Supplemental Information

Dynamic Role of SETD7 as a Transcriptional Activator of Cardiac Lineage Commitment

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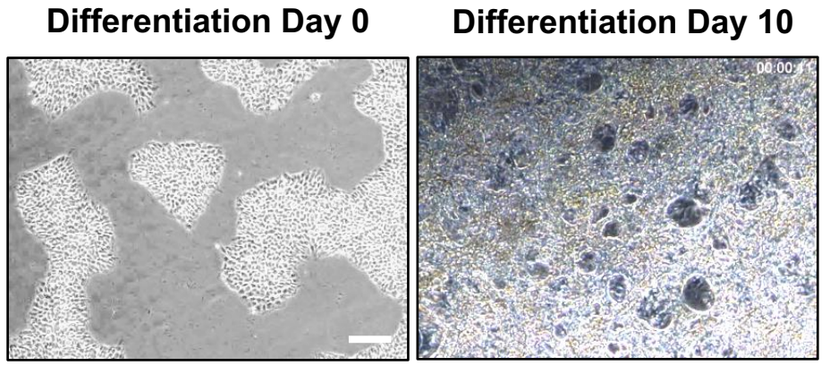
Figure S1

A



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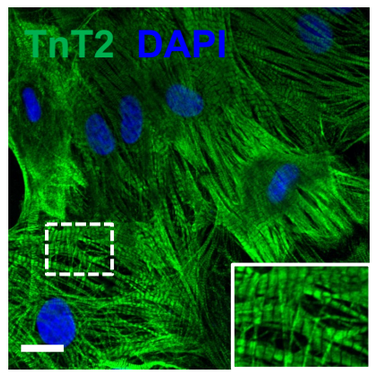

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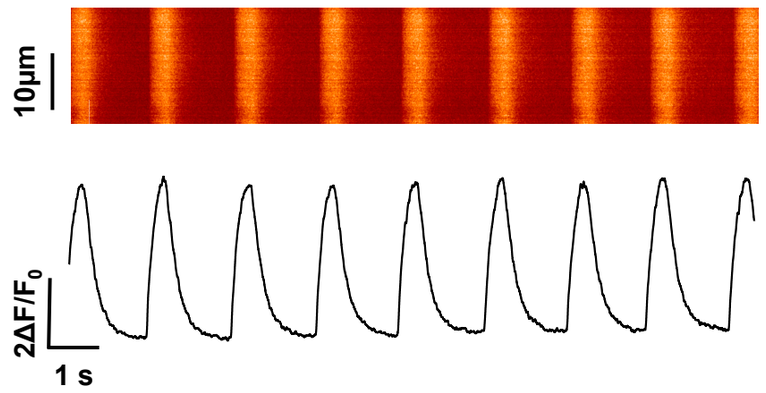

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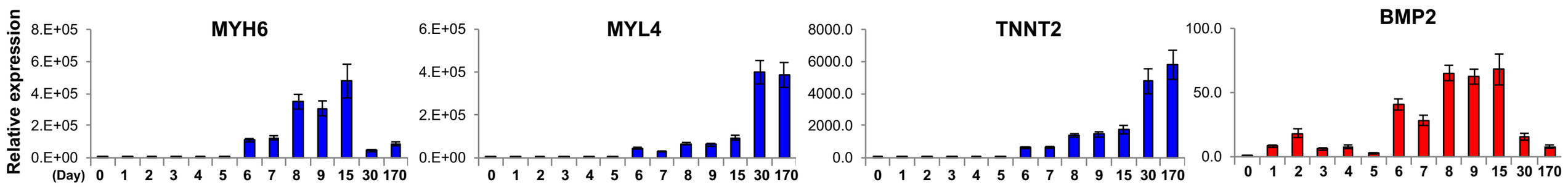

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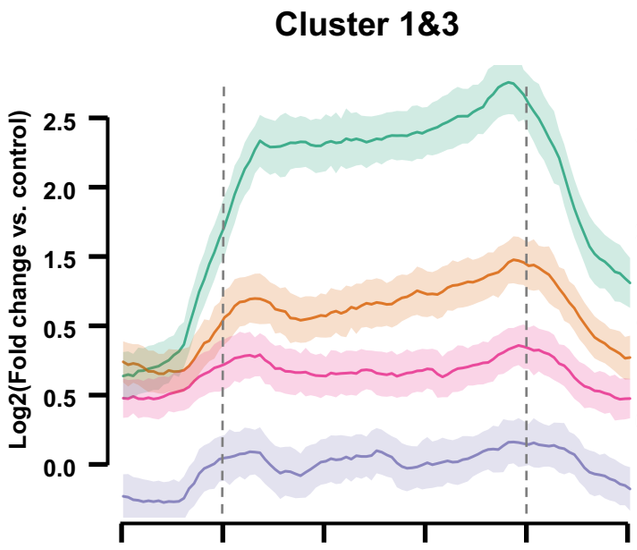

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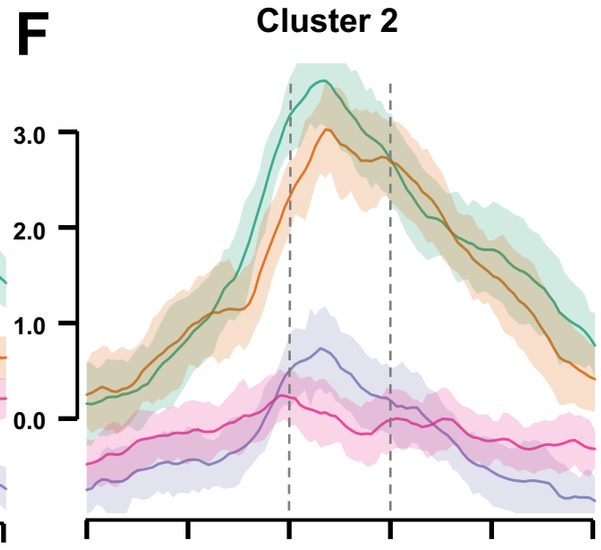

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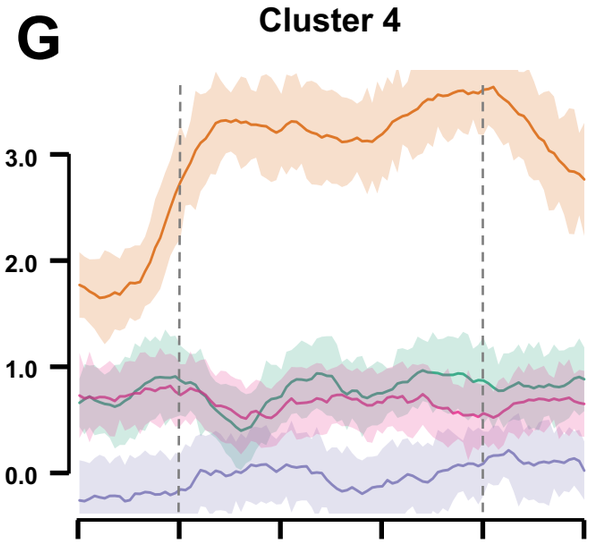

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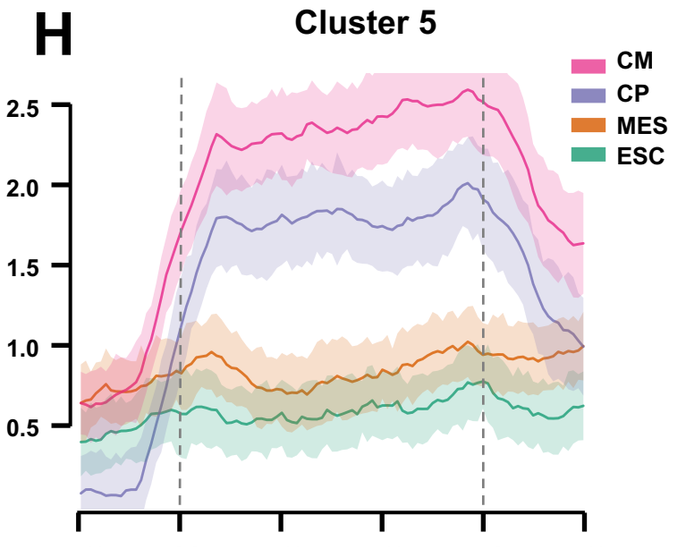

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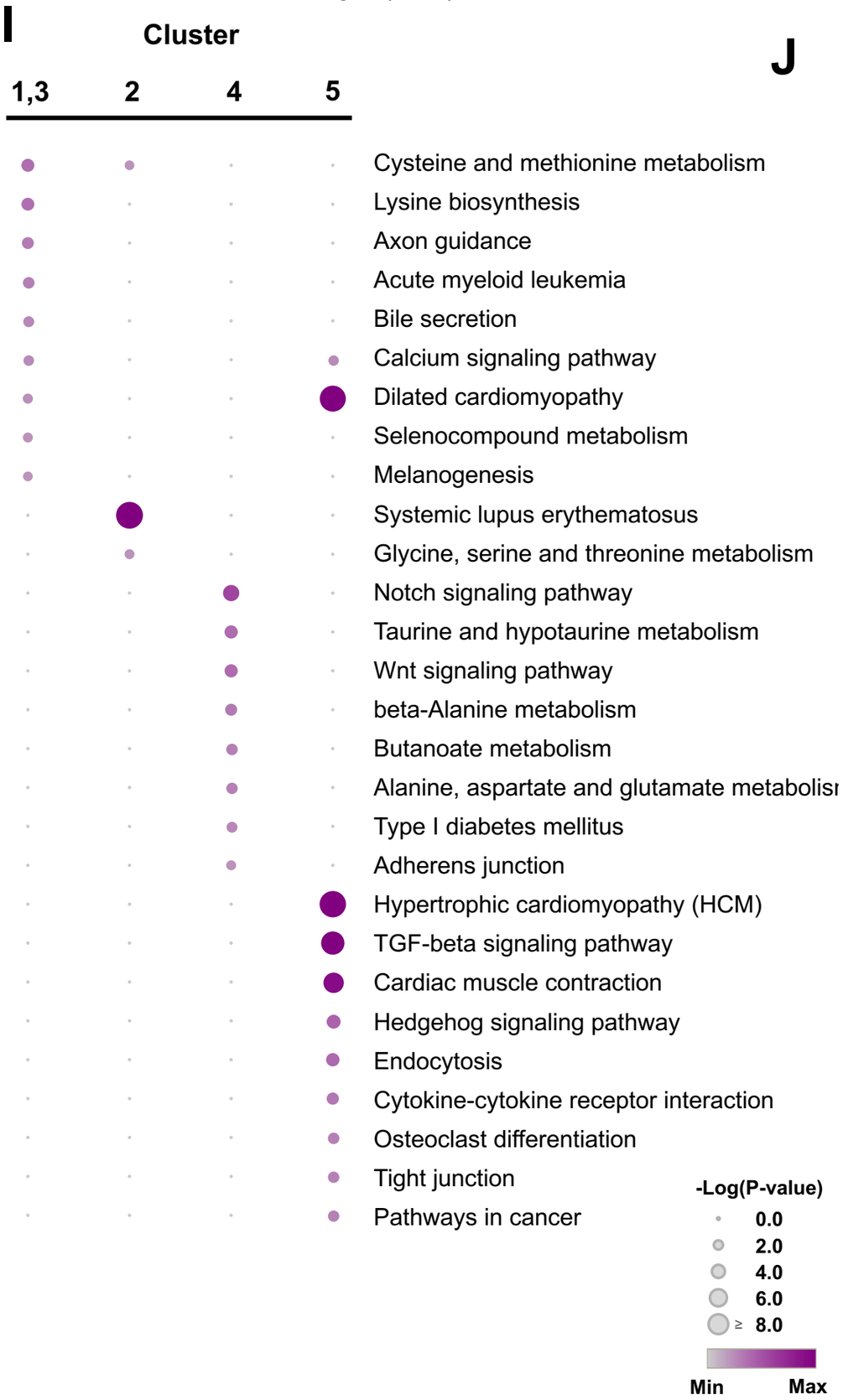


J

Figure S1 (Related to Figure 1). Dynamic enrichment pattern of SETD7 during CM differentiation.

(A) Representative microscopy images of H7 hESCs and H7 hESC-CMs at differentiation day 10. Scale bar, 100 uM

(B) Representative fluorescence microscopy images of H7 derived CMs. Green color represents cardiac Troponin T and blue color represents DAPI. Scale bar, 10 uM.

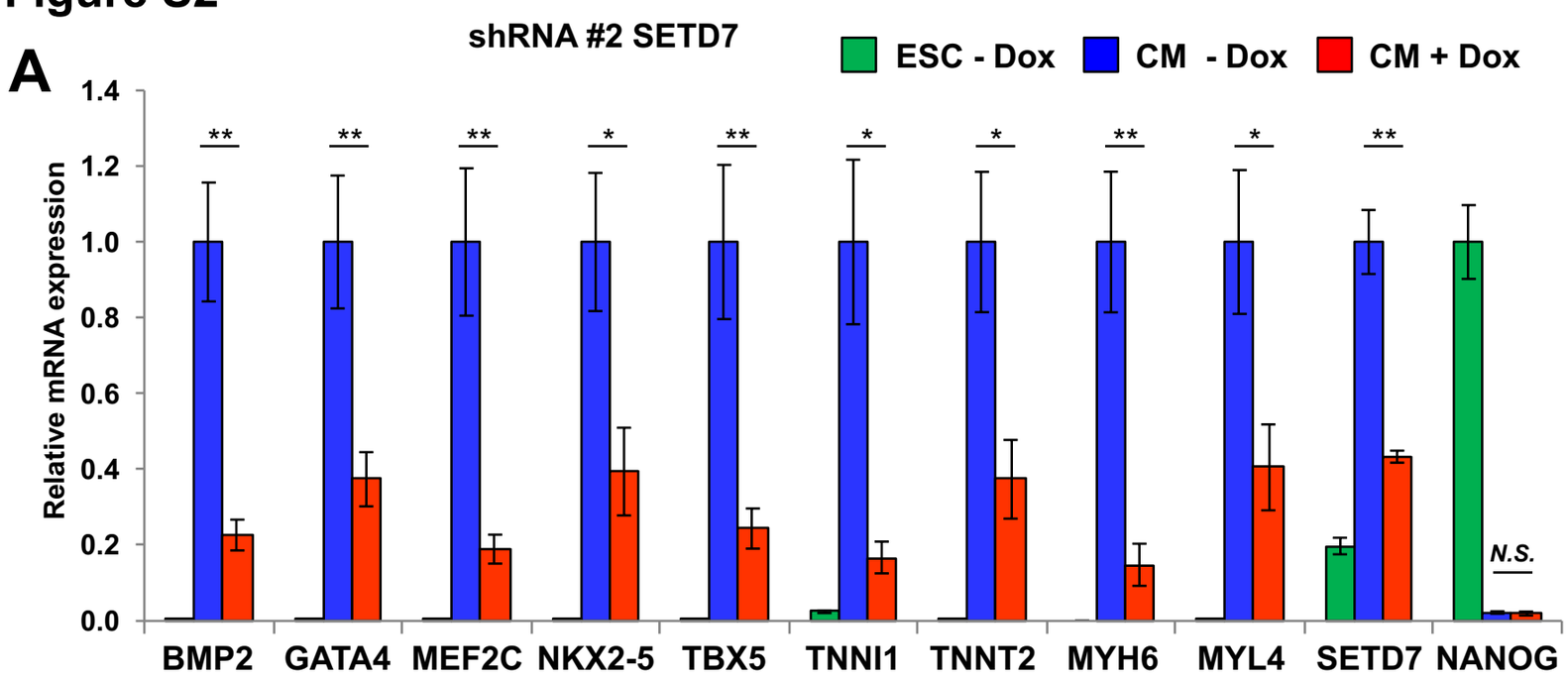
(C) Representative line-scan images and spontaneous calcium signaling of H7 hESC-CMs showed regular calcium transients.

