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Supplemental Material

BPDE, the Migration and Invasion of Human Trophoblast Cells, and Occurrence of Miscarriage in Humans: Roles of a Novel *lncRNA-HZ09*

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References

Additional File- Excel Document

Table S1. DNA sequences used for construction of pcDNA3.1 overexpression plasmids.

Plasmid name	Gene name	Sequence region
pcDNA3.1-HZ09	*Lnc-HZ09*	Full sequence, NCBI No. MW675687
pcDNA3.1-METTL3	*METTL3*	CDS region (NM_019852.5)
pcDNA3.1-MSX1	*MSX1*	CDS region (NM_002448.3)
pcDNA3.1-SP1	*SP1*	CDS region(NM_001251825.2)
pcDNA3.1-HuR	*HuR*	CDS region(NM_001419.3)

Table S2. RNA sequences used for RNA transfection.

Name	Sense (5'-3')	Antisense (5'-3')
si1-HZ09	CCACCUCUGUCUGUCUCUUTT	AAGAGACAGACAGAGGUGGTT
si2-HZ09	GCGAGUACUGAAUCCGGACTT	GUCCGGAUUCAGUACUCGCTT
si1-METTL3	GCACAUCCUACUCUUGUAATT	UUACAAGAGUAGGAUGUGCTT
si2-METTL3	GGAGAUCCUAGAGCUAUUATT	UAAUAGCUCUAGGAUCUCCTT
si1-MSX1	GCAUUUAGAUCUACACUCUTT	AGAGUGUAGAUCUAAAUGCTT
si2-MSX1	AGAGUGUAGAUCUAAAUGCTT	GCAUUUAGAUCUACACUCUTT
si1- SP1	GGCUCGAAGUAGCAGCACATT	UGUGCUGCUACUUCGAGCCTT
si2-SP1	GCAAGUUCUGACAGGACUATT	UAGUCCUGUCAGAACUUGCTT
si1-HuR	GACGCCAACUUGUACAUCATT	UGAUGUACAAGUUGGCGUCTT
si2- HuR	GAGUUACGAAGCCUGUUCATT	UGAACAGGCUUCGUAACUCTT
si1-PLD1	GCAAGUUAAGAGGAAAUUCTT	GAAUUUCCUCUUAACUUGCTT
si2-PLD1	GCGUCUACAUCCCAACAUATT	UAUGUUGGGAUGUAGACGCTT
NC	UUCUCCGAACGUGUCACGUTT	ACGUGACACGUUCGGAGAATT

Table S3. PCR protocols for RACE assays

Step	PCR		
	CYCLE (25 cycles)		
	Denature	Anneal	Extend
Temp.	94 ℃	68 ℃	72 ℃
Time	30 sec	30 sec	3 min

Table S4. Primer sequences used for RACE assays.

Name	Primers for RACE (5'-3')
3'-RACE of *lnc-HZ09* (human)	CGTTGGTCTAGGGGTATGATTCTCG
5'-RACE of *lnc-HZ09* (human)	AGAAGGTAGGAGAGG

Table S5. The full-length sequence of lnc-HZ09

Name	Nucleotide sequence (5’-3’)
lnc-HZ09	CGTTGGTCTAGGGGTATGATTCTCGCTTCGGGTGTGAGAGGTCCCGGG TTCAAATCCCGGACGAGCCCACTTCATTTTCGCCCAATAAGGTTTAAG GATATTTAAAGTATTTCCCCAAATAGTCATACCCAAAAATATGTACTTTC ACCACATCAACAGTAGAATTGTCTAAGCAGGCGAGTACTGAATCCGGA CTTCTTCTAAGGGCAGACACCGGAATGTTGATGCGGCTGCAGCTGCGC TCTCCCACCTCTGTCTGTCTCTTCTCAAACCCCGAAAGGAAGACCGTG GGCAGCTCGTGCGCGCGTGCGTGTGCGTGTTTAACCGAGTGCTGGAG GGGAAACGTGAGTTGCAGTCAACCGGTTA

Table S6. RT-qPCR programs

	1	2			3		
Step	Hot-Start DNA Polymerase Activation	PCR			Melt Curve		
	HOLD	CYCLE (40 cycles)			CYCLE (1 cycle)		
		Denature	Anneal	Extend			
Temp.	95 ℃	95 ℃	50-60 ℃	72 ℃	95 ℃	60 ℃	95 ℃
Time	10 min	15 sec	30 sec	30 sec	15 sec	60 sec	15 sec

Table S7. Primer sequences used for RT-qPCR analysis (Sangon Biotech, Shanghai, China)**.**

species	Gene	Forward (5'-3')	Reverse (5'-3')
Human	*lnc-HZ09*	GGCAGACACCGGAATGTTGA	TGCAACTCACGTTTCCCCTC
	MSX1	ACACAAGACGAACCGTAAGCC	CACATGGGCCGTGTAGAGTC
	METTL3	TTGTCTCCAACCTTCCGTAGT	CCAGATCAGAGAGGTGGTGTAG
	PLD1	TTTAGGAGGCAAAACGTCAGAG	TCATACCTTCTATGCCCTTTGGT
	SP1	TGGCAGCAGTACCAATGGC	CCAGGTAGTCCTGTCAGAACTT
	RAC1	ATGTCCGTGCAAAGTGGTATC	CTCGGATCGCTTCGTCAAACA
	CDC42	CCATCGGAATATGTACCGACTG	CTCAGCGGTCGTAATCTGTCA
	GAPDH	CTCCTACATCACCAAGGACAAG	GCAGCTGTGACACACAGTA
Mouse	*Pld1*	GCCGCAGATATGAGCAACCT	GGATGTTGGGCTCCTTAAATCCT
	Sp1	AGGGTCCGAGTCAGTCAGG	AGGGTCCGAGTCAGTCAGG
	Rac1	GTCCGTGCAAAGTGGTATCCT	GCACCGATCTCTTTCGCCAT
	Cdc42	CCCATCGGAATATGTACCAACTG	CGGTCGTAGTCTGTCATAATCCT
	Gapdh	TGGCCTTCCGTGTTCCTAC	GAGTTGCTGTTGAAGTCGCA

Table S8. Primer sequences used in various RT-qPCR assays (Sangon Biotech, Shanghai, China)**.**

Assay	Gene	Forward (5'-3')	Reverse (5'-3')
ChIP	*lnc-HZ09*	GGAGACAGTTGCAGGGAAGT	TTTAGGCCCCTTCAGTGCTC
	PLD1	TGGCCTTCCGTGTTCCTAC	GAGTTGCTGTTGAAGTCGCA
	GAPDH	AATGGACAACTGGTCGTGGAC	CCCTCCAGGGGATCTGTTTG
RIP	*lnc-HZ09*	GGCAGACACCGGAATGTTGA	TGCAACTCACGTTTCCCCTC
	PLD1	TTTAGGAGGCAAAACGTCAGAG	TCATACCTTCTATGCCCTTTGGT
	GAPDH	TGTGGGCATCAATGGATTTGG	ACACCATGTATTCCGGGTCAAT
MeRIP	*lnc-HZ09*	GTCTAAGCAGGCGAGTACTGA	CTGCAGCCGCATCAACATTC
	GAPDH	CTCACCGGATGCACCAATGTT	CGCGTTGCTCACAATGTTCAT

Table S9. DNA sequence in pGEM-T used for transcription of antisense sequence of *lnc-HZ09* or PLD1 mRNA

Name	Nucleotide sequence (5'-3')
lnc-HZ09 antisense	TAACCGGTTGACTGCAACTCACGTTTCCCCTCCAGCACTCGGTTAAACACGCACACGC ACGCGCGCACGAGCTGCCCACGGTCTTCCTTTCGGGGTTTGAGAAGAGACAGACAGA GGTGGGAGAGCGCAGCTGCAGCCGCATCAACATTCCGGTGTCTGCCCTTAGAAGAAGT CCGGATTCAGTACTCGCCTGCTTAGACAATTCTACTGTTGATGTGGTGAAAGTACATATT TTTGGGTATGACTATTTGGGGAAATACTTTAAATATCCTTAAACCTTATTGGGCGAAAAT GAAGTGGGCTCGTCCGGGATTTGAACCCGGGACCTCTCACACCCGAAGCGAGAATCAT ACCCCTAGACCAACG
PLD1 mRNA antisense	TTAAGTCCAAACCTCCATGGGCACTATGGCCTCTTTGGTCCCAACAGAAGGCAGTAGG CTTTCTTCAGACAAGAAATAAAAGGGGAATTGCACCAAAAATCCACGGATCTTCTTCA GTTCCTCCTCAGCTCGAATGGGATCTTCCTTAGCTAATACGGGCTTGTTTATAAAGTCTC TCAGCTGAATTAAATTGTGTACTTCATCATTGGGAAGGCACCGGAAAACCTTGTCATAA ATTGTAGCATTTCGAGCTGCTGTTGAAACCCACACCTCCTTGAAGAATTTGTCACTCAC TGGATCCTGAATGTCCTCACTTGGGTCATCAAGATAGCCAAGGACAACCCTAAAGCACT

GTAGCCGAAGTCCTCGGGCAAACCGGCCAGCTTGGTACTCTTTTCCATCCATTACTGAA
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TGAGTGTTATAAATAGCAGAGAAAGGGATATACACTTCTTGTATCTTGGGATCGCTGGG
AGACACGTCGTAGTCTACCTCCTCTCCCTCAAAGTGGAGTTCCCGCGTGTCCAGATTTT
CTATGATATTACTCATGTCAGCAGCAATTTTCTGCAGTGCAGAGGTATTTACCCGTGGCT
CGTTTTTCAGTGACAT

Table S10. Protein-coding potential of *lnc-HZ09* predicted by CPAT software[a]

Sequence Name	RNA size	Ficket Score	Hexamer Score	Coding Probability	Coding Label
lnc-HZ09	366	0.98	0.026	0.036[b]	No[c]

[a]The protein-coding potential of *lnc-HZ09* predicted by CAPT software (http://lilab.research.bcm.edu/cpat/index.php)

[b]The protein coding probability value of *lnc-HZ09* ＜ 0.364, indicating no coding capbility..

