Figure S1

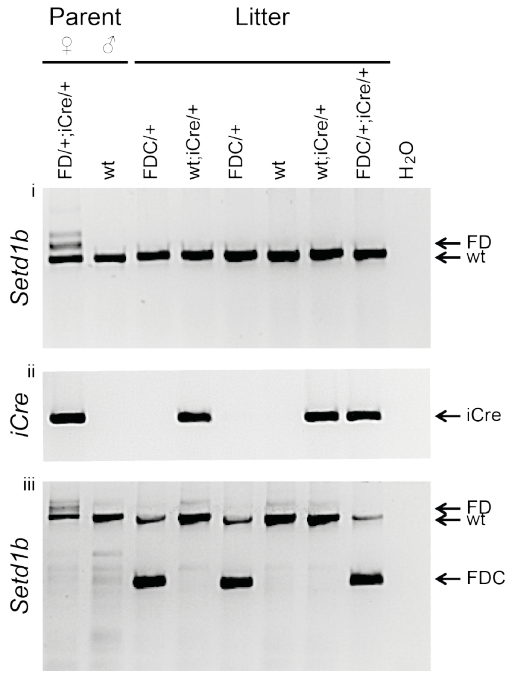

Figure S1. Recombination of the *Setd1b* conditional allele using *Gdf9iCre* as a deleter strain. Female mice of the genotype *Setd1b*$^{FD/+;Gdf9iCre/+}$ (control) were crossed to C57/BL6 males and the offspring were genotyped from tail biopsies. (i) The genotype of the *Setd1b*^{FD} allele was identified with primers flanking the downstream loxP site. The wild type band is 507 bp and the FD band is 696 bp. (ii) Genotyping for iCre. The expected size of the PCR product is 230 bp. Despite the Cre-negative genotype of pup 1 and 3, they display the recombined allele, possibly due to diffusion of Cre protein. (iii) The recombined *Setd1b*^{FDC} allele was identified by PCR with primers upstream of the 5' FRT site and downstream of the 3' loxP site to generate the FD band at 1695 bp. The wild type band is 1305 bp and the FDC band is 390 bp.

