

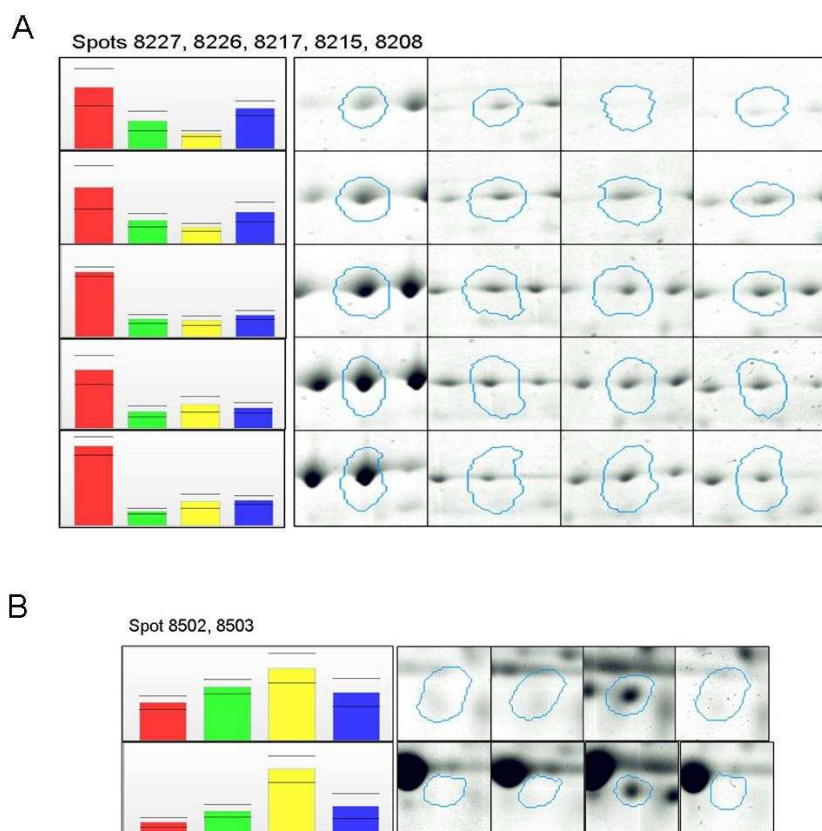
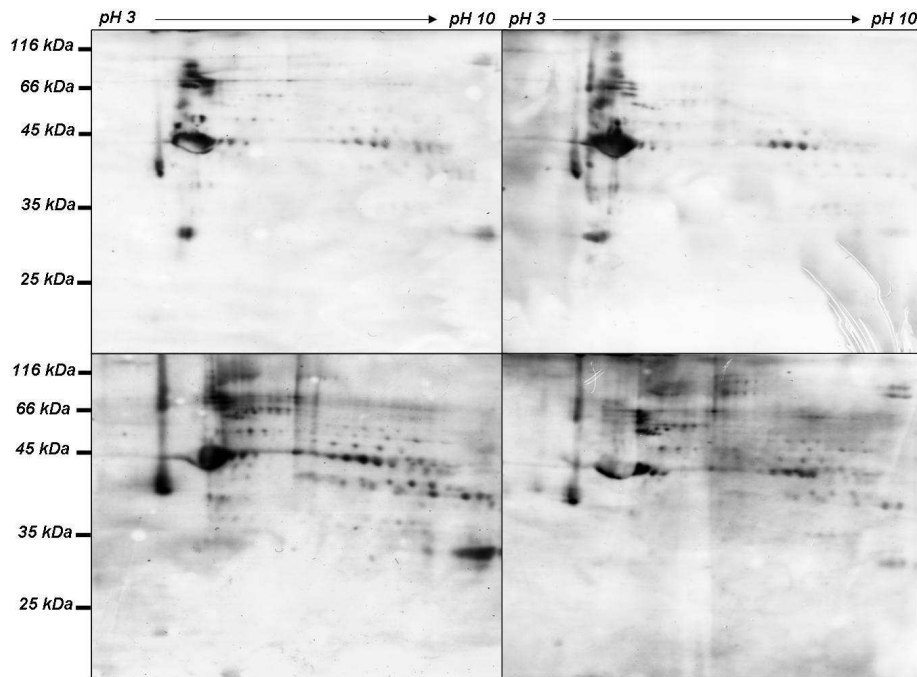


