

**Rational design of engineered H-ferritin nanoparticles with improved siRNA delivery efficacy across an *in vitro* model of mouse blood-brain barrier (BBB)**

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## **Protein and nucleic acid sequences**

Molecular weight (MW) and isoelectric point (pI) of proteins were calculated using the SIB Bioinformatics Resource Portal tool (<http://web.expasy.org/protparam/>).

### **Ferritin Heavy Chain (FTH1, HFn, No His6 tag)**

(182residues, MW = 21,094, pI = 5.30)

**TTASTSQVRQNYHQDSEAAINRQINLELYASYVYLSMSYYFDRDDVALKNFAKY  
FLHQSHREERHAEKLMKLNQRGGRIFLQDIKKPDCDDWESGLNAMECALHL  
EKNVNQSLLELHKLATDKNDPHLCDFIETHYLNEQVKAIKELGDHVTNLRKMG  
APESGLAEYLFDKHTLGSDNES**

**HFn1 (His6 tag)** (188 residues, MW = 22,191, pI = 8.92)

**HHHHHHTTASTSQVRQNYHQDSEAAINRQINLELYASYVYLSMSYYFRRDDVAL  
KNFAKYFLHQSHRERRHARKLMKLNQRGGRIFLQDIKKPDCDDWESGLNAM  
ECALHLEKNVNQSLLELHKLATDKNDPHLCRFIRTHYLREQVKAIKELGDHVT  
NLRKMGAPESGLAEYLFRKHTLGSDNES**

**HFn2 (His6 tag)** (188 residues, MW = 22,149, pI = 8.71)

**HHHHHHTTASTSQVRQNYHQDSEAAINRQINLELYASYVYLSMSYYFRRDDVAL  
KNFAKYFLHQSHRERRHARKLMKLNQRGGRIFLQDIKKPDCDDWESGLNAM  
ECALHLEKNVNQSLLELHKLATDKNDPHLCRFIRTHYLNEQVKAIKELGDHVT  
NLRKMGAPESGLAEYLFRKHTLGSDNES**

