

## **SUPPLEMENTAL INFORMATION**

### **MicroRNAs Contribute to the Maintenance of Cell Type-Specific Physiological Characteristics: miR-192 Targets Na<sup>+</sup>/K<sup>+</sup>-ATPase $\beta$ 1**

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## SUPPORTING INFORMATION – METHODS AND MATERIALS

***Isolation of nephron segments.*** Rats were anesthetized with an i.m. injection of ketamine (40 mg/kg), xylazine (8 mg/kg), and acepromazine (5 mg/kg) with supplementing anesthesia if needed. Left kidney was perfused with ~10ml cold dissection solution (HBSS, 20 mM Hepes, 5.5 mM D-glucose, 1 mg/ml BSA), followed by 10ml of digestion solution (HBSS, 20 mM Hepes, 5.5 mM D-glucose, 1500 U/ml collagenase). Kidney was removed, cortex and outer medulla separated, cut into pieces and transferred into separate beakers. 1ml of digestion solution was added and incubated at 37°C for 30min. During digestion, supernatant was periodically transferred into a new tube, while another 1ml of digestion solution was added into the beaker. Supernatant containing digested tissue was transferred into a Petri dish and microdissected under a LEICAM3Z stereomicroscope with dark-field illumination.

***Na<sup>+</sup>/K<sup>+</sup>-ATPase activity assay in crude membrane fractions.*** The assay was performed as described previously. (19,20) Cells were broken by sonication and centrifuged at 400 G for 4 min. The supernatant was transferred to another tube and further centrifuged at 40,000 g for 20 min. to isolate the crude membrane fraction. The pellet was resuspended in buffer containing (in mM) 300 D-mannitol, 10 Tris · Cl, and 1 EDTA, pH 7.4. Obtained aliquots were combined with the assay buffer containing (in mM) 100 NaCl, 20 KCl, 4 MgCl<sub>2</sub>, 100 Tris · Cl, and 2 Na<sub>2</sub> ·ATP, pH 7.4 for total ATPase activity measurement, or another containing (in mM) 120 NaCl, 4 MgCl<sub>2</sub>, 100 Tris · Cl, 1 ouabain, and 2 Na<sub>2</sub> ·ATP, pH 7.4 for ouabain-insensitive ATPase activity measurement.

The reaction was incubated at 37°C for 10 min, and stopped by returning it to ice and adding ice-cold TCA. Production of Pi was measured by Tausky and Shorr method. Nonenzymatic degradation of ATP was subtracted and Na<sup>+</sup>/K<sup>+</sup>-ATPase activity calculated as the difference between total ATPase activity and ouabain-insensitive ATPase activity.

***Primers used for cloning the 3'-UTR, 5'-UTR and promoter sequences, and introducing mutations or deletions.*** Description of the amplification products can be found in the text. In upper case letters is the sequence complementary with the amplification template; in lower case letters is the “overhang” sequence required for cloning purposes (sequence recognized by the restriction enzymes, listed in the last column). Underlined nucleotides or dashes mark the site of mutation or deletion, respectively.

