

## SUPPLEMENTARY INFORMATION

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### SUPPLEMENTARY FIGURE LEGENDS

**Figure S1.** The expression levels of *Setdb1* in mouse spermatogenic subpopulations in published RNA-seq, as shown in Dataset S1 by da Cruz *et al.* (2016), related to Figure 1.

**Figure S2.** The expression pattern of SETDB1 protein in *Setdb1* control and conditional knockout mice at 16 dpp testes, related to Figure 1.

**(A)** Histological sections of *Setdb1* control (+/-) and conditional knockout (CKO) testes at 16 dpp were stained with anti-SETDB1 (green) and TRA98 (red) antibodies. Arrows indicate SETDB1-expressing germ cells (TRA98 positive). Blue, DAPI. **(B)** Spermatocyte spreads from *Setdb1* control (+/-) and conditional knockout (CKO) testes at 16 dpp. Spermatocyte nuclei were stained with anti-SCP3 (green), SETDB1 (red), and centromere protein (CREST, gray) antibodies. The leptotene, zygotene, and pachytene stages of prophase I, respectively, are shown. Scale bars represent 5  $\mu$ m.

**Figure S3.** Phenotype of germline *Setdb1* knockout testes, related to Figure 1 and 2.

(A) No significant difference in testicular histology between *Setdb1* control and conditional knockout mice before 12 dpp. Histological sections were stained with hematoxylin and eosin. Testicular histology of *Setdb1* control (+/-) and conditional knockout (CKO) mice at 6, 8, and 10 dpp is shown. Scale bars represent 20  $\mu\text{m}$ . (B) Gross anatomy of testes from 5-week-old wildtype (+/+), *Setdb1* control (+/-), and *Setdb1* conditional knockout (CKO) mice. (C) Increased cell death in abolishing of SETDB1. TUNEL assay was performed using the testis sections from *Setdb1* control (+/-) and conditional knockout (CKO) mice at 16 dpp. Blue, DAPI; Green, TUNEL labeling. Scale bars represent 50  $\mu\text{m}$ .

**Figure S4.** Genome-wide map of H3K9me3 mark in the control and *Setdb1* mutant spermatocytes, related to Figure 6.

(A) Spearman correlation of all ChIP-Seq data on H3K9me3. (B) A genome-wide map of all H3K9me3 peaks in the control and *Setdb1* mutant spermatocytes.

**Figure S5.** Genome-wide map of H3K9me2 mark in the control and *Setdb1* mutant spermatocytes, related to Figure 6.

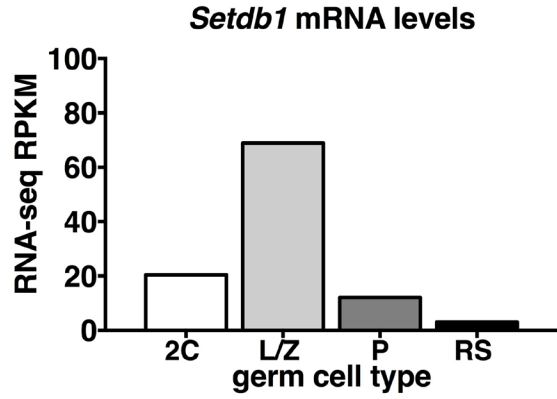
(A) Spearman correlation of all ChIP-Seq data on H3K9me2. (B) A genome-wide map of all H3K9me2 peaks in the control and *Setdb1* mutant spermatocytes.

**Figure S6.** Genome-wide map of the H3K27me3 mark in the control and *Setdb1* mutant spermatocytes, related to Figure 6.

