Theranostics
Submitted to The Forum for Diagnostics, Imaging, and Therapy Research

Supporting Information

Verification of Long-term Genetic Stability of hMSCs during Subculture after Internalization of Sunflower-type Nanoparticles (SF-NPs)

Ji Sun Park[1], Se Won Yi[1], Hye Jin Kim, Hyun Jung Oh, Jung Sun Lee, Min Yeon Go, Sung Han Shim*, Keun Hong Park*

[*] Prof. Keun-Hong Park, Dr. Ji Sun Park, Se Won Yi, Hye Jin Kim, Hyun Jung Oh, Jung Sun Lee
Laboratory of Nano-regenerative Medical Engineering, Department of Biomedical Science, College of Life Science, CHA University, 618, CHA Biocomplex, Sampyeong-Dong, Bundang-gu, Seongnam-si, 13488, Republic of Korea.
E-mail: pkh0410@cha.ac.kr

[*] Prof. Sung Han Shim and Min Yeon Go
Laboratory of Molecular Genetics, Department of Biomedical Science, College of Life Science, CHA University, 629, CHA Biocomplex, Sampyeong-Dong, Bundang-gu, Seongnam-si, 13488, Republic of Korea.
E-mail: shshim@cha.ac.kr

[1]These authors contributed equally to this work.

***Co-corresponding Authors:** Prof. Sung Han Shim (shshim@cha.ac.kr) and Prof. Keun-Hong Park (pkh0410@cha.ac.kr)

EXPERIMENTAL METHODS

Cytotoxicity of SF-NPs

The cytotoxicity of SF-NPs complexed with pDNA complexes was assessed using the LIVE/DEAD® Fixable Green Dead Cell Stain Kit (Molecular Probes Invitrogen, USA) and the Cell Counting Kit-8 (CCK-8) assay. The LIVE/DEAD kit is a two-color fluorescence cell viability assay that measures two recognized markers of cell viability, intracellular esterase activity and plasma membrane integrity. The optimal dyes are 2 μm calcein AM and 4 μm EthD-1; the former stains live cells green, whereas the latter stains dead cells red. Cell viability was observed by fluorescence microscopy (Zeiss). CCK-8 assays (Dojindo Molecular Technologies, Gaithersburg, MD, USA) were performed to monitor cellular activities. Briefly, CCK-8 solution (10 μl) was added to each sample, followed by incubation at 37 °C for 3 h. After the incubation, optical density (OD) at 450 nm was measured using a microplate reader.

Histological analysis in 3D pellets

SF-NP–treated T1 cells were detached after 6 h and pelleted; T4 cells were pelleted after three additional subculture passages. The T1 and T4 pellets were maintained in DMEM/High media for 21 days. Histological analyses were conducted on pellet aggregates fixed in 4% paraformaldehyde; sectioning and staining with Alcian blue and Safranin O were performed as outlined previously.[25]

