Supplementary materials

Fig. S1

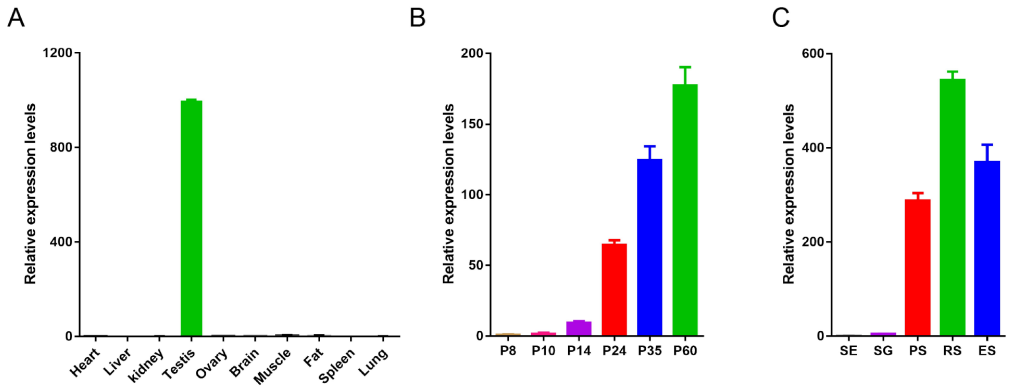

Fig. S1. (A) Quantitative RT-PCR analysis of *Sox30* mRNA transcripts in multiple tissues from adult mice. Gene expression was normalized to *Rplp0* (*36b4*). (B) Levels of *Sox30* mRNA in testis tissue collected postnatal day 8 (P8), P10, P24, P35 and P60. The expression level of *Sox30* mRNA at P7 was arbitrarily set as 1. Data presented are mean ± s.d. from three independent experiments.

Fig. S2

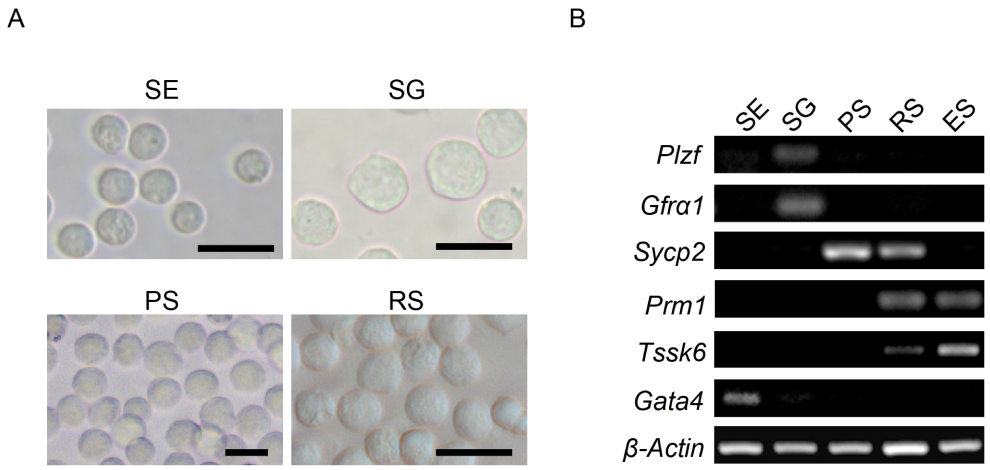

Fig. S2. (A) The purity of mouse testis cell populations collected using the STA-PUT method was confirmed by morphological appearance of spermatogonia (SG), pachytene spermatocytes (PS), round spermatids (RS), and elongating spermatids (ES). SE and SG were isolated from P6-8 mice, and PS and RS as well as elongating spermatids (ES) from 8-week-old mice. Scale bars, 25 μm. (B) To further validate the identity of cell populations collected via STA-PUT, expression of key marker genes was assessed by RT-PCR, including *Plzf*, *Gfrα1* (spermatogonia), *Sycp2* (meiotic spermatocytes), *Prm1* and *Tssk6* (postmeiotic cells) and *Gata4* (Sertoli cells). *Actin* served as a control.

