PNAS
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Supplementary Information for

Donor-derived spermatogenesis following stem cell transplantation in sterile *NANOS2* knockout males

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This PDF file includes:

Figures S1 to S7

Tables S1 to S7

Legends for Movies S1 and S2

Other supplementary materials for this manuscript include the following:

Movies S1 and S2

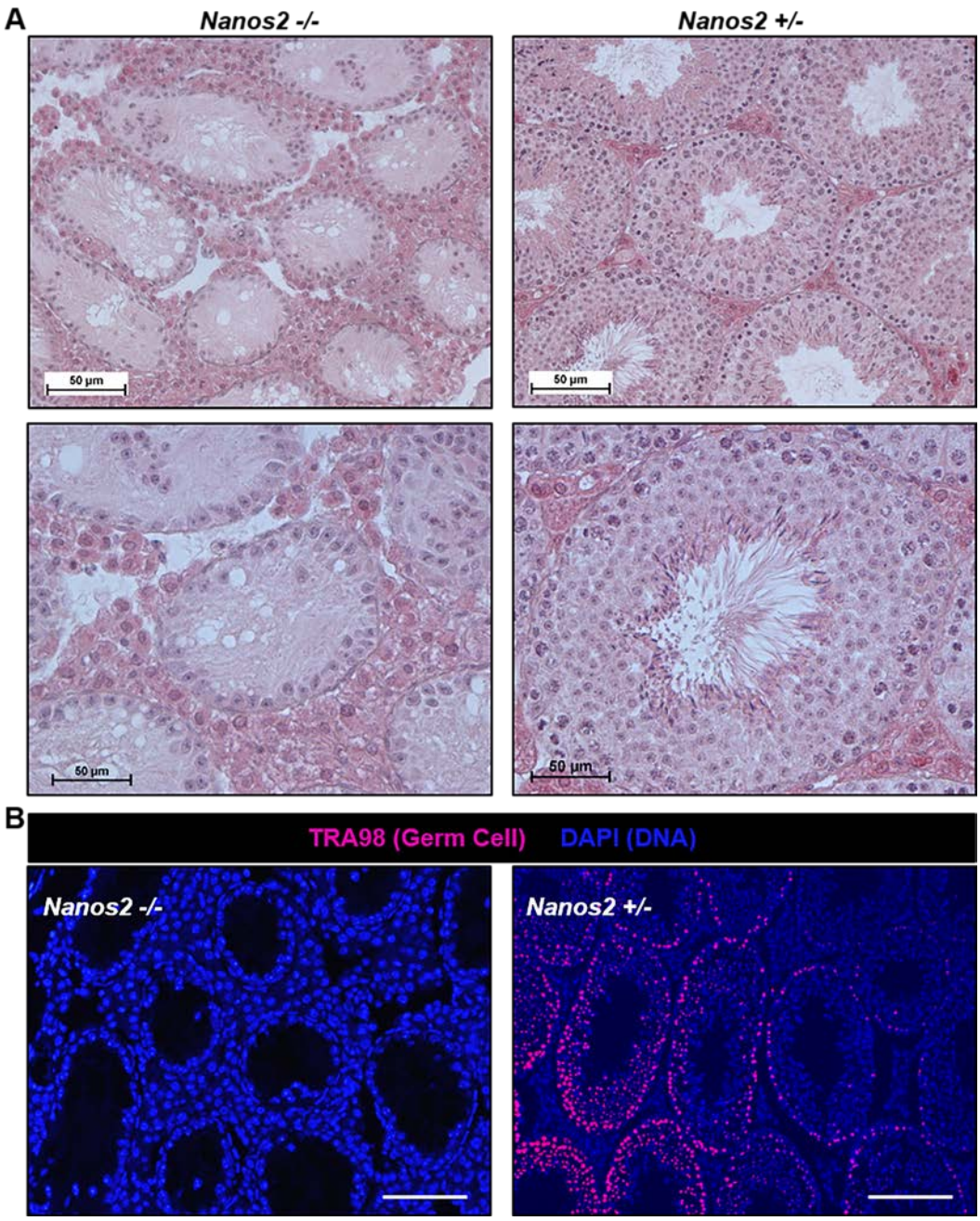

Fig. S1. Germline ablation in *Nanos2* knockout male mice. (*A*) Representative images of hematoxylin and eosin stained cross-sections from testes of adult (>2 months of age) *Nanos2 -/-* and *+/-* mice. (*B*) Representative images of immunofluorescent staining for the pan germ cell marker TRA98 in cross-sections of testes from adult *Nanos2 -/-* and *+/-* mice. DAPI was used to stain DNA. Bars are 50 μm.