[c]No protein coding ability of *lnc-HZ09* obtained from CAPT software.

Table S11. m6A modification site on *lnc-HZ09* identified by SRAMP software[a]

Position	Sequence context	Score (binary)	Score (knn)	Score (spectrum)	Score (combined)	Decision
194	AGCAG GCGAG UACUGAAUCC GGACU UCUUCUAAGG GCAGA CACCG	0.676	0.848	0.521	0.623	m6A site[b] (High confidence)

[a]m6A modification sites on the *lnc-HZ09* sequence were identified with SRAMP

software (http://www.cuilab.cn/sramp/).

[b]Higher confidence means a higher probability of m6A methylation.

Table S12. Univariate analysis of the parameters of the RM and HC groups (each n = 15).

Characteristic	RM[h]	HC[i]	P-value[j]
Age	27.6 ± 1.50[k]	27.4 ± 1.41	0.746
Gestational days (d)	50.5 ± 6.47	51.2 ± 7.33	0.784
Gestational week	7.89 ± 1.16	8.12 ± 1.26	0.607
BMI[a]	23.5 ± 2.37	23.2 ± 2.21	0.723
RBC[b] (10^{12}/L)	4.19 ± 0.20	4.18 ± 0.26	0.928
WBC[c] (10^{9}/L)	6.54 ± 1.27	6.72 ± 1.33	0.708
FSH[d] (U/mL)	6.24 ± 1.46	6.58 ± 1.13	0.482
PRL[e] (ng/mL)	57.1 ± 12.9	52.9 ± 15.60	0.428
LH[f] (U/L)	4.87 ± 3.24	5.03 ± 3.66	0.900
Hb[g] (g/L)	133 ± 6.43	131 ± 7.09	0.425
BPDE-DNA adduct levels (ng/μg)	12.2 ± 1.81	3.4 ± 1.89	<0.001

[a]BMI: Body Mass Index.

[b]RBC: Red blood cell.

[c]WBC: White blood cell.

[d]FSH: Follicle stimulating hormone.

[e]PRL: Prolactin.

[f]LH: Luteinizing hormone.

[g]Hb: Hemoglobin.

[h]RM: recurrent miscarriage group.

[i]HC: healthy control group.

[j]Chi-square test.

[k]mean ± standard deviation (n = 15 in each RM or HC group).

Table S13. Sequence conservation among various species[a]

			Human	Mouse	Rhesus	Dog	Elephant
mRNA	SP1	Per.Ident[b]	100%	87.8%	93.3%	91.5%	90.6%
	PLD1	Per.Ident[b]	100%	88.1%	94.5%	92.5%	91.6%
	RAC1	Per.Ident[b]	100%	100%	100%	100%	100%
	CDC42	Per.Ident[b]	100%	94.24%	95.29%	100%	94.24%
Protein	SP1	Per.Ident[b]	100%	87.80%	93.26%	91.53%	90.59%
	PLD1	Per.Ident[b]	100%	88.08%	94.51%	92.47%	91.63%
	RAC1	Per.Ident[b]	100%	100%	100%	100%	100%
	CDC42	Per.Ident[b]	100%	94.24%	95.29%	100%	94.24%

[a]Sequence conservation of SP1, PLD1, RAC1 and CDC42 was explored using NCBI Blast (https://blast.ncbi.nlm.nih.gov/Blast.cgi).

[b]Per.Ident means the ratio of the number of the matched ribonucleotides or amino acid residues to the total ribonucleotides or amino acid residues in the sequence.

Figure legends

Fig. S1

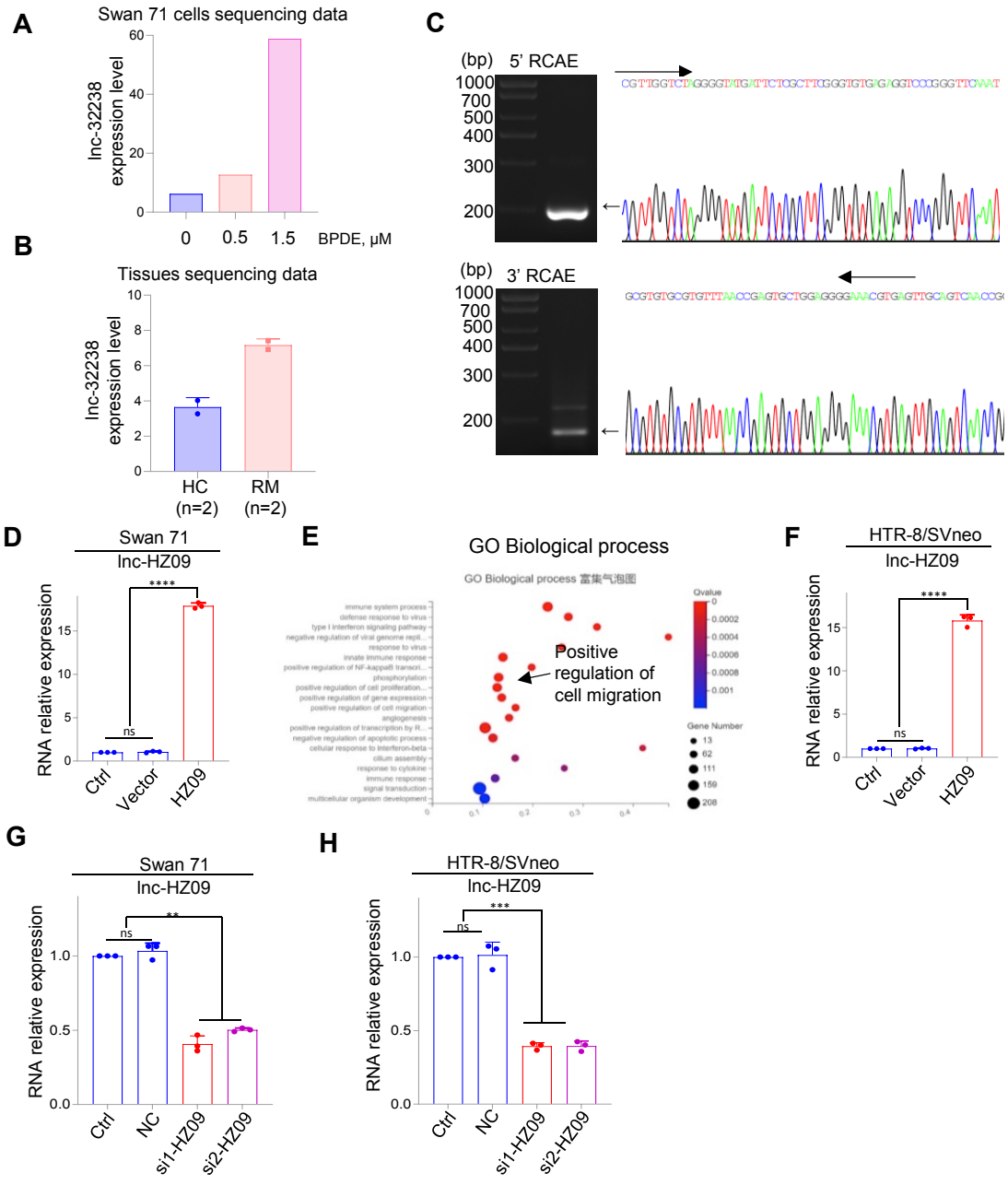

Fig. S1. Identification of lnc-HZ09 and overexpression or knockdown of lnc-HZ09 in human trophoblast cells.

(A) Lnc-32238 expressioin levels in sequencing data of BPDE-treated trophoblast cells. **(B)** Lnc-32238 expressioin levels in sequencing data of RM and HC villous

tissues. Data for figure S1A and B from Liang et al. 2021[1] **(C)** Rapid amplification of cDNA ends (RACE) and subsequent analysis of DNA product. **(D)** The efficiencies of overexpression of *lnc-HZ09* in Swan 71 cells, with empty vector (Vector) and untreated cells (Ctrl) as negative control (each n = 3). **(E)** GO biological process analysis of the differentially expressed mRNAs obtained from mRNA sequencing data of Swan 71 cells transfected with either *lnc-HZ09* overexpression plasmid or an empty control vector. **(F)** The efficiencies of overexpression of lnc-HZ09 in HTR-8/SVneo cells, with cells transfected with empty vector (Vector) as negative control (each n = 3). **(G-H)** The efficiencies of knockdown of lnc-HZ09 in Swan 71 (G) or HTR-8/SVneo (H) cells, with untreated cells (Ctrl) or cells transfected with siRNA (NC) as control (each n = 3).

