

**Experimental Study of the Biological Properties of Human Embryonic Stem
Cell-Derived Retinal Progenitor Cells**

Jingzhi Shao, ¹Peng-Yi Zhou, ²Guang-Hua Peng^{a,3}

¹Department of Ophthalmology, The First Affiliated Hospital of Zhengzhou University, Zhengzhou, Henan 450000, China. shaojingzhi@gs.zzu.edu.cn

²Department of Ophthalmology, The First Affiliated Hospital of Zhengzhou University, Zhengzhou, Henan 450003, China. zhoupengyi@zzu.edu.cn

³Department of Ophthalmology, The First Affiliated Hospital of Zhengzhou University, Zhengzhou 450000, China; Department of Ophthalmology, General Hospital of Chinese People's Liberation Army, Beijing 100853, China. ghp@zzu.edu.cn

^aCorresponding author: Guang-Hua Peng, email: ghp@zzu.edu.cn

[Supplemental materials]

Table S1. The Primer Sequences of Specific Genes in Differentiated Cells Used for Real-Time PCR Analysis

Gene	Primer sequences (5'-3')	Product length (bp)
<i>Pax6</i>	CCGTGGCTCGGCCTCATTTC(F) ACCGCTCCTCACTGGCCCATT(A)(R)	107
<i>Sox2</i>	CGCCCCCAGCAGACTTCA CA(F) CTCCTCTTTTGCACCCCTCCCATTT(R)	170
<i>Rax</i>	AACCGCACGACTTTCACCACG(F) TGCAGCTTCATGGAGGACACTTC(R)	200
<i>Nestin</i>	GAATCACTGAAGTCTGCGGGAC(F) TCCAGGAGTCTGAATGTCTCTTGG(R)	176
<i>Otx2</i>	ACCCGGTAGTGTGTCCCGCT(F) TCGCCGCTCTCTTCCAGGGT(R)	208
<i>Crx</i>	CCTTCTGACAGCTCGGTGTT(F) TGGTGTACTTCAGCGGTCAC(R)	149
<i>Recoverin</i>	GGAAAAGCGAGCCGAGAAGA(F) CCTGGGGTGGATGTGTGTGT(R)	282
<i>GAPDH</i>	AGCCTCCCGCTTCGCTCTCT(F) CCAGGCGCCCAATACGACCA(R)	141