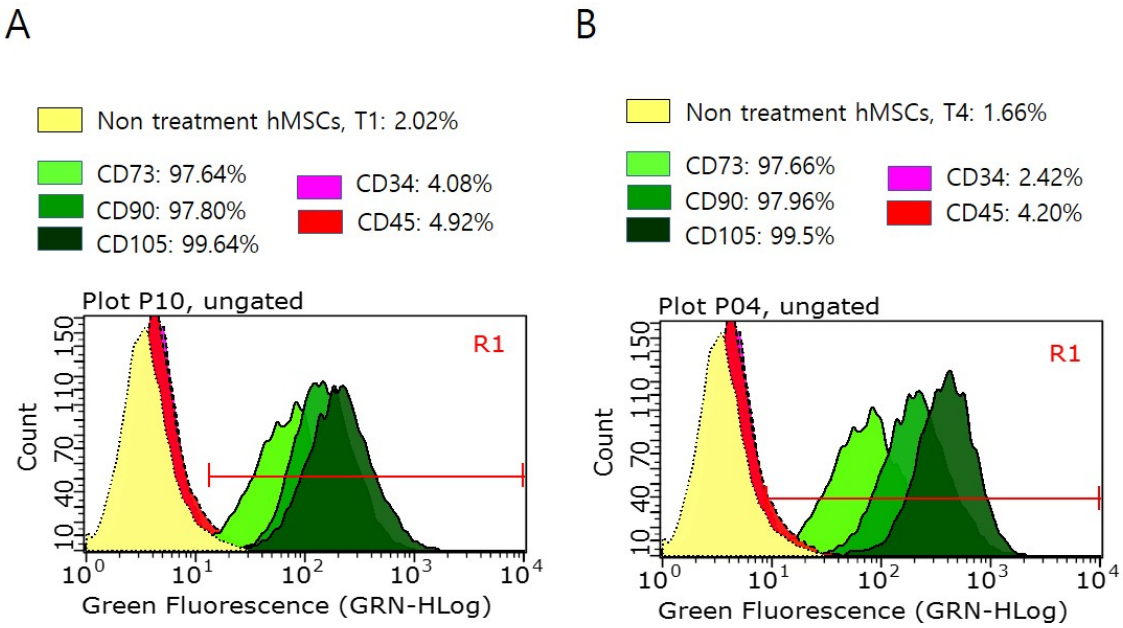


Figure S1. FACS analysis of the surface markers of hMSCs

the positive markers of hMSCs (CD73, CD90, and CD105) and the negative markers of hMSCs (CD34, and CD45) were detected by FACS analysis of immunostaining

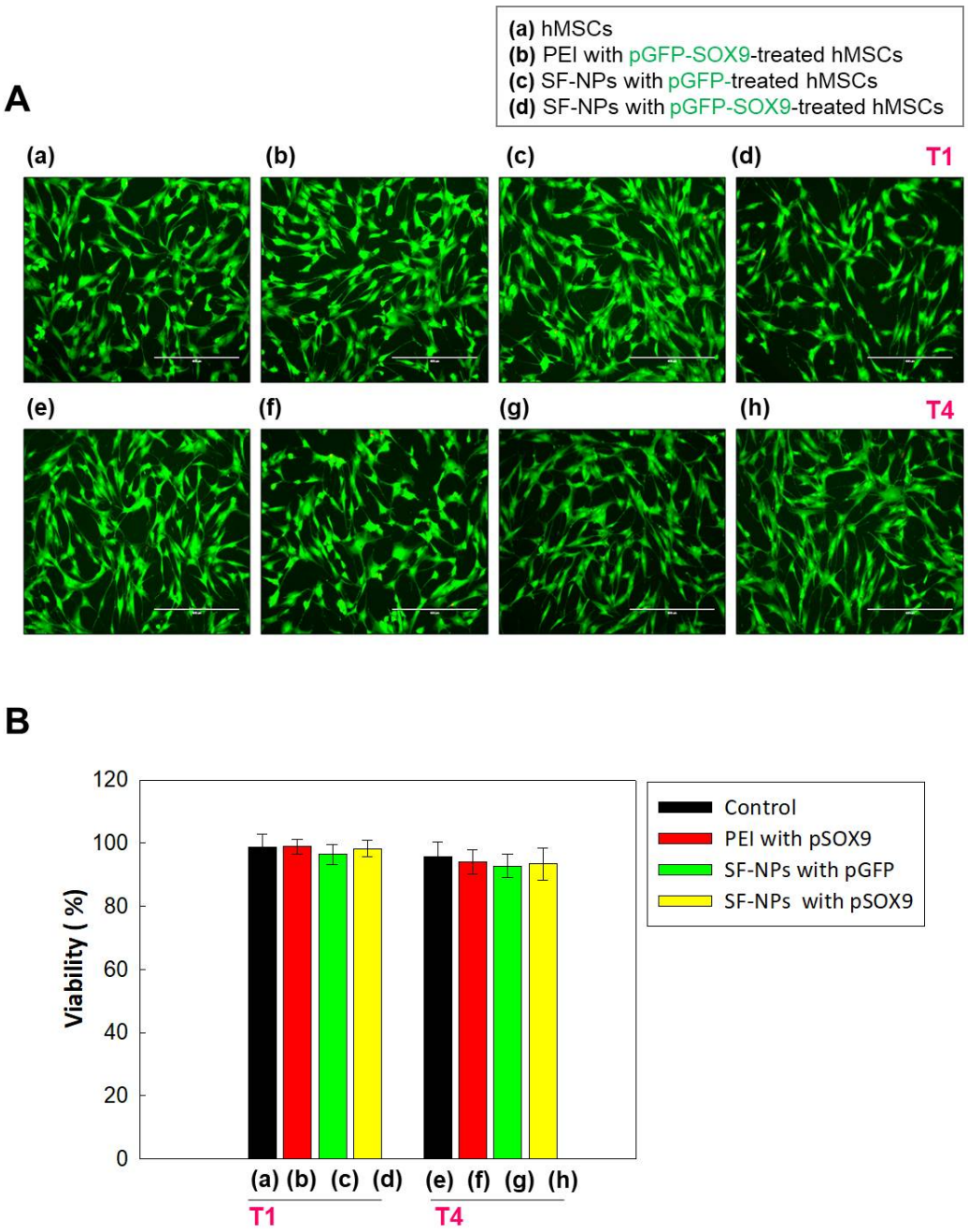


Figure S2. LIVE/DEAD analysis and CCK-8 assay for viability of hMSCs treated with several kinds of nanocarriers for 4h. A: LIVE/DEAD analysis of hMSCs. (a) and (e) Control; (b) and (f) PEI (5μg) complexed with *SOX9*; (c) and (g) SF-NPs complexed with *EGFP*; (d) and (h) SF-NPs complexed with *SOX9*. Bar, 400 μm. **B:** Cell viability of hMSCs treated with different carriers.

