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Supplemental Information

lnc-MAP3K13-7:1 Inhibits Ovarian GC Proliferation in PCOS via DNMT1 Downregulation-Mediated CDKN1A Promoter Hypomethylation

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Supplemental Figures and legends

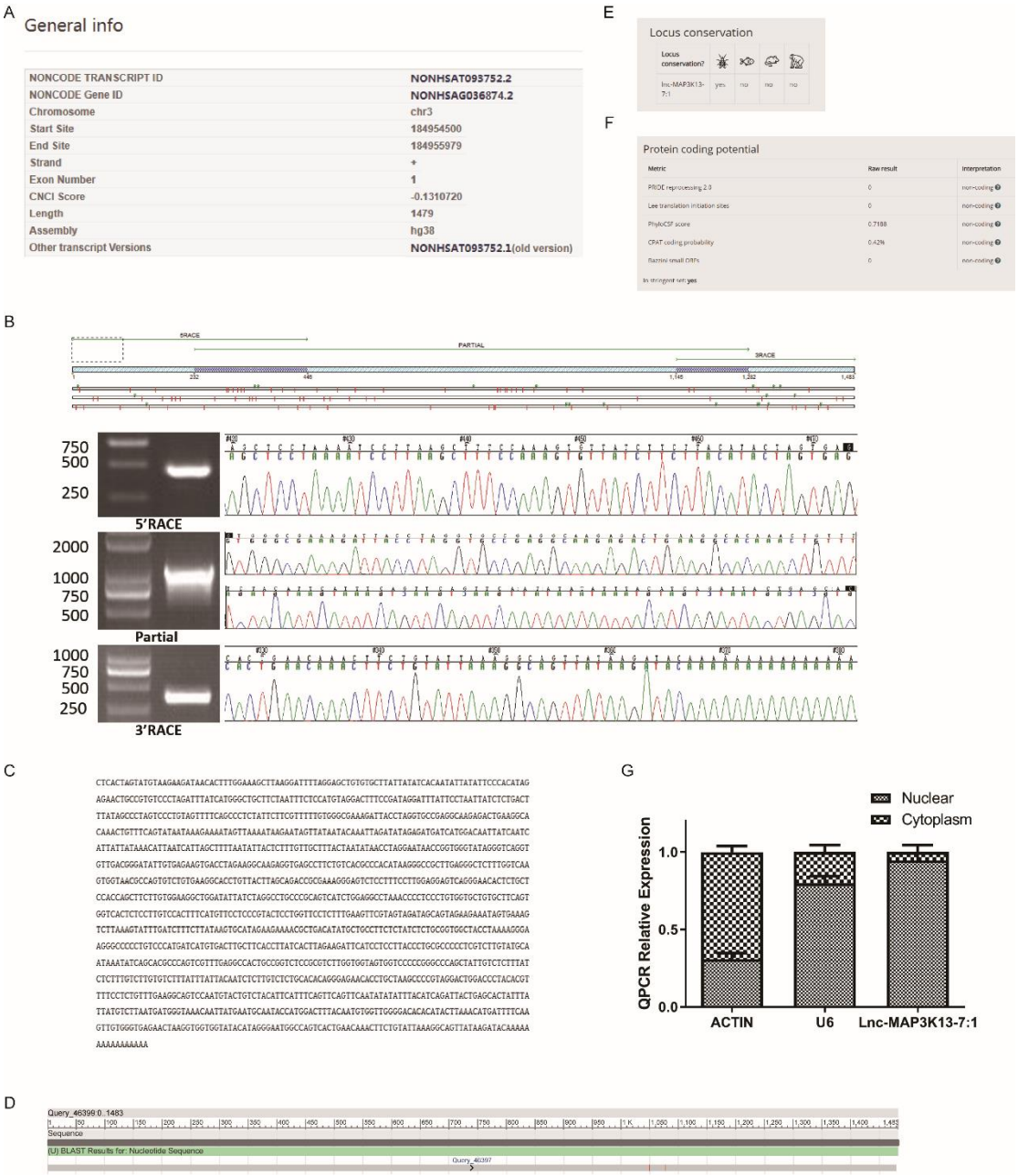

Figure S1. Characterization of lnc-MAP3K13-7:1

(A) General information of lnc-MAP3K13-7:1. Lnc-MAP3K13-7:1 is on chromosome 3, and is the transcription of the forward strand.

(B) Full‐length sequence of lnc-MAP3K13-7:1 was determined by 5′ and 3′ rapid amplification of cDNA ends (RACE) assays. Top, Schematic diagram of how the RACE fragments are assembled in the contig of lnc-MAP3K13-7:1. Left, gel electrophoresis images of PCR products from the 5′-RACE and 3′-RACE assays. Right, chromatograms of part of PCR products sequences, indicated the boundary between the primers and lnc-MAP3K13-7:1 sequences.

