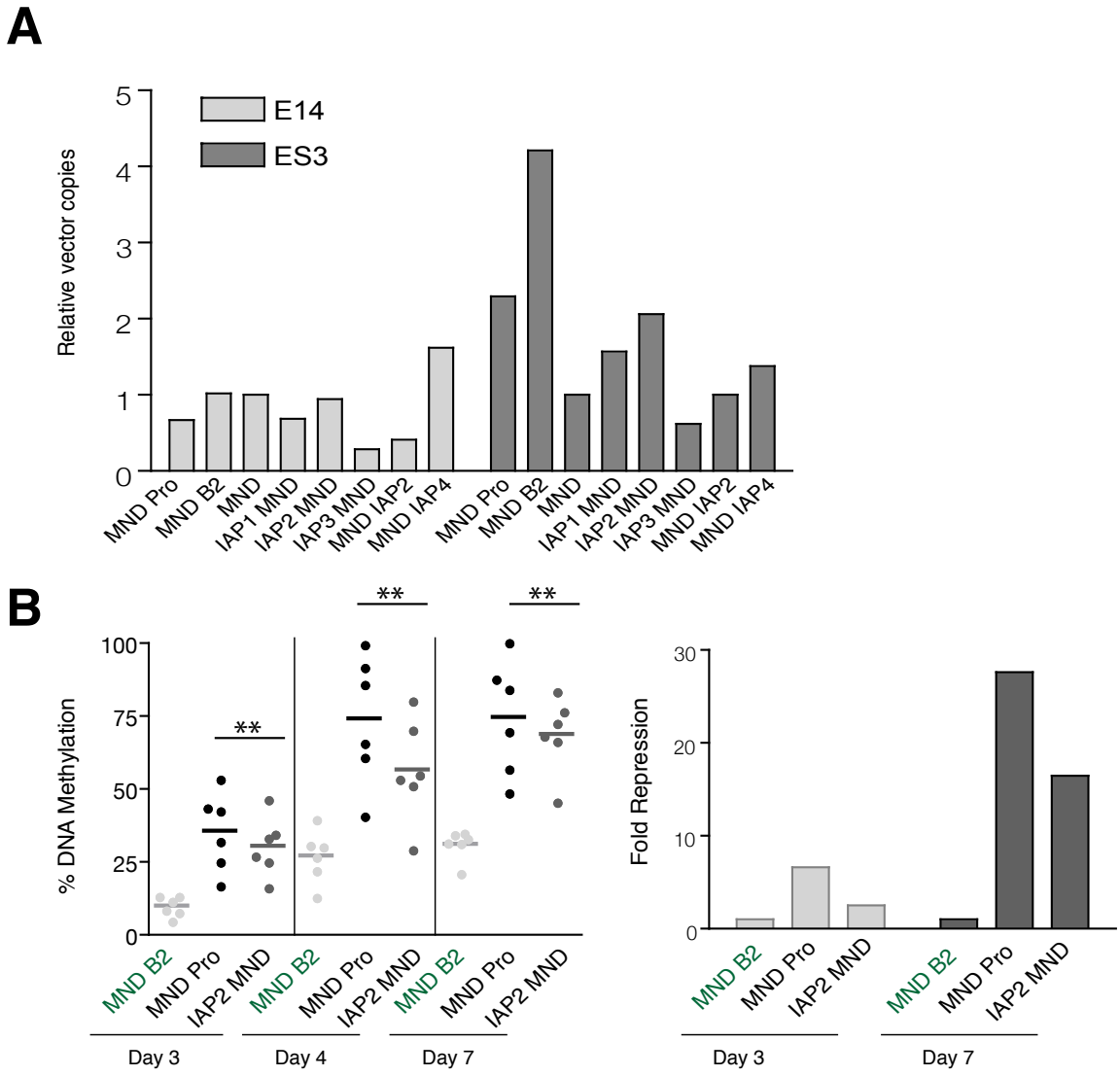

Fig. S1. ERV sequences can induce rapid *de novo* DNA methylation in ES cells. This relates to Fig. 1. (**A**) Genomic DNA samples from Fig. 1B,C (time-point day 4) were used to measure relative vector copies by Q-PCR, to verify that repressed vectors did not represent non-integrated samples. (**B**) *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: day 3, MND B2 versus MND Pro (0.0016) or versus IAP2 MND (0.0009); day 4, MND B2 versus MND Pro (0.0007) or versus IAP2 MND (0.0011); day 7, MND B2 versus MND Pro (0.0016) or versus IAP2 MND (0.0001).

