SUPPORTING INFORMATION

Integrating Epigenetic Modulators into NanoScript for Enhanced Chondrogenesis of Stem Cells

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MATERIALS AND METHODS

PEPTIDE SEQUENCES FOR STF MOLECULES: The transactivation peptide, having a sequence of OH-CGSDALDDFDLDMGSDLDDFDLDMGLGS-NH₂, was purchased from Invitrogen. The cell penetrating peptide (CPP), having a sequence of NH₂-CALNNAGRKKRRQRRR-OH, was purchased from GenScript.

SYNTHESIS OF HAIRPIN POLYAMIDES: All machine-assisted polyamide syntheses were performed on a PSSM-8 peptide synthesizer (Shimadzu, Kyoto) with a computer-assisted operation system at 40 mg of Fmoc-β-wang resin (ca. 0.6 mmol/g, 100~200 mesh) by Fmoc solid-phase chemistry. Reaction cycles were as follows: deblocking steps for 4 min x 2, 20% piperidine in DMF; coupling step for 60 min, corresponding carboxylic acids, HCTU (88 mg), diisopropylethylamine (DIEA) (36 μL), 1-methyl-2-pyrrolidone (NMP); washing steps for 1 min x 5, DMF. Each coupling reagents in steps were prepared in NMP solution of Fmoc-Py-COOH (77 mg), Fmoc-Im-COOH (77 mg), Fmoc-PyIm-COOH (70 mg), Fmoc-β-COOH (66 mg), Fmoc-γ-COOH (69 mg). All other couplings were carried out with single-couple cycles stirred by N₂ gas bubbling. Typically, resin (40 mg) was swollen in 1 mL of NMP in a 2.5-mL plastic reaction vessel for 30 min. 2-mL plastic centrifuge tubes with loading Fmoc-monomers with HCTU in NMP 1 mL were placed in programmed position. All lines were washed with NMP after solution transfers. After the completion of the synthesis by the peptide synthesizer, the resin was washed with DMF (1 mL x 2), methanol (1 mL x 2), and dried in a desiccator at room temperature in vacuo.

To synthesize the AcPyPyPy-β-PyImPy-γ-PyPyPy-β-PyImIm-β-NH₂ polyamide, a dried sample resin was cleaved with 0.5 ml of 3,3’-diamino-N-methyldipropylamine for 3 h at 45 °C. The reaction mixture was filtered, triturated from CH₂Cl₂-Et₂O, to yield Py-Im polyamide as a white-yellow crude powder. The crude was purified by HPLC (elution with trifluoroacetic acid and a 20-50% acetonitrile
linear gradient (0-30 min) at a flow rate of 3.0 mL min$^{-1}$ under 254 nm) as white powder (3.5 mg, 8% for 15 steps). ESI-TOF-MS (positive) m/z calcd for C$_{91}$H$_{113}$N$_{34}$O$_{17}$,$^{2+}$ [M+2H]$^{2+}$ 977.45; found 977.44. The preparation of mismatched polyamide, AcPyPyPy-β-PyImPy-γ-PyPyPy-β-PyPyIm-β-NH$_2$, was used by similar procedure, and confirmed by HPLC and Mass. ESI-TOF-MS (positive) m/z calcd for C$_{92}$H$_{114}$N$_{33}$O$_{17}$,$^{2+}$ [M+2H]$^{2+}$ 976.95; found 976.94.

SYNTHESIS OF CTB:

Synthesis of thiol-PEGylated CTB. (i) Preparation of heterobifunctional PEG linker: (a) (a-1) NaH, allyl bromide, DMF, 25 h, (a-2) TsCl, DMAP, Et$_3$N, CH$_2$Cl$_2$, 16 h. (ii) Synthesis of thiol-PEGylated CTB: (b) TFA/TFAA, acetone, 24 h; (c) 1, K$_2$CO$_3$, acetonitrile, 80°C, 24 h; (d) 4-chloro-2-(trifluoromethyl)aniline, DMPU, n-BuLi, THF, 80°C, 2 h; (e) AIBN, thioacetic acid, toluene, 100-110°C, 16 h; (f) Et$_2$SO$_4$, K$_2$CO$_3$, acetone, 48 h; (g) TBACN, MeOH, CHCl$_3$. 

