Supplemental Material

Fischer rats exhibit maladaptive structural and molecular right ventricular remodelling in severe pulmonary hypertension: a genetically prone model for right heart failure

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Results

Supplemental Table 1: ***Cardiac structural parameters by echocardiography***

Parameter/Group	**S-CTL**	**F-CTL**	**S-SUHx**	**F-SUHx**
N- value	5	5	14	13
Weight (g)	330±16	224±20^{+}	350±58	195±14*$^{\#}$
RVID-d (µm)	2410±628	1905±260	4110±507*	4062±445*
LVID-d (µm)	7545±210	6050±370^{+}	4973±538*	3523±401*$^{\#}$
RVID-d/BW (µm/g)	7.26±1.71	8.56±1.26	11.97±2.12*	21.05±3.66*$^{\#}$
LVID-d/BW (µm/g)	22.86±0.83	27.15±1.70^{+}	14.42±1.93*	18.14±2.35*$^{\#}$

*ANOVA with Tukey's post-hoc test. *$p<0.05$ vs CTL of same strain. $^{+}p<0.05$ vs S-CTL. $^{\#}p<0.05$ vs S-SUHx.*

Supplemental Table 2: Summary of differentially regulated genes in microarray analysis of RV in PH vs control

		Fischer		SD	
Gene	**Gene name**	**Fold change**	**adj p-value**	**Fold change**	**adj p-value**
Differentially regulated in either strain					
Nppb	natriuretic peptide B	12.6193	0.0065	5.9746	0.0833
Nppa	natriuretic peptide A	47.5618	0.0092	6.7470	0.4638
Hmox1	heme oxygenase 1	3.5358	0.0092	1.9637	0.2000
Crlf1	cytokine receptor like factor 1	4.4842	0.0154	2.5757	0.0500
Tnik	TRAF2 and NCK interacting kinase	0.3021	0.0154	0.5338	0.1472
Cyp2e1	cytochrome P450 family 2 subfamily E member 1	0.2938	0.0154	0.3305	0.0509
Gabrb2	gamma-aminobutyric acid type A receptor beta2 subunit	0.2310	0.0169	0.1897	0.0579
Nadk2	NAD kinase 2, mitochondrial	0.4095	0.0169	0.5344	0.1124
Epha7	EPH receptor A7	0.3951	0.0169	0.5941	0.3616
Lsmem1	leucine rich single-pass membrane protein 1	0.3975	0.0169	0.5851	0.2130
Abhd2	abhydrolase domain containing 2	2.5801	0.0169	1.8580	0.0581
Mir490	microRNA 490	0.2575	0.0169	0.3999	0.0880
Slc16a10	solute carrier family 16 member 10	0.4209	0.0172	0.4799	0.0541
Vwa8	von Willebrand factor A domain containing 8	0.4270	0.0172	0.5089	0.0581
Gpnmb	glycoprotein nmb	2.5486	0.0185	2.3586	0.0881
Gnao1	G protein subunit alpha o1	3.1942	0.0185	3.3488	0.1210
Syt14	synaptotagmin 14	0.4589	0.0187	0.5841	0.1266
Cd68	CD68 molecule	2.0678	0.0198	1.8196	0.1210
Ppm1k	protein phosphatase, Mg2+/Mn2+ dependent 1K	0.4623	0.0210	0.6887	0.1593
Nckap5	NCK associated protein 5	0.3588	0.0210	0.5747	0.0866
Stab1	stabilin 1	2.1801	0.0216	1.7006	0.1554
Acadsb	acyl-CoA dehydrogenase, short/branched chain	0.4789	0.0219	0.6194	0.0994
LOC100359438		0.4617	0.0219	0.7546	0.4779
Cd1d1		0.4610	0.0219	0.4295	0.1039
Isoc1	isochorismatase domain containing 1	0.4949	0.0225	0.6075	0.0637
Rimbp2	RIMS binding protein 2	0.4639	0.0232	0.6058	0.1310
Gpcpd1	glycerophosphocholine phosphodiesterase 1	0.3461	0.0233	0.4911	0.0541
Klrc3	killer cell lectin like receptor C3	0.4980	0.0250	0.5679	0.6643
Lgals3	galectin 3	2.2049	0.0257	1.7769	0.1168
Mcam	melanoma cell adhesion molecule	2.3836	0.0265	1.8103	0.0922
Ppp1r3a	protein phosphatase 1 regulatory subunit 3A	0.5093	0.0265	0.6923	0.2686
Klrd1	killer cell lectin like receptor D1	0.4406	0.0272	0.7043	0.3498
Gprin3	GPRIN family member 3	0.4687	0.0272	0.6638	0.1266
F2r	coagulation factor II thrombin receptor	2.1555	0.0272	1.5522	0.0848
Cds1	CDP-diacylglycerol synthase 1	0.3765	0.0272	0.5248	0.2057
Thrb	thyroid hormone receptor beta	0.5263	0.0280	0.6391	0.2081
Tgfb1i1	transforming growth factor beta 1 induced transcript 1	2.7864	0.0286	2.6509	0.0564
Decr1	2,4-dienoyl-CoA reductase 1	0.4151	0.0286	0.4458	0.0553
Mum1l1	MUM1 like 1	0.5212	0.0286	0.5951	0.1807
P2ry1	purinergic receptor P2Y1	0.5258	0.0286	0.6437	0.3664
Corin	corin, serine peptidase	0.5101	0.0286	0.4859	0.1185
Trabd2b	TraB domain containing 2B	0.5147	0.0288	0.7094	0.1954
Clic1	chloride intracellular channel 1	1.9957	0.0288	1.6340	0.0777
LOC102547230		0.4341	0.0291	0.6784	0.1614
Col6a6	collagen type VI alpha 6 chain	0.3902	0.0291	0.5727	0.1808
Erbb4	erb-b2 receptor tyrosine kinase 4	0.4365	0.0291	0.6410	0.1083
Pfkp	phosphofructokinase, platelet	2.5779	0.0291	2.1292	0.0507
Gadd45a	growth arrest and DNA damage inducible alpha	2.2398	0.0291	2.1031	0.0529
Grid1	glutamate ionotropic receptor delta type subunit 1	0.3503	0.0291	0.3708	0.0610
Tmem196	transmembrane protein 196	0.5239	0.0291	0.7424	0.2265
Kif20b	kinesin family member 20B	2.1659	0.0291	2.0602	0.2748
C1qc	complement C1q C chain	1.9959	0.0291	1.9067	0.1132
LOC498276		1.9087	0.0291	1.1401	0.7588
Ccl2	C-C motif chemokine ligand 2	2.4230	0.0291	2.6266	0.0627
Ednrb	endothelin receptor type B	1.9439	0.0292	1.7535	0.0564
Adamts1	ADAM metallopeptidase with thrombospondin type 1 motif 1	2.0433	0.0292	1.5864	0.1343
Tgfb1	transforming growth factor beta 1	1.9433	0.0292	1.7548	0.0564
Anxa2	annexin A2	2.2868	0.0292	1.8147	0.1086
Ptprd	protein tyrosine phosphatase, receptor type D	0.4680	0.0292	0.5454	0.0591
Gcnt1	glucosaminyl (N-acetyl) transferase 1, core 2	0.4588	0.0292	0.5844	0.1008
Piwil2	piwi like RNA-mediated gene silencing 2	0.4213	0.0292	0.4310	0.0686
Anxa5	annexin A5	2.0730	0.0292	1.7473	0.0690
Emc9	ER membrane protein complex subunit 9	0.5078	0.0292	0.6090	0.1168
Eng	endoglin	1.8556	0.0292	1.2453	0.4290
Ftl1		1.9465	0.0292	1.4752	0.3036

