Natural Polyphenols Augment Cytosolic Protein Delivery by a Functional Polymer

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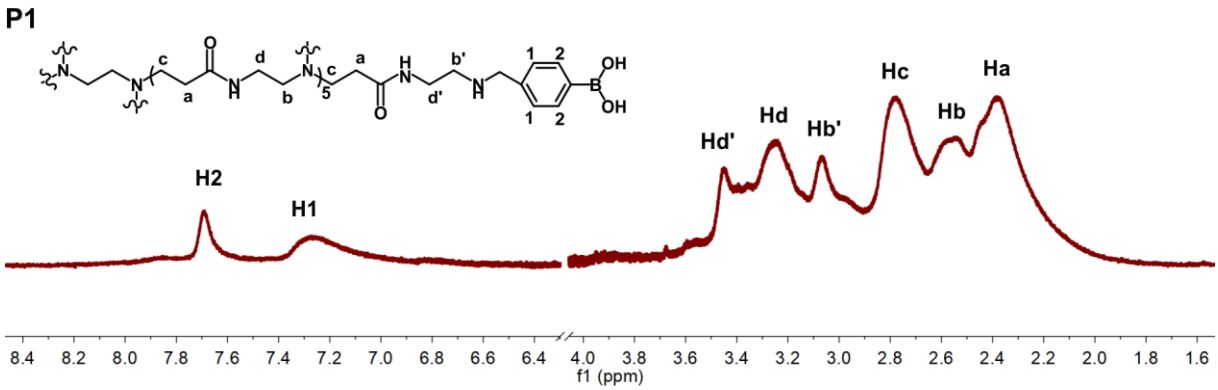

Figure S1. ^{1}H NMR spectrum of P1 in D_2O. An average number of 42 phenylboronic acid were modified on each G5 PAMAM dendrimer calculated according to the integrated peak area.

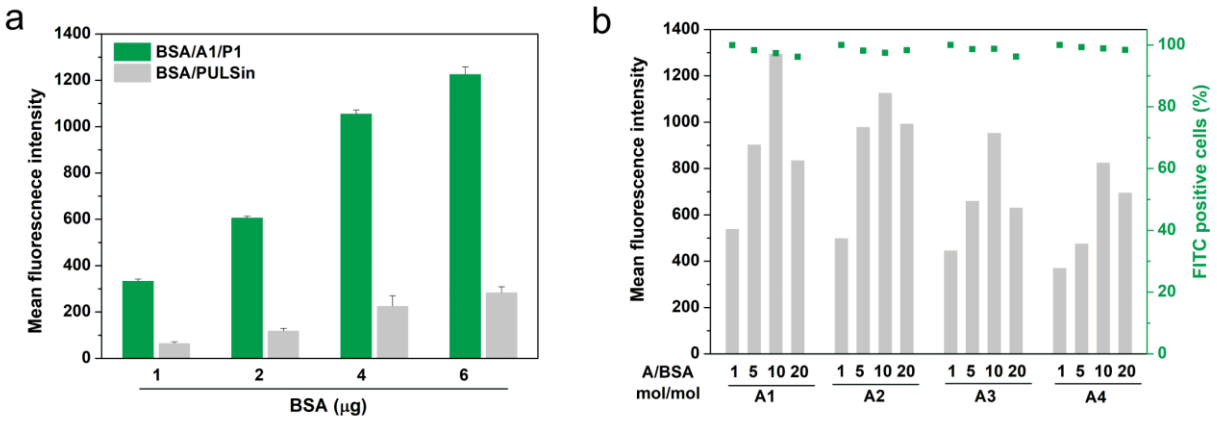

Figure S2. a. The efficiency of BSA/A1/P1 at different BSA doses. PULSin was measured as a control. The dosage of P1 in each well was 8 μg. **b.** Screening the optimal molar ratios of polyphenols (A1-A4) to BSA for cytosolic delivery. HeLa cells were treated with the complexes for 4 h. The doses of BSA and P1 in each well were 6 μg and 8 μg, respectively.

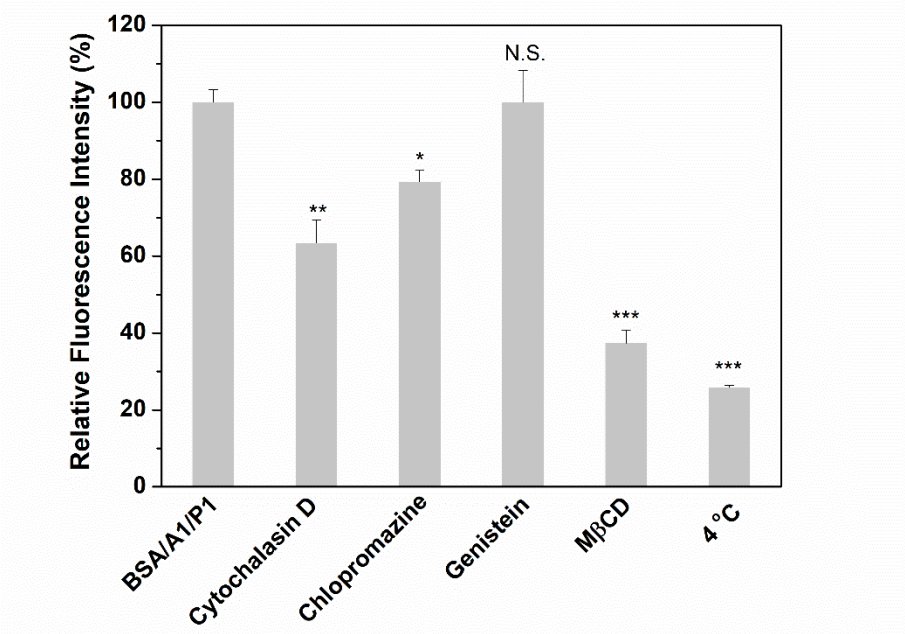

Figure S3. Intracellular uptake mechanism of the BSA/A1/P1 complexes. The Hela cells were treated with endocytosis inhibitors or incubated at 4 ^{o}C for 2 h. The concentrations of cytochalasin D, chlorpromazine, genistein and MβCD were 10 μM, 20 μM, 700 μM and 10 mM respectively. The cells were then treated with BSA/A1/P1 complexes for 4 h before flow cytometry measurement. The mean fluorescence intensity of the cells treated without any inhibitors was defined as 100%.

