Unique trophoblast chromatin environment mediated by the PcG protein SFMBT2

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ABSTRACT

Stem/progenitor cells are maintained by a chromatin environment, mediated in part by Polycomb group (PcG) proteins, which depress differentiation. The trophoblast-specific PcG protein SFMBT2 is known to be required for maintenance of trophoblast progenitors. Rather than binding to trophoblast-specific genes repressed in TSC, SFMBT2 is concentrated at chromocentres and regions rich in repetitive elements, specifically LINE sequences and major satellites, suggesting that it is involved in higher-order organization of the trophoblast genome. It is also found enriched at a subset of ncRNAs. Comparison of ChIP-seq datasets for other chromatin proteins reveals several stereotypical distribution patterns, suggesting that SFMBT2 interacts with several different types of chromatin complexes specific to the trophoblast lineage.

KEY WORDS: SFMBT2, Trophoblast, ChIP-seq, Placenta, Polycomb

INTRODUCTION

Epigenetic biochemistry is as old as cells themselves. It has had billions of years to diversify, which is why it is complex and as evolutionarily varied as it is conserved (Willbanks et al., 2016; Pikaard and Mittelsten Scheid, 2014; Skinner, 2011). Our understanding of the role played by epigenetics in life, the universe and everything, by comparison, is rudimentary, although the development of new tools has accelerated investigation beyond the blunt hammer of DNA methylation.

Pioneering genetic studies in fruit flies have revealed that gene silencing is rooted in complexes of chromatin proteins (Lewis, 1978; Struhl, 1981; Jürgens, 1985). One class that has received particular attention is the PcG of proteins, named after one of the first identified gene silencers, Polycomb, required to maintain posterior spatial expression of homeotic genes in fruit fly larvae (Lewis, 1949, 1978; Jürgens, 1985). The characteristic phenotype—heterozygous ectopic development of sex comb bristles and homozygous lethality due to ectopic expression of homeotic genes—is common to a particular class of proteins that constitute subunits of multi-protein Polycomb Repressive Complexes (PRC1 and PRC2), in addition to other complexes such as PrDUB (Polycomb repressive complex de-ubiquitinase) and PHO-RC (Pleiohomoic repressive complex) (Chittock et al., 2017; Schwartz and Pirrotta, 2013; Golabapour et al., 2013). In vertebrates, multiple flavours of PRCs have evolved in part as a result of two whole-genome duplication events (Dehal and Boore, 2005; Senthilkumar and Mishra, 2009). This greatly expands the possibilities with respect to developmental innovation.

One such innovation is the placenta. Extraembryonic membranes in egg-laying vertebrates evolved into complex structures in mammals that provide an interface between the developing embryo and the mother, a necessity dictated by the exceedingly small mammalian zygote; there is not enough mass in a mammalian egg to support development of an entity that can survive and feed. Mammalian development therefore takes place in a specialized extraembryonic environment. While there is growing epidemiological evidence that insults during fetal life can have lasting effects on health (Langley-Evans et al., 1999; Sasaki et al., 2014; Nakayama, 2017), there is surprisingly modest interest in examining the role played by the placenta in shaping the fetal developmental environment (Perez-Garcia et al., 2018; Price et al., 2016; Zhang et al., 2015; Suter et al., 2011; Banister et al., 2011).

One area that could benefit from more focused study is the epigenome of the placenta. The ENCODE project at present is greatly enriched by numerous studies of either embryonic stem cells or neural stem cells (Mouse ENCODE Consortium et al., 2012). Very little work has been done to date on the cells that form the placenta. The study we describe in this paper aims to fill some of that deficit.

Placentas are formed from specialized cells called trophoblast (Rossant and Cross, 2001; Cross et al., 2003; Cross, 1998). In mouse embryos, trophoblast cells are one of three early lineages to form before the embryo implants in the mother’s uterine wall, the other two lineages represented by embryonic cells and primitive endoderm cells (Cross, 1998; Cross et al., 1994). In mice, these three types of cell can be induced to form specialized stem or progenitor cells in vitro – trophoblast stem cells (TSC), embryonic stem cells (ESC) and extraembryonic stem cells (XEN) (Hemberger et al., 2004; Bibel et al., 2004; Kruithof-de Julio et al., 2011). One kind of genetic analysis that has helped define the molecular underpinnings of the trophoblast lineage is the ability of mutant embryos to make TSC. Our lab recently reported that the PcG gene Sfmbt2 is required for the establishment of TSC, indicating that it may form part of the epigenetic framework that supports trophoblast, and by extension development of the placenta (Miri et al., 2013). Sfmbt2 was designated a PcG gene because the fruit fly orthologue, dsfmbt, displays a classic Polycomb phenotype when mutated (Klymenko et al., 2006). Mammalian SFMBT2 can therefore be reasonably expected to form a complex with other chromatin proteins, and to associate with the genome.