Fig. S3

A

Sox30 founders by CRISPR/Cas9

ID	Gender	Nucleotide Sequence	Amino acid sequences	Fertility
		...CACCGGCCAGCACTAGCCAAAGCTAACCCAGCCGCCAACAATGCAGAAATC AGTGTCCAGCTCGGGTTA...	...HRPALAKANPAANNAEISVQLGL ...	
#1	♂	...CACCGGCCAG-----CCAAAGCTAACCCAGCCGCCAACAATGCAGAAATC AG------CTCGGGTTA...	...HRPA--KANPAANNAEISSGSTOP	Infertile
		...CACCGGCCAGCA---------AACCCAGCCGCCAACAATGCAGAAATCAGTGTC- GGCTCGGGTTA...	...HRPA---- NPAANNAEISVGSGSTOP	
#2	♂	...CAC------CACTAGCCAAAGCTAACCCAGCCGCCAACAATGCAGAAATC AGTGTCCAGCTCGGGTTA...	...H--HSTOP	Infertile
		...CACCGGCCAGCAC------AGCTAACCCAGCCGCCAACAATGCAGAAATC AGTGTCCAGCTCGGGTTA...	...HRPALSTOP	
#3	♂	...CACCGGCCAGCAA-AGCCAAAGCTAACCCAGCCGCCAACAATGCAGAAATCAG---- --CTCGGGTTA...	...HRPAKPKLTQPPTMQKSARVRVE QTSTOP	Infertile
#4	♂	...CACCGGCCAG----------CAGCTCGGGTTA...	...HRPAARVRVEQTSTOP	Fertile
		Wild type	Wild type	
#5	♀	...CA----------GCTAACCCAGCCGCCAACAATGCAGAAATC AGTGTCCAGCTCGGGTTA...	...QLTQPPTMQKSVSSSGSTOP	Fertile
		Wild type	Wild type	
#6	♀	...CACCGGCCAGCA---------AACCCAGCCGCCAACAATGCAGAAATC AGTGTCCAGCTCGGGTTA...	...HRPA----NPAANNAEISVQLGL...	Fertile
		Wild type	Wild type	
#7	♀	...CACCGGCCAGC----------TCGGGTTA...	...HRPARVRVEQTSTOP	Fertile
		...CACCGG----------GTTA...	...HRVRVEQTSTOP	
		...CA----------GCTAACCCAGCCGCCAACAATGCAGAAATC AGTGTCCAGCTCGGGTTA...	...QLTQPPTMQKSVSSSGSTOP	
		...CACCGGCCAGCA--------GCTAACCCAGCCGCCAACAATGCAGAAATC AGTGTCCAGCTCGGGTTA...	...HRPA---ANPAANNAEISVQLGL...	
		Wild type	Wild type	

