

Supplementary Material

Effects of bone morphogenetic protein 15 (*BMP15*) knockdown on porcine testis morphology and spermatogenesis

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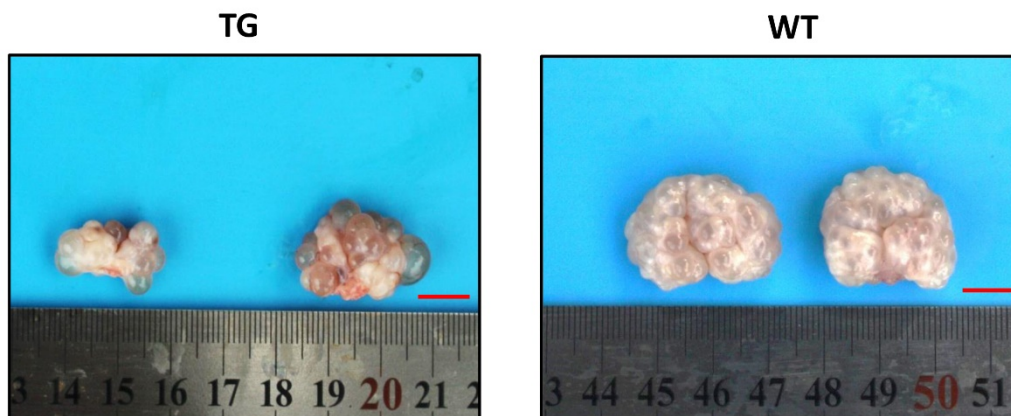
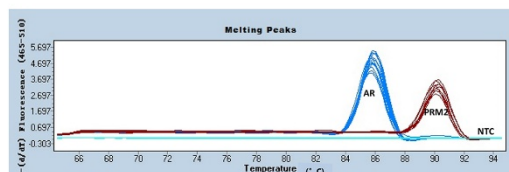
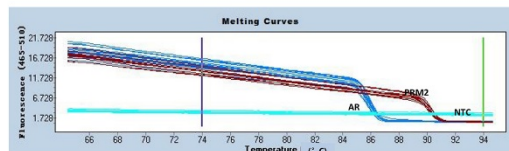
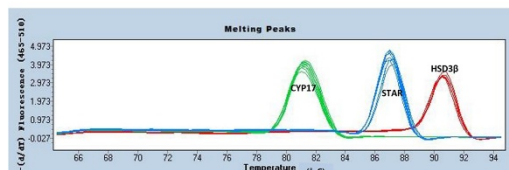
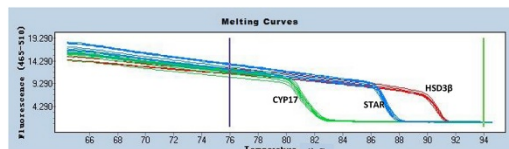
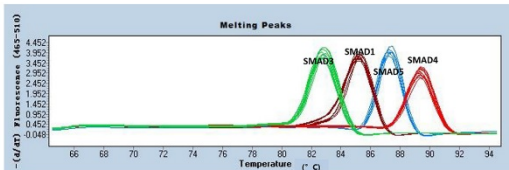
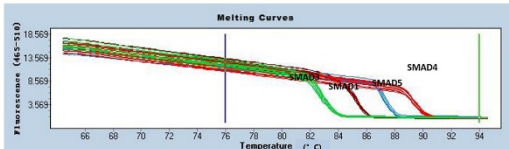
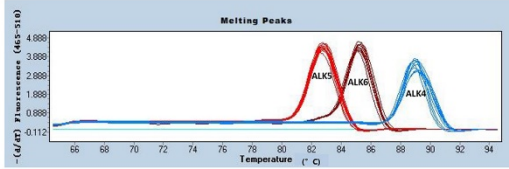
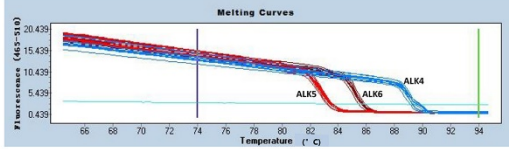
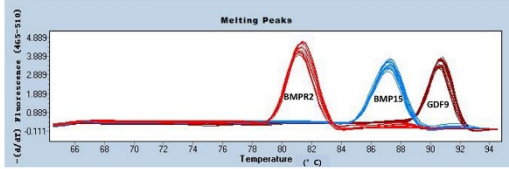
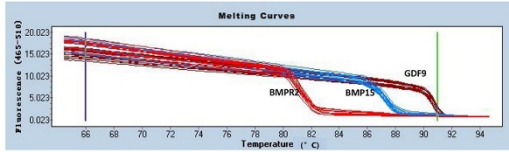


Fig. S1. Ovaries were collected from 365-day-old WT and TG sows. Scale bar = 1 cm.



