Supplementary figures

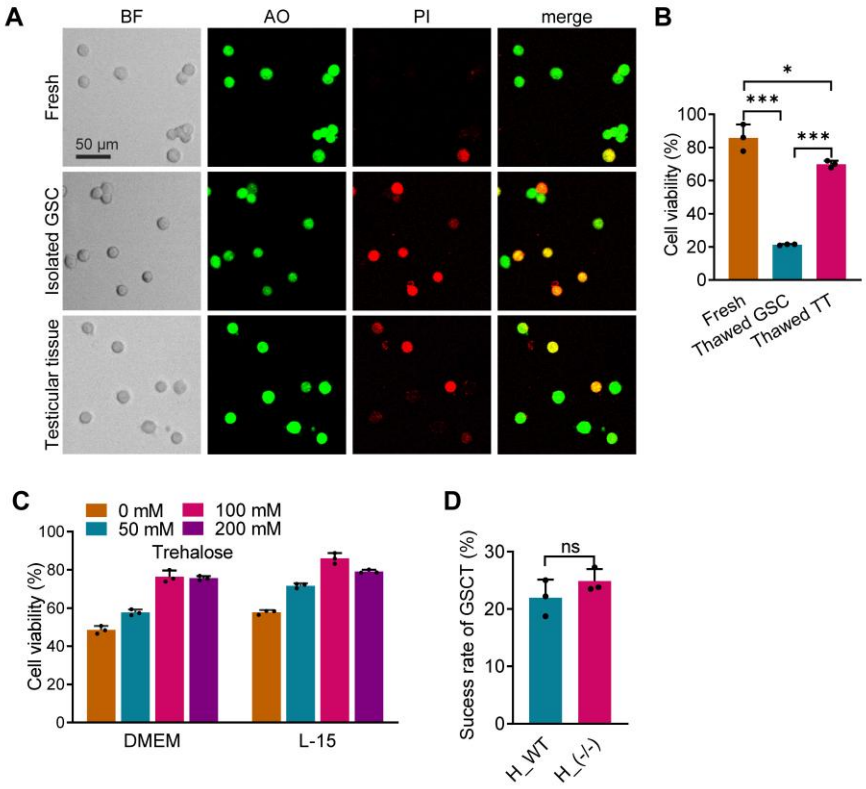

Figure S1 Optimization of GSC cryopreservation protocols

A, B: Viability assessment and statistical analysis of GSCs isolated from fresh groups, thawed GSCs and thawed TT by AO/PI staining. AO (green) stains all cells, while PI (red) stains dead cells. Scale bar: 50 μm. C: Comparison of GSC viability with varying concentrations of trehalose (0, 50, 100, 200 mM) added to DMEM and L-15 media. D: Success rates of *Tg(ddx4:EGFP)* GSCT to WT and *cyp11a2*$^{-/-}$ host. H_WT: Host _WT, H_(-/-): Host_*cyp11a2*$^{-/-}$, WT: wild type. All data are presented as mean values ± SEM, with *n*=3 for each group. Two-tailed Student's t-test was used to calculate the P values; *: *P*<0.05; ***: *P*<0.001, ns: no significant difference.

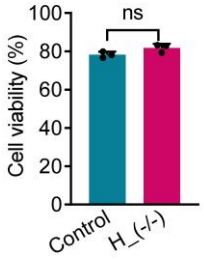

Figure S2 Cell viability assessment of control and host_*cyp11a2*$^{-/-}$

Cell viability assessment of isolated testicular cell from control and host_*cyp11a2*$^{-/-}$ testes after cryopreservation and thawing. All data are presented as mean values ± SEM, with *n*=3 for each group. Two-tailed Student's t-test was used to calculate the P values; ns: no significant difference.

