scientific reports

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LSD1 inhibits the invasion and migration of breast cancer through exosomes

Nan Zhang, Zhongyu Chen, Benkai Xin, Yueru Shi, Yutong Yao, Jingtong Yang, Xiaoyu Wang & Xin Hu✉

Metastasis accounts for almost 90% of breast cancer-related fatalities, making it frequent malignancy and the main reason of tumor mortality globally among women. LSD1 is a histone demethylase, which plays an important role in breast cancer. In order to explore the effect of LSD1 on invasion and migration of breast cancer, we treated breast cancer cells with MCF7 and T47D exosomes knocked down by LSD1, and the invasion and migration of breast cancer cells were significantly enhanced. This phenomenon indicates that LSD1 can inhibit the invasion and migration of breast cancer cells. miR-1290 expression was downregulated in LSD1 knockdown MCF7 exosomes. By analyzing the database of miR-1290 target gene NAT1, we verified that miR-1290 could regulate the expression of NAT1. These data provide fresh insights into the biology of breast cancer therapy by demonstrating how the epigenetic factor LSD1 stimulates the breast cancer cells' invasion and migration via controlling exosomal miRNA.

Keywords LSD1, Exosome, miR-1290

In women, the commonest cancer is breast cancer. It will represent 32% of all new cancer cases in the US in 2024 and increase at a pace of 0.6% annually[1]. One-quarter of all cancer cases and one-sixth of all cancer-related deaths among women globally are caused by breast cancer[2]. Although breast cancer's 5-year relative survival rate is now 90%, metastasis reduces survival chances to about 25%. Some increase in survival is noticed (from 75% for patients diagnosed in the mid-1970s to 90% for patients diagnosed between 2011 and 2017)[3,4]. Understanding the processes underlying breast cancer metastasis is crucial since it may lead to novel approaches to the disease's treatment.

It was not until the discovery of the histone lysine specific demethylase 1 (LSD1) that there was a renewed awareness of the fact that LSD1-mediated histone demethylation is a gradual, highly coordinated process. Lysine specific demethylase 1 (LSD1) removes methyl groups from monomethylated lysines on histone H3 and from dimethyllysine (H3K4me1/me2)[5–7]. Breast cancer cells' type and the stage of differentiation in which it is discovered determine the processes, including invasive metastasis and maintenance of breast cancer cell growth, in which LSD1 is involved[8]. It has been shown that LSD1 and SIN3A/HDAC complex synergistically inhibit the expression of a series of proto-oncogenes, such as CASP7, TGFB2, p21, etc. The LSD1 and SIN3A complexes are required for growth, epithelial characterisation, and the maintenance of drug sensitivity in breast cancer epithelial cells MCF7. And knockdown of LSD1 and SIN3A leads to increased invasion and migration of MDA-MB-231 cells.[9]. Moreover, earlier research has demonstrated that the binding of the LSD1/CoREST complex to the luminal-specific transcription factor GATA3 regulates luminal breast cancer cells, MCF7 invasion and migration and prevents tumor metastasis in mice with spontaneous breast cancer[6,10].

According to certain theories, cancer cells may be able to undergo an epithelial-mesenchymal transition (EMT) to separate from their primary tumor and enter the vasculature[11]. Epithelial cells are regulated by gene expression, modifying the expression of cytoskeletal and adhesion proteins as well as cell morphology to exhibit more invasive and migrating characteristics. Ultimately, epithelial cells transform into more motile mesenchymal cells, which are important for development and wound healing as well as unique characteristics of primary tumor formation and metastasis[12].

Exosomes are a type of extracellular vesicle that can have a diameter of 40–200 nm (or 30–150 nm). They are created when the cell membrane fuses with a multivesicular body[13] and contain several types of nucleic acids[14,15]. These exosomes are biochemically and functionally unique, and they have the ability to control

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target cell signaling pathways and protein production by being transported to receiver cells[16]. In the tumor microenvironment, exosomes contribute to intercellular communication. They also support the development and survival of tumor cells, metastasis, the shift from epithelial to mesenchymal tissue, angiogenesis, and immunosuppression[17–20]. Through exosomal transfer, breast cancer-derived miRNAs are essential for the genesis and progression of tumors, including the control of breast tumor growth and metastasis[21].

Breast, hepatocellular, and stomach cancers are just a few of the malignant tumor types where miR-1290 has been found. Several investigations demonstrate the diverse expression of miR-1290 and its correlation with various molecular models of breast cancer[23,24]. NAT1, or N-acetyltransferase 1, is an enzyme that breaks down xenobiotics in phase II[25,26]. High expression of NAT1 is strongly associated with breast cancer that is positive for the estrogen receptor.

The present study elucidated the involvement of exosomes in the invasive migration of breast cancer cells mediated by LSD1. Concurrently, we employed miRNA-seq to detect any differential expression of miR-1290 following LSD1 knockdown, identified the target gene NAT1 through database analysis, and confirmed the regulation of miRNA on the target gene. These findings shed light on the manner in which LSD1 controls breast cancer cells, invasion and migration through exosomes and offer novel therapeutic approaches for the disease.

Method

Cells culture

The ATCC provided the MCF7 and T47D cells. DMEM (Gibco, USA) was used to cultivate MCF7 cells. In order to cultivate MCF7, 10% FBS (BI, Israel) and 100 IU/ml penicillin and streptomycin (Solarbio, China) were added to DMEM. T47D cells were cultivated in RPMI-1640 media (Gibco, USA). 10% FBS (BI, Israel), 100 IU/ml penicillin and streptomycin (Solarbio, China), and 3.2 µg/ml insulin (Sigma, Saint Louis, USA) were added to the medium during T47D cell growth. Human embryonic kidney (HEK) 293 T cells were obtained from ATCC. 293 T cells were maintained with DMEM (Gibco, USA) supplemented with 10% FBS (BI, Israel) and 100 IU/ml penicillin and streptomycin (Solarbio, China).

The transfection of cells

Vectors and LSD1 siRNAs complied with previously published protocols[27]. Lipofectamine 3000 (Invitrogen USA), was used for the transfections. Utilizing RNAiMAX (Invitrogen, USA) for cell transfection, both miR-1290 mimics and inhibitor were employed. GenePharma (Suzhou, China) provided miR-1290 mimics and inhibitor.

Purification of exosomes

MCF7 cells were grown for 36 h in DMEM without fetal bovine serum. T47D cells were cultivated for 36 h in RPMI 1640 without fetal bovine serum. The conditioned medium was collected and centrifuged at 300×*g* for 10 min and the resulting supernatant was further centrifuged at 2000×*g* for 10 min to remove apoptotic bodies and large cellular debris. The conditioned medium was then centrifuged again at 2000×*g* for 10 min. Supernatants were filtered through 0.22-µm filters (Millipore, MA, USA) and centrifuged again at 5000×*g* to concentrate exosomes. The total exosome isolation reagent (Invitrogen, NY, USA) was added to the supernatant in one-half volume and the mixture was incubated at 4 °C overnight. The solution was then centrifuged at 10,000×*g* for 1 h, and the separated exosomes were obtained from the precipitate. For analysis of exosomes biomarker CD63 and TSG101, the exosome pellet was dissolved in radio immunoprecipitation assay (RIPA) lysis buffer for western blot.