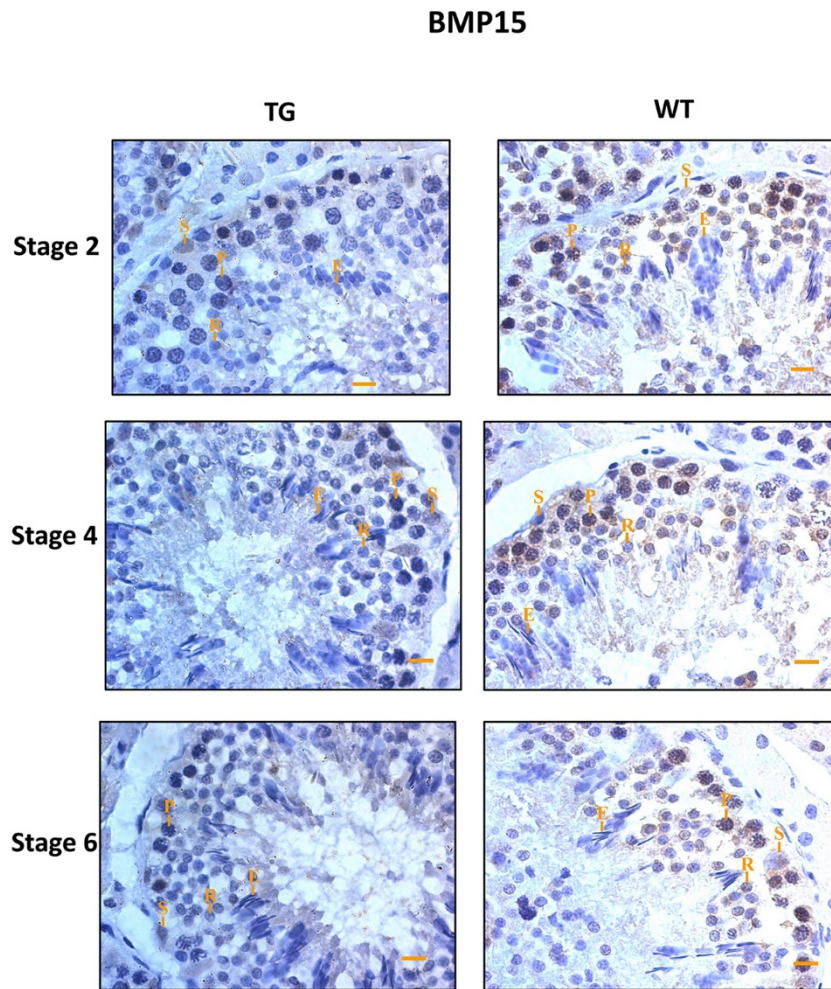


Fig. S5. Immunohistochemical analysis of BMP15 in different stages of the seminiferous cycle in adult boar testes. S, Sertoli cells; P, pachytene spermatocytes; R, round spermatids; E, elongated spermatids. Scale bar = 10 μ m.