(C) The full‐length sequence of lnc-MAP3K13-7:1 transcript was 1483 nucleotide.

(D) BLAST results for the sequence of lnc-MAP3K13-7:1 transcript. Green, sequence from RACE. Grey, reference sequence from NONCODE. Red vertical bar, the mismatches between the two sequences, one bar indicates one nucleotide mismatch.

(E, F) Lnc-MAP3K13-7:1 has high evolutionary conservation in humans and fruit flies. The protein coding potential of lnc-MAP3K13-7:1 under several metrics, indicates that lnc-MAP3K13-7:1 is a non-coding RNA.

(G) Quantitative RT-PCR analysis of lnc-MAP3K13-7:1 expression radio in nuclear and cytoplasm in untreated KGN cells using RNA cellular fractionation assay. ACTIN and U6 were used as cytoplasm RNA control and nuclear RNA control, respectively (n=4, mean ± SD).

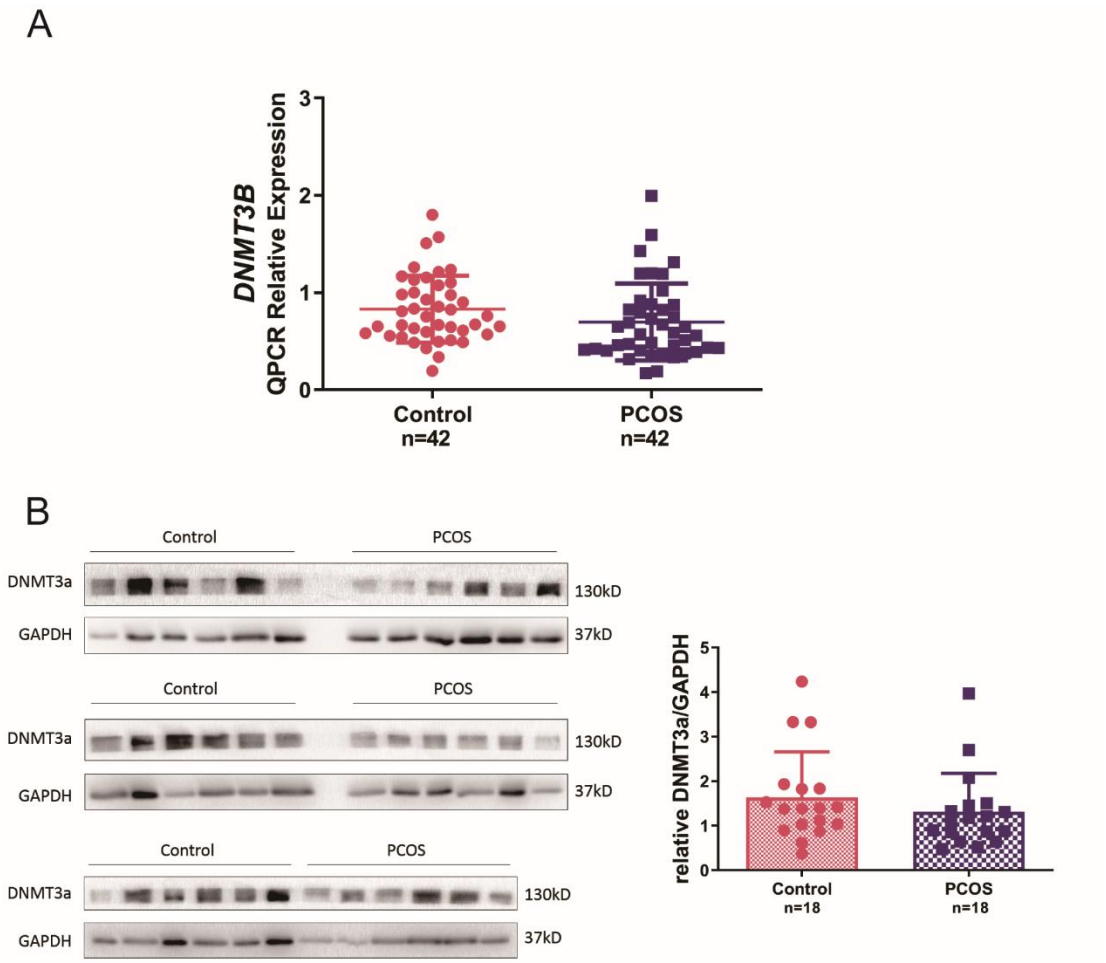

Figure S2. Expression of DNMT3A and DNMT3B in granulosa cells of patients with PCOS

(A) Quantitative RT-PCR analysis of *DNMT3B* expression in GCs of 42 PCOS patients and 42 normal controls which is the same cohort with Figure 1A (*P*=0.1072).

