Setd1a and NURF mediate chromatin dynamics and gene regulation during lineage commitment and differentiation

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Table S1. Primer sequences:

RT primer	sequence
mβ-major-globin	For: CACCTTTGCCAGCCTCAGTG Rev: GGTTTAGTGGTACTTGTGAGCC
mβH1-globin	For: AGTCCCCATGGAGTCAAAGA Rev: CTCAAGGAGACCTTTGCTCA
mεy-globin	For: AACCCTCATCAATGGCCTGTGG Rev: TCAGTGGTACTTGTGGGACAGC
mp4.2	For: GGGTGAGTAGACACTTGAAATCG Rev: GGGGCCTCTAAGGAGTTGTG
mNotch2	For: AACTGTCAGACCCTGGTGAAC Rev: CGACAAGTGTAGCCTCCAATC
mβ-actin	For: GTGGGCCGCTCTAGGCACCA Rev: TGGCCTTAGGGTGCAGGGGG
mTal1	For: GAGATTTCTGATGGTCCTCAC Rev: TCTTGCTTAGTTTCTTGTCTGG
mGata1	For: CAGGGATCCCATGGATTTTCCTGGTC Rev: TCCACAGTTCACACACTCTCTGGC
mKlf1	For: TCGCCGGAGACGCAGGCT Rev: CCCAGTCCTTGTGCAGGA
mBptf	For: TTCAGGAGCCATAGTACATACAG Rev: CAGAGCCGCACAGAAGTC

ChIP primer	sequence
mLCR5'	For: ACCCAATGGACTATCCCCTGTCTC Rev: GCCAATGTGATGAGTGCAGAGTTC
mHS2	For: TGCAGTACCACTG TCCAAGG Rev: ATCTGGCCACACACCCTAAG
mHS6	For: ATTCTCCTGCTGTATTCACTGG Rev: CCTCATCTTTCAACAATGCTCTG
mεy pro.	For: CAAAGAGAGTTTTTGTTGAAGGAGGAG Rev: AAAGTTCACCATGATGGCAAGTCTGG
mβH1pro.	For: CAAGGTCCAGGGTGAAGAATAAAAGG Rev: CAGGAGCCTAAGACATAGTGCATTAGC
mβ maj pro.	For: AAGCCTGATTCCGTAGAGCCACAC Rev: CCCACAGGCAAGAGACAGCAGC

mβ min pro.	For: TGCCCACTCTGTCCTCTC Rev: CCTGTGTAGATATGGTTGTCATC
mβ min 3'	For: AGCCCAAGAGCATTGAGTTGTATC Rev: TCAGACTGTGGAGGAGGAGGAG
mp4.2 pro	For: GCAGGTCATCTCCAAAGAGC Rev: CGAACCCAACTCTGAACCTC
mp4.2 3'UTR	For: TCTTTCCCTGGTGGCTATTG Rev: AGAGTACCCCCGAAAACACC
mNotch2 pro.	For: TGGATGGACGGATGAATG Rev: GAGACTGTCAACGGAAGG
mNotch2 3'UTR	For: GAATGTACGAGGACTGTT Rev: GAGGAAGTTGCCATAGTA
mMyoD	For: TGCCTGTCCAGCATAGTG Rev: ACTCTGGTGGTGCATCTG

3C primer	sequence
mHS2/3	ACATGAGGCTACTCTATTGTCAGACTGTGC
mHS2	ATGACTCAGCACTGCTGTGCTCAAGCC
mβH1pro.	TCAGGAATGTTCCCAACTTTCACTCAATTCCCC
mβ maj pro.	GGTGGAAGGGGGTATTATGAACATTCGG
m-3'distal	TAGCTGTGGAGAGCAGGAGGTCTGCTAATGCC
mHS2-BglII	For: TCCTACACATTAACGAGCCTCTG Rev: GCTAACCTCCTCTGCCCTTG
β maj-Bgl II	For: GCCTAGCCATAAAGATAGGATGAG Rev: AGCCACCAGCACTGTCTG
m β h1-Bgl II	For: AAGCCACAATATAGAATTACATCC Rev: AATGTCTCAGTCTCACAAGATG

Supplemental Figure Legends:

Figure S1. Loss of Setd1a in erythroid compartment resulted in impaired H3K4me3 and BPTF recruitment at erythroid gene promoters. ChIP analysis of H3K4me3 enrichment **(A),** ASH2L occupancy **(B),** and BPTF recruitment **(C)** at p4.2 promoter comparing *Setd1a*$^{fl/fl}$ and Setd1a CKO BM cells. Data are shown as mean ± SD. *P<0.05; **P<0.01.

Fig S2.Consistent colocalization of genome wide USF1 and USF2 binding. Two replicates of ChIP-seq analyses of USF1 and USF2 binding at the *β-globin* locus.

