Supplementary Materials

Molecular basis for Proline- and Arginine-Rich Peptide inhibition of proteasome

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Supplementary Text:

Structural model of PR11: NMR-based models of PR11 were calculated using distance geometry and simulated annealing, using NOE-based distance restraints and backbone dihedral angles measured from resolution-enhanced NOESY data (See Table S1). The NOE-based distance restraints were classified as strong, medium and weak, corresponding to upper limits of 2.8, 3.4, and 5.0 Å respectively. The torsion angle dynamics protocol of CNS (Ver. 1.1) was used to calculate 50 structures in each round. These structures were further refined using Cartesian dynamics. The final set of 25 lowest energy structures was used for further analyses. None of the 25 structures showed NOE violations greater than 0.5 Å or dihedral angle violations greater than 5°. Structures were analyzed using PROCHECK-NMR.

Table S1. PR11- Structural Statistics

RMSD from experimental restraints	
NOE based distance restraints, (Å)	0.0130 ± 0.0050
Dihedral angle restraints, (°)	0.0609 ± 0.1163
RMSD from ideal geometry	
Bonds, (Å)	0.0016 ± 0.00025
Angles, (°)	0.4585 ± 0.0119
Impropers, (°)	0.0858 ± 0.0102
Ramachandran Plot Statistics	
residues in most favored regions, %	60
residues in additional allowed regions, %	40
residues in generously allowed regions, %	0
residues in disallowed regions, %	0
NOE restraints	137
Strong	99
Medium	38
Diheral restraints	6
RMSD from the average structure	
Backbone	1.872 ± 0.3777
Heavy Atoms	3.892 ± 0.3577

