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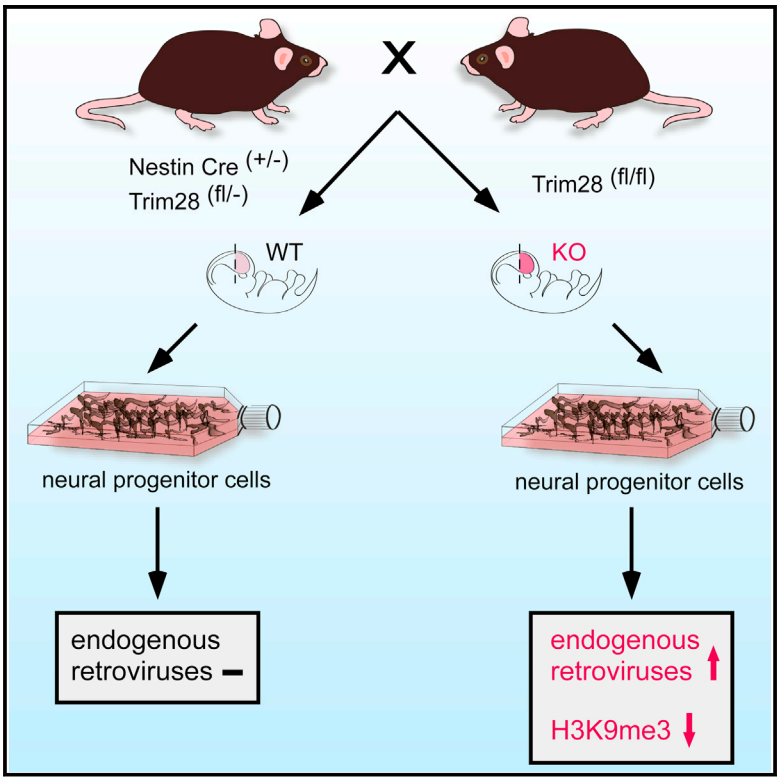
Report

Cell Reports

# TRIM28 Represses Transcription of Endogenous Retroviruses in Neural Progenitor Cells

## Graphical Abstract

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## In Brief

Endogenous retroviruses, which make up almost 10% of the mouse genome, are thought to be transcriptionally silenced by DNA methylation in adult tissues. Fasching et al. now show that endogenous retroviruses are controlled by TRIM28-mediated histone modifications in neural progenitor cells, suggesting a role for these elements in the control of transcriptional dynamics in the brain.

## Highlights

- Deletion of TRIM28 in NPCs results in transcriptional activation of ERVs
- ERVs are marked by H3K9me3 in NPCs, which is lost upon TRIM28 deletion
- Activation of ERVs in NPCs influences expression levels of nearby genes
- Activation of ERVs in NPCs results in the production of long noncoding RNAs

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# TRIM28 Represses Transcription of Endogenous Retroviruses in Neural Progenitor Cells

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## SUMMARY

**TRIM28 is a corepressor that mediates transcriptional silencing by establishing local heterochromatin. Here, we show that deletion of TRIM28 in neural progenitor cells (NPCs) results in high-level expression of two groups of endogenous retroviruses (ERVs): *IAP1* and *MMERVK10C*. We find that NPCs use TRIM28-mediated histone modifications to dynamically regulate transcription and silencing of ERVs, which is in contrast to other somatic cell types using DNA methylation. We also show that derepression of ERVs influences transcriptional dynamics in NPCs through the activation of nearby genes and the expression of long noncoding RNAs. These findings demonstrate a unique dynamic transcriptional regulation of ERVs in NPCs. Our results warrant future studies on the role of ERVs in the healthy and diseased brain.**

## INTRODUCTION

The mammalian brain is an extremely complex organ harboring more than a thousand different types of neurons that serve a wide variety of functions. How this complexity is achieved remains largely unknown. However, epigenetic mechanisms such as DNA methylation, histone modification, and noncoding RNAs are thought to be important in establishing a high diversity of gene expression from the same template, leading to a spatial pattern of transcription. How distinct transcriptional programs are established in different neuronal populations remains poorly understood, but one interesting recently proposed hypothesis suggests transposable elements (TEs) to be involved in this process (Muotri et al., 2007; Reilly et al., 2013). TEs are repetitive mobile genetic elements that were originally considered to be parasitic DNA without any function, popularly termed "junk DNA." Today, it is becoming increasingly clear that TEs can act as gene regulatory elements by serving as hubs for chromatin modifications and by acting as transcriptional start sites for noncoding RNAs. Consequently, TEs are very well suited to influence gene expression and may play an important role in controlling and fine-tuning gene networks in the brain (Jern and Coffin, 2008; Cowley and Oakey, 2013).

Retroviruses are found in most vertebrates and can transform their genetic material and integrate into the host genome as proviruses to produce new viruses. Occasionally, retroviruses infect germline cells allowing the integrated proviruses to be passed on to the offspring as an endogenous retrovirus (ERV). Around 8%–10% of the human and mouse genome are composed of this type of TE, and, despite up to millions of years since their integration in host germline, many ERVs contain sequences that can serve as transcriptional start sites or as *cis*-acting regulatory elements in the host genomes (Jern and Coffin, 2008). The large amount of ERVs in mammalian genomes suggest that they play important roles in the host organisms, for instance, by influencing gene regulatory networks (Kunarso et al., 2010; Feschotte, 2008), but ERVs have also been linked to diseases. In humans, aberrant expression of ERVs has been found in both cancer and brain disorders, although causality remains to be established (Jern and Coffin, 2008; Douville et al., 2011). Thus, ERVs may contribute both beneficial and detrimental effects, which have been balanced throughout evolution, to the host organism.

ERVs are silenced during the first few days of embryogenesis by TRIM28 (tripartite motif-containing protein 28, also known as KAP1 or TIF1beta), a transcriptional corepressor essential for early mouse development (Cammas et al., 2000; Rowe et al., 2010). During the extensive genome reprogramming that takes place at this period, TRIM28 is recruited to ERVs via sequence-specific Krüppel-associated box zinc-finger proteins (KRAB-ZFPs), a family of transcription factors that has undergone a rapid expansion in mammalian genomes in parallel with the expansion of ERVs (Wolf and Goff, 2009; Thomas and Schneider, 2011). TRIM28 then induces repressive histone modifications by recruiting multiprotein complexes including the H3K9 methyltransferase SETDB1 (also known as ESET), the histone deacetylase-containing NuRD complex, and heterochromatin protein 1 (HP1) (Schultz et al., 2002; Sripathy et al.,

2006). Deletion of *Trim28* or *Setdb1* in ESCs leads to loss of the H3K9me3-mark at ERVs, resulting in transcriptional activation of these elements (Matsui et al., 2010; Rowe et al., 2010).

