The battle inside our genome

Controlling transposable elements and the evolution of human gene regulatory networks

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Chapter 2

Generating a Knockdown of ZNF91 to Reactivate SVA Elements in Various Cell Types

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Abstract

SVA elements are an evolutionary young family of transposable elements (TEs) that possibly rewired gene regulatory networks by adding new cis-regulatory elements over the course of evolution. In most cases, however, SVA elements are epigenetically silenced by KRAB zinc finger proteins that co-evolved to repress them. Although ZNF91 was identified before as main candidate for SVA repression, little is known about how these elements are controlled in human cells. Therefore, we studied the transcriptional consequences of reduced ZNF91 levels in different cell types. First, we used lentiviral delivery of an shRNA against ZNF91 to knockdown ZNF91 in human embryonic stem cells (hESCs), that can be differentiated into any cell type. Although the shRNA was shown to effectively knockdown ZNF91 72 hours post-transduction, we were unable to expand hESC lines with a stable ZNF91 knockdown due to high levels of cell death. Next, transient transfection of hESCs and different neuronal(-like) cell types with siRNAs against ZNF91 resulted in a 40-80% knockdown. RNA sequencing of hESCs and SK-N-SH cells transfected with siRNAs against ZNF91 showed cell type specific effects of ZNF91 knockdown. Interestingly, in hESCs mainly genes orchestrating the response to a viral infection were upregulated, whereas genes associated with neuronal development were downregulated. Although SVA elements did not become transcriptionally active upon ZNF91 knockdown, we observed signs that SVA elements became derepressed. In SK-N-SH cells genes located in close vicinity of an SVA element showed a mild, but significant, upregulation. In hESCs we observed the induction of an SVA driven alternative transcript of HORMAD1. Because reduction of ZNF91 levels already seem to lead to SVA driven gene expression changes, we hypothesize that ZNF91 is essential for the control of these elements.
Introduction

Transposable elements (TEs) are mobile DNA elements that contributed to the expansion and innovation of the human genome over the course of evolution (Cordaux and Batzer 2009). Waves of TE insertions resulted in a wide variety of TE families in our genome. Although the majority of them are dead, there is a small group of active TEs. They accelerate(d) evolution by introducing novel transcription factor (TF) binding sites on a large scale, thereby rewiring gene regulatory networks (Sundaram et al. 2014; Trizzino et al. 2017, 2018; Pontis et al. 2019). The local chromatin state in combination with TF availability allows them to function as cell type specific gene regulators (Notwell et al. 2015; Chuong et al. 2016; Trizzino et al. 2017, 2018; Pontis et al. 2019). On top of that, they add a layer of species specific layer of gene regulation, as mainly evolutionary young TE families adopted cis-regulatory properties (Sundaram et al. 2014; Trizzino et al. 2017; Pontis et al. 2019). One of the most potent TE families is called SVA after its composite domains SINE-VNTR-Alu. They are a primate specific class of transposable elements (TEs) that show ~2700 fixed insertions in the human genome, but are still expanding through copy-paste activity (Xing et al. 2009). On top of that, they have been attributed strong gene regulatory properties in different cell types (Savage et al. 2013, 2014; Jacobs et al. 2014; Turelli et al. 2014; Trizzino et al. 2017, 2018; Pontis et al. 2019). In liver cells SVA elements are among the most important innovators of gene expression characterized by providing liver specific TF binding sites and induce high differential histone modification profiles in humans compared to other primate species (Trizzino et al. 2017, 2018). In naive human embryonic stem cells many SVA elements were bound by pluripotency controlling KLF4 and decorated with H3K27ac (Pontis et al. 2019). Most frequently, however, SVA elements are silenced through binding of members of the KRAB zinc finger (KZNF) gene family (Jacobs et al. 2014; Imbeault et al. 2017; Pontis et al. 2019) that recruit co-factor KAP1 (Nielsen et al. 1999; Sripathy et al. 2006) and repressive epigenetic modifiers (Schultz et al. 2001, 2002; Turelli et al. 2014). Note that this also enables passive species specific gene regulation, because novel SVA insertions induce local heterochromatin which can affect gene expression in the neighborhood (Lippman et al. 2004; Rebollo et al. 2011; Jacobs et al. 2014).

