Supplemental Figure legends:

Figure S1. RNA-seq analysis of LV, RV, SN and hiPSCs and the expression of EBF1 in different cell types

(A) Pearson correlation analysis for all 4 samples.

(B-F) Subcluster analysis based on gene expression profiling of LV, RV, SN and hiPSCs. Gene expression levels in the subcluster were up-regulated in LV/RV/SN (B), hiPSCs (C), SN (D), RV (E) or LV (F). Self-organizing maps (SOM) analysis was applied to the RNA-seq data to identify patterns of gene expression across 4 groups (LV, RV, SN and hiPSCs). GO analysis was run by the THE GENE ONTOLOGY RESOURCE (http://geneontology.org/).

(G) The *in vitro* model of human cardiac development. RNA-seq was performed to evaluate gene expression patterns during cardiac development. Cell samples on day 0 (hiPSCs), day 3 (mesodermal cells) and day 5 (cardiac progenitor cells) were collected for RNA-seq.

(H) Gene Ontology (GO) analysis of up-regulated genes during *in vitro* human cardiac development from (G).

(I) Heat map showing expression levels of cardiogenic TFs, which are reported to be crucial for cardiogenesis, during cardiac development.

(J) Statistic analysis of EBF1 protein expression pattern during cardiac development of hESCs. *$p < 0.05$.

(K) The *in vitro* model of human cardiac development and cardiomyocyte differentiation. Cardiomyocytes could be purified by using lactate. Flow cytometry was used to analyze EBF1 expression in non-purified cardiomyocytes and purified cardiomyocytes.

(L) Immunostaining of EBF1 in different mouse cardiac cell types, including cardiomyocytes ($CTNT^+$ cells), endothelial cells ($CD31^+$ cells) and smooth muscle cells (α-SMA^+ cells).

Figure S2. EBF1 knockout inhibits mesoderm differentiation and cardiomyocyte specification

(A) CRISPR/Cas9 to knock out EBF1 mediated by lentivirus vector (lentiCRISPRv2).

(B-C) RT-qPCR showing expression levels of *EBF1* (B), and the genes controlling mesoderm differentiation (*TBXT, MESP1)* (C). *p <0.05 (EBF1 gRNA2 vs. Control; EBF1 gRNA3 vs. Control).

(D) Flow cytometry showing the percentage of α-SMA^+ cells on day 7 post cardiac differentiation. *p <0.05 ($EBF1^{-/-}$ vs. WT).

Figure S3. EBF1, expressed in both human and mouse heart tissues, is potentially involved in cardiac function

(A) *EBF1* is expressed in different human tissues. Data was from NCBI database.

(B) *Ebf1* is expressed in different mouse tissues. Data was from NCBI database.

(C-D) Potential phenotypes of *Ebf1* knockout in mouse model. Magnetic Resonance Imaging (MRI) showed some cardiac phenotypes in mouse model (D). Data were from the public database (informatics.jax.org/marker/MGI:95275).

(E) Western blot showing the protein expression of cardiac fibrosis marker (COL1A1) and cardiac hypertrophy marker (NPPB) in 3-month-old mouse heart tissues.

Figure S4. EBF1 binds on upstream chromatin of hypertrophic markers NPPA/NPPB

(A-B) In situ Hi-C analysis showing the topologically associated domains (TADs) near *NPPB* transcriptional start site (TSS). Conserved and putative EBF1 binding sites near NPPB were presented. TADs were on chromosome 5 (chr5). Highlighted colorful boxes and lines showing the chromatin interactions. Several putative EBF1 binding sites, localized on upstream regions of NPPB loci, were found in the TADs. Data were from UCSC genome browser.

(C-E) ChIP-qPCR to evaluate the binding of EBF1 on *NPPB* chromatin. Different primers targeting to different regions were designed (C). ChIP-qPCR were conducted to detect binding of EBF1 on distal regions (D) and promoter regions (E). $p^{*}<0.05$ (anti-EBF1 vs. anti-IgG).

Figure S5. EBF1 binding motif analysis

(A) EBF1 potential binding motifs.

(B) The matrix of potential EBF1 binding motifs.

(C) NKX2-5 potential binding motif on EBF1 bound regions. NKX2-5 was enriched after analysis of ChIP-seq peaks by using transcription factors (TFs) binding motifs. This indicated that NKX2-5 may be a potential co-factor of EBF1.

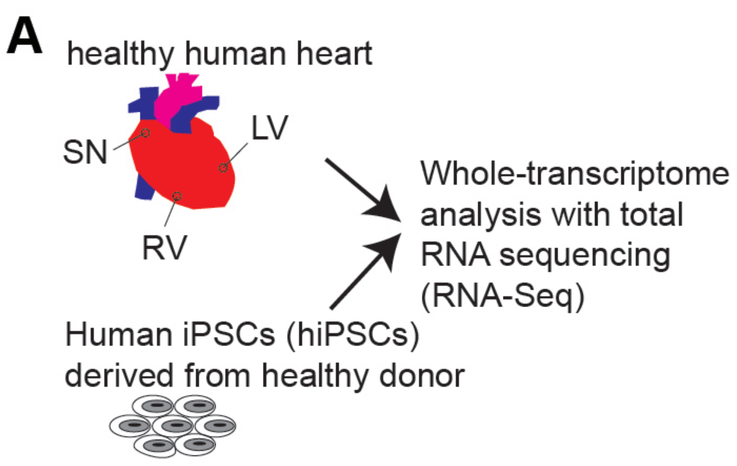
(D) Protein-protein interaction analysis of EBF1 and putative EBF1-interacting transcription factors (TFs) in cardiac system. The TFs were from the output of binding motif analyses in EBF1 bound regions in ChIP-seq. This data demonstrated that EBF1 may be involved in cardiac regulatory networks, by potentially interacting with some crucial cardiogenic TFs (such as NKX2-5).

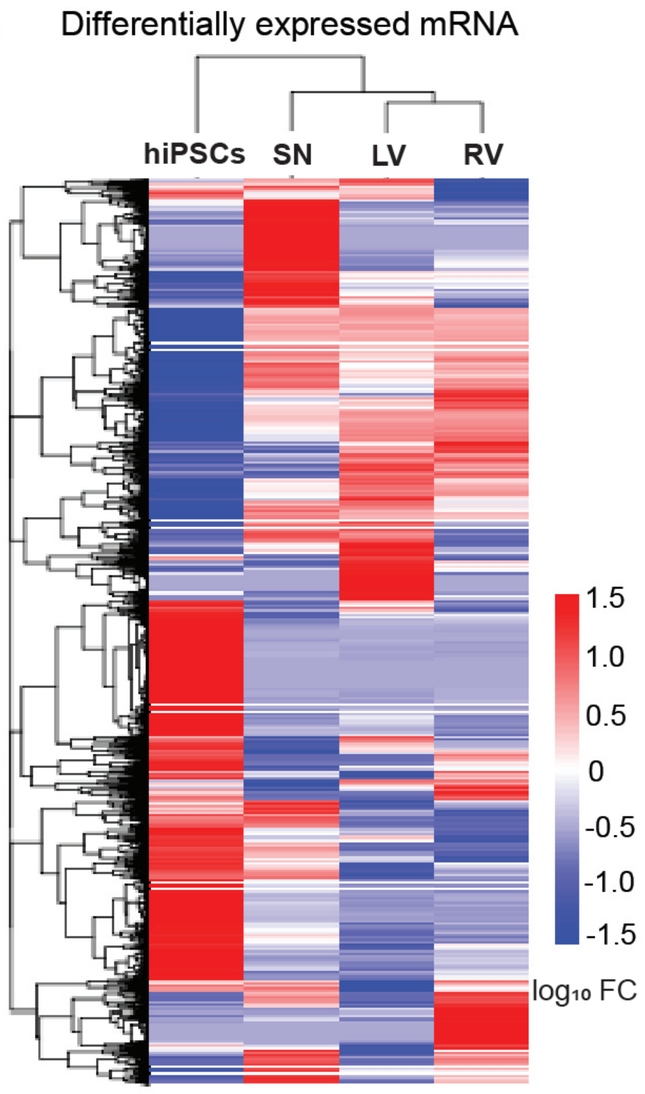
Figure S6. MEF2C promotes cardiac hypertrophy in hESC-derived cardiomyocytes

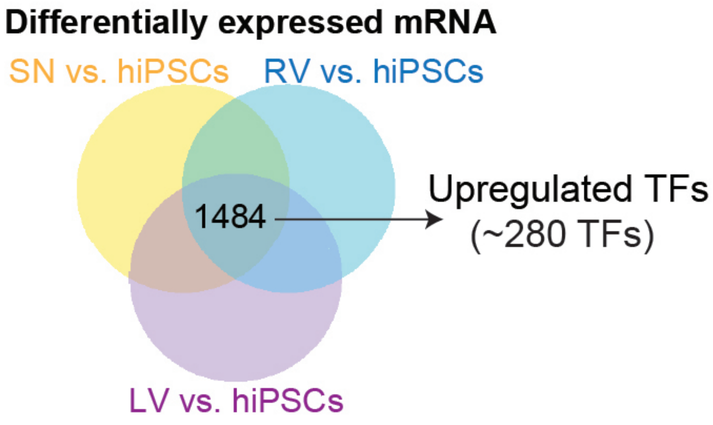
(A) Immunostaining showing the protein expression of cardiomyocyte marker (TNNT2, green color) and cardiac hypertrophy marker (NPPB, red color). $MEF2C^{OE}$, MEF2C overexpression by lentivirus infection. Control, blank lentivirus infection. Scale bar, 100 μm.

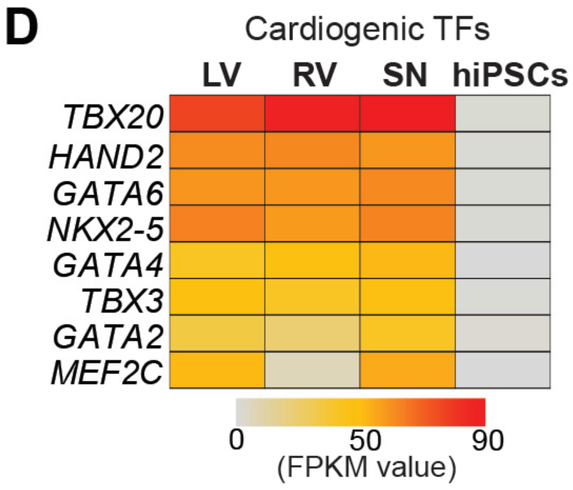
(B) The percentage of $NPPB^{+}$ hESC-derived cardiomyocytes (CMs). *p <0.05 ($MEF2C^{OE}$ vs. Control).

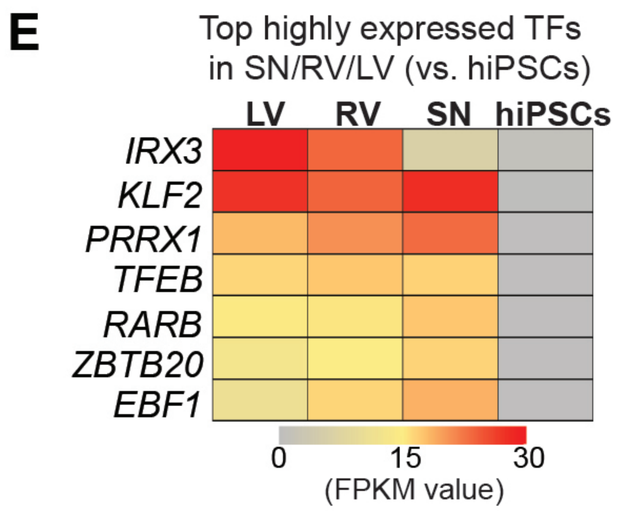
Figure 1

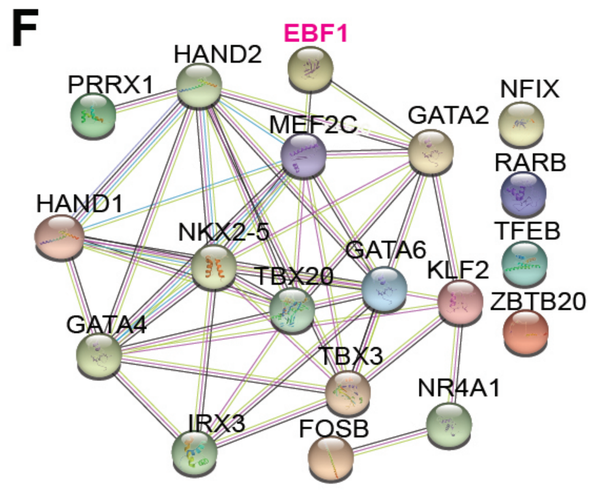

A
healthy human heart
SN
LV
RV
Whole-transcriptome analysis with total RNA sequencing (RNA-Seq)
Human iPSCs (hiPSCs) derived from healthy donor

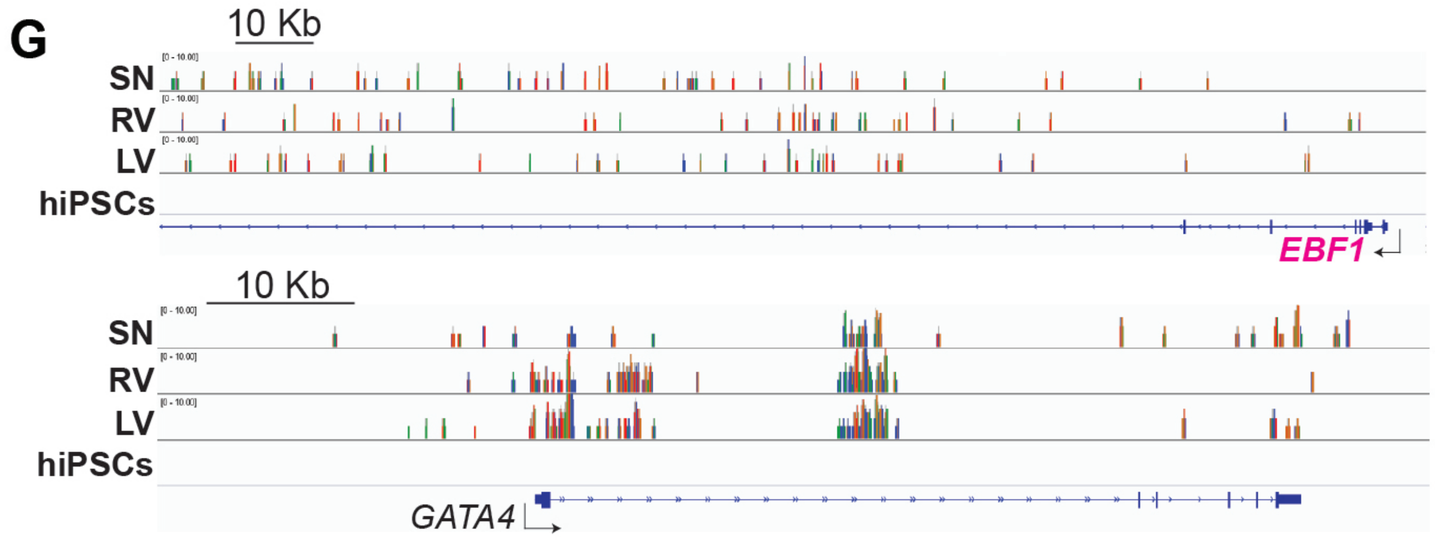

B
Differentially expressed mRNA
hiPSCs
SN
LV
RV
1.5
1.0
0.5
0
-0.5
-1.0
-1.5
log10 FC

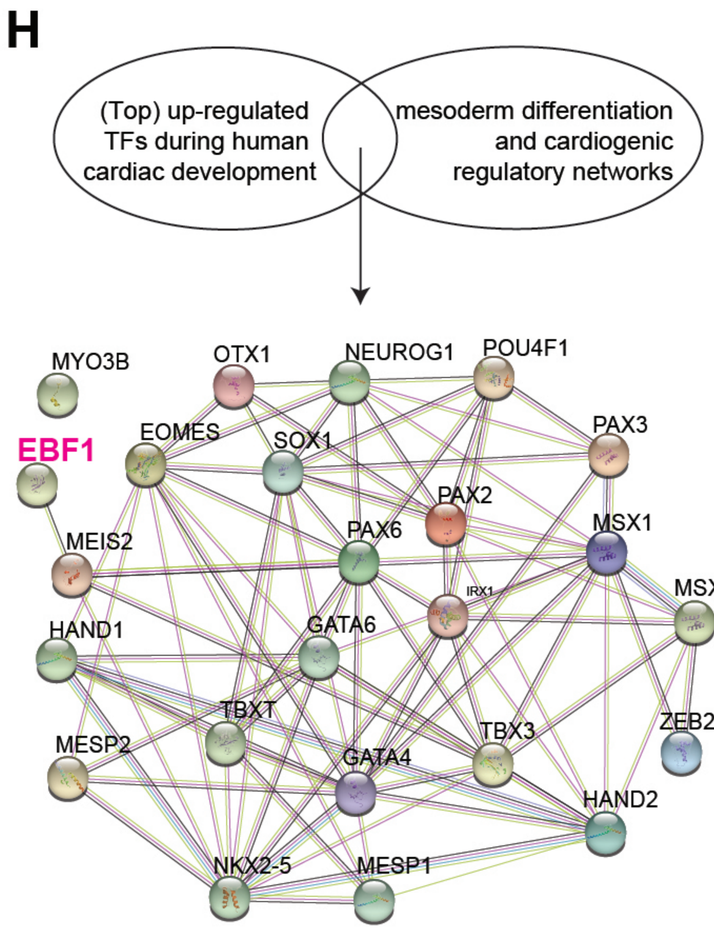

C
Differentially expressed mRNA
SN vs. hiPSCs
RV vs. hiPSCs
LV vs. hiPSCs
1484
Upregulated TFs
(~280 TFs)

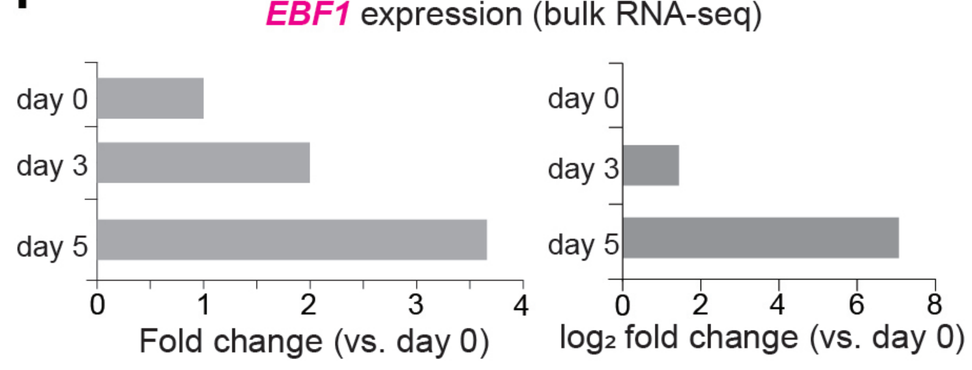

D
Cardiogenic TFs
LV
RV
SN
hiPSCs
TBX20
HAND2
GATA6
NKX2-5
GATA4
TBX3
GATA2
MEF2C
0
50
90
(FPKM value)

