Supporting Information

The polycomb group protein PCGF6 mediates germline gene silencing by recruiting histone-modifying proteins to target gene promoters

Mengjie Liu[1], Yaru Zhu[1], Fei Xing[2], Shuang Liu[1], Yin Xia[3], Qing Jiang[4], Jinzhong Qin[1]*

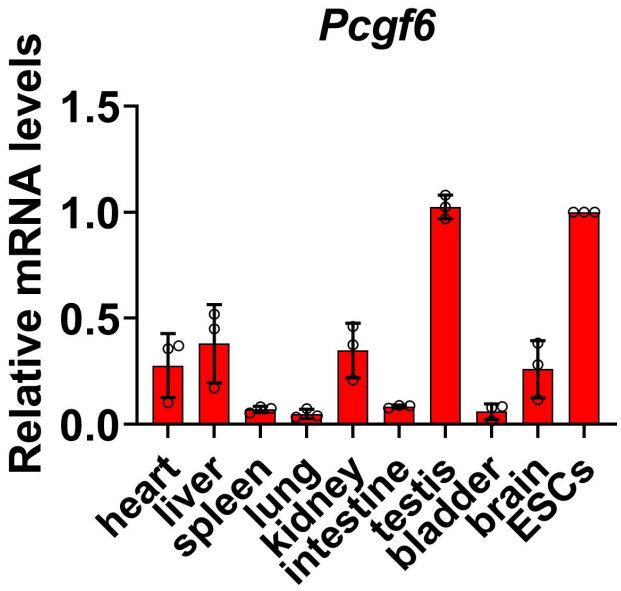

Figure S1. Transcriptional levels of *Pcgf6* in various tissues of wild-type mice. *Actin* was used as a control for normalization. The *Pcgf6* mRNA levels of wild-type ESCs were considered as 1. Data are from 3 biological replicates each with 3 technical replicates. Error bars are SD.

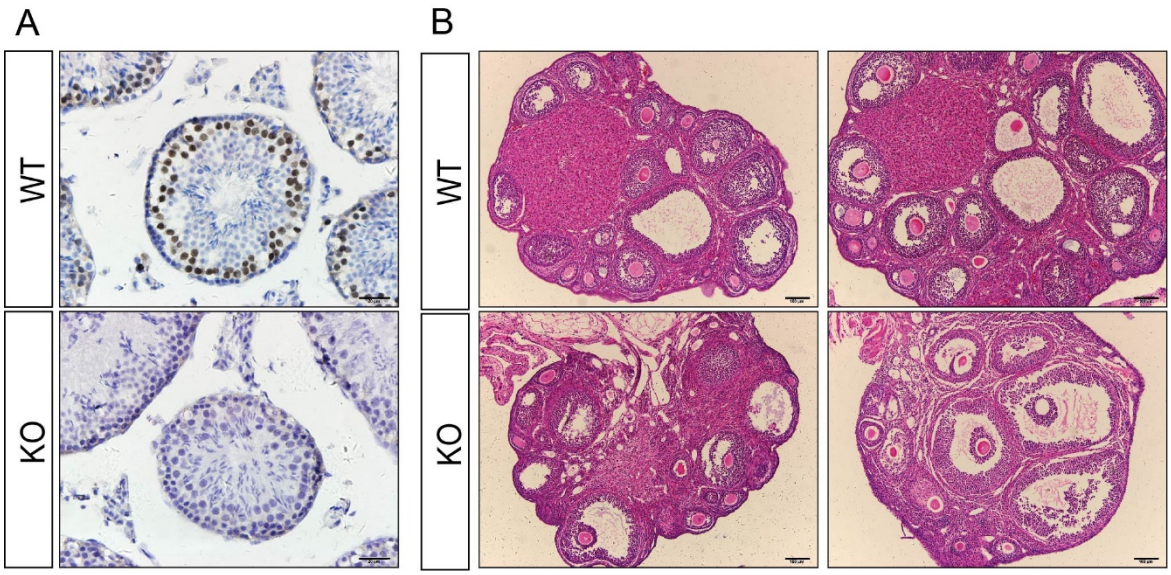

Figure S2. Staining of tissue sections. A. Immunohistochemistry staining of representative sections of the testes with the anti-Pcgf6 antibody. Scale bars represent 20 μm. B. HE-staining of representative sections of the ovaries of the WT and *Pcgf6*$^{-/-}$ mice. Scale bars represent 100 μm.

