

1      The X-linked intellectual disability gene KDM5C is a  
2      sex-biased brake against germline programs in somatic  
3      lineages

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## 10 Abstract

11 Mutations in numerous chromatin-modifying enzymes cause neurodevelopmental disorders (NDDs) with  
12 unknown mechanisms. Loss of repressive chromatin regulators can lead to the aberrant transcription of  
13 tissue-specific genes outside of their intended context, however the mechanisms and consequences of their  
14 dysregulation are largely unknown. Here, we examine how the X-linked intellectual disability gene lysine  
15 demethylase 5c (KDM5C), an eraser of histone 3 lysine 4 di and tri-methylation (H3K4me2/3), contributes  
16 to tissue identity. We found male *Kdm5c* knockout (-KO) mice, which recapitulate key human neurological  
17 phenotypes, aberrantly express many liver, muscle, ovary, and testis genes within the amygdala and  
18 hippocampus. Gonad-enriched genes misexpressed in the *Kdm5c*-KO brain are unique to germ cells,  
19 indicating an erosion of the soma-germline boundary. Germline genes are typically decommissioned in  
20 somatic lineages in the post-implantation epiblast, yet *Kdm5c*-KO epiblast-like cells (EpiLCs) aberrantly  
21 expressed key regulators of germline identity and meiosis, including *Dazl* and *Stra8*. Characterizing germline  
22 gene misexpression in males and female mutants revealed germline gene repression is sexually dimorphic,  
23 with female EpiLCs requiring a higher dose of KDM5C to maintain germline gene suppression. Using a  
24 comprehensive list of mouse germline-enriched genes, we found KDM5C is selectively recruited to a subset  
25 of germline gene promoters that contain CpG islands (CGIs) to facilitate DNA CpG methylation (CpGme)  
26 during ESC to EpiLC differentiation. However, late-stage spermatogenesis genes devoid of promoter CGIs  
27 can become expressed in *Kdm5c*-KO cells via ectopic activation by RFX transcription factors. Together,  
28 these data demonstrate KDM5C's fundamental role in tissue identity and indicate that KDM5C acts as a  
29 brake against runaway activation of germline developmental programs in somatic lineages.

## 30 Introduction

31 A single genome holds the instructions to generate the myriad of cell types found within an organism.  
32 This is, in part, accomplished by chromatin regulators that can either promote or impede lineage-specific  
33 gene expression through DNA and histone modifications<sup>1–5</sup>. Human genetic studies revealed mutations in  
34 chromatin regulators are a major cause of neurodevelopmental disorders (NDDs)<sup>6</sup> and many studies have  
35 identified their importance for regulating brain-specific transcriptional programs. Loss of chromatin regulators  
36 can also result in the ectopic expression of tissue-specific genes outside of their target environment, such  
37 as the misexpression of liver-specific genes within adult neurons<sup>7</sup>. However, the mechanisms underlying  
38 ectopic gene expression and its impact upon neurodevelopment are poorly understood.

39 To elucidate the role of tissue identity in chromatin-linked NDDs, it is essential to first characterize the  
40 nature of the dysregulated genes and the molecular mechanisms governing their de-repression. Here we  
41 focus on the X chromosome gene lysine demethylase 5C (KDM5C, also known as SMCX or JARID1C),  
42 which erases histone 3 lysine 4 di- and trimethylation (H3K4me2/3), a permissive chromatin modification  
43 enriched at gene promoters<sup>8</sup>. Pathogenic mutations in *KDM5C* cause Intellectual Developmental Disorder,  
44 X-linked, Syndromic, Claes-Jensen Type (MRXSCJ, OMIM: 300534). MRXSCJ is more common and severe  
45 in males and its neurological phenotypes include intellectual disability, seizures, aberrant aggression, and  
46 autistic behaviors<sup>9–11</sup>. Male *Kdm5c* knockout (-KO) mice recapitulate key MRXSCJ phenotypes, including  
47 hyperaggression, increased seizure propensity, social deficits, and learning impairments<sup>12–14</sup>. RNA sequenc-  
48 ing (RNA-seq) of the *Kdm5c*-KO hippocampus revealed ectopic expression of some germline genes within  
49 the brain<sup>13</sup>. However, it is unclear if other tissue-specific genes are aberrantly transcribed with KDM5C loss,  
50 at what point in development germline gene misexpression begins, and what mechanisms underlie their  
51 dysregulation.

52 Distinguishing between germ cells and somatic cells is a key feature of multicellularity<sup>15</sup> that occurs  
53 during early embryogenesis in many metazoans<sup>16</sup>. In mammals, chromatin regulators are crucial for  
54 decommissioning germline genes during the transition from naïve to primed pluripotency. Initially, germline  
55 gene promoters gain repressive histone H2A lysine 119 monoubiquitination (H2AK119ub1)<sup>17</sup> and histone  
56 H3 lysine 9 trimethylation (H3K9me3)<sup>17,18</sup> in embryonic stem cells (ESCs) and are then decorated with  
57 DNA CpG methylation (CpGme) in post-implantation epiblast cells<sup>18–21</sup>. The contribution of KDM5C to this  
58 process remains unclear. Additionally, studies on germline gene repression have primarily been conducted  
59 in males and focused on select marker genes important for early germ cell development, given the lack of a  
60 comprehensive list for germline-enriched genes. Therefore, it is unknown if the mechanism of repression  
61 differs between sexes or for different classes of germline genes, e.g. meiotic versus spermatid differentiation  
62 genes.

63 To illuminate KDM5C's role in tissue identity, here we characterized the aberrant expression of tissue-  
64 enriched genes within the *Kdm5c*-KO brain and epiblast-like stem cells (EpiLCs), an *in vitro* model of the

65 post-implantation embryo. We curated a list of mouse germline-enriched genes, which enabled genome-wide  
66 analysis of germline gene silencing mechanisms for the first time. Additionally, we characterized germline  
67 transcripts expressed in male and female *Kdm5c* mutants to illuminate the impact of sex upon germline  
68 gene suppression. Based on the data presented below, we propose KDM5C plays a fundamental, sexually  
69 dimorphic role in the development of tissue identity during early embryogenesis, including the establishment  
70 of the soma-germline boundary.

## 71 Results

### 72 Tissue-enriched genes are aberrantly expressed in the *Kdm5c*-KO brain

73 Previous RNA sequencing (RNA-seq) of the adult male *Kdm5c*-KO hippocampus revealed ectopic  
74 expression of some germline genes unique to the testis<sup>13</sup>. It is currently unknown if the testis is the only  
75 tissue type misexpressed in the *Kdm5c*-KO brain. We thus systematically tested whether other tissue-specific  
76 genes are misexpressed in the male brain with constitutive knockout of *Kdm5c* (*Kdm5c*<sup>-y</sup>, 5CKO)<sup>22</sup> by using  
77 a published list of mouse tissue-enriched genes<sup>23</sup>.

78 We found a large proportion of significantly upregulated genes (DESeq2<sup>24</sup>, log2 fold change > 0.5, q <  
79 0.1) within the male *Kdm5c*-KO amygdala and hippocampus are non-brain, tissue-specific genes (Amygdala:  
80 21/59 up DEGs, 35.59% ; Hippocampus: 48/183 up DEGs, 26.23%) (Figure 1A-B, Supplementary Table  
81 1). For both the amygdala and hippocampus, the majority of tissue-enriched differentially expressed genes  
82 (DEGs) were testis genes (Figure 1A-B). Even though the testis has the largest total number of tissue-  
83 enriched genes (2,496 genes) compared to any other tissue, testis-enriched DEGs were significantly enriched  
84 in both brain regions (Amygdala p = 1.83e-05, Odds Ratio = 5.13; Hippocampus p = 4.26e-11, Odds Ratio =  
85 4.45, Fisher's Exact Test). An example of a testis-enriched gene misexpressed in the *Kdm5c*-KO brain is  
86 *FK506 binding protein 6 (Fkbp6)*, a known regulator of PIWI-interacting RNAs (piRNAs) and meiosis<sup>25,26</sup>  
87 (Figure 1C).

88 Interestingly, we also observed significant enrichment of ovary-enriched genes in both the amygdala  
89 and hippocampus (Amygdala p = 0.00574, Odds Ratio = 18.7; Hippocampus p = 0.048, Odds Ratio = 5.88,  
90 Fisher's Exact Test) (Figure 1A-B). Ovary-enriched DEGs included *Zygotic arrest 1 (Zar1)*, which sequesters  
91 mRNAs in oocytes for meiotic maturation<sup>27</sup> (Figure 1D). Given that the *Kdm5c*-KO mice we analyzed are  
92 male, these data demonstrate that the ectopic expression of gonad-enriched genes is independent of  
93 organismal sex.

94 Although not consistent across brain regions, we also found significant enrichment of genes biased  
95 towards two non-gonadal tissues - the liver (Amygdala p = 0.04, Odds Ratio = 6.58, Fisher's Exact Test) and  
96 muscle (Hippocampus p = 0.01, Odds Ratio = 6.95, Fisher's Exact Test) (Figure 1A-B). *Apolipoprotein C-I*  
97 (*Apoc1*), a lipoprotein metabolism and transport gene, is among the liver-enriched DEG derepressed in both

98 the hippocampus and amygdala<sup>28</sup> and its brain overexpression has been implicated in Alzheimer's disease<sup>29</sup>  
99 (Figure 1E).

100 Our analysis of oligo(dT)-primed libraries<sup>22</sup> indicates aberrantly expressed mRNAs are polyadenylated  
101 and spliced into mature transcripts in the *Kdm5c*-KO brain (Figure 1C-E). Of note, we observed little to no  
102 dysregulation of brain-enriched genes (Amygdala p = 1, Odds Ratio = 1.22; Hippocampus p = 0.74, Odds  
103 Ratio = 1.22, Fisher's Exact Test), despite the fact these are brain samples and the brain has the second  
104 highest total number of tissue-enriched genes (708 genes). Altogether, these results suggest the aberrant  
105 expression of tissue-enriched genes within the brain is a major effect of KDM5C loss.

106 **Germline genes are misexpressed in the *Kdm5c*-KO brain**

107 *Kdm5c*-KO brain expresses testicular germline genes<sup>13</sup>, however the testis also contains somatic cells that  
108 support hormone production and germline functions. To determine if *Kdm5c*-KO results in ectopic expression  
109 of somatic testicular genes, we first evaluated the known functions of testicular DEGs through gene ontology.  
110 We found *Kdm5c*-KO testis-enriched DEGs had high enrichment of germline-relevant ontologies, including  
111 spermatid development (GO: 0007286, p.adjust = 6.2e-12) and sperm axoneme assembly (GO: 0007288,  
112 p.adjust = 2.45e-14) (Figure 2A, Supplementary Table 1).

113 We then evaluated *Kdm5c*-KO testicular DEG expression in wild-type testes versus testes with germ cell  
114 depletion<sup>30</sup>, which was accomplished by heterozygous *W* and *Wv* mutations in the enzymatic domain of *c-Kit*  
115 (*Kit*<sup>W/Wv</sup>)<sup>31</sup>. Almost all *Kdm5c*-KO testis-enriched DEGs lost expression with germ cell depletion (Figure 2B).  
116 We then assessed testis-enriched DEG expression in a published single cell RNA-seq dataset that identified  
117 cell type-specific markers within the testis<sup>32</sup>. Some *Kdm5c*-KO testis-enriched DEGs were classified as  
118 specific markers for different germ cell developmental stages (e.g. spermatogonia, spermatocytes, round  
119 spermatids, and elongating spermatids), yet none marked somatic cells (Figure 2C). Together, these data  
120 demonstrate that the *Kdm5c*-KO brain aberrantly expresses germline genes but not somatic testicular genes,  
121 reflecting an erosion of the soma-germline boundary.

122 As of yet, research on germline gene silencing mechanisms has focused on a handful of key genes rather  
123 than assessing germline gene suppression genome-wide, due to the lack of a comprehensive gene list.  
124 We therefore generated a list of mouse germline-enriched genes using RNA-seq datasets of *Kit*<sup>W/Wv</sup> mice  
125 that included males and females at embryonic day 12, 14, and 16<sup>33</sup> and adult male testes<sup>30</sup>. We defined  
126 genes as germline-enriched if their expression met the following criteria: 1) their expression is greater than  
127 1 FPKM in wild-type gonads 2) their expression in any non-gonadal tissue of adult wild type mice<sup>23</sup> does  
128 not exceed 20% of their maximum expression in the wild-type germline, and 3) their expression in the germ  
129 cell-depleted gonads, for any sex or time point, does not exceed 20% of their maximum expression in the  
130 wild-type germline. These criteria yielded 1,288 germline-enriched genes (Figure 2D), which was hereafter  
131 used as a resource to globally characterize germline gene misexpression with *Kdm5c* loss (Supplementary  
132 Table 2).

133 ***Kdm5c*-KO epiblast-like cells aberrantly express key regulators of germline identity**

134 Germ cells are typically distinguished from somatic cells soon after the embryo implants into the uterine  
135 wall<sup>34,35</sup>, when germline genes are silenced in epiblast stem cells that will form the somatic tissues<sup>36</sup>. This  
136 developmental time point can be modeled *in vitro* through differentiation of naïve embryonic stem cells  
137 (nESCs) into epiblast-like stem cells (EpiLCs) (Figure 3A)<sup>37,38</sup>. While some germline-enriched genes are  
138 also expressed in nESCs and in the 2-cell stage<sup>39–41</sup>, they are silenced as they differentiate into EpiLCs<sup>18,19</sup>.  
139 Therefore, we tested if KDM5C was necessary for the initial silencing of germline genes in somatic lineages  
140 by evaluating the impact of *Kdm5c* loss in male EpiLCs.

141 *Kdm5c*-KO cell morphology during ESC to EpiLC differentiation appeared normal (Figure 3B) and EpiLCs  
142 properly expressed markers of primed pluripotency, such as *Dnmt3b*, *Fgf5*, *Pou3f1*, and *Otx2* (Figure 3C). We  
143 then identified tissue-enriched DEGs in a RNA-seq dataset of wild-type and *Kdm5c*-KO EpiLCs<sup>42</sup> (DESeq2,  
144 log<sub>2</sub> fold change > 0.5, q < 0.1, Supplementary Table 3). Similar to the *Kdm5c*-KO brain, we observed  
145 general dysregulation of tissue-enriched genes, with the largest number of genes belonging to the brain and  
146 testis, although they were not significantly enriched (Figure 3D). Using our list of mouse germline-enriched  
147 genes assembled above, we identified 68 germline genes misexpressed in male *Kdm5c*-KO EpiLCs.

