Supporting Information

Substance P Containing Peptide Gene Delivery Vectors for Specifically Transfecting Glioma Cells Mediated by Neurokinin-1 Receptor

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Table S1. Percentages of α-helix conformation for peptide vectors at a concentration of 50 μM in TFE/PBS (50%, v/v) solution.

Figure S1. RP-HPLC of peptide vectors (P-01~P-08 and SP).

Figure S2. MALDI-TOF-MS of peptide vectors (P-01~P-08 and SP).

Figure S3. CD spectra of all peptide vectors at a concentration of 50 μM in TFE/PBS (50%, v/v) solution.

Figure S4. Agarose gel electrophoresis assays of P-01~P-08 at various N/P ratios.

Figure S5. The particle size distributions and the TEM images of the peptide/pGL3 complexes (P-01, P-03~P-08) at the N/P ratio of 10. The scale bar represents 100 nm.

Figure S6. Zeta potentials of the peptide/pGL3 complexes at N/P ratios from 4 to 12.

Figure S7. Cell viability of (A) U87, (B) 293T-NK1R, and (C) 293T cells after being treated with peptide/pGL3 complexes at various N/P ratios. The data are shown as mean ± SD (n = 5).

Figure S8. *In vitro* luciferase expression levels of peptide/pGL3 complexes in 293T cells at N/P ratios from 4 to 12. The data are shown as mean ± SD (n = 3). *$p < 0.05$; **$p < 0.01$; ***$p < 0.005$.

Figure S9. Cell viability of (A) U87, and (B) 293T-NK1R cells after being treated with free SP and P-02/pGL3 complexes in the presence of different amount of SP at an N/P ratio of 10 in (C) U87, and (D) 293T-NK1R cells. The data are shown as mean ± SD (n = 5).

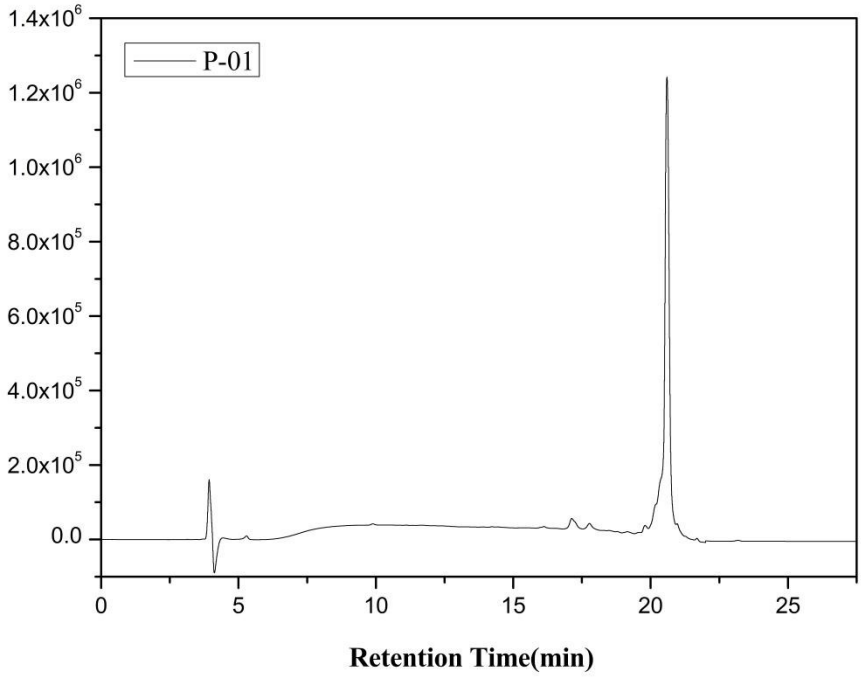
Figure S10. The mean fluorescence intensity of U87 (left), and 293T (right) cells in FACS assays. YOYO-1 was used to label the pGL3 plasmid.

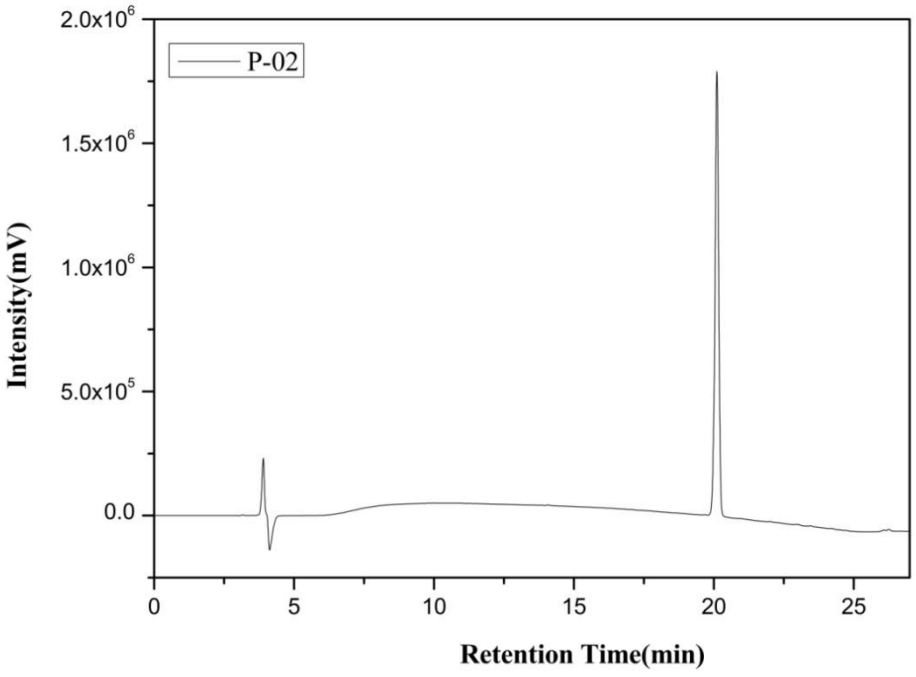
Figure S11. FACS assays of cellular uptake mechanisms of P-02/pGL3 complexes using free SP as a competitor at an N/P ratio of 10 in U87 cells. YOYO-1 was used to label the pGL3 plasmid.

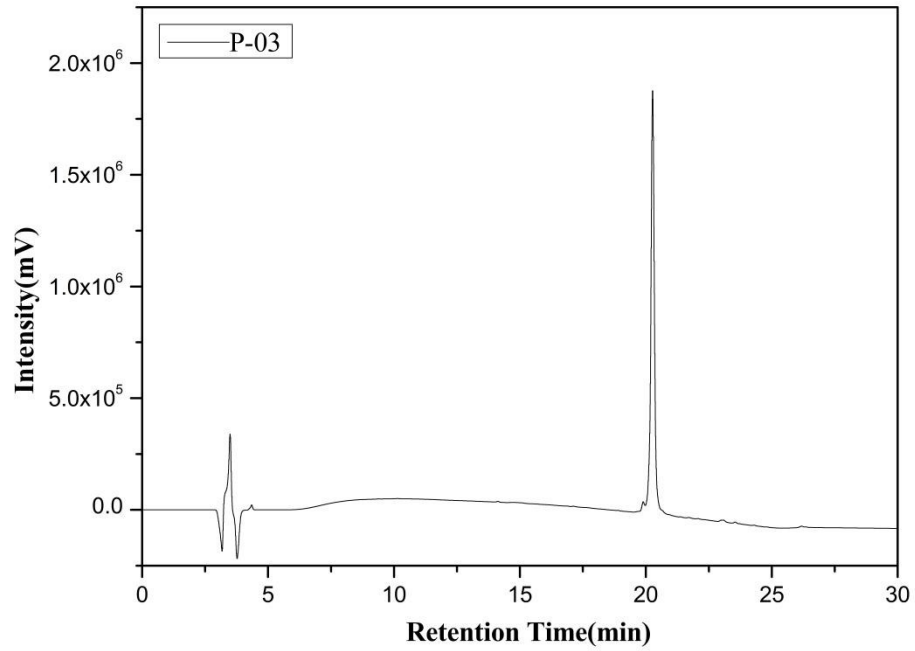
Figure S12. FACS assays of cellular uptake mechanisms of P-02/pGL3 complexes using different inhibitors for endocytosis at an N/P ratio of 10 in U87 cells. YOYO-1 was used to label the pGL3 plasmid.

