SUPPLEMENTARY INFORMATION

Cytosolic ME1 integrated with mitochondrial IDH2 supports tumor growth and metastasis

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Inventory of Supplementary information

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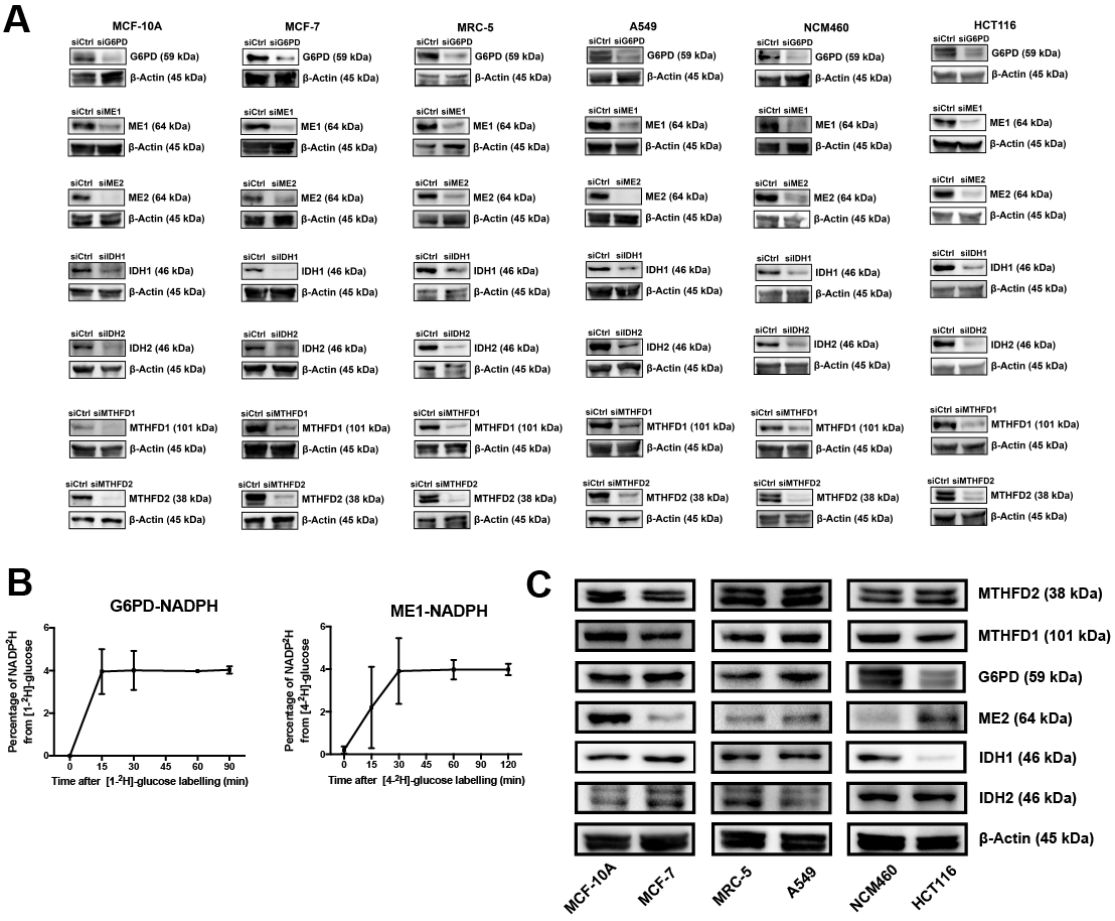


