

## Supporting Information

### Hydroxyethyl-functionalized ultrasmall chitosan nanoparticles as a gene delivery carrier

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Experimental Section

#### 1 Gel permeation Chromatography (GPC)

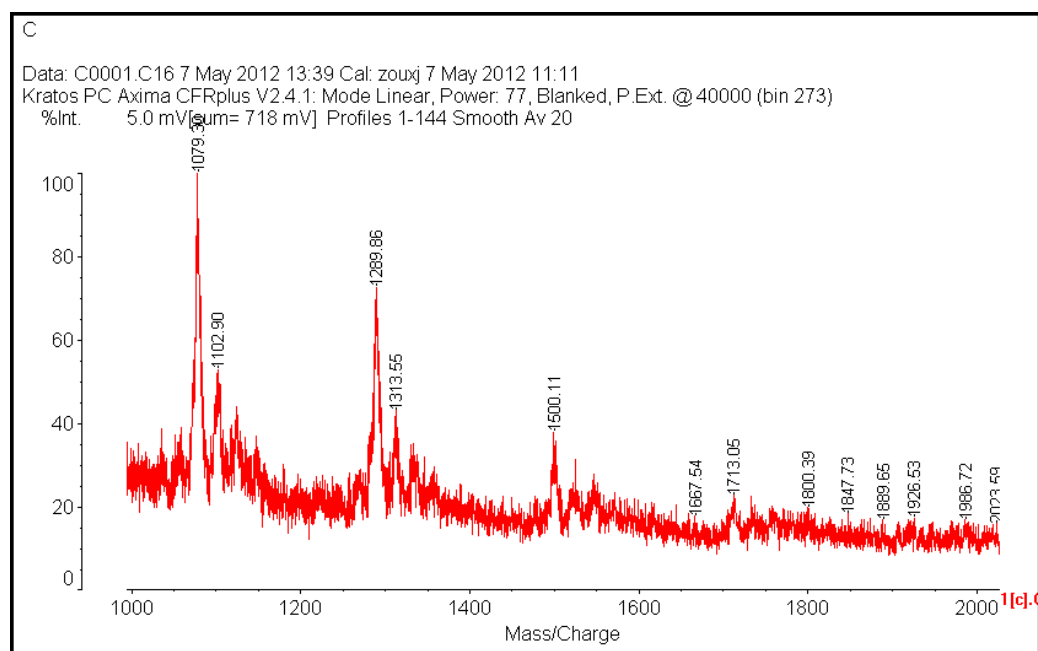
The molecular weight of HE-ULMWCh NPs was measured by GPC. Dextran with a molecular weight range from 1200 to 4300 (American Polymer Standards Corporation, Mentor, OH, USA) were used as standard samples to establish the universal calibration curve. The GPC equipment consists of Varion Prostar 350 RI Detector, 410 Autosampler, 210 Solvent Delivery, TSK-GEL G6000PWXL and GMPWXL Columns (Column temperature is 30°C) and Galaxie Chromatography workstation software (v 1.8.501.1). The concentration of HE-ULMWCh NPs solution was 1 mg/ml in 0.2 M CH<sub>3</sub>COOH/0.1 M CH<sub>3</sub>COONa. Injection volume was 50 µl, flow rate was 0.5 ml/min, and continuous phase was 0.2 M CH<sub>3</sub>COOH/0.1 M CH<sub>3</sub>COONa. All the solvents and solution was filtered through a 0.45 µm filter (Whatman Inc., Clifton, NJ, USA).

**Table S1.** Characterization of HE-ULMWCh NPs and HE-ULMWCh NPs /pDNA

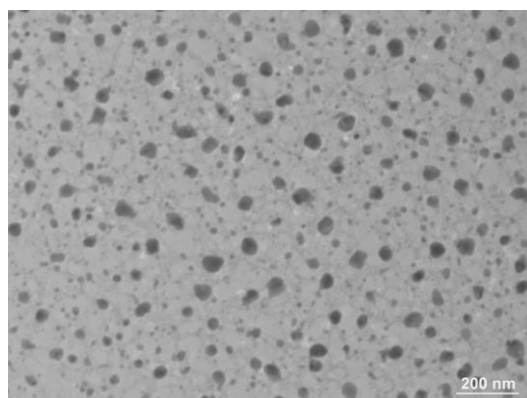
Formulation Drug	Mean diameter (nm)	Zeta Potential (mV)	PDI
HE-ULMWCh NPs	148±21nm	18.1±0.8mV	0.139
N/P=0.5	145±1nm	2.1±0.4mV	0.112
N/P=1	144±2nm	5.9±0.8mV	0.089
N/P=2	133±1nm	6.8±0.9mV	0.105
N/P=4	121±2nm	10.1±0.4mV	0.098
N/P=8	109±3nm	13.1±0.1mV	0.102
N/P=10	120±2nm	17.4±0.4mV	0.085

