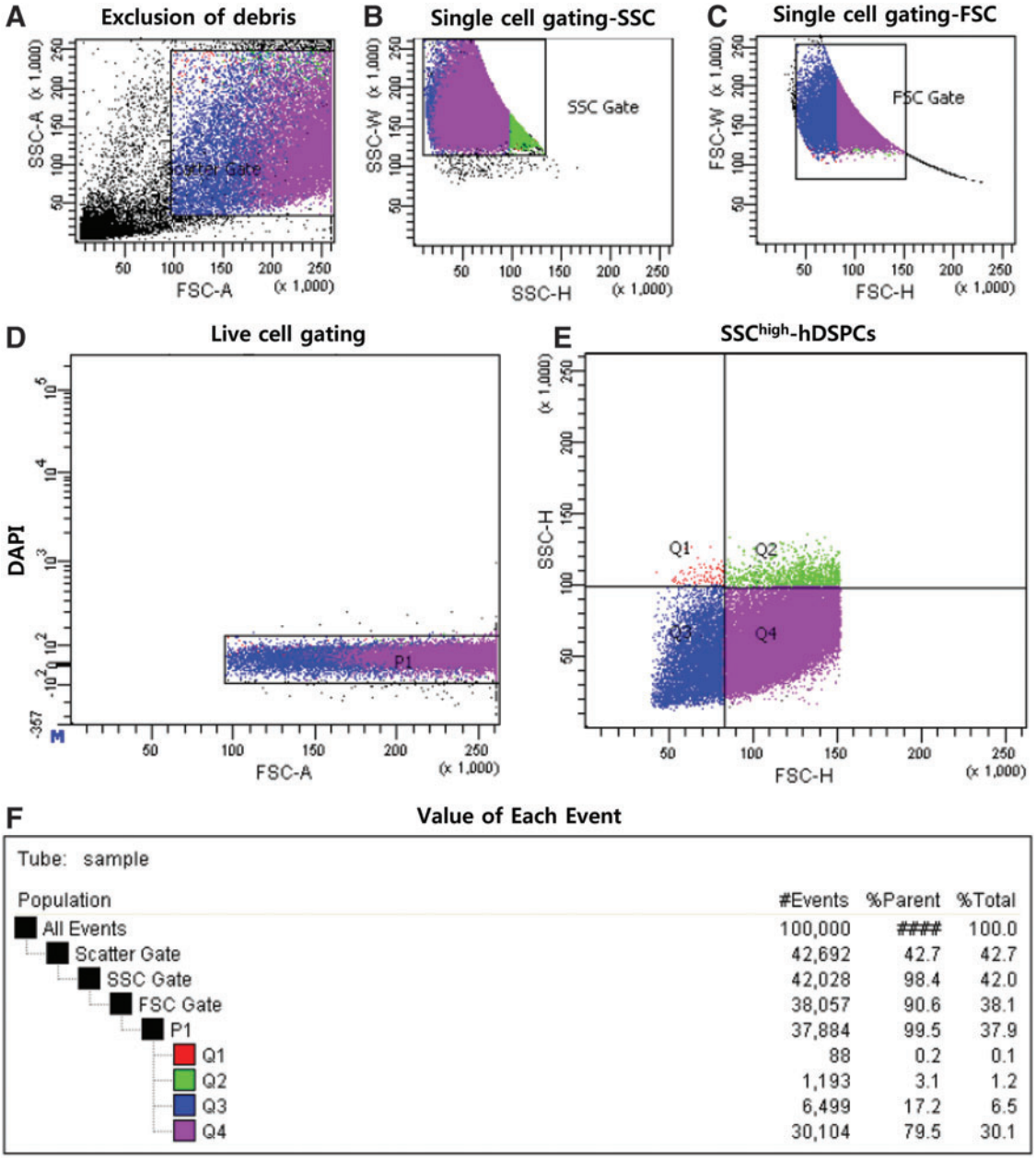
Supplementary Data

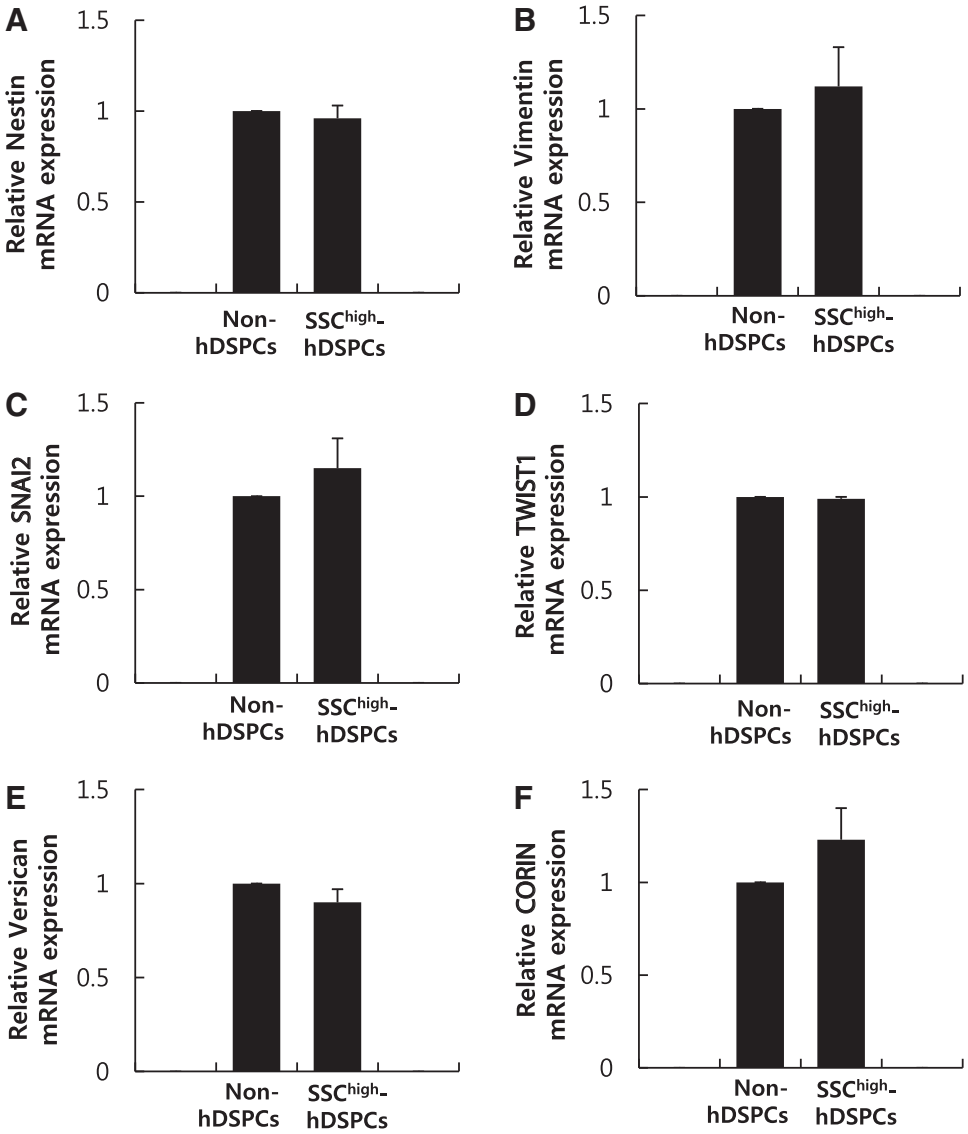
Supplementary Materials and Methods

For suspension culture of high side scatter (SSC^{high}) human dermal stem/progenitor cells (hDSPCs), the enriched SSC^{high}-hDSPCs were resuspended in the Dulbecco's modified Eagle's medium/F12 (3:1; Invitrogen) containing 100 U/mL penicillin and 100 μg/mL streptomycin, 40 ng/mL FGF2, 20 ng/mL EGF (from R&D Systems), and B27 (Invitrogen) and then transferred to a HydroCell™ 3.5-cm Dish (Nunc). The cells were grown at 37°C in 5% CO_2 for 3 days.

