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Supplementary Material

A genetic method for sex determination in *Ovis* spp. by interruption of the zinc finger protein, Y-linked (*ZFY*) gene on the Y chromosome

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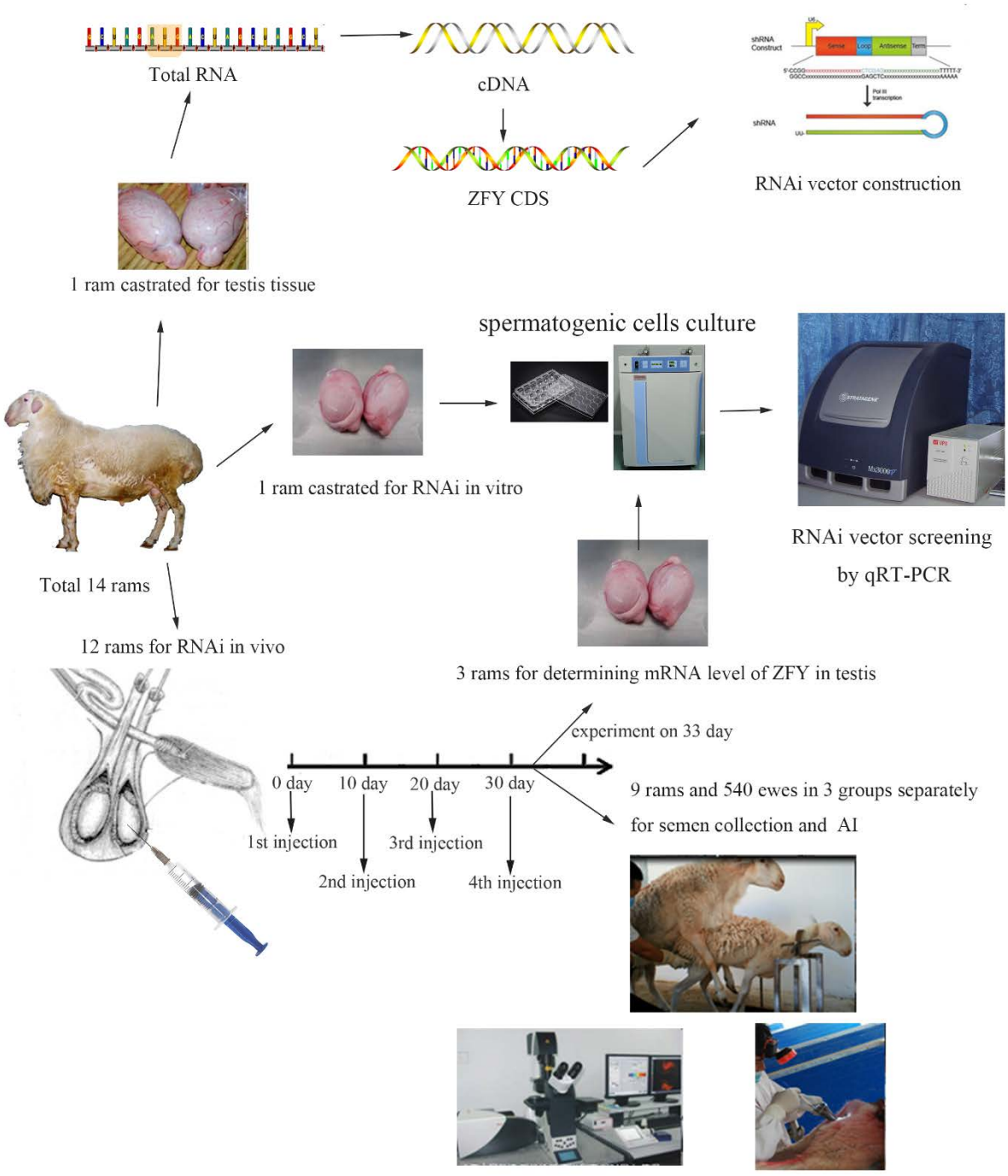

Fig. S1. Flowchart and timeline showing the number of animals per group when injections were administered, when semen was collected/AI performed, and when testicular tissues were collected for qRT-PCR analysis.