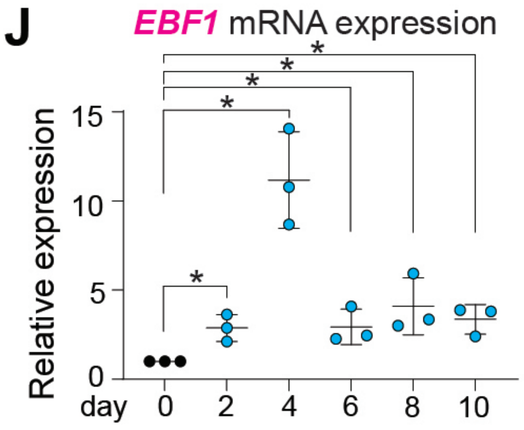

E
Top highly expressed TFs
in SN/RV/LV (vs. hiPSCs)
LV
RV
SN
hiPSCs
IRX3
KLF2
PRRX1
TFEB
RARB
ZBTB20
EBF1
0
15
30
(FPKM value)

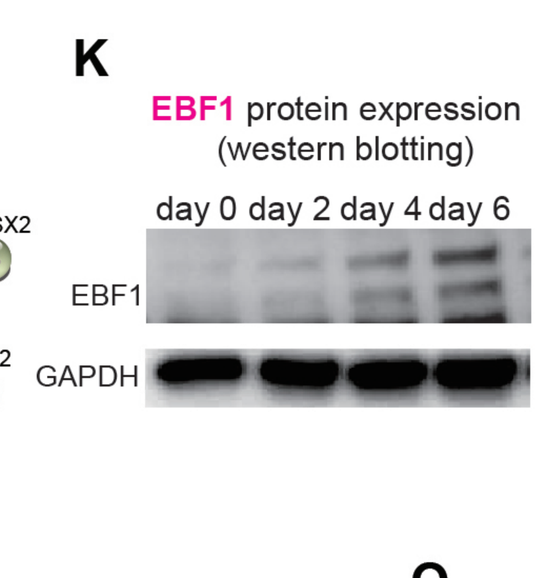

F
EBF1
HAND2
PRRX1
MEF2C
GATA2
NFIX
RARB
HAND1
NKX2-5
GATA6
TFEB
TBX20
KLF2
ZBTB20
GATA4
TBX3
NR4A1
IRX3
FOSB

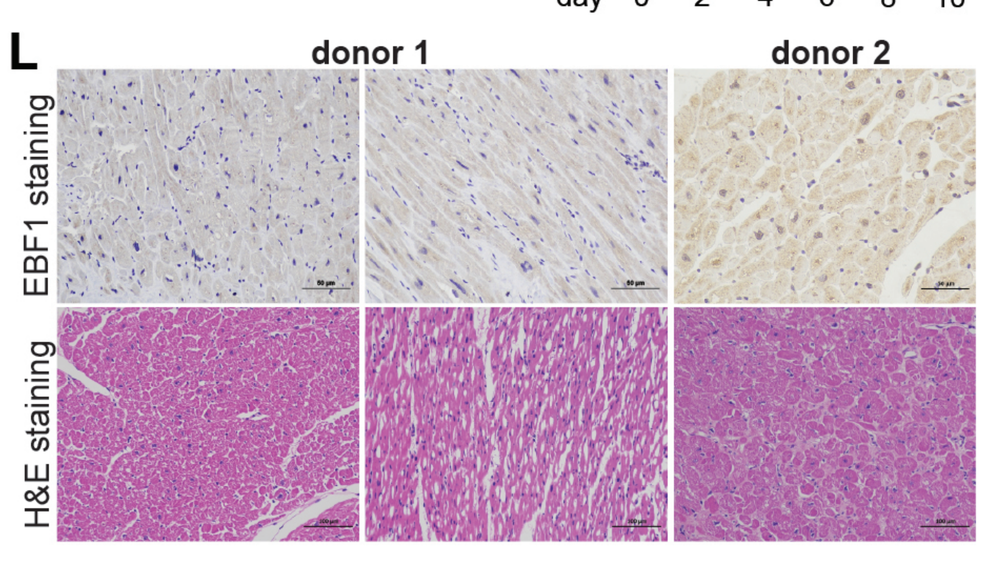

G
10 Kb
SN
RV
LV
hiPSCs
EBF1
10 Kb
SN
RV
LV
hiPSCs
GATA4

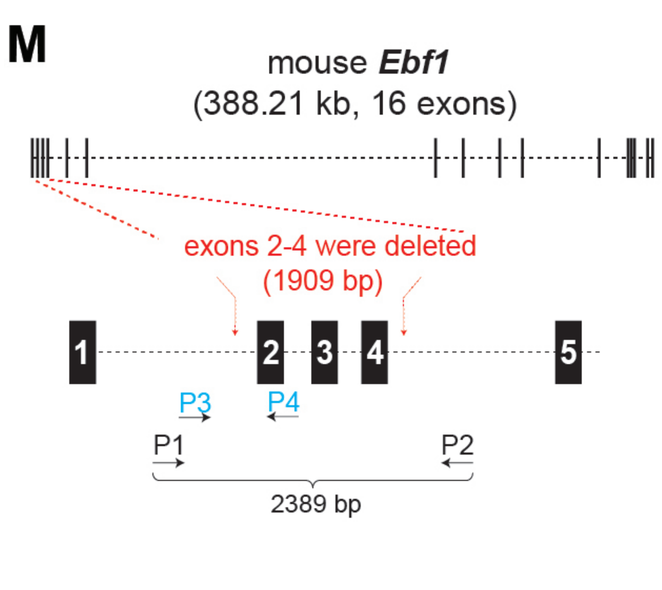

H
(Top) up-regulated TFs during human cardiac development
mesoderm differentiation and cardiogenic regulatory networks
MYO3B
OTX1
NEUROG1
POU4F1
EBF1
EOMES
SOX1
PAX3
PAX2
PAX6
MSX1
MEIS2
IRX1
MSX2
HAND1
GATA6
TBXT
ZEB2
MESP2
TBX3
GATA4
HAND2
NKX2-5
MESP1

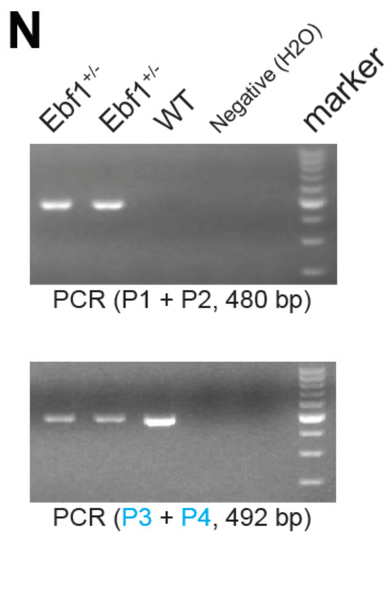

I
EBF1 expression (bulk RNA-seq)
day 0
day 3
day 5
0
1
2
3
4
Fold change (vs. day 0)
day 0
day 3
day 5
0
2
4
6
8
log2 fold change (vs. day 0)


J
EBF1 mRNA expression
Relative expression
15
10
5
0
day
0
2
4
6
8
10


K
EBF1 protein expression
(western blotting)
day 0 day 2 day 4 day 6
EBF1
GAPDH


L
donor 1
donor 2
EBF1 staining
H&E staining


M
mouse Ebf1
(388.21 kb, 16 exons)
exons 2-4 were deleted
(1909 bp)
1
2
3
4
5
P3
P4
P1
P2
2389 bp


N
Ebf1+/-
Ebf1+/-
WT
Negative (H2O)
marker
PCR (P1 + P2, 480 bp)
PCR (P3 + P4, 492 bp)

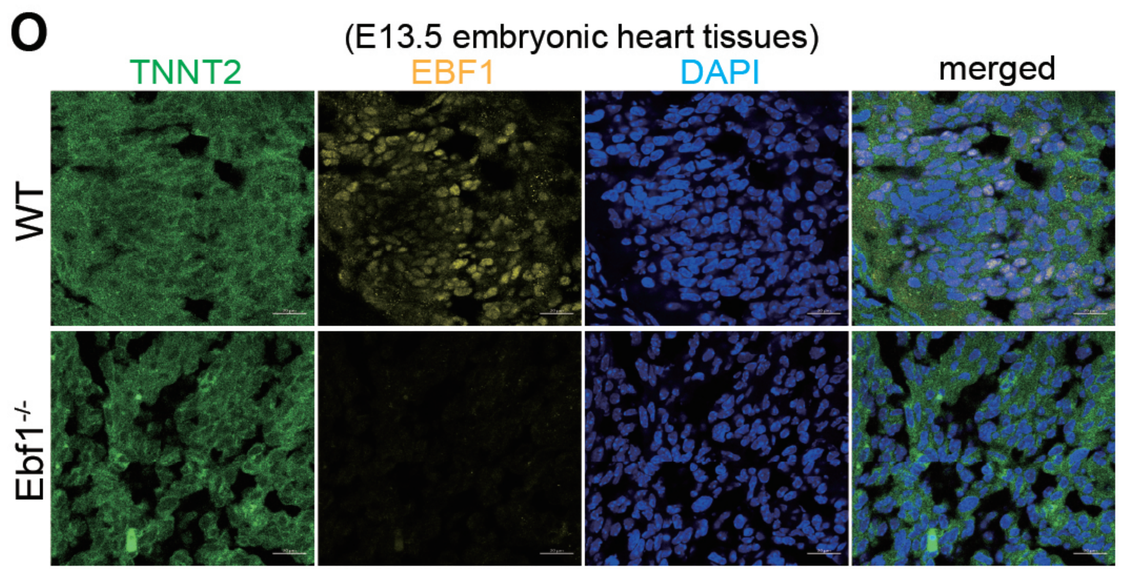

O
(E13.5 embryonic heart tissues)
TNNT2
EBF1
DAPI
merged
WT
Ebf1-/-

Figure 2

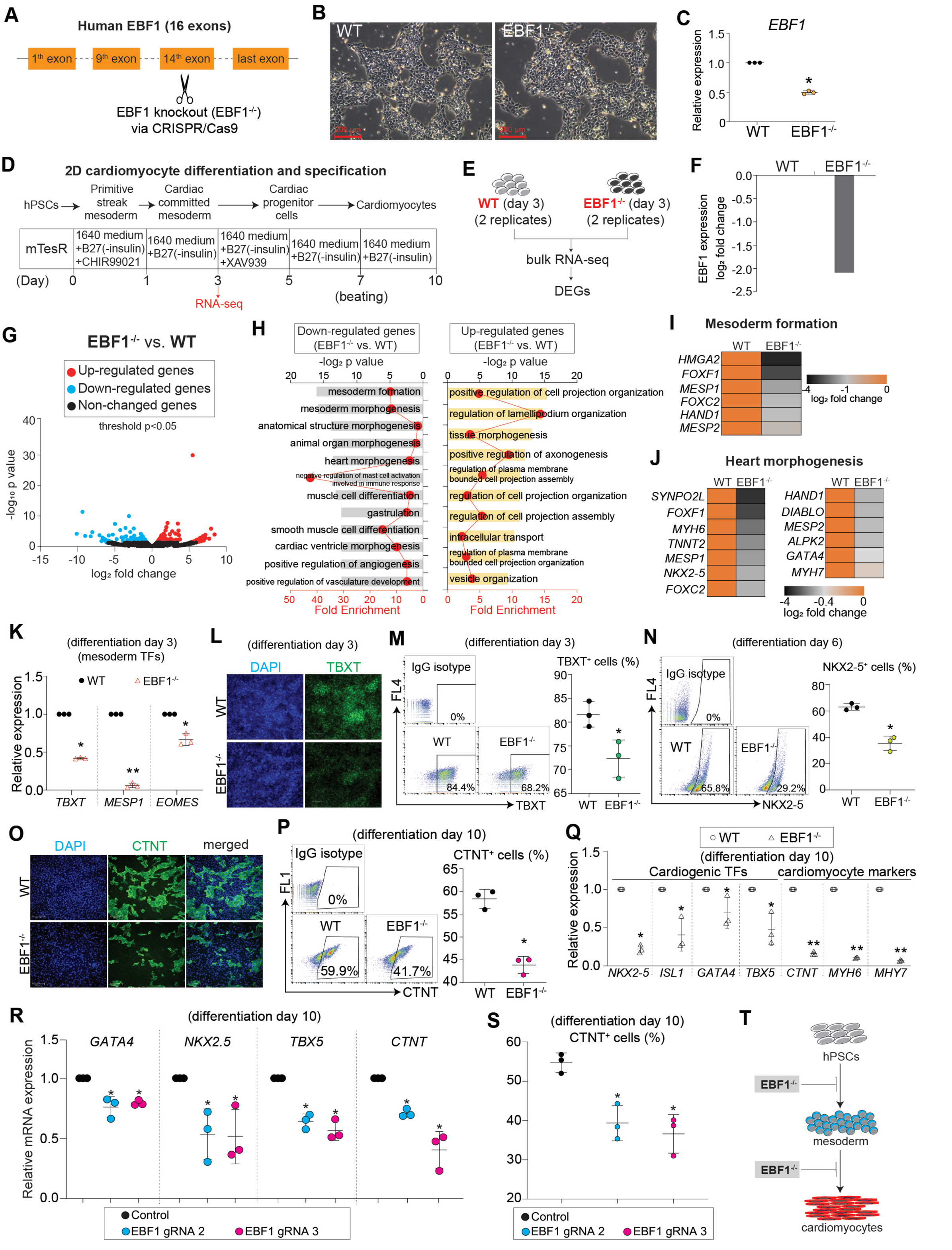
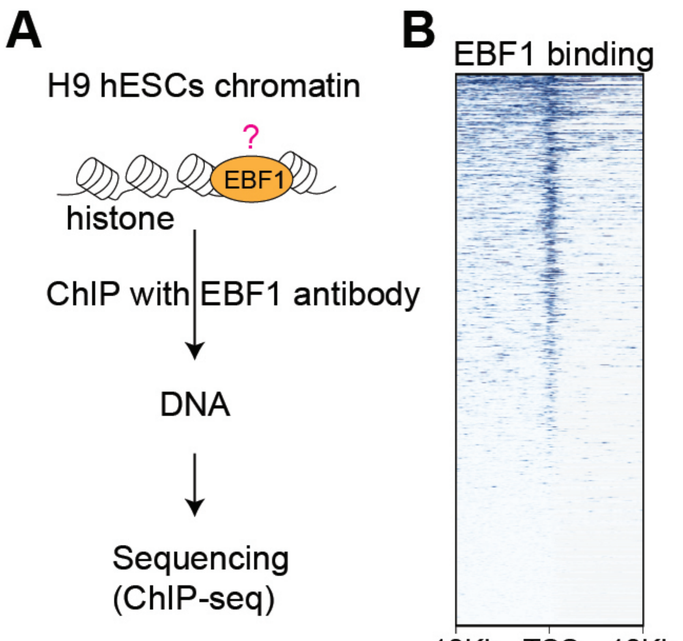
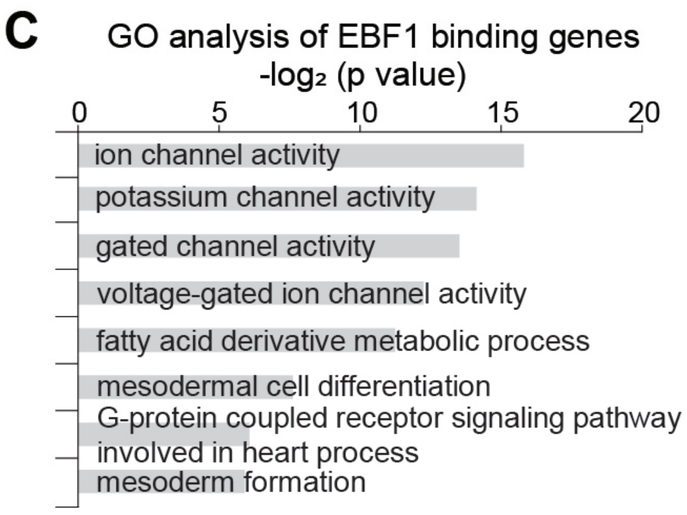
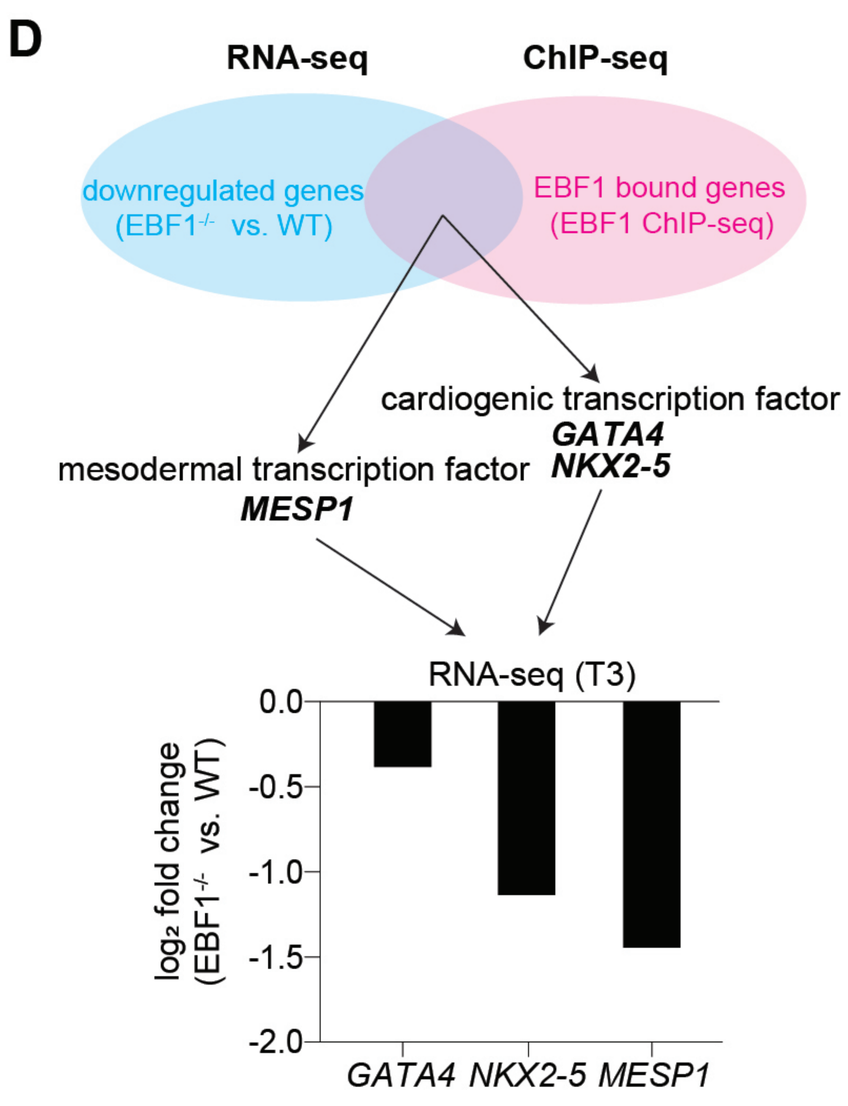

