# **­Dynamic chromatin accessibility in spermatogonial cells underlies changes in transcriptional programs from early postnatal to adult life**

Irina Lazar-Contes1\*, Deepak K. Tanwar1,2\*, Pierre-Luc Germain1,2, Isabelle M. Mansuy1#

1. Laboratory of Neuroepigenetics, Brain Research Institute, Medical Faculty of the University of Zurich, and ﻿Institute for Neuroscience, Department of Health Science and Technology of the ETH Zurich, Zurich, Switzerland
2. Statistical Bioinformatics Group, Swiss Institute of Bioinformatics, Zurich, Switzerland

\*Authors share equal contribution

#Correspondence: [mansuy@hifo.uzh.ch](mailto:mansuy@hifo.uzh.ch)

**Abstract**

In mammals, spermatogonial cells are undifferentiated male germ cells in testes that exit quiescence after birth, and self-renew or differentiate to produce spermatogenic cells and functional sperm across life. Recent rodent studies showed that spermatogonial cells have a highly dynamic transcriptome between early postnatal life and adulthood. However, what drives this developmental transition at the chromatin level is not known. We characterized chromatin accessibility in early postnatal and adult spermatogonial cells in mice using ATAC-seq, and integrated the data with a range of transcriptomic and epigenomic features across age. We show that extensive chromatin remodeling occurs in spermatogonial cells across postnatal development, and that this correlates with distinct gene expression profiles and transcription factor (TF) motif enrichment. We further identify genomic regions with significantly different chromatin accessibility in adult spermatogonial cells, that are marked by distinct histone modifications, and are situated in proximity of a transcription start site (TSS) of genes important for spermatogonial cell maintenance and proliferation. Some of the regions with increased accessibility in adult spermatogonial cells correspond to transposable element (TE) subtypes enriched in multiple TFs motifs and with increased RNA expression. Taken together, our results underscore the dynamic nature of open chromatin in spermatogonial cells across postnatal age, and reveal novel profiles of chromatin organization, histone modifications and gene expression between developing and adult spermatogonia stages.

# **Introduction**

Spermatogonial cells are the initiators and supporting foundation of spermatogenesis in various species, including mammals. In mice, they become active one to two days after birth, when they exit mitotic arrest and start dividing, to populate the basement membrane of seminiferous tubules. During the first week of postnatal life, a population of spermatogonial cells continues to proliferate and gives rise to undifferentiated Asingle (As), Apaired (Apr) and Aaligned (Aal) cells. The remaining spermatogonia differentiate to form chains of daughter cells that become primary and secondary spermatocytes around postnatal day (PND) 10 to 12. Spermatocytes undergo meiosis and give rise to haploid spermatids that develop into spermatozoa. Spermatozoa are then released in the lumen of the seminiferous tubules, and continue to mature in the epididymis until becoming capable of fertilization by PND 42-48 (Kubota and Brinster, 2018; Oatley and Griswold, 2017; De Rooij, 2017).

Recent work showed that distinct transcriptional profiles characterize spermatogonial cell populations in early postnatal life (Green et al., 2018; Hammoud et al., 2014, 2015; Hermann et al., 2018; Law et al., 2019). During the first week of postnatal development, spermatogonia display unique features necessary for the rapid establishment and expansion of the cell population along the basement membrane. This includes the high expression of gene programs involved in cell cycle regulation, stem cell proliferation, transcription and RNA (Grive et al., 2019). In comparison, in the adult testis, the focus lies in the maintenance of a steady cell population, which balances proliferation and differentiation capabilities to ensure sperm formation across life. Previous transcriptome datasets have revealed that adult spermatogonial cells prioritize pathways related to paracrine signaling and niche communication, as well as mitochondrial function and oxidative phosphorylation (Grive et al., 2019; Hermann et al., 2018). Concomitant with gene expression changes, histone tail modifications and DNA methylation differences in spermatogonial cells from distinct postnatal stages have also been described (Hammoud et al., 2014, 2015). However, little is known about the accessible chromatin landscape, and how it could facilitate the diverse transcriptome of spermatogonial cells during the transition from early postnatal to adult stage.

To investigate open chromatin reorganization in the transition from early postnatal to adult spermatogonia, we employed ﻿the Omni-ATAC protocol, an improved version of the Assay for Transposase-Accessible Chromatin with high-throughput sequencing (ATAC-seq) (Corces et al., 2017). Our results revealed extensive chromatin remodeling, in particular an increase in accessibility of open chromatin regions in adult spermatogonia compared to PND15 cells. We further integrated published chromatin immunoprecipitation sequencing (ChIP-seq), bisulfite sequencing (BS) and transcriptome profiles, and characterized novel histone signatures for the regions of differentially accessible chromatin associated with genes involved in distinct biological pathways. Lastly, by performing chromatin accessibility analysis at transposable elements (TEs), we described previously uncharacterized changes in accessibility at LTR and LINE L1 subtypes between developing and adult spermatogonial cells. Taken together our results suggest an important contribution of open chromatin reorganization to the diverse transcriptome of developing and adult spermatogonial cells.

# **Results**

## **FACS enriches spermatogonial cells from early postnatal and adult mouse testis**

We collected testes from 8 and 15 days old mouse pups (PND8 and 15), and from adult males at postnatal week (PNW) 20, and prepared cell suspensions by enzymatic digestion of the testes. To achieve high enrichment of spermatogonial cells in our cell populations, we employed fluorescence-activated cell sorting (FACS) using the surface phenotype established by Kubota et al. (Fig. S1A) (Kubota et al., 2004a). The enriched spermatogonial populations were further used for Omni-ATAC (n = 6 PND15 and n = 6 adult samples) and RNA-seq (n = 9 PND8 and n = 8 PND15 samples) approaches. To evaluate the purity of our sorted samples, we performed immunocytochemistry using PLZF, a well-established marker of undifferentiated spermatogonia (Costoya et al., 2004). This revealed that our FACS strategy generates cell populations that are 85-95% PLZF+,compared to only 3-6% PLZF+ cells identified in our samples before FACS (Fig. S1B). Using our newly generated RNA-seq data, we also examined the expression of known pluripotency, spermatogonial stem cell and somatic markers (Leydig and Sertoli cells). We observed that cells in our sorted preparations expressed high level of stem cell and undifferentiated spermatogonial markers, but low level of somatic cells markers, confirming spermatogonial cell enrichment (Fig. S1C).

## **Chromatin is remodeled in spermatogonial cells from early postnatal stage to adulthood**

Previous RNA-seq analyses in rodents showed that spermatogonial cell transcriptome changes during postnatal life, with distinct transcriptional programs revealed in neonatal, juvenile and adult stages (Grive et al., 2019; Hammoud et al., 2015). To determine if changes in chromatin organization underlie the dynamic transcriptome of spermatogonial cells as mice age, we profiled chromatin accessibility of spermatogonial cells at PND15 and from adult testis. We relied on the Omni-ATAC protocol, which shows a higher signal-to-noise ratio compared to the original ATAC-seq protocol, and can be performed from as low as few hundred cells (Corces et al., 2017). Accessible regions were identified by peak-calling on the merged nucleosome-free fragments (NFF) from all PND15 and adult samples. Following the removal of lowly enriched regions, we included 158,978 regions in our downstream analyses (see Methods section for details). Most of the Tn5-accesible regions were intergenic (38%), located in gene bodies (33%) or in proximity of a gene transcription starting site (TSS) (+/-1 kb from TSS, 28%) (Fig. S2A). Differential accessibility analysis revealed 3212 differentially accessible regions between PND15 and adult spermatogonia (FDR ≤ 0.05, abs Log2 fold change ≥ 1), with the majority of the regions showing a gain in accessibility in adult stage (Fig. 1A and Table S1). The regions of differential accessibility were predominantly localized in intergenic regions and introns (34% in introns and 45% in intergenic regions). Only 15% of all differentially accessible regions resided +/- 1kb from the TSS of a gene (Fig. 1B). To investigate the potential functional significance of the regions with differential accessibility, we performed Gene Ontology (GO) analysis using the Genomic Regions Enrichment of Annotations Tool (GREAT) (McLean et al., 2010). Overall, regions of increased accessibility in adult spermatogonia were associated with cell fate and stem cell population maintenance, protein metabolism and RNA metabolic processes (Fig. 1C and Table S1). Interestingly, when performing GREAT separately for regions gaining in accessibility depending on their genomic location (intergenic, gene bodies, +/- 1kb from TSS), we found that regions located in gene bodies (mainly introns) were enriched for GO terms related to reproduction and protein metabolism, whilst regions close to (+/- 1 kb), or overlapping TSSs of genes, were strongly enriched for cell fate specification and tissue morphogenesis (Fig. S2B and Table S1). In regions with decreased accessibility in adult spermatogonia, GO enrichment revealed multiple terms related to embryonic development (Fig. 1C and Table S1). Furthermore, we observed that the regions of less accessibility in adult spermatogonial cells were predominantly localized in intergenic regions (Table S1.

## **Differentially accessible chromatin regions associate with distinct gene expression dynamics and histone marks**

To better understand the chromatin accessibility – transcriptome relationships across postnatal age, we conducted RNA-seq on spermatogonial cells from PND8 and PND15 testis, and further used previously generated literature datasets from PND14 and postnatal week (PNW) 8 THY1+ spermatogonial cells (Hammoud et al., 2014, 2015). We found 719 differentially expressed genes (FDR ≤ 0.05, abs Log2 fold change ≥ 1) between PND8 and PND15 cells, indicative of dynamic transcriptional programs during early phases of spermatogonial cell proliferation and differentiation (Fig. 2A and Table S2). The comparison between PND14 and adult spermatogonia transcriptomes, while not affording the same type of analysis due to the low sample size, suggested even broader transcriptional changes taking place between early postnatal and steady -state adult spermatogonial cells (Log2CPM ≥ 1 and abs Log2 fold change ≥ 1) (Fig. 2B and Table S2). To identify the biological significance of these dynamic gene expression profiles, we conducted pathway analysis using Fast Gene Set Enrichment Analysis (FGSEA) (Korotkevich et al., 2016; Subramanian et al., 2005). Between PND8 and PND15 spermatogonia we revealed a downregulation of pathways related to RNA processing and splicing, cell cycle, redox homeostasis and protein catabolism, and an upregulation of terms associated with cellular transport, exocytosis and signal transduction (Fig. 2C and Table S2). GSEA from PND14 and adult spermatogonia revealed that, similarly to the transition from PND8 to PND15, pathways related to RNA processing, ribosome biogenesis, and cell cycle were downregulated in adult spermatogonia (Fig. 2D and Table S2). We also found a downregulation of pathways related to developmental programs and mitochondrial functions. In contrast, upregulated pathways were related to spermatogenesis, as well as numerous processes involving cytokine signaling (Fig. 2D and Table S2).