148 We then compared EpiLC germline DEGs to those expressed in the *Kdm5c*-KO brain to determine if  
149 germline genes are constitutively dysregulated or change over the course of development. The majority of  
150 germline DEGs were unique to either EpiLCs or the brain, with only *D1Pas1* and *Cyct* shared across all  
151 tissue/cell types (Figure 3E-F). EpiLC germline DEGs had particularly high enrichment of meiosis-related  
152 gene ontologies when compared to the brain (Figure 3G, Supplementary Table 3), such as meiotic cell  
153 cycle process (GO:1903046, p.adjust = 2.2e-07) and meiotic nuclear division (GO:0140013, p.adjust  
154 = 1.37e-07). While there was modest enrichment of meiotic gene ontologies in both brain regions, the  
155 *Kdm5c*-KO hippocampus primarily expressed late-stage spermatogenesis genes involved in sperm axoneme  
156 assembly (GO:0007288, p.adjust = 0.00621) and sperm motility (GO:0097722, p.adjust = 0.00612).

157 Notably, DEGs unique to *Kdm5c*-KO EpiLCs included key drivers of germline identity, such as *Stimulated*  
158 *by retinoic acid 8* (*Stra8*: log<sub>2</sub> fold change = 3.73, q = 2.17e-39) and *Deleted in azoospermia like* (*Dazl*:  
159 log<sub>2</sub> fold change = 3.36, q = 3.19e-12) (Figure 3H). These genes are typically expressed when a subset  
160 of epiblast stem cells become primordial germ cells (PGCs) and then again in mature germ cells to trigger  
161 meiotic gene expression programs<sup>43–45</sup>. Of note, some germline genes, including *Dazl*, are also expressed  
162 in the two-cell embryo<sup>40,46</sup>. However, we did not see derepression of two-cell stage-specific genes, like  
163 *Duxf3* (*Dux*) (log<sub>2</sub> fold change = -0.282, q = 0.337) and *Zscan4d* (log<sub>2</sub> fold change = 0.25, q = 0.381) (Figure  
164 3H, Supplementary Table 3), indicating *Kdm5c*-KO EpiLCs do not revert back to a 2-cell state. Altogether,  
165 *Kdm5c*-KO EpiLCs express key drivers of germline identity and meiosis while the brain primarily expresses  
166 spermiogenesis genes, indicating germline gene misexpression mirrors germline development during the  
167 progression of somatic development.

168 **Female epiblast-like cells have heightened germline gene misexpression with *Kdm5c***  
169 **loss**

170 It is currently unknown if the misexpression of germline genes is influenced by sex, as previous studies  
171 on germline gene repressors have focused on male cells<sup>17,18,20,47,48</sup>. Sex is particularly pertinent in the case  
172 of KDM5C because it partially escapes X chromosome inactivation (XCI), resulting in a higher dosage in  
173 females<sup>49–52</sup>. We therefore explored the impact of chromosomal sex upon germline gene suppression by  
174 comparing their dysregulation in male *Kdm5c* hemizygous knockout (*Kdm5c*<sup>-y</sup>, XY *Kdm5c*-KO, XY 5CKO),  
175 female homozygous knockout (*Kdm5c*<sup>-/-</sup>, XX *Kdm5c*-KO, XX 5CKO), and female heterozygous knockout  
176 (*Kdm5c*<sup>-/+</sup>, XX *Kdm5c*-HET, XX 5CHET) EpiLCs<sup>42</sup>.

177 In EpiLCs, homozygous and heterozygous *Kdm5c* knockout females expressed over double the number  
178 of germline-enriched genes than hemizygous males (Figure 4A, Supplementary Table 3). While the majority  
179 of germline DEGs in *Kdm5c*-KO males were also dysregulated in females (74%), many were sex-specific,  
180 such as *Tktl2* and *Esx1* (Figure 4B). We then compared the known functions of germline genes dysregulated  
181 uniquely in males and females or misexpressed in all samples (Figure 4C, Supplementary Table 3). Female-  
182 specific germline DEGs were enriched for meiotic (GO:0051321 - meiotic cell cycle) and flagellar (GO:  
183 0003341 - cilium movement) functions, while male-specific DEGs had roles in mitochondrial and cell signaling  
184 (GO:0070585 - protein localization to mitochondrion).

185 The majority of germline genes expressed in both sexes were more highly dysregulated in females  
186 compared to males (Figure 4D-F). This increased degree of dysregulation in females, along with the  
187 increased total number of germline genes, indicates females are more sensitive to losing KDM5C-mediated  
188 germline gene suppression. Heightened germline gene dysregulation in females could be due to impaired  
189 XCI in *Kdm5c* mutants<sup>42</sup>, as many spermatogenesis genes lie on the X chromosome<sup>53,54</sup>. However, female  
190 germline DEGs were not biased towards the X chromosome and females had a similar overall proportion  
191 of germline DEGs belonging to the X chromosome as males (XY *Kdm5c*-KO - 10.29%, XX *Kdm5c*-HET -  
192 7.43%, XX *Kdm5c*-KO - 10.59%) (Figure 4G). The majority of germline DEGs instead lie on autosomes for  
193 both male and female *Kdm5c* mutants (Figure 4G). Thus, while female EpiLCs are more prone to germline  
194 gene misexpression with KDM5C loss, it is likely independent of XCI defects.

195 **Germline gene misexpression in *Kdm5c* mutants is independent of germ cell sex**

196 Although many germline genes have shared functions in the male and female germline, e.g. PGC  
197 formation, meiosis, and genome defense, some have unique or sex-biased expression. Therefore, we  
198 wondered if *Kdm5c* mutant males would primarily express sperm genes while mutant females would primarily  
199 express egg genes. To comprehensively assess whether germline gene sex corresponds with *Kdm5c*  
200 mutant sex, we first filtered our list of germline-enriched genes for egg and sperm-biased genes (Figure 4,  
201 Supplementary Table 2). We defined germ cell sex-biased genes as those whose expression in the opposite

202 sex, at any time point, is no greater than 20% of the gene's maximum expression in a given sex. This  
203 criteria yielded 67 egg-biased, 1,024 sperm-biased, and 197 unbiased germline-enriched genes. We found  
204 regardless of sex, egg, sperm, and unbiased germline genes were dysregulated in all *Kdm5c* mutants at  
205 similar proportions (Figure 4I-J). Furthermore, germline genes dysregulated exclusively in either male or  
206 female mutants were also not biased towards their corresponding germ cell sex (Figure 4I). Altogether, these  
207 results demonstrate sex differences in germline gene dysregulation is not due to sex-specific activation of  
208 sperm or egg transcriptional programs.

## 209 **KDM5C binds to a subset of germline gene promoters during early embryogenesis**

210 KDM5C binds to the promoters of several germline genes in embryonic stem cells (ESCs) but not in  
211 neurons<sup>13,55</sup>. However, the lack of a comprehensive list of germline-enriched genes prohibited genome-wide  
212 characterization of KDM5C binding at germline gene promoters. Thus, it is unclear if KDM5C is enriched at  
213 germline gene promoters, what types of germline genes KDM5C regulates, and if its binding is maintained at  
214 any germline genes in neurons.

215 To address these questions, we analyzed KDM5C chromatin immunoprecipitation followed by DNA  
216 sequencing (ChIP-seq) datasets in EpiLCs<sup>42</sup> and primary forebrain neuron cultures (PNCs)<sup>12</sup> (MACS2 q <  
217 0.1, fold enrichment > 1, and removal of false-positive *Kdm5c*-KO peaks). EpiLCs had a higher total number  
218 of high-confidence KDM5C peaks than PNCs (EpiLCs: 5,808, PNCs: 1,276). KDM5C was primarily localized  
219 to gene promoters in both cell types (promoters = transcription start site (TSS) ± 500 bp, EpiLCs: 4,190,  
220 PNCs: 745), although PNCs showed increased localization to non-promoter regions (Figure 5A).

221 The majority of promoters bound by KDM5C in PNCs were also bound in EpiLCs (513 shared promoters),  
222 however a large portion of gene promoters were bound by KDM5C only in EpiLCs (3,677 EpiLC only  
223 promoters) (Figure 5B). Genes bound by KDM5C in both PNCs and EpiLCs were enriched for functions  
224 involving nucleic acid turnover, such as deoxyribonucleotide metabolic process (GO:0009262, p.adjust =  
225 8.28e-05) (Figure 5C, Supplementary Table 4). Germline ontologies were enriched only in EpiLC-specific,  
226 KDM5C-bound promoters, such as meiotic nuclear division (GO: 0007127 p.adjust = 6.77e-16) (Figure 5C).  
227 There were no significant ontologies for PNC-specific KDM5C target genes. Using our mouse germline gene  
228 list, we observed evident KDM5C signal around the TSS of many germline genes in EpiLCs, but not in PNCs  
229 (Figure 5D). Based on our ChIP-seq peak cut-off criteria, KDM5C was highly enriched at 211 germline gene  
230 promoters in EpiLCs (16.4% of all germline genes) (Figure 5E, Supplementary Table 2). Of note, KDM5C  
231 was only bound to about one third of RNA-seq DEG promoters unique to EpiLCs or the brain (EpiLC only  
232 DEGs: 34.9%, Brain only DEGs: 30%) (Supplementary Figure 1A-C). Representative examples of EpiLC  
233 DEGs bound and unbound by KDM5C in EpiLCs are *Dazl* and *Stra8*, respectively (Figure 5F). However,  
234 the four of the five germline genes dysregulated in both EpiLCs and the brain were bound by KDM5C in  
235 EpiLCs (*D1Pas1*, *Hsf2bp*, *Cyct*, and *Stk31*) (Supplementary Figure 1A). Together, these results demonstrate  
236 KDM5C is recruited to a subset of germline genes in EpiLCs, including meiotic genes, but does not directly

237 regulate germline genes in neurons. Furthermore, the majority of germline mRNAs expressed in *Kdm5c*-KO  
238 cells are dysregulated independent of direct KDM5C recruitment to their gene promoters, however genes  
239 dysregulated across *Kdm5c*-KO development are often direct KDM5C targets.

240 Many germline-specific genes are suppressed by the polycomb repressive complex 1.6 (PRC1.6), which  
241 contains the transcription factor heterodimers E2F/DP1 and MGA/MAX that respectively bind E2F and  
242 E-box motifs within germline gene promoters<sup>17,18,20,41,47,48,56–58</sup>. PRC1.6 members may recruit KDM5C to  
243 germline gene promoters<sup>13</sup>, given their association with KDM5C in HeLa cells and ESCs<sup>46,59</sup>. We thus  
244 used HOMER<sup>60</sup> to identify transcription factor motifs enriched at KDM5C-bound or unbound germline gene  
245 promoters (TSS ± 500 bp, q-value < 0.1, Supplementary Table 4). MAX and E2F6 binding sites were  
246 significantly enriched at germline genes bound by KDM5C in EpiLCs (MAX q-value: 0.0068, E2F6 q-value:  
247 0.0673, E2F q-value: 0.0917), but not at germline genes unbound by KDM5C (Figure 5G). One third of  
248 KDM5C-bound promoters contained the consensus sequence for either E2F6 (E2F, 5'-TCCCGC-3'), MGA  
249 (E-box, 5'-CACGTG-3'), or both, but only 17% of KDM5C-unbound genes contained these motifs (Figure 5H).  
250 KDM5C-unbound germline genes were instead enriched for multiple RFX transcription factor binding sites  
251 (RFX q-value < 0.0001, RFX2 q-value < 0.0001, RFX5 q-value < 0.0001) (Figure 5I, Supplementary figure  
252 1D). RFX transcription factors bind X-box motifs<sup>61</sup> to promote ciliogenesis<sup>62,63</sup> and among them is RFX2, a  
253 central regulator of post-meiotic spermatogenesis<sup>64,65</sup>. Although *Rfx2* is also not a direct target of KDM5C  
254 (Supplementary Figure 1E), RFX2 mRNA is derepressed in *Kdm5c*-KO EpiLCs (Figure 5J). Thus, RFX2 is a  
255 candidate transcription factor for driving the ectopic expression of many KDM5C-unbound germline genes in  
256 *Kdm5c*-KO cells.

## 257 **KDM5C is recruited to CpG islands at germline promoters to facilitate *de novo* DNA 258 methylation**

259 Previous work found two germline gene promoters have a marked reduction in DNA CpG methylation  
260 (CpGme) in the adult *Kdm5c*-KO hippocampus<sup>13</sup>. Since histone 3 lysine 4 di- and trimethylation (H3K4me2/3)  
261 impede *de novo* CpGme<sup>66,67</sup>, KDM5C's removal of H3K4me2/3 may be required to suppress germline  
262 genes. However, KDM5C's catalytic activity was recently shown to be dispensable for suppressing *Dazl* in  
263 undifferentiated ESCs<sup>46</sup>. To reconcile these observations, we hypothesized KDM5C erases H3K4me2/3 to  
264 promote the initial placement of CpGme at germline gene promoters in EpiLCs.

265 To test this hypothesis, we first characterized KDM5C's expression as naïve ESCs differentiate into  
266 EpiLCs (Figure 6A). While *Kdm5c* mRNA steadily decreased from 0 to 48 hours of differentiation (Figure  
267 6B), KDM5C protein initially increased from 0 to 24 hours and then decreased to near knockout levels by 48  
268 hours (Figure 6C). We then characterized KDM5C's substrates (H3K4me2/3) at germline gene promoters  
269 with *Kdm5c* loss using published ChIP-seq datasets<sup>22,42</sup>. *Kdm5c*-KO samples showed a marked increase in  
270 H3K4me2 in EpiLCs (Figure 6D) and H3K4me3 in the amygdala (Figure 6E) around the TSS of germline

271 genes. Together, these data suggest KDM5C acts during the transition between ESCs and EpiLCs to remove  
272 H3K4me2/3 at germline gene promoters.

273 Germline genes accumulate CpG methylation (CpGme) at CpG islands (CGIs) during the transition  
274 from naïve to primed pluripotency<sup>19,21,68</sup>. We first examined how many of our germline-enriched genes had  
275 promoter CGIs (TSS ± 500 bp) using the UCSC genome browser<sup>69</sup>. Notably, out of 1,288 germline-enriched  
276 genes, only 356 (27.64%) had promoter CGIs (Figure 6F, Supplementary Table 2). CGI-containing germline  
277 genes had higher enrichment of meiotic gene ontologies compared to CGI-free genes, including meiotic  
278 nuclear division (GO:0140013, p.adjust = 2.17e-12) and meiosis I (GO:0007127, p.adjust = 3.91e-10)  
279 (Figure 6G, Supplementary Table 5). Germline genes with promoter CGIs were more highly expressed than  
280 CGI-free genes across spermatogenesis stages, with highest expression in meiotic spermatocytes (Figure  
281 6H). Contrastingly, CGI-free genes only displayed substantial expression in post-meiotic round spermatids  
282 (Figure 6H). Although only a minor portion of germline gene promoters contained CGIs, CGIs strongly  
283 determined KDM5C's recruitment to germline genes ( $p = 2.37e-67$ , Odds Ratio = 17.8, Fisher's Exact Test),  
284 with 79.15% of KDM5C-bound germline gene promoters harboring CGIs (Figure 6F).