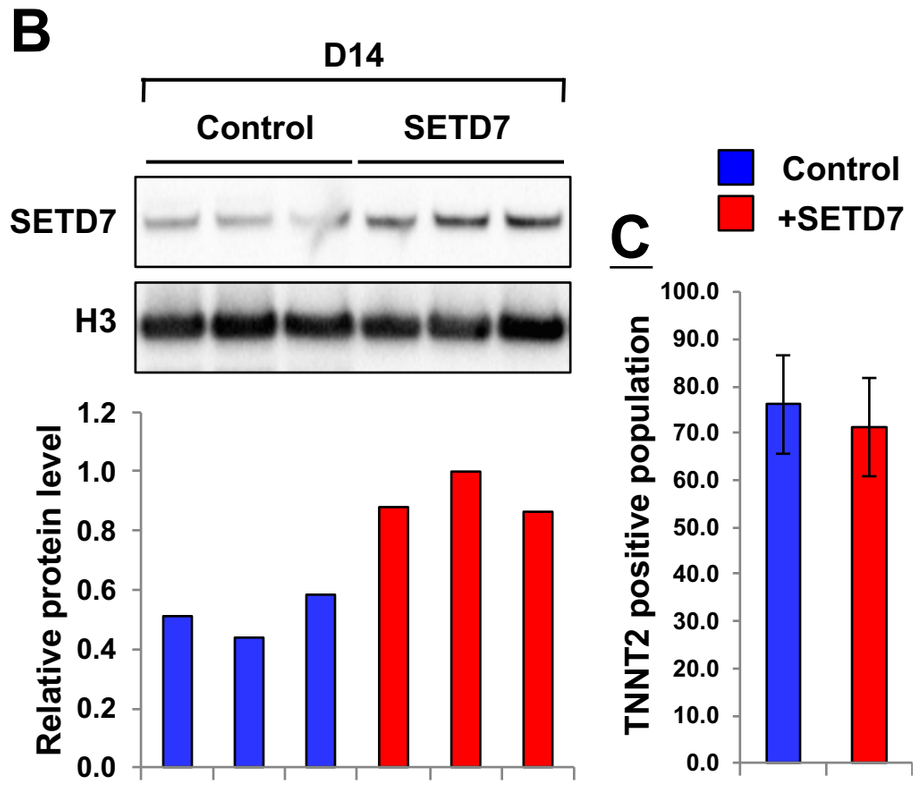
(D) The mRNA expression of MYH6, MYL4, TNNT2, TNNT1, BMP2, GATA4, MEF2C, TBX5, and NKX2-5 during CM differentiation was measured by RT-qPCR. Blue bar represents cardiac structure genes and red bar is cardiac transcription factors.

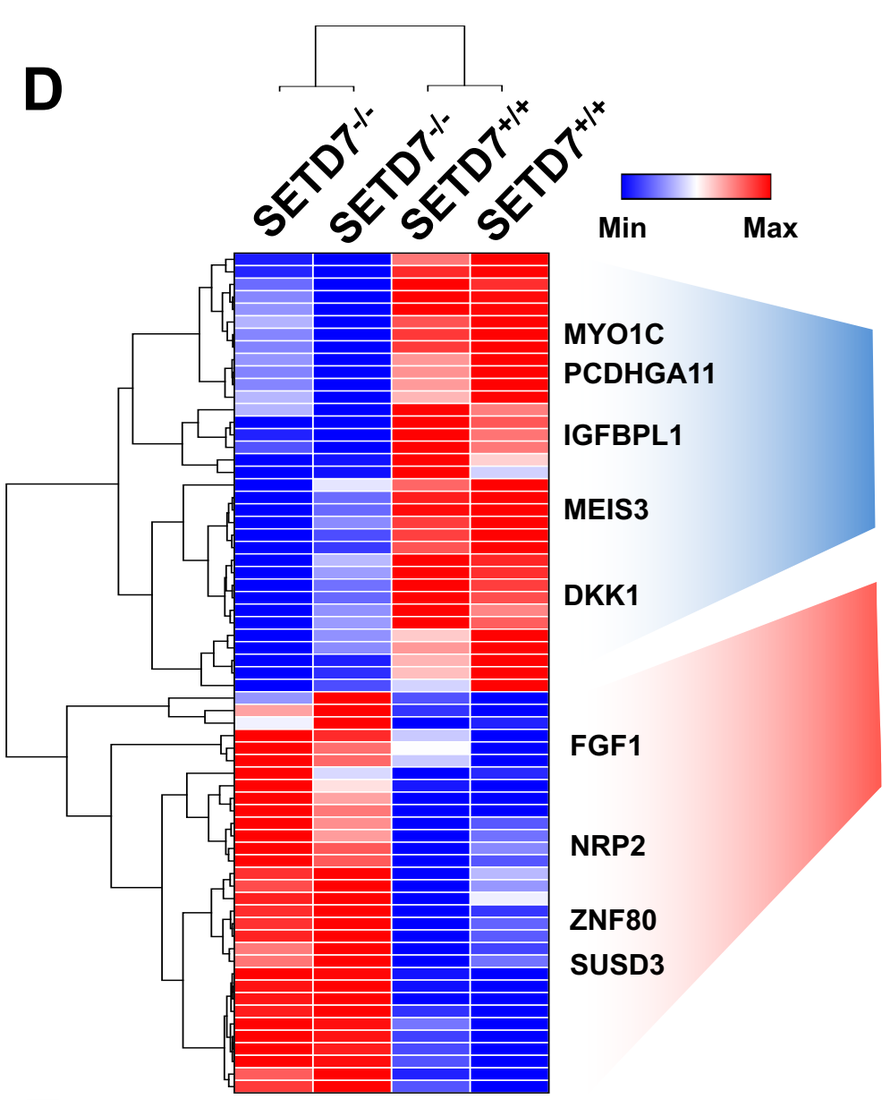
(E-H) The average profiles of SETD7 enrichment in gene body regions of target genes from each cluster.

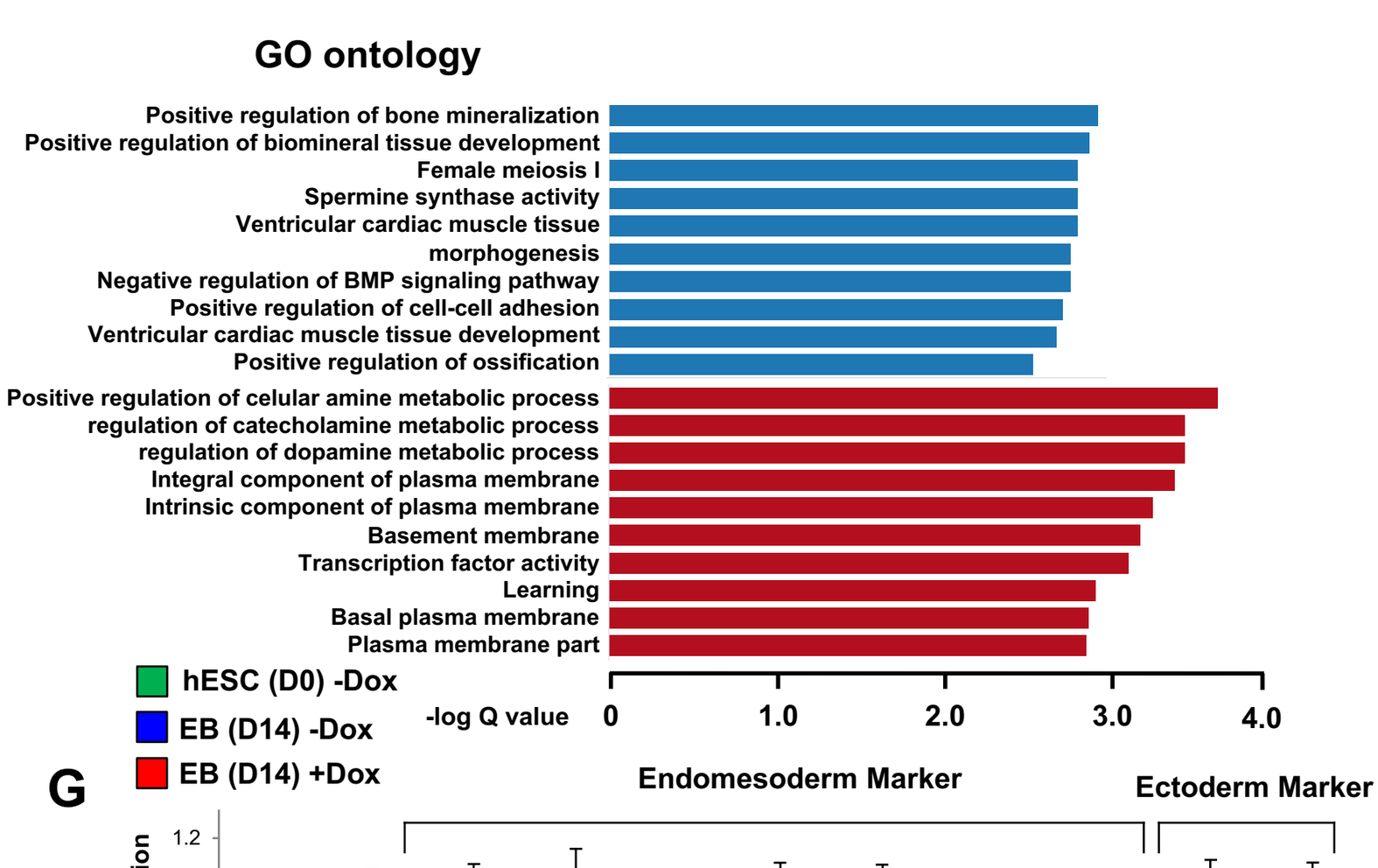
(I-J) KEGG and Reactome pathway analysis of each cluster. Color code indicates minus log transformed multiple testing adjusted P value of category enrichment.

Figure S2


A
shRNA #2 SETD7
ESC - Dox
CM - Dox
CM + Dox
Relative mRNA expression
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
**
**
**
*
**
*
*
**
*
**
N.S.
BMP2
GATA4
MEF2C
NKX2-5
TBX5
TNNI1
TNNT2
MYH6
MYL4
SETD7
NANOG


B
D14
Control
SETD7
SETD7
H3
Relative protein level
1.2
1.0
0.8
0.6
0.4
0.2
0.0
C
Control
+SETD7
TNNT2 positive population
100.0
90.0
80.0
70.0
60.0
50.0
40.0
30.0
20.0
10.0
0.0


D
SETD7-/-
SETD7-/-
SETD7+/+
SETD7+/+
Min
Max
MYO1C
PCDHGA11
IGFBPL1
MEIS3
DKK1
FGF1
NRP2
ZNF80
SUSD3

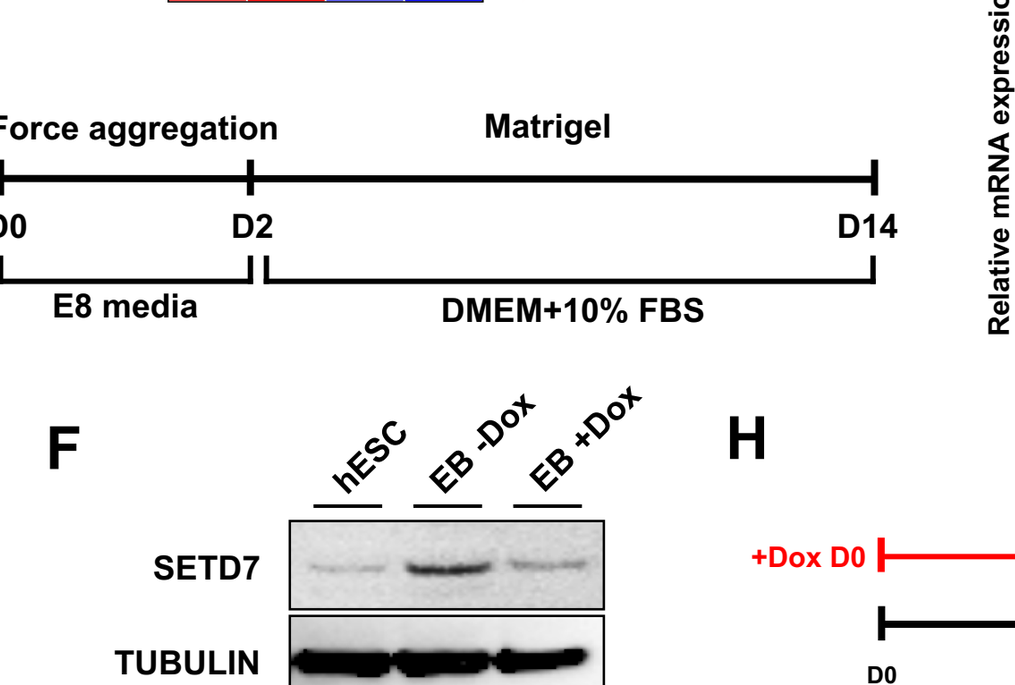

GO ontology
Positive regulation of bone mineralization
Positive regulation of biomineral tissue development
Female meiosis I
Spermine synthase activity
Ventricular cardiac muscle tissue
morphogenesis
Negative regulation of BMP signaling pathway
Positive regulation of cell-cell adhesion
Ventricular cardiac muscle tissue development
Positive regulation of ossification
Positive regulation of celular amine metabolic process
regulation of catecholamine metabolic process
regulation of dopamine metabolic process
Integral component of plasma membrane
Intrinsic component of plasma membrane
Basement membrane
Transcription factor activity
Learning
Basal plasma membrane
Plasma membrane part
hESC (D0) -Dox
EB (D14) -Dox
EB (D14) +Dox
-log Q value
0
1.0
2.0
3.0
4.0