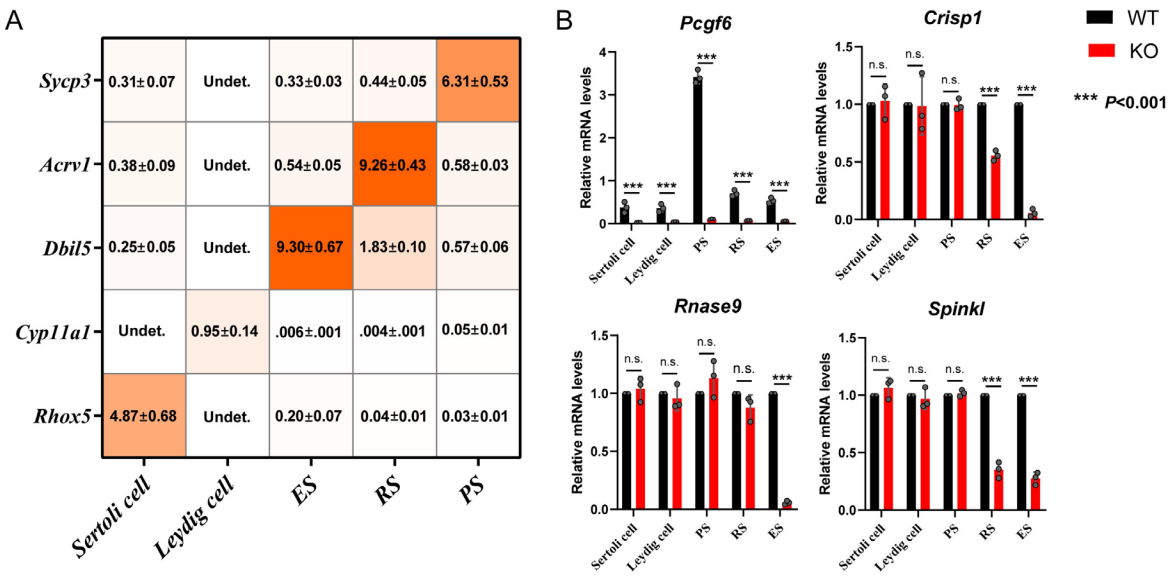

Figure S3. Transcriptional levels of *Pcgf6* targeted genes in various testicular cell types.

A. Expression levels of markers genes in purified cell populations. Values represent qRT-PCR analyses of RNA from purified mouse testicular cell populations with Sertoli, Leydig, and germ cell-specific markers, normalized to *Rps2* RNA. The values shown are representative of means ± SD of six individuals, relative to those for total testes RNA. Primer sequences are available in Supplementary Table S1. PS, pachytene spermatocyte; RS, round spermatid; ES, elongating spermatid; Undet., undetected. B. The relative mRNA levels of indicated genes in purified testicular cell populations of wildtype and *Pcgf6*$^{-/-}$ mice. Error bars indicate ± SD of 3 independent biological samples. n.s. means non-significant (two-tailed Student's t-test).

