SUPPORTING INFORMATION

Receptor-based Peptides for Inhibition of Leukotoxin Activity

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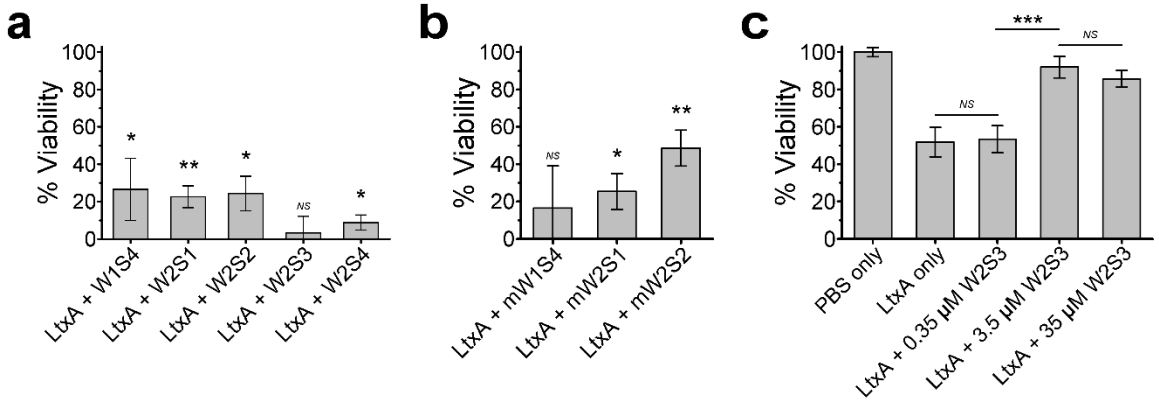

Figure S1. Normalized viability of THP-1 cells determined by Trypan blue exclusion. Simultaneous addition LtxA and (a) hCD11a peptides and (b) mCD11a peptides. All experimental conditions and concentrations of peptide and LtxA are identical to those used in Figure 2. (c) Viability of THP-1 cells after incubation with LtxA and increasing concentrations of W2S3 peptide, normalized to the PBS control. The level of significance was determined by a two-sample *t*-test. *NS*, $P > 0.05$; * $P \leq 0.01$; ** $P \leq 0.001$; *** $P \leq 0.0001$ versus LtxA control unless marked.