ENCODE datasets comprise, largely, whole-genome sequencing analyses of the occupation of sites by a variety of chromatin proteins (ChIP-seq) and chromosome conformation capture (Hi-C) (ENCODE Project Consortium et al., 2007; ENCODE Project Consortium et al., 2012; Mouse ENCODE Consortium et al., 2012;
Dixon et al., 2012). These have allowed investigators to develop novel hypotheses about genome function at a larger scale than was possible even 10 years ago (e.g. topologically associating domains, or TADs, and distal enhancers defined by transcription factor occupancy in specific cell types) (Pope et al., 2014; ENCODE Project Consortium et al., 2012; Zacher et al., 2017). Trophoblast cells are just beginning to acquire epigenomic descriptors. In our study, we analyze the occupancy of the genome in TSC by SFMBT2, a protein known from our genetic studies to be required for the maintenance of the stem cell pool. ChIP-seq analyses have revealed that SFMBT2 may play a unique role in the architecture of TSC, mainly through binding to specific repetitive elements, and association with pericentromeric heterochromatin.

RESULTS

Loss of SFMBT2 results in upregulation of genes

The mammalian SFMBT2 protein is orthologous with the fruit fly dSfmbt, which has been characterized as a Polycomb Group (PcG) protein because the null phenotype of mutants is classic Polycomb (Klymenko et al., 2006; Alfieri et al., 2013). It is therefore reasonable to assume that the mammalian SFMBT2 will act like other PcG proteins and be involved in transcriptional repression. This question was addressed by performing RNA-seq analysis of Sfmbt2−/− extraembryonic tissues (E7.5 ectoplacental cone and extraembryonic ectoderm). When compared with wild-type littermates, the mutant tissues displayed significant upregulation of 704 genes; in contrast, only 317 genes displayed downregulation in mutant tissues (Fig. 1). GO analysis suggests most genes affected by knockout of SFMBT2 have roles in developmental processes, cell organization and biogenesis, stress response, cell cycle and proliferation, and signal transduction. Only 20 of 704 genes (0.03%) of all significantly upregulated genes and 5 of 317 (0.02%) significantly downregulated genes have GO terminologies associated with placental development. This may in part reflect the underrepresentation of GO data associated with placenta. The stage of embryogenesis chosen, E7.5, represents a stage that precedes the most dramatic phenotypic differences between mutant and wild-type embryos, observed at E8.5 (Miri et al., 2013). At E7.5, mutant embryos are generally normal in appearance, although some may have reduced extraembryonic ectoderm. One would expect that loss of a tissue would be reflected in the transcriptome by loss of transcripts from that tissue. It is therefore interesting that so few genes display downregulation in mutant tissues.

ChIP-seq binding of SFMBT2 displays broad peaks

In fruit flies, PcG complexes bind to regulatory sequences called Polycomb Response Elements (PREs) (Khan et al., 2014; Orsi et al., 2014; Follmer et al., 2012). No clearly definitive PREs have been identified in mammalian cells, suggesting a different mechanism of regulation operates in the larger genomes of vertebrates. In order to assess whether SFMBT2 might be involved directly in transcriptional repression, we performed chromatin immunoprecipitation followed by next-generation sequencing in TSC, using both the antibody directed at endogenous SFMBT2 protein and antibody directed against FLAG; the latter was used on cells expressing a FLAG-tagged Sfmbt2 transgene.

