A novel miR-1291-ERRα-CPT1C axis modulates tumor cell proliferation, metabolism and tumorigenesis

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Supplementary Figure S1. The workflow of the Meta-analysis.
Supplementary Figure S2. (A) RT-qPCR analysis was used to determine the expression of miR-1291 in PANC-1 and MDA-MB-231 cells after transfection with miR-1291 plasmid, miR-1291
inhibitor as well as ST-miR1291 cells. The data are the mean ± SD (n = 6). (B) Expression of ERRα mRNA in PANC-1 and MDA-MB-231 cells after modulation of ERRα expression and activity with the pENTER-ERRα plasmid and agonist β-E2 (20 nM), respectively, as well as siRNA or chemical inhibitor XCT790 (20 μM). Data are mean ± SD (n = 6). (C) WST-8 assays were performed to examine the viability of PANC-1 and MDA-MB-231 cells after the addition of various concentrations of β-E2 or XCT790. Data are mean ± SD (n = 6). (D) Expression of CPT1C mRNA in PANC-1 and MDAMB-231 cells after modulation of CPT1C expression with the overexpression plasmid and siRNA. Data are mean ± SD (n = 6).
Supplementary Figure S3. (A) CPT1C 3’UTR luciferase reporter activity was assessed in HEK-293T cells after transfecting miR-1291. Data are mean ± SD (n = 5). (B) Detailed information on the possibility of miR-1291 combined with ERRα. (C) ERRα protein expression levels and mRNA levels of CPT1C were determined in PANC-1 and MDA-MB-231 cells after transfection with miR-1291 inhibitor. Data are mean ± SD (n = 3 for western-blot, n = 5 for qPCR). (D) Eight different ERRE regions predicted in the 3.0 kb CPT1C promoter were identified by a bioinformatics
analysis. The ERRE sequences are denoted as ERRE1, ERRE2, and ERRE3. (E) The efficacy of micrococcal nuclease to cutting the DNA fragments in ChIP assay.
Supplementary Figure S4. (A) Immunoblot analysis was used to determine cell cycle-related proteins, such as cyclin A/D/E in PANC-1 and MDA-MB-231 cells after transfection with miR-1291. The intensity of protein bands was assayed by Quantity One software and normalized to loading control. Data are mean ± SD (n = 3). (B) Western blot analysis was used to measure the protein expression of ERRα and CPT1C in PANC-1 and MDA-MB-231 cells after modulation of ERRα expression. Data are mean ± SD (n = 3). (C) The protein levels of cell cycle-related proteins, such as cyclin A/D/E were determined by immunoblot analysis in PANC-1 and MDA-MB-231 cells after transfection with ERRα siRNA. Data are mean ± SD (n = 3). (D) Western blot analysis was used to measure the protein expression of PGC-1α in PANC-1 and MDA-MB-231 cells after transfection with miR-1291 plasmid. Data are mean ± SD (n = 3).
Supplementary Figure S5. (A) WST-8 and BrdU assays were performed to examine the effect of high CPT1C expression on the viability and proliferation capacity of WT and ST-miR1291 PANC-
1cells. Data are mean ± SD (n = 5). (B) Glycolysis inhibition tests with 2-deoxyglucose and glucose deprivation tests with glucose were performed to measure the impact of overexpression of CPT1C on the anti-metabolic stress ability of WT and ST-miR1291 PANC-1 cells. Data are mean ± SD (n = 5). (C) WST-8 and BrdU assays were performed to examine the role of ERRα activation on the viability and proliferation capacity of WT and ST-miR1291 PANC-1 cells. Data are mean ± SD (n = 5). (D) Glycolysis inhibition tests with 2-deoxyglucose and glucose deprivation tests with glucose were conducted to measure the influence of increased ERRα expression on the anti-metabolic stress ability of WT and ST-miR1291 PANC-1 cells. Data are mean ± SD (n = 5). (E) The growth rates of ST-miR-1291 cells and WT cells in different time points. Data are mean ± SD (n = 5).
Supplementary Figure S6. (A) WST-8 and BrdU assays were performed to examine the effect of high CPT1C expression on the viability and proliferation capacity of WT and ST-miR1291 MDA-MB-231 cells. Data are mean ± SD (n = 5). (B) Glycolysis inhibition tests with 2-deoxyglucose and glucose deprivation tests with glucose were performed to measure the impact of overexpression of CPT1C on the anti-metabolic stress ability of WT and ST-miR1291 MDA-MB-231 cells. Data are mean ± SD (n = 5). (C) WST-8 and BrdU assays were performed to examine the role of ERRα activation on the viability and proliferation capacity of WT and ST-miR1291 MDA-MB-231 cells. Data are mean ± SD (n = 5). (D) Glycolysis inhibition tests with 2-
Deoxyglucose and glucose deprivation tests with glucose were conducted to measure the influence of increased ERRα expression on the anti-metabolic stress ability of WT and ST-miR1291 MDA-MB-231 cells. Data are mean ± SD (n = 5).
Supplementary Figure S7. (A) WST-8 and BrdU assays were performed to examine the effect of low CPT1C expression on the viability and proliferation capacity of WT and ST-miR1291 MDA-MB-231 cells. Data are mean ± SD (n = 5). (B) Glycolysis inhibition tests with 2-deoxyglucose and glucose deprivation tests with glucose were performed to measure the depletion of CPT1C expression on the anti-metabolic stress ability of WT and ST-miR1291 MDA-MB-231 cells. Data are mean ± SD (n = 5). (C) WST-8 and BrdU assays were performed to examine the influence of ERRα inhibition on the viability and proliferation capacity of WT
and ST-miR1291 MDA-MB-231 cells. Data are mean ± SD (n = 5). (D) Glycolysis inhibition tests with 2-deoxyglucose and glucose deprivation tests with glucose were performed to measure the impact of reduction of ERRα expression on the anti-metabolic stress ability of WT and ST-miR1291 MDA-MB-231 cells. Data are mean ± SD (n = 5).
### Table S1. Sequences of primers for quantitative RT-PCR analysis.

