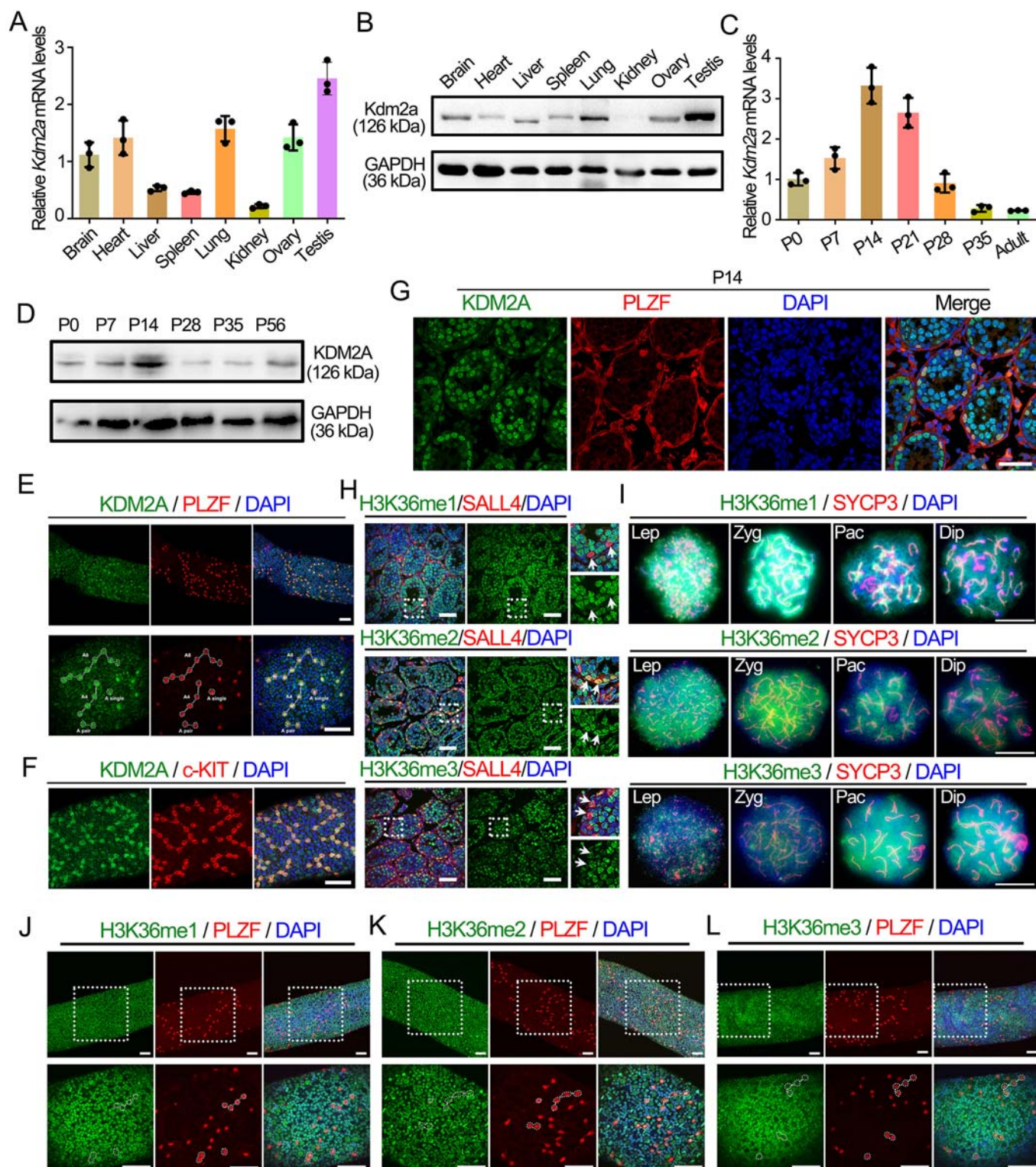
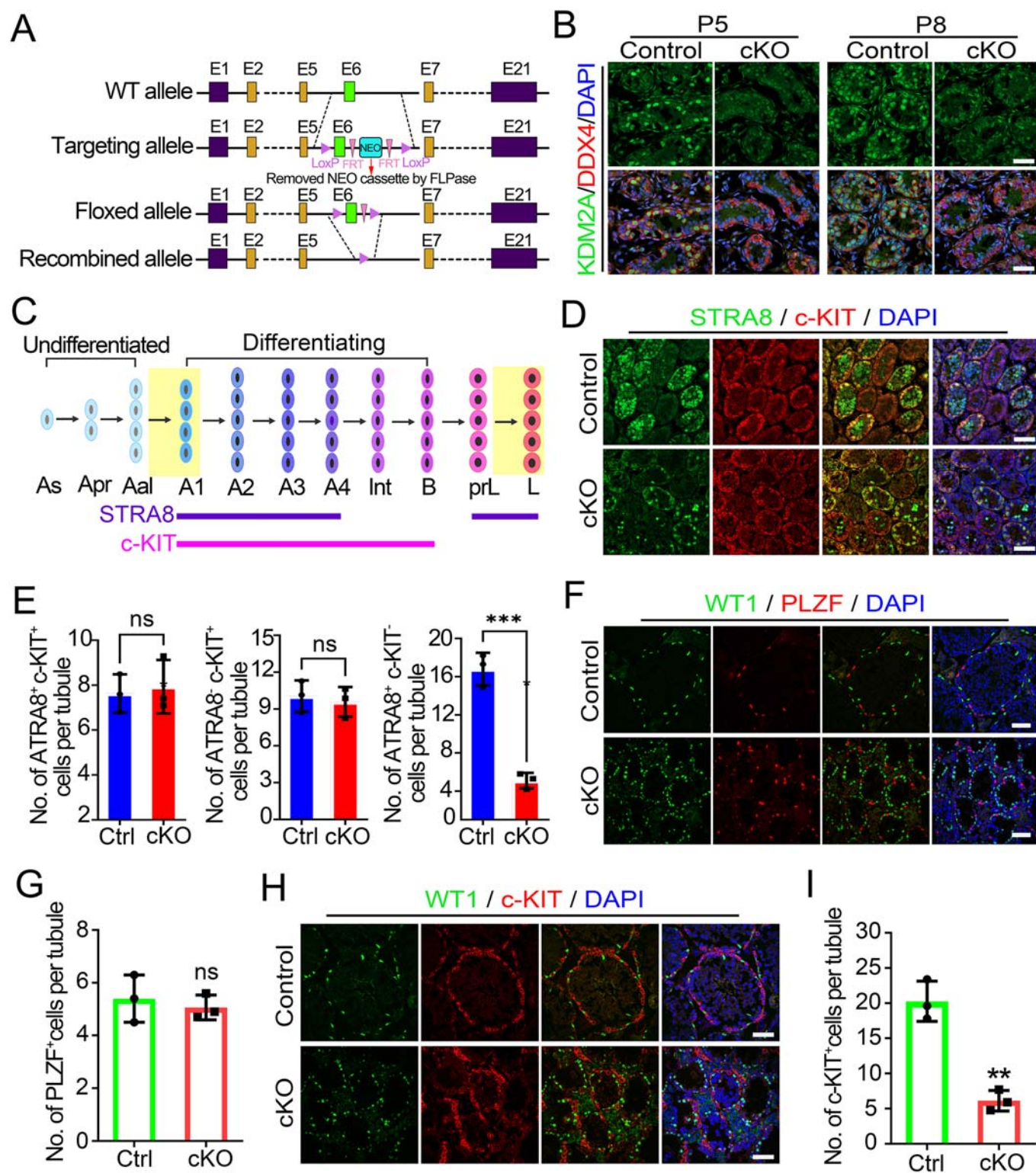


Expanded View Figures

Figure EV1. The expression pattern of KDM2A and H3K36me1/2/3 during spermatogenesis.

