Expanded View Figures

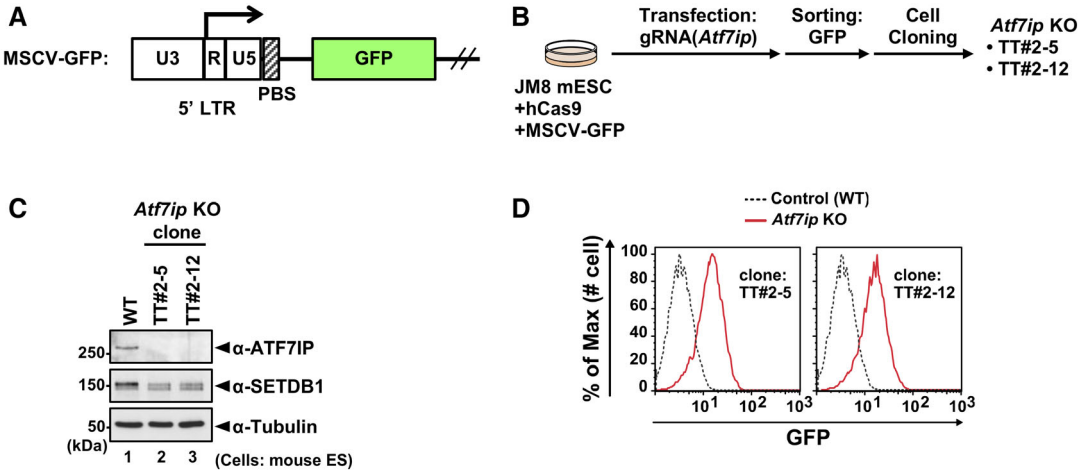


Figure EV1. Establishment and characterization of *Atf7ip* KO mESCs (related to Fig 1).

A The structure of MSCV-GFP reporter construct. PBS: primer binding site.

B Establishment of *Atf7ip* KO cell lines by CRISPR/Cas9 technology.

C Confirmation of a complete loss of ATF7IP protein and comparable expression of SETDB1 in the parental WT and established *Atf7ip* KO cell lines, TT#2-15 and TT#2-12, by WB analysis.

D Flow cytometric analysis shows that *Atf7ip* KO cell lines increase the expression of MSCV-GFP reporter.

Source data are available online for this figure.

