Supporting Information

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SI Materials and Methods

Isolation and Culturing of Bone Marrow-Derived Mesenchymal Stromal Cells. In short, aspirates were resuspended using a 20-gauge needle, plated at 500,000 cells per square centimeter, and cultured in mesenchymal stromal cell (MSC) proliferation medium consisting of α-MEM (Gibco), 10% heat-inactivated FBS (Biowhittaker), 0.2 mM ascorbic acid (Sigma), 2 mM L-glutamine (Gibco), 100 U/mL penicillin with 100 mg/mL streptomycin (Gibco), and 1 ng/mL basic FGF (Instruchemie). Chemicals were purchased from Sigma–Aldrich.

Chondrogenic Culture Medium. Chemical induction of chondrogenesis was accomplished by exposing passage 2 MSCs to DMEM high glucose that was supplemented with 100 μg/mL sodium pyruvate (Sigma), 0.2 mM ascorbic acid, 50 mg/mL insulin-transferrin-selenite (ITS + Premix; BD Biosciences), 100 U/mL penicillin, 100 μg/mL streptomycin, 0.1 μM dexamethasone, and 10 ng/mL TGF-β3 (R&D Systems). MSCs were cultured for up to 35 d under normoxic conditions (21% oxygen) or hypoxic conditions (2.5% oxygen), receiving biweekly medium refreshments.

Microarray Processing and Statistical Analysis. An Ovation PicoSL WTA System kit and Encore BiotinIL module (both from NuGEN) were used to generate biotinylated single stranded complementary DNA starting from 50 ng of total RNA. Seven hundred fifty nanograms of the obtained samples was hybridized onto Illumina HumanHT-12 v4 Expression BeadChips. The samples were scanned using an Illumina iScan array scanner. Gene expression profiling was performed using Illumina's Genomestudio v. 2010.3 software, with the default settings advised by Illumina. The raw fluorescence intensity values were normalized by applying quantile normalization. Differential gene expression was analyzed using the commercial software package Genespring, version 11.5.1 (Agilent Technologies). Genes with at least a twofold difference, being significantly differentially expressed according to a one-way ANOVA with a Benjamini–Hochberg false discovery rate correction and Tukey honestly significant difference post hoc test using a cutoff rate of $P = 0.05$, were selected. Changes of gene expression in annotated canonical pathways and biofunctions were visualized using Ingenuity Pathway Analysis software (Ingenuity Systems). The Search Tool for the Retrieval of Interacting Genes/Proteins (STRING) was used to investigate the predicted gene/gene interaction network (1). Clusters were formed using Markov clustering algorithms.

Quantitative Real-Time Reverse-Transcriptase PCR. One microgram of total RNA was reverse-transcribed using the iScript cDNA synthesis kit (BioRad), following manufacturer's instructions. Twenty nanograms of cDNA was amplified using Sensimix (Bioline) and the MyIQ Single-Color Real-Time PCR detection system (BioRad). Analysis was performed on the iQ5 optical system software (Biorad). The quantitative PCR amplifications were run under the following conditions: initial denaturation for 10 min at 95 °C and cycling 45 times at 95 °C for 15 s, 60 °C for 15 s, and 72 °C for 15 s, followed by a melting curve. Sequences of primers are available upon request. Data visualization was achieved using the program R (R Project) with the heat map script.

1. Wu L, et al. (2011) Trophic effects of mesenchymal stem cells increase chondrocyte proliferation and matrix formation. *Tissue Eng Part A* 17(9-10):1425–1436.

