SUPPLEMENTAL INFORMATION FOR GACITA ET AL. "GENETIC VARIATION IN ENHANCERS MODIFIES CARDIOMYOPATHY GENE EXPRESSION AND PROGRESSION"

EXPANDED METHODS

Epigenetic Datasets. For histone ChIP-Seq datasets and ATAC-seq datasets, the "fold change over negative control" bigwig file was used. For transcription factor Chip-seq datasets, peak bed files were used. For Homer computational predictions, a bed file representing the location of the transcription factor motif genome-wide was used. Files were imported into the UCSC genome browser for visualization. When necessary, datasets from mouse cells/tissues or hg38 were overlaid to hg19 using the UCSC liftover tool. For pcHiC data, the CHiCAGO pipeline raw output of three replicates of IPSC-CM promoter capture Hi-C data were downloaded.[19] Probe-probe interactions were filtered. 1kb was added to both ends of regions interacting with gene promoters. We intersected data from each replicate using bedtools and retained only genomic interactions that were present in at least two replicates.[14] Bed files representing pcHi-C interactions were visualized in the UCSC genome browser.

Enhancer constructs. Candidate enhancer regions were amplified from human genomic DNA using primers with a 5'-CACC overhang using Phusion High-Fidelity DNA polymerase (NEB). An aliquot of the PCR reaction was separated on a 1% agarose-TBE gel to confirm amplification, and the remaining reaction was purified using a PCR Purification Kit (Qiagen). In cases where PCR failed to generate an adequate product, the enhancer region sequence (matching hg19) was synthesized as a dsDNA gGlock gene fragment (IDT). Approximately 5ng of PCR product or gBlock was ligated into the pENTR/D-TOPO vector following manufacturer's instructions (ThermoFisher). The enhancer region was recombined into pGL4.23-GW (Addgene #60323) using LR Clonase II Enzyme mix (Thermo) with 150ng of each plasmid. EndoFree Maxipreps (Qiagen) were used to prepare DNA. Plasmids were confirmed using Sanger Sequencing. In all candidate enhancer plasmids, the enhancer sequence was located 125bp upstream of the minimal promoter sequence. PCR primers and the genomic regions amplified for each construct are shown in are shown in **Table II in the Supplement**.

Detailed HL-1 Luciferase Assay. Twenty-four hours before transfection, 140,000 HL-1 cells per well were plated on to a 12-well plate. On the day of transfection, HL-1 cells were transfected using Lipofecamine 3000 (Thermo Fisher) following manufacturer's instructions. Each well was transfected with 6μl of 0.15μM enhancer firefly luciferase plasmid, 50ng of pRL-SV40 (Promega), 2.5μl of Lipofecamine3000, and 6μl of P3000 in 100μl of Opti-MEM. Cells were allowed to incubate for 6-8 hours, following which half the media was replaced with Claycomb media. Forty-eight hours after transfection, the luciferase assay was performed with the Dual-Glo luciferase assay kit (Promega) according to manufacturer's instructions.

IPSC Reprogramming, Culturing, and IPSC-CM Differentiation. Human skin fibroblasts were obtained from Coriell (sample name GM03348, 10 year old male) and cultured in DMEM containing 10% FBS. Fibroblasts were re-programmed into induced pluripotent stem cells (IPSCs) via electroporation with pCXLE-hOCT3/4-shp53-F (Addgene plasmid 27077), pCXLE-hSK (Addgene plasmid 27078), and pCXLE-hUL (Addgene plasmid 27080) as described previously.[43] IPSCs were maintained on Matrigel-coated 6-well plates with mTeSR-1 (Stem Cell technologies, Cat#85850) and passaged as colonies every 5-7 days using ReLeSR (Stem Cell technologies, Cat#05872).

IPSCs were differentiated into cardiomyocytes (IPSC-CMs) using Wnt modulation as previously described.[11] Differentiation was conducted in CDM3 (RPMI 1640 with L-glutamine, 213 μg/mL L-asorbic acid 2-phosphate, 500μg/mL recombinant human albumin).[11] Cells were

grown to ~95% confluency and treated with 6μM - 10μM CHIR99021 for 24 hours and allowed to recover for 24 hours. Cells were treated with 2μM Wnt-C59 for 48 hours and then media was changed with CDM3 every two days until beating cardiomyocytes were obtained (~day 6-10). In order to prevent cell detachment, beating cardiomyocytes re-plated on to new plates using TrypLE (Thermo Fisher). Media was changed every two days until downstream assays were performed (~day 20).

CRISPr Enhancer Deletion in IPSCs. To delete enhancer regions, guides targeting the 5' and 3' end of enhancer regions were designed using CRISPOR.[44] Guides are shown in **Table II in the Supplement**. Guides were ligated into pSpCas9(BB)-2A-Puro (Addgene plasmid #62988) after the U6 promoter using either Bbs1 digestion and ligation or Gibson assembly. DNA preparations of plasmid were prepared using an EndoFree plasmid kit (Qiagen), and plasmid sequences were confirmed with Sanger sequencing. IPSCs were nucleofected using the Neon transfection system (Thermo Fisher). Briefly, GM03348 IPSCs were grown to ~70% confluency and treated with mTeSR-1 containing 2μM thiazovivin (TZV) for one hour. Cells were digested with TrypLE, collected and counted. 3.75 million IPSCs per nucleofection were pelleted at 300g for 3min. Cell pellets were resuspended in 125μl of buffer R and added to an Eppendorf tube containing 1.5μg or 2.5μg of each plasmid. Cells were nucleofected in the Neon system in a 100μl tip with the following settings: 1400 V, 20 ms, 2 pulses. Nucleofected cells were expelled into a single well of Matrigel-coated 6-well plate containing mTeSR-1 supplemented with ClonR (Stem Cell Technologies, Cat#05888) and 2μM TZV. For each round, a pSpCas9(BB)-2A-GFP (Addgene plasmid #48138) control was included. Twenty-four hours later, cells were treated with mTeSR-1 containing 0.15μg/mL puromycin. The next day, selection was continued with 0.2μg/mL puromycin until no viable cells were seen in the GFP control (~2-3 days). Cells were switched to mTeSR-1 supplemented with ClonR and 2μM TZV and media was changed daily until colonies appeared (5-7 days). Colonies were picked on to 96-well plates, expanded, and split on to two duplicate plates. The first plate was used for cryopreservation in 50% mTeSR-1/ClonR/2μM TZV and 50% KnockOut Serum replacement/25% DMSO. The second plate was processed for gDNA isolation using the DirectPCR lysis reagent (Viagen, Cat#301-C) following manufacturer's instructions. Colonies were screened for successful enhancer deletion using a 3-primer PCR approach. PCR products were cloned using the TOPO TA cloning kit (Thermo Fisher) and sequenced to determine alleles present. Positive colonies were thawed from the frozen plate, expanded, re-genotyped, and used for differentiation. In cases where no homozygous deletions were obtained, a heterozygous colony was treated with a second round of CRISPr editing.

IPSC Chromosome Analysis and CRIPSr-Off Target Analysis. IPSC Chromosome analysis was conducted using the hPSC genetic analysis kit (Stem Cell Technologies, Cat#07550) following manufacturer's instructions. IPSC lines must show no amplification or deletion in at least 8 of the 9 tested sites to pass our karyotypic quality control standards. We used the output from the CRISPOR[44] guide design tool to identify the most likely off target cut sites. We selected any regions with < 3 mismatches and additional off targets that were within or near genes important for cardiac function. Primers were designed to amplify putative off target sites and regions were amplified from gene edited cell gDNA. PCR products were purified using ExoSAp-IT (Thermo) or Ampure XP beads (Beckman Coulter) and sequenced with sanger sequencing. Primers used are available upon request. Sanger traces from unedited IPSCs were compared to gene edited lines to identify any off-target changes. Genotype of enhancer deleted cells are shown in **Table VI in the Supplement**. Off-target analysis is shown in **Table V in the Supplement**.

IPSC-CM RNA Extraction and qPCR. At ~day 10 of differentiation, 1 million IPSC-derived cardiomyocytes were plated on a well of 12-well plate. At ~day 20, cells were washed with PBS and 400μl of TRIzol (Thermo Fisher) was added directly to the well. Cells were collected into an Eppendorf tube using a cell scraper. Trizol was kept at -80°C until further processing. Six hundred μl of additional TRIzol was added to the cells and the entire sample was added to a tube containing 250μl of silica-zirconium beads. Tubes were placed in a bead beater homogenizer (BioSpec) for 1 minute and immediately cooled on ice. Samples were incubated at room temperature for 5min and then centrifuged at 12,000g for 5min to remove unhomogenized cell aggregates. Supernatant was transferred to a new tube and 200μl of chloroform was added. After vigorous shaking for 30 seconds followed by 10 min incubation with periodic shaking, samples were centrifuged at 12,000g for 15 min. The upper aqueous layer was added to an equal volume of fresh 70% ethanol and used an input to the Aurum Total RNA Mini Kit (Biorad). RNA was processed according to manufacturer's instructions including on-column DNase digestion. RNA was eluted twice with 30μl of warmed water and the concentration was measured using a nanodrop spectrophotometer.

The qScript cDNA SuperMix (Quantabio) was used to generate a 100ng cDNA library. A 1:10 dilution was used as a template in a 3-step SYBR-green qPCR region with a 57°C annealing temperature. We used a panel of primers targeting cardiomyocyte references genes (*TNNT2*, *MYBPC3*, *TNNI3*, *SLC8A1*, *MYOZ2* and *GAPDH*) that passed optimization studies confirming primer specificity and efficiency. For enhancer deletion measurements, changes in *MYH6* and *MYH7* expression were calculated using the delta-delta Cq method using the geometric mean expression of cardiomyocyte reference genes.

