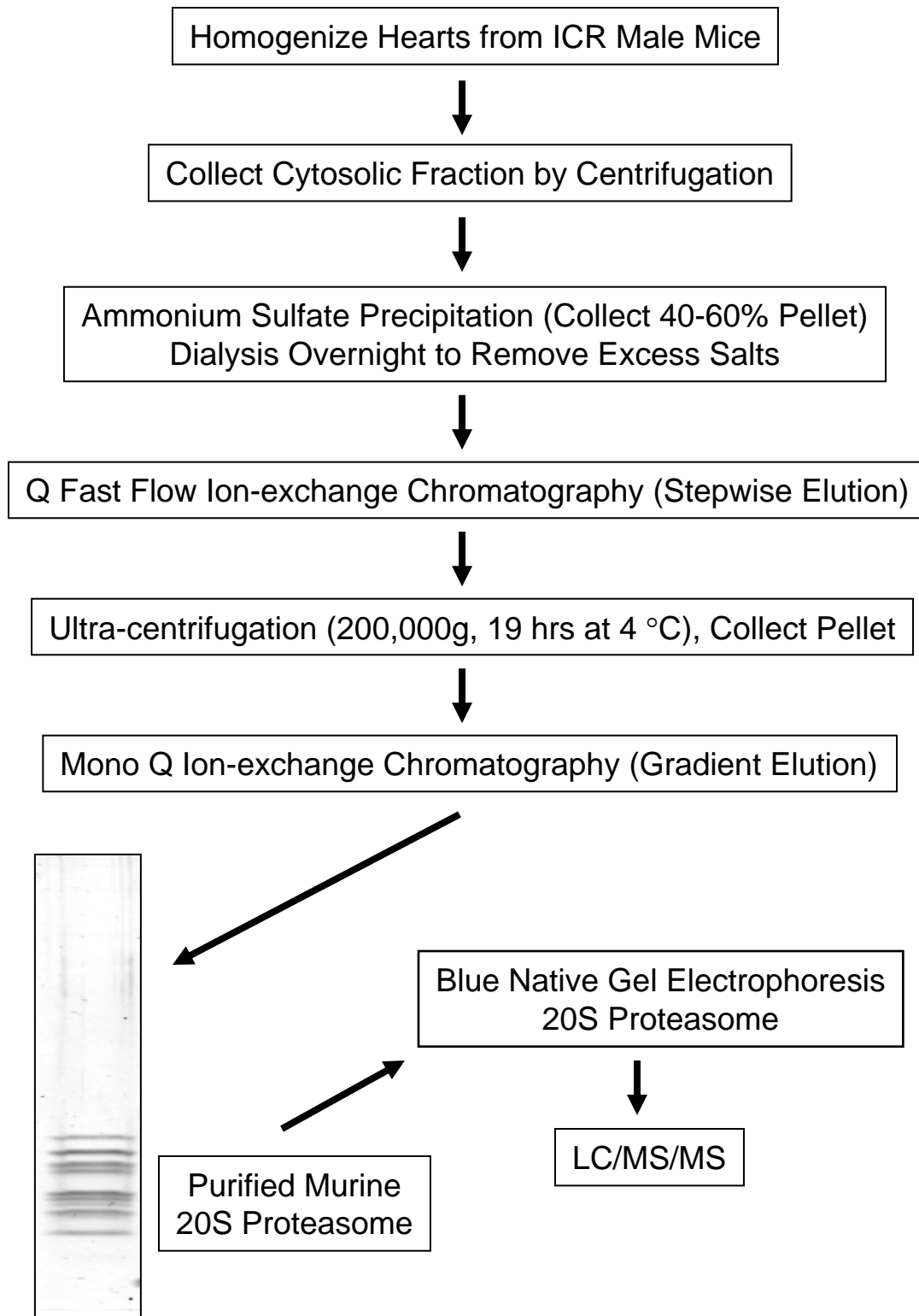


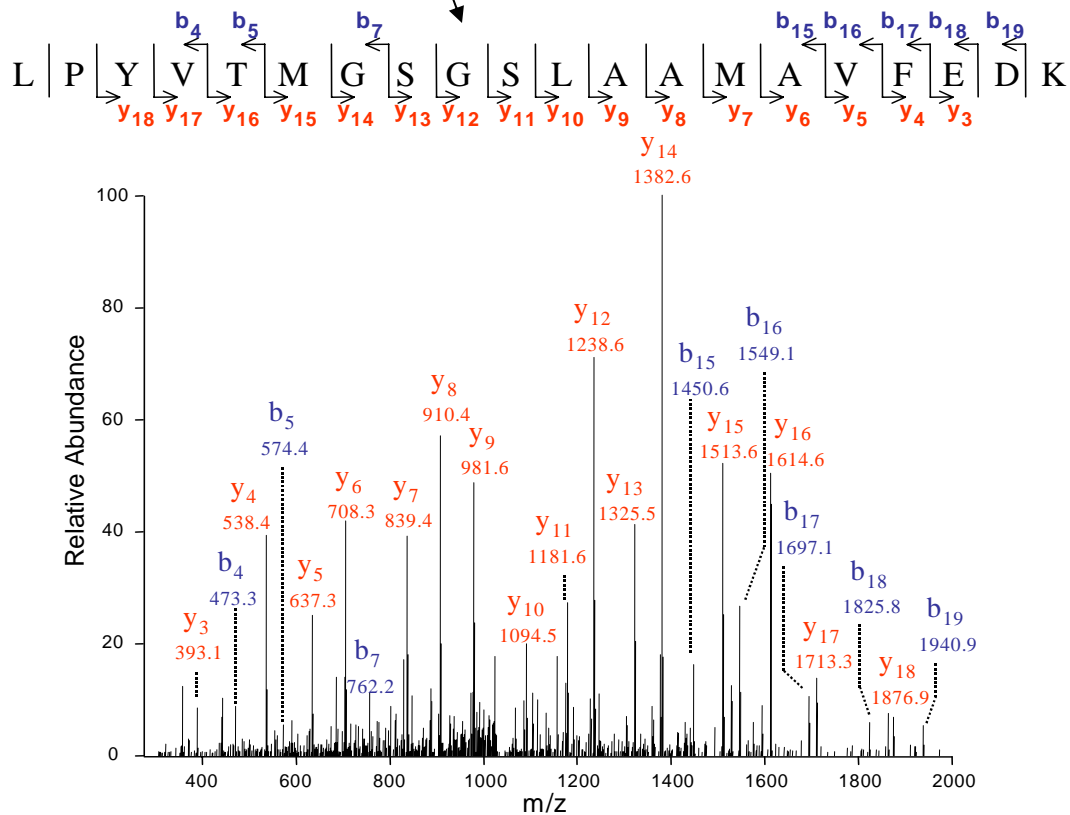
Supplemental Figure 1. Flow Chart Showing which Figures are Associated with the Main Sections of this Manuscript.