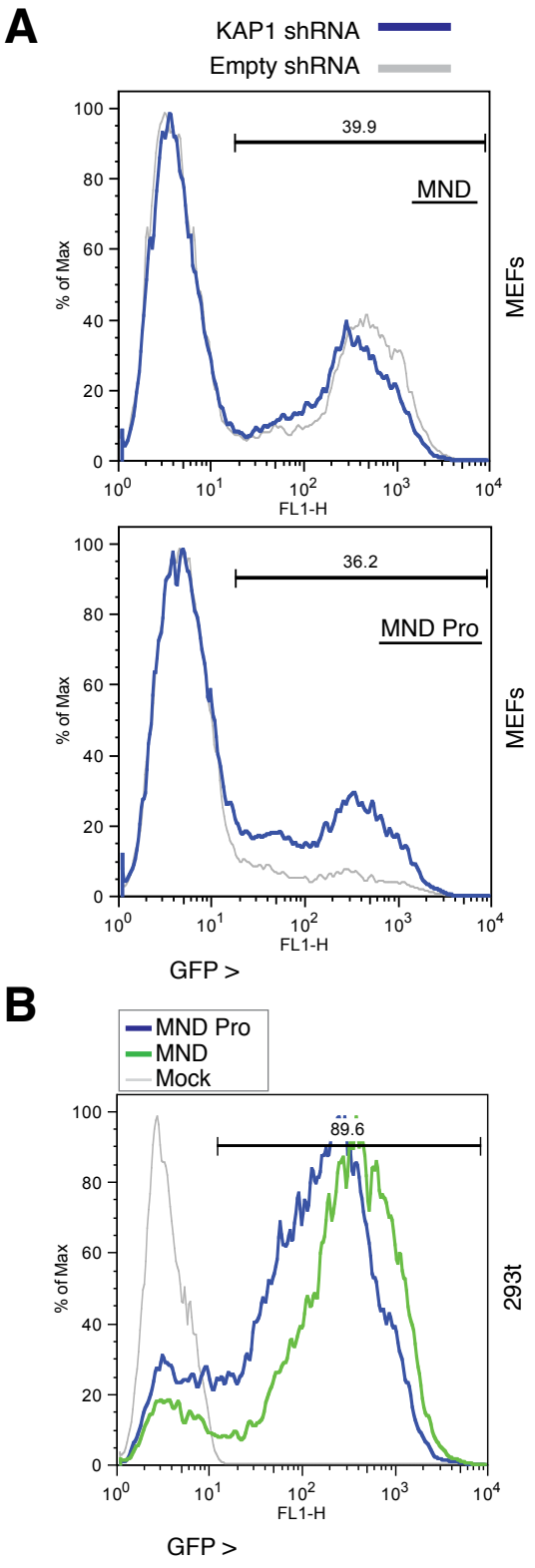

Fig. S2. ERV DNA methylation patterns are conditioned by KRAB-ZFP expression profiles. This relates to Fig. 2. Reversibility of MND Pro silencing in MEFs and 293t cells where levels of DNA methylation induced are very low. (**A**) MEFs shown in Fig. 2B transduced with either the MND or MND Pro vector 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 Fig. 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.

