Supplementary Methods

Orthotopic ovarian cancer model in nude mice

Eight-week-old female nude mice (BALB/c-derived nu/nu) were purchased from Shanghai Experimental Animal Center (Chinese Academy of Sciences, China) and maintained in a laminar-flow cabinet under specific pathogen-free conditions. All procedures involved in animal study were approved by the Institutional Committee on Animal Care, Nanjing Medical University. The mean weight of mice on arrival was 20 ± 2g (mean ± SD). The cells were cultured to 90% confluence, trypsinized, washed twice with 1× PBS buffer, and resuspended in serum-free medium. Mice were placed under general anesthesia; ovaries were exposed and injected with 20 μl resuspended cells (1×10^6 cells) through a sterile micro-syringe. Mice were divided into three groups, each group with 10 mice. Mice in control group were injected with OVCAR-3 cells. Mice in Ad-GFP group and Ad-CAT groups were injected with OVCAR-3 cells infected with adenovirus carrying GFP and catalase, respectively. The animals were sacrificed by decaptitation 30 days after cell implantation. Tumors and organs were removed. Each tissue was divided into two parts: one part was fixed in Bouin's fixative and paraffin-embedded, and the other part was snap frozen in liquid nitrogen and stored at -80°C for further analysis.

Luciferase reporter constructs and luc-activity assay

The 3'UTR-luciferase reporter constructs containing the 3'UTR regions of ERBB2 and ERBB3 with wild-type and mutant binding sites of miR-199a or miR-125b were amplified by PCR method using total cDNAs obtained from A2780 cells as temple. The

PCR products were cloned into the pMiR-luc reporter vector (Ambion) between Sac I and Hind III sites, immediately downstream of the luciferase gene. The mutant 3'UTR constructs were made by introducing four mismatch mutations into the putative seed regions of ERBB2 and ERBB3. All the constructs containing 3'UTR inserts were sequenced and verified. The cells were transiently co-transfected with luciferase (luc) reporters, β-gal plasmid and miRNA precursors using lipofectamine according to the manufacturer's instruction. The cells were harvested and lysed with reporter lysis buffer (Promega, Madison, WI) 48 h after the transfection. The luc activities in the cellular extracts were determined using the luc assay system (Promega). The relative luc activities were calculated by the ratio of luc/β-gal activity, and normalized to that of the control cells.

Targetsearch program

We developed a new algorithm "Targetsearch" by combining the matching seed-pairing program and free energy of the potential miRNA binding program adopted from publicly available search engines: microRNA, TargetScan, FindTar 3, and RNA 22. Targetsearch program only requires 6 matching miRNA:target seed-pairing, which could be separated by 0-3 bp non-matching sequence; and Free Minimum Energy < -8 for the potential microRNA binding.

Taqman qRT-PCR

Small RNAs were extracted from cultured cells using the mirVana miRNA isolation kit (Ambion, Austin, TX). Two-step Taqman real-time PCR analysis was performed to assess miRNA levels using Taqman miRNA reverse transcription kit and Taqman

universal PCR master mix (Applied Biosystem, Austin, TX, USA) in accordance with manufacturer's instructions. Normalization was performed using U6 RNA level.

Primers

Methylation-specific PCR primers:

MiR-199a-5p MF: 5'- ATTTCGTCGAGAAATTAGTGGTC -3'

MiR-199a-5p MR: 5'- AATCCTAAAAAATAAAAAAAACGAA -3'

MiR-199a-5p UF: 5'- ATTTTGTTGAGAAATTAGTGGTTGT -3'

MiR-199a-5p UR: 5'- AATCCTAAAAAATAAAAAAAACAAA -3'

MiR-125b MF: 5'- GATGGTGTTATAGGAGGTTGTGC -3'

MiR-125b MR: 5'- AAAAAAAACCAAAAAATAAAATTCGA -3'

MiR-125b UF: 5'- GATGGTGTTATAGGAGGTTGTGTG -3'

MiR-125b UR: 5'- AAAAAACCAAAAAATAAAATTCAAA -3'

