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Supplemental Information

PRMT5 regulates RNA m6A demethylation for doxorubicin sensitivity in breast cancer

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Supplementary Methods

1. Patient-derived xenograft (PDX) model

Two patient-derived xenograft models designated UM1 and UM2 were gifts from Professor Suling Liu (Fudan University Shanghai Cancer Center & Institutes of Biomedical Sciences & Cancer Institutes, Shanghai, China); the xenografts contained wild-type BRCA1 validated with whole-exon sequencing (Supplementary Table 2 and 3). Female BALB/c nude mice aged 5-6 weeks were bred and housed at the Laboratory Animal Center of Fourth Military Medical University. Mice were acclimated for 1 week in sterile laboratory cages with appropriate bedding material, food and water before experiments were performed. Tumor tissue samples from patients were disaggregated into small pieces under aseptic sterile conditions, resuspended in 100-200 of a 1:1 v/v mixture of cold DMEM:Matrigel™ (Fisher Scientific, USA) and kept on ice until subcutaneous implantation into female athymic nude mice. When tumor volumes reached 1,000 mm^3, the tumors were serially transplanted into new mice as before. When all tumor volumes reached 100-150 mm^3, the mice were treated with the indicated drugs. In the experiment, doxorubicin was administered once a week via intravenous tail vein injection at 2 mg/kg·body weight, and tadalafil was administered daily via oral gavage at 2 mg/kg·body weight. Tumor volume was measured every 3 days using a digital caliper, and the mice were sacrificed 27 days after injection. Tumor growth curves and harvested tumors are displayed.

2. siRNA design and transfection

All the siRNAs were ordered from GenePharma Company (China). Transfection was performed with Sage Lipoplus (Sagecreation, Beijing, China) following the manufacturer's protocols. The sequences for the siRNAs were as follows:

ALKBH7 1#, 5′-GCUCAGCCCGUUAUGACUUTT-3′;

ALKBH7-2#, 5′-GCCGCUACGAAUACGAUCATT-3′;

ALKBH5-1#, 5′- CUGCGCAACAAGUACUUCUTT-3′;

ALKBH5-2#, 5'-UCAGAUCGCCUGUCAGGAATT-3';

PRMT5-1#, 5′-CCGGACUUUGUGUGACUAUTT-3′;

PRMT5-2#, 5′- GGUGAACACAGUACUACAUTT -3′;

BRCA1-1#, 5′-GGAAAUGGCUGAACUAGAATT-3′;

BRCA1-2#, 5′-CCUUCUAACAGCUACCCUUTT-3′; and

negative control, 5′-UUCUCCGAACGUGUCACGUTT-3′.

3. Patient sample collection and genomic DNA and RNA extraction

All breast tumor tissue samples were obtained with informed consent under a protocol approved by the ethics committee of XiJing Hospital or TangDu Hospital of Fourth Military Medical University, China. The study complied with all relevant ethical regulations regarding research involving human participants. Fresh breast tumor tissue samples from patients treated with or without neoadjuvant therapy were separately dissected at the time of surgery and immediately transferred to liquid nitrogen until use. The tissue samples were homogenized in TRIzol reagent (Thermo Fisher, 15596018) with a Tissure-Tearor (BioSpec, 985-370). RNA was extracted following the manufacturer's instructions. For archival formalin-fixed, paraffin-embedded (FFPE) specimens, 10×10 μm^2 scrolls collected between July 2013 and December 2017 were taken from the Department of Pathology of XiJing Hospital. All diagnoses were made according to the Pathology and Genetics of Tumors of the Breast of the World Health Organization Classification of Tumors.

4. Lentivirus production, precipitation and infection

Lentiviruses for pCDH-PRMT5-wt/R368A, pCDH-ALKBH7-wt/3RK/3RA and corresponding controls were packaged with pMD2.G, pMDLg/pRRE and pRSVRev (Addgene). Briefly, 1 μg of pMD2.G, 3 μg of psPAX2 and 4 μg of construct for overexpression of specific genes were cotransfected into HEK-293T cells in 60-mm cell culture dishes with Sage Lipoplus (Sagecreation, Beijing, China) following the manufacturer's protocols. The lentiviral particles were harvested at 24 and 48 hours after transfection and filtered through 0.45-μm filters. Finally, the lentiviral particles, mixed with polybrene (Sigma, USA), were directly added to breast cancer cells and incubated at 37°C for 12-24 hours before they were washed out with PBS. Stable cell lines were generated by culturing the cells in medium containing the antibiotic puromycin (1 $mg \cdot ml^{-1}$).

