Downregulated IncRNA HOXA11-AS Affects Trophoblast Cell Proliferation and Migration by Regulating RND3 and HOXA7 Expression in PE

Yetao Xu,1,2,7 Dan Wu,1,7 Jie Liu,3,7 Shiyun Huang,1 Qing Zuo,1 Xi Xia,4 Ying Jiang,5 Sailan Wang,1 Yanzi Chen,6 Tianjun Wang,1 and Lizhou Sun1

1Department of Obstetrics and Gynecology, First Affiliated Hospital of Nanjing Medical University, Nanjing 210029, Jiangsu Province, China; 2Department of Obstetrics, Gynecology and Reproductive Sciences, Yale Stem Cell Center, Yale University School of Medicine, New Haven, CT 06510, USA; 3Department of Obstetrics and Gynecology and Reproduction Center, Xuzhou Maternity and Child Health Care Hospital, Xuzhou 221000, China; 4Department of Obstetrics and Gynecology, Center for Reproductive Medicine, Peking University Shenzhen Hospital, FuTian District, Shenzhen, Guangdong, China; 5Department of Obstetrics, Women’s Hospital, School of Medicine, Zhejiang University, Hangzhou, China; 6Department of Emergency, First Affiliated Hospital of Nanjing Medical University, Nanjing 210029, Jiangsu Province, China

The long noncoding RNA HOXA11-AS displays abnormal expression in numerous human diseases. However, its function and biological mechanisms remain unclear in preeclampsia (PE). In this study, we report that HOXA11-AS is significantly downregulated in preeclamptic placental tissues and could contribute to the occurrence and development of PE. Silencing of HOXA11-AS expression could significantly suppress trophoblast cell growth and migration, whereas HOXA11-AS overexpression facilitated cell growth in the HTR-8/SVneo, JEG3, and JAR cell lines. RNA-seq analysis also indicated that HOXA11-AS silencing preferentially regulated numerous genes associated with cell proliferation and cell migration. Mechanistic analyses showed that HOXA11-AS could recruit Ezh2 and Lsd1 protein and regulate RND3 mRNA expression in the nucleus. In the cytoplasm, HOXA11-AS modulates HOXA7 expression by sponged miR-15b-5p, affecting trophoblast cell proliferation. Together, these data confirm that aberrant expression of HOXA11-AS is involved in the occurrence and development of PE and may act as a prospective diagnosis and therapeutic target in PE.

INTRODUCTION
Preeclampsia (PE), characterized by blood pressure higher than 140/90 mmHg after 20 weeks of pregnancy, is a major contributor of pregnancy-related death and fetal morbidity. PE afflicts nearly 3%–5% of pregnancies, especially in developing countries.1,2 Despite the considerable morbidity and mortality, the cause of PE has been a mystery. Delivery of the placenta is the only known remedy for PE;3 other effective prevention strategies have not yet been developed. The preferential clinical treatment for PE is to use a combination of labetalol with magnesium sulfate to slow down the progression of this disorder and extend the pregnancy period. After in-depth study, PE, which results from the aberrant expression of numerous PE-associated genes,4–7 could be considered a heterogeneous disease with diverse clinical and molecular characteristics. Therefore, a deeper understanding of the biological mechanisms in PE might furnish more options for diagnosis and treatment.

Long noncoding RNAs (lncRNAs), which are longer than 200 bp with little or no protein-coding capacity, have intrinsic function as RNA.5,9 Recently, technological advances have allowed the analysis of lncRNAs in diverse human diseases. Emerging studies have demonstrated that lncRNAs have been implicated in a variety of biological and pathological processes, including cell differentiation,10 cell metabolism,11 immune response,12 and disease-associated development.13–15 Additionally, aberrant levels of lncRNAs have been reported that positively or negatively affect gene expression in diverse human diseases, including PE.16–19 Furthermore, a lot of studies have demonstrated that lncRNAs could act as significant regulatory molecules to regulate related gene expression at different levels, such as chromatin modification and transcriptional and post-transcriptional modification.16–20 For instance, the lncRNA CCAT1 modulates SPRY4 and HOXB13 expression by binding to SUV39H1 (suppressor of variegation 3–9 homolog 1) and EZH2 (enhancer of zeste 2 polycomb repressive complex 2 subunit) to affect cell growth and migration in esophageal squamous cell carcinoma.21 Apart from their role in gene expression regulation, lncRNAs can also crosstalk with associated gene expression by competing for shared microRNAs (miRNAs) at post-transcriptional levels to affect the occurrence and development of various diseases.22,23

HOXA11-AS, a 1,628-bp IncRNA gene located on chromosome 7p15.2, plays a significant role in various disorders.24–27 For instance,
HOXA11-AS can promote cell growth and invasion of gastric cancer by interacting with EZH2 and LSD1 (histone demethylase lysine-specific demethylase 1). In addition, HOXA11-AS can compete for shared miR-140-5p to promote glioma tumorigenesis. However, the biological functions of HOXA11-AS in PE remain unclear, which impels us to further explore the role and molecular mechanism of HOXA11-AS in PE.

In this study, we demonstrated that the expression level of HOXA11-AS was significantly downregulated in preeclamptic placental tissues compared with normal tissues. Furthermore, knockdown of HOXA11-AS could impair cell growth and migration in various trophoblast cell lines. Associated mechanistic exploration demonstrated that HOXA11-AS could exhibit different regulatory mechanisms in regulation of RND3 and HOXA7 expression in the nucleus and cytoplasm, thus being involved in the occurrence and development of PE. Unraveling the role of HOXA11-AS will provide novel insights for future PE treatments.

RESULTS

HOXA11-AS Is Downregulated in Human Preeclamptic Tissues

The expression level of HOXA11-AS was analyzed in 60 preeclamptic tissues and normal tissue samples by qRT-PCR. We found that the HOXA11-AS expression was significantly downregulated in preeclamptic tissues (Figure 1A). Furthermore, as shown in Figures 1B and 1C, HOXA11-AS expression levels also indicated a positive correlation with gestational age and the body weight of infants in the PE group. The detailed clinical characteristics of the patients who meet the criteria are listed in Table 1. In addition, we discovered that there were no significant differences between PE and the normal in gestational age and maternal age (p > 0.05). On the contrary, there were significant differences in systolic blood pressure, and body weight of infants between PE and the normal (p < 0.05).

