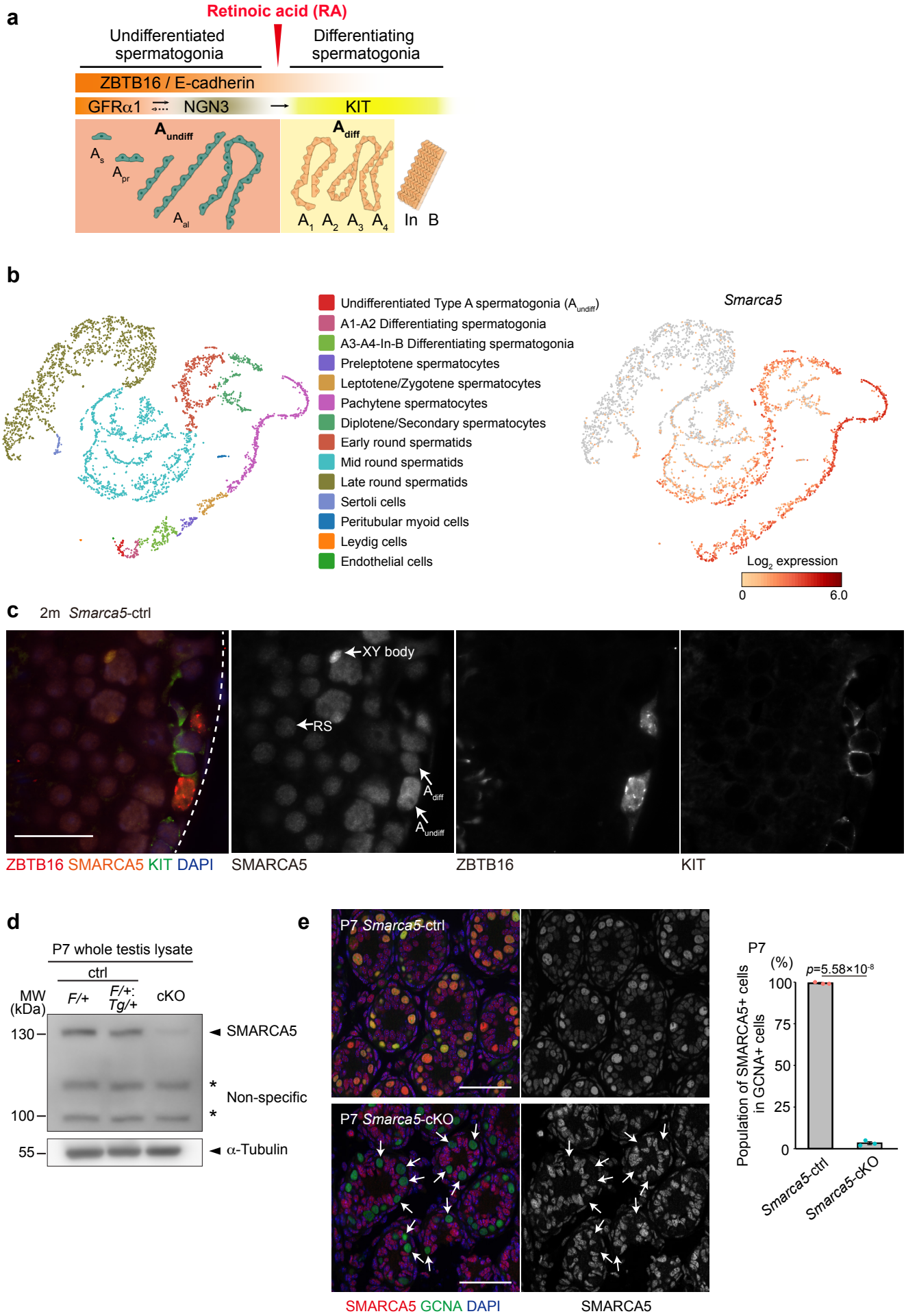

a
Retinoic acid (RA)
Undifferentiated spermatogonia
Differentiating spermatogonia
ZBTB16 / E-cadherin
GFRα1
NGN3
KIT
A_undiff
A_s
A_pr
A_al
A_diff
A_1 A_2 A_3 A_4
In B
b
Undifferentiated Type A spermatogonia (A_undiff)
A1-A2 Differentiating spermatogonia
A3-A4-In-B Differentiating spermatogonia
Preleptotene spermatocytes
Leptotene/Zygotene spermatocytes
Pachytene spermatocytes
Diplotene/Secondary spermatocytes
Early round spermatids
Mid round spermatids
Late round spermatids
Sertoli cells
Peritubular myoid cells
Leydig cells
Endothelial cells
Smarca5
Log2 expression
0
6.0
c
2m Smarca5-ctrl
XY body
RS
A_diff
A_undiff
ZBTB16 SMARCA5 KIT DAPI
SMARCA5
ZBTB16
KIT
d
P7 whole testis lysate
ctrl
MW (kDa)
F/+
F/+: Tg/+
cKO
130
100
55
SMARCA5
Non-specific