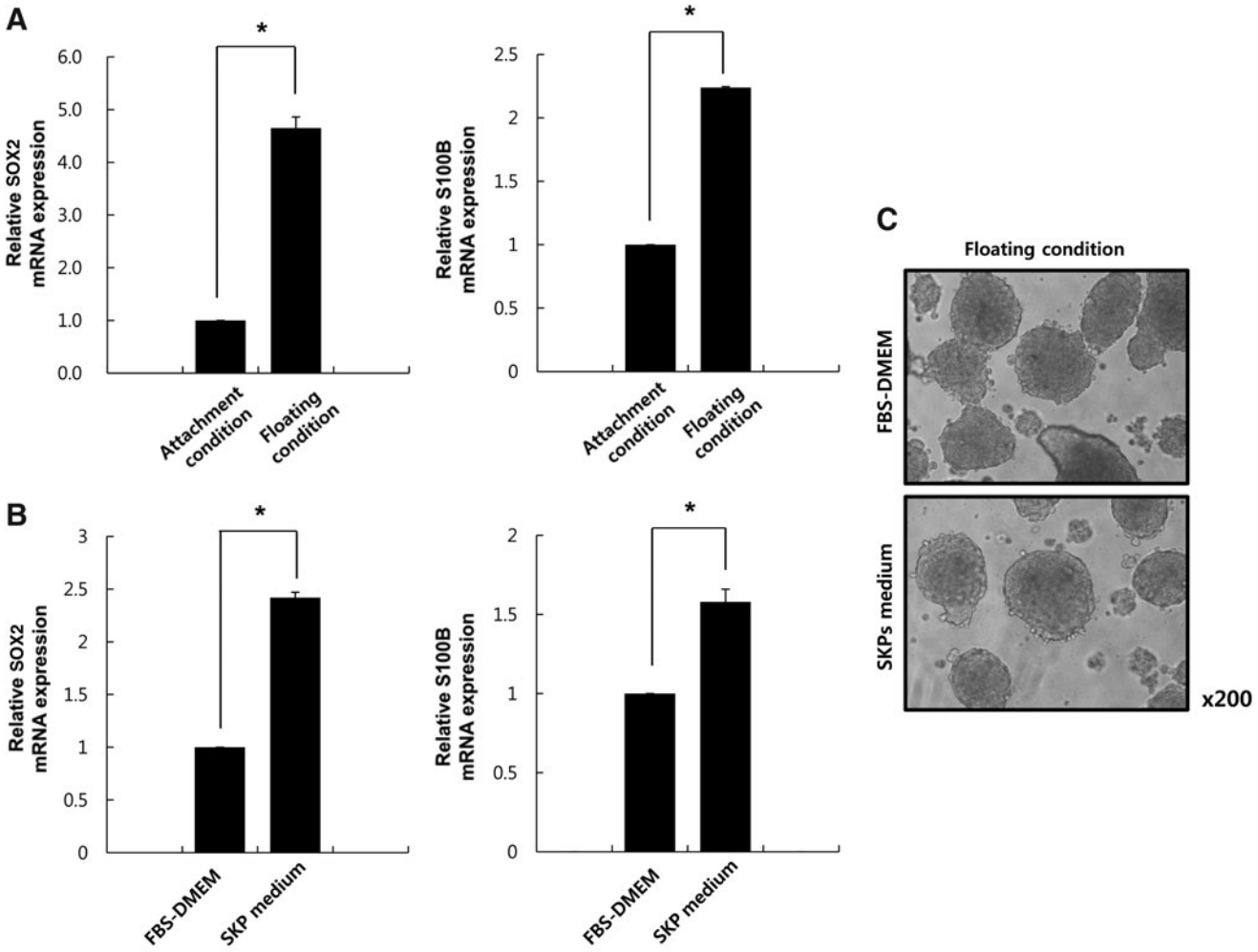


Population	#Events	%Parent	%Total
All Events	100,000	####	100.0
Scatter Gate	42,692	42.7	42.7
SSC Gate	42,028	98.4	42.0
FSC Gate	38,057	90.6	38.1
P1	37,884	99.5	37.9
Q1	88	0.2	0.1
Q2	1,193	3.1	1.2
Q3	6,499	17.2	6.5
Q4	30,104	79.5	30.1

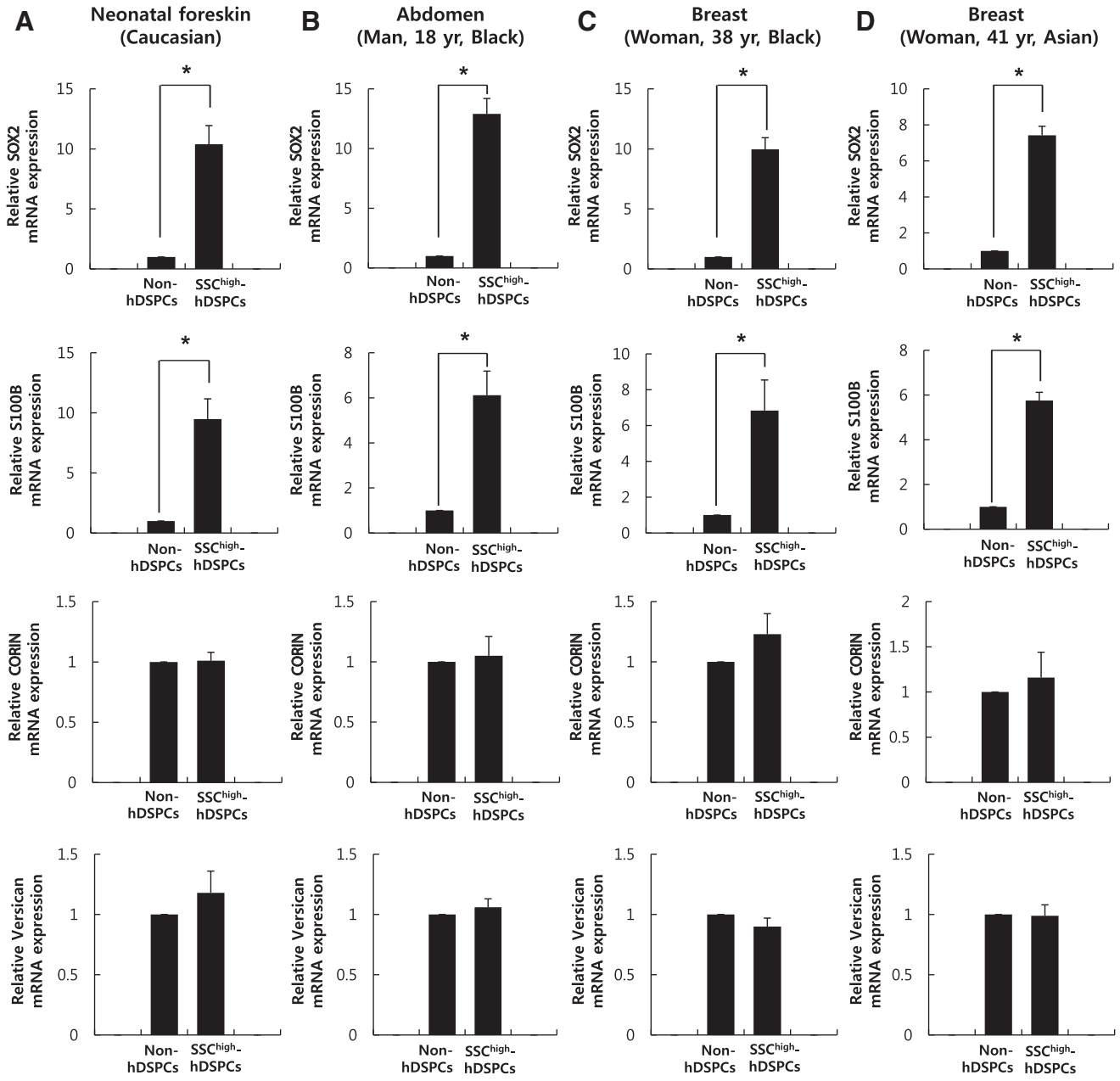
SUPPLEMENTARY FIG. S1. Purification of a SSC^{high}-hDSPC-enriched fraction. Cells were analyzed by flow cytometry as follows: **(A)** Cell debris was excluded at low and high extremes of FSC and SSC. **(B, C)** Doublets were excluded first by SSC and then by FSC-based time-of-flight analysis. **(D)** DAPI-positive (dead) cells were excluded. **(E)** Cells were divided into 4 groups (Q1: $FSC^{low}SSC^{high}$, Q2: $FSC^{high}SSC^{high}$, Q3: $FSC^{low}SSC^{low}$, and Q4: $FSC^{high}SSC^{low}$). **(F)** Both the cell number and percentage of each event versus percentage of total NHDFs are shown. A representative sample is shown ($n = 3$). DSPCs, human dermal stem/progenitor cells; FSC, forward scatter; SSC, side scatter; NHDFs, normal human dermal fibroblasts; DAPI, 4′,6-diamidino-2-phenylindole dihydrochloride.



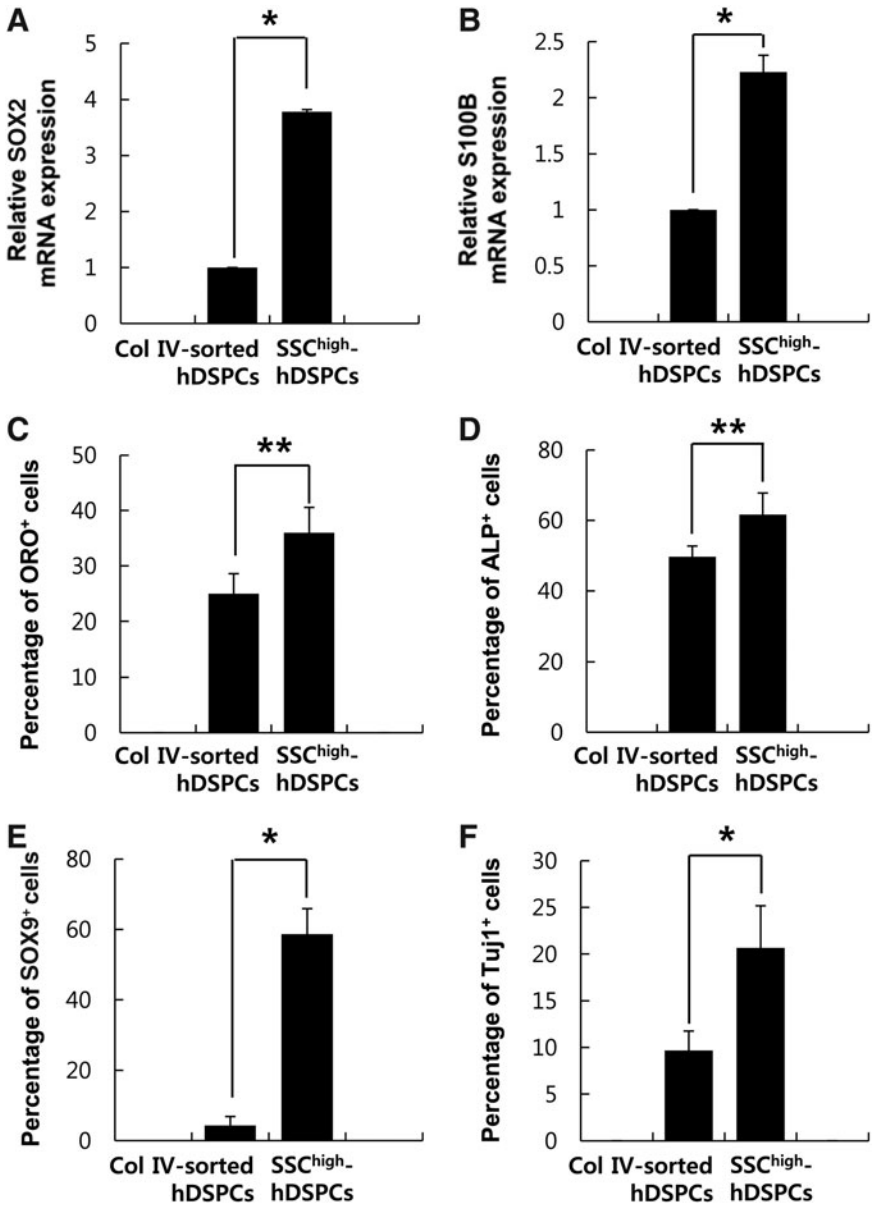
SUPPLEMENTARY FIG. S2. Characterization of SSC^{high}-hDSPCs. Real-time RT-PCR analysis of the SKP markers, **(A)** nestin, **(B)** vimentin, **(C)** *SNAI2* and **(D)** *TWIST1*, and the markers of dermal papillae, **(E)** versican and **(F)** *CORIN*. The data represent the mean ± SD of triplicate runs. RT-PCR, reverse transcription–polymerase chain reaction; SKPs, skin-derived progenitors; SD, standard deviation.



