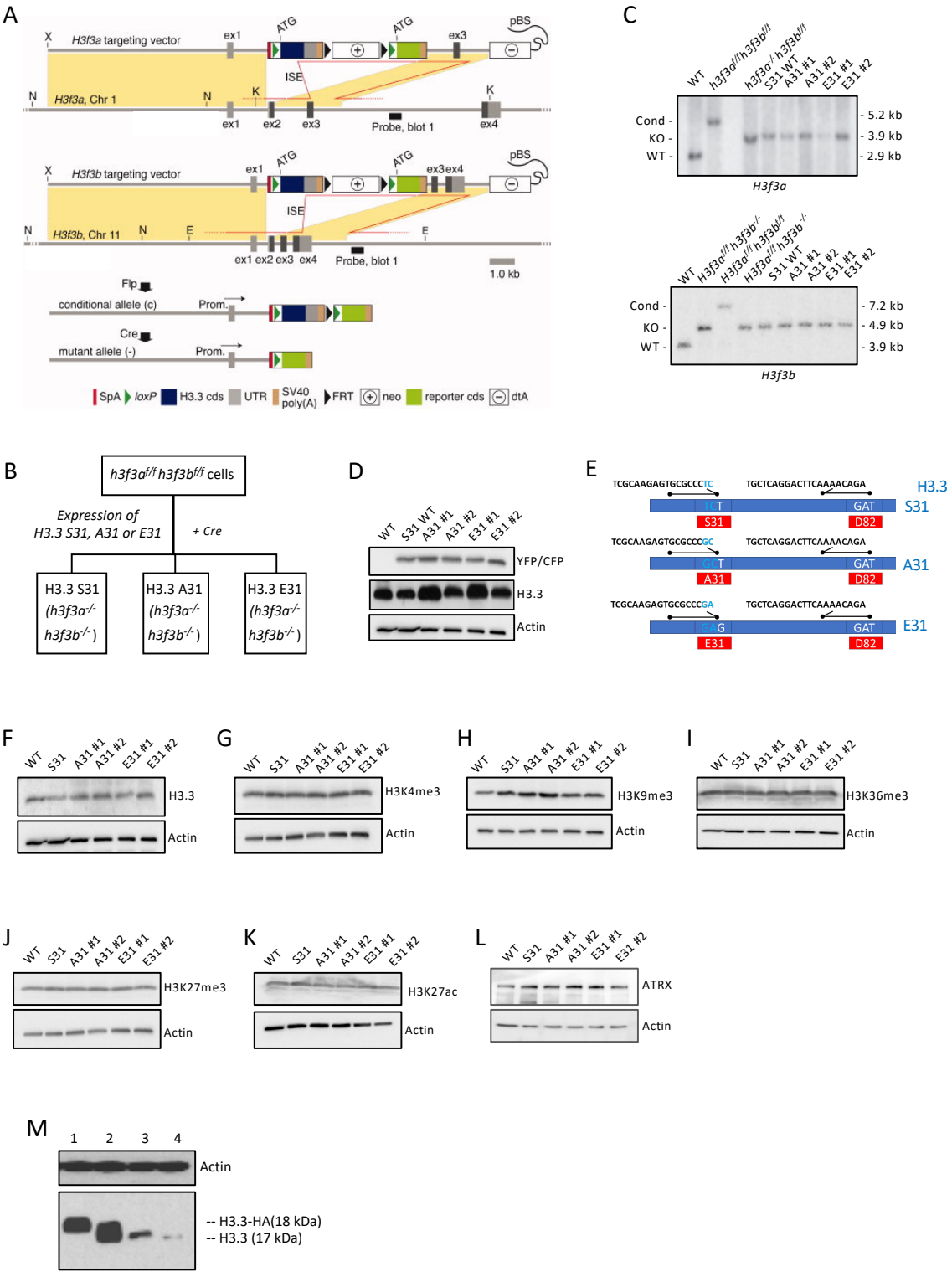
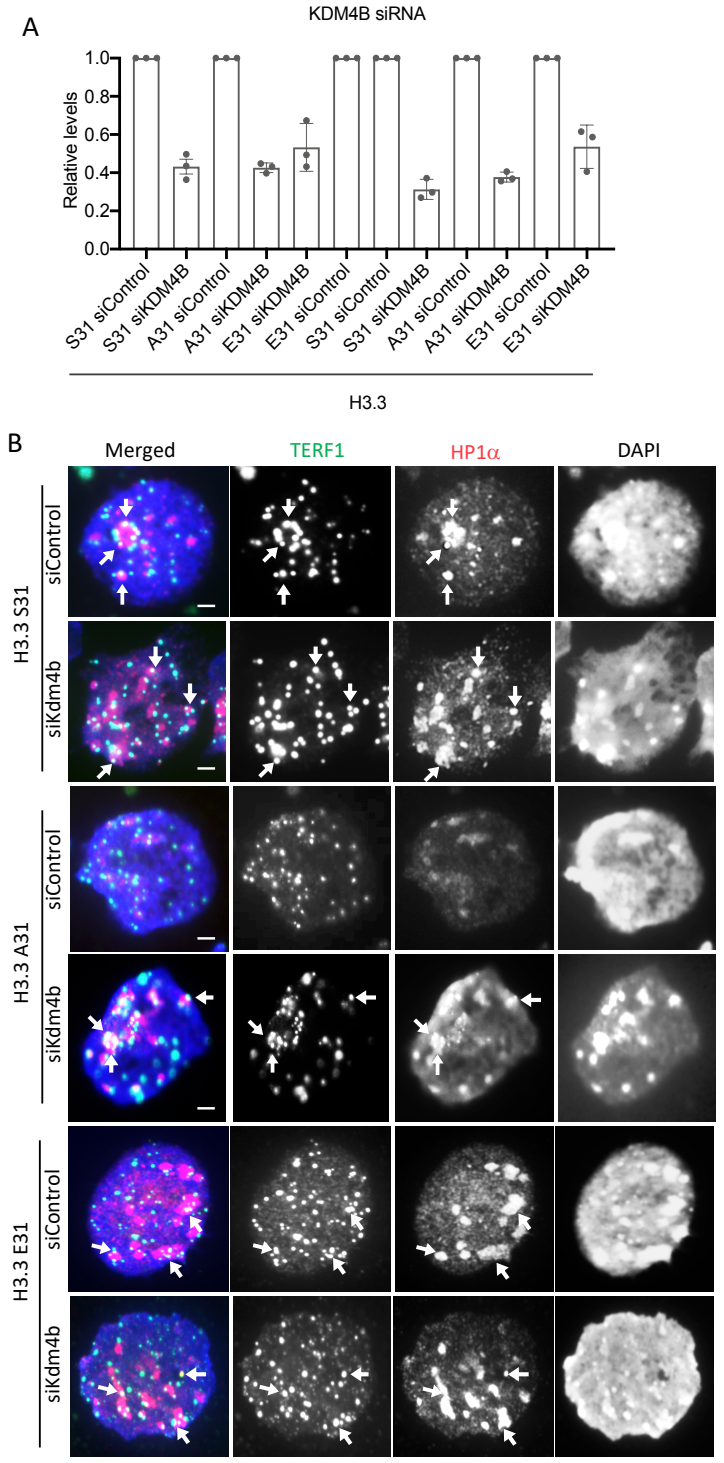
Suppl Figure 1. Targeting of *h3f3a* and *h3f3b*. **(A)** The targeting vector comprised of a neomycin selection cassette and a reporter coding sequence (cds) (yellow fluorescent protein (*yfp*) and cyan fluorescent protein (*cfp*) coding sequences for *H3f3a* and *H3f3b* targeting vectors, respectively). The targeting sequence substituted for much of exon 2 for *h3f3a* (encoding the first 17 amino acids) and all of exon 2 for *h3f3b* (encoding the first 41 amino acids). Exposure to Flp recombinase removed the *neo* selection cassette to produce the conditional alleles. The cassette arrangement and positioning of the *loxP* sites allows for conditional allelic replacement: on *Cre*-mediated excision of the H3.3 cds, an alternative cds is brought under control of the endogenous promoter, this being the *yfp* cds for *h3f3a*, and the *cfp* cds for *h3f3b*. The yellow shading marks the regions where strand exchange would be expected to occur. The red line marks a potential and undesired internal strand exchange (ISE) event occurring due to internal regions of homology. Such events would lead to loss of upstream targeting vector sequence including the *loxP* site. *SpA*, synthetic splice acceptor; *ex*, exon; *neo*, neomycin positive selection cassette; *dtA*, diphtheria toxin A chain negative selection cassette; E, *EcoRI*, K, *KpnI*; N, *NheI*; X, *XhoI* sites; *pBS*, pBluescript vector. **(B)** Generation of H3.3 S31, A31 and E31 mouse ES cell lines by exogenous expression of H3.3 S31, A31 and E31 in $h3f3a^{f/f}\,h3f3b^{f/f}$ line carrying conditional *h3f3a* and *h3f3b* alleles, followed by *Cre*-recombinase to excise H3.3 cds. **(C)** Southern blot analyses were performed using probes specific to either *h3f3a* (top) or *h3f3b* (bottom) genomic locus. In addition to wildtype (WT), $h3f3a^{f/f}\,h3f3b^{f/f}$, H3.3 S31, A31 and E31 mouse ES cell lines, $h3f3a^{-/f}\,h3f3b^{+/+}$ and $h3f3^{+/+}\,h3f3b^{-/-}$ cell lines were included as controls. For probing