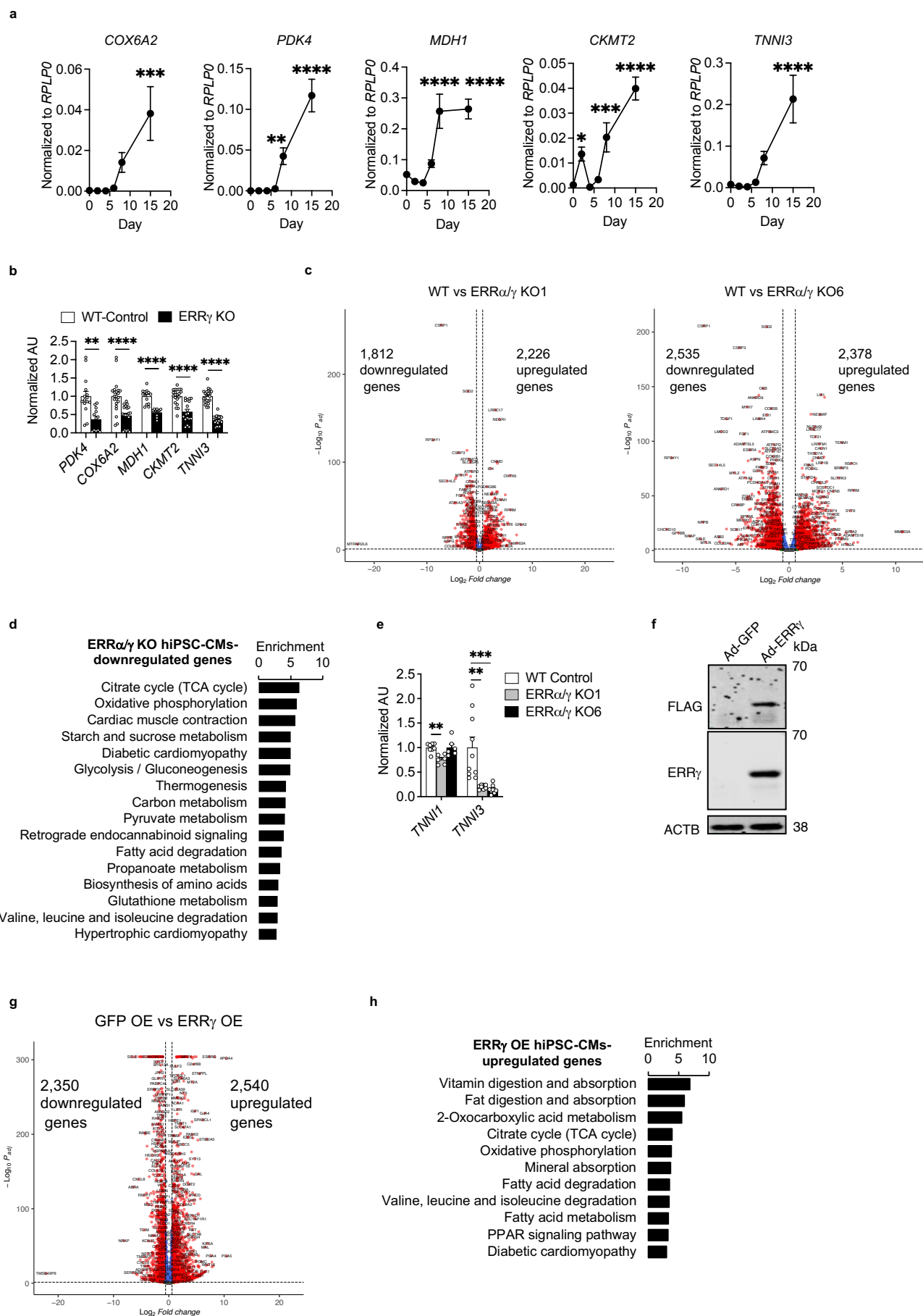


Supplementary Information

The Nuclear Receptor ERR Cooperates with the Cardiogenic Factor GATA4 to Orchestrate Cardiomyocyte Maturation

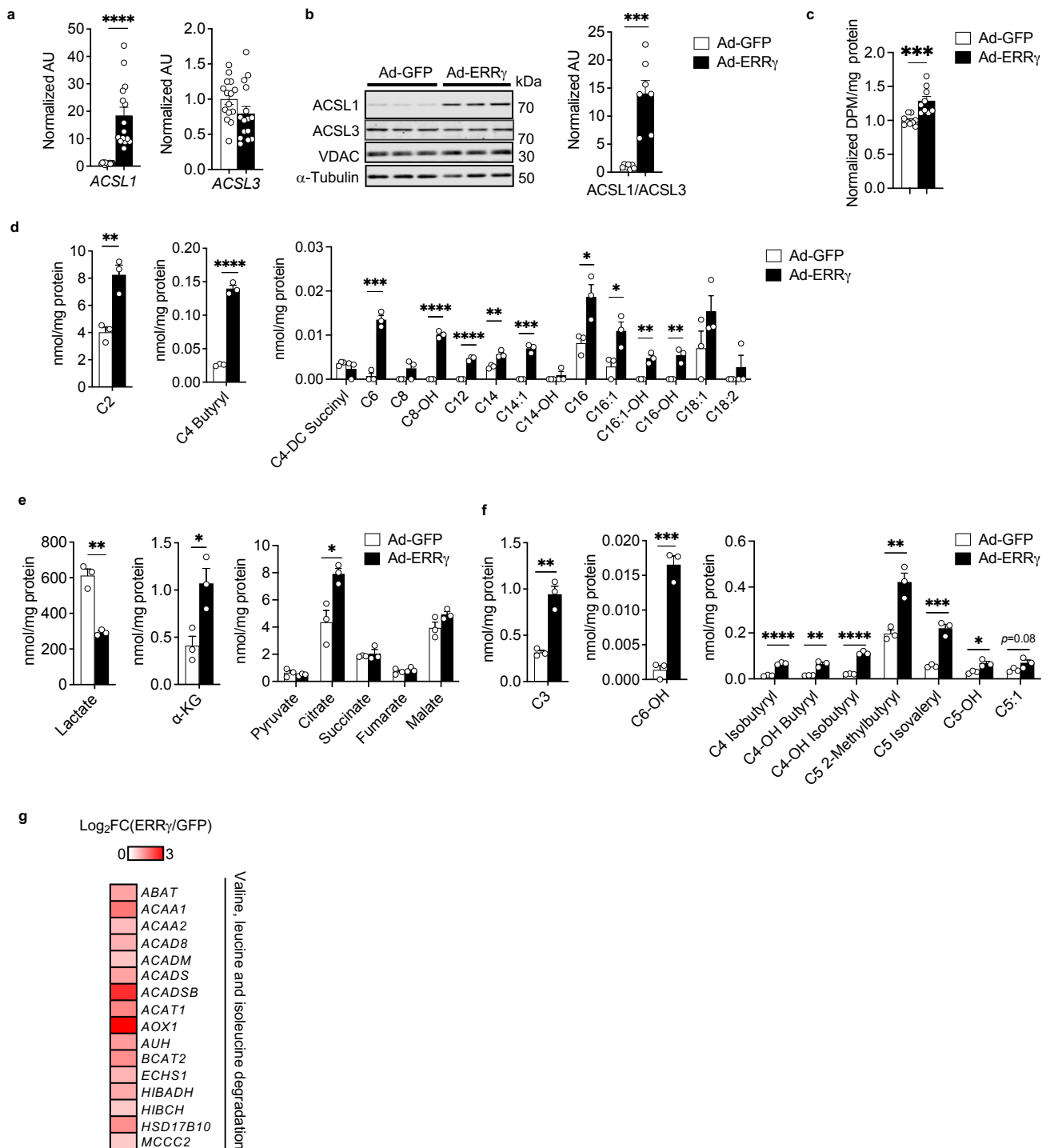
Tomoya Sakamoto, Kirill Batmanov, Shibiao Wan, Yuanjun Guo, Ling Lai, Rick B. Vega, and
Daniel P. Kelly

