Supplementary Figures, Table and legends:

Includes:

Figure S1.

(A) Western blot on nuclear extracts and Flag-IP from WT or EZH1-Flag mouse testes probed with anti-EZH1. (B) Volcano plot representation of EZH1 interactome from EZH1-Flag mice testes IP compared to WT. In red are all the core complex subunits, in green the cofactors and in blue GPIF. (C) Anti-FLAG WB analysis on nuclear extracts from Hela-S3 cells stably overexpressing *Mus Musculus* or *Homo Sapiens* GPIF and corresponding control; HDAC1 is used as a loading control. (D) Volcano plot representation of hGPIF interactome after Flag-IP on HeLa-S3 overexpressing a tagged version of the protein. Same color code as in (B), additional interactors in black. (E) Phylogenetic tree representing GPIF protein sequence across placental mammals (Phylogenetic Analysis by Maximum Likelihood (PAML) algorithm). (F) PRC2 components and cofactors expression in mouse somatic and germ cells [1]) (GSE89711). m: male, f: female, PGC: primordial germ cells, SOMA: Somatic cells (mean ± s.d., n= 3). (G) Expression of *GPIF*, *PIWIL2*, *EZH2*, *JARID2* in human PGCs and somatic cells at week 7 [2] (NCBI SRA: SRP057098; mean ± s.d., n≥ 3).

