Expanded View Figures

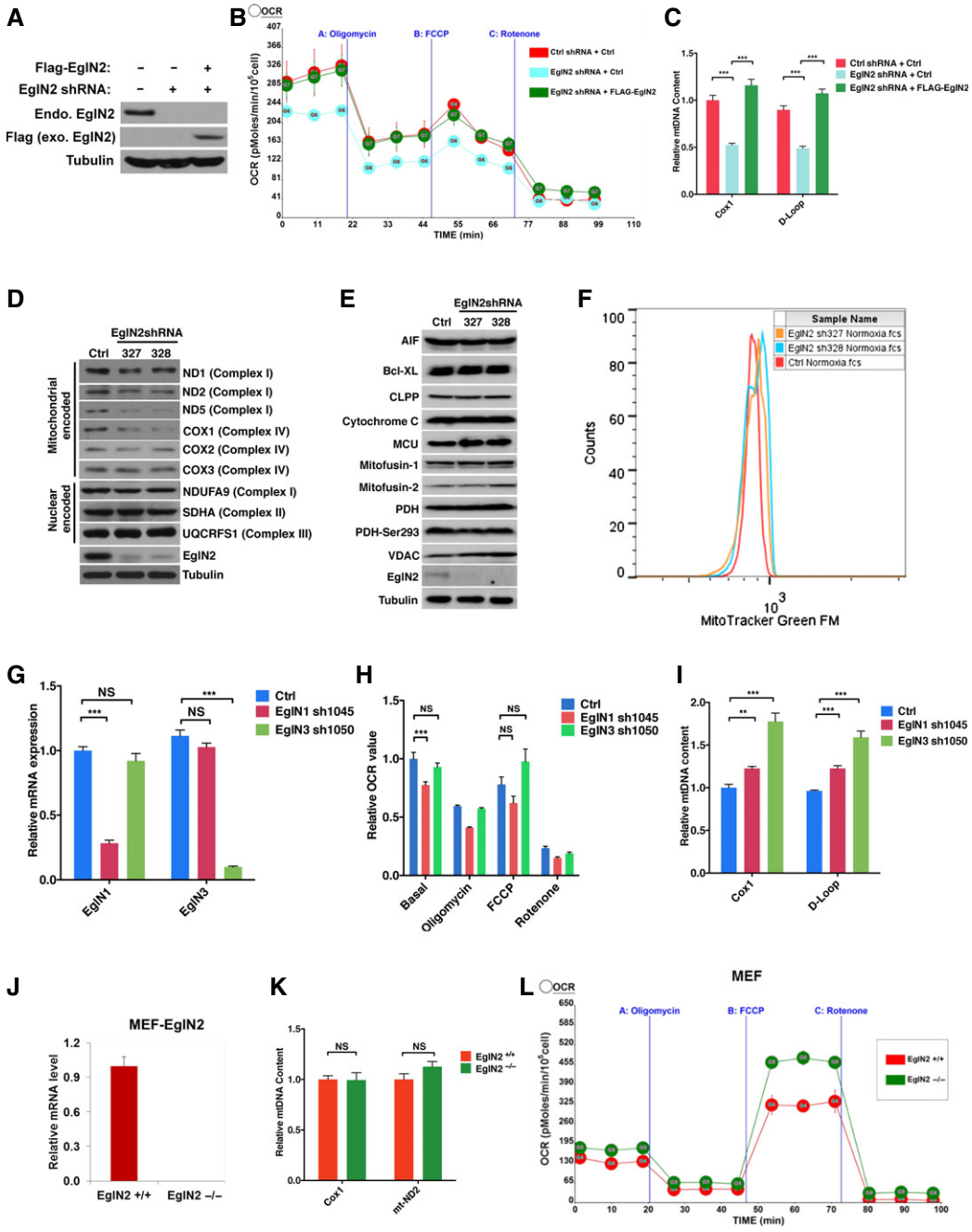

Figure EV1. EglN2 regulates mitochondrial function.

A–C Immunoblot of cell lysates (A), measurement of oxygen consumption rate (B), and qRT–PCR quantification of mtDNA (C) from T47D cells infected with lentivirus encoding either EglN2 shRNA or control (Ctrl) shRNA followed by another infection with lentivirus encoding either FLAG-EglN2 or control (Ctrl).