**HFn3 (His6 tag)** (188 residues, MW = 22,150, pI = 8.41)

**HHHHHHTTASTSQVRQNYHQDSEAAINRQINLELYASYVYLSMSYYFRRDDVAL  
KNFAKYFLHQSHRERRHARKLMKLNQRGGRIFLQDIKKPDCDDWESGLNAM**

**ECALHLEKNVNQSLLELHKLATDKNDPHLCDFIRTHYLREQVKAIKELGDHVT**

**NLRKMGAPESGLAEYLFRKHTLGSDNES**

**HFn4(His6 tag)** (188 residues, MW = 22,108, pI = 7.95)

**HHHHHHTTASTSQVRQNYHQDSEAAINRQINLELYASYVYLSMSYYFRRDDVAL**

**KNFAKYFLHQSHRERRHARKLMKLQNQRGGRIFLQDIKKPDCDDWESGLNAM**

**ECALHLEKNVNQSLLELHKLATDKNDPHLCDFIRTHYLNEQVKAIKELGDHVT**

**NLRKMGAPESGLAEYLFRKHTLGSDNES**

**HFn5(His6 tag)** (188 residues, MW = 22,123, pI = 7.42)

**HHHHHHTTASTSQVRQNYHQDSEAAINRQINLELYASYVYLSMSYYFRRDDVAL**

**KNFAKYFLHQSHRERRHARKLMKLQNQRGGRIFLQDIKKPDCDDWESGLNAM**

**ECALHLEKNVNQSLLELHKLATDKNDPHLCDFIETHYLREQVKAIKELGDHVT**

**NLRKMGAPESGLAEYLFRKHTLGSDNES**

**HFn6 (His6 tag)** (188 residues, MW = 22,123, pI = 7.42)

**HHHHHHTTASTSQVRQNYHQDSEAAINRQINLELYASYVYLSMSYYFRRDDVAL**

**KNFAKYFLHQSHRERRHARKLMKLQNQRGGRIFLQDIKKPDCDDWESGLNAM**

**ECALHLEKNVNQSLLELHKLATDKNDPHLCRFIETHYLREQVKAIKELGDHVT**

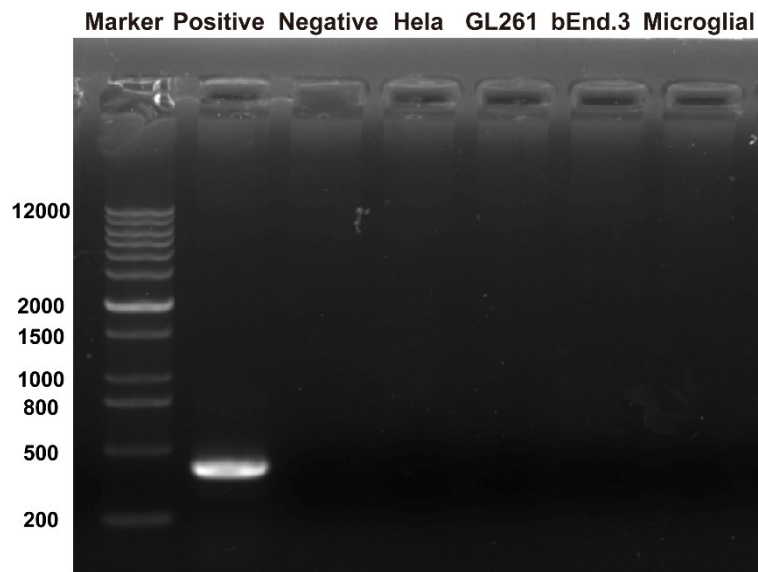
**NLRKMGAPESGLAEYLFDKHTLGSDNES**

## Supplementary Tables and Figures

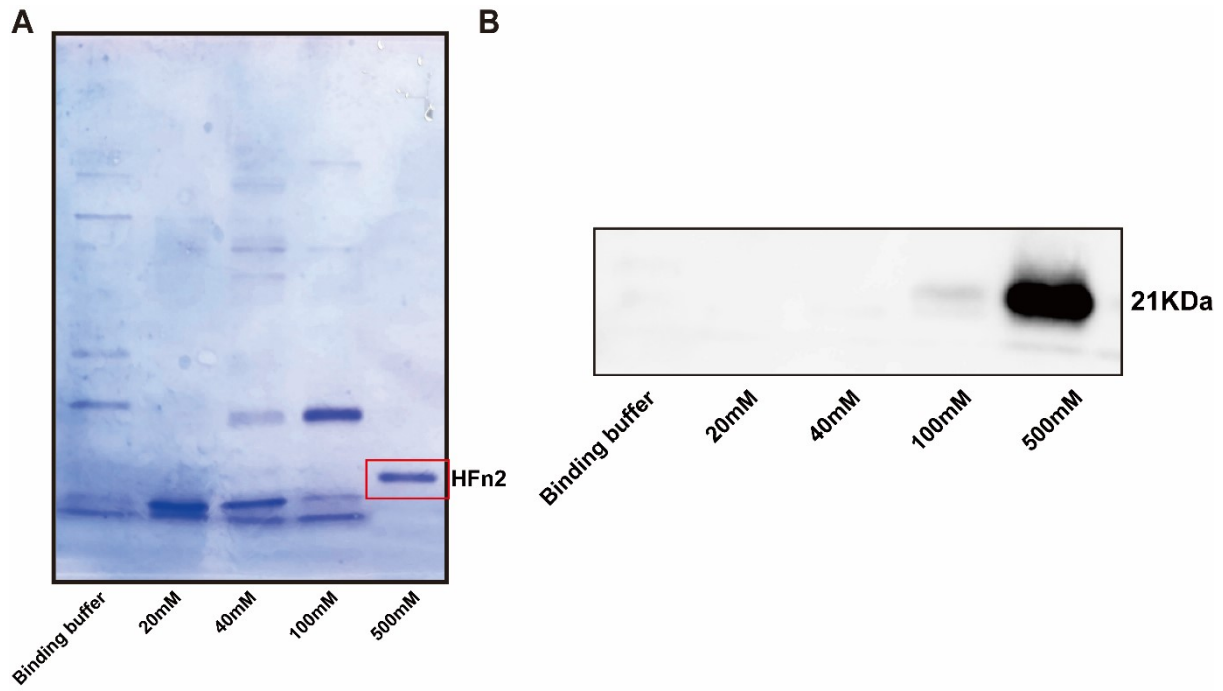
**Table S1. Oligonucleotide sequences**

<b>Identity</b>	<b>Length</b>	<b>Sequence</b>	<b>Required experiments</b>
siLuc	21	GCUUCAACGAGUACGACUUTT	Nuclease stability assay, luciferase knockdown
ssDNA-1	21	TCGTCGCGATGATGTGGCTTT	Arginine mutation of ASP42 for cloning HFn+
ssDNA-2	21	TCGTGAGAGGGAACATGCTGA	Arginine mutation of GLU61 for cloning HFn+
ssDNA-3	21	GGCGTCATGCTGAGAACTGA	Arginine mutation of GLU64 for cloning HFn+
ssDNA-4	23	TCGTAAACTGATGAAGCTGCAGA	Arginine mutation of GLU67 for cloning HFn+
ssDNA-5	21	TCGTAAGCACACCCTGGGAGA	Arginine mutation of ASP171 for cloning HFn+
ssDNA-6	24	TCGTACACATTACCTGAATGAGC  A	Arginine mutation of GLU134 for cloning HFn+
ssDNA-7	24	CGTTTCATTGAGACACATTACCT  G	Arginine mutation of ASP131 for cloning HFn+
ssDNA-8	22	GCGTGAGCAGGTGAAAGCCATC	Arginine mutation of ASP139 for cloning HFn+