Supplemental Figure 2. Diagrammatic Scheme Showing the Methodology Used to Determine the Proteins which Associate with the 20S Proteasome.

A

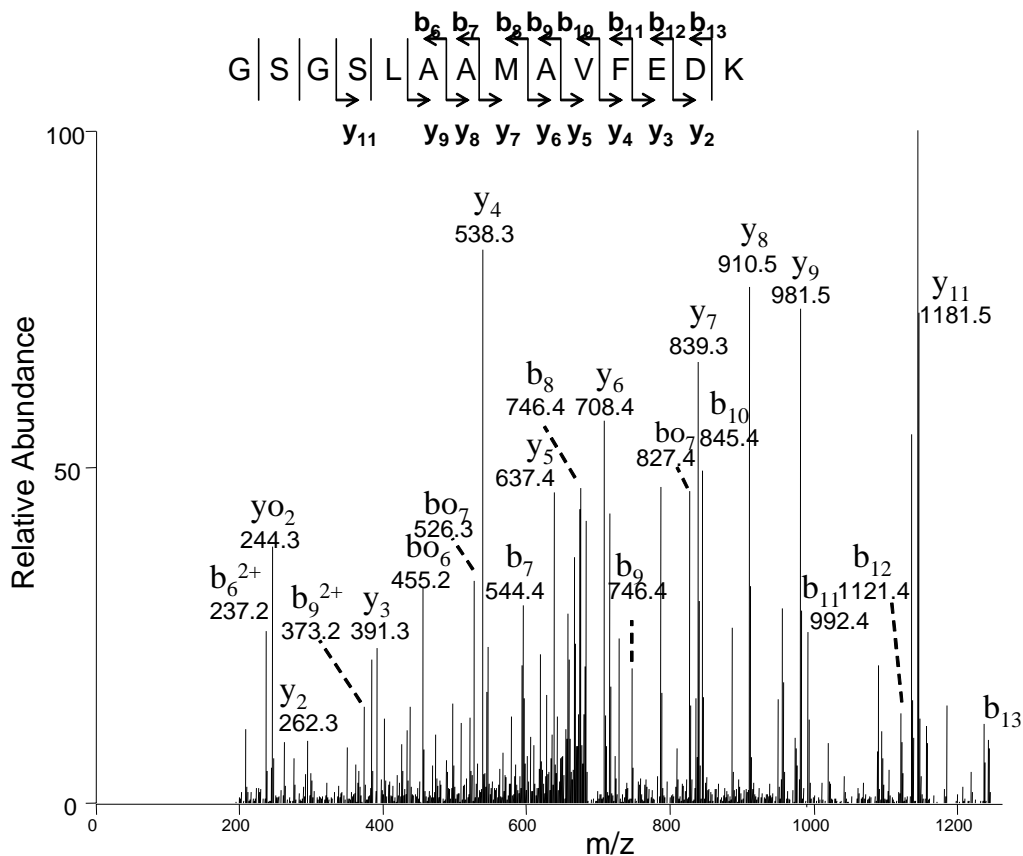
1	<u>Met</u>	<u>Ala</u>	<u>Ala</u>	<u>Val</u>	<u>Ser</u>	<u>Val</u>	<u>Tyr</u>	<u>Ala</u>	<u>Pro</u>	<u>Pro</u>	<u>Val</u>	<u>Gly</u>	<u>Gly</u>	<u>Phe</u>	<u>Ser</u>	15
16	<u>Phe</u>	<u>Asp</u>	<u>Asn</u>	<u>Cys</u>	<u>Arg</u>	<u>Arg</u>	<u>Asn</u>	<u>Ala</u>	<u>Val</u>	<u>Leu</u>	<u>Glu</u>	<u>Ala</u>	<u>Asp</u>	<u>Phe</u>	<u>Ala</u>	30
31	<u>Lys</u>	<u>Arg</u>	<u>Gly</u>	<u>Tyr</u>	<u>Lys</u>	<u>Leu</u>	<u>Pro</u>	<u>Lys</u>	<u>Val</u>	<u>Arg</u>	<u>Lys</u>	<u>Thr</u>	<u>Gly</u>	<u>Thr</u>	<u>Thr</u>	45
44	<u>Ile</u>	<u>Ala</u>	<u>Gly</u>	<u>Val</u>	<u>Val</u>	<u>Tyr</u>	<u>Lys</u>	<u>Asp</u>	<u>Gly</u>	<u>Ile</u>	<u>Val</u>	<u>Leu</u>	<u>Gly</u>	<u>Ala</u>	<u>Asp</u>	60
61	<u>Thr</u>	<u>Arg</u>	<u>Ala</u>	<u>Thr</u>	<u>Glu</u>	<u>Gly</u>	<u>Met</u>	<u>Val</u>	<u>Val</u>	<u>Ala</u>	<u>Asp</u>	<u>Lys</u>	<u>Asn</u>	<u>Cys</u>	<u>Ser</u>	75
76	<u>Lys</u>	<u>Ile</u>	<u>His</u>	<u>Phe</u>	<u>Ile</u>	<u>Ser</u>	<u>Pro</u>	<u>Asn</u>	<u>Ile</u>	<u>Tyr</u>	<u>Cys</u>	<u>Cys</u>	<u>Gly</u>	<u>Ala</u>	<u>Gly</u>	90
91	<u>Thr</u>	<u>Ala</u>	<u>Ala</u>	<u>Asp</u>	<u>Thr</u>	<u>Asp</u>	<u>Met</u>	<u>Thr</u>	<u>Thr</u>	<u>Gln</u>	<u>Leu</u>	<u>Ile</u>	<u>Ser</u>	<u>Ser</u>	<u>Asn</u>	105
106	<u>Leu</u>	<u>Glu</u>	<u>Leu</u>	<u>His</u>	<u>Ser</u>	<u>Leu</u>	<u>Ser</u>	<u>Thr</u>	<u>Gly</u>	<u>Arg</u>	<u>Leu</u>	<u>Pro</u>	<u>Arg</u>	<u>Val</u>	<u>Val</u>	120
121	<u>Thr</u>	<u>Ala</u>	<u>Asn</u>	<u>Arg</u>	<u>Met</u>	<u>Leu</u>	<u>Lys</u>	<u>Gln</u>	<u>Met</u>	<u>Leu</u>	<u>Phe</u>	<u>Arg</u>	<u>Tyr</u>	<u>Gln</u>	<u>Gly</u>	135
136	<u>Tyr</u>	<u>Ile</u>	<u>Gly</u>	<u>Ala</u>	<u>Ala</u>	<u>Leu</u>	<u>Val</u>	<u>Leu</u>	<u>Gly</u>	<u>Gly</u>	<u>Val</u>	<u>Asp</u>	<u>Val</u>	<u>Thr</u>	<u>Gly</u>	150
151	<u>Pro</u>	<u>His</u>	<u>Leu</u>	<u>Tyr</u>	<u>Ser</u>	<u>Ile</u>	<u>Tyr</u>	<u>Pro</u>	<u>His</u>	<u>Gly</u>	<u>Ser</u>	<u>Thr</u>	<u>Asp</u>	<u>Lys</u>	<u>Leu</u>	165
166	<u>Pro</u>	<u>Tyr</u>	<u>Val</u>	<u>Thr</u>	<u>Met</u>	<u>Gly</u>	<u>Ser</u>	<u>Gly</u>	<u>Ser</u>	<u>Leu</u>	<u>Ala</u>	<u>Ala</u>	<u>Met</u>	<u>Ala</u>	<u>Val</u>	180
181	<u>Phe</u>	<u>Glu</u>	<u>Asp</u>	<u>Lys</u>	<u>Phe</u>	<u>Arg</u>	<u>Pro</u>	<u>Asp</u>	<u>Met</u>	<u>Glu</u>	<u>Glu</u>	<u>Glu</u>	<u>Glu</u>	<u>Ala</u>	<u>Lys</u>	195
196	<u>Asn</u>	<u>Leu</u>	<u>Val</u>	<u>Ser</u>	<u>Glu</u>	<u>Ala</u>	<u>Ile</u>	<u>Ala</u>	<u>Ala</u>	<u>Gly</u>	<u>Ile</u>	<u>Phe</u>	<u>Asn</u>	<u>Asp</u>	<u>Leu</u>	210
211	<u>Gly</u>	<u>Ser</u>	<u>Gly</u>	<u>Ser</u>	<u>Asn</u>	<u>Ile</u>	<u>Asp</u>	<u>Leu</u>	<u>Cys</u>	<u>Val</u>	<u>Ile</u>	<u>Ser</u>	<u>Lys</u>	<u>Asn</u>	<u>Lys</u>	225
226	<u>Leu</u>	<u>Asp</u>	<u>Phe</u>	<u>Leu</u>	<u>Arg</u>	<u>Pro</u>	<u>Tyr</u>	<u>Thr</u>	<u>Val</u>	<u>Pro</u>	<u>Asn</u>	<u>Lys</u>	<u>Lys</u>	<u>Gly</u>	<u>Thr</u>	240
241	<u>Arg</u>	<u>Leu</u>	<u>Gly</u>	<u>Arg</u>	<u>Tyr</u>	<u>Arg</u>	<u>Cys</u>	<u>Glu</u>	<u>Lys</u>	<u>Gly</u>	<u>Thr</u>	<u>Thr</u>	<u>Ala</u>	<u>Val</u>	<u>Leu</u>	255
256	<u>Thr</u>	<u>Glu</u>	<u>Lys</u>	<u>Ile</u>	<u>Thr</u>	<u>Pro</u>	<u>Leu</u>	<u>Glu</u>	<u>Ile</u>	<u>Glu</u>	<u>Val</u>	<u>Leu</u>	<u>Glu</u>	<u>Glu</u>	<u>Thr</u>	270
271	<u>Val</u>	<u>Gln</u>	<u>Thr</u>	<u>Met</u>	<u>Asp</u>	<u>Thr</u>	<u>Ser</u>									



