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Supplemental Information

Epigenetic Inactivation of SOX30 Is Associated with Male Infertility and Offers a Therapy Target for Non-obstructive Azoospermia

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Supplemental tables

Table S1 Primer sequences were used in this study

Genes	Primer sequence	Primer purpose
Sox30-F	CCCATTCCACACTCACACGTCTA	RT-qPCR analysis
Sox30-R	AACCAAGACATTCTGGCATTGAACT	
Tnp1-F	ACAAGGGCGTCAAGAGAGGT	
Tnp1-R	CATCACAAGTGGGATCGGTA	
Prm2-F	GAAGGCGGAGGAGACACTC	
Prm2-R	GGGAGGCTTAGTGATGGTG	
Actin-F	GGAGATTACTGCTCTGGCTCCTA	RT-qPCR analysis (Internal control)
Actin-R	GACTCATCGTACTCCTGCTTGCTG	

Table S2 The detailed information of OA and NOA patients selected.

(XLS)

Table S3 The DMR-genelist in NOA testicular tissues

(XLS)

Table S4 Serious hyper-methylated sites of SOX30 promoter were detected in testicular tissues of NOA patients

SOX30 promoter region		Methylation		p-value
Start-End	CpG-position	OA	NOA	
Chr5: 157047686-157103488	157050543	0.091	1	8.38E-07
	157051907	0	1	1.84E-05
	157052725	0.333	0.913	0.000162
	157053392	0	0.786	0.002167
	157059318	0.200	1	0.000684
	157065261	0	1	0.000155
	157066415	0	0.867	0.000108
	157066472	0	0.760	0.000216
	157069417	0.069	1	1.45E-15
	157069480	0.037	1	4.26E-10
	157069741	0	0.833	9.12E-05
	157073956	0.281	1	2.92E-05
	157074092	0	0.818	0.000714
	157077021	0	0.605	4.30E-07
	157078082	0	0.736	4.01E-05
	157079312	0.023	0.821	2.15E-09
	157079404	0.125	0.668	3.26E-05
	157079440	0	0.779	1.35E-08
	157079468	0.262	0.836	7.17E-06
	157079520	0	1	3.56E-10
	157079825	0	1	7.65E-12
	157092982	0.238	1	0.001561
	157100554	0	0.555	0.000190
	157101015	0	1	1.34E-05
	157103143	0.411	1	4.70E-06

Table S5 Mutation or deletion in SOX30 was detected in NOA patients

Locus	Ref	NOA	Position type	Gene	SNP
Chr5 157065306	G	A	exonic	SOX30	Known SNP site rs35793864 G→A

Table S6 Correlations of SOX30 expression with clinicopathologic features in human NOA patients (n=58)

Clinical Feature	Total	SOX30 Expression		P value
		High (n=28)	Low (n=30)	
Smoking				
No	22	11	11	.830
Yes	34	16	18	
Drinking				
No	23	12	11	.630
Yes	35	16	19	
Obesity				
No	52	26	26	.669
Yes	5	2	3	
BMI				
<23	29	17	12	0.189
≥23	29	11	18	
Testicular volume				
<10 mL	28	7	21	**.001**
≥10 mL	30	21	9	
Semen volume				
<3 mL	28	16	12	.224
≥3 mL	27	11	16	
pH value				
<7.2	28	15	13	.688
≥7.3	27	13	14	
Liquefaction time				
≤20 min	46	22	24	.689
>20 min	11	6	5	

NOA specimens were classified into two groups according to the median score. The p values were measured with Pearson chi-square tests. All statistical tests are two sided. Some information of the patients is missing. For example smoking information of two patients, obesity information of one patient, pH value information and semen volume information of three patients, and liquefaction time information of one patient are missing.

Table S7 Fertility analysis was performed in different genotypes of mice

Genotypes	Sex	Mating	Fertility (%)
+/+ (n=30)	Male	mate with wild-type female mice	100% (30/30)
-/+ (n=50)	Male		100% (50/50)
-/- (n=30)	Male		**0.00% (0/30)**
+/+ (n=40)	Female	mate with wild-type male mice	100% (40/40)
-/+ (n=60)	Female		100% (60/60)
-/- (n=50)	Female		98% (49/50)

