Supporting Information:

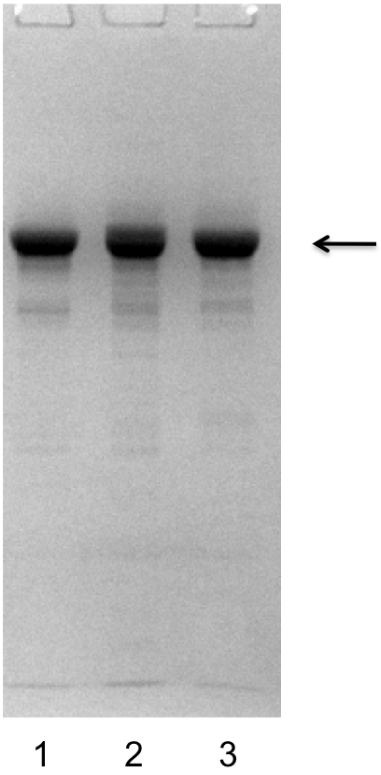

Figure S1. SDS-PAGE analysis of wild-type and his-tagged UTase/UR preparations. A 10% polyacrylamide gel was used, and approximately 2 μg of each protein was loaded. The arrow indicates the position of the UTase/UR band. Lane 1, wild-type enzyme; lane 2, his-tagged enzyme from strain UQ5516; lane 3, his-tagged enzyme from strain SA1.

