhD; Leigh M Marsh, PhD; Bence M Nagy, PhD; Philipp Douschan, MD; Kwame Atsina, MD; Todd M Kolb, MD, PhD; Stephen C Mathai, MD; Jody E Hooper, MD; Bahil Ghanim, MD, PhD; Walter Klepetko, MD; Friedrich Fruhwald, MD, PhD; Dirk Lassner, MD; Andrea Olschewski, MD; Horst Olschewski, MD; Paul M Hassoun, MD; and Grazyna Kwapiszewska, PhD

ONLINE DATA SUPPLEMENT

Online Data Supplement

Expanded methods

Human material

RV myocardial biopsies from patients with dilated cardiomyopathy were collected at the Medical University of Graz and underwent certified evaluation at the Institute for Cardiac Diagnostic and Therapy (Berlin, Germany) after written informed consent. Samples with no signs of viral or autoimmune disease were provided for research purposes.

Animal studies

Chronic hypoxia-induced pulmonary hypertension in mice was done as reported previously{{492 Crnkovic 2016;}}. Male CD1 mice at 10 weeks of age (Harlan Laboratories, Italy) were exposed for 5 weeks to normobaric hypoxia (10% oxygen) or normoxia. Chambers (Biospherix, USA) were supplemented with CO_2 absorber (GE Healthcare, Austria) and animals had ad libitum access to food and water.

Hydroxyproline assay

RV tissue samples minced in the 1 M NaCl buffer containing 0.05 M Tris, 1mM PMSF, 1 mM NEM, 5 mM EDTA, pH 7.5 were centrifuged at 13000 g for 15 minutes at 4°C to isolate non-crosslinked collagen. The pellet was resuspended in 0.5 M acetic acid and digested with 5 mg/mL pepsin for 24 h at 4°C followed by centrifugation at 13000 g for 15 minutes at 4°C to isolate crosslinked collagen. The obtained material was subjected to acid hydrolysis and hydroxyproline measurement using QuickZyme Hydroxyproline assay (QuickZyme Biosciences, USA). Data were normalized to the protein concentration of the tissue samples.

Capillary density

RV capillary density was determined with semiautomated image analysis (Visiomorph DP software; Visiopharm, Horshol, Denmark) on formalin-fixed, paraffin-embedded heart tissue stained against endothelial cell marker thrombomodulin as described previously{{492 Crnkovic 2016;}}.

Table E1. Clinical characteristics of patients used for right ventricle histologic assessment.

patient	sex	age	height (cm)	weight (kg)	diagnosis	mPAP (mmHg)
1	F	50	n.a.	n.a.	systemic sclerosis – pulmonary arterial hypertension	55
2	F	68	n.a.	n.a.	idiopathic pulmonary arterial hypertension	65
3	F	67	n.a.	n.a.	systemic sclerosis – pulmonary arterial hypertension	48
4	M	43	175	89	dilative cardiomyopathy	28
5	M	39	178	83	dilative cardiomyopathy	33
6	M	53	181	88	dilative cardiomyopathy	31
7	M	45	178	82	dilative cardiomyopathy	41
8	M	58	180	90	dilative cardiomyopathy	46
9	F	59	163	55	dilative cardiomyopathy	41
10	M	56	175	83	dilative cardiomyopathy	11
11	M	45	180	100	dilative cardiomyopathy	9
12	M	53	171	89	dilative cardiomyopathy	19
13	M	26	183	104	dilative cardiomyopathy	25

mPAP – mean pulmonary artery pressure; na – not available.

Table E2. Clinical characteristics of patients used for serum ELISA measurements of galectin-3.

	sex	age	mPAP* (mmHg)	PVR† (dyn s/cm^5)	PAWP‡ (mmHg)	CO$ (L/min)	CI§ (L/min/m^2)	NT-proBNP (pg/mL)	6MWD# (m)
LBI IPAH	8/2	57.5 (47.6-67.4)	41 (33-48)	485 (346-623)	7 (5-10)	5.66 (4.90-6.41)	3.03 (2.62-3.44)	544 (148-941)	389 (317-462)
USA IPAH	30/8	53.4 (48.8-58.1)	49 (44-54)	736 (600-871)	11 (10-13)	4.60 (4.12-5.09)	2.47 (2.22-2.71)	1663 (540-2787)	418 (380-455)
USA SSc-PAH	37/6	66.4 (62.4-70.4)	39 (34-43)	592 (428-756)	10 (9-12)	4.90 (4.21-5.58)	2.68 (2.36-3.00)	2374 (1129-3619)	340 (307-372)
LBI donors	10/5	48.8 (38.5-59.1)	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
USA donors	10/0	37.7 (30.5-44.9)	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.

Data are presented as mean (95% confidence interval).

§CI – cardiac index; $CO – cardiac output; IPAH – idiopathic pulmonary arterial hypertension; *mPAP – mean pulmonary artery pressure; ‡PAWP – pulmonary arterial wedge pressure; †PVR – pulmonary vascular resistance; #6MWD – six minute walk distance; n.a. – not available.

Table E3. Correlation of clinical parameters with galectin-3 serum levels.

	mPAP* (mmHg)	PVR† (dyn s/cm^5)	PAWP‡ (mmHg)	CO$ (L/min)	CI§ (L/min/m^2)	NT-proBNP (pg/mL)	6MWD# (m)
n (all PAH)	77	77	77	77	68	62	77
Spearman r	0.005683	0.1265	-0.06996	-0.2294	-0.09672	-0.02518	-0.005681
P value	0.9609	0.2728	0.5454	0.0448	0.4327	0.846	0.9609
n (all IPAH)	43	43	43	43	38	33	43
Spearman r	-0.1371	-0.1164	-0.03788	0.0614	0.1679	-0.04245	-0.1342
P value	0.3808	0.4572	0.8094	0.6957	0.3136	0.8146	0.3909
n (LBI IPAH)	10	10	10	10	10	10	10
Spearman r	-0.1515	-0.3333	0.4322	0.503	0.2364	-0.1152	-0.2606
P value	0.6821	0.3487	0.2182	0.144	0.5135	0.7589	0.4697
n (USA IPAH)	33	33	33	33	28	23	33
Spearman r	-0.05318	0.04579	0.02117	-0.1712	0.05003	-0.02273	-0.2189
P value	0.7688	0.8002	0.9069	0.3408	0.8004	0.918	0.2211
n (USA SSc-PAH)	34	34	34	34	30	29	34
Spearman r	0.128	0.3367	-0.09995	-0.5324	-0.399	0.03941	0.2597
P value	0.4708	0.0515	0.5738	0.0012	0.0289	0.8391	0.138

§CI – cardiac index; $CO – cardiac output; (I)PAH – (idiopathic) pulmonary arterial hypertension; *mPAP – mean pulmonary artery pressure; ‡PAWP – pulmonary arterial wedge pressure; †PVR – pulmonary vascular resistance; SSc-PAH – connective tissue disease associated PAH; #6MWD – six minute walk distance.

