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Supplemental Information

Increased expression of HOXA11-AS attenuates endometrial decidualization in recurrent implantation failure patients

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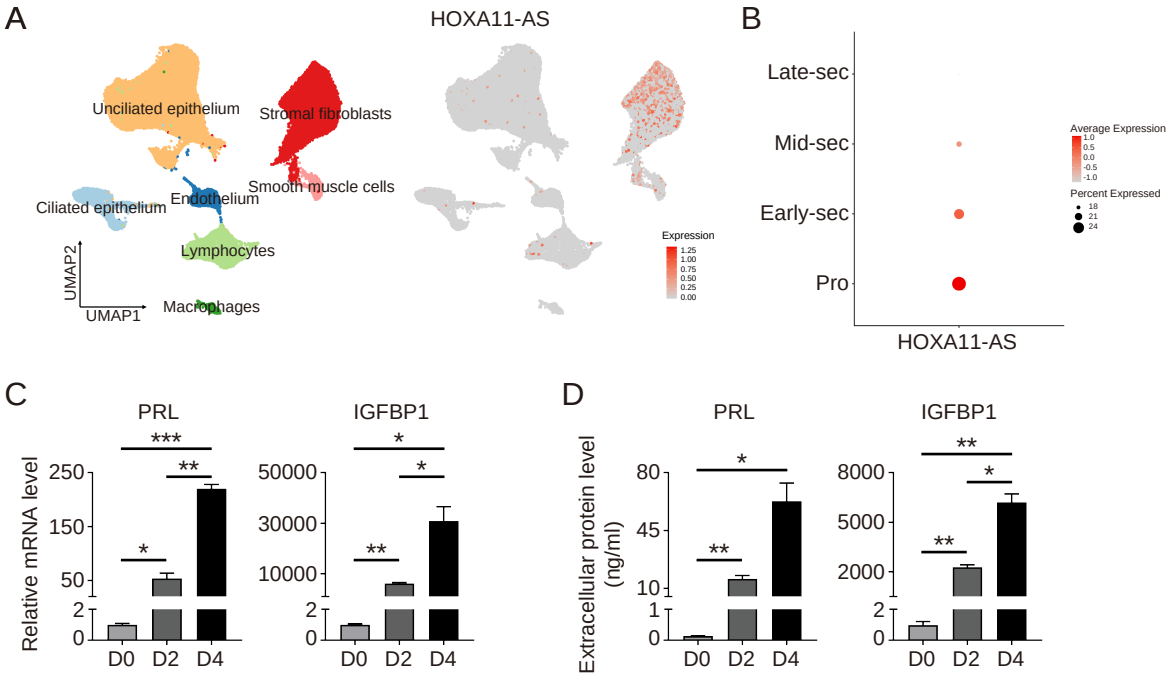


Figure S1. The expression of HOXA11-AS in single-cell dataset and the establishment of *in vitro* decidualization model

(A) Dimension reduction (uniform manifold approximation and projection (UMAP) on top PCs) and HOXA11-AS expression on all the single cells in 10× dataset. (B) Dynamics of HOXA11-AS stratified by stromal fibroblasts across menstrual cycle in 10× dataset. Pro: proliferative; sec: secretory. (C) qRT-PCR showing relative mRNA levels of two decidualization biomarkers PRL and IGFBP1 during *in vitro* decidualization (n=4, One-way ANOVA, Bonferroni test). (D) Extracellular protein levels of two decidualization biomarkers PRL and IGFBP1 during *in vitro* decidualization (n=4, One-way ANOVA, Bonferroni test). Error bars represent SEMs, and the data represent at least 3 independent experiments. ***: $p < 0.001$, **: $p < 0.01$, *: $p < 0.05$.