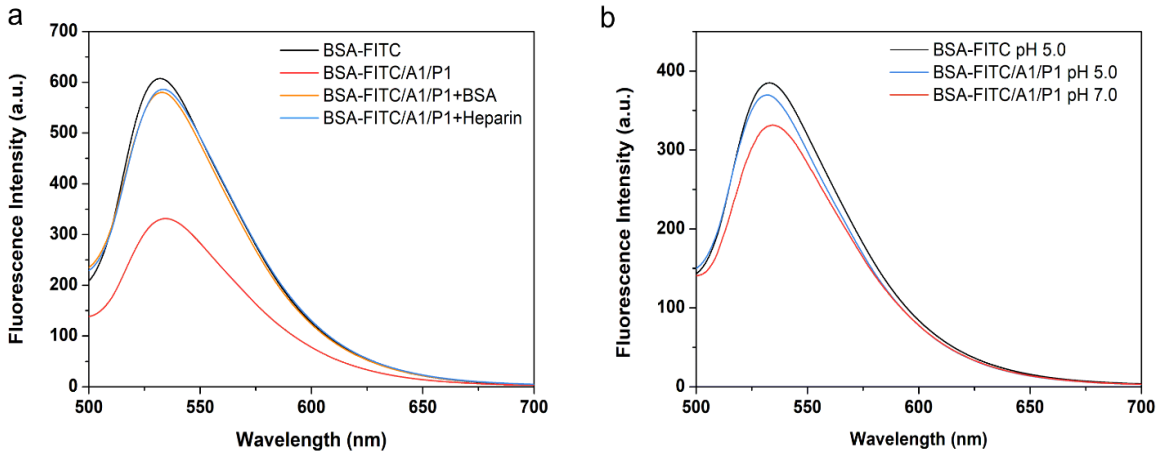

Figure S4. a, Fluorescence intensity of BSA-FITC/A1/P1 complexes in the presence of native BSA (0.1 mg/mL) or heparin sodium (0.5 mg/mL). Free BSA-FITC was measured as a control. **b**, Fluorescence intensity of BSA-FITC/A1/P1 complexes at pH 7.0 and pH 5.0. Free BSA-FITC at pH 5.0 was measured as a control. The concentrations of BSA-FITC and P1 were 6 and 8 μg/mL, respectively. The molar ratio of A1 to BSA-FITC is 10:1. The excitation and emission wavelengths were λ_{ex}=490 nm and λ_{em}=500-700 nm, respectively.

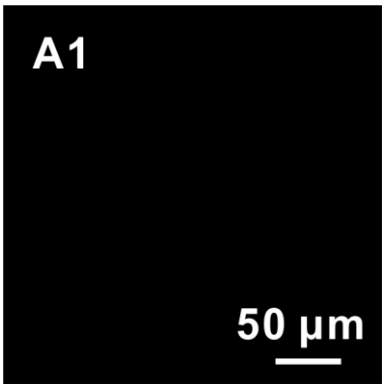

Figure S5. Fluorescence images of HeLa cells treated with BSA-FITC/A1 complexes without any polymer for 4 h. The concentrations of BSA and the polyphenols were equal to those in Figure 3.

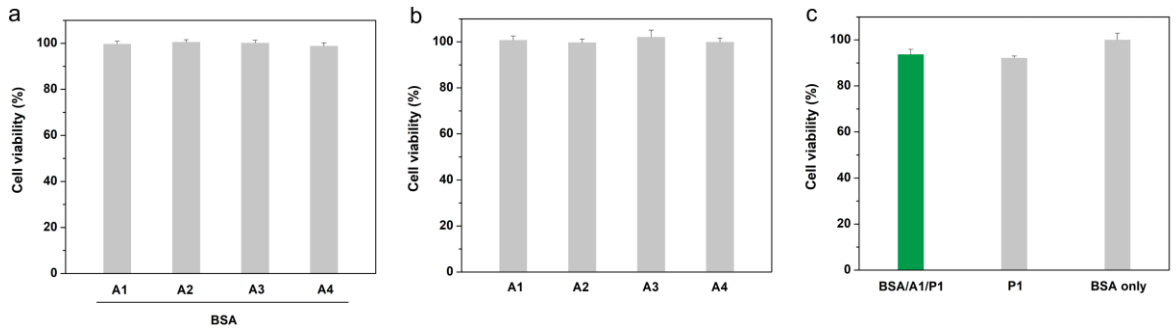

Figure S6. Cell viability of HeLa cells incubated with BSA/polyphenols complexes (**a**) and polyphenols only (**b**) at optimal cytosolic protein delivery conditions for 24 h. **c**. Cell viability of NIH3T3 cells treated with BSA/A1/P1 complex at optimal conditions. The free polymer P1 and BSA were tested as controls.

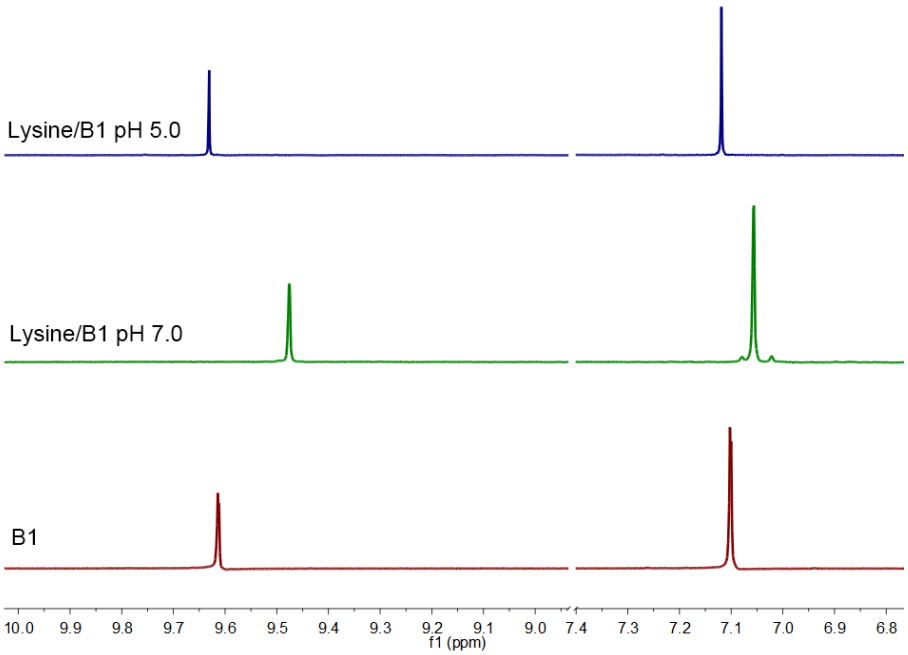

Figure S7. ^{1}H NMR of lysine incubated with B1 at pH 7.0 or 5.0. The molar ratio of B1 to lysine is 0.02:1.

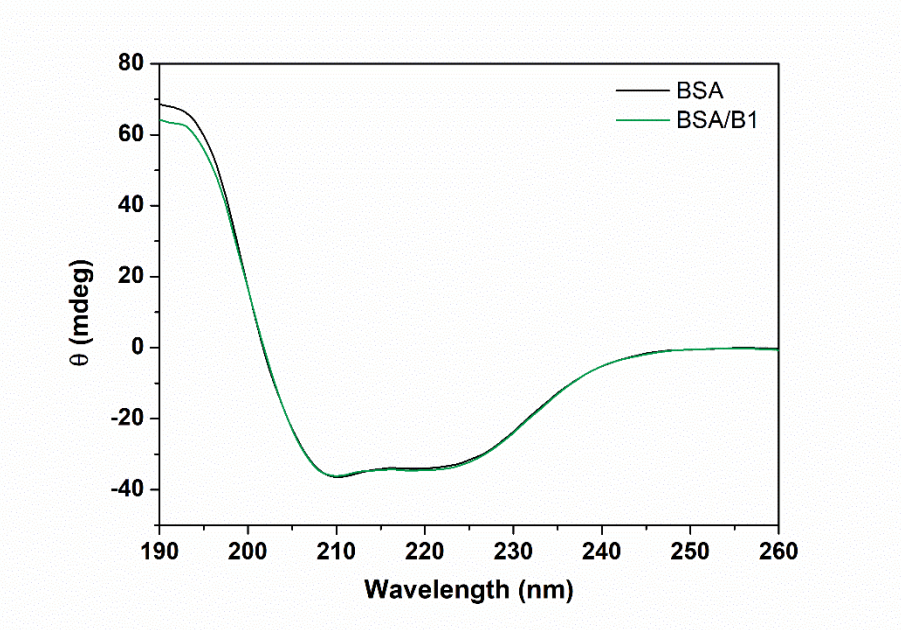

Figure S8. CD spectra of BSA and the BSA/B1 complex. The molar ratio of B1 to BSA was 50:1.