(B) Western blot analysis of DNMT3A protein expression in GCs of 18 PCOS and 18 normal controls which is the same cohort with Figure 1E (*P*=0.3059).

Data information: In (A, B), data are presented as mean ± SD. Student's *t*-test.

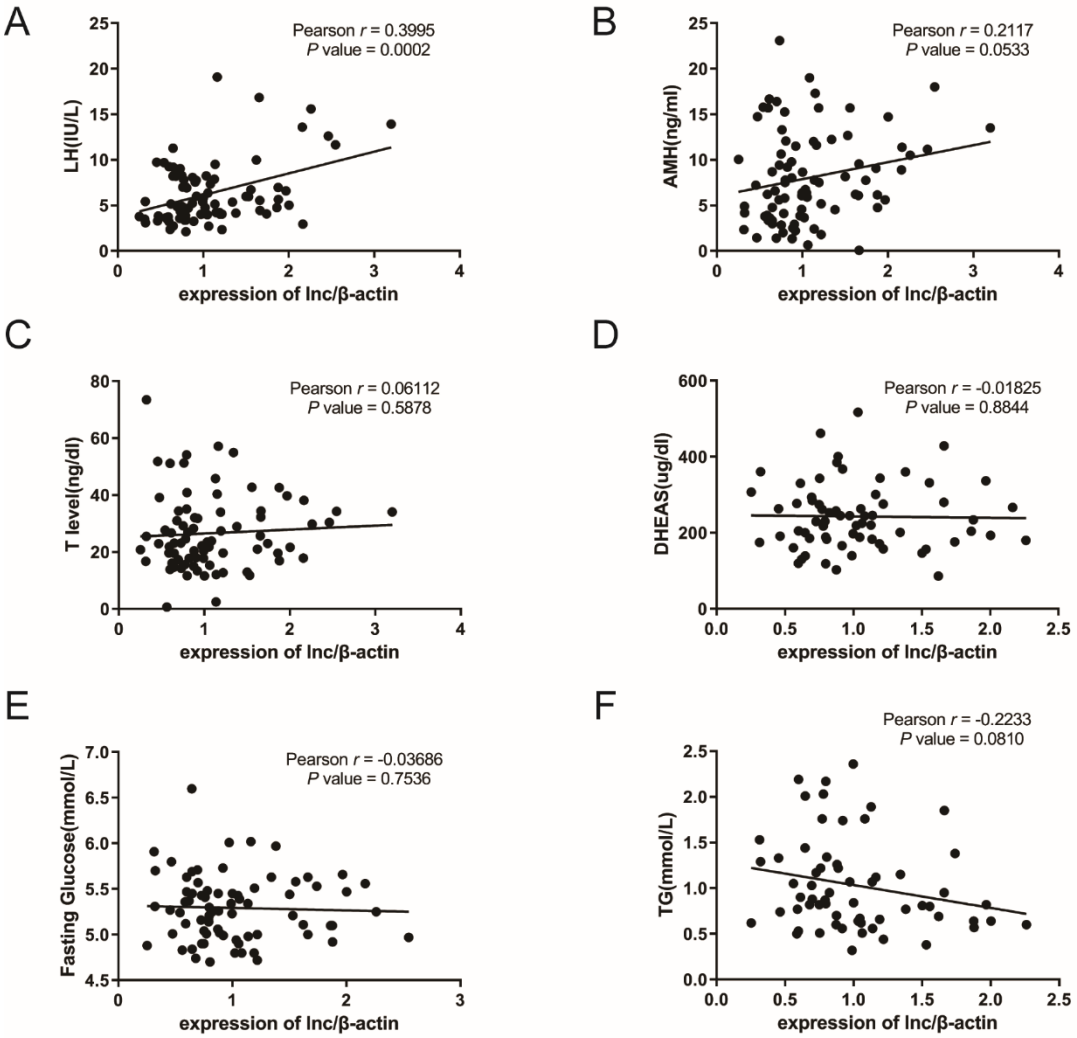

Figure S3. The correlation between the clinical indicators and the expression level of lnc-MAP3K13-7:1 in PCOS and controls

(A) The association between the expression of lnc-MAP3K13-7:1 and serum LH levels in 42 PCOS patients and 42 normal controls. The expression level was detected via Quantitative RT-PCR.

(B) The association between the expression of lnc-MAP3K13-7:1 and serum AMH levels in 42 PCOS patients and 42 normal controls.