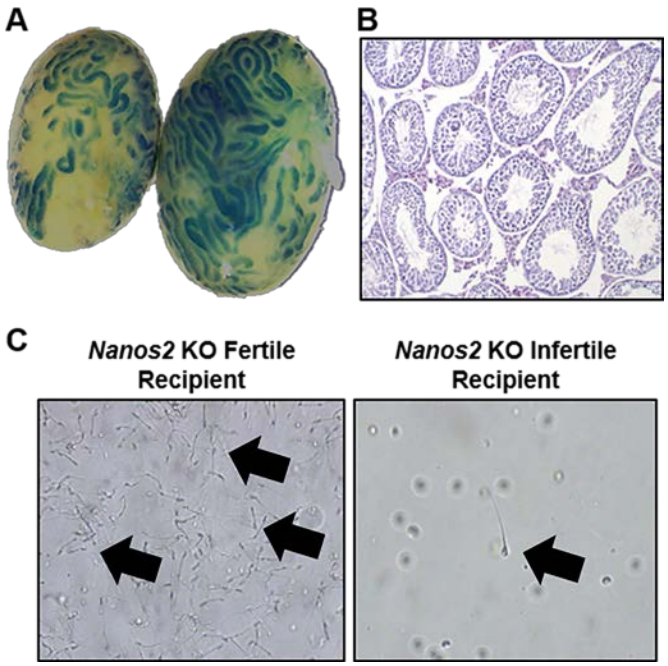

Fig. S2. Regeneration of donor-derived spermatogenesis in testes of *Nanos2* knockout recipient male mice that did not attain natural fertility. (*A*) Representative image of testes from a *Nanos2* knockout recipient stained with X-Gal 6 months after allogeneic SSCT. Donor-derived spermatogenesis is evidenced by intense blue staining. (*B*) Representative image of a hematoxylin and eosin stained cross-section from a *Nanos2* knockout recipient testis 6 months after allogeneic SSC transplantation. (*C*) Representative images of sperm (indicated by black arrows) in the epididymal flushing of a *Nanos2* knockout recipient 6 months after allogeneic SSC transplantation that did or did not attain fertility.

