**Supplemental Figure 1:** *Kdm1a* mutant ES cells have normal levels of DNMT1 and DNA methylation.

(A) The genetrap allele from ES line RRK075 allows the exploration of gene expression in heterozygous embryos and gene function in homozygotes and has the additional advantage of being “rescuable” by Cre-lox mediated excision. In this particular line, a β-geo stop cassette containing the engrailed splice acceptor site is inserted within intron 8 of *Kdm1a*. This insertion creates a fusion protein of β-geo to the first 8 exons of KDM1A, which truncates the majority of the catalytic amine oxidase domain. Excision of the splice acceptor site can then be carried out with the addition of Cre recombinase to generate the GT<sub>rescue</sub> allele. (B) Embryos derived from *Kdm1a* +/GT intercrosses were stained at E8.5 with X-gal in whole mount. (C) Immunofluorescence microscopy images of *Kdm1a* +/GT and GT/GT ES lines stained with indicated antibodies. (D) Methylated DNA IP was performed on *Kdm1a* +/GT (WT) or GT/GT (mutant) ES cells and the IP enriched and input samples were co-hybridized to a high density promoter tiling array. The normalized log<sub>2</sub> ratios of the wild type cells were then subtracted from that of the mutant cells, and the subtracted values were then summed across a 2kb region surrounding the transcriptional start site of genes on chromosome 19. (E-G) Bisulfite sequencing analysis of repetitive elements was performed on *Kdm1a* +/GT and GT/GT ES cells (E), *Kdm1a* +/GT and GT/GT blastocysts (F), or *Kdm1a* +/KO and KO/KO ES cells (G).

**Supplemental Figure 2:** Gene expression profiling of *Kdm1a* mutant ES cells.

(A) QRT-PCR was performed on independently derived *Kdm1a* GT/GT, +/GT, or +/+ ES lines with indicated primers and plotted relative to GAPDH with error bars representing standard deviation. (B) QRT-PCR was performed on three independently derived *Kdm1a* FL/FL ES cell lines transfected with GFP alone of Cre-GFP and subjected to FACS 24 hours later to collect GFP positive cells. After an additional 48 hours, RNA was prepared and QRT-PCR performed with indicated primers as in (A). (C) QRT-PCR was performed as in (A) with the addition of two clonal lines generated by transfection of *Kdm1a* GT/GT ES lines with Cre to rescue the GT allele. (D) Immunoblot analysis with the indicated antibodies was performed on *Kdm1a* +/+ (lane 1), +/GT (lane 2), GT/GT (lane 3), or a GT/GT clone transfected with Cre recombinase to rescue the GT allele (lane 4).
Supplemental Figure 3: KDM1A represses MERVL retroviruses and LTR enriched, segmentally duplicated genes.

(A-B) Distribution of the amount of LINE-1 elements per bp of gene (within a 10kb pad) for (A) all mouse genes tiled on the Affy Mouse 430 2.0 array and (B) KDM1A repressed genes. The mean is also displayed. (C-D) Distribution of the amount of LTR containing elements per bp of gene (within a 10kb pad) for (C) all mouse genes tiled on the Affy Mouse 430 2.0 array and (D) KDM1A repressed genes. (E) UCSC Genome Browser screenshot of mRNA-Seq reads from Kdm1a GT/GT and wild type ES cells aligning to the Gm428 cluster. Detected junctions are also displayed above the raw read alignments. Repeat Masker function is enabled to show locations of retrotransposable elements. (F) SNP plot of expressed MERVL elements from wild type and Kdm1a GT/GT ES cells. Tick lines indicate position of SNPs detected and total number of SNPs are displayed to the right.

Supplemental Figure 4: Endogenous retroviral LTRs are activated in Kdm1a mutant ES cells.

(A) Sequence Alignment of 4 different MERVL LTR-Gag clones utilized in luciferase reporter assays. (B) MERVL sequences as indicated were inserted upstream of luciferase and co-transfected with CMV βgal in triplicate into Kdm1a +/- or GT/GT ES cells. Relative luciferase activity was measured 24 hours later. Error bars represent S.E.M.

Supplemental Figure 5: Kdm1a mutant ES cells display histone modifications associated with transcription activation at target genes.

(A-D) ChIP on Chip analysis was performed on Kdm1a +/- or Kdm1a GT/GT ES cells with histone modification specific antibodies or methylated CpG antibodies (shown on the right). The normalized log2 intensity ratios were then displayed as individual tracks on the UCSC genome browser for Kdm1a +/- (red) and Kdm1a GT/GT (green) ES cells, which were then overlaid. Red and green overlapping signals are dark green. The log2 intensity ratios were then displayed on UCSC genome browser at the Af067061 gene (A) the Af067063 gene (B), or two MERVL retroviruses (C-D). The direction of the LTRs (5'-3') are indicated with an arrow.
**Supplemental Figure 6**: Epigenetic changes at a cluster of unknown genes upregulated in *Kdm1a* mutant ES cells. UCSC genome browser plot of mRNA-Seq and epigenetic marks. In black are the mRNA-Seq reads from *Kdm1a* GT/GT or WT/WT ES cells aligning to the cluster. In red are the subtracted (Mutant – Wild type) normalized log2 ChIP/Input intensity values for histone H3 AcK27, MonoMeK4, DiMeK4, and TriMeK4. Positive signals indicate an increase in the mutant. The Repeat Masker function is enabled to visualize the position of repetitive elements. The LTR elements within the cluster represent the ERVL and MaLR families.