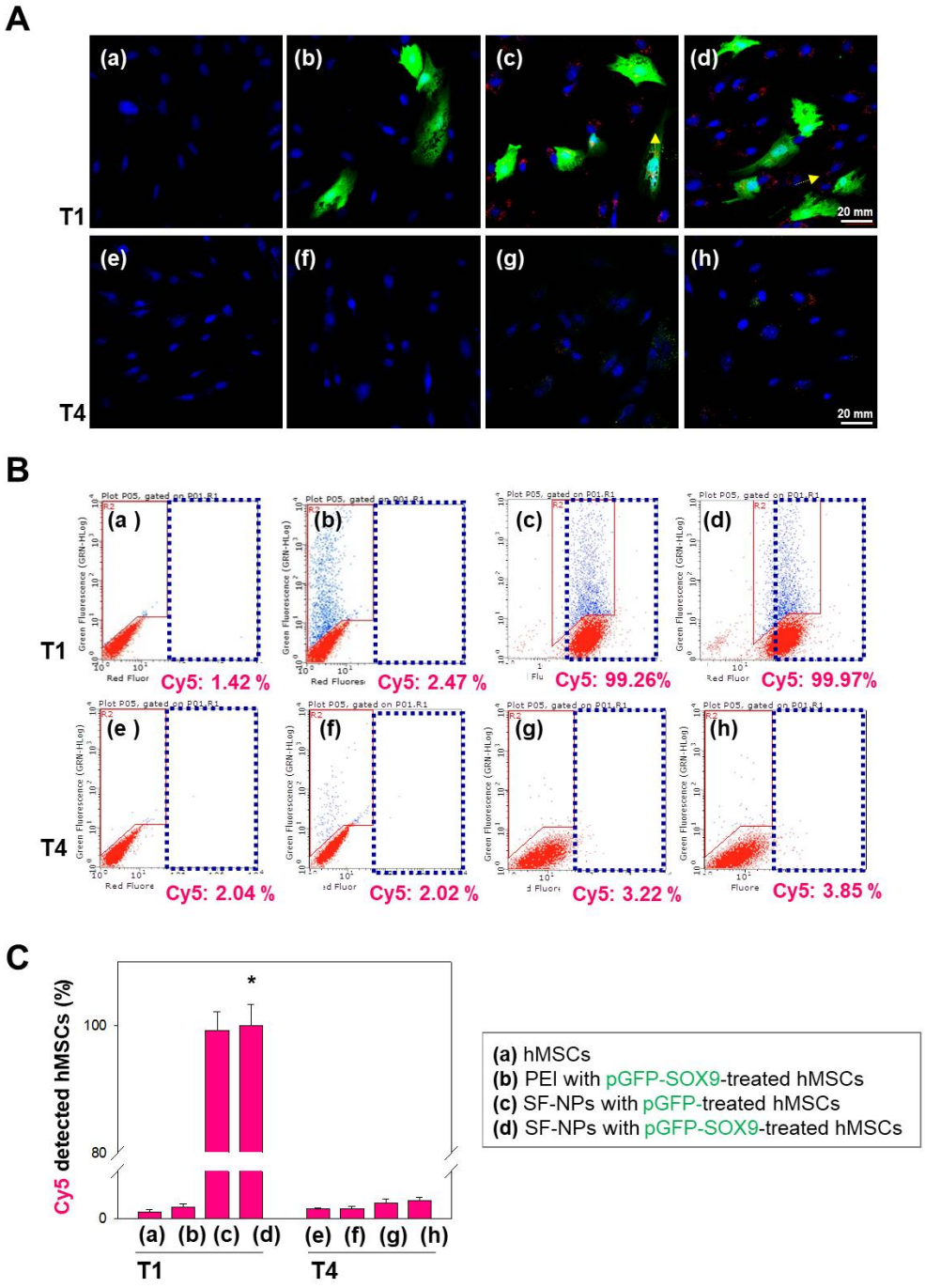


Figure S3. Gene expression of hMSCs transfected with SF-NPs 24h after transfection, as determined by confocal laser microscopy and FACS analysis. A: Confocal images of hMSCs. (a) and (e) Control; (b) and (f) PEI (5μg) complexed with *SOX9*; (c) and (g) SF-NPs complexed with *EGFP*; (d) and (h) SF-NPs complexed with *SOX9*. Bar, 100 μm. **B:** FACS analysis of hMSCs. (a) and (e) Control; (b) and (f) PEI (5μg) complexed with *SOX9*; (c) and (g) SF-NPs complexed with *EGFP*; (d) and (h) SF-NPs complexed with *SOX9*. **C:** The graph of FACS anlaysis * $p < 0.05$

