MATERIALS AND METHODS

Synthesis of Peptides: The corepressor peptide, with a sequence of NH_2-GWRPW-OH, was purchased from GenScript. The membrane penetrating peptide (MPP), with a sequence of NH_2-CALNNAGRKKRRQRRR-OH, was purchased from GenScript.

Synthesis of GFP and Sox9 Hairpin Polyamides: All hairpin polyamides were synthesized by adopting our previously published protocol.[1] The Py-Im (pyrrole [Py], imidazole [Im]) polyamide synthesis was machine assisted using a PSSM-8 peptide synthesizer (Shimadzu, Kyoto) with a computer-assisted operation system at 40 mg of Fmoc-β-Ala-Wang resin (ca. 0.55 mmol/g, 100-200 mesh, Novabiochem) by using Fmoc chemistry. The following synthetic procedure was performed: i) deblocking steps for 4 min (2 times), 20% piperidine in DMF; ii) coupling step for 60 min, using corresponding carboxylic acids, 1H-Benzotriazolium, 1-[bis(dimethylamino)methylene]-5chloro-hexafluorophosphate (1-),3-oxide (HCTU) (88 mg), diisopropylethylamine (DIEA) (36 μL); iii) washing steps for 1 min (5 times) in DMF. In the coupling step, each of the corresponding carboxylic acids were prepared in a 1-methyl-2-pyrrolidone solution of Fmoc-Py-COOH (77 mg), Fmoc-Im-COOH (77 mg), Fmoc-PyIm-COOH (100 mg), and Fmoc-γ-COOH (69 mg), with stirring by N_2 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. After each solution transfer, all lines were washed with DMF. After the completion of the synthesis by the last acetyl capping on the peptide synthesizer, the resin was washed with DMF (1 mL, 2 times) and methanol (1 mL, 2 times), and dried in a desiccator at room temperature *in vacuo*.

To synthesize the GFP polyamide with sequence PyPyPy-β-PyPyIm-γ-PyPyPy-β-PyImPy-β-Dp (γ is γ-aminobutyric acid, β is β-alanine, and Dp is dimethylaminopropylamide), a dried resin was cleaved with 0.4 ml of 3,3'-diamino-N-methyldipropylamine for 3 h at 45 ºC. Then the reaction mixture was filtered, triturated from CH_2Cl_2-Et_2O. This yielded a crude yellow powder. Purification of the crude was performed by flash column chromatography (elution with 0.1% trifluoroacetic acid in water and a 0-35% acetonitrile linear gradient (0-35 min) at a flow rate of 1.8 mL/min^{-1} under 254 nm). ESI-TOF-MS (positive) m/z calculated for $C_{92}H_{115}N_{33}O_{17}^{2+}$ $[M+2H]^{2+}$ 976.76; found 976.94.

To synthesize the Sox9 polyamide with sequence, AcPyPyPy-β-PyImPy-γ-PyPyPy-β-PyImIm-β-NH_2, a dried sample resin was cleaved with 0.4 ml of 3,3'-diamino-*N*-methyldipropylamine for 3 h at 45 ºC. The reaction mixture was filtered, triturated from CH_2Cl_2-

Et_2O, 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). ESI-TOF-MS (positive) m/z calculated for $C_{91}H_{113}N_{34}O_{17}^{2+}$ $[M+2H]^{2+}$ 977.45; found 977.44.

Synthesis of Magnetic Core-Shell Nanoparticles: Both the magnetic cores and core-shell particles were synthesized according to a previously reported protocol with slight modifications.[2] The 10 nm zinc doped iron oxide magnetic cores were synthesized by thermal decomposition. In a 100 mL 3-neck round bottom flask, 1.5174 mmol $Fe(Acac)_3$, 0.4825 mmol $FeCl_2$, 0.3338 mmol $ZnCl_2$, 10 mmol 1,2-hexadecandiol, 6 mmol oleic acid, 6 mmol oleylamine and 20 mL tri-n-octylamine were mixed at 150°C under vacuum for 45 min. The vacuum was then removed and the temperature was increased to 200°C at a rate of 4°C per min under dry air for 2 hr, and then further increased to 300°C for 30 min at rate of 4°C per min also under dry air. The reaction mixture was cooled to room temperature and the particles were purified by dispersing the reaction mixture in ethanol and centrifuging at 10,000 rpm several times to produce a dry pallet. The particles were then dispersed and stored in chloroform. The particles were characterized by dynamic light scattering (DLS) using a Malvern Instruments Zetasizer Nano ZS-90 and a Philips CM12 transition electron microscope (TEM).

The gold-coated magnetic nanoparticles (MNP@Au) were synthesized by reducing $AuCl_3$ on the 10 nm zinc-doped iron oxide magnetic cores. In a 50 mL 3-neck round bottom flask, 5 mgs of the 10 nm magnetic cores were mixed in 20 mL of tri-n-octylamaine and heated to 60°C under vacuum for 10 min to evaporate the chloroform. Upon cooling the reaction mixture to room temperature, 0.3mmol (100 µL) of oleylamine and 60 µl of a 5 mg/300 µL stock solution of $AuCl_3$ were added and heated to 70°C under vacuum to evaporate the solvents, after which the temperature was increase to 150°C at a rate of 10°C per min under atmosphere for 4 hr. The reaction mixture was then cooled to room temperature and centrifuged at 10,000 rpm to collect the particles. The particles were purified with chloroform and magnetically decanted several times. The purified particles were dispersed and stored in minimal amount of chloroform.

The chloroform dispersed MNP@Au were rendered water soluble by carrying out a ligand exchange in TMAOH and citrate buffer. A TMAOH solution was prepared by dissolving 0.09 g of trisodium citrate in 15 mL of 1 M TMAOH. The previously prepared MNP@Au particles were added to the TMAOH solution and sonicated using a probe sonicator for 30 min. The solution was magnetically decanted and the particles were purified several times using DI water and magnetic

decantation and finally dispersed in DI water. The citrate-capped core-shell particles were verified using a Cary US UV-Vis spectrometer and a Philips CM12 TEM.

Construction of NanoScript: We developed the NanoScript platform using a two-step method. First, the three amine terminated biomolecules (WRPW peptide, MPP peptide, and GFP/Sox9 polyamides) were conjugated to a linker molecule, SH-PEG-COOH (Thiol-PEG-Carboxy 1KDa [Creative PEGWorks, PBL-8073]). 50 mM of 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide) (EDC) (Sigma) and 50 mM of N-hydroxysuccinimide (NHS) (Acros Organics) was added to a solution of PEG (50 mM in EtOH), and placed on a shaker for 1 hr. A solution containing 10 molar excess of PEG-WRPW, PEG-MPP, and PEG-GFP/Sox9 polyamide (5 mM) with a mole ratio of 2:1:2 respectively was added drop-wise to the nanoparticle solution and allowed to stir for 2 hr. The functionalized nanoparticles (termed NanoScript) were filtered three times using a 10,000 MCFWO filter (Millipore) to remove unreacted molecules and to adjust the concentration.

