## *Supplementary Material*

1 Supplementary Materials and Methods

To further analyze the inhibitory effect of antisense RNA on the target gene, absolute quantitative PCR of *amh* was performed in 180-day-old Nile tilapia. The standard curve of *amh* was obtained by qRT-PCR amplification after doubling dilution of plasmid. The standard curve was y= -0.3091x + 10.563 (y: Ct value, x: initial copy number), and the regression coefficient $R^2$= 0.9998. The results showed that there was a good linear relationship between the initial template concentration and Ct value after 10-fold gradient dilution of standard substance concentration, and the standard curve established in this study could accurately reflect the amplification of the target gene fragment. According to the relationship between Ct value and gene copy number in the standard curve, the absolute expression level of *amh* in total RNA was calculated.

2 Supplementary Figures and Tables

2.1 Supplementary Figures

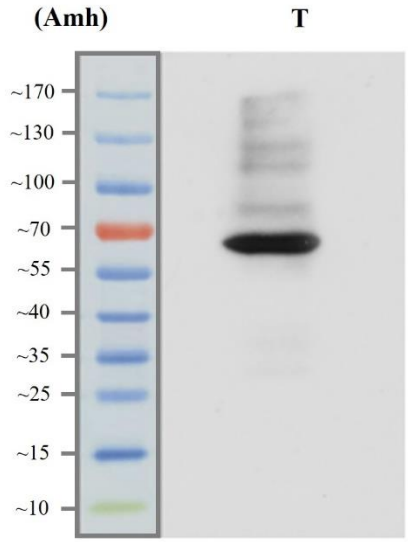


**Figure S1.** Protein marker map of Anti-Müllerian hormone in testis tissue of Nile tilapia.

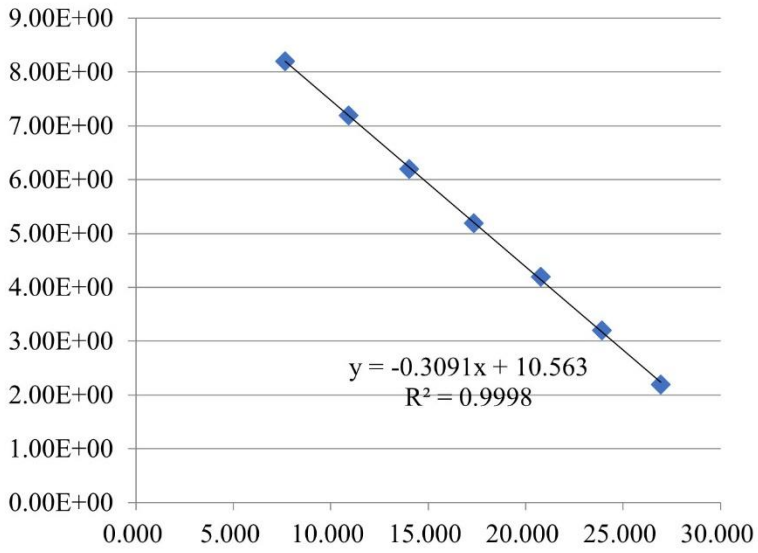


**Figure S2.** qRT-PCR standard curve in absolute quantification.

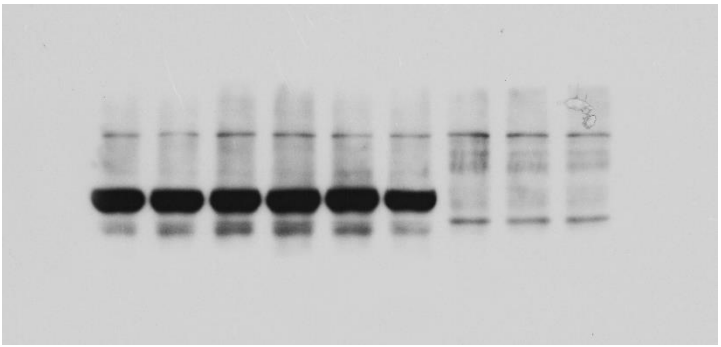

**Figure S3.** The blots for each independent biological replicate used in the analysis.

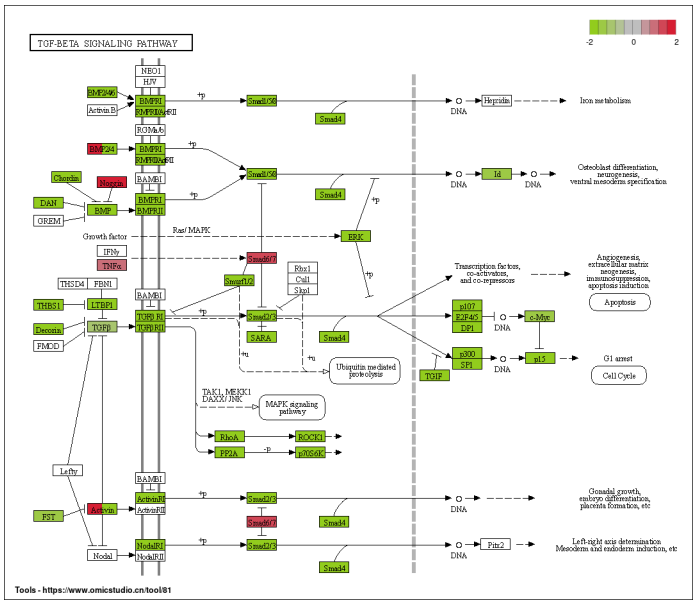


**Figure S4.** DEGs in TGF-beta signaling pathway. Dots: red, up-regulated DEGs in the TGF-beta

signaling pathway (treatment groups *vs.* control groups); green, down-regulated DEGs (treatment groups *vs.* control groups).

## 2.2 Supplementary Tables

**Table S1.** Dilution method of standard plasmid for absolute quantification

| Pipe number | Concentration (ng/μL) | Copy number (μL) |
|---|---|---|
| 1 | 1.00E+00 | 1.57E+08 |
| 2 | 1.00E-01 | 1.57E+07 |
| 3 | 1.00E-02 | 1.57E+06 |
| 4 | 1.00E-03 | 1.57E+05 |
| 5 | 1.00E-04 | 1.57E+04 |
| 6 | 1.00E-05 | 1.57E+03 |
| 7 | 1.00E-06 | 1.57E+02 |
| 8 | 0.00E+00 | 0.00E+00 |