

Supplementary Material

# Validation in an Independent Cohort of MiR-122, MiR-1271, and MiR-15b as Urinary Biomarkers for the Potential Early Diagnosis of Clear Cell Renal Cell Carcinoma

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**Table S1.** Ct values of miR-122-5p, miR-1271-5p, miR-15b-5p, Cel-miR-39-3p, miRTC, and miR-16-5p, obtained using the urine of HSs and patients with ccRCC.

	Ct Value	miR-122-5p	miR-1271-5p	miR-15b-5p	Cel-miR-39-3p	miRTC	miR-16-5p
ccRCC	ccRCC 1	40.00	38.10	32.85	28.41	32.19	39.21
	ccRCC 2	29.24	29.42	28.05	19.97	19.11	27.20
	ccRCC 3	34.76	30.02	35.24	22.85	28.94	30.69
	ccRCC 4	39.47	33.29	31.19	23.56	28.16	31.76
	ccRCC 5	35.30	29.53	37.15	30.10	29.32	27.04
	ccRCC 6	30.06	29.32	28.73	22.95	22.83	25.09
	ccRCC 7	36.62	31.08	40.00	23.80	23.95	31.21
	ccRCC 8	35.54	33.21	35.18	27.49	24.93	30.79
	ccRCC 9	33.75	30.70	33.38	29.05	26.81	30.14
	ccRCC 10	37.37	29.98	40.00	32.54	32.57	30.32
	ccRCC 11	38.63	31.31	38.45	24.82	32.45	37.21
	ccRCC 12	35.85	29.98	39.54	26.52	23.36	32.10
	ccRCC 13	40.00	32.33	39.32	23.67	26.55	30.10
	ccRCC 14	39.20	31.94	40.00	26.60	25.95	35.12
	ccRCC 15	38.95	30.35	38.63	26.76	30.19	30.60
	ccRCC 16	38.26	34.57	38.86	29.72	33.23	30.89
	ccRCC 17	31.14	29.58	28.03	34.16	31.09	29.30
	ccRCC 18	38.71	30.76	35.01	26.63	28.89	28.40
	ccRCC 19	25.50	24.51	28.01	23.24	24.02	23.43
	ccRCC 20	24.69	24.93	26.95	19.62	19.32	23.00
	ccRCC 21	31.20	28.12	25.59	21.51	21.04	25.45
	ccRCC 22	36.00	30.20	25.88	18.40	21.27	26.33
	ccRCC 23	35.31	29.28	26.12	20.90	23.80	30.24
	ccRCC 24	30.99	29.53	29.73	33.62	32.30	29.24
Mean ccRCC		34.86	30.50	33.41	25.70	26.76	29.79
SD ccRCC		4.43	2.77	5.31	4.38	4.42	3.87
HSs	HS 1	36.65	30.18	38.24	29.94	39.18	26.48
	HS 2	31.29	29.69	25.44	27.69	31.17	25.23
	HS 3	40.00	33.38	40.00	31.82	34.89	32.63
	HS 4	39.00	31.03	39.02	27.02	33.44	36.15
	HS 5	39.05	33.16	37.96	31.51	34.67	32.59

HS 6	40.00	36.37	40.00	23.18	28.40	38.06
HS 7	38.39	30.58	34.39	29.52	36.60	30.22
HS 8	34.93	32.23	29.67	30.32	37.72	28.57
HS 9	36.28	31.54	38.56	28.91	34.20	31.07
HS 10	35.92	31.15	38.84	25.09	26.54	29.08
HS 11	40.00	31.37	39.00	31.96	27.65	31.12
HS 12	40.00	28.87	38.36	28.26	24.23	35.32
HS 13	35.87	29.14	27.26	17.70	23.15	26.59
HS 14	31.19	32.67	30.98	30.60	39.72	33.10
HS 15	35.60	29.25	40.00	34.52	36.85	30.59
HS 16	37.86	30.40	35.31	31.30	37.11	31.92
HS 17	40.00	30.30	39.21	34.95	34.45	31.34
HS 18	34.55	33.38	38.32	20.77	32.07	28.54
HS 19	40.00	32.39	40.00	24.49	32.61	31.44
HS 20	40.00	31.66	26.74	32.35	31.28	28.17
HS 21	40.00	30.41	38.05	33.70	40.00	32.11
HS 22	33.80	34.35	40.00	24.37	27.65	39.51
HS 23	40.00	35.25	40.00	26.64	28.50	36.07
Mean HSs	37.41	31.68	36.32	28.55	32.70	31.56
SD HSs	2.86	1.97	4.78	4.40	4.90	3.66

