SUPPLEMENTAL MATERIAL

Figure S1. Homology model of Dhr1. Amino acids 375 to 1189 of Dhr1 were modeled on the structure of Prp43 (pdb 3KX2) using Phyre2 (Kelley, L.A., S. Mezulis, C.M. Yates, M.N. Wass, and M.J. Sternberg (2015) *Nat Protoc* **10**, 845-858). The structure of residues 1 to 374 and 1190 to 1267 is unknown. The model of Dhr1 contains two RecA-like domains (RecA1 and RecA2), a feature shared by DEAD-box and viral DExH helicases. The two RecA-like domains are involved in ATP binding and hydrolysis and duplex binding. RecA2 also contains a β-hairpin. Downstream of the RecA-like domains there is winged-helix domain (WHD) followed by a rachet domain, similar to the processive DNA helicase Ski2-like Hel308. The C-terminal region of Dhr1 contains a oligonucleotide/oligosaccharide-binding (OB) motif.

Figure S2. Expression level of Dhr1 and Utp14 two-hybrid proteins. (A) The protein level of Dhr1 truncated mutants. Gal4BD or Gal4AD fusions of either full-length DHR1 or truncated mutants were transformed in PJ69-4α. Cells were lysed with NaOH (41). The extract was separated with 8% SDS-PAGE, and subjected to western blotting using anti-myc or anti-HA antibodies. (B) The expression level of Utp14 truncated mutants. Gal4AD fusions of full-length UTP14 or truncated mutants were transformed in PJ69-4α. Cells were lysed with NaOH, the extracts were separated with 8% SDS-PAGE and subjected to western blotting. Protein was detected using anti-HA antibody.

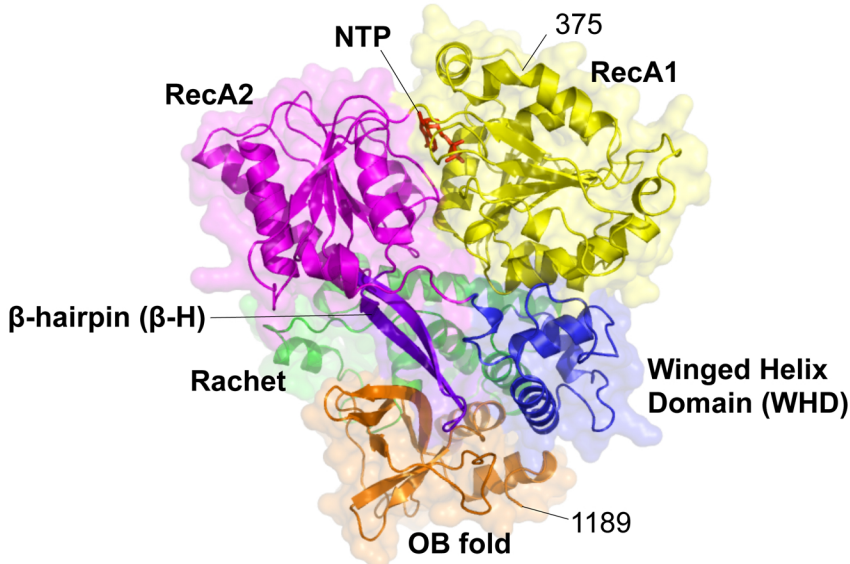
Figure S3. Complementation assay of truncated mutants of *DHR1* and *UTP14*. (A) Complementation of *DHR1* truncated mutants. Empty vector, full-length DHR1 or

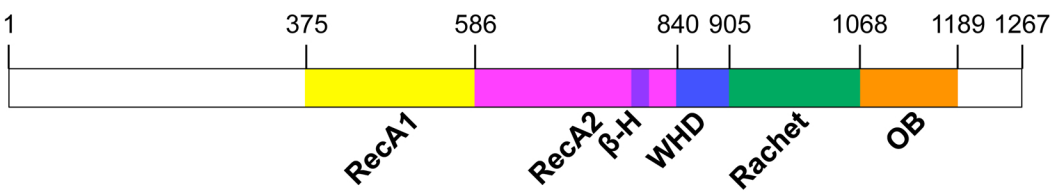
truncated mutants were transformed into P_{Gal1}-*DHR1* strain (AJY3711) and grown on SD Ura- glucose medium and SD Ura- galactose for 4 days at 30°C. (B) Complementation of *UTP14* truncated mutants. Empty vector, full-length *UTP14* or truncated mutants were transformed into P_{Gal1}-*UTP14* strain (AJY3243), grown on SD Ura- glucose medium and SD Ura- galactose for 4 days at 30°C.

