Figure S1

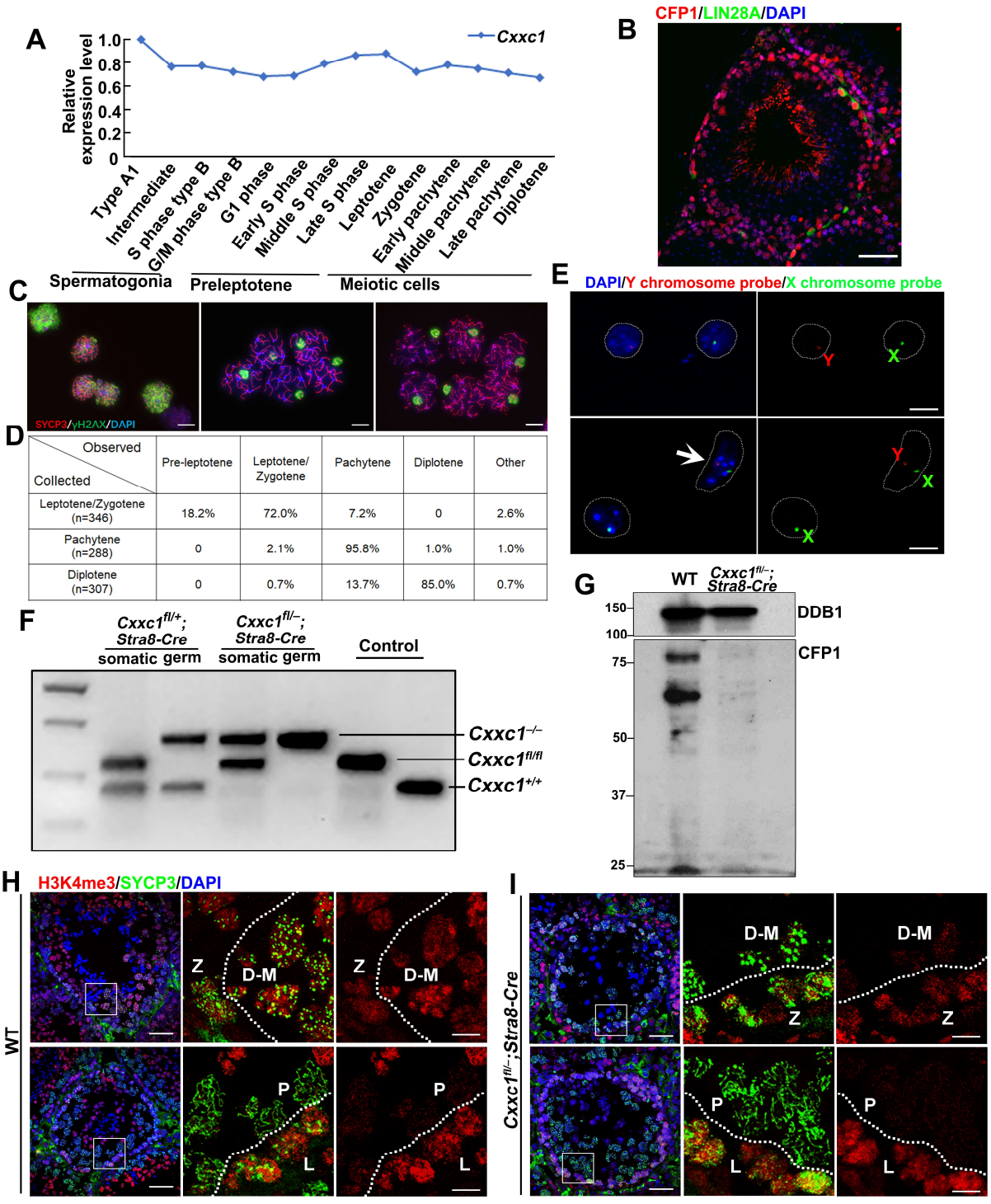

Figure S1. CFP1 is indispensable for H3K4me3 maintenance during spermatogenesis.