The summary data of these bar charts were shown in Excel Table S1. The RNA level in Ctrl group was set as '1' in all of RT-qPCR assays. Representative data in (C) represent three independent experiments. Data in (**B**) show mean ± SD of two samples. Data in (**D, F-H**) show mean ± SD of three independent experiments. Two-tailed Student's t-test for (**D, F**); one-way ANOVA analysis for (**G-H**); ns, non-significance; $^{**}p < 0.01$, $^{***}p < 0.001$, and $^{****}p < 0.0001$. Ctrl, untreated cells; Vector, cells transfected with empty vector of pcDNA3.1; NC, negative control of siRNA; si-HZ09, knockdown of lnc-HZ09; HZ09, overexpression of lnc-HZ09;

Fig. S2

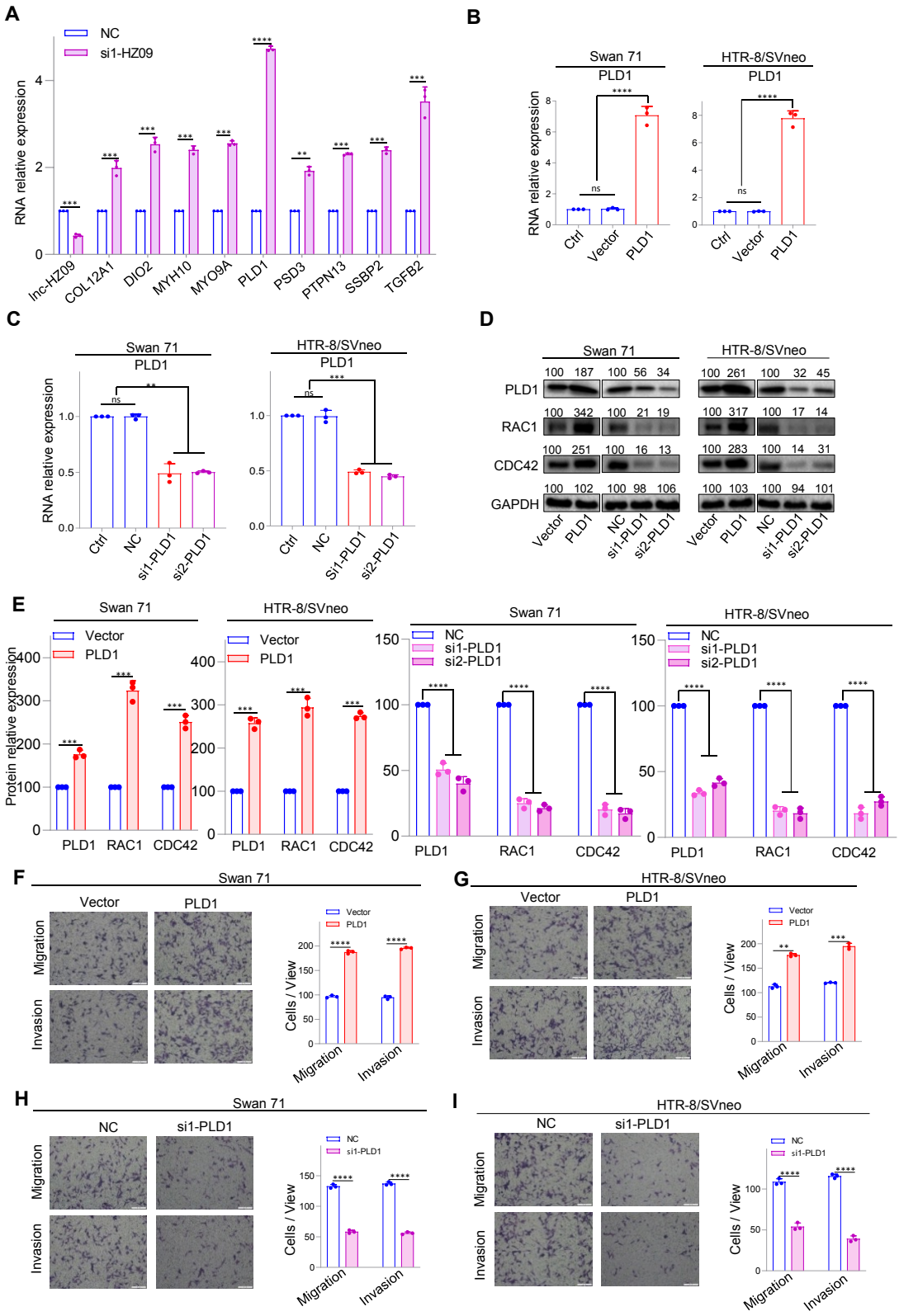

Fig. S2. Expression levels of nine mRNAs with knockdown of *lnc-HZ09* and the functions of PLD1 in human trophoblast cells.

(A) RT-qPCR analysis (each n = 3) of the nine mRNAs levels of nine genes in Swan 71 cells with knockdown of *lnc-HZ09*. **(B)** The efficiencies of overexpression of PLD1 in Swan 71 (left) or HTR-8/SVneo (right) cells, with empty vector (Vector) and untreated cells (Ctrl) as negative control (each n = 3). **(C)** The efficiencies of knockdown of PLD1 in Swan 71 (left) or HTR-8/SVneo (right) cells, with untreated cells (Ctrl) or cells transfected with negative control siRNA (NC) as control (each n = 3). **(D-E)** Representative western blot analysis of the protein levels of PLD1, RAC1 and CDC42 in Swan 71 or HTR-8/SVneo cells with overexpression or knockdown of PLD1, with GAPDH as internal standard (D). The relative intensity of each band was quantified and their mean ± SD of three replicates was shown in (E). **(F-I)** Transwell assay analysis of the migration and invasion of Swan 71 (F, H) or HTR-8/SVneo (G, I) cells with overexpression (F, G) or knockdown (H, I) of PLD1 (scale bar, 200 μm).

The summary data of these bar charts were shown in Excel Table S1. The RNA level in Ctrl group was set as '1' in all of RT-qPCR assays, and the band intensity in NC or Vector group was set as '100' in all of western blot assays. Representative data in (**D-I**) represent three independent experiments. Data in (**A-C, E-I**) show mean ± SD of three independent experiments. Two-tailed Student's t-test for (**A-C, E-I**); ns, non-significance; $^{**}p < 0.01$, $^{***}p < 0.001$, and $^{****}p < 0.0001$. Vector, empty vector of pcDNA3.1; PLD1, overexpression of PLD1; NC, negative control of siRNA; si-PLD1, knockdown of PLD1.