PKH67 and Phalloidin staining

For 5 min, exosomes of purified breast cancer cells were labeled by incubating them in PKH67 (Sigma-Aldrich) and diluent C. The addition of serum subsequently stopped the labeling. A 10 kDa filter (Millipore) was used to remove extra dye after the labeled exosomes were introduced to 5 mL of phosphate buffered saline (PBS). The cells were cultured with the PKH67-treated exosomes for a duration of 6 h. After 60 min of Phalloidin-iFluor 555 (Abcam, ab176756) staining the cells, PBS washing was performed, and DAPI counterstaining was performed. Last but not least, pictures were taken with the Olympus IX51 microscope (Olympus Corporation).

The test for wound healing

Grown MCF7 breast cancer cells (80–90% confluence) were moved to serum-free media for six hours. Using a 200 µL cannon tip, monolayers of cells were scraped vertically. Purified exosomes were then introduced to serum-free media and co-cultured with the cells. At 0, 24, and 48 h, images were acquired of cells after wound generation. As the ratio of the migrated distance to the beginning distance, the migration capability was examined.

Invasion study with Matrigel

In a Transwell (Corning, USA) system, precoated polycarbonate membranes containing matrigel (Corning, USA) were used for the upper chamber during invasion tests. Serum-free media was used to mesophilically resuspend the cells, which were then cautiously added to the upper chamber. After adding the medium containing 20% serum to the lower chamber, incubation (48 h at 37 °C) proceeded. The infiltrated cells in the lower membrane layer were fixed with 4% paraformaldehyde, stained with Giemsa for an hour at 37 °C, and the uninfiltrated cells in the upper membrane layer were carefully wiped off. The cells were then imaged using an inverted microscope, and the number of infiltrated cells was determined by counting five randomly chosen fields of view.

Real time polymerase chain reaction

TransGen Biotech, China's TransZol Up was used to extract the cells' total RNA, and Allsheng Instruments Co., Ltd., China's Nanodrop was used to measure the extracted RNA's concentration. Using TransGen Biotech's EasyScript® All-in-One First-Strand cDNA Synthesis SuperMix for RT-PCR, 1 μg of RNA was reverse transcribed into cDNA. One First-Strand cDNA Synthesis SuperMix for RT-PCR (TransGen Biotech, China) was utilized to transcribe 1 μg of RNA into cDNA. TransStart® Green qPCR SuperMix (TransGen Biotech, Beijing, China) was then used to evaluate the cDNA. TransGen Biotech, Beijing, China's TransStart® Green qPCR SuperMix was used to reverse transcribe the cDNA to cDNA. The primer sequences are listed in Tables 1 and 2.

Western blotting

Protease inhibitor containing RIPA lysis buffer was used to extract all of the proteins (BestBio, China). A PVDF membrane (Millipore) was electrically transferred with equal amounts of proteins separated by 10% SDS-PAGE. The membrane was then blocked for 30 min with 5% skim milk powder, and the antibody was incubated at 4 °C for a whole night. The next day, membranes were incubated with horseradish peroxidase (HRP)-labeled secondary antibody for one hour at room temperature. The antibody configuration was as follows: LSD1 (1:1000, Cell Signaling Technology, 2139S), GAPDH (1:5000, Bioworld, AP0063), CD63 (1:500, Abcam, ab59479), TSG101 (1:1000, Abcam, ab83), NAT1 (1:1000, Abcam, ab109114). The Tanon 5200 Chemiluminescent Imaging System (Tanon Science & Technology, Shanghai, China) was used to take the pictures.

Small RNA sequencing

The QIAGEN miRNeasy Mini Kit (Qiagen, Hilden, Germany) was used to extract the total RNA from the exosomes, with an Agilent Bioanalyzer 2100 (Agilent technologies, USA, Santa Clara) utilized to verify the quality of the RNA. Using QIAseq®, sequencing libraries were created. Per the manufacturer's instructions, the VAHTSTM Small RNA Library Prep Kit for Illumina (Vazyme, Nanjing, China) directives. The Illumina Nova seq platform (Illumina, San Diego, USA) was used for the sequencing. Small RNA-seq data were deposited in the GEO database under the accession number GSE266982.

Extraction and miRNA identification

The EasyPure® miRNA Kit (TransGen, Beijing, China's Biotech) was utilized to extract miRNAs from the exosomes. The miRNA reverse transcriptase and RT-PCR primers were manufactured and produced in Shanghai, China at GenePharma Co., Ltd. RT-PCR tests were synthesized in U6 and TransStart® Green qPCR SuperMix (TransGen Biotech, Beijing, China) was employed as the scrambled control for miRNA.

Examination of bioinformatics

For predicting miRNA's target genes, Targetscan (http://www.targetscan.org/), miR-Walk (http://mirwalk.umm.uni-heidelberg.de), and RNA22 (http://www.cm.jefferson.edu/rna22/) were utilized. The KEGG/GO pathway enrichment analysis was carried out using KOBAS, and the target genes' functions were annotated[28–30].

Luciferase reporter assay

The GP-miRGLO plasmid containing wild-type and mutant 3'UTR regions of NAT1 and miR-1290 mimics were co-transfected with Lipofectamine 3000 (Invitrogen, USA) to transfect the cells for 24 h. After 24 h, the luciferase activity was measured using the Dual Luciferase Reporter Analysis System (Promega, USA) to measure luciferase activity, followed by detection of fluorescence intensity in each group using a fluorescent microplate reader (Allsheng Instruments Co., Ltd., Hangzhou, China)..

Primers	Forward	Reverse
β-actin	ACCAACTGGGACGACATGGA	GGTCTCAAACATGATCTGGGTCAT
E-cadherin	GCCCCGCCTTATGATTCTC	GCCCCATTCGTTCAAGTAGTC
α-catenin	AGGAAGGCAACTCGACCTTT	TCCAGTAGCTTCTCCACGGT
ZEB2	AGCCTCTGTAGATGGTCCAGAAGAA	CACTGTACCATTGTTAATTGCGGTC
Snail1	CTTGTGTCTGCACGACCTGT	CTTCACATCCGAGTGGGTTT
NAT1	AGGGCAAAACGCTCAGAAATG	TCCTGAAGATTGCATCATGTCG

Table 1. RT-PCR primer sequences (all sequences from 5′ to 3′).