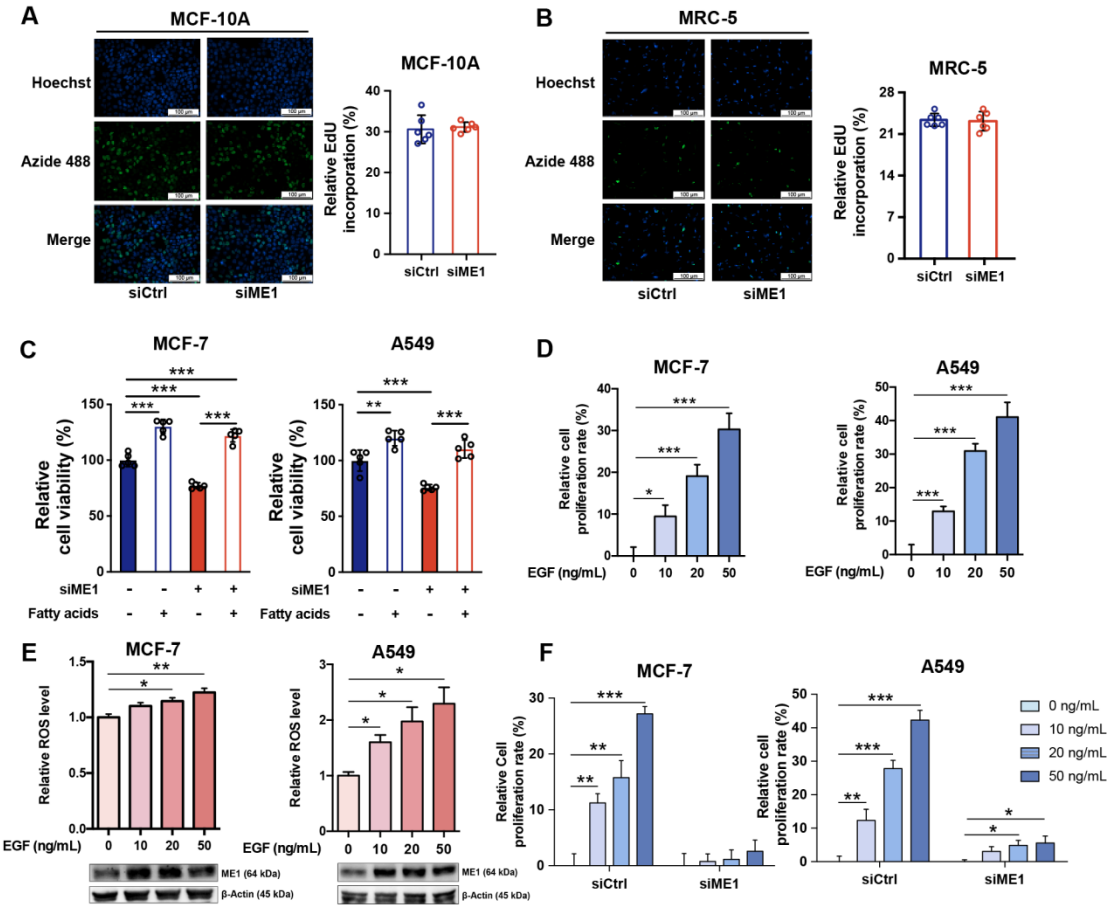
Supplementary Figure 1. Cancerous but not normal cells rely on ME1-mediated NADPH production. (Related to Figure 1)

(A) Immunoblot analysis validates the knockdown efficiency of G6PD, ME1, ME2, IDH1, IDH2, MTHFD1 and MTHFD2 with siRNAs in indicated cell lines. β-Actin was used as loading control.

(B) Kinetics of percentage of NADPH labeling from [1-^{2}H] glucose (10 mM) and [4-^{2}H] glucose (10 mM) (n=4).

(C) Immunoblot analysis of G6PD, ME2, IDH1, IDH2, MTHFD1 and MTHFD2 expression in indicated cell lines. The samples used here were the same as those of Figure 1G. β-Actin was used as the loading control.

Results are shown as mean ± SD. Experiments in A and B were conducted once, and C were repeated at least twice.



Supplementary Figure 2. ME1 is pivotal for supporting cancer cell growth. (Related to Figure 2)

(A) EdU incorporation assay of MCF-10A cells with control or ME1 knockdown (n=6). Scale bar, 100 μm.

