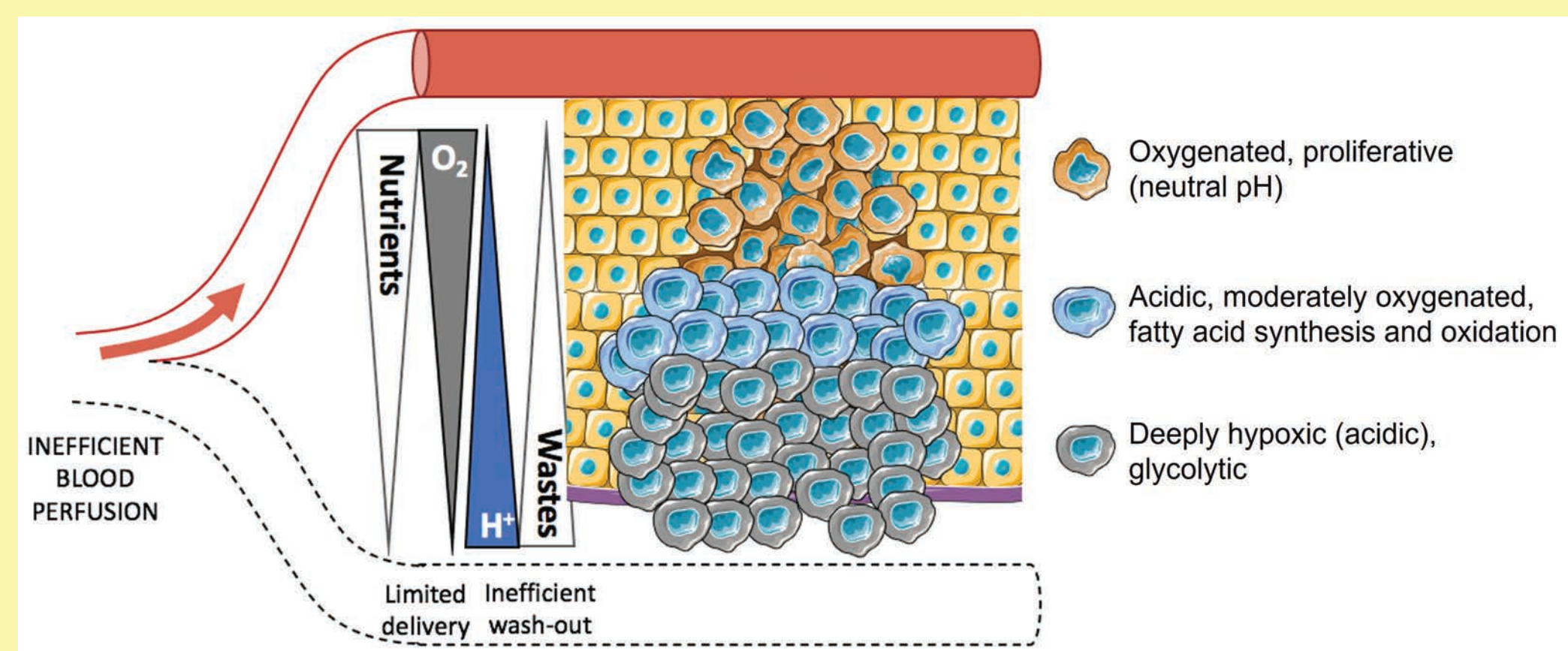


Deciphering the interplay between circadian rhythms and metabolic preferences in colorectal cancer cells and 3D spheroids

Léo Aubert, Kubra Ozkan, Sarah Sshr, Philippine Bruno, Céline Guilbaud, and Olivier Feron