E
Force aggregation
Matrigel
D0
D2
D14
E8 media
DMEM+10% FBS

G
Endomesoderm Marker
Ectoderm Marker
Relative mRNA expression
1.2
1.0
0.8
0.6
0.4
0.2
0.0
SETD7
GATA2
TAL1
SOX17
SOX9
T
LEF1
FOX2A
MIXL1
PAX6
SOX1

F
hESC
EB -Dox
EB +Dox
SETD7
TUBULIN

H
+Dox D5
+Dox D3
+Dox D0
D0
D2
D3
D5
D7
D10
D14
+CHIR
+IWR
B27-Insulin
B27+Insulin


I
Ecto
Endo
Meso
hESC-CM
Brain
Pancreas
Liver
Stomach
Kidney
Heart
Muscle
D0
D12
Fetus #1
SETD7
GAPDH
H3K36me3
H3

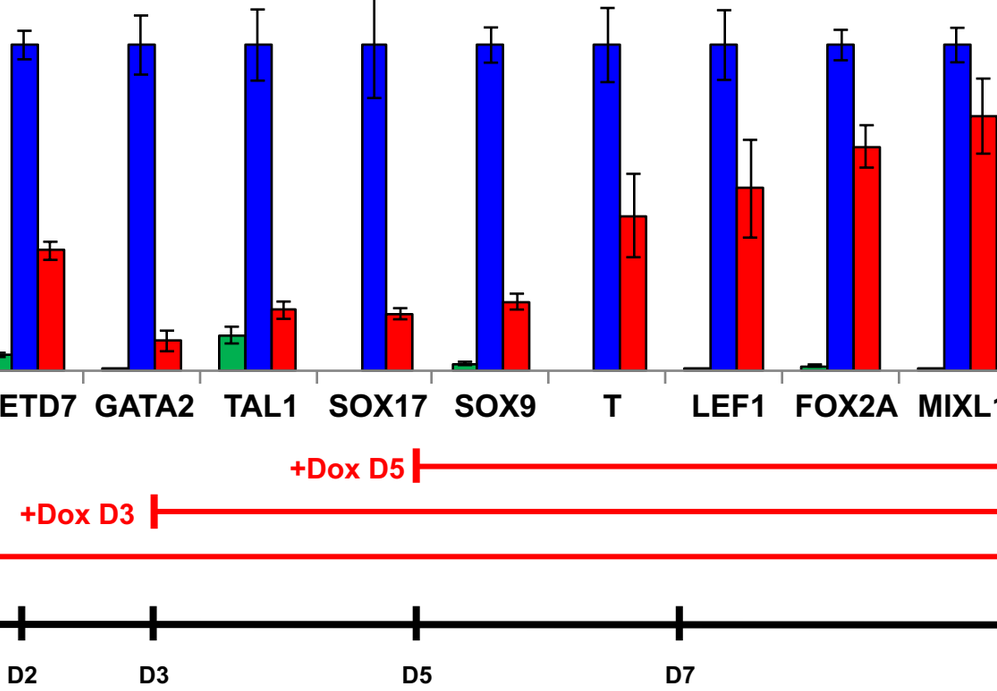

J
Ecto
Endo
Meso
hESC-CM
Brain
Pancreas
Liver
Stomach
Kidney
Heart
Muscle
D0
D12
Fetus #2
SETD7
GAPDH
H3K36me3
H3

Figure S2 (Related to Figure 2 and Figure 3). SETD7 is required for early cardiac lineage commitment.

(A) Real-time PCR analysis of cardiac genes and NANOG expression levels in in hESCs (H7) and hESC-CMs expressing doxycycline-inducible SETD7 shRNA or Scramble shRNA. Doxycycline was treated at differentiation day 0. The total RNA was extracted at differentiation day 10. One way ANOVA method and expressed as *P < 0.05; **P < 0.01; ***P < 0.005; (mean ± SEM; n=4).

(B) Immunoblot analysis of SETD7 protein levels in control and SETD7-overexpressed stable lines at day 14 of CM differentiation. The stable cell lines were generated using a lentiviral overexpression with puromycin selection step. The total lysates were extracted from 3 different experiments.

(C) Flow cytometry analysis of TNNT2 positive cells of control and SETD7-overexpressed stable lines at day 14 of CM differentiation (mean ± SEM; n=5).

(D) Heat-map of differentially expressed genes in mesoderm lineage cells derived from $SETD7^{+/+}$ or $SETD7^{-/-}$ (n=2). GO enrichment analysis of the differentially expressed genes. Color bar indicates multiple testing adjusted q value.

(E) Time line of EB formation and random differentiation.

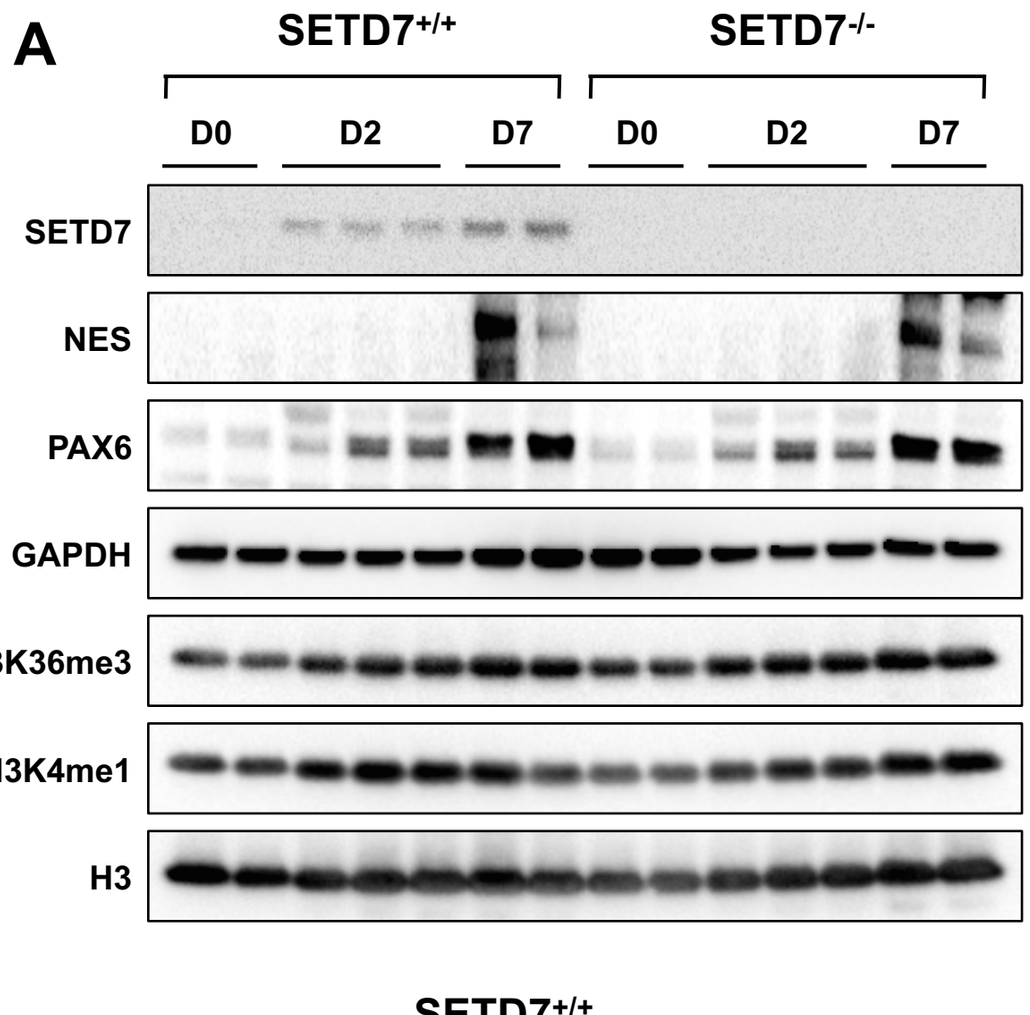
(F) Immunoblot analysis of cell lysate from hESCs or EBs (day 14) treated with or without doxycycline. Alpha tubulin was used as a loading control.

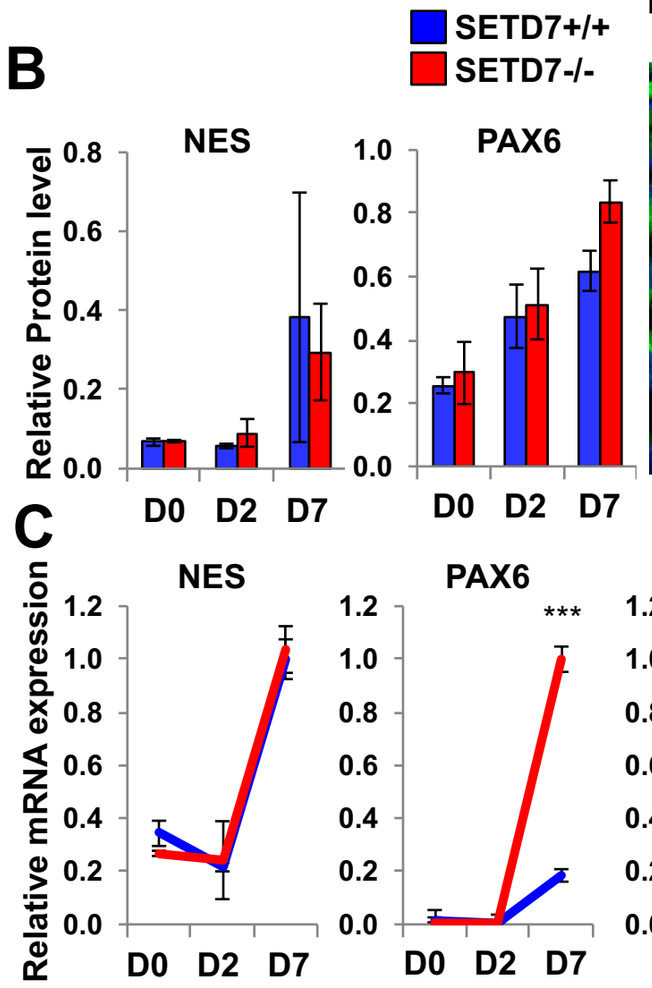
(G) mRNA level of endomesoderm or ectoderm maker genes in hESCs or EBs (day 14) treated with or without doxycycline.

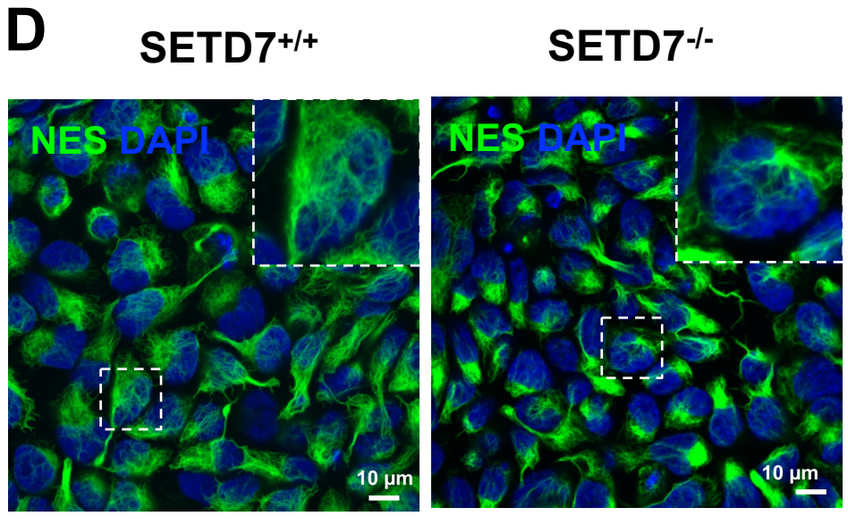
(H) Timeline of DOX treatment during CM differentiation.

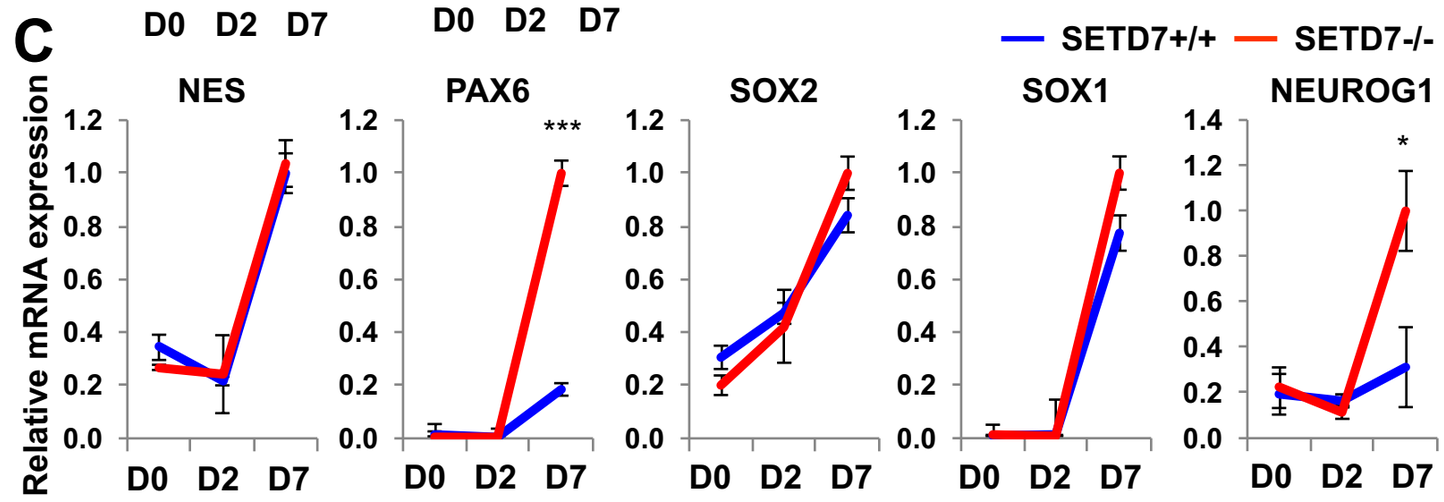
(I-J) Immunoblot analysis of SETD7, GAPDH, H3K36me3, and H3 levels in hESCs, hESC-CMs, and different tissues of human fetus. (Fetus-1: 16.4 week male; Fetus-2: 17.0 week female).

