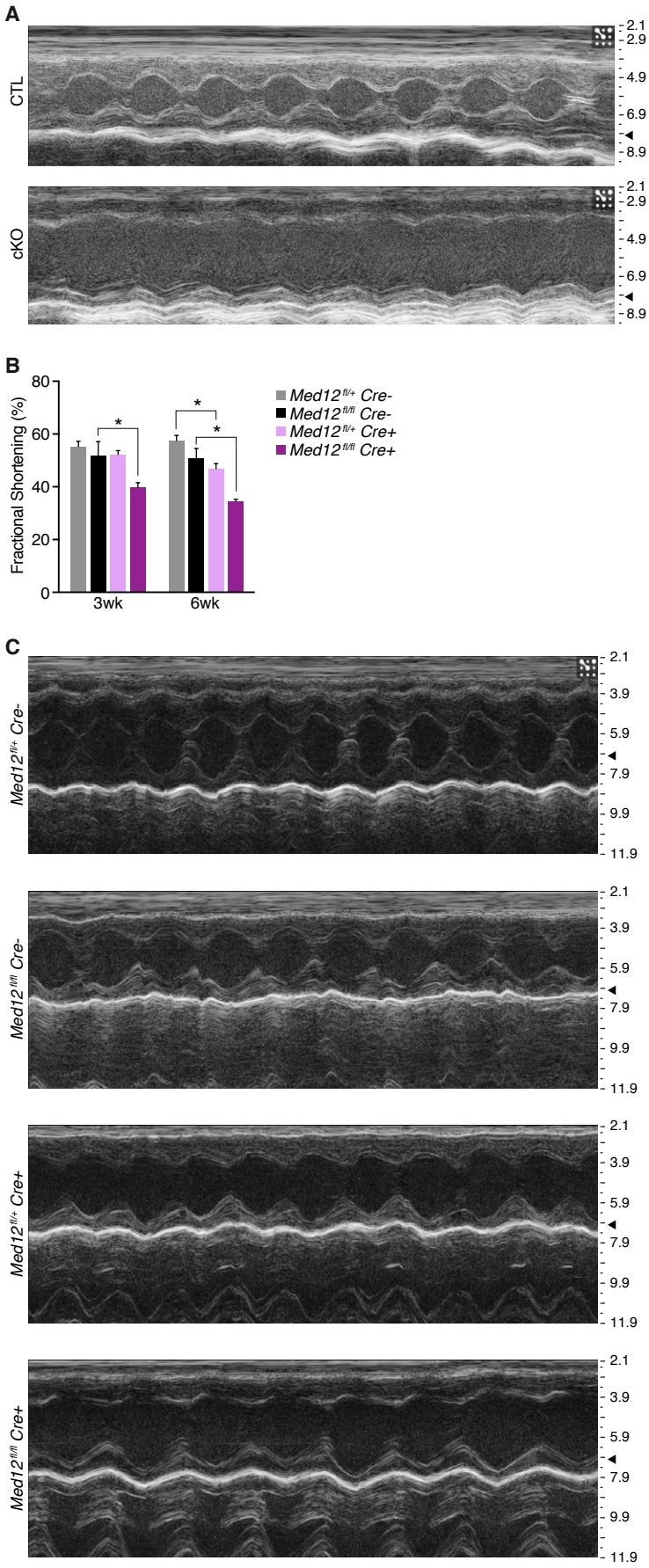
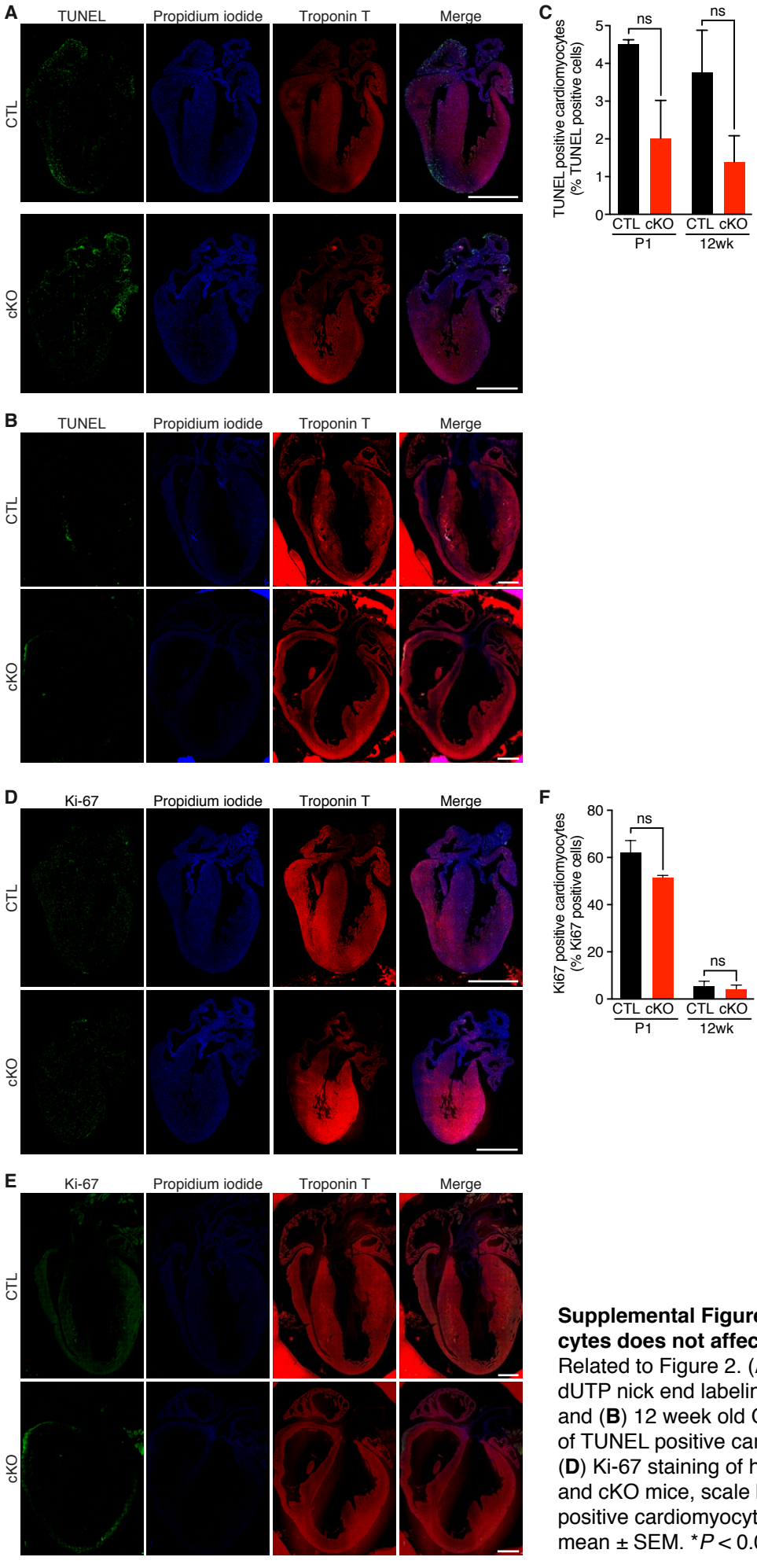
