Supplementary Information

Specific and efficient knockdown of intracellular miRNA and mRNA using partially neutralized phosphate-methylated DNA oligonucleic acid-loaded mesoporous silica nanoparticles

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Supplementary Data

Data 1 Calculation of N/P ratio

The materials of MSN are tetraethoxysilane (TEOS) and hexadecyl trimethyl ammonium bromide (CTAB). MSN was modified with polyethylene glycol (PEG) and polyethyleneimine (PEI).

The density of TEOS is 0.933 g/mL. The purity of TEOS is 98%. 1.25 mL TEOS was dissolved in 5 mL ethanol, and TEOS accounts for 0.2 (1.25 / 6.25). 2 mL of TEOS mixture was used in MSN synthesis.

The mass of TEOS:

$$0.2 \times 2\ \text{mL} \times 0.933\ \text{g/mL} \times 0.98 = 365.73\ \text{mg}$$

The density of PEG material was 1.076 g/L. 550 μL PEG was used in MSN synthesis. The mass of PEG:

$$550\ \mu\text{L} \times 1076\ \text{g/L} = 591800\ \mu\text{g} = 591.8\ \text{mg}$$

The density of PEI material was 0.92 g/mL. PEI was diluted to 50% (v/v). 20 μL of mixture was used in MSN synthesis.

The mass of PEI:

$$20\ \mu\text{L} \times 0.92\ \text{g/mL} \times 0.5 = 9.2\ \text{mg}$$

290 mg CTAB was used in MSN synthesis.

The mass ratio of PEI to MSN:

$$9.2\ \text{mg} / (9.2\ \text{mg} + 591.8\ \text{mg} + 290\ \text{mg} + 365.73\ \text{mg}) = 0.732\%\ ◦$$

The molecular weight of one PEI unit is 292 g/mol, and the average molecular weight of the entire PEI chain is 1650 g/mol. Consequently, the entire PEI chain is composed of 5.651 repeating units, with each unit containing three nitrogen atoms. Therefore, the entire PEI chain consists of 16.952 nitrogen atoms.

N/P ratio is the ratio of positively-chargeable polymer amine (N) groups to negatively-charged nucleic acid phosphate (P) groups.

The mass ratio of the MSN to the ASO probe used in ASO loading is 128:1 (w/w). PEI accounts for 0.732% of MSN (w/w). The average molecular weight of the entire PEI chain is 1650 g/mol containing 16.952 nitrogen atoms.

N:

$$(128 \times 0.00732) / (1650 \times 16.952)$$

The molecular weight of the ASO probe is 6702 g/mol containing 22 nucleotides.

P:

$$1 / 6702 \times 22$$

N/P ratio:

$$\frac{\frac{(128 \times 0.00732)}{(1650 \times 16.952)}}{\frac{1}{(6702 \times 22)}} = 4.94$$

Data 2 Calculation of RNA expression *via* the comparative Ct method

Ct values were determined by RT-qPCR. U6 was used as a housekeeping gene for miRNA studies. A control group represents a nontreatment group and an N4-mid nDNA group represents a group that cells treated with N4-mid nDNA-loaded MSN.

The Ct values of a control group are 17.4 for miR-21 and 13.6125 for U6.

The △Ct value of a control group:

$$17.4 - 13.6125 = 3.7875$$

The Ct values of an N4-mid nDNA group are 18.98 for miR-21 and 12.21 for U6.

The △Ct value of an N4-mid nDNA group:

$$18.98 - 12.21 = 5.77$$

The △△Ct value:

$$5.77 - 3.7875 = 1.9825$$

The comparative Ct method for quantitative gene expression:

$$2^{(-\triangle\triangle Ct)}$$

Expression of miR-21 in HCT116:

$$2^{(-\triangle\triangle Ct)} = 0.25 = 25\%$$

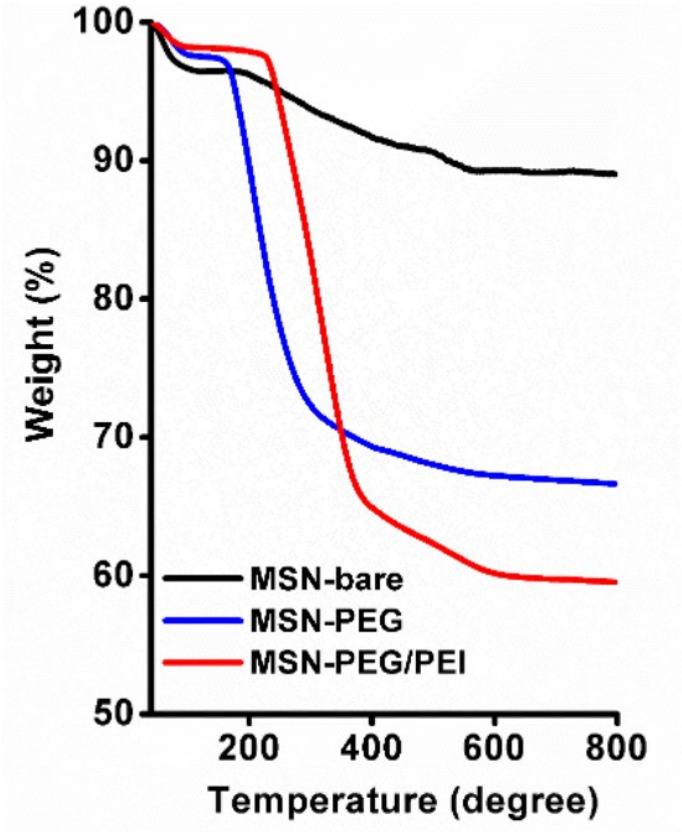
Supplementary Tables

Table S1 Oligonucleotide probe sequence of all probes used in this study.

Probes	Sequence (5' - 3')
miR-21 Scramble	CAT TAA TGT CGG ACA ACT CAAT
miR-21 DNA	TCA ACA TCA GTC TGA TAA GCTA
miR-21 N4-mid nDNA	TCA ACA **T**CA **G**TC **T**GA **T**AA GCTA
miR-21 N4-5' nDNA	T**C**A A**C**A T**C**A G**T**C TGA TAA GCTA
miR-21 N4-3' nDNA	TCA ACA TCA GT**C** TG**A** TA**A** GC**T**AG

*Bases with MPTE modifications are shown in boldface

Supplementary Figures



Weight Loss (%)[a-c]	Bare	PEG	PEG/PEI	PEG	PEI
25 nm MSN	7.46%	30.7%	38.58%	23.24%	0.42%

a. Weigh lost calculated from 150-800 degrees
b. Weight lost of PEG (blue) was subtract weight lost of MSN-bare
c. Weight lost of PEI (red) was subtract weight lost of MSN-bare and MSN-PEG

Figure S1 Thermogravimetric analysis (TGA). TGA was conducted to evaluate MSN before and after surface modification with PEI and PEG.