SDS-PAGE of Myosin Heavy Chain Isoforms. We prepared a 6.25% acrylamide/bis-acrylamide(99:1) resolving gel by combining 7.5mL of 25% Acrylamide/bis-acrylamide(99:1), 5.65mL of 2M Tris pH 8.8, 16.55mL of ddH20, 300μl of 10% SDS (w/v), 312μl 10% ammonium persulfate, and 12.5μl of TEMED. The resolving gel was allowed to polymerize for 1 hour at room temperature. A 5% acrylamide/bis-acrylamide (99:1) stacking gel was prepared by combining 2mL of 25% Acrylamide/bis-acrylamide(99:1), 2.5mL of 0.5M Tris pH 6.8, 5.325mL of ddH20, 100μl of 10% SDS (w/v), 90μl 10% ammonium persulfate, and 6μl of TEMED. The stacking gel was allowed to polymerize for 8 hours. Lysates of ~day 20 IPSC-CMs were prepared and protein concentrations were quantified with the Quick-Start Bradford Protein Assay (Bio-Rad). ~7μg of protein was mixed 1:1 with 2x Laemmli Sample Buffer containing β-mercaptoethanol. Samples were loaded into the SDS-polyacrylamide gel described above and separated at 13mA for 20min, and 15mA for 21 hours. After electrophoresis, gels were fixed with a 7% acetic acid/50% methanol solution for 1 hour at room temperature. Protein was visualized with the Sypro Ruby Protein Gel Stain (Thermo Fisher) following manufacturer's instructions. Quantification of band intensities was done using Fiji.[45]

Flow Cytometry Analysis of IPSC-CM Purity. At approximately day 20 of differentiation, IPSC-CMs were collected using TrypLE (Thermo Fisher). Cells were resuspended in 1mL of PBS and added to 1mL of 8% PFA in PBS for fixing. Cells were fixed at 37°C for 10min with shaking. Cells were collected by centrifugation at 600g for 5min and resuspended in 100μl ice-cold 90% methanol in PBS per 500,000 starting cells. Cells were stored at -20° C until further processing. On the day of flow, ~1 million cells were aliquoted into two tubes containing 2mL of 0.5mg/mL BSA in PBS and pelleted. One tube was resuspended in 100μl of PBS containing 1:200 dilution of *TNNT2*-Alexa Fluor 694 (BD Pharmingen #565744) and 1:200 *MYBPC3*-Alexa Fluor 488 (Santa Cruz Biotechnology #sc-137180 AF488) and the other tube was suspended in PBS alone. Cells were stained for 1 hour at room temperature. Four mL of 0.5mg/mL BSA in PBS was added to each tube and cells were pelleted. Cells were resuspended in 100μl in PBS

and analyzed on a flow cytometer. The percentage of TNNT2-positive cells was determined by using PBS only as a negative control.

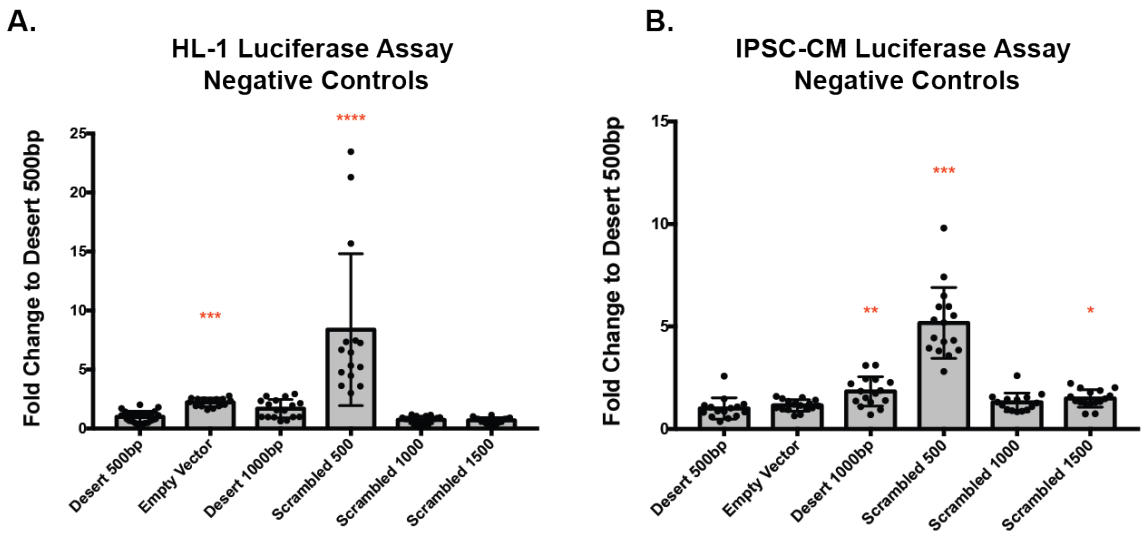

Figure I. Negative control region reporter assay activity in HL-1 cells and IPSC-CMs. HL-1 cells are a mouse atrial cell line [10]. Expression of *MYH6/7* differs between atrial and ventricles and between mouse and human ventricles [54]. **A.** Luciferase assay using HL-1 cells including multiple negative control regions. Average n=18 across three different days. **B.** Luciferase assay data for multiple negative control regions in IPSC-CMs. Average n=16 across two differentiations. Desert represents genomic regions with little or no evidence of enhancer function in left ventricle tissue. Scrambled represents randomly selected nucleotides. Significance vs desert 500bp determined by nonparametric one-way ANOVA with Dunn's multiple comparisons correction. *<0.03, **<0.0021, ***<0.0002, ****<0.0001.

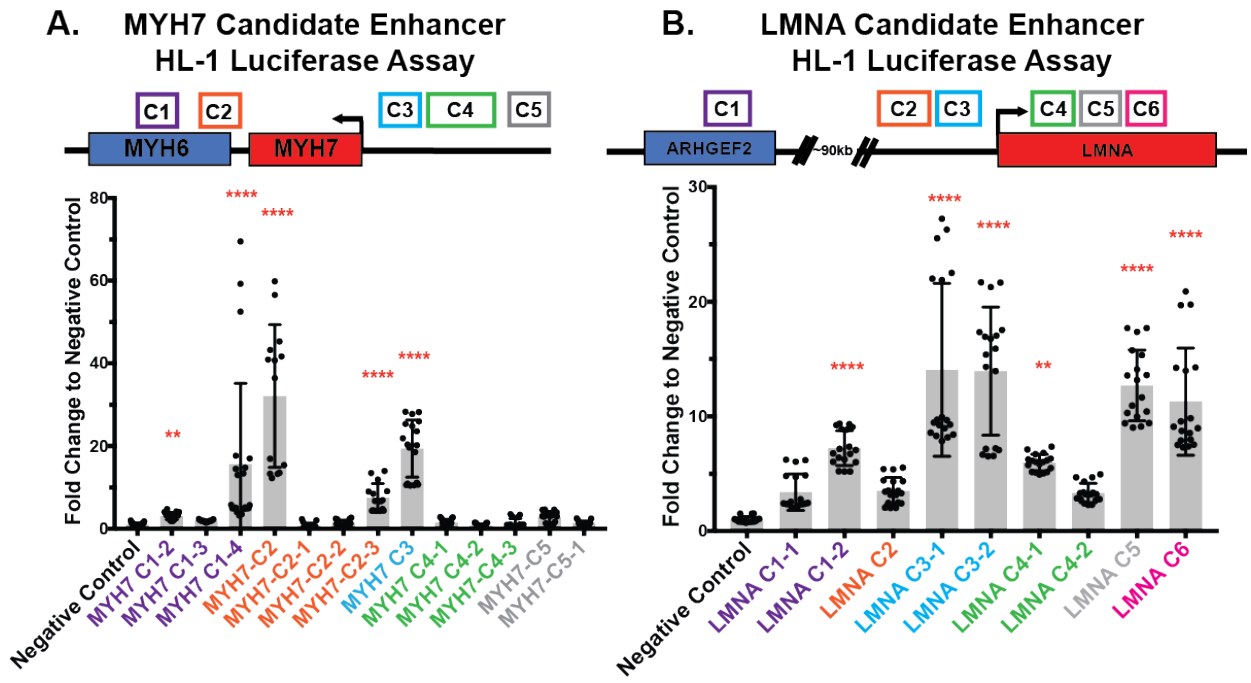

Figure II. Reporter assay for candidate enhancer regions of *MYH7* and *LMNA* in HL-1 cells. **A**. Above, color-coded schematic of candidate *MYH7* enhancers identified in figure 1. Below, data from luciferase reporter assay in HL-1s for full and partial candidate enhancer regions. **B.** Above, color-coded schematic of candidate *LMNA* enhancers identified in figure 1. Below, data from luciferase reporter assay in HL-1s for full and partial candidate enhancer regions. Data displayed as fold change to negative control genomic 500bp desert region with mean +/- SD. Average n=17 across three separate days. Significance vs negative control determined by nonparametric one-way ANOVA with Dunn's multiple comparisons correction, **<0.0021, ****< 0.0001.

