Supporting Information

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SI Materials and Methods

Mechanical Analysis. At set time points, samples were removed from the culture and the bulk mechanical properties of constructs were evaluated using a custom table-top testing device as described previously (1). Briefly, samples were first equilibrated under creep in an unconfined configuration to a tare load of 2 g by an impermeable loading platen in a loading chamber filled with PBS. From this offset, stress relaxation tests were performed with a single compression ramp at a rate of 10% per minute until reaching 10% strain. The equilibrium Young's modulus was determined from the equilibrium load obtained after 1,000 s of relaxation and the sample geometry at the time of testing.

Gene Expression Analysis. For gene expression analysis, samples were homogenized in TRIzol reagent (Invitrogen) with a tissue grinder, RNA was extracted according to the manufacturer's instructions, and the RNA concentration was determined using an ND-1000 spectrophotometer (Nanodrop Technologies). One microgram of RNA from each sample was reverse-transcribed into cDNA using reverse transcriptase (Superscript II; Invitrogen) and oligoDT (Invitrogen). PCR was performed on an Applied Biosystems 7300 Real-Time PCR system using Taqman primers and probes specific for GAPDH (housekeeping gene) and other genes of interest. Sequences of the primers and probes used are listed in Table S1. The relative gene expression was calculated using the $\Delta\Delta C_T$ method, where fold difference was calculated using the expression $2^{\Delta\Delta Ct}$. Each sample was internally normalized to GAPDH, and each group was normalized to the expression levels of mesenchymal stem cells (MSCs) at the time of encapsulation (i.e., after expansion and before differentiation). Relative expression levels greater than 1 represent up-regulation with culture, and relative expression levels less than 1 represent down-regulation of that gene compared with initially encapsulated MSCs.

Biochemical Analysis. One-half of each construct was weighed wet, lyophilized, reweighed dry, and digested in 0.5 mg/mL Proteinase-K (Fisher Scientific) at 56 °C for 16 h. The PicoGreen assay (Molecular Probes; Invitrogen) was used to quantify the DNA content of the constructs with Lambda phage DNA (0–1 mg/mL) as a standard (2). The glycosaminoglycan (GAG) content was measured using the dimethylmethylene blue (DMMB; Sigma Chemicals) dye-binding assay with shark chondroitin sulfate (0–50 mg/mL) as a standard (3). The overall collagen content was assessed by measuring the orthohydroxyproline (OHP) content via dimethylaminobenzaldehyde and chloramine T assay. Collagen content was calculated by assuming a 1:7.5 OHP-to-collagen mass ratio (4). The collagen and GAG contents were normalized to the disk wet weight.

Histological Analysis. The remaining halves of the constructs were fixed in 4% (wt/vol) formalin for 24 h, embedded in paraffin, and processed using standard histological procedures. The histological sections (8 μm thick) were immunostained for targets of interest using the Vectastain ABC kit and the DAB Substrate kit (Vector Labs). Briefly, sections were predigested in 0.5 mg/mL hyaluronidase for 30 min at 37 °C and incubated in 0.5 M acetic acid for 4 h at 4 °C to swell the samples before overnight incubation with primary antibodies at dilutions of 1:100, 1:200, and 1:3 for chondroitin sulfate (mouse monoclonal anti-chondroitin sulfate; Sigma), and type I (mouse monoclonal anti-collagen type 1; Sigma) and type II collagen antibodies (mouse monoclonal anti-collagen type II; Developmental Studies Hybridoma Bank), respectively. Nonimmune controls underwent the same procedure without primary antibody incubation. Images obtained from immunohistochemical staining were quantified by ImageJ to obtain mean staining intensity. For this purpose, each image was converted to an 8-bit image and inverted (Fig. S9). Mean intensity values within randomly placed square areas (200 × 200 μm) on the image were measured ($n = 4$).

Immunostaining. hMSCs were fixed in 4% (wt/vol) formalin for 10 min, washed [PBS containing 3% (wt/vol) BSA], permeabilized and blocked [PBS containing 0.3% Triton-X and 10% (vol/vol) goat serum], and incubated overnight at 4 °C with anti-CD44 (3/1,000 mouse mAb CD44; Abcam), anti-CD168 (3/1,000 rabbit mAb Cd168; Epitomics), or anti GC-4 (50 μg/mL; Sigma), which binds to the N-terminal half of the extracellular domain of human N-cadherin. Cells were then washed [PBS containing 1% (wt/vol) BSA] and incubated 2 h at room temperature with Alexa Fluor 488 anti-mouse or anti-rabbit secondary Ab (Invitrogen), both 1:200 dilution in PBS containing 1% (wt/vol) BSA. Cell nuclei were stained with DAPI.

