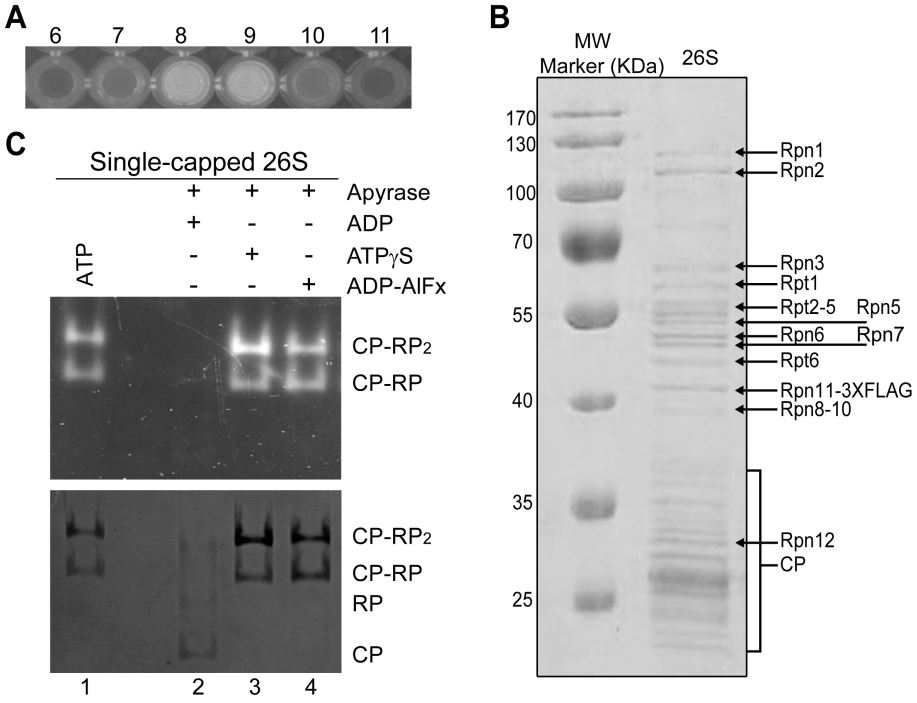

Supplementary information, Figure S1 Biochemical analyses of the yeast proteasome. (**A)** Rapid determination of the fractions containing active proteasome utilizing an activity assay conducted in a 96-well plate. Fractions 8 and 9 after glycerol density gradient centrifugation displayed fluorescence, ideal for cryo-EM sample preparation. (**B)** Identification of proteasome subunits by SDS-PAGE, followed by coomassie brilliant blue staining. All of the proteasome subunits were identified in the assay. **(C)** Verification of proteasome functionality in different nucleotide conditions. Purified 26S proteasome in the presence of ATP (originally presented in the purification buffer, lane 1), or in the presence of ATP analogues including ADP, ATPγS, and ADP-AlFx, respectively (lane 2-4). Samples were subjected to native PAGE and assayed for proteasome activity. The slower migration rate in the gel for ATP state (lane 1) may be due to the higher concentration of glycerol inherited from glycerol density gradient centrifugation (without buffer exchange that performed for the other samples). The weak intensity for RP-CP in lane 2 indicates ADP to be a poor stabilizer of the association of 19S with 20S. The existing of the double- and single-capped proteasome in both the ATPγS and the ADP-AlFx states indicates that nucleotide remains bound in the relevant pocket in these two states.