(C) The association between the expression of lnc-MAP3K13-7:1 and serum T levels in 42 PCOS patients and 42 normal controls.

(D) The association between the expression of lnc-MAP3K13-7:1 and serum DHEAS levels in 42 PCOS patients and 42 normal controls.

(E) The association between the expression of lnc-MAP3K13-7:1 and serum fasting glucose levels in 42 PCOS patients and 42 normal controls.

(F) The association between the expression of lnc-MAP3K13-7:1 and serum TG levels in 42 PCOS patients and 42 normal controls.

Data information: In (A–F), Pearson *r* and *P* value was shown on the image (Pearson correlation coefficient). LH: luteinizing hormone, AMH: anti-Müllerian hormone, T: testosterone, DHEAS: dehydroepiandrosterone sulfate, TG: triglyceride.

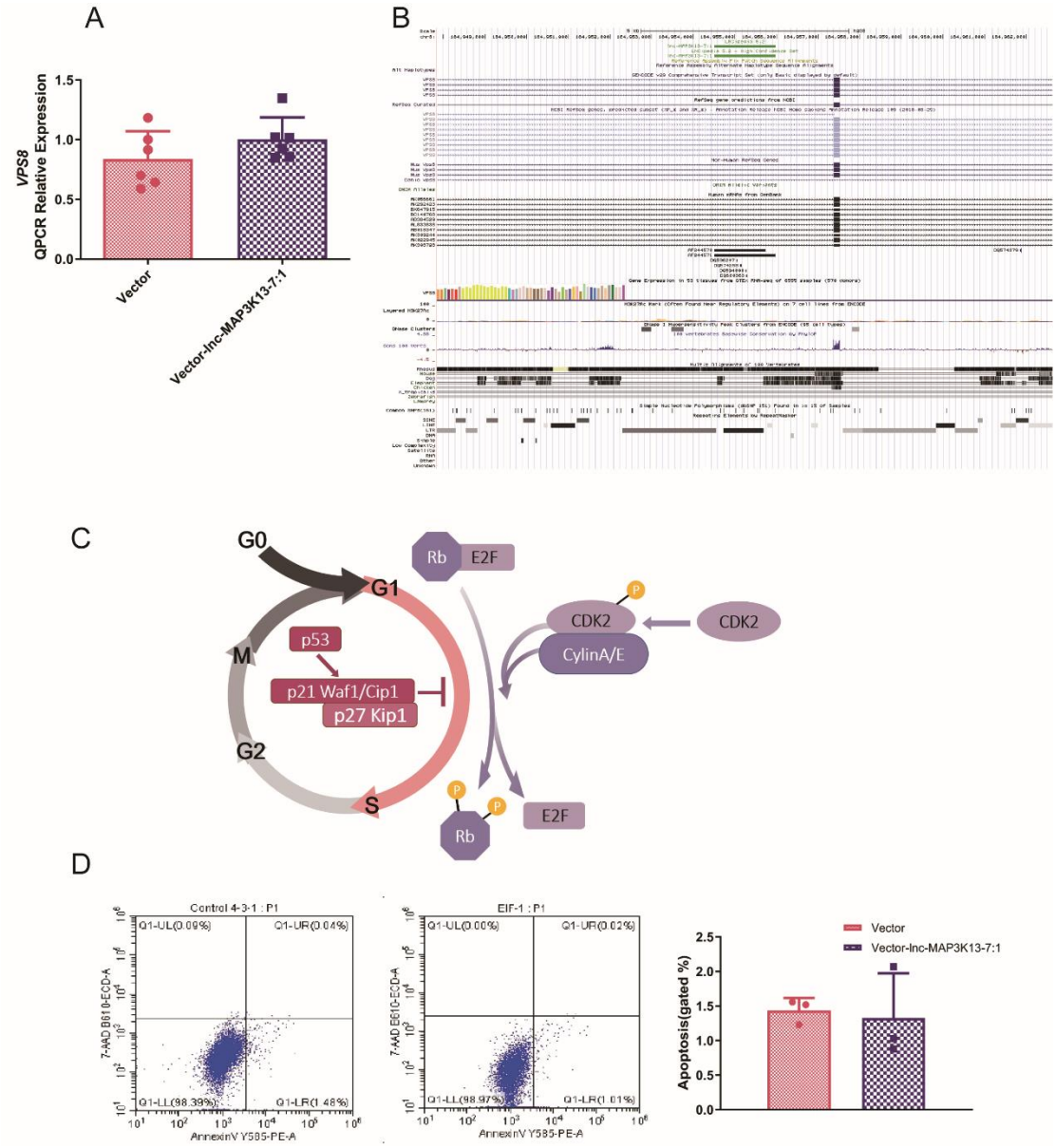

Figure S4. Overexpression of lnc-MAP3K13-7:1 did not affect *VPS8* expression and cell apoptosis

(A) Quantitative RT-PCR analysis of *VPS8* after transfection with lnc-MAP3K13-7:1 lentivirus for 72h (n=6, *P*=0.2052).