Flow Cytometry. For flow cytometry studies, hMSCs were first expanded on cell culture treated tissue culture plastic, detached from the substrate (via 0.1% trypsin), washed (PBS), and incubated with anti-CD44 (3/1,000 mouse mAb CD44; Abcam), anti-CD168 (3/1,000 rabbit mAb Cd168; Epitomics), or anti GC-4 (50 μg/mL; Sigma) on ice for 45 min. Immediately after antibody incubation, cells were washed twice and incubated with a secondary Ab (Alexa Fluor 488 anti-mouse or anti-rabbit; Invitrogen) in the same buffer (1/200) for 30 min on ice and assessed on a FACScan flow cytometer (80K cells, FACS Canto II; BD Biosciences). As a control, cells were incubated with secondary Ab alone.

1. Mauck RL, et al. (2000) Functional tissue engineering of articular cartilage through dynamic loading of chondrocyte-seeded agarose gels. *J Biomech Eng* 122(3):252–260.
2. McGowan KB, Kurtis MS, Lottman LM, Watson D, Sah RL (2002) Biochemical quantification of DNA in human articular and septal cartilage using PicoGreen and Hoechst 33258. *Osteoarthritis Cartilage* 10(7):580–587.
3. Farndale RW, Buttle DJ, Barrett AJ (1986) Improved quantitation and discrimination of sulphated glycosaminoglycans by use of dimethylmethylene blue. *Biochim Biophys Acta* 883(2):173–177.
4. Hollander AP, et al. (1994) Increased damage to type II collagen in osteoarthritic articular cartilage detected by a new immunoassay. *J Clin Invest* 93(4):1722–1732.

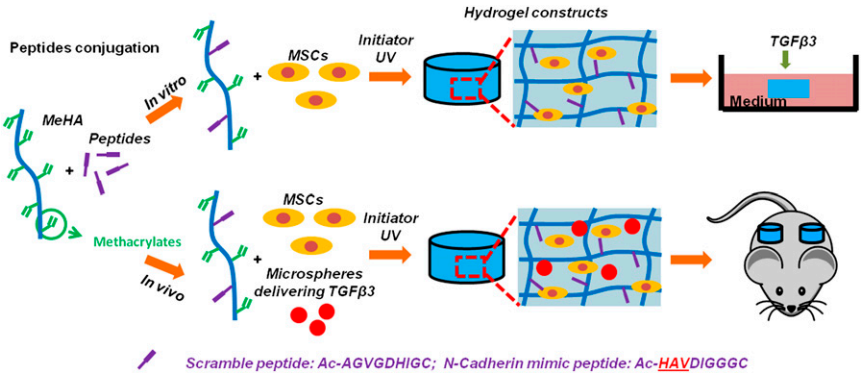

Fig. S1. N-cadherin mimetic or scrambled peptides were conjugated to methacrylated hyaluronic acid (MeHA) precursors via a Michael-type addition reaction. Subsequently, these modified macromers were used to photoencapsulate MSCs in peptide-modified MeHA hydrogels followed by in vitro culture in chondrogenic or growth media or in vivo s.c. implantation in nude mice.