However, KRAB-ZFP/TRIM28 histone-based repression of ERVs rapidly gives place to a more permanent silencing mechanism, as the TRIM28-mediated recruitment of de novo DNA methyltransferases leads to cytosine methylation at CpG dinucleotides (Ellis et al., 2007; Wiznerowicz et al., 2007; Rowe and Trono, 2011). The maintenance DNA methyltransferase complex then ensures that DNA methylation is maintained, alleviating the need for sequence-specific KRAB-ZFPs and TRIM28. In mouse embryonic fibroblasts as well as in all adult tissues examined so far, TRIM28 depletion has no impact on ERV silencing, which is instead released by drugs such as 5-azacytidine or by deletion of DNA methyltransferases (Jackson-Grusby et al., 2001; Hutnick et al., 2010).

DNA methylation has long been considered as a stable epigenetic mark resulting in maintenance of DNA-methylation patterns throughout the lifespan of an organism. However, several recent studies demonstrate a unique dynamic regulation of DNA-methylation patterns in the brain (Sweatt, 2013). There is also evidence that retroelements and transposons are highly active during brain development and in neural progenitor cells (NPCs) (Muotri et al., 2005, 2010; Baillie et al., 2011; Evrony et al., 2012; Li et al., 2013; Perrat et al., 2013). For example, *LINE-1* elements have been found to be transcriptionally active and to retrotranspose in NPCs (Muotri et al., 2005, 2010; Coufal et al., 2009). In addition, we have previously found that deletion of TRIM28 in postmitotic forebrain neurons results in complex behavioral alterations, including vulnerability to stress (Jakobsson et al., 2008). In the present work, we demonstrate that NPCs use TRIM28-mediated histone modifications to dynamically regulate the transcription and silencing of ERVs, rather than the DNA methylation at play in other somatic tissues. We also unveil that derepression of ERVs influences transcriptional dynamics in NPCs, by activating nearby genes and the expression of long noncoding RNAs (lncRNAs).

## RESULTS

### TRIM28-Deficient NPCs Express High Levels of ERVs

To investigate if TRIM28 contributes to ERV silencing in NPCs we established *Trim28*-deficient NPC cultures. We crossed transgenic *NestinCre* mice (Tronche et al., 1999) with mice carrying floxed *Trim28*-alleles (*Trim28*$^{fl/fl}$) (Weber et al., 2002), resulting in excision of *Trim28* in neural progenitors at the time when Nestin-expression is initiated, starting around embryonic day 10 (E10). At E13.5, we collected embryos, dissected the forebrain, and established NPC cultures from individual embryos (Figures 1A and 1B). We confirmed the deletion of *Trim28* by genotyping for the excised allele and by verifying the absence of TRIM28 protein (Figures 1C and 1D). We collected RNA from *Trim28*$^{-/-}$ NPCs and wild-type controls and performed RNA extraction followed by deep sequencing (RNA-seq). The resulting reads were mapped against reference sequences from Repbase, a database containing consensus sequences for known repetitive elements (Jurka et al., 2005). We found that several ERVs were highly upregulated in *Trim28*$^{-/-}$ NPCs, including, e.g., *Mus musculus ERV using tRNA*$^{Lys}$ *type 10C* (*MMERVK10C*) and *intracisternal A-particles class 1* (*IAP1*) (Figure 1E; Tables S1 and S2). Other retroelements such as *MusD* and *LINE-1* were modestly upregulated, whereas several other types of common repetitive elements were unaffected (Figure 1E; Tables S1 and S2).

We confirmed increased transcription of *MMERVK10C* and *IAP1* elements using quantitative RT-PCR (qRT-PCR) (Figure 1F). In contrast, when we used primer pairs designed to recognize the consensus sequence of the entire *IAP*-family, including more ancient *IAP* elements, we detected only a modest upregulation (Figure 1F). This finding is in line with the results of the RNA-seq, which indicated that only certain types of IAP elements were upregulated in *Trim28*$^{-/-}$ NPCs. Also in agreement with the RNA-seq, qRT-PCR analyses indicated that deletion of *Trim28* in NPCs only modestly increased the expression of other retroelements such as *LINE-1* or *MusD* (Figure 1F). We confirmed these results in cultures derived from two separate embryos (data not shown).

*Trim28*$^{-/-}$ NPCs proliferated at a similar rate compared to cells generated from wild-type and heterozygous siblings and could be expanded for more than 60 passages. However, we observed that *Trim28*$^{-/-}$ NPCs were growing in dense cluster-like formations, which seemed to attach less to the flask surface compared to the wild-type control. *Trim28*$^{-/-}$ NPCs could also be differentiated to both neurons and astrocytes suggesting that TRIM28 has no major influence on the self-renewal and differentiation of NPCs (Figures 1G and 1H).

### *MMERVK10C* Elements Are Controlled by TRIM28

The RNA-seq analysis indicated that *MMERVK10C* elements were among the most upregulated ERVs following *Trim28*-deletion in NPCs. *MMERVK10C* is a beta-like ERV similar to *HERVK* (*HML2*), one of the most recent ERVs to invade the human genome (Reichmann et al., 2012) (Belshaw et al., 2005). *MMERVK10C* sequences flanked by *RLTR10C* make up putative proviral sequences of around 8.4 kb. In the mouse genome, *MMERVK10C* is present in a few complete provirus loci (~20) and more than 1,000 incomplete loci (Reichmann et al., 2012). We performed sequence analysis of the *MMERVK10C* provirus for the presence of retroviral features using the RetroTector software (Sperber et al., 2007). Based on this analysis, we designed primers recognizing the *LTRs*, *gag*, *pol*, and *env* of the *MMERVK10C* provirus and investigated expression levels in *Trim28*$^{-/-}$ NPCs (schematics in Figure 2A). We found that transcripts over the entire region of the provirus were increased, including a massive expression of *env* sequences when compared to wild-type controls (170-fold; Figure 2B).

Ascertaining that the ERV induction observed in NPCs isolated from *Trim28*$^{-/-}$ animals was not secondary to more general developmental anomalies, knocking down TRIM28 in wild-type NPCs by lentivector-mediated RNA interference led to a marked upregulation of these retroelements (Figure 2C). Furthermore, increased ERV expression was detected in forebrain tissue from *Trim28*$^{-/-}$ embryos (Figure 2D).

