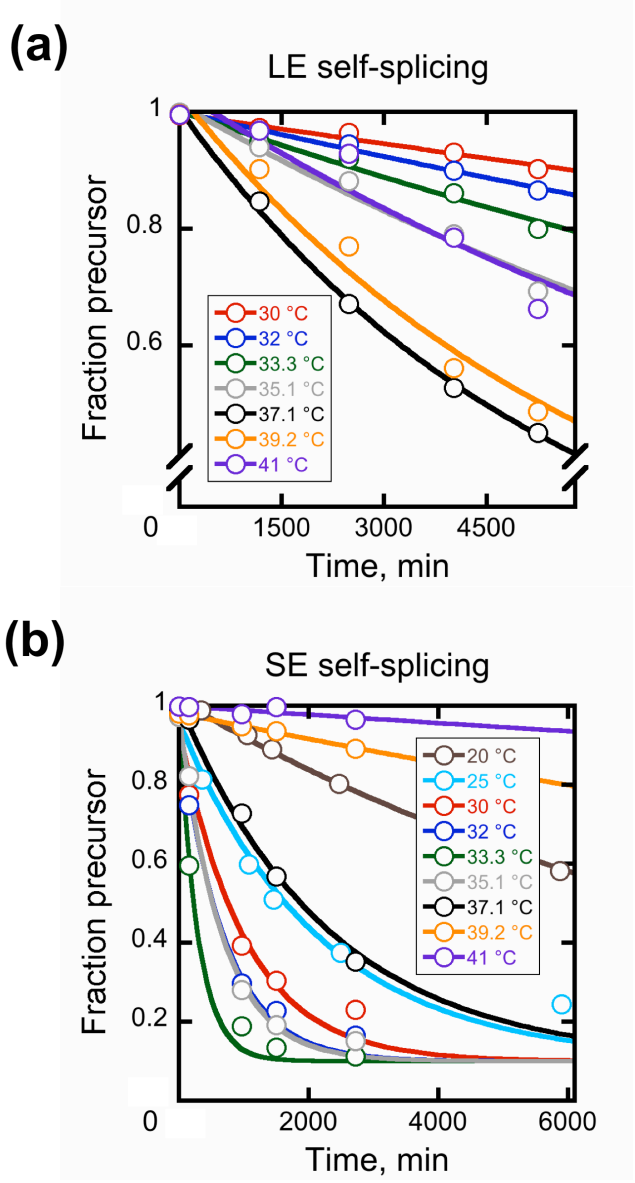

Fig. S1. Self-splicing of the LE construct (panel a) and SE construct (panel b) at different temperatures. Reactions were performed in a thermal cycler with a temperature gradient. Temperatures for each reaction were estimated by linear interpolation from the temperature settings. The fraction of precursor was determined by measuring the precursor band intensity relative to intensity at the positions of reaction products. For the LE construct, these products are the lariat intron, linear intron, and spliced exons. For the SE construct, the detectable products are the lariat intron and linear intron. At 41 °C, only upper limits were determined for the splicing rates because, although the fraction of precursor decreased over time, as indicated in the plots, additional products appeared alongside the expected ones, suggesting that alternative reactions and/or RNA degradation were occurring on the same time scale as splicing.