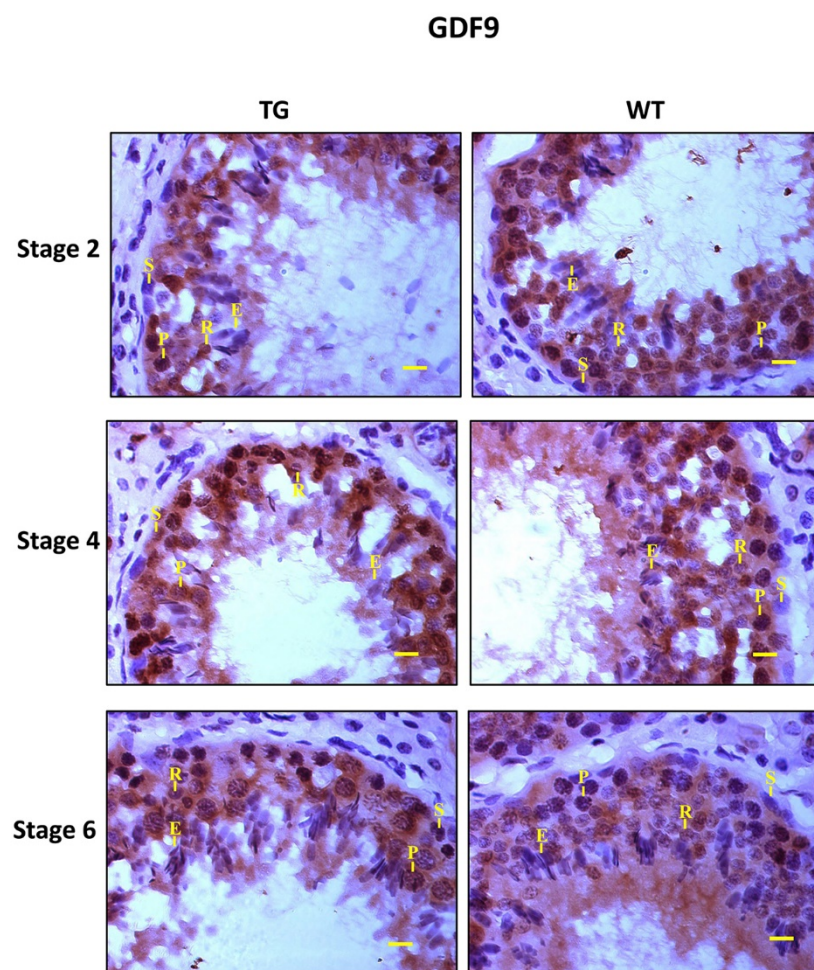


Fig. S6. Immunohistochemical analysis of GDF9 in different stages of the seminiferous cycle in adult boar testes. S, Sertoli cells; P, pachytene spermatocytes; R, round spermatids; E, elongated spermatids. Scale bar = 10 μ m.