A
Human EBF1 (16 exons)
1th exon
9th exon
14th exon
last exon
EBF1 knockout (EBF1-/-)
via CRISPR/Cas9
B
WT
EBF1-/-
200 μm
C
EBF1
Relative expression
WT
EBF1-/-
D
2D cardiomyocyte differentiation and specification
hPSCs
Primitive streak mesoderm
Cardiac committed mesoderm
Cardiac progenitor cells
Cardiomyocytes
mTesR
1640 medium +B27(-insulin) +CHIR99021
1640 medium +B27(-insulin)
1640 medium +B27(-insulin) +XAV939
1640 medium +B27(-insulin)
1640 medium +B27(-insulin)
(Day)
RNA-seq
(beating)
E
WT (day 3) (2 replicates)
EBF1-/- (day 3) (2 replicates)
bulk RNA-seq
DEGs
F
WT
EBF1-/-
EBF1 expression log2 fold change
G
EBF1-/- vs. WT
Up-regulated genes
Down-regulated genes
Non-changed genes
threshold p<0.05
-log10 p value
log2 fold change
H
Down-regulated genes (EBF1-/- vs. WT)
Up-regulated genes (EBF1-/- vs. WT)
-log2 p value
mesoderm formation
mesoderm morphogenesis
anatomical structure morphogenesis
animal organ morphogenesis
heart morphogenesis
negative regulation of mast cell activation involved in immune response
muscle cell differentiation
gastrulation
smooth muscle cell differentiation
cardiac ventricle morphogenesis
positive regulation of angiogenesis
positive regulation of vasculature development
positive regulation of cell projection organization
regulation of lamellipodium organization
tissue morphogenesis
positive regulation of axonogenesis
regulation of plasma membrane bounded cell projection assembly
regulation of cell projection organization
regulation of cell projection assembly
intracellular transport
regulation of plasma membrane bounded cell projection organization
vesicle organization
Fold Enrichment
I
Mesoderm formation
HMGA2
FOXF1
MESP1
FOXC2
HAND1
MESP2
log2 fold change
J
Heart morphogenesis
SYNPO2L
FOXF1
MYH6
TNNT2
MESP1
NKX2-5
FOXC2
HAND1
DIABLO
MESP2
ALPK2
GATA4
MYH7
K
(differentiation day 3)
(mesoderm TFs)
Relative expression
TBXT
MESP1
EOMES
L
(differentiation day 3)
DAPI
TBXT
M
(differentiation day 3)
IgG isotype
0%
84.4%
68.2%
FL4
TBXT
TBXT+ cells (%)
N
(differentiation day 6)
IgG isotype
0%
65.8%
29.2%
NKX2-5
NKX2-5+ cells (%)
O
DAPI
CTNT
merged
P
(differentiation day 10)
IgG isotype
0%
59.9%
41.7%
FL1
CTNT
CTNT+ cells (%)
Q
(differentiation day 10)
Cardiogenic TFs
cardiomyocyte markers
NKX2-5
ISL1
GATA4
TBX5
CTNT
MYH6
MHY7
R
(differentiation day 10)
GATA4
NKX2.5
TBX5
CTNT
Relative mRNA expression
Control
EBF1 gRNA 2
EBF1 gRNA 3
S
(differentiation day 10)
CTNT+ cells (%)
T
hPSCs
EBF1-/-
mesoderm
EBF1-/-
cardiomyocytes

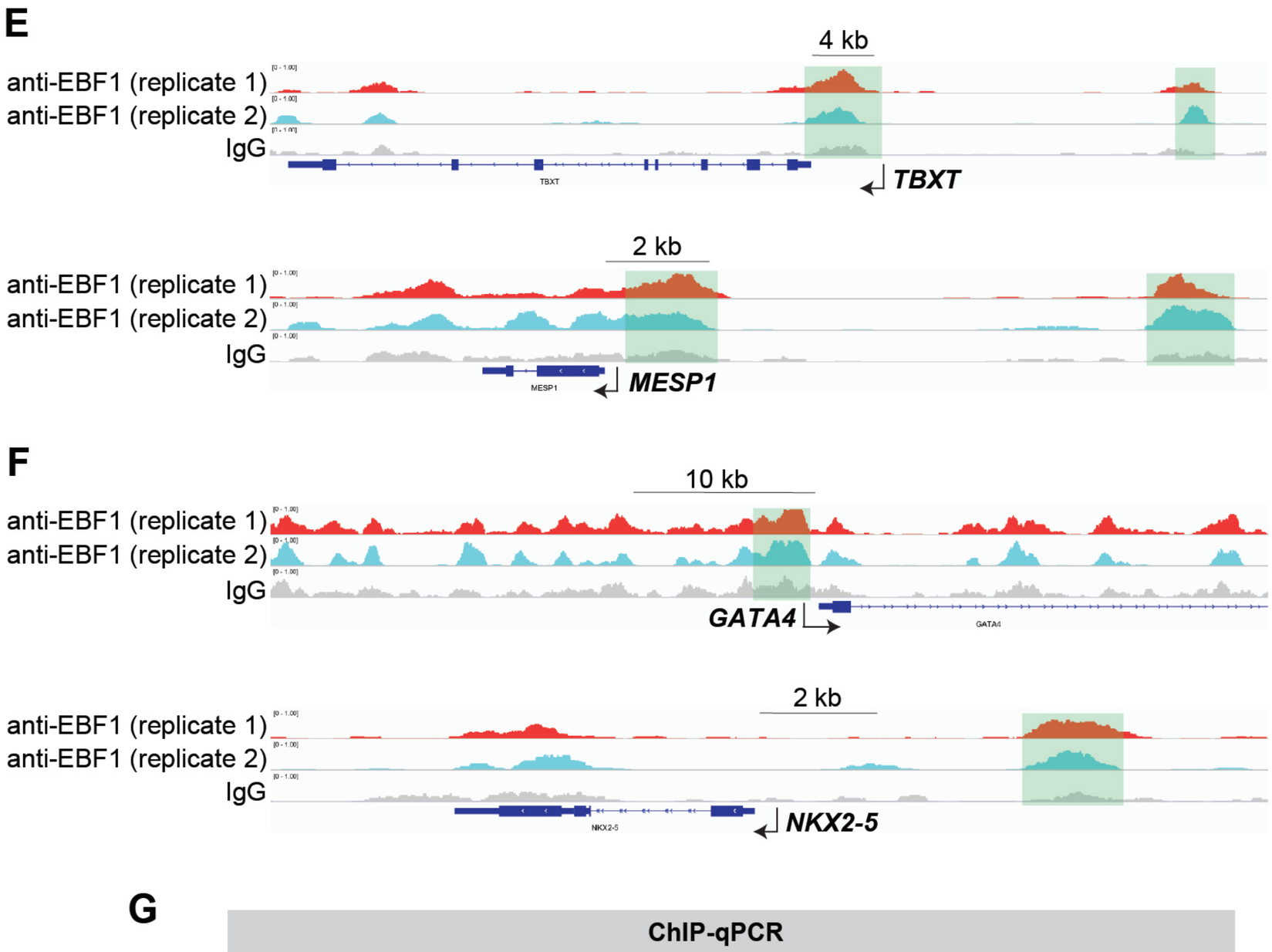
Figure 3

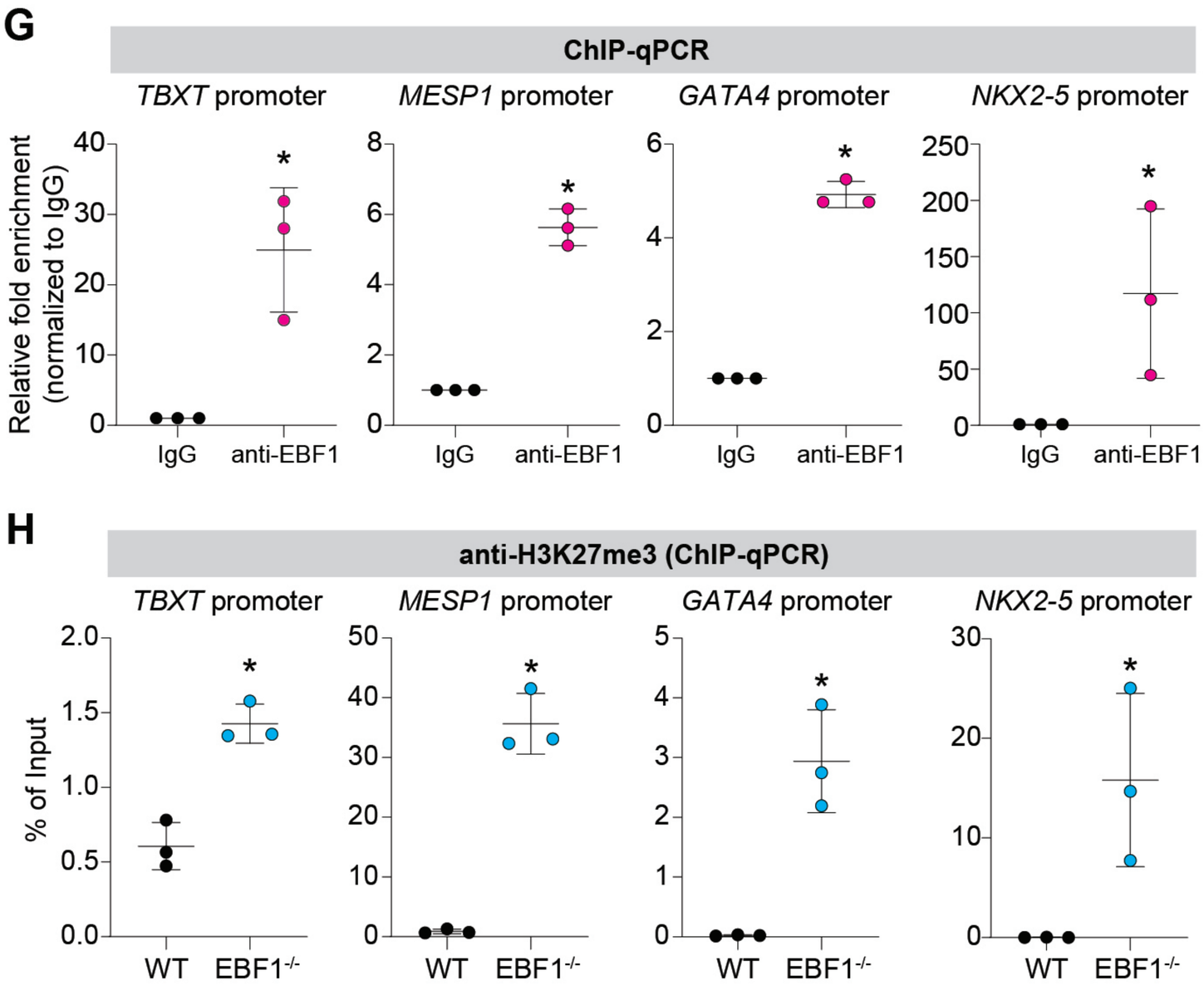

A
H9 hESCs chromatin
?
EBF1
histone
ChIP with EBF1 antibody
DNA
Sequencing
(ChIP-seq)

B
EBF1 binding
-10Kb
TSS
10Kb


C
GO analysis of EBF1 binding genes
-log2 (p value)
0
5
10
15
20
ion channel activity
potassium channel activity
gated channel activity
voltage-gated ion channel activity
fatty acid derivative metabolic process
mesodermal cell differentiation
G-protein coupled receptor signaling pathway
involved in heart process
mesoderm formation


D
RNA-seq
ChIP-seq
downregulated genes
(EBF1-/- vs. WT)
EBF1 bound genes
(EBF1 ChIP-seq)
cardiogenic transcription factor
GATA4
NKX2-5
mesodermal transcription factor
MESP1
RNA-seq (T3)
log2 fold change
(EBF1-/- vs. WT)
0.0
-0.5
-1.0
-1.5
-2.0
GATA4 NKX2-5 MESP1


E
4 kb
anti-EBF1 (replicate 1)
anti-EBF1 (replicate 2)
IgG
TBXT
TBXT
2 kb
MESP1
MESP1
F
10 kb
GATA4
GATA4
2 kb
NKX2-5
NKX2-5


G
ChIP-qPCR
TBXT promoter
MESP1 promoter
GATA4 promoter
NKX2-5 promoter
Relative fold enrichment
(normalized to IgG)
IgG
anti-EBF1
H
anti-H3K27me3 (ChIP-qPCR)
% of Input
WT
EBF1-/-

I
RNA-seq (T3)
log2 fold change
(EBF1-/+ vs. WT)
0.0
-0.5
-1.0
-1.5
-2.0
GATA5 GATA4 MESP2 HAND1 NKX2-5 MESP1

J
H3K27me3
EBF1
mesodermal TFs
cardiogenic TFs
Cardiac development
EBF1
H3K27me3
Repression
mesodermal TFs
cardiogenic TFs
Cardiac development

Figure 4

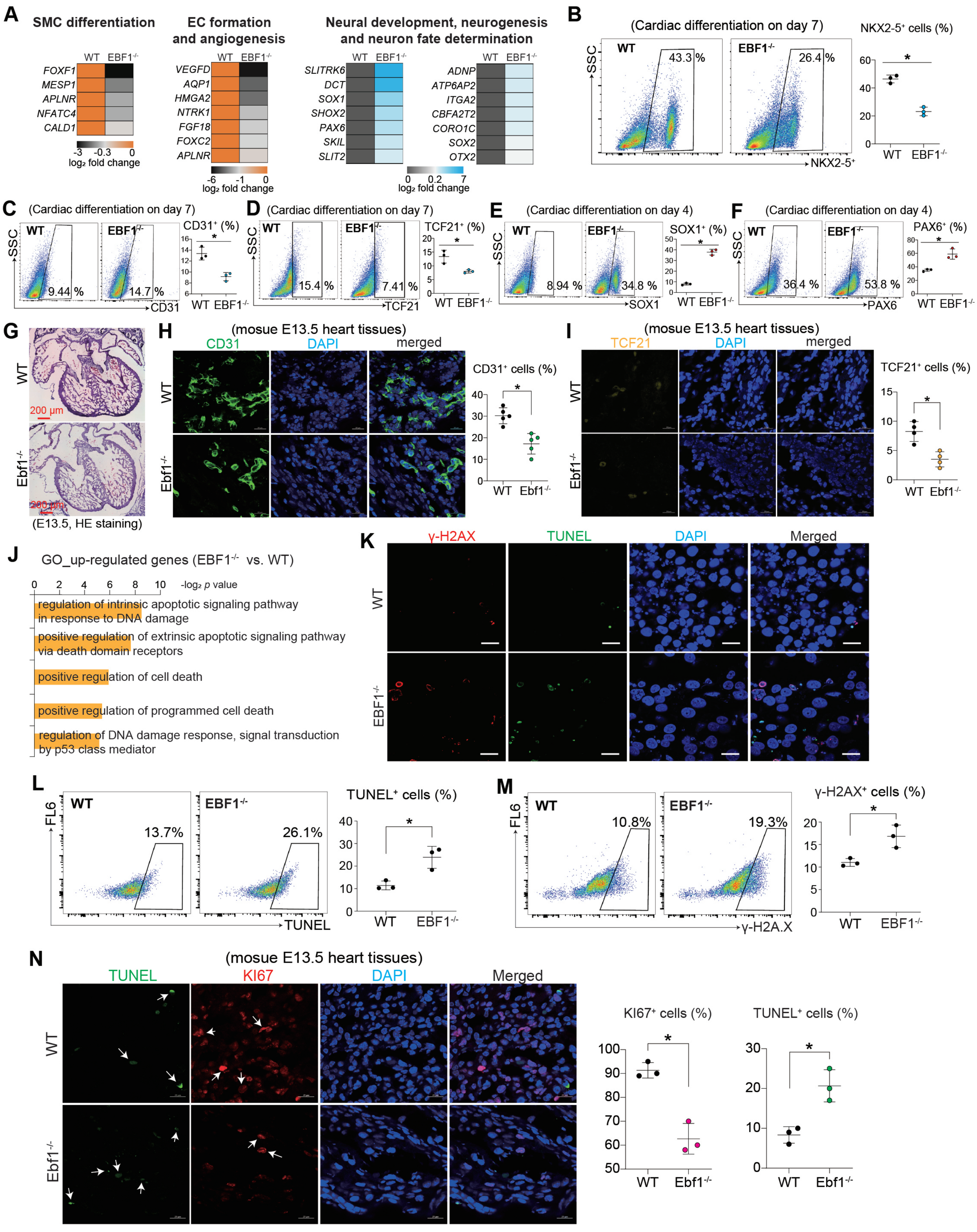
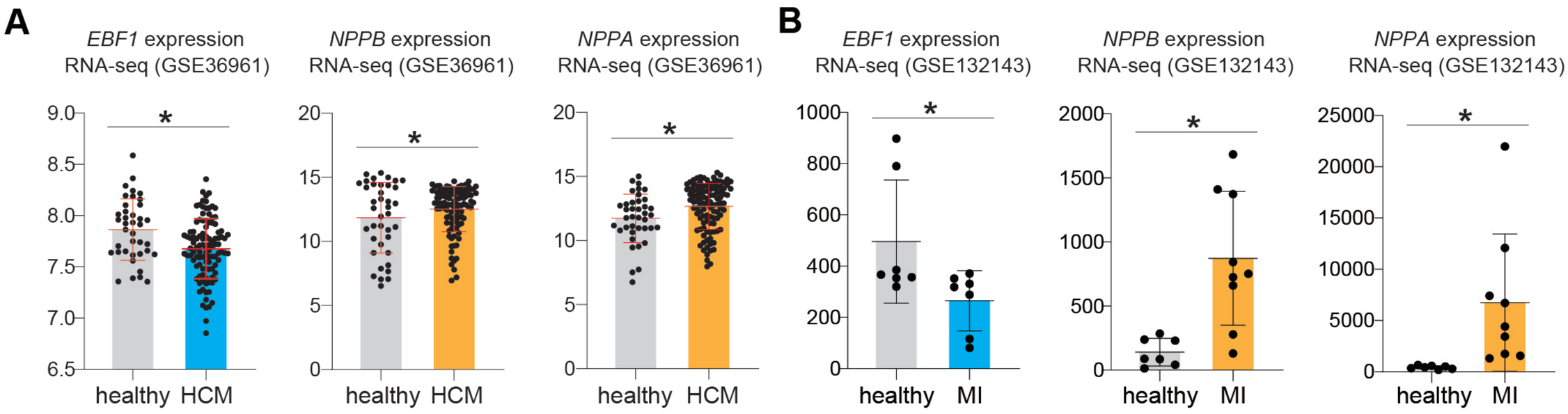
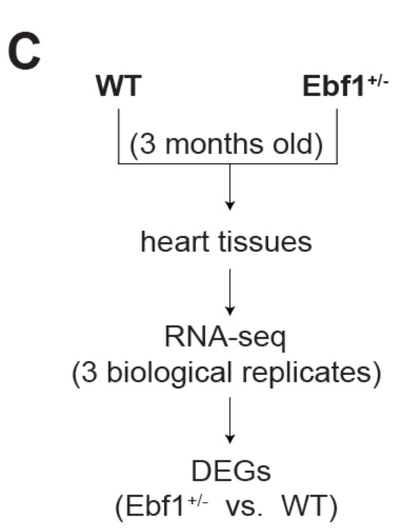
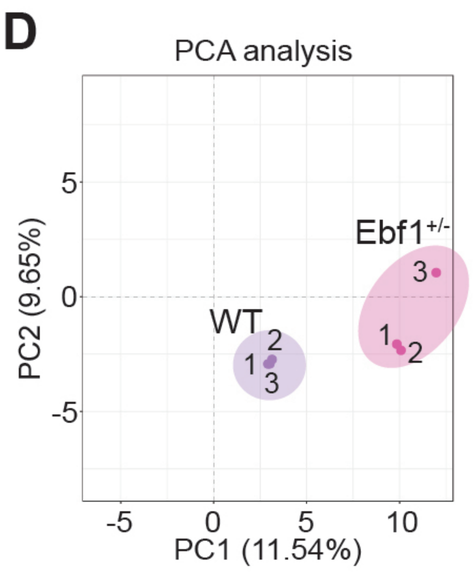
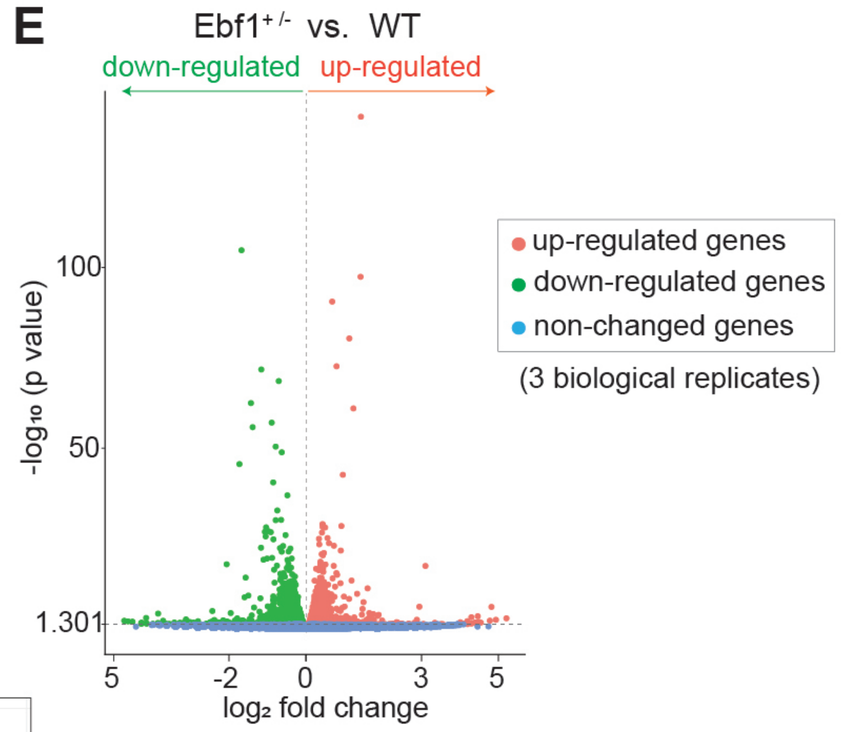