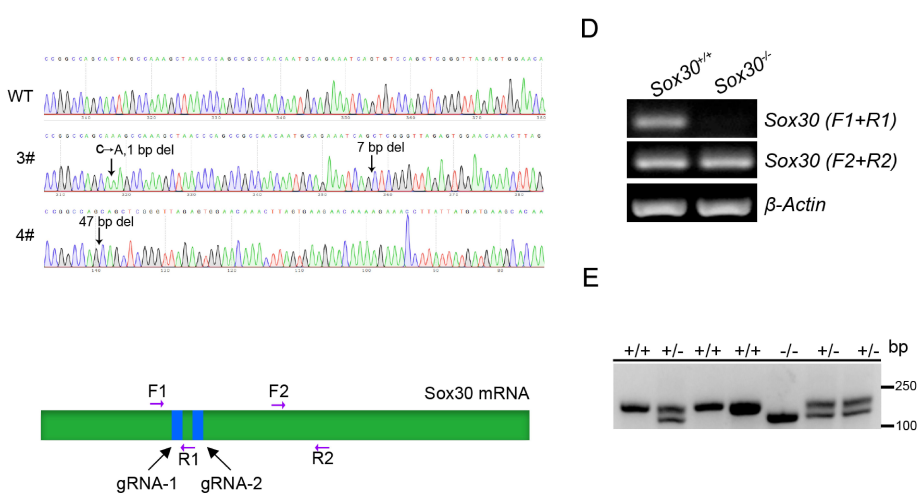

Fig. S3. Generation of *Sox30* mutant mice. (A) A summary of founders with mutated *Sox30* alleles following CRISPR/CAS9-mediated genome editing. (B) Examples of sequencing results from mutated-*Sox30* founders (founder #3 and #4). (C) Schematic diagram of the RT-PCR strategy used to identify wild-type and mutant *Sox30* transcripts. The location of primer pairs (F1/R1 and F2/R2) is shown relative to the targeting sites of sgRNA-1 and -2. (D) RT-PCR with primer pairs shown in (C) reveals the presence of full-length transcripts in testis from WT but not founder #4 (*Sox30*$^{-/-}$) mice, which only contain truncated *Sox30* mRNA. (E) Examples of PCR genotyping of the *Sox30* mutated region in WT, *Sox30*$^{+/-}$ and *Sox30*$^{-/-}$ mice.

Fig. S4

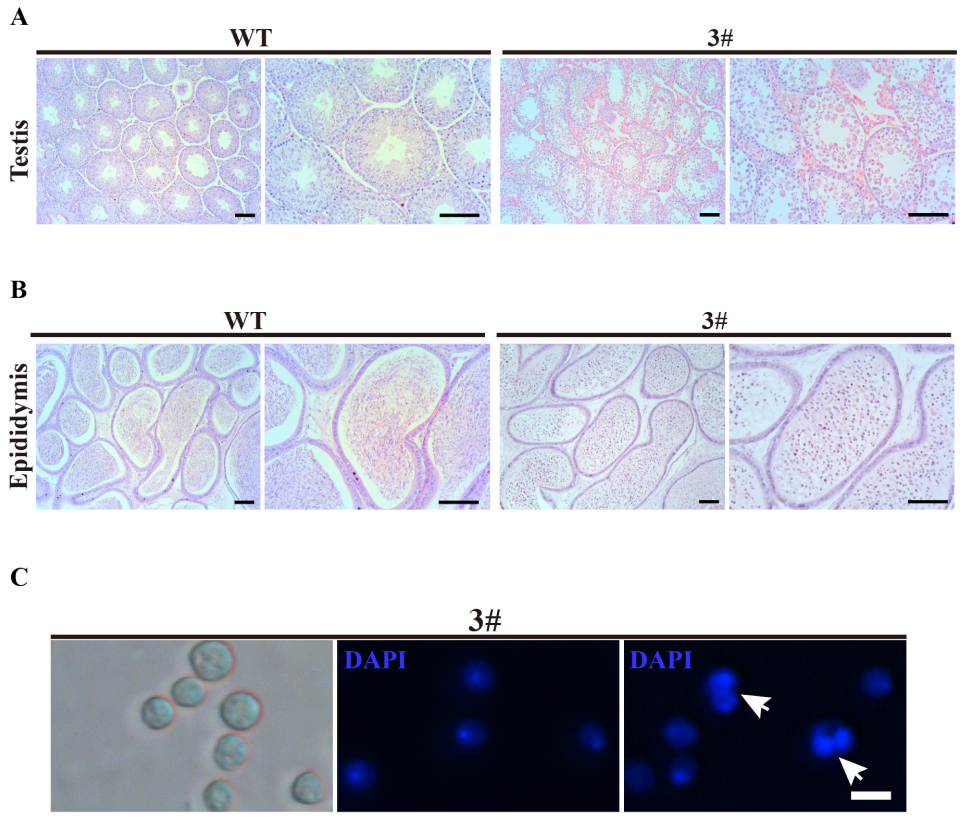

Fig. S4. Similar spermiogenic defects in founder mice with different biallelic *Sox30* mutations. (A-B) H&E stained tissue sections from the testis (A) and epididymis (B) of adult WT and founder #3 illustrate a similar phenotype in founder #3 and *Sox30*$^{-/-}$ mice derived from founder #4 (see Fig. 2I and J). Scale bars, 100 µm. (C) Differential interference contrast (DIC) microscopy and DAPI stained showing immature germ cells and spermatids with condensed nuclei (white arrow) in the epididymis from founder #3. Scale bars, 10 µm.

