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Supplemental Information

Circular RNA circRIMS1 Acts as a Sponge of miR-433-3p to Promote Bladder Cancer Progression by Regulating CCAR1 Expression

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Table S1. The sequences of primers and oligonucleotides used in this study

Primers	
circ-RIMS1 F	TACACACTGGAGCATAATGA
circ-RIMS1 R	AGAGTTATATTTTTCTTGTTCTGTT
Linear RIMS1 F	AGTTGTGGTATGATAAAGTGGGACA
Linear RIMS1 R	TTCGAGGACGTCCATCTACTCT
GAPDH F	GTCAAGGCTGAGAACGGGAA
GAPDH R	AAATGAGCCCCAGCCTTCTC
Myc F	GGCCCCCAAGGTAGTTATCC
Myc R	GTTTCCGCAACAAGTCCTCTTC
Birc5 F	GACCCACTTATTTCTGCCACATC
Birc5 R	GAGTACAGAGGCTGGAGTGCATT
CCAR1 F	CTGATGGCTAGCCCTAGTATGGA
CCAR1 R	TGCCTTTCATGCCCACTAAAA
CEP135 F	AGTTTGAGAGGGTTGTGGTGG
CEP135 R	TGTATCCTTCTCGTGGGAGGT
NEGR1 F	CCTCTTAACCCTCCAAGTACAGC
NEGR1 R	CCAGCCATCAGCACTTTCAG
Hsa-miR-433-3p	ATCATGATGGGCTCCTCGGTGT
Hsa-miR-3064-5p	TCTGGCTGTTGTGGTGTGCAA
Hsa-miR-301b-5p	GCTCTGACGAGGTTGCACTACT
Hsa-miR-892b	CACTGGCTCCTTTCTGGGTAGA
Hsa-miR-6803-5p	CTGGGGGTGGGGGGCTGGGCGT
Hsa-miR-4268	GGCTCCTCCTCTCAGGATGTG
Hsa-miR-6852-5p	CCCTGGGGTTCTGAGGACATG
Hsa-miR-331-3p	GCCCCTGGGCCTATCCTAGAA
Hsa-miR-4316	GGTGAGGCTAGCTGGTG
Hsa-miR-1468-5p	CTCCGTTTGCCTGTTTCGCTG
miRNA reverse	All-in-One miRNA qRT-PCR detection kit, GeneCopoeia, USA
siRNAs Targeting sequence	
Si circ-RIMS1 1#	CTTAGTCAAACAGAACAAGAA
Si circ-RIMS1 2#	GTCAAACAGAACAAGAAAAAT
Si circ-RIMS1 3#	TAGTCAAACAGAACAAGAAAA
Si CCAR1 1#	CCATCACTCCTTGGAGCAT
Si CCAR1 2#	CCACACAAACTCCAGCAAA
Si CCAR1 3#	CCAGCAAACTATCAGTTAA
Si CEP135 1#	TGGGTGTATACCTATGTTAATGA
Si CEP135 2#	TTGGAAAGACATAAAGAAGAAGT
Si CEP135 3#	CAGCAGAAAGAGATAAACTAAGT
Si NEGR1 1#	CTGTTCATCTATGATAGTCAACT
Si NEGR1 2#	TACAAGATTGTTGCAATTTCAGA
Si NEGR1 3#	ATCAAGTTAAACCATACACTATC

miRNAs mimics	
Hsa-miR-433-3p sense	AUCAUGAUGGGCUCCUCGGUGU
Hsa-miR-433-3p anti-sense	UAGUACUACCCGAGGAGCCACA
Hsa-miR-301b-5p sense	GCUCUGACGAGGUUGCACUACU
Hsa-miR-301b-5p anti-sense	CGAGACUGCUCCAACGUGAUGA
Hsa-miR-1468-5p sense	CUCCGUUUGCCUGUUUCGCUG
Hsa-miR-1468-5p anti-sense	GAGGCAAACGGACAAAGCGAC
Biotinylated probes	
Biotin-circ-RIMS1	GTTATATTTTTCTTGTTCTGTTTGACTAAGCTG
Probes for RNA Fluorescence in situ hybridization	
Hsa_circ_0132246-CY3	TTTTCTTGTTCTGTTTGACTA
Hsa-miR-433-3p-FITC	ACACCGAGGAGCCCATCATGAT