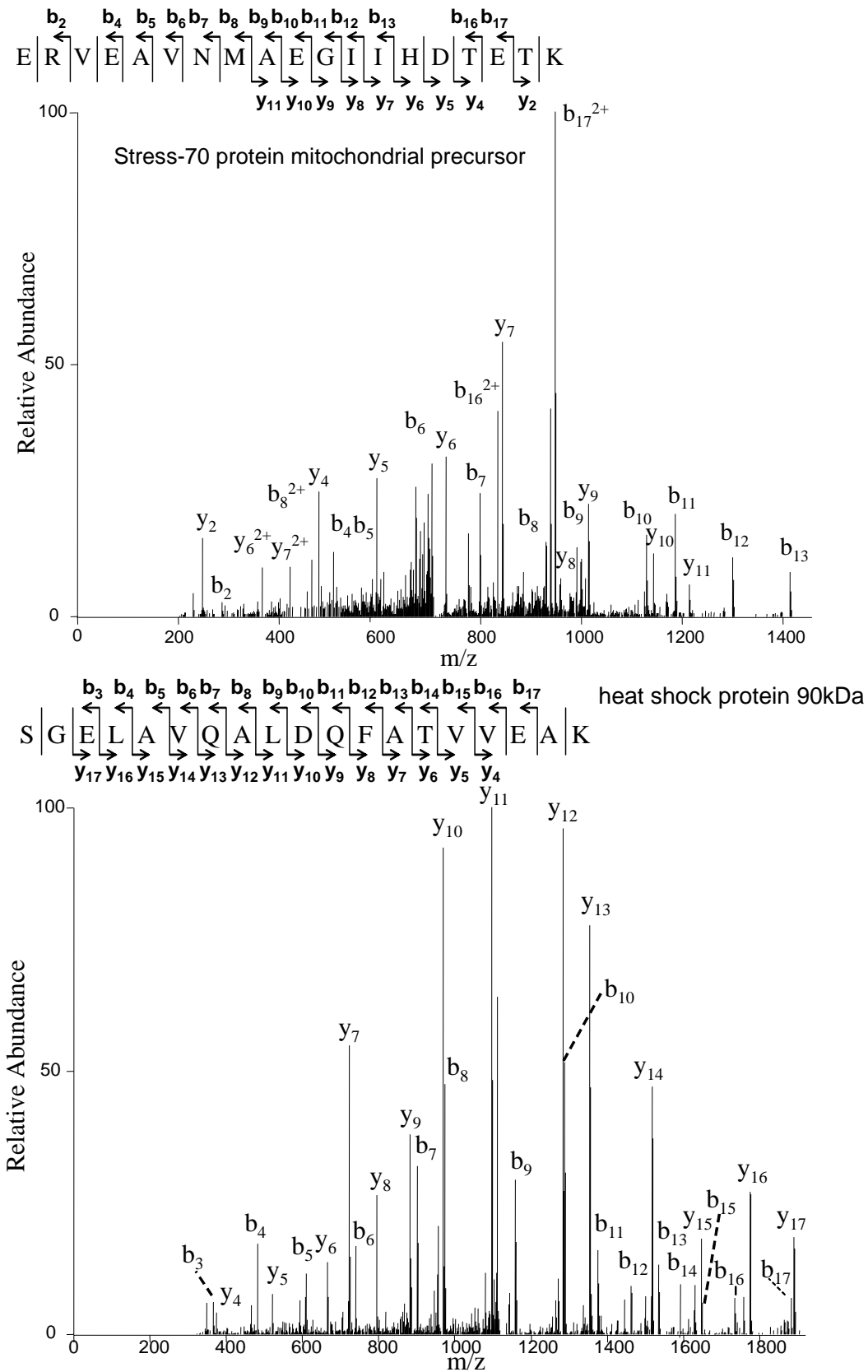
Supplemental Figure 3. Identification of a Proteasome Subunit from the BN-PAGE 20S band using LC/MS/MS analysis. Data were acquired on the LTQ (Thermo Fisher Scientific). A) Products of m/z 1067.04 are shown, and the observed product ions are indicated. The protein was identified as β 2 (PSMB7) with a molecular mass of 29.97 kDa and sequence coverage of 68.4%. The underlined residues 1-43 constitutes the propeptide which is absent in the catalytically active β 2 subunit. Peptides that were found by MS/MS are shown in red. One annotated peptide spectra from the heart proteasome is shown underlined in red.