Fig. S5

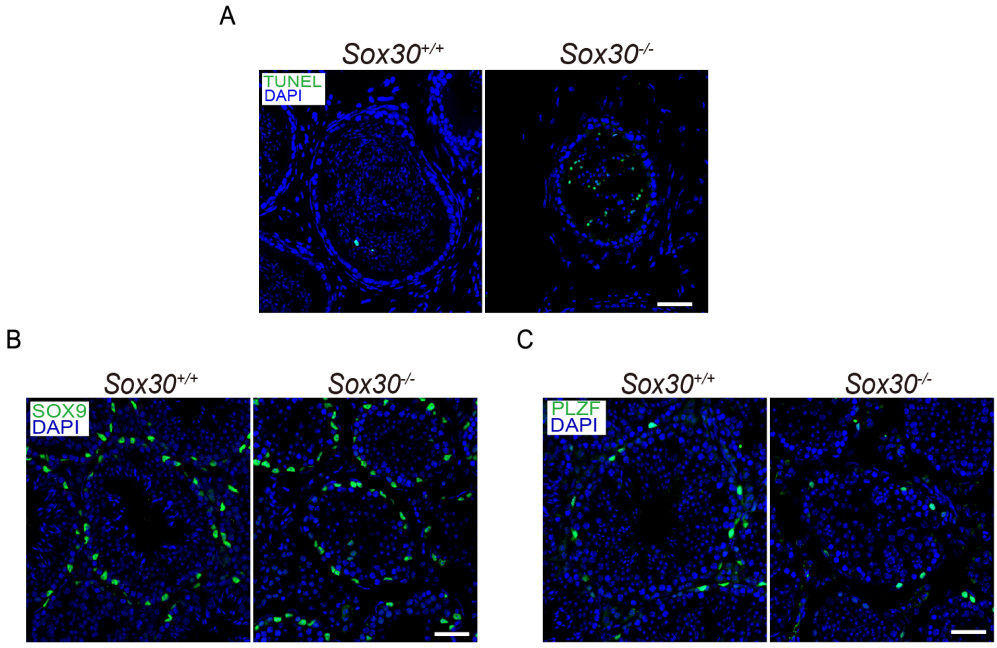

Fig. S5. Somatic cells and pre-meiotic germ cells are not affected by *Sox30* deficiency. (A) TUNEL assay in epididymal tissue sections from 8-week-old *Sox30*$^{+/+}$ and *Sox30*$^{-/-}$ males. Scale bars, 50 µm. Immunostaining of testis sections from adult WT and *Sox30*$^{-/-}$ mice with anti-Sox9 (B) and anti-PLZF (C) antibodies. Scale bars, 50 µm.

Fig. S6

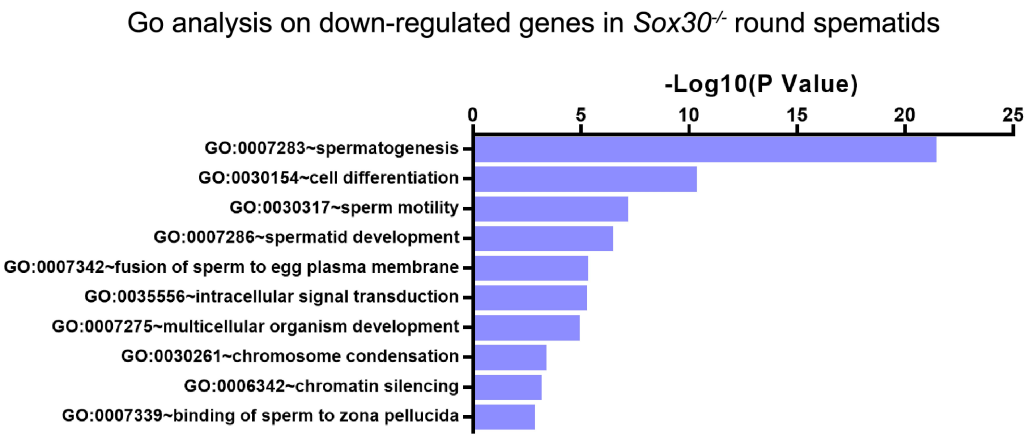

Fig. S6. GO term enrichment analysis for downregulated transcripts identified in round spermatids of *Sox30*$^{-/-}$ mice.

