Sirtuins' Deregulation in Bladder Cancer: SIRT7 Is Implicated in Tumor Progression Through Epithelial to Mesenchymal Transition Promotion

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Figure S1: Characterization of SIRT1, SIRT2, SIRT3, SIRT4 and SIRT5 (**a**), and SIRT6 and SIRT7 (**b**) in the TCGA bladder cancer cohort by quantitative RT-PCR. * p < 0.05, **** p < 0.0001 and ns—non significant. UCC—urothelial cell carcinoma, NB—normal bladder mucosae.



Figure S2: Characterization of Sirtuin family (SIRT1 to SIRT7) gene expression in bladder cancer tissues (**a**) and TCGA cohort (**b**) categorized by clinical grade. ** p < 0.01, *** p < 0.001, **** p < 0.0001 and ns – non significant. PLG – papillary low-grade, PHG – papillary high-grade, IHG – invasive high-grade.

SIRT7 Immunohistochemistry



Figure S3: Immunohistochemistry representative images for SIRT7 immunoexpression in normal urothelium and bladder urothelial carcinoma tissue sections.



Figure S4. Immunofluorescence representative images for SIRT7 immunoexpression in MGHU3, 5637 and J82 bladder cancer cell lines (100× magnification).





Figure S5: Immunofluorescence representative images for E-Cadherin and N-Cadherin immunoexpression in sh-CTRL and sh-SIRT7 MGHU3, 5637 and J82 bladder cancer cell lines (100× magnification).



Figure S6. Characterization of *CDH1* gene expression in the bladder cancer and normal mucosae tissue samples (**a**), and in non-muscle invasive and muscle-invasive bladder cancer cases (**b**), by quantitative RT-PCR. Characterization of *CDH2* gene expression in the bladder cancer and normal mucosae tissue samples (**c**), and in non-muscle invasive (NMIBC) and muscle-invasive bladder cancer (MIBC) cases (**d**), by quantitative RT-PCR. * p < 0.05, ** p < 0.01 and **** p < 0.0001.

MIBC



Figure S7. Heat map showing SIRT7 and EZH2 expression in muscle invasive bladder cancer (MIBC) cases (n = 26). Relative expression levels are depicted in colours from dark blue (lower expression), to dark yellow (higher expression) as shown in the colour bar bellow the graphic.

Gene	Reference ID
HPRT1	Hs01003267_m1
NCAD	Hs00354986_m1
SDHA	Hs00417200_m1
SIRT1	Hs01009005_m1
SIRT2	Hs00247263_m1
SIRT3	Hs00202030_m1
SIRT4	Hs00202033_m1
SIRT5	Hs00978335_m1
SIRT6	Hs00213036_m1
SIRT7	Hs01034735_m1

 $\textbf{Table S1.} \ Reference \ of \ TaqMan^{\$} \ gene \ expression \ assays \ for \ studied \ genes.$

Table S2. Primer sequences used in RT-PCR for studied genes.

Gene	Forward sequence (5'-3')	Reverse sequence (5'-3')	Annealing T. (°C)
BGUS	CTCATTTGGAATTTTGCCGATT	CCGAGTGAAGATCCCCTTTTTA	60
ECAD	CTTTGACGCCGAGAGCTACA	AAATTCACTCTGCCCAGGACG	64
EZH2	CCCTGACCTCTGTCTTACTTGTGGA	ACGTCAGATGGTGCCAGCAATA	60