Supplemental Figures and Figure legends

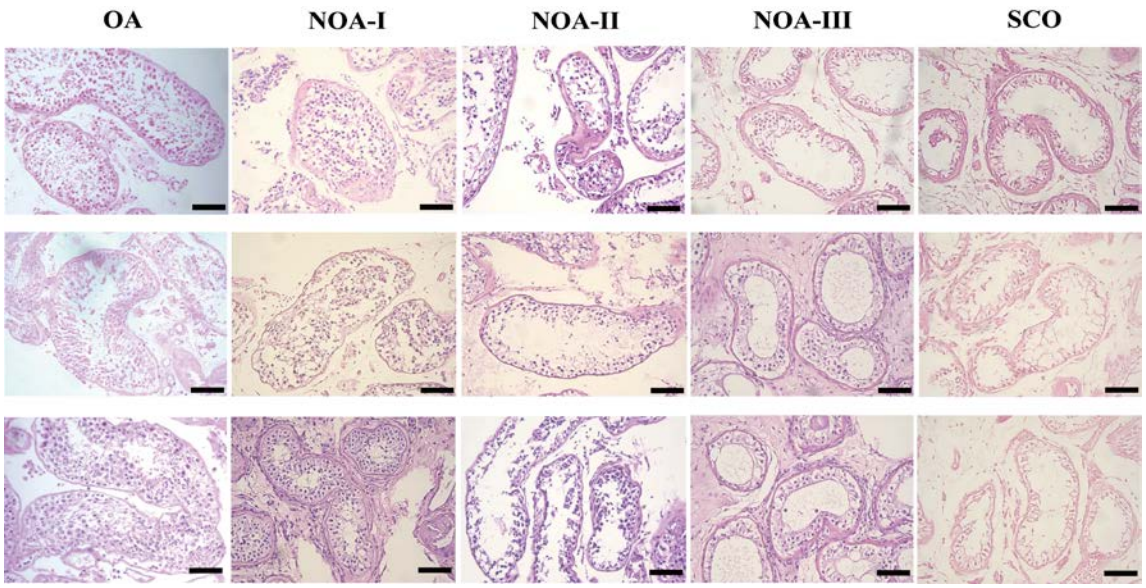

Figure S1 Histological pictures of OA and NOA patients analyzed were shown.

The SCO represents the Sertoli Cell-Only syndrome patients. Scale bars, 100 μm.

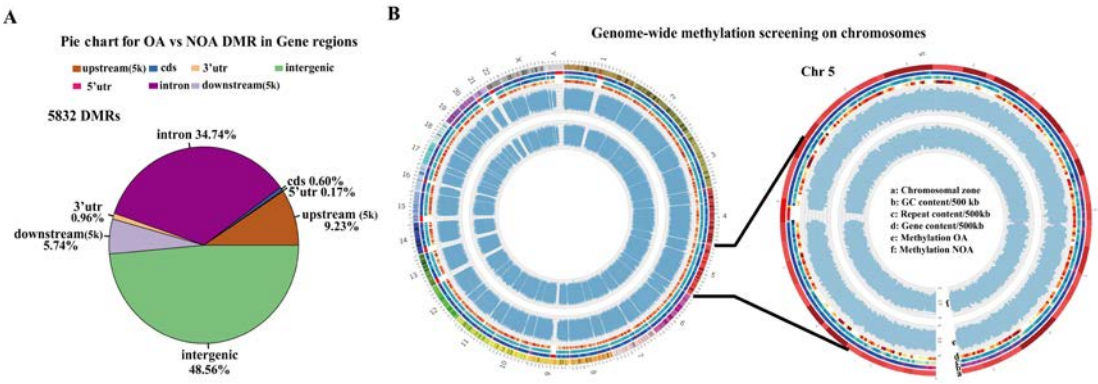

Figure S2 Different methylated genes on chromosomes were identified in NOA testicular tissues. (A) Distribution of the 5832 DMRs for NOA testicular tissues vs OA testicular tissues in different gene regions (B) Identification of different methylated genes on chromosome 5 (Chr5) in NOA samples using genome-wide methylation screening. Circos plot shows genome-wide and Chr 5 methylation pattern in OA and NOA samples. The chromosomal zone, GC content, repeated sequence content, gene sequence content, methylation level of OA and methylation level of NOA are presented from the outer circle to the inner circle in the Circos figure.