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All reactions were conducted in oven-baked glassware with magnetic stirring under nitrogen or argon atmosphere. All reagents and solvents (anhydrous) were purchased from Sigma-Aldrich, Acros Organics, Alfa Aesar and used without further purification. Analytical thin layer chromatography (TLC) was performed on silica gel matrix on TLC Al foil plates with fluorescent indicator 254 nm. Visualization was accomplished with UV light and iodine stain. Purification of reaction products was carried out by flash column chromatography over Teledyne Isco Redisep Rf silica gel (60 A, 40-60UM). The proton nuclear magnetic resonance (\(^1\)H-NMR) spectra were recorded on either a Varian-300 instrument (300 MHz), Varian-400 instrument (400 MHz), or Varian-500 instrument (500 MHz) spectrometer with a set temperature of 25°C. Chemical shifts (\(\delta\)) of the compounds were recorded in ppm corresponding to the internal standard, tetramethylsilane (TMS). NMR solvent standards were used as follows: for \(^1\)H-NMR, CDCl\(_3\) = 7.26 ppm. Data were reported as chemical shift, multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet), coupling constants \((J)\) in Hz, and integration. Mass spectroscopy was recorded on a Finnigan LCQ-DUO mass spectrometer.

**Compound 1: p-toluenesulfonyl tetraethylene glycol allyl ether (1).** Compound 1 was prepared according to literature with slightly modification.\(^{[1]}\) Tetraethylene glycol (32.11 g, 0.17 mol) was dissolved in 70 mL DMF and cooled to 0°C under inert atmosphere. NaH (60% in mineral oil, 1.32 g, 33 mmol) was added in portions. After addition, the reaction was stirred at room temperature for 1h. Then, allyl bromide (4 g, 33 mmol) was added dropwise. The mixture was allowed to proceed overnight. Then, the reaction was quenched with water, followed by washing with hexane and extracting with diethyl ether (more than 10 times) to yield colorless liquid oil (intermediate compound). The intermediate compound was used for next step without further purification. To an ice-cold solution of the intermediate compound (12.61 g, 54 mmol) in 100 mL dichloromethane, \(p\)-toluenesulfonate (30.78, 0.16 mol) was added. Then, Et\(_3\)N (73.41 mL, 0.54 mol) was added dropwise at 0°C using addition funnel.
The reaction was stirred overnight at ambient temperature. Upon completion, the reaction mixture was filtered and the precipitant was washed with aqueous saturated solution of NaHCO₃ and dried over MgSO₄. MgSO₄ was filtered out and the solution was concentrated in vacuo to yield brown crude mixture. The crude mixture was purified by column chromatography on silica gel using dichloromethane/ethyl acetate (4:1) as eluent to afford yellow liquid oil. Yield 30%. ¹H-NMR (300 MHz, CDCl₃): δ (ppm) 7.80 (d, J = 8.4 Hz, 2H), 7.34 (d, J = 8.6 Hz, 2H), 5.98-5.83 (m, 1H), 5.31 – 5.14 (m, 2H), 4.16 (t, 2H), 4.01 (dt, J = 5.7, 1.4 Hz, 2H), 3.72 – 3.54 (m, 14H), 2.44 (s, 3H); MS (m/z): calculated for C₁₈H₂₈O₇S, 388.16; found, 411.12 for [M+Na]⁺.