The dye-labeled NanoScript, used for tracking intracellular localization of NanoScript, was constructed by conjugating the Alexa Flour 568 (Invitrogen) fluorescent dye to the PEG molecules. Specifically, the free carboxy group on PEG was conjugated to the Alexa Flour 568 Hydrazide dye via EDC/NHS coupling as described above.

Characterization of NanoScript was performed using multiple methods. The nanoparticle concentration and confirmation of functionalized was obtained using UV-visible absorption spectra (Varian Cary 5000 UV Vis-NIR Spectrophotometer). Using Dynamic Light Scattering (Malvern Zetasizer Nano-ZS90), we determined the hydrodynamic size of NanoScript. The shape and monodisperse properties of the nanoparticles was confirmed using transmission electron microscopy (TEM). The nanoparticles were drop-cast on the Holey-carbon grids (Electron Microscopy Sciences), allowed to dry overnight under vacuum, and subsequently imaged using a JEOL JEM-2010F high-resolution TEM operated at an accelerating voltage of 200 kV.

SPR Binding Affinity: The SPR assays were performed using a BIACORE X instrument. The biotinylated hairpin DNAs that is complementary for each of the polyamides were purchased from JBioS (Tokyo, Japan) (See table below for the biotinylated hairpin DNA sequences).

For GFP Polyamide	3’-CCGA**GGTATACAA**CGGTTTTCCG**TTGTATACC**TCCG-Biotin-5’
For Sox9 Polyamide	3’-GCGT**GGTAACA**AGCTTTTGCT**TGTTACC**ACGC-Biotin-5’

The hairpin biotinylated DNA was immobilized to streptavidin-coated sensor chip SA to obtain the desired immobilization level (approximately 900 RU rise). SPR assays were performed using HBS-EP buffer (10 mM HEPES pH 7.4, 150 mM NaCl, 3 mM EDTA, and 0.005 % Surfactant P20)

with 0.1% DMSO at 25°C. A series of sample solutions with various concentrations were prepared in the buffer with 0.1 % DMSO and injected at a flow rate of 20 μl/min. To measure the rates of association (k_a), dissociation (k_d) and dissociation constant (K_D), data processing was performed with a fitting model using the BIAevaluation 4.1 program. The 1:1 binding with mass transfer was used for fitting the sensorgrams.

Rat Neural Stem Cell (rNSC) Culture and GFP Knockdown: The GFP-labeled rat neural stem cells (rNSCs) were purchased from Millipore and cultured according to the manufacturers' protocol. The culture media specific for rNSCs was purchased from Millipore. All cells were maintained at 37°C in a humidified incubator with 5% CO_2. All experiments were carried on cells between passage 3 and 5.

For GFP knockdown studies, the cultured rNSCs (75,000/well, 12-well plate) were transfected with the NanoScript-GFP constructs (1 nM) in growth medium, and the cell culture plates were placed on the Nd-Fe-B magnetic plates (OZ Biosciences, France) for 15 mins. After 4 hr, the cells were washed twice with PBS and fresh rNSC media (now without bFGF to stop proliferation) was added. The rNSCs were transfected on Day 0 and Day 2, and the GFP knockdown levels was imaged and analyzed on Day 2 and Day 4. Fresh media was exchanged every other day.

Human Neural Stem Cell (hNSC) Culture and Neuronal Differentiation: The human neural stem cell (hNSC) line was purchased from Millipore and cultured according to the manufacturer's protocol. All cells were maintained at 37°C in a humidified incubator with 5% CO_2. All experiments were carried on cells between passage 3 and 5. The hNSCs were seeded in plates (125,000/well, 12-well plate; and 62,500/well, 24-well plate) in hNSC specific media (from Millipore) supplemented with basic fibroblast growth factor (bFGF, 20 ng/mL) and epidermal growth factor (EGF, 20 ng/mL), 24 hr prior to experimentation.

To induce neuronal differentiation, the NanoScript-Sox9 constructs (1 nM) were added to the hNSCs with the culture plates placed a magnetic plate as described above, and after 4 hr, the cells were washed twice with PBS and fresh hNSC media (without growth factors to stop proliferation) was added. The hNSCs were transfected on Day 0 and Day 2, and the gene expression were analyzed on Day 3 (for the *Sox9* gene) and Day 5 (for the *Tuj1* gene) through qPCR. To quantify cell viability, a MTS assay (Promega) was performed on Day 5. For the study involving patch-clamp, the cells were seeded on cover slips with media exchanges occurring every other day.

Immunocytochemistry: All fluorescence images were obtained using a Nikon T2500 inverted fluorescence microscope. To investigate the nuclear localization of the dye-labeled NanoScript in hNSCs, 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 neuronal differentiation on Day 5, the hNSCs were 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 hr at room temperature. To study the extent of neuronal differentiation, the mouse monoclonal antibody against *Tuj1* (1:200 dilution, Covance MMS-435P) and a rabbit polyclonal antibody against *Sox9* (1:200 dilution, Abcam ab26414) 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 568 (1:100, Life Technologies) and DAPI (1:100, Life Technologies), in PBS containing 10% NGS, and washed three times thereafter.

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) following the manufacturers protocol. Primers sequences for the genes can be found in **Table S1**.

SEM Imaging Preparation: To investigate the morphology of neuronal differentiation, the basal medium of mature neurons was removed and the neurons were fixed for 15 minutes in Formalin solution (Sigma) followed by two PBS washes. The biological samples were then dehydrated in ethanol. The dehydration process entailed replacing PBS with 50% ethanol/water, 70% ethanol/water, 85% ethanol/water, 95% ethanol/water, and absolute ethanol for 10 minutes each in succession. The biological samples were then stored in absolute ethanol before transferring to critical point dryer to eliminate traces of ethanol. Then 20 nm of platinum was sputter coated onto the surface of biological samples after drying. Zeiss Sigma Field Emission-Scanning Electron Microscope (FE-SEM) was used to acquire the micrographs.

Calcium Imaging: Fluorescent calcium indicator dye Fluo4 AM (Life Technologies) was used for calcium imaging experiments. The dye was dissolved in DMSO and added to the cell culture to achieve a final concentration of 2 μM. Cells were incubated for 20 min with Fluo4 AM dye. Free dye was washed out with pre-warmed HBSS (Life Technologies). Cells then were incubated for 30 min in DMEM media (Life Technologies) for de-esterification of the Fluo4 AM dye. The media was replaced with pre-warmed HBSS during the imaging session. Images were acquired using a Zyla sCMOS camera (Andor) mounted on an Olympus IX71 using a 20x, 0.75 NA objective. Images were taken at 0.5 sec intervals for 1 min. Calcium imaging movies were displayed at 20 Hz. Images for calcium imaging were processed and intensity was quantified using the NIH ImageJ software.