(B) EdU incorporation assay of MRC-5 cells with control or ME1 knockdown (n=6). Scale bar, 100 μm.

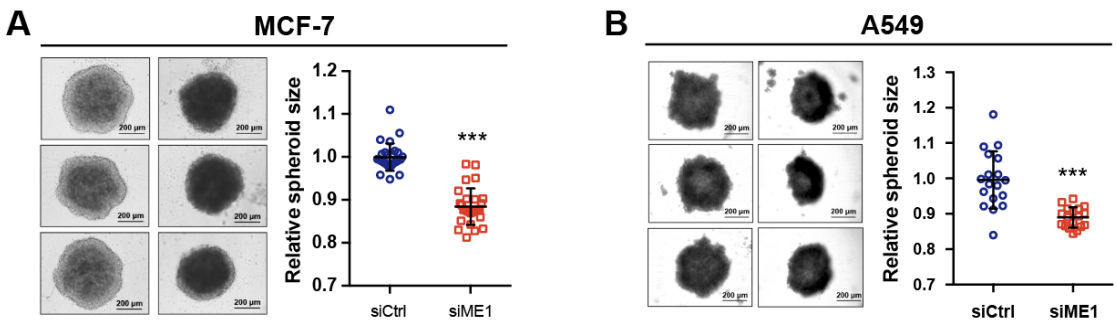
(C) Relative cell viability of MCF-7 and A549 cells with or without ME1 knockdown supplemented with palmitic acid (10 μM) and oleic acid (10 μM) for 24 h (n=5).

(D) Proliferation rate of MCF-7 and A549 cells after being treated with different concentrations of EGF for 24 h (n=10).

(E) Intracellular ROS level and ME1 protein level in MCF-7 and A549 cells after being treated with different concentrations of EGF for 24 h (n=3).

(F) Proliferation rate of MCF-7 and A549 cells with or without ME1 knockdown after being treated with different concentrations of EGF for 24 h (n=6).

