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Hypogonadotropic hypogonadism in male tilapia lacking a functional *rln3b* gene

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Introduction

- Relaxin 3 is a neuropeptide that plays a crucial role in animal reproductive function.
- Previous studies have confirmed that *rln3a* plays an important role in male reproduction of tilapia.
- However, the role of its paralogous gene *rln3b* in fish reproduction is still unclear.
- We aimed to investigate the regulatory effects of *rln3b* on the development and fertility of male tilapia testes.

Materials and Methods

- Constructed a homozygous mutant line of *rln3b* in Nile tilapia using CRISPR/Cas9 gene editing technology.
- Explored the effects of *rln3b* homozygous mutation on male testis development and male fertility through experiments such as H.E. staining, Real time PCR, immunohistochemistry, and EIA.

Results

- We have successfully constructed a homozygous mutant line of *rln3b* in Nile tilapia.

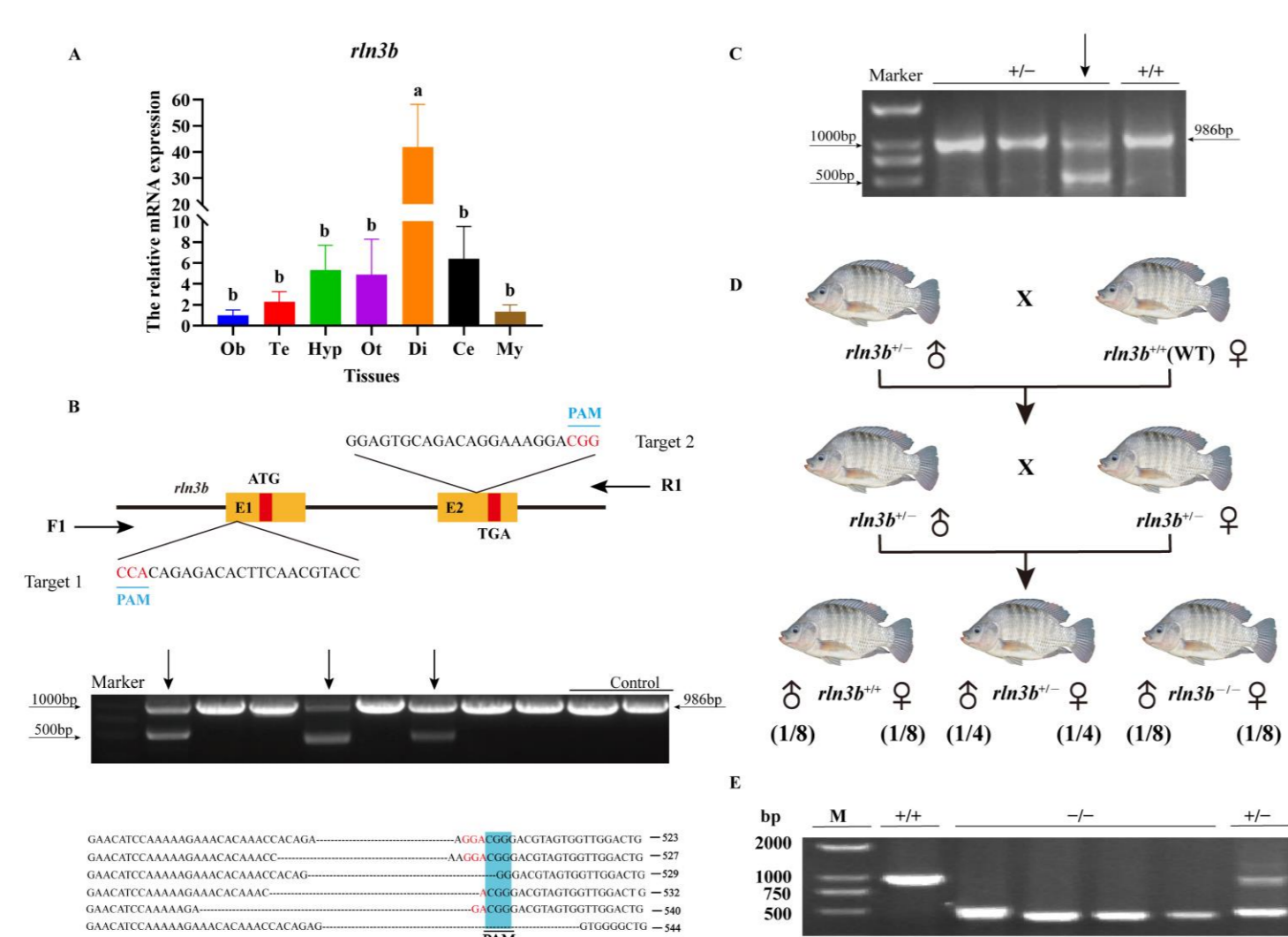


Fig.1 *rln3b* homozygous mutant (*rln3b*^{-/-}) in tilapia

- The *rln3b* mutation delays spermatogenesis and leads to abnormal testicular structure.