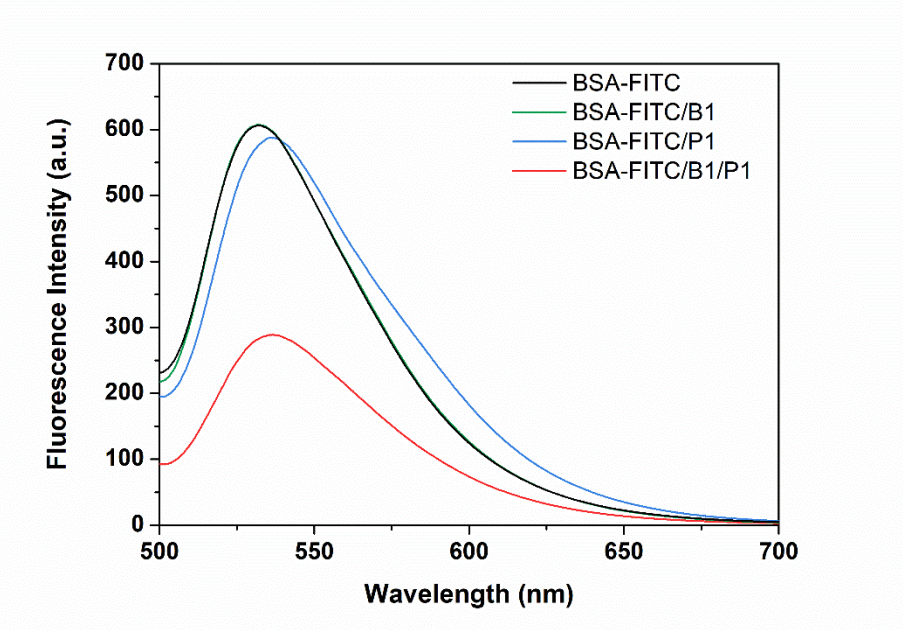

Figure S9. Fluorescence intensity of BSA-FITC, BSA-FITC/B1, BSA-FITC/P1 and BSA-FITC/B1/P1 complexes. The concentrations of BSA-FITC and P1 were 6 and 8 μg/mL, respectively. The molar ratio of B1 to BSA-FITC is 50:1. The excitation and emission wavelengths were λ_{ex}=490 nm and λ_{em}=500-700 nm, respectively.

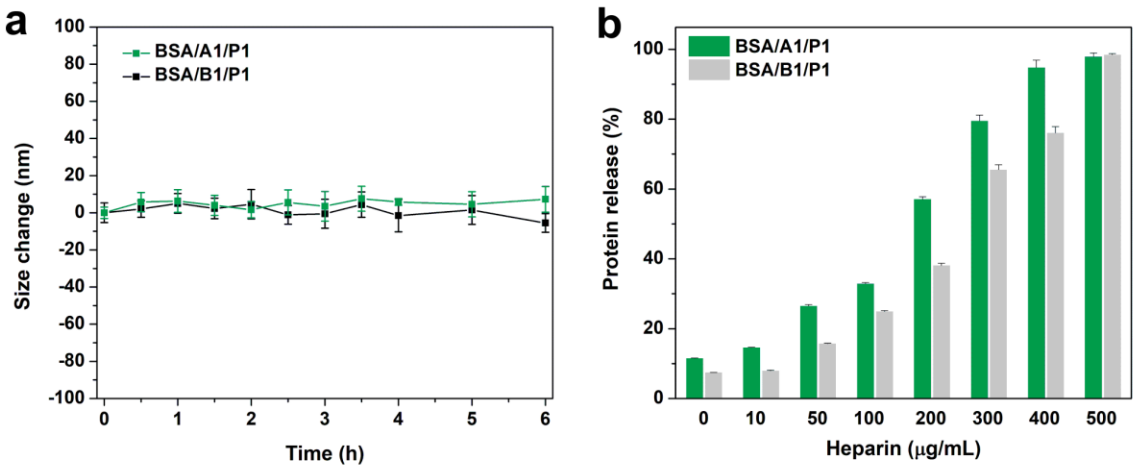

Figure S10. a. Sizes of the BSA/A1/P1 and BSA/B1/P1 complexes incubated for different periods. **b.** Protein release from the BSA/A1/P1 and BSA/B1/P1 complexes when added with different concentrations of heparin sodium. The released proteins in the presence of 500 μg/mL heparin sodium for the two complexes were set as 100%.

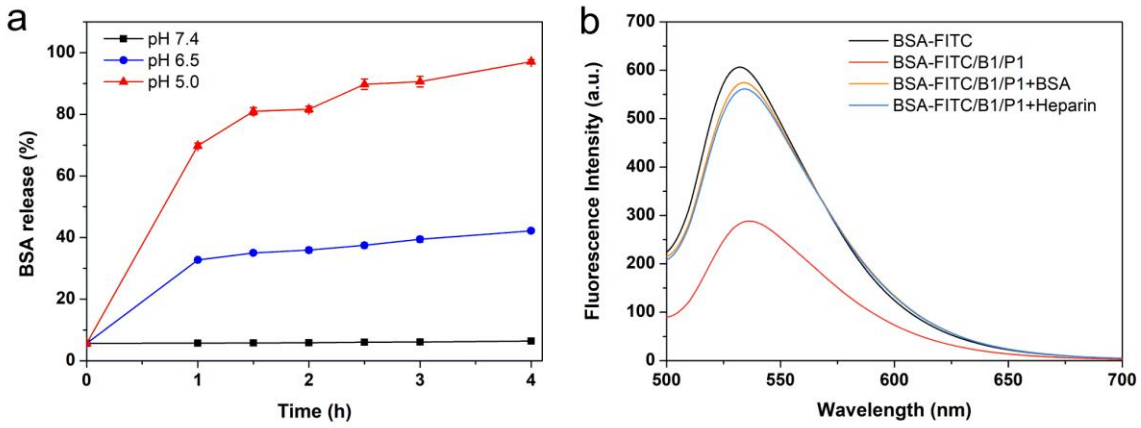

Figure S11. a. *In vitro* release kinetics of BSA-FITC from the complex under different pH conditions. **b.** Fluorescence intensity of BSA-FITC/B1/P1 complexes in the presence of native BSA (0.1mg/mL) or heparin sodium (0.5 mg/mL). Free BSA-FITC was measured as a control. The concentrations of BSA-FITC and P1 were 6 and 8 μg/mL, respectively. The molar ratio of A1 to BSA-FITC is 50:1. The excitation and emission wavelengths were λ_{ex}=490 nm and λ_{em}=500-700 nm, respectively.

