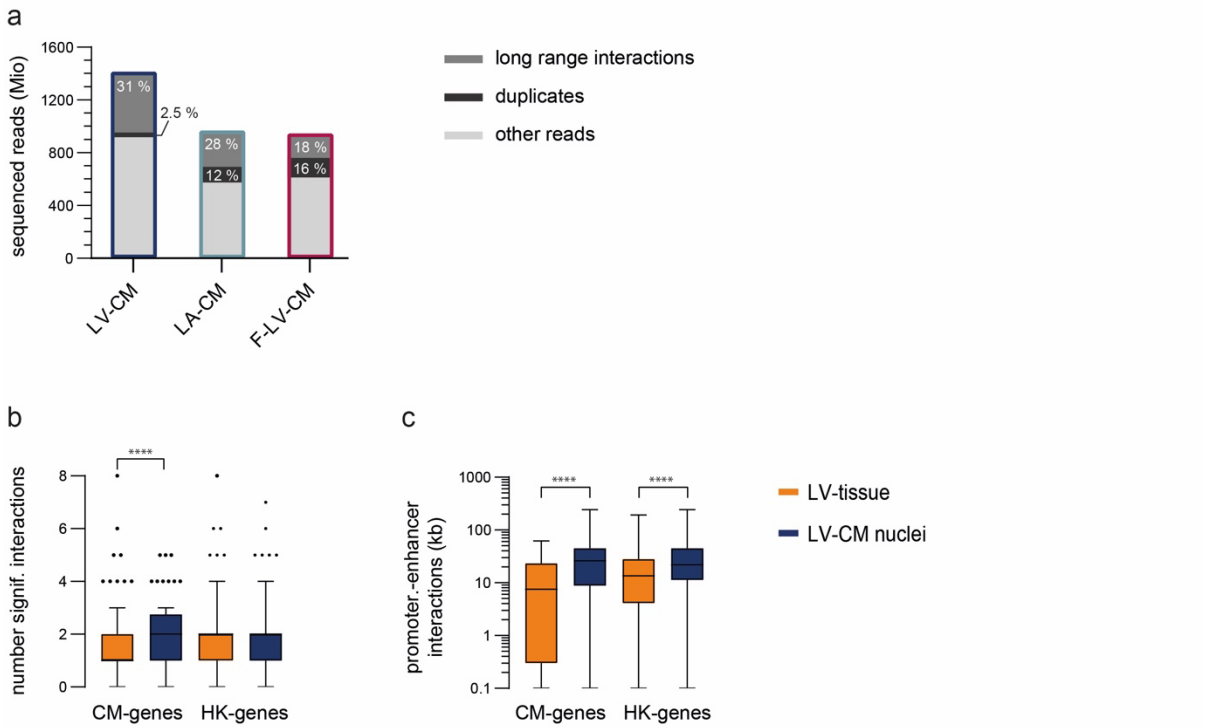