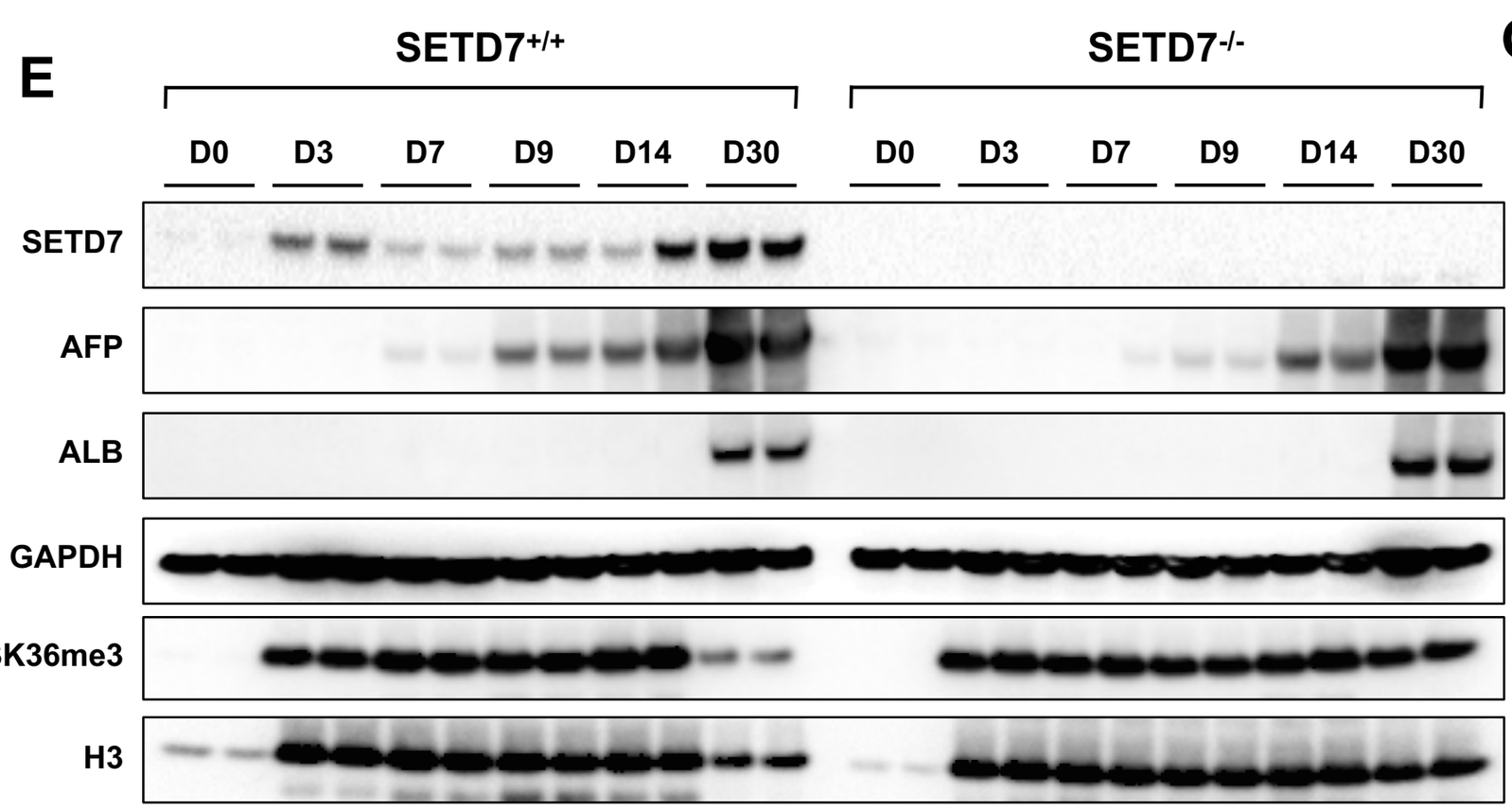
Figure S3

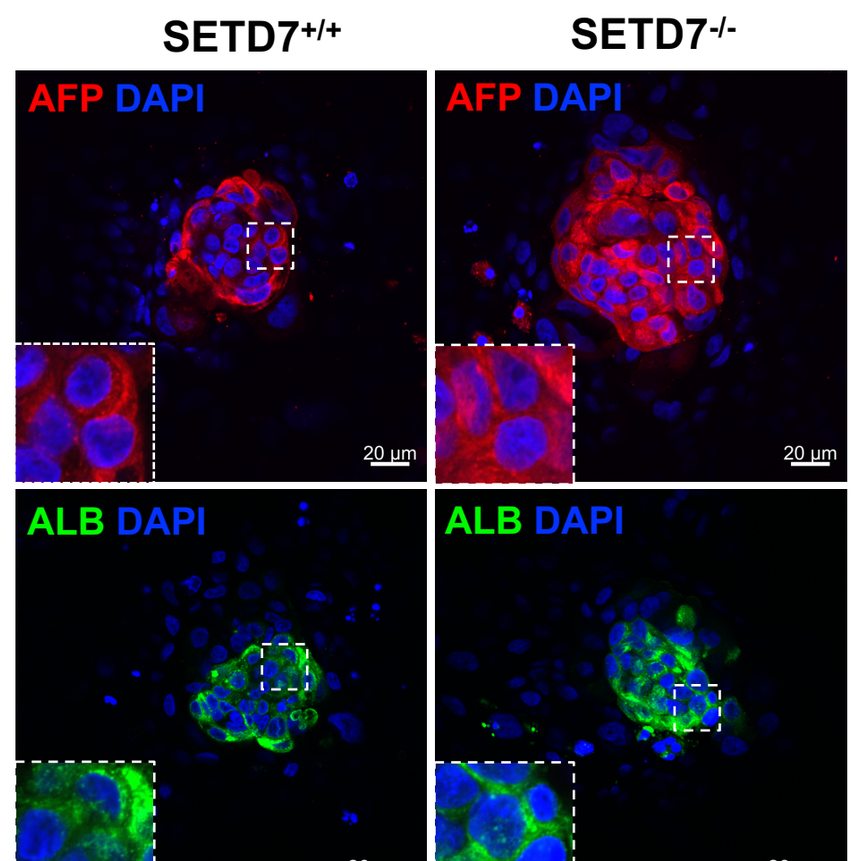

A
SETD7+/+
SETD7-/-
D0
D2
D7
D0
D2
D7
SETD7
NES
PAX6
GAPDH
H3K36me3
H3K4me1
H3

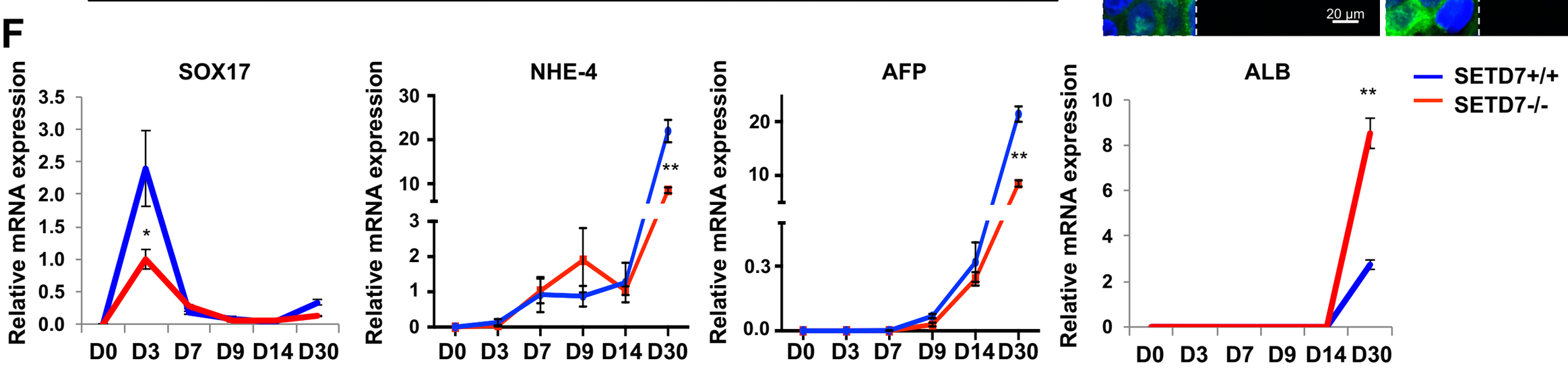

B
SETD7+/+
SETD7-/-
NES
PAX6
Relative Protein level
0.0
0.2
0.4
0.6
0.8
1.0
D0
D2
D7

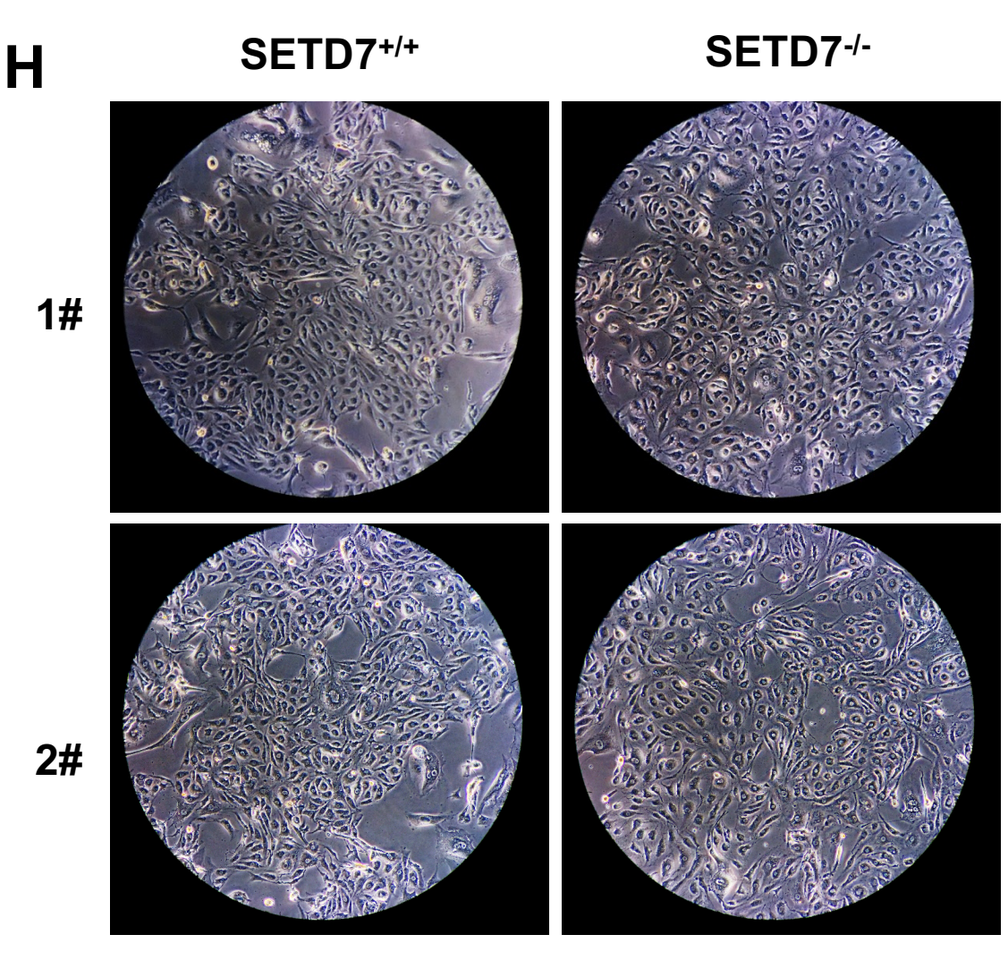

D
SETD7+/+
SETD7-/-
NES DAPI
NES DAPI
10 μm
10 μm


C
SETD7+/+
SETD7-/-
NES
PAX6
SOX2
SOX1
NEUROG1
Relative mRNA expression
***
*
D0
D2
D7


E
SETD7+/+
SETD7-/-
D0
D3
D7
D9
D14
D30
SETD7
AFP
ALB
GAPDH
H3K36me3
H3


G
SETD7+/+
SETD7-/-
AFP DAPI
AFP DAPI
ALB DAPI
ALB DAPI
20 μm
20 μm
20 μm
20 μm


F
SOX17
NHE-4
AFP
ALB
Relative mRNA expression
SETD7+/+
SETD7-/-
*
**
**
**
D0
D3
D7
D9
D14
D30