Figure S3. USF1, USF2, BPTF and H3K4me3 ChIP-seq signal are colocalized at transcription start sites (TSSs) and correlate with transcription levels in human primary erythroid cells. (A) Heatmaps represent USF1, USF2, BPTF and H3K4me3 ChIP enrichment at TSSs in R3/R4 erythroid cells. **(B)** Line-plots for USF1, USF2, BPTF and H3K4me3 ChIP enrichment at TSSs correlates with gene expression in R3/R4 erythroid cells (GEO accession number: GSE53983)**.**

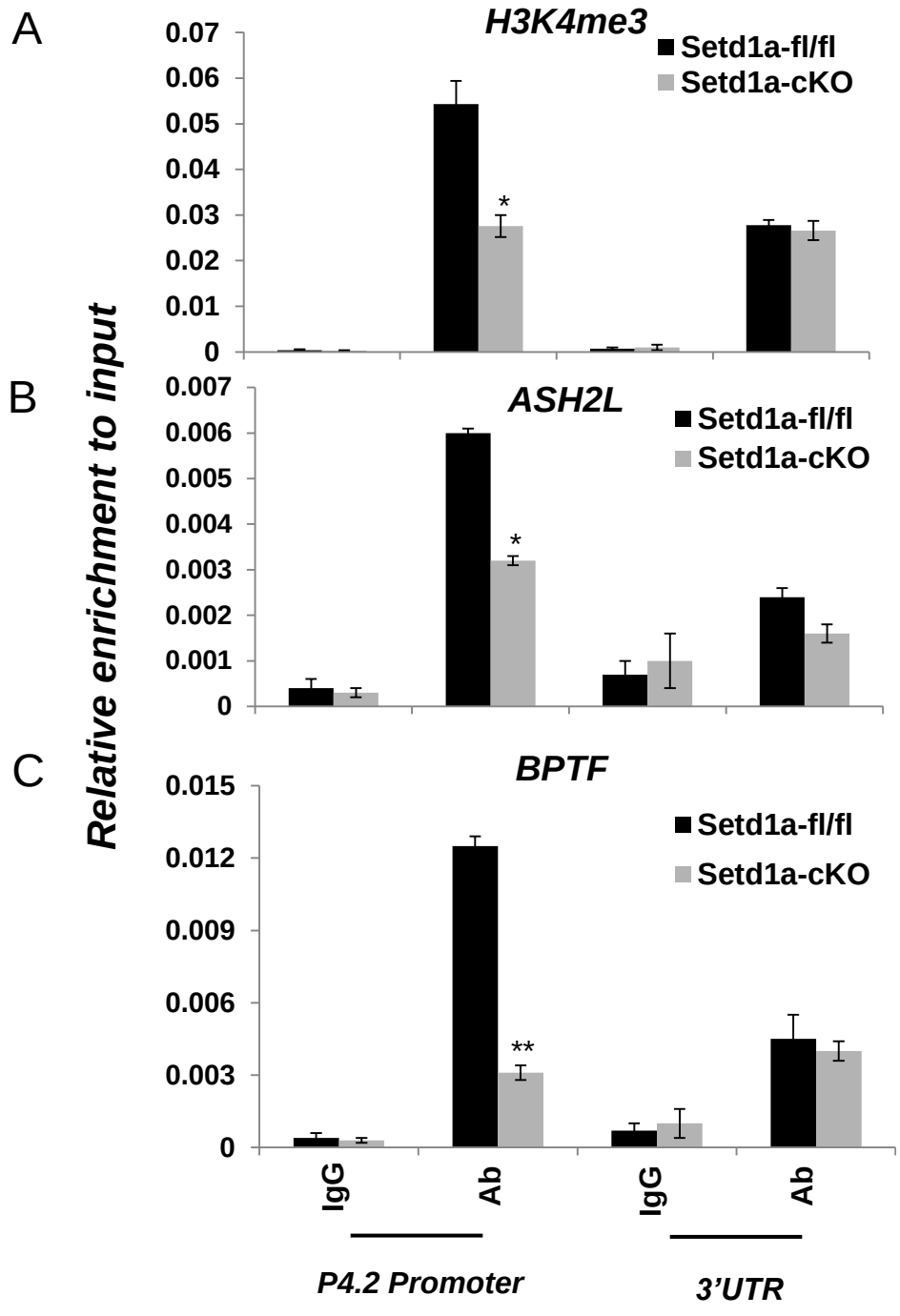
Figure S4. Directed differentiation of erythroid progenitors/precursors from murine embryonic stem cells (mESCs). (A) Schematic outline of induced erythroid differentiation of murine ES cells. **(B)** qRT-PCR analysis of the expression patterns of genes that are specifically expressed in definitive erythroid cells during EB differentiation. **(C)** qRT-PCR analysis of expression patterns of embryonic and adult globin genes during EB differentiation comparing the control and *Setd1a* KD cells. Data are shown as mean ± SD. *P<0.05; **P<0.01. **(D)** The 19-day differentiated EBs exhibited reddish color (Left). HEMA staining of erythroblast cells in differentiated ES cells at different days (Right).

