Epigenetic inactivation of Differentially Accessible Regions (DARs)

To select candidate DARs for functional validation I focused just on Category 1 type of DARs (Supplementary Table 3). These genomic regions show increased accessibility in the adult spermatogonia stage as compared with pups (PND15). Importantly, their linked genes also show an increase in transcription as evaluated by RNA-seq, suggesting that the increase in accessibility is linked to transcriptional activation. Among all regions, I decided to focus first on those ones showing the highest log fold change in accessibility. Then I took into consideration whether such DARs where located at the TSS or promoter. Based on such criteria I propose to inactive the DARs associated with *Gata2*, *Mroh2a* and *Chd2*.

Gata2

Gata2 is a transcription factor with critical functions in hematopoietic stem cell maintenance. Gata2 is also highly expressed in mouse spermatogonia stem cells suggesting a possible role of this transcription factor in spermatogonia stem cell maintenance. Interestingly, multiple genomic regions surrounding *Gata2* show an increase in chromatin accessibility in the adult spermatogonia stage (Figure 1). In total, nine genomic regions with differential accessibility are associated by linear proximity to *Gata2*. One of such regions (Figure 1; highlighted in red and red rectangle) overlap with the putative promoter of *Gata2*. Such region is also accessible in PND15 spermatogonia. Interestingly, most of the regions overlap with regions enriched for H3K4me3 in testis and with regions annotated as enhancers in different cell types in mouse strongly suggesting that such accessible sites are regulatory elements. I propose to inactivate five of DARs associated with Gata2 (Figure 1; highlighted). Such regions overlap with annotated enhancers, show genomic conservation with other species and 4/5 show log fold enrichment in adult spermatogonia of more than 2. A specific sgRNA will be selected for each region using CRISPOR. The 20-nucleotide sequence for each sgRNA will be cloned separately in the dCas9-KRAB-puro plasmid

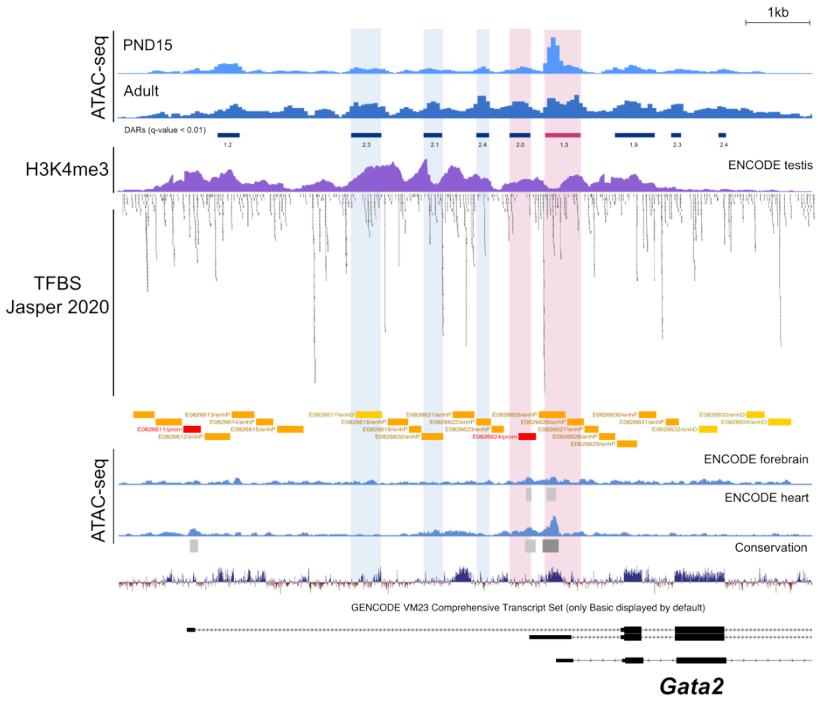

Figure 1. DARs associated to Gata2.

and transfected to GC-1 cells. After seven days of puromycin selection, cells will be harvest for RNA extraction. RT-qPCR experiments will be applied to characterize the affect in transcription and ATAC-qPCR experiments will be applied to evaluate changes in chromatin accessibility.

Mroh2a and *Chd2*

To make our functional validation more robust I also propose to inactive the DARs associated with *Mroh2a* and *Chd2*. The DAR associated with *Mroh2* is the one with the highest increase in accessibility in Category 1 class of DARs. While the function of the protein coded for such gene is still uncharacterized its paralog (Mroh2b) has important functions in spermatogenesis. The DAR associated with Mroh2 is located at the TSS of the gene (see attached figure). The DAR associated with Chd2 is also among the top 10 DARs with gain in accessibility in adult spermatogonia. Chd2 codes for a chromodomain-helicase-DNA binding protein. This protein is a chromatin remodeler with important functions in the control of gene expression. Furthermore, de-novo mutations in this gene have been associated with neurodevelopmental diseases.