The parallel evolution of KZNF genes and TE families together with evidence for the existence of specific KZNF-TE pairs is in support of an internal evolutionary arms race (Huntley et al. 2006; Thomas and Schneider 2011; Jacobs et al. 2014; Imbeault et al. 2017). Yet, how KZNF genes sense new TE invasions and how their response is mediated remains unclear. Possibly, they are controlled
by factors that are part of an innate immune response triggered by TE derived RNA molecules (Kauzlaric et al. 2017). Moreover, it is becoming increasingly clear that the function of KZNF genes is not limited to TE repression. They are associated with autoregulation by binding to itself and/or other members of the KZNF gene family (Frietze et al. 2010; Yan et al. 2017), gene regulation (Oleksiewicz et al. 2017; Yang et al. 2017; Chen et al. 2019, Farmiloe et al. In press), and maintenance of DNA methylation at imprinting control regions (Li et al. 2008; Riso et al. 2016; Takahashi et al. 2016, 2019). On top of that, KZNF genes show a highly dynamic expression pattern across cell types and developmental stages (Chapter 1) (Imbeault et al. 2017; Pontis et al. 2019).

Together, this suggests that SVA elements, and their partner KZNF(s) adopted functions that go beyond an evolutionary battle, making them indispensable for the host. This adds another layer of complexity, since KZNF expression and subsequent SVA repression need to be tightly controlled in a time and location dependent manner. Recently, the evolutionary young KZNF protein ZNF91 was identified as strong repressor of SVA elements when placed in a mouse cellular context (Jacobs et al. 2014). Little is known, however, about the role of ZNF91 in controlling SVA elements in human cells. We aimed to determine if ZNF91 is required for SVA control and elucidate gene regulatory properties of both ZNF91 and SVA elements. To this end we knocked down ZNF91 expression using shRNAs and siRNAs in different cell types.

**Results**

**Lentiviral delivery of shRNAs results in low survival of ZNF91 knockdown hESCs**

In order to understand the role of ZNF91 in regulating SVA elements and potentially other sequences, we aimed to knockdown ZNF91 in human embryonic stem cells (hESCs) using lentiviral delivery of an shRNA directed against ZNF91. The presence of GFP and a puromycin resistance cassette in the construct allowed us to select for transduced cells. First, hESCs were transduced with three different shRNAs, all targeting the 3' UTR of ZNF91 and one non-targeting control shRNA (Suppl Fig 1A). After 21 days of puromycin treatment transduced hESC colonies showed a very high percentage of GFP positive cells (Suppl Fig 1B). Unfortunately, qPCR showed that ZNF91 expression was not significantly reduced (Suppl Fig 1C). Noteworthy is that ZNF91 expression levels are highly variable, also in untransduced hESCs (Fig 1E and data not shown).
Generating a Knockdown of ZNF91 to Reactivate SVA Elements in Various Cell Types

