Supplementary information

# Efficient CRISPR-Cas9-based knockdown of RUNX2 to induce chondrogenic differentiation of stem cells

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## Materials and Methods

### T7E1 assay

Genomic DNA was extracted from control hMSCs and hMSCs transfected with CRISPR-Cas9 vectors #1 and #2. Mismatched duplex DNA was obtained by denaturation/renaturation of 25 µl of the genomic PCR samples using the following conditions: 95ºC for 10 min, 95ºC to 85ºC (-2.0ºC/s), 85ºC for 1 min, 85ºC to 75ºC (-0.3ºC/s), 75ºC for 1 min, 75ºC to 65ºC (-0.3ºC/s), 65ºC for 1 min, 65ºC to 55ºC (-0.3ºC/s), 55ºC for 1 min, 55ºC to 45ºC (-0.3ºC/s), 45ºC for 1 min, 45ºC to 35ºC (-0.3ºC/s), 35ºC for 1 min, 35ºC to 25ºC (-0.3ºC/s), 25ºC for 1 min, and hold at 4ºC. Each sample was supplemented with 3 µl of 10× NEB buffer 2, 0.3 µl of T7E1, and distilled water up to 30 µl. Digestion reactions were incubated for 1 h at 37ºC. T7E1-digested PCR products were analyzed by electrophoresis. The undigested PCR product migrated at 616 bp and the digestion products of CRISPR-Cas9 vectors #1 and #2 migrated at approximately 292/392 bp and 224/324 bp, respectively.

### Gel retardation assay

After complexation of DNPs, LPEI, and vectors, various amounts of each sample were loaded into a 1.5% agarose gel and stained with MG04 (Midori Green Advance; Nippon Genetics). Electrophoresis was performed in 0.5× TAE buffer at 100 V for 13 min. DNA bands were detected by a gel documentation imaging system (BR170-8265, Bio-Rad Laboratories).