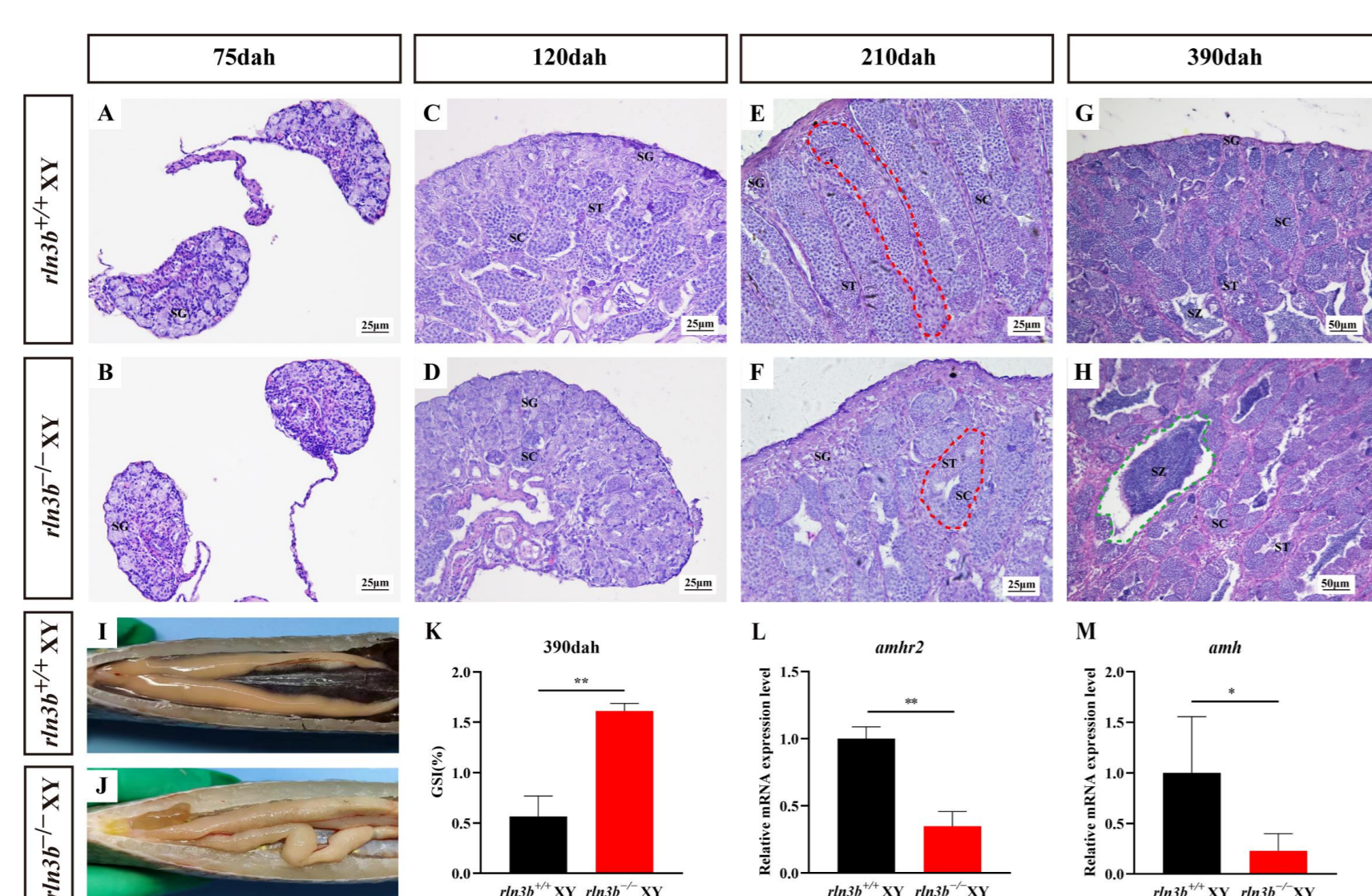


Fig.2 The effect of homozygous mutation of *rln3b* gene on the testis structure of male tilapia

Results

- The sperm tail of tilapia is curled and exhibits abnormal movement in *rln3b*^{-/-} tilapia.