Figure 1. Generation of ZNF91 knockdown hESCs through lentiviral transduction with shRNAs followed by FACS and clonal expansion
A) Experimental design to generate hESCs stably expressing shZNF91#6. B) GFP positive hESCs 72 hours after transduction with shZNF91#6. C) qPCR for ZNF91, expression relative to shControl samples (n=3). D) GFP positive hESCs clonally expanded after transduction with shZNF91#6. E) qPCR for ZNF91, expression relative to shControl line that was cultured in parallel (n=3).
Subsequently, we designed another shRNA (shZNF91#6) targeting the zinc finger coding exon 4, and obtained a new batch of lentivirus to transduce hESCs. Fluorescent activated cell sorting (FACS) was used to purify hESCs 72 hours post-transduction. We harvested cells directly after FACS and after clonal expansion (Fig 1A). 72 hours after transduction we observed a strong knockdown of ZNF91 expression (Fig 1B+C). However, ZNF91 expression levels were back at control levels in hESC lines that were clonally expanded and thus cultured for an extensive amount of time (Fig 1D+E). Noteworthy is that the amount of cell death increased dramatically with time after transduction. Of course this hampered the expansion procedure and only few clones survived the expansion. The high levels of cell death were not caused by the FACS procedure, because cell survival was also low when cells were selected with puromycin (data not shown). Important to note here is that no transduction with non-targeting shRNAs was done in parallel. To circumvent cell viability issues that were possibly related to ZNF91 knockdown we transduced TetR expressing hESCs with shZNF91 (Suppl Fig 2A). TetR represses shRNA expression and can be removed with doxycycline treatment, leading to the induction of the shRNA. However, we did not observe a reduction of ZNF91 expression after six days of doxycycline treatment in TetR hESCs transduced with shZNF91 (Suppl Fig 2B+C).

**Successful knockdown of ZNF91 using siRNA transfection in different cell types**

Next we used lipid based transient transfection of siRNAs against ZNF91 to establish a ZNF91 knockdown in hESCs (Fig 2A). We obtained siRNAs targeting the same sequence as shZNF91#6 and a pool with four different siRNAs (Suppl Table 1). Because the siRNA pool showed a stronger knockdown than siZNF91#6, we continued with them for all further experiments (Fig 2B). In our hands siRNA transfection was a much more efficient delivery method than lentiviral transduction, illustrated by high percentages of cells showing the fluorescently tagged reporter siGlo 48 hours after transfection and low amounts of cell death (Fig 2C+D). Even when the cells were cultured for a week after transfection, the amount of cell death was neglectable (data not shown). qPCR showed that in hESCs and a neuroblastoma cell line (SK-N-SH cells) ZNF91 expression was reduced to ~35% 48 hours after transfection (Fig 2C+D). Note that the transient nature of lipid based siRNA transfection makes this approach unsuitable to study long term effects of ZNF91 knockdown or use the hESCs for differentiation into other cell types. Therefore, we differentiated hESCs towards cortical and midbrain dopamine (mDA) neurons and transfected them...
Generating a Knockdown of ZNF91 to Reactivate SVA Elements in Various Cell Types

with siRNAs at the progenitor stage (Suppl Fig 3A). 48 hours post-transfection we isolated RNA and determined that ZNF91 expression levels were reduced with 80% in mDA progenitors and with 44% in cortical progenitors (Suppl Fig 3 B+C). Because the strong knockdown in mDA progenitors, we assessed SVA expression levels in this cell type. However, we observed no significant upregulation of SVA transcripts in mDA progenitors with SVA primers that target ~1200 insertions (Suppl Fig 3B). Taken together, transient transfection of siRNAs resulted in moderate to strong reduction of ZNF91 expression levels in various cell types.

Knockdown of ZNF91 mildly affects genes in close vicinity of SVA elements in SK-N-SH cells

Two replicates of hESCs and SK-N-SH cells transfected with siControl or siZNF91 were subjected to RNA sequencing. Differential expression revealed that ZNF91 expression was reduced ~50% in both cell types (Fig 3A). In
SK-N-SH cells, GO terms related to neuronal differentiation were strongly overrepresented among 2075 significantly upregulated genes (Fig 3B). Negative regulation of gene expression was the most relevant gene group overrepresented among the 1496 genes that were significantly downregulated (Fig 3B). In hESCs, however, neuronal differentiation, together with negative regulation of transcription were overrepresented among downregulated genes (Fig 3C). Genes associated with viral infections, on the other hand, were upregulated in hESCs (Fig 3C). Note that overall, we found a much lower number of differentially expressed genes in hESCs compared to SK-N-SH cells.