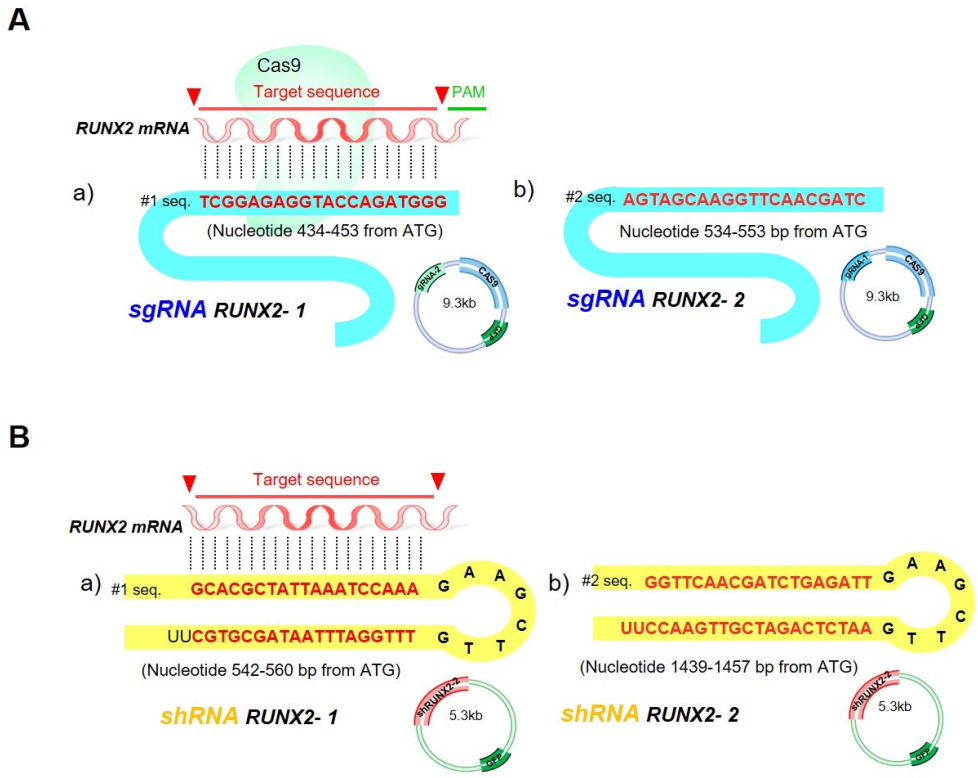


**Figure S1.** Schematic diagram of the complexation of CRISPR-Cas9 (A) and shRNA (B) vectors with NPs.

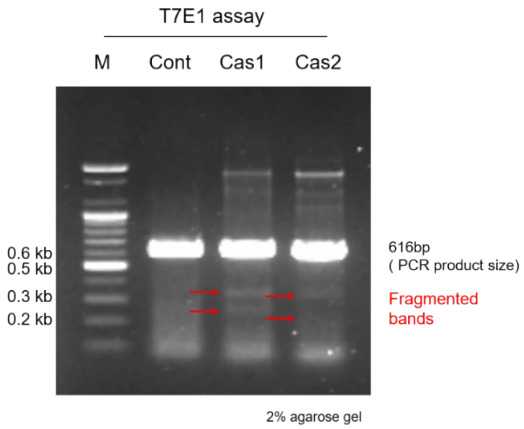


**Figure S2. T7E1 assay of CRISPR-Cas9 plasmids for knockdown of RUNX2**

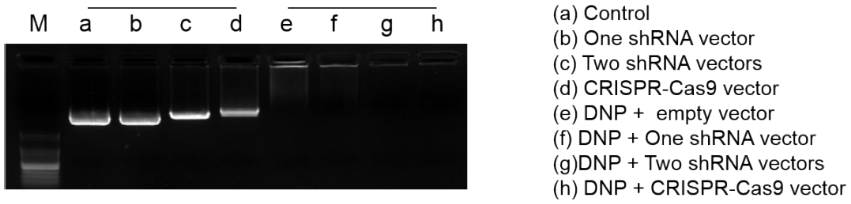


**Figure S3. Gel electrophoresis analysis of shP-NPs and CASP-NPs.**

Confirmation of complexation between DNPs and shRNA and CRISPR-Cas9 vectors.

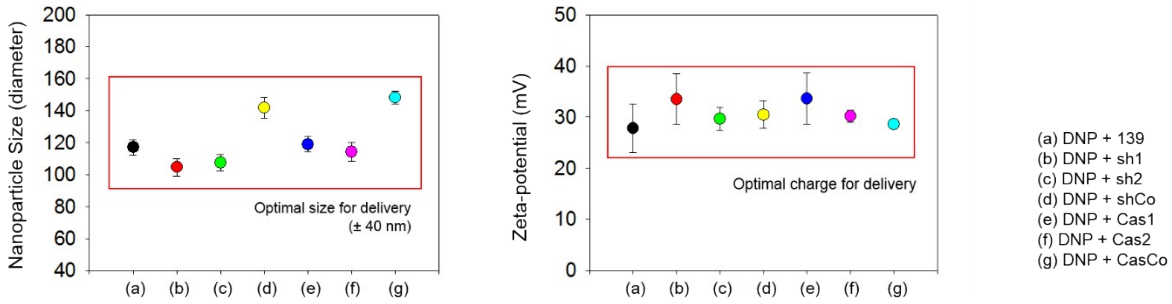


**Figure S4. DLS analysis of DNPs complexed with various vectors.**

DLS analysis of the sizes and zeta-potentials of DNPs complexed with various vectors.

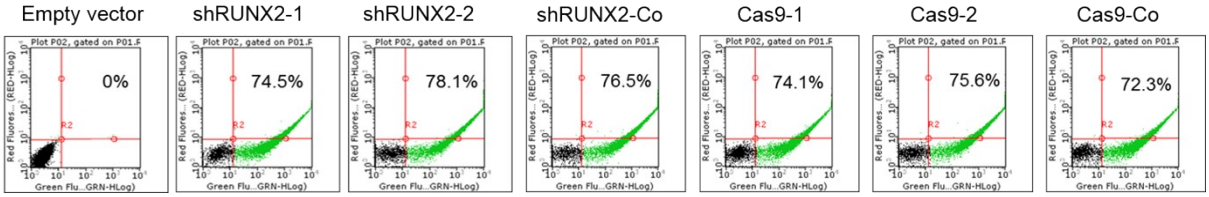


**Figure S5. FACS analysis of MG63 cells treated with shP-NPs and CASP-NPs.**

FACS analysis of the transfection efficiency of shP-NPs and CASP-NPs in MG63 cells.

The percentage of cells positive for GFP was determined.