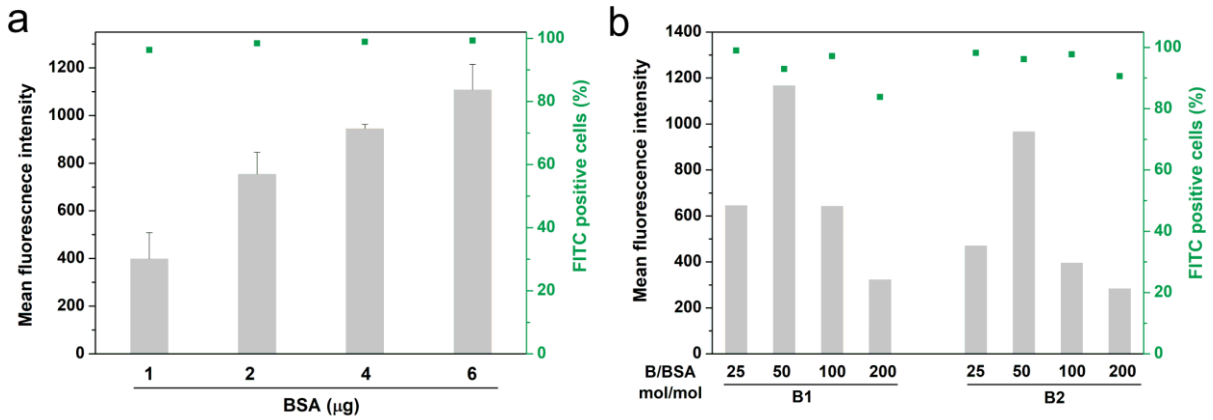

Figure S12. a. The efficiency of BSA/B1/P1 on HeLa cells at different BSA doses. **b.** Screening the optimal molar ratios of polyphenols (B1-B2) to BSA for cytosolic delivery. HeLa cells were treated with the complexes for 4 h. The doses of BSA and P1 in each well were 6 μg and 8 μg, respectively.

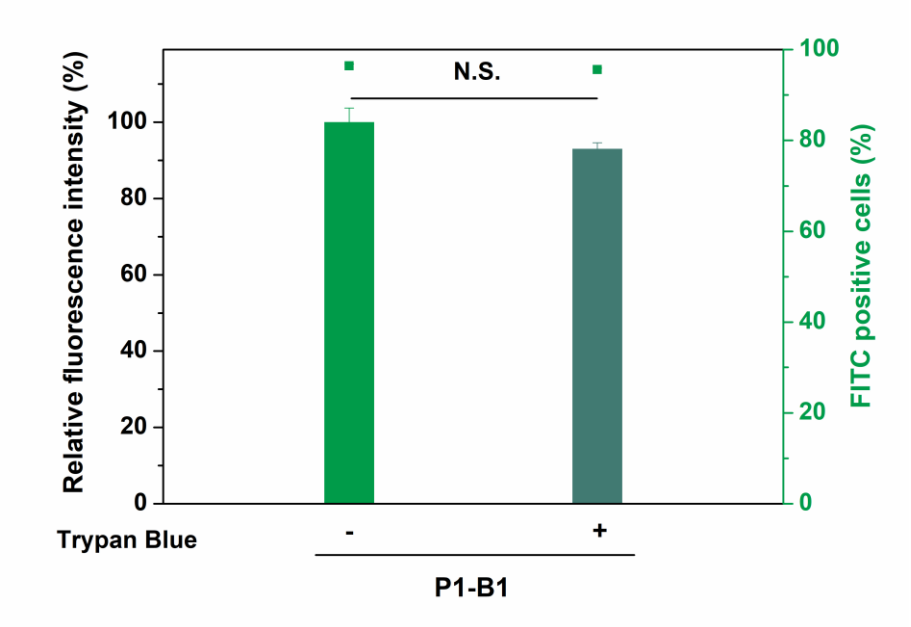

Figure S13. Relative fluorescence intensity of transduced cells before and after trypan blue quenching. The mean fluorescence intensity of cells treated without trypan blue was defined as 100%.

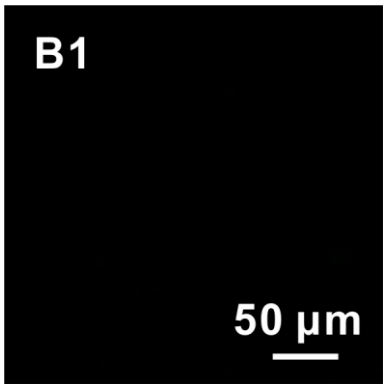

Figure S14. Fluorescence images of HeLa cells treated with BSA-FITC/B1 complexes without any polymer for 4 h. The concentrations of BSA and the polyphenols were equal to those in Figure 5.

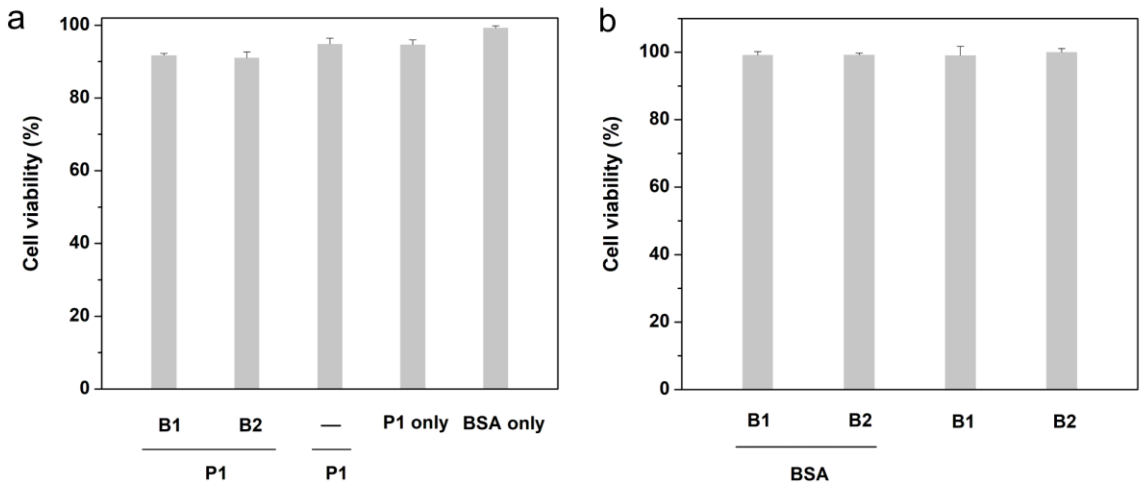

Figure S15. a, Cell viability of HeLa cells incubated with BSA/polyphenols/P1 complexes, BSA/P1 complexes, polymer only and BSA only. **b**, BSA/polyphenol complexes and polyphenols only at optimal cytosolic protein delivery conditions for 24 h.

