Description of Additional Supplementary Files

File Name: Supplementary Data 1
Description: Electrocardiogram parameters. ECG measurements, including QRS complex duration, PR interval and QRS and RR intervals (measured in milliseconds [ms]) from animals featured in Fig. 2e, 2i, 3a, and 3b. Corresponding p-values are provided for each measurement, determined by a two-tailed unpaired t test, reflecting the statistical significance of the data presented in the associated figures.

File Name: Supplementary Data 2
Description: Differentially expressed genes (DEGs) from bulk RNA-seq analysis. Genes differentially expressed in 21-day-old Dhx36Myh6 mouse hearts compared to WT littermates (refer to Supplementary Fig. 3). Differential expression was assessed using the Wilcoxon test, with p-values adjusted by Bonferroni correction. The table reports the average Log2 fold changes for the DEGs.

File Name: Supplementary Data 3
Description: List of Genes from snRNA-seq data identifying cell clusters in PD7 hearts, with a comparison to bulk RNA-seq data. The table highlights the most commonly expressed genes that characterize different cells clusters in PD7 hearts (see Supplementary Fig. 3), with a focus on genes specific to various cardiomyocyte clusters (Fig. 6). The table also includes a comparison of genes that are either commonly or uniquely deregulated between the snRNA-seq and bulk RNA-seq datasets.

File Name: Supplementary Data 4
Description: Differentially expressed genes between KO and WT cardiomyocytes (CM) from snRNA-seq data. The table list genes that are downregulated (blue) and upregulated (orange) in cKO CMs compared to WT, along with the percentage of CMs expressing each gene (pct.1 for WT and pct.2 for KO). Additionally, genes upregulated in the ventricular conduction system CMs(CM6,see Fig. 5 6) relative to the CM1 cluster are reported with their average expression levels (green). Differential expression was calculated using the Wilcoxon test, with p-values adjusted by Bonferroni correction. Average Log2 fold changes are also provided.

File Name: Supplementary Data 5
Description: Differentially accessible peaks (DAPs) between WT and cKO cardiomyocytes. The table lists chromosomic regions with open chromatin, along with the percentage of WT (pct.1) and cKO (pct.2) CMs exhibiting these open regions. It also includes a comparison of accessibility between WT and cKO, reported as average log2 fold changes (avg_log2FC). Adjusted p-values, using the Bonferroni correction are provided, obtained by a model from a logistic regression (LR). We show the statistically significant regions highlighted in bold. For a detailed description of the bioinformatics analysis, please refer to the Methods section.

File Name: Supplementary Data 6
Description: Intersection of DEGs with DAPs. This table lists the intersection of DEGs between WT and KO CMs with genes associated with differentially accessible chromatin regions, as identified in Supplementary table 5 are. The Statistical analysis are described in the Legends of

Supplementary tables 4 and 5.

File Name: Supplementary Data 7
Description: Transcription factor enrichment motifs in cardiomyocytes. The table presents the 25 motifs that are specifically enriched in CMs compared to other cardiac cell types, as well as the 11 motifs of transcription factors that are underrepresented in KO cardiomyocytes compared to WT. The enriched motifs p values were obtained from a hypergeometric test, to test the probability of observing the motif at the given frequency by chance, comparing with a background set of peaks matched for GC content, and using a Benjamini & Hochberg correction. Motif analysis was conducted 6 using DNA sequence motif information from the JASPAR datasets. For a detailed analysis, please refer to the Methods section. Supplementary table 8. DAPs linked to DEGs containing G-quadruplexes (G4s). List of chromatin regions with putative G4s (indicated as TRUE or FALSE) and their association with DAPs that are linked (TRUE) or not linked (FALSE) to DEGs in the snATAC-seq data. A total of 33 DAPs are linked to genes containing TRUE G4s. No Statistical analysis was involved in this table.

File Name: Supplementary Data 9
Description: G4 enrichment analysis in DAPs linked to DEGs. This table presents the results of G4 enrichment analysis, demonstrating a significant association between G4s and DAPs between WT and KO cardiomyocytes (I). The table also includes lists and the sequences of G4s overlapping DAPs (II) and the links between peaks and genes (III). For G4 enrichment: p-value obtained from a hypergeometric test. p-values for links between peaks and genes were obtained by correlation between gene expression and accessibility.

File Name: Supplementary Data 10
Description: Gene regulation by G4s in KO cardiomyocytes. This table categorizes genes based on their regulation by G4s. Genes identified as deregulated in KO cardiomyocytes have been organized into five distinct groups. These groups were defined based on their regulation status and functional relevance, as detailed in the Results section. No Statistical analysis was involved in this table. 7

File Name: Supplementary Data 11
Description: Primer Sequences for qPCR and Promoter cloning. This table lists the nucleotide sequences of oligonucleotide primers employed for qPCR and promoter cloning experiments in this study.