SUPPLEMENTAL MATERIAL

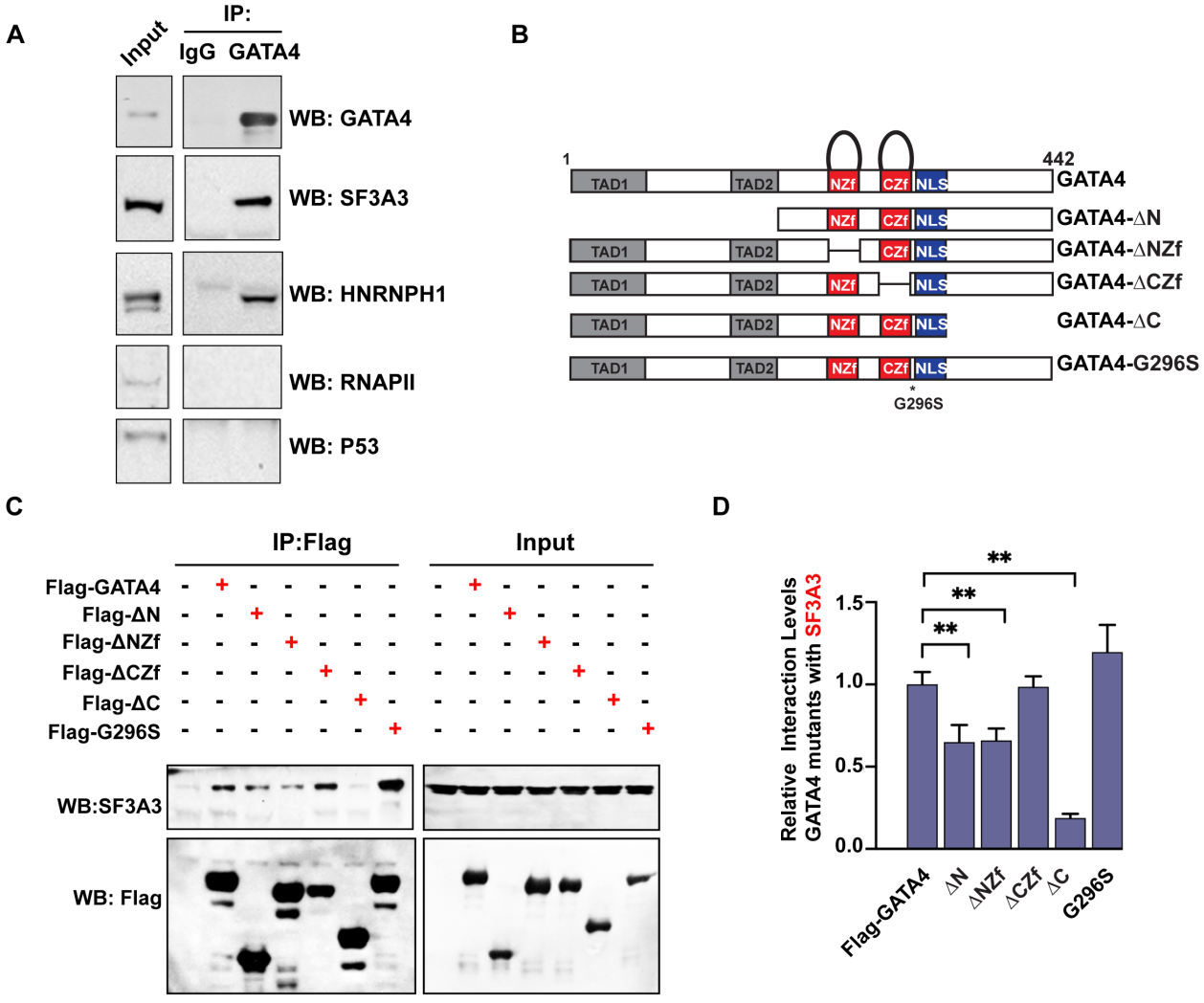


Figure S1. GATA4 protein interacts with RNA splicing proteins. **(A)** Western blot (WB) of splicing factors indicated after GATA4 immunoprecipitation (IP) using anti-GATA4 antibody in human iPS-CPCs. Protein expression in input lysate indicated. Cell lysate were treated with RNase and DNase before IP. (B) Domain architecture of wild-type GATA4 and deletion mutants. TAD, transcriptional activation domain; NZf, N-terminal Zinc finger ; CZf, C-terminal Zinc finger;