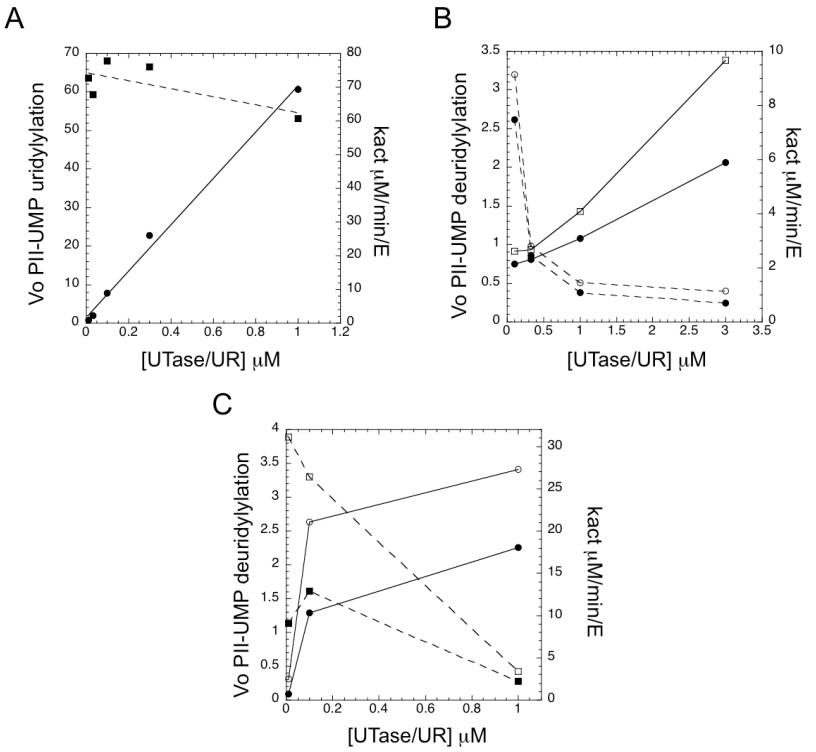

Figure S2. Characterization of the UT and UR activity assays. A. Characterization of the UT activity. Activity was measured as in Materials and Methods, with PII at 36 mM and enzyme concentration varied as indicated. ● and solid line, rate of PII uridylylation; ■ and dotted line, *kcat*. Note that *kact* was relatively independent of enzyme concentration. **B. Characterization of the UR activity using the standard protocol.** Activity was measured as in Materials and Methods with AMP-PNP in place of ATP, and reactions initially contained PII-UMP at 14.9 μM. Glutamine was present at 10 mM where indicated. ● and solid line, rate of PII-UMP deuridylylation in the absence of glutamine; ○, rate of PII-UMP deuridylylation in the presence of glutamine; ■ and dotted line, *kcat* in the absence of glutamine; □ and dotted line, *kcat* in the presence of glutamine. Clearly the *kact* was dependent on the enzyme concentration. C. Characterization of the UR activity using the TLC-based protocol. Activity was measured as in Materials and Methods; reaction mixtures initially contained 11.6 mM PII-UMP, and contained AMP-PNP at 0.5 mM in place of ATP. Glutamine was 10 mM when present. ● and solid line, rate of PII-UMP deuridylylation in

the absence of glutamine; ○, rate of PII-UMP deuridylylation in the presence of glutamine; ■ and dotted line, *kcat* in the absence of glutamine; □ and dotted line, *kcat* in the presence of glutamine. Using this assay method, *kcat* were fairly similar when enzyme was at 0.01 μM and 0.1 μM, but was dramatically lower when enzyme was at 1 μM.

Figure S3. Autoradiograph of a typical thin-layer chromatography separation used to quantify UMP produced in the UR activity assay. The autoradiograph is aligned with a copy of the PEI sheet, showing the pencil markings of the origin and nucleotide spots, as described in Materials and Methods. The positions of UTP, UDP, and UMP are shown with brackets. The samples on each end contained only PII-UMP (contained no enzyme); as shown the PII-UMP preparation had slight UTP contamination, which was well separated from UMP.