Next, we integrated the chromatin accessibility and transcriptome changes that characterize the transition from early postnatal to adult spermatogonial stage. Additionally, we mined previously published ChIP-seq and BS data from THY1+ spermatogonia and investigated histone mark (H3K4me3, H3K27ac, H3K27me3) distribution and DNAme patterns (Hammoud et al., 2014, 2015). Notably, ChIP-seq data was only available for adult spermatogonial cells (PNW8), whilst DNAme was available for PND7, PND14 and adult stages. Of note, THY1+ spermatogonia encompass a more heterogeneous population of cells than the one obtained with our sorting strategy. Therefore, we cannot exclude that the different proportions of spermatogonial cell subtypes present in the samples from literature can have an impact on our data interpretation. For integrating our ATAC-seq data with the RNA-seq, ChIP-seq and BS datasets, we first divided differentially-accessible regions into proximal (situated less than +/- 2.5 kb from a TSS) and distal (situated more than +/- 2.5 kb from a TSS), following ENCODE practice (Harrow et al., 2012; Myers et al., 2011; Thurman et al., 2012). We further divided proximal regions based on the change in expression of the nearest gene, leading to 6 distinct categories. The first 2 most abundant categories contained regions with increased chromatin accessibility and upregulated nearby genes - Category 1, and increased chromatin accessibility and downregulated nearby genes - Category 2. Category 3 and 4 comprised proximal regions of less chromatin accessibility for which gene expression was either downregulated or upregulated, respectively. Categories 5 and 6 were proximal regions with increased and decreased chromatin accessibility respectively, for which the expression of the nearest gene was not detected in spermatogonial cells (Fig. 3A and Table S3).

For a subset of regions in Category 1, ChIP-seq overlap identified active H3K4me3, H3K27ac or dual H3K4me3/H3K27ac modifications, and an overall lack of H3K27me3 (Fig. 3B and Table S3). Notably, several of the genes in Category 1 for which chromatin opening was also marked by histone modifications, are known regulators of stem cell potential. *Pdpk1* promoter region was marked by a dual H3K4me3/H3K27ac, while *Pdpk1* mRNA showed a slight upregulation in adult spermatogonia (Fig. S3A). *Pdpk1* (phosphoinositide-dependent protein kinase 1) is a glycolysis factor, important for spermatogonial stem cell self-renewal (Chen et al., 2020; Kanatsu-Shinohara et al., 2016). In contrast, *Gata2* promoter region was marked by a bivalent H3K4me3/H3K27me3 mark, while its expression showed a slight upregulation across testis maturation (Fig. S3). *Gata2* is a known target of NANOS2, an essential regulator of spermatogonial stem cell potential (Barrios et al., 2010; Sada et al., 2009). Other exemplary genes in Category 1 included pyruvate cellular carriers *Slc25a18, Slc23a1* and *Slc2a5*, suggesting differences in glycolysis regulation in adult spermatogonial cells (Table S3). Notably, we found an increased chromatin accessibility at the TSS of GDNF receptor *Gfra2* (Fig. 3C)*.* At mRNA level, *Gfra2* displayed a marked upregulation in adult spermatogonial cells, indicating an increased utilization of GFRA2 receptors in adult spermatogonial cells compared to early postnatal stages, in which GFRA1-mediated signaling is dominant (Fig. 3C) (Grive et al., 2019; Hammoud et al., 2015). We also observed an increased chromatin accessibility and upregulation of DNA damage responsive gene *Fus*, and the multifunctional redox gene *Prdx4* (Table S3)*.*

Interestingly, the highest number of differentially accessible chromatin regions were in Category 2, and included proximal regions with increased chromatin accessibility and decreased expression of nearby genes in adult spermatogonia, indicative of active repression taking place (Fig. 3A and Table S3). A subset of Category 2 regions was marked by H3K27me3 together with H3K4me3, and mainly associated with developmental genes such as *Satb1* and *Hmx1,* *(*Fig. S3B). Other developmental genes such as *Tbx4* also displayed a decreased accessibility and an increase in mRNA expression (Fig. 3C). GO enrichment analysis revealed that regions in Category 2 associated with regulation of cell cycle, RNA processing, DNA repair and cell division (Table S4). Such an example is is *Fgf8*, important for *Fgf8*-*Fgfr1* mediated maintenance of undifferentiated spermatogonia (Hasegawa and Saga, 2014). *Fgf8* showed increased chromatin accessibility close to its TSS and a downregulated expression in adult spermatogonia, in agreement with recent findings from scRNA-seq data suggesting a downregulation of *Fgfr1*-mediated signaling with age (Fig. S3B) (Grive et al., 2019; Hasegawa and Saga, 2014).

Regions in Category 3 displayed decreased chromatin accessibility and a downregulation of nearby genes in adult spermatogonia and were mostly depleted of any of the 3 histone marks investigated (Fig. 3A and 3B). GO enrichment on the nearby genes revealed an association with developmental processes and WNT signaling (Table S4). A notable example we identified in this category is *Pdgfra,* a gene involved in the hepatic stellate cell activation pathway, which was recently identified by scRNA-seq to be upregulated in spermatogonial stem cells in the immature testis compared to adult stage (Hermann et al., 2018). Pdgfra displayed a marked downregulation in adult spermatogonia, and a decrease in chromatin accessibility overlapping its TSS (Fig. 3C). Another example of a gene important for early postnatal spermatogonial cell establishment is Dap2ip, which we also found to carry a marked decrease in chromatin accessibility at its TSS and to decrease in expression in adult spermatogonial cells (Fig. S3C).Surprisingly, we also identified a category of proximal regions (Category 4) with decreased accessibility (<20 regions) at genes which were upregulated in adult spermatogonial cells and with previously uncharacterized roles in spermatogonial cells (Fig. 3A). Notably, DNAme profiles across postnatal stages did not show drastic changes across any of the 6 categories of proximal regions, suggesting a relatively stable DNAme profile in the transition from early postnatal to adult stage (Fig. S3D). Aside from proximal regions, we also identified numerous distal regions with differential chromatin accessibility between PND15 and adult (Fig. S4A). Similar to the proximal regions, accessibility in distal regions mainly increased in adult spermatogonial cells compared to early postnatal stage. When integrating the literature ChIP-seq data, we observed enrichment for H3K4me3, H3K27ac and H3K27me3 at a small number of the differentially accessible distal regions, indicative of potential regulatory roles (Fig. S4B and Table S3). Similar to proximal regions, DNAme levels did not display major changes in the transition from early postnatal to adult stage (Fig. S4B). Taken together, our data integration reveals novel associations between open chromatin regions of differential accessibility, histone marks and dynamically expressed genes in spermatogonial cells during testis maturation, and reveal how chromatin accessibility may contribute to the differential utilization of certain signaling pathways with age.

## **Accessibility changes at open chromatin regions are marked by binding sites for distinct families of transcription factors**

Previous work has underlined the importance of transcription factors (TFs) in establishing and maintaining transcriptome programs across the developmental trajectory of a cell population (Fushan et al., 2015; Shavlakadze et al., 2019). To investigate if the regions of different chromatin accessibility between PND15 and adult spermatogonia are enriched in regulatory elements such as TF binding motifs, we performed motif enrichment analysis using the Hypergeometric Optimization of Motif EnRichment (HOMER) tool (Heinz et al., 2010). In regions with increased chromatin accessibility, we identified 41 enriched TF motifs (q-value ≤ 0.05) (Fig. 4A). The top candidate list (q-value ≤ 0.0001) was dominated by members of the Fos/Jun family (FOS, FOSB, FOSL1 and FOSL2, JUN, JUNB and JUND) (Fig. 4B). Notably, at mRNA level, some of the TFs displayed age-specific differences (Log2CPM ≥ 1 and abs Log2 fold change ≥ 1): *Fos,* *Junb* and *Jund* were downregulated in adult spermatogonial cells (Fig. 4C). JUN, FOS and CREB are all part of the AP-1 (activating protein-1) superfamily, and play an important role in regulating cell proliferation and death, by mediating the senescence-associated chromatin and transcriptional landscape (Martínez-Zamudio et al., 2020; Shaulian and Karin, 2002).JUND and c-FOS specifically promote the proliferative potential of spermatogonial stem cells (He et al., 2008; Wang et al., 2018). USF1 and POU3F1, *2* factors important in the maintenance of the spermatogonial stem cell pool also displayed enriched binding motifs in the more accessible regions. However, their mRNA levels were downregulated in adult spermatogonial cells (Fig. 4C) (Faisal et al., 2019; Wu et al., 2010). POU3F1 is a GDNF-regulated TF, which has been shown to play an important role in promoting spermatogonial cell self-renewal capacity (Niu et al., 2011; Wu et al., 2010). Notably, *Pou3f1* downregulation in adult spermatogonial cells coincides with a marked downregulation of *Gfra1* and an upregulation of *Gfra2* (Fig. 3C and Table S2).TF motif analysis using HOMER also revealed enriched binding sites for retinoic acid receptors such as RXRα and RARα (Fig. 4A). Recently, expression of RA receptors *Rxrα* and *Rarα* was reported in the stem cell-containing population of spermatogonial cells in both pup and adult testis, together with evidence that their utilization in spermatogonial cells is vastly dependent on the niche microenvironment (Lord et al., 2018). To check if some of TF binding motifs exhibit are preferentially enriched in certain genomic locations, we performed motif enrichment analysis for more accessible chromatin regions situated in gene bodies, intergenic regions and in regions +/- 1kb from TSS. We identified several TF motifs specifically enriched in intergenic regions, specifically members of the ubiquitously expressed NF-Y complex, NF-YA, NF-YB and NF-YC (Fig. 4D). In mESCs, NF-Y TF family members located in distal regions facilitate a permissive chromatin conformation, and play an important role in the expression of core ESC pluripotency genes (Oldfield et al., 2014). Furthermore, NF-YA/B motif enrichment has also been found in regions of open chromatin in human spermatogonial cells (Guo et al., 2017).

