Supplemental Material

Methods

Human material

Biopsies on HCM patients were obtained as septal myomectomy specimens. Control samples were obtained from donor hearts not used for transplantation. Mutation and clinical data of patients are given in Table 1.

RNA sequencing

We performed RNA-seq on twenty-two human myocardial samples, eleven of which on diseased (HCM) and eleven in non-diseased state (Table 1, Supplemental Table 2). Prior to chromatin and RNA isolation, all frozen cardiac tissue biopsies were sectioned at the thickness of 10µm. RNA was isolated using Qiagen AllPrep Micro Kit according to the manufacturer's instructions. Sample quality assessed using the 2100 Bioanalyzer with a RNA 6000 Pico Kit (Agilent, Supplemental Table 2). After the selection of mRNA, libraries were prepared using the NEXTflex^{TM} Rapid RNA-seq Kit (Bio Scientific). Libraries were sequenced on the Nextseq500 platform (Illumina), producing single end reads of 75bp. Reads were aligned to the human reference genome GRCh37 using STAR v2.4.2a[1]. Picard's AddOrReplaceReadGroups v1.98 (http://broadinstitute.github.io/picard/) was used to add read groups to the BAM files, which were sorted with Sambamba v0.4.5[2] and transcript abundances were quantified with HTSeq-count v0.6.1p1[3] using the union mode. Subsequently, reads per kilobase million reads sequenced (RPKMs) were calculated with edgeR's RPKM function[4] (Supplemental Table 3). Genes with low counts (whose sum of all counts across samples included in the analysis was < 10) were removed. In order to obtain a

list of genes expressed in either HCM or control group, we categorized transcripts that are expressed as having >0.5 RPKM averaged across samples of each group (expected reads per kilobase of transcripts per million fragments sequenced). In order to obtain a list of differentially expressed genes between HCM and controls at FDR<0.05, we employed Deseq2 v1.10.1 package[5]. We calculated *p*-values using Wald statistics and corrected for multiple testing using the Benjamini-Hochberg method. We retrieved transcription start sites (TSS) of the obtained genes from Ensembl Genes 89 using Biomart (Human genes GRCh37.13)[6]. We defined promoter regions as ranging from -2500 to +2500 base-pairs (bp) from the TSS. Differentially regulated genes can be found in Supplemental Table 4.

Chromatin Immunoprecipitation and Sequencing

Chromatin immunoprecipitation and sequencing (ChIP-seq) using H3K27ac mark was performed on human myocardial samples from fourteen HCM patients and four control donors, partially matching the samples included in the RNA-seq experiment described above (Table 1). Chromatin was isolated from each sample using the MAGnify™ Chromatin Immunoprecipitation System kit (Life Technologies) according to the manufacturer's instructions. Immunoprecipitations were performed with antibody H3K27ac (ab4729, Abcam) for ChIP-seq as described previously[7]. ChIP DNA Clean & Concentrator kit (Zymo Research) was used to purify captured DNA fragments. Libraries were prepared using the NEXTflex™ Rapid DNA Sequencing Kit (Bioo Scientific) and sequenced on Illumina NextSeq500 sequencer. Alignment to the human reference genome (hg19) was performed using BWA v0.7.5a[8] . Duplicated, unmapped reads and reads with mapping quality less than 5 were removed using Samtools v1.3 [9]. Peak calling was performed using MACS v 2.1.0[10] using the respective input samples, -gsize=hg -nomodel parameters, and estimated fragment sizes (-extsize) predicted by PhantomPeakQualTools v1.1[11]. Epigenomic profiles were compared using DiffBind v2.2.12[12] to identify enhancer peaks that are quantitatively different

(FDR<0.05, Deseq2 algorithm) between control and HCM populations. Consensus peaksets were formed by peaks that overlapped in at least two samples. Differentially acetylated regions can be found in Supplemental Table 5.

Super-Enhancers

We identified super-enhancer regions on the 14 HCM H3K27ac datasets described previously by employing ROSE algorithm[13] using a stitching distance of 12.5kb. Peaks fully contained in the region spanning 2500bp upstream and downstream of an annotated TSS were excluded. HCM-specific super-enhancers were defined as super-enhancer in HCM not overlapping super-enhancers in the control septum samples. These can be found in Supplemental Table 6. BEDtools v2.17.0[14] and BEDOPS v2.4.35[15] were used for manipulation of bed-files. Super-enhancer regions from non-diseased human left ventricle (LV) were retrieved from the Super-Enhancer Archive (SEA)[16].

Open Chromatin

All datasets of regulatory features generated in this study were further narrowed down to regions overlapping maps of open chromatin in heart, in order to retain only the sequences accessible to transcription factors (TF). We retrieved peaks from the "open chromatin" tracks from ENCODE portal in heart tissue. These datasets were generated using DNaseI hypersensitivity or Formaldehyde-Assisted Isolation of Regulatory Elements (FAIRE) assays. For the DNaseI assays, 3 biological replicates from 3 non-diseased adult hearts were included. For the FAIRE assays, 2 biological replicates from 2 non-diseased adult hearts were included. A peaks file with GEO accession GSM1008559, identified as Heart_OC, was downloaded from UCSC Genome Browser (https://genome.ucsc.edu/).

Integrative analysis and statistical enrichment of QRS-associated variants on specific regulatory regions

We used FUMA[17] to expand the set of 52 lead SNPs to candidate SNPs in LD, using the following parameters: *p*-value of lead SNPs $<1x10^{-8}$, reference panel population 1000G Phase3 EUR, minor allele frequency ≥ 0.01, maximum distance between LD blocks to merge into a locus < 1000kb. We generated sets of LD variants in the following r^2 windows: 0.05 =< r^2 < 0.1, 0.1 =< r^2 < 0.2, 0.2 =< r^2 < 0.3, 0.3 =< r^2 < 0.4, 0.4 =< r^2 < 0.5, 0.5 =< r^2 < 0.6, 0.6 <= r^2 < 0.7, 0.7 <= r^2 < 0.8, 0.8 <= r^2 < 0.9 and 0.9 <= r^2 <= 1. We used bedtools v2.19.1[14] to identify candidate variants from the generated sets that overlap with differential regulatory regions of interest, including HCM-specific super-enhancers, LV-specific super-enhancers, regions differentially acetylated between HCM patients versus controls, and promoter regions of genes differentially expressed between HCM patients and controls. A SNP falling within coordinates of regulatory regions of interest was considered as overlapping SNP. To assess the significance and enrichment of the amount of overlaps found, we sampled 10,000 randomized sets of SNPs from the total set of HapMap phase 2 CEU SNPs[18]. HapMap SNPs were split into 5 equal minor allele frequency (MAF) bins ranging from 0=< MAF <= 0.5 and into 2 bins of distance (intronic and intergenic SNPs). Background distribution was sampled from these bins in order to account for similar MAF and distance to gene. We calculated the p-value using binominal cumulative distribution function b(x; n, p) (as done previously[19, 20]) using R[21] pbinom() function. We carried out permutation tests using overlaps with all promoters/enhancer elements identified in HCM and control hearts, and with regions resulting from differential analysis between HCM patients and controls.

Gene mapping

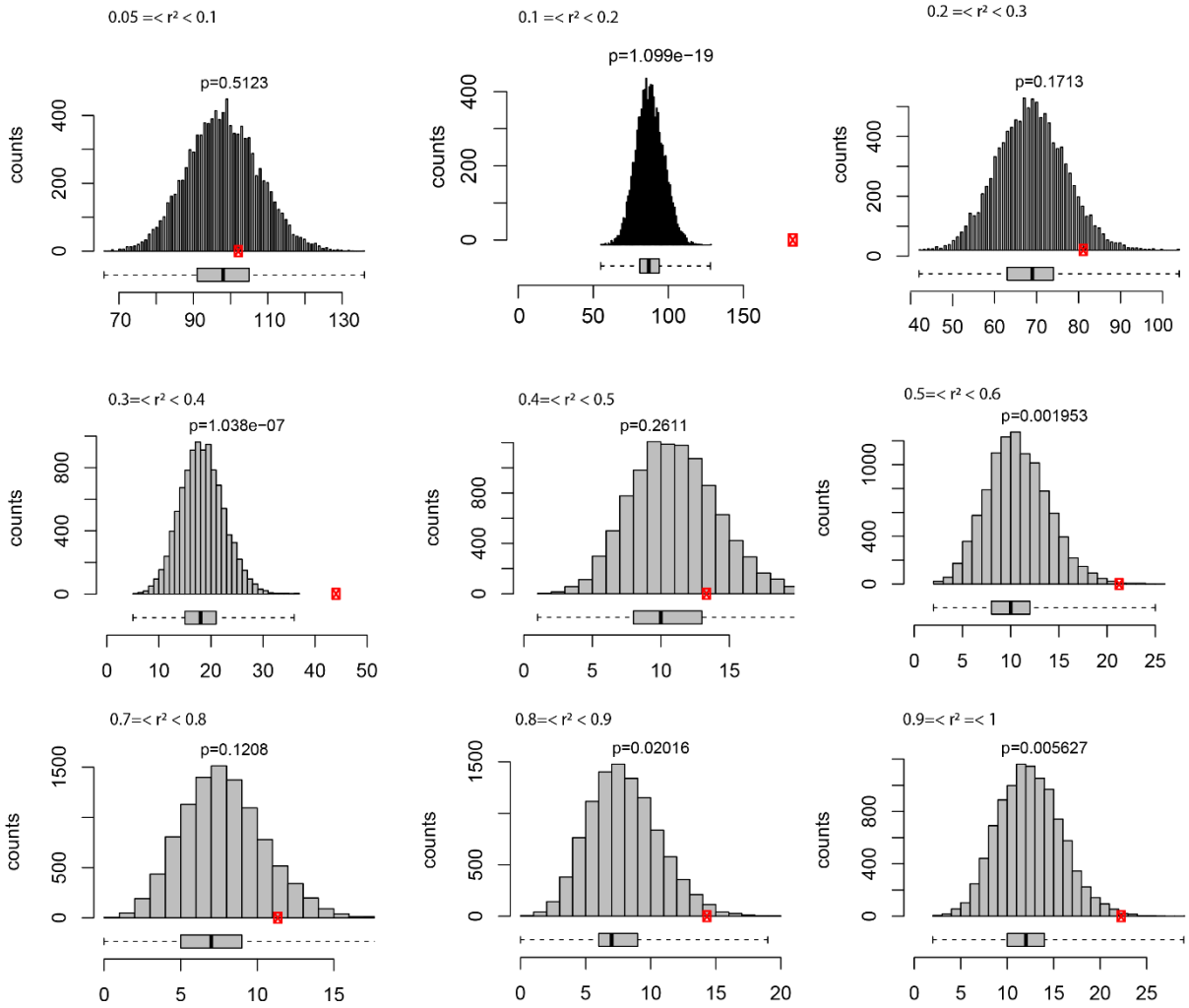
We used FUMA[17] for eQTL mapping, linking SNPs to genes whose expression is likely affected up to 1 Mb (cis-eQTL). Since eQTLs are highly tissue specific, LV was selected from GTEx portal. We defined significant eQTLs as FDR ≤ 0.05. The gene FDR is pre-calculated by GTEx and every gene-tissue pair has a defined p-value threshold for eQTLs based on permutation[17]. eQTL maximum *p*-value was defined as $\leq 1x10^{-3}$. In addition, we identified which of the three closest genes to QRS-candidate causative variants overlapping differential regulatory elements were differentially expressed.

Visualization of overlaps

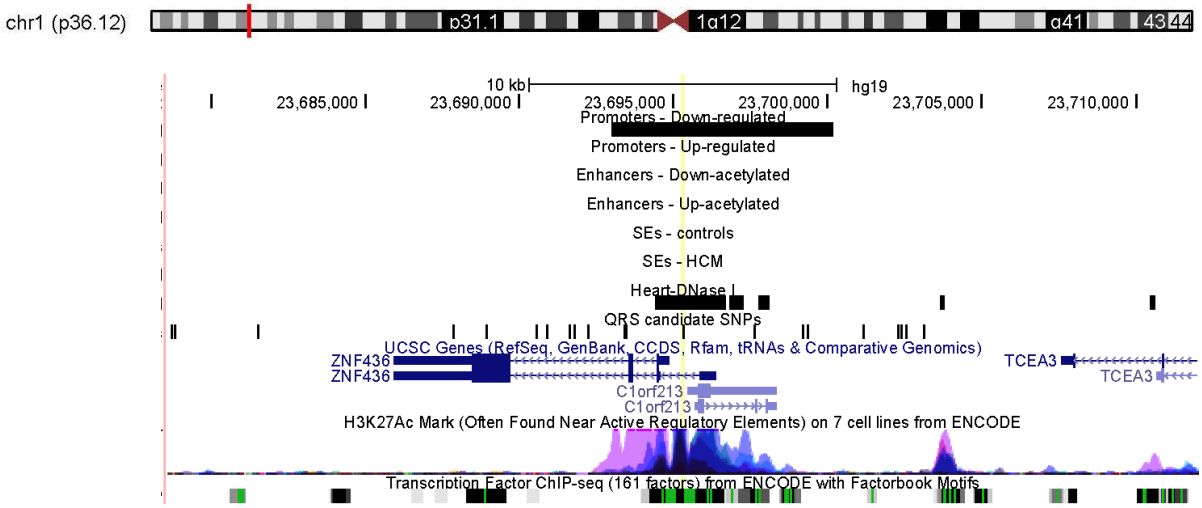
All 4620 QRS-associated candidate SNPs (52 lead SNPs and variants in LD $r^2>0.5$) were plotted on UCSC Genome Browser on Human GRCh37/hg19 assembly[22]. The following tracks were included in their full form (not filtered by open chromatin regions): down-regulated promoters in HCM versus controls, up-regulated promoters in HCM versus controls, down-acetylated regions in HCM versus controls, up-acetylated regions in HCM versus controls, super-enhancers specific to HCM (not overlapping super-enhancers found in the control group), LV super-enhancers from the Super-Enhancer Archive[16]. Finally, the heart DNase track from ENCODE[23], showing the regions to which differential regulatory elements were further narrowed down as regions of transcription factor binding. The 74 variants overlapping regulatory elements affected in HCM overlapping open chromatin in heart and spread through 20 QRS-associated loci can be seen on Supplemental Figures 2 to 21. Resolution is set to 10kb or 20kb (information present in each figure). UCSC genes, a layered mark of H3K27ac on 7 cell-lines and a transcription factor track from ENCODE are also shown.