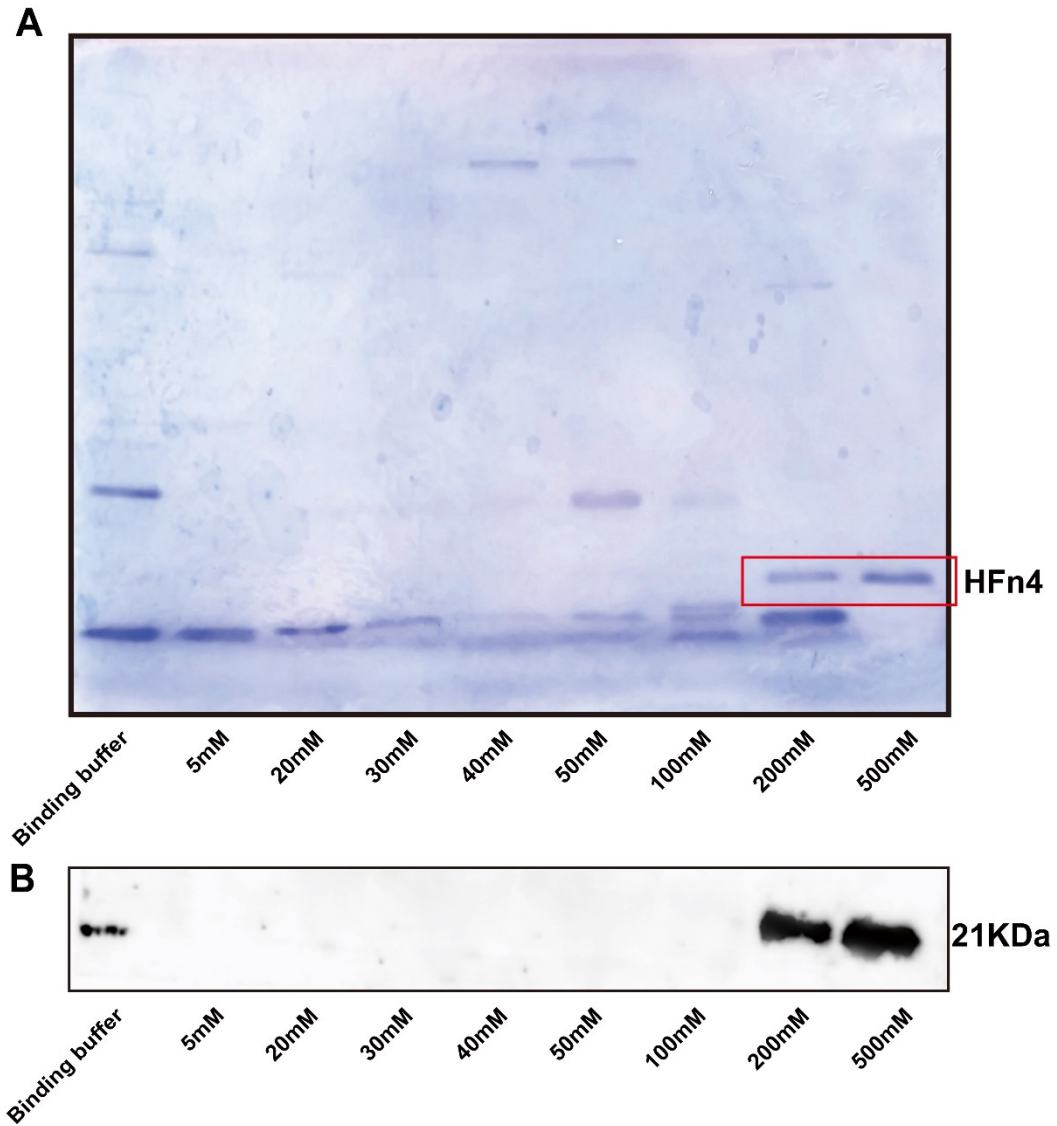
Note: ssDNA refers to the upstream primers of PCR designed for each arginine-mutated amino acid site of HFn during the gene site-directed mutation process.