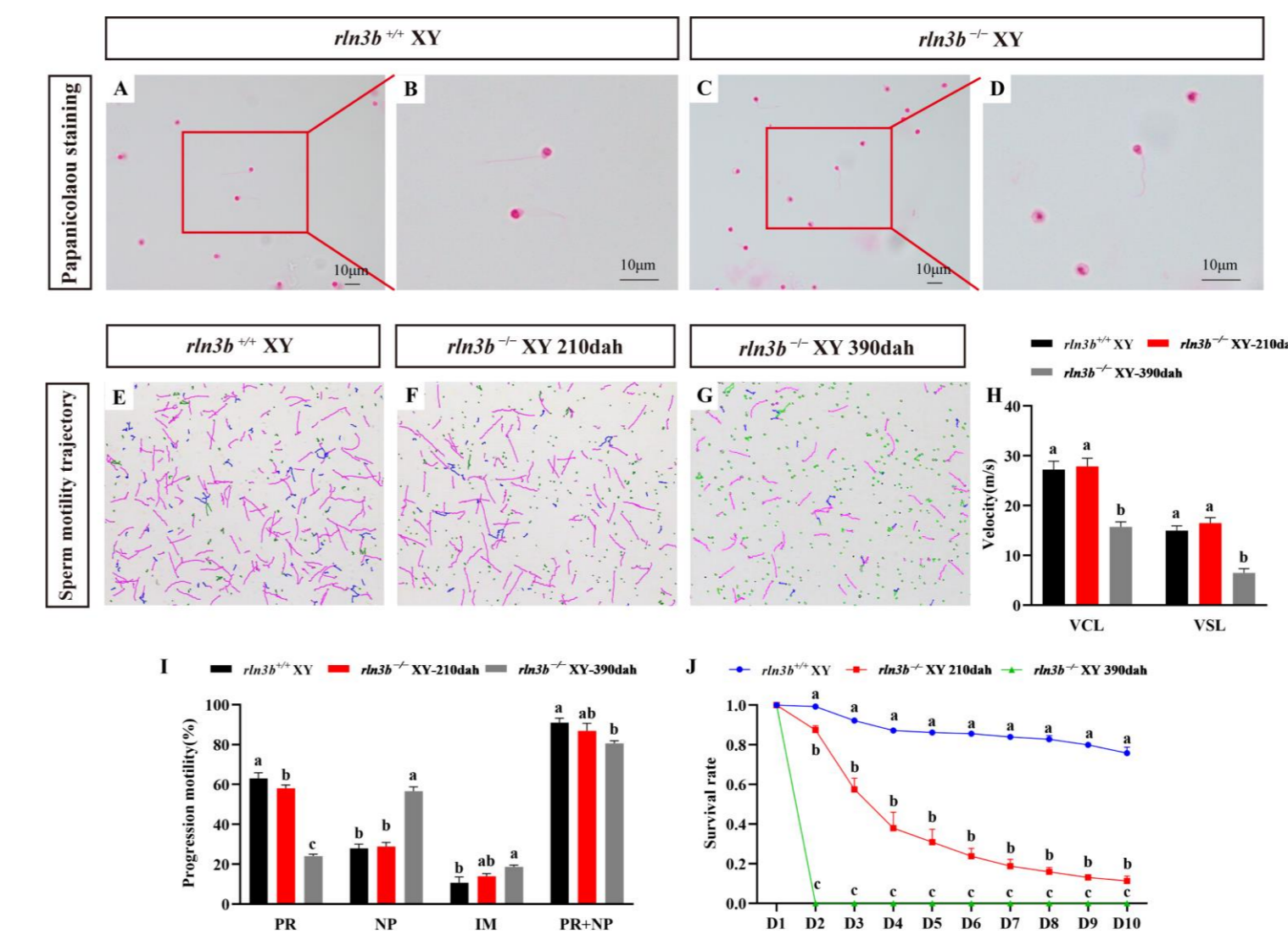


Fig.3 The effect of *rln3b* homozygous mutation on sperm motility and fertility in male fish

- The genes involved in steroid synthesis and partial androgen synthesis were first upregulated and then downregulated in *rln3b*^{-/-} tilapia.