**Table S2.** The particle size and zeta potential of FITC-HE-ULWMCh NPs.

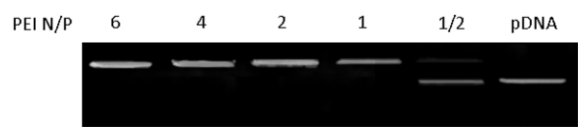
Nanoparticles	Mean diameter	Zeta potential	PDI
FITC-HE-ULWMCh NPs	143.7±3.7 nm	0.045±0.01	0.468±0.043



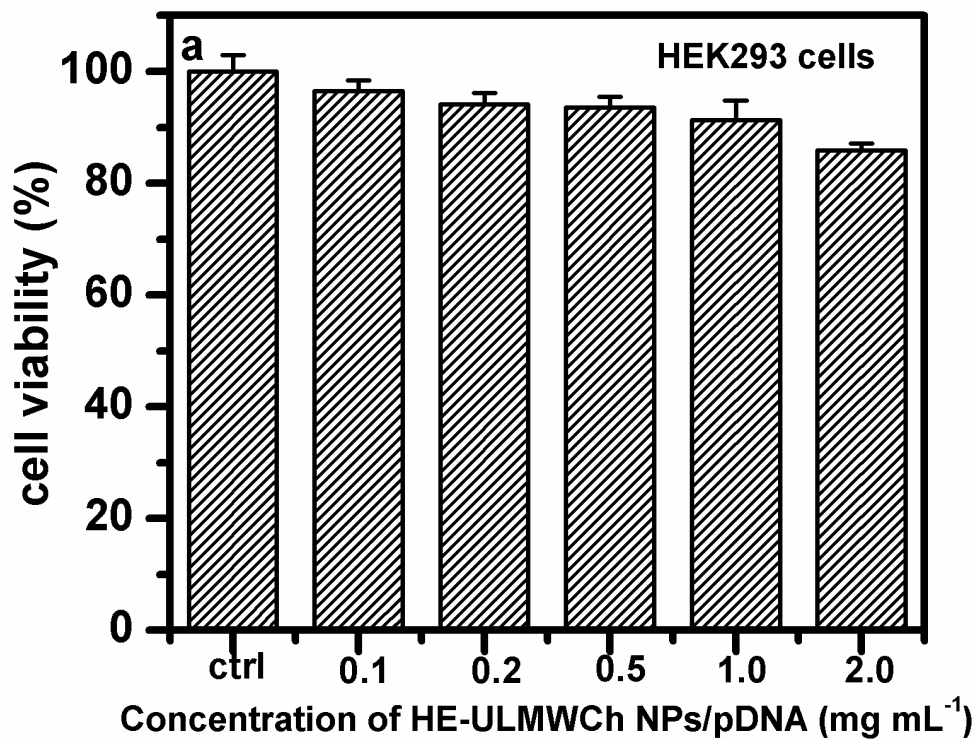
**Fig. S1.** The ESI spectrum of HE-ULWMCh oligmer. Mass peaks at 1500.11, 1289.86 and 1079.36 amu represent the HE-ULMWCh oligmer with 7 units, 6 units and 5 units, respectively.