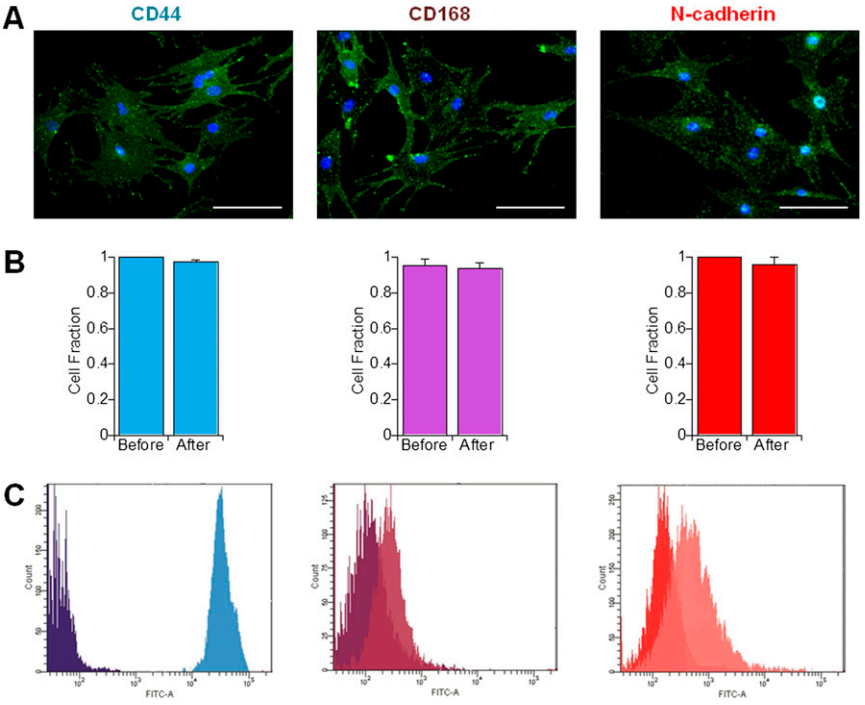

Fig. S2. Immunostaining and FACS. (*A*) Fluorescent microscopy images of human (h)MSCs replated and cultured for 4 h on tissue culture plastic (TCPS) immediately after trypsin treatment (cell detachment) after being expanded on TCPS. Cells were immunostained for CD44, CD168, or N-cadherin (green) and stained for cell nuclei (DAPI, blue). (Scale bars, 100 μm.) (*B*) Quantitative evaluation of immunostaining results from (*A*, Before) and Fig. 1 (After). Error bars denote SDs for $n = 3$. (*C*) Flow cytometry of hMSC surface expression of CD44, CD168, and N-cadherin immediately after cells were detached from the substrate and incubated with the antibody in suspension. The fraction of the antibody positive cells for CD168 or N-cadherin were significantly lower than the values obtained from immunostaining, which can be attributed to loss of these markers during the cell detachment process.

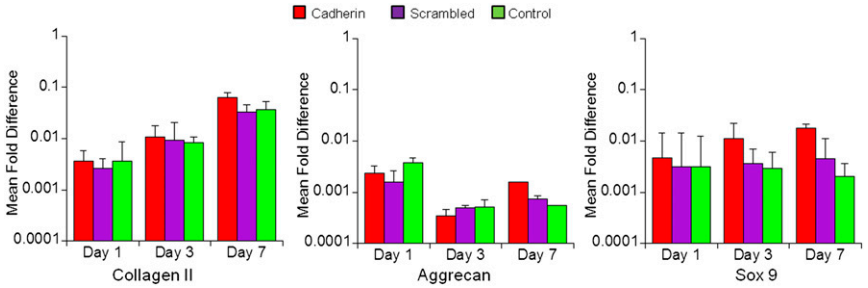

Fig. S3. Mean fold difference in gene expression (normalized to GAPDH and monolayer cells before encapsulation) of collagen II, aggrecan, and sex determining region Y-box 9 (Sox 9) by MSCs cultured in hyaluronic acid (HA) hydrogels [1.5% methacrylated (Me)HA, wt/vol, 10% methacrylate consumption with cadherin mimetic (Cadherin) or Scrambled peptides] after 1, 3, or 7 d of in vitro culture in growth media ($n = 3$). There were no statistical differences between groups at the same time point.

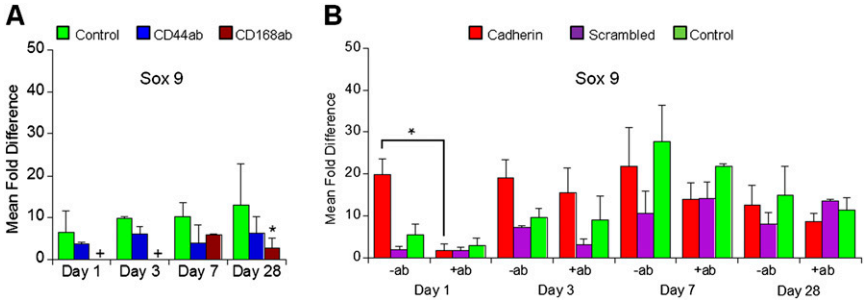

Fig. S4. (*A*) Mean fold difference in gene expression (normalized to GAPDH and monolayer cells before encapsulation) of Sox 9 by MSCs cultured in HA hydrogels (1.5% MeHA, wt/vol) after 1, 3, or 7 d of in vitro culture either untreated (Control) or after treatment with CD44 (CD44ab) or CD168 (CD16ab) antibodies before encapsulation. + indicates no amplified expression for the gene. (*B*) Mean fold change in gene expression (normalized to GAPDH and monolayer cells before encapsulation) of Sox 9 by MSCs cultured in HA hydrogels (1.5% MeHA wt/vol, 10% methacrylate consumption with Cadherin or Scrambled peptides) after 1, 3, 7, or 28 d of in vitro culture either untreated (–ab) or with treatment with the N-cadherin antibody before encapsulation (+ab). *$P < 0.05$ vs. +ab group of the same scaffold at the same culture time ($n = 4$).