Although regions of more accessible chromatin encompass the majority of the differentially accessible regions in adult spermatogonia compared to PND15, less accessible chromatin also displayed a high number of enriched TF binding motifs (Fig. 4A). Notably, almost all of these TF motifs were uniquely enriched in the regions of decreased chromatin accessibility and predominantly associated with developmental factors. Top hits included members of the FOX family (FOXO1, FOXO3, FOXP2, FOXK1, FOXA2) and members of the ETS and ETS-related families (ETS1, GABPA, ETV4, ELF1, ELF3) (Fig. 4B). The gene expression levels of most of these TFs were decreased in adult spermatogonial cells (Fig. 4A). FOXO1 is a pivotal regulator of the self-renewal and differentiation of spermatogonial stem cells in both pup and adult testis, via the PI3K-Akt signaling pathway (Chan et al., 2014; Goertz et al., 2011). The roles of the various ETS-related TFs in spermatogonial cells have not been clarified, however recently published data found ETV4 in the stem-cell enriched fraction of the spermatogonial population, particularly during the spermatogonial stem cell pool establishment immediately after birth (Cheng et al., 2020; Law et al., 2019). Motif enrichment analysis on the regions with decreased chromatin accessibility situated in gene body and intergenic regions revealed that TFs important in numerous developmental processes (FOXC1, FOXJ2, FOXM1, LHX6) were specifically enriched in intergenic regions of decreased chromatin accessibility (Fig. 4D). This is consistent with the association of intergenic regions with embryonic development-related GO terms that we previously detected (Fig. 1C). Our findings provide a detailed characterization of the enriched TF motifs present at the regions of dynamic accessibility between PND15 and adult spermatogonial populations and point towards novel candidate regulators of the differential transcriptome of pup and adult mouse spermatogonia.

## **Chromatin accessibility at transposable elements undergoes significant remodeling in the transition from early postnatal to adult spermatogonia**

Transposable elements (TEs) are under tight control in the germline, achieved through coordinated epigenetic mechanisms involving DNA methylation, chromatin silencing and the PIWI proteins – piRNA pathway (Deniz et al., 2019; Thompson et al., 2016). Recent evidence suggests an important role for long terminal repeat (LTR) - type elements, specifically for ERVKs, the youngest class of endogenous retroviruses (ERVs), in the transcriptional regulation of mRNAs and long non-coding RNAs (lncRNAs) during the mitosis-to-meiosis transition (Davis et al., 2017; Sakashita et al., 2020). Furthermore, accessibility analysis at LTRs in mitotic and meiotic germ cells, revealed a unique chromatin accessibility landscape in spermatogonial cells, compared to the rest of germ cells in the testis (Sakashita et al., 2020). To explore potential differences in TEs regulation driven by postnatal age, we compared the accessibility of TEs in PND15 and adult spermatogonia. For this purpose, we quantified the ATAC-seq reads overlapping TEs defined by UCSC RepeatMasker, and performed differential accessibility analysis at the subtype level (see Methods section). Our results revealed that the transition from PND15 to adult stage is accompanied by significant chromatin accessibility differences at 135 TE subtypes (Fig. 5A and 5B and Table S5). Although most of the differentially accessible TE subtypes displayed a decrease in chromatin accessibility between PND15 and adult stages (68,9%, 93/135) (Fig. 5A), we also observed 42 TE subtypes which increased in accessibility in adult spermatogonia (Fig. 5B). Of note, more accessible subtypes also displayed an increased expression in adult spermatogonia compared to early postnatal stage (Fig. 5B). TE loci within the subtypes harboring changes in chromatin accessibility were situated in intergenic and intronic regions (68% intergenic and 25% intronic), and around 6% were located in proximity of a gene (+/- 1kb from a TSS) (Fig. 5C). LTRs were the most abundant TEs to display changes in chromatin accessibility, specifically ERVK and ERV1 subtypes (Fig. 5A and 5B). Exemplary ERVK subtypes harboring less accessible chromatin included RLTR17, RLTR9A3, RLTR12B and RMER17B (Table S5). Enrichment of RLTR17 and RLTR9 repeats has been reported previously in mESCs, specifically at TFs important for pluripotency maintenance such as *Oct4* and *Nanog* (Fort et al., 2014). Interestingly, we identified the promoter region of the lncRNA *Lncenc1*, an important regulator of pluripotency in mESCs (Fort et al., 2014; Sun et al., 2018), harboring several LTR loci with decreased accessibility in our adult spermatogonia, with the RLTR17 locus falling within the TSS of *Lncenc1*. This decrease in accessibility correlated with a marked decrease in expression of *Lncenc1* in adult spermatogonia (Fig. 5D). *Lncenc1* (also known as *Platr18*) is part of the pluripotency-associated transcript (*Platr*) family of lncRNAs which were recently identified as potential regulators of the pluripotency-associated genes *Oct4*, *Nanog* and *Zfp42* in mESCs (Bergmann et al., 2015; Dann et al., 2008; Wu et al., 2018). We were also able to identify several other *Platr* genes, such as *Platr27* and *Platr14*, for which the TSS overlapped LTRs with reduced accessibility, RLTR17 and RLTR16B\_MM, respectively (Fig. 5D and Table S5). These 2 pluripotency-associated transcripts also showed a decrease in mRNA expression in adult spermatogonia, while their expression was unchanged between PND8 and PND15 (Fig. 5D and Table S5). The remaining LTR subtypes with decreased accessibility in adult spermatogonia belonged to the ERV1, ERVL and MaLR families (Fig. 5A). Only very few other non-LTR TEs showed a decrease in chromatin accessibility, with 7 DNA element subtypes, 2 Satellite subtypes and 1 LINE subtype, respectively (Fig. 5A and Table S5). Emerging evidence suggests an important contribution of TEs in providing tissue-specific substrates for TF binding (Fort et al., 2014; Sundaram and Wysocka, 2020; Sundaram et al., 2014). To investigate the regulatory potential of the less accessible LTR subtypes, we assessed the enrichment of TF motifs in these regions using HOMER. To do so, we focused on the family level and grouped together all LTR subtypes coming from one family (EVK, ERV1, ERVL and ERVL-MaLR families). Among the less accessible LTR families, ERVKs showed the highest number of enriched TF motifs in adult spermatogonial cells. Top hits included TFs with known regulatory roles in cell proliferation and differentiation such as FOXL1 and FOXQ1, stem cell maintenance factors ELF1, EBF1 and THAP11 and TFs important in spermatogenesis PBX3, ZNF143 and NFYA/B (Fig. 5E and S6A). ERVLs displayed motif enrichment for very few TFs, among which the previously undescribed ETV2, newly reported spermatogonial stem cell factor ZBTB7A and the testis-specific CTCF paralog CTCFL (Fig. 5E) (Green et al., 2018).

Among the TE subtypes which increased in chromatin accessibility, members of the ERVK, ERVL and ERV1 families were predominant (57,1%, 24/42) (Fig. 5B). Interestingly, we also found a considerable number of LINE L1 subtypes with increased chromatin accessibility in adult spermatogonial cells (Fig. 5B). When parsing the data for more accessible loci within the L1 subtypes, we found several L1 loci situated less than +/- 5 kb from the TSS of numerous olfactory (*Olfr*) genes. Most of them were located in *Olfr* gene clusters on chromosome 2, 7 and 11 (Table S5). Furthermore, the increase in accessibility of the L1 loci correlated with an increase in mRNA expression of the nearby *Olfr* gene in adult spermatogonial cells (Fig. 6A). Representative examples were *Olfr362* and *Olfr1307*, both situated in the *Olfr* gene cluster on Chr2 (Fig. 6B). Interestingly, when visualizing the data in IGV, we also observed that the *Olfr* gene cluster on chromosome 2 exhibited a higher density of L1 loci compared to neighboring regions (Fig. S6B). Similar to before, we performed TF motif enrichment analysis at the family level by grouping together all differentially accessible TE subtypes coming from one family. More accessible LINE L1s were highly enriched in TF motifs, particularly in multiple members of the ETS, E2F and FOX families (Fig. 6C). The most significant motifs belonged to spermatogonial stem cell maintenance and stem cell potential regulators FOXO1 and ZEB1, as well as TFs which have been recently associated with active enhancers of the stem cell-enriched population of spermatogonia such as ZBTB17 and KLF5 (Fig. 6C and S6C) (Cheng et al., 2020). More accessible ERV1s also displayed enrichment of several TF binding sites, including spermatogenesis-related TFs (PBX3, PRDM1, NFYA/B), hypoxia inducible HIF1A and cytokine regulators STAT5A/B, suggestive of different spermatogonial cell metabolic demands between early postnatal and adult stage (Fig. 6C and S6C). Overall, we provide an extensive characterization of the chromatin accessibility landscape of TEs in PND15 and adult spermatogonia, reveal differences in accessibility and TF motif landscape at distinct subtypes of TEs between these 2 timepoints, and suggest potential gene programs that may be regulated by these changes.

# **Discussion**

As initiators of the spermatogenic cascade, spermatogonial cells are essential in germ cell proliferation and differentiation throughout postnatal life. Although recent studies employing bulk and scRNA-seq have revealed distinct transcriptional signatures of spermatogonial cells across postnatal life, very few have focused on describing the underlying landscape of open chromatin, and the extent to which it can contribute to the gene expression dynamics (Grive et al., 2019; Hammoud et al., 2015; Hermann et al., 2018).