SUPPLEMENTARY FIG. S3. Comparison of conditions for maintenance of SSC^{high}-hDSPC stemness. Real-time RT-PCR analysis revealed upregulation of *SOX2* and *S100B* mRNA expression. **(A)** SSC^{high}-hDSPCs were grown in 10% FBS-DMEM under an adherent and floating condition. **(B)** SSC^{high}-hDSPCs on a HydroCell™ plate was maintained under the 10% FBS-DMEM and SKP medium, respectively. **(C)** Phase-contrast microscope images of SSC^{high}-hDSPCs on the HydroCell™ plate were shown under the 10% FBS-DMEM (*upper panel*) and SKP medium (*lower panel*), respectively. The data represent the mean ± SD of triplicate runs. $^*P < 0.01$. FBS, fetal bovine serum; DMEM, Dulbecco's modified Eagle's medium.



SUPPLEMENTARY FIG. S4. Characterization of SSC^{high}-hDSPCs from different origins. Real-time RT-PCR analysis of SKP markers, *SOX2* and *S100B*, and markers of dermal papillae, *CORIN* and versican. **(A)** Neonatal foreskin (Caucasian), **(B)** abdomen (man, 18 years, Black), **(C)** breast (woman, 38 years, Black), and **(D)** breast (woman, 41 years, Asian). The data represent the mean ± SD of independent experiments run in triplicate. *$P < 0.01$.



SUPPLEMENTARY FIG. S5. Comparison of collagen type IV-sorted hDSPCs and SSC^{high}-hDSPCs for stemness. **(A, B)** Real-time RT-PCR analysis of the SKP markers, *SOX2* and *S100B*. Histochemical or immunofluorescence staining analysis demonstrating the effectiveness of different conditions. **(C)** Oil-Red-O staining. **(D)** Alkaline phosphatase staining. **(E)** SOX9. **(F)** Tuj1. *$P < 0.01$, **$P < 0.05$.