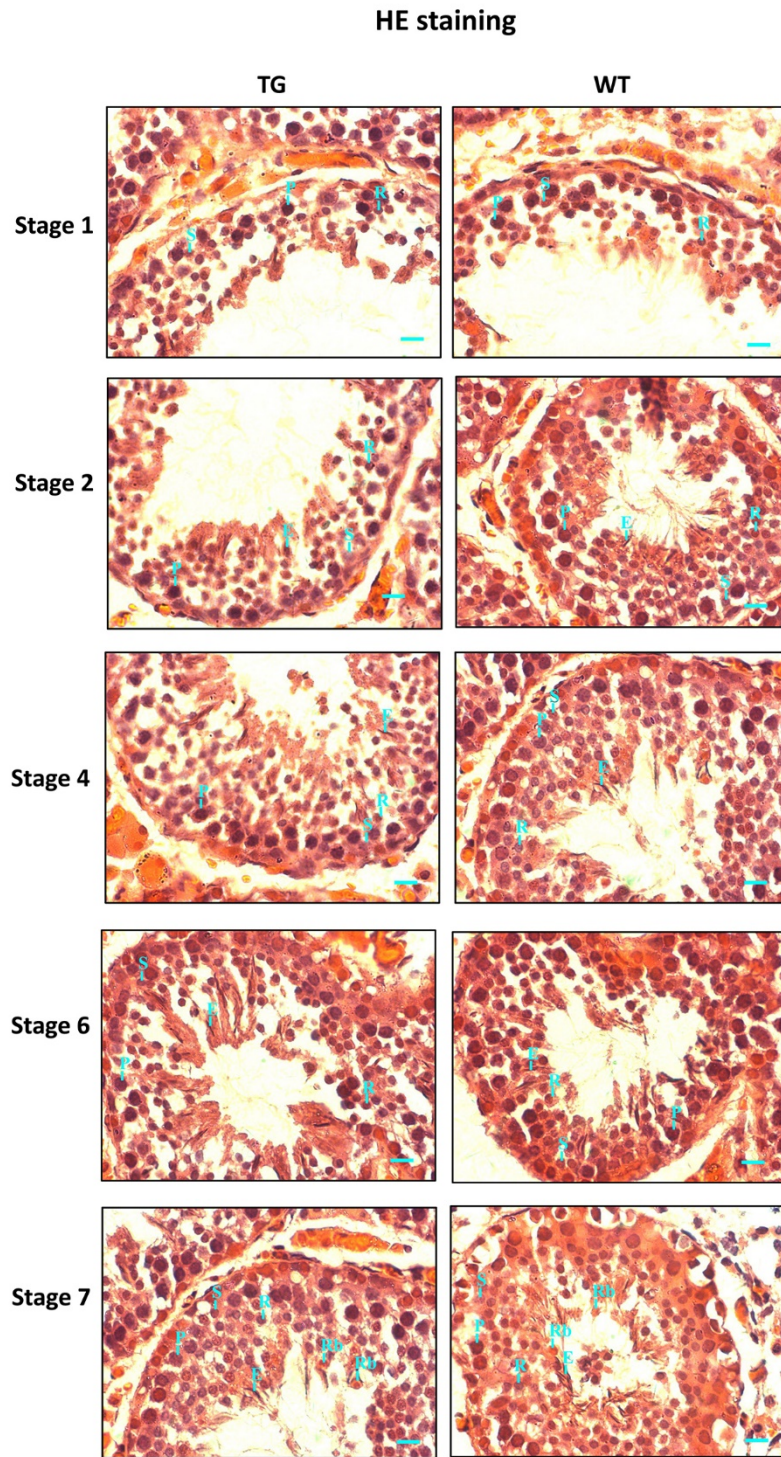


Fig. S7. HE stained TG and WT boar testicular sections at different stages of the seminiferous cycle. S, Sertoli cells; P, pachytene spermatocytes; R, round spermatids; E, elongated spermatids. Scale bar = 10 μ m.

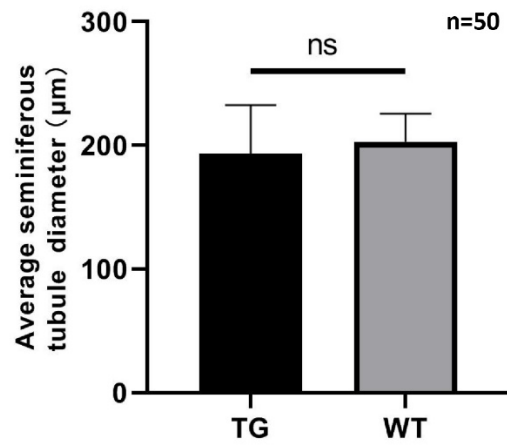


Fig. S8. Statistical analysis of seminiferous tubule diameter. Seminiferous tubule diameter was calculated as half of the sum of the major and minor axes in each sample. ns, no significant differences were observed.

ALK4

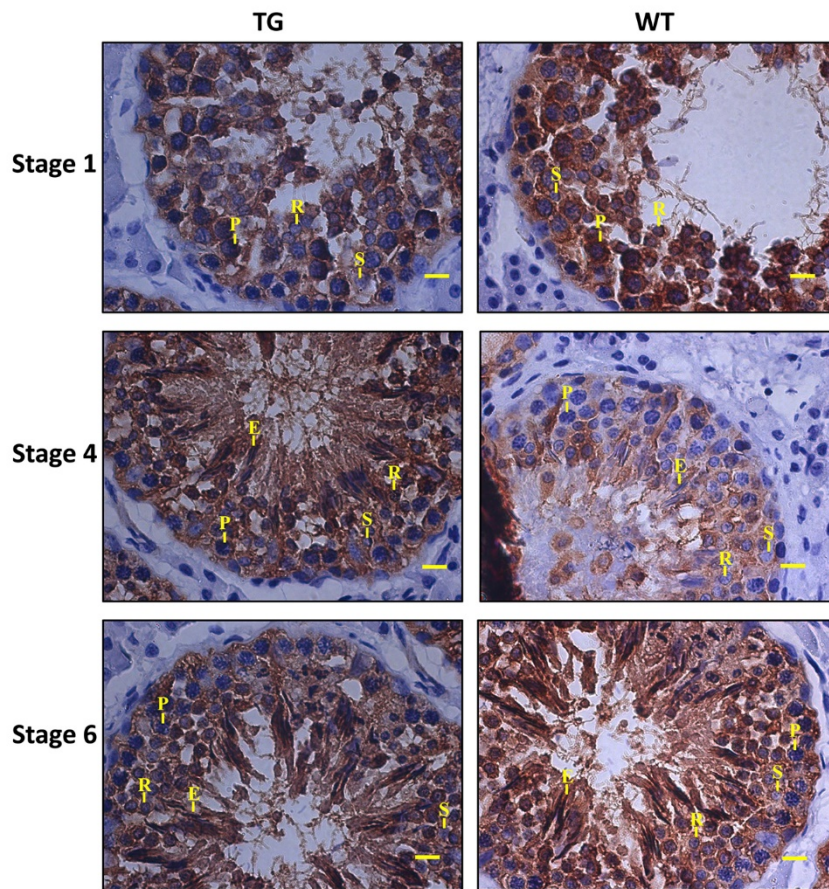


Fig. S9. Immunohistochemical analysis of ALK4 at different stages of the seminiferous cycle in adult boar testes. S, Sertoli cells; P, pachytene spermatocytes; R, round spermatids; E, elongated spermatids. Scale bar = 10 μ m.