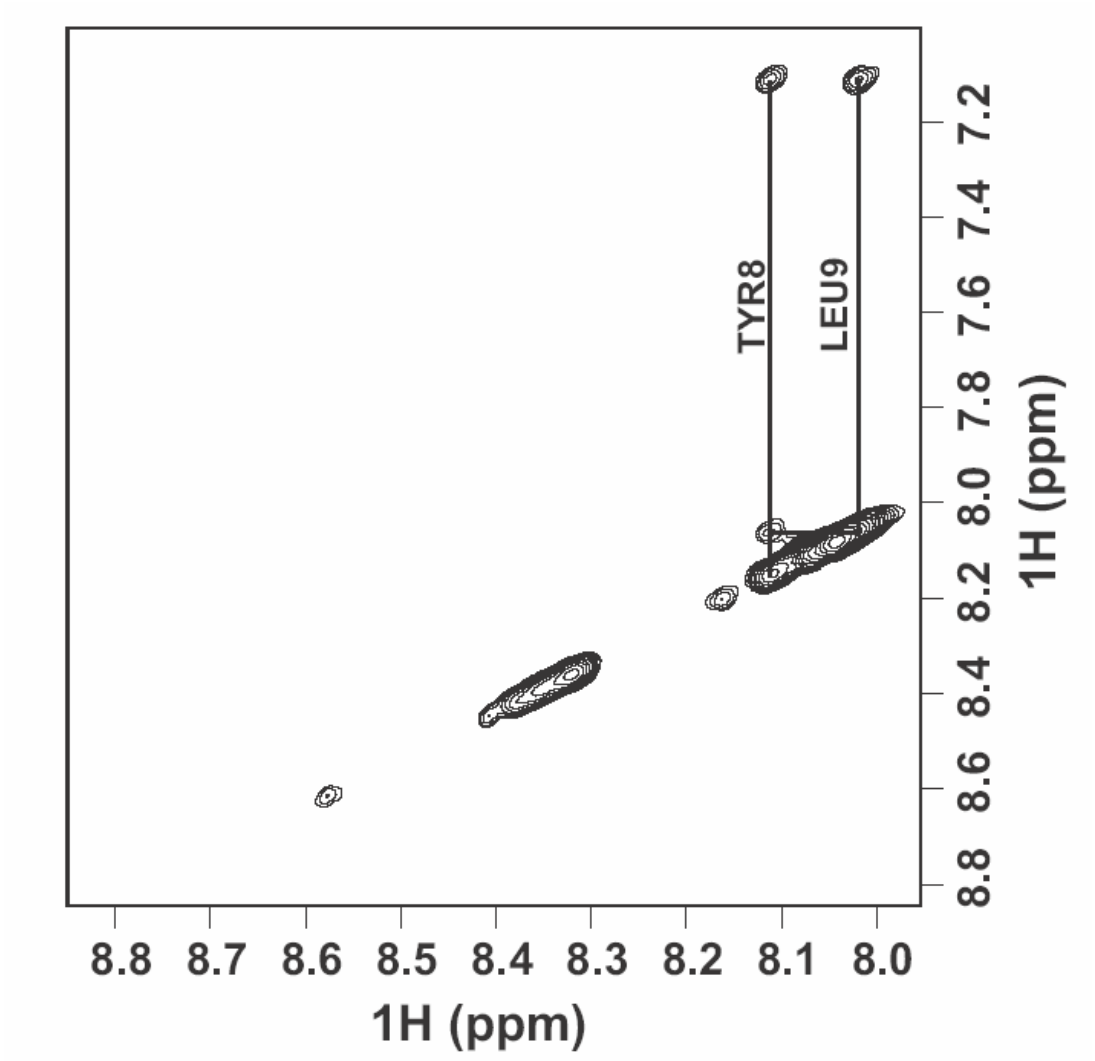

Figure S1. Amide region of NOESY spectrum. Cross peaks between amide protons of Tyr8 and Leu9 as well as toTyr8 side chain Hδ protons.

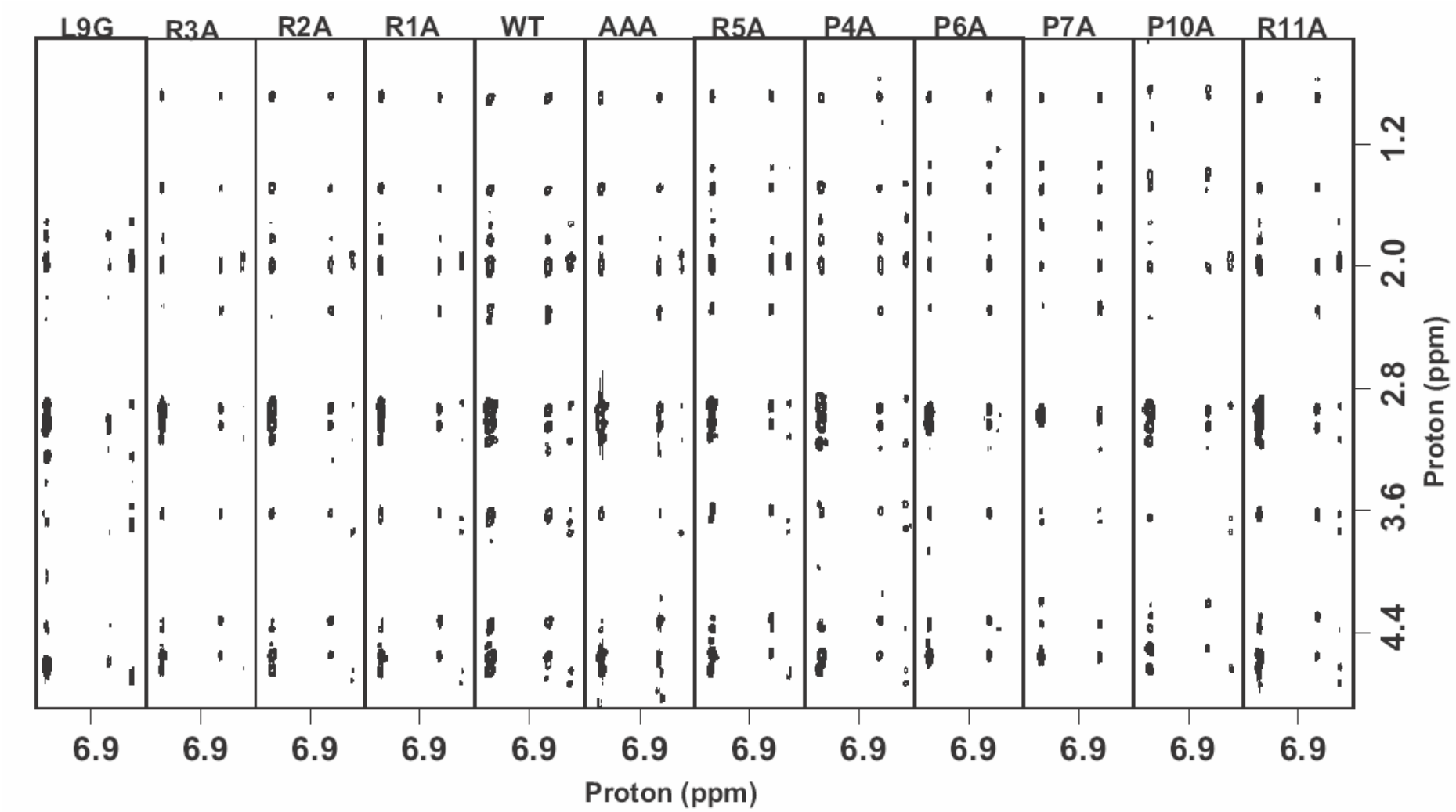

Figure S2. Aromatic region in the NOESY NMR spectra of PR11 and its mutants identify that Pro7 and Pro6 are responsible for Tyr8 side chain cross-peaks corresponding to minor (*cis*) conformers.

