

YMTHE, Volume 30

Supplemental Information

**The long non-coding RNA *NRON*
promotes the development of cardiac
hypertrophy in the murine heart**

Jeannine Hoepfner, Julia Leonardy, Dongchao Lu, Kevin Schmidt, Hannah J. Hunkler, Sinje Biß, Ariana Foinquinos, Ke Xiao, Kumarswamy Regalla, Deepak Ramanujam, Stefan Engelhardt, Christian Bär, and Thomas Thum

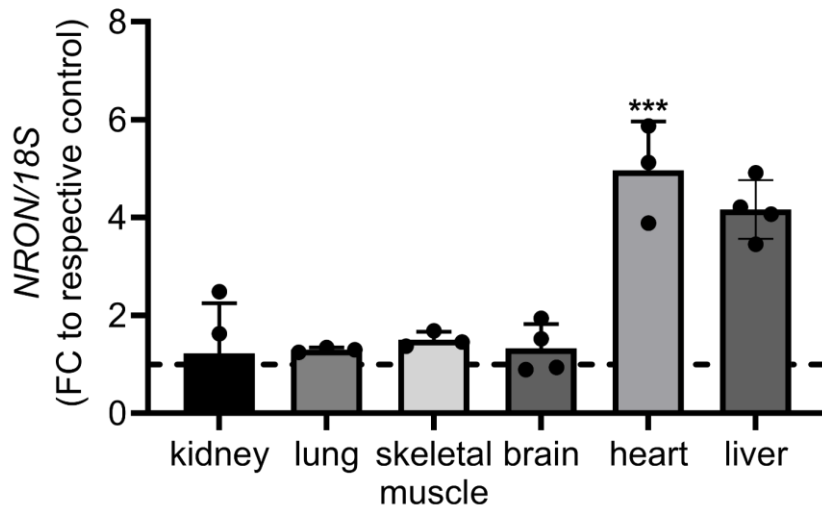


Figure S1: *NRON* level in different organs after AAV9-*NRON* administration. 1.5×10^{12} AAV9-*NRON* or AAV9-empty viral particles were injected. Mice were sacrificed after 6 weeks. *NRON* level was measured by qPCR relative to *18S* RNA. FC was calculated using *NRON* level in the respective AAV9-empty injected organ as control. All data are mean \pm S.D.; $n=3-4$. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; **** $P < 0.0001$; Student's t-test.

Table S1: Ct values of samples from figure 1 and 2. (100-500 ng RNA was used for cDNA synthesis according to the concentration of the sample).

Experiment	Sample	Ct value NRON	Ct value Housekeeper
Figure 1a	chromatin associated	27.89	
	cytoplasmic fraction	31.5	
	Nuclear soluble	33	
Figure 1b	CMC	29,46	21,93
	CF	30,65	21,56
	EC	32,13	22,22
Figure 1c	2w Sham	24,155	22,06
	2w TAC	25,17	21,44
	4w Sham	29,65	21,31
	4w TAC	29,73	21,45
	6w Sham	29,79	21,41
	6w TAC	29,95	21,37
	8w Sham	27,79	20,95
	8w TAC	28	20,84
	13w Sham	30,16	22,13
13w TAC	30,15	21,07	
Figure 2a	AAV9-Empty	29,02	10,75
	AAV9-NRON	27,11	11,01

Table S2: Echocardiography analysis after TAC surgery in AAV9-*NRON* injected mice.