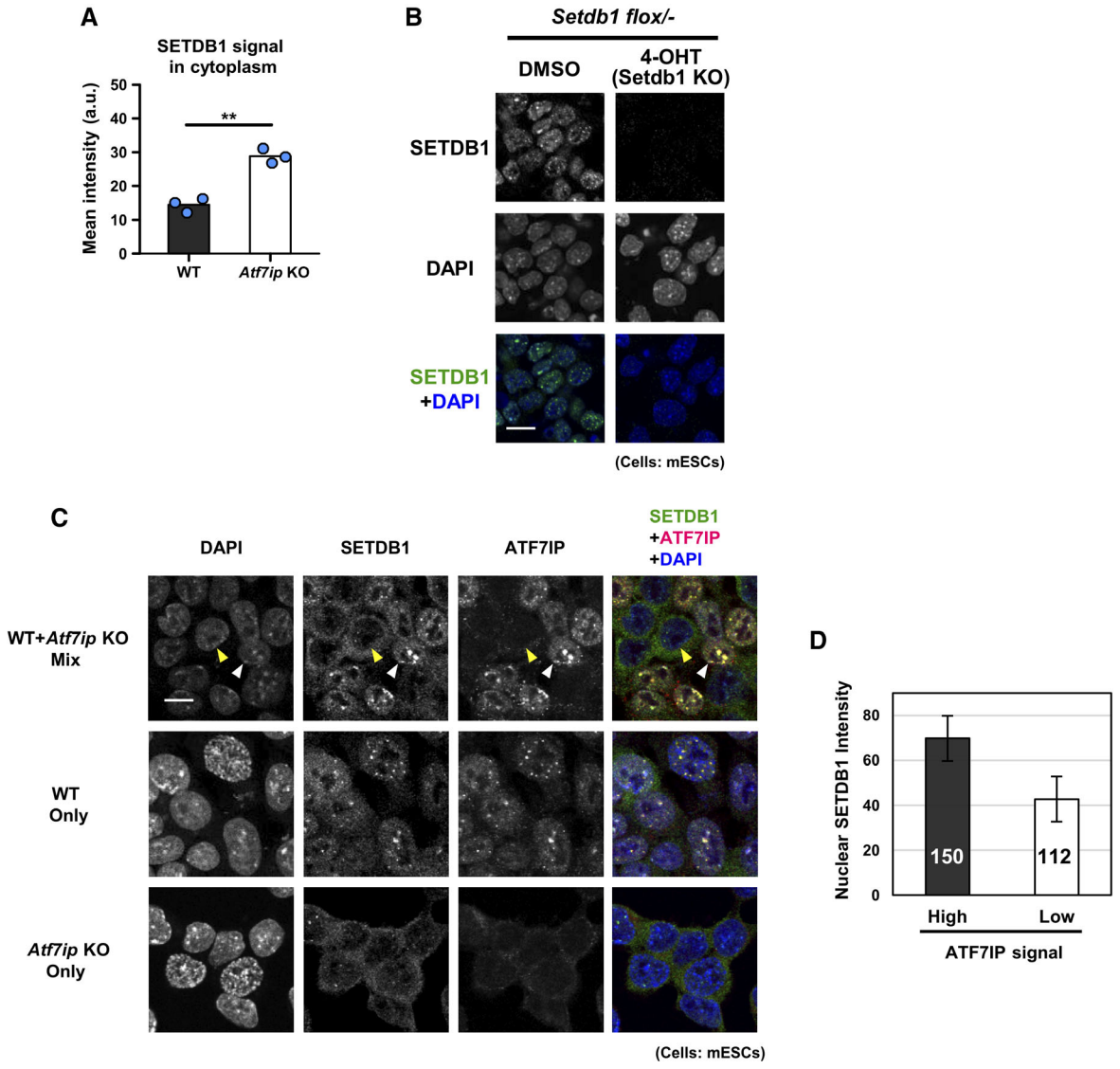


Figure EV2. Reduction of nuclear SETDB1 in *Atf7ip* KO mESCs (related to Fig 2).

A SETDB1 signal in the cytoplasm that is shown in Fig 2A was calculated. The mean from three independent experiments is shown as a bar graph with jittered points indicating the average % intensity of each experiment. Over 100 cells were analyzed per sample per experiment. $^{**}P < 0.001$ by unpaired Student's *t*-test.

B Validation of anti-SETDB1 antibody used in this study by IF analysis. Conditional *Setdb1* KO mESCs show no signal after 4-OHT treatment to deplete SETDB1. Scale bar: 7.33 μm.

C Immunostaining of ATF7IP as shown in Fig 2A was conducted using a mixture of WT and *Atf7ip* KO mESCs (1:1) (top) in addition to WT (middle) or *Atf7ip* KO (bottom) alone. The yellow and white arrowhead indicates AFP7IP signal high and low cell which are supposedly WT and *Atf7ip* KO cell, respectively. Scale bar: 10 μm.

D SETDB1 signal in the nucleus of ATF7IP high and low cells as shown in (C) top panel was calculated. The mean with SD is shown as a bar graph. Over 100 of each cell type (150 and 112 for ATF7IP high and low cells, respectively) was analyzed. Error bars: SD.