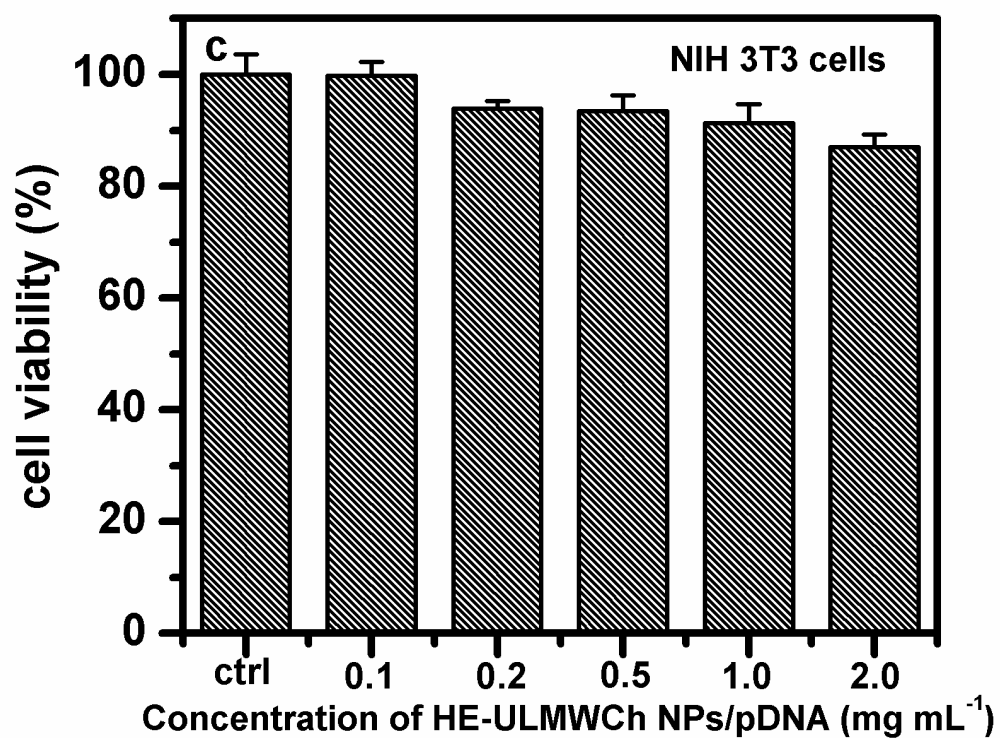
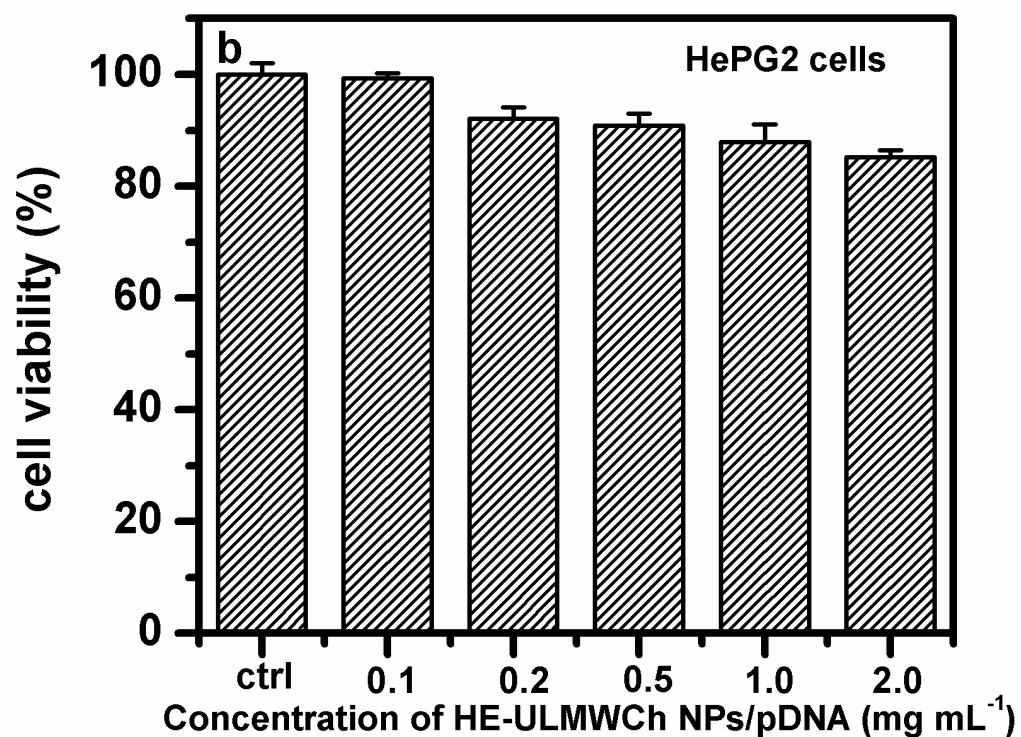


