

Expanded View Figures

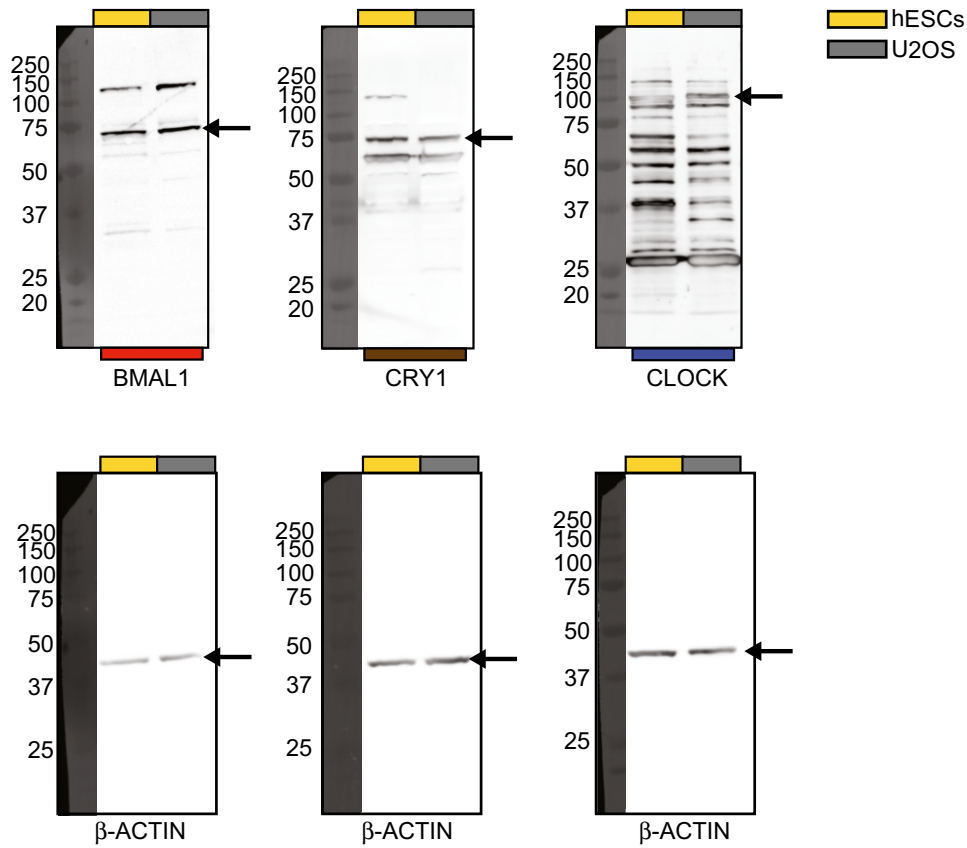


Figure EV1. Protein levels of clock genes in human ES cells versus U2OS cells.

Original Western blots for BMAL1, CRY1, and CLOCK protein.

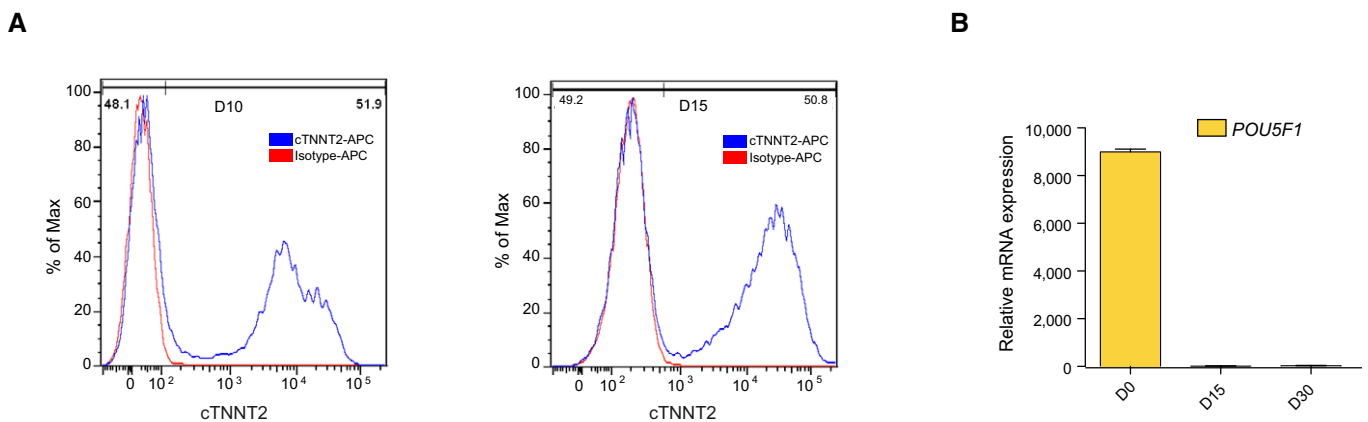


Figure EV2. Cardiomyocyte quantification and characterization by FACS and qRT-PCR.