Table E4. Antibodies used in the study.

antibody	producer	catalog number	dilution
galectin-3	R&D Systems	AF1197	IHC/IF 1:200; WB 1:600;
	Millipore	MABT51	IHC/IF 1:200
vimentin	Cell Signaling	3932	IHC/IF 1:50
		9856 (IF)	IHC/IF 1:50
platelet derived growth factor receptor alpha	Cell Signaling	3174	IHC/IF 1:100; WB 1:1000
	R&D Systems	AF1062	IHC/IF 1:100
alpha smooth muscle actin	Sigma	F3777	IHC/IF 1:100
	Sigma	C6198	IHC/IF 1:100
	Everest Biotech	A305053	IHC/IF 1:100
donkey anti-rabbit IgG Alexa Fluor 488/555 conjugate	ThermoFisher Scientific	A-21206 A-31572	IF 1:500
donkey anti-goat IgG Alexa Fluor 488/555/647 conjugate		A-11055 A-21432 A- 21447	
donkey anti-rat IgG Alexa Fluor 488/555 conjugate		A-21208	
GAPDH	Santa Cruz	SC-25778	WB 1:1000

IHC/IF – immunohistochemistry/immunofluorescence; WB – Western blotting.

Table E5. Sequences of primers used in the study.

primer	accession number	forward	reverse
human PBGD	NM_000190.3	TCGGAGCCATCTGCAAGCGG	GCCGGGTGTTGAGGTTTCCCC
human B2M	NM_004048.2	CCTGGAGGCTATCCAGCGTACTCC	TGTCGGATGGATGAAACCCAGACA
human galectin-3	NM_002306.3	CAGCCAAGTGCCACCGGAGC	GGCACAATCAGTGGCCCAGCAG
mouse PBGD	NM_013551.2	GCCAGAGAAAAGTGCCGTGGG	TCCGGAGGCGGGTGTTGAGG
mouse B2M	NM_009735.3	CGGCCTGTATGCTATCCAGAAAACC	TGTGAGGCGGGTGGAACTGTG
mouse galectin-3	NM_010705.3	GCACTACCCAGGAAAATGGCAGACA	CCCCAGGCTGGTTCCCCCAT
mouse Serca2a2	NM_009722.3	GGTCAACGAGAGCACGGGGC	GCCGGCAATTCGTTGGAGCC

Table E6. Non-invasive and invasive right ventricle functional parameters.

parameter	WT PAB (n=7)	GAL3 KO PAB (n=7)	p value
HR* (bpm)	378 ± 22	369 ± 28	0.567
gradient velocity (m/s)	2.59 ± 0.37	2.53 ± 0.62	0.876
TAPSE† (mm)	0.75 ± 0.15	0.62 ± 0.11	0.104
S'‡ (mm/s)	20.3 ± 4.3	19.0 ± 3.6	0.329
e'§ (mm/s)	16.2 ± 1.3	15.7 ± 2.6	0.931
a'‖ (mm/s)	27.9 ± 1.6	24.4 ± 2.8	0.064
cardiac output (mL/min)	13.4 ± 2.9	11.6 ± 3.2	0.534
HR* (bpm)	482 ± 18	483 ± 34	0.932
contractility index (1/s)	159 ± 30	181 ± 31	0.200
tau (ms)	53 ± 19	43 ± 16	0.249
pressure time index (mmHg s)	2.43 ± 0.51	2.12 ± 0.46	0.240

‖a' –late (atrial) peak of right ventricular relaxation velocity; §e' – early peak of right ventricular relaxation velocity; *HR – heart rate (beats per minute); ‡S' – velocity of the right ventricular contraction; †TAPSE – tricuspid annular plane systolic excursion; tau – time constant of monoexponential curve fitting model of RV diastolic pressure decay measured invasively. Data are given as mean ± SD; p value of Mann-Whitney test.

Table E7. Non-invasive and invasive right ventricle functional parameters.

parameter	vehicle PAB (n=8)	NacLac PAB (n=8)	p value
HR* (bpm)	413 ± 35	422 ± 18	0.792
gradient velocity (m/s)	2.72 ± 0.90	2.59 ± 0.19	0.165
TAPSE[†] (mm)	0.70 ± 0.17	0.73 ± 0.20	1.000
S'[‡] (mm/s)	22.1 ± 5.8	20.8 ± 4.4	0.282
e'[§] (mm/s)	19.3 ± 4.0	16.8 ± 2.8	0.137
a'[‖] (mm/s)	32.8 ± 7.8	29.2 ± 9.5	0.282
cardiac output (mL/min)	14.0 ± 2.9	14.3 ± 2.1	0.798
HR* (bpm)	451 ± 57	416 ± 51	0.959
contractility index (1/s)	164 ± 33	145 ± 27	0.235
tau (ms)	39 ± 14	37 ± 9	0.879
pressure time index (mmHg s)	2.61 ± 0.54	2.64 ± 0.59	0.879

[‖]a' –late (atrial) peak of right ventricular relaxation velocity; [§]e' – early peak of right ventricular relaxation velocity; *HR – heart rate (beats per minute); [‡]S' – velocity of the right ventricular contraction; [†]TAPSE – tricuspid annular plane systolic excursion; tau – time constant of monoexponential curve fitting model of RV diastolic pressure decay measured invasively. Data are given as mean ± SD; p value of Mann-Whitney test.