**Compound 2: 5-hydroxy-2,2-dimethyl-4H-benzo[d-1,3]dioxin-4-one (2).** Synthetic procedure of compound 2 was adopted from literature.² To a suspension of 2,6-dihydroxybenzoic acid (3 g) in trifluoroacetic acid (28.8 mL), trifluoroacetic anhydride (18 mL) and acetone (3.6 mL) were added at 0°C. The suspension was warmed slowly to room temperature. After 24 h, the clear yellow solution was observed, and then the volatiles were evaporated under reduced pressure. Saturated aqueous solution of NaHCO₃ was added to the residue. Then, the aqueous solution was extracted with three portions of ethyl acetate. The combined organic layers were washed with water and brine, and then dried over MgSO₄. The solvent was evaporated after filtrating MgSO₄ to afford a yellow crude mixture. Chromatography over silica gel with hexane/ethyl acetate (4:1) as the eluent gave white solid product. Yield 35%. ¹H-NMR (300 MHz, CDCl₃): δ (ppm) 10.34 (br, s, 1H), 7.41 (t, J = 8.3 Hz, 1H), 6.63 (dd, J = 8.5, 0.9 Hz, 1H), 6.44 (dd, J = 8.2, 0.9 Hz, 1H), 1.75 (s, 6H); MS (m/z): calculated for C₁₀H₁₀O₄, 194.06; found, 217.00 for [M+Na]⁺.

**Compound 3: 5-((3,6,9,12-tetraoxapentadec-14-en-1-yl)oxy)-2,2-dimethyl-4H-benzo[d-1,3]dioxin-4-one (3).** Compound 3 was synthesized according to literature.³ 5-hydroxy-2,2-dimethyl-4H-benzo[d-
1,3]dioxin-4-one 2 (0.45 g, 2.34 mmol) and p-toluenesulfonyl tetraethylene glycol allyl ether 3 (1 g, 2.57 mmol) were reacted in 15 mL acetonitrile in the presence of K₂CO₃ (0.36 g, 2.57 mmol). The reaction mixture was refluxed under inert atmosphere. After 24h, the mixture was allowed to cool down to room temperature. The mixture was diluted with diethyl ether. The solid byproduct was filtered out. The precipitant was concentrated in vacuo. The crude mixture was chromatographed on silica gel column using dichloromethane/ethyl acetate (4:1) as eluent to yield yellow liquid product. Yield. 99%.

¹H-NMR (500 MHz, CDCl₃): δ (ppm) 7.40 (t, J = 8.3 Hz, 1H); 6.62 (dd, J = 8.5, 0.9 Hz, 1H); 6.54 (dd, J = 8.1, 0.9 Hz, 1H); 5.96 – 5.84 (m, 1H); 5.31 – 5.13 (m, 2H); 4.22 (t, J = 5.0 Hz, 2H); 4.00 (dt, J = 5.7, 1.4 Hz, 2H); 3.95 (t, J = 5.0 Hz, 2H); 3.84 – 3.56 (m, 12H); 1.68 (s, 6H); MS (m/z): calculated for C₂₁H₃₀O₈, 410.19; found, 433.05 for [M+Na]⁺.

**Compound 4:** 2-((3,6,9,12-tetraoxapentadec-14-en-1-yl)oxy)-N-(4-chloro-3-(trifluoromethyphenyl)-6-hydroxybenzamide (4). The synthesis followed the reported protocol.[⁴] To a solution of 4-chloro-3-(trifluoromethyl)aniline (1.92 g, 9.79 mmol) and DMPU (2.46 g, 19.19 mmol) in 25 mL THF was added n-BuLi (3.92 mL of 2.5 M in hexane, 9.79 mmol) at 0°C. The reaction mixture was stirred at room temperature for 30 mins. In a separate flask, compound 3 (0.8 g, 1.95 mmol) was dissolved in 25 mL THF. The solution of compound 3 was then added to the aniline solution. After addition, the reaction was proceeded at 80°C for 2 h. Water was added to quench the reaction. Then, the aqueous layer was extracted with ethyl acetate. The combined organic layers were washed with 10% aqueous solution of hydrochloric acid, followed by water and brine, then dry over MgSO₄. After solvent evaporated, the crude mixture was purified by column chromatography (silica gel, 4:1 dichloromethane/ethyl acetate mixture) to yield yellow liquid product. Yield. 31%; ¹H-NMR (300 MHz, CDCl₃): δ (ppm) 13.32 (s, 1H), 10.69 (s, 1H), 8.07 (s, 1H), 7.95 (d, J = 8.7, 1H), 7.48 (d, J = 8.7 Hz, 1H), 7.31 (t, J = 8.4 Hz, 1H),
6.67 (d, J = 8.4 Hz, 1H), 6.42 (d, J = 7.6 Hz, 1H), 5.99 - 5.81 (m, 1H), 5.30 – 5.13 (m, 2H), 4.34 – 4.28 (m, 2H), 4.06 – 3.96 (m, 4H), 3.77 – 3.71 (m, 2H), 3.63 – 3.50 (m, 10H).