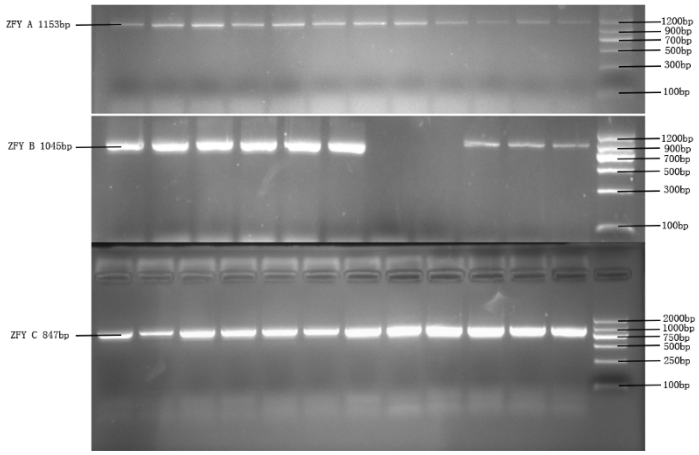

Fig. S2. Electrophoretogram of ZFY amplification results. Three fragments of the CDS sequence show that there is no false-positive and undesirable nonspecific banding. No bands were detected in lanes 7 and 8 from the mid panel in Figure S1B because the reaction mixtures had evaporated due to incomplete covering of the reaction tubes.