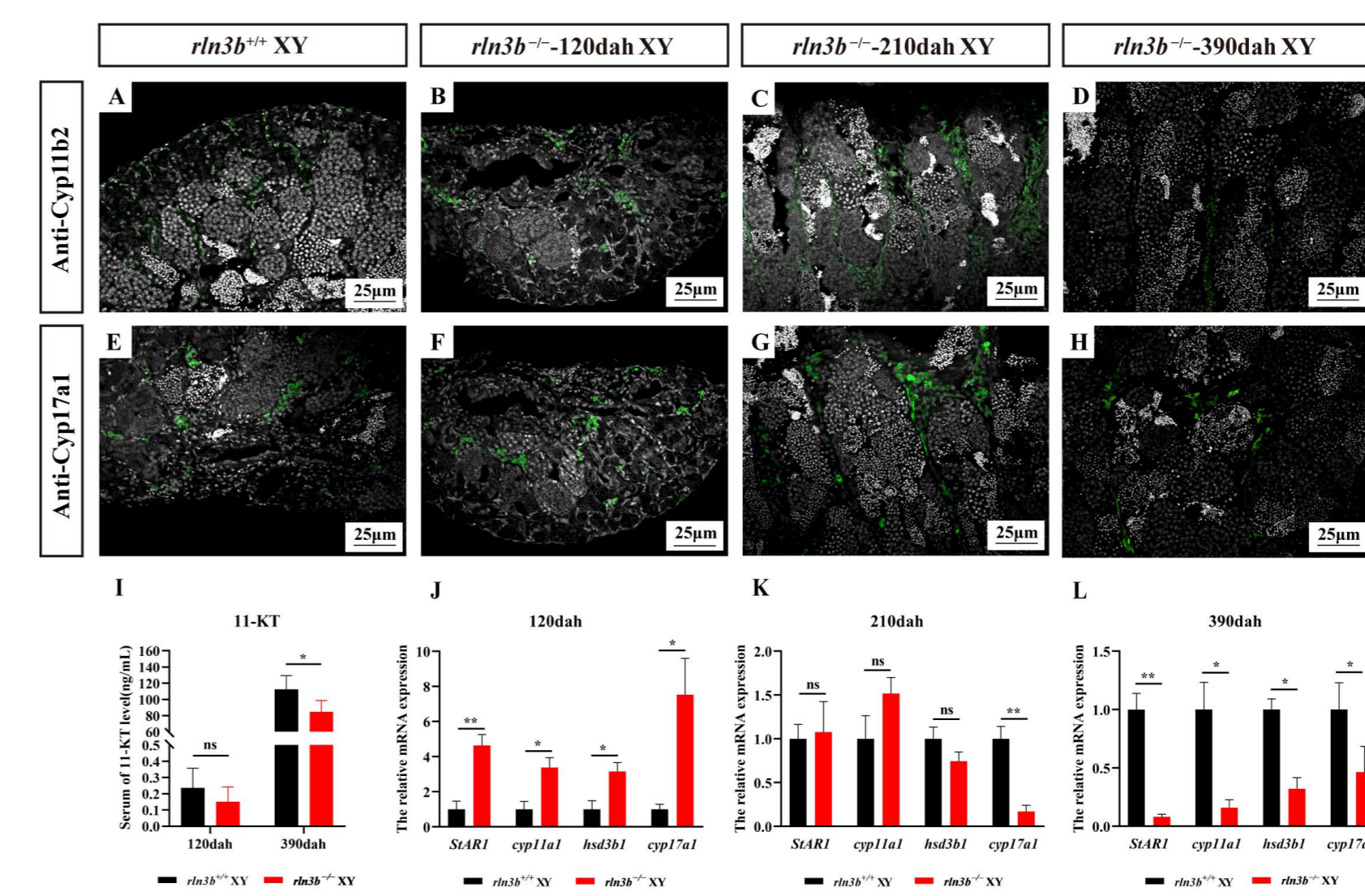


Fig.4 The effect of *rln3b* homozygous mutation on the expression of steroid and androgen synthesis genes