A
SMC differentiation
EC formation and angiogenesis
Neural development, neurogenesis and neuron fate determination
WT EBF1-/-
FOXF1
MESP1
APLNR
NFATC4
CALD1
-3 -0.3 0
log2 fold change
VEGFD
AQP1
HMGA2
NTRK1
FGF18
FOXC2
APLNR
-6 -1 0
log2 fold change
SLITRK6
DCT
SOX1
SHOX2
PAX6
SKIL
SLIT2
ADNP
ATP6AP2
ITGA2
CBFA2T2
CORO1C
SOX2
OTX2
0 0.2 7
log2 fold change
B
(Cardiac differentiation on day 7)
WT
43.3 %
EBF1-/-
26.4 %
SSC
NKX2-5+
NKX2-5+ cells (%)
WT EBF1-/-
C
(Cardiac differentiation on day 7)
WT
9.44 %
EBF1-/-
14.7 %
SSC
CD31
CD31+ (%)
WT EBF1-/-
D
(Cardiac differentiation on day 7)
WT
15.4 %
EBF1-/-
7.41 %
SSC
TCF21
TCF21+ (%)
WT EBF1-/-
E
(Cardiac differentiation on day 4)
WT
8.94 %
EBF1-/-
34.8 %
SSC
SOX1
SOX1+ (%)
WT EBF1-/-
F
(Cardiac differentiation on day 4)
WT
36.4 %
EBF1-/-
53.8 %
SSC
PAX6
PAX6+ (%)
WT EBF1-/-
G
WT
200 μm
Ebf1-/-
200 μm
(E13.5, HE staining)
H
(mosue E13.5 heart tissues)
CD31
DAPI
merged
WT
Ebf1-/-
CD31+ cells (%)
WT Ebf1-/-
I
(mosue E13.5 heart tissues)
TCF21
DAPI
merged
WT
Ebf1-/-
TCF21+ cells (%)
WT Ebf1-/-
J
GO_up-regulated genes (EBF1-/- vs. WT)
0 2 4 6 8 10
-log2 p value
regulation of intrinsic apoptotic signaling pathway in response to DNA damage
positive regulation of extrinsic apoptotic signaling pathway via death domain receptors
positive regulation of cell death
positive regulation of programmed cell death
regulation of DNA damage response, signal transduction by p53 class mediator
K
γ-H2AX
TUNEL
DAPI
Merged
WT
EBF1-/-
L
WT
13.7%
EBF1-/-
26.1%
FL6
TUNEL
TUNEL+ cells (%)
WT EBF1-/-
M
WT
10.8%
EBF1-/-
19.3%
FL6
γ-H2A.X
γ-H2AX+ cells (%)
WT EBF1-/-
N
(mosue E13.5 heart tissues)
TUNEL
KI67
DAPI
Merged
WT
Ebf1-/-
KI67+ cells (%)
WT Ebf1-/-
TUNEL+ cells (%)
WT Ebf1-/-

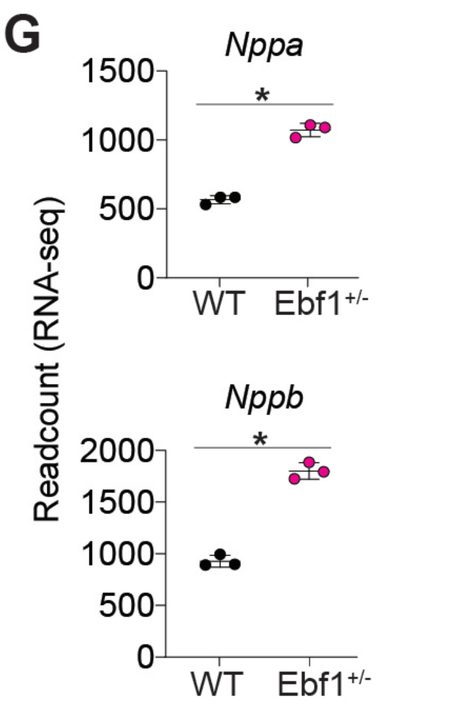
Figure 5

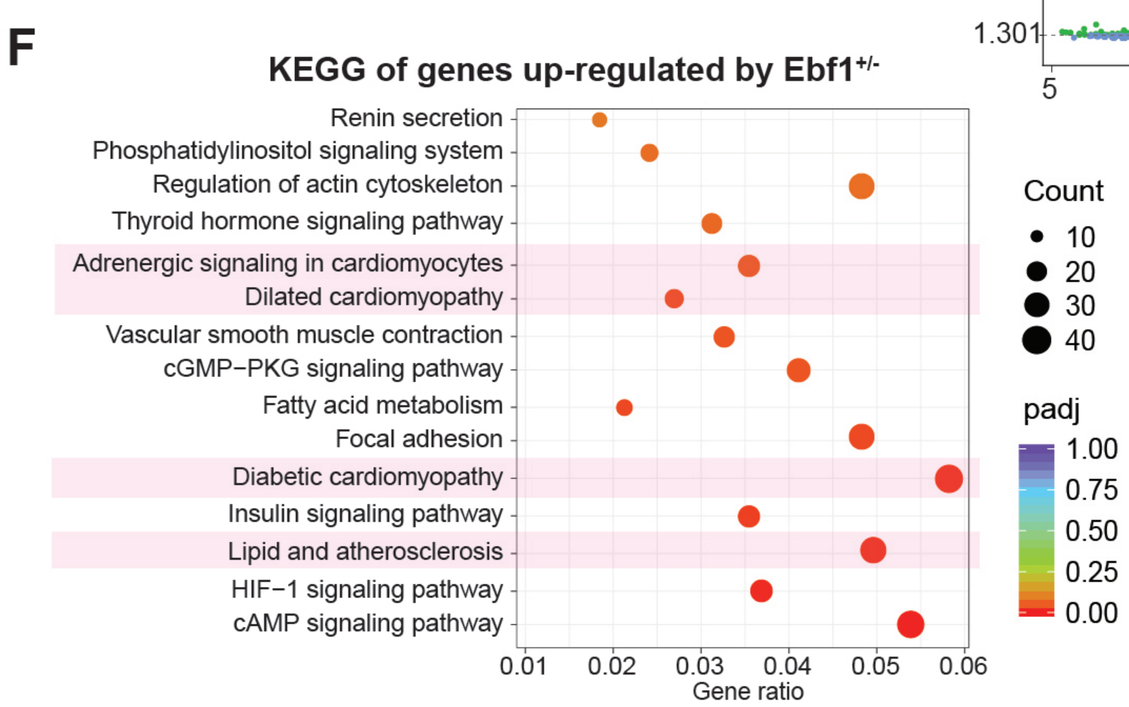

A
EBF1 expression
RNA-seq (GSE36961)
NPPB expression
RNA-seq (GSE36961)
NPPA expression
RNA-seq (GSE36961)
healthy HCM
B
EBF1 expression
RNA-seq (GSE132143)
NPPB expression
RNA-seq (GSE132143)
NPPA expression
RNA-seq (GSE132143)
healthy MI

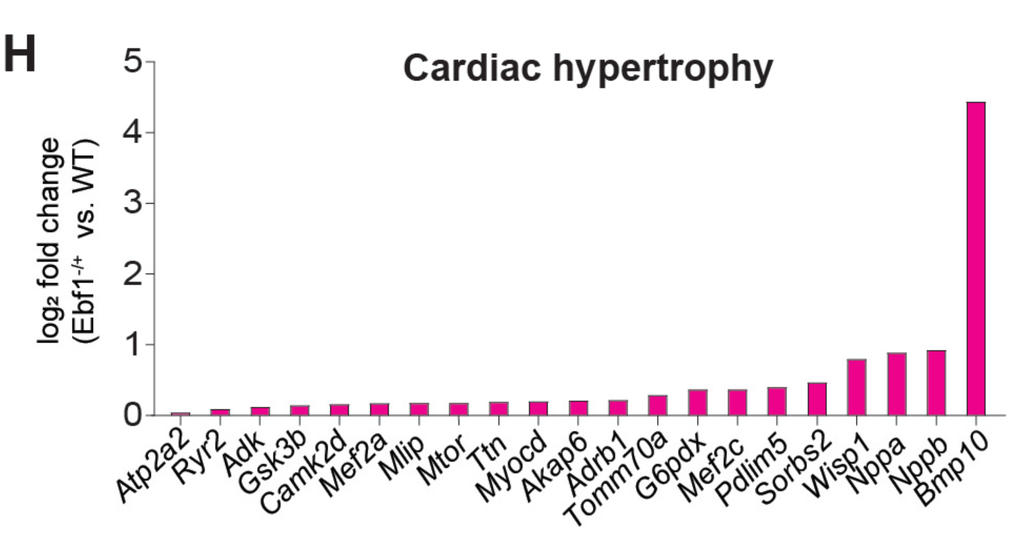

C
WT
Ebf1+/-
(3 months old)
heart tissues
RNA-seq
(3 biological replicates)
DEGs
(Ebf1+/- vs. WT)

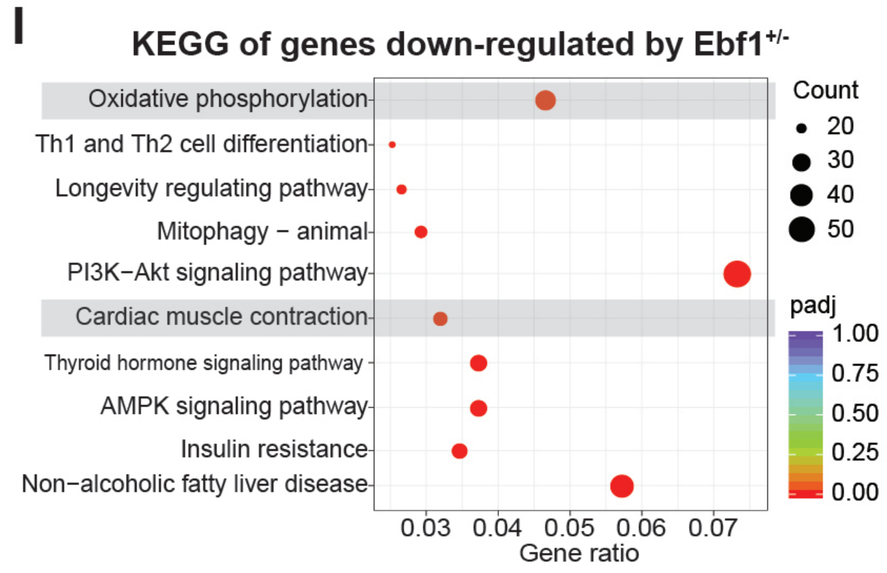

D
PCA analysis
Ebf1+/-
WT
PC2 (9.65%)
PC1 (11.54%)

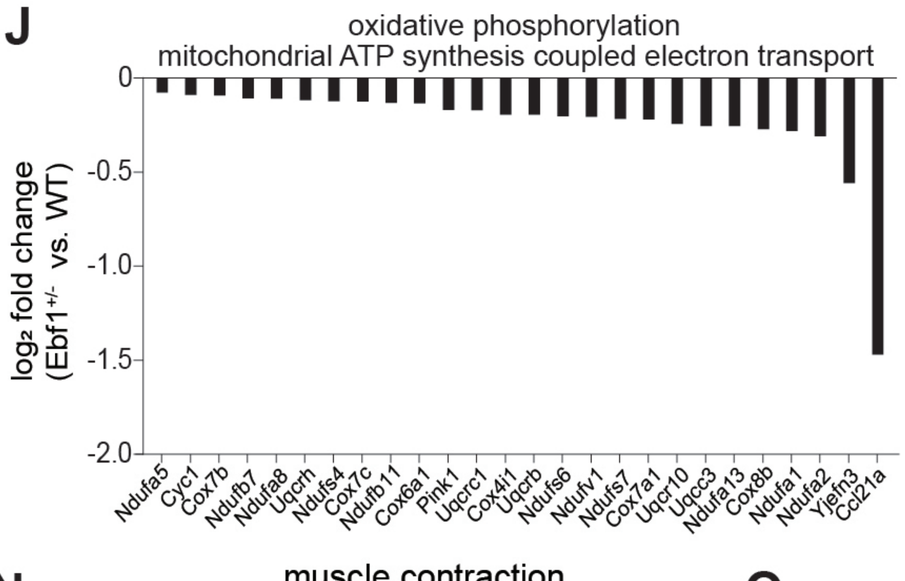

E
Ebf1+/- vs. WT
down-regulated
up-regulated
-log10 (p value)
log2 fold change
up-regulated genes
down-regulated genes
non-changed genes
(3 biological replicates)

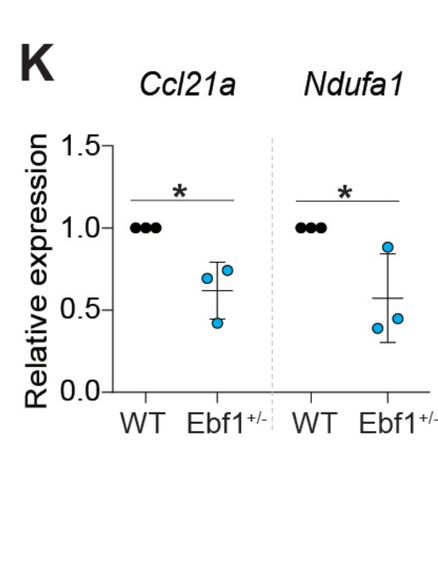

G
Nppa
Nppb
Readcount (RNA-seq)
WT
Ebf1+/-

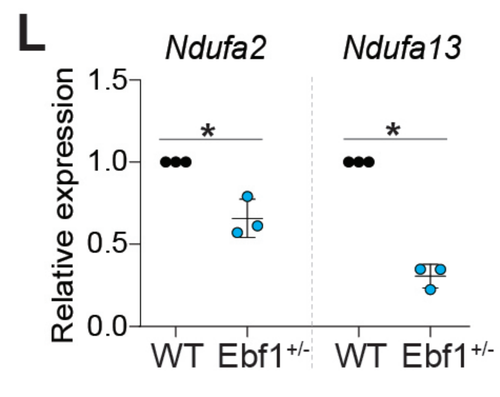

F
KEGG of genes up-regulated by Ebf1+/-
Renin secretion
Phosphatidylinositol signaling system
Regulation of actin cytoskeleton
Thyroid hormone signaling pathway
Adrenergic signaling in cardiomyocytes
Dilated cardiomyopathy
Vascular smooth muscle contraction
cGMP−PKG signaling pathway
Fatty acid metabolism
Focal adhesion
Diabetic cardiomyopathy
Insulin signaling pathway
Lipid and atherosclerosis
HIF−1 signaling pathway
cAMP signaling pathway
Gene ratio
Count
padj

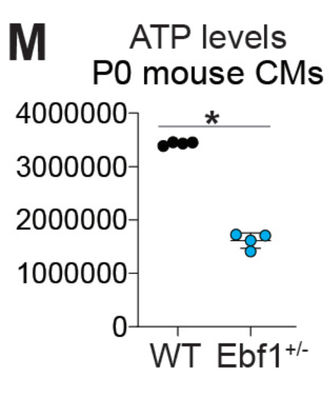

H
Cardiac hypertrophy
log2 fold change
(Ebf1-/+ vs. WT)
Atp2a2
Ryr2
Adk
Gsk3b
Camk2d
Mef2a
Mlip
Mtor
Ttn
Myocd
Akap6
Adrb1
Tomm70a
G6pdx
Mef2c
Pdlim5
Sorbs2
Wisp1
Nppa
Nppb
Bmp10

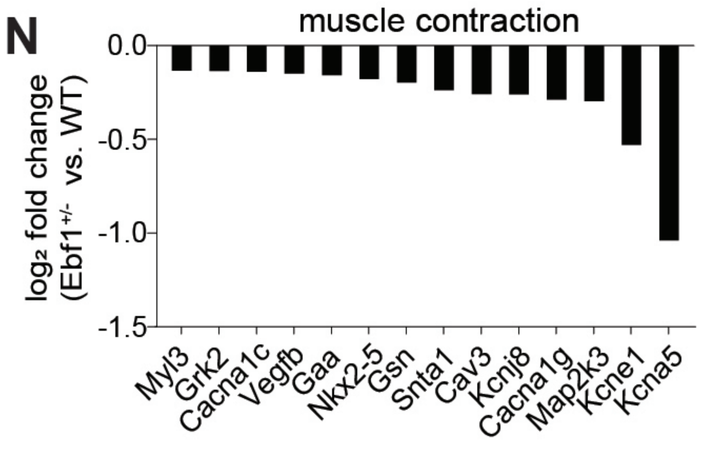

I
KEGG of genes down-regulated by Ebf1+/-
Oxidative phosphorylation
Th1 and Th2 cell differentiation
Longevity regulating pathway
Mitophagy − animal
PI3K−Akt signaling pathway
Cardiac muscle contraction
Thyroid hormone signaling pathway
AMPK signaling pathway
Insulin resistance
Non−alcoholic fatty liver disease
Gene ratio
Count
padj