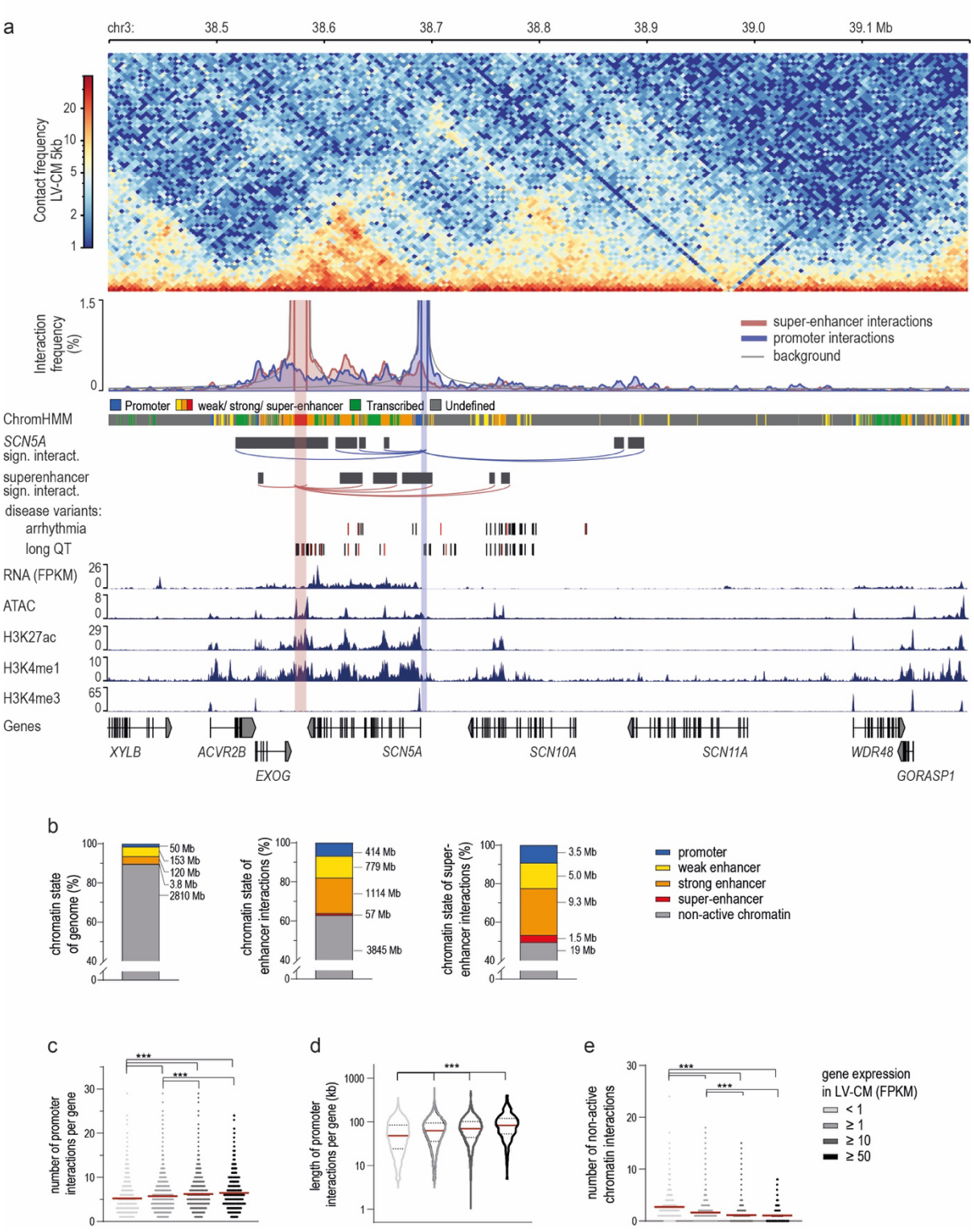
