Electronic supplementary information (ESI).

A. TEM images of the chitosan-coated magnetic composite particles

Chitosan-coated magnetic composite particles were stained with uranyl acetate and then examined and photographed in transmission electron microscopy (TEM, Hitachi-700, Hitachi Co., Japan) operated at 100 kV accelerating voltage, Fig. S1. The composite particles self-aggregated and form irregular clusters. The particle sizes were found consistent with the DLS results in Fig. 1(b).

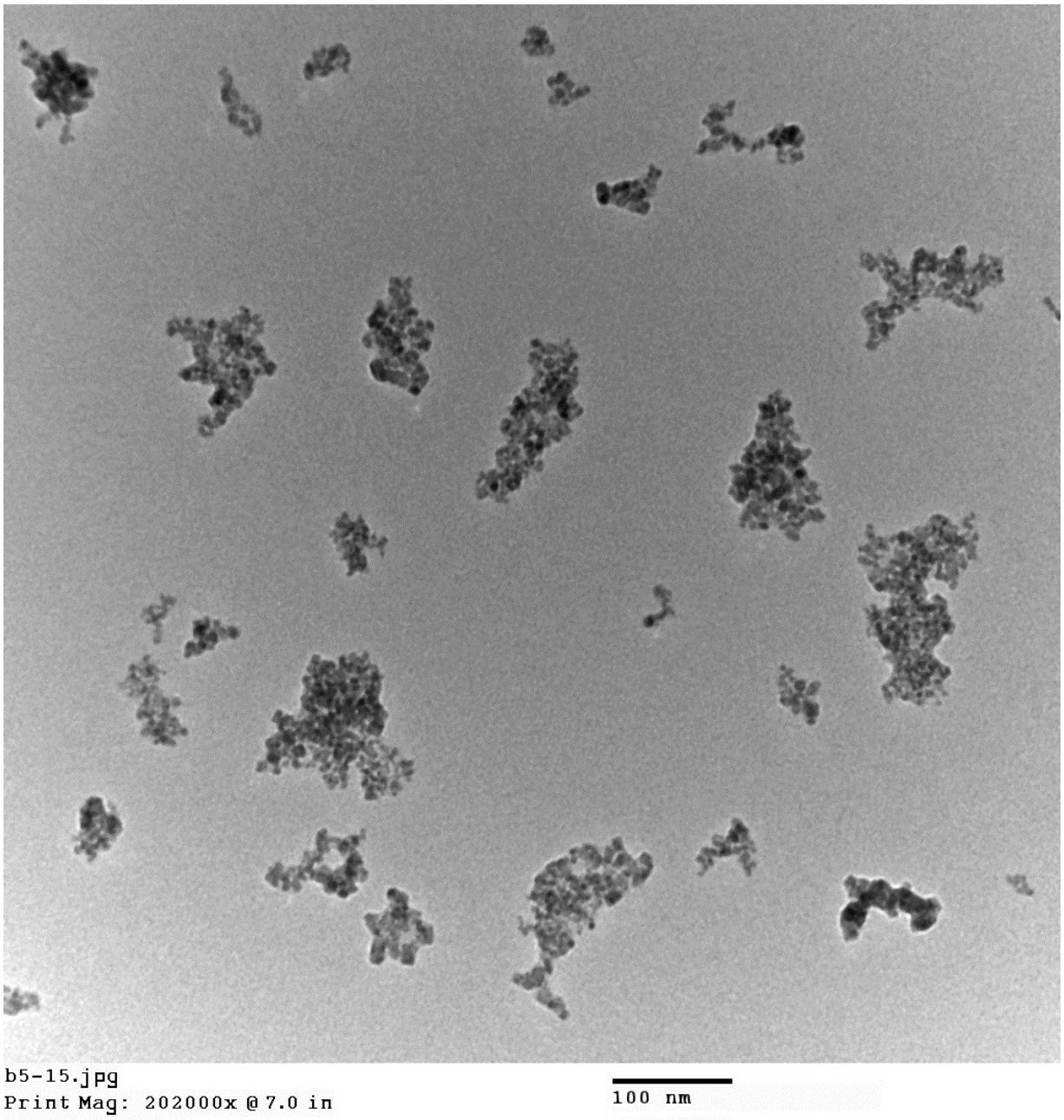

Figure S1. TEM image of chitosan-coated magnetic composite particles.

