Supplementary Information

- Eight Supplementary Figures and their legends
- Supplementary Table I: List of primers

Figure S1

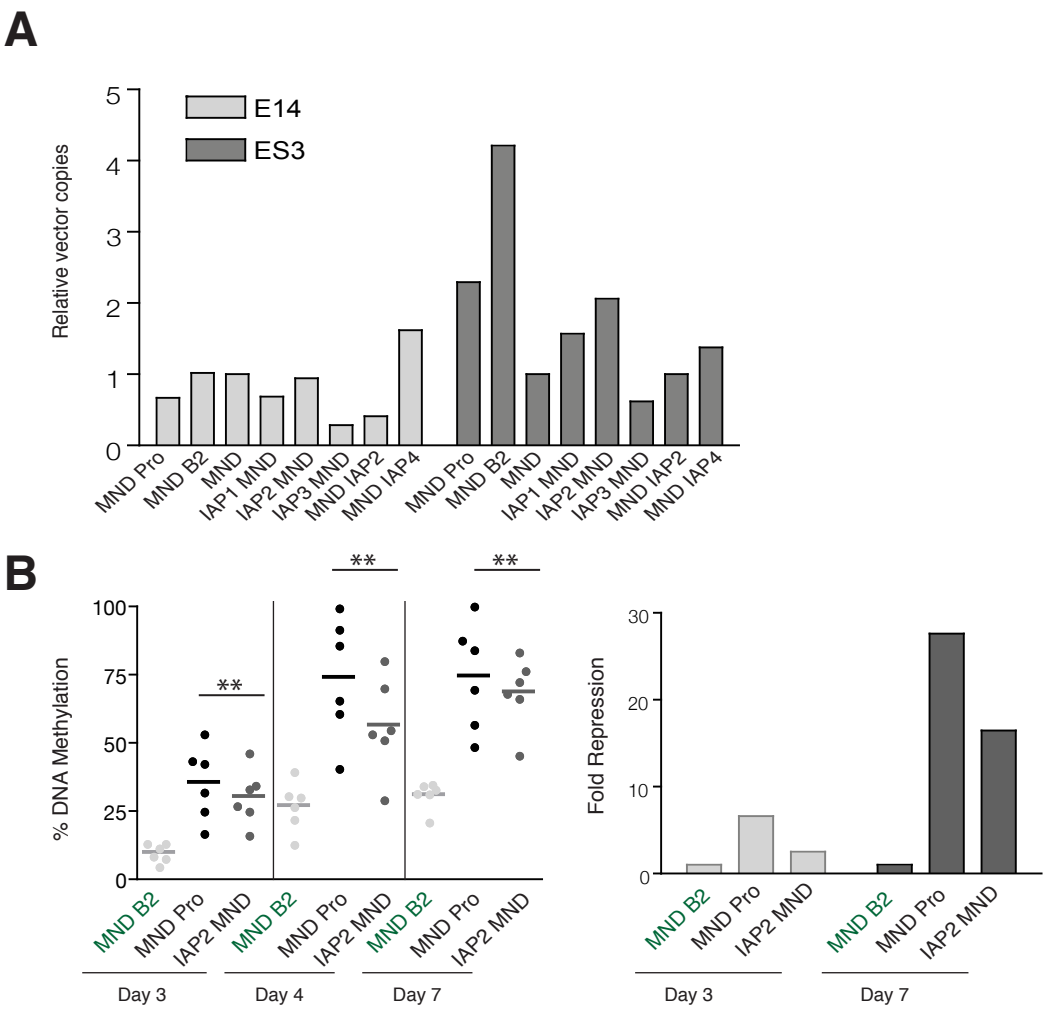
A
E14
ES3
Relative vector copies
0
1
2
3
4
5
MND Pro
MND B2
MND
IAP1 MND
IAP2 MND
IAP3 MND
MND IAP2
MND IAP4
B
% DNA Methylation
0
25
50
75
100
**
**
**
MND B2
MND Pro
IAP2 MND
Day 3
Day 4
Day 7
Fold Repression
0
10
20
30
MND B2
MND Pro
IAP2 MND
Day 3
Day 7