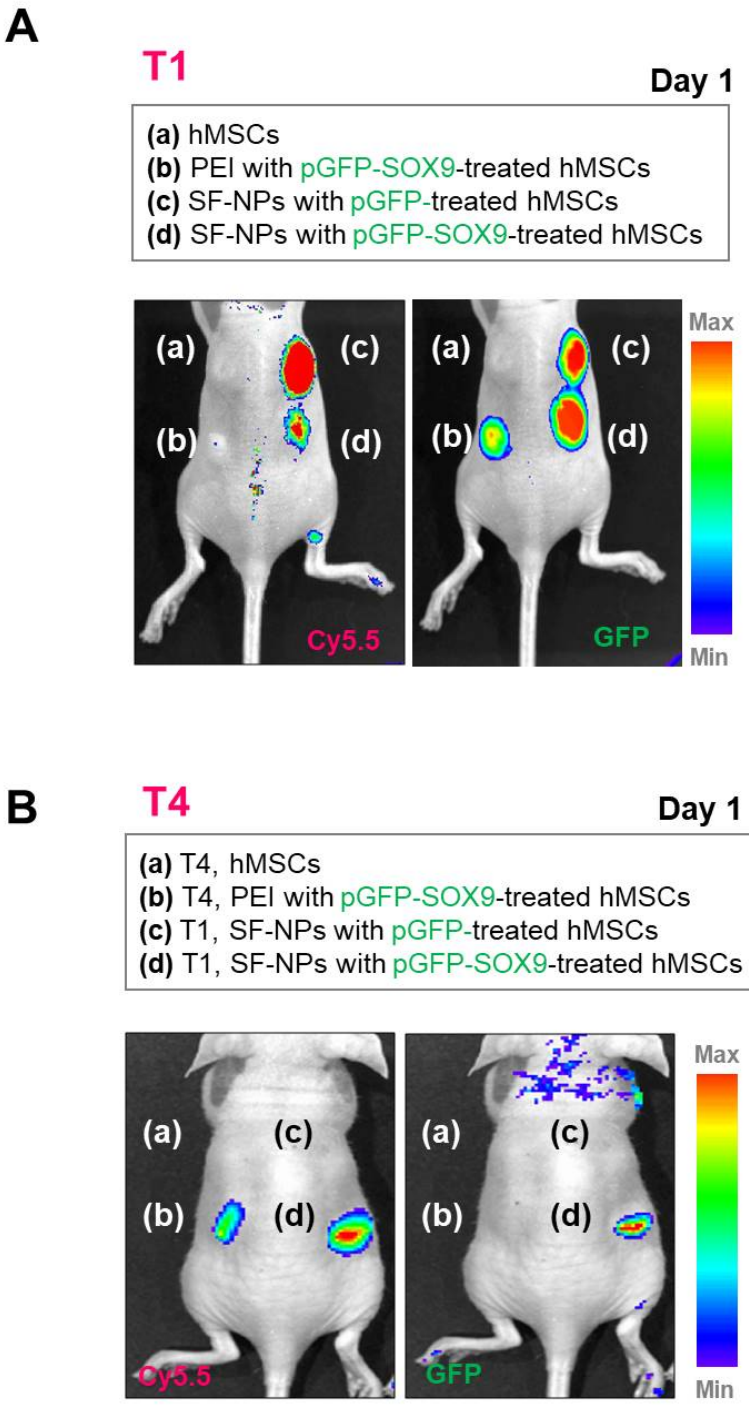


Figure S4. Bioimaging of hMSCs treated with SF-NPs 24h after transfection and transplanted into nude mice. A: Bioimaging of hMSCs treated with several kinds of carriers. (a) Control; (b) PEI (5μg) complexed with *SOX9*; (c) SF-NPs complexed with *EGFP*; (d) SF-NPs complexed with *SOX9*. **B:** Bioimaging of hMSCs treated with SF-NPs complexed with *SOX9* tagged with *EGFP*. (a) Control; (b) SF-NPs complexed with *SOX9* genes (T1 cells); (c) Control; (d) SF-NPs complexed with *SOX9* (T4 cells).

