

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

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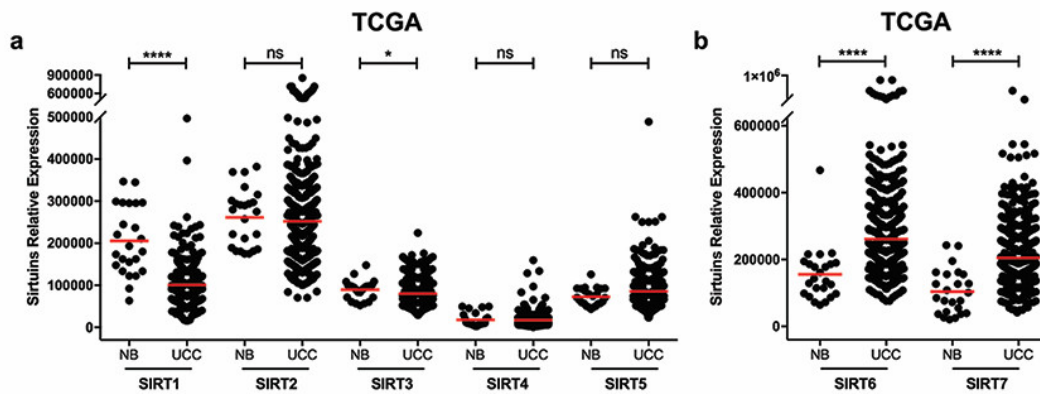


Figure S1: Characterization of SIRT1, SIRT2, SIRT3, SIRT4 and SIRT5 (a), and SIRT6 and SIRT7 (b) in the TCGA bladder cancer cohort by quantitative RT-PCR. * $p < 0.05$, **** $p < 0.0001$ and ns—non significant. UCC—urothelial cell carcinoma, NB—normal bladder mucosae.

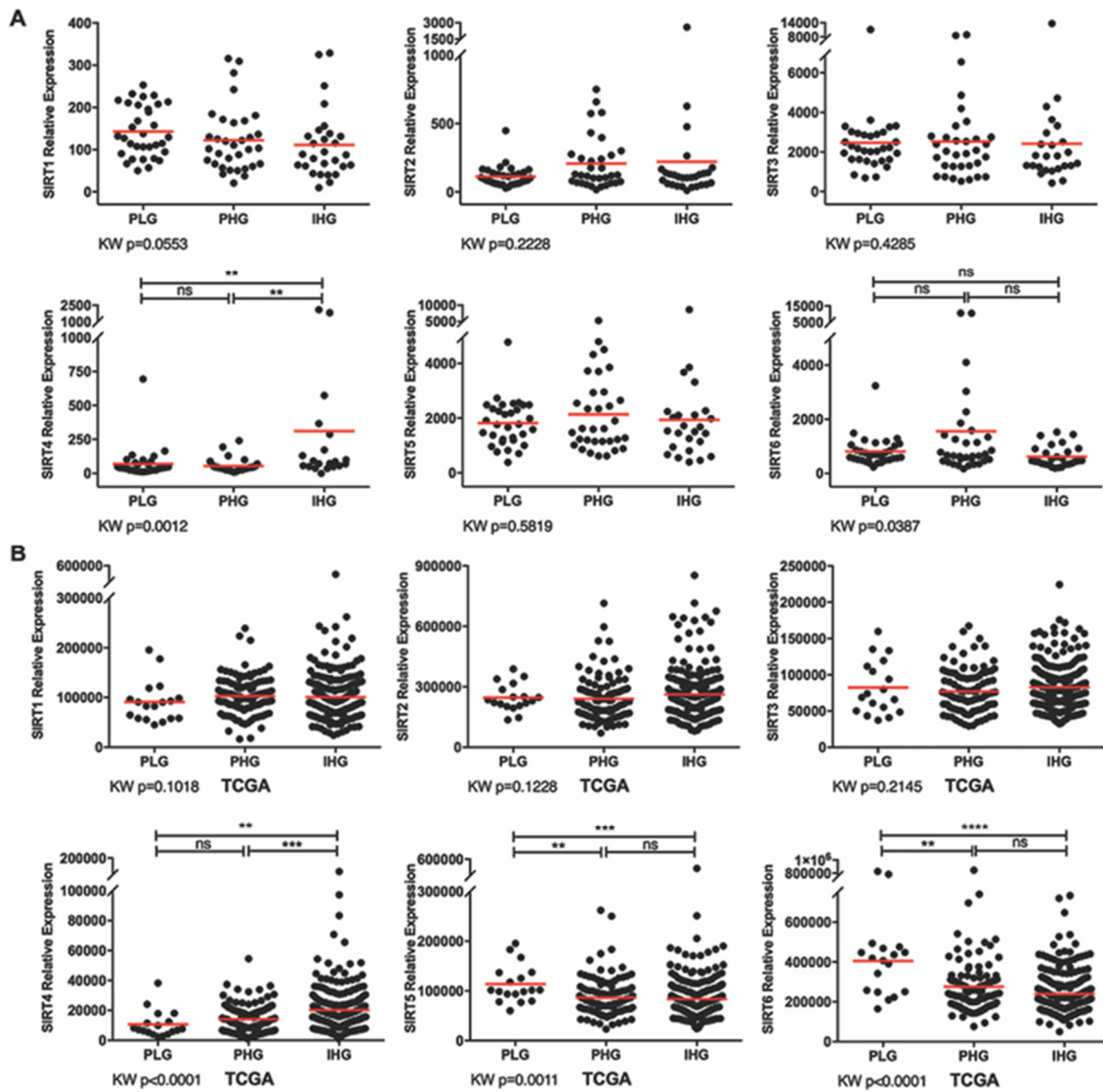
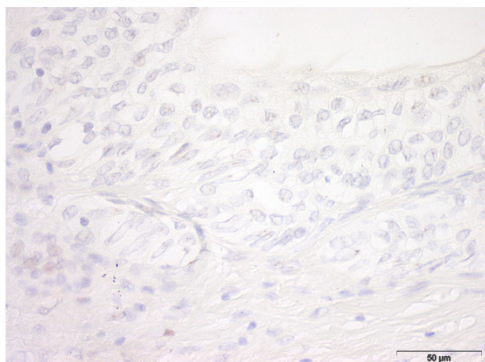
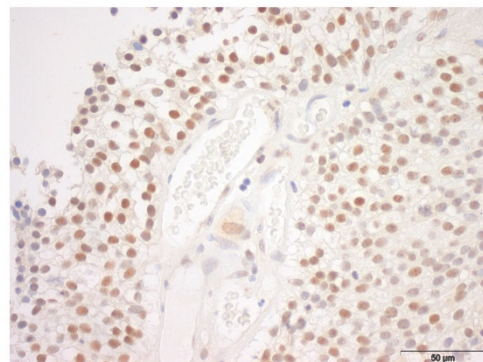