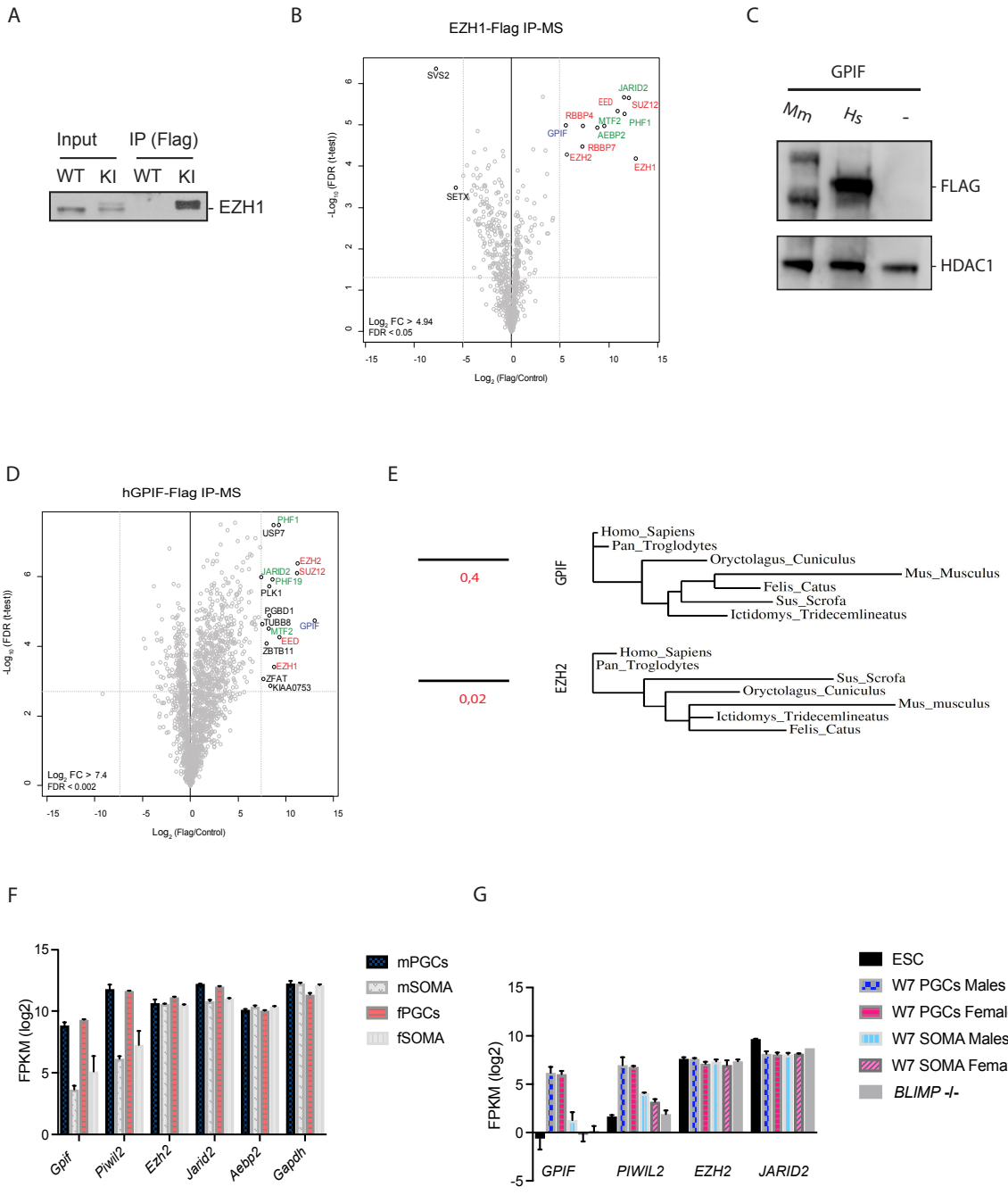

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Figure S2.

(A) Human Protein Atlas cell lines transcripts analysis. (B) Western blot analysis of H3K27me3 and H3 (loading control) on 293T nuclear extract and U2OS nuclear extracts WT, *EED* -/- and *GPIF* -/- (top panel). Bottom panel, same as above for U2OS extracts but loaded with more proteins to detect H3K27me3 in WT condition; (C) RT-qPCR to detect the overexpression of *GPIF* mutants in U2OS (control for figure 2C). (D) Different *GPIF* C-ter truncations were expressed in HEK-293 cells (top panel). Co-IP (Flag-IP) was analyzed by western blot with the specific antibodies indicated on the right (bottom panel). (E) Correlation heatmap for H3K27me3 ChIP-seq in U2OS WT, *EED* -/- and *GPIF* -/- (F) Heatmap representing H3K27me3 enrichment in the subset of genes that are downregulated upon knockout of *GPIF* (Bottom). Top is the heatmap quantification.