(B) Relative position of VPS8 gene and lnc-MAP3K13-7:1 shown in UCSC Genome Browser (http://genome.ucsc.edu/).

(C) The major regulators of the G1-to-S phase transition. In early G1, retinoblastoma protein (Rb) physically binds with E2F and blocks cell cycle progression. CDK2 can be activated by phosphorylation, after which it binds to cyclin A or cyclin E. Phosphorylation of Rb by CDK2-cyclin A/E complexes releases p-Rb from E2F, allowing the transcription of genes that enables cells to progression from G1 phase to S phase. In addition, p53-dependent p21 (Waf1/Cip1) and p53-independent and p27 (Kip1) induce cell cycle arrest by binding to cyclin-CDK complexes and inhibiting their catalytic activity.

(D) Annexin V-7AAD flow cytometry experiment of transfected vector or vector-lnc-MAP3K13-7:1 KGN cells. The KGN cells basically did not undergo apoptosis after lnc-MAP3K13-7:1 overexpression.

Data information: In (A, D), mean ± SD. Student's *t*-test.

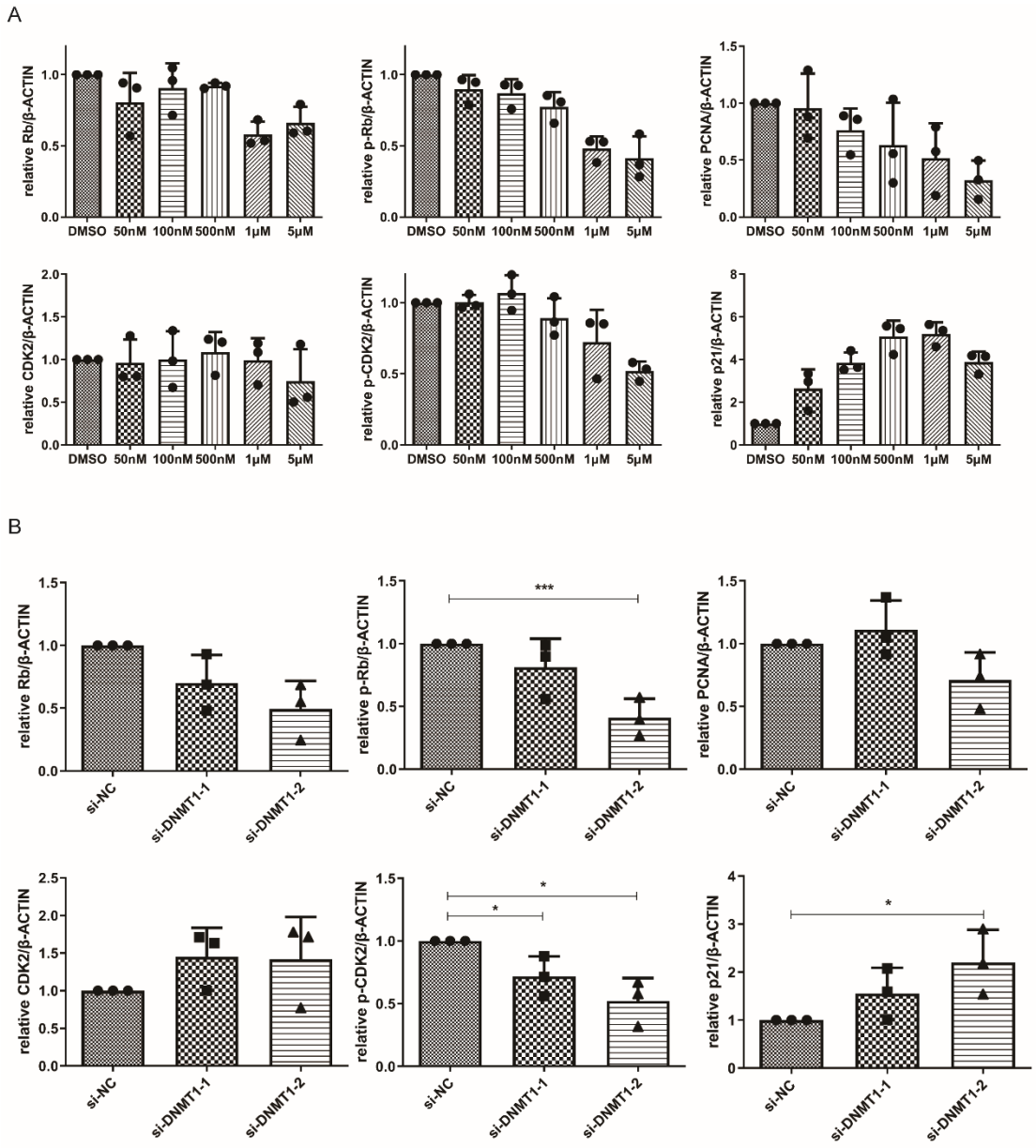