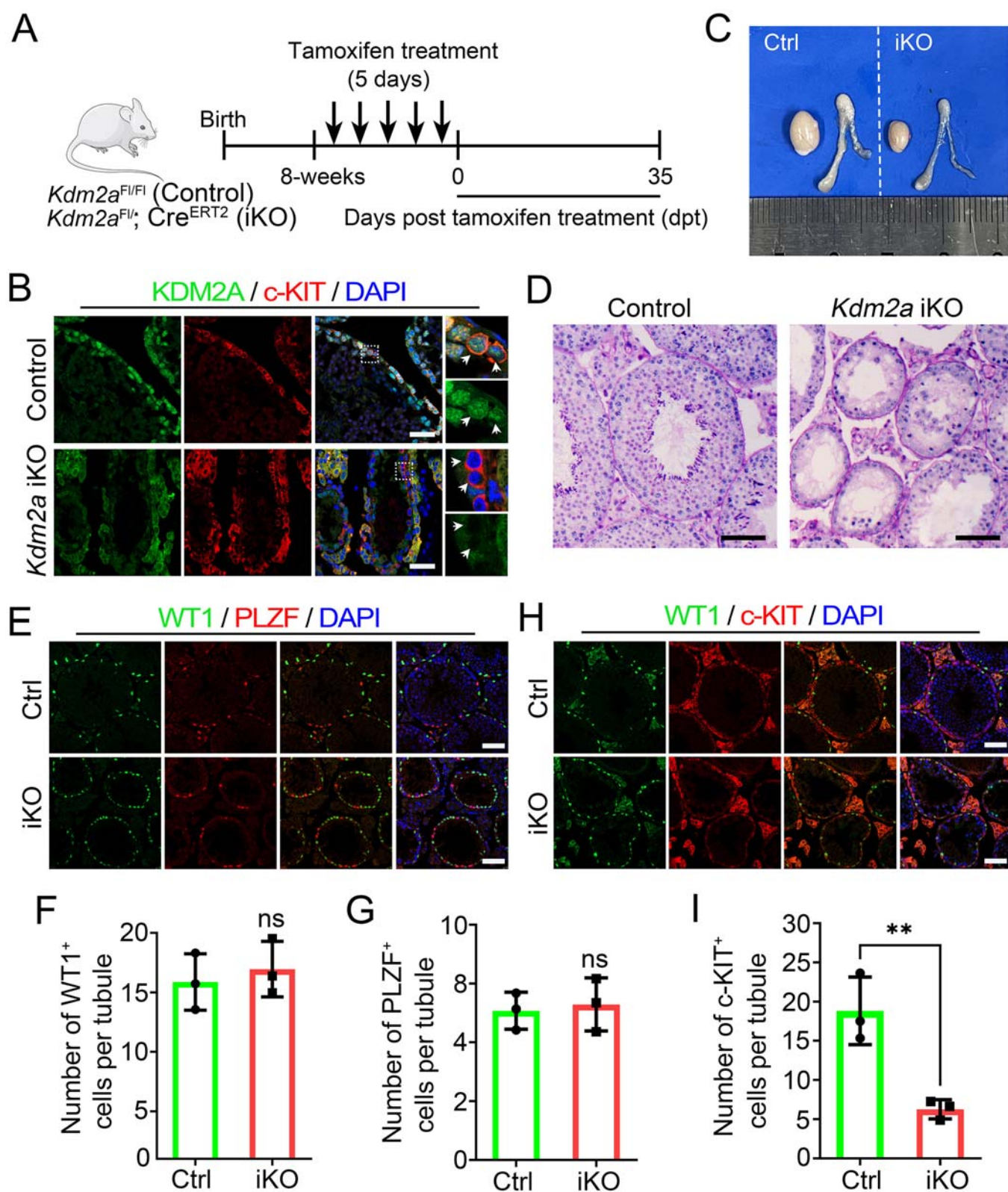
(A) RT-qPCR analyses of *Kdm2a* mRNA levels in various organs from wild-type (WT) adult mice. $n = 3$ (three biological replicates). Data are presented as the mean $\pm$ SD. (B) WB analyses the expression of KDM2A protein levels in multiple organs from WT adult mice. GAPDH was used as a loading control. Biologically independent mice ($n = 3$) performed three independent experiments. Data are presented as the mean $\pm$ SD. (C) RT-qPCR analyses of *Kdm2a* mRNA levels in WT testes at different developmental stages including postnatal day 0 (P0), P7, P14, P21, P28, P35, and adult. $n = 3$ (three biological replicates). (D) WB analyses the KDM2A protein levels in developing WT testes. GAPDH served as a loading control. Biologically independent mice ($n = 3$) performed three separate experiments. (E) Whole-mount staining of seminiferous tubules from adult WT testis with antibodies against KDM2A and PLZF. Nuclei were stained with DAPI. Scale bars = 50 μ m. Examples of A_{single} (As), A_{paired} (Apr), and A_{aligned} (Aal(4), Aal(8)) undifferentiated spermatogonia in adult testes (dotted circles). (F) same as (E) for adult WT testes, but with antibodies against KDM2A and c-KIT. Nuclei were stained with DAPI. Scale bars = 50 μ m. (G) Co-Immunofluorescent staining of KDM2A and PLZF on testis sections from WT mice at P14. Nuclei were stained with DAPI. Scale bars = 50 μ m. (H) Co-Immunofluorescent staining of SALL4 with H3K36me1 (upper), H3K36me2 (middle), and H3K36me3 (lower) on testis sections from WT mice at P14, respectively. Nuclei were stained with DAPI. Scale bars = 50 μ m. The white arrows indicate the SALL4⁺ spermatogonia. (I) Co-Immunofluorescent staining of SYCP3 with H3K36me1 (upper), H3K36me2 (middle), and H3K36me3 (lower) on surface-spread spermatocytes from WT mice at P21, respectively. Nuclei were stained with DAPI. Leptotene (Lep), Zygotene (Zyg), Pachytene (Pac), and Diplotene (Dip) spermatocytes are shown. Scale bars = 5 μ m. (J-L) Co-Immunofluorescent staining of PLZF with H3K36me1 (J), H3K36me2 (K), and H3K36me3 (L) on whole-mount testes seminiferous tubules from adult WT mice. Nuclei were stained with DAPI. Scale bars = 50 μ m. Source data are available online for this figure.





