YMTHE, Volume 31

# **Supplemental Information**

# Silencing mouse circular RNA circSlc8a1

## by circular antisense cA-circSlc8a1 induces

# cardiac hepatopathy

Nan Wu, Feiya Li, Weining Yang, William W. Du, Faryal Mehwish Awan, Chao Zhang, Juanjuan Lyu, Sema Misir, Kaixuan Zeng, Esra Eshaghi, and Burton B. Yang



# Supplementary Fig. S1. Generation of *circSlc8a1* knockdown mice by using circular antisense-*circSlc8a1* (*cA-circSlc8a1* or *cA-circSlc*) expression construct

- (a) The expression levels of linear Slc8a1 mRNA in different organs. n=12
- (b) The *cA-circSlc8a1* construct was transfected into human HEK293T cells. The expression of *cA-circSlc8a1* (left) and *Slc8a1* mRNA (right) with or without RNase R treatment were determined by using mouse specific primers. n=6, \*\*<0.01 vs vector control.</p>
- (c) The cA-circSlc8a1 construct was transfected into human HEK293T cells. The RNAs from vector or cA-circSlc8a1 transfected cells were reverse transcribed using random or oligo(dT) primers followed by qPCR using mouse specific primers. n=6, \*\*<0.01 vs vector control.</p>
- (d) The levels of unbound *circSlc8a1* and *Slc8a1* mRNA in HL-1 cells transfected with *cA-circSlc8a1*. n=6, \*\*<0.01 vs vector control.
- (e) The construct were used to generate *cA-circSlc8a1* transgenic mice. The 5' and 3' sequences of *cA-circSlc8a1* and the heart promoter sequence are shown.
- (f) Genotyping that validated the establishment of cA-circSLC8A1 (+) transgenic mice.
- (g) The levels of the SLC8A1 protein in the heart of cA-circSlc8a1 transgenic mice.
- (h) Representative photographs of *cA-circSlc8a1* (+) transgenic mice compared to the age-matched negative mice.



Supplementary Fig. S2. Analysis of heart function and fibrosis in the cA-circSlc8a1 transgenic mice.

(a) The heart/tibia length ratio of cA-circSlc8a1(+) mice was significantly higher compared to litter match negative mice. n=10, \*\* p<0.01 vs negative.

(b) Echocardiography of cA-circSlc8a1(+) mice showed elevated left ventricular end-diastolic diameter (LVEDD) compared to litter match negative mice.

(c) Echocardiography of *cA-circSlc8a1*(+) mice showed elevated left ventricular end-systolic dimension (LVESD) compared to litter match negative mice.

(d) Echocardiography of cA-circSlc8a1(+) mice showed reduced LVEDD-LVESD compared to litter match negative mice.

(e) Quantification of fibrosis area observed by Masson trichrome staining (left), Sirius red staining (right). n=4, \*\* p<0.01 vs negative.

(f) Quantification of cross-section area of cardiomyocytes by WGA staining. n=15, \*\* p<0.01 vs negative.

(g) RNAs isolated from heart tissues of mice aged 6-12 month old were subjected to real-time qPCR to measure levels of ANP (left) and BNP (right). n=14, \*\* p<0.01.



### Supplementary Fig. S3. Transgenic mice with heart-specific expression of cA-circSlc8a1 develop hepatic steatosis.

- (a) Quantification of lipid content observed by Oil-Red-O staining. n=6, \*\* p<0.01 vs negative.
- (b) Hepatic fibrosis/cirrhosis was developed in some of the *cA-circSlc8a1* mice (2 out of 14) at late stage, which examined by Masson trichrome staining.
- (c) The expression of *cA-circSlc8a1* precursor in different organs. n=4, \*\* p<0.01 vs negative.
- (d) The levels of cytokines measured by real-time PCR. \* p<0.05 vs negative



Supplementary Fig. S4. Generation of circSlc8a1 transgenic mice and validation of the cardiac protective effects of circSlc8a1.

- (a) The *circSlc8a1* constructs were transfected into human cell line HEK293T. The levels of *circSlc8a1* (left) and *Slc8a1* mRNA (right) with or without RNase R treatment were determined by using mouse specific primers respectively. n=4, \*\*<0.01 vs vector control.</p>
- (b) The *circSlc8a1* constructs were transfected into human cell line HEK293T. The RNA were reverse transcribed by using random or oligo(dT) primers. The levels of *circSlc8a1* were determined by using mouse specific primers. n=6, \*\*<0.01 vs vector control.
- (c) Genotyping that validated the establishment of *circSlc8a1* (+) transgenic mice.
- (d) Quantification of fibrosis area observed by Masson trichrome staining (left) and Sirius red staining (right). n=6, \*\* p<0.01
- (e) Quantification of cross-section area of cardiomyocytes by WGA staining. n=15, \*\* p<0.01



# Supplementary Fig. S5. The *circSlc8a1* protect the heart from cardiac hypertrophy, cardiac fibrosis and impaired heart function.

