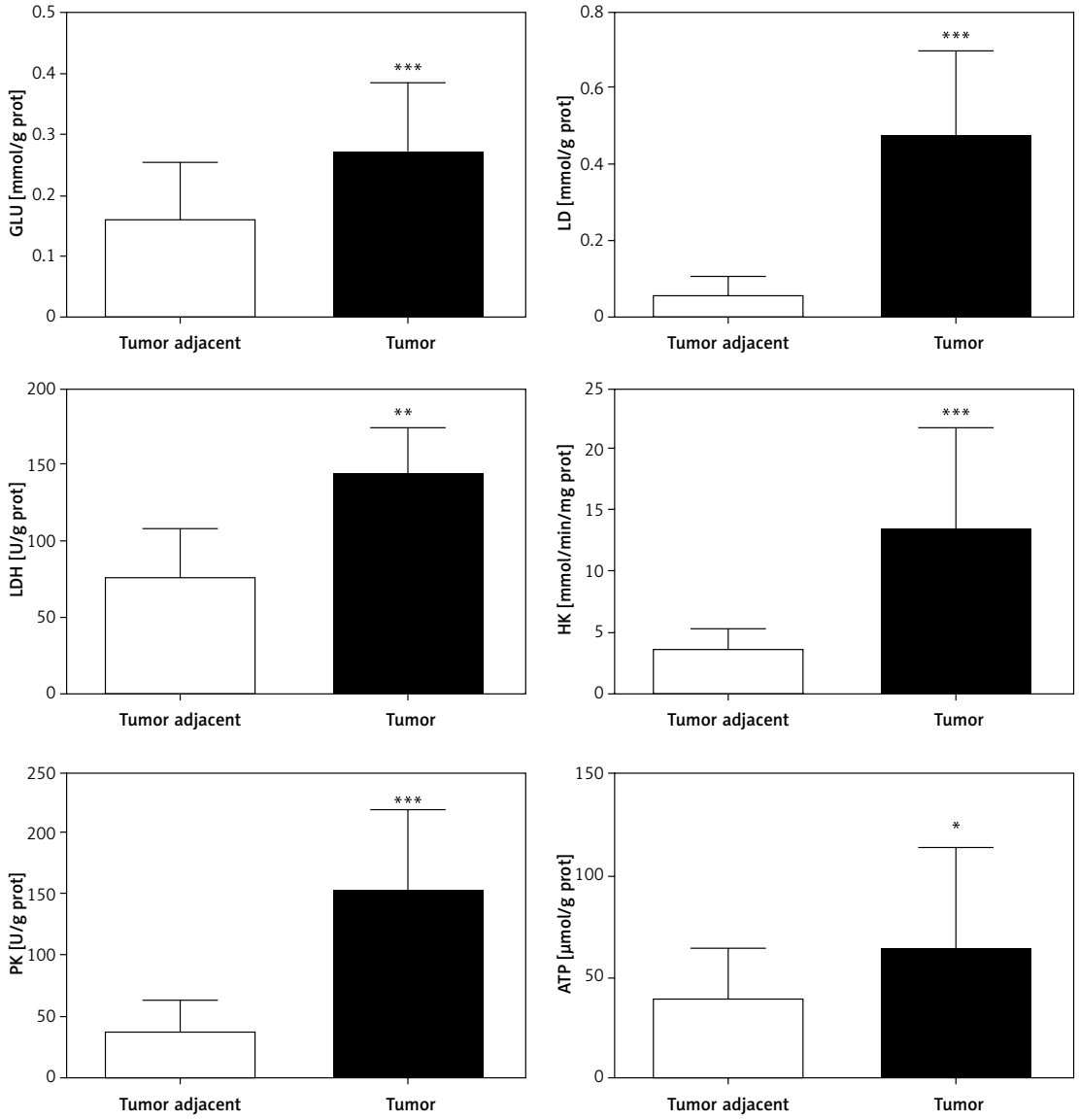
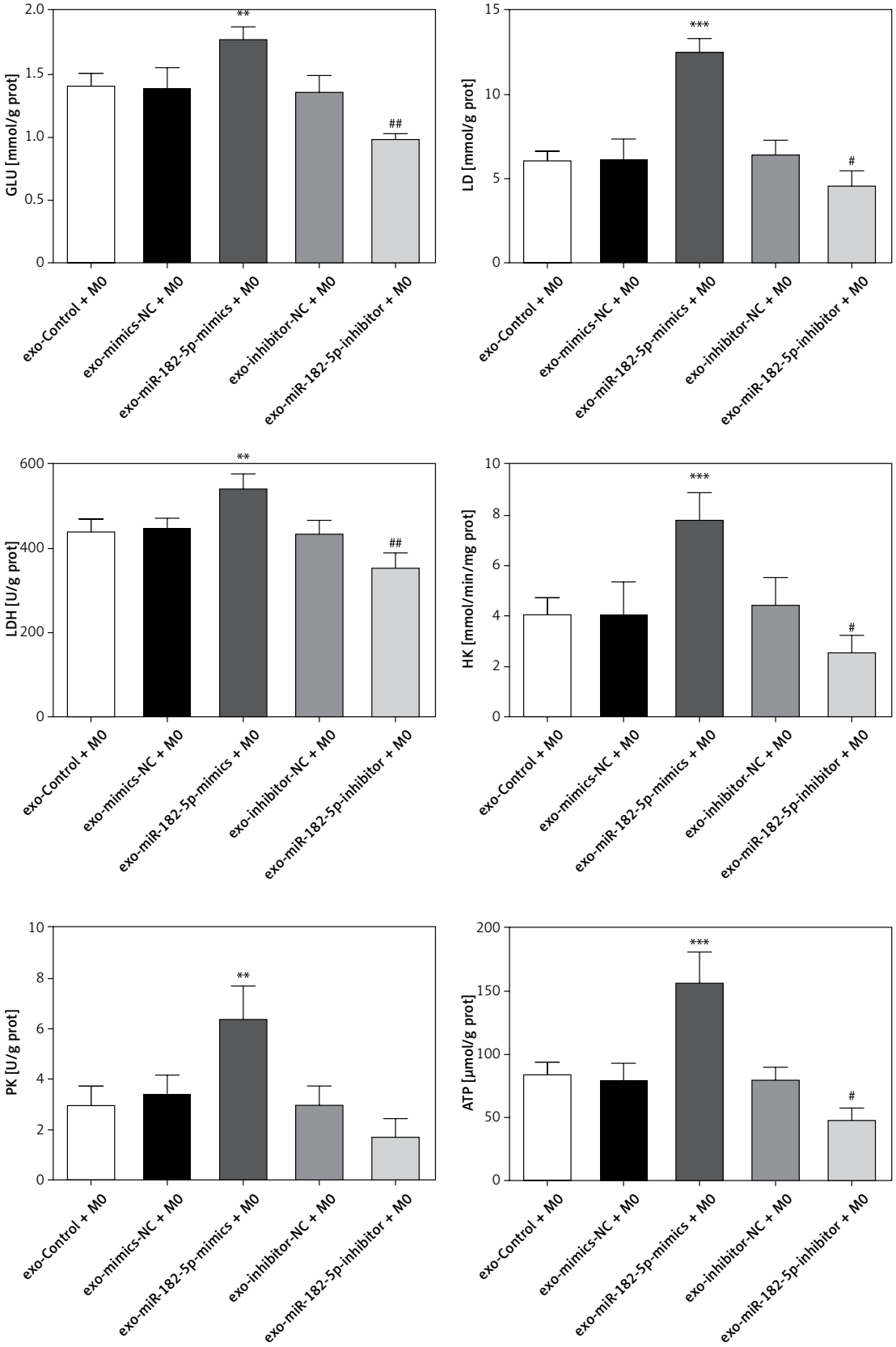