Bisulfate-sequencing PCR primers:

MiR-199a-5p F: 5'- GCGGAGCTCTGTTATATTTGGAATTGTTTATAGTG -3'

MiR-199a-5p R: 5'- GCGAAGCTTAAAAATCCTAAAAAATAAAAAAAAC-3'

MiR-125b F: 5'- GCGGAGCTCGGGATGGTGTTATAGGAGGTTG -3'

MiR-125b R: 5'- GCGAAGCTTACACAAACTCAAAAATACCCAAATT -3'

ChIP assay primers:

MiR-199a-5p F: ACAGTGCGATTCCGCCGAGAA

MiR-199a-5p R: GGGTTTGGGATCGTGGCTTCT

MiR-125b F: TAGCCTCCGAATCCTACT

MiR-125b R: GACTCAGAAATGCCCAAG

Immunohistochemistry

Tumor tissues were sliced and fixed with Bouin's solution (saturated picric acid 300 ml, formaldehyde 100 ml, glacial acetic acid 20 ml) for 24 h, and processed by conventional paraffin-embedded method. The paraffin-embedded tumor tissues (5μm thick) were deparaffinized using xylene, then rehydrated in a series of increasing ethanol concentrations. Dako Envision two-step method of immunohistochemistry was used. In brief, the sections were washed three times in 1× PBS buffer, and incubated for 10 min in 0.3% hydrogen peroxide. Antigen retrieval was performed by incubating the sections in 10 mM citrate buffer (pH 6.0) in microwave for 15 min, followed by incubation with rabbit anti-human ERBB2 or mouse anti-human ERBB3 antibody (1:200) at 4°C overnight, respectively. After three washes with 1× PBS buffer, sections were incubated with HRP-labeled goat anti-rabbit antibodies (DAKO; Hamburg, Germany) for 30 minutes. The tumor tissue sections were incubated with 3,3'-diaminobenzidine (DAB) for 5~10min, and signals were detected under a light microscope. Sections incubated with 1× PBS buffer instead of the primary antibodies were used as a negative control.

Legends to supplementary figures

Fig S1 OVCAR-3 cells were treated with catalase (3000U/ml), DPI (1μM), rotenone (2.5μM) for 12h, respectively. Then the intracellular ROS were stained using DCFH-DA as described in Materials and Methods. Fluorescent images were captured with a fluorescent microscope. The corresponding phase contrast images are shown on the bottom panel. Bar: 50 μm. Fluorescent intensity signals were quantified using ImageJ

system, and normalized to that of control. Each experiment was performed three times independently with three replicates for each treatment. *indicates significant decrease compared with the control (by Student's *t*-test, $P<0.05$).

