

Supplementary information:

Downregulation of miR-320a/383-sponge-like long non-coding RNA NLC1-C (narcolepsy candidate-region 1 genes) is associated with male infertility and promotes testicular embryonal carcinoma cell proliferation

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Supplementary Figure S1

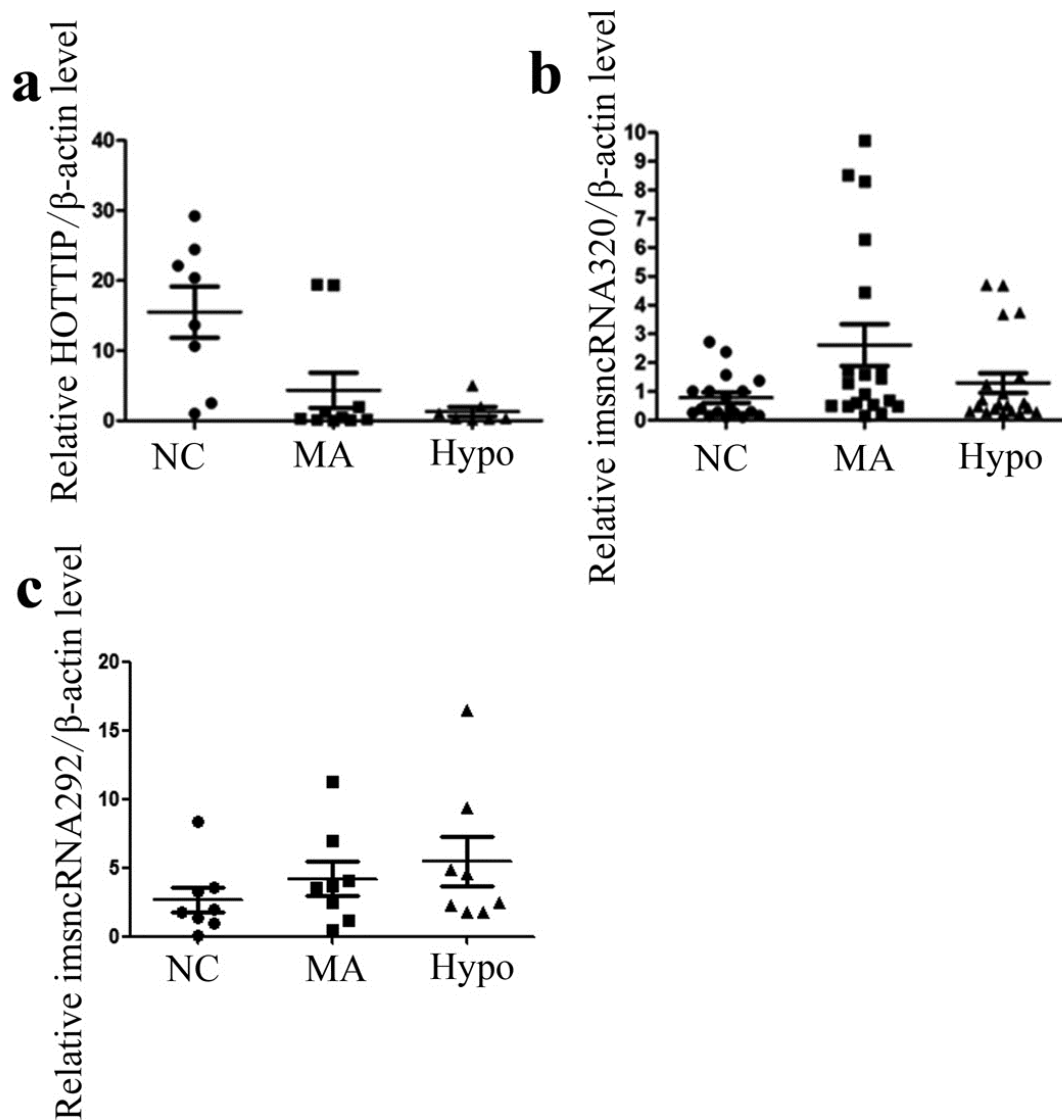


Figure S1. Confirmation of microarray data by quantitative real-time PCR. Quantitative real-time PCR analysis confirmed microarray data: lncRNA HOTTIP was down-regulated and imsnRNA320 and imsnRNA292 was up-regulated in NOA (including MA and Hypo) patients. NOA : non-obstructive azoospermia; MA: maturation arrest; Hypo: Hypospermatogenesis. Error bars indicate the SEM.