MS (m/z): calculated for C_{25}H_{29}ClF_{3}NO_{7}, 547.16; found, 570.1 for [M+Na]^+.

**Compound 5: S-(1-(2-((4-chloro-3-(trifluoromethyl)phenyl)carbamoyl)-3-hydroxyphenoxy)-3,6,9,12-tetraoxapentadecan-15-yl) ethanethioate (5).**

To a solution of compound 4 (0.34 g, 0.61 mmol) in 30 mL toluene was added AIBN (0.03, 0.18 mmol) and purged with argon. Thioacetic acid (0.23 mL, 0.18 mmol) was added. The solution was stirred at 100-110 °C overnight under argon atmosphere. Upon completion, toluene was evaporated. The residue was re-dissolved in fresh toluene, and the procedure was repeated for 3 times. The crude mixture was then loaded into chromatographic column over silica gel using dichloromethane, following by 15:1 and 10:1 dichloromethane/ethyl acetate mixture) to yield yellow liquid oil. Yield. 65%. ^1H-NMR (300 MHz, CDCl3): δ (ppm) 10.69 (s, 1H), 8.05 (d, J = 2.2 Hz, 1H), 7.97 (dd, J = 8.7, 2.3 Hz, 1H), 7.49 (d, J = 8.9 Hz, 1H), 7.32 (t, J = 8.3 Hz, 1H), 6.69 (dd, J = 8.4, 1.0 Hz, 1H), 6.43 (dd, 1H), 4.32 (t, J = 4.4 Hz, 2H), 4.02 (t, J = 4.7 Hz, 2H), 3.80 – 3.38 (m, 14H), 2.92 (t, J = 7.1 Hz, 2H), 2.31 (s, 3H), 1.91 – 1.74 (m, 2H);

MS (m/z): calculated for C_{27}H_{33}ClF_{3}NO_{8}S, 623.16; found, 646.0 for [M+Na]^+.

**Compound 6: S-(1-(2-((4-chloro-3-(trifluoromethyl)phenyl)carbamoyl)-3-ethoxyphenoxy)-3,6,9,12-tetraoxapentadecan-15-yl) ethanethioate (6).** Following the protocol for alkylation from literature[^4], a suspension of S-(1-(2-((4-chloro-3-(trifluoromethyl)phenyl)carbamoyl)-3-hydroxyphenoxy)-3,6,9,12-tetraoxapentadecan-15-yl) ethanethioate (0.25 g, 0.4 mmol) and K₂CO₃ (0.14 g, 0.99 mmol) in 10 mL acetone was added Et₂SO₄ (77.8 uL, 0.6 mmol). The reaction was stirred for 48h at ambient temperature. The reaction was added aqueous saturated solution of NH₄Cl and extracted with diethyl ether. The combined organic layers were washed with brine and dried over MgSO₄. After filtration, solvent was
evaporated under reduced pressure. The flash column chromatography was used to purify the mixture using dichloromethane/ethyl acetate (4:1) as eluent to afford yellow liquid oil. Yield. 84%. $^1$H-NMR (500 MHz, CDCl$_3$): $\delta$ (ppm) 8.61 (s, 1H); 8.02 – 7.89 (m, 2H); 7.37 (d, $J$ = 8.6 Hz, 1H); 7.19 (d, $J$ = 8.7 Hz, 1H); 6.50 (m, 2H); 4.14 (t, 2H); 4.00 (quartet, $J$ = 7.0 Hz, 2H); 3.71 (m, 2H), 3.58 – 3.37 (m, 12H); 3.34 (t, $J$ = 6.2 Hz, 2H); 2.82 (t, $J$ = 7.2 Hz, 2H); 2.23 (s, 3H); 1.72 (quintet, $J$ = 6.7 Hz, 2H); 1.30 (t, $J$ = 6.9 Hz, 3H); MS (m/z): calculated for C$_{29}$H$_{37}$ClF$_3$NO$_8$S, 651.19; found, 674.7 for [M+Na]$^+$. 

