Glycolytic reprogramming through PCK2 regulates tumor Initiation of Prostate Cancer Cells

SUPPLEMENTARY MATERIALS

Supplementary Figure 1: Isolation and characterization of a TIC-enriched single-cell clone from PC3/M cells (A) Morphology of the PC3/M-ML clone and PC3/M-EL clone (upper), and the first spheres cultured from these cells (bottom). Scale bar, 100 μm. (B) E-cadherin expression level in the PC3/M-ML clone and PC3/M-EL clone, detected by immunoblotting. (C) Numbers of spheres cultured from the PC3/M-ML and PC3/M-EL clones. (D) Representative flow cytometry results of CD44+/CD24-TICs in the PC3/M-ML and PC3/M-EL clones. *: p < 0.05; **: p < 0.01.
Supplementary Figure 2: Enhanced glycolysis and PKM2 detection in the PC3/M-EL clone. (A) Glucose consumption in the PC3/M-ML and PC3/M-EL clones. (B) Lactate production by the PC3/M-ML and PC3/M-EL clones. (C) PKM2 expression in Du145-derived and PC3/M-derived clones detected by immunoblotting. *: p < 0.05.
Supplementary Figure 3: High PCK2 expression in TIC-enriched prostate cancer cells is responsible for the enhanced glycolysis. (A and B) PCK2 expression in PC3/M-ML and PC3/M-EL clones detected by qPCR and immunoblotting. (C) PCK2 knockdown efficiency in PC3/M-EL cells, as detected by immunoblotting. (D) Glucose consumption in the scramble control and PCK2-knockdown PC3/M-EL clone. (E) Lactate production by the scramble control and PCK2-knockdown PC3/M-EL clone. (F) Quantification of cell viability after two days of glucose deprivation (W/O Gluc). FM: full medium. **: p < 0.01.

Supplementary Figure 4: PCK2 and PKM2 regulate PEP accumulation. Quantification of cellular PEP level in scramble control, and in PCK2- and PKM2-knockdown Du145-EL cells. *: p < 0.05; **: p < 0.01.
Supplementary Figure 5: PCK2 and PKM2 differentially regulate the TICs in PC3/M-EL cells. (A) Representative flow cytometry results of CD44+/CD24- TICs analyzed in scramble control and PCK2-knockdown PC3/M-EL cells. (B) Quantification of CD44+/CD24- TICs in scramble control and PCK2-knockdown PC3/M-EL cells. (C) Representative sphere formation results from scramble control and PCK2-knockdown PC3/M-EL cells. Scale bar: 100 μm. (D) Quantification of the spheres in scramble control and PCK2-knockdown PC3/M-EL cells. (E) PKM2 knockdown efficiency in PC3/M-EL cells, as detected by immunoblotting. (F) Glucose consumption detected in scramble control and PKM2-knockdown PC3/M-EL cells. (G) Lactate production detected in scramble control and PKM2-knockdown PC3/M-EL cells. (H) Representative flow cytometry results of CD44+/CD24- TICs in scramble control and PKM2-knockdown PC3/M-EL cells. (I) Quantification of the CD44+/CD24- TICs in scramble control and PKM2-knockdown PC3/M-EL cells. **: p < 0.01.
Supplementary Figure 6: PCK2 and PKM2 regulate cellular ROS in PC3/M-EL cells. (A) Representative flow cytometry results of the H$_2$O$_2$ level detected by DCFH-DA staining in scramble control, and in PCK2- and PKM2-knockdown PC3/M-EL cells. (B) Quantification of the H$_2$O$_2$ level in scramble control, and in PCK2- and PKM2-knockdown PC3/M-EL cells. MFI: mean fluorescence intensity, **: p < 0.01 (compared to scramble control). (C) Representative flow cytometry results of the O$_2^-$ level detected by DHE staining in scramble control, and in PCK2- and PKM2-knockdown PC3/M-EL cells. (D) Quantification of the O$_2^-$ level in scramble control, and in PCK2- and PKM2-knockdown PC3/M-EL cells. MFI: mean fluorescence intensity, **: p < 0.01 (compared to scramble control). (E) Quantification of the CD44+/CD24- TICs in scramble control and PCK2-knockdown PC3/M-EL cells after being treated with 1 mM Tempol for two days. **: p < 0.01. (F) Quantification of the CD44+/CD24- TICs in scramble control and PKM2-knockdown PC3/M-EL cells after being treated with 10 μM 6-AN for two days.
Supplementary Figure 7: PCK2 regulates cellular acetylation. (A) Protein acetylation in Du145-EL scramble and PCK2i-1 cells detected by immunofluorescence staining. Scale bar, 50 μm. (B) Representative flow cytometry results of CD44+/CD24- TICs in SAHA- and CI994-treated Du145-EL cells.