	Sham AAV9-empty	Sham AAV9- <i>NRON</i>	TAC AAV9-empty	TAC AAV9- <i>NRON</i>
Ejection fraction [%]	80.87 ± 8.66	72.3 ± 10.96	69.89 ± 13.79	64.85 ± 18.24
Fractional shortening [%]	49.56 ± 9.36	41.64 ± 8.66	39.84 ± 11.09	36.29 ± 12.3
LV mass [mg]	138.3 ± 37.86	136.9 ± 13.26	165.2 ± 38.39	184 ± 33.14
LV mass corr [mg]	110.6 ± 30.29	109.5 ± 10.61	132.2 ± 30.72	147.2 ± 26.51
IVS;d [mm]	1.2 ± 0.12	1.16 ± 0.12	1.34 ± 0.26	1.2 ± 0.17
IVS;s [mm]	1.79 ± 0.19	1.51 ± 0.17	1.7 ± 0.34	1.64 ± 0.21
LVID;d [mm]	3.53 ± 0.29	3.8 ± 0.29	3.48 ± 0.3	3.75 ± 0.52
LVID;s [mm]	1.76 ± 0.37	2.21 ± 0.53	2.08 ± 0.52	2.38 ± 0.79
LVPW;d [mm]	0.86 ± 0.3	0.79 ± 0.16	1.06 ± 0.26	1.16 ± 0.23
LVPW;s [mm]	1.27 ± 0.33	1.12 ± 0.31	1.42 ± 0.31	1.53 ± 0.37
diameter;s [mm]	1.76 ± 0.4	2.25 ± 0.51	2.09 ± 0.5	2.14 ± 0.28
diameter;d [mm]	3.48 ± 0.25	3.82 ± 0.23	3.44 ± 0.29	3.72 ± 0.5
volume;s [uL]	9.96 ± 5.52	18.53 ± 10.75	15.55 ± 8.52	15.62 ± 5.25
volume;d [uL]	50.69 ± 8.65	63.06 ± 8.92	49.39 ± 9.41	53.17 ± 7.07

Echocardiography parameters are displayed as mean ± SD. LV mass, left ventricular mass; LV mass corr, left ventricular mass corrected; IVS;d, intraventricular septum thickness in diastole; IVS;s, intraventricular septum thickness in systole ; LVID;d, left ventricular internal diameter in diastole; LVID;s, left ventricular internal diameter in systole; LVPW;d, left ventricular posterior wall thickness in diastole; LVPW;s, left ventricular posterior wall thickness in systole; diameter;s, systolic diameter; diameter;d, diastolic diameter; volume;s, systolic volume; volume;d, diastolic volume.

Table S3: Echocardiography analysis after TAC surgery in *NRON*-KO mice.