**Supplemental Figure 7**: Genomewide MeDIP ChiP analysis of *Kdm1a* mutant ES cells at ERVs and retrotransposons.

(A-D) MeDIP Chip analysis was performed on *Kdm1a* +/-GT and GT/GT ES cells using a whole genome promoter array. Normalized MeDIP/Input signals in GT/GT (Y axis) and and +/-GT (X-axis) ES cells were plotted for all (A) ORR1B1, (B) MT2C, (C) MT2B, and (D) MERVL elements tiled on the array. Dotted line shows the position where MeDIP/Input signals were identical between the two samples.

**Supplemental Figure 8**: *Kdm1a* regulated ERVs and cellular genes are activated without global loss of DNA methylation. (A) Bisulfite converted DNA from indicated wild type and *Kdm1a* mutant ES lines were amplified by PCR using primers against the Zfp352 promoter of MERVL regions as indicated. Amplified DNA was then left undigested (U) or digested (D) with the indicated restriction enzymes and run on an agarose gel and visualized using ethidium bromide staining. (B-C) *Kdm1a* +/- or GT/GT ES cells were treated with TSA or 5Aza for 24 hours. QRT-PCR was performed on cDNA generated by polydT priming using primers specific for (C) IAP retroviruses or (D) Similar to Tho4, and normalized to Gapdh. The fold change was then plotted relative to untreated *Kdm1a* +/- ES cells with error bars representing s.d.

**Supplemental Figure 9**: *Kdm1a* mutant cells have increased potency to generate extra-embryonic lineages.
(A-L) *Kdm1a* FL/FL Cre-ERT ES cells were treated with vehicle (black) or 4OHT (grey) and maintained for 48 hours before growing in bacterial grade dishes in the absence of Lif to induce differentiation. EBs were harvested at multiple timepoints and subject to QRT-PCR with the indicated primers. Error bars represent s.d.

(M-N) *Kdm1a* FL/FL Cre-ERT ES cells were treated with vehicle (black) or 4OHT (grey) and maintained for 48 hours before growing in bacterial grade dishes in the absence of Lif to induce differentiation. After two days, cells were treated with RA and smoothened agonist to induce motor neuron differentiation. (M) After 4 days, wild type and mutant EBs were mixed and immunostained with KDM1A (green) and Tuj1 (red) antibodies or (N) were immunostained with the motor neuron marker HB9 (green) and TUJ1 (red).

**Supplemental Table Legends**

**Supplemental Table 1:** Summary of *Kdm1a* mutant crosses and outcomes.

**Supplemental Table 2:** Microarray analysis of *Kdm1a* mutant ES cells. Affymetrix microarray analysis was performed on 3 independently derived *Kdm1a* FL/FL ES cell lines transfected with GFP alone or Cre-GFP (to delete the floxed *Kdm1a* allele) after enrichment of GFP+ cells using FACS. A second microarray comparison was performed on 4 independently derived *Kdm1a* GT/GT ES cell lines and 4 wild type ES lines derived from +/+ and +/-GT littermates. Mis-expressed genes, average fold change, and p values were determined using Vampire. Fold change represents average normalized expression in the mutant / average normalized expression in wild type.

**Supplemental Table 3:** List of genes mis-expressed in both *Kdm1a* GT/GT and *Kdm1a* FL/FL ES cells transfected with Cre recombinase as determined by affymetrix gene expression profiling and Vampire analysis. Fold change indicates expression ratio in *Kdm1a* FL/FL ES cells transfected with Cre-GFP / cells transfected with GFP alone. LTR elements within 2kb of the transcriptional start site are shown. Genes overlapping
segmental duplication on build 36 are indicated. Gene copy numbers are estimated based on searching Homologene databases for putative orthologues.

**Supplemental Table 4:** Summary table of primers used in this study.

**Supplemental Methods**

**Primary antibodies used for immunoblots:** rabbit anti KDM1A, Abcam ab17721, 1:1000; rabbit anti DNMT1, 1:1000, gift from Guoliang Xu; rabbit anti CoREST, Upstate 07-455, 1:2000; rabbit anti GAPDH, Santa Cruz sc25-778, 1:1000; mouse anti OCT3/4, Santa Cruz sc-5279, 1:1000; rabbit anti HDAC1, Novus NB 500-124, 1:10,000; mouse anti RNAPII, Covance clone 8WG16, 1:1000; mouse anti KAP1, 20C1, Abcam ab22553, 1:1000, rabbit anti MERVL Gag, 1:2000, gift from Thierry Heidmann.

**Primary antibodies used for IF:** rabbit anti KDM1A, Abcam ab17721, 1:1000; mouse anti OCT3/4, Santa Cruz sc-5279, 1:1000; mouse anti KDM1A, Sigma L7293, 1:1000; rabbit anti DNMT1, Santa Cruz sc-20701, 1:500; rabbit anti MERVL-GAG, Heidmann lab, 1:2000; rat anti E-CADHERIN, Abcam ab11512, 1:500; mouse anti KAP1, 20C1, Abcam ab22553, 1:1000, rabbit anti collagen, Millipore Ab756P, 1:1000.

**Primary antibodies used for ChIP:** H3K4me1 (Abcam ab8895), H3K9me1 (Abcam ab8896), H3K9me2 (Abcam ab1220), and H3K27A (Abcam ab4729), H3K4me2 (Millipore 05-790) and H3K4me3 (Millipore 04-745).
**A**

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**B**

Graph showing relative expression of IAP under different conditions.

**C**

Graph showing similar expression to Tho4 under different conditions.