Table E8. Non-invasive and invasive right ventricle functional parameters.

parameter	chow PAB (n=6)	pirfenidone PAB (n=8)	p value
HR* (bpm)	427 ± 26	446 ± 57	0.662
gradient velocity (m/s)	2.85 ± 0.20	2.93 ± 0.14	0.534
TAPSE† (mm)	0.59 ± 0.15	0.63 ± 0.16	0.445
S'‡ (mm/s)	17.1 ± 4.0	17.6 ± 2.8	1.000
e'§ (mm/s)	14.9 ± 4.4	15.1 ± 3.4	0.841
a'‖ (mm/s)	28.7 ± 7.2	28.2 ± 4.0	0.841
cardiac output (mL/min)	12.8 ± 2.0	12.3 ± 2.4	0.755
HR* (bpm)	484 ± 60	506 ± 40	0.423
contractility index (1/s)	166 ± 55	158 ± 43	0.762
tau (ms)	41 ± 39	28 ± 9	0.370
pressure time index (mmHg s)	2.14 ± 0.45	2.51 ± 0.74	0.367

‖a' –late (atrial) peak of right ventricular relaxation velocity; §e' – early peak of right ventricular relaxation velocity; *HR – heart rate (beats per minute); ‡S' – velocity of the right ventricular contraction; †TAPSE – tricuspid annular plane systolic excursion; tau – time constant of monoexponential curve fitting model of RV diastolic pressure decay measured invasively. Data are given as mean ± SD; p value of Mann-Whitney test.

Figure E1. Right ventricle (RV) fibrosis is characterized by expansion of PDGFRα-(platelet-derived growth factor receptor alpha)-expressing cells. Representative immunofluorescence co-staining of the RV fibrotic regions against vimentin, PDGFRα, and αSMA (alpha smooth muscle actin) with DAPI as nuclear counterstain. n=4 mice/group. Hearts were collected from mice 21 days after pulmonary artery banding (PAB) or sham operation. Scale bars 20 μm. PDGFRα antibody specificity testing using blocking peptide. Scale bars: 200 μm.

Figure E2. Accumulation of galectin-3 in RV fibrotic regions. (A) Representative immunohistochemical localization of galectin-3 expression in the RV from rats treated with monocrotaline (MCT, scale bar: 100 μm) Sugen5416. Hearts were collected 28 days after MCT injection or 6 weeks after Sugen5416 application (including 3 weeks hypoxia exposure followed by 3 weeks normoxia). (B) Western blot detection of galectin-3 protein expression in the RV; n=6-8 rats/group. (C) Quantitative PCR determination of galectin-3 mRNA expression in the RV. Student's t-test with Welch's correction, *$p<0.05$. (D) Quantitative PCR determination of galectin-3 mRNA expression in the RV from normoxia or chronic hypoxia-exposed (21 days) mice. (E) Representative immunofluorescent staining of RV fibrotic regions against galectin-3, vimentin, PDGFRα (platelet-derived growth factor receptor alpha), and αSMA (alpha smooth muscle actin); n=4 mice/group (3 weeks PAB). Scale bar: 20 μm. Immunofluorescent staining of PAB hearts against galectin-3 in wild type (WT) and galectin-3 knockout (GAL3 KO). Scale bar: 20 μm.

Figure E3. Morphological and molecular correlates of anti-fibrotic therapy. (A) Quantification of right ventricular (RV) fibrosis by hydroxyproline assay. (B) Weight ration of RV to left ventricle and septum (Fulton index). (C,D) Assessment of RV capillary density. Hearts

were collected from vehicle and NacLac-treated mice or (D) wild type (WT) and galectin-3 knockout (GAL3 KO) 21 days after pulmonary artery banding (PAB) or sham operation and stained against endothelial marker thrombomodulin. Two-way ANOVA with Bonferroni post-test (A,C,D). Mann-Whitney test (B), * $p<0.05$.

Figure E4. Correlation of fibrosis with right ventricular diastolic parameters. Fibrosis was assessed 21 days after pulmonary artery banding operation in controls or treated mice. Tau (time constant of monoexponential curve fitting model of RV diastolic pressure decay) and right ventricle end diastolic pressure (RVEDP) were measured invasively. (A,B) Wild type or galectin-3 knock-out mice. (C,D) Mice treated with vehicle or galectin-3 inhibitor (NacLac). (E,F) Mice treated with chow diet or pirfenidone-containing diet. (G) Correlation of tau and RV fibrosis area on pooled samples. Non-parametric correlation (Spearman). Linear regression (full line) with 95% confidence intervals (dashed lines).

Figure E5. Correlation of fibrosis with non-invasive right ventricular function parameters. Fibrosis was assessed 21 days after pulmonary artery banding operation in controls or treated mice. Cardiac output (CO), tricuspid annular plane systolic excursion (TAPSE), and right ventricular relaxation velocity (e') were measured by echocardiography. (A-C) Wild type or galectin-3 knock-out mice. (D-F) Mice treated with vehicle or galectin-3 inhibitor (NacLac). (G-I) Mice treated with chow diet or pirfenidone-containing diet. Non-parametric correlation (Spearman). Correlation of (J) CO and (K) e' with RV fibrosis area on pooled samples. Linear regression (full line) with 95% confidence intervals (dashed lines).

Figure E6. Functional consequences of chronic pulmonary artery banding (PAB). (A) Comparison of right ventricular (RV) fibrosis (Sirius red staining) between 3 weeks and 8 weeks PAB model. Two-way ANOVA with Bonferroni post-test, *$p<0.05$. Serial echocardiographic assessment of (B) cardiac output (CO) and (C) tricuspid annular plane systolic excursion

(TAPSE) following 3 and 8 weeks PAB. Paired two-way ANOVA, *p<0.05. Correlation of RV fibrosis with (D) RV end diastolic pressure, (E) cardiac output, and (F) tau. Effect of NacLac and pirfenidone treatment in chronic PAB model (8 weeks) on (G) RV fibrosis level, (H) RV systolic pressure, and (I) RV end diastolic pressure. Two-way ANOVA with Bonferroni post-test, p<0.05.