Supplementary Figures 1-8
Supplementary Tables 1-5



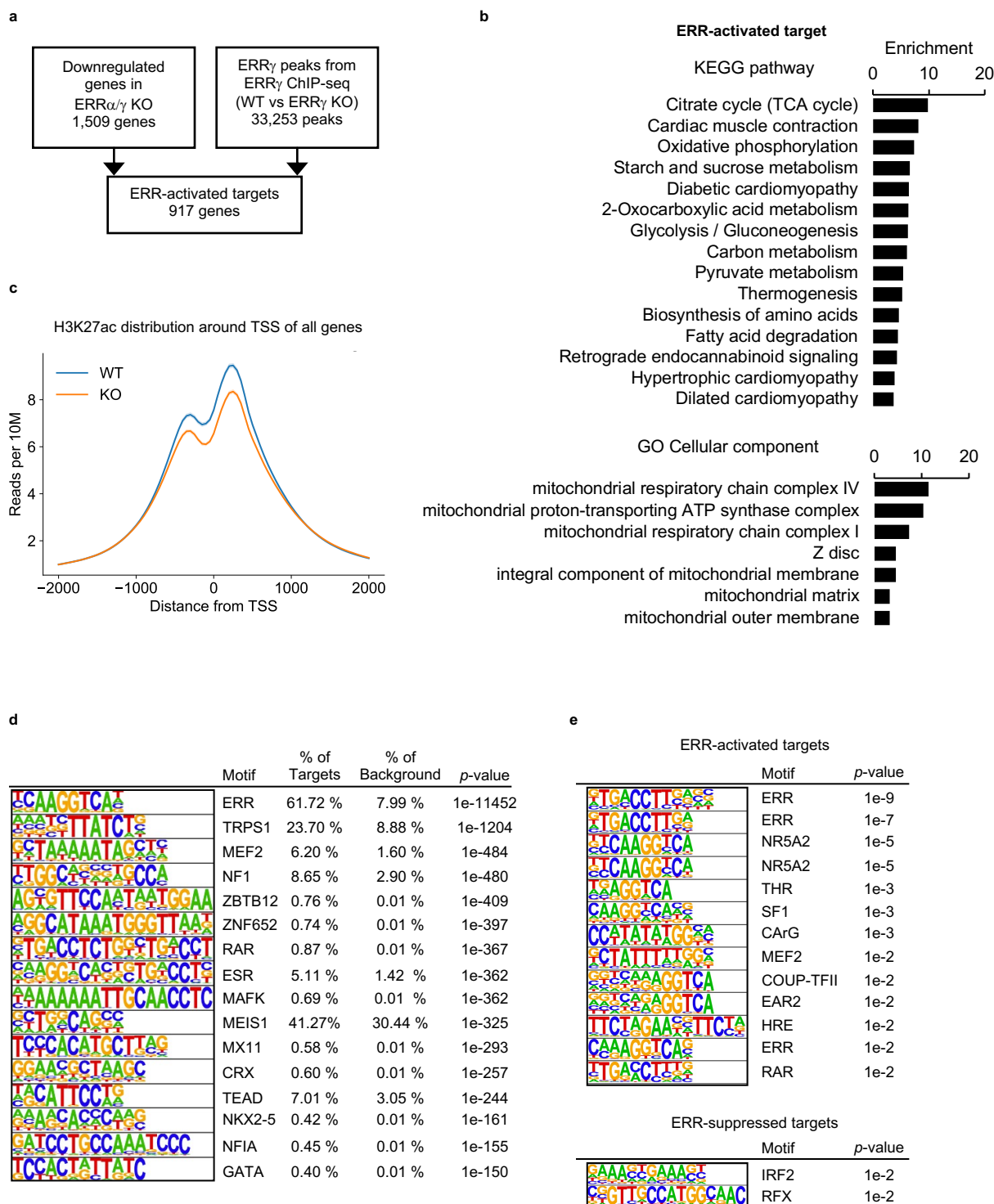
Supplementary Figure 1. ERR signaling is an activator for both cardiac energy metabolic and structural pathways in hiPSC-CMs.

(a) Real-time quantitative polymerase chain reaction (RT-qPCR)-determined levels of the designated mRNAs during human induced pluripotent stem cell-derived cardiomyocyte (hiPSC-CM) differentiation. Levels of indicated genes are shown as arbitrary units (AU) normalized to *RPLP0* levels at each timepoint. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$ vs Day 0; one-way ANOVA followed by Dunnett's multiple comparison test. Day 0 and 2, $n = 7$; Day 4, 6, 8, and 15, $n = 6$. (b) The mRNA levels of indicated genes determined by RT-qPCR in wild-type (WT)-Control and $ERR\gamma$ knockout (KO) hiPSC-CMs. ** $p < 0.01$ or **** $p < 0.0001$, WT-Control vs $ERR\gamma$ KO, two-tailed student's t -test. *PDK4* (WT-Control, $n = 15$ and $ERR\gamma$ KO, $n = 12$); *COX6A2* (WT-Control, $n = 22$ and $ERR\gamma$ KO, $n = 19$); *MDH1* (WT-Control, $n = 14$ and $ERR\gamma$ KO, $n = 11$); *CKMT2* (WT-Control, $n = 21$ and $ERR\gamma$ KO, $n = 18$); *TNNI3* (WT-Control, $n = 22$ and $ERR\gamma$ KO, $n = 19$). (c) Volcano plot of RNA-sequencing (RNA-seq) data showing regulated genes (red) in each $ERR\alpha/\gamma$ KO hiPSC-CM line compared to wild-type control cells (|Fold change| > 1.5 and Benjamini-Hochberg false discovery rate < 0.05). (d) Bars represent enrichment score of Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway terms using transcripts downregulated in $ERR\alpha/\gamma$ KO hiPSC-CMs compared to WT-control. (e) Bar graphs represent mRNA expression levels of *TNNI1* (WT, $n = 8$; $ERR\alpha/\gamma$ KO1 or 6, $n = 6$) and *TNNI3* (WT, $n = 10$; $ERR\alpha/\gamma$ KO1 or 6, $n = 8$) by RT-qPCR. ** $p < 0.01$, *** $p < 0.001$, one-way ANOVA followed by Dunnett's multiple comparison test. (f) Representative immunoblot images of FLAG, $ERR\gamma$, and ACTB levels in hiPSC-CMs following the infection of adenovirus expressing GFP or $ERR\gamma$ (Ad-GFP or Ad- $ERR\gamma$). ACTB was used as a loading control. FLAG expression indicates the levels of overexpressed FLAG-tagged $ERR\gamma$. The protein levels were assessed 48 hours after the adenoviral infection. All graphs in a, b and e represent the means $\pm$ SEM. n denotes independent biological replicates. (g) Volcano plot of RNA-seq data showing regulated genes (red) in $ERR\gamma$ overexpression (OE) hiPSC-CMs compared to GFP OE hiPSC-CMs (|Fold change| > 1.5 and adjusted p -values < 0.05). (h) Bars represent enrichment score of KEGG pathway terms using transcripts upregulated in $ERR\gamma$ OE hiPSC-CMs compared to GFP control.



Supplementary Figure 2. Forced expression of ERRγ drives mitochondrial oxidative flux in hiPSC-CMs.

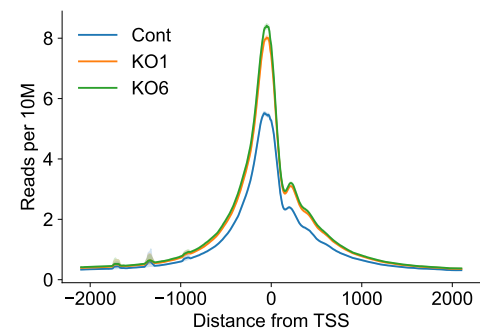
(a) The mRNA levels of *ACSL1* and *ACSL3* determined by real-time quantitative polymerase chain reaction (RT-qPCR) in human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs) following the infection of adenovirus expressing GFP or ERRγ (Ad-GFP; n=15 or Ad-ERRγ; n=15). (b) Representative immunoblot images of ACSL1, ACSL3, VDAC and α-Tubulin levels. VDAC was used as an internal control for mitochondrial content and α-Tubulin was used as a loading control. Bar graphs represents the ratio of ACSL1/3 protein expression normalized to Ad-GFP; n=7. (c) ³H-Palmitate oxidation rates measured in Ad-ERRγ or Ad-GFP. Bars represent mean disintegrations per minute (DPM) normalized to that of Ad-GFP control; n=3. (d) Levels of FAO-derived acylcarnitine species isolated from hiPSC-CMs following Ad-GFP (n=3) or Ad-ERRγ (n=3) were measured by targeted, quantitative metabolomics. (e) Levels of organic acids isolated from hiPSC-CMs following infection by Ad-GFP (n=3) or Ad-ERRγ (n=3) were measured by targeted, quantitative metabolomics. α-KG denotes α-Ketoglutarate. (f) Levels of branched-chain amino acid (BCAA)-catabolism-derived acylcarnitine species isolated from hiPSC-CMs following Ad-GFP (n=3) or Ad-ERRγ (n=3) were measured by targeted, quantitative metabolomics. All data represent the mean ± SEM. n denotes independent biological replicates. *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001 vs Ad-GFP, two-tailed student's t-test. (g) Heat map representing log₂ fold-change (FC) of mRNA levels of genes involved in BCAA catabolism ["Valine, leucine and isoleucine degradation" in Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway] from RNA-sequencing analysis performed in hiPSC-CMs following Ad-ERRγ compared to Ad-GFP.