Figure S2

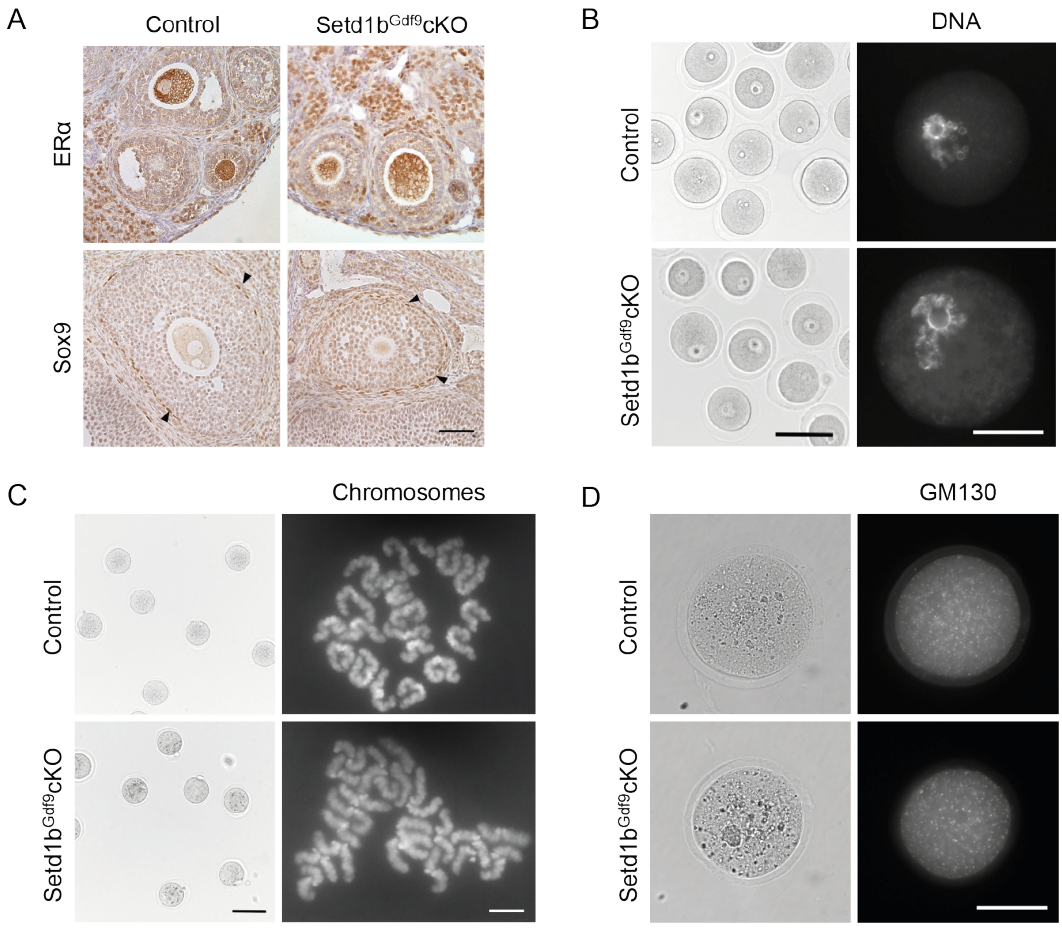

Figure S2. Ovarian marker molecules, MII oocyte chromosome number, Golgi complex distribution and periovulatory chromatin rearrangement are normal. (A) Ovarian morphology and germ cell development assessed by immunohistochemistry with antibodies against ERα and Sox9. 23 week old mice were sacrificed, ovaries fixed, paraffin embedded and further processed for immunohistochemistry with antibodies against ERα and Sox9. Scale bar: 50 µm. ERα is expressed in the oocyte and in stromal cells of the ovary. Sox9 is predominantly expressed in theca cells and to a lesser extent in granulosa cells and oocytes. Both ovaries of control and *Setd1b*^{Gdf9}cKO mice displayed comparable expression of these markers. (B) Periovulatory oocytes display a surrounded nucleolus. DIC (left) and fluorescence microscopy (right) analysis of periovulatory oocytes stained with DAPI to visualize DNA. A total of 70 oocytes of both genotypes were analysed in four independent experiments. Scale bar for DIC images: 100 µm, scale bar for fluorescence images: 50 µm. (C) Metaphase II chromosomes. Chromosome spreads were prepared using MII oocytes from control and *Setd1b*^{Gdf9}cKO mice. A total of 134 (control) and 105 (*Setd1b*^{Gdf9}cKO) MII oocytes were evaluated in three independent experiments. Chromosomes with two chromatids are shown. Scale bar for DIC images: 100 µm, scale bar for fluorescent images: 10 µm. (D) Golgi complex distribution in MII oocytes. DIC (left) and fluorescence microscopy (right) analysis of MII oocytes stained with GM130 to visualize Golgi complexes. A total of 45 (control) and 28 (*Setd1b*^{Gdf9}cKO) oocytes were evaluated. Scale bar: 50 µm.

Figure S3

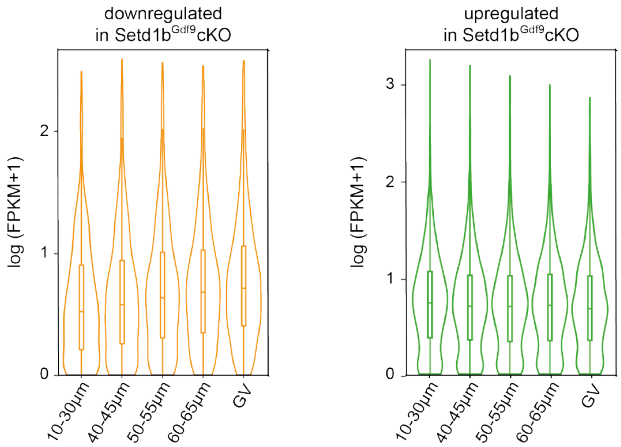

Figure S3. Violin plot representation of absolute expression levels of genes down- and up-regulated in *Setd1b*^{Gdf9}cKO during oocyte growth in wild-type oocytes. Shape of the plot shows Kernel density estimation, i.e., the probability density of the data at the different values. Horizontal lines correspond to the median, boxes represent the interquartile range and vertical lines adjacent values, i.e., the minimum and maximum values in the data within the 1.5 x interquartile range distance from the value of the first and third quartile, respectively.