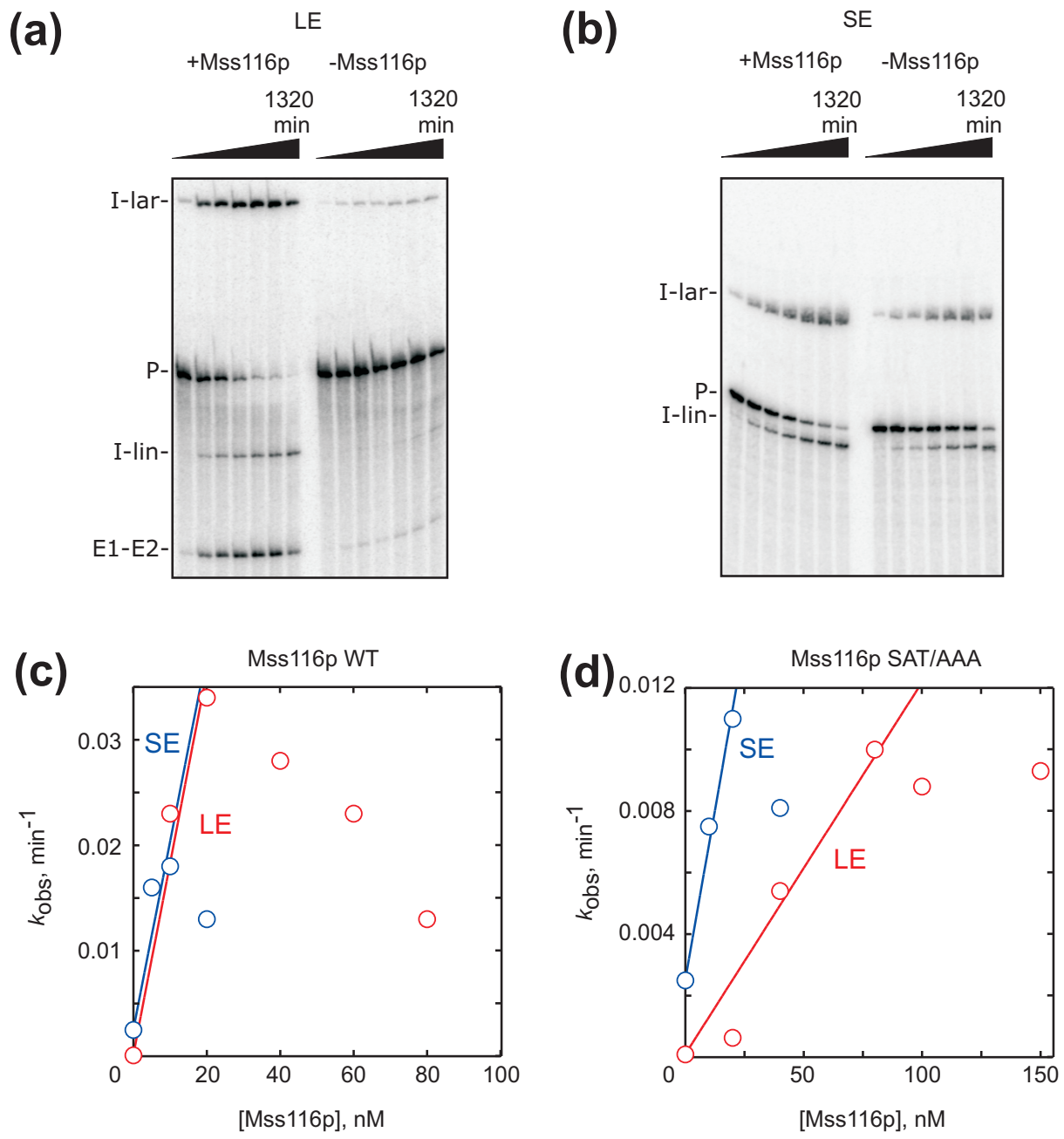

Fig. S2. Mss116p-stimulated splicing of LE and SE constructs in Tris buffer conditions (10 mM Tris-Cl, pH 7.5, 100 mM KCl, 10% glycerol), with 10 nM RNA. (a and b) Gel images of splicing in the presence and absence of Mss116p for the LE construct (40 nM Mss116p, panel a) and the SE construct (10 nM Mss116p, panel b). (c) Concentration dependences of Mss116p stimulation for the LE and SE constructs. Rate constants from this experiment were 1.8×10^6 M^{-1} min^{-1} for the LE construct and $\geq 1.8 \times 10^6$ M^{-1} min^{-1} for the SE construct. (d) Concentration dependences for Mss116p SAT/AAA for splicing of the LE and SE constructs under Tris buffer conditions. Rate constants were 1.2×10^5 M^{-1} min^{-1} for the LE construct and 4.4×10^5 M^{-1} min^{-1} for the SE construct.