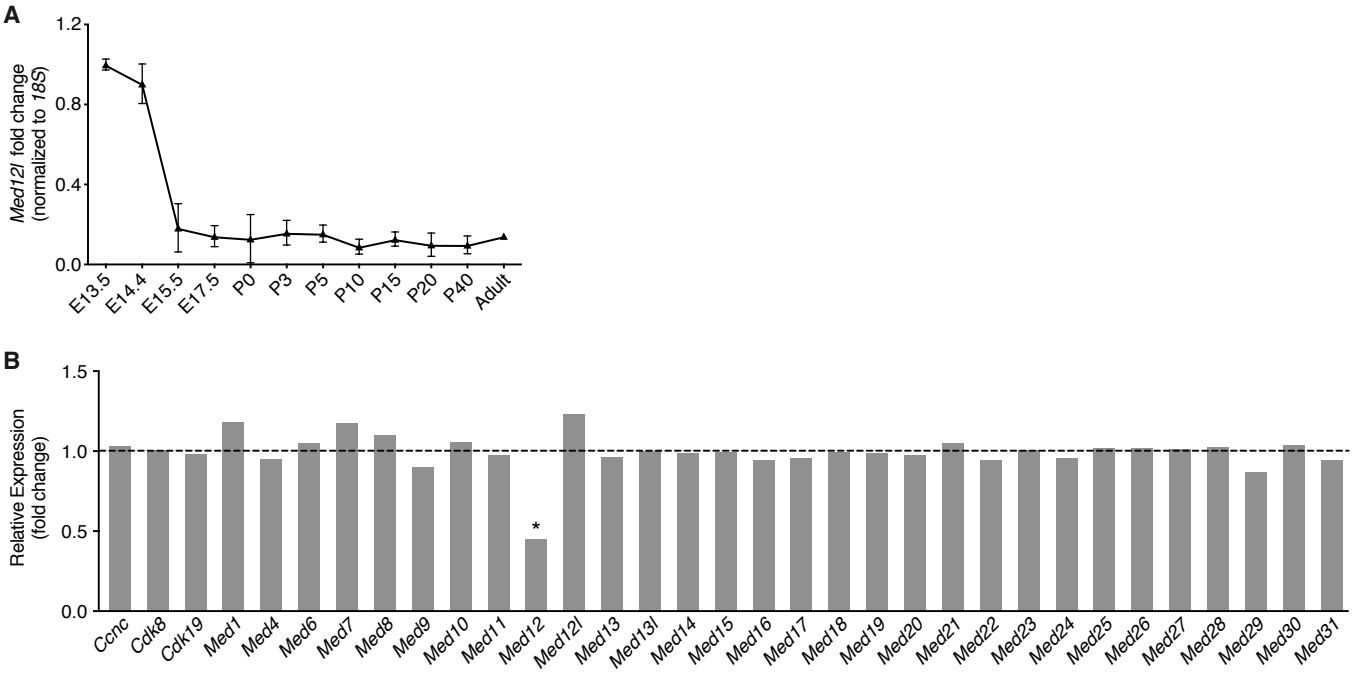