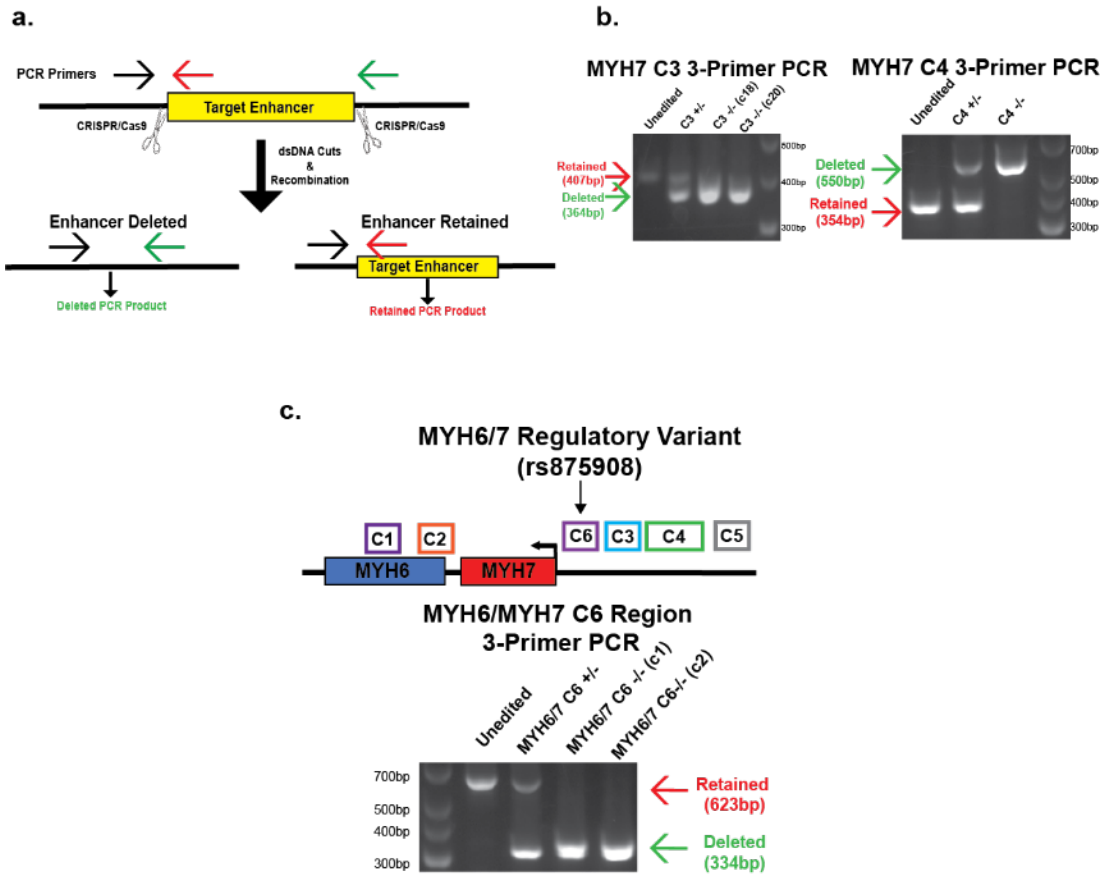

Figure III. CRISPr-Cas9 enhancer deletion strategy successfully removes *MYH7* enhancer regions. A. Schematic of CRISPr-Cas9 deletion strategy and PCR primers used for genotyping. **B.** Agarose gels of 3-pimer PCRs on genomic DNA from IPSCs treated with guides targeting *MYH7* candidate enhancers 3 and 4 demonstrating successful knockout. **C.** Top, schematic representation of the location of the MYH6/7 regulatory variant. Bottom, agarose gel of 3-pimer PCR on genomic DNA from IPSCs treated with guides targeting the region overlapping the MYH6/7 regulatory variant showing successful deletion.

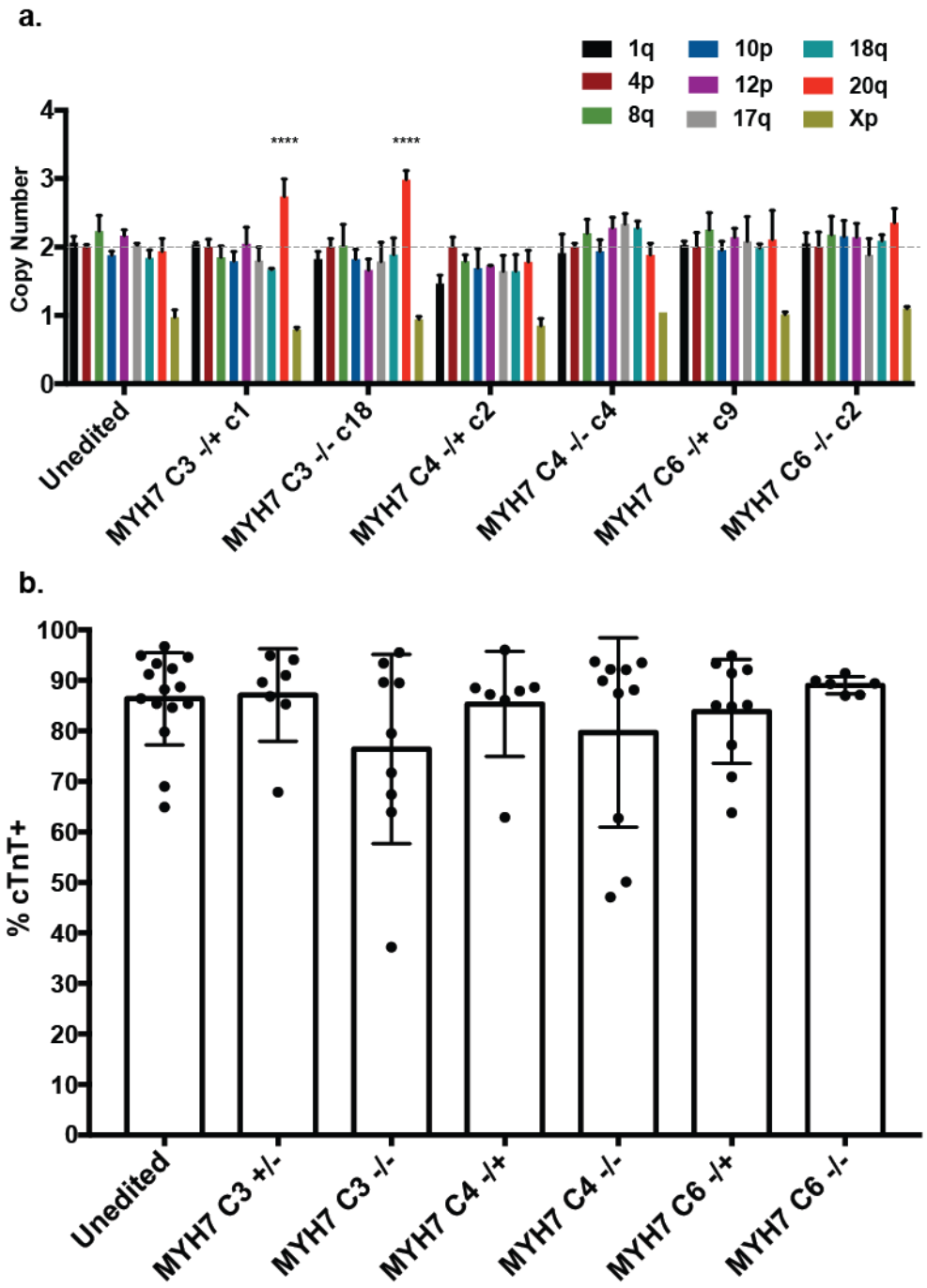

Figure IV. Validation of gene edited iPSCs and IPSC-CMs. Nonhomologous end joining CRISPr-Cas9 was used to generate guided deletions in iPSCs. Resulting clones were treated isolated analyzed for common chromosomal rearrangements. **A.** Results from the hPSC genetic analysis test kit (Stem Cell Technologies) assaying common chromosomal rearrangements in CRISPr treated IPSCs. **B.** IPSC-CM purity measurements evaluating the percent cardiac troponin T (cTNT) cells across different enhancer deletion lines. cTnT, cardiac troponin T. No significant differences were found between unedited and CRISPr treated cells by one-way ANOVA.

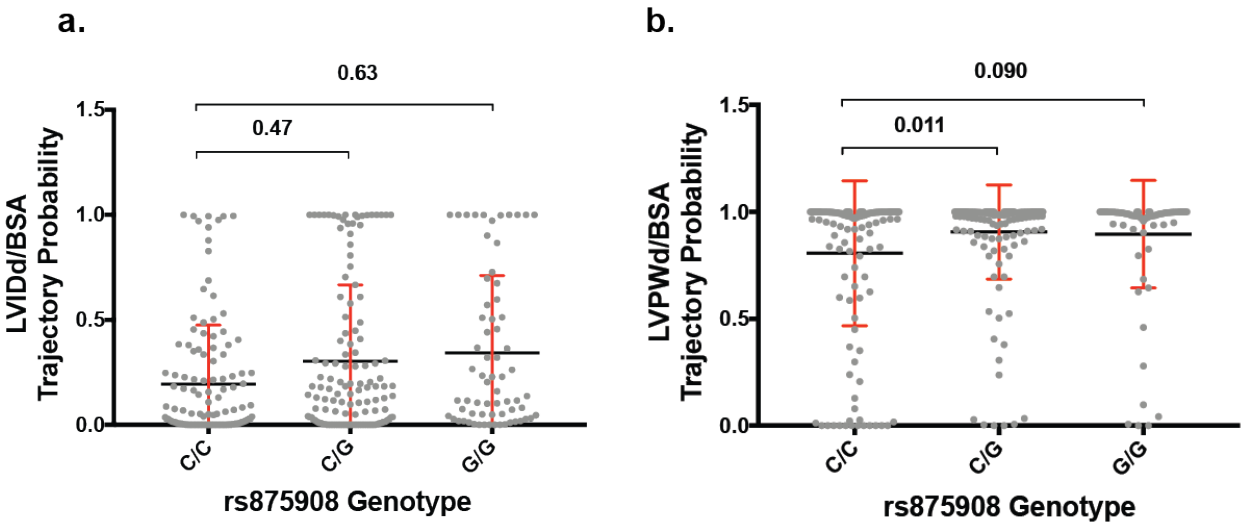

Figure V. Phenotypic regressions using the NU genomes cohort. A. Association of variant status with LVIDd/BSA over time in the NU genomes cohort (n=387). **B.** Association of variant genotype with LVPWd/BSA overtime in the NU genomes cohort. Significance determined using a linear regression model corrected for race and sex. LVIDd/BSA, left ventricular internal diameter during diastole corrected for body surface area. LVPWd/BSA, left ventricular posterior wall thickness during diastole corrected for body surface area.