Amplification Product	Primer	Primer Sequence	Restriction Enzyme
Atp1b1 P+5'-UTR	sense	5'-tttat ctcgag GTCGCTTCAGGGACAGGCTTTT	<i>Xho</i> I
	anti-sense	5'-aattc aagctt GCCTACCACCAATCCCCAACAC	<i>Hind</i> III
Atp1b1 P+5'-UTR mut	sense	5'-GTCCATCTCGCCAGAGCAGATAGCAGGCAGAGTGG	N/A
	anti-sense	5'-CCACTCTGCCTGCTATCTGCTCTGGCGAGATGGAC	N/A
Atp1b1 P+5'-UTR del	sense	5'-GTCCATCTCGCCAG-----TAGCAGGCAGAGTGG	N/A
	anti-sense	5'-CCACTCTGCCTGCTA-----CTGGCGAGATGGAC	N/A
Atp1b1 Promoter	sense	5'-tttat ctcgag GGTCGCTTCAGGGACAGGCTTTTC	<i>Xho</i> I
	anti-sense	5'-tttat aagctt GCGCGCAGGCCAGCAGCAGGAGAA	<i>Hind</i> III
Atp1b1 3'-UTR	sense	5'-atata gagctc TCACAAGCACAAATCTTTCCCACT	<i>Sac</i> I
	anti-sense	5'-ttcct aagctt TGTCCATTGTGTTAACCTGACTGA	<i>Hind</i> III
Atp1b1 3'-UTR partial	sense	5'-ttcta aagctt TATGGGACCTACTTAATCT	<i>Hind</i> III
	anti-sense	5'-ttcga actagt TGACTTTGGTTTATTTTCATACA	<i>Spe</i> I
Atp1b1 3'-UTR mut	sense	5-CACTAGCTTCTGCATTTAATAGGCTCGAATGTAAATTTAAAGTGTAGC	N/A
	anti-sense	5-GCTACACTTTAAATTTACATTCGAGCCTATTAATGCAGAAGCTAGTG	N/A
Romk2 3'-UTR	sense	5'-ttcga actagt AGGACCTAAGGGTTGACTGTGTTC	<i>Spe</i> I
	anti-sense	5'-ttcga gagctc TTTTCAAGGTGAATTTGGAATGA	<i>Sac</i> I
Nkcc2 3'-UTR	sense	5'-ttcga actagt AGGGATGAGAAATGTTGTACCTTG	<i>Spe</i> I
	anti-sense	5'-ttata gccgac TGCCAAAGCAAACAAACTTAGTG	<i>Nae</i> I
Umod 3'-UTR	sense	5'-atata actagt GGTGTGGCTCCCAGTTCACTTCCT	<i>Spe</i> I
	anti-sense	5'-ttcga aagctt AACTTATTATTTGATACATTCCT	<i>Hind</i> III

**UTR reporter assay.** The assay was performed as described previously. (13,14,15) HeLa or 3T3-L1 cells were cultured in 96-well, clear plates (Greiner Bio-One) with DMEM and 10% FBS. When 80%-90% confluent, cells were co-transfected with the UTR reporter construct (100 ng per well), a pMIR-REPORT  $\beta$ -gal plasmid (50 ng per well) and pre-miR or control oligonucleotides (10 pmol per well), using Lipofectamine 2000 (Invitrogen). Twenty four hours after transfection, luminescence from luciferase and  $\beta$ -galactosidase activities were measured using the Dual-Light system (ABI) and following the manufacturer's protocol for microplate assay. Luminescence signals were acquired using high-throughput Analyst HT 96.384 microplate reader (Molecular Devices).  $\beta$ -galactosidase activity was used to normalize luciferase signals as control for transfection efficiency and cell density.

**Atp1b1 promoter construct and promoter activity assay.** We followed the approach described previously. (21) We used the pGL4.81 reporter vector containing Renilla luciferase gene (Promega) in which we inserted the 1kb sequence upstream of the transcription start site of the Atp1b1 gene. Cloning primers are listed in the table above. Human renal epithelial cells (HRE) were cultured in 96-well plates with REGM medium, were transfected with the Atp1b1 promoter-pGL4.81 vector, the pGL2-control vector and pre-miR oligonucleotides, as described above. 24 hours after transfection, luciferase activity was determined using the Dual Luciferase Reporter Assay System (Promega) and following the manufacturer's protocol. pGL2-control containing the firefly luciferase gene was used to normalize the Renilla luciferase activity to control for transfection efficiency and cell density.

## SUPPORTING INFORMATION – TABLES

**Table S1. miRNA expression profiles in glomeruli, proximal convoluted tubule (PCT) and medullary thick**

**ascending limb (mTAL).** The table lists relative expression levels (and their standard error, SEM) of 118 miRNAs and their fold difference in the three nephron segments.