α-Tubulin
e
P7 Smarca5-ctrl
P7 Smarca5-cKO
SMARCA5 GCNA DAPI
SMARCA5
P7
(%)
p=5.58×10-8
Population of SMARCA5+ cells in GCNA+ cells
100
75
50
25
0
Smarca5-ctrl
Smarca5-cKO

Extended Data Figure 1. SMARCA5 expression during spermatogenesis and its deletion in the germline

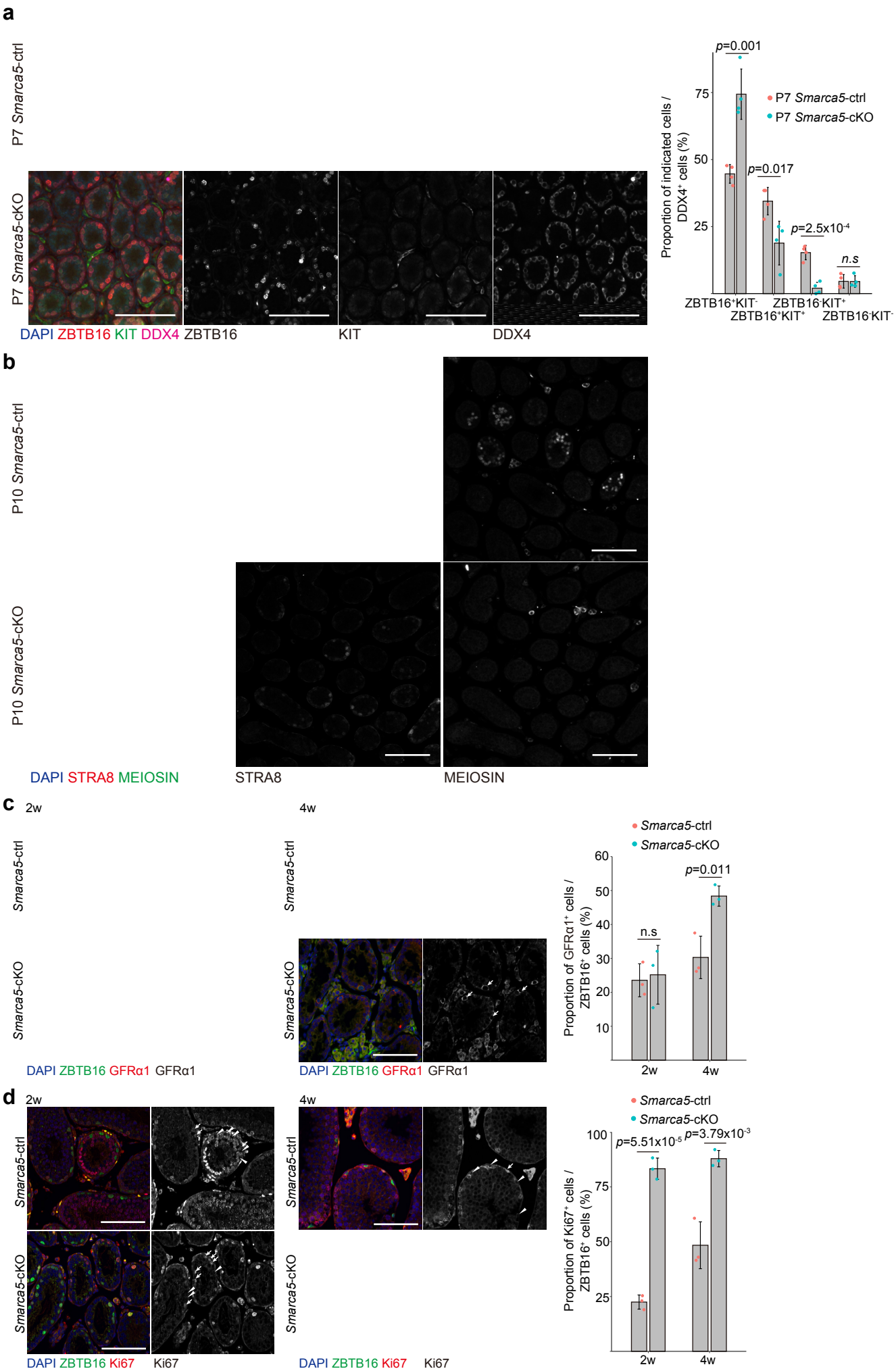
a. Schematic representation of the spermatogonial hierarchy and marker gene expression (created with BioRender.com). Spermatogonia are classified as type A, intermediate (In), or type B. Type A includes undifferentiated spermatogonia (A_{undiff}, including A_{single} (A_s), A_{paired}(A_{pr}) and $A_{aligned}$(A_{al})), and early differentiating spermatogonia (A_{diff}, including A_1, A_2, A_3 and A_4), while In and type B represent later stages of differentiating spermatogonia.

