Supplementary Information

Tissue engineering of human hair follicles using a biomimetic developmental approach

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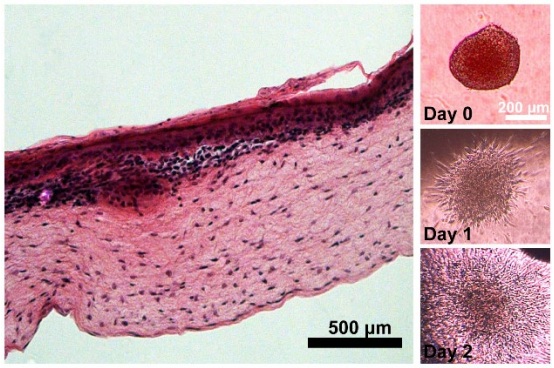
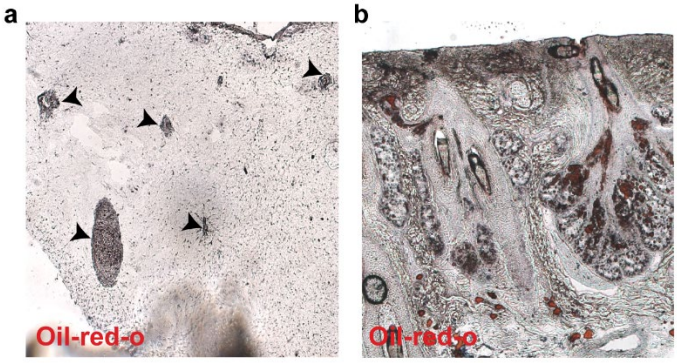
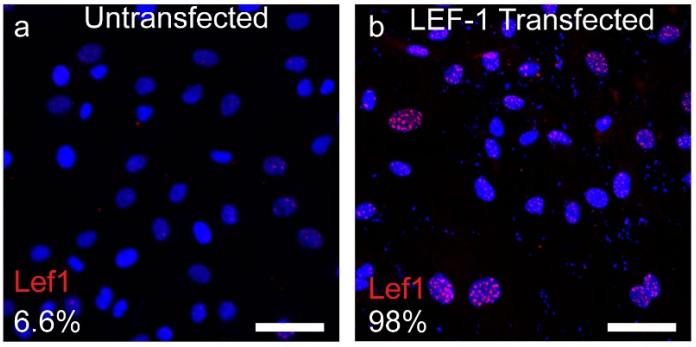


