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 1.76×10^6 for NTC cells and 1.74×10^6 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.

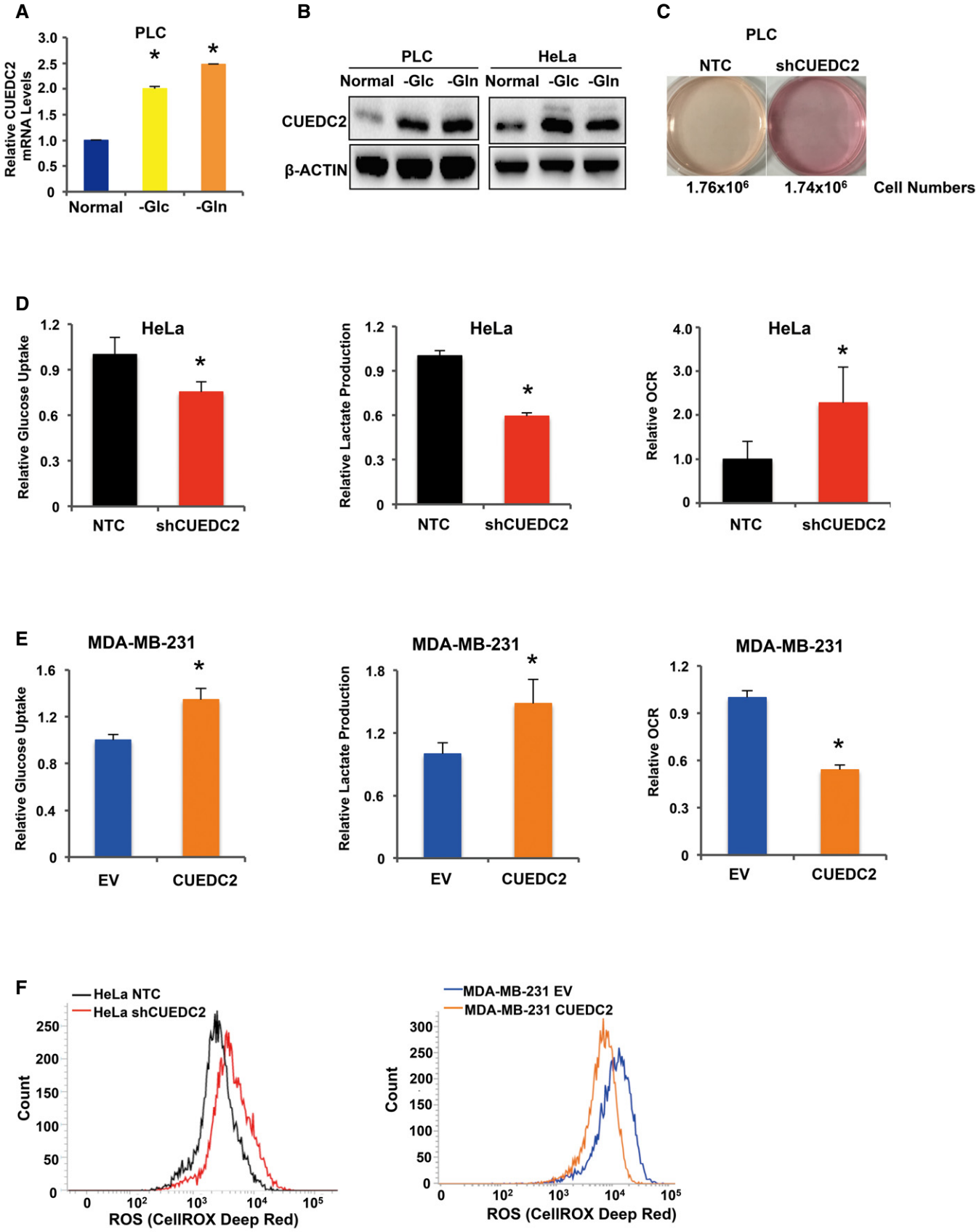

Figure EV1.