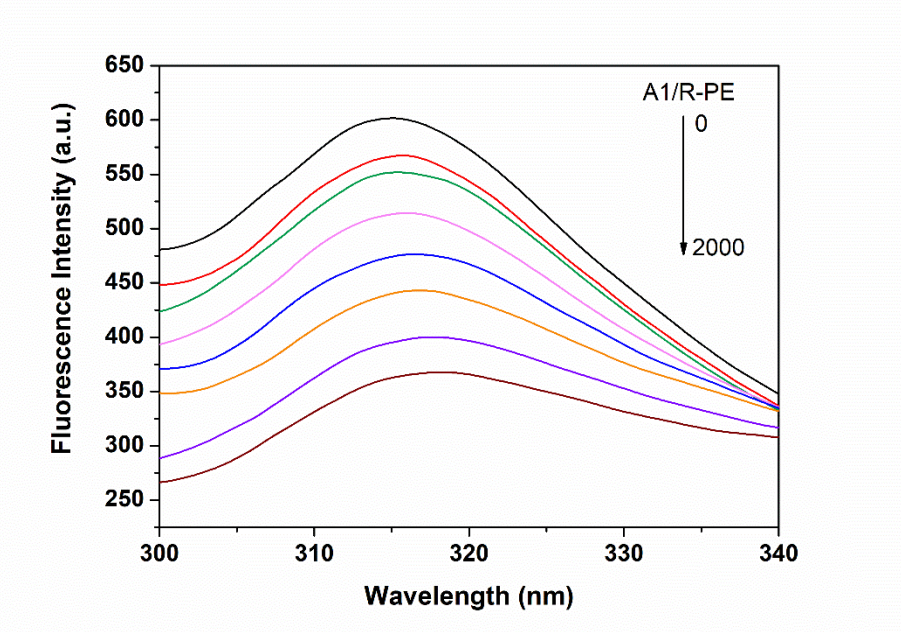

Figure S16. Quenching of R-PE fluorescence in the presence of A1. The molar ratios of A1 to R-PE were 0:1, 100:1, 200:1, 400:1, 800:1, 1200:1, 1600:1 and 2000:1, respectively. The excitation and emission wavelengths were λ_{ex}=280 nm and λ_{em}=300-340 nm, respectively.

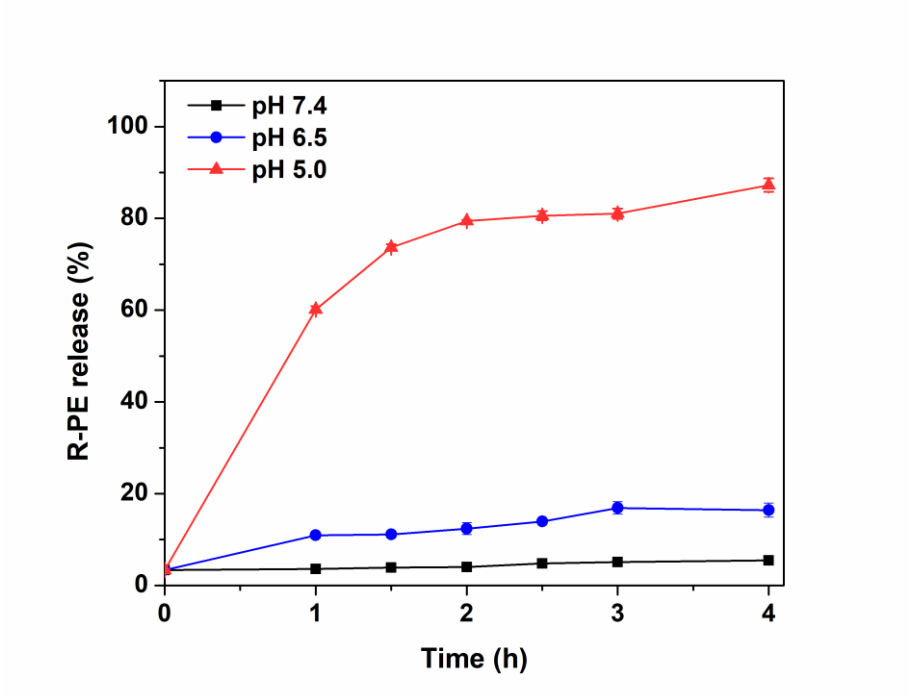

Figure S17. *In vitro* release kinetics R-PE from the complex under different pH conditions.

Figure S18. Fluorescence images of HeLa cells treated with R-PE/A1 or R-PE/B1 complexes without any polymer for 4 h. The concentrations of R-PE and the polyphenols were equal to those in Figure 6.

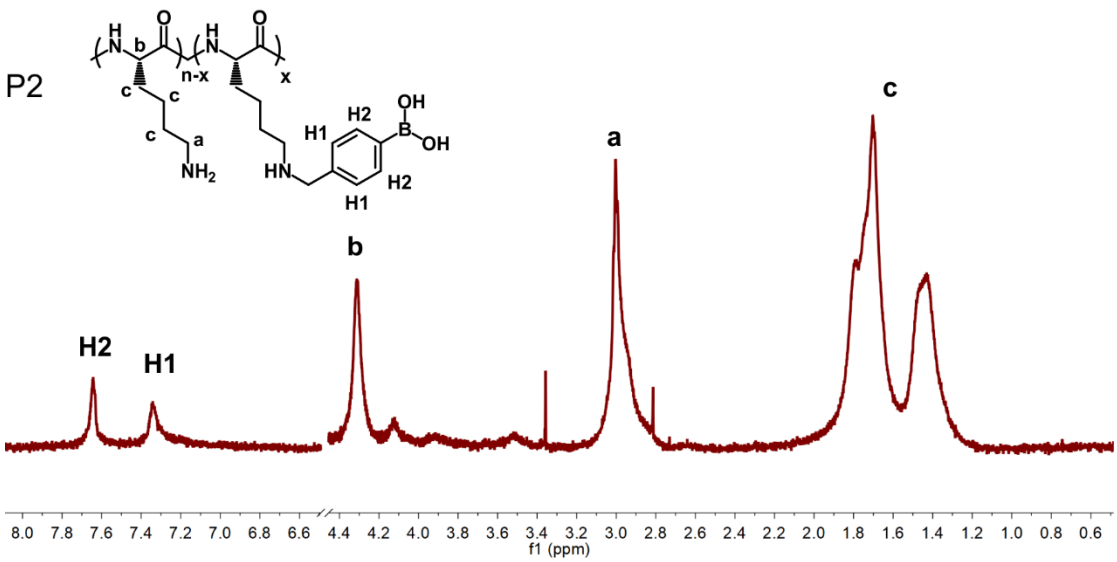

Figure S19. ^{1}H NMR spectrum of P2 in D_2O. An average number of 22 phenylboronic acid were modified on each poly-$_L$-lysine as calculated according to the integrated peak area.