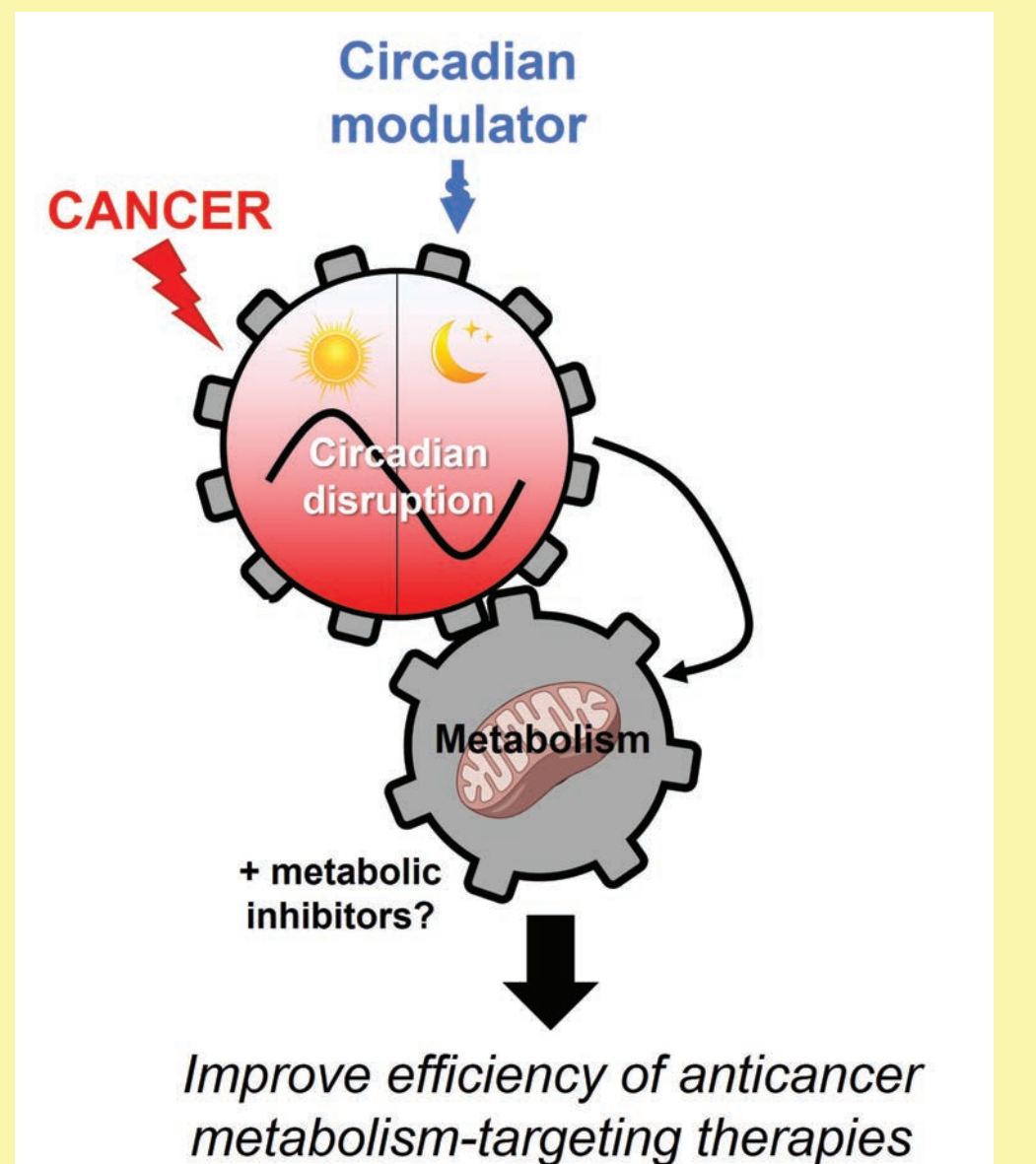
Cancer Translational Research Lab, Pole of Pharmacology and Therapeutics (FATH), Institute of Experimental and Clinical Research (IREC), University of Louvain, 57B Avenue Hippocrate, B-1200 Brussels, BELGIUM