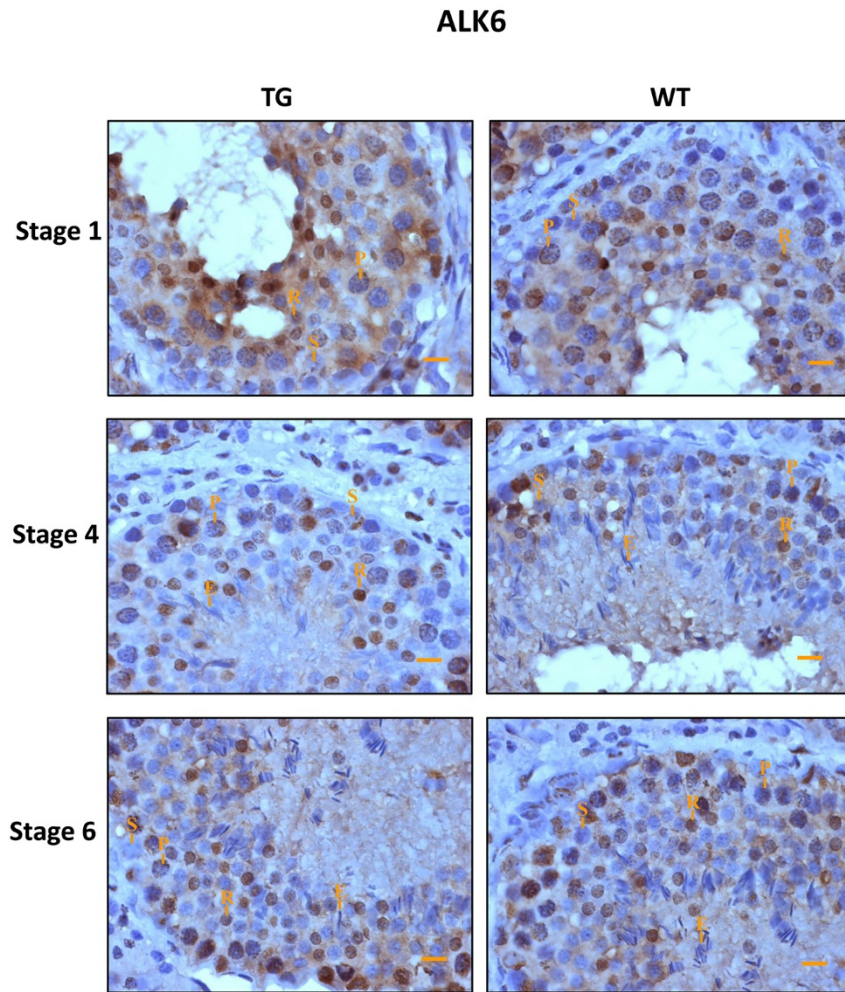


Fig. S10. Immunohistochemical analysis of ALK6 at different stages of the seminiferous cycle in adult boar testes. S, Sertoli cells; P, pachytene spermatocytes; R, round spermatids; E, elongated spermatids. Scale bar = 10 μ m.