				SIRT7 film					LMNB1 film			5
Cell Line	Replicate	Band OD	Inverted	Background OD	Inverted	Net	Band OD	Inverted	Background OD	Inverted	Net	Ratio
0	1	193,40	61,60	200,98	54,02	7,58	179,91	75,09	212,41	42,59	32,500	0,233
, HUC	2	180,81	74,19	189,41	65,59	8,60	177,43	77,57	210,90	44,10	33,470	0,257
5	3	194,35	60,65	201,28	53,72	6,93	181,27	73,73	212,05	42,95	30,780	0,225
3	1	170,27	84,73	201,77	53,23	31,50	177,91	77,09	212,92	42,08	35,010	0,900
GHD	2	160,05	94,95	189,19	65,81	29,14	179,25	75,75	209,28	45,72	30,030	0,970
4v.	3	168,89	86,11	202,08	52,92	33,19	181,14	73,86	212,94	42,06	31,800	1,044
1000	1	152,47	102,53	200,39	54,61	47,92	180,11	74,89	213,01	41,99	32,900	1,457
.31	2	150,90	104,10	190,42	64,58	39,52	178,41	76,59	209,79	45,21	31,380	1,259
3-	3	167,35	87,65	201,60	53,40	34,25	180,62	74,38	211,38	43,62	30,760	1,113
	1	171,12	83,88	199,98	55,02	28,86	179,32	75,68	212,69	42,31	33,370	0,865
-82	2	163,58	91,42	188,99	66,01	25,41	179,26	75,74	210,37	44,63	31,110	0,817
	3	174,10	80,90	201,04	53,96	26,94	182,98	72,02	212,59	42,41	29,610	0,910
12	1	178,08	76,92	200,59	54,41	22,51	180,31	74,69	211,03	43,97	30,720	0,733
24	2	168,92	86,08	189,08	65,92	20,16	177,39	77,61	209,82	45,18	32,430	0,622
	3	180,39	74,61	200,03	54,97	19,64	181,01	73,99	213,93	41,07	32,920	0,597
.0	1	201,13	53,87	201,37	53,63	0,24	179,38	75,62	212,46	42,54	33,080	0,007
SU	2	188,01	66,99	188,27	66,73	0,26	178,91	76,09	209,01	45,99	30,100	0,009
10	3	202,71	52,29	202,86	52,14	0,15	179,95	75,05	211,93	43,07	31,980	0,005

	2				SIRT7 film					LMNB1 film						
	Cell Line	Replicate	Band OD	Inverted	Background OD	Inverted	Net	Band OD	Inverted	Background OD	Inverted	Net	Ratio	Mean Control	Fold Variation	Mean Fol
	the	1	126,36	128,64	191,73	63,27	65,37	109,83	145,17	231,32	23,68	121,48	0,538	0,586	0,918	1,000
	an	2	131,03	123,97	203,28	51,72	72,25	115,07	139,94	231,05	23,95	115,99	0,623		1,063	
2	ansu.	3	127,45	127,55	199,12	55,88	71,67	110,89	144,11	230,91	24,09	120,02	0,597		1,019	
10.	1	1	172,11	82,89	187,29	67,71	15,18	119,89	135,11	232,79	22,21	112,91	0,134		0,229	0,280
	SIRC	2	175,27	79,73	204,53	50,47	29,26	106,00	149,00	230,33	24,67	124,33	0,235		0,402	
	Str	3	177,35	77,65	191,79	63,21	14,44	109,42	145,58	228,19	26,81	118,77	0,122		0,207	
	A	1	123,91	131,09	198,36	56,64	74,45	112,91	142,09	220,07	34,93	107,16	0,695	0,708	0,982	1,000
	CIT	2	127,10	127,90	200,72	54,28	73,62	115,02	139,98	218,34	36,66	103,32	0,713		1,007	
S	\$	3	124,56	130,44	195,27	59,73	70,71	123,13	131,87	221,90	33,10	98,77	0,716		1,012	
Gr	4	1	183,40	71,60	199,14	55,86	15,74	121,82	133,18	215,29	39,71	93,47	0,168		0,238	0,208
	SIR	2	181,09	73,91	194,25	60,75	13,16	118,29	136,71	222,39	32,61	104,10	0,126		0,179	
	4	3	181,55	73,45	194,78	60,22	13,23	120,04	134,96	221,06	33,94	101,02	0,131		0,185	
	~	1	136,38	118,62	197,58	57,42	61,20	134,23	120,77	227,62	27,39	93,38	0,655	0,603	1,087	1,000
	Car	2	125,10	129,90	185,46	69,54	60,36	122,29	132,71	228,41	26,59	106,12	0,569		0,944	
1	\$	3	131,93	123,07	189,21	65,79	57,28	131,72	123,28	229,81	25,19	98,09	0,584		0,969	
50	4	1	171,10	83,91	190,73	64,27	19,64	131,46	123,54	229,19	25,81	97,73	0,201		0,333	0,458
	SIR	2	162,42	92,58	187,15	67,85	24,73	127,40	127,60	225,27	29,73	97,87	0,253		0,419	
	ST	3	155.81	99.19	191.92	63.08	36.11	130.62	124.38	227.01	27.99	96.39	0.375		0.622	

