

Figure S1: Cell localization of *ly75*, *thy1*, and *dmc1* in adult testis of orange-spotted grouper. (A–C) Antisense probe signals of *ly75*, *thy1*, and *dmc1*. (D–F) Sense probe signals of *ly75*, *thy1*, and *dmc1*. Sg, Spermatogonium; Sc, Spermatocyte; St, spermatid. Scale Bars: 20 μ m.

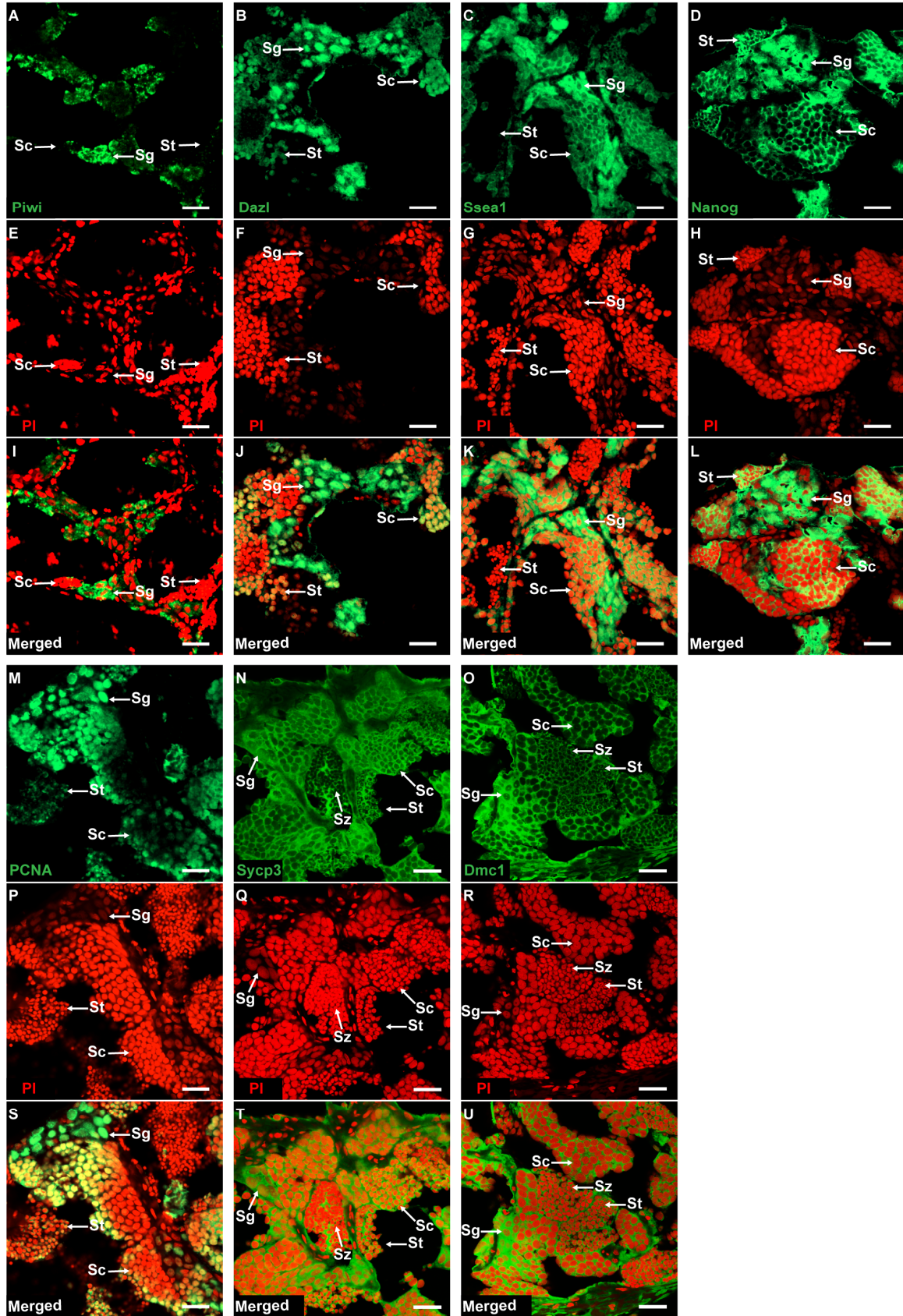


Figure S2: Fluorescent immunostaining of antibodies in adult testis of orange-spotted grouper. (A–D and M–O) Fluorescence signals of Piwi, Dazl, Ssea1, Nanog, PCNA, Sycp3, and Dmc1. (E–H and P–R) Nuclei are counterstained with PI. (I–L and S–U) Merge images. Sg, Spermatogonium; Sc,

Spermatocyte; St, spermatid; Sz, spermatozoa. Scale Bars: 20 μ m.