Fig. S7

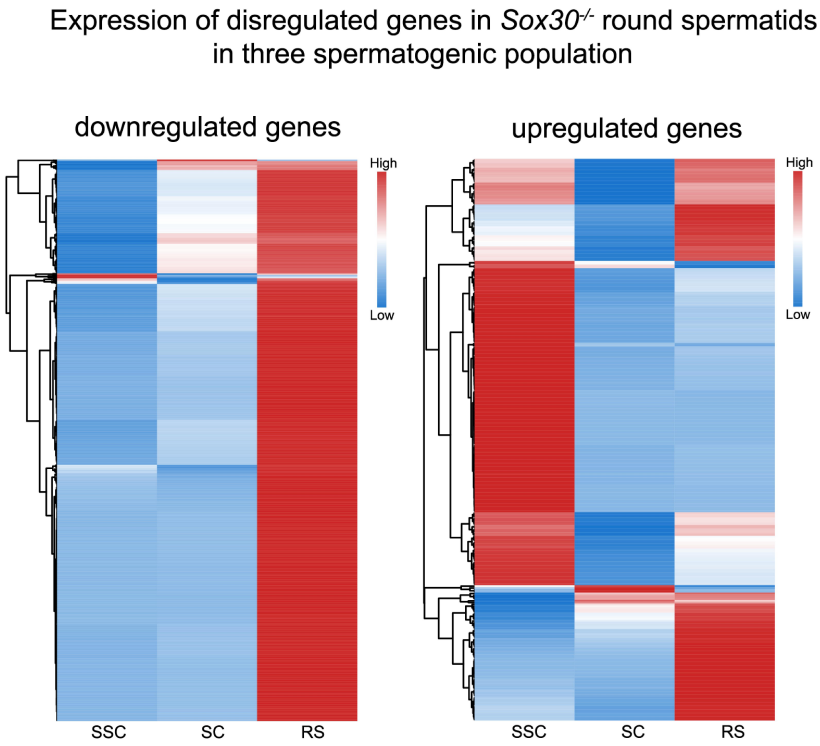

Fig. S7. Genes upregulated or downregulated in *Sox30*$^{-/-}$ round spermatids were used to produce heatmaps showing their expression pattern in stage-specific spermatogenic populations. SSC, spermatogonial stem cells; SC, pachytene spermatocytes; RS, round spermatids.