NLS, nuclear localization signal domain. (C,D) Co-IP assay of GATA4 mutants with SF3A3. Immunoblot (IB) of SF3A3 after GATA4 IP using anti-Flag antibody in GATA4 knockout HEK293T cells expressing Flag-GATA4 mutants. Protein expression in input lysate indicated. Cell lysates were treated with RNase and DNase before IP. (D) Quantification of relative interaction levels between GATA4 mutants and SF3A3. Data are shown as means ± SEM (n=3). One-way ANOVA coupled with a Tukey test was used to assess significance. (**P < 0.001).

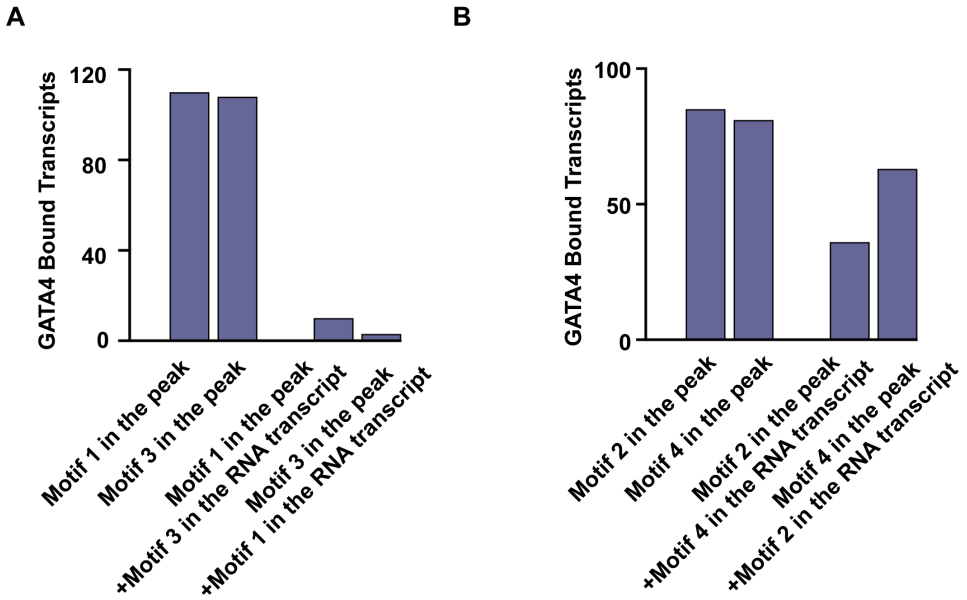


Figure. S2. GATA4-bound transcripts containing indicated motifs in eCLIP peak or throughout transcript.

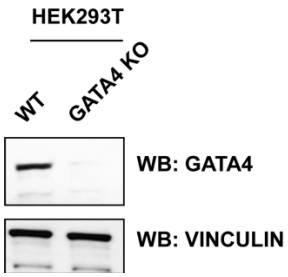


Figure S3. Protein levels of GATA4 in HEK293T or GATA4-KO HEK293T cells.