Figure S1: Selected areas of the colloidal Coomassie stained 2D-gels for spots corresponding to muscle isoform of glycogen phosphorylase (A, spots 11 to 7 from top to down from Fig. 3, left panel) and fructose 1,6-bisphosphate aldolase (B, spot 12 on top, spot 13 at bottom from Fig. 3, right panel) from control animals and animals 3, 9 or 24 h after X-ray irradiation (from left to right). The left bars indicate the spot volumes (intensities) of the circled spot areas used by Delta2D for quantification of the control (red bar), 3 h (green bar), 9 h (yellow bar) and 24 h (blue bar) samples. The corresponding gels are shown in Figure 2 and the protein identification in Tables 1 and 2.



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Figure S2: Western-blots probed with anti-DNP antibodies to detect DNPH-reactive carbonyl groups in skeletal muscle protein preparations. The control (A), 3 h (B), 9 h (C) and 24 h (D) samples (10 μ g protein each) were separated by 2-DE and semidry blotted on PVDF membranes. After IEF the carbonylated proteins were derivatized with DNPH in the strips (7 cm, 3-10 NL) for 20 min before the strips were transferred to SDS-PAGE. The PVDF membrane was first probed with a goat anti-DNP polyclonal serum, and after washing, incubated with donkey anti-goat IgG Ab conjugated with POD. The membranes were stained with the ECL Advance[®] western blot detection kit.

This figure shows the original images displayed in Figure 4 without markings showing which spots were carbonylated.

Table S1: This table refers to table 1 of the main document and contains the original Delta2D values, i.e. ratio, student-t test (values show the probability in % that the null hypothesis (groups are equal) is false), relative standard deviation (RSD). The gel image is shown in Fig. 3, left panel.

	Control	3h	9h	24h	Spot #
Heat shock cognate 71kDa protein					
Ratio		0.49	0.29	0.75	Spot 1
t-test		92.36	99.8	96.77	
RSD	10.65	58.03	30.46	3.89	
Skeletal muscle-specific alpha-actinin 3					
Ratio		0.24	0.13	0.47	Spot 2
t-test		98.03	98.78	91.56	
RSD	27.46	34.22	45.59	38.37	
Ratio		0.2	0.26	0.5	Spot 3
t-test		99.99	99.85	99.75	
RSD	5.67	16.16	47.91	17.8	
Ratio		0.19	0.29	0.59	Spot 4
t-test		99.84	99.54	97.84	
RSD	14.63	15.17	32.74	10.48	
Serum albumin precursor					
Ratio		0.54	0.29	0.61	Spot 5
t-test		84.87	97.91	90.09	
RSD	21.78	55.33	57.94	23.61	
Myosin binding protein H					
Ratio		0.36	0.42	0.4	Spot 6
t-test		99.91	97.54	99.83	
RSD	9.48	9.71	51.66	15.12	
Glycogen phosphorylase, muscle form					
Ratio		0.45	0.24	0.66	Spot 7
t-test		91.64	97.57	79.15	
RSD	30.01	35.29	20.79	18.38	
Ratio		0.41	0.29	0.56	Spot 8
t-test		88.94	93.57	77.83	
RSD	38.98	28.6	24.47	31.46	
Ratio		0.27	0.25	0.33	Spot 9
t-test		99.95	99.95	99.93	
RSD	7.28	26.45	29.72	21.11	
Ratio		0.29	0.41	0.35	Spot 10
t-test		98.16	95.85	97.57	
RSD	24.62	31.85	33.73	25.71	
Ratio		0.18	0.3	0.32	Spot 11
t-test		99.94	99.76	99.83	
RSD	11.48	19.37	29.08	17.67	
Aconitate hydratase (aconitase)					
Ratio		0.3	0.47	0.68	Spot 12
t-test		98.92	96.92	80.77	
RSD	20.16	29.9	23.1	30.04	
Ratio		0.24	0.44	0.57	Spot 13
t-test		99.38	97.97	94.13	
RSD	20.03	16.65	17.25	21.3	
Collagen alpha-1 chain					
Ratio		0.06	0.05	0.09	Spot 14
t-test		98.72	98.78	98.59	
RSD	30.85	29	63.9	39.59	
Ratio		0.1	0.02	0.09	Spot 15
t-test		90.3	92.15	90.5	
RSD	58.97	41.67	71.4	49.29	
Ratio		0.16	0.12	0.13	Spot 16