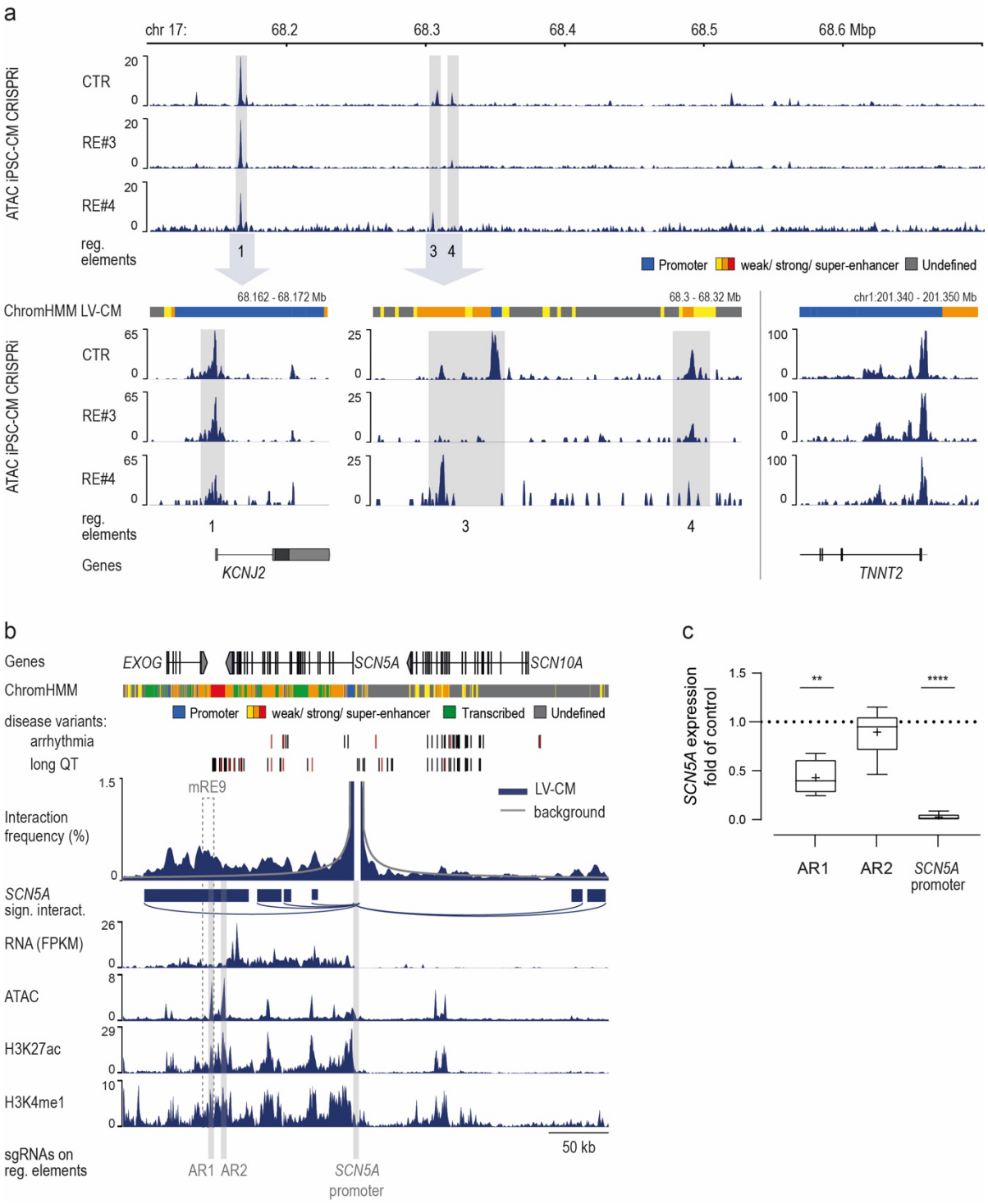
Supplemental Material