D–F Immunoblot of cell lysates with indicated antibodies (D, E) and measurement of mitochondrial mass with MitoTracker Green staining followed by flow cytometry (F) from T47D cells infected with lentivirus encoding EglN2 shRNA (327 or 328) or control shRNA (Ctrl).

G–I qRT–PCR of mRNA (G), measurement of oxygen consumption rate (H), and qRT–PCR quantification of mtDNA (I) from T47D cells infected with lentivirus encoding either EglN1 shRNA (1045), EglN3 shRNA (1050), or control (Ctrl) shRNA.

J–L qRT–PCR of mRNA (J), measurement of oxygen consumption rate (K), and qRT–PCR quantification of mtDNA (L) from EglN2 KO or WT MEF cells.

Data information: Two-tailed Student's *t*-test was used to examine the *P*-values from at least three replicate experiments. Error bars represent SEM. $^{**}P < 0.01$, $^{***}P < 0.005$. NS denotes not significant.

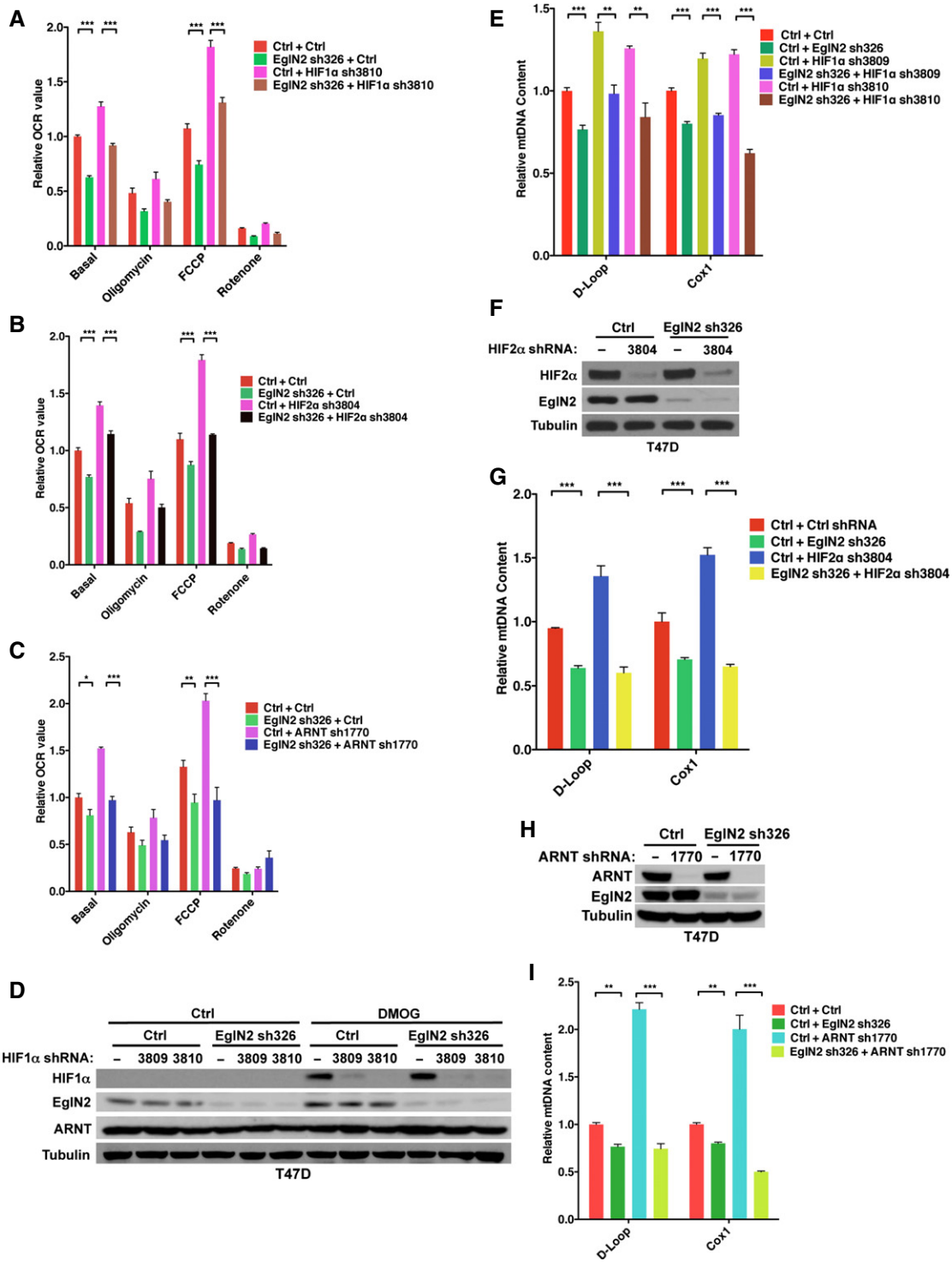

Figure EV2. EgIN2 regulates mitochondrial function in a HIF1/2α-independent manner.

A–C Measurement of oxygen consumption from MCF7 cells infected with lentivirus encoding either HIF1 shRNAs (3809, 3810) (A), HIF2 shRNA (3804) (B), ARNT shRNA (1770) (C), or control (Ctrl) shRNA followed by another infection with lentivirus encoding either EgIN2 shRNA or control (Ctrl) shRNA.

D–I Immunoblot of cell lysates (D, F and H) and qRT–PCR quantification of mtDNA (E, G and I) from T47D cells infected with lentivirus encoding either HIF1α shRNAs (3809, 3810) (D), HIF2α shRNA (3804) (F), ARNT shRNA (1770) (H), or control (–) followed by another infection with lentivirus encoding either EgIN2 shRNA (326) or control (Ctrl) shRNA.

Data information: Two-tailed Student's *t*-test was used to examine the *P*-values from at least three replicate experiments. Error bars represent SEM. $*P < 0.05$, $**P < 0.01$, $***P < 0.005$.