Supplemental Figures and Tables

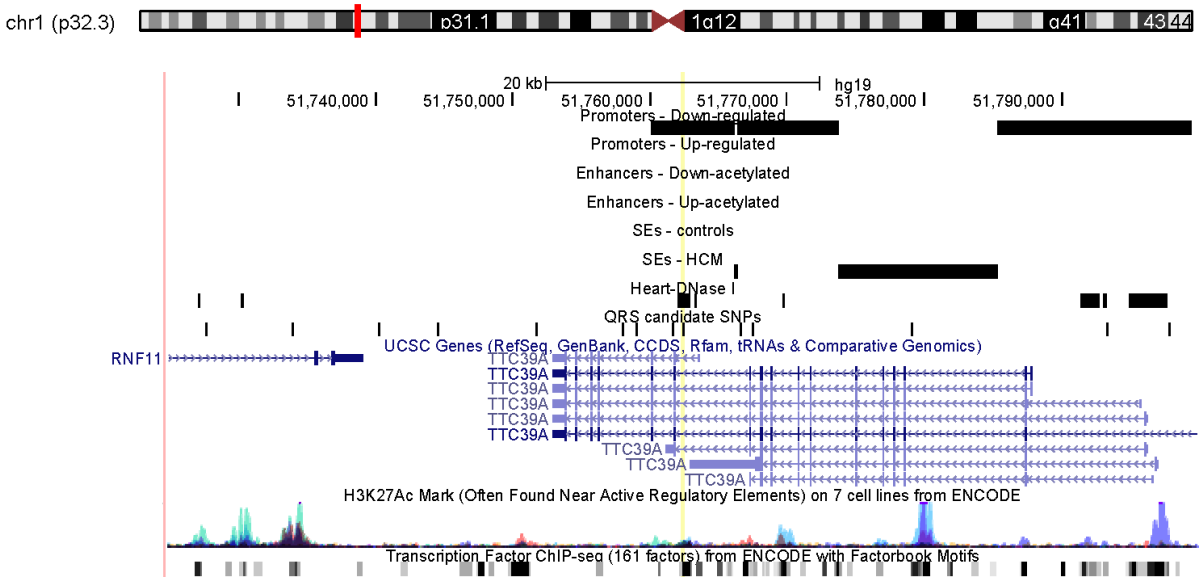
Supplemental Figures



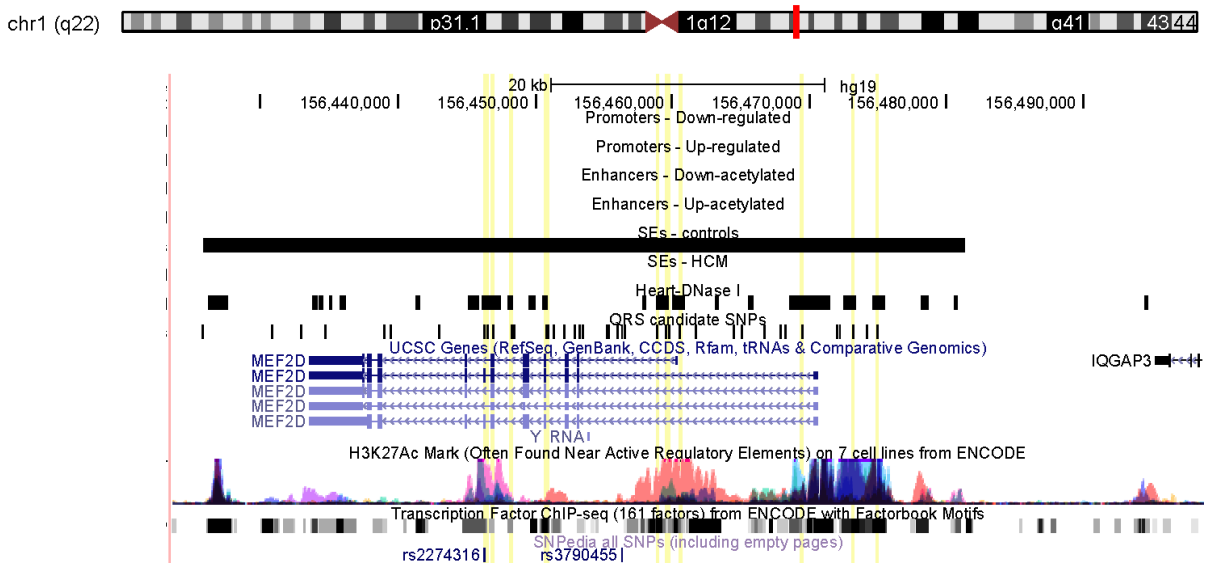
Supplemental Figure 1. Mean number of differential regulatory elements overlapping with QRS-associated loci (red circle) belonging to 9 windows of LD r^2 values, compared with 10,000 matched control sets (gray bars).



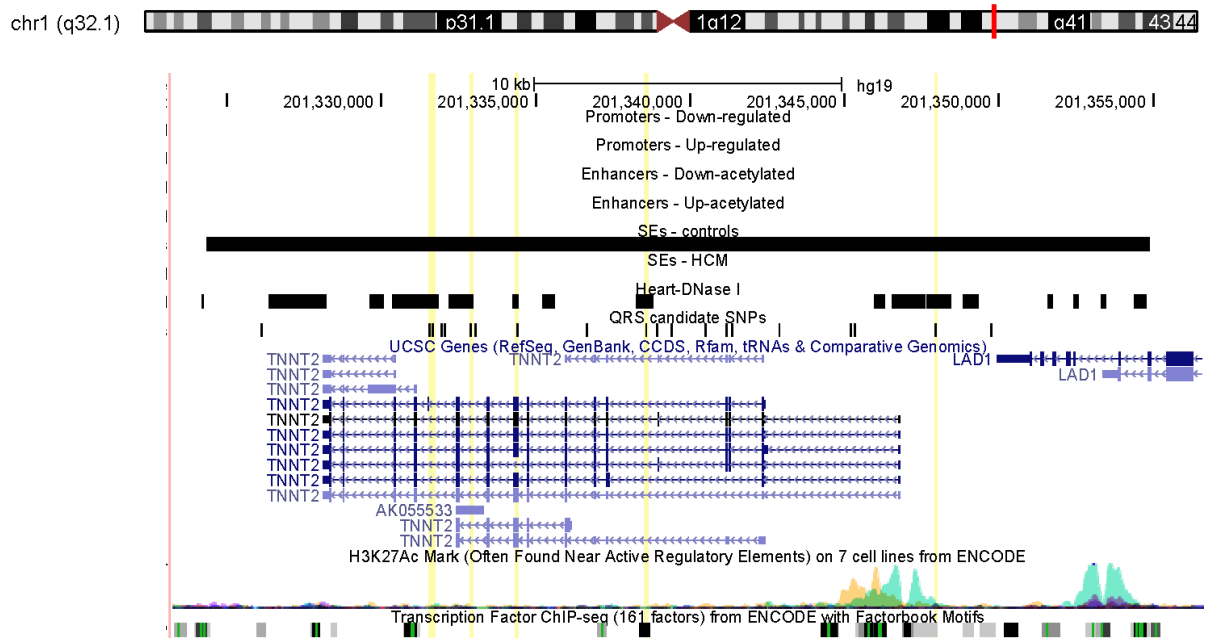
Supplemental Figure 2. Loci of lead QRS-associated SNP rs2849028, in which variant rs680386, in LD r^2=0.6, overlaps (in yellow) a down-regulated promoter on gene *ZNF436*.



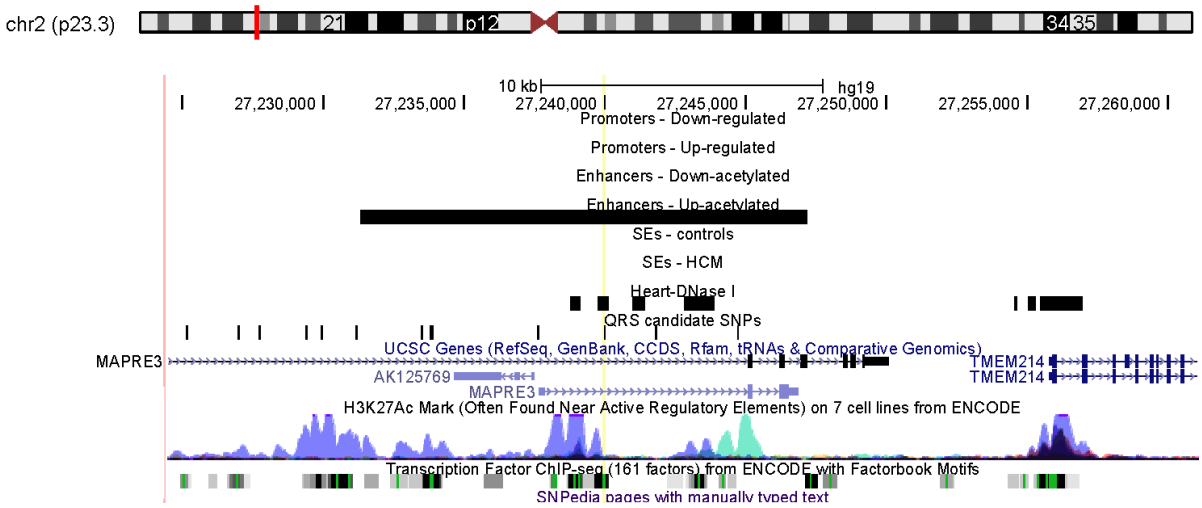
Supplemental Figure 3. Loci of lead QRS-associated SNP rs17391905, in which variant rs72694106, in LD r^2=0.6, overlaps (in yellow) a down-regulated promoter on gene *TTC39A*.



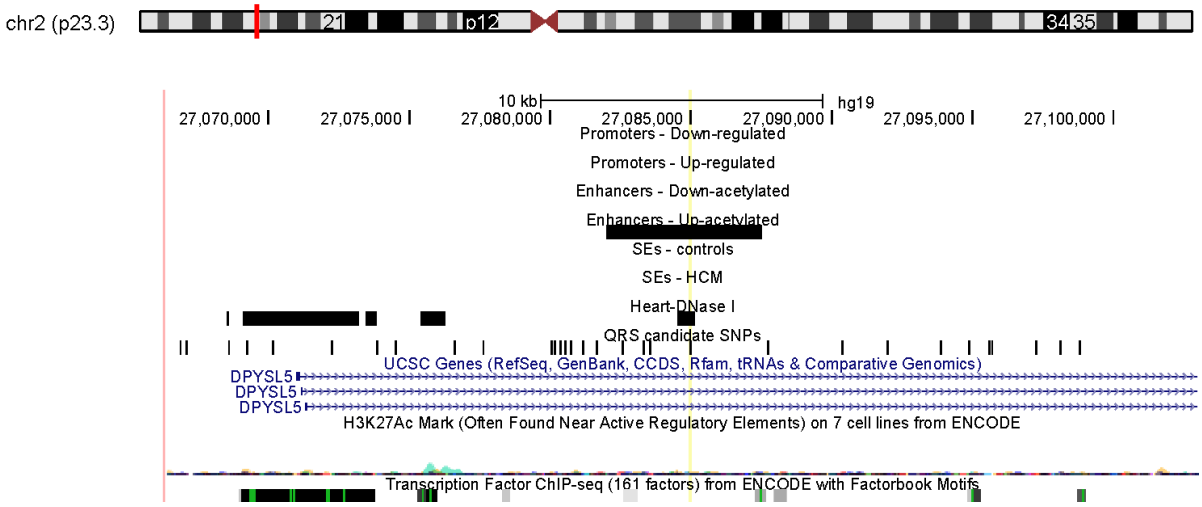
Supplemental Figure 4. Loci of lead QRS-associated SNP rs2274317, in which variants rs2274316, rs12131289, rs2274317, rs4450010, rs1185700, rs1925950, rs2274319, rs3790457, rs12038396, rs3790458, rs746527, rs3790461, rs12136856 and rs538050729, in LD r^2>0.6, overlap (in yellow) an LV super-enhancer.