b. t-SNE plot of single-cell RNA-seq data from adult testis,[35] showing identification of cell clusters (left) and *Smarca5* expression across clusters (right).

c. Immunostaining of SMARCA5, ZBTB16, and KIT in 2-month-old *Smarca5*-ctrl testes. In the SMARCA5-stained panel, representative A_{undiff}, A_{diff}, XY body, and round spermatid (RS) are indicated by arrows. Scale bar, 25 μm.

d. Western blot analysis of testicular lysate obtained from *Smarca5*$^{F/+}$, *Smarca5*$^{F/+}$; *Ddx4*-Cre^{+} (*Smarca5*-ctrl) and *Smarca5*$^{F/F}$; *Ddx4*-Cre^{+} (*Smarca5*-cKO) at P7. Asterisks indicate the positions of non-specific bands.

e.Testis sections of *Smarca5*-cKO and control littermates at P7 stained with DAPI and antibodies against SMARCA5 and GCNA (germ cell marker). Scale bar, 100 μm. The graph shows the percentage of SMARCA5^{+} cell in GCNA^{+} cells. Error bars represent mean ± s.d. 100 GCNA^{+} cells for each samples were counted. Independent values obtained from individual mice are shown as dots. SMARCA5^{-}GCNA^{+} cells are shown with arrows. Statistical significance was assessed using a two-tailed unpaired Student's t-test assuming equal variances (n = 3 per group).