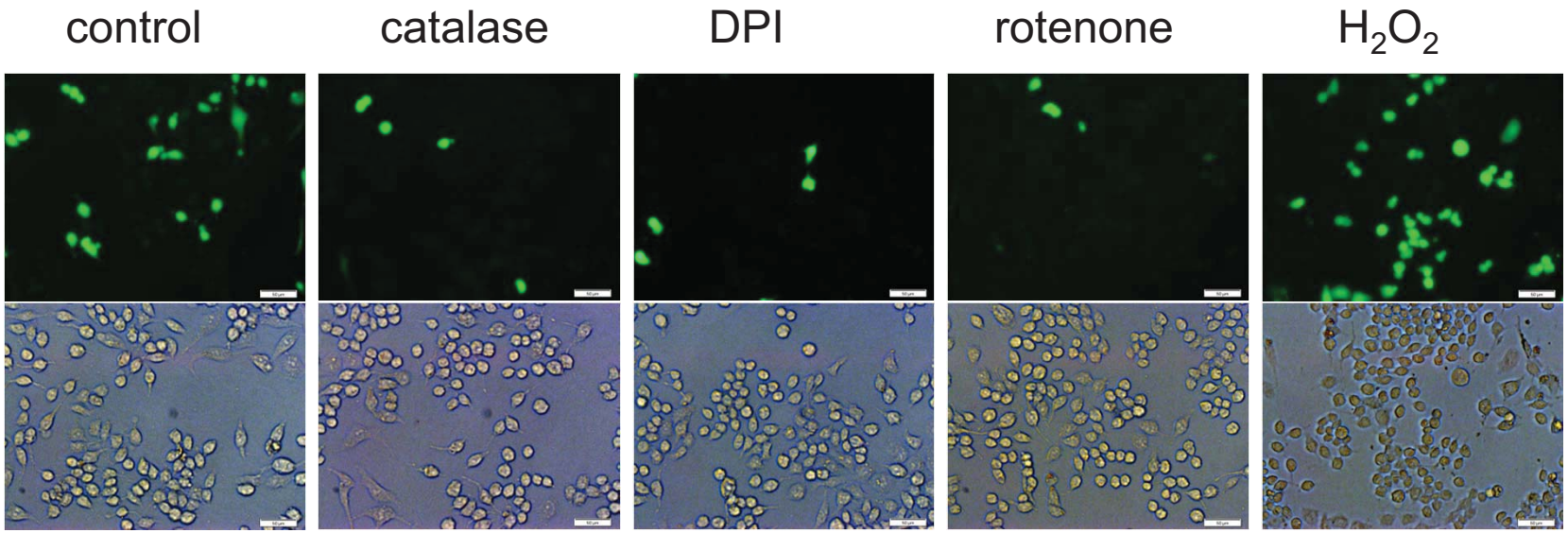
Fig S2 Eight-week-old female nude mice (BALB/c-derived nu/nu) were used to establish orthotopic ovarian cancer model. OVCAR-3 cells infected with or without adenovirus carrying GFP or catalase, were implanted into ovaries of nude mice. One month later, mice were sacrificed and tumors were removed. The representative images of tumors in each group are shown. Bar: 1 cm. Tumor weight was presented as mean ± SE (n=6~8). * indicates significant decrease compared with control group (by Student's *t*-test, $P<0.05$).

Fig S3 A2780 and OVCAR-3 cells were treated with catalase (3000 U/ml) or DPI (1 μM) for 12 h. Quantitive RT-PCR was performed to detect the levels of miR-125b, miR-125a, miR-143, miR-199a and miR-145 in the cells by Taqman miRNA assay. Results were normalized to U6 expression level and represented as mean ± SE from three independent replicates. *indicates significant decrease compared with the control (by Student's *t*-test, $P<0.05$).

Fig S4 (A) OVCAR-3 cells were transfected with pre-miR-control, pre-miR-199a or pre-miR-125b for 72 h as indicated. Taqman RT-PCR was performed to assess the miRNA expression levels. (B) Establishment of stable OVCAR-3 cells overexpressing miR-199a, miR-125b or negative control miRNA precursor, separately. Taqman RT-PCR was performed to assess the miRNA expression levels. Results were normalized to U6 expression level and represented as mean ± SE from three independent replicates. (C) Establishment of stable OVCAR-3 cell line overexpressing ERBB2 or ERBB3 using pReceiver-Lv105 vector, respectively. The protein levels of ERBB2 and ERBB3 were

evaluated by immunoblotting. **indicates significant decrease compared with the control (by Student's *t*-test, P<0.01).

Fig S5 (A) The OVCAR-3 cells were transfected with 50 nM of a siRNA scramble control or two unrelated siRNAs (siRNA1 and siRNA2) against DNMT1 for 72h. The DNMT1 protein levels were detected by immunoblotting. (B) miR-199a and miR-125b expression levels were analyzed by Taqman qRT-PCR. Results were normalized to U6 expression level and represented as mean ± SE from three independent replicates. * indicates significant decrease compared with the scrambled control (by Student's *t*-test, P<0.05).