Fig. S3

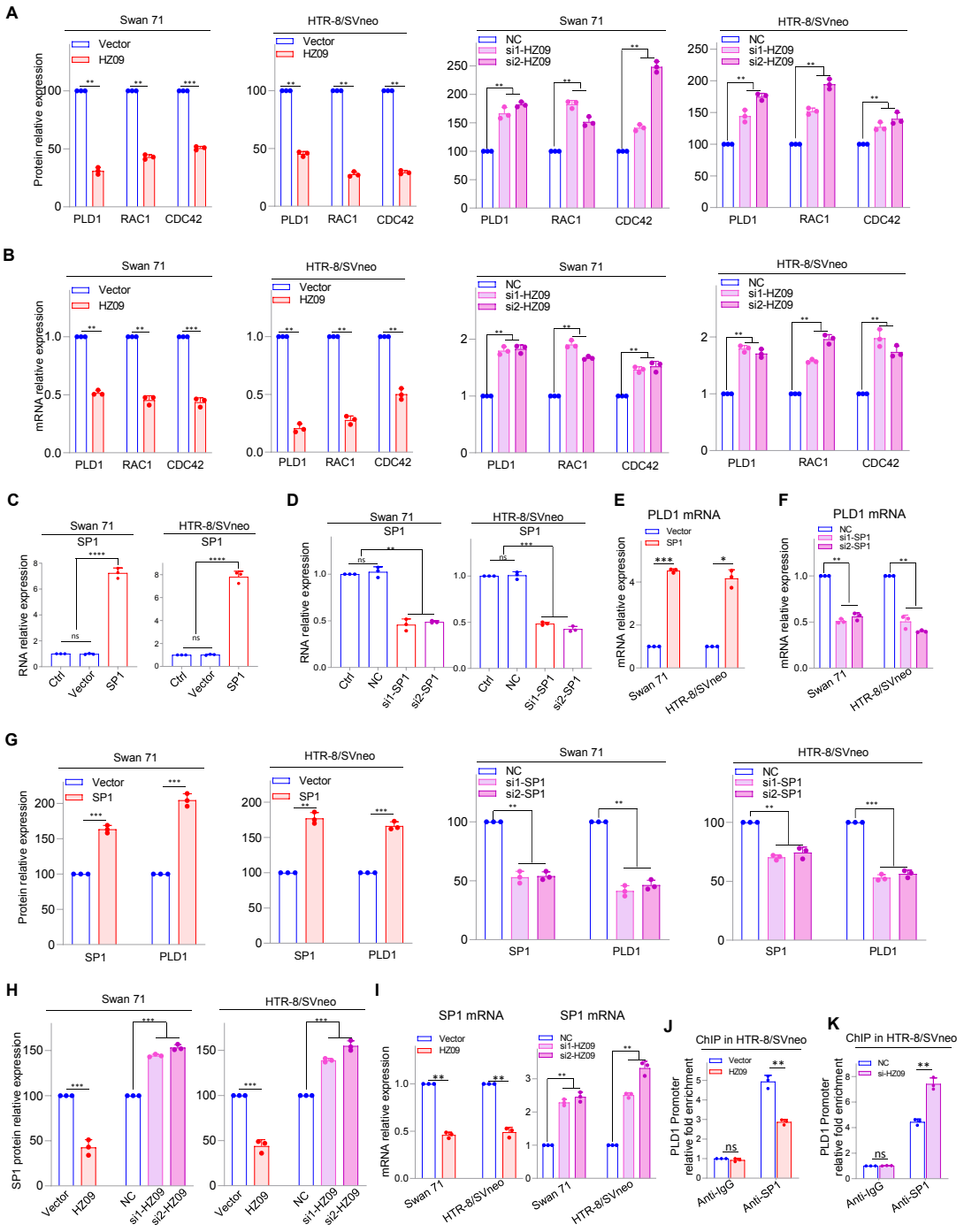

Fig. S3. Expression levels of members of the PLD1/RAC1/CDC42 pathway regulated by *lnc-HZ09* in human trophoblast cells.

(A) The relative intensity of each protein band of PLD1, RAC1 and CDC42 in Swan 71 or HTR-8/SVneo cells with overexpression or knockdown of *lnc-HZ09* (each n =

3). **(B)** RT-qPCR analysis (each n = 3) of the mRNA levels of PLD1, RAC1 and CDC42 in Swan 71 or HTR-8/SVneo cells with overexpression or knockdown of *lnc-HZ09*. **(C)** The efficiencies of overexpression of SP1 in Swan 71 (left) or HTR-8/SVneo (right) cells, with cells transfected with empty vector (Vector) as negative control (each n = 3). **(D)** The efficiencies of knockdown of SP1 in Swan 71 (left) or HTR-8/SVneo (right) cells, with untreated cells (Ctrl) or cells transfected with negative control siRNA (NC) as control (each n = 3). **(E-F)** RT-qPCR analysis (each n = 3) of the mRNA levels of PLD1 in Swan 71 or HTR-8/SVneo cells with overexpression (E) or knockdown (F) of SP1. **(G)** The relative intensity of each protein band of SP1 and PLD1 in Swan 71 or HTR-8/SVneo cells with overexpression or knockdown of SP1. **(H)** The relative intensity of protein band of SP1 in Swan 71 or HTR-8/SVneo cells with overexpression or knockdown of *lnc-HZ09*. **(I)** RT-qPCR analysis (each n = 3) of the mRNA levels of SP1 in Swan 71 or HTR-8/SVneo cells with overexpression or knockdown of *lnc-HZ09*. **(J-K)** SP1 ChIP assay (each n = 3) analysis of the relative enrichment of SP1 in the promoter region of PLD1 gene in HTR-8/SVneo cells with overexpression or knockdown of *lnc-HZ09*.

The summary data of these bar charts were shown in Excel Table S1. The RNA level in Ctrl or NC/Vector group was set as '1' in all of RT-qPCR assays; the DNA level in IgG group was set as '1' in all of ChIP assays; and the band intensity in NC/Vector group was set as '100' in all of western blot assays. Representative data in (**A-K**) represent three independent experiments. Data in (**A -K**) show mean ± SD

of three independent experiments. Two-tailed Student's t-test for (**A-K**); ns, non-significance; $^{**}p < 0.01$, $^{***}p < 0.001$, and $^{****}p < 0.0001$. Vector, empty vector of pcDNA3.1; HZ09, overexpression of *lnc-HZ09*; SP1, overexpression of SP1; NC, negative control of siRNA; si-HZ09, knockdown of *lnc-HZ09*; si-SP1, knockdown of SP1.

Fig. S4

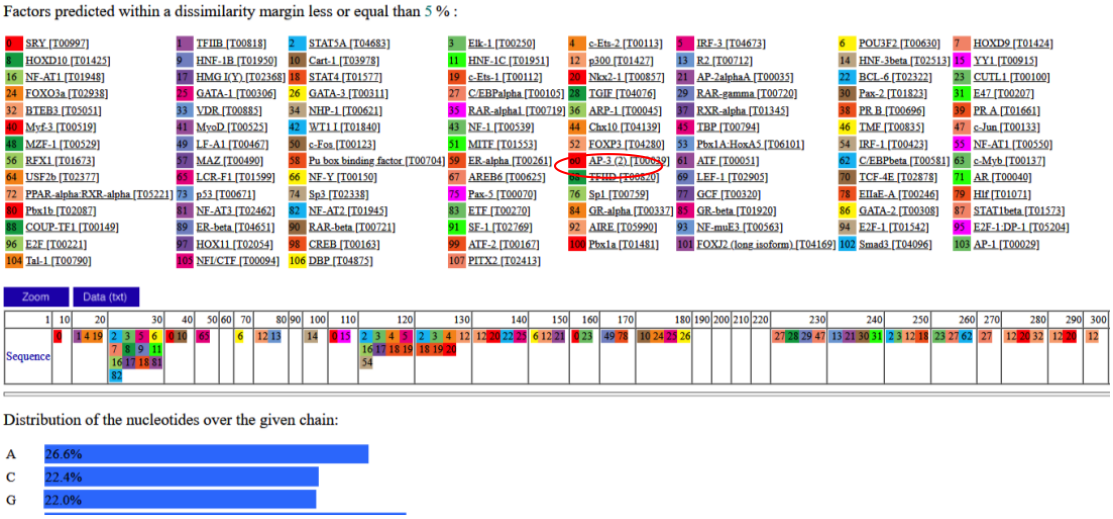

Fig. S4. Transcription factors of PLD1 predicted by PROMO software.

Fig. S5

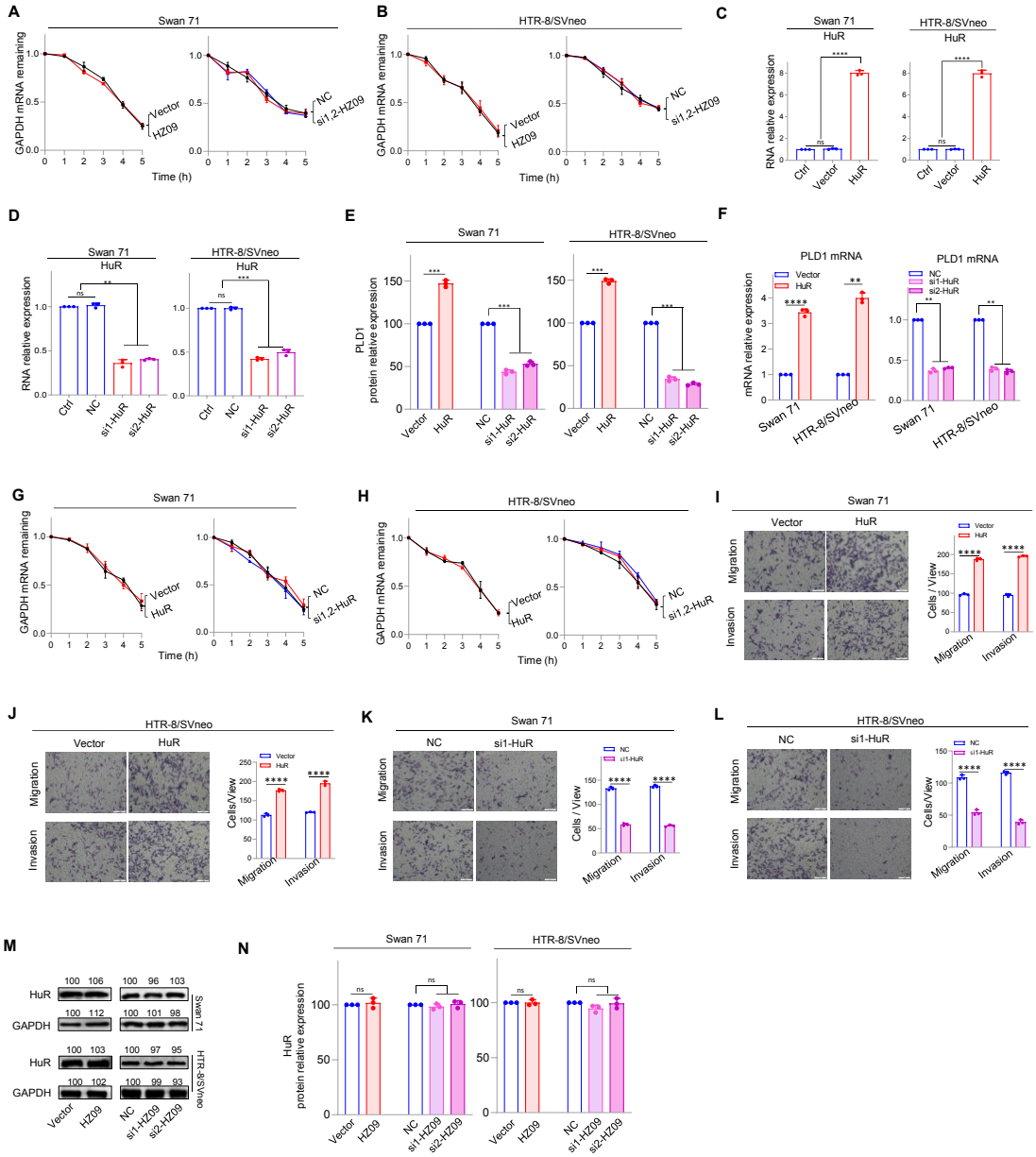

Fig. S5. PLD1 mRNA stability regulated by *Lnc-HZ09* in human trophoblast cells.