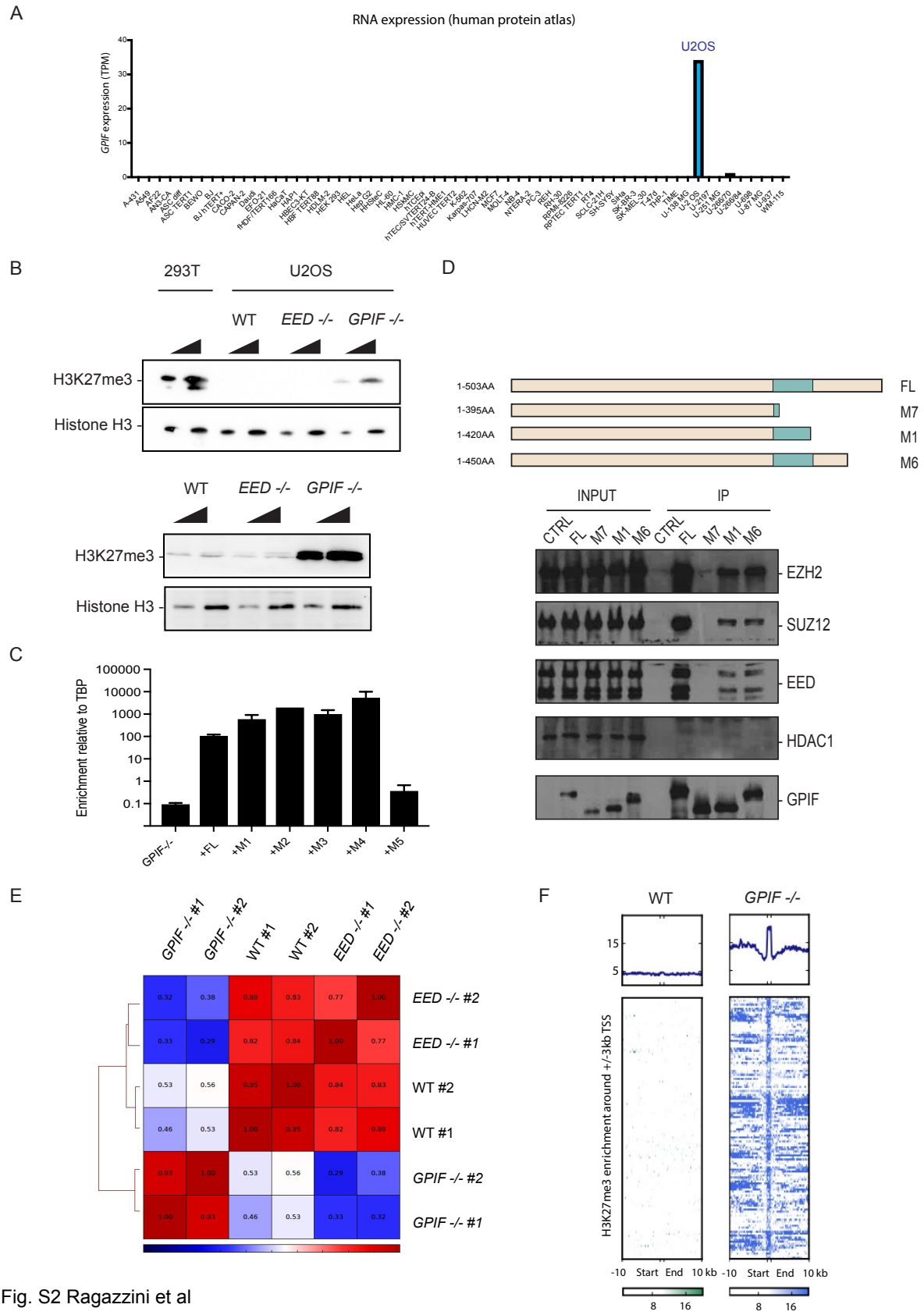

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Figure S3 (related to figure 3).

(A) Left: Scheme for hGPIF purification from Sf-9 insect cells. Right: Coomassie staining of purified protein. (B) HKMT assay performed with rPRC2-EZH2 on native nucleosomes purified from HeLa in presence of increasing amount of GPIF. (C) Flag-IPs on extracts of U2OS (WT, WT + Flag-EZH2, GPIF -/- + Flag-EZH2) analyzed by western blot and probed with an antibody recognizing EZH2. D) Volcano plot representation of normalized mass spectrometry data after Flag-IP in U2OS+ Flag-EZH2 [3], U2OS *GPIF* -/- Flag-EZH2 (Right), same color code as in Fig.1 and S1.