Figure S2: Characterization of Sirtuin family (SIRT1 to SIRT7) gene expression in bladder cancer tissues (a) and TCGA cohort (b) categorized by clinical grade. ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$ and ns – non significant. PLG – papillary low-grade, PHG – papillary high-grade, IHG – invasive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

SIRT7 Immunohistochemistry



Normal Urothelium



Bladder Carcinoma

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

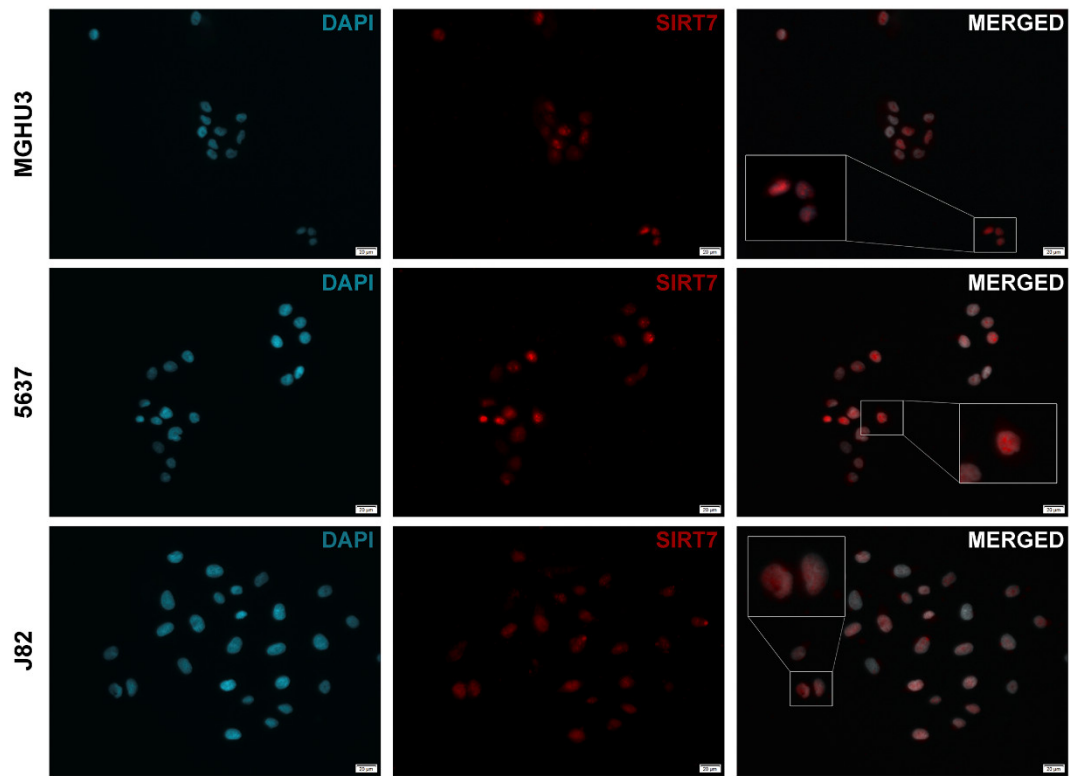


Figure S4. Immunofluorescence representative images for SIRT7 immunoexpression in MGHU3, 5637 and J82 bladder cancer cell lines (100× magnification).