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.

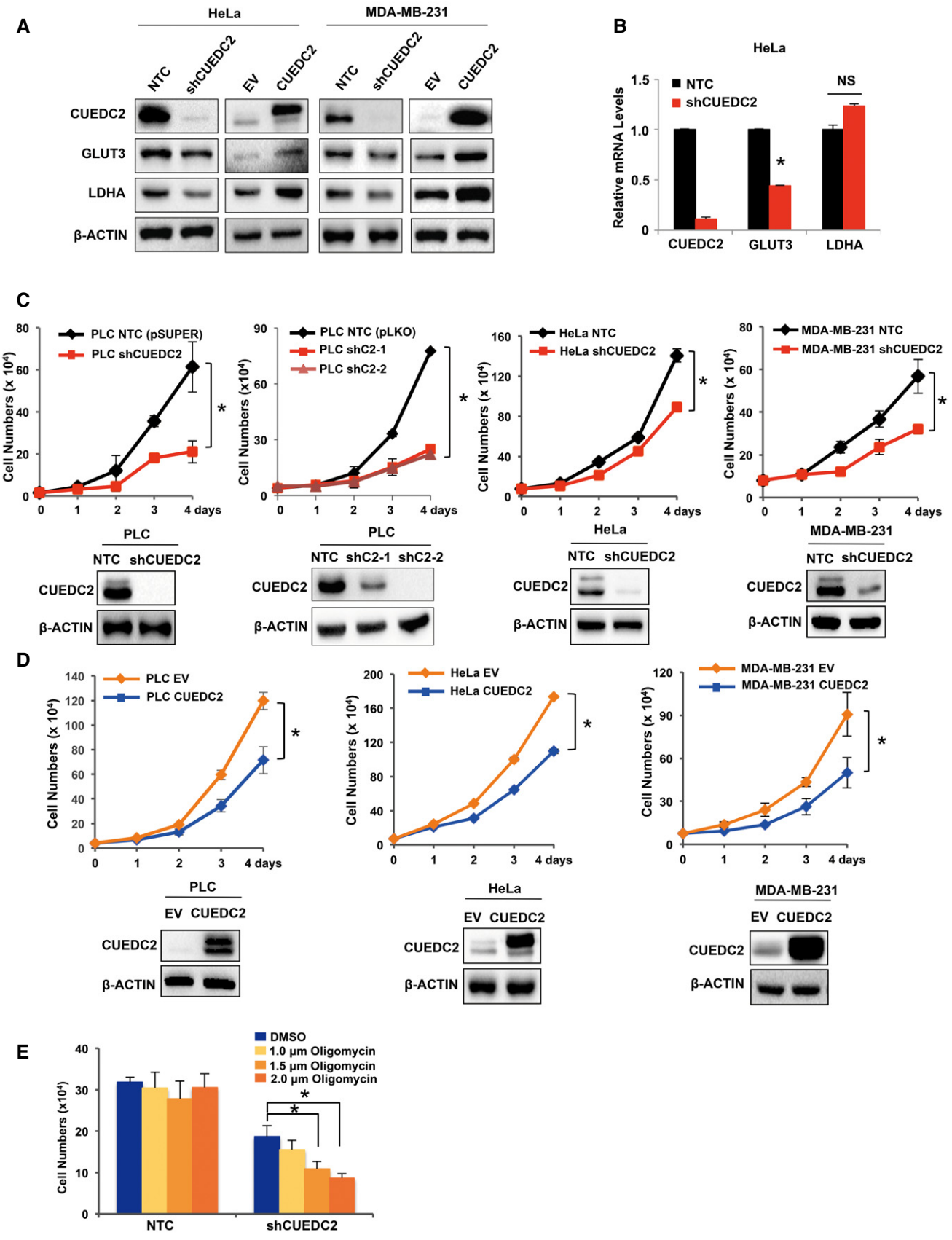

Figure EV2.