	2w Sham <i>NRON</i> +/+	2w Sham <i>NRON</i> -/-	2w TAC <i>NRON</i> +/+	2w TAC <i>NRON</i> -/-
Ejection fraction [%]	53.97 ± 8.96	62.46 ± 14.63	49.3 ± 9.43	65.38 ± 9.88
Fractional shortening [%]	27.63 ± 5.54	34.27 ± 10.7	24.92 ± 6.15	35.77 ± 7.46
LV mass [mg]	95.21 ± 15.88	105.5 ± 22.31	133.6 ± 28.02	115.1 ± 23.62
LV mass corr [mg]	76.17 ± 12.7	84.4 ± 17.85	106.8 ± 22.41	92.05 ± 18.89
IVS;d [mm]	0.79 ± 0.17	0.76 ± 0.08	0.93 ± 0.11	0.9 ± 0.13
IVS;s [mm]	0.98 ± 0.21	0.96 ± 0.13	1.14 ± 0.15	1.07 ± 0.14
LVID;d [mm]	3.72 ± 0.38	3.88 ± 0.38	4.04 ± 0.36	3.69 ± 0.28
LVID;s [mm]	2.7 ± 0.41	2.61 ± 0.61	3.04 ± 0.41	2.39 ± 0.41
LVPW;d [mm]	0.75 ± 0.21	0.79 ± 0.13	0.81 ± 0.14	0.82 ± 0.06
LVPW;s [mm]	0.87 ± 0.18	0.96 ± 0.1	0.99 ± 0.14	1.12 ± 0.15
diameter;s [mm]	2.71 ± 0.41	2.57 ± 0.59	3.07 ± 0.42	2.38 ± 0.42
diameter;d [mm]	3.74 ± 0.34	3.87 ± 0.38	4.08 ± 0.36	3.68 ± 0.29
volume;s [uL]	28.38 ± 10.44	25.76 ± 13.74	38.23 ± 11.69	20.75 ± 8.46
volume;d [uL]	60.22 ± 12.7	65.58 ± 15.5	74.37 ± 15.6	57.96 ± 10.56
	6w Sham <i>NRON</i> +/+	6w Sham <i>NRON</i> -/-	6w TAC <i>NRON</i> +/+	6w TAC <i>NRON</i> -/-
Ejection fraction [%]	52.04 ± 11.47	65.83 ± 6.01	42.54 ± 9.46	59.29 ± 8.93
Fractional shortening [%]	26.72 ± 7.34	35.93 ± 4.59	21.03 ± 5.44	31.24 ± 6.13
LV mass [mg]	96.6 ± 11.9	97.31 ± 16.22	168.6 ± 28.69	121.9 ± 17.14
LV mass corr [mg]	77.28 ± 9.52	77.84 ± 12.98	134.9 ± 22.95	97.55 ± 13.71
IVS;d [mm]	0.75 ± 0.1	0.81 ± 0.13	1.04 ± 0.09	1 ± 0.15
IVS;s [mm]	0.89 ± 0.13	0.99 ± 0.09	1.21 ± 0.14	1.11 ± 0.26
LVID;d [mm]	4.05 ± 0.37	3.89 ± 0.32	4.38 ± 0.24	3.76 ± 0.36
LVID;s [mm]	3 ± 0.57	2.5 ± 0.22	3.48 ± 0.3	2.61 ± 0.42
LVPW;d [mm]	0.64 ± 0.08	0.61 ± 0.1	0.86 ± 0.25	0.89 ± 0.2
LVPW;s [mm]	0.87 ± 0.15	0.86 ± 0.15	0.99 ± 0.23	1.09 ± 0.21
diameter;s [mm]	3.01 ± 0.54	2.5 ± 0.22	3.52 ± 0.31	2.61 ± 0.43
diameter;d [mm]	4.08 ± 0.37	3.9 ± 0.32	4.45 ± 0.23	3.77 ± 0.37
volume;s [uL]	36.93 ± 15.28	22.55 ± 4.8	52.05 ± 11.16	25.79 ± 10.46
volume;d [uL]	74.17 ± 15.86	66.63 ± 12.76	90.41 ± 10.54	61.73 ± 14.83
	13w Sham <i>NRON</i> +/+	13w Sham <i>NRON</i> -/-	13w TAC <i>NRON</i> +/+	13w TAC <i>NRON</i> -/-
Ejection fraction [%]	64.24 ± 7.56	60.43 ± 4.95	39.95 ± 7.97	44.05 ± 5.48
Fractional shortening [%]	34.92 ± 5.4	32 ± 3.48	19.54 ± 4.47	21.66 ± 3.16
LV mass [mg]	89.58 ± 15.97	96.69 ± 15.81	192.5 ± 51.47	138.7 ± 36.69
LV mass corr [mg]	71.66 ± 12.78	77.35 ± 12.65	154 ± 41.18	111 ± 29.36
IVS;d [mm]	0.71 ± 0.07	0.74 ± 0.12	0.96 ± 0.16	0.9 ± 0.16
IVS;s [mm]	0.93 ± 0.08	0.82 ± 0.07	1.13 ± 0.08	1.01 ± 0.19
LVID;d [mm]	4.14 ± 0.34	3.99 ± 0.3	4.71 ± 0.46	4.14 ± 0.48
LVID;s [mm]	2.72 ± 0.45	2.77 ± 0.17	3.8 ± 0.54	3.2 ± 0.43
LVPW;d [mm]	0.6 ± 0.09	0.6 ± 0.06	0.95 ± 0.15	0.88 ± 0.18
LVPW;s [mm]	0.88 ± 0.09	0.81 ± 0.11	1.05 ± 0.15	0.94 ± 0.27
diameter;s [mm]	2.7 ± 0.42	2.79 ± 0.18	3.76 ± 0.5	3.3 ± 0.38
diameter;d [mm]	4.13 ± 0.32	4.1 ± 0.2	4.66 ± 0.44	4.22 ± 0.45
volume;s [uL]	28.1 ± 10.94	29.46 ± 4.58	61.83 ± 19.8	45.11 ± 13.16
volume;d [uL]	76.37 ± 13.88	74.56 ± 8.68	101.5 ± 22.99	80.44 ± 20.9

