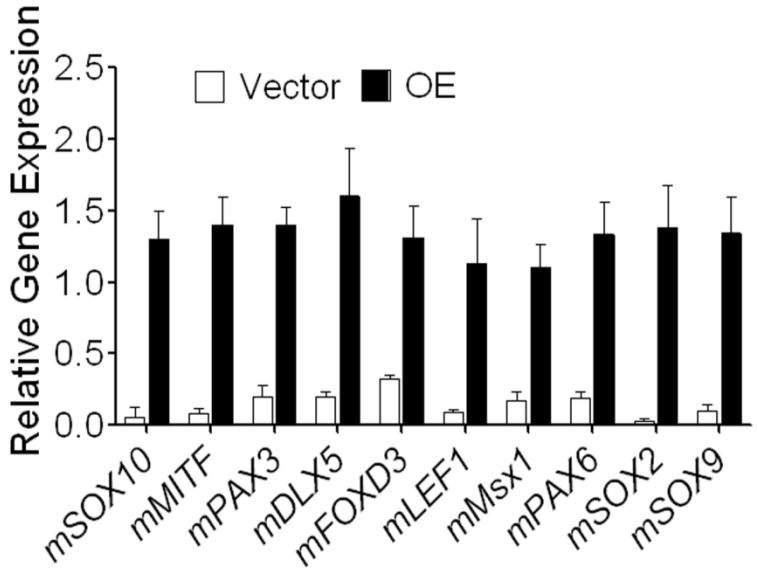
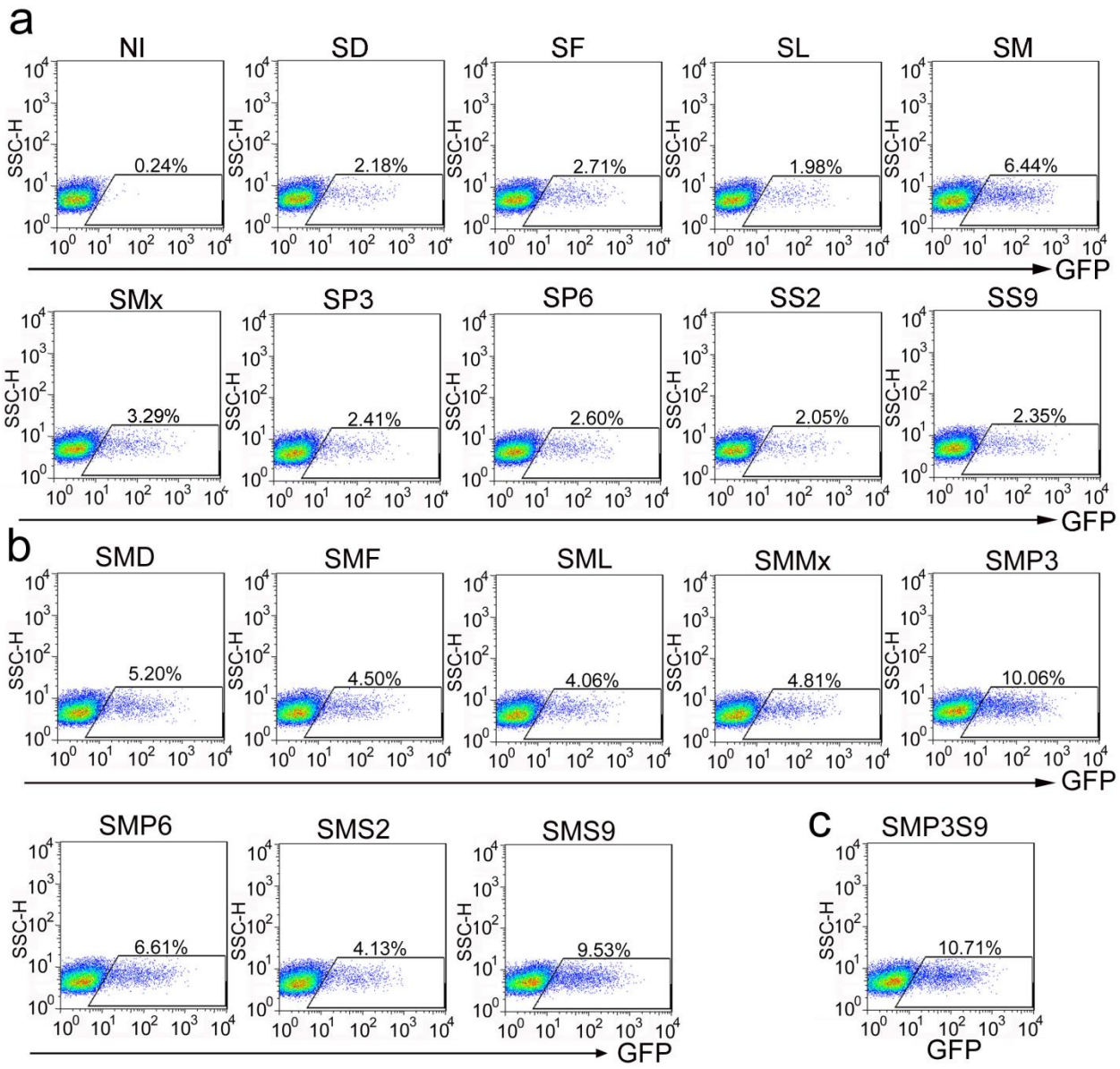
Supplementary Information

