Supplementary Materials

Identification of a boron nitride nanosphere-binding peptide for the intracellular delivery of CpG oligodeoxynucleotides

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Figure Legends

Supplementary Figure 1. Yield (output phages /input phages) against the rounds of panning. The phages with higher affinity for BNNS had been successfully concentrated in the phage pool.

Supplementary Figure 2. Amino acid frequencies in the BNNS-binding peptide sequence, as compared to the original phage library.

Supplementary Figure 3. Fluorescence microscopy images of the FITC-labeled peptides binding to BNNS. In the control group, the BNNS were incubated without peptides.

Supplementary Figure 4. Fluorescence emission spectra of BNNS, BP7 and BNNS/BP7 complex in TBS buffer.

Supplementary Figure 5. Cytotoxicity, cell uptake of the BNNS/BP7 complexs to HEK293XL-null and Hela cells. (a) Viabilities of 293XL-null and Hela cells measured by a water-soluble tetrazolium salt assay against BNNS and BNNS/BP7 complexes. Concentrations of the nanospheres: 0 μg/mL (Red), 25μg/mL (Cyan), 50μg/mL (Blue), 75μg/mL (Olive), 100μg/mL (Yellow). (b) Confocal microscopy images of HEK293XL-null and Hela cells after 24 h of incubation with BNNS/BP7 complexes. Data presented as mean ± SD (n =5)

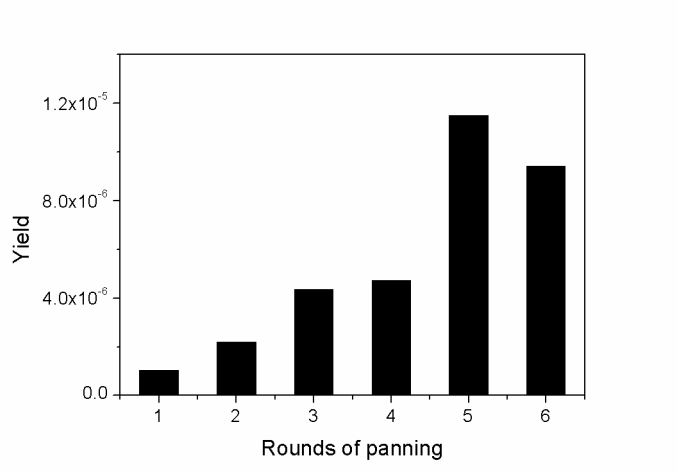
Supplementary Figure 6. Loading capacity of the BP7 mutants–CpG ODN conjugate on BNNS, denoted as μg CpG ODNs loaded on 1 mg BNNS. M1 (BP7-Y8A) and M2 (BP7-L10A) are mutants of BP7 whose tyrosine (Y8) and leucine (L10) at eighth and tenth positions from N-terminal were replaced by alanine (A), respectively. Loading capacity of the BP7–CpG ODN conjugate on BNNS is shown in Fig. 6a. Data presented as mean ± SD (n = 3).

Supplementary Figure 7. Zeta potentials of BNNS, BNNS/BP7, BNNS/CpG ODNs, BNNS/BP7-CpG ODNs complexs in TBS buffer (pH 7.4). Data presented as mean ± SD (n =6)

Supplementary Figure 8. IFN-α induction from PBMCs stimulated by CpG ODNs. M1 (BP7-Y8A), M2 (BP7-L10A). The concentration of the BNNS was about 87μg/mL. PTO-2216 is positive control. Data presented as mean ± SD (n =3). The symbol # means not detectable (below detection limit).

Supplementary Figure 9. Cytokine induction from PBMCs stimulated by BP7 mutants–CpG ODN conjugate–loaded BNNS. (a) IL-6 production. (b) TNF-α production. Loaded BNNS (87 μg/mL) was incubated with PBMCs for 8 h (TNF-α) and 24 h (IL-6) respectively, M1 (BP7-Y8A) and M2 (BP7-L10A) are mutants of BP7 whose tyrosine (Y8) and leucine (L10) at eighth and tenth positions from N-terminal were replaced by alanine (A), respectively. The levels of IL-6

and TNF-α induced by BNNS/BP7-CpG ODNs are shown in Fig. 7. Data are presented as mean ± SD (n = 3).