Figure S5. The quantifications of western blot analysis in Figure 3D, 3H

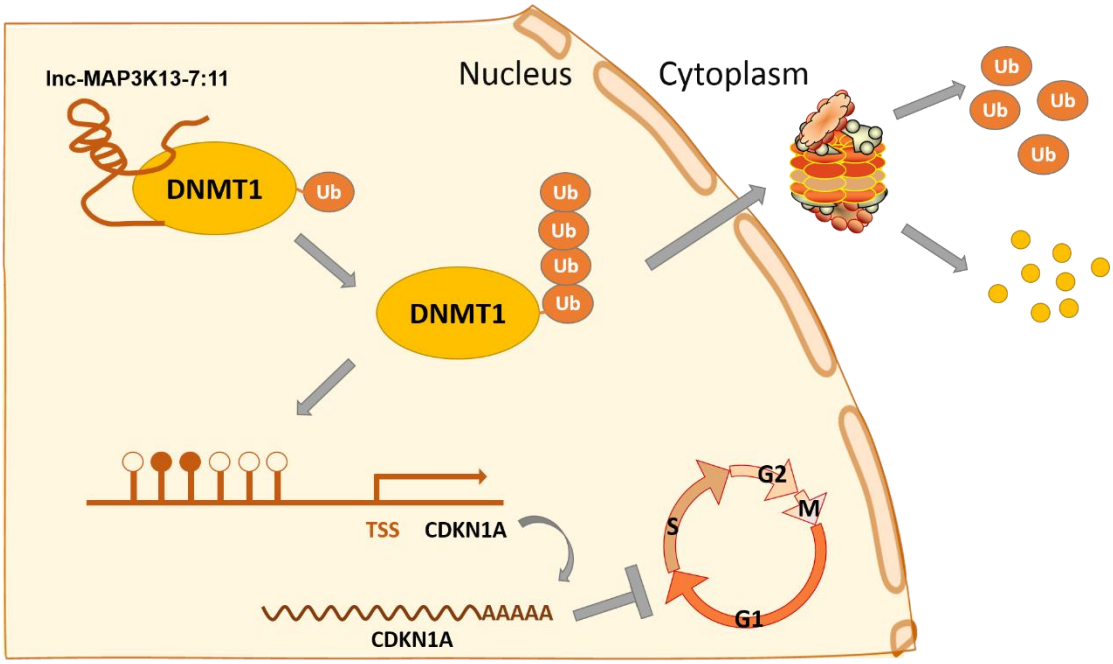

Figure S6. A proposed model illustrating regulation of CDKN1A expression by lnc-MAP3K13-7:1‐ dependent DNMT1 ubiquitination.

Supplemental Tables

Table S1 Clinical and biochemical indicators of women with and without PCOS involved in RT-PCR

	PCOS（n=42）	Control（n=42）	P value
Age（years）	28.64±2.87	29.07±2.65	0.4794
BMI（kg/m^2）	22.87±2.92	22.62±3.23	0.7181
Basal FSH（IU/L）	5.99±1.39	6.34±1.49	0.2772
Basal LH（IU/L）	7.71±3.94	4.94±1.93	< 0.0001
LH/FSH	1.31±0.71	0.79±0.29	< 0.0001
T（ng/dL）	33.84±14.25	19.96±7.45	< 0.0001
AMH（ng/mL）	11.13±4.47	4.95±3.30	< 0.0001
Fasting blood-glucose（mmol/L）	5.33±0.44	5.29±0.34	0.7048
HOMA-IR	2.86±1.34		

Data are presented as mean±SD, BMI: Body mass index, LH: luteinizing hormone, FSH: follicle stimulating hormone, T: testosterone, AMH: anti-Müllerian hormone, HOMA-IR: Fasting blood-glucose (mmol/L)*fasting insulin (uU/ml)/22.5.