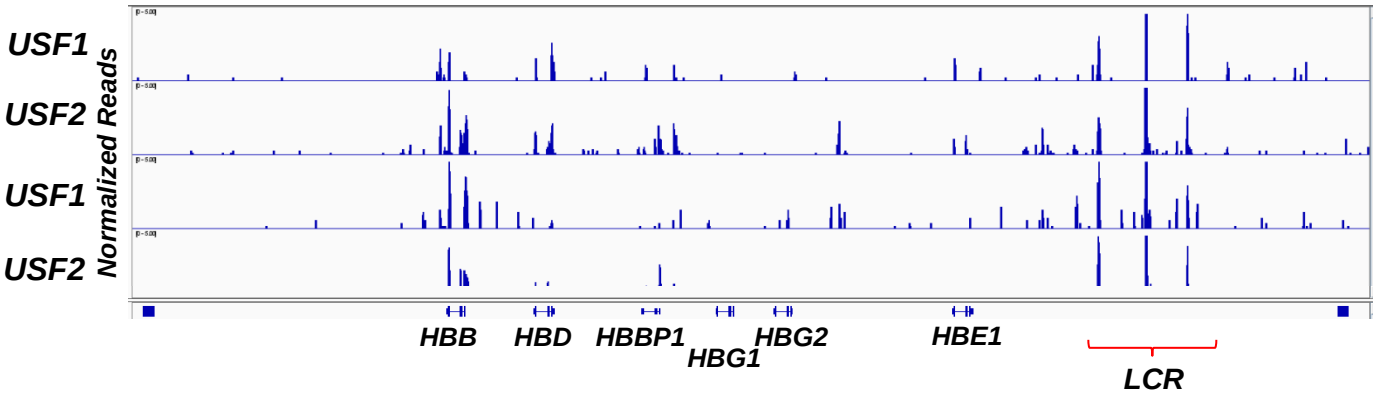
Figure S5. Depletion of *Setd1a* suppresses erythroid gene p4.2 and Notch2 transcription. (A) Stable ESC clones harboring vector control or shSet1d1a-expressing constructs were induced with EPO and other cytokines for erythroid differentiation and total RNA was extracted at day 0, 4, and 19. The *p4.2* (Top) and *Notch2* (Bottom) transcripts were analyzed by qRT-PCR and normalized with *β-actin*. **(B)** ChIP analysis of H3K4me3 on *p4.2* (Top) and *Notch2* (Bottom)

promoters at day 19 in control and *Setd1a* KD cells. Data are shown as mean ± SD. *P<0.05; **P<0.01.

Figure S6. Setd1a is required for *β-globin* transcription and chromatin accessibility at the *β-globin* locus during MEL cell differentiation. (A) Western blot of Setd1a expression in *Setd1a* KD MEL cells compared to control, *β-actin*, as a loading control. **(B)** qRT-PCR analysis of expression levels of genes required for erythroid differentiation, *Tal1* and *p4.2*, upon the Setd1a KD. **(C)** *β-major globin* transcripts were analyzed by qRT-PCR and normalized with *β-actin* in *Setd1a* KD MEL cells upon DMSO differentiation for 5 days. **(D and E)** ChIP analysis of H3K4me3 **(D)** and SNF2L recruitment **(E)** over the *β-globin* locus at days 5 upon *Setd1a* KD. **(F)** FAIRE assay on *β-globin* locus at days 5 upon *Setd1a* KD MEL cells. Data are shown as mean ± SD. *P<0.05; **P<0.01.

Figure S7. Loss of Setd1a disrupts enhancer /promoter interaction over *β*-globin locus during MEL cells differentiation. (A) and **(B)** The 3C analysis of the interaction between the HS2/3 or HS2 enhancer and *β-globin* promoters in the *β-globin* locus at DMSO-induced MEL cells upon *Setd1a* KD. Data are shown as PCR quantitation of the 3C products. Data are shown as mean ± SEM. *P<0.05; **P<0.01.


