

Figure S4. Expression of Png1 in *png1* Δ **expressing preRTA.** (A) Analysis of viability of *png1* Δ and BY4743 co-expressing preRTA and *PNG1* driven by the constitutive *GPD1* promoter. A series of 10-fold dilutions were spotted on a glucose plate at 0 and 24 h post induction in galactose media. The CFU/ml was calculated based on the analysis of at least three different transformants. (B) Immunoblot analysis of *png1* Δ and BY4743 co-expressing preRTA and *PNG1* or harboring the *PNG1* vector. Total protein isolated at 6 hpi was separated on a 10% SDS-polyacrylamide gel and probed with monoclonal anti-RTA (1:5000). The blot was reprobed with anti-HA (1:1000) to detect the expression of C-terminal HA tagged Png1. The ER membrane marker Dpm1p and cytosolic marker Pgk1p were used as loading controls. (C) Ribosome depurination by preRTA in *png1* Δ and BY4743 transformed with *PNG1* was analyzed by qRT-PCR at 2, 4 and 6 hpi.