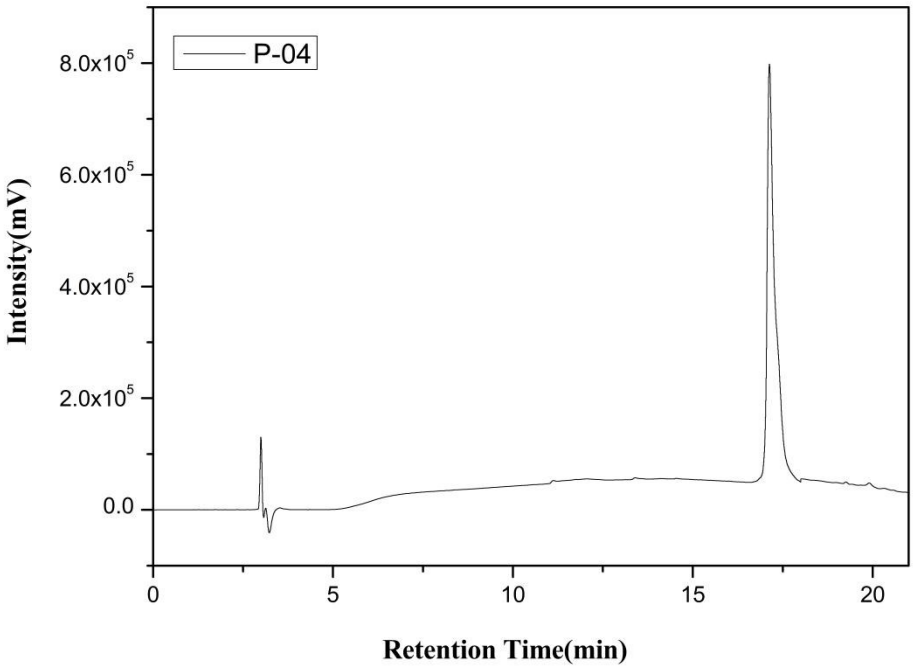
Table S1. Percentages of α-helix conformation for peptide vectors at a concentration of 50 μM in TFE/PBS (50%, v/v) solution.

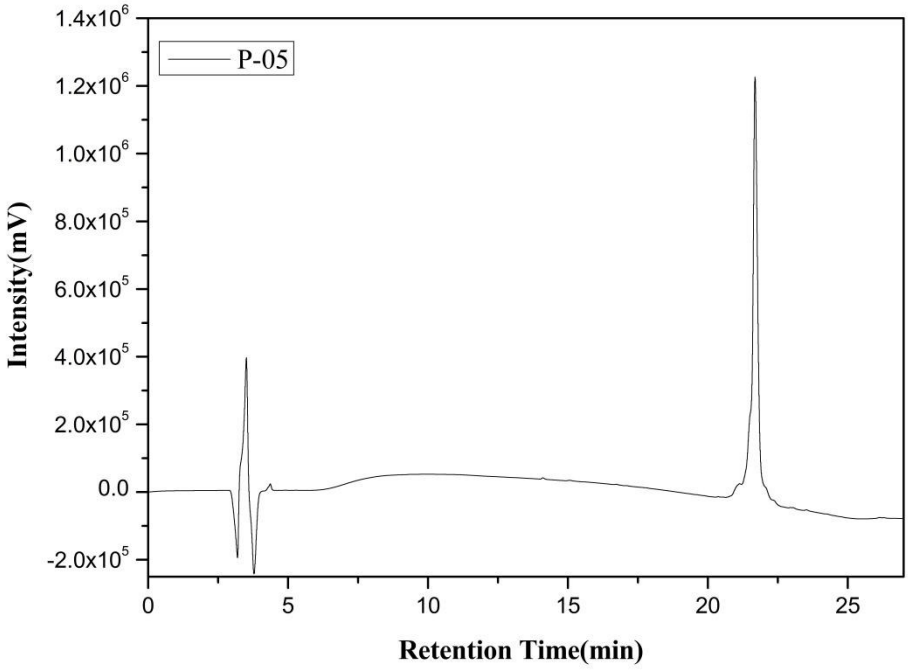
Compounds	Percentage of α-helix (%)
P-01	26.01
P-02	30.49
P-03	35.25
P-04	21.66
P-05	22.53
P-06	24.38
P-07	37.52
P-08	29.32


P-01
1.4x10^6
1.2x10^6
1.0x10^6
8.0x10^5
6.0x10^5
4.0x10^5
2.0x10^5
0.0
0
5
10
15
20
25
Retention Time(min)


P-02
2.0x10^6
1.5x10^6
1.0x10^6
5.0x10^5
0.0
Intensity(mV)
0
5
10
15
20
25
Retention Time(min)


P-03
2.0x10^6
1.5x10^6
1.0x10^6
5.0x10^5
0.0
Intensity(mV)
0
5
10
15
20
25
30
Retention Time(min)


P-04
8.0x10^5
6.0x10^5
4.0x10^5
2.0x10^5
0.0
Intensity(mV)
0
5
10
15
20
Retention Time(min)


