Identification of long non-coding RNAs that stimulate cell survival in bladder cancer

Supplementary Materials

Supplementary Table 1: Primers, antisense oligonucleotide (ASO) and siRNAs sequences

Primers

Gene	Forward primer (5′→3′)	Reverse primer (5′→3′)
ACTB	CGTCTTCCCCTCCATCGTG	GATGGGGTACTTCAGGGTGA
CAT266	GCTCATTGCCCTCAAGTGTT	AGGACTGCCTGAGACCATGA
CAT1297	TGGGATTACAAAATGCGTGA	CATGGCAGCAGCAAGTAGAA
CAT1647	GGCTAACAGATGCCCAGATG	AACTGGCCACAGCAGAGATT
HP1BP3	TGGAATATGCAATCTTGTCTGC	GAACCCTTTCCCAGAGATCTG
RNU6	CGCTTCGGCAGCACATATAC	AGGGGCCATGCTAATCTTCT

Antisense oligonucleotides

Name	Target sequence (5′→3′)	Antisense oligo (5′→3′)
CAT266-ASO1	CAACAAUGGAGCCCCAAGUG	CACTTGGGGCTCCATTGTTG
CAT266-ASO2	ACAUUGGCAUUAGCCUUAUC	GATAAGGCTAATGCCAATGT
CAT1297-ASO1	GAAAACAAUUCACCUCUUGC	GCAAGAGGTGAATTGTTTTC
CAT1297-ASO2	GUUCUCUACAACCUCCACCU	AGGTGGAGGTTGTAGAGAAC
CAT1647-ASO1	UCACUGUCUCUUACUAAAGC	GCTTTAGTAAGAGACAGTGA
CAT1647-ASO2	GAUUCAAUUGCUCACCUCUU	AAGAGGTGAGCAATTGAATC
CTRL-ASO1 (38)	*none*	AAGCGCGCACCAGCGCCTCC
CTRL-ASO2 (39)	*none*	CCTTCCCTGAAGGTTCCTCC

siRNAs

Name	Sense siRNA (5′→3′)	Antisense siRNA (5′→3′)
CAT266-siRNA	GCUGGAAUUACAGGUAUAATT	UUAUACCUGUAAUUCCAGCTT
CAT1297-siRNA1	CUCAUGACAACUCUUAUUUTT	AAAUAAGAGUUGUCAUGAGTT
CAT1297-siRNA2	GAAACACACACACACUCUUTT	AAGAGUGUGUGUGUGUUUCTT
CAT1647-siRNA1	ACUGUCUCUUACUAAAGCUTT	AGCUUUAGUAAGAGACAGUTT
CAT1647-siRNA2	GGAAACACCUUCACAGACUTT	AGUCUGUGAAGGUGUUUCCTT
CTRL-siRNA 1	Silencer Negative Control No.1	(AM4611, Ambion)

Supplementary Table 2: Clinical characteristics of patients used for validation

	NMIBC *n* = 18		**MIBC *n* = 47**
Age (average, range)	61 (50–77)	**Age** (average, range)	67 (37–84)
Gender		**Gender**	
female	4	female	15
male	14	male	32
Stage		**Stage**	
pTa	9	pT2	11
pT1	7	pT3	22
CIS	2	pT4	12
		unknown	2
Grade		**Grade**	
G1	1	G2	5
G2	9	G3	36
G3	6	unknown	6
unknown	2		

Supplementary Table 3: The 25 most up-regulated lncRNAs in MIBC identified based on average expression levels using MiTranscriptome data