Gene	Gene name	Fischer		SD	
		Fold change	adj p-value	Fold change	adj p-value
Arhgap26	Rho GTPase activating protein 26	0.4501	0.0292	0.5790	0.1266
Flrt2	fibronectin leucine rich transmembrane protein 2	3.1795	0.0292	1.9982	0.0553
Klhl38	kelch like family member 38	0.3990	0.0292	0.5813	0.1953
Sptlc2	serine palmitoyltransferase long chain base subunit 2	2.0422	0.0293	1.7637	0.0534
Osbpl6	oxysterol binding protein like 6	0.3474	0.0293	0.4955	0.1953
Pank1	pantothenate kinase 1	0.5387	0.0293	0.6471	0.2363
Slc16a7	solute carrier family 16 member 7	0.4054	0.0293	0.6193	0.0880
Sphkap	SPHK1 interactor, AKAP domain containing	0.5516	0.0293	1.1640	0.8203
Ank2	ankyrin 2	0.5531	0.0293	0.6038	0.0882
Ms4a7	membrane spanning 4-domains A7	2.1003	0.0294	1.8086	0.2073
Slc22a5	solute carrier family 22 member 5	0.5633	0.0301	0.5655	0.0509
Cthrc1	collagen triple helix repeat containing 1	2.4018	0.0301	2.3199	0.0580
Loxl1	lysyl oxidase like 1	3.0915	0.0301	2.7693	0.0541
Sqle	squalene epoxidase	2.1549	0.0301	1.5470	0.2766
Klrb1c		0.3473	0.0301	0.4938	0.0813
Fam13a	family with sequence similarity 13 member A	0.5732	0.0304	0.6874	0.2592
Rp1	RP1, axonemal microtubule associated	0.4705	0.0305	0.5643	0.1986
Amd1	adenosylmethionine decarboxylase 1	0.5261	0.0305	0.5684	0.0650
Emp1	epithelial membrane protein 1	2.4771	0.0305	1.8590	0.1283
Myoc	myocilin	0.4173	0.0320	0.4500	0.1593
Rai14	retinoic acid induced 14	1.7871	0.0322	1.3930	0.1457
Ppm1l	protein phosphatase, Mg2+/Mn2+ dependent 1L	0.5750	0.0322	0.5991	0.0848
Mpeg1	macrophage expressed 1	1.9393	0.0322	1.9467	0.0564
Tgm2	transglutaminase 2	2.6232	0.0322	1.9559	0.0564
Hook1	hook microtubule tethering protein 1	0.4693	0.0322	0.5573	0.0637
Baz1a	bromodomain adjacent to zinc finger domain 1A	1.7083	0.0322	1.3791	0.2085
Ghr	growth hormone receptor	0.5459	0.0322	0.6841	0.1492
Hsdl2	hydroxysteroid dehydrogenase like 2	0.5144	0.0323	0.5890	0.1100
Ccr2	C-C motif chemokine receptor 2	0.2631	0.0323	0.8702	0.7665
LOC367515		0.4163	0.0323	0.4570	0.2091
Lama5	laminin subunit alpha 5	2.0897	0.0323	1.5082	0.1123
Lxn	latexin	2.0314	0.0323	1.5483	0.0922
C5ar1	complement C5a receptor 1	1.7979	0.0323	1.7283	0.1853
Tox	thymocyte selection associated high mobility group box	0.4667	0.0327	0.6375	0.1135
Dynap	dynactin associated protein	0.1679	0.0327	0.4446	0.1644
Map2k6	mitogen-activated protein kinase kinase 6	0.4174	0.0327	0.4613	0.1277
Il1r1	interleukin 1 receptor type 1	2.1224	0.0327	2.0167	0.0507
Perp	PERP, TP53 apoptosis effector	0.3210	0.0327	0.5573	0.0564
LOC681266		1.8653	0.0327	1.5956	0.1155
Sat1	spermidine/spermine N1-acetyltransferase 1	1.7843	0.0327	1.2346	0.3859
Ndrg4	NDRG family member 4	1.8221	0.0327	1.7620	0.1100
Cyp4b1	cytochrome P450 family 4 subfamily B member 1	0.4816	0.0327	0.6240	0.1791
Pcnt	pericentrin	0.4805	0.0329	0.5088	0.0762
Foxs1	forkhead box S1	1.8334	0.0330	1.0405	0.9181
Cd86	CD86 molecule	2.1621	0.0331	2.2042	0.0749
Hpgds	hematopoietic prostaglandin D synthase	2.0509	0.0341	1.8519	0.4206
Fign	fidgetin, microtubule severing factor	0.4712	0.0346	0.7347	0.3627
Ecm1	extracellular matrix protein 1	2.2444	0.0349	1.7361	0.2583
Eno2	enolase 2	2.0413	0.0349	1.1961	0.7852
Asb15	ankyrin repeat and SOCS box containing 15	0.4252	0.0351	0.5054	0.0512
RragB	Ras related GTP binding B	0.5592	0.0356	0.7563	0.3097
Acot2	acyl-CoA thioesterase 2	0.4934	0.0361	0.5096	0.0994
RGD1561161		0.5498	0.0361	0.7491	0.2429
Oxr1	oxidation resistance 1	0.5818	0.0361	0.7129	0.1464
Cidea	cell death-inducing DFFA-like effector a	0.5688	0.0361	0.6675	0.1449
Iqub	IQ motif and ubiquitin domain containing	0.4598	0.0361	0.5597	0.2132
Odc1	ornithine decarboxylase 1	1.7727	0.0361	1.2598	0.2845
Prodh	proline dehydrogenase 1	0.2257	0.0361	0.3272	0.0570
Ptgis	prostaglandin I2 synthase	2.0164	0.0362	1.6421	0.1423
Bche	butyrylcholinesterase	0.5872	0.0362	0.6227	0.0690
Ppp1r3d	protein phosphatase 1 regulatory subunit 3D	0.4800	0.0362	0.5668	0.0690
Cdh13	cadherin 13	1.9121	0.0362	1.6179	0.1875
Hspb3	heat shock protein family B (small) member 3	2.2230	0.0362	2.1580	0.0564
Fam46c	family with sequence similarity 46 member C	2.5381	0.0362	1.5855	0.1667
Phkg1	phosphorylase kinase catalytic subunit gamma 1	0.4477	0.0362	0.3786	0.1932
Lmcd1	LIM and cysteine rich domains 1	1.9741	0.0362	1.5145	0.1604
Tmem65	transmembrane protein 65	0.5401	0.0362	0.6660	0.1083
Klrk1	killer cell lectin like receptor K1	0.4881	0.0362	0.9005	0.7189
Ppfibp2	PPFIA binding protein 2	0.5066	0.0362	0.6690	0.2821
Islr	immunoglobulin superfamily containing leucine rich repeat	3.1070	0.0362	2.8632	0.0849

Gene	Gene name	Fischer Fold change	adj p-value	SD Fold change	adj p-value
LOC100362543		1.8105	0.0362	1.0076	0.9909
C1qa	complement C1q A chain	1.7697	0.0364	1.6708	0.2643
Sesn1	sestrin 1	0.5471	0.0364	0.6208	0.0762
LOC102552996		0.3779	0.0364	0.4230	0.0677
Unc5b	unc-5 netrin receptor B	2.4247	0.0366	1.7126	0.2323
Meis1	Meis homeobox 1	0.5786	0.0370	0.6180	0.0847
Clec7a	C-type lectin domain family 7 member A	1.9574	0.0370	2.0257	0.0709
As3mt	arsenite methyltransferase	0.5786	0.0371	0.7430	0.2741
Coq10b	coenzyme Q10B	1.6322	0.0371	1.6419	0.1133
Tbc1d4	TBC1 domain family member 4	0.5570	0.0374	0.6745	0.1114
Junb	JunB proto-oncogene, AP-1 transcription factor subunit	2.0690	0.0379	1.4858	0.4009
Avpi1	arginine vasopressin induced 1	2.1510	0.0380	1.9527	0.0963
LOC102547145		0.5260	0.0391	0.4740	0.0690
Srpx2	sushi repeat containing protein, X-linked 2	1.9295	0.0393	2.1069	0.0708
Ly86	lymphocyte antigen 86	2.1621	0.0394	1.8611	0.0564
Itga9	integrin subunit alpha 9	2.1157	0.0400	1.9086	0.0677
Ccdc28a	coiled-coil domain containing 28A	0.5700	0.0400	0.6854	0.3192
Iqgap2	IQ motif containing GTPase activating protein 2	0.5428	0.0400	0.4149	0.0599
Cers4	ceramide synthase 4	0.5569	0.0400	0.6403	0.1100
Sult1a1	sulfotransferase family 1A member 1	0.4401	0.0400	0.3987	0.0534
Slc27a1	solute carrier family 27 member 1	0.5628	0.0400	0.6002	0.0815
Efcab6	EF-hand calcium binding domain 6	2.0039	0.0400	2.1749	0.1911
Tagln2	transgelin 2	1.8354	0.0400	1.3734	0.2813
Ldlr	low density lipoprotein receptor	1.8171	0.0400	1.6550	0.2305
B3galt2	beta-1,3-galactosyltransferase 2	1.9858	0.0400	1.8765	0.2581
Sema3d	semaphorin 3D	1.9688	0.0400	1.4336	0.2150
Usp2	ubiquitin specific peptidase 2	0.5857	0.0400	0.7713	0.2813
Fzd2	frizzled class receptor 2	1.8169	0.0404	1.3394	0.2313
Plat	plasminogen activator, tissue type	2.0445	0.0404	1.1778	0.6730
Depdc1	DEP domain containing 1	2.2562	0.0408	1.4354	0.2718
Cd2	CD2 molecule	0.4858	0.0408	0.5881	0.2021
LOC102555672		0.5237	0.0408	0.6845	0.2256
Vash2	vasohibin 2	2.2300	0.0408	1.8462	0.0587
LOC691325		0.4863	0.0408	0.6113	0.5106
Gamt	guanidinoacetate N-methyltransferase	0.5395	0.0408	0.4704	0.0507
LOC685505		0.5202	0.0409	0.8508	0.7242
Acsl1	acyl-CoA synthetase long-chain family member 1	0.5407	0.0409	0.5964	0.0583
Herc3	HECT and RLD domain containing E3 ubiquitin protein ligase 3	0.5818	0.0409	0.6338	0.0708
RGD1311756		0.5685	0.0411	0.6728	0.1979
Ftl1l1		1.8416	0.0411	1.4486	0.3458
Mir32	microRNA 32	0.5535	0.0420	0.6622	0.3126
Tnfaip6	TNF alpha induced protein 6	2.0238	0.0426	1.1497	0.6728
Rhoq	ras homolog family member Q	2.2006	0.0426	1.9639	0.0762
Chi3l1	chitinase 3 like 1	3.6964	0.0427	2.5238	0.1078
Zfpm2	zinc finger protein, FOG family member 2	0.6203	0.0430	0.7231	0.2148
C1qb	complement C1q B chain	1.9006	0.0430	2.2538	0.1182
Ccnd1	cyclin D1	2.0780	0.0430	1.5976	0.1453
Hspa2	heat shock protein family A (Hsp70) member 2	1.7197	0.0430	1.1436	0.8045
Cnst	consortin, connexin sorting protein	0.5511	0.0430	0.6398	0.1283
Gcsam	germinal center associated signaling and motility	0.4676	0.0430	0.7320	0.2544
Gba3	glucosylceramidase beta 3 (gene/pseudogene)	3.2596	0.0430	2.4416	0.0599
Kcnd3	potassium voltage-gated channel subfamily D member 3	0.6145	0.0430	0.8157	0.4720
Cd55	CD55 molecule (Cromer blood group)	2.1780	0.0430	2.2225	0.0509
Il1rn	interleukin 1 receptor antagonist	1.8775	0.0430	1.9872	0.0690
Adamtsl2	ADAMTS like 2	1.9368	0.0430	1.7726	0.1660
Trpc6	transient receptor potential cation channel subfamily C member 6	2.4916	0.0430	2.0454	0.0902
Ankrd1	ankyrin repeat domain 1	1.7234	0.0430	1.4945	0.1008
Alk	anaplastic lymphoma receptor tyrosine kinase	1.6514	0.0430	0.8994	0.7564
Dip2c	disco interacting protein 2 homolog C	0.5769	0.0430	0.5966	0.1011
Mical2	microtubule associated monooxygenase, calponin and LIM domain containing 2	1.7775	0.0430	1.6038	0.0916
Pik3ip1	phosphoinositide-3-kinase interacting protein 1	0.5748	0.0430	0.8054	0.5728
Stx11	syntaxin 11	2.5853	0.0430	2.5679	0.0583
Dcxr	dicarbonyl and L-xylulose reductase	0.5728	0.0430	0.5156	0.1644
Id4	inhibitor of DNA binding 4, HLH protein	1.6095	0.0430	1.2531	0.6149
RGD1563400		1.8654	0.0431	1.5826	0.0849
RGD1566399		0.5795	0.0431	0.7189	0.1729
Ttk	TTK protein kinase	2.0962	0.0431	1.7726	0.3890
Pde3a	phosphodiesterase 3A	0.5911	0.0431	0.6059	0.0690
Tax1bp3	Tax1 binding protein 3	1.6857	0.0431	1.3386	0.3984
Hadhb	hydroxyacyl-CoA dehydrogenase/3-ketoacyl-CoA thiolase/enoyl-CoA hydratase	0.5156	0.0431	0.5896	0.1100

