



**Figure S2. The RTA variant preS215F is not a substrate for Png1.** (A) The viability of BY4743 and *png1*Δ expressing preS215F. A series of 10-fold dilutions were spotted on glucose and galactose plates after overnight growth in glucose. (B) Immunoblot analysis of BY4743 and *png1*Δ expressing preS215F. The membrane fraction (M) and cytosol fraction (C) isolated at 6 and 24 hpi were separated on a 10% SDS-polyacrylamide gel and probed with monoclonal anti-RTA (1:5000). The ER membrane marker Dpm1p and cytosolic marker Pgk1p were used as loading controls. (C) Ribosome depurination by preS215F expressed in BY4743 and *png1*Δ transformed with preS215F by qRT-PCR at 2, 4, and 6 hpi.