Introduction

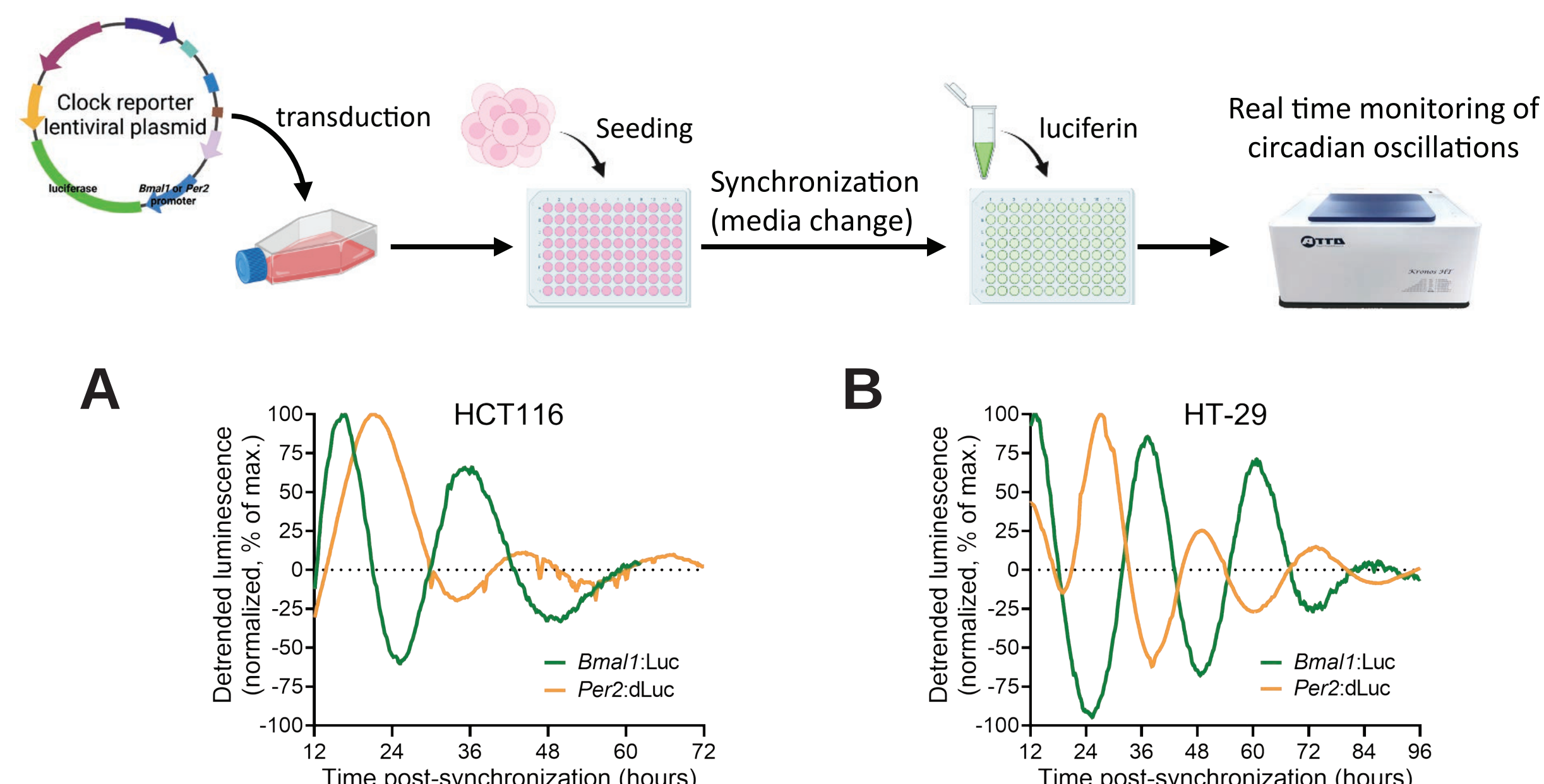


Background - While disruption of circadian rhythms has been associated with an increased risk of metabolic disorders, including cancer, much less is known about how alterations in clock machinery influence the progression of established tumors. **The interplay between circadian rhythms and metabolism makes this question highly relevant considering the growing interest for tumor bioenergetics.** We have previously documented that tumor acidosis shifts cancer cell metabolic preference from glucose to lipids, and that this rewiring significantly contributes to cancer progression (Corbet et al., Cell Metabolism 2016; Nature Comm. 2020). Recapitulating this intermediate cell compartment consisting of acidic, non-hypoxic cells in 3D tumor spheroids offers an attractive model to study the regulation of fatty acid metabolism in tumors and optimize therapeutic strategies.

Aim of the study - To examine whether tackling the circadian clock machinery may impact on cancer metabolism and progression