Supplementary Figure S2

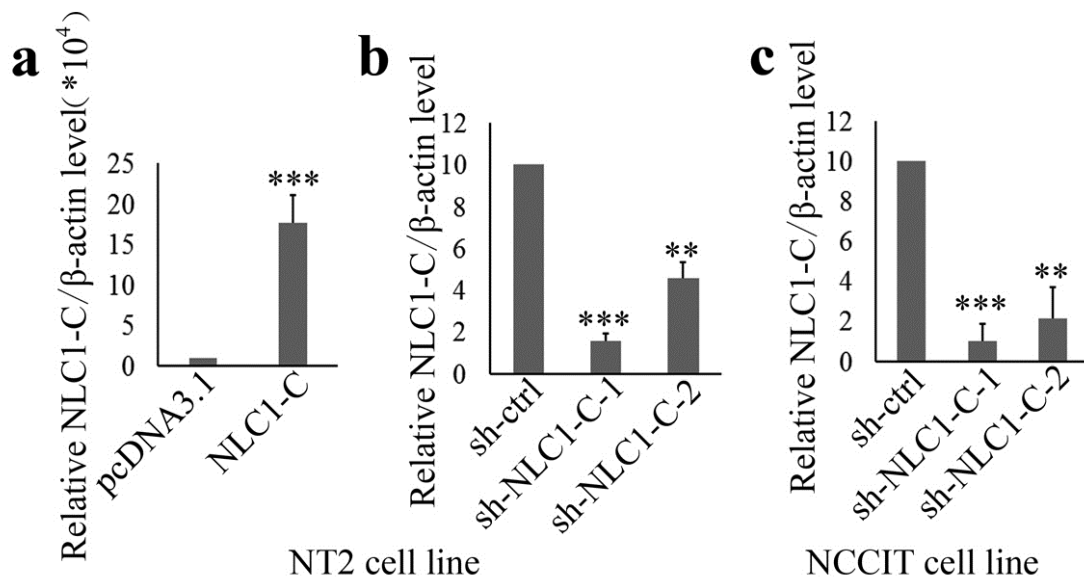


Figure S2. Real-time PCR analysis of NLC1-C expression in NT2 and NCCIT cells. a.

Real-time PCR analysis of NLC1-C expression in NT2 cells when transfected with pcDNA3.1 or pcDNA3.1-NLC1-C (NLC1-C) for 48h. b and c. Real-time PCR analysis of NLC1-C expression in NT2 (b) and NCCIT (c) cells when infected with control shRNA or NLC1-C-1/2 shRNA lentiviral particles culture for 48h.

