SUPPLEMENTARY INFORMATION

Supplementary Figures 1 – 4

Supplementary Movie 1

Top view of morph between WT and L81Y density-modified maps

Supplementary Movie 2

Side view of morph between WT and L81Y density-modified maps

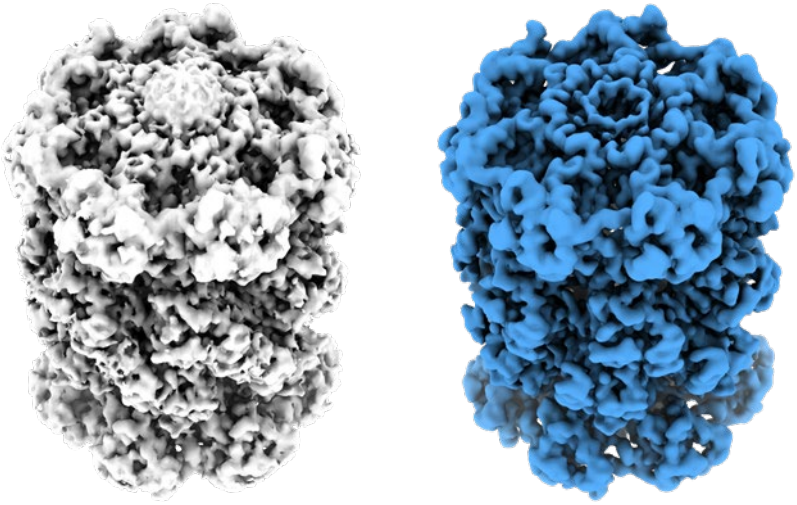
Supplementary Movie 3

Tilted view of morph between WT and L81Y unsharpened maps

Supplementary Movie 4

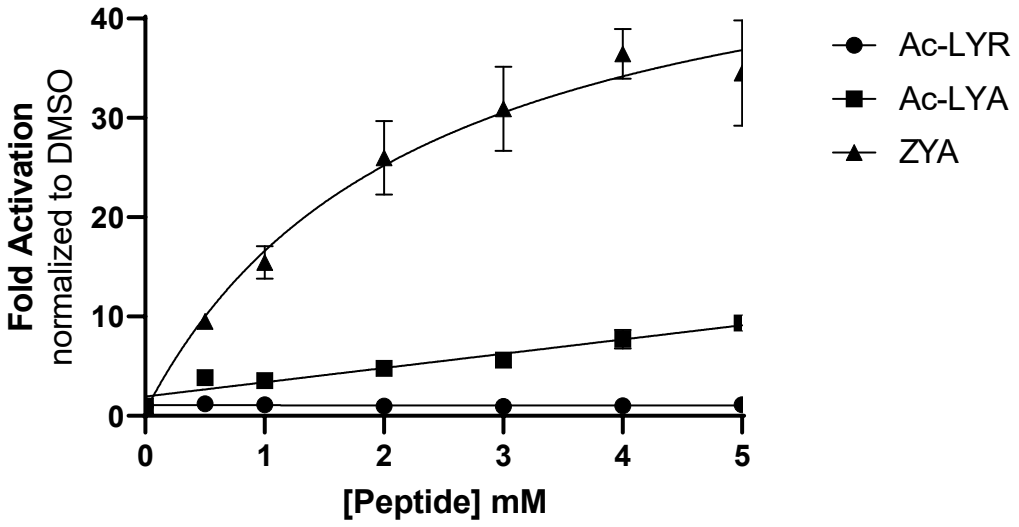
Top view of morph between maps, EMD-9215 and EMD-9221, showing primarily the 20S gate