Primers	Forward	Reverse
U6	ATTGGAACGA TACAGAGAAGATT	GGAACGCTTCACGAATTTG
miR-1290	GCGCGTGGATTTTTGGAT	AGTGCAGGGTCCGAGGTATT

Table 2. miRNAs RT-PCR primer sequences (all sequences from 5′ to 3′).

Examinations of statistics

The information shown is the average ± standard deviation from a minimum of three different experiments. The statistical analysis software program GraphPad Prism 8.0 was utilized for all data analysis. $P < 0.05$ was regarded as statistically significant, and Student's t test was used for the statistical analyses.

Results

Isolation, characterization, and uptake of exosomes from LSD1 knockdown breast cancer cells

We first created LSD1 knockdown and rescue MCF7 and T47D breast cancer cells by transient transfection in order to examine whether LSD1 controls breast cancer invasion and migration through exosomes. The results of the Western Blot analysis demonstrated that LSD1 was successfully knockdown and rescued in MCF7 and T47D cells (Fig. 1A, B). The exosome markers CD63 and TSG101 in the enriched exosomes were then identified by Western Blot after we had enriched exosomes from the media of both cell cultures (Fig. 1C, D). Afterwards we analyzed the size of the exosomes by NTA at approximately 150 nm and the exosomes were about 6×10^9 particles/mL (Fig. S1). To validate the ability of the enriched exosomes from MCF7 and T47D cells to penetrate the target cell MCF7, we co-incubated the enriched exosomes with MCF7 after performing cell membrane staining with PKH67. The immunofluorescence results demonstrated that the exosomes from MCF7 and T47D cells could effectively penetrate the target cell MCF7 (Fig. 1E, F). Exosomes entering the target cells were detected to be localized in the cytoplasm and nucleus by staining the cytoskeleton with phalloidin (Fig. 1G, H). The results of the above experiments showed that exosomes were successfully recovered from MCF7 and T47D, two types of breast cancer cells in which LSD1 was knocked down and rescued, and that exosomes were able to enter the target cells MCF7.

LSD1 controls exosome-mediated breast cancer's invasion and migration

We isolated exosomes from two breast cancer cell lines, LSD1 knockdown and rescue, and provided MCF7 cells for co-culture concurrently to validate the function of LSD1 in controlling breast cancer invasion and migration through exosomes. The results of the wound healing assay demonstrated that exosomes from breast cancer cells with LSD1 knockdown, MCF7 (Fig. 2A, B), and T47D (Fig. 2C, D), were able to considerably accelerate the migration of breast cancer cells. Exosomes from LSD1 knockdown MCF7 (Fig. 2E, F) and T47D (Fig. 2G, H) breast cancer cells were then found to considerably enhance MCF7 cell invasion, according to the Transwell assay results. It is believed that cancer cells activate epithelial mesenchymal transition (EMT), which causes epithelial cells to lose polarity, have reduced adhesion ability, and acquire the motility and migration properties of mesenchymal cells[31,32]. The transcription of the epithelial marker E-cadherin and the related adhesion gene α-catenin were repressed, and the treated MCF7 cells showed increased expression of the marker genes for EMT, ZEB2 and Snail1 (Fig. 2I, J). Our findings demonstrate that LSD1 knockdown exosomes enhance MCF7 cell invasion and migration, as well as modulate the expression of genes involved in cell–cell adhesion regulation and cell-transmitter metabolism.

Differentiated miRNAs in exosomes following LSD1 knockdown examined using miRNA-seq

We have undertaken miRNA high-throughput sequencing studies on LSD1 knockdown and rescue exosomes to explore whether LSD1 knockdown is involved in exosome-regulated breast cancer invasion and migration by modifying which micro RNAs in exosomes. To identify differentially expressed miRNA, we employed fold change (FC) ≥ 2 and $p < 0.05$ as the cutoff value. The sequencing results revealed that 38 miRNAs were located at the intersection of altered miRNA expression in the exosomes of the LSD1 KD vs Rescue group and the Control vs LSD1 KD group (Fig. 3A). 13 of these miRNAs were expressed in the LSD1 KD vs. Control group while none were expressed in the LSD1 KD vs. Rescue group. It is very possible that the expression of LSD1 influenced the alterations in these miRNA levels. (Fig. 3B) displays the outcomes of the heatmap mapping of these 13 miRNAs. A study found a favorable correlation between the tumor grade of breast cancer that was ERα-positive and miR-1290[24]. miR-1290's expression in exosomes was then confirmed, and it was found that exosomal miR-1290 expression was lowered upon LSD1 knockdown and restored during LSD1 rescue. (Fig. 3C). Using the cell invasion and migration assays, we then looked into the function of miR-1290 in MCF7. MiR-1290 overexpression was seen to hinder cell migration in miR-1290 mimics' transfected cells (Fig. 3D, E). The findings of transfection of miR-1290 inhibitor demonstrated that it facilitated cell migration and reduced the expression of miR-1290 in cells (Fig. 3D, E). In MCF7 transfected with miR-1290 mimics, cell invasion was consistently decreased (Fig. 3F, G), whereas cell invasion was increased by transfection with miR-1290 inhibitors (Fig. 3F, G). These findings imply that varying levels of miR-1290 in exosomes following LSD1 knockdown may have an impact on MCF7 invasion and migration.

LSD1-knockdown exosomes from breast cancer cells influence cell invasion and migration through miR-1290

For demonstrating miR-1290's function in LSD1 deleted breast cancer cells' exosomes, we first used immunofluorescence to establish that miR-1290 tagged with Cy3 could enter the exosomes and function (Fig. 4A). And following the transfection of miR-1290 mimics, we confirmed by RT-PCR that there was a rise in miR-1290 expression in exosomes (Fig. 4B). Next, we enriched the exosomes for cell wound healing assays after knocking down LSD1 in MCF7, knocking down LSD1 while transfected with miR-1290 mimics, and transfected with miR-1290 inhibitors in MCF7, respectively. The results of the cell scratch assay demonstrated that the LSD1 KD + miR-1290 mimics group's migration ability was significantly lower than that of the LSD1 KD group, and that the Control group's migration ability was also lower than that of the Control + miR-1290 inhibitor group (Fig. 4C, E). We then conducted the same experiments in T47D, and the results again demonstrated significant differences

