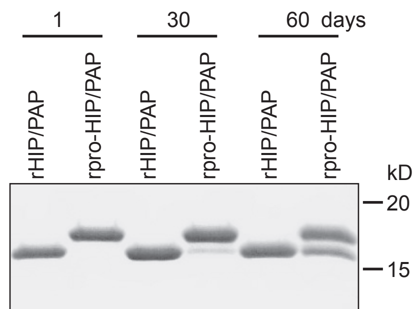
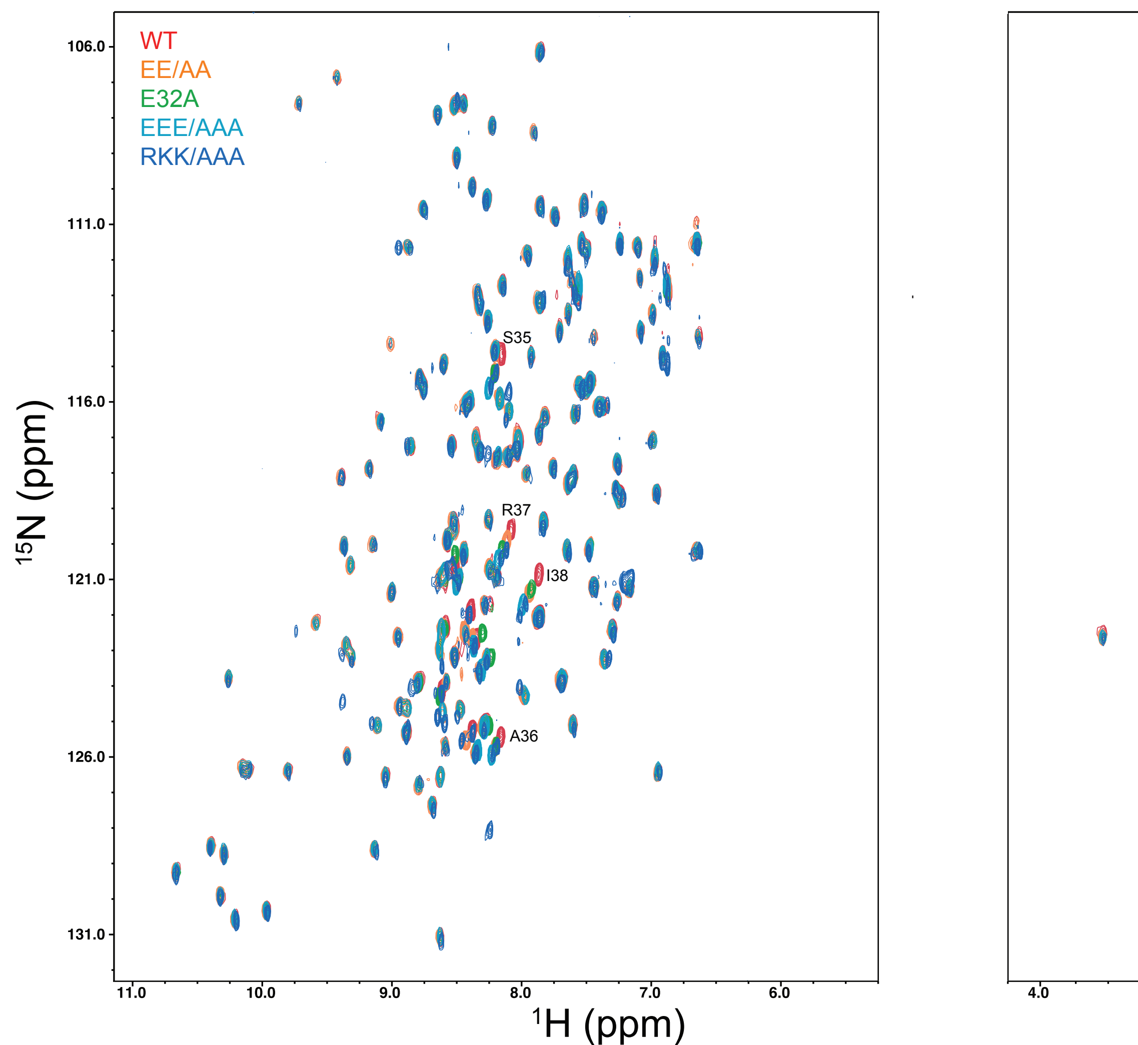
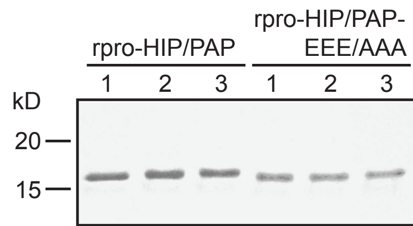
Supplementary Data



Supplementary Figure 1: rpro-HIP/PAP undergoes N-terminal cleavage during storage. rpro-HIP/PAP and rHIP/PAP were purified as outlined in Materials and Methods and stored at 4°C for the indicated periods of time. 5 μg of protein was analyzed by SDS-PAGE on a 15% gel and stained with Coomassie blue.



Supplementary Figure 2. Chemical shift comparison between pro-HIP/PAP and activating pro-HIP/PAP mutants. Superimposed $^{15}N/^{1}H$ HSQC spectra of ^{15}N labeled rpro-HIP/PAP and activating rpro-HIP/PAP mutations reveal colinear chemical shift perturbations among four residues surrounding the trypsin cleavage site. Detailed views are shown in Figure 5. The water signal at ~4.7 ppm has been omitted.



Supplementary Figure 3: rpro-HIP/PAP is not proteolytically processed during *in vitro* bactericidal assays. Wild-type rpro-HIP/PAP and the activated variant rpro-HIP/PAP-EEE/AAA were analyzed by SDS-PAGE following antibacterial assays with *Listeria monocytogenes* as the target organism. Assays were performed as outlined in Figure 2. 1, protein prior to incubation in the antibacterial assay; 2, protein mock-incubated at $37^{o}C$ for 2 hours; 3, protein after a 2 hour incubation in antibacterial assays. The results indicate that activating rpro-HIP/PAP mutations do not result in proteolytic processing during antibacterial assays.