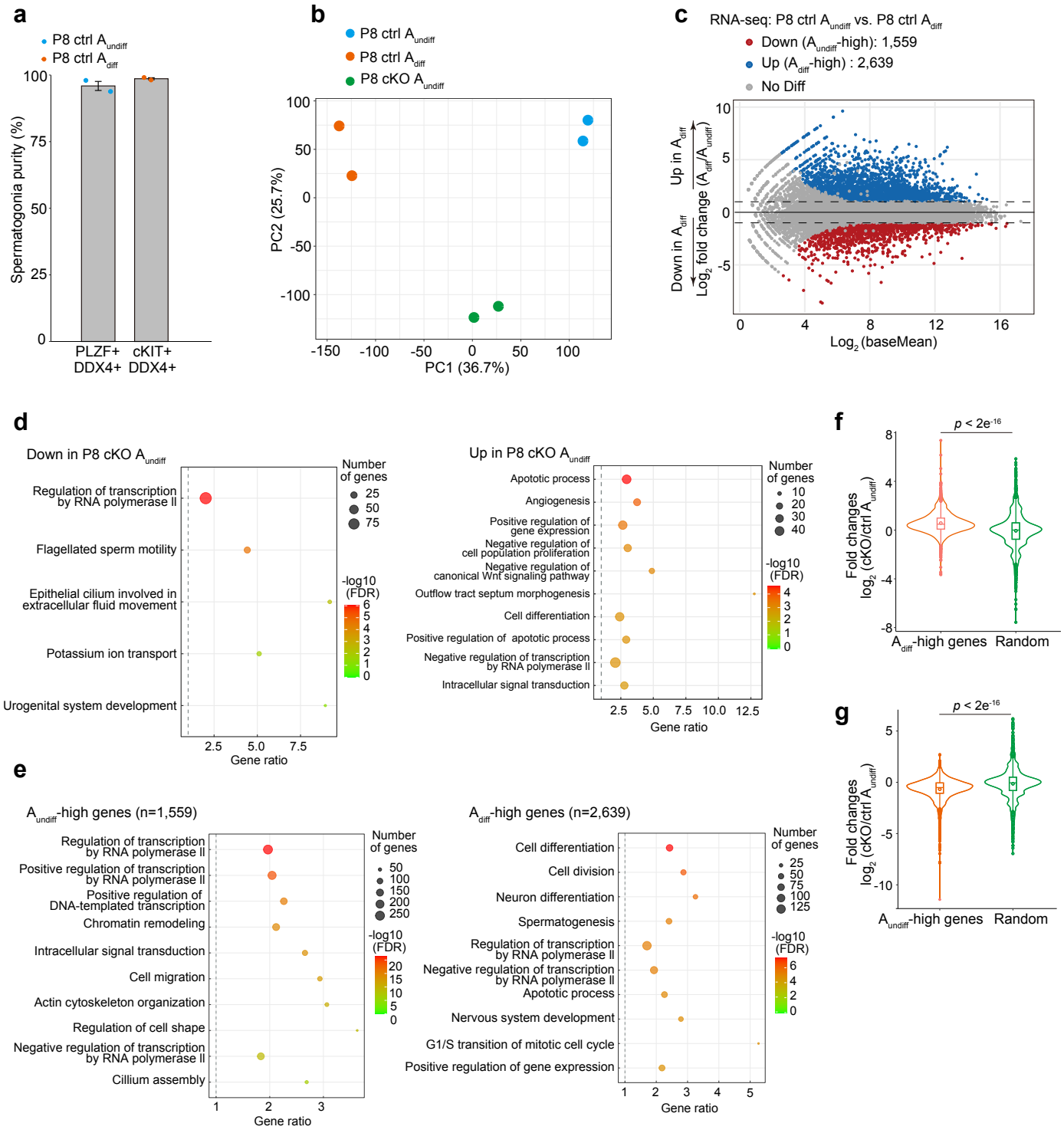

a
P7 Smarca5-ctrl
P7 Smarca5-cKO
DAPI ZBTB16 KIT DDX4
ZBTB16
KIT
DDX4
p=0.001
p=0.017
p=2.5x10-4
n.s
Proportion of indicated cells / DDX4+ cells (%)
P7 Smarca5-ctrl
P7 Smarca5-cKO
ZBTB16+KIT-
ZBTB16+KIT+
ZBTB16-KIT+
ZBTB16-KIT-
b
P10 Smarca5-ctrl
P10 Smarca5-cKO
DAPI STRA8 MEIOSIN
STRA8
MEIOSIN
c
2w
4w
Smarca5-ctrl
Smarca5-cKO
DAPI ZBTB16 GFRα1
GFRα1
Smarca5-ctrl
Smarca5-cKO
p=0.011
n.s
Proportion of GFRα1+ cells / ZBTB16+ cells (%)
d
2w
4w
Smarca5-ctrl
Smarca5-cKO
DAPI ZBTB16 Ki67
Ki67
p=5.51x10-5
p=3.79x10-3
Proportion of Ki67+ cells / ZBTB16+ cells (%)

Extended Data Figure 2. Immunohistochemical analysis of *Smarca5*-cKO testis sections.

a. Testis sections from *Smarca5*-cKO and control littermates at P7 stained with DAPI and antibodies against ZBTB16, KIT, and DDX4. Scale bars, 100 μm. The graph shows the percentage of $ZBTB16^+$ and/or KIT^+ cells among $DDX4^+$ germ cells. Error bars represent mean ± s.d. At least 180 $DDX4^+$ germ cells were counted per sample. Data points represent values from individual mice. Statistical significance was assessed using a two-tailed unpaired Student's t-test assuming equal variances (n = 4 per group).

b. Testis sections from *Smarca5*-cKO and control littermates at P10 stained with DAPI and antibodies against STRA8 and MEIOSIN (a marker of preleptotene spermatocytes). Scale bars, 100 μm.

c, d. Testis sections from *Smarca5*-cKO and control littermates at 2 and 4 weeks of age stained with DAPI and antibodies against ZBTB16 and Ki67 (**c**) or GFRα1 (**d**). Scale bars, 100 μm. Graphs show the percentage of $Ki67^+$ (**c**) or $GFR\alpha1^+$ (**d**) cells among $ZBTB16^+$ cells. Error bars represent mean ± s.d. At least 120 $ZBTB16^+$ cells were counted per sample. Data points represent individual mice. Statistical significance was determined using a two-tailed unpaired Student's t-test assuming equal variances (n = 3 per group). $Ki67^+$ and $GFR\alpha1^+$ cells are indicated by arrows; negative cells are indicated by arrowheads.



Extended Data Figure 3. RNA-seq analysis of A_{undiff} and A_{diff}.

a. Quantification of the purity of isolated spermatogonia. Purity values from two independent experiments are shown as individual dots.