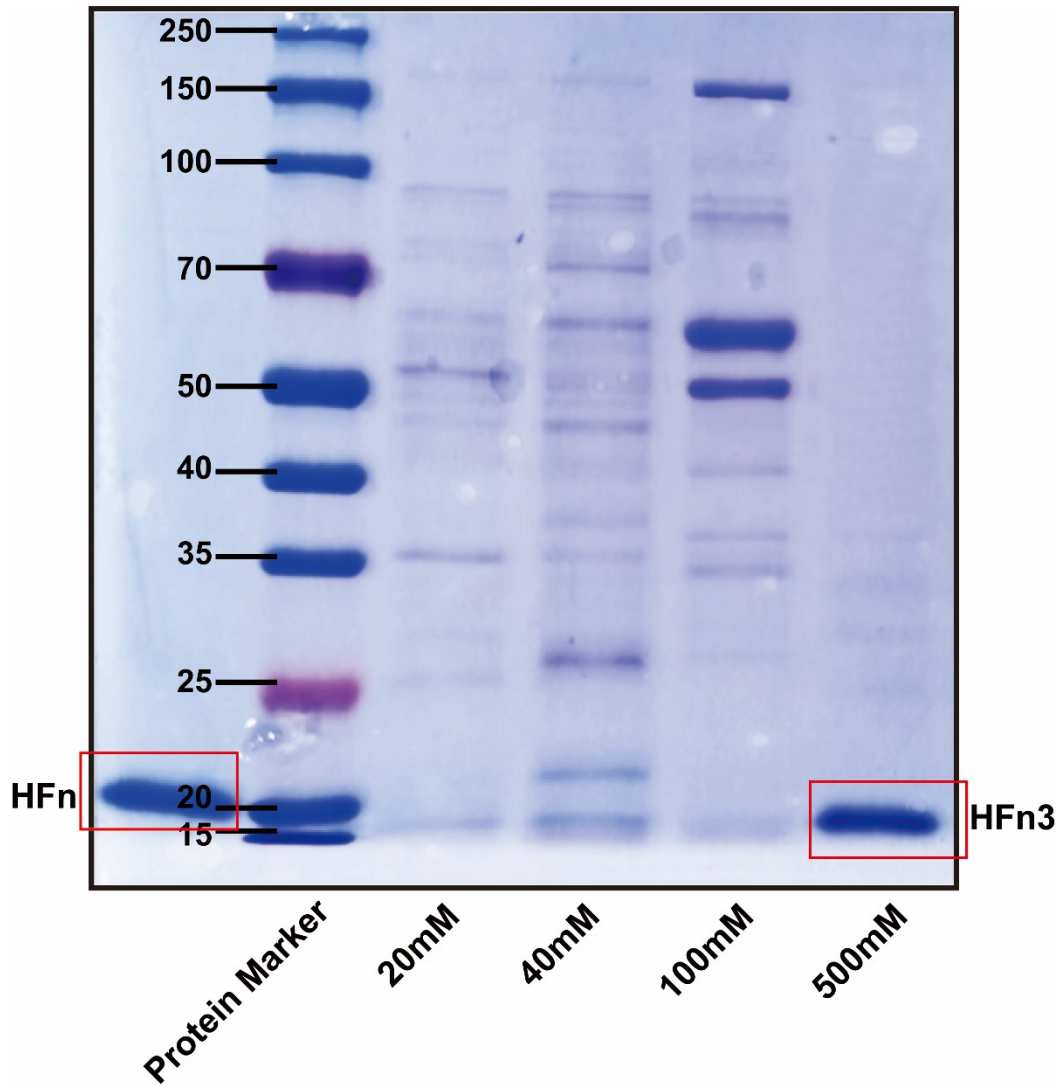
**Fig. S1** Mycoplasma PCR detection to check the mycoplasma contamination of HeLa cells, GL261 cells, bEnd.3 cells and primary microglial cells.



**Fig. S2** (A) The purification of HFn2 was analyzed by 12% SDS-PAGE. Lane 1: unbound HFn2 lysate eluted by binding buffer; Lanes 2-5: HFn2 lysate eluted by 20 mM, 40 mM, 100 mM, and 500 mM imidazole respectively. (B) The western blot result of HFn2 lysate from various concentrations of imidazole by HFn antibody.