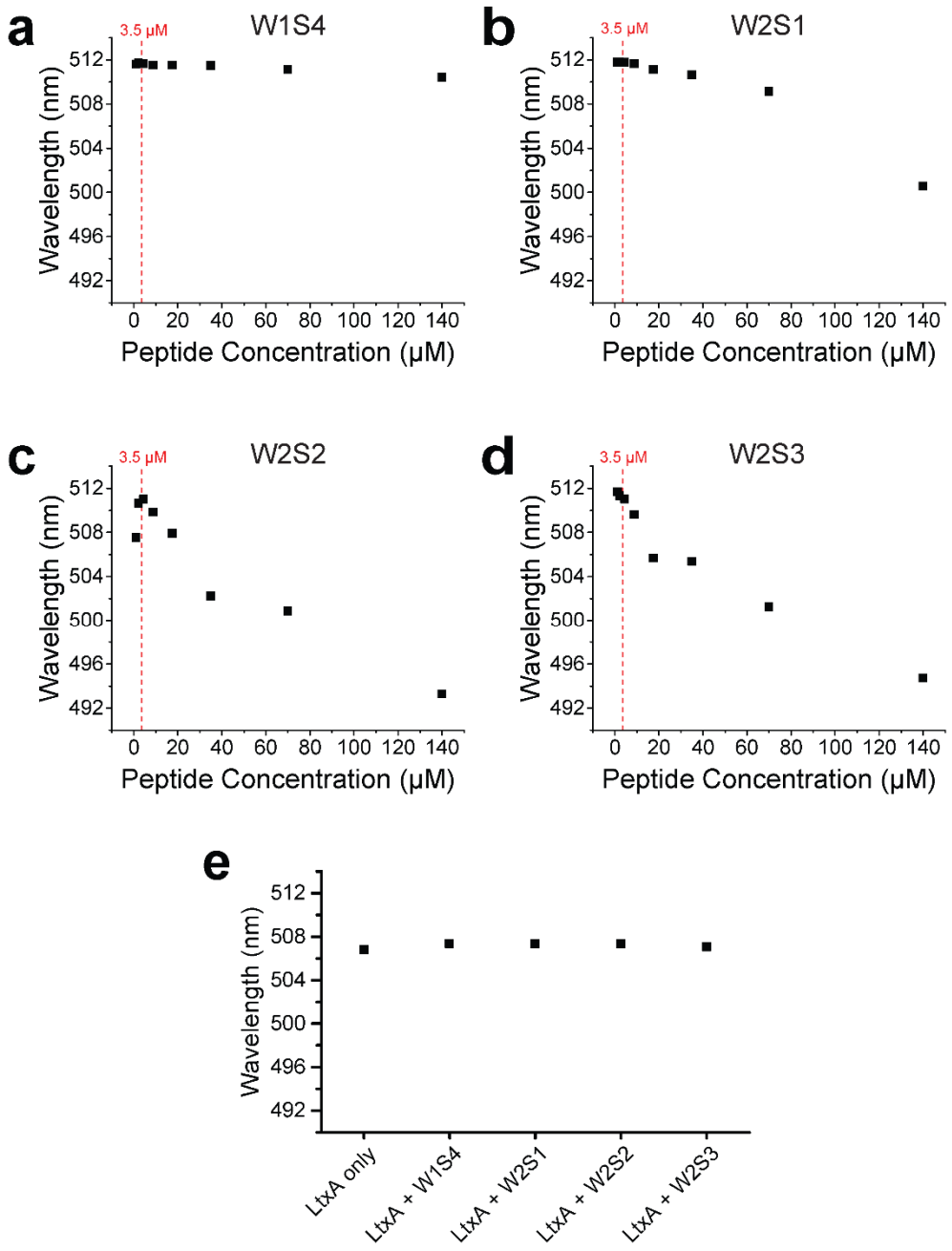

Figure S2. Fluorescence emission peak of 8-anilino-1-naphthalenesulfonic acid (ANS) excited at 350 nm for increasing concentrations of peptides (a) W1S4, (b) W2S1, (c) W2S2 and (d) W2S3. Red dotted line indicates the 3.5 µM peptide concentration used in cytotoxicity investigations. (e) Fluorescence peak of ANS excited at 350 nm for 3.5 µM LtxA incubated with 3.5 µM peptide for 1 h at 37 °C.