5. RNA extraction and quantitative RT-PCR analysis

Total RNA was isolated using TRIzol reagent. For mRNA expression, 2,000 ng of RNA was reverse transcribed into cDNA in a total reaction volume of 20 μl with Takara's RT kit according to the manufacturer's instructions. Real-time quantitative PCR (qPCR) analysis was performed with 2 μl of cDNA using SYBR green PCR master mix (Yeasen, China) in a LightCycler 96 real-time PCR instrument (Roche, CH). β-actin was used as an internal control. Each sample was run in triplicate. The primers used

for RT-qPCR were as follows:

β-actin-For, 5’-CATGTACGTTGCTATCCAGGC-3’;

β-actin-Rev, 5’-CTCCTTAATGTCACGCACGAT-3’;

BRCA1-For, 5’-TTGTTACAAATCACCCCTCAAGG-3’;

BRCA1-Rev, 5’-CCCTGATACTTTTCTGGATGCC-3’;

BARD1-For, 5’-GGTATCCTTCTGTAGCCAACCA-3’;

BARD1-Rev, 5’-GGAGCCACTTGCTAGTAAGTCT-3’;

BRIP1-For, 5’-GGAAACAGTCAAGAGTCATCGAA-3’;

BRIP1-Rev, 5’-TCTGAGCAATCTGCTTGTGTG-3’;

PRMT5-For, 5’-CTGAATTGCGTCCCCGAAATA-3’;

PRMT5-Rev, 5’-AGGTTCCTGAATGAACTCCCT-3’;

ALKBH7-For, 5’-AGCGTTATGCGGCTGGTG-3’;

ALKBH7-Rev, 5’-TCTTCATCCCGAAGGATCTCA-3’;

HPRT1-For, 5’-CATTATGCTGAGGATTTGGAAAGG-3’;

HPRT1-Rev, 5’-CTTGAGCACACAGAGGGCTACA-3’;

BRCA1-1 3'UTR-For, 5’-CTGGGAGCTCCTCTCACTCT-3’;

BRCA1-1 3’UTR-Rev, 5’-GGACCCTTGCATAGCCAGAA-3’;

BRCA1-4 3'UTR-For, 5’-CCTGGGAGCTCCTCTCACTC-3’; and

BRCA1-4 3'UTR-Rev, 5’-TGGCAGATTTCCAAGGGAGAC-3’.

6. **Immunofluorescence**

For staining of RPA foci, cells were pre-extracted 2 times with CSK-R buffer (Britton et al., 2013) for 3 min before fixation with 2% paraformaldehyde (PFA) in CSK buffer for 10 min. For ALKBH5, PRMT5, and ALKBH7 analysis, cells were fixed with 4% PFA. Fixed cells were permeabilized with 0.5% Triton-X in PBS for 5 min and incubated with primary antibodies overnight at 4℃. After washing with cold PBS 3 times, a goat anti-rabbit IgG-Cy3 antibody (BA1032, Boster, China) was added to the cells and incubated for 1 hour at 37℃. In the dark, the cells were then counterstained with 4’,6-diamidino-2-phenylindole (DAPI; Yeasen, China), and images were captured using a BX51 fluorescence microscope (Olympus) and a CS-60M digital camera (BITRAN) or an LSM 510 META (Zeiss).

7. Immunohistochemistry and tissue microarray analysis

Nude mice were sacrificed to collect tumors for further analyses. Portions of the tumors were collected, fixed in formalin, embedded in paraffin and sectioned. Nude mouse samples and tissue microarrays were stained with an anti-PRMT5 antibody (1:100, Abcam, USA), anti-γH2AX antibody (1:100, Cell Signaling Technology, USA), anti-Ki-67 antibody (1:100, Cell Signaling Technology, USA), anti-ALKBH7 antibody (1:50, Abcam, USA), anti-RPA antibody (1:100, Abcam, USA), and anti-BRCA1 antibody (1:100, Abcam, USA) overnight at 4°C. Subsequent steps were performed using the Polymer Detection System for Immuno-Histological Staining (ZSGB-BIO, PV-9000, Beijing, China) according to the manufacturer's instructions. Images were processed with a Pannoramic MIDI (3D HISTECH, HU); all the dark brown tissue in a tissue section was considered strongly positive staining, brown yellow was considered moderately positive staining, light yellow was considered weakly positive staining, and blue tissue was considered negative. For each tissue sample, the numbers and percentages of strongly positive, moderately positive, weakly positive and negative cells were identified and analyzed, and a histochemistry score (H-score) method was performed. H-score = $\sum$ (PI × I) = (percentage of cells with weak intensity × 1) + (percentage of cells with moderate intensity × 2) + (percentage of cells with strong intensity × 3), where PI represents the percentage of all cells that were positive in the section and I stands for the staining intensity.