Figure S1, related to Figure 1: **ERV sequences can induce rapid *de novo* DNA methylation in ES cells**

A) Genomic DNA samples from Figure 1B-C (time-point day 4) were used to measure relative vector copies by Q-PCR, to verify that repressed vectors did not represent non-integrated samples. **B)** *De novo* methylation and repression are rapid and progressive thereafter. Left: ERV-containing (Pro or IAP2) vectors or a control vector (B2) were measured for their levels of promoter methylation at 3, 4 or 7 days post transduction. Right: In parallel, fold repression was recorded at days 3 and 7 post transduction. Left p values: Day3: MND B2 vs. MND Pro (0.0016) or vs. IAP2 MND (0.0009); Day4: MND B2 vs. MND Pro (0.0007) or vs. IAP2 MND (0.0011); Day7: MND B2 vs. MND Pro (0.0016) or vs. IAP2 MND (0.0001).

Figure S2

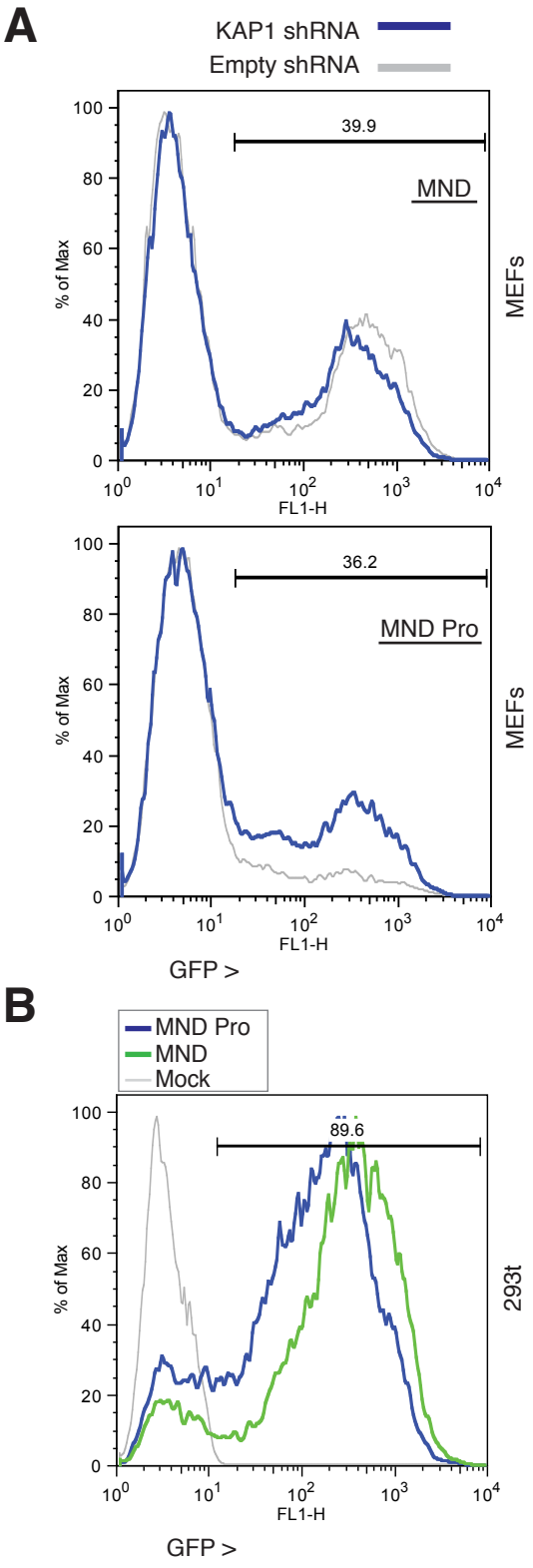
A
KAP1 shRNA
Empty shRNA
39.9
MND
% of Max
MEFs
FL1-H
36.2
MND Pro
GFP >
B
MND Pro
MND
Mock
89.6
293t