J
oxidative phosphorylation
mitochondrial ATP synthesis coupled electron transport
log2 fold change
(Ebf1+/- vs. WT)
Ndufa5
Cyc1
Cox7b
Ndufb7
Ndufa8
Uqcrh
Ndufs4
Cox7c
Ndufb11
Cox6a1
Pink1
Uqcrc1
Cox4i1
Uqcrb
Ndufs6
Ndufv1
Ndufs7
Cox7a1
Uqcr10
Uqcc3
Ndufa13
Cox8b
Ndufa1
Ndufa2
Yjefn3
Ccl21a


K
Ccl21a
Ndufa1
Relative expression
WT
Ebf1+/-


L
Ndufa2
Ndufa13
Relative expression
WT
Ebf1+/-


M
ATP levels
P0 mouse CMs
WT
Ebf1+/-


N
muscle contraction
log2 fold change
(Ebf1+/- vs. WT)
Myl3
Grk2
Cacna1c
Vegfb
Gaa
Nkx2-5
Gsn
Snta1
Cav3
Kcnj8
Cacna1g
Map2k3
Kcne1
Kcna5

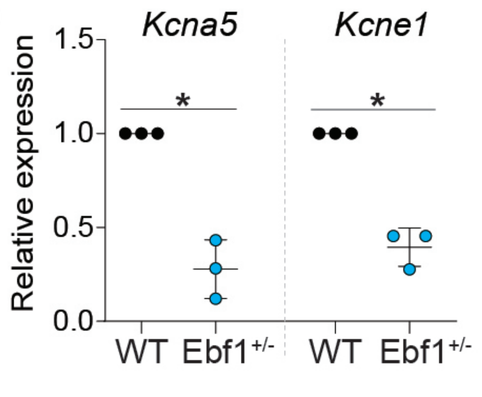

O
Kcna5
Kcne1
Relative expression
WT
Ebf1+/-

Figure 6

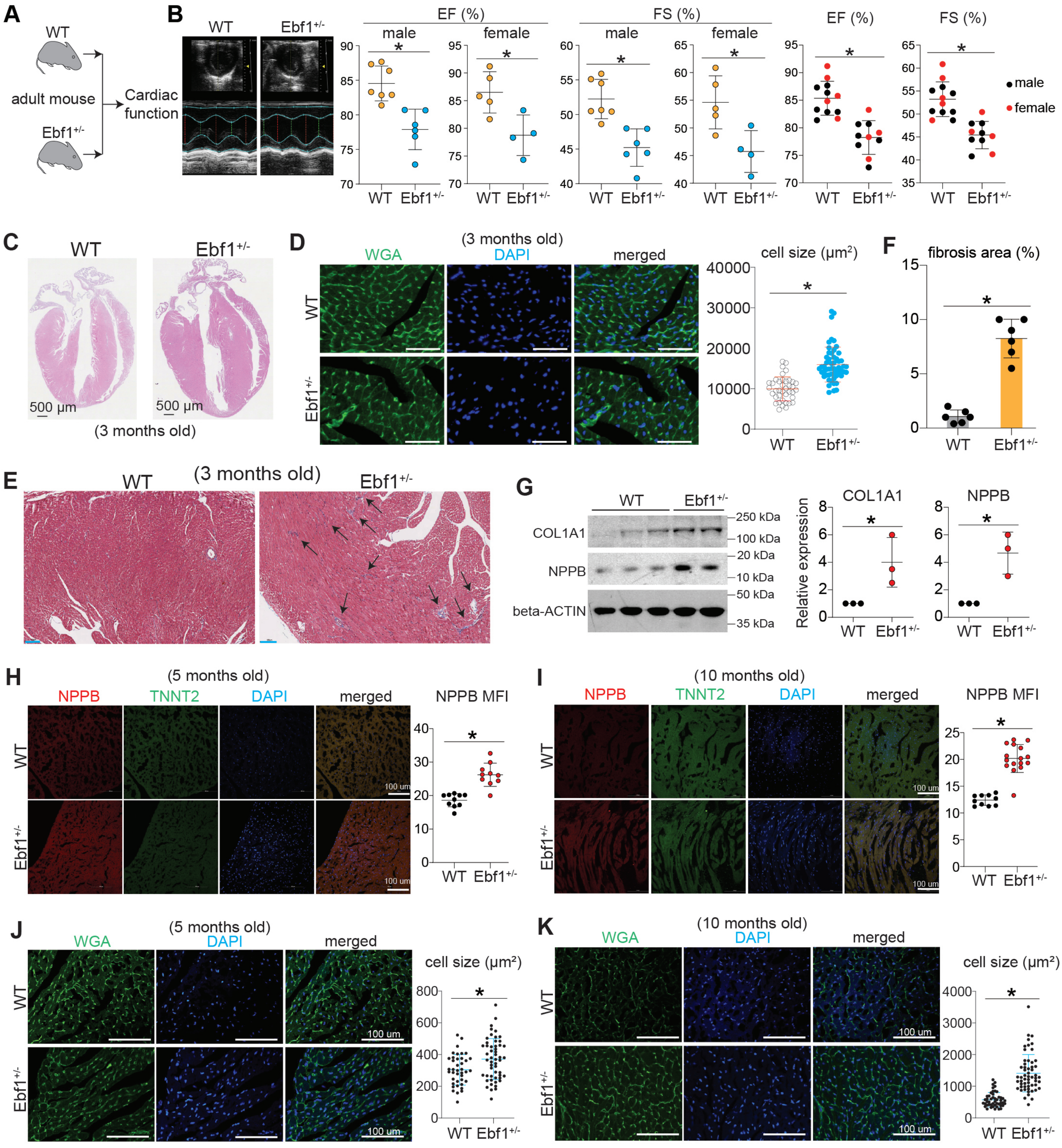
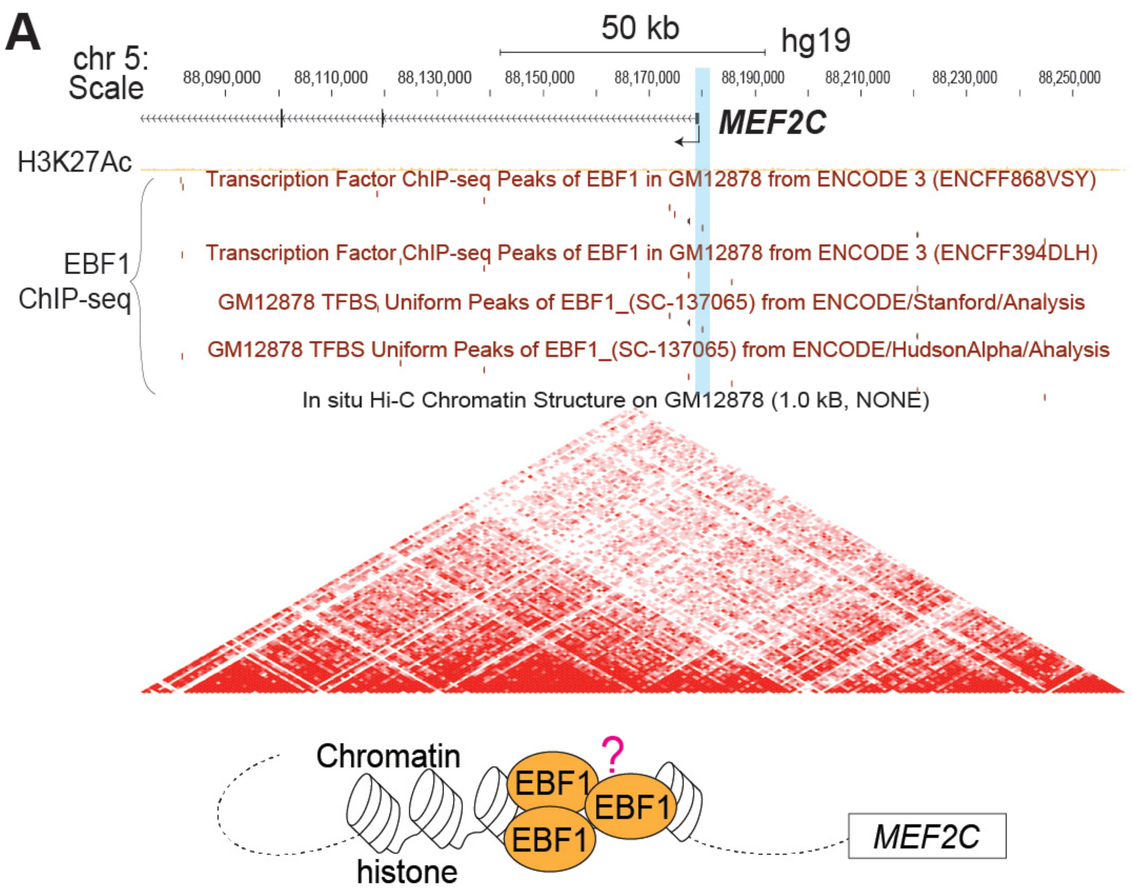
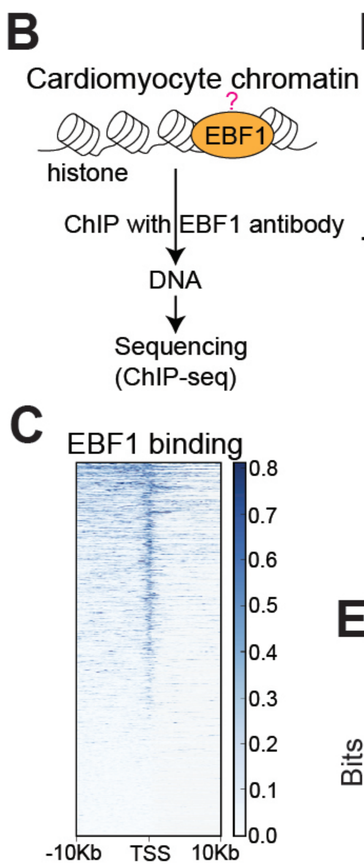
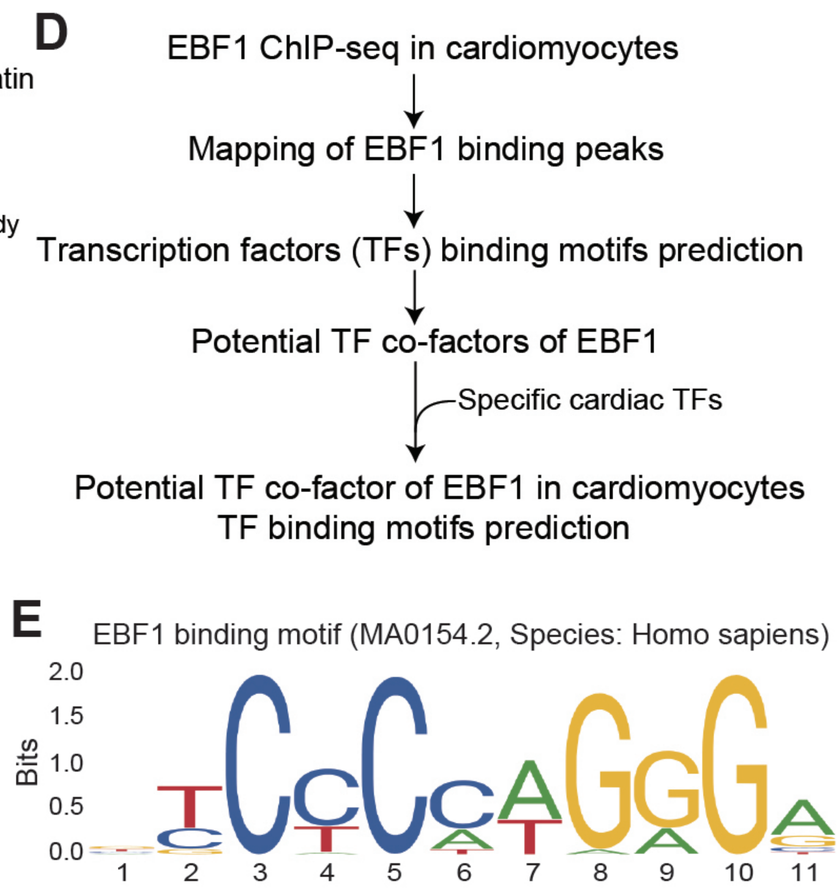
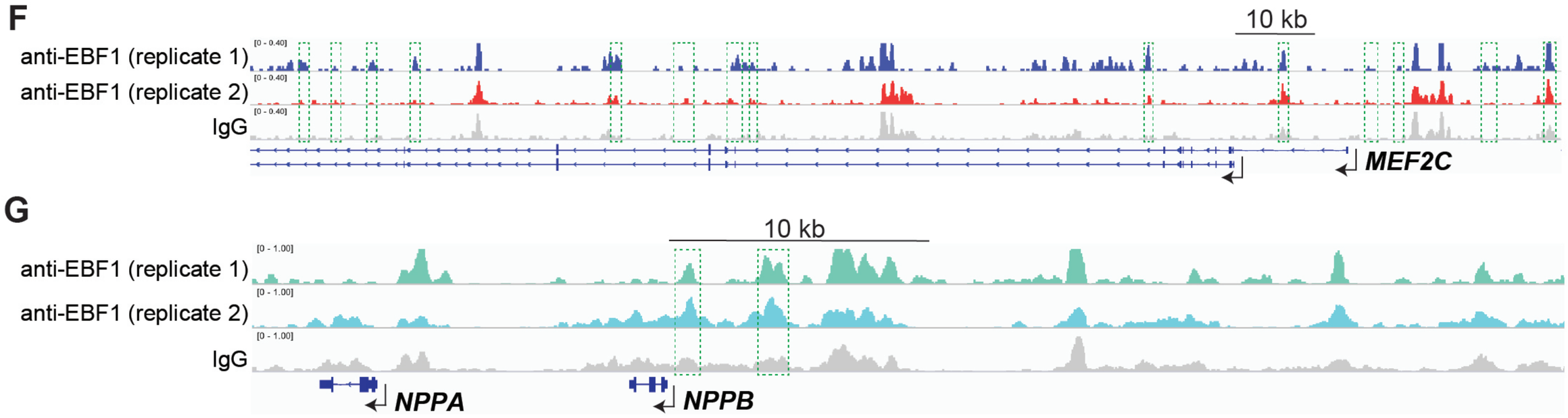

A
WT
adult mouse
Ebf1+/-
Cardiac function
B
WT
Ebf1+/-
EF (%)
male
female
FS (%)
male
female
EF (%)
FS (%)
male
female
C
WT
Ebf1+/-
500 μm
500 μm
(3 months old)
D
(3 months old)
WGA
DAPI
merged
WT
Ebf1+/-
cell size (μm²)
F
fibrosis area (%)
E
WT
(3 months old)
Ebf1+/-
G
WT
Ebf1+/-
COL1A1
NPPB
beta-ACTIN
250 kDa
100 kDa
20 kDa
10 kDa
50 kDa
35 kDa
Relative expression
COL1A1
NPPB
H
(5 months old)
NPPB
TNNT2
DAPI
merged
NPPB MFI
100 um
I
(10 months old)
NPPB
TNNT2
DAPI
merged
NPPB MFI
100 um
J
(5 months old)
WGA
DAPI
merged
cell size (μm²)
100 um
K
(10 months old)
WGA
DAPI
merged
cell size (μm²)
100 um

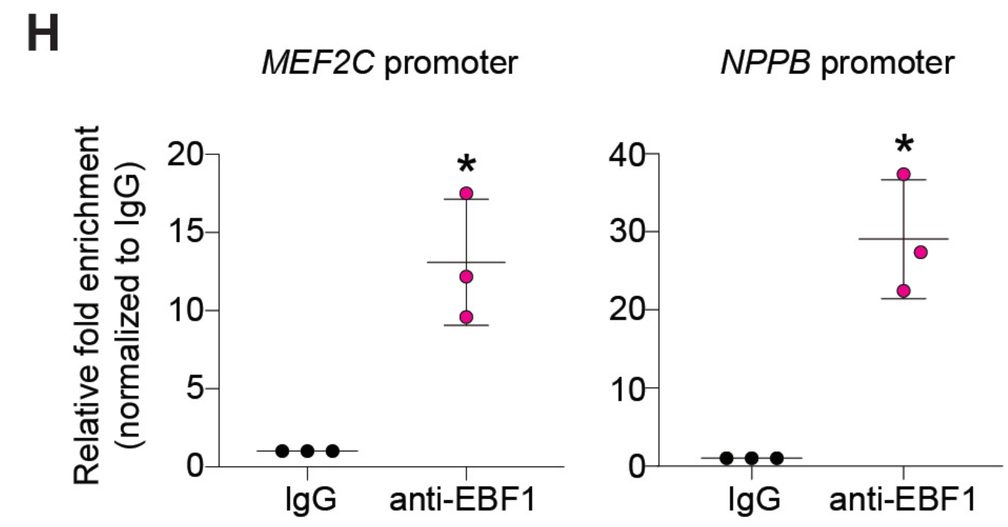
Figure 7

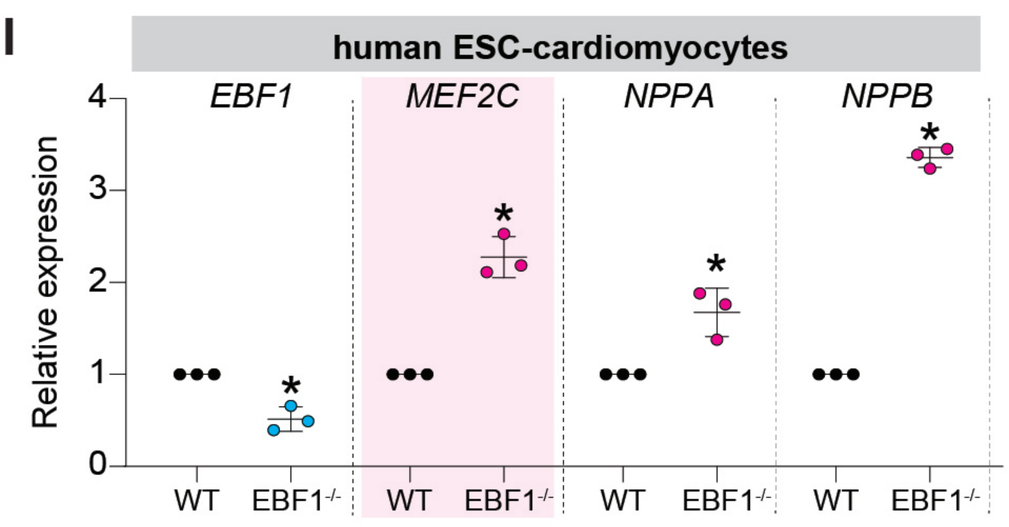

A
50 kb
hg19
chr 5:
Scale
88,090,000
88,110,000
88,130,000
88,150,000
88,170,000
88,190,000
88,210,000
88,230,000
88,250,000
MEF2C
H3K27Ac
Transcription Factor ChIP-seq Peaks of EBF1 in GM12878 from ENCODE 3 (ENCFF868VSY)
EBF1 ChIP-seq
Transcription Factor ChIP-seq Peaks of EBF1 in GM12878 from ENCODE 3 (ENCFF394DLH)
GM12878 TFBS Uniform Peaks of EBF1_(SC-137065) from ENCODE/Stanford/Analysis
GM12878 TFBS Uniform Peaks of EBF1_(SC-137065) from ENCODE/HudsonAlpha/Analysis
In situ Hi-C Chromatin Structure on GM12878 (1.0 kB, NONE)
Chromatin
EBF1
EBF1
EBF1
?
histone
MEF2C