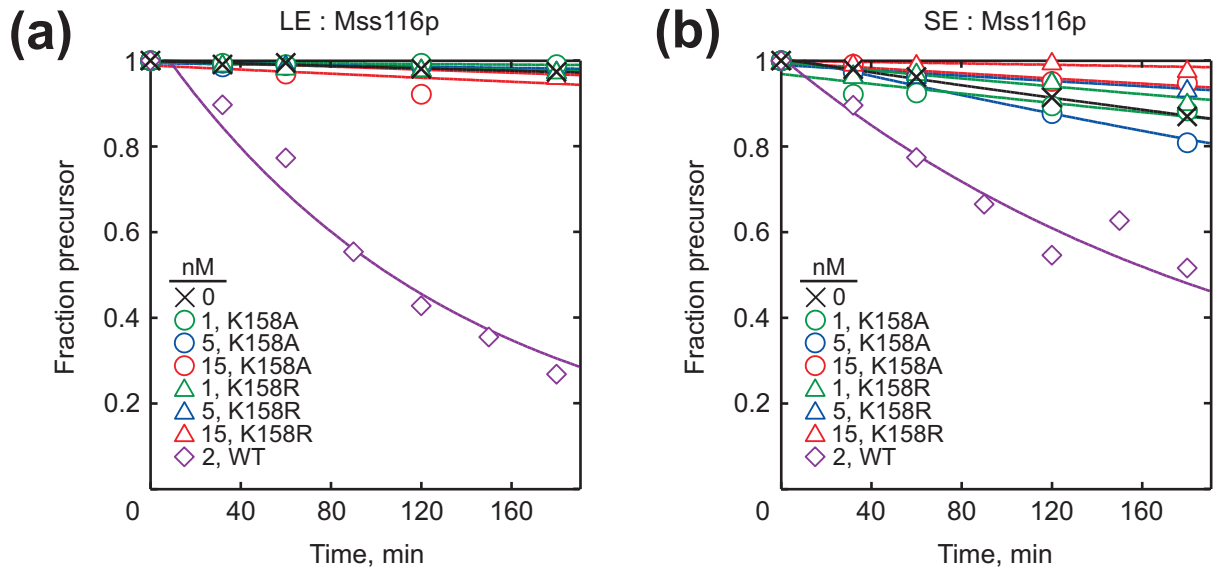

Fig. S3. Mss116p motif I mutants K158A and K158R in splicing reactions of the LE and SE constructs (panels a and b, respectively). All reactions included 1 mM ATP and were performed under standard near-physiological conditions at 30 °C (50 mM Na-MOPS, pH 7.0, 100 mM KCl, 8 mM $MgCl_2$, and 10% Mss116p storage buffer). Reactions in the absence of Mss116p (black x's) and in the presence of WT Mss116p and 1 mM ATP (purple diamonds) were performed side-by-side and gave the same rates within error as equivalent reactions shown in Fig. 3.