Initial data analysis with MACS2 did not generate any obvious discrete binding of SFMBT2 to the genome. Given that previous studies showed an interaction between SFMBT2 and modified histones, we subjected the data to analysis with SICER and found that SFMBT2 binds to the genome in a manner similar to that observed for histones, i.e. in modified and broad peaks. Many of

Fig. 1. RNA-seq analysis of Sfmbt2 null extraembryonic tissues from E7.5 embryos. Approximately 70% of significantly differentially expressed genes identified in Sfmbt2gt/gt extraembryonic tissues were upregulated (green) compared to wild type (wt; P<0.05). Few significantly downregulated genes (red) were identified in these tissues. Expression levels of each gene in genetrap mutants (Sfmbt2gt/gt) were mapped relative to their wild-type counterparts.
these peaks map to regions rich in repetitive elements, in particular LINE sequences (Fig. 2A). Validation of association with LINE elements was performed by qPCR (Fig. 2B). No obvious peaks were located close to any of the de-repressed genes in mutant extraembryonic tissues.

SFMBT2 associates with centromeric major satellite sequences and other repetitive elements

Immunohistochemistry in TSC clearly showed an association of SFMBT2 with centromeric regions (Fig. S1B). Because of the repetitive nature of centromeres in mammalian genomes, mapping is problematic. However, during the quality control step in which adapter sequences are removed, we noted that a significant enrichment of major satellite sequences was found in the non-adapter overrepresented sequence files in the MN-digested samples of both endogenous and FLAG immunoprecipitates (Table S2). Enrichment was confirmed by qPCR using published primer sequences (Fig. 3).

Binding of SFMBT2 to LINE elements and major satellite sequences prompted us to look at other classes of repetitive DNA. We used regioneR to visualize the distribution of SFMBT2 in relation to: LINE elements (Fig. 4), low complexity DNA, LTRs, ncRNA, satellite sequences, simple repeats and SINE elements (Fig. S2). Statistically significant patterns were generated for each of these classes of repetitive DNA sequence. Interestingly, association with ncRNAs was quite common (Fig. 5). A statistical analysis of broad peaks using bedtools revealed that between approximately 26% and 46% of SFMBT2 peaks associated with lncRNAs (Table 1), many of which were pseudogenes embedded in regions rich in other repetitive elements, making the design of appropriate qPCR primers problematical.

The observation of SFMBT2 peaks at repetitive elements prompted us to look at expression of LINE sequences in Sfmbt2 null extraembryonic tissues. Interestingly, only six displayed statistically significant (modest) changes in mutant tissues, and of those five were reduced (Fig. 6; Table S3). We can’t say whether this reflects loss of regulation (positive or negative), or loss of cell types; while Sfmbt2 null embryos at E7.5 are largely normal in appearance, it is not unusual to find some with reduced extraembryonic ectoderm.

Fig. 2. Association of SFMBT2 with major satellite sequences. (A) An example of a called SFMBT2 peak localized to a region enriched for major satellite sequences. The peak was called using SICER with a FDR of 0.01. Track colours are as follows: MN endogenous SFMBT2 (bnm_peak), pink; MN FLAG-SFMBT2 (bsm_peak), orange; sonicated FLAG-SFMBT2 (bss_peak), brown. Peaks for sonicated endogenous SFMBT2 (bns_peak) were not called for this region, likely due to the samples being prepared and sequenced separately. (B) Major satellite sequences were significantly enriched in ChIP DNA of MN FLAG-SFMBT2 samples relative to wild type when equal amounts of DNA were used in qPCR (P<0.005).

Fig. 3. Association of SFMBT2 with LINE elements. Endogenous and FLAG-SFMBT2 ChIP exhibit enrichment of LINE elements relative to input approximately 1.8- to 2.0-fold even when two different primers were used. Sonicated samples likely display greater fold enrichment due to larger DNA fragments.
SFMBT2 distribution linked to stereotypical histone marks

The association of SFMBT2 with DNA features such as repetitive and major satellite sequences prompted us to examine the relationship with other histone marks in TSC. Publicly available ChIPseq data for several histone variants were mapped using SEQminer onto the SICER-defined SFMBT2 peaks. Histone H3 displayed a stereotypical distribution surrounding the middle of SFMBT2 peaks, with three strong peaks on one side within about 2.5 kb, and a fourth peak on the other side at about the same distance (Fig. 7). The strongest signal is seen in the H3K4me3 dataset. A similar pattern is observed in the CTCF dataset, while both RNA polymerase II (PolII) and H3K27ac appear to pile up over the SFMBT2 peaks (Fig. 8). These observations are unexpected, given that H3K4Me3, H3K27ac and PolII are generally associated with

Fig. 4. Read distribution of SFMBT2 peaks at LINE elements across the genome. RegionR analysis revealed similar patterns of endogenous (pink) and FLAG-SFMBT2 (blue) read distributions are observed across known LINE genomic coordinates from 5000 bp upstream to 5000 bp downstream of the centre of the specified LINE coordinates.