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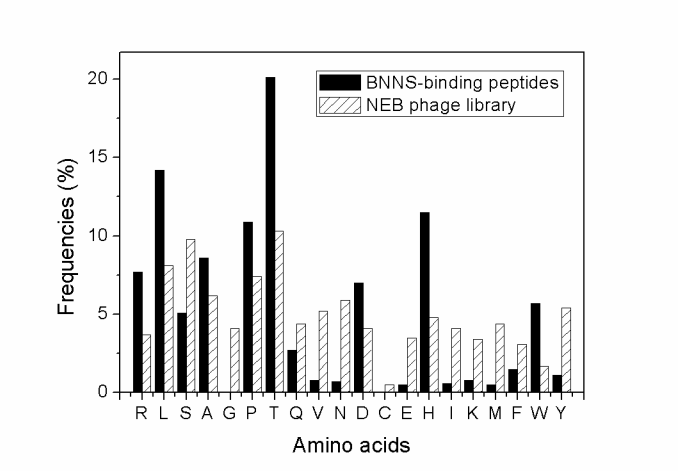

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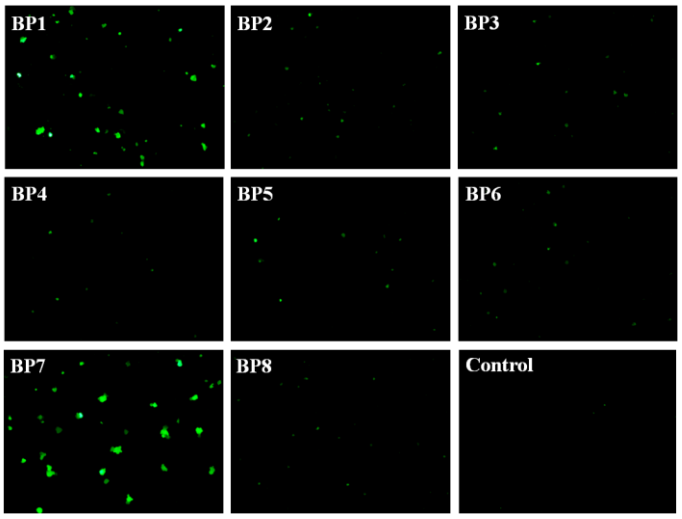

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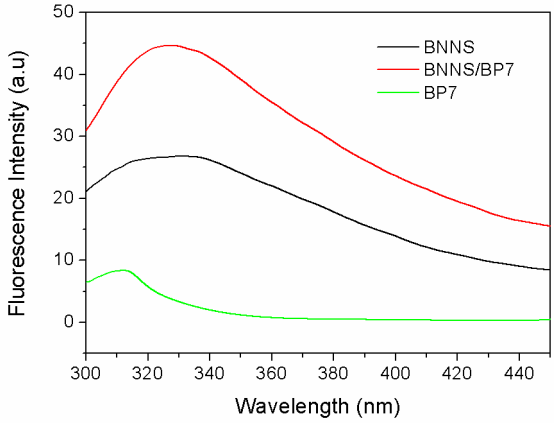

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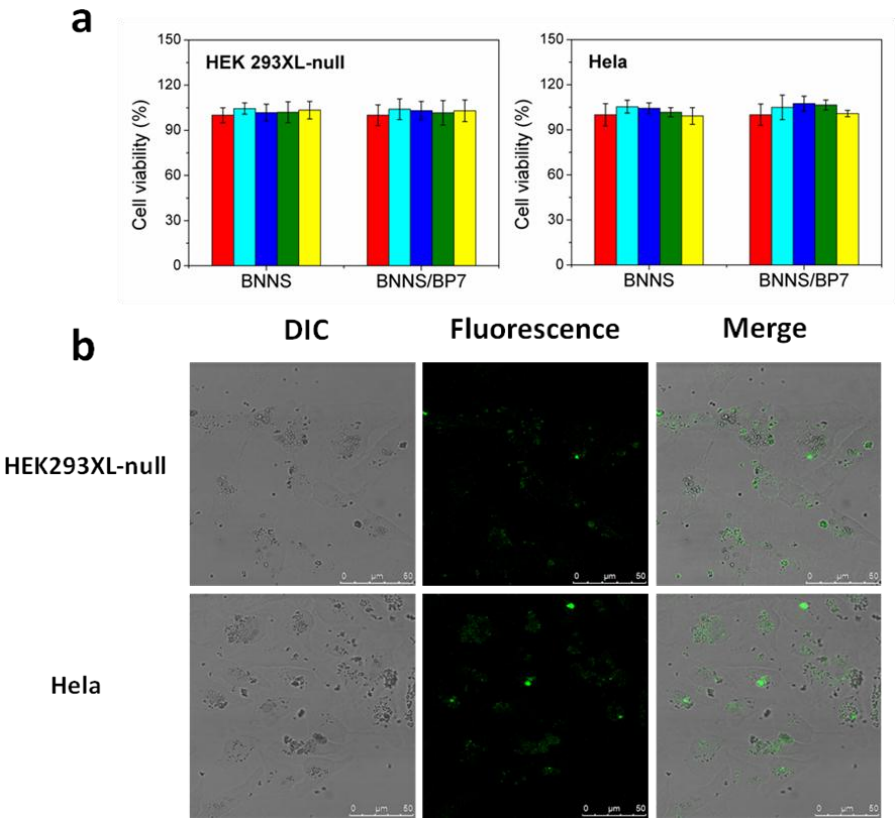