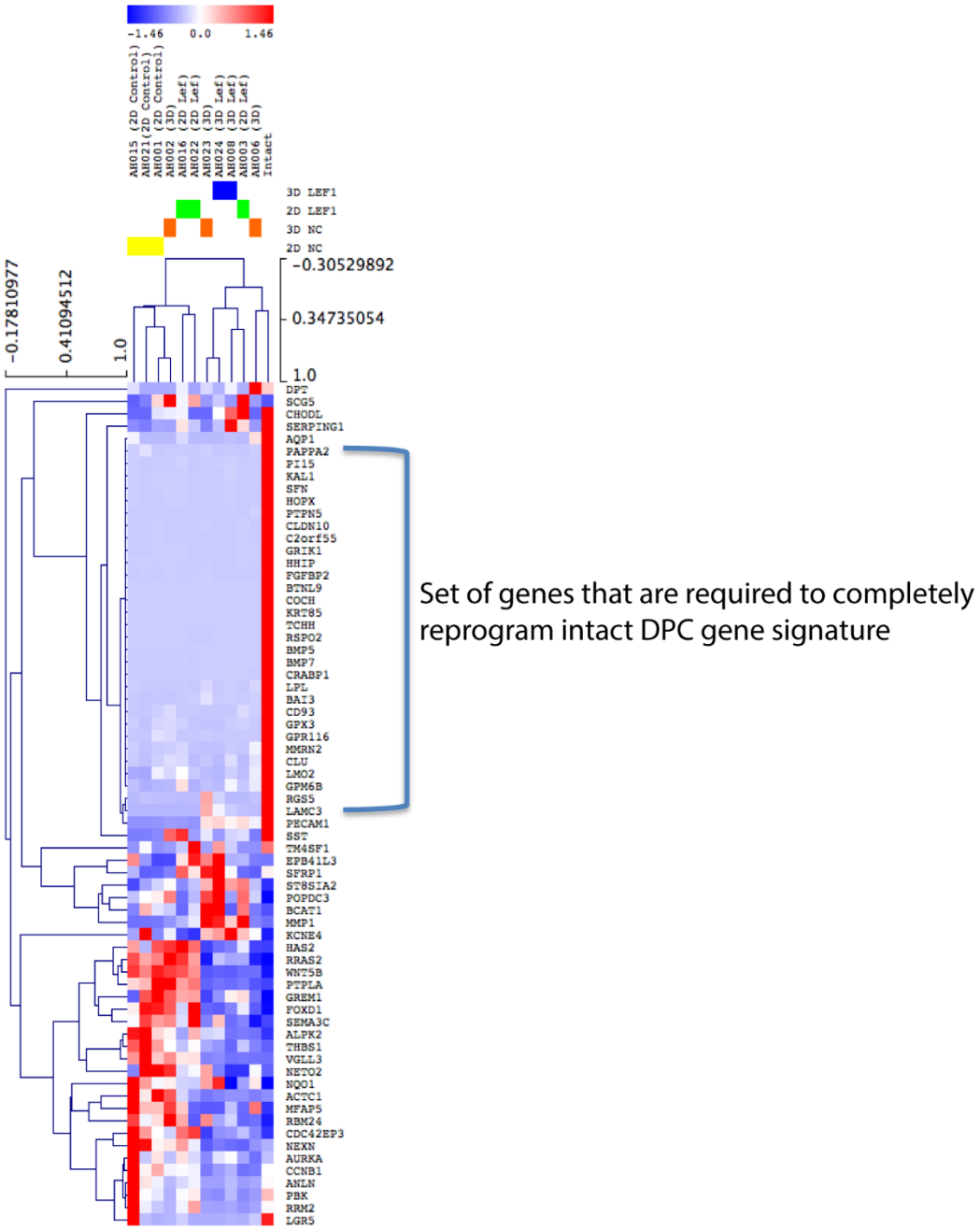
Supplementary Figure 1. Regression of DPC spheroids in HSCs. DPC spheroids were produced by the hanging drop method and embedded in HSCs where they gradually regressed and dispersed into the dermal compartment in several days. Scale bars: 500 µm (left panel) 200 µm (right panel)



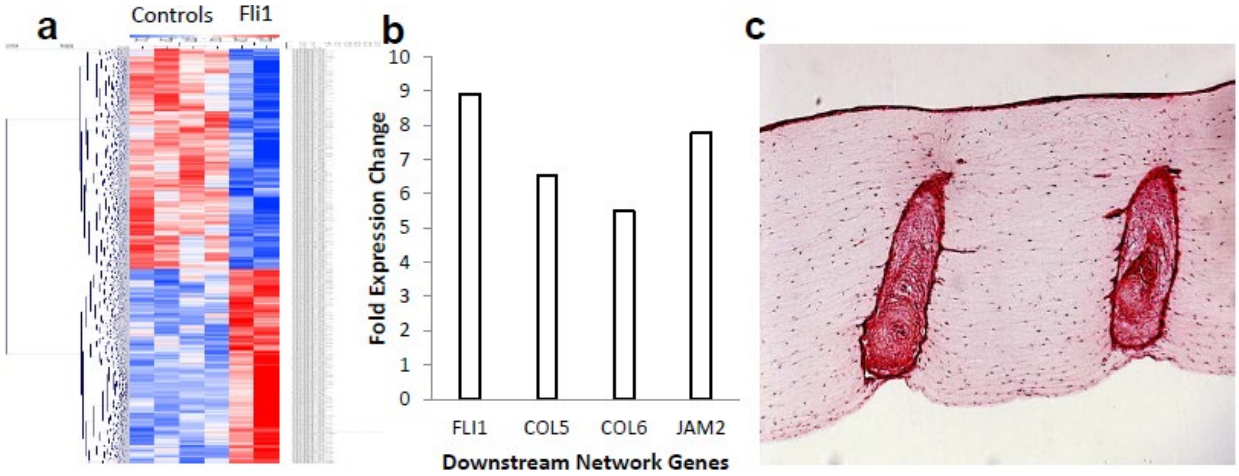
Supplementary Figure 2. Sebocyte differentiation is not induced in HSCs. Histological sections of HSCs (arrowheads indicate HF) **(a)** and human scalp tissue **(b)** stained with Oil-red-O.