- (a) The levels of heart failure markers ANP (left) and BNP (right) were remarkably increased in the heart of negative mice by TAC surgery, while were significantly lower in the circSlc8a1 (+) mice compared to the litter match negative mice after TAC surgery. n=15, \*\* p<0.01.</p>
- (b) The levels of cardiac fibrosis markers collagen-I (left) and collagen-III (right) were remarkably increased in the heart of negative mice by TAC surgery, while were significantly lower in the circSlc8a1-transgenic mice compared to the litter match negative mice after TAC surgery. n=15, \*\* p<0.01.</p>
- (c-f) The circSlc8a1(+) mice subjected to TAC prevented the impair of heart function that validated by (c) LVEDD, (d) LVESD, (e) LVEDD-LVESD, and (f) left ventricular pressure (dp/dt ). n=10



### Supplementary Fig. S6. Generation of double transgenic mice with ectopic expression of both circSlc8a1 and cA-circSlc8a1.

- (a) Genotyping that validated the establishment of *circSlc8a1* (+) transgenic mice.
- (b) The expression levels of *circSlc8a1* precursor in negative (Neg), *circSlc8a1* (+) transgenic mice, *cA-circSlc8a1* (+) transgenic mice, or double transgenic mice. n=10, \*\*<0.01 vs negative mice.
- (c) The expression levels of *cA-circSlc8a1* precursor in negative (Neg), *ccircSlc8a1* (+) transgenic mice, *cA-circSlc8a1* (+) transgenic mice, or double transgenic mice. n=10, \*\*<0.01 vs negative mice.
- (d) The expression levels of *Slc8a1 mRNA* in negative (Neg), *ccircSlc8a1* (+) transgenic mice, *cA-circSlc8a1* (+) transgenic mice, or double transgenic mice. n=10
- (e) Quantification of cross-section area of cardiomyocytes by WGA staining. n=10, \*\* p<0.01
- (f) Quantification of lipid content observed by Oil Red O. n=10, \*\* p<0.01



# Supplementary Fig. S7. The *circSlc8a1* protected heart function via binding with mitochondrial proteins.

- (a-c) The density of the bands in Western blot images presented in Fig.7a-c regarding the *circSlc8a1* pull down proteins was analysis. \*\* p<0.01, n=3-4
- (d-f) The density of the bands in Western blot images presented in Fig.7d-f regarding the mitochondrial translocation of proteins was analysis. \*\* p<0.01, n=4-6



### Supplementary Fig. S8. Co-localization of circSlc8a1 with ATPB, COX5B, and NDUFS2.

Fig (a) In situ hybridization of *circSlc8a1* and immunofluorescence staining of ATPB, COX5B, and NDUFS2 in the *cA-circSlc8A1* transgenic and litter-matched negative mice. Representative photographs show co-localization of *circSlc8a1* with these mitochondrial proteins. (b) Quantitation of the staining. \*\*, p<0.01.