B

1	<u>Met Ala Ala Val Ser Val Tyr Ala Pro Pro Val Gly Gly Phe Ser</u>	15
16	<u>Phe Asp Asn Cys Arg Arg Asn Ala Val Leu Glu Ala Asp Phe Ala</u>	30
31	<u>Lys Arg Gly Tyr Lys Leu Pro Lys Val Arg Lys Thr Gly Thr Thr</u>	45
44	<u>Ile Ala Gly Val Val Tyr Lys Asp Gly Ile Val Leu Gly Ala Asp</u>	60
61	<u>Thr Arg Ala Thr Glu Gly Met Val Val Ala Asp Lys Asn Cys Ser</u>	75
76	<u>Lys Ile His Phe Ile Ser Pro Asn Ile Tyr Cys Cys Gly Ala Gly</u>	90
91	<u>Thr Ala Ala Asp Thr Asp Met Thr Thr Gln Leu Ile Ser Ser Asn</u>	105
106	<u>Leu Glu Leu His Ser Leu Ser Thr Gly Arg Leu Pro Arg Val Val</u>	120
121	<u>Thr Ala Asn Arg Met Leu Lys Gln Met Leu Phe Arg Tyr Gln Gly</u>	135
136	<u>Tyr Ile Gly Ala Ala Leu Val Leu Gly Gly Val Asp Val Thr Gly</u>	150
151	<u>Pro His Leu Tyr Ser Ile Tyr Pro His Gly Ser Thr Asp Lys Leu</u>	165
166	<u>Pro Tyr Val Thr Met Gly Ser Gly Ser Leu Ala Ala Met Ala Val</u>	180
181	<u>Phe Glu Asp Lys Phe Arg Pro Asp Met Glu Glu Glu Glu Ala Lys</u>	195
196	<u>Asn Leu Val Ser Glu Ala Ile Ala Ala Gly Ile Phe Asn Asp Leu</u>	210
211	<u>Gly Ser Gly Ser Asn Ile Asp Leu Cys Val Ile Ser Lys Asn Lys</u>	225
226	<u>Leu Asp Phe Leu Arg Pro Tyr Thr Val Pro Asn Lys Lys Gly Thr</u>	240
241	<u>Arg Leu Gly Arg Tyr Arg Cys Glu Lys Gly Thr Thr Ala Val Leu</u>	255
256	<u>Thr Glu Lys Ile Thr Pro Leu Glu Ile Glu Val Leu Glu Glu Thr</u>	270
271	<u>Val Gln Thr Met Asp Thr Ser</u>	

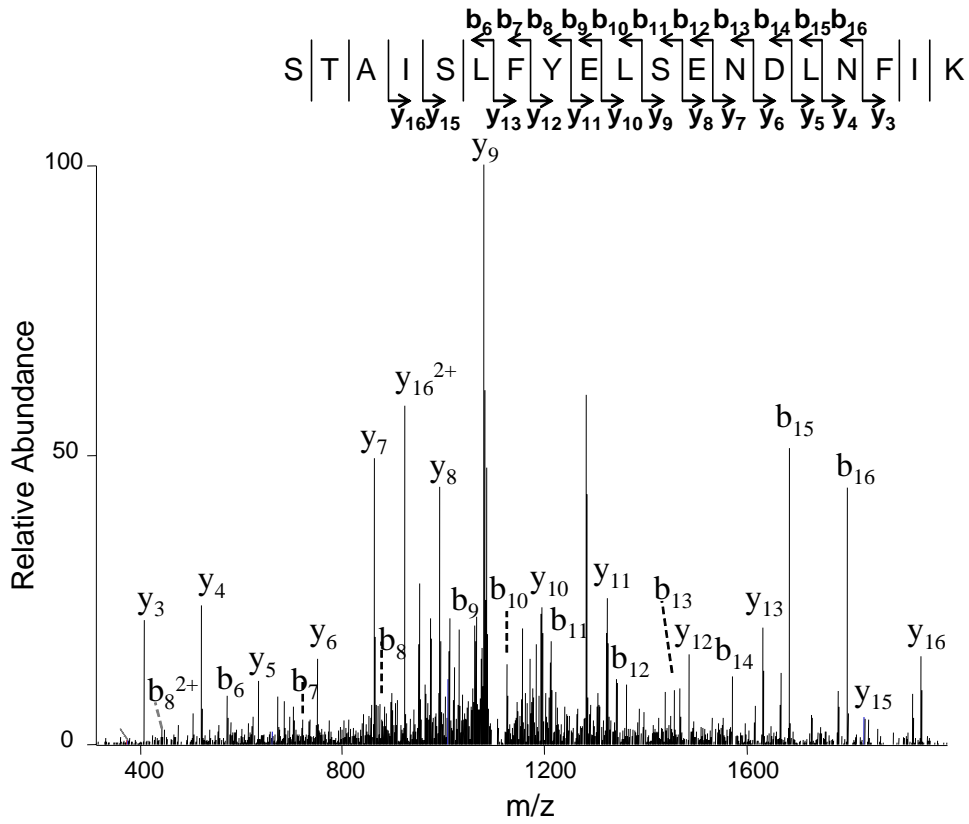


Supplemental Figure 3. Identification of a Heart Proteasome Subunit from the BN-PAGE 20S Band Using LC/MS/MS Analysis. Data were acquired on the LTQ (Thermo Fisher Scientific). B) The protein was identified as liver β 2 (PSMB7) with a molecular mass of 29.97 kDa and sequence coverage of 54.2%. One annotated peptide spectra from liver is shown underlined in red.

