Short hairpin RNA (shRNA) mediated knockdown

Constructs used for shRNA expression were generated by subcloning shRNA oligonucleotides (Table S1) into the pSuper.retro.puro vector following the manufacturer's instructions (Oligoengine, Seattle, WA). Retrovirus generation and infections were performed as described previously[1]. In brief, Phoenix A cells were cotransfected with 15 μg of shRNA vectors and 2.5 μg of expression plasmids for gag-pol and env using the calcium phosphate transfection procedure. The recombinant retroviruses were collected 48 hours after transfection by centrifugation of the culture supernatant at 3000 rpm for 20 min at 4°C, followed by filtration through a cellulose acetate membrane with a 0.45 μm pore size. To generate stable knockdown cells, 5×10^5 exponentially-growing cells were co-cultured with virus particles in the presence of 4 μg/mL polybrene followed by puromycin selection (1 μg /mL) for 2 weeks. Several single clones were selected, further expanded, and confirmed by western blot analysis.

Barrier analysis

The barrier assay was performed by seeding 2×10^4 cells/ml of the stable pSuper or knockdown clones in the growth medium with or without hygromycin selection (1 μg/μl) in 6-well plates. The medium was changed every 4 days for 20 days. The cells were subjected to fluorescence-activated cell sorting (FACS) analysis. Approximately 5×10^5 cells were collected by centrifugation at 1000 rpm for 5 min at 4°C, washed twice with 2% FBS/PBS, and stained with

phycoerythrin (PE)-conjugated anti-human CD25 (IL2R) antibody (eBioscience, San Diego, CA) for 15 min at room temperature. Immunostained cells were then washed twice with 2% FBS/PBS before analyzed using Accuri C6 Flow Cytometer (Accuri Cytometers, Ann Arbor, MI). A total of 10, 000 events were counted and the unstained cells were used to set markers for the autofluorescence negative control.

Purification of USF1 complexes and histone methyltransferase (HMT) assay

Purification of the USF1 containing complex was performed using stably transduced HeLa S3 cells with an N-terminal FLAG-hemagglutinin (HA) double-tagged chicken USF1 construct as described previously [2]. Briefly, Nuclear extracts from 8L of culture were purified with anti-FLAG antibody-conjugated agarose (Sigma) packed immunoaffinity column and then eluted with 200 μl of washing buffer plus 50 μg/ml FLAG peptide.

The eluted USF1 complex was incubated with histone H3 tails with or without tri-methylated Lys 4 residue in a total volume of 30 μl containing 20 mM Tris-HCl (pH 8.0), 4 mM EDTA, 1 mM PMSF, 0.5 mM DTT, and 1 μl of [^{3}H]S-adenosylmethionine (15 Ci/mmol; PerkinElmer, Waltham, MA) at 30°C for 1 h. Reaction products were resolved on 4-12% Bis-Tris Gel (Invitrogen) and visualized following autoradiography of dried gels.

Nucleosome sliding assays

Nucleosome sliding assays were performed essentially as described previously [3]. Briefly, core histones were isolated from HeLa cells according to a standard protocol. A 194 bp fragment with a strong nucleosome positioning sequence was generated by PCR amplification of the plasmid

pGEM-3Z-601 (34-227 bp) using the sequence-specific primers (Table S1). For reconstitution of mononucleosome, 5 μg of the 194 bp DNA fragment was incubated in a 1:1 ratio (w/w) with HeLa core histones in 50 μL of 2 M NaCl, 10 mM Tris-HCl (pH 8.0), 1 mM EDTA, 0.5 mM benzamidine and 100 μg/ml BSA. The reaction was dialyzed sequentially for 1 hr at 4 °C against 1.25, 1.0, 0.75 M NaCl and then against TE (10 mM Tris-HCl [pH 8.0], 0.5 mM EDTA). Nucleosome sliding assays were performed at 37°C for 30 min in a standard volume of 10 μl containing 10 mM Tris-HCl (pH 7.6), 50 mM NaCl, 3 mM $MgCl_2$, 1 mM β-mercaptoethanol, 0.1 μg/μl BSA, and 1 mM ATP. About 100-200 ng DNA equivalent of assembled mononucleosomes were incubated with either purified NURF or USF1 containing complexes and stopped by adding 2.5 μl of loading buffer (10 mM Tris-HCl [pH 7.6], 1 mM EDTA, 60% sucrose, 0.01 % xylene cyanol). Monocleosomes waere resolved on a native 6% PAGE, stained with ethidium bromide and analyzed by a Typhoon 9410 imager (Molecular Dynamics) using Image-Quant (GE Healthcare).

Immunoprecipitation and Western blot analysis

Immunoprecipitation and western blot analysis was carried out as described previously [4]. Briefly, nuclear extracts were prepared as described above and pre-cleared for 2 hours at 4°C by incubation with normal rabbit IgG (Bethyl Laboratories) and protein A-Sepharose 4B (PharmaciaBiotech) to prevent nonspecific binding. Lysates were then incubated with anti-USF1, anti-SET1 antibody, or normal rabbit IgG for overnight at 4°C. The immune complexes were recovered with protein A-Sepharose 4B and washed four times with RIPA buffer containing 10 mM Tris-HCl, pH 8.0, 140 mM NaCl, 0.025% NaN3, 0.5% Triton X-100,1% sodium deoxycholate, 0.5 mM DTT and 0.2 mM PMSF. For immunoblot analysis, immunoprecipitated

complexes were fractionated by SDS-PAGE, electrotransferred to polyvinylidene difluoride membranes and then blotted with specific primary antibodies. Proteins were visualized by enhanced chemiluminescence (Pierce, Rockford, IL).