Table S3:

Echocardiography parameters are displayed as mean \pm SD. LV mass, left ventricular mass; LV mass corr, left ventricular mass corrected; IVS;d, intraventricular septum thickness in diastole; IVS;s, intraventricular septum thickness in systole ; LVID;d, left ventricular internal diameter in diastole; LVID;s, left ventricular internal diameter in systole; LVPW;d, left ventricular posterior wall thickness in diastole; LVPW;s, left ventricular posterior wall thickness in systole; diameter;s, systolic diameter; diameter;d, diastolic diameter; volume;s, systolic volume; volume;d, diastolic volume.

Table S4: Top 100 deregulated mRNAs.

Gene name	Gene description	Sham WT vs TAC WT		TAC WT vs TAC KO	
		fold change	p-value	fold change	p-value
Gm4544	predicted gene 4544	-1.75	1.74E-14	1.72	1.75E-14
Tgfb2	transforming growth factor, beta 2	1.97	8.12E-39	-1.61	6.70E-23
Nppa	natriuretic peptide type A	2.62	1.04E-40	-1.56	6.84E-31
Ppm1e	protein phosphatase 1E (PP2C domain containing)	1.55	1.76E-32	-1.57	3.84E-30
Syndig1	synapse differentiation inducing 1	2.64	5.05E-27	-1.55	5.56E-11
Scd4	stearoyl-coenzyme A desaturase 4	-1.51	9.55E-12	1.44	2.35E-09
Aldob	aldolase B, fructose-bisphosphate	-1.87	6.34E-14	1.42	1.50E-09
Dok5	docking protein 5	1.36	2.34E-11	-1.32	8.89E-10
Acot1	acyl-CoA thioesterase 1	-1.38	2.23E-17	1.31	2.04E-14
Gm13054	predicted gene 13054	2.36	2.28E-27	-1.26	1.09E-08
Ankrd1	ankyrin repeat domain 1 (cardiac muscle)	2.04	2.12E-23	-1.24	3.15E-15
Lad1	ladinin	-1.23	4.08E-10	1.24	8.93E-10
Gm40841	predicted gene, 40841	-1.94	1.50E-15	1.21	6.43E-07
Lgi1	leucine-rich repeat LGI family, member 1	-1.23	7.04E-12	1.21	1.04E-11
Nuak1	NUAK family, SNF1-like kinase, 1	1.45	1.58E-41	-1.20	2.85E-17
Mybl1	myeloblastosis oncogene-like 1	1.18	1.07E-10	-1.23	1.73E-11
Apold1	apolipoprotein L domain containing 1	1.20	5.70E-16	-1.18	3.31E-14
Frat1	frequently rearranged in advanced T cell lymphomas	-1.38	1.74E-10	1.18	8.46E-08
Lrrc4b	leucine rich repeat containing 4B	-1.18	7.02E-18	1.19	7.75E-14
Gm24474	predicted gene, 24474	-1.19	3.21E-08	1.18	4.30E-08
Gm19277	predicted gene, 19277	-1.41	1.01E-08	1.17	1.68E-06
Ctgf	connective tissue growth factor	2.50	1.60E-78	-1.14	3.47E-07
Gm29773	predicted gene, 29773	3.23	4.70E-42	-1.13	8.56E-07
Gpr3	G-protein coupled receptor 3	1.21	1.86E-06	-1.13	2.04E-06
