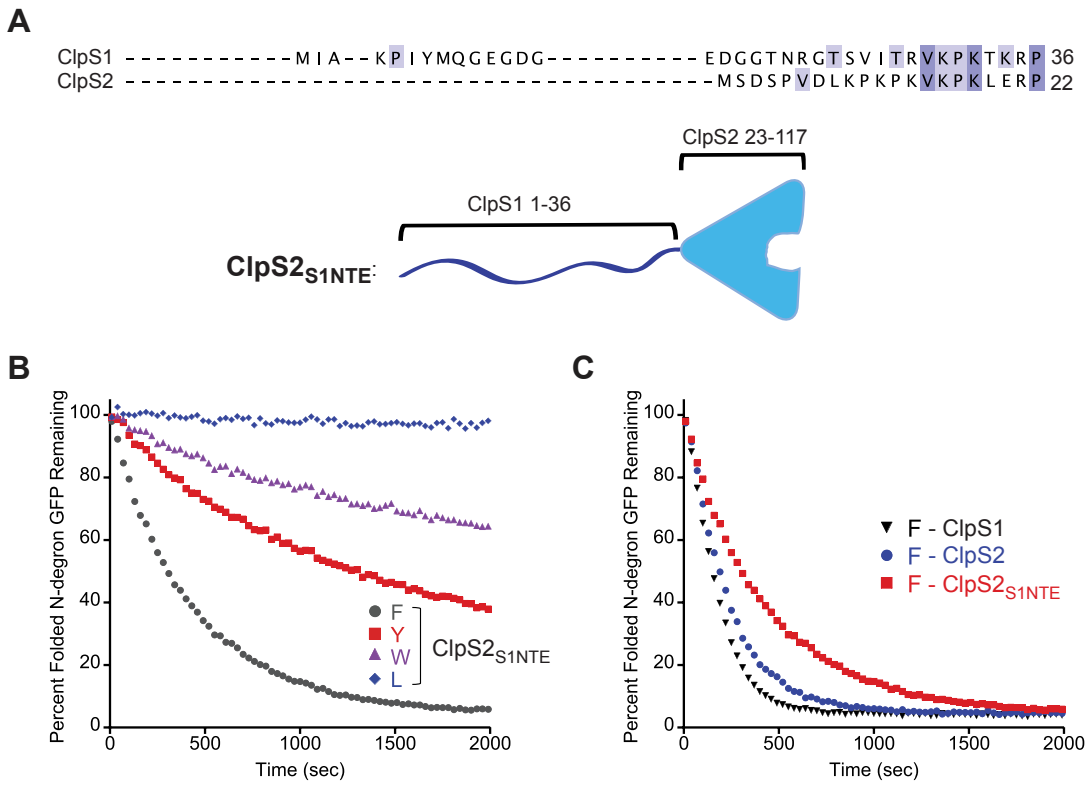

Figure S1, Related to Figure 4. A. Top: Sequence alignment of *A. tumefaciens* ClpS1 and ClpS2 NTEs. The alignment is colored by conservation from lower conservation (light blue) to higher conservation (dark blue) and was constructed using Clustal Omega (Sievers et al., 2011) and Jalview (Waterhouse et al., 2009). Bottom: Diagram of the $ClpS2_{S1NTE}$ mutant. B. *In vitro* unfolding of N-degron GFP (1 μM) by $ClpA_6$ (0.5 μM) and $ClpS2_{S1NTE}$ (2 μM). See Fig. 4A for more experimental detail. C. Comparison of *in vitro* unfolding of F-GFP (1 μM) by $ClpA_6$ (0.5 μM) and ClpS1, ClpS2, or $ClpS2_{S1NTE}$. The $ClpS2_{S1NTE}$ data is the same as in panel B. Data are averages of 3 technical replicates and are representative of 3 independent experiments.

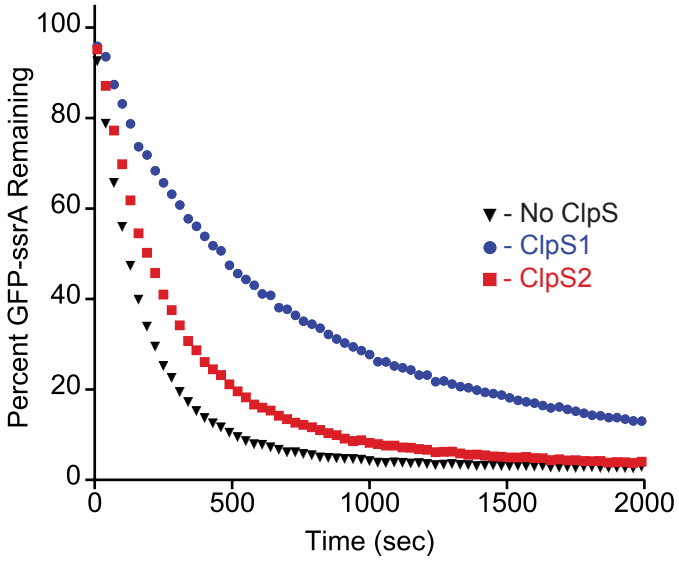

Figure S2, Related to Figure 4. *In vitro* GFP-ssrA (1 μM) degradation in the presence of *E. coli* $ClpA_6$ (0.2 μM), *E. coli* $ClpP_{14}$ (0.4 μM), and *A. tumefaciens* ClpS1 or ClpS2 (2 μM). Data are averages of 3 technical replicates and are representative of 3 independent experiments.