285 To assess how KDM5C loss impacts initial CpGme placement at germline gene promoters, we performed  
286 whole genome bisulfite sequencing (WGBS) in male wild-type and *Kdm5c*-KO ESCs and 96-hour extend  
287 EpiLCs (exEpiLCs), when germline genes reach peak methylation levels<sup>18</sup> (Figure 6I). We first identified  
288 which germline gene promoters significantly gained CpGme in wild-type cells during nESC to exEpiLCs  
289 differentiation (methylKit<sup>70</sup>,  $q < 0.01$ ,  $|methylation\ difference| > 25\%$ , TSS ± 500 bp). In wild-type cells, the  
290 majority of germline genes gained substantial CpGme at their promoter during differentiation (60.08%),  
291 regardless if their promoter contained a CGI (Figure 6J, Supplementary Table 5).

292 We then identified promoters differentially methylated in wild-type versus *Kdm5c*-KO exEpiLCs (methylKit,  
293  $q < 0.01$ ,  $|methylation\ difference| > 25\%$ , TSS ± 500 bp, Supplementary Table 5). Of the 48,882 promoters  
294 assessed, 274 promoters were significantly hypomethylated and 377 promoters were significantly hyper-  
295 methylated with KDM5C loss (Supplementary Figure 2A). Many promoters hyper- and hypomethylated  
296 in *Kdm5c*-KO exEpiLCs belonged to genes with unknown functions. However, 10.22% of hypomethyl-  
297 lated promoters belonged to germline genes and germline-relevant ontologies like meiotic nuclear division  
298 (GO:0140013, p.adjust = 0.012) are significantly enriched (Supplementary Figure 2B, Supplementary Table  
299 5). Approximately half of all germline gene promoters hypomethylated in *Kdm5c*-KO exEpiLCs are direct  
300 targets of KDM5C in EpiLCs (13 out of 28 hypomethylated promoters).

301 Promoters that showed the most robust loss of CpGme in *Kdm5c*-KO exEpiLCs (lowest q-values) harbored  
302 CGIs (Figure 6K). CGI promoters, but not CGI-free promoters, had a significant reduction in CpGme with  
303 KDM5C loss as a whole (Figure 6L) (Non-CGI promoters  $p = 0.0846$ , CGI promoters  $p = 0.0081$ , Mann-  
304 Whitney U test). Significantly hypomethylated promoters included germline genes consistently dysregulated  
305 across multiple *Kdm5c*-KO RNA-seq datasets<sup>13</sup>, such as *D1Pas1* (methylation difference = -60.03%, q-value  
306 = 3.26e-153) and *Naa11* (methylation difference = -42.45%, q-value = 1.44e-38) (Figure 6M). Surprisingly,

307 we observed only a modest reduction in CpGme at *Dazl*'s promoter (methylation difference = -6.525%,  
308 q-value = 0.0159) (Figure 6N). Altogether, these results demonstrate KDM5C is recruited to germline gene  
309 CGIs in EpiLCs to promote CpGme at germline gene promoters. Furthermore, this suggests while KDM5C's  
310 catalytic activity is required for the repression of some germline genes, CpGme can be placed at others even  
311 with elevated H3K4me2/3 around the TSS.

## 312 Discussion

313 In the above study, we demonstrate KDM5C's pivotal role in the development of tissue identity. We first  
314 characterized tissue-enriched genes expressed within the mouse *Kdm5c*-KO brain and identified substantial  
315 derepression of testis, liver, muscle, and ovary-enriched genes. Testis genes significantly enriched within the  
316 *Kdm5c*-KO amygdala and hippocampus are specific to the germline and absent in somatic cells. *Kdm5c*-KO  
317 epiblast-like cells (EpiLCs) aberrantly express key drivers of germline identity and meiosis, including *Dazl* and  
318 *Stra8*, while the adult brain primarily expresses genes important for late spermatogenesis. We demonstrated  
319 that although sex did not influence whether sperm or egg-specific genes were misexpressed, female EpiLCs  
320 have heightened germline gene de-repression with KDM5C loss. Germline genes can become aberrantly  
321 expressed in *Kdm5c*-KO cells via indirect mechanisms, such as activation through ectopic RFX transcription  
322 factors. Finally, we found KDM5C is dynamically regulated during ESC to EpiLC differentiation to promote  
323 long-term germline gene silencing through DNA methylation at CpG islands. Therefore, we propose KDM5C  
324 plays a fundamental role in the development of tissue identity during early embryogenesis, including the  
325 establishment of the soma-germline boundary. By systematically characterizing KDM5C's role in germline  
326 gene repression, we unveiled unique mechanisms governing the misexpression of distinct germline gene  
327 classes within somatic lineages. Ultimately, these data provide molecular footholds which can be exploited to  
328 test the overarching contribution of ectopic germline gene expression upon neurodevelopment.

329 By comparing *Kdm5c* mutant males and females, we revealed germline gene suppression is sexually  
330 dimorphic. Female EpiLCs are more severely impacted by loss of KDM5C-mediated germline gene sup-  
331 pression, yet this difference is not due to the large number of germline genes on the X chromosome<sup>53,54</sup>.  
332 Heightened germline gene misexpression in females may be related to females having a higher dose of  
333 KDM5C than males, due to its escape from XCI<sup>49–52</sup>. Intriguingly, heterozygous knockout females (*Kdm5c*<sup>-/+</sup>)  
334 also had over double the number of germline DEGs than hemizygous knockout males (*Kdm5c*<sup>-/Y</sup>), even  
335 though their expression of KDM5C should be roughly equivalent to that of wild-type males (*Kdm5c*<sup>+/Y</sup>). Males  
336 could partially compensate for KDM5C's loss via the Y-chromosome homolog, KDM5D. However, KDM5D  
337 exhibits weaker demethylase activity than KDM5C<sup>8</sup> and has not been reported to regulate germline gene  
338 expression. Nevertheless, these results demonstrate germline gene silencing mechanisms differ between  
339 males and females, which warrants further study to elucidate the biological ramifications and underlying  
340 mechanisms.

341 We found KDM5C is largely dispensable for promoting normal gene expression during development, yet  
342 is critical for suppressing ectopic developmental programs. It is important to note that while we highlighted  
343 KDM5C's repression of germline genes, some germline-enriched genes like *Dazl* are also expressed at the 2-  
344 cell stage and in the inner cell mass/naïve ESCs for their role in pluripotency and self-renewal<sup>41,46,71,72</sup>. These  
345 "self-renewal" germline genes are then silenced during ESC differentiation into epiblast stem cells/EpiLCs<sup>18,19</sup>.  
346 We found that while *Kdm5c*-KO EpiLCs express *Dazl*, they did not express 2-cell-specific genes like *Zscan4c*.  
347 These data suggest the 2-cell-like state reported in *Kdm5c*-KO ESCs<sup>46</sup> likely reflects KDM5C's primary  
348 role in germline gene repression. Germline gene misexpression in *Kdm5c*-KO EpiLCs may indicate they  
349 are differentiating into primordial germ cell-like cells (PGCLCs)<sup>34,35,37</sup>. Yet, *Kdm5c*-KO EpiLCs had normal  
350 cellular morphology and properly expressed markers for primed pluripotency, including *Otx2* which blocks  
351 EpiLC differentiation into PGCs/PGCLCs<sup>73</sup>. In addition to unimpaired EpiLC differentiation, *Kdm5c*-KO gross  
352 brain morphology is overall normal<sup>12</sup> and hardly any brain-specific genes were significantly dysregulated in  
353 the amygdala and hippocampus. Thus, ectopic germline gene expression occurs in conjunction with overall  
354 proper somatic differentiation in *Kdm5c*-KO animals.

355 Our work provides novel insight into the cross-talk between H3K4me2/3 and CpGme, which are gen-  
356 erally mutually exclusive<sup>74</sup>. In EpiLCs, loss of KDM5C binding at a subset of germline gene promoters,  
357 e.g. *D1Pas1*, strongly impaired promoter CGI methylation and resulted in their long-lasting de-repression into  
358 adulthood. Removal of H3K4me2/3 at CGIs is a plausible mechanism for KDM5C-mediated germline gene  
359 suppression<sup>13,55</sup>, given H3K4me2/3 can oppose DNMT3 activity<sup>66,67</sup>. However, emerging work indicates  
360 many histone-modifying enzymes have non-catalytic functions that influence gene expression, sometimes  
361 even more potently than their catalytic roles<sup>75,76</sup>. Indeed, KDM5C's catalytic activity was recently found to be  
362 dispensible for repressing *Dazl* in ESCs<sup>46</sup>. In our study, *Dazl*'s promoter still gained CpGme in *Kdm5c*-KO  
363 exEpiLCs, even with elevated H3K4me2. *Dazl* and a few other germline genes employ multiple repressive  
364 mechanisms to facilitate CpGme, such as DNMT3A/B recruitment via E2F6 and MGA<sup>17,18,47,48</sup>. This suggests  
365 alternative silencing mechanisms are sufficient to recruit DNMT3s to some germline CGIs, while others may  
366 require KDM5C-mediated H3K4me removal to overcome promoter CGI escape from CpGme<sup>74,77</sup>. These  
367 results also suggest the requirement for KDM5C's catalytic activity can change depending upon the locus  
368 and developmental stage, even for the same class of genes. However, further experiments are required to  
369 determine if catalytically inactive KDM5C can suppress germline genes at later developmental stages.

370 By generating a comprehensive list of mouse germline-enriched genes, we revealed distinct derepressive  
371 mechanisms governing early versus late-stage germline programs. Previous work on germline gene silencing  
372 has focused on genes with promoter CGIs<sup>19,74</sup>, and indeed the majority of KDM5C targets in EpiLCs were  
373 germ cell identity genes harboring CGIs. However, over 70% of germline-enriched gene promoters lacked  
374 CGIs, including the many KDM5C-unbound germline genes that are de-repressed in *Kdm5c*-KO cells. CGI-  
375 free, KDM5C-unbound germline genes were primarily late-stage spermatogenesis genes and significantly  
376 enriched for RFX2 binding sites, a central regulator of spermiogenesis<sup>64,65</sup>. These data suggest that once

377 activated during early embryogenesis, drivers of germline gene expression like *Rfx2*, *Stra8*, and *Dazl* turn  
378 on downstream germline programs, ultimately culminating in the expression of spermiogenesis genes in  
379 the adult *Kdm5c*-KO brain. Therefore, we propose KDM5C is recruited via promoter CGIs to act as a brake  
380 against runaway activation of germline-specific programs.

381 The above work provides the mechanistic foundation for KDM5C-mediated repression of tissue and  
382 germline-specific genes. However, the contribution of these ectopic, tissue-specific genes towards *Kdm5c*-  
383 KO neurological impairments is still unknown. In addition to germline genes, we also identified significant  
384 enrichment of muscle and liver-enriched transcripts within the *Kdm5c*-KO brain. Intriguingly, select liver and  
385 muscle-enriched DEGs do have known roles within the brain, such as the liver-enriched lipid metabolism  
386 gene *Apolipoprotein C-I (Apoc1)*<sup>28</sup>. *APOC1* dysregulation is implicated in Alzheimer's disease in humans<sup>29</sup>  
387 and overexpression of *Apoc1* in the mouse brain can impair learning and memory<sup>78</sup>. KDM5C may therefore  
388 be crucial for neurodevelopment by fine-tuning the expression of tissue-enriched, dosage-sensitive genes  
389 like *Apoc1*.

390 Given that germline genes have no known functions within the brain, their impact upon neurodevelopment  
391 is currently unknown. In *C. elegans*, somatic misexpression of germline genes via loss of *Retinoblastoma*  
392 (*Rb*) homologs results in enhanced piRNA signaling and ectopic P granule formation in neurons<sup>79,80</sup>. Ectopic  
393 testicular germline transcripts have also been observed in a variety of cancers, including brain tumors in  
394 *Drosophila* and mammals<sup>81,82</sup> and shown to promote cancer progression<sup>83–85</sup>. Intriguingly, mouse models  
395 and human cells for other chromatin-linked NDDs also display impaired soma-germline demarcation<sup>86–88</sup>,  
396 such as mutations in DNA methyltransferase 3b (DNMT3B), H3K9me1/2 methyltransferases G9A/GLP,  
397 and methyl-CpG -binding protein 2 (MECP2). Recently, the transcription factor ZMYM2 (ZNF198), whose  
398 mutation causes neurodevelopmental-craniofacial syndrome with variable renal and cardiac abnormalities  
399 (OMIM #619522), was also shown to repress germline genes by promoting H3K4 methylation removal and  
400 CpGme<sup>89</sup>. Thus, KDM5C is among a growing cohort of neurodevelopmental disorders that have erosion of  
401 the germline-soma boundary. Further research is required to determine the impact of these germline genes  
402 upon neuronal functions and the extent to which this phenomenon occurs in humans.

## 403 Materials and Methods

### 404 Classifying tissue-enriched and germline-enriched genes

405 Tissue-enriched differentially expressd genes (DEGs) were determined by their classification in a previ-  
406 ously published dataset from 17 male and female mouse tissues<sup>23</sup>. This study defined tissue expression as  
407 greater than 1 Fragments Per Kilobase of transcript per Million mapped read (FPKM) and tissue enrichment  
408 as at least 4-fold higher expression than any other tissue.

409 We curated a list of germline-enriched genes using an RNA-seq dataset from wild-type and germline-

410 depleted (*Kit*<sup>W/W<sup>v</sup></sup>) male and female mouse embryos from embryonic day 12, 14, and 16<sup>33</sup>, as well as adult  
411 male testes<sup>30</sup>. Germline-enriched genes met the following criteria: 1) their expression is greater than 1  
412 FPKM in wild-type germline 2) their expression in any wild-type somatic tissues<sup>23</sup> does not exceed 20%  
413 of maximum expression in wild-type germline, and 3) their expression in the germ cell-depleted (*Kit*<sup>W/W<sup>v</sup></sup>)  
414 germline, for any sex or time point, does not exceed 20% of maximum expression in wild-type germline. We  
415 defined sperm and egg-biased genes as those whose expression in the opposite sex, at any time point, is no  
416 greater than 20% of the gene's maximum expression in a given sex. Genes that did not meet this threshold  
417 for either sex were classified as 'unbiased'.

## 418 Cell culture

419 We utilized our previously established cultures of male wild-type and *Kdm5c* knockout (-KO)  
420 embryonic stem cells<sup>42</sup>. Sex was confirmed by genotyping *Uba1/Uba1y* on the X and Y chromo-  
421 somes with the following primers: 5'-TGGATGGTGTGCCAATG-3', 5'-CACCTGCACGTTGCCCTT-  
422 3'. Deletion of *Kdm5c* exons 11 and 12, which destabilize KDM5C protein<sup>12</sup>, was confirmed  
423 through the primers 5'-ATGCCCATATTAAGAGTCCCTG-3', 5'-TCTGCCTTGATGGACTGTT-3', and  
424 5'-GGTTCTAACACTCACATAGTG-3'.