Next we explored if these effects could be related to activated SVA elements as a consequence of reduced ZNF91 levels. First we looked at SVA expression, but we did not observe upregulated SVA transcripts in ZNF91 knockdown hESCs or SK-N-SH cells (data not shown). However, it is possible that SVA elements are epigenetically activated but lack (detectable) transcripts (Pontis et al 2019). Therefore, we looked at expression changes of genes neighboring SVA elements (within 25kb distance). In SK-N-SH cells we observed a mild, but significant, upregulation of genes that are located next to an SVA insertion compared to a set of randomly selected genes, whereas these genes were unaffected in hESCs (Fig 4A+B). On the contrary, in hESCs we observed that an alternative HORMAD1 transcript, starting in an upstream SVA-F, was induced upon ZNF91 knockdown (Fig 4C).

Taken together, we show that knockdown of ZNF91 results various gene expression changes that may be the consequence of reduced repression on SVA elements.
**Discussion**

Here we set out to elucidate the role of ZNF91 in controlling SVA elements by interfering with ZNF91 expression levels using shRNAs and siRNAs. First we attempted to generate hESC lines that stably express a ZNF91 targeting or non-targeting shRNA. This would allow us to differentiate ZNF91 knockdown hESCs into any desirable cell type. Although we showed that the ZNF91 targeting shRNA was successful in reducing ZNF91 levels, it was difficult to expand transduced hESCs due to high levels of cell death. We can only guess what caused the high levels of cell death upon lentiviral transduction of shRNAs against ZNF91, but most
likely an unknown technical problem related to the transduction procedure. It seems unlikely that the lentivirus itself caused cytotoxicity, since we observed similar levels of cell death with two different batches of lentivirus (one commercially bought and one in house produced). We cannot rule out, however, that it was a consequence of secondary effects of the integration of the ZNF91-shRNA construct because a parallel transduction with a non-targeting shRNA was lacking. The fact that the few GFP positive clones that survived probably lacked shRNA expression is in support of this. Because hESCs transfected with siRNAs targeting the same sequence as the shRNA displayed a negligible amount of cell death, it seems unlikely that the cell death is a direct consequence of ZNF91 knockdown. RNA seq analysis of hESCs and SK-N-SH cells transfected with siRNAs against ZNF91 or non-targeting siRNAs revealed a cell type specific effect of ZNF91 knockdown. In SK-N-SH cells it seems that ZNF91 knockdown resulted in differentiation towards a neuronal-like phenotype. SK-N-SH cells are often used as a model for human neurons because they can be easily differentiated into neuronal-like cells (Preis et al. 1988). However, this makes it difficult to distinguish direct and indirect effects caused by ZNF91.

Collectively, our results suggest that SVA repression is reduced upon ZNF91 knockdown. Although transcriptional activity of SVA elements was virtually undetectable, we observed a mild upregulation of genes that are located close to an SVA. This suggests that SVA elements have become epigenetically active, exposing their cis-regulatory potential. Indeed, SVA transcripts are usually lowly expressed, quickly degraded and only a small fraction of epigenetically active SVA elements also become transcribed (Pontis et al. 2019). Yet, the identification of a transcript driven by an SVA-F suggests that the repression of SVA elements is reduced. Reduction of ZNF91 levels may not be enough to activate SVA elements on a large scale, but loss of repression could make the surrounding genomic region more permissive for transcription. The opposite has been shown by Jacobs et al (2014): overexpression of ZNF91 negatively affects gene expression in close vicinity of SVA elements on chromosome 11 that lack repression in a mouse cellular context. Profiling of repressive and active histone marks could give more insight into SVA activity upon ZNF91 knockdown. Assuming that (some) SVA elements become activated it is tempting to speculate that the large fraction of upregulated genes in siZNF91 hESCs associated with a cellular response to a viral infection is a reaction to SVA activation. This is supported by recent data showing that activated TEs upon KAP1 knockout trigger an innate immune response (Tie et al. 2018).