ChIP analysis

Nuclei from 6C2 or K562 cells were prepared in lysis buffer containing 10 mM Tris-HCl (pH 7.5), 10 mM NaCl, 3 mM MgCl2, 0.4% NP-40 and protease inhibitors. Before MNase digestion, nuclei were pelleted and resuspended in the same lysis buffer plus 1 mM CaCl2. The A260 value was adjusted to 1.25 and the resuspended nuclei were digested with 0.1 and 0.2 U/ μl MNase (Worthington) for 10 min at 37°C. The reaction was stopped by adding EDTA (pH 8.0) to a final concentration of 10 mM, and the suspension was centrifuged at 2500 rpm for 5 min, retaining supernatant (S1). The nuclei pellet was then resuspended in lysis buffer plus 0.25 mM EDTA, incubated on ice for 15 min, and recentrifuged at 10,000 rpm for 10 min after passing four times through a 20-gauge needle. The supernatant was combined with S1 and incubated with 150 mM NaCl for 20 min at 4°C to remove histones H1/H5, followed by centrifugation at 10, 000 rpm for 15 min. Nucleosomes were purified on a 5%–30% sucrose gradient containing 80 mM NaCl, 10 mM Tris-HCl (pH 7.5), 0.2 mM EDTA and protease inhibitors. Fractions containing di- and tri-nucleosomes were pooled, desalted using Econo-Pac 10DG columns (Bio-Rad), and exchanged into fractionation buffer (50 mM NaCl, 10 mM Tris pH 7.5, 1mM EDTA and Protease Inhibitors). 20 μg of nucleosomes were pre-cleared with protein A agarose at 4°C for 1 hour, followed by incubation with 10 μg of specific antibody at 4 °C for 1 hour. 50 μl of protein A agarose was added and incubated at 4°C overnight. Immunoprecipitated chromatin was collected, washed 5 times with fractionation buffer and then eluted with the same fractionation

buffer supplemented with 1.5% SDS followed by 0.5% SDS. Two elutes were combined and digested with Proteinase K at 42°C for 1 hour. DNA was extracted by phenol/chloroform and precipitated for qPCR analysis.

For ChIP analysis on cross-linked chromatin, cells were cross-linked with sequential ethylene glycol bis(succinimidyl succinate) (Sigma) (1.5 mM) for 30 min, followed by 1% formaldehyde for 10 min. Reactions were stopped by adding glycine to a final concentration of 0.125M. Cells were lysed twice in lysis buffer (10 mM Tris-HCl (pH 7.5), 10 mM NaCl, 3 mM MgCl2, 0.4% NP-40 and protease inhibitors) and then resuspended in chromatin sonication buffer containing 50 mM Tris (pH 8.0), 10 mM EDTA, 1% SDS, and protease inhibitors. Chromatin was sheared by sonication to generate 200 to 800-bp DNA fragments using a Bioruptor UCD 200 (Diagenode, Denville, NJ). Chromatin preparations were then incubated with specific antibody and immune complexes collected with protein A agarose and washed with low salt buffer, high-salt, LiCl buffer and TE buffer. After reverse cross-linking, the immunoprecipitated DNA was purified by phenol/chloroform extraction and ethanol precipitation.

Realtime qPCR analysis was done on a Bio-Rad CFX96 Real-Time PCR Detection System. The relative concentrations of target sequences in the immunoprecipitated (IP) compared to input (Ref) fractions were measured by SYBR green fluorescence. Analysis of dissociation curves showed that primer pairs yielded single products. The relative enrichment was calculated using the following equation: $2^{Ct(Ref) - Ct(IP)}$, where *Ct(Ref)* is the *Ct* of the input DNA and *Ct(IP)* is the *Ct* of ChIP DNA. Primer sequences are listed in Table S1.

Nucleosome scanning assay

Mononucleosomes were prepared as described above. Briefly, nuclei were isolated, digested with MNase for 10 min at 37°C and the reaction was stopped by adding EDTA (pH 8.0) to a final concentration of 10 mM. Fractions of mononucleosomes were purified on a 5-30% sucrose gradient and then deproteinized by Proteinase K digestion in the presence of 0.03% of SDS at 37 °C for 30 min. The DNA was then extracted with phenol-chloroform, followed by ethanol precipitation. The resulting mononucleosomal DNA was analyzed by a set of overlapping primer pairs covering the 5'HS4 insulator region (Supplemental Table 1) by using a CFX96 Real-Time PCR Detection System (Bio-Rad). Additionally, the value obtained for the 16kb condensed region (designated primer pair 16kb) was arbitrarily set to "1", and all of the other nucleosomal DNA enrichment values are presented relative to this standard. Primer sequences are listed in supplemental Table 1.

Table S1. Primer sequences

	Primer	*Primer sequences*
shRNA	chicken Set1	Target: 5'- GAGGAGAACTGGAGGAGAA-3'
	chicken Bptf-1	Target: 5'- CAGCAAACCTAGAGAATTT-3'
	chicken Bptf-2	Target: 5' GGAGAGGAATATAGAGTAA-3'
	human SET1	Target: 5'- GGAAAGAGCCATCGGAAAT-3'
Nucleosome sliding assay	601	For: TCACACCGAGTTCATCCCTTATGT Rev: ACAGGATGTATATATCTGACACGT
RT-PCR	cBptf	For: GAGGAGGACGACGATGAC Rev: ACCAGAGCAGCACAGAAG
	cGapdh	For: TGCAGGTGCTGAGTATGTTGTGGA Rev: CCACAACACGGTTGCTGTATCCAA
	α-spectrin	For: CATGAGCCCAAGTTTGAGAAG Rev: GCAGTATAGTCCTCCAGTGAGACA
	β-actin	For: GCACAGAGCCTCGCCTTT Rev: CGGCGATATCATCATCCAT
ChIP-human	HS2	For: TGTGTGTCTCCATTAGTGACCTCCC Rev: TTTTGCCATCTGCCCTGTAAGC
	3'HS1	For: TCACTGAAGTAGGGAGGGAAGAA Rev: AAGGTCATTCCTTTAATGGTCTTTTC
	Spectrin 183	For: GCCGGATCCATGTCTTCTAAAGATAATGTCGATTG Rev: GGGGGATCCGGTTTAGAACCTGGCAAGATAA

