

# SnoRNA ENST00000391318 Suppressed Endometrial Receptivity by Competitively Binding To miRNA-3928-5p To Regulate CDH11 in Women in a GnRH Antagonist Stimulation Protocol

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## Research

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# Abstract

## Background

To determine the small nucleolar RNA (snoRNA) expression profiles in the endometrium during the implantation phase and identify their potential biological functions.

## Methods

We used RNA sequencing to analyze the expression changes of snoRNAs in the endometrium of women during the implantation phase of the natural cycle and in the GnRH antagonist stimulation protocol. The expression of snoRNA ENST00000391318 was manipulated in endometrial cells by lentivirus. CCK-8, clone formation, flow cytometry, scratch and Transwell assays were used to detect the effects of ENST00000391318 on the proliferation, apoptosis, migration and invasion of endometrial cells. RNA pulldown and luciferase reporter gene experiments were used to detect the binding of ENST00000391318 to miRNA.

## Results

Endometrial SNORNA ENST00000391318 was upregulated in the GnRH antagonist stimulation protocol. SNORNA ENST00000391318 overexpression inhibited cell proliferation and the cell cycle in Ishikawa cells, and SNORNA ENST00000391318 knockdown promoted cell proliferation and the cell cycle in Ishikawa cells. SNORNA ENST00000391318 overexpression inhibited cell migration and invasion in Ishikawa cells. SNORNA ENST00000391318 inhibited the expression of endometrium receptivity factor. miRNA-3928-5p is a target of SNORNA ENST00000391318 in Ishikawa cells. SNORNA ENST00000391318 regulates the expression of CDH11 through interaction with miRNA-3928-5p in Ishikawa cells.

## Conclusions

The ENST00000391318/miR-3928-5p/CDH11 signaling pathway can regulate the receptivity of the endometrium for patients in the GnRH antagonist stimulation protocol. SNORNA ENST00000391318 downregulates the function of miR-3928-5p through a ceRNA mechanism to upregulate the expression of CDH11. SNORNA ENST00000391318 downregulates the receptivity of the endometrium by upregulating the expression of CDH11.

## Introduction

Endometrial receptivity is a complex process that provides an opportunity for embryos to attach, invade and develop, eventually forming a new individual [1; 2]. In most normal women, the implantation time in

the secretory phase was prolonged by 3–6 days. In some inflammatory or anatomical conditions, this window is narrowed or moved to prevent normal implantation, leading to infertility or abortion [3]. The quality of the embryo and endometrium are among the factors that commonly affect normal implantation and pregnancy [3; 4].

Endometrial receptivity (ER) is a time limit for endometrial epithelial cells (EEs) to acquire functional and transient ovarian hormone-dependent status, thus allowing blastocyst adhesion [5]. This period, known as the "implantation window," opens 4–5 days after progesterone production or administration and closes after 9–10 days [6]. Implantation failure is now recognized as an important component of failed assisted reproduction treatment (ART). How to accurately assess ER to better improve ER and increase the pregnancy rate of IVF is a challenge. In addition, how to combine various evaluation indicators to judge ER to accurately predict the implantation window, reasonably select the best time for embryo transfer, and improve the pregnancy outcome of ART is still a clinical problem that needs to be solved urgently. A scientific understanding of the process of endometrial receptivity is the basis for understanding human reproduction, but thus far, none of the proposed biochemical indicators of endometrial receptivity have been proven to be clinically useful. Uterine function, especially the mechanism of regulating endometrial receptivity, has been extensively studied in the past two decades. These studies are mainly carried out from the perspective of morphology and biochemistry and, recently, cellular and molecular biological analysis. Most of these studies focus on specific molecules or some members of specific families, such as integrins, mucins, cytokine cytoskeleton-related proteins and others. The most widely accepted biochemical marker is a specific pattern of endometrial adhesion molecules, namely, membrane glycoprotein integrin, which mediates adhesion to the extracellular matrix. In addition, mucin MUC1 is considered to be a key molecule with important functions during embryo implantation [7; 8]. Paracrine autocrine cytokine systems, such as the interleukin-1 system and leukemia inhibitory factor, also seem to control at least part of the adhesion phase of implantation.

Small nucleolar RNA (snoRNA) is an important participant in the regulation of gene expression in human cells [9]. The standard functions of box C/D and box H/ACA snoRNAs are posttranscriptional modifications of ribosomal RNA (rRNA), which are 2-O-methylation and pseudouridine, respectively [10]. A series of independent studies have shown that snoRNA and other noncoding RNAs can be used as sources of various short regulated RNAs [11]. Some snoRNAs and their fragments can also participate in the regulation of mRNA selective splicing and posttranscriptional modification [12]. Changes in the expression of snoRNA in human cells affect many important cellular processes. The levels of snoRNA in human cells, serum and plasma mean that they are promising targets for the diagnosis and treatment of human pathology [13]. SnoRNAs represent a class of regulatory RNAs responsible for the posttranscriptional maturation of ribosomal RNAs (rRNAs). According to their structure and main function, small nucleolar RNAs can be divided into two families: box C/D snoRNAs and box H/ACA snoRNAs. Box C/D snoRNAs are responsible for 2'-O-methylation, and the latter family guides the pseudouridine acidification of nucleotides.

At present, there are no reports on the relationship between snoRNA and endometrial receptivity. In this study, we will use high-throughput sequencing to analyze the regulatory role of snoRNA in endometrial receptivity from women in the GnRH antagonist stimulation protocol.

## **Materials And Methods**

### **Participants and Samples**

Samples were collected after patients gave written informed consent. The study was approved by the ethics committee of Zhongshan Hospital, Fudan University (Shanghai, People's Republic of China) and was performed in compliance with the Population and Family Planning Law of the People's Republic of China.

The endometrium for this study was collected from 3 participants who underwent in vitro fertilization (IVF) for an indication of tubal factor infertility at the Reproductive Center, Zhongshan Hospital, Fudan University.

### **Treatment Protocol**

Endometrial specimens were obtained from pipe suction curettage on day LH + 7 in a natural cycle. The next cycle included three patients who received the antagonist stimulation protocol. Briefly, on cycle day 3, ovarian stimulation was started by the daily injection of recombinant FSH (r-FSH) (Gonal-F; Merck Serono). Gonadotropin-releasing hormone (GnRH) antagonist (Ganirelix, 0.25 mg; MSD) injection was started on day 5 or 6 of stimulation. When at least three follicles had reached 17 mm or two follicles had reached 18 mm in diameter, an intramuscular injection of 5,000 IU of human chorionic gonadotropin (hCG) was used to trigger final oocyte maturation. Vaginal utrogestan (Capsugel) at 600 mg/d and oral dydrogesterone (Abbott) at 20 mg/d were given for luteal support beginning on the day of oocyte retrieval. The endometrium was collected on the 5th day after oocyte retrieval from patients. All specimens were frozen at  $-80^{\circ}\text{C}$  until subsequent analysis.

