



Figure S2. CLOCK regulates GSC metabolism.

(A) Transcriptomic profiling in ishControl and ishCLOCK GSC272 cells. GSEA analysis shows top ten downregulated hallmark pathways in ishCLOCK GSC272 cells comparing to ishControl cells. Blue bars indicate the signatures relate to metabolism.

(B) Schematic representation of the key steps and related enzymes (marked in red) in TCA cycle (*lower panel*), fatty acid biosynthesis (*left panel*) and glycolysis (*right panel*).

(C) qRT-PCR shows the expression of key enzymes involved in glycolysis, TCA cycle and fatty acid biosynthesis in ishControl and ishCLOCK GSC272 cells. n = 3 biological replicates; * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ vs ishControl.

(D) Inhibition of PGM1 and ACACA impairs GSC tumorsphere formation. Representative images (*left panel*) and quantification (*right panel*) of GSC272 treated with PGM1 inhibitor (PGM11i) lithium (20 mM) and ACACA inhibitor (ACACAi) CP-640186 (10 μ M). Scale bar, 50 μ m; n = 3 biological replicates; *** $P < 0.001$.

(E) Inhibition of PGM1 and ACACA impairs CLOCK overexpression induced GSC tumorsphere formation. Representative images (*left panel*) and quantification (*right panel*) of control GSC17 or CLOCK overexpressed (OE) GSC17 treated with lithium (20 mM) or CP-640186 (10 μ M). Scale bar, 50 μ m; n = 3 biological replicates; ** $P < 0.01$ and *** $P < 0.001$.