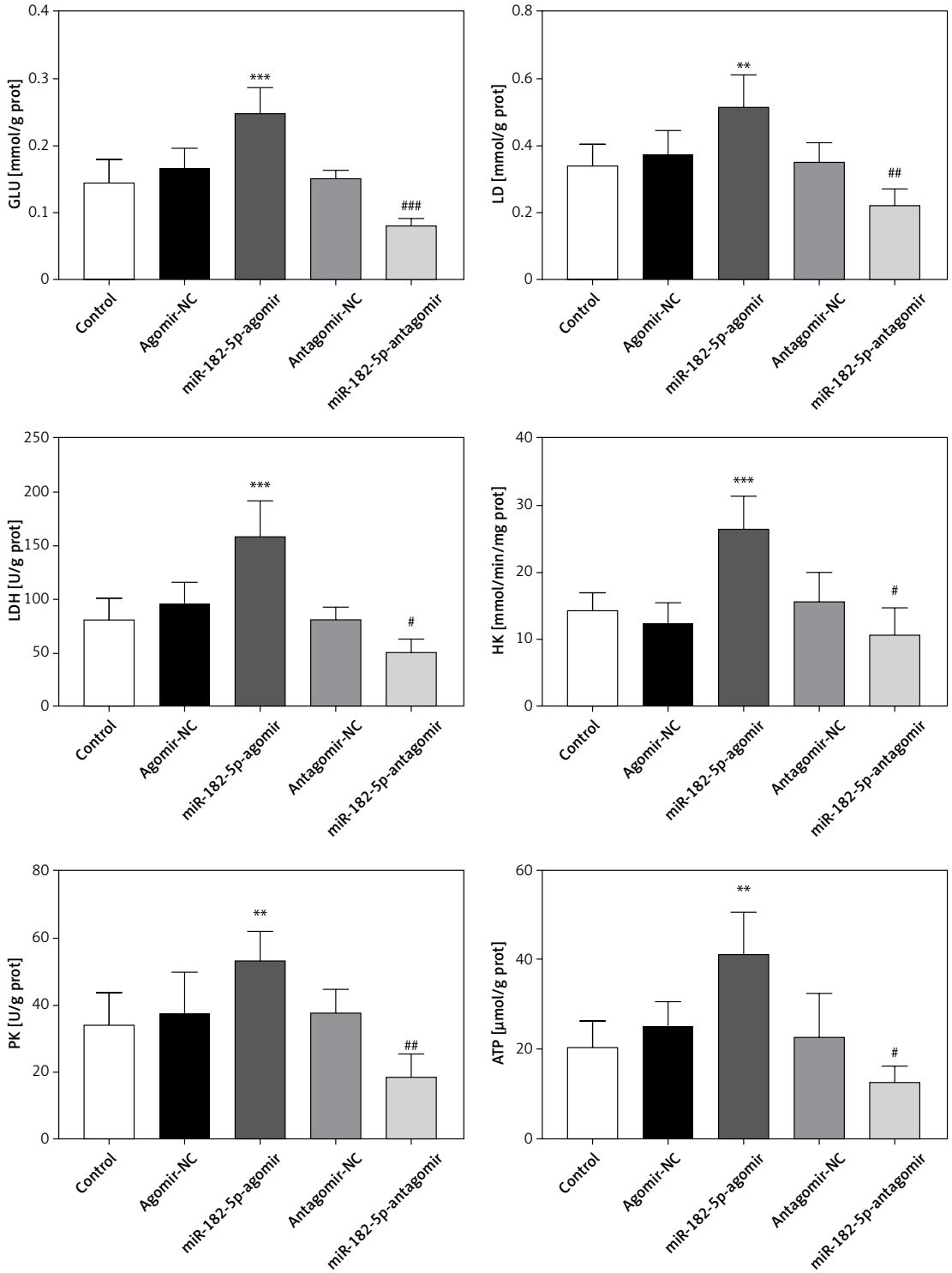