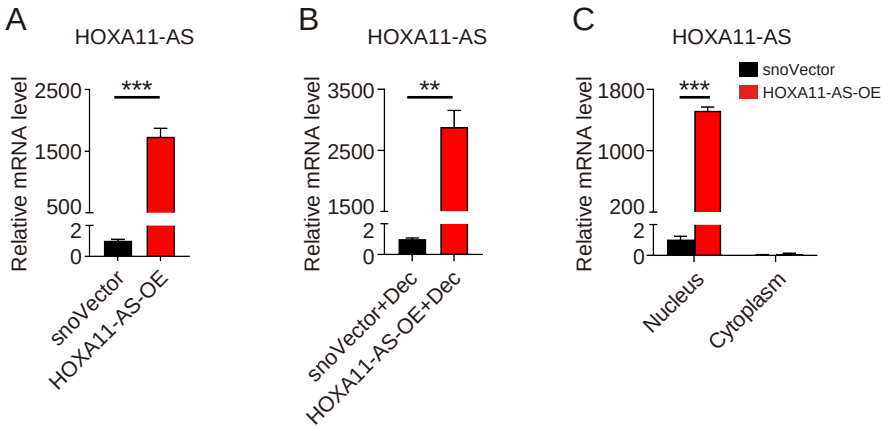
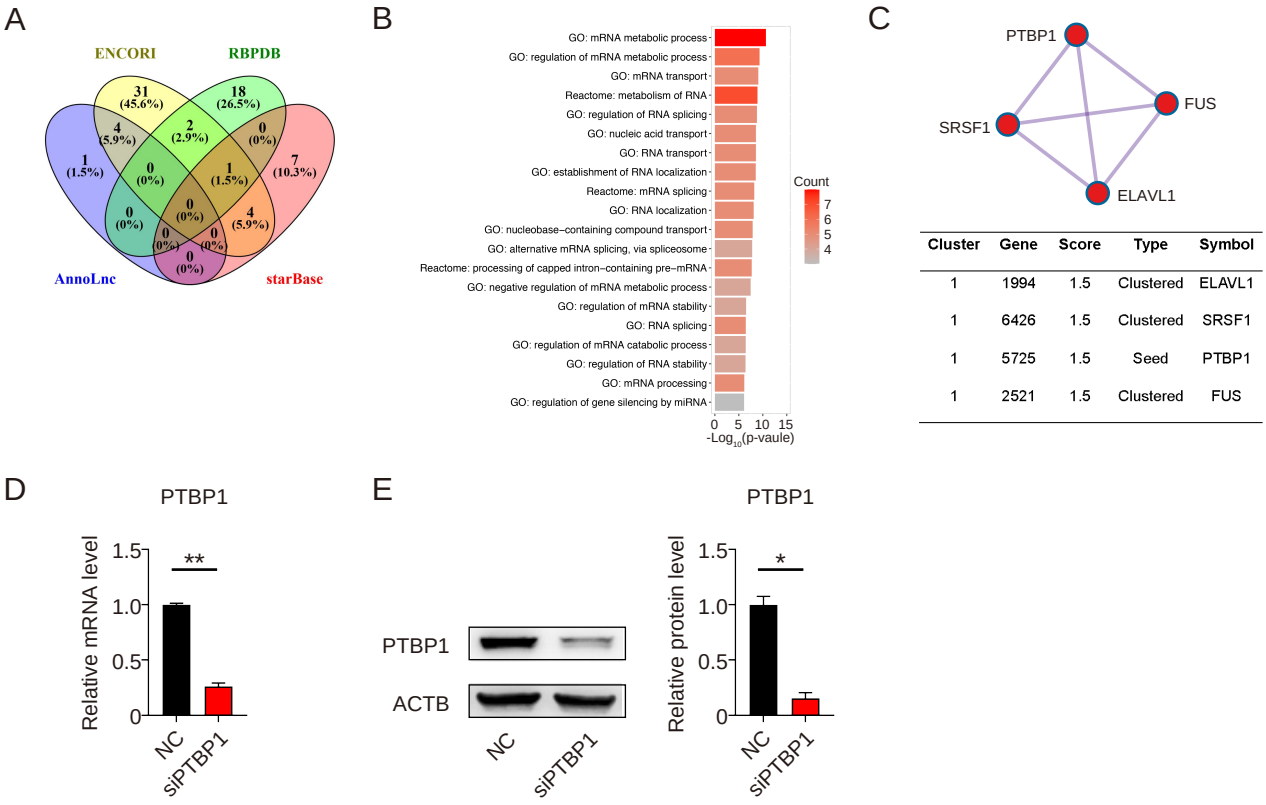