Sup	lemetary Table 1: Protein identified by mass spectomatry analysis						
#	Identified Proteins (750)	Accession	⊆	Weight	circNed	circSI C8A1	Fold change
-	structures estimated by the mitrochandrial OS=Mus musculus (Mause) OX=10090 GN=Ath5fth PE=1 SV=2	P56480	Atn5f1b	56 kDa	662 99	1 208 20	182
	ATD symmetry and the mitrochardial OS=Mus musculus (Mariae) OX=10000 GN = 4475 ATD = 7.5.2.2.4.1.4.1.4.1.4.1.4.1.4.1.4.1.4.1.4.1	003265	Ath5f1a	60 kDa	284.4	494.04	1.74
1 (7	Methylymetry of carbonic and anticometry of the microbardial OS=Mile microbardial OS=Mile microbard (http://www		Mcrch	61 kDa	00 00	7 86	1 30
~	mengrationary or conservations and animity intervienting of many measure (mouse) (N. 1900, 011, 1910). MAPU interviews originations 75 kDs interviews miscobradial OC-Mus musculus (Musco) (N. 1900, 011, 1911, 1911,		Miduife 1		164 26	261.07	150
						10.102	
0 0			ndediv		00.100	00.101	00.1
jo I	ATP synthase-coupling factor 6, mitochondrial OS=Mus musculus (Mouse) OX=10090 GN=AppPr FE=1 SV=1	P9/450	Atpopt	12 KUa	97.79	11/.23	<b>c</b> 0.7
_	NADH denydrogenase [ubiquinone] flavoprotein 1, mitochondrial OS=Mus musculus (Mouse) OX=10090 GN=NdurV1 PE=1 SV=1	Q91Y10	Ndutv1	51 KDa	/2.948	117.23	1.61
œ	Alpha-actinin-2 OS=Mus musculus (Mouse) OX=10090 GN=Actn2 PE=1 SV=2	Q9JI91	Actn2	104 kDa	87.722	114.84	1.31
б	Succinate dehydrogenase [ubiquinone] flavoprotein subunit, mitochondrial OS=Mus musculus (Mouse) OX=10090 GN=Sdha PE=1 SV=1	Q8K2B3	Sdha	73 kDa	65.561	96.894	1.48
10	NADH dehydrogenase [ubiquinone] iron-sulfur protein 2, mitochondrial OS=Mus musculus (Mouse) OX=10090 GN=Ndufs2 PE=1 SV=1	Q91WD5	Ndufs2	53 kDa	45.246	95.698	2.12
11	Trifunctional enzyme subunit aloha mitochondrial OS=Mus musculus (Mouse) OX=10090 GN=Hacha PE=1 SV=1	<b>OBBMS1</b>	Hadha	83 kDa	30.472	92,109	3.02
; ;	Beneficial of the statement of the statement of the main statement of the statement of t	001740	Dood		EE ADO	00 574	1 50
2 !		C47160			00.400	170.00	00.1
13	Caveolae-associated protein 2 OS=Mus musculus (Mouse) OX=10090 GN=Cavin2 PE=1 SV=3	Q63918	Cavin2	47 kDa	63.714	86.128	1.35
14	NADH dehydrogenase [ubiquinone] iron-sulfur protein 3, mitochondrial OS=Mus musculus (Mouse) OX=10090 GN=Ndufs3 PE=1 SV=2	Q9DCT2	Ndufs3	30 kDa	42.476	71.774	1.69
15	ATP synthase subunit delta. mitochondrial OS=Mus musculus (Mouse) OX=10090 GN=Ato5ftd PE=1 SV=1	Q9D3D9	Atp5f1d	18 kDa	39.706	70.577	1.78
4	NADH deividrozonase fubiciuinonal fevrorotain 2 mitrochondrial OS=Mus musculus (Mouse) OX=10000 GN=Ndi 6/2 DE=1 SV=2		Ndufu	27 kDa	48 016	60 381	1 44
2 [							Ē
2 !		Eactita	NI03	23 KU3	5	920.20	1
18	NADH denyarogenase [ubiquinone] iron-suitur protein 6, mitochondrial OS=Mus musculus (Mouse) OX=10090 GN=Ndurs6 PE=1 SV=2	P52503	Nduts6	13 KUa	35.089	52.634	1.50
19	Trifunctional enzyme subunit beta, mitochondrial OS=Mus musculus (Mouse) OX=10090 GN=Hadhb PE=1 SV=1	Q99JY0	Hadhb	51 kDa	17.544	50.241	2.86
20	ATP synthase subunit O, mitochondrial OS=Mus musculus (Mouse) OX=10090 GN=Atp5po PE=1 SV=1	Q9DB20	Atp5po	23 kDa	30.472	49.045	1.61