Gene	Gene name	Fischer Fold change	adj p-value	SD Fold change	adj p-value
	(trifunctional protein), beta subunit				
Ywhag	tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein gamma	1.6019	0.0431	1.5089	0.1372
Pcmtd2	protein-L-isoaspartate (D-aspartate) O-methyltransferase domain containing 2	0.6111	0.0431	0.6668	0.1593
Adam1a	ADAM metallopeptidase domain 1A (pseudogene)	0.5637	0.0431	0.6560	0.1147
Idi1	isopentenyl-diphosphate delta isomerase 1	1.8086	0.0432	1.5498	0.1054
Smim19	small integral membrane protein 19	0.6286	0.0432	0.7805	0.2465
Fhl1	four and a half LIM domains 1	1.8109	0.0432	1.8584	0.0826
Scn3b	sodium voltage-gated channel beta subunit 3	4.9263	0.0432	4.0952	0.0564
Ucp3	uncoupling protein 3	0.5402	0.0432	0.4852	0.1986
LOC100360507		0.3470	0.0432	0.4618	0.0595
Cecr2	CECR2, histone acetyl-lysine reader	0.5280	0.0432	0.8546	0.4706
Maoa	monoamine oxidase A	2.7086	0.0432	2.6604	0.1266
Inpp4b	inositol polyphosphate-4-phosphatase type II B	0.5832	0.0432	0.6497	0.1283
Slc30a3	solute carrier family 30 member 3	1.7877	0.0432	1.1824	0.5721
Mfap5	microfibrillar associated protein 5	2.5310	0.0432	1.8905	0.1822
Cyb5b	cytochrome b5 type B	0.5977	0.0432	0.6025	0.0745
Mkx	mohawk homeobox	0.3716	0.0432	0.5581	0.1724
Hpd	4-hydroxyphenylpyruvate dioxygenase	0.5097	0.0432	0.7040	0.3932
Inpp5j	inositol polyphosphate-5-phosphatase J	1.7940	0.0432	1.4286	0.2618
Cd151	CD151 molecule (Raph blood group)	1.7044	0.0433	1.3413	0.2778
Tfdp2	transcription factor Dp-2	0.5909	0.0433	0.6689	0.1135
Ckb	creatine kinase B	2.0692	0.0433	1.3041	0.4051
Hist2h2bb	histone cluster 2 H2B family member b (pseudogene)	1.6798	0.0433	1.2991	0.4878
Zc3h6	zinc finger CCCH-type containing 6	0.5810	0.0433	0.6929	0.2595
Fbp2	fructose-bisphosphatase 2	0.5185	0.0433	0.6454	0.3914
Car4	carbonic anhydrase 4	0.5373	0.0433	0.7146	0.2939
Cnn3	calponin 3	1.6463	0.0433	1.4083	0.1745
Cep70	centrosomal protein 70	0.5513	0.0433	0.7431	0.3629
Rps6ka5	ribosomal protein S6 kinase A5	0.5553	0.0433	0.7109	0.3237
Edn1	endothelin 1	2.2115	0.0433	1.3092	0.3475
Uck2	uridine-cytidine kinase 2	1.6499	0.0433	1.2368	0.4720
Foxp2	forkhead box P2	0.5641	0.0433	0.6718	0.2570
Plk2	polo like kinase 2	2.2053	0.0437	1.8946	0.0690
Dirc2	disrupted in renal carcinoma 2	0.6000	0.0438	0.6392	0.0787
Thap4	THAP domain containing 4	0.5781	0.0443	0.6867	0.1701
Tet1	tet methylcytosine dioxygenase 1	0.5569	0.0445	0.7292	0.2845
Gfpt2	glutamine-fructose-6-phosphate transaminase 2	2.0138	0.0445	1.7986	0.1100
Tecrl	trans-2,3-enoyl-CoA reductase-like	0.5453	0.0446	0.6602	0.1370
Acot11	acyl-CoA thioesterase 11	0.6083	0.0446	0.7376	0.1946
Ube2q2l	ubiquitin conjugating enzyme E2 Q2 like	1.6740	0.0446	1.5306	0.2268
Pcp4l1	Purkinje cell protein 4 like 1	0.6121	0.0446	0.7912	0.4170
Fgf7	fibroblast growth factor 7	0.4164	0.0446	1.1174	0.8840
Bcat2	branched chain amino acid transaminase 2	0.5283	0.0447	0.4722	0.0627
Gem	GTP binding protein overexpressed in skeletal muscle	2.0273	0.0447	1.3833	0.3096
Epha3	EPH receptor A3	0.4144	0.0447	0.6100	0.1384
Ctsz	cathepsin Z	1.7250	0.0449	1.4547	0.2012
Dnajb5	DnaJ heat shock protein family (Hsp40) member B5	1.6924	0.0449	1.3241	0.2623
Mitf	melanogenesis associated transcription factor	0.6244	0.0449	0.7452	0.1821
Sh3pxd2b	SH3 and PX domains 2B	2.0287	0.0449	1.4692	0.1132
Fundc1	FUN14 domain containing 1	0.5994	0.0449	0.6424	0.1038
Gramd1b	GRAM domain containing 1B	0.6237	0.0449	0.8246	0.4929
LOC102551257		0.5839	0.0453	0.9896	0.9919
Erc1	ELKS/RAB6-interacting/CAST family member 1	0.6294	0.0453	0.6861	0.1923
Pls3	plastin 3	1.7026	0.0456	1.4876	0.1147
Fam81a	family with sequence similarity 81 member A	0.5034	0.0456	0.5230	0.1464
Fibin	fin bud initiation factor homolog (zebrafish)	4.4079	0.0456	2.9882	0.0971
Slc25a34	solute carrier family 25 member 34	0.5640	0.0456	0.6949	0.1302
Lamb3	laminin subunit beta 3	2.3516	0.0456	1.6636	0.1132
Ctsa	cathepsin A	1.6322	0.0456	1.3181	0.3022
Lpcat3	lysophosphatidylcholine acyltransferase 3	0.5549	0.0456	0.5883	0.0541
Slamf8	SLAM family member 8	1.7277	0.0456	1.4198	0.4202
Tpm3	tropomyosin 3	1.5621	0.0456	1.3975	0.2813
Litaf	lipopolysaccharide induced TNF factor	1.7941	0.0458	1.4420	0.2030
Ccdc28b	coiled-coil domain containing 28B	0.6025	0.0458	0.6698	0.0838
Pnp	purine nucleoside phosphorylase	1.8572	0.0458	1.5731	0.1986
Gstz1	glutathione S-transferase zeta 1	0.3416	0.0458	0.3193	0.0529
Dcaf11	DDB1 and CUL4 associated factor 11	0.6299	0.0462	0.6825	0.1152
Klrb1a		0.4479	0.0464	0.5254	0.1231
Cdkal1	CDK5 regulatory subunit associated protein 1 like 1	0.5920	0.0464	0.8218	0.6087

Gene	Gene name	Fischer Fold change	adj p-value	SD Fold change	adj p-value
RGD1305184		0.3534	0.0464	0.3026	0.0813
Ccdc85a	coiled-coil domain containing 85A	0.5072	0.0464	0.5855	0.1548
Il7r	interleukin 7 receptor	1.8120	0.0464	1.7763	0.0690
Baalc	brain and acute leukemia, cytoplasmic	0.4976	0.0464	0.7791	0.3012
Magix	MAGI family member, X-linked	0.5074	0.0464	0.5478	0.0595
Flnc	filamin C	1.6929	0.0472	1.7886	0.0553
Sema4d	semaphorin 4D	0.5060	0.0472	0.6202	0.0833
RGD1562667		0.5004	0.0472	0.8776	0.6924
Csf2rb	colony stimulating factor 2 receptor beta common subunit	1.6623	0.0472	1.1842	0.6920
RGD1566189		1.8958	0.0472	1.5528	0.2848
Slitrk1	SLIT and NTRK like family member 1	0.5715	0.0474	0.7519	0.5645
Ctsc	cathepsin C	0.5919	0.0474	0.6528	0.1006
Aldh6a1	aldehyde dehydrogenase 6 family member A1	0.6127	0.0474	0.6792	0.1155
Med21	mediator complex subunit 21	1.5595	0.0475	1.6007	0.0748
Egln3	egl-9 family hypoxia inducible factor 3	1.6414	0.0475	1.5696	0.1372
Disp1	dispatched RND transporter family member 1	0.6090	0.0477	0.8011	0.3892
Hmcn2	hemicentin 2	1.9314	0.0477	1.6644	0.1086
Fads3	fatty acid desaturase 3	1.6264	0.0477	1.3444	0.2649
Hadha	hydroxyacyl-CoA dehydrogenase/3-ketoacyl-CoA thiolase/enoyl-CoA hydratase (trifunctional protein), alpha subunit	0.5781	0.0477	0.5877	0.0570
Fcgr1a	Fc fragment of IgG receptor Ia	1.9368	0.0477	1.8465	0.1369
Iscu	iron-sulfur cluster assembly enzyme	1.6353	0.0477	1.5050	0.1622
Lipe	lipase E, hormone sensitive type	0.5737	0.0477	0.6316	0.1302
Adcy6	adenylate cyclase 6	0.6064	0.0477	0.6092	0.0591
Grn	granulin precursor	1.6460	0.0477	1.4517	0.3914
RGD1311251		0.5574	0.0477	0.9958	0.9923
Adcy1	adenylate cyclase 1	0.4542	0.0477	0.4073	0.0690
Pcdh7	protocadherin 7	0.6001	0.0477	0.6518	0.2091
Vom1r47		1.6251	0.0477	0.8817	0.7573
Gria1	glutamate ionotropic receptor AMPA type subunit 1	0.5874	0.0477	0.8105	0.6507
LOC102555217		0.6386	0.0478	1.0296	0.9724
Cyp2j4	cytochrome P450 family 2 subfamily J member 4	0.5471	0.0482	0.6544	0.1468
Lancl1	LanC like 1	0.6091	0.0483	0.7195	0.1604
Slamf9	SLAM family member 9	2.0779	0.0484	1.8273	0.2232
Cd69	CD69 molecule	0.4899	0.0484	0.6739	0.2848
LOC100360205		0.5781	0.0484	0.9881	0.9754
Fxyd6	FXYD domain containing ion transport regulator 6	2.1596	0.0484	1.7110	0.0749
Ctsl	cathepsin L	1.5508	0.0486	1.4783	0.1529
Dlgap1	DLG associated protein 1	0.6011	0.0486	0.6854	0.2683
Htatip2	HIV-1 Tat interactive protein 2	1.5989	0.0489	1.2813	0.3142
Atg10	autophagy related 10	0.6463	0.0489	0.7319	0.1576
Ncapg	non-SMC condensin I complex subunit G	2.0647	0.0489	1.4956	0.4178
Ppargc1a	PPARG coactivator 1 alpha	0.5387	0.0495	0.6473	0.0847
Sh3bgrl3	SH3 domain binding glutamate rich protein like 3	1.6459	0.0496	1.2880	0.4354
Clnk	cytokine dependent hematopoietic cell linker	0.5944	0.0496	0.7224	0.3428
Fcer1g	Fc fragment of IgE receptor Ig	1.6729	0.0498	1.9070	0.0623
Hfe2	hemochromatosis type 2 (juvenile)	0.4598	0.0564	0.4152	0.0214
Lsamp	limbic system-associated membrane protein	0.2445	0.0569	0.2545	0.0058
Abca5	ATP binding cassette subfamily A member 5	3.0678	0.0582	2.6446	0.0321
Plod2	procollagen-lysine,2-oxoglutarate 5-dioxygenase 2	2.7881	0.0607	3.3632	0.0236
Rxrg	retinoid X receptor gamma	0.4570	0.0610	0.4502	0.0277
Klrc1	killer cell lectin like receptor C1	0.5227	0.0616	0.4838	0.0443
Kcna2	potassium voltage-gated channel subfamily A member 2	0.3121	0.0652	0.4640	0.0456
Ephx2	epoxide hydrolase 2	0.6024	0.0652	0.4348	0.0443
Crat	carnitine O-acetyltransferase	0.5657	0.0652	0.5435	0.0443
Asb4	ankyrin repeat and SOCS box containing 4	0.4474	0.0675	0.4280	0.0371
Aqp7	aquaporin 7	0.2366	0.0696	0.1947	0.0455
Fitm1	fat storage inducing transmembrane protein 1	0.5480	0.0696	0.4984	0.0321
Pi16	peptidase inhibitor 16	2.4583	0.0698	2.7178	0.0472
Fkbp4	FK506 binding protein 4	0.4960	0.0702	0.4556	0.0236
Hadh	hydroxyacyl-CoA dehydrogenase	0.4212	0.0750	0.4196	0.0277
Mybl1	MYB proto-oncogene like 1	2.3660	0.0752	3.1589	0.0419
Tmod4	tropomodulin 4	0.3977	0.0758	0.3910	0.0236
Dhrs7c	dehydrogenase/reductase 7C	0.2363	0.0768	0.2433	0.0122
Macrod1	MACRO domain containing 1	0.4885	0.0824	0.4411	0.0277
Uap1	UDP-N-acetylglucosamine pyrophosphorylase 1	2.2610	0.0824	2.0719	0.0491
Kcnd2	potassium voltage-gated channel subfamily D member 2	0.3095	0.0845	0.3031	0.0122
Clcn4	chloride voltage-gated channel 4	0.5265	0.0845	0.4822	0.0258
Fap	fibroblast activation protein alpha	2.3651	0.0877	2.8794	0.0122
Cxcr4	C-X-C motif chemokine receptor 4	2.3851	0.0902	2.0009	0.0410