8. Dot-blot assay for m6A

Total RNA was isolated from cells with TRIzol reagent according to the manufacturer's instructions or from fresh tissue samples with a Tissure-Tearor and quantified by UV spectrophotometry (NanoDrop 2000, Thermo, USA). mRNA was extracted from the isolated total RNA using the Hieff NGS™ mRNA Isolation Master Kit (12603ES96, Yeasen, China). Briefly, the mRNA samples were loaded onto an Amersham Hybond-N+ membrane (RPN119B, GE Healthcare, USA) with a Bio-Dot Apparatus (170-6545, Bio-Rad) and UV crosslinked to the membrane with a UV crosslinker (Bio-Rad, USA). Then, the membrane was blocked with 5% nonfat dry milk (in 1X PBST) for 1-2 hours at room temperature and incubated with a specific anti-m6A antibody (1:1,000, 202003, Synaptic Systems, DE) overnight at 4°C. Then, an HRP-conjugated goat anti-rabbit IgG antibody (7074P, Cell Signaling Technology, USA) was added to the blots and incubated for 1 hour at room temperature, and the membrane was developed with Amersham ECL Prime Western Blotting Detection Reagent (IC-5009, BioCytoSci, TX, USA).

9. MeRIP-qPCR

Real-time qPCR was performed to assess the relative abundance of a selected mRNA in samples immunoprecipitated with an anti-m6A antibody. Here, the housekeeping gene Hypoxanthine Phosphoribosyltransferase 1 (HPRT1) was chosen as an internal control according to a previous report indicating that HPRT1 mRNA does not have m6A peaks in m6A profiling data (Wang et al., 2014). Two micrograms of mRNA was incubated with 4 μg of anti-m6A antibody (Synaptic Systems) and diluted in 500 μL of IP buffer (150 mM NaCl; 0.1% NP-40; 10 mM Tris, pH 7.4; and 100 U RNase inhibitor). The mixture was rotated at 4°C for 4 hours, and Dynabeads® Protein A (ThermoFisher Scientific) was added and rotated for another 2 hours at 4°C. RNA was extracted from the beads by acid phenol/chloroform extraction, and the RNA concentration was measured with the Qubit® RNA HS Assay Kit (Thermo Fisher Scientific). RT-qPCR was performed on a Roche LightCycle® 96 system (Roche) by using HieffTM qPCR SYBR Green Master Mix (Yeasen, China) with 200 ng of total RNA and m6A-IP mRNA as the template. mRNA expression was calculated from the number of amplification cycles (C_q). Relative mRNA expression was calculated as the value of Cq in the m6A IP portion divided by the value of Cq in the input portion (C_qIP/$C_q$input).

10. Measurement of mRNA stability

Cells were exposed to different treatments for 24 hours and then treated with vehicle or flavopiridol (MedChemExpress, MCE) at a concentration of 0.8 μM for 3 hours (MDA-MB-231) or 3.2 μM for 6 hours (T47-D), followed by RNA extraction and RT-qPCR as described earlier. HPRT1 was used as an internal control for mRNA stability measurement.

11. Immunoblotting (western blotting) and immunoprecipitation

Cells were harvested and washed twice with ice-cold PBS and lysed with RIPA buffer (Beyotime Biotechnology, China) containing a protease inhibitor cocktail (Sigma, USA). Cell extracts were centrifuged for 15 min at 10,000 x g, and supernatants were collected. The protein concentration was measured using Beyotime Biotechnology Protein Assay Reagent (Beyotime Biotechnology, China). The lysates were resolved by SDS-PAGE and transferred to nitrocellulose membranes (Millipore, USA). The membranes were blocked for 1 hour with 5% nonfat milk in Tris-buffered saline containing 0.1% Tween 20 at room temperature and incubated overnight at 4°C with an anti-PRMT5 antibody (Abcam, USA),

