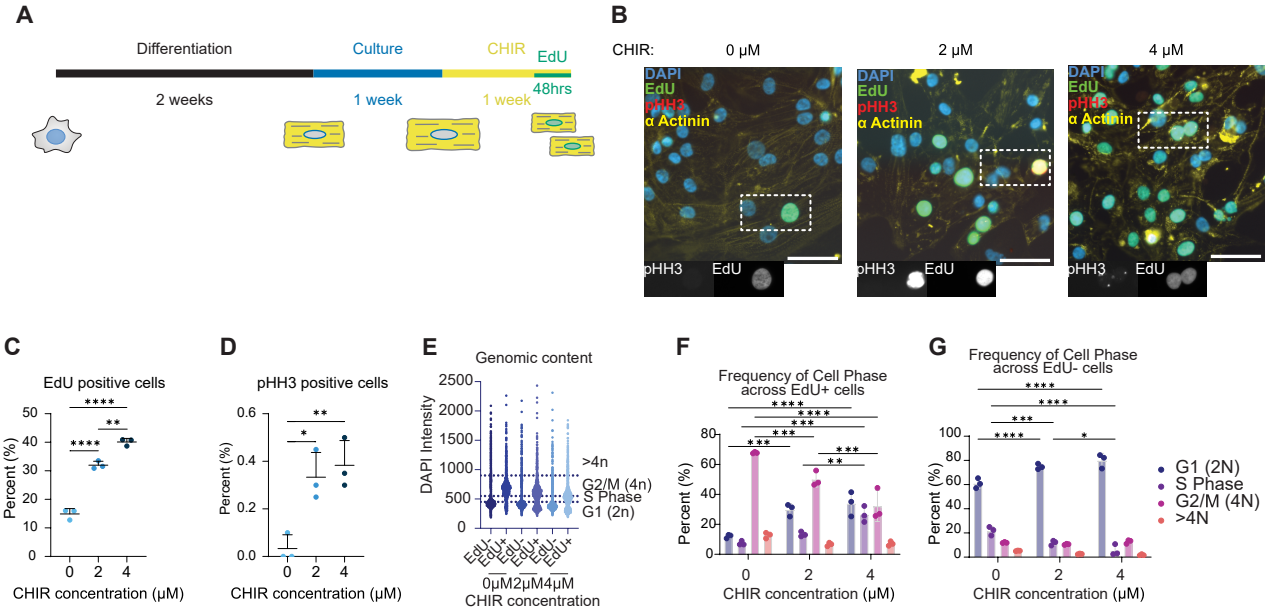


Fig. S1 WNT activation promotes immature phenotypes in cultured iPSC-CMs.
A) The experimental timeline. **B**) Representative image of the iPSC-CMs: DAPI (blue), pHH3 (red), EdU (green), and α Actinin (yellow). Scale bar=50 μM. **C-D**) Frequency of proliferation markers, EdU (**C**) and pHH3 (**D**). **E-G**) Distribution of genomic content (**E**) and cell cycle stage across EdU+ (**F**) and EdU- (**G**). A one-way ANOVA with Tukey's post hoc test was used to compare the percentages of EdU+ and pHH3+ cells. A two-way ANOVA with Tukey's post hoc test was used for statistical analysis was used for **F-G**. (*Padj<0.05, **Padj<0.01, ***Padj<0.001, ****Padj<0.0001). **See also Supplemental Table S1**.