A
H3K4me3
Setd1a-fl/fl
Setd1a-cKO
0.07
0.06
0.05
0.04
0.03
0.02
0.01
0
*
B
ASH2L
Setd1a-fl/fl
Setd1a-cKO
0.007
0.006
0.005
0.004
0.003
0.002
0.001
0
*
C
BPTF
Setd1a-fl/fl
Setd1a-cKO
0.015
0.012
0.009
0.006
0.003
0
**
Relative enrichment to input
IgG
Ab
IgG
Ab
P4.2 Promoter
3'UTR


USF1
USF2
USF1
USF2
Normalized Reads
HBB
HBD
HBBP1
HBG1
HBG2
HBE1
LCR

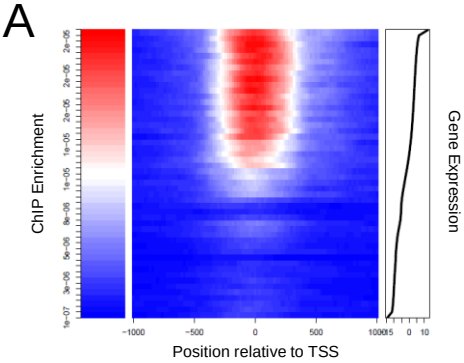
Li_Figure S3

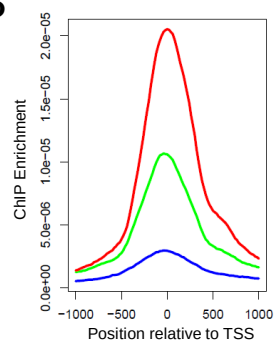
A

B

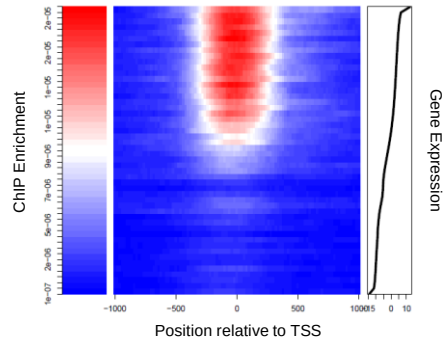
High expression
Moderate expression
Low expression