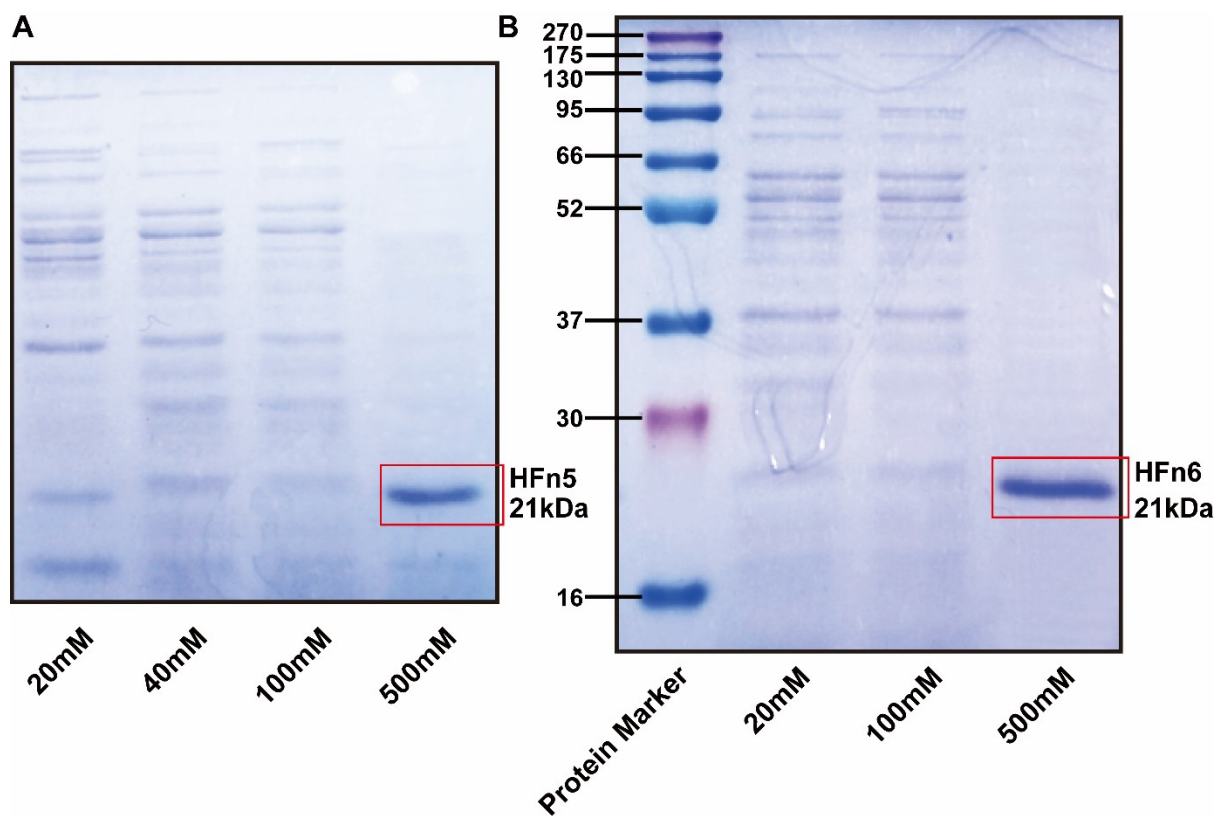


**Fig. S3** (A) The purification of HFn4 was analyzed by 12% SDS-PAGE. Lane 1: unbound HFn2 lysate eluted by binding buffer; Lanes 2-9: HFn4 lysate eluted by 5 mM, 20 mM, 30 mM, 40 mM, 50 mM, 100 mM, 200 mM, and 500 mM imidazole respectively. (B) The western blot result of HFn4 lysate from various concentrations of imidazole by HFn antibody.