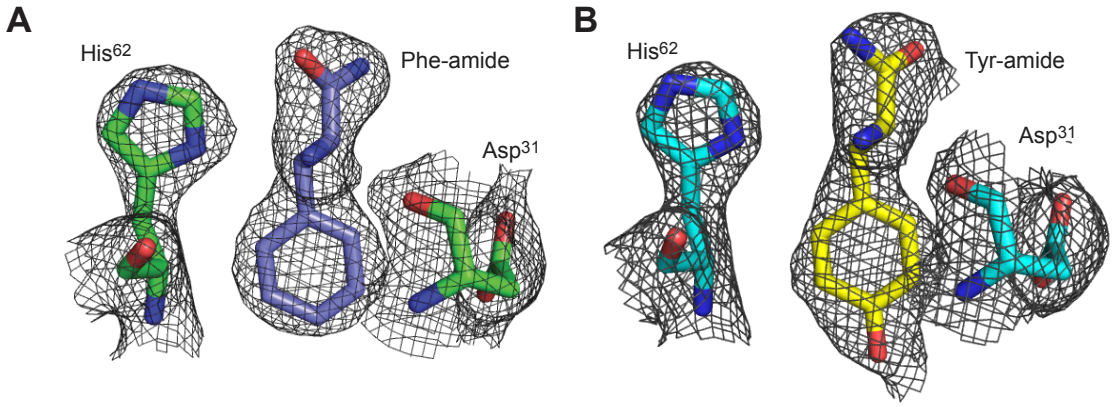

Figure S3, Related to Figure 5. A. $2F_0$-F_C electron density map, contoured at 1.25 σ, for bound Phe-amide, His^{62}, and Asp^{31} in Chain B of the Phe-bound structure. B. $2F_0$-F_C electron density map, contoured at 1.25 σ, for bound Tyr-amide, His^{62}, and Asp^{31} in Chain B of the Tyr-bound structure.

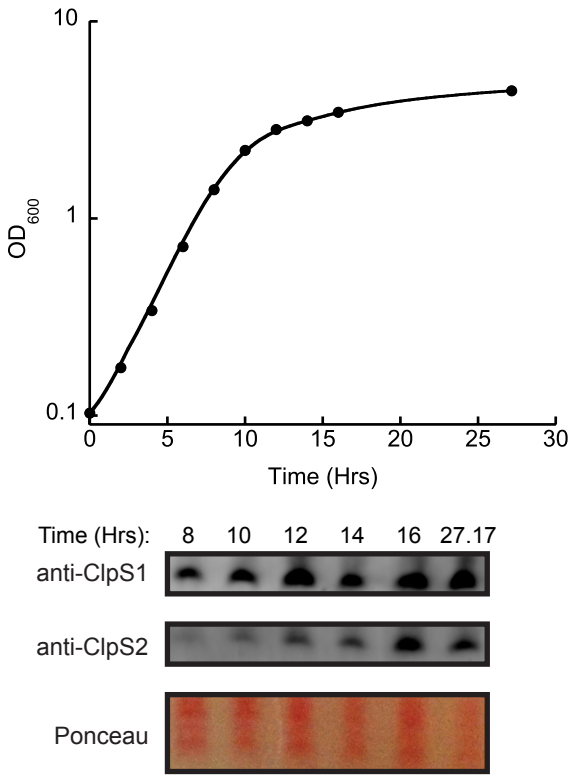

Figure S4, Related to Figure 8. Western blots probing ClpS1 and ClpS2 protein levels at the transition between exponential and stationary phase. Top: Growth curve of *A. tumefaciens* GV3101. Bottom: Selected slices of western blots using anti-ClpS1 or anti-ClpS2 polyclonal antibodies. Ponceau staining of total protein is shown as a loading control.

Supplemental Methods

Strains, Plasmids, and Proteins
$ClpS2_{S1NTE}$ was cloned from ClpS2 and ClpS1 ORFs using standard molecular biology techniques. $ClpS2_{S1NTE}$ was expressed and purified as described in the main text materials and methods. Superfolder GFP-ssrA (Nager et al., 2011) was a gift from Amaris Torres-Delgado.

Unfolding and Degradation Assays
N-degron GFP unfolding assays were carried out as described in main text material and methods. GFP-ssrA degradation experiments were carried out as described in the main text material and methods, using GFP-ssrA instead of N-degron GFP.

Supplemental References

Nager, A.R., Baker, T.A., and Sauer, R.T. (2011). Stepwise Unfolding of a β Barrel Protein by the AAA+ ClpXP Protease. J. Mol. Biol. *413*.