A: Relative expression level of *Cxxc1* from spermatogonia to meiotic cells from published single-cell RNA-seq dataset (Chen et al., 2018). **B:** Immunofluorescent co-staining of LIN28A and CFP1 in testes of 12-week-old WT mice. DNA was counterstained with DAPI. Scale bar = 50 μM. **C:** Representative images showed the sorted Leptotene/Zygotene (L/Z), Pachytene (P) and Diplotene (D) spermatocytes. The spread nuclei were double labeled with γH2AX (green) and SYCP3 (red) and co-stained with DAPI (blue). Scale bars = 20 μm. **D:** Percent purity quantification based on immunofluorescence analysis after cell sorting. Cell purity was calculated as (cell type observed/total cells) × 100%. **E:** Representative images of FISH assay of X and Y chromosome on FACS-isolated diploid cells. The white arrow indicated a somatic cell with both X and Y chromosome and the others were secondary spermatocytes with either X or Y chromosome. Scale bars = 20 μm. **F:** Genotyping of somatic cells and Cre-mediated recombined germ cells in heterozygous and knockout mice. **G:** Western blot using an antibody targeting CFP1 N-terminal region in spermatogenic cells isolated from adult WT and *Cxxc1*$^{fl/-}$*;Stra8-Cre* mice. **H and I:** Left tri-color panel, Immunofluorescent co-staining of SYCP3 and H3K4me3 in testes of 12-week-old WT and *Cxxc1*$^{fl/-}$*;Stra8-Cre* mice. DNA was counterstained with DAPI. Scale bar = 50 μM. Right two panels are enlarged images. Scale bar = 200 μM.

