

**Supplemental Material to:**

**THE C-TERMINUS OF RPT3, AN ATPase SUBUNIT OF PA700 (19S) REGULATORY COMPLEX, IS ESSENTIAL FOR 26S PROTEASOME ASSEMBLY BUT NOT FOR ACTIVATION**

**Brajesh Kumar, Young-Chan Kim and George N. DeMartino**

**Table 1. Identification of 20S proteasome subunit  $\alpha$ 1 (PSMA6) as the crosslinked product of the Dopa-Rpt3 C-terminal peptide.**

Crosslinking of 20S proteasome with the Dopa-Rpt3 C-terminal peptide was conducted as described in the text. The area containing fluorescent cross-linked spots from Figure 3C and the biotin cross-linked spots from Figure 3F were excised and subjected to mass spectrometry. Mass spectrometry was conducted by the Protein Core Facility, UT Southwestern Medical Center. The excised area was subjected to in-gel trypsin digestion. The tryptic peptide mixture was subjected to nano LC/MS/MS analysis. Nano-HPLC/electrospray mass spectrometry was performed on a ThermoFinnigan LTQ instrument, coupled with an Agilent 1100 Series HPLC system. Separation was achieved with a 75-micron (inside diameter) C18 reverse-phase chromatography column. The resulting files were searched against NCBI-nr protein sequence databases through the Mascot search engine (Matrix Science). Results from Figure 3C are shown in 1A. Results from Figure 3F are shown in 1B.

**1A.**

Match to: gi|8394076

Score: 109

Sequence coverage: 19 %

<b>Residue number</b>	<b>Sequence</b>	<b>Mr (experimental)</b>	<b>Mr (calculated)</b>	<b>Delta</b>	<b>Miss</b>	<b>Ion Score</b>
12-21	R.HITIFSPEGR.L	1159.50	1155.60	3.90	0	23
22-30	R.LYQVEYAFK.A	1161.45	1159.59	1.86	0	29
31-43	K.AINQGGLTSVAVR.G	1282.18	1284.71	-2.54	0	56
72-88	K.ITENIGCVMTGMTADSR.S	1811.69	1813.80	-2.12	0	5

Peptides of the  $\alpha$ 1 subunit of 20S proteasome were similarly identified in two additional independent crosslinking experiments. No peptides were identified when a similar analysis was conducted with samples crosslinked with Dopa-Rpt3 (-3C) peptide.

**1B.**

Match to: gi|8394076

Score: 123

Sequence coverage: 9 %

<b>Residue number</b>	<b>Sequence</b>	<b>Mr (experimental)</b>	<b>Mr (calculated)</b>	<b>Delta</b>	<b>Miss</b>	<b>Ion Score</b>
12-21	R.HITIFSPEGR.L	1157.35	1155.60	1.75	0	33
31-43	K.AINQGGLTSVAVR.G	1286.56	1284.71	1.84	0	63

Peptides of the  $\alpha$ 1 subunit of 20S proteasome were similarly identified in two additional independent crosslinking experiments. No peptides were identified when a similar analysis was conducted with samples crosslinked with Dopa-Rpt3 (-3C) peptide.