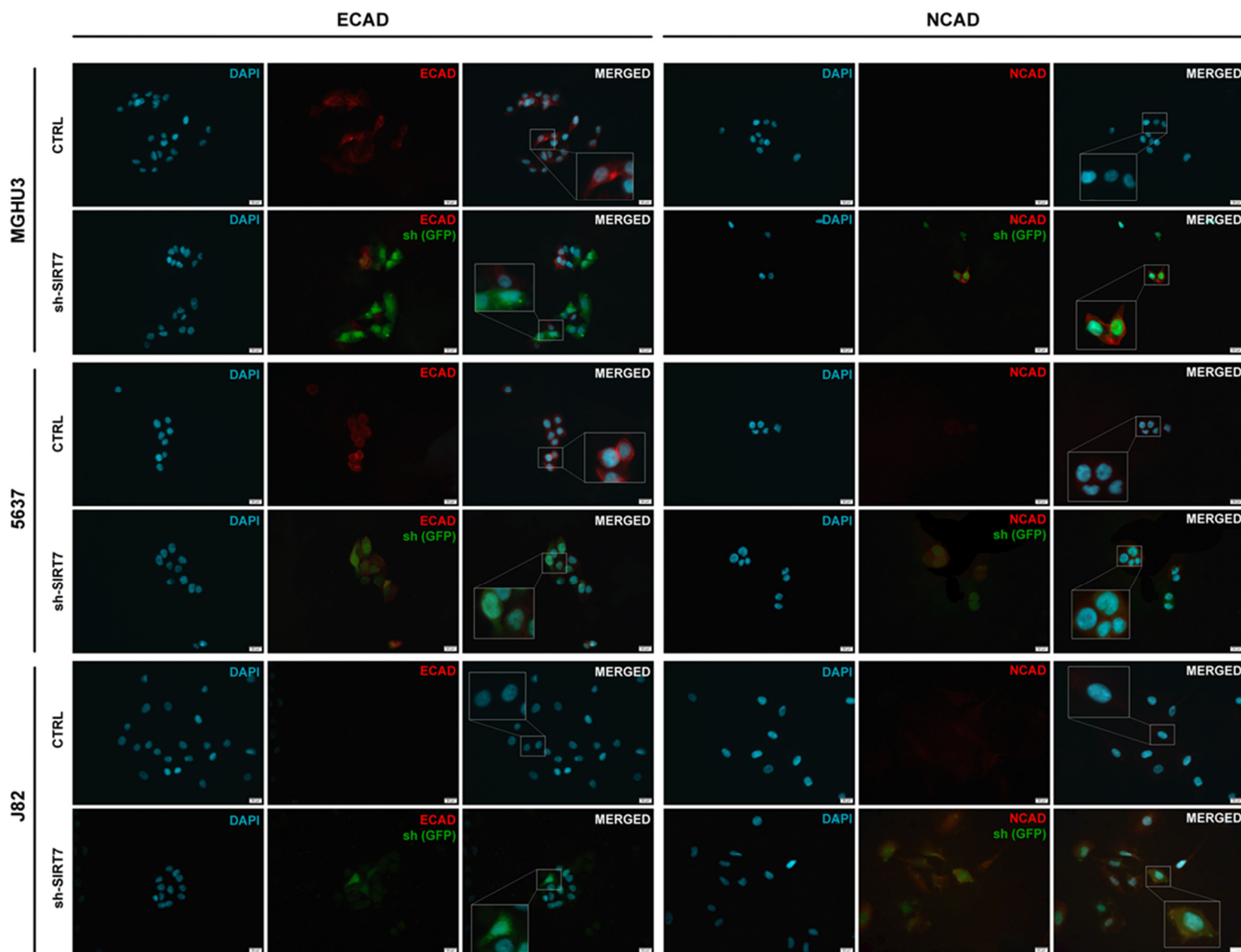


Figure S5: Immunofluorescence representative images for E-Cadherin and N-Cadherin immunoexpression in sh-CTRL and sh-SIRT7 MGHU3, 5637 and J82 bladder cancer cell lines (100× magnification).

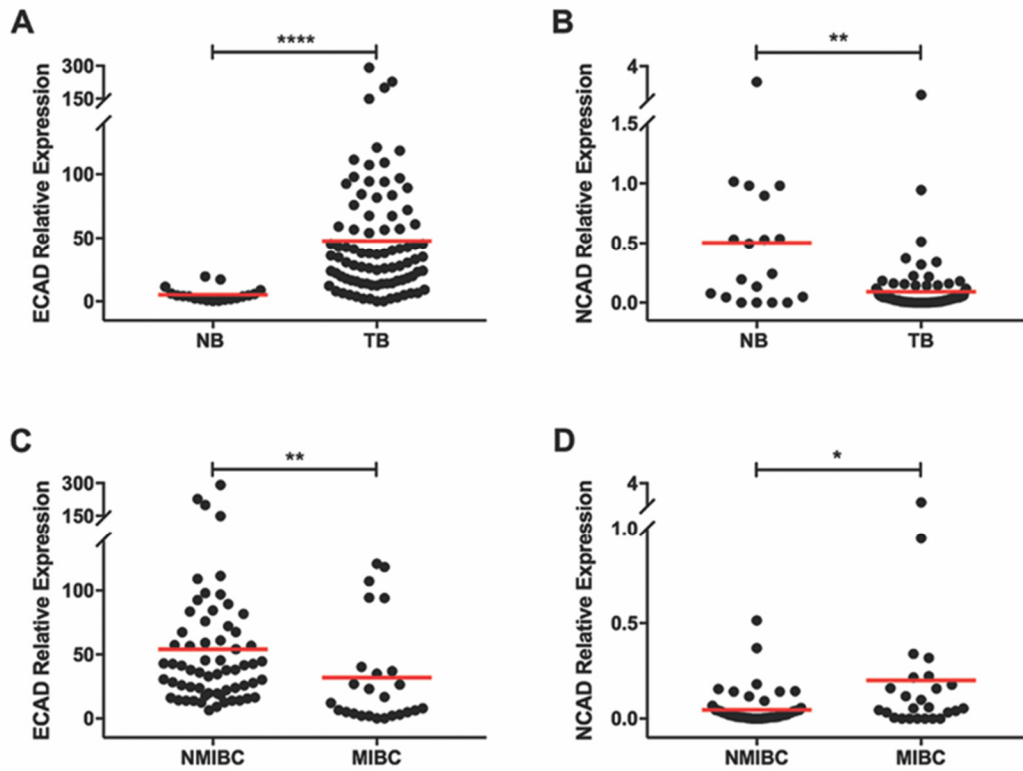


Figure S6. Characterization of *CDH1* gene expression in the bladder cancer and normal mucosae tissue samples (a), and in non-muscle invasive bladder cancer cases (b), by quantitative RT-PCR. Characterization of *CDH2* gene expression in the bladder cancer and normal mucosae tissue samples (c), and in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