ICP-OES Measurement: After hNSCs were seeded in a 12-well plate (125,000 cells/well), 1 nM of NanoScript was added. After 6 hours, the cells were washed with PBS and detached using Accutase (Gibco). After centrifugation, the supernatant was removed and 25 μL of lysis buffer was added. Then 180 μL of aqua regia (caution: extremely reactive) was added and allowed to sit overnight in an eppendorf tube (Note: Immediately after closing the cap of the eppendorf tube, puncture a small hole in the cap to prevent pressure buildup within the tube). The next day, the solution was transferred to 5.8 mL of distilled water (6 mL total volume with 3 % aqua regia). Cellular uptake experiments were performed three times and each replicate was measured for gold, iron, and zinc content three times by Inductively coupled plasma optical emission spectroscopy (Perkin Elmer Optima 7300 DV), operating under normal conditions.

Cell Transmission Electron Microscopy (TEM): hNSCs cells were cultured with NanoScripts using the same method as above. 24 hr post-transfection, the cells were trypsinized and fixed with Trump's Fixative (Electron Microscopy Sciences) for 1 hr, washed with sodium cacodylate buffer (Electron Microscopy Sciences), suspended in a 1% osmium tetroxide solution for 1 hr, washed with water, and then progressively dehydrated with ethanol (50, 70, 80, 95, 100%) . Then the cells were embedded in epoxy resin using the Low Viscosity Embedding Media Spurr's Kit (Electron Microscopy Sciences) following the manufacturer's protocol. The images were obtained with the JEOL 100CX TEM.

SUPPORTING FIGURES AND TABLES

Figure S1 – GFP Promoter Sequence

The GFP-labeled rat neural stem cells (rNSCs) were commercially purchased and the first 700 base pairs upstream from the GFP promoter region was provided by the vender. This GFP sequence contains several motifs (highlighted in green) which complement the 5'-CANNTG-3' target sequence of the GFP polyamide.

GAAGAACACCTGCCTGCAGGAAGTGATGAGCTACAAGGTCAACCTGGAGGGCATCGTGAAC
AACCACGTCTTTACCATGGAGGGCTGCGGCAAGGGCAACATCCTGTTCGGCAACCAATTGGT
GCAGATCCGCGTGACCAAGGGCGCCCCCCTGCCCTTCGCCTTCGACATCGTGAGCCCCGCCT
TCCAGTACGGCAACCGTACGTTCACAAAGTACCCCAACGACATCAGCGACTACTTCATCCAG
AGCTTCCCCGCCGGCTTCATGTACGAGCGCACCCTGCGCTACGAGGACGGCGGCCTGGTGGA
GATCCGCAGCGACATCAACCTGATCGAGGACAAGTTCGTGTACCGCGTGGAGTACAAGGGC
AGCAACTTCCCCGACGACGGGCCCGTGATGCAGAAGACCATCCTGGGCATCGAGCCCAGCT
TCGAGGCCATGTACATGAACAACGGCGTGCTGGTGGGCGAGGTCATCCTGGTGTACAAGCT
GAACAGCGGCAAGTACTACAGCTGCCACATGAAGACCCTGATGAAGAGCAAGGGCGTGGTC
AAGGAGTTCCCCAGCTACCACTTCATCCAGCACCGCCTGGAGAAGACCTACGTGGAGGACG
GCGGCTTCGTGGAGCAGCACGAGACCGCCATCGCCCAGATGACCAGCATCGGCAAGCCCCT
GGGATCTCTGCACGAGTGGGTGTAG

Figure S2 – Structure and Binding Affinity for GFP Polyamide

(a) The hairpin polyamide specific for GFP was synthesized to target the GFP promoter 5'-CANNTG-3', with the pyrrole group targeting the A-T base pairs and the imidazole group targeting the G-C base pairs. **(b)** Surface plasmon resonance (SPR) sensograms show the binding chatacteristis of the GFP polyamide to the target GFP sequence, and reveals a tight nanomolar binding affinity of 9.0×10^{-9}. The equilibrium constant (K_D), which is indicative of the binding affinity, was determined by the ratio of the dissociation constant (k_d) to the association constant (k_a).

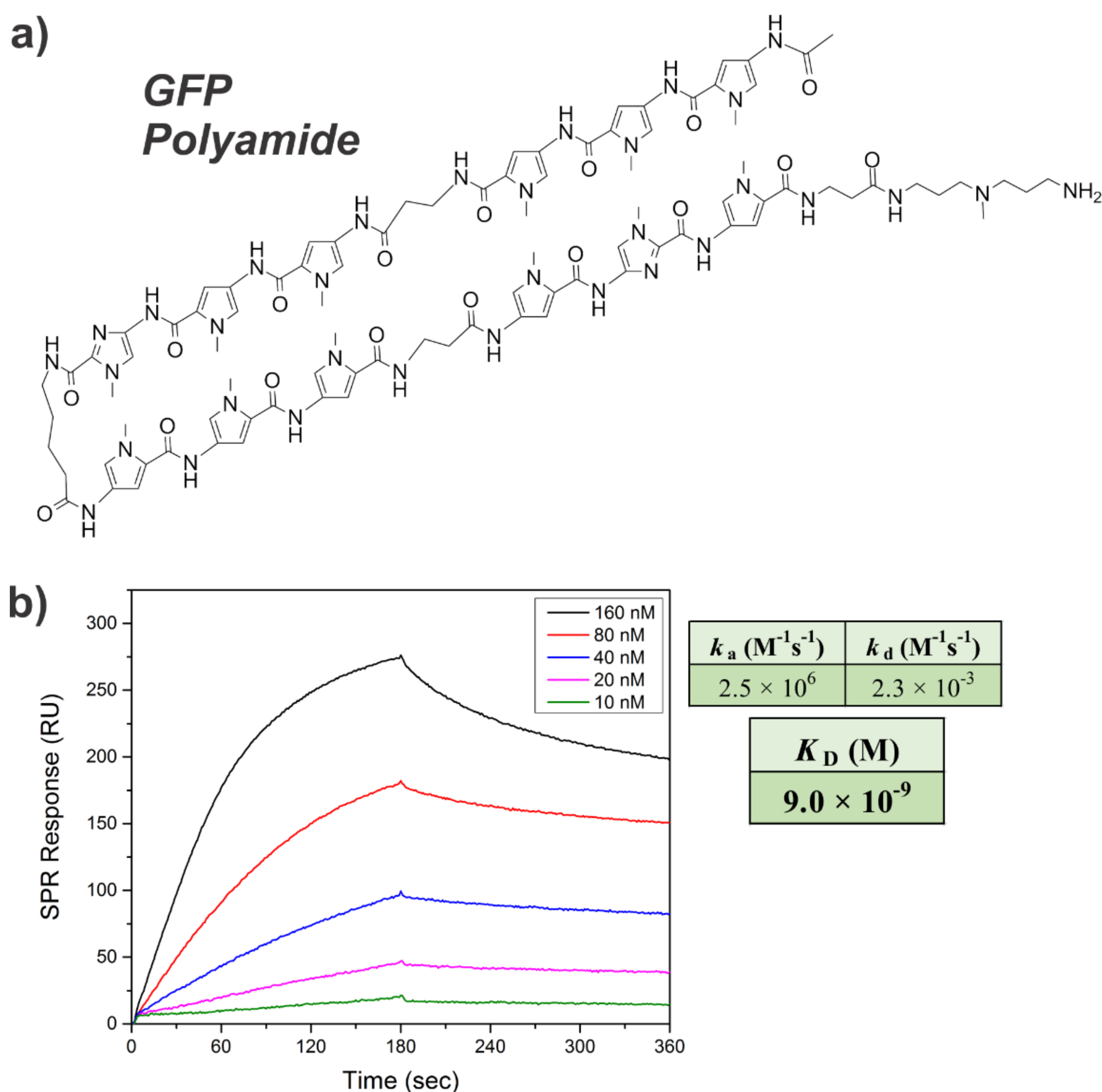
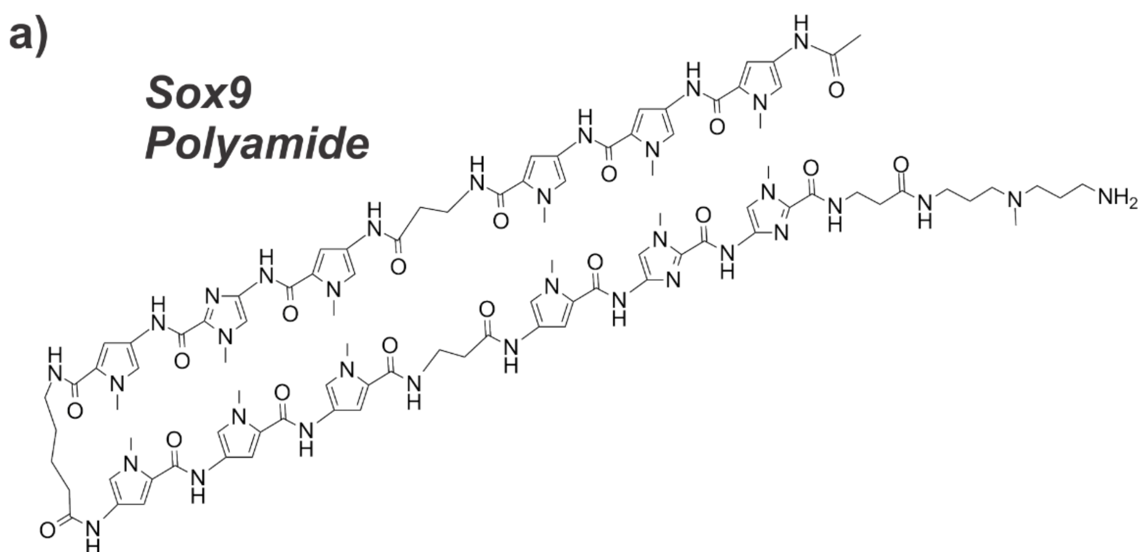


