

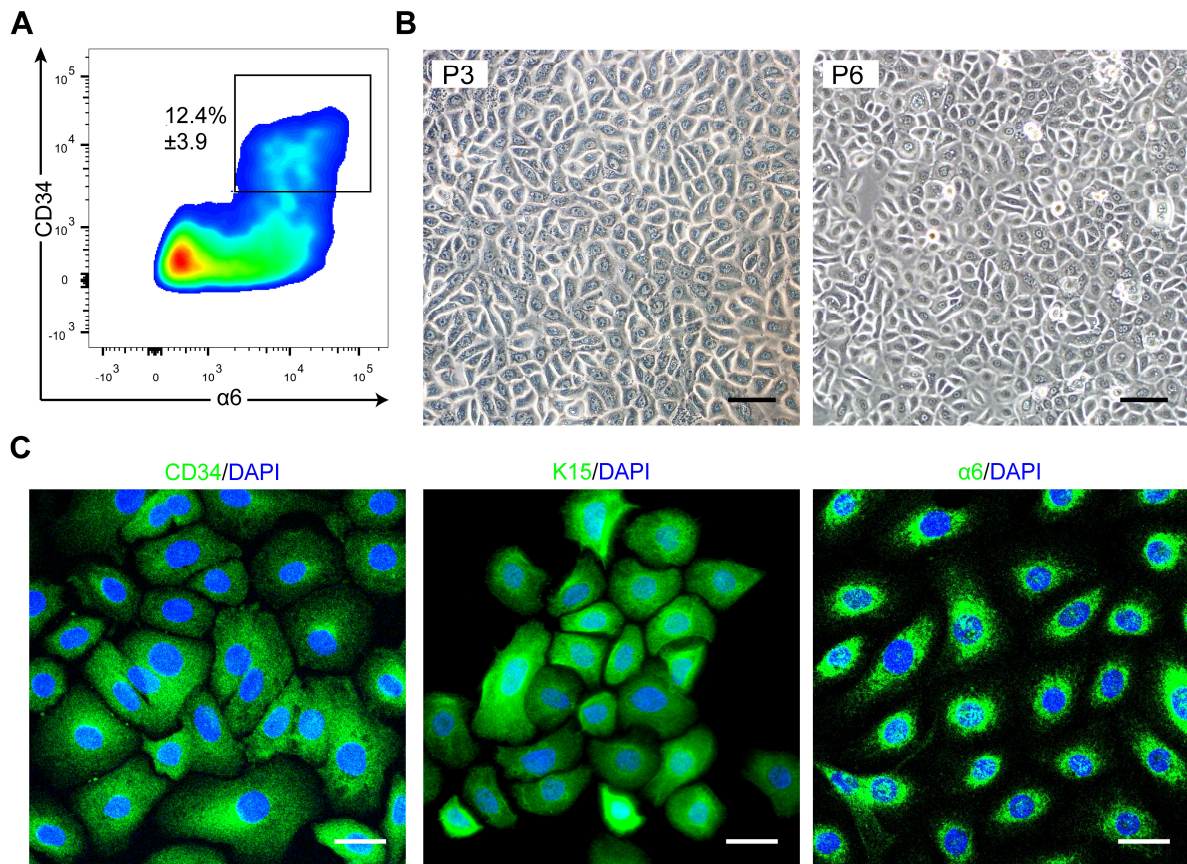
## Supplementary Material

### I. Supplementary Tables

**Supplementary Table S1.** Primers used for quantitative real-time polymerase chain reaction

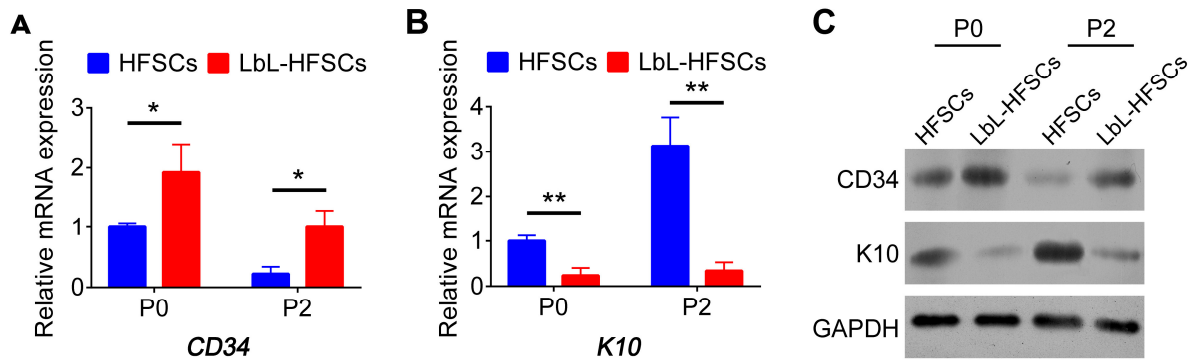
Gene name	Sequences (5' to 3')	
	Forward primer	Reverse primer
<i>CD34</i>	GAGCCACCAGAGCTATTCCC	CCAAGATGGCCAGCAGAACT
<i>K10</i>	AACTGACAATGCCAACGTGC	TAGGTAGGCCAGCTCTTCGT
<i>Lgr5</i>	CCAGTGTTGTGCATTTGGGG	CTAGCAAGGGGATTGTGGCA
<i>Tmeff1</i>	CAGTATCGGCCAG- ATGTGAAAGAT	TGCCTCCGCCCTCTATTGTT
<i>ID2</i>	ATCCCCCAGAACAAGAAGGT	TGTCCAGGTCTCTGGTGATG
<i>ID3</i>	GCATGGATGAGCTTCGATCT	ACCAGCGTGTGCTAGCTCTT