| Gene Name   | Gene ID | Species | Specificity | Sequences of Primers            |
|-------------|---------|---------|-------------|----------------------------------|
| β-actin     | 60      | Human   | forward 5’- | forward 5’- CCCTGCAACATGCACAGG-3’ |
|             |         |         | reverse 3’-  | reverse 5’- GCACAGAGGCTCGCT-3’   |
| ERRα        | 2101    | Human   | forward 5’- | forward 5’- AGGGTTCTCCGGGAGACAG-3’ |
|             |         |         | reverse 3’-  | reverse 5’- TCACAGGATGCCACACC-3’ |
| CPT1C       | 126129  | Human   | forward 5’- | forward 5’- GGATGGCCTGAAAGAGGA-3’ |
|             |         |         | reverse 3’-  | reverse 5’- TCCTGGAAAAAGGCATCT-3’ |
| PGC-1α      | 10891   | Human   | forward 5’- | forward 5’- TCTGAGTCTTGATGGAGTCAT-3’ |
|             |         |         | reverse 3’-  | reverse 5’- CCAAGTCGTTACATCTAGT-3’ |
| GLS         | 2744    | Human   | forward 5’- | forward 5’- AGGGTCTGTTACCTAGCT-3’ |
|             |         |         | reverse 3’-  | reverse 5’- ACGTCGCAATCCTGAGAT-3’ |
| Perlipin-1  | 5346    | Human   | forward 5’- | forward 5’- TGGCAAATGCCCTAGGAGG-3’ |
|             |         |         | reverse 3’-  | reverse 5’- AGGGCGGGGATTTTCT-3’   |
| STARS       | 137735  | Human   | forward 5’- | forward 5’- AGCAGTGGGCGAATGAGAC-3’ |
|             |         |         | reverse 3’-  | reverse 5’- GTGATTTGGTATGGAGCTG-3’ |
| SPP1        | 6696    | Human   | forward 5’- | forward 5’- CTCCATTGACCTCAAGCAG-3’ |
|             |         |         | reverse 3’-  | reverse 5’- CATCCTCCTTTCCAGTGT-3’ |
| EGF         | 1950    | Human   | forward 5’- | forward 5’- CCCGTCGAAAGACAGATTTG-3’ |
|             |         |         | reverse 3’-  | reverse 5’- GGGTGTCGGCATCCACAGCAG-3’ |
| TFF1        | 7031    | Human   | forward 5’- | forward 5’- AGGAACACGGGAGTGACAA-3’ |
|             |         |         | reverse 3’-  | reverse 5’- TATGCTCGTGGTAGTACCGG-3’ |
| NRF-1       | 4899    | Human   | forward 5’- | forward 5’- ATGGCGTCTCTCCGAGCA-3’ |
|             |         |         | reverse 3’-  | reverse 5’- TCCGCCCTATAAACGATTTG-3’ |
| TFAM        | 7019    | Human   | forward 5’- | forward 5’- CCCAGTGAGCTTTGGTGCC-3’ |
|             |         |         | reverse 3’-  | reverse 5’- GCGGTCATGTACTTCCTGT-3’ |
| CYBA        | 1535    | Human   | forward 5’- | forward 5’- AGGGTCTGACCTGTCGCT-3’ |
|             |         |         | reverse 3’-  | reverse 5’- GGGTGTCGGCATCCACAGCAG-3’ |
Table S2. Sequences of primers for miRNA quantitative RT-PCR analysis.