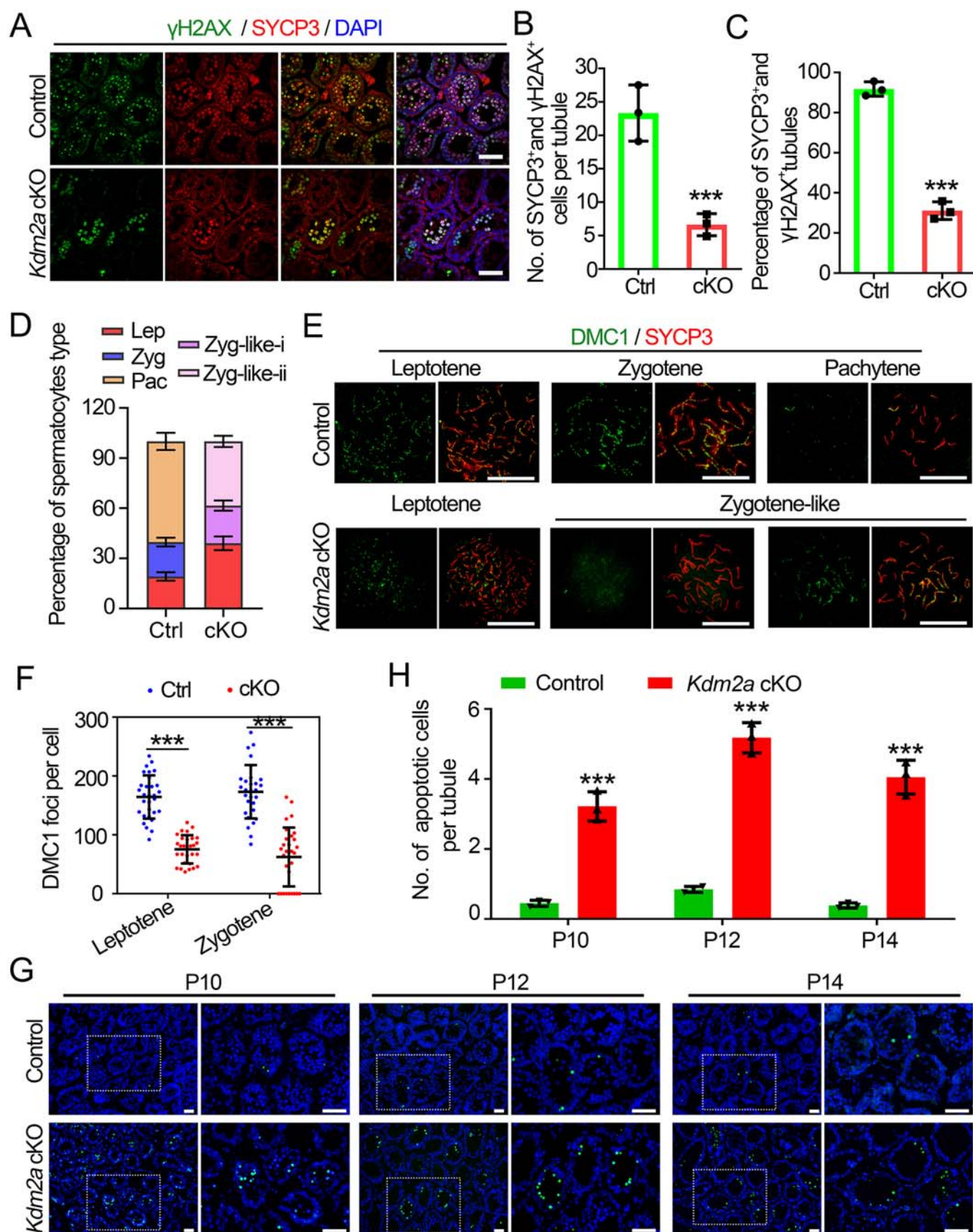
◀ **Figure EV2. *Kdm2a* deletion results in defective differentiation of adult spermatogonia.**

(A) Schematic diagram of the targeting strategy used to generate floxed *Kdm2a* allele by homologous recombination in mouse embryonic stem cells. (B) Immunofluorescence (IF) staining of KDM2A and DDX4 in control and *Kdm2a* cKO testes of P5 and P8 mice. Scale bars = 50 μ m. (C) The Schematic diagram of spermatogonial differentiation process. As, A-single; Apr, A-paired; Aal, A-aligned; Int, intermediary spermatogonia; B, B spermatogonia; prL, preleptotene spermatocytes; and L, leptotene spermatocytes. (D) Co-immunofluorescent staining of STRA8 with c-KIT on testis sections from P10 control and *Kdm2a* cKO mice. Nuclei were stained with DAPI. Scale bars = 50 μ m. (E) The quantifications of STRA8⁺ c-KIT⁺ or STRA8⁺ c-KIT⁺ or STRA8⁺ c-KIT⁻ cells per tubule. Data were presented as the mean $\pm$ SD. $n = 3$ biological replicates. P value was calculated using a two-tailed Student's t -test. ns, not significant. *** $P = 0.0004$. (F) Co-immunofluorescent staining of WT1 with PLZF on