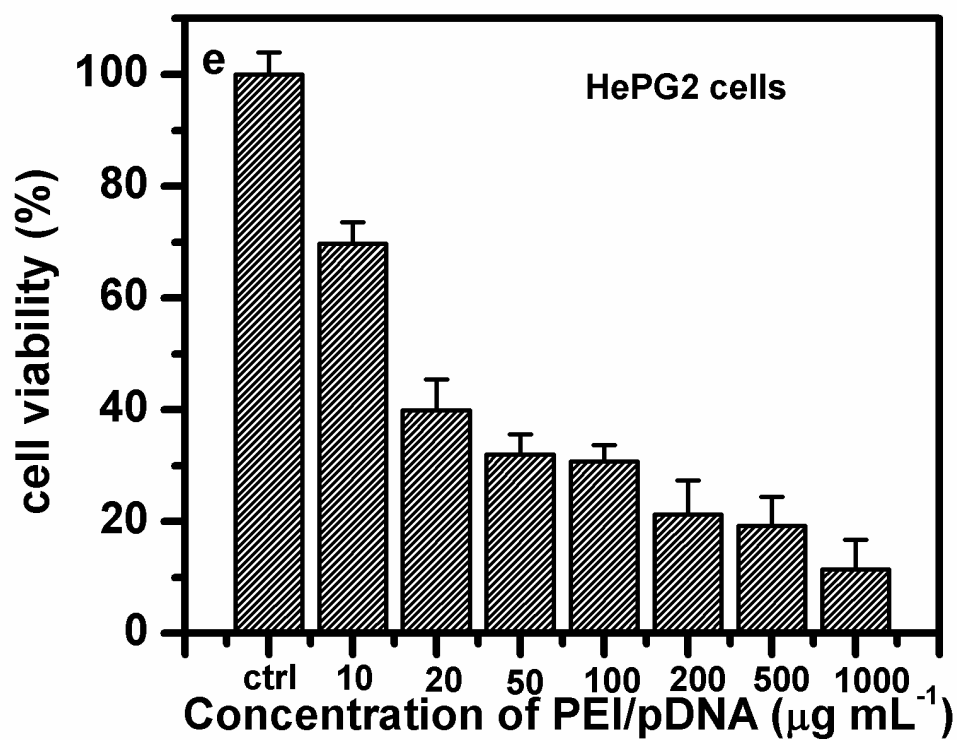
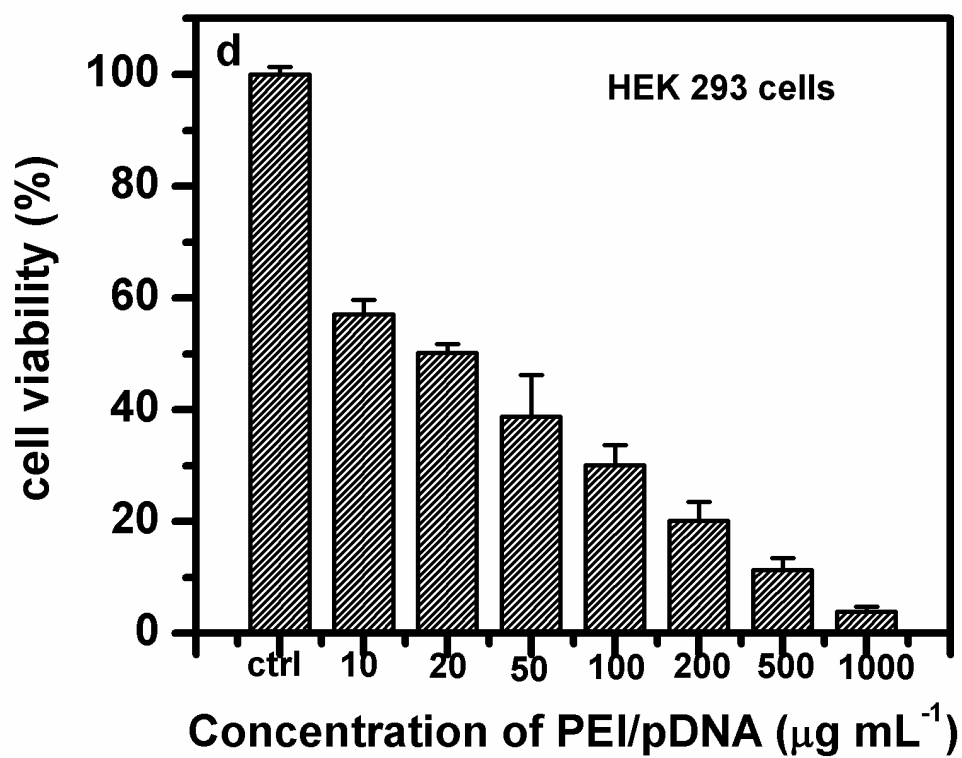
**Fig. S2.** The TEM spectra of redissolved HE-ULWMCh NPs after lyophilization.