Suppl. Fig. 1: Comparison of Hi-C mapping results and promoter interactions in left ventricular tissue and LV-CM nuclei. a Mapping statistics of Hi-C data. **b, c** Mean number of promoter-interactions (**b**) and promoter-enhancer interactions (**c**) for CM-specific genes and housekeeping genes (CM-genes and HK-genes) in LV-tissue and LV-CM nuclei. ****p = 0.0001, paired Wilcoxon test.

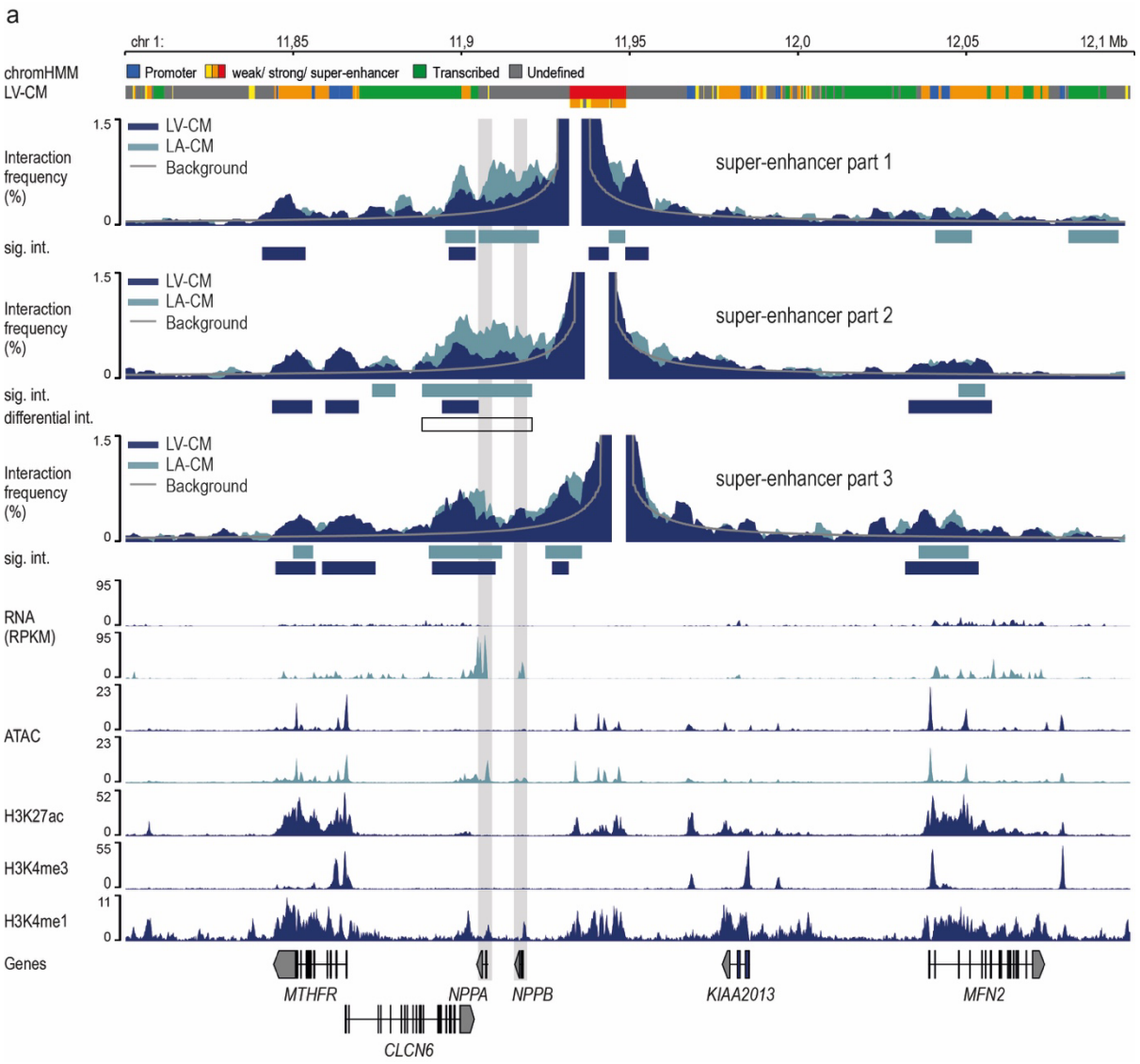


Suppl. Fig. 2: Promoter and enhancer interactions in left ventricular cardiac myocytes. a Heatmap of chromatin interactions, GWAS-associated variants for long QT and cardiac arrythmia (lead-SNPs = red, proxy-SNPs = black) and original traces for gene expression and chromatin accessibility and histone modifications of human LV-CM. Virtual viewpoint interactions of the *SCN5A* promoter (Sodium Voltage-Gated Channel Alpha Subunit 5) and neighboring super-enhancer as well as chromatin states are annotated. **b** Percentage of promoter, enhancer, super-enhancer and non-regulatory elements in the human genome (left), in enhancer interactions (middle) and in super-enhancer interactions (right). **c,d,e** Number of promoter interactions (**c**), length pf promoter interactions (**d**) and number of promoter interactions outside of promoter and enhancer regions (non-active regulatory elements) (**e**) were classified according to gene expression levels. ***P = 0.0001, ANOVA/ Kruskal-Wallis.