Fig. 5. Association of SFMBT2 with ncRNAs. SFMBT2 peaks are mapped to a pericentromeric region enriched for major satellite sequences, which also encode for a large cluster of lncRNAs.
transcriptionally active chromatin, while SFMBT2 appears at least superficially to be transcriptionally repressive (see RNAseq section). Histone H2A also displays a stereotypical arrangement close to SFMBT2 peaks (Fig. S3).

DISCUSSION

Progenitor cells allow a tissue to bulk up during development or repair itself following injury. Mammals are particularly dependent on stem/progenitor cells during development because embryogenesis is accompanied by an increase in mass, unlike, for example, insect or amphibian embryos. The variable size of different mammalian species may reflect, at least in part, the renewal capacity of their stem/progenitor cell populations.

The placenta, a highly specialized organ, is dependent, at least in rodents, on the integrity of the trophoblast progenitor population. Reduced numbers of TSC in embryos leads to a reduced placenta and embryonic death. Establishment of TSC has been studied extensively (Ohinata and Tsukiyama, 2014; Rossant and Cross, 2001); however, maintenance of TSC is less well understood. SFMBT2, a PcG protein, is required to maintain the TS compartment.

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Sfmbt2 mutant embryos establish a TS cell compartment, as measured by CDX2 positive cells, but the cell numbers are reduced and placenta growth does not proceed past E8.5 (Miri et al., 2013). Its loss results in de-repression of a suite of genes and premature differentiation of existing TSC into a small placenta. SFMBT2, a PcG protein, is required to maintain the TS compartment.

Fig. 6. Altered expression of LINE elements in Sfmbt2 null tissue. RNA-seq data from Sfmbt2 null versus wild-type E7.5 extraembryonic tissues were subjected to RepEnrich2 analysis to assess the expression of LINE elements. Six elements were found to be altered in mutant tissues. (****FDR ≤ 0.00005; ***FDR ≤ 0.005; **FDR ≤ 0.05).

Table 1. Frequency of overlap between called peaks and known lncRNAs

| Sample         | # Peaks | # IncRNAs | % Peaks associated with IncRNA |
|----------------|---------|-----------|-------------------------------|
| MN endogenous  | 12,350  | 37,669    | 46.06                         |
| Sonicated endogenous | 2170    | 5673      | 37.50                         |
| MN FLAG        | 23,393  | 63,585    | 33.63                         |
| Sonicated FLAG | 16,087  | 37,781    | 26.44                         |

Fig. 7. Histone variant association with SFMBT2 peaks. Histones exhibit three distinct patterns of distribution across called endogenous SFMBT2 peak coordinates. The distribution of ChIP-seq reads associated with each histone modification was visualized from 5000 bp upstream to 5000 bp downstream of the centre of called SFMBT2 peaks.
with altered expression in mutant tissues, five of six display downregulation rather than upregulation, suggests they may be doing something else in trophoblast cells. The localization of SFMBT2 at pericentromeric regions in mitotic cells and chromocentres in interphase cells suggests a strong interaction with heterochromatic elements. At the same time, the stereotypical pile-up of active chromatin marks such as H3K4me3 and H3K27ac to regions flanking SFMBT2 peaks suggests that in TSC, SFMBT2 may be part of the architectural components that maintain a poised chromatin state, characteristic of other stem/progenitor cells but specific in mechanism to trophoblast, and that reduction of SFMBT2 may allow opening up of chromatin during the differentiation process (Fig. 9). The stereotypical association of CTCF with SFMBT2 peaks supports this view, given the role this boundary-element protein plays in the maintenance of stem cell integrity. Phenotypic and biochemical analysis of SFMBT2-interacting proteins in trophoblast stem cells may provide further insight into the mechanism by which this chromatin protein regulates placenta development.