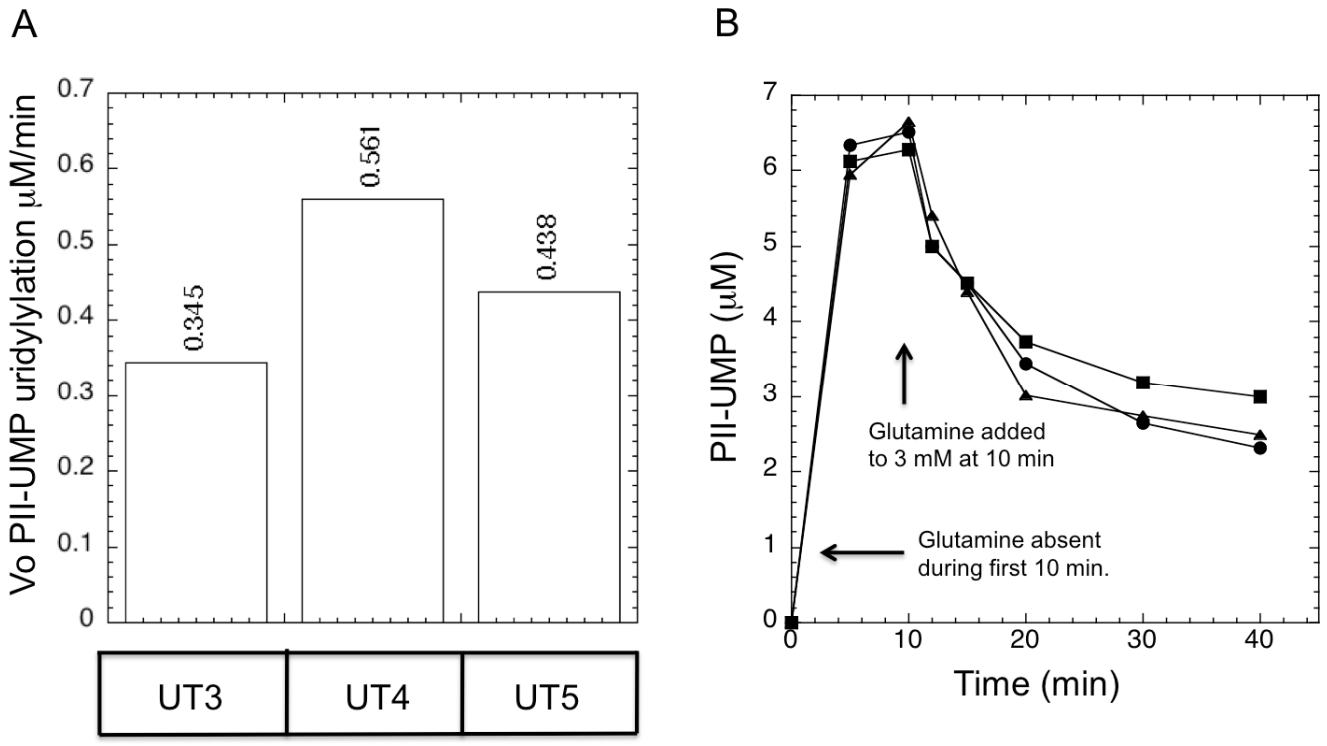

Figure S4. Comparisons of three different preparations of wild-type UTase/UR. Three different preparations of the wild-type enzyme, prepared over a two year period, were compared in side by side experiments to assess reproducibility of purification results. **A. Comparison of UTase/UR activity in the presence of glutamine.** Since the his-tagged enzyme preparation were defecting in glutamine inhibition of the UT activity, we characterized UT activity of three different preparation of the wild-type enzyme in the presence of 10 mM glutamine. Assays were performed as in Materials and Methods and contained 36 μM PII and 0.5 μM enzyme. As shown, the three enzyme preparation behaved quite similarly. **B. Comparison of the behavior of reconstituted covalent modification cycles containing three different preparations of wild-type UTase/UR.** The experiment is identical to that shown in Fig 1C of the main text, and reaction mixtures contained 0.2 μM enzyme and initially contained 2.26 μM PII-UMP. Glutamine was added at 10

min, as indicated with an arrow. As shown, the response to addition of glutamine was similar for all three preparations of the wild-type enzyme.

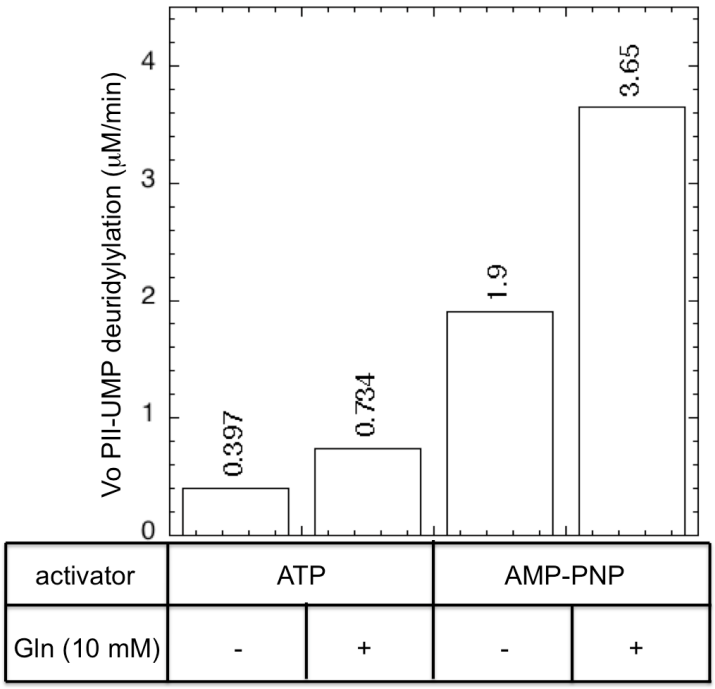

Figure S5. Effect of AMP-PNP on the rate of the UR reaction. UR assays were conducted using the TLC-based protocol (Materials and Methods); reactions contained 0.1 mM wild-type enzyme and PII-UMP was initially present at 10.33 mM. Glutamine when present was at 10 mM; ATP and AMP-PNP were present as indicated at 0.5 mM. As shown, faster rates of PII deuridylylation were obtained in the presence of AMP-PNP.