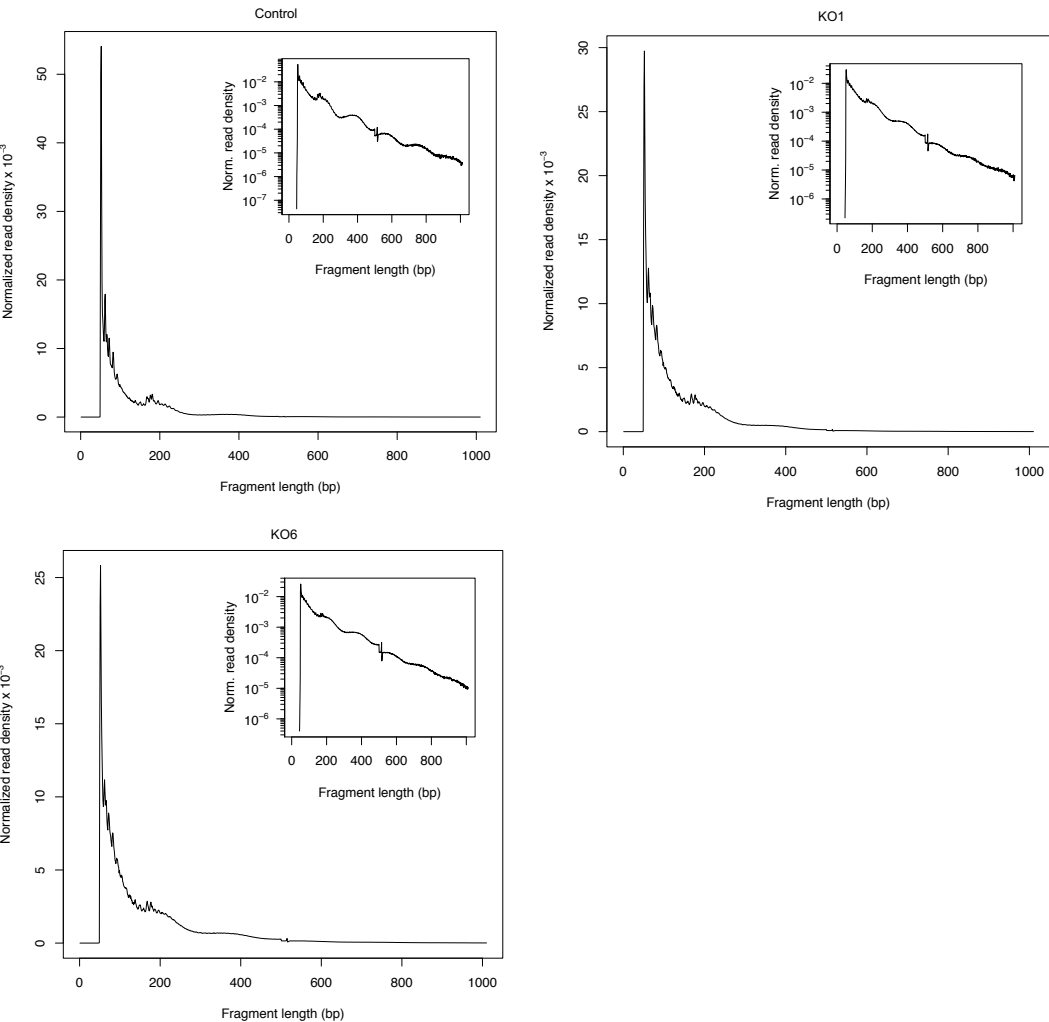
Supplementary Figure 3. ERRγ directly activates the transcription of genes coding cardiac metabolic and structural proteins.

(a) Schematic indicates the intersection analysis with RNA-sequencing (RNA-seq) in ERRα/γ knockout (KO) human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs; GSE165963) and published ERRγ chromatin immunoprecipitation sequencing (ChIP-seq) in hiPSC-CMs (GSE113784). (b) Bar graphs represent enrichment score of Kyoto Encyclopedia of Genes and Genomes (KEGG) and Gene Ontology (GO) Cellular Component pathway terms using the ERR-activated genes defined by the intersection analysis. (c) Aggregation plots represent H3K27ac ChIP-seq signals around transcription start sites (TSS) in WT and ERRγ KO hiPSC-CMs. Significant enrichment of H3K27ac ChIP-seq signals were observed in both groups. (d) De novo motif enrichment results within ERRγ peaks in which H3K27ac deposition was not altered by ERRγ KO. (e) Known motif enrichment results are presented for each ERR-activated and ERR-suppressed targets. The *p*-values were calculated with Fisher's exact test in d and e.

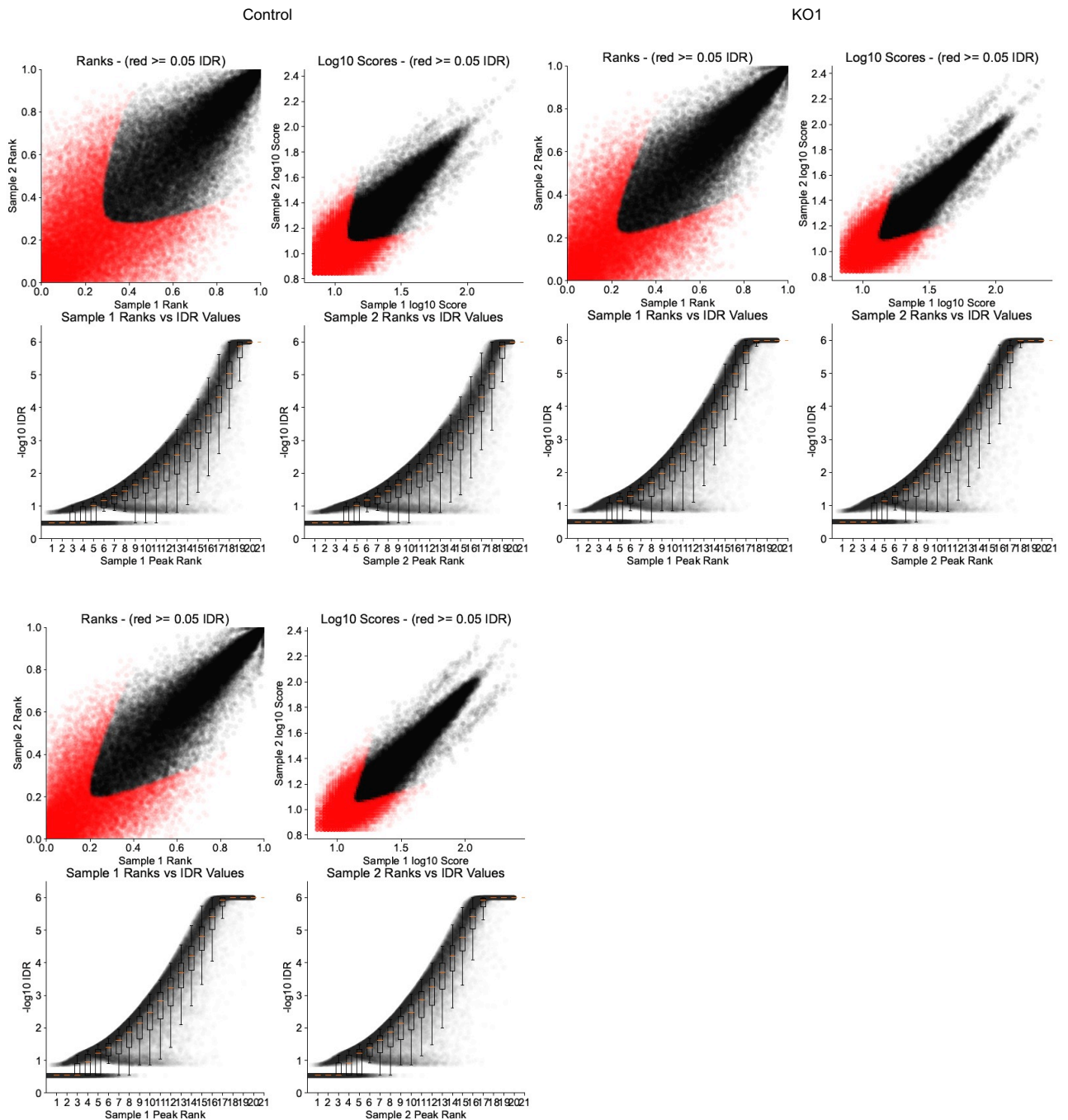
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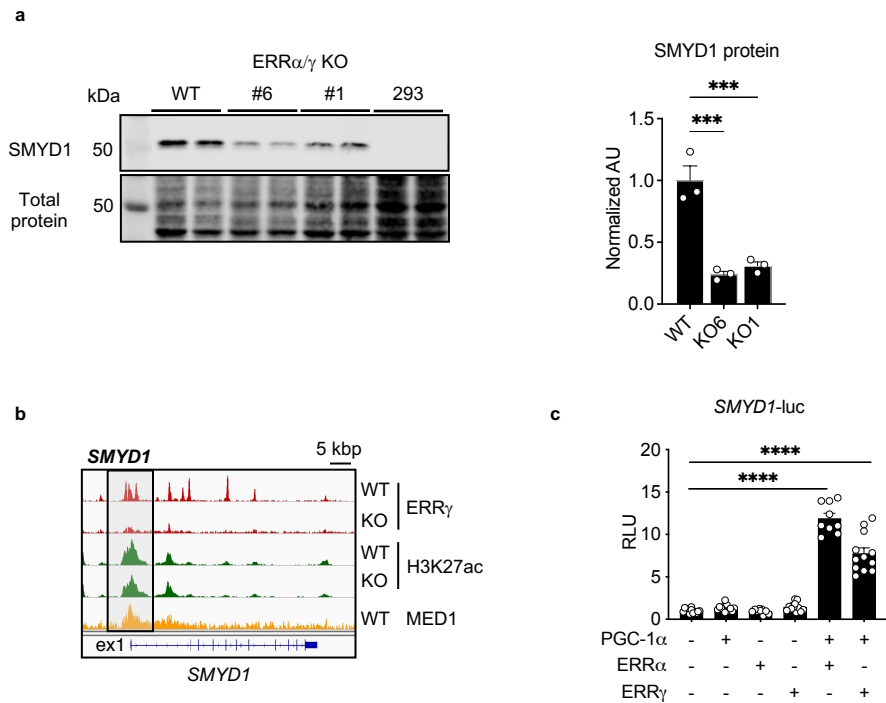
b