Figure S3 – Structure and Binding Affinity for Sox9 Polyamide

(a) The hairpin polyamide specific for Sox9 was synthesized to target the Sox9 promoter 5'-ACAATGG-3', with the pyrrole group targeting the A-T base pairs and the imidazole group targeting the G-C base pairs. **(b)** SPR sensograms show the binding characteristics of the Sox9 polyamide to the target Sox9 sequence, and reveals a tight binding affinity of 5.4×10^{-8}. The equilibrium constant (K_D), was determined by the ratio of the dissociation constant (k_d) to the association constant (k_a).



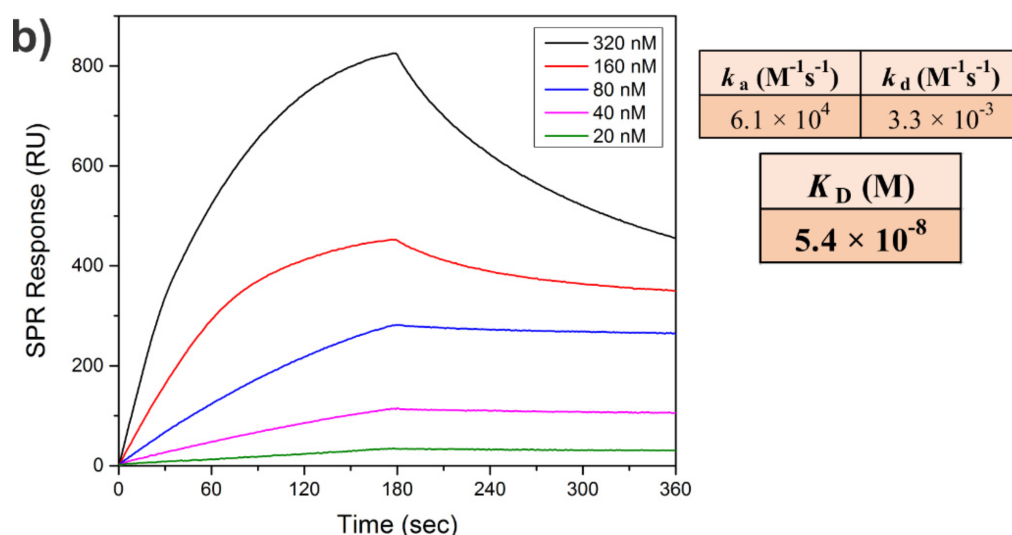


Figure S4 – High Resolution Microscopy of MCNP

A high resolution TEM image of an MCNP reveals the total size to be 17.3 nm. Specifically, based on changes in the refractive shades between the gold (Au) shell and magnetic core, we approximated the magnetic core diameter to be 13.9 nm and the Au shell thickness to be 1.7 nm.