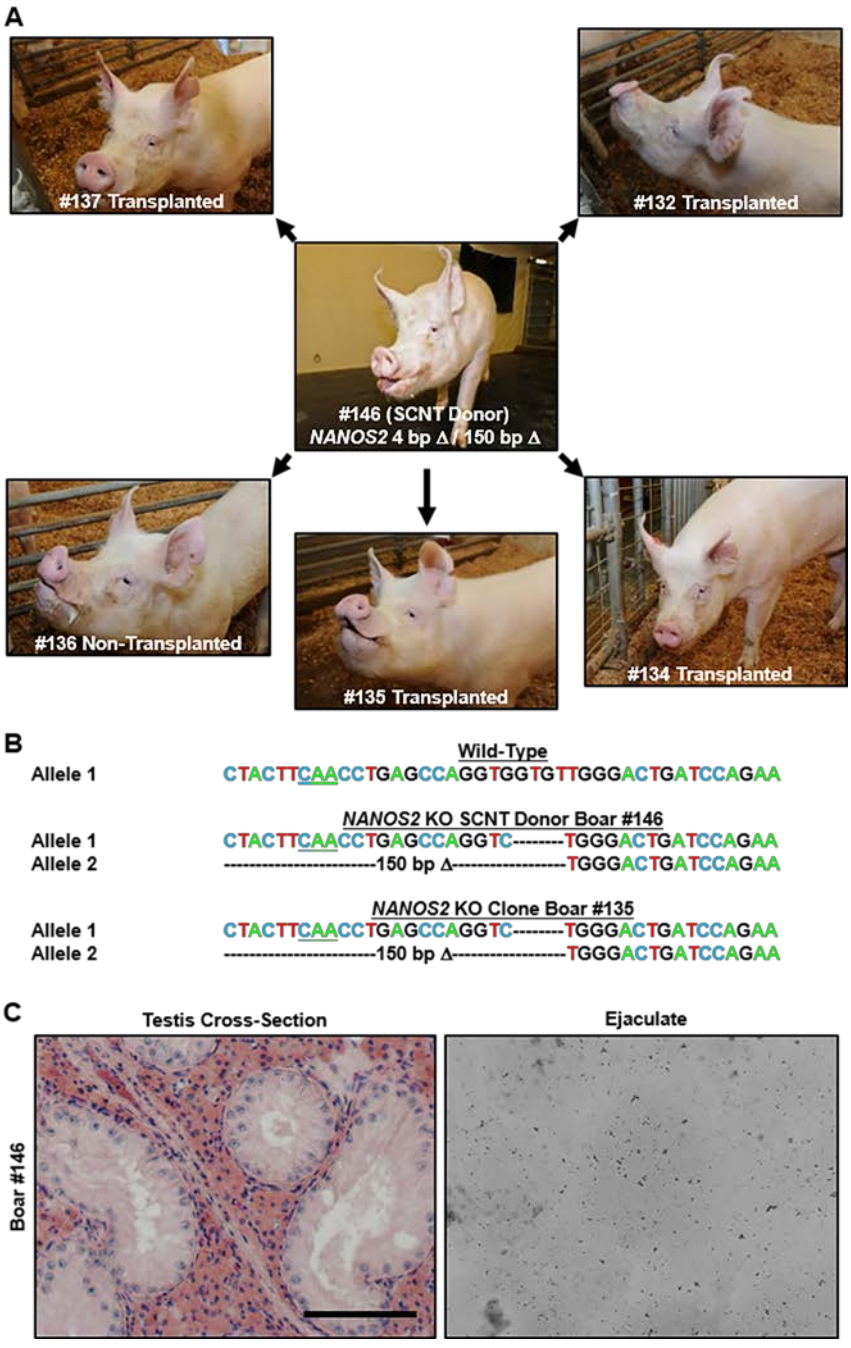

Fig. S3. Generation of *NANOS2* knockout male pigs via somatic cell nuclear transfer. (*A*) Images of boar #146 generated in previous studies (Park et al., 2017) that possessed 4 bp and 150 bp *NANOS2* deletion (Δ) alleles from CRISPR-Cas9 gene editing and 5 clones (#s 132, 134, 135, 136, and 137) produced by somatic cell nuclear transfer from skin fibroblasts of #146. Note that a sixth clone (#133) is not pictured. (*B*) Outcomes of DNA sequencing analysis confirming the *NANOS2* inactivation mutations in the cloned boars. (*C*) Representative images of testis cross-sections and ejaculate samples from the cloned *NANOS2* knockout boars demonstrating germline ablation and azoospermia.

