Supporting Information

Videos
Sample video data of Cy3-ssrA binding events on wild-type ClpA (WT_ATP.avi, WT_ATPgS.avi), and Y540A ClpA (Y540A_ATP.avi; Y540A_ATPgS.avi), in the presence of ATP and ATPγS are available as accompanying supporting information.

ClpA Autodegradation in Presence of ATPγS
To verify that formation of the observed autodegradation product is dependent upon ATP hydrolysis, we repeated the gel-based experiment in the presence of the non-hydrolyzable ATP analog ATPγS. The autodegradation product is not observed under these conditions (Fig S-1).

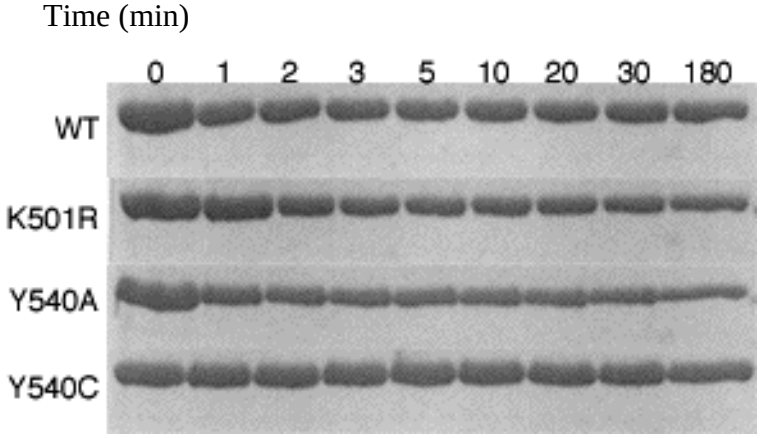

Fig S-1. ClpA bands from gel electrophoresis after incubation for the indicated time with ClpP and ATPγS. Reactions were carried out as described in Materials and Methods.

Complete Gel Images
Full gels from which relevant bands were exised are shown in Figures S-2 and S-3.

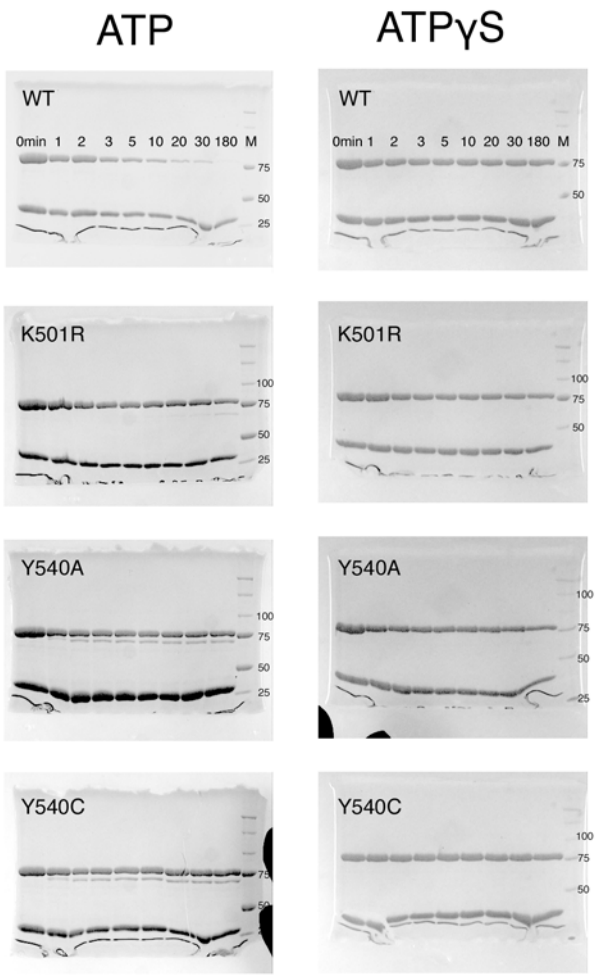

Figure S-2. Complete gel images for mutant and wild-type ClpA autodegradation in the presence of ATP and ATPγS. The bands that appear at the bottom of the gels are creatine phosphokinase. The lane containing protein molecular weight standards is indicated by M, with the relevant molar masses given in kilodaltons.