	Glom				PCT				mTAL			
	Expressi on	SEM	Fold differen ce vs. PCT	Fold differen ce vs. mTAL	Expressi on	SEM	Fold differen ce vs. Glom	Fold differen ce vs. mTAL	Expressi on	SEM	Fold differen ce vs. Glom	Fold differen ce vs. PCT
<b>let-7a</b>	121.13	21.59	0.92	0.75	130.97	47.09	1.08	0.81	161.40	56.41	1.33	1.23
<b>let-7b</b>	447.90	20.07	1.53	0.63	292.94	35.41	0.65	0.41	709.84	83.32	1.58	2.42
<b>let-7c</b>	541.47	34.85	1.05	0.58	517.23	60.42	0.96	0.55	936.07	37.88	1.73	1.81
<b>let-7d</b>	28.80	8.27	0.74	0.84	39.09	13.69	1.36	1.14	34.28	2.95	1.19	0.88
<b>let-7e</b>	118.75	3.55	1.29	0.69	92.15	17.77	0.78	0.54	172.03	13.10	1.45	1.87
<b>let-7f</b>	0.25	0.08	0.30	0.52	0.86	0.49	3.39	1.77	0.48	0.14	1.92	0.57
<b>let-7i</b>	41.43	13.54	1.21	1.07	34.35	4.90	0.83	0.89	38.64	10.98	0.93	1.13
<b>miR-100</b>	304.01	58.32	0.94	0.25	323.35	39.54	1.06	0.26	1233.24	253.9	4.06	3.81
<b>miR-103</b>	13.52	6.87	0.44	0.46	31.04	10.98	2.30	1.06	29.29	10.84	2.17	0.94
<b>miR-106b</b>	9.38	3.99	0.94	0.66	10.01	3.04	1.07	0.70	14.24	5.21	1.52	1.42
<b>miR-122</b>	0.04	0.03	1.14	2.57	0.03	0.01	0.88	2.25	0.01	0.00	0.39	0.44
<b>miR-124</b>	0.18	0.07	0.46	0.96	0.39	0.14	2.18	2.08	0.19	0.05	1.05	0.48
<b>miR-125a- 5p</b>	234.31	39.24	0.80	0.72	293.46	41.93	1.25	0.90	325.80	42.68	1.39	1.11

		183.4										
<b>miR-126</b>	1676.89	1	6.96	7.03	240.96	35.22	0.14	1.01	238.44	31.39	0.14	0.99
<b>miR-130a</b>	5.75	3.76	1.10	0.62	5.24	2.94	0.91	0.56	9.31	4.21	1.62	1.78
<b>miR-130b</b>	2.21	1.57	0.64	1.10	3.44	2.58	1.56	1.71	2.01	0.82	0.91	0.58
<b>miR-132</b>	2.81	0.32	11.30	3.27	0.25	0.05	0.09	0.29	0.86	0.03	0.31	3.46
<b>miR-133b</b>	0.01	0.00	0.22	0.06	0.05	0.04	4.51	0.27	0.20	0.09	16.41	3.64
<b>miR-134</b>	0.26	0.08	0.06	1.24	4.15	0.39	16.15	20.07	0.21	0.06	0.80	0.05
<b>miR-135a</b>	1.02	0.17	0.76	1.08	1.34	0.20	1.31	1.42	0.94	0.34	0.92	0.70
<b>miR-135b</b>	0.11	0.04	0.70	0.64	0.15	0.02	1.42	0.91	0.17	0.08	1.56	1.10
<b>miR-137</b>	0.01	0.01	6.02	1.18	0.00	0.00	0.17	0.20	0.01	0.01	0.85	5.11
<b>miR-138</b>	2.62	1.33	4.47	0.72	0.59	0.22	0.22	0.16	3.63	1.29	1.39	6.20
<b>miR-139-5p</b>	61.60	12.09	9.03	8.64	6.82	1.43	0.11	0.96	7.13	0.08	0.12	1.05
<b>miR-141</b>	0.30	0.08	0.71	0.02	0.43	0.17	1.40	0.03	14.80	0.42	48.65	34.78
<b>miR-142-3p</b>	1.74	0.81	0.53	0.36	3.26	1.10	1.87	0.68	4.77	1.87	2.74	1.47
<b>miR-142-5p</b>	0.25	0.14	0.60	0.66	0.41	0.15	1.66	1.09	0.37	0.12	1.52	0.92
<b>miR-144</b>	0.01	0.00	1.40	0.76	0.01	0.00	0.71	0.54	0.01	0.00	1.32	1.85
<b>miR-146a</b>	117.95	7.92	4.53	3.98	26.04	7.00	0.22	0.88	29.64	3.86	0.25	1.14
<b>miR-150</b>	140.19	29.99	15.14	5.33	9.26	1.27	0.07	0.35	26.33	1.34	0.19	2.84
<b>miR-152</b>	8.19	1.10	1.75	0.34	4.68	0.55	0.57	0.19	24.28	0.83	2.96	5.19
<b>miR-15b</b>	31.67	3.71	0.52	0.94	61.48	7.55	1.94	1.82	33.75	5.40	1.07	0.55
<b>miR-16</b>	475.28	52.86	0.46	1.70	1024.63	53.15	2.16	3.68	278.76	4.79	0.59	0.27
<b>miR-17</b>	7.31	2.61	0.50	0.22	14.49	2.21	1.98	0.43	33.62	7.84	4.60	2.32
<b>miR-181a</b>	13.76	6.80	0.91	1.15	15.20	3.53	1.10	1.27	11.99	4.32	0.87	0.79
<b>miR-181b</b>	7.89	1.86	0.51	1.06	15.47	2.55	1.96	2.08	7.45	2.05	0.94	0.48
<b>miR-181c</b>	9.33	4.68	1.10	0.71	8.52	3.13	0.91	0.65	13.08	4.90	1.40	1.53
<b>miR-181d</b>	2.02	0.86	0.74	0.41	2.74	0.48	1.36	0.56	4.88	1.47	2.42	1.78
<b>miR-183</b>	0.28	0.12	0.18	0.16	1.54	0.15	5.43	0.87	1.79	0.09	6.28	1.16
<b>miR-184</b>	0.74	0.14	1.91	14.95	0.39	0.16	0.52	7.85	0.05	0.01	0.07	0.13

