- Supplement The X-linked intellectual disability gene KDM5C
- is a sex-biased brake against germline programs in somatic

3 lineages

Supplementary Tables

Supplementary table 1: Misexpression of tissue-enriched genes within the *Kdm5c*-KO brain. 1)

Genes upregulated in the Kdm5c-KO hippocampus, with tissue-enriched genes annotated 2) Same as 1

but for the Kdm5c-KO amygdala 3) Results of the Fisher exact test for enrichment tissue-specific genes. 4)

9 enrichPlot gene ontology results of testis-enriched genes misexpressed in the amygdala or hippocampus.

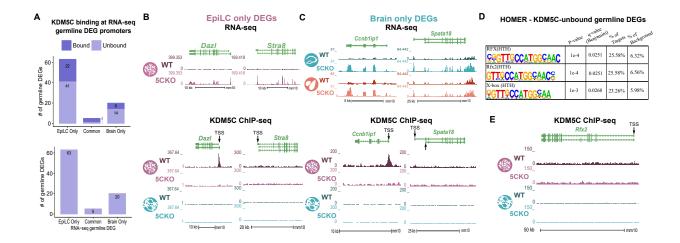
Supplementary table 2: Mouse germline-enriched genes. List of mouse germline-enriched genes identified in this study (see Methods). Includes whether germline gene promoters have KDM5C ChIP-seq peaks and CpG islands.

Supplementary table 3: Germline gene misexpression in *Kdm5c* mutant EpiLCs. 1) Genes upregulated in male *Kdm5c*-KO (*Kdm5c*-/y) epiblast-like cells (EpiLCs), with annotations for tissue-enriched genes. 2) Results of Fisher exact test on XY EpiLC tissue-enriched genes. 3) enrichPlot gene ontology results of germline-enriched genes misexpressed in male *Kdm5c*-KO EpiLCs, amygdala, and hippocampus. 4) Germline genes misexpressed in XY *Kdm5c*-KO (*Kdm5c*-/y), XX *Kdm5c*-KO (*Kdm5c*-/-), and XX *Kdm5c*-HET (*Kdm5c*-/-) EpiLCs. 5) enrichPlot gene ontology results of germline genes misexpressed in male versus female *Kdm5c* mutant EpiLCs

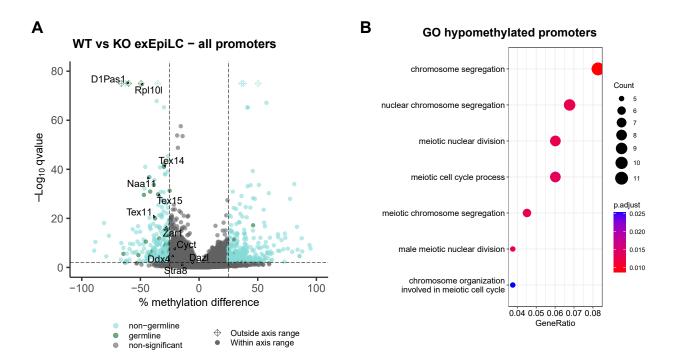
Supplementary table 4: KDM5C ChIP-seq in EpiLCs and PNCs. 1) enrichPlot gene ontology results of KDM5C-bound promoters (see Methods) in epiblast-like cells (EpiLCs) and forebrain primary neuron cultures (PNCs). 2) HOMER known motif analysis of germline gene promoters (TSS \pm 500 bp) bound by KDM5C . 3) HOMER known motif analysis of germline genes not bound by KDM5C.

Supplementary table 5: Germline gene CpG islands and promoter CpG methylation. 1) enrichPlot gene ontology results of germline genes with and without CpG islands (CGIs) within their promoter (TSS \pm 500 bp). 2) methylKit whole genome bisulfite sequencing (WGBS) results table comparing germline gene promoter CpG methylation (TSS \pm 500 bp) in male wild-type ESCs versus wild-type extended EpiLCs (exEpiLCs). 3) methylKit WGBS results table comparing germline gene promoter CpG methylation in male Kdm5c-KO versus wild-type exEpiLCs. 4) enrichPlot gene ontology results of promoters hypomethylated in

- *Kdm5c*-KO exEpiLCs
- 31 Supplementary Figures



Supplementary Figure 1: KDM5C binds to a subset of germline RNA-seq differentially expressed genes. A. Bar graph of the number of germline-enriched DEGs with promoter KDM5C ChIP-seq peaks in wild-type EpiLCs (Top) and PNCs (Bottom). RNA-seq DEGs were classified as shared betwen EpiLCs and the brain (Common), unique to EpiLCs (EpiLC Only), or unique to one or multiple brain regions (Brain Only). B. Average bigwigs of two example RNA-seq DEGs dysregulated in EpiLCs but not the brain, *Dazl* and *Stra8*. Top is the RNA-seq tracks for wild-type (WT) and *Kdm5c*-KO (5CKO) EpiLCs, bottom is the KDM5C ChIP-seq tracks, with the annotated transcription start site (TSS) for each gene. C. Same as B but for two example DEGs only dysregualted in the brain and not expressed in EpiLCs, *Ccnb1ip1* and *Spata18*. D. HOMER motif analysis of all KDM5C-unbound germline DEGs shows significant enrichment of multiple RFX members and their X-box motif. E. KDM5C ChIP-seq shows no KDM5C accumulation at the *Rfx2* promoter in EpiLCs or PNCs.



Supplementary Figure 2: Loss of KDM5C impairs CpG methylation of germline gene promoters. A. Volcano plot of whole genome bisulfite sequencing (WGBS) for all gene promoters in wild-type (WT) versus *Kdm5c*-KO extended EpiLCs (exEpiLCs). Significantly differentially methylated promoters (q < 0.01, |methylation difference| > 25%). Germline promoters highlighted in green, non-germline promoters in light blue, non-significant promoters in gray. B. enrichPlot gene ontology of all promoters significantly hypomethylated in *Kdm5c*-KO exEpiLCs.