Fig. S8

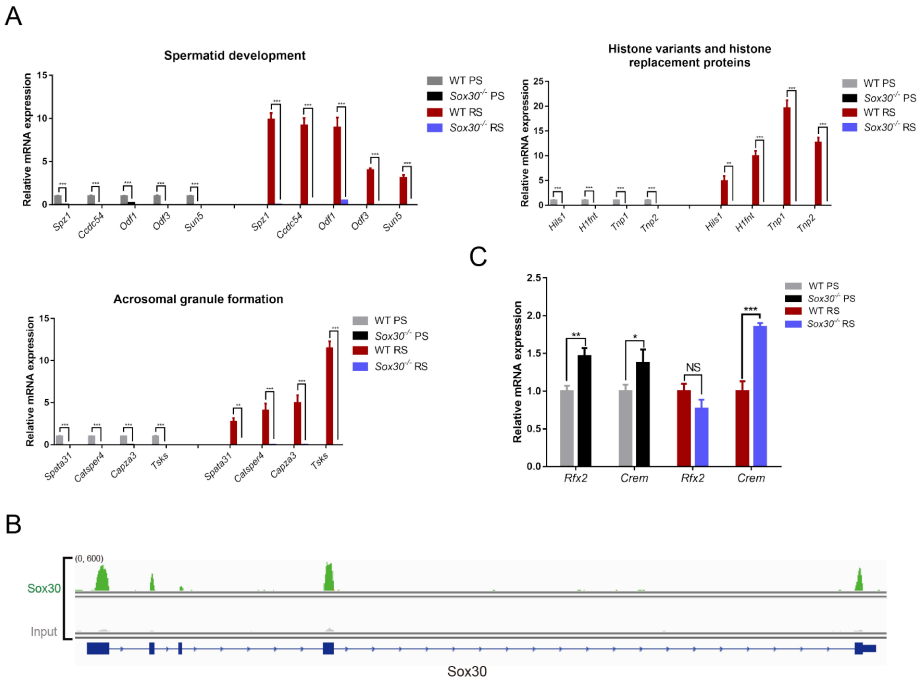

Fig. S8. (A) Q-PCR validation for downregulated transcripts in *Sox30*$^{-/-}$ pachytene spermatocytes and round spermatids. Pachytene spermatocytes were isolated for 8-week-old wild-type and *Sox30*$^{-/-}$ mice by STA-PUT method. WT, n=6; *Sox30*$^{-/-}$, n=8. Data presented are mean ± s.d. Student's t test. *P<0. 05, **P<0. 01 and ***P<0. 001. (B) Genome browser view of Sox30 ChIP-seq reads on the promoter of Sox30. (C) Q-PCR analysis of *Crem* and *Rfx2* mRNA levels in wild-type and *Sox30*$^{-/-}$ PS and RS.