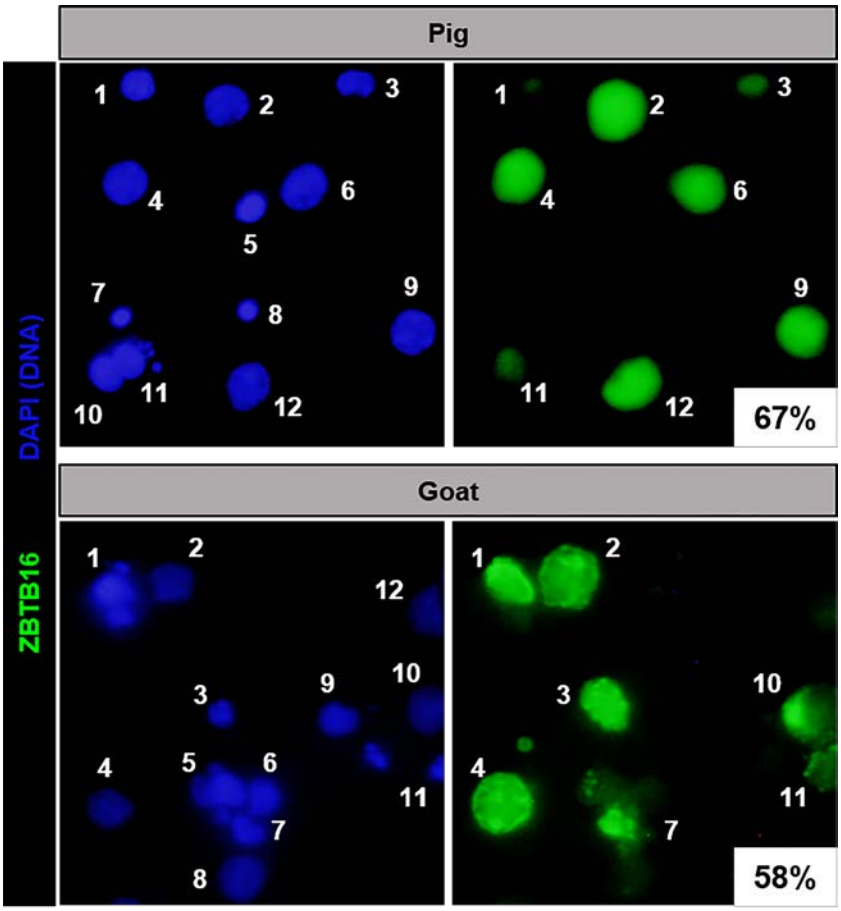

Fig. S4. Enrichment of donor pig and goat testis cell suspension for spermatogonia. Representative images of immunocytochemical staining for cells expressing the spermatogonial marker ZBTB16 in the unselected total testis cell population from a 3 month old donor boar or buck and following multiparameter selection. The percentage of the population that was calculated to be ZBTB16+ is indicated in each image. DAPI was used to stain for DNA in all cells. Bars are 50 μm.

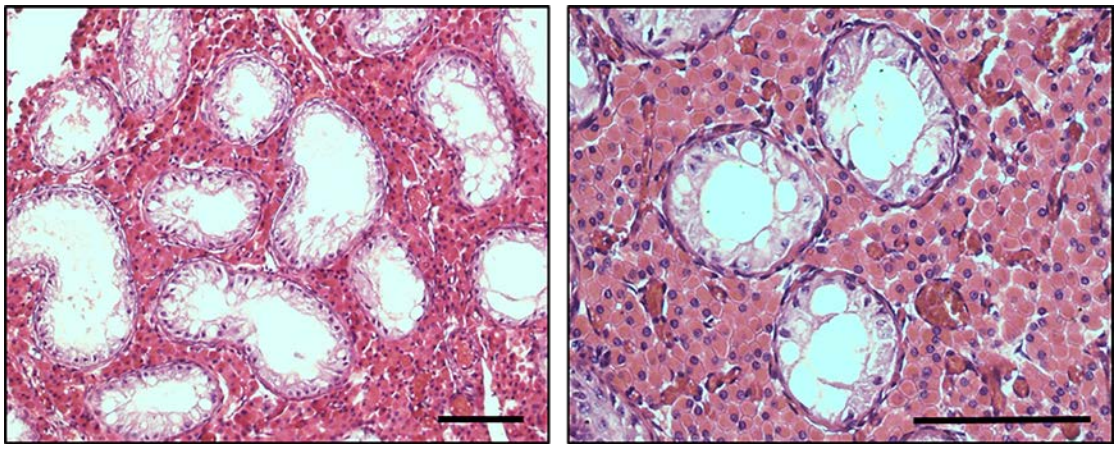

Fig. S5. Persistent germline ablation in *NANOS2* knockout boars not subjected to SSCT. Representative images of hematoxylin and eosin stained cross-sections from testes of *NANOS2* knockout boar #136 at 3 years of age who was never subjected to transplantation with donor spermatogonia. Bars are 50 μm.