Transcript[#]	Name	Average expression		T/N ratio	Median expression		T/N ratio	*P*-value[$]
		Benign (N)	Cancer (T)		Benign (N)	Cancer (T)		
T102457	***CAT1647.6***	0	0.155	> 100	0	0.069	> 100	< 0.001
T047629	***CAT1297.4***	0	0.128	> 100	0	0.077	> 100	< 0.001
T078005	***CAT1507***	0.001	0.296	> 100	0	0.096	> 100	< 0.001
T290558	***THCAT68.1***	0.002	0.128	60.98	0	0.042	> 100	< 0.001
T253942	***CAT467.2***	0.002	0.132	53.84	0	0.053	> 100	< 0.001
T235647	***CAT2260.3***	0.003	0.108	38.80	0	0.045	> 100	< 0.001
T108430	***CAT1695***	0.005	0.138	29.88	0	0.020	> 100	< 0.001
T047638	***LACAT97***	0.030	0.777	26.03	0	0.474	> 100	< 0.001
T065634	***CAT1409***	0.005	0.139	25.92	0	0.068	> 100	< 0.001
T207213	***CAT367***	0.035	0.887	25.31	0.011	0.367	31.71	< 0.001
T247881	***LSCAT103***	0.007	0.174	24.44	0	0.029	> 100	< 0.001
T318530	***CAT904.4***	0.006	0.147	24.44	0	0.075	> 100	< 0.001
T087060	***CAT1573***	0.006	0.137	23.60	0	0.024	> 100	< 0.001
T362906	***CAT1167***	0.005	0.114	23.50	0	0.019	> 100	< 0.001
T256721	***CAT491.2***	0.005	0.104	22.13	0	0.056	> 100	< 0.001
T074211	***LSCAT131***	0.005	0.114	21.28	0	0.055	> 100	< 0.001
T108891	***BRCAT116***	0.008	0.166	19.50	0	0.048	> 100	< 0.001
T196786	***MIR4435–1HG.1***	0.008	0.159	19.47	0	0.032	> 100	< 0.001
T078121	***LSCAT167***	0.013	0.242	18.70	0	0.142	> 100	< 0.001
T332213	***LSCAT55***	0.007	0.126	18.69	0	0.049	> 100	< 0.001
T100203	***STCAT7***	0.059	1.075	18.11	0	0.349	> 100	< 0.001
T220458	***CAT2182***	0.012	0.212	18.06	0.002	0.145	65.69	< 0.001
T332495	***CAT971***	0.016	0.281	17.56	0.008	0.129	16.21	< 0.001
T286785	***LINC00491.3***	0.008	0.132	17.34	0	0.063	> 100	< 0.001
T255259	***CAT475.3***	0.009	0.148	17.08	0	0.054	> 100	< 0.001
T192655	***CAT266.3****	0.26	1.84	6.95	0	1.49	> 100	< 0.001

[#]MiTranscriptome identifier; [$]unpaired *t*-test; [*]selected based on the highest T/N ratio calculated using median expression. The lncRNAs with average expression in cancer higher than 0.1 FPKM are shown.

Supplementary Table 4: Expression levels of 11 bladder cancer-associated lncRNAs that are described in literature, and for which expression data are available in the MiTranscriptome dataset

Transcript	**Ref**	**Average expression***		**T/N ratio**	**Median expression***		**T/N ratio**	***P*-value$^{\$}$**
		Benign (N)	**Cancer (T)**		**Benign (N)**	**Cancer (T)**		
ANRIL	(17)	0.01	0.166	14.27	0.005	0.04	7.02	< 0.001
SChLAP1	(18)	0.06	0.31	5.1	0	0	1	0.014
HIF1A-AS2	(19)	0.02	0.07	3.5	0.02	0.02	–1.20	0.001
BC072678	(20)	0.05	0.18	3.49	0.04	0.06	1.58	< 0.001
PCAT1	(21)	0.01	0.04	2.94	0.01	0.02	1.97	< 0.001
TINCR	(22)	2.06	5.59	2.71	0.59	3.42	5.72	< 0.001
MALAT1.2	(23)	4.63	12.16	2.62	0.08	5.81	78.32	0.033
HOTAIR	(40)	0.005	0.009	1.94	0	0	1	0.133
TUG1.4	(24)	11.99	22.93	1.91	8.89	18.34	2.06	< 0.001
ZEB2NAT	(41)	0.05	0.02	-3,16	0.05	0	> 100	0.001
HOXD-AS1	(42)	1.49	1.18	-1.26	0.25	0.52	–2.1	0.631
NEAT1	(43)	11.22	8.76	-1.28	4.75	2.16	2.19	0.585

*MiTranscriptome expression data; $^{\$}$unpaired *t*-test.

Supplementary Table 5: Correlation of clinico-pathological parameters with expression levels of the three identified MIBC-associated lncRNAs

