SUPPLEMENTARY DATA

Archaeal RNA ligase is a homodimeric protein that catalyzes intramolecular ligation of single stranded RNA and DNA.

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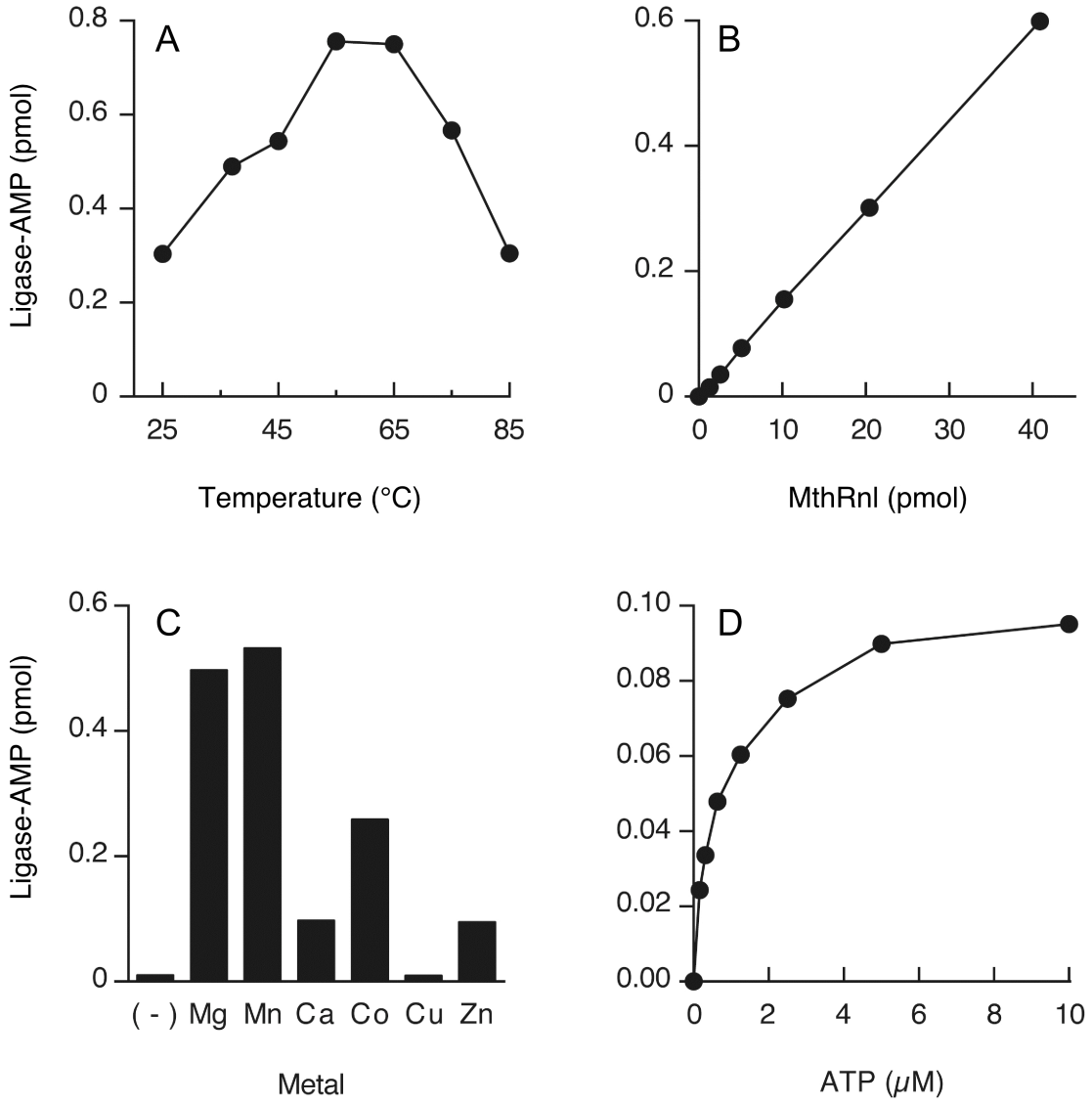

Figure S1. Characterization of ligase-AMP formation. (A) Temperature dependence. Standard adenylyltransferase reaction mixtures contained 40 pmol of MthRnl. Reactions were incubated for 5 min at the indicated temperature. Formation of ligase-AMP is plotted as a function of reaction temperature. (B) Protein titration. Standard adenylyltransferase reaction mixtures contained indicated amount of MthRnl. The extent of ligase-AMP is plotted as a function of input protein. (C) Metal dependence. Standard adenylyltransferase reaction mixtures contained 40 pmol of MthRnl and 2 mM $MgCl_2$, $MnCl_2$, $CaCl_2$, $CoCl_2$, $CuCl_2$ or $ZnCl_2$. Control reaction without metal is shown in lane indicated by (-). (D) ATP dependence. Standard adenylyltransferase mixtures contained 5 pmol of MthRnl with indicated concentration of [α^{32}P]-ATP. The extent of ligase-AMP is plotted as a function of ATP concentration.