<b>miR-185</b>	2.85	1.28	0.13	0.35	22.58	7.31	7.91	2.77	8.14	2.86	2.85	0.36
<b>miR-186</b>	6.20	2.39	0.26	0.35	23.66	3.66	3.82	1.32	17.88	5.74	2.88	0.76
<b>miR-187</b>	0.41	0.15	0.53	0.46	0.78	0.39	1.90	0.86	0.91	0.25	2.19	1.16
<b>miR-18a</b>	0.84	0.30	0.40	0.16	2.08	0.17	2.47	0.40	5.18	1.41	6.16	2.49
<b>miR-190</b>	1.35	0.23	0.14	0.08	9.59	1.46	7.11	0.60	15.97	4.14	11.84	1.67
<b>miR-191</b>	78.66	0.28	0.46	0.81	172.66	13.71	2.19	1.78	97.24	0.56	1.24	0.56
<b>miR-192</b>	27.63	5.26	0.04	1.88	625.04	85.32	22.63	42.63	14.66	3.47	0.53	0.02
<b>miR-194</b>	25.91	5.63	0.04	2.22	624.67	66.91	24.11	53.62	11.65	0.65	0.45	0.02
<b>miR-195</b>	327.34	23.10	0.74	2.29	443.58	55.79	1.36	3.11	142.64	16.97	0.44	0.32
<b>miR-196a</b>	18.17	2.70	0.79	0.97	22.85	1.94	1.26	1.22	18.80	5.11	1.04	0.82
<b>miR-196b</b>	28.35	0.65	0.83	1.78	34.02	0.25	1.20	2.14	15.93	4.13	0.56	0.47
<b>miR-199a-3p</b>	18.17	2.28	0.63	0.44	28.76	3.91	1.58	0.69	41.72	6.23	2.30	1.45
<b>miR-19a</b>	8.31	3.90	0.72	0.16	11.55	4.35	1.39	0.22	51.90	18.29	6.24	4.49
<b>miR-19b</b>	12.71	5.63	0.76	0.17	16.73	5.75	1.32	0.22	76.60	27.16	6.03	4.58
<b>miR-203</b>	3.16	0.41	0.11	0.22	29.28	4.88	9.26	2.04	14.34	2.10	4.53	0.49
<b>miR-205</b>	3.06	1.02	1.93	0.65	1.59	0.20	0.52	0.33	4.74	2.34	1.55	2.99
<b>miR-206</b>	0.34	0.14	0.44	0.23	0.77	0.14	2.25	0.53	1.46	0.16	4.26	1.89
<b>miR-208</b>	0.06	0.02	1.38	1.57	0.04	0.02	0.73	1.14	0.04	0.02	0.64	0.88
<b>miR-20a</b>	9.86	2.38	0.43	0.16	22.99	1.11	2.33	0.37	62.04	7.23	6.29	2.70
						164.0						
<b>miR-21</b>	80.45	8.06	0.10	0.37	845.89	1	10.52	3.84	220.29	27.52	2.74	0.26
<b>miR-210</b>	0.84	0.45	0.37	0.23	2.27	0.89	2.69	0.61	3.72	1.28	4.40	1.64
<b>miR-218</b>	1.81	0.37	0.05	0.56	36.33	6.08	20.05	11.22	3.24	0.21	1.79	0.09
<b>miR-219-2-3p</b>	0.12	0.06	0.32	0.30	0.38	0.13	3.08	0.91	0.42	0.15	3.39	1.10
<b>miR-219-5p</b>	0.13	0.04	0.56	0.12	0.23	0.15	1.79	0.21	1.10	0.54	8.56	4.78
<b>miR-22</b>	78.00	38.77	0.37	0.73	208.04	69.25	2.67	1.94	107.19	40.98	1.37	0.52
<b>miR-222</b>	1.19	0.52	4.80	0.17	0.25	0.14	0.21	0.04	6.99	2.17	5.90	28.28
<b>miR-23a</b>	1021.79	130.4	7.67	1.90	133.28	25.08	0.13	0.25	536.73	59.26	0.53	4.03