BMPR2

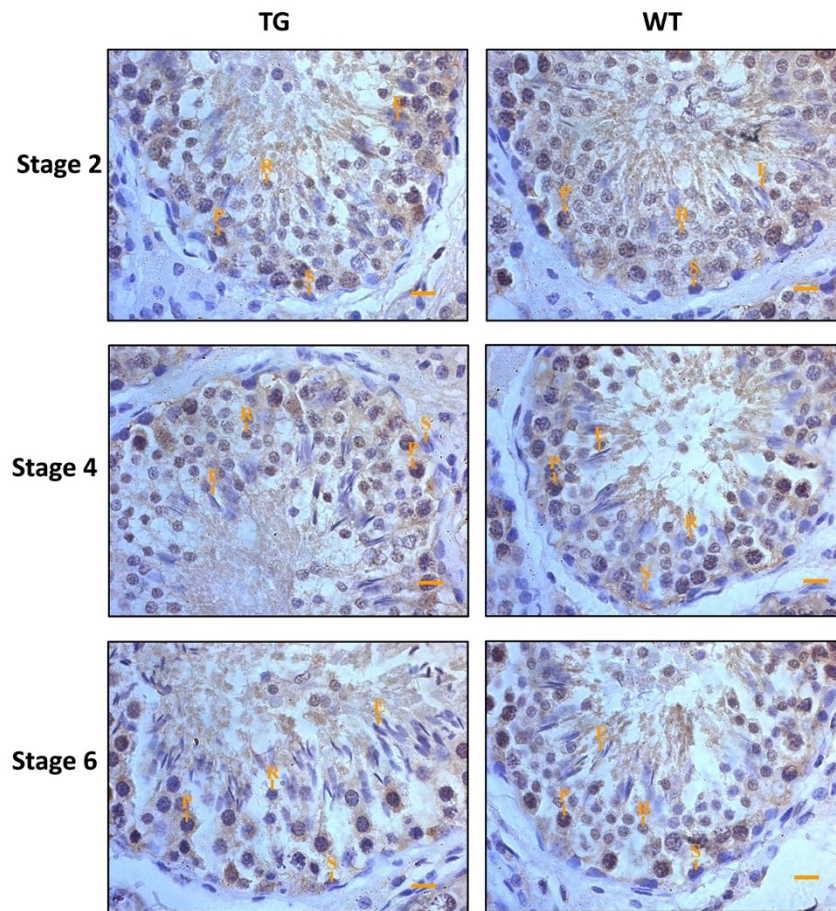


Fig. S11. Immunohistochemical analysis of BMPR2 in different stages of the seminiferous cycle in adult boar testes. S, Sertoli cells; P, pachytene spermatocytes; R, round spermatids; E, elongated spermatids. Scale bar = 10 μ m.

SMAD2/3

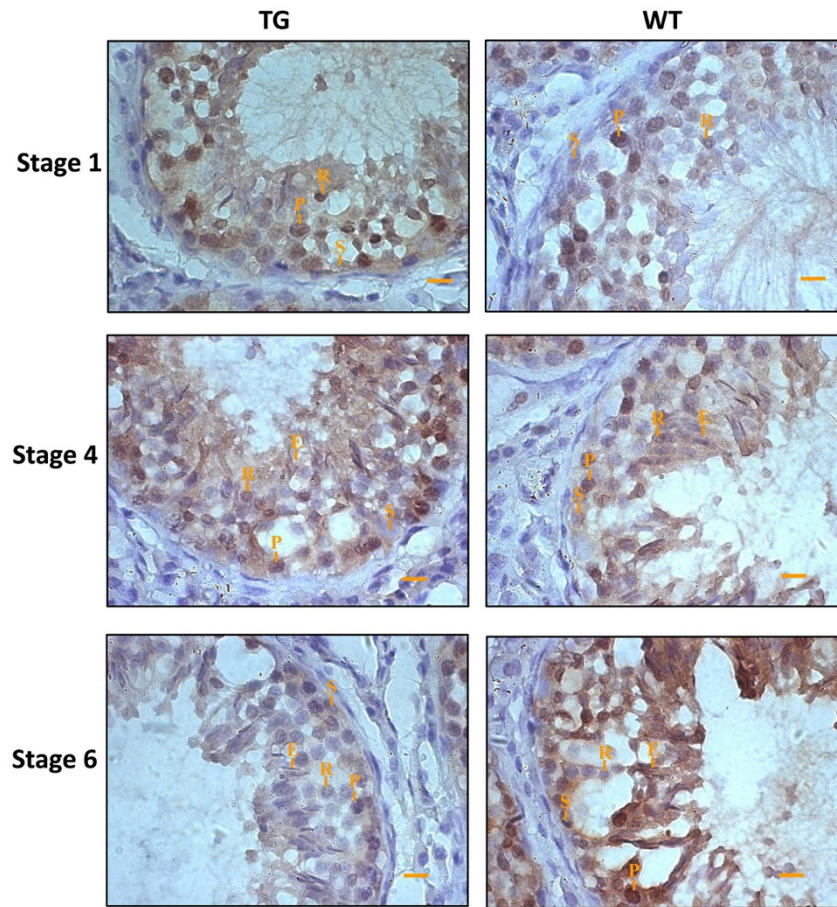


Fig. S12. Immunohistochemical analysis of SMAD2/3 in different stages of the seminiferous cycle in adult boar testes. S, Sertoli cells; P, pachytene spermatocytes; R, round spermatids; E, elongated spermatids. Scale bar = 10 μm.