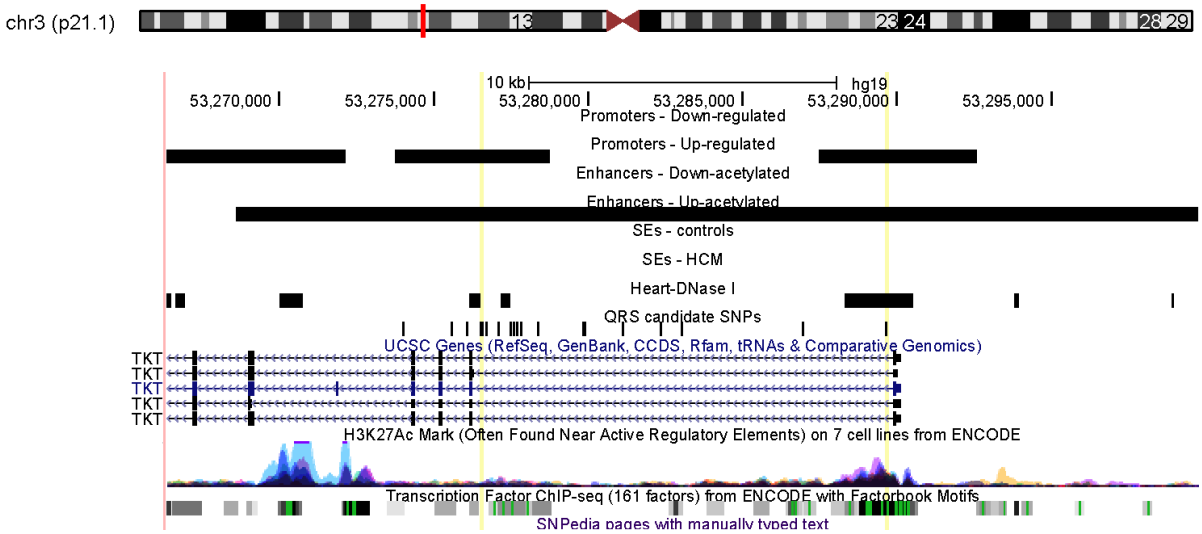
Supplemental Figure 5. Loci of lead QRS-associated SNP rs10920184, in which variants rs1104859, rs2365652, rs1892026, rs3729547, rs10800776, rs10920183 and rs4915232, in LD r^2>0.6, overlap (in yellow) an LV super-enhancer.



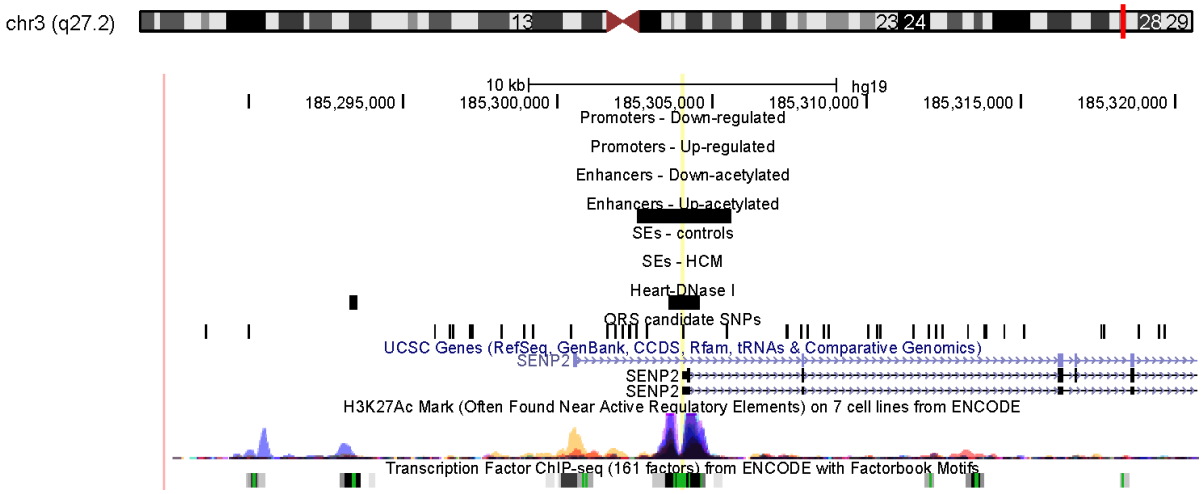
Supplemental Figure 6a. Loci of lead QRS-associated SNP rs6710065, in which variants rs2252860, rs2304337, in LD r^2>0.7, overlap (in yellow) an LV super-enhancer.



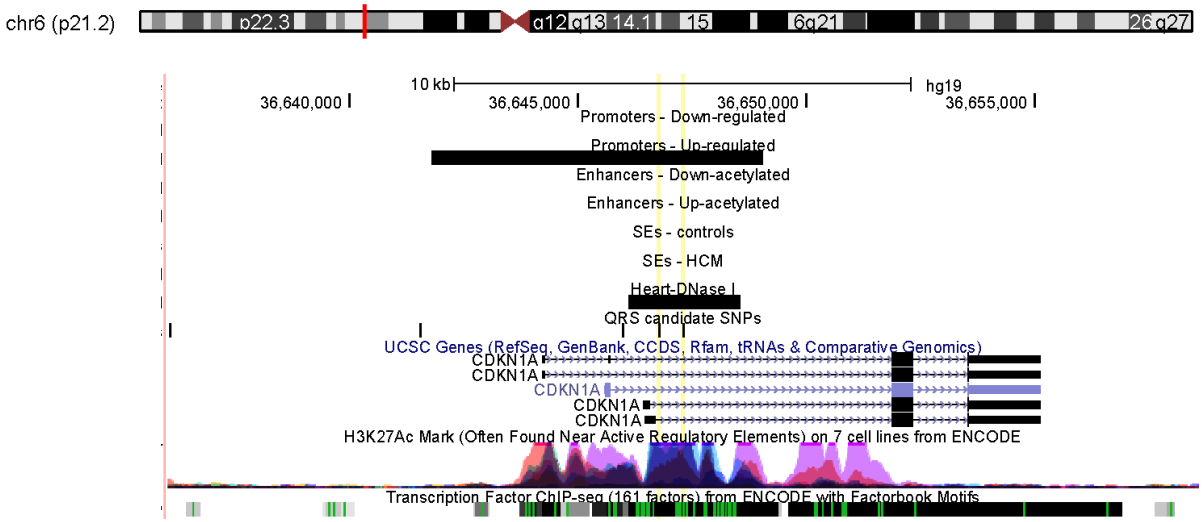
Supplemental Figure 6b. Loci of lead QRS-associated SNP rs6710065, in which variants rs34902032 and rs11677841, in LD r^2>0.5, overlap (in yellow) an up-acetylated enhancer.



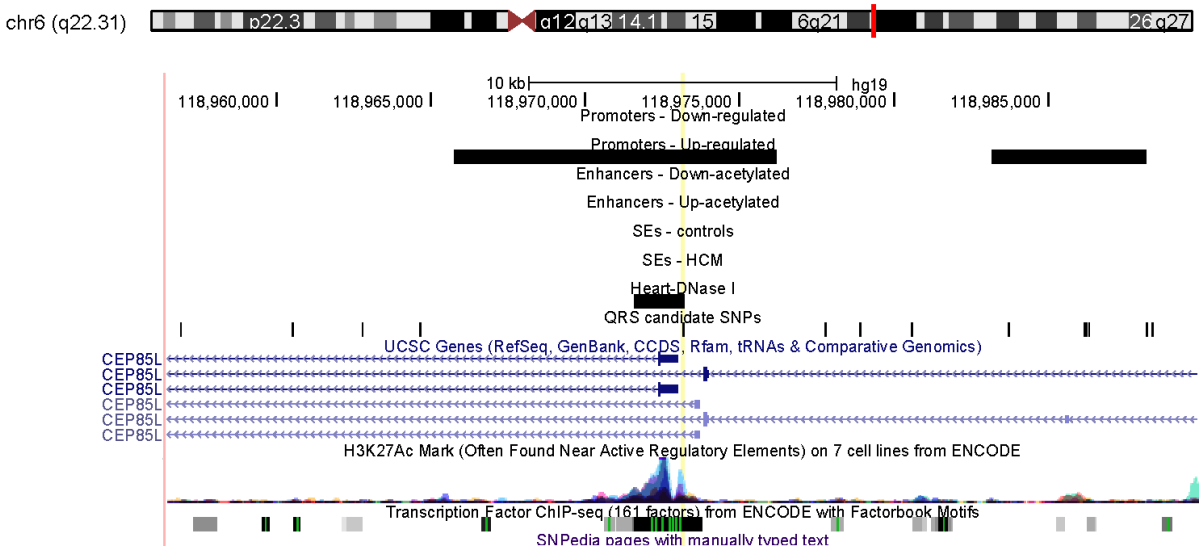
Supplemental Figure 7. Loci of lead QRS-associated SNP rs4687718, in which variants rs968702 and rs62256006, in LD r^2>0.6, overlap (in yellow) an up-regulated promoter on gene *TKT*, as well as up-acetylated regions.



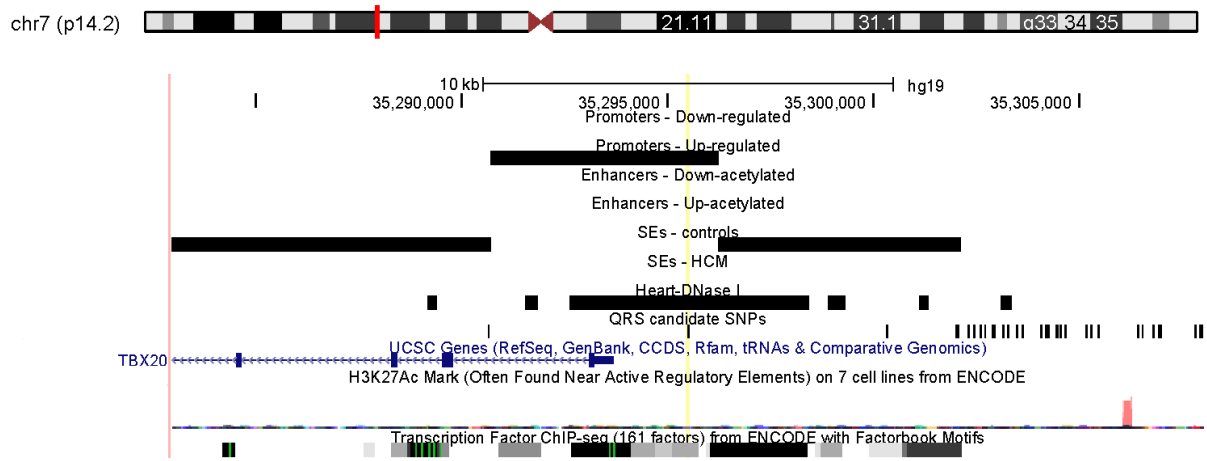
Supplemental Figure 8. Loci of lead QRS-associated SNP rs4687718, in which variants rs968702 and rs62256006, in LD r^2>0.6, overlap (in yellow) an up-regulated promoter as well as up-acetylated regions.



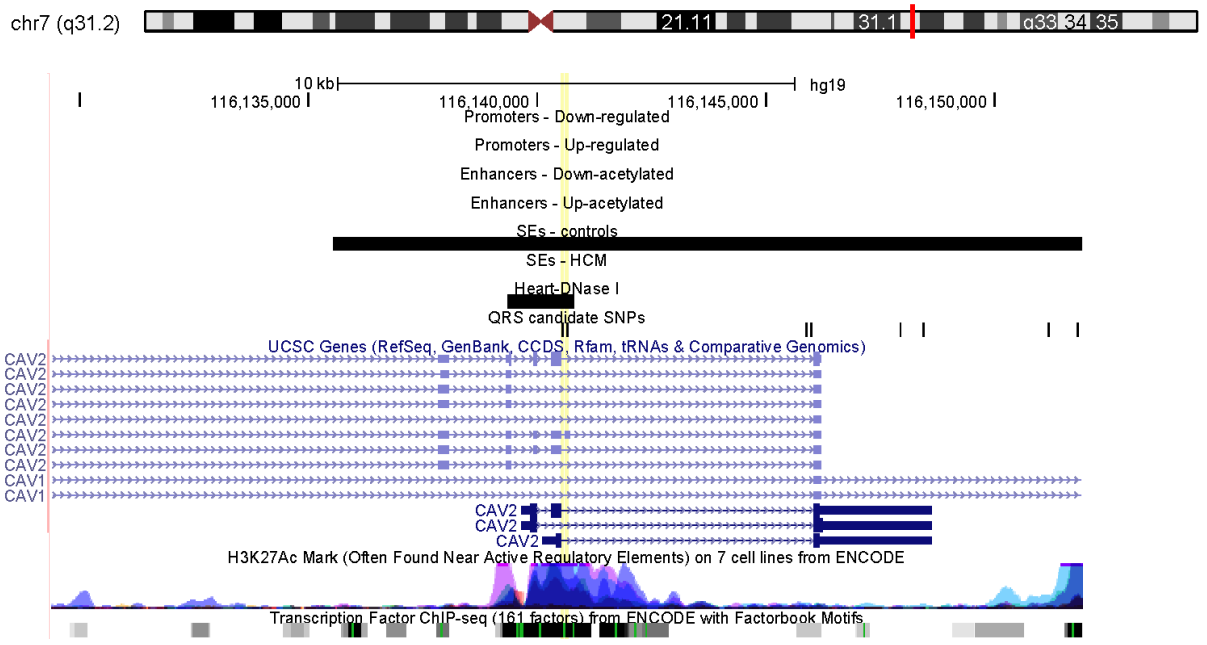
Supplemental Figure 9. Loci of lead QRS-associated SNP rs1321311, in which variants rs146170154 and rs3176326, in LD r^2>0.7, overlap (in yellow) an up-regulated on gene *CDKN1A*.