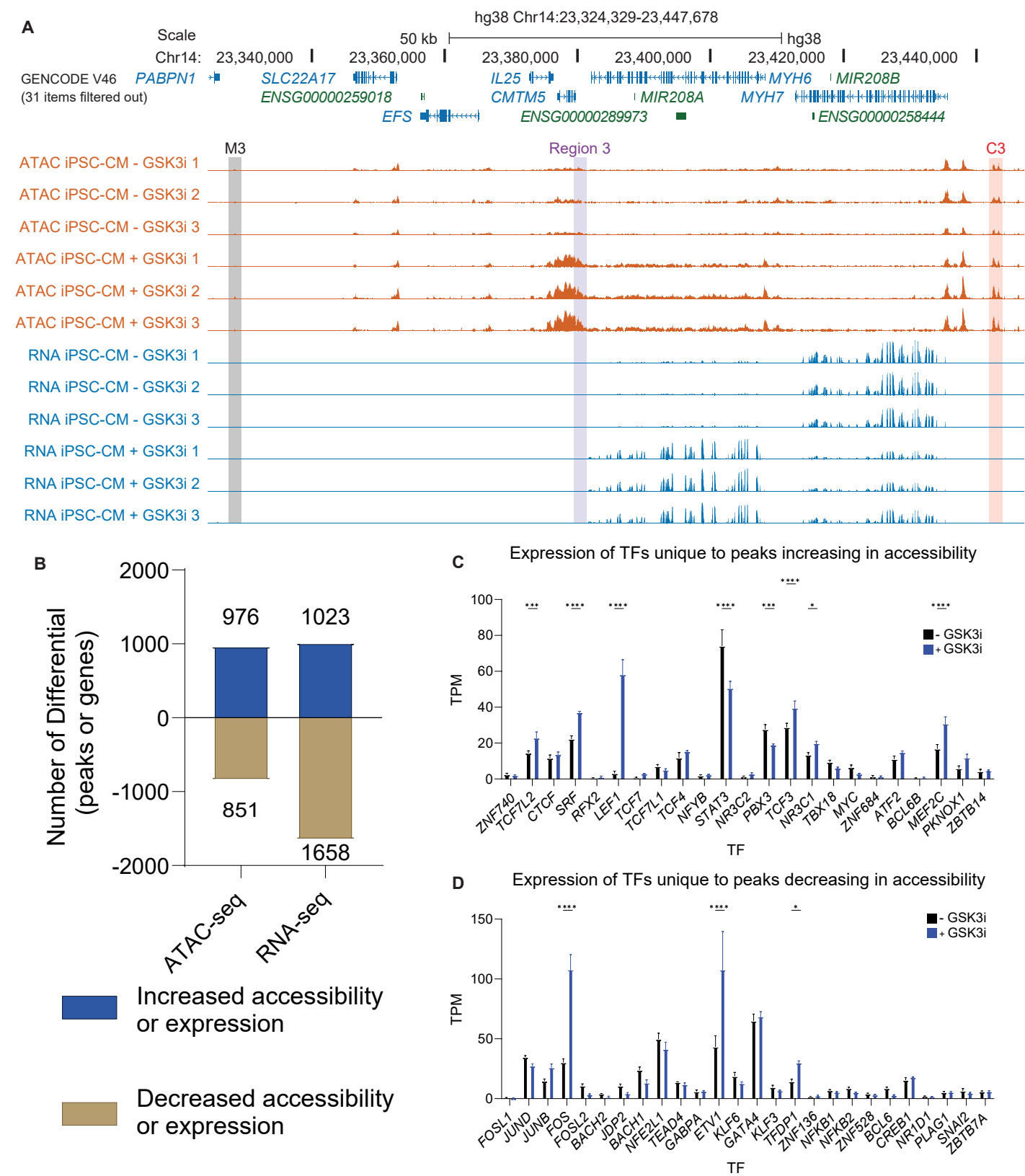


Fig. S2 GSK3 inhibition reproducibly alters gene expression and chromatin accessibility

A) Visualization of ATAC-seq (orange) and RNA-seq (blue) profiles surrounding the *MYH6* locus. GSK3 inhibition leads to a reproducible increase in chromatin accessibility overlapping the cCRE region 3' of the *MYH6* gene body. Additionally, there is a consistent shift in gene expression from *MYH7* to *MYH6* following GSK3i treatment. **B**) Number of differentially accessible ATAC peaks and differentially expressed genes following GSK3 inhibition. **C-D**) TPM of transcription factors with motifs unique to ATAC peaks, showing increased (**C**) or decreased (**D**) accessibility. Only motifs with an FDR < 0.1 were considered. See also **Fig. 1**.

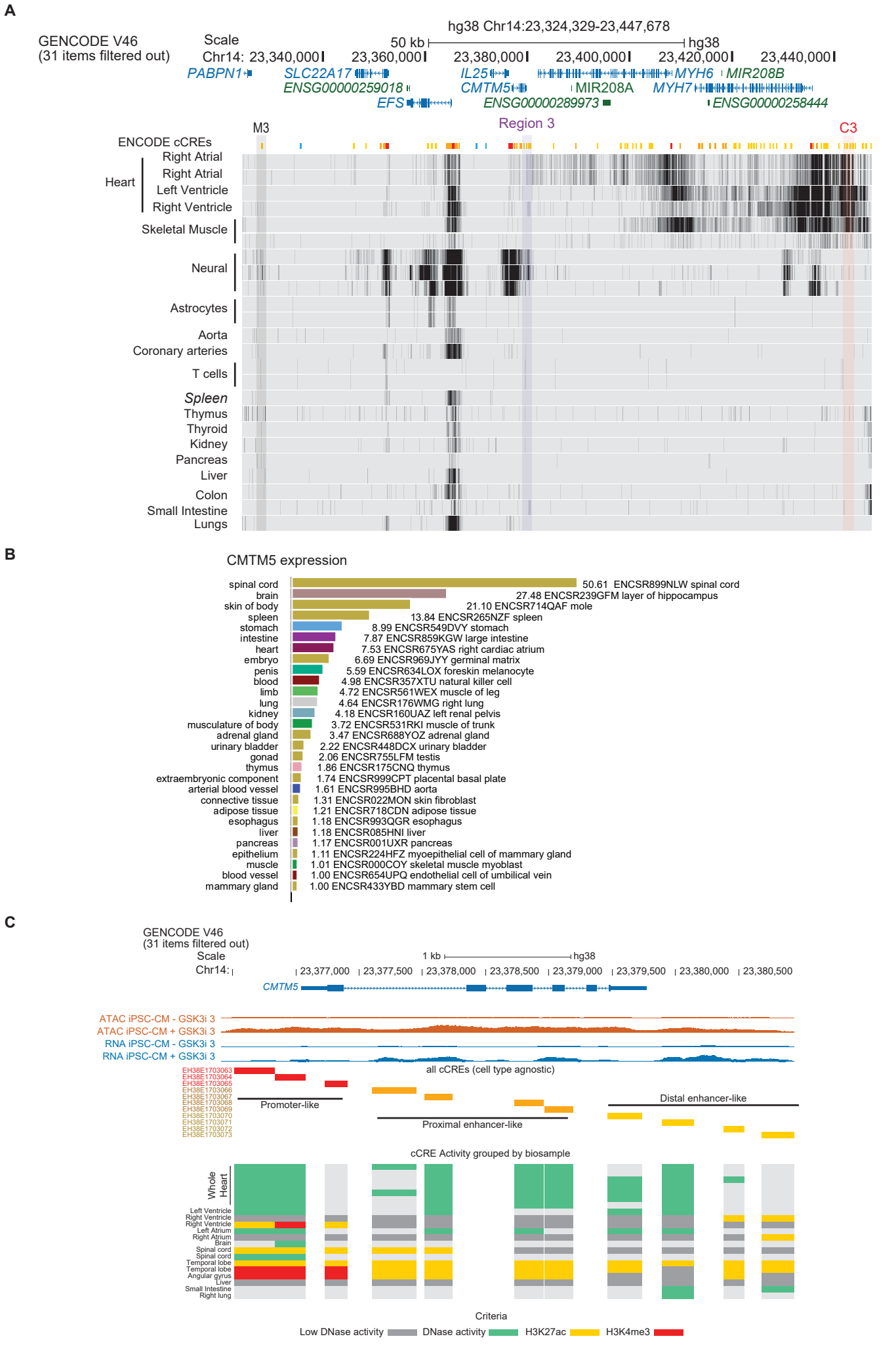


Fig. S3 ENCODE cCRE activity across assorted human tissue types

A) Visualization of ENCODE H3K27ac data around the *MYH6* locus in various human tissues. **B)** Expression levels of *CMTM5* across ENCODE tissue and primary cell samples, shown as TPM + 0.01. **C**) Visualization of ENCODE data indicating cCRE (enhancer) activity across selected tissue types. The first four rows show tracks generated from our ATAC-seq (orange) and RNA-seq (blue) data. Following these, ENCODE cCREs overlapping Regions R1-R3 are displayed with their classifications. These enhancers overlap RNA-seq signals in our iPSC-CM, which increase after GSK3i treatment. Lastly, the summary of cCRE activity supporting cCRE activity across various tissue types. See also Fig. 3