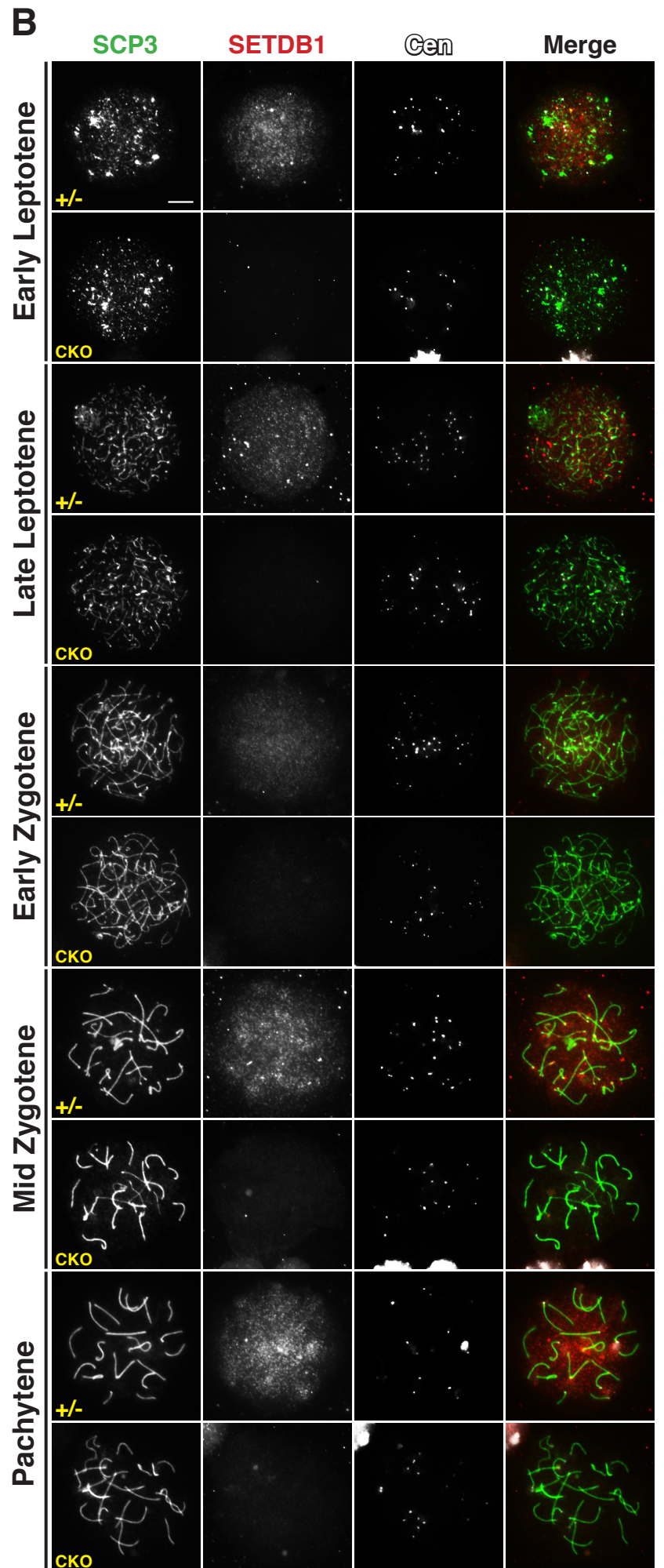
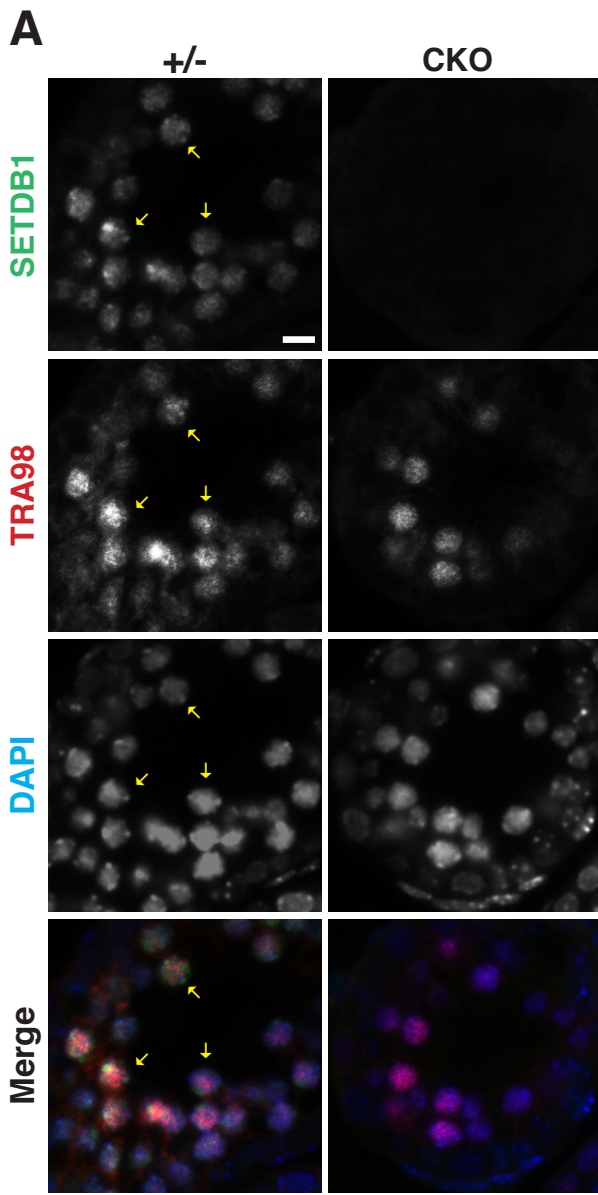
(A) Spearman correlation of all ChIP-Seq data on H3K27me3. (B) A genome-wide map of all H3K27me3 peaks in the control and *Setdb1* mutant spermatocytes.