Figure S2

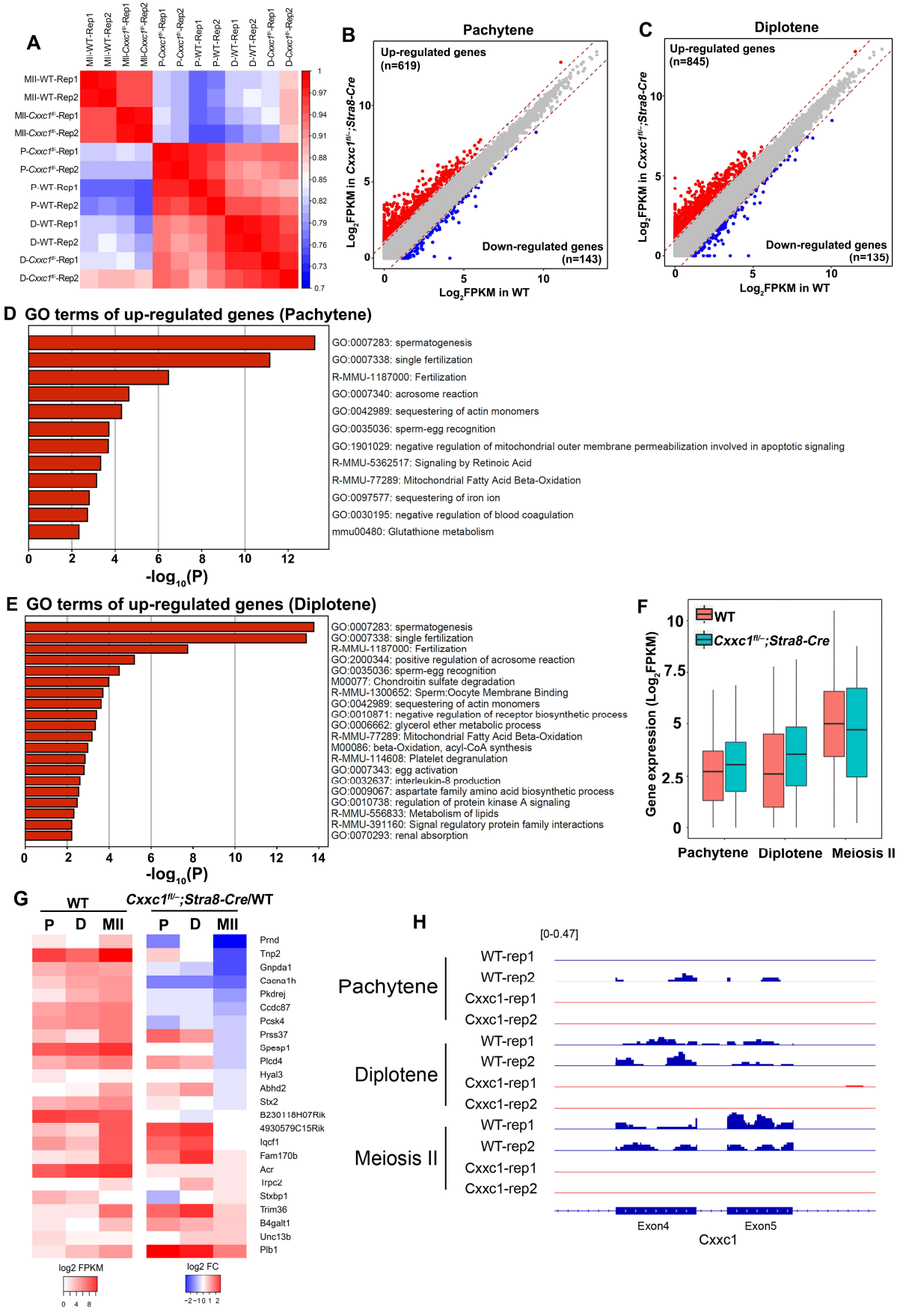

Figure S2. RNA-seq analyses of pachytene, diplotene and MII spermatocytes derived from WT, *Cxxc1* cKO mice

A: Heatmap of spearman correlation coefficients of total transcripts among WT and *Cxxc1*-null spermatocytes at different stages. **B and C:** Scatter plot comparing transcripts between WT and *Cxxc1* cKO spermatocytes at pachytene (B) and diplotene (C) stages. Genes up-regulated (FC>2.0) and down-regulated (FC<2.0) in *Cxxc1* cKO spermatocytes are colored with red and blue, respectively. **D and E:** Gene ontology analysis of up-regulated genes in *Cxxc1*-null spermatocytes at pachytene (D) and diplotene (E) stages. **F and G:** Box plot and heatmap showing varying gene expressive level of acrosome-reaction genes (GO: 0007340) among WT and *Cxxc1*-null spermatocytes at different stages. **H:** Snapshot of RNA-seq data showing the deletion of *Cxxc1* exons.