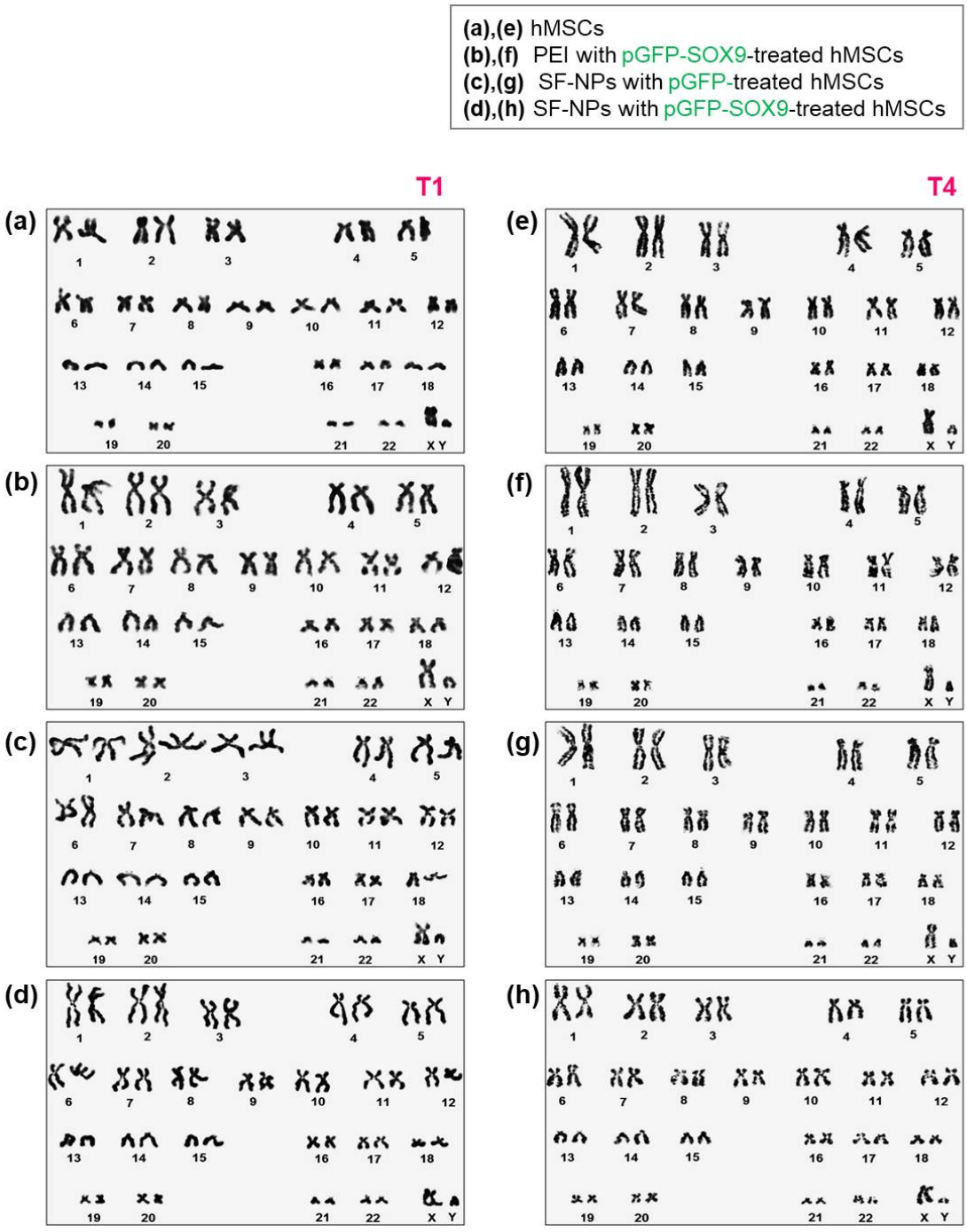


Figure S5. Karyotype results of of hMSCs. All samples show normal male chromosome constitution, 46,XY.

Theranostics
Submitted to The Forum for Diagnostics, Imaging, and Therapy Research

