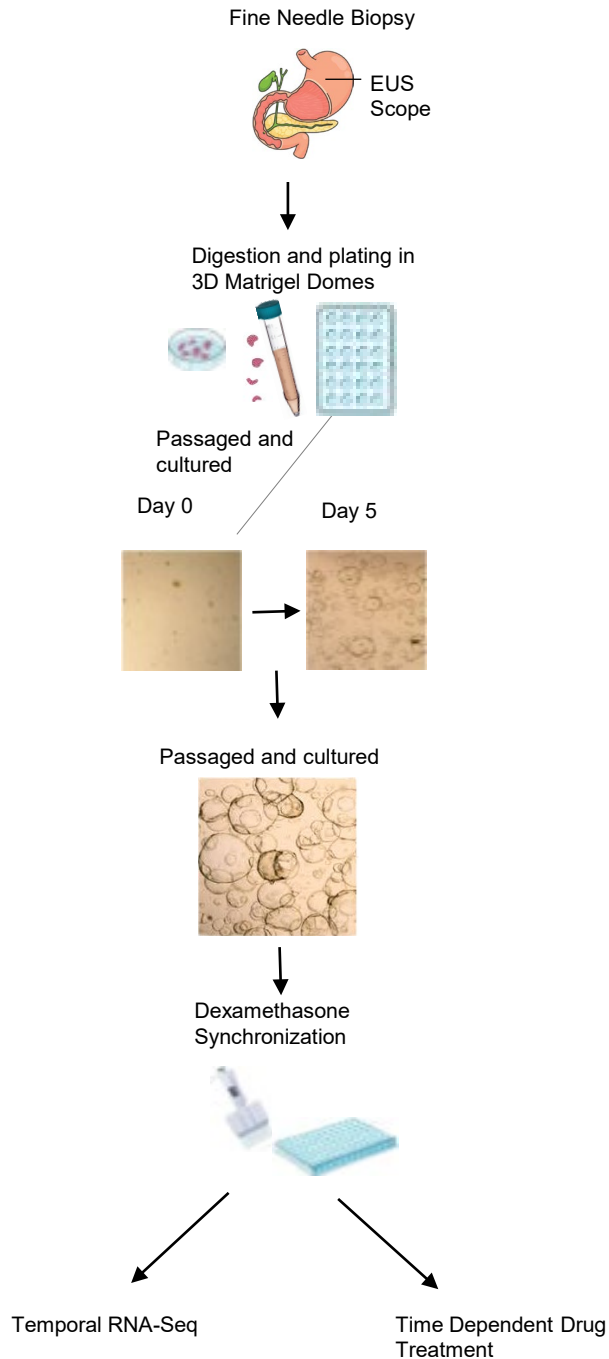
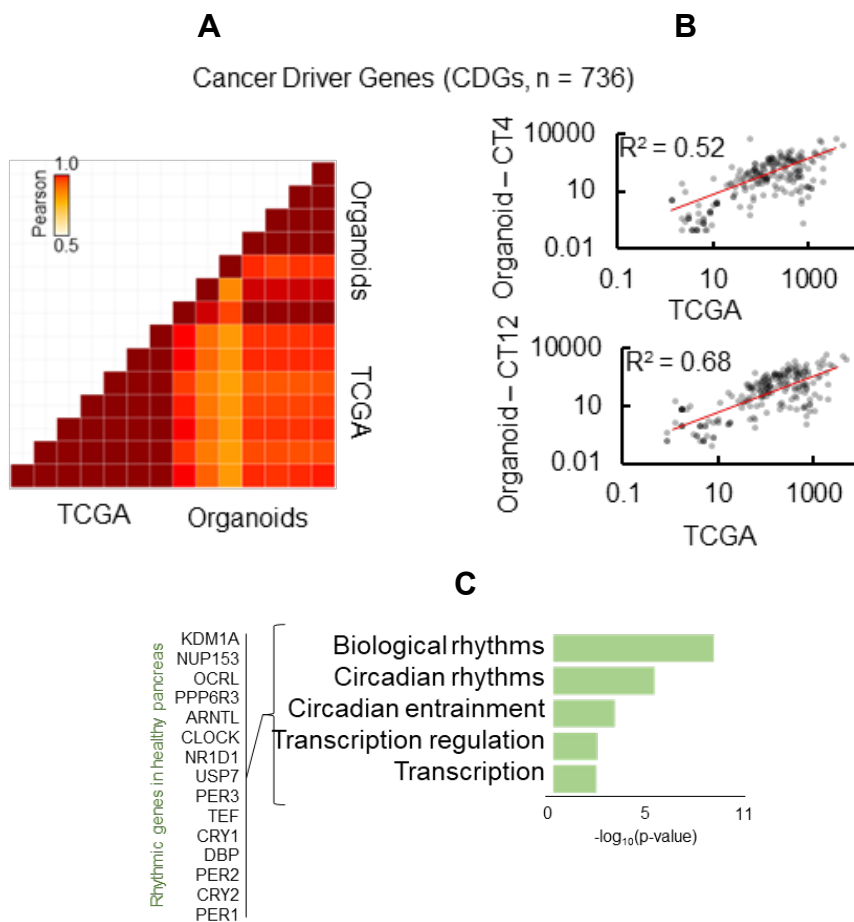