**Table S2.** Ct and  $\Delta$ Ct of the seven parameters #1, #2, #3, #4, #5, #6, and #7.  $\Delta$ Ct (parameters #2, #3, #4, #5, #6, and #7) were calculated using data reported in Table S1.

Parameter	# 1	# 2	# 3	# 4	# 5	# 6	# 7
Parameter Description	miR-1271-5p	miR-122-5p/miR-16-5p	miR-122-5p/miRTC	miR-1271-5p/miR-16-5p	miR-1271-5p/miRTC	miR-15b-5p/miRTC	miR-15b-5p/Cel-miR-39-3p
	Ct	$\Delta$ Ct	$\Delta$ Ct	$\Delta$ Ct	$\Delta$ Ct	$\Delta$ Ct	$\Delta$ Ct
ccRCC	ccRCC 1	38.10	0.79	7.81	- 1.11	5.91	4.44
	ccRCC 2	29.42	2.04	10.13	2.22	10.31	8.08
	ccRCC 3	30.02	4.07	5.82	-0.67	1.08	12.39
	ccRCC 4	33.29	7.71	11.31	1.53	5.13	7.63
	ccRCC 5	29.53	8.27	5.98	2.49	0.21	7.05
	ccRCC 6	29.32	4.97	7.23	4.23	6.49	5.78
	ccRCC 7	31.08	5.41	12.67	-0.13	7.13	16.20
	ccRCC 8	33.21	4.75	10.61	2.42	8.28	7.69
	ccRCC 9	30.70	3.61	6.94	0.56	3.89	4.33
	ccRCC 10	29.98	7.05	4.80	-0.34	-2.59	7.46
	ccRCC 11	31.31	1.42	6.18	-5.90	-1.14	13.63
	ccRCC 12	29.98	3.75	12.49	-2.12	6.62	13.02
	ccRCC 13	32.33	9.90	13.45	2.23	5.78	15.65
	ccRCC 14	31.94	4.08	13.25	-3.18	5.99	13.40
	ccRCC 15	30.35	8.35	8.76	-0.25	0.16	11.87
	ccRCC 16	34.57	7.37	5.03	3.68	1.34	9.14
	ccRCC 17	29.58	1.84	0.05	0.28	-1.51	-6.13
	ccRCC 18	30.76	10.31	9.82	2.36	1.87	8.38
	ccRCC 19	24.51	2.07	1.48	1.08	0.49	4.77
	ccRCC 20	24.93	1.69	5.37	1.93	5.61	7.33
	ccRCC 21	28.12	5.75	10.16	2.67	7.08	4.08
	ccRCC 22	30.20	9.67	14.73	3.87	8.93	7.48
	ccRCC 23	29.28	5.07	11.51	-0.96	5.48	5.22
	ccRCC 24	29.53	1.75	-1.31	0.29	-2.77	-3.89
Mean ccRCC		30.50	5.07	8.09	0.72	3.74	7.71
SD ccRCC		2.77	2.94	4.27	2.37	3.83	5.33
HSs	HS 1	30.18	10.17	-2.53	3.70	-9.00	8.30
	HS 2	29.69	6.06	0.12	4.46	-1.48	-2.25
	HS 3	33.38	7.37	5.11	0.75	-1.51	8.18
	HS 4	31.03	2.85	5.56	-5.12	-2.41	12.00