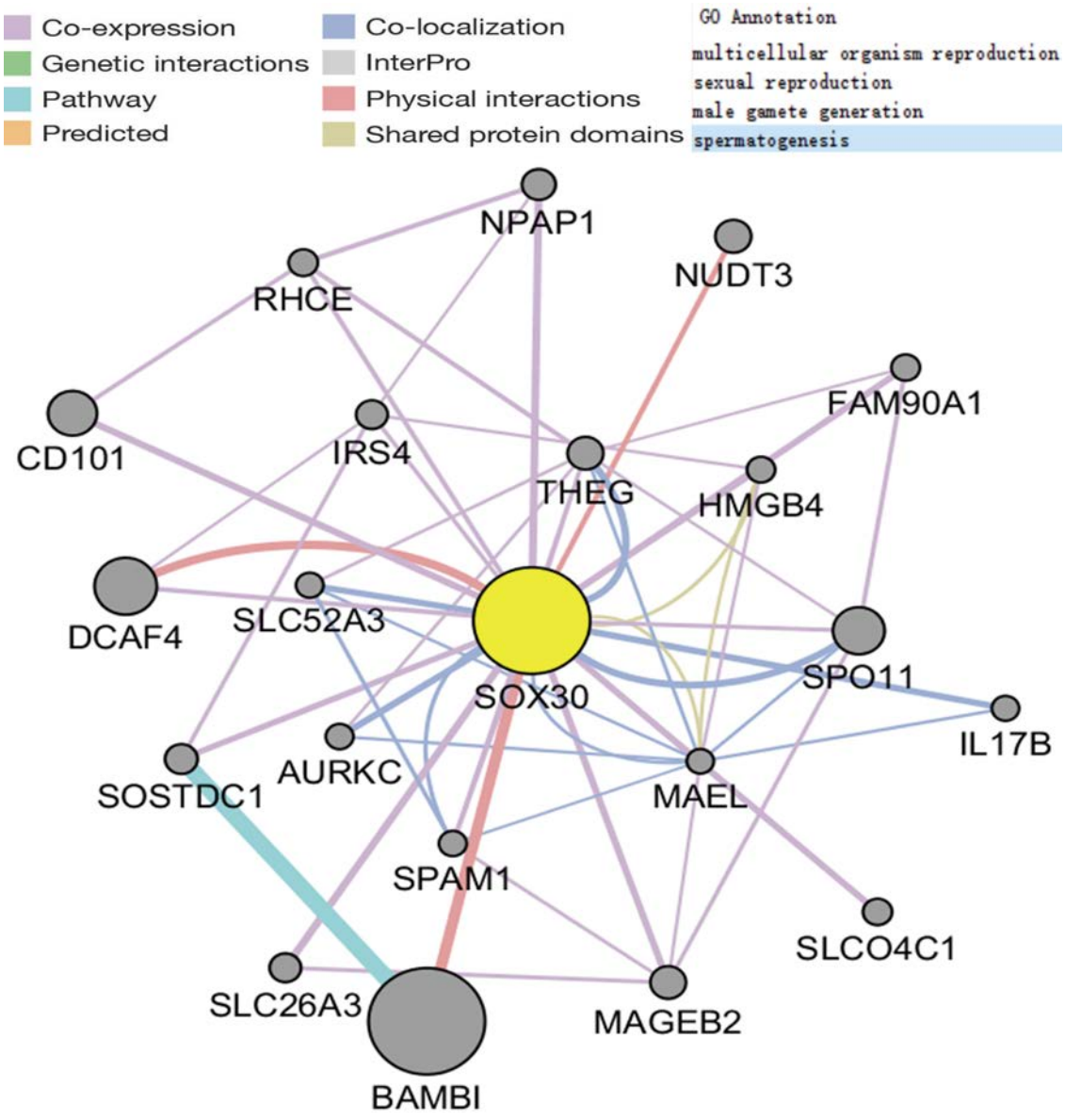

Figure S3 The network of SOX30-regulated genes from gene expression profiling of patients' testicular tissues. The interactions of SOX30 and SOX30-regulated genes were visualized as a network using cytoscape. The mainly involved biological processes are sexual reproduction, gamete generation and spermatogenesis.