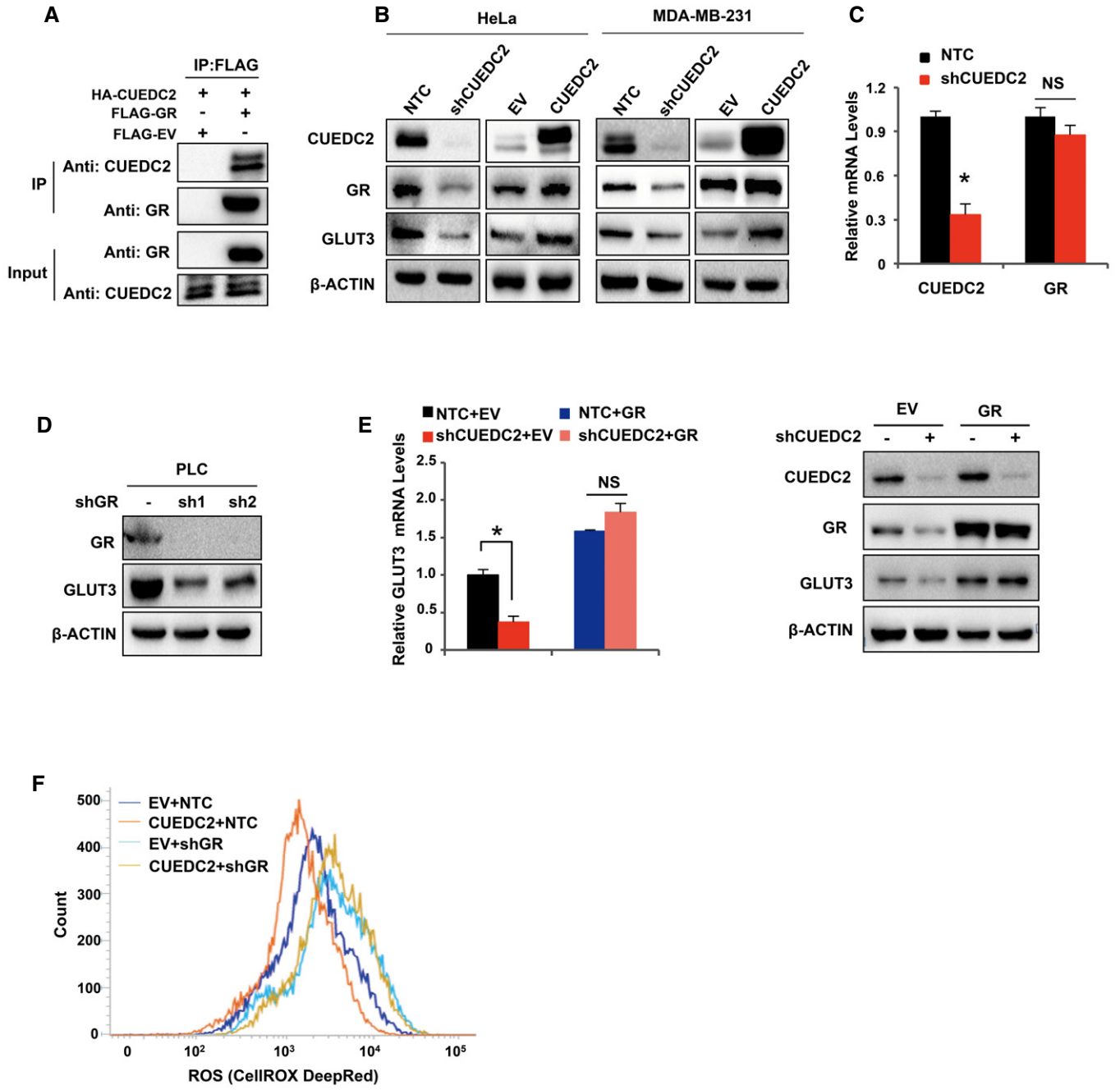

Figure EV3. CUEDC2 regulates GLUT3 via GR.

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.