Figure S2, related to Figure 2: **ERV DNA methylation patterns are conditioned by KRAB-ZFP expression profiles**

Reversibility of MND Pro silencing in MEFs and 293t cells where levels of DNA methylation induced are very low. **A)** MEFs shown in Figure 2B transduced with either the MND or MND Pro vector were cultured for 2 weeks and then transduced with shRNA vectors that were Puromycin selected (either against KAP1 or with an empty vector control) as stated. One week later, GFP was analysed by flow cytometry. **B)** 293t cells from Figure 2E transduced with either the MND or MND Pro vectors and transfected with ZFP809 and then washed and maintained for 5 days longer to allow loss of ZFP809 expression and reversibility of Pro repression.

Figure S3

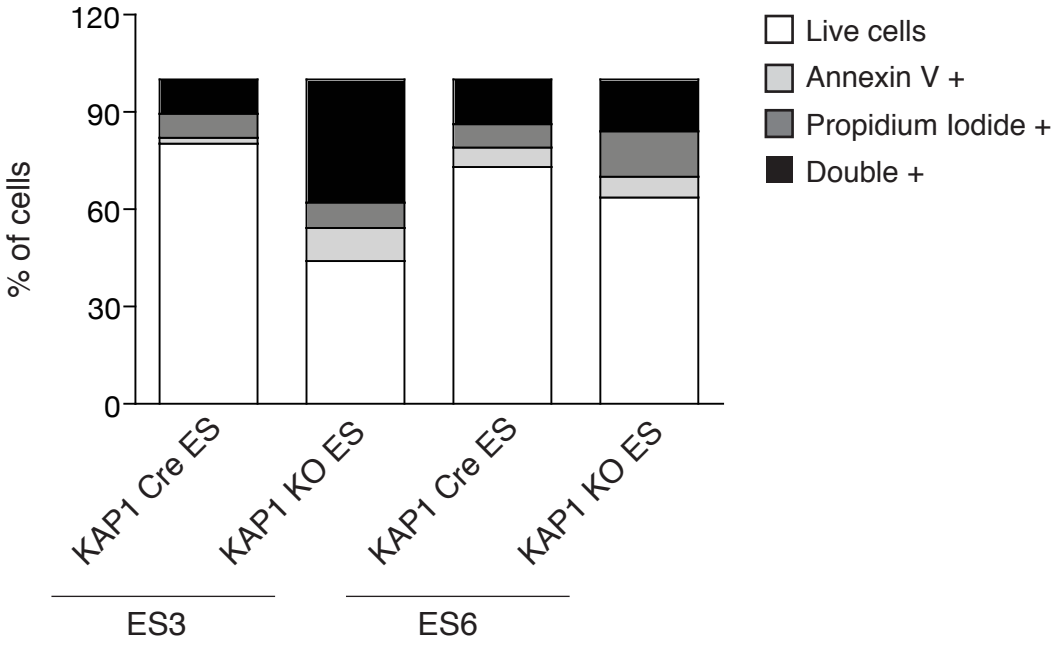
120
90
60
30
0
% of cells
Live cells
Annexin V +
Propidium Iodide +
Double +
KAP1 Cre ES
KAP1 KO ES
KAP1 Cre ES
KAP1 KO ES
ES3
ES6

Figure S3, related to Figure 3: **KAP1 and ESET are required for *de novo* DNA methylation of ERVs**

KAP1 knockout is lethal in ES cells around 4-5 days post *Kap1*- excision. Cells were therefore assessed for DNA methylation at 3 days post *Kap1*-excision (see Figure 3). Here, *Kap1 LoxP*-flanked cells were transduced with a 4-OHT inducible Cre vector in the absence (KAP1 Cre ES) or presence (KAP1 KO ES) of 4-OHT. Two cell lines are shown (ES3 and ES6). Four days post *Kap1*- excision, cells were harvested, stained and assessed for cell death by flow cytometry. Note that cell death is underestimated since some cells die and detach before harvesting.