Figure S4

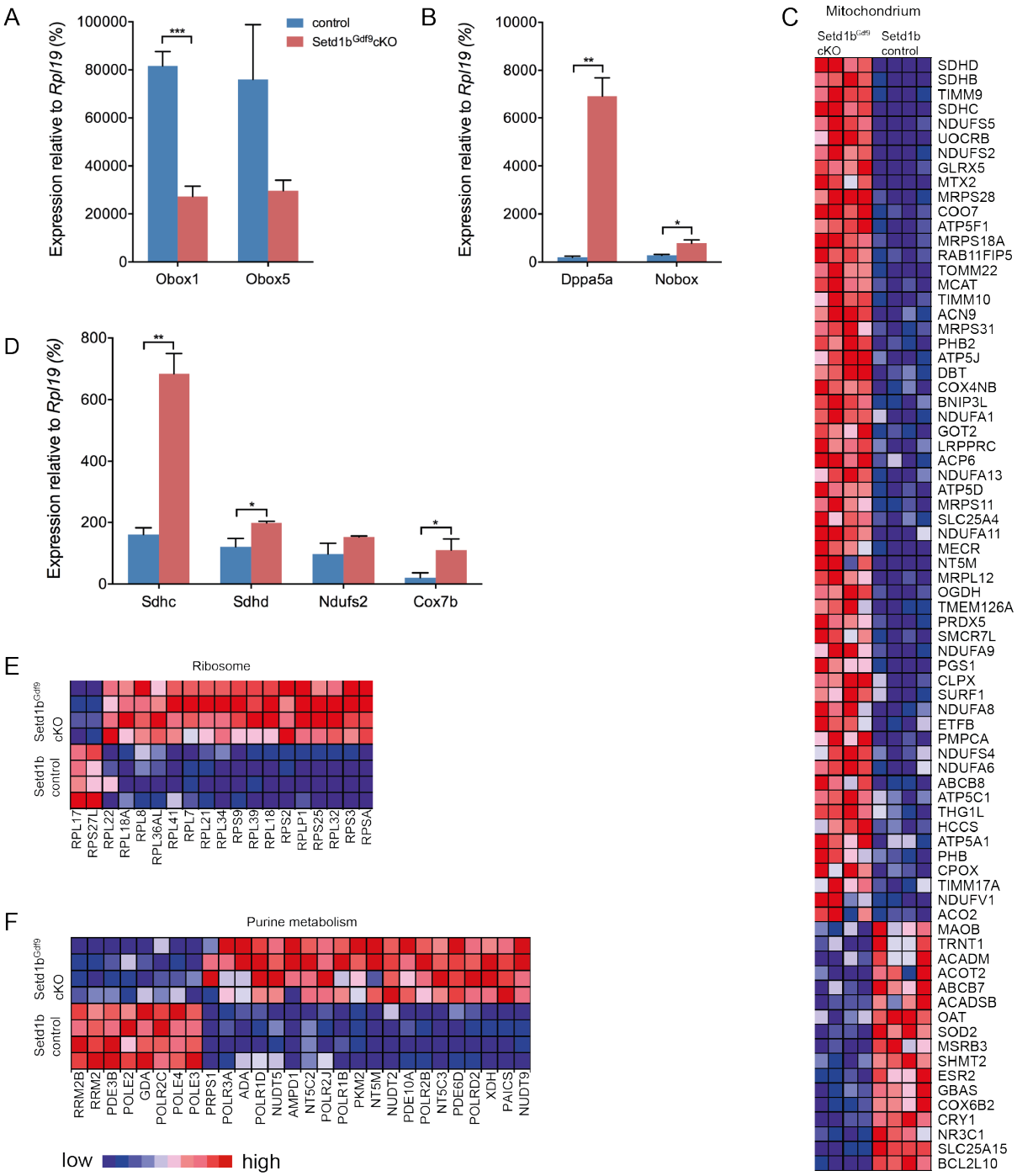

Figure S4. Oocyte-specific deletion of Setd1b influences the expression of mitochondrial genes. (A and B) To validate the RNA-seq results, quantitative RT–PCR was performed on control and *Setd1b*^{Gdf9}cKO MII oocytes for selected genes essential for oocyte regulatory pathways. Relative quantities of mRNA were normalized against *Rpl19*. (C) Gene set enrichment analysis (GSEA) heatmap of control versus *Setd1b*^{Gdf9}cKO oocytes for the term mitochondrium. (D) To validate the RNA-seq results, quantitative RT–PCR was performed on control and *Setd1b*^{Gdf9}cKO MII oocytes for selected genes essential for oxidative phosphorylation. Relative quantities of mRNA were normalized against *Rpl19*. Mean + SD is shown; n = 3. Statistical significance was tested using the unpaired t test with Welch's correction: *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$. (E and F) Gene set enrichment analysis (GSEA) heatmaps of control versus *Setd1b*^{Gdf9}cKO oocytes for genes responsible for ribosome biogenesis and purine metabolism.

