Supplementary Figure Legends

Figure S1. MBP-dDis3 is active in a range of reducing and pH conditions. *(A)* dDis3 activity in reducing conditions. Ribonuclease activity was assessed in buffer containing 75 mM KCl, 40 µM $MgCl_2$, 10 mM tris, pH 8.0, and the designated 2-mercaptoethanol concentrations. Reactions were stopped and products were analyzed after 60 minutes. *(B)* dDis3 activity in various pH conditions. Reactions here also contained 75 mM KCl, 40 µM $MgCl_2$, and 10 mM tris at the designated pH values. Again, reactions were incubated for 60 minutes.

Figure S2. dDis3 is active in the presence of various monovalent cations. (*A*) Ribonuclease activity of dDis3 in monovalent cation-containing buffer. Assays were performed as described in Materials and Methods. Here, the reaction buffer either contained 10 mM tris, pH 8.0 alone or tris and 75 mM of the monovalent cation indicated. Additionally, 1 mM 2-mercaptoethanol was present in the buffer of one experiment as indicated (labeled K^+ + 2Me). (*B*) Quantification of dDis3 exoribonuclease activity. The amounts of RNA remaining at each time point were quantified by densiometry using ImageQuant software. To obtain % polyU remaining, each lane was normalized to the zero time point lane for that particular reaction; this method of quantification was used for the remaining experiments. The MBP control line (•) represents data averaged for all of the reaction conditions (14 experiments). The MBP control data has also been separated for each reaction condition, which can be seen in Figure S4. The remaining lines on the graph are as follows: Tris ■; KCl ▲; KCl + 2-Me ▼; NaCl ◆; NH_4Cl ○; CsCl △; LiCl □. These represent data averaged for two independent experiments.

Figure S3. dDis3 is activated by divalent cations. (*A*) dDis3 activity in divalent ion-containing buffer. The reaction buffer for the first panel contained 10 mM tris, pH 8.0, 75 mM KCl, 40 µM $MnCl_2$, and 1 mM 2-mercaptonethanol (labeled "Mn^{2+}/K^+"). The reaction buffer for the two remaining experiments contained 10 mM tris, pH 8.0, and 40 µM of either divalent cation, as indicated. (*B*) Quantification of dDis3 activity. % polyU remaining for the MBP control reactions again represents data averaged for all of the reaction conditions shown (6

experiments total). See Supplemental Figure S4 for MBP control data from each individual reaction condition. For MBP-dDis3 reactions, averages of two independent experiments for each reaction condition are shown.

Figure S4. MBP is not active on polyU under any condition. MBP was incubated with 5'-end labeled RNA for 60 minutes in reaction buffer containing 10 mM tris, pH 8.0 with or without monovalent cation or divalent cations added to the reaction buffer, as specified. % polyU was calculated as described in Figure S2. Data shown represents averages of two independent experiments for each condition. (*A*) MBP activity in monovalent cation-containing buffer. Tris ■; KCl ▲; KCl + 2-Me▼; NaCl ◆; NH_4Cl ○; CsCl △; LiCl □. (*B*) MBP activity in divalent ion-containing buffer. $MnCl_2$ + KCl ●; $MnCl_2$ ■ ; $MgCl_2$ ▲.

Figure S5. dDis3 is inactive in the presence of chelating agent. Assays were carried out essentially as described before. Here, however, proteins were incubated with polyU RNA for 10 minutes total. Reactions on the left were incubated in buffer containing 10 mM tris, pH 8.0, 75 mM KCl, and 40 μM $MgCl_2$. Bracket indicates reaction products. Reactions on the right were incubated in buffer containing the same components plus 5 mM EDTA. Images are representative of two independent experiments.