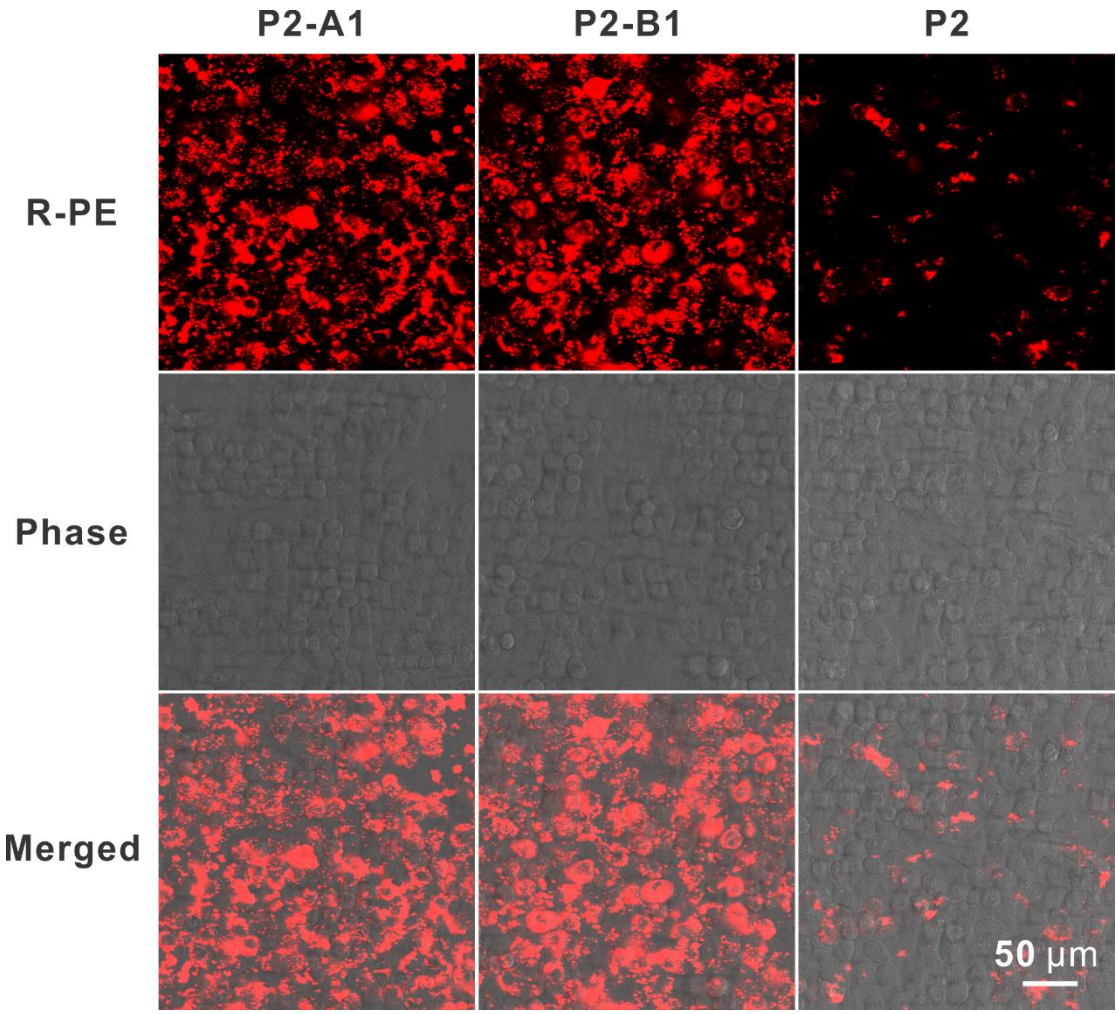

Figure S20. Cytosolic delivery of R-PE into HeLa cells by P2 in the presence of polyphenols A1 or B1 for 4 h. The doses of R-PE and polymer P2 in each well were 1 μg and 3 μg, respectively. The molar ratios of A1 and B1 to R-PE are 200:1 and 1000:1, respectively.

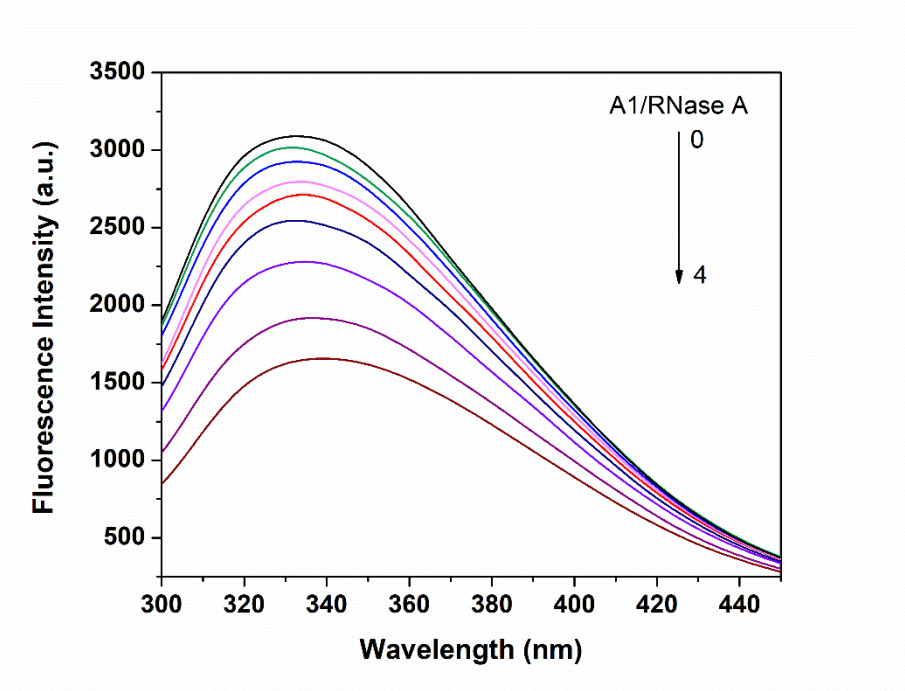

Figure S21. Quenching of RNase A fluorescence in the presence of A1. The molar ratios of A1 to RNase A were 0:1, 0.2:1, 0.4:1, 0.8:1, 1:1, 1.5:1, 2:1, 3:1 and 4:1, respectively. The excitation and emission wavelengths were λ_{ex}=280 nm and λ_{em}=300-450 nm, respectively.