Figure E7. Correlation of galectin-3 levels with right ventricular (RV) fibrosis, mean pulmonary artery pressure (mPAP), and N-terminal pro-B type natriuretic peptide (NT-proBNP) levels. (A) Immunohistochemical staining of the RV against vimentin, platelet-derived growth factor receptor alpha (PDGFRα), alpha smooth muscle actin (αSMA), and galectin-3 on RV biopsy samples from patients with pulmonary hypertension (group 2 PH). Fibrotic regions were detected using Sirius red stain. Arrows depict positively stained regions/cells, arrowheads mark cardiac vessels. Scale bar: 500 μm (50 μm in magnified regions). (B) Fibrosis and galectin-3 scoring was performed on the RV biopsy samples from patients with or without pulmonary hypertension (group 2 PH).

Figure E1.

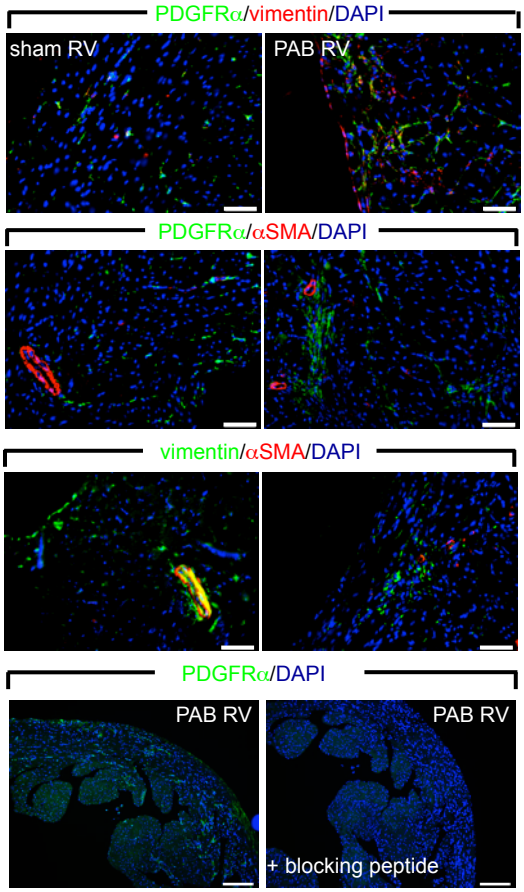
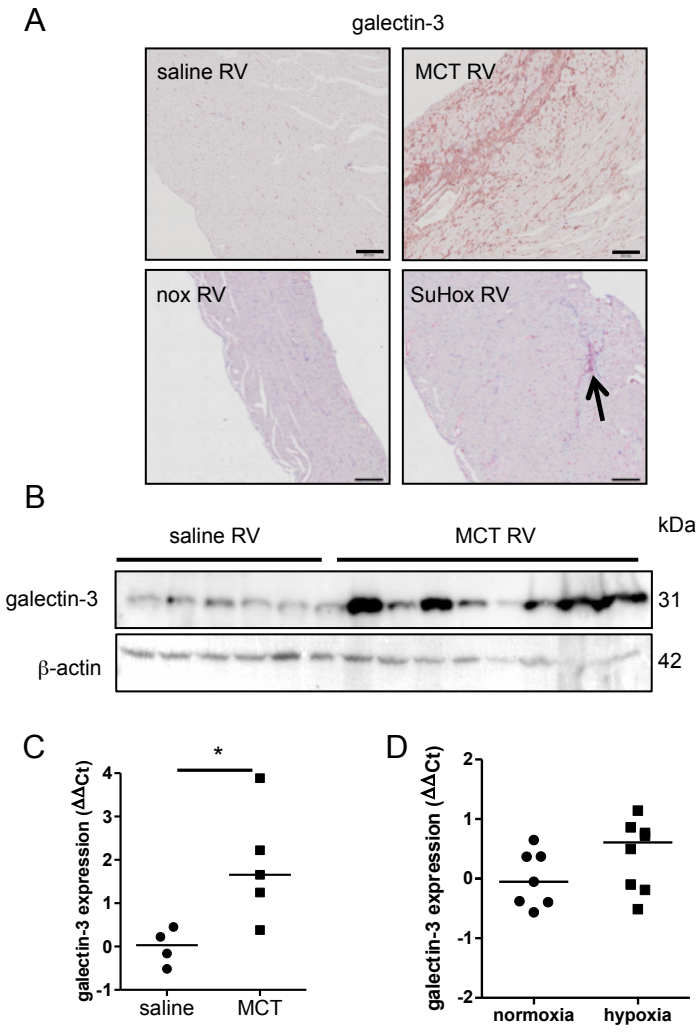

PDGFRα/vimentin/DAPI
sham RV
PAB RV
PDGFRα/αSMA/DAPI
vimentin/αSMA/DAPI
PDGFRα/DAPI
PAB RV
PAB RV
+ blocking peptide

Figure E2.


A
galectin-3
saline RV
MCT RV
nox RV
SuHox RV
B
saline RV
MCT RV
kDa
galectin-3
31
β-actin
42
C
galectin-3 expression (ΔΔCt)
saline
MCT
*
D
galectin-3 expression (ΔΔCt)
normoxia
hypoxia