	3 region	For: TGAGTGGCTGGCTTATACCC Rev: CAGCTCTCTTCACCCCAAGA
Nucleosome scanning assay	16 kb	For: CAGCAGACGCTGTGGTGAA Rev: CTTGCAGGATGCAGACTGGA
	up-2	For: CCCCAAAGTGAAATCATG Rev: GGATTGAAGGATGCTGAG
	up-4	For: CTCTGTGCTCAGCATCCTTCAAT Rev: CCTTTCGGCACTTTCTTCCTTT
	up-5	For: GCACCTCCTCTGCAAAAC Rev: CCTTTCGGCACTTTCTTCCTTT
	up-6	For: CCTCCAGGAAACCACTCG Rev: TTCTTCAACCTTTCGGCACT
	up-7	For: CCTCTCCCGGAGACCTTA Rev: AGCCTGCTTCTTTTCTTCAAC
	up-9	For: AAAGGAAGAAAGTGCCGAAAG Rev: TTCCAGGACCTTCCAGGA
	1	For: AGAAGCAGGCTTTCCTGGAAG Rev: GGGAGGGACGTAATTACATCC
	4	For: AAAGCCCCCAGGGATGTAAT Rev: GGGCGGCTCGCTGCTG
	5	For: CGCTAGGGGGCAGCAG Rev: GATCCCGTGCCACCTTC
	N6	For: CCCCGCATCCCCGAG Rev: GAAAGCGATCCCGTGC
	7	For: GCAGCGTGCGGGGACAGC Rev: GCGTTCAGAGGAAAGCGATC
	9	For: CGGGGAAGGTGGCACGGGATC Rev: CTCAAAGAGCAGCGAGAAGCGTTC
	10	For: TTCCTCTGAACGCTTCTC Rev: CTGTCATTCTAAATCTCTCTTTC
	12	For: TTCTCGCTGCTCTTTGAG Rev: CCAGGCCGTTCTATGATTC
	15	For: CTTTAGGCTGAAAGAGAGATTTAG Rev: CACTGTGCTCCTTTGC
	17	For: TGCTATGTGCAGGGTCAT Rev: GCATTCAAGGCCAGGCTG
	18	For: CACATCCAGCCTGGCCTT Rev: ACTGAACAGGTTGCCCAA
	19	For: CATCCACAGCCTCCTTGG Rev: GAGGTTTGGGTTGGATATGAG

Figure S1. USF1 forms a complex with hSET1 and NURF complexes. Nuclear extracts from HeLa cells expressing Flag-USF1 were fractionated through a Sephacryl S-300 HR column. The fractions were collected and analyzed by western blotting with antibodies against

(A) USF1, **(B)** USF2, **(C)** hSET1, **(D)** HCF1, **(E)** ASH2L, and **(F)** WDR5. The hSET1 complex comigrates with USF1 at the size of ~1.8 MDa. **(G and H)** Endogenous USF1 associates with SET1 and NURF complexes in erythroid cells. Nuclear extracts from MEL (G) and 6C2 cells were immunoprecipitated with USF1 or SET1 antibodies and analyzed by WB for the components of the SET1 complex.

Figure S2. (A) USF1-associated protein complexes isolated from Flag-USF1 expressed HeLa cells were fractionated through a Superose-6 column and the co-migration of protein complexes were analyzed by WB of each fraction. **(B)** The USF1 complexes containing fractions from superpose-6 column were collected and analyzed by western blotting with antibodies against USF1, USF2, hSET1, ASH2L, RBBP5, WDR5, and SNF2L. The USF1 complex containing hSET1 and NURF migrates at a size of ~1.8 MDa.

Figure S3. Enrichment of ASH2L and RBBP5 across the β-globin locus. ChIP analysis in chicken erythroid progenitor 6C2 cells. **(A)** Schematic representation of the chicken β-globin cluster. Open arrows indicate locations of primer sets used in ChIP analysis. **(B and C)** ChIP analysis of ASH2L **(B)** and RBBP5 **(C)**. **(D)** Western blotting analysis of BPTF protein level in 6C2, K562 and HeLa cells.

Figure S4. NURF containing protein complexes are responsible for chromatin barrier activity of the 5'HS4 inasulator. (A) Flow cytometry (FACS) analysis of transgenic IL2R expression was determined in the uninsulated IL2R transgene stably integrated into 6C2 genome, Clone 9. **(B)** The 809 cells contain four copies of the IL-2R transgene flanked

by the 5'HS4 insulators and expression of IL-2R is maintained in this fully insulated cell line. **(C and D)** Flow cytometry (FACS) analysis of transgenic IL2R expression was determined in the 809 clone transduced with vector control **(C)** and BPTF shRNA **(D)**. **(E)** Quantitative RT-PCR of BPTF mRNA levels isolated from vector control transfected and shBPTF transfected cells (Clone A8).

Figure S5. Comparison of Chip enrichments between IgG and H3K4me2 or H3K9me2 in the β-globin locus (A) and the IL-2R transgene flanked by the 5'HS4 insulator (B) in 6C2 cells. (C) Comparison between IgG and H3K4me2. **(D)** Comparison between IgG and H3K9me2.

Figure S6. KD of BPTF has a moderate effect on active histone modifications. (A) Schematic representation of the chicken β-globin cluster. Open arrows indicate locations of primer sets used in ChIP analysis. **(B and C)** ChIP analysis of H3K4me2 **(B)** and H3K9K14ac **(C)** at the endogenous 5'HS4 sequences in wild-type (white bars) or BPTF knockdown (black bars) cells. Relative enrichments were normalized to those observed at the HSA element to adjust for IP efficiency. **(D)** ChIP analysis of SET1 at the endogenous HSA and 5'HS4 sequences in wild-type (white bars) or BPTF knockdown (black bars) cells. **(E)** Schematic representation of the insulated IL-2R transgene, which are present in for copies in the 6C2 genome (line 809), and the position of PCR primer sets used in ChIP analysis. **(F and G)** ChIP analysis of H3K4me2 **(F)** and H3K9K14ac **(G)** at the flanking 5'HS4 sites and at the integrated IL2R transgenes comparing the pSuper control transfected and BPTF KD 809 cells.