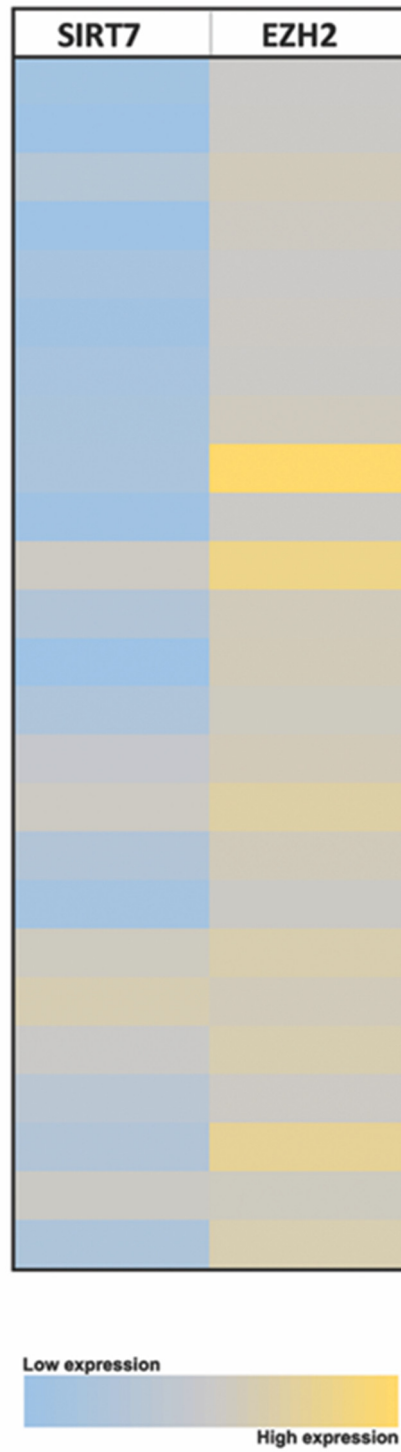


Figure S7. Heat map showing SIRT7 and EZH2 expression in muscle invasive bladder cancer (MIBC) cases ($n = 26$). Relative expression levels are depicted in colours from dark blue (lower expression), to dark yellow (higher expression) as shown in the colour bar below the graphic.

Table S1. Reference of TaqMan® gene expression assays for studied genes.

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

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

Gene	Forward sequence (5'-3')	Reverse sequence (5'-3')	Annealing T. (°C)
<i>BGUS</i>	CTCATTGGAATTTGCCGATT	CCGAGTGAAGATCCCCTTTTA	60
<i>ECAD</i>	CTTGACGCCGAGAGCTACA	AAATTCACTCTGCCCAGGACG	64
<i>EZH2</i>	CCCTGACCTCTGTCTTACTTGTGGA	ACGTCAGATGGTGCCAGCAATA	60

5637	ECAD film					Actin film					Ratios			
	Band OD	inverted	background OD	inverted	Net	Band OD	inverted	background OD	inverted	Net	ECAD/ACTIN			
sh-CTRL	(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
	(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
sh-SIRT7	(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
	(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	NCAD film					Actin film					Ratios			
	Band OD	inverted	background OD	inverted	Net	Band OD	inverted	background OD	inverted	Net	NCAD/ACTIN			
sh-CTRL	(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
	(2)	150,44	104,56	193,89	61,11	43,45	173,95	81,05	192,47	62,53	18,52	2,346		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
sh-SIRT7	(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
	(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