c

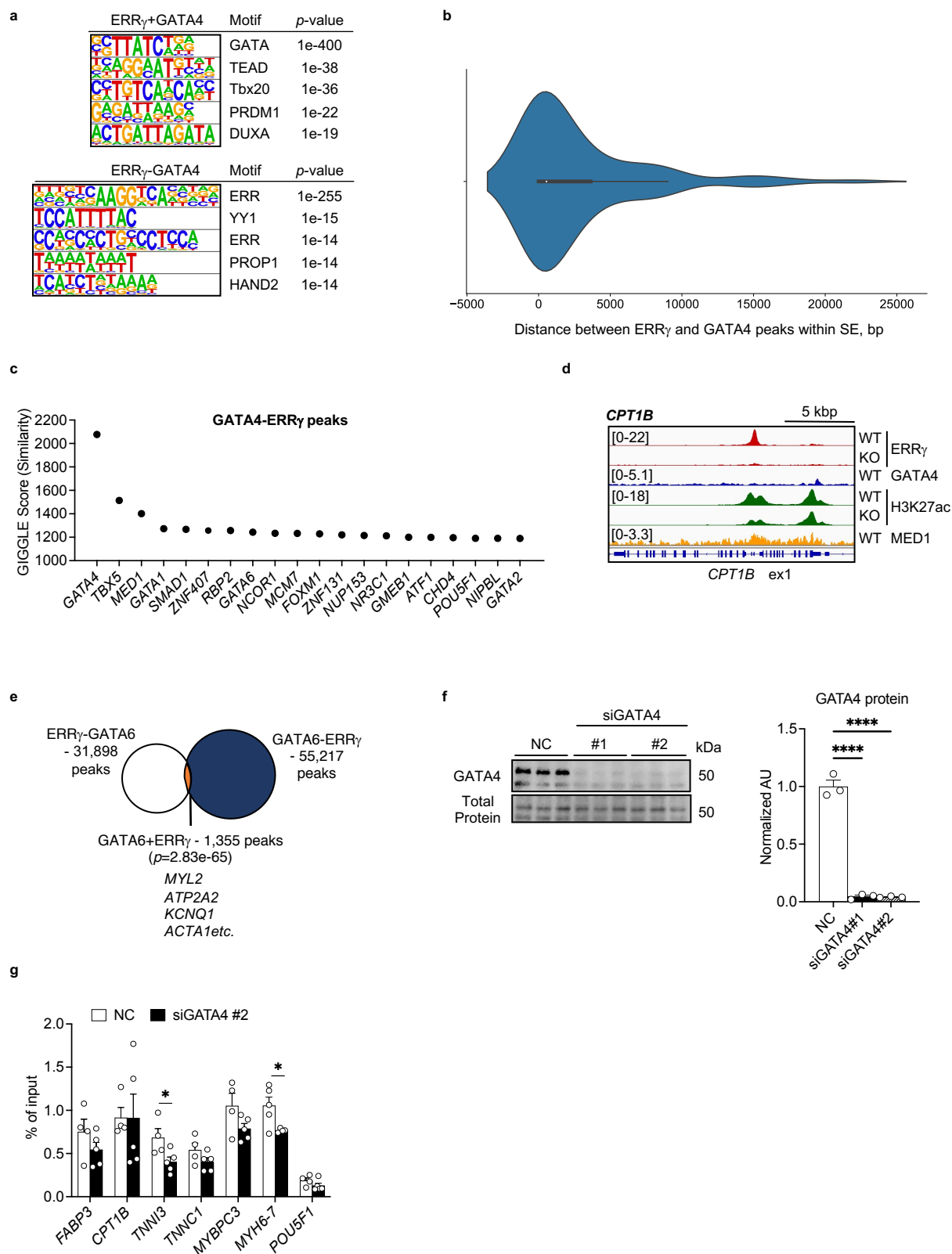


Supplementary Figure 4. Evaluation of the data quality of ATAC-seq conducted on WT and $ERR\alpha/\gamma$ KO hiPSC-CMs. We evaluated our assay for transposase accessible chromatin with high-throughput sequencing (ATAC-seq) data according to Encyclopedia of DNA Elements (ENCODE) data quality standards for ATAC-Seq (<https://www.encodeproject.org/atac-seq/>). (a) Transcriptional start site (TSS) enrichment values in ATAC-seq data from wild-type control (Cont) and $ERR\alpha/\gamma$ KO hiPSC-CMs derived from two distinct lines (KO1 and KO6) are presented. Ideal TSS enrichment is > 10 -fold over flanking regions. (b) Representative fragment size distribution of each data. Nucleosome-free region (< 100 bp) and fragments spanning one or multiple nucleosomes are visible as peaks on the fragment size distribution plot. (c) Replicate concordance was measured by calculating Irreproducible Discovery Rate (IDR) values. The experiment passes the ENCODE criteria if both rescue, and self consistency ratios are less than 2. IDR Self consistency ratio: Cont= 1.01998451, KO1= 1.07301239, and KO6= 1.19442941; IDR Rescue ratio: Cont= 1.272725, KO1= 1.161522, and KO6= 1.145185.



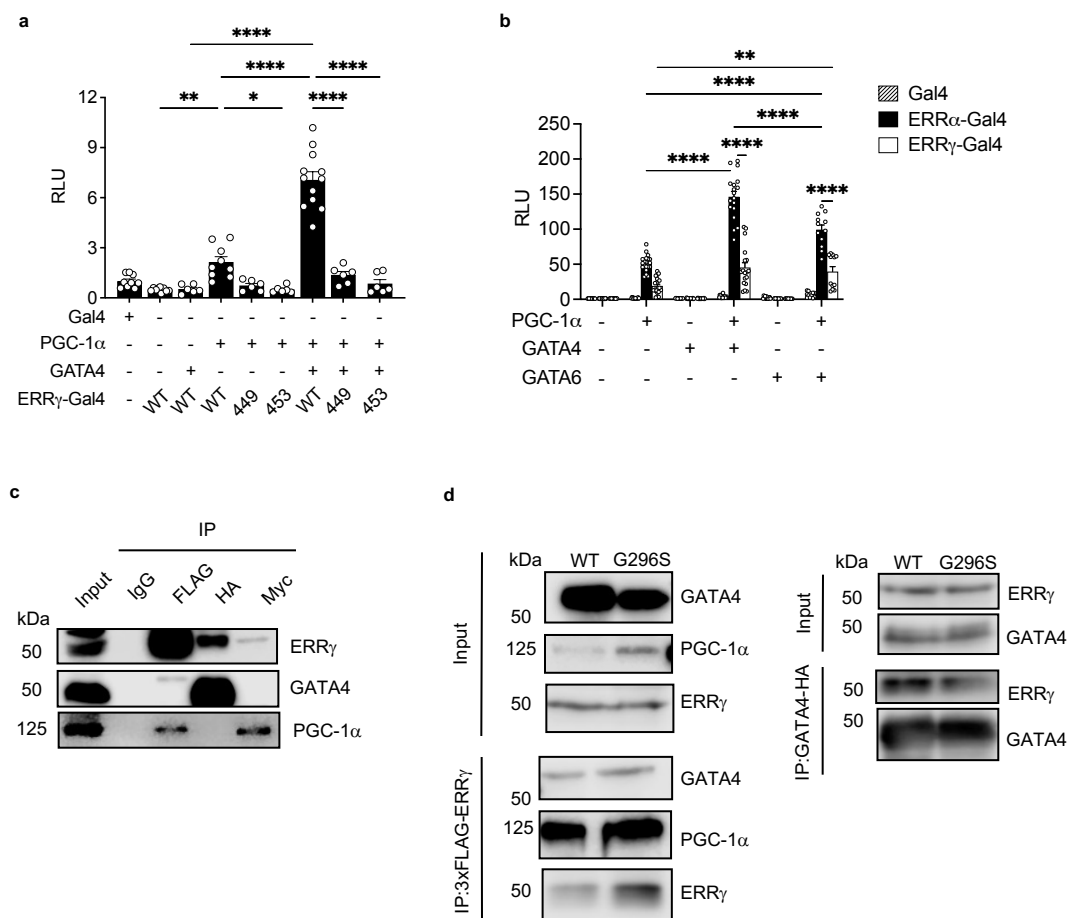
Supplementary Figure 5. ERR α/γ directly regulates the expression level of SMYD1 in hiPSC-CMs.