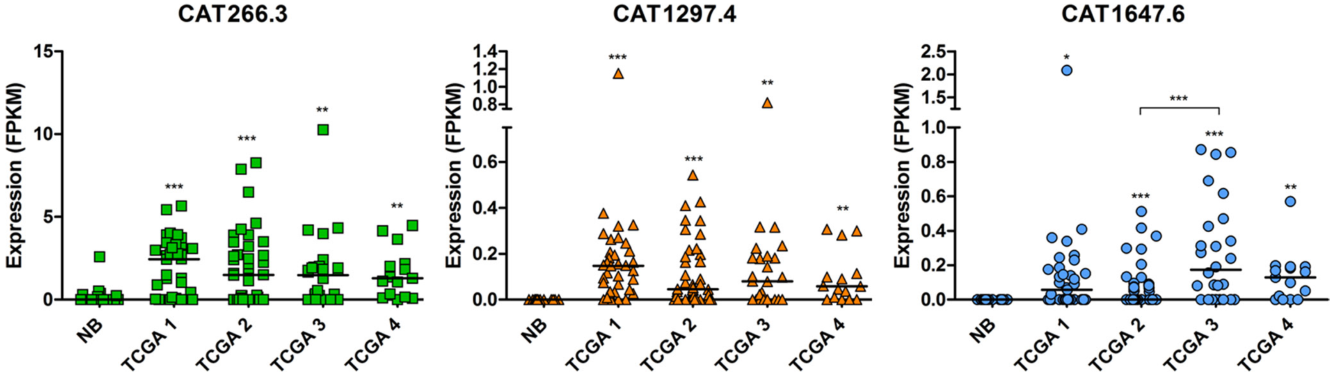
Parameter	No*	*CAT266*		*CAT1297*		*CAT1647*	
		Average expression	***P*-value$**	**Average expression**	***P*-value$**	**Average expression**	***P*-value$**
Age (years)							
< 60	31	1.601	0.407	0.111	0.426	0.115	0.210
> 60	91	1.922		0.134		0.164	
Sex							
Female	34	2.169	0.259	0.079	0.008	0.125	0.311
Male	88	1.713		0.147		0.166	
Lymph nodes							
Positive (N1–3)	40	2.049	0.412	0.130	0.940	0.176	0.745
Negative (N0)	71	1.722		0.129		0.155	
Stage							
pT2	30	1.979	0.746	0.147	0.718	0.288	**0.015**
pT3	62	1.938		0.117		0.117	
pT4	16	1.529		0.123		0.117	
Grade							
low	6	1.184	0.411	0.110	0.867	0.049	**0.027**
high	115	1.863		0,120		0.144	
Subtype							
Papillary	33	1.916	0.882	0.166	0.226	0.208	0.354
Non-papillary	85	1,856		0.115		0.138	
Smoking							
No smokers	28	1.717	0.308	0.097	0.127	0.091	0.147
Current/former smokers	88	2.156		0.139		0.175	

*not all data available for all patients; $unpaired *t*-test.

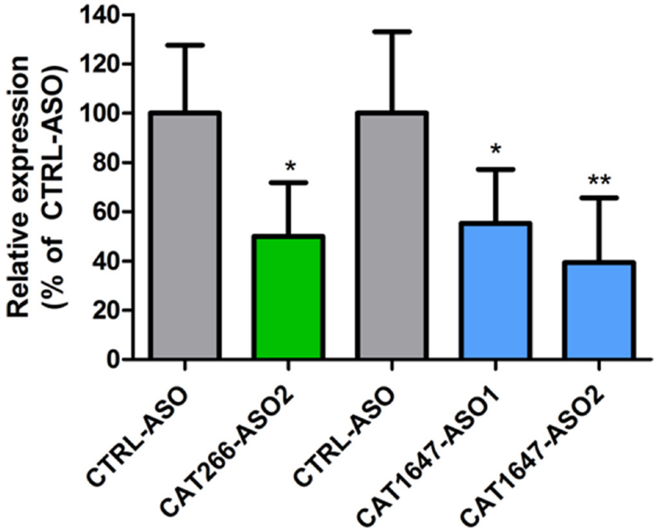
Supplementary Table 6: Univariate and multivariate analysis of prognostic factors in MIBC for overall survival

Prognostic factor	Univariate analysis			Multivariate analysis		
	Exp (B)	**95% CI**	***P* value**	**Exp (B)**	**95% CI**	***P* value**
CAT1297 expression	0.487	0.292–0.812	0.006	0.508	0.284–0.909	0.022
Age	1.027	1.002–1.053	0.036	1.034	1.004–1.064	0.026
Gender	0.967	0.553–1.691	0.906	0.719	0.374–1.380	0.321
Stage	1.952	1.242–3.067	0.004	1.975	1.188–3.285	0.009
Lymph node metastasis*	1.725	1.020–2.919	0.042	1.434	0.374–2.478	0.197

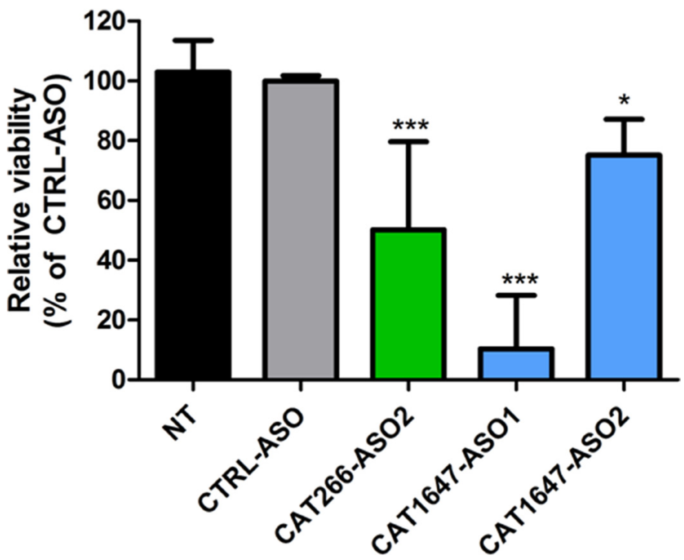
*At the time of diagnosis, CI-confidence intervals.