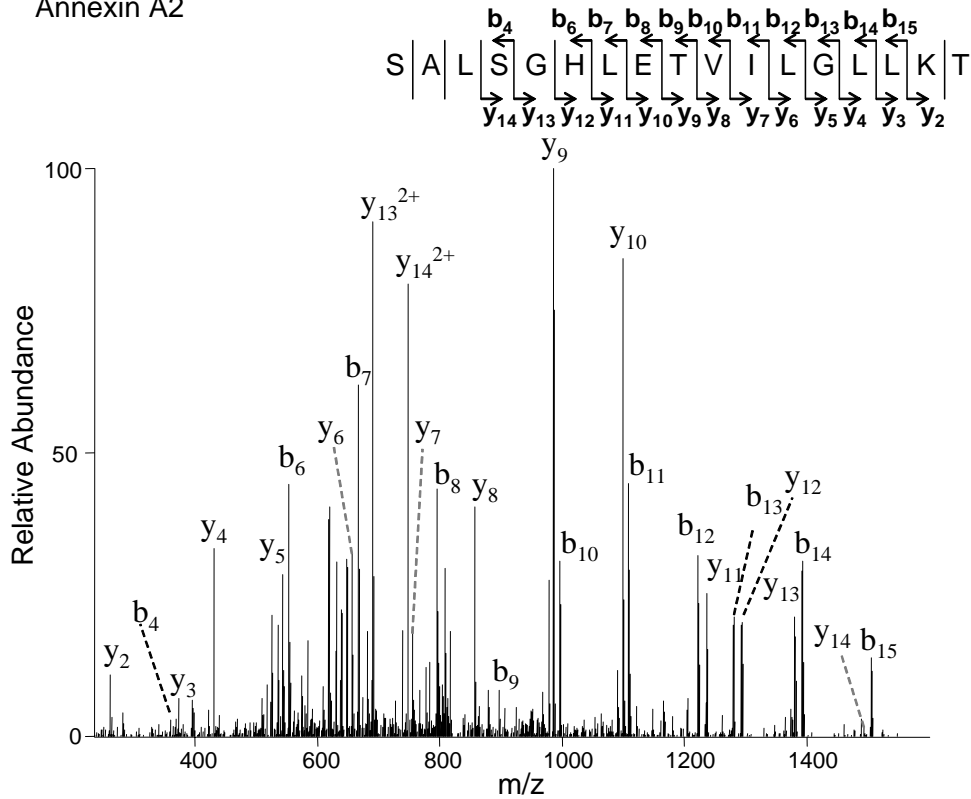


Supplemental Figure 4. Mass Spectra of Associating Partners of the 20S Proteasome.

Elongation Factor 2

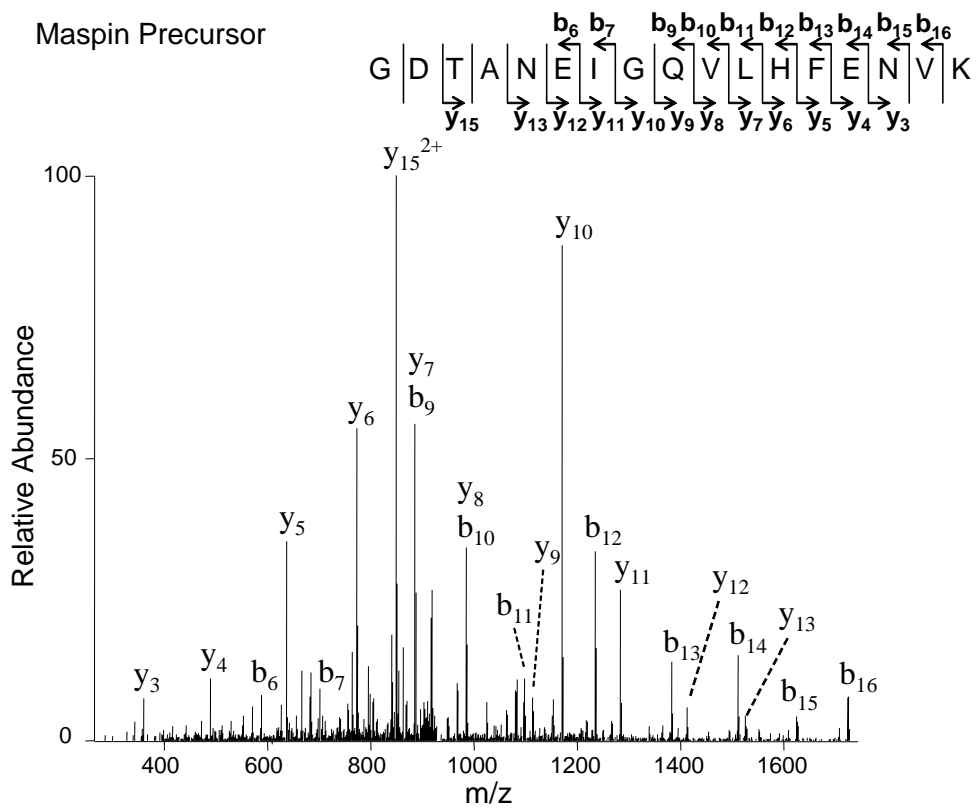


Annexin A2



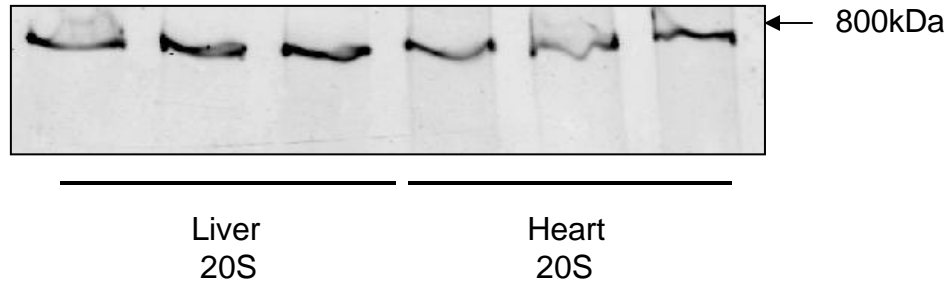
Supplemental Figure 4. Mass Spectra of Associating Partners of the 20S Proteasome.

M8:00058-MCP-R1

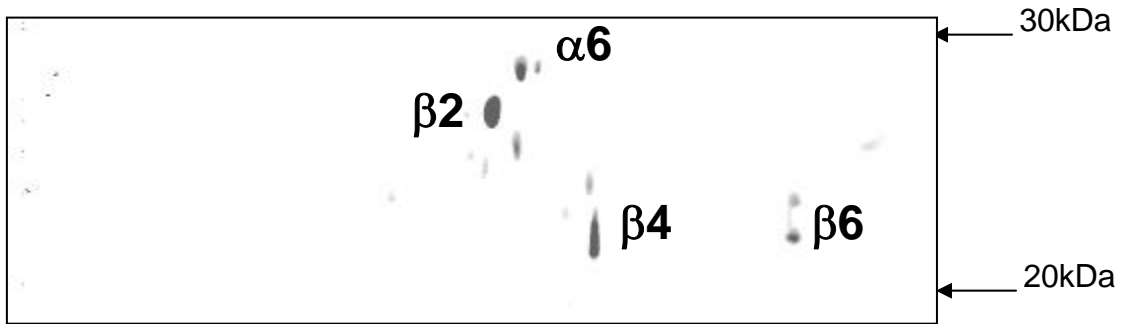


Supplemental Figure 4. Mass Spectra of Associating Partners of the 20S Proteasome.