Our ATAC-seq revealed a reorganization of open chromatin in adult spermatogonia compared to the PND15 cell population. This striking reorganization of accessible chromatin, which we detected mainly in intergenic and intronic regions, may be indicative of novel regulatory regions governing at least in part the vast transcriptome changes that spermatogonial cells undergo in their transition from early postnatal to adult stage (Grive et al., 2019; Hammoud et al., 2015). Indeed, we found that the regions of differential accessibility were associated with distinct gene pathways, with morphogenesis and developmental pathways associated to the regions of decreased chromatin accessibility, while regions of increased chromatin openness were enriched for DNA repair pathways, stem cell maintenance, RNA processing and protein metabolic processes. Furthermore, we identified numerous enriched TF motifs in the regions of differential accessibility, indicative of their potential regulatory role. AP-1 TFs with previously described roles in spermatogonial cell proliferation such as JUND and c-FOS, and pluripotency factors such as POU3F1, displayed enriched binding sites in the regions of increased chromatin accessibility. In contrast, FOX and ETS TF motifs, known regulators of developmental pathways, mainly mapped to regions which decreased in accessibility in adult spermatogonial cells. For some of the enriched TF motifs, a preference for certain genomic locations was evident: NF-YA and B binding sites exhibited enrichment specifically in intergenic regions of more accessible chromatin which interestingly, were also associated with spermatogenesis-related pathways. NF-YA/B also localized in intergenic regions of open chromatin in humans spermatogonial cells (Guo et al., 2017), prompting additional investigation of their roles in regulating spermatogonial cell programs, with potential consequences for sperm formation.

Our comparison of the gene expression changes from PND8 to PND15 spermatogonial cells confirmed the dynamic transcriptome associated with developing spermatogonial cell states (Grive et al., 2019; Hammoud et al., 2015). Notably, PND15 corresponds to the maturation of Sertoli cell niche in the mouse testis (Flickinger, 1967; Shinohara et al., 2001). Upregulation of pathways associated with signal transduction and cellular transport in PND15 spermatogonia suggests an increased cellular communication with the somatic niche, compared to the first week of postnatal development. We complemented these findings with literature RNA-seq data from PND14 and adult spermatogonia, and confirmed further transcriptome changes in signaling pathways related to mitochondria, developmental processes and cell-to-cell signaling. Furthermore, we revealed numerous pathways related to cytokine signaling upregulated in adult spermatogonia, which point to the recently suggested role of testis resident macrophages in maintaining and regulating spermatogonial proliferation (DeFalco et al., 2015; Garbuzov et al., 2018). These findings suggest that, as the testis matures and the somatic niche develops, spermatogonial cells rely more on paracrine signaling and undergo vast changes in gene expression programs.

To obtain a comprehensive profile of the chromatin and the transcriptome differences between early postnatal and adult spermatogonial cells, we have integrated the chromatin accessibility and gene expression, with known histone H3 modifications and global DNAme patterns of THY1+ spermatogonial cells from (Hammoud et al., 2014, 2015). This allowed us to identify 4 distinct categories of differentially accessible chromatin regions for which the nearest gene was dynamically expressed between early postnatal and adult stages. Interestingly, we found a similar number of genes with increased chromatin accessibility around their TSS which exhibited either an up- or a downregulated expression between pup and adult spermatogonia. In the category of upregulated genes with increased nearby chromatin accessibility, we identified several factors associated with redox processes, mitochondria function and cell proliferation. In contrast, genes marked by an increase in chromatin accessibility and downregulated expression in adult spermatogonia comprised factors important for cell cycle, RNA processing and developmental genes, suggesting that active repression is taking place at these genes (Starks et al., 2019). For some of the developmental genes, more accessible chromatin was also marked by a bivalent H3K4me3/H3K27me3, indicative of a poised state. Notably, previous findings in THY1+ adult spermatogonial cells and in sperm also revealed a poised state at promoters of developmental genes (Erkek et al., 2013; Hammoud et al., 2014; Jung et al., 2017). Therefore, our findings suggest that open chromatin reorganization may contribute to the poised status that is already established at the spermatogonial cell stage for certain developmental genes. We also identified a category of regions for which the decrease in chromatin accessibility correlated with a decreased expression, category which also included developmental factors. The stable methylation patterns we detected at the differentially accessible chromatin regions, in both distal and proximal genomic regions, suggest a minimal impact for DNAme in regulating gene expression dynamics of spermatogonial cells across postnatal age.

Lastly, by investigating chromatin accessibility specifically at TEs, we revealed that distinct TE subtypes undergo changes in chromatin accessibility between PND15 and adult spermatogonial populations. ERVK and ERV1 subtypes were the most abundant categories of TEs to become less accessible in adult spermatogonia, whilst LINE L1 subtypes gained in accessibility. Although the majority of these TEs resided in intergenic and intronic regions, we were able to detect specific loci belonging to the differentially accessible ERVK and LINE L1 subtypes, which localized nearby TSS of distinct gene families. RLTR17, one of the LTR subtypes with decreased chromatin accessibility in adult spermatogonial cells, overlapped the TSS of several downregulated long-non coding RNAs from the *Platr* family. *Platr* genes, including the ones identified in our study, *Lncenc1* and *Platr14,* areLTR-associated long non-coding RNAsimportant for embryonic stem cell gene expression programs (Bergmann et al., 2015). Interestingly, RLTR17 has also been previously linked to pluripotency maintenance. In mouse embryonic stem cells, RLTR17 is highly expressed and enriched in open chromatin regions, and has been shown to provide binding sites for pluripotency factors Oct4 and Nanog (Fort et al., 2014). On the basis of these findings, we suggest that RLTR17 chromatin organization may play a significant role in regulating pluripotency programs between early postnatal and adult spermatogonial cells. In contrast to the decreased accessibility of LTRs, LINE L1 subtypes displayed an increase in chromatin accessibility in adult spermatogonial cells. Some of these L1 loci were situated in the vicinity of olfactory receptor genes with upregulated mRNA expression in adult spermatogonia. Recent findings in mouse and human embryonic stem cells have suggested a non-random genomic localization for L1 elements, specifically at genes which encode proteins with specialized functions (Lu et al., 2020). Among these, the *Olfr* gene family was the most enriched in L1 elements (Lu et al., 2020) . Although their role in spermatogonial cells is currently not established, Olfr proteins have been implicated in the swimming behavior of sperm (Fukuda and Touhara, 2005; Vanderhaeghen et al., 1997). Given their dynamic regulation across age in spermatogonial cells, we speculate that *Olfr* genes could play additional roles in spermatogenesis, other than in sperm physiology. This data together with the high number of enriched TF motifs identified at the differentially accessible ERVKs and LINE L1 elements, underscores previously undescribed regulatory roles for chromatin organization of TEs in spermatogonial cells during the transition from developing to adult stages (Sundaram and Wysocka, 2020; Sundaram et al., 2014).

One limitation of our study is the incomplete purification achieved using FACS, which doesn’t fully remove other testis cell types from our cell preparations. Therefore, we cannot entirely exclude the influence of contaminating cells on some of the transcriptome and chromatin accessibility data interpretation. Secondly, differences can also stem from the literature datasets which involve similar but not identically enriched populations of spermatogonial cells. Nevertheless, by comparing open chromatin landscape between developing and adult spermatogonial cells, our results reveal for the first time that there is an age-dependent dynamic reorganization of chromatin accessibility in spermatogonial cells. By integrating this newly generated data with gene expression profiles and known histone modifications, we provide novel insight into the chromatin - transcriptome dynamics of mouse spermatogonial cells between developing and adult stages and compile an information-rich resource for further germline studies.

# **Methods**

## **Mouse husbandry**

Male C57Bl/6J mice were purchased from Janvier Laboratories (France) and bred in-house to generate male mice used for experiments. All animals were kept on a reversed 12-h light/12-h dark cycle in a temperature- and humidity-controlled facility, with food (M/R Haltung Extrudat, Provimi Kliba SA, Switzerland) and water provided ad libitum. Cages were changed once weekly. Animals from 2 independent breedings were used for the experiments.

## **Germ cells isolation**

Germ cells were isolated from male mice at postnatal day (PND) 8 or 15 for RNA-seq and ATAC-seq experiments, and adults at 20 weeks of age (PNW20) for ATAC-seq. Testicular single-cell suspensions were prepared as previously described with slight modifications (Kubota et al., 2004a, 2004b). For preparations using PND8 and PND15 pups, testes from 2 animals were pooled for each sample. Pup testes were collected in sterile HBSS on ice. Tunica albuginea was gently removed from each testis, making sure to keep the seminiferous tubules as intact as possible. Tubules were enzymatically digested in 0.25% trypsin-EDTA (ThermoFisher Scientific) and 7mg/ml DNase I (Sigma-Aldrich) solution for 5 min at 37oC. The suspension was vigorously pipetted up and down 10 times and incubated again for 3 min at 37oC. The digestion was stopped by adding 10% fetal bovine serum (ThermoFisher Scientific) and the cells were passed through a 20μm-pore-size cell strainer (Miltenyi Biotec) and pelleted by centrifugation at 600g for 7 min at 4oC. Cells were resuspended in PBS-S (PBS with 1% PBS, 10 mM HEPES, 1 mM pyruvate, 1mg/ml glucose, 50 units/ml penicillin and 50 μg/ml streptomycin) and used for sorting. For preparations from adult testis, one adult male was used for each sample. The tunica was removed and seminiferous tubules were digested in 2 steps. The first consisted in an incubation in 1mg/ml collagenase type IV (Sigma-Aldrich) for 5 min at 37oC and vigorous swirling until the tubules were completely separated. Then tubules were placed on ice for 5 min to sediment, the supernatant removed and washed with HBSS. Washing/sedimentation steps were repeated 3 times and were necessary to remove interstitial cells. After the last washing step, sedimented tubule fragments were digested again with 0.25% trypsin-EDTA and 7mg/ml DNase I solution, and the digestion was stopped by adding 10% FBS. The resulting single-cell suspension was filtered through a 20μm strainer (Corning Life Sciences) and washed with HBSS. After centrifugation at 600g for 7 min at 4oC, the cells were resuspended in PBS-S, layered on a 30% Percoll solution (Sigma-Aldrich) and centrifuged at 600g for 8 min at 4oC without braking. The top 2 layers (HBSS and Percoll) were removed and the cell pellets resuspended in PBS-S and used for sorting.