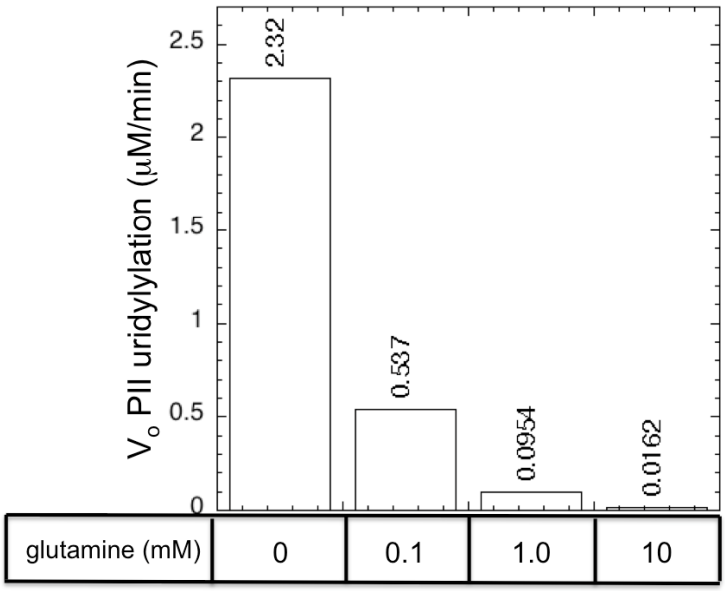

Figure S6. Glutamine inhibition of the UT activity in the presence of AMP-PNP. UT activity was measured as in Materials and Methods in reaction mixtures that contained 0.5 mM AMP-PNP in place of ATP, 5 μM PII and 0.05 μM wild-type enzyme.