The strongest association, as measured by visualization, is with chromocentres. Our ChIP-seq data contain elements from pericentromeric DNA, e.g. major satellite sequences, and while independent validation using qPCR was successful, it was modest at best, probably a reflection of the technical problems associated with analysing highly repetitive DNA. Indeed, centromeres are one of the last frontiers in genome analysis (Jain et al., 2018), their mysteries camouflaged by their repeats. ChIP-seq datasets typically exclude these regions because they are unmappable. This makes pursuit of a structural role in TS cell centromere function highly challenging. Advances in technologies aimed at studying the architectural organization of genomes in undifferentiated, differentiated and abnormal cells has revealed that large distances in the genome are bridged by loops that are defined by Topological Associating Domains (TADs) (Pombo and Dillon, 2015; Dekker and Misteli, 2015).
2015; Dixon et al., 2012). These features emerge from analysis of multiple chromatin marks in whole genome datasets. Trophoblast stem cells are currently poorly represented in the ENCODE database. The addition of SFMBT2 distribution in trophoblast stem cells reported here will build on a small but growing set of parameters that will allow more comprehensive analysis of this lineage.

**MATERIALS AND METHODS**

**FLAG-tagged TSC**

The lentiviral construct expressing myc-tagged Sfmbt2 with a linked GFP transgene (Miri et al., 2013) was modified by substitution of the myc-tag with a triple FLAG tag, following digestion of the lentiviral plasmid with BstBI and XbaI, and ligation of a FLAG containing oligonucleotide with engineered sticky ends. Lentiviruses were produced as described (Miri et al., 2013) and used to infect (C57BL6×Castaneus) F1 TSC at early passage, kindly supplied by Dr Terry Magnuson (Department of Genetics, University of North Carolina at Chapel Hill, USA).

GFP-expressing cells were sorted by fluorescence-activated cell sorting (FACS) for three different levels of GFP: strong, medium and weak. The resulting cell pools were expanded into ‘bulk’ cell lines. The experiments described in this paper were generated with the strongly expressing TSC cell line, designated 7H2-1. Immunohistochemistry revealed that FLAG distribution was indistinguishable from endogenous SFMBT2 (Fig. S1A). Following fixation in neutral buffer 10% formalin (Sigma-Aldrich, #HT501320) for 15 min, cells were rinsed with phosphate buffered saline (PBS) and permeabilized in 0.3% Triton-X for 15 min. Blocking was done in 10% goat serum and 0.1% Triton-X in PBS for 1 h at room temperature. Antibodies were diluted in antibody dilution buffer consisting of 5% goat serum and 0.1% Triton-X in PBS. Washes were performed in PBS and 0.05% Tween20. Counter staining was done using 4',6-diamidino-2-phenylindole (DAPI) at a concentration of 0.1 µg/ml in PBS. Human anti-CREST antibody was purchased from Antibodies Incorporated (#15-234); anti-FLAG antibody was purchased from Sigma-Aldrich (M2, #F3165); anti-SFMBT2 antibody was produced in-house (Miri et al., 2013).

**Chromatin immunoprecipitation**

**Harvesting TSC**

A minimum of 10⁷ cells are required for a standard ChIP experiment coupled with next-generation sequencing (ChIP-seq). TSC form colonies in culture with SFMBT2 distribution in trophoblast stem cells are currently poorly represented in the ENCODE database. The addition of SFMBT2 distribution in trophoblast stem cells reported here will build on a small but growing set of parameters that will allow more comprehensive analysis of this lineage. Chromatin immunoprecipitation (ChIP) was performed as previously described (Tsankov et al., 2015) until the wash procedure. Pellets were resuspended in 1 ml lysis buffer and incubated on ice for 1 h. Chromatin fragmentation was achieved using 418 units of micrococcal nuclease (MN) with incubation at 37°C for 2.5 h. Digestion was inhibited by addition of EGTA until a final concentration of 50 mM. Washes, elution and DNA purification steps were performed as in SChIP.

**Library preparation and high-throughput sequencing**

ChIP and total input DNA was sent to The Centre for Applied Genomics (TCAG) for library preparation and subsequent next-generation sequencing. Equal amounts (10 ng) of input and ChIP DNA of sonicated endogenous SFMBT2 samples was supplied for library preparation using the Illumina TruSeq protocol. Sonicated FLAG-SFMBT2 and all MN ChIP samples were prepared using the NEB Ultra DNA library preparation protocol. The NEB Ultra DNA library preparation protocol was used because of low ChIP DNA yields. Although all libraries were subject to paired-end sequencing on the Illumina HiSeq 2500 platform, sonicated endogenous SFMBT2 libraries were sequenced at a different time.