anti-β-actin antibody (Cell Signaling Technology, USA), anti-ALKBH5 antibody (Proteintech), anti-ALKBH7 antibody (Proteintech), anti-FTO antibody (Abcam), anti-α-tubulin antibody (Cell Signaling Technology), anti-Lamin A/C antibody (Proteintech), anti-FLAG antibody (Sigma), anti-MYC tag antibody (Cell Signaling Technology), anti-HA antibody (Cell Signaling Technology) or anti-Fbw7α antibody (Abcam). The membranes were washed for 30 min with Tris-buffered saline containing 0.1% Tween 20 and incubated for 1 hour with appropriate secondary antibodies conjugated to horseradish peroxidase at room temperature, and signals were detected using Amersham ECL Prime Western Blotting Detection Reagent. For the immunoprecipitation assay, cells were lysed in NP-40 buffer (Beyotime Biotechnology, China) supplemented with a protease inhibitor cocktail (Sigma). The cell lysates were incubated with primary antibodies overnight at 4°C on a shaker. Protein G/A-agarose beads (Biamake, USA) were then added, and the reaction mixtures were further incubated at 4°C for 2 hours. After five washes with NP-40 buffer, complexes were released from the beads by boiling for 8 min in SDS-PAGE loading buffer. Immunoblotting was performed to detect the expression of the proteins of interest.

12. MeRIP-seq and data analysis

Total RNA was extracted from breast cancer cells with TRIzol (Invitrogen). Then, mRNA sequencing and m6A sequencing were simultaneously performed (Shanghai Jiayin Biotechnology Ltd., Shanghai, China). In brief, total RNA was isolated and fragmented into ~100-nucleotide fragments. Approximately 5% of the fragmented RNA was used as input RNA, and the remaining RNA was analyzed by immunoprecipitation using affinity-purified anti-m6A polyclonal antibodies (ABE572, Millipore, Germany). The immunoprecipitated RNA was analyzed through high-throughput sequencing on an Illumina Novaseq 6000 platform.

MeRIP-seq reads were aligned to the human genome hg38 by using STAR (version 2.4.2a) with the reference annotation GENCODE version 25. For the transcriptome-based peak caller MeTPeak, the m6A peak region summit was defined as the site with the highest fragment pileup ratio between the IP and Input. The top 5,000 peaks were chosen for motif analysis with MEME. Peaks falling in mRNA were assigned to 5 nonoverlapping regions with R, 5'UTR, CDS, and 3'UTR. Peaks were annotated by the function of annotate peak of chromatin immunoprecipitation (ChIP) seeker. Gene Ontology (GO) analysis was performed to elucidate the biological implications of unique genes in the significant or representative profiles of the genes in the experiment. Fisher's exact test was applied to identify the

significant GO categories, and a false discovery rate (FDR) was used to correct the p-values. Pathway analysis was used to determine the significant pathways of the genes according to the Kyoto Encyclopedia of Genes and Genomes (KEGG) database. We used Fisher's exact test to select the significant pathways, and the threshold of significance was defined by the P-value and FDR.

13. Mass Spectrometry

Lysis buffer was made from PBS buffer, pH 7.2, with the addition of 2% SDS, 10% glycerol, 10 mM dithiothreitol, 1 mM EDTA, and a protease inhibitor mixture (Roche Applied Science). The total amount of protein in samples was estimated on a Coomassie blue-stained SDS gel by comparison with a standard protein marker with a known concentration. For mass spectrometry (MS) analysis, the proteins in each sample were separated on a 12% SDS gel (1.0-mm thick) and stained with Coomassie blue G-250. The entire lane was cut into 15 pieces, followed by in-gel trypsin digestion. Protein digestion was performed according to the FASP procedure described by Wisniewski, Zougman. Liquid chromatography (LC)-MS/MS measurements were performed on an Easy-nano-LC (Thermo Fisher Scientific) coupled to a Q Exactive mass spectrometer (Thermo Fisher Scientific). Peptides were separated on a reverse-phase column (15 cm, 75-μm inner diameter and 3-μm Reprosil resin) using a 100-min gradient of water-acetonitrile. All MS measurements were performed in the positive ion mode. Each scan cycle consisted of one full scan mass spectrum (m/z 300-1800) followed by 20 MS/MS events of the most intense ions with the following dynamic exclusion settings: repeat count 2, repeat duration 30 seconds, and exclusion duration 90 seconds. The samples were loaded onto the trap column first with a 10 μl/min flow rate, and then the desalted samples were eluted at a flow rate of 1,200 nL/min in MDLC by applying a linear gradient of 0-50% B for 60 min. The Q Exactive mass spectrometer was used for the MS/MS experiment with an ion transfer capillary of 160°C and ISpary voltage of 3 kV. The normalized collision energy was 35. All DTA files were created using Bioworks Browser rev. 3.1 (Thermo Electron, San Jose, CA, USA) with a precursor mass tolerance of 1.4 Da, threshold of 100, and minimum ion count of 10. The acquired MS/MS spectra were searched against the concatenated target/reverse Glycine_max database using the SEQUEST search engine. The target database contained Glycine_max protein sequences (80,292 entries) downloaded on 05/20/2010 from the NCBI database. Searches were performed in the trypsin enzyme parameter in the software. Methionine oxidation was specified as only a differential modification, and cystine carbamidomethyl was the fixed modification. All output results were combined using in-house