		Fischer		**SD**	
Gene	**Gene name**	**Fold change**	**adj p-value**	**Fold change**	**adj p-value**
Clec5a	C-type lectin domain family 5 member A	1.8990	0.0939	2.8644	0.0277
Art1	ADP-ribosyltransferase 1	0.3555	0.1077	0.2052	0.0132
Fitm2	fat storage inducing transmembrane protein 2	0.5290	0.1101	0.4200	0.0491
Tnfsf18	tumor necrosis factor superfamily member 18	2.1695	0.1174	2.3636	0.0323
Fam111a	family with sequence similarity 111 member A	2.0780	0.1226	2.6403	0.0277
Foxo1	forkhead box O1	0.6902	0.1385	0.5417	0.0491
Hhatl	hedgehog acyltransferase-like	0.5383	0.1387	0.4082	0.0142
Sbsn	suprabasin	2.6016	0.1445	3.0569	0.0277
Pfkm	phosphofructokinase, muscle	0.5536	0.1451	0.5077	0.0410
Depdc7	DEP domain containing 7	2.0160	0.1554	2.7717	0.0319
Rpl3l	ribosomal protein L3 like	0.4694	0.1722	0.5327	0.0320
Xpr1	xenotropic and polytropic retrovirus receptor 1	1.9265	0.1931	1.9420	0.0319
Fndc5	fibronectin type III domain containing 5	0.6285	0.2257	0.4931	0.0296
Aoah	acyloxyacyl hydrolase	1.2598	0.4124	2.0271	0.0410
LOC102554467		0.7951	0.5535	0.3352	0.0258
Zim1		0.8618	0.7738	0.4213	0.0277
Fam110c	family with sequence similarity 110 member C	1.0046	0.9916	2.4596	0.0154

Upregulated in both strains

Gene	Gene name	Fold change	adj p-value	Fold change	adj p-value
Cyp1a1	cytochrome P450 family 1 subfamily A member 1	18.5171	0.0007	19.9919	0.0014
Tceal7	transcription elongation factor A like 7	11.1439	0.0007	9.0831	0.0115
Rcan1	regulator of calcineurin 1	3.1554	0.0092	2.4327	0.0258
Tnfrsf12a	TNF receptor superfamily member 12A	4.0118	0.0092	3.2911	0.0154
Mest	mesoderm specific transcript	3.0619	0.0092	2.4075	0.0410
Acta1	actin, alpha 1, skeletal muscle	11.1305	0.0097	5.5002	0.0455
Amica1	junction adhesion molecule like	3.4190	0.0097	2.4180	0.0258
Akip1	A-kinase interacting protein 1	6.5008	0.0154	6.5742	0.0036
Ankrd23	ankyrin repeat domain 23	7.9228	0.0159	10.4485	0.0122
Bex1	brain expressed X-linked 1	6.6050	0.0172	7.3223	0.0122
Myot	myotilin	2.5365	0.0174	2.9235	0.0277
Fxyd5	FXYD domain containing ion transport regulator 5	3.0398	0.0174	2.5173	0.0190
Tlr5	toll like receptor 5	2.2476	0.0187	2.3341	0.0456
Ncam1	neural cell adhesion molecule 1	6.9153	0.0187	7.4160	0.0190
Tgfb2	transforming growth factor beta 2	6.2014	0.0209	4.7572	0.0400
Ccnd2	cyclin D2	2.2115	0.0219	2.2701	0.0367
Cyp1b1	cytochrome P450 family 1 subfamily B member 1	5.8359	0.0219	4.2054	0.0256
Tsc22d1	TSC22 domain family member 1	2.7726	0.0225	2.5858	0.0132
Osmr	oncostatin M receptor	2.2309	0.0232	2.1514	0.0296
Thbs1	thrombospondin 1	5.0405	0.0232	3.1856	0.0442
Ahrr	aryl-hydrocarbon receptor repressor	2.7523	0.0272	2.3674	0.0410
Fgf23	fibroblast growth factor 23	9.1111	0.0272	4.5384	0.0277
Ifitm1	interferon induced transmembrane protein 1	2.6934	0.0272	2.0651	0.0320
Rnd1	Rho family GTPase 1	4.9707	0.0272	4.2722	0.0230
RGD1559864		2.7574	0.0274	2.1238	0.0410
Gclc	glutamate-cysteine ligase catalytic subunit	2.0524	0.0291	2.0330	0.0491
Postn	periostin	8.8373	0.0291	7.0604	0.0491
Enah	enabled homolog (Drosophila)	2.7077	0.0291	2.4483	0.0277
LOC100910669		2.1784	0.0292	2.5888	0.0443
Ctgf	connective tissue growth factor	4.8038	0.0292	3.5118	0.0367
Tmem100	transmembrane protein 100	3.3493	0.0312	3.6365	0.0236
Col8a1	collagen type VIII alpha 1 chain	6.1455	0.0318	6.4619	0.0251
C3	complement C3	2.1784	0.0322	1.9973	0.0410
Timp1	TIMP metallopeptidase inhibitor 1	3.1414	0.0327	3.2529	0.0122
Olr1	oxidized low density lipoprotein receptor 1	5.4220	0.0338	8.2207	0.0122
Wbp5	transcription elongation factor A like 9	1.9770	0.0361	1.9143	0.0320
RGD1311744		4.5079	0.0361	3.5994	0.0277
Thbs4	thrombospondin 4	16.5124	0.0362	10.1139	0.0125
Prss23	protease, serine 23	2.3515	0.0420	2.5018	0.0431
Cx3cr1	C-X3-C motif chemokine receptor 1	2.3420	0.0430	2.4459	0.0376
Xirp2	xin actin binding repeat containing 2	2.6386	0.0431	3.0736	0.0151
Cilp	cartilage intermediate layer protein	6.6490	0.0444	6.7025	0.0132
Ltbp2	latent transforming growth factor beta binding protein 2	7.4955	0.0446	6.1763	0.0368
Loxl2	lysyl oxidase like 2	2.5768	0.0449	2.3845	0.0304
MGC105649		2.5626	0.0456	2.6759	0.0258
Cxcl16	C-X-C motif chemokine ligand 16	2.2244	0.0456	2.5248	0.0154
Sorbs2	sorbin and SH3 domain containing 2	1.7747	0.0472	1.9178	0.0367
Serpine2	serpin family E member 2	3.7100	0.0477	3.4697	0.0236
Nupr1	nuclear protein 1, transcriptional regulator	3.6261	0.0484	2.6827	0.0277

Gene	**Gene name**	**Fischer** **Fold change**	**adj p-value**	**SD** **Fold change**	**adj p-value**
Downregulated in both strains					
Gene	Gene name	Fold change	adj p-value	Fold change	adj p-value
Cacng6	calcium voltage-gated channel auxiliary subunit gamma 6	0.2624	0.0092	0.3896	0.0258
Kir3dl1	killer cell immunoglobulin like receptor, three Ig domains and long cytoplasmic tail 1	0.2832	0.0092	0.4109	0.0258
Ddit4l	DNA damage inducible transcript 4 like	0.2523	0.0154	0.1839	0.0036
Klhl33	kelch like family member 33	0.3387	0.0169	0.2926	0.0122
Gpam	glycerol-3-phosphate acyltransferase, mitochondrial	0.3731	0.0169	0.4247	0.0435
Angpt1	angiopoietin 1	0.1475	0.0169	0.2580	0.0266
Ros1	ROS proto-oncogene 1, receptor tyrosine kinase	0.3535	0.0288	0.4413	0.0407
Kcnk2	potassium two pore domain channel subfamily K member 2	0.2946	0.0288	0.2547	0.0189
Scn4b	sodium voltage-gated channel beta subunit 4	0.2675	0.0291	0.2761	0.0218
Deptor	DEP domain containing MTOR interacting protein	0.5285	0.0291	0.5071	0.0312
Atp6ap1l	ATPase H+ transporting accessory protein 1 like	0.1626	0.0292	0.2850	0.0236
Stc2	stanniocalcin 2	0.4889	0.0292	0.4642	0.0277
Pcdh11x	protocadherin 11 X-linked	0.3645	0.0292	0.4130	0.0154
Nudt4	nudix hydrolase 4	0.4203	0.0293	0.3905	0.0277
Slc25a42	solute carrier family 25 member 42	0.5063	0.0322	0.4913	0.0320
Cxadr	coxsackie virus and adenovirus receptor	0.4989	0.0323	0.4568	0.0277
Acacb	acetyl-CoA carboxylase beta	0.4314	0.0327	0.5231	0.0367
Tmem116	transmembrane protein 116	0.5546	0.0356	0.5792	0.0491
Atp1a2	ATPase Na+/K+ transporting subunit alpha 2	0.3742	0.0362	0.3195	0.0320
Myo16	myosin XVI	0.2895	0.0362	0.5090	0.0407
Eci1	enoyl-CoA delta isomerase 1	0.4324	0.0362	0.4091	0.0420
Myo10	myosin X	0.5215	0.0362	0.5310	0.0320
Acsf2	acyl-CoA synthetase family member 2	0.4230	0.0387	0.5071	0.0456
Mlycd	malonyl-CoA decarboxylase	0.5195	0.0430	0.5251	0.0457
Ech1	enoyl-CoA hydratase 1	0.3300	0.0431	0.3291	0.0400
Ano5	anoctamin 5	0.4978	0.0433	0.1547	0.0058
Il2rb	interleukin 2 receptor subunit beta	0.4767	0.0437	0.3749	0.0132
Selenbp1	selenium binding protein 1	0.4853	0.0443	0.4630	0.0420
Adra1b	adrenoceptor alpha 1B	0.4688	0.0456	0.5236	0.0320
Rbfox1	RNA binding protein, fox-1 homolog 1	0.3527	0.0458	0.4154	0.0265
Lpin1	lipin 1	0.5031	0.0469	0.4324	0.0320
Cdnf	cerebral dopamine neurotrophic factor	0.4915	0.0472	0.4990	0.0457