Figure S4

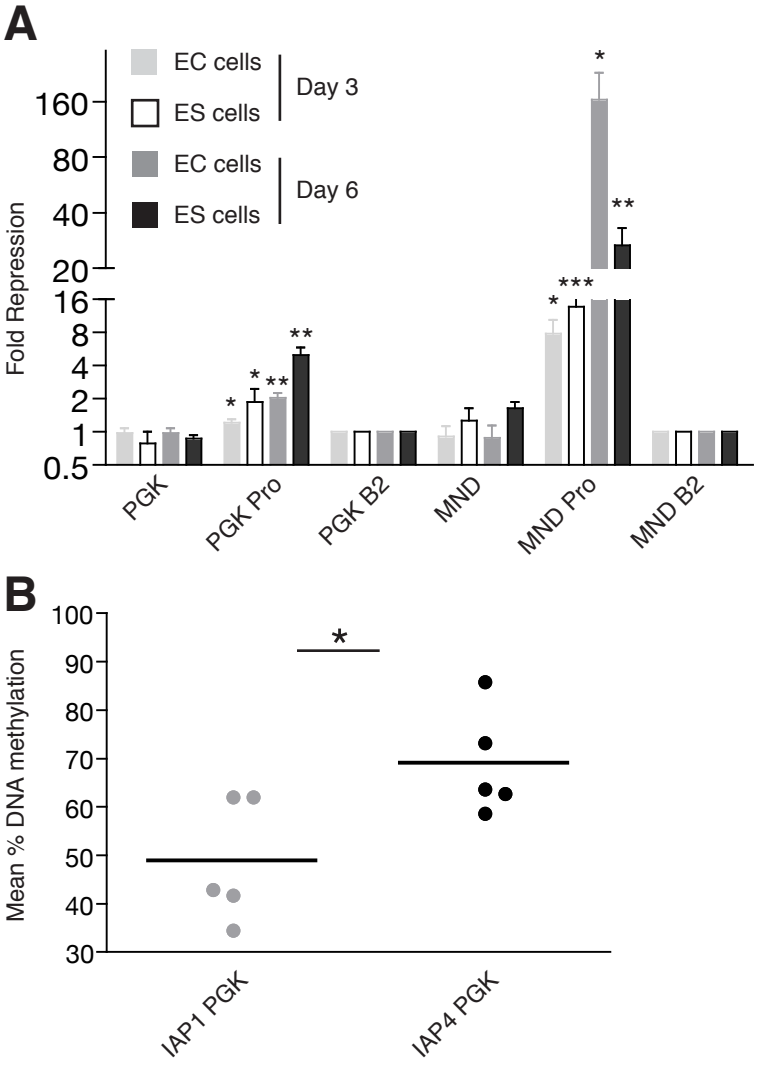
A
EC cells
ES cells
Day 3
EC cells
ES cells
Day 6
Fold Repression
160
80
40
20
16
8
4
2
1
0.5
PGK
PGK Pro
PGK B2
MND
MND Pro
MND B2
B
Mean % DNA methylation
100
90
80
70
60
50
40
30
IAP1 PGK
IAP4 PGK

Figure S4, related to Figure 5: **ERV sequences can induce repression and DNA methylation of a cellular promoter**

