Supplemental Material

A novel mechanism of RNA repair by RtcB via sequential 2',3'-cyclic phosphodiesterase and 3'-phosphate/5'-hydroxyl ligation reactions

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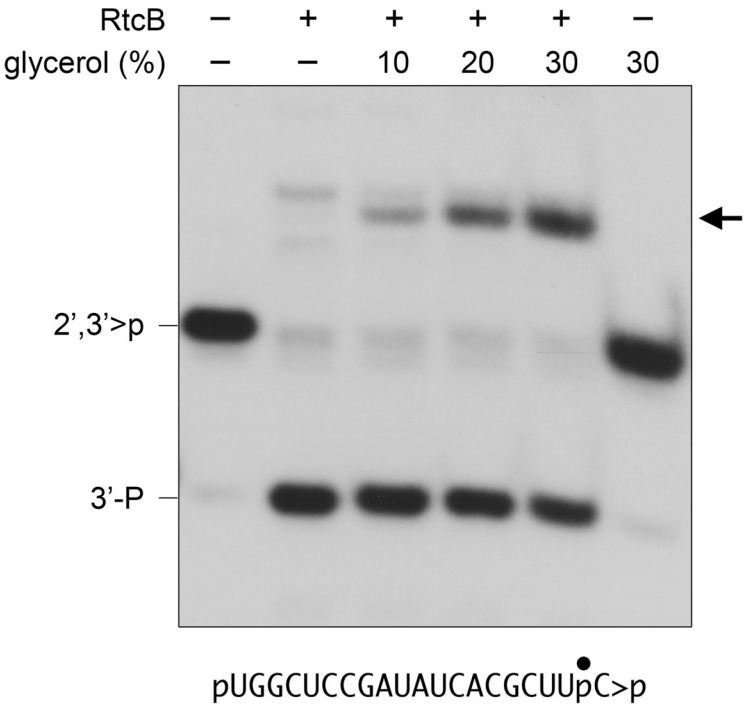

Figure S1. **Glycerol as a nucleophile for the RtcB CPDase reaction**. Reaction mixtures (10 μl) containing 50 mM Tris-HCl (pH 8.0), 0.1 mM GTP, 2 mM $MnCl_2$, 20 nM ^{32}P-labeled 20-mer pRNA>p as shown, and 1 μM RtcB (where indicated by +) were supplemented with 10%, 20%, or 30% (v/v) glycerol. The reaction mixtures were incubated at 37°C for 30 min, then quenched with EDTA. The products were digested with RNase T1 and then resolved by PAGE. A novel radiolabeled T1 fragment (denoted by the arrow at *right*), formation of which was RtcB-dependent and proportional to glycerol concentration, is a putative glycerololysis reaction product, $_{HO}$CUU**p**C$_{3'}$p-glycerol.

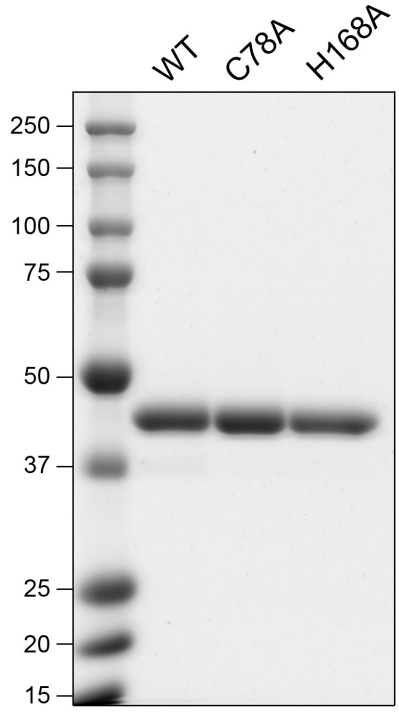

Figure S2. **Purification of wild-type RtcB and mutants C78A and H168A**. Aliquots (4.5 µg) of the Superdex 200 preparations of the indicated RtcB proteins were analyzed by SDS-PAGE. The polypeptides were visualized by staining with Coomassie blue dye. The positions and sizes (kDa) of marker polypeptides are indicated at left.

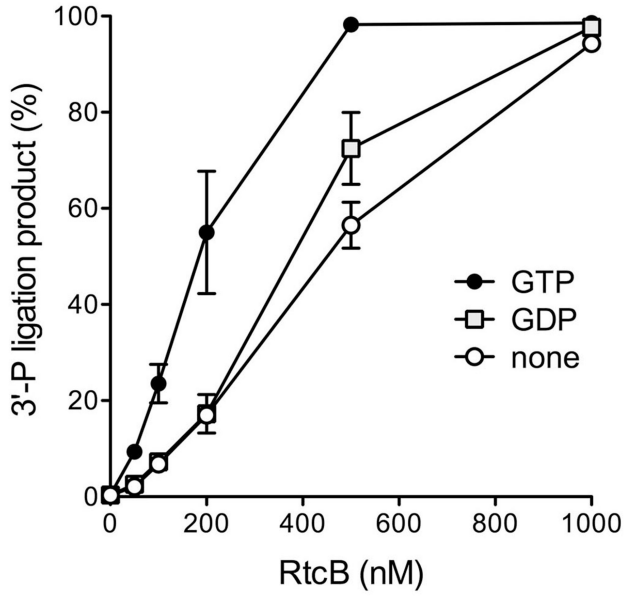

Figure S3. **GTP stimulates 3'-phosphate ligation activity**. Ligase reaction mixtures (10 µl) containing 50 mM Tris-HCl (pH 8.0), 2 mM $MnCl_2$, 20 nM ^{32}P-labeled 20-mer $_{HO}$RNAp, either 0, 50, 100, 200, 500 or 1000 nM RtcB, and either 0.1 mM GTP, 0.1 mM GDP or no added nucleotide (none) were incubated at 37°C for 30 min. The products were digested with RNase T1 and then analyzed by PAGE. The extents of product formation are plotted as a function of enzyme concentration. Each datum is the average of three separate RtcB titration experiments ±SEM.