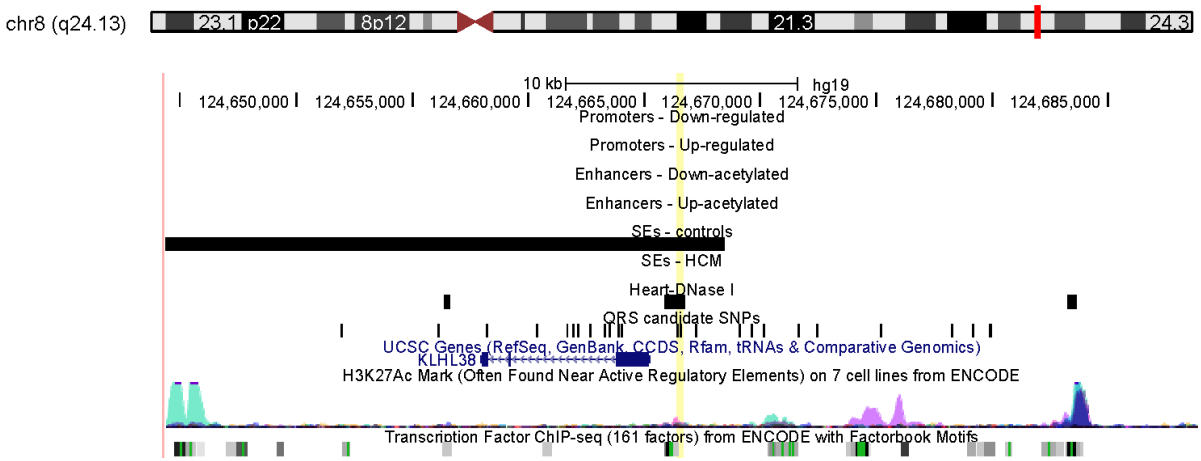
Supplemental Figure 10. Loci of lead QRS-associated SNP rs11153730, in which variant rs6913012, in LD r^2=0.7, overlap (in yellow) an up-regulated on gene *CEP85L*.



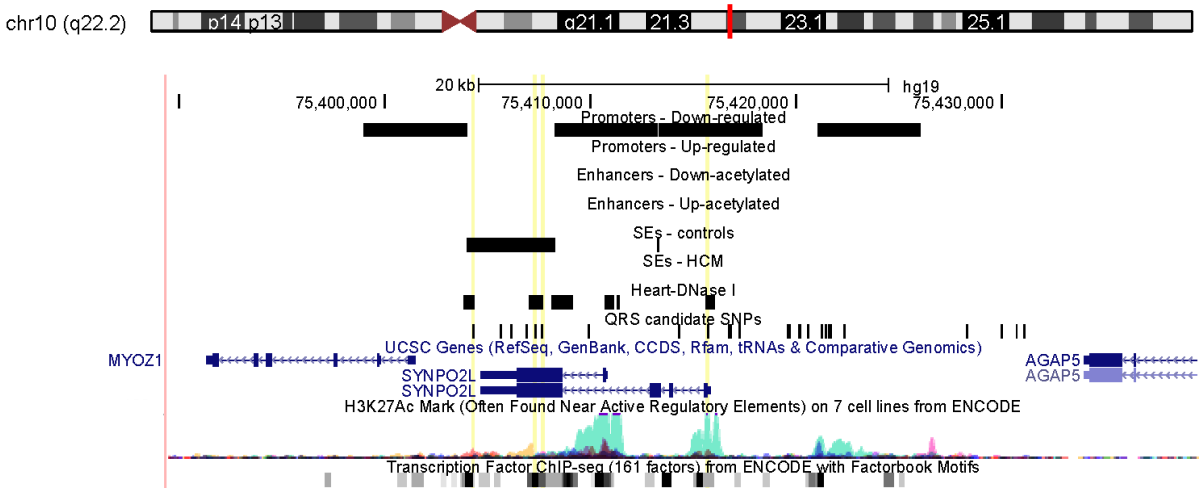
Supplemental Figure 11. Loci of lead QRS-associated SNP rs1419856, in which variant rs1003549, in LD r^2=0.7, overlap (in yellow) an up-regulated on gene *TBX20*.



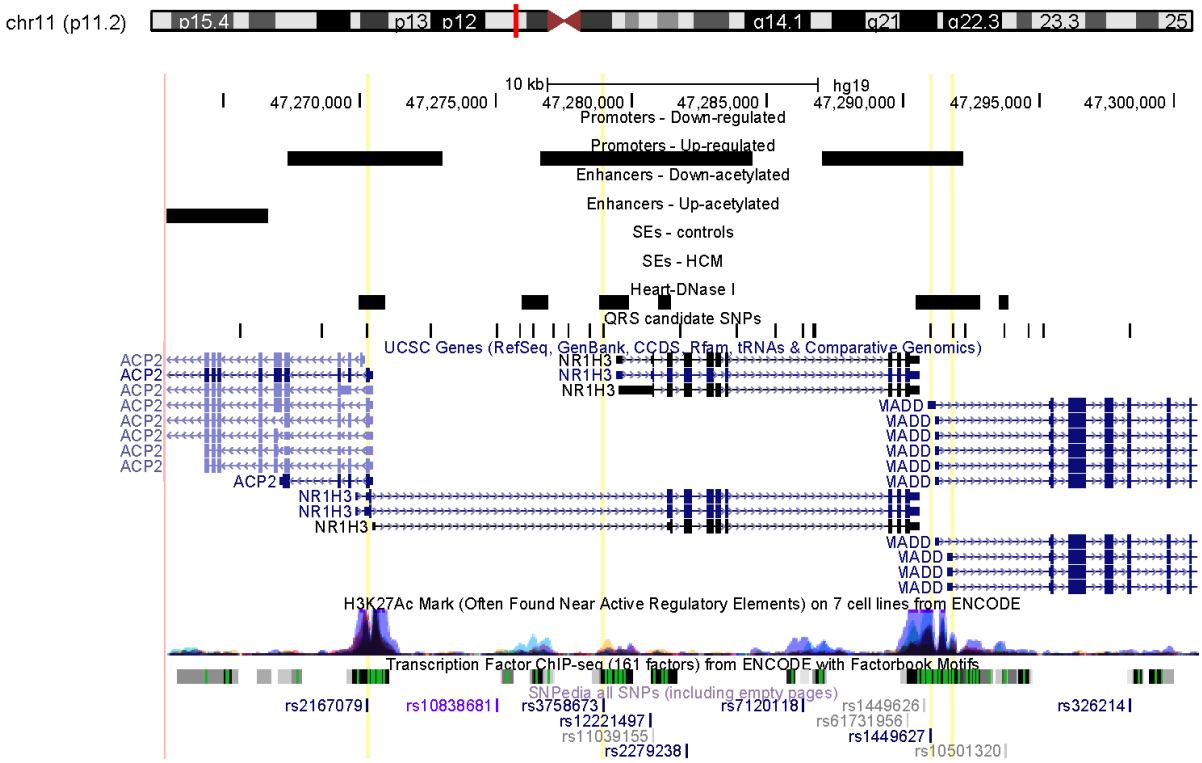
Supplemental Figure 12. Loci of lead QRS-associated SNP rs11773845, in which variants rs2270188 and rs2270189, in LD r^2=0.5, overlap (in yellow) an LV super-enhancer.



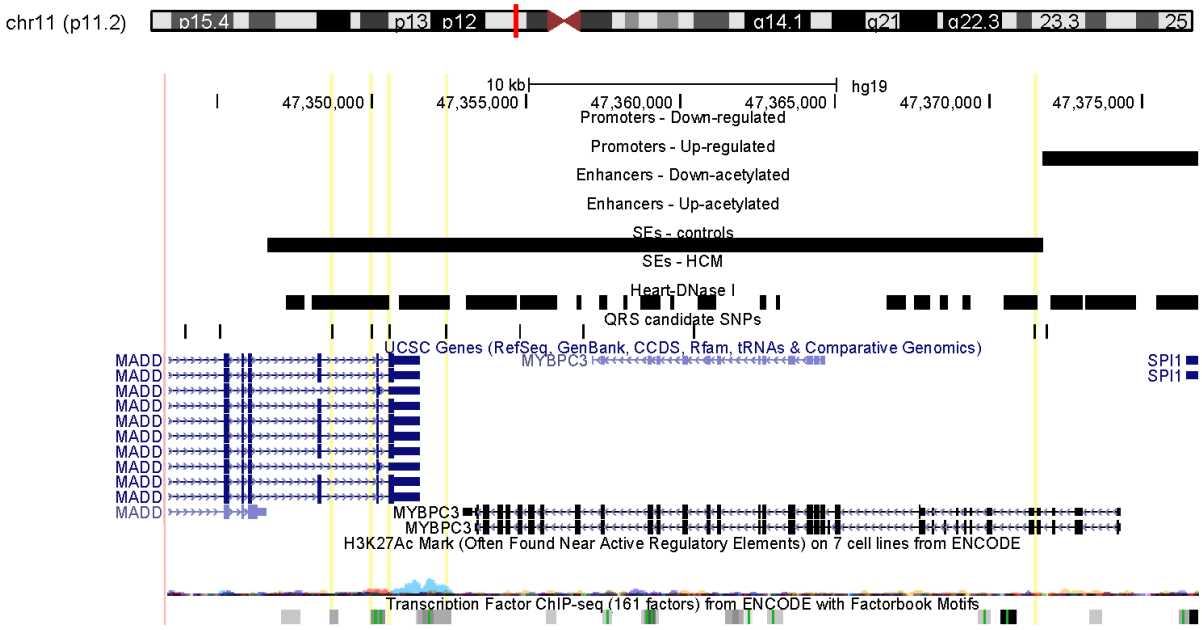
Supplemental Figure 13. Loci of lead QRS-associated SNP rs4367519, in which variant rs143950919, in LD r^2=1, as well as lead SNP rs4367519 itself, overlap (in yellow) an LV super-enhancer.



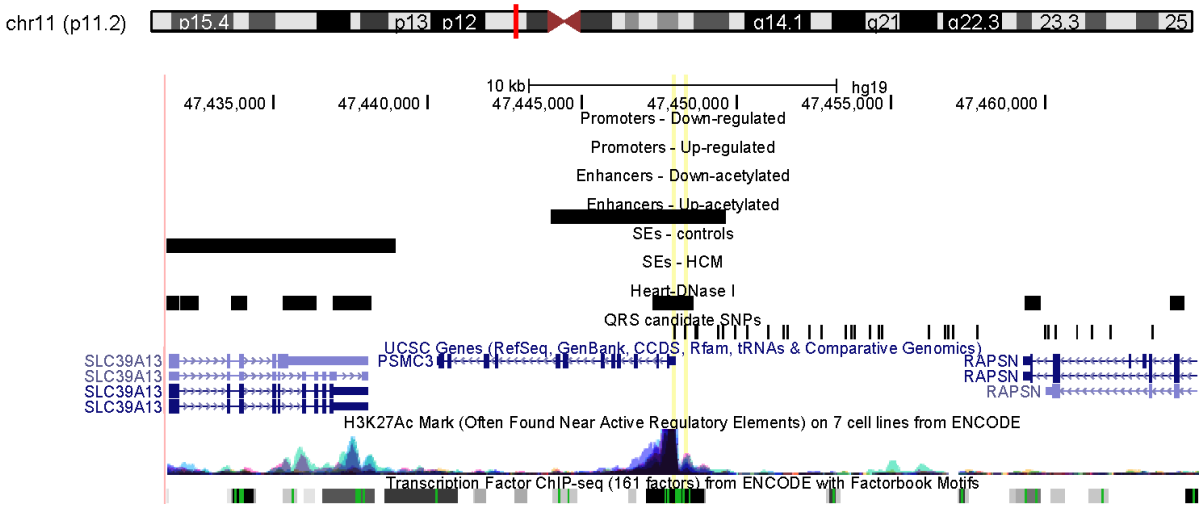
Supplemental Figure 14. Loci of lead QRS-associated SNP rs7099599, in which variants rs11000728, rs3812629 and rs4746139, in LD r^2=0.8, overlap (in yellow) an LV super-enhancer, while rs60632610, in LD r^2=0.9, overlaps the down-regulated promoter (and exon) region of *SYNPO2L*.



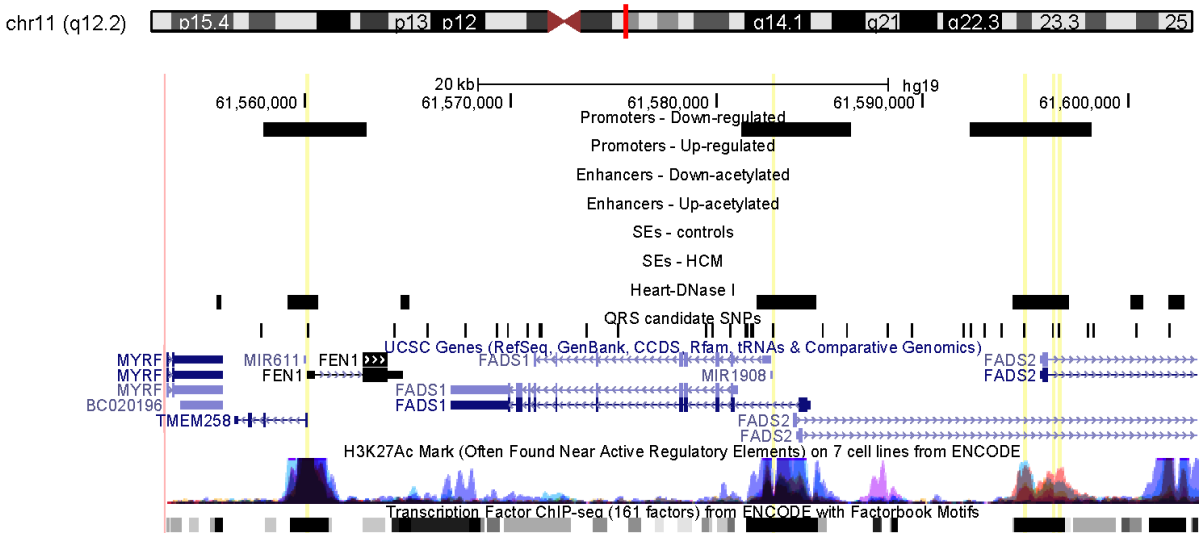
Supplemental Figure 15a. Loci of lead QRS-associated SNP rs2269434, in which variants rs2167079, rs3758673, rs1449627 and rs142960070, in LD r^2=0.8, overlap (in yellow) up-regulated promoter regions.