Grip1	glutamate receptor interacting protein 1	1.49	1.39E-09	-1.11	1.43E-06
Angpt1	angiopoietin 1	-1.91	6.35E-35	1.11	5.61E-12
Slc22a4	solute carrier family 22 (organic cation transporter), member 4	1.51	3.57E-49	-1.11	4.88E-21
Edn3	endothelin 3	1.74	3.79E-23	-1.10	2.56E-09
Hmgcs2	3-hydroxy-3-methylglutaryl-Coenzyme A synthase 2	-1.09	1.30E-05	1.22	7.54E-09
AC165271.1	novel transcript, antisense to Kcnj6	2.08	1.03E-24	-1.09	2.64E-08
Tg	thyroglobulin	-1.09	5.54E-07	1.32	2.15E-10
Hspa1a	heat shock protein 1A	1.84	3.10E-13	-1.07	1.25E-05
Pou3f1	POU domain, class 3, transcription factor 1	1.34	7.97E-09	-1.06	3.63E-06
Wnk4	WNK lysine deficient protein kinase 4	-1.06	6.04E-12	1.13	1.08E-11
Hsp90aa1	heat shock protein 90, alpha (cytosolic), class A member 1	1.06	1.47E-23	-1.06	4.39E-15
A730036117Rik	RIKEN cDNA A730036117 gene	1.70	2.50E-12	-1.05	2.96E-06
Hbegf	heparin-binding EGF-like growth factor	1.75	1.28E-30	-1.05	1.68E-06
2310039L15Rik	RIKEN cDNA 2310039L15 gene	-1.03	1.11E-10	1.30	1.13E-17
Uck11os	uridine-cytidine kinase 1-like 1, opposite strand	-1.61	5.41E-15	1.03	2.19E-05
Nmrk2	nicotinamide riboside kinase 2	1.69	3.03E-11	-1.02	3.05E-05
Tent5a	terminal nucleotidyltransferase 5A	1.09	1.41E-20	-1.01	1.27E-14
Shisa6	shisa family member 6	-1.01	9.54E-06	1.10	7.58E-07
Fbp2	fructose bisphosphatase 2	-1.03	1.47E-12	1.01	2.70E-10
Synpo2l	synaptopodin 2-like	1.70	3.38E-17	-1.00	1.71E-06
Shisa3	shisa family member 3	1.00	4.67E-07	-1.07	1.15E-06
Gal3st3	galactose-3-O-sulfotransferase 3	-1.35	1.51E-29	0.99	2.38E-09
Clec18a	C-type lectin domain family 18, member A	-1.37	8.92E-09	0.99	2.19E-05
Sox9	SRY (sex determining region Y)-box 9	1.41	4.79E-23	-0.98	5.43E-10
Serpinb1c	serine (or cysteine) peptidase inhibitor, clade B, member 1c	3.11	7.13E-52	-0.98	4.77E-05
Ripor2	RHO family interacting cell polarization regulator 2	-0.97	1.87E-11	1.12	1.15E-11
Nr4a3	nuclear receptor subfamily 4, group A, member 3	1.38	1.67E-18	-0.97	2.19E-08
Resf1	retroelement silencing factor 1	1.38	1.33E-35	-0.97	3.18E-08
Enah	ENAH actin regulator	1.44	7.61E-55	-0.97	1.24E-14
Gm10635	predicted gene 10635	-1.75	6.89E-16	0.96	2.20E-05
Car9	carbonic anhydrase 9	1.21	1.79E-06	-0.96	8.16E-05
Clcn1	chloride channel, voltage-sensitive 1	-1.68	1.41E-32	0.96	7.23E-10
Xirp2	xin actin-binding repeat containing 2	1.75	1.97E-40	-0.95	1.55E-09