H
SETD7+/+
SETD7-/-
1#
2#

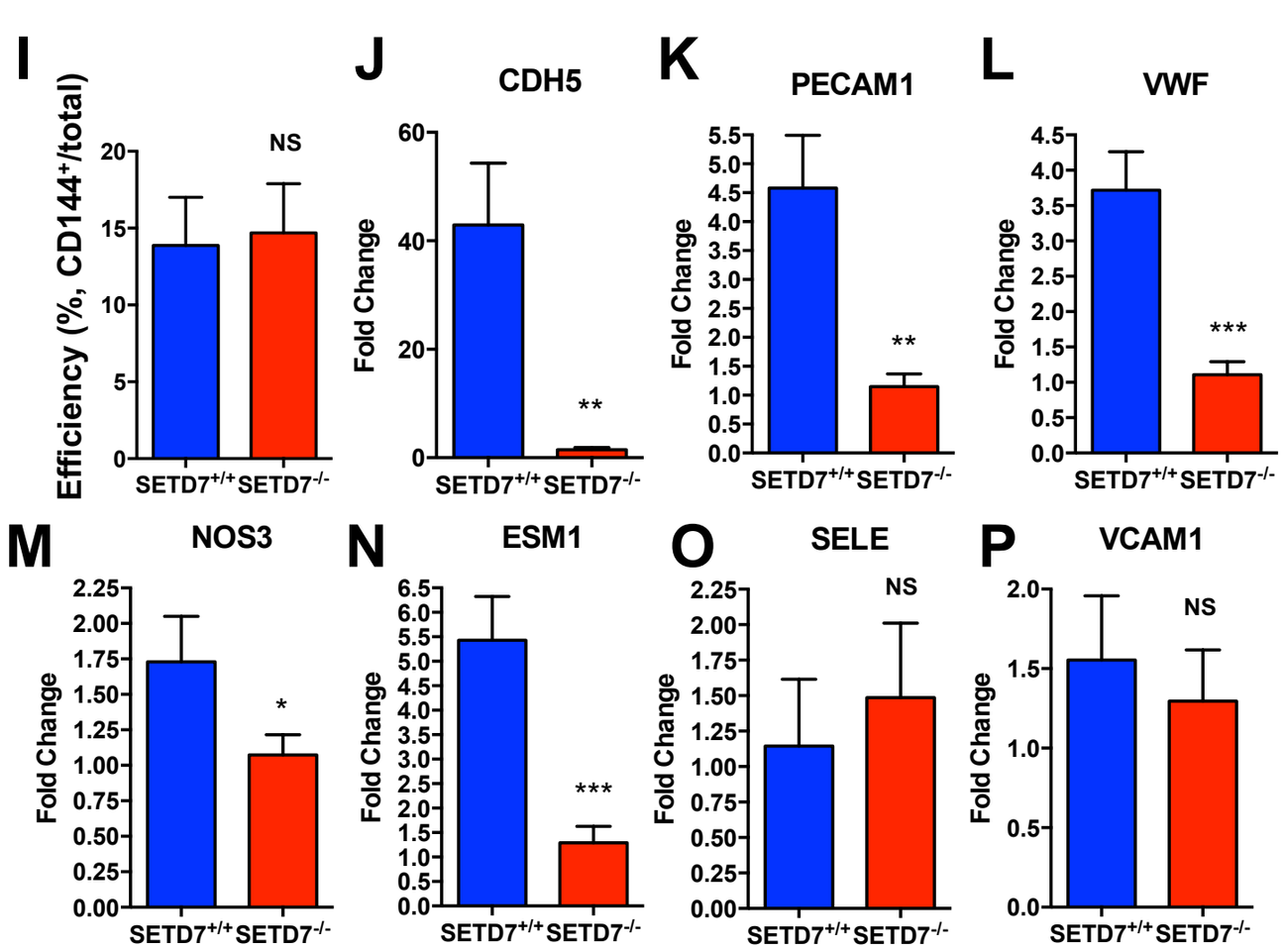

I
Efficiency (%, CD144+/total)
NS
J
CDH5
Fold Change
**
K
PECAM1
**
L
VWF
***
M
NOS3
*
N
ESM1
***
O
SELE
NS
P
VCAM1
NS
SETD7+/+
SETD7-/-

Figure S3 (Related to Figure 3). Effects of SETD7 on lineage-specific gene expression of non-cardiomyocyte cell types.

(A) Immunoblot analysis of SETD7, NES, PAX6, GAPDH, H3K36me3, H3Kme1, and H3 level at different time points of NPC differentiation. The total lysates were extracted from 2~3 different experiments. NPC differentiation was performed according to the manufacturer's protocol.

(B) Relative signal intensities of NES and PAX protein level in Figure A.

(C) Real-time PCR analysis of NES, PAX6, SOX2, SOX1, and NEUROG1 expression during NPC differentiation. Statistical significance was analyzed using the one way ANOVA. $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.005$; (mean ± SEM; n=3).

(D) Immunostaining of NES (green) and DAPI (blue) in $SETD7^{+/+}$ and $SETD7^{-/-}$-derived NPCs.

(E) Immunoblot analysis of SETD7, AFP, ALB, GAPDH, H3K36me3, and H3 level at different time points of hepatocyte differentiation. The total lysates were extracted from 2 different experiments. Hepatocyte differentiation was performed according to previously published protocol (Song et al., 2009).

(F) Real-time PCR analysis of SOX17, NHE-4, AFP, and ALB expression during hepatocyte differentiation. Statistical significance was analyzed using the one way ANOVA. $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.005$; (mean ± SEM; n=3).

(G) Immunostaining of AFP (red) and ALB (green) in $SETD7^{+/+}$ and $SETD7^{-/-}$-derived hepatocyte-like cells.

(H) Representative images of bona fide ECs derived from $SETD7^{+/+}$ and $SETD7^{-/-}$ lines. EC differentiation was performed according to previous papers (Gu et al., 2017; Hu et al., 2016).

(I) Cell count before (total) and after ($CD144^{+}$) magnetic-activated cell sorting (MACS).

(J-O) Real-time PCR analysis of CDH5, PECAM1, VWF, NOS3, ESM1, SELE, and VCAM1 of $SETD7^{+/+}$ and $SETD7^{-/-}$-derived ECs after MACS. Statistical significance was analyzed using the one way ANOVA. $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.005$; (mean ± SEM; n=3).

Figure S4

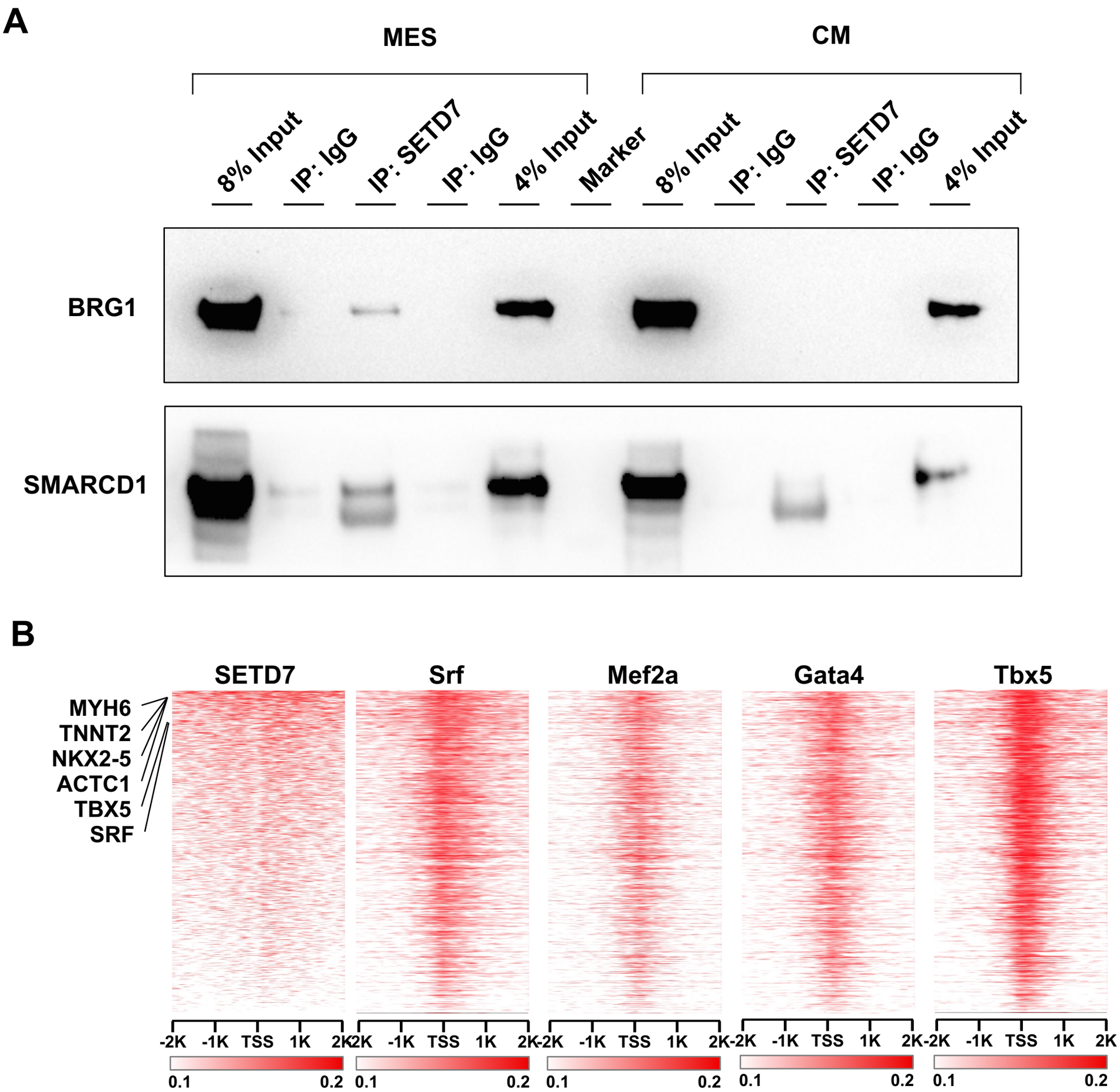
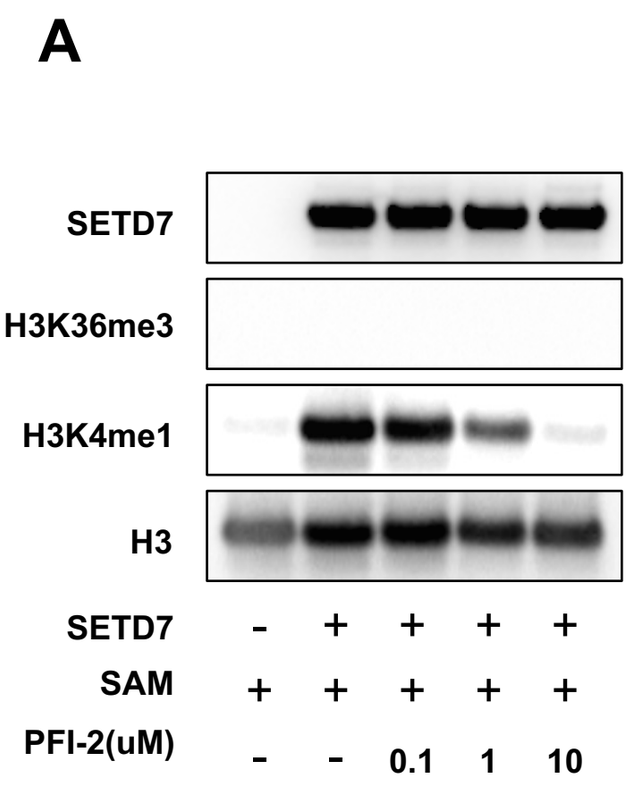


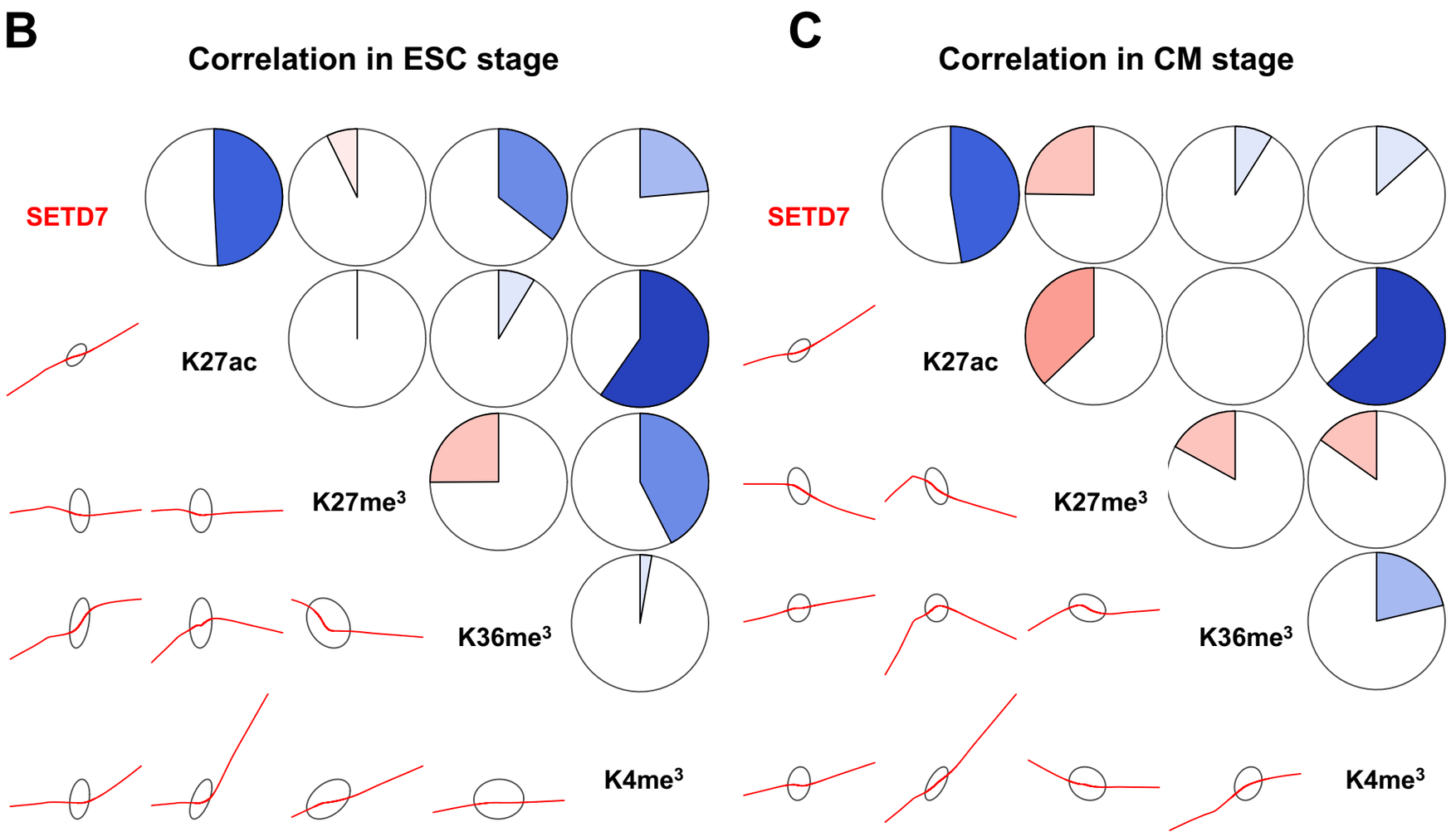
Figure S4 (Related to Figure 4). Identification of co-factors of SETD7 during CM differentiation.