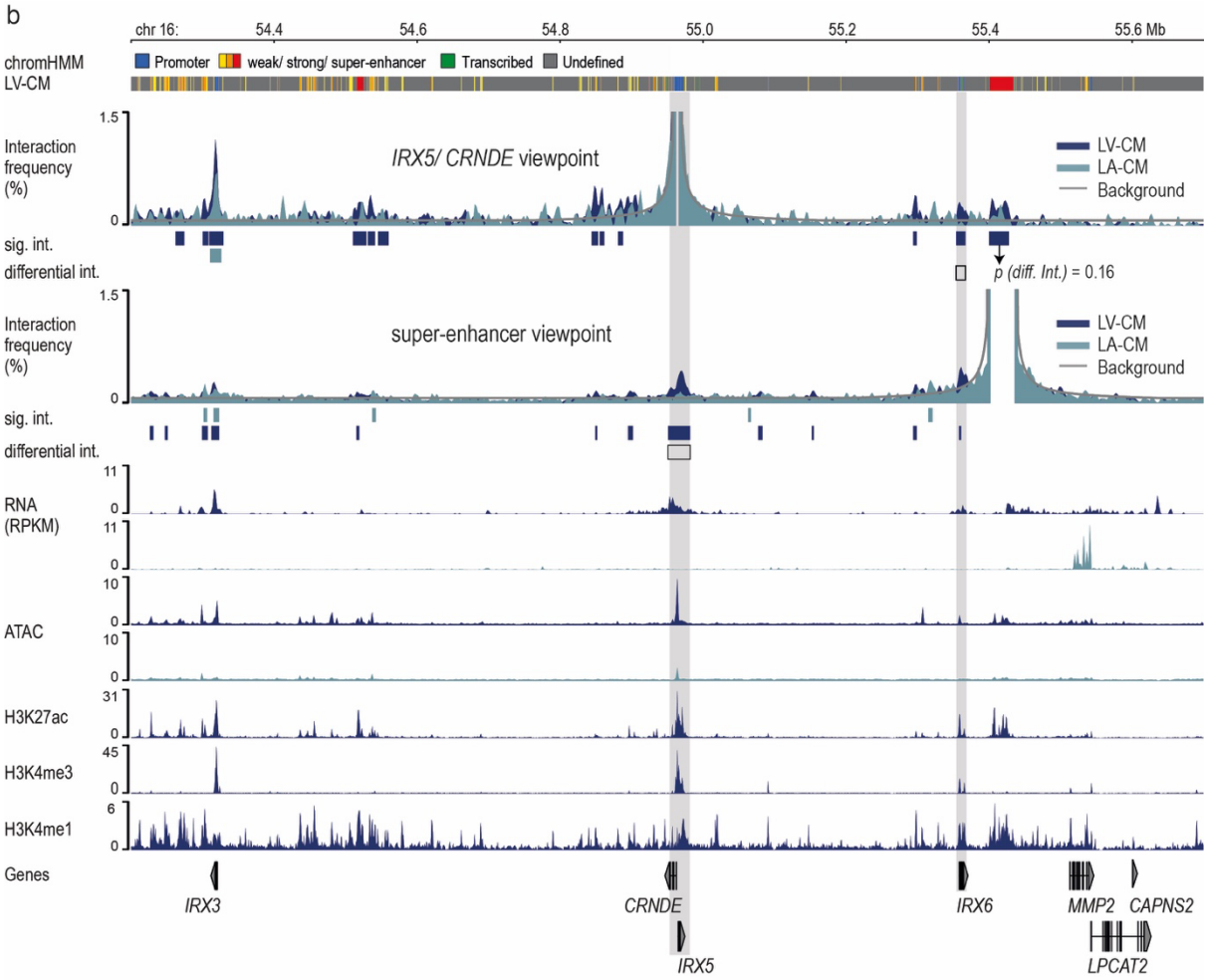
Suppl. Fig. 3: CRISPRi-mediated silencing of regulatory elements. a Depicted is the genomic locus containing the *KCNJ2* gene in iPSC-CM and the corresponding ATAC traces after CRISPRi for control (CTR) and for functional enhancer elements (RE#3 and RE#4). Grey areas highlight the sgRNA localization. The level of ATAC signal is adjusted to the maximum signal for the cardiac specific promoter of Troponin T2 (*TNNT2*) in percent. **b** Chromatin interactions, GWAS-associated variants for long QT and cardiac arrythmia (lead-SNPs = red, proxy-SNPs = black) and original traces for gene expression and chromatin accessibility of human LV-CM for *SCN5A* are depicted. Virtual viewpoint interactions of the *SCN5A* promoter as well as chromatin states are annotated. Grey areas AR1 and AR2 highlight the sgRNA localization. The region mRE9 depicts the human homologue of a domain tested by Man et al., 2019, *Nature Communications* in mouse. **c** *SCN5A* gene expression analysis after CRISPRi-mediated silencing of the accessible regions AR1, AR2 and of the *SCN5A* promoter. Shown are data from n ≥ 4 biological replicates, mean ± S.E.M., **p = 0.01, ***p = 0.001, ANOVA.