P-05
Intensity(mV)
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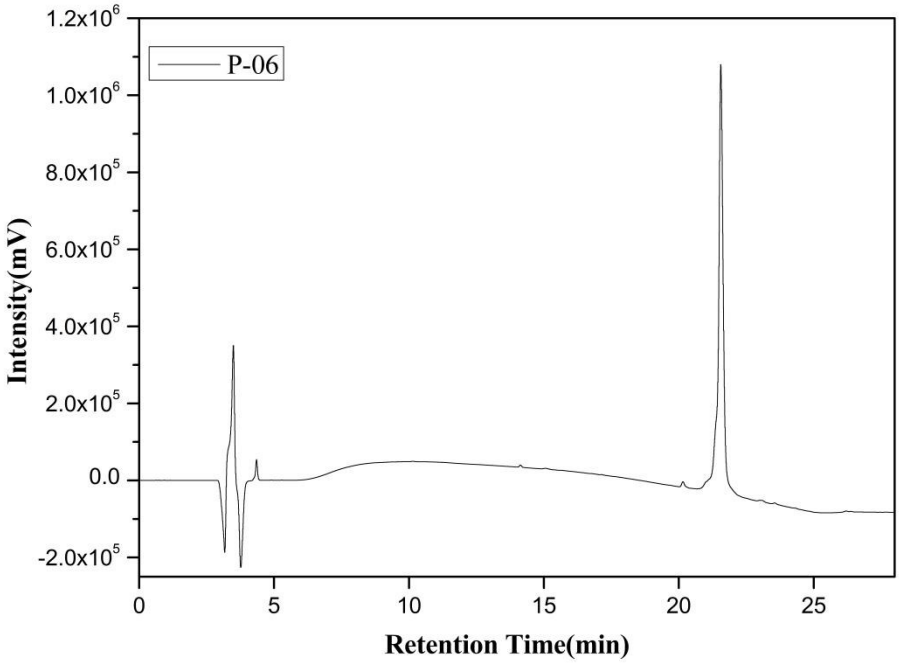
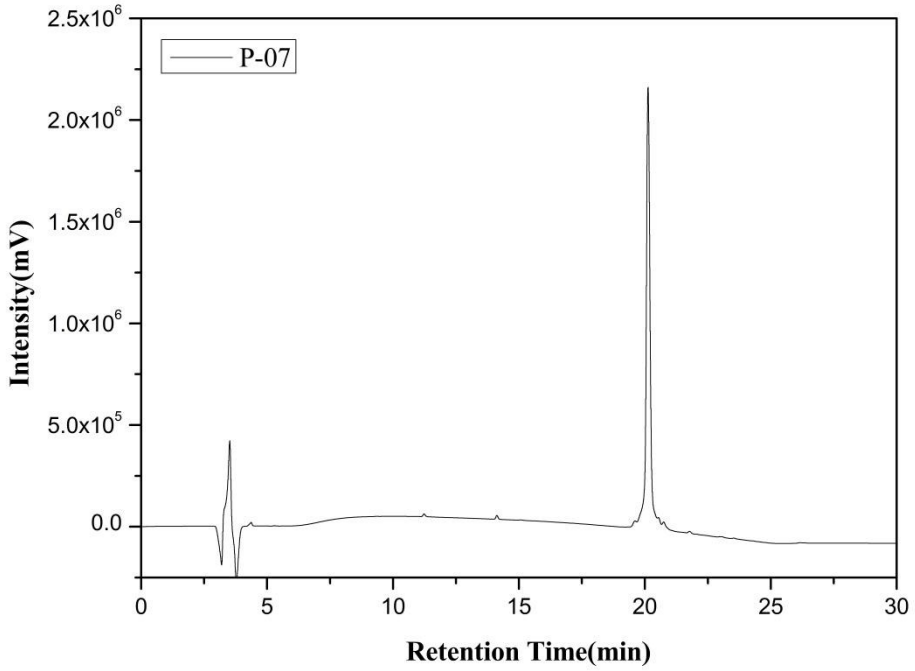
1.2x10^6
1.0x10^6
8.0x10^5
6.0x10^5
4.0x10^5
2.0x10^5
0.0
-2.0x10^5
0
5
10
15
20
25
Retention Time(min)


P-06
Intensity(mV)
1.2x10^6
1.0x10^6
8.0x10^5
6.0x10^5
4.0x10^5
2.0x10^5
0.0
-2.0x10^5
0
5
10
15
20
25
Retention Time(min)


P-07
Intensity(mV)
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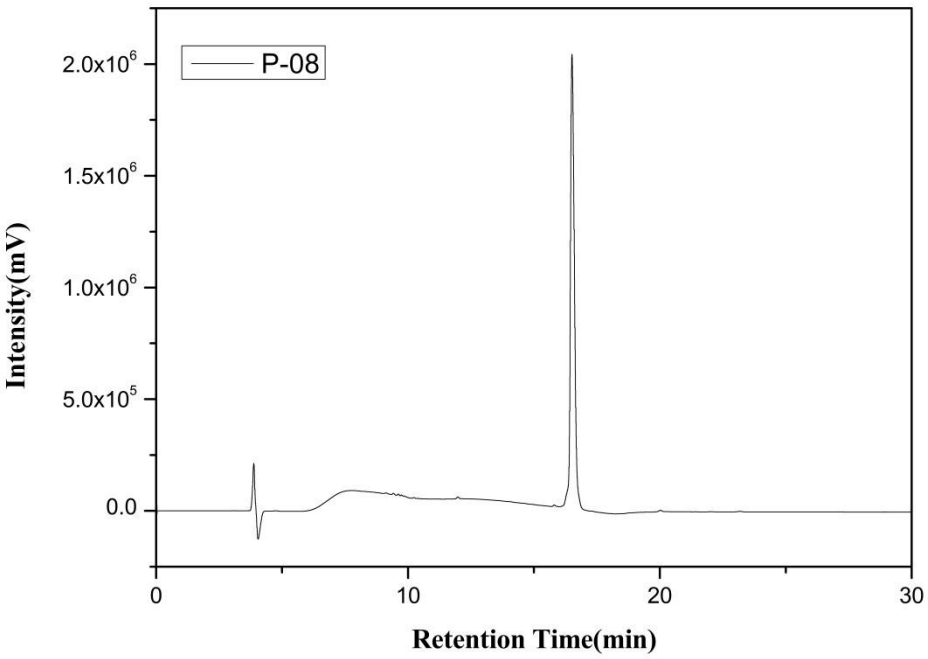
2.0x10^6
1.5x10^6
1.0x10^6
5.0x10^5
0.0
0
5
10
15
20
25
30
Retention Time(min)


P-08
Intensity(mV)
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1.5x10^6
1.0x10^6
5.0x10^5
0.0
0
10
20
30
Retention Time(min)