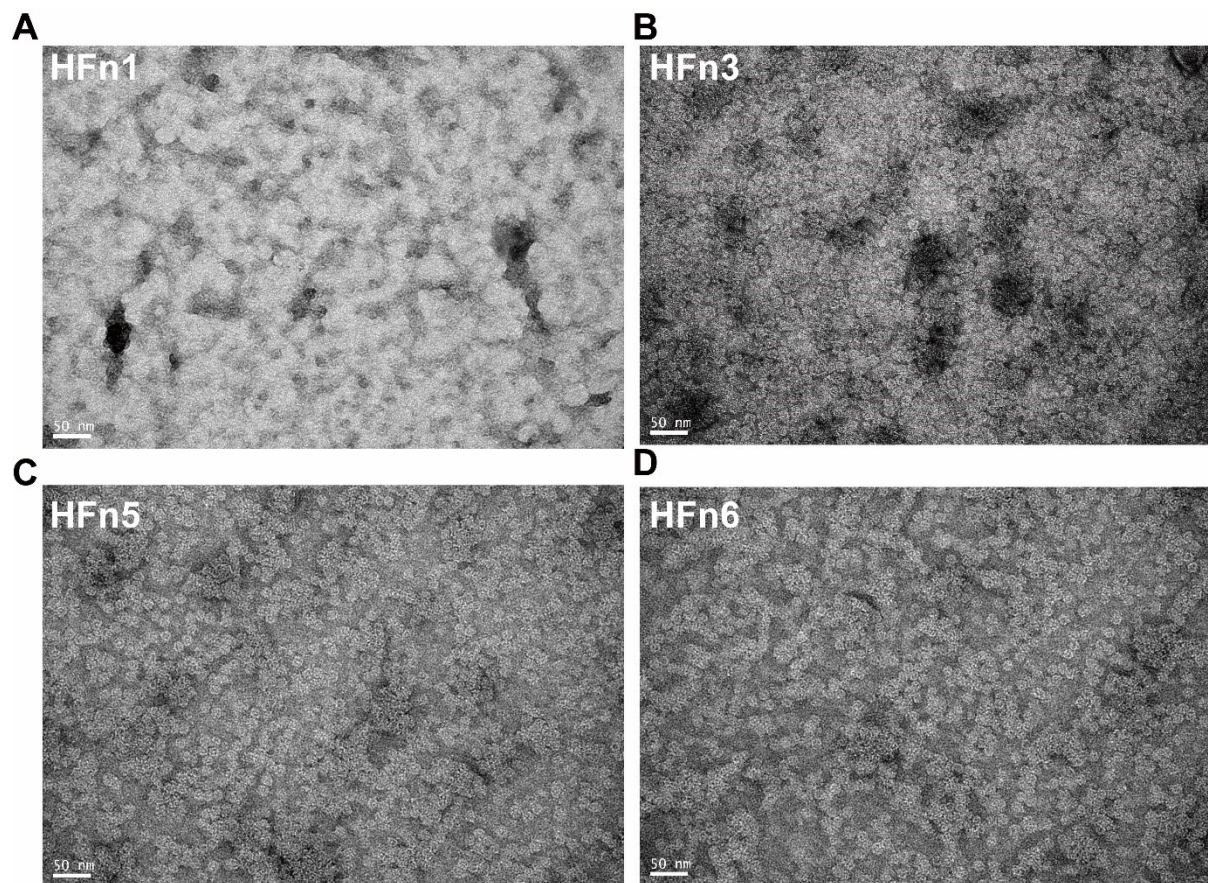


**Fig. S4** The purification of HFn3 was analyzed by 12% SDS-PAGE. Lane 1: HFn purified by SEC; Lane 2: protein marker; Lanes 3-6: HFn3 lysate eluted by 20 mM, 40 mM, 100 mM, and 500 mM imidazole respectively.