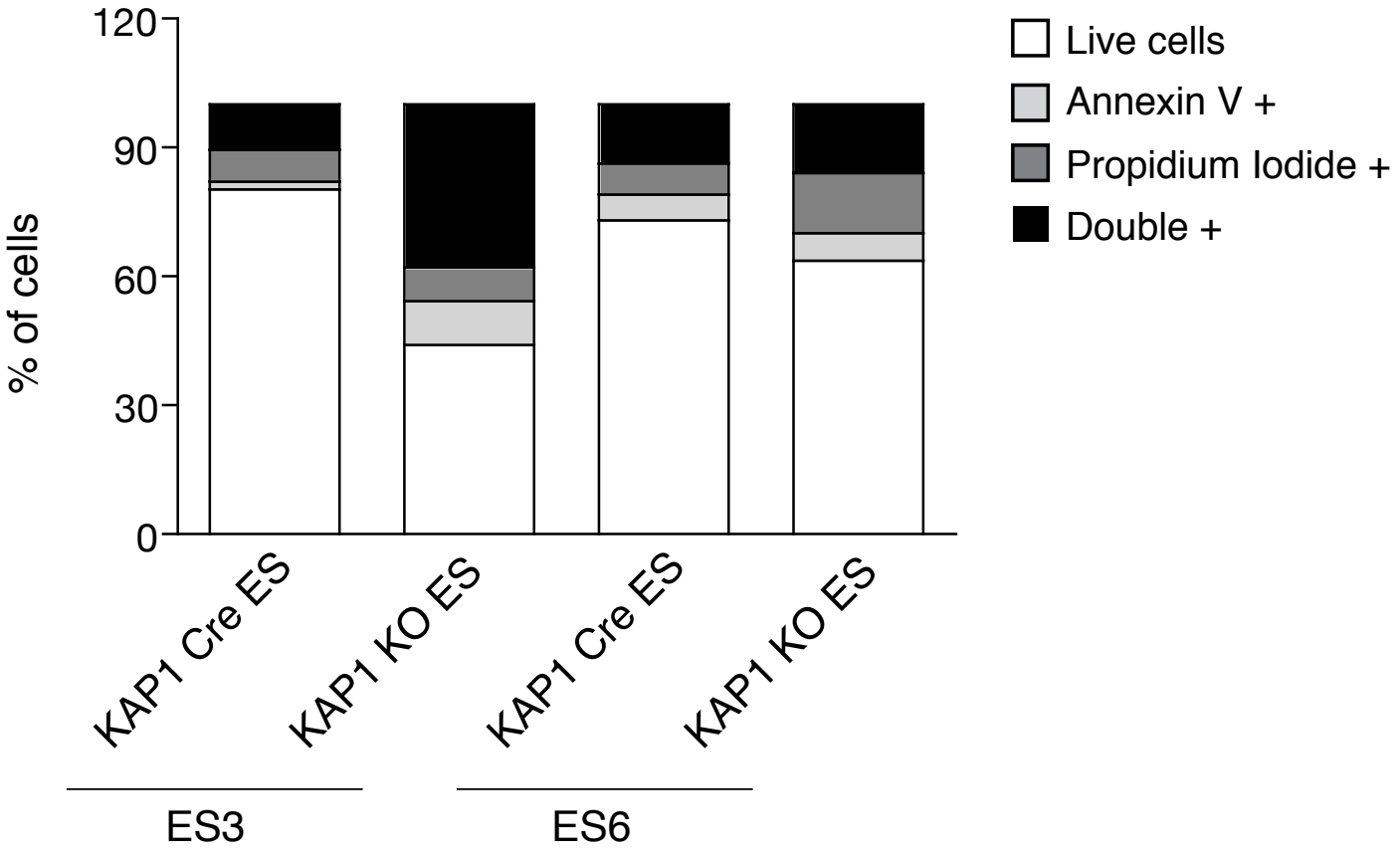

Fig. S3. KAP1 and ESET are required for *de novo* DNA methylation of ERVs. This relates to Fig. 3. KAP1 knockout is lethal in ES cells around 4-5 days post-*Kap1* excision. Cells were therefore assessed for DNA methylation at 3 days post-*Kap1* excision (see Fig. 3). Here, *Kap1 LoxP*-flanked cells were 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 as some cells die and detach before harvesting.