Figure S7. Knock down of hSET1 impairs erythroid differentiation and β-globin transcription (A) ChIP analysis of the levels of H3K4me3 mark at the proximal promoter of the α-spectrin gene comparing WT and hSET1 KD K562 cells. **(B)** Chip analysis of TAF3 binding at the α-spectrin promoter in WT and hSET1 KD K562 cells. **(C)** Stable MEL clones harboring control and SET1 shRNA-expressing constructs were incubated with 1.5% DMSO and hemoglobinization was assayed by benzidine staining at day 3 and 5. (D) Total RNA was extracted from Stable MEL clones harboring control and SET1 shRNA-expressing constructs and incubating with 1.5% DMSO for 5 days. The β-globin primary transcripts were analyzed by real-time RT-qPCR and normalized to β-actin. Significant difference by Student *t* test: **P<.01*. Shown are the mean ± SDM of 3 indepenent experiments.

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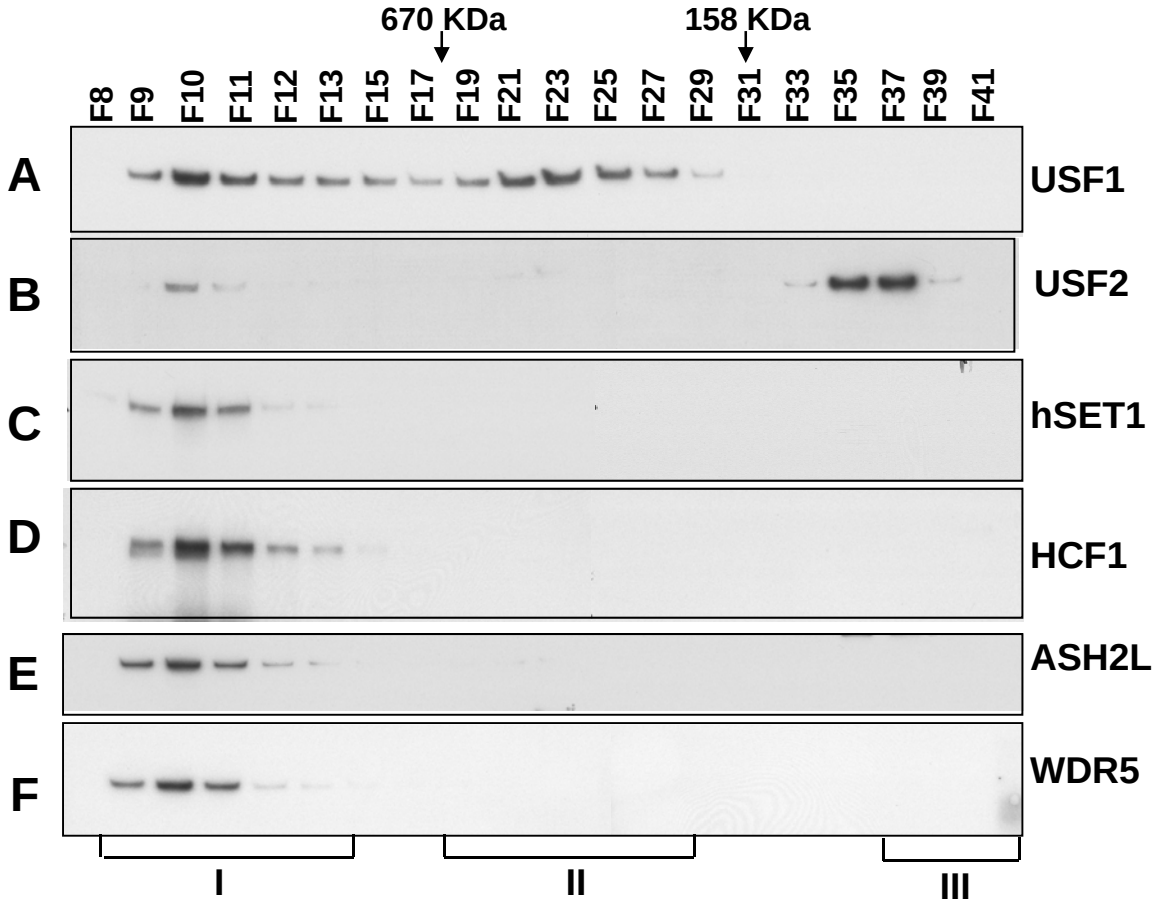
Figure S1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158 KDa
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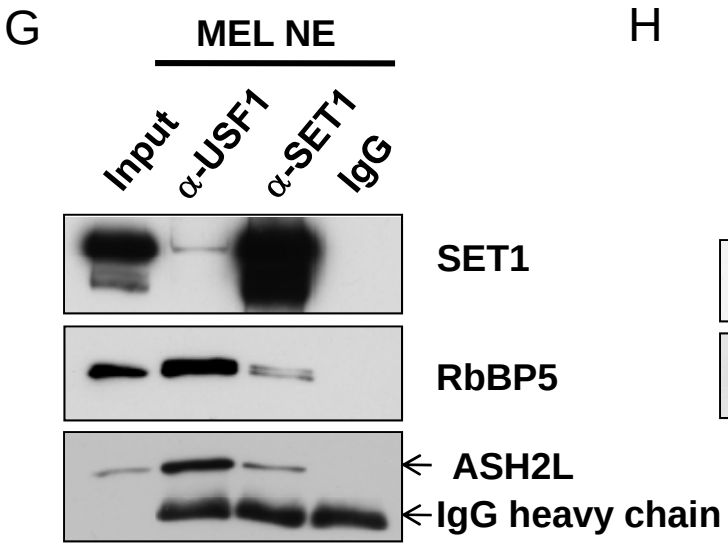
ASH2L
F
WDR5
I
II
III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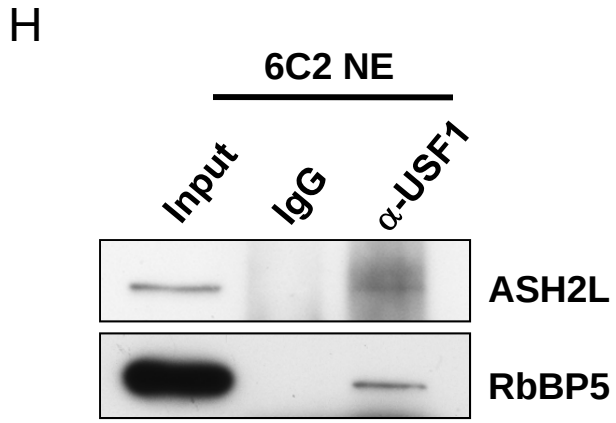
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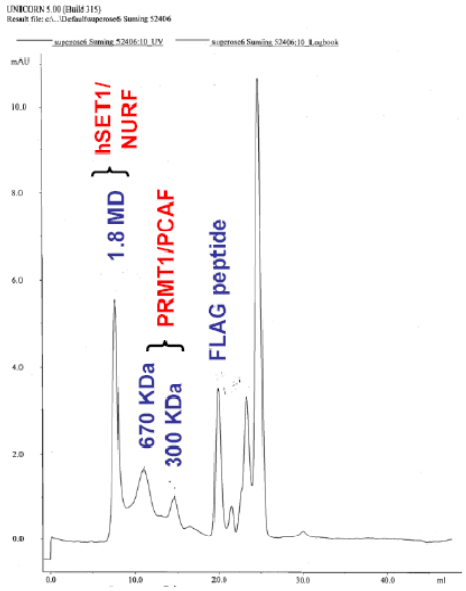
Figure S2