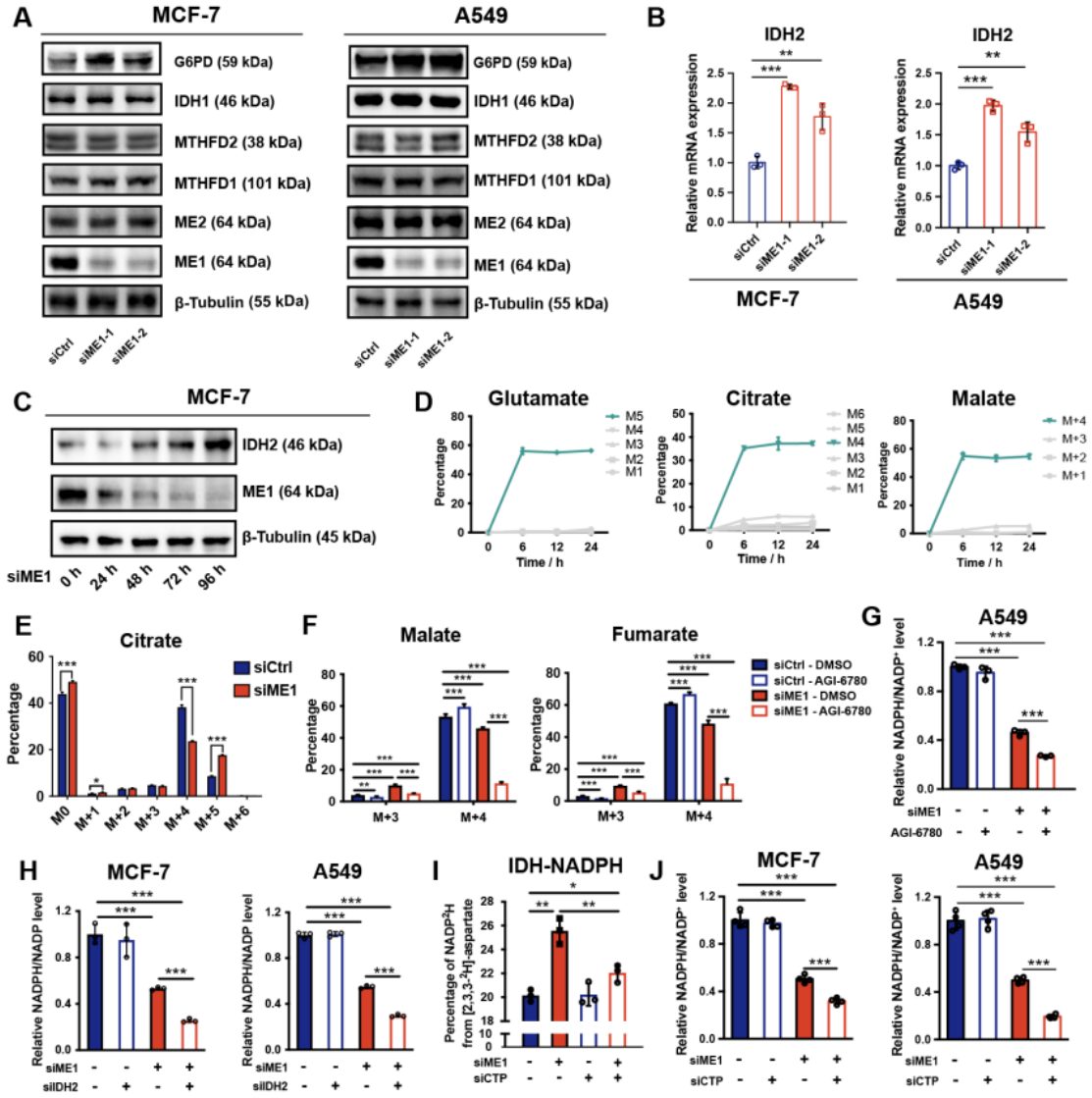
Results are shown as mean ± SD. Experiments in A, B and F were conducted once, and all the other experiments were repeated at least twice. Statistical significance was determined by Student's t-test, * $p<0.05$, ** $p<0.01$, *** $p<0.001$.



Supplementary Figure 3. ME1 regulates cancer cell migration and anchorage-independent survival. (Related to Figure 3)

(A) Spheroid diameter of MCF-7 (n=28) and (B) A549 (n=17) cells with control and ME1 knockdown. Scale bar, 200 μm.

Results are shown as mean ± SD. Data represents at least two independent experiments. Statistical significance was determined by Student's t-test, ***p<0.001.



Supplementary Figure 4. ME1 interference adaptively activates IDH2-mediated NADPH compensation. (Related to Figure 4)

(A) Immunoblot analysis of G6PD, IDH1, MTHFD1, MTHFD2, ME1 and ME2 expression in MCF-7 and A549 cells with control or ME1 knockdown. The samples used here were the same as those of Figure 4C. β-Tubulin was used as the loading control.

(B) mRNA level of IDH2 in MCF-7 and A549 cells with control or ME1 knockdown (n=3). β-Actin was used for normalization.

(C) Immunoblot analysis of IDH2 in MCF-7 cells with ME1 knockdown cultured for indicated time. β-Tubulin was used as the loading control.

(D) Kinetics of glutamate, citrate and malate labeling with 2 mM [U-^{13}C] glutamine in MCF-7 cells (n=3).

(E) Mass isotopologue analysis of citrate in A549 cells cultured with 2 mM [U-^{13}C] glutamine for 24 h (n=5).

(F) m+3 and m+4 isotopologues of malate and fumarate in MCF-7 cells treated as indicated and cultured in medium containing 2 mM [U-^{13}C] glutamine for 24 h (n=3 for the

siME1-DMSO group and n=5 for others). The concentration of AGI-6780 used in this study is 2.5 μM.