of *h3f3a* targeting, the genomic DNA was digested with *KpnI* enzyme, followed by Southern blot transfer and hybridisation. The WT cells gave a single 2.9 kb band, while $h3f3a^{f/f}\,h3f3b^{f/f}$ cells showed a 5.2 kb band. Successful deletion of *h3f3a* cds in H3.3 S31, A31, E31 and $h3f3a^{-/f}\,h3f3b^{+/+}$ mouse ES cell lines resulted in a 3.9 kb band. For probing of *h3f3b* targeting, the genomic DNA was digested with *EcoRI* enzyme, followed by Southern blot transfer and hybridisation. The WT cells gave a single 3.9 kb band, while $h3f3a^{f/f}\,h3f3b^{f/f}$ cells showed a 7.2 kb band. Successful deletion of *h3f3b* cds in H3.3 S31, A31, E31 and $h3f3^{+/+}\,h3f3b^{-/-}$ mouse ES cell lines resulted in a 4.9 kb band. **(D)** Western blot analyses of cell lysates extracted from wildtype, H3.3 S31, A31 and E31 H3.3 mouse ES cell lines. *Cre*-recombinase mediated excision of H3.3 cds resulted in expression of YFP/CFP fluorescent proteins in H.3 S31, A31 and E31 expressing cells. H3.3 protein levels were comparable between the different clones. Actin was used as a loading control. **(E)** Schematic diagram showing specific primers used in qPCR to assess expression of H3.3 S31, A31 and E31 expression. (**F-L**) Western blot analyses of H3.3 S31, A31 and E31 H3.3 mouse ES cell lines with antibodies against H3.3, H3K4me3, H3K9me3, H3K36me3, H3K27me3 and H3K27ac. (**M**) Western blot analyses of mouse ES cell lysates extracted from H3.3 knockout ($h3f3a^{-/-}h3f3b^{-/-}$) expressing only exogenous HA-H3.3 (lane 1, 18 KDa), WT ($h3f3a^{+/+}h3f3b^{+/+}$) expressing both endogenous H3.3 and exogenous HA-H3.3 (lane 2, 17 KDa and 18 KDa bands, respectively), wildtype cells expressing only endogenous H3.3 (lane 3, 17 KDa) and H3.3B knockout ($h3f3^{+/+}\,h3f3b^{-/-}$) cells expressing a lower level of endogenous H3.3 (lane 4). Compared to WT H3.3 S31 cells (lane 3), H3.3B knockout ($h3f3^{+/+}\,h3f3b^{-/}$) cells showed a reduced H3.3 protein level given they only expressed H3.3 from the H3.3A genes (53). Actin was used as a loading control.