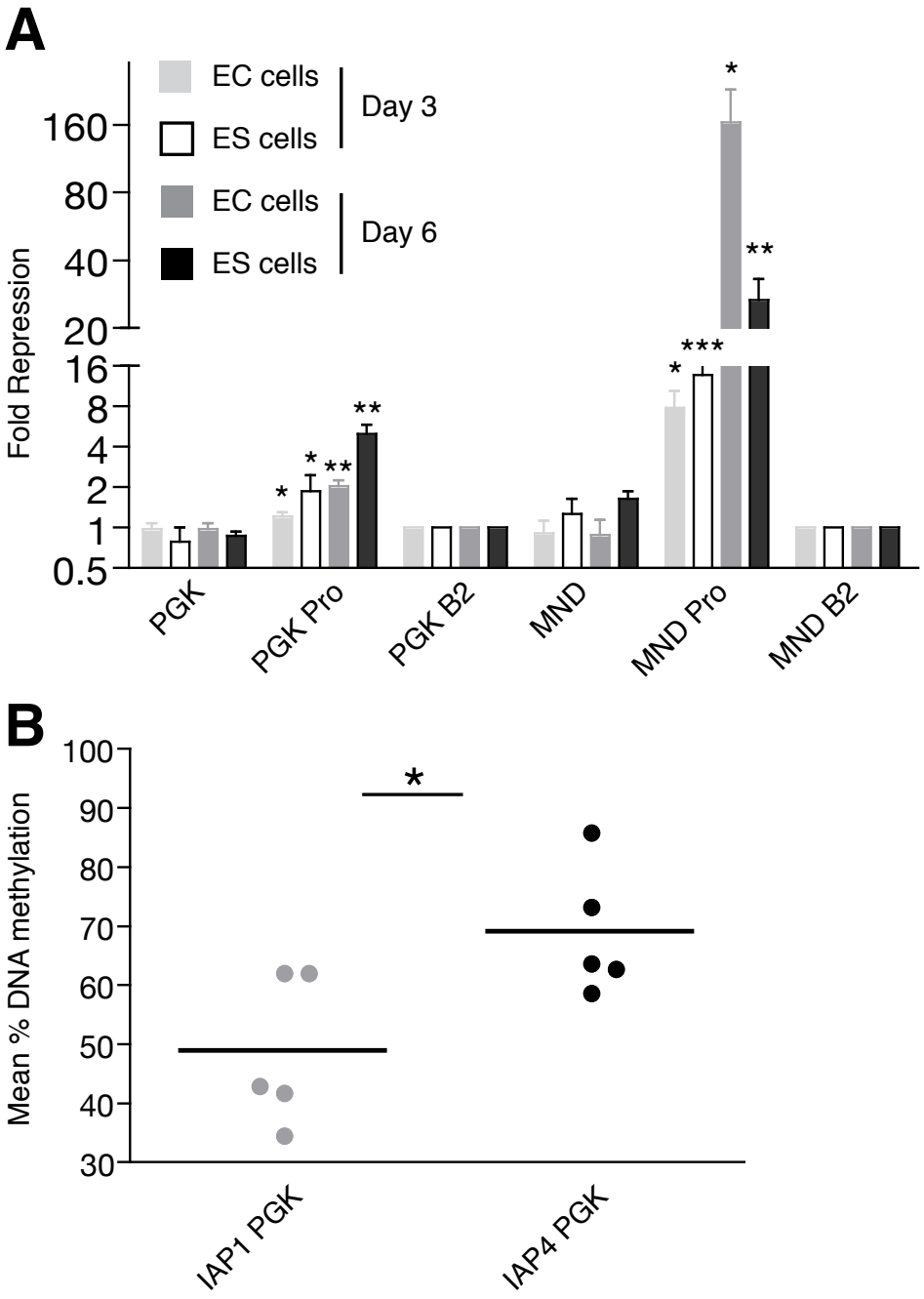

Fig. S4. ERV sequences can induce repression and DNA methylation of a cellular promoter. This relates to Fig. 5. (**A**) Side-by-side comparison of repression of the MND promoter versus the cellular promoter PGK at day 3 and day 6 post-vector transduction of F9 EC cells and ES cells. Results were normalized to expression in 3T3 cells and fold repression of PGK vectors was normalized to the PGK B2 control vector, whereas 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 versus the PGK control: EC day 3, *P*=0.0492; ES day 3, *P*=0.0406; EC day 6, *P*=0.0016; ES day 6, *P*=0.0012. For the MND Pro vector versus the MND control: EC day 3, *P*=0.0119; ES day 3, *P*=0.0009; EC day 6, *P*=0.0125; ES day 6, *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 Fig. 1) PGK that escapes repression. Five embryos per group were assessed for DNA methylation of the PGK promoter. *P*=0.0270.