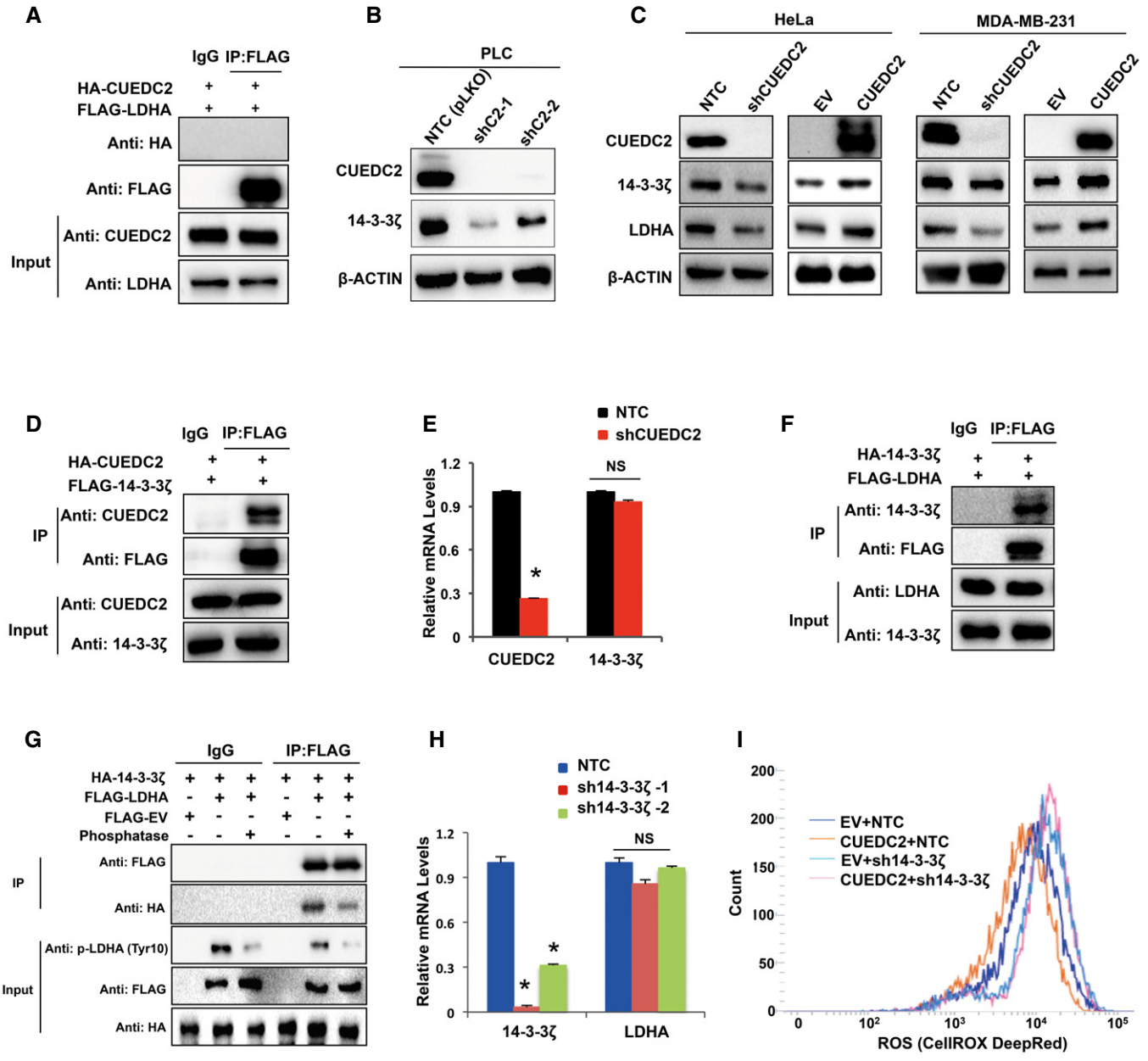

Figure EV4.

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.