Supplementary Figures

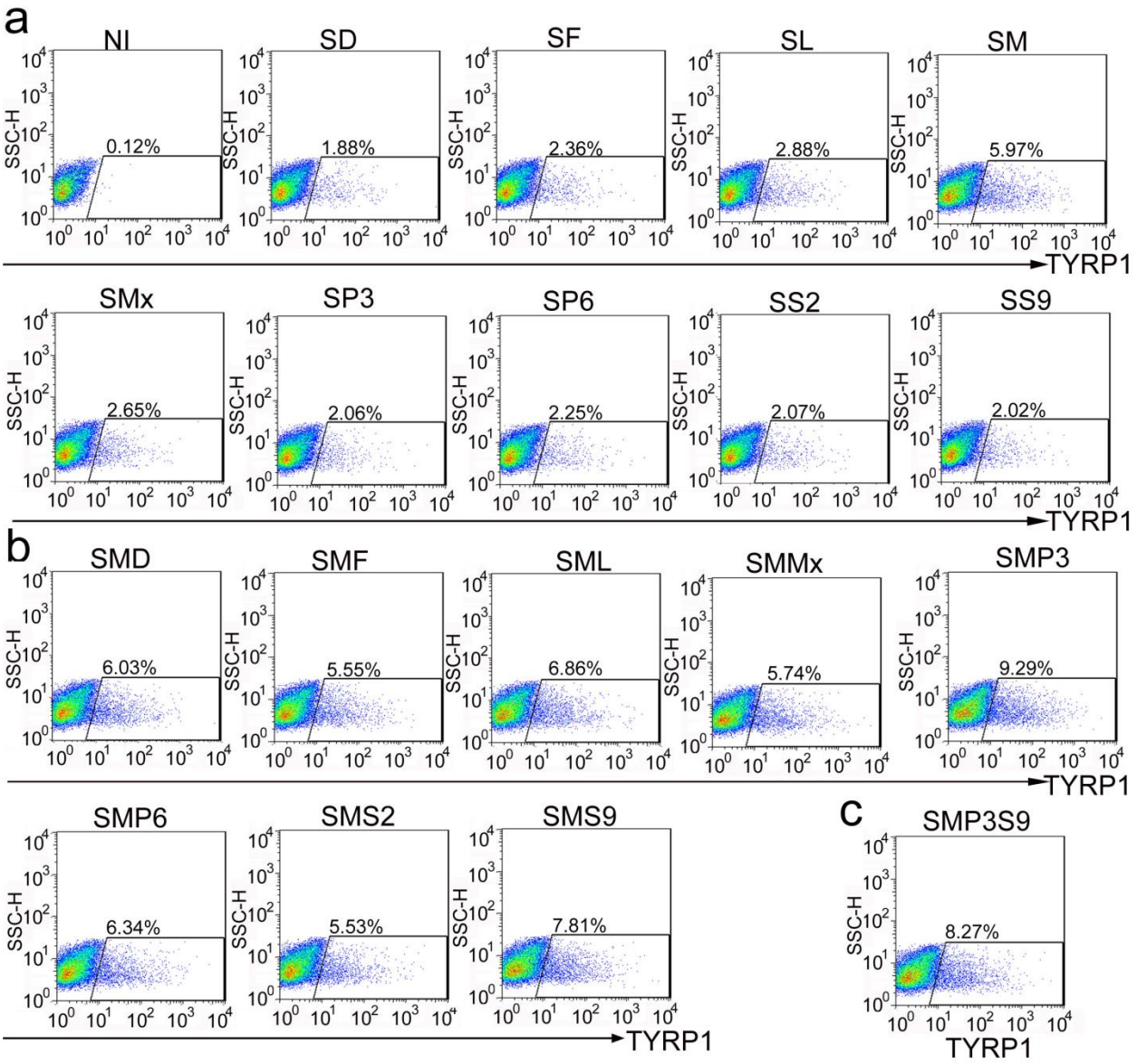


Supplementary Figure 1. qRT-PCR analysis of the expression of candidate factors including *SOX2*, *MITF*, *PAX3*, *DLX5*, *FOXD3*, *LEF1*, *MSX1*, *PAX6*, *SOX2* and *SOX9* in fibroblasts after infection. *mSOX2*, *mMITF*, *mPAX3*, *mDLX5*, *mFOXD3*, *mLEF1*, *mMSX1*, *mPAX6*, *mSOX2* and *mSOX9* mean that these genes are mouse origin. OE represents fibroblasts infected with candidate factors; vector represents fibroblasts infected with empty vectors. Data shown are mean ± SD of the expression from three independent experiments.



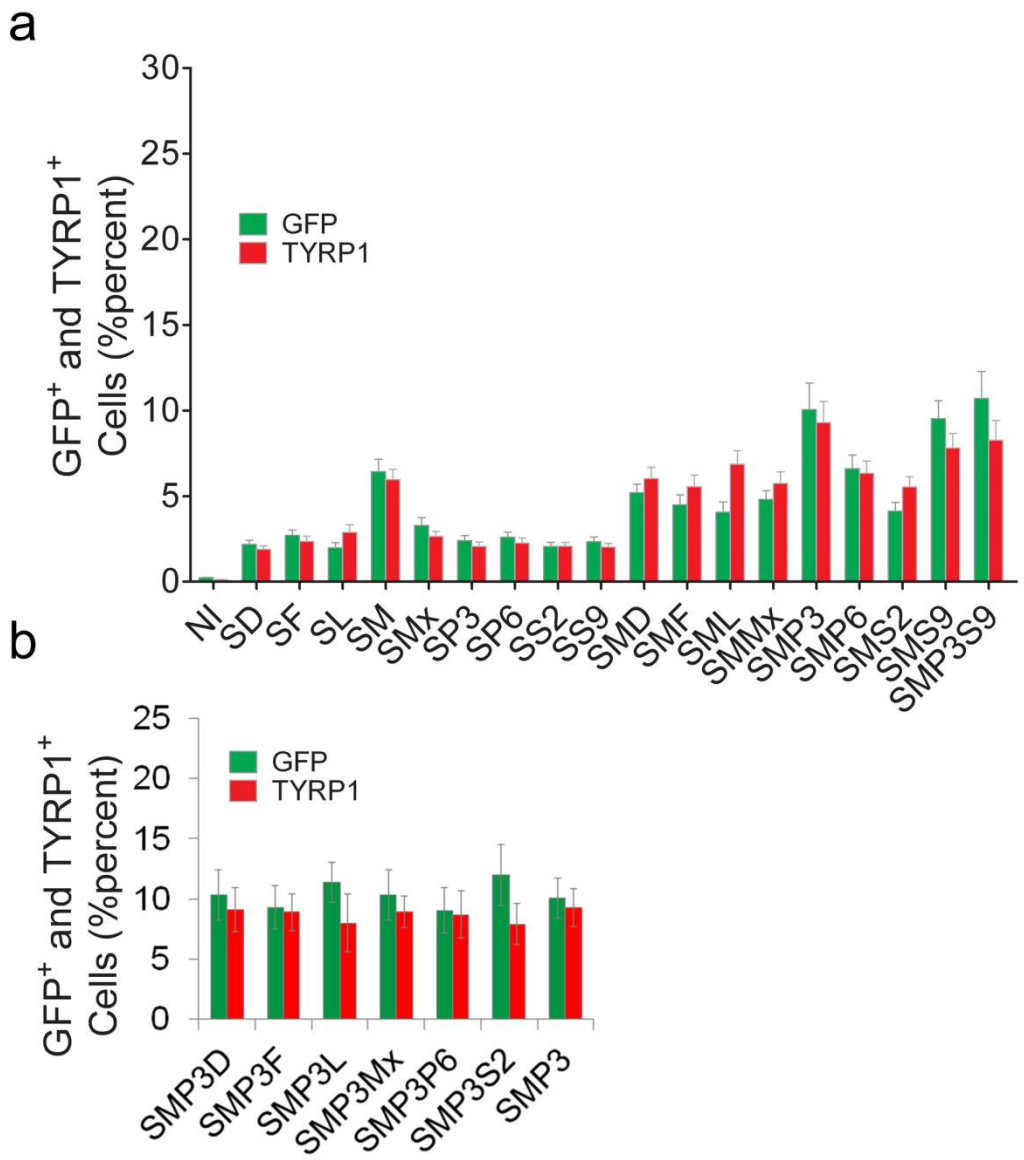
Supplementary Figure 2. Flow cytometric analysis of the percentage of GFP positive (GFP^+) cells after infection.