Supplemental Table 3. GO Term enrichment analysis for SD subset

GO term	Count	P-Value	Genes
Embryogenesis	3	0.032027262	XPR1, FOXO1, HHATL

Supplemental Table 4. GO Term enrichment analysis for both strains subset

GO term	Count	P-Value	Genes
Fatty acid metabolism	5	0.00725223	ECH1, CYP1B1, CYP1A1, ACACB, ACSF2
Cell communication	10	0.03060527	NCAM1, AHRR, LTBP2, CTGF, PCDH11X, FGF23, ANGPT1, COL8A1, AMICA1, KIR3DL1
Lipid, fatty acid and steroid metabolism	8	0.031723598	ECH1, CYP1B1, NUDT4, CYP1A1, ACACB, GPAM, LPIN1, ACSF2
Immunity and defense	11	0.04201403	IL2RB, AHRR, IFITM1, C3, CXCL16, CX3CR1, SELENBP1, THBS1, CXADR, KIR3DL1, THBS4
Receptor protein tyrosine kinase signaling pathway	4	0.047277736	CTGF, FGF23, ANGPT1, ROS1
Developmental processes	14	0.058482062	LTBP2, PCDH11X, RCAN1, POSTN, TLR5, LPIN1, TGFB2, TIMP1, NCAM1, ADRA1B, ANGPT1, THBS1, ROS1, THBS4
Cytokine and chemokine mediated signaling pathway	4	0.061125532	IL2RB, OSMR, CX3CR1, TLR5
Other developmental process	3	0.062572317	THBS1, TIMP1, THBS4

Supplemental Table 5. Baseline angiogenic gene expression in RV of Fischer vs SD rats.

Genes	Gene name	Fold Change	p-value
Up-regulated genes			
Cxcl9	Chemokine (C-X-C motif) ligand 9	2.7403	0.0007
Tymp	Thymidine phosphorylase	1.5117	0.0108
Ccl2	Chemokine (C-C motif) ligand 2	2.1864	0.0235
Angpt1	Angiopoietin 1	1.9241	0.0274
Timp2	metallopeptidase inhibitor 2	1.2288	0.0279
Fgf2	Fibroblast growth factor 2	1.6755	0.0308
Egf	Epidermal growth factor	1.9414	0.0351
Id1	Inhibitor of DNA binding 1	1.3386	0.0398
Vegfc	Vascular endothelial growth factor C	1.3973	0.0407
Fn1	Fibronectin 1	1.3312	0.0435
Tnf	Tumor necrosis factor (TNF superfamily, member 2)	2.3042	0.0457
Serpin1	Serpin peptidase inhibitor, clade E (nexin, plasminogen activator inhibitor type 1), member 1	1.6995	0.0552
Itgav	Integrin, alpha V	1.2264	0.0702
Pecam1	Platelet/endothelial cell adhesion molecule 1	1.2259	0.1365
F3	Coagulation factor III (thromboplastin, tissue factor)	1.3163	0.1598
Plau	Plasminogen activator, urokinase	1.5155	0.1610
Itga1	Integrin, alpha 5 (fibronectin receptor, alpha polypeptide)	1.2771	0.1981
Pdgfb	Platelet-derived growth factor beta polypeptide (simian sarcoma viral (v-sis) oncogene homolog)	1.1683	0.2119
Mapk14	Mitogen activated protein kinase 14	1.2602	0.2158
Pdgfa	Platelet-derived growth factor alpha polypeptide	1.1631	0.2281
Vegfa	Vascular endothelial growth factor A	1.2601	0.2295
Hif1a	Hypoxia-inducible factor 1, alpha subunit (basic helix-loop-helix transcription factor)	1.3209	0.2305
Epas1	Endothelial PAS domain protein 1	1.1778	0.2465
Pgf	Placental growth factor	1.2320	0.2751
Efna1	Ephrin A1	1.2069	0.2819
Vegfb	Vascular endothelial growth factor B	1.2003	0.3225
Erbb2	V-erb-b2 erythroblastic leukemia viral oncogene homolog 2, neuro/glioblastoma derived oncogene homolog (avian)	1.2253	0.3232
Il1b	Interleukin 1 beta	1.5163	0.3928
Eng	Endoglin	1.1375	0.4442
Mdk	Midkine	1.1446	0.4494
Flt1	Fms-related tyrosine kinase 1	1.1094	0.4499
Timp1	metallopeptidase inhibitor 1	1.1329	0.4741

Tgfb1	Transforming growth factor, beta receptor 1	1.1096	0.4790
Thbs1	Thrombospondin 1	1.1433	0.5190
Fgf1	Fibroblast growth factor 1	1.1487	0.5246
Jag1	Jagged 1	1.0542	0.6990
Hgf	Hepatocyte growth factor	1.0584	0.7390
Sphk1	Sphingosine kinase 1	1.1245	0.8130
Nrp1	Neuropilin 1	1.0383	0.8598
Plg	Plasminogen	1.0185	0.9625
Mmp2	Matrix metallopeptidase 2	1.0087	0.9782
Down-regulated genes			
Edn1	Endothelin 1	0.5029	0.0120
Tgfb3	Transforming growth factor, beta receptor 3	0.7895	0.0135
Cdh5	Cadherin 5	0.7139	0.0198
Timp3	metallopeptidase inhibitor 3	0.8594	0.0265
Figf	C-fos induced growth factor	0.5192	0.0429
Ptgs1	Prostaglandin-endoperoxide synthase 1	0.6824	0.1048
Igf1	Insulin-like growth factor 1	0.7624	0.1057
Nrp2	Neuropilin 2	0.8206	0.1836
Anpep	Alanyl (membrane) aminopeptidase	0.8957	0.2219
Serpinf1	Serpin peptidase inhibitor, clade F (alpha-2 antiplasmin, pigment epithelium derived factor), member 1	0.8987	0.2346
Akt1	V-akt murine thymoma viral oncogene homolog 1	0.8554	0.3300
Nos3	Nitric oxide synthase 3, endothelial cell	0.8973	0.3898
Ang1	Angiogenin, ribonuclease, RNase A family, 5	0.8150	0.4004
Tie1	Tyrosine kinase with immunoglobulin-like and EGF-like domains 1	0.8983	0.4228
Mmp14	Matrix metallopeptidase 14 (membrane-inserted)	0.9256	0.4289
Col4a3	Collagen, type IV, alpha 3	0.9116	0.4470
Tgfb2	Transforming growth factor, beta receptor 2	0.9213	0.5209
Ctgf	Connective tissue growth factor	0.9400	0.6147
Mmp19	Matrix metallopeptidase 19	0.9536	0.6477
Fgfr3	Fibroblast growth factor receptor 3	0.9511	0.6717
Kdr	Kinase insert domain receptor	0.9581	0.7085
Itgb3	Integrin, beta 3	0.9644	0.7314
S1pr1	Sphingosine-1-phosphate receptor 1	0.9683	0.7653
Col18a1	Collagen, type XVIII, alpha 1	0.9663	0.7660
Ptk2	protein tyrosine kinase 2	0.9980	0.9235
Tek	tyrosine kinase, endothelial	0.9955	0.9367
Angpt2	Angiopoietin 2	0.9970	0.9384
Tgfbr1	Transforming growth factor, beta receptor 1	0.9982	0.9684
Adgrb1	Brain-specific angiogenesis inhibitor 1	UD	

Cxcl1	Chemokine (C-X-C motif) ligand 1 (melanoma growth stimulating activity, alpha)	UD
Cxcl2	Chemokine (C-X-C motif) ligand 2	UD
F2	Coagulation factor II (thrombin)	UD
Fgf6	Fibroblast growth factor 6	UD
Ifna1	Interferon-alpha 1	UD
Ifnb1	Interferon beta 1, fibroblast	UD
Ifng	Interferon gamma	UD
Il6	Interleukin 6	UD
Lect1	Leukocyte cell derived chemotaxin 1	UD
Lep	Leptin	UD
Mmp3	Matrix metallopeptidase 3	UD
Mmp9	Matrix metallopeptidase 9	UD
Serpinb5	Serpin peptidase inhibitor, clade B (ovalbumin), member 5	UD
Tgfa	Transforming growth factor alpha	UD

Note: UD- Undetectable, Cq>35.

Fold change in baseline angiogenic genes expression in the RV of Fischer and SD rats measured by quantitative RT-PCR. N=4 per group using beta-actin as housekeeping gene.

Supplemental Table 6. Fold change in angiogenic gene expression in RV of both rat strains compared to vehicle.