	HS 5	33.16	6.46	4.38	0.57	-1.51	3.29	6.45
	HS 6	36.37	1.94	11.60	-1.69	7.97	11.60	16.82
	HS 7	30.58	8.17	1.79	0.36	-6.02	-2.21	4.87
	HS 8	32.23	6.36	-2.79	3.66	-5.49	-8.05	-0.65
	HS 9	31.54	5.21	2.08	0.47	-2.66	4.36	9.65
	HS 10	31.15	6.84	9.38	2.07	4.61	12.30	13.75
	HS 11	31.37	8.88	12.35	0.25	3.72	11.35	7.04
	HS 12	28.87	4.68	15.77	-6.45	4.64	14.13	10.10
	HS 13	29.14	9.28	12.72	2.55	5.99	4.11	9.56
	HS 14	32.67	-1.91	-8.53	-0.43	-7.05	-8.74	0.38
	HS 15	29.25	5.01	-1.25	-1.34	-7.60	3.15	5.48
	HS 16	30.40	5.94	0.75	-1.52	-6.71	-1.80	4.01
	HS 17	30.30	8.66	5.55	-1.04	-4.15	4.76	4.26
	HS 18	33.38	6.01	2.48	4.84	1.31	6.25	17.55
	HS 19	32.39	8.56	7.39	0.95	-0.22	7.39	15.51
	HS 20	31.66	11.83	8.72	3.50	0.38	-4.54	-5.61
	HS 21	30.41	7.89	0	-1.70	-9.59	-1.95	4.35
	HS 22	34.35	-5.71	6.15	-5.16	6.70	12.35	15.63
	HS 23	35.25	3.93	11.50	-0.82	6.75	11.50	13.36
	Mean HSs	31.68	5.85	4.71	0.12	-1.01	3.62	7.77
	SD HSs	1.97	3.86	5.98	3.03	5.45	6.86	6.24
	Mean ccRCC/Mean HSs	1.18	0.78	-3.39	-0.59	-4.75	-3.03	0.06
	Passed KS test	No	Yes	Yes	Yes	No	Yes	Yes
	<i>p</i> value	0.0645	0.4408	0.0300	0.4578	0.0025	0.0881	0.9704
values shown by ccRCC patients	Range-SD	27.73	2.13	3.82	-1.65	-0.09	1.71	2.38
	Range + SD	33.27	8.01	12.37	3.08	7.57	11.59	13.04

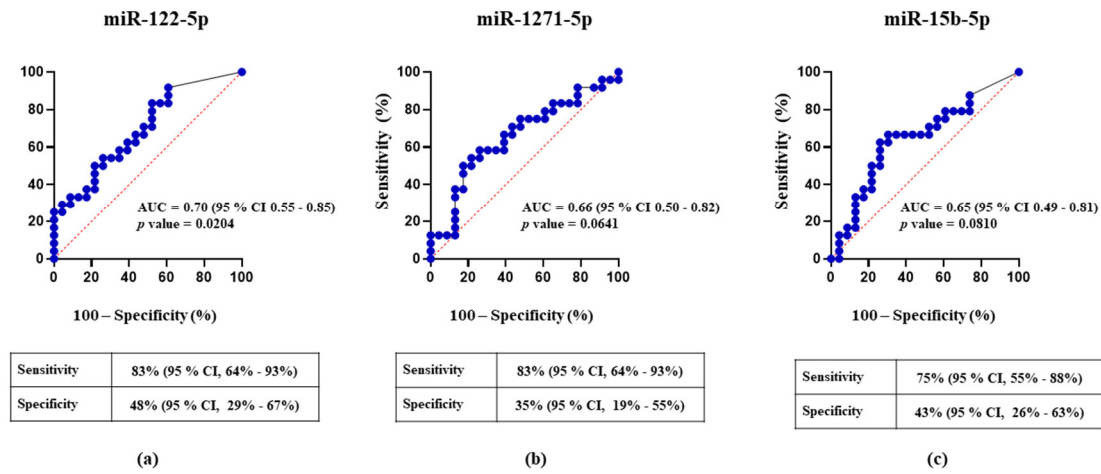
**Table S3.** Derivative of Ct and  $\Delta$ Ct values of the seven parameters obtained as described in the Materials and Methods section and their sum. In bold the values which are below the disease cut-off (-14.76).