TTFs derived from Tyrosinase-CreER/Gt(ROSA)26Sor$^{tm4(ACTB-tdTomato,-EGFP)Luo/J}$ mice were infected with viruses containing different combinations of candidate factors. Flow cytometric analysis was performed 5 days after infection. GFP^+ cells were analyzed when the TTFs were infected with viruses carrying 2 different candidate factors (a); 3 different candidate factors (b) or 4 different candidate factors (c). NI: vector only; S: SOX10; D: DLX5; F: FOXD3; L: LEF1; M: MITF; Mx: MSX1; P3:PAX3; P6: PAX6; S2:SOX2; S9: SOX9.

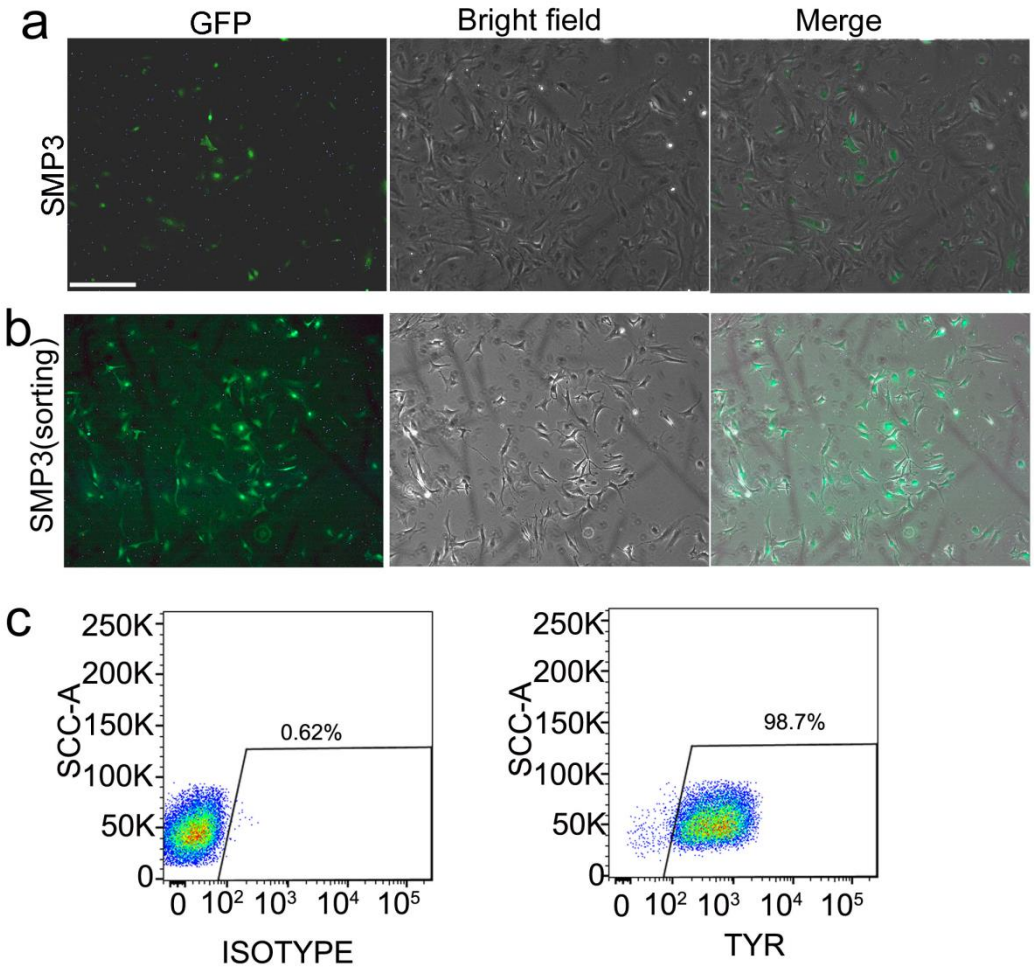


Supplementary Figure 3. Flow cytometric analysis of the percentage of TYRP1 positive ($TYRP1^{+}$) cells after infection.