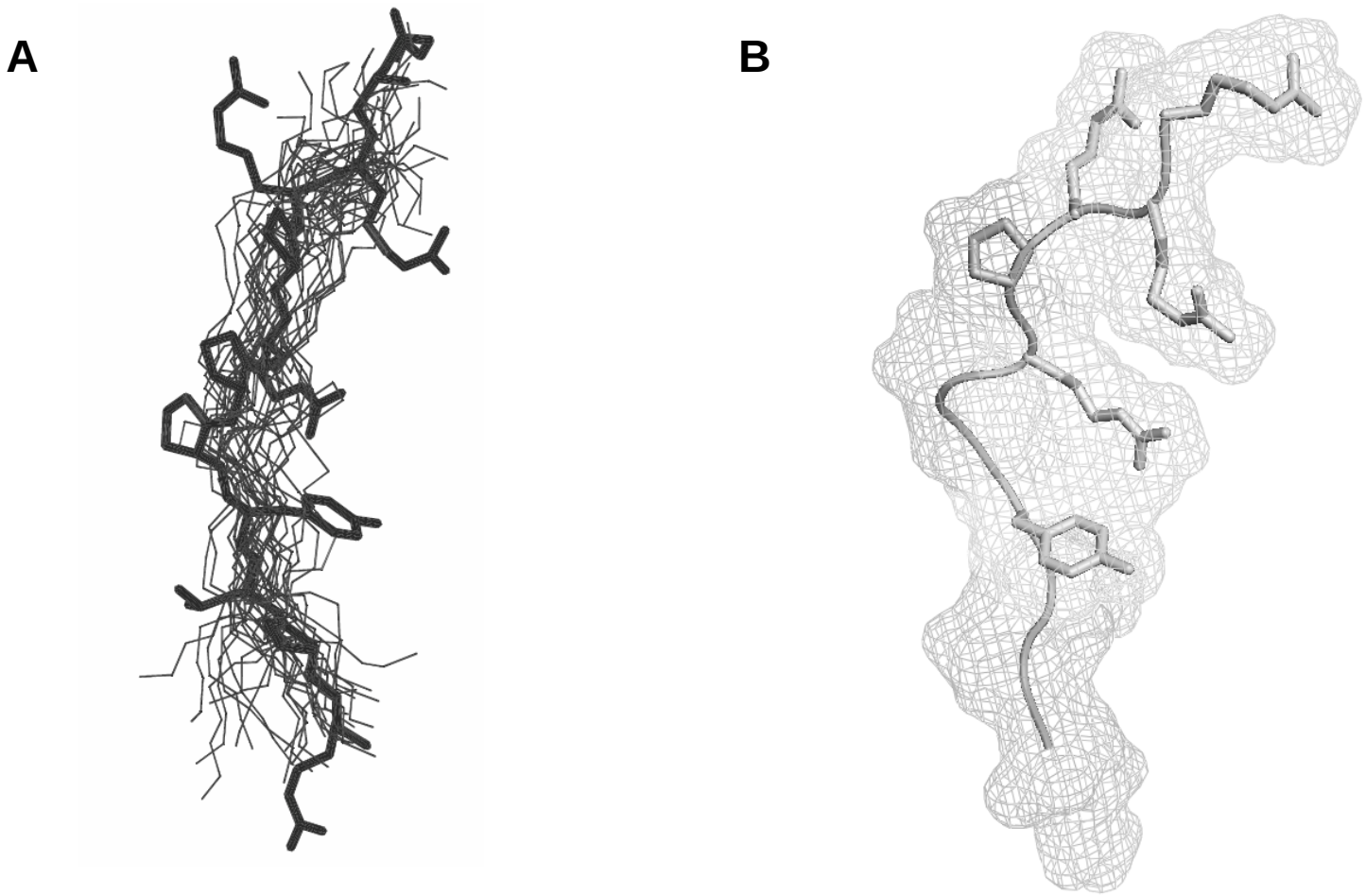

Figure S3. A NMR-based model of the all-trans PR11.
a. Backbone atoms of a bundle of 25 structural models of PR11 computed using limited NMR-derived restraints and the representative PR11 model (bold) is the average of the 25 structures. Average root-mean-squared-distance for the superposition of backbone C_α atoms of the individual models on to the averaged structure (bold) is 1.87 Å.
b. Side chains of amino acids that are critical to PR11 activity are shown on the representative, average, PR11 structural model.