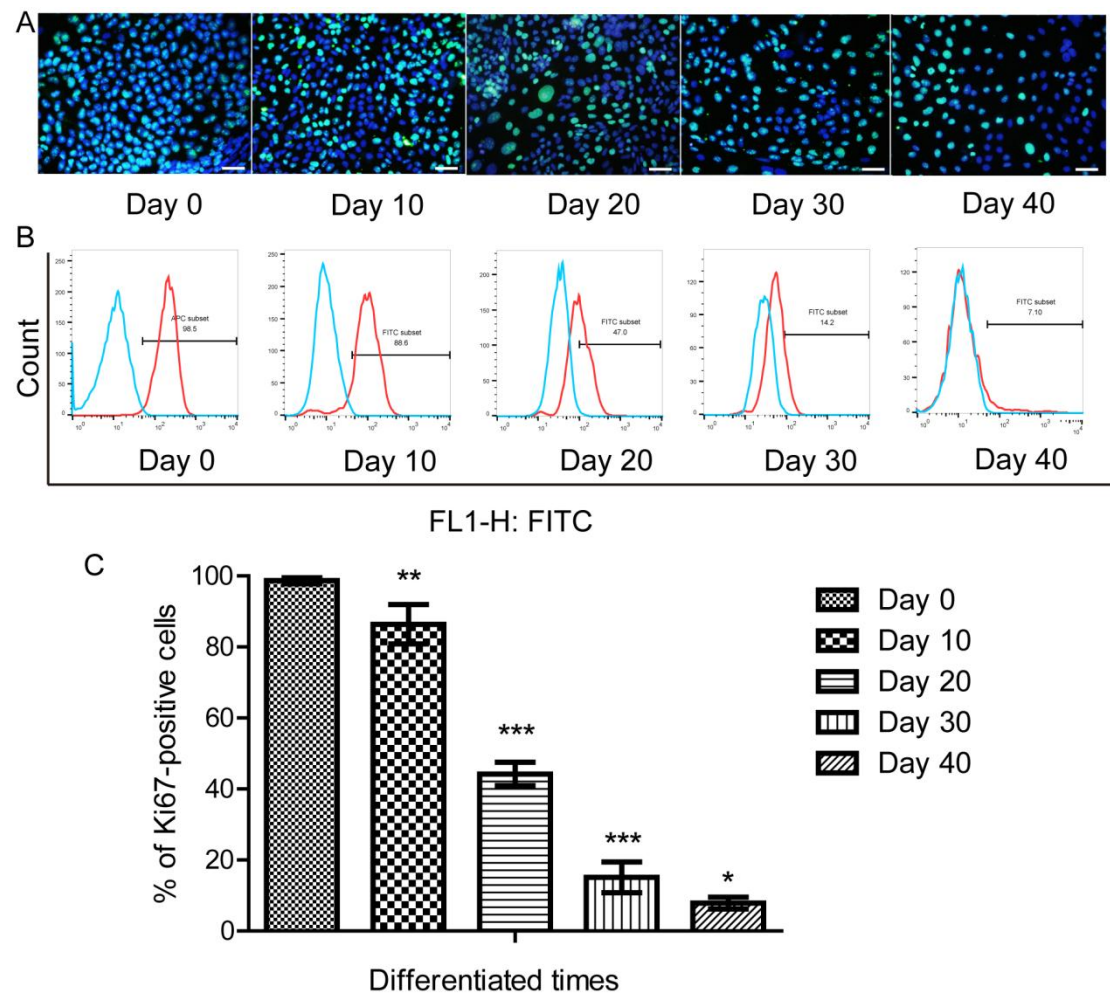


Figure. S1 The expression of protein Ki67 on day 0, 10, 20, 30 and 40 of differentiation. (A) The expression of protein Ki67 decreased over time evaluated by immunofluorescence. (B) Staining of Ki67 was evaluated by using flow cytometry. Blue line, isotype. (C) Statistical analysis of expression of Ki67. Cell nuclei are shown in 6-diamidino-2-phenylindole (DAPI), blue. Data from at least three independent experiments are represented as the mean $\pm$ SD. Scale bar = 100 μ m. * P <0.05, ** P <0.01, *** P <0.001, versus with data of previous time point.

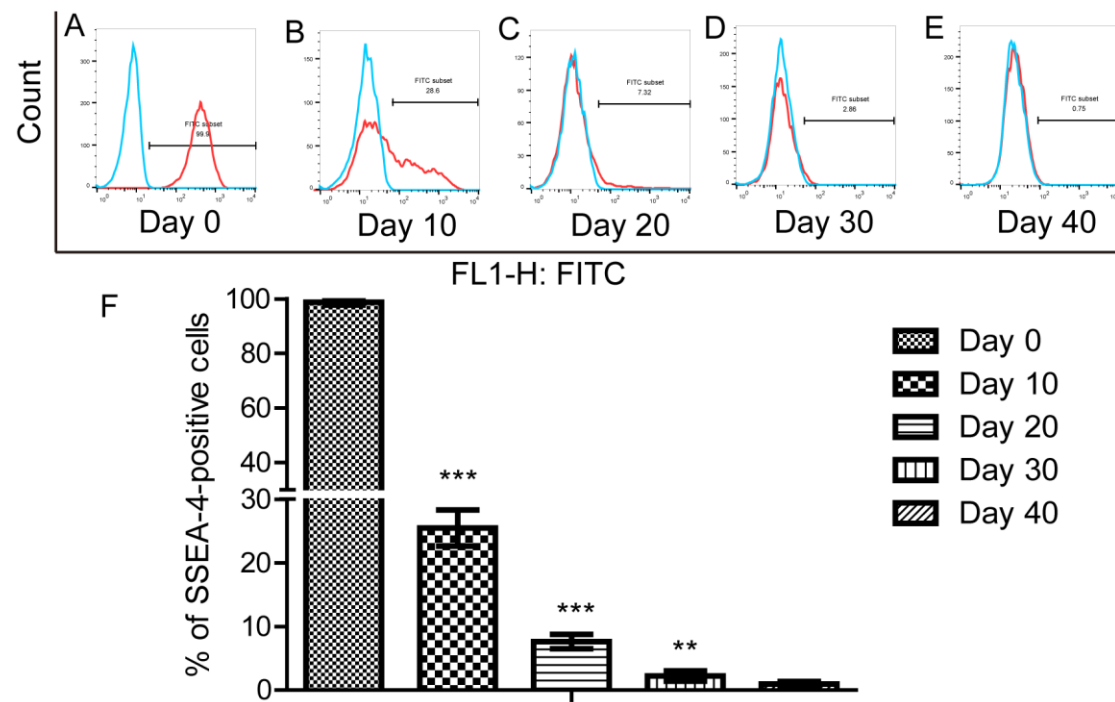


Figure. S2 The expression of protein SSEA-4 at day 0, 10, 20, 30 and 40 of differentiation. (A) The expression of protein SSEA-4 decreased over time evaluated by flow cytometry. (B) Statistical analysis of expression of SSEA-4. Data from at least three independent experiments are represented as the mean $\pm$ SD. ** $P < 0.01$, *** $P < 0.001$, versus with data of previous time point.