**Figure S7.** Distribution of control-specific H3K9me3 (A and C) and *Setdb1*-mutant-specific (B and D) peaks in spermatocytes with regard to genes vs intergenic regions (A and B) and transposons (TE) vs genes (C and D), related to Figure 6.

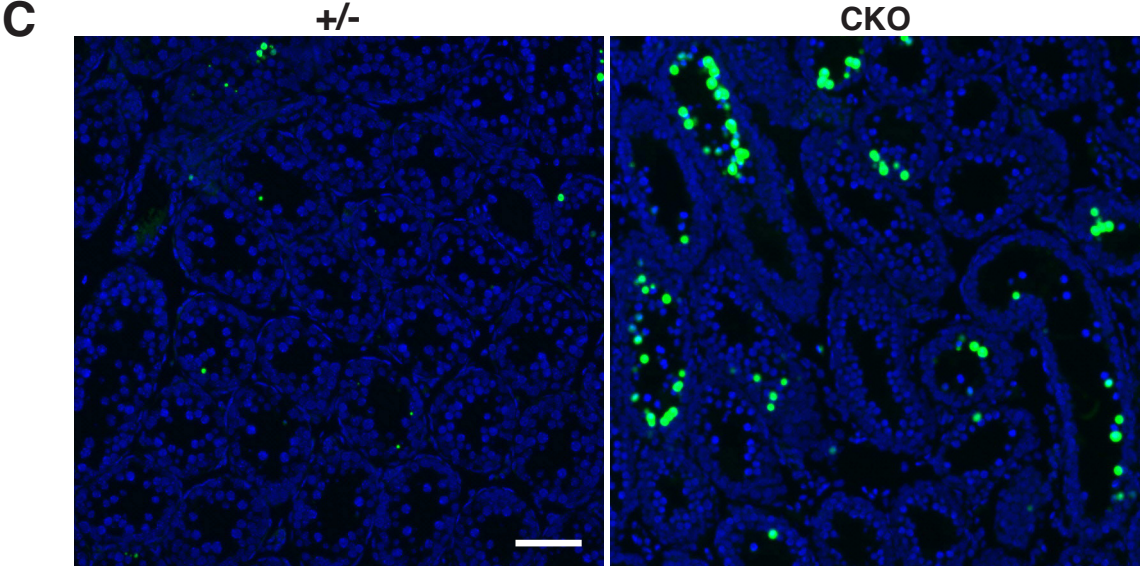
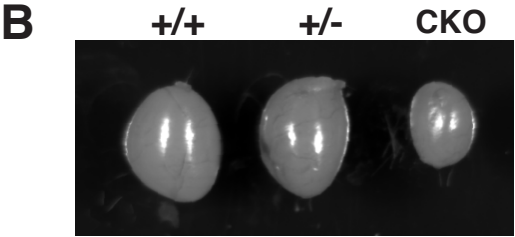
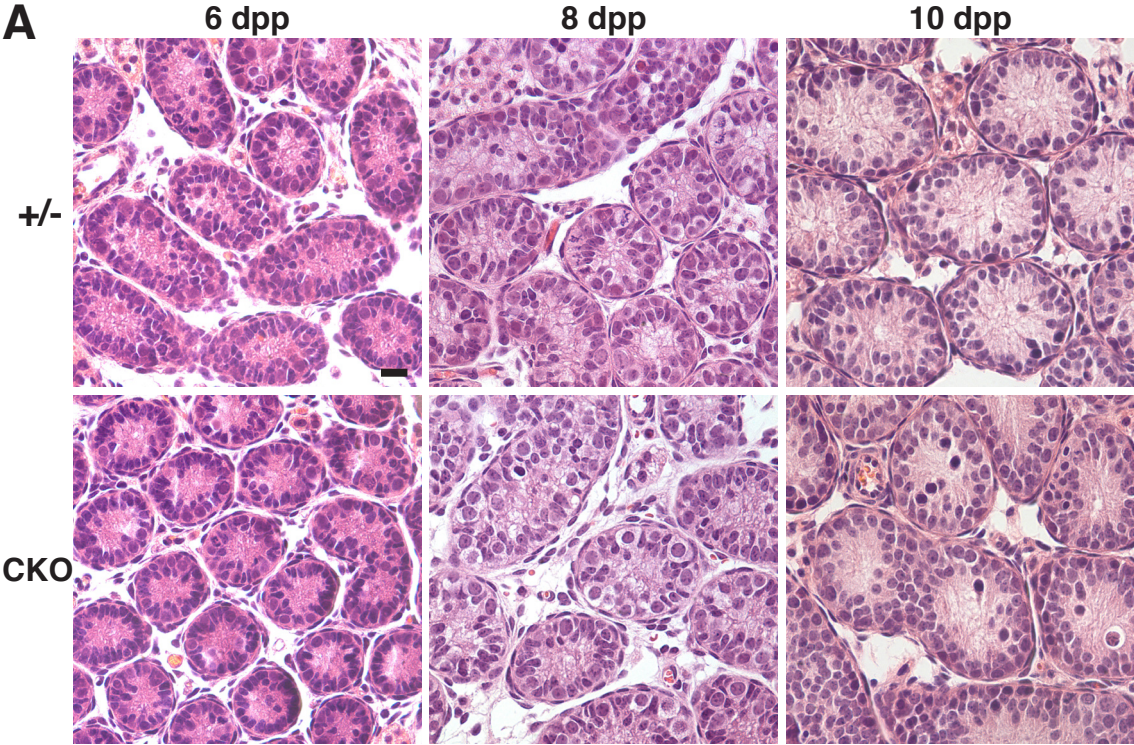
# Supplementary Figure 1