B. Zeta potentials of TIPs

The thymine-imprinted polymers (TIPs) composite nanoparticles before and after template removal were also examined by a zeta potential analyzer (90Plus, Brookhaven Instruments Co., New York). Both mean size and zeta potential measurements showed the TIPs composites nanoparticles are stable in PBS for at least three weeks, which agreed with literature reported for ~70% deacetylated chitosan.[1, 2] However, Leong's group reported that aggregation of nanoparticles or protein adsorption to the nanoparticles might occur when they are exposed to a buffer with relatively high ionic strength and/or serum.[2]

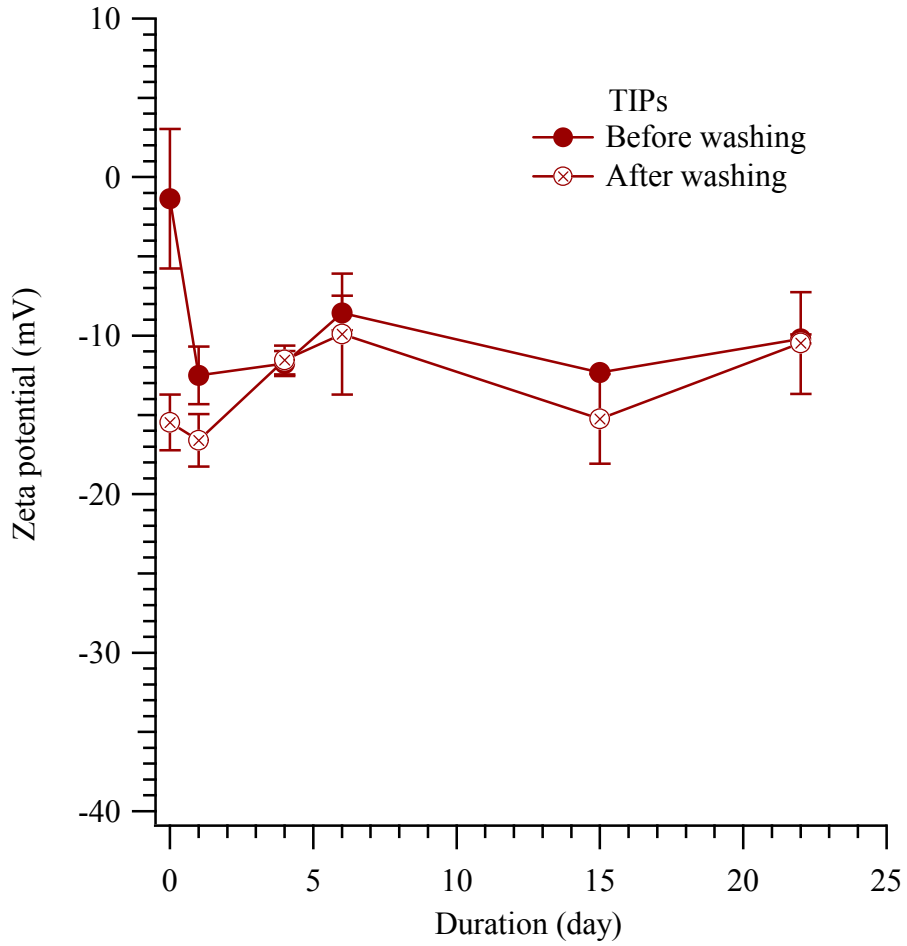

Figure S2. The Zeta potential of TIPs before and after template removal over three weeks.