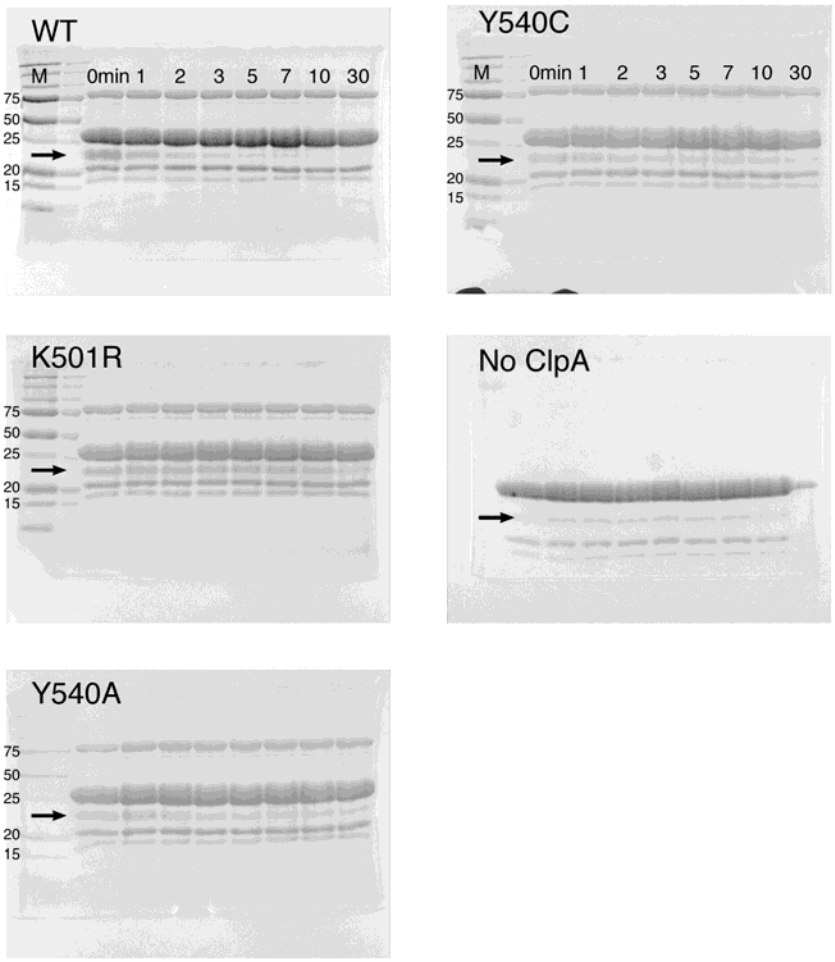

Figure S-3. Complete gel images for casein degradation by ClpAP in the presence of ATP. The lane containing protein molecular weight standards is indicated by M, with the relevant molar masses given in kilodaltons. Casein (~23 kDa) bands on each gel are indicated by an arrow. Other proteins visible on the gels are ClpA (~80 kDa), creatine phosphokinase (~28 kDa), an impurity in the creatine phosphokinase (~21 kDa), and ClpP (~15 kDa).

Peptide Map of Intact ClpA Y540A and Its Autodegradation Product

Figure S-4 shows the signal strength of the ionized peptides detected for the two samples. The peptides detected for the autodegradation product are of approximately the same strength as those detected for the intact ClpA. This suggests that the lack of signal in the N-terminal region of the autodegradation product is not due to lower signal intensity.

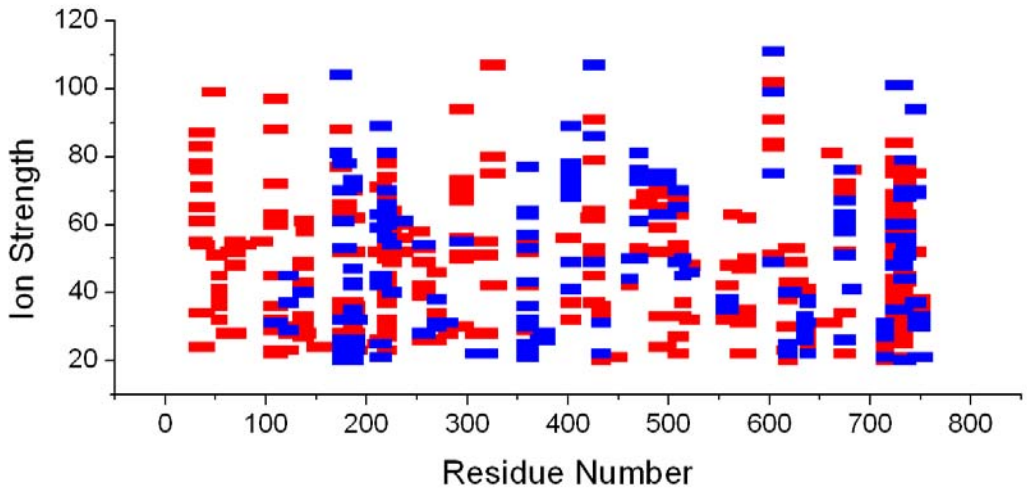

Figure S-4. Signal strength of detected peptides. Red marks indicate peptides detected from the intact ClpA sample; blue marks indicate peptides detected from the autodegradation product.