Supplementary Figure 2

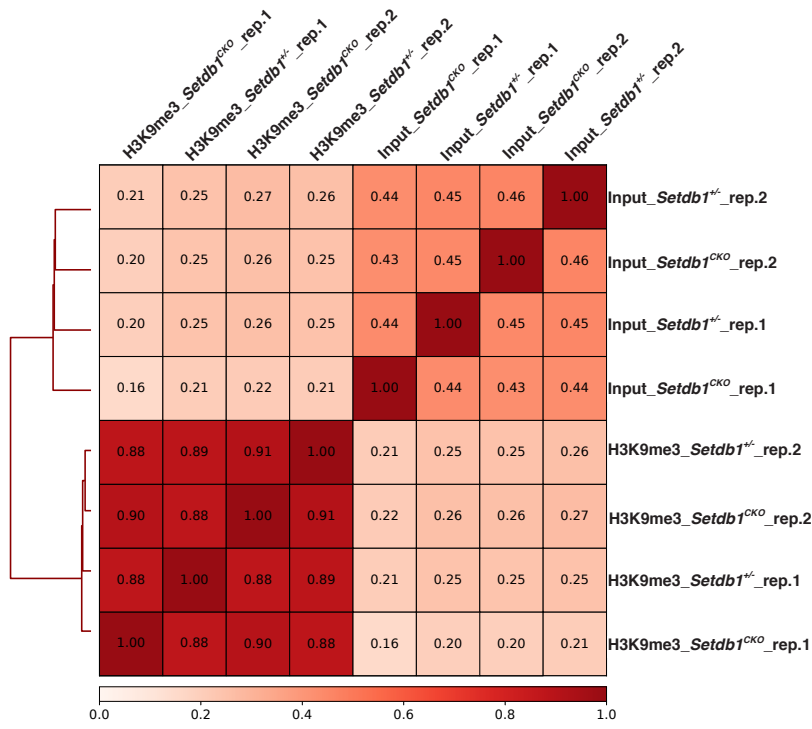


Supplementary Figure 3

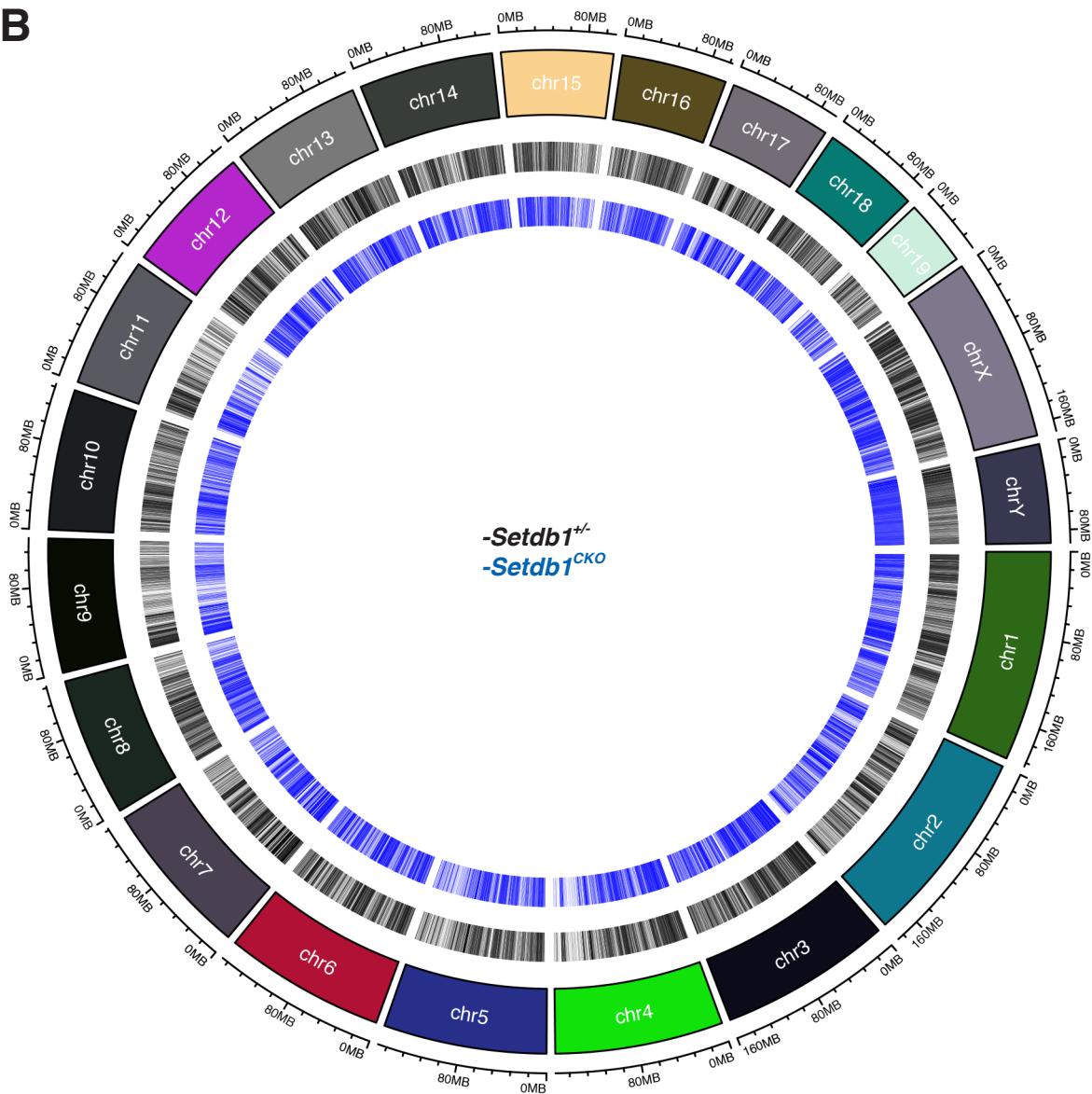


