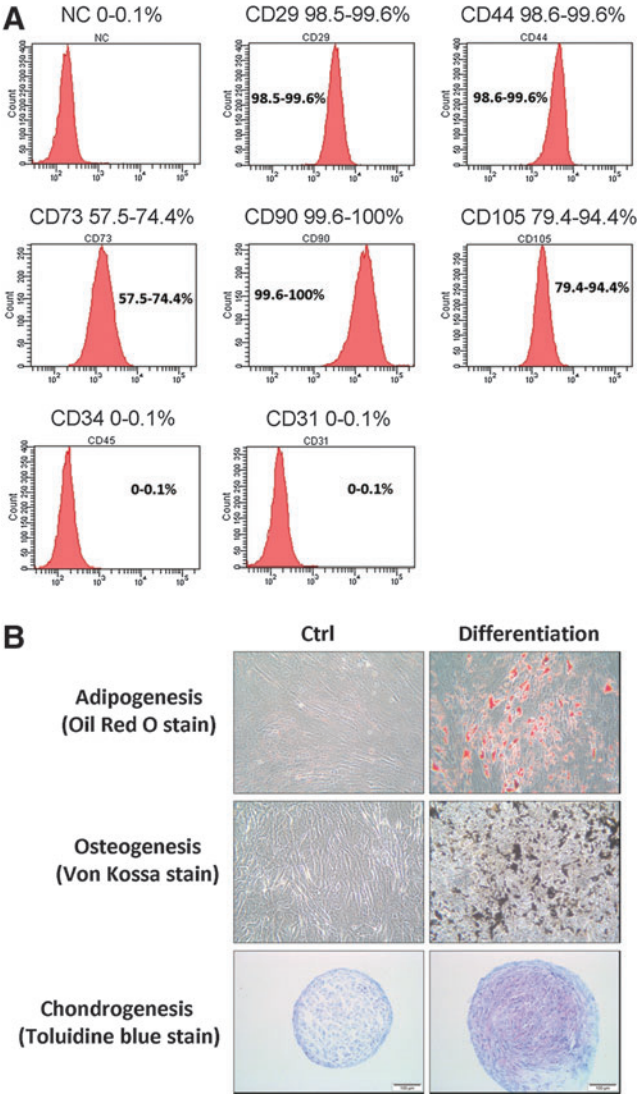
# Supplementary Data

## Supplementary Materials and Methods

### Materials

#### *Definition of cell surface markers*

CD29 and CD44 are adhesion molecules that connect extracellular matrix to cytoskeleton (1) (2). CD73 has tissue protective mechanism against hypoxia, ischemia, and inflammation (3). CD90 is involved in recruitment of inflammatory cells (4). CD105 is a member of the transforming growth factor beta receptor family that binds TGF-β1 and -β3 (5).




**SUPPLEMENTARY FIG. S1.** Characterization of isolated human adipose-derived stem cell (hASC**). (A)** Scatter plots of flow cytometry analysis showing the expression profiles of stem cell markers CD29, CD44, CD73, CD90, and CD105 and nonstem cell markers CD45 and CD31 in isolated hASC. **(B)** Isolated hASC exhibited multi-lineage differentiation potential when cultured under specific inducing conditions: adipogenic differentiation detected by Oil red O staining; osteogenic differentiation detected by Von Kossa staining; and chondrogenic differentiation detected by toluidine blue staining. Scale bar = 100 μm.

#### *The siRNA targeting sequences for human FOXO1A and FOXO3A*

ON-TARGETplus SMARTpool siRNA for human forkhead box O (FOXO)1A:

Target sequence 1: GGACAACAACAGUAAAUUU
Target sequence 2: UGACUUGGAUGGCAUGUUC
Target sequence 3: GAGGUAUGAGUCAGUAUAA
Target sequence 4: GCGCUUAGACUGUGACAUG

ON-TARGETplus SMARTpool siRNA for human FOXO3A:

Target sequence 1: UAACUUUGAUUCCCUCAUC
Target sequence 2: CGAAUCAGCUGACGACAGU
Target sequence 3: GUACUCAACUAGUGCAAAC
Target sequence 4: GCACAGAGUUGGAUGAAGU

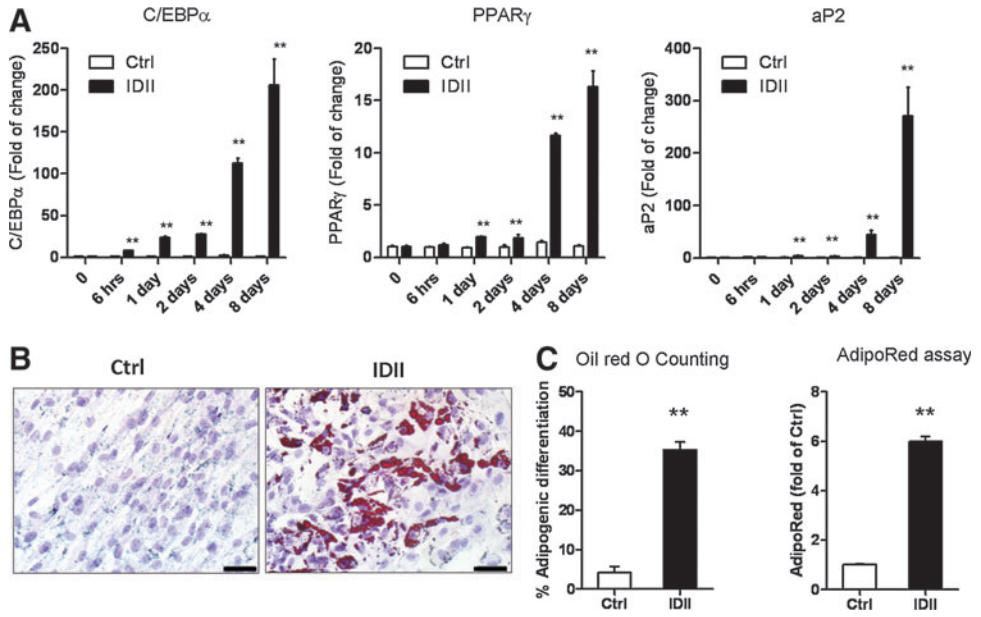
#### *The sequence of primers for human PPARγ, C/EBPα, aP2, and GAPDH*