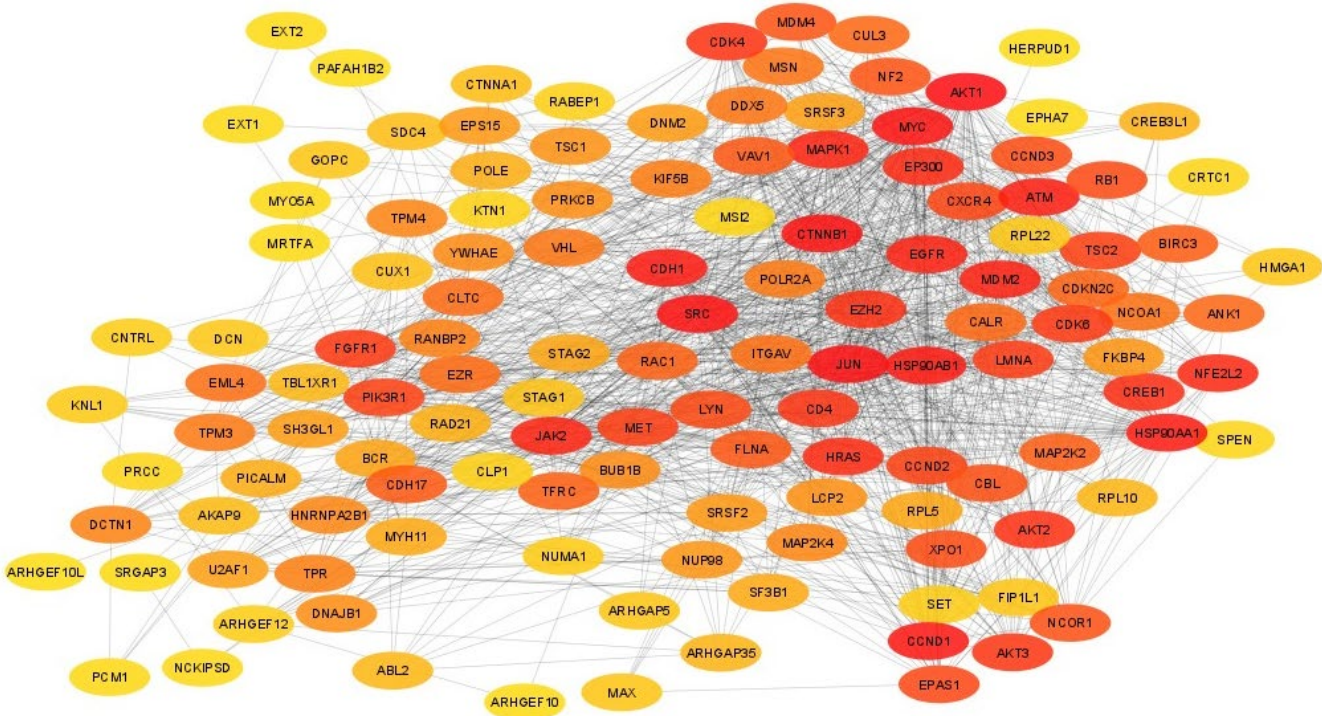


Supplementary Figure 1: Workflow describing the generation, characterization, and multiplex-drug screening assay of PDOs from EUS FNA biopsies. Temporal RNA-Seq and timed drug treatments were carried out in parallel to identify chronotherapeutic targets.