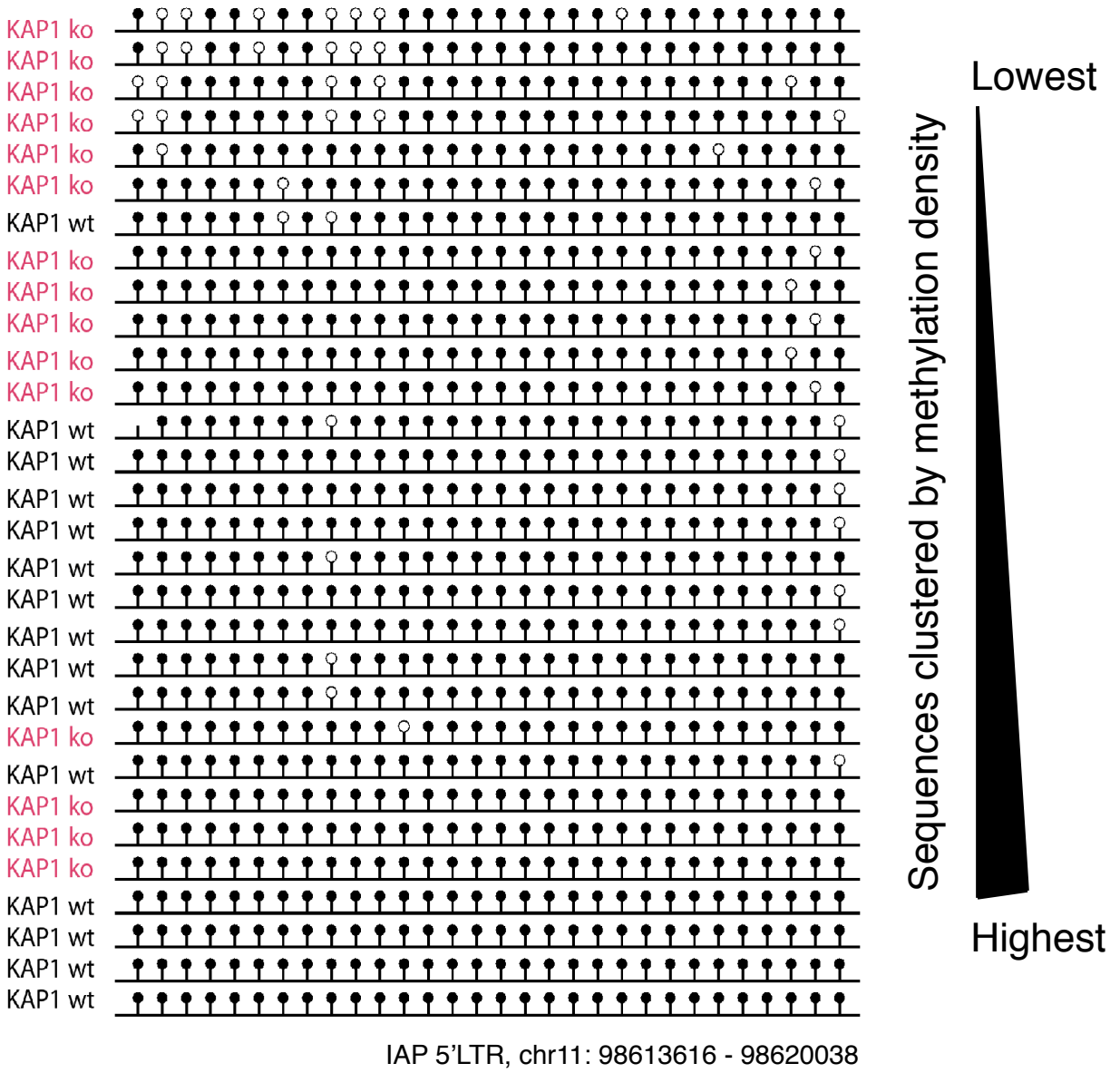

Fig. S5. KAP1 shapes DNA methylation of endogenous retroviruses in ES cells and embryos. This relates to Fig. 6. Bisulphite sequencing with TOPO cloning showing the difference in DNA methylation status between molecules at one IAP locus. KAP1 knockout (ko) ES cell samples shown in red and wild-type (wt) ones in black. Sequences are ordered depending on methylation density. Unfilled and filled lollipops represent unmethylated and methylated CpGs, respectively.