In ESCs, TRIM28 controls ERV expression via histone modifications including H3K9 trimethylation (Rowe et al., 2010), whereas it is DNA methylation that instead prevails in somatic

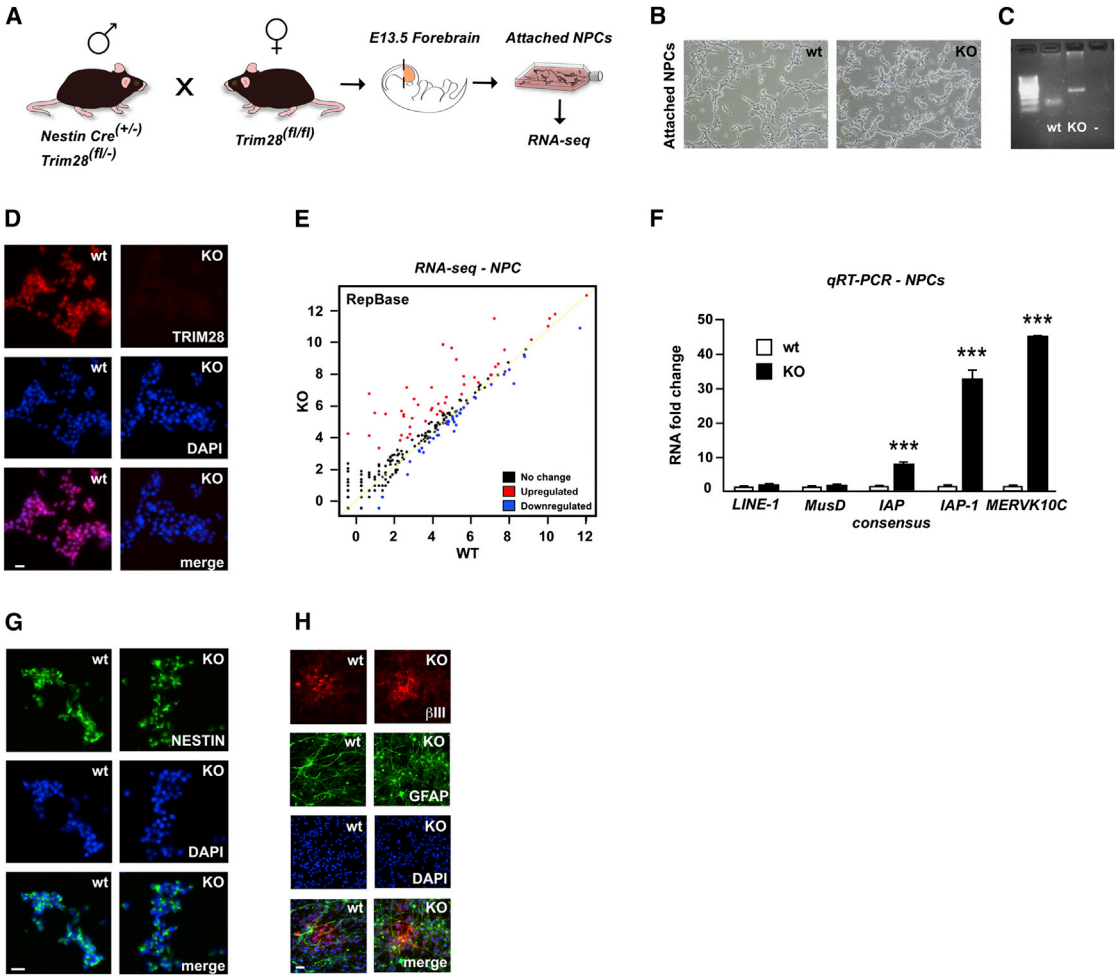


**Figure 1. Establishment of *Trim28*-Deficient Neural Progenitor Cultures**

(A) Illustration of the experimental approach.

(B) Representative images of early passage *Trim28*$^{-/-}$ NPCs.

(C) PCR analysis of genomic DNA from wild-type and *Trim28*$^{-/-}$ NPCs demonstrates the presence of the 152 and 290 bp products corresponding to *loxP*-flanked or excised *Trim28*, respectively.

(D) Verification of a complete lack of TRIM28 protein via immunocytochemistry.

(E) RNA-seq analysis. The graph shows KO samples plotted versus wild-type samples, where each dot represents a Repbase sequence.

(F) qRT-PCR of RNA isolated from wild-type and *Trim28*$^{-/-}$ NPCs.

(G) *Trim28*-deficient NPCs display a homogenous expression of NESTIN.

(H) Immunofluorescent analysis of differentiated NPCs.

Data are presented as mean of relative values ± SEM. $^{**}p < 0.01$, $^{***}p < 0.001$, Student's t test. Scale bars, 200 (A) and 50 (B) μm. See also Tables S1 and S2.

tissues. In NPCs, we found that the *MMERVK10C* provirus was enriched in H3K9me3, and that this repressive mark was markedly reduced in *Trim28*$^{-/-}$ NPCs (Figure 2E).

Because *MMERVK10C* appeared to be under TRIM28 control in NPCs, we hypothesized that at least a proportion of these retroelements escaped DNA methylation in these cells. To probe this issue, we examined the DNA methylation status of full-length *MMERVK10C*, which were among the most highly upregulated retroelements in *Trim28*$^{-/-}$ NPCs. Bisulfite sequencing of a CpG-island located in the 3′ region of *MMERVK10C* revealed several clones with some unmethylated CpGs (17% unmethylated CpGs, Figure 2F) in NPCs, whereas this region was almost fully methylated in DNA extracted from mouse tail (7% unmethylated CpGs, Figure 2F, Fisher's exact test one-sided $p < 0.05$). Moreover, we found no difference in the level of CpG methylation between wild-type and *Trim28*$^{-/-}$ NPCs. In summary, these data suggest that a proportion of the *MMERVK10C* elements are spared from undergoing DNA methylation specifically in NPCs during early development.

## Increased Expression of *IAP1* Results in ERV-Derived Protein Expression

*IAP1* elements, which lose H3K9me3 marks and were also highly upregulated in *Trim28*$^{-/-}$ NPCs (Figures 3A and 3B), are