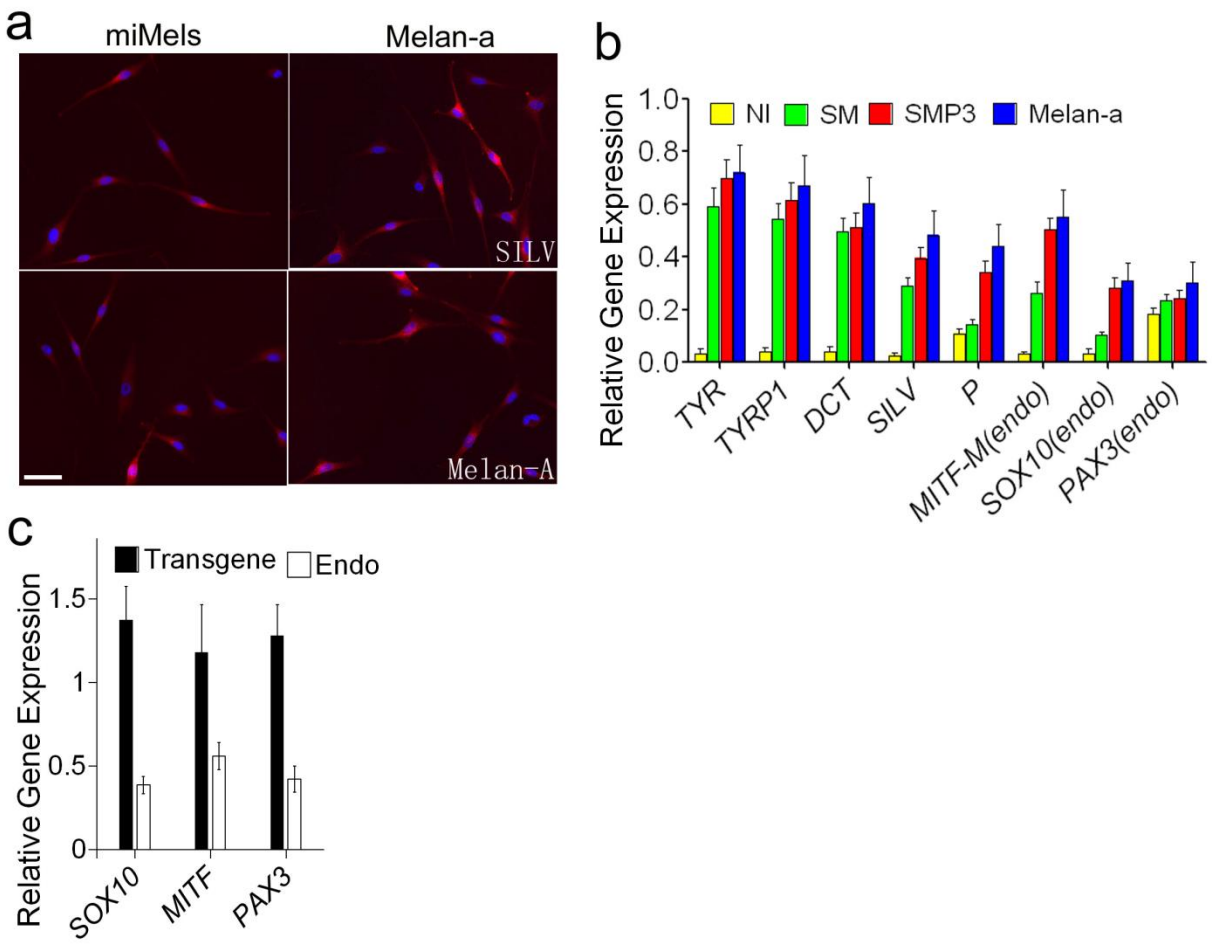
TTFs derived from Tyrosinase-CreER/Gt(ROSA)26Sor$^{tm4(ACTB-tdTomato,-EGFP)Luo/J}$ mice were infected with viruses carrying different combinations of the candidate factors. Flow cytometric analysis was performed 5 days after infection. $TYRP1^{+}$ cell population were analyzed when TTFs were infected with virus containing 2 different candidate factors(a) ; 3 different candidate factors (b) or 4 different candidate factors (c).



Supplementary Figure 4. a. Quantification of GFP^+ and $TYRP1^+$ cells by flow cytometric analysis as shown in Supplementary Figure 2 and 3. Data shown are mean ± SD from three independent experiments. b. Quantification of GFP^+ and $TYRP1^+$ cells by flow cytometric analysis of SMP3 combination grouped with the fourth factor. Data shown are mean ± SD from three independent experiments.