**RNA-seq**

Sfmbt2 null embryos were generated by inter-crossing heterozygous males and females from the Sfmbt2 gene trap (gt) colony (Miri et al., 2013). Embryos at E7.5 of development were individually dissected into either embryo (DNA extraction buffer – genotyping) or extraembryonic (RNA extraction buffer) portions. Following genotype identification, homozygous mutant or wild-type RNA extracts from 10–20 embryos were pooled and processed for RNA-seq by TCAG using the Illumina TruSeq protocol and paired end next-generation sequencing on the Illumina HiSeq 2500 platform. Two biological replicates of each were analyzed. Animals used in this study were housed in the Biological Sciences Facility of the Faculty of Arts and Sciences at the University of Toronto, and procedures were approved by the Local Animal Care Committee that oversees protocol design to ensure compliance with standards set out by the Canadian Council on Animal Care.

**Bioinformatics ChIPseq**

Illumina fastq data files acquired from sequencing by TCAG necessitated the use of computational programs for data analysis due to the dense information content. All bioinformatics programs were run using an Ubuntu 14.04 OS.

**Quality assessment and adaptor trimming**

The wrapper tool TrimGalore was used to facilitate quality assessment and adaptor trimming (Krueger, 2015). FastQC was used for assessing the quality of sequencing reads, identification of adaptor sequences, and identification of non-adaptor overrepresented sequences (Andrews, 2010).
Adaptor sequences of paired-end reads were trimmed using CutAdapt (Martin, 2011). Quality of trimmed reads was then reassessed to ensure removal of adaptor sequences.

**Genome alignment and peak calling**
Adaptor-trimmed sequences were aligned to the mm10 *Mus musculus* C57Bl6 genome annotation via Bowtie2 with the quality-check filter implemented (Langmead and Salzberg, 2012). Narrow peaks were called using the MACS2 program with a false discovery rate (FDR) of 0.01 (Zhang et al., 2008). Broad peaks were called using SICER v1.1 with a FDR of 0.01, a window of 200 bp, and a gap of 1000 bp (Zang et al., 2009). Prior to invoking SICER, modifications were made to the GenomeData.py code for inclusion of the mm10 genome. A statistical comparison of genome-wide count distributions was performed using the University of California Santa Cruz (UCSC) utility wigCorrelate (Table S1) (Lee et al., 2011). Due to high correlation between replicates, replicates were pooled and subject to peak calling. All subsequent analyses were performed using SICER-called peak sets.

**Assessing peak conservation across samples**
The R package DiffBind was used for comparison of called peaks across samples (Stark and Brown, 2011). DiffBind enabled depiction of similarity between called peaks in a heatmap and generated a consensus peak set. Venn diagrams were generated for visualization of peak conservation in the consensus peak set for each pooled sample and conserved peaks between samples. The fraction of total reads found in called peaks (FRIP) was also calculated.

**Statistical assessment of SFMBT2 peak association with genomic features**
The R package regionR was used to assess the association between SFMBT2 peak sets with genomic repeats and ncRNA (Gel et al., 2016). Genomic coordinates for mm10 repeats were acquired from RepeatMasker through the UCSC table browser function. Coordinates in the genomic repeat regions were binned according their respective repeat families. Information in each repeat family bin was then formatted into BED files for use in regionR. The same approach was used for ncRNA coordinates acquired from the NONCODE website (Bu et al., 2012). 1000 permutation tests were run per sample with a seed number of 1.

**Assessing distribution of available ChIP-seq reads across supplied coordinates**
Publicly available ChIP-seq data in TSC were downloaded from NCBI GEO. The associated accession numbers are: CTCF (GSM989939), RNA polymerase II (GSM967644), H3K4me1 (GSM1035385), H3K4me2 (GSM967645), H3K4me3 (GSM1035382), H3K27ac (GSM967654), H3K27me3 (GSM967649), H3K36me3 (GSM967646), Total H3 (GSM967647), and Total H2A (GSM1015786, GSM1015787, GSM1015788, H3K36me3 (GSM1035383), H4K20me1 (GSM967655). All available mm9 BED files were converted to mm10 through the UCSC liftOver utility with automated processing of headers in command-line. NCBI accession numbers with no available BED files were processed from SRA files. SRA files were processed using the ratoolkit to generate fastq files which were subsequently mapped to the mm10 genome using Bowtie2.