**Compound 7: N-(4-chloro-3-(trifluoromethyl)phenyl)-2-ethoxy-6-((15-mercapto-3,6,9,12-tetraoxapentadecyl)oxy)benzamide (7).** The protocol for deprotection of thioacetate was adopted from a reported procedure$^{[5]}$. Briefly, the thioacetate-protected PEG-CTB (21.3 mg, 0.03 mmol) was dissolved in 1 mL chloroform/methanol (1:1) mixture. The reaction vessel was purged with argon before addition of tetrabutylammonium cyanide (8.7 mg, 0.03 mmol). The reaction mixture proceeded for 24h. The reaction was monitored by mass spectrometry because the TLC spots of the starting materials and the product gave spots on the same Rf value. After reaction completed, the reaction mixture was partitioned with chloroform and water. The aqueous layer was then extracted with chloroform. The combined organic layers were washed with saturated NH$_4$Cl solution and dried over MgSO$_4$. The purification was employed through flash column chromatography on silica gel using hexane/ethyl acetate (1:1) as the eluting solvent. Yield. 83%. $^1$H-NMR (400 MHz, CDCl$_3$): $\delta$ (ppm) 8.56 (s, 1H), 7.94 (m, 2H), 7.38 (d, $J$ = 8.5 Hz, 1H), 7.20 (t, $J$ = 8.3 Hz, 1H), 6.50 (dd, $J$ = 8.3, 3.9 Hz, 2H), 4.26 – 4.08 (m, 2H), 4.00 (quartet, $J$ = 6.9 Hz, 2H), 3.78 – 3.65 (m, 2H), 3.62 – 3.28 (m, 14H), 2.49 (quartet, $J$ = 7.3 Hz, 2H), 1.74 (quintet, $J$ = 6.4 Hz, 2H), 1.35 – 1.23 (m, 4H); MS (m/z): calculated for C$_{27}$H$_{35}$ClF$_3$NO$_8$S, 609.18; found, 632.4 for [M+Na]$^+$. 

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**SPR ANALYSIS:** The Surface Plasmon Resonance (SPR) assays were performed using a BIACORE X instrument as described before.[6] Biotinylated hairpin DNAs were purchased from JBioS (Tokyo, Japan) and the sequences are shown in Figure S1. Hairpin biotinylated DNA are immobilized to streptavidin-coated sensor chip SA as described before and SPR assays were carried out using HBS-EP buffer (10 mM HEPES pH 7.4, 150 mM NaCl, 3mM EDTA, and 0.005 % Surfactant P20) with 0.1 % DMSO at 25 °C. A series of sample solutions with various concentrations (320 nM- 20 nM) were prepared in the buffer with 0.1 % DMSO and injected at a flow rate of 20 µl/min. The rates of association (ka), dissociation (kd) and the dissociation constant (KD), were analyzed with an appropriate fitting model using the BIAevaluation 4.1 program as mentioned before and the 1:1 binding with mass transfer was used for fitting the sensorgrams.