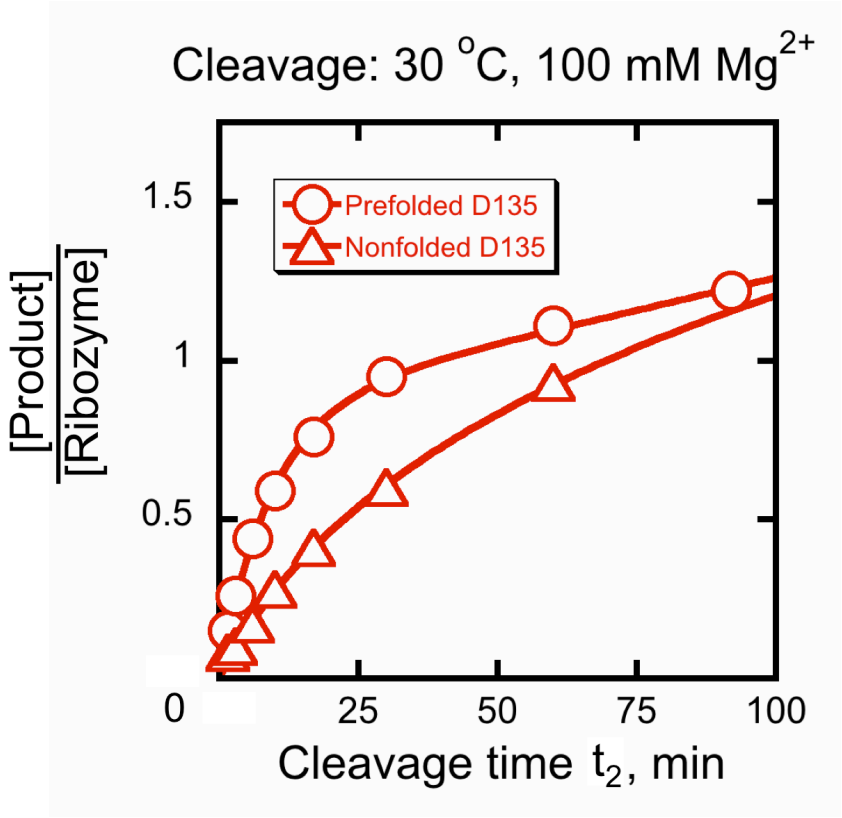

Fig. S4. Testing 30 °C and 100 mM Mg^{2+} for stage 2 in the discontinuous catalytic activity assay. The amount of rapid substrate cleavage from ribozyme transferred directly from buffer solution lacking Mg^{2+} at 30 °C (triangles, the nonfolded reaction) is not much less than that from an equivalent amount of ribozyme that was prefolded (42 °C, 100 mM Mg^{2+}, 20 min) before being transferred to stage 2. A comparison of this plot with Fig. 5b demonstrates that the lower temperature in that experiment for stage 2, 15 °C, is more effective for blocking folding of D135 on the time scale of substrate cleavage by the native ribozyme. Thus, the lower temperature was used for stage 2 in all subsequent experiments.