In summary, we showed that ZNF91 knockdown has a cell type specific effect on
gene expression. Yet, in both cell types we observed signs that SVA repression is reduced, positively affecting genes nearby. Together these results contribute to our understanding of the complex battle between SVA elements and KZNF genes.

**Materials and Methods**

**Cell culture**
SK-N-SH cells were cultured in MEM (Invitrogen) supplemented with 10% FBS (Invitrogen) and antibiotics and passaged at 1:8 ratio twice a week with 0.25% trypsin and 0.5% EDTA (Invitrogen).

H9 human embryonic stem cells were grown on matrigel (Corning) coated dishes. They were cultured in hESC medium that was incubated with mouse embryonic fibroblasts (MEFs) for 24 hours. hESC medium consisted of: DMEM/F12 supplemented with 2mM L-glutamine (Invitrogen), 20% knockout serum replacement (Gibco), penicillin/streptomycin (Invitrogen), non-essential amino acids (Invitrogen), 0.1mM -2-mercaptoethanol (Invitrogen). Medium was supplemented with basic fibroblast growth factor (Sigma, 8ng/μl) and changed daily to secure pluripotency of hESCs. For maintenance of the culture, cells were grown in colonies and passaged manually by cutting the colonies with a needle. For transfection and clonal expansion hESCs were grown as single cells and passaged using accutase (sigma) at ratios 1:4-1:10. For single cell culturing, culture medium was supplemented with ROCK inhibitor Thiazovivin (2μM, Sigma) 1hr before disociation and during plating.

**Neuronal differentiations**
The mDA differentiation protocol by Doi et al. (2014) was used with slight adaptations. In short, hESCs are seeded as single cells using accutase and grown until 100% confluency. Then hESC culture medium was changed to differentiation medium containing; GMEM (Invitrogen), 8% KOSR (Invitrogen), 0.1mM non-essential amino acids (Invitrogen), sodium pyruvate (Invitrogen), and 0.1 mM 2-2-mercaptoethanol (Invitrogen). This was considered D0 of differentiation. To induce a neuronal faith LDN193189 (0.1 μM, Sigma) was added from D0-D11 and SB431542 (10 μM, Sigma) from D0-D5. A midbrain dopamine identity was induced by the addition of purmorphamine (2 μM, Sigma) and FGF8 (100ng/ml, R&D) from D1-D7 and CHIR99021 (3 μM, Sigma) from D3-D12. From D13 onwards neuronal maturation medium was used consisting of: neurobasal medium (Invitrogen), B27 without vitamin A (Invitrogen), Ascorbic acid (0.2mM, Sigma),
BDNF (20ng/ml, R&D), GDNF (10ng/ml, R&D), Dibutyryl cAMP (0.4mM, Sigma). mDA progenitors were replated (80,000 cells/cm2) at D11 for siRNA transfection. For cortical differentiation full grown hESC colonies (2-3mm in size) were lifted off in one piece from the MEF dish using a cell scraper and transferred to an ultra low attachment plate (Corning) in neuronal differentiation medium containing: hESC culture medium supplemented with sodium pyruvate (Invitrogen) without additives such as bFGF. After 24 hours embryo bodies (EBs) were exposed to neuronal differentiation medium supplemented with: 10 μM SB431542 (Sigma), 1 μM Cyclopamine (Sigma), 1 μM Dorsomorphin (Sigma), 3 μM IWR (Sigma), this is considered day 0 (D0). Cortical progenitors were differentiated in 3D and plated on dishes coated with matrigel at day 10 and transfected at day 15.

**Lentiviral transduction of shRNAs**

hESCs were seeded as tiny colonies or single cells (20,000 cells/cm2) and transduced at ~70% confluency. Lentivirus carrying ShZNF91#1-3 or the non-targeting shControl were commercially bought at Amsbio. 25μl lentivirus/cm2 with titers around 1 x 10^8 IFU/ml was used for transduction and polybrene was (8μg/ml) added to enhance transduction efficiency. Puromycin (500ng/ml, Sigma) was added to the medium from day 4 to 11 post transduction. Subsequently, hESCs were expanded for approximately two weeks before cells were harvested for RNA isolation.