SMAD1/5/8

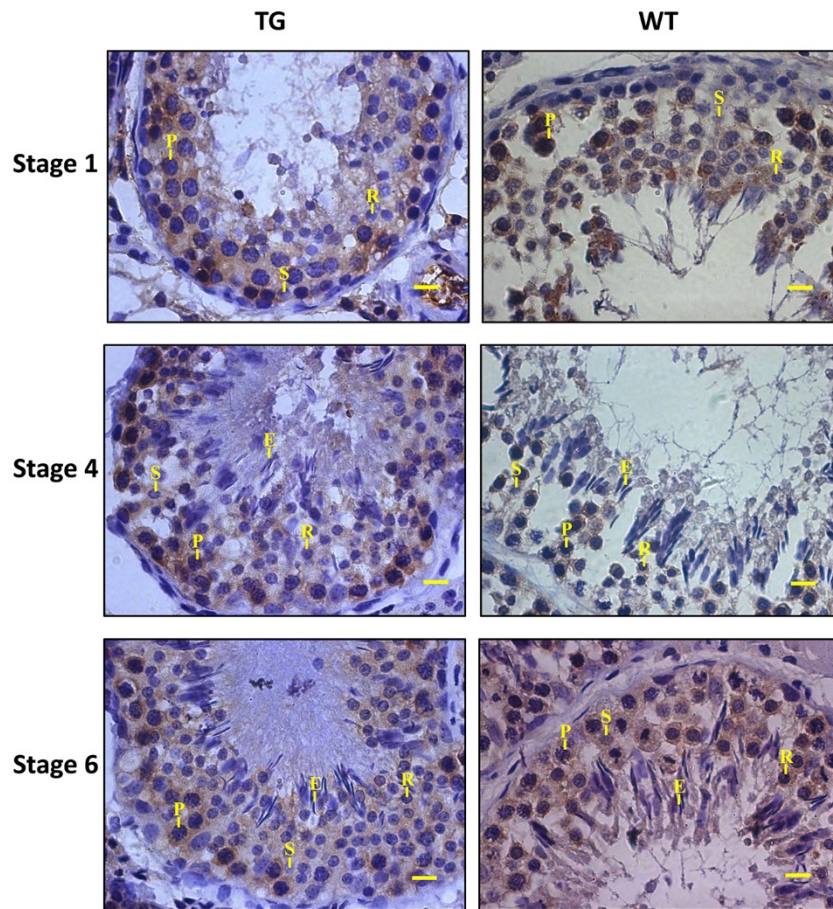


Fig. S13. Immunohistochemical analysis of SMAD1/5/8 in different stages of the seminiferous cycle in adult boar testes. S, Sertoli cells; P, pachytene spermatocytes; R, round spermatids; E, elongated spermatids. Scale bar = 10 μ m.

Table S1. Summary of generation of transgenic pigs through SCNT (only on pregnant recipients)

Experiment	Name of donor cell lines	No. transferred embryos	Name of recipient	Litter size	No. born alive	No. born alive/ No. transferred embryo (%)	Name of alive pigs (gender)
1	DBEF-4-2 (51)	85	318	4	4	4/85 (4.7%)	T-177 (male)
2	DBEF-4-2	118	364	2	2	2/118 (1.7%)	T-179 (female)
3	DBEF-3-1 (M)	92	384	6	5	5/92 (5.4%)	-
4	318-4	96	312	2	2	2/96 (2.1%)	T-262 (male) T-263 (male)
5	DBEF3-1-13	90	210	4	4	4/90 (4.4%)	T-300 (male) T-301 (male) T-302 (male) T-303 (male)
6	DBEF3-1-13	74	391	5	5	5/74 (6.8%)	T-295 (male) T-296 (male)

							T-298 (male)
							T-299 (male)
7	364-2	179	273	3	3	3/179 (1.7%)	T-304 (male)
							T-306 (male)
Total	-	734	7	26	25	25/734 (3.4%)	-