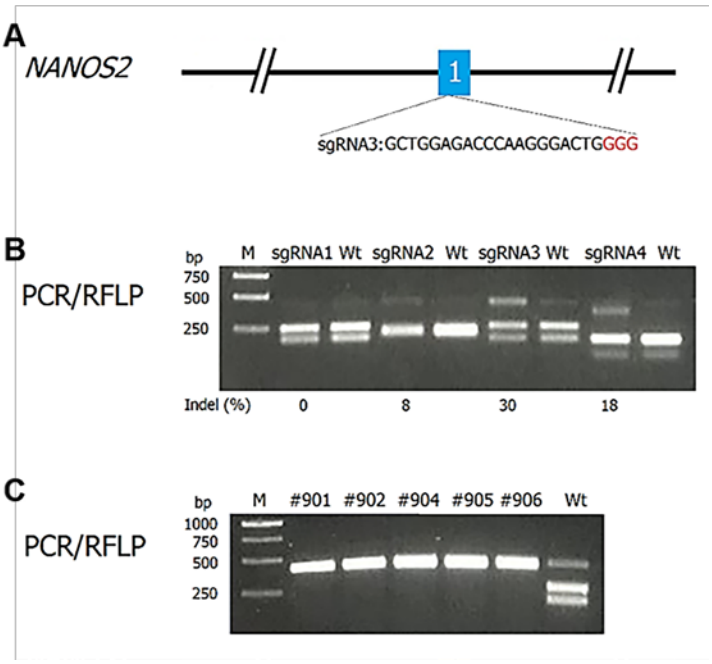

Fig. S6. CRISPR-Cas9 gene editing strategy for mutation of *NANOS2* in goat fetal fibroblasts. (*A*) Schematic of the sgRNA design to target the single exonic *NANOS2* gene in the caprine genome. The PAM sequence is highlighted in red. (*B*) Representative image of an agarose gel to visualize PCR products from amplified gDNA of CRISPR-Cas9 treated fetal fibroblast for the *NANOS2* gene followed by restriction enzyme digestion to assess editing efficiency. (*C*) Representative image of an agarose gel to visualize restriction enzyme digested PCR products from amplified gDNA of 5 bucks that were generated by somatic cell nuclear transfer with fetal fibroblast possessing CRISPR-Cas9 mutated *NANOS2* alleles. gDNA from a wild-type (Wt) buck was used as a positive control for restriction fragment length polymorphism (RFLP) assessment.