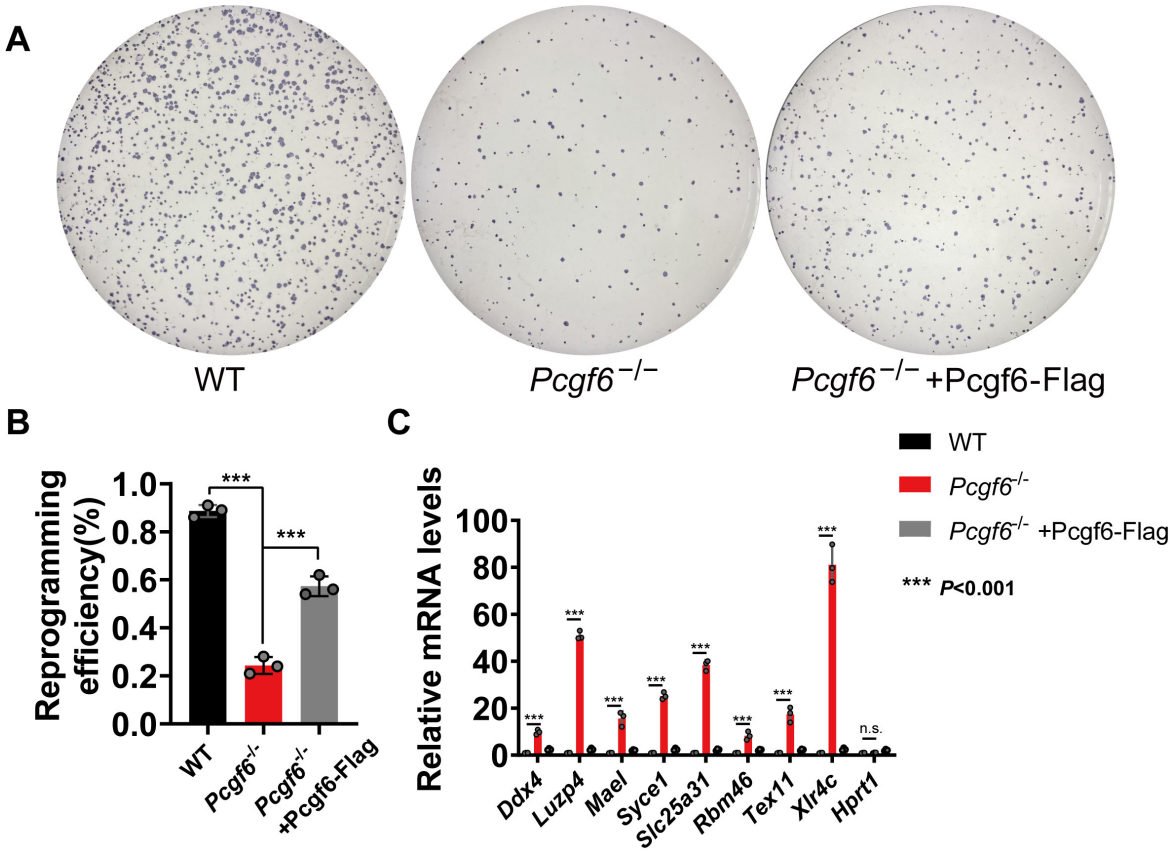

Figure S4. Deficiency of *Pcgf6* decreases iPS colony numbers. A. Effects of *Pcgf6* knockout on somatic cell reprograming. iPS cell colonies were visualized in 60 mm dishes by AP staining at day 21. B. Quantification (average and SD) of iPS cell colonies from three independent experiments at day 21. C. Relative expression of the indicated germline-related genes in MEFs of the indicated genotypes determined by qRT-PCR. *Actin* was used as a control for normalization. Error bars indicate ±SD of 3 independent experiments.