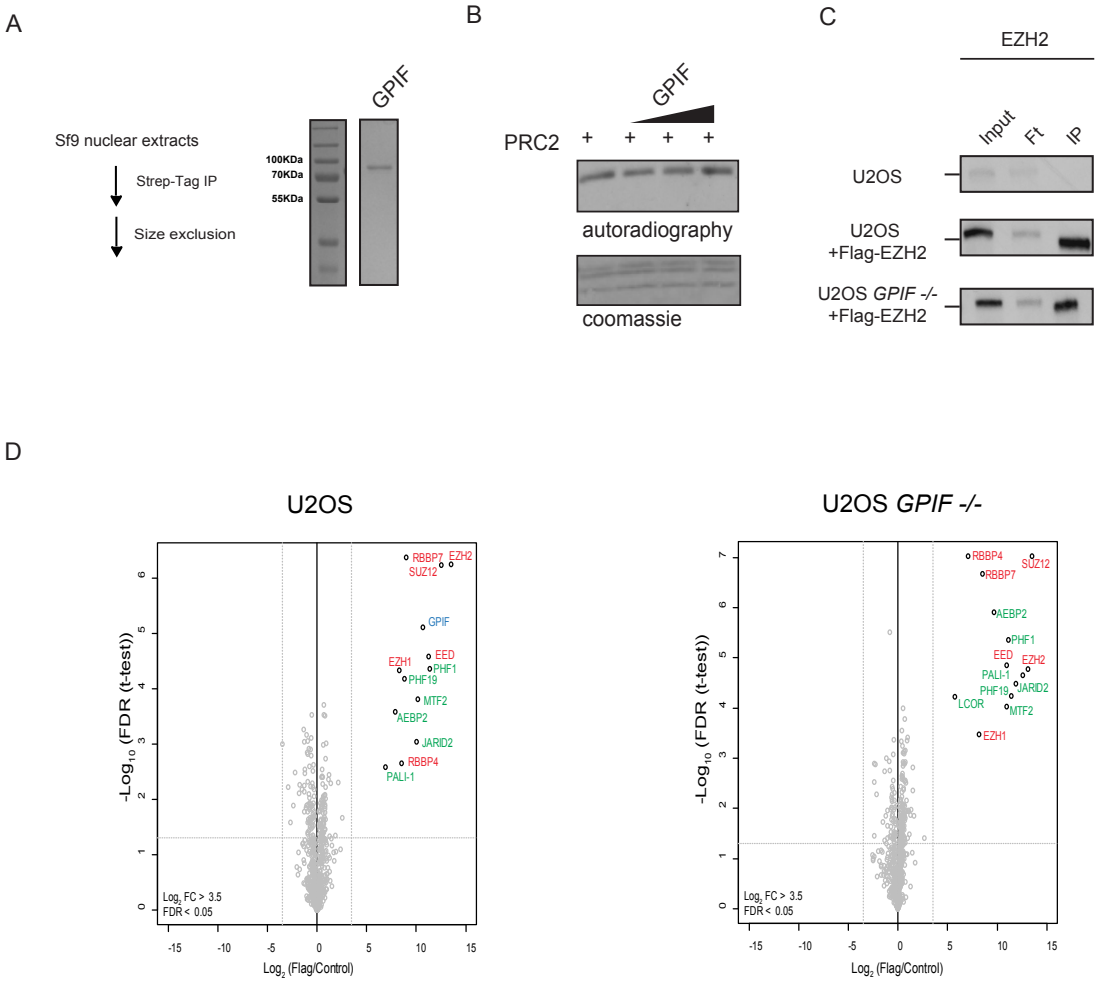

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Figure S4.

(A) Schematic representation of *Gpif* locus, scissors indicate the deletion generated by genome editing in mice. At the bottom is a representative sequencing of this locus in founders. (B) *Nudt10*, *Gpif*, *Nudt11* and *Ezh2* mRNA relative abundancy normalized to *Gapdh* in whole testis from adult male mice WT and *Gpif* -/- (mean ± s.d., n= 3). (C) WB analysis with mouse anti-GPIF on WT and *Gpif* -/- testis nuclear extracts. Arrows indicate specific signal. (D) *Nudt10*, *Gpif* and *Nudt11* mRNA normalized to *Gapdh* mRNA in whole ovaries from adult female mice WT and *Gpif* -/- (mean ± s.d., n= 3). (E) RT-qPCR analysis of *Gpif* and *Ezh2* mRNA expression during spermatogenesis. *Gpif* and *Ezh2* mRNA are normalized to *Tbp*. The different spermatogenic populations (undifferentiated spermatogonia kit-, differentiating spermatogonia kit+, 4N, 2N, N) have been sorted by FACS (mean ± s.d., n= 2). (G) Mice testis absolute weight (mg) (mean ±SD, n≥ 3). (F) Western blot analysis of H3K27me3 and H3 levels on whole testis extracts of WT; *Dnmt3l* -/- and *Gpif* -/-; *Dnmt3 l*-/- mice.