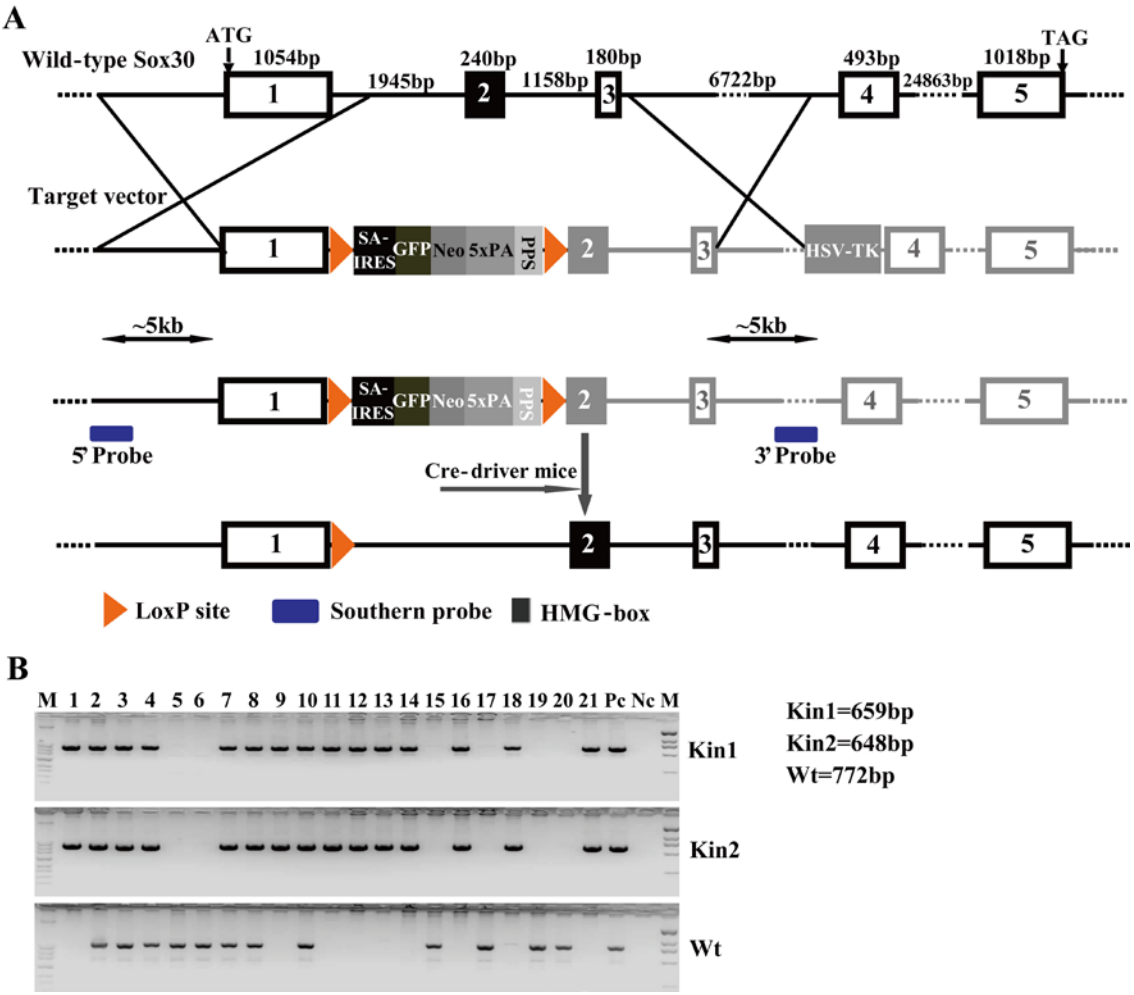

Figure S4 Generation of *Sox30*-null mice by homologous recombination was performed. (A) Targeted disruption of the mouse Sox30 gene generated by a knockin (kin) strategy. The LoxP-SA-IRES-GFP-NEO-STOP (poly A)-PPS-LoxP cassette was introduced to *Sox30* between Exon1 and Exon2 by homologous recombination. The numbered boxes denote Sox30 exons. The black box represents the HMG-box domain. (B) The genotypes analyzed by RT-PCR are shown. M represents DNA marker. Pc represents positive control. Nc represents negative control. Wt/Wt (+/+), Kin/Wt (-/+) and Kin/Kin (-/-) represent $Sox30^{+/+}$, $Sox30^{-/+}$ and $Sox30^{-/-}$ mice, respectively. Kin1 and Kin2 are the abbreviations of knockin primer-1 and knockin primer-2, two pairs of primers with different size to determine the genotypes of gene knockout.