**SYNTHESIS OF GOLD NANOPARTICLES:** The gold nanoparticles with an approximate diameter of 9 nm were prepared by the Ferns method of citrate reduction of HAuCl4 following establish protocols and a previous report. First, the glassware was cleaned in aqua regia (3 HCl: 1 HNO3, handle with extreme caution!), and oven dried after washing with water. While stirring, a 100 mL aqueous solution of 1mM HAuCl4 was heated to a reflux. Then 16 mL of 1% (by weight) sodium citrate was quickly added, resulting in a change in solution color from yellow to ruby red. After the color change, the solution was refluxed for another 5 minutes, then cooled to room temperature and filtered using a 0.45 µm syringe filter. Characterization of the AuNPs was performed using transmission electron microscopy (TEM) and dynamic light scattering (DLS), and concentration was obtained using UV-vis spectroscopy. The gold nanoparticle concentration was obtained using UV-visible absorption spectra (Varian Cary 5000 UVVis-NIR Spectrophotometer). Second, using Dynamic Light Scattering (Malvern Zetasizer Nano-ZS90), we measured the hydrodynamic size and zeta potential (surface charge) of NanoScript.
CONSTRUCTION OF NANOSCRIPT: NanoScript was constructed using a two-step method. First, the NH2 terminated STF molecules (hairpin polyamide [DBD], transactivation peptide [AD], and TAT peptide [CPP]) were conjugated to linker molecule, SH-PEG-COOH (Thiol-PEG-Carboxy 1KDa [Creative PEGWorks, PBL-8073]). The PEG molecule was dissolved into a 50 mM solution in ethanol. Then, 50 mM of 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide) (EDC) (Sigma) and 50 mM of N-hydroxysuccinimide (NHS) (Acros Organics) was added to this solution and then placed on a shaker for 1 hr. Afterwards, a 5 mM solution of the STF molecule was added to the solution and allowed to react at room temperature for 2 hr. The solution was kept on a shaker during this time, and the percent yield for this EDC/NHS reaction was 96%.[7] The three STF molecules conjugated with PEG (PEG-Sox9-DBD, PEG-AD, and PEG-CPP), were coated on 10 nm AuNPs. A solution containing 10 molar excess of 20% PEG-CPP, 30% PEG-DBD, 25% CTB, and 25% PEG-AD was added drop-wise to the AuNP solution and allowed to stir for 2hr. The functionalized AuNPs (termed NanoScript) were filtered three times using a 10,000 MCWO filter (Millipore) to remove unreacted molecule and to adjust the volume to the desired final concentration. The dye-labeled NanoScript, used for tracking intracellular localization of NanoScript, was constructed by conjugating Alexa Flour 594 (Invitrogen) fluorescent dye to the AD (the transactivation peptide). Specifically, the free carboxyl group on PEG-AD was further conjugated to the Alexa Flour 594 Hydrazide dye via EDC/NHS coupling as described above.

HAT ASSAY: To perform the HAT assay and quantify the increase of HAT activity, we purchased two specialized kits. The first is the Nuclear/Cytosol Fractionation Kit (BioVision K266-100) to collect and purify the nuclear extract. The second is the HAT Activity Fluorometric Assay Kit (BioVision K334-100) to quantify the HAT activity in the samples. Both kits were carried out using the manufacturer’s protocol.
ADMSC CULTURE AND CHONDROGENIC DIFFERENTIATION: Commercially available human adipose-derived mesenchymal stem cells (ADMSCs) (ATCELLs™, American CryoStem) were expanded using the manufacture’s protocol using standard 0.5% FBS growth media. The ADMSCs were expanded on fibronectin (2 μg/cm2, Millipore) coated culture dishes, and maintained at 37°C in a humidified incubator with 5% CO2. For consistency, all experiments were carried out on cells between passages 2 to 4. For chondrogenic differentiation experiments, ADMSCs were seeded in a 12-well plate at a density of 50,000 cells per well. After 24 hr, a 1 nM of NanoScript-Sox9 was incubated with the cells for 4hr, after which, the cells were washed twice with PBS and fresh commercially-available chondrogenic media (ACSelerate CY™ [media formulation is kept confidential by the company], American CryoStem) was added. After 48hrs, the ADMSCs were re-transfected with 1 nM of NanoScript-MRF for 4 hr, washed twice with PBS, and given fresh chondrogenic media. The cells were allowed to differentiate for 7 days with chondrogenic media changes every other day. All conditions and controls were transfected with 1 nM of the constructs.