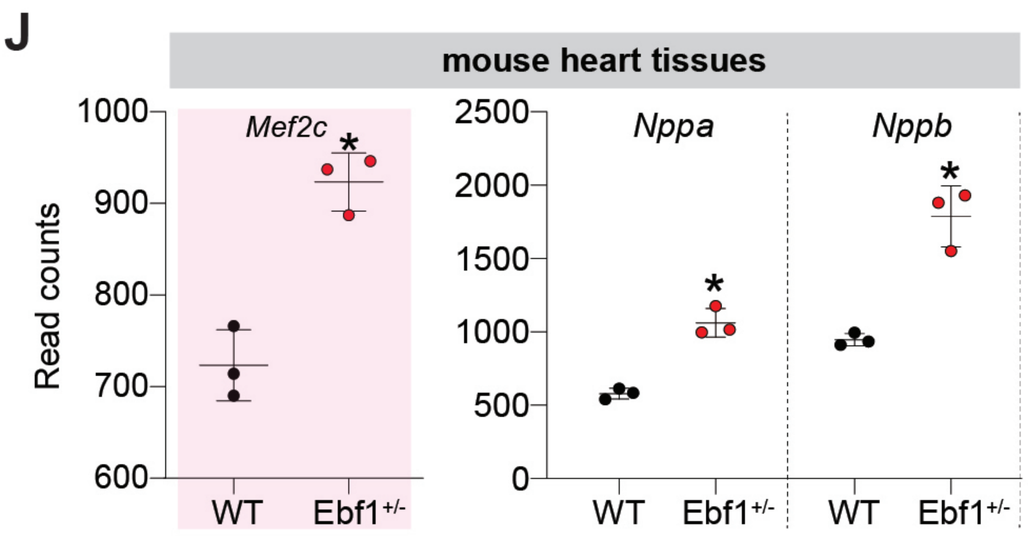

B
Cardiomyocyte chromatin
?
EBF1
histone
ChIP with EBF1 antibody
DNA
Sequencing
(ChIP-seq)
C
EBF1 binding
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
-10Kb
TSS
10Kb

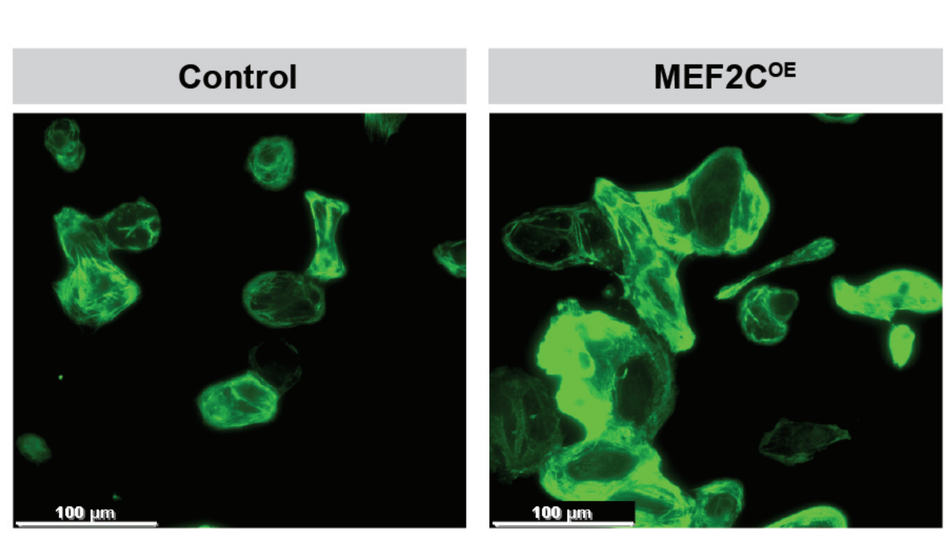

D
EBF1 ChIP-seq in cardiomyocytes
Mapping of EBF1 binding peaks
Transcription factors (TFs) binding motifs prediction
Potential TF co-factors of EBF1
Specific cardiac TFs
Potential TF co-factor of EBF1 in cardiomyocytes
TF binding motifs prediction
E
EBF1 binding motif (MA0154.2, Species: Homo sapiens)
Bits
2.0
1.5
1.0
0.5
0.0
1
2
3
4
5
6
7
8
9
10
11

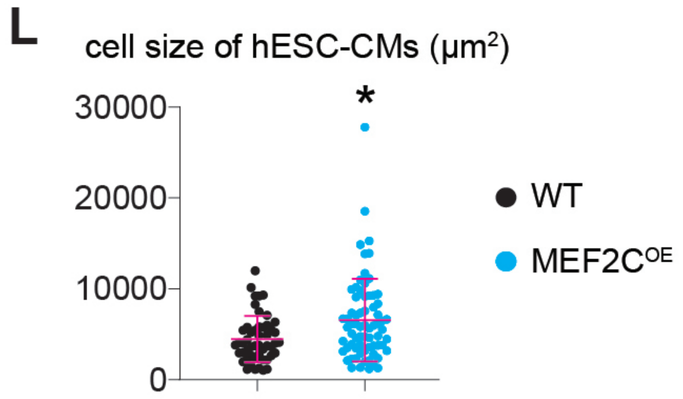

F
10 kb
anti-EBF1 (replicate 1)
anti-EBF1 (replicate 2)
IgG
[0 - 0.40]
MEF2C
G
10 kb
anti-EBF1 (replicate 1)
anti-EBF1 (replicate 2)
IgG
[0 - 1.00]
NPPA
NPPB

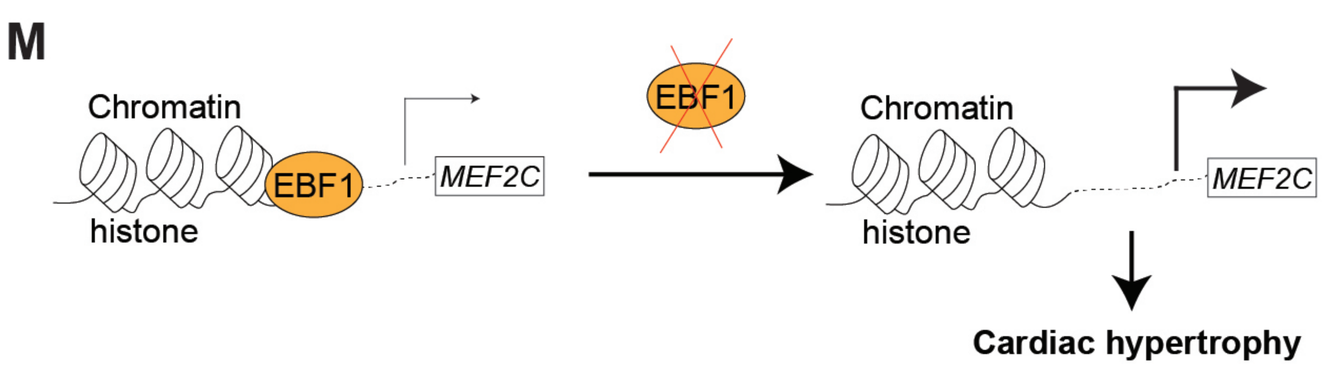

H
MEF2C promoter
NPPB promoter
Relative fold enrichment
(normalized to IgG)
IgG
anti-EBF1
IgG
anti-EBF1


I
human ESC-cardiomyocytes
EBF1
MEF2C
NPPA
NPPB
Relative expression
WT
EBF1-/-
WT
EBF1-/-
WT
EBF1-/-
WT
EBF1-/-

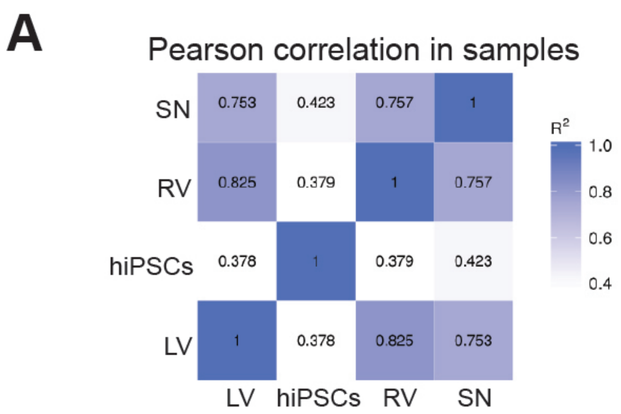

J
mouse heart tissues
Mef2c
Nppa
Nppb
Read counts
WT
Ebf1+/-
WT
Ebf1+/-
WT
Ebf1+/-