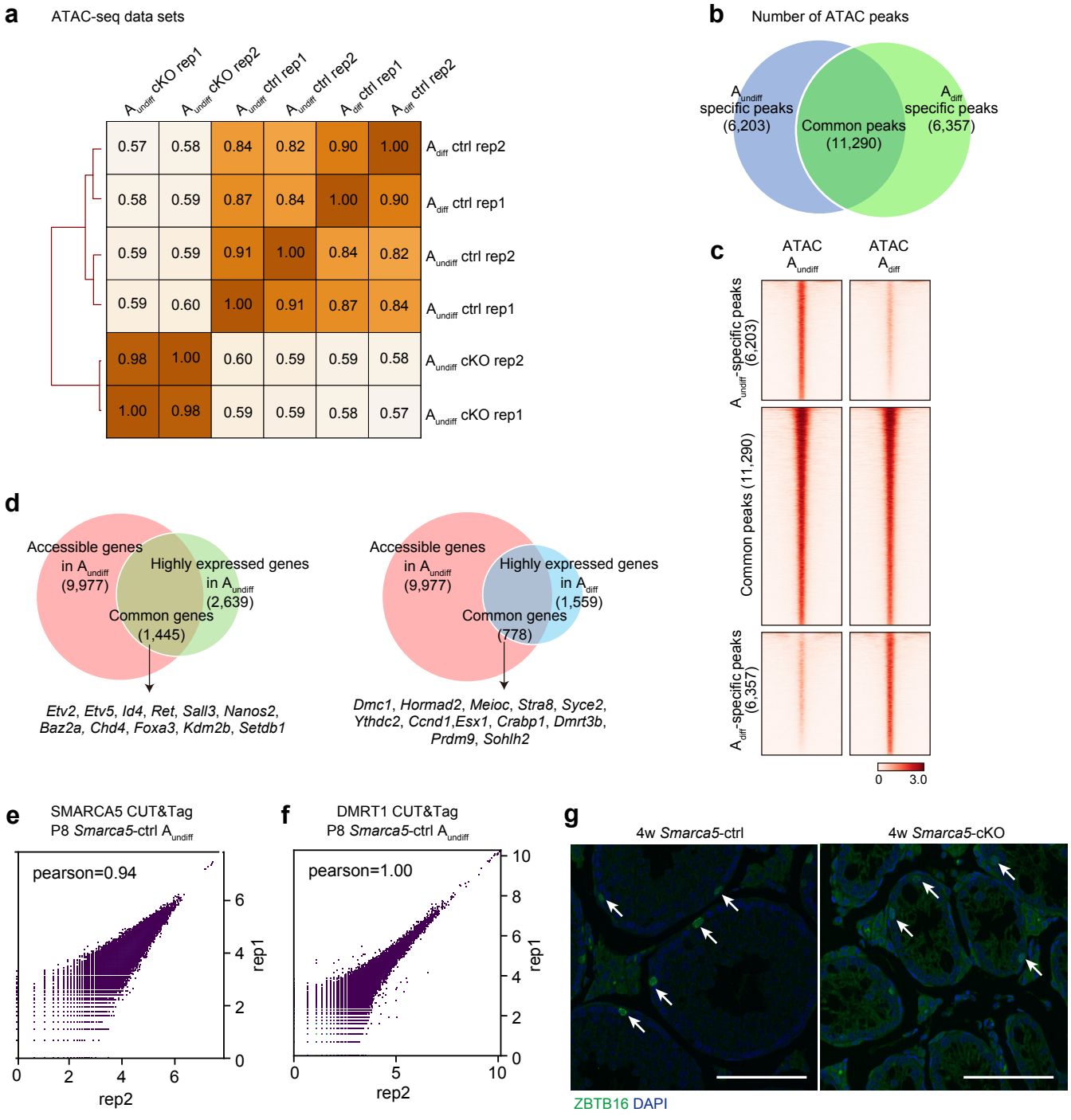
b. Principal component analysis (PCA) plot showing biological replicates of RNA-seq samples.

c. Transcriptomic comparison between A_{undiff} and A_{diff} populations. Differentially expressed genes (DEGs) are defined as those with Log_2 fold change > 2, *Padj* < 0.05, based on a binomial test with Benjamini-Hochberg correction.

d. Gene Ontology (GO) analysis of genes downregulated (left) and upregulated (right) in *Smarca5*-cKO A_{undiff} compared to *Smarca5*-ctrl A_{undiff}.

e. GO analysis of genes downregulated (A_{undiff}-high genes: left) and upregulated (A_{diff}-high genes: right) in *Smarca5*-ctrl A_{diff} compared to ctrl A_{undiff}.

f, g. Violin plots showing log_2 fold change in *Smarca5*-cKO A_{undiff} compared to ctrl A_{undiff} within gene groups highly expressed in A_{diff} (A_{diff}-high genes: n = 2,639; **f)** or A_{undiff} (A_{undiff}-high genes: n = 1,559; **g**). For comparison, log_2 fold change values from randomly selected genes are also shown. Box plots indicate the 25th, median, and 75th percentiles; dots within the box represent the mean. Statistical significance was based on Levene's test and Wilcoxon rank sum test.



Extended Data Figure 4. ATAC-seq and CUT&Tag analysis of A_{undiff} and A_{diff}.

a. Heatmap showing Pearson correlation coefficients among each cell type based on ATAC-seq profiles. Two biological replicates were merged for downstream analysis.

