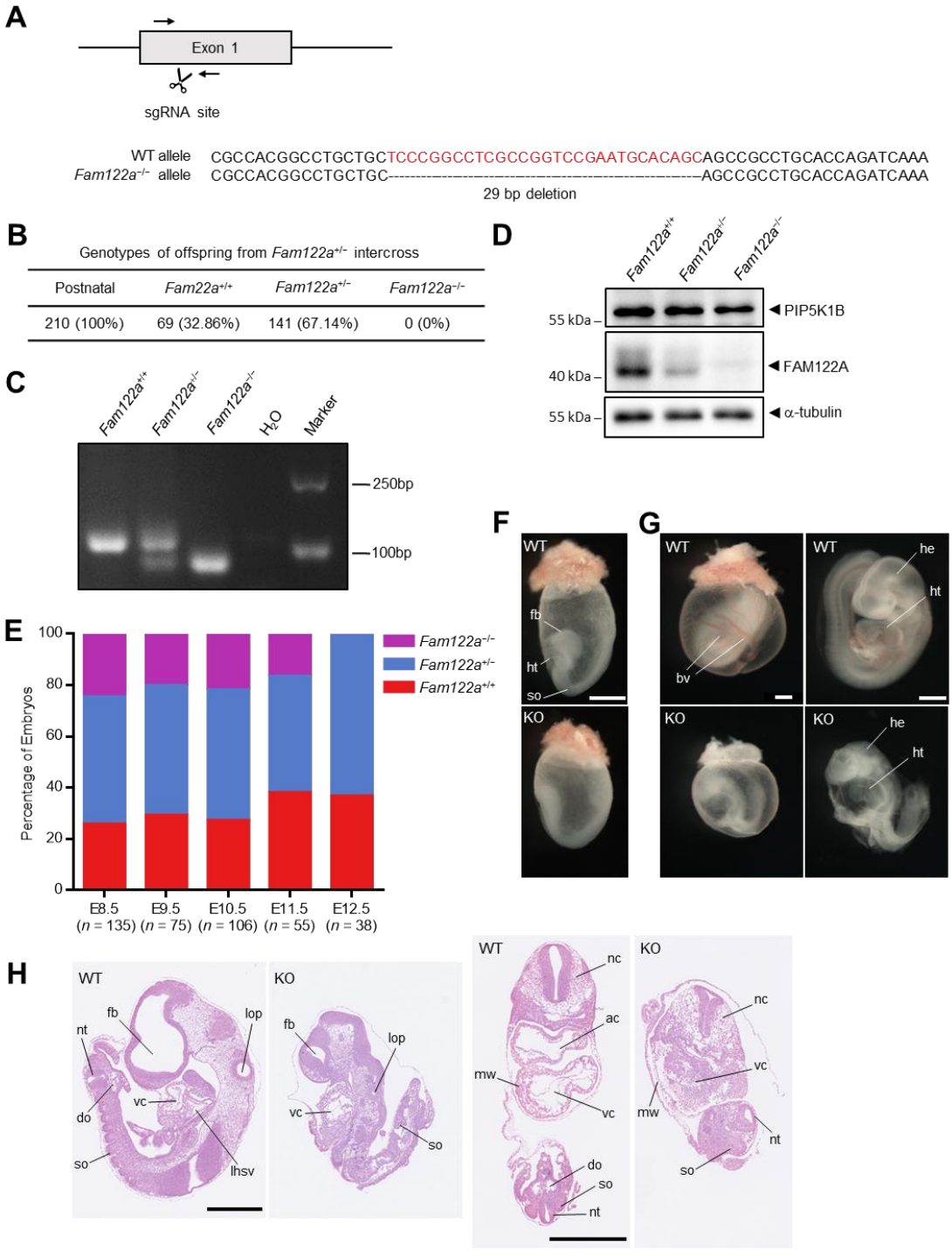
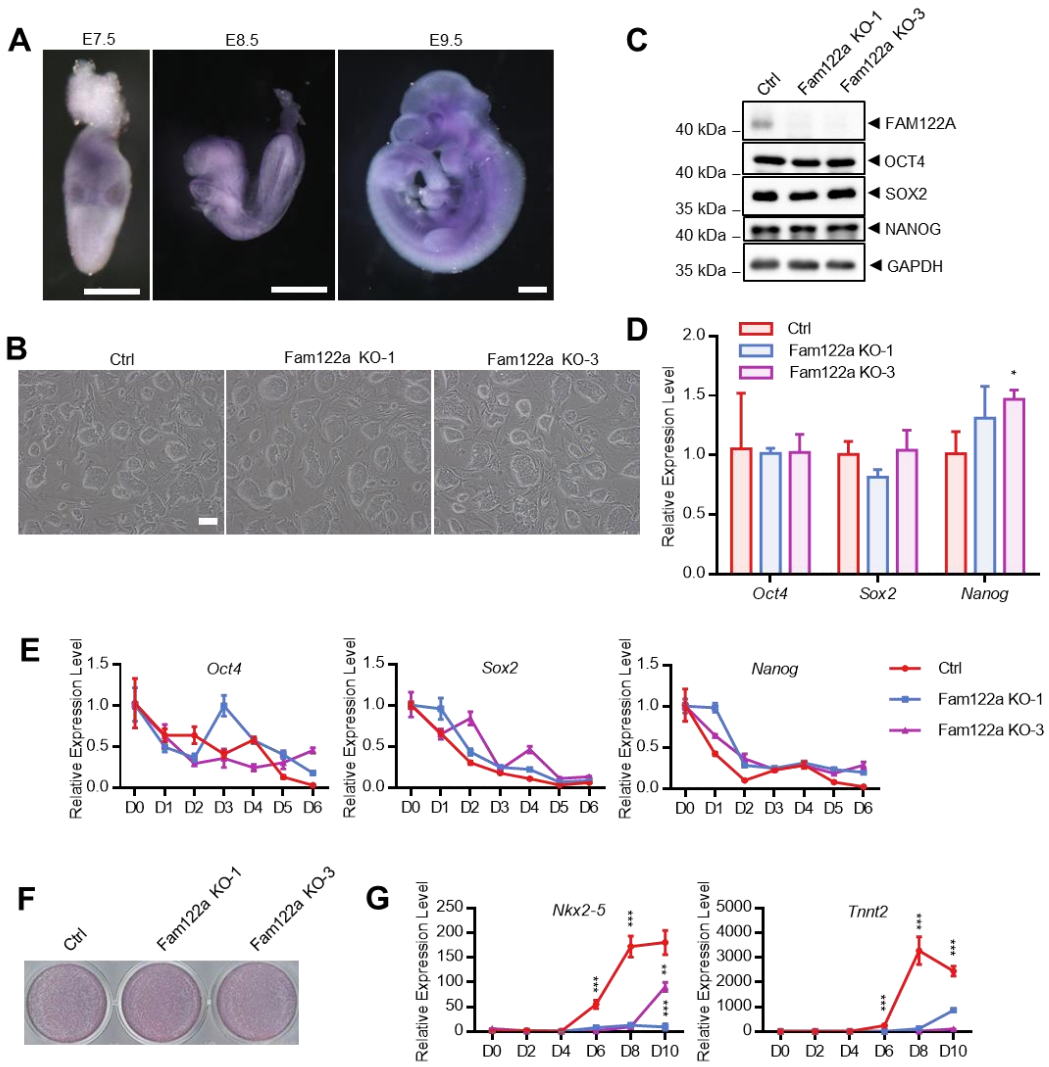
Supplementary Materials for FAM122A is required for mesendodermal and cardiac differentiation of embryonic stem cells