21	Alnha-crystallin Bichain OS=Mus musculus (Mouse) OX=10090 GN=Cryab PE=1 SV=2	P23927	Crvah	20 kDa	30 472	47 849	1.57
60	Costruction and the section OS=Mits miscritics (Mories) OX=10000 GN=Crone PE=1 SV=3	OB9079	Cone	35 kDa	22 161	46.653	2 11
1 6	oddania prava stavani sprava OC makavana SIC - 5000 OL -0000 OL -0000 II 10 - 10 - 10 - 10 - 10 - 10 - 10 - 1		Nidi ifeo		20 640	AC CES	1
52	NADH denyarogenase (upiquinone) iron-suirur protein s, mitocrionarial OS=Mus musculus (mouse) OA=10090 GN=Nadus	M8K3J 1	Nautso	24 KUa	24.044	40.003	00.1
24	Methylcrotonoyl-CoA carboxylase subunit alpha, mitochondrial OS=Mus musculus (Mouse) OX=10090 GN=Mccc1 PE=1 SV=2	Q99MR8	Mccc1	79 kDa	30.472	46.653	1.53
25	Lipoamide acyltransferase component of branched-chain alpha-keto acid dehydrogenase complex, mitochondrial OS=Mus musculus (Mouse) OX=10090 GN=Dbt	t PE=1 P53395	Dbt	53 kDa	31.395	43.064	1.37
26	Casein kinase II subunit beta OS=Mus musculus (Mouse) OX=10090 GN=Csnk2b PE=1 SV=1	P67871	Csnk2b	25 kDa	24.932	39.475	1.58
27	Control marcon or control in the marcon control of the marcon of the mar	D10K3K	Coveh		17 544	38 770	2 18
17			nexon		44C. / I	20.21 8	<b>7</b> .10
28	Casein kinase II subunit alpha OS=Mus musculus (Mouse) OX=10090 GN=Csnk2a1 PE=1 SV=2	Q60737	Csnk2a1	45 kDa	21.238	38.279	1.80
29	Serine beta-lactamase-like protein LACTB, mitochondrial OS=Mus musculus (Mouse) OX=10090 GN=Lactb PE=1 SV=1	Q9EP89	Lactb	61 kDa	19.391	28.709	1.48
30	Histone H4 OS=Mus musculus (Mouse) OX=10090 GN=H4f16 PE=1 SV=2	P62806	H4f16	11 kDa	19.391	27.513	1.42
5	ATD survhasse suithunit ramma minimum OS=Mus musculus (Morise) OY=10000 GN=Ath6ftc DE=1 GV=1	001//00	Atn6f1 c	33 kDa	17 544	<b>JE 101</b>	1 43
- 0						121.02	2
32	I ranscriptional activator protein Pur-alpha OS=Mus musculus (Mouse) OX=10090 GN=Pura PE=1 SV=1	P42669	Pura	35 KDa	10.15/	22.728	2.24
33	RNA-binding motif protein, X chromosome OS=Mus musculus (Mouse) OX=10090 GN=Rbmx PE=1 SV=1	Q9WV02	Rbmx	42 kDa	14.774	21.532	1.46
34	2-oxoisovalerate dehydrogenase subunit alpha, mitochondrial OS=Mus musculus (Mouse) OX=10090 GN=Bckdha PE=1 SV=1	P50136	Bckdha	50 kDa	16.621	21.532	1.30
35	Pentatricopeptide repeat domain-containing protein 3, mitochondrial OS=Mus musculus (Mouse) OX=10090 GN=Ptcd3 PE=1 SV=2	Q14C51	Ptcd3	78 kDa	16.621	21.532	1.30
36	NADH dehvdronenase [ubinitionne] 1 aleha subcomblex subunit 5 OS=Mus musculus (Mouse) 0 X=10090 GN=Ndufa5 PE=1 SV=3	09CPP6	Ndufa5	13 kDa	12 927	20.336	1.57
37	Adamidate kinase is construme 1 OS=Mire musculus (Monitea) OX=10000 GN=4k1 BE=1 SV=1	CORNYS	<u>Δ</u> k1	20 kDa	12 027	10 14	1 48
200	radigitate minae navolarije 100 - musi muzavdarg (morae) (M. 1970) 101 - 101 - 101 - 101 - 101 - 101 - 101 - 10 Statisticanda indensis 200 milionarchadici (ISC-Milio musaculus (Maneo) V 40000 f.M. 1940 - 101 - 101 - 101 -		Mrne 22		14 774	111	0, 1
8	zeu nodorna pretan otz, imouninalia do Oroman indexedua (menedoro) Antina pzz. FT. over 10×−1 Anti Ark Antina pretan otz, imouninalia Adviso Antina Marsino, AZ-4 MOR (AR-Antina pzz. FT. 0×−1					16 747	00.1
50			Acau IO		0 000	14/01	00
40		ESCUTU	RIT/8	112 KUa	9.2339	1.00.01	20.1