<i>t-test</i>		84.79	86.25	85.9	
<i>RSD</i>	66.82	49.8	73.23	63.3	
Pyruvate kinase isozymes M1/M2					
Ratio		0.44	0.76	0.63	Spot 17
<i>t-test</i>		98.53	88.64	95.15	
<i>RSD</i>	14.1	30.2	12.23	18.66	
Troponin T, fast skeletal muscle troponin					
Ratio		0.35	0.62	0.53	Spot 18
<i>t-test</i>		97.97	96.11	98.61	
<i>RSD</i>	14.75	57.73	16.34	10.44	
LIM domain-binding protein 3					
Ratio		0.78	0.8	0.56	Spot 19
<i>t-test</i>		75.52	98.57	99.12	
<i>RSD</i>	1.93	29.91	8.28	23.14	
Ratio		0.86	0.67	0.53	
<i>t-test</i>		73.01	99.07	99.11	
<i>RSD</i>	7.59	16.14	9.61	21.77	
Ratio		0.88	0.7	0.41	Spot 21
<i>t-test</i>		54.46	91.32	97.63	
<i>RSD</i>	12.35	18.01	20.52	47.59	
NADH-ubiquinone oxidoreductase 75 kDa subunit					
Ratio		0.3	0.1	1.22	Spot 11
<i>t-test</i>		99.65	99.96	92.79	
<i>RSD</i>	10.68	38.75	42.16	6.12	

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Comment about the t-test calculation provided by the Delta 2D software package, as provided here an in the following tables:

Delta2D computes t-test values for certain pairs of replicate groups. The test is a unpaired two-sided student's t-test, with the assumptions of equal unknown variance and unpaired samples. The null hypothesis is that both groups have equal means. Delta2D computes the p-value, which is the probability that a test statistic at least as significant as the one observed would be obtained assuming that the null hypothesis were true (i.e. the differences in quantities are the result of mere chance). The value q shown in the statistics table is derived from the p-value as follows:

$$q = (1 - p) \cdot 100$$

So higher values mean a higher probability that the null hypothesis is false. The value q is the t-test value shown for the test applied to every group vs. the first one displayed in the project manager.

Table S2: This table refers to table 2 of the main document and contains the original Delta2D

values, i.e. ratio, student-t (values show the probability in % that the null hypothesis (groups are equal) is false), relative standard deviation (RSD). The gel image is shown in Fig. 3, right panel.