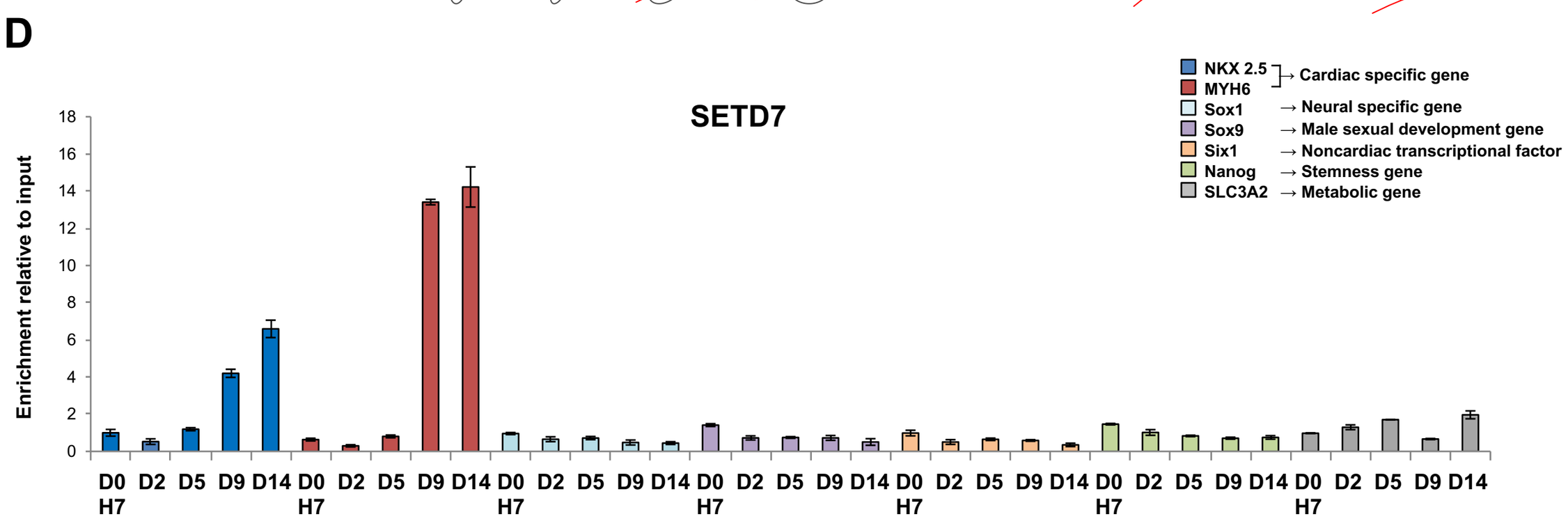
(A) Immunoblot analysis of SETD7 immunoprecipitates (IP line) and cell lysates (Input line) from mesoderm lineage and CM lineage cells derived from hESCs (H7).

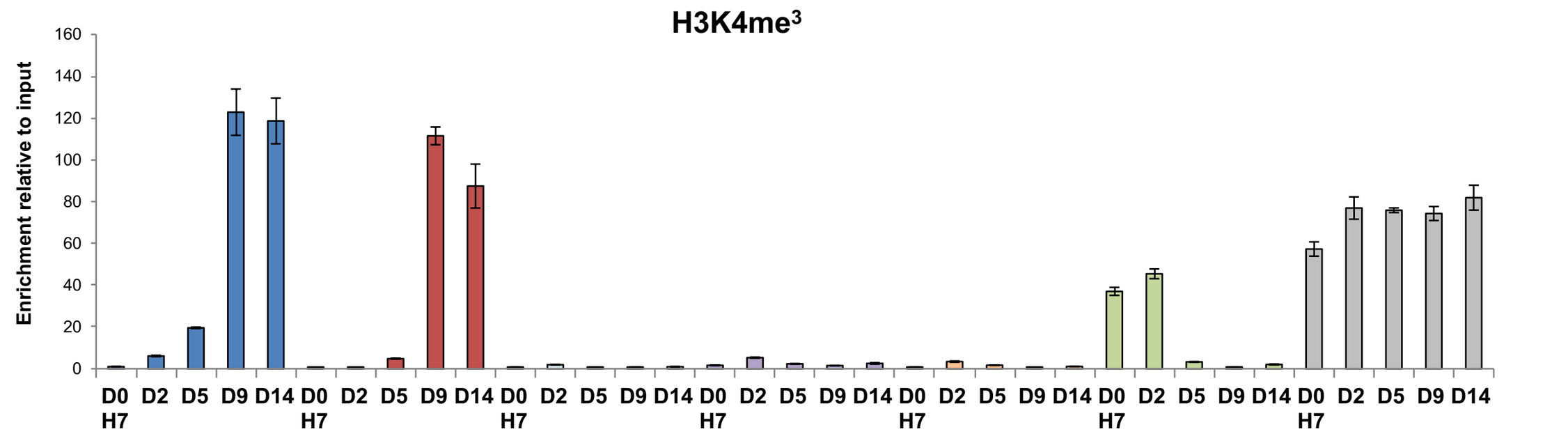
(B) Heatmaps showing relative enrichment levels of SETD7 (in human) and Srf, Mef2a, Gata4, and Tbx5 (in mouse), on TSS of ortholog coding genes in CMs. The heatmaps were ranked by the enrichment of SETD7. ChIP-seq data were adapted from a previous study (He et al., 2011).

Figure S5


A
SETD7
H3K36me3
H3K4me1
H3
SETD7 - + + + +
SAM + + + + +
PFI-2(uM) - - 0.1 1 10


B
Correlation in ESC stage
SETD7
K27ac
K27me3
K36me3
K4me3
C
Correlation in CM stage
SETD7
K27ac
K27me3
K36me3
K4me3


D
SETD7
NKX 2.5
MYH6
Cardiac specific gene
Sox1 → Neural specific gene
Sox9 → Male sexual development gene
Six1 → Noncardiac transcriptional factor
Nanog → Stemness gene
SLC3A2 → Metabolic gene
Enrichment relative to input
0 2 4 6 8 10 12 14 16 18
D0 H7 D2 D5 D9 D14


E
H3K4me3
Enrichment relative to input
0 20 40 60 80 100 120 140 160
D0 H7 D2 D5 D9 D14

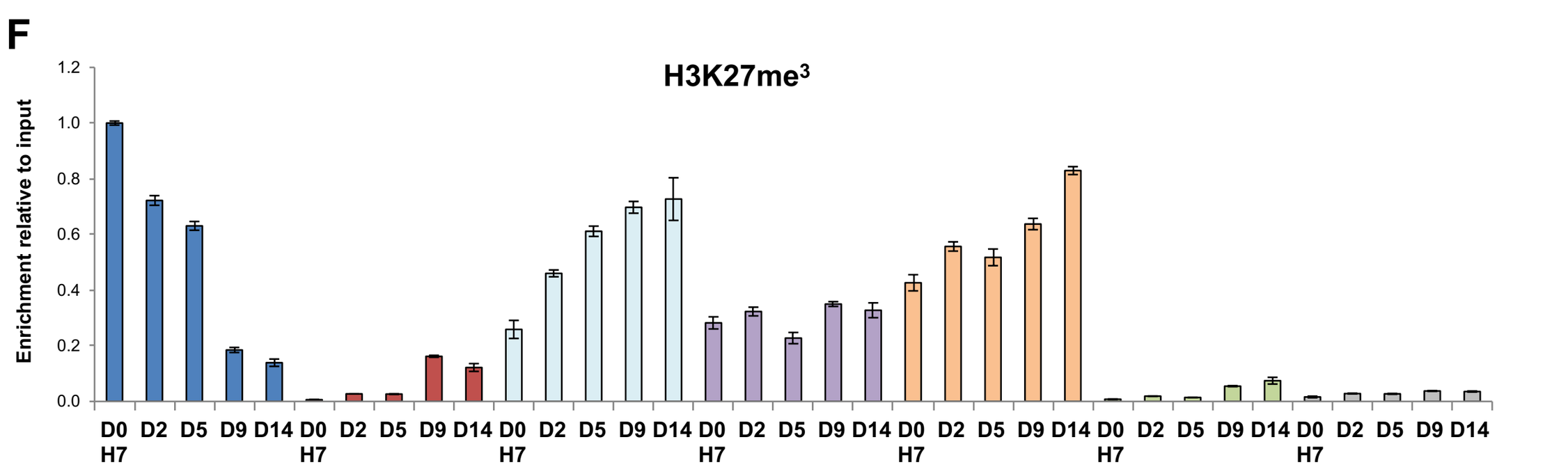

F
H3K27me3
Enrichment relative to input
0.0 0.2 0.4 0.6 0.8 1.0 1.2
D0 H7 D2 D5 D9 D14

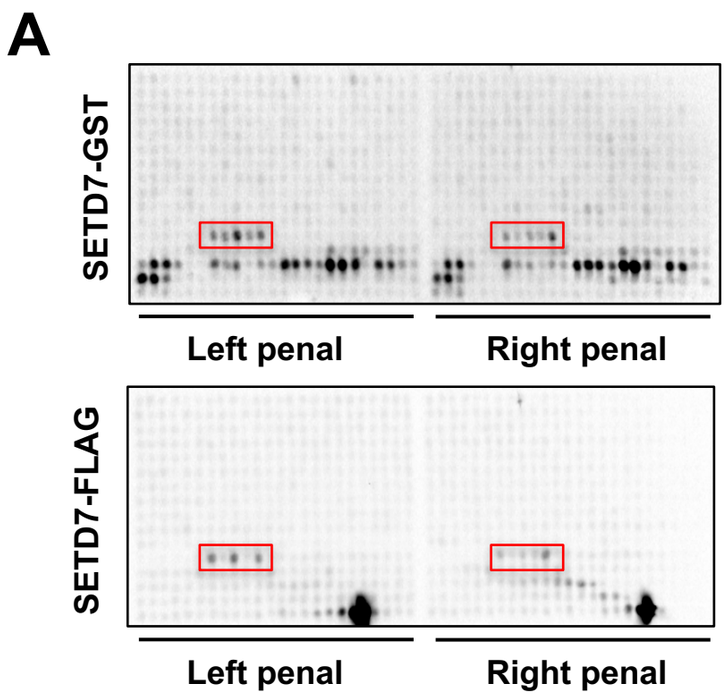
Figure S5 (Related to Figure 5). Correlation between the SETD7 enrichment and several histone markers at each stage of CM differentiation.

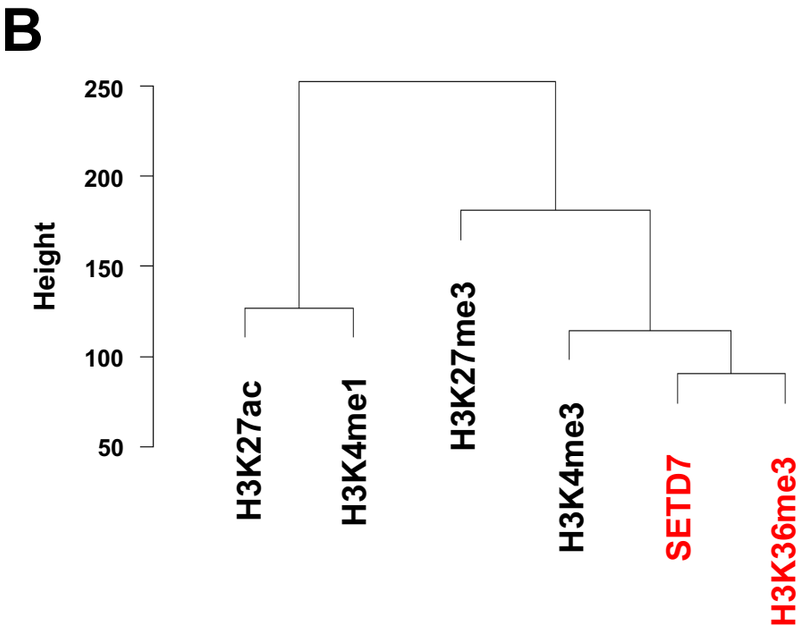
(A) Immunoblot analysis of SETD7, H3K4me1, and H3K36me3. *In vitro* methylation assay was performed with histone 3 and active SETD7-FLAG protein. PFI-2 and DMSO were treated as indicated.

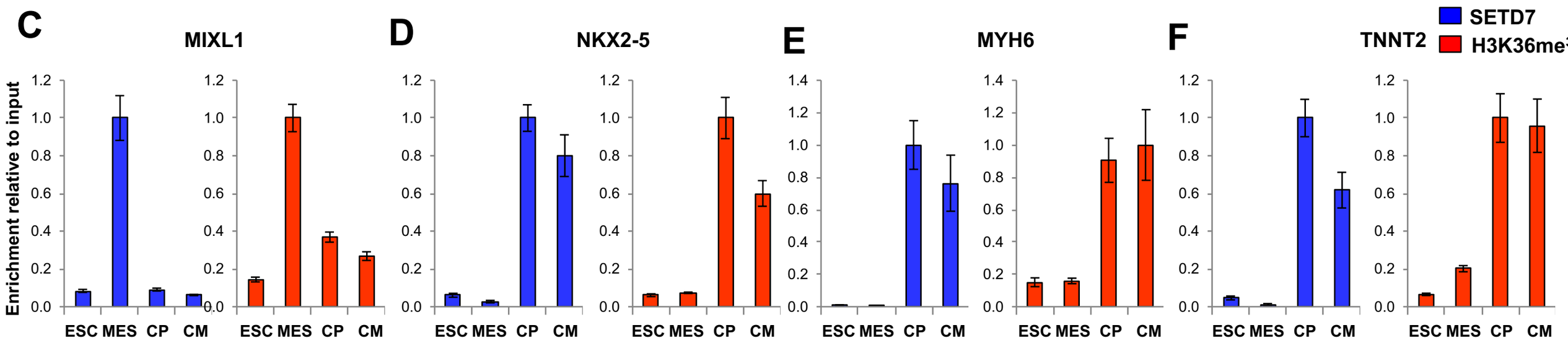
(B-C) Corrgram package analysis was used to calculate correlation/r-values. The piechart where the intersection represents the correlation value between the two factors/marks. The size of the shaded region within the pie reflects correlation/r-values (bigger shaded region represents higher correlation or r-value), whereas the color of the shaded region represents the direction of correlation (blue reflects positive correlation and red reflects negative correlation). The lower panel represents the confidence ellipses and smoothed lines.