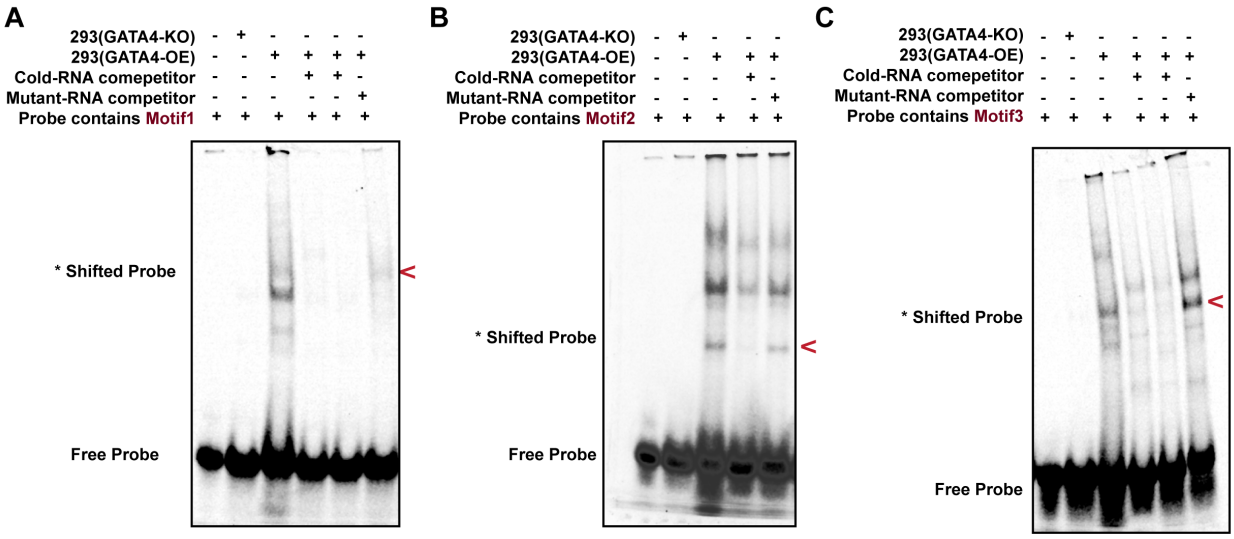


Figure. S4. GATA4 binds to RNA with consensus motifs. (A to C), EMSAs were performed by adding GATA4-KO or GATA4-OE HEK293T cell lysate to an IRDye 800 labeled GATA4-motif-sequence containing RNA probe or annealed paired probes, as indicated in the figure. Competitive gel-shift assays were performed by incubating HEK293T GATA4-OE lysate, and IRDye 800 marked GATA4-peak-sequence containing RNA probe in the presence of the unlabeled competitor RNA or unlabeled mutant-RNA competitor. Observed specific shifted bands are labeled on the right (Red arrow).

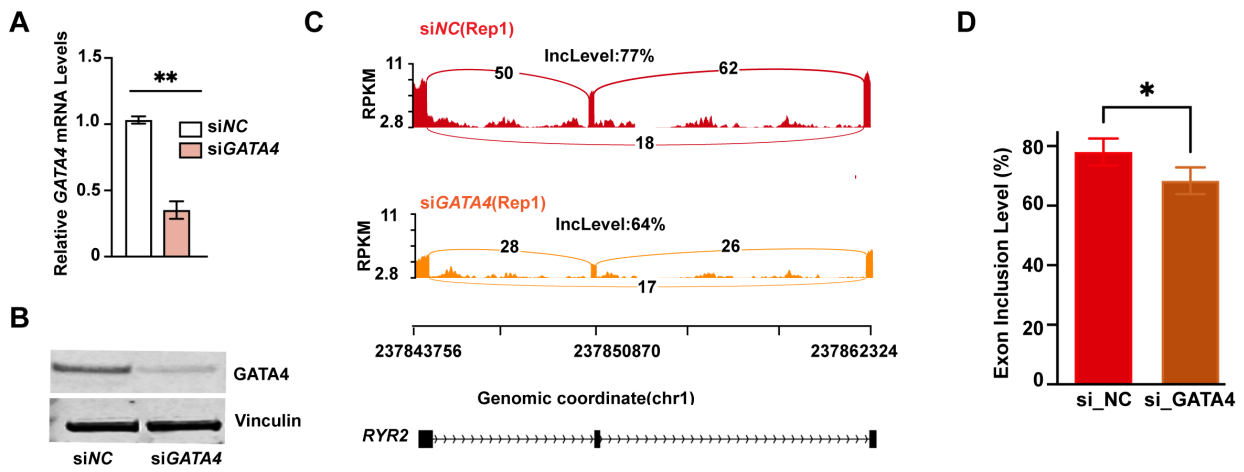


Figure. S5. GATA4 knockdown in human iPS-CPCs induces alternative splicing changes. RNA **(A)** and protein **(B)** levels of GATA4 upon GATA4-knockdown by siRNA for 48hrs. Data are shown as means ± SEM (n=3). Statistical significance was assessed using Student's *t* test.**P < 0.001. **(C and D)** Representative Sashimi plots depicting alternative splicing pattern of *RYR2* in hCPCs with siRNA targeting GATA4 (siGATA4) or negative control siRNA (siNC) and exon inclusion levels in each condition across four replicates (Rep). Data are shown as means ± SEM (n=4). Statistical significance was assessed using Student's *t* test.*P < 0.05.