HOXA11-AS Regulates Trophoblast Cell Proliferation and Migration In Vitro

Because human IncRNAs play essential roles in various cellular processes, we detected the expression of HOXA11-AS in four trophoblast cell lines and another two cell lines related to pregnancy, including HTR-8/SVneo, BeWo, JEG-3 and JAR, WISH, and HUVEC-C. As shown in Figure 1D, we found that the relative HOXA11-AS level in HTR-8/SVneo cells was higher than that in other cell lines, whereas the expression levels of HOXA11-AS in the BeWo, JEG3, and JAR cell lines were relatively lower compared with those in the WISH and HUVEC-C cell lines.

To explore the potential role of HOXA11-AS in trophoblast cells, we used an overexpression and knockdown model of HOXA11-AS in vitro. It was found that the expression levels of HOXA11-AS were exogenously influenced by specific small interfering RNAs (siRNAs) and overexpression plasmids in the HTR-8/SVneo, JEG3, and JAR cell lines (Figures 1E and 1F). Then we performed 3-(4,5)-dimethylthiazol-2-yl)-2,5-di-phenyltetrazolium bromide (MTT) and colony formation assays to illustrate the effect of HOXA11-AS...
on the proliferation of HTR-8/SVneo, JEG3, and JAR trophoblast cells. The resulting data revealed that silencing of HOXA11-AS significantly retarded cell growth compared with controls, whereas upregulation of HOXA11-AS could enhance cell proliferation (Figures 2A and 2B). In addition, ethynyl deoxyuridine (EdU) staining assays and bromodeoxyuridine (BrdU) assays also demonstrated that HOXA11-AS knockdown inhibited trophoblast cell proliferation; however, HOXA11-AS overexpression boosted the rate of proliferating trophoblast cells (Figures 2C and 2D). These data indicate that downregulated HOXA11-AS might play a role as a suppressor in the inhibition of trophoblast cell proliferation.

Furthermore, transwell assays confirmed that silencing of HOXA11-AS significantly inhibited trophoblast cell migration and invasion compared with si-normal control (NC) treatment (Figure 3A). In contrast, upregulated HOXA11-AS could stimulate cell migration and invasion (Figure 3A).

### The Effect of HOXA11-AS on the Cell Cycle and Apoptosis

#### In Vitro

Because cell proliferation assays cannot thoroughly reflect cell cycle changes, we next performed flow cytometry analysis to detect whether cell cycle progression was affected after HOXA11-AS knockdown. The results revealed that cells transfected with specific siRNAs promoted cell accumulation in G0–G1 phase compared with those treated with si-normal control (NC). In contrast, HOXA11-AS overexpression could reduce cell cycle accumulation in G0–G1 phase (Figure 3B).

Also, flow cytometry assays were performed to investigate whether knockdown of HOXA11-AS affected cell apoptosis. The results showed that the ratio of total apoptotic cells was dramatically increased in HTR-8/SVneo cells transfected with siRNAs (Figure 3C). Similarly, we conducted flow cytometry assays to detect the protein expression level of cleaved caspase, which further confirmed that the apoptosis level was increased after HOXA11-AS knockdown (Figure 3D). These findings suggest that HOXA11-AS promotes proliferation and inhibits apoptosis in trophoblast cells.

### Gene Expression Profiling

To investigate the HOXA11-AS-associated pathway on an unbiased basis in PE, we conducted RNA sequencing (RNA-seq) and evaluated the gene expression profiles of HTR-8/SVneo cells transfected with siRNAs against HOXA11-AS. After knockdown of HOXA11-AS, 131 mRNAs showed at least a 2-fold increased abundance, whereas a total (≤2-fold) of 99 genes showed decreased abundance (Figure 4A). Gene ontology (GO) analysis showed that many significant biological processes were involved in cell proliferation, migration, as well as apoptosis (Figure 4B). Among all enriched genes, there are many well-known proliferation-related and migration-associated genes, such as TNFSF9, TFPI2, CA9, IFTTM1, TMEM158, RND3, ESM1, NAMPT1, HOXA7, PSAT1, C4A, MEST, OLR1, etc. We suspected that some altered genes might induce the occurrence and development of PE. The expression changes of these genes, therefore, were selectively demonstrated by qRT-PCR in HOXA11-AS-depleted and/or HOXA11-AS-overexpressing HTR-8/SVneo cells and HOXA11-AS-overexpressing JAR cells (Figures 4C and 4D). RND3 and HOXA7 have been identified as candidate factors involved in cell proliferation, apoptosis, and migration; therefore, we selected RND3 and HOXA7 for further study.

### HOXA11-AS Could Recruit Lsd1 and Ezh2 in the Nucleus, Epigenetically Silencing RND3

To further explore the potential biological mechanisms of HOXA11-AS-mediated regulation in trophoblast cells, we first performed subcellular fractionation assays to assess the distribution of HOXA11-AS in nuclear and cytoplasmic fractions in HTR/SVneo, JEG-3, and JAR cells. As shown in Figure 5A, approximately 70% of HOXA11-AS is located in the trophoblast nucleus, and 30% of HOXA11-AS is in the cytoplasm. Therefore, these findings indicated that HOXA11-AS might play an essential role in transcriptional regulation. We employed bioinformatics analysis to predict possible RNA binding proteins, including Ezhi (trimethylation of histone 3 lysine 27 [H3K27me3]), Suz12 (H3K27me3), Lsd1 (dimethylation of histone 3 lysine 4 [H3K4me2]), Dnmt1, Hur, Stau1, and Aog2 (http:// pridb.gdcb.iastate.edu/RPISeq/references.php). As shown in Figure 5B, HOXA11-AS may interact with Ago2, Ezhi2 (H3K27me3), and Lsd1 (H3K4me2) in trophoblast cells. Previous studies have reported that HOXA11-AS can recruit Ezhi2 and Lsd1 to epigenetically silenced targets in many tumor cell lines.