(a) Representative images of SMYD1 immunoblot. Total protein staining is used as a loading control. Bars represent the quantification of SMYD1 protein expression levels in wild-type control (WT; n=3) and ERR α/γ knockout (KO; n=3) human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs). *** p <0.001, one-way ANOVA, Dunnett's multiple comparison test. Whole cell lysate of AD-293 cells was used as the negative control for SMYD1 protein expression. (b) Genome browser of *SMYD1* locus to show ERR γ (GSE113784), H3K27ac (GSE165965), and MED1 (GSE85631) peaks. (c) The marked region (chr2:88066119-88069666) in (b) was cloned into pGL3 reporter plasmid. Bar graphs represent relative light unit (RLU) derived from *SMYD1*-luc reporter construct. GFP, PGC-1 α , ERR γ , and PGC-1 α /ERR γ , n=12; ERR α and PGC-1 α /ERR α , n=9. One-way ANOVA followed by Dunnett's multiple comparison test was used. **** p <0.0001 compared to GFP transfected group. n denotes independent biological replicates. Data are presented as the means $\pm$ SEM.



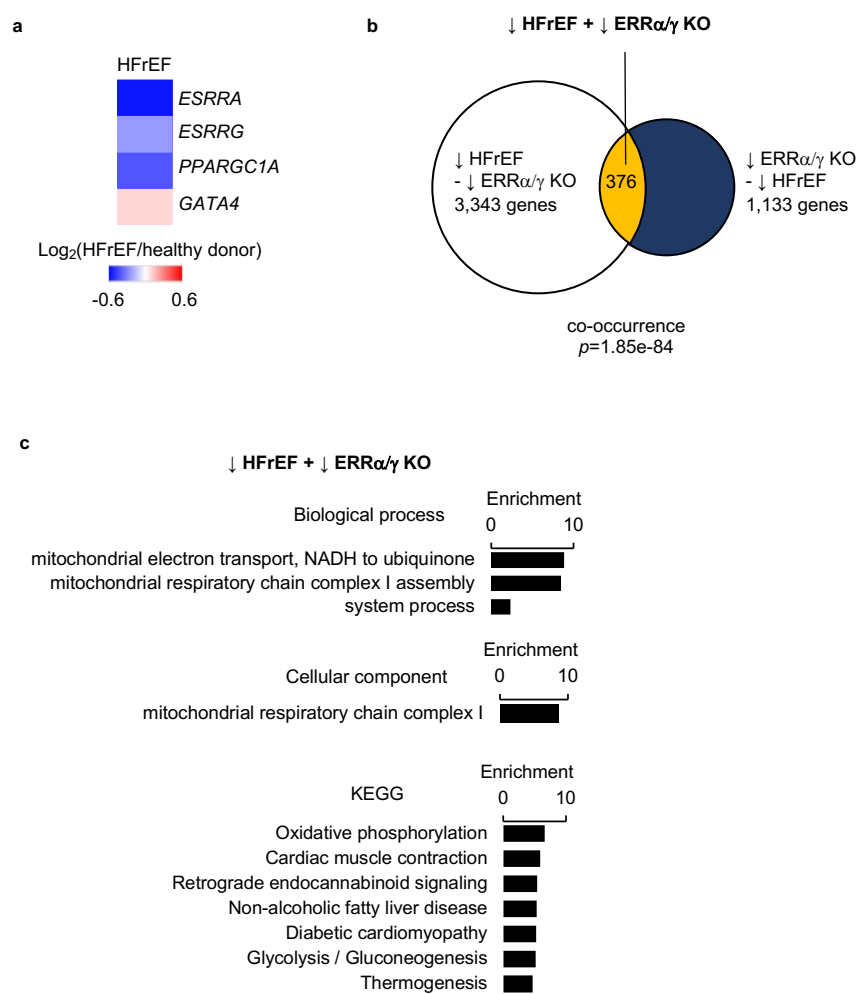
Supplementary Figure 6. GATA4 but not GATA6 often colocalizes with ERR γ on genes coding cardiac-enriched structural proteins.

(a) Differential motif analysis of ERR γ +GATA4 peaks vs. ERR γ -GATA4 peaks (top) and ERR γ -GATA4 peaks vs. ERR γ +GATA4 peaks (bottom). The p -values were calculated with Fisher's exact test. (b) The violin plot shows smoothed density (in arbitrary units) of the distribution of distances between ERR γ and GATA4 peaks within a single super-enhancer (SE) in human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs). White dot: median; horizontal black box: 25-75 percentile; whiskers: 1.5 interquartile range; blue area: width proportional to the density of points in the vicinity of the X coordinate. The sample number = 99, independent SEs containing both ERR γ and GATA4 peaks. (c) Top 20 transcription factors significantly overlapped with GATA4 peaks without ERR γ peaks (GATA4-ERR γ) in Cistrome Data Base Toolkit analysis (<http://dbtoolkit.cistrome.org/>) were plotted. Y axis represents the GIGGLE score which means the similarity between published data sets and given peak set. (d) Genomic browser track of *CPT1B* locus as an ERR γ target. (e) Venn diagram indicates the overlapped peaks from published GATA6 chromatin immunoprecipitation sequencing (ChIP-seq) and ERR γ ChIP-seq (GSE113784) in hiPSC-CMs. GATA6 and ERR γ -shared peaks (GATA6+ERR γ) are highlighted by orange. The p -value was calculated with Fisher's exact test. (f) Representative immunoblot image of GATA4 in hiPSC-CMs transfected with negative control (NC) or two distinct siRNAs targeting GATA4 (siGATA4#1 or #2). Total protein staining was used as a loading control. Bar graphs represent the quantification of GATA4 protein amount ($n=3$). **** $p < 0.0001$ vs NC; one-way ANOVA followed by Dunnett's multiple comparison test. (g) Levels of ERR γ occupation on the indicated targets in negative control (NC, $n=4$) or siGATA4 #2 ($n=5$)-transfected hiPSC-CMs as measured by ChIP-quantitative polymerase chain reaction. * $p < 0.05$ vs WT, two-tailed student's t -test. Stem cell enhancer region on *POU5F1* was used as a negative control. All bars in f and g represent the means $\pm$ SEM. n denotes independent biological replicates in f and g.



Supplementary Figure 7. The ERRγ-PGC-1α cooperates with GATA4.

(a) A series of ERRγ-Gal4 activation function 2 (AF2) mutant reporter experiments were performed in AD-293 cells with PGC-1α and GATA4. 449 and 453 represent L449A-F450A and M453A-L454A AF2 mutants respectively. Bar graphs represent relative light unit (RLU) from pG5luc construct and the means $\pm$ SEM. Gal4, ERRγ-Gal4, and PGC-1α/ERRγ-Gal4, $n=9$; PGC-1α/GATA4/ERRγ-Gal4, $n=12$; PGC-1α/ERRγ L449A-F450A-Gal4, PGC-1α/ERRγ M453A-L454A-Gal4, PGC-1α/GATA4/ERRγ L449A-F450A-Gal4, and PGC-1α/GATA4/ERRγ M453A-L454A-Gal4, $n=6$. $*p<0.05$, $**p<0.01$, $****p<0.0001$, one-way ANOVA followed by Tukey's multiple comparisons test. (b) ERRα or ERRγ-Gal4 system was employed to examine the interaction of GATA4 or 6. Gal4 with or without PGC-1α, $n=18$; ERRα-Gal4, or ERRγ-Gal4 with or without PGC-1α, $n=21$; Gal4 and GATA4 with or without PGC-1α, $n=15$; ERRα-Gal4, or ERRγ-Gal4 and GATA4 with or without PGC-1α, $n=18$; Gal4, ERRα-Gal4, or ERRγ-Gal4 and GATA6 with or without PGC-1α, $n=12$. Bars represent RLU and the means $\pm$ SEM. $**p<0.01$, $****p<0.0001$, two-way ANOVA followed by Tukey's multiple comparisons test. n denotes independent biological replicates. (c) Representative immunoblot immunoprecipitation (IP) images to show overexpressed PGC-1α tagged with Myc/His, ERRγ tagged with 3xFLAG, GATA4 tagged with human influenza hemagglutinin (HA) in AD293 cells. (d) Representative immunoblot IP with anti-FLAG or anti-HA images to show overexpressed ERRγ, PGC-1α, and WT or G296S GATA4 in AD293 cells.