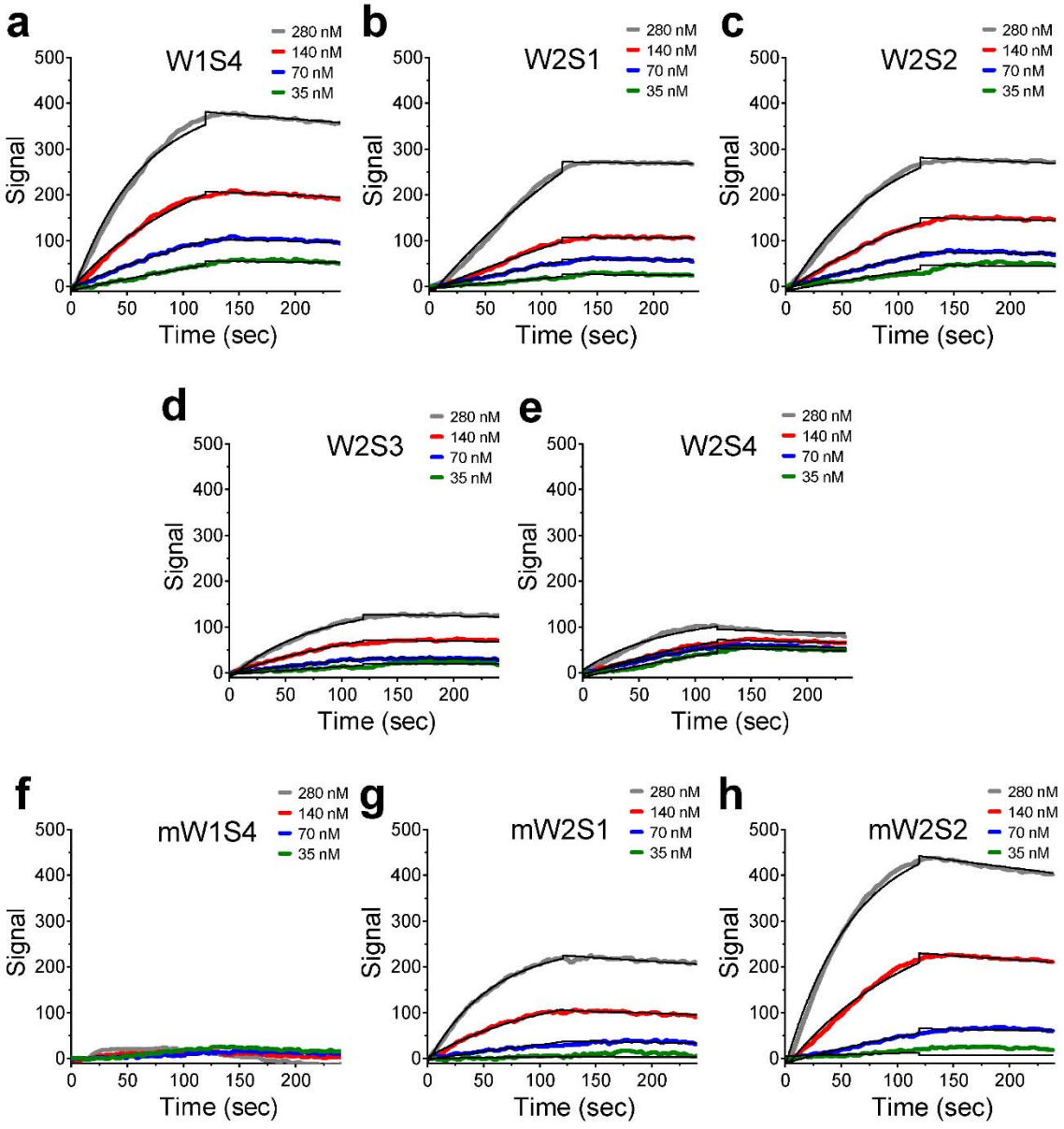

Figure S3. SPR sensorgrams of LtxA binding to hCD11a peptides (a) W1S4, (b) W2S1, (c) W2S2, (d) W2S3, (e) W2S4 and mCD11a peptides (f) mW1S4, (g) mW2S1, (h) mW2S2. Curve fitting to a 1:1 Langmuir binding model with TraceDrawer shown in black.

Table S1. The association (k_a) and dissociation (k_d) rate constants of the LtxA-peptide interactions.

peptide	k_a ($\times 10^4$ $M^{-1} \cdot s^{-1}$)	k_d ($\times 10^{-4}$ s^{-1})
W1S4	5.71 ± 0.002	5.22 ± 0.091
W2S1	1.45 ± 0.001	1.11 ± 0.178
W2S2	3.96 ± 0.002	3.56 ± 0.023
W2S3	4.75 ± 0.005	3.58 ± 0.015
W2S4	4.59 ± 0.008	8.32 ± 0.035
mW1S4	No binding detected	
mW2S1	5.97 ± 0.01	7.46 ± 0.015
mW2S2	5.64 ± 0.065	7.35 ± 0.114

Errors are from the TraceDrawer fitting results.

