Elevated temperatures or inclusion of the arginine analog canavanine can stress the proteolytic system in yeast by increasing the level of unfolded or aberrantly formed proteins. For example, loss of the 20S assembly chaperone Ump1 caused slow growth at 37° (row 5, YPAD 37°). While neither elevated temperature nor canavanine alone caused a noticeable defect in growth for a *blm10*-Δ mutant, growth on a low level of canavanine at 37° was significantly impaired (compare *blm10*-Δ with WT on the 1.5 μg/ml canavanine plate incubated at 37°). Further, combining both *blm10*-Δ and *ump1*-Δ deletions caused an enhanced growth defect relative to the *ump1*-Δ strain on YPAD at 37°. These observations demonstrate that cells lacking Blm10 have impaired ability to respond to proteolytic stress, possibly due to inadequate proteasome assembly.

It was recently reported that combining *blm10*-Δ with a deletion of the C-terminal 19 residues of the 20S subunit Pre4 (β7) caused strong temperature sensitivity (Marques et al., 2007). We have been unable to reproduce this result using strains in the A364a background, as single and double mutants each grew at equivalent rates at 37° (rows 3 and 4 in the figure) or at 38° (not shown), the maximal permissive temperature for this strain background. To determine whether this difference is due to the different strain backgrounds used, we obtained the strains used by Marques et al. (2007) in the JD47-13c background. After switching the mating type of one strain we performed a genetic cross to generate double *blm10*-Δ *pre4*-Δ*CT* mutants by segregation, instead of the procedure described previously that involved sequential integration of mutations (Marques et al., 2007). Once again, none of the double mutants isolated from the cross displayed temperature sensitivity. Because the *pre4*-Δ*CT* allele used by Marques et al. was not marked, we scored it using a PCR test and verified a subset of the results by DNA sequencing. To further confirm this result, we introduced a similar *pre4*-Δ*CT* mutation into JD47-13c but this time with the *URA3* gene inserted adjacent to the deletion. This allowed a much larger number of double mutant *blm10*-Δ *pre4*-Δ*CT* segregants to be identified and tested, but all of these also proved to be temperature resistant. We were therefore unable to observe a synthetic growth defect or temperature sensitivity for *blm10*-Δ *pre4*-Δ*CT* combinations in either of two genetic strain backgrounds. The pre4-ΔCT strains we constructed in the A364a background do display resistance to 4-nitroquinoline 1-oxide, a phenotype associated with several proteasome assembly defects (Le Tallec et al., 2007), consistent with suboptimal proteasome formation. However, this phenotype is also unaffected by loss of Blm10 (compare rows 3 and 4).