Supplementary Figure 8. Assessment of the PGC-1 α /ERR/GATA4 transcriptional circuitry in human heart disease. (a) The heatmap represents Log_2 [heart failure with reduced ejection fraction (HFrEF)/donor control] of the indicated genes in the published RNA-sequencing (RNA-seq) dataset (<https://zenodo.org/record/4114617#.YYGfOL3MLUJ>) from right ventricle samples of HFrEF and donor control. Changes in *ESRRA*, *ESRRG*, and *PPARGC1A* levels were significant (Benjamini-Hochberg false discovery rate<0.05). (b) Venn diagram shows the intersection of significantly downregulated genes in *ERR* α/γ KO hiPSC-CMs and HFrEF. The p -value was calculated with Fisher's exact test. (c) Bar graphs represent significantly enriched Gene Ontology (GO) Biological process, GO Cellular component, and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway terms with the commonly downregulated genes (highlighted with yellow in b) in *ERR* α/γ KO hiPSC-CMs and HFrEF.

Supplementary Table 1. Row-wise z-scores of FPKM values of the annotated-genes associated with both cardiac SE and ERR γ binding sites in hiPSC-CMs.

Gene name	WT_1	WT_2	WT_3	KO1_1	KO1_2	KO6_1	KO6_2	KO6_3
SRSF2	1.491202282	-0.953351718	-0.639713543	1.00264854	-1.739789238	0.522664046	0.384906515	-0.068566885
ZFAND5	1.216273898	-0.596917315	0.814566932	0.182971716	-2.250302801	0.04044577	0.039278929	0.632685268
MAT2A	2.133536182	-0.447481997	0.093134491	0.210309413	-1.772523267	0.076683037	-0.191788069	-0.101869789
KLF9	1.107925236	0.31611589	0.966167675	0.453406243	0.901625399	-1.279778666	-1.380213996	-1.085247781
STOM	0.273283718	0.643426137	1.631014632	0.724979589	0.291327624	-1.223681419	-1.218273792	-1.120276489
GADD45A	1.273400705	-0.841289086	1.441412201	1.133231071	-0.928995305	-0.651077695	-0.667753252	-0.758928639
CDC42EP3	1.239275613	0.041032701	1.009167961	1.276386677	-0.248927347	-1.304634837	-0.891661797	-1.120638972
MICAL3	2.359127081	-0.207803242	-0.053959728	-1.239560972	0.185660165	-0.748823915	0.179254214	-0.473893603
BOLA1	0.513116465	1.675159504	1.106222275	-1.327505423	0.167422199	-0.214192967	-0.843881576	-1.076340478
SRL	0.870999996	1.509117226	1.069454938	-0.522421626	0.221822941	-1.095984237	-0.718682413	-1.334306826
IRF2BPL	0.687892181	1.519458387	1.134310021	-0.512785928	0.371702952	-1.309837558	-1.061893497	-0.828846557
MYH6	0.862482219	1.18568879	1.571818633	-0.910822398	-0.026975306	-1.099343177	-0.581582689	-1.001266072
IRF2BP2	0.608031832	0.724279667	1.891639316	-1.05551168	0.418841489	-0.803129595	-0.866883675	-0.917267354
ARID1A	0.61387756	1.348007049	1.504784579	-1.415752418	-0.896138469	-0.654252297	-0.551034041	0.050508037
LMOD2	0.539801858	0.753356775	2.175412309	-0.659407689	-0.675256063	-0.712196994	-0.712445144	-0.709265054
FILIP1	0.915069311	-0.162611692	2.20092709	-1.013256186	-0.072282478	-0.838907976	-0.39808751	-0.630850558
LMO7	1.403073967	-0.378867861	1.895570663	-1.193597509	-0.203006629	-0.649500939	-0.324434274	-0.549228417
RND3	1.574556659	0.297921078	1.433726039	0.09807875	-0.257730335	-1.053811924	-1.053411173	-1.039329094
NUAK1	1.557838073	0.188278693	1.532252503	0.089627761	-0.336562716	-0.968636411	-0.966755905	-1.067841198
SAMD4A	1.230119768	0.000202847	1.964850592	-0.13988311	-0.317107571	-0.969514541	-0.917052741	-0.851510041
SCHIP1	1.369411032	0.184033356	1.86679998	-0.292782268	-0.549191486	-0.864455909	-0.820125581	-0.893689124
ADAM11	1.495209005	-0.061353317	1.857250837	-0.448305863	-0.451877076	-0.780852953	-0.796697599	-0.813373034
PFDN1	1.493100118	0.454089059	1.676608134	-0.555724697	-0.511619823	-0.892924702	-0.801029595	-0.862480495
JPH2	1.632500457	0.461289047	1.549835449	-0.67301446	-0.547173172	-0.846401308	-0.725663432	-0.851372441
SYNP2L	1.563121787	0.413175893	1.655760694	-0.674555767	-0.697828932	-0.765339286	-0.7387934	-0.755539078
CENPN	1.275809697	0.635016447	1.776439411	-0.582404504	-0.813983967	-0.560053342	-0.861759296	-0.869064447
XIRP1	1.295210934	0.531267896	1.824570385	-0.563376534	-0.696013461	-0.826029308	-0.756592988	-0.809036925
MYL12A	1.420611797	0.605528282	1.675475412	-0.550771448	-0.659814788	-0.811374552	-0.827052387	-0.852602316
CMYA5	1.152996947	0.572065475	1.912559662	-0.745176941	-0.733530154	-0.813471402	-0.785092981	-0.560350607
DAPK3	1.070126881	0.382551823	2.036100931	-0.422603636	-0.613176594	-0.781368809	-0.802205009	-0.811380506
TIN	1.589671336	0.337689485	1.624152096	-1.067573451	-0.50948733	-0.779257874	-0.586131655	-0.609062606
SLMAP	1.43009841	0.12572858	1.887001416	-0.762593828	-0.676068054	-0.648565064	-0.669001368	-0.686600091
ANKRD1	1.527422151	0.048875468	1.828541968	-0.645978102	-0.663659282	-0.697755107	-0.70018976	-0.697257336
SLC38A1	2.335401895	0.134654624	0.709989419	-0.624808372	-0.66315269	-0.619418753	-0.637919238	-0.634746885
TNNC1	2.022070763	0.429769467	1.093215423	-0.785563786	-0.721364274	-0.658344016	-0.624267903	-0.755515674
PALM2AKAP2	2.062648084	0.359585331	1.07269444	-0.817635865	-0.721035827	-0.668045714	-0.621097828	-0.667112622
FSD2	2.170370078	0.360572174	0.870083356	-0.661872008	-0.915304547	-0.501437263	-0.641794307	-0.680617483
MYL3	2.136870611	0.42519886	0.910051452	-0.701191802	-0.791647695	-0.645055565	-0.678164031	-0.65606183
FLNC	2.180073608	0.278654702	0.901040657	-0.432889795	-0.634672617	-0.82862841	-0.661440238	-0.802137907
NAV1	2.177045813	0.395221444	0.843878735	-0.734414266	-0.493255307	-0.74986166	-0.640642314	-0.797972446
SH3BGR	2.052903969	0.549648783	0.920546799	-0.550913467	-0.45020214	-0.809971279	-0.791468251	-0.920544416
RAPGEF2	1.937012825	0.019546535	1.40493295	-0.58449645	-0.737619749	-0.671244597	-0.655937184	-0.712194329
SLC20A2	1.859591816	0.270413428	1.402057248	-0.676707128	-0.678700628	-0.731113401	-0.672067767	-0.773473569
ACTC1	1.902715233	0.334410468	1.315440959	-0.673114569	-0.706497873	-0.754286216	-0.638694597	-0.779973404
BMPR1A	1.972000705	-0.160788587	1.373192627	-0.369074386	-0.484565086	-0.857640235	-0.800153285	-0.672971754
RBM20	2.125255227	-0.006098742	1.123901248	-0.509874264	-0.508015842	-0.863885291	-0.559801292	-0.801481044
MYLK3	1.451488215	1.08899163	1.283803539	-0.618482067	-0.458030992	-0.833595572	-0.771058104	-0.945314183
PGM1	1.490191364	1.066501022	1.286537427	-0.769655442	-0.676729127	-0.806571869	-0.792098163	-0.888175211
HSPB7	1.472033998	1.105751346	1.261110611	-0.65521292	-0.612708162	-0.870090278	-0.773782842	-0.927101752
ANKMY2	1.250648294	1.116279916	1.480024788	-0.757509686	-0.605541061	-0.805976912	-0.866423689	-0.811501651
WIPI1	1.125500789	0.278035395	1.436842702	-0.563039345	-0.721594965	-0.910403371	-0.909951235	-0.735389969
PRKAR1A	1.636689294	0.582308291	1.476908419	-0.821313401	-0.920103708	-0.619053064	-0.618529152	-0.71690668
LRRIIP1	1.652374203	0.85726383	1.279880152	-0.768585822	-0.757099669	-0.880927129	-0.707175825	-0.675729741
FABP3	1.543053395	0.997291731	1.293991981	-0.736681151	-0.853650616	-0.743011193	-0.749022425	-0.751970932
SMYD1	1.667242691	0.728340825	1.354984133	-0.741035574	-0.66638837	-0.827787285	-0.640956444	-0.874399976
ACTN2	1.625679054	0.708734579	1.423277019	-0.723887326	-0.765492728	-0.775945459	-0.704437919	-0.787928123
MXRA7	1.685627337	0.65802829	1.388639743	-0.811878335	-0.736258611	-0.769595608	-0.675516842	-0.739520519
TRIM55	1.483444237	0.900044617	1.432716472	-0.871498972	-0.716183413	-0.703585448	-0.705646247	-0.819291246
MYH7	1.508001439	0.886248528	1.422285926	-0.765756123	-0.710033647	-0.784489838	-0.759735035	-0.796521252
CSRP3	1.526730063	0.749481546	1.500545173	-0.71586581	-0.735779651	-0.779769095	-0.769436313	-0.774185142
BZW2	1.411986151	0.848666119	1.54556296	-0.796466659	-0.791250639	-0.756172659	-0.716229794	-0.745095478
STK39	1.903997807	0.56940156	1.166681416	-0.776456562	-0.876865254	-0.642318677	-0.697892029	-0.646548261
ROR1	1.796808273	0.719836524	1.20777368	-0.791706934	-0.746041495	-0.680168647	-0.83637032	-0.670131082
PYGB	1.929536061	0.553157024	1.140763301	-0.698047314	-0.637175711	-0.790683828	-0.626717751	-0.870831782
PTGAB3	1.948263303	0.709052908	0.977185674	-0.62726881	-0.614693129	-0.937351451	-0.589943517	-0.865244705
AFG3L2	1.767035865	0.841692828	1.141317097	-0.624291919	-0.82987182	-0.692821052	-0.693823046	-0.909237953
POPDCC	1.786561391	0.547154204	1.318497804	-0.552075347	-0.572687226	-0.873887553	-0.76842428	-0.885138993
CMC2	1.752104804	0.770394372	1.222196805	-0.640737701	-0.645112855	-0.842511155	-0.803290821	-0.813043448
PLN	1.751818882	0.763644097	1.226718679	-0.694968877	-0.59171085	-0.8302193	-0.81296442	-0.812318211
KIF13A	1.700975066	0.920749619	1.127676004	-0.167305019	-0.53467815	-0.843944092	-0.667016622	-0.636456804
ST6GAL1	1.832808644	0.927188865	0.956571612	-1.025699509	-0.635095246	-0.717974387	-0.682259784	-0.655540195
TNNT2	1.774003463	0.988629439	0.944097084	-0.653909518	-0.428772244	-0.88185312	-0.674280213	-1.067914891
SORBS2	1.672428636	0.918235661	1.168377431	-0.861873909	-0.4294365	-0.901265091	-0.664299151	-0.902167078
LDB3	1.884399987	0.557701222	1.105640897	-0.624117928	-0.233529323	-0.951605884	-0.703746153	-1.03474282
AMOTL2	1.436350081	0.889218453	1.361658977	-0.394764629	-0.265880483	-1.084061345	-0.928486405	-1.014034649
JMDJ6	1.525391834	0.083925586	1.649779577	-0.856868153	-1.224605009	-0.790195308	-0.119471153	-0.267957375
MYL4	2.218626241	-0.102559277	0.794593982	-0.624140929	-1.27274592	-0.329379899	-0.13765285	-0.546741348
RBM24	1.944844071	-0.301548081	1.060443068	-1.319064432	-1.032881607	-0.162688366	0.180209039	-0.369313693
MTHFR	0.629434037	2.126171467	-0.169494477	0.480728401	-0.179794522	-1.007287918	-0.870303623	-1.009453366
NKX2-5	0.550838394	2.06638068	0.488595174	-0.266852288	-0.256362925	-1.08931509	-0.134570496	-1.358754994
H4C9	-0.485872288	1.755198789	-1.032061077	-0.484985292	1.374649801	-0.933655639	-0.158854327	0.330447661
TXNIP	-0.432735507	1.618472939	0.247124811	-0.80071021	1.656729102	-0.763301332	-0.763384972	-0.76219483
POLR3GL	-0.181084673	1.948338524	0.698642964	-1.296131431	0.733386353	-0.92143719	-0.231682726	-0.750031822
FOS	-0.006523142	-0.39518158	-0.063246404	-0.280309411	2.576472172	-0.553280931	-0.626053935	-0.65187677
FOX P1	-0.130673033	-1.327724128	-0.443536819	-0.900770525	2.208388419	0.432367936	0.351227728	-0.180179578
JOSD2	-1.544028028	0.042657651	0.109471474	1.912156006	0.570125969	-0.293015787	0.3813913	-1.17758333
JPH1	0.210565949	-1.150364224	0.071404372	2.418528463	-0.36988			