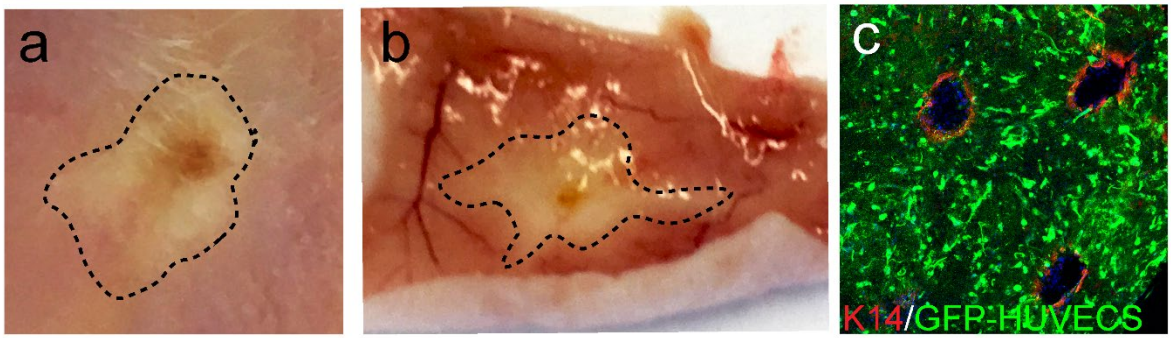
Supplementary Figure 3. Transfection efficiency of Lef-1. Untransfected **(a)** and Lef-1 transfected **(b)** DPCs in 2D culture stained with Lef-1 antibody (red) and DAPI (blue). Scale bars: 10 µm



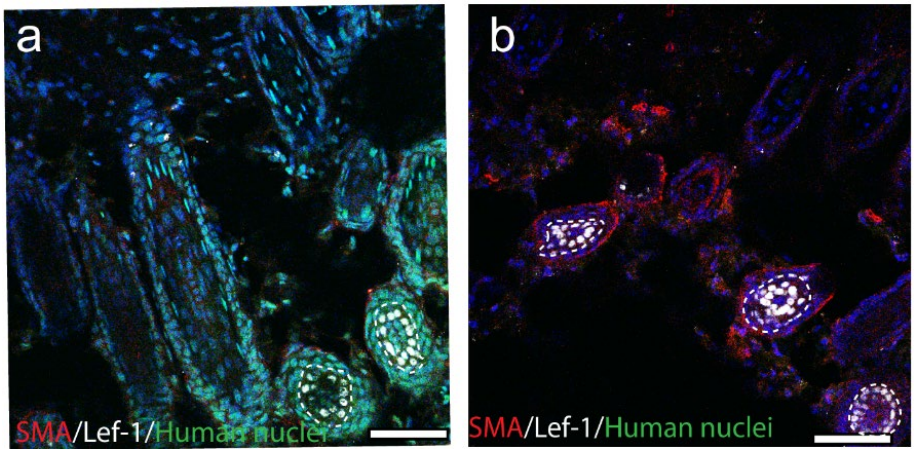
Supplementary Figure 4. Heatmap and hierarchical clustering analyses of RNA-sequencing data of DPCs cultured in different conditions. 3D spheroid culture and Lef-1 overexpression both significantly restored intact DPC gene signature.