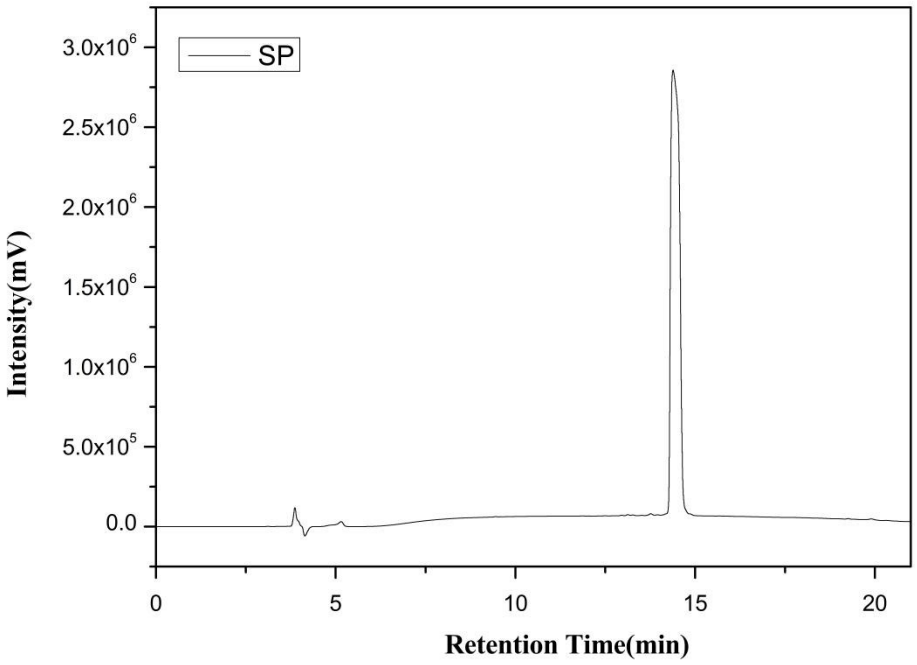
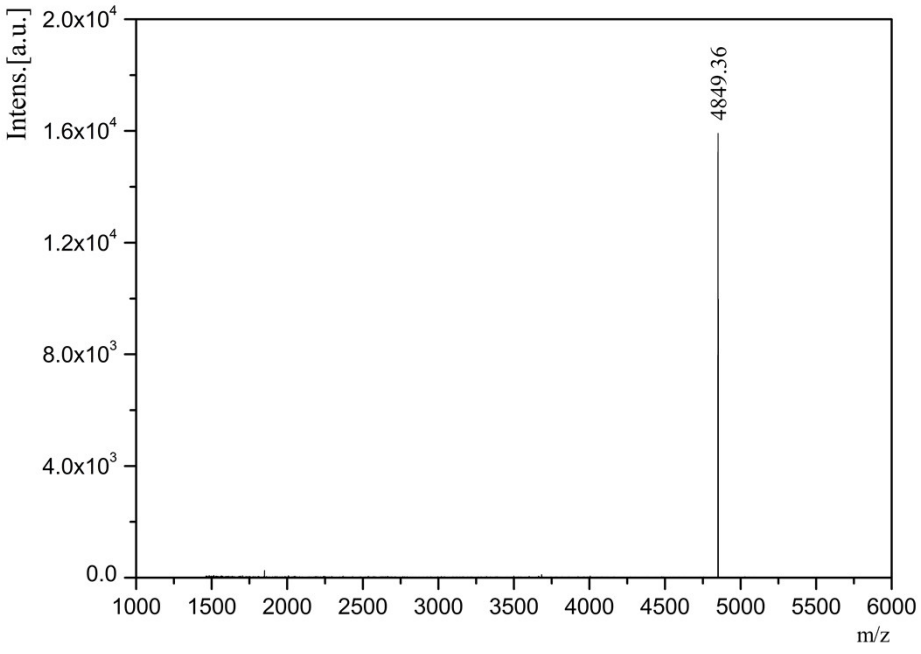
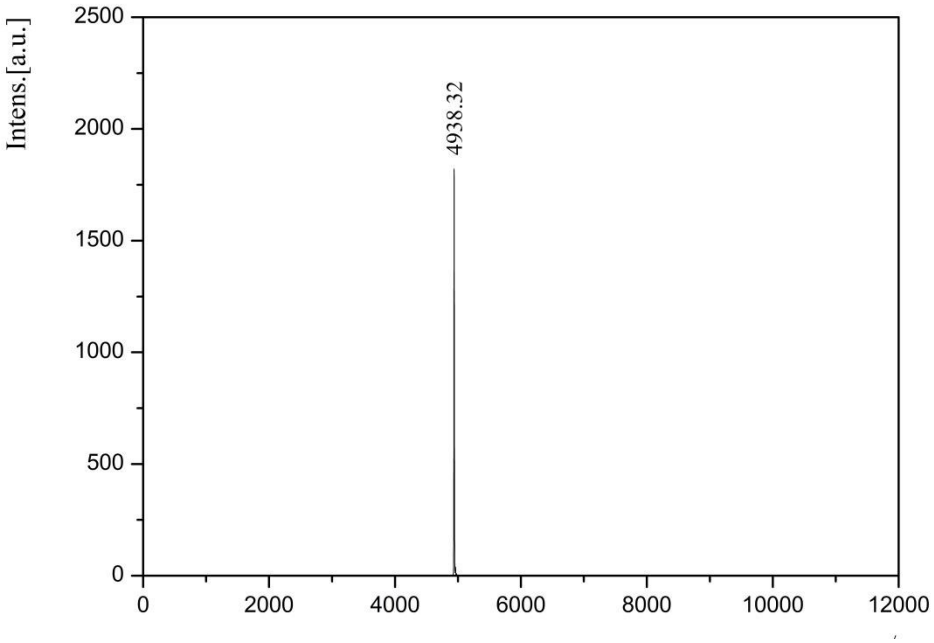


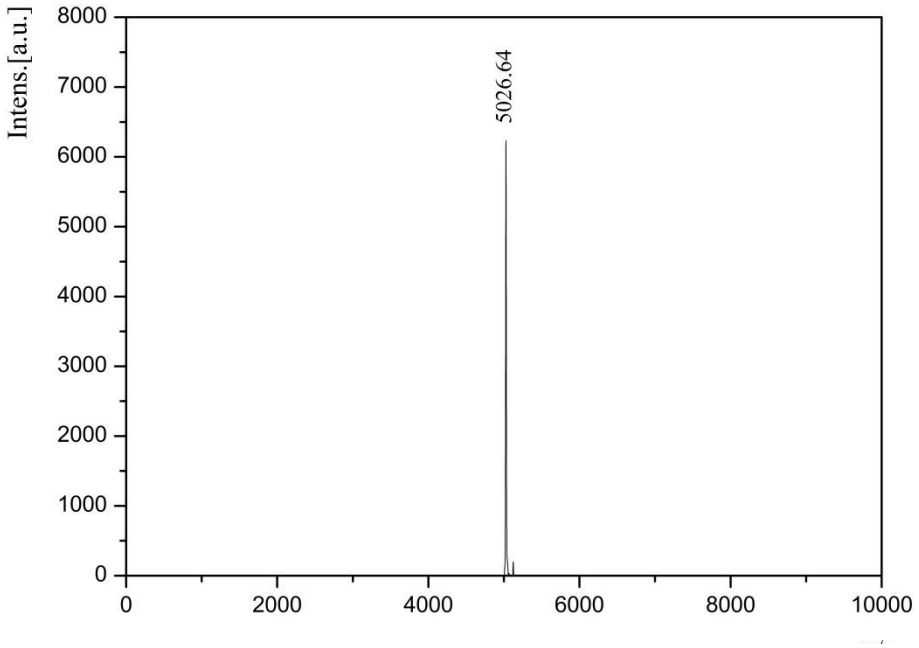
Figure S1. RP-HPLC of peptide vectors (P-01~P-08 and SP).



P-01


Intens.[a.u.]
2500
2000
1500
1000
500
0
4938.32
0
2000
4000
6000
8000
10000
12000

P-02


Intens.[a.u.]
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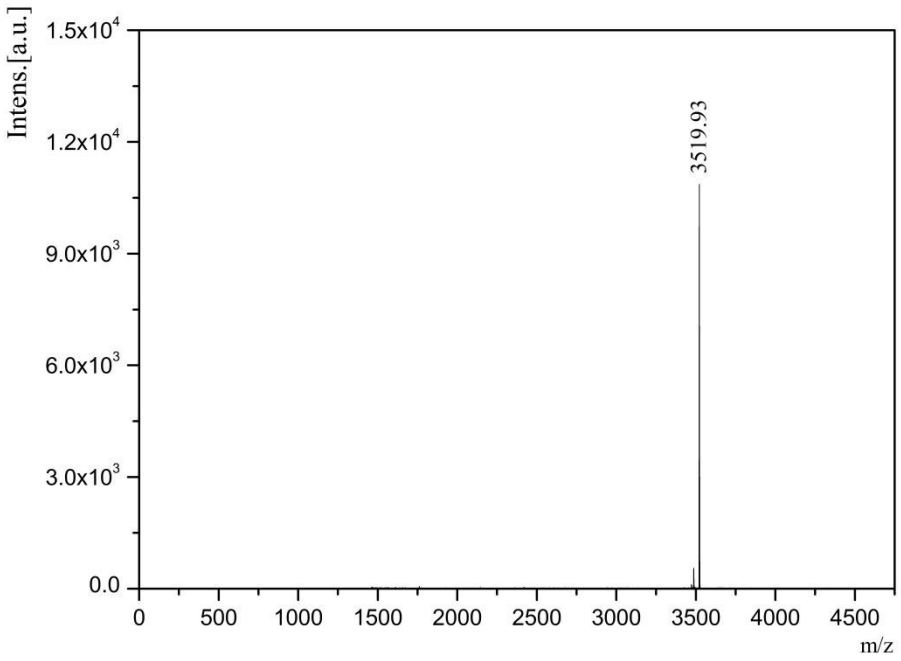
7000
6000
5000
4000
3000
2000
1000
0
5026.64
0
2000
4000
6000
8000
10000