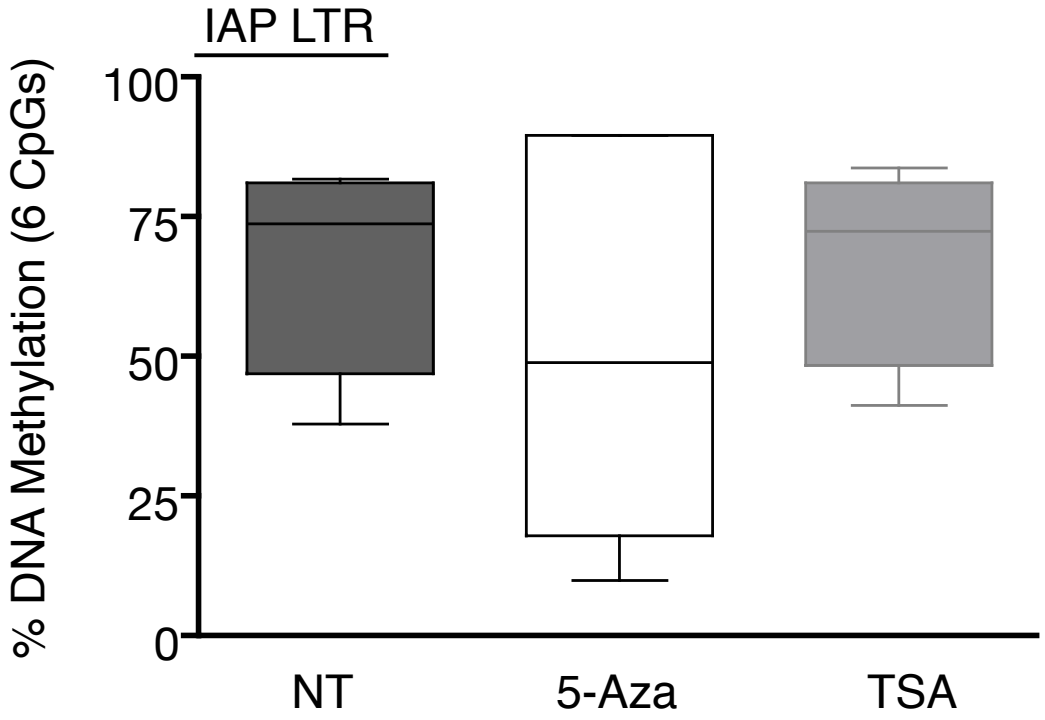

Fig. S6. DNA methylation of introduced ERV sequences and resident ERVs becomes crucial late in development. This relates to Fig. 7. MEFs from Fig. 7A,B (line PGK Pro 1) were used for DNA methylation analysis by bisulphite pyrosequencing at endogenous IAPs in parallel to the expression analysis shown in Fig. 7B. DNA methylation was reduced in the 5-Aza-treated group.