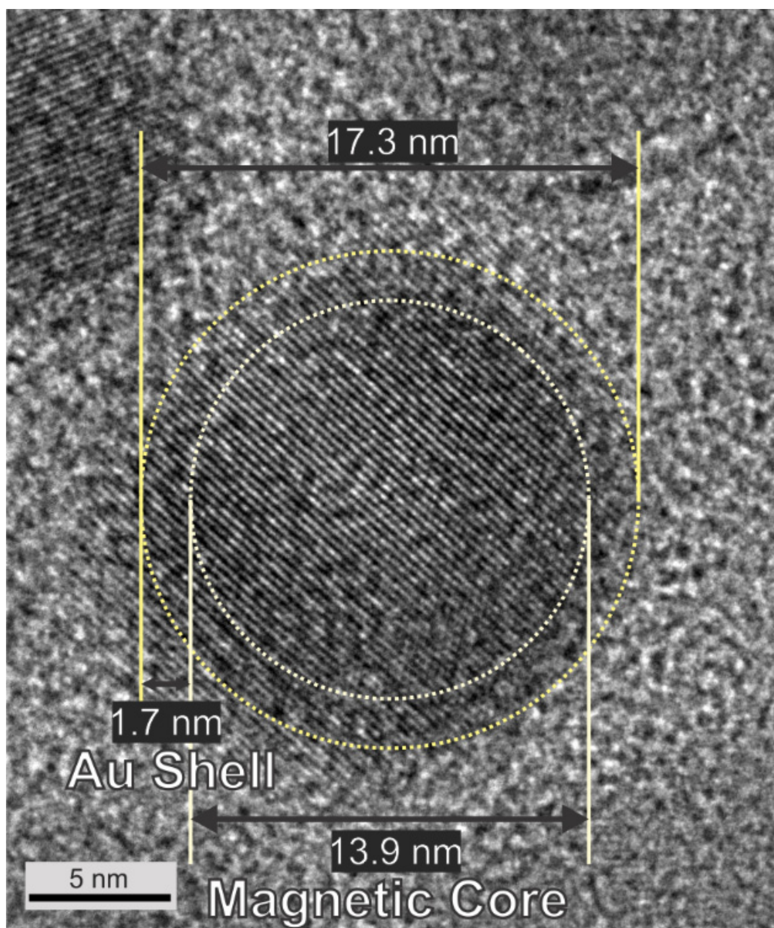


Figure S5 – UV Absorbance of NanoScript

To confirm the functionalization of the magnetic core-shell nanoparticles (MCNPs), we performed UV absorbance. The unmodified gold-shell MCNPs showed a characteristic peak at 524 nm. Adsorption of the biomolecules alters the surface plasmon resonance AuNPs,[3] and hence, we observed a shift in the absorbance peak of the NanoScript-GFP and NanoScript-Sox9 to 530 and 531 nm respectively.

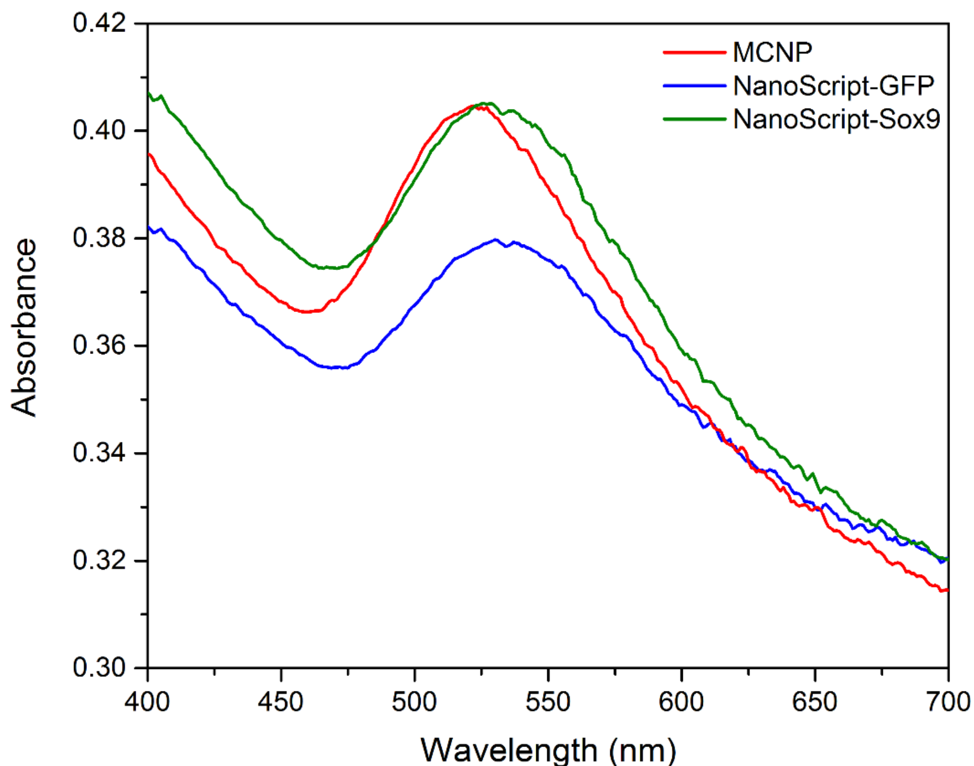


Figure S6 – Intracellular Localization of MCNPs

The intracellular localization of NanoScript was evaluated using fluorescence imaging. Results show that NanoScript (with the MMP and magnetic field application) is able to enter the nucleus. If the magnet is removed the nanoparticle (with the MPP) can moderately enter the cell due to the MPP peptide, but if the MPP is removed from the nanoparticle, also no particles enter the cell. (scale bar = 20 μm)

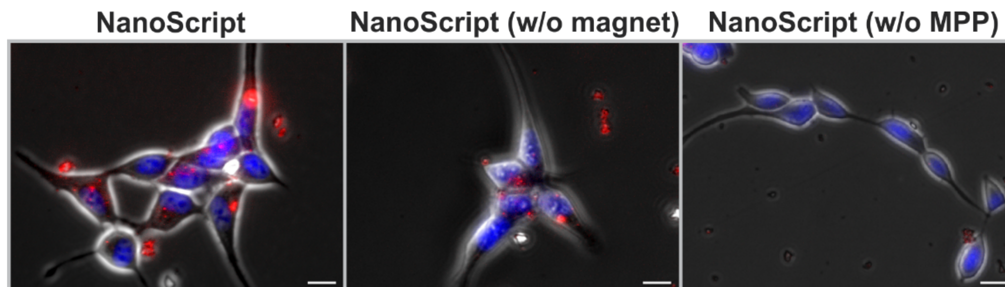


Figure S7 – Cellular Cross-Section TEM

The NanoScript was transfected into the hNSCs, and after 24 hours, we performed transmission electron microscopy (TEM) on the cellular cross sections. The NanoScript particles (indicated by yellow arrows) were found to be present in both the nucleus (purple outline) and the cytoplasm.