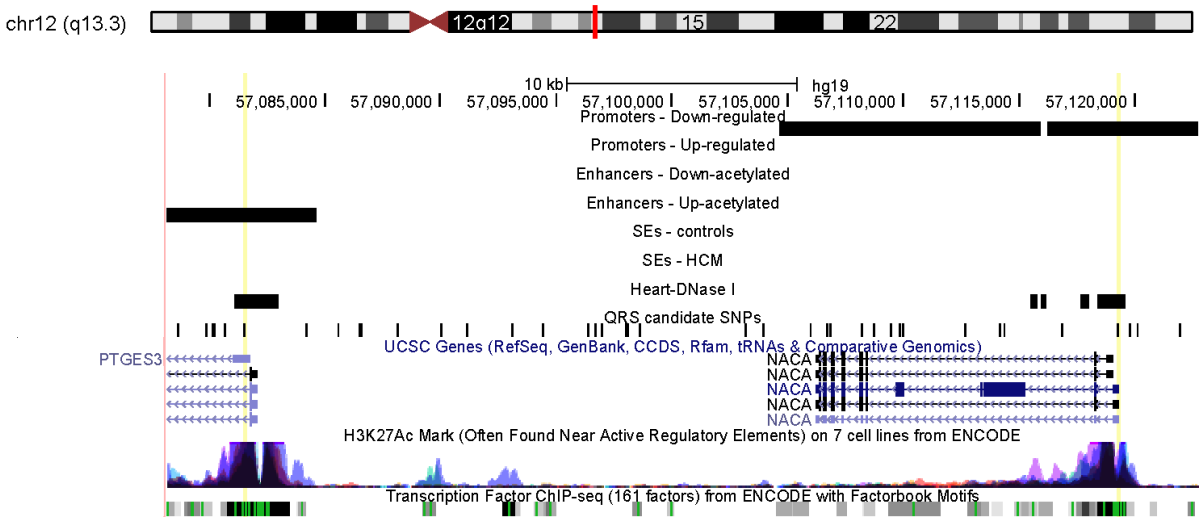
Supplemental Figure 15b. Loci of lead QRS-associated SNP rs2269434, in which variants rs3781622, rs753993, rs10838693, rs4752825 and rs11570050, in LD r^2>0.5, overlap (in yellow) an LV super-enhancer.



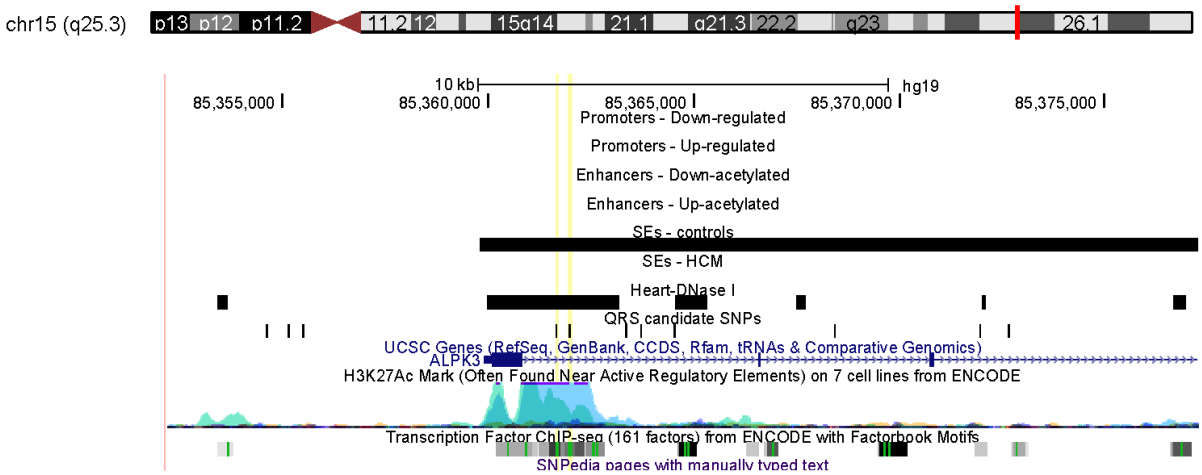
Supplemental Figure 15c. Loci of lead QRS-associated SNP rs2269434, in which variants rs7948705 and rs11307826, in LD r^2=0.6, overlap (in yellow) an up-acetylated region.



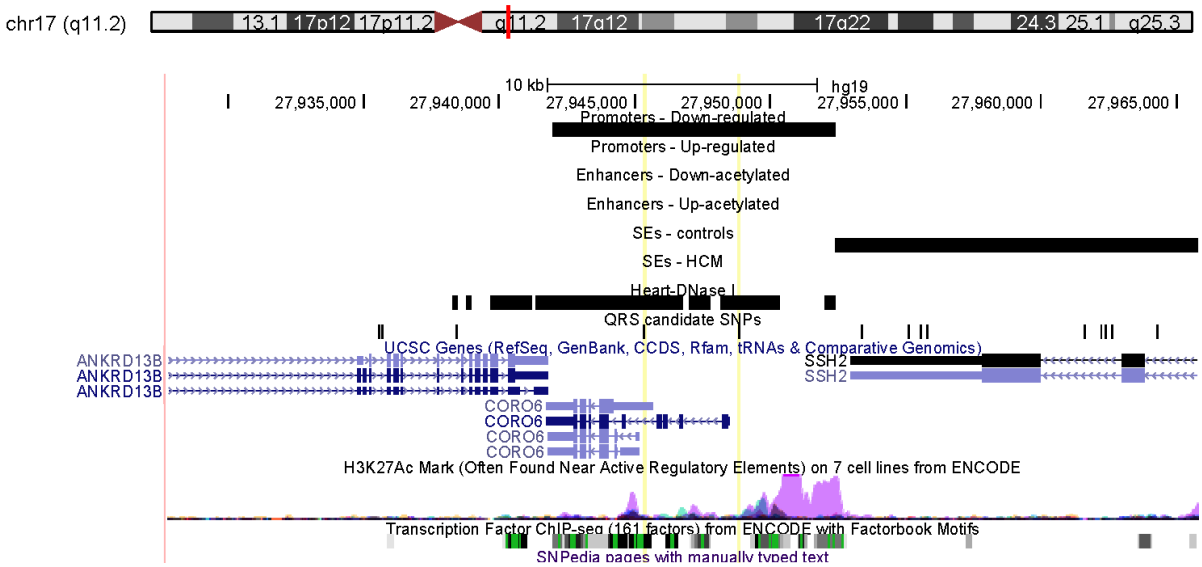
Supplemental Figure 16. Loci of lead QRS-associated SNP rs174577, in which variants rs174538, rs174561, rs3834458, rs5792235 and rs99780, in LD r^2>0.8, overlap (in yellow) up-regulated promoter regions.



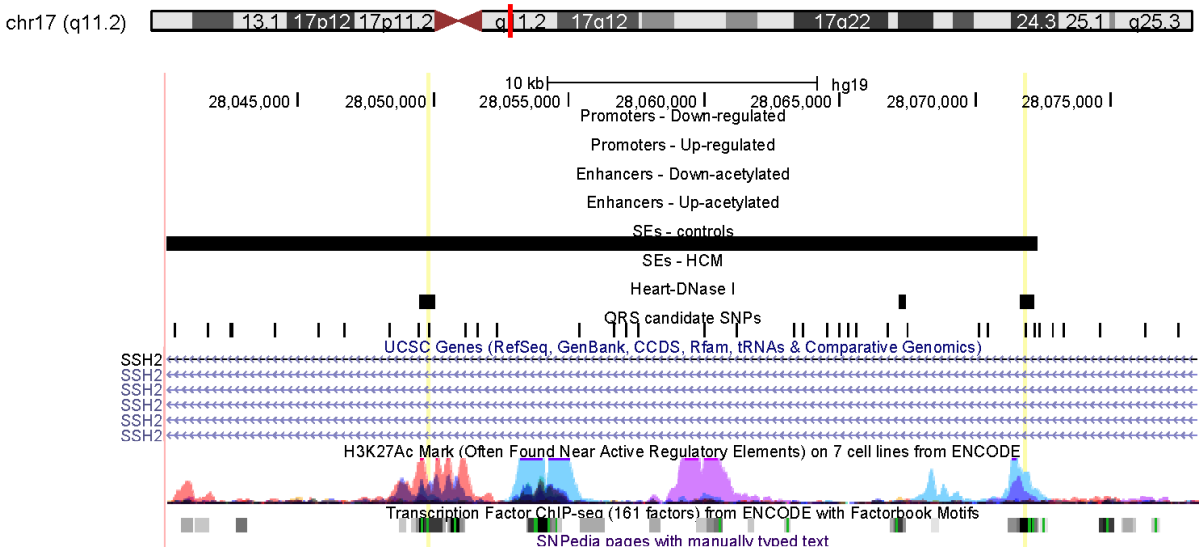
Supplemental Figure 17. Loci of lead QRS-associated SNP rs2926743, in which variants rs2958153, in LD r^2=0.9, overlaps (in yellow) an up-acetylated region, while rs3214051, in LD r^2=0.6, overlaps a down-regulated promoter.



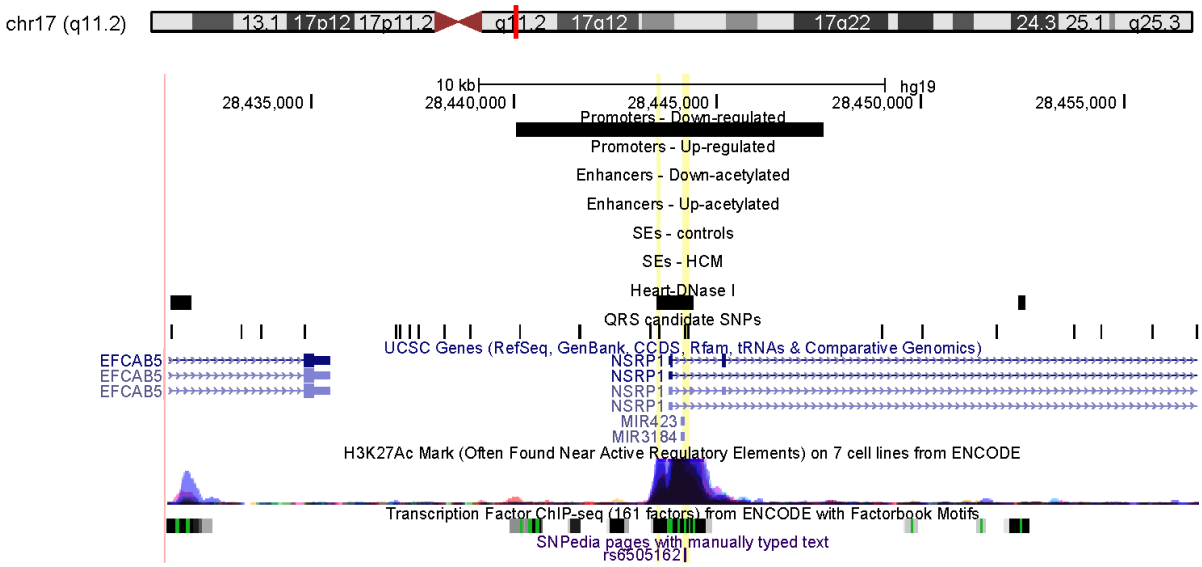
Supplemental Figure 18. Loci of lead QRS-associated SNP rs7183401, in which variants rs8039472, rs4633690, and rs2879828, in LD r^2>0.8, overlap (in yellow) an LV super-enhancer.