Figure S3

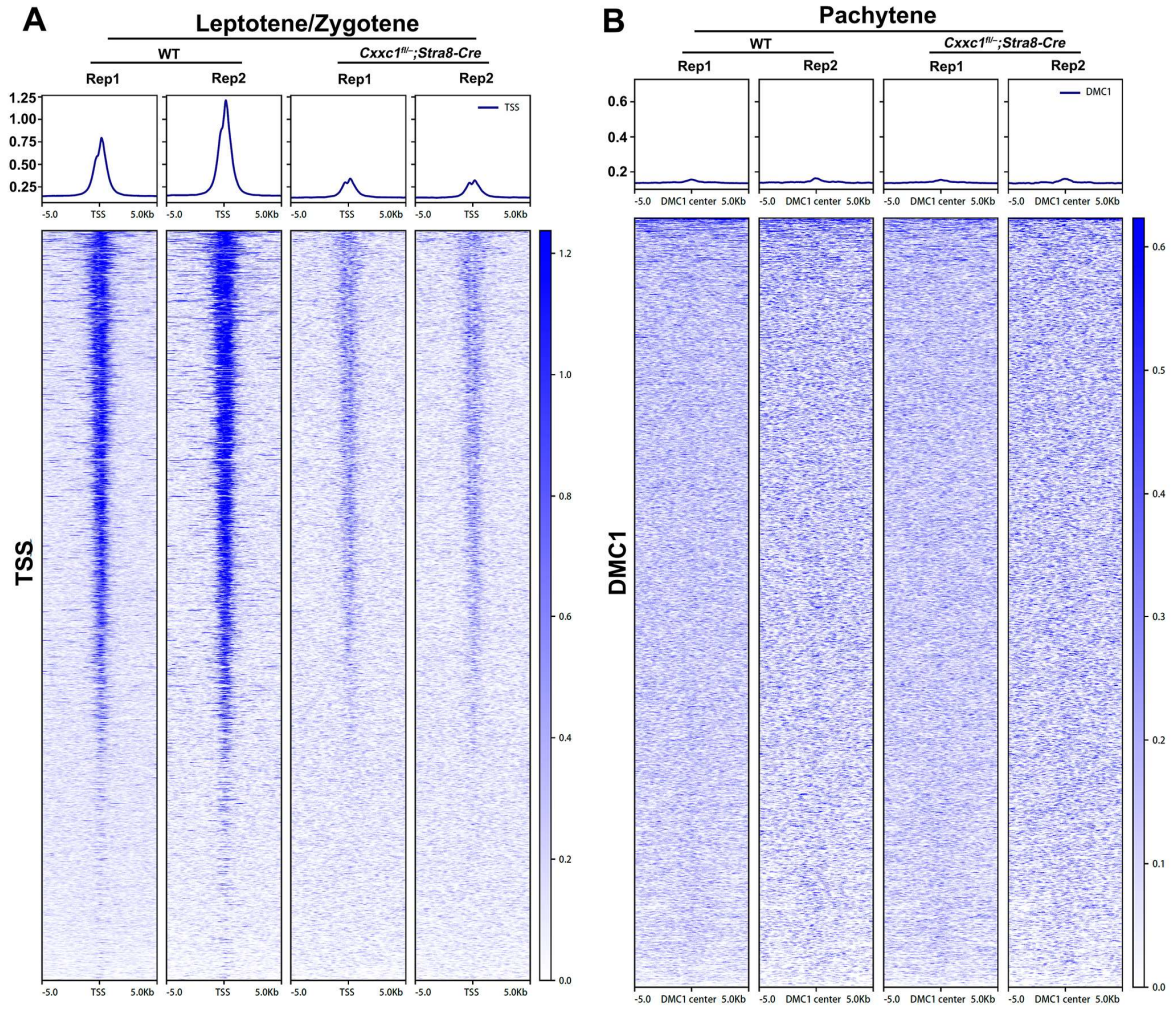

Figure S3. H3K4me3 ChIP-seq analyses of leptotene/zygotene and pachytene spermatocytes derived from WT, *Cxxc1* cKO mice

A: Heatmap of H3K4me3 levels in WT and *Cxxc1*-null leptotene/zygotene spermatocytes at the TSS regions. **B:** Heatmap of H3K4me3 levels in WT and *Cxxc1*-null pachytene spermatocytes at the centers of DMC1-binding sites.

Table S1. Antibody information

Protein name	Manufacture (catalogue number)	Applications (working dilution)	Website Link
H3K4me1	Cell Signaling (5326)	WB (1:1000)	http://www.cst-c.com.cn/products/primary-antibodies/mono-methyl-histone-h3-lys4-d1a9-xp-rabbit-ab/5326?_=1508331672321&Ntt=&tahead=true
H3K4me2	Cell Signaling (9725)	WB (1:1000)	http://www.cst-c.com.cn/products/primary-antibodies/di-methyl-histone-h3-lys4-c64g9-rabbit-mab/9725?_=1508331697704&Ntt=H3K4me&tahead=true
H3K4me3	Abcam (ab8580)	IF (1:400) WB (1:1000)	http://www.abcam.com/histone-h3-tri-methyl-k4-antibody-chip-grade-ab8580.html
α-Tubulin	Sigma (F2168)	WB (1:1000)	http://www.sigmaaldrich.com/catalog/product/sigma/f2168?lang=zh&region=CN
CFP1	Abcam (ab198977)	WB (1:500); IF (1:100) IHC(1:100)	http://www.abcam.com/cgbp-antibody-epr19199-ab198977.html
CFP1-N terminus	Abcam (ab189829)	WB (1:1000)	https://www.abcam.com/CGBP-antibody-N-terminal-ab189829.html?utm_source=labome&utm_medium=paid_referral&utm_term=1RY_CGBP_ab189829
SYCP3	Homemade	IF (1:400)	Immunogen: Full length of mouse SYCP3
SYCP1	Abcam (ab15087)	IF (1:200)	https://www.citeab.com/antibodies/771942-ab15087-anti-scp1-antibody
H2AK119ub1	Cell Signaling (8240)	WB (1:2000)	https://www.cst-c.com.cn/products/primary-antibodies/ubiquityl-histone-h2a-lys119-d27c4-xp-rabbit-mab/8240?site-search-type=Products
H3K27me3	Abcam (ab9733)	WB (1:1000)	https://www.cst-c.com.cn/products/primary-antibodies/tri-methyl-histone-h3-lys27-c36b11-rabbit-mab/9733?site-search-type=Products
MVH	Abcam (ab13840)	IF (1: 200); IHC (1: 400)	http://www.abcam.com/ddx4--mvhantibody-ab13840.html