A FACS staining for cTNNT2 in D10 and D15 human ES cell-derived cardiac cells. IgG₁ isotype was used as a control.

B *POU5F1* mRNA expression levels in human ES cells and D15 as well as D30 cardiac cells, as measured by qRT-PCR. Expression levels were normalized to a non-oscillatory housekeeping gene (*PPA*). Data are represented as mean ± s.e.m. of three independent replicates.

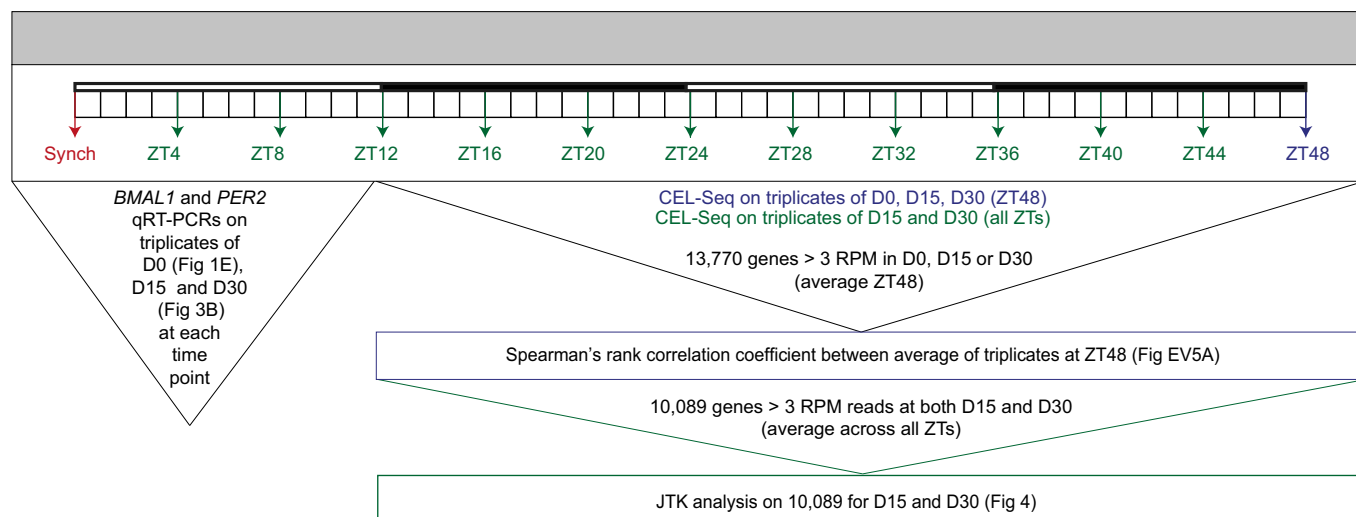


Figure EV3. Setup of CEL-Seq experiment.

Schematic of RNA samples that were processed for qRT-PCR and/or CEL-Seq. ZT: Zeitgeber and RPM: reads per million.