To examine the interaction probabilities of HOXA11-AS with target proteins, we performed RNA immunoprecipitation (RIP) assays with these antibodies. There was a substantial enrichment in RIPs of Ezhi2, Lsd1, and Aog2 in HTR-8/SVneo cells (Figure 5C). We also conducted RIP assays in JEG3 and IAR cells that were transfected with an overexpression plasmid of HOXA11-AS. Our results demonstrated that HOXA11-AS directly interacted with Ezhi2, Lsd1, and Aog2 (Figure 5C). Furthermore, RNA pulldown assays further confirmed that HOXA11-AS could interact with Ezhi2, Lsd1, and Aog2 in

| Table 1. Clinical Characteristics of Preeclamptic and Normal Pregnancies |
|-----------------|-----------------|-----------------|
| Variable        | PE (n = 60)     | Normal (n = 60) |
| Maternal age (year) | 32.23 ± 4.526   | 34.62 ± 3.376   |
| p Value (Normal versus Preeclamptic) | p > 0.05         | p < 0.05         |
| Maternal weight (kg) | 75.99 ± 10.995  | 74.46 ± 7.979   |
| Smoking         | 0               | 0               |
| p Value (Normal versus Preeclamptic) | p > 0.05         | p > 0.05         |
| Systolic blood pressure (mmHg) | 162.83 ± 15.103 | 116.6 ± 7.983   |
| p Value (Normal versus Preeclamptic) | p < 0.01         | p < 0.01         |
| Diastolic blood pressure (mmHg) | 106.417 ± 10.803 | 71.54 ± 8.767   |
| p Value (Normal versus Preeclamptic) | p < 0.01         | p < 0.01         |
| Proteinuria (g/day) | >0.3            | <0.3            |
| p Value (Normal versus Preeclamptic) | p > 0.05         | p < 0.05         |
| Body weight of infant (g) | 2,287.33 ± 908.078 | 3,385.42 ± 372.269 |
| p Value (Normal versus Preeclamptic) | p > 0.05         | p < 0.05         |
| Gestational age (weeks) | 33.93 ± 3.231   | 38.38 ± 0.958   |
| p Value (Normal versus Preeclamptic) | p < 0.05         | p < 0.05         |
HTR-8/SVneo cells (Figure 5D). These data demonstrated that HOXA11-AS could directly bind Lsd1, Ezh2, and Ago2 in trophoblast cells. Previous studies have reported that Lsd1 and Ezh2 are negative regulators of transcription via H3K4me2 and H3K27me3, respectively. Therefore, we further explored the mechanism of correlation among Ezh2, Lsd1, and HOXA11-AS using experimental methods.

We first suppressed the expression of Lsd1 and Ezh2 with effective siRNAs. The protein level of RND3 was significantly upregulated after transfection with EZH2 siRNAs and/or LSD1 siRNAs in HTR-8/SVneo cells (Figure 5E). Then we hypothesized that HOXA11-AS may recruit Ezh2 and Lsd1 to the RND3 promoter region, resulting in H3K27me3 and/or H3K4me2 in this region. Therefore, we performed chromatin immunoprecipitation (ChIP) assays to detect the enrichment of Ezh2 and H3K27me3 and Lsd1 and H3K4me2 in the promoter region of RND3. As shown in Figure 5F, the results showed that Lsd1 and Ezh2 protein could be directly recruited to the promoter region of the RND3 gene.
silencing HOXA11-AS. Silencing of HOXA11-AS further suppressed Ezh2-mediated H3K27me3 demethylation and Lsd1-mediated H3K4me2 demethylation.

Our previous studies have shown that the expression level of RND3 is significantly increased in preeclamptic placental tissues compared with the controls. In this study, we also found that overexpression of RND3 could inhibit cell proliferation in the HTR-8/SVneo and JEG3 cell lines (Figures 6A–6C). Overexpression of RND3 could also partly reverse HOXA11-AS-mediated growth promotion (Figures 6D and 6E). Together, these data suggest that HOXA11-AS-mediated cell growth could be reversed partly through epigenetic suppression of RND3 by binding to Ezh2 and Lsd1 in the nucleus of trophoblast cells.

HOXA11-AS Promotes HOXA7 Expression by Sponged miR-15b-5p, Affecting Trophoblast Cell Proliferation

Based on the RNA-seq analysis, numerous genes affecting cell phenotype were downregulated after silencing of HOXA11-AS. HOXA7, part of the cluster on chromosome 7, could promote cell proliferation and migration in various cell lines. We next performed western blotting assays to further confirm the RNA-seq results. As shown in Figure 7A, we found that the protein level of HOXA7 was significantly upregulated after HOXA11-AS overexpression, whereas the opposite

Figure 3. HOXA11-AS Regulates Trophoblast Cell Migration, the Cell Cycle, and Apoptosis In Vitro

(A) Trophoblast cells were treated with specific HOXA11-AS siRNAs and/or an overexpression plasmid. Transwell assays were used to investigate the changes in migratory and invasion abilities of trophoblast cells after transfection. (B) Cell cycle analysis by flow cytometry in HTR-8/SVneo and JEG-3 cells. (C) Flow cytometry was used to detect the apoptotic rates of cells. LR, early apoptotic cells; UR, terminal apoptotic cells. (D) Flow cytometry assays to detect the protein expression level of cleaved caspase after transfection with siRNAs against HOXA11-AS in HTR-8/SVneo cells. All experiments were performed in biological triplicates with three technical replicates. *p < 0.05; **p < 0.01; n.s., not significant.)
result was found after HOXA11-AS knockdown in HTR-8/SVneo cells. An increasing number of studies have reported that lncRNAs could compete for specific miRNAs in the cytoplasm to mediate mRNA expression, further affecting cell phenotype. There is evidence that miRNAs are found predominantly in the cytoplasm by binding to Ago2, which is the fundamental element of the RNA-induced silencing complex. Based on subcellular fractionation assays and RIP assays (Figure 5C), we found that 30% of HOXA11-AS is distributed in the cytoplasm and that HOXA11-AS could interact with Ago2 protein. Then we hypothesized that HOXA11-AS modulates HOXA7 expression levels by sponging specific miRNA in HTR-8/SVneo cells.

To validate this concept, we first conducted bioinformatics analysis and found that miR-15b-5p was predicted to bind to the 3’ UTR of the downstream target gene of both HOXA11-AS and HOXA7 (Figure 7B). Then we performed qRT-PCR, and the results showed that silencing of HOXA11-AS significantly upregulated the expression of miR-15b-5p; in contrast, the miR-15b-5p level was decreased after HOXA11-AS overexpression. Further experiments indicated that the RNA and protein levels of HOXA11-AS and HOXA7 were significantly reduced after transfection with miR-15b-5p mimics (Figure 7C). Next we further explored the functions of miR-15b-5p in trophoblast cells. Diverse luciferase genes, including HOXA11-AS, mutant HOXA11-AS, the 3’ UTR of HOXA7, and the mutant 3’ UTR of HOXA7, were cloned and then co-transfected with miR-15b-5p in HTR-8/SVneo cells. Interestingly, we found that the relative luciferase activity of reporters of HOXA11-AS and the 3’ UTR of HOXA7 were significantly abolished after treatment with miR-15b-5p (Figure 7D). In contrast, the relative luciferase activity on mutant reporters of both HOXA11-AS and the 3’ UTR of HOXA7 showed no effect after treatment with miR-15b-5p (Figure 7D). Therefore, these data demonstrate that miR-15b-5p can bind to both HOXA11-AS and the HOXA7 gene. Functional research further indicated that overexpression of miR-15b-5p could stimulate trophoblast cell growth and migration (Figure S1). In parallel, MTT and BrdU assays also indicated that knockdown of HOXA7 could inhibit proliferation in HTR-8/SVneo cells and that silencing of HOXA7 could reverse HOXA11-AS-induced cell proliferation (Figure 7E).
downregulation of HOXA11-AS is positively related to early termination of pregnancy, which further suggests that downregulation of HOXA11-AS might lead to severe PE. Our data also revealed that knockdown of HOXA11-AS could impair trophoblast cell proliferation and migration in vitro, whereas HOXA11-AS overexpression could promote cell proliferation and migration. To investigate the HOXA11-AS -related pathway and downstream genes in PE, we conducted RNA transcriptome sequencing after transfecting a target cell line with specific siRNAs against HOXA11-AS; GO analysis suggested that gene expression profiles were primarily proliferation- and