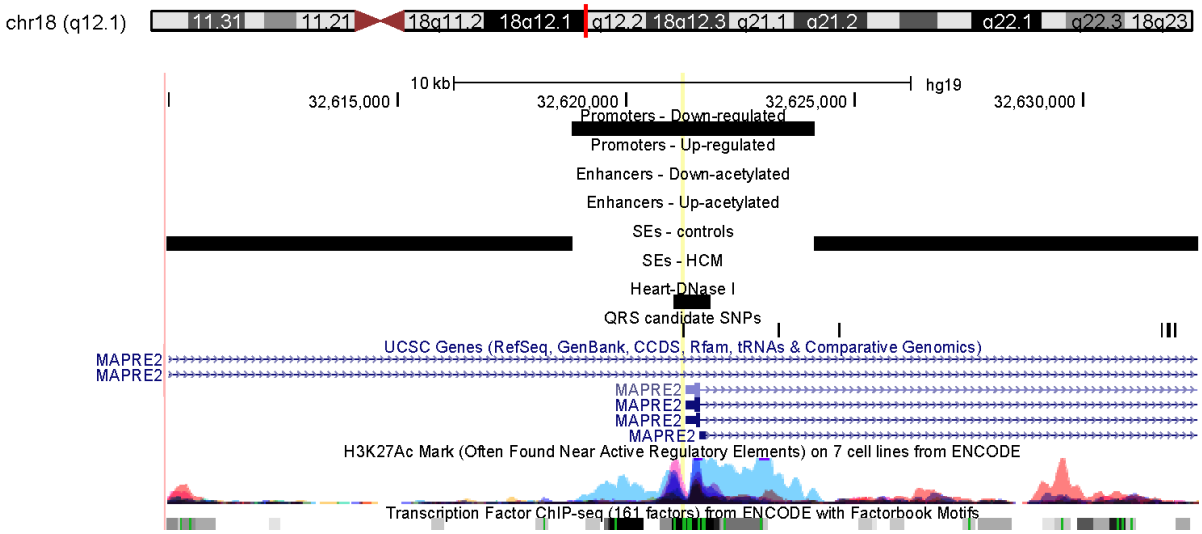
Supplemental Figure 19a. Loci of lead QRS-associated SNP rs7211246, in which variants rs871014 and rs3760456, in LD r^2>0.5, overlap (in yellow) an up-regulated promoter.



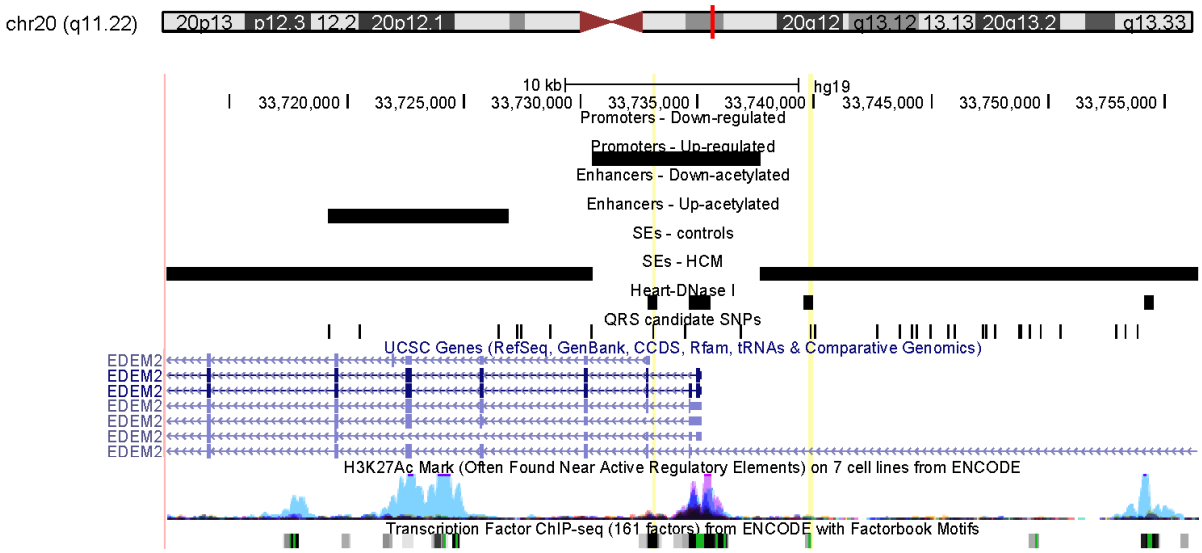
Supplemental Figure 19b. Loci of lead QRS-associated SNP rs7211246, in which variants rs2617865 and rs2628179, in LD r^2>0.7, overlap (in yellow) an LV super-enhancer.



Supplemental Figure 19c. Loci of lead QRS-associated SNP rs7211246, in which variants rs4795529, rs6505162 and rs8067576, in LD r^2>0.8, overlap (in yellow) an LV super-enhancer.



Supplemental Figure 20. Loci of lead QRS-associated SNP rs617759, in which variants rs530081, in LD r^2=0.6, overlaps (in yellow) a down-regulated promoter on gene *MAPRE2*.



Supplemental Figure 21. Loci of lead QRS-associated SNP rs2025096, in which variant rs6060266, in LD r^2=0.6, overlaps (in yellow) an up-regulated promoter on gene *EDEM2*, while variants rs111275419 and rs6058194, in LD r^2=0.6, overlap (in yellow) an HCM super-enhancer.

1.1 Supplemental Tables

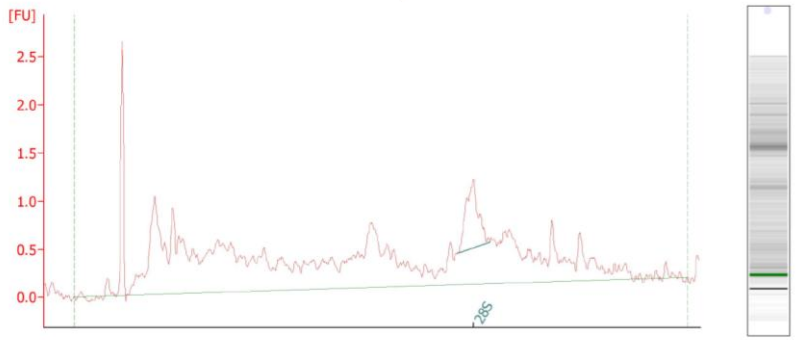
Supplemental Table 1. Overview of the 20 QRS-associated loci overlapping regulatory regions of interest. NEA: non-effect allele. EA: effect allele. SE: super-enhancer.

rsID	chr	pos (hg19)	NEA	EA	MAF	Function	CADD	GWAS p-value	r^2	Lead SNP	promoters down	promoters up	H3K27ac down	H3K27ac up	SE HCM	SE LV	Nearest gene 1	Nearest gene 2	Nearest gene 3	Nearest gene down-regulated in HCM	Nearest gene up-regulated in HCM	eQTL gene
rs680386	chr1	23695275	G	T	0.1889	intronic	5.7		0.6	rs2849028	X						ZNF436	C1orf213	TCEA3	ZNF436		TCEA3
rs72694106	chr1	51762346	G	A	0.02386	intronic	4.7		0.6	rs17391905	X						TTC39A	RNF11	EPS15	TTC39A		
rs2274316	chr1	1.56E+08	A	C	0.329	intronic	12.3		1.0	rs2274317						X	MEF2D	C1orf61	IQGAP3			MEF2D
rs12131289	chr1	1.56E+08	T	C	0.329	intronic	11.4		1.0	rs2274317						X	MEF2D	C1orf61	IQGAP3			MEF2D
rs2274317	chr1	1.56E+08	C	T	0.328	exonic		1.82E-11	1.0	rs2274317						X	MEF2D	C1orf61	IQGAP3			MEF2D
rs4450010	chr1	1.56E+08	G	T	0.328	intronic			1.0	rs2274317						X	MEF2D	IQGAP3	C1orf61			MEF2D
rs1185700	chr1	1.56E+08	G	A	0.2386	exonic	14.7		0.6	rs2274317						X	MEF2D	IQGAP3	C1orf61			
rs1925950	chr1	1.56E+08	A	G	0.327	exonic	11.7		1.0	rs2274317						X	MEF2D	IQGAP3	C1orf61			MEF2D
rs2274319	chr1	1.56E+08	C	T	0.325	intronic	2.2		1.0	rs2274317						X	MEF2D	IQGAP3	C1orf61			
rs3790457	chr1	1.56E+08	T	C	0.329	intronic	12.0		1.0	rs2274317						X	MEF2D	IQGAP3	C1orf61			MEF2D
rs12038396	chr1	1.56E+08	T	C	0.329	intronic	7.3		1.0	rs2274317						X	MEF2D	IQGAP3	C1orf61			MEF2D
rs3790458	chr1	1.56E+08	A	T	0.2356	intronic	9.6		0.6	rs2274317						X	MEF2D	IQGAP3	C1orf61			
rs746527	chr1	1.56E+08	C	T	0.2356	intronic	15.7		0.6	rs2274317						X	MEF2D	IQGAP3	C1orf61			
rs3790461	chr1	1.56E+08	G	A	0.2356	intronic	7.9		0.6	rs2274317						X	MEF2D	IQGAP3	C1orf61			
rs12136856	chr1	1.56E+08	G	C	0.327	intergenic	12.5		1.0	rs2274317						X	MEF2D	IQGAP3	C1orf61			
rs538050729	chr1	1.56E+08	GC	G	0.3489	intergenic	12.6		0.9	rs2274317						X	MEF2D	IQGAP3	C1orf61			
rs1104859	chr1	2.01E+08	G	T	0.2555	intronic	16.3		0.6	rs10920184						X	TNNT2	LAD1	PKP1			
rs2365652	chr1	2.01E+08	A	C	0.3499	intronic	5.1		1.0	rs10920184						X	TNNT2	LAD1	PKP1			
rs1892026	chr1	2.01E+08	T	G	0.2565	intronic	2.1		0.6	rs10920184						X	TNNT2	LAD1	PKP1			
rs3729547	chr1	2.01E+08	A	G	0.2584	exonic	14.2		0.6	rs10920184						X	TNNT2	LAD1	PKP1			
rs10800776	chr1	2.01E+08	C	T	0.2565	intronic	0.6		0.6	rs10920184						X	TNNT2	LAD1	TNNI1			
rs10920183	chr1	2.01E+08	G	A	0.3499	intronic	1.8		1.0	rs10920184						X	TNNT2	LAD1	TNNI1			
rs4915232	chr1	2.01E+08	T	C	0.4254	intronic	2.1		0.7	rs10920184						X	LAD1	TNNT2	TNNI1			
rs2252860	chr2	26987481	A	G	0.4066	intronic	7.6		0.7	rs6710065	X						SLC35F6	CENPA	KCNK3	CENPA	KCNK3	KHK
rs2304337	chr2	26987488	G	C	0.4056	intronic	5.9		0.7	rs6710065	X						SLC35F6	CENPA	KCNK3	CENPA	KCNK3	KHK

rs34902032	chr2	27084942	TC	T	0.3787	intronic	1.6		0.9	rs6710065				X		DPYSL5	CENPA	SLC35F6	CENPA		KHK
rs11677841	chr2	27239949	T	G	0.3817	intronic	1.3		0.5	rs6710065				X		MAPRE3	TMEM214	AGBL5	AGBL5		KHK
rs968702	chr3	53276485	G	C	0.1779	intronic	0.5		0.7	rs4687718		X		X		TKT	DCP1A	PRKCD		PRKCD,TKT	
rs62256006	chr3	53289634	G	A	0.164	intronic	12.3		0.6	rs4687718		X		X		TKT	DCP1A	PRKCD		PRKCD,TKT	
rs17523471	chr3	1.85E+08	T	C	0.3231	UTR5	4.4		1.0	rs10937226				X		SENP2	LIPH	IGF2BP2	LIPH		
rs146170154	chr6	36646768	C	CTA	0.173	intronic	8.4		0.7	rs1321311		X				CDKN1A	RAB44	CPNE5		CPNE5,CDKN1A	
rs3176326	chr6	36647289	G	A	0.173	intronic	7.9		0.7	rs1321311		X				CDKN1A	RAB44	CPNE5		CPNE5,CDKN1A	
rs6913012	chr6	1.19E+08	A	G	0.4632	intronic	8.4		0.7	rs11153730		X				CEP85L	PLN	MCM9		CEP85L	
rs1003549	chr7	35295491	T	C	0.1292	intergenic	0.8		0.7	rs1419856		X				TBX20	DPY19L1	HERPUD2		TBX20,DPY19L1	
rs2270188	chr7	1.16E+08	T	G	0.4811	UTR3	11.8		0.5	rs11773845					X	CAV2	CAV1	MET			
rs2270189	chr7	1.16E+08	G	A	0.4811	UTR3	7.8		0.5	rs11773845					X	CAV2	CAV1	MET			
rs4367519	chr8	1.25E+08	C	T	0.04573	ncRNA_intronic	8.9	4.15E-11	1.0	rs4367519					X	KLHL38	ANXA13	FBXO32	FBXO32		
rs143950919	chr8	1.25E+08	T	TA	0.04573	ncRNA_intronic	2.5		1.0	rs4367519					X	KLHL38	ANXA13	FBXO32	FBXO32		
rs11000728	chr10	75404300	C	G	0.1342	downstream	5.6		0.8	rs7099599					X	SYNPO2L	MYOZ1	USP54	SYNPO2L,MYOZ1		FUT11
rs3812629	chr10	75407290	G	A	0.1332	exonic			0.8	rs7099599					X	SYNPO2L	MYOZ1	USP54	SYNPO2L,MYOZ1		FUT11
rs4746139	chr10	75407649	A	C	0.1332	exonic	0.0		0.8	rs7099599					X	SYNPO2L	MYOZ1	USP54	SYNPO2L,MYOZ1		
rs60632610	chr10	75415677	C	T	0.1372	exonic	32.0		0.9	rs7099599	X					SYNPO2L	MYOZ1	AGAP5	SYNPO2L,MYOZ1		FUT11
rs2167079	chr11	47270255	C	T	0.3002	exonic	22.6		0.8	rs2269434		X				ACP2	NR1H3	DDB2		NR1H3	ACP2
rs3758673	chr11	47278917	C	T	0.2972	intronic	6.7		0.8	rs2269434		X				NR1H3	ACP2	MADD		NR1H3	ACP2
rs1449627	chr11	47290984	T	G	0.3072	UTR5	6.5		0.8	rs2269434		X				MADD	NR1H3	ACP2		NR1H3	ACP2
rs142960070	chr11	47291817	T	TG	0.3072	intronic	6.6		0.8	rs2269434		X				MADD	NR1H3	ACP2		NR1H3	ACP2
rs3781622	chr11	47348702	T	C	0.3072	intronic	1.4		0.8	rs2269434					X	MADD	MYBPC3	SPI1		MYBPC3	ACP2
rs753993	chr11	47349969	C	A	0.2028	intronic	13.1		0.5	rs2269434					X	MADD	MYBPC3	SPI1		MYBPC3	ACP2,MADD
rs10838693	chr11	47350553	G	C	0.3101	intronic			0.9	rs2269434					X	MADD	MYBPC3	SPI1		MYBPC3	ACP2
rs4752825	chr11	47352409	G	A	0.3072	downstream	4.0		0.8	rs2269434					X	MYBPC3	MADD	SPI1			ACP2
rs11570050	chr11	47371484	A	AG	0.2942	intronic	1.0		0.6	rs2269434					X	MYBPC3	SPI1	MADD			
rs7948705	chr11	47447955	C	G	0.2932	UTR5	8.9		0.6	rs2269434				X		PSMC3	SLC39A13	RAPSN			
rs11307826	chr11	47448334	CA	C	0.2932	upstream	10.4		0.6	rs2269434				X		PSMC3	SLC39A13	RAPSN			
rs174538	chr11	61560081	G	A	0.3131	UTR5	9.2		0.8	rs174577	X					TMEM258	FEN1	FADS2	FADS2		FADS1, FADS2
rs174561	chr11	61582708	T	C	0.3032	ncRNA_exonic	7.9		0.8	rs174577	X					FADS2	FADS1	FEN1	FADS2		FADS1, FADS2
rs3834458	chr11	61594920	CT	C	0.3459	Intronic	10.9		0.9	rs174577	X					FADS2	FADS1	FEN1	FADS2		FADS1, FADS2