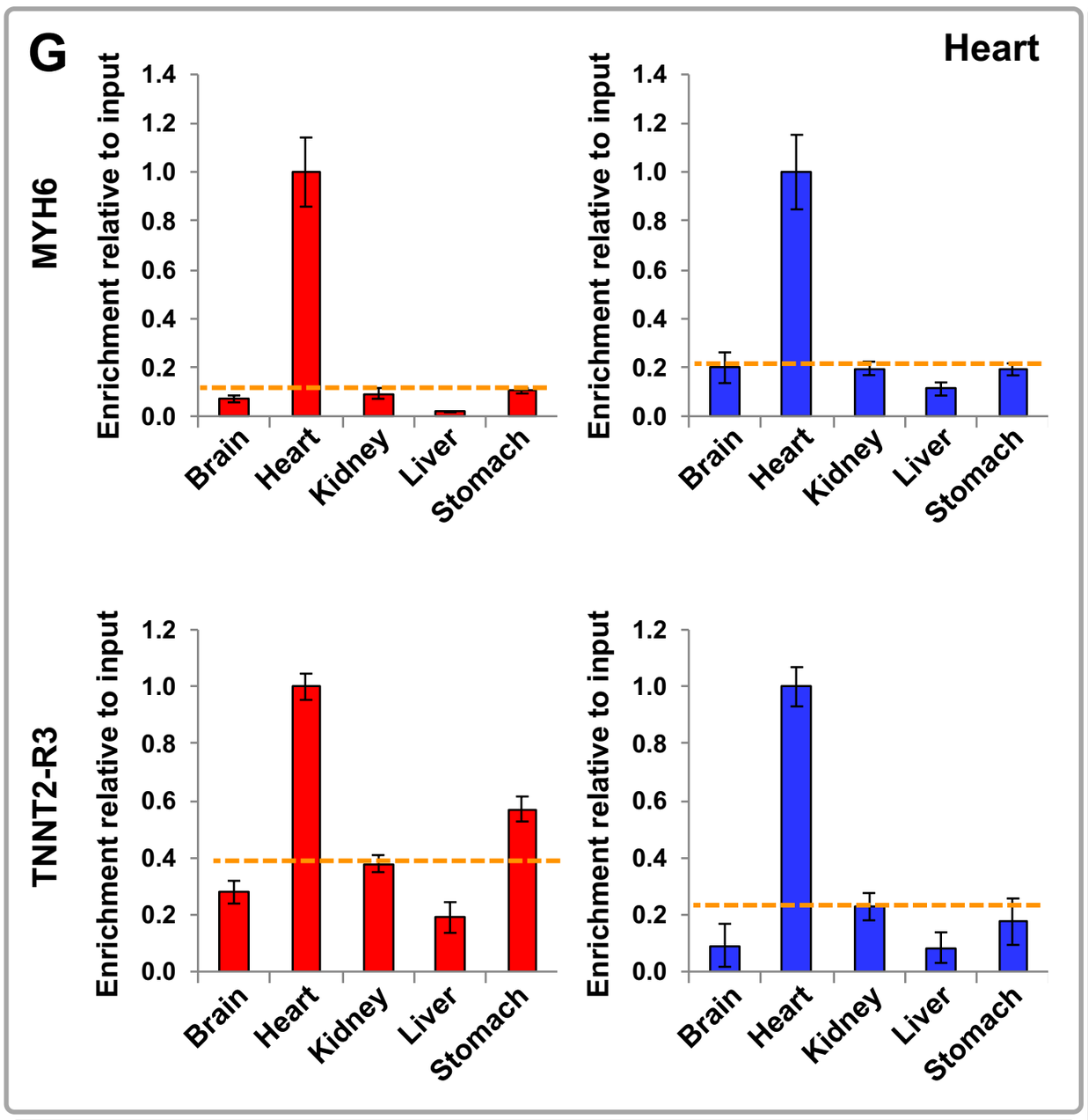
(D-F) ChIP-qPCR assay of SETD7, H3K4me3, and H3K27me3 enrichment at promotor region of several lineage specific genes during CM differentiation.

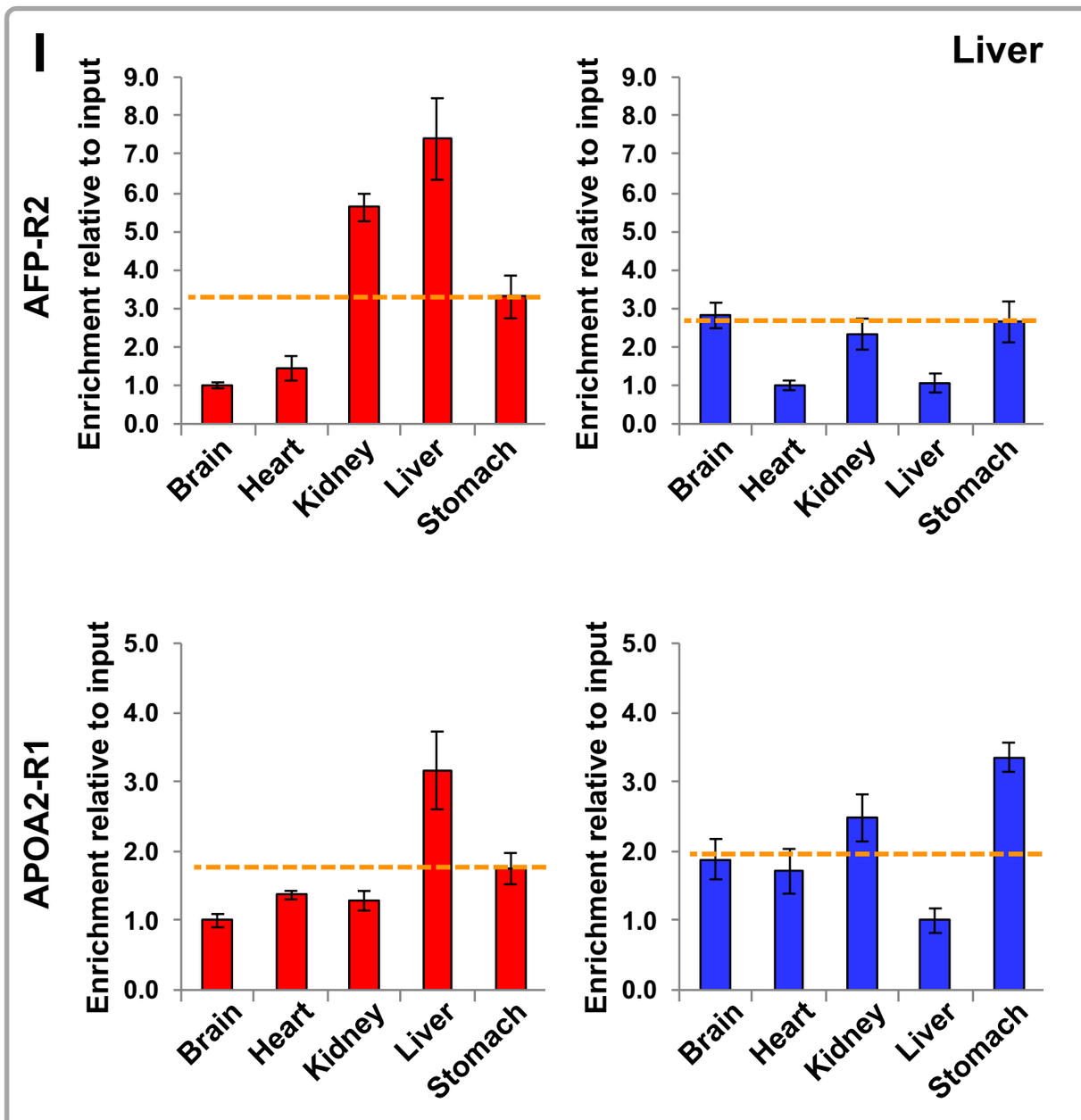
Figure S6

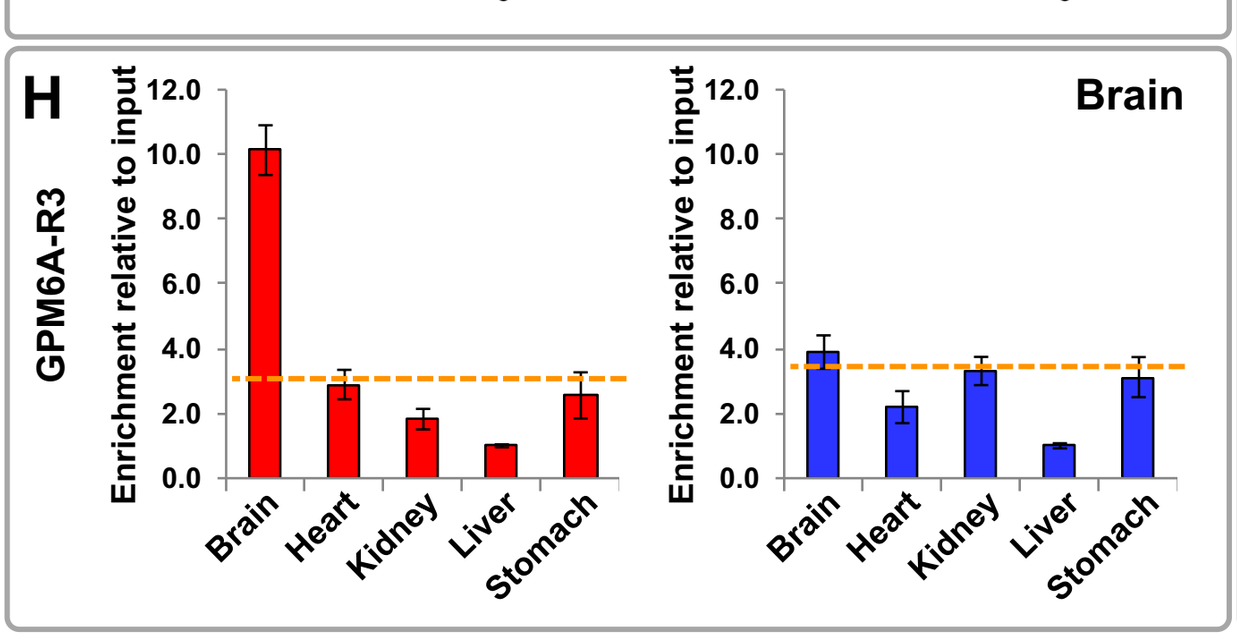

A
SETD7-GST
Left penal
Right penal
SETD7-FLAG
Left penal
Right penal


B
Height
250
200
150
100
50
H3K27ac
H3K4me1
H3K27me3
H3K4me3
SETD7
H3K36me3


C
MIXL1
D
NKX2-5
E
MYH6
F
TNNT2
SETD7
H3K36me3
Enrichment relative to input
ESC MES CP CM


G
Heart
MYH6
TNNT2-R3
Enrichment relative to input
Brain
Heart
Kidney
Liver
Stomach


I
Liver
AFP-R2
APOA2-R1
Enrichment relative to input
Brain
Heart
Kidney
Liver
Stomach


H
GPM6A-R3
Brain
Enrichment relative to input
Brain
Heart
Kidney
Liver
Stomach

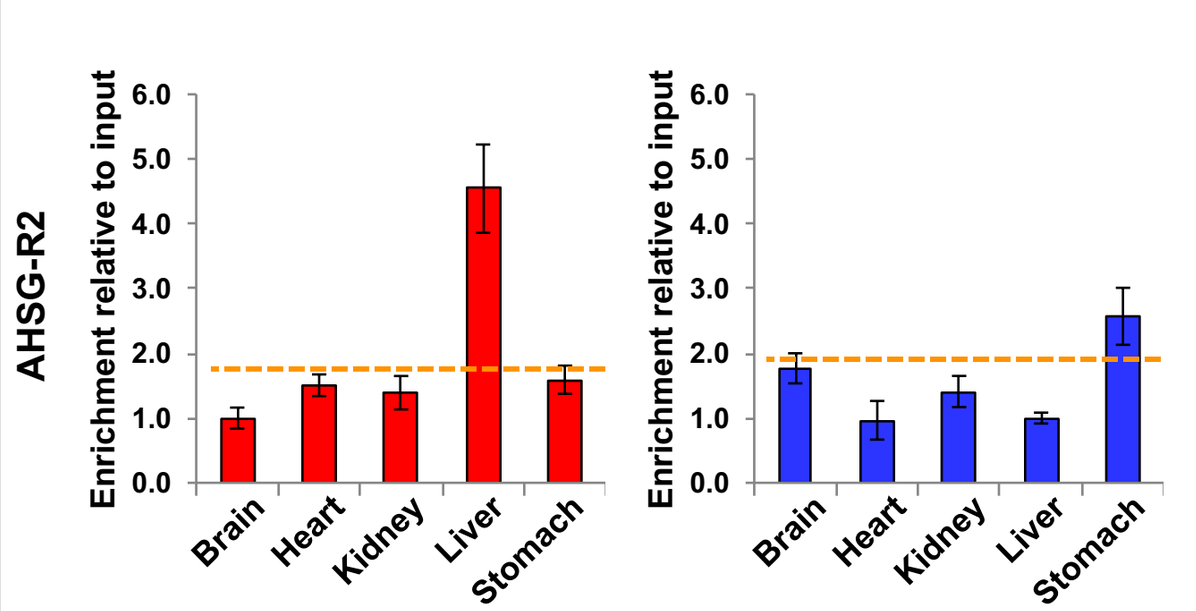

AHSG-R2
Enrichment relative to input
Brain
Heart
Kidney
Liver
Stomach

Figure S6 (Related to Figure 5 and Figure 6). SETD7 associates with H3K36me3 on its target genes during CM differentiation.