Supplementary Figure S1. Embryonic lethality and severe defects of cardiovascular development occurred in *Fam122a* knockout mice.
(A) Schematic of *Fam122a* knockout mice. The site of sgRNA is indicated in the exon. PCR primers used for genotyping are shown as horizontal arrows. *Fam122a* KO allele is 29bp deleted compared to WT allele, causing frameshift mutation.

(B) Genotypes of offspring from *Fam122a*$^{+/-}$ intercross.
(C) Gel image of genotyping PCR results.
(D) Western blot analysis of indicated proteins from E9.5 embryos.
(E) Percentages of embryos in the indicated day.
(F) Lateral view of E8.5 embryos. Somite (so), forebrain (fb) and heart (ht) are indicated in WT embryo.
(G) Lateral view of E9.5 embryos with or without yolk sac. Bv, blood vessel; he, head; ht, heart.
(H) Haematoxylin and eosin (H&E)-stained sagittal and transverse sections of E9.5 embryos. Fb, forebrain; lop, left otic pit; nt, neural tube; do, dorsal aorta; vc, ventricular chamber; lhsv, left horn of sinus venosus; so, somite; nc, neural crest; ac, atrial chamber; mw, myocardial wall.
Scale bars, 500 μm (F-H).



Supplementary Figure S2. Pluripotency not affected in *Fam122a* knockout mESCs.

(A) Whole-mount *in situ* hybridization of *Fam122a* in mouse embryos at indicated time. Scale bars, 500μm.

(B) The colony morphologies of control and *Fam122a* KO mESCs. Scale bar, 100 μm.

(C) Western blot analysis of pluripotency markers in indicated mESCs.

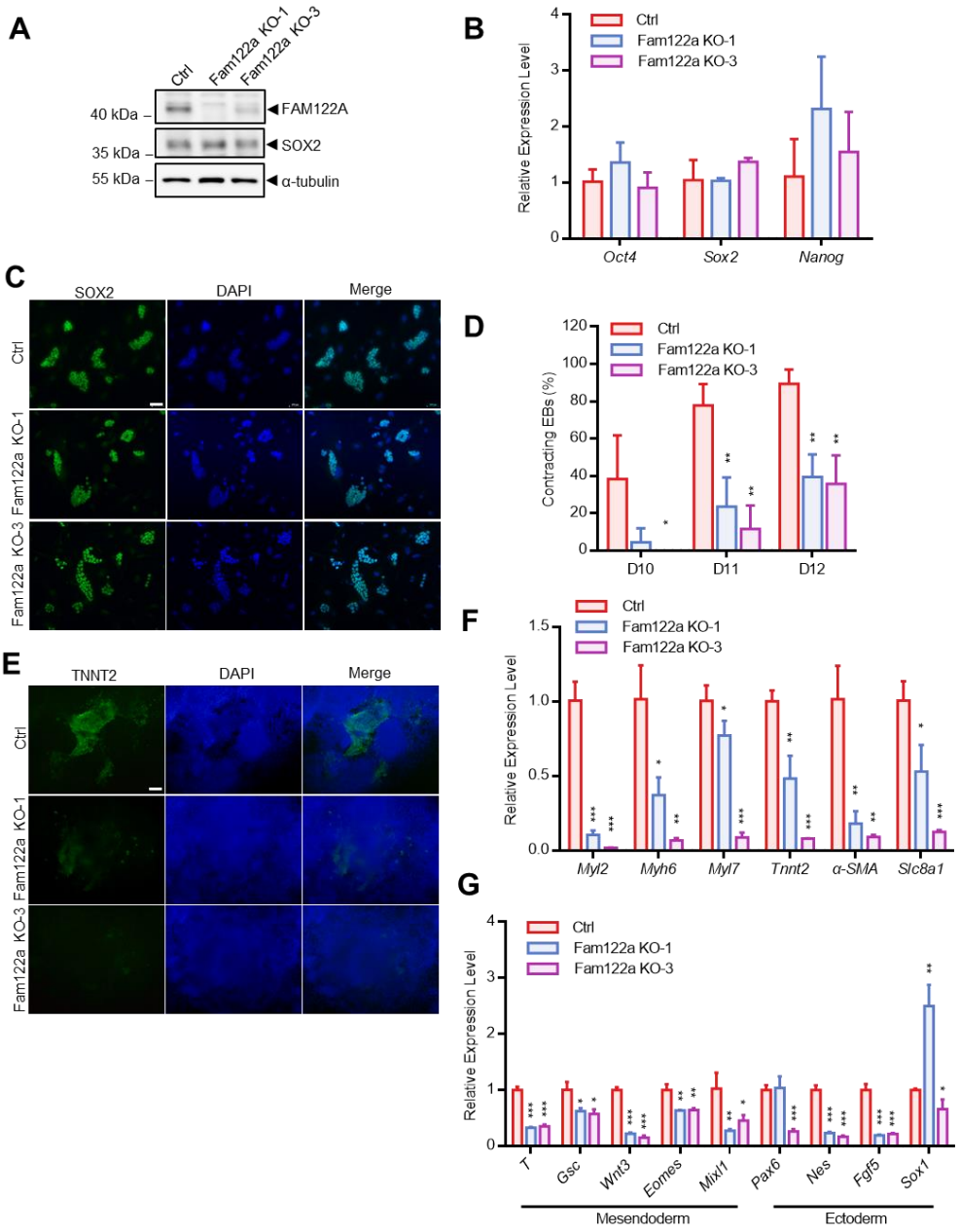
(D) qRT-PCR analysis of pluripotency marker genes in control and *Fam122a* KO mESCs.