A_F



B

USF associated hSET1 and NURF complexes

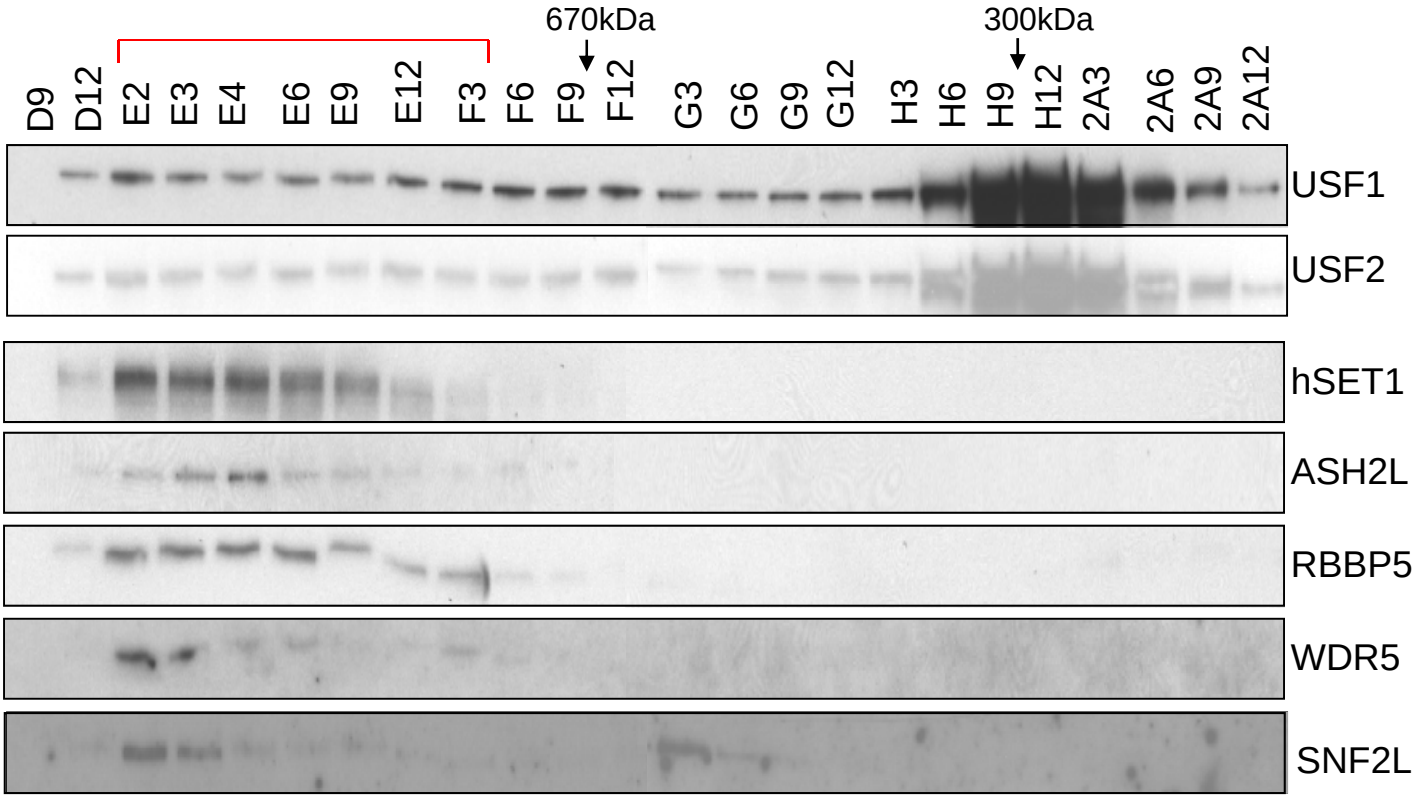
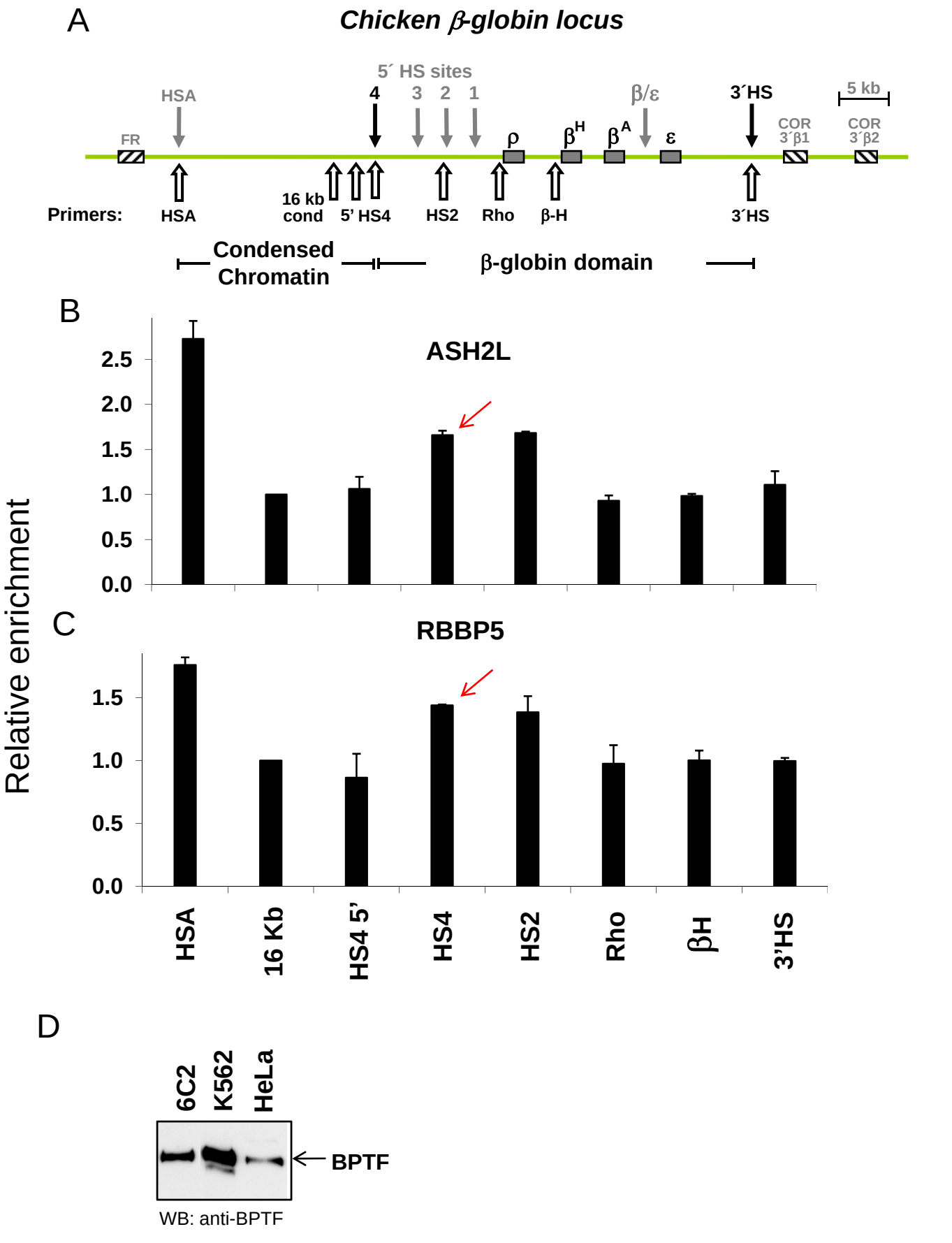