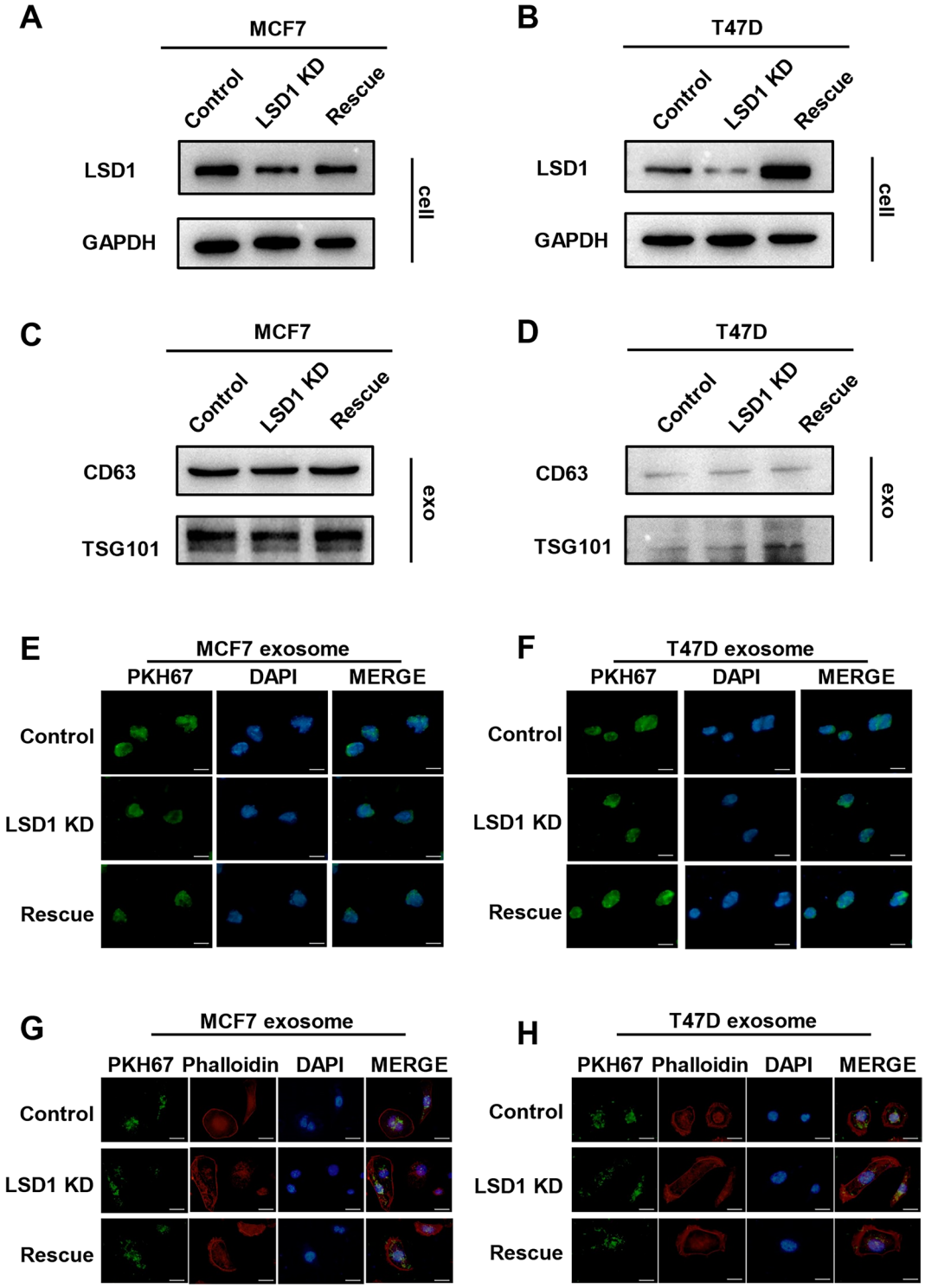

Fig. 1. Exosomes from MCF7 and T47D cells can be taken up by MCF7 cells. LSD1 knockdown was achieved by transfecting LSD1 siRNA into MCF7 (**A**) and T47D (**B**) cells. To restore LSD1 expression, the pcDNA3.1-LSD1 plasmid was transfected into MCF7 cells based on the knockdown, and the Western Blot results showed levels of LSD1 in MCF7 (**A**) and T47D (**B**). Western Blot results for the expression of exosomal markers TSG101 and CD63 in MCF7 (**C**) and T47D (**D**) show simultaneous enrichment of Control, LSD1 KD, and exosomes from Rescue. Immunofluorescence pictures of exosomes tagged with PKH67 that MCF7 (**E**) and T47D (**F**) cells can effectively internalize (scale bar = 10 μm; green: PKH67; blue: DAPI). Immunofluorescence pictures of the cytoskeleton labeled with ghost pen cyclic peptide, showing exosomes located in the cytoplasm and nucleus of MCF7 (**G**) and T47D (**H**) (Phalloidin is red, DAPI is blue, and PKH67 is green; scale bar = 10 μm).