Table S4

Efnb3	ephrin B3	-1.13	7.32E-18	0.95	4.56E-14
Adcy8	adenylate cyclase 8	-0.95	5.46E-05	0.95	4.18E-05
Abra	actin-binding Rho activating protein	2.10	6.29E-52	-0.95	2.17E-05
Klhl33	kelch-like 33	-1.69	1.08E-31	0.95	7.74E-08
Mrgprh	MAS-related GPR, member H	-1.41	3.44E-17	0.94	3.08E-06
Hsbp111	heat shock factor binding protein 1-like 1	-0.93	5.77E-06	0.96	1.48E-06
Nr4a1	nuclear receptor subfamily 4, group A, member 1	0.93	1.20E-04	-0.98	3.19E-06
Gm45457	predicted gene 45457	0.93	2.01E-06	-1.34	2.96E-13
Gm43660	predicted gene 43660	1.15	7.64E-15	-0.93	2.40E-06
Otud1	OTU domain containing 1	1.21	1.25E-09	-0.92	9.88E-05
Acta1	actin, alpha 1, skeletal muscle	3.02	2.76E-45	-0.92	3.57E-10
Gm11992	predicted gene 11992	-0.92	2.90E-04	1.27	1.72E-07
Efemp1	epidermal growth factor-containing fibulin-like extracellular matrix protein 1	-1.11	4.51E-10	0.92	1.14E-07
2310016G11Rik	RIKEN cDNA 2310016G11 gene	-1.27	2.72E-07	0.91	1.65E-04
Ucp3	uncoupling protein 3 (mitochondrial, proton carrier)	-0.90	2.06E-04	1.28	7.57E-14
Gcnt1	glucosaminyl (N-acetyl) transferase 1, core 2	1.10	2.42E-15	-0.89	1.23E-10
Xirp1	xin actin-binding repeat containing 1	1.38	4.10E-54	-0.89	7.72E-11
P3h2	proyl 3-hydroxylase 2	1.28	2.90E-16	-0.89	1.94E-08
Alox5	arachidonate 5-lipoxygenase	-0.89	2.83E-07	0.96	8.92E-10
Lman1l	lectin, mannose-binding 1 like	2.75	4.36E-73	-0.89	1.13E-06
Cabcoco1	ciliary associated calcium binding coiled-coil 1	-1.00	1.17E-11	0.89	6.25E-09
Cadm4	cell adhesion molecule 4	-0.89	2.06E-16	1.16	1.73E-23
Gm8113	predicted gene 8113	-1.16	1.13E-06	0.88	2.14E-04
Tnnt3	troponin T3, skeletal, fast	1.01	7.14E-05	-0.88	2.95E-04
Gm36569	predicted gene, 36569	1.07	2.80E-06	-0.88	1.98E-04
Etv4	ets variant 4	1.31	2.08E-08	-0.88	7.25E-05
Fam198b	family with sequence similarity 198, member B	0.88	2.18E-15	-1.05	2.53E-19
Gdf15	growth differentiation factor 15	2.30	1.76E-21	-0.88	2.61E-04
Gpr22	G protein-coupled receptor 22	-1.76	4.38E-29	0.88	9.30E-07
Hspa1b	heat shock protein 1B	1.83	3.55E-14	-0.87	3.47E-04
Klhl38	kelch-like 38	-0.87	3.66E-04	1.24	4.07E-08
Cplx3	complexin 3	1.42	1.09E-08	-0.87	3.36E-04
Cngeb3	cyclic nucleotide gated channel beta 3	-0.87	2.12E-04	1.33	5.73E-09
Gm11382	predicted pseudogene 11382	1.53	1.85E-09	-0.86	2.97E-04
P4ha1	procollagen-proline, 2-oxoglutarate 4-dioxygenase (proline 4-hydroxylase), alpha 1 polypeptide	0.86	5.14E-18	-0.95	2.80E-17
Gm31663	predicted gene, 31663	-0.86	2.89E-06	1.02	1.75E-08
Stc1	stanniocalcin 1	0.88	7.35E-09	-0.86	1.21E-06
Bcl2	B cell leukemia/lymphoma 2	0.86	3.51E-14	-1.07	6.39E-25
Kcnv2	potassium channel, subfamily V, member 2	-0.96	5.19E-05	0.85	2.76E-05
Retnla	resistin like alpha	-1.38	2.91E-09	0.85	2.65E-04
Nppb	natriuretic peptide type B	2.15	1.55E-27	-0.85	7.54E-05
Hspa1l	heat shock protein 1-like	1.36	1.15E-38	-0.85	1.23E-13
Fhl1	four and a half LIM domains 1	1.22	5.56E-07	-0.84	3.40E-04