### **Small RNA sequencing analysis**

The quality of the original sequencing data was assessed by FastQC, cutadapt was used to remove the adaptor, trimmomatic was used to remove the low-quality bases at both ends, and reads were filtered. Then, blastn was used to compare reads with sRNA, tRNA, snRNA, and snoRNA sequences in the Rfam database, count the number and percentage of reads on the alignment, and filter out the reads on the alignment. The bowtie program was used to compare the reads with the exon and intron sequences of the species as well as compare the number and percentage of reads on the comparison and filter out the reads on the exon of the comparison that cannot be compared with the intron. Again, bowtie was used to align the reads with the species reference genome sequence, count the number and percentage of reads on the comparison, and filter out the reads that cannot be compared. Sequencing was performed by the Sangon Biotech (Shanghai) Co., Ltd.

## Cell culture

Ishikawa cells were purchased from the Cell Bank of the Chinese Academy Sciences (Shanghai, China). The cells were cultured with DMEM high glucose medium containing 10% FBS and 1% antibiotics (100 U/ml penicillin and 100 µg/ml streptomycin) at 37°C in a humidified atmosphere containing 5% CO<sub>2</sub>. When the fusion degree reached 90%, the cells were subcultured.

## Quantitative real-time polymerase chain reaction (qRT-PCR)

The total RNA were isolated from Ishikawa cells according to the manufacturer's instruction of TRIzol® Reagent (Invitrogen, Carlsbad, CA, USA). According to the manufacturer's instructions, 1 µg of total RNA was reverse transcribed into cDNA using primescript RT Master Mix kit (RR036A, Takara, Dalian, China). To quantitate ENST00000391318 and CDH11 expression, qPCR was carried out with a SYBR® Premix Ex Taq II Kit (RR420A, Takara, Dalian, China). The expression levels of ENST00000391318 and CDH11 were normalized to GAPDH expression. QPCR was performed using the miScript SYBR Green PCR Kit (Qiagen, Hilden, Germany) to determine miR-3928-5p expression. The expression level of miR-3928-5p was normalized to U6 expression. All of the data were analyzed by the  $2^{-\Delta\Delta Ct}$  method.

## Cell proliferation assay

Ishikawa cells were seeded into 96 well culture plate according to the density of 4000 cells/ well. Ishikawa cells were divided into four groups: pLVX-vector group, pLVX- ENST00000391318, pLKO.1-vector group and pLKO.1- ENST00000391318, the activity of Ishikawa cells was detected by CCK-8 at 12h, 24h, 36h and 48h after inoculation, and 10 µl CCK-8 solution was added to each well. After incubation in 37 °C incubator for 2h, the absorbance at 450nm was detected by microplate reader.

## Wound healing assay

The Ishikawa cells were seeded into 6-well culture plate according to 50000 cells/well. When the cells grew to 100% healing degree, they were scratched with 1 ml blue tips. After the scratch, the medium and suspension cells were sucked off, and then the serum-free medium was replaced and take photos at 0 h. Then the cells were cultured for 24 h to take photos. The cell mobility was calculated by comparing the healing degrees of 0 h and 24 h.

## Cell invasion assay

After mixing DMEM medium and Matrigel 1:1, 50 µl was evenly spread in Transwell chamber and placed in 37 °C incubator for 45 min. The cells were divided into four groups: pLVX-vector group, pLVX- ENST00000391318, pLKO.1-vector group and pLKO.1- ENST00000391318. Cells were inoculated into Transwell upper chamber according to the density of 20000 cells/well. The upper chamber of Transwell was serum-free medium, and 700 µl medium containing 5% serum was added into the lower chamber. The cells in the upper chamber were removed after 12 h in the incubator. The cells at the bottom of Transwell were stained with crystal violet and counted after taking pictures.

## Cell cycle analysis

The cell cycle distribution was determined by using the Cell Cycle Analysis Kit (Beyotime, China) according to the instructions. Cells were seeded onto 6-well plates and transfected with NC shRNA or ENST00000391318 shRNA. After 48 h, the cells were harvested and fixed with precooled 70% ethanol at 4 °C for 12 h. The fixed cells were suspended in staining buffer, mixed with 25 µL of propidium iodide staining solution and 10 µL of RNase A and incubated in the dark for 30 min. Flow cytometry detection was immediately performed.

## Western blot

The cell protein was extracted from RIPA protein lysate and lysed on 30min, then centrifuged at 1000r/min, and the supernatant was extracted. The protein concentration was detected by BCA solution and 10% SDS-PAGE gel was distributed. The protein was transferred to PVDF membrane and sealed with 5% BSA for 1 h. then the antibody was incubated at 4 °C overnight. After tbst cleaning, HRP labeled antibody was incubated for 1 h. tbst was washed and then developed. Finally, the protein bands were visualized using an ECL Kit (Solarbio, China). GAPDH served as an internal control. LIF antibody (#ab113262, Abcam, 1:1000), an ITGB3 antibody (#ab119992, Abcam, 1:1000), a DKK1 antibody (#ab109416, Abcam, 1:1000), and a claudin-4 antibody (#ab53156, Abcam, 1:1000) were used.

## Statistical analysis

Statistical analysis was performed using GraphPad Prism 8.0 software. All results are expressed as the mean ± standard deviation (SD).  $p < 0.05$  indicated statistical significance. The experiments were repeated at least three times.

# Results

## Endometrial SNORNA ENST00000391318 was upregulated in the GnRH antagonist stimulation protocol

A SNORNA profile was established, showing the analysis of the differential endometrial SNORNA expression in the natural cycle and GnRH antagonist protocol (Fig. 1A and B). Then, qRT-PCR was carried out to verify the expression of upregulated and downregulated SNORNA in a heatmap (Fig. 1A and B). The results indicated that the endometrial expression of ENST00000391318 was significantly higher with the GnRH antagonist protocol (Fig. 1C).

## SNORNA ENST00000391318 regulates cell proliferation and the cell cycle in Ishikawa cells

ENST00000391318 in Ishikawa cells was significantly overexpressed by transfection with pLVX-ENST00000391318 and knocked down by transfection with pLKO.1-ENST00000391318 (Fig. 2A). E2 and P4 could decrease the expression of snoRNA ENST00000391318 in Ishikawa cells (Fig. 2B). Through the CCK-8 assay (Fig. 2C) and colony formation assay (Fig. 2E), cell proliferation was analyzed, which showed that ENST00000391318 overexpression inhibited the proliferation of Ishikawa cells, while

ENST00000391318 knockdown promoted the proliferation of Ishikawa cells. However, cell apoptosis was not affected by the overexpression or knockdown of ENST00000391318 (Fig. 2D). In the flow cytometry analysis, the cell cycle of Ishikawa cells was inhibited by the overexpression of ENST00000391318 and promoted by the knockdown of ENST00000391318 (Fig. 2F).