Supplementary Figure S3

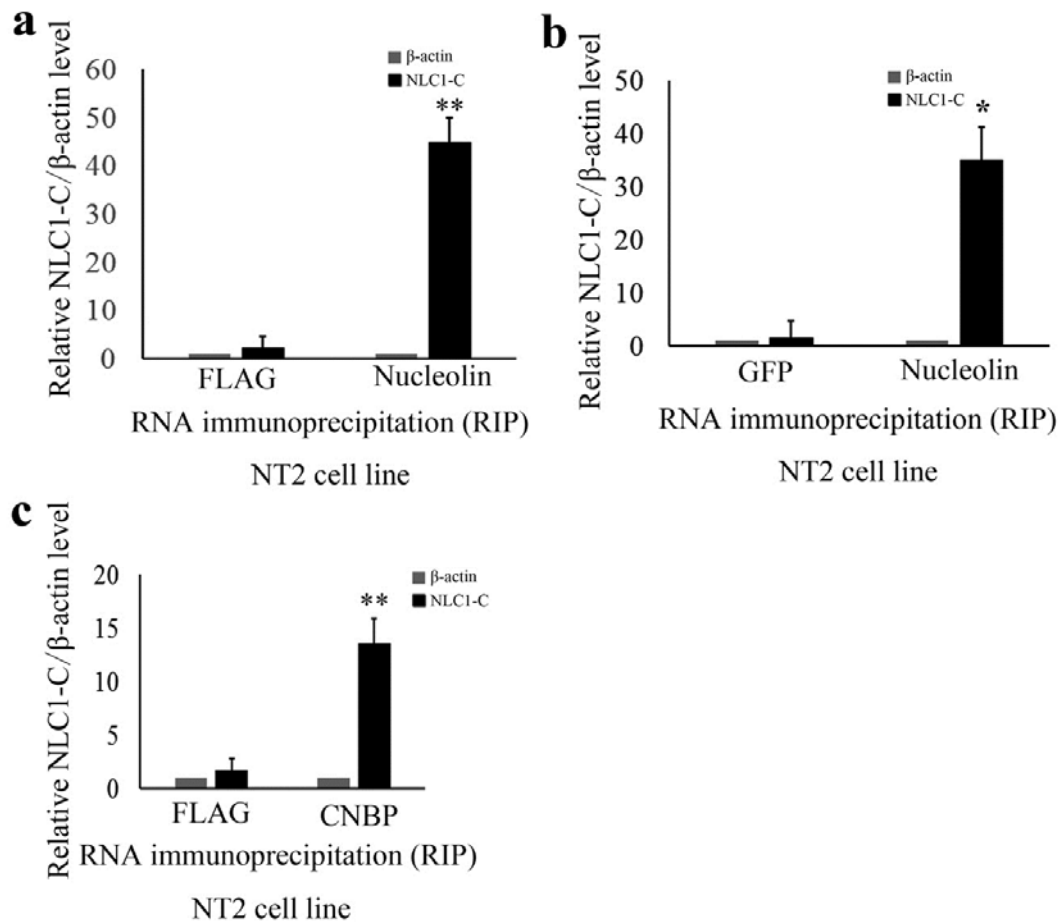


Figure S3. Nucleolin IP and CNBP IP specifically retrieves NLC1-C RNA in vitro.

a, b and c. The indicated plasmids were transfected into NT2 cells for 48 h. Lysates were prepared from these cells. RNAs that were co-precipitated with FLAG, GFP were detected by qRT-PCR using primers for NLC1-C and β -actin (as negative control). IP enrichment was determined as the amount of RNA associated to the various proteins. Data are from one of three independent experiments and are represented as mean \pm s.d. with n=3. Data (mean \pm s.d, n = 3 replicates) is relative to mock-IP (FLAG or GFP)

Supplementary Figure S4

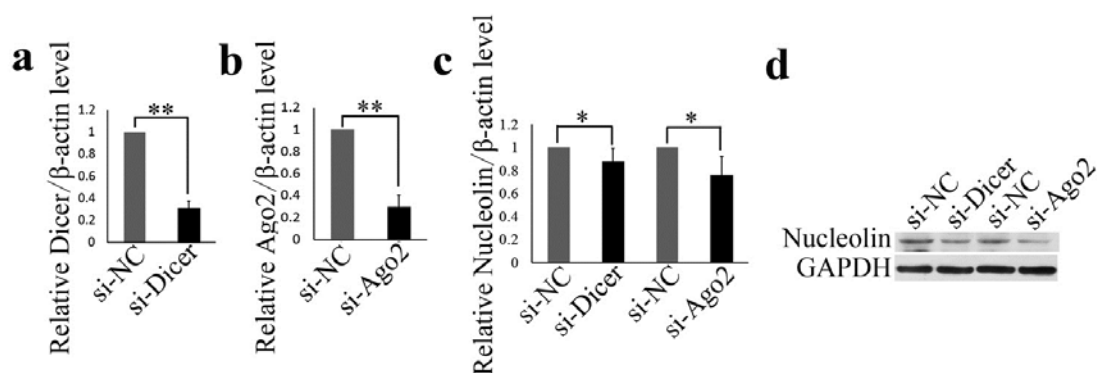


Figure S4. Knockdown of Dicer and Ago2 decreased Nucleolin expression. a, b ,c and d. NT2 cells were transfected with control siRNA (si-NC) or Dicer or Ago2 siRNAs separately. The expression of Dicer(a), Ago2(b), Nucleolin(c and d) were detected by quantitative real-time PCR and western blot after transfection for 48 h. GAPDH was used as internal reference. Data are represented as mean±s.d. from three independent experiments. *P<0.05 or **P<0.01 compared with control RNA.