SeqMiner was used for read pile-up assessments across specified genomic coordinates (Ye et al., 2014). The shell command for invoking SeqMiner was modified from `java -Xmx2000 m -jar seqMINER.jar` to `java -Xmx15000 m -jar seqMINER.jar` for increased memory usage. xGenomic coordinates of called SFMBT2 peaks or repetitive elements in BED format were supplied as a reference file. Aligned read files for histone or protein distributions of interest were in BED or BAM format. A seed value of 1 was used for all analyses and analysis window was ±5000 bp from the centre of coordinates supplied in the reference file.

**Bioinformatics RNAseq**
Fasta files acquired from TCAG were analyzed according to the protocol described by Trapnell et al. (2012). Reads were aligned to the C57Bl6 mm10 genome using TopHat2 and subsequent analysis was performed using the Tuxedo suite (i.e. Cufflinks, Cuffmerge, Cuffdiff and the R package CummeRbnd). Data were further analyzed using SeqMonk. General Gene Ontology (GO) analysis was performed using the GO analysis tool at Mouse Genome Informatics (MGI) website (www.informatics.jax.org). Significantly differentially expressed genes identified by CuffDiff were cross-analyzed with a list of genes associated with placenta-specific GO terminologies acquired from EMBL-EBI’s QuickGO (www.ebi.ac.uk/QuickGO).

**RepEnrich2 analysis for differential expression of LINE elements in RNA-seq**
An updated method of RepEnrich (i.e. RepEnrich2) as detailed by Criscione et al. (2014) was applied for the analysis (https://github.com/neretlib/RepEnrich2). Of note, all available python codes were converted from python 2.7 to python 3.6 for use. To reiterate, a bed file detailing the mm10 coordinates of known repetitive LINE elements was extracted from the UCSC genome browser. RNA-seq data of replicate *Sfmbt2* null and wild-type extraembryonic tissues were mapped to the mm10 reference genome. RepEnrich2_subset.py was executed to parse aligned sequences into uniquely and multi-mapping files. RepEnrich2.py was then applied using the custom mm10 LINE bed file for all samples. Differential enrichment analysis of the RepEnrich2 output was performed using the R package ‘edgeR’.

**Validation ChIP qPCR**
Standard qPCR was performed using 20 pg of sonicated FLAG-SFMBT2 template whereas 50 pg of MN-digested FLAG-SFMBT2 template was used. Standards used consisted of serial dilutions of their respective total input fractions. qPCR was performed using the WISENT advanced qPCR mastermix with supergreen lo-trox reagent (WISENT Bioproducts, #800-435-UL). Anniming temperatures for all qPCRs was set at 57°C with 40 amplification cycles.

**Primer design**
Common peaks called across pooled ChIP samples were identified as potential targets. Genomic sequences were then subject to primer design using the Primer3 program. Potential amplicons corresponding to each putative primer set were identified by Primer-BLAST. Primer pairs which generate a single amplicon within 1000 bp were then tested by end-point PCR using input template. Only primers that gave rise to a single distinct amplicon were used for qPCR. Negative targets were designed in the same manner for regions where no fold enrichment over input was observed.

**Major satellite qPCR**
Primers used for major satellite qPCR were previously published by Martens et al. (2005). qPCR could only be performed on MN-digested FLAG-SFMBT2 samples; 0.5 pg of template was amplified with 1 µM primer.

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**Competing interests**
The authors declare no competing or financial interests.

**Author contributions**
Conceptualization: S.V.; Methodology: P.T., K.M.; Software: P.T.; Validation: P.T.; Formal analysis: P.T.; Investigation: P.T., S.V.; Resources: P.T., K.M., S.V.; Data curation: P.T.; Writing - original draft: P.T., S.V.; Writing - review & editing: P.T., S.V.; Visualization: P.T., K.M.; Supervision: S.V.; Project administration: S.V.; Funding acquisition: S.V.