### **SNORNA ENST00000391318 overexpression inhibited cell migration and invasion in Ishikawa cells**

The wound healing assay showed that the cell migration ability was inhibited and promoted by the overexpression and knockdown of ENST00000391318, respectively (Fig. 3A). Transwell assays showed that the cell invasion ability was inhibited and promoted by the overexpression and knockdown of ENST00000391318, respectively (Fig. 3B).

### **Snorna Enst00000391318 Inhibited The Expression Of Endometrium Receptivity Factor**

qPCR detected that the mRNA expression of LIF, ITGB3, DKK1 and Claudinlp4 in Ishikawa cells was suppressed by ENST00000391318 overexpression and enhanced by ENST00000391318 knockdown (Fig. 4A-D). Western blot analysis detected that the protein expression of LIF, ITGB3, DKK1 and Claudinlp4 in Ishikawa cells was suppressed by ENST00000391318 overexpression and enhanced by ENST00000391318 knockdown (Fig. 4E).

### **miRNA-3928-5p is a target of SNORNA ENST00000391318 in Ishikawa cells**

One of the important mechanisms of snoRNA in affecting the functions of cells is to regulate the function of miRNA through the ceRNA mechanism. Therefore, we predicted the miRNAs that could bind to ENST00000391318 using the bioinformatics software MIRDB (<http://mirdb.org/index.html>). An ENST00000391318 RNA pulldown experiment was performed to detect the miRNA bound with ENST00000391318, and the results showed that miR-3928-5p was significantly enriched in the MS2bs-ENST00000391318 group (Fig. 5A). Through luciferase reporter gene detection, miR-3928-5p mimics significantly inhibited the activity of the WT luciferase reporter gene; however, miR-3928-5p mimics had no effect on the activity of the mut luciferase reporter gene (Fig. 5B). It was found that ENST00000391318 was significantly enriched in the biotin-miR-3928-5p group by the miRNA pull-down assay (Fig. 5C). CCK-8 assays showed that 3928-5p mimics significantly promoted the proliferation of Ishikawa cells, while miR-3928-5p inhibitors significantly inhibited the proliferation of Ishikawa cells (Fig. 5D). Transwell assays showed that miR-3928-5p mimics significantly promoted the invasion of Ishikawa cells, while miR-6768-3p inhibitors significantly inhibited the invasion of Ishikawa cells (Fig. 5E and 5F). To determine whether miR-3928-5p mediates the regulatory effect of ENST00000391318 on the invasion of Ishikawa cells, we inhibited the function of miR-3928-5p using miR-3928-5p inhibitors. When the function of miR-3928-5p was inhibited, the regulatory effect of ENST00000391318 on the invasion of Ishikawa cells was decreased (Fig. 5G and 5H).

### **SNORNA ENST00000391318 regulates the expression of CDH11 through miRNA-3928-5p in Ishikawa cells**

The target gene of miR-3928-5p was analyzed by using the bioinformatics software TargetScan. The results showed that miR-3928-5p could bind to the CDH11 mRNA 3'-UTR (Fig. 6A). qRT-PCR showed that miR-3928-5p mimics and miR-3928-5p inhibitors had no effect on the expression of CDH11 (Fig. 6B). It was found that miR-3928-5p mimics significantly inhibited the activity of the CDH11-WT-3'-UTR luciferase reporter construct but had no effect on the activity of the CDH11-mut-3'-UTR luciferase reporter construct (Fig. 6C). Western blot analysis showed that miR-3928-5p mimics could inhibit the expression of CDH11 protein, while miR-3928-5p inhibitors could promote the expression of CDH11 protein (Fig. 6D). The overexpression of ENST00000391318 promoted the expression of CDH11, and silencing of miR-3928-5p by miR-3928-5p inhibitors significantly inhibited the regulatory effect of ENST00000391318 on the protein expression of CDH11 (Fig. 6E).

## Discussion

Endometrial receptivity is a complex phenomenon that plays an important role in infertility. Although embryo quality can be evaluated for successful implantation, endometrial receptivity remains an unknown factor [1; 14]. GnRH antagonist protocol is one of the most commonly used regimens in IVF. We found that endometrial snoRNA ENST00000391318 which is closely related to endometrial receptivity was upregulated in the GnRH antagonist protocol. The process of embryo implantation includes a series of processes, such as positioning, adhesion, invasion, and placental angiogenesis. Our research found that snoRNA ENST00000391318 inhibits the proliferation, migration and invasion ability of endometrial cells.

Posttranscriptional modification of RNA and the control of mRNA stability and translation are important components of gene expression regulation in human cells [10]. Small nucleolar RNAs and their functional fragments play an important role in these processes: they direct the nucleotide modification of rRNA and snRNA, influence the alternative splicing of complementary pre-mRNA, and control the translation and stability of mRNA through a RISC-dependent pathway. The destruction of snoRNA expression may be caused by external factors and intracellular signaling cascades, leading to physiological changes at the cellular level, organ dysfunction and various diseases [17]. The structure, expression pattern and intracellular localization of snoRNAs have regulatory significance and are considered diagnostic markers of pathology. Further understanding of snoRNA expression and its functional mechanism will provide new possibilities for the development of human disease diagnosis systems and new treatment methods. Comparing snoRNA expression in the GnRH antagonist regimen group with that in the natural cycle group by small RNA sequencing, we found that some snoRNA expression was changed and identified with qRT-PCR. We found that the change in snoRNA ENST00000391318 expression was mostly significant. Therefore, we will focus on the relationship between snoRNA ENST00000391318 and endometrial receptivity. We found that snoRNA ENST00000391318 could regulate the expression of endometrial receptivity factors, and thus, snoRNA ENST00000391318 may be a key gene regulating endometrial receptivity. To study the mechanism by which snoRNA enst00000391318 regulates endometrial receptivity, we verified the binding of snoRNA ENST00000391318 with miR-3928-5p by bioinformatics analysis and experimental verification. This is the first report of the relationship between snoRNA and

endometrial receptivity. In this study, we found that the snoRNA ENST00000391318 can downregulate the function of miR-3928-5p through the ceRNA mechanism, thereby upregulating the expression of CDH11. The CDH11 gene, located on chromosome 16q22.1, is a member of the cadherin superfamily. Calcium-dependent intercellular adhesion molecules in this family are essential in cell adhesion, proliferation and invasion. CDH11 participates in mir-27b-induced epithelial mesenchymal transition (EMT) by regulating the expression of E-cadherin, vimentin and N-cadherin [18]. CDH11 is closely related to cell proliferation and migration [19]. The mechanism of CDH11 in regulating endometrial cell proliferation, invasion and endometrial receptivity needs to be further explored in a follow-up study.

## Conclusion

We used high-throughput sequencing to analyze the relationship between snoRNAs and endometrial receptivity. For the first time, we reported the role of snoRNAs in endometrial receptivity. Furthermore, we explained the mechanism by which snoRNA ENST00000391318 regulates endometrial receptivity.