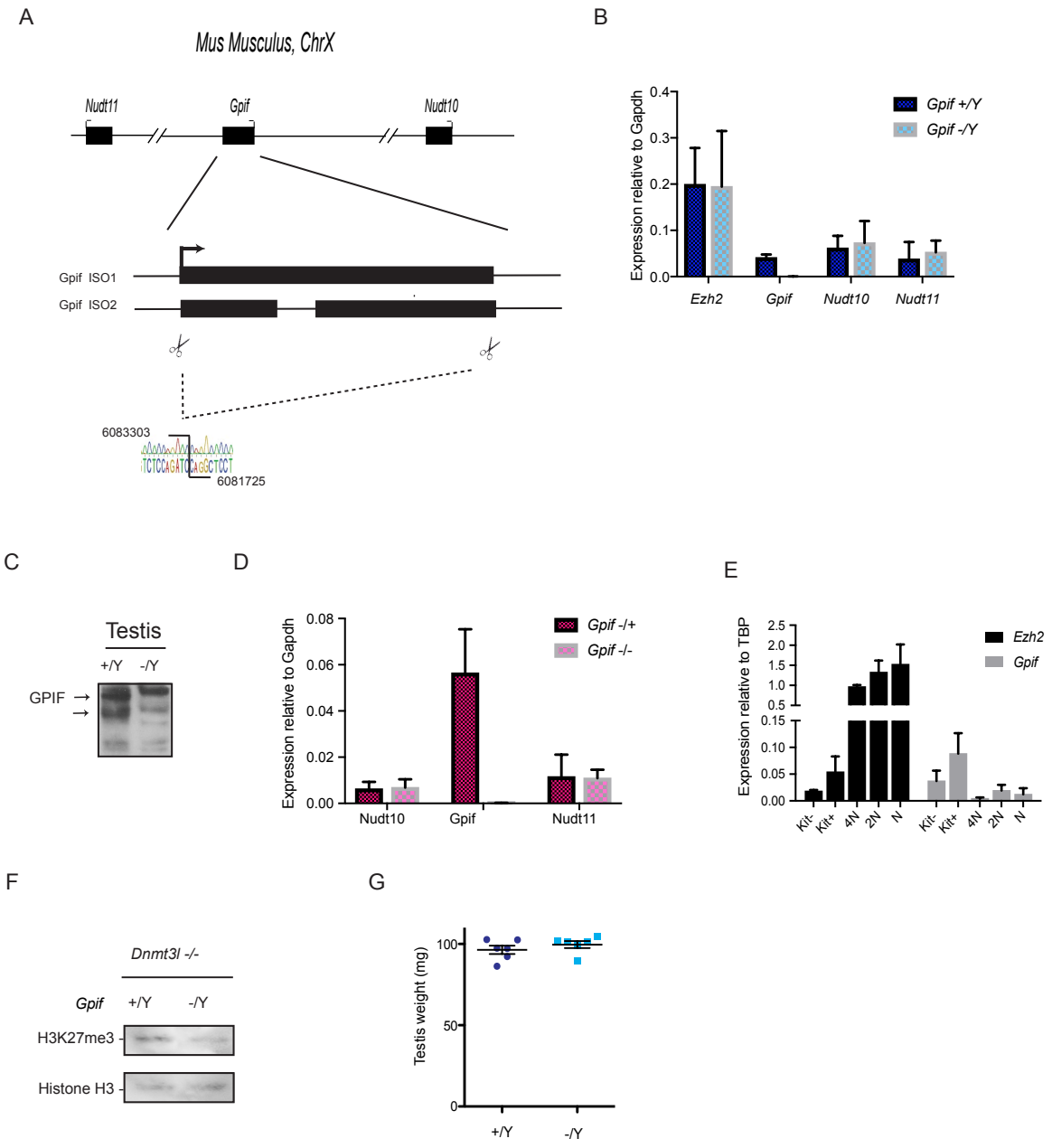

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Figure S5.