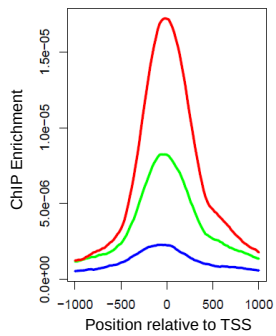
USF1



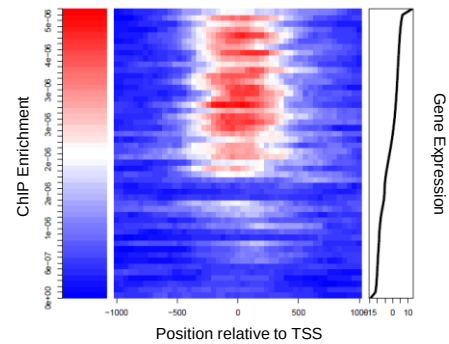


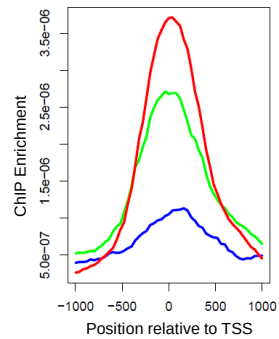
USF2



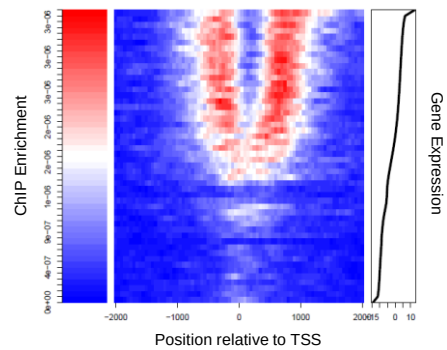


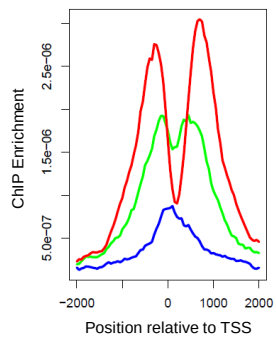
BPTF



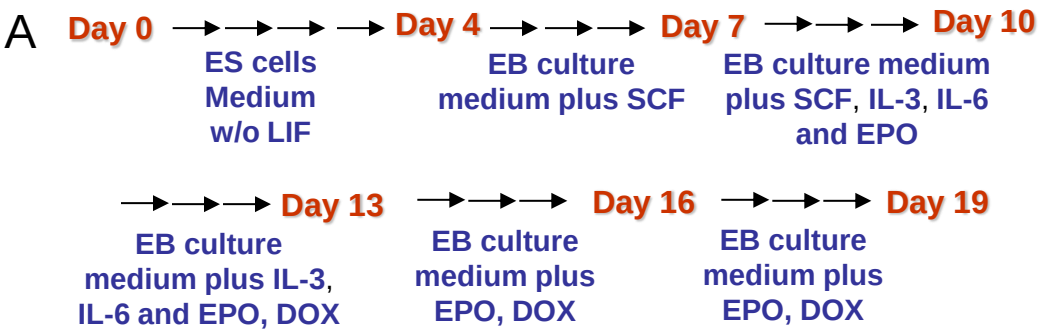


H3K4me3

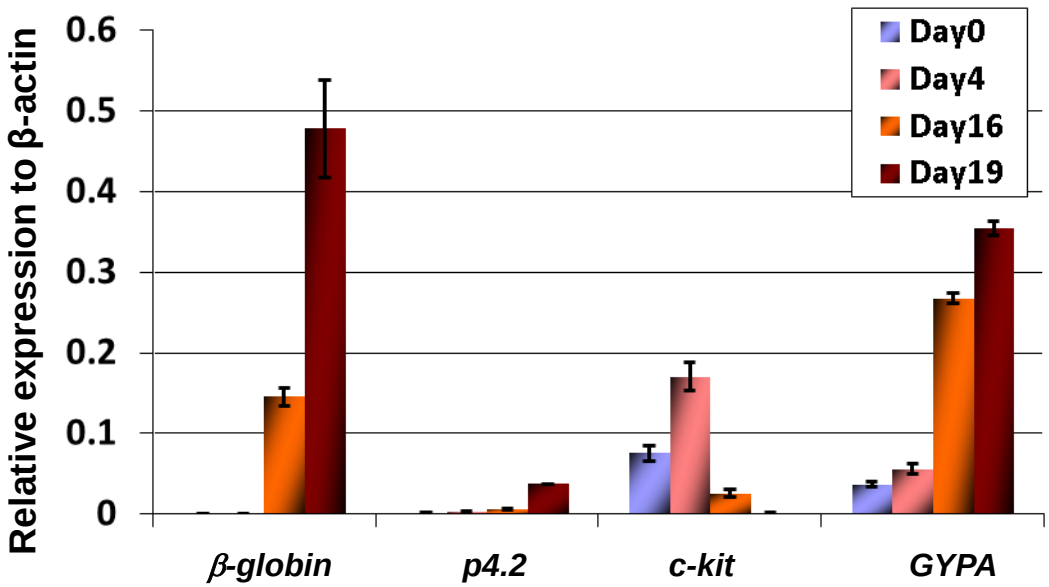




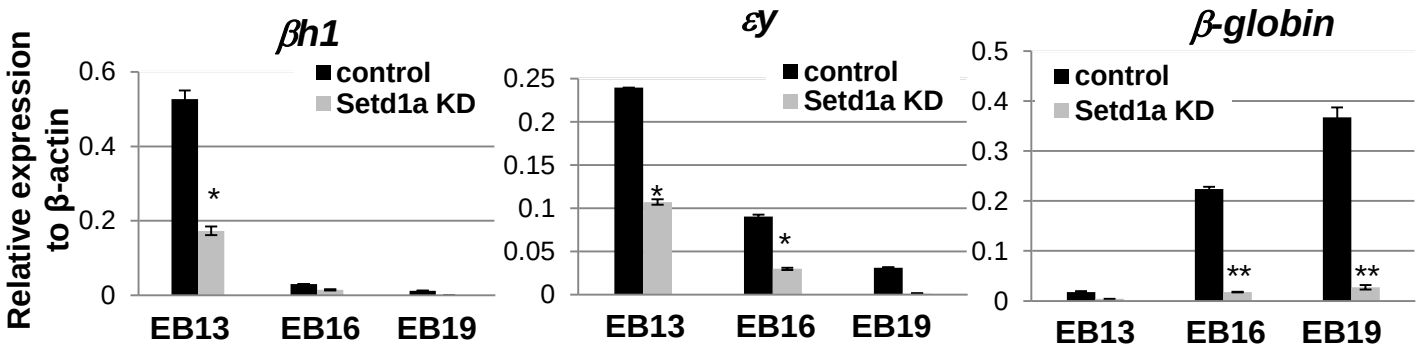
A


Day 0
ES cells Medium w/o LIF
Day 4
EB culture medium plus SCF
Day 7
EB culture medium plus SCF, IL-3, IL-6 and EPO
Day 10
Day 13
EB culture medium plus IL-3, IL-6 and EPO, DOX
Day 16
EB culture medium plus EPO, DOX
Day 19
EB culture medium plus EPO, DOX

B

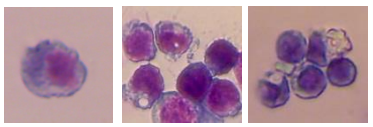

Relative expression to β-actin
0.6
0.5
0.4
0.3
0.2
0.1
0
Day0
Day4
Day16
Day19
β-globin
p4.2
c-kit
GYPA