# Supplementary Figure 4

**A**

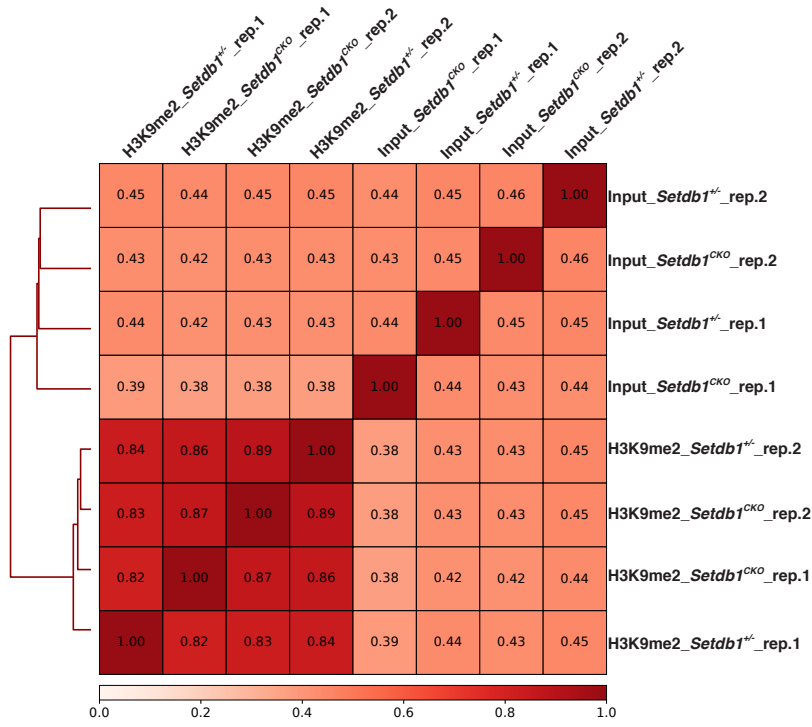


**B**