Figure S-5 (next page) shows peptides detected after Glu-C and tryptic digests of intact ClpA Y540A (orange and red, respectively) and its autodegradation product (light blue and dark blue, respectivey) cut from polyacrylamide gels. The first peptide from the intact ClpA begins at position 29, whereas the first peptide from the autodegradation product begins at residue 103. Position numbers are listed at the left of each row; these do not take into account the N-terminal 9-amino acid FLAG sequence shown in parentheses. Every tenth position is shown in boldface.

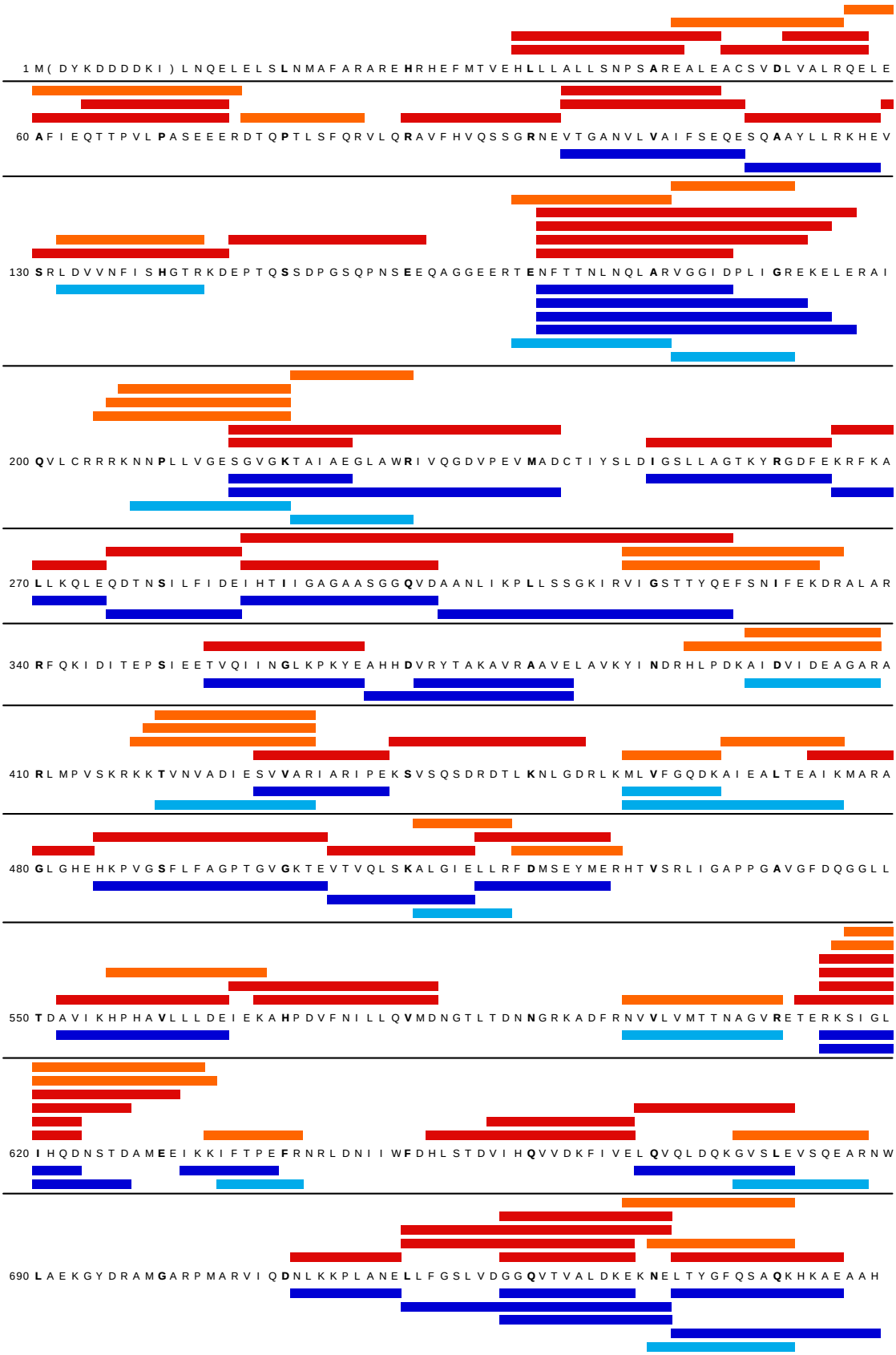

Figure S-5. Peptide map of intact ClpA Y540A and its autodegradation product.