Supplementary Information

Identification and Functional Analysis of E3 Ubiquitin Ligase *g2e3* in Chinese Tongue Sole, *Cynoglossus Semilaevis*

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I. siRNA synthesis

Method selection: In our study, siRNA was synthesized by chemical synthesis method due to its high specificity, stability and reproducibility. This method ensures the effectiveness and targeting of siRNA by precisely controlling the synthesis of RNA sequences.

Chemical reagents and service providers: We selected Sangon Co., Ltd. (Shanghai, China) for the custom synthesis of siRNA. The company ensured the accuracy and purity of the siRNA sequences. All siRNA molecules were purified by HPLC and their sequence correctness was verified by mass spectrometry.

Sequence information:

Primer name	sequence (5′ to 3′)
g2e3-835-F	GCAACAAUCAGGACAACUUTT
g2e3-835-R	AAGUUGUCCUGAUUGUUGCTT
g2e3-1562-F	CCGUGAAGAUCUCUACUUUTT
g2e3-1562-R	AAAGUAGAGAUCUUCACGGTT
g2e3-1928-F	GCAGACGUUGGGUGUCUUUTT
g2e3-1928-R	AAAGACACCCAACGUCUGCTT

II. siRNA transfection

Transfection reagent: We chose riboFECTTM CP Transfection Kit (Ribobio, Guangzhou, China) as the transfection reagent. This reagent is known for its high efficiency, low toxicity and widely applicable cell types, and has been widely used in siRNA transfection experiments for a variety of cells.

Cell type: This study used the ovarian cell line (CO) and testicular cell line (CT) of *Cynoglossus semilaevis*.

Transfection concentration: Prior to dispensing the mixture into each well of a 12-well plate, we prepared a solution by diluting 3 μL of 20 μM siRNA with 60 μL of CP buffer, followed by the incorporation of 5 μL of CP reagent.

Transfection time: siRNA was mixed with transfection reagents and added into the cell culture medium, and continued to incubate for 48 hours to achieve the best transfection effect.

Evaluation of transfection efficiency: We evaluated the transfection efficiency of siRNA and the knockdown effect on *Cs-g2e3* genes by qPCR method. The results showed that the selected transfection conditions were able to effectively introduce siRNA into the target cells and significantly down-regulate the expression level of the *Cs-g2e3* genes, and the knockdown efficiencies all reached more than 70%.