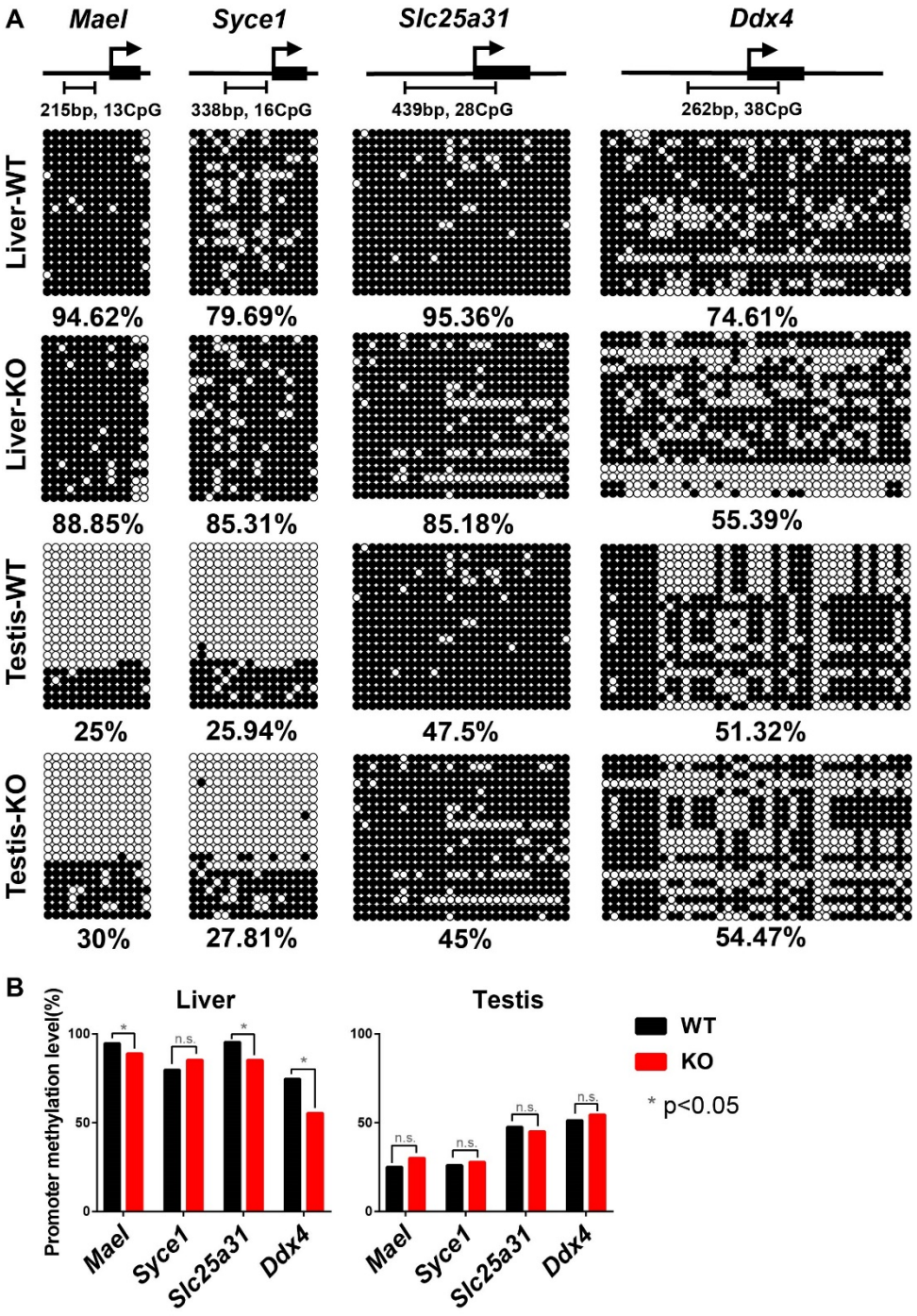

Figure S5. DNA methylation levels of the germline genes are modestly decreased in the liver after Pcgf6 depletion.

A. Methylation status of CGI promoters of four up-regulated germline genes in the liver of *Pcgf6*$^{-/-}$ mice. Testis was used as a control. Cartoons demonstrate locations of CGIs relative to TSSs (arrows). CpG nucleotides are shown by black (methylated) or white (unmethylated) circles. Methylation extent is given as a percentage of methylated CpG. B. Bar graph ($\chi 2$ test) of the CpG methylation levels.