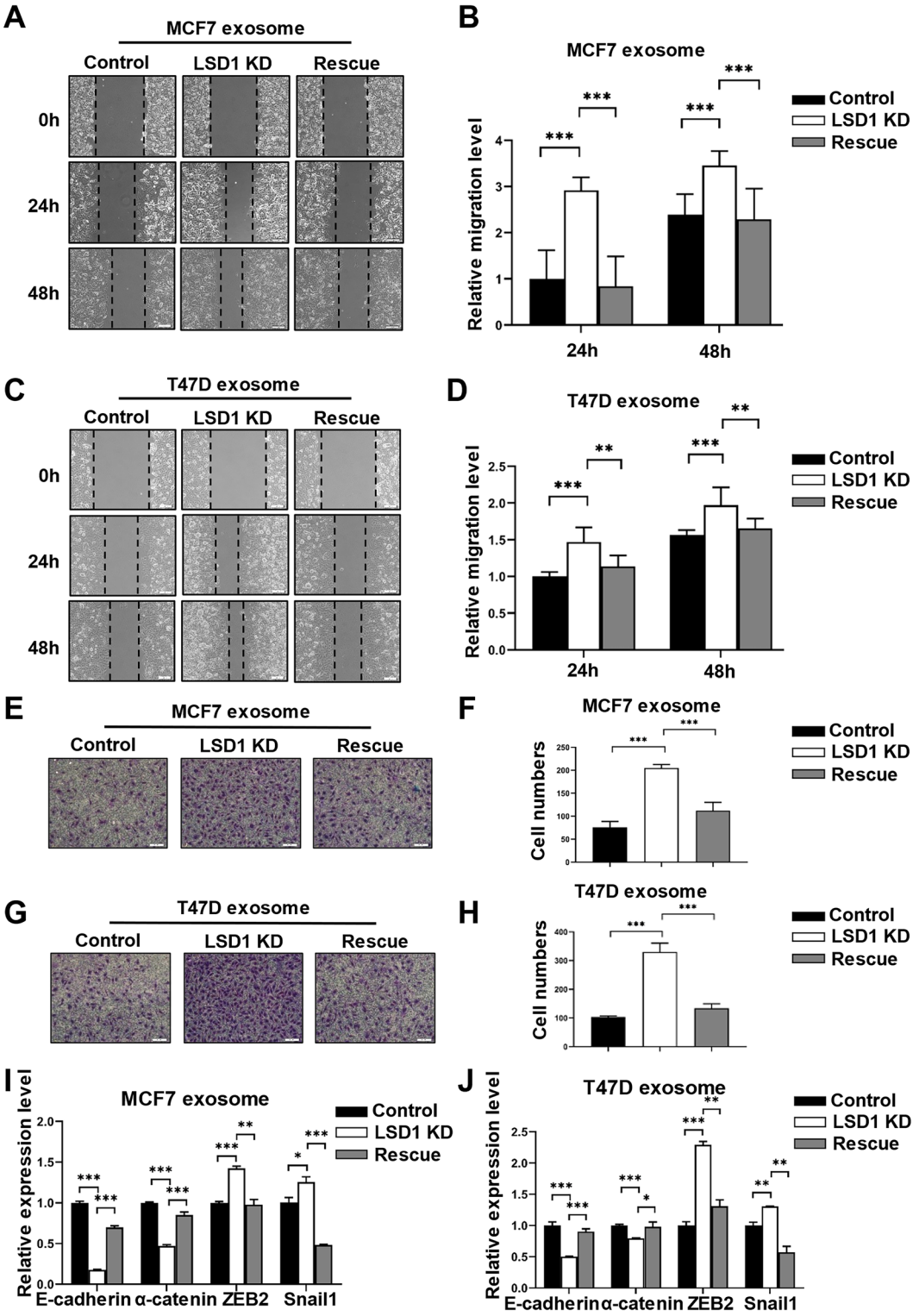

Fig. 2. LSD1 regulates breast cancer invasion and migration via exosomes. Using the wound healing assay, the cell migration rate (**B, D**) of MCF7 treated with exosomes of MCF7 (A) and T47D (C) cells was assessed. Scale bar: 100 μm. After incubating with exosomes of MCF7 (**E,F**) and T47D (**G,H**), cells were planted on Matrigel-coated transwell inserts and the invasion experiment was conducted. Bars of scale: 50 μm. MCF7 cells were incubated with exosomes from T47D (**J**) and MCF7 (**I**) cells, and the mRNA expression of E-cadherin, α-catenin, ZEB2, and Snail1 was examined using RT-PCR findings. t test without pairing, *P < 0.05, **P < 0.01, ***P < 0.001.

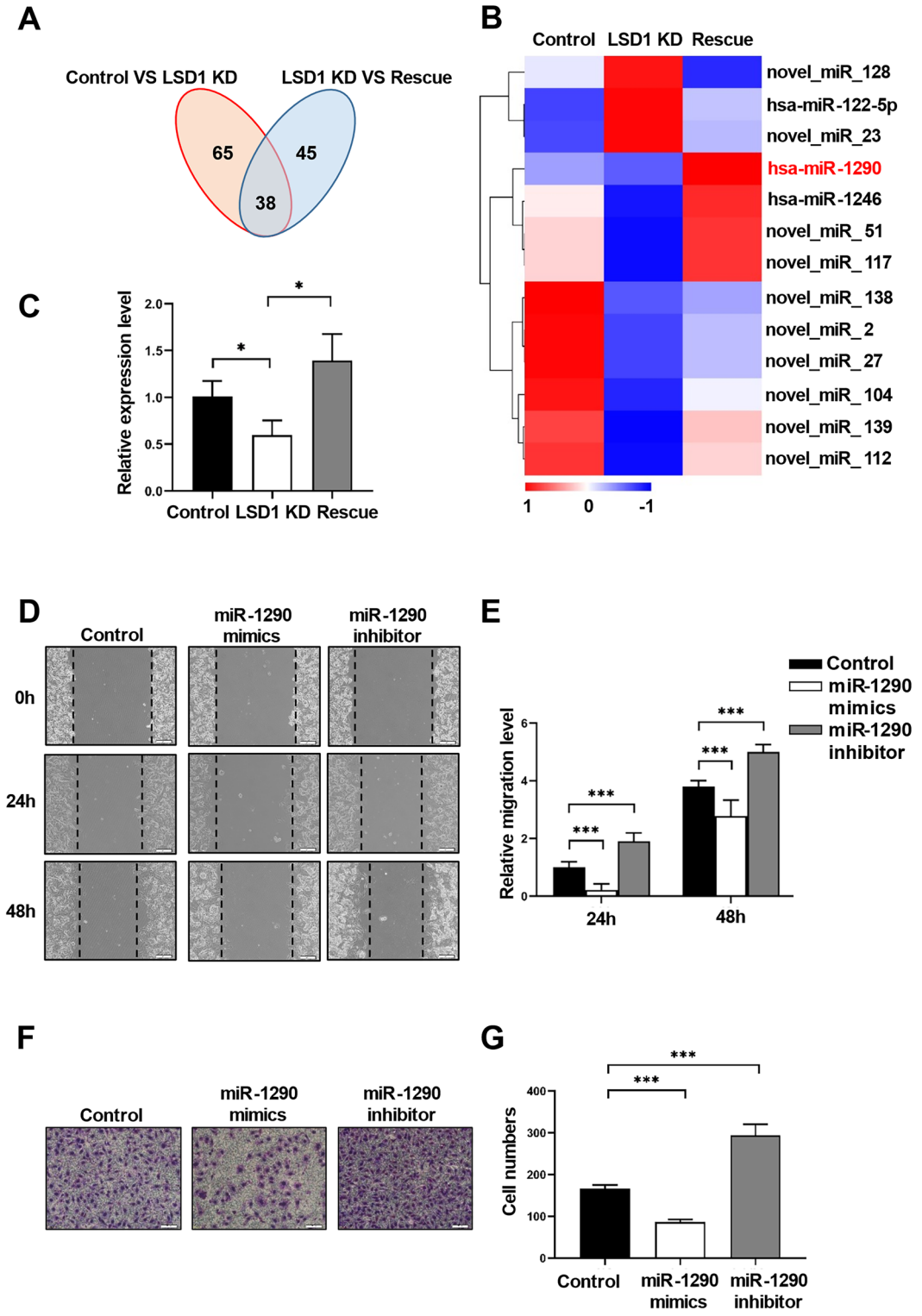

Fig. 3. Differentiated miRNAs in exosomes following LSD1 knockdown examined using miRNA-seq. miRNA-seq sequencing technique to analyze differential miRNAs in exosomes after LSD1 knockdown and effect on invasive migration. Venn plots (**A**) showing the quantity of miRNAs that differed in expression between the exosomes of the Control and LSD1 KD groups, as well as between the LSD1 KD and Rescue groups. (**B**) A heat map displaying 13 miRNAs that exhibit opposing expression trends in the groups who receive LSD1 KD, Rescue, and LSD1 KD. (**C**) The miR-1290 highlighted is expressed differently in exosomes of LSD1 KD, Rescue, and Control. Transfection of MCF7 cells with miR-1290 mimics and inhibitors for 24 and 48 h was used for wound healing (**D,E**) and transwell (**F,G**) assays. Scale bar: 100 μm. T-test without pairing, *P < 0.05, **P < 0.01, ***P < 0.001.