(A-B) The mRNA stability of GAPDH (each n = 3) in Swan 71 (A) or HTR-8/SVneo (B) cells with overexpression or knockdown of *lnc-HZ09*. **(C)** The efficiencies of overexpression of HuR in Swan 71 (left) or HTR-8/SVneo (right) cells, with cells transfected with empty vector (Vector) or untreated cells (Ctrl) as negative control

(each n = 3). **(D)** The efficiencies of knockdown of HuR in Swan 71 (left) or HTR-8/SVneo (right) cells, with untreated cells (Ctrl) or cells transfected with negative control siRNA (NC) as control (each n = 3). **(E)** The relative intensity of protein band of PLD1 in Swan 71 or HTR-8/SVneo cells with overexpression or knockdown of HuR. **(F)** RT-qPCR analysis (each n = 3) of the mRNA levels of PLD1 in Swan 71 or HTR-8/SVneo cells with overexpression or knockdown of HuR. **(G-H)** The mRNA stability of GAPDH (each n = 3) in Swan 71 (G) or HTR-8/SVneo (H) cells with overexpression or knockdown of HuR. **(I-L)** Transwell assay analysis of the migration and invasion of Swan 71 (I and K) or HTR-8/SVneo (J and L) cells with overexpression or knockdown of HuR (scale bar, 200 μm) and quantitation of the numbers of cells/view. (**M-N)** Western blot analysis of the protein levels of HuR in Swan 71 or HTR-8/SVneo cells with overexpression or knockdown of *lnc-HZ09* (M). The relative intensity of each band was quantified and their mean ± SD of three replicates was shown in (N).

The summary data of these bar charts and diagrams were shown in Excel Table S1. The RNA level in Ctrl or NC/Vector group was set as '1' in all of RT-qPCR and mRNA stability assays; and the band intensity in NC/Vector group was set as '100' in all of western blot assays. Representative data in (**I-M**) represent three independent experiments. Data in (**A-L, N**) show mean ± SD of three independent experiments. Two-tailed Student's t-test for (**C-F, I-L, N**); ns, non-significance; $^{**}p < 0.01$, $^{***}p < 0.001$, and $^{****}p < 0.0001$. Vector, empty vector of pcDNA3.1; HZ09,

overexpression of *lnc-HZ09*; NC, negative control of siRNA; si-HZ09, knockdown of *lnc-HZ09*; HuR, overexpression of HuR; si-HuR, knockdown of HuR.

Fig. S6

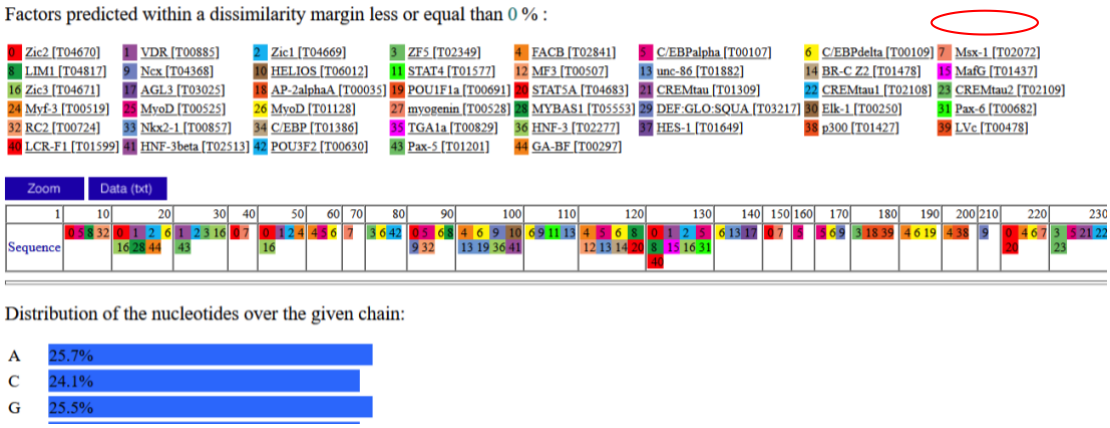

Fig. S6. Transcription factors of *lnc-HZ09* predicted by PROMO software.

Fig. S7

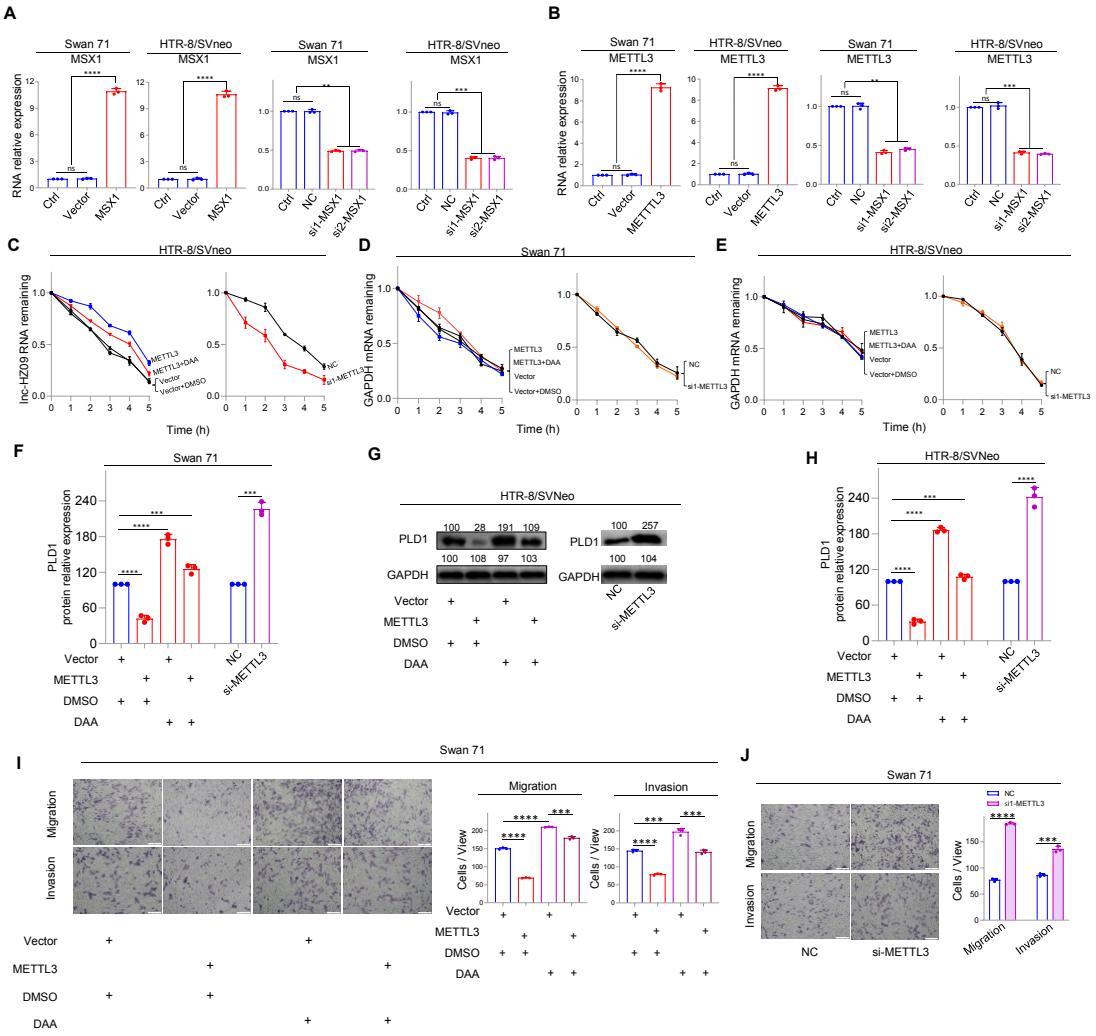

Fig.S7. METTL3-mediated m6A RNA methylation on *lnc-HZ09* regulated PLD1 expression level and migration and invasion of human trophoblast cells.