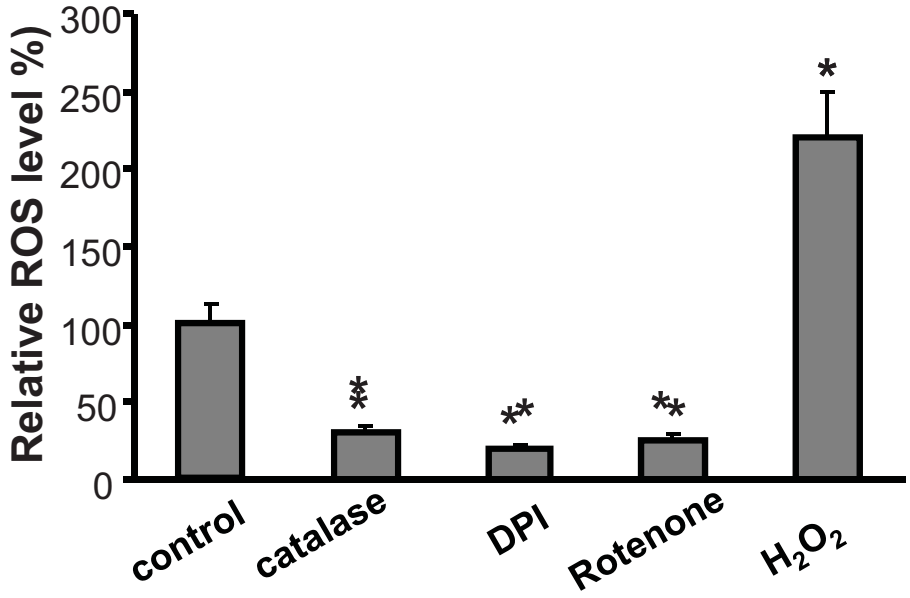


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Fig S1

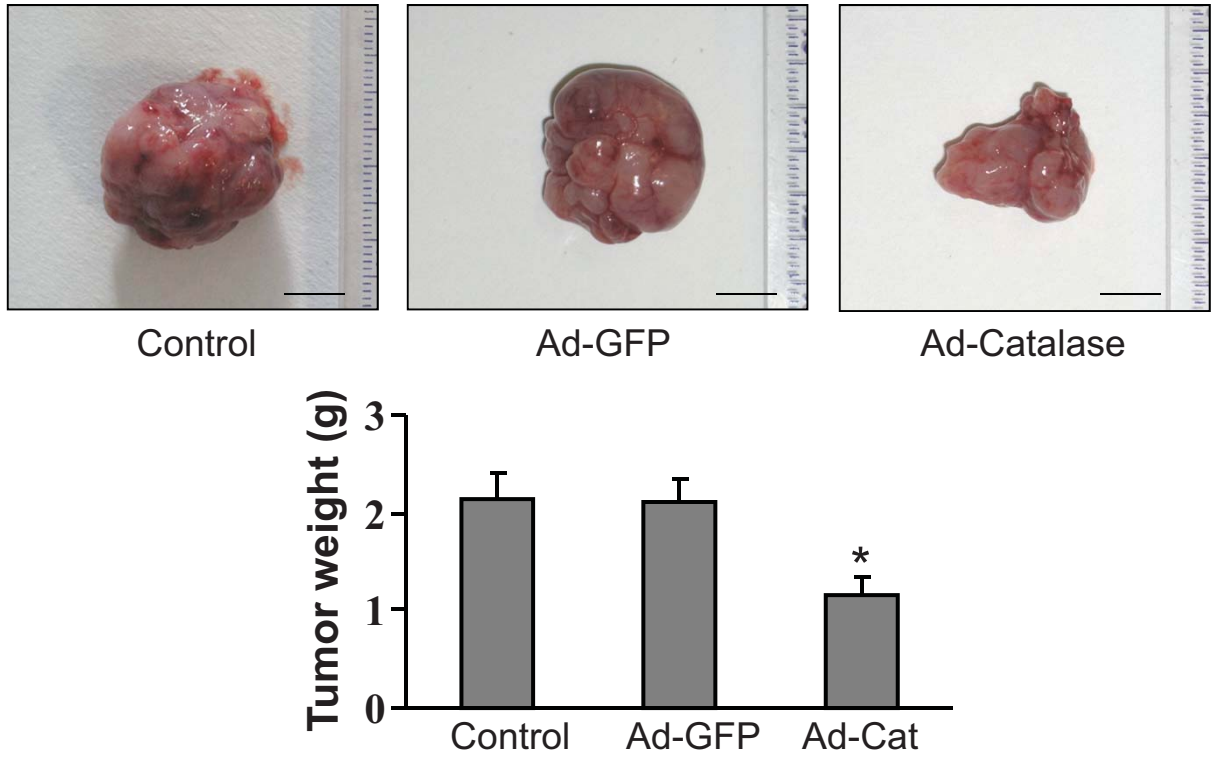