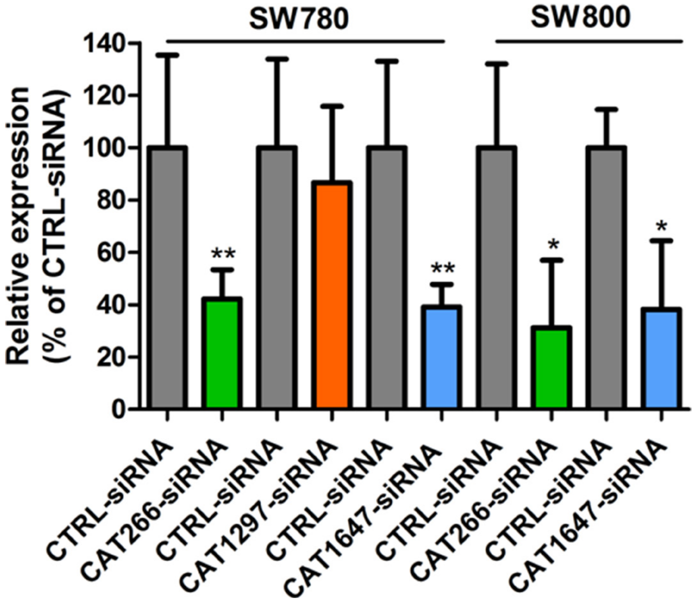
Supplementary Figure 1: Expression of *CAT266, CAT1297* and *CAT1647* in normal bladder (NB) and MIBC tumors divided into TCGA clusters (TCGA 1-4; unpaired *t* -test, *$p < 0.05$; **$p < 0.01$, ***$p < 0.001$; lines represent the median).



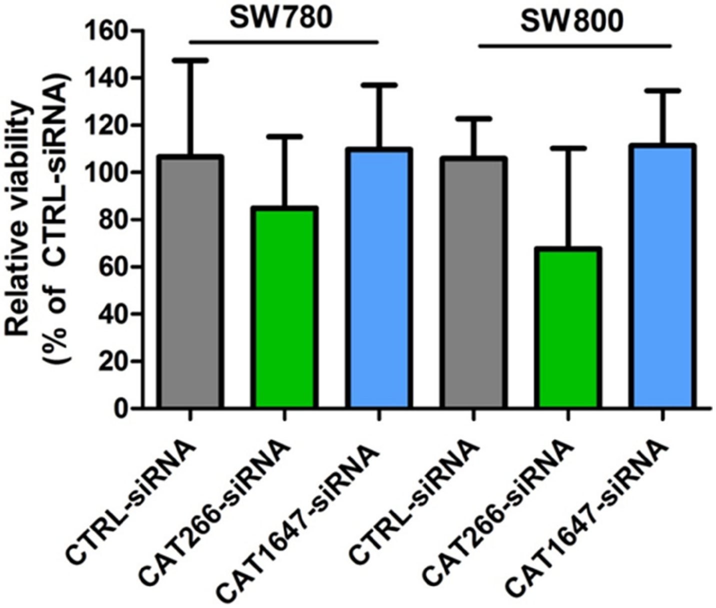
Supplementary Figure 2: Knockdown of lncRNAs expression in SW800 bladder cancer cell line (expressing *CAT266* and *CAT1647*) using ASOs. All data are compared to control ASO-transfected cells. (*t*-test, *$p < 0.05$, ***$p < 0.001$, bars represent the mean ± SD).



Supplementary Figure 3: The effect of *CAT266* and *CAT1647* ASO-mediated knockdown in SW800 cells on cell viability. All data are compared to control ASO-transfected cells. (*t*-test, *$p < 0.05$, ***$p < 0.001$, bars represent the mean ± SD).



Supplementary Figure 4: Knockdown of lncRNAs expression using siRNAs targeting *CAT266* (CAT266-siRNA), *CAT1297* (CAT1297-siRNA1 and CAT1297-siRNA2 mix) and CAT1647 (CAT1647-siRNA1 and CAT1647-siRNA2 mix) in SW780 and SW800 bladder cancer cell lines. All data are compared to control siRNA-transfected cells (*t*-test, *$p < 0.05$; **$p < 0.01$, bars represent the mean ± SD).



Supplementary Figure 5: The effect of *CAT266* (CAT266-siRNA1) and *CAT1647* (CAT1647-siRNA1 and CAT1647-siRNA2 mix) siRNA-mediated knockdown on cell viability in SW780 and SW800 bladder cancer cell lines. All data are compared to control siRNA-transfected cells (*t* -test, bars represent the mean ± SD).