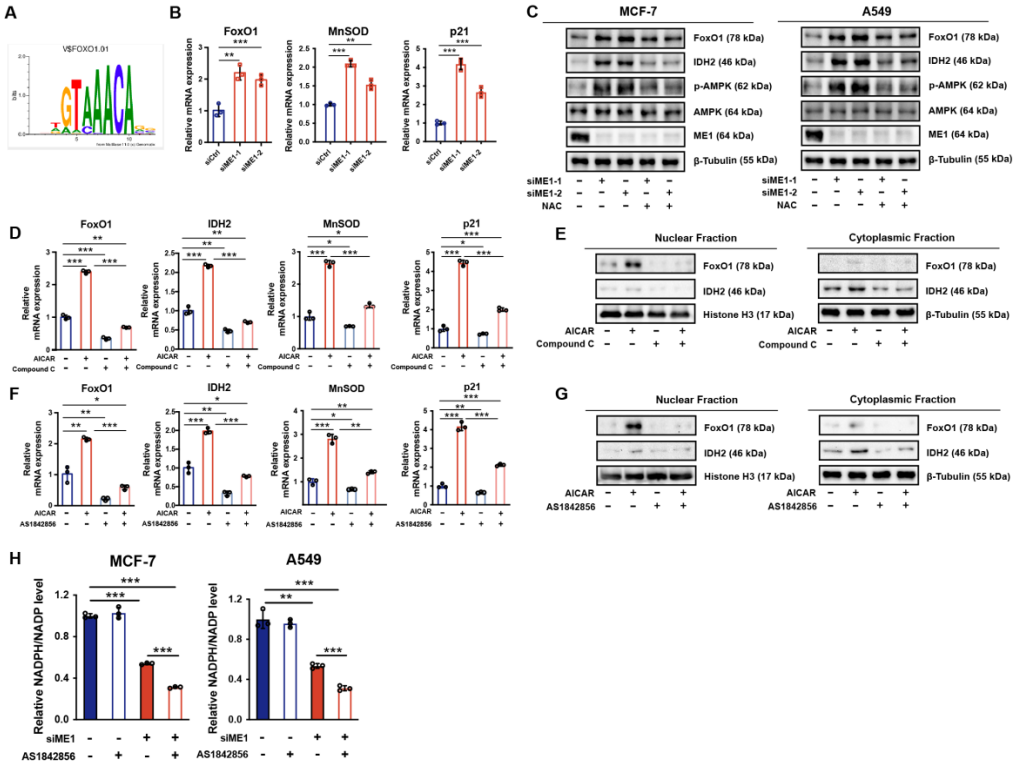
(G) Effect of combinatorial targeting ME1 and IDH2 (2.5 μM AGI-6780 for 24 h) on relative NADPH/$NADP^+$ ratio in A549 cells (n=3).

(H) Effect of combinatorial targeting ME1 and IDH2 via siRNAs on relative NADPH/$NADP^+$ ratio in MCF-7 and A549 cells (n=3).

(I)Percentage of $NADP^2H$ labeling from 2 mM [2,3,3-^{2}H] aspartate for 24 h in MCF-7 cells with control transfection or ME1, CTP knockdown or both (n=3).

(J) Effect of combinatorial targeting ME1 and CTP on relative NADPH/$NADP^+$ ratio in MCF-7 and A549 cells (n=4).

Results are shown as mean ± SD. Experiments in D, E and I were conducted once and all the other experiments were repeated at least twice. Statistical significance was determined by Student's t-test, * $p<0.05$, ** $p<0.01$, *** $p<0.001$.



Supplementary Figure 5. AMPK-FoxO1 axis underlies adaptive IDH2 upregulation. (Related to Figure 5)

(A) FoxO1 DNA-binding sites are present in the human IDH2 promoter region.

(B) mRNA level of FoxO1, MnSOD and p21 in MCF-7 cells with control and ME1 knockdown (n=3). β-Actin was used for normalization.