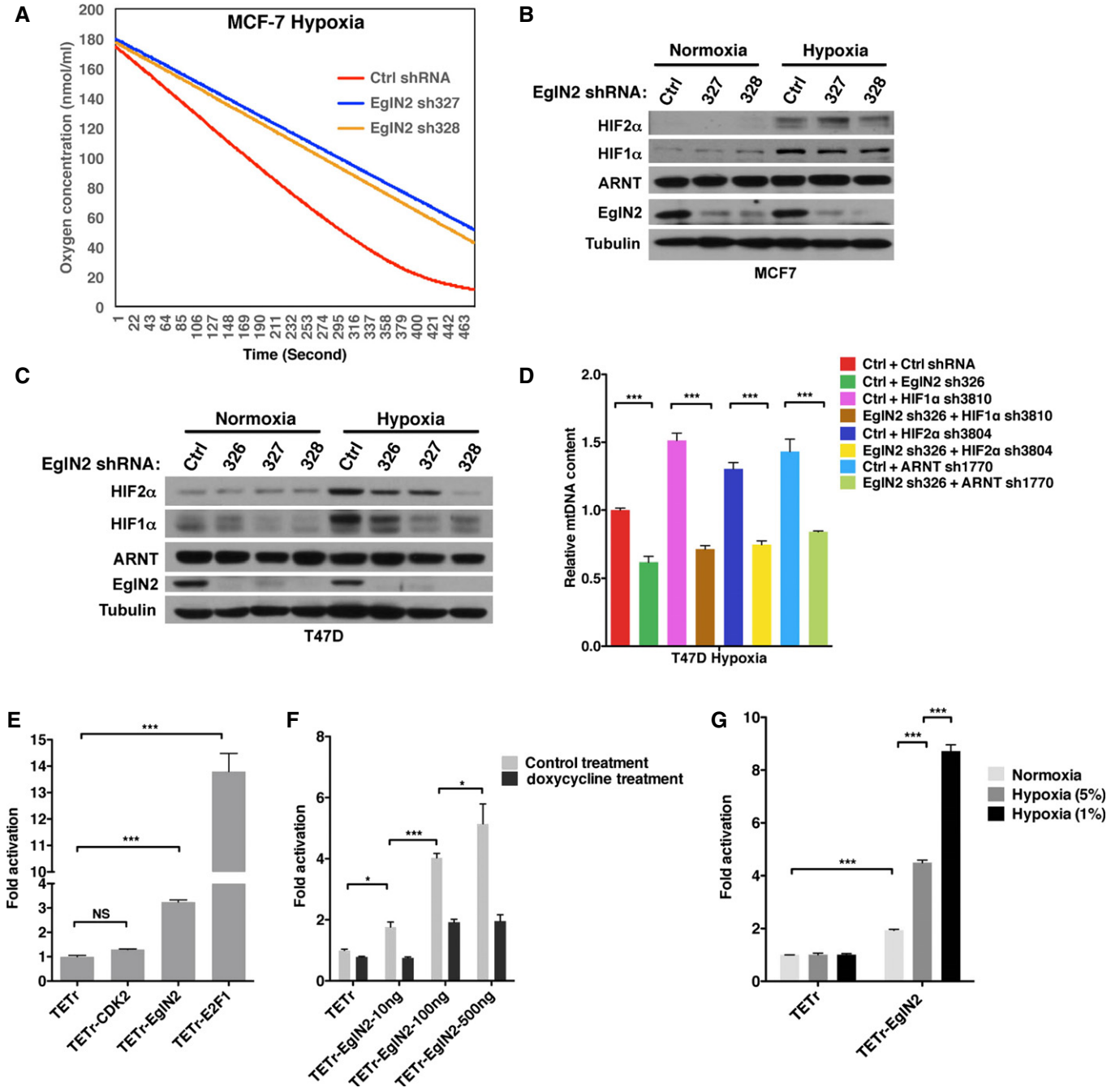

Figure EV3. EglN2 binds to chromatin and regulates mitochondrial function under hypoxia.

A Measurement of oxygen consumption by an oxytherm electrode unit as a function of time for MCF7 cells infected with lentivirus encoding EglN2 shRNA (327 or 328) or control shRNA treated with hypoxia (5% O_2).

B, C Immunoblot from MCF7 (B) or T47D (C) cells infected with lentivirus encoding EglN2 shRNA (326, 327, or 328) or control shRNA (Ctrl) with or without hypoxia (5% O_2) treatment.

D qRT–PCR quantification of mtDNA from MCF7 cells infected with lentivirus encoding HIF1α shRNA (3810), HIF2α shRNA (3804), ARNT shRNA (1770), or control (–) followed by another infection with lentivirus encoding either EglN2 shRNA (326) or control (Ctrl) shRNA with hypoxia (5% O_2) treatment.

E–G Determination of luciferase activity in 293FT cells with plasmids encoding the indicated TETr-fusion proteins.

Data information: Two-tailed Student's *t*-test was used to examine the *P*-values from at least three replicate experiments. Error bars represent SEM. $*P < 0.05$, $***P < 0.005$. NS denotes not significant.