## List Of Abbreviations

snoRNA, small nucleolar RNA;

GnRH, Gonadotropin-releasing hormone;

CeRNA, competing endogenous RNAs;

EMT, Epithelial mesenchymal transition;

CDH11, Cadherin 11;

miRNA, MicroRNA;

LIF, Leukemia inhibitory factor;

ITGB3, Integrin subunit beta 3;

DKK1, Dickkopf WNT signaling pathway inhibitor 1;

qRT-PCR, Quantitative real-time polymerase chain reaction;

## Declarations

### Ethics approval and consent to participate

Samples were collected after patients gave written informed consent. The study was approved by the ethics committee of Zhongshan Hospital, Fudan University (Shanghai, People's Republic of China) and

was performed in compliance with the Population and Family Planning Law of the People's Republic of China.

### **Consent for publication**

Not applicable

### **Availability of data and materials**

The original contributions presented in the study are included in the article, further inquiries can be directed to the corresponding author/s.

### **Competing interests**

The authors report no conflicts of interest in this work.

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### **Authors' contributions**

Xiaowei Ji and Xi Dong: conception and design; Xiaowei Ji, Suying Liu and Miao Liu: experiments and/or data analysis; Lin Wang: intellectual input and supervision; Jun Xu, Xutong Zou, Liying He, Wei Chen and Xi Dong: article writing.

### **Acknowledgements**

Not applicable

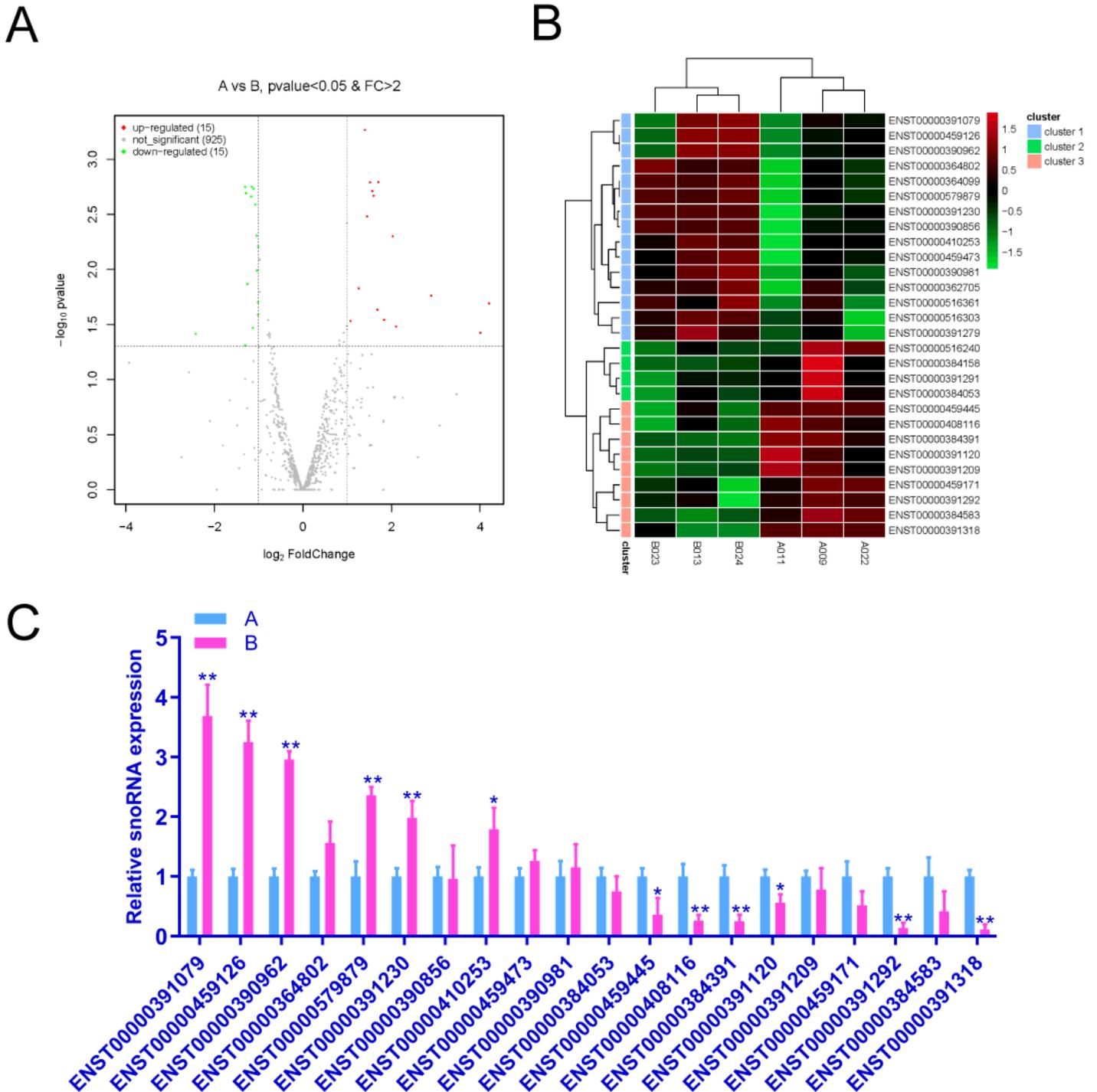
## **References**

1. Lessey BA, Young SL. What exactly is endometrial receptivity? *Fertility and sterility* 111 (2019) 611–617. doi: 10.1016/j.fertnstert.2019.02.009.
2. Munro MG. Uterine polyps, adenomyosis, leiomyomas, and endometrial receptivity. *Fertility sterility*. 2019;111:629–40. doi:10.1016/j.fertnstert.2019.02.008.
3. Altmae S, Aghajanova L. Growth Hormone and Endometrial Receptivity. *Front Endocrinol*. 2019;10:653. doi:10.3389/fendo.2019.00653.
4. Paulson RJ. Introduction: Endometrial receptivity: evaluation, induction and inhibition. *Fertility sterility*. 2019;111:609–10. doi:10.1016/j.fertnstert.2019.02.029.
5. Karizbodagh MP, Rashidi B, Sahebkar A, Masoudifar A, Mirzaei H. Implantation Window and Angiogenesis. *Journal of cellular biochemistry*. 2017;118:4141–51. doi:10.1002/jcb.26088.