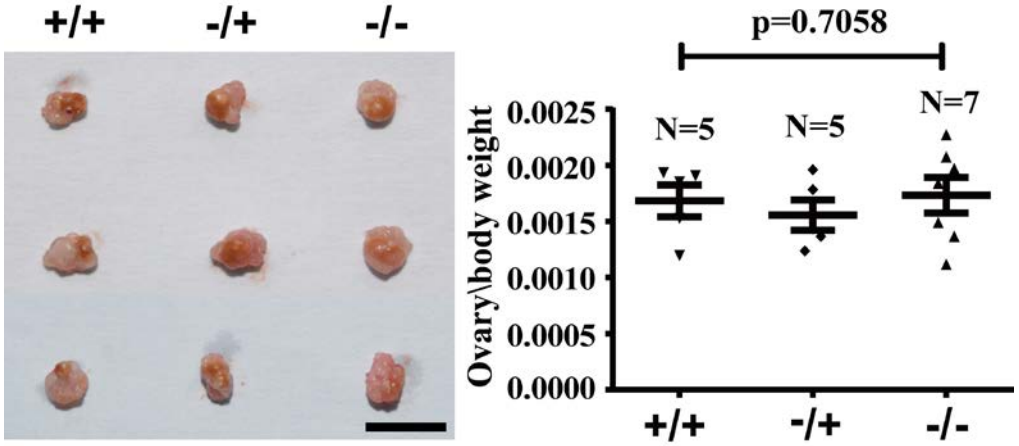

Figure S5 The morphology and weight of ovaries were evaluated in Sox30$^{+/+}$, Sox30$^{-/+}$ and Sox30$^{-/-}$ mice. Sox30$^{+/+}$ n=5, Sox30$^{-/+}$ n=5 and Sox30$^{-/-}$ n=7. Scale bar represents 0.5 cm.