## **Spermatogonial cells enrichment by FACS**

For pup testis, dissociated cells were stained with BV421-conjugated anti-β2M, biotin-conjugated anti-THY1 (53-2.1), and PE-conjugated anti-αv-integrin (RMV-7) antibodies. THY1 was detected by staining with Alexa Fluor 488-Sav. For adult testes, cells were stained with anti-α6-integrin (CD49f; GoH3), BV421-conjugated anti-β2 microglobulin (β2M; S19.8), and R-phycoerythrin (PE)-conjugated anti-THY1 (CD90.2; 30H-12) antibodies. α6-Integrin was detected by Alexa Fluor 488-SAv after staining with biotin-conjugated rat anti-mouse IgG1/2a (G28-5) antibody. Prior to FACS, 1 μg/ml propidium iodide (Sigma) was added to the cell suspensions to discriminate dead cells. All antibody incubations were performed in PBS-S for at least 30 min at 4oC followed by washing in PBS-S. Antibodies were obtained from BD Biosciences (San Jose, United States) unless otherwise stated. Cell sorting was performed at 4oC on a FACS Aria III 5L using an 85μm nozzle at the Cytometry Facility of University of Zurich. For RNA-seq on PND8 and PND15 spermatogonia, cells were collected in 1.5 ml Eppendorf tubes in 500 μL PBS-S, immediately pelleted by centrifugation and snap frozen in liquid N2. Cell pellets were stored at -80oC until RNA extraction. For OmniATAC on PND15 spermatogonia, 25’000 cells were collected in a separate tube, pelleted by centrifugation and immediately processed using the OmniATAC library preparation protocol (Corces et al., 2017). For OmniATAC on adult spermatogonia, 5000 cells from each animal were collected in a separate tube and further processed using the same protocol.

## **Immunocytochemistry**

The protocol used for assessing spermatogonial cell enrichment after sorting was kindly provided by the Oatley Lab at Washington State University, Pullman, USA (Yang et al., 2013). Briefly, 30,000-50,000 cells were adhered to poly-L-Lysine coated coverslips (Corning Life Sciences) in 24-well plates for 1 h. Cells were fixed in freshly prepared 4% PFA for 10 min at room temperature then washed in PBS with 0.1% Triton X-100 (PBS-T). Non-specific antibody binding was blocked by incubation with 10% normal goat serum for 1 h at room temperature. Cells were incubated overnight at 4oC with mouse anti-PLZF (0.2 μg/ml, Active Motif, clone 2A9) primary antibody. Alexa488 goat anti-mouse IgG (1 µg/mL, ThermoFisher Scientific) was used for secondary labelling at 4oC for 1 h. Coverslips were washed 3x and mounted onto glass slides with VectaShield mounting medium containing DAPI (Vector Laboratories) and examined by fluorescence microscopy. Stem cell enrichment was determined by counting PLZF+ cells in 10 random fields of view from each coverslip and dividing by the total number of cells present in the field of view (DAPI-stained nuclei).

## **RNA extraction and RNA-seq library preparation**

For RNA-seq on PND8 and PND15 spermatogonial cells, total RNA was extracted from sorted cells using the AllPrep RNA/DNA Micro kit (Qiagen). RNA quality was assessed using a Bioanalyzer 2100 (Agilent Technologies). Samples were quantified using Qubit RNA HS Assay (ThermoFisher Scientific). 10 ng of total RNA from each sample were used to prepare total long RNA sequencing libraries using the SMARTer® Stranded Total RNA-Seq Kit v2 - Pico Input Mammalian (Takara Bio USA, Inc.) at the Functional Genomics Center (FGC) Zurich, according to the manufacturer’s instructions. The number of biological replicates sequenced at each timepoint was 8 for PND8 and 9 for PND15 spermatogonia.

**Omni-ATAC library preparation and sequencing**

Chromatin accessibility was profiled from PND15 and adult spermatogonial cells. The libraries were prepared according to the Omni-ATAC protocol, starting from 25 000 PND15 and 5000 adult sorted spermatogonia, respectively (Corces et al., 2017). Briefly, sorted cells were lysed in cold lysis buffer (10 mM Tris-HCl pH 7.4, 10 mM NaCl, 3 mM MgCl2, 0.1% NP40, 0.1% Tween-20, and 0.01% digitonin) and the nuclei were pelleted and transposed using the Nextera Tn5 (Illumina) for 30 min at 37oC in a thermomixer with shaking at 1000 rpm. The transposed fragments were purified using the MinElute Reaction Cleanup Kit (Qiagen). Following purification, libraries were generated by PCR amplification using the NEBNext High-Fidelity 2X PCR Master Mix (New England Biolabs), and purified using Agencourt AMPure XP magnetic beads (Beckman Coulter) in order to remove primer dimers (78bp) and large fragments of 1000-10,000 bp in length. Library quality was assessed on an Agilent High Sensitivity DNA chip using the Bioanalyzer 2100 (Agilent Technologies). 6 samples were sequenced from PND15 and 5 from adult spermatogonial cells.

## **High-throughput sequencing data analysis**

*Data availability:*the datasets used in this study are available from the following GEO accessions: **GSE\_\_\_\_\_**, GSE49621, GSE49622, GSE62355, and GSE49623. An overview of the datasets included in the study is shown in the following table:

|  |  |  |
| --- | --- | --- |
| Source | \*Seq | Stages (n) |
| GSE\_\_\_\_\_ | RNA-seq | PND8 (8), PND15 (9) |
| GSM1525703 | RNA-seq | PND14 (1) |
| GSM1415671 | RNA-seq | PNW8 (1) |
| GSE\_\_\_\_\_ | ATAC-seq | PND15 (6), PNW20 (5) |
| GSM1202705 | ChIP-seq (H3K4me3) | PNW8 (1) |
| GSM1202708 | ChIP-seq (H3K27me3) | PNW8 (1) |
| GSM1202713 | ChIP-seq (H3K27ac) | PNW8 (1) |
| GSM1202723 | ChIP-seq (Input) | PNW8 (1) |
| GSE49623 | BS-seq | PND7 (1), PND14 (1), PNW8 (7) |

## **RNA sequencing (RNA-seq)**

*Quality control and alignment:*Single-end (SE) sequencing was performed on the PND8, and PND15 spermatogonia samples on the Illumina HiSeq4000 at the FGC Zurich. PND8 raw data (FASTQ files) was merged from two individual runs. For the analysis of literature RNA-seq data, FASTQ files of PND14 and PNW8 samples were obtained using fastq-dump (version 2.10.8). Quality assessment of the FASTQ files was performed using FastQC (Andrews et al., 2012) (version 0.11.8). TrimGalore (Krueger, 2015) (version 0.6.2) was used to trim adapters and low-quality ends from reads with Phred score less than 30 (-q 30), and for discarding trimmed reads shorter than 30 bp (--length 30). Trimmed reads were then pseudo-aligned using Salmon (Patro et al., 2017) (version 0.9.1) with automatic detection of the library type (-l A), correcting for sequence-specific bias (--seqBias) and correcting for fragment GC bias correction (--gcBias) on a transcript index prepared for the Mouse genome (GRCm38) from GENCODE (version M18) (Harrow et al., 2012), with additional piRNA precursors and transposable elements (concatenated by family) from Repeat Masker as in (Gapp et al., 2018).

*Downstream analysis:*the downstream analysis was performed using R (R Core Team, [2020](#ref-r_2020)) (version 3.6.2), using packages from The Comprehensive R Archive Network (CRAN) ([https://cran.r-project.org](https://cran.r-project.org/)) and Bioconductor (Huber et al., 2015). Pre-filtering of genes was performed using the filterByExpr function from edgeR (Robinson et al., 2009) (version 3.28.1) with a design matrix and requiring at least 15 counts (min.counts = 15). Normalization factors were obtained using TMM normalization (Robinson and Oshlack, 2010) from the edgeR package and differential gene expression (DGE) analysis was performed using the limma-voom (Law et al., 2014) pipeline from limma (Ritchie et al., 2015) (version 3.42.2). The Log2 fold change between PNW8 and PND14 spermatogonial samples was calculated by subtracting Log2 normalized expression values of PND14 from PNW8 samples. Gene ontology (GO) analysis was performed on expressed genes with fGSEA (version 1.15.2), using fGSEAMultilevel function on sets with 10 to 1000 annotated genes (minSize = 10, maxSize = 1000), and p-values boundary of 1E-100 (eps = 1e-100) (Korotkevich et al., 2016). For the PND15 vs PND8 comparison, genes were pre-ranked using their t-statistic; for the PNW8 vs PND14 comparison the Log2 fold change was used due to the lack of multiple replicates for each timepoint. REVIGO was used to summarize the GO terms obtained following fGSEA (Supek et al., 2011).