6. Casper RF, Yanushpolsky EH. Optimal endometrial preparation for frozen embryo transfer cycles: window of implantation and progesterone support. *Fertility and sterility* 105 (2016) 867 – 72. doi: 10.1016/j.fertnstert.2016.01.006.
7. Wu F, Chen X, Liu Y, Liang B, Xu H, Li TC, Wang CC. Decreased MUC1 in endometrium is an independent receptivity marker in recurrent implantation failure during implantation window. *Reprod Biol Endocrinol*. 2018;16:60. doi:10.1186/s12958-018-0379-1.
8. Li DD, Yue L, Yang ZQ, Zheng LW, Guo B. Evidence For Hmgn2 Involvement in Mouse Embryo Implantation and Decidualization. *Cellular physiology and biochemistry: international journal of experimental cellular physiology, biochemistry, and pharmacology* 44 (2017) 1681–1695. doi: 10.1159/000485775.
9. Zhou F, Liu Y, Rohde C, Pauli C, Gerloff D, Kohn M, Misiak D, Baumer N, Cui C, Gollner S, Oellerich T, Serve H, Garcia-Cuellar MP, Slany R, Maciejewski JP, Przychodzen B, Seliger B, Klein HU, Bartenhagen C, Berdel WE, Dugas M, Taketo MM, Farouq D, Schwartz S, Regev A, Hebert J, Sauvageau G, Pabst C, Huttelmaier S. and C. Muller-Tidow, AML1-ETO requires enhanced C/D box snoRNA/RNP formation to induce self-renewal and leukaemia. *Nat Cell Biol*. 2017;19:844–55. doi:10.1038/ncb3563.
10. Huang C, Shi J, Guo Y, Huang W, Huang S, Ming S, Wu X, Zhang R, Ding J, Zhao W, Jia J, Huang X, Xiang AP, Shi Y, Yao C. A snoRNA modulates mRNA 3' end processing and regulates the expression of a subset of mRNAs. *Nucleic acids research*. 2017;45:8647–60. doi:10.1093/nar/gkx651.
11. Romano G, Veneziano D, Acunzo M, Croce CM. Small non-coding RNA and cancer. *Carcinogenesis*. 2017;38:485–91. doi:10.1093/carcin/bgx026.
12. Lykke-Andersen S, Ardal BK, Hollensen AK, Damgaard CK, Jensen TH. Box C/D snoRNP Autoregulation by a cis-Acting snoRNA in the NOP56 Pre-mRNA. *Molecular cell*. 2018;72:99–111 e5. doi:10.1016/j.molcel.2018.08.017.
13. Sundar IK, Li D, Rahman I. Small RNA-sequence analysis of plasma-derived extracellular vesicle miRNAs in smokers and patients with chronic obstructive pulmonary disease as circulating biomarkers. *Journal of extracellular vesicles*. 2019;8:1684816. doi:10.1080/20013078.2019.1684816.
14. Craciunas L, Gallos I, Chu J, Bourne T, Quenby S, Brosens JJ, Coomarasamy A. Conventional and modern markers of endometrial receptivity: a systematic review and meta-analysis. *Hum Reprod Update*. 2019;25:202–23. doi:10.1093/humupd/dmy044.
15. Dvoran M, Vodicka J, Dostal J, Hajduch M, Dzubak P, Peskova M, Pilka R. Implantation and diagnostics of endometrial receptivity. *Ceska Gynekol* 83 291–298. doi.
16. Demiral I, Dogan M, Bastu E, Buyru F. Genomic, proteomic and lipidomic evaluation of endometrial receptivity. *Turk J Obstet Gynecol*. 2015;12:237–43. doi:10.4274/tjod.98475.
17. Becker D, Hirsch AG, Bender L, Lingner T, Salinas G, Krebber H. Nuclear Pre-snRNA Export Is an Essential Quality Assurance Mechanism for Functional Spliceosomes. *Cell reports*. 2019;27:3199–214 e3. doi:10.1016/j.celrep.2019.05.031.

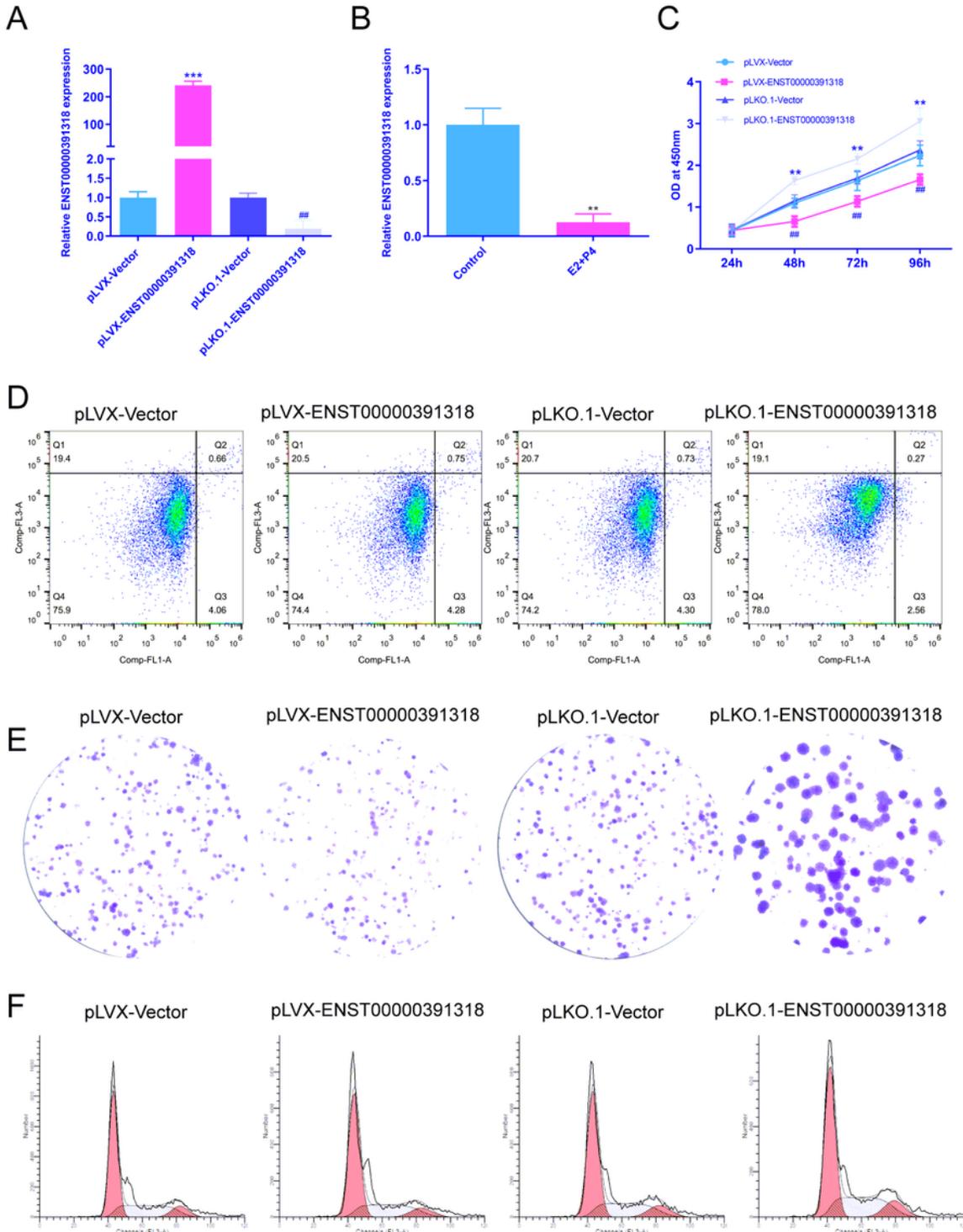
18. Yao J, Deng B, Zheng L, Dou L, Guo Y, Guo K. miR-27b is upregulated in cervical carcinogenesis and promotes cell growth and invasion by regulating CDH11 and epithelial-mesenchymal transition. *Oncol Rep.* 2016;35:1645–51. doi:10.3892/or.2015.4500.
19. Piao S, Inglehart RC, Scanlon CS, Russo N, Banerjee R, D'Silva NJ. CDH11 inhibits proliferation and invasion in head and neck cancer. *J Oral Pathol Med.* 2017;46:89–97. doi:10.1111/jop.12471.