P-03


Intens.[a.u.]
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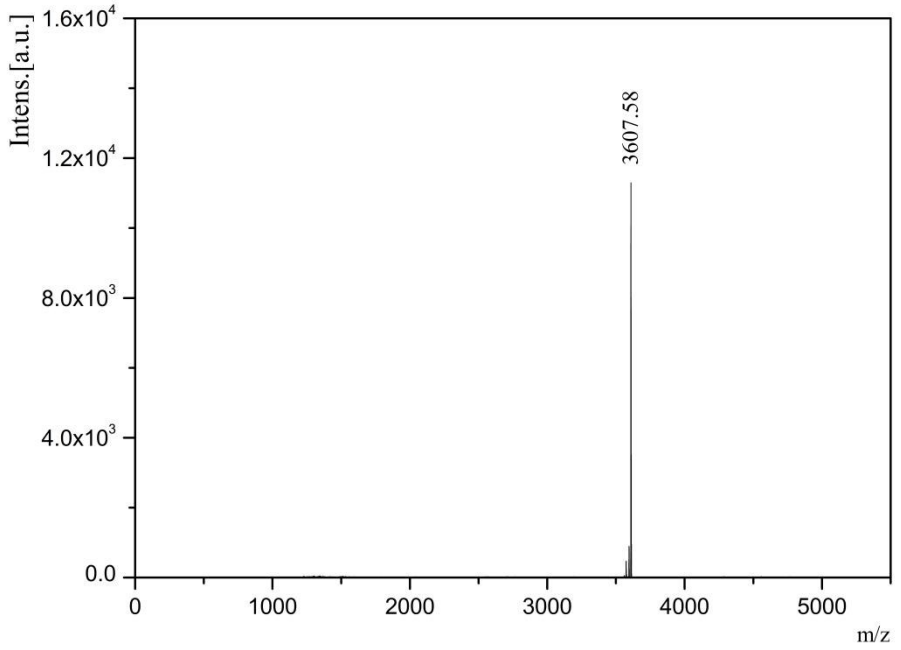
1.2x10^4
9.0x10^3
6.0x10^3
3.0x10^3
0.0
3519.93
0
500
1000
1500
2000
2500
3000
3500
4000
4500
m/z

P-04


Intens.[a.u.]
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1.2x10^4
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4.0x10^3
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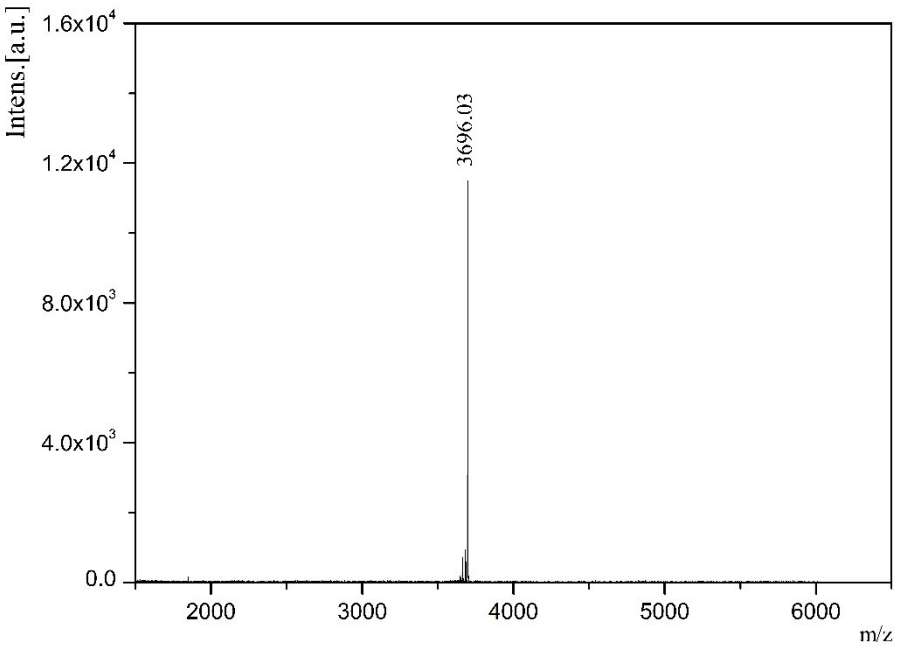
0
1000
2000
3000
4000
5000
m/z

P-05


Intens.[a.u.]
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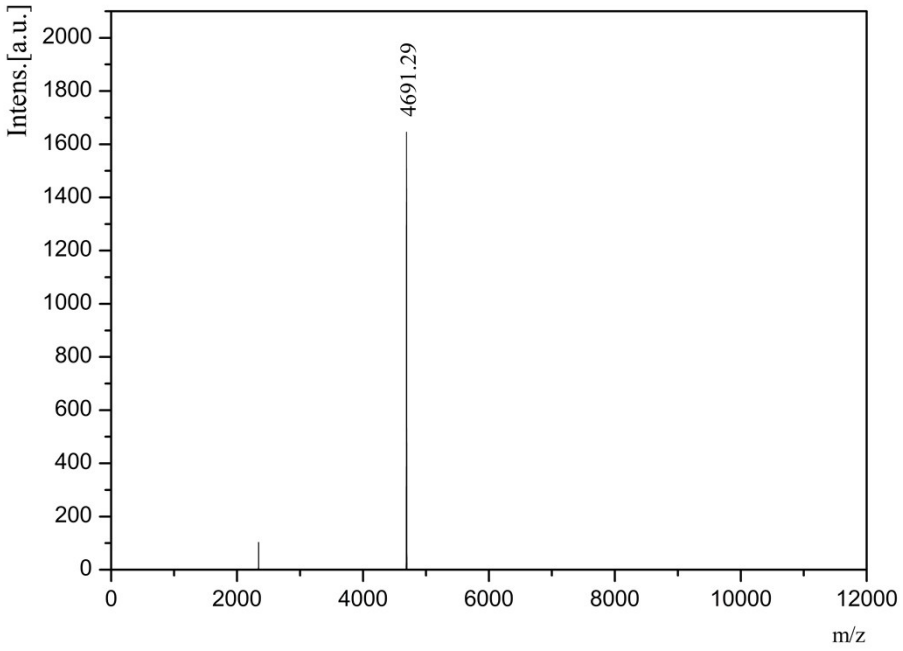
4.0x10^3
0.0
3696.03
2000
3000
4000
5000
6000
m/z