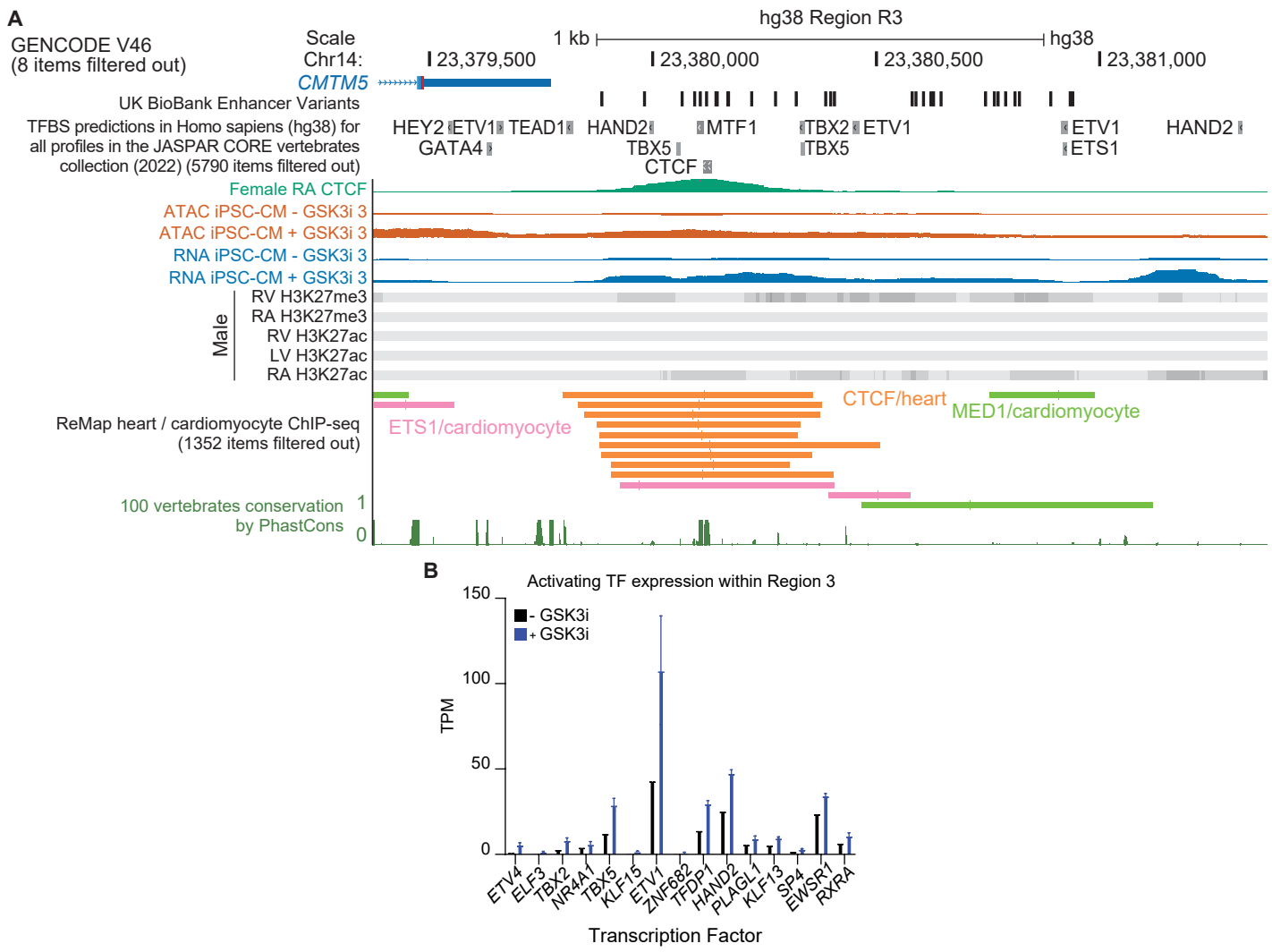


Fig. S4 Epigenetic landscape myosin enhancer R3, C3, and M3 in the human heart

A) Visualization of epigenetic datasets surrounding region R3. Datasets include CTCF-ChIP (green), ATAC-seq profiles from iPSC-CMs and single-cell ATAC-seq in human hearts (orange), RNA-seq profiles in iPSC-CMs (blue), histone ChIP-seq density plots, and ReMap ChIP-seq data from cardiac-related studies. The cCRE regions are highlighted as follows: M3 (grey), R3 (purple), and C3 (orange). **B**) TPM values for transcription factors (TFs) with binding sites within R3 (FDR < 0.01) that significantly increase in expression following GSK3i treatment (Padj< 0.01 and log2(FC) > 1). **C-D**) Visualization of epigenetic datasets surrounding Region C3 (**C**) and M3 (**D**). Datasets include CTCF-ChIP (green), ATAC-seq profiles in iPSC-CMs and single-cells ATAC-seq human hearts (orange), RNA-seq profiles in iPSC-CMs (blue), histone ChIP-seq density plots, and ReMap ChIP-seq data from cardiac-related studies. See also **Fig. 2**.