Table S2. Primers used for PCR or qRT-PCR analysis

Primer*	Sequence (5'to 3')	Amplicon size (bp)	Accession ID of target gene	Location
shRNA F	CTATTTCCCATGATTCCTTC	1381	-	-
shRNA R	ATCAGAGCAGCCGATTGT			-
probe F	CGATACAAGGCTGTTAGAGAGA	398	-	-
probe R	TCAACCCTATCTCGGTCTATTC			-
ALK4 F	CGGGGGATCCAGGCTCT	184	100152462	Exon1, 2
ALK4 R	CGGAGCTCAGGCAGTAGAAG			Exon2
ALK5 F	CCGTCACAGAGACCACAGAC	188	396665	Exon2
ALK5 R	TCCTGGAAAAGGACCAACAGT			Exon2, 3
ALK6 F	AGAGAGTGAACGCCCTCTGAA	195	396691	Exon2
ALK6 R	TCAAGGAAGTTTGCTTCTGGT			Exon3, 4
AR F	ATGACACTGGGAGGTTTCCG	194	397582	Exon3, 4
AR R	CACTGGCTGTACATTCGGGA			Exon5
BMP15 F	AAACAGCAGAGGAAGCCA	175	448811	Exon1
BMP15 R	GCCAGGGCCCTCTGA			Exon1, 2
BMPR2 F	GGATGCTGACAGGAGATCGT	197	100127483	Exon4, 5
BMPR2 R	CTGGCGGTTTGCAAAGGAAA			Exon6
GAPDH F	TCGGAGTGAACGGATTTGGC	189	396823	Exon2, 3
GAPDH R	TGACAAGCTTCCCGTTCTCC			Exon4
GDF9 F	GACCAGGTGACAGGAACCCTT	197	414285	Exon1, 2
GDF9 R	GGAGCTTTAGGCAGAGTCTTGT			Exon2
PRM2 F	CAGCAGCGCGAAAACGAG	193	397486	Exon1
PRM2 R	TGATCCGTCTGCAGCCTCTG			Exon1, 2
SMAD1 F	AGCCAACCCGGTGCCTGA	170	397016	Exon1,2
SMAD1 R	CCTCGTGGACGGAGAAACAA			Exon2
SMAD2 F	TCGAAAAGGACTGCCACACG	177	100155304	Exon2
SMAD2 R	ACTGGAGGCAAACTGGTGT			Exon2, 3
SMAD3 F	CTGGGCTGGAAGAAGGGTG	172	397260	Exon1
SMAD3 R	CATCCAGGGACCTGGGGA			Exon1, 2
SMAD5 F	CGGTCACTCGGCCGCT	198	100624262	Exon1
SMAD5 R	CATTGGTTCAAGTCGGGC			Exon1, 2
STAR F	AGACTTTGTGAGTGTGCGCT	186	396597	Exon3, 4
STAR R	CTGAGCAGCCAGGTGAGTTT			Exon4

CYP17 F	CCAGGATGCTATCGACCAGAA			Exon7
CYP17 R	AATTCGCCAATGCTGGAGTC	168	403330	Exon7, 8
HSD3B1 F	ATTTCTCGGTGCCCAGGTTT			Exon2
HSD3B1 R	TCTTGCTCTGGAGCTTAGAAAA	184	445539	Exon2, 3

* Primers were synthesized by Synbio Technologies (Suzhou, China).

Table S3. Information of antibodies used in western blot and immunohistochemistry assay

Antibody	Application	Brands	Product code	Dilution rate*
BMP15	WB	abcam	ab198226	1:800
GDF9	WB	abcam	ab93892	1:800
p-Smad1/5	WB	CST	9516S	1:1000
p-smad2/3	WB	abcam	ab5203	1:1000
ALK4	WB	abcam	ab109300	1:1000
BMPR2	WB	abcam	ab96826	1:1000
ALK6	WB	abcam	ab175385	1:200
HSD3B2	WB	abcam	ab154385	1:1000
Protamine2	WB	abcam	ab190791	1:1000
AR	WB	Santa cruz	sc816	1:200
BMP15	IHC	Eterlife	EL166380	1:200
GDF9	IHC	abcam	ab93892	1:200
ALK4	IHC	abcam	ab109300	1:200
BMPR2	IHC	abcam	ab96826	1:200
ALK6	IHC	abcam	ab175385	1:100
SMAD2/3	IHC	Santa cruz	sc8332	1:500
SMAD1/5/8	IHC	Santa cruz	sc6031R	1:500
HSD3B2	IHC	abcam	ab154385	1:100
AR	IHC	Santa cruz	sc816	1:50

* Antibody was diluted in TBS solution containing 3% BSA. The dilution rate of each antibody was adjusted according to both product specifications and optimization of the experimental procedures.

Table S4. Statistical analysis of litter size and total number born alive of sows mated with F0 or F1 transgenic boar

TNB: Total number born; NBA: Number born alive. Data are presented as mean \pm s.e.m.

Boar	F0 (<i>n</i>=1)	F1 (<i>n</i>=1)
Sow	<i>n</i>=19	<i>n</i>=8
TNB	9.21\pm0.66	10.75\pm0.68
NBA	8.26\pm0.72	8.37\pm0.66