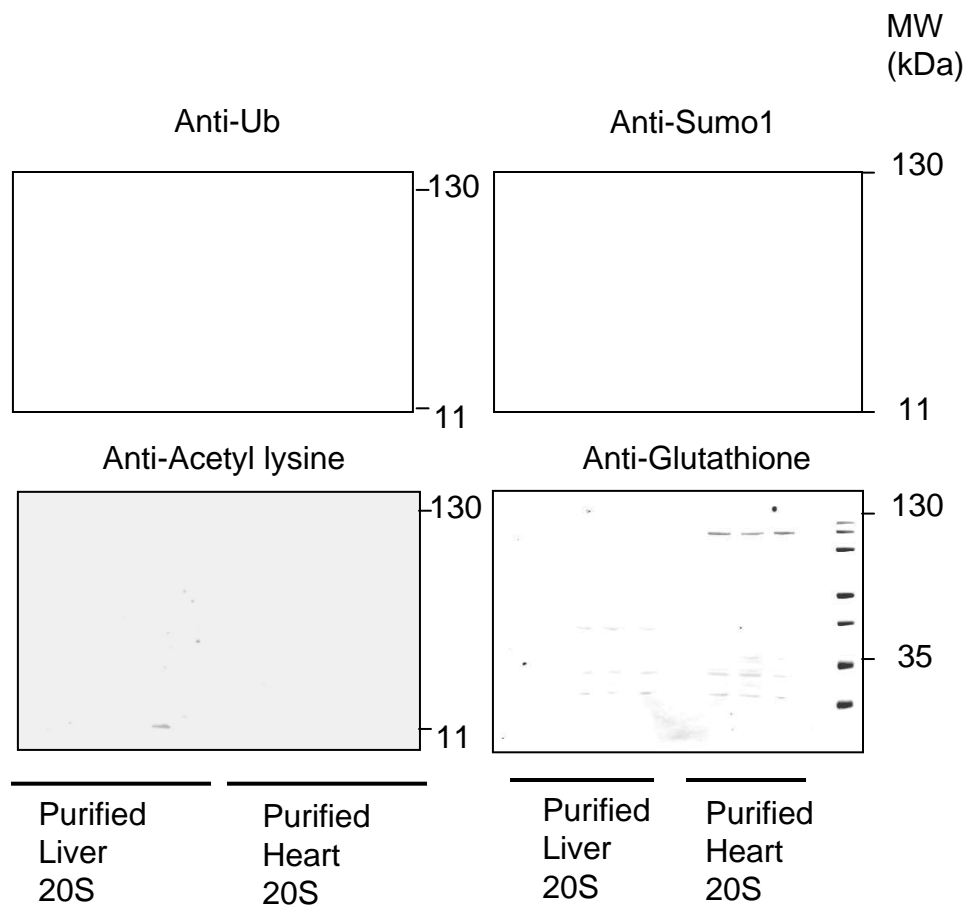
Anti-Methylarginine Native Gel



Anti-dimethyl-lysine Liver 2DE

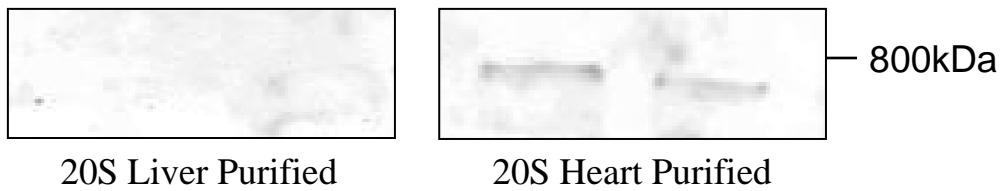


Supplemental Figure 5. Identification of Proteasome Modification by Methyl Groups. Upper Gel shows a native gel of purified 20S proteasomes from heart and liver which are immunoblotted with anti-methyl-arginine. Lower figure shows a two-dimensional gel of the purified liver 20S proteasome immunoblotted with anti-methyl-lysine.

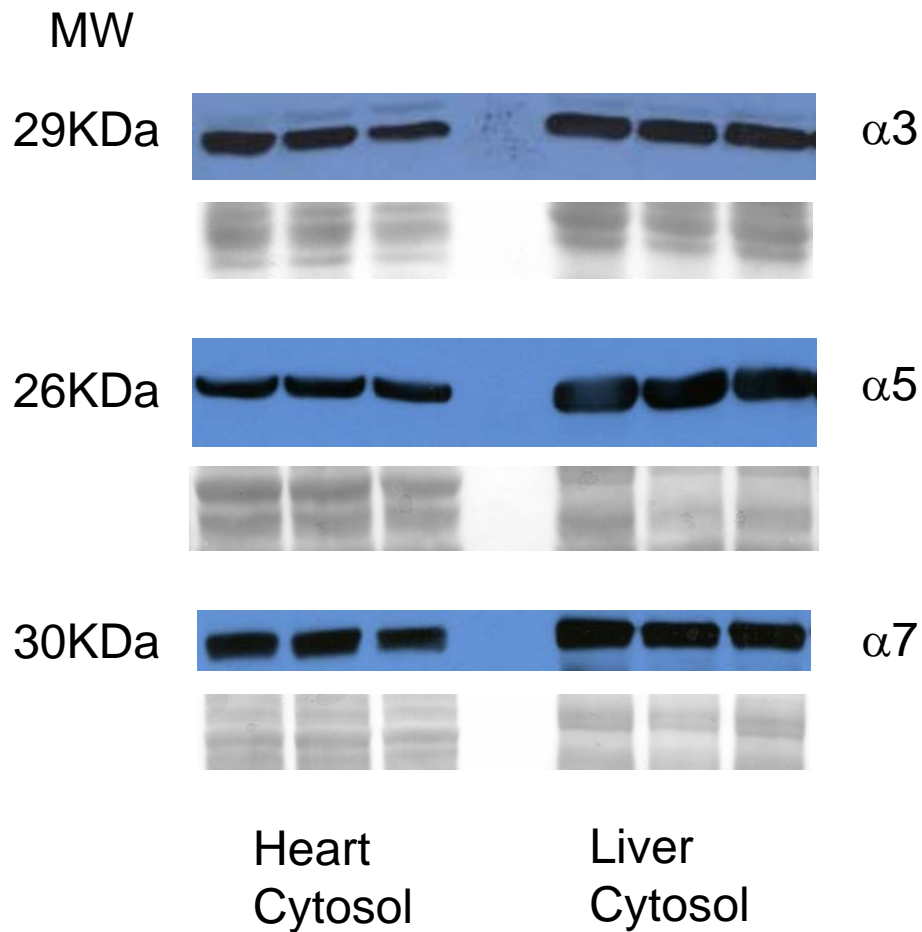


Supplemental Figure 6. Comparison of Post-Translational Modifications on Purified 20S Proteasomes from Heart and Liver. Western blot of purified 20S proteasomes from Heart and Liver with anti-ubiquitin, anti-Sumo-1, anti-glutathione, and anti-acetyl-lysine. Each lane contained 1 μ g of heart proteasome or 1 μ g of liver proteasome. The Nedd8 that is associated with the proteasome is not free.