Fig. S9

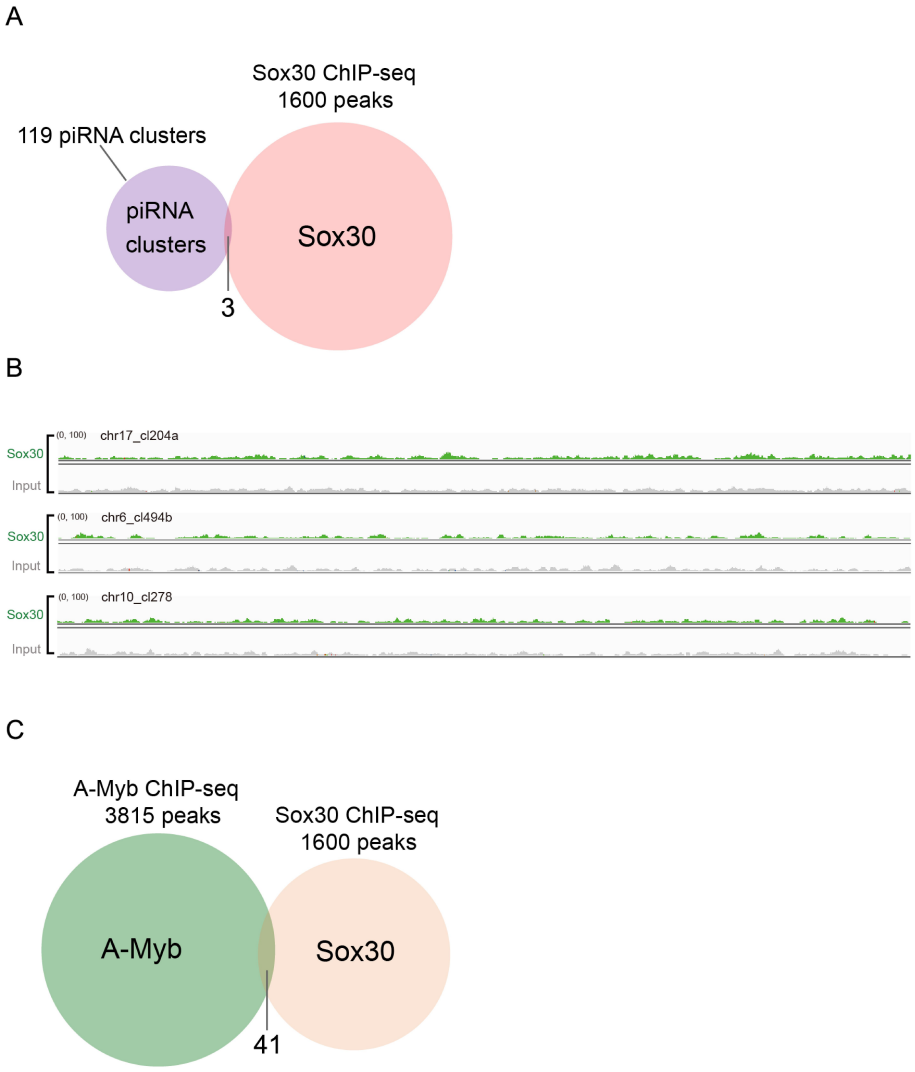

Fig. S9. (A) Overlapping binding sites of Sox30 ChIP-seq peaks and 119 annotated pachytene piRNA clusters in testis. (B) Genome browser view of Sox30 ChIP-seq reads on representative examples of top 10 pachytene piRNA clusters. (C) Overlapping binding sites of Sox30 and A-Myb ChIP-seq peaks in testis.

Table S1. Differentially expressed genes list in *Sox30*-deficient testes at postnatal day 21. RNA were extracted from three individual wild-type and *Sox30*$^{-/-}$ mice and deep-sequenced separately. P<0.05, fold change >2.

Click here to Download Table S1

Table S2. Differentially expressed genes list in *Sox30*-deficient pachytene spermatocytes. FDR<0.01, fold change >2. Pachytene spermatocytes were isolated for 8-week-old wild-type and *Sox30*$^{-/-}$ mice by STA-PUT method. WT, n=6; *Sox30*$^{-/-}$, n=8.

Click here to Download Table S2

Table S3. Differentially expressed genes list in *Sox30*-deficient round spermatids. FDR<0.01, fold change >2. Round spermatids were isolated for 8-week-old wild-type and *Sox30*$^{-/-}$ mice by STA-PUT method. WT, n=6; *Sox30*$^{-/-}$, n=8.

Click here to Download Table S3

Table S4. Sox30 bound genomic loci in mouse testes at P28. Excel table showing annotation of binding peaks of Sox30.

Click here to Download Table S4

Table S5. *De novo* motif analysis of Sox30 ChIP-seq with MEME algorithm. The motif prediction MEME were used to obtain enriched binding motif of Sox30. E-value is indicated.

MEME motif	E-value
	7.2e-147
	2.4e-030
	5.6e-009

Table S6. Excel list of genes bound by Sox30 in their promoters and exhibiting differential expression in *Sox30*$^{-/-}$ pachytene spermatocytes and round spermatids, related to the Venn diagram in Fig. 6E-F. Sheet 1: genes with Sox30 ChIP-seq peaks at promoters; Sheet 2: genes downregulated in *Sox30*$^{-/-}$ PS (FDR<0.01, fold change >2) bound by Sox30 at promoters; Sheet 3: genes downregulated in *Sox30*$^{-/-}$ RS (FDR<0.01, fold change >2) bound by Sox30 at promoters; sheet 4: genes upregulated in *Sox30*$^{-/-}$ PS (FDR<0.01, fold change >2) bound by Sox30 at promoters; sheet 5: genes upregulated in *Sox30*$^{-/-}$ RS (FDR<0.01, fold change >2) bound by Sox30 at promoters.

Click here to Download Table S6

Table S7. Primer sequences for RT-PCR and ChIP-qPCR.