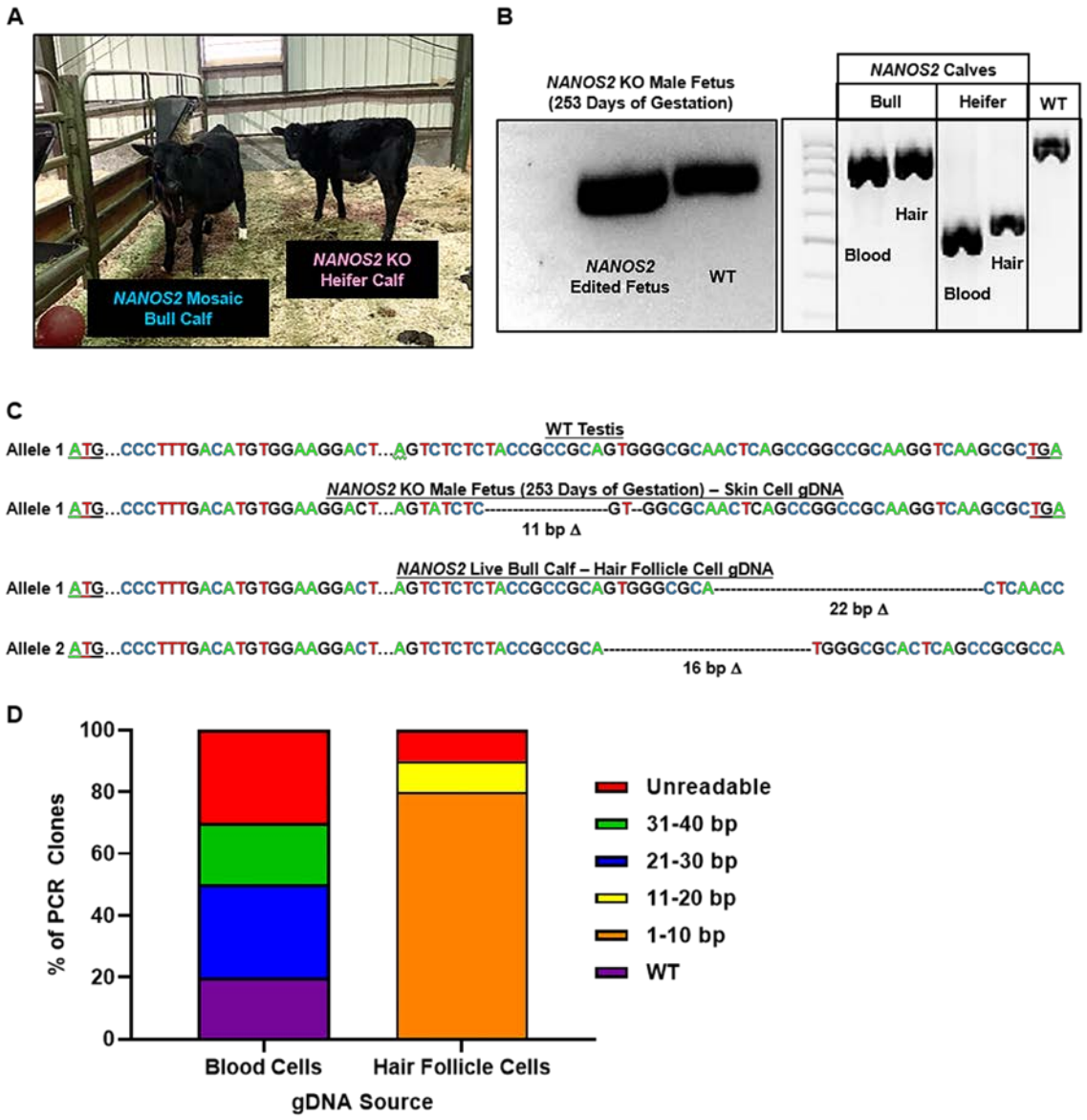

Fig. S7. Generation of *NANOS2* mutant cattle by CRISPR-Cas9 editing of zygotes and embryo transfer. (*A*) Representative image of a *NANOS2* knockout (KO) heifer calf and *NANOS2* mosaic bull calf. (*B* and *C*) Representative images of agarose gels to visualize amplicons from PCR based genotyping analysis for the *NANOS2* gene (*B*) and outputs from Sanger sequencing of the amplicons (*C*). Gentotyping was conducted with gDNA isolated from a stillborn male fetus at gestational day 253, a live born heifer calf, and a live born bull calf. gDNA from wild-type cattle (WT) was used as a control. (*D*) Quantification of the frequency of different *NANOS2* edited alleles in cloned PCR genotyping amplicons using gDNA from either hair follicles or skin epithelial cells of the live born mosaic bull calf. Data are presented as the percentage of clones with a certain edited from n=10 clones analyzed for each source of gDNA.

Movie S1. Video demonstrating motility of donor-derived sperm in the ejaculate of a *NANOS2* knockout recipient boar 110 days after allogeneic SSC transplantation.

Movie S2. Video demonstrating motility of donor-derived sperm in the ejaculate of a *NANOS2* knockout recipient buck 150 days after allogeneic SSC transplantation.

Table S1. Fertility of *Nanos2* +/- and -/- mice.

Mouse ID	Gender	*Nanos2* Genotype	Number of Litters	Avg. Pups/Litter
MKO1	Male	-/-	0	-
MKO2	Male	-/-	0	-
MKO3	Male	-/-	0	-
MHET1	Male	+/-	3	11.7
MHET2	Male	+/-	3	15.3
MHET3	Male	+/-	3	15.0
FKO1	Female	-/-	2	13.5
FKO2	Female	-/-	2	12.5
FKO3	Female	-/-	2	14.5
FHET1	Female	+/-	2	14.5
FHET2	Female	+/-	2	14
FHET3	Female	+/-	2	11.5

Table S2. Fertility attainment following allogeneic donor spermatogonial stem cell transplantation in *Nanos2* knockout recipient mice.

