Supplementary Material

Controlled Delivery of an Anti-Inflammatory Toxin to Macrophages by Mutagenesis and Nanoparticle Modification

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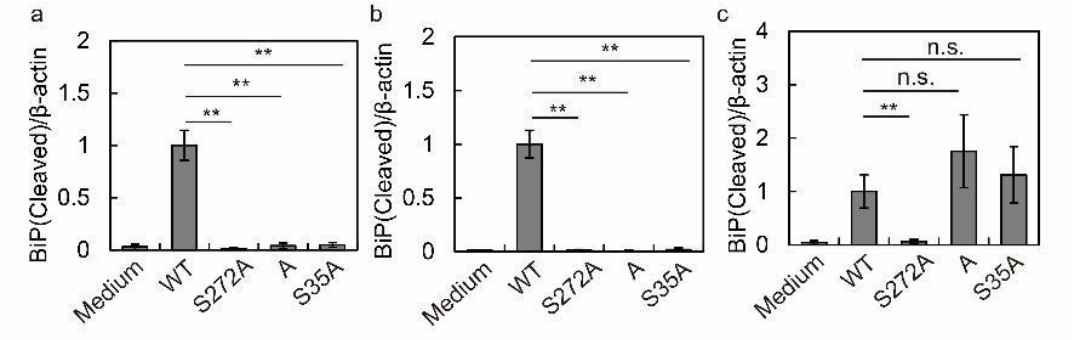

Figure S1. Densitometry of BiP cleavage in (**a**) J774.1 cells, (**b**) HeLa cells, and (**c**) a HeLa cell lysate. The cells and cell lysate were treated with 5 μg/mL toxin for 1 h. After treatment, the cells and cell lysate were incubated in SDS sample buffer and then subjected to Western blotting and densitometry. Data are expressed as means ± S.D. (n = 3). Statistical analyses were performed using Student's t-test. n.s., not significant. $^{**}p < 0.01$.

