

Supplementary Material File

This file contains three supplemental figures and corresponding figure legends.

**EVIDENCE FOR MULTIPLE MODES OF NEUTROPHIL SERINE PROTEASE
RECOGNITION BY THE EAP FAMILY
OF STAPHYLOCOCCAL INNATE IMMUNE EVASION PROTEINS**

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Running Head: Inhibition of Neutrophil Serine Proteases by EapH1 and EapH2

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Figure S1

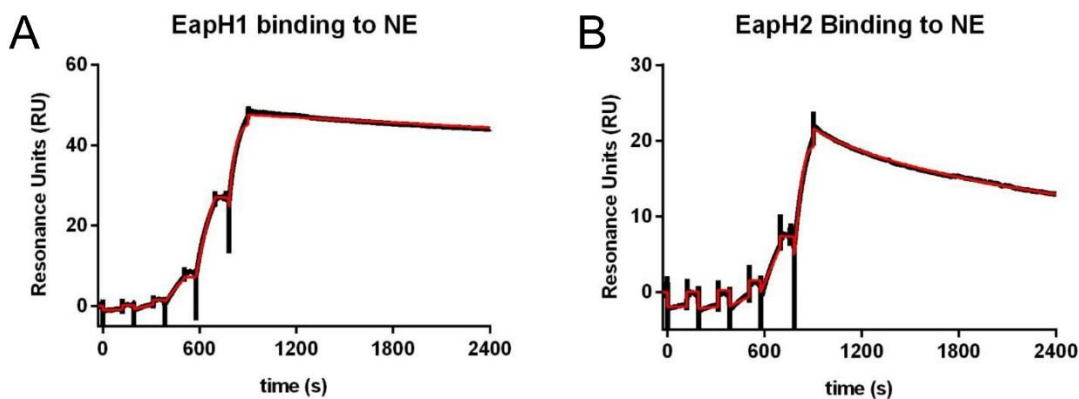


Figure S1. Characterization of EapH1 and EapH2 Binding to NE by a Single-Cycle Kinetic Approach. EapH1 (A) and EapH2 (B) samples at concentrations representing sequential 5-fold increases were injected over an NE surface, followed by a single 60 min dissociation phase. The reference subtracted sensorgrams are shown in black, while the results of curve fitting to a Langmuir binding model are shown in red. To facilitate representation of the comparatively brief association phase, only the first 25 min of the dissociation phase are shown in both sensorgrams. Curve fitting parameters are found in Table 1.

Figure S2

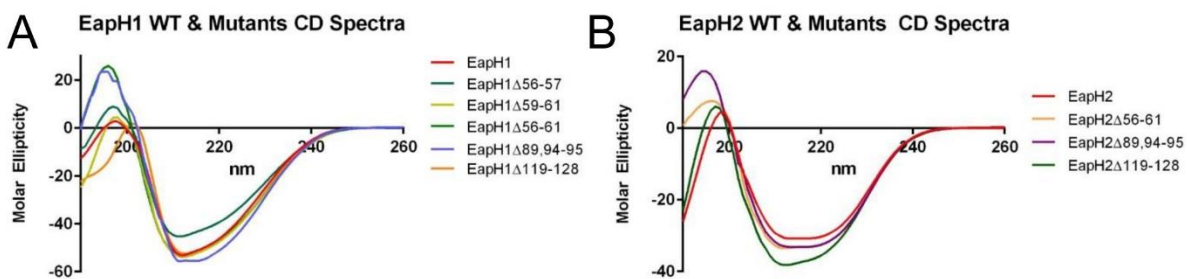


Figure S2. Circular Dichroism Spectra of EapH1, EapH2, and Various Site-Directed Mutants Thereof. CD spectra for EapH1 and its mutants (A) and EapH2 and its mutants (B) were acquired as described in Experimental Procedures. A legend is inset for both panels.

Figure S3

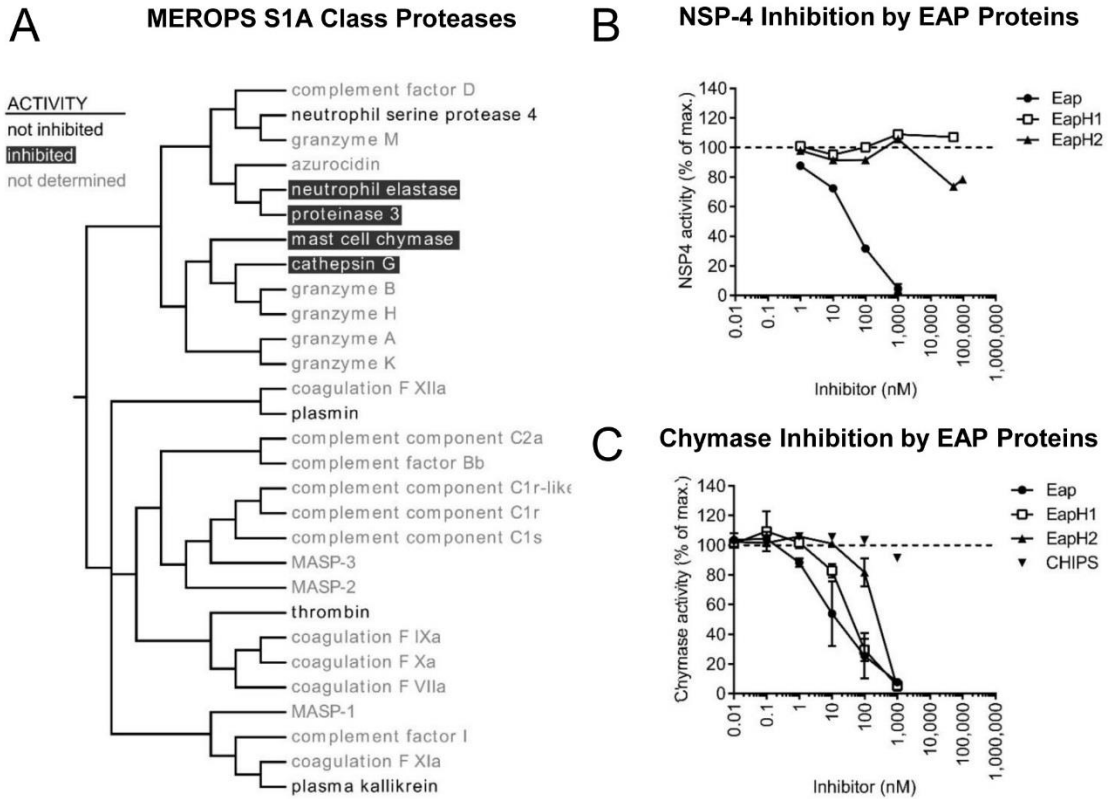


Figure S3. The Protease Inhibitory Activity of *S. aureus* EAP Domains Extends to NSP-4 and Mast Cell Chymase. (A) A phylogenetic tree of human immune proteases within MEROPS class S1A, based on the sequence of their protease domains. A legend is inset and reflects whether or not individual proteases have been tested for inhibition by *S. aureus* EAP domains and to what extent inhibition occurs. (B) Residual activity of NSP-4 upon incubation with *S. aureus* Eap, EapH1, or EapH2. A representative plot is shown from two repeats. (C) Residual activity of Mast Cell Chymase upon incubation with the *S. aureus* Eap, EapH1, EapH2, or a negative control protein, CHIPS.