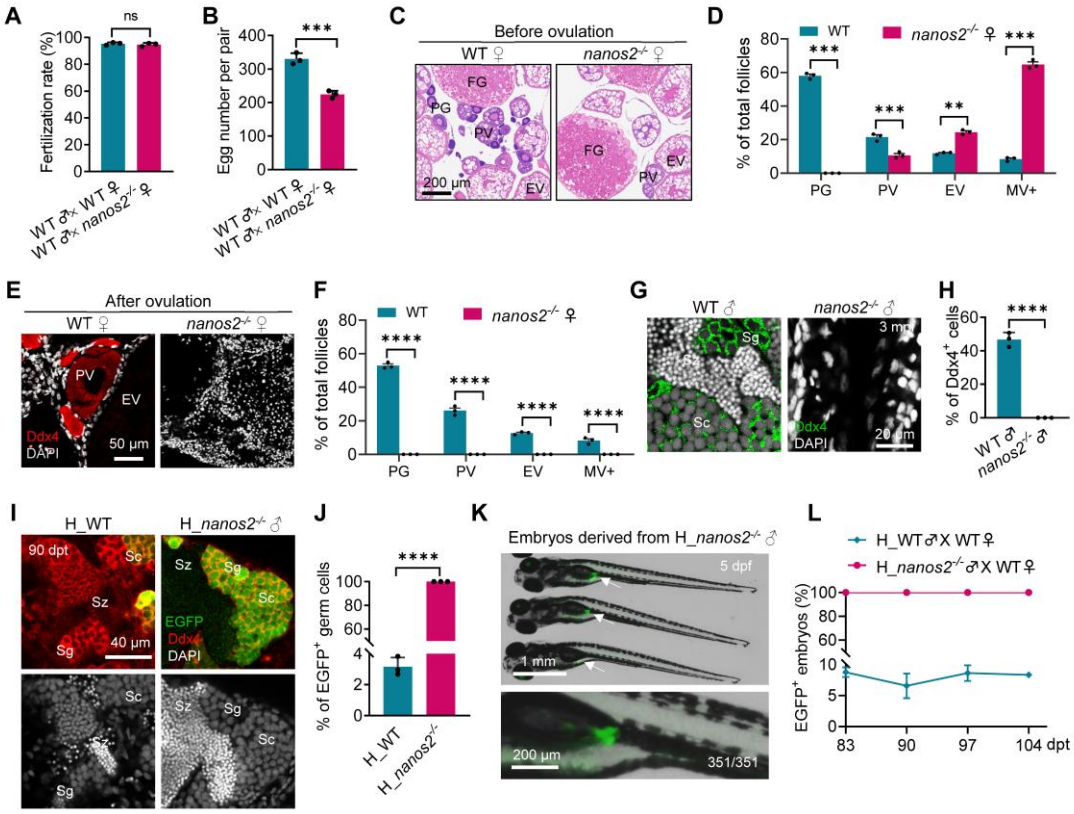

Figure S3 The *nanos2*$^{-/-}$ hosts enable efficient generation of donor-derived functional sperm from cryopreserved eGSCs

A, B: Fertilization rates and egg numbers in WT and *nanos2*$^{-/-}$ females. Egg production in *nanos2*$^{-/-}$ females was significantly lower than in WT females. C, D: Histological analysis and oocyte proportion statistics at different developmental stages in WT and *nanos2*$^{-/-}$ females prior to their first spawning (3 mpf, n=3). Scale bar: 200 μm. E: Immunofluorescence analysis with Ddx4 antibody and oocyte proportion statistics at different developmental stages after ovulation in WT and *nanos2*$^{-/-}$ females (after 1-2 spawnings, n=3). After two spawnings, *nanos2*$^{-/-}$ females were completely devoid of oocytes. Red represents Ddx4-positive cells, and gray represents DAPI staining. Scale bar: 50 μm. G, H: Immunofluorescence detection of

Ddx4 antibody and statistical analysis of Ddx4-positive germ cell proportions in WT and *nanos2*$^{-/-}$ males (3 mpf, n=3). Green represents Ddx4-positive cells, and gray represents DAPI staining. Scale bar: 20 μm. I: Immunofluorescence analysis of donor-derived germ cell differentiation in host_*nanos2*$^{-/-}$ testes at 90 dpt, showing colocalization of EGFP (green, from donor *Tg(ddx4: EGFP-UTRnanos3)*) with Ddx4 (red) and DAPI (gray). Scale bar: 50 μm. J: Statistical comparison of the proportion of EGFP-positive germ cells in testes of WT and *nanos2*$^{-/-}$ hosts. K: Localization of donor-derived EGFP fluorescence in gonadal ridges of F1 embryos at 5 dpf from *nanos2*$^{-/-}$ male hosts (white arrows). Scale bars: up: 1 mm, down: 200 μm. L: Statistical analysis of EGFP-positive embryos derived from WT and *nanos2*$^{-/-}$ male hosts during four consecutive spawning events. WT: wild type, dpf: day post fertilization, mpf: month post-fertilization, PG: Primary oocyte, PV: Pre-vitellogenic, EV: Early vitellogenic, MV: Midvitellogenic stage, FG: Full-grown stage. Sg: spermatogonium, Sc: spermatocyte, Sz: spermatozoa. H_WT: Host_WT, H_*nanos2*$^{-/-}$: Host_*nanos2*$^{-/-}$. All data are presented as mean values ± SEM, with *n*=3 for each group. Two-tailed Student's t-test was used to calculate the P values; **: *P*<0. 01, ***: *P*<0.001, ****: *P*<0.0001, ns: no significant difference.