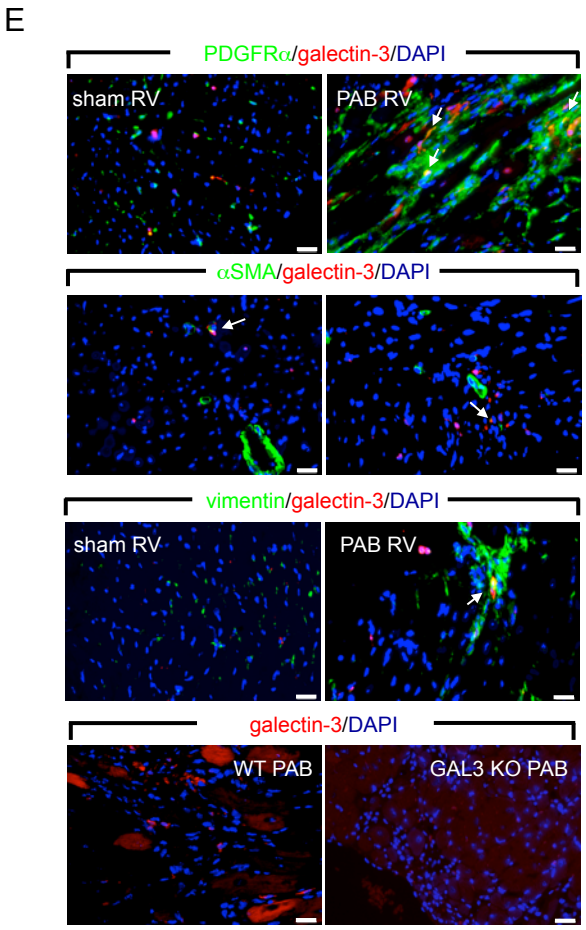

E
PDGFRα/galectin-3/DAPI
sham RV
PAB RV
αSMA/galectin-3/DAPI
vimentin/galectin-3/DAPI
sham RV
PAB RV
galectin-3/DAPI
WT PAB
GAL3 KO PAB

Figure E3.

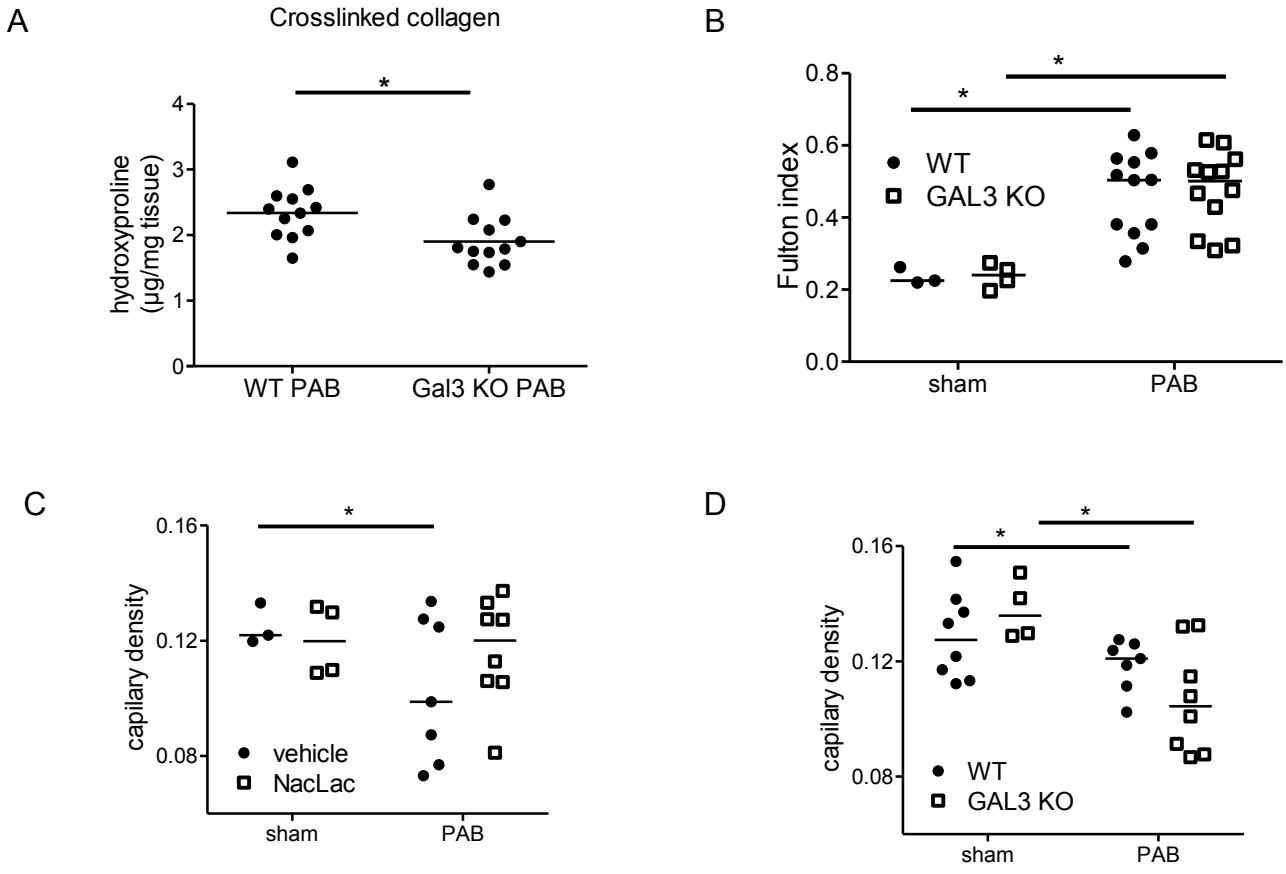
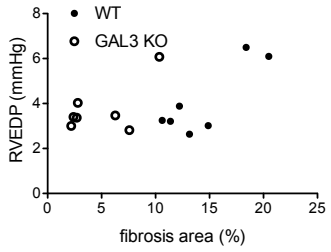

A
Crosslinked collagen
hydroxyproline (µg/mg tissue)
0
1
2
3
4
*
WT PAB
Gal3 KO PAB
B
Fulton index
0.0
0.2
0.4
0.6
0.8
*
*
WT
GAL3 KO
sham
PAB
C
capilary density
0.08
0.12
0.16
*
vehicle
NacLac
sham
PAB
D
capilary density
0.08
0.12
0.16
*
*
WT
GAL3 KO
sham
PAB

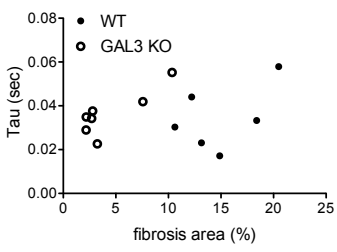
Figure E4.