P-06


Intens.[a.u.]
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1800
1600
1400
1200
1000
800
600
400
200
0
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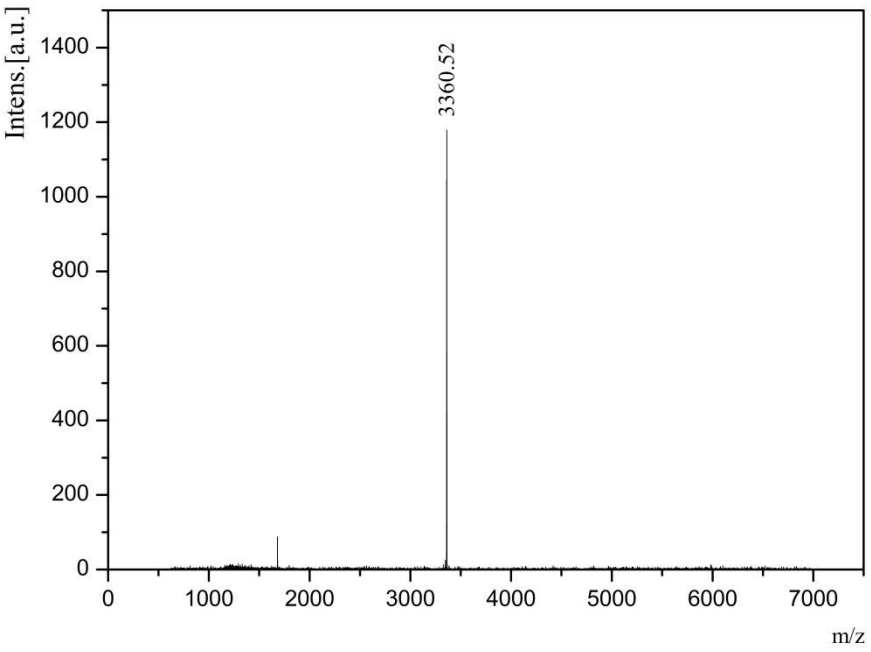
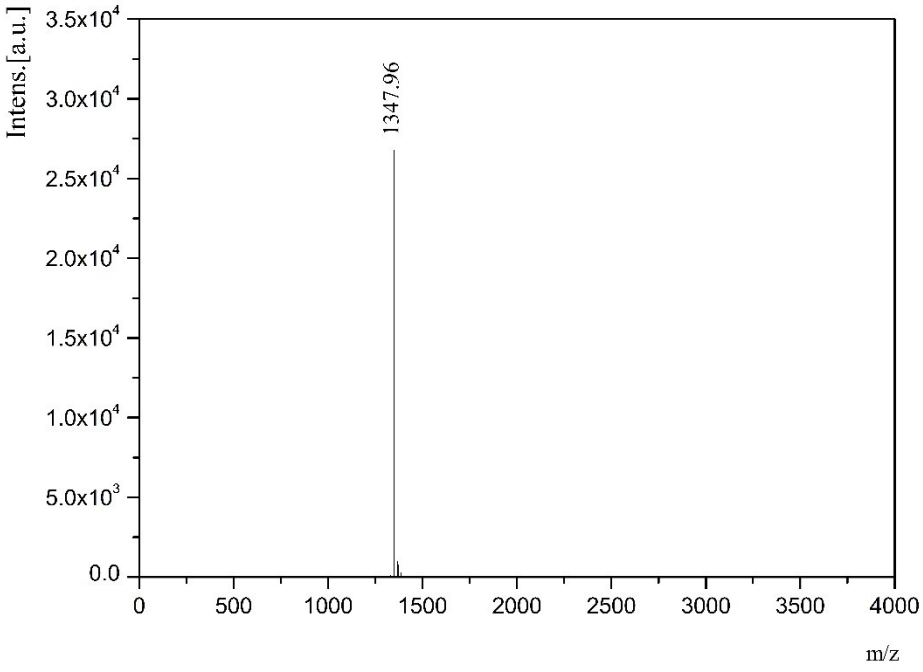
0
2000
4000
6000
8000
10000
12000
m/z

P-07



P-08



SP

Figure S2. MALDI-TOF-MS of peptide vectors (P-01~P-08 and SP).

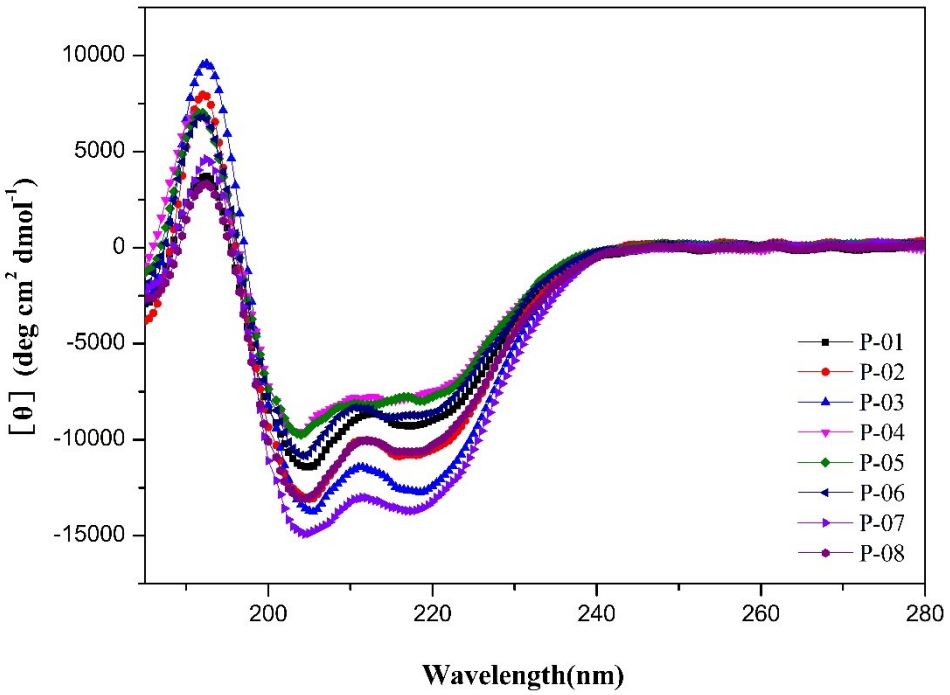


Figure S3. CD spectra of all peptide vectors at a concentration of 50 μM in TFE/PBS (50%, v/v) solution.

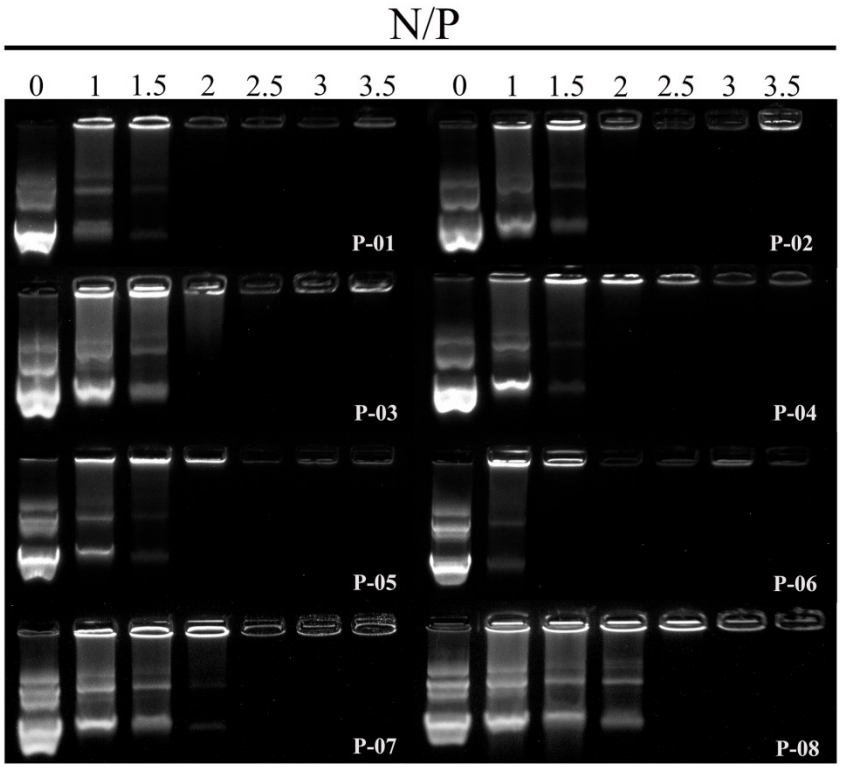


Figure S4. Agarose gel electrophoresis assays of P-01~P-08 at various N/P ratios.