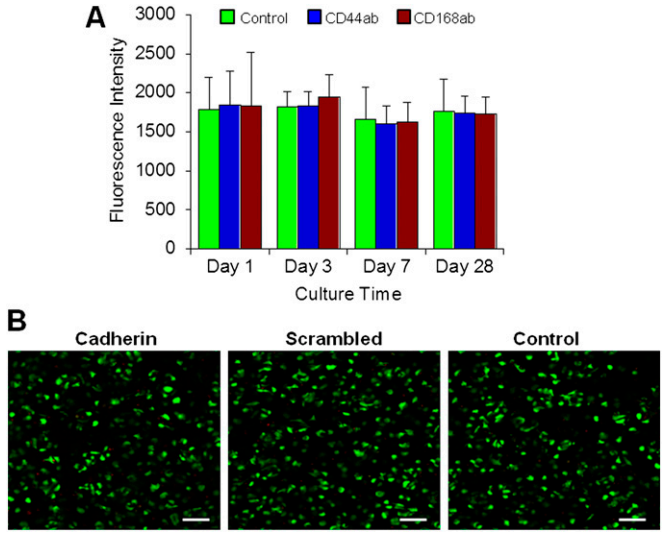

Fig. S5. (*A*) Alamar blue assay results to investigate cell survival for hMSCs treated with CD44 or CD168 compared with untreated cells during culture in chondrogenic media. For this purpose, three groups of hMSCs including control (without any antibody treatment) and CD44- and CD168-treated cells were encapsulated in MeHA hydrogels and cultured for 28 d in chondrogenic media. At days 1, 3, 7, and 28 samples were incubated with Alamar blue assay (10 vol%) for 4 h, and the corresponding fluorescence intensity was measured via plate reader. Error bars denote SDs for $n = 3$ samples. There were no statistical differences between groups. (*B*) Staining for hMSC viability after 28 d of in vitro culture, illustrating primarily live cells. Green, live cells; red, dead cells. (Scale bars, 100 μm.)

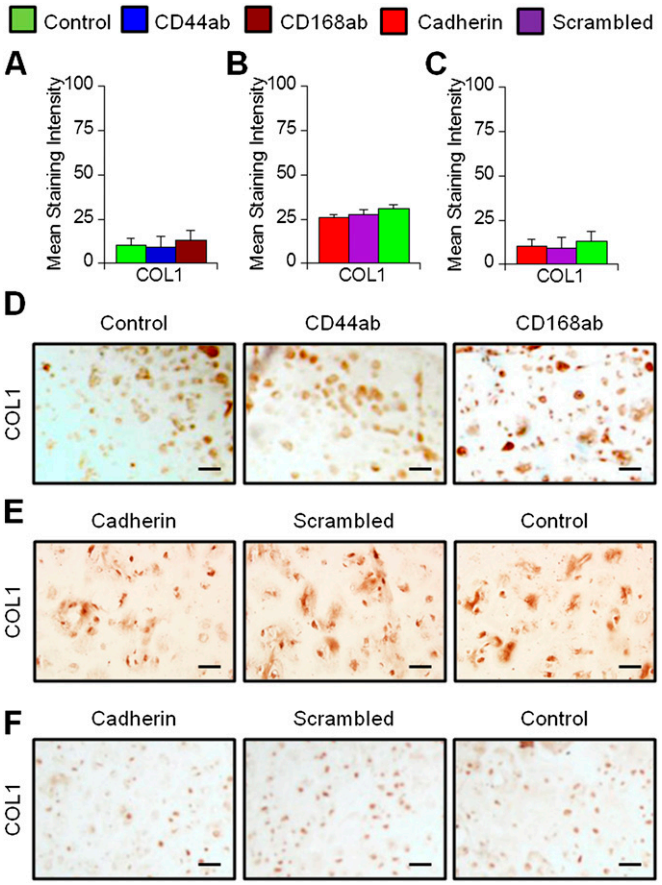

Fig. S6. Quantification (*A*–*C*) and images (*D*–*F*) for immunohistochemical staining of type I collagen (COL1) of MSC-laden HA hydrogel constructs after 28 d of in vitro culture (*A* and *B*; *D* and *E*) and s.c. implantation in nude mice (*C* and *F*). Comparison of COL1 expression for hMSCs with no treatment (Control) or treatment with CD44 (CD44ab) or CD168 (CD16ab) antibodies before encapsulation (*A* and *D*). Comparison of COL1 expression for hMSCs cultured in Cadherin, Scrambled, and control hydrogels (*B* and *E* for in vitro, *C* and *F* for in vivo). $n = 4$. (Scale bars, 50 μm.)