## **Assay for Transposase-Accessible Chromatin using sequening (Omni-ATAC)**

*Quality control, alignment, and peak calling:*Paired-end (PE) sequencing was performed on PND15 and adult spermatogonial cell samples on the Illumina HiSeq2500 platform at the FGC Zurich. FASTQ files were assessed for quality using FastQC (Andrews et al., 2012) (version 0.11.8). Quality control (QC) was performed using TrimGalore (Krueger, 2015) (version 0.6.2) in PE mode (--paired), trimming adapters, low-quality ends (-q 30) and discarding reads that become shorter than 30 bp after trimming (--length 30). Alignment on the GRCm38 genome was performed using Bowtie2 (Langmead and Salzberg, 2012) (version 2.3.5) with the following parameters: fragments up to 2 kb were allowed to align (-X 2000), entire read alignment (--end-to-end), suppressing unpaired alignments for paired reads (--no-mixed), suppressing discordant alignments for paired reads (--no-discordant) and minimum acceptable alignment score with respect to the read length (--score-min L,-0.4,-0.4). Using alignmentSieve (version 3.3.1) from deepTools (Ramirez et al., 2016) (version 3.4.3), aligned data (BAM files) were adjusted for the read start sites to represent the center of the transposon cutting event (--ATACshift), and filtered for reads with a high mapping quality (--minMappingQuality 30). Reads mapping to the mitochondrial chromosome and ENCODE blacklisted regions were filtered out. To call nucleosome-free regions, all aligned files were merged within groups (PND15 and adult), sorted, and indexed using SAMtools (Li et al., 2009) (version 0.1.19), and nucleosome-free fragments (NFFs) were obtained by selecting alignments with a template length between 40 and 140 inclusively. Peak calling (identifying areas in a genome that have been enriched for transcription factors) on the NFFs was performed using MACS2 (Zhang et al., 2008) (version 2.2.7.1) with mouse genome size (-g 2744254612) and PE BAM file format (-f BAMPE).

*Differential accessibility analysis:*The downstream analysis was performed in R (version 3.6.2), using packages from CRAN ([https://cran.r-project.org](https://cran.r-project.org/)) and Bioconductor (Huber et al., 2015). The peaks were annotated based on overlap with GENCODE (version M18) (Harrow et al., 2012) transcript, and/or the distance to the nearest transcription start site (available at the following link: https://github.com/mansuylab/SC\_postnatal\_adult/bin/annoPeaks.R). The number of extended reads overlapping in the peak regions was calculated using the csaw package (Lun and Smyth, 2015) (version 1.20.0). Peak regions which did not have at least 15 reads in at least 40% of the samples were filtered out. Normalization factors were obtained on the filtered peak regions using the TMM normalization method (Robinson and Oshlack, 2010) and differential analysis on the peaks (adults vs PND15) was performed using the Genewise Negative Binomial Generalized Linear Models with Quasi-likelihood (glmQLFit) Tests from the edgeR package (Robinson et al., 2009) (version 3.28.1). Peak regions which had an absolute Log2 fold change ≥ 1 and an FDR ≤ 0.05 were categorized as differentially accessible regions. GO analysis was performed on DARs with the rGREAT package (Zuguang, 2020) (version 1.18.0), which is a wrapper around the GREAT tool (McLean et al., 2010) (version 4.0). Transcription factor motif enrichment analysis was performed using the marge package (Amezquita, 2018) (version 0.0.4.9999), which is a wrapper around the Homer tool (Heinz et al., 2010) (version 4.11.1).

*Differential accessibility analysis at transposable elements:* TE gene transfer format (GTF) file was obtained from [http://labshare.cshl.edu/shares/mhammelllab/www-data/TEtranscripts/TE\_GTF/mm10\_rmsk\_TE.gtf.gz](http://labshare.cshl.edu/shares/mhammelllab/www-data/TEtranscripts/TE_GTF/mm10_rmsk_TE.gtf.gz%20on%2003.02.2020) on 03.02.2020. The GTF file provides hierarchical information about TEs: **Class** (level 1, eg. LTR), **Family** (level 2, eg. LTR  L1), **Subtype** (level 3, eg. LTR  L1  L1\_Rod), and **Locus** (level 4, eg. LTR  L1  L1\_Rod  L1\_Rod\_dup1). TE loci were annotated based on overlap with GENCODE (version M18) as described above for ATAC-seq peaks. Filtered BAM files (without reads mapping to blacklisted or mitochondrial regions) were used for analyzing TEs. Mapped reads were assigned to TEs using featureCounts from the R package Rsubread (Liao et al., 2019) (version 2.0.1) and were summarized to Subtypes (level 3), allowing for multi-overlap with fractional counts, while ignoring duplicates. The number of extended reads overlapping at the TE loci were obtained using the csaw package (Lun and Smyth, 2015) (version 1.20.0). Subtypes which did not have at least 15 reads, and loci which did not have at least 5 reads in at least 40% of the samples, were filtered out. Normalization and differential accessibility analysis were performed as described above. Subtypes which had an absolute Log2 fold change ≥ 0.5 and an FDR ≤ 0.05 were categorized as differentially accessible subtypes and the loci with an absolute Log2 fold change ≥ 1 and an FDR ≤ 0.05 were categorized as differentially accessible loci. For further downstream data analysis, only the differentially accessible loci of differentially accessible subtypes were considered. GO and motif enrichment analysis were performed as described above.

## **Chromatin Immunnoprecipitation sequencing (ChIP-seq)**

*Quality control, alignment, and peak calling:*ChIP-Seq SE data for PNDW8 (adults) were obtained from GEO accession GSE49621 (Hammoud et al., 2014). FASTQ files were obtained using fastq-dump (version 2.10.8), and different runs were merged. The FASTQ files were assessed for quality using FastQC (Andrews et al., 2012) (version 0.11.8). Quality control (QC) was performed using TrimGalore (Andrews et al., 2012) (version 0.6.0), trimming adapters, low-quality ends (-q 30) and discarding trimmed reads shorter than 30 bp (--length 30). Alignment to the GRCm38 genome was performed using Bowtie2 (Langmead and Salzberg, 2012) (version 2.3.5). Reads with more than 3 mismatches were removed from the aligned data, as suggested in (Royo et al., 2016), and reads with low mapping quality (--minMappingQuality 30) or mapping to the mitochondrial chromosome or aforementioned blacklisted regions were filtered out. Peak calling was performed using MACS2 (Zhang et al., 2008) (version 2.2.7.1) with mouse genome size (-g 2744254612) and SE BAM file format (-f BAM).

## **Bisulfite sequencing (BS)**

*Quality control and alignment:*BS paired-end data for PND7, PND14, and PNW8 (adults) were obtained from GEO accession GSE49623 (Hammoud et al., 2015). FASTQ files were obtained using fastq-dump (version 2.10.8), and different runs were merged. FASTQ files were assessed for quality using FastQC (Andrews et al., 2012) (version 0.11.8). QC was performed using TrimGalore (Krueger, 2015) (version 0.6.4\_dev) in PE mode (--paired), trimming adapters, low-quality ends (-q 30) and discarding trimmed reads shorter than 30 bp (--length 30). Alignment of the QC data was performed using Bismark (Krueger and Andrews, 2011) (version 0.22.3) on a GRCm38 index built using bismark\_genome\_preparation (version 0.17.0). Methylation information for individual cytosines was extracted using the bismark\_methylation\_extractor tool from the Bismark package (version 0.22.3).

**Figures**

All figures in this study were generated using ggplot2 (Wickham, 2016), EnrichedHeatmap (Gu et al., 2018), and ComplexHeatmap (Gu et al., 2016) packages, and using base plotting functions in R. Genomic tracks were generated in IGV and color coded in Inkscape.

**Authors contribution:** ILC and IMM designed and conceived the study. ILC performed all RNA-seq, ICC and ATAC-seq experiments. DKT analyzed the RNA-seq, ATAC-seq, ChIP-seq and BS data, with significant support from PLG. ILC and DKT prepared figures. ILC interpreted the data with significant input from DKT, PLG and IMM. ILC and DKT wrote the manuscript, with significant input from PLG and IMM. ﻿All authors read and accepted the final version of the manuscript.

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**Data and materials availability:** Repository accession numbers will be available at publication or by request to the corresponding author. The code employed for the data analysis is available from <https://github.com/mansuylab/SC_postnatal_adult>.

**References**

Amezquita, R.A. (2018). marge: An API for Analysis of Motifs Using HOMER in R.

Andrews, S., Krueger, F., Segonds-Pichon, A., Biggins, L., Krueger, C., and Wingett, S. (2012). FastQC. A quality control tool for high throughput sequence data.

Barrios, F., Filipponi, D., Pellegrini, M., Paronetto, M.P., Di Siena, S., Geremia, R., Rossi, P., De Felici, M., Jannini, E.A., and Dolci, S. (2010). Opposing effects of retinoic acid and FGF9 on Nanos2 expression and meiotic entry of mouse germ cells. J. Cell Sci. *123*, 871–880.

Bergmann, J.H., Li, J., Eckersley-Maslin, M.A., Rigo, F., Freier, S.M., and Spector, D.L. (2015). Regulation of the ESC transcriptome by nuclear long noncoding RNAs. Genome Res. *25*, 1336–1346.

Chan, F., Oatley, M.J., Kaucher, A. V, Yang, Q.-E., Bieberich, C.J., Shashikant, C.S., and Oatley, J.M. (2014). Functional and molecular features of the Id4+ germline stem cell population in mouse testes. Genes Dev. *28*, 1351–1362.

Chen, W., Zhang, Z., Chang, C., Yang, Z., Wang, P., Fu, H., Wei, X., Chen, E., Tan, S., Huang, W., et al. (2020). A bioenergetic shift is required for spermatogonial differentiation. Cell Discov. *6*, 1–17.

Cheng, K., Chen, I.-C., Eric Cheng, C.-H., Mutoji, K., Hale, B.J., Hermann, B.P., Geyer, C.B., Oatley, J.M., and McCarrey, J.R. (2020). Unique Epigenetic Programming Distinguishes Regenerative Spermatogonial Stem Cells in the Developing Mouse Testis. IScience *23*, 101596.

Corces, M.R., Trevino, A.E., Hamilton, E.G., Greenside, P.G., Sinnott-Armstrong, N.A., Vesuna, S., Satpathy, A.T., Rubin, A.J., Montine, K.S., Wu, B., et al. (2017). An improved ATAC-seq protocol reduces background and enables interrogation of frozen tissues. Nat. Methods *14*, 959–962.