Supplementary Figure S5

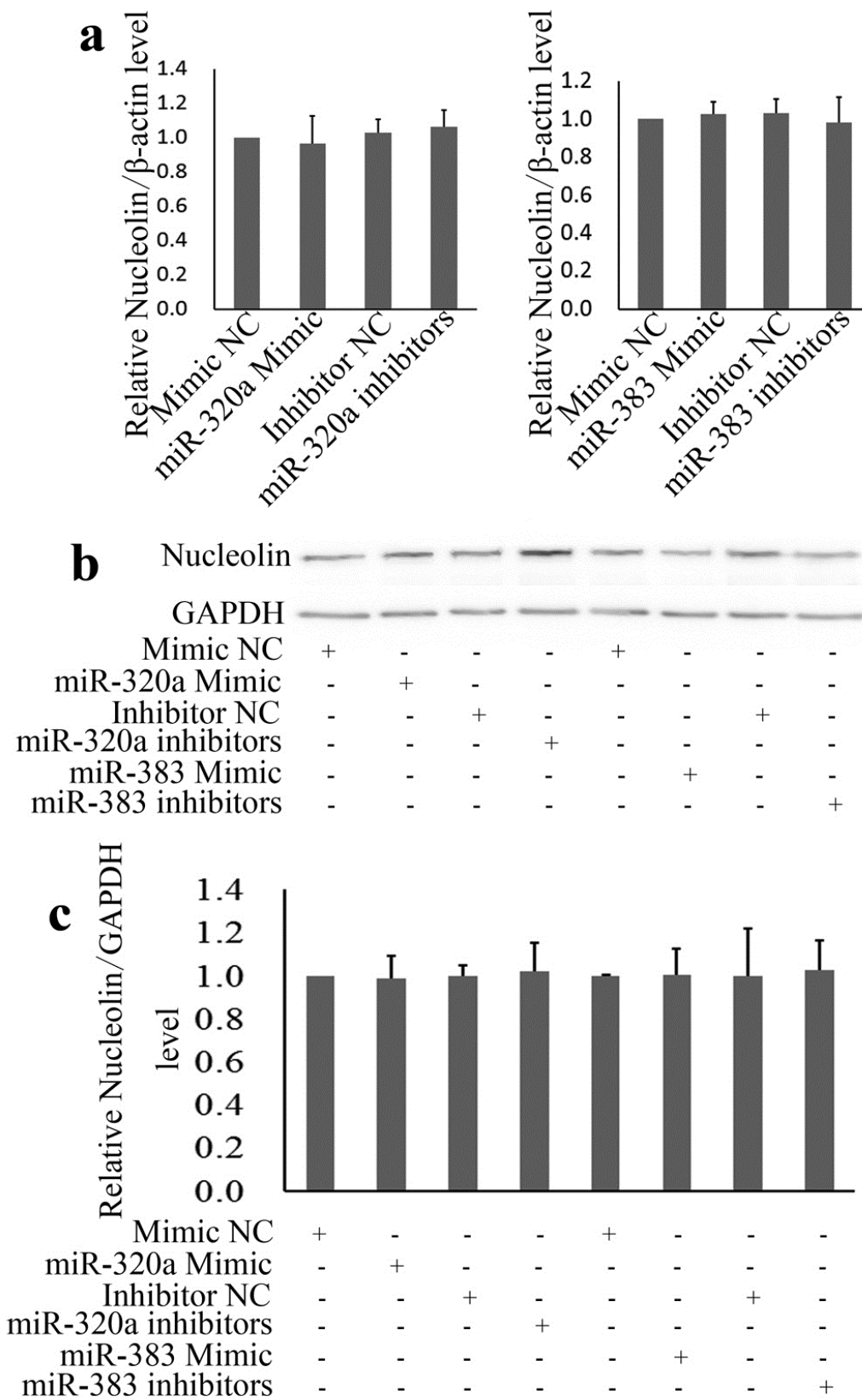


Figure S5. Nucleolin was not the target of miR-320a and miR-383. a and b. Quantitative

real-time PCR(a) and western blot(b)analysis of Nucleolin expression after NT2 cells were transfected with indicated miRNAs for 48 h and the corresponding densitometric analysis (c).

Data are represented as mean \pm s.d. from three independent experiments.

Supplementary Table S1. List of primer pairs for construction of vectors.

Name of gene	Sequence (5' to 3')	Annealing temperature (°C)
NLC1-C-pcDNA3.1	Forward:ATTAGGATCCTGAAGTGCTGACGGGTGGAGCGA Reward:CGCTGCGGCCGCTTCCCACTGACTTGTATATTT	58
NLC1-C (ISH, sense)	Forward:ACTGAAGCTTCTCTATGACCTCATCCATGGGAAA Reward:AGCTGAATTCTCCCACTGACTTGTATATTTTCA	65
NLC1-C (ISH, antisense)	Forward:ACTGAAGCTTTGGAGCCTCATTCACTGTGAAGT Reward:AGCTGAATTCTCTATGACCTCATCCATGGGAAA	64
NLC1-C (Pulldown)	Forward:ACTAAAGCTTTGAAGTGCTGACGGGTGGAGCGA Reward:ACGAATCTTTCCCACTGACTTGTATATTTTCA	60
NLC1-C (miR-320a binding site mutation)	Forward:ATGGAAGAGTGCTAACTATAGGTGGGGAGCACATGTGGTGAAGAT Reward:TAGTTAGACTCTTCCATCTGTGGGGTCTGCCCTACT	65
NLC1-C (miR-383 binding site mutation)	Forward:TTGACATACGAGAGACTAGATCAGTCACTTCACAGTGAATGAGGCT Reward:ATCTAGTCTCTCGTATGTCAACCTGCTCCTTCAGGTCCCTCT	64
NLC1-C-pZW1-snovector	Forward: ATTGGAATTCTGAAGTGCTGACGGGTGGAG Reward: ACGGGGTACCTTCCCACTGACTTGTATAT	62
Nucleolin-FLAG	Forward:AGCTGAATTCATGGTGAAGCTCGCGAAGGCAGGTAATAAAA Reward: TGCAGTCGACCTATTCAAACCTTCGTCTTCTTTCTTGT	58

Supplementary Table S2. List of primer pairs for real-time PCR and PCR.