(E) Time course analysis of *Oct4*, *Sox2* and *Nanog* expression during mESC differentiation.

(F) Images of alkaline phosphatase-stained colonies in control and *Fam122a* KO mESCs.

(G) Time course analysis of *Nkx2-5* and *Tnnt2* expression during mESC differentiation.

In (D) and (G), data represent means ± s.d. from $n = 3$ independent experiments, and P values were calculated by two-tailed unpaired t-test (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$).



Supplementary Figure S3. Cardiac differentiation defect in *Fam122a* knockout TC1 mESCs.

(A) Western blot analysis of FAM122A knockout effect in TC1 mESCs.

(B) qRT-PCR analysis of pluripotency marker genes in Fam122a KO TC1 mESCs.

(C) SOX2 imumunostaining of control and Fam122a KO mESCs. Scale bar, 100 μm.

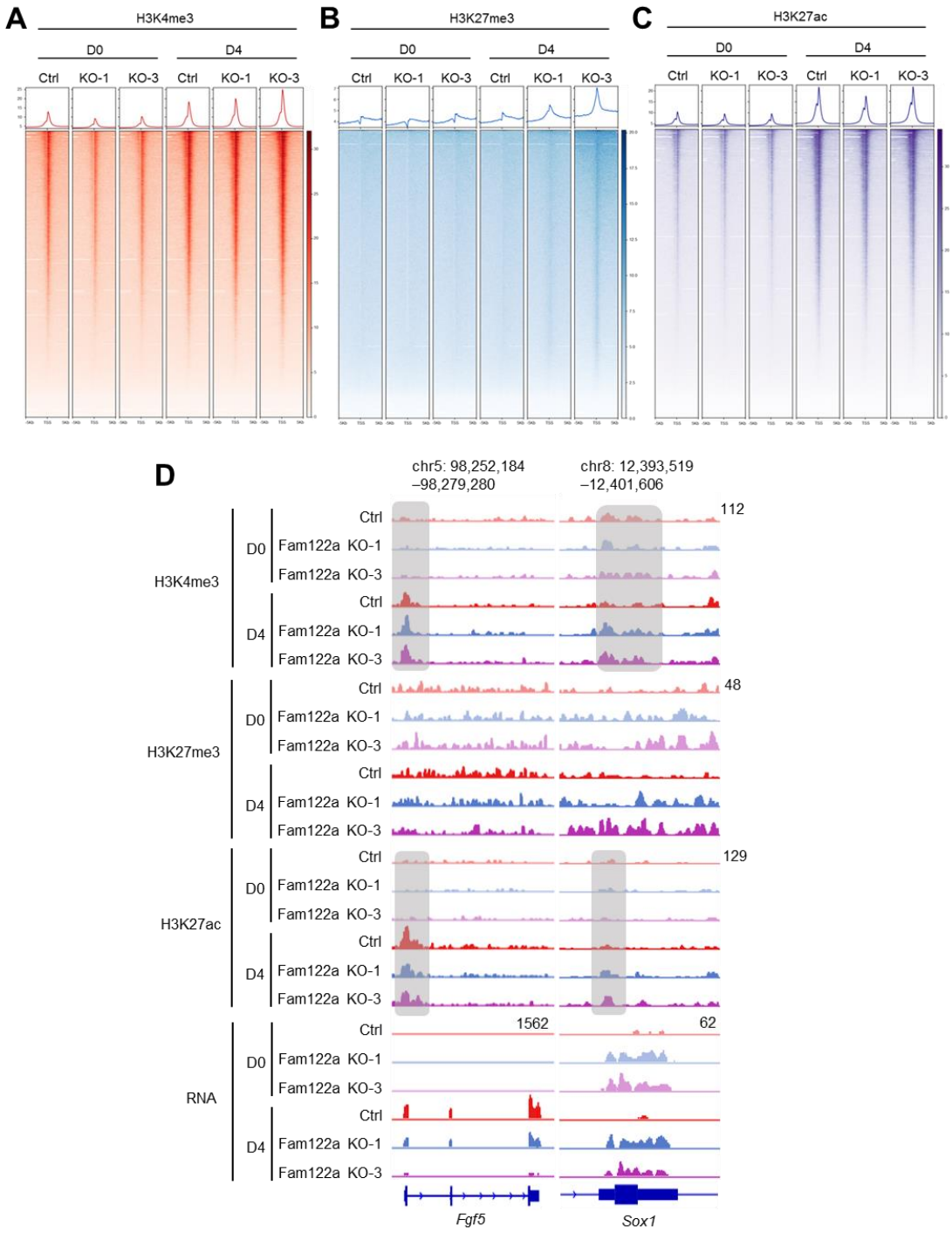
(D) Percentages of contracting EBs from D10 to D12 in differentiated TC1 mESCs.

(E) TNNT2 imumunostaining on D12 in differentiated TC1 mESCs. Scale bar, 100 μm.

(F) Expression analysis of cardiomyocyte marker genes on D12 of TC1 mESCs.

(G) qRT-PCR analysis of three germ layer marker genes on D4 of TC1 EBs.