Costoya, J.A., Hobbs, R.M., Barna, M., Cattoretti, G., Manova, K., Sukhwani, M., Orwig, K.E., Wolgemuth, D.J., and Pandolfi, P.P. (2004). Essential role of Plzf in maintenance of spermatogonial stem cells. Nat. Genet. *36*, 653–659.

Dann, C.T., Alvarado, A.L., Molyneux, L.A., Denard, B.S., Garbers, D.L., and Porteus, M.H. (2008). Spermatogonial Stem Cell Self-Renewal Requires OCT4, a Factor Downregulated During Retinoic Acid-Induced Differentiation. Stem Cells *26*, 2928–2937.

Davis, M.P., Carrieri, C., Saini, H.K., Dongen, S., Leonardi, T., Bussotti, G., Monahan, J.M., Auchynnikava, T., Bitetti, A., Rappsilber, J., et al. (2017). Transposon‐driven transcription is a conserved feature of vertebrate spermatogenesis and transcript evolution. EMBO Rep. *18*, 1231–1247.

DeFalco, T., Potter, S.J., Williams, A. V., Waller, B., Kan, M.J., and Capel, B. (2015). Macrophages Contribute to the Spermatogonial Niche in the Adult Testis. Cell Rep. *12*, 1107–1119.

Deniz, Ö., Frost, J.M., and Branco, M.R. (2019). Regulation of transposable elements by DNA modifications. Nat. Rev. Genet. *20*, 417–431.

Erkek, S., Hisano, M., Liang, C.Y., Gill, M., Murr, R., Dieker, J., Schübeler, D., Vlag, J. Van Der, Stadler, M.B., and Peters, A.H.F.M. (2013). Molecular determinants of nucleosome retention at CpG-rich sequences in mouse spermatozoa. Nat. Struct. Mol. Biol. *20*, 868–875.

Faisal, I., Cisneros-Montalvo, S., Hamer, G., Tuominen, M.M., Laurila, P.-P., Tumiati, M., Jauhiainen, M., Kotaja, N., Toppari, J., Mäkelä, J.-A., et al. (2019). Transcription Factor USF1 Is Required for Maintenance of Germline Stem Cells in Male Mice. Endocrinology *160*, 1119–1136.

Flickinger, C.J. (1967). The postnatal development of the Sertoli cells of the mouse. Zeitschrift Für Zellforsch. Und Mikroskopische Anat. *78*, 92–113.

Fort, A., Hashimoto, K., Yamada, D., Salimullah, M., Keya, C.A., Saxena, A., Bonetti, A., Voineagu, I., Bertin, N., Kratz, A., et al. (2014). Deep transcriptome profiling of mammalian stem cells supports a regulatory role for retrotransposons in pluripotency maintenance. Nat. Genet. *46*, 558–566.

Fukuda, N., and Touhara, K. (2005). Developmental expression patterns of testicular olfactory receptor genes during mouse spermatogenesis. Genes to Cells *11*, 71–81.

Fushan, A.A., Turanov, A.A., Lee, S.G., Kim, E.B., Lobanov, A. V., Yim, S.H., Buffenstein, R., Lee, S.R., Chang, K.T., Rhee, H., et al. (2015). Gene expression defines natural changes in mammalian lifespan. Aging Cell *14*, 352–365.

Gapp, K., van Steenwyk, G., Germain, P.L., Matsushima, W., Rudolph, K.L.M., Manuella, F., Roszkowski, M., Vernaz, G., Ghosh, T., Pelczar, P., et al. (2018). Alterations in sperm long RNA contribute to the epigenetic inheritance of the effects of postnatal trauma. Mol. Psychiatry *25*, 2162–2174.

Garbuzov, A., Pech, M.F., Hasegawa, K., Sukhwani, M., Zhang, R.J., Orwig, K.E., and Artandi, S.E. (2018). Purification of GFRα1+ and GFRα1– Spermatogonial Stem Cells Reveals a Niche-Dependent Mechanism for Fate Determination. Stem Cell Reports *10*, 553–567.

Goertz, M.J., Wu, Z., Gallardo, T.D., Hamra, F.K., and Castrillon, D.H. (2011). Foxo1 is required in mouse spermatogonial stem cells for their maintenance and the initiation of spermatogenesis. J. Clin. Invest. *121*, 3456–3466.

Green, C.D., Ma, Q., Manske, G.L., Shami, A.N., Zheng, X., Marini, S., Moritz, L., Sultan, C., Gurczynski, S.J., Moore, B.B., et al. (2018). A Comprehensive Roadmap of Murine Spermatogenesis Defined by Single-Cell RNA-Seq. Dev. Cell *46*, 651-667.e10.

Grive, K.J., Hu, Y., Shu, E., Grimson, A., Elemento, O., Grenier, J.K., and Cohen, P.E. (2019). Dynamic transcriptome profiles within spermatogonial and spermatocyte populations during postnatal testis maturation revealed by single-cell sequencing. PLoS Genet. *15*, e1007810.

Gu, Z., Eils, R., and Schlesner, M. (2016). Complex heatmaps reveal patterns and correlations in multidimensional genomic data. Bioinformatics *32*, 2847–2849.

Gu, Z., Eils, R., Schlesner, M., and Ishaque, N. (2018). EnrichedHeatmap: An R/Bioconductor package for comprehensive visualization of genomic signal associations. BMC Genomics *19*, 234.

Guo, J., Grow, E.J., Yi, C., Goriely, A., Hotaling, J.M., and Cairns Correspondence, B.R. (2017). Chromatin and Single-Cell RNA-Seq Profiling Reveal Dynamic Signaling and Metabolic Transitions during Human Spermatogonial Stem Cell Development. *21*, 533–546.

Hammoud, S.S., Low, D.H.P., Yi, C., Carrell, D.T., Guccione, E., and Cairns, B.R. (2014). Chromatin and Transcription Transitions of Mammalian Adult Germline Stem Cells and Spermatogenesis. Cell Stem Cell *15*, 239–253.

Hammoud, S.S., Low, D.H.P., Yi, C., Lee, C.L., Oatley, J.M., Payne, C.J., Carrell, D.T., Guccione, E., and Cairns, B.R. (2015). Transcription and imprinting dynamics in developing postnatal male germline stem cells. Genes Dev. *29*, 2312–2324.

Harrow, J., Frankish, A., Gonzalez, J.M., Tapanari, E., Diekhans, M., Kokocinski, F., Aken, B.L., Barrell, D., Zadissa, A., Searle, S., et al. (2012). GENCODE: The reference human genome annotation for the ENCODE project. Genome Res. *22*, 1760–1774.

Hasegawa, K., and Saga, Y. (2014). FGF8-FGFR1 signaling acts as a niche factor for maintaining undifferentiated spermatogonia in the mouse. Biol. Reprod. *91*, 145–146.

He, Z., Jiang, J., Kokkinaki, M., Golestaneh, N., Hofmann, M.-C., and Dym, M. (2008). Gdnf Upregulates c-Fos Transcription via the Ras/Erk1/2 Pathway to Promote Mouse Spermatogonial Stem Cell Proliferation. Stem Cells *26*, 266–278.

Heinz, S., Benner, C., Spann, N., Bertolino, E., Lin, Y.C., Laslo, P., Cheng, J.X., Murre, C., Singh, H., and Glass, C.K. (2010). Simple Combinations of Lineage-Determining Transcription Factors Prime cis-Regulatory Elements Required for Macrophage and B Cell Identities. Mol. Cell *38*, 576–589.

Hermann, B.P., Cheng, K., Singh, A., Roa-De La Cruz, L., Mutoji, K.N., Chen, I.-C., Gildersleeve, H., Lehle, J.D., Mayo, M., Westernströer, B., et al. (2018). The Mammalian Spermatogenesis Single-Cell Transcriptome, from Spermatogonial Stem Cells to Spermatids. Cell Rep. *25*, 1650-1667.e8.

Huber, W., Carey, V.J., Gentleman, R., Anders, S., Carlson, M., Carvalho, B.S., Bravo, H.C., Davis, S., Gatto, L., Girke, T., et al. (2015). Orchestrating high-throughput genomic analysis with Bioconductor. Nat. Methods *12*, 115–121.

Jung, Y.H., Sauria, M.E.G., Lyu, X., Cheema, M.S., Ausio, J., Taylor, J., and Corces, V.G. (2017). Chromatin States in Mouse Sperm Correlate with Embryonic and Adult Regulatory Landscapes. Cell Rep. *18*, 1366–1382.

Kanatsu-Shinohara, M., Tanaka, T., Ogonuki, N., Ogura, A., Morimoto, H., Cheng, P.F., Eisenman, R.N., Trumpp, A., and Shinohara, T. (2016). Myc/Mycn-mediated glycolysis enhances mouse spermatogonial stem cell self-renewal. Genes Dev. *30*, 2637–2648.

Korotkevich, G., Sukhov, V., and Sergushichev, A. (2016). Fast gene set enrichment analysis. BioRxiv 060012.

Krueger, F. (2015). Trim Galore. A wrapper tool around Cutadapt and FastQC to consistently apply quality and adapter trimming to FastQ files, www.bioinformatics.babraham.ac.uk/projects/trim\_galore/.

Krueger, F., and Andrews, S.R. (2011). Bismark: a flexible aligner and methylation caller for Bisulfite-Seq applications. Bioinformatics *27*, 1571–1572.

Kubota, H., and Brinster, R.L. (2018). Spermatogonial stem cells. Biol. Reprod. *99*, 52–74.

Kubota, H., Avarbock, M.R., and Brinster, R.L. (2004a). Culture Conditions and Single Growth Factors Affect Fate Determination of Mouse Spermatogonial Stem Cells1. Biol. Reprod. *71*, 722–731.

Kubota, H., Avarbock, M.R., and Brinster, R.L. (2004b). Growth factors essential for self-renewal and expansion of mouse spermatogonial stem cells. Proc. Natl. Acad. Sci. U. S. A. *101*, 16489–16494.

Langmead, B., and Salzberg, S.L. (2012). Fast gapped-read alignment with Bowtie 2. Nat. Methods *9*, 357–359.