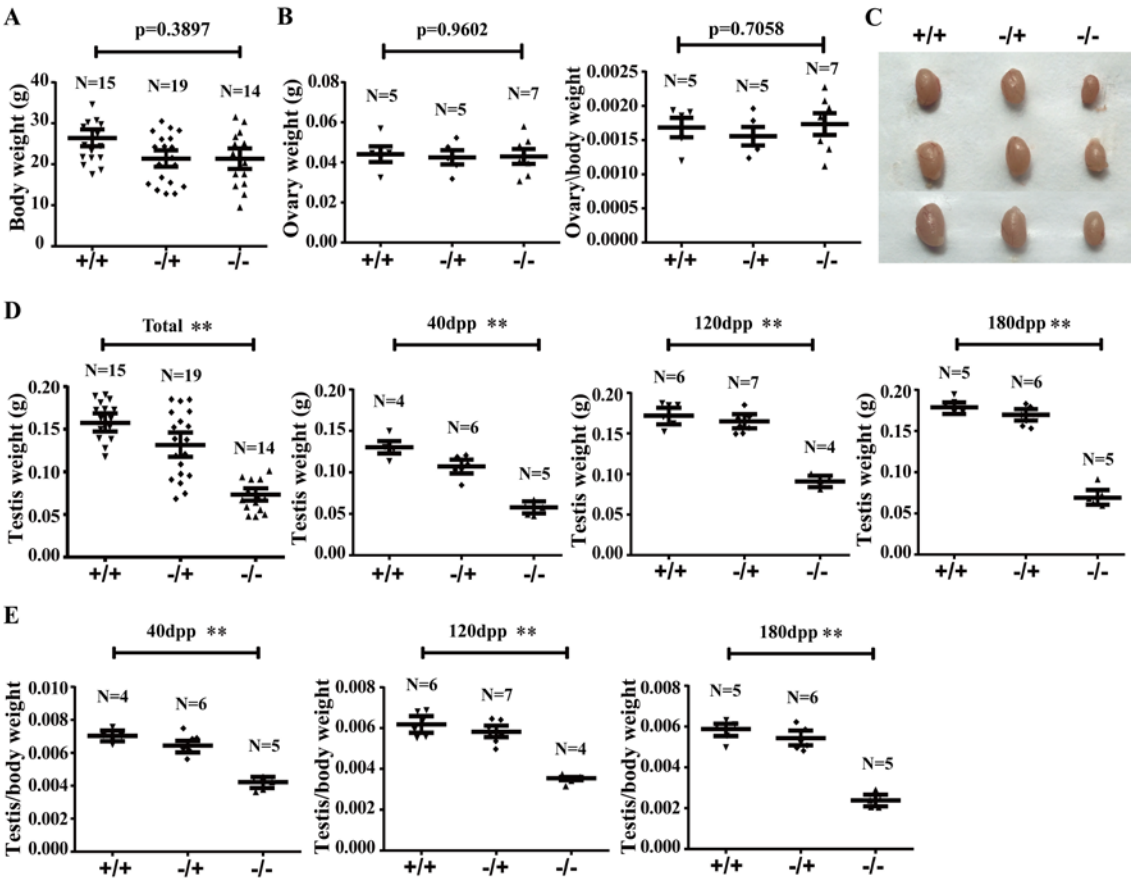

Figure S6 Sox30 is specifically essential for testis development. (A) The body weights of Sox30$^{+/+}$, Sox30$^{-/+}$ and Sox30$^{-/-}$ mice. (B) The ovary weights of Sox30$^{+/+}$, Sox30$^{-/+}$ and Sox30$^{-/-}$ mice. (C) The morphologies of the testes from Sox30$^{+/+}$, Sox30$^{-/+}$ and Sox30$^{-/-}$ mice at 40 dpp. (D) The weights of the testes from Sox30$^{+/+}$, Sox30$^{-/+}$ and Sox30$^{-/-}$ mice at different developmental stages (40, 120 and 180 dpp [days post-partum]). The "**" represent p value less than 0.01. (E) The testis/body weights of Sox30$^{+/+}$, Sox30$^{-/+}$ and Sox30$^{-/-}$ mice at different development stages. The "**" represent p value less than 0.01.

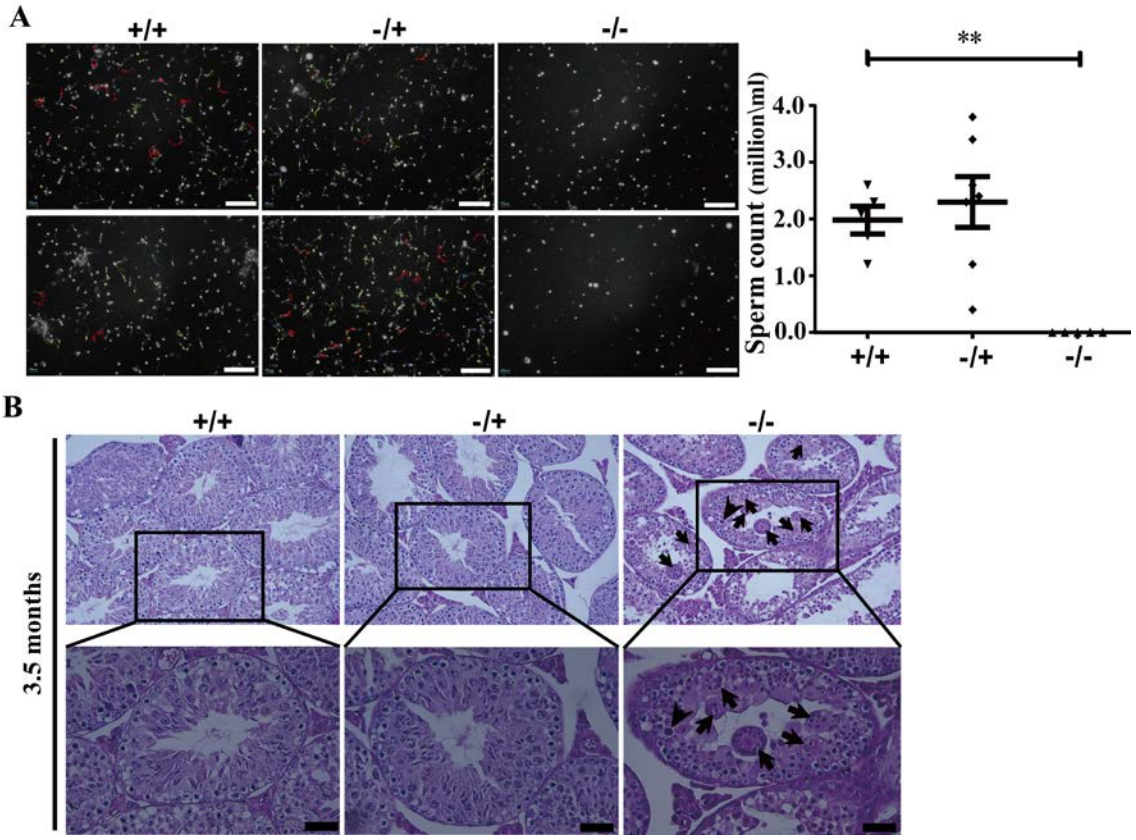

Figure S7 Sox30 deletion caused scarce spermatids and complete absence of spermatozoa. (A) Analyses of spermatozoa in the epididymides of $Sox30^{+/+}$, $Sox30^{-/+}$ and $Sox30^{-/-}$ mice by sperm class analyzer (SCA) system. Scale bars, 50μm. The "**" represent p value less than 0.01. (B) H&E staining of the testis sections of $Sox30^{+/+}$, $Sox30^{-/+}$ and $Sox30^{-/-}$ mice at 3.5 months stage. The arrows with long tail indicate multi-nucleated spermatogenic cells. The arrows with short tail indicate apoptotic spermatogenic cells. Scale bars, 50μm.