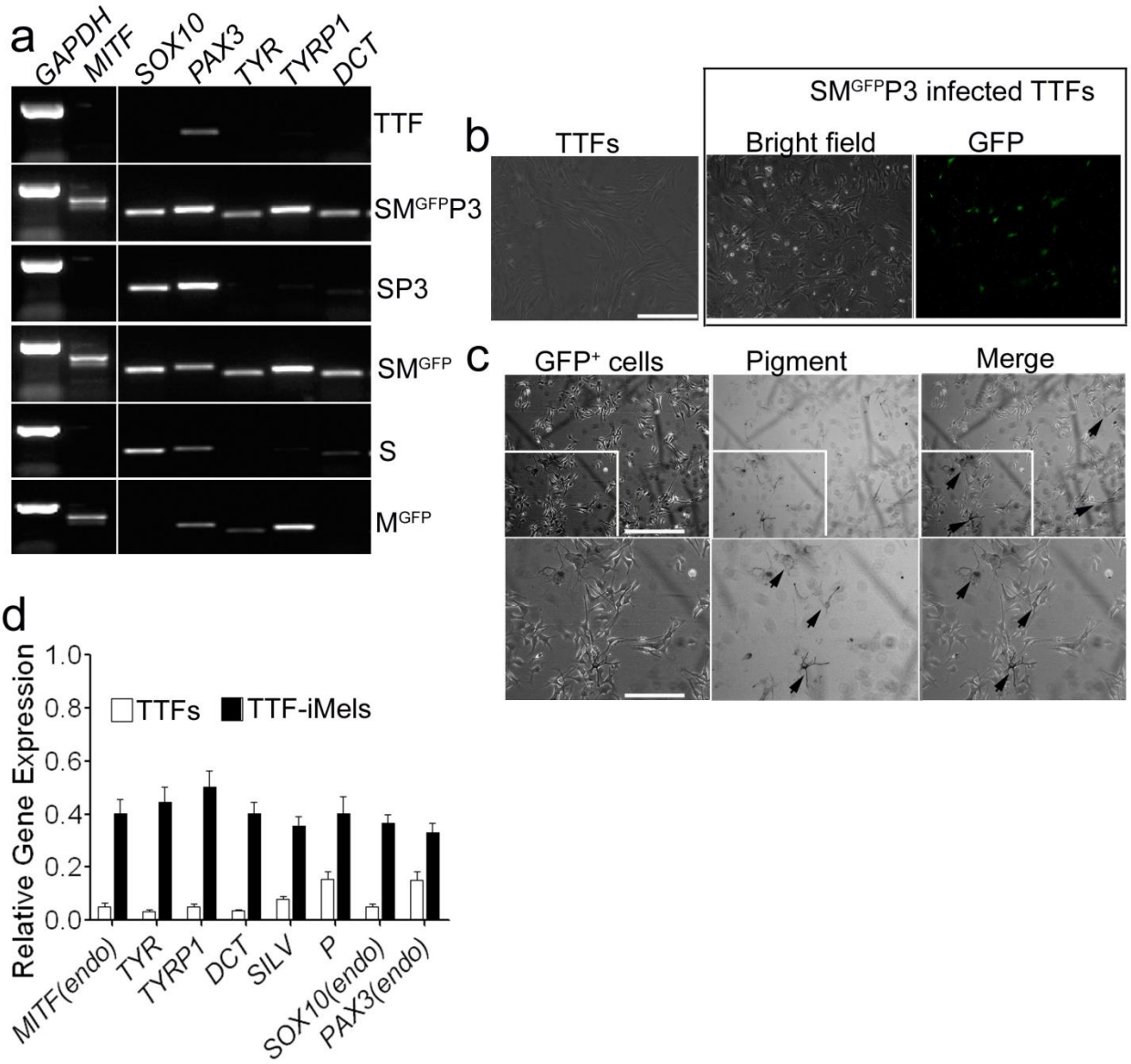


Supplementary Figure 5. Detection of GFP$^+$ cells after directed reprogrammed by SMP3. a and b. Morphology of GFP$^+$ cells after direct reprogrammed by SMP3. Representative images of SMP3-infected TTFs derived from Tyrosinase-CreER/Gt(ROSA)$^{26Sortm4(ACTB-tdTomato,-EGFP)Luo/J}$ mice . GFP$^+$ cells were detected among the SMP3 infected cells and photographed at Day 14 (a). GFP$^+$ cells were purified after FACS sorting and these cells showed typical melanocytic morphology (b). Scale bar, 50 μm. c. Flow cytometry analysis of the percentage of TYR+ cells among GFP$^+$ cells. TTFs derived from Tyrosinase-CreER/Gt(ROSA)26Sor$^{tm4(ACTB-tdTomato,-EGFP)Luo/J}$ mice were infected with SMP3 for 14 days. TYR+ cells were gated from the GFP+ cells. Representative results are from 3 independent experiments.



Supplementary Figure 6. Characterization of directly reprogrammed mouse iMels (miMels).

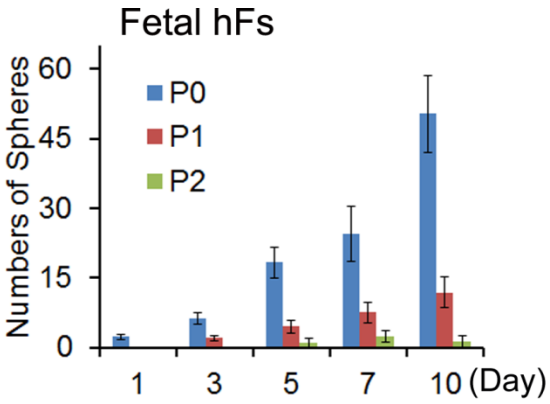
a. Immunocytochemical staining of miMels. TTFs derived from Tyrosinase-CreER/Gt(ROSA)26Sor$^{tm4(ACTB-tdTomato,-EGFP)Luo/J}$ mice were reprogrammed by SMP3. After direct reprogramming, miMels were stained with antibodies specific for SILV or Melan-A. Melan-a melanocytes were used as a positive control. Scale bar, 30 μm. b. qRT-PCR analysis of melanocyte specific markers, such as *TYR*,*TYRP1*,*DCT* and *SILV*, and endogenous expression of *SOX10*, *MITF* and *PAX3* in TTFs infected with viruses packaged with vector only (NI), SM and SMP3. Melan-a melanocytes were used as a positive control. Data shown are mean ± SD of the expression from three independent experiments. c. qRT-PCR analysis of transgenic and endogenous *SOX10*, *MITF* and PAX3 expression in SMP3-infected TTFs. Data shown are mean ± SD of the expression from three independent experiments.



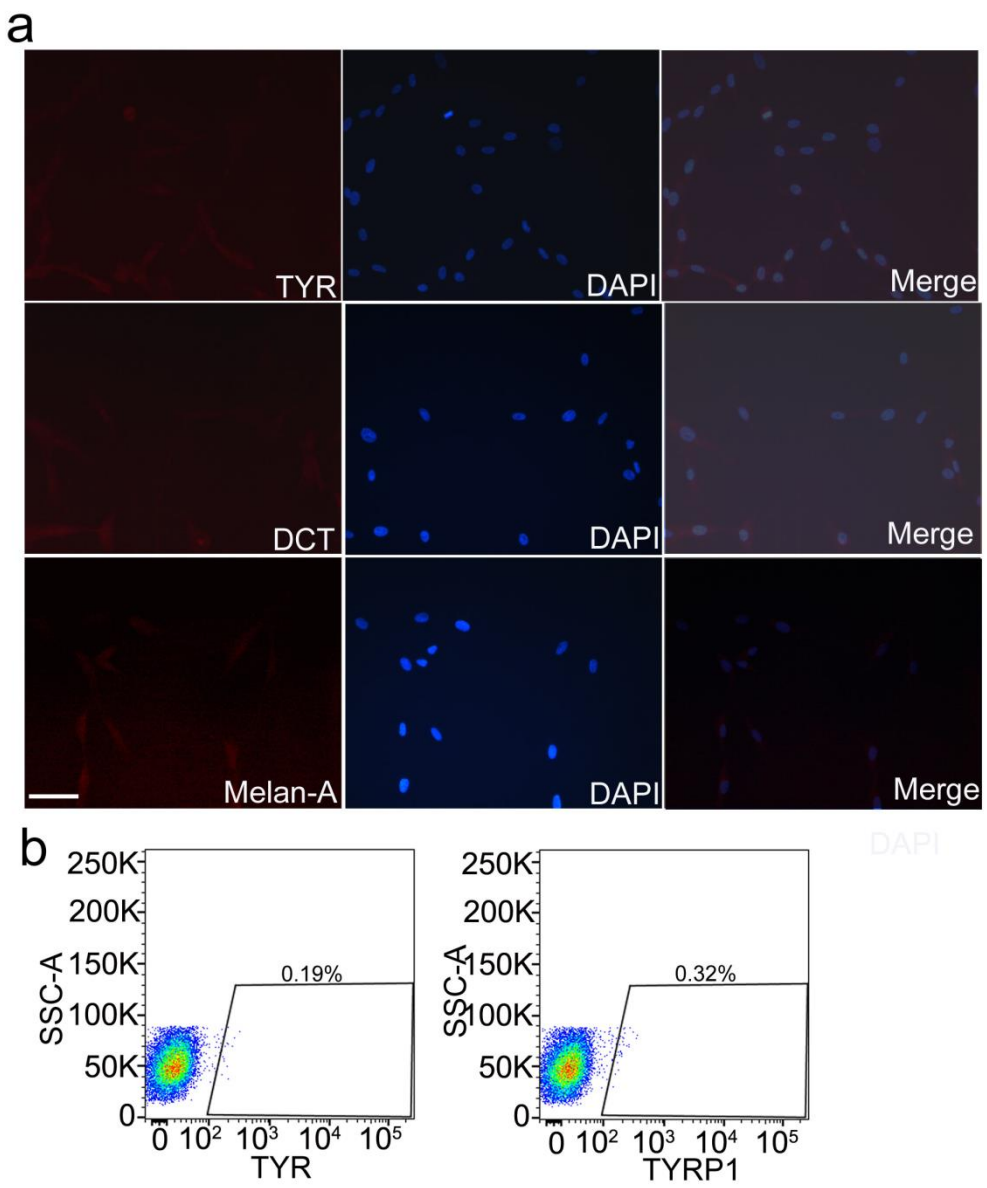
Supplementary Figure 7. Reprogramming of mouse TTFs to miMels.