| Name                        | Sequences of Primers                                                                 |
|-----------------------------|--------------------------------------------------------------------------------------|
| MiRNA Stem-loop Primer      | 5'-GTCGTATCCAGTGACGGGTCCGAGGT                                                      |
|                             | ATTCGCACTGGGATACGACACTGCT-3'                                                        |
| U6-F                        | forward 5'-CTCGCTTCGGCAGCACA-3'                                                     |
| U6-R                        | reverse 5'-AACGCTTCACGAATTGCGT-3'                                                   |
| miR-1291-F                  | forward 5'-CGTGCCCTGACCTGAAGACC-3'                                                 |
| miR-1291-F                  | reverse 5'-AGTCAGGCTCCGAGGTATT-3'                                                  |

Table S3. Sequences of primers for ChIP-qPCR analysis.

| Name                        | Sequences of Primers                                                                 |
|-----------------------------|--------------------------------------------------------------------------------------|
| ERR-CPT1C-chip-1F           | forward 5'-GAATGGCTTTGCGCTTAAGG-3'                                                 |
| ERR-CPT1C-chip-1R           | reverse 5'-AGTTGCACGTGACGTTGTAAGC-3'                                               |
| ERR-CPT1C-chip-2F           | forward 5'-TTCTGCGATCTGCAGCCTCC-3'                                                 |
| ERR-CPT1C-chip-2R           | reverse 5'-TCGAGTGTCTGGGAGG-3'                                                      |
| ERR-CPT1C-chip-3F           | forward 5'-TGGCGCCGCTTGAGGCG-3'                                                    |
| ERR-CPT1C-chip-3R           | reverse 5'-AGTACGGAGAGAATGCTGGAGAGC-3'                                             |
| ERR-CPT1C-chip-4F           | forward 5'-GGACCAGGCTGGCCGAA-3'                                                    |
| ERR-CPT1C-chip-4R           | reverse 5'-ACTTCCGAGGAGAGCAG-3'                                                    |
| CANCER       | GSE      | GPL | PMID     | YEAR | Samples Number | NORMAL | CANCER |
|--------------|----------|-----|----------|------|----------------|--------|--------|
| BREAT CANCER | GSE10780 | GPL570 | 19266279 | 2009 | 101            | 42     |
|              | GSE10810 | GPL570 | 20029976 | 2009 | 21             | 37     |
|              | GSE15852 | GPL96  | 20097481 | 2009 | 43             | 43     |
|              | GSE20437 | GPL96  | 20197764 | 2010 | 24             | 18     |
|              | GSE22544 | GPL570 | 20799942 | 2010 | 4              | 16     |
|              | GSE25407 | GPL570 | 21118987 | 2010 | 5              | 5      |
|              | GSE29431 | GPL570 | #N/A     | 2011 | 12             | 54     |
|              | GSE42568 | GPL570 | 23740839 | 2013 | 17             | 104    |
|              | GSE5764  | GPL570 | 17389037 | 2007 | 20             | 10     |
|              | GSE61304 | GPL570 | #N/A     | 2015 | 4              | 58     |
|              | GSE7904  | GPL570 | 16473279 | 2007 | 19             | 43     |
|              | GSE9574  | GPL96  | 18058819 | 2007 | 15             | 14     |
| PANCREATIC CANCER | GSE15471 | GPL570 | 19260470 | 2009 | 42             | 36     |
|              | GSE18670 | GPL570 | 23157946 | 2012 | 6              | 18     |
|              | GSE19650 | GPL570 | 20955708 | 2010 | 7              | 15     |
|              | GSE22780 | GPL570 | #N/A     | 2011 | 8              | 8      |
|              | GSE27890 | GPL570 | #N/A     | 2014 | 4              | 6      |
|              | GSE46234 | GPL570 | #N/A     | 2017 | 4              | 4      |