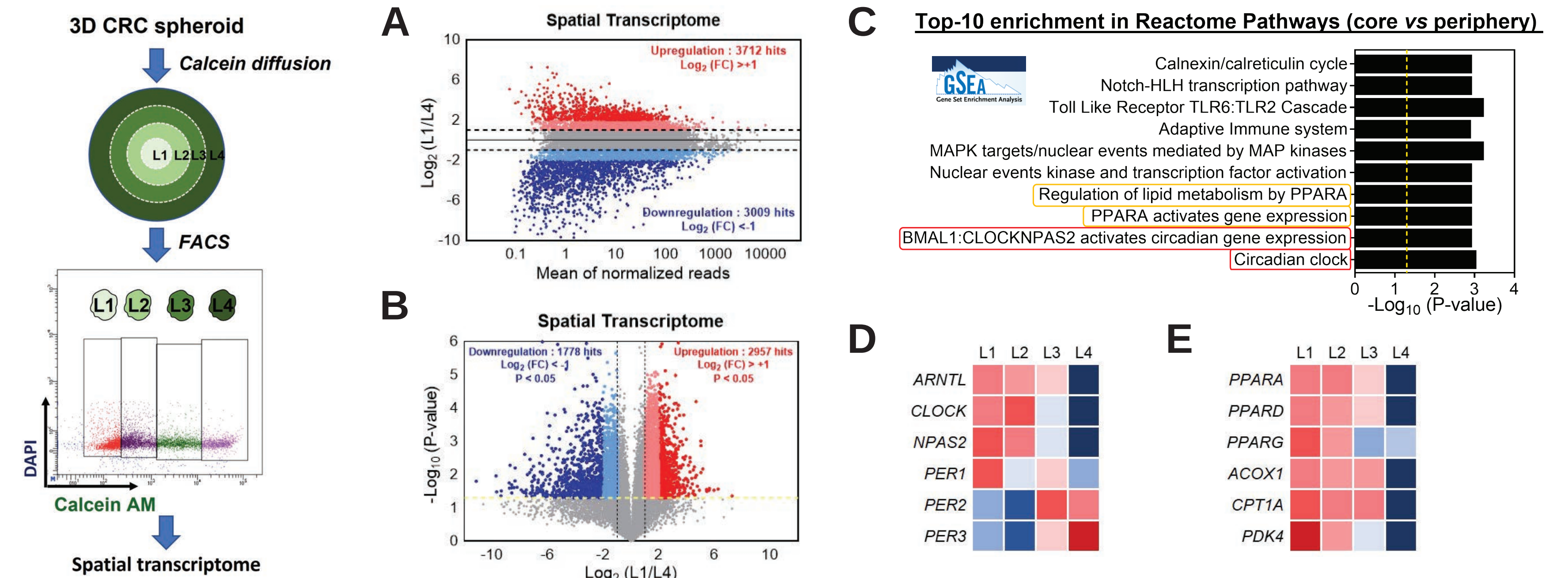


Colorectal cancer cells exhibit circadian oscillations



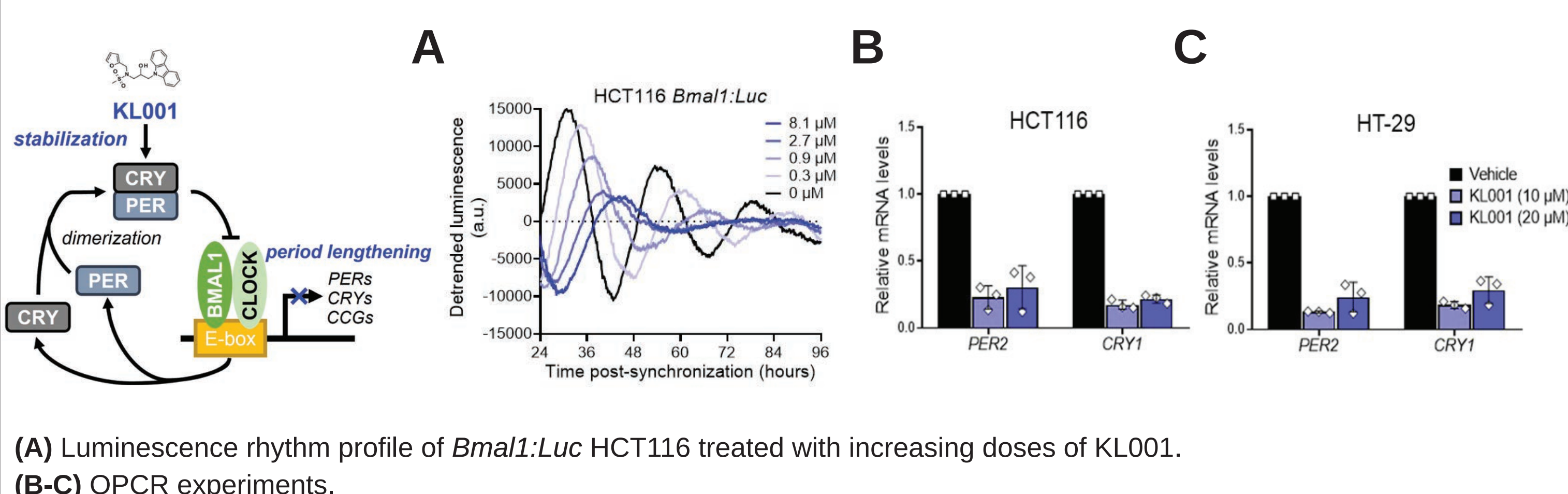
(A-B) Luminescence rhythm profiles of *Bmal1:Luc* versus *Per2:dluc* HCT116 (B) or HT-29 (C) CRC cells were monitored using KRONOS HT system. Data were detrended and normalized to the maximum of luminescence.