Figure 5. HOXA11-AS Can Recruit EZH2 and LSD1 to Silence RND3 Expression
(A) Cell fractionation assays indicated that HOXA11-AS is mostly located in the nucleus. GAPDH and U1 acted as markers of the cytoplasm and nucleus, respectively. (B) Bioinformatics were used to predict the possibility of interaction of HOXA11-AS. Predictions with probabilities >0.5 were considered positive. RIPSeq predictions are based on random forest (RF) or support vector machine (SVM). (C) RIP experiments were performed, and the coprecipitated RNA was detected by qRT-PCR. (D) In vitro-transcribed pulldown assays showed that HOXA11-AS could retrieve EZH2 and LSD1 in HTR-8/SVneo cells but not G9a. G9a was a negative control. (E) Western blot assays detected the expression of RND3 after silenced EZH2 after si-RNA transfection in HTR-8/SVneo cells. (F) The enrichment of EZH2/H3K27me3 and LSD1/H3K4me2 in the promoter regions of RND3 was identified via ChIP assays, and this enrichment was decreased after HOXA11-AS knockdown in the HTR-8/SVneo cell line. Antibody directed against immunoglobulin G (IgG) was used as a negative control. Values represent the mean ± SEM from three independent experiments. **p < 0.01, *p < 0.05.
migration-associated. Previous studies have determined that many lncRNAs can bind with various chromatin-modifying enzymes to regulate related gene expression at the epigenetic level. For instance, the pseudogene DUXAP10 promotes an aggressive phenotype through binding with LSD1 to repress LATS2 and RRAD in non-small-cell lung cancer, and the lncRNA TUG1 is involved in cell proliferation of small-cell lung cancer by regulating LIMK2 via EZH2. Our resulting data also revealed that HOXA11-AS could recruit and bind to two histone methylation modification complexes, including EZH2 and LSD1 in the nucleus, silencing genes expression. Further experiments were performed, indicating that these targets were affected by promoter H3K4me2 demethylation and H3K27me3 demethylation in trophoblast cells.

RND3/RhoE is a small GTPase that can exhibit biological functions as a suppressor gene in numerous diseases, inhibiting multiple cellular processes such as actin cytoskeleton dynamics, the cell cycle, migration, invasion, and apoptosis. However, the functions of RND3 in the pathological process of PE is still unclear. Our results revealed that HOXA11-AS can contribute to the downregulated expression of RND3 by histone methylation in trophoblast cells. These results suggest that HOXA11-AS can bind to Ezh2 and Lsd1, epigenetically silencing RND3 in the nucleus of trophoblast cells.

HOX genes, a highly conserved subgroup of the homeobox superfamily, are spatially and temporally regulated during embryonic development. Abnormal expression of HOXB7 and HOXB13 has been reported to regulate many processes, including apoptosis, receptor signaling, and differentiation, in a myriad of disorders. In our study, we found that low levels of HOXA7 could affect proliferation in trophoblast cell lines, which implies that the post-transcriptional regulation of HOXA7 is partly mediated by HOXA11-AS in development of PE through sponging miR-15b-5p in the cytoplasm, accelerating trophoblast cell growth. Furthermore, Sun et al. have
demonstrated that HOXA11-AS can promote gastric cancer tumorigenesis through sponging miR-1297. These findings demonstrated that HOXA11-AS simultaneously competes for miR-1297 and miR-15b-5p.

In brief, HOXA11-AS facilitates cell proliferation and migration by epigenetically regulating RND3 in the nucleus, and it can promote trophoblast cell growth through sponging miR-15b-5p in the cytoplasm. Our results indicate that HOXA11-AS can exhibit diverse biological regulatory mechanisms in PE (Figure 8). However, there are still many gaps in our current understanding of HOXA11-AS function and its biological mechanism in PE. Further studies, including clinical trials and animal research, are needed to elucidate whether HOXA11-AS might act as a prospective diagnosis and therapeutic target for PE.

MATERIALS AND METHODS

Tissue Samples and Ethics Statement

60 PE patients were selected for this study at the obstetrical department of the First Affiliated Hospital of Nanjing Medical University. Briefly, placenta tissue samples (about 1 cm × 1 cm × 1 cm in size) were taken from the central area of the placenta’s maternal surface to avoid necrosis and calcification and placed in embedding molds that contained optimal cutting temperature (OCT) medium, frozen over a dry ice or ethanol slurry, stored at −80°C, and subsequently used for RNA and protein extraction. All patients provided written informed consent. The clinical characteristics of the PE patients are shown in Table 1. This research was authorized by the Ethics Board of the First Affiliated Hospital of Nanjing Medical University, China, and it was performed in compliance with the Declaration of Helsinki principles.

Cell Culture

The HTR-8, JEG3, and JAR cell lines were purchased from the Institute of the Chinese Academy of Sciences (Shanghai, China). HTR-8 and JAR cells were cultured in RPMI 1640 medium (Gibco, Nanjing, China) supplemented with 10% fetal bovine serum (FBS) (Gibco, BRL, Invitrogen, Carlsbad, CA, USA), 100 U/mL penicillin, and 100 mg/mL streptomycin. JEG3 cells were cultured in MEM with 10% FBS. All cell lines were cultured in humidified air at 37°C/5% CO₂.