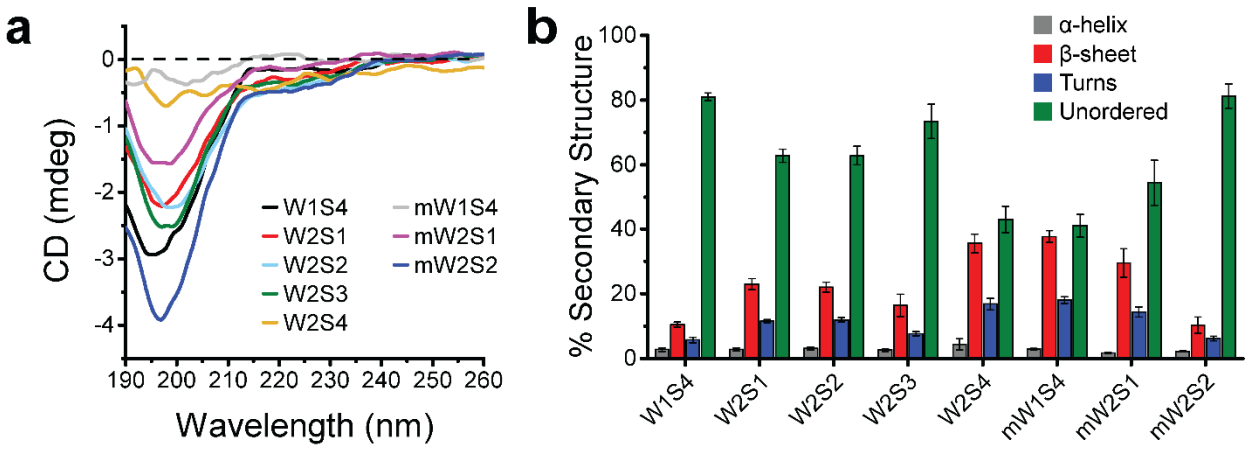

Figure S4. Circular dichroism (CD) analysis of peptide structure. (a) CD spectra for each of the peptides. (b) Analysis with DICHROWEB reveals that the peptides have mostly an unordered conformation.