Supplementary Figure S1. Biochemical indicators' transformation of glycolysis in clinical samples. The biochemical indicators of glycolysis, including glucose (GLU), lactate (LD), lactate dehydrogenase (LDH), hexokinase (HK), pyruvate kinase (PK), and adenosine triphosphate (ATP), were notably elevated in 30 TNBC patients compared to adjacent normal tissue. Numeric data are expressed as mean ± SEM. *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$



Supplementary Figure S2. Biochemical indicators' transformation in macrophages. The biochemical indicators of glycolysis in macrophages were significantly increased according to exosomal miR-182-5p mimics, and decreased according to exosomal miR-182-5p inhibitor. *exo-miR-182-5p mimics compared to exo-mimics-NC. #exo-miR-182-5p inhibitor compared to exo-inhibitor-NC. Numeric data are expressed as mean ± SEM. $^{\#}p < 0.05$; $^{**/\#\#}p < 0.01$; $^{***}p < 0.001$



Supplementary Figure S3. Biochemical indicators' transformation in nude-mouse graft model. The biochemical indicators of glycolysis in the nude-mouse graft model were notably increased according to miR-182-5p-agmir, and decreased according to miR-182-5p-antagomir. *miR-182-5p-agomir compared to agomir-NC. #miR-182-5p-antagomir compared to antagomir-NC. Numeric data are expressed as mean ± SEM. $^{\#}p < 0.05$; $^{**/\#\#}p < 0.01$; $^{***/\#\#\#}p < 0.001$

Supplementary files

Cell culture and macrophage differentiation

Human breast cancer cell line MDA-MB-231, human breast epithelial cell line MCF-10A, human monocytic leukemia cell line THP-1, and human kidney epithelial cell line 293T were obtained from the Cell Bank of the Chinese Academy of Sciences. Lipofectamine 3000 (13778-030, Thermo Fisher) was used to transfect the miR-182-5p mimics, miR-182-5p inhibitor, or oligonucleotide inhibitor into MDA-MB-231. Follow-up experiments were generally carried out around 24 h after the transfection.

For macrophage differentiation, THP1 cells were stimulated with 100 ng/ml PMA for 48 h to obtain M0 macrophages. Then M0 macrophages were co-cultured with breast cancer cell-derived EVs.