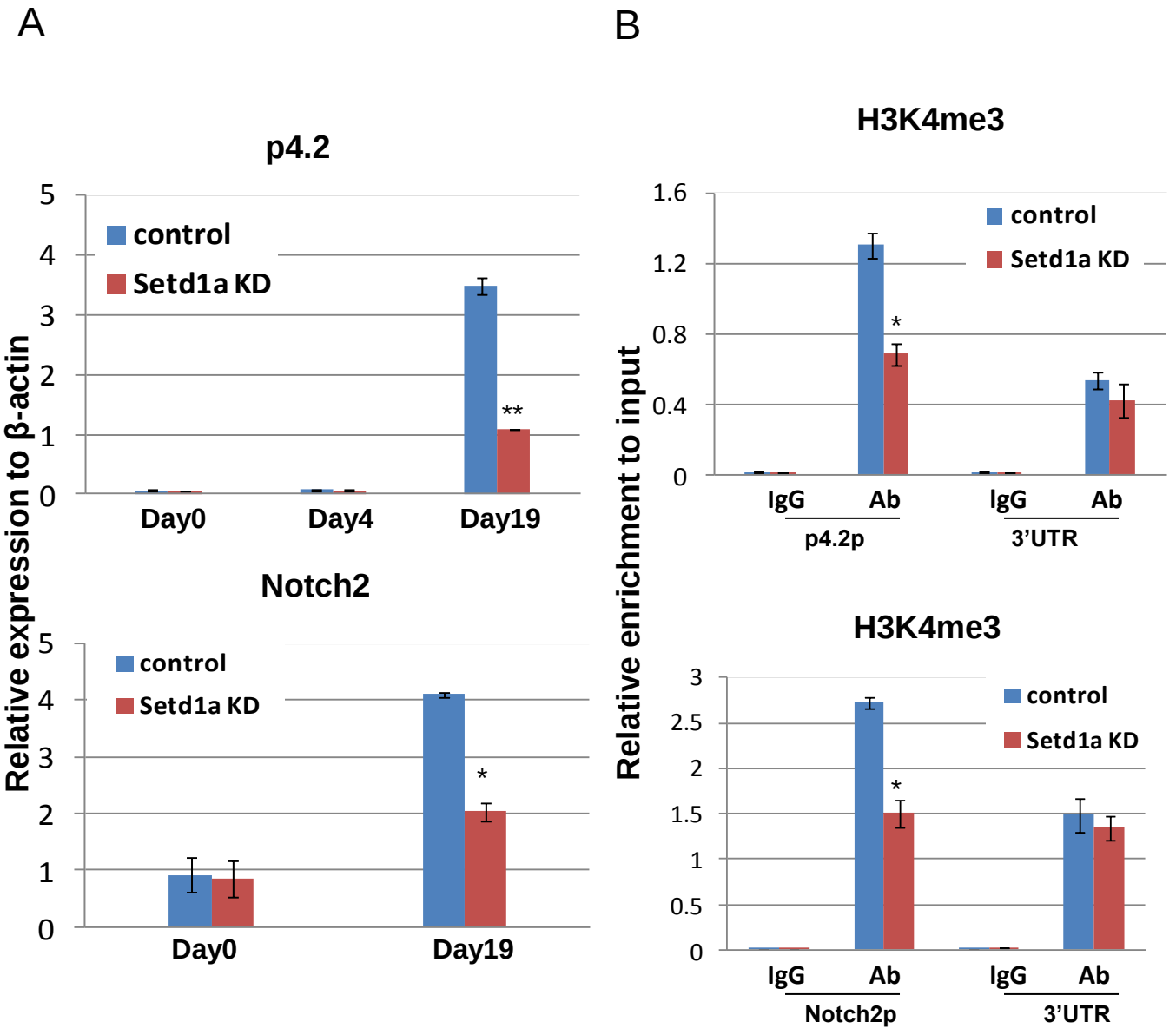
C

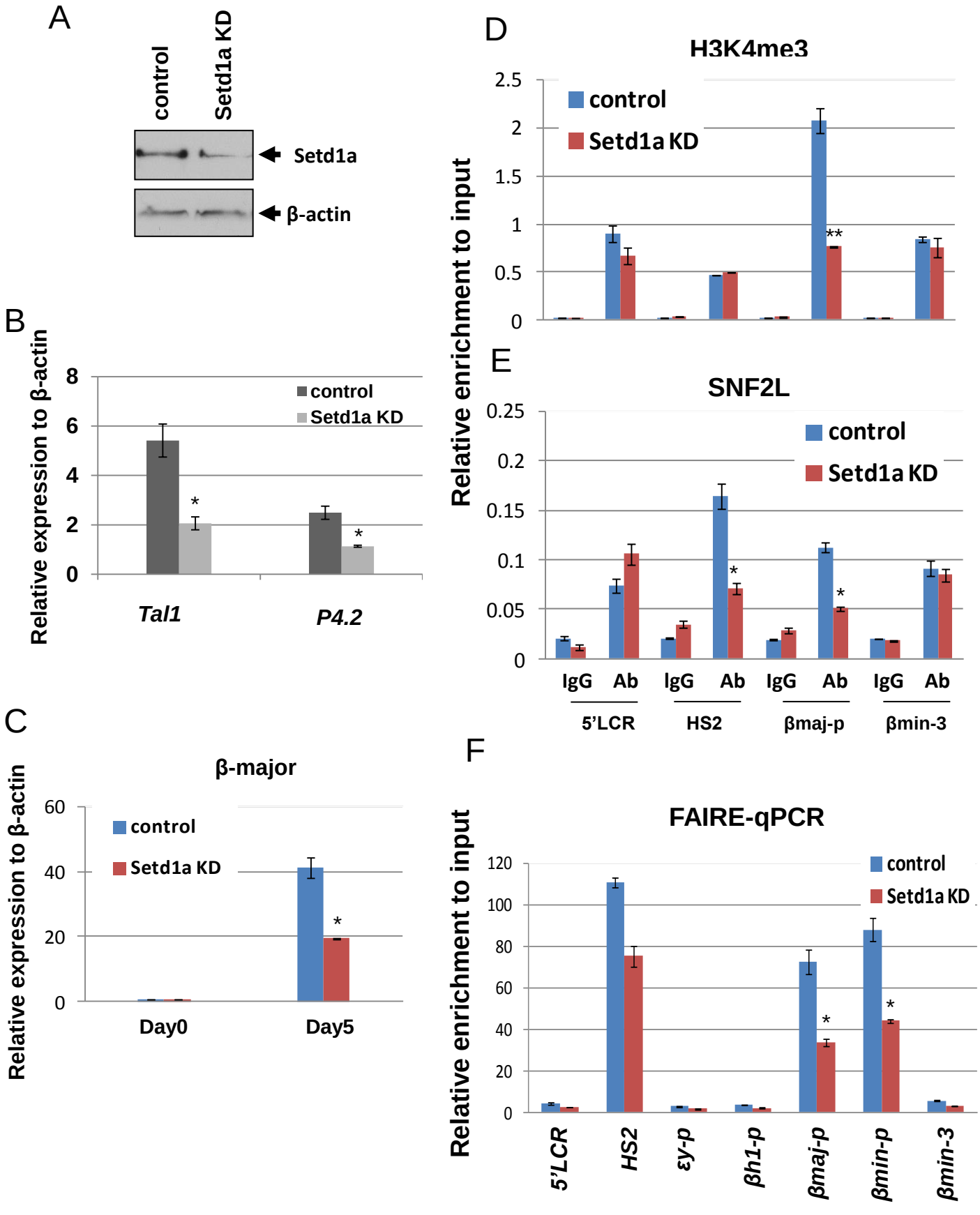

Relative expression to β-actin
βh1
control
Setd1a KD
0.6
0.4
0.2
0
*
EB13
EB16
EB19
εy
control
Setd1a KD
0.25
0.2
0.15
0.1
0.05
0
*
*
EB13
EB16
EB19
β-globin
control
Setd1a KD
0.5
0.4
0.3
0.2
0.1
0
**
**
EB13
EB16
EB19