A



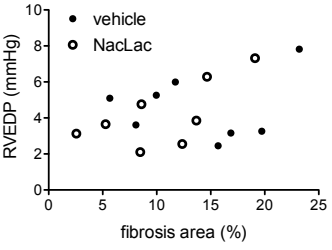
	WT	GAL3 KO	pooled
Number of XY Pairs	7	7	14
Spearman r	0.3929	0.3929	0.2352
95% confidence interval	0.3956	0.3956	-0.3530 to 0.6901
P value (two-tailed)	ns	ns	0.4183

B



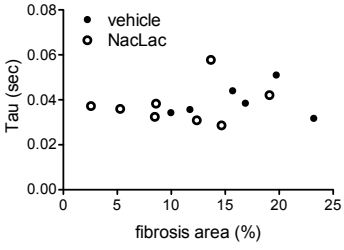
	WT	GAL3 KO	pooled
Number of XY Pairs	6	7	13
Spearman r	0.3143	0.6071	-0.1374
95% confidence interval	0.5639	0.1667	-0.6507 to 0.4621
P value (two-tailed)	ns	ns	0.6545

C



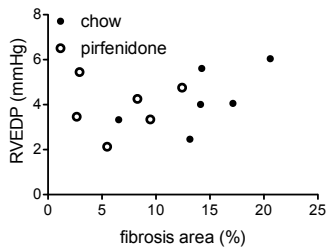
	vehicle	NacLac	pooled
Number of XY Pairs	8	8	16
Spearman r	-0.02381	0.6905	0.2765
95% confidence interval	0.9768	0.0694	-0.2692 to 0.6877
P value (two-tailed)	ns	ns	0.2999

D



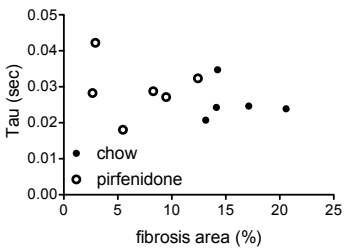
	vehicle	NacLac	pooled
Number of XY Pairs	6	8	14
Spearman r	0.08571	0.1190	0.2264
95% confidence interval	0.9194	0.7930	-0.3612 to 0.6852
P value (two-tailed)	ns	ns	0.4364

E



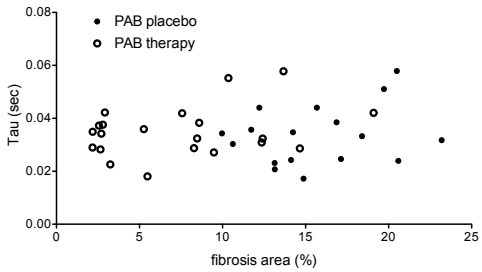
	chow	pirfenidone	pooled
Number of XY Pairs	6	6	12
Spearman r	0.8857	0.02857	0.4266
95% confidence interval	0.0333	1.0000	-0.2138 to 0.8105
P value (two-tailed)	*	ns	0.1667

F



	chow	pirfenidone	pooled
Number of XY Pairs	5	6	11
Spearman r	0.3000	0.02857	-0.2636
95% confidence interval	0.6833	1.0000	-0.7546 to 0.4166
P value (two-tailed)	ns	ns	0.4348

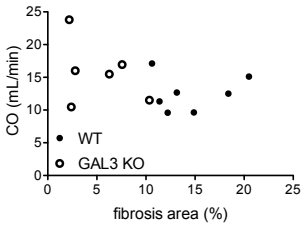
G



	PAB placebo	PAB therapy	PAB
Number of XY Pairs	17	21	38
Spearman r	0.1373	0.1584	-0.04432
95% confidence interval	-0.3810 to 0.5900	-0.3058 to 0.5618	-0.3675 to 0.2884
P value (two-tailed)	0.5991	0.4927	0.7916

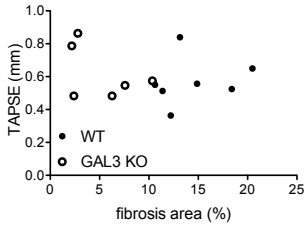
Figure E5.

A



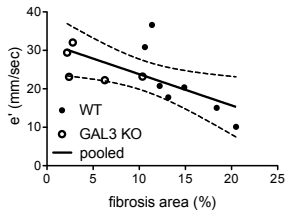
	WT	GAL3 KO	pooled
Number of XY Pairs	7	6	13
Spearman r	0.0	-0.2571	-0.4066
95% confidence interval	1.0365	0.6583	-0.7894 to 0.2039
P value (two-tailed)	ns	ns	0.1680

B



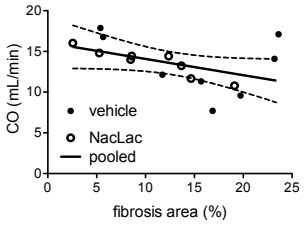
	WT	GAL3 KO	pooled
Number of XY Pairs	7	6	13
Spearman r	0.4286	-0.1449	0.0
95% confidence interval	0.3536	0.8028	-0.5637 to 0.5637
P value (two-tailed)	ns	ns	1.0000

C



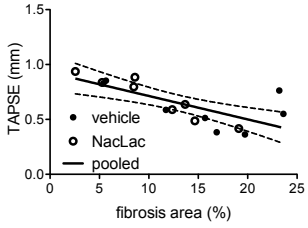
	WT	GAL3 KO	pooled
Number of XY Pairs	7	5	12
Spearman r	-0.9286	-0.3000	-0.7133
95% confidence interval	0.0067	0.6833	-0.9165 to -0.2175
P value (two-tailed)	**	ns	0.0118

D



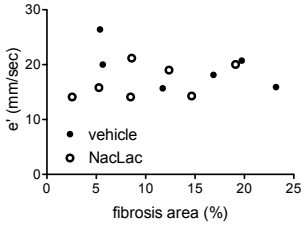
	vehicle	NacLac	pooled
Number of XY Pairs	8	8	16
Spearman r	-0.2381	-0.9286	-0.5176
95% confidence interval	0.5821	0.0022	-0.8120 to -0.01331
P value (two-tailed)	ns	**	0.0400

