Supplementary Material

The collectin SP-A and its trimeric recombinant fragment protect alveolar epithelial cells from the cytotoxic and proinflammatory effects of human cathelicidin *in vitro*

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Running Title: SP-A protects from LL-37 cytotoxicity

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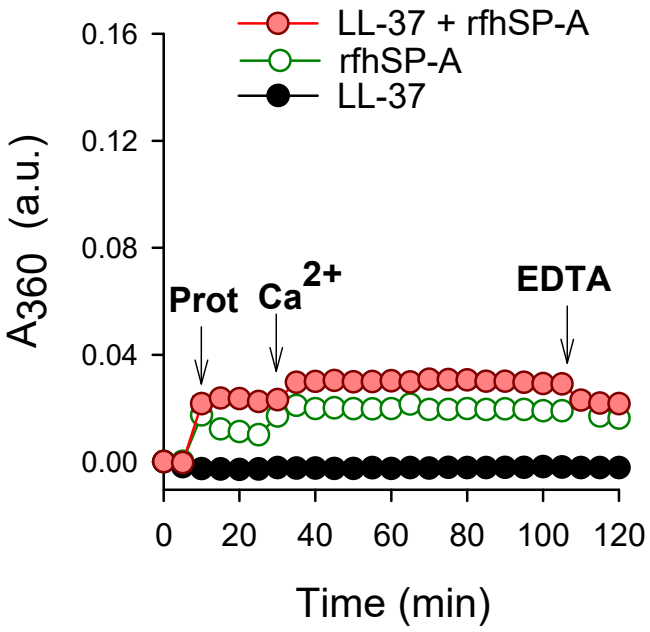

Figure S1. rfhSP-A (0.45 μM), which lacks the N-terminal domain and the collagen domain, could not undergo Ca^{2+}-dependent self-aggregation either in the presence or absence of LL-37 (1.1 μM). Results are shown as means ± SD of three independent experiments.