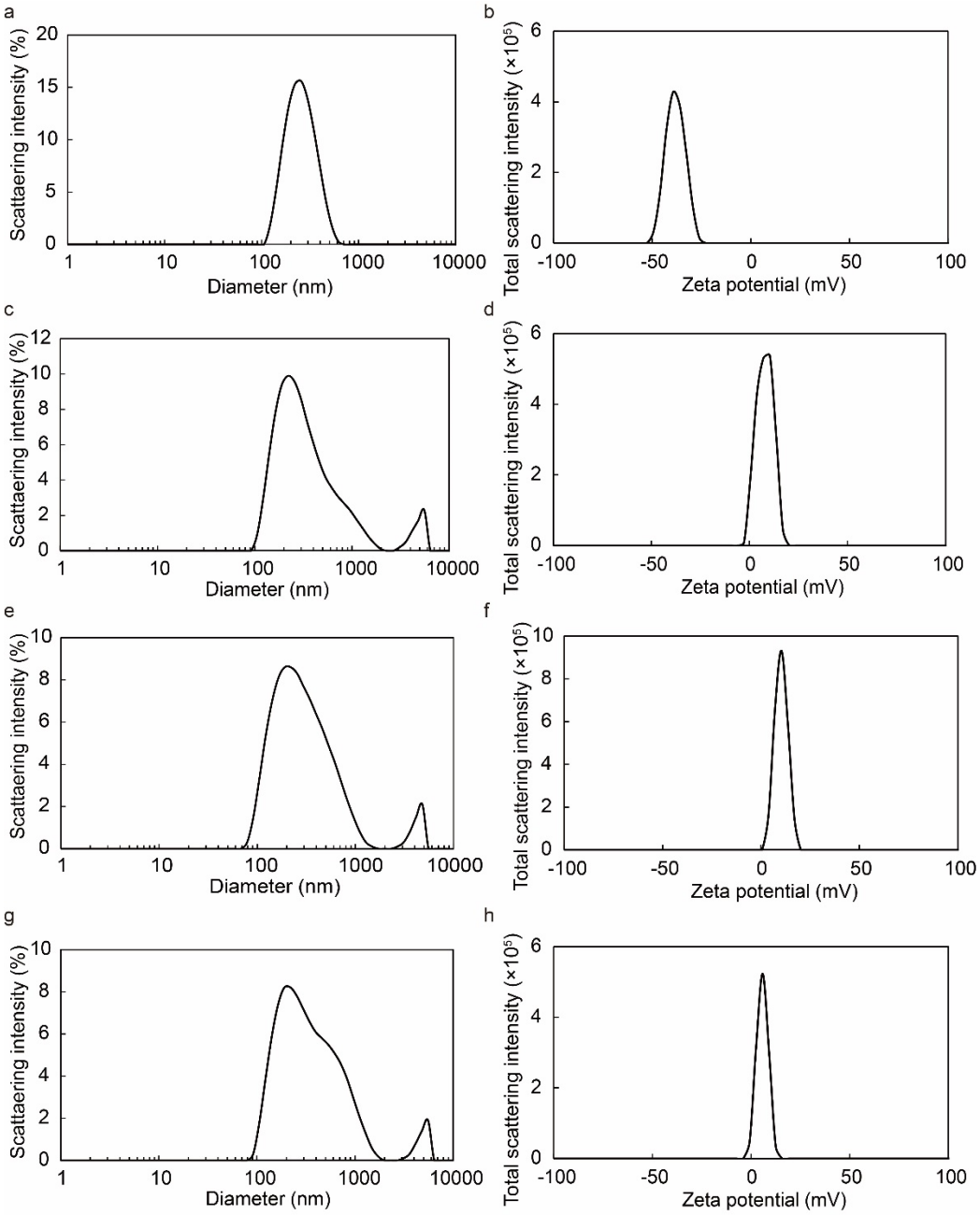

Figure S2. Characterization of PLGA NPs. Dynamic light scattering of (**a**) PLGA NPs, (**c**) WT-PLGA NPs, (**e**) A-PLGA NPs and (**g**) S35A-PLGA NPs in water. Zeta potentials of (**b**) PLGA NPs, (**d**) WT-PLGA NPs, (**f**) A-PLGA NPs and (**h**) S35A-PLGA NPs were measured at room temperature.