- RLN3b is an important upstream regulatory peptide hormone in the HPG reproductive regulatory network

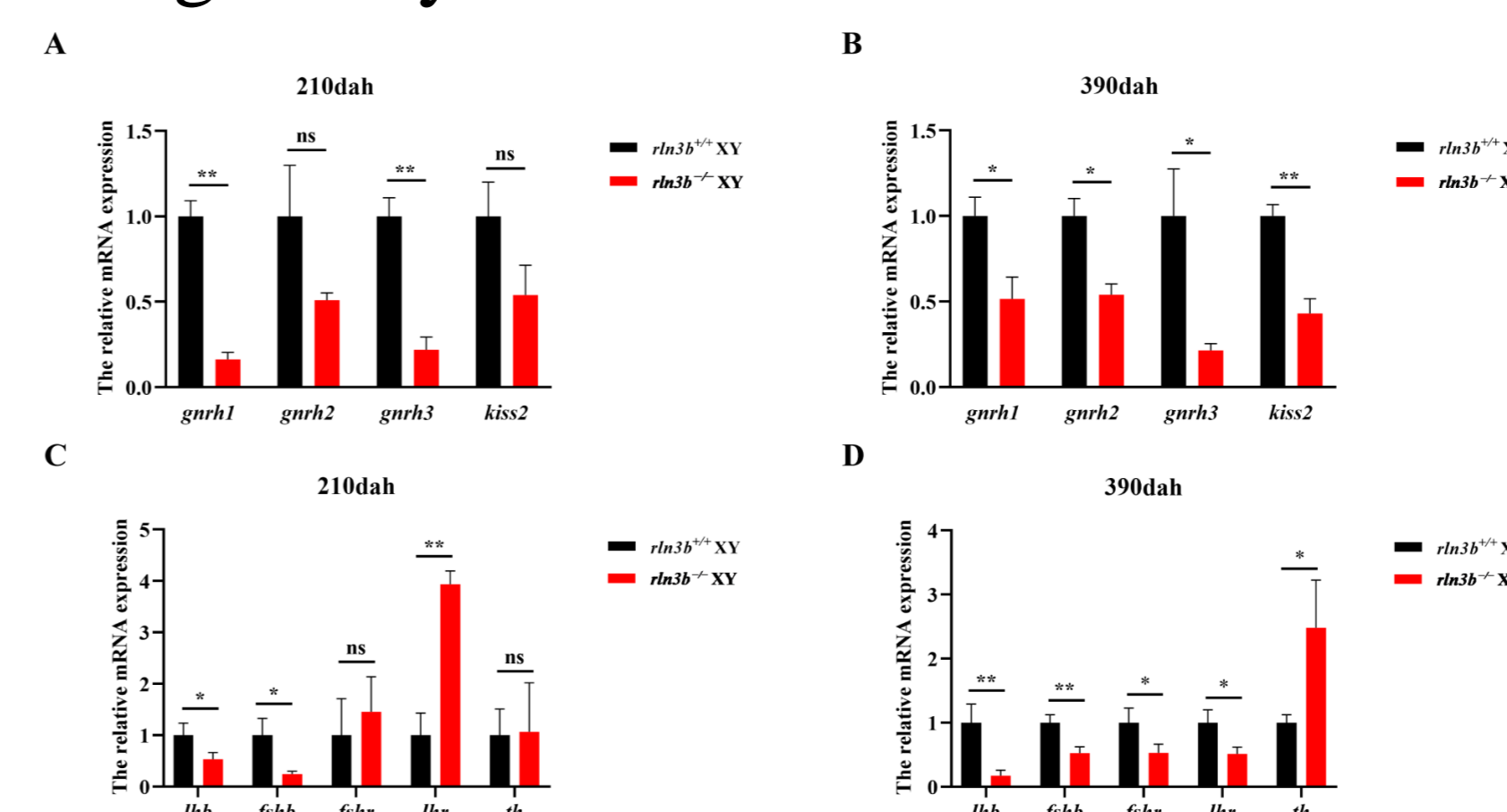


Fig.5 The effect of *rln3b* homozygous mutation on the expression of key regulatory genes of HPG axis

Conclusion

Rln3b, as an upstream regulatory factor of the HPG axis, regulates the development of male tilapia testes, sperm motility, and fertility. This is of great significance for cultivating high-yield fish varieties with controllable fertility in the future

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