Circadian clock is altered in the core of 3D CRC spheroids



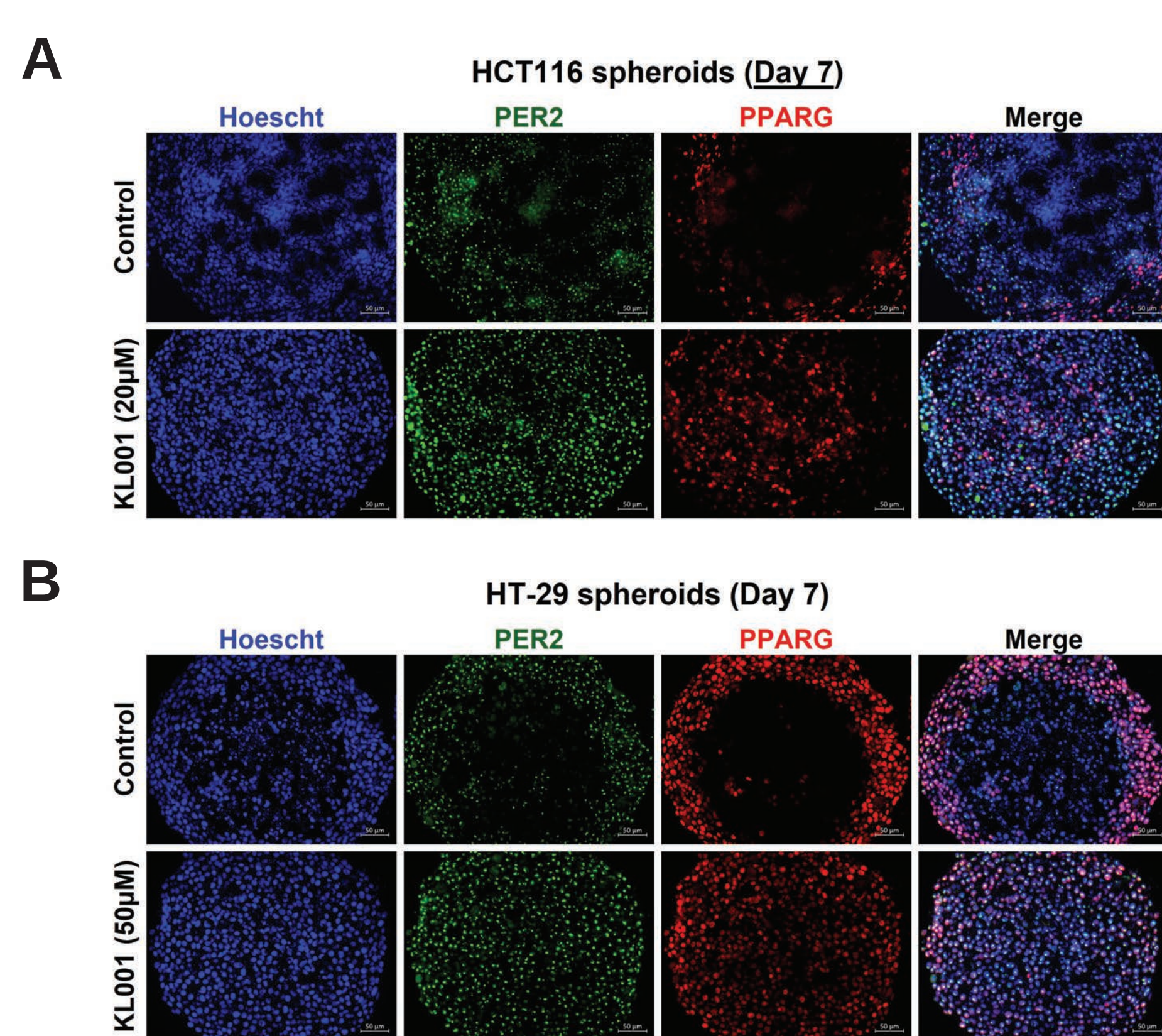
(A) MA plot and (B) Volcano plot depicting the fold-changes of significant DEGs between the core (L1) and the periphery (L4) from CRC 3D spheroid. (C-E) Parametric Gene Set Enrichment Analysis (PGSEA) (C) and z-score heat-maps showing the relative expression of several clock (D) and PPAR (E) genes across the different layers of 3D CRC spheroid.