## Figures



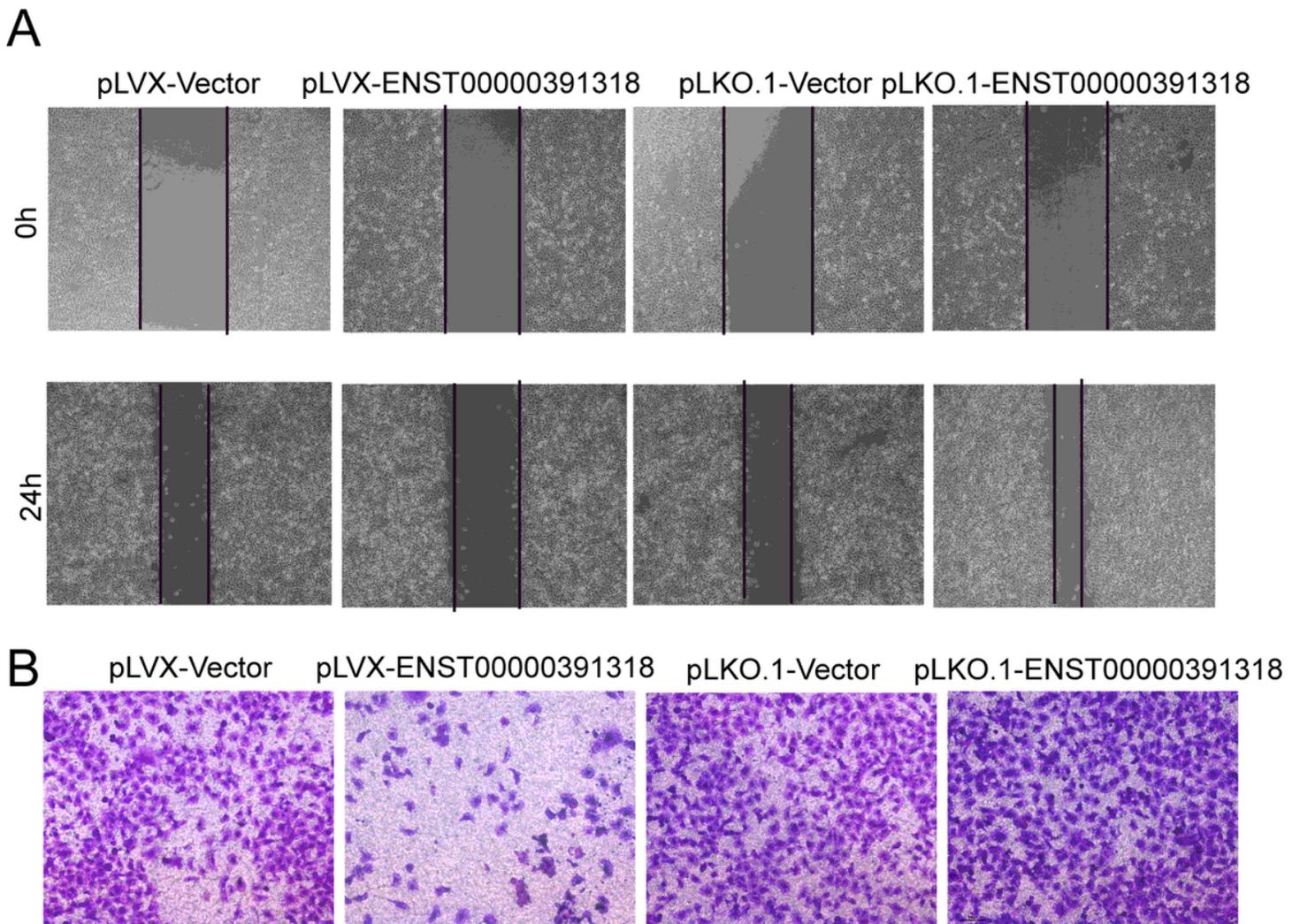
**Figure 1**

Endometrial SNORNA ENST00000391318 was upregulated in the GnRH antagonist protocol. (A) Volcano map indicating the expression of endometrial SNORNA in the GnRH antagonist protocol and natural cycle. A Group GnRH antagonist protocol B Group Natural cycle. (B) Heatmap indicating the expressions of endometrial SNORNA in the GnRH antagonist protocol and natural cycle. (C) qPCR detecting the expressions of endometrial SNORNA in the GnRH antagonist protocol and natural cycle.