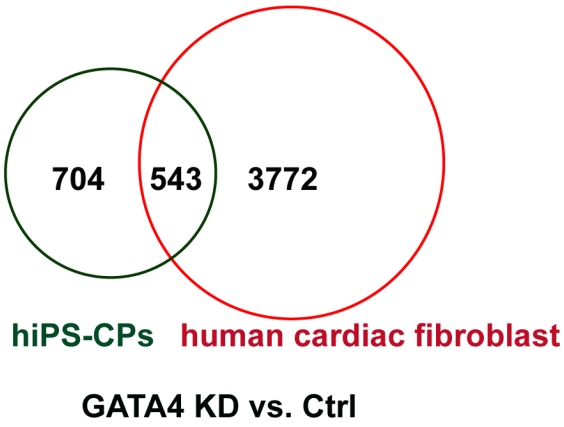
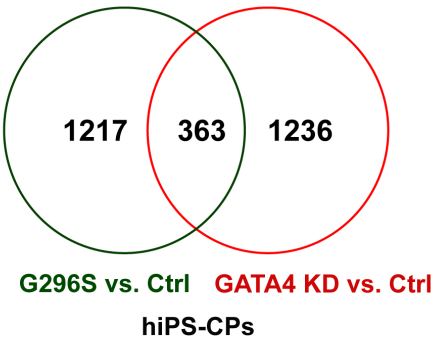


Figure S6. Intersection of alternatively spliced events upon GATA4-KD in hiPS-CPs (FDR <0.05, junction counts ≥5) and in cardiac fibroblasts (FDR <0.05, junction counts ≥5).

A



B

GO Biological Process

GO Terms	p.Adj
Cytosolic Calcium ion transport	0.027
Sarcomere organization	0.027
Cardiac muscle contraction	0.029
Transmembrane receptor protein tyrosine phosphatase signaling	0.047

Figure. S7. GATA4 G296S disease-causing missense variant induces alternative splicing changes in human iPS-CPs. (A) Intersection of alternatively spliced events upon GATA4-KD defined by rMATs (FDR <0.05, junction counts ≥5) and induced by the GATA4 G296S missense

variant (FDR <0.05, junction counts ≥5) in hiPS-CPs (n=3). (B) GO biological process analysis of genes affected by alternative splicing events upon GATA4KD and by the GATA4 G296S variant.

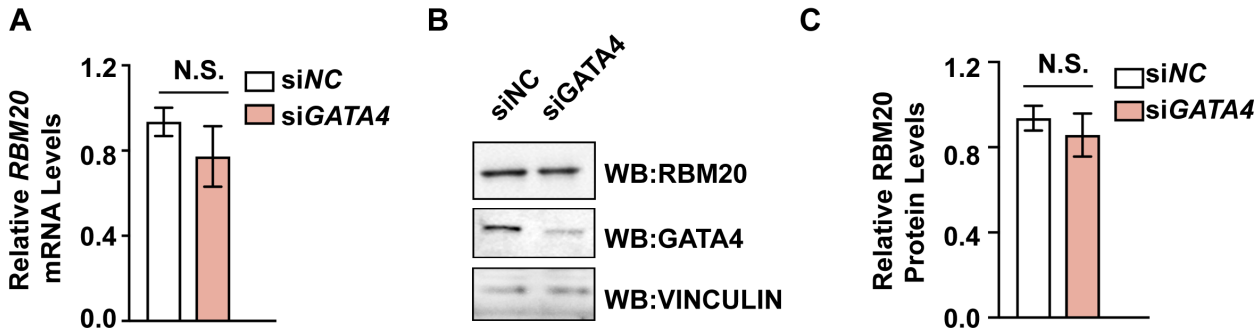


Figure S8. RBM20 RNA and protein levels upon GATA4 knockdown. (A) qPCR for RBM20 RNA transcripts 48 hours after siRNA knockdown of GATA4 (siGATA4) or negative control (siNC). (B) Representative Western blot (WB) of RBM20, GATA4 or Vinculin after siGATA4 treatment. (C) Quantification of RBM20 protein levels from Western blot. Data are shown as means ± SEM (n=3). Statistical significance was assessed using Student's t test. N.S., non-significant.