Law, C.W., Chen, Y., Shi, W., and Smyth, G.K. (2014). voom: precision weights unlock linear model analysis tools for RNA-seq read counts. Genome Biol. *15*, R29.

Law, N.C., Oatley, M.J., and Oatley, J.M. (2019). Developmental kinetics and transcriptome dynamics of stem cell specification in the spermatogenic lineage. Nat. Commun. *10*, 2787.

Li, H., Handsaker, B., Wysoker, A., Fennell, T., Ruan, J., Homer, N., Marth, G., Abecasis, G., and Durbin, R. (2009). The Sequence Alignment/Map format and SAMtools. Bioinformatics *25*, 2078–2079.

Liao, Y., Smyth, G.K., and Shi, W. (2019). The R package Rsubread is easier, faster, cheaper and better for alignment and quantification of RNA sequencing reads. Nucleic Acids Res. *47*, e47–e47.

Lord, T., Oatley, M.J., and Oatley, J.M. (2018). Testicular Architecture Is Critical for Mediation of Retinoic Acid Responsiveness by Undifferentiated Spermatogonial Subtypes in the Mouse. Stem Cell Reports *10*, 538–552.

Lu, J.Y., Shao, W., Chang, L., Yin, Y., Li, T., Zhang, H., Hong, Y., Percharde, M., Guo, L., Wu, Z., et al. (2020). Genomic Repeats Categorize Genes with Distinct Functions for Orchestrated Regulation. Cell Rep. *30*, 3296-3311.e5.

Lun, A.T.L., and Smyth, G.K. (2015). csaw: a Bioconductor package for differential binding analysis of ChIP-seq data using sliding windows. Nucleic Acids Res. *44*, e45.

Martínez-Zamudio, R.I., Roux, P.F., de Freitas, J.A.N.L.F., Robinson, L., Doré, G., Sun, B., Belenki, D., Milanovic, M., Herbig, U., Schmitt, C.A., et al. (2020). AP-1 imprints a reversible transcriptional programme of senescent cells. Nat. Cell Biol. *22*, 842–855.

McLean, C.Y., Bristor, D., Hiller, M., Clarke, S.L., Schaar, B.T., Lowe, C.B., Wenger, A.M., and Bejerano, G. (2010). GREAT improves functional interpretation of cis-regulatory regions. Nat. Biotechnol. *28*, 495–501.

Myers, R.M., Stamatoyannopoulos, J., Snyder, M., Dunham, I., Hardison, R.C., Bernstein, B.E., Gingeras, T.R., Kent, W.J., Birney, E., Wold, B., et al. (2011). A user’s guide to the Encyclopedia of DNA elements (ENCODE). PLoS Biol. *9*, e1001046.

Niu, Z., Goodyear, S.M., Rao, S., Wu, X., Tobias, J.W., Avarbock, M.R., and Brinster, R.L. (2011). MicroRNA-21 regulates the self-renewal of mouse spermatogonial stem cells. Proc. Natl. Acad. Sci. U. S. A. *108*, 12740–12745.

Oatley, J.M., and Griswold, M.D. (2017). The biology of mammalian spermatogonia.

Oldfield, A.J., Yang, P., Conway, A.E., Cinghu, S., Freudenberg, J.M., Yellaboina, S., and Jothi, R. (2014). Histone-Fold Domain Protein NF-Y Promotes Chromatin Accessibility for Cell Type-Specific Master Transcription Factors. Mol. Cell *55*, 708–722.

Patro, R., Duggal, G., Love, M.I., Irizarry, R.A., and Kingsford, C. (2017). Salmon provides fast and bias-aware quantification of transcript expression. Nat. Methods *14*, 417–419.

Ramirez, F., Ryan, D.P., Grüning, B., Bhardwaj, V., Kilpert, F., Richter, A.S., Heyne, S., Dündar, F., and Manke, T. (2016). deepTools2: a next generation web server for deep-sequencing data analysis. Nucleic Acids Res. *44*, W160--W165.

Ritchie, M.E., Phipson, B., Wu, D., Hu, Y., Law, C.W., Shi, W., and Smyth, G.K. (2015). limma powers differential expression analyses for RNA-sequencing and microarray studies. Nucleic Acids Res. *43*, e47--e47.

Robinson, M.D., and Oshlack, A. (2010). A scaling normalization method for differential expression analysis of RNA-seq data. Genome Biol. *11*, R25.

Robinson, M.D., McCarthy, D.J., and Smyth, G.K. (2009). edgeR: a Bioconductor package for differential expression analysis of digital gene expression data. Bioinformatics *26*, 139–140.

De Rooij, D.G. (2017). The nature and dynamics of spermatogonial stem cells. Dev. *144*, 3022–3030.

Royo, H., Stadler, M., and Peters, A. (2016). Alternative Computational Analysis Shows No Evidence for Nucleosome Enrichment at Repetitive Sequences in Mammalian Spermatozoa. Dev. Cell *37*, 98–104.

Sada, A., Suzuki, A., Suzuki, H., and Saga, Y. (2009). The RNA-binding protein NANOS2 is required to maintain murine spermatogonia! Stem Cells. Science (80-. ). *325*, 1394–1398.

Sakashita, A., Maezawa, S., Alavattam, K., Yukawa, M., Barski, A., Pavlicev, M., and Namekawa, S. (2020). Endogenous retroviruses drive species-specific germline transcriptomes in mammals. BioRxiv 2020.03.11.987230.

Shaulian, E., and Karin, M. (2002). AP-1 as a regulator of cell life and death. Nat. Cell Biol. *4*, E131–E136.

Shavlakadze, T., Morris, M., Fang, J., Wang, S.X., Zhu, J., Zhou, W., Tse, H.W., Mondragon-Gonzalez, R., Roma, G., and Glass, D.J. (2019). Age-Related Gene Expression Signature in Rats Demonstrate Early, Late, and Linear Transcriptional Changes from Multiple Tissues. Cell Rep. *28*, 3263-3273.e3.

Shinohara, T., Orwig, K.E., Avarbock, M.R., and Brinster, R.L. (2001). Remodeling of the postnatal mouse testis is accompanied by dramatic changes in stem cell number and niche accessibility. Proc. Natl. Acad. Sci. U. S. A. *98*, 6186–6191.

Starks, R.R., Biswas, A., Jain, A., and Tuteja, G. (2019). Combined analysis of dissimilar promoter accessibility and gene expression profiles identifies tissue-specific genes and actively repressed networks. Epigenetics and Chromatin *12*, 16.

Subramanian, A., Tamayo, P., Mootha, V.K., Mukherjee, S., Ebert, B.L., Gillette, M.A., Paulovich, A., Pomeroy, S.L., Golub, T.R., Lander, E.S., et al. (2005). Gene set enrichment analysis: A knowledge-based approach for interpreting genome-wide expression profiles. Proc. Natl. Acad. Sci. U. S. A. *102*, 15545–15550.

Sun, Z., Zhu, M., Lv, P., Cheng, L., Wang, Q., Tian, P., Yan, Z., and Wen, B. (2018). The Long Noncoding RNA Lncenc1 Maintains Naive States of Mouse ESCs by Promoting the Glycolysis Pathway. Stem Cell Reports *11*, 741–755.

Sundaram, V., and Wysocka, J. (2020). Transposable elements as a potent source of diverse cis-regulatory sequences in mammalian genomes. Philos. Trans. R. Soc. B Biol. Sci. *375*.

Sundaram, V., Cheng, Y., Ma, Z., Li, D., Xing, X., Edge, P., Snyder, M.P., and Wang, T. (2014). Widespread contribution of transposable elements to the innovation of gene regulatory networks. Genome Res. *24*, 1963–1976.

Supek, F., Bošnjak, M., Škunca, N., and Šmuc, T. (2011). REVIGO Summarizes and Visualizes Long Lists of Gene Ontology Terms. PLoS One *6*, e21800.

Thompson, P.J., Macfarlan, T.S., and Lorincz, M.C. (2016). Long Terminal Repeats: From Parasitic Elements to Building Blocks of the Transcriptional Regulatory Repertoire. Mol. Cell *62*, 766–776.

Thurman, R.E., Rynes, E., Humbert, R., Vierstra, J., Maurano, M.T., Haugen, E., Sheffield, N.C., Stergachis, A.B., Wang, H., Vernot, B., et al. (2012). The accessible chromatin landscape of the human genome. Nature *489*, 75–82.

Vanderhaeghen, P., Schurmans, S., Vassart, G., and Parmentier, M. (1997). Specific repertoire of olfactory receptor genes in the male germ cells of several mammalian species. Genomics *39*, 239–246.

Wang, M., Liu, X., Chang, G., Chen, Y., An, G., Yan, L., Gao, S., Xu, Y., Cui, Y., Dong, J., et al. (2018). Single-Cell RNA Sequencing Analysis Reveals Sequential Cell Fate Transition during Human Spermatogenesis. Cell Stem Cell *23*, 599-614.e4.

Wickham, H. (2016). ggplot2: Elegant Graphics for Data Analysis (Springer-Verlag New York).

Wu, F., Liu, Y., Wu, Q., Li, D., Zhang, L., Wu, X., Wang, R., Zhang, D., Gao, S., and Li, W. (2018). Long non-coding RNAs potentially function synergistically in the cellular reprogramming of SCNT embryos. BMC Genomics *19*, 631.

Wu, X., Oatley, J.M., Oatley, M.J., Kaucher, A. V., Avarbock, M.R., and Brinster, R.L. (2010). The POU Domain Transcription Factor POU3F1 Is an Important Intrinsic Regulator of GDNF-Induced Survival and Self-Renewal of Mouse Spermatogonial Stem Cells1. Biol. Reprod. *82*, 1103–1111.

Yang, Q.-E., Racicot, K.E., Kaucher, A. V, Oatley, M.J., and Oatley, J.M. (2013). MicroRNAs 221 and 222 regulate the undifferentiated state in mammalian male germ cells. Development *140*, 280–290.

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

Zuguang, G. (2020). No Title. Https://Github.Com/Jokergoo/RGREAT.