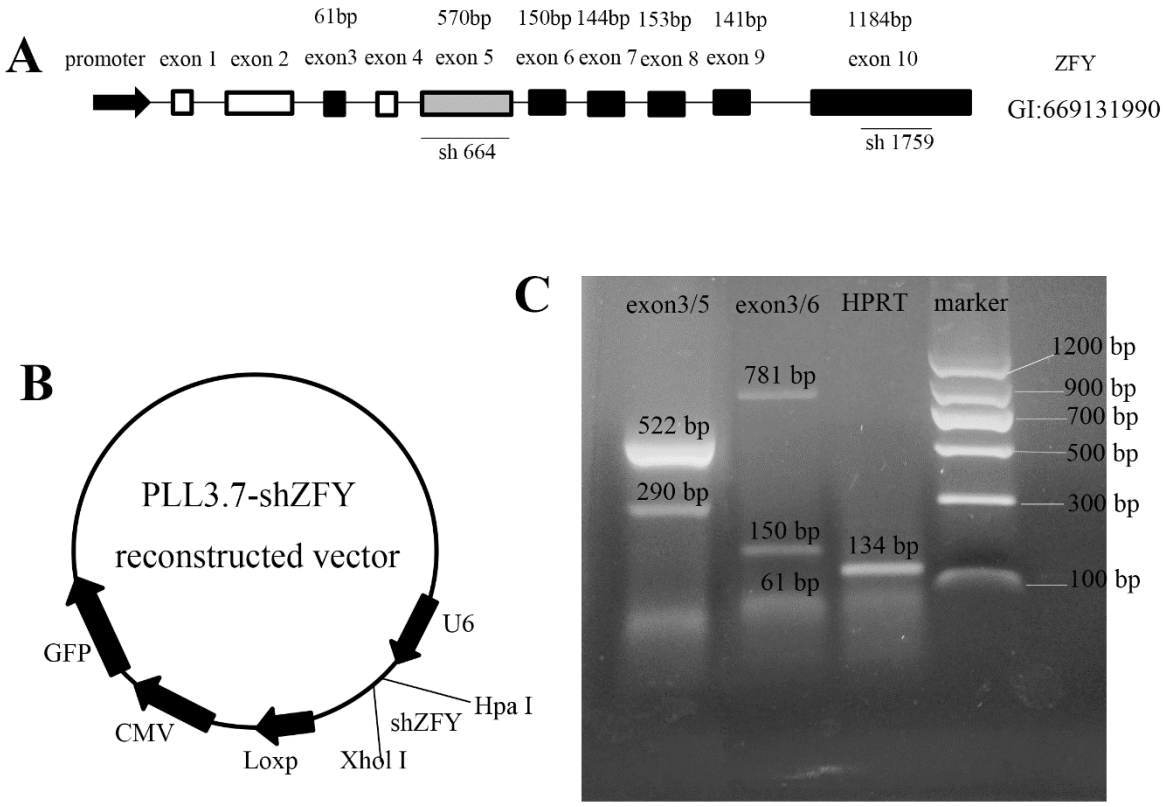

Fig. S3. Structure of the ZFY gene (Gene ID: 669131990) and shZFY constructs. (A) Exons are represented by boxes: coding exons are shaded , exons contain splice variants are grey and non-coding are Blank. Introns and exons are not to scale. (B) The location of the sequences used to produce the shZFYconstructs is indicated beneath the schematic of the ZFY gene. Upper left insert: schematic of the plasmid construct injected to produce shZFY-interrupted sperms in Hu sheep testis. (C) RT-PCR analysis of mRNA from normal sheep testis with primers in exon 3/6 and exon 3/5 of the different ZFY transcripts.