Top 100 of significantly different mRNAs with reverse regulation between the three groups (Sham WT, TAC WT and TAC KO) and a minimum FC of 1.5 are displayed. Fold change and p-value is given for Sham WT vs TAC WT and TAC WT vs TAC KO. One-way Anova.

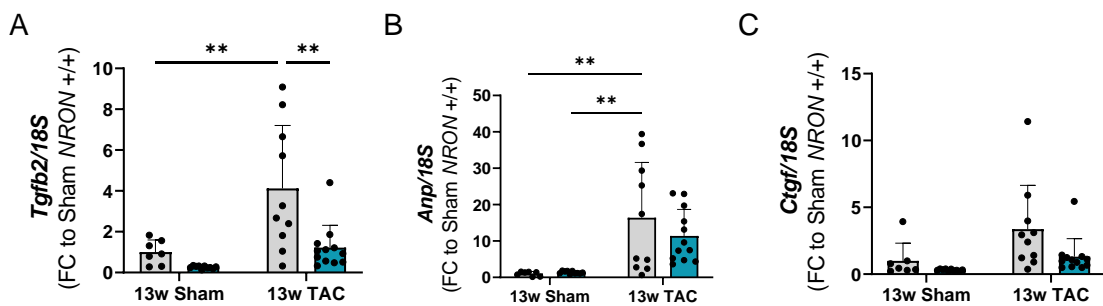


Figure S2: Validation of targets from transcriptomic data. RNA level of (A) *Tgfb2*, (B) *Anp* and (C) *Ctgf* mRNA after 13 weeks of surgery was measured by qPCR and normalized to 18S RNA. All data are mean \pm S.D.; n=7-12. *P<0.05; **P<0.01; ***P<0.001; ****P<0.0001. Two-way ANOVA with Tukey's multiple comparisons test

Table S5: Primer sequences used for gene expression analysis.

Gene	Forward primer sequence	Reverse primer sequence
<i>Gapdh</i>	5'TTCACCACCATGGAGAAGGC3'	5'GGCATGGACTGTGGTCATGA3'
<i>ActB</i>	5'ATCAAGATCATTGCTCCTCCTG3'	5'AGGGTGTAACACGCAGCTCA3'
<i>18S</i>	5'GTAACCCGTTGAACCCATT3'	5'CCATCCAATCGGTAGTAGCG3'
<i>Nron</i>	5'AATGGTGCAGCTCGGATTAC3'	5'GGGAAGGGTTAAGGTGGTA3'
<i>Bnp</i>	5'CTGAAGGTGCTGTCCCAGAT3'	5'GTTCTTTTGTGAGGCCTTGG3'
<i>Mcip1.4</i>	5'AGGGACTTTAGCTACAATTT3'	5'TATGTTCTGAAGAGGGATT3'
<i>Xist</i>	5'TCATCCGCTTGC GTTCATAG3'	5'GAGATCAGTGCTGGCTAAATCAGA3'