A
H3f3a targeting vector
H3f3a, Chr 1
ISE
Probe, blot 1
H3f3b targeting vector
H3f3b, Chr 11
1.0 kb
Flp
conditional allele (c)
Prom.
Cre
mutant allele (-)
SpA loxP H3.3 cds UTR SV40 poly(A) FRT neo reporter cds dtA
C
WT
h3f3a f/f h3f3b f/f
h3f3a -/- h3f3b f/f
S31 WT
A31 #1
A31 #2
E31 #1
E31 #2
Cond -
KO -
WT -
- 5.2 kb
- 3.9 kb
- 2.9 kb
H3f3a
h3f3a f/f h3f3b -/-
h3f3a f/f h3f3b f/f
h3f3a f/f h3f3b -/-
- 7.2 kb
- 4.9 kb
- 3.9 kb
H3f3b
B
h3f3a f/f h3f3b f/f cells
Expression of H3.3 S31, A31 or E31
+ Cre
H3.3 S31 (h3f3a -/- h3f3b -/-)
H3.3 A31 (h3f3a -/- h3f3b -/-)
H3.3 E31 (h3f3a -/- h3f3b -/-)
D
YFP/CFP
H3.3
Actin
E
TCGCAAGAGTGCGCCCTC
TGCTCAGGACTTCAAAACAGA
H3.3 S31
TCT
GAT
S31
D82
TCGCAAGAGTGCGCCGC
A31
GCT
TCGCAAGAGTGCGCCGA
E31
GCG
F
H3.3
Actin
G
H3K4me3
H
H3K9me3
I
H3K36me3
J
H3K27me3
K
H3K27ac
L
ATRX
M
1 2 3 4
Actin
-- H3.3-HA(18 kDa)
-- H3.3 (17 kDa)