Figure S2. Overexpression efficiency of HOXA11-AS in hESCs

(A) qRT-PCR assays were conducted to detect HOXA11-AS expression in hESCs transfected with snoVector or HOXA11-AS nuclear overexpression plasmid (HOXA11-AS-OE) (n=7, Student's t-test). (B) qRT-PCR assays were conducted to detect the overexpression efficiency of HOXA11-AS in hESCs under *in vitro* decidualization for 4 days (n=4, Student's t-test). Dec: decidualization. (C) qRT-PCR assays were performed to examine HOXA11-AS expression in the nuclear and cytoplasmic fractions of hESCs transfected with snoVector or HOXA11-AS-OE (n=3, Student's t-test). Error bars represent SEMs, and the data represent at least 3 independent experiments. ***: $p < 0.001$, **: $p < 0.01$.



Cluster	Gene	Score	Type	Symbol
1	1994	1.5	Clustered	ELAVL1
1	6426	1.5	Clustered	SRSF1
1	5725	1.5	Seed	PTBP1
1	2521	1.5	Clustered	FUS

Figure S3. Prediction of potential binding proteins of HOXA11-AS

(A) Venn diagram illustrating the intersections of potential interacting proteins of HOXA11-AS predicted by Annolnc, ENCORI, RBPDB, and starBase. (B) Pathway enrichment analysis of 11 potential interacting proteins at least shared by two databases of Annolnc, ENCORI, RBPDB, and starBase. (C) Four proteins (ELAVL1, SRSF1, PTBP1, and FUS) aggregated into one MCODE network using Metascape software. In particular, the PTBP1 protein played a seed role in the network. (D) qRT-PCR showing relative PTBP1 mRNA levels after transfection with NC or siPTBP1 (n=3, Student's t-test). (E) Representative Western blotting images and statistical analysis of PTBP1 after transfection with NC or siPTBP1 (n=3, Student's t-test). Error bars represent SEMs, and the data represent at least 3 independent experiments. **: $p < 0.01$, *: $p < 0.05$.

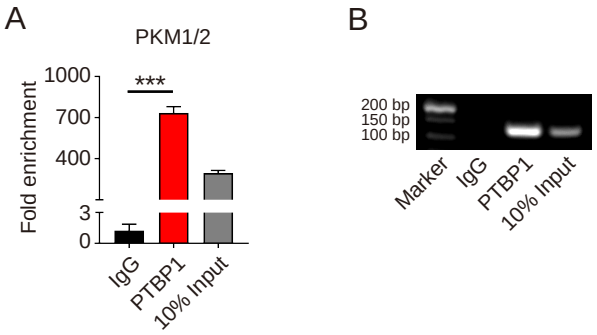


Figure S4. PTBP1 binds to PKM1/2 mRNA

(A) Enrichment of PKM1/2 mRNA by PTBP1 RIP in hESCs was determined by qRT-PCR. Normal rabbit Immunoglobulin G (IgG) was used as a negative control (n=3, One-way ANOVA, Bonferroni test). (B) Agarose gel electrophoresis of RIP qRT-PCR products. Error bars represent SEMs, and the data represent at least 3 independent experiments. ***: $p < 0.001$.