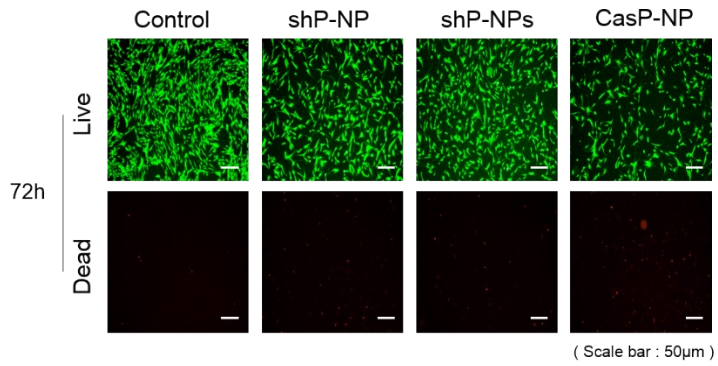


**Figure S6. Investigation of the cytotoxic effects of shPNP, shPNPs, and CasPNP on MG63 cells after 72 h by the LIVE/DEAD assay.**

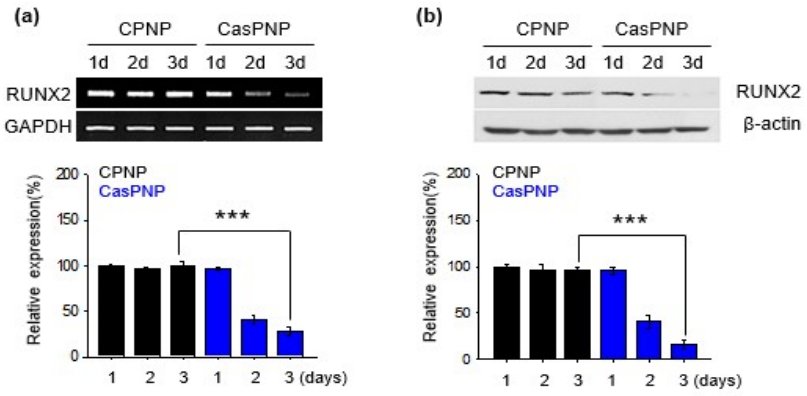


**Figure S7.** Analysis of mRNA and protein expression of RUNX2 over time in MG63 cells treated with control NPs (CPNP) and CasPNP by RT-PCR (a) and Western blotting (b).

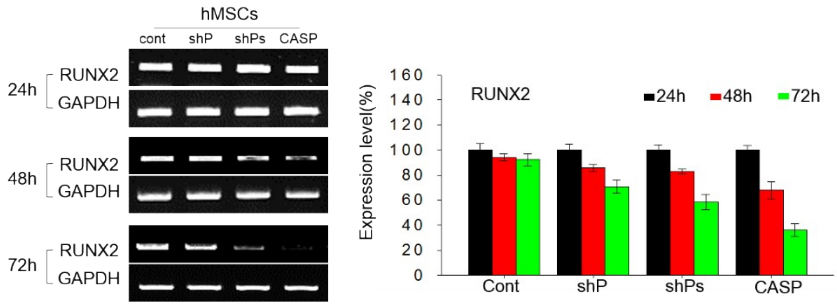


**Figure S8. Effects of shPNP, shPNPs, and CasPNP on mRNA expression of RUNX2 in hMSCs.**

RT-PCR analysis of the mRNA levels of RUNX2 in hMSCs treated with shP-NP, shP-NPs, and CASP-NP for 24, 48, and 72 h.

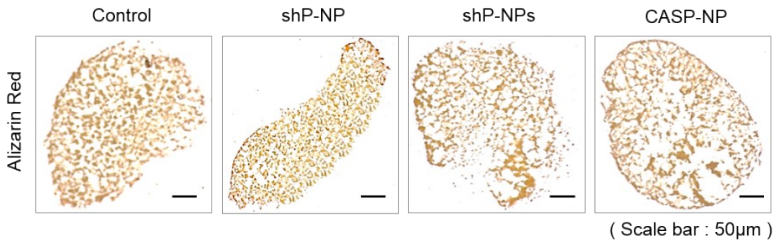


**Figure S9. Assessment of inorganic mineralization in hMSCs treated with shP-NP, shP-NPs, and CASP-NP by Alizarin Red staining.**