Table S2 Clinical and biochemical indicators of women with and without PCOS involved in western blot assay

	PCOS（n=15）	Control（n=15）	P value
Age（years）	28.40±3.46	29.47±3.00	0.3745
BMI（kg/m^2）	24.05±2.71	22.32±2.55	0.0814
Basal FSH（IU/L）	5.68±0.80	6.36±1.01	0.0768
Basal LH（IU/L）	8.71±4.46	5.26±1.38	0.0079
LH/FSH	1.52±0.70	0.87±0.29	0.0022
T（ng/dL）	33.71±14.22	16.85±6.85	0.0003
AMH（ng/mL）	8.81±4.93	2.22±0.63	< 0.0001
Fasting blood-glucose（mmol/L）	5.58±0.92	5.26±0.43	0.2268
HOMA-IR	4.75±2.53		

Data are presented as mean±SD, BMI: Body mass index, LH: luteinizing hormone, FSH: follicle stimulating hormone, T: testosterone, AMH: anti-Müllerian hormone, HOMA-IR: Fasting blood-glucose (mmol/L)*fasting insulin (uU/ml)/22.5.

Table S3 Clinical and biochemical indicators of women with and without PCOS involved in Methylated DNA Quantification Kit

	PCOS（n=25）	Control（n=25）	P value
Age（years）	28.44±3.45	28.92±3.23	0.6139
BMI（kg/m^2）	24.13±3.22	22.94±3.37	0.2093
Basal FSH（IU/L）	5.49±1.20	6.15±1.56	0.0993
Basal LH（IU/L）	7.64±4.00	4.90±1.92	0.0033
LH/FSH	1.42±0.73	0.80±0.28	0.0003
T（ng/dL）	40.81±15.33	18.98±6.74	< 0.0001
AMH（ng/mL）	10.96±4.75	4.78±3.20	< 0.0001
Fasting blood-glucose（mmol/L）	5.46±0.65	5.34±0.31	0.4399
HOMA-IR	3.61±2.80		

Data are presented as mean±SD, BMI: Body mass index, LH: luteinizing hormone, FSH: follicle stimulating hormone, T: testosterone, AMH: anti-Müllerian hormone, HOMA-IR: Fasting blood-glucose (mmol/L)*fasting insulin (uU/ml)/22.5.

Table S4 Primers used in 5’ and 3’ RACE analysis

Oligo	Sequence 5’-3’
5’lnc-MAP3K13-R1	GGCTCACCTCTTGCCTTCTAGGTCACT
5’lnc-MAP3K13-R2	CCACCGGTTATTCCTAGGTTATATTAG
3’lnc-MAP3K13-F1	GGCCCAGCTATTGTCTCTTTATCTCTTTGTC
3’lnc-MAP3K13-F2	CTGCACACAGGGAGAACACCTGCTAAG
mlnc-MAP3K13-F	GTGGGCGAAAGATTACCTAG
mlnc-MAP3K13-R	GTGCTCAGTAATCTGATGTAAAT

5’lnc-MAP3K13-R, reverse primer for gene specific primer of 5’RACE; 3’lnc-MAP3K13-F, forward primer for gene specific primer of 3’RACE; UPM, universal primer mix; mlnc-MAP3K13-F, forward primer for full length of lnc-MAP3K13-7:1; mlnc-MAP3K13-R, reverse primer for full length of lnc-MAP3K13-7:1.

Table S5 siRNA sequences used in cell knockdown assay

Oligo Name	Sequence（5'-3'）		position
si-DNMT1-1	sense	GUCCCAAUAUGGCCAUGAAdTdT	2298
	antisense	UUCAUGGCCAUAUUGGGACdTdT	
si-DNMT1-2	sense	CCACAGAUUUCUGAUGAAAdTdT	1151
	antisense	UUUCAUCAGAAAUCUGUGGdTdT	
si-DNMT1-3	sense	GAGGCCUAUAAUGCAAAGAdTdT	3485
	antisense	UCUUUGCAUUAUAGGCCUCdTdT	