KL001 modulates circadian oscillations in CRC cells

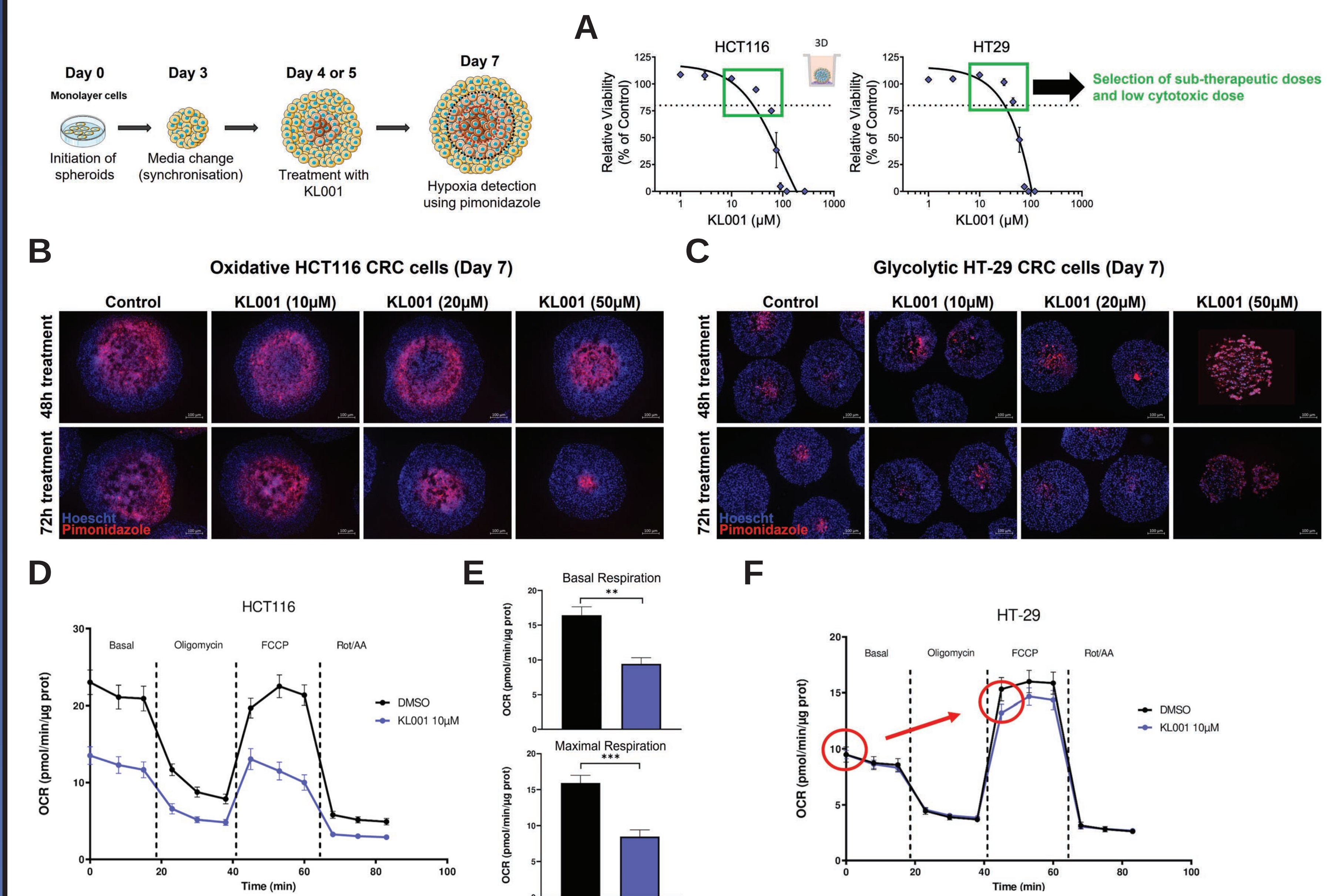


(A) Luminescence rhythm profile of *Bmal1:Luc* HCT116 treated with increasing doses of KL001. (B-C) QPCR experiments.