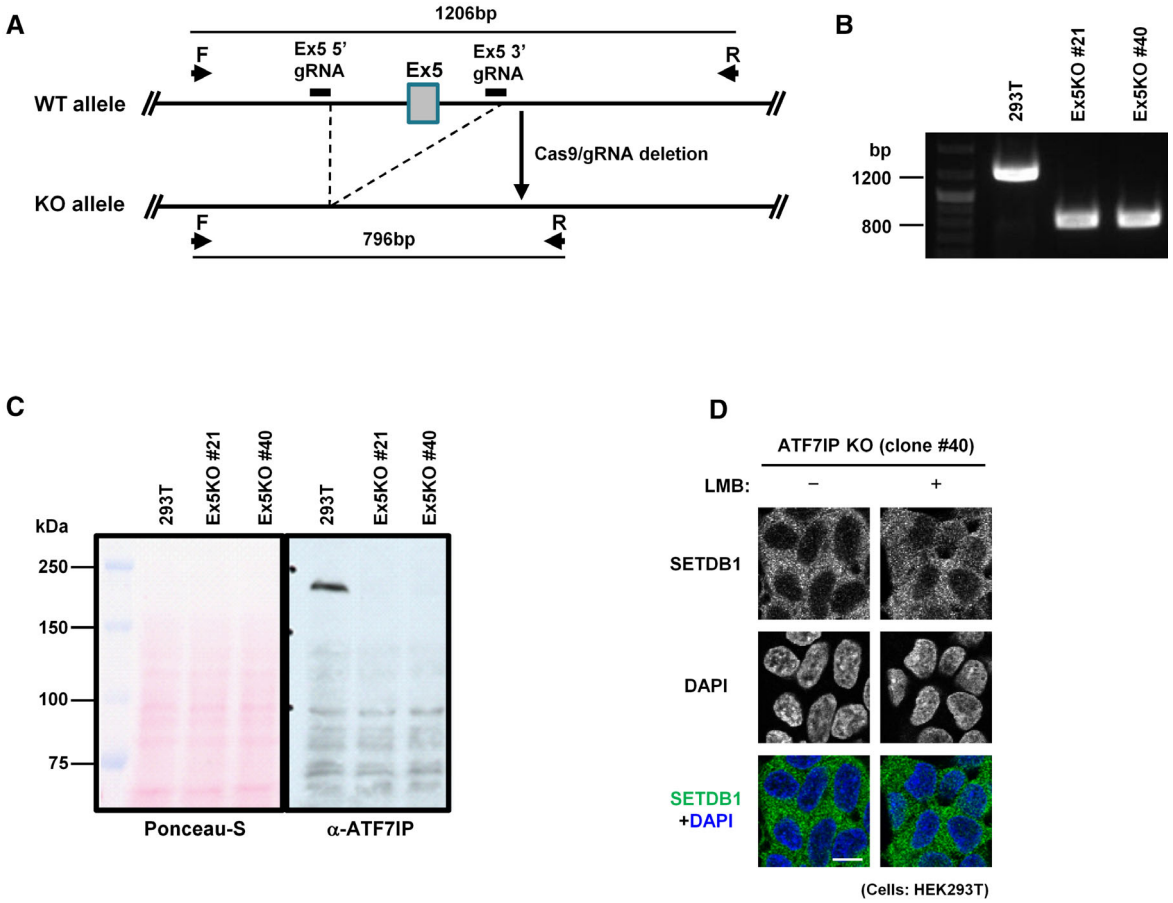

A
1206bp
F
Ex5 5'
gRNA
Ex5
Ex5 3'
gRNA
R
WT allele
Cas9/gRNA deletion
KO allele
F
796bp
R
B
293T
Ex5KO #21
Ex5KO #40
bp
1200
800
C
293T
Ex5KO #21
Ex5KO #40
293T
Ex5KO #21
Ex5KO #40
kDa
250
150
100
75
Ponceau-S
α-ATF7IP
D
ATF7IP KO (clone #40)
LMB:
–
+
SETDB1
DAPI
SETDB1
+DAPI
(Cells: HEK293T)
E
WT
ATF7IP KO
LMB:
bortezomib:
kDa
250
150
α-Ub
250
150
100
75
50
Ponceau-S
150
α-SETDB1
250
150
100
75
50
Ponceau-S

Figure EV3.

Figure EV3. Establishment and characterization of *ATF7IP* KO HEK293T cells (related to Fig 3).

A *ATF7IP* KO strategy by the CRISPR/Cas9 system. If Exon 5(Ex5) of *ATF7IP* is deleted between the 5′ and 3′ gRNA target sequences, PCR with primer F and R amplifies about 800-bp DNA fragment from the KO allele vs. 1,200 bp from WT allele.

B PCR validation result of two independent *ATF7IP* KO HEK293T cell clones, #21 and #40.

C Confirmation of no ATF7IP protein expression in two *ATF7IP* KO HEK293T cell clones, #21 and #40. Ponceau-S staining of same blotted membrane used for ATF7IP detection. The representative data of two independent experiments are shown.