41	Protein TSSC4 OS=Mus musculus (Mouse) OX=10090 GN=1ssc4 PE=1 SV=1	Q9JHE7	Tssc4	34 kDa	9.2339	15.551	1.68
42	Hemoglobin subunit alpha OS=Mus musculus (Mouse) OX=10090 GN=Hba PE=1 SV=2	P01942	Hba	15 kDa	12.004	15.551	1.30
43	(E3-independent) E2 ubiquitin-conjugating enzyme UBE20 OS=Mus musculus (Mouse) OX=10090 GN=Ube2o PE=1 SV=3	Q6ZPJ3	Ube2o	141 kDa	8.3105	14.355	1.73
44	Peroxiredoxin-1 OS=Mus musculus (Mouse) OX=10090 GN=Prdx1 PE=1 SV=1	P35700	Prdx1	22 kDa	9.2339	14.355	1.55
45	60S acidic ribosomal protein P2 OS=Mus musculus (Mouse) OX=10090 GN=Rolp2 PE=1 SV=3	P99027	Rolo2	12 kDa	10.157	14.355	1.41
46	Transcriptional activator procheta OS=Mus musculus (Mouse) OX=10090 GN=Purb PE=1 SV=3	035295	Purb	34 kDa	9 2339	13 158	1 42
2.4	Politochalitia, 4 October Marines (NY=41000) GN=7-0000 CN=2-0000 CN=2-000		- meC	17 kDa	5 5403	11 062	2.16
F	our instanti - O - The instantian instantian (CP-Mi is missified in 10-1). Orthoferen kind analow substantian (CP-Mi is missified in 10-1).		llash		C.CT00	11 067	4
2 9						700011	
49		P.10853	HZDC/	14 KUa	0.403/	206.11	C8.1
50	cAMP-dependent protein kinase type II-alpha regulatory subunit OS=Mus musculus (Mouse) OX=10090 GN=PrkarZa PE=1	P1236/	Prkarža	45 kDa	5.5403	10.766	1.94
51	AP-3 complex subunit sigma-2 OS=Mus musculus (Mouse) OX=10090 GN=Ap3s2 PE=1 SV=1	Q8BSZ2	Ap3s2	22 kDa	7.3871	10.766	1.46
52	Elongation factor 1-alpha 2 OS≡Mus musculus (Mouse) OX=10090 GN=Eef1a2 PE=1 SV=1	P62631	Eef1a2	50 kDa	7.3871	10.766	1.46
53	Small nuclear ribonucleoprotein Sm D2 OS=Mus musculus (Mouse) OX=10090 GN=Snrpd2 PE=1 SV=1	P62317	Snrpd2	14 kDa	8.3105	10.766	1.30
54	Enhancer of rudimentary homolog OS=Mus musculus (Mouse) OX=10090 GN=Erh PE=1 SV=1	P84089	Erh	12 kDa	5.5403	9.5698	1.73
55	14-3-3 protein aamma OS=Mus musculus (Mouse) OX=10090 GN=Ywhaa PE=1 SV=2	P61982	Ywhag	28 kDa	6.4637	9.5698	1.48
56	Beta-enclase OS=Mus musculus (Mouse) OX=10090 GN=Enc3 PE=1 SV=3	P21550	Eno3	47 kDa	6.4637	9.5698	1.48
22	Liner Antonina, 20 Cartaine Manuschi DX241000 (All-Inho Pert 2) (2) (2)	09FT78	Chul.	75 kDa	6 4637	9.5698	1 48
a d	usinsuppliming of the international production (DAV regional Ottophic) TeV (DV event) biotecome interface interface (DAV event) (DV		Drho1	173 100	6 A627	0.5608	87.1
	Nuovolle-billulla puelli i Loo-tuka illuskula (nuovo CH-1000 CH-1000 CH-1000 CH-1000 CH-1000 CH-1000 CH-1000 CH Statio-Neto-statio anderio abcondenano 24 actività rubu inferenzi inferenzi CH-1000 CH-1000 CH-1000 CH-1000 CH-		Dentro	26 1/10	0.4001	0.5000	01.1
20 20			Pppzca	30 KUa	0.403/	8.3090 7 7000	0471
00		0.12 111	Hypk	15 KUa	1.38/1	9.5698	1.30
61	Complement C1q and tumor necrosis ractor-related protein 9 US=Mus musculus (Mouse) UX=10090 GN=C1qtrift PE=1 SV=1	Q4ZJN1	C1qtnf9	35 KDa	3.6936	8.3/36	17.7
62	NADH dehydrogenase [ubiquinone] 1 beta subcomplex subunit 11, mitochondrial OS=Mus musculus (Mouse) 0X=10090 GN=Ndurb11 PE=1 SV=2	009111	Ndufb11	17 kDa	5.5403	8.3736	1.51
63	2-oxoisovalerate dehydrogenase subunit beta, mitochondrial OS=Mus musculus (Mouse) OX=10090 GN=Bckdhb PE=1 SV=2	Q6P3A8	Bckdhb	43 kDa	6.4637	8.3736	1.30