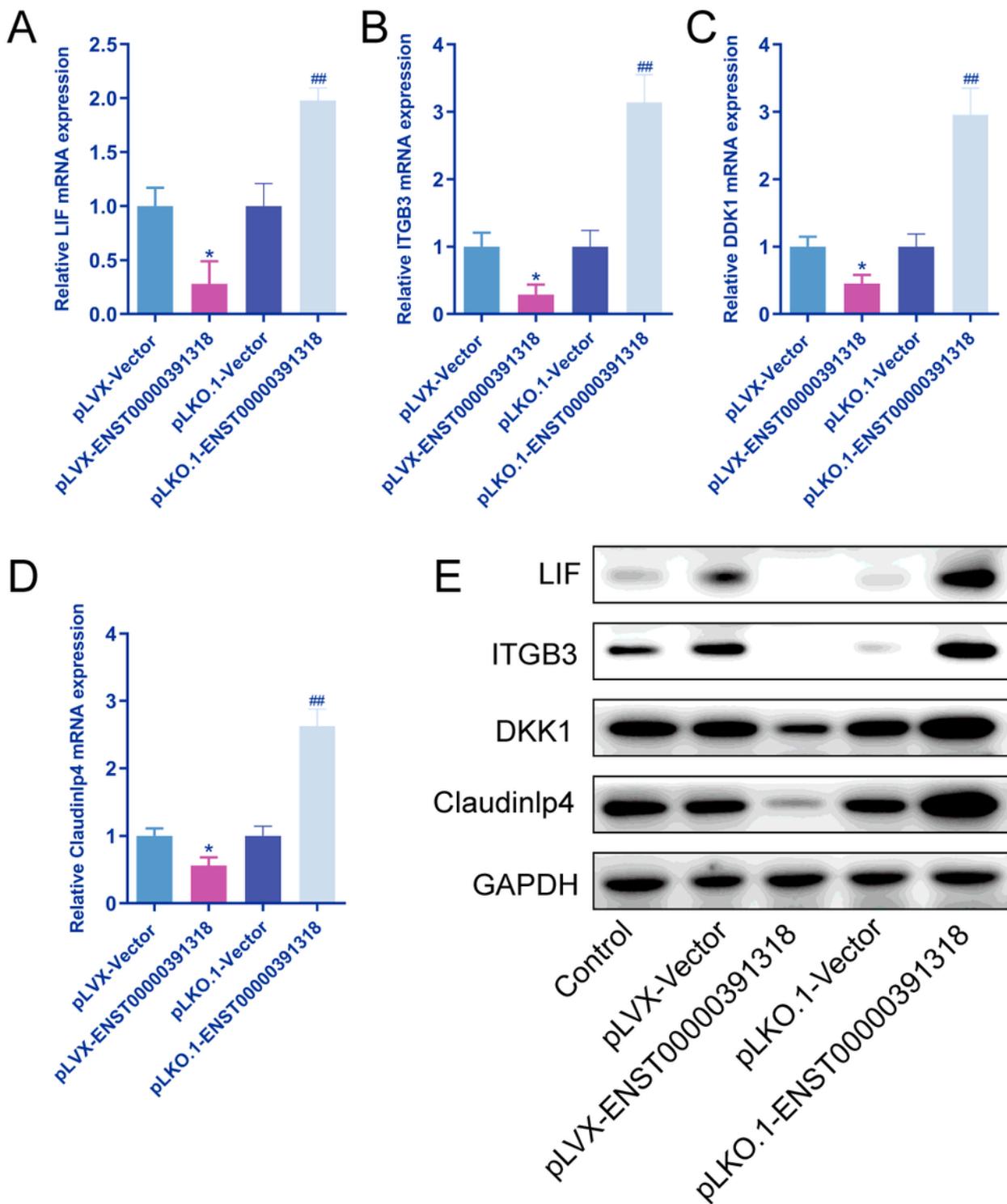
**Figure 2**

SNORNA ENST00000391318 inhibited the proliferation of Ishikawa cells. (A) qPCR was used to detect the expression of ENST00000391318 in Ishikawa cells transfected with lentivirus. (B) qPCR was used to detect the expression of ENST00000391318 in Ishikawa cells with or without E2 and P4 stimulation. (C) The CCK-8 assay was used to detect the proliferation of Ishikawa cells with ENST00000391318 overexpression or knockdown. (D) Flow cytometry detected the apoptosis of Ishikawa cells with ENST00000391318 overexpression or knockdown. (E) The clone formation assay was used to detect the proliferation of Ishikawa cells with ENST00000391318 overexpression or knockdown. (F) Flow cytometry detected the cell cycle of Ishikawa cells with ENST00000391318 overexpression or knockdown.



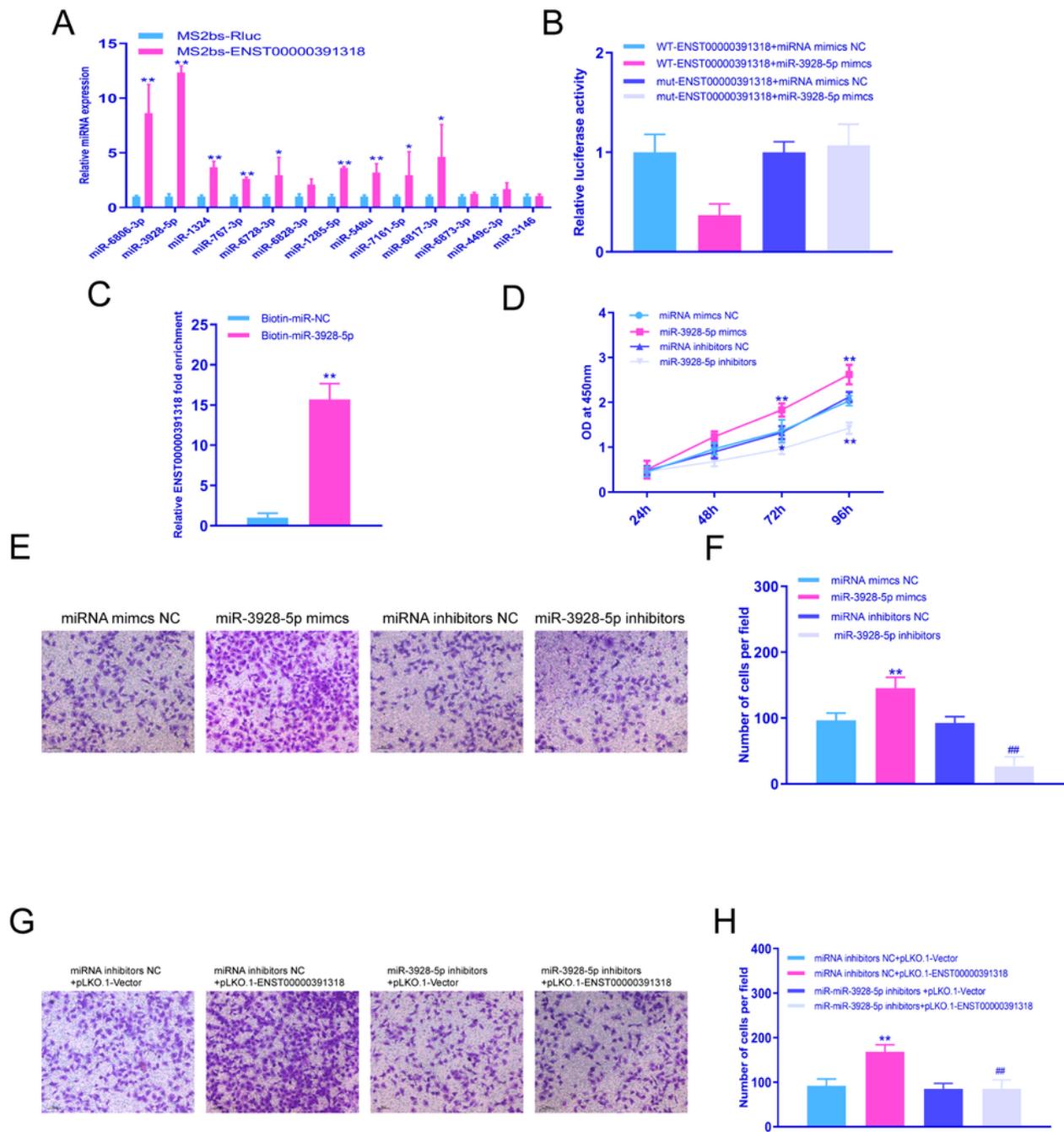
**Figure 3**

SNORNA ENST00000391318 inhibited the migration and invasion of Ishikawa cells. (A) Scratch assays detected the migration of Ishikawa cells with ENST00000391318 overexpression or knockdown. (B) The Transwell assay detected the invasion of Ishikawa cells with ENST00000391318 overexpression or knockdown.