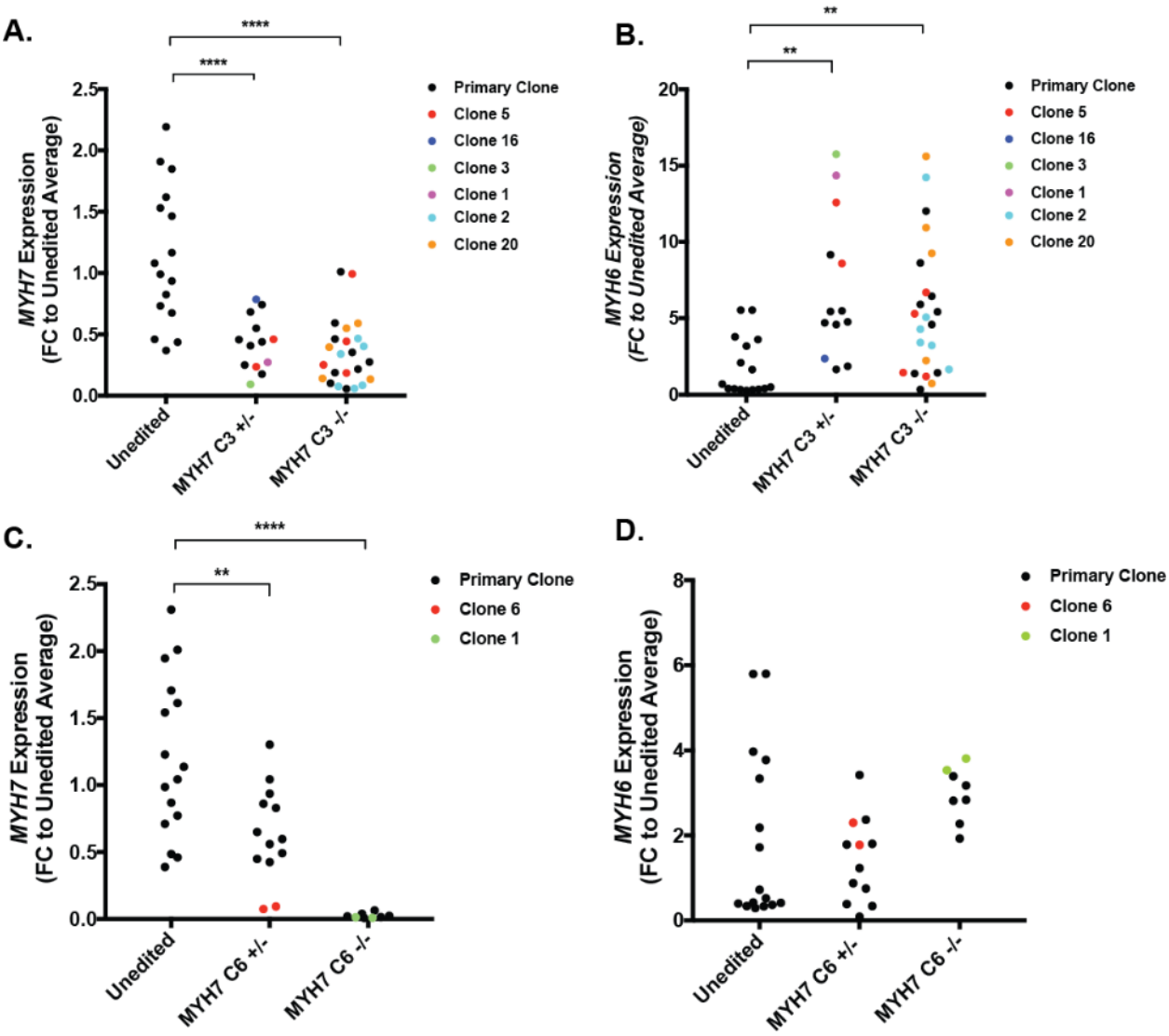

Figure VI. Deletion of the MYH7-C3 and MYH7-C6 enhancer affects *MYH7* and *MYH6* expression levels across multiple clones. **A.** Reduction of *MYH7* mRNA expression in *MYH7*-C3-deleted cells was observed across multiple independent clones. **B.** Increase of *MYH6* expression in *MYH7*-C3-deleted cells was observed across multiple independent clones. **C.** Reduction of *MYH7* mRNA expression in *MYH7*-C6-deleted cells was observed across multiple clones. **D.** Changes in *MYH6* mRNA expression in *MYH7*-C6-deleted cells are consistent across multiple independent clones. All data shown as mean ±SD. * determined by one-way ANOVA with Dunnett's multiple comparisons test. **<0.0021, ****<0.0001.

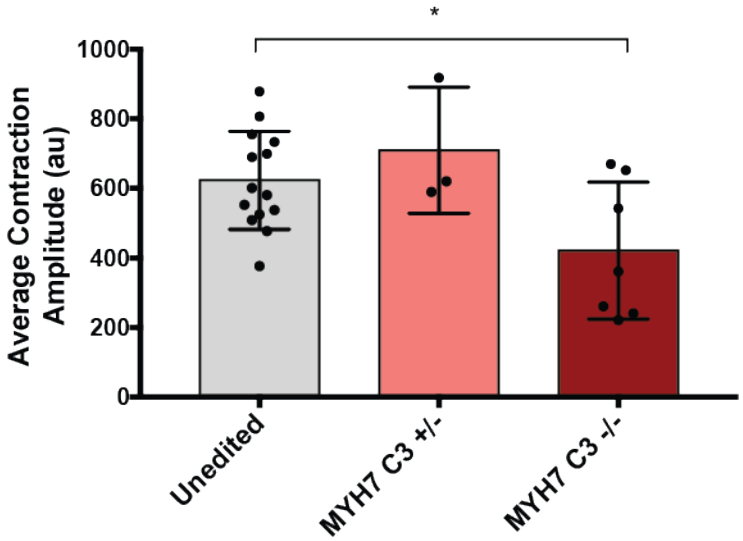

Figure VII. Deletion of the *MYH7*-C3 enhancer produces hyperdynamic function in engineered heart tissues. Average contraction amplitude measurements of EHT contractions containing unedited or *MYH7*-C3 deleted cells showed a decrease in contraction amplitude in *MYH7*-C3 deleted EHTs. Each point represents the average time to peak measurement of a single EHT across multiple contractions (unedited n=14, *MYH7* $C3^{+/-}$ n=3, *MYH7* $C3^{-/-}$ n=7.) All data shown as mean ±SD. * determined by one-way ANOVA with Dunnett's multiple comparisons correction. *<0.03.

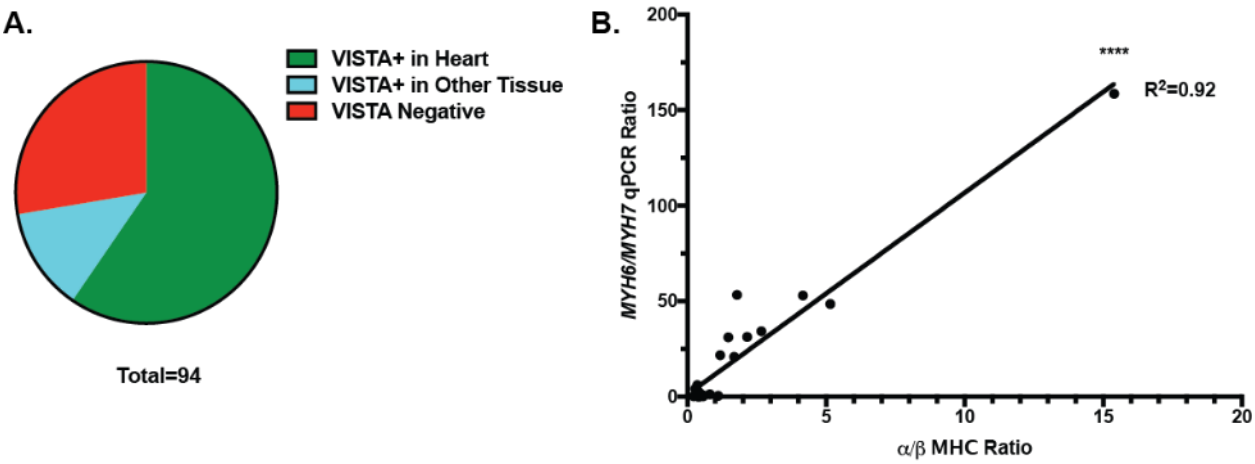

Figure VIII. Regulatory Variant VISTA Overlap and myosin heavy chain RNA and protein level correlations. A. Overlap between variants identified by our pipeline and the enhancer regions tested in the VISTA database. **B.** Regression between the *MYH6/MYH7* ratio determined by qPCR and the α/β MHC ratio determined by SDS-PAGE. Significance determined by linear regression. **** <0.0001.