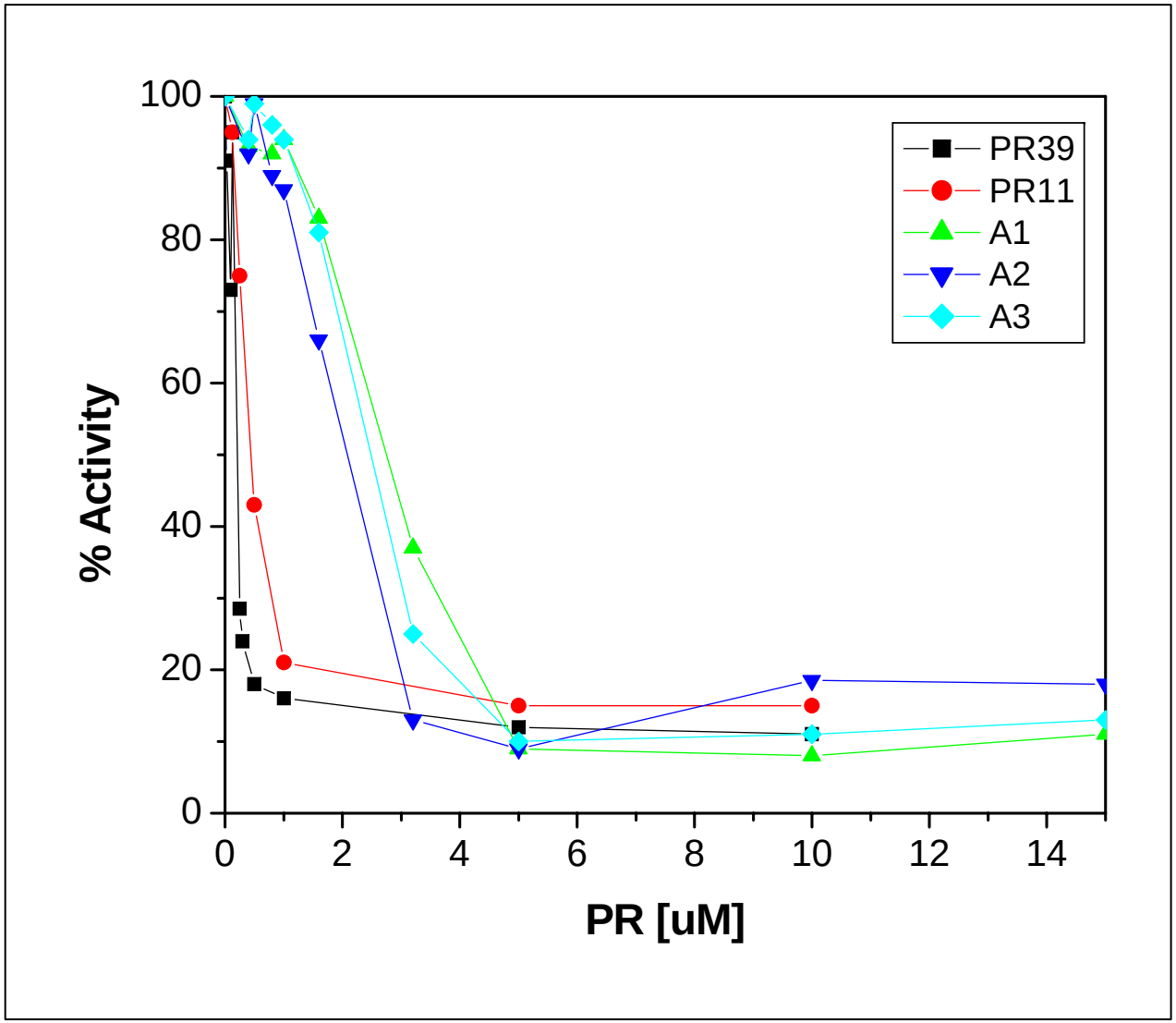

Figure S4. Inhibition of human 20S proteasome activity by PR-peptides.
The chymotrypsin-like activity of 20S proteasome was measured as a function of increasing peptide concentration. Figure shows that PR-peptides maximally inhibit about 90% of h20S activity.