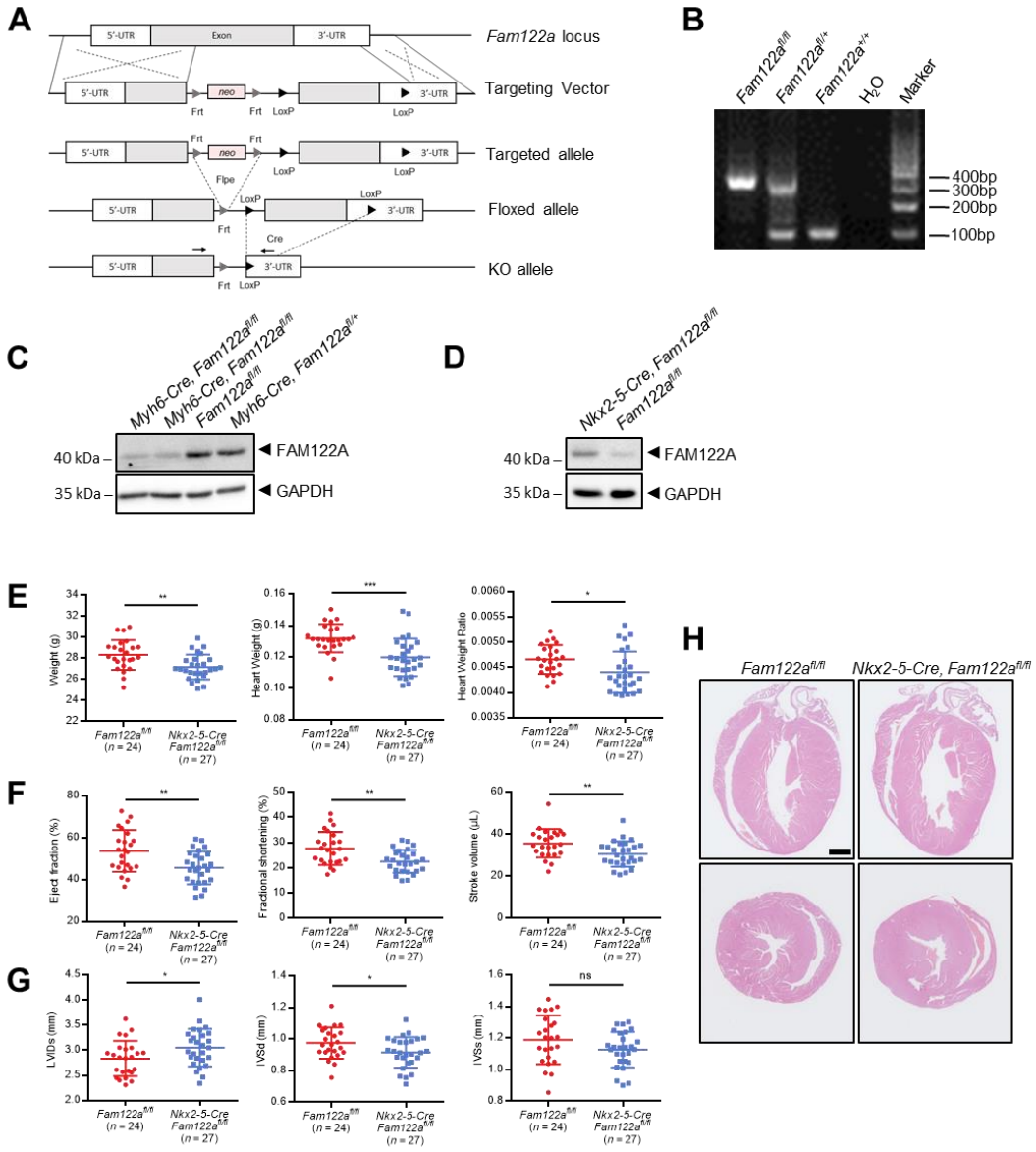
In (B), (D), (F) and (G), data represent means ± s.d. from $n = 3$ independent experiments, and P values were calculated by two-tailed unpaired t-test (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$).



Supplementary Figure S4. Histone modification regulated in *Fam122a* knockout mESCs.

(A-C) Heatmaps showing H3K4me3 (A), H3K27me3 (B) and H3K27ac (C) enrichment in D0 and D4 control and *Fam122a* knockout mESCs.

(D) Genome browser views of H3K4me3, H3K27me3 and H3K27ac ChIP-seq signals as well as RNA expression in D0 and D4 control and *Fam122a* knockout mESCs at ectoderm genes *Fgf5* and *Sox1*.



Supplementary Figure S5. Cardiac function attenuated in *Nkx2-5*-Cre *Fam122a* CKO mice.

(A) Schematic of *Fam122a* conditional knockout mice.

(B) Gel image of *Fam122a* loxp genotyping PCR results.

(C and D) Western blot analysis of FAM122A expression in heart tissues from *Myh6-Cre Fam122a* CKO mice (C) and *Nkx2-5 CreFam122a* CKO mice (D).

(E) Body weight, heart weight and heart weight ratio analyses in control (n = 24) and *Fam122a* CKO (n = 27) mice.

(F) Echocardiography analyses for the EF, FS and SV in control (n = 24) and *Fam122a* CKO (n = 27) mice.

(G) Echocardiography analyses for the LVID and IVS in diastole or systole in control (n = 24) and *Fam122a* CKO (n = 27) mice.

(H) H&E-stained sagittal or transverse sections of heart in control and *Fam122a* CKO mice. Scale bar, 1 mm.

In (E), (F) and (G), data represent means ± s.d. and P values were calculated by two-tailed unpaired t-test (* P< 0.05, ** P< 0.01, *** P< 0.001, ns, no significance).