Figure S4. 6xHis-*UTP14* complements the function of *UTP14*. Empty vector (pRS416), untagged *UTP14* (pAJ1919) and 6xHis-*UTP14* (pAJ3331) were transformed in P_{Gal1}-*UTP14* strain (AJY3243) and grown on SD Ura- glucose medium and SD Ura- galactose for 3 days at 30°C.

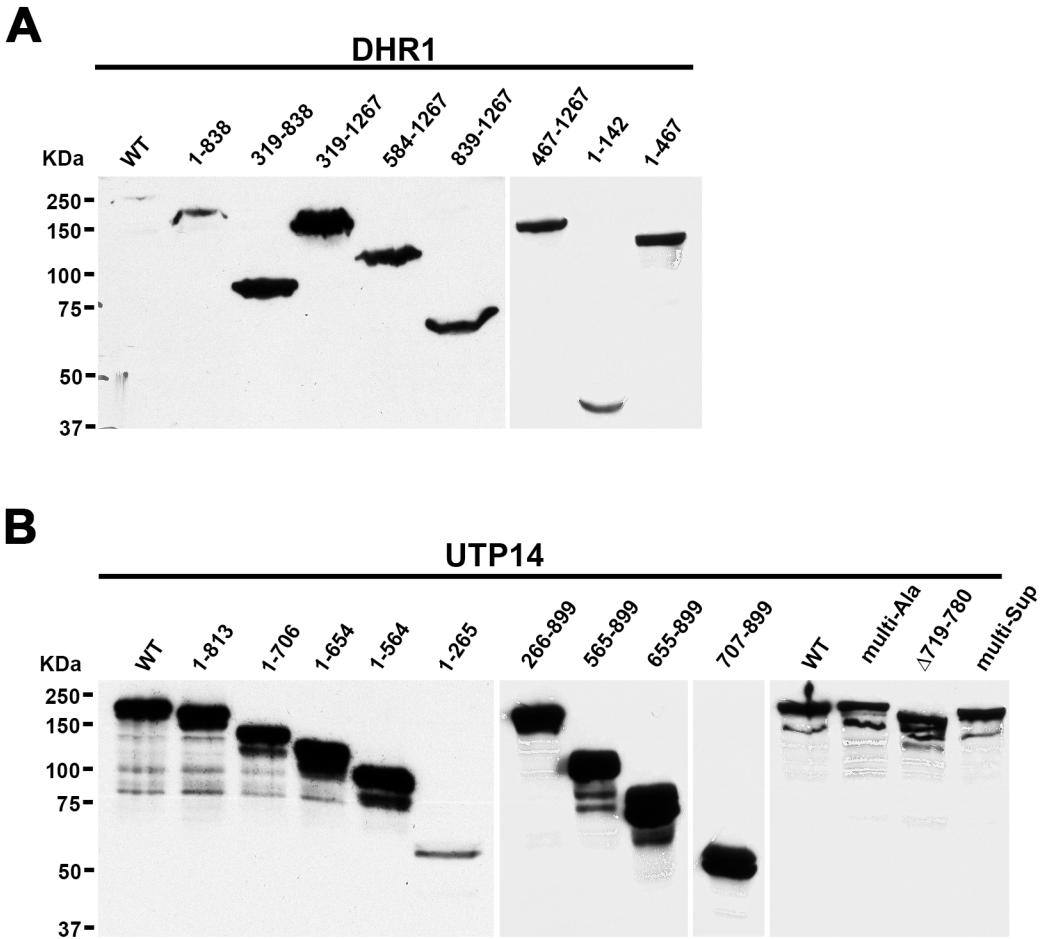
Figure S5. Initial velocities of P_i released after addition of 1 mM ATP at room temperature in the presence or absence of the poly(A) added to either Dhr1, Dhr1 with Utp14, Utp14, or mock with other reagents described in Materials and Methods. In the activity units of $min^{-1}protein^{-1}$, protein refers to either Dhr1, Utp14 or the complex of the two. Protein concentrations were 50 nM Dhr1 in the presence or absence of 200 nM Utp14.