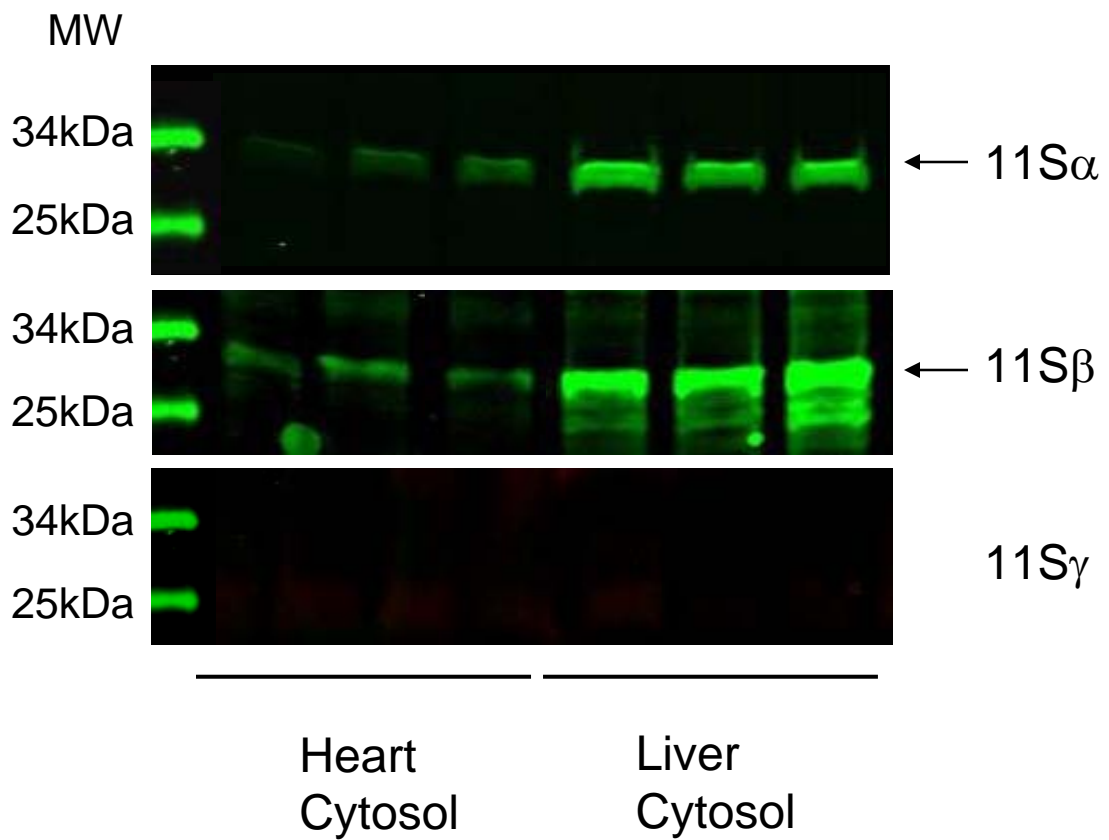
Anti-ZFH4



Supplementary Figure 7. Validating the presence of Zinc Finger Homeodomain 4 protein in Purified Cardiac Proteasomes. Purified liver and heart 20S proteasomes were run on 6% Native Gel and immunoblotted with anti zinc finger homeodomain 4 (anti ZFH4).



Supplementary Figure 8. Expression of Alpha Subunits in Heart and Liver Cytosol Fractions. Comparison of total subunit content by Western blotting. Ponceau S (PS) stained loading controls displayed below respective blot.



Supplemental Figure 9. Comparison of Free and Assembled 20S Proteasome Subunits in Heart and Liver by Western Blotting. Comparison of 11S isoforms in cytosolic fractions from heart and liver.

20S	Heart	Liver	Figure
Molecular Composition		Higher β 1i, β 2i, β 5i	2 A.,B; 3B
Complex Assembly	Higher β 2i	Higher β 2, β 5, β 1i, β 5i	3 A.,C.
Post-Translational Modification		Higher Phosphorylation	4 A.
Functional Associating Partner	PP1 ZFHX4*	CKII	7 A-D
Regulatory Complex		11S α , β	Supp. 8
Proteolytic Function	Higher Chymotrypsin	Higher Caspase/Trypsin	6 A.,B.

Supplemental Table 1. Contrasting Proteome Biology of 20S Proteasomes between Heart and Liver. Profile delineating the major differences in composition, activity and regulation of the 20S proteasomes found between the murine heart and liver. *Functional significance not yet determined.

Subunit Name	IPI accession number	Liver 20S Sequence Coverage	S.D.	Heart 20S Sequence Coverage	S.D.
α -1	IPI00131845	61.4	4.6	69.7	2.9
α -2	IPI00318970	62.4	13.2	53.2	5.0
α -3	IPI00277001	54.7	9.0	54.5	5.2
α -4	IPI00131406	56.2	3.4	55.0	8.5
α -5	IPI00131407	53.9	2.0	68.5	2.7
α -6	IPI00283862	50.3	2.8	52.7	0.8
α -7	IPI00331644	39.4	9.9	41.9	3.2
β -1	IPI00119239	64.7	6.9	65.0	2.7
β -2	IPI00136483	49.9	12.5	61.5	18.9
β -3	IPI00314467	49.6	6.3	44.6	8.4
β -4	IPI00128945	59.2	1.3	53.9	3.8
β -5	IPI00317902	50.2	3.9	56.1	1.8
β -6	IPI00113845	56.7	0.8	64.4	4.8
β -7	IPI00129512	55.9	6.0	55.6	11.2
β -1i	IPI00309379	57.0	8.3	27.4	9.2
β -2i	IPI00316736	40.9	4.8	50.1	6.9
β -5i	IPI00116712	52.6	2.5	45.1	2.4
Average		53.8	5.8	54.1	5.8

Supplemental Table 2. Sequence Coverage of the 20S Proteasome Subunits. Data were obtained by displaying native 20S complexes on blue-native PAGE, excise the intact proteasome complexes from the gel and analyze by LC-MS/MS. Each result is the average of three different 20S preparations. Results are shown as the mean \pm SD.