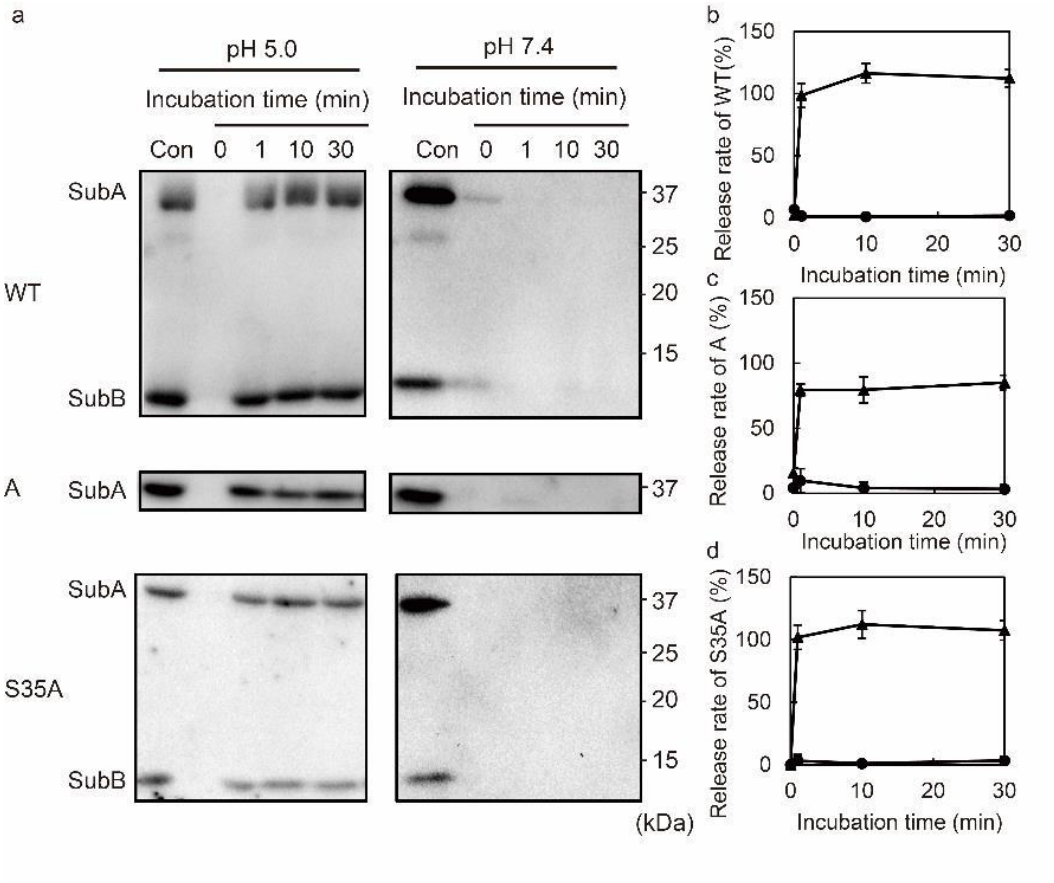

Figure S3. pH-responsive dissociation of His-tagged WT, A, and S35A from PLGANPs. (**a**) Toxin-modified PLGA NPs were treated for 0–30 min with buffers at the indicated pH value. After centrifugation, the supernatant was analyzed by Western blotting and densitometry. Quantification of released (**b**) WT, (**c**) A, and (**d**) S35A by densitometry (●: pH 5.0; ▲: pH 7.4). Data are expressed as means ± S.D. (n = 3).

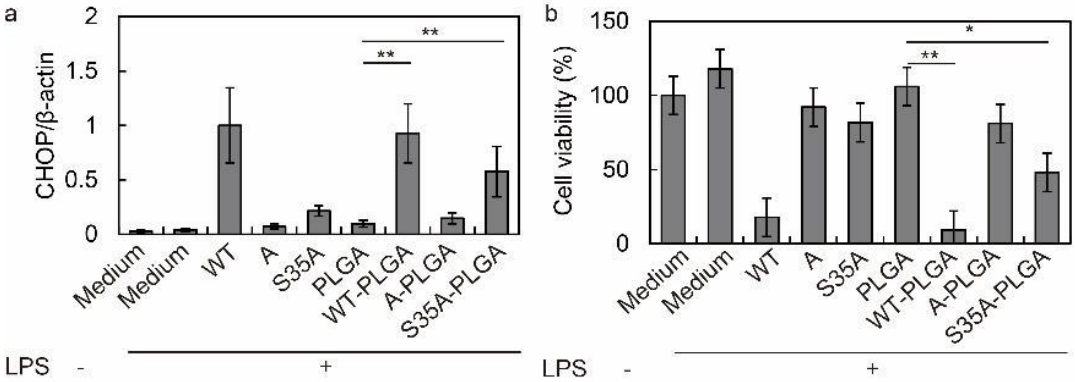

Figure S4. Evaluation of ER stress and cytotoxicity in J774.1 cells. The cells were treated with or without LPS (100 ng/mL) in the presence 5 μg/mL toxin and 20 μg/mL PLGA NPs for 8 and 24 h. After 8 h, the cells were lysed in SDS sample buffer and then subjected to Western blotting and densitometry. Additionally, the cells were subjected to MTT assays at 24 h of treatment. (**a**) Densitometry of CHOP expression at 8 h. (**b**) Evaluation of cell viability by MTT assays. Data are expressed as means ± S.D. (n = 3). Statistical analyses were performed using Student's t-test. *$p < 0.05$, **$p < 0.01$.