		Assession		Melocular			
#	Identified Proteins (750)	Number	9	Weight	circNed	circSLC8A1	Fold change
64	recontinue i recontra (rec) ATP summers submit residen mitro-bondrial OS=Mus musculus (Mouse) OX=10090 GN=Ath6fte PE=1 SV=2	P56382	Atn5f1e	6 kDa	6 4637	8 3736	130
. 59	structure of the material previous and the material of the material previous of the material of the	P20108	Prdx3	28 kDa	6.4637	8.3736	130
29	ring counting periods periods to concentration of the machine in the machine induced (machine) and the machine induced (machine) (machin	O3LIER3	Duffen		CUTT C	7 1774	2 50
22	r by Draining second of the mission of the down of the second of the sec	DE0743	An2e1	17 kDa	3 6036	71774	1 94
89	restrictions of the mission of the measure of the m	OBOVG0	Ndro 2	41 kDa	3,6936	7 1774	194
200	r room instruction Communication (non-construction) (Annues) Oxi-1000 (Annues) (Ann		Sref	31 kDa	3.6936	7 1774	194
02	den index gammer in spring i accord i occurato indexatora (macator) occuratora i entra contra accordante accord Prese ambediar processioni accorde da Occi-Amber anicentinis (Annices) OXY=100000 GN=Dertrada DE=4 6V/=1	COR1C7	DrnfAlla		A 617	7 1774	
2 1	r term styr processing accurate a removal of OC-man interaction (mode). OX-mode OF-1 processing accurate a removal of the mice of the other other of the other oth		Sorhe 2	132 kDa	1617	A771 7	- 100- 777
	outini ana orio domain-tontaning proteini z OO-muoda inteoducia (nouted) ON-10000 DN-001021 - 1012 Transferi anti domain-tonteinina antichia Rustine Manuesi (Natries) ON-10000 DN-001021 - 1012	E00710	Ondhl	117 LDa	1617	A774 7	
12	na insve⊥py vontain sontaaning protein Or-musi insoeden (Norosey / Arr 1000 Oth-Ogain E-1 04-1 Diensverbad arotabis K minokondrial OS-Mire musicalije (Marinea) OY-41000 DR-Gibk BE-1 421-2				5 5403	A774 7	00- 1
2 7					0.010		00.1
4 1		02021		23 KUa	0.5	1.100.C	
75	Elongation factor 1-beta OS=Mus musculus (Mouse) OX=10090 GN=Eer1b PE=1 SV=5	O70251	Eef1b	25 kDa	1.8468	5.9811	3.24
76	ATP synthase subunit f, mitochondrial OS=Mus musculus (Mouse) OX=10090 GN=Atp5mf PE=1 SV=3	P56135	Atp5mf	10 kDa	2.7702	5.9811	2.16
22	NADH dehydrogenase [ubiquinone] 1 alpha subcomplex assembly factor 3 OS=Mus musculus (Mouse) OX=10090 GN=Ndufat3 PE=1 SV=1	Q9JKL4	Ndufaf3	21 kDa	2.7702	5.9811	2.16
. 82	Transformer-2 protein homolog beta OS=Mus musculus (Mouse) OX=10090 GN=Tra2b PE=1 SV=1	P62996	Tra2b	34 kDa	2.7702	5.9811	2.16
19	Lipoprotein lipase OS=Mus musculus (Mouse) OX=10090 GN=LpI PE=1 SV=3	P11152	LpI	53 kDa	3.6936	5.9811	1.62
80	NADH dehydrogenase [ubiquinone] 1 alpha subcomplex subunit 2 OS=Mus musculus (Mouse) OX=10090 GN=Ndufa2 PE=1 SV=3	Q9CQ75	Ndufa2	11 kDa	4.617	5.9811	1.30
81	Cvtochrome c1. heme protein. mitochondrial OS=Mus musculus (Mouse) OX=10090 GN=Cvc1 PE=1 SV=1	Q9D0M3	Cvc1	35 kDa	0	4.7849	
82	ADH dehvdropenase fubianinonei 1 beta subcomplex subhunit 9 OS=Mus musculus (Mouse) OX=10090 GN=Ndrubo PE=1 SV=3	09CO.IB	Ndufb9	22 kDa		4.7849	
5 68	triant and an entry international contract account of the mission of the mission of the matter of the mission of the mission of the mission o	00CP03	Tomm22	16 kDa	1 8468	4 7849	2 59
3 2	Mitch Johana in their receptor sociality of Antizz Institutionaly OC-Mitors (Modes) (Modes) (AC-1009) OC-10111112 ET-21-05-0 Mitch Johana Constructional A alpha antipromotica antiproiti 7 OC-Mitor nurse (Modes) (OC-1000) OC-1011112 ET-2				00400-1	A 70A0	2.03 7 FD
5 8	rvotni engluigeriase turquininet i attina succumptica sucurus intescuts (mouse) XA-10000 XH-10040 XH-10040 XH-1 2885 antonomo ana Attanon rendritare unburgi 0 OS−Mini enviroilin (Marineo IX-40000 XH-10040 XH-10040 XH-10040		Demol		00400-1 CU22 C	A 70AD	00.7 72
000	20- priessonie fort-state regulatory sourcini a OCinto into-cuto for the Court of		Nhao		2011.2	4./043	C/.1
0 0		060667			2011.2	4./043	C/.1
20	Historie H.Z.A. IVPE Z-A. OS=Mus musculus (Mouse) OX=1.0090 (BN=HISIZATE) PE=1 SV=3	(10000/	HISTZNZAA1	14 KUa	2.1/12	4./849	1./3
88 8	Serinelarginine-rich splicing factor 1 OS=Mus musculus (Mouse) OX=10090 GN=Sist1 PE=1 SV=3	Q6PDM2	Srsf1	28 kDa	2.7702	4.7849	1.73