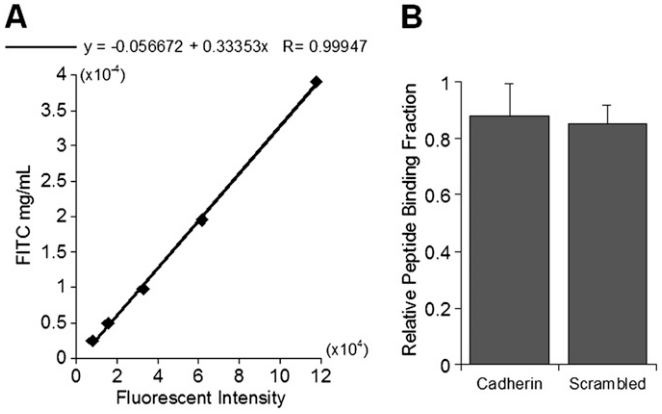

Fig. S7. (*A*) Fluorescence standard curve of the FITC tag. FITC was conjugated to Cadherin and Scrambled peptides using a FluoroTag FITC conjugation kit (Sigma). The molar ratio of FITC to peptide in the conjugates was calculated according to the manufacturer's protocol. MeHA macromers were modified with FITC-conjugated or original peptides (Cadherin and Scrambled) with a theoretical 10% consumption of the methacrylates. Hydrogels fabricated from these macromers were equilibrated in PBS to ensure removal of the unbound peptides and then solubilized via addition of hyaluronidases overnight at 37 °C. (*B*) The relative peptide binding fractions for FITC-conjugated Cadherin and Scrambled peptides were determined by measuring the fluorescence intensity at 490/525 nm (excitation/emission) after blanking with solubilized hydrogels formed from non-FITC conjugated peptides.

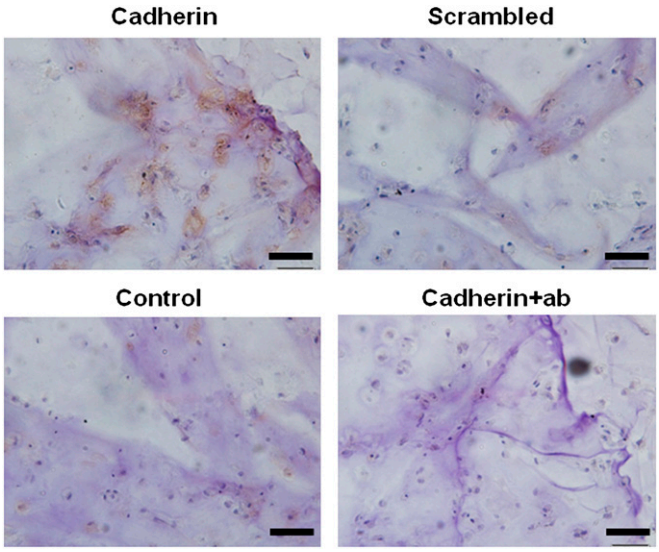

Fig. S8. Immunohistochemical staining of hydrogel sections for β-catenin on day 7; ab indicates treatment of hMSCs with N-cadherin antibody GC-4 before encapsulation. (Scale bars, 50 μm.)

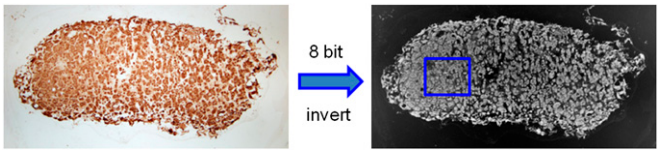

Fig. S9. Quantification of immunohistochemical staining. Images were first converted to 8-bit and then inverted. Mean staining intensity within randomly placed square areas (200 × 200 μm) on the image ($n = 4$) was measured via ImageJ.

Table S1. Sequences of primers and probes used for real-time PCR

Gene	Forward primer	Reverse primer	Probe
GAPDH	AGGGCTGCTTTTAACTCTGGTAAA	GAATTTGCCATGGGTGGAAT	CCTCAACTACATGGTTTAC
COL1	AGGACAAGAGGCATGTCTGGTT	GGACATCAGGCGCAGGAA	TTCCAGTTCGAGTATGGC
COL2	GGCAATAGCAGGTTCACGTACA	CGATAACAGTCTTGCCCCACTT	CTGCACGAAACATAC
Aggrecan	TCGAGGACAGCGAGGCC	TCGAGGGTGTAGCGTGTAGAGA	ATGGAACACGATGCCTTTCACCACGA