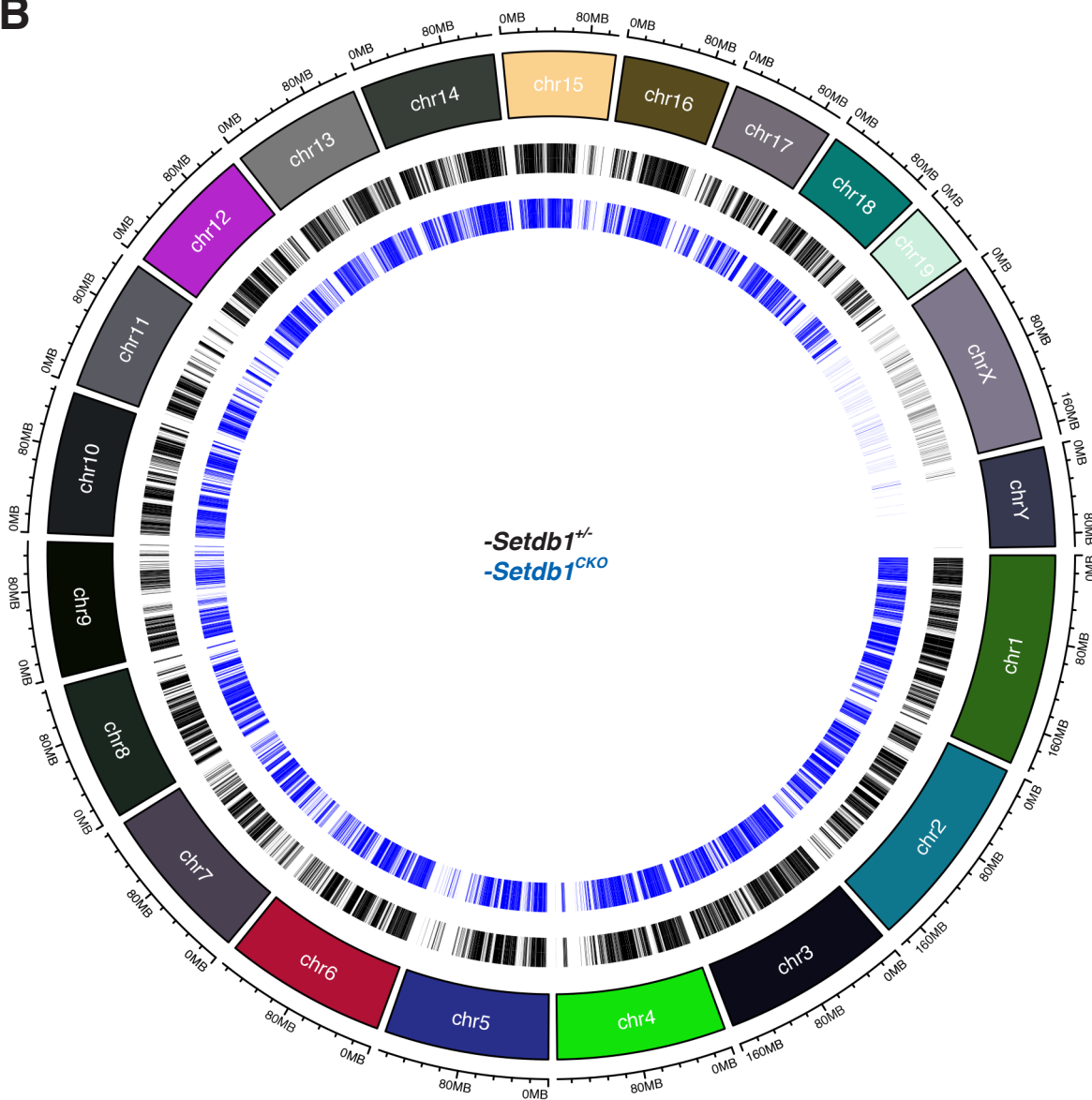


# Supplementary Figure 5

**A**



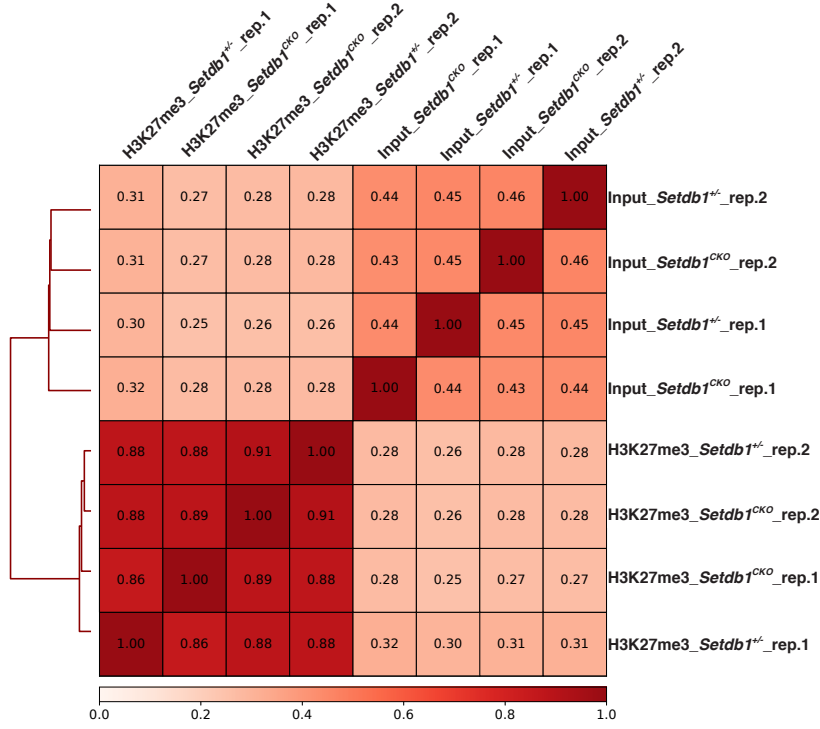