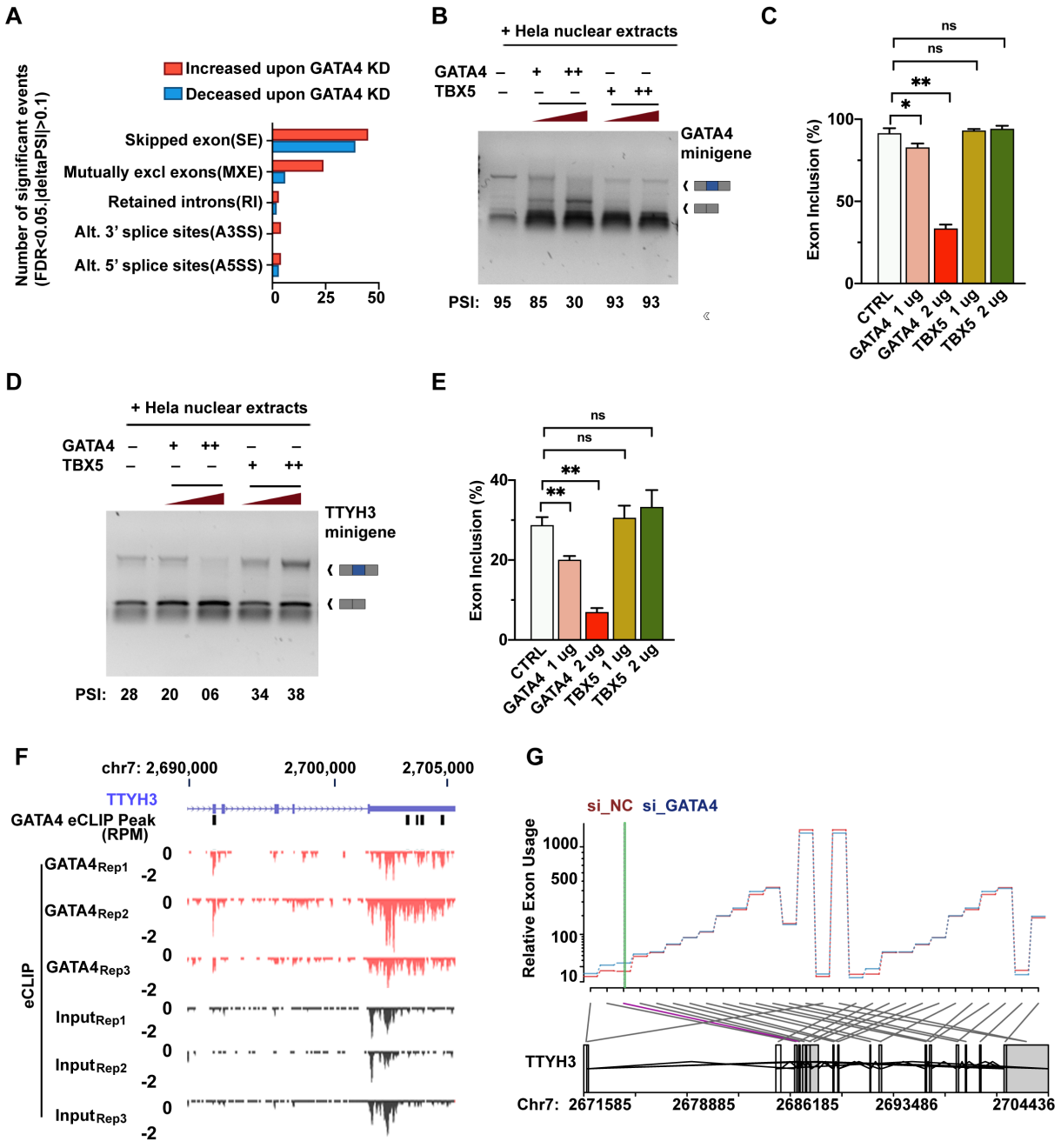


Figure. S9. GATA4 regulates mRNA splicing through direct interaction with mRNA. (A) Breakdown of the alternative splicing events in 89 genes (genes that are differentially spliced and have RNA with GATA4 eCLIP binding peaks) by event type. **(B)** *In vitro* splicing of *GATA4*