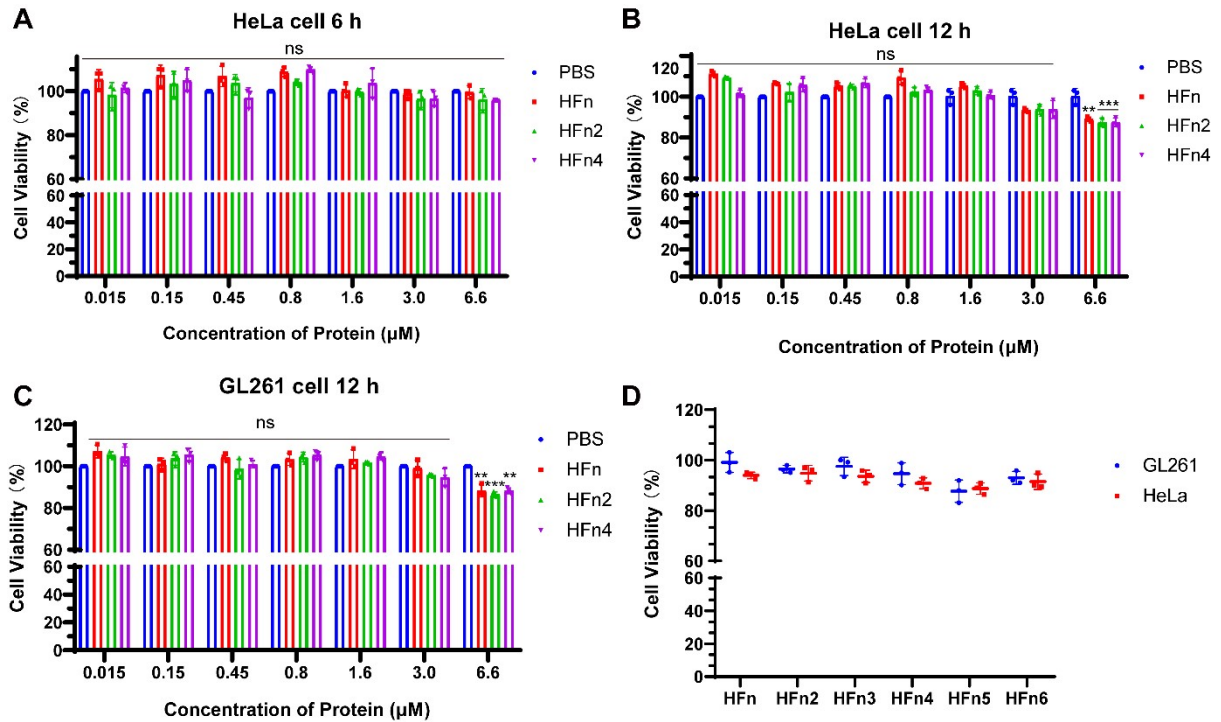




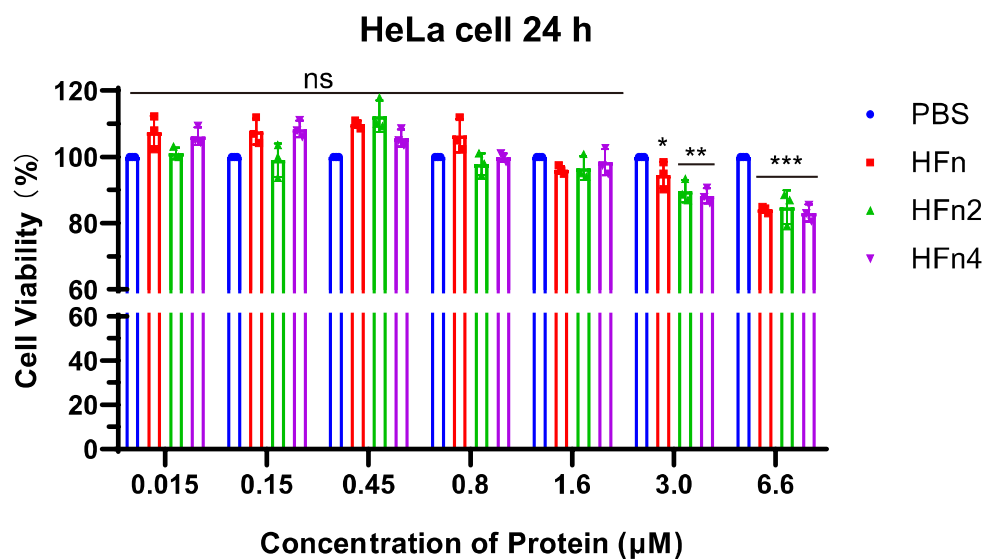
**Fig. S5** (A) The purification of HFn5 was analyzed by 12% SDS-PAGE. Lanes 1-4: HFn5 lysate eluted with 20 mM, 40 mM, 100 mM, and 500 mM imidazole, respectively. (B) The purification of HFn6 was analyzed by 12% SDS-PAGE. Lane 1: protein marker; Lanes 2-4: HFn6 lysate eluted with 20 mM, 100 mM, and 500 mM imidazole respectively.



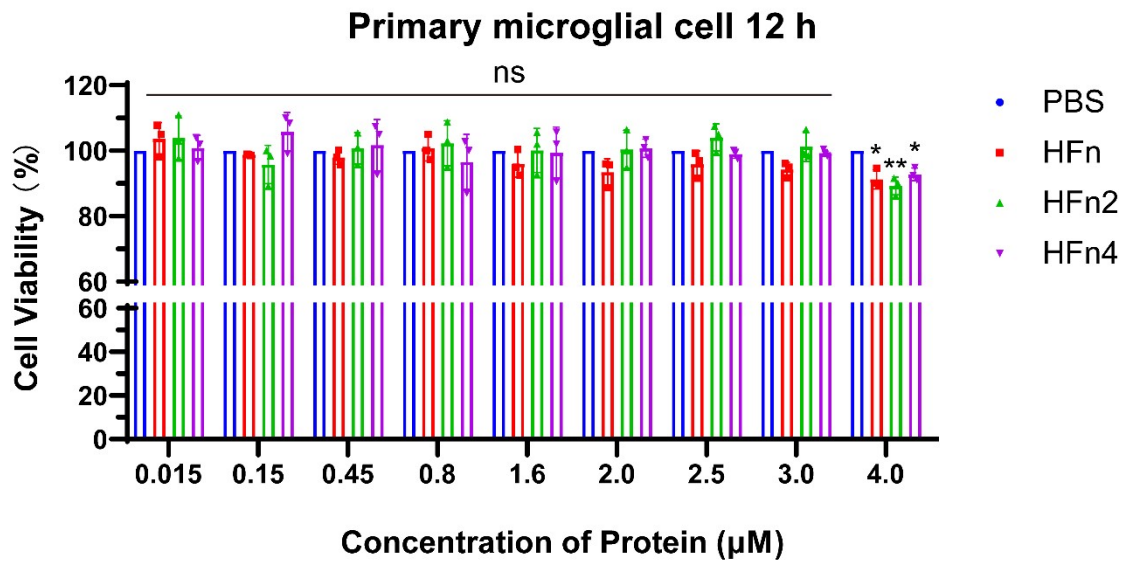
**Fig. S6** TEM photographs of (A) HFn1, (B) HFn3, (C) HFn5, and (D) HFn6 NPs in PBS buffer (0.05 M  $\text{PO}_4^{3-}$ , 0.15 M NaCl). Scale bars: 50 nm.