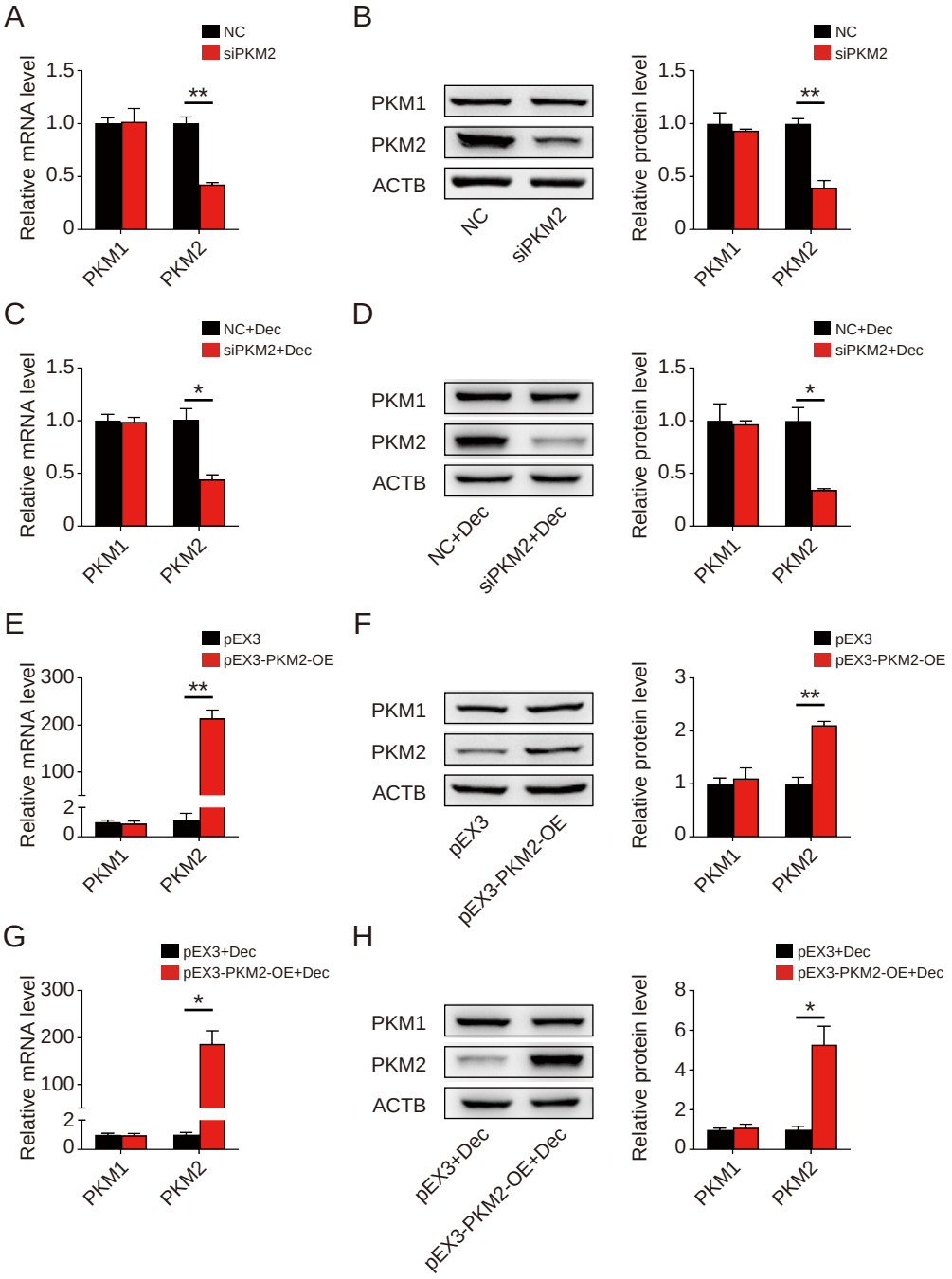


Figure S5. Knockdown and overexpression efficiency of PKM2 in hESCs

(A) qRT-PCR showing relative PKM1 and PKM2 mRNA levels after transfection with NC or siPKM2 (n=4, Student's t-test). (B) Representative Western blotting images and statistical analysis of PKM1 and PKM2 in hESCs after transfection with NC or siPKM2 (n=4, Student's t-test). (C) qRT-PCR showing relative PKM1 and PKM2 mRNA levels after transfection with NC or siPKM2 under *in vitro* decidualization for 4 days (n=3, Student's t-test). Dec: decidualization. (D) Representative Western blotting images and statistical analysis of PKM1 and PKM2 in hESCs after transfection with NC or siPKM2 under *in vitro* decidualization for 4 days (n=3, Student's t-test). (E) qRT-PCR results of relative PKM1 and PKM2 mRNA levels after transfection with pEX3 or pEX3-PKM2-OE (n=3, Student's