minigene reporter transcripts in Hela nuclear-extracts, with or without the addition of GATA4 or TBX5. **(C)** Quantification of exon inclusion level in (b). Data are shown as means ± SEM (n=3). One-way ANOVA coupled with a Tukey test was used to assess significance. ns, non-significant.*P < 0.05, **P < 0.001. **(D)** *In vitro* splicing of *TTYH3* minigene reporter transcripts in Hela nuclear-extracts, with or without the addition of GATA4 or TBX5. **(E)** Quantification of Exon inclusion level in (D). One-way ANOVA coupled with a Tukey test was used to assess significance. ns, non-significant . *P < 0.05, **P < 0.001. **(F),** Integrated genome viewer tracks centered on gene *TTYH3*. OmniCLIP identified peak regions indicated as black box above.**(G)** Representative relative exon usage plots depicting alternative *TTYH3* splicing pattern in hCPCs with green lines indicating change in exon inclusion upon GATA4 knockdown with siRNA compared to negative control siRNA.

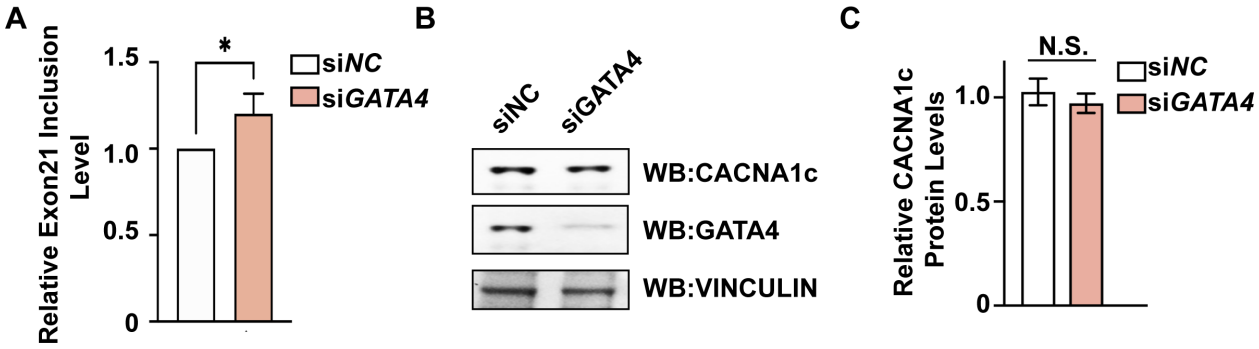


Figure S10. CACNA1c relative exon 21 inclusion upon GATA4 knockdown in human iPS-CPs. (A) Relative exon 21 inclusion upon GATA4 knockdown for 48 hours (siGATA4) compared to siRNA control (siNC). Statistical significance was assessed using Student's t test. *P < 0.05 (B) Representative Western blot of CACNA1c with or without GATA4 siRNA, with Vinculin as a positive control. (C) Quantification of CACNA1c protein levels from Western blots. Data are

shown as means ± SEM (n=3). Statistical significance was assessed using Student's t test. N.S., non-significant.

Table S1. List of GATA4, RbFOX2 eCLIP peaks identified in human cardiac progenitors.

Table S2. List of alternative splicing (AS) events upon GATA4 knockdown in human cardiac progenitors.

Table S3. List of alternative splicing (AS) events upon GATA4 knockdown in human cardiac fibroblasts.

Table S4. List of alternative splicing (AS) events in human iPS-CPs with GATA4 G296S disease-causing missense variant compared with WT.

Table S5. List of differentially expressed genes upon GATA4 knockdown in human cardiac progenitors.

Table S6. List of human proteins known to be involved in RNA binding or splicing.

Table S7. List of genes from integrating of splicing pattern alterations upon GATA4 knockdown with GATA4-eCLIP data, and GATA4-chromatin immunoprecipitation (ChIP) datasets in human cardiac progenitors.

Table S8. List of 40 differentially spliced exons upon GATA4 knockdown, which were also significantly different between hearts of patients with dilated cardiomyopathy and healthy donors.