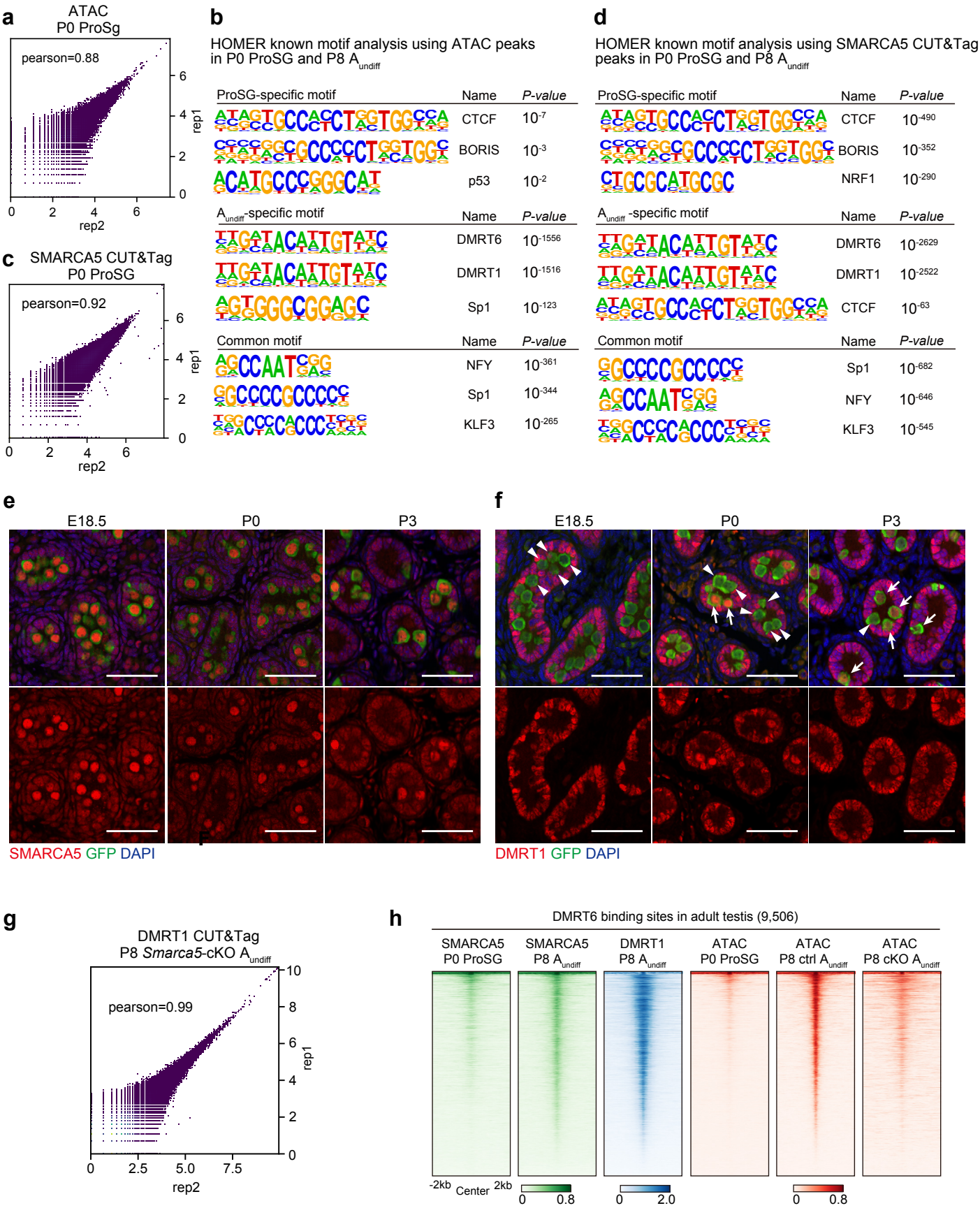
b. Venn diagram showing the overlap of ATAC-seq peaks between A_{undiff} and A_{diff}.