t-test). (F) Representative Western blotting images and statistical analysis of PKM1 and PKM2 after transfection with pEX3 or pEX3-PKM2-OE (n=3, Student's t-test). (G) qRT-PCR results of relative PKM1 and PKM2 mRNA levels after transfection with pEX3 or pEX3-PKM2-OE under *in vitro* decidualization for 4 days (n=3, Student's t-test). (H) Representative Western blotting images and statistical analysis of PKM1 and PKM2 after transfection with pEX3 or pEX3-PKM2-OE under *in vitro* decidualization for 4 days (n=3, Student's t-test). Error bars represent SEMs, and the data represent at least 3 independent experiments. **: $p < 0.01$, *: $p < 0.05$.

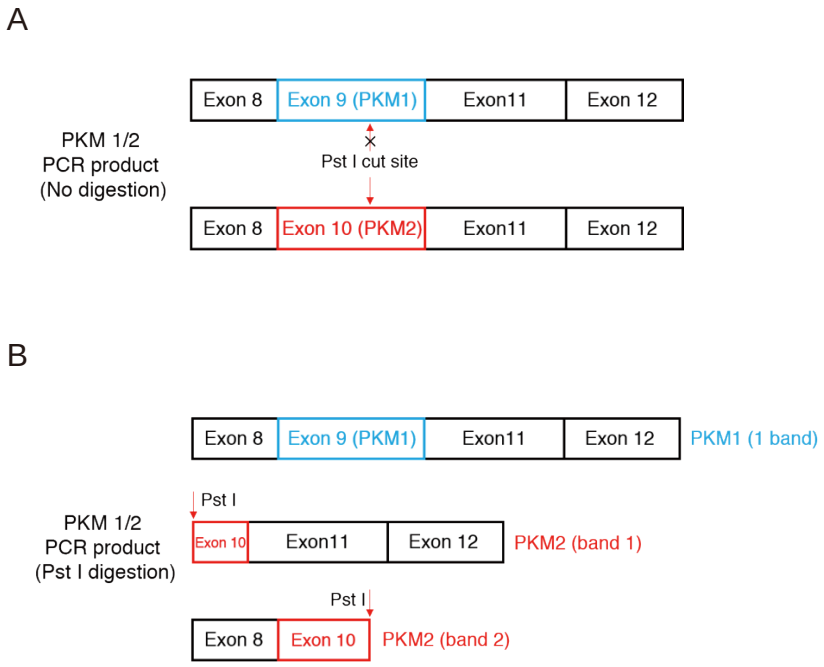


Figure S6. The schematic diagram of PKM alternative splicing analysis
(A) PKM 1/2 PCR product using universal primers without PstI digestion. (B) one band for PKM1 and two bands for PKM2 after PstI digestion.

