

Supplemental Material

**RtcB, a novel RNA ligase, can catalyze tRNA splicing  
and *HAC1* mRNA splicing *in vivo***

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Supplemental Figures S1 and S2.

## Supplemental Figure 1

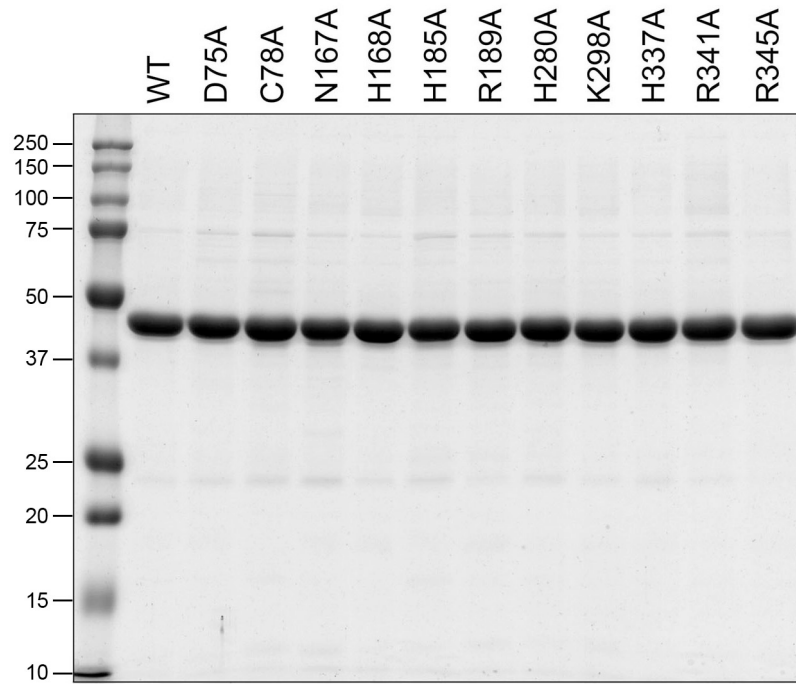


Fig. S1. **Alanine scanning mutagenesis of *E. coli* RtcB.** Aliquots (4.5  $\mu$ g) of recombinant wild-type (WT) RtcB and the indicated RtcB-Ala mutants were analyzed by SDS-PAGE. The Coomassie blue-stained gel is shown. The positions and sizes (kDa) of marker proteins are indicated on the *left*.

## Supplemental Figure 2

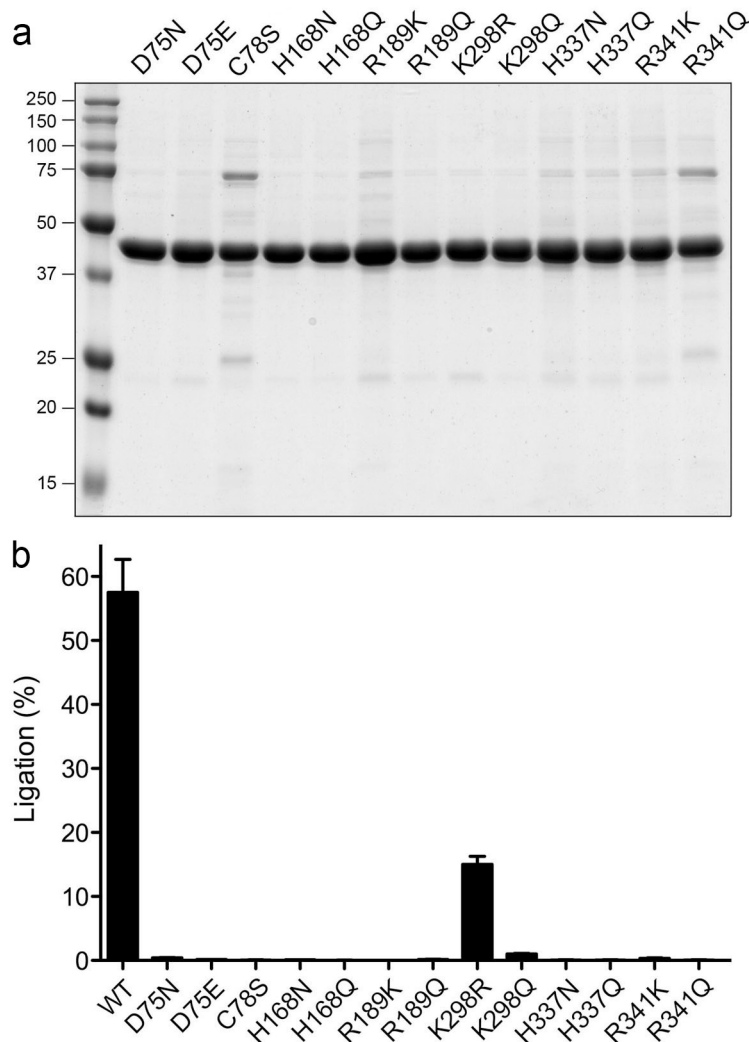


Fig. S2. **Effects of conservative mutations on RtcB activity.** (a) Aliquots (4.5  $\mu$ g) of recombinant RtcB mutants as specified were analyzed by SDS-PAGE. The Coomassie blue-stained gel is shown. The positions and sizes (kDa) of marker proteins are indicated on the *left*. (b) RNA ligase reaction mixtures (10  $\mu$ l) containing 50 mM Tris-HCl (pH 8.0), 2 mM  $MnCl_2$ , 100  $\mu$ M GTP, 100 nM of 5'- $^{32}P$ -labeled broken RNA stem-loop substrate, and 2  $\mu$ M wild-type or mutant RtcB as specified were incubated for 30 min at 37°C. The extents of conversion of the radiolabeled 19-mer substrate strand into sealed 39-mer product (% ligation) are plotted. Each datum is the average of three separate ligation experiments  $\pm$ SEM.