A) Side-by-side comparison of repression of the MND promoter vs. the cellular promoter PGK at day3 and day6 post vector transduction of F9 EC cells and ES cells. Results were normalized to expression in 3T3 cells and fold repression of PGK vectors was normalized to the PGK B2 control vector, while MND vectors were normalized to the MND B2 control vector. Bars show means and s.d. of triplicate infections. P values (unpaired two-tailed *t* tests) for the PGK Pro vector vs. the PGK control: EC day3, p=0.0492; ES day3, p=0.0406; EC day6, p=0.0016; ES day6, p=0.0012. For the MND Pro vector vs. the MND control: EC day3, p=0.0119; ES day3, p=0.0009; EC day6, p=0.0125; ES day6, p=0.0026. **B)** ERV sequences can direct *de novo* methylation of the PGK promoter *in vivo*. Lentiviral transgenesis was performed with either the IAP4 PGK vector or a control vector, IAP1 (see Figure 1) PGK that escapes repression. 5 embryos per group were assessed for DNA methylation of the PGK promoter. P=0.0270.

Figure S5

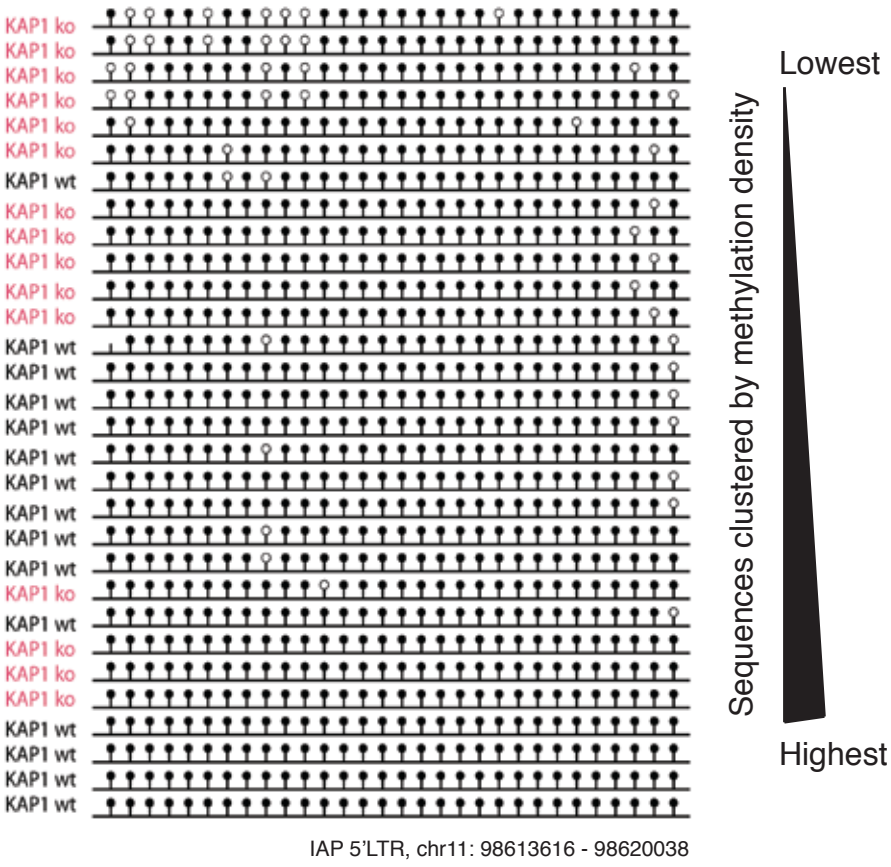
KAP1 ko
KAP1 ko
KAP1 ko
KAP1 ko
KAP1 ko
KAP1 ko
KAP1 wt
KAP1 ko
KAP1 ko
KAP1 ko
KAP1 ko
KAP1 ko
KAP1 wt
KAP1 wt
KAP1 wt
KAP1 wt
KAP1 wt
KAP1 wt
KAP1 wt
KAP1 wt
KAP1 wt
KAP1 ko
KAP1 wt
KAP1 ko
KAP1 ko
KAP1 ko
KAP1 wt
KAP1 wt
KAP1 wt
KAP1 wt
Lowest
Sequences clustered by methylation density
Highest
IAP 5'LTR, chr11: 98613616 - 98620038

Figure S5, related to Figure 6: **KAP1 shapes DNA methylation of endogenous retroviruses in ES cells and embryos**

Bisulphite sequencing with TOPO cloning showing the difference in DNA methylation status between molecules at one IAP locus. KAP1 knockout (ko) ES cell samples shown in red and wildtype (wt) ones in black. Sequences are ordered depending on methylation density. Unfilled and filled lollipops represent unmethylated and methylated CpGs respectively.