IMMUNOCYTOCHEMISTRY: To investigate the nuclear localization of the dye-labeled NanoScript in ADMSC cells, the media was removed and the cells were fixed for 15 minutes in formalin (Sigma) followed by two washes with PBS. The nucleus was stained with DAPI (Life Technologies) for 30 minutes and then washed with PBS three times. To investigate the extent of chondrogenic differentiation on Day 7, the media was removed and fixed with formalin for 15 minutes and then washed twice with PBS. Cells were then permeabilized with 0.1% Triton X-100 in PBS for 10 minutes and non-specific binding was blocked with 5% normal goat serum (NGS, Life Technologies) in PBS for 1 hour at room temperature. To study the extent of chondrogenic differentiation, the primary mouse antibody against Collagen II (1:200 dilution, Santa Cruz Biotechnologies) and the primary rabbit antibody against Aggrecan (1:200 dilution, Abcam) was used. Following the manufacturer’s protocol, the fixed samples
were incubated overnight at 4°C in a solution of these antibodies in PBS containing 10% NGS. After washing three times with PBS, the samples were incubated for 1 hr at room temperature in a solution of anti-mouse secondary antibody labeled with Alexa Flour 647 (1:100, Life Technologies), anti-rabbit secondary antibody labeled with Alexa Flour 546 (1:100, Life Technologies), and DAPI (1:100, Life Technologies), in PBS containing 10% NGS. After washing thrice, all the samples were imaged using the Nikon T2500 inverted fluorescence microscope.

**PCR ANALYSIS:** Total RNA was extracted with TRIzol reagent (Invitrogen) and was reverse transcribed to cDNA with Superscript III Reverse Transcriptase (Invitrogen). Conventional quantitative RT-PCR was performed using a SYBR Green PCR Master Mix (Applied Biosystems) on a StepOnePlus Real-time PCR System (Applied Biosystems). Primer sequences are included in the Supplemental Information (Table S1).
FIGURE S1: Sox9 Polyamide Structure and Binding Affinity. 

A) The Sox9 polyamide, with imidazole (red) and pyrrole (blue) motifs arranged in a specific sequence to complement the 5’-ACAATGG-3’ Sox9 response element. B) As deduced from the corresponding SPR sensograms, the Sox9 polyamide has a high binding affinity to the target Sox9 DNA sequence of 5.4×10^{-8}. Furthermore, this result was compared to a scrambled polyamide (SRY polyamide) and a scrambled DNA sequence (SRY sequence).[8] The SRY polyamide had a significantly lower binding affinity to the target Sox9 sequence, while the Sox9 polyamide also had a significantly lower binding affinity to the target SRY sequence, thus confirming specificity and high binding affinity of the Sox9 polyamide to its target sequence.
FIGURE S2: Small Molecules Conjugated on NanoScript-Sox9. Unmodified, citrate-stabilized 10 nm gold nanoparticles (AuNPs) show a characteristic peak at 517 nm. After functionalization with the small molecules, the successive shift in the absorbance peak to 532 nm suggests attachment of biomolecules on the AuNP surface.
FIGURE S3: Hydrodynamic Diameter of NanoScript-Sox9. Using dynamic light scattering, the hydrodynamic diameter of NanoScript-Sox9 was determined to be 57.9 nm.

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<th>Hydrodynamic Diameter (nm)</th>
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<tr>
<td>Unmodified AuNPs</td>
<td>9.8 ± 0.3</td>
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<tr>
<td>NanoScript-Sox9</td>
<td>57.9 ± 8.1</td>
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FIGURE S4: NanoScript Enters ADMSCs and Localizes in the Nucleus: A) After the ADMSCs were incubated with NanoScript-Sox9, 48 hr post-transfection, fluorescence microscopy was used to evaluate intracellular localization of NanoScript. The phase image shows the morphology of the ADMSCs. The combination of Hoechst (blue), dye-labeled NanoScript-Sox9 (red, Alexa Flour 594), and the merged fluorescence images, suggest that NanoScript-Sox9 in uptaken and is localized inside the nucleus. (Scale bar = 25 μm). B) To quantify the percentage of cells that have uptaken NanoScript, we randomly took 6 images (which contained a total of 61 cells) and counted the number of cells with fluorescence overlap.