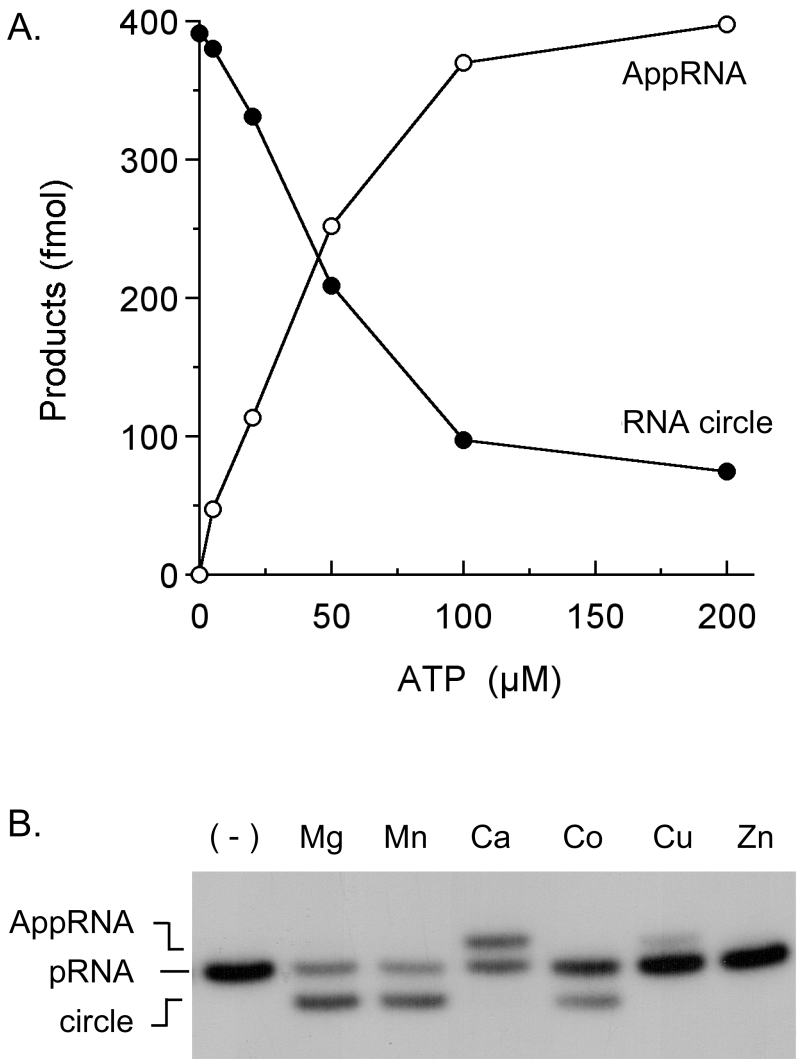

Figure S2. Effects of ATP and divalent cations on ligation. (A) Effect of ATP. Standard ligation assay containing 5 pmol of MthRnl and indicated concentration of ATP were incubated for 30 min at 65°C. The levels of RNA circle and AppRNA are plotted as functions of ATP concentration. (B) Metal specificity. Standard ligation assay contained 5 pmol of MthRnl and 0.5 mM $MgCl_2$, $MnCl_2$, $CaCl_2$, $CoCl_2$, $CuCl_2$ or $ZnCl_2$, were incubated for 30 min at 65°C. Control reaction without metal, is shown in lane indicated by (-). Products were analyzed by denaturing PAGE and visualized by autoradiography.