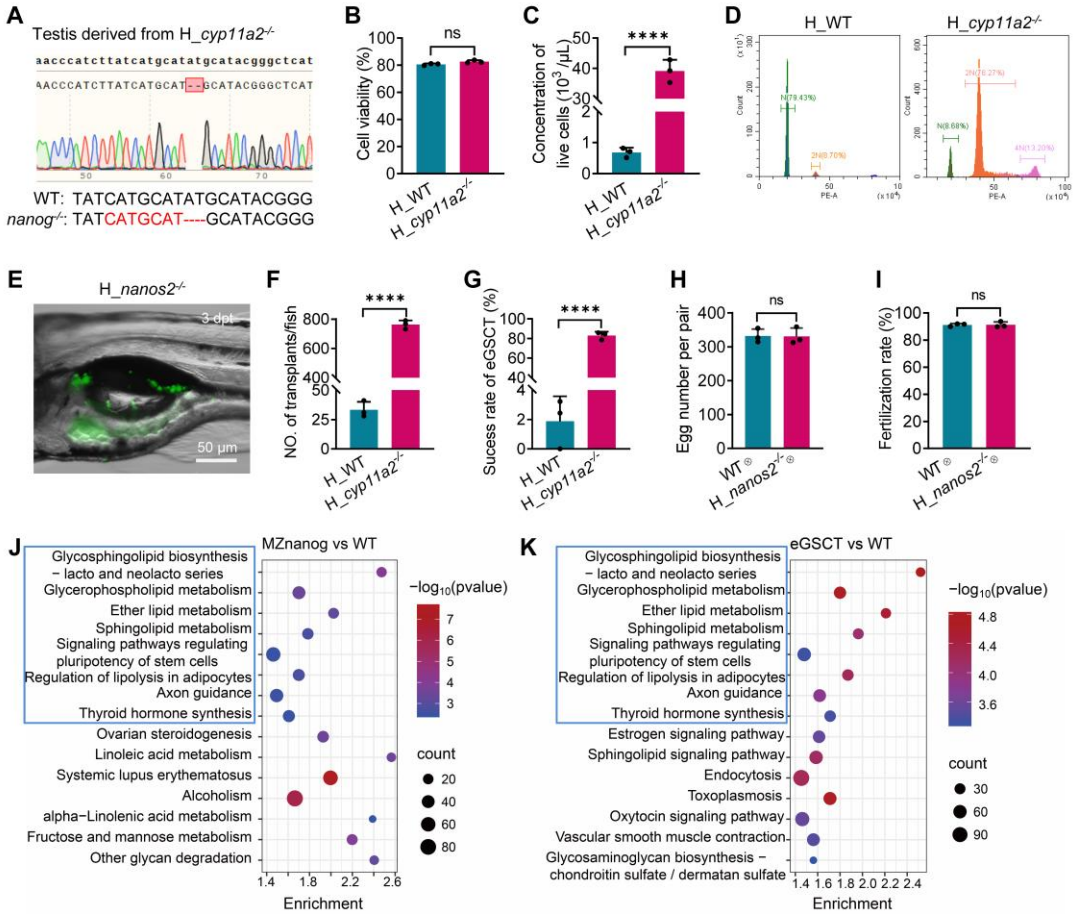

Figure S4 MZ*nanog* were recovered using *nanos2*$^{-/-}$ hosts from cryopreserved eGSCs

A: Genotyping of testes from host _*cyp11a2*$^{-/-}$ mutants. Sequencing confirmed that the testes in host _*cyp11a2*$^{-/-}$ are derived from donor *nanog*$^{-/-}$. B, C: Analysis of cell viability and live cell concentration following the thawing of testicular cells from host_WT and host_*cyp11a2*$^{-/-}$. D: Haplotype analysis of testicular cells from host_WT and host_*cyp11a2*$^{-/-}$ after thawing. E: Donor cell colonization in *nanos2*$^{+/-}$ self-crossed offspring hosts at 3dpt. Scale bars: 50 μm. F, G: Statistical analysis of transplantable host numbers and transplantation success rates using testicular cells of *nanog*$^{-/-}$ from host_WT and host_*cyp11a2*$^{-/-}$. H, I: Statistical analysis of egg production and fertilization rates from self-crossed WT and host_*nanos2*$^{-/-}$. J: KEGG pathway enrichment analysis of differentially expressed genes between MZnanog and WT, and between MZnanog_eGSCT and WT (top 15 pathways). The blue box indicates

the same 8 signaling pathways in both groups. WT: wild-type, dpt: day post transplantation, H_WT: Host_WT, H_cyp11a2$^{-/-}$: Host_*cyp11a2*$^{-/-}$, H_*nanos2*$^{-/-}$: Host_*nanos2*$^{-/-}$. All data are presented as mean values ± SEM. Two-tailed Student's t-test was used to calculate the P values; ****: *P*<0.0001, ns: no significant difference.

Table S1 Primers used for genotyping

Primers	Sequences (5' to 3')
cyp11a2-HRM-F	ACAAAAGTCTTCTTTCAGTGGGTC
cyp11a2-HRM-R	TTCAGTGAAGACACAGACTGATCC
nanog-F	CAGGTGATGTAAATGGGTCGGTA
nanog-R	TATCGCGTCGAGATGTACGCATG
nanos2-F	GACGGCTGTTTCCTGATG
nanos2-R	GCTACTGTTCTTCTTTGTGATG