E



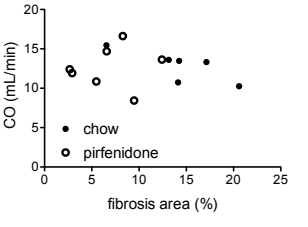
	vehicle	NacLac	pooled
Number of XY Pairs	8	8	16
Spearman r	-0.5238	-0.9048	-0.7794
95% confidence interval	0.1966	0.0046	-0.9222 to -0.4495
P value (two-tailed)	ns	**	0.0004

F



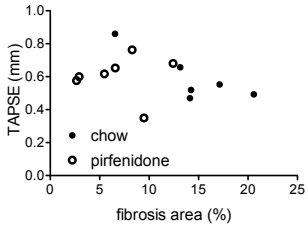
	vehicle	NacLac	pooled
Number of XY Pairs	6	7	13
Spearman r	-0.4286	0.5406	0.2091
95% confidence interval	0.4194	0.2357	-0.4020 to 0.6913
P value (two-tailed)	ns	ns	0.4930

G



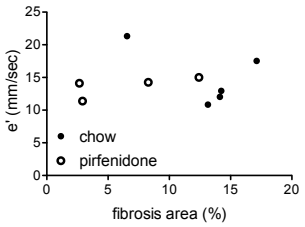
	chow	pirfenidone	pooled
Number of XY Pairs	6	7	13
Spearman r	-0.8286	0.1429	-0.1758
95% confidence interval	0.0583	0.7825	-0.6729 to 0.4306
P value (two-tailed)	ns	ns	0.5656

H



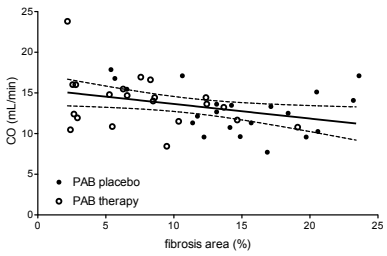
	chow	pirfenidone	pooled
Number of XY Pairs	6	7	13
Spearman r	-0.6000	0.3929	-0.4176
95% confidence interval	0.2417	0.3956	-0.7943 to 0.1911
P value (two-tailed)	ns	ns	0.1557

I



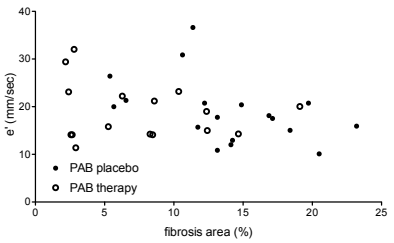
	chow	pirfenidone	pooled
Number of XY Pairs	5	4	9
Spearman r	0.0	0.8000	0.01667
P value (two-tailed)	1.0500	0.3333	0.9816
P value summary	ns	ns	ns

J



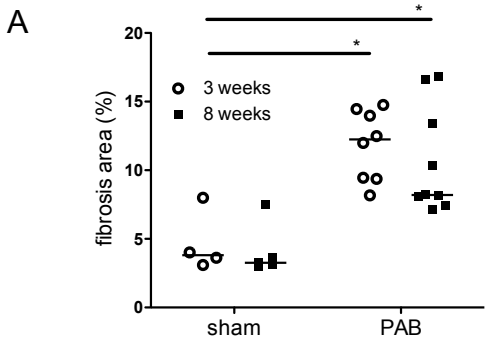
	PAB placebo	PAB therapy	PAB
Number of XY Pairs	21	21	42
Spearman r	-0.2528	-0.3584	-0.3668
95% confidence interval	-0.6256 to 0.2139	-0.6915 to 0.1003	-0.6094 to -0.06143
P value (two-tailed)	0.2688	0.1106	0.0169

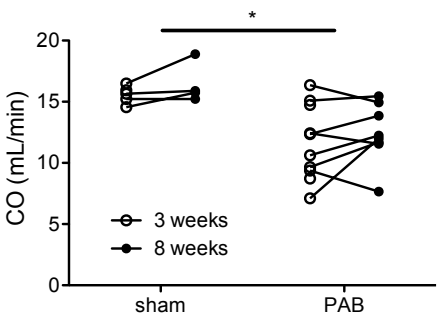
K

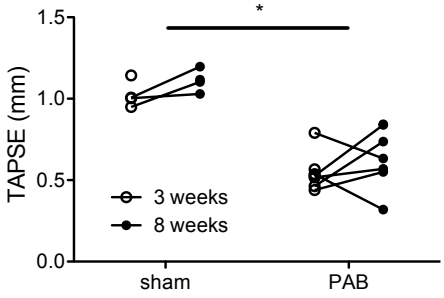


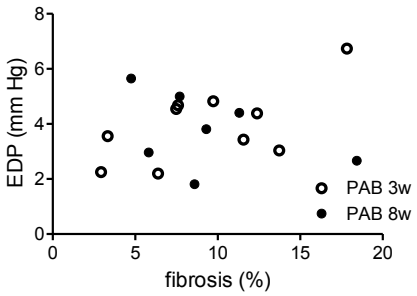
	PAB placebo	PAB therapy	PAB
Number of XY Pairs	18	16	34
Spearman r	-0.5555	-0.07965	-0.2705
95% confidence interval	-0.8169 to -0.1048	-0.5646 to 0.4462	-0.5649 to 0.08487
P value (two-tailed)	0.0167	0.7694	0.1217

Figure E6.