34



35

36 **Suppl. Fig. 4: legend on the following page**



Suppl. Fig. 4: Representative super-enhancer-promoter interactions for LV-CM and LA-CM. a Virtual viewpoints were calculated for three parts of a representative super-enhancer highlighted in Fig. 4a (red) for left ventricular and left atrial CMs. Differential interaction analysis (empty squares) was performed for interacting regions. **b** Virtual viewpoints were calculated for *IRX5, CRNDE* and the nearby super-enhancer (top third in Fig. 4a) for left ventricular and left atrial CMs. The promoters of the expressed transcripts *CRNDE* and *IRX5* are offset by < 600 bp and all significant interactions are identical. Differentia interaction analysis (empty squares) was performed for interacting regions.

Suppl. Table 1: Sample characteristics

Samples Id	group	Age	Details	EF (%)	NYHA	Sex	Myocarditis or acute infection	Medication	Comorbidities
LV-CM_1	non-failing	47 years	car accident	-	-	male	no	-	no pathological cardiac characteristics
LV-CM_2	non-failing	50 years	accident (drunk)	-	-	male	no	-	non-obstructive coronary heart disease
LV-CM_3	non-failing	46 years	stroke	-	-	male	no	-	no pathological cardiac characteristics
LA-CM_1	non-failing	61 years	MKP (mitral valvuloplasty)	-	-	male	no	-	mitral valve insufficiency
LA-CM_2	non-failing	64 years	MKP (mitral valvuloplasty)	-	-	male	no	-	mitral valve insufficiency
LA-CM_3	non-failing	54 years	MKP (mitral valvuloplasty)	-	-	male	no	-	mitral valve insufficiency, atrial fibrillation (intermediate)
F-LV-CM_1	failing	61 years	Terminal heart failure (HTX)	15	3	male	no	digitoxin, amiodarone, molsidomine, spironolactone	ventricular arrhythmia, renal insufficiency, hyperuricemia, hypercholesterolemia, valvular insufficiency (M I-II)
F-LV-CM_2	failing	60 years	Explanted heart (HTX)	25	3	male	no	losartan, digitoxin, sotalol, furosemide, isosorbide dinitrate, pravastatin, phenprocoumon	pulmonary hypertension, stroke history, implanted defibrillator, HIS ablation (AF), coronary heart disease (LAD stent), valvular insufficiency (MI II)
F-LV-CM_3	failing	56 years	Explanted heart (LVAD)	20	4	male	no	ACE inhibitor, digitoxin, amiodarone, furosemide	COPD, pulmonary hypertension, valvular insufficiency (AI I, MI I, PI I)

Suppl. Table 2: Read statistics

Samples Hi-C	number of nuclei	sequencing depth (Mio reads)	long-range interactions (% of total reads)	duplicates (% of total reads)
LV-CM_1	225418	432,3	29,175	
LV-CM_2	107081	446,7	31,85	
LV-CM_3	102593	507,2	34,175	
Total: LV-CM_1-3		1385,8	31,5	2,5
LA-CM_1	57000	35,5	22,2	
LA-CM_2	51000	479,7	29,5	
LA-CM_3	28000	440,4	28,3	
Total: LA-CM_1-3		955,5	27,8	12,4
F-LV-CM_1	33000	244,5	19,9	
F-LV-CM_2	41000	147,7	21,0	
F-LV-CM_3	51000	536,2	16,8	
Total: F-LV-CM_1-3		928,5	18,3	16,2