Supplementary Table 1 shows the row-wise z-scores of fragments per kilobase of exon per million reads mapped (FPKM) values of the annotated-genes associated with both cardiac super-enhancer regions (SEs) defined by MED1 chromatin immunoprecipitation sequencing (ChIP-seq; GSE85631) and ERR γ binding sites defined by ERR γ ChIP-seq (GSE113784) in human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs). The heatmap in Fig. 3c was generated with the z-score presented in this table.

Supplementary Table 2. DNA oligonucleotide sequences for cloning

Cloning primer	Forward	Reverse
pcDNA3.1 (-)		
3xFLAG-ERR α	GCCCTCTAGACTCGAGCCACCATGGACTACAAAGACCAT GACGGTGATTATAAAGATCATGACATCGATTACAAGGAT GACGATGACAAGTCCAGCCAGGTGGTGGGCATTGAGC	GTGGCGGCCGCTCGATCAGTCCATCATGGCCTCG
3xFLAG-ERR γ	GCCCTCTAGACTCGAGCCACCATGGACTACAAAGACCAT GACGGTGATTATAAAGATCATGACATCGATTACAAGGAT GACGATGACAAGGATTCCGGTAG	GTGGCGGCCGCTCGATCAGACCTTGGCCTCCAAC
GATA4-HA	GCCCTCTAGACTCGAGCCACCATGTATCAGAGCTTGGC CATG	GTGGCGGCCGCTCGATTAAGCGTAATCTGGAACATCGTAT GGGTACGCAGTGATTATGTC
GATA6-HA	GCCCTCTAGACTCGAGCCACCATGGCCTTGACTGACGG CGG	GTGGCGGCCGCTCGATCAAGCGTAATCTGGAACATCGTAT GGGTAGGCCAGGCCAGGGC
pGL3-Basic	Forward	Reverse
<i>TNNI3</i>	CTAGCCCGGGCTCGAGCCTGGACTCTTGGGTCTGAG	GATCGCAGATCTCGAGGCGAGGTTCCCTAGCCTG
<i>TNNI3</i> promoter vector for ERRE deletion	GCCTCCTGCCATTCCCGG	CAGACTAAATATACTGTCACCTCC
ERRE-deleted fragment	AGTATATTAGTCTGCCTTTATCTCAGGTCTCAAGCAGC CTAGCCCGGGCTCGATTAAGGTACGAATTATTATACATT T	GGAATGGCAGGAGGCGGGGCGTTTGGAGGGTCA GATCGCAGATCTCGACAGCGCCAGCACGAAGGT
<i>COX6A2</i>	CTAGCCCGGGCTCGATCGAAGGTGTTTTGGGAGATTCA	GATCGCAGATCTCGAGGAATGAAAGCCCATTTCACTTT
<i>SMYD1</i>		
pGL4.24	Forward	Reverse
<i>MYH6-7</i> enhancer	GCTCGCTAGCCTCGATACCAGTGTGCTTAAAAACCCAGG	TCTTGATATCCTCGAGTAGGCAACAGATACACACATTATG
pAdTrack-CMV	Forward	Reverse
FLAG-ERR γ	GAAGGATCCGCCACCATGGACTACAAAGACGATGACGA CAAGGATTCGGTAGAACTTTGC	CGGTAGAATTCTCAGACCTTGGCCTCCAACATTTTC
Adeno-X CMV	Forward	Reverse
GATA4-HA	GTAACCTATAACGGTCATGTATCAGAGCTTGCCATGGCC GCCAACCAC	ATTACCTCTTTCTCCTTAAGCGTAATCTGGAACATCGTATG GGTACGCAGTGATTATGTC