425 Embryonic stem cells (ESCs) and epiblast-like cells were cultured using previously established  
426 methods<sup>38</sup>. Briefly, ESCs were initially cultured in primed ESC (pESC) media consisting of KnockOut  
427 DMEM (Gibco#10829–018), fetal bovine serum (Gibco#A5209501), KnockOut serum replacement  
428 (Invitrogen#10828–028), Glutamax (Gibco#35050-061), Anti-Anti (Gibco#15240-062), MEM Non-essential  
429 amino acids (Gibco#11140-050), and beta-mercaptoethanol (Sigma#M7522). They were then transitioned  
430 into ground-state, "naïve" ESCs (nESCs) by culturing for four passages in N2B27 media containing  
431 DMEM/F12 (Gibco#11330–032), Neurobasal media (Gibco#21103–049), Gluamax (Gibco#35050-061),  
432 Anti-Anti (Gibco#15240-062), N2 supplement (Invitrogen#17502048), and B27 supplement without vitamin  
433 A (Invitrogen#12587-010), and beta-mercaptoethanol (Sigma#M7522). Both pESC and nESC media  
434 were supplemented with 3  $\mu$ M GSK3 inhibitor CHIR99021 (Sigma #SML1046-5MG), 1  $\mu$ M MEK inhibitor  
435 PD0325901 (Sigma #PZ0162-5MG), and 1,000 units/mL leukemia inhibitory factor (LIF, Millipore#ESG1107).

436 nESCs were differentiated into epiblast-like cells (EpiLCs, 48 hours) and extendend EpiLCs (exEpiLCs,  
437 96 hours) by culturing in N2B27 media containing DMEM/F12, Neurobasal media, Gluamax, Anti-Anti, N2  
438 supplement, B27 supplement (Invitrogen#17504044), and beta-mercaptoethanol supplemented with 10  
439 ng/mL fibroblast growth factor 2 (FGF2, R&D Biotechne 233-FB), and 20 ng/mL activin A (R&D Biotechne  
440 338AC050CF), as previously described<sup>38</sup>.

441 **Real time quantitative PCR (RT-qPCR)**

442 nESCs were differentiated into EpiLCs as described above. Cells were lysed with Tri-reagent BD (Sigma  
443 #T3809) at 0, 24, and 48 hours of differentiation. RNA was phase separated with 0.1  $\mu$ L/ $\mu$ L 1-bromo-3-  
444 chloropropane (Sigma #B9673) and then precipitated with isopropanol (Sigma #I9516) and ethanol puri-  
445 fied. For each sample, 2  $\mu$ g of RNA was reverse transcribed using the ProtoScript II Reverse transcriptase kit  
446 from New England Biolabs (NEB #M0368S) and primed with oligo dT. Expression of *Kdm5c* was detected us-  
447 ing the primers 5'-CCCATGGAGGCCAGAGAATAAG-3' 5'-CTCAGCGGATAAGAGAATTGCTAC-3' and nor-  
448 malized to TBP using the primers 5'-TTCAGAGGATGCTCTAGGGAAGA-3' 5'-CTGTGGAGTAAGTCCTGTGCC-  
449 3' with the Power SYBR™ Green PCR Master Mix (ThermoFisher #4367659).

450 **Western Blot**

451 Total protein was extracted during nESC to EpiLC differentiation at 0, 24, and 48 hours by sonicating cells  
452 at 20% amplitude for 15 seconds in 2X SDS sample buffer, then boiling at 100 °C for 10 minutes. Proteins  
453 were separated on a 7.5% SDS page gel, transferred overnight onto a fluorescent membrane, blotted for  
454 rabbit anti-KDM5C (in house, 1:500) and mouse anti-DAXX (Santa Cruz #(H-7): sc-8043, 1:500), and then  
455 imaged using the LiCor Odyssey CLx system. Band intensity was quantified using ImageJ.

456 **RNA sequencing (RNA-seq) data analysis**

457 After ensuring read quality via FastQC (v0.11.8), reads were then mapped to the mm10 *Mus musculus*  
458 genome (Gencode) using STAR (v2.5.3a), during which we removed duplicates and kept only uniquely  
459 mapped reads. Count files were generated by FeatureCounts (Subread v1.5.0), and BAM files were  
460 converted to bigwigs using deeptools (v3.1.3) and visualized by the UCSC genome browser<sup>69</sup>. RStudio  
461 (v3.6.0) was then used to analyze counts files by DESeq2 (v1.26.0)<sup>24</sup> to identify differentially expressed  
462 genes (DEGs) with a q-value (p-adjusted via FDR/Benjamini–Hochberg correction) less than 0.1 and a log2  
463 fold change greater than 0.5. For all DESeq2 analyses, log2 fold changes were calculated with IfcShrink  
464 using the ashR package<sup>90</sup>. MA-plots were generated by ggpibr (v0.6.0), and Eulerr diagrams were generated  
465 by eulerr (v6.1.1). Boxplots and scatterplots were generated by ggpibr (v0.6.0) and ggplot2 (v3.3.2). The  
466 Upset plot was generated via the package UpSetR (v1.4.0)<sup>91</sup>. Gene ontology (GO) analyses were performed  
467 by the R package enrichPlot (v1.16.2) using the biological processes setting and compareCluster.

468 **Chromatin immunoprecipitation followed by DNA sequencing (ChIP-seq) data analysis**

469 ChIP-seq reads were aligned to mm10 using Bowtie1 (v1.1.2) allowing up to two mismatches. Only  
470 uniquely mapped reads were used for analysis. Peaks were called using MACS2 software (v2.2.9.1) using  
471 input BAM files for normalization, with filters for a q-value < 0.1 and a fold enrichment > 1. We removed

472 “black-listed” genomic regions that often give aberrant signals. Common peak sets were obtained in R via  
473 DiffBind<sup>92</sup> (v3.6.5). In the case of KDM5C ChIP-seq, *Kdm5c*-KO false-positive peaks were then removed from  
474 wild-type samples using bedtools (v2.25.0). Peak proximity to genomic loci was determined by ChIPSeeker<sup>93</sup>  
475 (v1.32.1). Gene ontology (GO) analyses were performed by the R package enrichPlot (v1.16.2) using the  
476 biological processes setting and compareCluster. Enriched motifs were identified using HOMER<sup>60</sup> to search  
477 for known motifs within 500 base pairs up and downstream of the transcription start site. Average binding  
478 across genes was visualized using deeptools (v3.1.3). Bigwigs were visualized using the UCSC genome  
479 browser<sup>69</sup>.

## 480 CpG island (CGI) analysis

481 Locations of CpG islands were determined through the mm10 UCSC genome browser CpG island track<sup>69</sup>,  
482 which classified CGIs as regions that have greater than 50% GC content, are larger than 200 base pairs,  
483 and have a ratio of CG dinucleotides observed over the expected amount greater than 0.6. CGI genomic  
484 coordinates were then annotated using ChIPseeker<sup>93</sup> (v1.32.1) and filtered for ones that lie within promoters  
485 of germline-enriched genes (TSS ± 500).

## 486 Whole genome bisulfite sequencing (WGBS)

487 Genomic DNA (gDNA) from male naïve ESCs and extended EpiLCs was extracted using the Wizard  
488 Genomic DNA Purification Kit (Promega A1120), following the instructions for Tissue Culture Cells. gDNA  
489 from two wild-types and two *Kdm5c*-KOs of each cell type was sent to Novogene for WGBS using the  
490 Illumina NovaSeq X Plus platform and sequenced for 150 bp paired-end reads (PE150). All samples had  
491 greater than 99% bisulfite conversion rates. Reads were adapter and quality trimmed with Trim Galore  
492 (v0.6.10) and aligned to the mm10 genome using Bismark<sup>94</sup> (v0.22.1). Analysis of differential methylation at  
493 gene promoters was performed using methylKit<sup>70</sup> (v1.28.0) with a minimum coverage of 3 paired reads, a  
494 percentage greater than 25% or less than -25%, and q-value less than 0.01. methylKit was also used to  
495 calculate average percentage methylation at germline gene promoters. Methylation bedgraph tracks were  
496 generated via Bismark and visualized using the UCSC genome browser<sup>69</sup>.

## 497 Data availability

### 498 WGBS in wild-type and *Kdm5c*-KO ESCs and exEpiLCs

499 Raw fastq files are deposited in the Sequence Read Archive (SRA) <https://www.ncbi.nlm.nih.gov/sra>  
500 under the bioProject PRJNA1165148.

501 **Published datasets**

502 All published datasets are available at the Gene Expression Omnibus (GEO) <https://www.ncbi.nlm.nih.gov/geo>. Published RNA-seq datasets analyzed in this study included the male wild-type and *Kdm5c*-KO  
503 adult amygdala and hippocampus<sup>22</sup>, available at GEO: GSE127722. Male and female wild-type, *Kdm5c*-KO,  
504 and *Kdm5c*-HET EpiLCs<sup>42</sup> are available at GEO: GSE96797.

505 Previously published ChIP-seq experiments included KDM5C binding in wild-type and *Kdm5c*-KO  
506 EpiLCs<sup>42</sup> (available at GEO: GSE96797) and mouse primary neuron cultures (PNCs) from the cortex  
507 and hippocampus<sup>12</sup> (available at GEO: GSE61036). ChIP-seq of histone 3 lysine 4 dimethylation (H3K4me2)  
508 in male wild-type and *Kdm5c*-KO EpiLCs<sup>42</sup> is also available at GEO: GSE96797. ChIP-seq of histone 3 lysine  
509 4 trimethylation (H3K4me3) in wild-type and *Kdm5c*-KO male amygdala<sup>22</sup> are available at GEO: GSE127817.  
510

511 **Data analysis**

512 Scripts used to generate the results, tables, and figures of this study are available via the GitHub  
513 repository: [https://github.com/kbonefas/KDM5C\\_Germ\\_Mechanism](https://github.com/kbonefas/KDM5C_Germ_Mechanism)

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527 **Author contributions**

528 K.M.B. and S.I. conceived the study and designed the experiments. I.V. generated the ESC and exEpiLC  
529 WGBS data. K.M.B performed all data analysis and all other experiments. The manuscript was written by  
530 K.M.B and S.I. and edited by K.M.B, S.I., and I.V.

531 **References**

- 532 1. Strahl, B.D., and Allis, C.D. (2000). The language of covalent histone modifications. *Nature* **403**,  
533 41–45. <https://doi.org/10.1038/47412>.
- 534 2. Jenuwein, T., and Allis, C.D. (2001). Translating the Histone Code. *Science* **293**, 1074–1080.  
535 <https://doi.org/10.1126/science.1063127>.
- 536 3. Lewis, E.B. (1978). A gene complex controlling segmentation in *Drosophila*. *Nature* **276**, 565–570.  
537 <https://doi.org/10.1038/276565a0>.
- 538 4. Kennison, J.A., and Tamkun, J.W. (1988). Dosage-dependent modifiers of polycomb and antennapedia  
539 mutations in *Drosophila*. *Proc. Natl. Acad. Sci. U.S.A.* **85**, 8136–8140. <https://doi.org/10.1073/pnas.8>  
5. Kassis, J.A., Kennison, J.A., and Tamkun, J.W. (2017). Polycomb and Trithorax Group Genes in  
541 *Drosophila*. *Genetics* **206**, 1699–1725. <https://doi.org/10.1534/genetics.115.185116>.
- 542 6. Gabriele, M., Lopez Tobon, A., D'Agostino, G., and Testa, G. (2018). The chromatin basis of  
543 neurodevelopmental disorders: Rethinking dysfunction along the molecular and temporal axes. *Prog  
Neuropsychopharmacol Biol Psychiatry* **84**, 306–327. <https://doi.org/10.1016/j.pnpbp.2017.12.013>.
- 544 7. Schaefer, A., Sampath, S.C., Intrator, A., Min, A., Gertler, T.S., Surmeier, D.J., Tarakhovsky, A.,  
545 and Greengard, P. (2009). Control of cognition and adaptive behavior by the GLP/G9a epigenetic  
suppressor complex. *Neuron* **64**, 678–691. <https://doi.org/10.1016/j.neuron.2009.11.019>.
- 546 8. Iwase, S., Lan, F., Bayliss, P., De La Torre-Ubieta, L., Huarte, M., Qi, H.H., Whetstine, J.R., Bonni, A.,  
Roberts, T.M., and Shi, Y. (2007). The X-Linked Mental Retardation Gene SMCX/JARID1C Defines a  
Family of Histone H3 Lysine 4 Demethylases. *Cell* **128**, 1077–1088. [547](https://doi.org/10.1016/j.cell.200)  
7.02.017.
- 548 9. Claes, S., Devriendt, K., Van Goethem, G., Roelen, L., Meireleire, J., Raeymaekers, P., Cassiman,  
J.J., and Fryns, J.P. (2000). Novel syndromic form of X-linked complicated spastic paraparesia. *Am J  
549 Med Genet* **94**, 1–4.
- 550 10. Jensen, L.R., Amende, M., Gurok, U., Moser, B., Gimmel, V., Tzschach, A., Janecke, A.R., Tariverdian,  
G., Chelly, J., Fryns, J.P., et al. (2005). Mutations in the JARID1C gene, which is involved in  
transcriptional regulation and chromatin remodeling, cause X-linked mental retardation. *Am J Hum  
551 Genet* **76**, 227–236. <https://doi.org/10.1086/427563>.
- 552 11. Carmignac, V., Nambot, S., Lehalle, D., Callier, P., Moortgat, S., Benoit, V., Ghoumid, J., Delobel,  
B., Smol, T., Thuillier, C., et al. (2020). Further delineation of the female phenotype with KDM5C  
553 disease causing variants: 19 new individuals and review of the literature. *Clin Genet* **98**, 43–55.  
<https://doi.org/10.1111/cge.13755>.