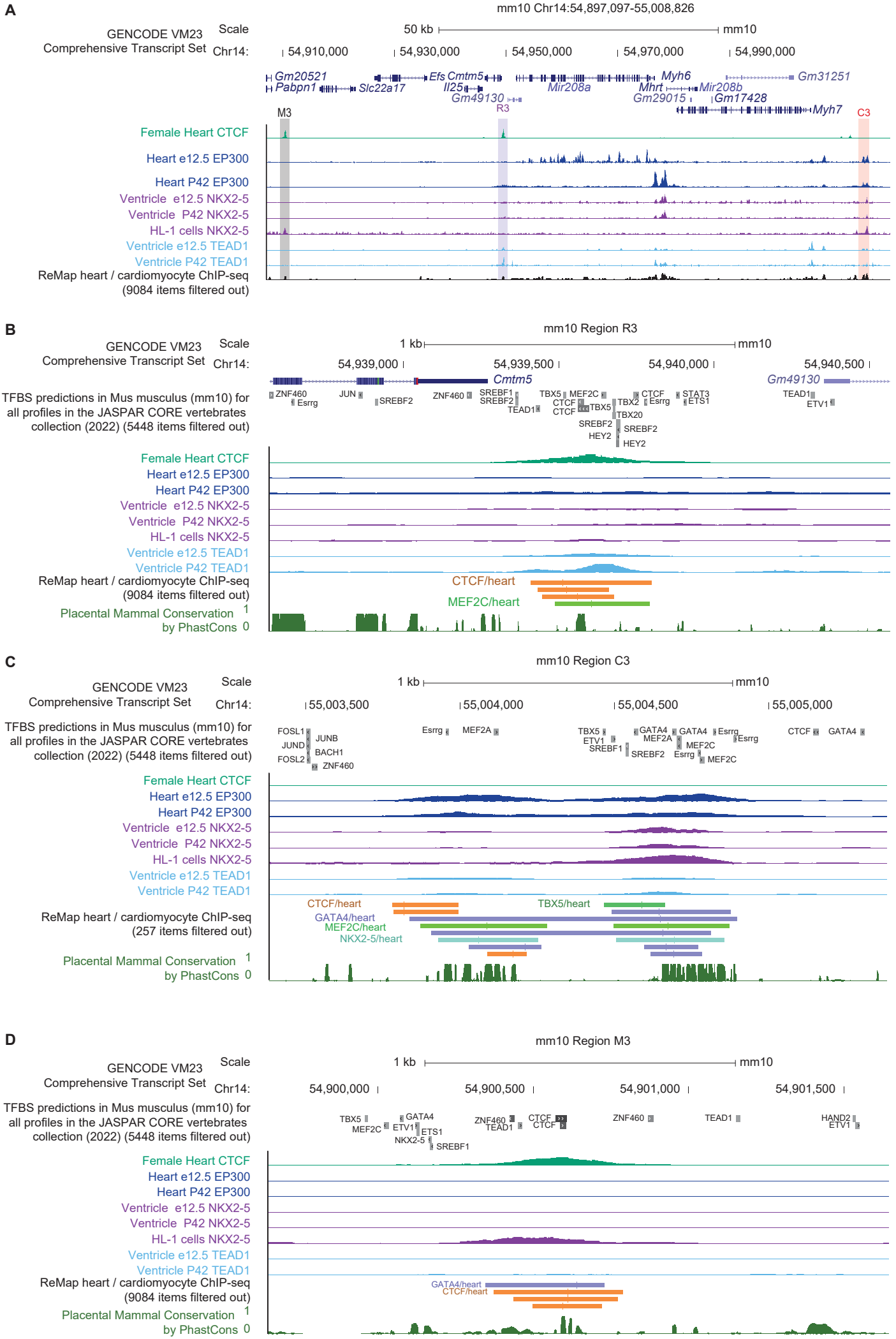
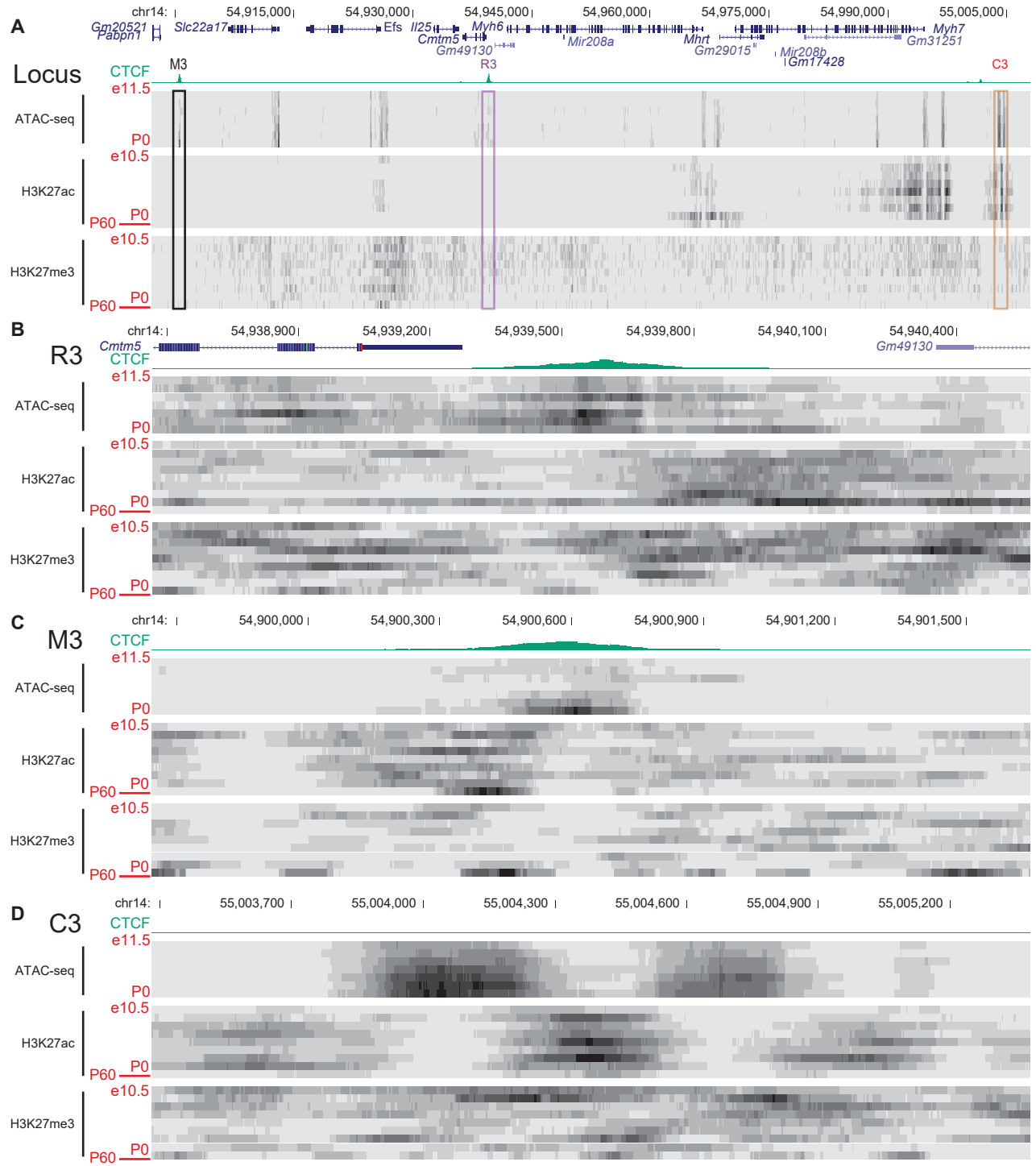