Plasmid Construction

Full-length cDNA of HOXA11-AS (1,628 bp, NR_002795.2), RND3 sequence (2807bp, NR_001254738) were synthesized and cloned into the pcDNA3.1(+) plasmid vector (Invitrogen). The resulting plasmids as well as the empty pcDNA3.1(+) vector were transfected into HTR-8/SVneo, JEG3, and JAR cells on 6-well plates and/or 24-well plates.

Cell Transfection

Lipofectamine 2000 and/or Lipofectamine 3000 transfection reagents (Life Technologies, Invitrogen, USA) were used to transfect the trophoblast cell lines with siRNAs targeting HOXA11-AS mRNA according to the manufacturer’s protocol. All siRNA sequences are
listed in Table S1. Interference target sequences of HOXA7 and RND3 were purchased from Invitrogen. The transfected cells, on 6-well plates, were harvested for further experiments 48 hr post-transfection.

RNA Extraction and qRT-PCR Analyses
Total RNA from each treatment was extracted using TRIzol reagent (Thermo Fisher Scientific), and qRT-PCR analyses were conducted using SYBR Green Master Mix (TaKaRa Bio, Otsu, Japan) according to the protocol. The sequences of specific primers used are shown in Table S1.

Subcellular Fractionation Location
The nuclear and cytosolic fractions were separated and purified using the PARIS kit (Life Technologies, Carlsbad, CA, USA) according to the manufacturer’s manual.

Cell Viability Assays
Cell viability was detected with an MTT kit (Sigma) following the manual. For the colony formation assay, 600, 800, or 1,000 cells treated with siRNAs and/or plasmid were plated on 6-well plates and maintained in proper medium containing 10% FBS for 10–14 days, during which the medium was replaced every 4 days. Colonies were then fixed with methanol and stained with 0.1% crystal violet (Sigma) in PBS for 30 min. Colony formation was determined by counting the number of stained colonies.

BrdU experiments were performed using a BrdU cell proliferation assay kit (Millipore, catalog no. 2750) following the protocol. The higher optical density (OD) reading represents the higher BrdU concentration in each sample.

The EdU assay was implemented as a complementary method to authenticate the proliferation level. We exploited the 5-ethyl-2-deoxyuridine labeling and detection kit (Ribobio, Guangzhou, China) to evaluate cell proliferation, following the manufacturer’s manual.

Flow Cytometric Analysis of Cell Cycle and Apoptosis
After transfecting cells with siRNAs or plasmid, we performed fluorescein isothiocyanate (FITC)-Annexin V and propidium iodide (PI) staining using the FITC-Annexin V Apoptosis Detection Kit (BD Biosciences, Franklin Lakes, NJ, USA) following the manufacturer’s instructions. Cell cycle level was determined by propidium iodide staining using the Cycle Test Plus DNA Reagent Kit (BD Biosciences, Franklin Lakes, NJ, USA), following the protocol, and analysis by FACScan. The ratio of the cells in G0/G1, S and G2/M phase were calculated and compared.

Transwell Assays
Cell Migration and invasion were detected and analyzed by transwell assays, as reported previously by Xu et al.34 24-well chambers were placed into the upper chamber of an insert with an 8-μm pore size polycarbonate membrane (Millipore, Billerica, MA, USA). Medium containing 15% FBS was added to the lower chamber. After incubation for 24–48 hr, the cells on the upper membrane were removed with a cotton swab. Cells that migrated or invaded through the polycarbonate membrane were stained with methanol and 0.1% crystal violet. The experiments were conducted three times.

Western Blot Assays
WB assays were conducted as reported previously by Xu et al.34 and the following primary antibodies were used: anti-EZH2, anti-LSD1, and anti-AGO2 (Millipore, USA) and anti-RND3 and anti-HOXA7 from Proteintech (Wuhan, China). GAPDH (Cell Signaling Technology, San Jose, CA, USA) antibody was used as the control.

RNA-Seq Bioinformatic Analysis
The RNA-seq experiments were conducted by the Wuhan Genomics Institute (Wuhan, China). Culture cells that were treated were extracted. To establish the mRNA-seq library, the cDNAs were fragmented by nebulization following the protocol.

ChIP
ChIP assays were conducted as described previously by Xu et al.34 According to the manual, experiments were performed using the EZ-CHIP kit (Millipore, USA). Relevant antibodies against H3K27me3, H3K4me2, and other target proteins were purchased from Millipore. The primer sequences of gene promoters are summarized in Table S1. Immunoprecipitated DNA were detected and analyzed by qRT-PCR. Experiments were repeated three times.

RIP Assays
RIP experiments were performed following the protocol of the Magna RIP RNA-Binding Protein Immunoprecipitation Kit (Millipore, USA). Antibodies, including EZH2, SUZ12, DNMT3a, DNMT3b, LSD1, AGO2, and STAU1, were purchased from Millipore.

Luciferase Reporter Assays
Luciferase reporter assays were performed as described previously by Zhang et al.11 HOXA11-AS and HOXA7 3′ UTR cDNA fragments

Figure 8. Proposed Model of Mediation by HOXA11-AS in Proliferation and Migration Progression of PE
were amplified by PCR assays and then subcloned downstream of the luciferase genes in the pGL3 plasmid. Mutant of plasmids, such as pGL3-HOXA7-3’ UTR MUT and pGL3-HOXA11-AS-MUT, were obtained by platinum pfX DNA polymerase according to the protocol. Luciferase activity was measured by the Dual-Luciferase Reporter Assay System (Promega, Madison, WI, USA). Briefly, 1 × 105 HTR-8/SVneo cells were plated in 24-well plates for 36 hr. 48 hr post-transfection, the cells were retrieved and lysed for further experiments. The relative luciferase activity was normalized with *Renilla* luciferase activity.

**Statistical Analysis**

All statistical analyses in our experiment were performed using SPSS 20.0 software (IBM, SPSS, USA). The resulting data are represented as the mean ± SD. Statistical significance was ascribed at *p* < 0.05 or **p* < 0.01. Each experiment was repeated at least three times independently.

**SUPPLEMENTAL INFORMATION**

Supplemental Information includes one figure, one table, and two data files and can be found with this article online at [https://doi.org/10.1016/j.omtn.2018.05.007](https://doi.org/10.1016/j.omtn.2018.05.007).

**AUTHOR CONTRIBUTIONS**

Y.X., D.W., and J.L. performed most experiments. S.H., Q.Z., and Y.J. collected clinic tissues and analyzed data. X.X., Y.C., and T.W. conducted some experiments. Y.X. and L.S. designed the project and edited the manuscript.