Figure S6

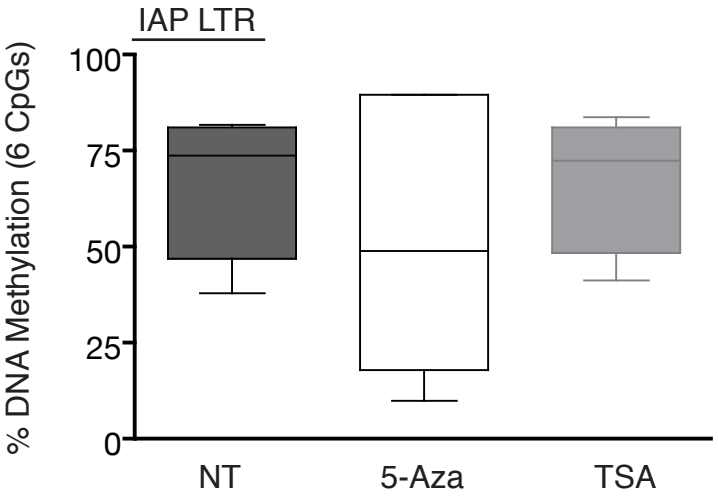

Figure S6, related to Figure 7: **DNA methylation of introduced ERV sequences and resident ERVs becomes critical late in development**

MEFs from Figure 7AB (line PGK Pro 1.) were used for DNA methylation analysis by bisulphite pyrosequencing at endogenous IAPs in parallel to the expression analysis shown in Figure 7B. DNA methylation was reduced in the 5-Aza treated group.

Figure S7

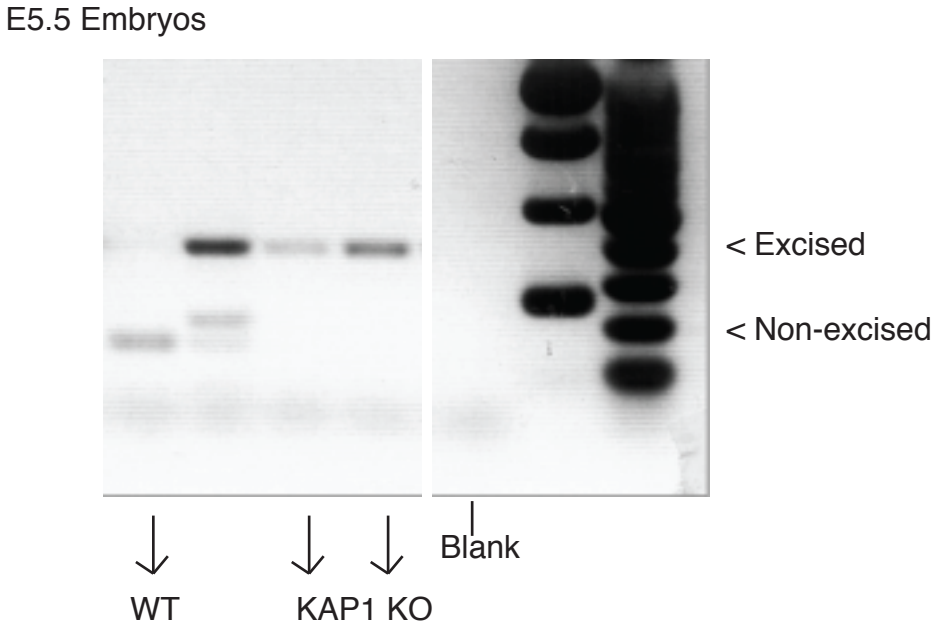

Figure S7, related to Figure 6: **KAP1 shapes DNA methylation of endogenous retroviruses in ES cells and embryos**