(C) Immunoblot analysis of FoxO1, p-AMPK and AMPK expression in MCF-7 and A549 cells with control or ME1 knockdown and treated with or without NAC (10 mM) for 24 h. β-Tubulin was used as the loading control.

(D) mRNA levels of FoxO1, IDH2, MnSOD and p21 in MCF-7 cells treated with AICAR (1 mM), Compound C (5 μM) or both for 24 h (n=3). β-Actin was used for normalization.

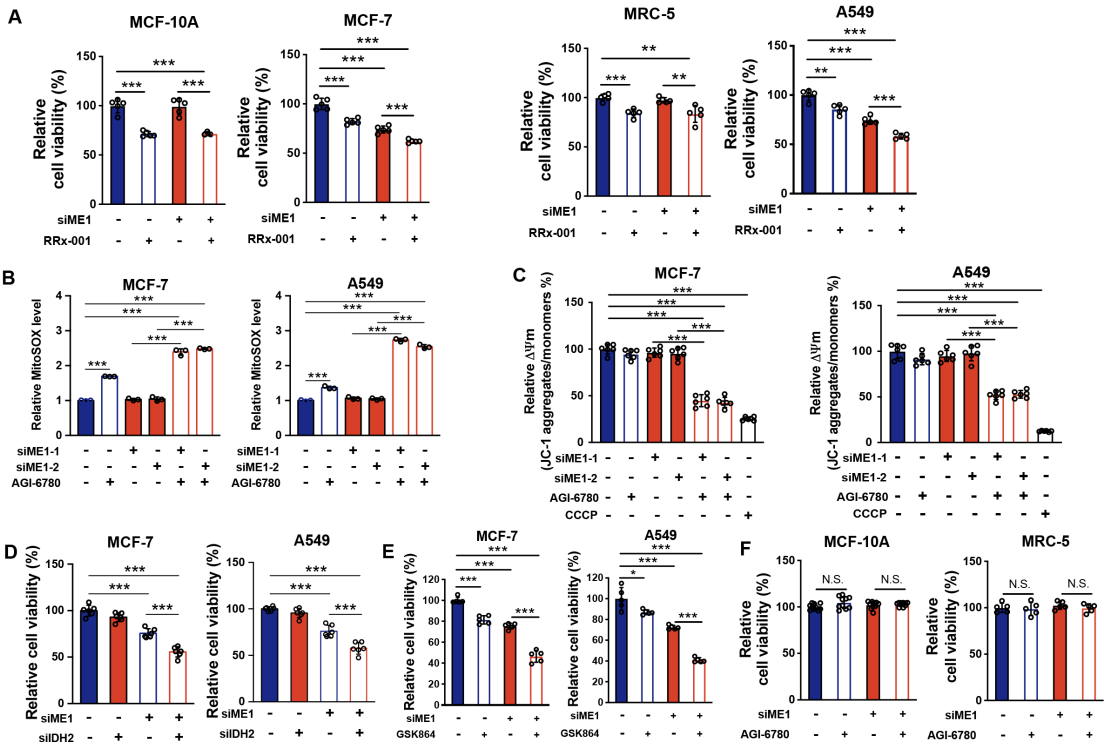
(E) Immunoblotting analysis of nuclear and cytosolic FoxO1 and IDH2 expression in MCF-7 cells treated with AICAR (1 mM), Compound C (5 μM) or both for 24 h. Histone H3 was used as nuclear loading control and β-Tubulin was used as cytosolic loading control.

(F) mRNA levels of FoxO1, IDH2, MnSOD and p21 in MCF-7 cells treated with AICAR (1 mM), AS1842656 (0.2 μM) or both for 24 h (n=3). β-Actin was used for normalization.

(G) Immunoblotting analysis of nuclear and cytosolic FoxO1 and IDH2 expression in MCF-7 cells treated with AICAR (1 mM), AS1842656 (0.2 μM) or both for 24 h. Histone H3 was used as nuclear loading control and β-Tubulin was used as cytosolic loading control.