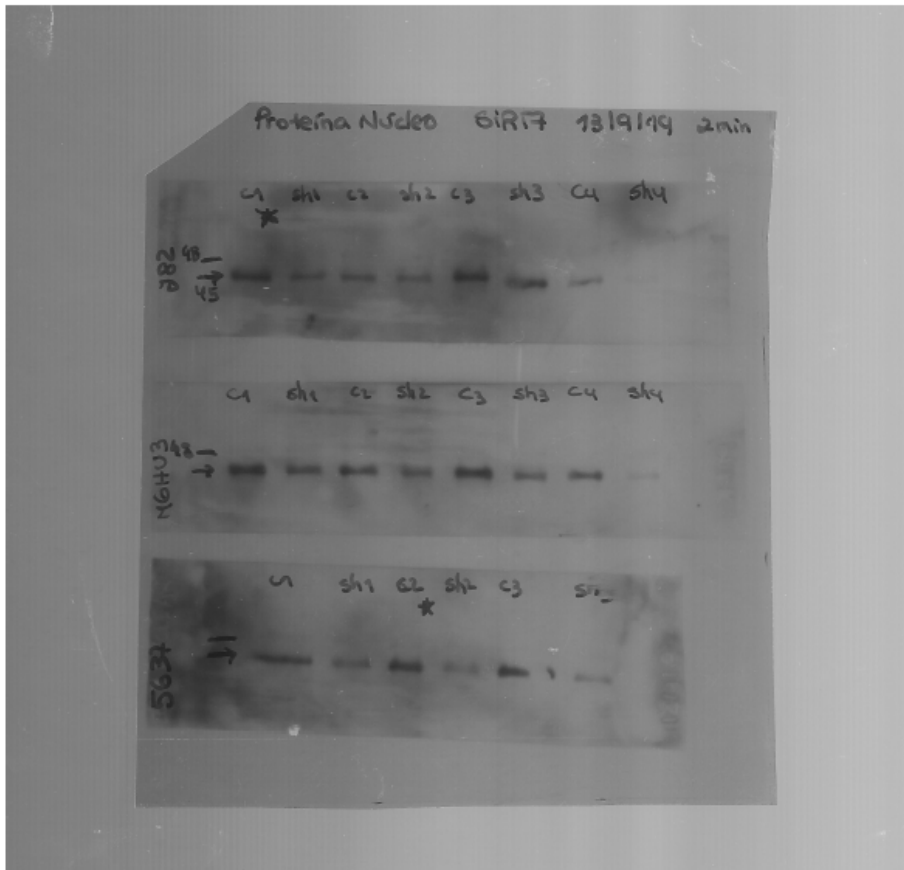
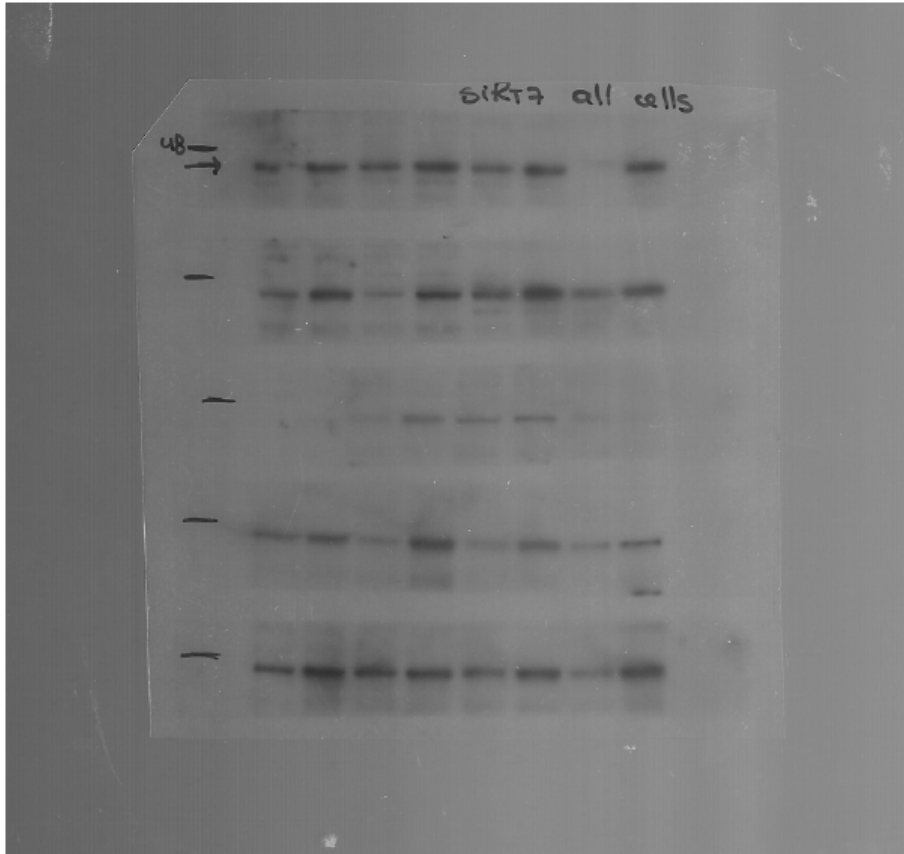
5637	SLUG film					Actin film					Ratios			
	Band OD	inverted	background OD	inverted	Net	Band OD	inverted	background OD	inverted	Net	SLUG/ACTIN			
sh-CTRL	(1)	157,99	97,01	200,44	54,56	42,45	175,31	79,69	191,33	63,67	16,02	2,650	2,691	0,985
	(2)	162,41	92,59	205,79	49,21	43,38	173,95	81,05	192,47	62,53	18,52	2,342		0,871
	(3)	160,87	94,13	203,12	51,88	42,25	177,56	77,44	191,28	63,72	13,72	3,079		1,145
sh-SIRT7	(1)	125,71	129,29	203,57	51,43	77,86	172,34	82,66	190,21	64,79	17,87	4,357		1,619
	(2)	107,22	147,78	200,91	54,09	93,69	169,27	85,73	191,08	63,92	21,81	4,296		1,597
	(3)	116,58	138,42	200,32	54,68	83,74	173,88	81,12	192,90	62,10	19,02	4,403		1,636