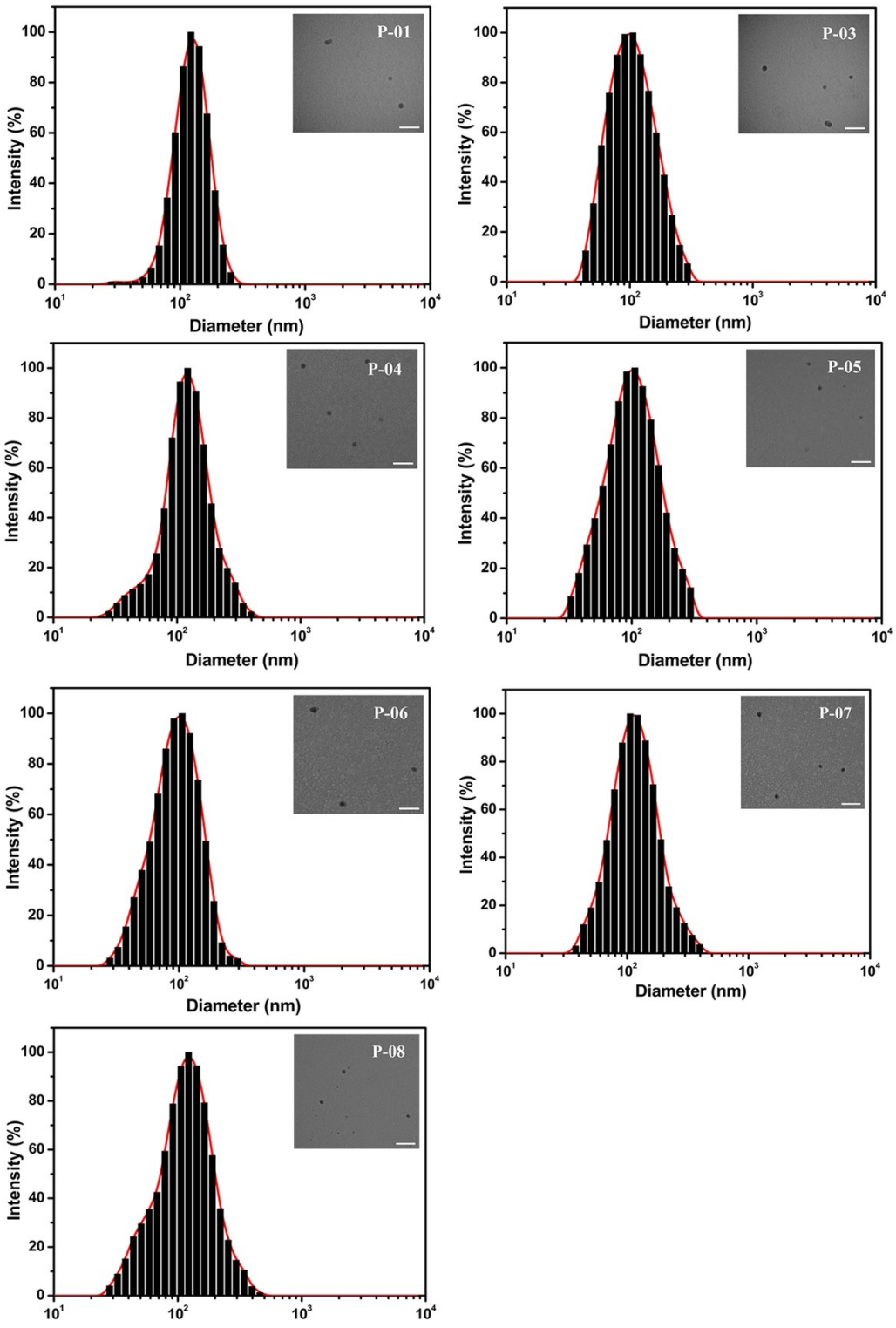


Figure S5. The particle size distributions and the TEM images of the peptide/pGL3 complexes (P-01, P-03~P-08) at the N/P ratio of 10. The scale bar represents 100 nm.

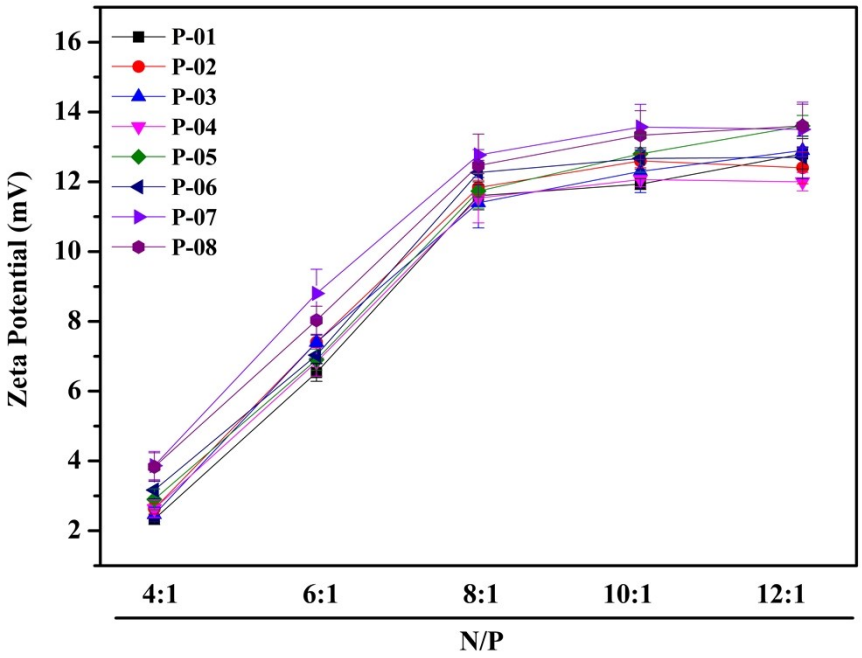


Figure S6. Zeta potentials of the peptide/pGL3 complexes at N/P ratios from 4 to 12.

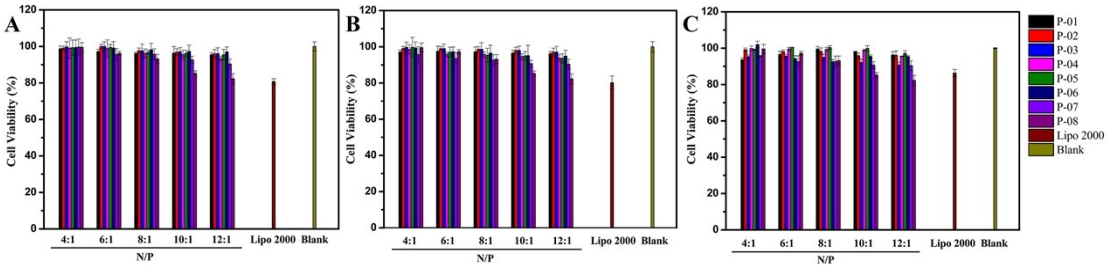


Figure S7. Cell viability of (A) U87, (B) 293T-NK1R, and (C) 293T cells after being treated with peptide/pGL3 complexes at various N/P ratios. The data are shown as mean ± SD (n = 5).

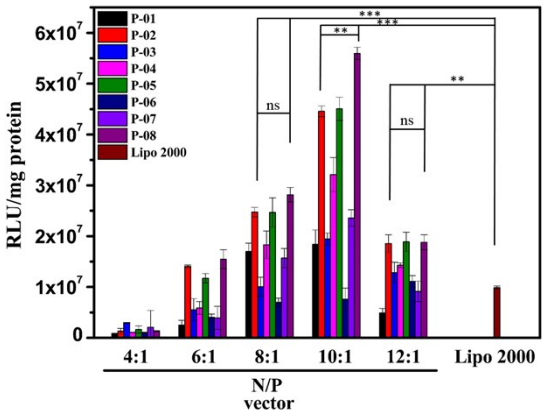


Figure S8. *In vitro* luciferase expression levels of peptide/pGL3 complexes in 293T cells at N/P ratios from 4 to 12. The data are shown as mean ± SD (n = 3). *p < 0.05; **p < 0.01; ***p < 0.005.