Supplemental Figure 1



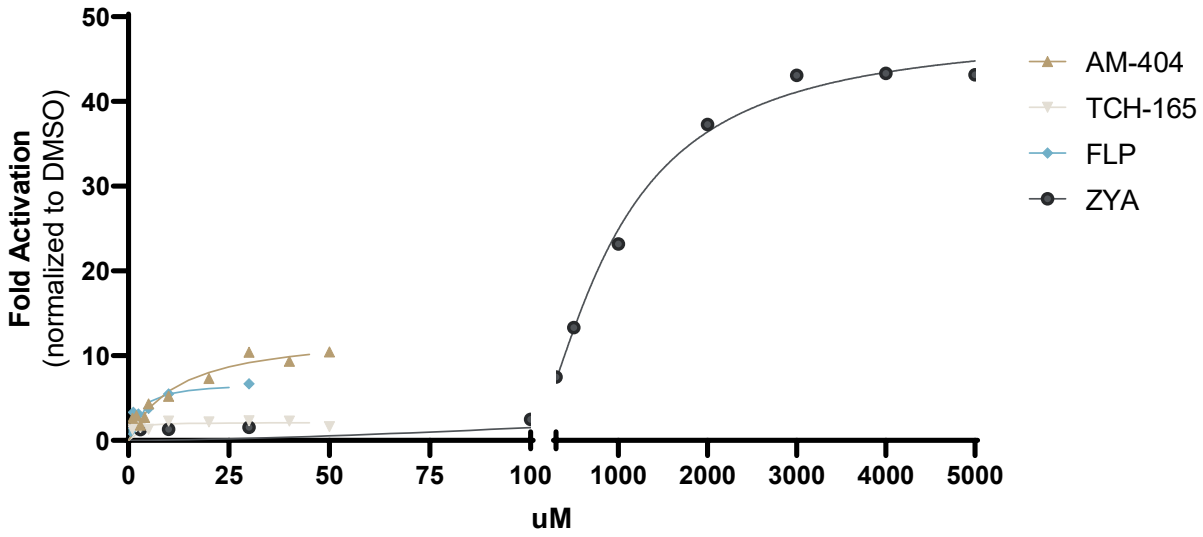
Similar to Figure 3C except angled to show the differences in density corresponding to the gate of WT (light gray) and L81Y (blue) T20S.

Supplemental Figure 2



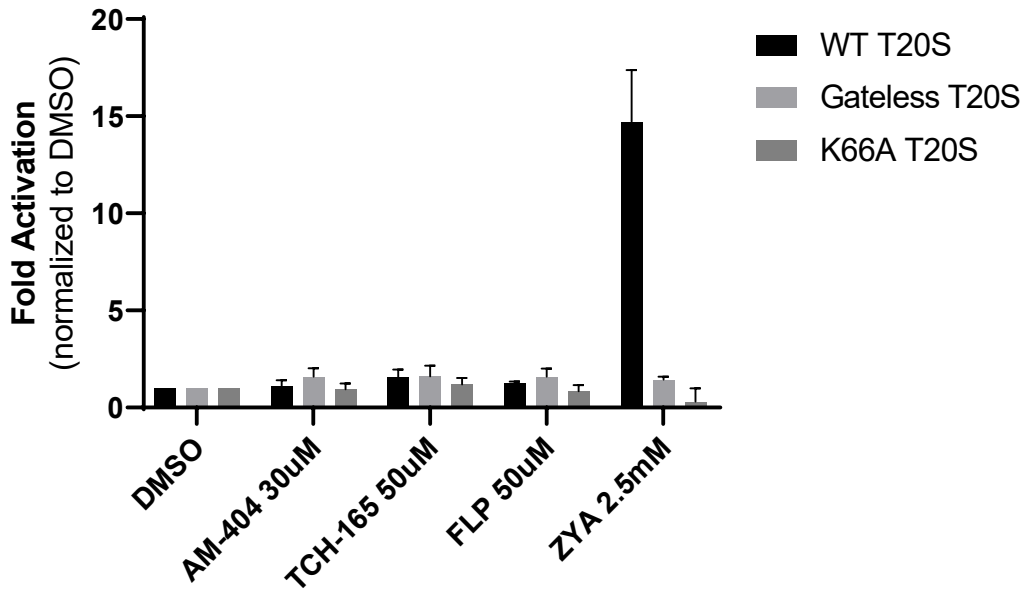
Same as Fig 4B except peptides were incubated at indicated concentrations with mammalian proteasomes (0.5nM) and nLPnLD-amc. Proteasome activity is normalized to DMSO. Experiment performed in triplicate. Error bars represent ± standard deviation.

Supplemental Figure 3



Compounds incubated with human 20S proteasomes (0.5nM) at indicated concentrations and suc-LLVY-amc. Proteasome activity is normalized to DMSO. Fits were generated in Prism using the Michaelis Menten equation for all compounds except ZYA, which was analyzed using the Hill slope equation. Data are representative of 2-3 independent experiments performed in singlicate.

Supplemental Figure 4



Compounds incubated with T20S at saturating concentrations as indicated. Proteasome activity was measured by degradation of LFP and normalized to DMSO controls. Data was representative of 3 independent experiments performed in singlicate. Error bars represent ± standard deviation