KL001 redistributes PER2 and PPARG expression in 3D CRC

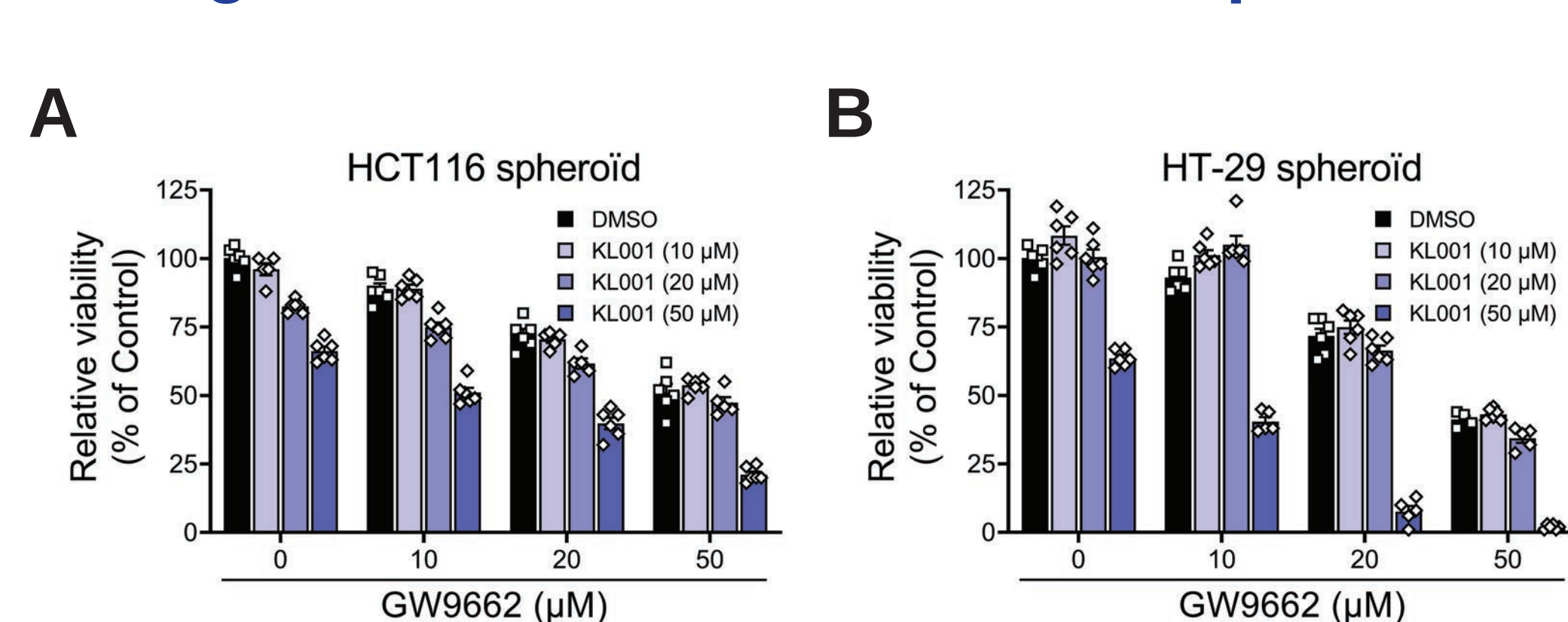


KL001 dose-dependently inhibits the growth of 3D CRC spheroids and reduces the extent of hypoxia through a direct inhibition of cell respiration



(A) 3D-CellTiterGlow measurements. (B-C) Pimonidazole staining of hypoxic spheroid areas. (D-F) Seahorse measurements of O₂ consumption rate (OCR).

Combining KL001 with PPARG antagonist shows more pronounced growth inhibition in 3D CRC spheroids



(A-B) 3D-CellTiterGlow assay measurements.

Conclusion

Altogether, our data shed a new light on the tumor expression of clock genes and the possibility to impact cancer cell growth and lipid metabolism by pharmacologically modulating the activity of major regulators of circadian rhythms.