## II. Supplementary Figures and Legends

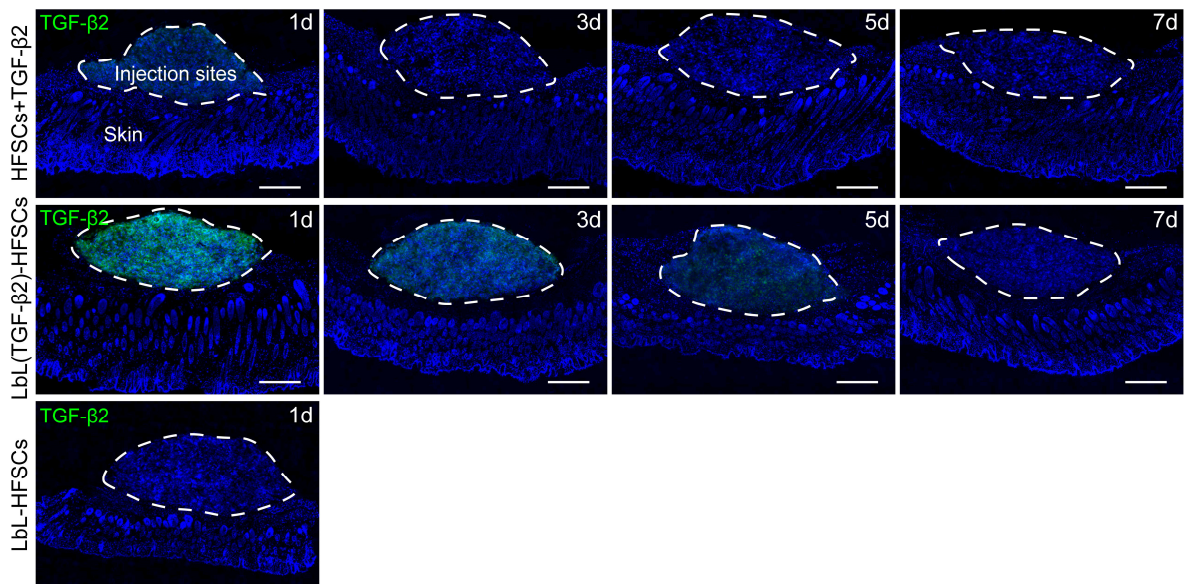


**Figure S1. Purification, culturing, and identification of hair follicle stem cells (HFSCs).**

(A) FACS of freshly isolated vibrissae HFSCs demonstrating the ratio of  $CD34^+ \alpha 6^+$  HFSCs being  $12.4 \pm 3.9\%$  ( $\pm$  SD). (B) FACS-purified HFSCs were cultured using layer-by-layer (LbL) coating and examined by microscopy at passage 3 (p3) and passage 6 (p6). Scale bars: 100  $\mu$ m. (C) LbL-coated HFSCs cultured for 7 days at P1 were immunofluorescent stained for stem cell markers CD34, cytokeratin 15 (K15), and integrin  $\alpha 6$  ( $\alpha 6$ ). All three proteins were positively expressed. CD34, K15, and  $\alpha 6$  (green); DAPI (blue); Scale bars: 20  $\mu$ m.



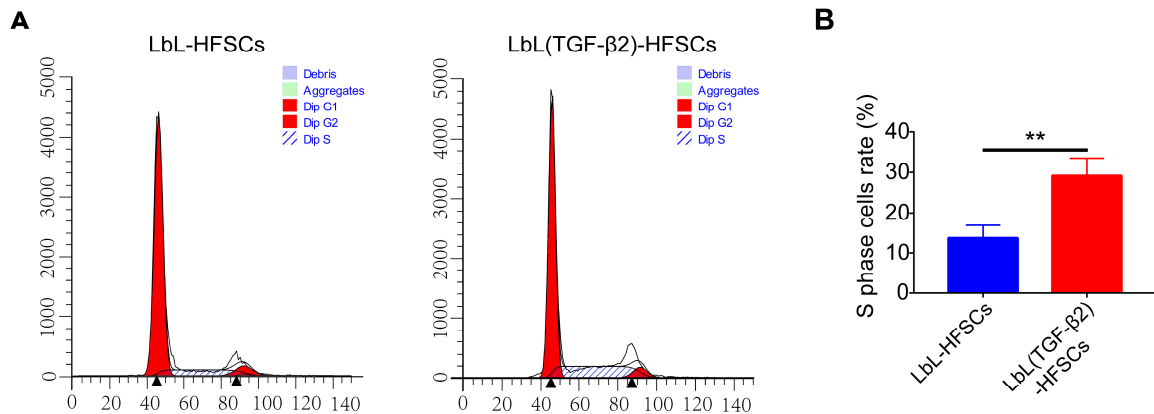
**Figure S2. Stem cell properties of layer-by-layer (LbL)-coated hair follicle stem cells (HFSCs).** (A–B) Quantitative reverse transcription-polymerase chain reaction (qRT-PCR) analysis of *CD34* and *K10* mRNA expression. The results show that in P0 or P2, the expression of *CD34* in LbL-HFSCs was significantly higher than HFSCs, while the expression of *K10* was significantly lower. (B) Western blot analysis of CD34 and K10 protein expression. LbL-HFSCs exhibited higher expression of CD34 and lower expression of K10 in P0 or P2. \* $p < 0.05$ ; \*\* $p < 0.01$ .