3D pellet culture/ 3 weeks

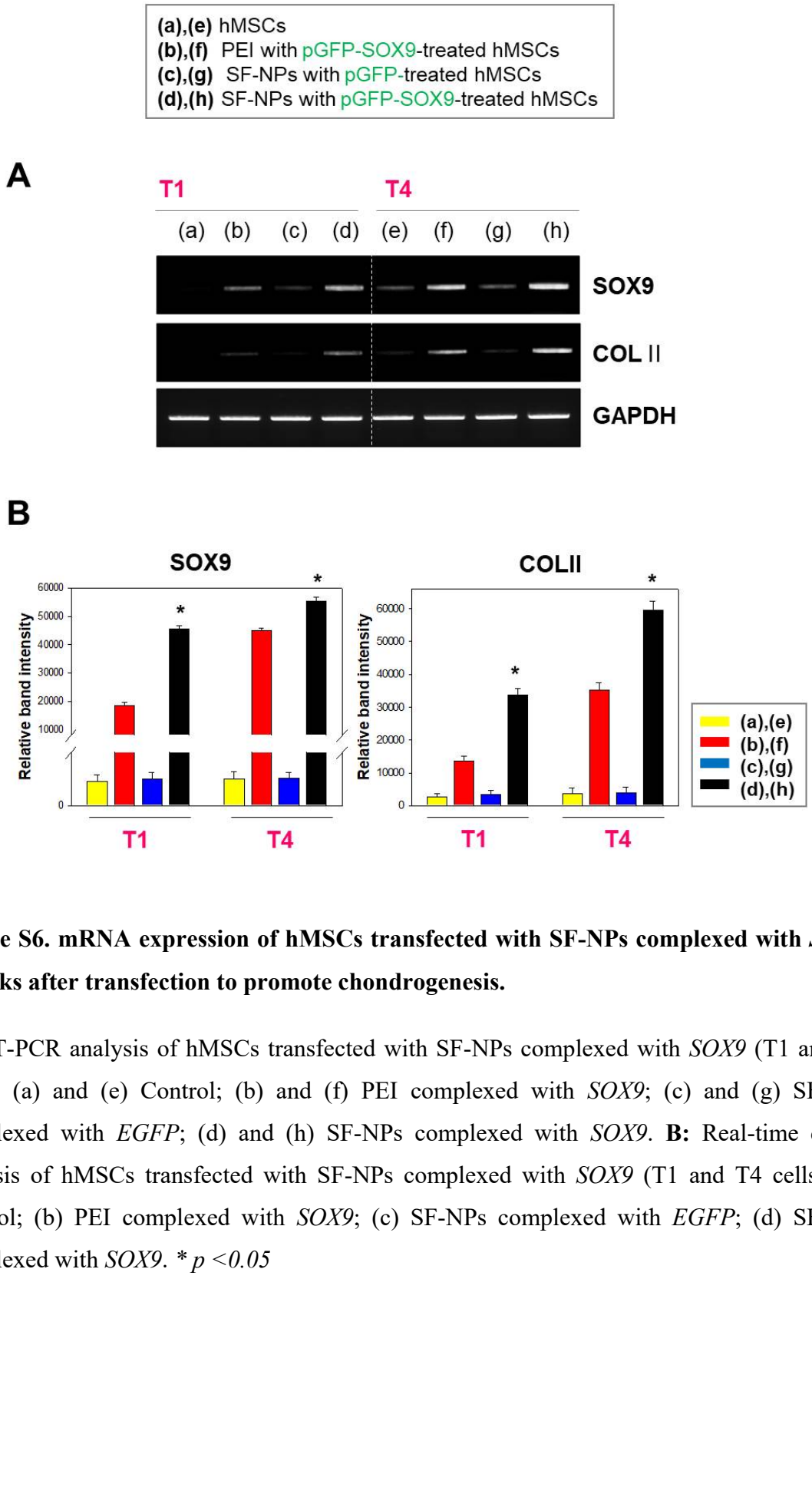


Figure S6. mRNA expression of hMSCs transfected with SF-NPs complexed with *SOX9* 3 weeks after transfection to promote chondrogenesis.

A: RT-PCR analysis of hMSCs transfected with SF-NPs complexed with *SOX9* (T1 and T4 cells). (a) and (e) Control; (b) and (f) PEI complexed with *SOX9*; (c) and (g) SF-NPs complexed with *EGFP*; (d) and (h) SF-NPs complexed with *SOX9*. **B:** Real-time qPCR analysis of hMSCs transfected with SF-NPs complexed with *SOX9* (T1 and T4 cells). (a) Control; (b) PEI complexed with *SOX9*; (c) SF-NPs complexed with *EGFP*; (d) SF-NPs complexed with *SOX9*. * $p < 0.05$

Theranostics
Submitted to The Forum for Diagnostics, Imaging, and Therapy Research