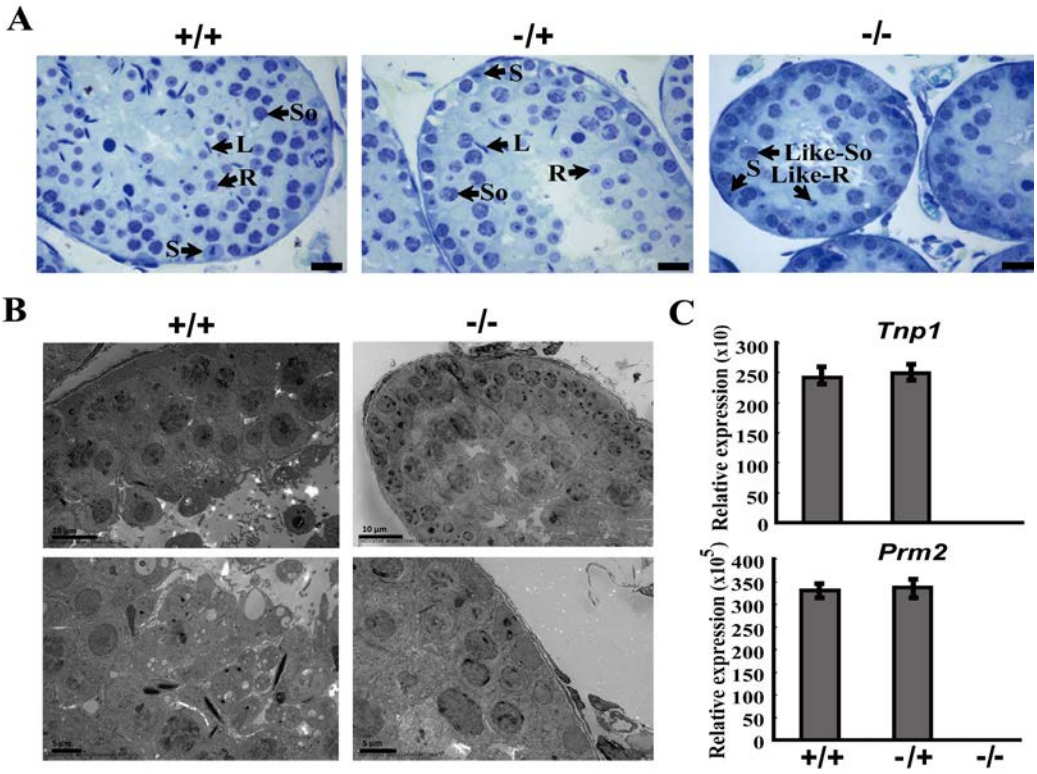

Figure S8 Sox30 deletion caused scarce spermatids and complete absence of spermatozoa. (A) Semithin histological sections stained with toluidine blue demonstrate the presence of spermatocyte nuclei in testes of $Sox30^{+/+}$, $Sox30^{-/+}$ and $Sox30^{-/-}$ mice. So represents spermatocyte. S represents sertoli cell. R represents round spermatid. L represents long spermatozoa. (B) Morphological examinations of the testes of $Sox30^{+/+}$ and $Sox30^{-/-}$ mice by transmission electron microscopy (TEM). (C) The expression analyses of spermatid markers in the testes of $Sox30^{+/+}$, $Sox30^{-/+}$ and $Sox30^{-/-}$ mice.