a. RT-PCR analysis of melanocyte markers in TTFs after infection with different combinations of transcription factors. Adult TTFs from C57B6 mice were infected with different combination of SOX10 (S), MITF^{GFP} (M^{GFP}) and PAX3 (P3). TTFs were collected for RT-PCR analysis at Day 5 after infection. b and c. Morphologies of TTFs and SM^{GFP}P3 infected TTFs. TTFs were infected with viruses carrying SOX10/ MITF^{GFP}/PAX3 (SM^{GFP}P3), cultured for 21 days and photographed (b). TTF culture was used as a negative control (b). Scale bar, 50 μm. GFP^{+} cells were sorted out and cultured for additional 14 days. These cells showed typical melanocyte morphology and

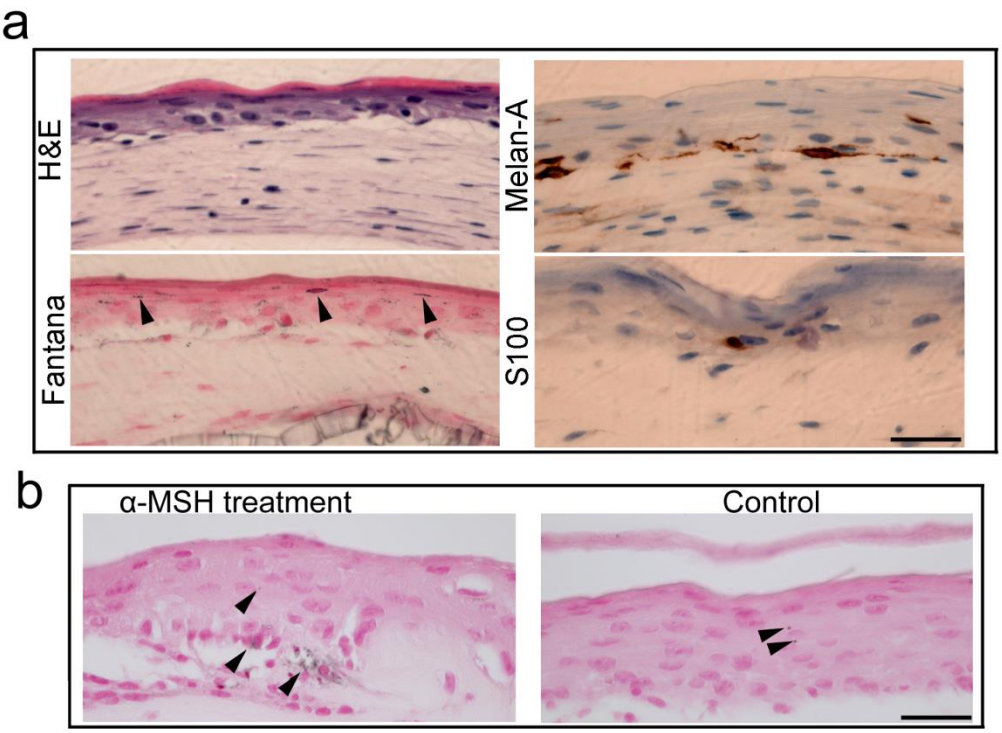
pigmentation (c). Arrow heads point to pigmentation. Scale bar in upper panels, 50 μm; Scale bar in lower panels, 25 μm. d. qRT-PCR analyses of the melanocyte markers in TTFs and iMels derived from TTFs (TTF-iMels). *MITF* (endo), *TYR*, *TYRP1*, *DCT*, *P*, *SOX10* (endo) and *PAX3* (endo) were analyzed in TTFs and TTF-iMels. Data shown are mean ± SD of the expression from three independent experiments.



Supplementary Figure 8. Sphere formation capacity of human fetal primary fibroblasts. Sphere formation capacity of the Passage 0 (P0), Passage 1 (P1) and Passage 2 (P2) human fetal primary fibroblasts (Fetal hFs) isolated from fetal skin.