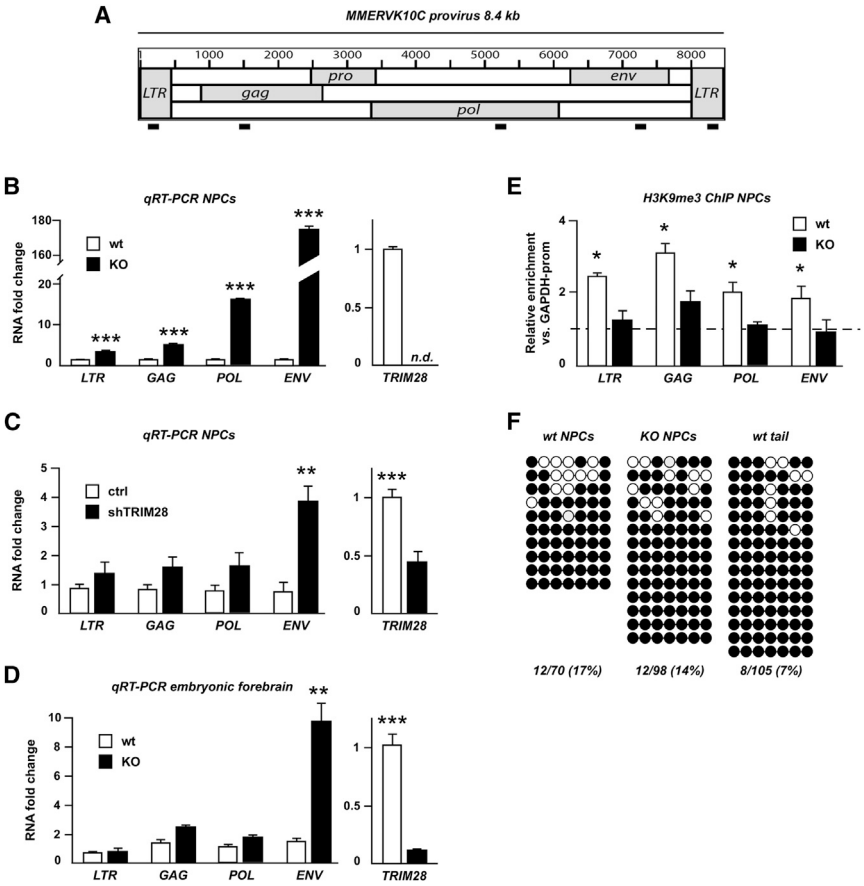


**Figure 2. Analysis of the Putative *MMERVK10C* Provirus**

(A) Schematic drawing of the *MMERVK10C* provirus and approximate primer positions.

(B) Quantitative analysis of transcript levels of different regions of the *MMERVK10C* provirus in *Trim28*$^{-/-}$ and wild-type NPCs.

(C) qRT-PCR analysis of *MMERVK10C* following TRIM28-shRNA knockdown.

(D) qRT-PCR analysis of E13.5 forebrain dissected from intercrosses of *NestinCre Trim28*$^{floxed}$ mice.

(E) ChIP for H3K9me3 in *Trim28*$^{-/-}$ and wild-type NPCs.

(F) Bisulfite sequencing analysis of the 3′ end region of *MMERVK10C*. Empty and full circles represent unmethylated and methylated CpGs, respectively.

Data are presented as mean of relative values ± SEM. *$p < 0.05$, Student's t test.

internalized *env*-lacking mouse ERVs that demonstrate a large degree of polymorphism among different mouse strains and maintain the capacity to retrotranspose. Using immunocytochemistry with an IAP-specific antibody, we found a uniform, high-level IAP-gag expression located to the cytoplasm in *Trim28*$^{-/-}$ NPCs (Figure 3C).

Taken together, these data demonstrate that deletion of TRIM28 in NPCs results in a massive transcriptional increase of ERVs, including *MMERVK10C* and *IAP1*. NPCs thus appear to constitute a cellular environment distinct from that of other somatic cells studied so far, with the TRIM28-induced histone-based repressive mechanism playing a role in ERV control.

## Activation of ERVs Correlates with Increased Transcription of Nearby Genes

The ability of ERVs to attract transcription factors and silencing complexes has led to a reassessment of their role in the host genome. ERVs are now considered to be important transcriptional regulatory elements that shape and influence gene expression during early development (Isbel and Whitelaw, 2012). For example, we have recently found that TRIM28 controls the expression of developmental genes by repressing ERV-associated enhancers in pluripotent cells (Rowe et al., 2013). Twenty-six *MMERVK10C* proviruses and 361 *IAP* proviruses that were upregulated in *Trim28*$^{-/-}$ NPCs were mapped to precise genomic locations (Figure S1). Out of these 387 proviruses, 90 were situated close to genes (<50 kb). We found that 25 of those genes (28%) demonstrated significantly increased expression, whereas expression of only six of them was decreased (7%) (Figure 4A). We also found that those 90 genes located close to upregulated ERVs (ERV-up genes) were on average 3-fold upregulated in *Trim28*$^{-/-}$ cells (Figure 4B). In contrast, a random selection of ERVs that was not upregulated in *Trim28*$^{-/-}$ cells (n = 129, *MMERVK10C* and *IAP1* elements) did not affect nearby genes (ERVs-ctrl genes, n = 50, Figure 4B). Interestingly, we also found that ERV-up genes were expressed at low levels in wild-type cells (Figure 4C), which is in agreement with a model where ERVs mediate repressive regulation of nearby genes caused by the attraction of the TRIM28 silencing complex to ERV sequences. We validated the increased expression of five ERV-up genes in *Trim28*$^{-/-}$ cells using qRT-PCR (Figure 4D).

## ERVs Produce Long Noncoding RNAs

We looked in detail at *BC048671*, which is a protein-coding transcript that is induced in *Trim28*$^{-/-}$ NPCs but completely absent in wild-type NPCs. *BC048671* is located 5 kb downstream of an *IAP* element, which is also highly upregulated in *Trim28*$^{-/-}$ NPCs. The RNA-seq data show that transcriptional initiation at the *IAP* element results in the formation of a long transcript (>10 kb) that extends into the coding sequence of *BC048671* (Figure 4E). The presence of high levels of this long transcript was verified using qRT-PCR primers located both upstream and within the coding sequence of *BC048671* (Figure 4F). Thus, readthrough of an ERV-derived transcript into another locus is likely to be one of several mechanisms by which nearby gene expression can be affected (see also Figure S2). This finding supports the notion that a general feature of ERVs might

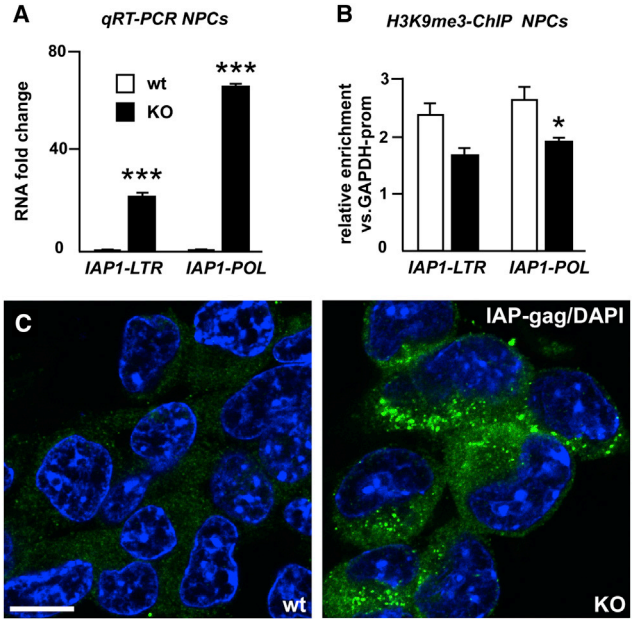


**Figure 3. Analysis of *IAP1* Expression**

(A) Quantitative analysis of transcript levels of different regions of *IAP1* provirus in *Trim28*$^{-/-}$ and wild-type NPCs.