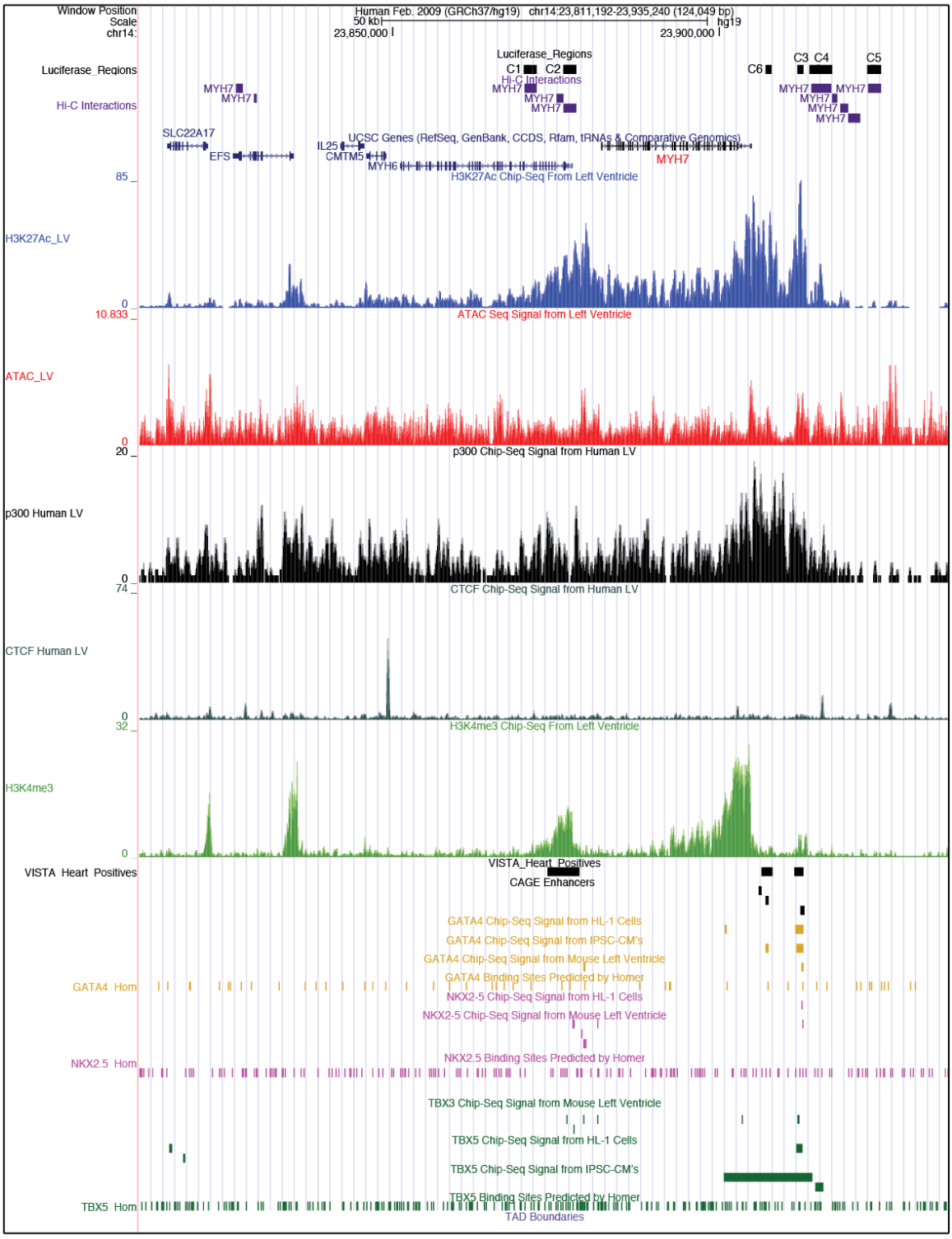

Figure IX. Full map of putative *MYH7* Enhancers.

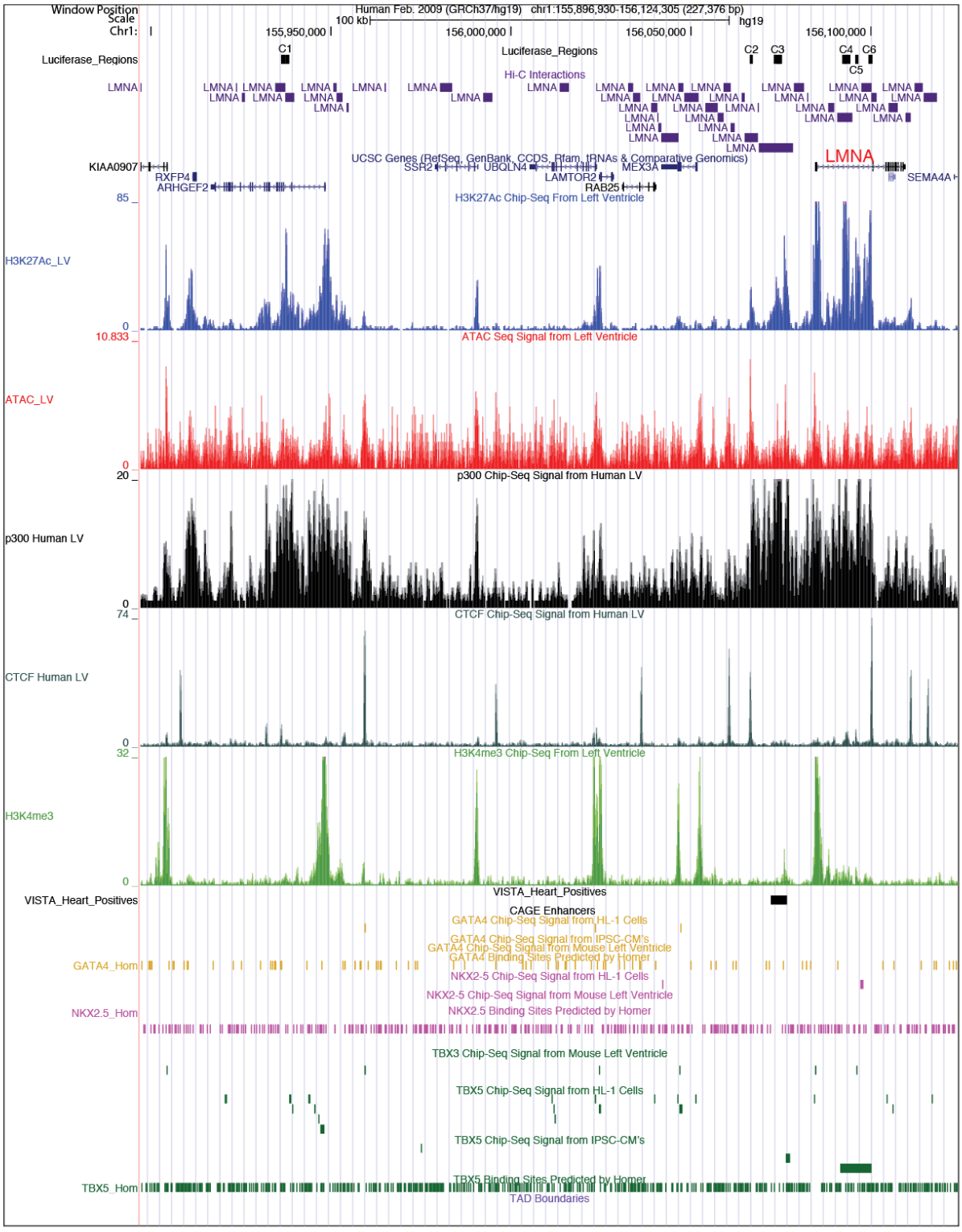

Figure X. Full map of putative *LMNA* Enhancers.

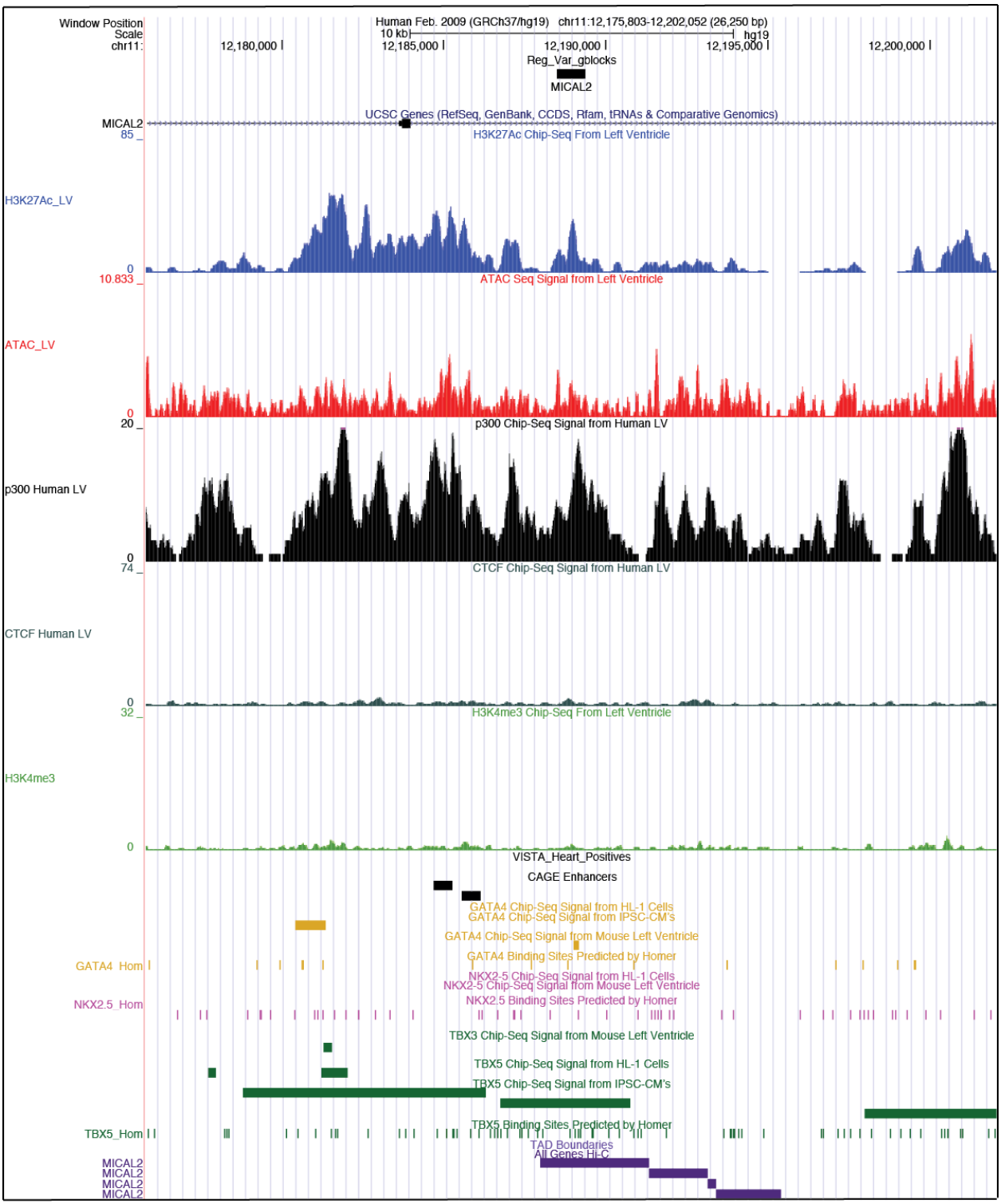

Figure XI. Full map of *MICAL2* Regulatory Variant.