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Fig S2

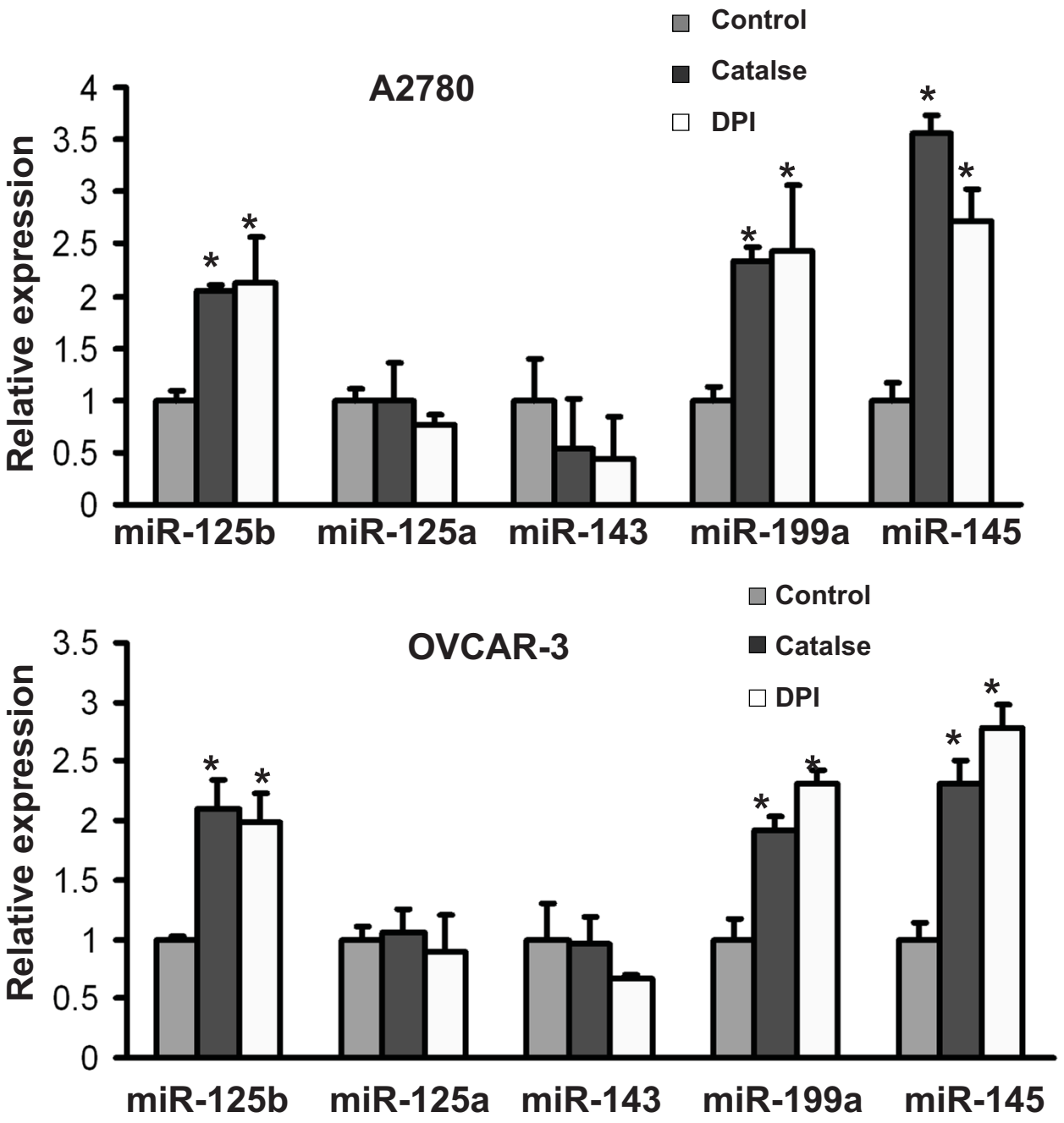