(A) Non-surrounded Nuclei (NSN) WT and *Gpif* -/- oocytes were fixed and stained for H3K27me3 (green in merged). DNA was stained with DAPI (blue in merged). IF quantification is indicated on the right, *n* number of oocytes analyzed, H3K27me3 intensities are normalized to DAPI. (B) Same as in (A) but Surrounded Nuclei (SN) oocytes were probed for H3K4me3.

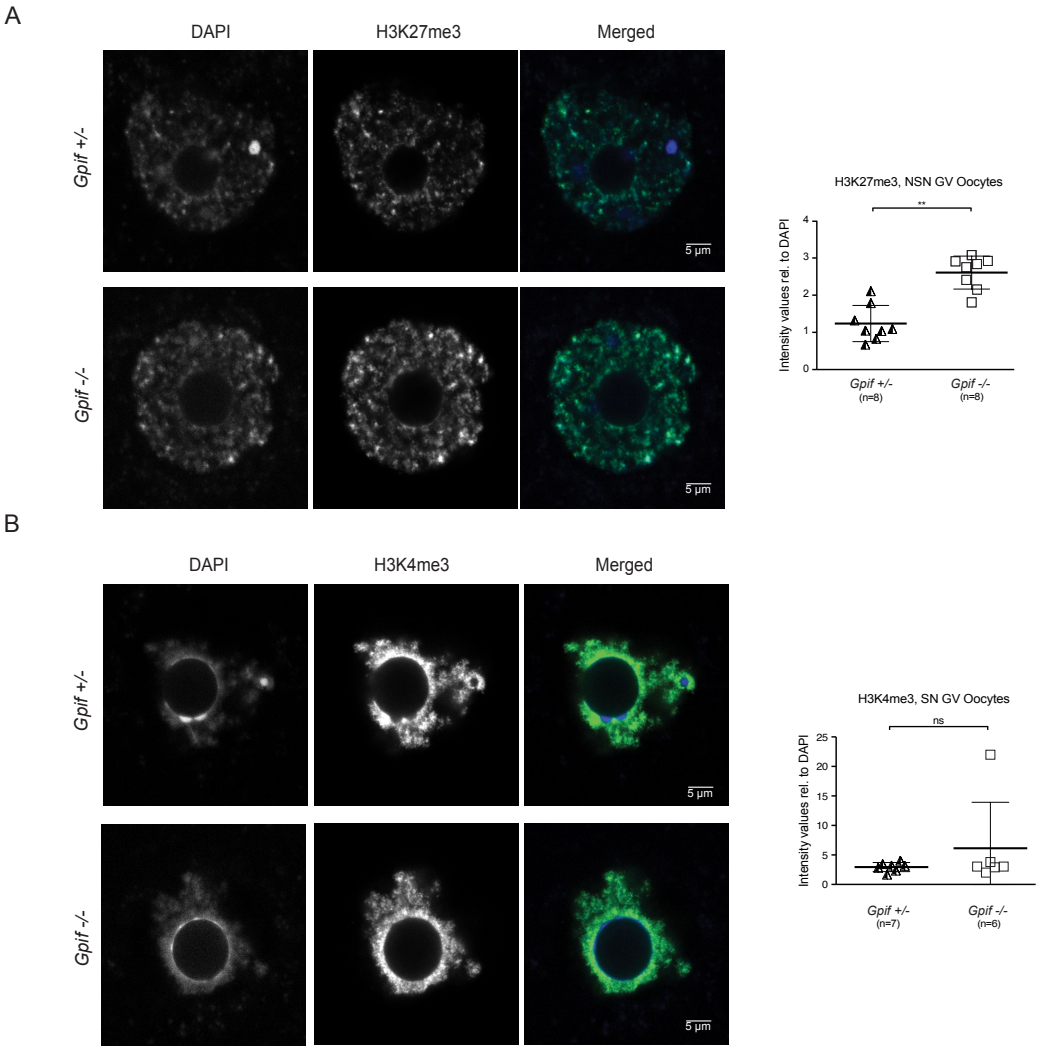

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Table S1.
Primer and gRNA sequences.