c. Heatmaps showing ATAC-seq enrichment in A_{undiff} and A_{diff} at A_{undiff}-specific, common, or A_{diff}-specific peaks.

d. Venn diagrams showing the overlap between accessible genes in A_{undiff} (n = 9,977) and genes highly expressed in A_{undiff} compared to A_{undiff} (left), or highly expressed in A_{diff} compared to A_{diff} (right). Genes located within ±10 kb of ATAC-seq peaks in A_{undiff} were defined as accessible.

e, f. Scatter plots showing Pearson correlation between two biological replicates for SMARCA5 CUT&Tag (**e**) and DMRT1 CUT&Tag (**f**) in P8 *Smarca5*-ctrl A_{undiff}. Two replicates were merged for downstream analysis.

g. Testis sections from *Smarca5*-cKO and control littermates at 4 weeks stained with DAPI and antibodies against ZBTB16. Arrows indicate ZBTB16^{+} cells. Scale bars, 100 μm.


a
ATAC
P0 ProSg
pearson=0.88
rep1
rep2
c
SMARCA5 CUT&Tag
P0 ProSG
pearson=0.92
rep1
rep2
b
HOMER known motif analysis using ATAC peaks in P0 ProSG and P8 A undiff
ProSG-specific motif
Name
P-value
CTCF
10-7
BORIS
10-3
p53
10-2
A undiff -specific motif
Name
P-value
DMRT6
10-1556
DMRT1
10-1516
Sp1
10-123
Common motif
Name
P-value
NFY
10-361
Sp1
10-344
KLF3
10-265
d
HOMER known motif analysis using SMARCA5 CUT&Tag peaks in P0 ProSG and P8 A undiff
ProSG-specific motif
Name
P-value
CTCF
10-490
BORIS
10-352
NRF1
10-290
A undiff -specific motif
Name
P-value
DMRT6
10-2629
DMRT1
10-2522
CTCF
10-63
Common motif
Name
P-value
Sp1
10-682
NFY
10-646
KLF3
10-545
e
E18.5
P0
P3
SMARCA5 GFP DAPI
f
E18.5
P0
P3
DMRT1 GFP DAPI
g
DMRT1 CUT&Tag
P8 Smarca5-cKO A undiff
pearson=0.99
rep1
rep2
h
DMRT6 binding sites in adult testis (9,506)
SMARCA5 P0 ProSG
SMARCA5 P8 A undiff
DMRT1 P8 A undiff
ATAC P0 ProSG
ATAC P8 ctrl A undiff
ATAC P8 cKO A undiff
-2kb
Center
2kb
0
0.8
0
2.0
0
0.8

Extended Data Figure 5. ATAC-seq and CUT&Tag analysis of ProSG and A_{undiff}.

a, c. Scatter plot showing Pearson correlation between two biological replicates of ATAC-seq (**a**) and SMARCA5 CUT&Tag (**c**) in P0 ProSG. Replicates were merged for downstream analysis.

b, d. HOMER known motif analysis of ATAC-seq peaks (**b**) and SMARCA5 CUT&Tag peaks (**d**) at ProSG-specific, A_{undiff}-specific, and common sites.

e, f. Testis sections from *Stalla*-GFP mice stained with DAPI and antibodies against GFP and SMARCA5 (**e**) or DMRT1 (**f**) at E18.5, P0, and P3. $DMRT1^{+}$ germ cells are indicated by arrows; $DMRT1^{-}$ cells are indicated by arrowheads. Scale bars, 100 μm.

g. Scatter plot showing Pearson correlation between two biological replicates of DMRT1 CUT&Tag in P8 *Smarca5*-cKO A_{undiff}.

h. Heatmap showing SMARCA5, DMRT1, and ATAC enrichment in ProSG and A_{undiff} at DMRT6-binding sites identified in the adult testis (n = 9,506).