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		178.1										
<b>miR-23b</b>	1084.37	4	6.15	3.05	176.31	25.74	0.16	0.50	355.59	35.32	0.33	2.02
		106.0										
<b>miR-24</b>	471.84	6	4.13	2.38	114.23	17.74	0.24	0.58	198.53	23.62	0.42	1.74
<b>miR-25</b>	35.09	7.46	1.15	1.08	30.62	4.77	0.87	0.94	32.64	4.99	0.93	1.07
<b>miR-26a</b>	635.97	44.97	1.37	1.02	465.80	43.08	0.73	0.75	623.37	26.88	0.98	1.34
<b>miR-26b</b>	74.70	7.51	0.45	0.81	165.30	12.74	2.21	1.78	92.72	19.62	1.24	0.56
<b>miR-27a</b>	249.46	47.13	4.06	1.45	61.51	5.28	0.25	0.36	171.45	38.09	0.69	2.79
<b>miR-27b</b>	394.42	27.47	2.32	1.95	169.83	12.06	0.43	0.84	202.27	12.05	0.51	1.19
<b>miR-29a</b>	127.24	47.76	0.52	0.64	243.30	63.85	1.91	1.22	199.07	55.65	1.56	0.82
<b>miR-29b</b>	0.52	0.29	0.25	0.18	2.06	0.68	3.99	0.73	2.83	1.34	5.46	1.37
<b>miR-29c</b>	46.94	19.18	0.40	0.96	117.92	45.61	2.51	2.42	48.76	15.12	1.04	0.41
<b>miR-301a</b>	1.10	0.51	0.32	0.24	3.41	1.47	3.09	0.76	4.51	1.89	4.10	1.32
<b>miR-30a</b>	160.45	8.98	0.37	0.32	437.21	15.24	2.72	0.86	506.21	26.01	3.15	1.16
<b>miR-30c</b>	263.87	13.89	0.46	0.30	579.74	41.39	2.20	0.67	870.45	22.69	3.30	1.50
<b>miR-30d</b>	70.95	11.68	0.41	0.47	175.11	6.37	2.47	1.17	149.69	5.98	2.11	0.85
<b>miR-30e</b>	20.80	8.66	0.18	0.13	116.39	39.21	5.60	0.71	163.78	43.33	7.87	1.41
<b>miR-32</b>	0.13	0.07	0.18	0.08	0.76	0.50	5.69	0.46	1.65	0.75	12.29	2.16
<b>miR-320</b>	13.77	4.01	1.09	0.49	12.62	2.49	0.92	0.45	28.10	6.42	2.04	2.23
<b>miR-324-</b>												
<b>5p</b>	1.84	0.92	0.12	0.35	15.21	6.21	8.28	2.88	5.29	1.88	2.88	0.35
<b>miR-335</b>	0.00	0.00	0.63	0.37	0.01	0.00	1.59	0.58	0.01	0.00	2.73	1.72
<b>miR-339-</b>												
<b>5p</b>	6.44	2.26	0.21	0.55	30.00	5.69	4.66	2.55	11.75	3.34	1.83	0.39
<b>miR-342-</b>												
<b>3p</b>	8.84	1.28	0.19	1.06	47.20	5.74	5.34	5.66	8.33	0.21	0.94	0.18
<b>miR-34a</b>	1.01	0.52	1.60	3.15	0.63	0.24	0.62	1.96	0.32	0.20	0.32	0.51
<b>miR-363</b>	0.20	0.09	0.32	2.48	0.61	0.01	3.11	7.72	0.08	0.03	0.40	0.13
<b>miR-365</b>	25.99	1.71	1.09	4.57	23.83	1.33	0.92	4.19	5.68	0.17	0.22	0.24
<b>miR-369-</b>	0.10	0.08	0.03	10.50	3.00	0.42	30.31	318.40	0.01	0.01	0.10	0.00