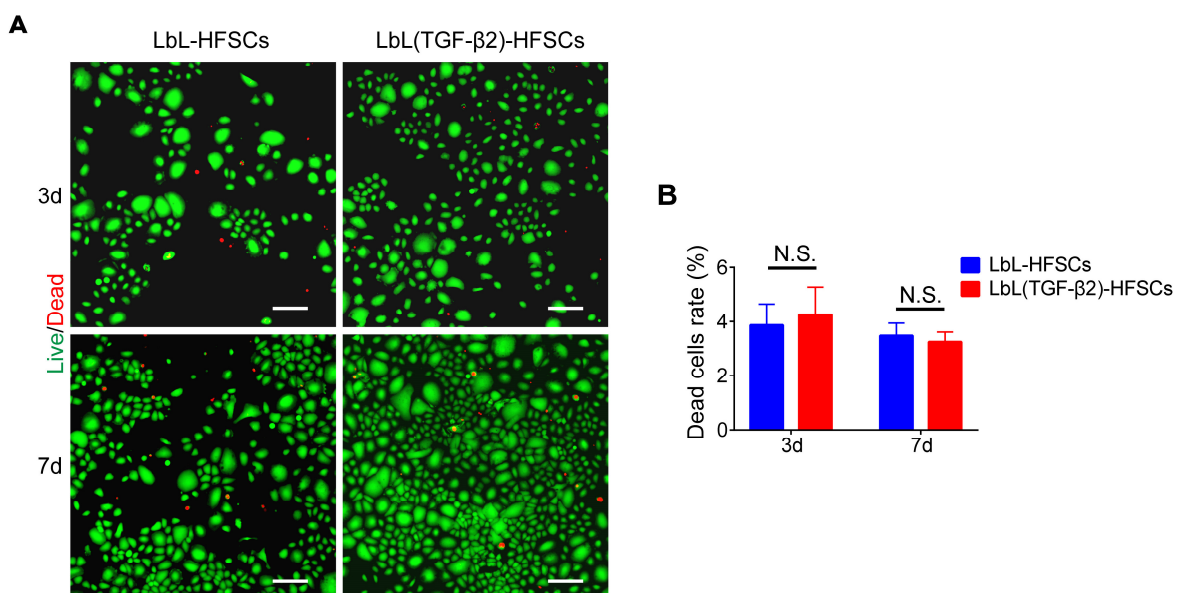
**Figure S3. Layer-by-layer (LbL) coating maintained transforming growth factor (TGF)-β2 *in vivo*.** *In vivo* skin sections around the injection sites showed LbL coating maintained TGF-β2 for about 7 days, while in HFSCs + TGF-β2 a weak fluorescence

intensity was detected only on the first day. Additionally, LbL-HFSCs did not exhibit green fluorescence, indicating that the fluorescence was an accurate readout of TGF- $\beta$ 2 activity.

TGF- $\beta$ 2 (green); Scale bars: 500  $\mu$ m.



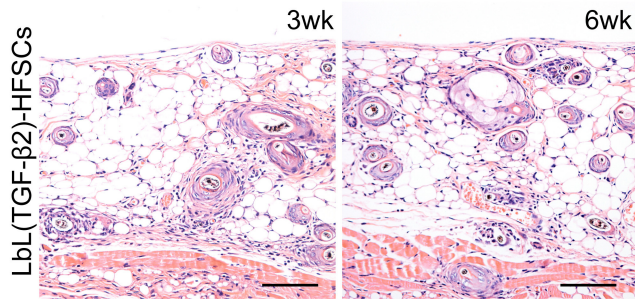
**Figure S4. Cell cycle analysis of cell proliferation.** (A) A cell cycle kit was used to detect the cell proliferation of layer-by-layer hair follicle stem cells (LbL-HFSCs) and LbL(TGF- $\beta$ 2)-HFSCs cell proliferation after 7 days of culturing. (B) The proportion of S-phase cells for LbL(TGF- $\beta$ 2)-HFSCs was significantly higher than that for LbL-HFSCs. \*\* $p < 0.01$ .



**Figure S5. Live/death staining analysis of cell viability.** (A) Live/death staining was performed to examine the viability of transforming growth factor (TGF)- $\beta$ 2 unloaded and



loaded layer-by-layer hair follicle stem cells (LbL-HFSCs) after 7 days of culturing. Live (green); Dead (red); Scale bars: 100  $\mu\text{m}$ . (B) There was no significant difference in the proportion of dead cells in the LbL-HFSCs and LbL(TGF- $\beta$ 2)-HFSCs. NS, not significant.



**Figure S6. H&E staining to detect the safety of prolonging release of transforming growth factor (TGF)- $\beta$ 2 *in vivo*.** After 3 wk or 6 wk of LbL (TGF- $\beta$ 2) -HFSCs transplantation *in vivo*, no tumorigenesis or fibrosis was observed with H&E staining.