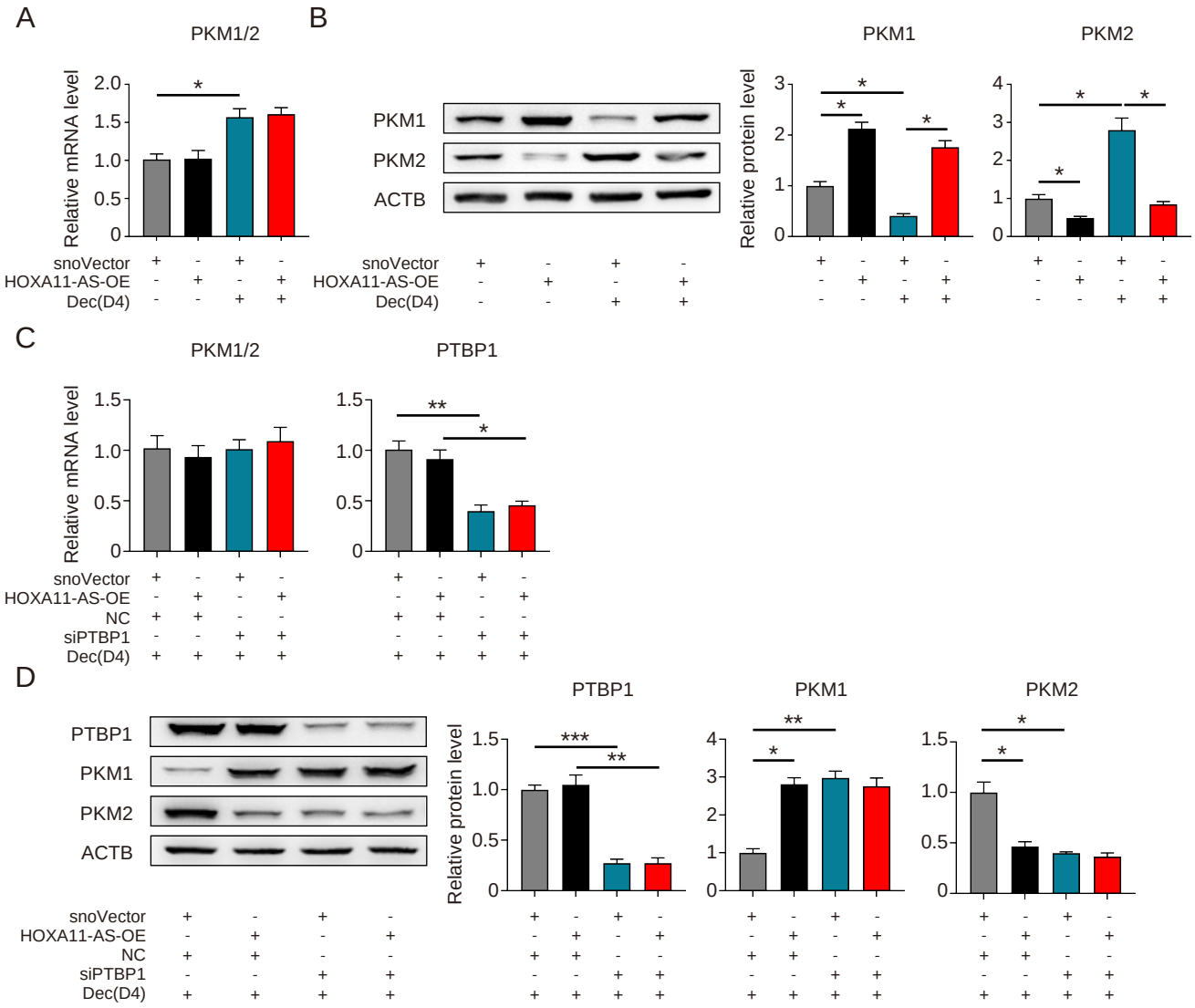


Figure S7. HOXA11-AS regulates PKM1 and PKM2 dependent on PTBP1

(A) qRT-PCR showing relative mRNA levels of PKM1/2 after transfection with snoVector or HOXA11-AS-OE with or without *in vitro* decidualization (Dec) for 4 days (n=7, One-way ANOVA, Bonferroni test). (B) Representative Western blotting images and statistical analysis of PKM1 and PKM2 after transfection with snoVector or HOXA11-AS-OE with or without *in vitro* decidualization for 4 days. (n=4, One-way ANOVA, Bonferroni test). (C) qRT-PCR showing relative mRNA levels of PKM1/2 and PTBP1 after PTBP1 knockdown or HOXA11-AS overexpression as indicated with *in vitro* decidualization for 4 days (n=4, One-way ANOVA, Bonferroni test). (D) Representative Western blotting images and statistical analysis of PTBP1, PKM1 and PKM2 after PTBP1 knockdown or HOXA11-AS overexpression as indicated with *in vitro* decidualization for 4 days (n=4, One-way ANOVA, Bonferroni test). Error bars represent SEMs, and the data represent at least 3 independent experiments. ***: $p < 0.001$, **: $p < 0.01$, *: $p < 0.05$.

Table S1. Potential HOXA11-AS interacting proteins predicted by 4 databases

Database (number)	Potential interacting proteins
AnnoLnc (5)	FMR1, PTBP1, CPSF1, CSTF2T, SRSF1
ENCORI (42)	CNBP, CSTF2T, DDX54, DGCR8, DICER1, DKC1, EIF4A3, ELAVL1, EWSR1, FBL, FMR1, FUS, HNRNPA1, HNRNPC, HNRNPK, IGF2BP3, KHDRBS2, LARP4B, LIN28, LIN28A, MOV10, NOP56, NOP58, NUMA1, PTBP1, QKI, RANGAP1, RBFOX2, RBM10, RNF219, RTCB, SRSF1, SRSF3, SRSF7, TIAL1, U2AF2, UPF1, VIM, YTHDC1, YTHDF1, ZFP36, ZNF184