Supplementary Figure 2: Validation of pancreatic derived organoid (PDOs) gene expression pattern. A) Comparison of normalized read counts (TPMs) from PDOs and selected TCGA datasets (n = 7). Note the high pairwise correlation among all the samples. B) Scatter plot of normalized read counts (TPMs) from organoids at specific time points and TCGA datasets (average TPM from n = 7). C) (Left) List of 15 genes that were rhythmic in both PDOs and healthy pancreas. (Right) Pathway enrichment of genes that were rhythmic in both PDOs and healthy pancreas.

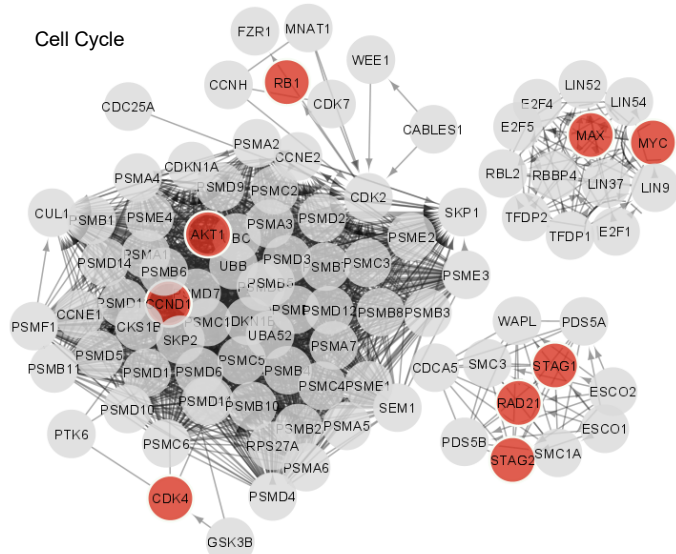
A



Cancer driver genes ($p: 8 \times 10^{-12}$)