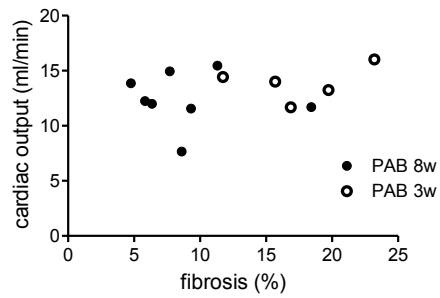




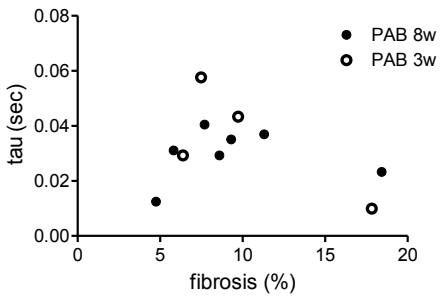




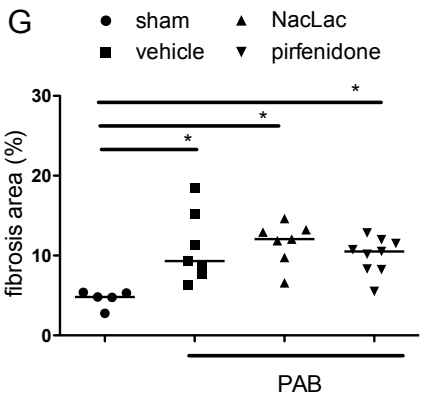
	PAB 3w	PAB 8w	pooled
Number of XY Pairs	10	7	17
Spearman r	0.4545	-0.4643	0.1201
95% confidence interval	0.1912	0.3024	-0.3959 to 0.5784
P value (two-tailed)	ns	ns	0.6461

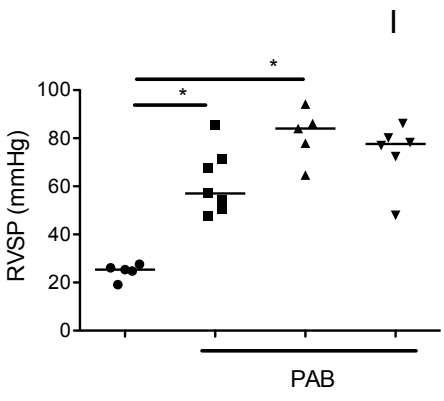


	PAB 3w	PAB 8w	pooled
Number of XY Pairs	5	8	13
Spearman r	0.1000	-0.2143	0.1703
95% confidence interval	0.9500	0.6191	-0.4352 to 0.6697
P value (two-tailed)	ns	ns	0.5780



	PAB 3w	PAB 8w	pooled
Number of XY Pairs	4	7	11
Spearman r	-0.4000	0.1786	-0.09091
95% confidence interval	0.7500	0.7131	-0.6667 to 0.5528
P value (two-tailed)	ns	ns	0.7964





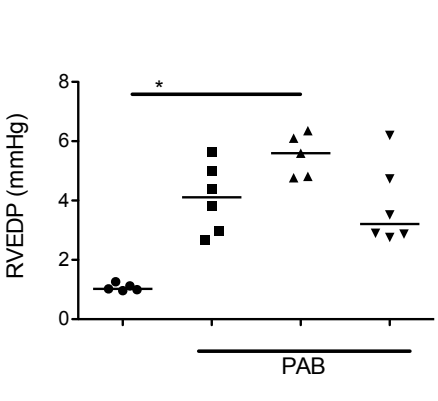
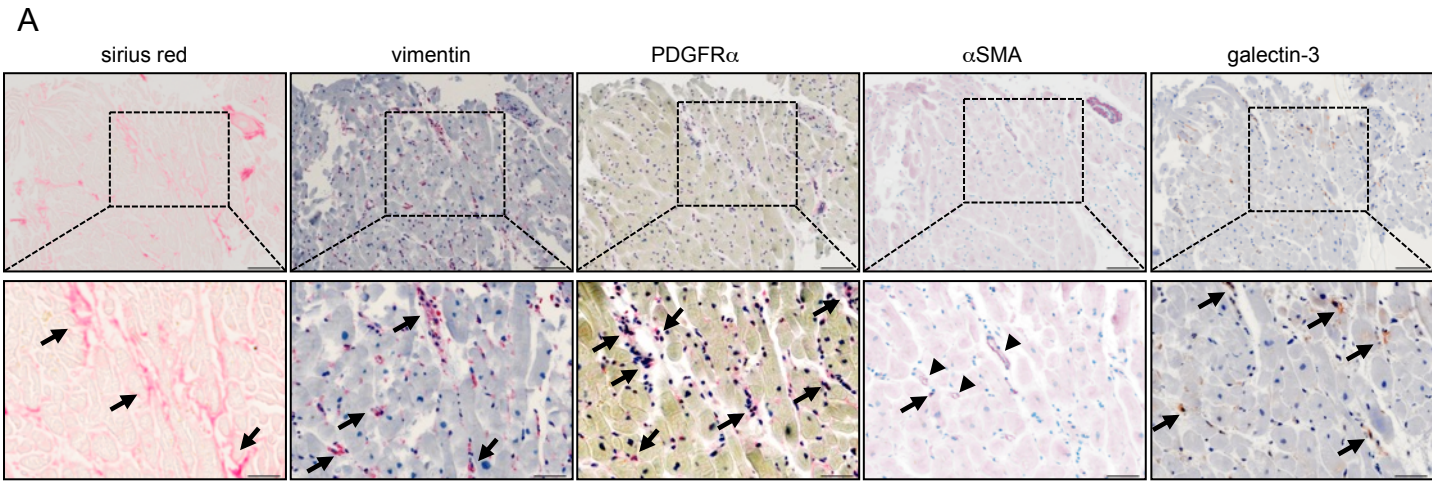
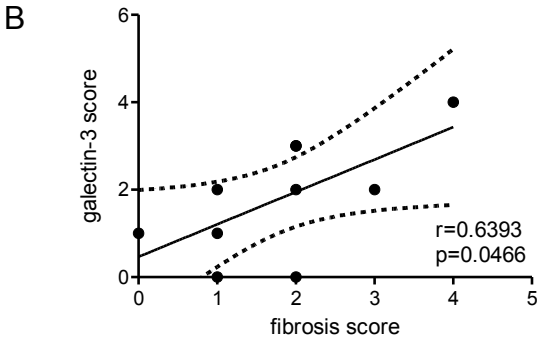


Figure E7.


A
sirius red
vimentin
PDGFRα
αSMA
galectin-3


B
galectin-3 score
fibrosis score
r=0.6393
p=0.0466
0
2
4
6
0
1
2
3
4
5