Fig. S5 Epigenetic landscape myosin enhancer R3, C3, and M3 in the mouse heart

A-C) Visualization of epigenetic datasets surrounding the *Myh6* locus (**A**), Region R3 (**B**), Region C3 (**C**), Region M3 (**D**). Data include CTCF-ChIP (green), EP300-ChIP (dark blue), NKX2-5 (purple), TEAD1 (light blue), and ReMap ChIP-seq data from cardiac-related studies. The cCRE regions are highlighted as follows: M3 (grey), R3 (purple), and C3 (orange). See also **Fig. 2**.



E

Mouse

Regulatory Element	ATAC-seq (heart)	H3K27ac	H3K27me3
R3	Maintained through life	High at P0	High e10.5 to e16.5
C3	Increases with development	High e10.5 to e16.5	High at e10.5
M3	Increases with development	High at P60	High at P60

Fig. S6 Visualization of region activity over the development of a mouse

A-D) Visualization of mouse heart (mm10) ATAC-seq (e11.5 to e16.4, and P0), H3K27ac ChIP-seq (e10.5 to e16.5, P0, and P60), and H3K27me3 ChIP-seq (e10.5 to e16.5, P0, and P60). Regions visualized are as follows the *Myh6* locus (**A**), R3 (**B**), M3 (**C**), and C3 (**D**). **E**) Table summarizing the data in panels a to d. See also **Fig. 2**.

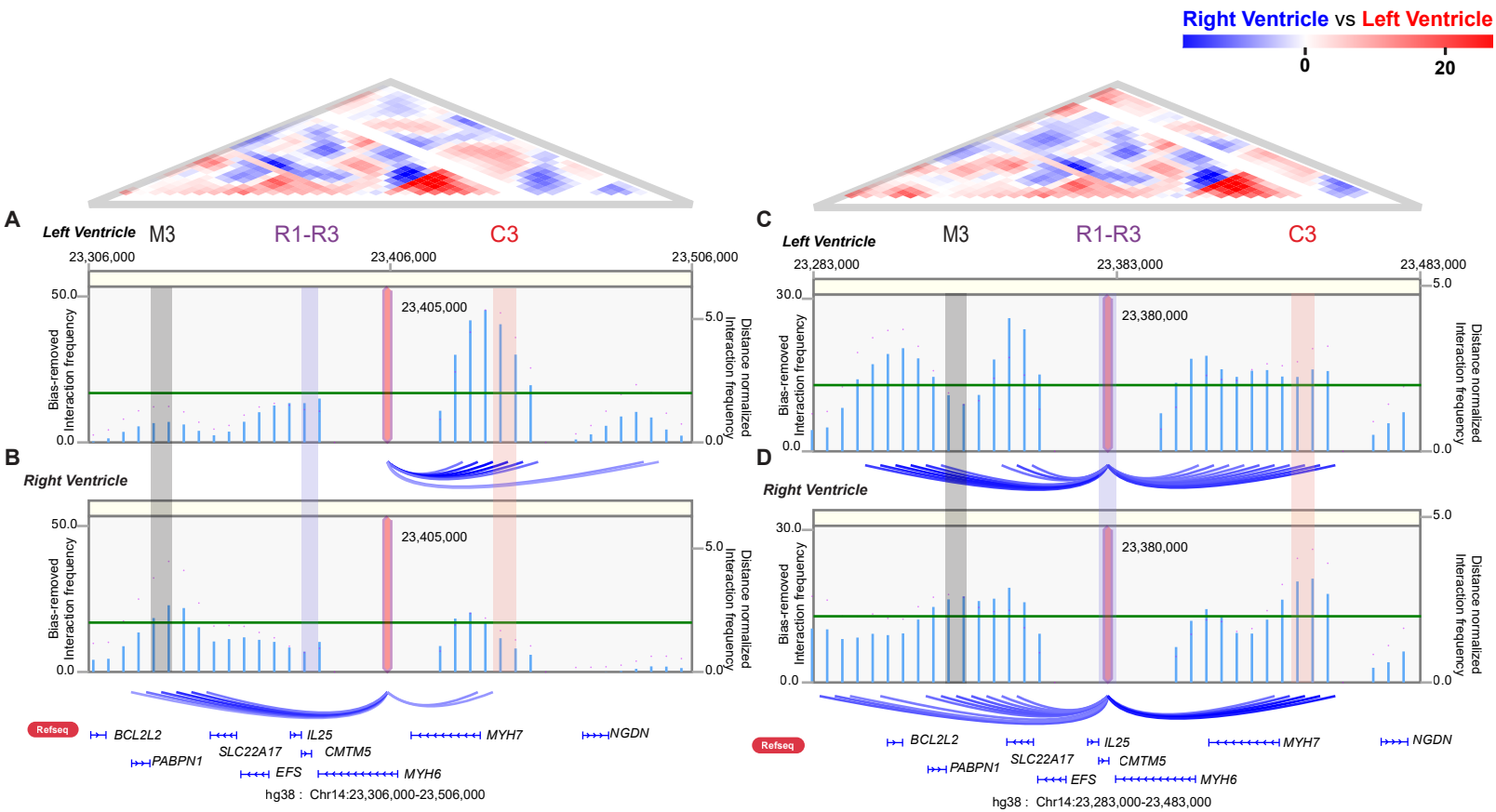


Fig. S7 Chromatin looping data for the MYH6 promoter and R1-R3 in the Left and Right ventricle chambers of the heart.