(A) The efficiencies of overexpression or knockdown of MSX1 in Swan 71 or HTR-8/SVneo cells, with untreated cells (Ctrl), cells transfected with empty vector (Vector) or negative control of siRNA (NC) as control, respectively (each n = 3). **(B)** The efficiencies of overexpression or knockdown of METTL3 in Swan 71 or HTR-8/SVneo cells, with untreated cells (Ctrl), cells transfected with empty vector (Vector) or negative control of siRNA (NC) as control (each n = 3). **(C)** The RNA

stability of *lnc-HZ09* (each n = 3) in HTR-8/SVneo cells with overexpression or knockdown of METTL3, or overexpression of METTL3 together with DAA treatment. **(D-E)** The mRNA stability of GAPDH (each n = 3) in Swan 71 (D) or HTR-8/SVneo (E) cells with overexpression or knockdown of METTL3, or overexpression of METTL3 together with DAA treatment. **(F)** The relative intensity of each protein band of PLD1 in Swan 71 cells with overexpression or knockdown of METTL3, or with overexpression of METTL3 together with DAA treatment. **(G-H)** Representative western blot analysis of the protein levels of PLD1 in HTR-8/SVneo cells with overexpression or knockdown of METTL3, or with overexpression of METTL3 together with DAA treatment (G). The relative intensity of each band was quantified and their mean ± SD of three replicates was shown in (H). **(I-J)** Transwell assay analysis of the migration and invasion of Swan 71 or HTR-8/SVneo cells with overexpression or knockdown of METTL3, or with overexpression of METTL3 together with DAA treatment (scale bar, 200 μm). The number of cells/view in transwell assays were counted.

The summary data of these bar charts and diagrams were shown in Excel Table S1. The RNA level in Ctrl or NC/Vector group was set as '1' in all of RT-qPCR and mRNA stability assays; and the protein band intensity in NC/Vector group was set as '100' in all of western blot assays. Representative data in (**G-J**) represent three independent experiments. Data in (**A-F, H, I, J**) show mean ± SD of three independent experiments. Two-tailed Student's t-test for (**A-B, I, J**); one-way ANOVA analysis for (**F, H**); ns, non-significance; $^{**}p < 0.01$, $^{***}p < 0.001$, and

****$p < 0.0001$. Vector, empty vector of pcDNA3.1; METTL3, overexpression of METTL3; NC, negative control of siRNA; si-METTL3, knockdown of METTL3.

Fig. S8

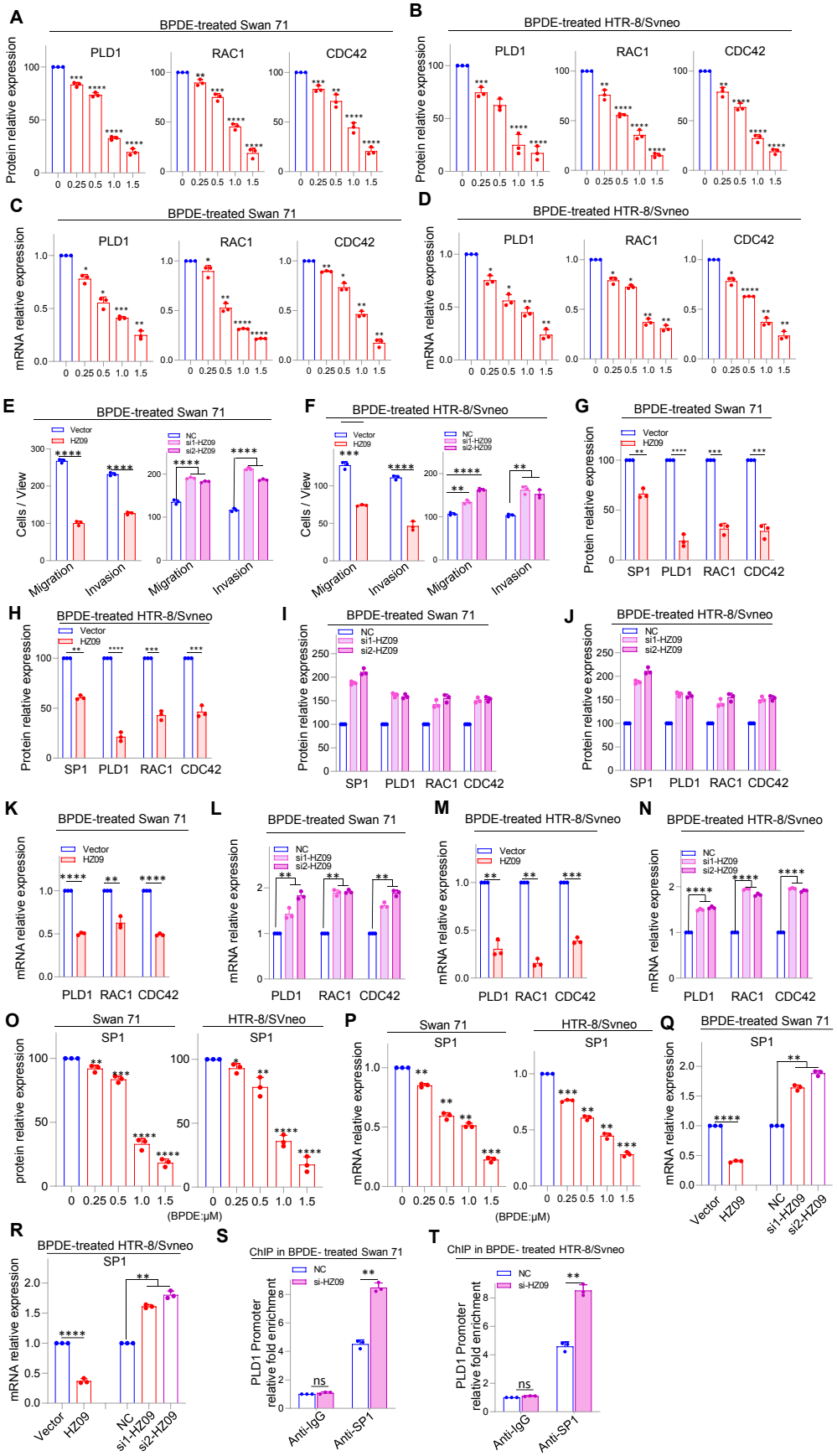
A
BPDE-treated Swan 71
PLD1
RAC1
CDC42
Protein relative expression
B
BPDE-treated HTR-8/Svneo
C
mRNA relative expression
D
E
Cells / View
Vector
HZ09
NC
si1-HZ09
si2-HZ09
Migration
Invasion
F
G
SP1
H
I
J
K
L
M
N
O
Swan 71
HTR-8/SVneo
protein relative expression
(BPDE:μM)
P
Q
R
S
ChIP in BPDE- treated Swan 71
PLD1 Promoter relative fold enrichment
si-HZ09
Anti-IgG
Anti-SP1
ns
T
ChIP in BPDE- treated HTR-8/Svneo

Fig. S8. Expression levels of members of PLD1/RAC1/CDC42 pathway regulated by BPDE or *lnc-HZ09* in BPDE-exposed human trophoblast cells.

(A-B) The relative intensity of each protein band of PLD1 in BPDE-treated Swan 71 (A) or HTR-8/SVneo (B) cells. **(C-D)** RT-qPCR analysis (each n = 3) of the mRNA levels of PLD1, RAC1 and CDC42 in BPDE-treated Swan 71 (C) and HTR-8/SVneo (D) cells. **(E-F)** Quantitative analysis of the number of cells/view in transwell assays using 0.5 μM BPDE-treated Swan 71 (E) or HTR-8/SVneo (F) cells with overexpression or knockdown of *lnc-HZ09*. **(G-J)** The relative intensity of each protein band of SP1, PLD1, RAC1 and CDC42 in 0.5 μM BPDE-treated Swan 71 or HTR-8/SVneo cells with overexpression or knockdown of *lnc-HZ09*. **(K-N)** RT-qPCR analysis (each n = 3) of the mRNA levels of PLD1, RAC1 and CDC42 in 0.5 μM BPDE-treated Swan 71 or HTR-8/SVneo cells with overexpression or knockdown of *lnc-HZ09*. **(O)** The relative intensity of each protein band of SP1 in BPDE-treated Swan 71 or HTR-8/SVneo cells. **(P)** RT-qPCR analysis (each n = 3) of the mRNA levels of SP1 in BPDE-treated Swan 71 or HTR-8/SVneo cells. **(Q-R)** RT-qPCR analysis (each n = 3) of the mRNA levels of SP1 in 0.5 μM BPDE-treated Swan 71 or HTR-8/SVneo cells with overexpression or knockdown of *lnc-HZ09*. **(S-T)** SP1 ChIP assay analysis (each n = 3) of the relative enrichment of SP1 in the promoter region of PLD1 gene with knockdown of *lnc-HZ09* in 0.5 μM BPDE-treated Swan 71 or HTR-8/SVneo cells.

The summary data of these bar charts were shown in Excel Table S1. The RNA level in Ctrl or NC/Vector group was set as '1' in all of RT-qPCR assays; the DNA

level in IgG group was set as '1' in all of ChIP assays; and the protein band intensity in NC/Vector group was set to '100' in all of western blot assays. Data in (**A-T**) show mean ± SD of three independent experiments. Two-tailed Student's t-test for (**E-N, Q-T**); one-way ANOVA analysis for (**A-D, O-P**); ns, non-significance; $^{**}p < 0.01$, $^{***}p < 0.001$, and $^{****}p < 0.0001$. Vector, empty vector of pcDNA3.1; HZ09, overexpression of *lnc-HZ09*; NC, negative control of siRNA; si-HZ09, knockdown of *lnc-HZ09*.