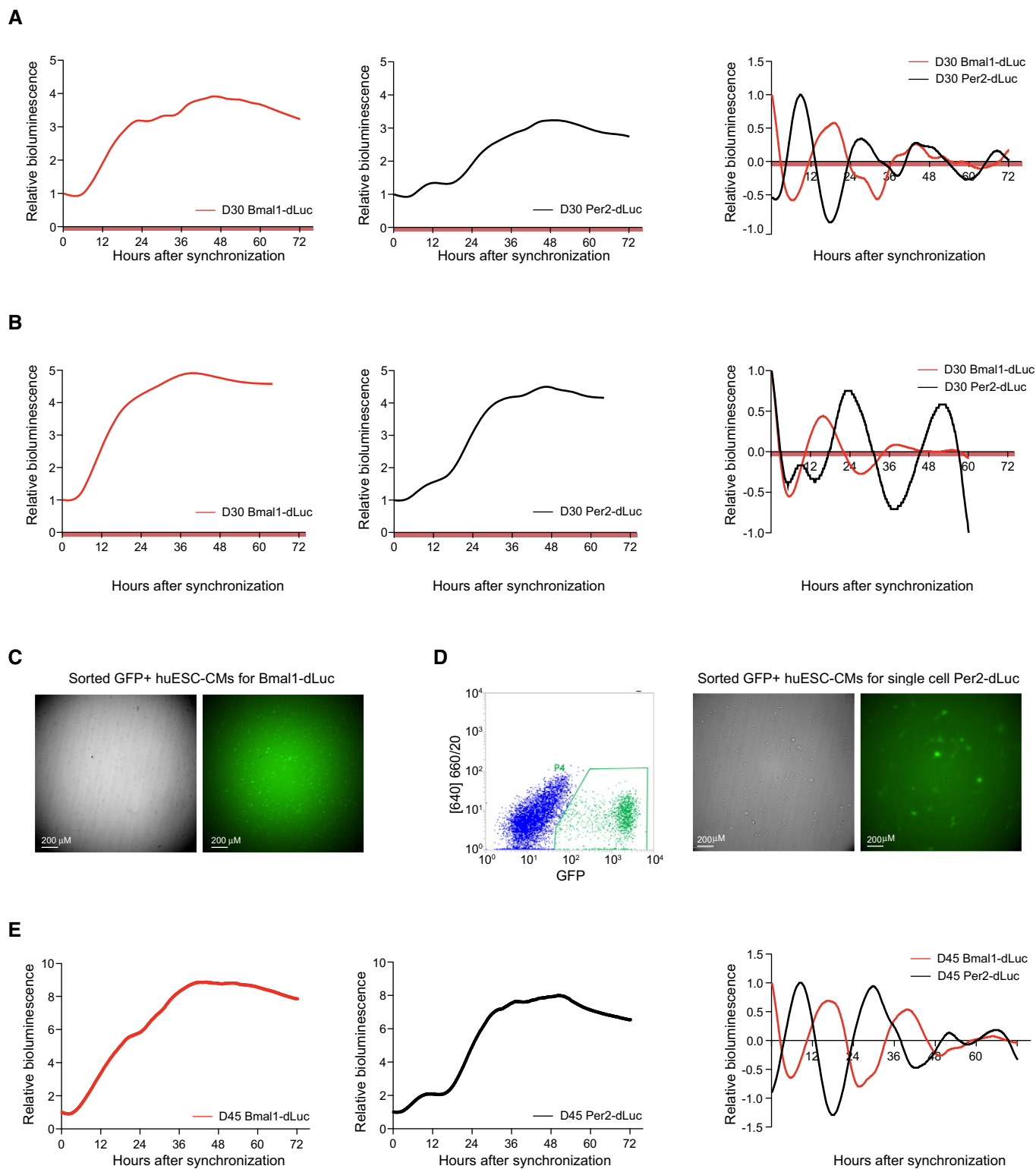


Figure EV4.

Figure EV4. Human ES cell-derived cardiomyocytes show circadian oscillation in Bmal1- and Per2-dLuc.

- A Promoter-based destabilized luciferase (dLuc) reporter assay of the Bmal1 (red) and Per2 (black) promoter in synchronized human ES cell-derived cardiac cells at D30 (replicate for Fig 3D). Values are relative to T0. Right: Detrended Bmal1-dLuc and Per2-dLuc Luciferase signal. Measurements were performed using a LumiCycle32.
- B Similar analysis as in (A) on an additional independent replicate.
- C Brightfield and fluorescent image of D30 GFP⁺ sorted human ES cell-derived cardiomyocytes used for analysis in Fig 3F (left/red).
- D FACS plot, brightfield, and fluorescent image of D30 GFP⁺ sorted human ES cell-derived cardiomyocytes used for single-cell bioluminescent analysis of Per2-dLuc signal in Fig 3F (right/black) and Fig 3G and H.
- E Similar analysis as in (A) for D45 human ES cell-derived cardiac cells.

Figure EV5. CEL-Seq-based characterization of transcriptome maturation and association between read coverage and oscillatory expression.

- A Spearman's rank correlation with average linkage based on average RPM-normalized CEL-Seq read counts of three ZT48 replicates. Genes ($n = 13,770$) with an average ZT48 expression level of more than 3 RPM (reads per million) at one stage (either D0, D15, or D30) were used for analysis. Spearman's rank correlation coefficients are indicated in white.
- B Heatmap of average ZT48 CEL-Seq read counts for 13,770 genes used in (A). Genes were sorted according to human ES cell expression from high (top) to low (bottom). Log₂ RPM values were plotted.
- C MYH7/MYH6 CEL-Seq read count ratio in D15 and D30 human ES cell-derived cardiac cultures (average of three ZT48 replicates) as a measure for maturation.
- D Heatmaps of average ZT48 CEL-Seq read counts in human ES cells (D0) and human ES cell-derived cardiac cells (D15 and D30). The left heatmap shows pluripotency markers, the two middle heatmaps depict cardiomyocyte and cardiomyocyte maturation markers, and the right panel comprises core circadian clock genes. Log₂ RPM values were plotted.
- E Boxplots depict average log₂ RPM-normalized CEL-Seq counts across all time points for D15 and D30 oscillatory and non-oscillatory genes. 10,089 genes with average RPM values above 3 across all time points (ZT4-ZT48) on which JTK analysis was done in both D15 and D30 were used. Bottom and top of the boxes are the first and third quartiles. The line within the boxes represents the median and whiskers denote the interval within 1.5× the interquartile range from the median. Outliers are plotted as dots.
- F Boxplots depict average log₂ RPM-normalized CEL-Seq counts across all time points (ZT4-ZT48) for D15-specific, D30-specific, and shared cardiac oscillators. Boxplot representation is as described in (E).