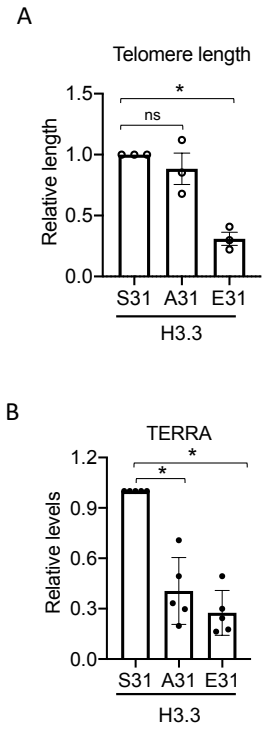
Suppl
Fig 1

Suppl Figure 2. KDM4B knockdown in H3.3 A31 mouse ES cells restores ATRX binding, HP1α binding at telomeres. (A) Quantitative RT-PCR analyses of Kdm4b expression levels in H3.3 S31, A31 and E31 mouse ES cells, 48 hours post-transfection of either control siRNA (siControl) or *Kdm4b* specific siRNA (siKdm4b). Real time RT-PCR analysis showing reduction of *Kdm4b* RNA transcripts in *Kdm4b* knockdown samples. Primers used were Kdm4b exons 5, 6/7 and 7/8 specific primers. **(B)** Immunostaining of HP1α (red) and TERF1 (green; marker for telomere) in H3.3 S31, A31 and E31 cells 48 hours post-transfection of either control siRNA (siControl) or *Kdm4b* specific siRNA (siKdm4b). Prominent HP1α staining was found at telomeres in both H3.3 S31 and E31 cell lines subjected to either siControl or siKdm4b knockdown. An increased binding of HP1α to telomeres (indicated by TERF1; arrows) was found in H3.3 A31 cells, following the knockdown of *Kdm4b* expression (indicated by arrows).



Suppl Fig 2

Suppl Figure 3. Telomere length (A) and TERRA transcript level (B) analyses of WT (wildtype) H3.3 S31, and mutant A31 and E31 ES cells (n=3 independent experiments, mean ± SEM, n=3).