Figure S3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Chicken β-globin locus
5´ HS sites
HSA
4
3
2
1
β/ε
3´HS
5 kb
FR
ρ
β^H
β^A
ε
COR 3´β1
COR 3´β2
Primers:
HSA
16 kb cond
5' HS4
HS2
Rho
β-H
3´HS
Condensed Chromatin
β-globin domain
B
ASH2L
2.5
2.0
1.5
1.0
0.5
0.0
C
RBBP5
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Figure S4

A

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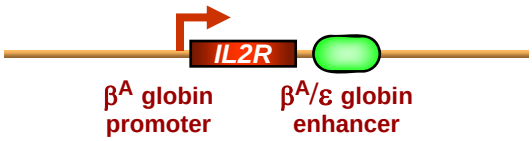

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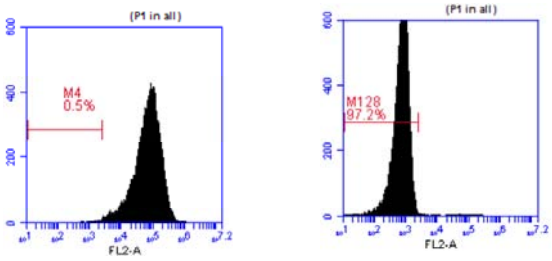

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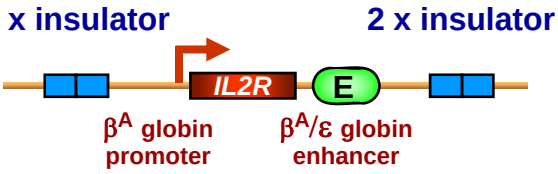

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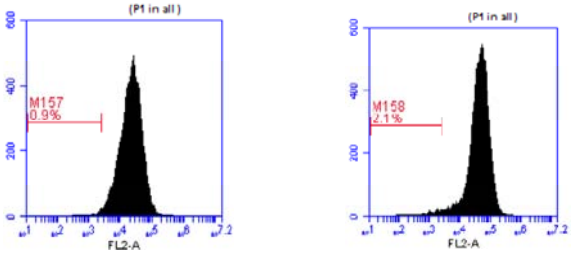

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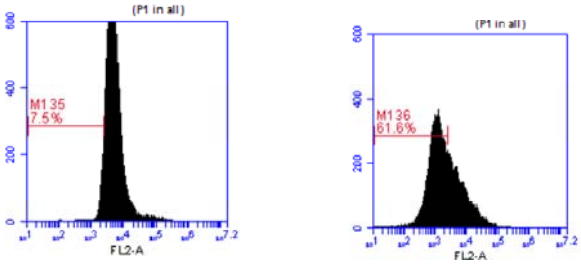