D

EB 4 days
EB19 days

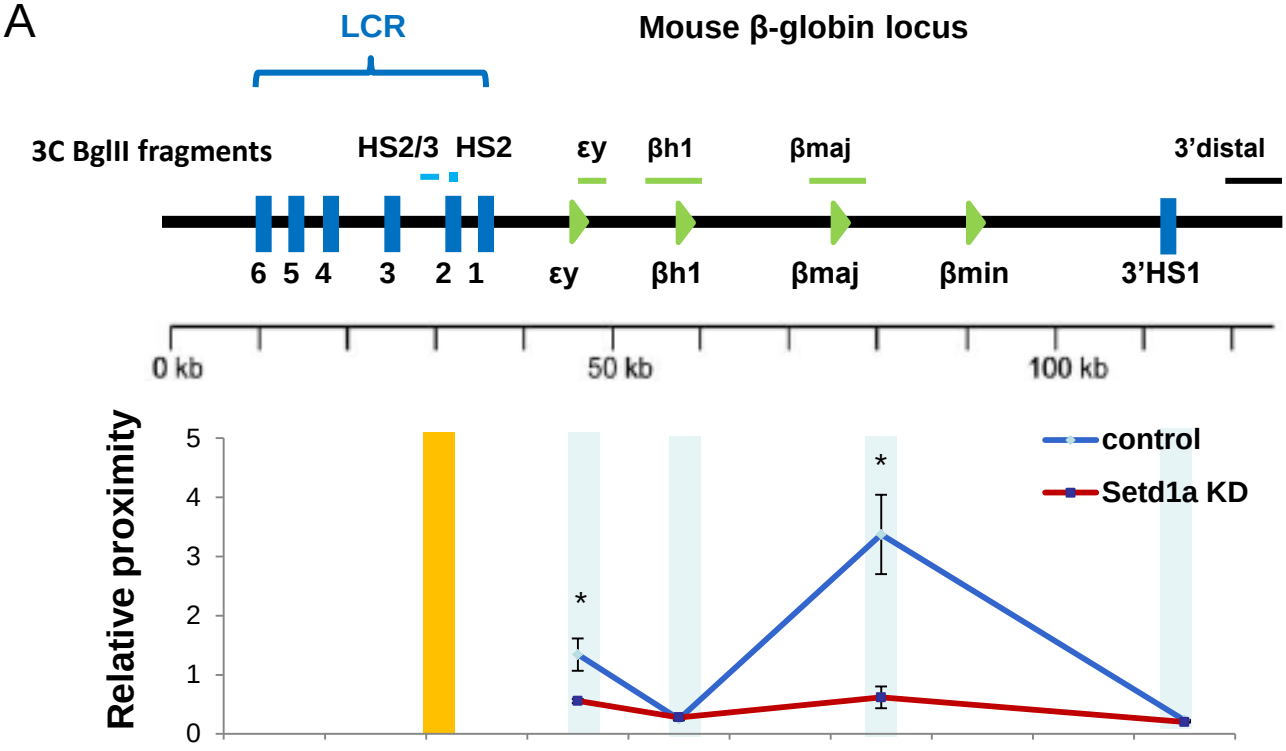
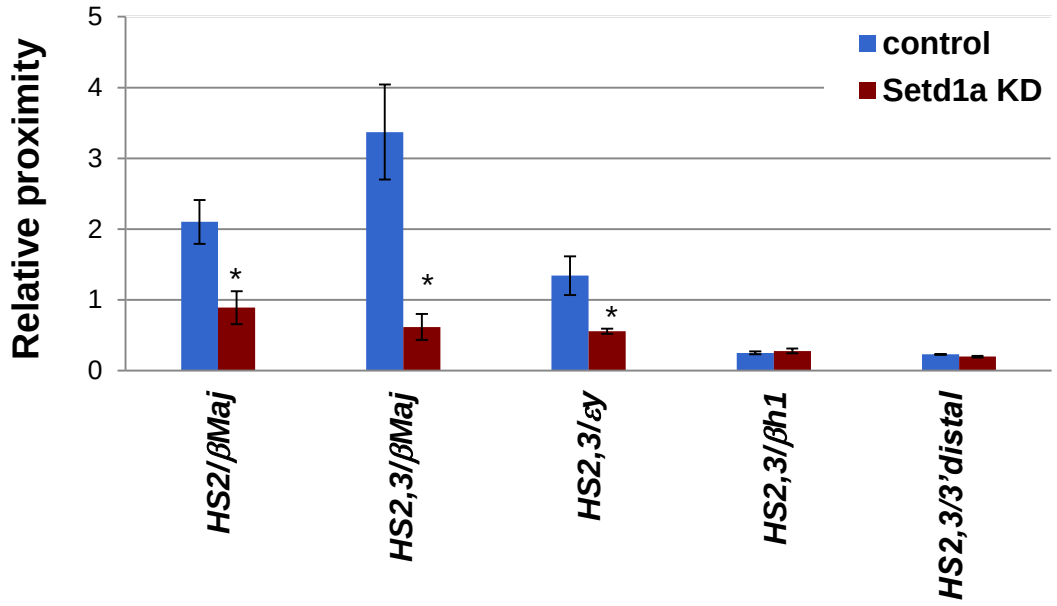

EB13
EB16
EB19


A
p4.2
control
Setd1a KD
Relative expression to β-actin
5
4
3
2
1
0
**
Day0
Day4
Day19
Notch2
control
Setd1a KD
5
4
3
2
1
0
*
Day0
Day19
B
H3K4me3
control
Setd1a KD
Relative enrichment to input
1.6
1.2
0.8
0.4
0
*
IgG
Ab
IgG
Ab
p4.2p
3'UTR
H3K4me3
control
Setd1a KD
3
2.5
2
1.5
1
0.5
0
*
IgG
Ab
IgG
Ab
Notch2p
3'UTR


A
control
Setd1a KD
Setd1a
β-actin
B
Relative expression to β-actin
control
Setd1a KD
Tal1
P4.2
C
β-major
Relative expression to β-actin
control
Setd1a KD
Day0
Day5
D
H3K4me3
Relative enrichment to input
control
Setd1a KD
E
SNF2L
Relative enrichment to input
control
Setd1a KD
IgG
Ab
5'LCR
HS2
βmaj-p
βmin-3
F
FAIRE-qPCR
Relative enrichment to input
control
Setd1a KD
5'LCR
HS2
εy-p
βh1-p
βmaj-p
βmin-p
βmin-3

Li_Figure S7

A



B