MGHU3	1 2			ECAD film					Actin film			Ratios		
		Band OD	inverted	background OD	inverted	Net	Band OD	inverted	background OD	inverted	Net		ECAD/ACTIN	
	(1)	216.70	38.30	233.91	21.09	17.21	159.28	95.72	170.91	84.09	11.63	1.480	2.976	0.469
sh-CTRL	(2)	117,20	137,80	191,99	63,01	74,80	161,42	93,58	179,95	75,05	18,53	4,036		1,279
	(3)	193,91	61,09	226,72	28,28	32,82	159,93	95,07	169,54	85,46	9,61	3,413		1,082
	(1)	221,92	33,08	251,34	3,66	29,42	155,83	99,17	171,40	83,60	15,57	1,890		0,599
sh-SIRT7	(2)	203,85	51,15	229,95	25,05	26,10	157,73	97,27	171,08	83,92	13,36	1,954		0,619
	(3)	207,68	47,32	240,11	14,89	32,43	154,34	100,66	170,41	84,59	16,07	2,018		0,639
											-			
MGHU3				NCAD film					Actin film				Ratios	
		Band OD	inverted	background OD	inverted	Net	Band OD	inverted	background OD	inverted	Net		NCAD/ACTIN	
POLICY CONTRACTOR	(1)	195,12	59,88	238,63	16,37	43,51	159,28	95,72	170,91	84,09	11,63	3,742	3,156	1,257
sh-CTRL	(2)	168,54	86,46	223,185	31,82	54,65	161,42	93,58	179,95	75,05	18,53	2,949		0,991
	(3)	185,14	69,86	211,837	43,16	26,69	159,93	95,07	169,54	85,46	9,61	2,777		0,933
	(1)	165,81	89,19	253,91	1,09	88,10	155,83	99,17	171,40	83,60	15,57	5,658		1,793
sh-SIRT7	(2)	170,73	84,27	245,33	9,67	74,60	157,73	97,27	171,08	83,92	13,36	5,586		1,770
	(3)	153,56	101,44	240,11	14,89	86,55	154,34	100,66	170,41	84,59	16,07	5,385		1,706
	5 5						-							
MGHU3		3		SLUG film		3			Actin film				Ratios	
		Band OD	inverted	background OD	inverted	Net	Band OD	inverted	background OD	inverted	Net		SLUG/ACTIN	
	(1)	193,82	61,18	194,76	60,24	0,94	159,28	95,72	170,91	84,09	11,63	0,081	1,991	0,041
sh-CTRL	(2)	133,47	121,53	202,54	52,46	69,07	161,42	93,58	179,95	75,05	18,53	3,727		1,872
	(3)	1/9,64	/5,30	200,46	54,54	20,81	159,93	95,07	169,54	85,46	9,61	2,165		1,087
1 (1977)	(1)	129,70	125,30	185,24	69,76	55,55	155,83	99,17	1/1,40	83,60	15,57	3,568		1,792
sn-SIR17	(2)	134,51	120,49	185,11	/1,89	48,61	157,73	97,27	171,08	83,92	15,30	3,639		1,828
	(5)	107,27	07,75	240,23	0,77	30,30	104,04	100,00	170,41	04,35	10,07	3,000		1,042
MGHU3	2	8		SNAIL film					Actin film				Ratios	
		Band OD	inverted	background OD	inverted	Net	Band OD	inverted	background OD	inverted	Net		SNAIL/ACTIN	
	(1)	208.21	46.79	252 52	2.48	44.31	159.28	95.72	170.91	84.09	11.63	3,811	5 921	0.644
sh-CTRL	(2)	134.76	120.24	253.67	1.33	118.91	161.42	93.58	179.95	75.05	18.53	6,417	2,221	1.084
Constant Constant of Constant	(3)	178,10	76,90	250,54	4.46	72,44	159,93	95,07	169,54	85,46	9,61	7,535		1,273
	(1)	148.16	106.84	250,74	4.26	102.58	155.83	99.17	171.40	83.60	15.57	6,589		1.113
sh-SIRT7	(2)	158,70	96.30	252.91	2.09	94.21	157,73	97.27	171.08	83.92	13.36	7.054		1.191

