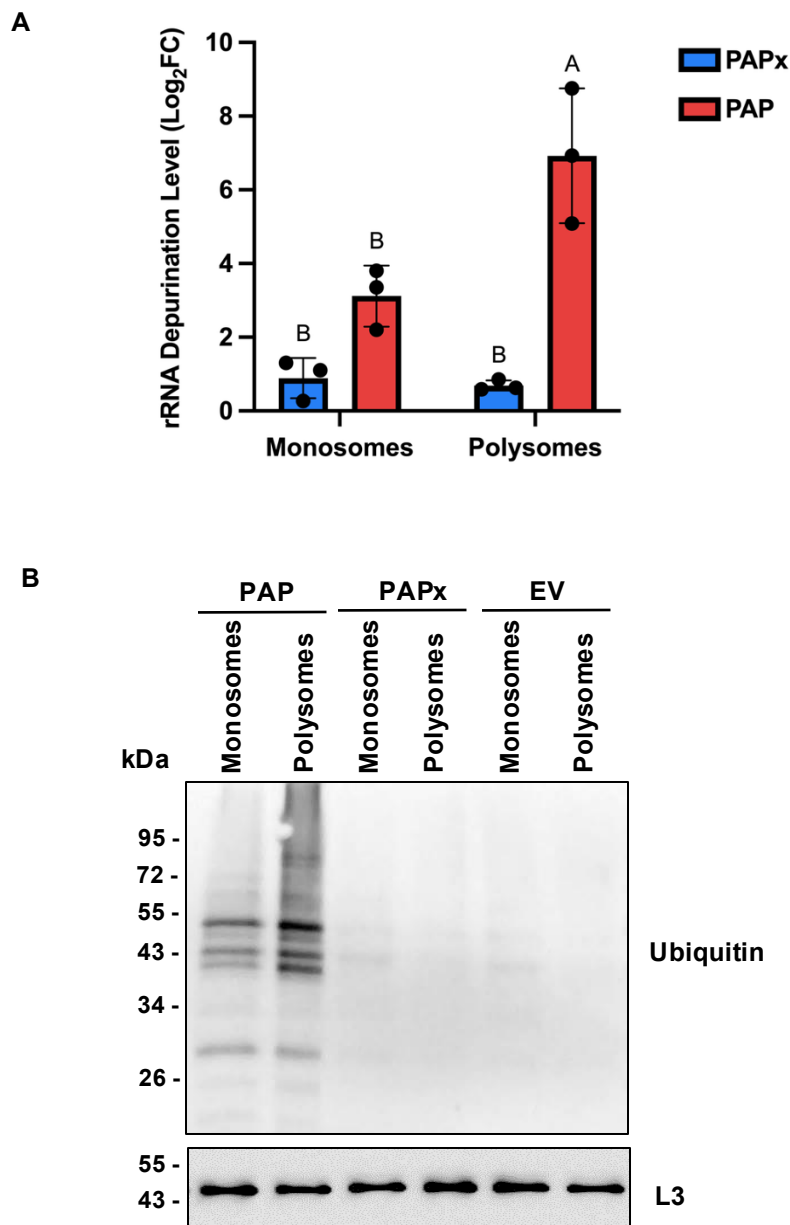




Supplemental Figure S3. rRNA depurination correlates with ubiquitination of ribosomes. (A) Total RNA (500 ng) was isolated from monosome and pooled polysome fractions from WT BY4741 cells induced to express PAP, PAPx or EV for 6 hours. RT-qPCR was performed to measure “reference” and “target” cDNA levels as illustrated in Supplemental Figure S1. Fold changes for PAP and PAPx are shown relative to EV. Statistical significance of differences in level of rRNA depurination was determined by two-way ANOVA with Tukey’s multiple comparisons test. Bars represent means $\pm$ SEM of three independent biological replicates (n=3). The different letters on bars represent statistically significant differences at $p < 0.05$. (B) Monosomes and pooled polysome fractions from the same samples were analyzed for ubiquitination by immunoblotting. Equal amounts of proteins (20 μ g) were separated through 12% SDS PAGE, transferred to nitrocellulose and probed with a polyclonal ubiquitin antibody (1:1000). A monoclonal antibody against ribosomal protein L3 (43 kDa; 1:5000) was used as a loading control. The blots shown are representative of three independent biological replicates (n=3).