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**Data availability**
High throughput sequence data have been deposited in GEO under reference numbers GSE117880, GSE115087 and GSE117879.
Schwartz, Y. B. and Pirrotta, V. (2013). A new world of Polycombs: unexpected partnerships and emerging functions. *Nat. Rev. Genet.* **14**, 853-864. doi:10.1038/nrg3603

Senthilkumar, R. and Mishra, R. K. (2009). Novel motifs distinguish multiple homologues of Polycomb in vertebrates: expansion and diversification of the epigenetic toolkit. *BMC Genomics* **10**, 549. doi:10.1186/1471-2164-10-549

Simmons, D. G., Fortier, A. L. and Cross, J. C. (2007). Diverse subtypes and developmental origins of trophoblast giant cells in the mouse placenta. *Dev. Biol.* **304**, 567-578. doi:10.1016/j.ydbio.2007.01.009

Skinner, M. K. (2011). Role of epigenetics in developmental biology and transgenerational inheritance. *Birth Defects Res. C Embryo Today* **93**, 51-55. doi:10.1002/bdrc.20199

Stark, R. and Brown, G. (2011). https://bioconductor.statistik.tu-dortmund.de/packages/2.13/bioc/vignettes/DiffBind/inst/doc/DiffBind.pdf.

Struhl, G. (1981). A gene product required for correct initiation of segmental determination in *Drosophila*. *Nature* **293**, 36-41. doi:10.1038/293036a0

Suter, M., Ma, J., Harris, A. S., Patterson, L., Brown, K. A., Shope, C., Showalter, L., Abramovic, A. and Aagaard-Tillery, K. M. (2011). Maternal tobacco use modestly alters correlated epigenome-wide placental DNA methylation and gene expression. *Epigenetics* **6**, 1284-1294. doi:10.4161/epi.6.11.17819

Trapnell, C., Roberts, A., Goff, L., Pertea, G., Kim, D., Kelley, D. R., Pimentel, H., Salzberg, S. L., Rinn, J. L. and Pachter, L. (2012). Differential gene and transcript expression analysis of RNA-seq experiments with TopHat and Cufflinks. *Nat. Protoc.* **7**, 562-578. doi:10.1038/nprot.2012.016

Tsankov, A. M., Gu, H., Akopian, V., Ziller, M. J., Donaghey, J., Amit, I., Gnirke, A. and Meissner, A. (2015). Transcription factor binding dynamics during human ES cell differentiation. *Nature* **518**, 344-349. doi:10.1038/nature14233

Willbanks, A., Leary, M., Greenshields, M., Tyminski, C., Heerboth, S., Lapinska, K., Haskins, K. and Sarkar, S. (2016). The evolution of epigenetics: From prokaryotes to humans and its biological consequences. *Genet. Epigenet.* **8**, 25-36. doi:10.4137/EG.101863

Ye, T., Ravens, S., Krebs, A. R. and Tora, L. (2014). Interpreting and visualizing ChIP-seq data with the seqMINER software: stem cell transcriptional networks. *Methods Mol. Biol.* **1150**, 141-152. doi:10.1007/978-1-4939-0512-6_8

Zacher, B., Michel, M., Schwabl, B., Cramer, P., Tresch, A. and Gagneur, J. (2017). Accurate promoter and enhancer identification in 127 ENCODE and roadmapp epigenomics cell types and cells by GenoSTAN. *PLoS ONE* **12**, e0169249. doi:10.1371/journal.pone.0169249

Zhang, C., Schones, D. E., Zeng, C., Cui, K., Zhao, K. and Peng, W. (2009). A clustering approach for identification of enriched domains from histone modification ChIP-Seq data. *Bioinformatics* **25**, 1952-1958. doi:10.1093/bioinformatics/btp340

Zhang, Y., Liu, T., Meyer, C. A., Eeckhoute, J., Johnson, D. S., Bernstein, B. E., Nusbaum, C., Myers, R. M., Brown, M., Li, W. et al. (2008). Model-based Analysis of ChIP-Seq (MACS). *Genome Biol.* **9**, R137. doi:10.1186/gb-2008-9-9-r137

Zhang, X., Pei, L., Li, R., Zhang, W., Yang, H., Li, Y., Guo, Y., Tan, P., Han, J. J., Zheng, X. et al. (2015). Spina bifida in fetus is associated with an altered pattern of DNA methylation in placenta. *J. Hum. Genet.* **60**, 605-611. doi:10.1038/jhg.2015.80