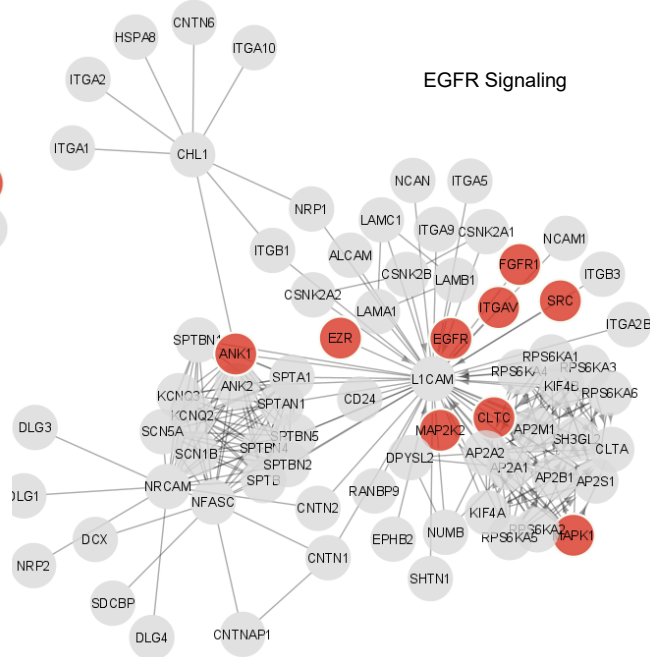
B

Cell Cycle

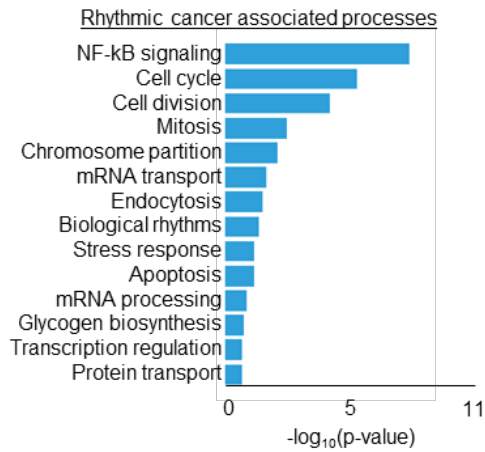


C

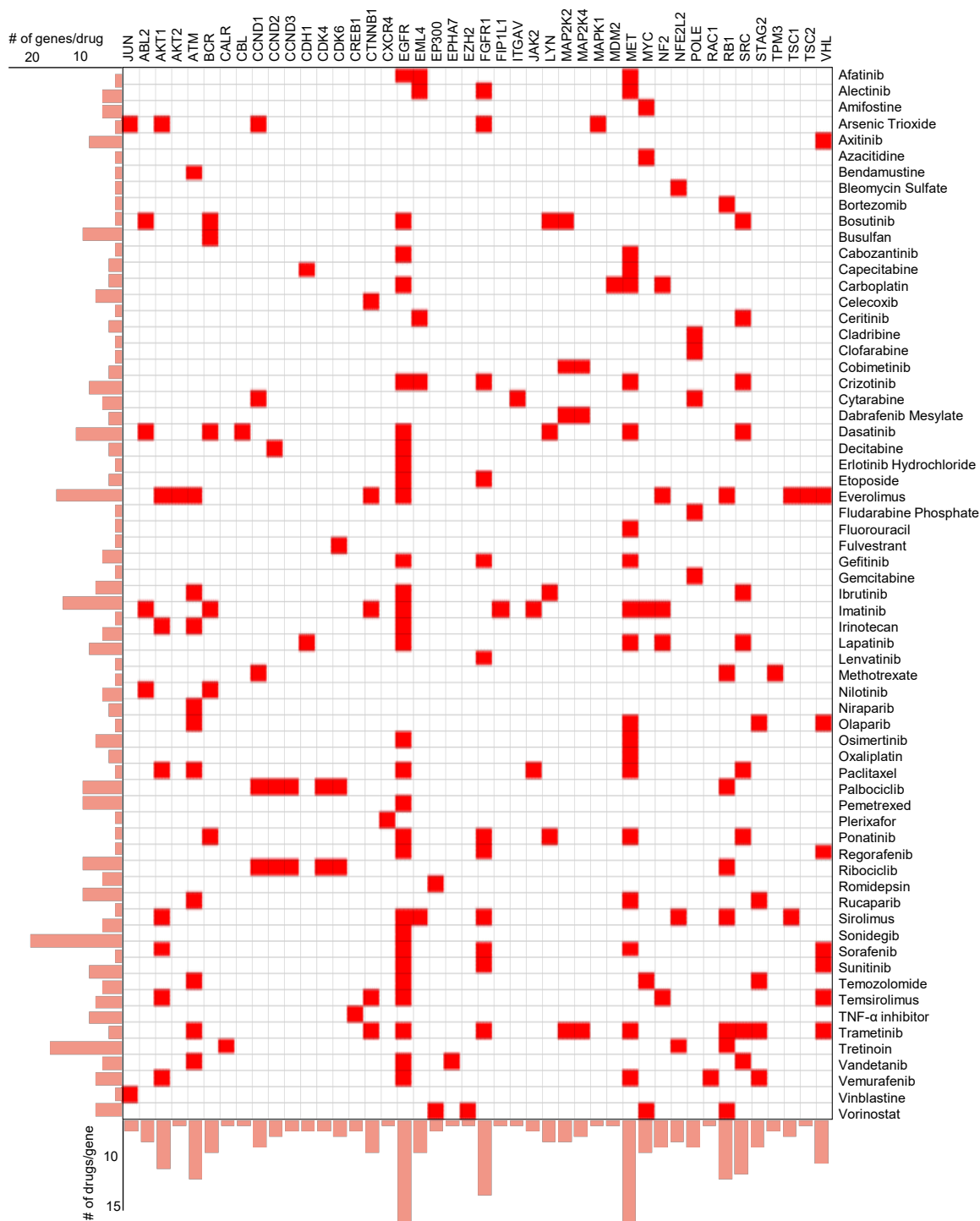
EGFR Signaling



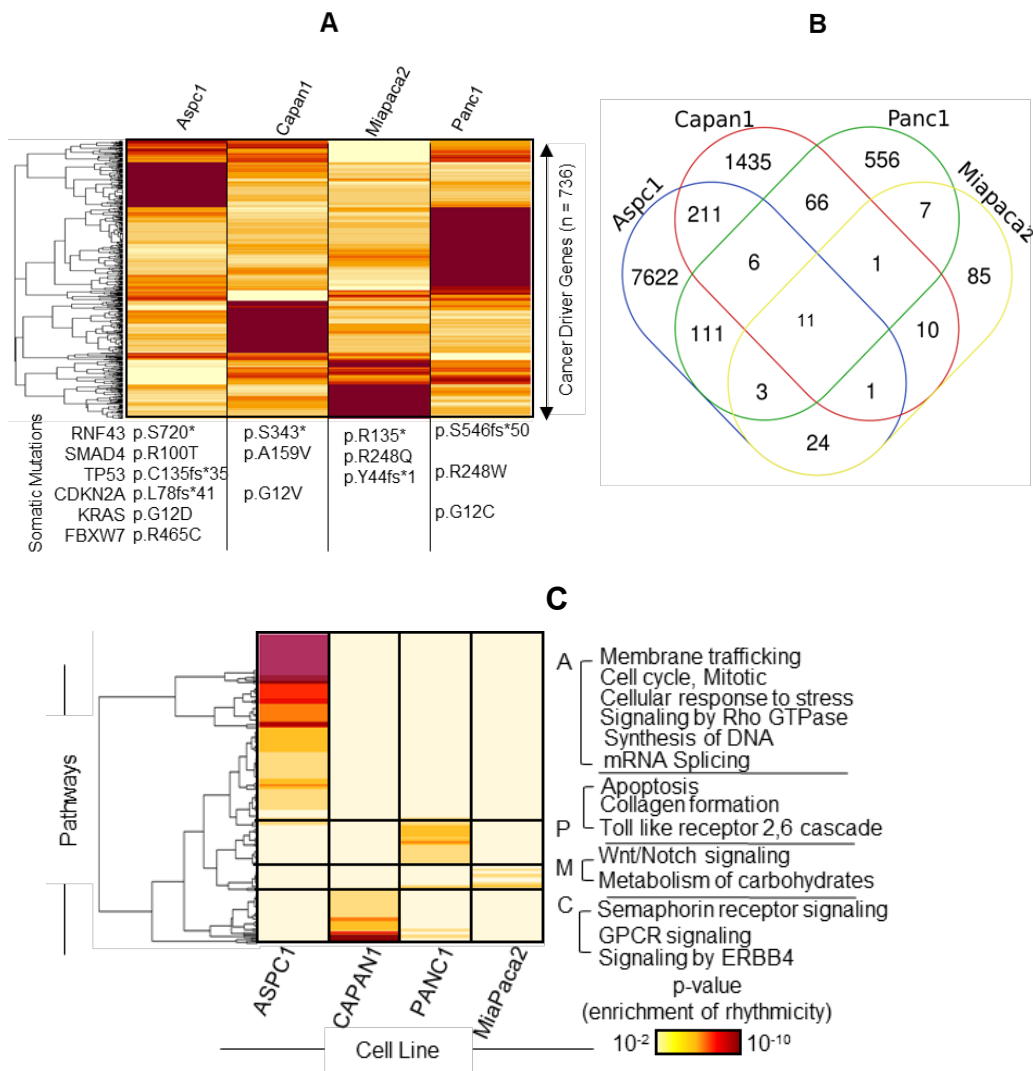
Supplementary Figure 3: A) Interaction network of rhythmic cancer driver genes based on the network connectivity parameter (the maximum clique centrality or MCC). Node color corresponds to MCC value, with red being the highest. B) Network of cell cycle with rhythmic cancer driver genes red color coded. C) Network of EGFR signaling with rhythmic cancer driver genes red color coded.