- 554 12. Iwase, S., Brookes, E., Agarwal, S., Badeaux, A.I., Ito, H., Vallianatos, C.N., Tomassy, G.S., Kasza, T.,  
Lin, G., Thompson, A., et al. (2016). A Mouse Model of X-linked Intellectual Disability Associated with  
Impaired Removal of Histone Methylation. *Cell Reports* *14*, 1000–1009. <https://doi.org/10.1016/j.celrep.2015.12.091>.
- 555 13. Scandaglia, M., Lopez-Atalaya, J.P., Medrano-Fernandez, A., Lopez-Cascales, M.T., Del Blanco, B.,  
Lipinski, M., Benito, E., Olivares, R., Iwase, S., Shi, Y., et al. (2017). Loss of Kdm5c Causes Spurious  
Transcription and Prevents the Fine-Tuning of Activity-Regulated Enhancers in Neurons. *Cell Rep* *21*,  
47–59. <https://doi.org/10.1016/j.celrep.2017.09.014>.
- 556 14. Bonefas, K.M., Vallianatos, C.N., Raines, B., Tronson, N.C., and Iwase, S. (2023). Sexually Dimorphic  
Alterations in the Transcriptome and Behavior with Loss of Histone Demethylase KDM5C. *Cells* *12*,  
637. <https://doi.org/10.3390/cells12040637>.
- 557 15. Devlin, D.K., Ganley, A.R.D., and Takeuchi, N. (2023). A pan-metazoan view of germline-soma  
distinction challenges our understanding of how the metazoan germline evolves. *Current Opinion in  
Systems Biology* *36*, 100486. <https://doi.org/10.1016/j.coisb.2023.100486>.
- 558 16. Lehmann, R. (2012). Germline Stem Cells: Origin and Destiny. *Cell Stem Cell* *10*, 729–739.  
<https://doi.org/10.1016/j.stem.2012.05.016>.
- 559 17. Endoh, M., Endo, T.A., Shinga, J., Hayashi, K., Farcas, A., Ma, K.W., Ito, S., Sharif, J., Endoh, T.,  
Onaga, N., et al. (2017). PCGF6-PRC1 suppresses premature differentiation of mouse embryonic  
stem cells by regulating germ cell-related genes. *eLife* *6*. <https://doi.org/10.7554/eLife.21064>.
- 560 18. Mochizuki, K., Sharif, J., Shirane, K., Uranishi, K., Bogutz, A.B., Janssen, S.M., Suzuki, A., Okuda,  
A., Koseki, H., and Lorincz, M.C. (2021). Repression of germline genes by PRC1.6 and SETDB1  
in the early embryo precedes DNA methylation-mediated silencing. *Nat Commun* *12*, 7020. <https://doi.org/10.1038/s41467-021-27345-x>.
- 561 19. Borgel, J., Guibert, S., Li, Y., Chiba, H., Schübeler, D., Sasaki, H., Forné, T., and Weber, M. (2010).  
Targets and dynamics of promoter DNA methylation during early mouse development. *Nat Genet* *42*,  
1093–1100. <https://doi.org/10.1038/ng.708>.
- 562 20. Velasco, G., Hubé, F., Rollin, J., Neuillet, D., Philippe, C., Bouzinba-Segard, H., Galvani, A., Viegas-  
Péquignot, E., and Francastel, C. (2010). Dnmt3b recruitment through E2F6 transcriptional repressor  
mediates germ-line gene silencing in murine somatic tissues. *Proc Natl Acad Sci U S A* *107*, 9281–  
9286. <https://doi.org/10.1073/pnas.1000473107>.
- 563 21. Hackett, J.A., Reddington, J.P., Nestor, C.E., Dunican, D.S., Branco, M.R., Reichmann, J., Reik,  
W., Surani, M.A., Adams, I.R., and Meehan, R.R. (2012). Promoter DNA methylation couples  
genome-defence mechanisms to epigenetic reprogramming in the mouse germline. *Development*  
139, 3623–3632. <https://doi.org/10.1242/dev.081661>.
- 564 573

- 574 22. Vallianatos, C.N., Raines, B., Porter, R.S., Bonefas, K.M., Wu, M.C., Garay, P.M., Collette, K.M.,  
Seo, Y.A., Dou, Y., Keegan, C.E., et al. (2020). Mutually suppressive roles of KMT2A and KDM5C  
in behaviour, neuronal structure, and histone H3K4 methylation. *Commun Biol* 3, 278. <https://doi.org/10.1038/s42003-020-1001-6>.  
575
- 576 23. Li, B., Qing, T., Zhu, J., Wen, Z., Yu, Y., Fukumura, R., Zheng, Y., Gondo, Y., and Shi, L. (2017). A  
Comprehensive Mouse Transcriptomic BodyMap across 17 Tissues by RNA-seq. *Sci Rep* 7, 4200.  
577 <https://doi.org/10.1038/s41598-017-04520-z>.
- 578 24. Love, M.I., Huber, W., and Anders, S. (2014). Moderated estimation of fold change and dispersion for  
579 RNA-seq data with DESeq2. *Genome Biol* 15, 550. <https://doi.org/10.1186/s13059-014-0550-8>.
- 580 25. Crackower, M.A., Kolas, N.K., Noguchi, J., Sarao, R., Kikuchi, K., Kaneko, H., Kobayashi, E., Kawai, Y.,  
581 Kozieradzki, I., Landers, R., et al. (2003). Essential Role of Fkbp6 in Male Fertility and Homologous  
Chromosome Pairing in Meiosis. *Science* 300, 1291–1295. <https://doi.org/10.1126/science.1083022>.
- 582 26. Xiol, J., Cora, E., Koglgruber, R., Chuma, S., Subramanian, S., Hosokawa, M., Reuter, M., Yang, Z.,  
Berninger, P., Palencia, A., et al. (2012). A Role for Fkbp6 and the Chaperone Machinery in piRNA  
583 Amplification and Transposon Silencing. *Molecular Cell* 47, 970–979. <https://doi.org/10.1016/j.molcel.2012.07.019>.
- 584 27. Cheng, S., Altmeppen, G., So, C., Welp, L.M., Penir, S., Ruhwedel, T., Menelaou, K., Harasimov, K.,  
Stützer, A., Blayney, M., et al. (2022). Mammalian oocytes store mRNAs in a mitochondria-associated  
585 membraneless compartment. *Science* 378, eabq4835. <https://doi.org/10.1126/science.abq4835>.
- 586 28. Rouland, A., Masson, D., Lagrost, L., Vergès, B., Gautier, T., and Bouillet, B. (2022). Role of  
587 apolipoprotein C1 in lipoprotein metabolism, atherosclerosis and diabetes: A systematic review.  
*Cardiovasc Diabetol* 21, 272. <https://doi.org/10.1186/s12933-022-01703-5>.
- 588 29. Leduc, V., Jasmin-Bélanger, S., and Poirier, J. (2010). APOE and cholesterol homeostasis in  
589 Alzheimer's disease. *Trends in Molecular Medicine* 16, 469–477. <https://doi.org/10.1016/j.molmed.2010.07.008>.
- 590 30. Mueller, J.L., Skaletsky, H., Brown, L.G., Zaghlul, S., Rock, S., Graves, T., Auger, K., Warren,  
591 W.C., Wilson, R.K., and Page, D.C. (2013). Independent specialization of the human and mouse X  
chromosomes for the male germ line. *Nat Genet* 45, 1083–1087. <https://doi.org/10.1038/ng.2705>.
- 592 31. Handel, M.A., and Eppig, J.J. (1979). Sertoli Cell Differentiation in the Testes of Mice Genetically  
Deficient in Germ Cells. *Biology of Reproduction* 20, 1031–1038. <https://doi.org/10.1095/biolreprod20.5.1031>.
- 593 32. 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. <https://doi.org/10.1016/j.devcel.2018.07.025>.

- 596 33. Soh, Y.Q., Junker, J.P., Gill, M.E., Mueller, J.L., van Oudenaarden, A., and Page, D.C. (2015). A  
597 Gene Regulatory Program for Meiotic Prophase in the Fetal Ovary. *PLoS Genet* **11**, e1005531.  
<https://doi.org/10.1371/journal.pgen.1005531>.
- 598 34. Magnúsdóttir, E., and Surani, M.A. (2014). How to make a primordial germ cell. *Development* **141**,  
599 245–252. <https://doi.org/10.1242/dev.098269>.
- 600 35. Günesdogan, U., Magnúsdóttir, E., and Surani, M.A. (2014). Primordial germ cell specification: A  
601 context-dependent cellular differentiation event [corrected]. *Philos Trans R Soc Lond B Biol Sci* **369**.  
<https://doi.org/10.1098/rstb.2013.0543>.
- 602 36. Bardot, E.S., and Hadjantonakis, A.-K. (2020). Mouse gastrulation: Coordination of tissue patterning,  
603 specification and diversification of cell fate. *Mechanisms of Development* **163**, 103617. <https://doi.org/10.1016/j.mod.2020.103617>.
- 604 37. Hayashi, K., Ohta, H., Kurimoto, K., Aramaki, S., and Saitou, M. (2011). Reconstitution of the  
605 mouse germ cell specification pathway in culture by pluripotent stem cells. *Cell* **146**, 519–532.  
<https://doi.org/10.1016/j.cell.2011.06.052>.
- 606 38. Samanta, M., and Kalantry, S. (2020). Generating primed pluripotent epiblast stem cells: A methodol-  
607 ogy chapter. *Curr Top Dev Biol* **138**, 139–174. <https://doi.org/10.1016/bs.ctdb.2020.01.005>.
- 608 39. Welling, M., Chen, H., Muñoz, J., Musheev, M.U., Kester, L., Junker, J.P., Mischerikow, N., Arbab, M.,  
609 Kuijk, E., Silberstein, L., et al. (2015). DAZL regulates Tet1 translation in murine embryonic stem cells.  
*EMBO Reports* **16**, 791–802. <https://doi.org/10.15252/embr.201540538>.
- 610 40. Macfarlan, T.S., Gifford, W.D., Driscoll, S., Lettieri, K., Rowe, H.M., Bonanomi, D., Firth, A., Singer, O.,  
611 Trono, D., and Pfaff, S.L. (2012). Embryonic stem cell potency fluctuates with endogenous retrovirus  
activity. *Nature* **487**, 57–63. <https://doi.org/10.1038/nature11244>.
- 612 41. Suzuki, A., Hirasaki, M., Hishida, T., Wu, J., Okamura, D., Ueda, A., Nishimoto, M., Nakachi, Y.,  
613 Mizuno, Y., Okazaki, Y., et al. (2016). Loss of MAX results in meiotic entry in mouse embryonic and  
germline stem cells. *Nat Commun* **7**, 11056. <https://doi.org/10.1038/ncomms11056>.
- 614 42. Samanta, M.K., Gayen, S., Harris, C., Maclary, E., Murata-Nakamura, Y., Malcore, R.M., Porter, R.S.,  
615 Garay, P.M., Vallianatos, C.N., Samollow, P.B., et al. (2022). Activation of Xist by an evolutionarily  
conserved function of KDM5C demethylase. *Nat Commun* **13**, 2602. <https://doi.org/10.1038/s41467-022-30352-1>.
- 616 43. Koubova, J., Menke, D.B., Zhou, Q., Capel, B., Griswold, M.D., and Page, D.C. (2006). Retinoic  
617 acid regulates sex-specific timing of meiotic initiation in mice. *Proc. Natl. Acad. Sci. U.S.A.* **103**,  
2474–2479. <https://doi.org/10.1073/pnas.0510813103>.

- 618 44. Lin, Y., Gill, M.E., Koubova, J., and Page, D.C. (2008). Germ Cell-Intrinsic and -Extrinsic Factors  
619 Govern Meiotic Initiation in Mouse Embryos. *Science* *322*, 1685–1687. <https://doi.org/10.1126/science.1166340>.
- 620 45. Endo, T., Mikedis, M.M., Nicholls, P.K., Page, D.C., and De Rooij, D.G. (2019). Retinoic Acid and Germ  
621 Cell Development in the Ovary and Testis. *Biomolecules* *9*, 775. <https://doi.org/10.3390/biom9120775>.
- 622 46. Gupta, N., Yakhou, L., Albert, J.R., Azogui, A., Ferry, L., Kirsh, O., Miura, F., Battault, S., Yamaguchi,  
623 K., Laisné, M., et al. (2023). A genome-wide screen reveals new regulators of the 2-cell-like cell state.  
*Nat Struct Mol Biol*. <https://doi.org/10.1038/s41594-023-01038-z>.
- 624 47. Pohlers, M., Truss, M., Frede, U., Scholz, A., Strehle, M., Kuban, R.-J., Hoffmann, B., Morkel, M.,  
Birchmeier, C., and Hagemeier, C. (2005). A Role for E2F6 in the Restriction of Male-Germ-Cell-  
625 Specific Gene Expression. *Current Biology* *15*, 1051–1057. <https://doi.org/10.1016/j.cub.2005.04.060>.
- 626 48. Dahlet, T., Truss, M., Frede, U., Al Adhami, H., Bardet, A.F., Dumas, M., Vallet, J., Chicher, J.,  
Hammann, P., Kottnik, S., et al. (2021). E2F6 initiates stable epigenetic silencing of germline genes  
627 during embryonic development. *Nat Commun* *12*, 3582. <https://doi.org/10.1038/s41467-021-23596-w>.
- 628 49. Agulnik, A.I., Mitchell, M.J., Mattei, M.G., Borsani, G., Avner, P.A., Lerner, J.L., and Bishop, C.E.  
(1994). A novel X gene with a widely transcribed Y-linked homologue escapes X-inactivation in mouse  
629 and human. *Hum Mol Genet* *3*, 879–884. <https://doi.org/10.1093/hmg/3.6.879>.
- 630 50. Carrel, L., Hunt, P.A., and Willard, H.F. (1996). Tissue and lineage-specific variation in inactive  
X chromosome expression of the murine Smcx gene. *Hum Mol Genet* *5*, 1361–1366. <https://doi.org/10.1093/hmg/5.9.1361>.
- 632 51. Sheardown, S., Norris, D., Fisher, A., and Brockdorff, N. (1996). The mouse Smcx gene exhibits  
developmental and tissue specific variation in degree of escape from X inactivation. *Hum Mol Genet*  
633 *5*, 1355–1360. <https://doi.org/10.1093/hmg/5.9.1355>.
- 634 52. Xu, J., Deng, X., and Disteche, C.M. (2008). Sex-Specific Expression of the X-Linked Histone  
Demethylase Gene Jarid1c in Brain. *PLoS ONE* *3*, e2553. <https://doi.org/10.1371/journal.pone.0002553>.
- 636 53. Wang, P.J., McCarrey, J.R., Yang, F., and Page, D.C. (2001). An abundance of X-linked genes  
637 expressed in spermatogonia. *Nat Genet* *27*, 422–426. <https://doi.org/10.1038/86927>.
- 638 54. Khil, P.P., Smirnova, N.A., Romanienko, P.J., and Camerini-Otero, R.D. (2004). The mouse X  
chromosome is enriched for sex-biased genes not subject to selection by meiotic sex chromosome  
639 inactivation. *Nat Genet* *36*, 642–646. <https://doi.org/10.1038/ng1368>.