Supplementary Table 3. DNA oligonucleotide sequences for mutagenesis

Mutagenesis	Forward	Reverse
GATA4 mutants		
pcDNA3.1 (-)-GATA4 S52F	GGGCCTGTTCTACCTCCAGGGCGGAGGC	AGGTAGAACAGGCCCCAGCACGGAGG
pcDNA3.1 (-)-GATA4 P163S	GCCCCTACTCGGCTTACATGGCCGACG	AAGCCGAGTAGGGGCTGGAGTAGGAGC
pcDNA3.1 (-)-GATA4 E216D	GGCAGAGACTGTGTCAACTGTGGGGCTATGTC	GACACAGTCTCTGCCTTCTGAGAAGTCGTC
pcDNA3.1 (-)-GATA4 G296C	ATGCCTGCTGCCTCTACATGAAGCTCCACGG	AGAGGCAGCAGGCATTGCACACAGGC
pcDNA3.1 (-)-GATA4 G296S	ATGCCTGCAGCCTCTACATGAAGCTCCACGG	AGAGGCTGCAGGCATTGCACACAGGC
pcDNA3.1 (-)-GATA4 E395RfsX44	GCAGCAGCAGGAGATGCGTCCCATCAAGACG	ATCTCCTGCTGCTGCTGGTGGTGGC
pcDNA3.1 (-)-GATA4 L403M	TCTCGGCCATGAAGCTCTCCCCACAAGGC	GCTTCATGGCCGAGAGGACAGGGTGG
pcDNA3.1 (-)-GATA4 N-zinc Δ	GCAGAGAGGGCCTCTACCACAAGATGAACG	AGAGGCCCTCTCTGCCTTCTGAGAAGTCG
pcDNA3.1 (-)-GATA4 C-zinc Δ	GCCTCTCCGGCCTCTACATGAAGCTCCACG	AGAGGCCGGAGAGGCCCACTCGGCG
<i>TNNI3</i> -luc mutant	Forward	Reverse
pGL3-Basic- <i>TNNI3</i> promoter GATA binding sites deletion	GATCCAGCTGGGACCCTGAAGGTCACC	GGTCCCAGCTGGATCACCAGCCACC
ERR γ -Gal4 mutant	Forward	Reverse
ERR γ L449A-F450A-Gal4	ACAAAGCAGCATTGGAATGCTGGAGGCC	CCAATGCTGCTTTGTGCATGGGCACTTTGCC
ERR γ M453A-L454A-Gal4	TGGAAGCAGCAGAGGCCAAGGTCTGACGTC	CCTCTGCTGCTTCCAAAAAAGTTTGTGCATGGGC

Supplementary Table 4. DNA oligonucleotide sequences for qPCR and ChIP-qPCR

qPCR	Forward	Reverse
<i>ACADM</i>	TCCAGATCCTAAAGCTCCTGC	ACATCGCTGGCCCATGTTTA
<i>ACSL1</i>	ACTTGGGAAGGATTCTGGTCTG	GGCCTTTTCGCCCTTCATTGT
<i>ACSL3</i>	TGGAGAGTTTGAACCCGATGG	AGAAACATATTCGCCCTGCCTGT
<i>ACTG1</i>	CCGAGCCGTGTTTCCTTCC	GCCATGCTCAATGGGGTACT
<i>ANKRD1</i>	CGTGGAGGAAACCTGGATGTT	GTGCTGAGCAACTTATCTCGG
<i>ATP1A3</i>	TGACCTGCGGATCATCTCAG	GAGTCTGGGGCTCGGATTC
<i>ATP5B</i>	TGGCCACTGACATGGGTACTA	GGGTCAGTCAAGTCATCAGCA
<i>BCL6</i>	GTTGTGGACACTTGCCGGAA	CTCTTCACGAGGAGGCTTGAT
<i>CKMT2</i>	AGAAAGTGTGTGCCGAGGTC	ATGCAGTTGTTGTGCTTGCG
<i>COX6A2</i>	TTCCGTCCCTACCAACACCT	GGGTTACGCTGGCTATTGTG
<i>CPT1B</i>	GTGAGTGACTGGTGGGAAGAGTA	TGCACGTCTGTATTCTTGATGAG
<i>CSRP3</i>	GTGCCATCTGTGGGAAGAGT	TGTGTAAGGCCTCCAAACCC
<i>ESRRA</i>	AGCGAGAGGAGTATGTTCTAC	AGCCTCGGCATCTTC
<i>ESRRB</i>	TGCGAGATCACCAACGGAG	GCATCCCCACTTTGAGGCAT
<i>ESRRG</i>	TTGGTGGCTGAACCGGA	CACACAGTGTAGTGAGGGCT
<i>FABP3</i>	CACAGCACCTTCAAGAACACA	AGTTTCCTCCATCCAGTGTC
<i>GATA4</i>	GTG TCC CAG ACG TTC TCA GTC	GGG AGA CGC ATA GCC TTG T
<i>GATA6</i>	CTGCGGGCTCTACAGCAAG	GTGGGCACAGGACAATCCAAG
<i>KCNQ1</i>	ACCATCGGGCCACCATTAAAG	CGCGCTTGCTGGAATTTCTT
<i>LMOD2</i>	TCATGGAAGCAATTCGGGGA	TTATCGCAGGGCTTCTGGAAC
<i>MDH1</i>	TGCTGTCTATCAAGGCTCGAA	CTCCCTCTGGGGTTCCAAAC
<i>MEF2C</i>	GACCTCACGTCTGGTGCGAC	TGCTTGTCATATTCTTGTTCAAGTT
<i>MYBPC3</i>	GGTGGAGTTTGAGTGTGAAGTA	TGAACCGGTATTTGAAGGTCTC
<i>MYH7</i>	CTGTCCAAGTTCGCCAAGGT	ATTCAAGCCCTTCGTGCCAA
<i>MYL3</i>	GCCAAAGACAGGAAGAGCTCAAT	CCTCATAGGTGCCTGTGTCC
<i>PDK4</i>	GGAGCATTTCTCGCGCTACA	ACAGGCAATTCTTGTCGCAAA
<i>PLN</i>	GACAGAGTCAGAAAATCCCCA	AAAAGCTGGCAGCCAATATGAG
<i>PPARGC1A</i>	AGTGACCAATCAGAAATAATCCAATCAG	TGAGGACTGCAGCAAGTTTG
<i>PPARGC1A variant 8</i>	TCTGGGTGGACTCAAGTGGT	TCCAAAGGATGTCCTTACCTCAAA
<i>PPARGC1A variants 1-7</i>	AGAGGCAGAAGGCAATTGAAGA	GTCCGTGTTGTGTCAGGTCT
<i>PPARGC1B</i>	CAGCCACTCGAAGGAACCTCA	CGGATGCTTGGCGTTCTG
<i>RPLP0</i>	TCTACAACCCTGAAGTGCTTGAT	GATAGAATGGGGTACTGATGCAA
<i>RYR2</i>	AGCCCTGAAGAATCGGCAAA	GACGTGCAAAACGGTCTATGC
<i>TNNC1</i>	TGGTTCGGTGCATGAAGGAC	GTGCATGTAGCCATCAGCATT
<i>TNNI1</i>	CTCCTCTGGAAGTGGGATTA	GCCTCATGGGTGGATAGA
<i>TNNI3</i>	GTGAAGAAGGAGGACACCGAG	CAAACCTTTTCTTGCGGCC
<i>TTN</i>	CCCCATCGCCCATAGACAC	CCACGTAGCCCTCTTGCTTC
ChIP-qPCR	Forward	Reverse
<i>ATP5B</i>	CGGCTCCAGGCATCCTTTTAACACCAC	CGGCGGTCCATCCTGGTAAGTGCTTTT
<i>CPT1B</i>	GTTTCTGTGCGGTGAAGTT	CATCGGTGACCTTTTCCCTA
<i>FABP3</i>	TCTTGACCTTGCCTTGGGAC	GTCTGACCTTGCTCTGCAGT
<i>MYBPC3</i>	CTCTCTGCGTCCCTGACCT	TTGATTCTCGGTTTCTTTTCC
<i>POU5F1</i>	CCCCAGGACAGAACCATCAC	GGTGTGGAGATTCAGCCAA
<i>TNNC1</i>	AGGAGTGAGGGAGGCTGAG	CTATCCTGGGTGGTGCTGAC
<i>TNNI3</i>	CGTTTGGAGGGTCAGTGAG	CTGTGTCCTCGCCCTTTATC
<i>MYH6-7 enhancer</i>	CTCTAGTGCACCCCTTCACC	ATCTGCTAACCACGGTGTC
<i>NPPA-NPPB enhancer</i>	CTTGGTGACATCTGGGCTTT	CTCACCACGTGTGACGAAG

Supplementary Table 5. DNA oligonucleotide sequences for guide RNA

plentiCRISPRv2	Forward	Reverse
gRNA targeting rodent <i>Esrra</i>	CACCGCAGACCAGGCAGAGCGTTT	AAACAAACGCCTCTGCCTGGTCTGC
gRNA targeting rodent <i>Esrrg</i>	CACCGGTGGGTCTTCCGACGCCAGT	AAACACTGGCGTCGGAAGACCCACC
Non targeting control (NTC)	CACCGATTGTTGACCGTCTACGGG	AAACCCCGTAGACGGTCGAACAATC
pLV hU6-sgRNA hUbC-dCas9-KRAB-T2a-Puro	Forward	Reverse
gRNA targeting human <i>PPARGC1A</i>	CACCGGTGTGTGCTCTGTGTCACTG	AAACCAGTGACACAGAGCACACACC