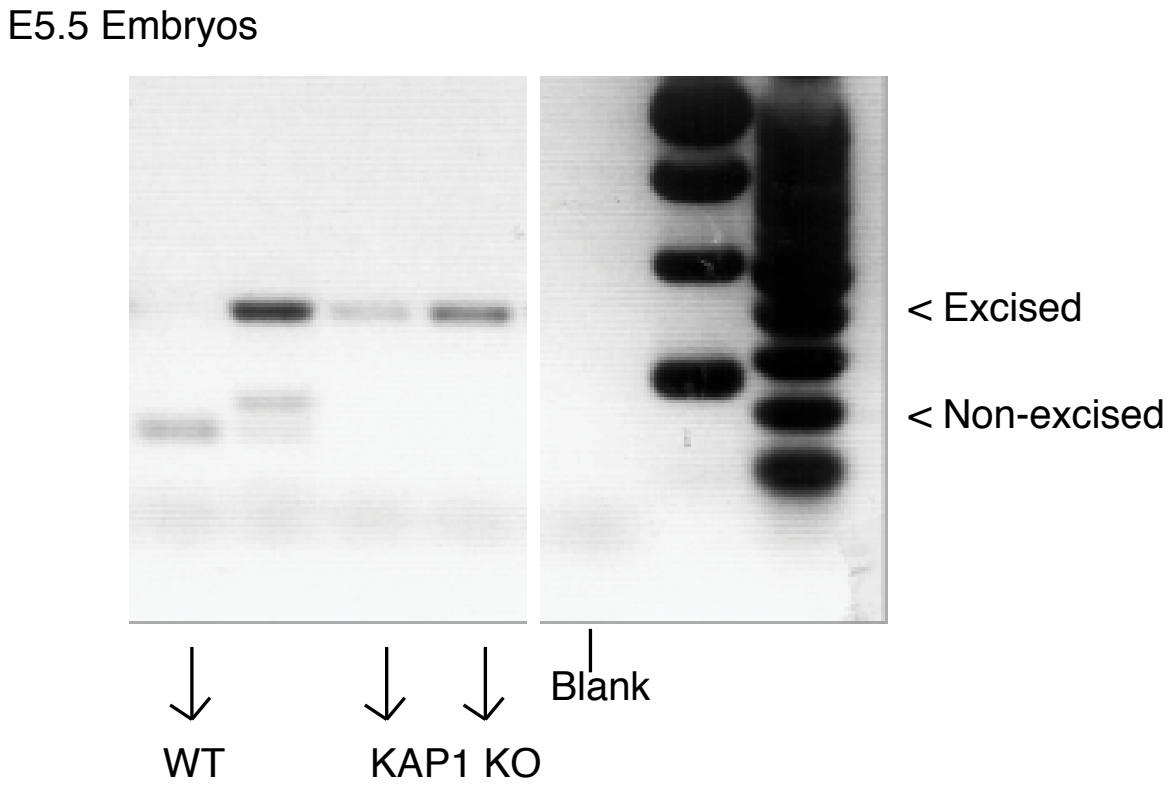

Fig. S7. KAP1 shapes DNA methylation of endogenous retroviruses in ES cells and embryos. This relates to Fig. 6. *Kap1* heterozygous mice were crossed and embryos dissected at E5.5 to measure DNA methylation of endogenous IAPs. Here, the results of the genotyping that was carried out by PCR with a mix of three primers (see Fig. 3) are shown. The 171 and 390 bp products represent *loxP*-flanked or excised *Kap1*, respectively. Embryos selected for analysis (two knockouts and one wild-type embryo) are labelled. The image shows two parts of the same gel but all at the same exposure and analysis settings.