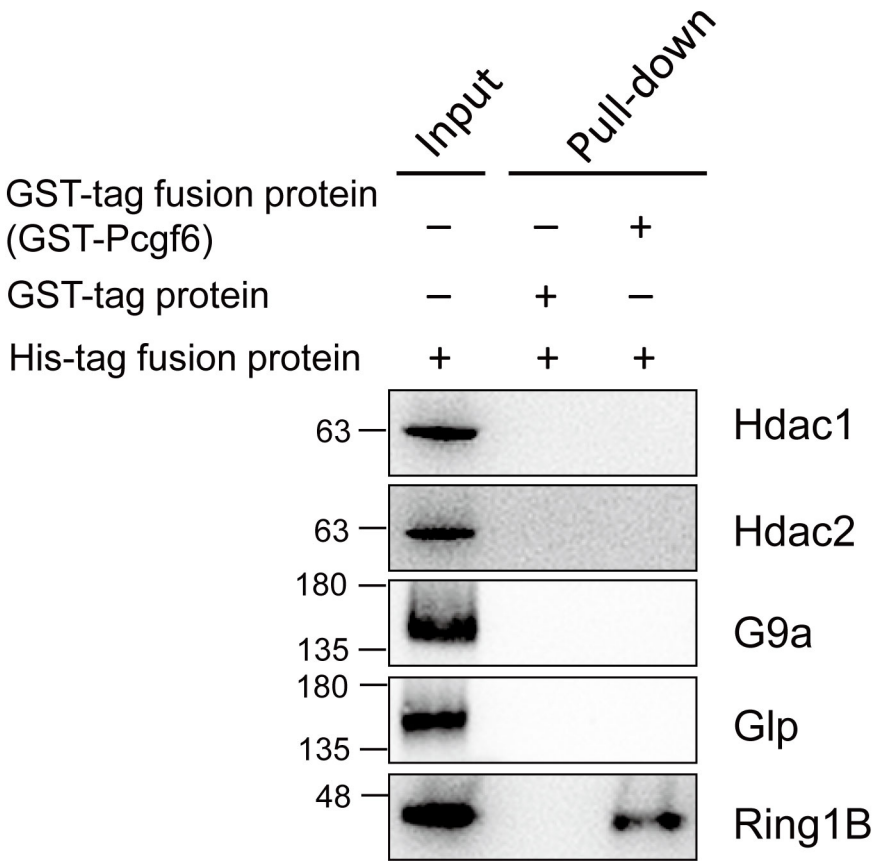

Figure S6. Pcgf6 does not directly interact with Hdac1/2 and G9a/Glp. His-tag fusion proteins were probed for interaction with GST-Pcgf6 in pull-down assays. GST tag alone is used as a negative control. The specific interaction of those proteins with GST-Pcgf6 was detected by Western blot with specific antibodies for Hdac1, Hdac2, G9a, Glp or Ring1B respectively. 10% of the fusion protein used in each assay was loaded as input. Assays were done independently for each protein. The results are representative of two independent experiments.

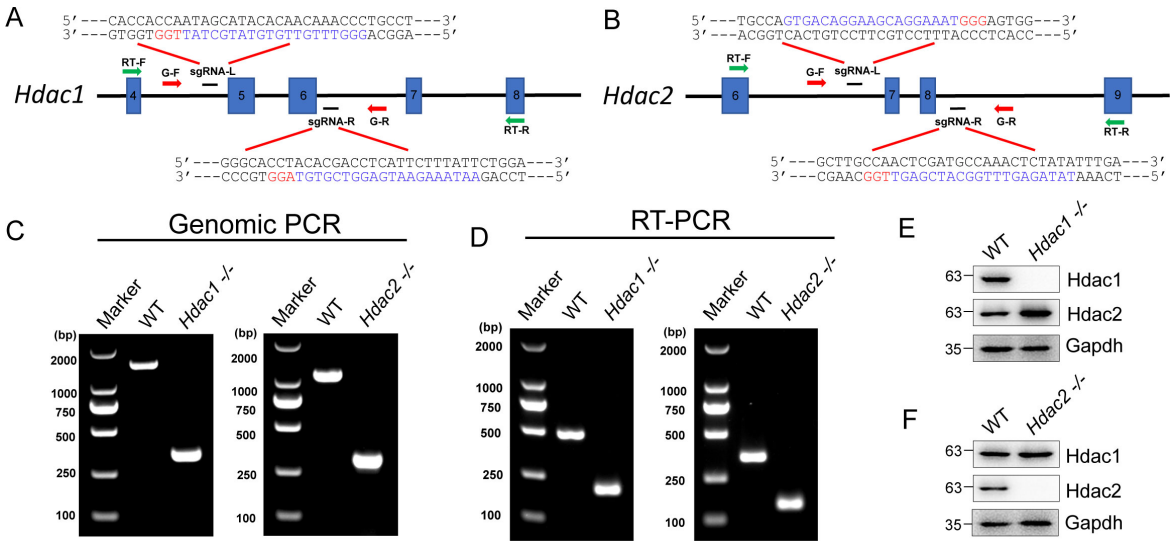

Figure S7. Generation of *Hdac1* or *Hdac2* knockout ESCs. A, B. The schematic of *Hdac1* and *Hdac2* gene targeting strategies. The exons 5-6 of *Hdac1* or the exons 7-8 of *Hdac2* were deleted by a pair of sgRNAs, respectively. The location of genomic PCR primers was shown by red arrows, and RT-PCR primers were represented by green arrows. C. Genomic PCR for analysis of *Hdac1/2* gene status. D. RT-PCR analysis for residual *Hdac1/2* mRNA. E, F. The absence of the proteins Hdac1 or Hdac2 in knockout ESCs analyzed by Western blot. Gapdh served as a loading control.