**ACKNOWLEDGMENTS**

This study was supported by the National Scientific Foundation of China (81471508 and 81771603), the Traditional Chinese Medicine Project of Jiangsu Province (ZX2016D2), the Natural Science Foundation of Jiangsu Province (projects BK20161061 and BK20171502), Key Disciplines of 13th Fifth-year Strong and Healthy Engineering in Jiangsu Province, and the Research Team of Female Reproductive Health and Fertility Preservation (SZSM201612065).

**REFERENCES**

1. Powe, C.E., Levine, R.J., and Karamanchi, S.A. (2011). Preeclampsia, a disease of the maternal endothelium: the role of antiangiogenic factors and implications for later cardiovascular disease. Circulation 123, 2856–2869.
2. MacKay, A.P., Berg, C.J., and Atrash, H.K. (2001). Pregnancy-related mortality from reproductive health and fertility preservation (SZSM201612065).
3. Bharadwaj, S., Bhat, V.B., Vickneswaran, V., Adhisivam, B., Zachariah, B., and Habeebullah, S. (2018). Oxidative stress in preeclamptic mother - newborn dyads. J. Reprod. Med. Fetal Neonatal Med. 31, 1548–1553.
4. Lu, L., Hou, Z., Li, L., Yang, Y., Wang, X., Zhang, B., Ren, M., Zhao, D., Miao, Z., Yu, L., and Yao, Y. (2014). Methylation pattern of H19 exon 1 is closely related to preeclampsia and trophoblast abnormalities. Int. J. Mol. Med. 34, 765–771.
5. Bourque, D.K., Avila, L., Péñaherrera, M., von Dadeletz, P., and Robinson, W.P. (2010). Decreased placental methylation at the H19/IGF2 imprinting control region is associated with normotensive intrauterine growth restriction but not preeclampsia. Placenta 31, 197–202.
6. Yu, L., Chen, M., Zhao, D., Yi, P., Lu, L., Han, J., Zheng, X., Zhou, Y., and Li, L. (2009). The H19 gene imprinting in normal pregnancy and pre-eclampsia. Placenta 30, 443–447.
7. Zhao, D., Li, L., Yu, L.L., Lu, L.S., Han, J., Chen, X.Y., and Zhou, Y.G. (2009). [H19 expression in placenta with pre-eclampsia]. Zhonghua Fu Chan Ke Za Zhi 44, 87–90.
8. Ponting, C.P., Oliver, P.L., and Reik, W. (2009). Evolution and functions of long non-coding RNAs. Cell 136, 629–641.
9. Mercer, T.R., Dinger, M.E., and Mattick, J.S. (2009). Long non-coding RNAs: insights into functions. Nat. Rev. Genet. 10, 155–159.
10. Kretz, M., Siprashvili, Z., Chu, C., Webster, D.E., Zehnder, A., Qu, K., Lee, C.S., Flockhart, R.J., Groff, A.F., Chow, J., et al. (2013). Control of somatic tissue differentiation by the long non-coding RNA TINCR. Nature 493, 231–235.
11. Hung, C.L., Wang, L.Y., Yu, Y.L., Chen, H.W., Srivastava, S., Petrovics, G., and Kung, H.J. (2014). A long noncoding RNA connects c-Myc to tumor metabolism. Proc. Natl. Acad. Sci. USA 111, 18697–18702.
12. Wang, P., Xue, Y., Han, Y., Lin, L., Wu, C., Xu, S., Jiang, Z., Xu, J., Liu, Q., and Cao, X. (2014). The STAT3-binding long noncoding RNA Inc-DC controls human dendritic cell differentiation. Science 344, 310–313.
13. Gupta, R.A., Shah, N., Wang, K.C., Kim, J., Horlings, H.M., Wong, D.J., Tsai, M.C., Hung, T., Argani, P., Rinn, J.L., et al. (2010). Long non-coding RNA HOTAIR programs chromatin state to promote cancer metastasis. Nature 464, 1071–1076.
14. Zou, Y., Jiang, Z., Yu, X., Sun, M., Zhang, Y., Zuo, Q., Zhou, J., Yang, N., Han, P., Ge, Z., et al. (2013). Upregulation of long noncoding RNA SRY:ITI modulates proliferation, migration, apoptosis, and network formation in trophoblast cells. HTR-8/SVneo. PLoS ONE 8, e79598.
15. Fu, X., Ravindranath, L., Tran, N., Petrovics, G., and Srivastava, S. (2006). Regulation of apoptosis by a prostate-specific and prostate cancer-associated noncoding gene, PCGEM1. DNA Cell Biol. 25, 135–141.
16. Chen, H., Meng, T., Liu, X., Sun, M.-P., Tong, C., Liu, J., Wang, H., and Du, J. (2015). Long non-coding RNA MALAT-1 is downregulated in preeclampsia and regulates proliferation, apoptosis, migration and invasion of JEG-3 trophoblast cells. Int. J. Clin. Exp. Pathol. 8, 12718–12727.
17. Cao, C., Li, J., Li, J., Liu, L., Cheng, X., and Jia, R. (2017). Long Non-Coding RNA Uc.187 Is Upregulated in Preeclampsia and Modulates Proliferation, Apoptosis, and Invasion of HTR-8/SVneo Trophoblast Cells. J. Cell. Biochem. 118, 1462–1470.
18. Song, X., Rui, C., Meng, L., Zhang, R., Shen, R., Ding, H., Li, J., Li, J., and Long, W. (2017). Long non-coding RNA RPAID regulates the invasion and apoptosis of trophoblast cell lines via complement protein C1q. Oncotarget 8, 7535–7546.
19. Cox, B., Leavey, K., Noi, U., Wong, F., and Kingdom, J. (2015). Placental transcriptome in development and pathology: expression, function, and methods of analysis. Am. J. Obstet. Gynecol. 213 (4, Suppl), S138–S151.
20. Wilusz, J.E., Sunwoo, H., and Spector, D.L. (2009). Long noncoding RNAs: functional surprises from the RNA world. Genes Dev. 23, 1494–1504.
21. Zhang, E., Han, L., Yin, D., He, X., Hong, L., Si, X., Qiu, M., Xu, T., De, W., Xu, L., et al. (2017). H3K27 acetylation activated-long non-coding RNA CAT1 affects cell proliferation and migration by regulating SPARC and HOXB13 expression in esophageal squamous carcinoma. Nucleic Acids Res. 45, 3086–3101.
22. Cesana, M., Cacciarelli, D., Lagnini, I., Santini, T., Sthandier, O., Chinappi, M., Tramontano, A., and Bozzone, I. (2011). A long noncoding RNA controls muscle differentiation by functioning as a competing endogenous RNA. Cell 147, 358–369.
23. Khan, I., Kerwin, J., Owen, K., and Griner, E. (2015). Reproducibility Project: Cancer B: Correction: Registered report: A coding-independent function of gene and pseudogene mRNA regulates tumour biology. eLife 4, e08245.