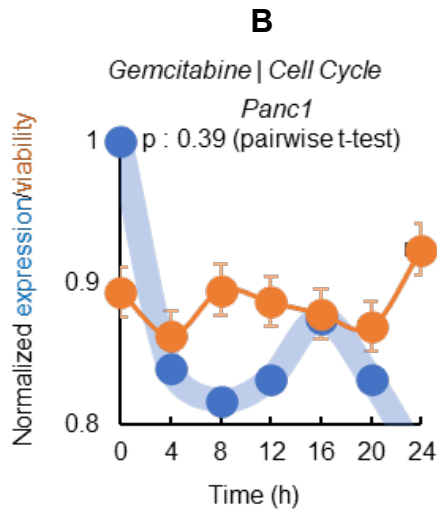
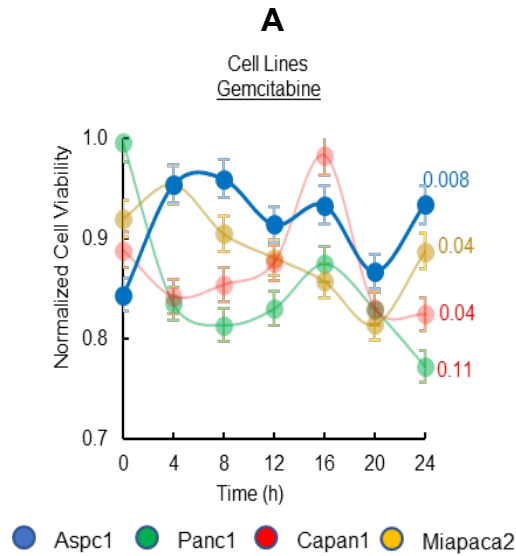
Supplementary Figure 4: Most enriched biological pathways for rhythmic cancer driver genes (r-CDGs, n = 127) in PDOs.



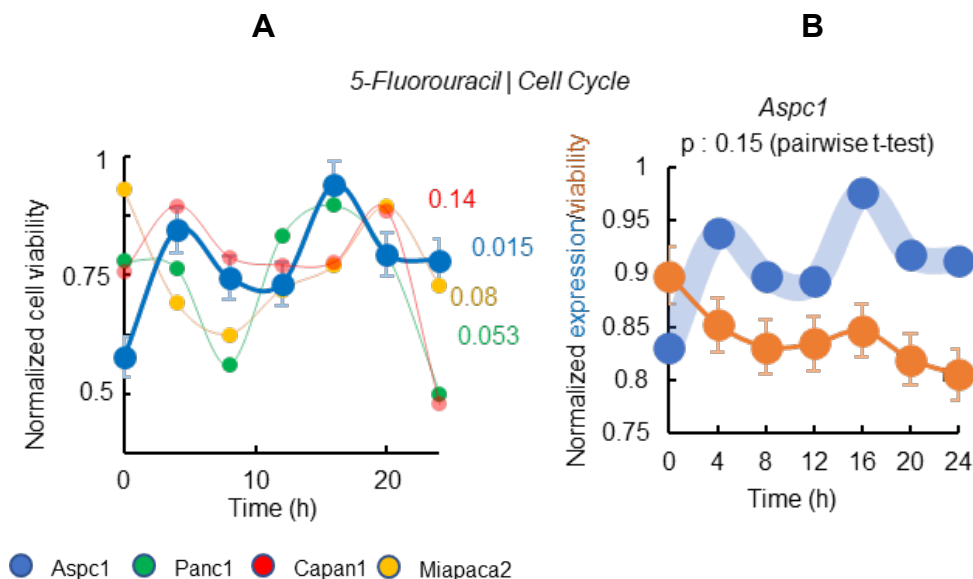
Supplementary Figure 6: Known FDA-approved anticancer drugs against top rhythmic cancer driver genes (r-CDGs) that could be potential chronotherapy targets. Bars represent number of drugs known for each r-CDG. Top drugs in each category are labeled on top of the bars.