A-B Visualization of chromatin looping between the promoter of MYH6 and the surrounding genomic environment in the left ventricle chamber **A** and the right ventricle chamber **B**. In the left ventricle, MYH6 predominantly loops to the C3 enhancer, while in the right ventricle, MYH6 predominantly loops to the M3 enhancer. **C-D** Visualization of chromatin looping between the promoter of MYH6 and the surrounding genomic environment in the left ventricle chamber **C** and the right ventricle chamber **D**. In both chambers,R1-3 loops in both directions; however, R1-R3 exhibits fewer interactions with MYH6 in the right ventricle chamber compared to the left ventricle chamber. See also **Fig. 2**.

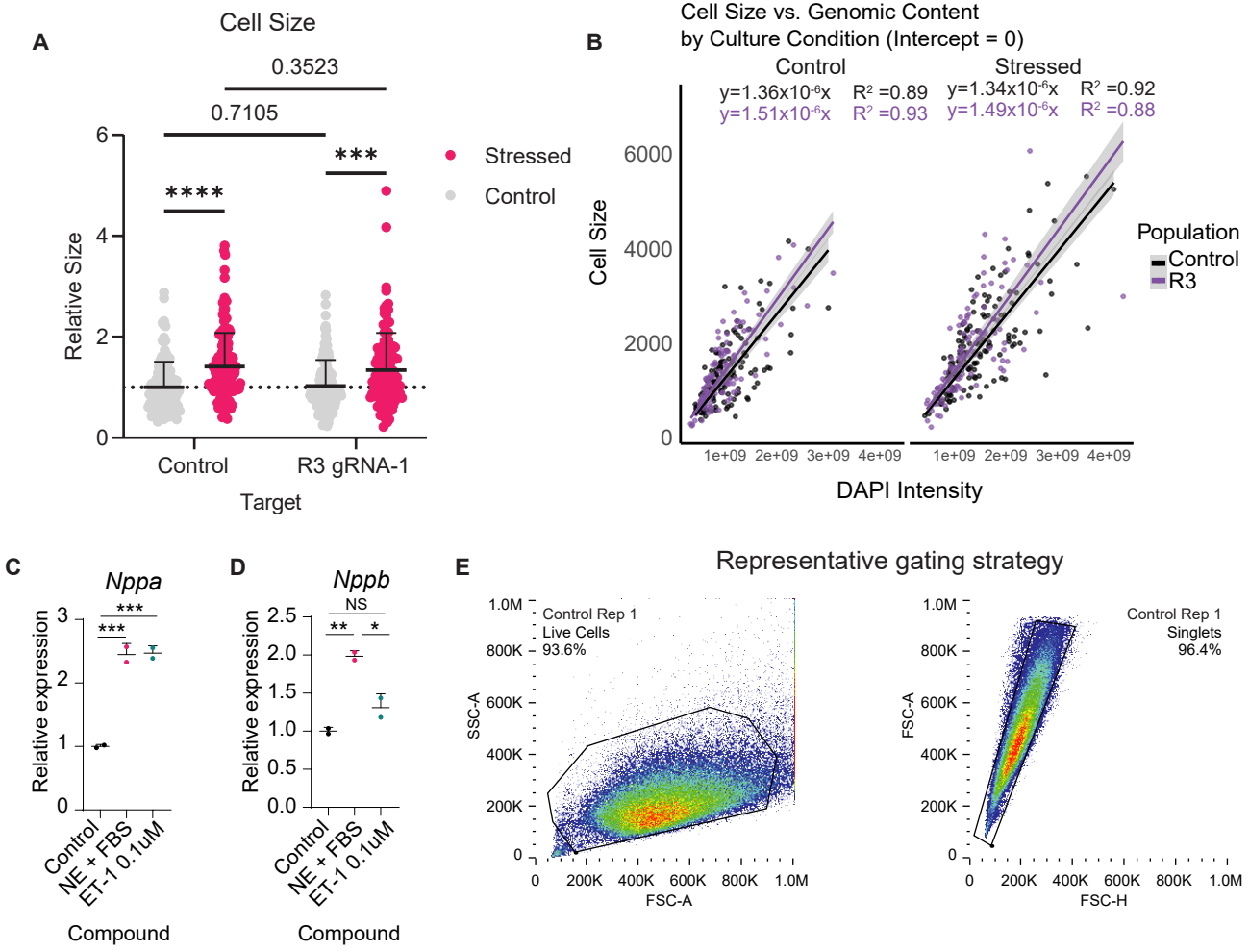


Fig. S8 Analysis of functional measurements of cellular response to stress

A) Normalized cell size for all cells measured of human iPSC-CMs represented 20 days following activation of each designated target ± ET-1 1μM for the last 48 hours of culture, n = 3 replicates. Statistics were calculated with a two-way ANOVA with Fisher's LSD. **B**) Visualization of individual cell size vs the genomic content (DAPI Intensity) within each cell separated by culture condition (Control = left, Stress = right) and target (Control = black, R3 = purple). Formulas for each linear regression with corresponding R^2 values are displayed. **C-D**) RT-qPCR analysis of *Nppa* (**C**) and *Nppb* (**D**) expression in HL-1 mouse atrial cardiomyocytes 48 hours after treatment with the indicated compounds. Relative expression is normalized to *TBP* and control (n = 2 replicates, mean ± SD). Statistical comparisons were made using dCt values (normalized to TBP) and one-way ANOVA with Tukey's post hoc test. Significant Padj values: *Nppa*: Control vs. NE+FBS (P_{adj} = 0.0009), Control vs. ET-1 (P_{adj} = 0.009), NE+FBS vs. ET-1 (P_{adj} = 0.9828); *Nppb*: Control vs. NE+FBS (P_{adj} = 0.0089), Control vs. ET-1 (P_{adj} = 0.1104), NE+FBS vs. ET-1 (P_{adj} = 0.0346). **E**) Gating strategy for cell size measurement: live cells were gated based on FSC-A and SSC-A, followed by a singlet gate to isolate the subpopulation of living cells, and median FSC-A per replicate was measured. See also **Fig. 3**.