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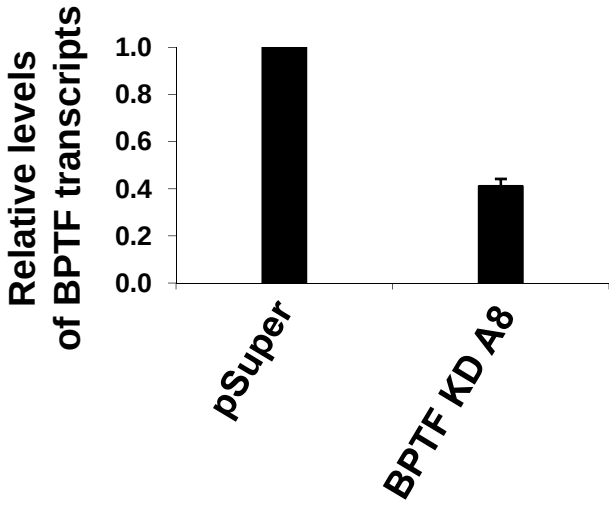
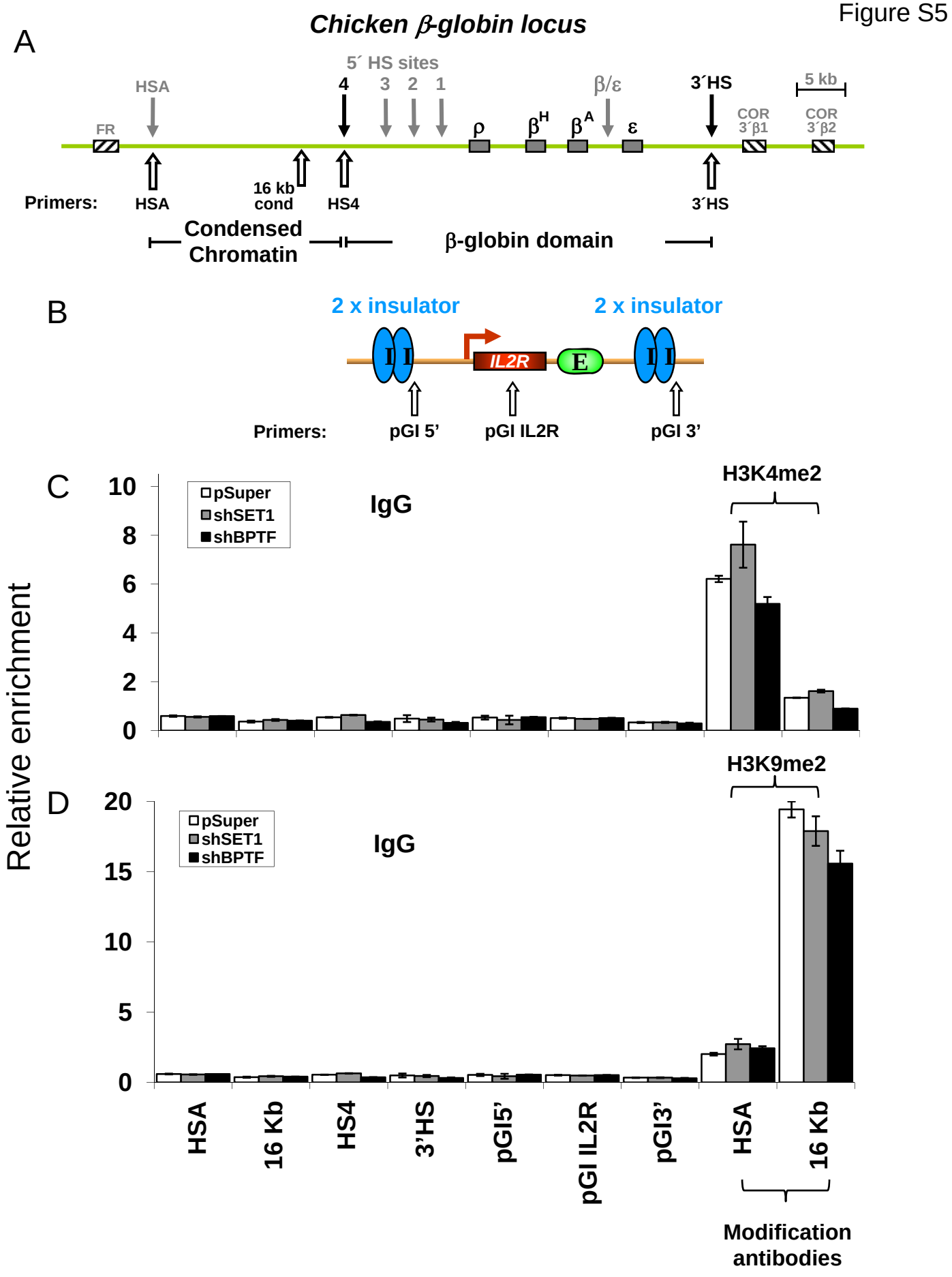