Proteasome Subunit	Peptide	M.W.	Z	XCorr	Delta Cn	Probability
$\alpha 1$	K.AINQGGLTSSVAVR.G	1285.46	2	3.96	0.48	3.24E-04
$\alpha 2$	R.GYSFSLTTFSPSGK.L	1478.62	2	4.02	0.49	4.15E-04
	K.LAQQYYLVYQEPIPTAQLVQR.V	2521.88	3	5.53	0.32	2.46E-06
	K.LVQIEYALAAVAGGAPSVGIK.A	2027.38	2	5.80	0.45	2.60E-05
$\alpha 3$	K.HYGFQLYQSDPSGNYGGWK.A	2204.33	2	4.72	0.54	1.27E-04
	K.RPFGVSLLYIGWDK.H	1650.93	2	4.37	0.43	1.33E-04
$\alpha 4$	K.ALLEVVQSGGK.N	1100.28	2	3.75	0.25	1.11E-05
	K.LTVEDPVTVEYITR.F	1634.84	2	5.02	0.42	5.65E-04
	R.RPFGISALIVGFDFDGTTPR.L	2065.35	3	4.09	0.39	2.50E-07
$\alpha 5$	R.LFQVEYAIEAIK.L	1423.66	2	5.06	0.35	3.37E-05
	R.PFGVALLFGGVDEK.G	1448.68	2	5.69	0.48	6.35E-10
	K.LGSTAIGIQTSEGVCLAVEK.R	2033.32	2	5.52	0.49	4.11E-04
$\beta 1$	R.LAAIQESGVER.Q	1172.3	2	3.69	0.24	6.41E-05
$\beta 3$	R.DAVSGMGVIVHVIEK.D	1553.84	2	4.54	0.42	2.16E-04
	R.LYIGLAGLATDVQTVAGR.L	1889.17	3	4.79	0.35	2.12E-08
$\beta 4$	R.NGYELSPTAAANFTR.R	1611.72	2	4.39	0.34	4.28E-05
$\beta 5$	R.DAYSGGAVNLYHVR.E	1521.64	2	4.38	0.37	2.81E-08
	K.FLHGIVIVAADSR.A	1284.48	2	3.16	0.42	1.25E-04
$\beta 6$	K.GAVYSFDPVGSYQR.D	1545.66	2	4.92	0.46	4.37E-06
	K.TVIGCSGFHGDCLTLTK.I	1866.14	2	4.78	0.50	7.08E-04
	R.TQNPMVTGTSLVGVK.F	1531.79	2	3.34	0.35	4.98E-04
$\beta 1i$	R.DGSSGGVIYLVTTAAGVDHR.V	2088.29	2	4.91	0.49	4.80E-07
	K.YREDLLAHLIVAGWDQR.E	2055.31	3	4.72	0.25	9.69E-08
$\beta 5i$	R.QDLSPEEAYDLGR.R	1492.56	2	4.30	0.39	6.87E-06
	K.VESSDVSDLLYK.Y	1354.47	2	4.51	0.38	6.08E-06
	R.ATAGSYISLR.M	1125.24	2	3.56	0.49	7.22E-04

Supplemental Table 3.
Properties of the Peptides Used for the O¹⁸ Quantitative Analysis as Identified by MS/MS.

M8:00058-MCP-R1

Antibody	Heart	Liver
$\beta 1$	1 ± 0.15	0.94 ± 0.11
$\beta 2$	1 ± 0.09	0.95 ± 0.08
$\beta 5$	1 ± 0.12	0.80 ± 0.10
$\beta 1i$	1 ± 0.30	2.50 ± 0.35
$\beta 2i$	1 ± 0.22	1.95 ± 0.40
$\beta 5i$	1 ± 0.27	2.10 ± 0.29

Supplementary Table 4.
Relative Concentration of Proteasome subunits in Heart and Liver
Purified 20S Proteasomes as Imaged by the Odyssey Infrared
Scanning of the Immunoblots Presented in Figure 3A.

Assembly	Liver	Heart
$\beta 1$	2.58 ± 0.40	3.66 ± 0.79
$\beta 2$	1.04 ± 0.08	0.18 ± 0.02
$\beta 5$	0.26 ± 0.04	0.04 ± 0.01
$\beta 1i$	0.10 ± 0.01	0.04 ± 0.01
$\beta 2i$	1.31 ± 0.01	1.92 ± 0.08
$\beta 5i$	1.02 ± 0.33	0.13 ± 0.03

Supplemental Table 5.

Assembly Index For the 20S Catalytic Subunits in Liver and Heart. Values obtained by the Odyssey infrared scanning of the immunoblots presented in Figure 3A.

Protein Name	IPI accession number	Molecular Weight (kDa)	Average Liver 20S Sequence Coverage (average no of peptides)	Average Heart 20S Sequence Coverage (average no of peptides)
Heat shock Protein 90 alpha	IPI00330804	84604	4% (2)	4% (3)
Protein phosphatase 2A	IPI00310091	65150	2.3% (2)	4.8% (3)
Elongation factor 2	IPI00466069	95122	2.2% (3)	2.2% (3)
Casein kinase II beta subunit	IPI00126762	25268	13% (2)	13% (2)
Stress-70 mitochondrial precursor	IPI00133903	73483	5.2% (2)	8.5% (3)
Elongation translation initiation factor 3 subunit 10	IPI00129276	161852	2.2% (2)	2.2% (2)
Delta-5 Desaturase	IPI00468859	52317	4.3% (2)	4.3% (3)
Protein phosphatase 1	IPI00311873	37032	8.5% (2)	8.5% (2)
Nedd-8	IPI00127021	8972	17.3% (3)	17.3% (2)
Annexin A2	IPI00468203	38521	Not found	4.7 (3)

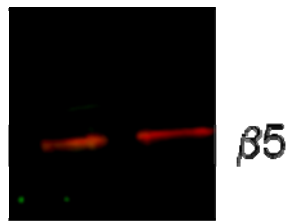
Supplementary Table 6.
Proteasome Associating Partners found by LC-MS/MS.
These proteins were found identified in more than one purified proteasome fraction.

M8:00058-MCP-R1

Protein Name	IPI accession number	Molecular Weight (kDa)	Liver 20S Sequence Coverage (no of peptides)	Heart 20S Sequence Coverage (no of peptides)
Calpain 2 catalytic subunit	IPI00308938	79691	4.7% (3)	4.7% (2)
Zinc Finger Homeobox Protein 4	IPI00122043	392323	Not found	0.06% (2)
Maspin precursor 6 (Serpin B5 Precursor)	IPI00229963	42084.6	Not found	4.5% (5)
5,6-Dihydroxyindole-2-carboxylic acid oxidase precursor	IPI00125149	60723	3.2% (3)	Not found
PRA1 family protein 3	IPI00119024	21543	10.1% (3)	Not found
Protein kinase A	IPI00169788	45589	3.5% (2)	3.5% (2)

Supplementary Table 7.
Proteasome Associating Partners Found by LC-MS/MS.
These proteins were found identified in only one replicate of the heart and liver preparations.

Protein Name	IPI Accession number	Molecular Weight (kDa)	Unique Peptides/Total number of peptides
Stress-70 protein, mitochondrial precursor	IPI00133903	73483	9/17
Heat shock protein HSP 90-alpha	IPI00330804	84604	8/13
Protein phosphatase 2A	IPI00310091	65150	2/1
Protein phosphatase 1	IPI00311873	37032	3/2
Casein kinase II	IPI00126762	25268	1/1



Supplementary Table 8.

MS/MS Identification of Co-immunoprecipitated Associating Partners of the Cardiac 20S Proteasomes Using Anti- α 3. Isolation of proteasome complexes by immunoprecipitation confirmed by immunoblotting for β 5 proteasome subunit (image below).

M8:00058-MCP-R1