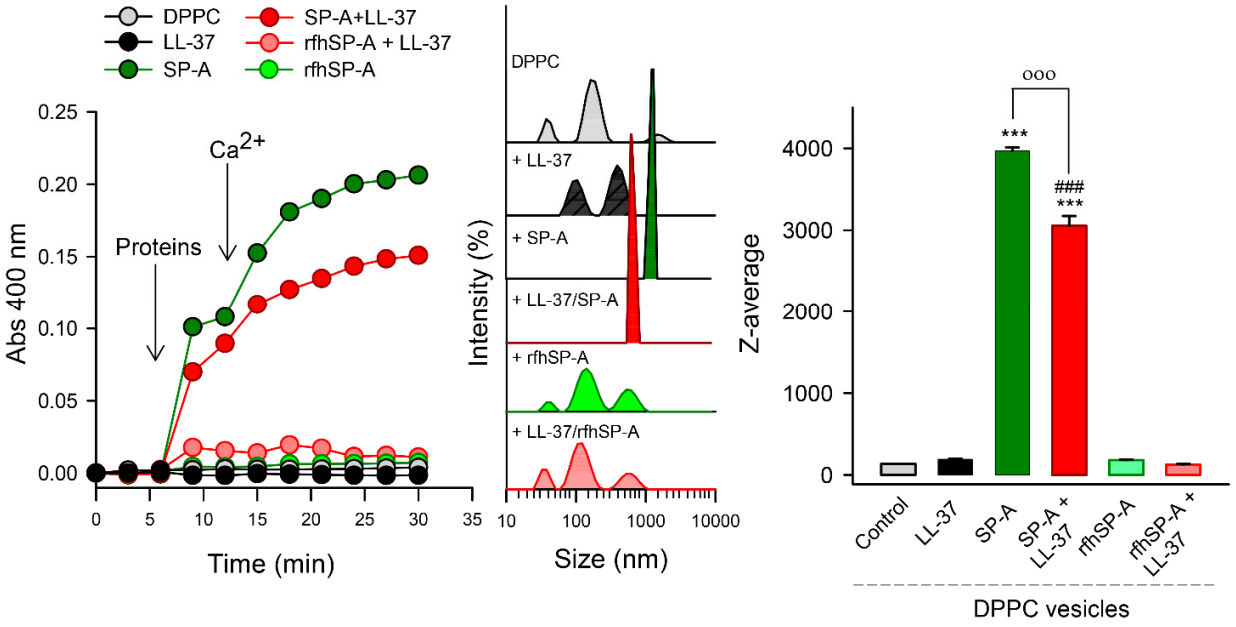

Figure S2. Ca^{2+}-dependent DPPC vesicle aggregation induced by SP-A, LL-37, rfhSP-A, and combinations thereof. DPPC vesicles (50 µg/ml) in 5 mM Tris-HCl buffer, pH 7.4, containing 150 mM NaCl and 0.1 mM EDTA were used. The left panel represent one representative experiment of three DPPC vesicle aggregation induced by proteins measured by turbidity at 400 nm. The central panel shows the samples used for turbidity measurements analyzed by DLS. The right panel shows the Z-average measurements of these samples. Results are the mean ± SD of three experiments. For statistical analysis, ANOVA followed by Bonferroni multiple comparison test was used. *** $p<0.001$ when protein-treated samples are compared with the control (DPPC vesicles). ^{ooo} $p<0.001$ when samples with SP-A are compared with SP-A/LL-37 samples. $^{###}$ $p<0.01$ when SP-A/LL-37 and LL-37 samples are compared. The concentrations of proteins were 75 nM SP-A, 1.1 µM LL-37, and 0.43 µM rfhSP-A. The concentration of Ca^{2+} was 2.5 mM.

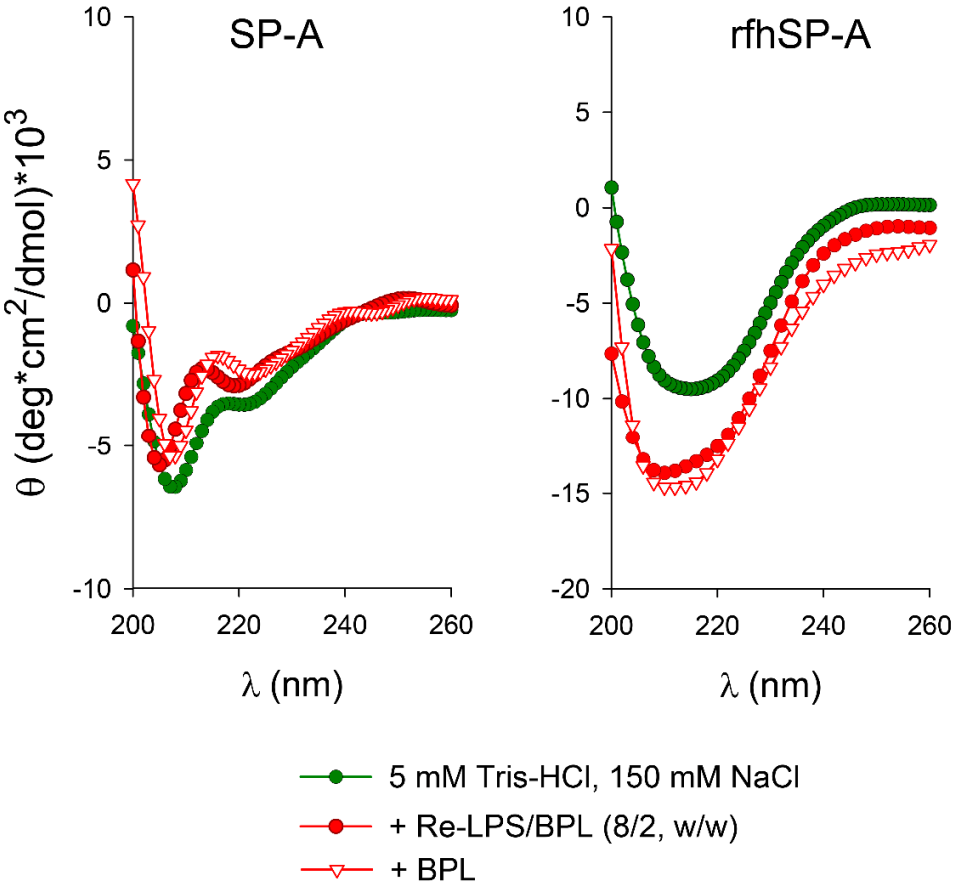

Figure S3. Secondary structure of SP-A and rfhSP-A in the presence and absence of bacterial membranes. Changes in the molar ellipticity of SP-A and rfhSP-A in the absence (green circles) and presence of lipid vesicles mimicking the outer membrane (red circles) composed of Re-LPS/BPL (8:2, w/w) (1 mg/ml) and inner membrane (open triangles) (BPL: POPE/POPG/CL, 67/23/10, w/w/w)(1 mg/ml) of Gram-negative bacteria. Measurements were performed at 25 °C in 5mM Tris-HCl, 150mM NaCl buffer, pH 7.4. Protein concentrations were 75 µg/ml for SP-A, and rfhSP-A. A representative experiment of three are shown.