	Control	3h	9h	24h	Spot #
Myosin light chain 3, skeletal muscle isoform					
Ratio		2.73	7.14	2.53	Spot 1
t-test		99.3	96.23	67.94	
RSD	42.23	8.22	39.31	74.4	
Myosin regulatory light chain 2, skeletal muscle isoform					
Ratio		1.97	1.93	1.46	Spot 2
t-test		99.99	99.6	93.4	
RSD	8.12	0.81	10.64	16.7	
Ratio		3.52	3.79	2.33	Spot 3
t-test		99.94	99.28	86.59	
RSD	22.77	8.07	19.72	41.95	
Myosin light chain 1, slow-twitch muscle B/ventricular isoform					
Ratio		3	2.65	1.3	Spot 4
t-test		99.99	99.99	41.49	
RSD	14.89	3.7	0.82	54.47	
Troponin T, slow skeletal muscle troponin					
Ratio		2.35	1.86	1.63	Spot 5
t-test		99.87	99.45	88.44	
RSD	11.16	5.13	10.38	26.53	
Ratio		1.78	1.94	1.36	Spot 6
t-test		96.29	99.57	54.67	
RSD	8.15	19.63	11.03	44.44	
Myosin light polypeptide 3					
Ratio		2.38	1.68	1.87	Spot 7
t-test		98.68	90.66	94.7	
RSD	20.02	17.41	90.66	94.7	
Ratio		4.33	2.97	2.35	Spot 8
t-test		99.75	98.88	94.22	
RSD	44.23	12.46	14.9	24.32	
Probable C→U-editing enzyme APOBEC-2					
Ratio		1.54	1.24	1.45	Spot 9
t-test		74.75	86.47	91.75	
RSD	13.36	35.89	9.94	16.8	
Actin, alpha skeletal muscle					
Ratio		1.2	2.67	1.85	Spot 10
t-test		56.11	97.63	89.98	
RSD	17.2	23.38	24.05	29.06	
ATP synthase subunit alpha					
Ratio		1.1	1.44	1.75	Spot 12
t-test		25.61	76.64	97.38	
RSD	27.09	27.75	24.71	8.55	
Fructose-bisphosphate aldolase A					
Ratio		1.42	1.91	1.28	Spot 13
t-test		91.76	96.06	60.45	
RSD	17.48	13.48	20.48	29.01	
Ratio		1.84	4.9	2.19	Spot 14
t-test		89.11	99.41	83.96	
RSD	33.83	25.42	19.83	41.95	

Deleted: High molecular weight actin aggregates

Deleted: NADH-ubiquinone oxidoreductase 75 kDa subunit [1]

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Table S3: Identification of proteins down-regulated by oxidative stress in spots 1 to 21 of the 2-DE presented in Fig. 3 (left panel) by peptide mass fingerprinting (PMF) and tandem mass spectrometry (MS/MS). Samples were digested with trypsin and analyzed on a MALDI-TOF/TOF-MS. Scores provided by MASCOT for the PMF and the resulting sequence coverage, as well as the sequences identified by MS/MS, are shown.

Spot N	Identified protein	Swiss-Prot ID	Mascot score	Matched peptides	Sequence coverage	MW	pI	MS/MS
1	<i>Heat shock cognate 71 kDa protein</i>	HSP7C_RAT	219	27	45%	70827	5.37	<u>138-155</u> (1982.05) <u>172-188</u> (1788.04) <u>300-311</u> (1480.79) <u>516-529</u> (1689.1)
2	<i>Skeletal muscle-specific alpha-actinin 3</i>	Q8R4I6_RAT	335	79	73%	102948	5.31	<u>728-739</u> (1372.9) <u>416-424</u> (1070.6) <u>516-529</u> (1689.1)
3	<i>Skeletal muscle-specific alpha-actinin 3</i>	Q8R4I6_RAT	324	78	74%	102948	5.31	<u>728-739</u> (1372.9) <u>416-424</u> (1070.6) <u>516-529</u> (1689.1)
4	<i>Skeletal muscle-specific alpha-actinin 3</i>	Q8R4I6_RAT	332	84	71%	102948	5.31	<u>728-739</u> (1372.9) <u>416-424</u> (1070.6) <u>516-529</u> (1689.1)
5	<i>Serum albumin precursor</i>	ALBU_RAT	151	41	62%	68686	6.09	<u>585-602</u> (1948.94) <u>104-117</u> (1684.76) <u>439-452</u> (1439.81)
6*	<i>Myosin binding protein H</i>	MYBPH_RAT	611	12	39%	52624	6.00	<u>5-23</u> (1828.87) <u>24-49</u> (4459.13) <u>264-281</u> (1847.08) <u>279-293</u> (1841.22)
7	<i>Glycogen phosphorylase, muscle form</i>	PYGM_RAT	189	54	56%	97212	6.91	<u>400-410</u> (1427.02) <u>643-650</u> (1053.75) <u>440-458</u> (2007.29)
8	<i>Glycogen phosphorylase, muscle form</i>	PYGM_RAT	196	45	46%	97212	6.91	<u>193-206</u> (1690.03) <u>400-410</u> (1426.9) <u>400-410</u> (1427.02)
9	<i>Glycogen phosphorylase, muscle form</i>	PYGM_RAT	174	45	45%	97212	6.91	<u>643-650</u> (1053.75) <u>440-458</u> (2007.29)