Table S2. Detailed information of our own 20 bladder cancer patients is listed

Patient number	Age at surgery	Gender	Grade	T	N	M	AJCC clinical stage
1	53	Male	High	T4b	N2	M0	4
2	67	Male	High	T4a	N1	M0	4
3	40	Male	High	T2b	N3	M0	4
4	52	Male	Low	Tis	N0	M0	Ois
5	58	Female	Low	Tis	N0	M0	Ois
6	59	Female	High	T1	N0	M0	1
7	78	Male	Low	Tis	N0	M0	Ois
8	81	Male	High	T1	N0	M0	1
9	68	Female	Low	Tis	N0	M0	Ois
10	66	Male	High	T1	N0	M0	1
11	68	Male	High	T2a	N0	M0	2
12	73	Male	High	T4b	N1	M0	4
13	61	Female	Low	Tis	N0	M0	Ois
14	62	Female	High	T2	N0	M0	2
15	68	Male	High	T1	N0	M0	1
16	84	Female	High	T2a	N0	M0	2
17	88	Male	High	Tis	N0	M0	Ois
18	86	Male	High	T2	N0	M0	2
19	84	Male	High	T2	N0	M0	2
20	48	Male	High	T2b	N0	M0	2

Table S3. Correlation of circRIMS1 expression with clinicopathologic features of our own bladder cancer patients

Characteristics	Number of cases	circRIMS1 expression in tumor tissue		P Value
		Low	High	
Age (year)				
<60	6	3	3	> 0.9999
≥60	14	7	7	
Gender				
Female	6	5	1	0.1409
Male	14	5	9	
T stage				
Tis-T_1	10	8	2	**0.0230**
T_2-T_4	10	2	8	
N stage				
N0	16	10	6	0.0867
N_1+N_2+N_3	4	0	4	
Grade				
Low	5	5	0	**0.0325**
High	15	5	10	

The bold P value is less than 0.05, which has statistically significant.

Table S4. Detailed information of 60 bladder cancer cases for CCAR1 IHC assay

Patient number	Age at surgery	Gender	Grade	T	N	M	AJCC clinical stage
1	76	Male	High	T2	N1	M0	4
2	67	Male	High	Tis	N0	M0	Ois
3	82	Male	High	T3	N0	M0	3
4	82	Male	High	T2	N0	M0	2
5	62	Male	High	T2	N0	M0	2
6	80	Male	High	T2	N0	M0	2
7	50	Male	High	T3	N0	M0	3
8	59	Male	High	T3	N0	M0	3

9	66	Male	High	T2	N0	M0	2
10	76	Male	High	T3	N0	M0	3
11	67	Male	High	T2	N0	M0	2
12	83	Male	High	T1	N0	M0	1
13	81	Male	Low	T2	N0	M0	2
14	75	Male	High	T3	N1	M0	4
15	71	Male	High	T4	N1	M0	4
16	75	Male	High	T3	N0	M0	3
17	72	Female	High	T2	N0	M0	2
18	66	Female	High	T2	N0	M0	2
19	67	Male	High	T2	N1	M0	4
20	58	Male	High	T1	N0	M0	1
21	77	Male	High	T1	N0	M0	1
22	68	Male	High	T3	N0	M0	3
23	61	Male	High	T1	N0	M0	1
24	58	Female	High	T3	N0	M0	3
25	73	Male	High	T1	N0	M0	1
26	42	Female	High	T3	N0	M0	3
27	57	Male	High	T2	N0	M0	2
28	55	Male	High	T3	N0	M0	3
29	75	Male	Low	T1	N0	M0	1
30	73	Male	High	T3	N0	M0	3
31	77	Male	High	T3	N1	M0	4
32	57	Male	High	Tis	N0	M0	Ois
33	78	Male	High	T1	N0	M0	1
34	74	Male	High	T1	N0	M0	1
35	72	Female	Low	Tis	N0	M0	Ois
36	65	Female	High	T3	N0	M0	3
37	59	Male	Low	T4	N0	M0	3
38	75	Male	High	T3	N0	M0	3
39	55	Male	High	T3	N0	M0	3
40	57	Male	High	T3	N0	M0	3
41	61	Male	High	T3	N1	M0	4
42	79	Male	High	T3	N0	M0	3
43	77	Male	High	T1	N0	M0	1
44	48	Male	High	Tis	N0	M0	Ois
45	72	Female	Low	Tis	N0	M0	Ois
46	85	Female	High	T3	N0	M0	3
47	76	Male	High	T1	N0	M0	1
48	61	Male	Low	T1	N0	M0	1
49	75	Male	High	Tis	N0	M0	Ois
50	66	Female	Low	Tis	N0	M0	Ois
51	84	Male	High	T3	N0	M0	3