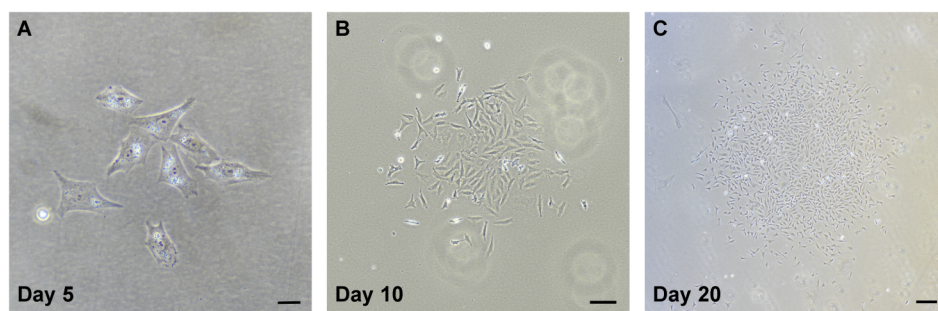


Figure S3: Derivation of a single colony from a single cell in GPT line. (A) A small colony after five days of culture. (B) A distinct colony after 10 days of culture. (C) A large colony containing hundreds of cells after 20 days of culture. Scale Bars: 20 μ m in A; 50 μ m in B; 200 μ m in C.