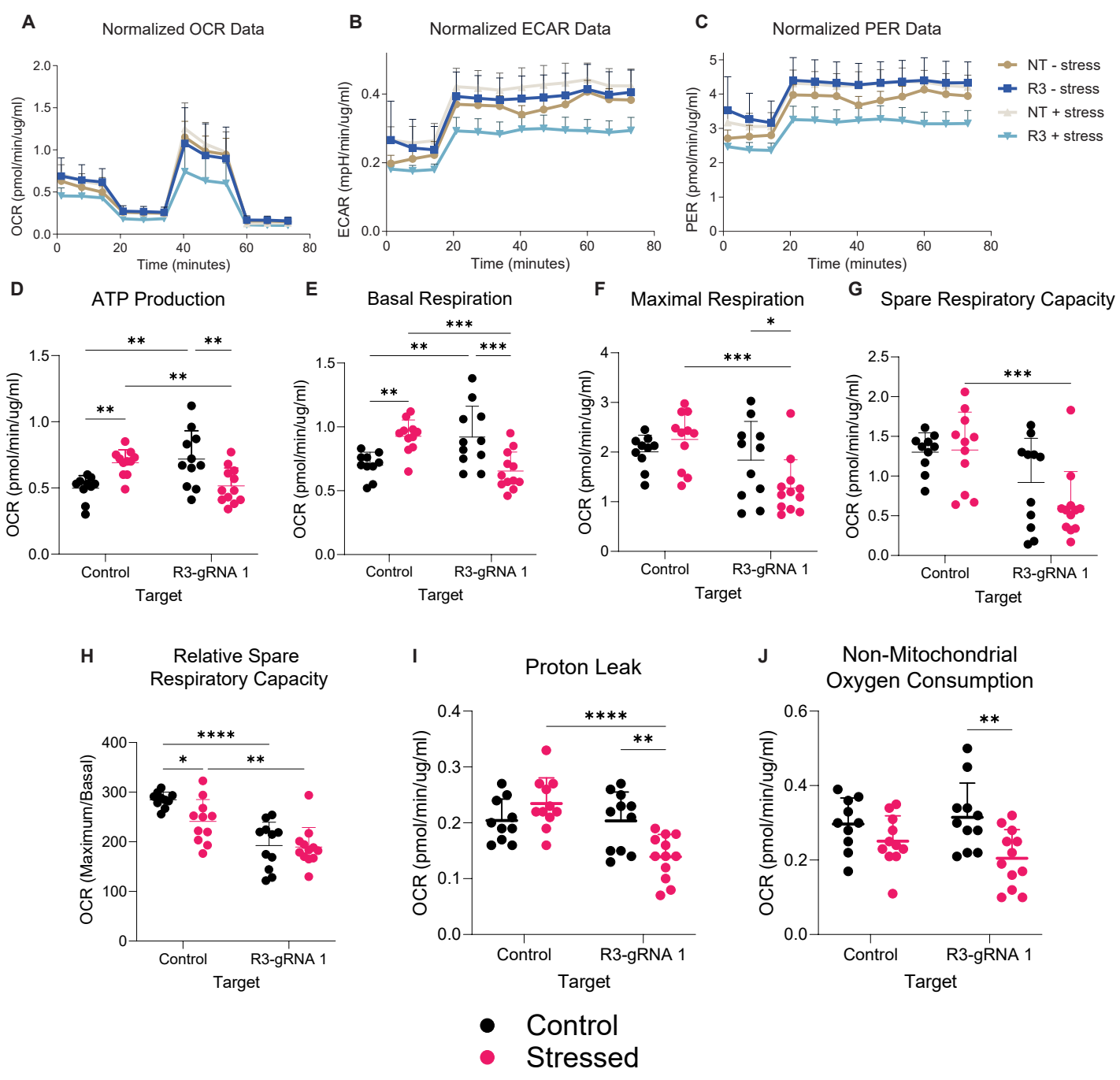


Fig. S9 iPSC-CM metabolism assessment through the Mito Stress test

A-C) Normalized measurements throughout the test for OCR (**A**), ECAR (**B**), and PER (**C**), grouped by culture conditions. **D**) Metabolic metrics derived from the Mito Stress test for each culture condition (n = 10 to 12 wells across three transductions for each control or R3 gRNA. Measurements for each condition are as follows: ATP Production (**D**), Basal Respiration (**E**), Maximal Respiration (**F**), Spare Respiratory Capacity (**G**), Relative Spare Respiratory Capacity (**H**), Proton Leak (**I**), and Non-Mitochondrial Oxygen Consumption (**J**). Statistics were calculated with a two-way ANOVA with Fisher's LSD (*Padj < 0.05, **Padj < 0.01, ***Padj < 0.001, ****Padj < 0.0001).