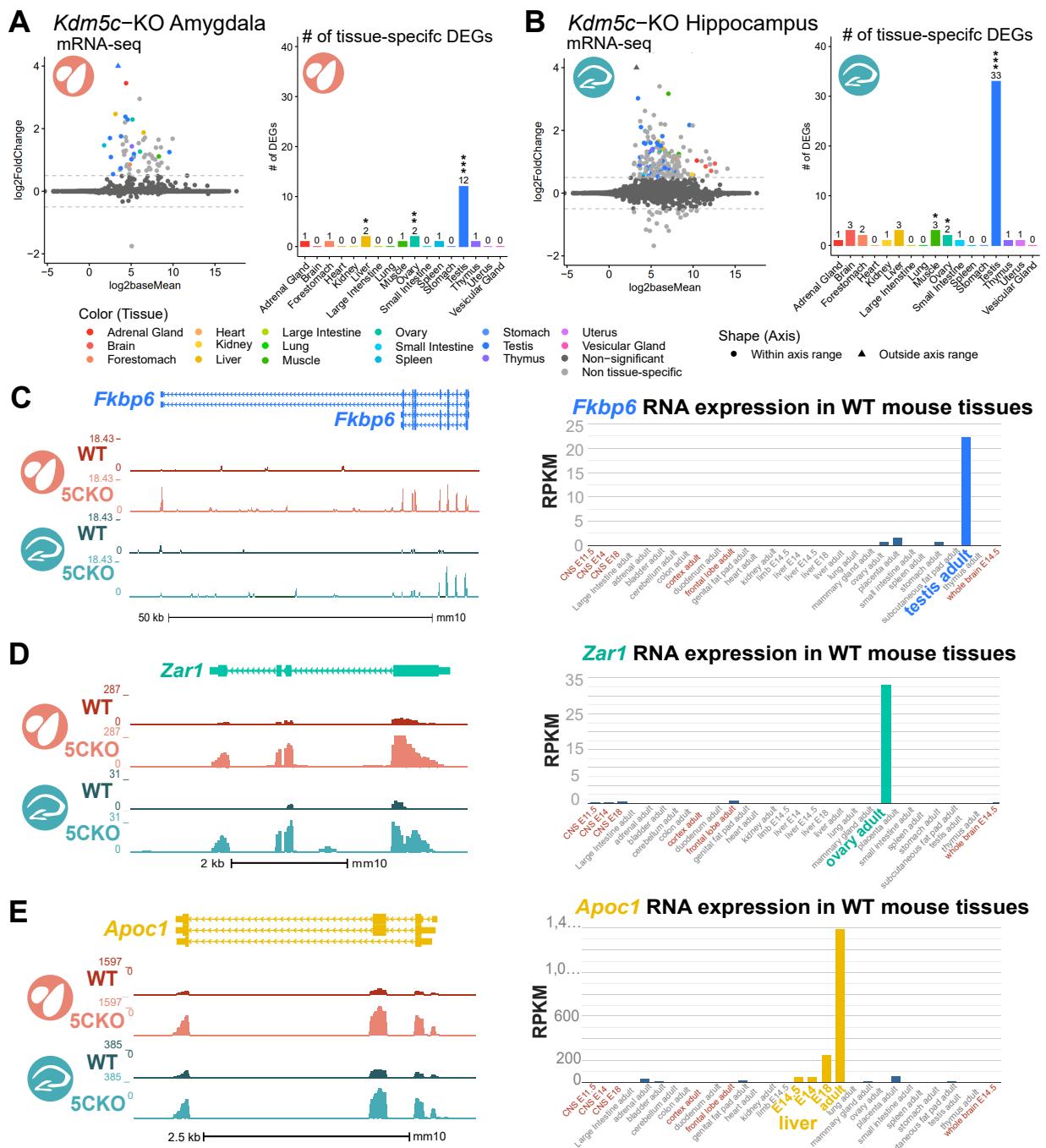
- 640 55. Al Adhami, H., Vallet, J., Schaal, C., Schumacher, P., Bardet, A.F., Dumas, M., Chicher, J., Hammann, P., Daujat, S., and Weber, M. (2023). Systematic identification of factors involved in the silencing of germline genes in mouse embryonic stem cells. *Nucleic Acids Research* *51*, 3130–3149. <https://doi.org/10.1093/nar/gkad071>.
- 641
- 642 56. Hurlin, P.J. (1999). Mga, a dual-specificity transcription factor that interacts with Max and contains a T-domain DNA-binding motif. *The EMBO Journal* *18*, 7019–7028. <https://doi.org/10.1093/emboj/18.24.7019>.
- 643
- 644 57. Stielow, B., Finkernagel, F., Stiewe, T., Nist, A., and Suske, G. (2018). MGA, L3MBTL2 and E2F6 determine genomic binding of the non-canonical Polycomb repressive complex PRC1.6. *PLoS Genet* *14*, e1007193. <https://doi.org/10.1371/journal.pgen.1007193>.
- 645
- 646 58. Tatsumi, D., Hayashi, Y., Endo, M., Kobayashi, H., Yoshioka, T., Kiso, K., Kanno, S., Nakai, Y., Maeda, I., Mochizuki, K., et al. (2018). DNMTs and SETDB1 function as co-repressors in MAX-mediated repression of germ cell-related genes in mouse embryonic stem cells. *PLoS ONE* *13*, e0205969. <https://doi.org/10.1371/journal.pone.0205969>.
- 647
- 648 59. Tahiliani, M., Mei, P., Fang, R., Leonor, T., Rutenberg, M., Shimizu, F., Li, J., Rao, A., and Shi, Y. (2007). The histone H3K4 demethylase SMCX links REST target genes to X-linked mental retardation. *Nature* *447*, 601–605. <https://doi.org/10.1038/nature05823>.
- 649
- 650 60. 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. *Molecular Cell* *38*, 576–589. <https://doi.org/10.1016/j.molcel.2010.05.004>.
- 651
- 652 61. Gajiwala, K.S., Chen, H., Cornille, F., Roques, B.P., Reith, W., Mach, B., and Burley, S.K. (2000). Structure of the winged-helix protein hRFX1 reveals a new mode of DNA binding. *Nature* *403*, 916–921. <https://doi.org/10.1038/35002634>.
- 653
- 654 62. Swoboda, P., Adler, H.T., and Thomas, J.H. (2000). The RFX-Type Transcription Factor DAF-19 Regulates Sensory Neuron Cilium Formation in *C. elegans*. *Molecular Cell* *5*, 411–421. [https://doi.org/10.1016/S1097-2765\(00\)80436-0](https://doi.org/10.1016/S1097-2765(00)80436-0).
- 655
- 656 63. Ashique, A.M., Choe, Y., Karlen, M., May, S.R., Phamluong, K., Solloway, M.J., Ericson, J., and Peterson, A.S. (2009). The Rfx4 Transcription Factor Modulates Shh Signaling by Regional Control of Ciliogenesis. *Sci. Signal.* *2*. <https://doi.org/10.1126/scisignal.2000602>.
- 657
- 658 64. Kistler, W.S., Baas, D., Lemeille, S., Paschaki, M., Seguin-Estevez, Q., Barras, E., Ma, W., Duteyrat, J.-L., Morlé, L., Durand, B., et al. (2015). RFX2 Is a Major Transcriptional Regulator of Spermiogenesis. *PLoS Genet* *11*, e1005368. <https://doi.org/10.1371/journal.pgen.1005368>.
- 659

- 660 65. Wu, Y., Hu, X., Li, Z., Wang, M., Li, S., Wang, X., Lin, X., Liao, S., Zhang, Z., Feng, X., et al.  
(2016). Transcription Factor RFX2 Is a Key Regulator of Mouse Spermiogenesis. *Sci Rep* 6, 20435.  
661 <https://doi.org/10.1038/srep20435>.
- 662 66. Otani, J., Nankumo, T., Arita, K., Inamoto, S., Ariyoshi, M., and Shirakawa, M. (2009). Structural basis  
for recognition of H3K4 methylation status by the DNA methyltransferase 3A ATRX–DNMT3–DNMT3L  
663 domain. *EMBO Reports* 10, 1235–1241. <https://doi.org/10.1038/embor.2009.218>.
- 664 67. Guo, X., Wang, L., Li, J., Ding, Z., Xiao, J., Yin, X., He, S., Shi, P., Dong, L., Li, G., et al. (2015).  
Structural insight into autoinhibition and histone H3-induced activation of DNMT3A. *Nature* 517,  
665 640–644. <https://doi.org/10.1038/nature13899>.
- 666 68. Meissner, A., Mikkelsen, T.S., Gu, H., Wernig, M., Hanna, J., Sivachenko, A., Zhang, X., Bernstein,  
B.E., Nusbaum, C., Jaffe, D.B., et al. (2008). Genome-scale DNA methylation maps of pluripotent and  
667 differentiated cells. *Nature* 454, 766–770. <https://doi.org/10.1038/nature07107>.
- 668 69. Nassar, L.R., Barber, G.P., Benet-Pagès, A., Casper, J., Clawson, H., Diekhans, M., Fischer, C.,  
Gonzalez, J.N., Hinrichs, A.S., Lee, B.T., et al. (2023). The UCSC Genome Browser database: 2023  
669 update. *Nucleic Acids Research* 51, D1188–D1195. <https://doi.org/10.1093/nar/gkac1072>.
- 670 70. Akalin, A., Kormaksson, M., Li, S., Garrett-Bakelman, F.E., Figueiroa, M.E., Melnick, A., and Mason,  
C.E. (2012). methylKit: A comprehensive R package for the analysis of genome-wide DNA methylation  
671 profiles. *Genome Biol* 13, R87. <https://doi.org/10.1186/gb-2012-13-10-r87>.
- 672 71. Torres-Padilla, M.-E. (2020). On transposons and totipotency. *Philos Trans R Soc Lond B Biol Sci*  
673 375, 20190339. <https://doi.org/10.1098/rstb.2019.0339>.
- 674 72. Yang, M., Yu, H., Yu, X., Liang, S., Hu, Y., Luo, Y., Izsvák, Z., Sun, C., and Wang, J. (2022). Chemical-  
induced chromatin remodeling reprograms mouse ESCs to totipotent-like stem cells. *Cell Stem Cell*  
675 29, 400–418.e13. <https://doi.org/10.1016/j.stem.2022.01.010>.
- 676 73. Zhang, J., Zhang, M., Acampora, D., Vojtek, M., Yuan, D., Simeone, A., and Chambers, I. (2018).  
OTX2 restricts entry to the mouse germline. *Nature* 562, 595–599. [018-0581-5](https://doi.org/10.1038/s41586-<br/>677 018-0581-5).
- 678 74. Weber, M., Hellmann, I., Stadler, M.B., Ramos, L., Pääbo, S., Rebhan, M., and Schübeler, D. (2007).  
Distribution, silencing potential and evolutionary impact of promoter DNA methylation in the human  
679 genome. *Nat Genet* 39, 457–466. <https://doi.org/10.1038/ng1990>.
- 680 75. Aubert, Y., Egolf, S., and Capell, B.C. (2019). The Unexpected Noncatalytic Roles of Histone Modifiers  
in Development and Disease. *Trends in Genetics* 35, 645–657. <https://doi.org/10.1016/j.tig.2019.06.004>.

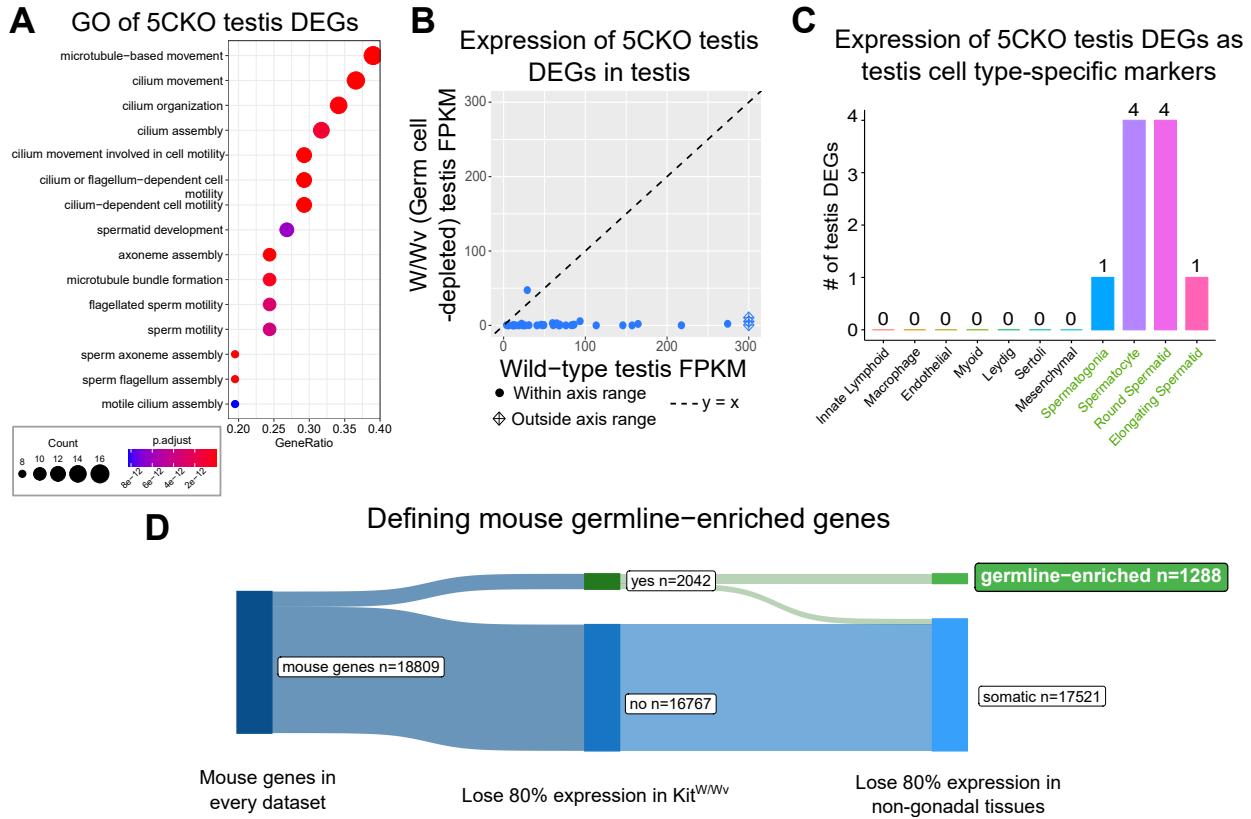
- 682 76. Morgan, M.A.J., and Shilatifard, A. (2020). Reevaluating the roles of histone-modifying enzymes  
and their associated chromatin modifications in transcriptional regulation. *Nat Genet* 52, 1271–1281.  
<https://doi.org/10.1038/s41588-020-00736-4>.
- 683
- 684 77. Long, H.K., King, H.W., Patient, R.K., Odom, D.T., and Klose, R.J. (2016). Protection of CpG  
islands from DNA methylation is DNA-encoded and evolutionarily conserved. *Nucleic Acids Res* 44,  
6693–6706. <https://doi.org/10.1093/nar/gkw258>.
- 685
- 686 78. Abildayeva, K., Berbée, J.F.P., Blokland, A., Jansen, P.J., Hoek, F.J., Meijer, O., Lütjohann, D., Gautier,  
T., Pillot, T., De Vente, J., et al. (2008). Human apolipoprotein C-I expression in mice impairs learning  
and memory functions. *Journal of Lipid Research* 49, 856–869. <https://doi.org/10.1194/jlr.M700518JLR200>.
- 687
- 688 79. Wang, D., Kennedy, S., Conte, D., Kim, J.K., Gabel, H.W., Kamath, R.S., Mello, C.C., and Ruvkun,  
G. (2005). Somatic misexpression of germline P granules and enhanced RNA interference in  
retinoblastoma pathway mutants. *Nature* 436, 593–597. <https://doi.org/10.1038/nature04010>.
- 689
- 690 80. Wu, X., Shi, Z., Cui, M., Han, M., and Ruvkun, G. (2012). Repression of germline RNAi pathways  
in somatic cells by retinoblastoma pathway chromatin complexes. *PLoS Genet* 8, e1002542. <https://doi.org/10.1371/journal.pgen.1002542>.
- 691
- 692 81. Nielsen, A.Y., and Gjerstorff, M.F. (2016). Ectopic Expression of Testis Germ Cell Proteins in Cancer  
and Its Potential Role in Genomic Instability. *Int J Mol Sci* 17. <https://doi.org/10.3390/ijms17060890>.
- 693
- 694 82. Adebayo Babatunde, K., Najafi, A., Salehipour, P., Modarressi, M.H., and Mobasheri, M.B. (2017).  
Cancer/Testis genes in relation to sperm biology and function. *Iranian Journal of Basic Medical  
Sciences* 20. <https://doi.org/10.22038/ijbms.2017.9259>.
- 695
- 696 83. Janic, A., Mendizabal, L., Llamazares, S., Rossell, D., and Gonzalez, C. (2010). Ectopic expression  
of germline genes drives malignant brain tumor growth in *Drosophila*. *Science* 330, 1824–1827.  
<https://doi.org/10.1126/science.1195481>.
- 697
- 698 84. Ghafouri-Fard, S., and Modarressi, M.-H. (2012). Expression of Cancer–Testis Genes in Brain Tumors:  
Implications for Cancer Immunotherapy. *Immunotherapy* 4, 59–75. <https://doi.org/10.2217/imt.11.145>.
- 699
- 700 85. Nin, D.S., and Deng, L.-W. (2023). Biology of Cancer-Testis Antigens and Their Therapeutic Implications  
in Cancer. *Cells* 12, 926. <https://doi.org/10.3390/cells12060926>.
- 701
- 702 86. Bonefas, K.M., and Iwase, S. (2021). Soma-to-germline transformation in chromatin-linked neurode-  
velopmental disorders? *FEBS J.* <https://doi.org/10.1111/febs.16196>.
- 703
- 704 87. Velasco, G., Walton, E.L., Sterlin, D., Hédouin, S., Nitta, H., Ito, Y., Fouyssac, F., Mégarbané, A.,  
Sasaki, H., Picard, C., et al. (2014). Germline genes hypomethylation and expression define a  
molecular signature in peripheral blood of ICF patients: Implications for diagnosis and etiology.  
*Orphanet J Rare Dis* 9, 56. <https://doi.org/10.1186/1750-1172-9-56>.
- 705