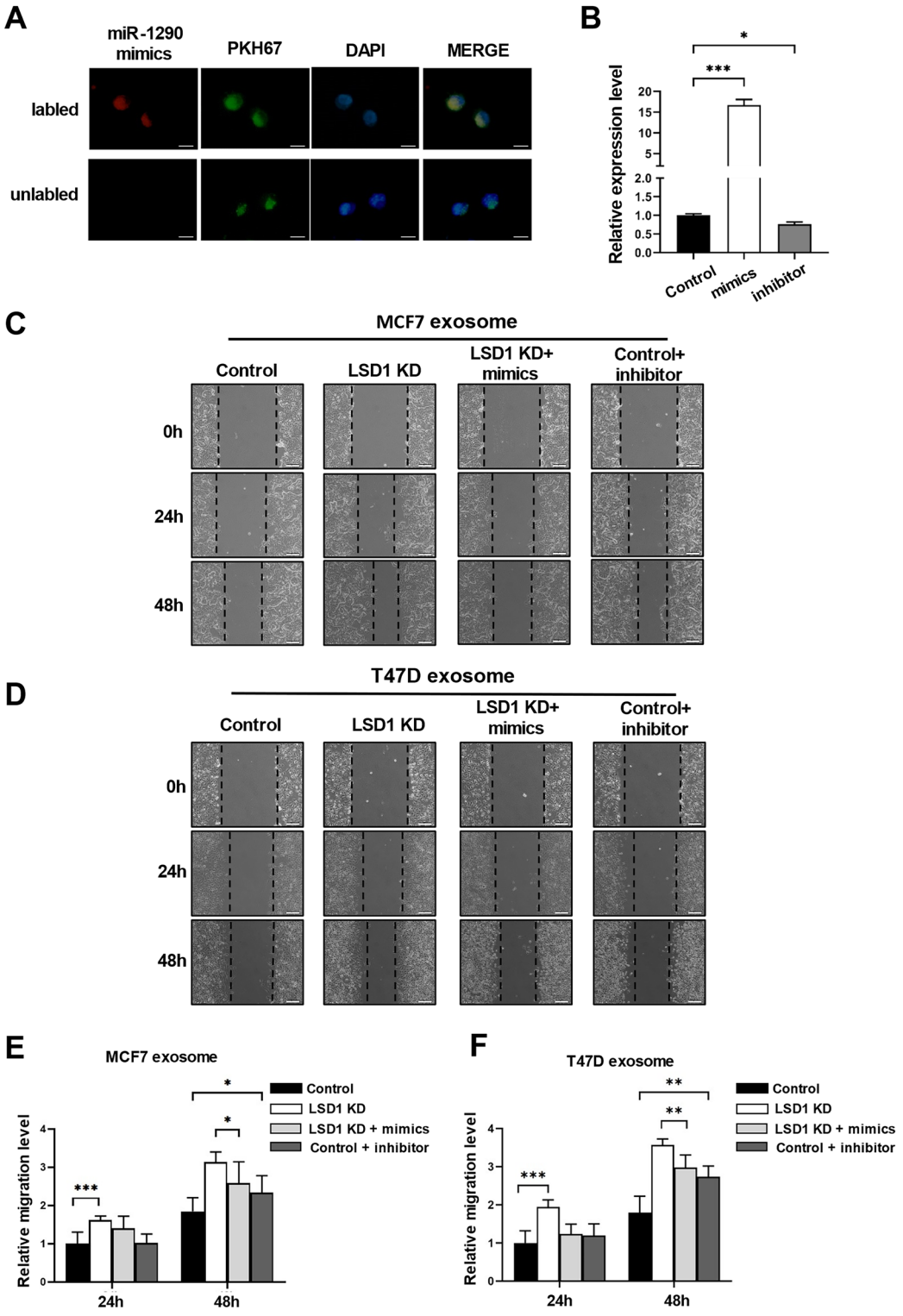

Fig. 4. Knockdown of LSD1 in breast cancer cell exosomes affects cell invasion and migration via miR-1290. (**A**) Immunofluorescence pictures of Cy3-labeled miR-1290 that can be accessed by exosomes (DAPI, green, PKH67, and red, miR-1290; scale bar = 10 μm). (**B**) Real-time PCR analysis confirmed that exosomes express miR-1290. The wound healing experiment was used to observe the exosome-treated MCF7 cell migration assay, as well as the evaluation of cell migration rate following transfection with miR-1290 mimics following LSD1 knockdown in MCF7 (**C,E**) and T47D (**D,F**) cells and control transfection with the inhibitor. T-test without pairing, Scale bar: 100 μm. *P < 0.05, **P < 0.01, ***P < 0.001.

in the migration abilities between the LSD1 KD group and the LSD1 KD + miR-1290 mimics group. The Control group's migration ability was also lower than that of the Control + miR-1290 inhibitor group. (Fig. 4D, F). The aforementioned experimental findings showed that miR-1290-mediated cell invasion and migration were impacted by breast cancer cell exosomes that knocked down LSD1.

miR-1290 regulates the target molecule NAT1

Next, we used Targetscan, RNA22, and miRWalk databases to estimate the target genes of miR-1290. A total of 1617 putative target genes were identified (Fig. 5A). Further KEGG analysis revealed that the putative target genes of miR-1290 concentrated in pathways linked to cell adhesion, influencing cell invasion and migration, and cancer-related pathways (Fig. 5B). Additionally, GO enrichment analysis revealed that the target genes of miR-1290 were enriched in cell adhesion and migration regulatory pathways (Fig. 5C). According to predictions, NAT1 could be a target gene for miR-1290, and it bonded to the 3'UTR region of NAT1 (Fig. 5D). To investigate how miR-1290 affected the expression of NAT1, the potential miR-1290 binding sites on the 3'UTR regions of NAT1 were mutated, and the mutated and wild-type 3'UTR regions were cloned into luciferase reporter vector pmirGLO. These luciferase reporter constructs and miR-1290 were transfected into 293 T cells. miR-1290 significantly reduced the luciferase activities from the pmirGLO-NAT1-wt constructs, and did not affect the luciferase activities from the pmirGLO-NAT1-mut constructs (Fig. 5D). MiR-1290 mimics and inhibitor were then transfected into the MCF7 breast cancer cell line. Following the overexpression and knockdown of miR-1290, NAT1 in cells was confirmed (Fig. 5E, F). The findings demonstrated that NAT1 expression rose following miR-1290 knockdown and fell following miR-1290 overexpression. NAT1 might be a target gene for miR-1290.

In conclusion, the exocytosis of breast cancer cells with LSD1 knockdown increases cell invasion and migration by reducing the expression of miR-1290, and it validates that NAT1 is a target gene of miR-1290 (Fig. 6). Created with BioRender.com.

In breast cancer cells, down-regulation of LSD1 leads to down-regulation of miR-1290 in exosomes, which promotes EMT, invasion and migration of breast cancer cells by targeting NAT1.