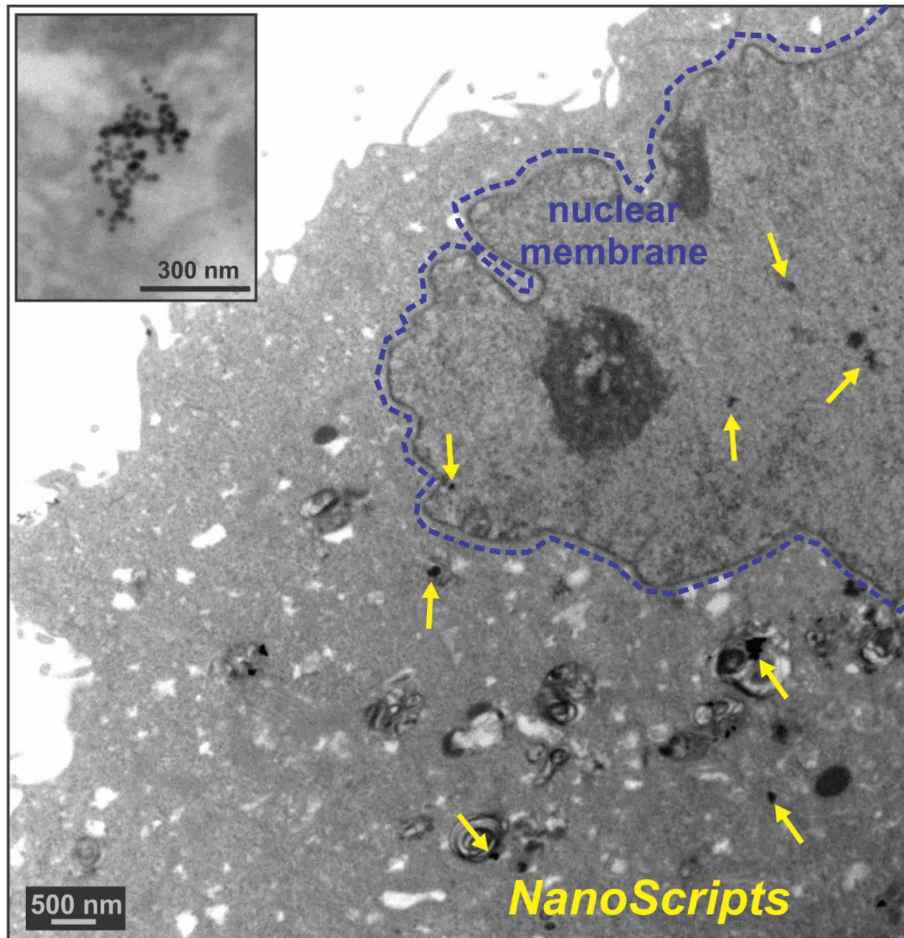


Figure S8 – ICP-OES Quantification of MCNP Cellular Uptake

To evaluate the cellular uptake of MCNPs in hNSCs, we performed inductively coupled plasma optical emission spectrometry (ICP-OES). After 6 hours of transfection, we observed that magnetic nanoparticles (MCNPs) are most readily uptaken when the MPP and magnetic field application is present. Without the magnet, about half the MCNP enter the cell, and if the MPP is removed, there is minimal uptake. The units in the vertical axis are nanograms of MCNPs uptaken per well (ng/well); each well contained 125,000 cells at the time of measurement.

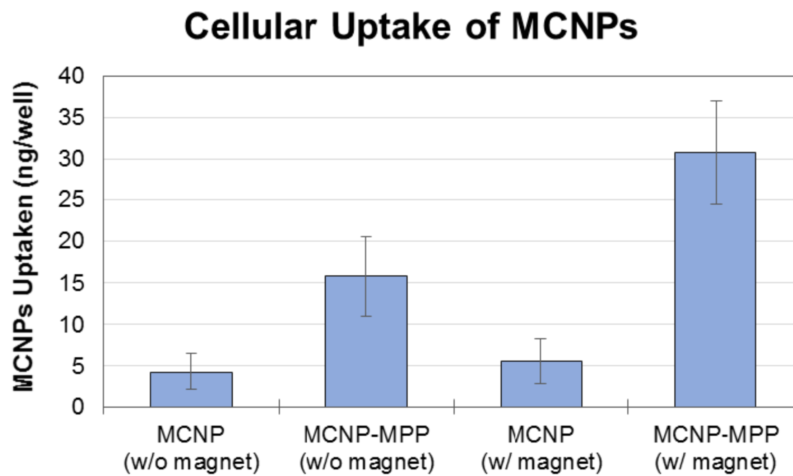


Figure S9 – GFP Knockdown of NanoScript Controls

The GFP knockdown of NanoScript controls was evaluated and we found that the MPP, either alone or on the MCNP, itself has almost no direct influence on GFP knockdown. Moreover, in the absence of the magnet the NanoScript-Sox9 was able to induce about 21% GFP knockdown (which is roughly half as compared to the presence of the magnet). The GFP knockdown values are the mean of six images and are relative to the untreated controls.

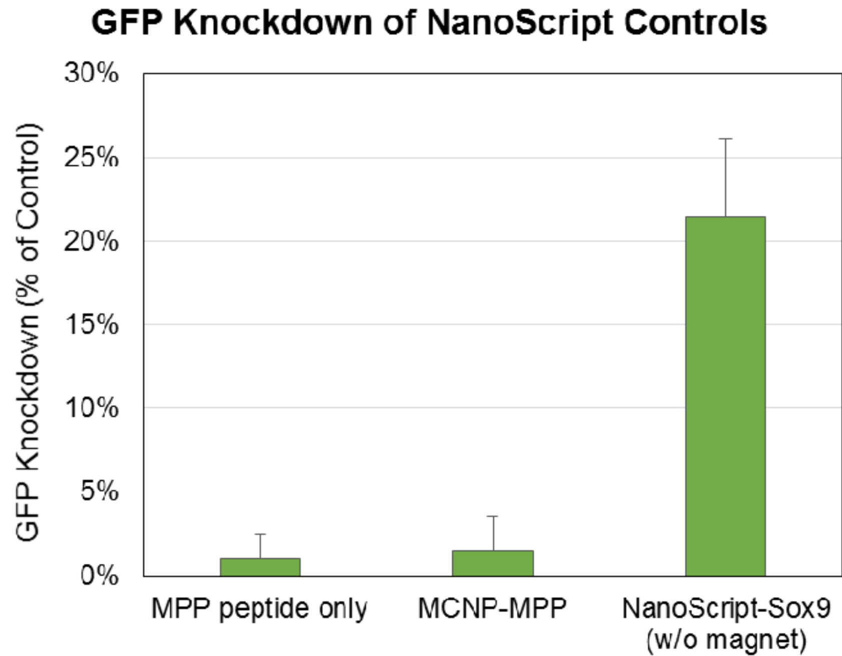


Figure S10 – mRNA Expression of GFP Knockdown

The GFP expression of NanoScript-transfected rNSCs was quantified on Day 4 with qPCR and results showed a similar trend as in the images of Figure 3. GFP mRNA levels are relative to the untreated control.

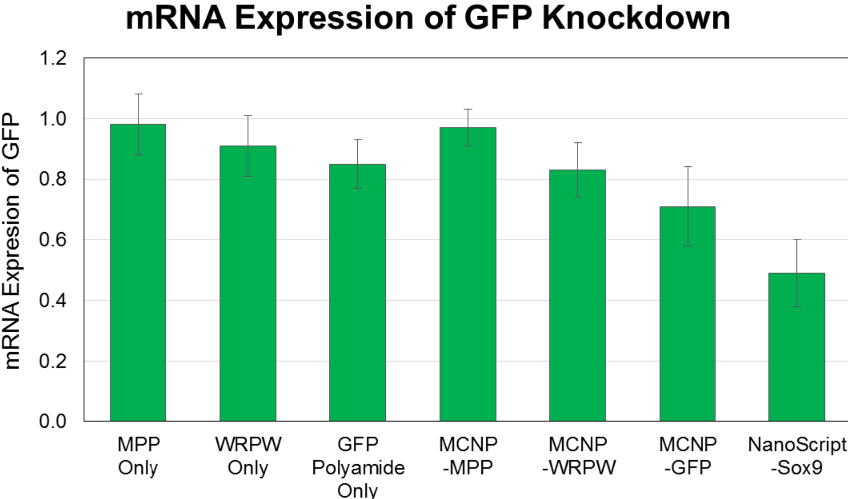


Figure S11 – Gene Expression of Control Conditions

To evaluate the effect of control conditions including Sox9 Polyamide only and WRPW Peptide only, we performed qPCR to measure the expression levels of *Sox9* and *Tuj1* at Day 3 and Day 5 respectively. Results showed that expression of Sox9 was minimally repressed and Tuj1 was minimally overexpressed. This trend follows previous literature wherein greater levels of Sox9 repression correlates to greater levels of Tuj1 overexpression. Expression levels were calculated by normalizing to the control and standard error is from three independent experiments.