Dhr1 homology model

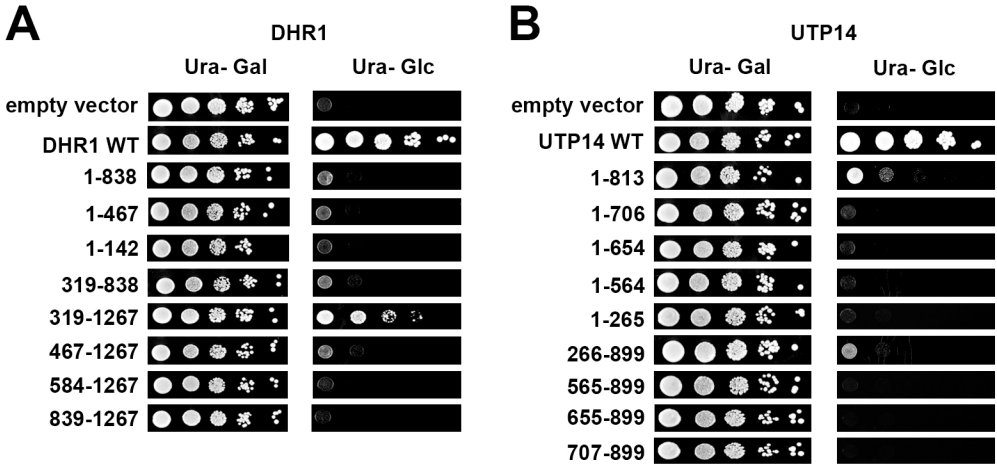




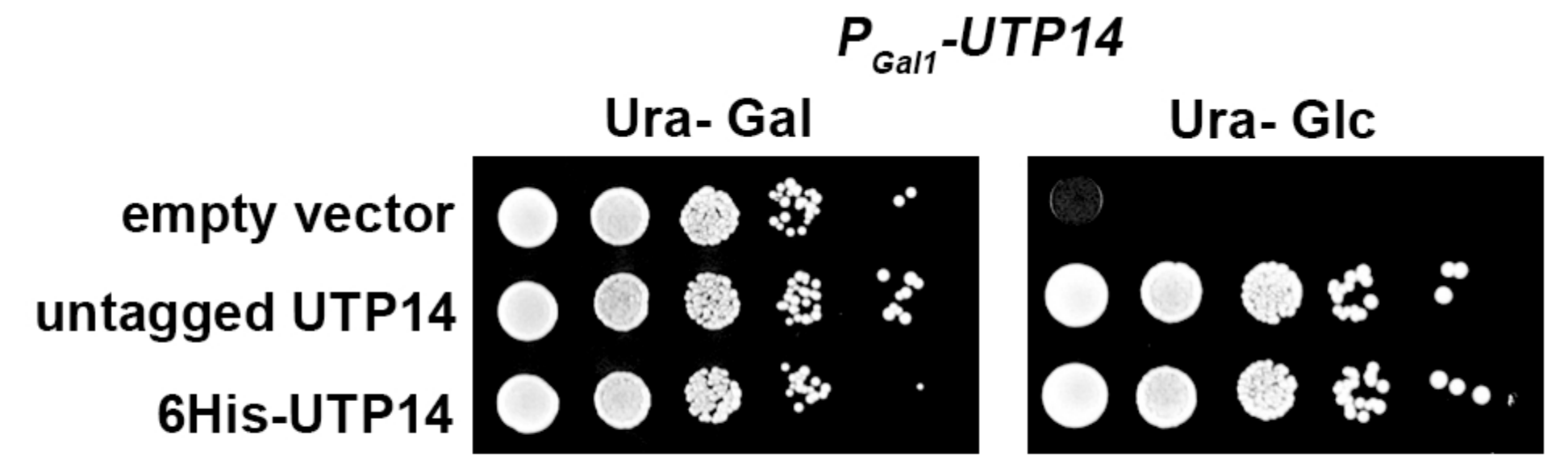
Supplemental Figure S1, Zhu et al