C. Control experiment of NIPs and untreated controls.

Cells treated with non-imprinted particles (NIPs) also had gene expression indistinguishable from controls (as shown in Fig. S3). The cells were harvested after NIPs treatment on the same, second, fourth and sixth days.

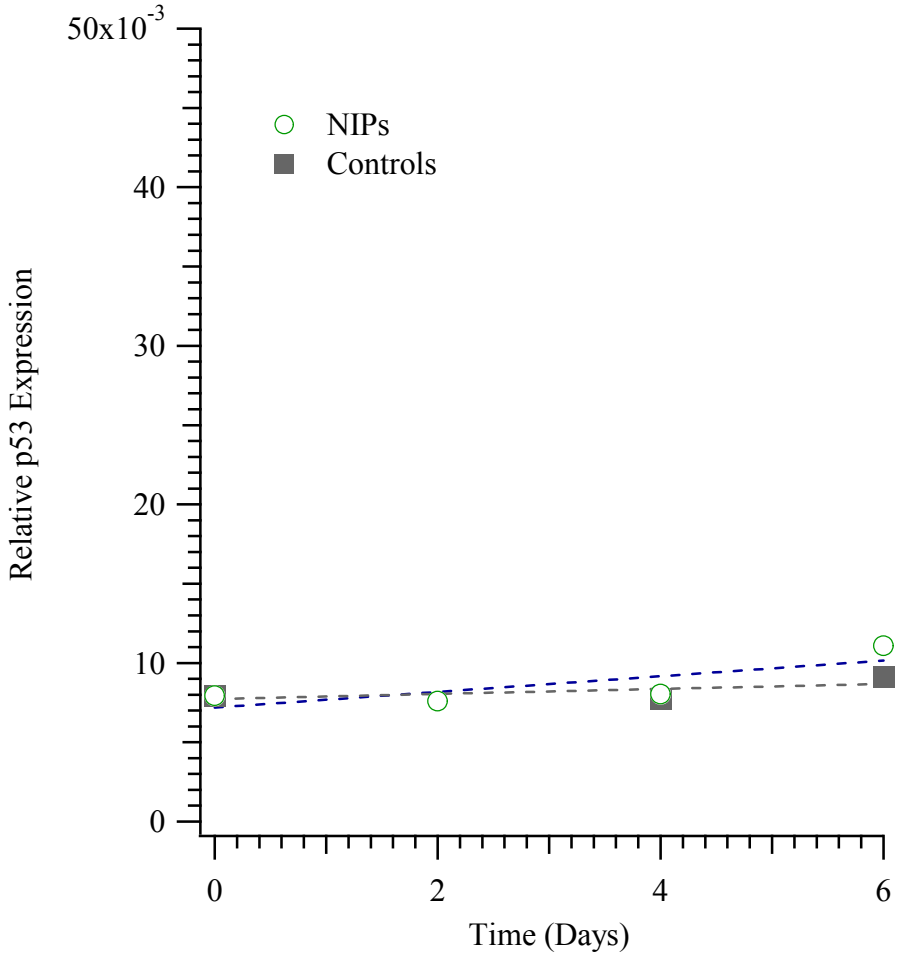

Figure S3. The relative p53 expression of HepG2 cells treated with NIPs and untreated controls. The dosage was 100 μg particles/mL

D. Experimental

Reagents and chemicals.

Thymine (≧99%,), adenine (≧99%, HPLC), cytosine (≧99%, HPLC) and chitosan (#C3646, from shrimp shells, ≥75% deacetylated), fetal bovine serum (#12003C) and minimum essential medium Eagle, 3-(4,5-dimethyl-2-thiazolyl)-2,5- diphenyl-2H-tetrazolium bromide (MTT)) were purchased from Sigma-Aldrich Co. (St. Louis, MO). Iron (III) chloride 6-hydrate (97%) and iron (II) sulphate 7-hydrate (99.0%) were from Panreac (Barcelona, Spain). Dimethyl sulfoxide (DMSO) and acetic acid (ACS grade) were from J. T. Baker (Phillipsburg, NJ). All chemicals were used as received unless otherwise mentioned.