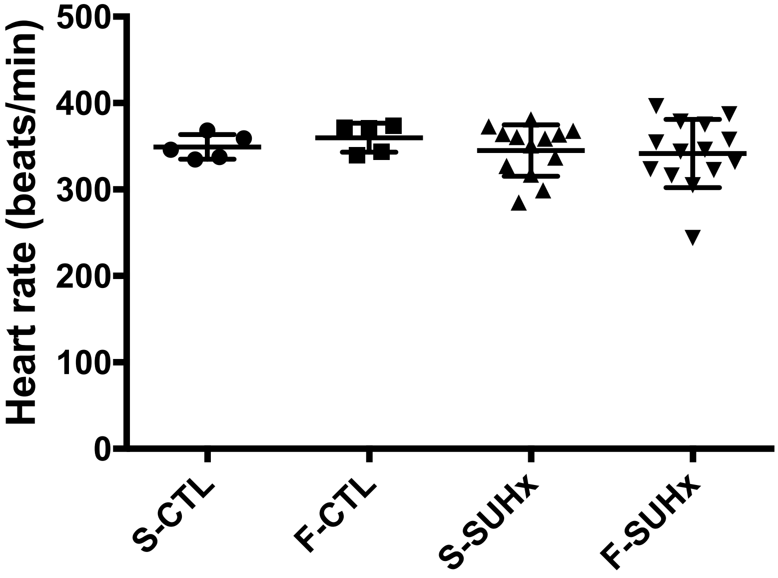
		Fischer		SD	
Gene	**Gene name**	**Fold change**	**p-value**	**Fold change**	**p-value**
Differently regulated genes in either strain					
Vegfc	Vascular endothelial growth factor C	0.5038	0.0045	0.9919	0.9086
Ptgs1	Prostaglandin-endoperoxide synthase 1	1.8385	0.0056	0.9588	0.7228
Fn1	Fibronectin 1	1.8559	0.0071	1.5590	0.0718
Edn1	Endothelin 1	2.2538	0.0199	1.2359	0.1722
Mmp14	Matrix metallopeptidase 14 (membrane-inserted)	0.6985	0.0274	0.8150	0.0823
Tgfbr1	Transforming growth factor, beta receptor 1	0.7141	0.0298	0.8800	0.1027
Tnf	Tumor necrosis factor (TNF superfamily, member 2)	0.4590	0.0427	0.9092	0.3677
Vegfa	Vascular endothelial growth factor A	0.5956	0.0510	0.6065	0.0040
Pecam1	Platelet/endothelial cell adhesion molecule 1	0.6923	0.0546	0.6640	0.0020
Ang1	Angiogenin, ribonuclease, RNase A family, 5	0.5470	0.0705	0.4783	0.0294
Tymp	Thymidine phosphorylase	0.7453	0.0944	0.7541	0.0153
Fgf1	Fibroblast growth factor 1	0.6604	0.0965	0.6406	0.0050
Nos3	Nitric oxide synthase 3, endothelial cell	0.7472	0.1027	0.6469	0.0059
Pdgfb	Platelet-derived growth factor beta polypeptide (simian sarcoma viral (v-sis) oncogene homolog)	0.7532	0.1230	0.6071	0.0001
Figf	C-fos induced growth factor	1.4159	0.1646	1.7456	0.0285
Serpin1	Serpin peptidase inhibitor, clade E (nexin, plasminogen activator inhibitor type1), member 1	1.6212	0.1668	2.2051	0.0019
Angpt2	Angiopoietin 2	1.0225	0.9287	0.7238	0.0040
Up-regulated genes in both the strains					
Thbs1	Thrombospondin 1	4.5011	0.0006	3.2311	0.0103
Timp1	metallopeptidase inhibitor 1	2.9759	0.0016	2.9892	0.0000
Tgfb2	Transforming growth factor, beta receptor 2	6.1616	0.0089	4.2252	0.0003
Ctgf	Connective tissue growth factor	7.6548	0.0155	6.1891	0.0003
Ccl2	Chemokine (C-C motif) ligand 2	1.6799	0.0164	2.7391	0.0011
Serpinf1	Serpin peptidase inhibitor, clade F (alpha-2 antiplasmin, pigment epithelium derived factor), member 1	1.9996	0.0166	1.8559	0.0002
Sphk1	Sphingosine kinase 1	4.0936	0.0260	2.8188	0.0003
Tgfb1	Transforming growth factor, beta receptor 1	1.4564	0.0352	1.3692	0.0094
Eng	Endoglin	1.3109	0.1426	1.1139	0.1590
Itgb3	Integrin, beta 3	1.2237	0.2329	0.8932	0.3257

Col18a1	Collagen, type XVIII, alpha 1	1.2452	0.3091	1.2161	0.3751
Tgfb3	Transforming growth factor, beta receptor 3	1.2035	0.6953	0.7700	0.0815
S1pr1	Sphingosine-1-phosphate receptor 1	1.0559	0.8415	0.9048	0.2170
Down-regulated genes in both the strains					
Angpt1	Angiopoietin 1	0.0675	0.0004	0.1493	0.0025
Epas1	Endothelial PAS domain protein 1	0.3536	0.0008	0.4584	0.0003
Mapk14	Mitogen activated protein kinase 14	0.4547	0.0028	0.5769	0.0070
Pdgfa	Platelet-derived growth factor alpha polypeptide	0.4841	0.0028	0.5653	0.0001
F3	Coagulation factor III (thromboplastin, tissue factor)	0.3796	0.0067	0.4835	0.0025
Erbb2	V-erb-b2 erythroblastic leukemia viral oncogene homolog 2, neuro/glioblastoma derived oncogene homolog (avian)	0.4532	0.0068	0.6054	0.0077
Vegfb	Vascular endothelial growth factor B	0.4437	0.0071	0.5246	0.0009
Efna1	Ephrin A1	0.4483	0.0074	0.5457	0.0001
Egf	Epidermal growth factor	0.3910	0.0087	0.6171	0.0410
Anpep	Alanyl (membrane) aminopeptidase	0.6472	0.0133	0.7150	0.0111
Akt1	V-akt murine thymoma viral oncogene homolog 1	0.4791	0.0133	0.6037	0.0004
Ptk2	protein tyrosine kinase 2	0.6770	0.0137	0.6929	0.0108
Timp3	metallopeptidase inhibitor 3	0.7475	0.0164	0.6485	0.0011
Tek	tyrosine kinase, endothelial	0.5565	0.0175	0.5643	0.0020
Flt1	Fms-related tyrosine kinase 1	0.5934	0.0186	0.6933	0.0039
Cdh5	Cadherin 5	0.5988	0.0186	0.6593	0.0056
Jag1	Jagged 1	0.5006	0.0284	0.6925	0.0056
Hif1a	Hypoxia-inducible factor 1, alpha subunit (basic helix-loop-helix transcription factor)	0.5127	0.0288	0.6997	0.0148
Col4a3	Collagen, type IV, alpha 3	0.6726	0.0332	0.6696	0.0249
Pgf	Placental growth factor	0.5984	0.0459	0.5437	0.0054
Timp2	metallopeptidase inhibitor 2	0.8105	0.0503	1.0329	0.7315
Cxcl9	Chemokine (C-X-C motif) ligand 9	0.6016	0.0544	0.5832	0.0601
Igf1	Insulin-like growth factor 1	0.7577	0.0665	0.7807	0.1014
Itgav	Integrin, alpha V	0.7660	0.0793	1.0125	0.8125
Hgf	Hepatocyte growth factor	0.7324	0.0834	0.8754	0.2322
Fgfr3	Fibroblast growth factor receptor 3	0.7204	0.0902	0.7682	0.1406
Id1	Inhibitor of DNA binding 1	0.7664	0.1110	0.9136	0.3198
Fgf2	Fibroblast growth factor 2	0.7227	0.1486	0.9463	0.5781
Tie1	Tyrosine kinase with immunoglobulin-like and EGF-like domains 1	0.7721	0.1668	0.8051	0.1008

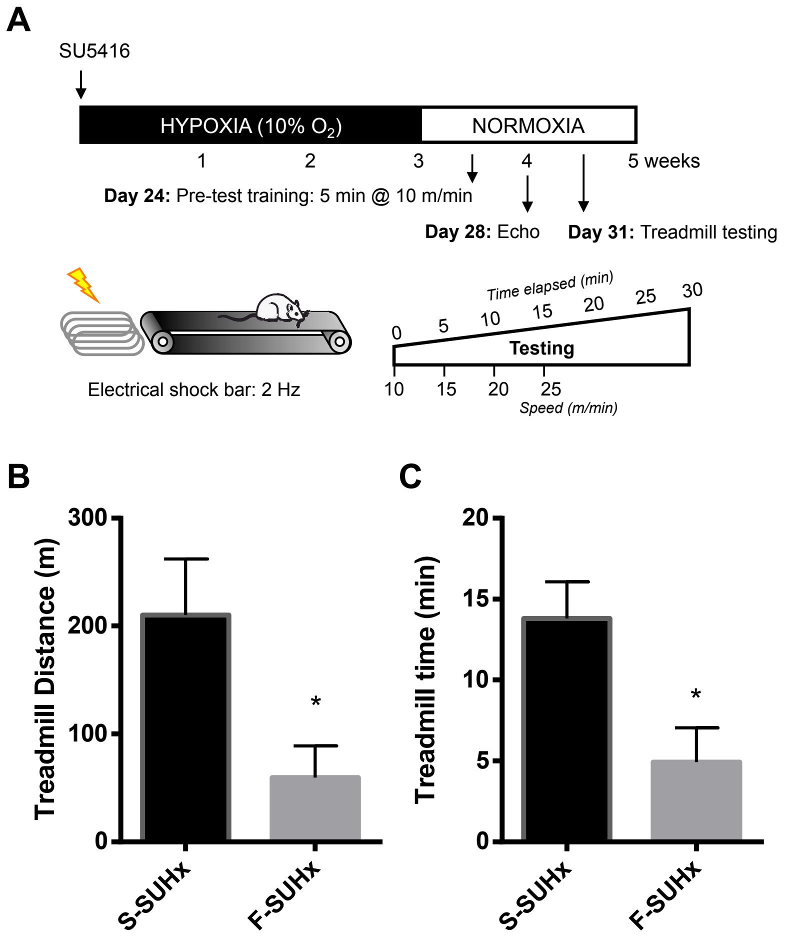
Nrp1	Neuropilin 1	0.8036	0.2355	0.8088	0.0651
Itga1	Integrin, alpha 5 (fibronectin receptor, alpha polypeptide)	0.8088	0.2444	1.1269	0.2325
Mmp2	Matrix metallopeptidase 2	0.8606	0.2745	0.9404	0.5291
Plg	Plasminogen	0.8329	0.3869	0.9165	0.5298
Kdr	Kinase insert domain receptor	0.8347	0.4109	0.8211	0.0842
Il1b	Interleukin 1 beta	0.8706	0.4468	1.5450	0.4679
Nrp2	Neuropilin 2	0.9238	0.5872	0.8856	0.2934
Mdk	Midkine	0.9712	0.7165	1.2978	0.1503
Mmp19	Matrix metallopeptidase 19	0.9778	0.7918	0.9715	0.7299
Plau	Plasminogen activator, urokinase	0.9835	0.8094	1.0521	0.8072
Adgrb1	Brain-specific angiogenesis inhibitor 1	UD		UD	
Cxcl1	Chemokine (C-X-C motif) ligand 1 (melanoma growth stimulating activity, alpha)	UD		0.7147	0.3784
Cxcl2	Chemokine (C-X-C motif) ligand 2	UD		UD	
F2	Coagulation factor II (thrombin)	UD		UD	
Fgf6	Fibroblast growth factor 6	UD		UD	
Ifna1	Interferon-alpha 1	UD		UD	
Ifnb1	Interferon beta 1, fibroblast	UD		UD	
Il6	Interleukin 6	UD		UD	
Lect1	Leukocyte cell derived chemotaxin 1	UD		UD	
Lep	Leptin	UD		UD	
Mmp3	Matrix metallopeptidase 3	UD		UD	
Mmp9	Matrix metallopeptidase 9	UD		UD	
Serpinb5	Serpin peptidase inhibitor, clade B (ovalbumin), member 5	UD		UD	
Tgfa	Transforming growth factor alpha	UD		3.3091	0.0998
Ifng	Interferon gamma	0.4063	0.0216	UD	

Note: UD- Undetectable, Cq>35.