52	75	Male	High	T2	N1	M0	4
53	78	Male	Low	Tis	N0	M0	Ois
54	44	Male	High	T3	N0	M0	3
55	77	Female	High	T3	N0	M0	3
56	71	Male	High	T3	N0	M0	3
57	62	Male	High	T2	N0	M0	2
58	59	Male	Low	T3	N0	M0	3
59	64	Male	Low	T2	N1	M0	4
60	67	Male	High	T1	N0	M0	1

Table S5. Correlation of CCAR1 expression with clinicopathologic features of 60 bladder cancer patients

Characteristics	Number of cases	CCAR1 expression in tumor tissue		P Value
		Low score	High score	
Age (year)				
<60	14	5	9	0.3604
≥60	46	25	21	
Gender				
Female	10	6	4	0.7306
Male	50	24	26	
T stage				
Tis-T_1	20	15	5	**0.0127**
T_2-T_4	40	15	25	
N stage				
N_0	52	27	25	0.7065
N_1	8	3	5	
Grade				
Low	10	8	2	0.0797
High	50	22	28	

The bold P value is less than 0.05, which has statistically significant.

Figure S1. The efficiency of siRNAs for CCAR1, CEP135 and NEGR1 in T24 and EJ.

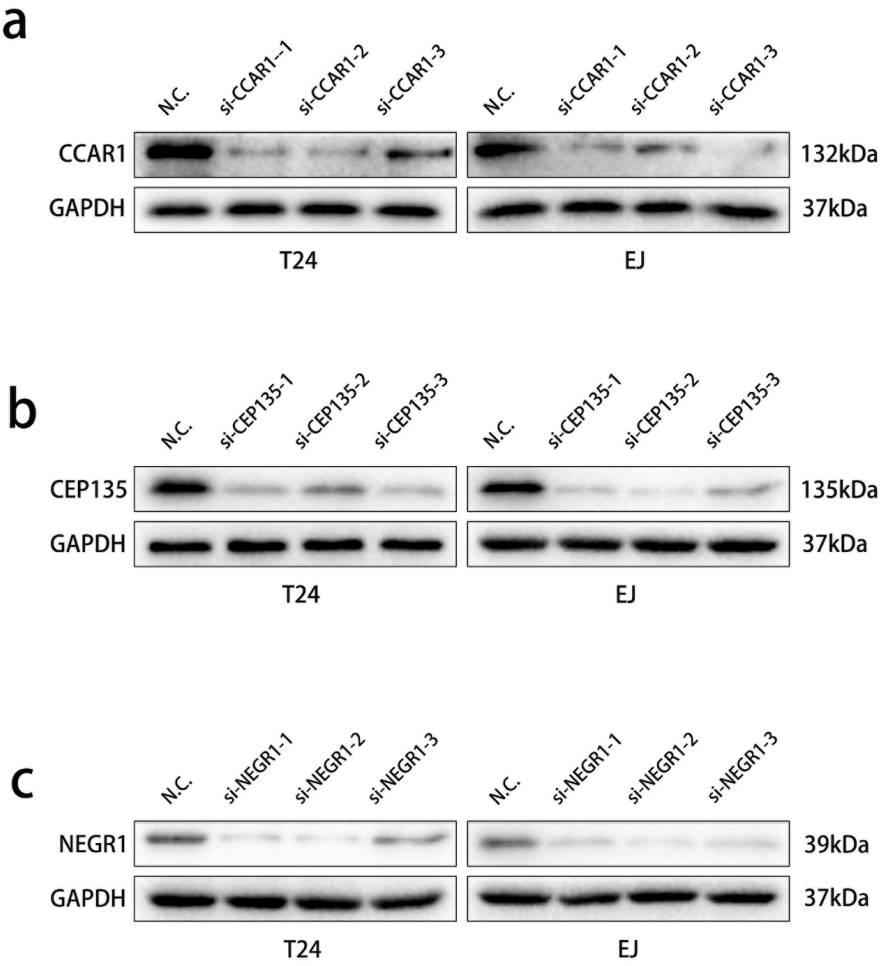

Figure S1. The efficiency of siRNAs for CCAR1, CEP135 and NEGR1 in T24 and EJ. **a-c** The alterations of CCAR1, CEP135 and NEGR1 in T24 and EJ cells stably transfected with N.C. or siRNAs were determined by western blotting respectively.

Figure S2. CCAR1 is regulated by miR-433-3p.

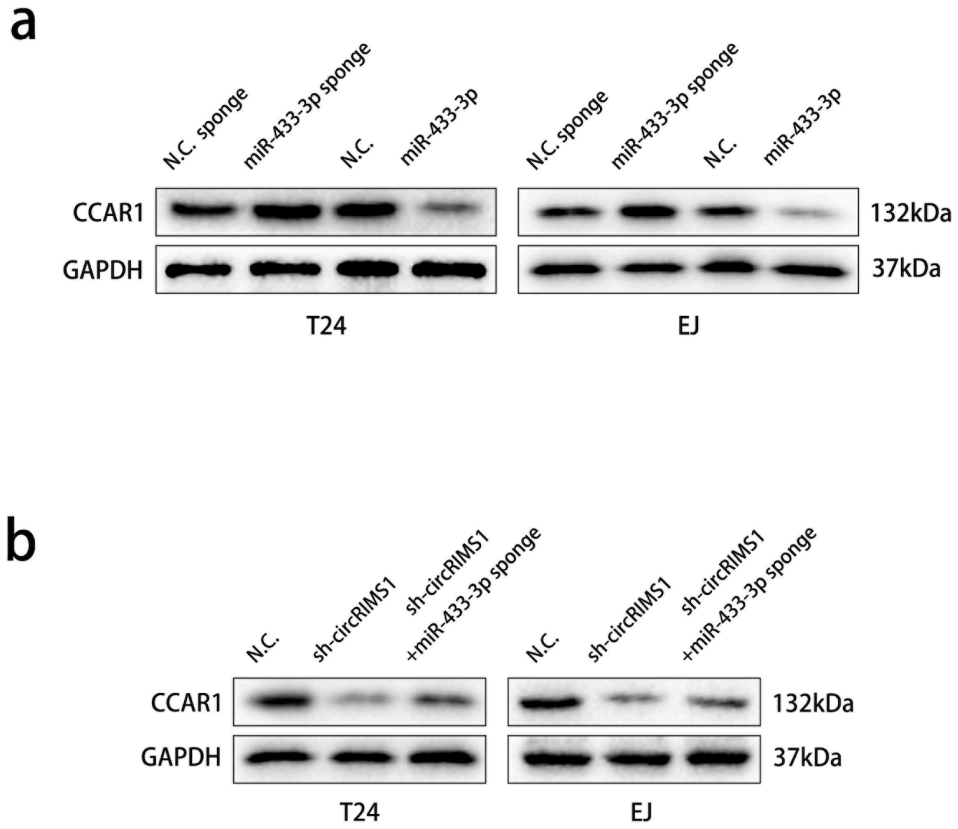

Figure S2. CCAR1 is regulated by miR-433-3p. **a.** The protein level of CCAR1 of bladder cancer cells transfected miR-433-3p sponge (or N.C. sponge) or pre-miR-433-3p (or N.C.) were respectively evaluated by western blotting. **b.** T24 and EJ cells were transfected with NC or sh-circRIMS1 or cotransfected with sh-circRIMS1 and miR-433-3p sponge. CCAR1 level was detected by western blotting.