Table S6 Primer sequences and amplification conditions used in RT-PCR analysis

Target genes	Primer sequences(5’ → 3’)	Amplification condition
lnc-MAP3K13-7:1	F:5’-AAATATCAGCACGCCCAGTC-3’	
	R:5’-TGTAGGGTCCAGTCCTACGG-3’	
lnc-MAP3K13-7:1	F:5’-GCAAGAGGTGAGCCTTCTGT-3’	
	R:5’-AAGCTGGTGGAGCAGAGTGT-3’	
VPS8	F:5’-GCTGAGGAGCTGGAGAGAGA-3’	
	R:5’-CTGAAAATGCTGCTCCATCA-3’	
NEAT1	F:5’-CTTCCTCCCTTTAACTTATCCATTCAC-3’	
	R:5’-CTCTTCCTCCACCATTACCAACAATAC-3’	Stage 1: 95 ℃, 10s
UI	F:5’-GGGAGATACCATGATCACGAAGGT-3’	Stage 2: 95 ℃, 10s 60 ℃, 20s 72 ℃, 20s
	R:5’-CCACAAATTATGCAGTCGAGTTTCCC-3’	Number of cycles: 40
ACTB	F:5’-CTCCATCCTGGCCTCGCTGT-3’	Stage 3: 95 ℃, 5s
	R:5’-GCTGTCACCTTCACCGTTCC-3’	65 ℃, 1min
DNMT1	F:5’-GTGGGGGACTGTGTCTCTGT-3’	4°C, ∞
	R:5’-TGAAAGCTGCATGTCCTCAC-3’	
DNMT3A	F:5’-AGCCCAAGGTCAAGGAGATT-3’	
	R:5’-CAGCAGATGGTGCAGTAGGA-3’	
DNMT3B	F:5’-TTGAATATGAAGCCCCCAAG-3’	
	R:5’-GGTTCCAACAGCAATGGACT-3’	
CDKN1A	F:5’-AAGACCATGTGGACCTGTCACTGT-3’	
	R:5’-AGGGCTTCCTCTTGGAGAAGATCA-3’	
TP53	F:5’-GTTCCGAGAGCTGAATGAGG-3’	
	R:5’-TCTGAGTCAGGCCCTTCTGT-3’	
CDKN1B	F:5’-ATGTCAAACGTGCGAGTGTC-3’	
	R:5’-TCTCTGCAGTGCTTCTCCAA-3’	
U6	F:5’-GGAACGATACAGAGAAGATTAGC-3’	
	R:5’-TGGAACGCTTCACGAATTTGCG-3’	

VPS8: Vacuolar protein sorting-associated protein 8; NEAT1: Nuclear paraspeckle assembly transcript 1; U1: Uridine-rich 1 small nuclear RNA; *ACTB*: Actin Beta; *DNMT1*: DNA (cytosine-5-)-methyltransferase 1; *DNMT3A*: DNA (cytosine-5-)-methyltransferase 3A; *DNMT3B*: DNA (cytosine-5-)-methyltransferase 3B; *CDKN1A*: Cyclin Dependent Kinase Inhibitor 1A; *TP53*: Tumor protein 53; *CDKN1B*: Cyclin Dependent Kinase Inhibitor 1B; U6: Uridine-rich 1 small nuclear RNA.

Table S7 Primary antibodies used in western blot analysis

Peptide/ protein target	Host	Dilution used	Manufacturer
GAPDH	Mouse Monoclonal	1:5000	Proteintech Group Inc
β-Actin	Mouse Monoclonal	1:3000	Proteintech Group Inc
DNMT1	Rabbit Polyclonal	1:1000 for WB 5μg for each IP sample	Abcam
DNMT3a	Rabbit Monoclonal	1:1000	Abcam
Rb	Mouse Monoclonal	1:2000	Cell Signaling Technology
Phospho-Rb(Ser807/811)	Rabbit Monoclonal	1:1000	Cell Signaling Technology
Cyclin E1	Mouse Monoclonal	1:500	Proteintech Group Inc
PCNA	Rabbit Monoclonal	1:1000	Cell Signaling Technology
CDK2	Mouse Monoclonal	1:1000	Cell Signaling Technology
Phospho-CDK2(Thr160)	Rabbit Monoclonal	1:1000	Cell Signaling Technology
p21(Waf1/Cip1)	Rabbit Monoclonal	1:1000	Cell Signaling Technology
Ubiquitin	Mouse Monoclonal	1:1000	Cell Signaling Technology

Table S8 Primers of pyrosequencing in CDKN1A promoter region

Primer Set			**Score: 74** **Quality: Medium**		
Primer	**Id**	**Sequence**	**Nt**	**Tm, ℃**	**%GC**
PCR	F1	AGGAATTGATTTAGGTAGTTGTTTATAT	28	56.0	25.0
PCR	R1	ACTAAAACTCCACCAAACTCAACTAACTC	29	58.6	37.9
Sequencing	S1	ATTGATTTAGGTAGTTGTTTATATT	25	42.7	20.0
Target Polymorphisms	Position1, Position2, Position3, Position4, Position5, Position6, Position7, Position8, Position9, Position10, Position11, Position12, Position13, Position14, Position15, Position16				
Sequence to Analyze	TTAGTTGGYG TAGTTTAGYG YGGTTTTGAT ATATAATYGT TTYGTTYGGG TTYGTTTTAA GGAGGYGGGA TTYGYGTTYG GTTTATYGYG TYGTTYGGGA TYGTTT				
Amplicon length	198				