D SETDB1 cellular localization in another *ATF7IP* KO HEK239T cell clone, #40 without or with LMB treatment for 5 h. The representative data of multiple independent experiments are shown. Scale bar: 10 μm.

E Impact of bortezomib treatment on cellular protein stability. Same number of WT or *ATF7IP* KO HEK293T cells was aliquoted into the 1.5-ml tubes and untreated or treated with LMB (10 ng/ml) or/and bortezomib (100 nM) for 5 h before analysis which is same condition as used for Fig 3E. Ponceau-S staining of same blotted membrane used for poly-Ub and SETDB1 detection, respectively. The representative data of two independent experiments are shown.

Source data are available online for this figure.

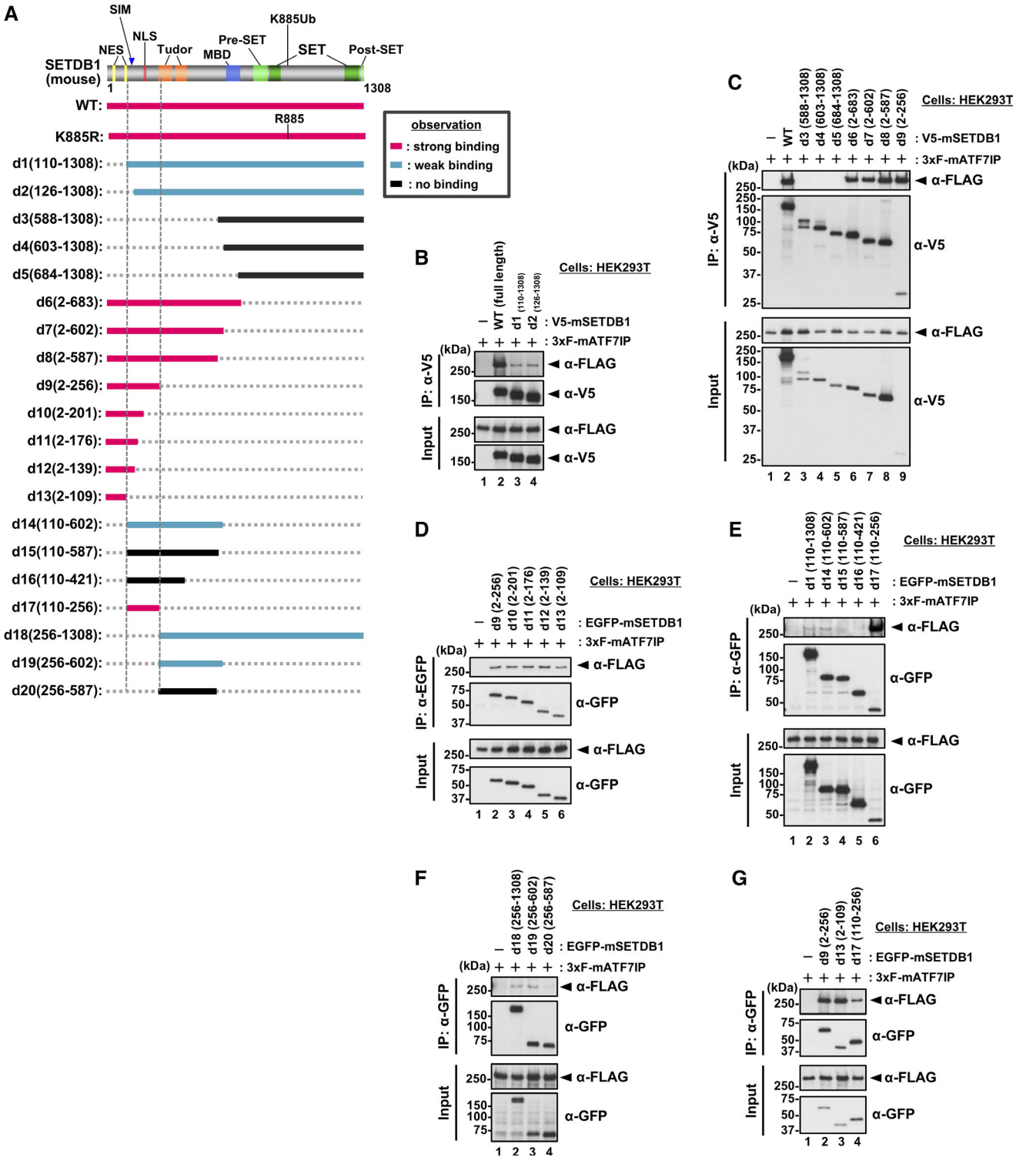
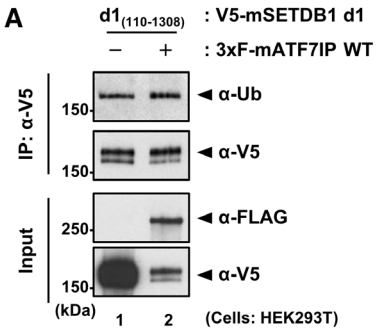