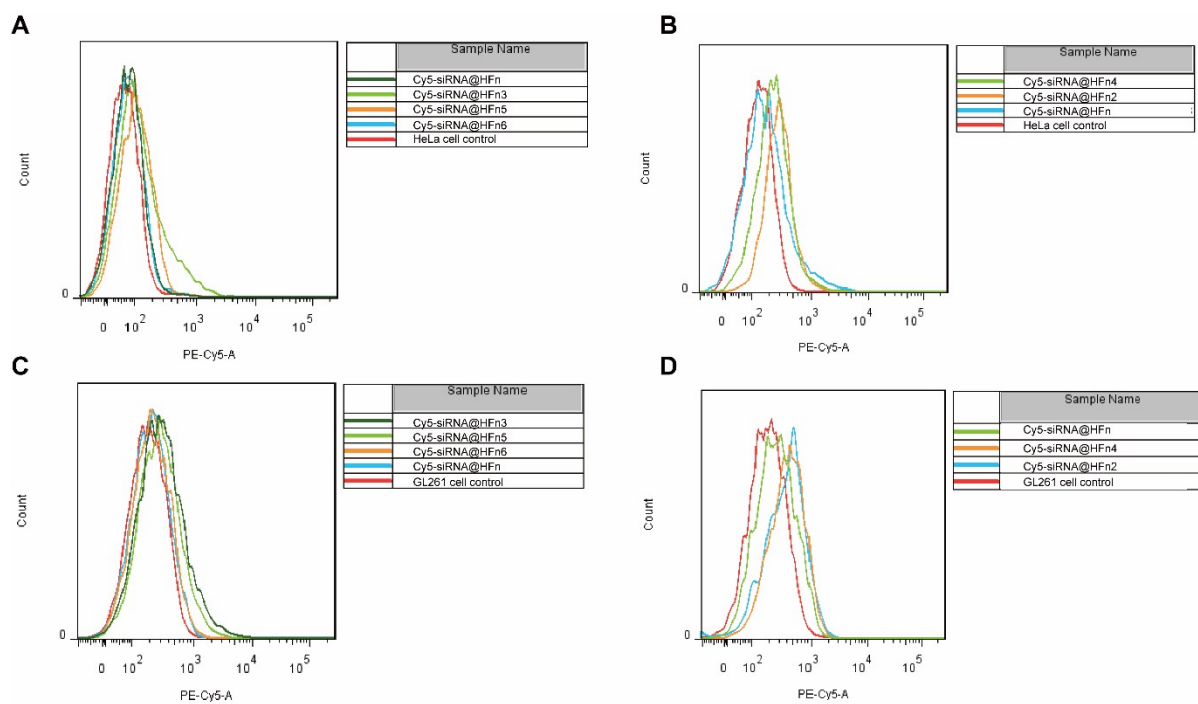
**Fig. S7** Cytotoxicity evaluation of HFfn, HFfn2, and HFfn4 blank vehicles in HeLa cells after (A) 6 h and (B) 12 h of co-incubation at concentrations ranging from 0.015  $\mu\text{M}$  to 6.6  $\mu\text{M}$ . *In vitro* cell viability of (C) GL261 cells after 12 h of exposure to HFfn, HFfn2, and HFfn4 NPs at the same concentration range. (D) Cytotoxicity of both HFfn+ and HFfn blank vehicles in GL261 and HeLa cells after 12 h of co-incubation at a concentration of 3.0  $\mu\text{M}$ . Data are represented as the mean  $\pm$  SD ( $n = 3$ ;  $p > 0.05$  (ns) vs. HFfn). Significant differences were assessed using a one-way ANOVA with the Turkey test (A-D). Data in (A-D) are presented as mean  $\pm$  SD from the second repeat.



**Fig. S8** Cytotoxicity evaluation of HFfn, HFfn2 and HFfn4 blank vehicles in HeLa cells after incubation for 24 h at a concentration range from 0.015  $\mu\text{M}$  to 6.6  $\mu\text{M}$  ( $n=3$ ;  $p > 0.05$  (ns),  $*p < 0.05$ ,  $**p < 0.01$ ,  $***p < 0.001$  vs. HFfn). Significant differences were assessed using a one-way ANOVA with the Turkey test. Data is presented as mean  $\pm$  SD from the second repeat.



**Fig. S9** Cytotoxicity evaluation of HFfn, HFfn2 and HFfn4 blank vehicles in primary microglial cells after incubation for 12 h at a concentration range from 0.015 µM to 4.0 µM ( $n=3$ ;  $p > 0.05$  (ns),  $*p < 0.05$ ,  $**p < 0.01$  vs. HFfn). Significant differences were assessed using a one-way ANOVA with the Turkey test. Data is presented as mean  $\pm$  SD from the second repeat.



**Fig. S10** Flow cytometry analysis of HeLa cells induced by HFn@Cy5-siRNA and HFn+@Cy5-siRNA NPs for 12 h. (A), (B) HFn/HFn+@Cy5-siRNA NPs at a concentration of 1.5  $\mu$ M (1:8 ratio) in HeLa cells and (C), (D) GL261 cells ( $n=3$ ).