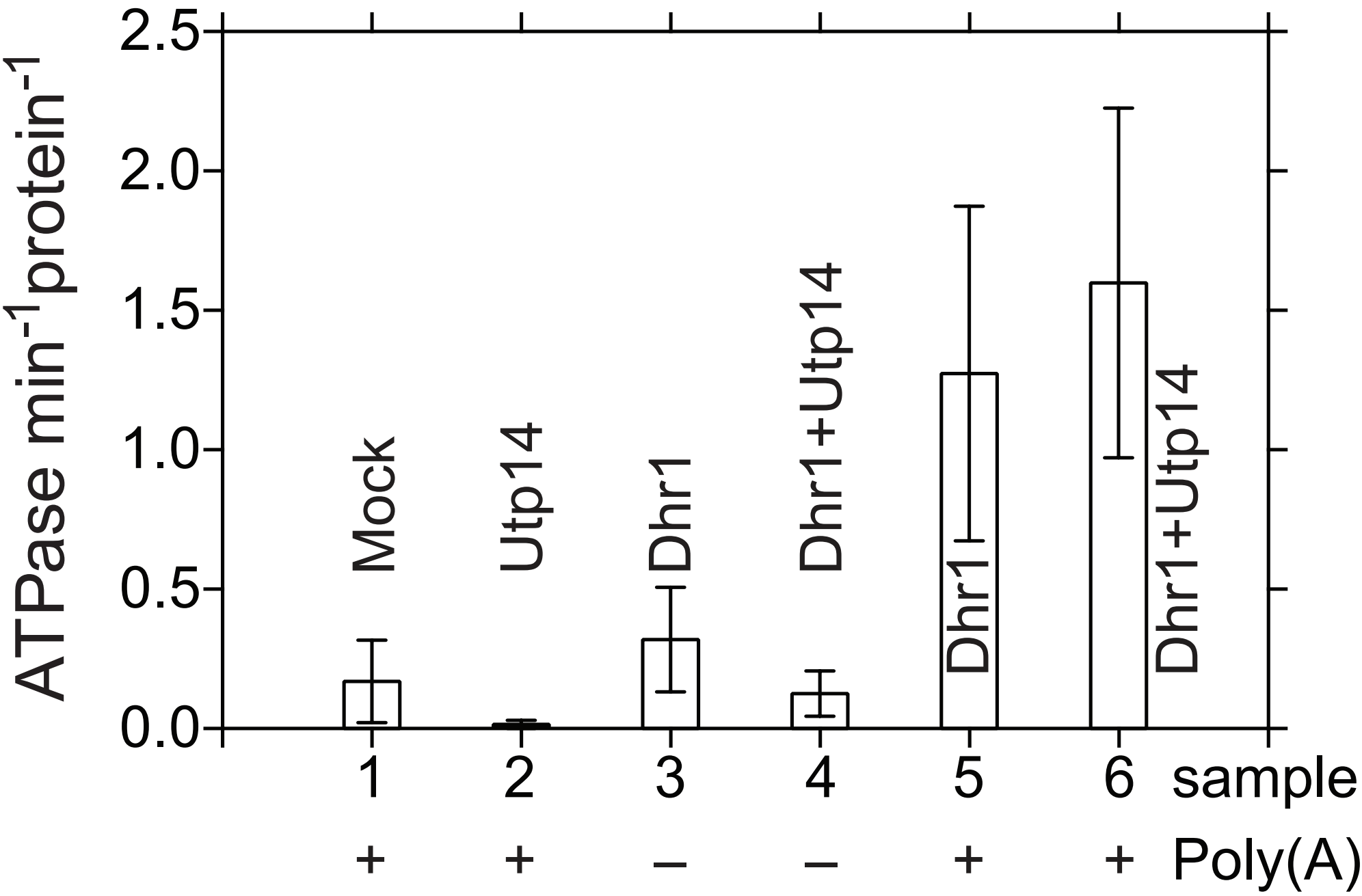
Supplemental Figure S2, Zhu et al



Supplemental Figure S3 Zhu et al



Supplemental Figure S4, Zhu et al



Supplemental Figure S5, Zhu et al