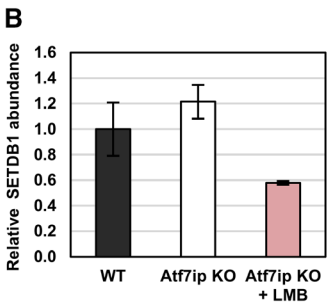
Figure EV4. Mapping analyses for the identification of ATF7IP-interacting region of SETDB1 (related to Fig 4).

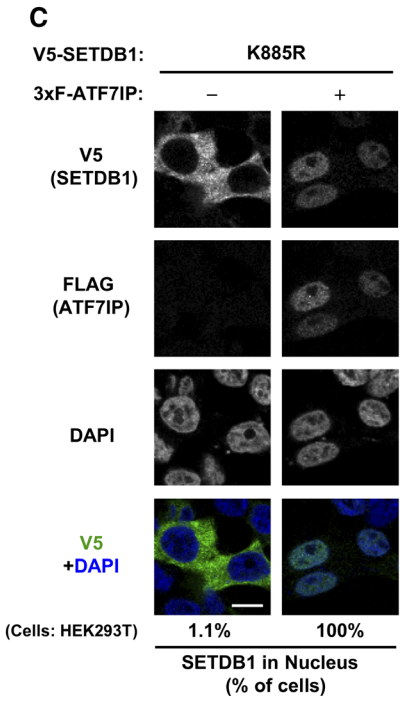
A Summary of the co-IP experiments. After the transfection, V5-SETDB1 was immune-purified with anti-V5 antibody and used for WB analysis. Location of estimated NES and NLS motifs is shown [23].

B–G HEK293T cells were co-transfected with 3xFLAG-ATF7IP and V5-tagged or EGFP-fused SETDB1 WT or deletion mutants as shown in (A). After IP with anti-V5 antibody (B and C) or anti-GFP antibody (D–G), the bound ATF7IP was detected by WB analysis. The residues 2–109 (d13) of SETDB1 are mostly sufficient for the interaction with ATF7IP (G).

Source data are available online for this figure.







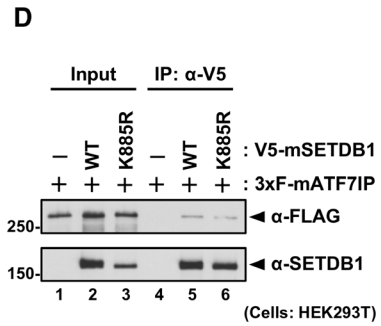


Figure EV5. Characterization of SETDB1 d1 and K885 mutant, and SETDB1 stability in *Atf7ip* KO treated with LMB (related to Figs 4 and 5).

A V5-SETDB1 d1 mutant expression with or without 3xF-ATF7IP co-expression in HEK293T cells as shown in Fig 4B.

B Measurement of total SETDB1 signals of WT, *Atf7ip* KO, and *Atf7ip* KO treated with LMB shown in Fig 5C top panel. Data from three different samples in a single experiment. Error bars: SD.

C HEK293T cells were transfected with V5-SETDB1 K885 mutant and either empty or 3xF-ATF7IP expressing vector. Over 50 transfected cells per sample were analyzed. The representative data of two independent experiments are shown. Scale bar: 10 μm.

D HEK293T cells were transfected with 3xF-ATF7IP and either empty, V5-SETDB1 WT, or K885 mutant. After V5-IP, the bound 3xF-ATF7IP was detected by WB analysis.

Source data are available online for this figure.