**Figure 4**

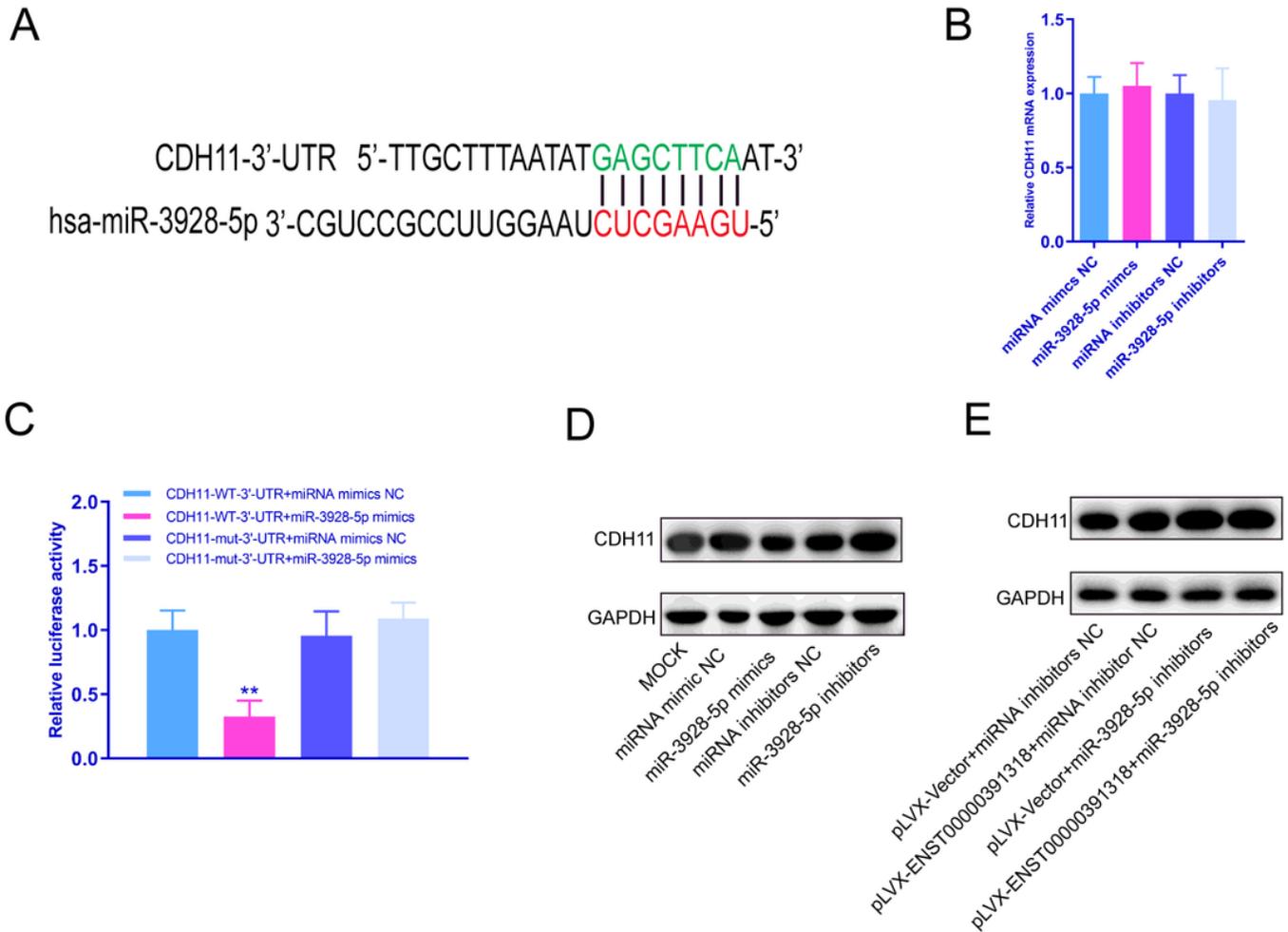
SNORNA ENST00000391318 inhibited the expression of receptivity factors. qPCR detected the mRNA expressions of LIF (A), ITGB3 (B), DKK1 (C) and Claudinlp4 (D) in Ishikawa cells with ENST00000391318 overexpression or knockdown. (E) Western blot analysis of the protein expressions of LIF, ITGB3, DKK1 and Claudinlp4 in Ishikawa cells with ENST00000391318 overexpression or knockdown.



**Figure 5**

miRNA-3928-5p is a target of ENST00000391318 in Ishikawa cells. (A) RNA pull-down experiment of ENST00000391318 to detect the miRNA bound with ENST00000391318. (B) The luciferase reporter assay detected the interaction of miR-3928-5p with ENST00000391318. (C) ENST00000391318 was found to be significantly enriched in the biotin-miR-3928-5p group by miRNA pull-down, detecting the interaction of miR-miR-3928-5p with ENST00000391318. (D) The CCK-8 assay detected the effect of miR-

3928-5p on the proliferation of Ishikawa cells. (E) Transwell assays detected the effect of miR-3928-5p on the invasion of Ishikawa cells. (F) Quantitative analysis of the effect of miR-6768-3p on the invasion of Ishikawa cells. (G) Transwell assays detected the effect of ENST00000391318 on the invasion of Ishikawa cells with miR-3928-5p inhibition. (H) Quantitative analysis of the effect of ENST00000391318 on the invasion of Ishikawa cells with miR-3928-5p inhibition. \* indicates  $P < 0.05$ , \*\* indicates  $P < 0.01$ .



**Figure 6**

ENST00000391318 regulates the expression of CDH11 through miR-3928-5p in Ishikawa cells. (A) TargetScan analysis showing the interaction of miR-3928-5p and the CDH11 mRNA 3'-UTR. (B) qRT-PCR detected the effect of miR-3928-5p on the expression of CDH11. (C) The luciferase reporter assay detected the interaction of miR-3928-5p with the CDH11 mRNA 3'-UTR. (D) Western blot analysis detected the effect of miR-3928-5p on the expression of the CDH11 protein. (E) Western blot analysis detected the effect of ENST00000391318 on the expression of CDH11 protein in cells with miR-3928-5p inhibition. \* indicates  $P < 0.05$ , \*\* indicates  $P < 0.01$ .