24. Yu, J., Hong, J.F., Kang, J., Liao, L.H., and Li, C.D. (2017). Promotion of lncRNA HOXA11-AS on the proliferation of hepatocellular carcinoma by regulating the expression of LATS1. Eur. Rev. Med. Pharmacol. Sci. 21, 3402–3411.
25. Lu, Q., Zhao, N., Zha, G., Wang, H., Tong, Q., and Xin, S. (2017). LncRNA HOXA11-AS Exerts Oncogenic Functions by Repressing p21 and miR-124 in Uveal Melanoma. DNA Cell Biol. 36, 837–844.
26. Zhang, Y., Chen, W.J., Gan, T.Q., Zhang, X.L., Xie, Z.C., Ye, Z.H., Deng, Y., Wang, Z.F., Cai, K.T., Li, S.K., et al. (2017). Clinical Significance and Effect of lncRNA
33. Brockdorff, N. (2013). Noncoding RNA and Polycomb recruitment. RNA 19, 439–442.
34. Volkel, P., Dupret, B., Le Bourhis, X., and Angrand, P.O. (2015). Diverse involvement of EZH2 in cancer epigenetics. Am. J. Transl. Res. 7, 175–193.
35. Shin, J., Ming, G.L., and Song, H. (2015). Molecular toggle switch of histone demethylase LSD1. Mol. Cell 57, 949–956.
36. Xu, Y., Ge, Z., Zhang, E., Zou, Q., Huang, S., Yang, N., Wu, D., Zhang, Y., Chen, Y., Xu, H., et al. (2017). The IncRNA TUG1 modulates proliferation in trophoblast cells via epigenetic suppression of RND3. Cell Death Dis. 8, e13104.
37. Tang, B., Qi, G., Sun, X., Tang, F., Yuan, S., Wang, Z., Liang, X., Li, B., Yu, S., Liu, J., et al. (2016). HOXA7 plays a critical role in metastasis of liver cancer associated with activation of Snail. Mol. Cancer 15, 57.
38. Zhang, Y., Cheng, J.C., Huang, H.F., and Leung, P.C. (2013). Homeobox A7 stimulates breast cancer cell proliferation by up-regulating estrogen receptor-alpha. Biochim. Biophys. Res. Commun. 440, 652–657.
39. Li, Y., Yang, X.H., Fang, S.J., Qin, C.F., Sun, R.L., Liu, Z.Y., Jiang, B.Y., Wu, X., and Li, G. (2015). HOXA7 stimulates human hepatocellular carcinoma proliferation through cyclin E1/CDK2. Oncol. Rep. 33, 990–996.
40. Leroy, P., Berto, F., Bourget, I., and Rossi, B. (2004). Down-regulation of HOX A7 is required for cell adhesion and migration on fibronectin during early H1-60 monocyte differentiation. J. Leukoc. Biol. 75, 680–688.
41. Zuo, Q., Huang, S., Zou, Y., Xu, Y., Jiang, Z., Zou, S., Xu, H., and Sun, L. (2016). The Lnc RNA SPRY4-I71 Modulates Trophoblast Cell Invasion and Migration by Affecting the Epithelial-Mesenchymal Transition. Sci. Rep. 6, 37183.
42. Zhang, Y., Zou, Y., Wang, W., Zuo, Q., Jiang, Z., Sun, M., De, W., and Sun, L. (2015). Down-regulated long non-coding RNA MEG3 and its effect on promoting apoptosis and suppressing migration of trophoblast cells. J. Cell. Biochem. 116, 542–550.
43. Liu, X., Chen, H., Kong, W., Zhang, Y., Cao, L., Gao, L., and Zhou, R. (2017). Down-regulated long non-coding RNA ABT in preeclampsia and its effect on suppressing migration, proliferation, and tube formation of trophoblast cells. Gene 612, 80–87.
44. Marchese, F.P., and Huarte, M. (2014). Long non-coding RNAs and chromatin modifiers: their place in the epigenetic code. Epigenetics 9, 21–26.
45. Wei, C.C., Nie, F.Q., Jiang, L.L., Chen, Q.N., Chen, Z.Y., Chen, X., Pan, X., Liu, Z.L., Lu, B.B., and Wang, Z.X. (2017). The pseudogene DUXAP10 promotes an aggressive phenotype through binding with LSD1 and repressing LATS2 and RRAD in non small cell lung cancer. Oncotarget 8, 5233–5246.
46. Niu, Y., Ma, F., Huang, W., Fang, S., Li, M., Wei, T., and Guo, L. (2017). Long non-coding RNA TUG1 is involved in cell growth and chemoresistance of small cell lung cancer by regulating LIMK2b via EZH2. Mol. Cancer 16, 5.
47. Hernández-Sánchez, M., Poch, E., Guasch, R.M., Ortega, J., López-Almela, L., Palmero, I., and Pérez-Roger, I. (2015). RhoE is required for contact inhibition and negatively regulates tumor initiation and progression. Oncotarget 6, 17479–17490.
48. Riento, K., Guasch, R.M., Garg, R., Jin, B., and Ridley, A.J. (2003). RhoE binds to ROCK I and inhibits downstream signaling. Mol. Cell. Biol. 23, 4219–4229.
49. Katoh, H., Harada, A., Mori, K., and Negishi, M. (2002). Socius is a novel Rnd GTPase-interacting protein involved in disassembly of actin stress fibers. Mol. Cell. Biol. 22, 2952–2964.
50. Lin, X., Liu, B., Yang, X., Yue, X., Diao, L., Wang, J., and Chang, J. (2013). Genetic deletion of Rnd3 results in aqueductal stenosis leading to hydrocephalus through up-regulation of Notch signaling. Proc. Natl. Acad. Sci. USA 110, 8236–8241.
51. Xia, H., Li, M., Chen, L., Leng, W., Yuan, D., Pang, X., Chen, L., Li, R., Tang, Q., and Bi, F. (2013). Suppression of RND3 activity by AES downregulation promotes cancer cell proliferation and invasion. Int. J. Mol. Med. 31, 1081–1086.
52. Ongusha, P.P., Kim, H.G., Boswell, S.A., Ridley, A.J., Der, C.J., Dotto, G.P., Kim, Y.B., Aaronson, S.A., and Lee, S.W. (2016). RhoE Is a Pro-Survival p53 Target Gene That Inhibits ROCK I-Mediated Apoptosis in Response to Genotoxic Stress. Curr. Biol. 26, 2221–2222.
53. Pearson, J.C., Lemons, D., and McGinnis, W. (2005). Modulating Hox gene functions during animal body patterning. Nat. Rev. Genet. 6, 893–904.
54. Soret, C., Martin, E., Duluc, I., Dantzer, F., Vanier, M., Gross, I., Freund, J.N., and Domon-Dell, C. (2016). Distinct mechanisms for opposite functions of homeoproteins Gdx2 and HoxB7 in double-strand break DNA repair in colon cancer cells. Cancer Lett. 374, 208–215.
55. Kim, Y.R., Kang, T.W., To, P.K., Xu, K.J., Cho, Y.S., Jung, C., and Kim, M.S. (2016). HOXB13-mediated suppression of p21WAF1/CIP1 regulates JNK/c-Jun signaling in prostate cancer cells. Oncol. Rep. 35, 2011–2016.
56. Shah, N., and Sukumar, S. (2010). The Hox genes and their roles in oncogenesis. Nat. Rev. Cancer 10, 361–371.
Supplementary Figure 1