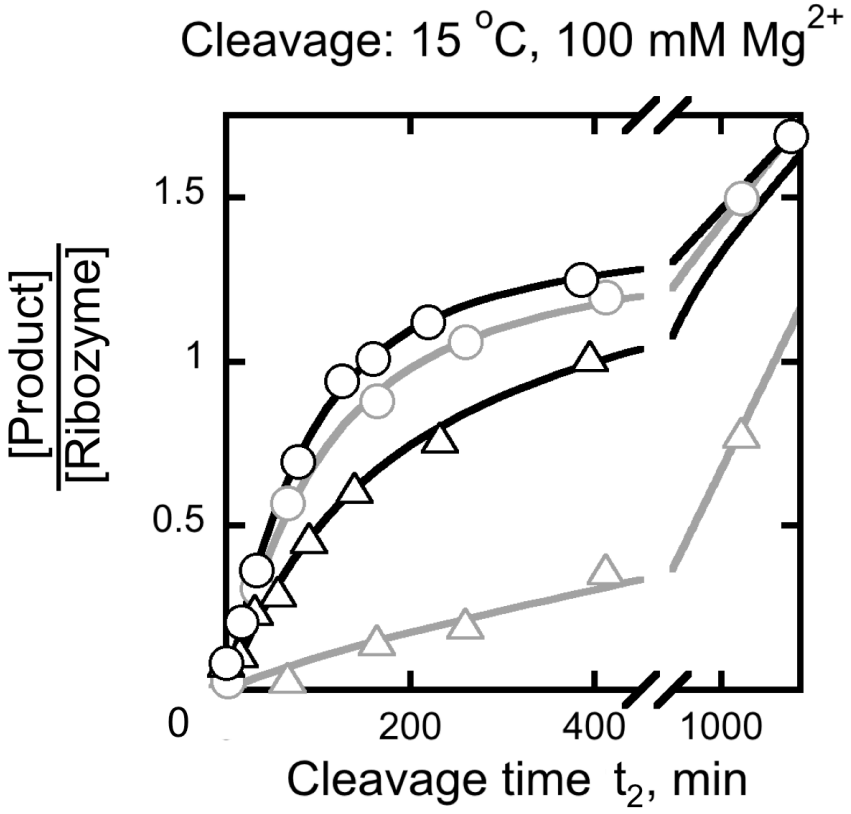

Fig. S5. Prefolded and nonfolded reactions for monitoring D135 RNA folding at 42 °C. Black symbols and curves show reactions that were transferred from 42 °C into standard stage 2 reaction conditions (15 °C, 100 mM Mg^{2+}, 80 mM Na-HEPES, pH 8.1). The circles show the progress of substrate cleavage for a reaction that was first prefolded (42 °C, 100 mM Mg^{2+}, 50 mM Na-MOPS, pH 7.0, 20 min) and then transferred, and the triangles show results from a reaction that was transferred from 42 °C (80 mM Na-HEPES, pH 8.1) to the stage 2 conditions without prefolding. HEPES buffer was used here in analogy to previous work.[49] The gray symbols show analogous reactions that were transferred from 30 °C (50 mM Na-MOPS, pH 7.0, ± 100 mM Mg^{2+}), either with or without prefolding, reprinted from Fig. 5b for comparison. The prefolded reactions are essentially the same, as expected, indicating nearly 100% native ribozyme, but the nonfolded reaction transferred from 42 °C gives substantially more cleavage than the equivalent reaction transferred from 30 °C (black vs gray triangles), despite the essentially identical conditions in stage 2 for the two reactions.