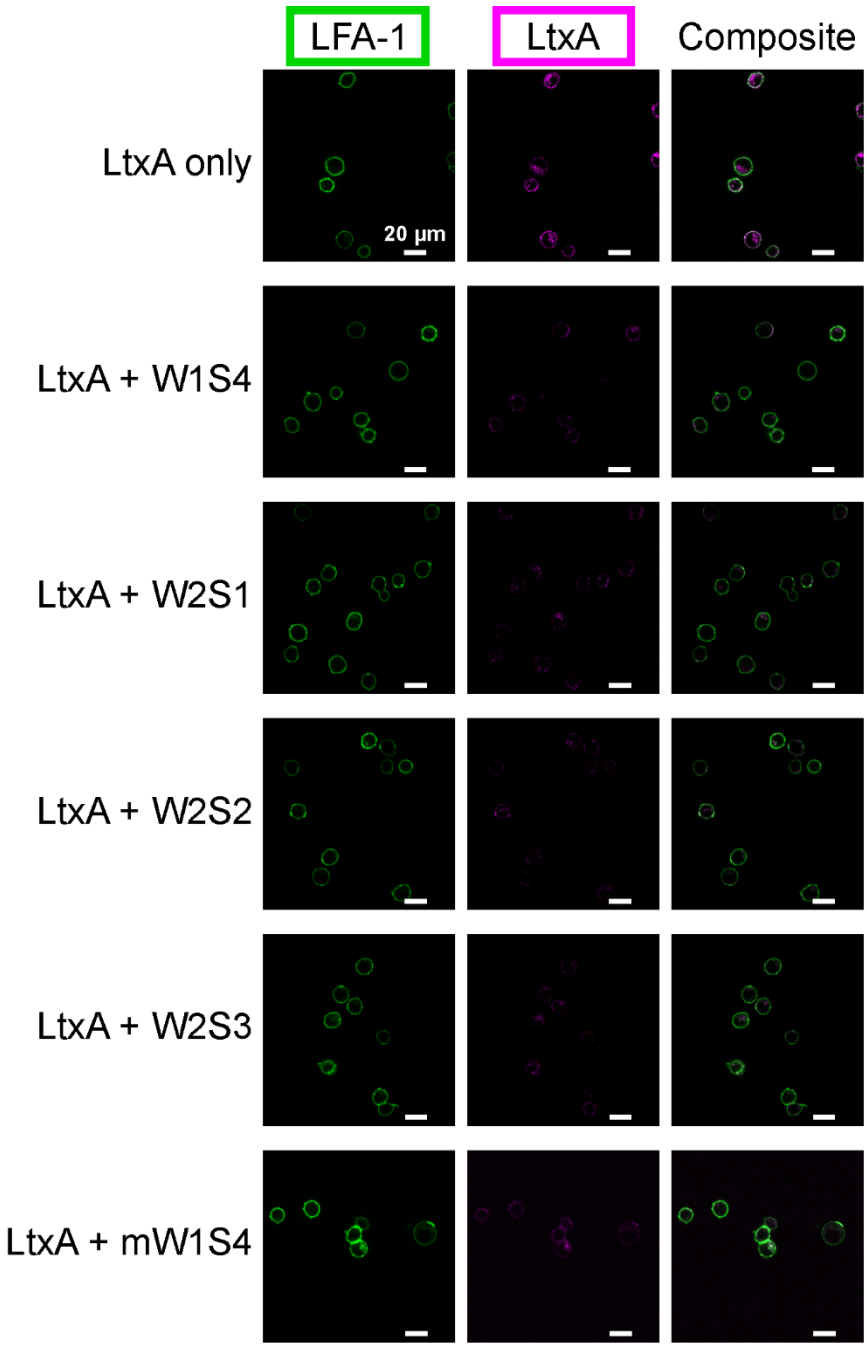

Figure S5. Representative confocal microscopy images of the channels comprising the composite images shown in Figure 4. Left column: FITC-labeled LFA-1 on THP-1 cells (green). Middle column: LtxA labeled with Alexa Fluor 647 (magenta) after pretreatment of the toxin with the peptides. Right column: composite images. Scale bar: 20 µm.

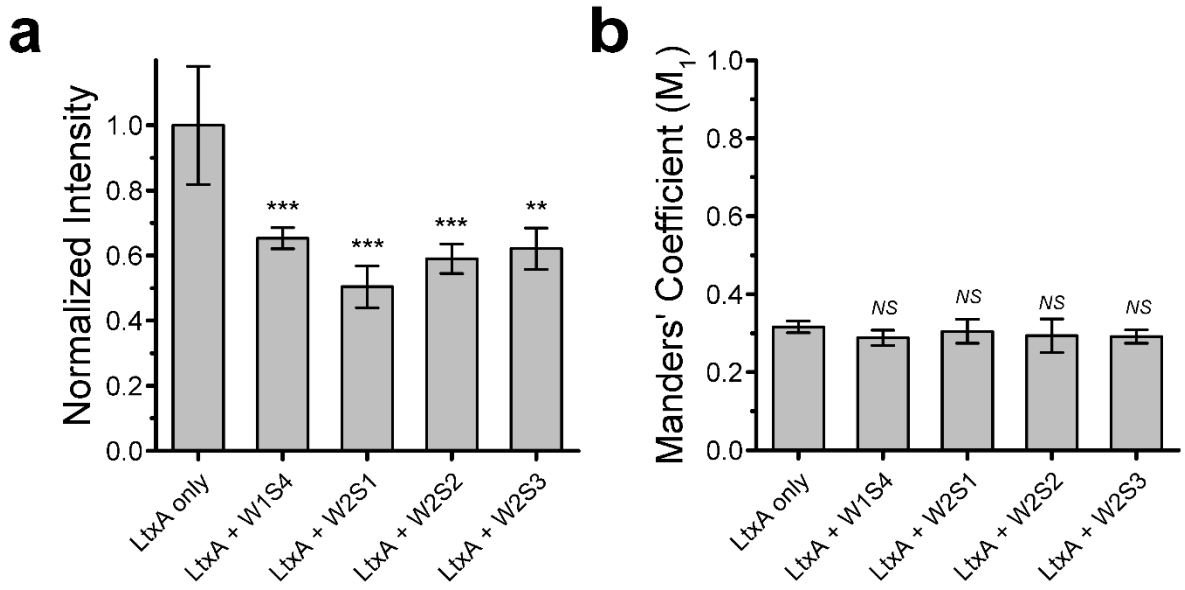

Figure S6. (a) Mean fluorescence of LtxA-AF647 in THP-1 cells normalized to LtxA control. Error bars represent the standard deviation of the mean intensities of 4 - 6 images of cells represented in Figure S5. (b) Mean Manders' coefficient M_1 of labeled LFA-1 and labeled LtxA from simultaneous addition of LtxA and peptide. Error bars represent the standard deviation of the mean M_1. The level of significance was determined by a two-sample *t*-test. *NS*, $P > 0.05$; ** $P \leq 0.001$; *** $P \leq 0.0001$ vs LtxA control.