68	60S acidic ribosomal protein P1 OS=Mus musculus (Mouse) OX=10090 GN=Rpjp1 PE=1 SV=1	P47955	Rplp1	11 kDa	3.6936	4.7849	1.30
06	Cytochrome c oxidase subunit 7A1, mitochondrial OS=Mus musculus (Mouse) OX=10090 GN=Cox7a1 PE=1 SV=1	P56392	Cox7a1	9 kDa	3.6936	4.7849	1.30
91	DNA-directed RNA polymerases I, II, and III subunit RPABC3 OS=Mus musculus (Mouse) OX=10090 GN=Polr2h PE=1 SV=3	Q923G2	Polr2h	17 kDa	3.6936	4.7849	1.30
92	Long-chain specific acyl-CoA dehydrogenase, mitochondrial OS=Mus musculus (Mouse) OX=10090 GN=Acadl PE=1 SV=2	P51174	Acadl	48 kDa	3.6936	4.7849	1.30
93	Metaxin-2 OS=Mus musculus (Mouse) OX=10090 GN=Mtx2 PE=1 SV=1	088441	Mtx2	30 kDa	3.6936	4.7849	1.30
94	Scaffold attachment factor B1 OS=Mus musculus (Mouse) OX=10090 GN=Safb PE=1 SV=2	D3YXK2	Safb	105 kDa	3.6936	4.7849	1.30
95	Serine/threonine-protein phosphatase 2A 55 KDa regulatory subunit B alpha isoform OS=Mus musculus (Mouse) OX=10090 GN=Ppp2r2a PE=1 SV=1	Q6P1F6	Ppp2r2a	52 kDa	3.6936	4.7849	1.30
96	Desmoplakin OS=Mus musculus (Mouse) OX=10090 GN=Dsp PE=1 SV=1	E9Q557	Dsp	333 kDa	0	3.5887	
97	Guanine nucleotide-binding protein G(I)/G(S)/G(T) subunit beta-1 OS=Mus musculus (Mouse) OX=10090 GN=Gnb1 PE=1 SV=3	P62874	Gnb1	37 kDa	0	3.5887	
86	Kinesin heavy chain isoform 5C OS=Mus musculus (Mouse) OX=10090 GN=Kif5c PE=1 SV=3	P28738	Kif5c	109 kDa	0	3.5887	
66	Chromobox protein homolog 3 OS=Mus musculus (Mouse) OX=10090 GN=Cbx3 PE=1 SV=2	P23198	Cbx3	21 kDa	1.8468	3.5887	1.94
100	Eukaryotic translation initiation factor 3 subunit F OS=Mus musculus (Mouse) OX=10090 GN=Erf3f PE=1 SV=2	Q9DCH4	Eif3f	38 kDa	1.8468	3.5887	1.94
101	KH domain-containing, RNA-binding, signal transduction-associated protein 1 OS-Mus musculus (Mouse) OX=10090 GN=Khdrbs1 PE=1 SV=2	Q60749	Khdrbs1	48 kDa	1.8468	3.5887	1.94
102		P/0202	LXN	25 KDa	1.8468	3.5887	1.94
103		000760		184 KUa	1.8408	3.588/	1.94
104	101 small inclear inconcreptioner A OS-MMS incussion SATE 10890 GNS-SATE 2045	U02189	Surpa	32 KUa	1.8468	3.5887	1.94
CO 4	or diameter of the second modes (Area 10000 GN=00 FT=1 SV=2 Are diameter of the second modes (Area 10000 GN=00 FT=1 SV=2				0.400	3.300/ 2.200 C	-34
	4-0 incontral protein oz 1. OG-Mitra muzertular (Moures) OY = 1000 ON = 1-3 OV = 1 Mutro de Anadrez Stevensianes De Mitra muzertular (Moures) OY = 10000 GN = 5-3 OV = 1		Arach	9 NUA 976 L/Da		2.3323 7 3075	
801	reventroor country for a CO-mine intervent (mouse) CA-mouse (March 1000 CH-mouse) Activitient IM montain 1 OS=Mine muscrifice (Marines) OY=10000 GN=Ablim FE1 SV-1	ORK4G5	Ahlim1	97 kDa		2 3025	
109	Actin-related profession 203 complex submit? OS=Musc misculus (Muse) (X=1000 GN=Arm? PE=1 SV=3	OBCVB6	Arnc.2	34 kDa	0 0	2 3925	
110	roun interation of the activity of a mount of the activity of	D3Z609	Bin2	53 kDa	0 0	2.3925	
11	cardiaerin-13 OSEaMis misculus (Mouse) OX=10000 GN=Cardiaeri	O9WTR5	Cdh13	78 kDa	, 0	2.3925	
112	COPPENDING CONTRACTION OF A CONTRACTION OF	Q8VBV7	Cops8	23 kDa	0 0	2.3925	
113	E3 ubiquitin-protein ligase RMND5A OS=Mus musculus (Mouse) OX=10090 GN=Rmnd5a PE=1 SV=2	Q80YQ8	Rmnd5a	- 44 kDa	0	2.3925	
114	Glutaryl-CoA dehydrogenase, mitochondrial OS=Mus musculus (Mouse) OX=10090 GN=Gcdh PE=1 SV=2	Q60759	Gcdh	49 kDa	0	2.3925	
115	Histidine-rich calcium-binding protein OS=Mus musculus (Mouse) OX=10090 GN=Hrc PE=1 SV=1	G5E8J6	Hrc	85 kDa	0	2.3925	
116	NADH dehydrogenase [ubiquinone] 1 alpha subcomplex subunit 9, mitochondrial OS=Mus musculus (Mouse) OX=10090 GN=Ndufa9 PE=1 SV=2	Q9DC69	Ndufa9	43 kDa	0	2.3925	
117	Predicted gene 11639 OS=Mus musculus (Mouse) OX=10090 GN=Gm11639 PE=1 SV=1	A0A1D5RLM8-DEC	О		0	2.3925	
118	Protein phosphatase 1A OS-Mus muscuus (Mouse) OX=10090 GN=Pointa PE=1 SV=1	P49443	Ppm1a	42 kDa	0 0	2.3925	
8118	rubose-priospirate on pipospiratinases OG-musi mucacuju studiosej VG-ar pipos de Partija III PE-1 SV-1 Romantonajio 2 OG-Mini emicandira (Manaci) OX-40000 GNI-Sumao BE-14 GV-1		Suppo 111	30 KUa 117 L/Do		2.3323	
104	Diraptoppoult≠ Con-tunes intextuates (wurdes CA+ todos One-Vorpus) Er E-1 o V-2 Diraptopro-assessinated interimiti shiba OS=Mire mirerulus (Mories) OX=10000 (M=Serf DE=4 KV=1		Ser1	3.0 kDa		2 3025	
i		× · · · · · · · · · · · · · · · · · · ·		1111	<b>,</b>		