Figure S5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βA
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COR 3´β1
COR 3´β2
Primers:
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16 kb cond
HS4
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2 x insulator
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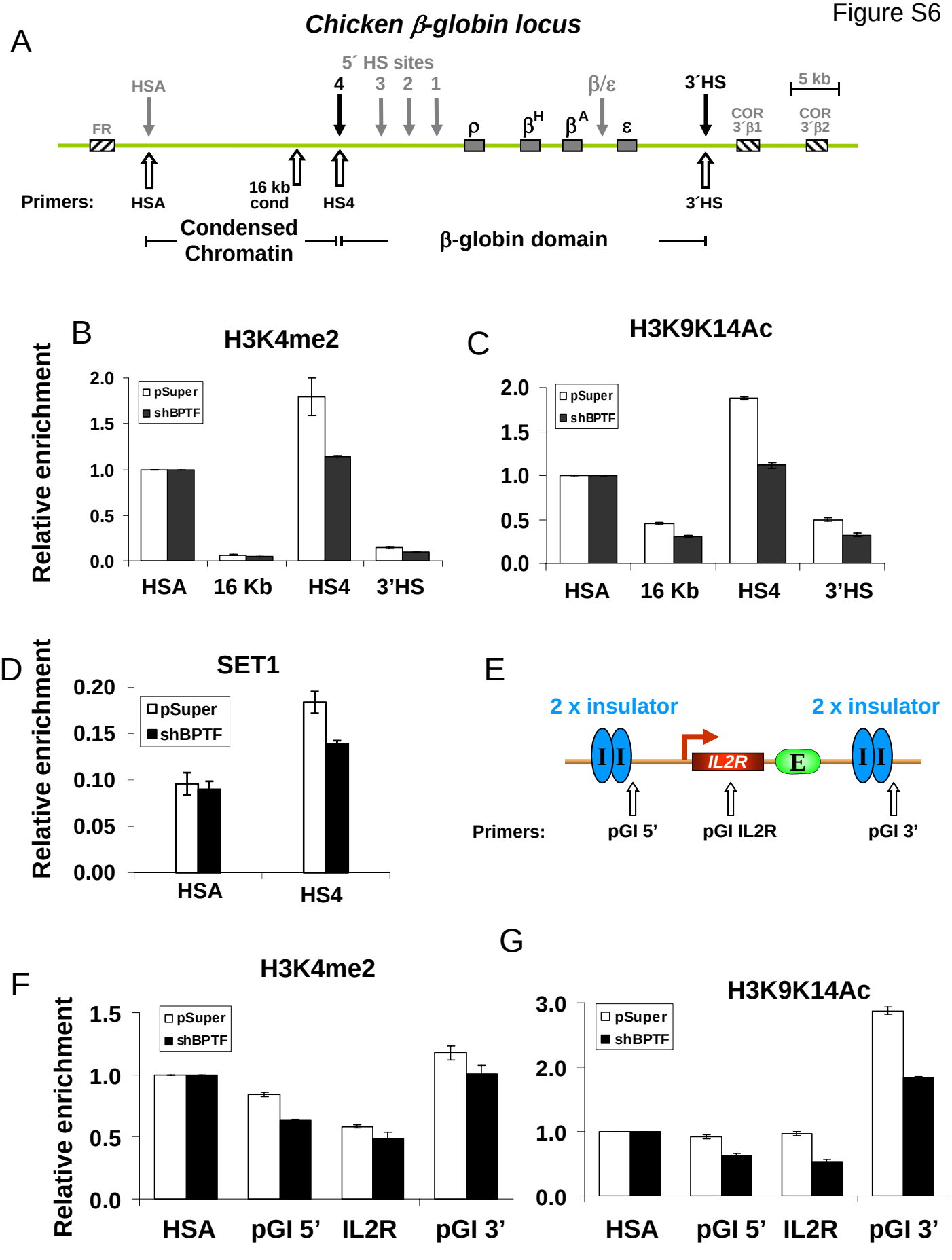
Figure S6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COR 3´β1
COR 3´β2
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HS4
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SET1
E
2 x insulator
2 x insulator
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Figure S7

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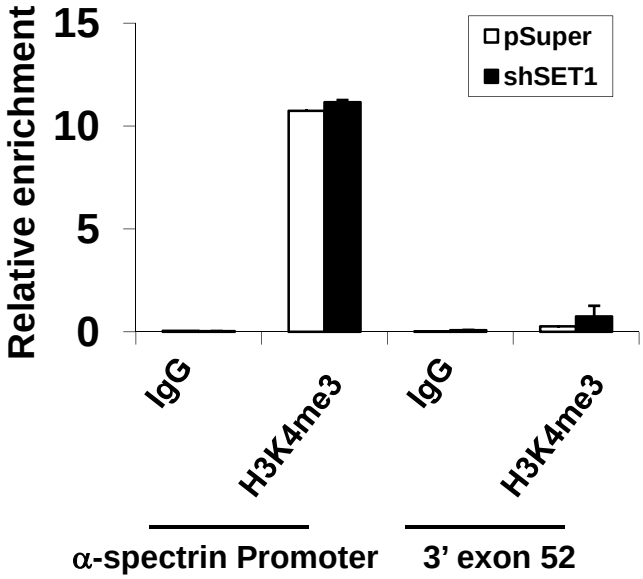

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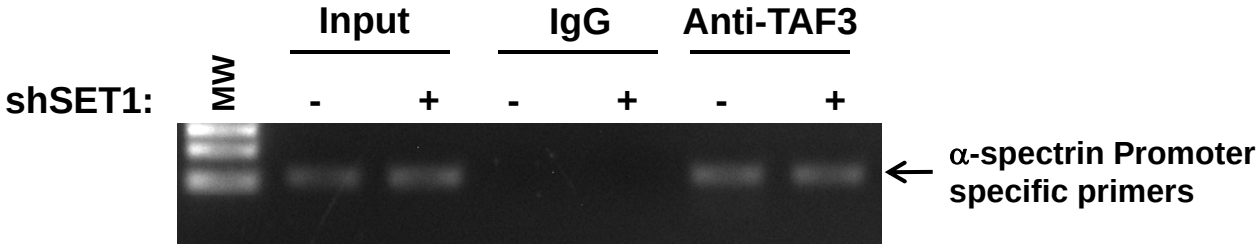

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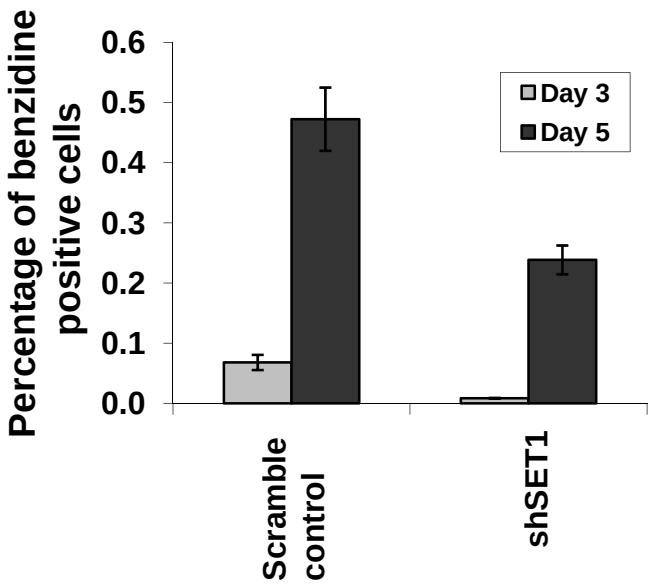

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