<b>3p</b>												
<b>miR-369-5p</b>	0.06	0.03	0.08	7.17	0.67	0.19	11.79	84.56	0.01	0.01	0.14	0.01
<b>miR-375</b>	0.59	0.24	0.44	0.18	1.33	0.31	2.26	0.41	3.21	0.69	5.46	2.42
<b>miR-378</b>	32.05	12.09	0.14	0.16	222.47	26.04	6.94	1.13	196.66	54.56	6.14	0.88
<b>miR-379</b>	0.10	0.04	0.05	0.27	2.17	0.32	21.63	5.82	0.37	0.28	3.72	0.17
<b>miR-382</b>	0.28	0.10	0.07	1.31	4.28	0.83	15.16	19.85	0.22	0.04	0.76	0.05
<b>miR-410</b>	0.09	0.05	0.14	0.85	0.64	0.15	7.29	6.17	0.10	0.03	1.18	0.16
<b>miR-411</b>	0.08	0.01	0.04	0.44	1.91	0.29	22.68	10.08	0.19	0.08	2.25	0.10
<b>miR-412</b>	3.18	0.77	1.73	0.53	1.83	0.35	0.58	0.31	5.97	4.16	1.88	3.25
<b>miR-425</b>	2.94	0.74	0.28	0.45	10.65	1.41	3.63	1.65	6.47	1.35	2.20	0.61
<b>miR-431</b>	0.19	0.04	0.47	0.53	0.40	0.20	2.12	1.12	0.36	0.13	1.90	0.90
<b>miR-433</b>	0.14	0.06	0.10	0.59	1.39	0.66	10.10	5.91	0.24	0.07	1.71	0.17
<b>miR-448</b>	0.02	0.01	0.34	1.28	0.06	0.03	2.97	3.81	0.02	0.00	0.78	0.26
<b>miR-449a</b>	0.05	0.02	0.41	0.50	0.12	0.05	2.44	1.21	0.10	0.05	2.02	0.83
<b>miR-484</b>	0.25	0.10	0.14	0.36	1.81	0.62	7.09	2.52	0.72	0.31	2.81	0.40
<b>miR-495</b>	0.03	0.01	0.03	1.75	1.24	0.12	38.09	66.84	0.02	0.00	0.57	0.01
<b>miR-532-3p</b>	4.54	0.42	1.00	0.85	4.55	0.41	1.00	0.85	5.37	0.61	1.18	1.18
<b>miR-542-3p</b>	1.50	0.16	2.41	5.49	0.62	0.07	0.42	2.28	0.27	0.03	0.18	0.44
<b>miR-652</b>	12.04	4.51	1.06	0.59	11.34	2.44	0.94	0.56	20.34	5.36	1.69	1.79
<b>miR-758</b>	1.55	0.34	3.25	2.48	0.48	0.10	0.31	0.76	0.63	0.35	0.40	1.31
<b>miR-873</b>	0.03	0.02	0.23	0.38	0.15	0.03	4.41	1.68	0.09	0.04	2.63	0.60
<b>miR-877</b>	0.82	0.26	0.22	0.29	3.66	1.26	4.45	1.31	2.80	0.75	3.40	0.76
<b>miR-9</b>	0.35	0.04	0.62	0.04	0.56	0.08	1.61	0.06	8.96	1.83	25.82	16.06
<b>miR-93</b>	7.53	2.29	0.47	0.55	16.15	2.64	2.14	1.19	13.58	3.80	1.80	0.84
<b>miR-96</b>	0.23	0.12	0.21	0.16	1.10	0.25	4.85	0.75	1.46	0.52	6.43	1.33
<b>miR-98</b>	0.60	0.20	0.36	0.40	1.64	0.69	2.75	1.10	1.49	0.57	2.49	0.91
<b>miR-99a</b>	45.62	9.16	0.26	0.27	178.86	10.80	3.92	1.07	167.06	16.35	3.66	0.93

**Table S2. Proteins known to be differentially enriched in proximal convoluted tubule (PCT) and medullary thick ascending limb (mTAL).** See the main text for more information. Protein expression range is described as very low abundance (-/+ ) to highly abundant (+++).