Note: Red labeled proteins are mitochondrial proteins.

# Supplementary Table S2: Primer information.

Name		Sequence
circSlc8a1/cA-circSlc8a1-1	Forward:	5'-ctattgaaggcacagcccgagg-3'
	Reverse:	5'-gtacaataagacttccaactgc-3'
circSlc8a1/cA-circSlc8a1-2	Forward:	5'-acagcccgaggtggaggggaagac-3'
	Reverse:	5'-ggtgggagacttaatcgaagcatg-3'
<i>Slc8a1</i> mRNA	Forward:	5' gagcagccttcagagctggtcgg-3'
	Reverse:	5'-gtacaataagacttccaactgc-3'
RNU6	Forward:	5'-gtgctcgcttcggcagcacata-3'
	Reverse:	5'-tggaacgcttcacgaatttgcg-3'
Gapdh	Forward:	5'-cccacttgaagggtggagcc-3'
	Reverse:	5'-acaatcttgagtgagttgtc-3'
cA-circSlc8a1 precursor	Forward:	5'-acagcccgaggtggaggggaagac-3'
	Reverse:	5'-gagctgtcagagatttctccaacc-3'
cA-circSlc8a1 genotyping	Forward:	5'gggcccgcctcgagacacttagccgtgttctt-3'
	Reverse:	5'-ggtgggagacttaatcgaagcatg-3'
circSlc8a1 genotyping	Forward:	5'-tgtggagagctcgaattccagaa-3
	Reverse:	5'-gcatgcatagatcttacggaaaacggcagaag-3'
collagen1a1	Forward:	5'-gaatggagatgatggggaagctgg-3'
	Reverse:	5'-catctcctttggcaccatccaaac-3'
collagen3a1	Forward:	5'-gtaaagaagtctctgaagctgatg-3'
	Reverse:	5'-gcgatatctatgatgggtagtctc-3'
ANP	Forward:	5'-gcggtgtccaacacagatctgatg-3'
	Reverse:	5'-ggtgggttgacctccccagtccag-3'
BNP	Forward:	5'-gagcaattcaagatgcaggtgagc-3'
	Reverse:	5'-cacacacctgaatcccccatcc-3'

### **Supplementary Table S3**

### **Construct sequences**

#### cA-circSlc8a1

agcaggcatatgggatgggatataaaggggctggagcactggaggactgtcagagatttetccaacccgtcgacacacttagccgtgttctttgcactttctgcatgtcccccgtctggcctggcctggcctggcctggcctggcctcccagtggcttccccagtgg atgetetegeteacgtgagteacgggtteeteaaatgtaaagatgeetgeatggteateateaaaaatggttatggtggeagtgetgggtgaceeaagaeaageaattgaagaagegtgattggattetagtatgecaetettetgaaaeatetgaagagactetgacattgetaagatgeacaaggaagttteatettetteaaagatateateateaatgatgeeaactetgattteettetgggteteecetggtttgaagateacagteeetteegtgaatteataateagaeeeageattggetgtgeegtettetg etgteteetteatgtteaatgateateeceettgtaceggeeetgtacegettgtagacataettgtaaaagagaageegeetgtetgetaceeaegegaacacaaegeagatgggaaagaagaaagaaagaaagaageageetteeeagaeettee caacteccaggagagctgacagacaagattatataaagccaggtataggcaaagatgctccaggctgctgtcacaaagaacacacgcagatgcttgatcttccttgtctctccatcaggggaccacgtaaacacaggagtgcgattatgatgaacatgtta a agg cag cact to ccac gat gg tg ctg gg acc cag gt ccct g cg gt gaag tt at gg ccg cacact tc a at gac tg ac cag gag cag a agt ccc agg g cc cac gg t c cac g gt to cac gat to tc cac gat gat ccc agg g cc cac g gt ccc agg t cc cac gat to tc cac gat ccc agg g cc cac gat ccc agg g cc cac gat ccc agg t ccc aggtegtettggtggtetetecatteggtttetttategttatttetttetettgagaggtgatgaecetetatagaggaeataaaceggtetgeaataatagaaaceteeaaggaaeatgtagaecatagaecatagaecatagaecatttggteetetagaagatgatgaecatagaecatttggteetetagaagatgatgaecatagaecatttggteetetagaagatgatgaecatagaeecatagaecatagaecatagaeecatagaeecatagaeecatagaeecatagaeecatagaeecatagaeecatagaeecatagacgccctttctcactggttctctcttctgccgttttccgtaggatcct

#### circSlc8a1

#### pre-cA-circSlc. cA-circSlc8a1-intron mutation (producing pre-cA-circSlc8a1).

#### 

#### pre-circSlc. circSlc8a1-intron mutation (producing pre-circSlc8a1).

#### Probe sequences

circSlc8a1 probe: 5' agacttccaactgctccaacactatttcatcgttgaa-3' cA-circSlc8a1 probe: 5' cctgtggagagctcgaattccagaacgatgaaatagtgtt-3'