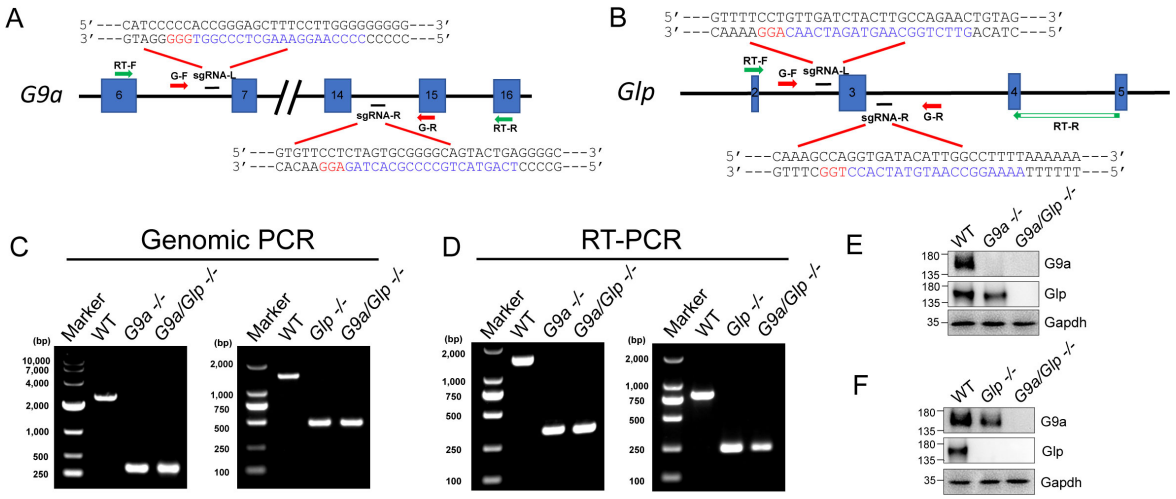

Figure S8. Generation of *G9a* and *Glp* knockout ESCs. A, B. The schematic of *G9a* and *Glp* gene targeting strategies. The exons 7-14 of *G9a* or the exons 3 of *Glp* were deleted by a pair of sgRNAs, respectively. The locations of genomic PCR primers were showed by red arrows, and RT-PCR primers were represented by green arrows. C. Genomic PCR for analysis of *G9a* and *Glp* gene status. D. RT-PCR analysis for residual *G9a* and *Glp* mRNA. E, F. The absence of the proteins G9a or Glp in knock-out ESCs analyzed by Western blot. Gapdh was used as a loading control.

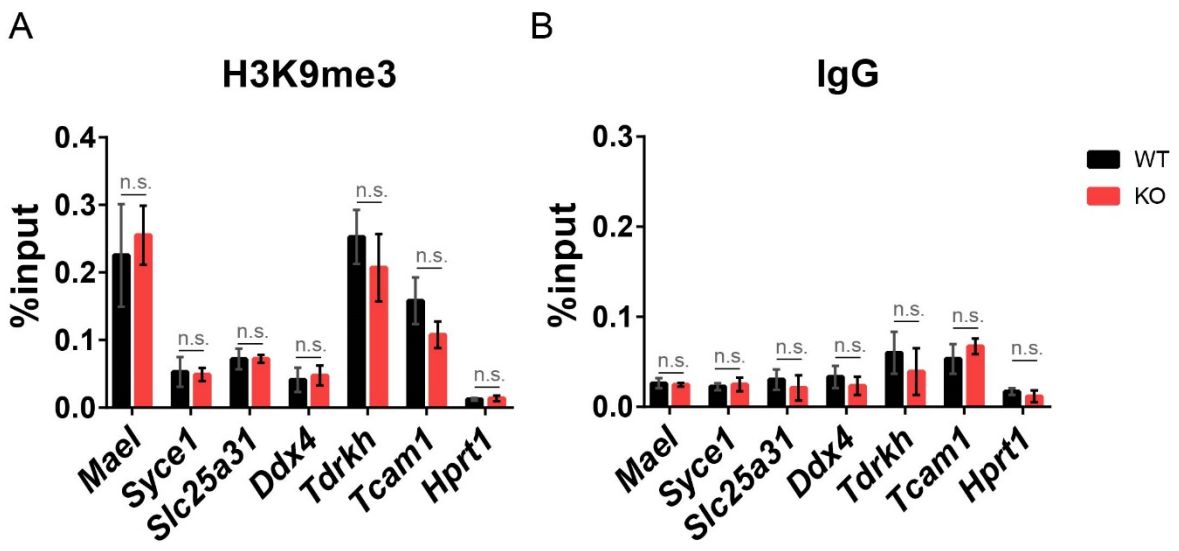

Figure S9. ChIP of H3K9me3 followed by qPCR analysis in ESCs of indicated genotypes. Purified rabbit IgG was used as a negative control. Error bars indicate ± SD of 3 biologically repeats. n.s. means non-significant (two-tailed Student's t-test).