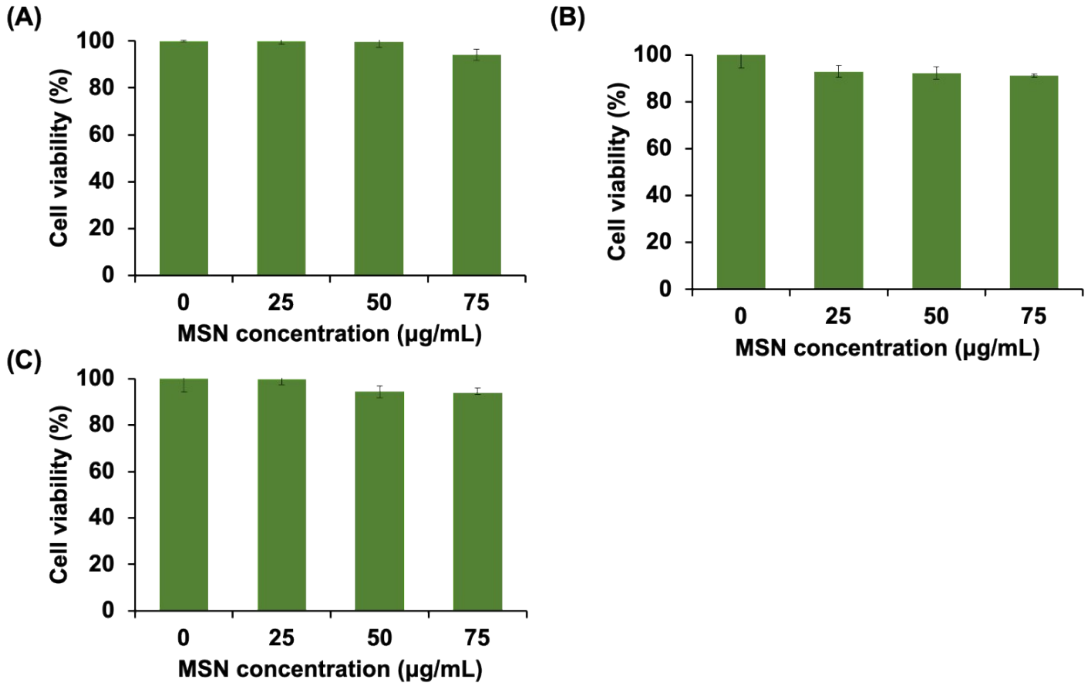


Figure S2 Cell viability of MSN-treated HCT116 cells. HCT116 were treated with MSN for (A) 24 h, (B) 48 h, and (C) 72 h incubation and cell viability was determined by cell viability assay. The MSN concentration used in *in vitro* assessment is 64 μg/mL for 75 nM ASO probe concentration. All results are expressed as the mean ± S.D. (n = 4)

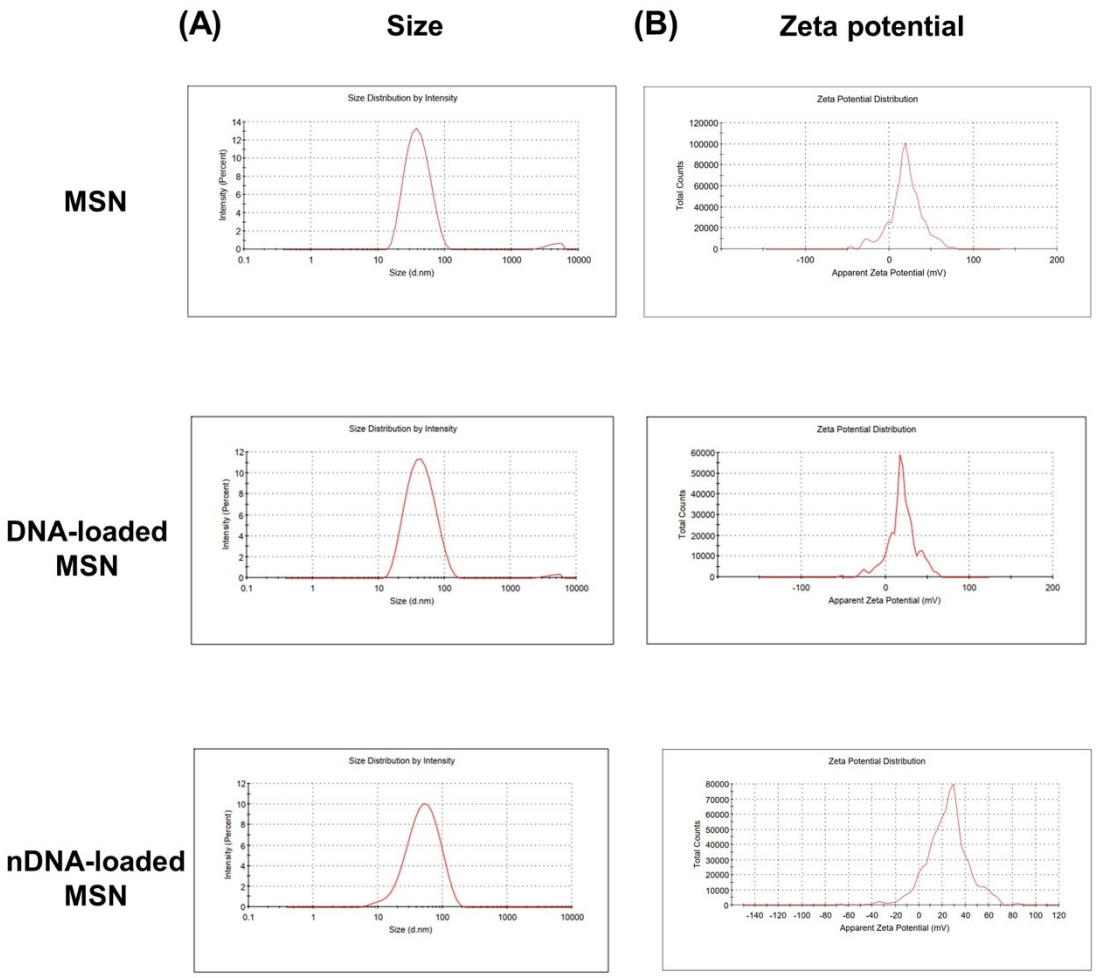


Figure S3 Distribution of hydrodynamic diameter and ζ-potential of MSNs in water. (A) Size distribution by intensity and (B) ζ potential distribution.