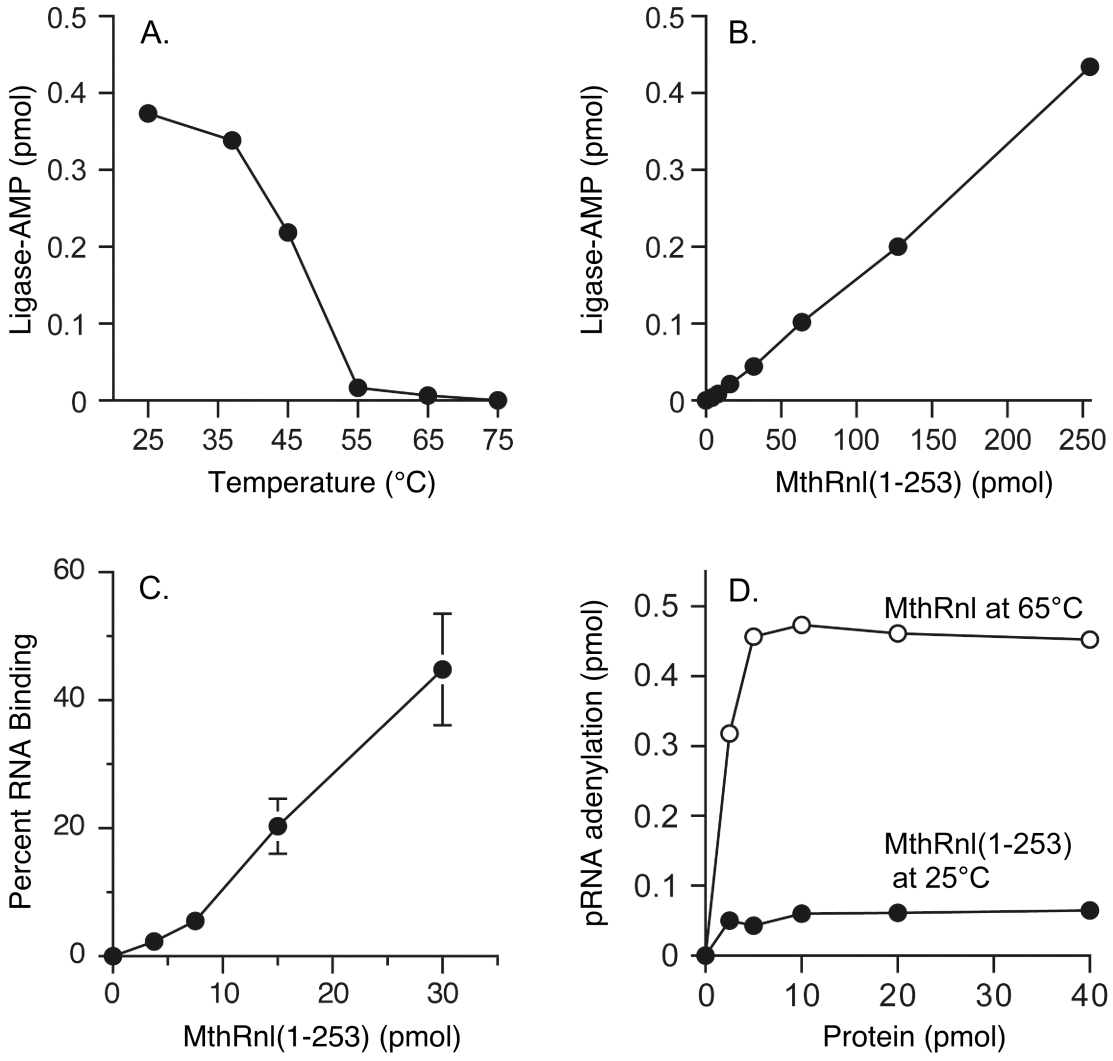

Figure S3. Characterization of MthRnl(1-253) (A) Effect of temperature on EpA formation as described in Fig 5C. The extent of MthRnl(1-253)-AMP was plotted as function of temperature. The data shown represent the average of two separate experiments (B) Protein titration. Standard adenylyltransferase reaction mixtures containing indicated amount of MthRnl(1-253) were incubated at 25°C for 5 min. The extent of MthRnl(1-253)-AMP is plotted as a function of input protein. (C) RNA binding by MthRnl(1-253) as described in Figure 5E. Percentage protein-pRNA complex formed was plotted as a function of input protein. The data shown represent the average of three separate binding experiments. Standard error bars are included for each datum point. (D) Ligation assay. Standard ligation assay was performed with 100 μM of ATP and indicated amount of either MthRnl(1-253) for 30 min at 25°C (closed circle) or full-length MthRnl for 30 min at 65°C (open circle). The levels of AppRNA are plotted as function of protein concentration.