HepG2 cell line (#60025) was purchased from Bioresource Collection and Research Center (BCRC), Hsinchu, Taiwan. The culture medium for HepG2 cell lines contains Eagle's Minimum Essential Medium (EMEM including salts, L-glutamin and non-essential amino acids), sodium bicarbonate ($NaHCO_3$) 1.5 g/L, sodium pyrurate 1mM (c.a. 0.11g/L), 10% v/v of fetal bovine serum (FBS) and 1% v/v of antibiotics (penicillin and streptomycin).

The KingFisher Total RNA Kit (Cat. #: 97020196, Thermo Scientific, Vantaa, Finland) includes KingFisher magnetic beads 3.1 mL, rDNase 3vials, rDNase buffer 35 mL, reducing agent (tris(2-carboxyethyl)phosphine, TCEP) 1 vial, lysis buffer 40 mL, binding buffer 75 mL, wash buffer I 65 mL, wash buffer II 200 mL, elution buffer 20 mL and RNase-free water 120 mL. The Deoxy+ real-time 2x SYBR green RT-PCR kit contains (1) RealStart Taq DNA polymerase; (2) Reverse transcriptases; (3) dATP , dCTP , dGTP , dTTP mix; (4) 5 mM $MgCl_2$; (5) SYBR® Green I and (6) ROX was purchased from Yeastern Biotech Co., Ltd, Taiwan. Diethylpyrocarbonate (DEPC) water was purchased from Protech Technology Enterprise Co, Ltd., Taiwan.

Formation of composite molecularly imprinted chitosan nanoparticles.

Magnetic nanoparticles were prepared for the later formation of composite particles, using the Massart method: co-precipitation of a mixture of iron (III) chloride 6-hydrate and iron (II) sulphate 7-hydrate by sodium hydroxide. The magnetite was then mixed with oleic acid for better dispersion and repeatedly washed while adsorbed on a magnetic plate, and then freeze-dried overnight. The synthesis of magnetic thymine-imprinted (TIPs) and non-imprinted (NIPs) chitosan nanoparticles included three steps (Scheme 1): (1) Magnetic nanoparticles 20 mg were added to 1 mL thymine/chitosan solution (0.1 and 20 mg/mL, respectively, in acetic acid); (2) dropwise dispersion of the chitosan/magnetic particle solution in 10 mL deionized (DI) water; and then (3) removal of the template molecules and chitosan particles without encapsulated magnetic nanoparticles using a magnetic plate and washing with deionized water 10 mins for three times to remove the template molecules

(Fig.1 (a)). All composite nanoparticles were equilibrated with deionized water overnight before use. The NIPs were prepared identically, except that the template was omitted.

Raman Scattering and Size distribution of the Thymine-imprinted Chitosan Nanoparticles.

The *magnetic*, thymine- and non-imprinted nanoparticles at 1 mg/mL were washed gently with DI water, and a 1 μL sample was added to the sample holder for the measurement of Raman shift by a Raman microscope (NTEGRA Spectra, NT-MDT Co., Moscow, Russia). The Raman microscope is equipped with a 532 nm laser.

Magnetic (MNPs) and thymine-imprinted polymers (TIPs) composite nanoparticles were monitored by a dynamic light scattering (DLS) particle sizer (90Plus, Brookhaven Instruments Co., New York). The measurement of the particle size distribution was set at 25^{o}C with 3 minutes duration data collection at 90° detection angle. The average count rate of the background was 15 kcps and that of each measurement was between 20~ 500 kcps.