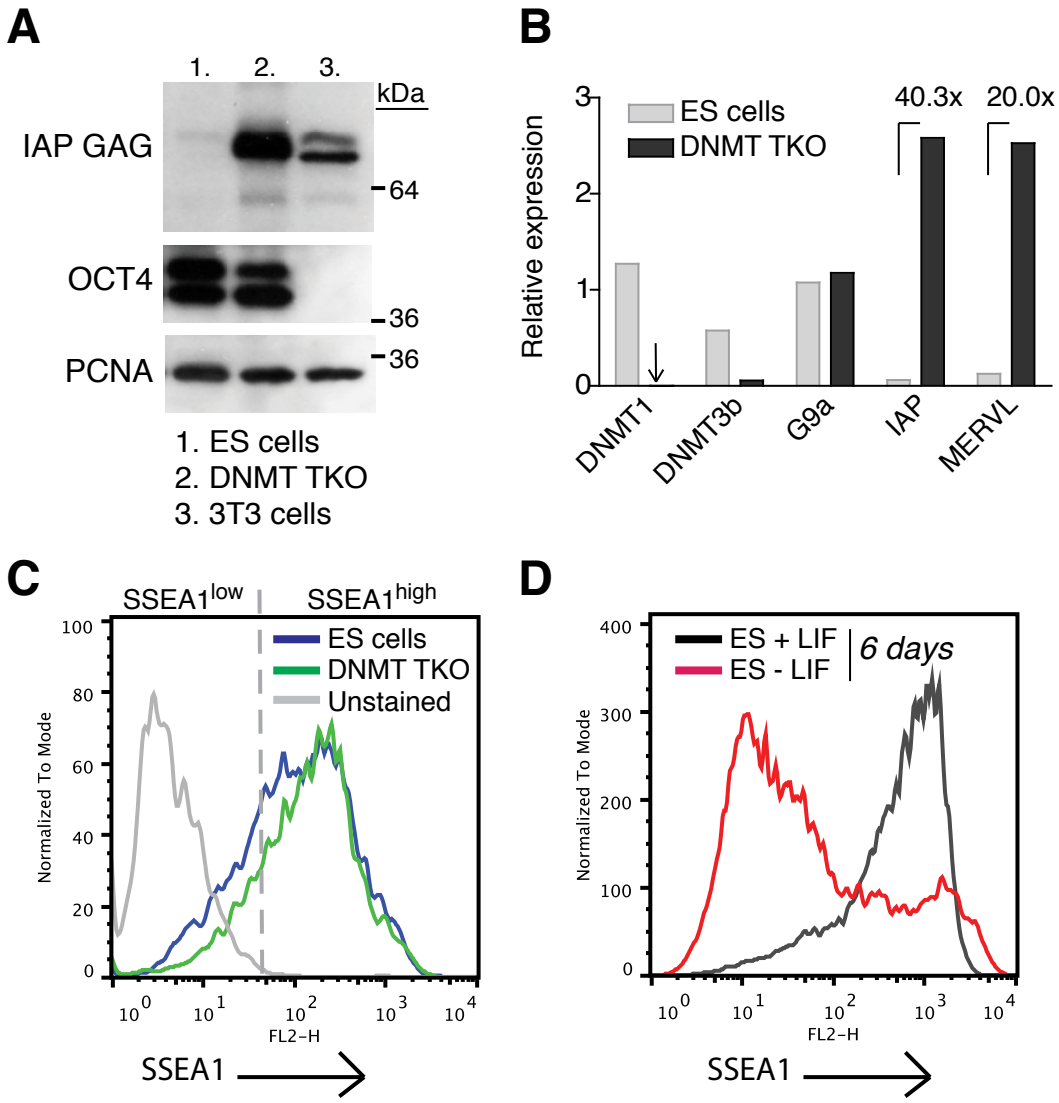

Fig. S8. DNA methylation of introduced ERV sequences and resident ERVs becomes crucial late in development. This relates to Fig. 7. (**A**) Accumulation of IAP GAG p73 in DNA methyltransferase triple knockout ES cells (DNMT TKO). 3T3 cells were a positive control as they overexpress IAP GAG. Global OCT4 protein levels were comparable between wild-type 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 whereas DNMT1 and DNMT3B were verified to be absent in DNMT TKO cells. (**C**) Wild-type and DNMT TKO ES cells were stained with an anti-SSEA1 antibody or left unstained, and histogram results were overlaid to verify that cells were largely undifferentiated. (**D**) In another experiment, ES cells were cultured for 6 days in the presence or absence of LIF, and then stained as in C to verify that cells downregulate SSEA1 upon differentiation in order to validate its use as a marker of undifferentiation in C.

Table S1. 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