software named buildsummary. The filter was set to FDR≤0.01.

14. Nuclear and cytoplasmic protein extraction

A nuclear and cytoplasmic protein extraction assay was performed using the Minute™ Cytoplasmic and Nuclear Extraction Kit for Cells (SC-003, Invent Biotechnologies, Inc., USA) according to the manufacturer's instructions. Briefly, cells were washed twice with cold PBS, and the buffer was aspirated completely. Appropriate amounts of cytoplasmic extraction buffer were added and swirled to distribute the lysis buffer over the entire surface of tissue cultures, and then the tissue cultures were placed on ice for 5 min. The lysed cells were scraped with a transfer pipette, and the cell lysates were transferred to a prechilled 1.5-ml microcentrifuge tube. The tube was vortexed vigorously for 15 seconds and centrifuged for 5 min at top speed in a microcentrifuge at 4°C. The supernatant (cytosolic fraction) was transferred to a fresh prechilled 1.5-ml tube. Appropriate amounts of nuclear extraction buffer were added to the pellet, and the tube was vortexed vigorously for 15 seconds and incubated on ice for 1 min. The 15-second vortex and 1-min incubation protocol was repeated 4 times. Then, the nuclear extract was immediately transferred to a prechilled filter cartridge with a collection tube and centrifuged at top speed (14,000-16,000 x g) in a microcentrifuge for 30 seconds to collect the supernatant of the nuclear extract. Immunoblotting was performed to detect protein expression in the nucleus and cytoplasm.

15. Comet assay

Cell samples were washed twice with PBS, and trypsin was added. After the cell digestion was complete, a small amount of PBS was added and gently pipetted evenly. The cells were placed into a centrifuge tube and centrifuged at 1,000 rpm for 3 min. The supernatant was discarded, and the cells were washed twice with PBS. Three different agarose layers were prepared with 1%, 0.8% and 0.5% low-melting-point agarose and then covered with a cover glass until solidified. The coverslip was removed, and the slide was immersed in the newly prepared cell lysate and lysed at 4°C for at least 2 hours. The slides were removed and rinsed twice with PBS to remove the high-concentration salt on the surface of the slides. Then, they were placed in a horizontal electrophoresis tank, and the newly configured alkaline electrophoretic buffer was poured over 0.25 cm of the gel surface with alkali untwisting for 20 min. After electrophoresis at 25 V, 300 mA for 20 min, the slides were neutralized with Tris-HCl (pH 7.5) for 15 min. Then, 50 μl of 30 μg/mL ethidium bromide (EB) solution was added to each slide, covered with a cover slip, protected from light, and stained for 20 min in the dark prior to observation. After EB staining,

the samples were observed under a fluorescence microscope as soon as possible, and the electrophoresis pattern was imaged. For the cells of each tissue section, the comet image was analyzed using Comet Assay Software Pect (CASP 1.2.3 beta 1, USA), and the tailing cells were analyzed according to the DNA content of the tail of the comet (TDNA%).

16. The Cancer Genome Atlas (TCGA) data analysis

FPKM data for breast cancer (https://portal.gdc.cancer.gov/) including data for 1,109 breast cancer samples and 113 paracancer samples was downloaded from the TCGA. The relationship between PRMT5 and BRCA1 gene expression in breast cancer patients was analyzed, and the correlation coefficient R was calculated by the Pearson method. At the same time, by comparing the 113 paracancer samples and 1,109 cancer samples, PRMT5 was found to be highly expressed in the cancer samples using the Wilcox test statistical method. Then, Perl language was used to extract patient survival times and survival statuses, and the survival information was merged with the gene expression data. The survminer R package was used to determine the cutoff value for gene expression, and then the Kaplan-Meier survival curve was plotted. For BRCA1 mutations in breast cancer (https://www.cbioportal.org/), we selected the Breast Invasive Carcinoma Breast (TCGA PanCan 2018) dataset and viewed gene amplification, mutation, and deletion information.