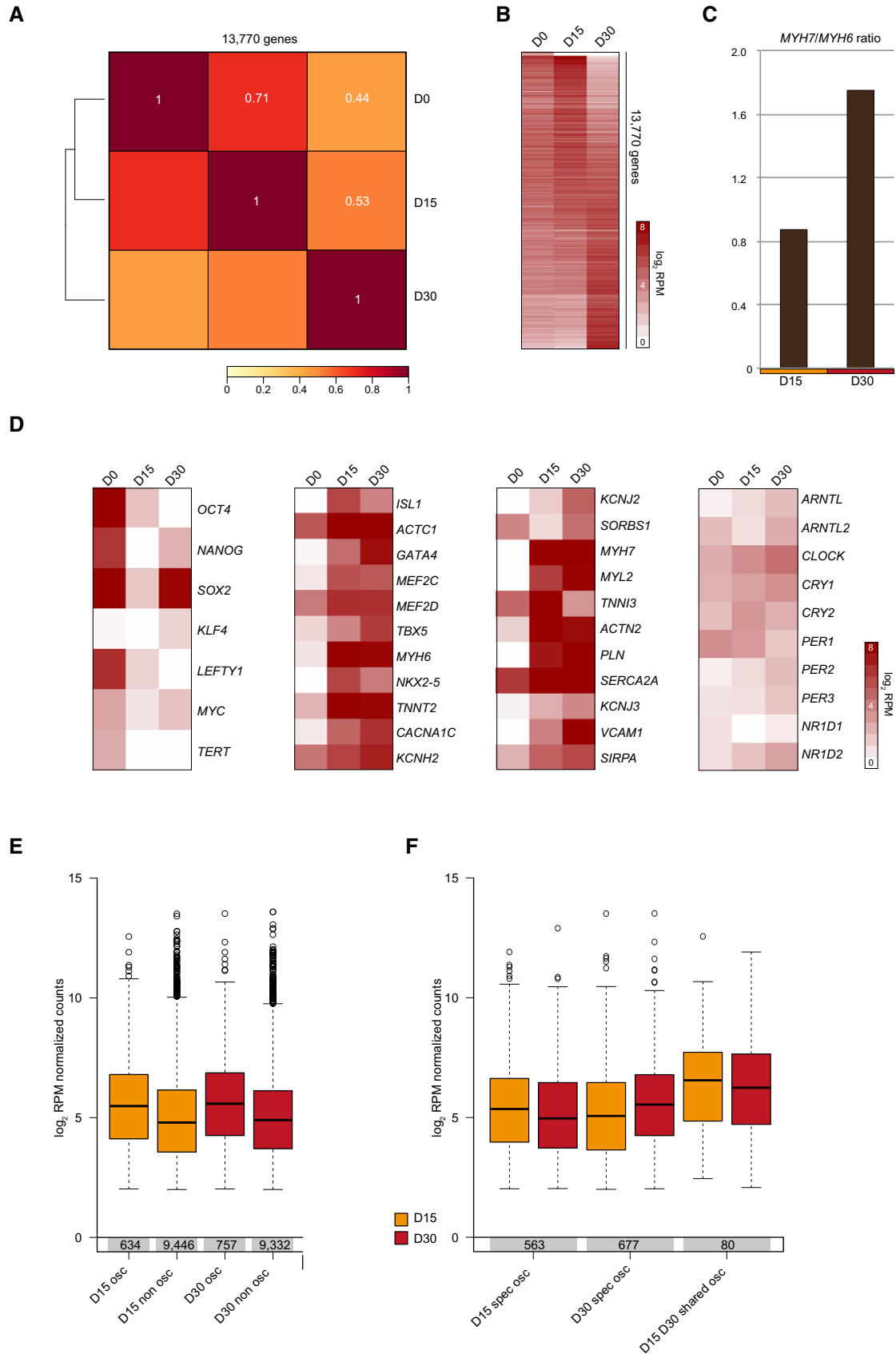


Figure EV5.