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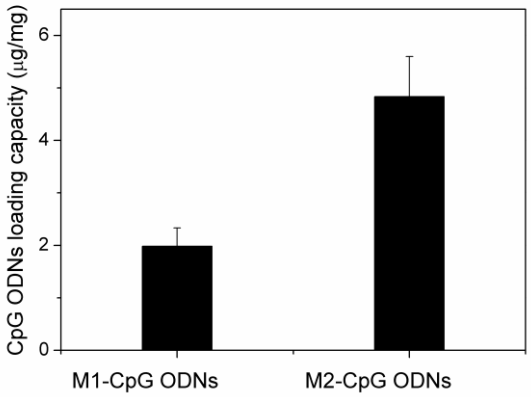

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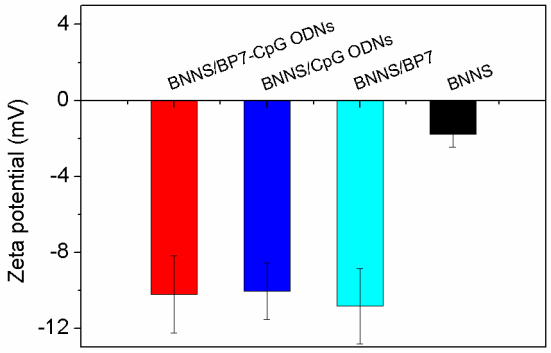

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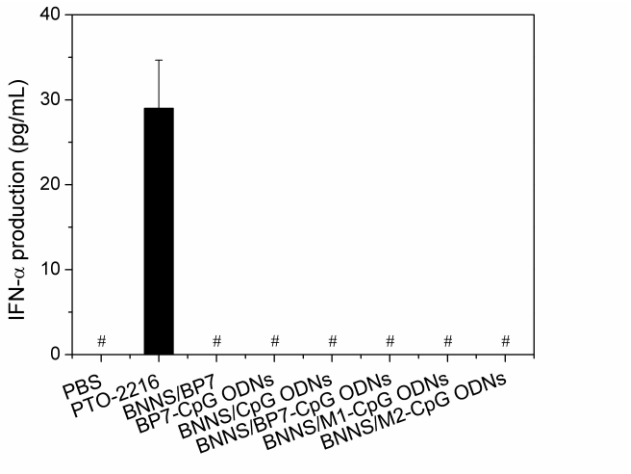

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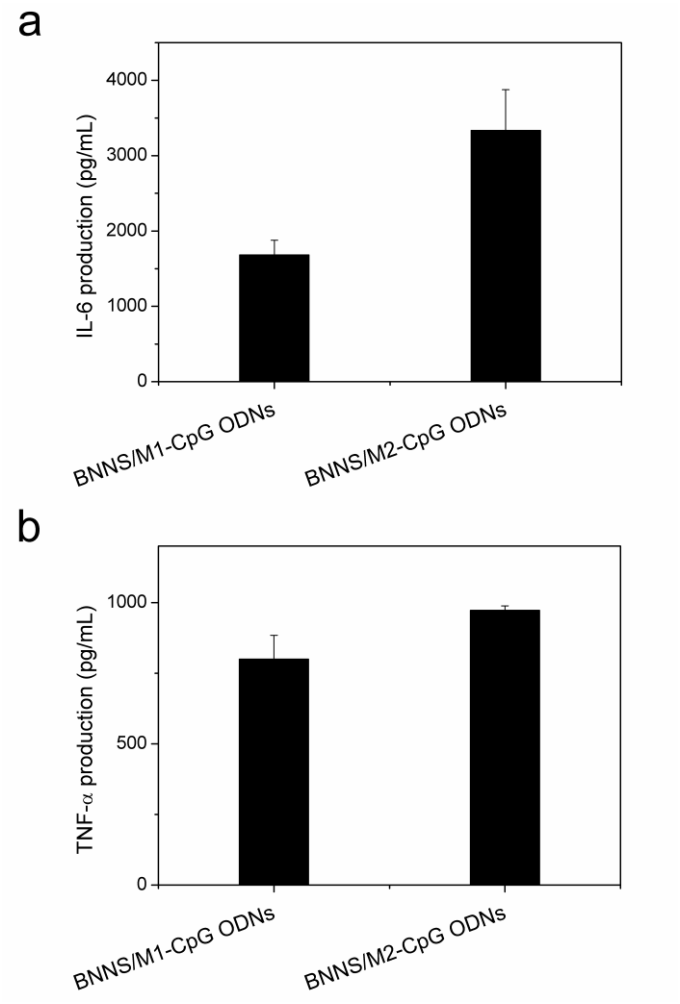

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