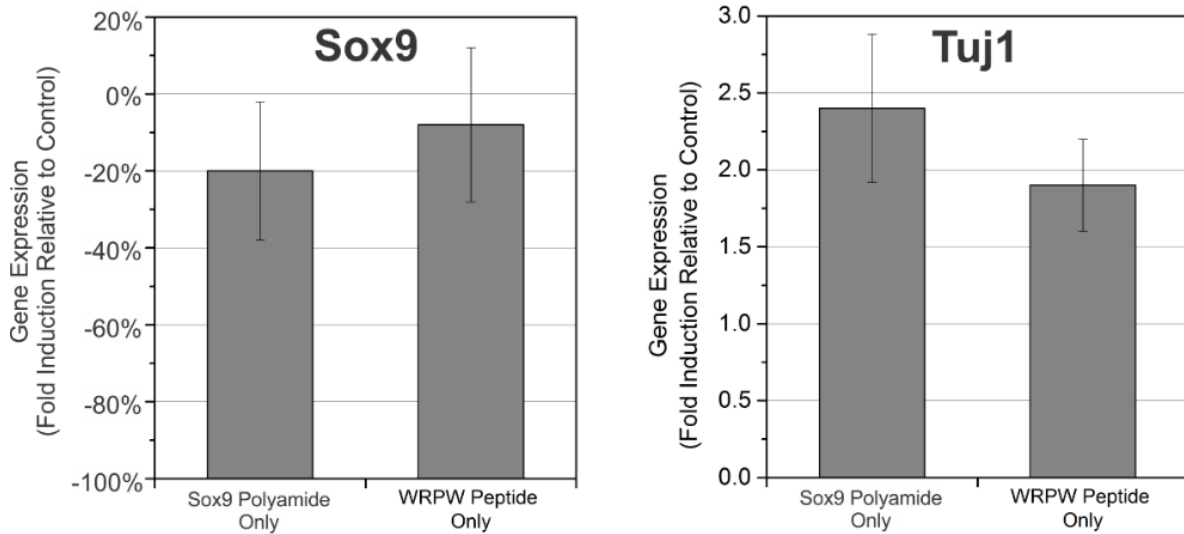


Figure S12 – Immunostaining of Sox9

The expression of Sox9 protein was evaluated via immunostaining and revealed a decreasing trend of Sox9 expression (green, right column) when the NanoScript-Sox9 was applied. (scale bar = 40 μm)

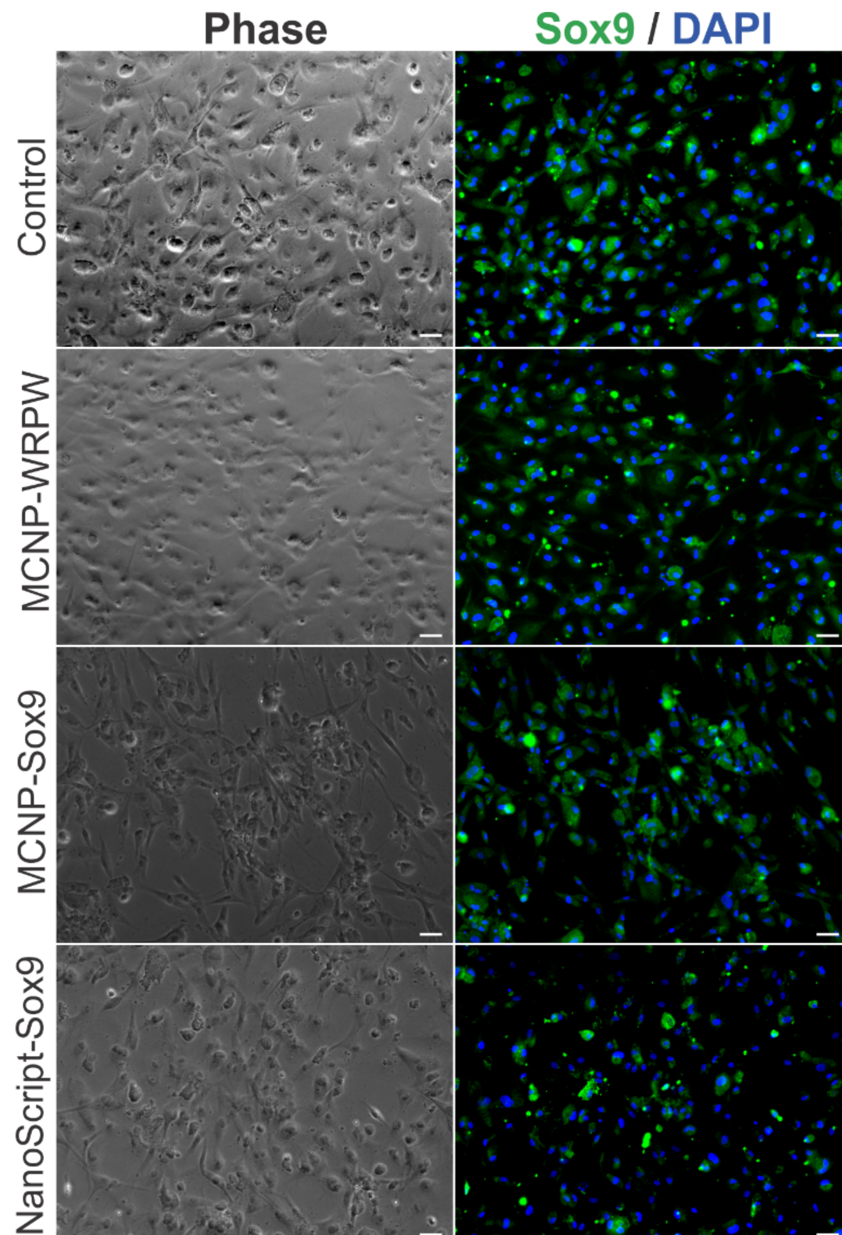


Figure S13 – Neuronal Morphology

We performed scanning electron microscopy (SEM) 5 days post-transfection to visualize the characteristic cellular morphology of the induced neurons (scale bar = 10 μm).