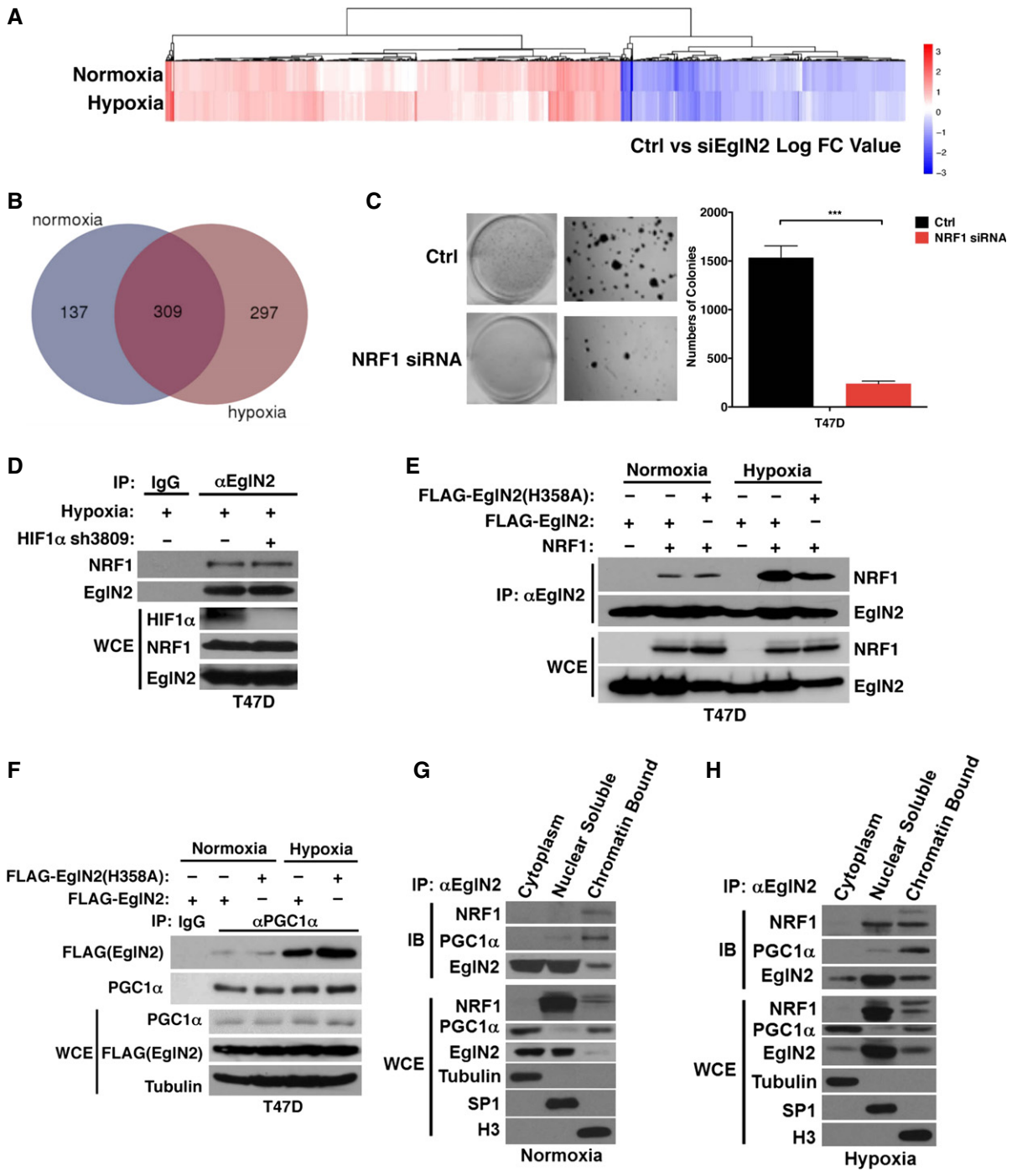

Figure EV4. EgIN2 binds to NRF1 and PGC1α complex on chromatin.

A, B Analysis and comparison of gene expression profiling (siCtrl vs siEgIN2) between normoxia and hypoxia (1% O_2) in T47D.

C Representative images (left) and quantification (right) of anchorage-independent growth assay from T47D cells transfected with NRF1 siRNA or control siRNA (Ctrl). Two-tail Student's *t*-test was used to examine the *P*-values from at least three replicate experiments. Error bars represent SEM. ***$P < 0.005$.

D Immunoblot (IB) assays of whole-cell extracts (WCE) and immunoprecipitation (IP) of T47D (5×10^7) cells infected with lentivirus encoding either HIF1 shRNA or control (—) with hypoxia (5% O_2) treatment.

E, F Immunoblot (IB) assays of whole-cell extracts (WCE) and immunoprecipitation (IP) of T47D (1×10^7) cells infected with lentivirus encoding either FLAG-EgIN2, FLAG-EgIN2 H358A, or control (—) followed by transfection with either NRF1 or empty control with or without hypoxia (5% O_2) treatment.

G, H Cell fractionation using subcellular protein fractionation kit followed by immunoblot and immunoprecipitation (IP) of T47D (5×10^7) cells under normoxia (G) and hypoxia (5% O_2) (H).