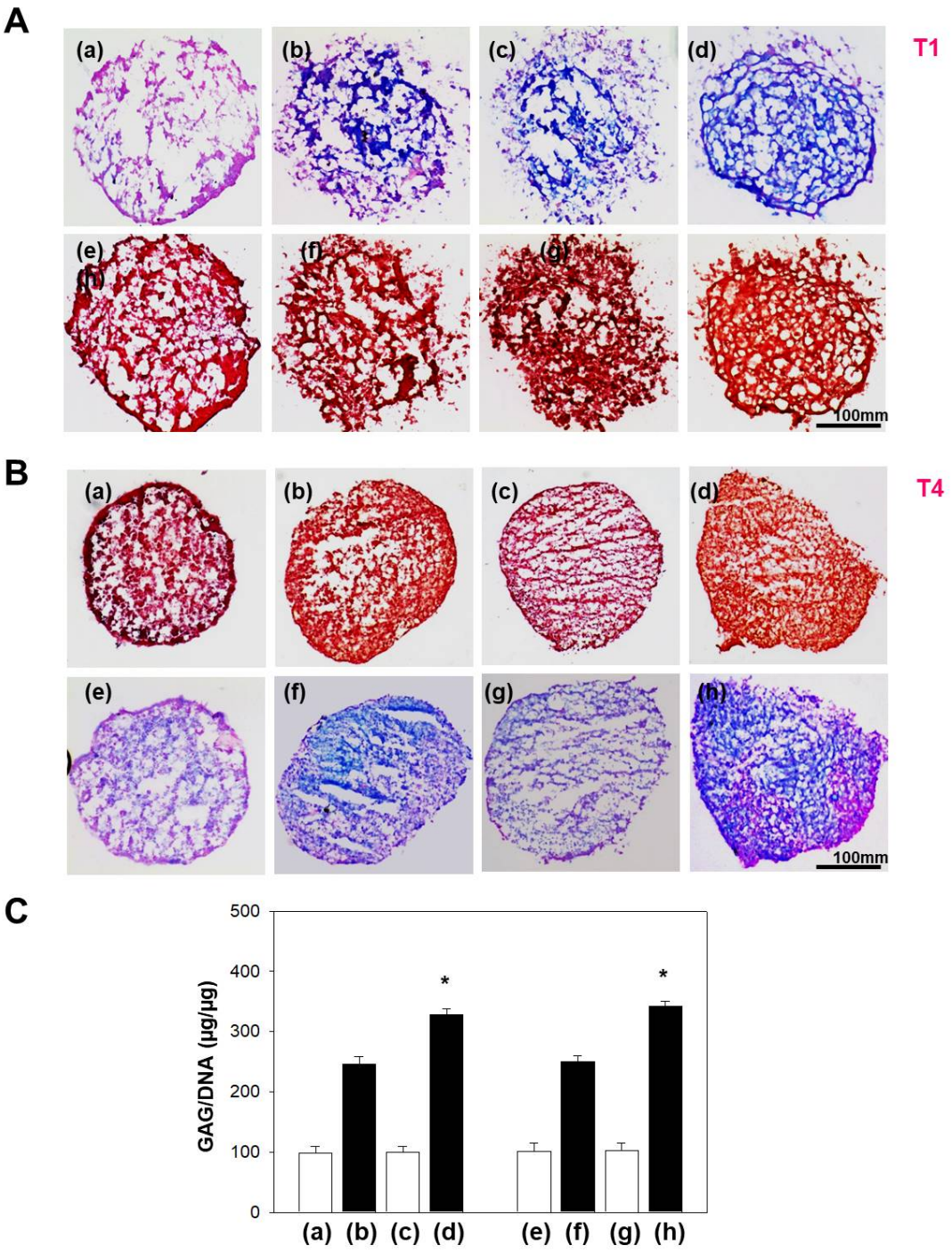
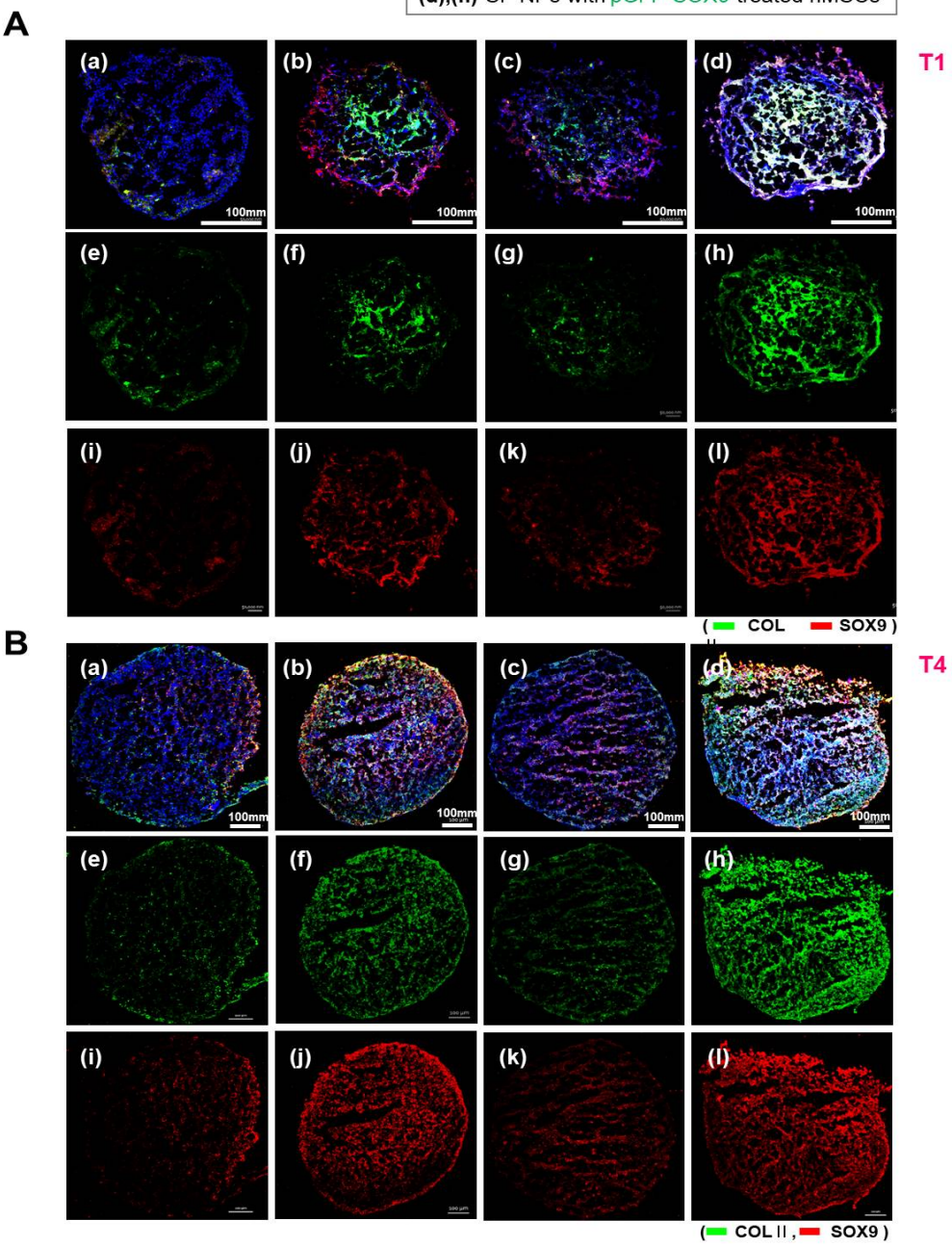