Suppl
Fig 3

Supplementary table 1

Primer sequences for PCR amplification and RT-qPCR

	Primer sequences for PCR amplification and RT-qPCR analysis	
H3f3a	*5′* GTCGAGCAGTGGGATAGTGTC *3′*	*5′* GACTTTTTAGGCTTTTAGATCAGATTA CAG *3’*
H3f3b	*5′*- GGAGTGCTAGTGTGCATAAAT *3’*	*5′* CCAATAACCAACATGCTCCAA *3′*
H33 S31	5’ TCGCAAGAGTGCGCCC**TC** 3’	5’ TCTGTTTTGAAGTCCTGAGCA 3’
H33 A31	5’ TCGCAAGAGTGCGCCC**GC** 3’	5’ TCTGTTTTGAAGTCCTGAGCA 3’
H33 E31	5’ TCGCAAGAGTGCGCCC**GA** 3’	5’ TCTGTTTTGAAGTCCTGAGCA 3’
Kdm4B ex5	5’ GGCGTGAATACACCCTACTT 3’	5’ GCAGGTAGTTGATGCTGTAGA 3’
Kdm4B ex6/7	5’CCTGGCCATAGGCTTCTTC 3’	5’CGTACTTCTTCAGGATGATGGG 3’
Kdm4B ex7/8	5’ GCCTTCCTAAGGCACAAGAT 3’	5’CCCAGCTTCCTGTGTAATCC 3’
FOXP3	5’ TGAGGGAAAGAGCAAAGGAGTG 3’	5’ GCATCCTGCAGAGAGCTAAGAGT 3’
GAPDH	5’ GTGGAGTCTACTGGTGTCTTC 3’	5’ GGTTCACACCCATCACAAAC 3’
Telomere (TERRA)	5’ CGGTTTGTTTGGGTTTGGGTTTGG GTTTGGGTTTGGGTT 3’	5’ GGCTTGCCTTACCCTTACCCTTACCCTT ACCCTTACCCT 3’
36B4	5’ ACTGGTCTAGGACCCGAGAAG 3’	5’ TCAATGGTGCCTCTGGAGATT 3’
Yq satellite (Yr_f and Yr_r)	5’ ATACTACTCTAGAATACTGCATGC 3’	5’ TGTAGTCATCAAATGGTTTCCAAG 3’

Supplementary table 2

List of antibodies used

Antibodies	Source	Antibodies	Source
H3	Abcam, ab1791	ATRX	Santa Cruz Biotechnologies, sc15408
H3.3	Abcam, ab176840	KDM4B	Abcam, ab191434
H3K4me3	Abcam, ab8580	PML	Millipore, MAB3738
H3K9me3	Abcam, ab8898	TERF1	Alpha Diagnostics, TRF12-S
H3K27me3	Merck Millipore, 7447	HP1α	Merck Millipore, MAB3584
H3K36me3	Abcam, ab9050	Flag	Sigma, F1804
H3K27Ac	Merck Millipore, 7360	GFP	Roche, 11814460001
γH2A.X/phospho-H2A.X (Ser139)	Merck Millipore, JBW301	β Actin (AC15)	Santa Cruz Biotechnology, sc6987
Donkey anti-Mouse IgG, HRP conjugate	Merck Millipore, AP192P	Donkey anti-Mouse IgG (H+L), Alexa Fluor 488	Invitrogen, A-21202
Goat anti Rabbit IgG, HRP conjugate	Merck Millipore, AP187P	Donkey anti-Rabbit IgG (H+L), Alexa Fluor 488	Invitrogen, A-21206
Donkey anti-Mouse IgG (H+L), Alexa Fluor 594	Invitrogen, A-21203	Chicken anti-Rabbit IgG (H+L), Alexa Fluor 594	Invitrogen, A-21442

Supplementary table 3

Amino acid sequences for the short peptides used

Peptides	Amino acid sequences
H3.3 S31 K36me3	ATKAARKSAPSTGGVK(Me3) KPHRYRPG-GK(Biotin)
H3.3 S31Ph K36me3	ATKAARKSAPS(Ph)TGGVK(Me3) KPHRYRPG-GK(Biotin)
H3.3 A31 K36me3	ATKAARKSAPATGGVK(Me3) KPHRYRPG-GK(Biotin)
H3.3 E31 K36me3	ATKAARKSAPETGGVK(Me3) KPHRYRPG-GK(Biotin)

Supplementary table 4

Oligonucleotide sequences of siRNA used

siRNA	Sense	Anti-sense
Kdm4b-mus-2703	5’ GCAGCGAUGGAAACUGAAATT 3’	3’ UUUCAGUUUCCAUCGCUGCTT 5’
Kdm4b-mus-2933	5’ GCCAGAUCGUCAUCACCAATT 3’	3’ UUGGUGAUGACGAUCUGGCTT 5’
Kdm4b-mus-3478	5’CCUGUCCCACUGUAGGUAATT 3’	3’ UUACCUACAGUGGGACAGGTT 5’
Kdm4b-mus-4552	5’ CCUCCAGUUCAGUAUCAAUTT 3’	3’ AUUGAUACUGAACUGGAGGTT 5’