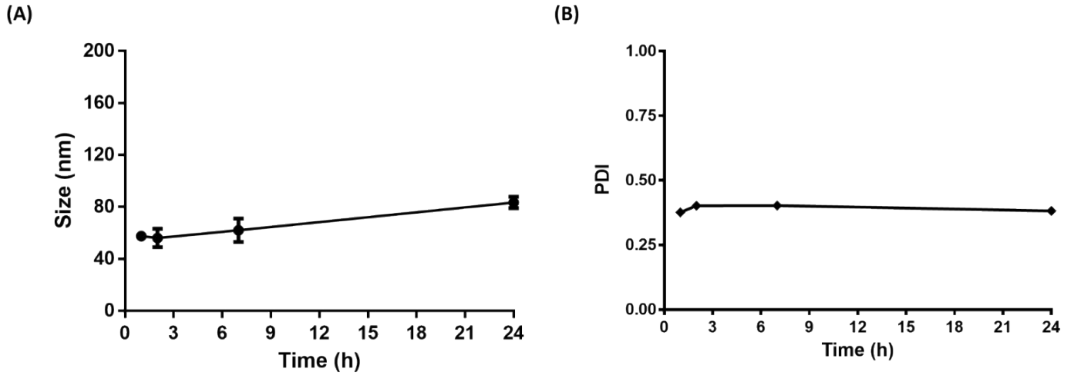


Figure S4 Serum stability of probe-loaded MSN. Serum stability of probe-loaded MSN measured by (A) particle size and (B) PDI over 24 hours in McCoy' 5A medium containing 10% serum. The results are expressed as the mean ± S.D. (n = 3)

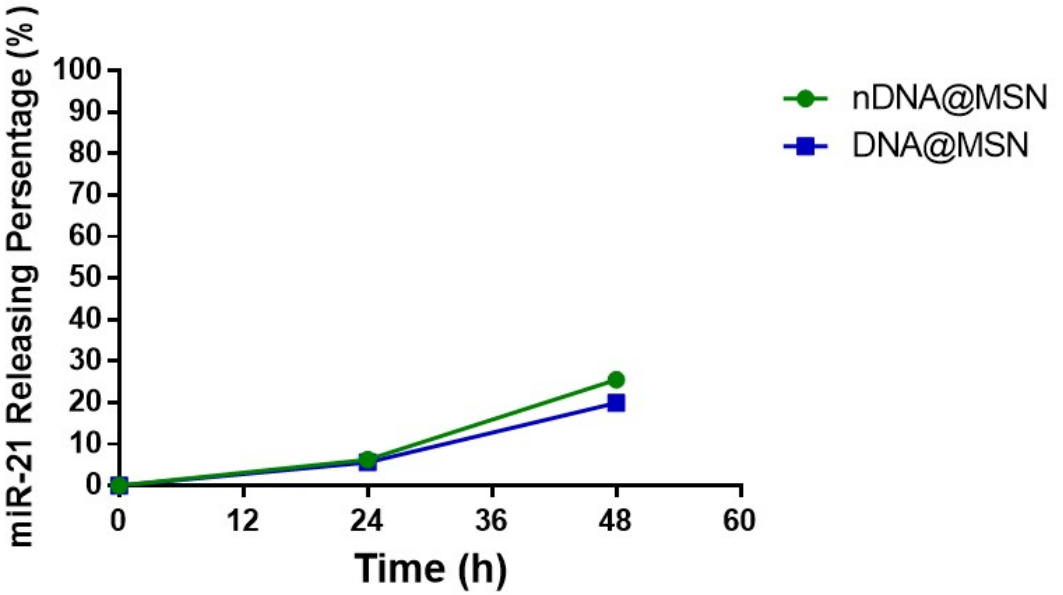


Figure S5 Release test of nDNA- and DNA-loaded MSN. Release profiles of nDNA and DNA from nDNA- and DNA-loaded MSN during 48 h. The results are expressed as the mean ± S.D. (n = 3)