	Parameter	# 1 <sup>1</sup>	# 2 <sup>1</sup>	# 3 <sup>2</sup>	# 4 <sup>2</sup>	# 5 <sup>2</sup>	# 6 <sup>2</sup>	# 7 <sup>1</sup>	Sum of parameters	Diseased cut-off value
	ccRCC 3	-3.25	-3.94	-2.00	-0.98	-1.17	-4.59	-0.65	<b>-16.57</b>	
	ccRCC 4	0.02	-0.30	-7.49	-3.18	-5.22	-1.32	-5.41	<b>22.89</b>	
	ccRCC 5	-3.74	0.26	-2.16	-4.41	-0.30	-6.12	-5.98	<b>-22.19</b>	
	ccRCC 6	-3.95	-3.04	-3.41	-5.88	-6.58	-4.19	-7.26	<b>-34.30</b>	
	ccRCC 7	-2.19	-2.60	-8.85	-1.52	-7.22	-14.34	3.16	<b>-33.55</b>	
	ccRCC 8	-0.06	-3.26	-6.79	-4.07	-8.37	-8.54	-5.35	<b>-36.43</b>	
S	ccRCC 9	-2.57	-4.40	-3.12	-2.21	-3.98	-4.86	-8.71	<b>-29.84</b>	
A	ccRCC 10	-3.29	-0.96	-0.98	-1.31	2.50	-5.72	-5.58	<b>-15.33</b>	
M	ccRCC 11	-1.96	-6.59	-2.36	4.25	1.05	-4.29	0.59	-9.30	
P	ccRCC 12	-3.29	-4.26	-8.67	0.47	-6.71	-14.47	-0.02	<b>-36.94</b>	
L	ccRCC 13	-0.94	1.89	-9.63	-3.88	-5.87	-11.06	2.61	<b>-26.87</b>	
E	ccRCC 14	-1.33	-3.92	-9.43	1.53	-6.08	-12.34	0.36	<b>-31.21</b>	<b>-14.76</b>
	ccRCC 15	-2.92	0.34	-4.94	-1.40	-0.25	-6.73	-1.17	<b>-17.06</b>	
C	ccRCC 16	1.30	-0.64	-1.21	-5.33	-1.43	-3.92	-3.90	<b>-15.12</b>	
O	ccRCC 17	-3.69	-6.17	3.77	-1.93	1.42	4.77	-19.17	<b>-20.99</b>	
D	ccRCC 18	-2.51	2.30	-6.00	-4.01	-1.96	-4.41	-4.66	<b>-21.24</b>	
E	ccRCC 19	-8.76	-5.94	2.34	-2.73	-0.58	-2.28	-8.27	<b>-26.21</b>	
	ccRCC 20	-8.34	-6.32	-1.55	-3.58	-5.70	-5.92	-5.71	<b>-37.11</b>	
	ccRCC 21	-5.15	-2.26	-6.34	-4.32	-7.17	-2.84	-8.96	<b>-37.03</b>	
	ccRCC 22	-3.07	1.66	-10.91	-5.52	-9.02	-2.90	-5.56	<b>-35.31</b>	
	ccRCC 23	-3.99	-2.94	-7.69	-0.69	-5.57	-0.61	-7.82	<b>-29.30</b>	
	ccRCC 24	-3.74	-6.26	5.13	-1.94	2.68	4.28	-16.93	<b>-16.77</b>	
	HS 1	-3.09	2.16	6.35	-5.35	8.90	2.64	-4.73	6.89	