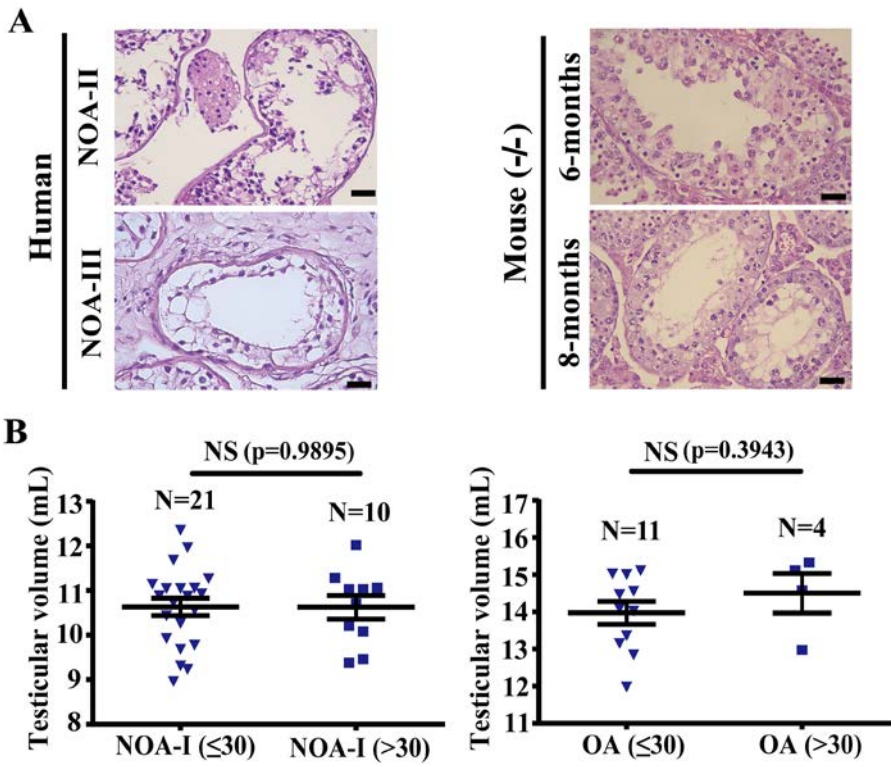

Figure S9 The pathology and testicular size of Sox30$^{-/-}$ mice simulates those of NOA patients. (A) H&E staining of testes from human NOA patients and Sox30$^{-/-}$ mice at 6-month or 8-month developmental stage were compared. Almost only spermatocyte-like, spermatogonia and sertoli cells were observed in 6-month old Sox30-deficient mice. A few spermatocyte-like, and almost only spermatogonia and sertoli cells were found in 8-month old Sox30-deficient mice. Scale bars are 50 μm. (B) The testicular volume of NOA and OA patients at different ages were compared. The NOA-Ⅰ (≤30) represents NOA-Ⅰ patients who are less than and equal to 30 years, and the NOA-Ⅰ (>30) represents NOA-Ⅰ patients who are greater than 30 years. The OA (≤30) represents OA patients who are less than and equal to 30 years, and the OA (>30) represents OA patients who are greater than 30 years.

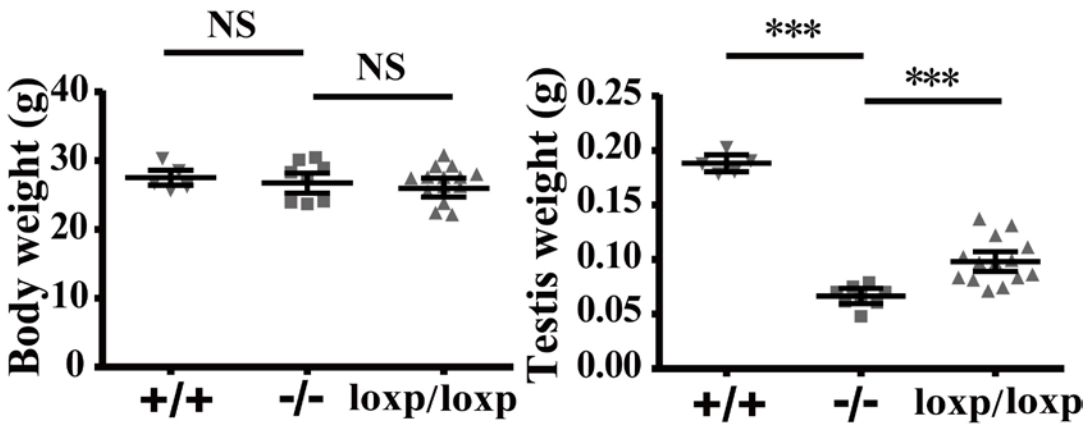

Figure S10 The weight of bodies and testes was analyzed in Sox30$^{+/+}$, Sox30$^{-/-}$ and Sox30$^{loxp/loxp}$ male mice. +/+, Sox30$^{+/+}$ mice injected with tam; -/-, Sox30$^{-/-}$ mice injected with solvent; loxp/loxp, Sox30$^{-/-}$ mice injected with tam. The "NS" represent no significant. The "***" represent p value less than 0.001. The "**" represent p value less than 0.01.