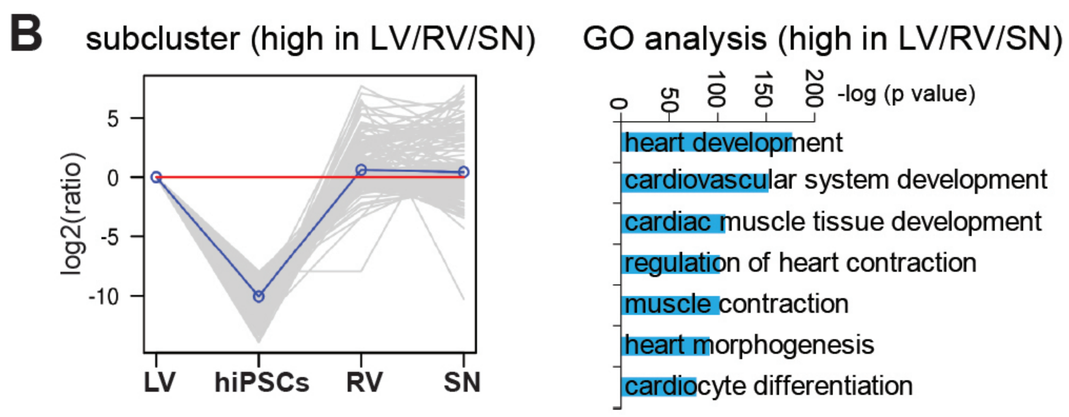

K
Control
MEF2COE
100 μm
100 μm

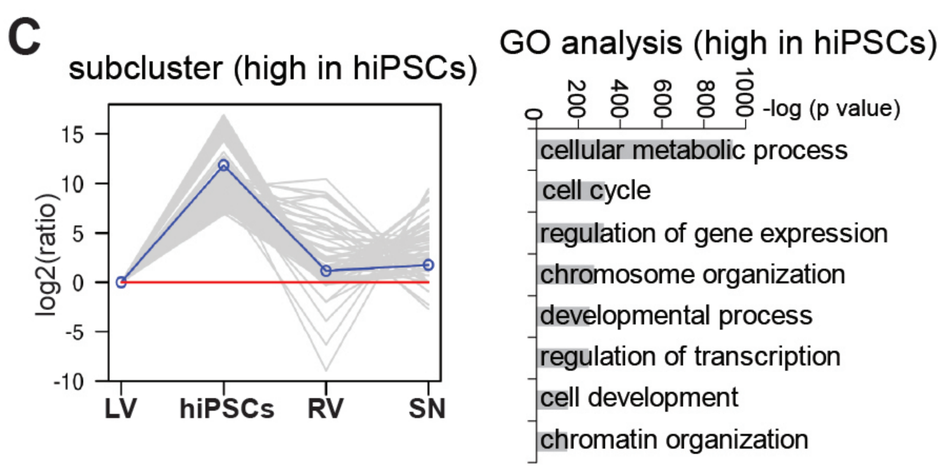

L
cell size of hESC-CMs (μm2)
WT
MEF2COE

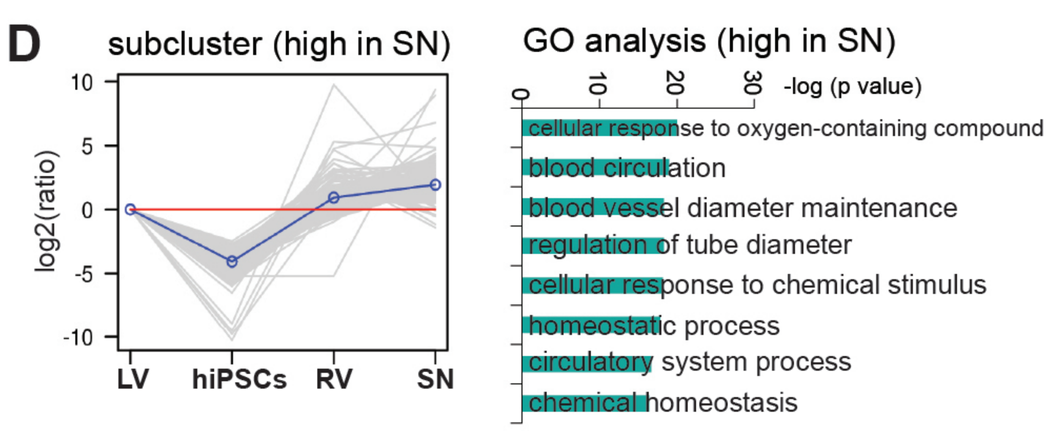

M
Chromatin
EBF1
MEF2C
histone
EBF1
Chromatin
MEF2C
histone
Cardiac hypertrophy

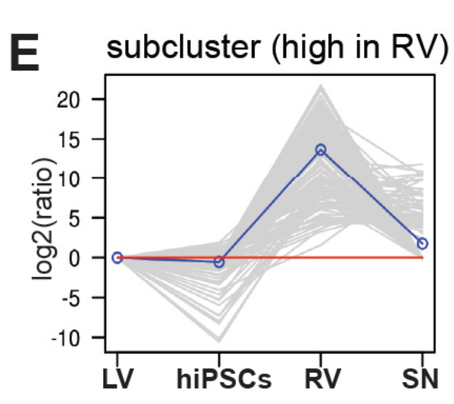
Supplemental Figure 1

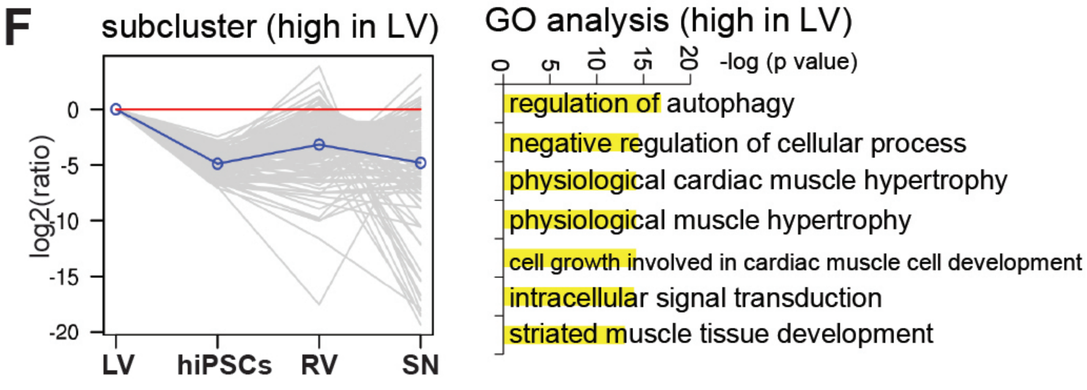


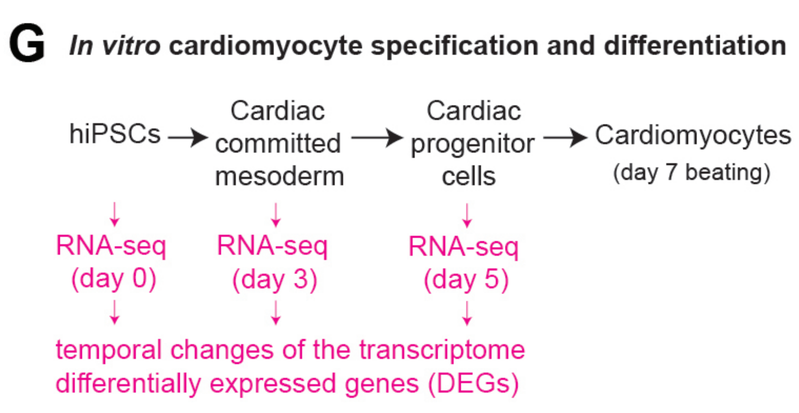


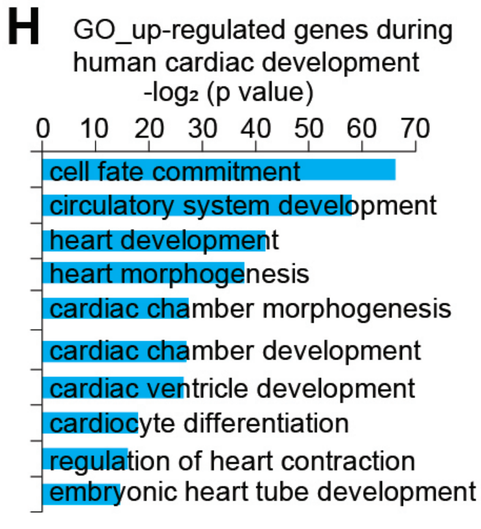


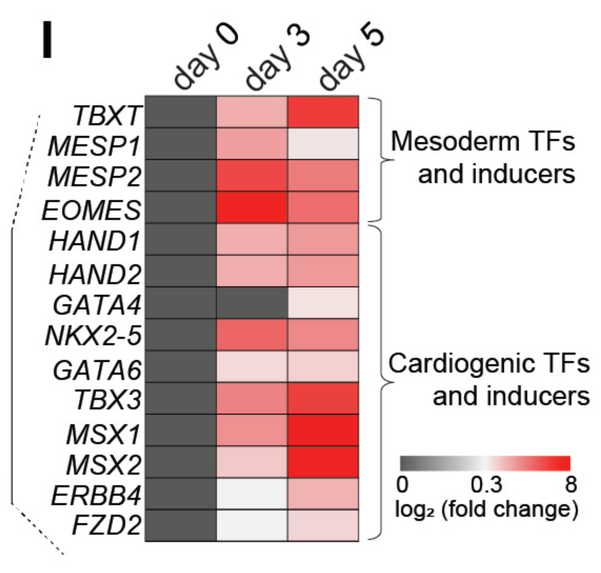


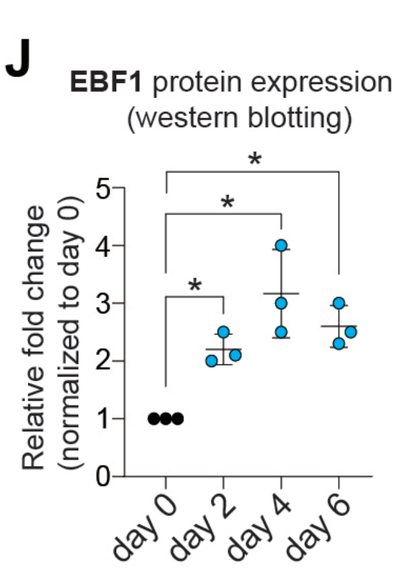


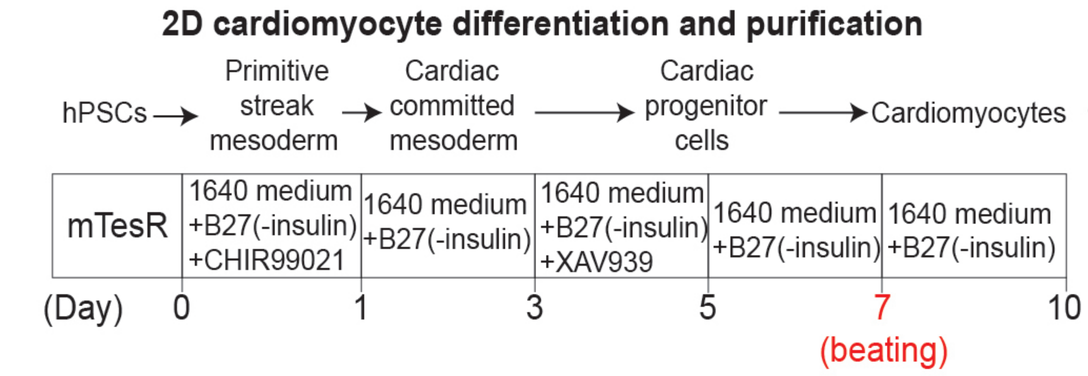


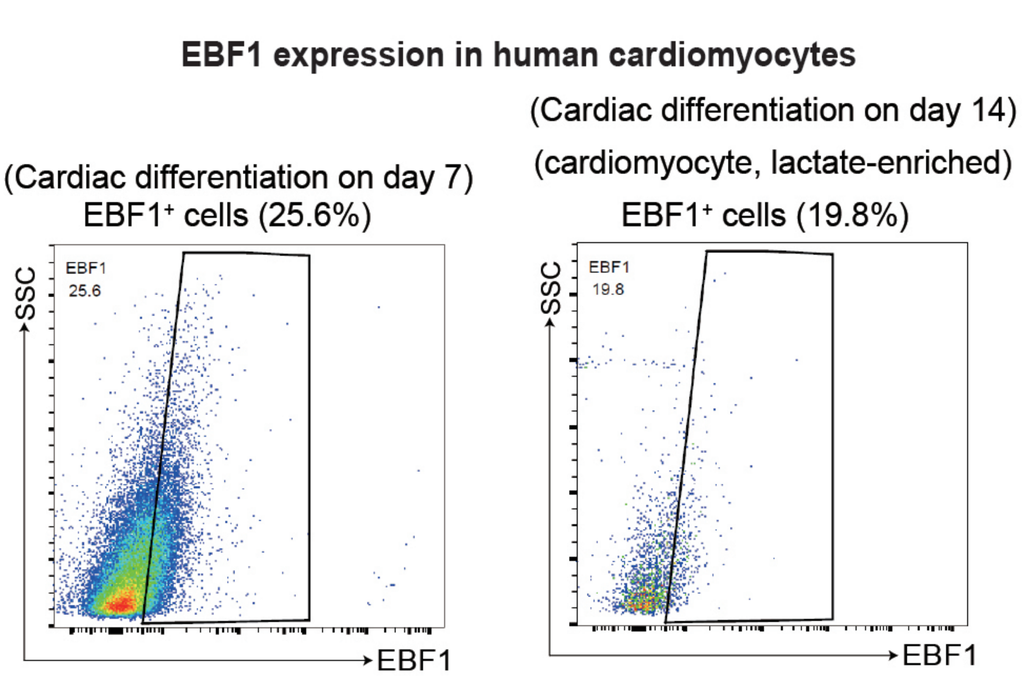


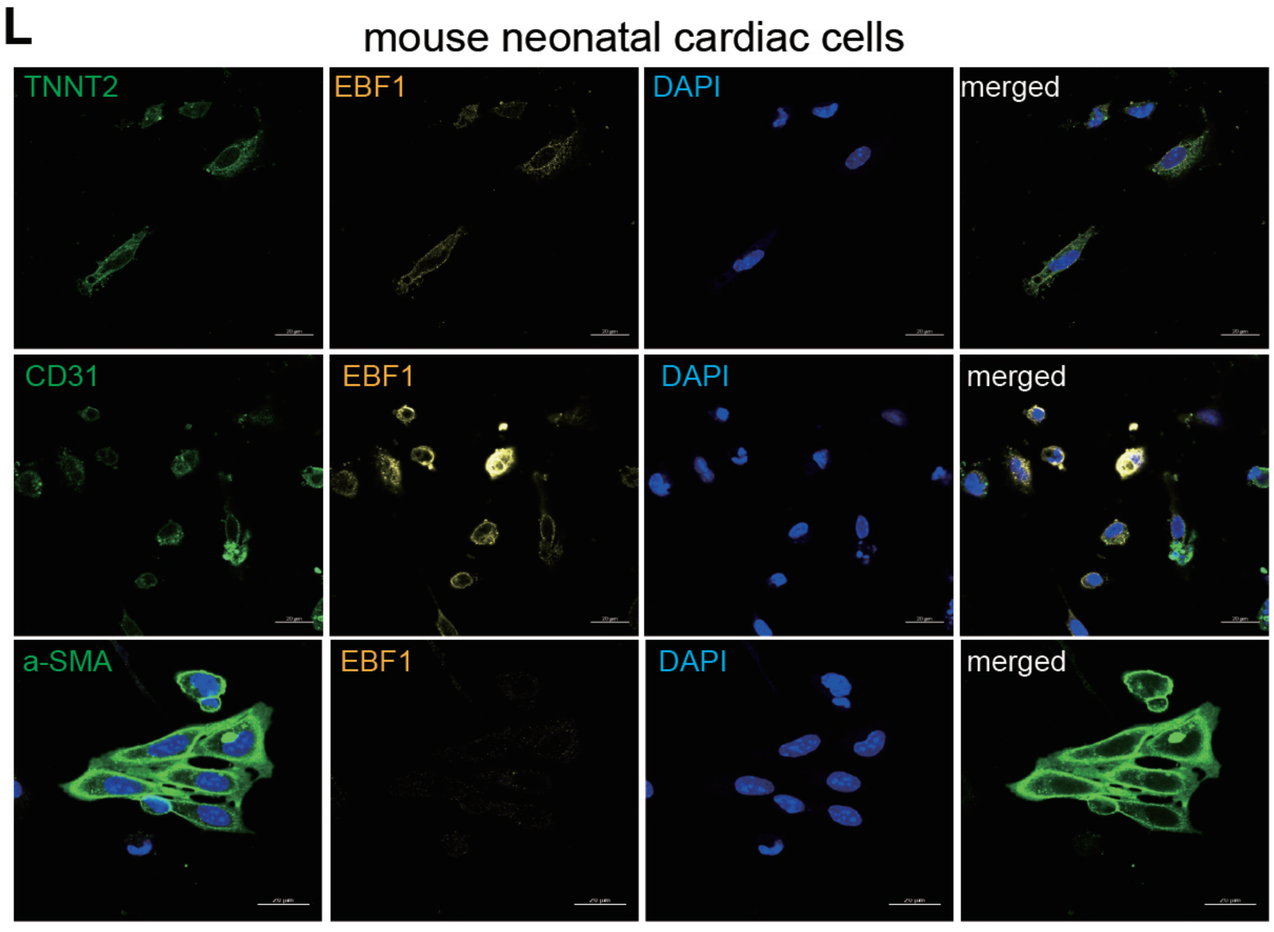




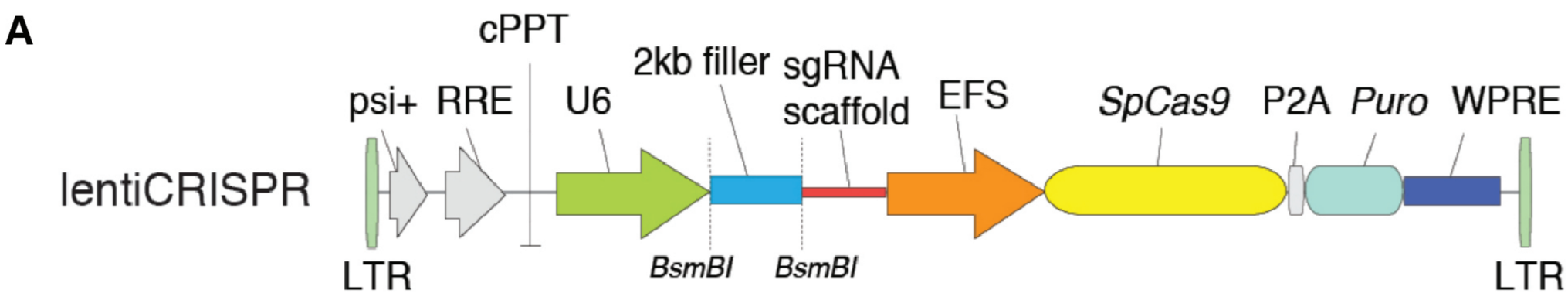




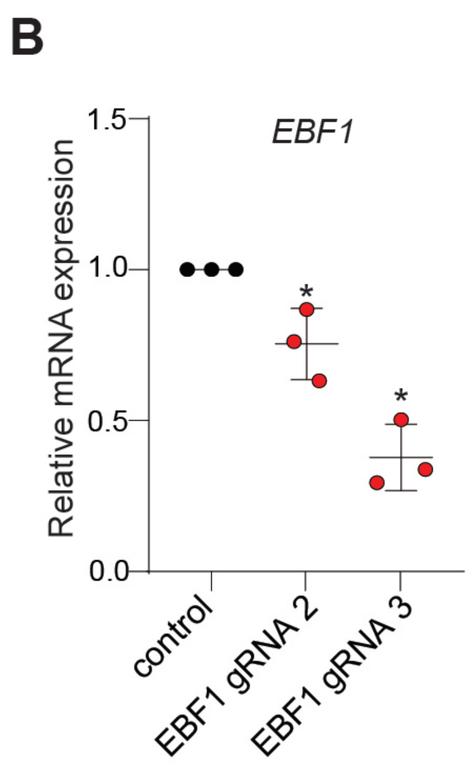


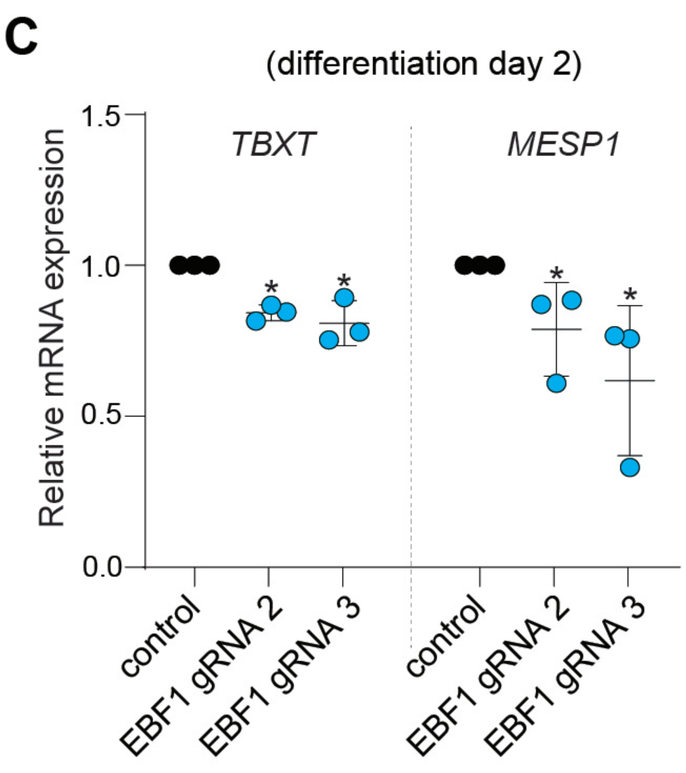


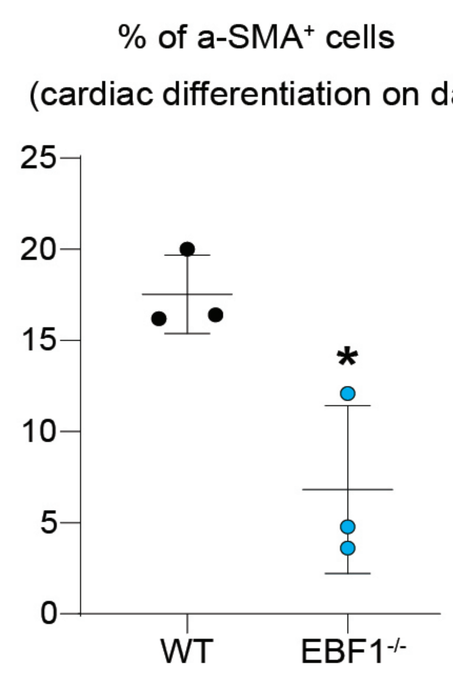
Supplemental Figure 2



lentiCRISPRv2 lenti-virus vector (expressing gRNA and spCas9 protein)