- 706 88. Walton, E.L., Francastel, C., and Velasco, G. (2014). Dnmt3b Prefers Germ Line Genes and Cen-  
707 tromeric Regions: Lessons from the ICF Syndrome and Cancer and Implications for Diseases. *Biology*  
708 (Basel) 3, 578–605. <https://doi.org/10.3390/biology3030578>.
- 709 89. Graham-Paquin, A.-L., Saini, D., Sirois, J., Hossain, I., Katz, M.S., Zhuang, Q.K.-W., Kwon, S.Y.,  
Yamanaka, Y., Bourque, G., Bouchard, M., et al. (2023). ZMYM2 is essential for methylation of  
germline genes and active transposons in embryonic development. *Nucleic Acids Research*, gkad540.  
<https://doi.org/10.1093/nar/gkad540>.
- 710 90. Stephens, M. (2016). False discovery rates: A new deal. *Biostat*, kxw041. <https://doi.org/10.1093/biostatistics/kxw041>.
- 711 91. Conway, J.R., Lex, A., and Gehlenborg, N. (2017). UpSetR: An R package for the visualization of  
intersecting sets and their properties. *Bioinformatics* 33, 2938–2940. <https://doi.org/10.1093/bioinformatics/btx364>.
- 712 92. Ross-Innes, C.S., Stark, R., Teschendorff, A.E., Holmes, K.A., Ali, H.R., Dunning, M.J., Brown, G.D.,  
Gojis, O., Ellis, I.O., Green, A.R., et al. (2012). Differential oestrogen receptor binding is associated  
713 with clinical outcome in breast cancer. *Nature* 481, 389–393. <https://doi.org/10.1038/nature10730>.
- 714 93. Yu, G., Wang, L.G., and He, Q.Y. (2015). ChIPseeker: An R/Bioconductor package for ChIP peak  
annotation, comparison and visualization. *Bioinformatics* 31, 2382–2383. <https://doi.org/10.1093/bioinformatics/btv145>.
- 715 94. Krueger, F., and Andrews, S.R. (2011). Bismark: A flexible aligner and methylation caller for Bisulfite-  
716 Seq applications. *Bioinformatics* 27, 1571–1572. <https://doi.org/10.1093/bioinformatics/btr167>.

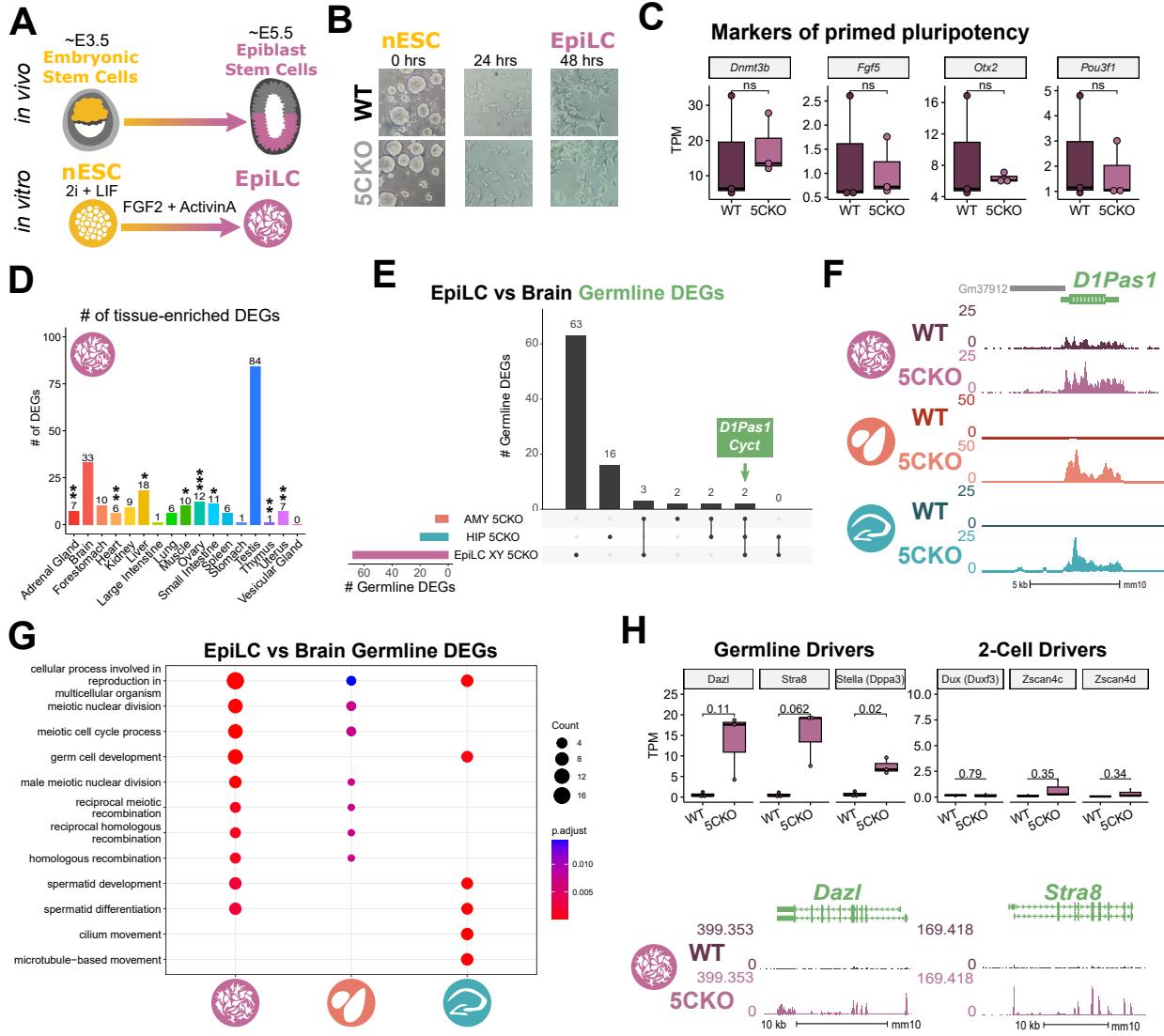
720 **Figures and Tables**



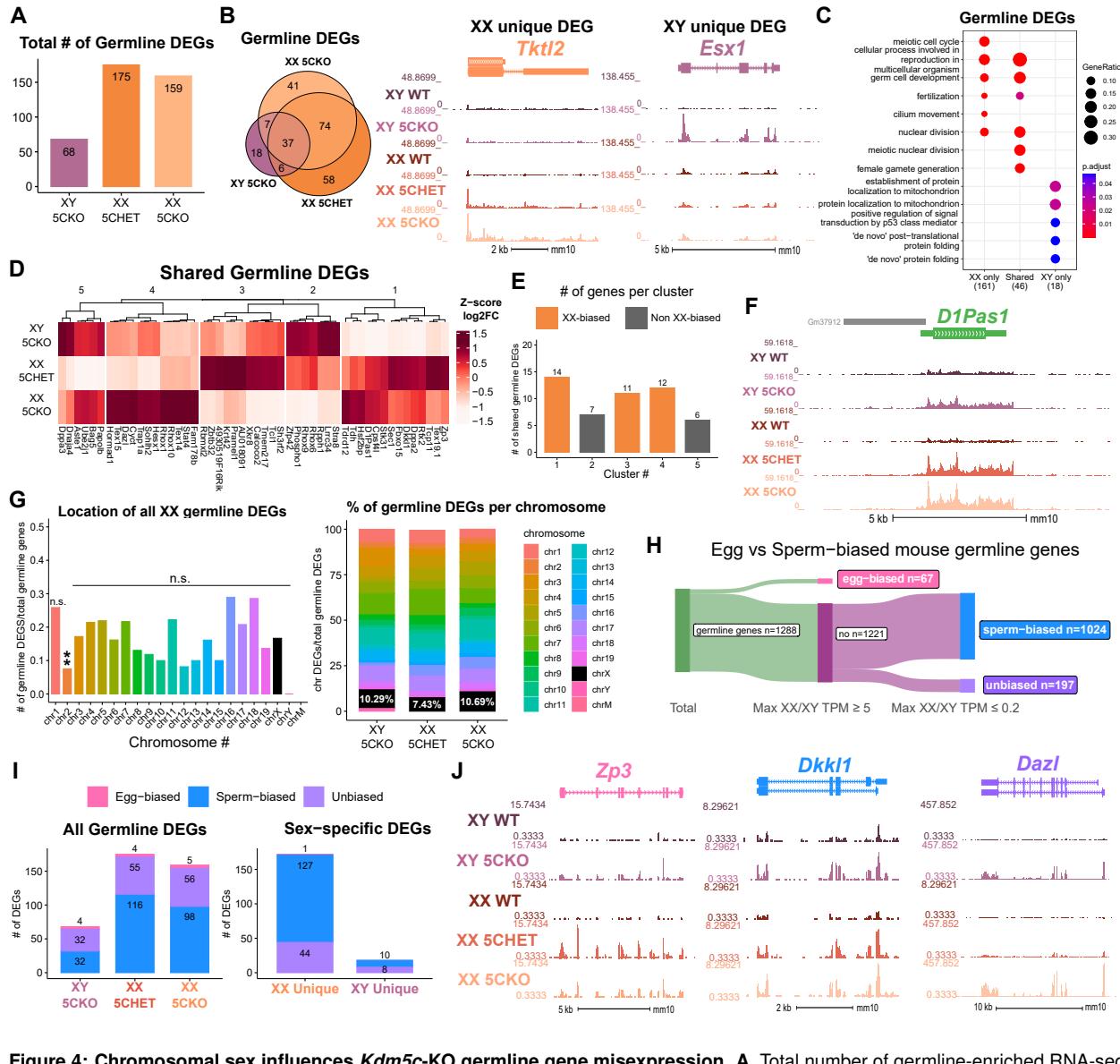
**Figure 1: Tissue-enriched genes are misexpressed in the *Kdm5c*-KO brain.** **A-B.** Expression of tissue-enriched genes (Li et al 2017) in the male *Kdm5c*-KO amygdala (A) and hippocampus (B). Left - MA plot of mRNA-sequencing. Right - Number of tissue-enriched differentially expressed genes (DEGs). \* p<0.05, \*\* p<0.01, \*\*\* p<0.001, Fisher's Exact Test. **C.** Left - UCSC browser view of an example aberrantly expressed testis-enriched DEG, *FK506 binding protein 6* (*Fkbp6*) in the wild-type (WT) and *Kdm5c*-KO (5CKO) amygdala (red) and hippocampus (teal) (Average, n = 4). Right - Expression of *Cyct* in wild-type tissues from NCBI Gene, with testis highlighted in blue and brain tissues highlighted in red. **D.** Left - UCSC browser view of an example ovary-enriched DEG, *Zygotic arrest 1* (*Zar1*). Right - Expression of *Zar1* in wild-type tissues from NCBI Gene, with ovary highlighted in teal and brain tissues highlighted in red. **E.** Left - UCSC browser view of an example liver-enriched DEG, *Apolipoprotein C-I* (*Apoc1*). Right - Expression of *Apoc1* in wild-type tissues from NCBI Gene, with liver highlighted in orange and brain tissues highlighted in red.