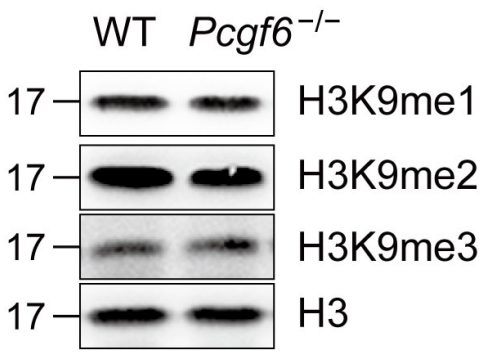

Figure S10. Western blot showing the methylation levels of H3K9 in WT and *Pcgf6*$^{-/-}$ ESCs.

Table S1. List of Primers Used in this Study.

Using	Target	Forward	Reverse
Genomic PCR	*Pcgf6*	TGGGGAGGGAAGTTAAGAGG	R1: TGAGCCCAAGAGTGTTGGTA R2: CTTGAACTCACGGCAATCCT
	Hdac1	CTTATGGTGCACATGCAGACG	GATCAGGAGGCCGTGACAAT
	Hdac2	AGCAGAAGAAGGCCTAGTCG	TCAGACAACAAGGTACAGGTC
	G9a	GCTTTTCGTGGAGTGTGGTT	AGTTGGGGTCCAGGTTGTC
	Glp	ATGCCAGAAGAAAGCATTGG	ACTTCACTCAATGCCCAGGA
RT-PCR	*Hdac1*	TGAGGACTGTCCGGTGTTTG	CACTGCACTAGGCTGGAACA
	Hdac2	CAACAGATCGCGTGATGACC	CGGATTGTGTAGCCTCCTCC
	G9a	GAACTCTGGTAGCCTGTCCG	GTTCACCACGGCCTCCATTA
	Glp	AGTTCTGGCCAAGCAAGAGA	ACAAAAGGCAAGCAAGCTGT
qRT-PCR	*Pcgf6*	GCCAGTCCCTTCAAGCAAAG	GCCCGATAGTTGCTTCTCCT
	Ddx4	TGTCAGACGCTCAACAGGAT	ACTGGATTGGGAGCTTGTGA
	Luzp4	CAGACGTGACAGTAGGGGAA	GGCCATGAGATCTGCCAATG
	mael	TCTAAGACCTGGGTGCGAAG	ACTGTTGCTGGCCTCATAGT
	Rbm46	ATTGCAAATGGATCCCAGAG	TCCCAGAAGAGAGGGTAGCA
	Syce1	CTGAGGTTGGATTTTGAAGA	TCCTTGCTGCTGTCCAAAAC
	Slc25a31	CGATCCGGTGTCTTTCTCGA	ACATTTGCCAAATTGCCACG
	Tex11	CCTGAGGCTGACTTGACCAT	GGGGAGCCTCATCAACATATC
	Hprt1	TCAGTCAACGGGGGACATAAA	GGGGCTGTACTGCTTAACCAG
	Xlr4c	CCACTTCTTGAAAGTCCAGCA	TCAGAGAGTTTTCCAGCCTGT
	Xlr4b	GAAACAATCTGGCCCTTGAA	CTTCGCTCATGCTGGACTTT
	β-actin	AGCCATGTACGTAGCCATCC	CTCTCAGCTGTGGTGGTGAA
	Mageb4	TACTGCTGGCTTCCCTCAAA	TCTCCCTTAGATGCTGGCTG
	Zscan4c	ACTCAGGAGCTGCAAAGTCT	ACCATGACAGGAGGCTTCAA
	Tdrkh	AATCATAGGGAGAGGCGGTG	ACTGATTGGCTGCTTTCGTG
	Taf7l	GCATGGCATTACTCCACCAC	GGCATGGACCCTTGACTTTC
	Hprt1	TCAGTCAACGGGGGACATAAA	GGGGCTGTACTGCTTAACCAG