Recipient ID	Age at Transplant (Days)	Donor Cells Transplanted (Left/Right)		Days to First Offspring	# of Litters	Total Offspring	Avg. Pups / Litter	LacZ+ Offspring
2654	28	$7.5X10^4$	0	113	5	11	2.2	11
2757	21	0	$3X10^4$	81	14	45	3.2	45
2761	21	$5X10^4$	$5X10^4$	90	14	55	3.9	55
2751	21	0	$3X10^4$	-	-	-		-
2767	42	$8X10^4$	0	-	-	-		-
2670	43	$5X10^4$	0	-	-	-		-

Table S3. Donor spermatogenic lineage regeneration following allogeneic donor spermatogonial stem cell transplantation in *NANOS2* knockout recipient boars.

Recipient ID	Transplant 1 (4 Months of Age) Donor Cells Transplanted (Left/Right)[a]		Days to Round Cells	Days to Sperm	Transplant 2 (14 Months of Age) Donor Cells Transplanted (Left/Right)[b]		Days to Round Cells	Days to Sperm
132	$4.3X10^6$	-	-	-	$4.0 X 10^6$	$4.0 X 10^6$	-	-
133	-	-	-	-	-	-	-	-
134	0	$4.3X10^6$	-	-	$4.0 X 10^6$	$3.0 X 10^6$	90	-
135	$4.3X10^6$	$4.3X10^6$	-	-	$4.3 X 10^6$	$4.3 X 10^6$	90	110
136 (control)	0	0	-	-	-	-	-	-
137	$4.3X10^6$	$4.3X10^6$	-	-	$5.0 X 10^6$	$3 X 10^6$	-	-

[a]Single cell suspension of total testis cell population and 1 ml volume per testis.
[b]Single cell suspension of isolated spermatogonia and 4 ml volume per testis.

Table S4. Genotypic and phenotypic data for *NANOS2* knockout bucks.

Animal ID	Fetal Fibroblast Clone	Allelic Mutations	Birth Weight (kg)	Weaning Weight (kg)
901	C82-2	16 bp Δ / 16 bp Δ	6.8	35.0
902	C82-2	16 bp Δ / 16 bp Δ	5.3	27.3
904	C25-1	16 bp Δ / 16 bp Δ	4.0	20.9
905	C25-1	16 bp Δ / 16 bp Δ	4.0	22.3
906	C25-1	16 bp Δ / 16 bp Δ	4.6	24.1

Table S5. Donor spermatogenic lineage regeneration following allogeneic donor spermatogonial stem cell transplantation in *NANOS2* knockout recipient bucks.

Recipient ID	Age at Transplant (Months)	Donor Cells Transplanted (Left/Right)		Days to Round Cells	Days to Sperm
901	4	1.5×10^6	0	85	-
902	4	2×10^6	0	85	136
904	4	2×10^6	2×10^6	-	-
905	4	1.5×10^6	0	-	-
906	4	2×10^6	0	85	-

Table S6. Embryo transfer details for generation of NANOS2 gene edited cattle.

Recipient Cow ID	# Embryos Transferred	Embryo Stage	Day 30 Pregnancy	Day 60 Pregnancy	Outcome
RR1	2	Blastocyst	No	No	
RR2	2	Blastocyst	Yes	No	
RR3	2	Blastocyst	Yes	No	
RR4	2	Blastocyst	No	No	
RR5	2	Blastocyst	No	No	
RR6	2	Blastocyst	Yes	No	
RR7	2	Blastocyst	No	No	
RR8	2	Blastocyst	Yes	Yes	Pre-term stillborn @ GD 253 *NANOS2* KO Bull Calf
210	2	Blastocyst	Yes	Yes	Birth of live *NANOS2* KO Heifer Calf
227	2	Blastocyst	No	No	
309	2	Blastocyst	Yes	No	
323	2	Blastocyst	No	No	
338	2	Blastocyst	No	No	
409	2	Blastocyst	Yes	No	
806	2	Blastocyst	No	No	
830	2	Blastocyst	Yes	Yes	Birth of Live *NANOS2* Mosaic Bull Calf

Table S7. Phenotypic data for live born *NANOS2* gene edited cattle.

Animal ID	Genotype	Birth Weight (kg)	Weaning Weight (kg)	Germline
Heifer Calf (Marta)	*NANOS2 -/-*	38	118	Intact
Bull Calf (Messi)	*NANOS2* Mosaic	50	155	Ablation