Supplemental Figure 1. Purified $CRAC^{336WT}$ peptide characterized by reversed-phase HPLC and MALDI-TOF MS. (A) MALDI-TOF spectrum of the purified CRAC peptide. The calculated molecular weight of the CRAC peptide = 2512.9 g/mol. (B) An analytical HPLC chromatograph exhibiting the CRAC peptide peak at 12.498 minutes.

Supplemental Figure 2. Isothermal Titration Calorimetry Experiments. (A) Types of ITC Experiments Performed. A 10 mM of 100% POPC liposomes were titrated into 100 µM of LtxA. LtxA interacts with one set of *n* sites (POPC lipid molecules). **B** 10 mM of 60% POPC/40% Chol liposomes were titrated into 100 μ M of LtxA. LtxA interacts with two sets of *n* sites (POPC or Chol lipid molecules). C 7.65 mM of CRAC^{336WT} was titrated into 2 mM 100% POPC. CRAC^{336WT} interacts with one set of *n* sites (POPC lipid molecules). **D** 7.65 mM of CRAC^{336WT} was titrated into 2 mM 60% POPC/40% sterol liposomes. CRAC^{336WT} interacts with two sets of *n* sites (POPC or sterol lipid molecules). (B) Raw ITC Heats of Injection for LtxA and POPC, POPC/Chol. ITC measurements were performed at 30 °C in a Low Volume Nano ITC. 50 µL of a liposome solution (10 mM) was injected into a cell containing 100 µM LtxA. Triangles depict each 1 µL injection of 100% POPC titrated into LtxA. Circles depict each 1 µL injection of 60% POPC/40% Chol titrated into LtxA. Lines of best fit are shown, with long dashes depicting POPC/Chol and short dashes depicting POPC. (C) Raw ITC Heats of Injection for CRAC^{336WT} and POPC, POPC/Chol. ITC measurements were performed at 30 °C in a Low Volume Nano ITC. 50 µL of CRAC^{336WT} (7.65 mM) was injected into a cell containing 2 mM liposome solution. Inverted triangles depict each 2.5 µL injection of CRAC^{336WT} titrated into 100% POPC. Squares depict each 2.5 µL injection of CRAC^{336WT} titrated into 60% POPC/40% Chol. Lines of best fit are shown, with long dashes depicting POPC/Chol and short dashes depicting POPC. (D) Raw ITC Heats of Injection for CRAC^{336SCR} and POPC, POPC/Chol. ITC measurements were performed at 30 °C in a Low Volume Nano ITC. 50 µL of CRAC^{336WT} (7.65 mM) was injected into a cell containing 2 mM liposome solution. Squares depict each 2.5 µL injection of CRAC^{336SCR} titrated into 100% POPC. Circles depict each 2.5 µL injection of CRAC336SCR titrated into 60% POPC/40% Chol. Lines of best fit are shown, with short dashes depicting POPC/Chol and long dashes depicting POPC.

Supplemental Figure 3. Raw CD Data. Spectral scans were performed in 10 mM phosphate buffer, using a peptide concentration of 0.25 mg/mL. To ensure that the spectra represent the structure of only bound peptide, unbound peptide was removed using centrifugal filters. Mean residue ellipticity was calculated for each spectrum.

Supplemental Figure 4. Isothermal Titration Calorimetry Experiments with Various Sterols. Raw ITC Heats of Injection. ITC measurements were performed at 30 °C in a Low Volume Nano ITC. 50 μ L of CRAC^{336WT} (7.65 mM) was injected into a cell containing 2 mM liposome solution. Triangles depict each 2.5 μ L injection of CRAC^{336WT} titrated into 60% POPC/40% DHC. Squares depict each 2.5 μ L injection of CRAC^{336WT} titrated into 60% POPC/40% Desmo. Diamonds depict each 2.5 μ L injection of CRAC^{336WT} titrated into 60% POPC/40% Desmo. Diamonds depict each 2.5 μ L injection of CRAC^{336WT} titrated into 60% POPC/40% Desmo. Lines of best fit are shown, with long dashes depicting POPC/Desmo, short dashes depicting POPC/DHC, and combined long and short dashes depicting POPC/CC.

FIGURES



Supplemental Figure 1



Supplemental Figure 2



Supplemental Figure 3



Supplemental Figure 4