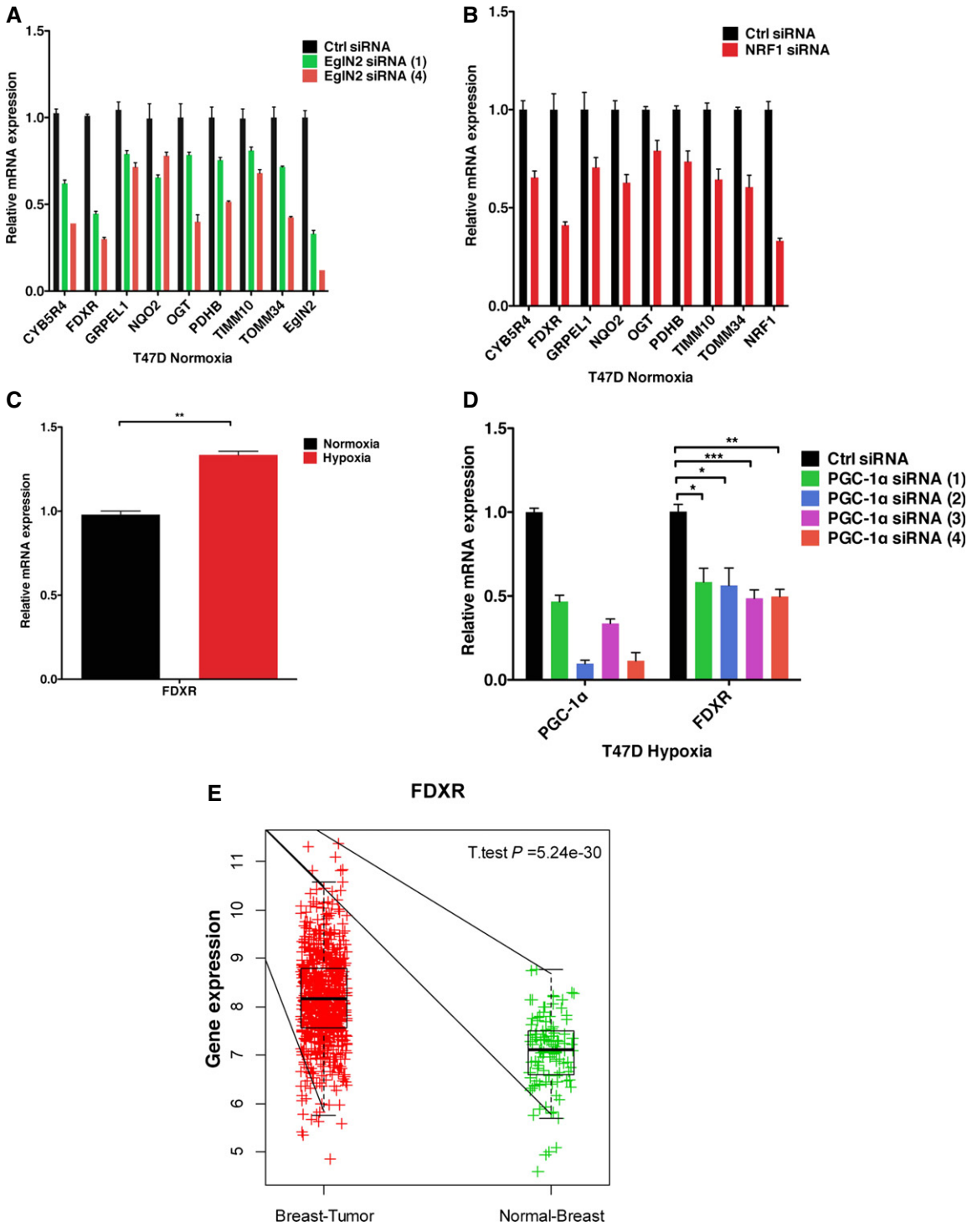

Figure EV5. EglN2/NRF1/PGC1α regulates FDXR in breast cancer.

A, B qRT–PCR of mRNA from T47D transfected with either EglN2 siRNA (1, 4) (A) or NRF1 pool siRNA (B) under normoxia.

C qRT–PCR of mRNA from T47D with or without hypoxia (5% O_2) treatment.

D qRT–PCR of mRNA from T47D cells transfected with either PGC-1α siRNAs (1, 2, 3, and 4) or control (Ctrl) followed by hypoxia (5% O_2) treatment for 16 h.

E FDXR regulates ERα-positive breast tumorigenesis. Unpaired two-sample *t*-test comparing expression of FDXR in the TCGA dataset between the indicated patients.

Data information: Two-tailed Student's *t*-test was used to examine the *P*-values from at least three replicate experiments. Error bars represent SEM. $*P < 0.05$, $**P < 0.01$, $***P < 0.005$.