EV purification

Breast cancer cell-derived EVs were isolated by differential centrifugation. Briefly, 8 ml of cell supernatant from transfected MDA-MB-231 cells was collected and filtered through a 0.22-μm filter. An equal volume of XBP was added, and the mixture was mixed five times. The mixture was then transferred to an exoEasy tube and centrifuged twice at 500 g for 3 min. Subsequently, 10 ml of buffer XWP was added, followed by centrifugation at 2,810 g for 7 min to remove the residual buffer. Next, 600 μl of XE was added to the membrane and incubated for 3 min, then centrifuged at 500 g for 5 min to collect the eluate in a new collection tube. Finally, the eluate was re-added to the rotating column membrane, incubated for 3 min, and centrifuged at 2,810 g for 8 min to collect the exosomes in a new centrifuge tube.

Cell proliferation, migration, and invasion

Cell proliferation was analyzed using the CCK-8 assay. Briefly, a total of 5×10^3 MDA-MB-231 cells were seeded in 96-well Costar plates and co-cultured with 5×10^3 M0 cells containing different exo-miRNAs for 24 h. The plates were incubated for 1 h after adding 10 μl of CCK-8 solution. The absorbance was measured at 450 nm using a microplate reader.

Cell migration and invasion assays were performed using Matrigel Basement Membrane Matrix (354234, Corning). After being starved for 12 h, a total of 1×10^6 transfected MDA-MB-231 cells were seeded in the upper chambers in serum-free DMEM, while the lower chambers were loaded with DMEM containing 10% FBS. After 48 h, non-migrating cells on the upper chambers were removed with a cotton swab, and cells that invaded through the Matrigel layer to the underside of the membrane were stained and counted. Cell migration assays were performed similarly, but without Matrigel.

Constructs and reagents

The miR-182-5p mimics, miR-182-5p inhibitor, miR-182-5p agomir, miR-182-5p antagomir, and the negative control oligonucleotides were purchased from GenePharma.

For western blotting and flow cytometric analyses, the following antibodies were used: β-actin (100166-MM10, Sinobiological), Notch1 (ab52627, Abcam), HES1 (BM4488, Boster), Hexokinase 2 (HK2, A01389, Boster), PKM2 (4053s, CST), PE Anti-Human CD86 Antibody (E-AB-F1012D, Elabscience), APC Anti-Human CD206 Antibody (E-AB-F1161E, Elabscience), FITC Anti-Mouse F4/80 Antibody (E-AB-F0995C, Elabscience), PE Anti-Mouse CD86 Antibody (E-AB-F0994D, Elabscience), APC Anti-Mouse CD206 Antibody (E-AB-F1135E, Elabscience).

For IHC analyses, the following antibodies were used: Notch1 (20687-1-AP, Proteintech), HES1 (BM4488, Boster), HK2 (22029-1-AP, Proteintech), PKM2 (15822-1-AP, Proteintech).

Enzyme-linked immunoassay (ELISA) kits were purchased from Nanjing Jiancheng and used to analyze the levels of PK (A076-1-1), GLU (A154-1-1), LD (A019-2-1), LDH (A020-2), ATP (A095-1-1), HK (A077-3), and ROS (E004-1-1). Human TNF-α (EK182) and Human IL-10 (EK110/2) were purchased from Liankebio.

microRNA and mRNA detection

Total RNAs were extracted using TRIzol reagent (Invitrogen, 15596018), and mature microRNAs were reverse-transcribed using the TaqMan MicroRNA Reverse Transcription Kit (4366597, Invitrogen). Subsequently, the TaqMan MicroRNA Assay Kit (#000597, ABI) was used for qPCR detection. The data were normalized to U6 expression (Supplementary Table SI).

Luciferase reporter assays

For Notch1 3′UTR assays, 293T cells were co-transfected with either the miR-182-5p mimics or a negative control, along with the psiCHECK-2 vector containing the Notch1 3′UTR or its mutation at the end of the Renilla luciferase coding sequence. Lysates were collected 72 h after transfection. Renilla luciferase activities were normalized to firefly luciferase activities to assess 3′UTR activities.

Supplementary Table SI. qPCR primers for miRNA/mRNA

Primer	5′ to 3′
hsa-miR-182-5p F	agtttggcaatggtagaactc
hsa-miR-182-5p R	gtccagttttttttttttttttagtgtg
Notch1 F1	GAGGCGTGGCAGACTATGC
Notch1 R1	CTTGTACTCCGTCAGCGTGA
Hes1 F1	TCAACACGACACCGGATAAAC
Hes1 R1	GCCGCGAGCTATCTTTCTTCA
HK2 F2	TGCCACCAGACTAAACTAGACG
HK2 R2	CCCGTGCCCACAATGAGAC
PKM2 F1	ATGTCGAAGCCCCATAGTGAA
PKM2 R1	TGGGTGGTGAATCAATGTCCA
hsa GAPDH_F	GGAGCGAGATCCCTCCAAAAT
hsa GAPDH_R	GGCTGTTGTCATACTTCTCATGG
mouse GAPDH F1	AGGTCGGTGTGAACGGATTTG
mouse GAPDH R1	TGTAGACCATGTAGTTGAGGTCA