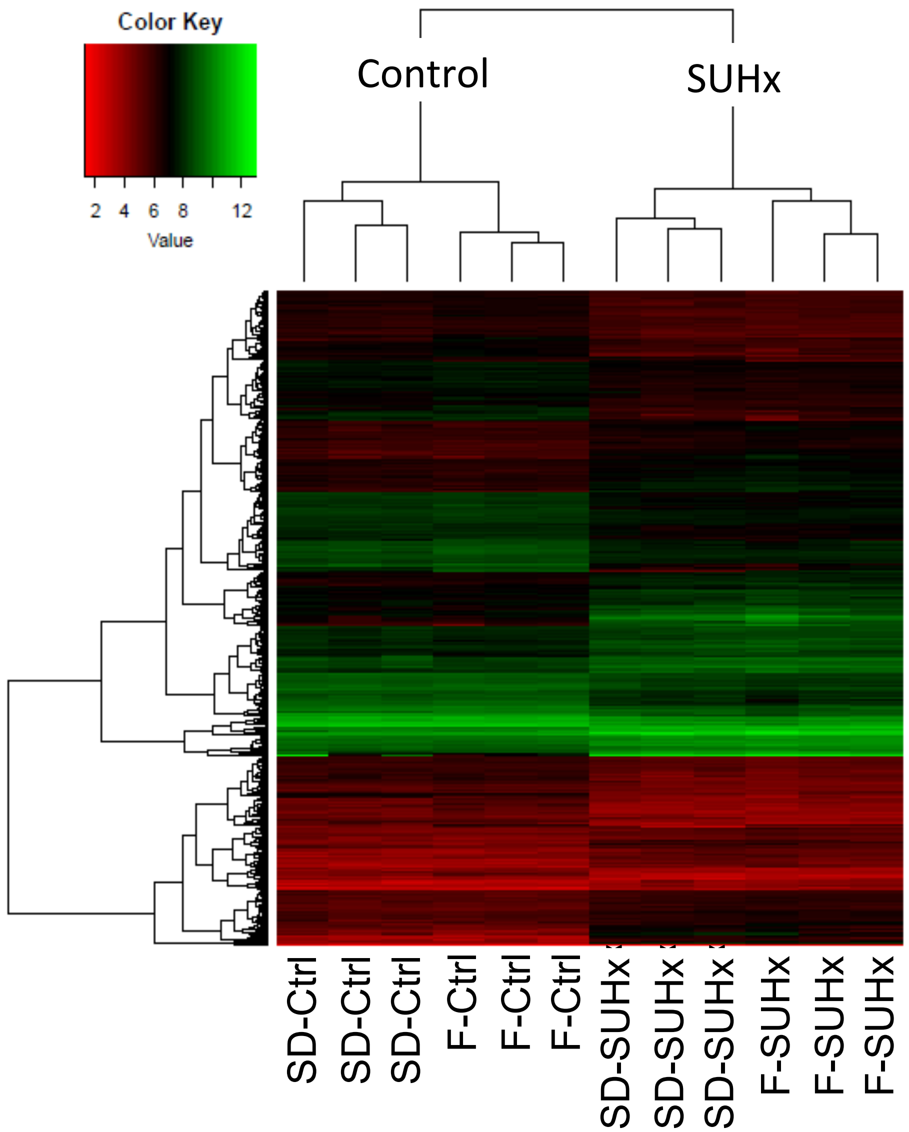
Fold change in angiogenic genes expression in the RV of SUHx Fischer and SD rats relative to vehicle control of respective group. Gene expression was measured by quantitative RT-PCR, N=4 per group using beta-actin as housekeeping gene.



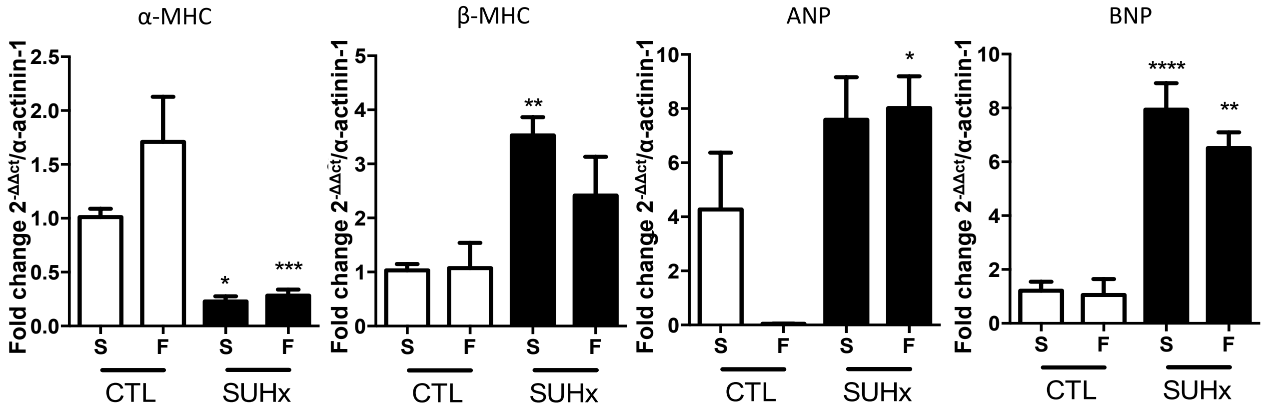
Supplemental Figure 1: Heart rate variability. Bar graph demonstrating heart rate of Fischer control, SD control, SD SUHx and Fischer SUHx rats, as determined by echocardiography.



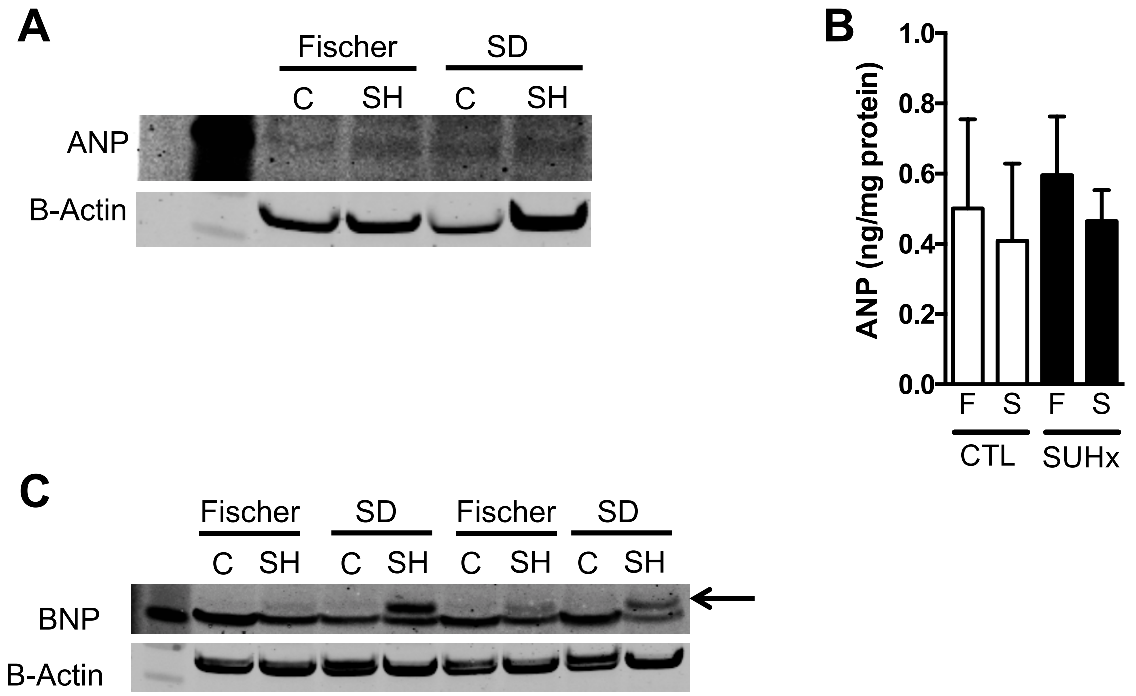
Supplemental Figure 2: Exercise tolerance testing at 4 weeks post SU. (A) Timeline of treadmill exercise testing and echocardiography. Training is performed 7 days prior to treadmill testing (top). Schematic of treadmill configuration with electric stimulus at the rear of the treadmill belt (bottom left). Treadmill testing protocol (bottom right) begins at a speed of 5 m/min, after which the speed is increased by 5 m/min every 5 min until a maximum speed of 25 m/min is reached. Exercise capacity in Fischer vs SD SUHx rats as determined by treadmill (B) distance and (C) time. *$p<0.05$ by Student's t-test. n=4 in each group



Supplemental Figure 3: Heatmap demonstrating differentially expressed genes in RV of SUHx Sprague-Dawley or Fischer CDF rats compared to respective controls.



Supplemental Figure 4. Right ventricular expression of genes involved in cardiac remodeling in Fischer and SD rats with or without SUHx measured by quantitative RT-PCR. *,**,***,**** p<0.05, 0.01, 0.001, 0.0001 vs control. n = 3-6 per group using alpha-actinin-1 as housekeeping gene



Supplemental Figure 5: Natriuretic peptide expression in RV. A. Representative image of a western-blot demonstrating ANP protein expression in RV of SD and Fischer +/- SUHx; n=3-4 per group. B. ANP levels in the RV (n=3-5 per group) of SD and Fischer +/- SUHx. C. Representative image of a western-blot showing BNP protein expression in RV of SD and Fischer +/- SUHx; n=3-4 per group.

Methods

Ethics

All study protocols were approved by the animal ethics and research committee (University of Ottawa, Ontario, Canada) and conducted according to guidelines from the Canadian Council for the Care of on Animal Care (CCAC).

SU5416+chronic hypoxia model of PAH

Male Sprague Dawley (SD, Harlan laboratories, Indianapolis, IND, USA) and Fischer (CDF, Charles River, Montreal, QC, Canada) rats weighing 125-200 g were used for this study. PH was induced by a single subcutaneous injection of SU5416 (SU:3-(3,5-dimethyl-1H-pyrrol-2-ylmethylene)-1,3-dihydroindol-2-one)) (Tocris, Bristol, United Kingdom) suspended in 0.5% vehicle of CMC (0.5% carboxymethylcellulose sodium, 0.9% sodium chloride, 0.4% Tween 80, 0.9% benzyl alcohol in deionized water) as previously described[1, 2]. Immediately after SU injection, rats were exposed to chronic hypoxia (8.5-10% O_2) in hypoxic chamber system using controlled nitrogen gas release (Biospherix, Lacona, NY, USA) for 3 weeks (SU5416-hypoxia; SUHx). Control rats received CMC vehicle and remained at room air (21% O_2) until end study at 4 or 7 weeks.