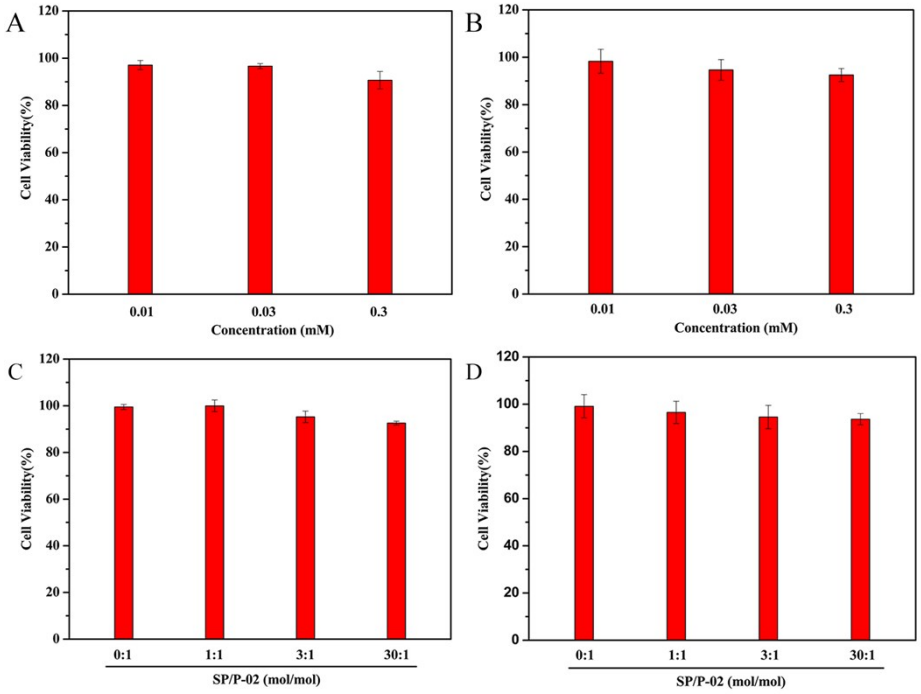


Figure S9. Cell viability of U87 (A, C) and 293T-NK1R (B, D) cells after being treated with free SP (A, B) and P-02/pGL3 complexes in the presence of different concentration of SP at an N/P ratio of 10 (C, D). The data are shown as mean ± SD (n = 5).

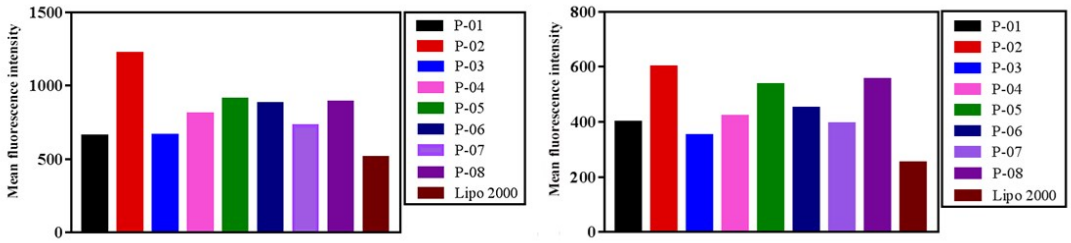


Figure S10. The mean fluorescence intensity of U87 (left) and 293T (right) cells in FACS assays. YOYO-1 was used to label the pGL3 plasmid.

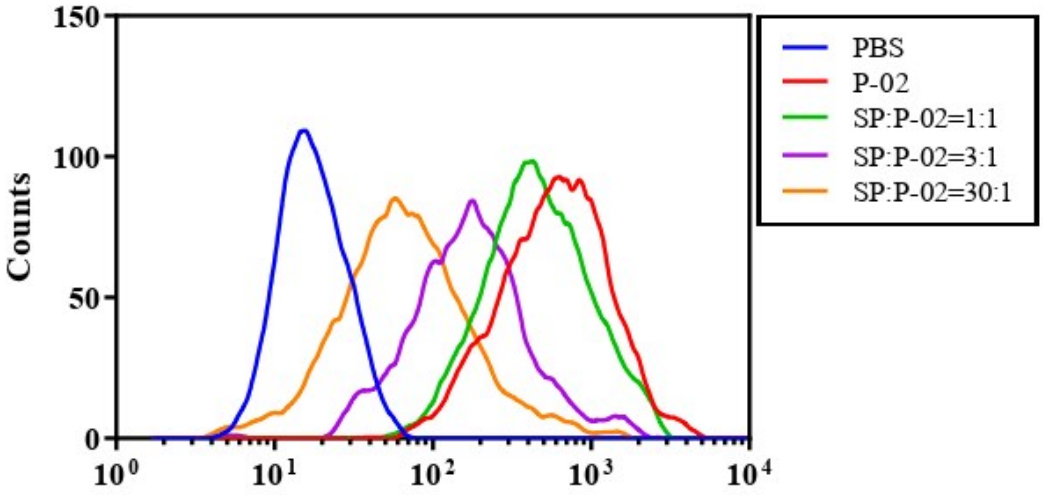


Figure S11. FACS assays of cellular uptake mechanisms of P-02/pGL3 complexes using free SP as a competitor at an N/P ratio of 10 in U87 cells. YOYO-1 was used to label the pGL3 plasmid.

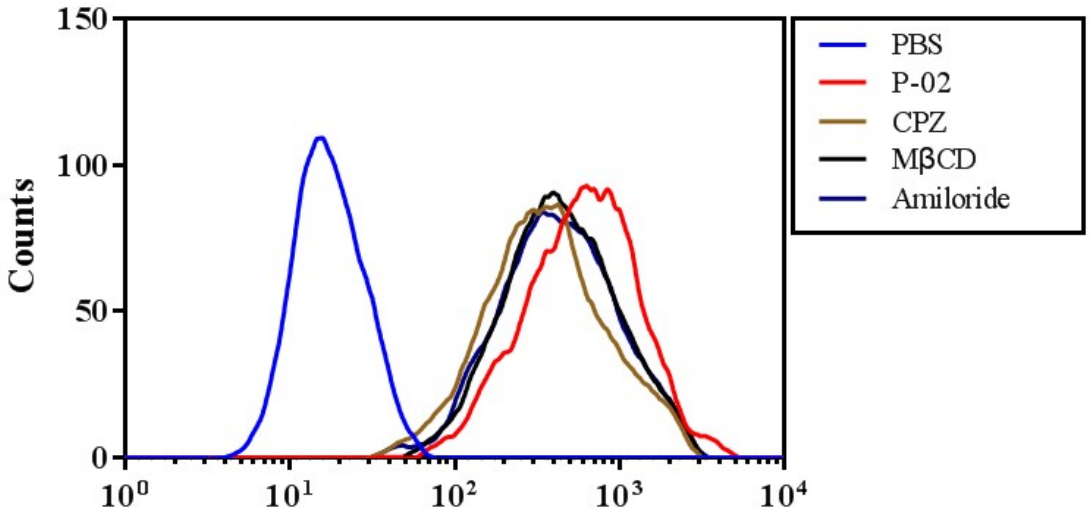


Figure S12. FACS assays of cellular uptake mechanisms of P-02/pGL3 complexes using different inhibitors for endocytosis at an N/P ratio of 10 in U87 cells. YOYO-1 was used to label the pGL3 plasmid.