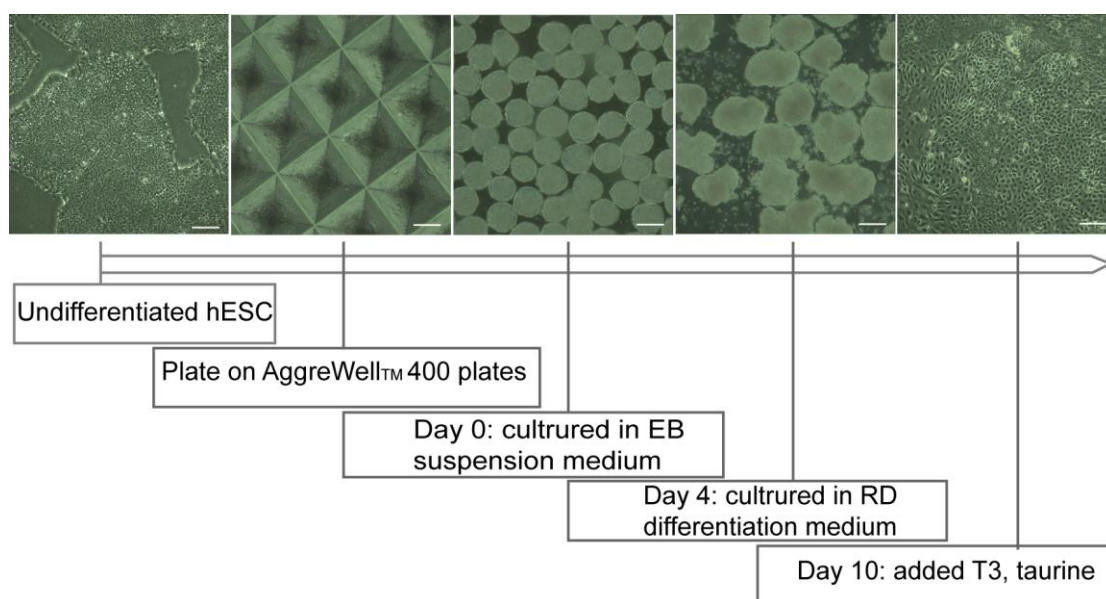


Figure. S3 Flow chart of hESC differentiation.

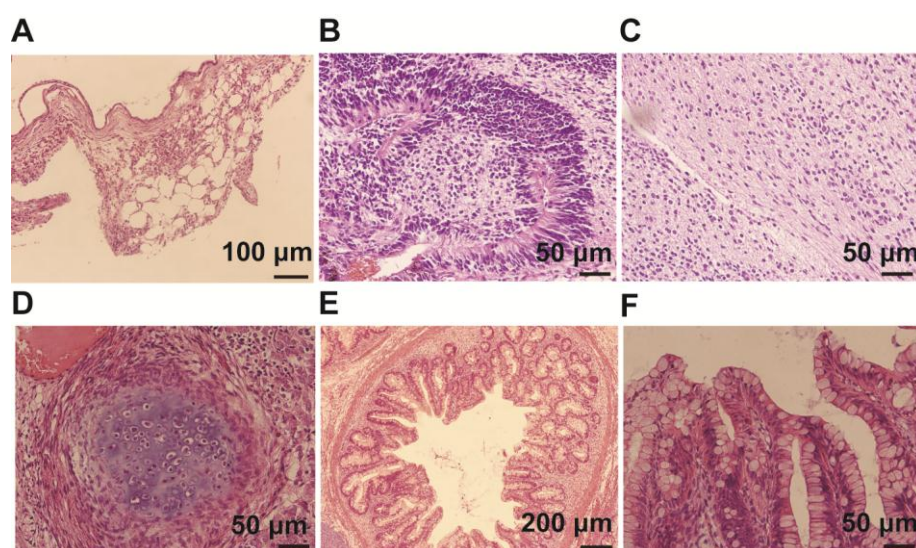


Figure. S4 Hematoxylin-eosin assay of teratoma. (A) Epithelium that was defined as ectoderm. (B and C) Neural tissues which were defined as ectoderm. (D) Cartilage, fibrous tissues and erythrocyte which were defined as mesoderm. (E) Circular muscle and gut-like epithelium with mucous-containing cells which were defined as endoderm. (F) Gut-like epithelium, which was defined as endoderm.