testis sections from adult control and *Kdm2a* cKO mice. Nuclei were stained with DAPI. Scale bars = 50 μ m. (G) Quantification of PLZF⁺ cells per tubule for (F). Data were presented as the mean $\pm$ SD. $n = 3$ biological replicates. P value was calculated using a two-tailed Student's t -test. ns, not significant. (H) Co-immunofluorescent staining of WT1 with c-KIT on testis sections from adult control and *Kdm2a* cKO mice. Nuclei were stained with DAPI. Scale bars = 50 μ m. (I) Quantification of c-Kit⁺ cells per tubule for (H). Data were presented as the mean $\pm$ SD. $n = 3$ biological replicates. P value was calculated using a two-tailed Student's t -test. ns, not significant. ** $P = 0.0016$. Source data are available online for this figure.



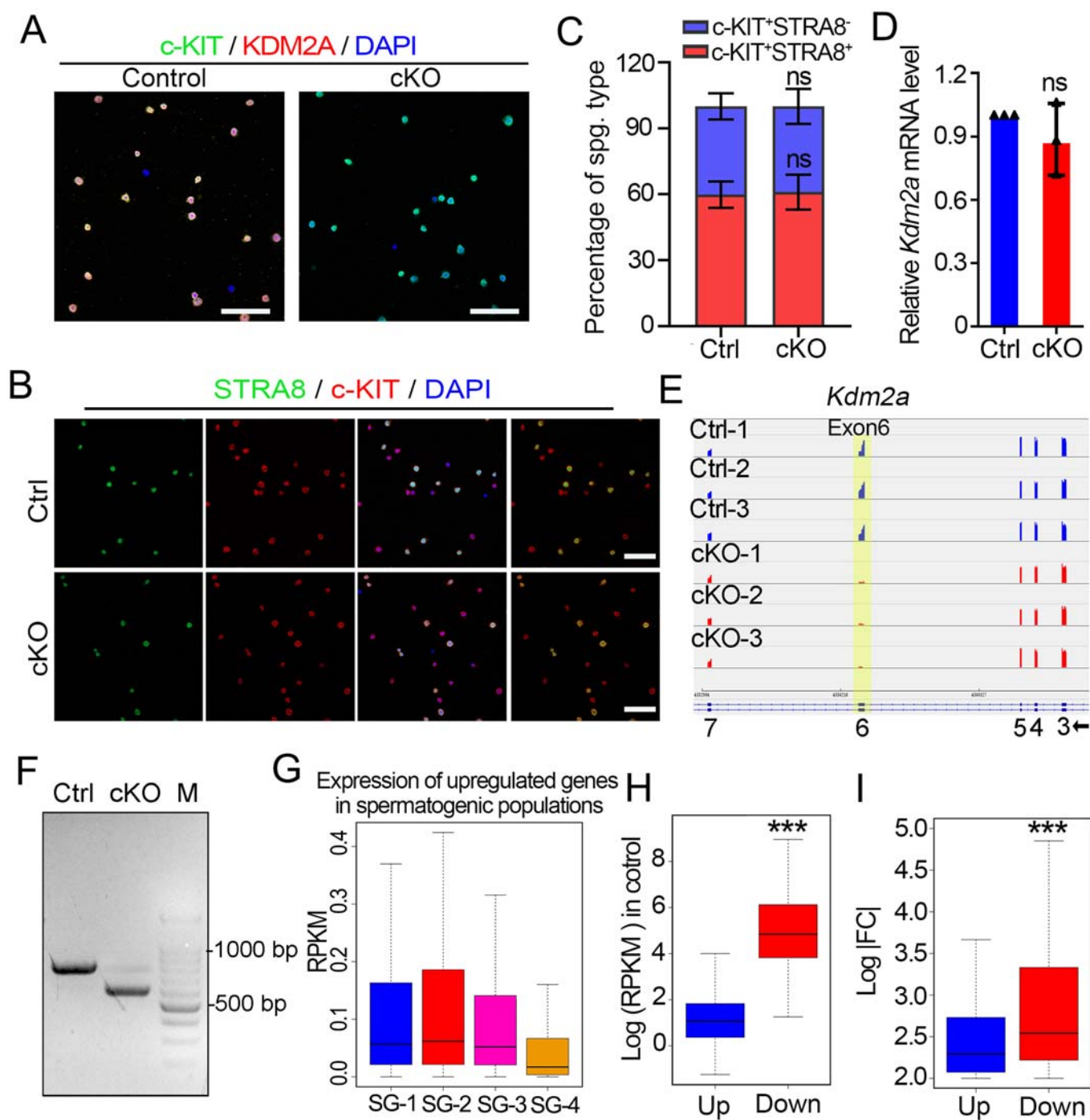
◀ **Figure EV3. Tamoxifen-induced *Kdm2a* deletion in adult mice affects spermatogonial differentiation.**

(A) Regimen of tamoxifen treatment in adult (2 M) *Kdm2a^{fl/-}* Ddx4-Cre^{ERT2} male mice. (B) Immunofluorescence (IF) staining of KDM2A and c-KIT in control and *Kdm2a* iKO testes. Arrowheads indicate KDM2A signals in the c-KIT positive cells. Nuclei were stained with DAPI. Enlarged images are shown in right panels. Scale bars = 50 μ m. (C) Gross morphology of testes and epididymides from control and *Kdm2a* iKO mice. (D) Periodic acid-Schiff (PAS) staining of testes from control and *Kdm2a*-iKO mice. Scale bars = 50 μ m. (E) Co-immunofluorescent staining of WT1 with PLZF on testis sections from adult control and *Kdm2a* iKO mice. Nuclei were stained with DAPI. Scale bars = 50 μ m. (F, G) Quantification of WT1⁺ and PLZF⁺ (G) cells per tubule for (E). Data were presented as the mean $\pm$ SD. *n* = 3 biological replicates. *P* value was calculated using a two-tailed Student's *t*-test. ns, not significant. (H) Co-immunofluorescent staining of WT1 with c-KIT on testis sections from adult control and *Kdm2a* iKO mice. Nuclei were stained with DAPI. Scale bars = 50 μ m. (I) Quantification of c-Kit⁺ cells per tubule for (H). Data were presented as the mean $\pm$ SD. *n* = 3 biological replicates. *P* value was calculated using a two-tailed Student's *t*-test. ns, not significant. ***P* = 0.0083. Source data are available online for this figure.