Protein, differentially enriched in PCT/mTAL	Gene symbol	UniProt ID	Species	PCT	mTAL	Reference	PMID #
Anion exchange protein 2	Slc4a2	P23347	rat	-/+	+++	Alper 1997	9362338
						Nelsen 1993	7678419
Aquaporin 1	Aqp1	P29975	rat	+		Sabolic 1992	1282299
Aquaporin 7	Aqp7	P56403	rat	+		Nejsum 2000	11027658
Aquaporin 8	Aqp8	P56405	rat	+		Elkjaer 2001	11704555
Aquaporin 11	Aqp11	Q8BHH1	mouse	+		Morishita 2005	16107722
Carbonic anhydrase 4	Ca4	P48284	rat	++	+++	Brown 1990	2120700
	CD2AP	Q9Y5K6	human				
CD2-associated protein	Cd2ap	Q9JLQ0	mouse	+		Li 2000	10997929
Cubilin	Cubn	O70244	rat	+		Christensen 2002	11994745
Extracellular Ca <sup>2+</sup> sensing receptor	Casr	P48442	rat	++	++	Riccardi 1998	9530279
H <sup>+</sup> -translocating ATPase			rat	++	+	Bastani 1991	1829094

Inward rectifier K <sup>+</sup> channel, isoform B	Kcnj1	P35560	rat		+++	Mennitt 1997 Boim 1995	9402083 7611454
LDL receptor-related protein 2 (Megalin)	Lrp2	P98158	rat	+		Christensen 2002	11994745
Na <sup>+</sup> /H <sup>+</sup> exchanger 1	SLC9A1	P23791	rabbit	+		Biemesderfer 1993	8238556
	Slc9a3	P26433	rat			Amemiya 1995	8569082
Na <sup>+</sup> /H <sup>+</sup> exchanger 3	SLC9A3	P26432	rabbit	++	+	Biemesderfer 1993	8238556
Na <sup>+</sup> /HCO <sub>3</sub> <sup>-</sup> cotransporter 1	Slc4a4	Q9JI66	rat			Schmitt 1999	9887077
	SLC4A4	Q9XSZ4	rabbit	+		Maunsbach 2000	11095641
Na <sup>+</sup> /HCO <sub>3</sub> <sup>-</sup> cotransporter 3	Slc4a7	Q9R1N3	rat		+	Vorum 2000	11053051
Na <sup>+</sup> /K <sup>+</sup> -transporting ATPase alpha 1	Atp1a1	P06685	rat	+	++	McDonough 1994	7943283
Na <sup>+</sup> /K <sup>+</sup> -transporting ATPase beta 1	Atp1b1	P07340	rat	+	+++	McDonough 1994	7943283
Na <sup>+</sup> /K <sup>+</sup> /Cl <sup>-</sup> cotransporter 2	Slc12a1	P55016	rat		+	Kaplan 1996 Nielsen 1998	8770947 9843905
Organic anion transporter 1	SLC22A6	Q4U2R8	human			Hosoyamada 1999	9887087
	Slc22a6	O35956	rat	+		Tojo 1999	10073596
Organic cation transporter 1	Slc22a1	Q63089	rat	+++		Karbach 2000	10997918
Organic cation transporter 2	Slc22a2	Q9R0W2	rat	+		Karbach 2000	10997918
	UMOD	P07911	human			Sikri 1981	7028707
Uromodulin	Umod	P27590	rat		+	Bachmann 1985	3910623

**Table S3. Several combinations of three of the five miRNAs showing the largest fold enrichment in PCT or mTAL had one or more shared predicted target genes.** Target prediction was based on TargetScan.

miRNAs enriched in PCT	Shared predicted target genes	
miR-194, miR-369-3p, miR-495	ACVR2B	activin A receptor, type IIB
	ARHGAP5	Rho GTPase activating protein 5
	ARID4A	AT rich interactive domain 4A (Rbp1 like)
	BICD2	bicaudal D homolog 2
	CASK	calcium/calmodulin-dependent serine protein kinase (MAGUK family)
	CEP350	centrosomal protein 350
	DSEL	dermatan sulfate epimerase-like
	FOXO1	forkhead box O1
	LPHN2	latrophilin 2
	MGAT4A	mannosyl (alpha-1,3-)-glycoprotein beta-1,4-N-acetylglucosaminyltransferase, isozyme A
	ONECUT2	one cut homeobox 2
	PHF21A	PHD finger protein 21A
	PPARGC1A	peroxisome proliferator-activated receptor gamma, coactivator 1 alpha
	SATB1	SATB homeobox 1
	SP3	Sp3 transcription factor
	ZFAND5	zinc finger, AN1-type domain 5
	ZFHX4	zinc finger homeobox 4
	ZNF516	zinc finger protein 516
	NR2F2	nuclear receptor subfamily 2, group F, member 2
	miR-192, miR-369-3p, miR-495	CHD7
CUX1		cut-like homeobox 1
NIPBL		Nipped-B homolog
WNK1		WNK lysine deficient protein kinase 1