Fig. S9

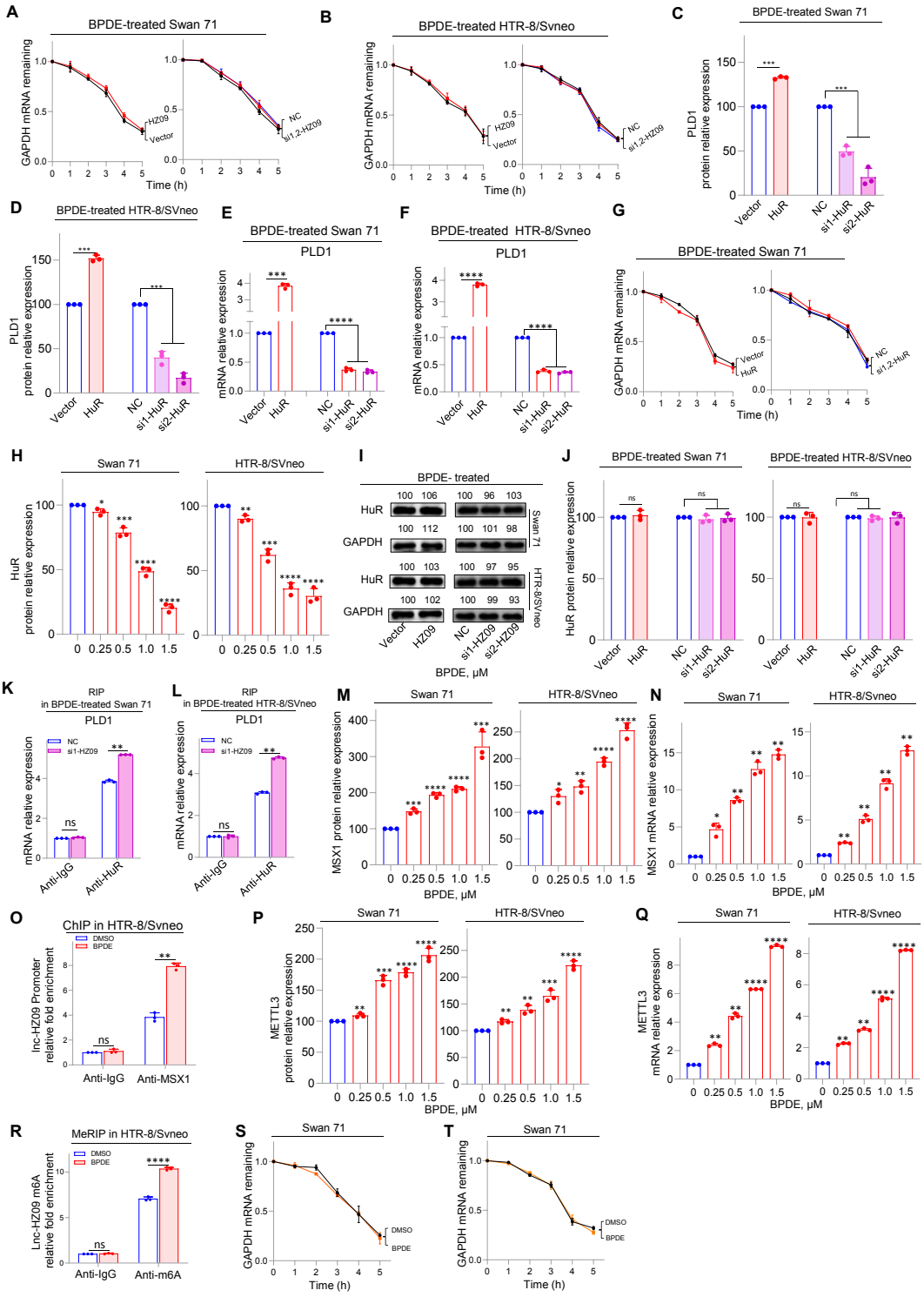

Fig. S9. PLD1 and *lnc-HZ09* RNA stability in BPDE-exposed human trophoblast cells.

(A-B) The mRNA stability of GAPDH (each n = 3) in 0.5 μM BPDE-treated Swan 71 (A) or HTR-8/SVneo (B) cells with overexpression or knockdown of *lnc-HZ09*. **(C-D)** The relative intensity of each protein band of PLD1 in 0.5 μM BPDE-treated Swan 71 or HTR-8/SVneo cells with overexpression or knockdown of HuR. **(E-F)** RT-qPCR analysis (each n = 3) of the mRNA levels of PLD1 in 0.5 μM BPDE-treated Swan 71 or HTR-8/SVneo cells with overexpression or knockdown of HuR. **(G)** The mRNA stability of GAPDH (each n = 3) in 0.5 μM BPDE-treated Swan 71 cells with overexpression or knockdown of HuR. **(H)** The relative intensity of each protein band of HuR in BPDE-treated Swan 71 or HTR-8/SVneo cells. (**I-J)** Representative western blot analysis of the protein levels of HuR in 0.5 μM BPDE-treated Swan 71 or HTR-8/SVneo cells with overexpression or knockdown of *lnc-HZ09* (I). The relative intensity of each band was quantified and their mean ± SD of three replicates was shown in (J). **(K-L)** RIP assay analysis (each n = 3) of the relative levels of PLD1 mRNA that was pulled down by HuR protein in 0.5 μM BPDE-treated Swan 71 or HTR-8/SVneo cells with knockdown of *lnc-HZ09*. **(M)** The relative intensity of each protein band of MSX1 in BPDE-treated Swan 71 or HTR-8/SVneo cells. **(N)** RT-qPCR analysis (each n = 3) of the mRNA levels of MSX1 in BPDE-treated Swan 71 or HTR-8/SVneo cells. **(O)** MSX1 ChIP assay analysis (each n = 3) of the relative enrichment of MSX1 in the promoter region of *lnc-HZ09* in untreated or 0.5 μM BPDE-treated HTR-8/SVneo cells. **(P)** The relative intensity of each protein band of METTL3 in BPDE-treated Swan 71 or HTR-8/SVneo cells. **(Q)** RT-qPCR analysis (each n = 3) of the mRNA levels of

METTL-3 in BPDE-treated Swan 71 or HTR-8/SVneo cells. **(R)** MeRIP assay analysis (each n = 3) of the relative levels of m6A RNA methylation on *lnc-HZ09* in untreated or 0.5 μM BPDE-treated HTR-8/SVneo cells. **(S)** The mRNA stability of GAPDH (each n = 3) in untreated or 0.5 μM BPDE-treated Swan 71 cells, as negative control for *lnc-HZ09* stability assays. **(T)** The mRNA stability of GAPDH (each n = 3) in untreated or 0.5 μM BPDE-treated Swan 71 cells, as negative control for PLD1 mRNA stability assays.

The summary data of these bar charts and diagrams were shown in Excel Table S1. The mRNA level in NC/Vector group was set as '1' in all of RT-qPCR and mRNA stability assays; the DNA or RNA level in IgG group was set as '1' in all of ChIP, RIP and MeRIP assays; and the protein band intensity in NC/Vector group was set as '100' in all of western blot assays. Representative data in **(I)** represent three independent experiments. Data in **(A-H, J-T)** show mean ± SD of three independent experiments. Two-tailed Student's t-test for (**C-F, K-L, O, R**); one-way ANOVA analysis for (**H, M-N, P-Q**); ns, non-significance; $^{**}p < 0.01$, $^{***}p < 0.001$, and $^{****}p < 0.0001$. Vector, empty vector of pcDNA3.1; HZ09, overexpression of *lnc-HZ09*; NC, negative control of siRNA; si-HZ09, knockdown of *lnc-HZ09*; HuR, overexpression of HuR; si-HuR, knockdown of HuR; BPDE: 0.5 μM BPDE-treated cells.