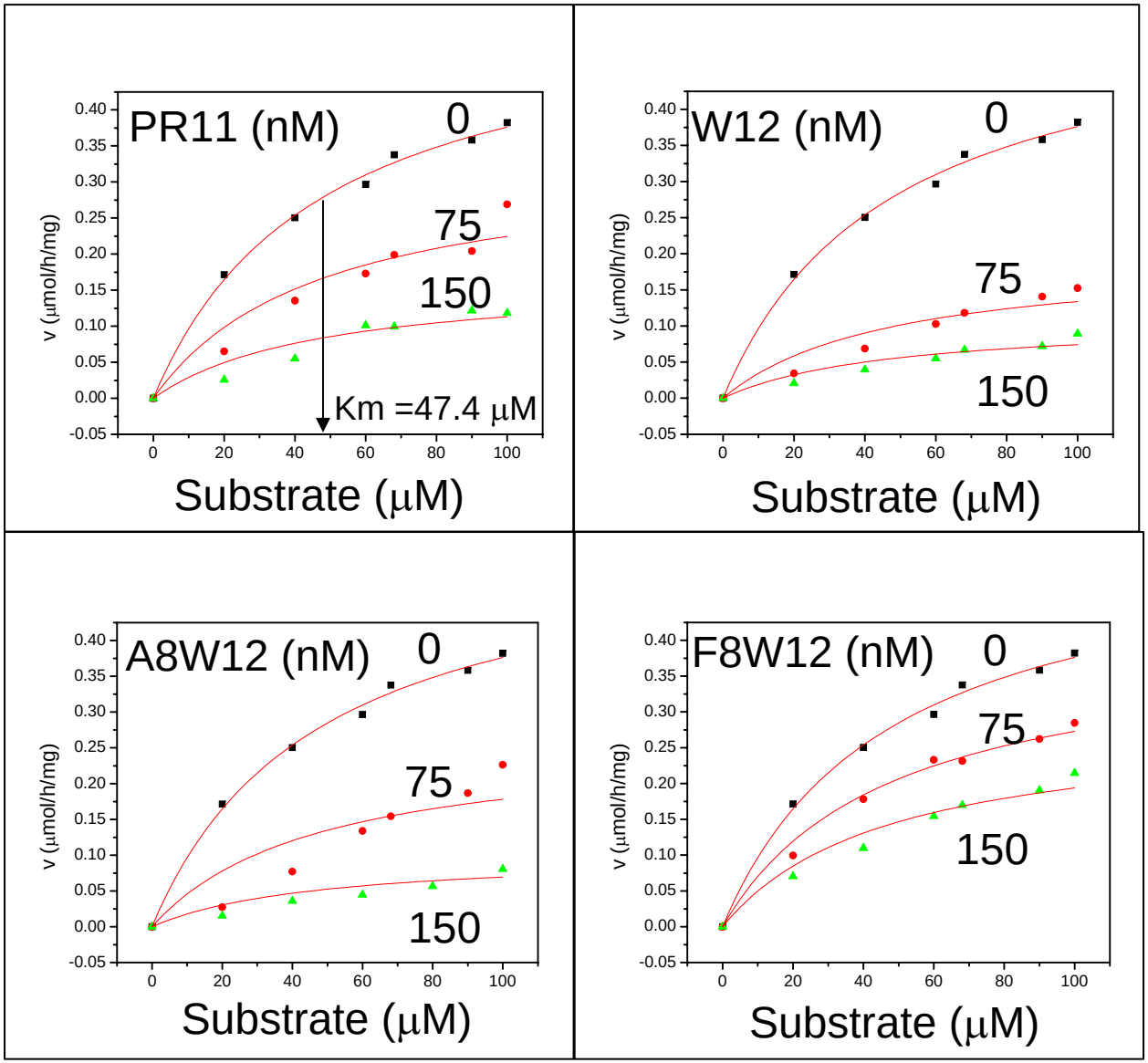

Figure S5. Inhibition constants (Ki) of PR11 and its most active mutants.
The chymotrypsin-like activity of 20S proteasome (at 5 nM concentration) was measured at 37 ºC as a function of increasing substrate concentration. Initial velocity is plotted against substrate concentration. Non-linear least squares fits to Michaelis-Menton equation are shown as red lines. PR-peptide concentration is shown on each curve.