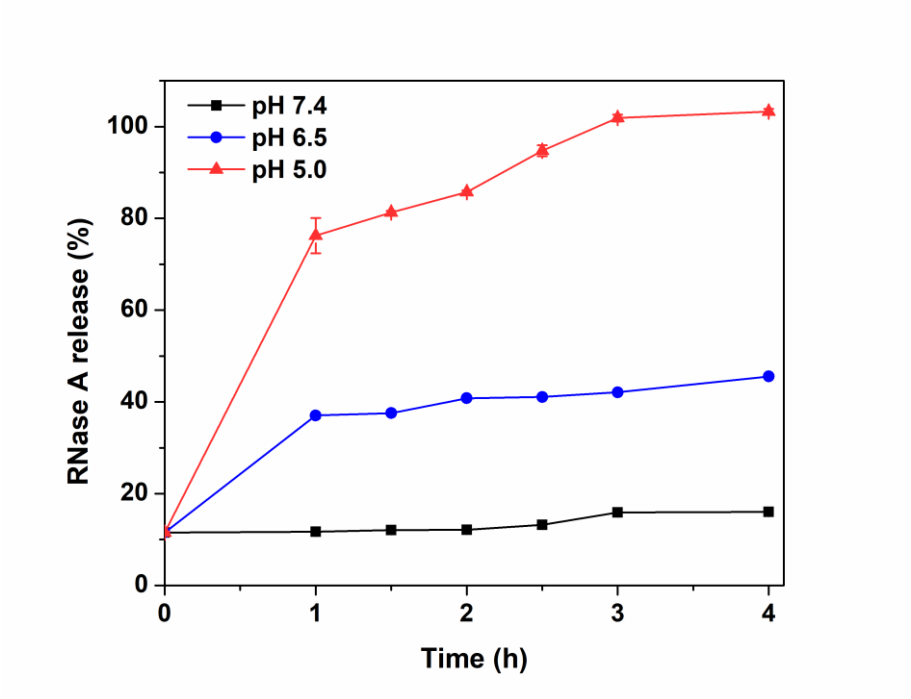

Figure S22. *In vitro* release kinetics of RNase A from the complex under different pH conditions.

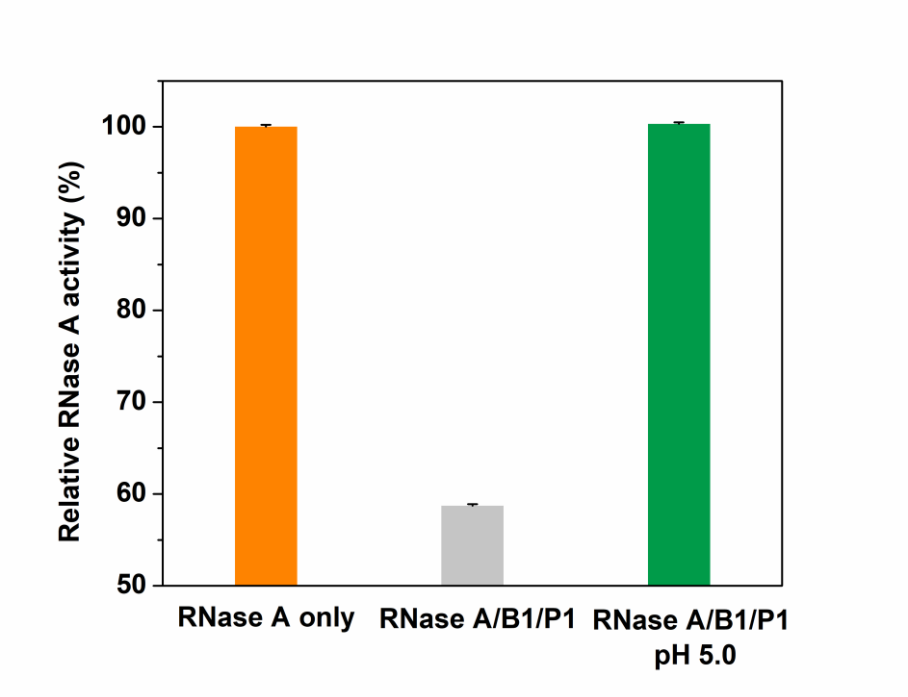

Figure S23. Relative RNase A activity determined by the RNaseAlert Kit. The recovery of RNase activity for the RNase A/B1/P1 complex was observed in an acidic buffer. The enzyme activity of native RNase A was defined as 100%.

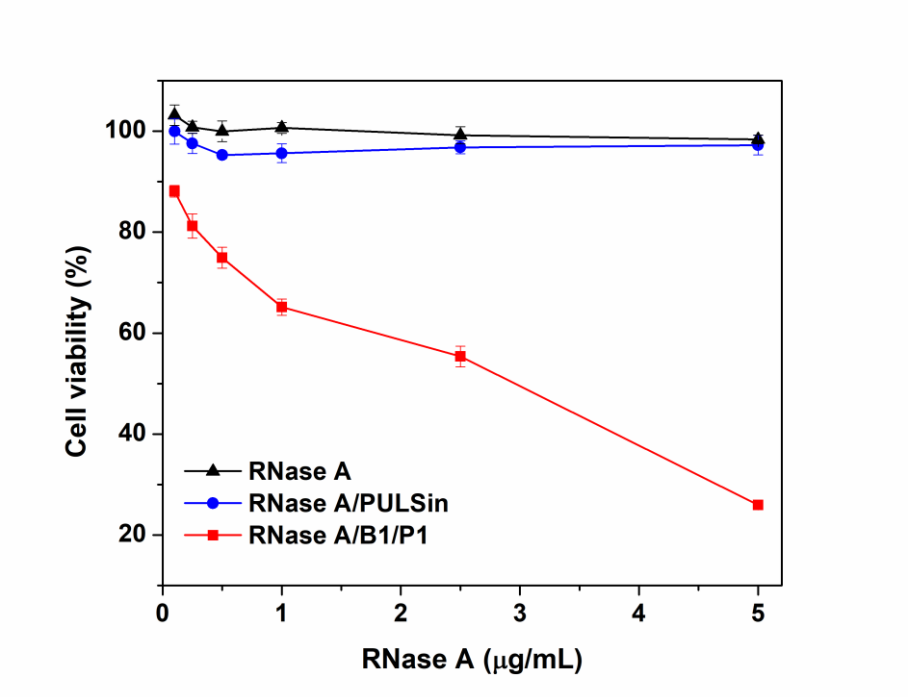

Figure S24. Cytotoxicity of the RNase A/B1/P1 complexes on MDA-MB-231 cells at different RNase A concentrations determined by an MTT assay. Free RNase A and PULSin were tested as controls.