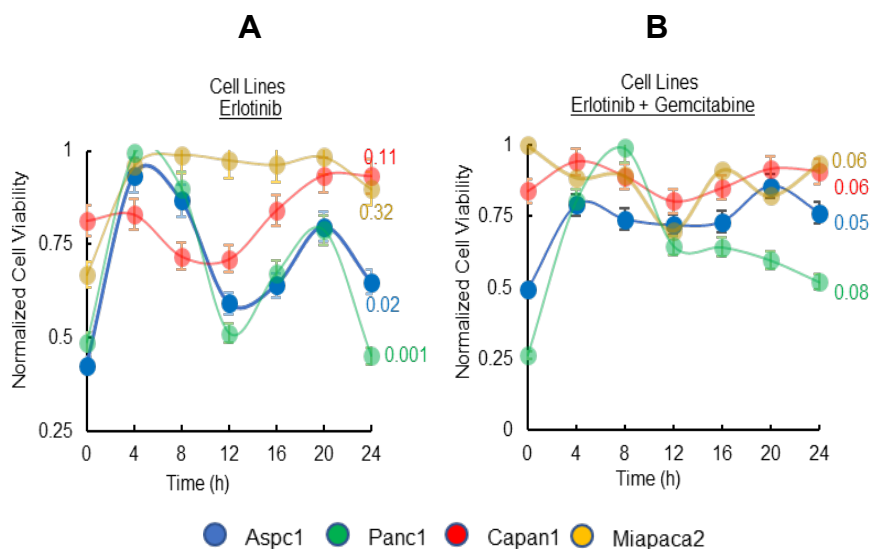
Supplementary Figure 7: B) Molecular signatures for the PDA cell lines used in this study, reflecting the two most common PDA molecular subtypes: squamous (PANC1 and MIAPACA2) and progenitor (PANC1 and MIAPACA2) subtypes (71, 72). Heatmap describes the normalized expression levels (TPM) obtained from cell model passports database. Genes below the heatmap reflect cancer driver genes (CDGs) with cell line specific somatic mutations. ASPC1 and CAPAN1 closely resemble the progenitor subtype, while mimics the squamous subtypes B) Venn diagram showing rhythmic genes in each cell line. C) Enrichment of rhythmic genes in biological pathways in each cell line.



Supplementary Figure 8: A) Temporal drug efficacies for Gemcitabine in 4 cell lines. Colors represent the cell lines. Y-axis is the normalized cell viability for each line. Each datapoint represents an average of 3 replicates. B) Comparison of the amplitude of composite gene expression of cell cycle hub genes and gemcitabine efficacy in PANC1 cell line. p-value corresponds to the significance of the effect of time of therapy on viability of each cell line. Error bars represent standard deviation from 3 independent experiments.



Supplementary Figure 9: Temporal drug efficacies of PDA drugs. A) B) Temporal changes in the normalized cell viability of 5-Fluorouracil (5-FU) in PDA cell lines. C) Comparison of the amplitude of composite gene expression of cell cycle hub genes and 5-FU efficacy in ASPC1 cell line. Colors represent the cell lines. Y-axis is the normalized cell viability for each line. Each datapoint represents an average of 3 replicates. p-value corresponds to the significance of the effect of time of therapy on viability of each cell line. Error bars represent standard deviation from 3 independent experiments.



Supplementary Figure 10: Temporal drug efficacies for A) Erlotinib and B) Erlotinib + Gemcitabine in 4 cell lines. Colors represent the cell lines. Y-axis is the normalized cell viability for each line. Each datapoint represents an average of 3 replicates. p-value corresponds to the significance of the effect of time of therapy on viability of each cell line. Error bars represent standard deviation from 3 independent experiments.