miR-192, miR-194, miR-495	C1orf21	chromosome 1 open reading frame 21
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<b>miRNAs enriched in mTAL</b>	<b>Shared predicted target genes</b>	
miR-9, miR-23a, miR-152	ABCA1	ATP-binding cassette, sub-family A (ABC1), member 1
	ABL2	v-abl Abelson murine leukemia viral oncogene homolog 2
	AMMECR1	Alport syndrome, mental retardation, midface hypoplasia and elliptocytosis chromosomal region gene 1
	ANKRD52	ankyrin repeat domain 52
	BCL11A	B-cell CLL/lymphoma 11A (zinc finger protein)
	CCDC6	coiled-coil domain containing 6
	CPEB4	cytoplasmic polyadenylation element binding protein 4
	CTTNBP2NL	CTTNBP2 N-terminal like
	EPAS1	endothelial PAS domain protein 1
	FBN1	fibrillin 1
	FMR1	fragile X mental retardation 1
	GPRC5B	G protein-coupled receptor, family C, group 5, member B
	KLHL18	kelch-like 18 (Drosophila)
	PIK3R3	phosphoinositide-3-kinase, regulatory subunit 3 (gamma)
	RAI14	retinoic acid induced 14
	SBNO1	strawberry notch homolog 1 (Drosophila)
	SH2B3	SH2B adaptor protein 3
	SIK1	salt-inducible kinase 1
	STAM	signal transducing adaptor molecule (SH3 domain and ITAM motif) 1
	SYNJ1	synaptojanin 1
	ZBTB44	zinc finger and BTB domain containing 44
	ZFH3	zinc finger homeobox 3
miR-23a, miR-132, miR-152	ACVR2B	activin A receptor, type IIB

	ATXN1	ataxin 1
	CBLL1	Cas-Br-M (murine) ecotropic retroviral transforming sequence-like 1
	ETNK1	ethanolamine kinase 1
	ETV1	ets variant 1
	MEX3C	mex-3 homolog C (C. elegans)
	QKI	quaking homolog, KH domain RNA binding (mouse)
	TMED7	transmembrane emp24 protein transport domain containing 7
	ZNF238	zinc finger protein 238
miR-9, miR-23a, miR-132	ADAMTS6	ADAM metallopeptidase with thrombospondin type 1 motif, 6
	BNC2	basonuclin 2
	BTBD7	BTB (POZ) domain containing 7
	CBFA2T2	core-binding factor, runt domain, alpha subunit 2; translocated to, 2
	CCNT2	cyclin T2
	CHSY1	chondroitin sulfate synthase 1
	DYRK2	dual-specificity tyrosine-(Y)-phosphorylation regulated kinase 2
	FOXP2	forkhead box P2
	GSK3B	glycogen synthase kinase 3 beta
	ISL1	ISL LIM homeobox 1
	LIN28B	lin-28 homolog B (C. elegans)
	MAP3K3	mitogen-activated protein kinase kinase kinase 3
	MEX3A	mex-3 homolog A (C. elegans)
	NFIB	nuclear factor I/B
	ZNF395	zinc finger protein 395
miR-9, miR-132, miR-152	C18orf25	chromosome 18 open reading frame 25
	PHIP	pleckstrin homology domain interacting protein
	SOS1	son of sevenless homolog 1 (Drosophila)
	ZNF704	zinc finger protein 704

miR-23a, miR-100, miR-152	PPP1CB	protein phosphatase 1, catalytic subunit, beta isozyme
miR-9, miR-100, miR-152	RMND5A	required for meiotic nuclear division 5 homolog A ( <i>S. cerevisiae</i> )
miR-9, miR-100, miR-132	SMARCA5	SWI/SNF related, matrix associated, actin dependent regulator of chromatin, subfamily a, member 5
miR-9, miR-23a, miR-100	THAP2	THAP domain containing, apoptosis associated protein 2