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Fig S3

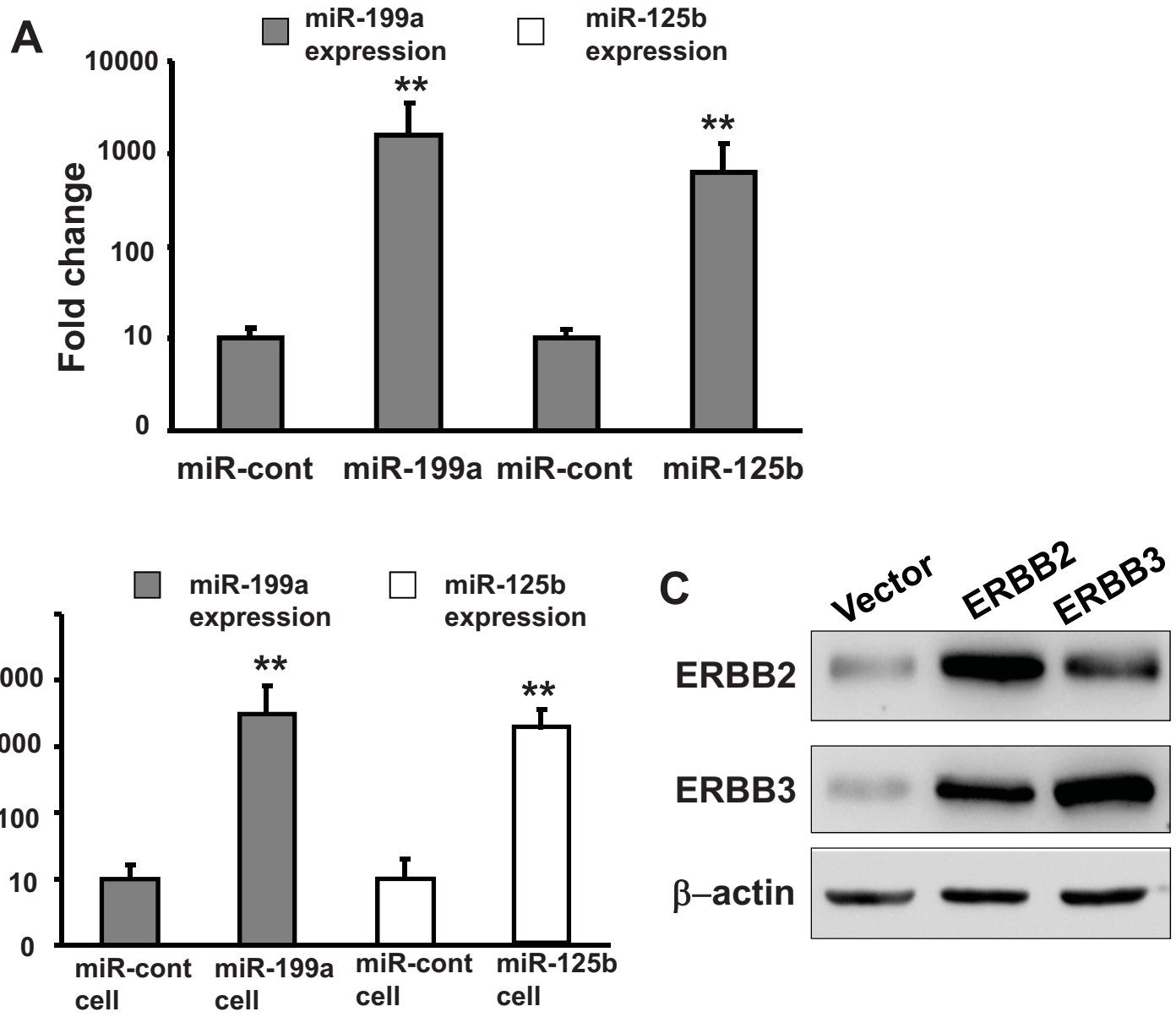

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