Lentivirus carrying shZNF91#6 was generated according to standard protocol and used for transduction at an MOI of 1. 72 hours post transduction GFP positive cells were sorted with a FACS Aria III using a 100 μm nozzle and collected for RNA isolation or plated for expansion.

To generate a doxycycline inducible ZNF91 knockdown hESC line, hESCs were first transduced with an TetR-RFP-BSD construct and selected with blasticidin according the same procedure as shZNF91#1-3. Then TetR expressing hESCs were transduced with shZNF91#6 and selected for GFP positive cells with puromycin. shRNA expression was induced by treating the cells for 6 days with doxycycline (500ng/ml, Sigma). GFP positive cells were sorted and isolated for RNA.

**siRNA transfection**

hESCs were seeded as single cells (20,000 cells/cm2) one day before transfection to ensure they reached 70-80% confluency at transfection. To increase transfection efficiency, hESCs were first incubated in PBS for 5 minutes to increase spacing between cells. Subsequently, they were starved in opti-MEM (Invitrogen) supplemented with basic fibroblast growth factor (Sigma, 8ng/μl) and ROCK
inhibitor Thiazovivin (2μM, Sigma) for 4 hours. A confluent 10cm dish of SK-N-SH cells were used to seed cells at 1:3 dilution one day prior transfection. Cortical progenitors were plated at D10, by mechanical dissociation of organoids, (~14 organoids per well in a 24wp) on matrigel coated dishes and transfected at D17. mDA progenitors were replated at D11 (80.000 cells/cm²) on matrigel coated dishes and transfected at D14. siRNA transfection was done according to the protocol supplied with DharmaFECT 1 transfection reagent. Briefly, for a 24 well plate, per well 75nM-100nM siRNA was diluted in basal medium in a total volume of 25μl, which was incubated for 5 minutes before being mixed 1:1 with a mixture consisting of 0.9μl DharmaFECT1 and 24.1μl DMEM/F12. This was incubated for 20 minutes at room temperature and then added to the cells in a final volume of 250μl normal culture medium per well. A single and pool of siRNAs against ZNF91 was compared to a non-targeting control (Suppl Table 1). On top of that, a fluorescently tagged siRNA (siGLO) was transfected in a separate well to determine transfection efficiency.

**Lentiviral vectors and siRNA sequences**

shRNAs were designed and cloned into pLenti-H1-shRNA-RSV-(GFP-puro) transfer vectors at Amsbio (Suppl. Table 1). The TeTR expression construct was also purchased at Amsbio and had the following composition: LVP459-EF1a-TetR-RSV-(RFP-BSD).

A custom siRNA with the same sequence as shZNF91#6 was ordered together with a predesigned smart pool siRNAs against ZNF91 (Dharmacon, **Suppl Table 1**).

**qPCR and RNA sequencing**

Two replicates of siControl and siZNF91 transfected hESCs and SK-N-SH cells were used for RNA sequencing. Total RNA was isolated using Trizol according manufacturer’s protocol, followed by a DNAse treatment and RNA purification using the RNA clean and concentrator kit (Zymo research). 10ng RNA of three replicates per condition was used to profile ZNF91 expression with the QuantiTect SYBR green kit (Qiagen). Beta-actin was included as reference gene (primers in **Suppl Table 2**). For data analysis we used the 2–∆∆Ct method and displayed ZNF91 or SVA expression of ZNF91 knockdown samples relative to control.