(A) HTR-8/SVneo

- Cell vitality (OD 490 nm) over incubation time (h)

(B) JEG3

- BrdU concentration (OD 490 nm) over incubation time (h)

(C) Images showing cell migration

- mimics NC vs. miR-15b-5p mimics
**Supplementary Figure 1.** Overexpression of miR-15b-5p could stimulate trophoblast cell growth and migration. (A) MTT assays were performed to detect cell viability of miR-15b-5p mimics-transfected trophoblast Cells. (B) BrdU assays were used to identify cell growth after transfection in HTR-8/SVneo and JEG3 cell lines. (C) For cell migration analyses, Migration were allowed to occur for 24 h. Quantitative analysis are on the below. Numbers are mean ± SD. **p < 0.01, *p<0.05.
Supplementary table S1  sequence (5'-3')

| Gene   | Forward Primer   | Reverse Primer   |
|--------|------------------|------------------|
| GAPDH | GGGAGCCAAAAGGGTCAT | GAGTCCCTTCCACGATACCAA |
| EZH2  | TGCACATCCTGACTTCTGTG | AAGGGCATTCACCAACTCC |
| TFPI2 | CTGGGGGCTGTGATTCTGC | TCTCCGCGTTATTTTCTGGTG |
| TNFSF9| GGCTGGAGTCTACTATGTCTTCT | ACCTCGGTGAAGGGAGTCC |
| CA9   | GGATCTACCTACTGTTGAGGCT | CATAGCGCCAATGACTCTGGT |
| IFITM1| CCAAGGTCCACCGTGATTAAC | ACCAGTTCAAGAAGGGGTGTT |
| NAMPT | CGGCAGAAGCCGAGTTCAA | GCTTGTGTTGGGGATATTGT |
| TMEM158| CTGAACCGTAAGCCCATTGAG | CGCTCCACACCACGATGAC |
| ESMT1 | TGCCGCACTCAGTGTTTCAAC | TGCCGCACTCAGTGTTTCAAC |
| HOXA7 | TCCTATGATGGAGGCTGCTT | CAAGAAGTCGGCTCGGCATT |
| PSAT1 | TGCCGCACTCAGTGTTTCAAC | TGCCGCACTCAGTGTTTCAAC |
PSAT1 R  GCAATTCGCCAACAAGATTCT
CPA4 F   GCGACCTAAACACCTCAAC
CPA4 R   ATGCCGCTAAACTGTTGTC
MEST F   AGTTGTGCTTTTACACGTTTTC
MEST R   CAAAGGCCAATCAACCAGATGAA
OLR1 F   TTGCTGGGATTAGTGAGTGACC
OLR1 R   GCTTTGCTCTTGTTAGGAGGT
LSD1 F   GTGGGAGAGTTGCCACATTTC
LSD1 R   TGACCACAGCCATAGGATTCC
U1 snRNA-F GGGAGATACCAGATCGACGAAGGT
U1 snRNA-R CCACAAATTATGCGAGTGATTTC

RND3-F1   CTGTACCACCCCTCGAAAC
RND3-R1   GTGCACGCTAGCTAGACTGCC
TFPI2-F1   GCCTTTGAGGATTCTGGGGG
TFPI2-R1   GGCTACAGGAGACGAAAGG
TFPI2-F2   TTTGAGGATTCTGGGGGCTC
TFPI2-R2   GGGAGACGAAAGGAGCAAT
TFPI2-F3   CTTTGAGGATTCTGGGGGCTC
TFPI2-R3   TGGGAGACGAAAGGAGCAAA
TFPI2-F4   AGAGCCTTTGAGGATTCTGGG
TFPI2-R4   TTGGCTACATGGGAGACGAAG
HOXA11-AS F1   AGAAATCTGGACCCGAGACG
HOXA11-AS R1   CGTCAGCTTACGTCTCCAAA
HOXA11-AS F2   ATGTTAGAGGAGCAGGGAACCA
HOXA11-AS R2   CCCTGCTAAGGATGGGATAG
HOXA11-AS F3   GGGACCACGCTCATCAAAATC
HOXA11-AS R3   GCACCTCCCTGCTAAGGAT

si-LSD1#CAACCTCTCAGAAGATGAGTATTAT
si-EZH2#   GAGGUUCAGACGAGCUGAUUU
si-FOXP1

si-HOXA11-AS 1# 5’-
UUACACCGGCGAUUACGUGCUUUCG-3’
si-HOXA11-AS 2# 5’-
AGGCCAAGUCCGAGUUCAUUUCU-3’
si-HOXA11-AS 3# 5’-
AAAGGCUUCUUUCCACAGCCUUU-3’
HOXA11-AS probe sequences

```
GCCTCAATTGCATGGTTTCCCGAGTCCTCAGC
```

si-RND3 1# 5’-GGACACUUCGGGUUCUCCUUACUAU-3’
si-RND3 2# 5’-CCCUGAUUCCGGAUGCUUGCUGAUU-3’
si-RND3 3# 5’-CAAACAGAUUGGAGCAGCUACUUUAU-3’

si-HOXA7 1# 5’-CCGTTCCGGGCTTATACAATGTCAA-3’
si-HOXA7 2# 5’-TCCTTTGCTCCCCAACTCAGAGAA-3’
si-HOXA7 3# 5’-TCGACCCTTCCGGCTTATACAGTAATG-3’