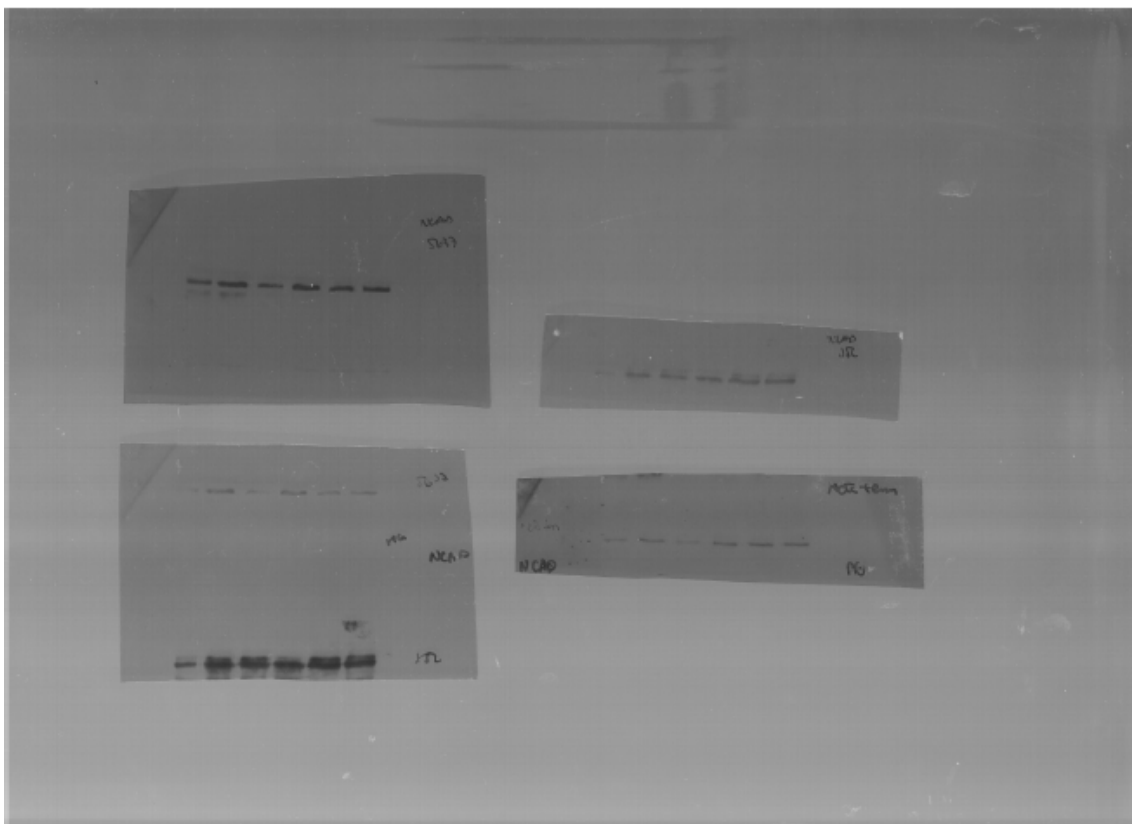
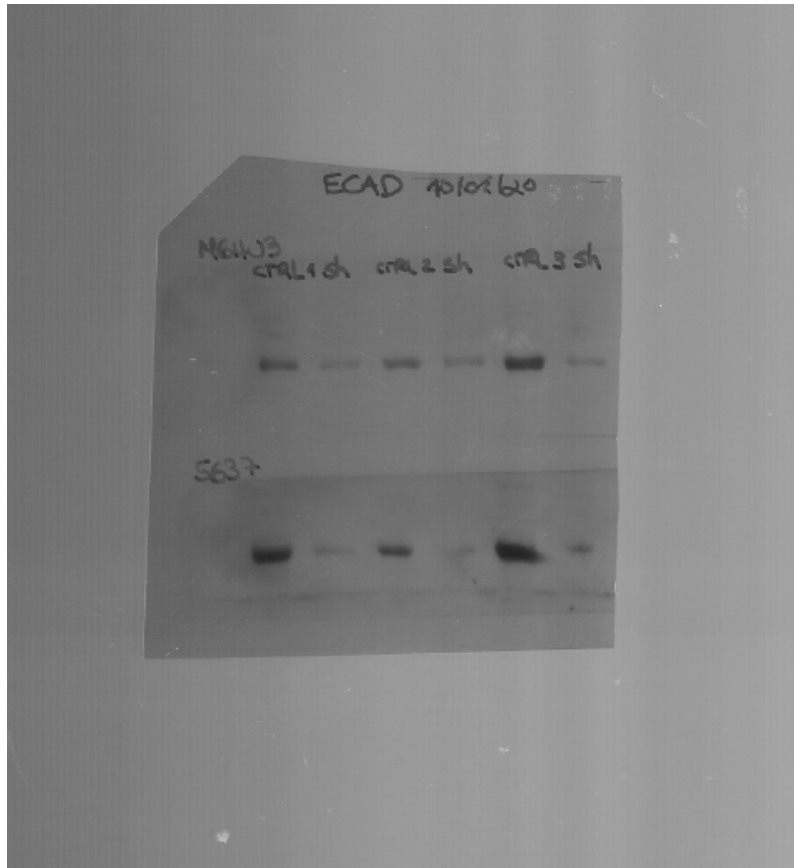
5637	SNAIL film					Actin film					Ratios			
	Band OD	inverted	background OD	inverted	Net	Band OD	inverted	background OD	inverted	Net	SNAIL/ACTIN			
sh-CTRL	(1)	169,59	85,41	245,93	9,07	76,34	175,31	79,69	191,33	63,67	16,02	4,765	4,873	0,978
	(2)	161,33	93,67	243,60	11,40	82,27	173,95	81,05	192,47	62,53	18,52	5,442		0,912
	(3)	171,20	83,80	245,44	9,56	74,24	177,56	77,44	191,28	63,72	13,72	4,411		1,110
sh-SIRT7	(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
	(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