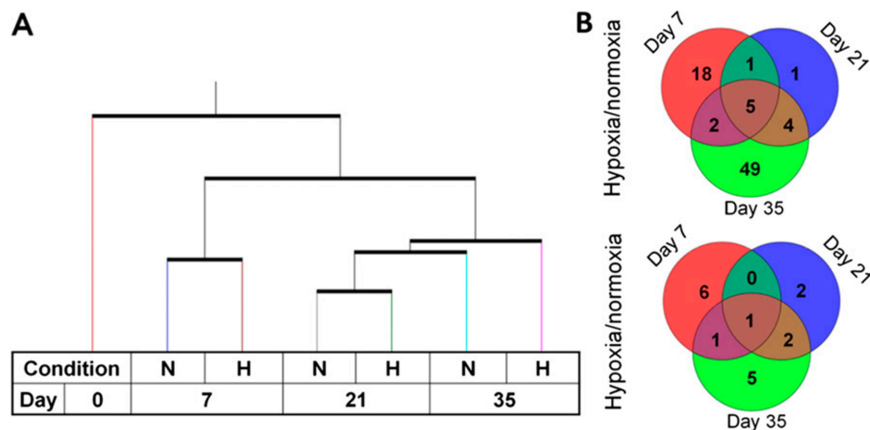

Fig. S1. Clustering of the whole-genome gene expression analysis of chondrogenically differentiated MSCs in either normoxic (N) or hypoxic (H) conditions. (*A*) Normalized microarray data were hierarchically clustered. (*B*) Venn diagrams of genes of which the transcription was at least twofold up- or down-regulated in a significant manner between normoxic and hypoxic culture conditions at day 7, day 21, or day 35.

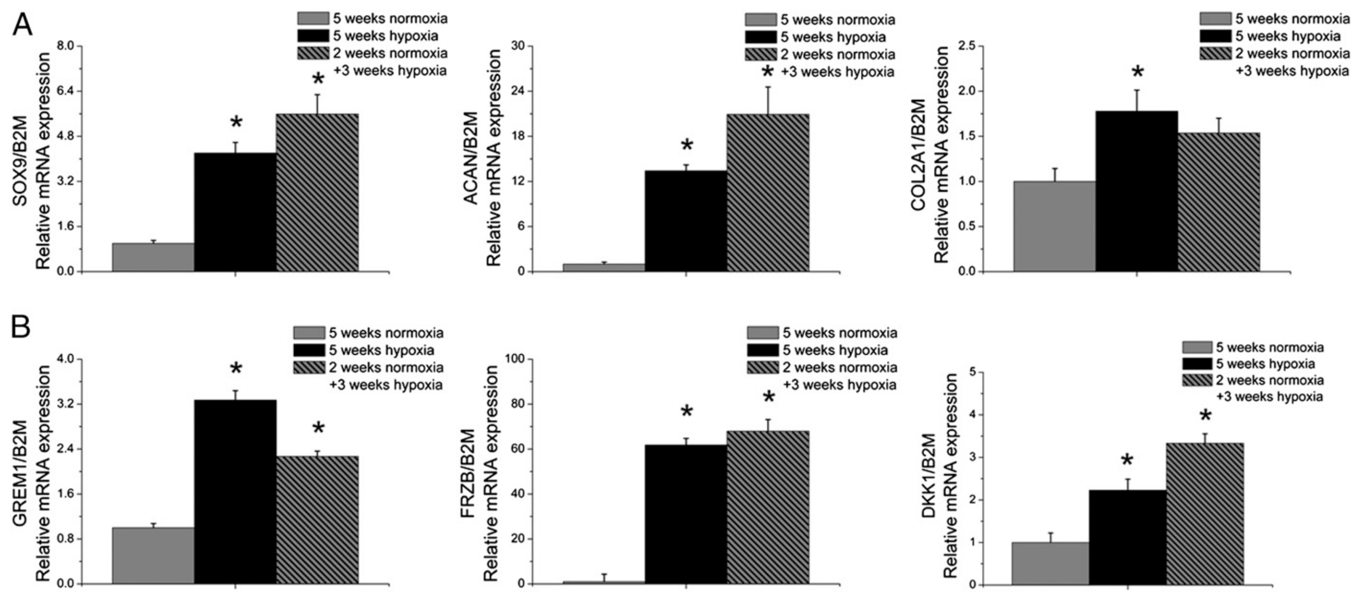

Fig. S2. MSCs underwent chondrogenic differentiation for 5 wk in normoxia, 5 in wk in hypoxia, or 2 wk in normoxia followed by 3 wk in hypoxia. mRNA levels of articular cartilage matrix components sex-determining region Y-box 9 (*SOX9*), aggrecan (*ACAN*), and collagen type II (*COL2A1*) (*A*) and the secreted antagonists gremlin 1 (*GREM1*), frizzled-related protein (*FRZB*), and Dickkopf WNT signaling pathway inhibitor 1 (*DKK1*) (*B*) were investigated using quantitative PCR analysis. Data represent the mean of three donors, each measured in triplicate ± SD. *$P < 0.05$. B2M, beta-2 microglobulin.