(B) ChIP for H3K9me3 in *Trim28*$^{-/-}$ and wild-type NPCs.

(C) Confocal analysis of immunofluorescence staining for IAP-gag on *Trim28*$^{-/-}$ and wild-type NPCs. Scale bar, 10 μm.

Data are presented as mean of relative values ± SEM. *$p < 0.05$, Student's t test.

be to act as transcriptional start sites for long noncoding RNAs (lncRNAs). Indeed, when we scrutinized ERV elements located in gene free regions, we found that both *IAP* and *MMERVK10C* elements serve as start sites for lncRNAs (Figures 4G and 4I). Using qRT-PCR, we confirmed high-level expression of two ERV-derived lncRNAs in *Trim28*$^{-/-}$ NPCs (Figures 4H and 4J). The length of the ERV-derived lncRNAs did in many cases extend 25 kb (Figure 4K). These data demonstrate that derepression of ERVs in NPCs can result in the expression of multiple lncRNAs. The functional role of lncRNAs in NPCs remains largely unexplored, but they are thought to play important regulatory roles and have been implicated as scaffolds for nuclear protein complexes and as antisense transcripts in the control of epigenetic pathways (Guttman and Rinn, 2012).

## DISCUSSION

In pluripotent stem cells, TRIM28 is a master corepressor of retroelements including ERVs (Matsui et al., 2010; Rowe et al., 2010). When these cells differentiate into various somatic cell types, DNA methylation is instated on ERV sequences, which ultimately results in stable silencing that is no longer dependent on TRIM28 (Wiznerowicz et al., 2007; Rowe et al., 2013). Thus, when TRIM28 is deleted from various somatic cell types such as fibroblasts, hepatocytes, and white blood cells, no increased ERV expression is detected (Rowe et al., 2010; Bojkowska et al., 2012; Santoni de Sio et al., 2012a, 2012b). Here, we describe an exception to this rule. When TRIM28 is deleted in NPCs, several ERVs become highly expressed. This finding unravels a unique transcriptional regulation of ERVs in NPCs.

ERVs regulated by TRIM28 in NPCs are recent invaders of the mouse genome. *IAP1* is the most recent member of the well-studied IAP ERVs (Qin et al., 2010). IAPs are ERVs that have lost the *env* gene and adopted an intracellular life cycle (Ribet et al., 2008). *IAP1* has been shown to retrotranspose and has distinct integration patterns in different strains of laboratory mice (Li et al., 2012). *MMERVK10C*, another ERV massively upregulated in *Trim28*$^{-/-}$ NPCs, is poorly characterized, and it is unclear if it is still endowed with retrotransposition potential, whether on its own or with the support of factors provided in *trans*. A previous study that analyzed the structure of *MMERVK10C* elements in the mouse genome found that the majority of these elements have 3′ deletions removing the start of the *gag* open reading frame as well as the major part of *env* (Reichmann et al., 2012). Our data demonstrate that, in NPCs, TRIM28 controls the rare copies of *env*-containing *MMERVK10C* elements, which are most likely to be the youngest ones, raising the possibility that these recent invaders of the mouse genome contain *cis*-acting genomic elements that allow them to escape DNA methylation in NPCs.

The classic view of repetitive mobile genetic elements as parasitic DNA without beneficial function to the host is challenged in many ways. There are a number of recent studies indicating that transposable elements (TEs) play important roles in establishing and rewiring gene networks (Kunarso et al., 2010; Chuong et al., 2013). TEs have been shown to act as enhancers, repressors, and alternative promoters. In addition, TEs can affect splicing patterns and produce peptides with important functional roles (Jern and Coffin, 2008). In this study, we demonstrate that activated ERVs can influence gene expression of nearby genes, such as *BC048671*, and serve as start sites for lncRNAs. Taken together, our findings indicate that ERVs participate in the control of gene networks in the brain.

We have previously demonstrated that deletion of *Trim28* in postmitotic forebrain neurons results in complex behavioral changes (Jakobsson et al., 2008). In addition, heterozygous germline deletion of *Trim28* has been described to result in abnormal behavioral phenotypes (Whitelaw et al., 2010). In this study, we found that deletion of *Trim28* during brain development is lethal (Figure S3). In addition, we also noted that heterozygous deletion of *Trim28* during brain development resulted in behavioral changes characterized by hyperactivity (Figure S3). Together, these findings demonstrate that disruption of TRIM28 levels in the mouse brain results in behavioral changes that are similar to impairments found in humans with certain psychiatric disorders. With this in mind, it is noteworthy that increased levels of ERV transcripts have been detected in patients with several neurological and psychiatric disorders (Jeong et al., 2010; Douville et al., 2011; Li et al., 2012; Karlsson et al., 2001). The significance of these findings has been questioned because the human genome does not appear to harbor ERVs with known retrotransposing capacity (Jern and Coffin, 2008). However, the increasing evidence that derepression of