rs5792235	chr11	61596322	CA	C	0.3469	intronic	9.4	0.9	rs174577	X						FADS2	FADS1	FEN1	FADS2		FADS1, FADS2
rs99780	chr11	61596633	C	T	0.3579	intronic:intronic	8.6	1.0	rs174577	X						FADS2	FADS1	FEN1	FADS2		FADS1, FADS2
rs2958153	chr12	57081517	G	A	0.2684	intronic	5.9	0.9	rs2926743				X			PTGES3	NACA	ATP5B	NACA		
rs3214051	chr12	57119236	A	G	0.3728	UTR5	8.2	0.6	rs2926743	X						NACA	PRIM1	HSD17B6	NACA		
rs8039472	chr15	85361644	G	A	0.4881	intronic	5.7	0.8	rs7183401						X	ALPK3	ZNF592	SLC28A1			
rs4633690	chr15	85361960	C	T	0.4533	intronic	18.3	0.9	rs7183401						X	ALPK3	ZNF592	SLC28A1			
rs2879828	chr15	85361977	G	T	0.4523	intronic	15.8	1.0	rs7183401						X	ALPK3	ZNF592	SLC28A1			
rs871014	chr17	27945339	C	T	0.492	ncRNA_intronic	7.2	0.5	rs7211246	X						CORO6	ANKRD13B	SSH2	CORO6		
rs3760456	chr17	27948844	C	T	0.4463	ncRNA_exonic	7.4	0.6	rs7211246	X						CORO6	SSH2	ANKRD13B	CORO6		
rs2617865	chr17	28049804	T	G	0.4742	intronic	15.5	0.8	rs7211246						X	SSH2	CORO6	ANKRD13B	CORO6		
rs2628179	chr17	28071796	C	G	0.4612	intronic	5.3	0.7	rs7211246						X	SSH2	CORO6	ANKRD13B	CORO6		
rs4795529	chr17	28443549	A	G	0.4652	ncRNA_intronic	5.5	0.9	rs7211246	X						NSRP1	EFCAB5	SLC6A4	NSRP1		
rs6505162	chr17	28444183	A	C	0.4414	ncRNA_exonic:ncRNA_exonic		0.8	rs7211246	X						NSRP1	EFCAB5	SLC6A4	NSRP1		
rs8067576	chr17	28444254	T	A	0.4354	ncRNA_intronic	15.6	1.0	rs7211246	X						NSRP1	EFCAB5	SLC6A4	NSRP1		
rs530081	chr18	32621231	G	A	0.33	intronic		0.6	rs617759	X						MAPRE2	DTNA	ZNF397	MAPRE2		
rs6060266	chr20	33733078	T	C	0.2266	intronic	9.1	0.6	rs2025096			X				EDEM2	PROCR	TRPC4AP		PROCR,EDEM2	PROCR,EDEM2
rs111275419	chr20	33739804	CCCAT	C	0.2286	intronic	15.8	0.6	rs2025096					X		EDEM2	PROCR	TRPC4AP		PROCR,EDEM2	PROCR,EDEM2
rs6058194	chr20	33739831	G	A	0.2276	intronic	20.1	0.6	rs2025096					X		EDEM2	PROCR	TRPC4AP		PROCR,EDEM2	PROCR,EDEM2

Supplemental Table 2. RNA-seq quality control measures.

Sample name	Number of input reads	Uniquely mapped reads %	Overall quality assessment
HCM_1	18147501	88.07%	
HCM_3	17069996	89.39%	
HCM_5	14616054	87.30%	

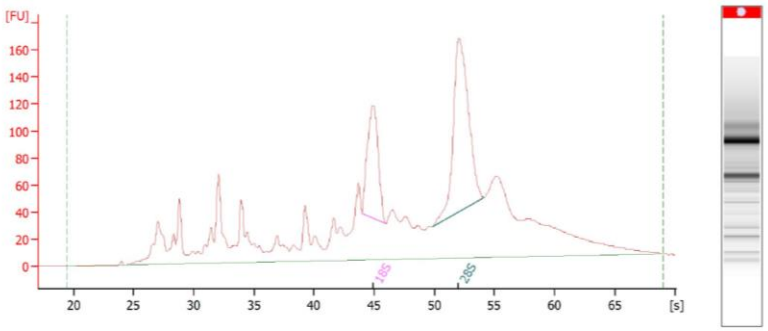
Overall Results for sample

RNA Area:	35.1	RNA Integrity Number (RIN):	2.7 (B.02.08)
RNA Concentration:	559 pg/µl	Result Flagging Color:	
rRNA Ratio [28s / 18s]:	0.0	Result Flagging Label:	RIN: 2.70

Fragment table for sample

Name	Start Time [s]	End Time [s]	Area	% of total Area
28S	42.73	44.93	1.6	4.5

HCM_6	17987731	86.79%



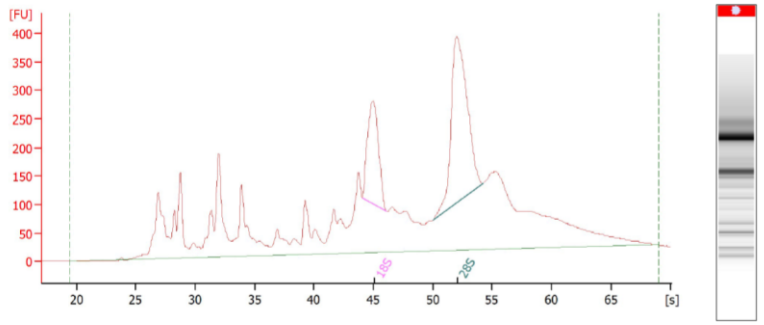
Overall Results for sample

RNA Area:	2,611.1	RNA Integrity Number (RIN):	7.1 (B.02.08)
RNA Concentration:	789,798 pg/µl	Result Flagging Color:	
rRNA Ratio [28s / 18s]:	1.8	Result Flagging Label:	RIN: 7.10

Fragment table for sample

Name	Start Time [s]	End Time [s]	Area	% of total Area
18S	44.00	45.95	194.1	7.4
28S	49.85	54.10	355.7	13.6

HCM_7	16020022	87.72%



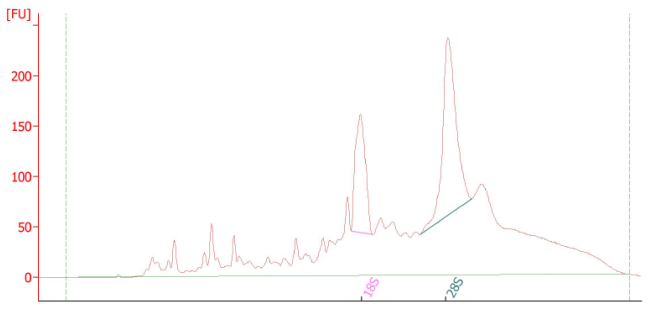
Overall Results for sample

RNA Area:	6,674.7	RNA Integrity Number (RIN):	6.9 (B.02.08)
RNA Concentration:	2,018,930 pg/µl	Result Flagging Color:	
rRNA Ratio [28s / 18s]:	2.0	Result Flagging Label:	RIN: 6.90

Fragment table for sample

Name	Start Time [s]	End Time [s]	Area	% of total Area
18S	44.05	46.10	438.3	6.6
28S	50.00	54.20	859.1	12.9

HCM_8	15764338	85.76%



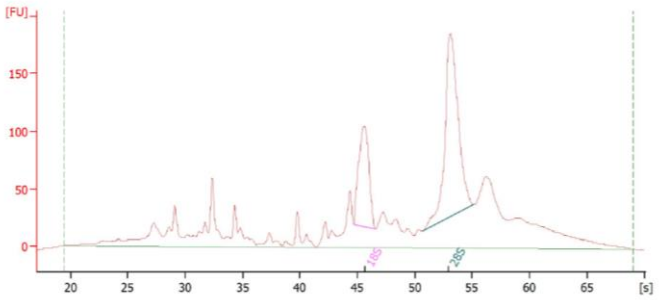
Overall Results for sample

RNA Area:	3,511.5	RNA Integrity Number (RIN):	7.1 (B.02.08)
RNA Concentration:	17,631 pg/µl	Result Flagging Color:	
rRNA Ratio [28s / 18s]:	1.7	Result Flagging Label:	RIN: 7.10

Fragment table for sample

Name	Start Time [s]	End Time [s]	Area	% of total Area
18S	40.67	42.40	265.0	7.5
28S	46.19	50.34	449.6	12.8

HCM_9	17169707	87.59%



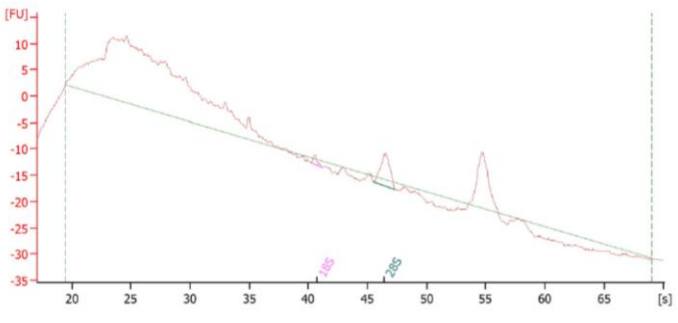
Overall Results for sample

RNA Area:	2,214.9	RNA Integrity Number (RIN):	7.9 (B.02.08)
RNA Concentration:	669,953 pg/µl	Result Flagging Color:	
rRNA Ratio [28s / 18s]:	2.0	Result Flagging Label:	RIN: 7.90

Fragment table for sample

Name	Start Time [s]	End Time [s]	Area	% of total Area
18S	44.70	46.60	198.1	8.9
28S	50.70	55.15	397.9	18.0

HCM_10	16954945	85.81%



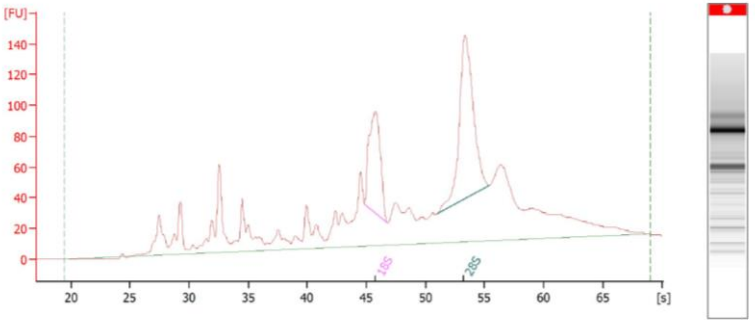
Overall Results for sample

RNA Area:	449.9	RNA Integrity Number (RIN):	N/A (B.02.08)
RNA Concentration:	136,074 pg/µl	Result Flagging Color:	
rRNA Ratio [28s / 18s]:	7.2	Result Flagging Label:	RIN N/A

Fragment table for sample

Name	Start Time [s]	End Time [s]	Area	% of total Area
18S	40.20	41.20	1.7	0.4
28S	45.50	47.35	12.0	2.7