Suppl. table 3: sgRNA sequences

target gene	name sgRNA	target chr	chr start	chr end	sgRNA sequence
KCNJ2	RE#1; KCNJ2 prom.	chr17	68165551	68165571	GCCGGCCCCGGGAACCGCGG
		chr17	68165643	68165663	GGACCGCCTCAAAAGAGCCC
		chr17	68165472	68165491	GGCCGGGCTCCGAGGGTGGA
	RE#2	chr17	68205175	68205194	GCATAGCAGAGTGCTGAAGT
		chr17	68205247	68205266	GACTAATGAAAATGCTTCAA
		chr17	68205355	68205374	GCTGCCTGAATCAAGCATTG
	RE#3	chr17	68303674	68303694	GGTGATTCAGAGCCAAAGCA
		chr17	68303821	68303841	GCAGTGTTCCAGGGTACCCC
		chr17	68303886	68303905	GTGAAATAACTCTTGAGTGA
		chr17	68306465	68306484	GCCGCTAGTCATGCTTCTGT
		chr17	68306588	68306607	GCATTTGCTCCTAAGGCCTC
		chr17	68306699	68306718	GGGAACTGGATATATTTGCT
	RE#4	chr17	68317134	68317153	GGGAATCTTGTTAAGTGAAG
		chr17	68317218	68317237	GCTTTCTGGAGCTAGACCAC
		chr17	68317289	68317308	GGGTAATGCTTCGGAAGTCA
	RE#5	chr17	68472518	68472537	GAAAAATAGAGATGTATTAA
		chr17	68472700	68472719	GACTTTTGTGATCTAATAGA
		chr17	68472801	68472820	GTGTTACCTTTGTACATAGT
		chr17	68475014	68475033	GATACATATCACTATTCAAA
		chr17	68475134	68475153	GAGTACGCAGGAAACCATCA
	RE#6	chr17	68518038	68518057	GGCTGAGTGACCAATTTATC
		chr17	68518190	68518209	GTAGGTTCAACAGAATCATG
		chr17	68518338	68518357	GGGTTATCCTGCAAAAGCTA
		chr17	68523601	68523620	GCTGACGCTGCATATCAGGC
		chr17	68523778	68523797	GCTGCCATTAGTGGTAGACT
		chr17	68523918	68523937	GAACAGAAATAATAAAAGAA
	RE#7	chr17	68549193	68549212	GTAATACATTGAATGATGCC
		chr17	68549243	68549262	GTCTCCCAGACACAACAAAG
		chr17	68548687	68548707	TCTCCCTAAGCACGCTTGAA
		chr17	68559429	68559448	GACCCATCTTTGAGAGAGCC
		chr17	68559541	68559560	GATGGACAGGGGCTGTTTTA
		chr17	68559611	68559630	GACTATAATTGGCATTTGCT
	RE#8	chr17	68617891	68617910	GCTGTGCCTTTCCCCAGAAG
		chr17	68618004	68618023	GAGCAAGATACCAGGGCAGA
		chr17	68618536	68618555	GAGGAAAATATTTGAACGGA
		chr17	68618681	68618700	GGCTACTACTTCCCGAAGAA
SCN5A	SCN5A prom.	chr3	38691135	38691154	GGCGGCGCCCGTAGGATGCA
		chr3	38691251	38691270	GGCCCCGCCTGGGTTCGGCC
		chr3	38691299	38691318	GCCCTGGGCGCGTCGCTCTG
	AR1	chr3	38574992	38575011	GGTGTCGCAGACGGGAGATA
		chr3	38575050	38575069	GGCCTGTCACAAGGCGGCCG
		chr3	38575147	38575166	GTCTAGTCTGACAGGGCAAT
	AR2	chr3	38585189	38585208	GGCCACGAGACTCAAAGCCC
		chr3	38585275	38585294	GACGTCAGCTCTCACCCTGG
		chr3	38585430	38585449	GCAAGGCTGCAGCTGTCACT
control regions	sgRNA_control_1				GGGTGTCTTGGCTCCAGGTC
	sgRNA_control_2				GCGTGGATAAACTGCCCGTA
	sgRNA_control_3				GGGGGACCACTATATCACTG
	sgRNA_control_4				GATCACGCCCGGAAACCTGG