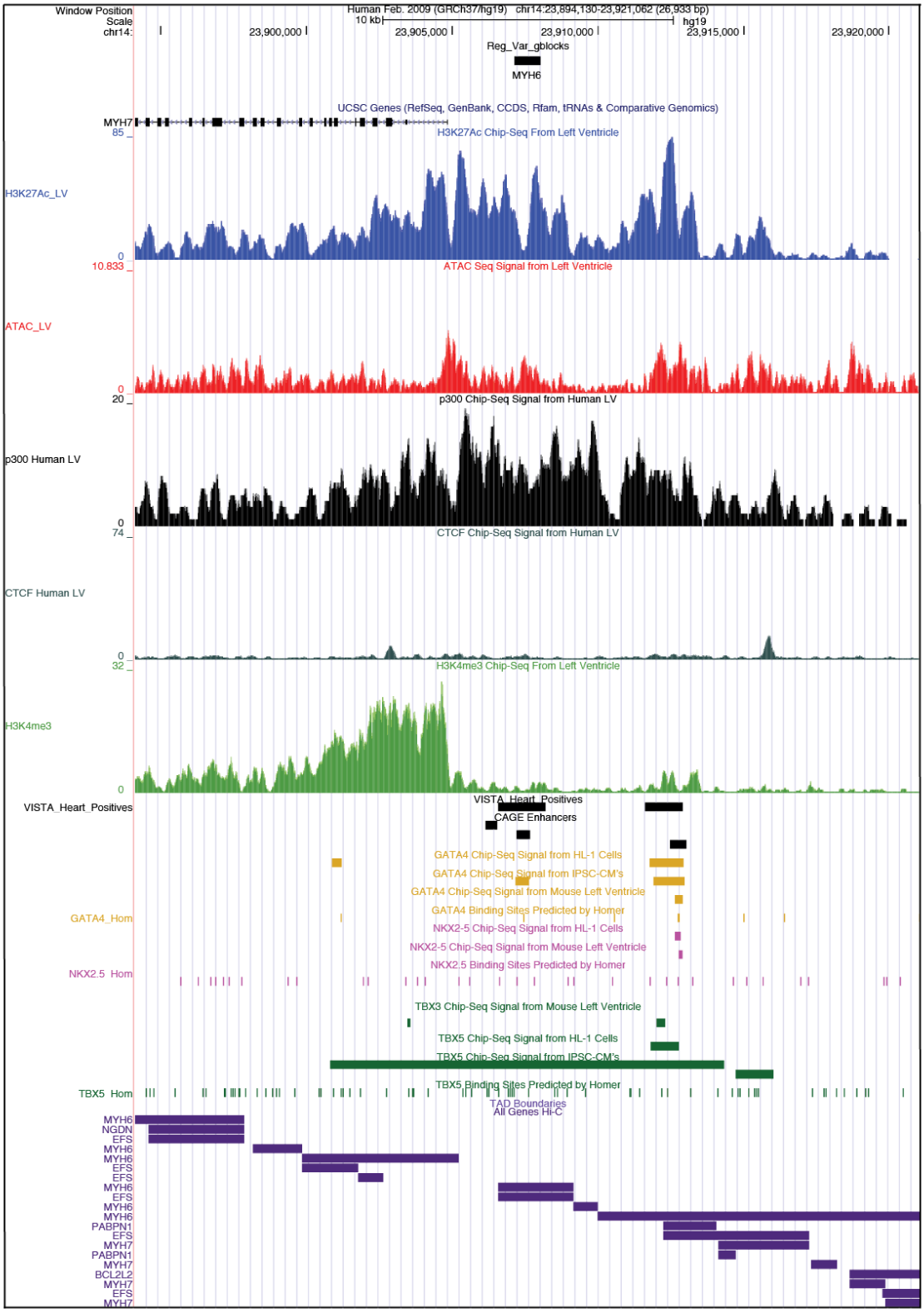

Figure XII. Full map of *MYH6/7* Regulatory Variant

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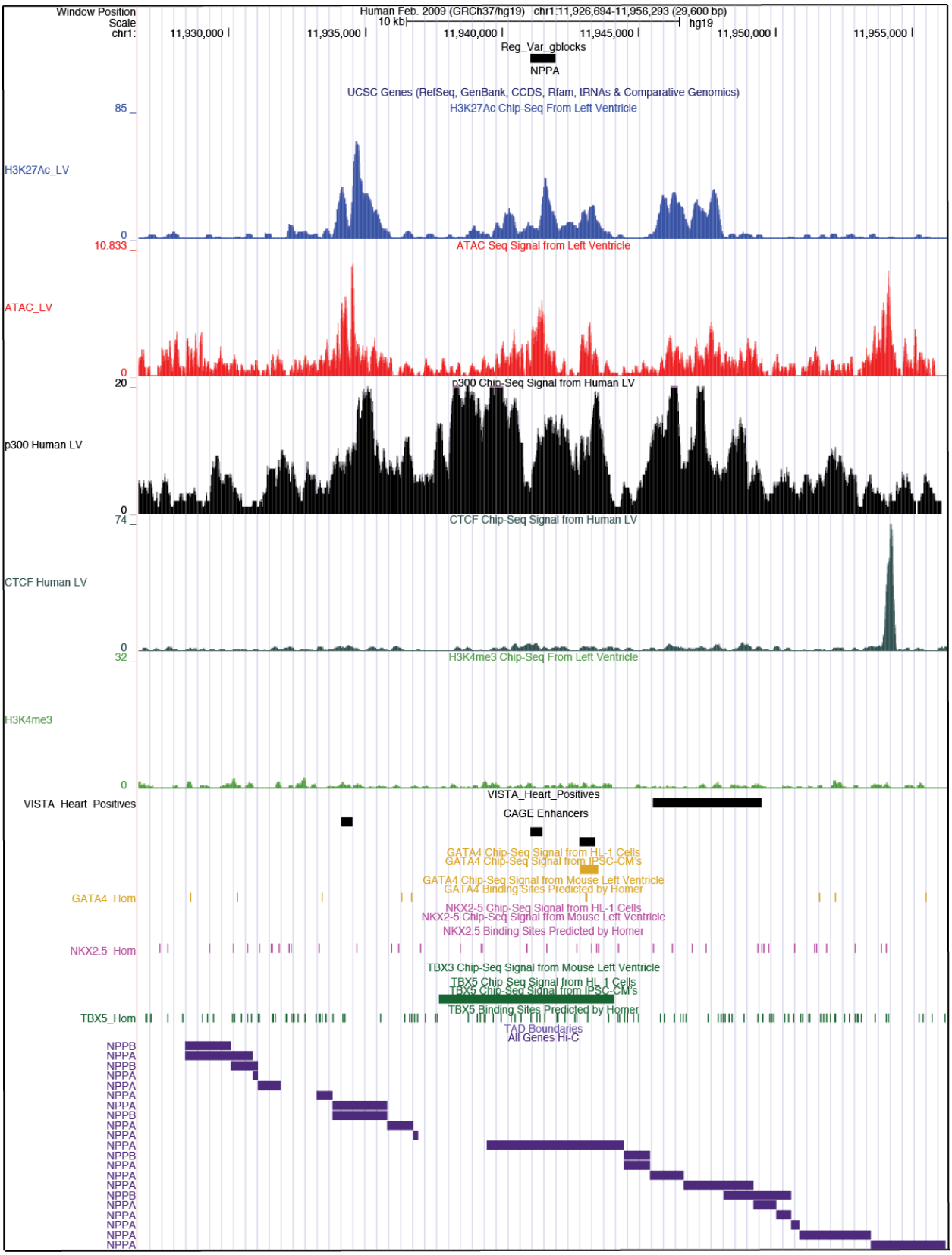

Figure XIII. Full map of *NPPA* Regulatory Variant.