HCM_11	16177356	85.31%

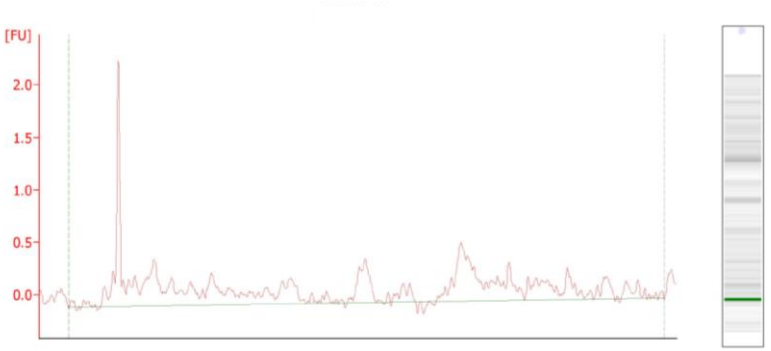
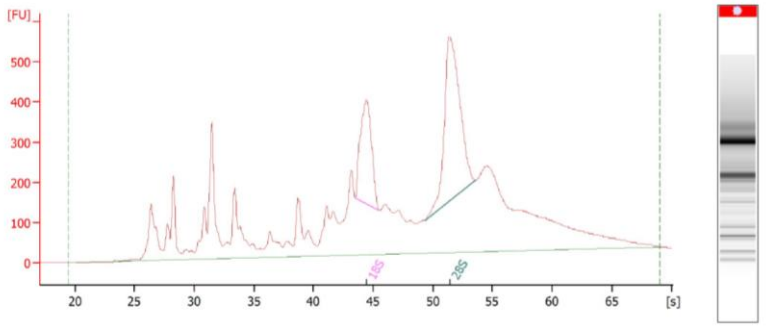


Overall Results for sample

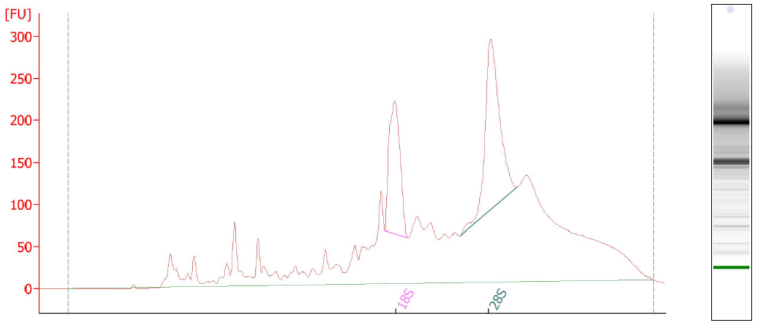
RNA Area:	1,931.8	RNA Integrity Number (RIN):	N/A (B.02.08)
RNA Concentration:	584,334 pg/µl	Result Flagging Color:	
rRNA Ratio [28s / 18s]:	1.7	Result Flagging Label:	RIN N/A

Fragment table for sample

Name	Start Time [s]	End Time [s]	Area	% of total Area
18S	44.85	46.80	160.1	8.3
28S	50.95	55.40	271.6	14.1

HCM_12	17130387	88.70%	 **Overall Results for sample** RNA Area: 15.6 — RNA Integrity Number (RIN): 1.9 (B.02.08) RNA Concentration: 248 pg/µl — Result Flagging Color: rRNA Ratio [28s / 18s]: 0.0 — Result Flagging Label: RIN: 1.90
HCM_14	16904754	85.43%	 **Overall Results for sample** RNA Area: 9,660.9 — RNA Integrity Number (RIN): 6.8 (B.02.08) RNA Concentration: 2,922,198 pg/µl — Result Flagging Color: rRNA Ratio [28s / 18s]: 1.9 — Result Flagging Label: RIN: 6.80 **Fragment table for sample** **Name** **Start Time [s]** **End Time [s]** **Area** **% of total Area** 18S 43.50 45.50 636.6 6.6 28S 49.35 53.55 1,178.9 12.2
CONTROL_RNA_1†	17324505	55.79%	--

CONTROL_RNA_2	16741601	89.21%
CONTROL_RNA_3†	16142540	90.28%
CONTROL_RNA_4†	21129003	87.89%
CONTROL_RNA_5	16650887	86.20%



Overall Results for sample

RNA Area:	4,520.5	RNA Integrity Number (RIN):	6.8 (B.02.08)
RNA Concentration:	13,847 pg/µl	Result Flagging Color:	
rRNA Ratio [28s / 18s]:	1.4	Result Flagging Label:	RIN: 6.80

Fragment table for sample

Name	Start Time [s]	End Time [s]	Area	% of total Area
18S	40.70	42.46	361.4	8.0
28S	46.33	50.56	512.0	11.3

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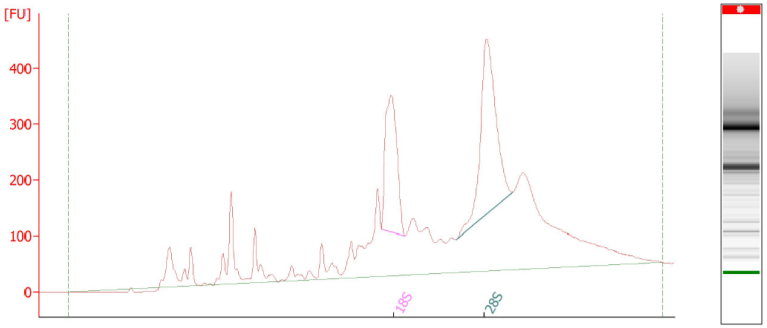
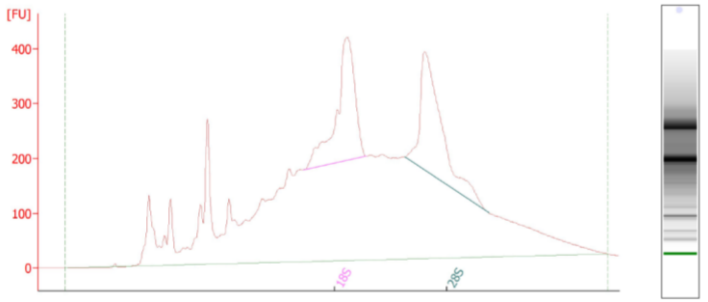
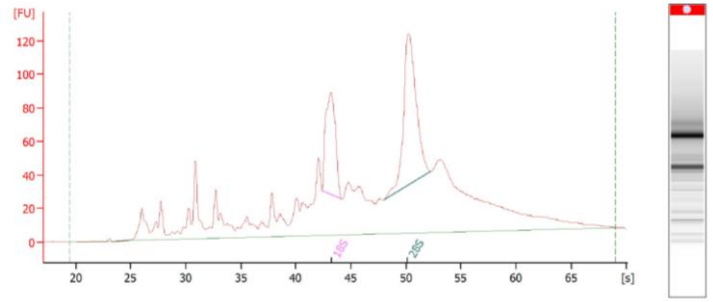


Overall Results for sample

RNA Area:	5,805.4	RNA Integrity Number (RIN):	N/A (B.02.08)
RNA Concentration:	17,783 pg/µl	Result Flagging Color:	
rRNA Ratio [28s / 18s]:	1.4	Result Flagging Label:	RIN N/A

Fragment table for sample

Name	Start Time [s]	End Time [s]	Area	% of total Area
18S	40.62	42.35	590.4	10.2
28S	46.18	50.32	841.2	14.5

CONTROL_RNA_6	18140395	86.31%	
CONTROL_RNA_7†	19381202	86.81%	--
CONTROL_RNA_8	17889557	88.43%	
CONTROL_RNA_9†	16682602	84.56%	--
CONTROL_RNA_10†	15953144	86.26%	--

Overall Results for sample

RNA Area:	11,912.3	RNA Integrity Number (RIN):	6.1 (B.02.08)
RNA Concentration:	75,352 pg/µl	Result Flagging Color:	
rRNA Ratio [28s / 18s]:	1.0	Result Flagging Label:	RIN: 6.10

Fragment table for sample

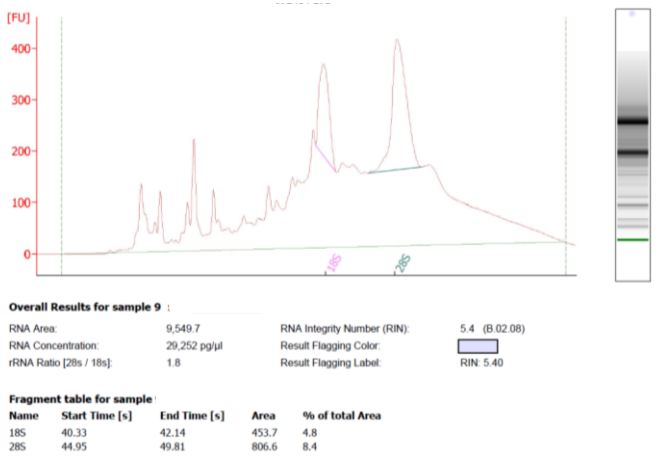
Name	Start Time [s]	End Time [s]	Area	% of total Area
18S	37.69	42.99	982.2	8.2
28S	46.15	53.19	943.5	7.9

Overall Results for sample :

RNA Area:	1,883.8	RNA Integrity Number (RIN):	7 (B.02.08)
RNA Concentration:	569,812 pg/µl	Result Flagging Color:	
rRNA Ratio [28s / 18s]:	1.6	Result Flagging Label:	RIN:7

Fragment table for sample

Name	Start Time [s]	End Time [s]	Area	% of total Area
18S	42.35	44.15	144.1	7.7
28S	48.00	52.30	237.3	12.6

CONTROL_RNA_11	16622707	85.93%	

†: due to very limited size, all tissues were used for RNA-seq and no RNA left for other purposes.

Supplemental Table 3. Raw read counts and Reads Per Kilobase per Million (RPKM) values from the RNA-seq analysis in HCM patients and controls.

Available as .xlsx file.

Supplemental Table 4. Differentially regulated genes identified by RNA-seq experiment in HCM patients versus controls.

Available as .xlsx file.

Supplemental Table 5. Differentially acetylated regions identified by ChIP-seq experiment in HCM patients versus controls (positions in hg19).

Available as .xlsx file.

Supplemental Table 6. HCM-specific super-enhancer regions, not overlapping with LV super-enhancers (positions in hg19).

Available as .xlsx file.

Supplemental Table 7. Summary of the functional annotation using differential regulatory elements in HCM patients versus controls.

Locus	Lead QRS-associated SNP	Genes from original study	# LD SNPs overlapping exons with CADD > 12.37	# LD SNPs overlapping differentially regulated promoters in HCM x controls	# LD SNPs overlapping differentially acetylated regions in HCM x controls	# LD SNPs overlapping HCM or LV super-enhancers	Genes from this study
1p36.12	rs2849028	ZNF436; C1orf213		1			ZNF436; TCEA3
1p32.3	rs17391905	CDKN2C		1			TTC39A
1p31.3	rs2207790	NFIA					
1p13.1	rs12039739	CASQ2					
1q22	rs2274317	MEF2D	1			14	MEF2D
1q23.3	rs12036340	OLFML2B					
1q32.1	rs10920184	TNNT2	1			7	TNNT2
1q32.1	rs4288653	PLEKHA6					
2p23.3	rs6710065	DPYSL5	1	2	2		CENPA; AGBL5; KCNK3; KHK
2p22.2	rs3770770	STRN					
2q31.2	rs3816849	TTN					
3p22.2	rs6801957	SCN10A					
3p21.1	rs4687718	TKT		2	2		PRKCD;TKT
3p14.1	rs2242285	LRIG1					
3p14.1	rs13314892	MITF					
3q27.2	rs10937226	SENP2			1		LIPH
4p15.31	rs1344852	SLIT2					
5q33.2	rs13185595	HAND1					
6p21.31	rs1321311	CDKN1A		2			CPNE5; CDKN1A
6p21.1	rs1015150	TFEB					
6q22.31	rs11153730	PLN;SLC35F1;CEP85L		1			CEP85L
7p14.3	rs1419856	TBX20		1			TBX20; DPY19L1
7p12.3	rs6968945	TNS3					
7q31.2	rs11773845	CAV1				2	
8q24.13	rs4367519	FBXO32;KLHL38	1				

8q24.13	rs10105974	MTSS1					
10q21.1	rs1733724	DKK1					
10q21.3	rs12414364;rs10509289	CTNNA3					
10q22.2	rs7099599	SYNPO2L;SEC24C;CAMK2G	2	1		3	SYNPO2L; MYOZ1; FUT11
10q25.2	rs7918405	VTI1A					
11p11.2	rs2269434	ACP2;MADD;MYBPC3;NR1H3	1	4	2	5	ACP2; MADD; MYBPC3; NR1H3
11q12.2	rs174577	FADS2;TMEM258		5			FADS1; FADS2
12q13.13	rs736825	HOXC5;HOXC4;HOXC6					
12q13.3	rs2926743	NACA	3	1	1		
12q24.21	rs7132327	TBX3					
13q14.13	rs1408224	LRCH1					
13q22.1	rs728926	KLF12					
14q24.2	rs12880291	SIPA1L1					
15q25.3	rs7183401	ALPK3				3	
15q26.3	rs8038015	IGF1R					
16q23.3	rs6565060	CDH13					
17q11.2	rs7211246	NSRP1;EFCAB5		5		2	NSRP1; CORO6
17q21.31	rs242562	MAPT					
17q24.2	rs9912468	PRKCA					
18q12.1	rs617759	MAPRE2		1			MAPRE2
18q12.2	rs879568	FHOD3	3				
18q12.3	rs10853525	SETBP1					
20p12.3	rs3929778	BMP2					
20q11.22	rs2025096	MYH7B;GSS;EDEM2	1			2	PROCR; EDEM2
21q21.1	rs7283707	USP25					
21q21.3	rs13047360	ADAMTS5					

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