**B**



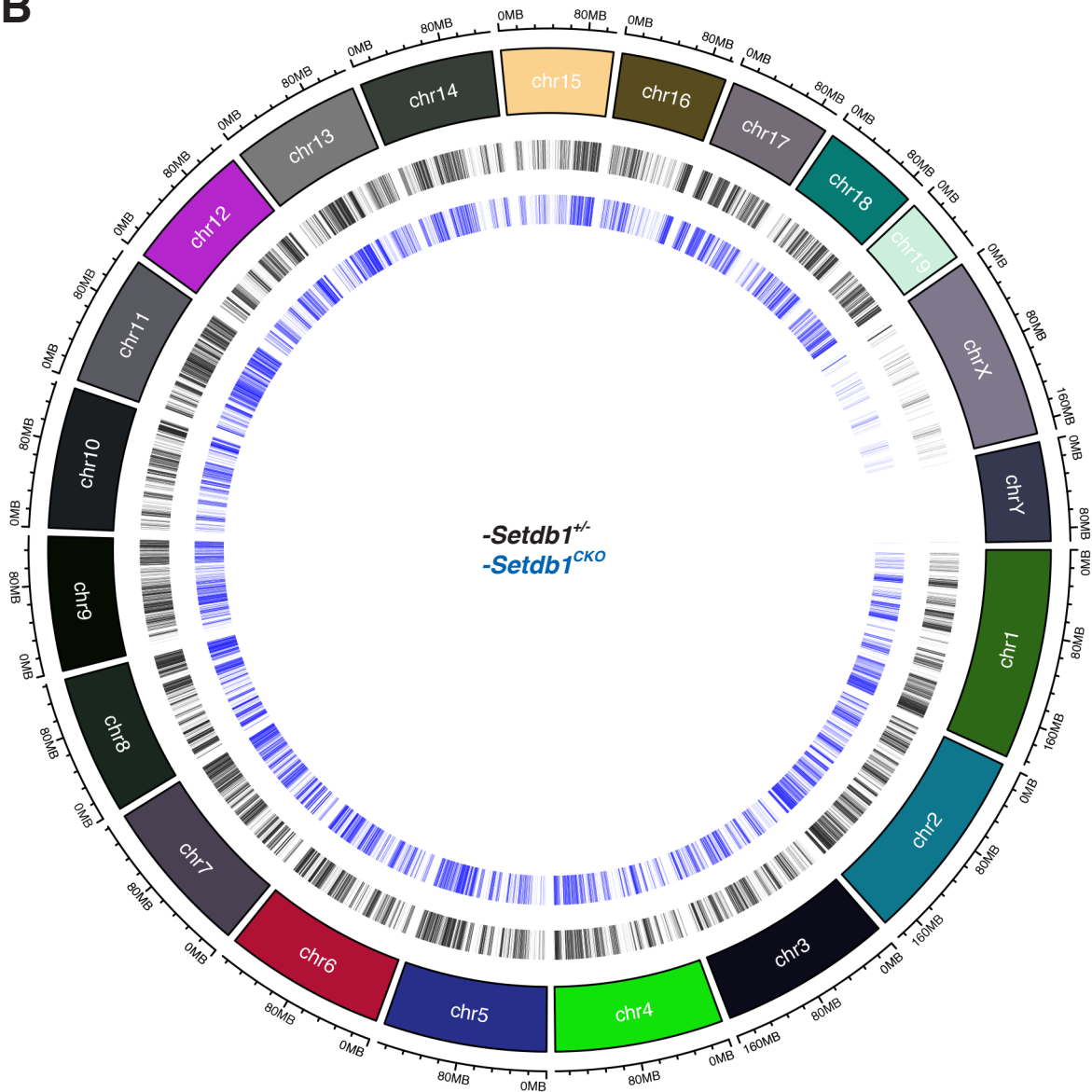


# Supplementary Figure 6

**A**



**B**



# Supplementary Figure 7