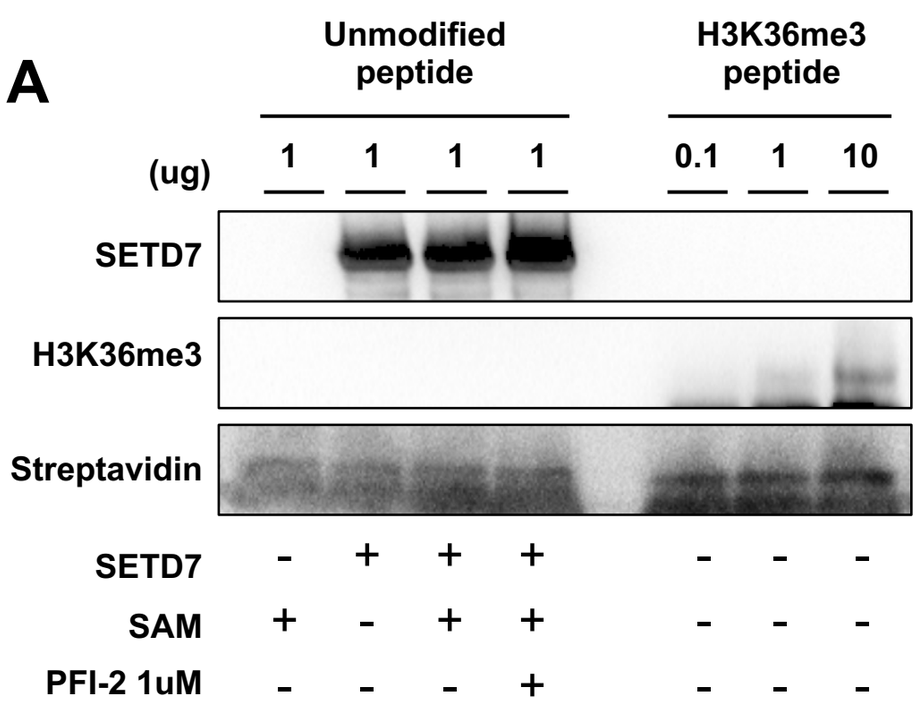
(A) Peptide array of 384 different histone modification with SETD7 proteins conjugated with two different tags. Red box showed the position of unmethylated H3K36, H3K36me, H3K36me2, H3K36me3, and H3K36Ac.

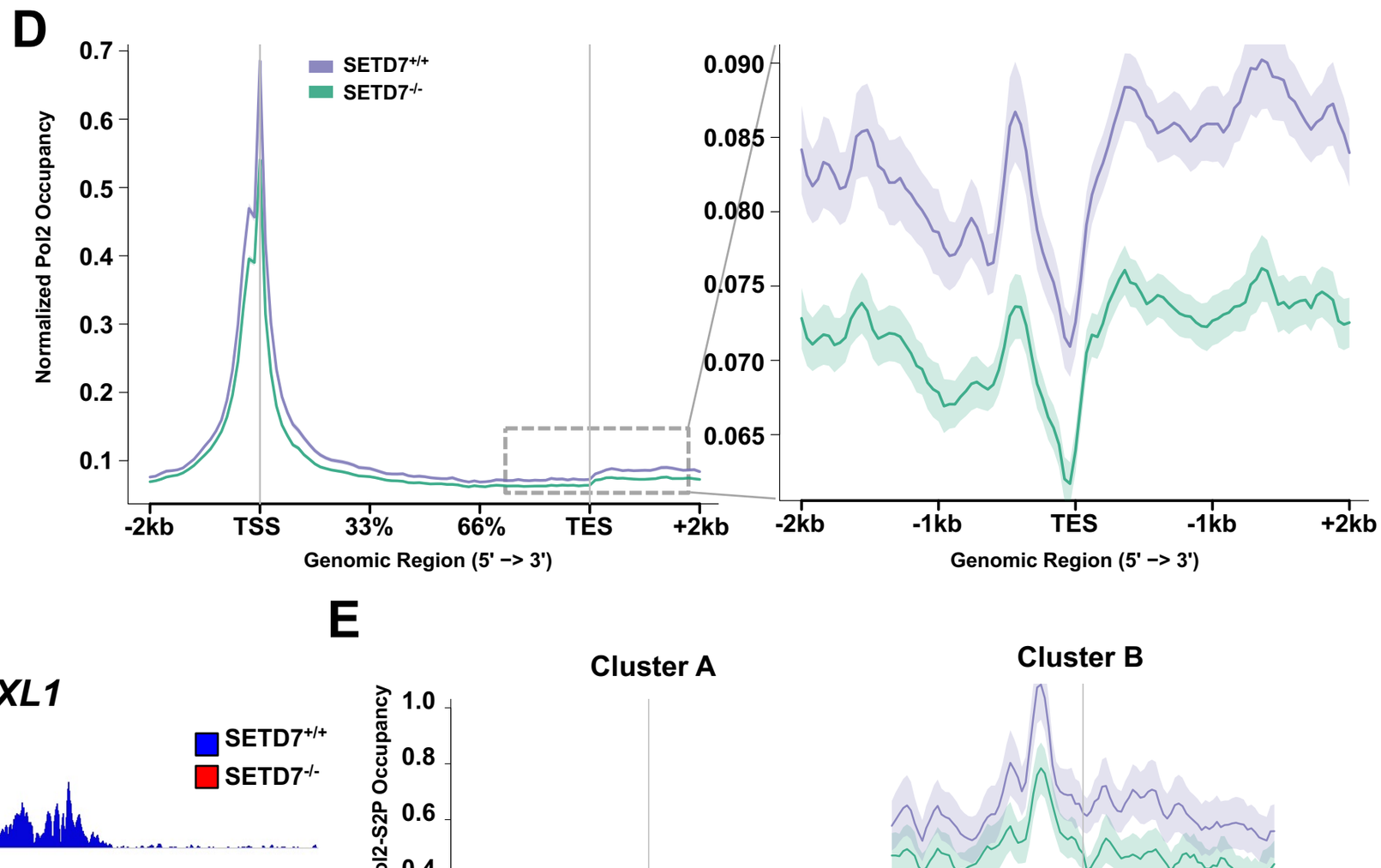
(B) Hierarchical clustering analysis based on the enrichment of histone marks and SETD7 of genebodies of coding genes.

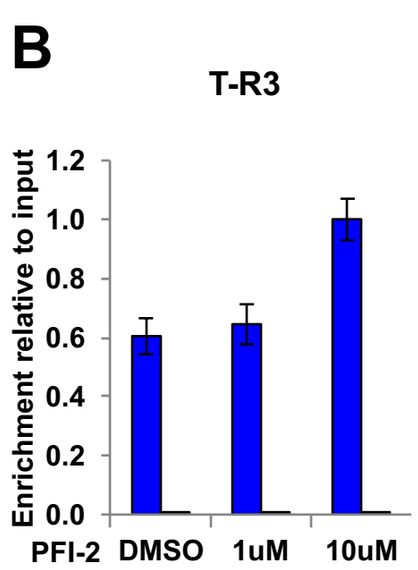
(C-F) ChIP-qPCR assay of SETD7 and H3K36me3 enrichment at several target genes during CM differentiation. Primer targets gene-body region of each gene.

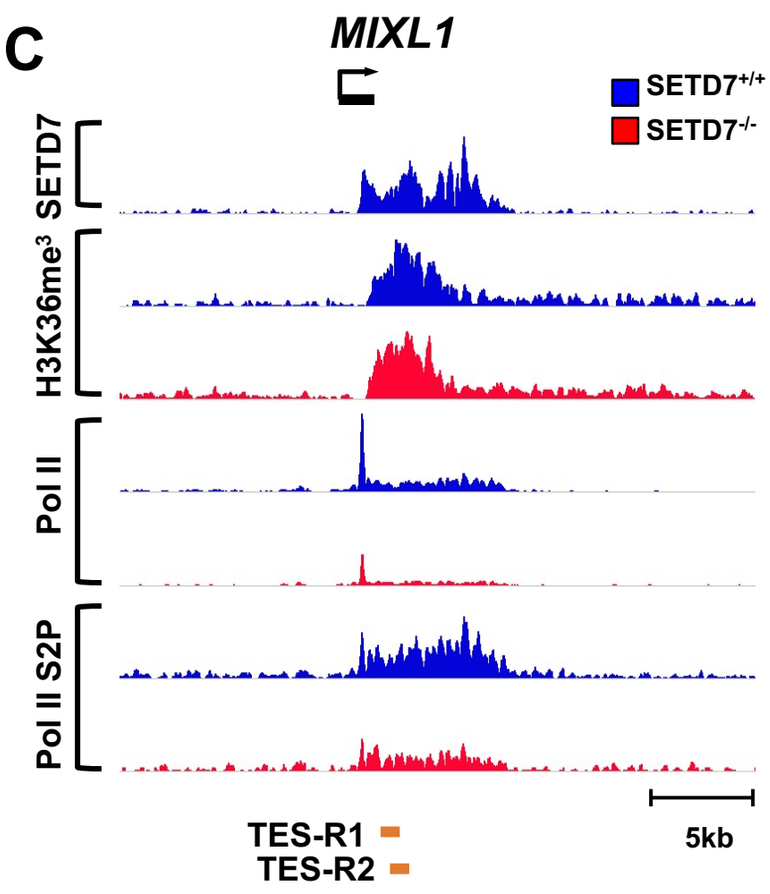
(G-I) ChIP-qPCR assay of SETD7 and H3K36me3 enrichment at tissue specific genes in different organs of human fetus (Fetus-1: 16.4 week male).

Figure S7


A
Unmodified peptide
H3K36me3 peptide
(ug)
1 1 1 1
0.1 1 10
SETD7
H3K36me3
Streptavidin
SETD7 - + + + - - -
SAM + - + + - - -
PFI-2 1uM - - - + - - -


D
Normalized Pol2 Occupancy
SETD7+/+
SETD7-/-
-2kb TSS 33% 66% TES +2kb
Genomic Region (5' -> 3')
-2kb -1kb TES -1kb +2kb
Genomic Region (5' -> 3')


B
T-R3
Enrichment relative to input
PFI-2 DMSO 1uM 10uM
MIXL1-R2
Enrichment relative to input
PFI-2 DMSO 1uM 10uM
H3K36me3
IgG


C
MIXL1
SETD7+/+
SETD7-/-
SETD7
H3K36me3
Pol II
Pol II S2P
TES-R1
TES-R2
5kb

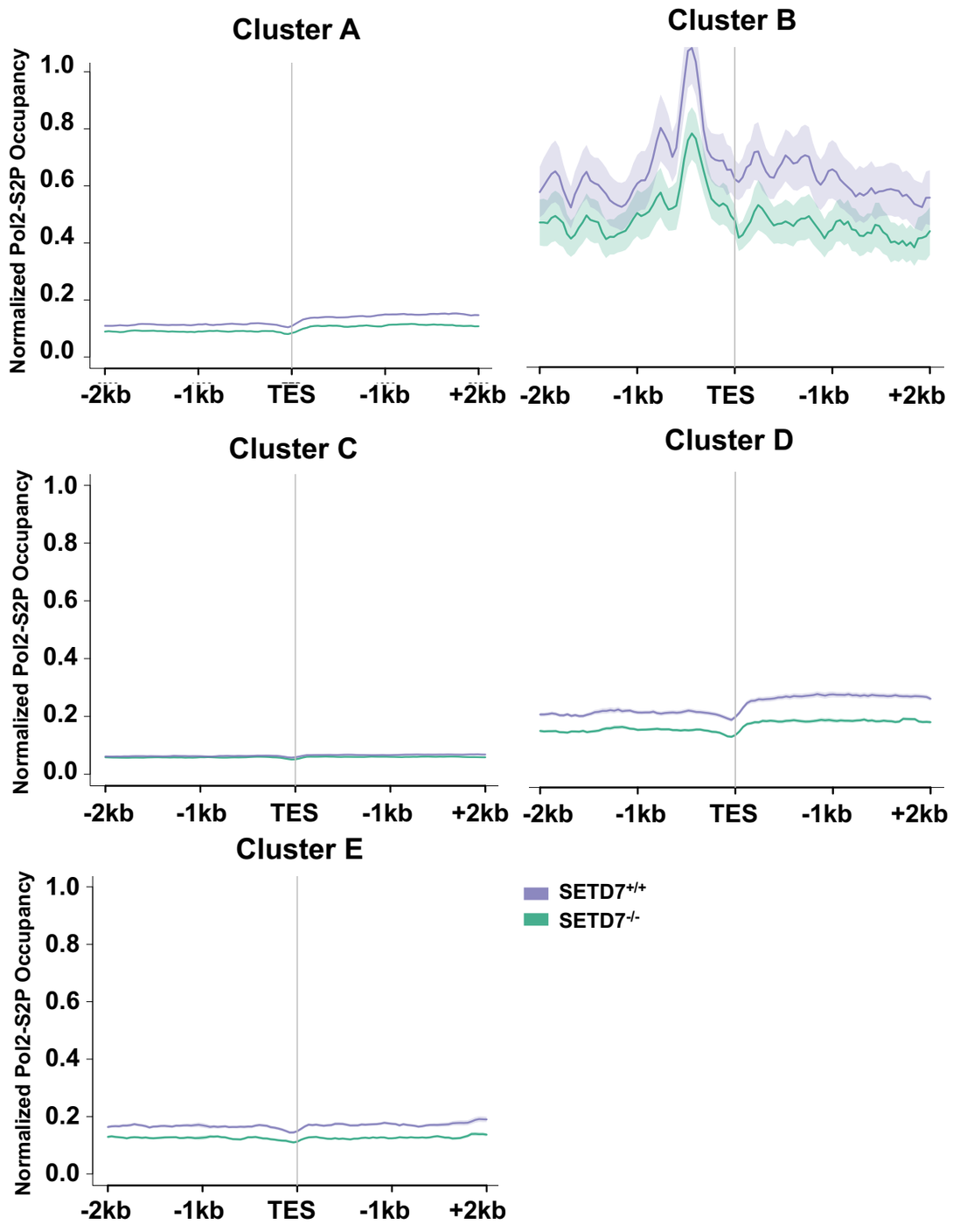

E
Cluster A
Cluster B
Cluster C
Cluster D
Cluster E
Normalized Pol2-S2P Occupancy
-2kb -1kb TES -1kb +2kb
SETD7+/+
SETD7-/-

Figure S7 (Related to Figure 6). SETD7 is not necessary for methylation of H3K36, but required for Pol II-mediated gene transcription.

(A) Immunoblot analysis of SETD7 and H3K36me3. *In vitro* methylation assay was performed with histone peptides (biotin-conjugated; H3 a.a. 22-44) and active SETD7-FLAG protein. H3K36me3 peptide were used as positive control.

(B) ChIP-qPCR analysis of H3K36 enrichment at gene-body regions of T and MIXL1 genes at mesoderm stage. Statistical significance was analyzed using the one way ANOVA. *P<0.05; **P < 0.01; (mean ± SEM; n=3).

(C) Genome browser screenshots of SETD7, H3K36me3, Pol II, and PolII-S2P ChIP-seq profiles on MIXL1 gene at mesoderm lineage of $SETD7^{+/+}$ and $SETD^{-/-}$ lines.

(D) The genome-wide average profiles of Pol II enrichment in gene body region.

(E) The genome-wide average profiles of Pol II-S2P enrichment in gene body region of each cluster showed in Figure 5J.