Discussion

Breast cancer is a prevalent malignant tumor in women that makes up 20% of all malignant tumors. It has a significant effect on patient survival[33], and research has shown that LSD1, the first histone demethylase to be discovered, plays a crucial role in the invasive migration of breast cancer. Through the positive regulation of GATA-binding protein 3 (GATA3) and the inhibition of TRIM37 (tripartite motif-containing 37) expression, it has been discovered that LSD1 inhibits the invasion, migration, and metastasis of luminal breast cancer cells[6]. LSD1 plays different roles in different cancers. It has been shown that LSD1 is involved in the development of a variety of solid tumors, such as breast, bladder, prostate and non-small cell carcinomas. It is associated with cancer proliferation, migration, invasion and metastasis[34]. Some studies have shown that LSD1 is overexpressed in a variety of tumor cells and is also associated with poor tumor prognosis, but it has also been shown that down-regulation of LSD1 inhibits the oncogenic role of TRIM37 in breast cancer[6].

We have uncovered the mechanism by which LSD1 prevents breast cancer cells from migrating invasively through exosomes. LSD1 KD exosomes stimulated the invasive migration of MCF7 breast cancer cells while modifying the expression of genes linked to cellular EMT. Subsequent investigations revealed that LSD1 controls the miRNA content in the exosomes of breast cancer cells, hence playing a significant role in cell invasion and migration. The expression of miR-1290 was found to be reduced in exosomes of LSD1 KD, as shown by high-throughput sequencing of miRNAs in exosomes. This lowered miR-1290 may influence invasive cell migration and interact with the target gene NAT1 (Fig. 6).

Intercellular signaling regulators are molecules that carry information from one cell to another and are found in large quantities in exosomes. As a signaling molecule, microRNA (miRNA) is a non-coding RNA that is typically loaded into exosomes[35]. It has been shown that miRNAs are assigned to exosomes in a regulated manner[36]. In our sequencing data, it was shown that downregulation of LSD1 caused changes in miRNA expression in exosomes of breast cancer cells. And it was also shown that LSD1 deletion caused downregulation of miR-145-5p in exosomes, which affected gastric cancer metastasis[37]. So downregulation of LSD1 regulates cancer metastasis by modulating changes in levels of contents in exosomes.

Exosomes have been linked to several stages in the metastatic spread of breast cancer, including invasion, extravasation, MET, colonization, migration, and EMT, according to research conducted in recent years[38]. Exosomes have a crucial role in the promotion of cancer invasion and migration. They are also engaged in a number of important activities, including drug resistance, tumor growth, angiogenesis, tumor microenvironment formation, cell invasion, and metastasis[39–41]. Furthermore, miRNAs contained in exosomes have a direct bearing on the advancement of breast cancer since they are implicated in numerous cancer processes and have distinct functions during the course of the disease's development. MiR-7-5p targets the RYK gene and influences the phosphorylation of JNK and c-Jun proteins, which increases the expression of EMT transcription factors including ZEB1 and affects breast cancer cells' invasion and migration in exosomes[42]. MiR-516a-3p was found to decrease the Pygo2/Wnt/β-catenin signaling pathway, hence inhibiting the proliferation, metastasis, and EMT of breast cancer cells[43]. Our investigation revealed that the miRNA content contained in MCF7 cells' exosomes at LSD1 KD was changed, with a reduction in miR-1290. miR-1290 has been found in a number of malignant tumors, including breast cancer[22]. Several investigations have also revealed that there is variability in miR-1290 expression and that it is connected to several molecular models[23,24]. It has been demonstrated by in vitro research that miR-1290 can prevent breast cancer cells from migrating invasively. KEGG and GO studies revealed that the target genes of miR-1290 were connected to the regulatory pathways of cell adhesion and migration after additional investigation into the function and target genes of the protein. According to predictions, NAT1 binds