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<th>Percentage of ADMSCs with Fluorescence Overlap</th>
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<td>51.6 % ± 5.4%</td>
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FIGURE S5: Modified CTB Retains HAT Functionality: To ensure that PEG modification of CTB does not influence its function of inducing HAT activity, we compared HAT activity of unmodified CTB versus PEG-modified CTB. After 48 hours, the HAT activity of cells exposed to both molecules was almost identical; thus indicating that modification to the ortho-position on CTB does not affect the functionality of the molecule.
FIGURE S6: Effect of Gold Nanoparticle CTB Ratios on HAT Activity: The ratio of CTB on the gold nanoparticle (AuNP) can play a role on HAT activity. When the ratio of CTB was varied, we observed an increase in HAT activity in correlation to increasing CTB ratios.
FIGURE S7: NanoScript-Sox9 Concentration Induction of HAT Activity: Various concentrations of NanoScript-Sox9 were incubated with ADMSCs and HAT activity was tested after 24 hours. We observed a concentration-dependent increase of HAT activity.
FIGURE S8: Cell Viability of NanoScript-Sox9 Treated ADMSCs: After the adipose-derived mesenchymal stem cells (ADMSCs) were treated by NanoScript-Sox9 constructs, we performed MTS cell viability assays on Day 7. We observed that all conditions showed high viability as compared to the control.
FIGURE S9: Influence of Free CTB on Gene Expression: The NanoScript-Sox9 (w/o CTB) with free CTB was incubated in ADMSCs and qPCR performed after 7 days to evaluate if free CTB can induce gene expression without being attached to the nanoparticle. The expression of all three chondrogenic genes, Sox9, Aggrecan, and Collagen II, are similar to that of NanoScript-Sox9 (w/o CTB) as shown in Figure 3e. This indicates that for enhanced gene expression, the CTB must be conjugated to the nanoparticle.
FIGURE S10: Gene Expression Levels of Housekeeping Genes: We tested the expression levels of several housekeeping genes (GAPDH, β-actin, and HPRT1),[^9] after 7 days post-transfection. When compared to the untreated control, the expression of these housekeeping gene remained unchanged.

![Expression of Housekeeping Genes](image)

[^9]: Source of the gene expression data.
**TABLE S1: PRIMER SEQUENCES AND THE EXPECTED BAND SIZE.**

<table>
<thead>
<tr>
<th>Target</th>
<th>Forward Primer (5’ – 3’)</th>
<th>Reverse Primer (5’ – 3’)</th>
<th>Expected Size (bp)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aggrecan</td>
<td>CCCCTGCTATTTTATAGGCACC</td>
<td>GACACACGGCTCCACTTGAT</td>
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<tr>
<td>Collagen II</td>
<td>TGGGACCGCCATGAGGTGTCTTCT</td>
<td>TGGGAGCCAGATTCCTGTCT</td>
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<tr>
<td>Sox9</td>
<td>ATCACCCGCTCAGTACGA</td>
<td>GTGGCTGTAGTAGGGAGCTGG</td>
<td>60</td>
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<tr>
<td>GAPDH</td>
<td>CATGTTCAATATGATTCACC</td>
<td>GATGGGATTTCCATTGATGAC</td>
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<tr>
<td>β-actin</td>
<td>CATGTACGTTGCTATCCAGGC</td>
<td>CTCCTTAATGTCACGCAGAT</td>
<td>250</td>
</tr>
<tr>
<td>HPRT1</td>
<td>CCTGGCGTCGTATGATTAGTGTG</td>
<td>AGACGTTTCAGTCTGTCCATGA</td>
<td>131</td>
</tr>
</tbody>
</table>
REFERENCES FOR SUPPORTING INFORMATION