RBPDB (21)	NCL, SNRPA, NONO, PABPC1, RBMY1A1, a2bp1, EIF4B, FUS, Pum2, SFRS9, MBNL1, Vtsl, KHSRP, YBX1, YTHDC1, RBMX, SFRS13A, RBM4, SFRS1, ELAVL1, KHDRBS3
starBase (12)	HuR, PTB, IGF2BP3, eIF4AIII, DGCR8, FMRP, FUS, C22ORF28, FUS-mutant, U2AF65, TIAL1, UPF1

Table S2. Baseline characteristics of LH+2 and LH+7 patients

Clinical features	LH+2 (n=4)	LH+7 (n=4)	p value
Age (years)	26.00 ± 3.65	29.25 ± 2.99	0.217
BMI (kg/m^2)	21.25 ± 1.60	20.06 ± 1.31	0.295
Basal FSH level (IU/L)	5.80 ± 1.55	7.58 ± 2.37	0.257
Basal LH level (IU/L)	3.65 ± 0.85	3.83 ± 1.39	0.827
Basal E2 level (pg/ml)	68.04 ± 26.14	54.50 ± 35.52	0.562

The data are presented as mean ± SD and analyzed with Student's t-test. BMI, body mass index; FSH, follicle stimulating hormone; LH, luteinizing hormone; E2, estradiol.

Table S3. Baseline characteristics of control and recurrent implantation failure patients

Clinical features	CTRL (n=11)	RIF (n=11)	p value
Age (years)	27.64 ± 1.91	28.55 ± 2.30	0.325
BMI (kg/m^2)	20.63 ± 2.54	21.98 ± 2.44	0.219
Basal FSH level (IU/L)	6.17 ± 0.88	5.75 ± 1.49	0.432
Basal LH level (IU/L)	4.50 ± 1.46	5.44 ± 2.13	0.241
Basal E2 level (pg/ml)	38.42 ± 8.98	36.92 ± 10.18	0.718

The data are presented as mean ± SD and analyzed with Student's t-test. CTRL, control; RIF, recurrent implantation failure; BMI, body mass index; FSH, follicle stimulating hormone; LH, luteinizing hormone; E2, estradiol.