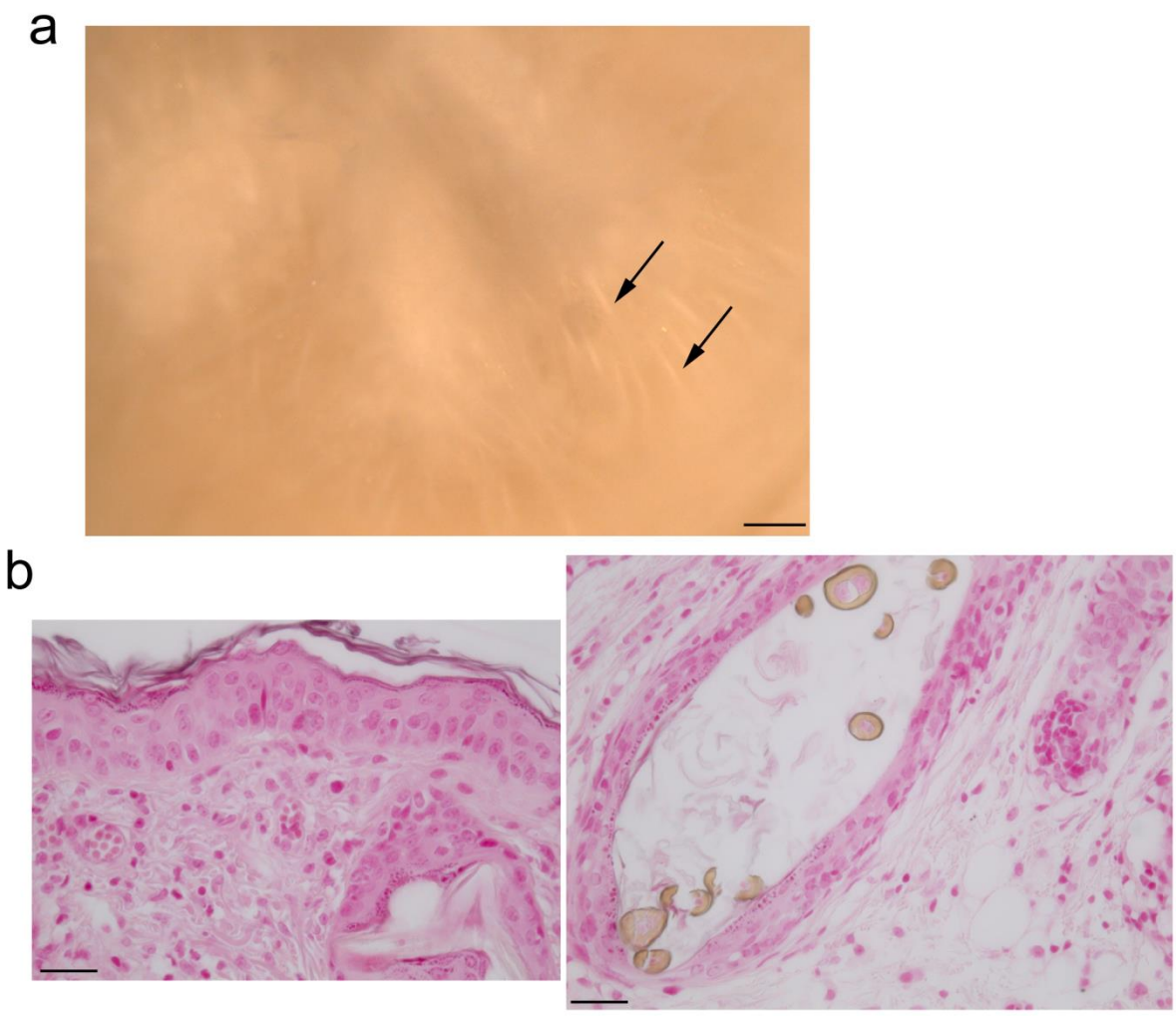


Supplementary Figure 9. Melanocytic marker expression in human fetal fibroblasts.
P2 human fetal fibroblasts (fetal hFs) were cultured in the induction medium for 40 days. a. Immunocytochemical staining analysis of TYR, DCT and Melan-A in fetal hFs. Fetal hFs were negative for these markers. Scale bar, 25 μm. b. Flow cytometric analysis of the percentage of TYR^+ and $TYRP1^+$ cells in the fibroblasts. Representative results are from three independent experiments.

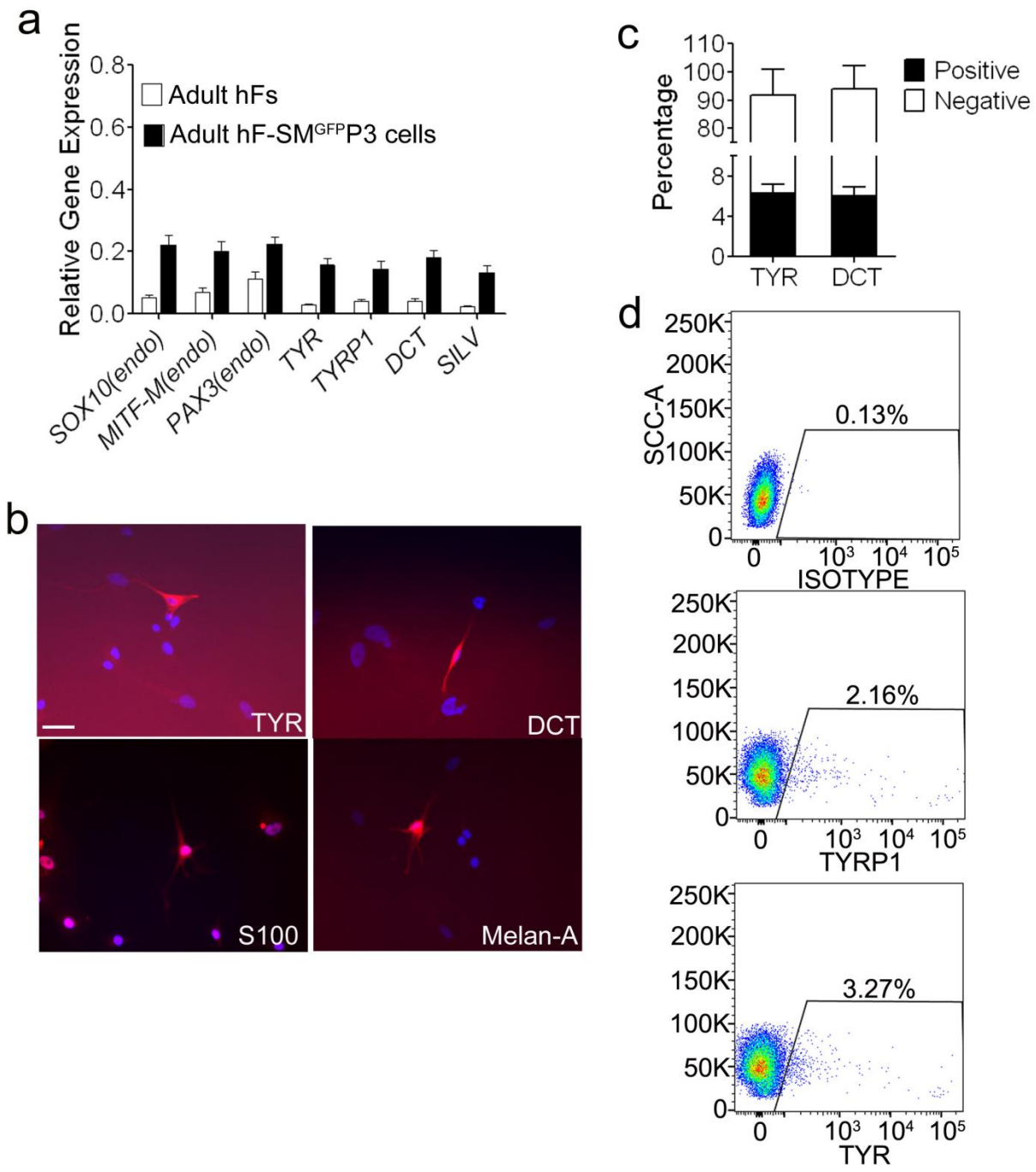


Supplementary Figure 10. In vitro functional analysis fo hiMels using 3D skin reconstruction assay.

a. hiMels derived from human fetal fibroblast in 3D skin equivalents. 3D skin equivalents were constructed using foreskin keratinocytes and hiMels. Fontana-Mason staining of 3D skin equivalents showed melanin pigment in keratinocytes, indicating transfer of pigment from melanocytes to keratinocytes. Arrow heads point to pigments in the keratinocytes. Melan-A and S100 stained scattered melanocytes in the dermal epidermal junction. Scale bar, 30 μm. b. hiMels derived from human fetal fibroblasts pigment production in response to MSH stimulation. α-MSH was applied on the top of the epidermis.The skin reconstruct was harvested 3 weeks after α-MSH stimulation. Fontana-Mason staining of the α-MSH-treated (left panel) and control (right panel) 3D skin reconstructs. Arrow heads point to pigments. Scale bar, 30 μm.