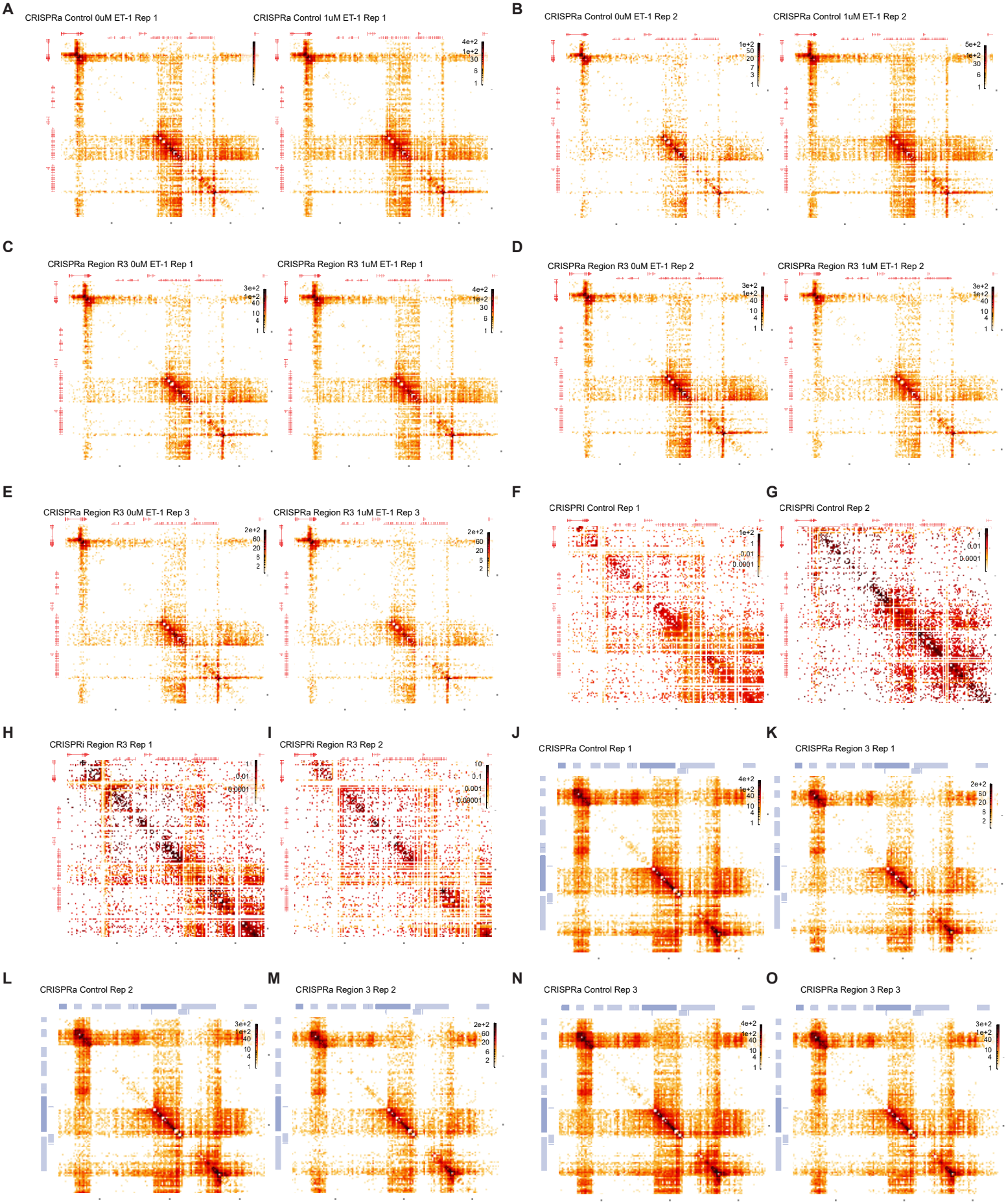


Fig. S10 Visualization of targeted enrichment of HiCAR libraries in iPSC-CMs and HL-1 cells

A-B) Contact heatmaps (hg38) at 1kb bin resolution, illustrating targeted enrichment of HiCAR libraries in iPSC-CMs transduced with ^{VP64}dCas9^{VP64} + Control gRNA +/- ET1 treatment for 72hrs. **A**) Replicate 1. **B**) Replicate 2. **C-E**) Contact heatmaps (hg38) at 1kb bin resolution, illustrating targeted enrichment of HiCAR libraries in iPSC-CMs transduced with ^{VP64}dCas9^{VP64} + Region R3 gRNA-1 ± ET1 treatment for 72hrs. **C**) Replicate 1. **D**) Replicate 2. **E**) Replicate 3. **F-I**) Contact heatmaps (hg38) at 1 kb bin resolution, illustrating targeted enrichment of HiCAR libraries in iPSC-CMs transduced with dCas9KRAB + gRNA. **F**) Control gRNA replicate 1. **G**) Control gRNA replicate 2. **H**) Control gRNA replicate 1. **I**) R3 gRNA replicate 2. **J-O**) Contact heatmaps (mm39) at 1 kb bin resolution, illustrating targeted enrichment of HiCAR libraries in HL-1 cells transduced with VP64dCas9VP64 + gRNA. **J,L,N**) Control gRNA replicates 1 to 3. **K,M,O**) Region R3 gRNA-2 replicates 1 to 3. Genomic range: Chr14:23,303,865-23,451,365 in hg38 coordinates and Chr14:55,111,190-55,258,690 in mm39 coordinates. See also **Fig. 5**.

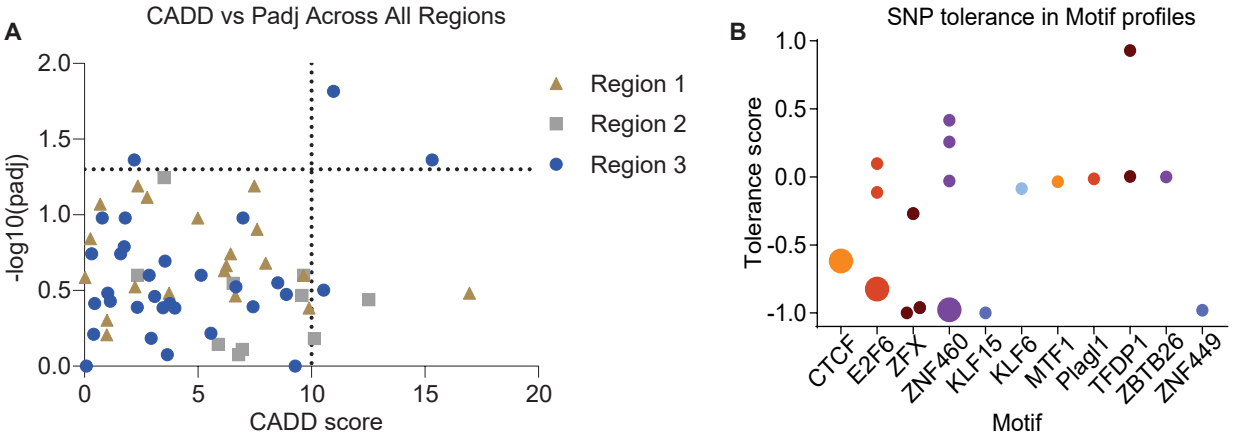


Fig. S11 Analysis of genetic variants within the myosin enhancer.
A) Visualization of CADD scores for variants within regions R1 - R3 found in subjects from the UKBiobank. Padj was determined by a Fisher's exact test on the frequency of genetic variants. **B**) Tolerance scores for genetic variants within motif profiles found in R3 from human participants in the UK Biobank. Enlarged dots correspond to variants with Padj <0.05. See also **Supplemental Table S5**.