Figure S7. Histological analysis of hMSCs transfected with SF-NPs complexed with *SOX9* 3 weeks after transfection to promote chondrogenesis.

A: T1 cells; **B:** T4 cells. In both panels: (a) and (e) Control; (b) and (f) PEI complexed with *SOX9*; (c) and (g) SF-NPs complexed with *EGFP*; (d) and (h) SF-NPs complexed with *SOX9*. Bar, 100 μm, **C:** GAG assay * $p < 0.05$

Theranostics
Submitted to The Forum for Diagnostics, Imaging, and Therapy Research

(a),(e) hMSCs
(b),(f) PEI with pGFP-SOX9-treated hMSCs
(c),(g) SF-NPs with pGFP-treated hMSCs
(d),(h) SF-NPs with pGFP-SOX9-treated hMSCs



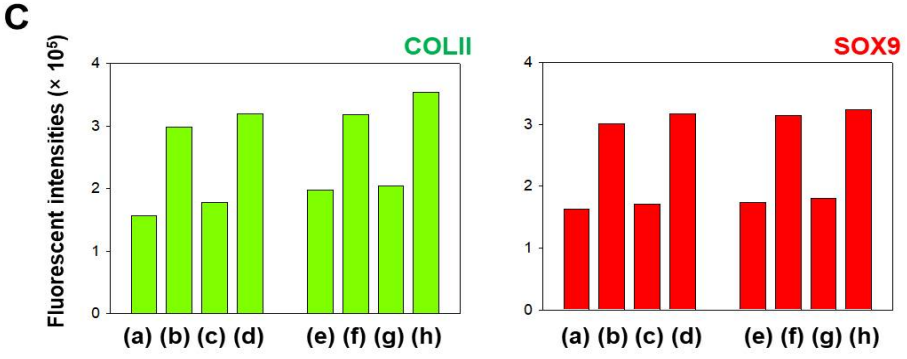


Figure S8. hMSCs transfected with SF-NPs complexed with *SOX9* genes 3 weeks after transfection.

A: T1 cells; **B:** T4 cells. In both panels: (a), (e), and (i), Control; (b), (f), and (j) PEI complexed with *SOX9*; (c), (g), and (k) SF-NPs complexed with *EGFP*; (d), (h), and (i) SF-NPs complexed with *SOX9*. (e)–(l) Immunofluorescence analysis of COL II (e–h) and SOX9 (i–l). Bar, 100 μm, . **C:** The graph of the fluorescent insensities * $p < 0.05$