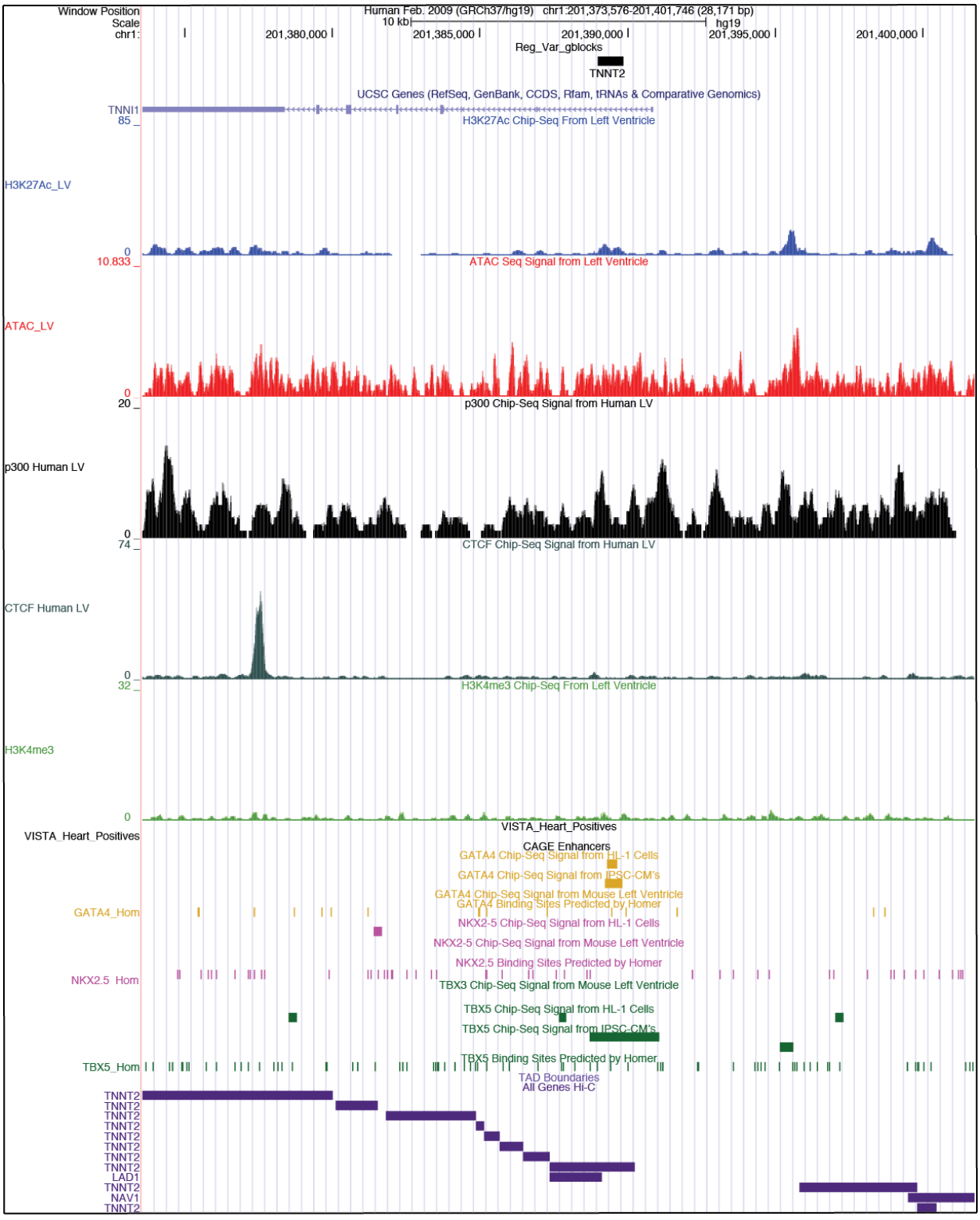

Figure XIV. Full map of *TNNT2* Regulatory Variant.

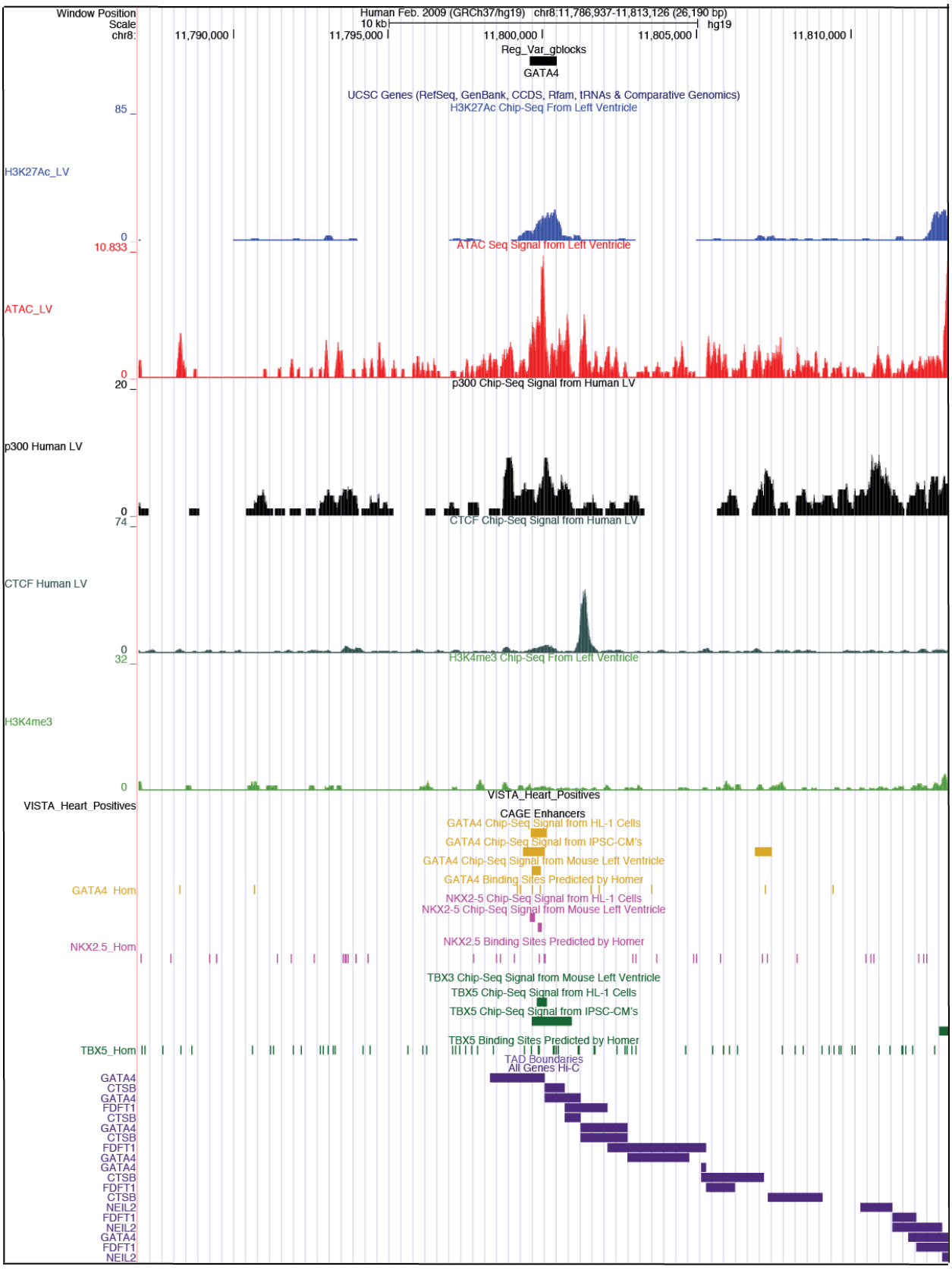

Figure XV. Full map of *GATA4* Regulatory Variant.

Table I. Datasets used for epigenomic identification of candidate enhancers

Target	Dataset	Accession Number	Reference
H3K27Ac Histone Modification	Human LV- ChiP-Seq	ENCSR150QXE	Roadmap Epigenomics Consortium, et al. 2015[46]
H3K4me3 Histone Modifications	Human LV- ChiP-Seq	ENCFF045RCM	Roadmap Epigenomics Consortium, et al. 2015[46]
Open Chromatin	Human LV-ATAC-Seq	ENCFF148ZMS	ENCODE Project. 2018[47]
	IPSC-CM- ATAC-Seq	GSE85330	Liu, Q. et al. 2017[48]
p300	Human LV- ChiP-Seq	GSE32587	May, D. et al. 2012[49]
CTCF	Human LV- ChiP-Seq	ENCFF482ZNO	ENCODE Project. 2018[47]
Promoter Interactions	IPSC-CM Promoter-Capture Hi-C	E-MTAB-6014	Montefiori, L. et al. 2018[19]
TAD Boundaries	Human LV- Hi-C	GSE58752	Leung, D. et al. 2015[21]
GATA4 Binding Sites	IPSC-CM-ChIP-Seq	GSM2280004	Ang, Y. et al. 2016[50]
	HL-1- ChIP-Seq	GSM558904	He, A. et al. 2011
	Mouse LV- ChIP-Seq	GSM862697	van den Boogaard, M. et al. 2012[51]
	Computational Predictions	HOMER	Heinz, S. et al. 2010[16]
TBX5/3 Binding Sites	IPSC-CM-ChIP-Seq	GSM2280011	Ang, Y. et al. 2016[50]
	HL-1- ChIP-Seq	GSM558908	He, A. et al. 2011[52]
	Mouse LV- ChIP-Seq	GSM862695	van den Boogaard, M. et al. 2012[51]
	Computational Predictions	HOMER	Heinz, S. et al. 2010[16]
NKX2.5 Binding Sites	HL-1- ChIP-Seq	GSM558906	He, A. et al. 2011[52]
	Mouse LV- ChIP-Seq	GSM862698	van den Boogaard, M. et al. 2012[51]
	Computational Predictions	HOMER	Heinz, S. et al. 2010[16]
eRNA Expression	Human LV-CAGE-Seq	GSE147236	Gacita, A. et al. 2020[53]
Experimentally Validated Heart Enhancers	Reporter Expression in Transgenic Mouse Embryos	VISTA Enhancer Browser	Visel, A. Et al. 2007[22]