5637	r - 1			ECAD film					Actin film		1		Ratios			
		Band OD	inverted	background OD	inverted	Net	Band OD	inverted	background OD	inverted	Net		ECAD/ACTIN			
	(1)	180,89	74,11	230,77	24,23	49,88	175,31	79,69	191,33	63,67	16,02	3,114	2,802	1,111		
sh-CTRL	(2)	154,13	100,88	227,56	27,44	73,44	173,95	81,05	192,47	62,53	18,52	3,965		1,415		
	(3)	212,40	42,60	230,62	24,38	18,22	177,56	77,44	191,28	63,72	13,72	1,328		0,474		
	(1)	206,51	48,49	231,23	23,77	24,72	172,34	82,66	190,21	64,79	17,87	1,383		0,494		
sh-SIRT7	(2)	202,67	52,33	233,71	21,29	31,04	169,27	85,73	191,08	63,92	21,81	1,423		0,508		
	(3)	205,21	49,79	230,89	24,11	25,68	173,88	81,12	192,90	62,10	19,02	1,350		0,482		
5637	6			NCAD film			Actin film						Ratios			
		Band OD	inverted	background OD	inverted	Net	Band OD	inverted	background OD	inverted	Net		NCAD/ACTIN			
	(1)	143.79	111.21	191.34	63.66	47.55	175.31	79.69	191.33	63.67	16.02	2.968	2.801	1.059		
sh-CTRL	(2)	150,44	104,56	193,89	61,11	43,45	173,95	81,05	192,47	62,53	18,52	2,346	1	0,837		
	(3)	149,27	105,73	191,66	63,34	42,39	177,56	77,44	191,28	63,72	13,72	3,090		1,103		
	(1)	113,83	141,17	191,21	63,79	77,38	172,34	82,66	190,21	64,79	17,87	4,330		1,546		
sh-SIRT7	(2)	102,74	152,26	192,99	62,01	90,25	169,27	85,73	191,08	63,92	21,81	4,138		1,477		
	(3)	114,01	140,99	192,17	62,83	78,16	173,88	81,12	192,90	62,10	19,02	4,109		1,467		
5607		-		anno di									D .:			
5037		B		SLUG NIM		Net	Devel OD		Actin film		Net		Ratios			
r	(4)	Band OD	inverted	background OD	Inverted	Net	Band OD	Inverted	background OD	inverted	Net	2,650	SLUG/ACTIN	0.005		
ch CTDI	(1)	157,99	97,01	200,44	54,50	42,45	172.05	79,69	191,55	63,67	10,02	2,650	2,691	0,985		
SIFCIRE	(2)	162,41	92,59	205,79	49,21	45,56	173,95	77.44	192,47	63 72	18,52	2,542		1 1 4 5		
	(1)	125 71	120.20	203,12	51.00	77.96	172.34	92.66	100.21	64.70	17.97	4 357		1,145		
ch-STPT7	(1)	107.22	147 78	203,57	54.00	03.60	160.27	85.73	190,21	63.02	21.81	4,337		1,015		
SILDIKIZ	(2)	116 58	138.42	200,31	54.68	83 74	173.88	81 12	192.90	62 10	19.02	4 403		1,537		
	(0)								,	,						
5637	1 - I			SNAIL film					Actin film		S.	8	Ratios			
		Band OD	inverted	background OD	inverted	Net	Band OD	inverted	background OD	inverted	Net		SNAIL/ACTIN			
	(1)	169,59	85,41	245,93	9,07	76,34	175,31	79,69	191,33	63,67	16,02	4,765	4,873	0,978		
sh-CTRL	(2)	161,33	93,67	243,60	11,40	82,27	173,95	81,05	192,47	62,53	18,52	4,442		0,912		
	(3)	171,20	83,80	245,44	9,56	74,24	177,56	77,44	191,28	63,72	13,72	5,411		1,110		
	(1)	89,90	165,10	243,71	11,29	153,81	172,34	82,66	190,21	64,79	17,87	8,607		1,766		
sh-SIRT7	(2)	60,61	194,39	245,99	9,01	185,38	169,27	85,73	191,08	63,92	21,81	8,500		1,744		
	(3)	79,55	175,45	241,87	13,13	162,32	173,88	81,12	192,90	62,10	19,02	8,534		1,751		