Table S4. Primer sequences

Gene name (species)	Forward primer sequence (5'-3')	Backward primer sequence (5'-3')
ACTB (Homo sapiens)	CATGTACGTTGCTATCCAGGC	CTCCTTAATGTCACGCACGAT
HOXA11-AS (Homo sapiens)	CACCCATCTGCCTGGTCTTCTG	GGCTAAGCTCGGCTGTTGGAC
EPCAM (Homo sapiens)	AATCGTCAATGCCAGTGTACTT	TCTCATCGCAGTCAGGATCATAA
VIM (Homo sapiens)	AGTCCACTGAGTACCGGAGAC	CATTTCACGCATCTGGCGTTC
U6 (Homo sapiens)	CTCGCTTCGGCAGCACA	AACGCTTCACGAATTTGCGT
PRL (Homo sapiens)	GGAGCAAGCCCAACAGATGAA	GGCTCATTCCAGGATCGCAAT
IGFBP1 (Homo sapiens)	TTGGGACGCCATCAGTACCTA	TTGGCTAAACTCTCTACGACTCT
HOXA10 (Homo sapiens)	CTTCCGAGAGCAGCAAAGCCTC	TCCAGTGTCTGGTGCTTCGTGT
HOXA11 (Homo sapiens)	TGCCAAGTTGTACTTACTACGTC	GTTGGAGGAGTAGGAGTATGTCA
EMX2 (Homo sapiens)	GCTCATCCACCGCTACCGATAT	TTCTCAAAGGCGTGTTCCAGCC
ITGB3 (Homo sapiens)	CATGGATTCCAGCAATGTCCTCC	TTGAGGCAGGTGGCATTGAAGG
LIF (Homo sapiens)	AGATCAGGAGCCAACTGGCACA	GCCACATAGCTTGTCCAGGTTG
MMP2 (Homo sapiens)	AGCGAGTGGATGCCGCCTTTAA	CATTCCAGGCATCTGCGATGAG
MMP9 (Homo sapiens)	GCCACTACTGTGCCTTTGAGTC	CCCTCAGAGAATCGCCAGTACT
PTBP1 (Homo sapiens)	AGCGCGTGAAGATCCTGTTC	CAGGGGTGAGTTGCCGTAG
PKM1/2 (Homo sapiens)	CATTGATTCACCACCCATCA	AGACGAGCCACATTCATTCC
PKM1 (Homo sapiens)	GGACTATCCTCTGGAGGCTGTG	CATGAGGTCTGTGGAGTGACTTG
PKM2 (Homo sapiens)	TCTGGAGAAACAGCCAAAGGG	GGGGTCGCTGGTAATGGG
PKM (Homo sapiens) for PCR and PstI digestion	GAGAAACAGCCAAAGGGGACTATC	CATCACGGCACAGGAACAACAC

Table S5. siRNAs sequences used for transfection

Gene name (species)	Sense（5'-3'）	Antisense（5'-3'）
NC (Homo sapiens)	UUCUCCGAACGUGUCACGUTT	ACGUGACACGUUCGGAGAATT
PKM2 (Homo sapiens)	UCCUUCAAGUGCUGCAGUGTT	CACUGCAGCACUUGAAGGATT
PTBP1 (Homo sapiens)	GCACAGUGUUGAAGAUCAUTT	AUGAUCUUCAACACUGUGCTT

Table S6. Primary antibodies used for Western blotting

Protein	Manufacturer	Catalog number	Dilution
HOXA10	Santa Cruz Biotechnology	sc-17158	1:200
HOXA11	Santa Cruz Biotechnology	sc-393440	1:100
PTBP1	Cell Signaling Technology	57246	1:2000
Lamin A/C	Cell Signaling Technology	4777	1:2000
PKM1	Cell Signaling Technology	7067	1:1000
PKM2	Cell Signaling Technology	4053	1:1000
GAPDH	Proteintech Group Inc	60004-1-Ig	1:10000
ACTB	Proteintech Group Inc	20536-1-AP	1:10000