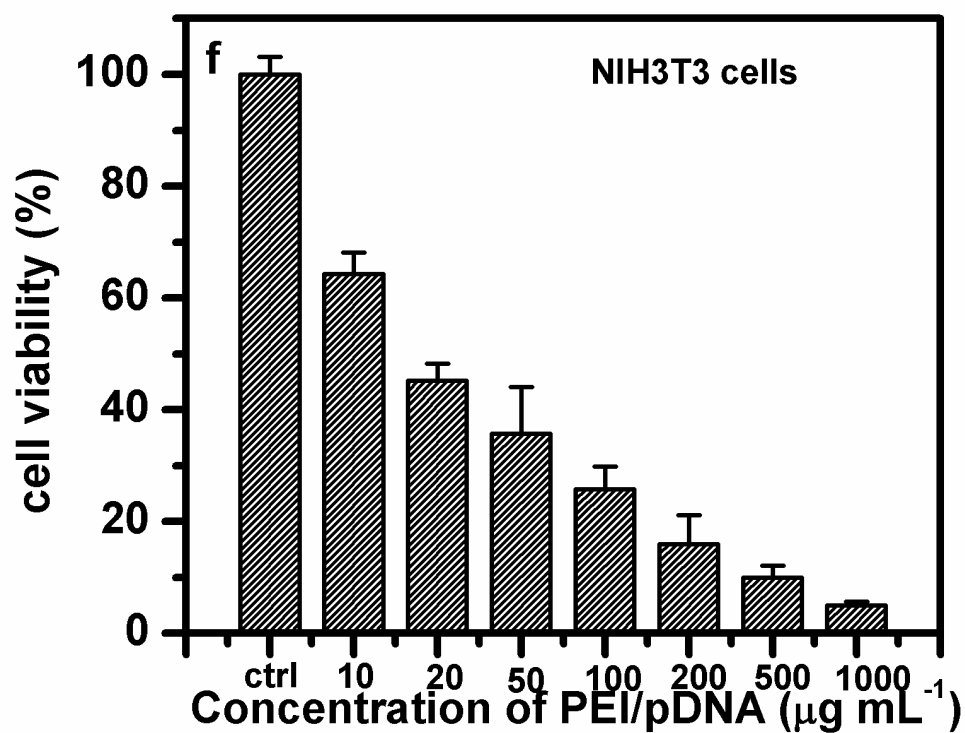


**Fig. S3.** The gel retardation assay for PEI/pDNA.

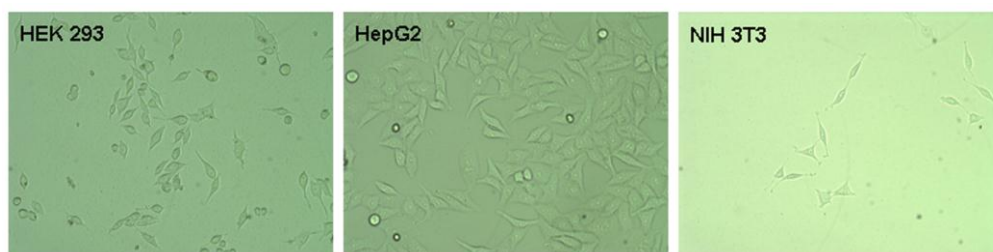








**Fig. S4** The cell viability assay of HE-ULMWCh NPs/pDNA and PEI/pDNA transfected NIH3T3, HePG2, and HEK 293 cells at the optimal N/P ratio (the concentration refers to the concentration of pDNA).



**Fig. S5** The bright field of HEK 293, HepG2, NIH3T3 cells treated by HE-ULMWCh NPs/pEGFP (N/P=10).