◀ **Figure EV4. *Kdm2a* deletion results in defective meiosis initiation and progression.**

(A) Co-immunostaining of SYCP3 with γ H2AX in testis sections from control and *Kdm2a* cKO mice at P14. Nuclei were stained with DAPI. Scale bars = 50 μ m. (B, C) The quantifications of SYCP3⁺ and γ H2AX⁺ cells per tubule (B) and the percentage of SYCP3⁺ and γ H2AX⁺ tubule (C) are shown. Data were presented as the mean $\pm$ SD. $n = 3$ biological replicates. P value was calculated using a two-tailed Student's t -test. ns, not significant. For (B), *** $P = 0.003$; For (C), *** $P < 0.0001$. (D) The percentage of spermatocytes at the leptotene (Lep), zygotene (Zyg), and pachytene (Pac) stages for (Fig. 3D). Data were presented as the mean $\pm$ SD. $n = 3$ biological replicates. (E) Spermatocyte spreads from control and *Kdm2a* cKO testes at P18 were co-stained SYCP3 and DMC1. Scale bars = 50 μ m. (F) A scatter plot shows the number of DMC1 foci per cell on SYCP3 axes in leptotene and zygotene spermatocytes from control and *Kdm2a* cKO mice, respectively. Data are presented as the mean $\pm$ SD. A total of $n = 30$ control leptotema, $n = 29$ *Kdm2a* cKO leptotema, $n = 29$ control zygonema, and $n = 30$ *Kdm2a* cKO zygonema were counted from three biologically independent mice for each genotype. P value was calculated using a two-tailed Mann-Whitney U -test. left to right: *** $P < 0.0001$, *** $P < 0.0001$. (G) TUNEL staining of testis sections from control and *Kdm2a* cKO mice at P10, P12, and P14. Nuclei were stained with DAPI. Scale bars = 50 μ m. (H) Quantification of apoptotic cells per tubule for (G). Data were presented as the mean $\pm$ SD. $n = 3$ biological replicates. P value was calculated using a two-tailed Student's t -test. left to right: *** $P = 0.0003$, *** $P < 0.0001$, *** $P = 0.0002$. Source data are available online for this figure.



◀ **Figure EV5. Verification of KDM2A deletion in isolated c-KIT cells from *Kdm2a* cKO testes.**

(A) Co-immunofluorescence staining of anti-c-KIT with anti-KDM2A on isolated differentiated spermatogonia from control and KDM2A cKO mice. Nuclei were stained with DAPI. Scale bars = 50 μ m. (B) Co-immunofluorescence staining of anti-STRAB with anti-c-KIT on isolated differentiated spermatogonia from P10 control and *Kdm2a* cKO mice. Nuclei were stained with DAPI. Scale bars = 50 μ m. (C) The percentage of c-KIT⁺ STRAB⁺ and c-KIT⁺ STRAB⁻ spermatogonia from control and *Kdm2a* cKO mice. Data were presented as the mean $\pm$ SD. $n = 3$ biological replicates. P value was calculated using a two-tailed Student's t -test. ns, not significant. (D) RT-qPCR analyses of *Kdm2a* mRNA levels in isolated differentiated spermatogonia from P10 control and *Kdm2a* cKO mice. Data were presented as the mean $\pm$ SD. $n = 3$ biological replicates. P value was calculated using a two-tailed Student's t -test. ns, not significant. (E) Genome browser tracks depicting peaks of the region containing exon6 of *Kdm2a* based on RNA-seq. (F) RT-PCR analyses of *Kdm2a* mRNA in isolated c-KIT spermatogonia from control and *Kdm2a* cKO mice. (G) Expression levels (RPKM) of the upregulated 1934 genes in different types of spermatogonia (SG1-4) were reanalyzed with the previously published data of stage-specific bulk RNA-seq ($N = 3$ biologically independent samples). Whiskers indicate min and max. Bounds of box indicate 25th and 75th percentiles quantile with median. SPG 1, 2, 3, 4 correspond to SSCs (spermatogenic stem cells), undifferentiated spermatogonia, early differentiating spermatogonia and late differentiating spermatogonia, respectively. (H) Expression levels (RPKM) of the 1934 upregulated and 1922 downregulated genes in control (c-KIT⁺ cells of P10 control mice) are shown by box-whisker plot (whiskers indicate min and max. Bounds of box indicate 25th and 75th percentiles quantile with median). Data were presented as the mean $\pm$ SD. P value was calculated using an unpaired two-tailed Student's t -test. *** $P < 0.0001$. (I) Fold change of the 1934 upregulated and 1922 downregulated genes from RNA-seq are shown by box-whisker plot (whiskers indicate min and max. Bounds of box indicate 25th and 75th percentiles quantile with median). Data were presented as the mean $\pm$ SD. P value was calculated using an unpaired two-tailed Student's t -test. *** $P < 0.0001$. Source data are available online for this figure.