17. Dual-luciferase reporter and mutagenesis assays

The mRNA fragments of the BRCA1-3'UTR containing the wild-type m6A motifs or mutant motifs (m6A was replaced by G) were subcloned downstream of firefly luciferase in the pMIR-Report Luciferase vector (ThermoFisher, USA). For a dual-luciferase reporter assay, 100 ng of wild-type or mutant BRCA1-3'UTR, 300 ng of pCDH-PRMT5 (or pCDH vector), and 20 ng of pRL-TK (Renilla luciferase control reporter vector) were cotransfected into HEK-293T cells in 24-well plates. Relative luciferase activities were determined 48 hours post transfection with the Dual Luciferase Reporter Gene Assay Kit (11402ES60, Yeasen, China). Each group was repeated in triplicate.

(1) BRCA1-3'UTR with wild-type m6A sites:

TGACTGCAGCCAGCCACAGGTACAGAGCCACAGGACCCCAAGAATGAGCTTACAAAGTGG
CCTTTCCAGGCCCTGGGAGCTCCTCTCACTCTTCAGTCCTTCTACTGTCCTGGCTACTAAAT
ATTTTATGTACATCAGCCTGAAAA**GGACT**TCTGGCTATGCAAGGGTCCCTTAAAGATTTTCT

GCTTGAAGTCTCCCTTGGAAATCTGCCATGAGCACAAAATTATGGTAATTTTTCACCTGAGA

AGATTTTAAAACCATTTAAACGCCACCAATTGAGCAAGATGCTGATTCATTATTTATCAGCCC

TATTCTTTCTATTCAGGCTGTTGTTGGCTTAGGGCTGGAAGCACAGAGTGGCTTGGCCTCAA

GAGAATAGCTGGTTTCCCTAAGTTTACTTCTCTAAAACCCTGTGTTCACAAAGGCAGAGAG

TCAGACCCTTCAATGGAAGGAGAGTGCTTGGGATCGATTATGTGACTTAAAGTCAGAATAG

TCCTTGGGCAGTTCTCAAATGTTGGAGTGGAACATTGGGGAGGAAATTCTGAGGCAGGTAT

TAGAAATGAAAAGGAAACTTGAAACCTGGGCATGGTGGCTCACGCCTGTAATCCCAGCACT

TTGGGAGGCCAAGGTGGGCAGATCACTGGAGGTCAGGAGTTCGAAACCAGCCTGGCCAAC

ATGGTGAAACCCCATCTCTACTAAAAATACAGAAATTAGCCGGTCATGGTGGTGGACACCT

GTAATCCCAGCTACTCAGGTGGCTAAGGCAGGAGAATCACTTCAGCCCGGGAGGTGGAGG

TTGCAGTGAGCCAAGATCATACCACGGCACTCCAGCCTGGGTGACAGTGAGACTGTGGCTC

AAAAAAAAAAAAAAAAAAAGGAAAATGAAACTAGAAGAGATTTCTAAAAGTCTGAGATAT

ATTTGCTAGATTTCTAAAGAATGTGTTCTAAAACAGCAGAAGATTTTCAAGAACCGGTTTCC

AAAGACAGTCTTCTAATTCCTCATTAGTAATAAGTAAAATGTTTATTGTTGTAGCTCTGGTAT

ATAATCCATTCCTCTTAAAATATAAGACCTCTGGCATGAATATTTCATATCTATAAAATGACAG

ATCCCACCAGGAAGGAAGCTGTTGCTTTCTTTGAGGTGATTTTTTTCCTTTGCTCCCTGTTG

CTGAAACCATACAGCTTCATAAATAATTTTGCTTGCTGAAGGAAGAAAAAGTGTTTTTCATA

AACCCATTATCCAGGACTGTTTATAGCTGTTGGAA**GG<u>A</u>CT**AGGTCTTCCCTAGCCCCCCCAG

TGTGCAAGGGCAGTGAAGACTTGATTGTACAAAATACGTTTTGTAAATGTTGTGCTGTTAAC

ACTGCAAATAAACTTGGTAGCAAACACTTCC

(2) BRCA1-3'UTR with mutant-1 m6A sites:

TGACTGCAGCCAGCCACAGGTACAGAGCCACAGGACCCCAAGAATGAGCTTACAAAGTGG

CCTTTCCAGGCCCTGGGAGCTCCTCTCACTCTTCAGTCCTTCTACTGTCCTGGCTACTAAAT

ATTTTATGTACATCAGCCTGAAAA**GG<u>G</u>CT**TCTGGCTATGCAAGGGTCCCTTAAAGATTTTCT

GCTTGAAGTCTCCCTTGGAAATCTGCCATGAGCACAAAATTATGGTAATTTTTCACCTGAGA

AGATTTTAAAACCATTTAAACGCCACCAATTGAGCAAGATGCTGATTCATTATTTATCAGCCC

TATTCTTTCTATTCAGGCTGTTGTTGGCTTAGGGCTGGAAGCACAGAGTGGCTTGGCCTCAA

GAGAATAGCTGGTTTCCCTAAGTTTACTTCTCTAAAACCCTGTGTTCACAAAGGCAGAGAG

TCAGACCCTTCAATGGAAGGAGAGTGCTTGGGATCGATTATGTGACTTAAAGTCAGAATAG

TCCTTGGGCAGTTCTCAAATGTTGGAGTGGAACATTGGGGAGGAAATTCTGAGGCAGGTAT

TAGAAATGAAAAGGAAACTTGAAACCTGGGCATGGTGGCTCACGCCTGTAATCCCAGCACT

TTGGGAGGCCAAGGTGGGCAGATCACTGGAGGTCAGGAGTTCGAAACCAGCCTGGCCAAC

ATGGTGAAACCCCATCTCTACTAAAAATACAGAAATTAGCCGGTCATGGTGGTGGACACCT

GTAATCCCAGCTACTCAGGTGGCTAAGGCAGGAGAATCACTTCAGCCCGGGAGGTGGAGG

TTGCAGTGAGCCAAGATCATACCACGGCACTCCAGCCTGGGTGACAGTGAGACTGTGGCTC

AAAAAAAAAAAAAAAAAAAGGAAAATGAAACTAGAAGAGATTTCTAAAAGTCTGAGATAT

ATTTGCTAGATTTCTAAAGAATGTGTTCTAAAACAGCAGAAGATTTTCAAGAACCGGTTTCC

AAAGACAGTCTTCTAATTCCTCATTAGTAATAAGTAAAATGTTTATTGTTGTAGCTCTGGTAT

ATAATCCATTCCTCTTAAAATATAAGACCTCTGGCATGAATATTTCATATCTATAAAATGACAG

ATCCCACCAGGAAGGAAGCTGTTGCTTTCTTTGAGGTGATTTTTTTCCTTTGCTCCCTGTTG

CTGAAACCATACAGCTTCATAAATAATTTTGCTTGCTGAAGGAAGAAAAAGTGTTTTTCATA

AACCCATTATCCAGGACTGTTTATAGCTGTTGGAA**GG<u>A</u>CT**AGGTCTTCCCTAGCCCCCCCAG

TGTGCAAGGGCAGTGAAGACTTGATTGTACAAAATACGTTTTGTAAATGTTGTGCTGTTAAC

ACTGCAAATAAACTTGGTAGCAAACACTTCC

(3) BRCA1-3'UTR with mutant-2 m6A sites:

TGACTGCAGCCAGCCACAGGTACAGAGCCACAGGACCCCAAGAATGAGCTTACAAAGTGG

CCTTTCCAGGCCCTGGGAGCTCCTCTCACTCTTCAGTCCTTCTACTGTCCTGGCTACTAAAT

ATTTTATGTACATCAGCCTGAAAA**GG<u>A</u>CT**TCTGGCTATGCAAGGGTCCCTTAAAGATTTTCT

GCTTGAAGTCTCCCTTGGAAATCTGCCATGAGCACAAAATTATGGTAATTTTTCACCTGAGA

AGATTTTAAAACCATTTAAACGCCACCAATTGAGCAAGATGCTGATTCATTATTTATCAGCCC

TATTCTTTCTATTCAGGCTGTTGTTGGCTTAGGGCTGGAAGCACAGAGTGGCTTGGCCTCAA

GAGAATAGCTGGTTTCCCTAAGTTTACTTCTCTAAAACCCTGTGTTCACAAAGGCAGAGAG

TCAGACCCTTCAATGGAAGGAGAGTGCTTGGGATCGATTATGTGACTTAAAGTCAGAATAG

TCCTTGGGCAGTTCTCAAATGTTGGAGTGGAACATTGGGGAGGAAATTCTGAGGCAGGTAT

TAGAAATGAAAAGGAAACTTGAAACCTGGGCATGGTGGCTCACGCCTGTAATCCCAGCACT

TTGGGAGGCCAAGGTGGGCAGATCACTGGAGGTCAGGAGTTCGAAACCAGCCTGGCCAAC

ATGGTGAAACCCCATCTCTACTAAAAATACAGAAATTAGCCGGTCATGGTGGTGGACACCT

GTAATCCCAGCTACTCAGGTGGCTAAGGCAGGAGAATCACTTCAGCCCGGGAGGTGGAGG

TTGCAGTGAGCCAAGATCATACCACGGCACTCCAGCCTGGGTGACAGTGAGACTGTGGCTC

AAAAAAAAAAAAAAAAAAAGGAAAATGAAACTAGAAGAGATTTCTAAAAGTCTGAGATAT

ATTTGCTAGATTTCTAAAGAATGTGTTCTAAAACAGCAGAAGATTTTCAAGAACCGGTTTCC

AAAGACAGTCTTCTAATTCCTCATTAGTAATAAGTAAAATGTTTATTGTTGTAGCTCTGGTAT

ATAATCCATTCCTCTTAAAATATAAGACCTCTGGCATGAATATTTCATATCTATAAAATGACAG

ATCCCACCAGGAAGGAAGCTGTTGCTTTCTTTGAGGTGATTTTTTTCCTTTGCTCCCTGTTG

CTGAAACCATACAGCTTCATAAATAATTTTGCTTGCTGAAGGAAGAAAAAGTGTTTTTCATA

AACCCATTATCCAGGACTGTTTATAGCTGTTGGAA**GGGCT**AGGTCTTCCCTAGCCCCCCCA

GTGTGCAAGGGCAGTGAAGACTTGATTGTACAAAATACGTTTTGTAAATGTTGTGCTGTTAA

CACTGCAAATAAACTTGGTAGCAAACACTTCC

(4) BRCA1-3'UTR with mutant-3 m6A sites:

TGACTGCAGCCAGCCACAGGTACAGAGCCACAGGACCCCAAGAATGAGCTTACAAAGTGG

CCTTTCCAGGCCCTGGGAGCTCCTCTCACTCTTCAGTCCTTCTACTGTCCTGGCTACTAAAT

ATTTTATGTACATCAGCCTGAAAA**GGGCT**TCTGGCTATGCAAGGGTCCCTTAAAGATTTTCT

GCTTGAAGTCTCCCTTGGAAATCTGCCATGAGCACAAAATTATGGTAATTTTTCACCTGAGA

AGATTTTAAAACCATTTAAACGCCACCAATTGAGCAAGATGCTGATTCATTATTTATCAGCCC

TATTCTTTCTATTCAGGCTGTTGTTGGCTTAGGGCTGGAAGCACAGAGTGGCTTGGCCTCAA

GAGAATAGCTGGTTTCCCTAAGTTTACTTCTCTAAAACCCTGTGTTCACAAAGGCAGAGAG

TCAGACCCTTCAATGGAAGGAGAGTGCTTGGGATCGATTATGTGACTTAAAGTCAGAATAG

TCCTTGGGCAGTTCTCAAATGTTGGAGTGGAACATTGGGGAGGAAATTCTGAGGCAGGTAT

TAGAAATGAAAAGGAAACTTGAAACCTGGGCATGGTGGCTCACGCCTGTAATCCCAGCACT

TTGGGAGGCCAAGGTGGGCAGATCACTGGAGGTCAGGAGTTCGAAACCAGCCTGGCCAAC

ATGGTGAAACCCCATCTCTACTAAAAATACAGAAATTAGCCGGTCATGGTGGTGGACACCT

GTAATCCCAGCTACTCAGGTGGCTAAGGCAGGAGAATCACTTCAGCCCGGGAGGTGGAGG

TTGCAGTGAGCCAAGATCATACCACGGCACTCCAGCCTGGGTGACAGTGAGACTGTGGCTC

AAAAAAAAAAAAAAAAAAAGGAAAATGAAACTAGAAGAGATTTCTAAAAGTCTGAGATAT

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AACCCATTATCCAGGACTGTTTATAGCTGTTGGAA**GGGCT**AGGTCTTCCCTAGCCCCCCCA

GTGTGCAAGGGCAGTGAAGACTTGATTGTACAAAATACGTTTTGTAAATGTTGTGCTGTTAA

CACTGCAAATAAACTTGGTAGCAAACACTTCC