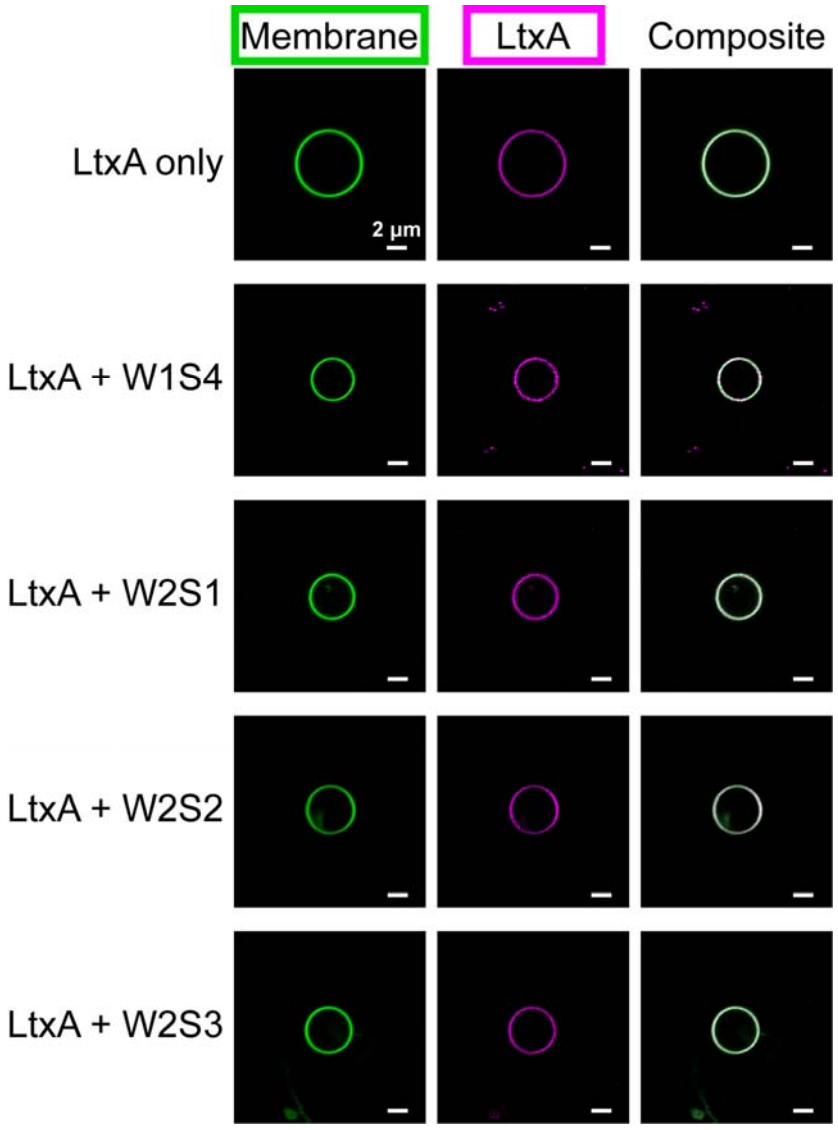

Figure S7. Representative confocal microscopy images of the channels comprising the GUV composite images shown in Figure 5. Left column: fluorescent GUVs (green). Middle column: LtxA labeled with Alexa Fluor 647 (magenta) after pretreatment of the toxin with the peptides. Right column: composite images. Scale bar: 2 µm.