Kap1 heterozygous mice were crossed and embryos dissected at E5.5 to measure DNA methylation of endogenous IAPs. Here the results of the genotyping that was done by PCR with a mix of three primers (see Figure 3) are shown. 171- and 390-bp products represent *loxP*-flanked or excised *Kap1*, respectively. Embryos selected for analysis (two knockouts and one WT embryo) are labelled. The image shows two parts of the same gel but all at the same exposure and analysis settings.

Figure S8

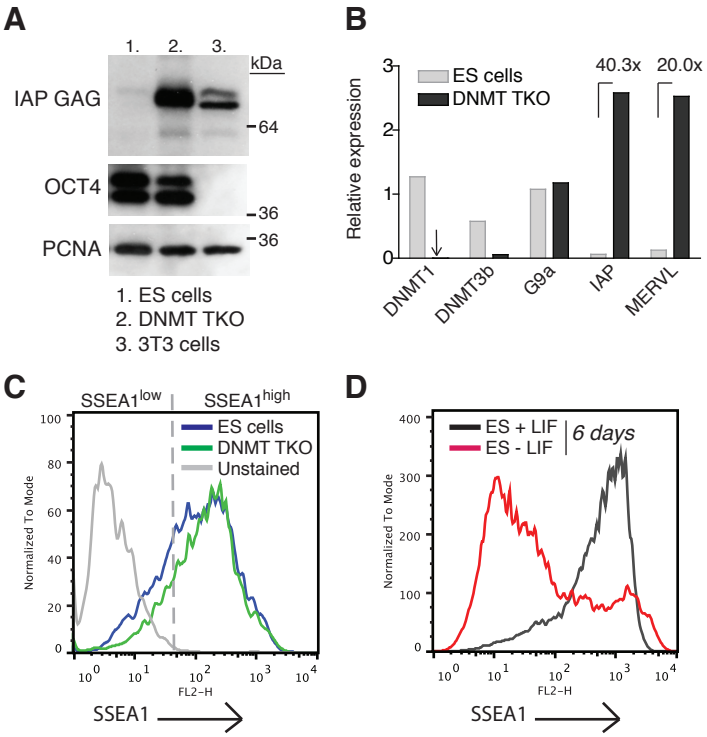
A
1. 2. 3.
kDa
IAP GAG
64
OCT4
36
PCNA
36
1. ES cells
2. DNMT TKO
3. 3T3 cells
B
ES cells
DNMT TKO
40.3x
20.0x
Relative expression
DNMT1
DNMT3b
G9a
IAP
MERVL
C
SSEA1low
SSEA1high
ES cells
DNMT TKO
Unstained
Normalized To Mode
FL2-H
SSEA1
D
ES + LIF
ES - LIF
6 days
Normalized To Mode
FL2-H
SSEA1

Figure S8, related to Figure 7: **DNA methylation of introduced ERV sequences and resident ERVs becomes critical late in development**

A) Accumulation of IAP GAG p73 in DNA methyltransferase triple knockout ES cells (DNMT TKO). 3T3 cells were a positive control since they overexpress IAP GAG. Global OCT4 protein levels were comparable between WT and TKO ES cells. **B)** qRT-PCR showing upregulation of IAP and MERVL transcripts in DNMT TKO cells. Samples were normalized to *Gapdh* and G9a levels were also similar between samples while DNMT1 and 3B were verified to be absent in DNMT TKO cells. **C)** WT and DNMT TKO ES cells were stained with an anti-SSEA1 antibody or left unstained and histogram results overlaid to verify that cells were largely undifferentiated. **D)** In another experiment, ES cells were cultured for six days in the presence or absence of LIF and then stained as in C) above to verify that cells downregulate SSEA1 upon differentiation to validate its use as a marker of undifferentiation in C).

