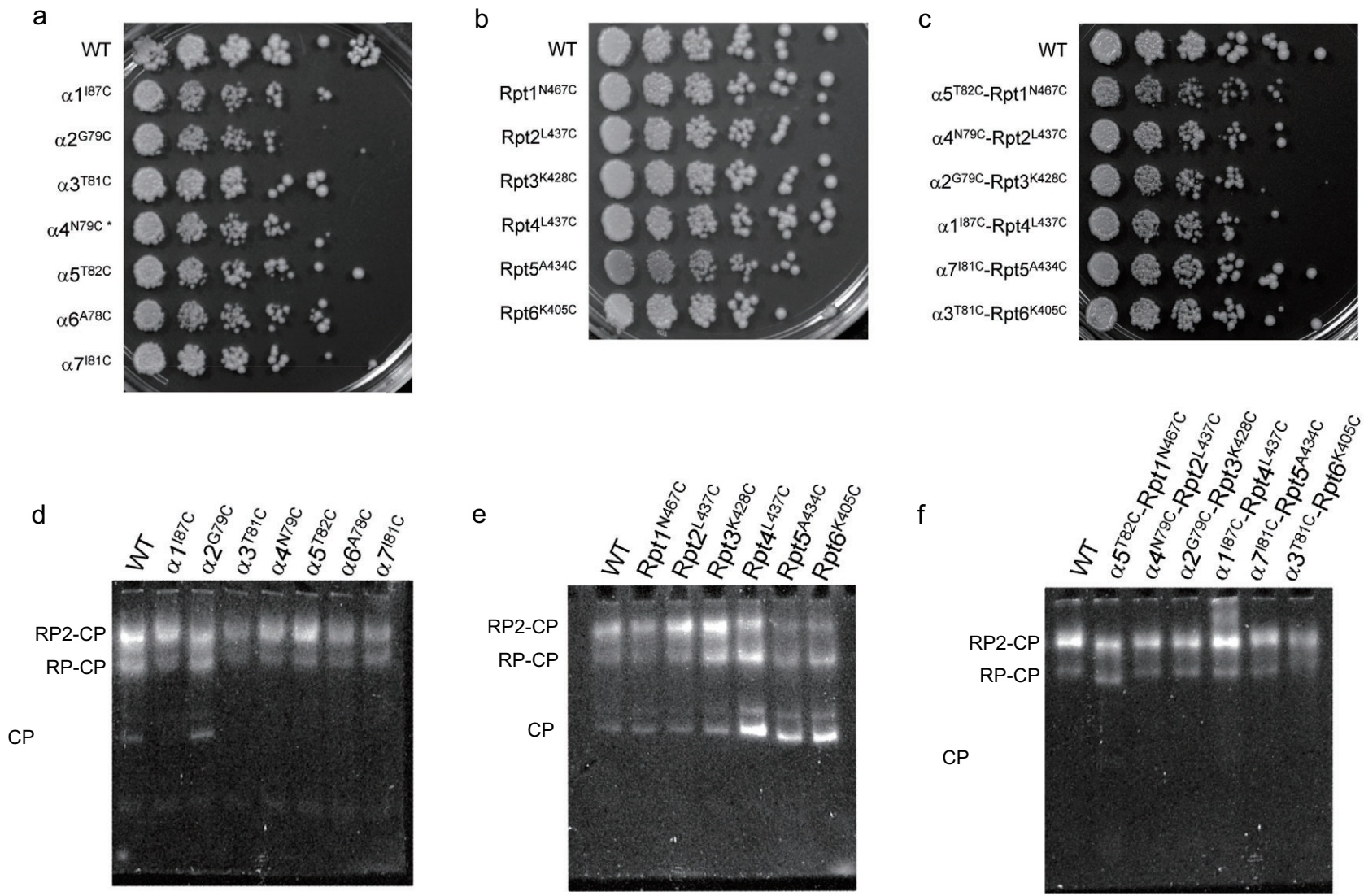

Supplementary figure 2. Evaluation of the effects of introduced cysteines on growth rate and on structural stability of the proteasome. Growth assays of wild type and mutant strains bearing introduced cysteines in α subunits (**a**), Rpt proteins (**b**), and six identified Rpt–α pairs (**c**). Spot assays of cell growth were carried out with 0.05 OD_{600} of cells grown in YPD, resuspended in 250 µl of H_2O, and serially diluted in five-fold increments. The cells were then spotted on YPD plates followed by incubation at 30°C for 48 hr. Note that the RPN11–TevProA allele was not present in these strains. The α4N79C mutant used in all these studies is produced from a strain expressing α4C32A C46A (TG644), which shows the same growth phenotype as wild type under various conditions (Fig. S2 and data not shown). (**d–f**) Total cell lysates of the same set of strains were analyzed by native PAGE followed by overlay assay with LLVY–AMC. Early stationary phase cells were lysed by grinding under liquid N_2. 50 µg of protein were loaded for each strain. Several of the strains are mildly hypomorphic, which is most readily indicated by the enhanced levels of CP in extracts of Rpt4-Rpt6 (ref. 1).