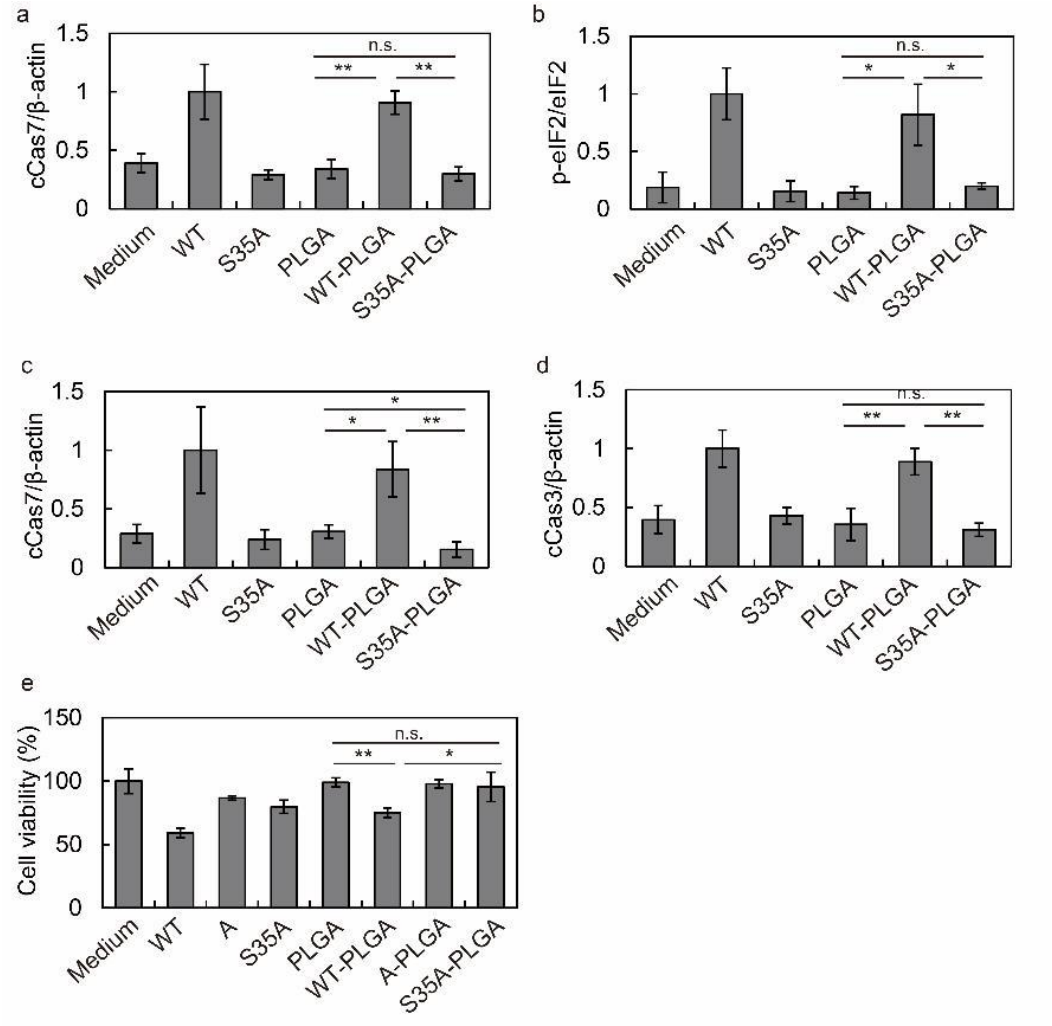

Figure S5. Evaluation of ER stress and apoptosis induction in HeLa cells. The cells were treated with or without 5 μg/mL toxin and 20 μg/mL PLGA NPs for 3, 8, and 24 h. After 3 and 8 h, the cells were lysed in SDS sample buffer and subjected to Western blotting and densitometry. Additionally, cell viability was evaluated by MTT assays at 24 h of treatment. (**a**) Densitometry of caspase 7 activation at 3 h. (**b**) Densitometry of eIF2 phosphorylation at 3 h. (**c**) Densitometry of caspase 7 activation at 8 h. (**d**) Densitometry of caspase 3 activation at 8 h. (**e**) Evaluation of cell viability by MTT assays. Data are expressed as means ± S.D. (n = 3). Statistical analyses were performed using Student's t-test. n.s., not significant. *$p < 0.05$, **$p < 0.01$.