Supplementary Table I: Primer sequences

qRT-PCR primers	
GFP_F	CTGCTGCCCGACAACCAC
GFP_R	ACCATGTGATCGCGCTTCTC
GFP_probe	CCAGTCCGCCCTGAGCAAAGACC
Titin_F	TTCAGTCATGCTGCTAGCGC
Titin_R	AAAACGAGCAGTGACGTGAGC
Titin_probe	TGCACGGAAGCGTCTCGTCTCAGTC
Actin_F	TAGGCACCAGGGTGTGATGG
Actin_R	CATGGCTGGGGTGTTGAAGG
KAP1_F	CGGAAATGTGAGCGTGTTCTC
KAP1_R	CGGTAGCCAGCTGATGCAA
Gapdh_F	TCCATGACAACTTTGGCATTG
Gapdh_R	CAGTCTTCTGGGTGGCAGTGA
G9A_F	AGACAGCCCGTGGGTGAA
G9A_R	CCCTCGGAGGCTCTCGTT
DNMT1_F	CCAGGCATTTCGGCTGAA
DNMT1_R	CGTTGCAGTCCTCTGTGAACA
DNMT3b_F	AACTCCATCAGACAGGGCAAA
DNMT3b_R	CGTCCTTGCCATTCATGACTAC
ESET_F	TGGCAACAGCGGTTCAGA
ESET_R	CAGAAGTTATCATCAGAGCTGTCATCA
Zfp809_F	AATTTGGAGCGTGGATTTGG
Zfp809_R	GGGAGGCTCCTGCTTGAAG
EeF1a1_F	AGCAAAAATGACCCACCAATG
EeF1a1_R	GGCCTGGATGGTTCAGGATA
EeF1a1_probe	CACCTGAGCAGTGAAGCCAGCTGCTT
qPCR copy number primers	
HIV_GAG_F	GGAGCTAGAACGATTCGCAGTTA
HIV_GAG_R	GGTGTAGCTGTCCCAGTATTTGTC
HIV_GAG_probe	ACAGCCTTCTGATGTTTCTAACAGGCCAGG
GFP_F	CTGCTGCCCGACAACCAC
GFP_R	ACCATGTGATCGCGCTTCTC
GFP_probe	CCAGTCCGCCCTGAGCAAAGACC
Titin_F	TTCAGTCATGCTGCTAGCGC
Titin_R	AAAACGAGCAGTGACGTGAGC
Titin_probe	TGCACGGAAGCGTCTCGTCTCAGTC
Bisulphite pyrosequencing primers	
Oct4_Promoter_F	AGGGGTGAGAGGATTTTGAA
Oct4_Promoter_biotin_R	CCACCCTCTAACCTTAACCT
Oct4_Promoter_seq	GGTTGAAAATGAAGGTTT
IAP LTR_biotin_F	GGTTTTGGAATGAGGGATTTT
IAP LTR_R	CTCTACTCCATATACTCTACCTTC
IAP LTR_seq	ATACTCTACCTTCCCC

IAP 5'UTR_F	GGGTTGTAGTTAATTAGGGAGTGATA
IAP 5'UTR_biotin_R	ACAATTAAATCCTTCTTAACAATCTACTT
IAP 5'UTR_seq	ATTTTGGTTTGTTGTGT
MND_F	TTAGATGTTTTTAGGGTGTTTTAAGGA
MND_biotin_R	TCACTCAAAAAAAACCCTCCCAAAAAA
MND_seq	TGATTTTGTGTTTTATTTGAATTAA
hPGK_F	GGTTGGGGTTGAGTTTTTTTTAAGGTA
hPGK_biotin_R	CTAAACAACCCCTATTAACCACAACCCAT
hPGK_seq	GTTTTTAAGTAGGGAAGGTTTT