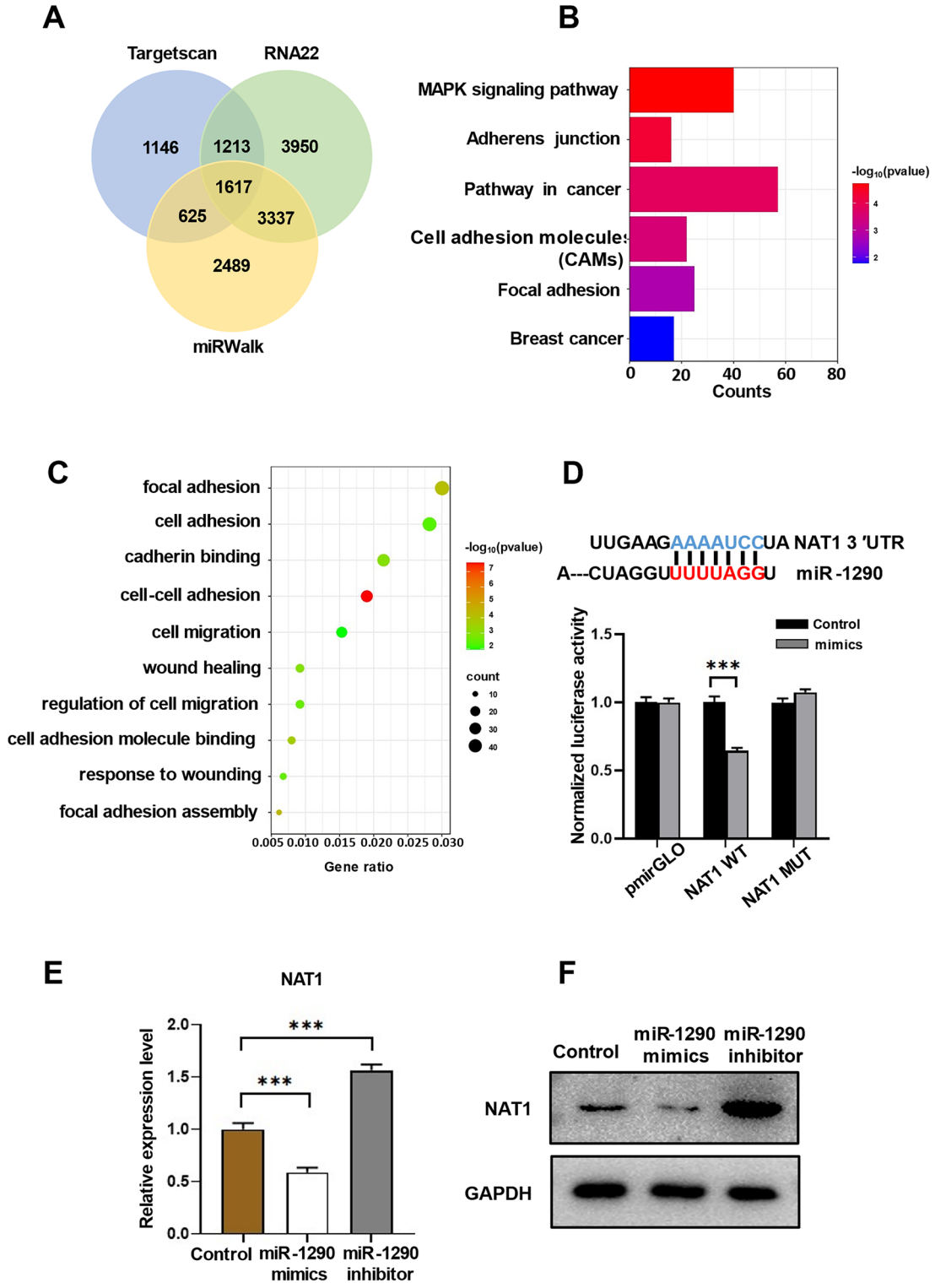

Fig. 5. miR-1290 regulates the target molecule NAT1. (**A**) Veen plot of the total number of target genes predicted by miRWalk, RNA22, and Targetscan for miR-1290. (**B**) KEGG (https://www.kegg.jp/kegg/kegg1.html) pathway analysis of 1617 miR-1290 target genes associated with RNA22, Targetscan, and miRWalk predictions. (**D**) Schematic depiction of binding locations to miR-1290 in the NAT1 3' UTR. Measure the luciferase activity of NAT1 in 293 T cells using miR-1290 mimics and luciferase expression constructs with wild-type or mutant 3'UTR, (**C**) GO enrichment analysis of 1617 miR-1290 target genes predicted by Targetscan, RNA22, and miRWalk. The expression of NAT1 in MCF7 cells following transfection with inhibitor, control, and miR-1290 mimics was demonstrated by RT-PCR (**E**) and Western Blot (**F**) analysis. ***$P < 0.001$ for the unpaired t-test.

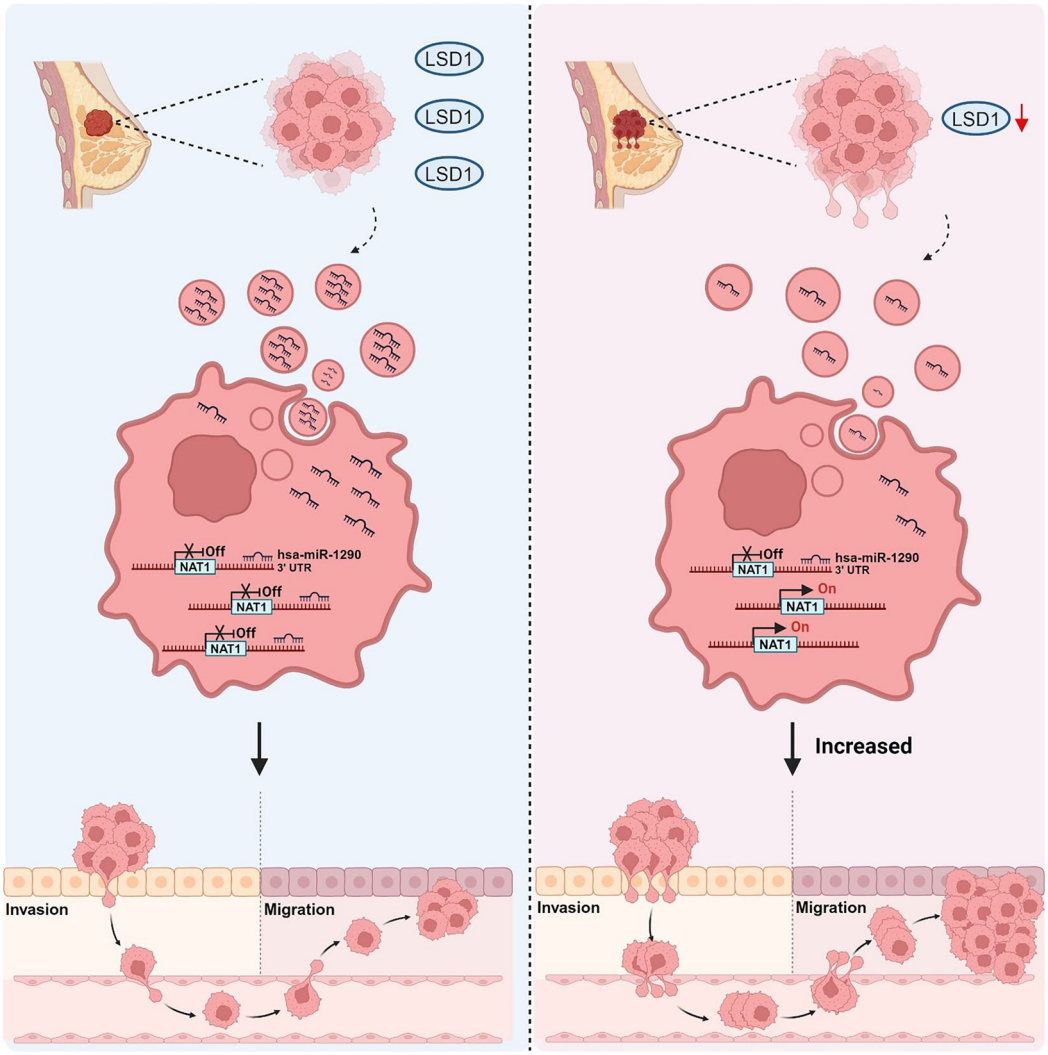

Fig. 6. Schematic diagram of LSD1 regulation of breast cancer invasive migration via exosomes.

to the 3'UTR region of NAT1 and may be a possible target gene for miR-1290. Exosomes are a crucial conduit for cellular communication. Since the miRNAs located within them do not degrade in the bloodstream, they may be useful for the early detection of malignancies[44]. The variability of miR-1290, however, suggests that it may be a useful biomarker for the early detection of estrogen receptor α (ERα)-positive breast cancer.

Our findings demonstrate, in summary, that downregulation of LSD1 in breast cancer cells increases invasive migration in estrogen receptor α (ERα)-positive breast cancer by causing downregulation of miR-1290 in the cells' exosomes. Our research broadens the understanding of the function of epigenetic changes in cancer spread.

Data availability

The Small RNA-seq data during the current study are available in the GEO repository, GSE266982.

Received: 10 June 2024; Accepted: 27 August 2024
Published online: 06 September 2024

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Funding
This work was supported by Jilin Scientific and Technological Development Program [20230508066RC], Special Project for Health Research Talents of Jilin Province [2022scz04], Norman Bethune Program of Jilin University [2022B06] and Innovation and Entrepreneurship Talent Funding Project of Jilin province [2023QN05].

Competing interests
The authors declare no competing interests.

Additional information
Supplementary Information The online version contains supplementary material available at https://doi.org/10.1038/s41598-024-71353-y.

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