Figure S5

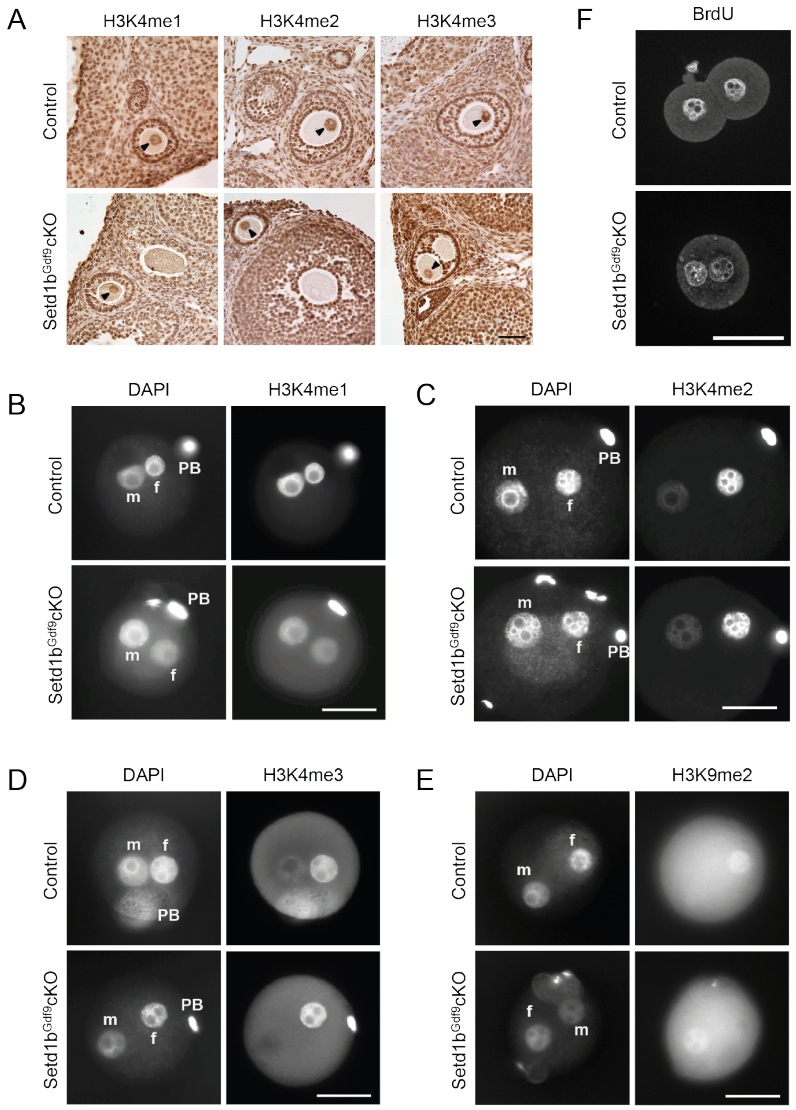

Figure S5. *Setd1b*^{Gdf9}cKO oocytes and zygotes show no defect in bulk H3K4 methylation. (A) Control and *Setd1b*^{Gdf9}cKO ovaries were sectioned and stained with antibodies against H3K4 mono-, di- and trimethylation as indicated. Arrowheads denote the oocyte nucleus. Scale bar: 50 µm. Representative micrographs showing fluorescence microscopy analysis of H3K4me1 (B) H3K4me2 (C) H3K4me3 (D) and H3K9me2 (E). The female pronucleus (f) displays a comparable level of H3K4me1, H3K4me2 and H3K4me3. The male pronucleus (m) is negative for H3K4me2, H3K4me3 and H3K9me2. Polar bodies (PB) stain with all antibodies used. Note in C the presence of multiple sperm heads (hook-shaped structures in the perivitelline space). A total of 30 (control) and 37 (*Setd1b*^{Gdf9}cKO) zygotes were evaluated for H3K4me1. A total of 72 (control) and 65 (*Setd1b*^{Gdf9}cKO) zygotes were evaluated in two independent experiments for H3K4me2. A total of 94 (control) and 28 (*Setd1b*^{Gdf9}cKO) zygotes were evaluated in two independent experiments for H3K4me3. A total of 54 (control) and 52 (*Setd1b*^{Gdf9}cKO) zygotes were evaluated in two independent experiments for H3K9me2. Scale bars: 50 µm. (F) Representative images showing BrdU labeling. Zygotes were incubated with BrdU until 44 h post-hCG. A total of 56 (control) and 40 (*Setd1b*^{Gdf9}cKO) zygotes were evaluated in two independent experiments. Scale bar: 50 µm.