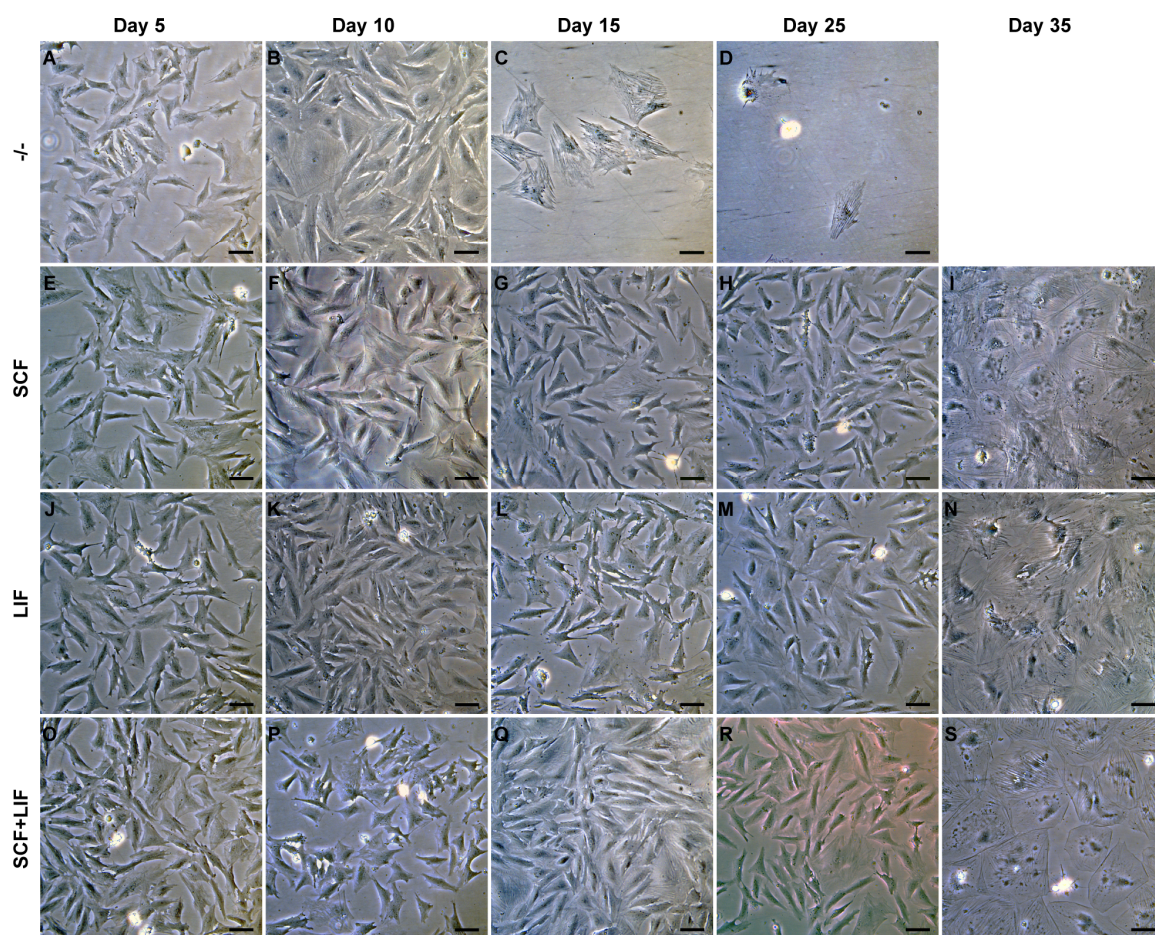


Figure S4: Prolonged cultivation of GPT cells under the lack of bFGF. (A–D) All GPT cells would differentiate into large epithelial-like cells and gradually die out during 25 days of culture under the lack of bFGF, LIF, and SCF (-/-). (E–H, J–M, and O–R) GPT cells consisted of some polygonal-like cells and many epithelial-like cells during 25 days of culture in the ESM media containing SCF and/or LIF. (I, N and S) All GPT cells transformed into very large epithelial-like cells and gradually died after 35 days of culture in the ESM media containing SCF and/or LIF. Scale Bars: 50 μ m.

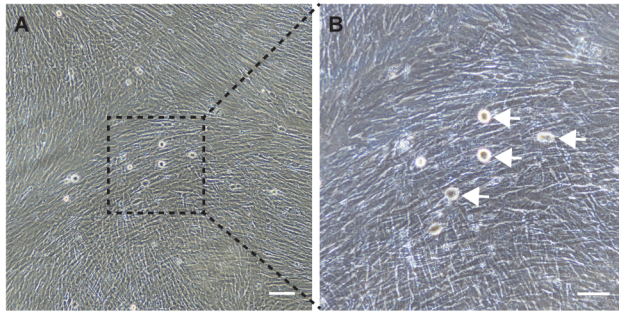


Figure S5: Morphology of GPT cells under a condition of high cell confluence. (A) GPT cells were cultured for 14 days without subculture and (B) generated a few spherical cells (Arrows). Scale Bars: 50 μm in A; 20 μm in B.

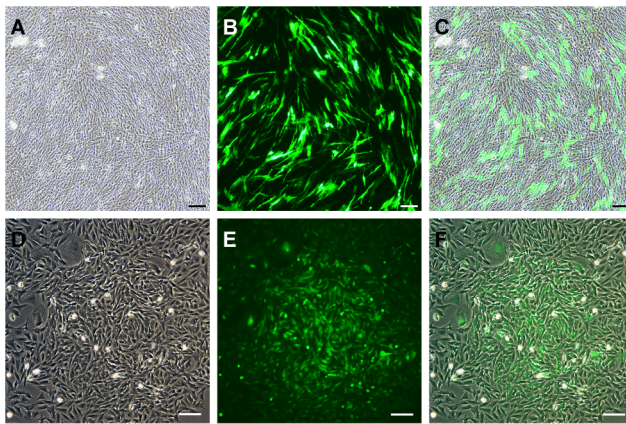


Figure S6: Establishment of a GPT cell line stably expressing green fluorescence protein. (A–C) Bright-field image, fluorescent image, and merged image of GPT cells after 48 hours of culture following electrotransfection with pEGFP-N3 plasmid. (D–F) Bright-field image, fluorescent image, and merged image of GPT cells after G418 resistance screening. Scale Bars: 200 μm in A–C; 100 μm in D–F.