Name of gene	Forward primer sequence (5' to 3')	Reverse primer sequence (5' to 3')
β-actin(human)	TGGCACCCAGCACAATGAA	CTAAGTCATAGCCGCTAGAA
NLC1-C	TGCTTGACTTTCAAGAGGTAA	GCTCTCAGTCAGCAGACTT
Nucleolin	CGTTCGGGCAAGGATAGTTAC	GGCATCCTCCTCACTGTTGAAG
Ims320	ATTTGAGGTATAAAAATTATAACTAT	TTATAAGTCATTCTGTCACCTTCTG
HOTTIP	CCTAAAGCCACGCTTCTTTG	TGCAGGCTGGAGATCCTACT
pri-miR-383	CTTTCCAAGAGTTTCACT	CCACTCCAGTCCACCAAAT
pri-miR-320a	TGGGCCACAGTATTTATCAGGCGGCGCTT	AGCGCCGAGCATAGCGTAGCCAGACCA
miR-320 promoter	CACAGAGCCTCGAATGCCATACCTAGGATT CATC	ACCTCATCCTTTTTCGCCCTCTCAACCCAGC TTT
SGCZ promoter	AAACTATAAAGAAGTAGGGGGAGAGACAG CTGTC	CAGTGGGAAAAGAAGATAATCAAGTCAGA AGGG

Supplementary Table S3. Sequence of oligonucleotides.

Oligonucleotides	Sequence (5' to 3')
Inhibitor NC	CAGUACUUUUGUGUAGUACAA
has-miR-320a inhibitors	UCGCCCUCUCAACCCAGCUUUU
has-miR-383 inhibitors	AGCCACAAUCACCUUCUGAUCU
Mimics NC	UUCUCCGAACGUGUCACGUTT ACGUGACACGUUCGGAGAATT
hsa-miR-320a mimics	AAAAGCUGGGUUGAGAGGGCGA GCCUCUCAACCCAGCUUUUUU
hsa-miR-383 mimics	AGAUCAGAAGGUGAUUGUGGCU CCACAAUCACCUUCUGAUCUUU
LNA miR-320	TCGCCCTCTCAACCCAGCTTTT
LNA miR-383	AGCCACAATCACCTTCTGATCT
NLC1-C-PLKO.1-1	GCTGGCTCACCTGGCACTGCA
NLC1-C-PLKO.1-2	GACCGTCCCTGGCCGCAAGTG
NLC1-C (RNA-FISH)-sense	UCAAGAGGUAUUUCCGAGCUCUGCUCUGAGGCCU
NLC1-C (RNA-FISH)-antisense	AGGCCUCAGAGCAGAGCUCGGAAUUUACCUUUGA

Supplementary Table S4. List of primary antibodies and secondary antibodies.

Antigen	WB dilutions	Antibody name	Catalog number	Company
GAPDH	1:1000	GAPDH (14C10) Rabbit mAb	#2118	cell signaling technology
Nucleolin	1:1000	Rb-pAb to Nucleolin	ab70493	abcam
c-Myc	1:200	c-Myc Rabbit Ab	#9402	cell signaling technology
Rabbit IgG secondary antibody	1:6000	Rabbit IgG secondary antibody - H&L	ab6802	abcam
Mouse IgG secondary antibody	1:6000	Mouse IgG secondary antibody - H&L	ab6819	Abcam
PARP	1:200	PARP (46D11) Rabbit mAb	#9532S	cell signaling technology
Caspase-3	1:6000	Caspase-3 (8G10) Rabbit mAb	#9665S	cell signaling technology
Caspase-8	1:6000	Caspase-8 (D35G2) Rabbit mAb	#4790S	cell signaling technology
Caspase-9	1:6000	Cleaved Caspase-9 (Asp330) (D2D4) Rabbit mAb	#7237S	cell signaling technology
Anti-Digaoxigenin -Fluorescein, Fab fragments	1:250	Anti-Digaoxigenin -Fluorescein, Fab fragments	11207741910	Roche