Binding Kinetics of Magnetic Thymine-imprinted Chitosan Nanoparticle

The rebinding measurements of the thymine to the thymine-imprinted (TIPs) or non-imprinted (NIPs) chitosan nanoparticles were performed with 1 mL 0.1 mg/mL of cytosine solution, which was dissolved in the phosphate buffer saline (PBS). These solutions were added to 1 mL of MMIP particles (15 mg) in deionized water on a magnetic plate for 30 min (Scheme 1(b)). The resultant solution was passed through a 0.22-μm filter (ADVANGENE, USA) and then subjected to high-pressure liquid chromatography (HPLC, Hitachi, Tokyo, Japan) for analysis of the three nucleobases. The separation was performed on a NUCLEODUR® C18 ec column (50 μm, 25 cm x 4.6 mm I.D., MACHEREY-NAGEL GmbH & Co. KG, Düren, Germany). The sample (20 μL) was eluted with a mobile phase composed of 20 mM (NaH_2PO_4: Na_2HPO_4 = 38:62). The flow rate and detection wavelengths were set to be 1.0 mL/min and 254 nm (adenine, thymine), and 266 nm (cytosine). The retention time for the adenine, thymine and cytosine is 15.4, 4.5 and 14.7 min, respectively.

Cytotoxicity Test of HepG2 Cells with Magnetic Chitosan Nanoparticles.

The MTT test has used as a rapid and sensitive method for screening nanoparticle effects on HepG2 cells. About twenty thousand HepG2 cells were injected to the 96 wells culture plate and incubated for 24 hrs. MTT solution (50 μL) was added to each well and interacts with cells for 3 h. DMSO 50 μL was added after all medium was removed. Detection and reference wavelengths 570 and 620 nm, respectively, were measured with an ELISA reader (Power Wave HT340, BioTek, Winooski, USA). Effective adsorption is defined as the

difference of the adsorptions at the detection and reference wavelengths. The cell viability (%) was then calculated from the ratio of effective adsorption of treated to control samples.

p53 Gene Expression of HepG2 Cell Line Treated with Magnetic Chitosan Nanoparticles.

The sequence (5'- 3') of primers for p53 and GAPDH genes: p53, Forward: ACCTATGGAAACTACTTCCTG (SIGMA SG001500962-001); Reverse: ACCATTGTTCAATATCGTCC (SIGMA SG00150963-001). GAPDH, Forward: ACAGTTGCCATGTAGACC (SIGMA SG00141886-001); Reverse: TTTTTGGTTGAGCACAGG (SIGMA SG00141885-001). The total RNA from the HepG2 cultured for one day on the substrates was purified using the KingFisher Total RNA Kit and the KingFisher mL magnetic particle processors, both from Thermo Scientific (Vantaa, Finland). RNA extraction was done following the KingFisher kit protocol. The concentration of cellular RNA was quantified by determining the absorbance maximum at the wavelength of 260 and 280 nm to give the optimum OD between 1.6 to 2.0 in a UV/Vis spectrometer (Lambda 35, PerkinElmer, Wellesley MA). Complementary DNA was obtained by mixing 1 µL of total RNA and 19 µL of reaction mixture including 10 µL of Deoxy+ real-time 2x SYBR green RT-PCR premix, 7 µL of diethylpyrocarbonate (DEPC) water and 1 µL of forward and reverse primers (Yao-Hong Biotechnology Inc., HPLC grade, New Taipei City, Taiwan). Finally, the mixture was kept at 48 °C for 30 min and then incubated at 90 °C for 10 min. The real-time PCR was performed in a PikoReal real-time PCR system (Thermo Scientific, Vantaa, Finland). Relative gene expression was determined using a $\Delta\Delta$Cq method[3] and normalized to a reference gene (GAPDH) and to a treatment control (HepG2 only).

Reference

1. T. Kiang, J. Wen, H. W. Lim and K. W. Leong, *Biomaterials*, 2004, **25**, 5293-5301.
2. H.-Q. Mao, K. Roy, V. L. Troung-Le, K. A. Janes, K. Y. Lin, Y. Wang, J. T. August and K. W. Leong, *Journal of Controlled Release*, 2001, **70**, 399-421.
3. M. W. Pfaffl, *Nucleic acids research*, 2001, **29**, e45-e45.