γH2AX	Cell Signaling (9718S)	IF (1: 400)	https://www.cellsignal.com/products/primary-antibodies/phospho-histone-h2a-xser139-20e3-rabbitmab/9718?N=4294956287&Ntt=h2a.x&fromPage=plp
MLH1	Proteintech (11697-1-AP)	IF (1: 100)	https://www.ptglab.com/products/MLH1-Antibody-11697-1-AP.htm
CREST	Fitzgerald Industries International (70R-21494)	IF (1:100)	https://www.fitzgerald-fii.com/crest-antibody-70r-21494.html
DMC1	Proteintech (13714-1-AP)	IF (1: 100)	https://www.ptglab.com/Products/DMC1-Antibody-13714-1-AP.htm
Rad51	Abcam (ab176458)	IF (1: 100)	http://www.abcam.com/rpa70-antibodyab87272.html
PNA	Sigma (L7381)	IF (1: 500)	https://www.sigmaaldrich.com/catalog/product/sigma/l7381?lang=zh&region=CN
Lin28a	R&D Systems	IF (1: 200)	https://www.rndsystems.com/cn/products/human-lin-28a-antibody_af3757

Table S2. Summary of sequencing information

Type	Sample	Total reads	Mapping efficiency	Uniquely mapping efficiency	Correlation
RNA-seq	P-WT-Rep1	16,984,226	88.41%	78.72%	0.965
	P-WT-Rep2	20,610,839	88.12%	81.88%	
	P-*Cxxc1*$^{fl/-}$-Rep1	16,179,726	90.22%	82.18%	0.980
	P- *Cxxc1*$^{fl/-}$-Rep2	22,555,557	86.69%	78.11%	
	D-WT-Rep1	20,124,165	92.72%	86.80%	0.978
	D-WT-Rep2	21,454,127	89.78%	84.51%	
	D- *Cxxc1*$^{fl/-}$-Rep1	20,723,889	93.36%	87.26%	0.971
	D- *Cxxc1*$^{fl/-}$-Rep2	19,643,176	91.66%	85.87%	
	MII-WT-Rep1	19,902,160	91.86%	86.38%	0.980
	MII-WT-Rep2	20,810,855	90.34%	85.60%	
	MII- *Cxxc1*$^{fl/-}$-Rep1	19,101,599	92.93%	87.52%	0.978
	MII- *Cxxc1*$^{fl/-}$-Rep2	17,072,060	91.80%	85.57%	
ChIP-seq	L/Z-WT-Rep1	27,322,505	94.73%	67.77%	0.94
	L/Z-WT-Rep2	14,196,091	94.17%	69.14%	
	L/Z-*Cxxc1*$^{fl/-}$-Rep1	13,905,388	85.25%	61.81%	0.97
	L/Z- *Cxxc1*$^{fl/-}$-Rep2	14,497,563	93.52%	67.58%	
	P-WT-Rep1	26,641,654	96.38%	66.95%	0.91
	P-WT-Rep2	11,196,777	94.27%	67.16%	
	P- *Cxxc1*$^{fl/-}$-Rep1	24,615,804	96.32%	65.40%	0.89
	P- *Cxxc1*$^{fl/-}$-Rep2	11,252,776	91.04%	64.83%	