Figure S6

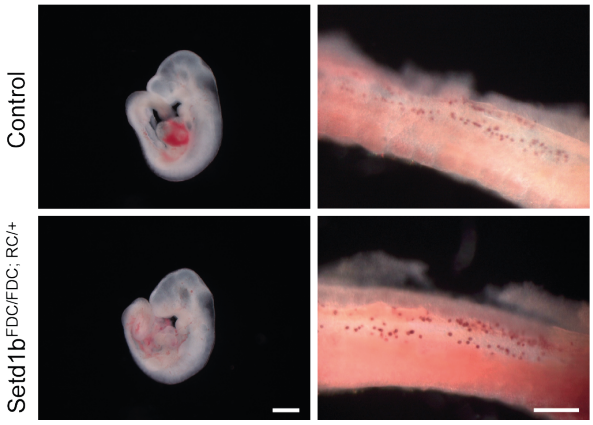

Figure S6. Setd1b does not control germ cell number. *Setd1bFD/FD;RC/+* and *Setd1bFD/+;RC/+* embryos induced with tamoxifen at E6.5 were dissected at E10.5. Scale bar: 1 mm. Genital ridges of females were stained for alkaline phosphatase expression. Scale bar: 200 µm.

Table S1. Primers for genotyping and qRT-PCR

Primer pairs	Sequence (5'-3')	Product size (bp)
Genotyping of Setd1bD allele		
Setd1b_ex5	GAAACTCGCATGCGCTTCTAC	507 [wt];
Setd1b_loxP2	AGTTCATACTGTGGCTGAATGG	696 [FD]
Genotyping of Setd1bFDC allele		
Setd1b_flp	GGGTGGAGAGGGAAAGAAAAG	1305 [wt]; 1695 [FD];
Setd1b_loxP2	AGTTCATACTGTGGCTGAATGG	390 [FDC]
Genotyping of iCre		
iCre	AACCTGAGGATGTGAGGGACTA	230
	GTCAAAGTCAGTGCGTTCAAAG	
Genotyping of Cre		
Cre	GCCTGCATTACCGGTCGATGCAACGA	700
	GTGGCAGATGGCGCGGCAACACCATT	
sex-specific PCR		
Ube1x	TGGTCTGGACCCAAACGCTGTCCACA	198+217 ♂
	GGCAGCAGCCATCACATAATCCAGATG	217 ♀
qRT-PCR		
Rpl19	CTGATCAAGGATGGGCTGATC	147
	CTTCTCAGGCATCCGAGCATT	
Setd1b	CTGTTGGTGAGCTGGATGCTA	172
	CTGGAGTAAGCTGTGTCTTGG	
Sdhc	GTCTCTCTTTTTGGCCTGTCG	194
	GGTATTGCCAGGCCTTTTCCT	
Sdhd	CTGGGTACTTGAATCCCTGCT	194
	AGGTCAAAGCTGAGAGTGCCA	
Ndufs2	CGTTTACGACCAGGTGGAGTT	166
	GGACACTTTGGCGTCATCAAC	
Cox7b	GCATTCAGCAAGTGGTGGCAA	203
	GCACGACTACTGATCTCTCCA	
Dppa5a	TGGATGCTTCAGTCCATGGCT	125
	AGGACTGGAAACTGGCTTCAC	
Obox1	GCTGGCACTATCAGTTGGTGT	126
	GAGCTTCCATTTGACTCTGGC	
Obox5	GCTGGCAGTATCAGTTGGTGT	151
	ATGGGTTGACTCAGAAACCGC	
Nobox	CAGTGGAGACTCATCACAGGA	271
	CTCTACTCTAGTGCCTTCGAG	

Table S2. Primary antibodies

Antibodies	Source	Identifier	Dilution
Rabbit anti-Ki67	Leica	NCL Ki67-p	1:2200 IHC
Rabbit anti-Sox9	Millipore	AB5535	1:3000 IHC
Rabbit anti-Ddx4	Abcam	ab13840	1:1000 IHC
Rat anti-Zp2	Santa Cruz	sc-32752	1:100 IHC/IF
Rabbit anti-ERα	Santa Cruz	sc-543	1:250 IHC
Rabbit anti-H3K4me1	Diagenode	C15410037 lot A1657D	1:1000 IHC 1:100 IF
Rabbit anti-H3K4me2	Diagenode	pAb-035-050 lot 936-0014P	1:1000 IHC 1:100 IF
Rabbit anti-H3K4me3	Abcam	ab8580 lot GR188707-1	1:500 IHC 1:50 IF
Rabbit anti-H3K9me2	Millipore	07-441	1:150
Mouse anti-GM130	BD Biosciences	610822	1:200 IF
Rabbit anti-Tomm20	Biorbyt	orb128858	1:100 IF
Mouse anti-α-tubulin FITC	Sigma-Aldrich	F2168	1:40 IF
Rat anti-BrdU FITC	Abcam	ab74545	1:50 IF

Table S3

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Table S4

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Table S5

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