Supplementary Figure 11. Skin reconstitution assays using MITF-induced fibroblasts. P2 human fetal fibroblasts were infected with MITF and cultured for 50 days. These MITF induced cells were used in the skin reconstitution assays. a. White hair follicles and hair shafts were observed at the site of injection, and photographed from the underside of the skin. Arrow heads point to the hair shafts. Scale bar, 2mm. b. Fantana-Mason staining of the reconstructed skin did not show any pigment in the hair follicle or epidermis. Scale bar, 50 µm.



Supplementary Figure 12. Characterization of SM^{GFP}P3 infected human adult fibroblast. a. qRT-PCR analysis of melanocytic markers in human adult fibroblasts (adult hFs) and SM^{GFP}P3 infected adult hFs (adult hF-SM^{GFP}P3 cells). Adult hF-SM^{GFP}P3 cells were cultured for 15 days under selection of G418 and sorted for qRT-PCR analysis. The melanocytic markers included *MITF* (endo), *TYR*, *TYRP1*, *DCT*, *P*, *SOX10* (endo) and *PAX3* (endo). Data shown are mean ± SD of the expression from three independent

experiments. b. Immunostaining analysis of TYR, DCT, S100 and Melan-A in $SM^{GFP}P3$ infected adult hFs. Adult hFs were reprogrammed using $SM^{GFP}P3$ and stained for TYR, DCT, S100 and Melan-A expression. Scale bar, 30 μm. c. Quantification of TYR^+ and DCT^+ cells by immunostaining analysis as described in b. Representative data are from three independent experiments. d. Flow cytometric analysis of the percentage of TYR^+ and $TYRP1^+$ cells in $SM^{GFP}P3$ reprogrammed adult hFs. Representative data are from 3 independent experiments.

Supplementary Tables

Supplementary Table 1

Transcription factors screened for melanocytic conversion of TTFs

Gene name	Genebank number
hSOX10	NM_006941
hMITF	NM_198159
hPAX3	NM_181459
mSOX10	NM_011437
mMITF	NM_001113198
mPAX3	NM_008781
mDLX5	NM_010056
mFOXD3	NM_010425
mLEF1	NM_010703
mMSX1	NM_010835
mPAX6	NM_001244200
mSOX2	NM_011443
mSOX9	NM_011448

Supplementary Table 2

Primer sequences

Gene	Forward sequence	Reverse sequence	Applications
MITF-M (mouse)	GCTGGAAATGCTAGAATACAG	TTCCAGGCTGATGATGTCATC	RT-PCR
Pax3(mouse)	ATGGTTGCGTCTCTAAGATCCTG	GCGTCCTTGAGCAATTTGTC	RT-PCR
Sox10 (mouse)	TTCAGGCTCACTACAAGAGTG	TCAGAGATGGCAGTGTAGAGG	RT-PCR
Tyr(mouse)	CTTCTTCTCCTCCTGGCAGATC	TGGGGGTTTTGGCTTTGTC	RT-PCR
TYRP1(mouse)	GCCCCAACTCTGTCTTTTCTCAAT	GATCGGCGTTATACCTCCTTAGC	RT-PCR
Dct(mouse)	GGACCGGCCCCGACTGTAATC	GTAGGGCAACGCAAAGGACTCAT	RT-PCR
Gpr143(mouse)	ACTGCAACTGGGTCCTGCAAC	TGGCAGCAAGAACACAATCCA	RT-PCR

Silv(mouse)	ATGCGCCTAGAGAACAAAGAC	TAGCAGGTTTGACGGTCAGC	RT-PCR
MITF-M (endo)	CGTGACCCTTTCTCCTGTAAG	TTATAAAATGGAAAGGGTTAGT	RT-PCR
PAX3(endo)	TCCAGCAGCAAAGCCCCAG	GTGAGCAGGCCCTTCTCAGGT	RT-PCR
SOX10 (endo)	AATAGGAGACAAAGGAGAGTG	CTTAAAATGTTGCATTTGTCT	RT-PCR
TYR	CAGCCCAGCATCATTCTTCTC	GGATTACGCCGTAAAGGTCCCTC	RT-PCR
TYRP1	CCTGCGTCTGGAGAAAGAC	GGATCCCATCAAGTCATCCGTG	RT-PCR
DCT	TCTGTTAGAGATACATTATTAG	GACTCATTGCCAATGAGTCGCT	RT-PCR
P	CCAGAGACTTGACTGCTGGAG	TGCCCATCTGGCAATACCT	RT-PCR
SILV	CATTCCTCACAAAAGGGAG	CGTGACCCTTTCTCCTGTAAG	RT-PCR
TYR	TCTGGGCTCTGAAGACAATCT	CAGTTAATAGACTACAAAACTAATG	ChIP
TYRP1	AAATATAAGATCTTATCATCAG	TTTTATTCTGTTATTCAACTGTT	ChIP
TYR	TAACGTGAGATATCCCCACAATG	TATCACATGTCTTGGCTGAGAC	Bisulfite sequencing
TYRP1	CATTTCCAATTTGGATGCTCT	TAAGTGCATGTGGATTGCTG	Bisulfite sequencing

Supplementary Table 3

Antibody Information

ANTIBODY NAME	APPLICATION	DILUTION	COMPANY
TYR	IMMUNOFLUORESCENCE & FLOW CYTOMETRY	1:200	ABCAM
TYRP1	IMMUNOFLUORESCENCE & FLOW CYTOMETRY	1:500	SIGMA
C-KIT	FLOW CYTOMETRY	1: 20	EBIOSCIENCE
PDGFRA	FLOW CYTOMETRY	1:20	BIOLEGNED
S100	IMMUNOFLUORESCENCE	1:500	DAKO
MELAN-A	IMMUNOFLUORESCENCE	1:500	DAKO
DCT	IMMUNOFLUORESCENCE	1:500	A GIFT FROM DR. V.J. HEARING
SILV	IMMUNOFLUORESCENCE	1:300	DAKO