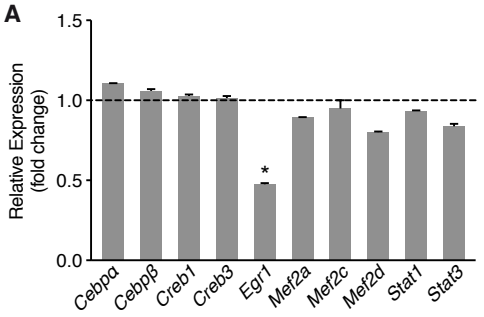
Supplemental Figure 1. Deletion of *Med12* in cardiomyocytes impairs cardiac function in male and female mice. Related to Figure 1. (**A**) Representative M-mode echocardiogram from 6 week old male mice. (**B**) Fractional shortening of female hearts, $n = 7$. (**C**) Representative M-mode echocardiogram from 6 week old female mice. Data are mean ± SEM. *$P < 0.05$ by one-way ANOVA with post-hoc Tukey Test.

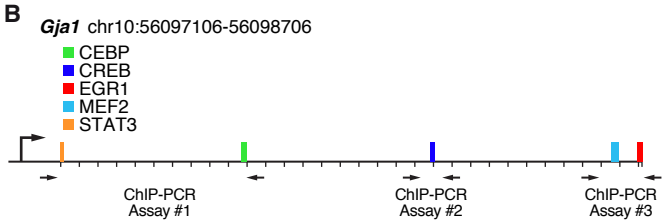


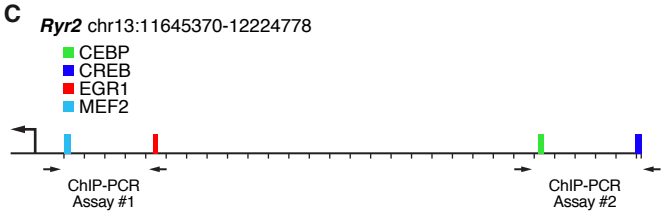
Supplemental Figure 2. Deletion of *Med12* in cardiomyocytes does not affect cardiomyocyte death or proliferation. Related to Figure 2. (**A**) Terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) staining of hearts from P1 and (**B**) 12 week old CTL and cKO mice. (**C**) Quantification of TUNEL positive cardiomyocytes, $n = 3$ layers from 3 mice. (**D**) Ki-67 staining of hearts from P1 and (**E**) 12 week old CTL and cKO mice, scale bars: 1mm. (**F**) Quantification of Ki-67 positive cardiomyocytes, $n = 3$ layers from 3 mice. Data are mean ± SEM. $^*P < 0.05$ by Student's T test.