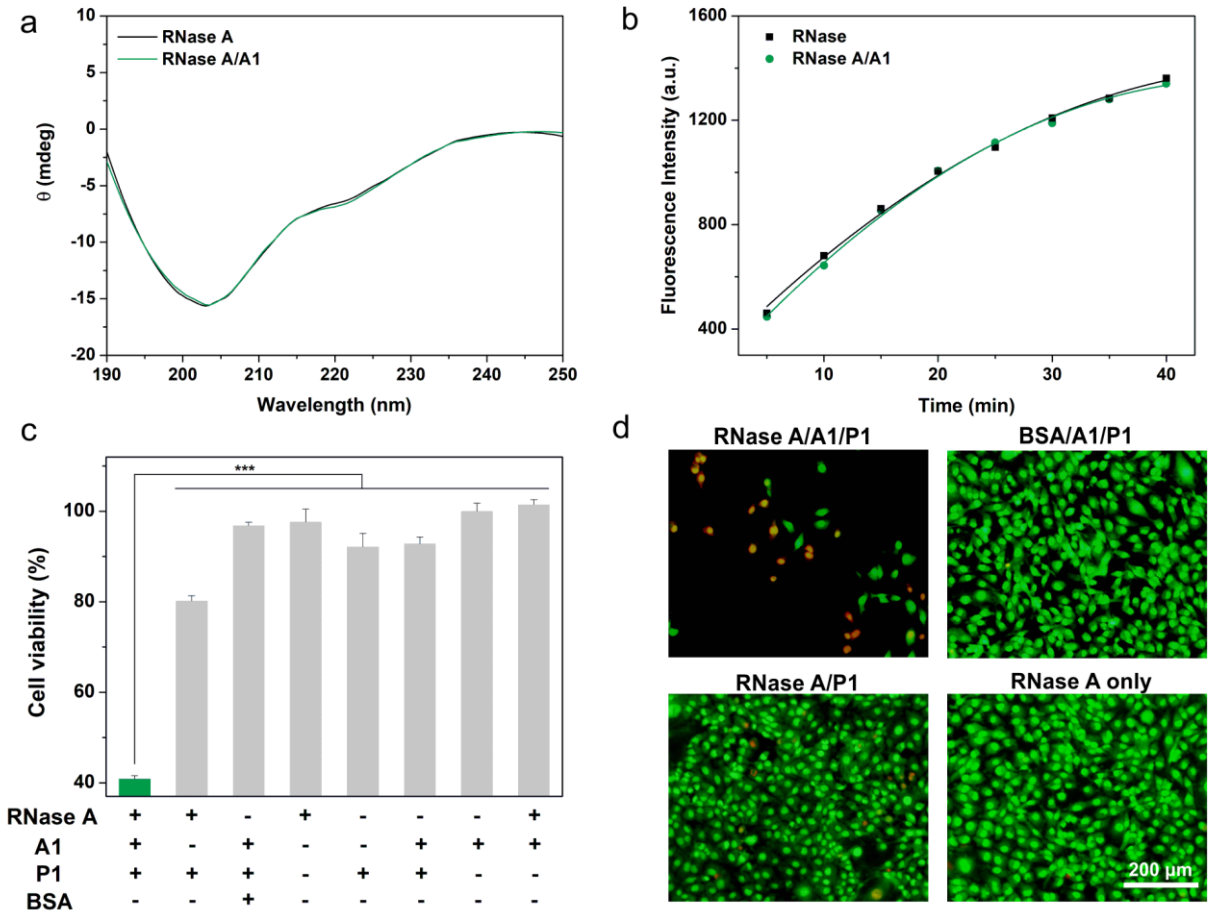

Figure S25. a, CD spectra of RNase A and RNase A/A1 complex. **b**, Enzymatic activity of native RNase A and RNase A/A1 complex. The molar ratio of A1 to RNase A was 0.2:1. **c**, Cytotoxicity of the RNase A/A1/P1 or RNase A/P1 complexes on MDA-MB-231 cells determined by MTT. BSA was used as a negative control and its concentration was equal to that of RNase A in the complex. **d**, AO/EB staining of the transduced MDA-MB-231 cells. The concentrations of RNase A and P1 were 25 and 6 μg/mL, respectively. The molar ratio of A1 to RNase A was 0.2:1.

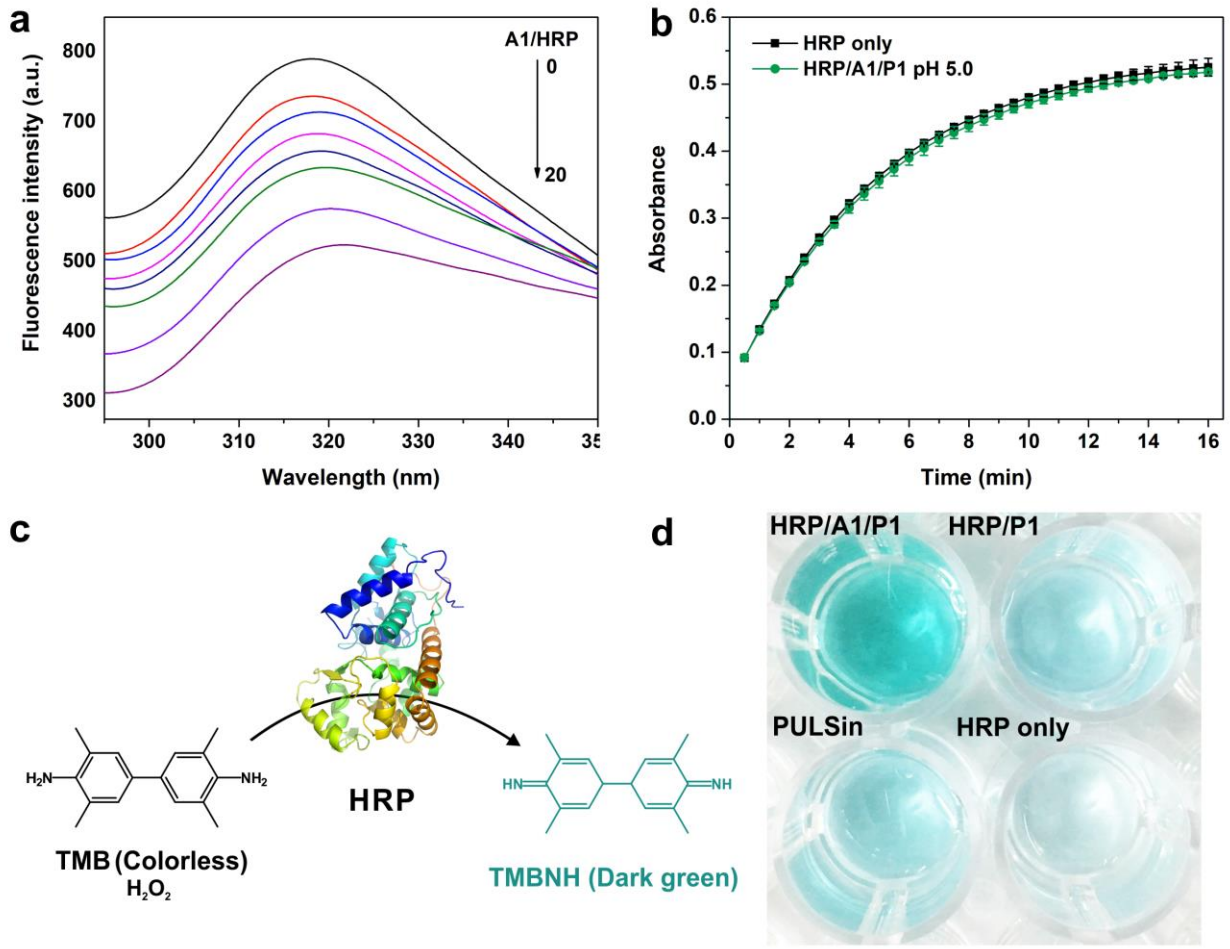

Figure S26. Cytosolic delivery of HRP/A1/P1 complex into HeLa cells. (a) Quenching of HRP fluorescence in the presence of A1. The molar ratios of A1 to HRP were 0:1, 2:1, 4:1, 6:1, 8:1, 10:1, 15:1, 20:1, respectively. (c) Enzymatic activity of free HRP and HRP/A1/P1 complex at pH 5.0. (c). HRP catalyzes the colorless substrate TMB into a green dye. (d) HRP activity in the transduced cells measured by a TMB assay. The doses of HRP and P1 in each well was 4 μg and 8 μg, respectively. The molar ratios of A1 to HRP was 10:1.