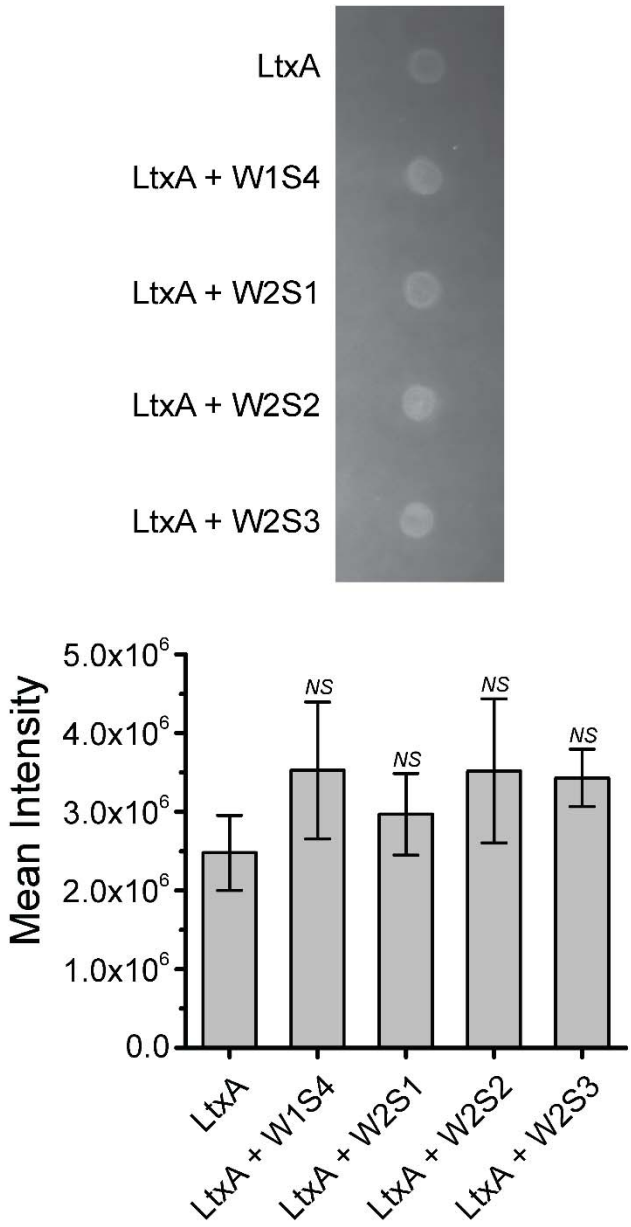

Figure S8. Quantification of dot blot to measure LtxA binding to cholesterol-containing liposomes. LtxA alone or with one of the peptides was spotted on a nitrocellulose membrane, and then incubated with fluorescently labeled cholesterol-containing liposomes. Binding of the toxin to the liposomes was quantified using the resulting fluorescence intensity of each spot. *NS, P* > 0.05 vs LtxA only control.

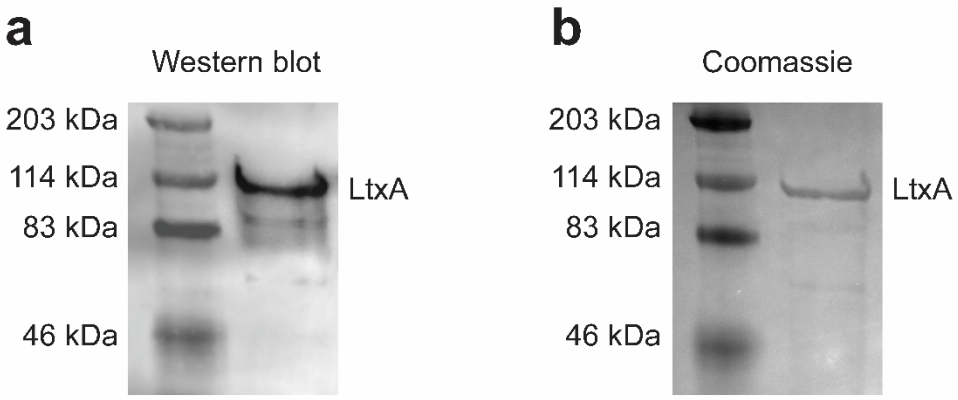

Figure S9. Western blot (a) and Coomassie staining (b) of purified LtxA. Standards are shown in lane 1 of both images.