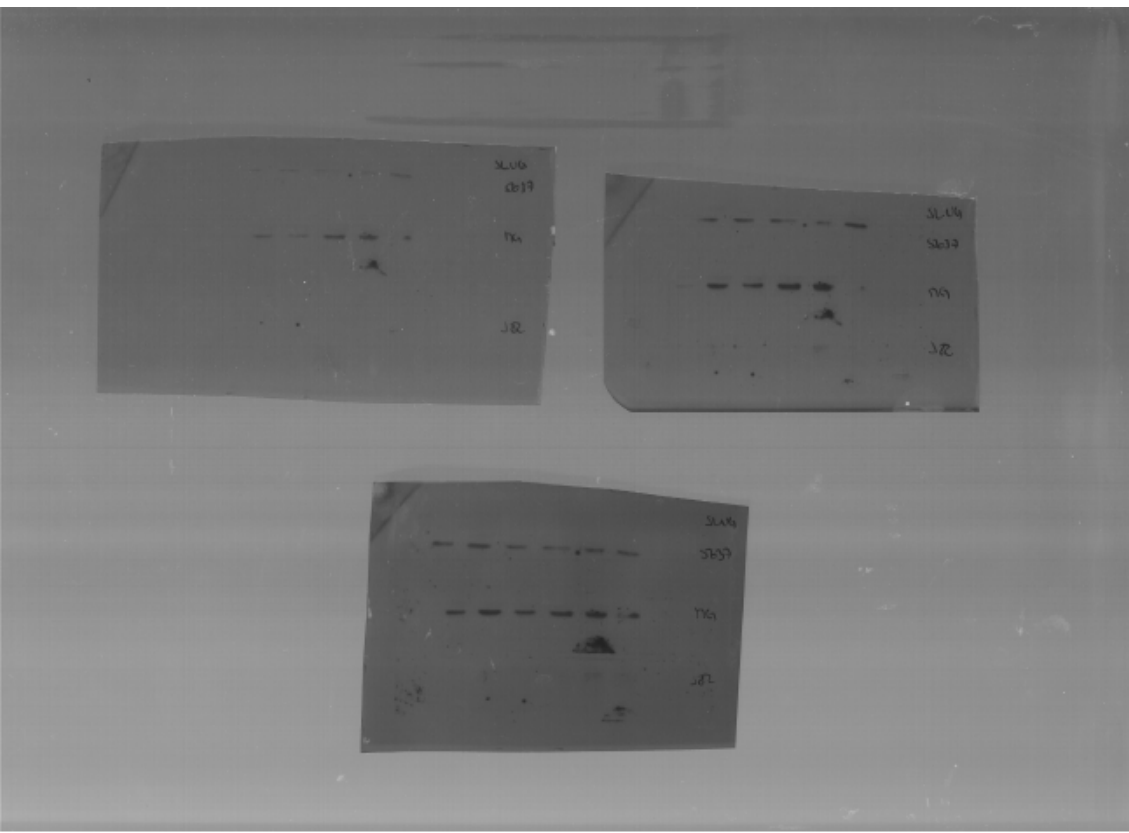
J82	NCAD film					Actin film					Ratios			
	Band OD	inverted	background OD	inverted	Net	Band OD	inverted	background OD	inverted	Net	NCAD/ACTIN			
sh-scramble	(1)	127,90	127,10	188,04	66,96	60,14	162,23	92,77	182,76	72,24	20,53	2,929	2,515	1,165
	(2)	134,65	120,35	190,21	64,79	55,56	155,12	99,88	183,94	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
sh-SIRT7	(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
	(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