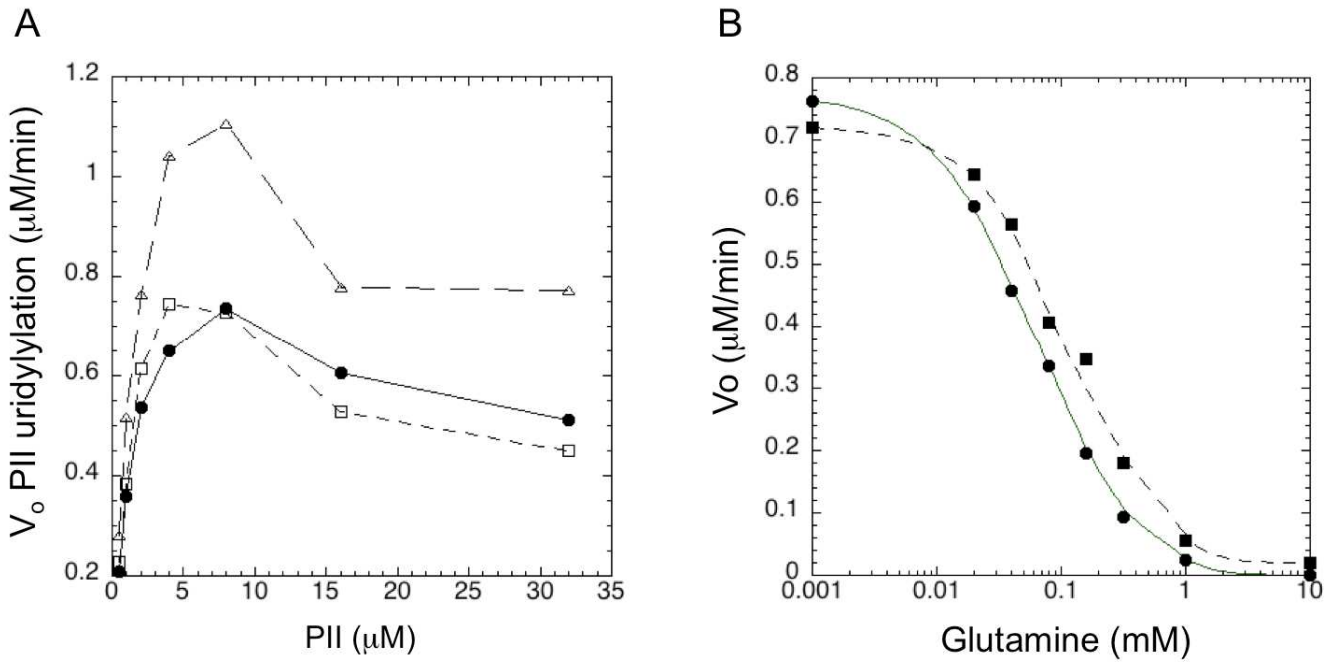

Figure S7. Kinetic parameters of wild-type and his-tagged enzymes. A. Determination of Km and Substrate inhibition of the UT activity. The initial rate of PII uridylylation was determined as in Materials and Methods, with enzyme at 0.01 μM. Solid line and ●; wild-type UTase/UR; dotted line and □, SA1; dotted line and △, UQ5516. Using the ascending phase of the curves, the Km for the wild-type and UQ5516 enzymes was estimated at ~ 1 mM PII, while the Km for the SA1 enzyme was estimated at ~ 1.2 mM. **B. Determination of glutamine inhibition constant.** The initial rate of PII uridylylation was determined in reaction mixtures that contained PII at 2 μM, enzyme at 0.01 μM, and glutamine as indicated. The glutamine inhibition constant for the wild-type enzyme was 0.06 mM; the inhibition constant for the UQ5516 enzyme was 0.14 mM. In a separate experiment, the glutamine Ki for the his-tagged enzyme preparation from strain SA1 was observed to be 0.15 mM (not depicted).

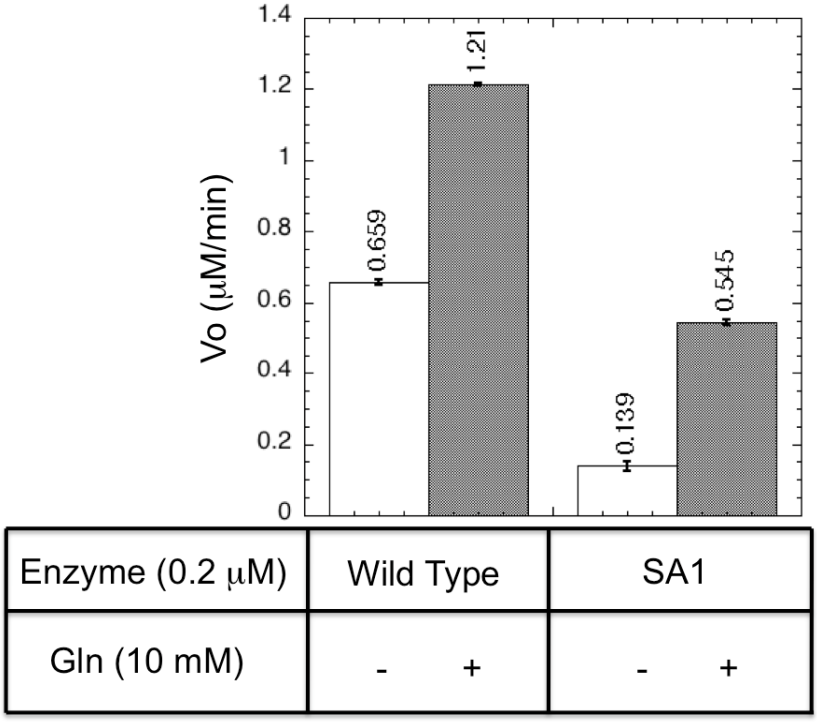

Figure S8. UR activity and its stimulation by glutamine in reaction mixtures containing AMP-PNP in place of ATP. UR activity was measured by the standard protocol as described in Materials and Methods; reaction mixtures contained 0.2 μM enzyme and initially contained 6 μM PII-UMP. Assays were performed side-by-side on two consecutive days, error bars indicate the standard deviation from the two experiments.