Fig. S10

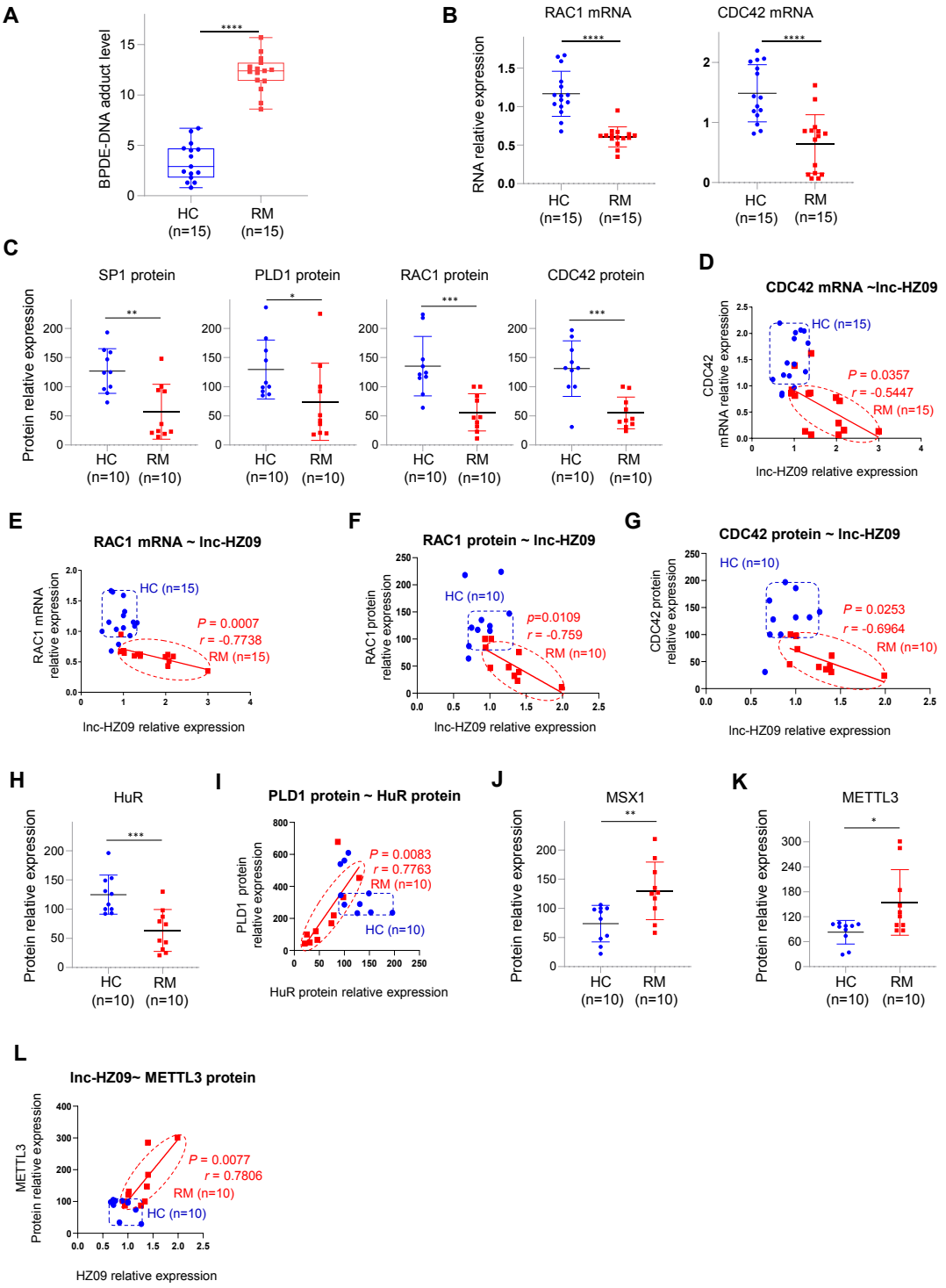

Fig. S10. The BPDE-DNA adduct levels and the correlation among various RNAs and proteins in HC and RM villous tissues.

(A) The BPDE-DNA adduct levels in HC (round) and RM (square) tissues (each n = 15). **(B)** RT-qPCR analysis of the mRNA levels of RAC1 and CDC42 in HC (round) and RM (square) tissues (each n = 15). **(C)** The relative intensity of each protein band of SP1, PLD1, RAC1 and CDC42 in HC (round) and RM (square) tissues (each n = 10). **(D-G)** The correlation between the mRNA or protein levels of CDC42 or RAC1 and *lnc-HZ09* levels in HC (round) and RM (square) groups (each n = 15 for mRNA and 10 for protein). **(H)** The relative intensity of each protein band of HuR in HC (round) and RM (square) tissues (each n = 10). **(I)** The correlation between the protein levels of PLD1 and HuR in HC (round) and RM (square) tissues (each n = 10). **(J-K)** The relative intensity of each protein band of MSX1 and METTL3 in HC (round) and RM (square) tissues (each n = 10). **(L)** The correlation between the levels of *lnc-HZ09* and the protein levels of METTL3 in HC (round) and RM (square) groups (each n = 10).

The summary data of these box and scatter plots were shown in Excel Table S1. The middle intensity value was set as '100' in all of western blot assays. Two-tailed Student's t-test for (**A-C, H, J-K**); Pearson analysis for **(D-G, I, L)**. RM, recurrent miscarriage group; HC, healthy control group; n, the number of biologically independent samples. $*p < 0.05$, $**p < 0.01$, $***p < 0.001$, and $****p < 0.0001$.

Fig. S11

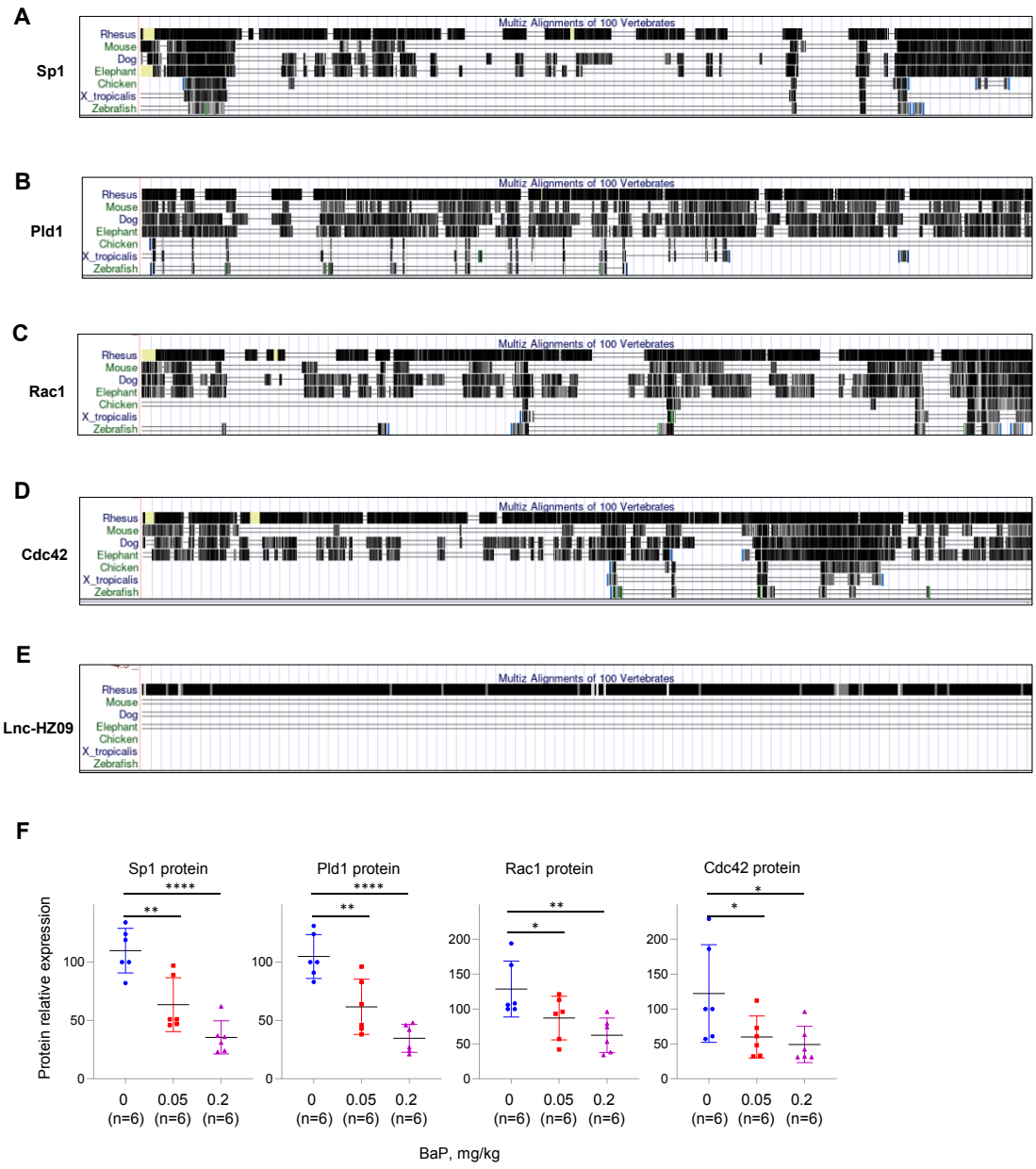

Fig. S11. Sequence alignment of various genes in different species and the protein levels of Sp1, Pld1, Rac1, and Cdc42 in BaP-treated mice.

(A-E) Sequence alignment of SP1 (A), PLD1 (B), RAC1 (C), CDC42 (D) and *lnc-HZ09* (E) in various species, as analyzed by UCSC (http://genome.ucsc.edu). **(F)** The relative intensity of each protein band of Sp1, Pld1, Rac1 and Cdc42 protein level in each BaP-treated mouse group (each n = 6).

The summary data of these scatter plots were shown in Excel Table S1. The middle intensity value was set as '100' in all of western blot assays. n, the number of biologically independent samples. *$p < 0.05$, **$p < 0.01$, and ****$p < 0.0001$.

References

1. Liang, T. *et al.* Novel lnc-HZ03 and miR-hz03 promote BPDE-induced human trophoblastic cell apoptosis and induce miscarriage by upregulating p53/SAT1 pathway. *Cell biology and toxicology*, doi:10.1007/s10565-021-09583-3 (2021).