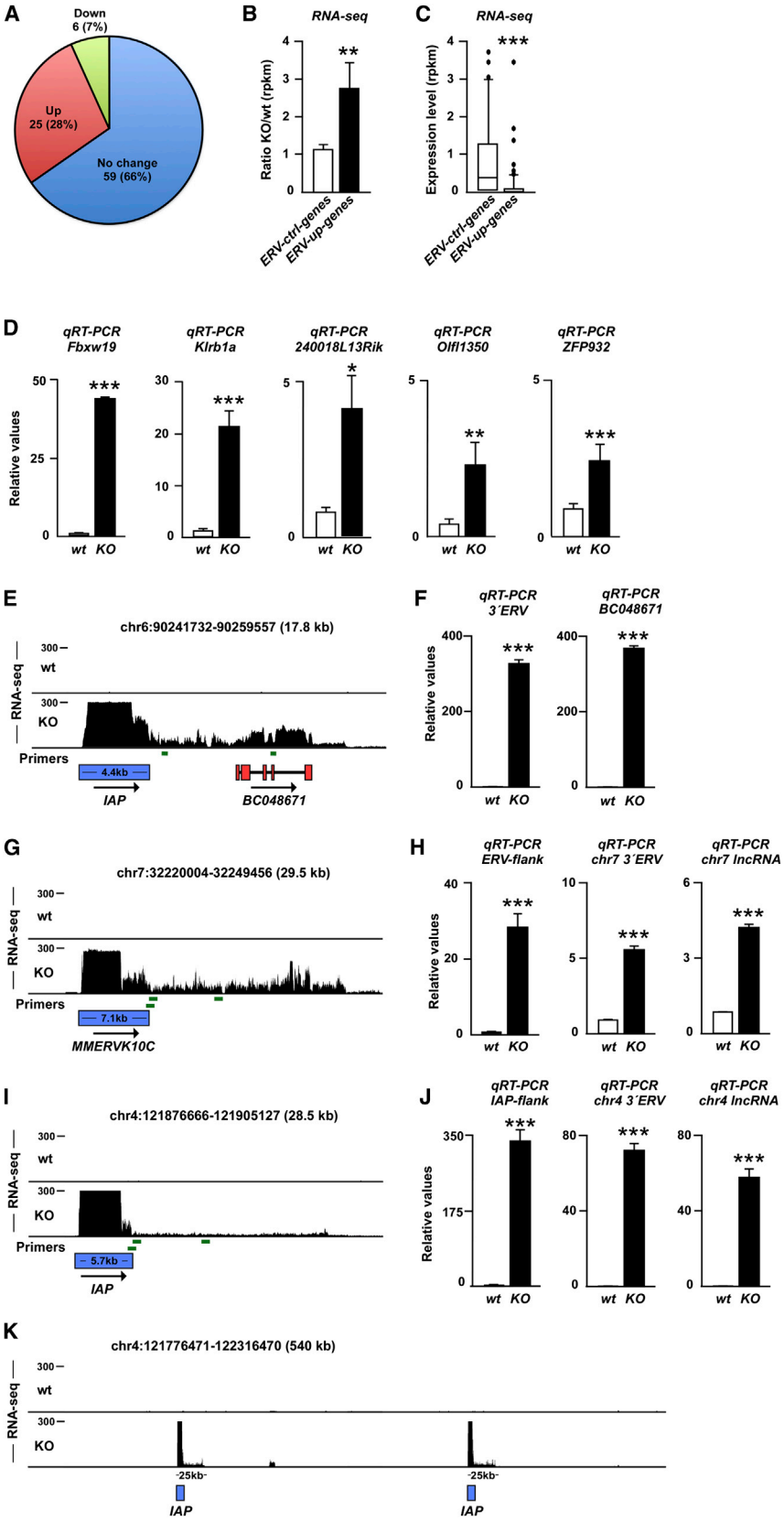


**Figure 4. Activation of ERVs Influences Expression of Nearby Genes and Results in the Expression of lncRNAs**

(A) Transcriptional change of genes located close (<50 kb) to ERVs in *Trim28*$^{-/-}$ NPCs.

(B) Mean transcriptional change of genes located to ERVs with increased transcription (ERV-up genes) and genes located close to unchanged ERVs (ERV-ctrl genes) in *Trim28*$^{-/-}$ NPCs.

(C) Absolute expression level of ERV-up genes and ERV-ctrl genes in wild-type NPCs.

(D) qRT-PCR of RNA isolated from wild-type and *Trim28*$^{-/-}$ NPCs.

(E) Screen shot from the USCS genome browser (mm9) showing induced transcription of *BC048671 in Trim28*$^{-/-}$ NPCs.

(G, I, and K) Activation of ERVs results in the expression of lncRNAs. Screen shot from the USCS genome browser (mm9).

(F, H, and J) qRT-PCR of RNA isolated from wild-type and *Trim28*$^{-/-}$ NPCs. Primers are indicated as green bars and include primers over the ERV junction as well as close and more distant from the 3′ end of the ERVs.

Data are presented as mean of relative values ± SEM. *$p < 0.05$, Student's t test. See also Figures S1 and S2.

ERVs influence gene networks, including the findings presented here, provides a potential mechanistic explanation for these observations.

In summary, our data suggest that ERVs may be involved in the regulation of gene expression in NPCs and may hereby offer a link between ERVs and brain disorders. It seems unlikely that behavioral phenotypes would arise from the derepression of a single ERV-induced gene. Instead, the presence of ERVs in multiple copies scattered throughout the genome allows for a powerful network-like control of gene expression, where dysregulation could result in widespread consequences. However, due to the large numbers of ERVs present in the mouse and human genome and their sequence variation, it is currently unfeasible to demonstrate a causal role for ERVs in controlling complex behavior or brain disorders using loss-of-function approaches, such as gene targeting and small hairpin RNA (shRNA) knockdown. Instead, improving our knowledge of critical host factors and networks controlling ERVs is essential to appreciate their impact on the genome and pathologies that may stem from their dysregulation. The demonstration that there is an ongoing dynamic TRIM28-mediated regulation of ERVs in NPCs is a step in this direction and warrants future studies of epigenetic and posttranscriptional regulation of ERVs in the healthy and diseased brain.

## EXPERIMENTAL PROCEDURES

Detailed experimental procedures can be found in the Supplemental Experimental Procedures.

### Procedures

#### *Transgenic Animals*

All animal-related procedures were approved by and conducted in accordance with the committee for use of laboratory animals at Lund University. NestinCre and floxed *Trim28* mice have been described previously (Weber et al., 2002; Tronche et al., 1999).

#### *Cell Culture*

NPC was established from embryonic day 13.5 (E13.5) forebrain and cultured as previously described (Conti et al., 2005).

#### *Immunofluorescence*

Immunofluorescence was performed as previously described (Thompson et al., 2005; Sachdeva et al., 2010).

#### *RNA Studies*

RNA-seq and qRT-PCR was performed as previously described (Rowe et al., 2010). The 50-base-paired end reads were mapped onto the RepBase version 16.08 (Jurka et al., 2005) and to the mouse genome (mm9) assembly. Mapping was done using the bowtie short read aligner (Langmead et al., 2009).

#### *Chromatin Immunoprecipitation*

Chromatin immunoprecipitation was performed with iDeal chromatin immunoprecipitation sequencing (ChIP-seq) kit (Diagenode) according to supplier's recommendations.

#### *DNA-Methylation Analysis*

Bisulfite sequencing was performed with the EpiTect bisulfite kit (QIAGEN) according to the supplier's recommendations. Sequence data were analyzed with the QUantification tool for Methylation Analysis (Kumaki et al., 2008).

#### *Statistical Analysis*

An unpaired t test was performed in order to test for statistical significance. Data are presented as mean ± SEM.

## ACCESSION NUMBERS

The RNA-seq data were deposited in the NCBI Gene Expression Omnibus and are available under accession number GSE45930.

## SUPPLEMENTAL INFORMATION

Supplemental Information includes Supplemental Experimental Procedures, three figures, and three tables and can be found with this article online at http://dx.doi.org/10.1016/j.celrep.2014.12.004.

## AUTHOR CONTRIBUTIONS

L.F., A.K., R.S., R.P., M.E.J., and C.M. designed and performed research and analyzed data. P.J., P.T., and D.T. designed research and analyzed data. F.C. contributed reagents. J.J. designed and coordinated the project and analyzed data. L.F. and J.J. wrote the paper, and all authors reviewed the manuscript.

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## REFERENCES

Baillie, J.K., Barnett, M.W., Upton, K.R., Gerhardt, D.J., Richmond, T.A., De Sapio, F., Brennan, P.M., Rizzu, P., Smith, S., Fell, M., et al. (2011). Somatic retrotransposition alters the genetic landscape of the human brain. Nature *479*, 534–537.