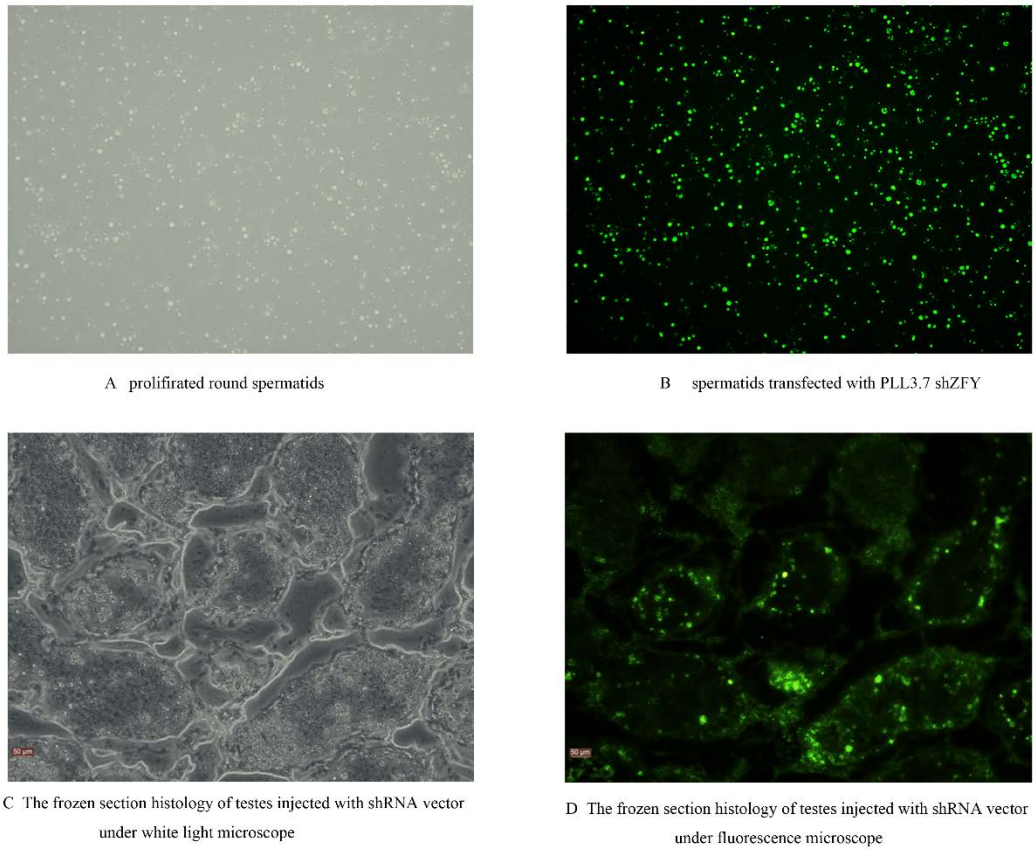

Fig. S4. The transfected efficiency of PLL3.7-shZFY recombined vector to *Ovis* spp. round spermatids. A indicates round spermatid extracted from Hu sheep testis. B represents spermatid transfected with PLL3.7-shZFY by using Lipofectamine® 3000. C displays the frozen section histology of testes injected with shRNA vector under white light microscope observation. D showes the frozen section histology of testes injected with shRNA vector under fluorescence microscope observation.

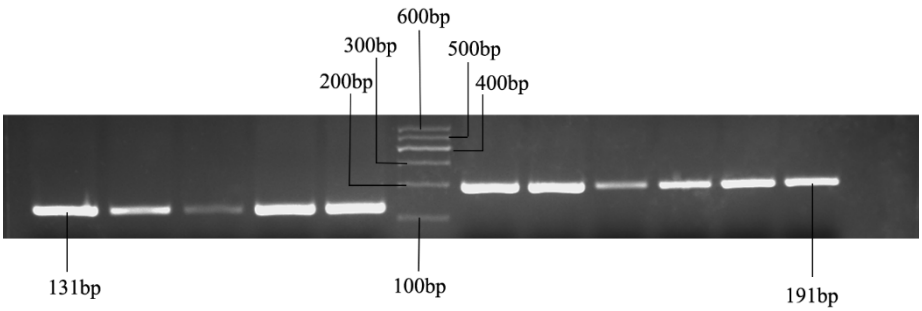

Fig. S5. The validation of primers by PCR amplification. No primer dimers or other non-specific amplification products were detected, indicating that that the primers and conditions for qRT-PCR were highly specific.

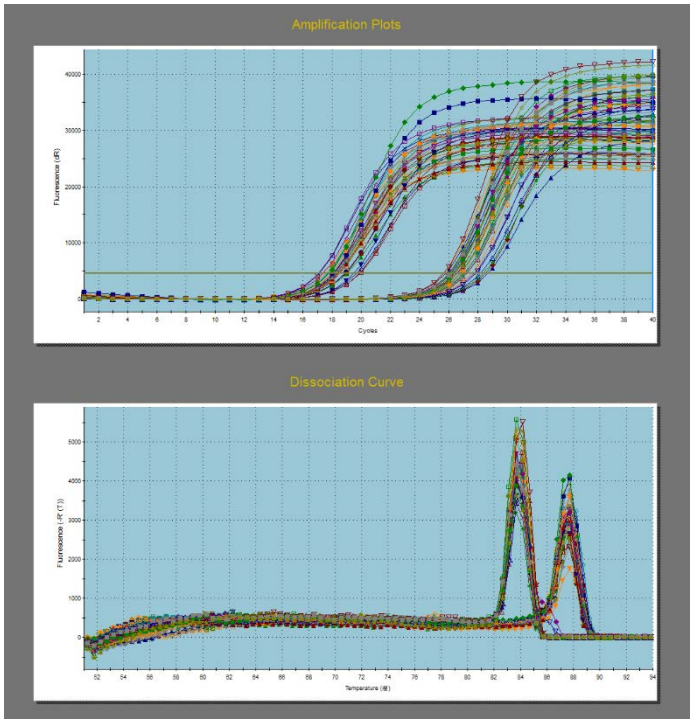

Fig. S6. The amplification plots and disassociation curve of primers used in qRT-PCR analysis. The validation indicates accurate data analysis.