J82				NCAD film					Actin film				Ratios	
		Band OD	inverted	background OD	inverted	Net	Band OD	inverted	background OD	inverted	Net	N	ICAD/ACTIN	
	(1)	127,90	127,10	188,04	66,96	60,14	162,23	92,77	182,76	72,24	20,53	2,929	2,515	1,165
sh-scramble	(2)	134,65	120,35	190,21	64,79	55,56	155,12	99,88	183,94	71,06	28,82	1,928		0,767
	(3)	131,19	123,81	189,14	65,86	57,95	160,44	94,56	182,01	72,99	21,57	2,687		1,068
	(1)	104,51	150,49	188,37	66,63	83,86	161,91	93,09	180,39	74,61	18,48	4,538		1,805
sh-SIRT7	(2)	81,12	173,88	186,29	68,71	105,17	159,30	95,70	182,22	72,78	22,92	4,589		1,825
	(3)	91,72	163,28	189,01	65,99	97,29	161,19	93,81	182,17	72,83	20,98	4,637		1,844

J82				SNAIL film			Actin film						Ratios		
		Band OD	inverted	background OD	inverted	Net	Band OD	inverted	background OD	inverted	Net		SNAIL/ACTIN		
	(1)	169,59	85,41	240,37	14,63	70,78	162,23	92,77	182,76	72,24	20,53	3,448	3,151	1,094	
sh-scramble	(2)	161,33	93,67	241,24	13,76	79,91	155,12	99,88	183,94	71,06	28,82	2,773		0,880	
	(3)	171,20	83,80	240,90	14,10	69,70	160,44	94,56	182,01	72,99	21,57	3,231		1,026	
1177 (1797) AND 1797	(1)	153,10	101,90	238,73	16,27	85,63	161,91	93,09	180,39	74,61	18,48	4,634		1,471	
sh-SIRT7	(2)	133,41	121,59	240,99	14,01	107,58	159,30	95,70	182,22	72,78	22,92	4,694		1,490	
	(3)	145,02	109,98	241,04	13,96	96,02	161,19	93,81	182,17	72,83	20,98	4,577		1,453	

5637				EZH2 film				LMNB1 film					Ratios		
CT (2)		Band OD	inverted	Background OD	inverted	Net	Band OD	inverted	Background OD	inverted	Net		EZH2/LMNB1		
2	(1)	180,89	74,11	222,99	32,01	42,10	182,30	72,70	219,01	35,99	36,71	1,147	0,989	1,160	
sh-CTRL	(2)	154,13	100,88	219,94	35,06	65,82	181,34	73,66	220,82	34,18	39,48	1,667		1,686	
	(3)	212,40	42,60	217,38	37,62	4,98	184,88	70,12	217,53	37,47	32,65	0,153		0,154	
	(1)	169,47	85,53	241,38	13,62	71,91	180,39	74,61	221,28	33,72	40,89	1,759		1,779	
sh-SIRT7	(2)	176,30	78,70	239,28	15,72	62,98	182,91	72,09	219,06	35,94	36,15	1,742		1,762	
	(3)	178,17	76,83	240,25	14,75	62,08	182,49	72,51	218,48	36,52	35,99	1,725		1,744	



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Figure S8. Detailed Information about Western Blot Figures 3, 4E and 5F.



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