Belshaw, R., Dawson, A.L., Woolven-Allen, J., Redding, J., Burt, A., and Tristem, M. (2005). Genomewide screening reveals high levels of insertional polymorphism in the human endogenous retrovirus family HERV-K(HML2): implications for present-day activity. J. Virol. *79*, 12507–12514.

Bojkowska, K., Aloisio, F., Cassano, M., Kapopoulou, A., Santoni de Sio, F., Zangger, N., Offner, S., Cartoni, C., Thomas, C., Quenneville, S., et al. (2012). Liver-specific ablation of Krüppel-associated box-associated protein 1 in mice leads to male-predominant hepatosteatosis and development of liver adenoma. Hepatology *56*, 1279–1290.

Cammas, F., Mark, M., Dollé, P., Dierich, A., Chambon, P., and Losson, R. (2000). Mice lacking the transcriptional corepressor TIF1beta are defective in early postimplantation development. Development *127*, 2955–2963.

Chuong, E.B., Rumi, M.A., Soares, M.J., and Baker, J.C. (2013). Endogenous retroviruses function as species-specific enhancer elements in the placenta. Nat. Genet. *45*, 325–329.

Conti, L., Pollard, S.M., Gorba, T., Reitano, E., Toselli, M., Biella, G., Sun, Y., Sanzone, S., Ying, Q.L., Cattaneo, E., and Smith, A. (2005). Niche-independent symmetrical self-renewal of a mammalian tissue stem cell. PLoS Biol. *3*, e283.

Coufal, N.G., Garcia-Perez, J.L., Peng, G.E., Yeo, G.W., Mu, Y., Lovci, M.T., Morell, M., O'Shea, K.S., Moran, J.V., and Gage, F.H. (2009). L1 retrotransposition in human neural progenitor cells. Nature *460*, 1127–1131.

Cowley, M., and Oakey, R.J. (2013). Transposable elements re-wire and fine-tune the transcriptome. PLoS Genet. *9*, e1003234.

Douville, R., Liu, J., Rothstein, J., and Nath, A. (2011). Identification of active loci of a human endogenous retrovirus in neurons of patients with amyotrophic lateral sclerosis. Ann. Neurol. *69*, 141–151.

Ellis, J., Hotta, A., and Rastegar, M. (2007). Retrovirus silencing by an epigenetic TRIM. Cell *131*, 13–14.

Evrony, G.D., Cai, X., Lee, E., Hills, L.B., Elhosary, P.C., Lehmann, H.S., Parker, J.J., Atabay, K.D., Gilmore, E.C., Poduri, A., et al. (2012). Single-neuron sequencing analysis of L1 retrotransposition and somatic mutation in the human brain. Cell *151*, 483–496.

Feschotte, C. (2008). Transposable elements and the evolution of regulatory networks. Nat. Rev. Genet. *9*, 397–405.

Guttman, M., and Rinn, J.L. (2012). Modular regulatory principles of large non-coding RNAs. Nature *482*, 339–346.

Hutnick, L.K., Huang, X., Loo, T.C., Ma, Z., and Fan, G. (2010). Repression of retrotransposal elements in mouse embryonic stem cells is primarily mediated by a DNA methylation-independent mechanism. J. Biol. Chem. *285*, 21082–21091.

Isbel, L., and Whitelaw, E. (2012). Endogenous retroviruses in mammals: an emerging picture of how ERVs modify expression of adjacent genes. Bioessays *34*, 734–738.

Jackson-Grusby, L., Beard, C., Possemato, R., Tudor, M., Fambrough, D., Csankovszki, G., Dausman, J., Lee, P., Wilson, C., Lander, E., and Jaenisch, R. (2001). Loss of genomic methylation causes p53-dependent apoptosis and epigenetic deregulation. Nat. Genet. *27*, 31–39.

Jakobsson, J., Cordero, M.I., Bisaz, R., Groner, A.C., Busskamp, V., Bensadoun, J.C., Cammas, F., Losson, R., Mansuy, I.M., Sandi, C., and Trono, D. (2008). KAP1-mediated epigenetic repression in the forebrain modulates behavioral vulnerability to stress. Neuron *60*, 818–831.

Jeong, B.H., Lee, Y.J., Carp, R.I., and Kim, Y.S. (2010). The prevalence of human endogenous retroviruses in cerebrospinal fluids from patients with sporadic Creutzfeldt-Jakob disease. J. Clin. Virol. *47*, 136–142.

Jern, P., and Coffin, J.M. (2008). Effects of retroviruses on host genome function. Annu. Rev. Genet. *42*, 709–732.

Jurka, J., Kapitonov, V.V., Pavlicek, A., Klonowski, P., Kohany, O., and Walichiewicz, J. (2005). Repbase Update, a database of eukaryotic repetitive elements. Cytogenet. Genome Res. *110*, 462–467.

Karlsson, H., Bachmann, S., Schröder, J., McArthur, J., Torrey, E.F., and Yolken, R.H. (2001). Retroviral RNA identified in the cerebrospinal fluids and brains of individuals with schizophrenia. Proc. Natl. Acad. Sci. USA *98*, 4634–4639.

Kumaki, Y., Oda, M., and Okano, M. (2008). QUMA: quantification tool for methylation analysis. Nucleic Acids Res. *36* (Web Server issue), W170-5.

Kunarso, G., Chia, N.Y., Jeyakani, J., Hwang, C., Lu, X., Chan, Y.S., Ng, H.H., and Bourque, G. (2010). Transposable elements have rewired the core regulatory network of human embryonic stem cells. Nat. Genet. *42*, 631–634.

Langmead, B., Trapnell, C., Pop, M., and Salzberg, S.L. (2009). Ultrafast and memory-efficient alignment of short DNA sequences to the human genome. Genome Biol. *10*, R25.

Li, J., Akagi, K., Hu, Y., Trivett, A.L., Hlynialuk, C.J., Swing, D.A., Volfovsky, N., Morgan, T.C., Golubeva, Y., Stephens, R.M., et al. (2012). Mouse endogenous retroviruses can trigger premature transcriptional termination at a distance. Genome Res. *22*, 870–884.

Li, W., Prazak, L., Chatterjee, N., Grüninger, S., Krug, L., Theodorou, D., and Dubnau, J. (2013). Activation of transposable elements during aging and neuronal decline in Drosophila. Nat. Neurosci. *16*, 529–531.

Matsui, T., Leung, D., Miyashita, H., Maksakova, I.A., Miyachi, H., Kimura, H., Tachibana, M., Lorincz, M.C., and Shinkai, Y. (2010). Proviral silencing in embryonic stem cells requires the histone methyltransferase ESET. Nature *464*, 927–931.

Muotri, A.R., Chu, V.T., Marchetto, M.C., Deng, W., Moran, J.V., and Gage, F.H. (2005). Somatic mosaicism in neuronal precursor cells mediated by L1 retrotransposition. Nature *435*, 903–910.