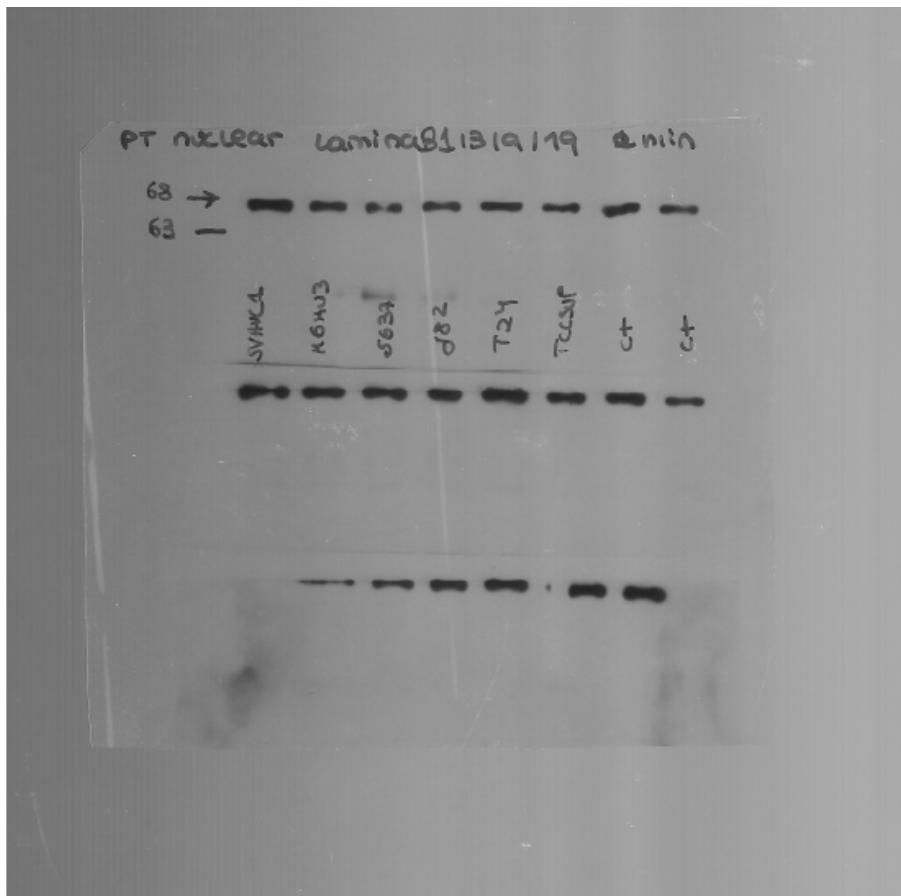
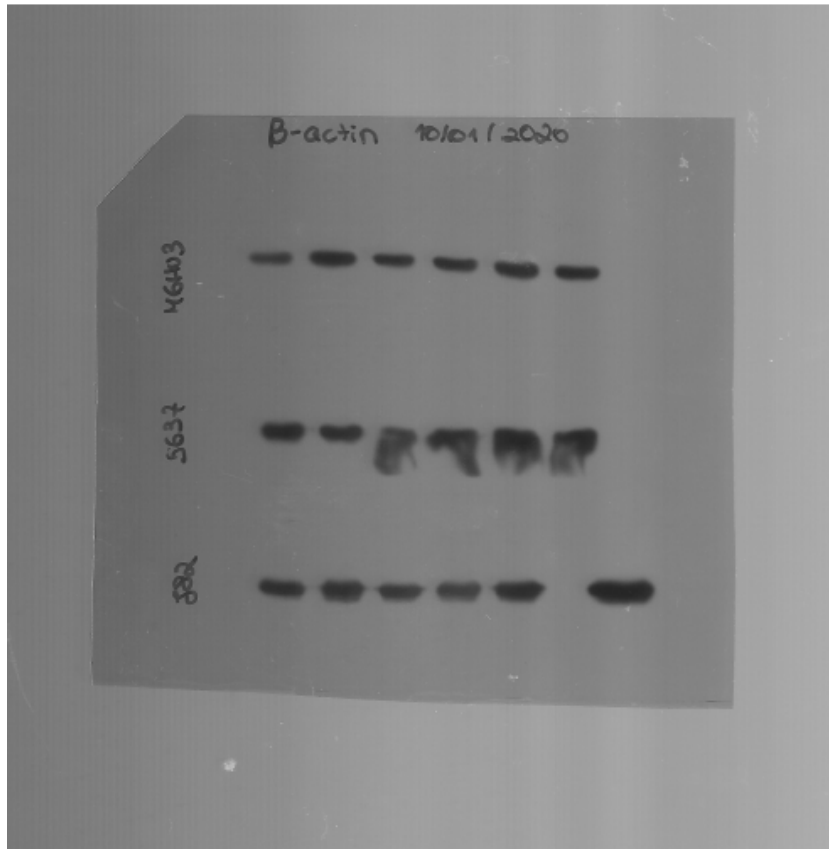
J82	SNAIL film					Actin film					Ratios			
	Band OD	inverted	background OD	inverted	Net	Band OD	inverted	background OD	inverted	Net	SNAIL/ACTIN			
sh-scramble	(1)	169,59	85,41	240,37	14,63	70,78	162,23	92,77	182,76	72,24	20,53	3,448	3,151	1,094
	(2)	161,33	93,67	241,24	13,76	79,91	155,12	99,88	183,94	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
sh-SIRT7	(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
	(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	EZHZ film					LMNB1 film					Ratios			
	Band OD	inverted	Background OD	inverted	Net	Band OD	inverted	Background OD	inverted	Net	EZHZ/LMNB1			
sh-CTRL	(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
	(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
sh-SIRT7	(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
	(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









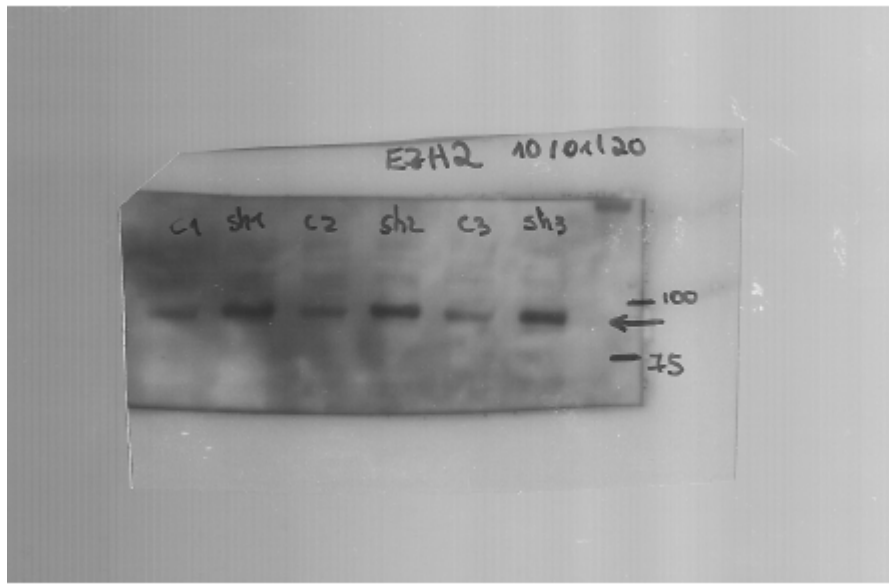


Figure S8. Detailed Information about Western Blot Figures 3, 4E and 5F.



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