	Acrv1	TCAGCAACTTTCAAGCGAGTAT	CTCCTGAAGAGTGCTCACCTG
	Cyp11a1	CCAGTGTCCCCATGCTCAAC	TGCATGGTCCTTCCAGGTCT
	Dbil5	CCCAGGGCGACTGTAACATC	GCAATGTAGATCCTCATGGCAT
	Rhox5	CACCAGGACCAAAGTGGCC	GGTATGGAAGCTGAGGGTT
	Rps2	CTGACTCCCGACCTCTGGAAA	GAGCCTGGGTCCTCTGAACA
	Sycp3	AGCCAGTAACCAGAAAATTGAGC	CCACTGCTGCAACACATTCATA
	Rnase9	CCGAAGCTTCAGGGGAACTA	ACAGCGCCCCTTATAGTGAA
	Crisp1	GCTGTTCTTCTTGGCTGCTG	CTGCCAGATGGAGAAACCAT
	Spinkl	TCTGACTTTGGTGCTTCTTCC	CTATCATCTGCACAAACTGGGT
Bisulfite sequencing	*Ddx4*	GGTTATGGAGTTAAGAGGTTT	ACCAAAACCAAACTTAAAAA
	Mael	GGAATGTGAAATATTTAGGAA	TATAAAAATAAAAACAACCTAAAACTAAAT
	Syce1	TTTGGTAATTGGGAATTGGG	AACTTCCAAACTATTAAACACTACACCTA
	Slc25a31	AGGAGTTAATAAGTTTAGGGTGAT	AACACCTACAAACCACACCAAC
CHIP-qPCR	*Ddx4*	CCTTGGAGAGAGAAACGGGA	GCGGGGACAACAAATAGCAT
	Mael	ATCACAGTGCTCTCCCCAC	CGCCCTTAGTAACCGAGCT
	Syce1	GCAGTGTCTAACAGCCTGGA	CCCTTCAGCCACTACCTCTG
	Slc25a31	ACACGTGTTATGGTCACATGC	GCCTTCTTTGAAGACTGCTTCT
	Tdrkh	GTGAGCGTTGATTGGTGAGG	TTCTCCCACACCCCAAACTT
	Tcam1	TGTTCACGCTTCTCCTCTCA	GAGTCAAAGCGGTCGTCTTG
	Hprt1	GAATCCTCTGGGAGACGACA	CGGAAAGCAGTGAGGTAAGC

Table S2. List of antibodies used in this study.

Description	Supplier	Product ID#	Applications
Anti-Pcgf6	Abcam	ab200038	WB, IP, CHIP
Anti-Ring1B	Proteintech	16031-1-AP	WB, CHIP
Anti-Rybp	Santa Cruz Biotechnology	sc-374235	WB
Anti-L3mbtl2	Santa Cruz Biotechnology	sc-365134	WB
Anti-Max	Santa Cruz Biotechnology	sc-197	WB
Anti-Hdac1	CST	5356	WB,CHIP
Anti-Hdac2	CST	5113	WB,CHIP
Anti-Hp1γ	CST	2619	WB
Anti-E2f6	Santa Cruz Biotechnology	sc-390022	WB
Anti-G9a	RnD Systems	PP-A8620A-00	WB,CHIP
Anti-Glp	RnD Systems	PP-B0422-00	WB, CHIP
Anti-Cbx8	CST	14696	WB
Anti-Ezh2	CST	5246	WB
Anti-H3k9me1	Millipore	17-680	WB,CHIP
Anti-H3k9me2	Millipore	17-648	WB,CHIP
Anti-H3k9me3	Millipore	17-625	WB,CHIP
Anti-H3k27ac	Abcam	Ab4729	WB
Anti-H2AK119ub1	CST	8240	ChIP
Anti-H3ac	Millipore	06-599	WB,CHIP
Anti-H4ac	Millipore	06-866	CHIP
Anti-H3	Millipore	07-690	WB
Anti-Gapdh	Santa Cruz Biotechnology	sc-47724	WB

goat anti-mouse lgG-HRP	Santa Cruz Biotechnology	sc-2005	WB
goat anti-rabbit lgG-HRP	Santa Cruz Biotechnology	sc-2004	WB

Table S3. The misregulated genes in the testis of *Pcgf6*$^{-/-}$ mice. Separate file (.xlsx)

Table S4. The misregulated genes in the liver of *Pcgf6*$^{-/-}$ mice. Separate file (.xlsx)

Table S5. The misregulated genes in the brain of *Pcgf6*$^{-/-}$ mice. Separate file (.xlsx)