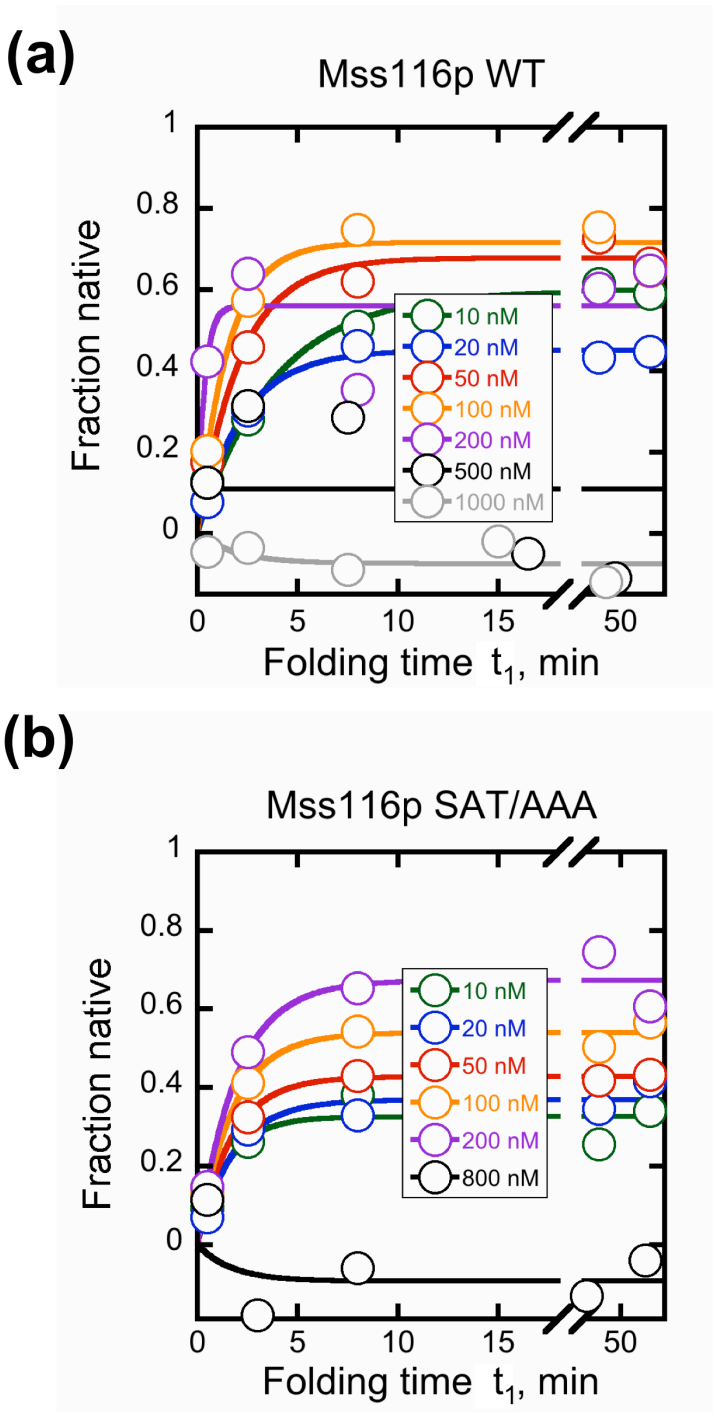

Fig. S6. Progress curves of D135 ribozyme folding (50 nM) in the presence of 1 mM ATP and various concentrations of Mss116p (panel a) or Mss116p SAT/AAA (panel b).