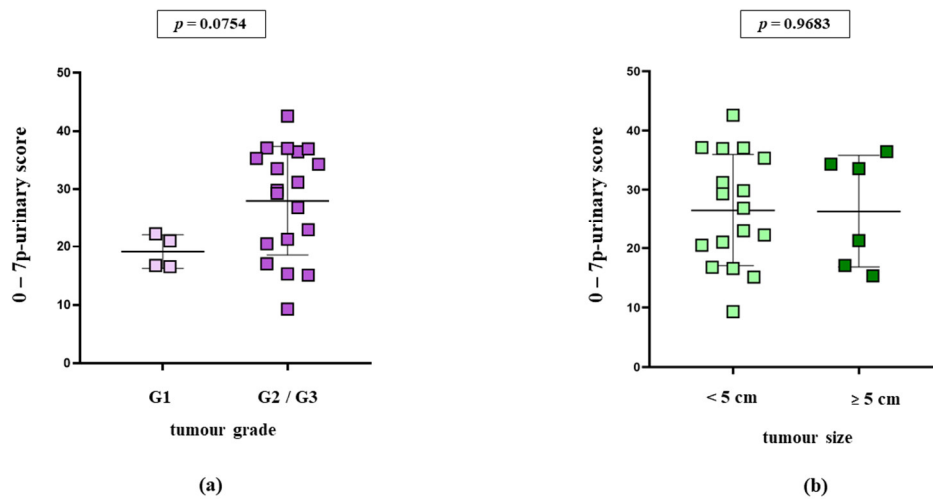
HS 2	-3.58	-1.95	3.70	-6.11	1.39	7.44	-15.29	-14.39
HS 3	0.11	-0.64	-1.29	-2.40	1.42	-3.40	-4.86	-11.05
HS 4	-2.24	-5.16	-1.74	3.47	2.32	-3.87	-1.04	-8.25
HS 5	-0.11	-1.55	-0.56	-2.22	1.42	-1.58	-6.59	-11.18
HS 6	3.10	-6.07	-7.78	0.04	-8.06	-9.89	3.78	<b>-24.87</b>
HS 7	-2.69	0.16	2.04	-2.01	5.93	3.92	-8.17	-0.82
HS 8	-1.04	-1.65	6.61	-5.31	5.40	9.76	-13.69	0.09
HS 9	-1.73	-2.80	1.74	-2.12	2.57	-2.65	-3.39	-8.37
HS 10	-2.12	-1.17	-5.56	-3.72	-4.70	-10.59	0.71	<b>-27.14</b>
HS 11	-1.90	0.87	-8.53	-1.90	-3.81	-9.64	-6.00	<b>-30.90</b>
HS 12	-4.40	-3.32	-11.95	4.80	-4.73	-12.42	-2.94	<b>-34.96</b>
HS 13	-4.13	1.27	-8.90	-4.20	-6.08	-2.40	-3.48	<b>-27.91</b>
HS 14	-0.60	-9.92	12.35	-1.22	6.96	10.45	-12.66	5.37
HS 15	-4.02	-3.00	5.07	-0.31	7.51	-1.44	-7.56	-3.74
HS 16	-2.87	-2.07	3.07	-0.13	6.62	3.51	-9.03	-0.89
HS 17	-2.97	0.65	-1.73	-0.61	4.06	-3.05	-8.78	-12.42
HS 18	0.11	-2.00	1.34	-6.49	-1.40	-4.54	4.51	-8.46
HS 19	-0.88	0.55	-3.57	-2.60	0.13	-5.68	2.47	-9.57
HS 20	-1.61	3.82	-4.90	-5.15	-0.47	6.25	-18.65	<b>-20.70</b>
HS 21	-2.86	-0.12	3.82	0.05	9.50	3.66	-8.69	5.37
HS 22	1.08	-13.72	-2.33	3.51	-6.79	-10.64	2.59	<b>-26.29</b>
HS 23	1.98	-4.08	-7.68	-0.83	-6.84	-9.79	0.32	<b>-26.91</b>

<sup>1</sup> Values were calculated with the following formula: subject Ct (or ΔCt) -[mean Ct (or ΔCt) of ccRCC + SD of ccRCC].

<sup>2</sup> Values were calculated with the following formula: [mean Ct (or ΔCt) of ccRCC -SD of ccRCC] -subject Ct (or ΔCt).



**Figure S1.** ROC curves of miR-122-5p (a), miR-1271-5p (b), and miR-15b-5p (c).



**Figure S2.** Lack of difference of the 7p-urinary scores between ccRCC tumors of different grades and sizes. (a) The 7p-urinary score mean of patients with grade 1 (G1) ccRCC is not significantly different (unpaired t-test) from that of patients with ccRCC with grade 2 and grade 3 (G2 – G3). (b) The 7p-urinary score mean of patients with ccRCC with a size smaller than 5 cm is not significantly different (unpaired t-test) from that of patients with a ccRCC size greater than 5 cm.