Non-invasive assessment by echocardiography

Echocardiography was performed using the Vevo2100 ultrasonography system (VisualSonics, Toronto, ON, Canada). Rats were anesthetized using 2-3% inhaled isoflurane. Using short-axis M-mode echocardiography, RV chamber size was expressed as the ratio of end-diastolic RV to LV internal diameter (RVID-d/LVID-d) obtained. RV free wall (RVFW) thickness was measured as the distance between the epicardium and the endocardium of the right ventricular wall. Pulsed-wave Doppler mode was used to assess pulmonary artery flow in the parasternal long axis view. Cardiac output (CO) was calculated using the pulmonary artery velocity time integral PA-VTI method as previously described[3], where pulmonary artery cross section area (PA_{CSA}) = π ($(PA_{diameter}/2)^2$), stroke volume (SV) = PA_{CSA} x PA-VTI, and CO = SV x heart rate.

Cardiac magnetic resonance imaging (MRI)

Cardiac magnetic resonance imaging was performed using the 7T GE/Agilent MRI system at the uOttawa preclinical imaging core. Rats were anaesthetized using 2% inhaled isoflurane. Axial, two-chamber, and short-axis localizer scans were first performed, followed by short-axis cine. For the short-axis cine, ECG gating was used with 30 reconstructed cardiac phases and 2 views per segment. The field of view, matrix size, and slice thickness were 7 cm, 256x192, and 1.5 mm, respectively. Using short-axis cine MRI, RV and LV volumes were calculated by summation of the endocardial volume of sequential stacks during diastole or systole using ImageJ. RV ejection fraction was calculated as (end diastolic volume – end systolic volume)/end diastolic volume x 100%.

Right heart catheterization

Rats were anesthetized by an intraperitoneal injection of xylazine (7 mg/kg) and ketamine (35 mg/kg). High-fidelity pressure catheters (Transonic-Scisense Inc., London, ON, Canada) were inserted into the right jugular vein and advanced through the superior vena cava and right atrium into the RV. Hemodynamic parameters were recorded and analyzed using the LabScribe3 software (iWorx, Dover, NH, USA).

Lung and heart Morphometric and Histological Measurements

Following right heart catheterization, rats were euthanized under anaesthesia by exsanguination and thorax was opened. The left lobe of the lung was inflated via the trachea with 50:50 OCT/saline solution (Tissue-Tek OCT; Qiagen, Mississauga, ON, Canada) and then removed. The left lobe and whole heart was then sectioned and fixed in 4% paraformaldehyde (PFA) for 48 h, rinsed and washed in PBS for overnight and stored in 70% ethanol until the day of paraffin embedding. Tissue blocks were sectioned 5 μm thickness with a microtome (Leica Microsystems, Concord, ON, Canada), placed onto poly-L-lysine-coated slides, dried at 37°C for 16 hours and then dewaxed and dehydrated through graded alcohols. For microscopy and quantitative morphometry of the lung, hematoxylin and eosin (H&E) staining was performed with standard protocols and data was analyzed using the Aperio Imagescope Plus software (Leica, Concord, ON, Canada). We counted small pulmonary arteries (outer diameter (OD) <200 μm) from two cross sections of left lobe per rat. Vessels were assessed for occlusive lesions using H and E staining and scored as follows: no evidence of neointimal formation (Grade 0); partial luminal occlusion (<50%, Grade 1); and severe luminal occlusion (>50%; Grade 2). Morphology of the heart and cardiomyocytes was assessed using Masson's Trichrome staining using standard protocols. For quantitative assessment of RV cardiomyocyte cross sectional area, the area of each cardiomyocyte was traced from each 3 random cross-sectional fields /animal (20X objective).

Immunohistochemistry

Paraffin heart sections were deparaffinized through graded alcohols. After antigen unmasking specimens were blocked in 2% normal goat serum in PBS-T. Endogenous biotin and avidin were blocked using the Vector Avidin/Biotin blocking kit (Vector Labs, Burlington, ON, Canada). Sections were stained with rabbit polyconal antibody to CD31 (Novus Biologicals, Oakville, ON, Canada) at 1:250 dilution for 1 hour at room temperature, followed by endogenous peroxidase quenching by incubation with 3% H_2O_2 for 15 minutes. Sections were then incubated with biotinylated goat anti-rabbit IgG antibody at 1:66 dilution (Vector Labs, Burlington, ON, Canada), followed by DAB staining using the VECTASTAIN ABC Elite kit (Vector Labs, Burlington, ON, Canada) according to manufacturer's protocol.

For natural cytotoxicity receptor-1 (NCR1), paraffin heart sections were stained with rabbit polyconal antibody to NCR1 (Abcam, ON, Canada) at 1:400 dilution followed by DAB staining using Rabbit specific HRP/DAB (ABC) Detection IHC Kit (Abcam, ON, Canada) according to manufacturer's protocol. Images were acquired using Nikon Eclipse TE2000 inverted microscopes using 10X and 40X objectives (Nikon C1si, Nikon Instruments Inc., NY, USA) and NCR1 positive cells were counted using the open-source softwares ImageJ (Fiji package). Total NCR1 positive cells per high magnification (40X) image were calculated and 5 images per sample were analyzed.

Fluorescence microangiography

Following exsanguination, residual blood was flushed using 60 mL of heparinized saline (10 U/mL) via jugular vein. The ascending aorta and pulmonary vein were ligated and an incision was made in the right atrium. A cannula was inserted into the aorta to deliver warm (37^oC) flush buffer (10 U/mL heparin, 0.0025 ng/mL sodium nitroprusside) to flush the coronary circulation by retrograde perfusion. Approximately 2 mL of pre-warmed 0.22 μm fluorescent microspheres dissolved in 2% low melting point agarose (Life Technologies, Burlington, ON, Canada) were perfused into the coronary circulation via the aorta. Immediately afterwards, the heart was placed on ice for 5 minutes to cast the fluorescent microspheres, and then fixed in 4% PFA for 48 h. Following a PBS wash, whole hearts were embedded in frozen OCT ((Tissue-Tek OCT; Qiagen, Mississauga, ON, Canada) and sectioned into 50 μm sections using a cryostat (Leica Microsystems, Concord, ON, Canada). Slides were washed in PBS, mounted with Vector Hardset mounting medium (Vector Labs, Burlington, ON, Canada) and coverslipped. The FMA sections were visualized using a laser confocal microscope (Zeiss LSM510), where 40 z-stacks at 20X objective per slide were acquired using a 1 μm step size. To quantify capillary volume, fluorescent pixels were thresholded based on a uniform pixel intensity to represent filled vessels using ImageJ (threshold=50). For each stack, the thresholded pixel area (capillary area, in μm^2) was multiplied by the stack depth (1 μm), where capillary Volume = capillary area x stack depth. The total capillary volume was represented by the summation of 40 stacks.

Microarray gene expression and analysis

RNA was isolated as described above and total RNA was assayed using Affymetrix Rat Gene 2.0ST and Affymetrix miRNA 4.0 gene chips. Annotations from Affymetrix (RaGene-2_0-st-v1.na35.rn5.transcript) were used to assign gene symbols. Fold change analyses were performed using the R Bioiconductor limma package, according to gene expression changes in PH vs control in SD and F subsets with cutoff values of adjusted p-value < 0.05 and fold-change > 3. Heat maps and hierarchical clustering were also constructed in R Bioiconductor. Fold change analyses used the limma package and PCA analysis used prcomp. For gene-gene ontology enrichment analysis, lists were subdivided into fold-changes common to both, unique to SD and unique to Fischer, and gene ontology was performed on each subset using the DAVID Functional Annotation tool (https://david.ncifcrf.gov/) using the PANTHER database for biological processes.

PCR array for angiogenic genes

RV tissue was snap frozen in liquid nitrogen and stored at -80° C until RNA isolation. Tissue homogenization was achieved using the TissueLyser-II system (Qiagen, Toronto, ON, Canada) at 30 Hz for 5 min followed by RNA isolation lysis using miRCURY RNA isolation kit (Exiqon, Woburn, MA, USA) according to manufacturer's protocol.

cDNA was prepared using RT^2 First Strand Kit (Qiagen, Toronto, ON, Canada) as per manufacturer's instructions. The cDNA was stored at -80° C until used for the RT^2 Profiler PCR array for angiogenic genes (Qiagen, Toronto, ON, Canada). Quantitative RT-PCR was performed using SYBR green qPCR mastermix (Qiagen, Toronto, ON, Canada) as per the manufacturer's instructions.

Quantitative real-time PCR

RNA was isolated as described above and converted to cDNA using the Taqman High Capacity cDNA Reverse Transcription kit (Applied Biosystems, Burlington, ON, Canada). Quantitative RT-PCR was performed using Taqman probes for rat α-myosin heavy chain (Myh6, Rn00691721_g1), β-myosin heavy chain (Myh7, Rn01488777_g1), atrial natiuretic peptide (Nppa, Rn00664637_g1), brain natiuretic peptide (Nppb, Rn04219558_g1) and α-actinin-1 (Actn1, Rn00667357_m1) (Applied Biosystems, Burlington, ON, Canada).

Atrial natriuretic peptide ELISA

Atrial natriuretic peptide in the RV homogenate and plasma was measured using ANP (NPPA) rat ELISA kit (Abcam, ON, Canada) as per manufacturer's instructions. Briefly, the RV was weighed and homogenized using 0.1% Triton-X 100 in PBS (pH 7.4) using the TissueLyser (Qiagen, ON, Canada) two cycles of 25hz for 3 min. The homogenate was centrifuged at 12000xg for 20 min at 4° C and the supernatant was collected. Protein concentration of the supernatant was measured by the DC Protein Assay (Bio-rad, ON, Canada) and 150 μg protein was used per test. For plasma, 50 μL of undiluted plasma was used per test. Samples were analyzed in triplicates.

Western blotting

RV lysates were prepared in CelLytic™ MT Cell Lysis Reagent (Sigma, ON, Canada) using the TissueLyser (Qiagen, ON, Canada) two cycles of 25hz for 3 min. The tissue lysate was then centrifuged at 12000xg for 10 min and supernatant was collected. Protein concentration of the protein extract was determined colorimetrically by the DC Protein Assay kit (Bio-rad, ON, Canada), using bovine serum albumin as standard. SDS-polyacrylamide gel electrophoresis of RV protein extract (50 μg) was performed with NuPAGE® Novex® 4-12% Bis-Tris Protein Gels (ThermoFisher Scientific, ON, Canada). Following transfer of the separated proteins to nitrocellulose membranes

(NOVEX iBLOT Gel transfer Stacks, ThermoFisher Scientific, ON, Canada), blots were blocked with 2% BSA in PBS-T (PBS containing 0.1% Tween 20, pH 7.4). After blocking, blots were incubated with primary antibodies to Atrial natriuretic peptide (Abcam, ON, Canada), B-type natriuretic peptide (EMD Millipore, ON, Canada) and β-actin (ThermoFisher Scientific, ON, Canada) for overnight at 4° C. Then the blots were washed for three times for 15 min with PBS-T and incubated with appropriate IRDye® anti-rabbit or anti-mouse secondary antibodies (LI-COR Biotechnology, NE, USA) in 2% BSA/PBS-T. Further the blots were washed for three times for 15 min with PBS-T and imaged with Odyssey® imaging system (LI-COR Biotechnology, NE, USA).

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