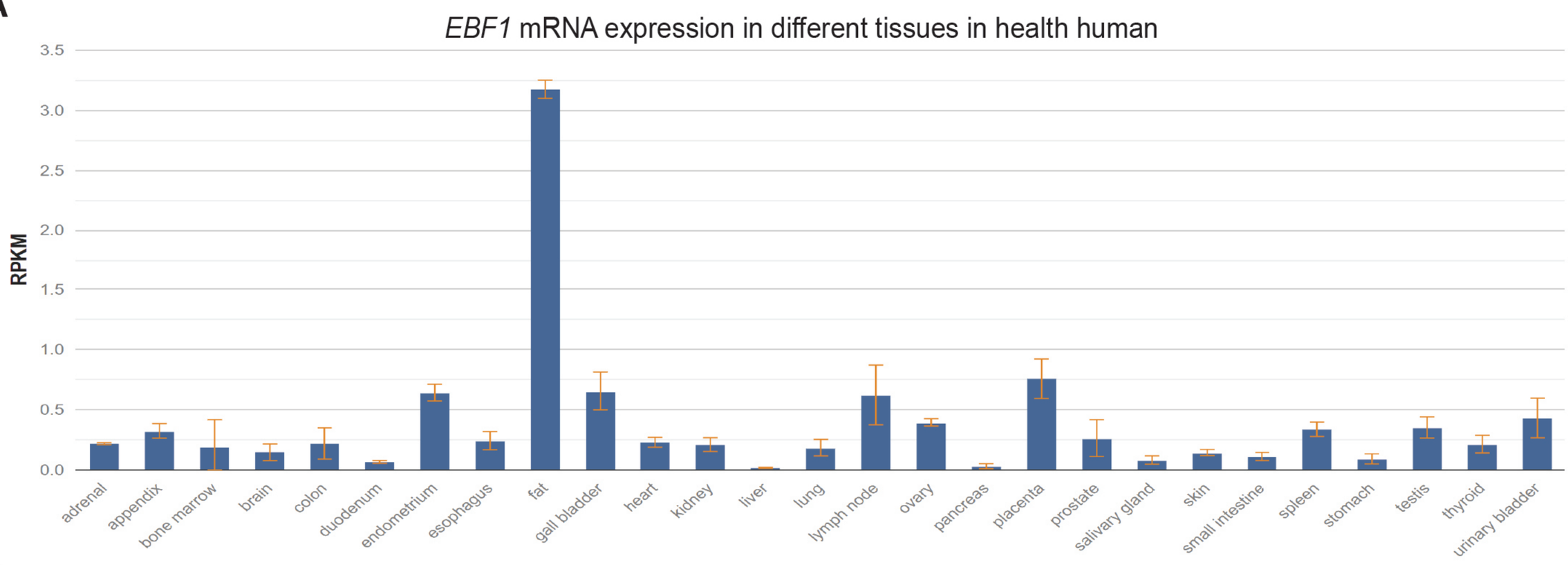




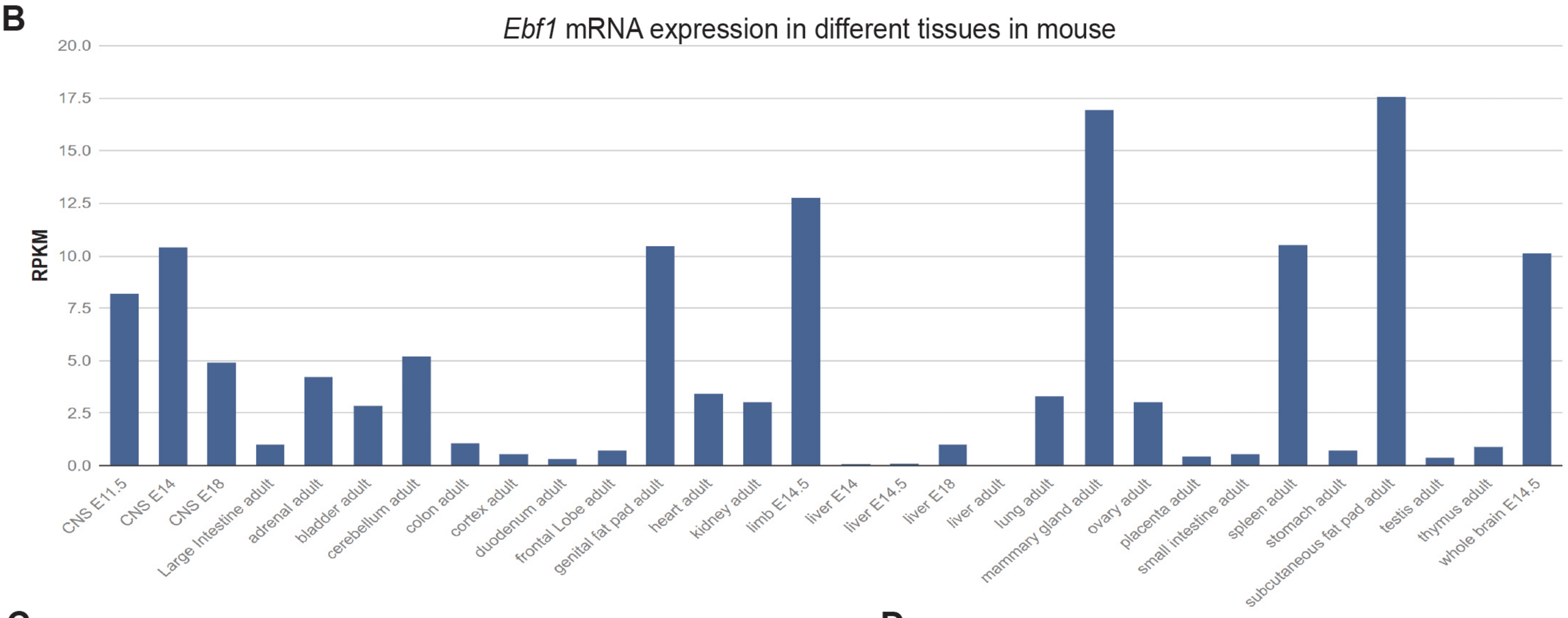


Supplemental Figure 3

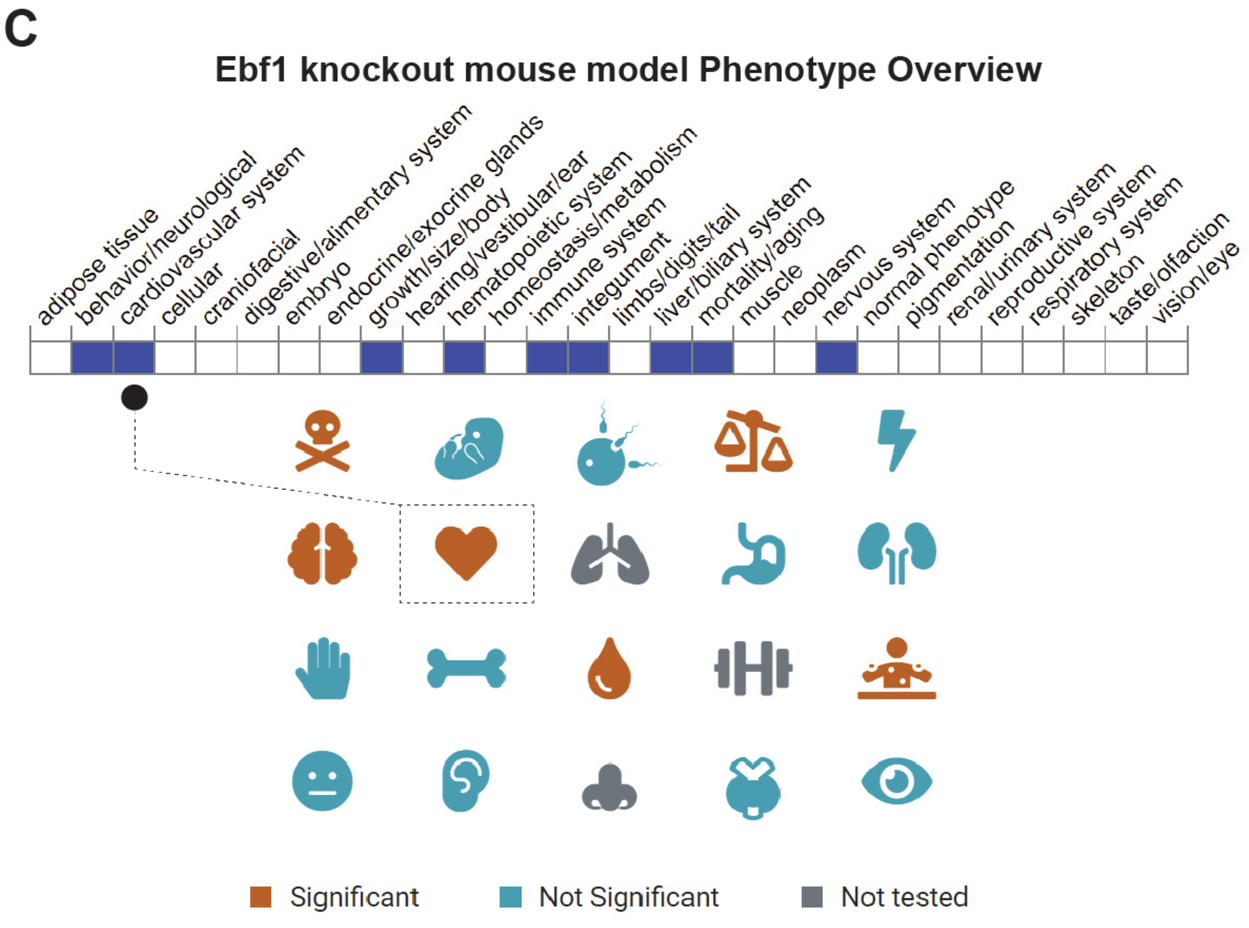
A



B



C

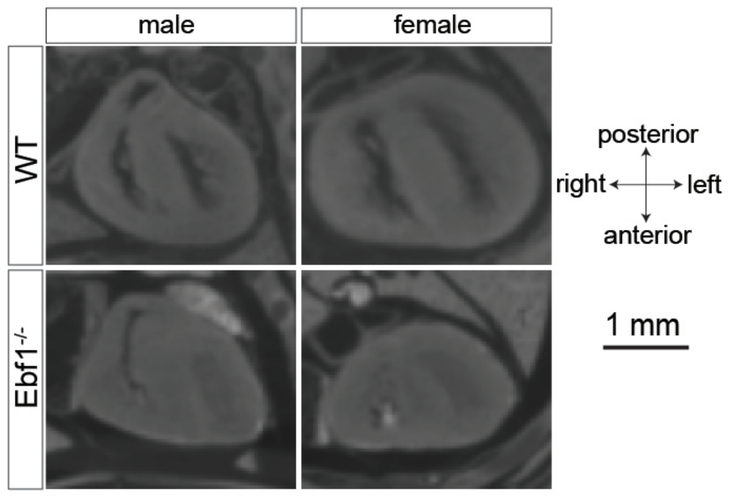


D

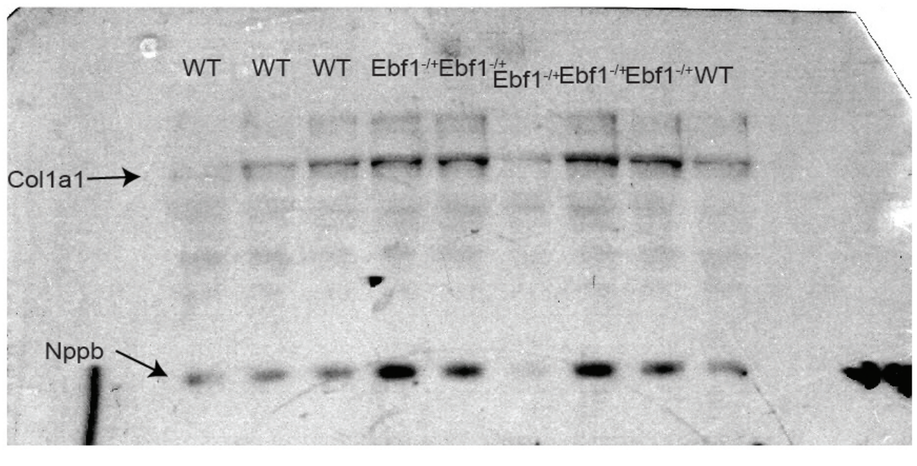
Cardiac phenotype overview of Ebf1 knockout mouse (vs. WT)

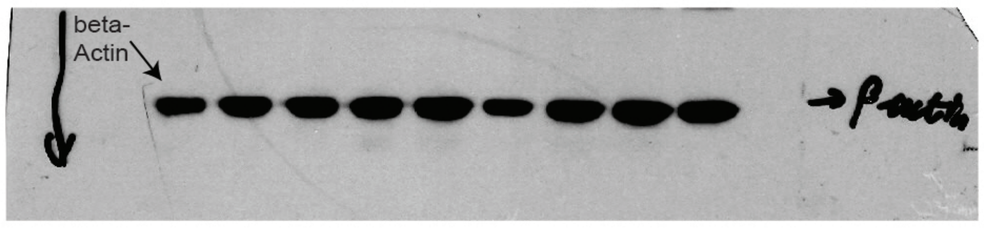
Genotype	Phenotype	Sex	Life Stage	P Value
-/-	preweaning lethality incomplete penetrance	male/female	early adult	0
+/-	abnormal heart morphology	male/female	late adult	0
+/-	small heart	female	late adult	0
+/-	enlarged heart	male	late adult	0

E18.5 embryonic hearts

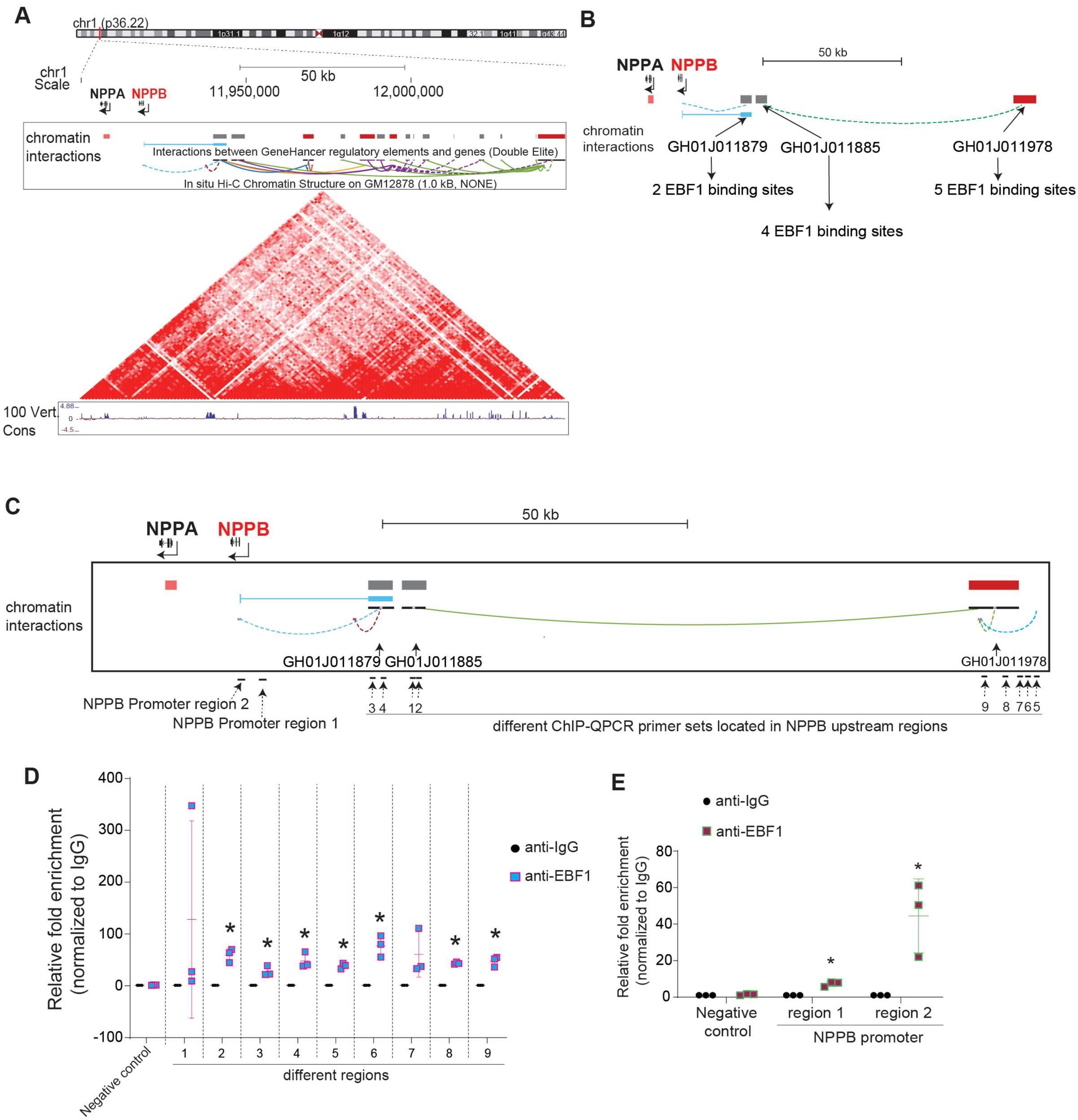


E



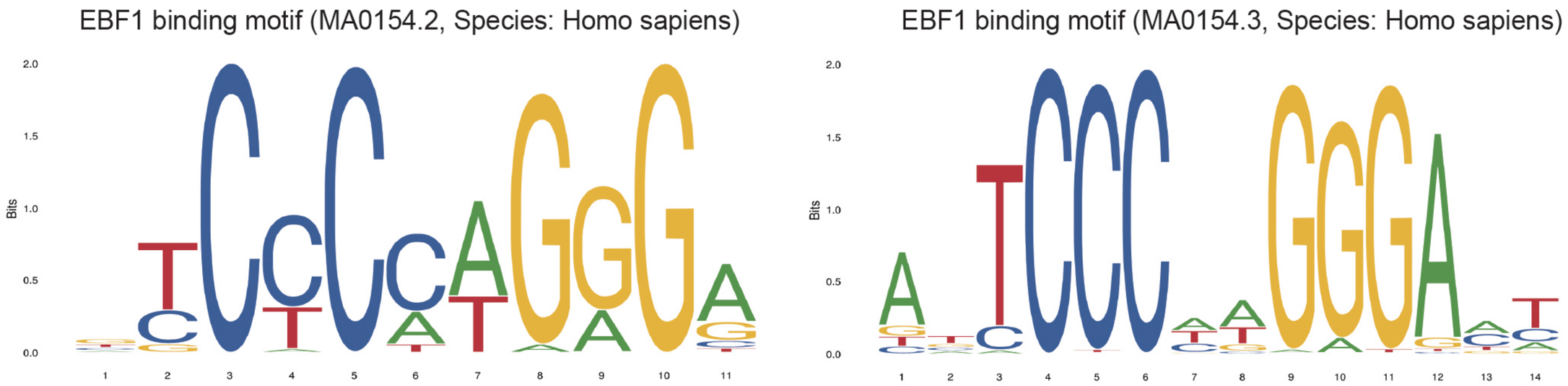


Supplemental Figure 4



Supplemental Figure 5

A



B **All EBF1 binding motifs Frequency matrix, Species: Homo sapiens**

>MA0154.2 EBF1

A [	4513	0	0	886	0	9191	21366	1085	8427	0	22124]
C [	7097	10267	33855	22776	33781	22317	0	0	0	0	2891]
G [	12581	2643	0	0	0	0	0	32770	25294	33855	7215]
T [	9664	20945	0	10193	74	2347	12489	0	134	0	1625]

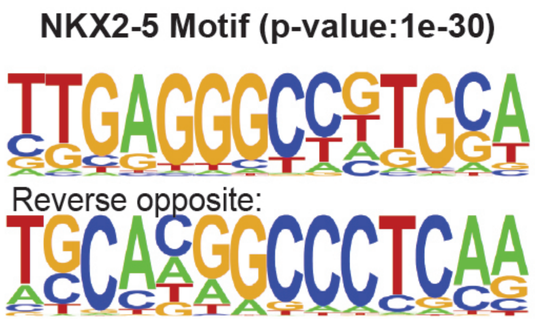
>MA0154.3 EBF1

A [	1110	257	37	0	3	0	562	777	21	107	6	1544	604	250]
C [	115	276	232	1544	1544	1544	391	77	6	8	0	17	586	364]
G [	166	334	0	3	4	0	63	208	1544	1544	1544	84	145	81]
T [	153	678	1544	1	18	6	529	482	0	0	22	24	209	849]

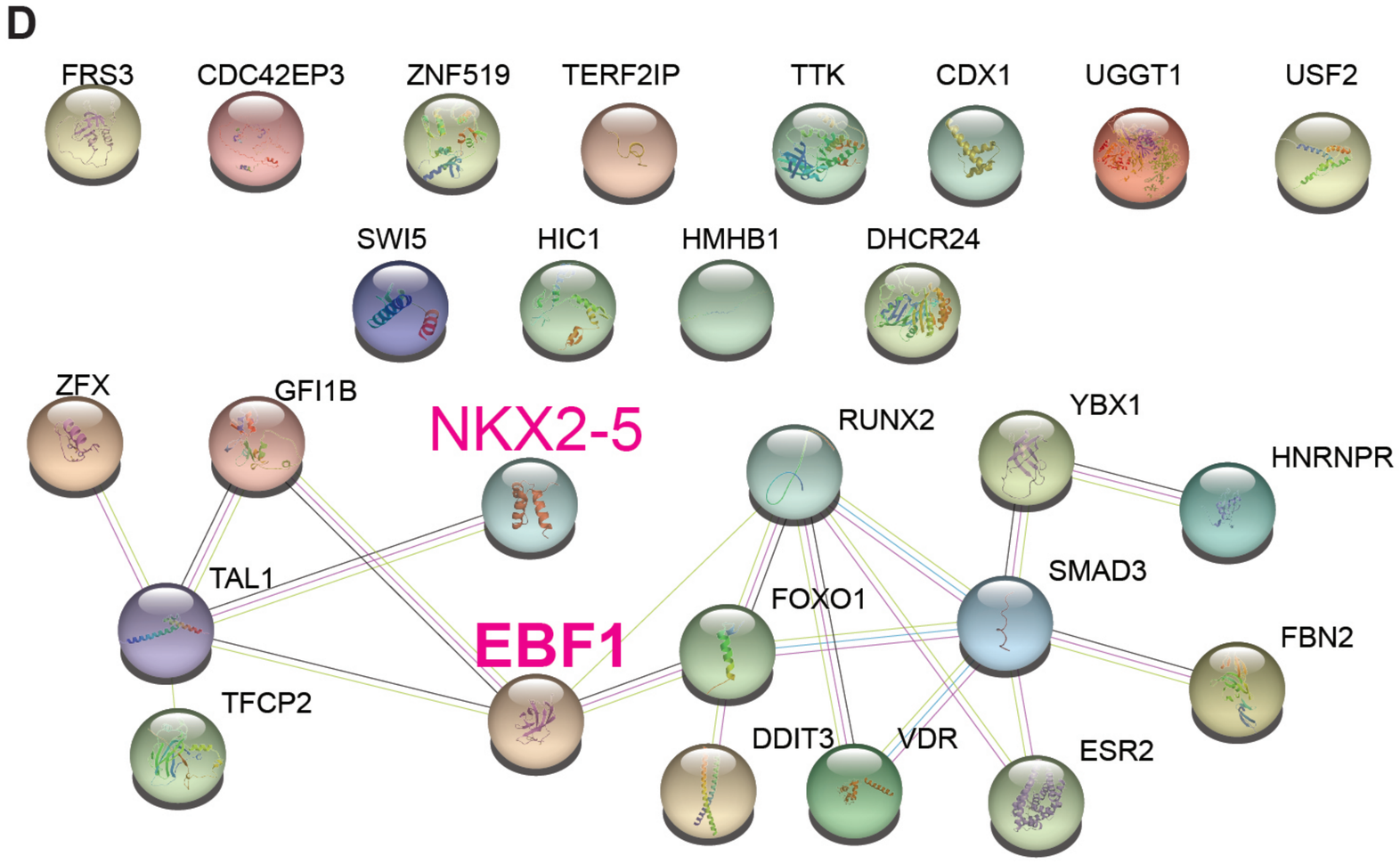
>MA0154.4 EBF1

A [	14160	11403	2352	1321	568	648	2539	34745	3502	2617	1515	8856	31636	11229	11903]
C [	8868	9554	4506	34665	43256	42562	39675	1707	814	495	455	2462	6355	13299	10836]
G [	10891	13481	5497	1920	371	443	862	1723	38728	41651	42789	32761	4957	9855	9173]
T [	11506	10987	33070	7519	1230	1772	2349	7250	2381	662	666	1346	2477	11042	13513]

C



D



Supplemental Figure 6