Muotri, A.R., Marchetto, M.C., Coufal, N.G., and Gage, F.H. (2007). The necessary junk: new functions for transposable elements. Hum. Mol. Genet. *16 Spec No. 2*, R159–R167.

Muotri, A.R., Marchetto, M.C., Coufal, N.G., Oefner, R., Yeo, G., Nakashima, K., and Gage, F.H. (2010). L1 retrotransposition in neurons is modulated by MeCP2. Nature *468*, 443–446.

Perrat, P.N., DasGupta, S., Wang, J., Theurkauf, W., Weng, Z., Rosbash, M., and Waddell, S. (2013). Transposition-driven genomic heterogeneity in the Drosophila brain. Science *340*, 91–95.

Qin, C., Wang, Z., Shang, J., Bekkari, K., Liu, R., Pacchione, S., McNulty, K.A., Ng, A., Barnum, J.E., and Storer, R.D. (2010). Intracisternal A particle genes: Distribution in the mouse genome, active subtypes, and potential roles as species-specific mediators of susceptibility to cancer. Mol. Carcinog. *49*, 54–67.

Reichmann, J., Crichton, J.H., Madej, M.J., Taggart, M., Gautier, P., Garcia-Perez, J.L., Meehan, R.R., and Adams, I.R. (2012). Microarray analysis of LTR retrotransposon silencing identifies Hdac1 as a regulator of retrotransposon expression in mouse embryonic stem cells. PLoS Comput. Biol. *8*, e1002486.

Reilly, M.T., Faulkner, G.J., Dubnau, J., Ponomarev, I., and Gage, F.H. (2013). The role of transposable elements in health and diseases of the central nervous system. J. Neurosci. *33*, 17577–17586.

Ribet, D., Harper, F., Dupressoir, A., Dewannieux, M., Pierron, G., and Heidmann, T. (2008). An infectious progenitor for the murine IAP retrotransposon: emergence of an intracellular genetic parasite from an ancient retrovirus. Genome Res. *18*, 597–609.

Rowe, H.M., and Trono, D. (2011). Dynamic control of endogenous retroviruses during development. Virology *411*, 273–287.

Rowe, H.M., Jakobsson, J., Mesnard, D., Rougemont, J., Reynard, S., Aktas, T., Maillard, P.V., Layard-Liesching, H., Verp, S., Marquis, J., et al. (2010). KAP1 controls endogenous retroviruses in embryonic stem cells. Nature *463*, 237–240.

Rowe, H.M., Kapopoulou, A., Corsinotti, A., Fasching, L., Macfarlan, T.S., Tarabay, Y., Viville, S., Jakobsson, J., Pfaff, S.L., and Trono, D. (2013). TRIM28 repression of retrotransposon-based enhancers is necessary to preserve transcriptional dynamics in embryonic stem cells. Genome Res. *23*, 452–461.

Sachdeva, R., Jönsson, M.E., Nelander, J., Kirkeby, A., Guibentif, C., Gentner, B., Naldini, L., Björklund, A., Parmar, M., and Jakobsson, J. (2010). Tracking differentiating neural progenitors in pluripotent cultures using microRNA-regulated lentiviral vectors. Proc. Natl. Acad. Sci. USA *107*, 11602–11607.

Santoni de Sio, F.R., Barde, I., Offner, S., Kapopoulou, A., Corsinotti, A., Bojkowska, K., Genolet, R., Thomas, J.H., Luescher, I.F., Pinschewer, D., et al. (2012a). KAP1 regulates gene networks controlling T-cell development and responsiveness. FASEB J. *26*, 4561–4575.

Santoni de Sio, F.R., Massacand, J., Barde, I., Offner, S., Corsinotti, A., Kapopoulou, A., Bojkowska, K., Dagklis, A., Fernandez, M., Ghia, P., et al. (2012b). KAP1 regulates gene networks controlling mouse B-lymphoid cell differentiation and function. Blood *119*, 4675–4685.

Schultz, D.C., Ayyanathan, K., Negorev, D., Maul, G.G., and Rauscher, F.J., 3rd. (2002). SETDB1: a novel KAP-1-associated histone H3, lysine 9-specific methyltransferase that contributes to HP1-mediated silencing of euchromatic genes by KRAB zinc-finger proteins. Genes Dev. *16*, 919–932.

Sperber, G.O., Airola, T., Jern, P., and Blomberg, J. (2007). Automated recognition of retroviral sequences in genomic data—RetroTector. Nucleic Acids Res. *35*, 4964–4976.

Sripathy, S.P., Stevens, J., and Schultz, D.C. (2006). The KAP1 corepressor functions to coordinate the assembly of de novo HP1-demarcated microenvironments of heterochromatin required for KRAB zinc finger protein-mediated transcriptional repression. Mol. Cell. Biol. *26*, 8623–8638.

Sweatt, J.D. (2013). The emerging field of neuroepigenetics. Neuron *80*, 624–632.

Thomas, J.H., and Schneider, S. (2011). Coevolution of retroelements and tandem zinc finger genes. Genome Res. *21*, 1800–1812.

Thompson, L., Barraud, P., Andersson, E., Kirik, D., and Björklund, A. (2005). Identification of dopaminergic neurons of nigral and ventral tegmental area

subtypes in grafts of fetal ventral mesencephalon based on cell morphology, protein expression, and efferent projections. J. Neurosci. *25*, 6467–6477.

Tronche, F., Kellendonk, C., Kretz, O., Gass, P., Anlag, K., Orban, P.C., Bock, R., Klein, R., and Schütz, G. (1999). Disruption of the glucocorticoid receptor gene in the nervous system results in reduced anxiety. Nat. Genet. *23*, 99–103.

Weber, P., Cammas, F., Gerard, C., Metzger, D., Chambon, P., Losson, R., and Mark, M. (2002). Germ cell expression of the transcriptional co-repressor TIF1beta is required for the maintenance of spermatogenesis in the mouse. Development *129*, 2329–2337.

Whitelaw, N.C., Chong, S., Morgan, D.K., Nestor, C., Bruxner, T.J., Ashe, A., Lambley, E., Meehan, R., and Whitelaw, E. (2010). Reduced levels of two modifiers of epigenetic gene silencing, Dnmt3a and Trim28, cause increased phenotypic noise. Genome Biol. *11*, R111.

Wiznerowicz, M., Jakobsson, J., Szulc, J., Liao, S., Quazzola, A., Beermann, F., Aebischer, P., and Trono, D. (2007). The Kruppel-associated box repressor domain can trigger de novo promoter methylation during mouse early embryogenesis. J. Biol. Chem. *282*, 34535–34541.

Wolf, D., and Goff, S.P. (2009). Embryonic stem cells use ZFP809 to silence retroviral DNAs. Nature *458*, 1201–1204.