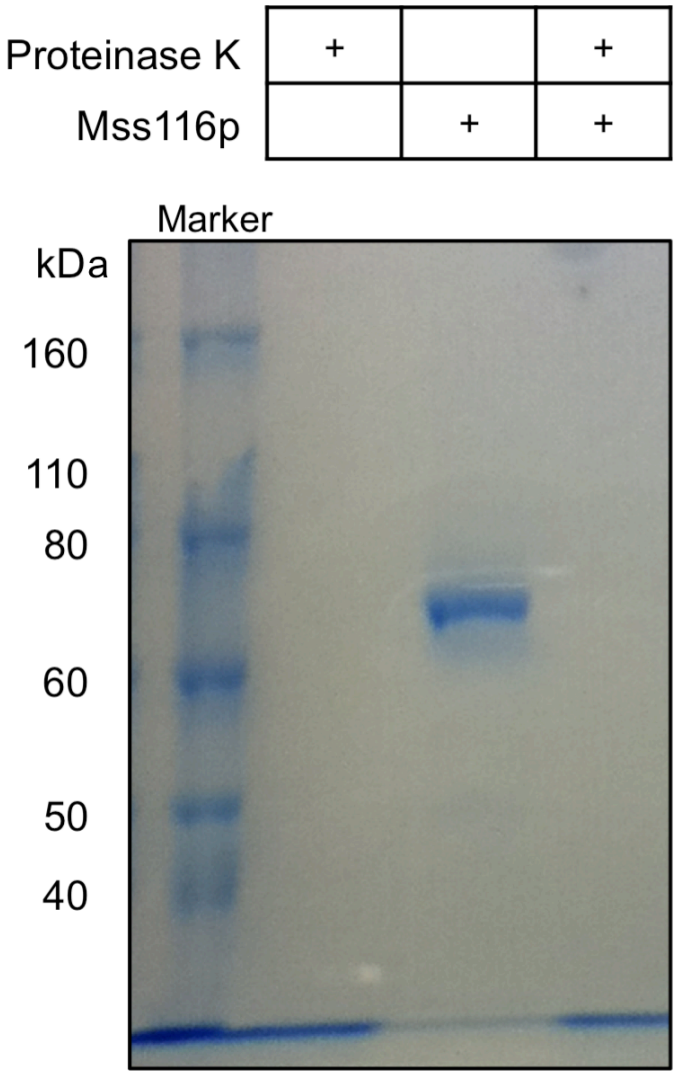

Fig. S7. Proteinase K digestion of Mss116p in the experiment shown in Fig. 7. When proteinase K was added to Mss116p in the stage 1 conditions (30 °C, 50 mM MOPS, pH 7.0, 8 mM Mg^{2+}), Mss116p became undetectable within 30 s (right lane). The second lane from the right shows an equivalent reaction to which proteinase K was omitted, and the third lane from the right shows an equivalent amount of proteinase K alone (at dye front).

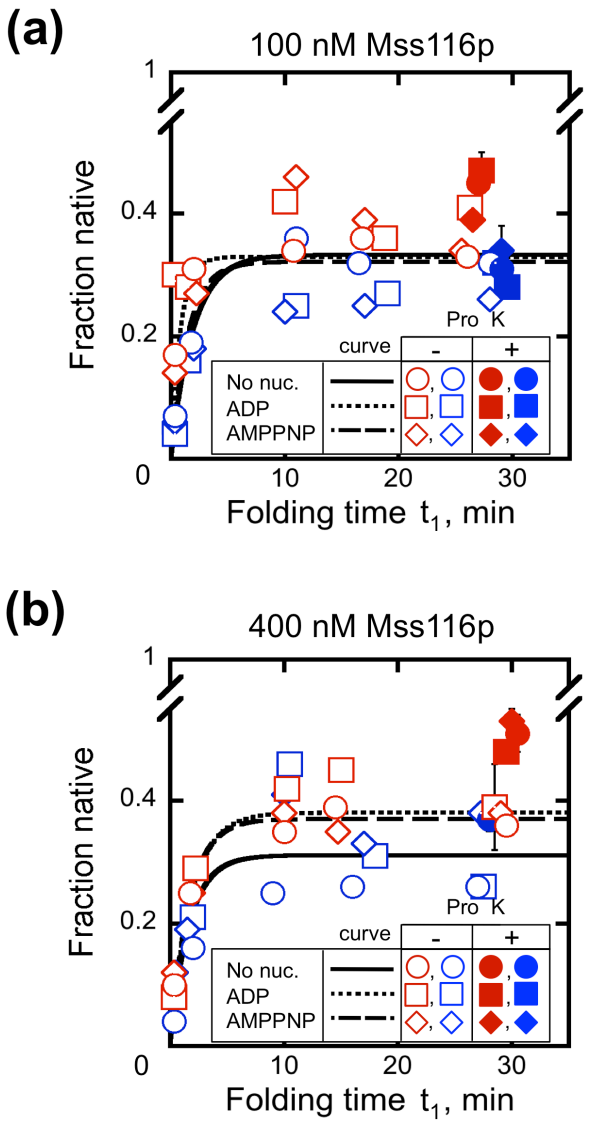

Fig. S8. Proteolysis of Mss116p after incubation with D135 RNA. Mss116p was present at 100 nM (panel a) or 400 nM (panel b). Nucleotides were present, as indicated, at 1 mM. Closed symbols show the fraction of native ribozyme for reactions in which proteinase K (1 mg/ml) added at the indicated time, followed by an additional incubation of 5 – 30 min before transfer to stage 2. The results did not depend on this incubation time, and the average values are shown. SDS (0.5%) was added immediately after proteinase K to ensure that peptide fragments were prevented from interacting with the ribozyme. Open symbols show equivalent reactions to which proteinase K was not added. Reactions in the absence of nucleotide (circles) were performed side-by-side for comparison and gave the same results within error as the equivalent reactions shown in Fig. 7. Results from two independent experiments are shown in red and blue.