Fig S4

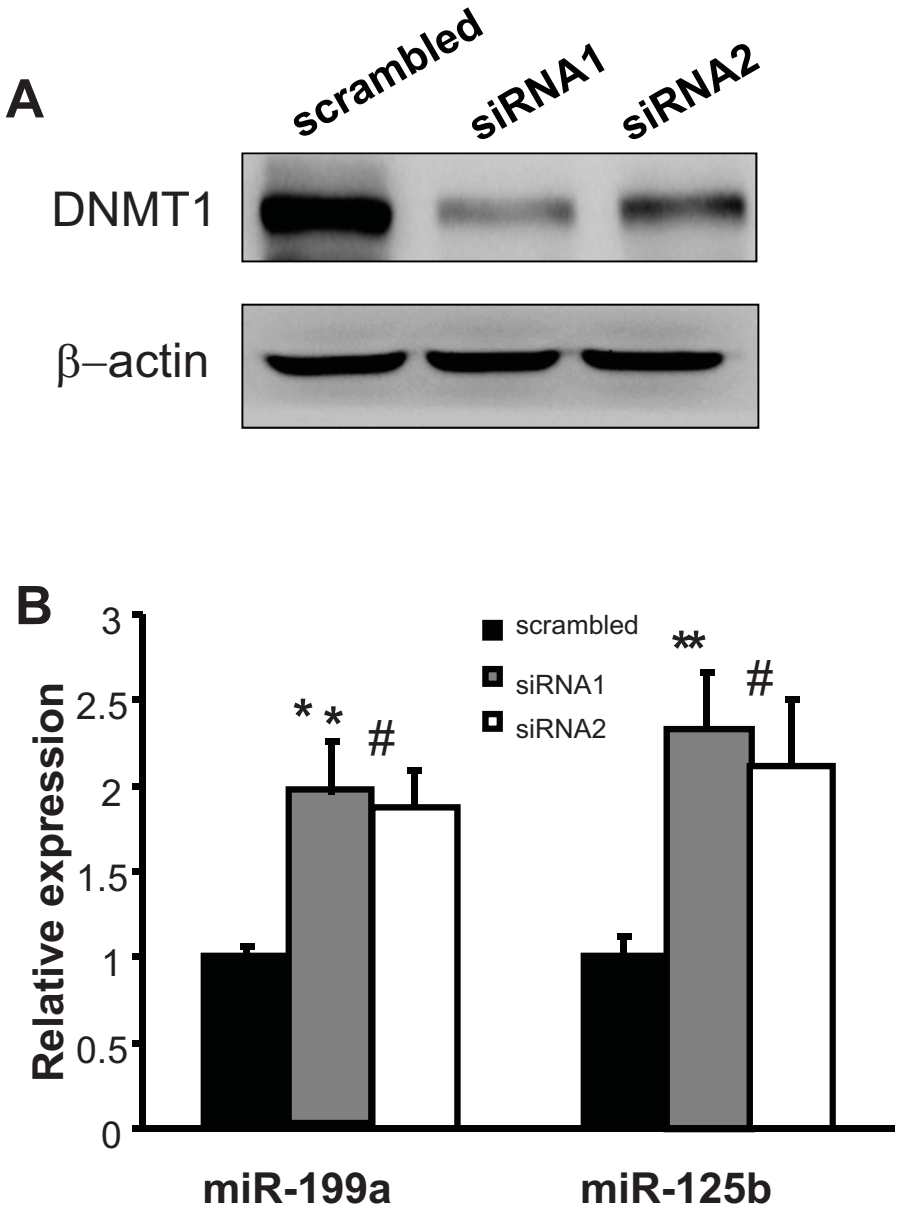

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Fig S5