Ribosomal RNA was depleted from total RNA with the rRNA depletion kit (NEB# E6310) and subsequently prepared for RNA seq with NEB Next Ultra Directional RNA Library Prep Kit (NEB #E7420) at GenomeScan. Samples were sequenced at 150 bp paired end at an illumina Hiseq 4000 device.
Mapping and quantification of RNA seq data
A snakemake (Köster and Rahmann 2012) pipeline was written to quality check, trim, map and quantify RNA seq data. Before running the pipeline read quality was assessed with FASTQC. The complete pipeline can be found at DOI:10.5281/zenodo.2581199. Below a short description of the separate steps composing the pipeline. Paired end Illumina RNA seq fastq files were used as input. Trimmomatic (Bolger et al. 2014) was used to clip adapter sequences and trim low quality reads. Reads were aligned to the human genome (Hg19 version) using STAR (Dobin et al. 2013) with default settings except: outFilter-MismatchNmax=2 and outFilterMultimapNmax=10, outWigType=bedGraph, outSAMtype=BAM SortedByCoordinate. Base-by-base coverage tracks were generated from each bedGraph file with bedGraphToBigWig. Data are displayed on the UCSC genome browser (session: http://genome-euro.ucsc.edu/s/ninaharing/Chapter_2). Raw read counts of genes were determined with FeatureCounts (Liao et al. 2014) using Hg19 KnownGenes.GTF from UCSC for annotation (downloaded 12th September 2016). Only properly paired reads were counted (-B) and the library was reversely stranded (-s 2). Genes were summarized at metalevel (default), whereas TEs were summarized at the feature level (-f). DEseq2 (Love et al. 2014) was used to normalize raw counts and perform principal component analysis of genes and TEs. R packages ggplot2 (Wickham 2009) and ggpubr were used to generate plots and perform statistical testing. Heatmaps were generated with MeV (vs 4.8.1).

GO term analysis
Gene Ontology analysis was performed with the online tool of the GO Consortium (http://geneontology.org/). Differentially up and downregulated genes were used separately as input list and compared to a reference list consisting of all analyzed genes with a baseMean>=30. GO enrichment for biological processes were obtained and ranked by FDR corrected p value (q value). Top 25 of GO terms were displayed for hESCs. Because of the high number of differentially expressed genes in SK-N-SH cells, many generic GO terms were enriched. Therefore, we displayed GO terms ranking 26-50 based on q value.

Acknowledgements
We thank Lars von Oerthel for his technical assistance with FACS experiments.
Supplemental Figure 1. Generation of ZNF91 knockdown hESCs through lentiviral transduction with shRNAs followed by puromycin selection.

A) Experimental design to generate hESCs stably expressing shRNAs. B) GFP expression in colonies of expanded hESC lines transduced with different shRNAs. C) qPCR for ZNF91, expression in shZNF91 transduced hESCs relative to shControl hESCs (n=3).
Supplemental figure 2. Generating an inducible ZNF91 knockdown hESC line. 
A) Experimental design to generate an inducible ZNF91 knockdown hESC line. B). GFP expression in TetR hESCs transduced with shZNF91#6 with or without doxycycline treatment. C) left: qPCR comparing ZNF91 expression levels of TetR hESCs transduced with shZNF91#6 to shControl hESCs. Right: qPCR comparing ZNF91 expression levels of TetR hESCs transduced with shZNF91#6 with and without doxycycline treatment.
Supplemental Figure 3. Transflecting cortical and midbrain dopaminergic (mDA) progenitors with siRNAs against ZNF91.

A) Experimental design of neuronal differentiations followed by siRNA transfection. B) Left: bright field picture of mDA progenitors at D14 of differentiation. Middle: qPCR of ZNF91 expression relative to in parallel transfected non-targeting siRNAs. Right: qPCR with primers targeting ~1200 SVA insertions. An unpaired two sided t-test was used to compare siZNF91 to siControl (p=0.22). C) Left: bright field picture of cortical progenitors at D17 of differentiation. Right: qPCR of ZNF91 expression relative to in parallel transfected non-targeting siRNAs.
Supplemental Table 1. shRNA and siRNA sequences used to target ZNF91

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Supplemental Table 2. qPCR primer sequences

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References


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