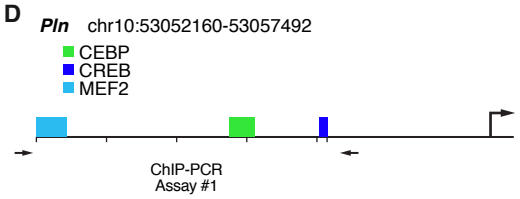


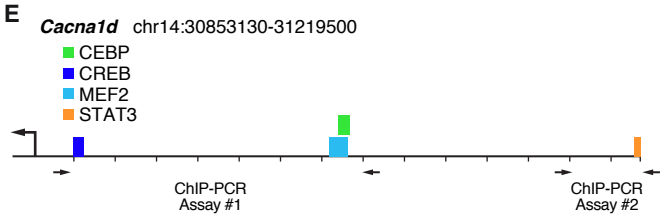
Supplemental Figure 3. Deletion of *Med12* in cardiomyocytes does not affect expression of other Mediator subunits. Related to Figure 3. (**A**) *Med12l* expression in ventricles during development and aging of mouse hearts, $n = 3$. (**B**) Expression of other Mediator components in *Med12cKO* hearts by RNA-seq, $n = 3$. Data are mean ± SEM.

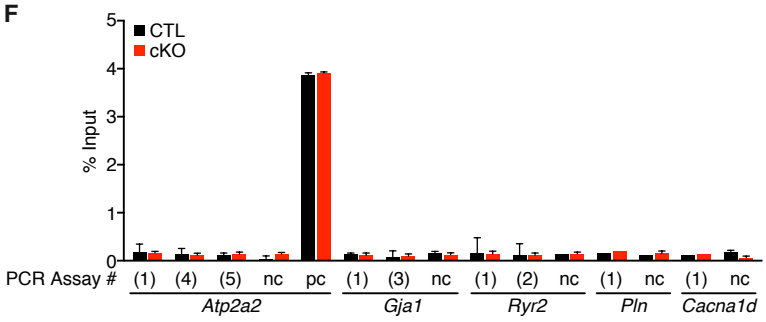


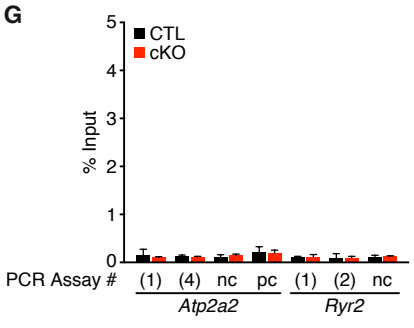




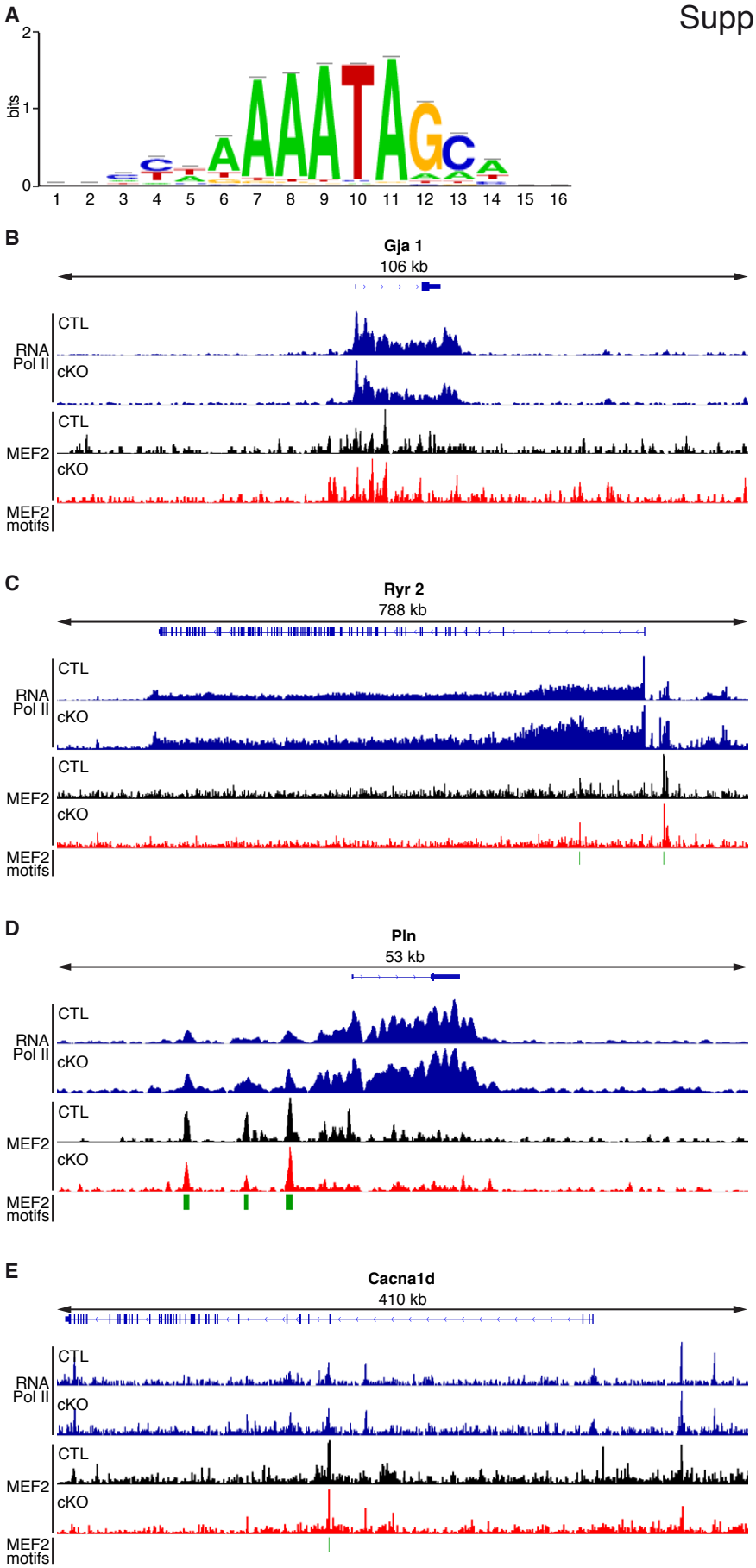








Supplemental Figure 4. MED12 regulates calcium handling genes in the heart, in part, through MEF2. Related to Figure 5. (**A**) Expression of transcription factors in *Med12cKO* hearts by RNA-seq, $n = 3$. (**B**) Chromosomal maps of *Gja1*, (**C**) *Ryr2*, (**D**) *Pln*, and (**E**) *Cacna1d* with TFBS and chromatin immunoprecipitation (ChIP)-PCR assay primers. (**F**) NFκB ChIP-PCR results for select calcium handling genes. PCR assay # corresponds to sequences on chromosomal maps for the indicated genes, regions devoid of transcription factor binding sites were used as negative controls (nc), and a region known to bind NFκB was used as a positive control (pc), $n = 4$. (**G**) MED12- NFκB ChIP-reChIP on *Atp2a2* and *Ryr2* promoters, $n = 4$. Data are mean ± SEM. $^*P < 0.05$ by Student's T test.



Supplemental Figure 5. Loss of MED12 does not affect MEF2 or RNA Polymerase II DNA binding. Related to Figure 5. (**A**) De novo motif discovery was performed using the top 8000 peaks from MEF2 ChIP-seq reads. The MEF2 motif was the top motif with MEF2 C having the highest Pearson correlation (0.945), k-mer sig=47.36, evalue=-4.4e-48.
(**B**) RNA polymerase II and MEF2 ChIP-seq signals at the *Gja1* locus (**C**) *Ryr2* locus, (**D**) *Pln* locus, and (**E**) *Cacna1d* locus in CTL and cKO ventricles. MEF2 motif locations are shown in green.