Name	Application	Sequence
mGapdh FW	RT-qPCR	AACAGCAACTCCCACTCTTC
mGapdh REV	RT-qPCR	TGGTCCAGGGTTTCTTACTC
mEzh2 FW	RT-qPCR	AATACATGTGCAGCTTTCTGTTC
mEzh2 REV	RT-qPCR	ACGAATTTTGTTGCCCTTTC
mAu022751 FW	RT-qPCR	TTCCGGAGTTGTACCTTTCG
mAu022751 REV	RT-qPCR	ACGTAAATTCCAGCCTGTGC
mNudt10 FW	RT-qPCR	AGAGAGCGAGCCCTAGTGAATGGA
mNudt10 REV	RT-qPCR	GAGCTCACCTGTGCTTCACAATTCC
mNudt11 FW	RT-qPCR	ACCGAGGCATGCTCAAGATCACA
mNudt11 REV	RT-qPCR	TGAGCGGTCTCCTTGGCAACCTTA
hVSX2 FW	ChIP-qPCR	AAGCGCTGAGCAAGCCCAAATC
hVSX2 REV	ChIP-qPCR	GCTCCTTGTTCAAGCCCAGGATCT
hDRGX FW	ChIP-qPCR	AAAACATCGCCGGCTGTCAGATCG
hDRGX REV	ChIP-qPCR	TCGCTGTTTGCTTTGCAGCCA
hKRT1 FW	ChIP-qPCR	TGGTCCTGCGCTGGTAGTTGATGA
hKRT1 REV	ChIP-qPCR	AGTTCCAGGTCTGGGTACCGAAGT
hMEGF11 FW	ChIP-qPCR	TGGTGCACTTGAACCAGTTGAGGA
hMEGF11 REV	ChIP-qPCR	TCCAGGAATCGTATGCACACCCCT
hSTAMBP FW	ChIP-qPCR	AAGGAGGCCTCGTGAGAGAT
hSTAMBP REV	ChIP-qPCR	CGGGTCACAATTCCTCCACA
hMCC FW	ChIP-qPCR	ACATGTGTCAACACTGGGCA
hMCC REV	ChIP-qPCR	TGGGGCATCCTCATTTTCCC
hGAPDH FW	ChIP-qPCR	CTTCAACAGCGACACCCACT
hGAPDH REV	ChIP-qPCR	GTGGTCCAGGGGTCTTACTC
hGPIF N-ter FW	RT-qPCR	ACCTCCGCCGCCATTTTCATCA
hGPIF N-ter REV	RT-qPCR	TCGGGCACCACACACCCAAAAA
hGPIF stretch FW	RT-qPCR	GCCTGTTTGGCATGCAGTCCGTAT
hGPIF stretch REV	RT-qPCR	ACTGCTGAGGGATGGGAAGGAAGA

hGAPDH REV	**ChIP-qPCR**	**GTGGTCCAGGGGTCTTACTC**
hGPIF N-ter FW	RT-qPCR	ACCTCCGCCGCCATTTTCATCA
hGPIF N-ter REV	RT-qPCR	TCGGGCACCACACACCCAAAAA
hGPIF stretch FW	RT-qPCR	GCCTGTTTGGCATGCAGTCCGTAT
hGPIF stretch REV	RT-qPCR	ACTGCTGAGGGATGGGAAGGAAGA

Supplemental References:

1. Percharde, M., Wong, P. & Ramalho-Santos, M. Global Hypertranscription in the Mouse Embryonic Germline. *Cell Rep* **19**, 1987-1996 (2017).
2. Tang, W.W. et al. A Unique Gene Regulatory Network Resets the Human Germline Epigenome for Development. *Cell* **161**, 1453-67 (2015).
3. Steger, D.J. et al. DOT1L/KMT4 recruitment and H3K79 methylation are ubiquitously coupled with gene transcription in mammalian cells. *Mol Cell Biol* **28**, 2825-39 (2008).