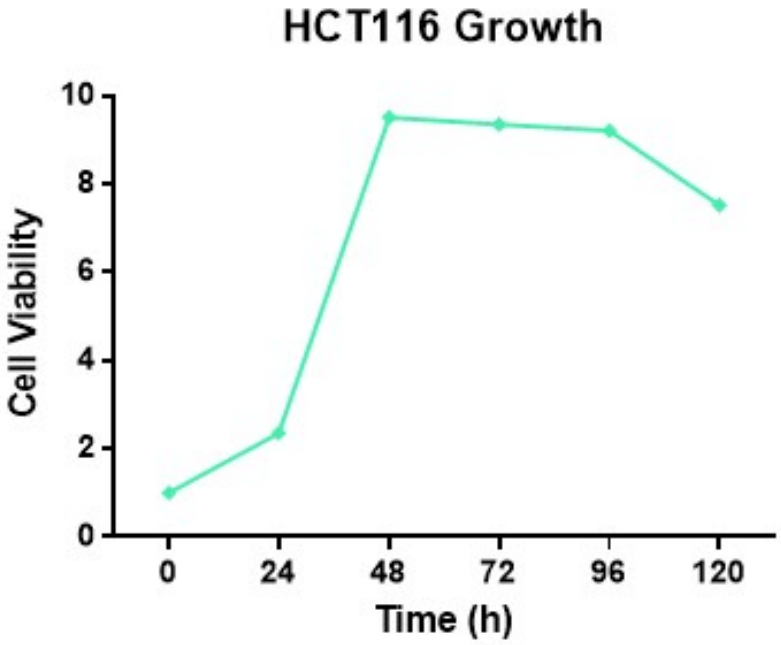


Figure S6 Cell growth assessment of HCT116 by cell viability assay. The cell viability decreased, and alterations in cell morphology were observed after 72 hours of incubation. All results are expressed as the mean ± S.D. (n = 4)

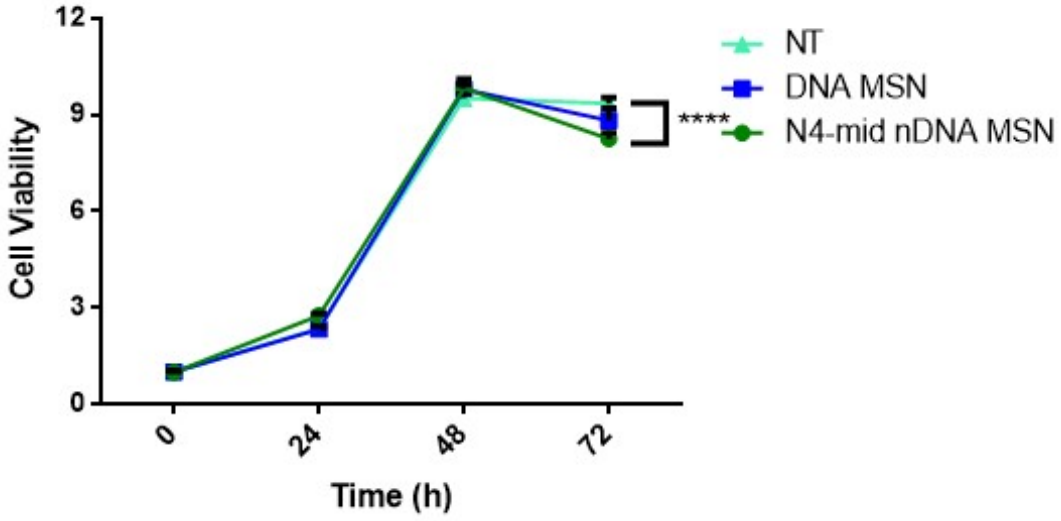


Figure S7 Cell growth suppression by N4-mid nDNA- and DNA-loaded MSN during a 72-hour incubation. Cells were treated at a probe concentration of 75 nM, and cell growth was observed by a cell viability assay. All results are expressed as the mean ± S.D. (n = 4), **** $p < 0.0001$ (two-way ANOVA with Tukey's multiple comparisons test)