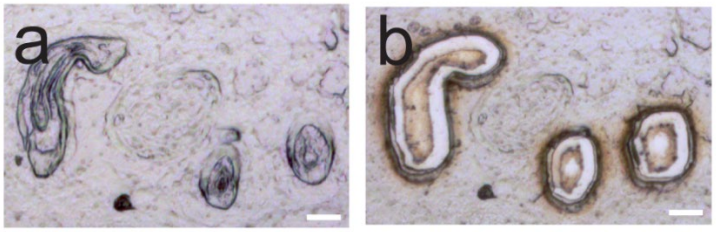
Supplementary Figure 5. Reprogramming of DPCs by Fli-1 overexpression. (a) Heat map of DPCs illustrating gene expression profiles of cultured DPCs (passage 3) from three different donors, empty-vector transfected control, and Fli1 overexpressed DPCs from 2 donors. **(b)** RT-PCR of Fli-1 overexpressed cells showed upregulation of Fli-1 downstream network genes as predicted previously by ARACNE algorithm. **(c)** H&E staining of HSCs containing DPCs overexpressing Fli-1.



Supplementary Figure 6. Necrosis in unvascularized hair-follicle-bearing grafts. (a) Top view of the grafted HSCs showing necrotic tissue at the center of the grafts. **(b)** Bottom view of the explanted HSCs (dashed circle) after 4 weeks showing the absence of blood vessels. **(c)** Encapsulated GFP-HUVECs are in close proximity to the K14-labeled hair follicles in HSCs.



Supplementary Figure 7. Dermal sheath cells are not present in engineered HFs. SMAα expression is absent in the engineered human HFs **(a)**, whereas mouse hair follicles express SMAα around the Lef-1-positive DPCs **(b)** (dashed circle indicates the DP compartment). Scale bars are 100 μm



Supplementary Figure 8. Laser-capture of engineered hair follicles. Hair follicles in histological sections **(a)** were microdissected using laser capture **(b)**.

Supplementary Table 1.

Primer	Forward Sequence	Reverse Sequence
K16	GACCGGCGGAGATGTGAAC	CTGCTCGTACTGGTCACGC
K17	GGTGGGTGGTGAGATCAATGT	CGGCATCCTTGCGGTTCTT
K25	ATGTCTCTTCGACTTTCCAGTGC	GCCACTTCCAATCCCTGAAAT
K75	CCCAGGTCGGTGACACATC	GCAATGTCCTCGTATTGTGCTTT
K71	GCTGCTTACGCCAATAAGGTG	ATCTGAGTGATCTCGGCTTCA
Wnt10a	GGAGACTCGCAACAAGATCCC	CGATGGCGTAGGCAAAAGC
Wnt10b	CATCCAGGCACGAATGCGA	CGGTTGTGGGTATCAATGAAGA
Lef-1	AGAACACCCCGATGACGGA	GGCATCATTATGTACCCGGAAT
Human specific primer 1	TATTGCAGCCCTAGCAGCACTCCA	AGAATGAGGAGGTCTGCGGC
Mouse specific primer 1	GCACTGAAAATGCTTAGATGGATAATTG	CCTCTCATAAACGGATGTCTAG
Human specific primer 2	TAGACATCGTACTACACGACACG	TCCAGGTTTATGGAGGGTTC
Mouse specific primer 2	ATTACAGCCGTACGCTCCTAT	CCCAAAGAATCAGAACAGATGC

Supplementary Table 2

Antibody	Company	Dilution	Catalog Number
Keratin 14	Covance	1:800	905301
Keratin 5	Covance	1:800	PRB-160P
Keratin 71	Covance	1:100	NBP2-14176
Keratin 75	Covance	1:100	NBP1-87845
AE13	Santa Cruz	1:100	sc-57012
AE15	Santa Cruz	1:100	sc-80607
Smooth muscle actin	Abcam	1:100	ab5694
CD133	Abcam	1:100	ab19898
Versican	Fisher Sci.	1:200	PA1-1748A
Ki67	Abcam	1:800	ab15580
Human nucleus	Millipore	1:100	MAB1281B
Lef-1	Santa Cruz	1:100	sc-8591