Table II. Luciferase constructs tested in IPSC-CMs and HL-1 cells

Region Name	Primers	Size (bp)	Coordinates (hg19)	Distance to TSS (bp)
MYH7-C1-2	AGTTCAGCCCCATGAGGTAG GGTACCGAGGCGAGGGATATGGTGAAGG	673	chr14:23870150-23870823	34,047
MYH7-C1-3	GGGTCAGGTCTTTCACAAGC TTTTCCTCCTGTGCCCAAGAC	698	chr14:23870761-23871458	33,412
MYH7-C1-4	TCTTGGGCACAGGAGGAAAATTC TCCCTTCCTCCATTCACCC	697	chr14:23871436-23872136	32,734
MYH7-C2	CTGGCCTTGGCTTTTCTCCAG CAAACCAGGGTGGCCTCAAG	2072	chr14:23876121-23878188	26,682
MYH7-C2-1	AAACCTCCTCTTACCTGGGC TTGGGGAACAGAAGGAGACC	694	chr14:23877446-23878141	26,729
MYH7-C2-2	GCCCTACTCACCTTCCCATTC TGCCTCTCTGCTTCTAACCC	838	chr14:23876221-23877058	27,812
MYH7-C2-3	ACCTGGTTATCCCTTCACGG TGTCACCTCCAGAGCCAAAGG	844	chr14:23876782-23877626	27,244
MYH7-C3	GBLOCK	961	chr14:23912000-23912961	-7,130
MYH7-C4-1	TGTTCACAATCCCATCCCCA AGTGGGTCTCTGAAAAGGCA	1400	chr14:23913940-23915344	-9,070
MYH7-C4-2	TGGCTGGATTCCTGATGTG CGGACTTTGCCCTTCATAGCACC	2209	chr14:23915187-23917391	-10,317
MYH7-C5	GCCAGAGGCTGAGCGTGAATTAG GCAATTTGAATATGATATGCCCAGG	2223	chr14:23922666-23924886	-17,796
MYH7-C5-1	GBLOCK	790	chr14:23923381-23924171	-18,511
LMNA-C1-1	CCTGTCCTGGAGTGGCTAAATC GGGCAGGGGTTAGAATTCCTG	1156	chr1:155937201-155938359	-146,102
LMNA-C1-2	CATTCGGACTCTCTCTCCCC TTTAGCCACTCCAGGACAGG	1210	chr1:155936009-155937220	-147,241
LMNA-C2	GTTAGGTGCCGGGTTTTCTG TGATATGTGCATGTACGGCG	928	chr14:23904382-23905597	-17,195
LMNA-C3-1	CTCTCTCGTCCATCCTCCAC GCTCCTCTTCGGGTCTTGAAAG	1108	chr1:156074366-156075480	-8,981
LMNA-C3-2	ACTCCTCTAACAGCTGTGGG CCCCTTGGTGAATGGATCCA	1199	chr1:156073216-156074415	-10,046
LMNA-C4-1	GAAAGGGATTGGAGCGGAAAG CAGCAGCCCCTTAACTCTC	1211	chr1:156092084-156093294	7,623
LMNA-C4-2	TAACACTGCCACCTTCTGC TTGGCTAGTCTGTGGGTCTG	1392	chr1:156093103-156094494	8,642
LMNA-C5	TGAGATCACCTGGGCGAC AGAAGGGCTGGGCATCCTG	850	chr1:156095724-156096574	11,263
LMNA-C6	CCAGAAAAGGTGAGGGAGGTG GGGAGGGCCTAGGTAGAAGAG	1101	chr1:156099538-156100640	15,077

Negative distances refer to upstream the transcriptional start site (TSS) and positive distances are downstream.

Table III. qPCR Primers and CRISPR Guides Used in this Study

Name	Sequence(s)	Experiments/Notes
MYH7_C3_KO_G1	GCCTAGAAGTCCGGACACCG	Guide used to remove C3 Enhancer
MYH7_C3_KO_G2	GTGGTGTGGAACAAAGCGAA	Guide used to remove C3 Enhancer
MYH7_C3_KO_Homo_G1	CCTAGAAGTCCGGACAACCG	Guide used to remove C3 Enhancer in het. IPSCs
MYH7_C3_KO_Homo_G2	TGGTGTGGAACAAAGCCGAA	Guide used to remove C3 Enhancer in het. IPSCs
MYH7_C4_KO_G1	ATGGGATTGTGAACAGCGGA	Guide used to remove C4 Enhancer
MYH7_C4_KO_G2	CGTGATTTGGACTGGCGATC	Guide used to remove C4 Enhancer
MYH7_C6_KO_G1	CAGAGCCTCCCAAACCCGAA	Guide used to remove C6 Enhancer
MYH7_C6_KO_G2	TTTGTGGGGAGTGACCGGTC	Guide used to remove C6 Enhancer
MYH7_C6_KO_Homo_G1	CAGAGCCTCCCAAACCGAA	Guide used to remove C4 Enhancer in het. IPSCs
MYH7_C3_3PrimerGenotyping_Mix	1.AAGACAGTGGAGTGACGAGG 2.AAAGACCTCTAGTGCACCCC 3.AGAAGAGAACGAAGCGGGAA	Primers used for genotyping C3 enhancer KO IPSCs
MYH7_C4_3PrimerGenotyping_Mix	1.GAGAGGGTGGAGGAGGGT 2.TGCATTCCAGGCTGAGTGA 3.CCCCTTGGTACTGTCCTCAC	Primers used for genotyping C4 enhancer KO IPSCs
MYH7_C6_3PrimerGenotyping_Mix	AAAGGGTGCTTGGGACGTAG CCTCACTCTCCCCACAAGG GCCTGAGTAGCCCTGGAAA	Primers used for genotyping C6 enhancer KO IPSCs
hsMYH7_qPCR	F.GCAGCTAAAGGTCAAGGCC R.AGCTACTCCTCATTCAAGCC	Gene expression in IPSC-CMs Efficiency= 1.04
hsMYH6_qPCR	F.AAGTCCTCCCTCAAGCTCATGGC R.ATTTTCCCGGTGGAGAGC	Gene expression in IPSC-CMs Efficiency= 0.96
hsTNNT2_qPCR	F.AGGAGACCAGGGCAGAAGATG R.CTGGGCTTTGGTTTGGACTCC	Gene expression in IPSC-CMs Efficiency=0.98
hsMYBPC3_qPCR	F.CCCCATCTGAGTACGAGCG R.AGCCAGTTCCACGGTCAG	Gene expression in IPSC-CMs Efficiency= 0.95
hsSLC8A1_qPCR	F.AGTGCTGGGGAAGATGATGACGACG R.AGGATGGAGACAATGAAACACGCCC	Gene expression in IPSC-CMs Efficiency= 1.02
hsTNNI3_qPCR	F.CGTGTGGACAAGGTGGATGA R.CCGCTTAAACTTGCCTCGAA	Gene expression in IPSC-CMs Efficiency=1.06
hsMYOZ2_qPCR	F.AACACCCCAGATCCACGAAG R.GCCTCTAAAAGCTCCGGATC	Gene expression in IPSC-CMs Efficiency=1.02
hsGAPDH_qPCR	F.GTGGACCTGACCTGCCGTCT R.GGAGGAGTGGGTGTCGCTGT	Gene expression in IPSC-CMs Efficiency= 0.96

Table IV. Genotypes of enhancer deleted cells as determined by Sanger sequencing.

Target	Clone	Genotype Call	Allele 1		Allele 2	
			Guide 1 Site	Guide 2 Site	Guide 1 Site	Guide 2 Site
MYH7 C3	1	Heterozygous	WT +1 (T)	WT +1 (C)	Deletion +0	Deletion +0
MYH7 C3	18	Homozygous	Deletion +2 (CT)	Deletion +2 (CT)	Deletion -1 (T)	Deletion -1 (T)
MYH7 C4	2	Heterozygous	WT +1 (G)	WT +0	Deletion -6	Deletion -3
MYH7 C4	4	Homozygous	Deletion +0	Deletion +1 (G)	Deletion +0	Deletion -10
MYH7 C6	9	Heterozygous	WT +1 (G)	WT +0	Deletion -9	Deletion -10
MYH7 C6	2	Homozygous	Deletion +0	Deletion +0	Deletion -9	Deletion -10

Allele changes are shown relative to predicted cut site (+1 means a 1bp insertion at the predicted cut site).

Table V. Off target analysis in CRISPr-treated IPSCs.

#	Guide	#Mismatches	Location (hg19)	Annotation (Gene)	Result
1	MYH7_C3_KO_G1	3	chr3:43948037-43948059-	Intergenic (RP4-672N11.1-RP4-555D20.3)	Negative
2	MYH7_C3_KO_G2	2	chr3:8242570-8242592:+	Intron (LMCD1-AS1)	Negative
3	MYH7_C3_KO_G2	3	chr2:196514628-196514650:-	Intron (SLC39A10)	Negative
4	MYH7_C3_KO_G2	3	chr4:25160613-25160635:-	Exon (SEPSECS)	Negative
5	MYH7_C3_KO_G2	4	chr2:179579180-179579202:-	Exon (TTN)	Negative
6	MYH7_C4_KO_G1	2	chr2:237534886-237534908:-	Intergenic (ACKR3-AC011286.1)	Negative
7	MYH7_C4_KO_G1	3	chr18:55971940-55971962-	Intron (NEDD4L)	Negative
8	MYH7_C4_KO_G1	3	chr1:237019608-237019630:+	Intron (MTR)	Negative
9	MYH7_C4_KO_G1	4	chrX:33011884-33011906:+	Intron (DMD)	Negative
10	MYH7_C4_KO_G2	3	chr5:37853503-37853525:+	Intron (GDNF-AS1)	Negative
11	MYH7_C4_KO_G2	3	chr2:43370103-43370125:-	Intergenic (AC093609.1-THADA)	Negative
12	MYH7_C6_KO_G1	3	chr2:19143341-19143363:+	Intergenic (AC106053.1-AC092594.1)	Negative
13	MYH7_C6_KO_G1	3	chr9:134519754-134519776:-	Intron (RAPGEF1)	Negative
14	MYH7_C6_KO_G2	3	chr22:18336723-18336745:+	Intron (MICAL3)	Negative
15	MYH7_C6_KO_G2	4	chr2:224012528-224012550+	Intron (KCNE4)	Negative
16	MYH7_C6_KO_G2	4	chr1:32712994-32713016:-	Exon (FAM167B)	Negative