Targeted gene	Forward (5' → 3')	Reverse (5' → 3')	Application
Sox30	ACCTGTCGGTGGGATCTCG	CAGCCTACAATCGTCCCTGG	qRT-PCR
Sox30	GCTCCAACTTAGAATGCTGAGT	AACTCCTCTCTGTGCTTCTCTT	Genotyping PCR
Sox30	GCCTTCTGTAAAATTGAAACCA	GTGCCAGTATGGGTCTGTCT	F1/R1
Sox30	AAGCACAGAGAGGAGTTTCCTG	CGGGAATTACCACAGAGTAGGT	F2/R2
pre-piR1	GTTAGCGAAGGACATTATTCTAACC	TGACATGAACACAGGTGCTCAGAT	RT-PCR
pre-piR2	CTATGCTTATGATGGCATTGGAGAG	TTCCAGTTCAACAGGGACACGGGAC	RT-PCR
pre-piR3	GTTCTCACTTTATCAGCTCTCAAG	TGAGAGTGGCATCTAAATGTTTAG	RT-PCR
pre-piLR	GTGAAGCTAAGGATGCTGGGATAG	ACAGGATGTCCCCTGAAATCAGTC	RT-PCR
Prepachytene cluster 10	GGCCATAGGTTAACTTTCAGAAGTC	CTATAACTGCAAGTTCAGGTGGACAG	RT-PCR
pri-let7g	GTACGGTGTGGACCTCATCA	TCTTGCTGTGTCCAGGAAAG	RT-PCR
β-actin	CCGTAAAGACCTCTATGCC	CTCAGTAACAGTCCGCCTA	RT-PCR
Spz1	ATGTCGGACACAGACAACTCA	GTGGTGGGAAGGAGTGGTAG	qRT-PCR
Ccdc54	CACACCAAAAGAGTAAGAGCTGC	TGGGTCGTACATTTCTGGTAAAC	qRT-PCR
Odf1	CCGCACTGAGTTGTCTTTTGG	GGGTGCATGTATAAGTCACACA	qRT-PCR
Odf3	AAGTTCAAGGCTCCACAGTACA	TTGATGCCAAAGGTGACAGTAG	qRT-PCR
Sun5	TGGATCCGACTGTGGAACTAC	GGTAGACTTTCTGGGCCAAAC	qRT-PCR
Hils1	GGTCCCAAGCCAGAGTGAG	AGCTTTCTTCAAGGTAGCAAGG	qRT-PCR
H1fnt	GGCGCAGAACTTACGATCCA	GACTTCCCCTCGTGGTGAG	qRT-PCR
Tnp1	ACCAGCCGCAAGCTAAAGAC	TTTCCTACTTTTCAGGACGCTC	qRT-PCR
Tnp2	GTGCACTCTCGACACTCACCT	AGCTACGCCTCTTAGCTCTGTG	qRT-PCR
Spata31	TCAGCTCCACTATATGGGCAA	CTTTTTCAGGCATTCTCCCCAA	qRT-PCR
Catsper4	GGTCGGCATGAGGAGCAAG	AATGGTGATAGCGTTGGACAG	qRT-PCR
Capza3	TCCACAGGCTCTTAGTCCAGG	GTTGCCGTCGATGCAGAGT	qRT-PCR
Tsks	GTGGTGGTGAAGACAATCTGG	GACCGCTTGAGGTTCAGGC	qRT-PCR
36B4	GCAGATCGGGTACCCAACTGTTG	CAGCAGCCGCAAATGCAGATG	qRT-PCR
RFX2	AGACCCTCAGCTTTACGCC	GTGCCACCTGGAGTCTCAAA	qRT-PCR
Crem	ATGTCTTGAAAATCGTGTGGCT	TGGCAATAAAGGTCTTTGAGGG	qRT-PCR
Line1	GAGAACATCGGCACAACAATC	TTTATTGGCGAGTTGAGACCA	qRT-PCR
IAP	CAGACTGGGAGGAAGAAGCA	ATTGTTCCCTCACTGGCAAA	qRT-PCR
Hils1	CTGTTGCCTCAGACCTTCCTAA	GGCTCAGAGACTGGTGAAGTTA	ChIP-qPCR
Tnp1	CAGCAAGGTGTAGCAAAGGTTA	CCGAAATGAGGGGCTTTG	ChIP-qPCR
Ccdc54	ACAGGAATGGTCACATGCAGTA	CCTGGTCACATCATTGAATACC	ChIP-qPCR
Tsks	CAGCACCTATTCTCCCCCTTAC	GGGGGAGACTCACCATCTACTC	ChIP-qPCR
Odf3	GACAATGACCTCTGGCCCAA	CTCGTGTGGTTGCTGGAGAT	ChIP-qPCR
Spz1	TGTCTGAGTTGTGGGACAGG	GGCCCCAGCTACTAGGTCTT	ChIP-qPCR
Plzf	GTAGGAAACCCGGGAAGGACT	TGCAGCAGCAACCAGAGAAC	ChIP-qPCR
Gapdh	CCTCTGCGCCCTTGAGCTAGGA	CACAAGAAGATGCGGCCGTCTC	ChIP-qPCR