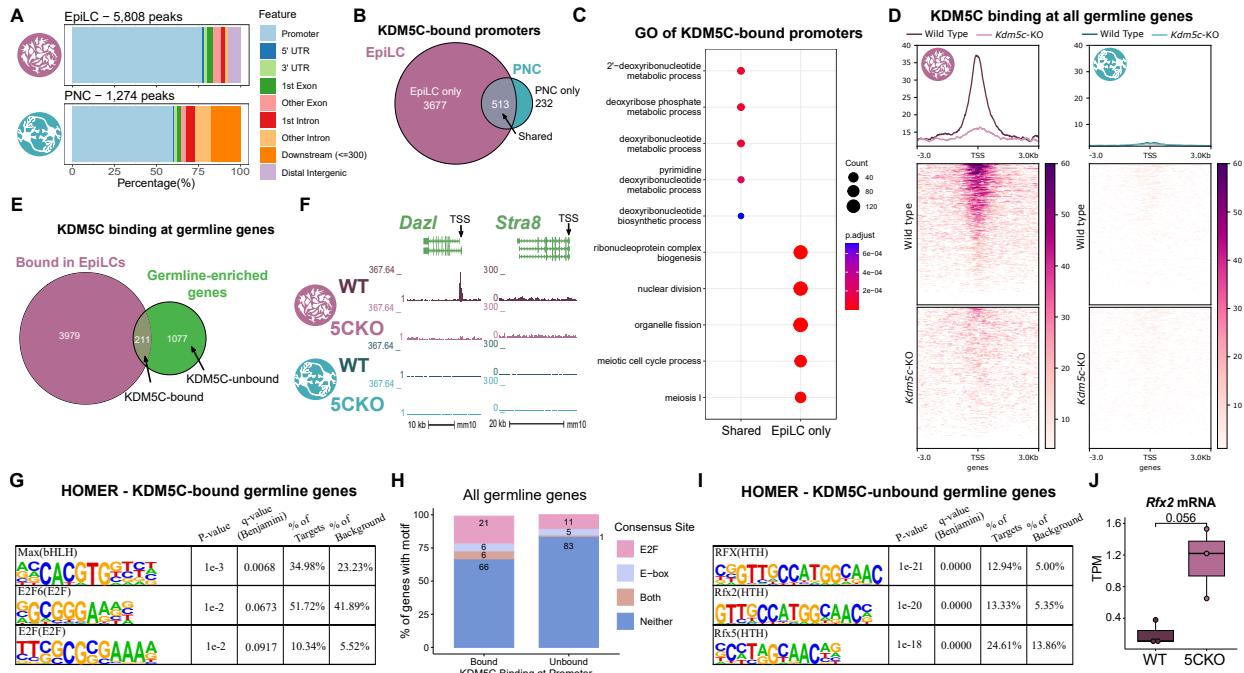
**Figure 2: Aberrant transcription of germline genes in the *Kdm5c*-KO in the brain.** **A.** enrichPlot gene ontology (GO) of *Kdm5c*-KO amygdala and hippocampus testis-enriched DEGs **B.** Expression of testis DEGs in wild-type (WT) versus germ cell-depleted (W/W<sup>v</sup>) testis (Mueller et al 2013). Expression is in Fragments Per Kilobase of transcript per Million mapped reads (FPKM). **C.** Number of testis DEGs that were classified as cell-type specific markers in a single cell RNA-seq dataset of the testis (Green et al 2018). Germline cell types are highlighted in green, somatic cell types in black. **D.** Sankey diagram of mouse genes filtered for germline enrichment based on their expression in wild-type and W/W<sup>v</sup> mice and in adult mouse non-gonadal tissues (Li et al 2017).



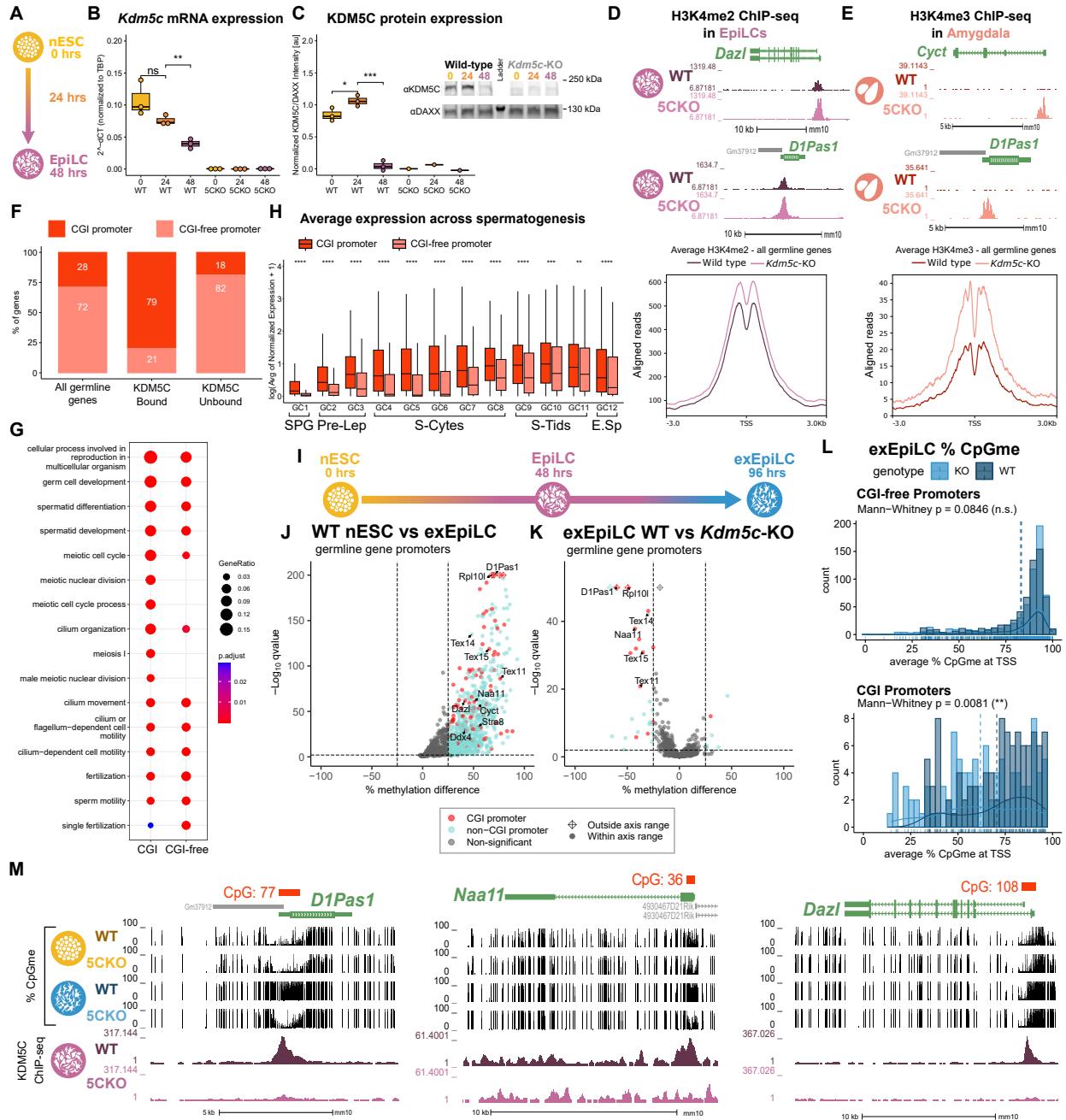
**Figure 3: Kdm5c-KO epiblast-like cells express key drivers of germline identity** **A.** Top - Diagram of *in vivo* differentiation of embryonic stem cells (ESCs) of the inner cell mass into epiblast stem cells. Bottom - *in vitro* differentiation of ESCs into epiblast-like cells (EpiLCs). **B.** Representative images of male wild-type (WT) and *Kdm5c*-KO (5CKO) cells during ESC to EpiLC differentiation. Brightfield images taken at 20X. **C.** No significant difference in primed pluripotency marker expression in wild-type versus *Kdm5c*-KO EpiLCs. Welch's t-test, expression in transcripts per million (TPM). **D.** Number of tissue-enriched differentially expressed genes (DEGs) in *Kdm5c*-KO EpiLCs. \*  $p < 0.05$ , \*\*  $p < 0.01$ , \*\*\*  $p < 0.001$ , Fisher's Exact Test. **E.** Upset plot displaying the overlap of germline DEGs expressed in *Kdm5c*-KO EpiLCs, amygdala (AMY), and hippocampus (HIP) RNA-seq datasets. **F.** UCSC browser view of an example germline gene, *D1Pas1*, that is dysregulated *Kdm5c*-KO EpiLCs (top, purple. Average,  $n = 3$ ), amygdala (middle, red. Average,  $n = 4$ ), and hippocampus (bottom, blue. Average,  $n = 4$ ). **G.** enrichPlot gene ontology analysis comparing enriched biological processes for *Kdm5c*-KO EpiLC, amygdala, and hippocampus germline DEGs. **H.** Top left - Example germline identity DEGs unique to EpiLCs. Top right - Example 2-cell genes that are not dysregulated in *Kdm5c*-KO EpiLCs. p-values for Welch's t-test. Bottom - UCSC browser view of *Dazl* and *Stra8* expression in wild-type and *Kdm5c*-KO EpiLCs (Average,  $n = 3$ ).



**Figure 4: Chromosomal sex influences *Kdm5c*-KO germline gene misexpression.** **A.** Total number of germline-enriched RNA-seq DEGs for male hemizygous *Kdm5c* knockout EpilCs (XY 5CKO, purple), female heterozygous *Kdm5c* knockout (XX 5CHET, orange), female homozygous *Kdm5c* knockout (XX 5CKO, light orange) EpilCs. **B.** Left - Eulerr overlap of *Kdm5c* mutant male and female EpilCs germline DEGs. Right - Example of germline DEGs unique to females or males, *Tktl2* and *Esx1*. **C.** enrichPlot gene ontology analysis comparing enriched biological processes for germline DEGs shared between *Kdm5c* mutant males and females (Shared), or unique to one sex (XX only or XY only). **D.** Heatmap of germline DEGs shared between male and female mutants. Color is the average log 2 fold change from sex-matched wild-type, z-scored across rows. **E.** Number of genes within each cluster from D. Clusters with higher expression in females compared to males (XX-biased) highlighted in orange. **F.** UCSC browser view of a male and female shared germline DEG *D1Pas1* that is more highly expressed in female mutants (Average, n = 3). **G.** Left - Number of all female germline DEGs located on each chromosome over the total number of germline-enriched genes on that chromosome. Right - Percentage of germline DEGs that lie on each chromosome for each *Kdm5c* mutant. X chromosome highlighted in black. **H.** Sankey diagram classifying egg-biased (pink) and sperm-biased (blue) and unbiased (purple) mouse germline-enriched genes. **I.** Number of egg, sperm, or unbiased germline DEGs for male and female *Kdm5c* mutants. **J.** UCSC browser view of egg-biased (*Zp3*), sperm-biased (*Dkk1*), and unbiased (*Dazl*) germline genes dysregulated in both male and female *Kdm5c* mutants (Average of n = 3).



**Figure 5: KDM5C binds to a subset of germline gene promoters during early embryogenesis.** **A.** ChIPseeker localization of KDM5C peaks at different genomic regions in EpiLCs (top) and hippocampal and cortex primary neuron cultures (PNCs, bottom). **B.** Overlap of genes with KDM5C bound to their promoters ( $TSS \pm 500$ ) in EpiLCs (purple) and PNCs (blue). **C.** Gene ontology (GO) comparison of genes with KDM5C bound to their promoter in EpiLCs and PNCs. Genes were classified as either bound in EpiLCs only (EpiLC only), unique to PNCs (PNC only, no significant ontologies) or bound in both PNCs and EpiLCs (Shared). **D.** Average KDM5C binding around the transcription start site (TSS) of all germline-enriched genes in EpiLCs (left) and PNCs (right). **E.** Eulerr overlap of germline-enriched genes (green) with significant KDM5C binding at their promoter in EpiLCs (purple). **F.** Example KDM5C ChIP-seq signal around the *Dazl* TSS but not *Stra8* in EpiLCs. **G.** HOMER motif analysis of all KDM5C-bound germline gene promoters, highlighting significant enrichment of MAX, E2F6, and E2F motifs. **H.** Number of all gene promoters bound or unbound by KDM5C with instances of the E2F or E-box consensus sequence. **I.** HOMER motif analysis of all KDM5C-unbound germline gene promoters, highlighting significant enrichment of RFX family transcription factor motifs. **J.** Expression of RNA-seq DEG *Rfx2* in wild-type and *Kdm5c*-KO EpiLCs. P-value of Welch's t-test, expression in transcripts per million (TPM).



**Figure 6: KDM5C promotes long-term silencing of germline genes via DNA methylation at CpG islands.** **A.** Diagram of embryonic stem cell (ESC) to epiblast-like cell (EpiLC) differentiation and collection time points. **B.** Real time quantitative PCR (RT-qPCR) of *Kdm5c* mRNA expression in wild-type (WT) and *Kdm5c*-KO (5CKO) ESCs at 0, 24, and 48 hours of differentiation into EpiLCs. Expression calculated in comparison to TBP mRNA expression ( $2^{-\Delta\Delta CT}$ ). \*  $p < 0.05$ , \*\*  $p < 0.01$ , \*\*\*  $p < 0.001$ , Welch's t-test. **C.** KDM5C protein expression normalized to DAXX. Quantified intensity using ImageJ (artificial units - au). Right - representative lanes of Western blot for KDM5C and DAXX. \*  $p < 0.05$ , \*\*  $p < 0.01$ , \*\*\*  $p < 0.001$ , Welch's t-test. **D.** Top - Representative UCSC browser view of histone 3 lysine 4 dimethylation (H3K4me2) ChIP-seq signal at two germline genes in wild-type and *Kdm5c*-KO EpiLCs. Bottom - Average H3K4me2 signal at the TSS of all germline-enriched genes in wild-type (dark purple) and *Kdm5c*-KO (light purple) EpiLCs. **E.** Top - Representative UCSC browser view of histone 3 lysine 4 trimethylation (H3K4me3) ChIP-seq signal at two germline genes in the wild-type (WT) and *Kdm5c*-KO (5CKO) adult amygdala. Bottom - Average H3K4me3 signal at the transcription start site (TSS) of all germline-enriched genes in wild-type (dark red) and *Kdm5c*-KO (light red) amygdala. **F.** Percentage of germline genes that harbor CpG islands (CGIs) in their promoters (CGI promoter, red), based on UCSC annotation. Left - percentage of all germline-enriched genes, middle - KDM5C-bound germline genes, and right - KDM5C-unbound germline genes. (Legend continued on next page.)

**Figure 6: KDM5C promotes long-term silencing of germline genes via DNA methylation at CpG islands.** (Legend continued.) **G.** enrichPlot gene ontology analysis of germline genes with (CGI-promoter) or without (CGI-free) CGIs in their promoter. **H.** Expression of germline genes with (CGI-promoter, red) or without (CGI-free, salmon) CGIs in their promoter across stages of spermatogenesis from Green et al 2018. SPG - spermatogonia, Pre-Lep - preleptotene spermatocytes, S-Cytes - meiotic spermatocytes, S-Tids - post-meiotic haploid round spermatids, E.Sp - elongating spermatids. Wilcoxon test, \*  $p < 0.05$ , \*\*  $p < 0.01$ , \*\*\*  $p < 0.001$ , \*\*\*\*  $p < 0.0001$ . **I.** Diagram of ESC to extended EpiLC (exEpiLC) differentiation. **J.** Volcano plot of whole genome bisulfite sequencing (WGBS) comparing CpG methylation (CpGme) at germline gene promoters ( $TSS \pm 500$ ) in wild-type (WT) ESCs versus exEpiLCs. Significantly differentially methylated promoters ( $q < 0.01$ ,  $|methylated difference| > 25\%$ ) with CGIs in red, CGI-free promoters in light blue **K.** Volcano plot of WGBS of wild-type (WT) versus *Kdm5c*-KO exEpiLCs for germline gene promoters. Promoters with CGIs in red, CGI-free promoters in light blue. **L.** Histogram of average percent CpGme at the promoter of germline genes with or without CGIs. Wild-type in navy and *Kdm5c*-KO (KO) in light blue. Dashed lines are average methylation for each genotype, p-values for Mann-Whitney U test. **M.** UCSC browser view of germline genes, showing UCSC annotated CGI with number of CpGs, representative CpGme in wild-type (WT) and *Kdm5c*-KO (5CKO) ESCs and exEpiLCs, and KDM5C ChIP-seq signal in EpiLCs.