Expanded View Figures

Figure EV1. CUEDC2 facilitates aerobic glycolysis in different types of cancer cell lines.

A, B mRNA (A) and protein (B) levels of CUEDC2 were determined by qRT–PCR and Western blot, respectively, in glucose- or glutamine-starved PLC cells (A, B) or HeLa cells (B).

C The medium color of cultured PLC cells expressing shCUEDC2 was much pinker than that of control cells expressing NTC. The cell numbers were $1.76 \times 10^6$ for NTC cells and $1.74 \times 10^6$ for shCUEDC2 cells, respectively.

D, E Cellular glucose uptake, lactate production, and O$_2$ consumption rate were measured in HeLa cells expressing shCUEDC2 (D) or MDA-MB-231 cells stably overexpressing HA-CUEDC2 (E).

F Cellular ROS levels were detected by flow cytometry using CellROX DeepRed staining in HeLa cells expressing shCUEDC2 and MDA-MB-231 cells overexpressing CUEDC2.

Data information: (A, D, E) Data are presented as mean (± SD), n = 3 in each group. *P < 0.05 as compared to normal group in (A), to NTC group in (D) and to EV group in (E), respectively. P was calculated by Student’s t-test. The representative results of three independent experiments are shown in (F). β-Actin served as loading control.

Source data are available online for this figure.
Xiuying Zhong et al

Figure EV1.
Figure EV2. CUEDC2 facilitates cancer cell growth, at least partially by enhancing Warburg effect.

A, B Protein (A) and mRNA (B) levels of GLUT3 and LDHA were determined by Western blot and qRT–PCR, respectively, in HeLa cells and MDA-MB-231 cells.

C, D Cell growth was determined by trypan blue counting in shCUEDC2s expressing (C) or HA-CUEDC2 overexpressing (D) PLC, HeLa, and MDA-MB-231 cells.

E The same numbers of PLC cells stably expressing NTC or shCUEDC2 were cultured for 60 h followed by treatment with or without indicated concentrations of oligomycin for 8 h. Cell numbers were determined by trypan blue counting.

Data information: (B–E) Data are presented as mean (± SD); n = 3 in each group. *P < 0.05 as compared to NTC group in (B, C), to EV group in (D), and to DMSO group in (E), respectively. P was calculated by Student's t-test. NS: Not significant between indicated groups. β-Actin served as loading control.

Source data are available online for this figure.
Xiuying Zhong et al  
CUEDC2 promotes the Warburg effect

Figure EV2.

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Figure EV3. CUEDC2 regulates GLUT3 via GR.

A Immunoprecipitation (IP) assay was performed with anti-FLAG antibody in 293T cells cotransfected with HA-CUEDC2, FLAG-GR, or FLAG-EV, followed by blotting with anti-CUEDC2 or anti-GR.

B Protein levels of GR and GLUT3 were determined by Western blot in HeLa cells and MDA-MB-231 cells expressing shCUEDC2 or overexpressing HA-CUEDC2.

C mRNA levels of CUEDC2 and GR were detected by qRT-PCR in PLC cells stably expressing shCUEDC2.

D The protein level of GLUT3 in PLC cells stably expressing shGRs was analyzed by Western blot.

E mRNA and protein levels of GLUT3 were determined by qRT-PCR and Western blot in PLC cells stably expressing shCUEDC2 with further overexpression of EV or HA-GR.

F Cellular ROS levels were detected by flow cytometry using CellROX DeepRed staining in PLC cells stably expressing CUEDC2 with further knockdown of GR by shRNAs.

Data information: (C and E) Data are presented as mean ± SD; n = 3 in each group. *P < 0.05 as compared to NTC group in (C), and to NTC + EV or to NTC + GR group in (E), respectively. P was calculated by Student’s t-test. NS: Not significant between indicated groups. The representative results of three independent experiments are shown in (F). β-Actin served as loading control.

Source data are available online for this figure.
Figure EV4.
Figure EV4. CUEDC2 regulates LDHA via 14-3-3ζ.

A Immunoprecipitation (IP) assay was performed with anti-FLAG antibody in HEK293T cells cotransfected with HA-CUEDC2 and FLAG-LDHA, followed by blotting with anti-HA and anti-FLAG.

B Protein levels of 14-3-3ζ were determined by Western blot in PLC cells expressing shCUEDC2s.

C Protein levels of 14-3-3ζ and LDHA were determined by Western blot in HeLa cells and MDA-MB-231 cells expressing shCUEDC2 or overexpressing HA-CUEDC2.

D Immunoprecipitation (IP) assay was performed using anti-FLAG antibody in HEK293T cells cotransfected with HA-CUEDC2 and FLAG-14-3-3ζ, followed by blotting with anti-CUEDC2 and anti-FLAG.

E mRNA levels of CUEDC2 and 14-3-3ζ were detected by qRT-PCR in PLC cells stably expressing shCUEDC2.

F Immunoprecipitation (IP) assay was performed using anti-FLAG antibody in HEK293T cells cotransfected with HA-14-3-3ζ and FLAG-LDHA, followed by blotting with anti-14-3-3ζ and anti-FLAG.

G Cell lysates from PLC cells cotransfected with HA-14-3-3ζ and FLAG-LDHA were treated with or without PP2A, followed by immunoprecipitation with anti-FLAG or IgG. The immunoprecipitates were blotted with anti-HA or anti-FLAG.

H mRNA levels of 14-3-3ζ and LDHA were detected by qRT-PCR in PLC cells stably expressing sh14-3-3ζ.

I Cellular ROS levels were detected by flow cytometry using CellROX DeepRed staining in PLC cells stably expressing CUEDC2 with further knockdown of 14-3-3ζ by shRNAs.

Data information: (E and H) Data are presented as mean (± SD); n = 3 in each group. *P < 0.05 as compared to NTC group. P was calculated by Student’s t-test. NS: Not significant between indicated groups. The representative results of three independent experiments are shown in (I). β-Actin served as loading control.

Source data are available online for this figure.