(H) Effect of combinatorial targeting ME1 and FoxO1 on NADPH/$NADP^+$ ratio in MCF-7 and A549 cells (n=3).

Results are shown as mean ± SD. Data represents at least two independent experiments except experiments in H were conducted once. Statistical significance was determined by Student's t-test, * $p<0.05$, ** $p<0.01$, *** $p<0.001$.



Supplementary Figure 6. ME1 and IDH2 synergistically induce cancer cell apoptosis. (Related to Figure 6)

(A) Effect of combinatorial targeting ME1 and G6PD (5 μM RRx-001 for 24 h) on relative cell viability in MCF-10A, MCF-7, MRC-5 and A549 cells (n=5).

(B) Effect of combinatorial targeting ME1 and IDH2 (2.5 μM AGI-6780 for 24 h) on mitochondrial ROS level in MCF-7 and A549 cells (n=3).

(C) Effect of combinatorial targeting ME1 and IDH2 (2.5 μM AGI-6780 for 24 h) on mitochondrial outer membrane permeabilization (MOMP) in MCF-7 and A549 cells (n=6). Cells treated with carbonyl cyanide 3-chlorophenylhydrazone (CCCP) (10 μM for 20 min) were used as a positive control.

(D) Viability of cells with control transfection, ME1, IDH2 knockdown and both in MCF-7 and A549 cells (n=6).

(E) Effect of combinatorial targeting ME1 and IDH1 (8 μM GSK864 for 24 h) on relative cell viability in MCF-7 and A549 cells (n=5).

(F) Effect of combinatorial targeting ME1 and IDH2 (2.5 μM AGI-6780 for 24 h) on relative cell viability of MCF-10A (n=10) and MRC-5 cells (n=5).

Results are shown as mean ± SD. Data represents at least two independent experiments. Statistical significance was determined by Student's t-test. * $p<0.05$, ** $p<0.01$, *** $p<0.001$, N.S., non-significant.

Supplementary Table 1. Clinical characters of the 12 breast cancer patients included in this study.

Patient ID	age	grade	ER	PR	HER2	Pathological type	Metastasis
1	70	2-3	90.0%	5%	-	Type B	Lymph node metastasis
2	44	3	80.0%	20.0%	+	Type B	-
3	41	2	90.0%	90.0%	-	Type B	-
4	77	2	90.0%	-	-	Type B	Lymph node metastasis
5	75	2	90.0%	90.0%	-	Type B	-
6	58	2	-	-	-	Triple negative	-
7	60	2-3	10.0%	-	+	Type B	-
8	76	2	90.0%	30.0%	-	Type B	+
9	49	2	80.0%	60.0%	-	Type B	+
10	74	2	90.0%	50.0%	-	Type B	-
11	62	3	-	-	-	Triple negative	-
12	49	2	90.0%	90.0%	-	Type A	+

Supplementary Table 2. Sequences of qPCR Primers used in this study.

ME1 forward primer	5’-ACAGATAATATTTTCCTCACT-3’
ME1 reverse primer	5’-CTACTGGTCAACTTTGGT-3’
IDH2 forward primer	5’-TACGGGTCATCTCATCACCA-3’
IDH2 reverse primer	5’-ACCTCGCAAGAGCAGCC-3’
FoxO1 forward primer	5’-GGATGTGCATTCTATGGTGTACC-3’
FoxO1 reverse primer	5’-TTTCGGGATTGCTTATCTCAGAC-3’
MnSOD forward primer	5’-TTTCAATAAGGAACGGGGACAC -3’
MnSOD reverse primer	5’- GTGCTCCCACACATCAATCC-3’
p21 forward primer	5’-GTGGACCTGGAGACTCTC-3’
p21 reverse primer	5’-TTCCTCTTGGAGAAGATCAG -3’
β-actin forward primer	5’-GCGTGACATTAAGGAGAAG-3’
β-actin reverse primer	5’-GAAGGAAGGCTGGAAGAG-3’