peroxisome proliferator-activated receptor γ (PPARγ) Forward: 5′-GCTGTGCAGGAGATCACAGA-3′
PPARγ Reverse: 5′GGGCTCCATAAAGTCACCAA-3′
C/EBPα Forward: 5′-AGTCGGTGGACAAGAACAGC-3′
C/EBPα Reverse: 5′-TTGTCACTGGTCAGCTCCAG-3′
aP2 Forward: 5′-CATGGCCAAACCTAACATGA-3′
aP2 Reverse: 5′-TACCAGGACACCCCCATCTA-3′
GAPDH Forward: 5′-GAGTCAACGGATTTGGTCGT-3′
GAPDH Reverse: 5′ TTGATTTTGGAGGGATCTCG-3′

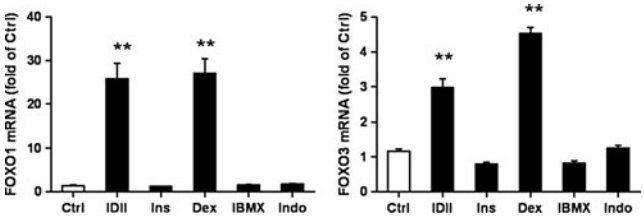
### Methods

#### *Osteogenic and chondrogenic differentiation of hASC*

To determine the differentiation ability of human adipose-derived stem cells (hASC), they were cultured under specific culture conditions. For osteogenic differentiation, confluent cells were cultured in the osteogenic medium: the high-glucose Dulbecco's modified Eagle's medium supplemented with 10% fetal calf serum, 1% antibiotic/antimycotic, 10 mM β-glycerophosphate, 100 nM dexamethasone, and 200 μM ascorbic acid-2-phosphate. After 4 weeks, von Kossa staining was used to assess the level of calcium precipitate. Briefly, cells were fixed in 4% paraformaldehyde and rinsed with distilled water before incubation with 5% silver nitrate under ultraviolet light for 3 h. Cells were then treated with 2.5% sodium thiosulfate for 5 min before the nuclei were counterstained with hematoxylin for 5 min. A 3-dimensional cell culture method (cell spheroids) was used to induce chondrogenic differentiation, where $2 \times 10^4$ cells were




**SUPPLEMENTARY FIG. S2.** IDII-induced adipogenic differentiation of hASC. **(A)** The gene expression of adipogenic markers C/EBPα, peroxisome proliferator-activated receptor γ (PPARγ), and adipocyte protein 2 (aP2) following 8 days of IDII induction by real-time polymerase chain reaction (PCR) analysis. **(B)** IDII-induced lipid accumulation was detected by Oil red O staining. Adipogenic differentiation rates were determined as the ratio of number of positively stained cells to total cell number in each photo and are shown in the bar graph. **(C)** Accumulation of intracellular triglycerides was determined by a conventional AdipoRed assay. Data are presented as mean ± standard error of the mean (SEM) from 3 experiments. $^{**}P < 0.01$ compared to the control (Ctrl) medium. Scale bar = 50 μm.




**SUPPLEMENTARY FIG. S3.** Effects of individual components of the IDII cocktail on expression of FOXOs in hASC. Cells were treated with insulin, dexamethasone, indomethacine, or IBMX for 8 days. The expression levels of forkhead box O (FOXO)1 and FOXO3 were measured by real-time PCR. Data are presented as mean ± SEM from 3 experiments. $^{**}P < 0.01$ compared to control (Ctrl).

resuspended in a chondrogenic medium (StemPro® chondrogenesis differentiation medium; GIBCO-Invitrogen, Carlsbad, CA) containing 20% methylcellulose to form a single spheroid in nonadherent U-shaped-bottom 96-well microplates (BD Biosciences, Mountain View, CA). After 2 weeks, spheroids were embedded in Optimal Cutting Temperature compound (Tissue-Tek® Sakura Finetek Inc., Torrance, CA) and snap-frozen by immersing in a dry ice-isopentane bath for 1 min. Frozen sections were cut at 10 μm thickness and stained for the presence of proteoglycan with 0.1% toluidine blue solution (pH 3.0) for 15 s. For each differentiation method, control cells were treated with the same procedure without using differentiation inducers. All induction medium and control medium were changed every 2 days in each induction of differentiation.

## References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