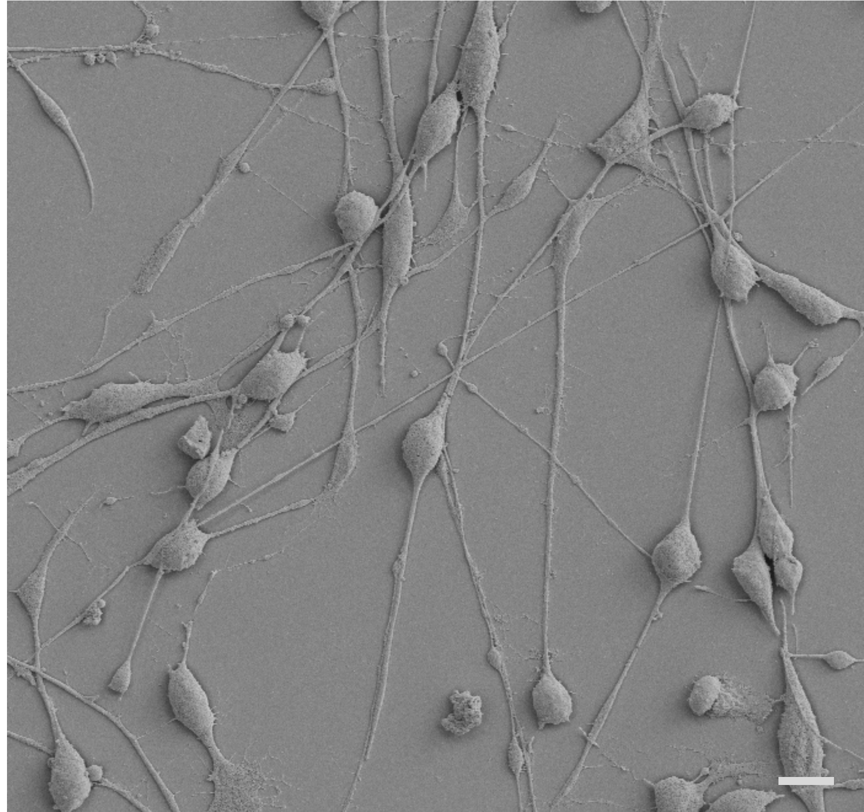


Figure S14 – NanoScript has High Cell Viability

To evaluate the biocompatibility of our NanoScript conditions, we performed a cell viability assay (PrestoBlue) and found that all the conditions had a high viability as compared to the untreated control. Percent viability is relative to the control and standard error is from three independent experiments.

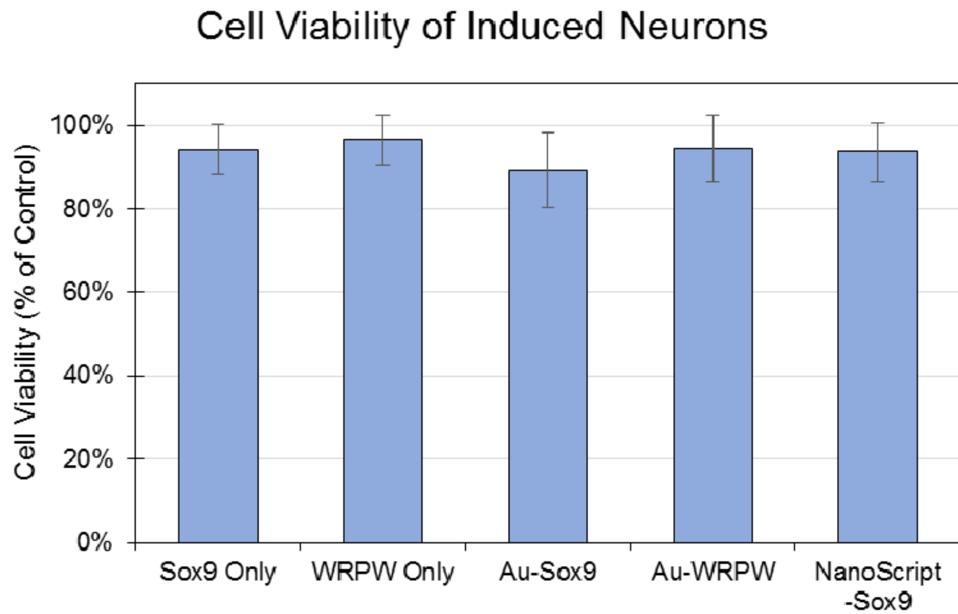
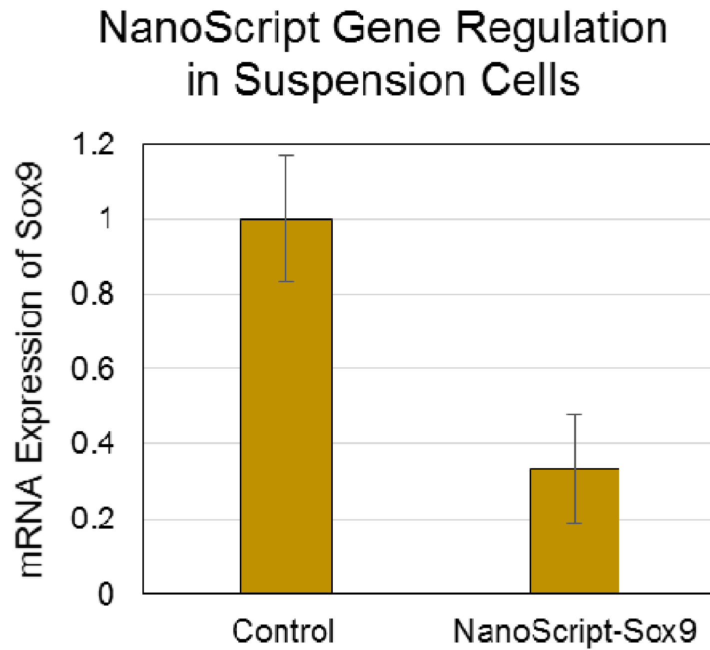


Figure S15 – NanoScript-based Gene Regulation in Suspension Cells.
The NanoScript-Sox9 platform was tested for its gene-regulating applicability in hNSCs suspension cells. The hNSCs were cultured in suspension and 3 days after NanoScript-Sox9 was transfected, we evaluated Sox9 expression levels. We found that NanoScript-Sox9 was able to knockdown Sox9; however further testing and optimization is ongoing to validate NanoScript's application for other cell types.



Video S1 – Movement of Intracellular Calcium Ions

Spontaneous activities in neuronal cell bodies were observed as a change in fluorescence of the Fluo4 dye (orange pseudocolor) due to changing levels of calcium ions. Movie represents 120 images acquired at 0.5 sec intervals for 1 min and displayed at 20 Hz.

Table S1 – Primer Sequences

Target	Forward Primer (5' – 3')	Reverse Primer (5' – 3')
Tuj1	GGCCGCAACCAAAATTCAGG	CAGCTCCGACAGATCCAGT
Sox9	ATCACCCGCTCACAGTACGA	GTGGCTGTAGTAGGAGCTGG
GFP	CCACATGAAGCAGCAGGACTT	GGTGCGCTCCTGGACGTA
GAPDH	CATGTTCCAATATGATTCCACC	GATGGGATTTCCATTGATGAC

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