10	<i>Glycogen phosphorylase, muscle form</i>	PYGM_RAT	131	51	58%	97212	6.91	<u>279-293</u> (1841.22) <u>400-410</u> (1427.02) <u>643-650</u> (1053.75) <u>193-206</u> (1690.03)
11	<i>Glycogen phosphorylase, muscle form</i>	PYGM_RAT	107	58	60%	97212	6.91	<u>400-410</u> (1426.9) <u>279-293</u> (1841.22) <u>412-424</u> (1464.00)
12	<i>Aconitate hydratase</i>	ACON_RAT	142	38	51%	85380	7.87	<u>634-648</u> (1602.06) <u>69-84</u> (1869.22) <u>143-160</u> (1953.12)
13	<i>Aconitate hydratase</i>	ACON_RAT	148	44	53%	85380	7.87	<u>69-84</u> (1869.02) <u>145-160</u> (1753.96) <u>542-563</u> 3Pox (2015.3)
14	<i>Collagen alpha-1 chain</i>	CO1A1_RAT	54	28	31%	137802	5.77	<u>1142-1157</u> 3Pox (1562.1) <u>387-404</u> 3Pox (1586.1) <u>542-563</u> 3Pox (2015.3)
15	<i>Collagen alpha-1 chain</i>	CO1A1_RAT	51	27	31%	137802	5.77	<u>1142-1157</u> 3Pox (1562.1) <u>387-404</u> 3Pox (1586.1) <u>542-563</u> 3Pox (2015.3)
16	<i>Collagen alpha-1 chain</i>	CO1A1_RAT	57	29	35%	137802	5.77	<u>1142-1157</u> 3Pox (1562.1) <u>387-404</u> 3Pox (1586.1) <u>74-89</u> (1883.9)
17	<i>Pyruvate kinase isozymes M1/M2</i>	KPYM_RAT	245	48	71%	57781	6.63	<u>279-294</u> (1821.92) <u>423-436</u> (1473.82) <u>92-104</u> (1666.93)
18	<i>Troponin T, fast skeletal muscle troponin</i>	TNNT3_RAT	61	22	67%	30732	6.19	<u>164-174</u> (1265.68) <u>49-62</u> (1632.89) <u>17-31</u> (1691.89)
19	<i>LIM domain-binding protein 3</i>	LDB3_MOUSE	147	36	72%	76432	7.96	<u>22-31</u> (1192.59) <u>71-82</u> (1323.70) <u>22-31</u> MetOx (1208.59)
20*	<i>LIM domain-binding protein 3</i>	LDB3_MOUSE	492	9	11%	76432	7.96	<u>233-248</u> (1674.88) <u>249-262</u> (1660.73)
21*	<i>LIM domain-binding protein 3</i>	LDB3_MOUSE	411	8	10%	76432	7.96	<u>17-31</u> MetOx (1691.89) <u>274-294</u> 2MetOx (2498.14)

249-263 (1816.84)

* - measurements were done by UPLC-Orbitrap MS/MS

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Table S4: Identification of proteins up-regulated by oxidative stress in spots 1 to 14 of the 2-DE presented in Fig. 3 (right panel) by peptide mass fingerprinting (PMF) and tandem mass spectrometry (MS/MS). Samples were digested with trypsin and analyzed on a MALDI-TOF/TOF-MS. Scores provided by MASCOT for the PMF and the resulting sequence coverage, as well as the sequences identified by MS/MS, are shown.

Spot N	Identified protein	Swiss-Prot ID	Mascot score	Matched peptides	Sequence coverage	MW	pI	MS/MS
1	<i>Myosin light chain 3, skeletal muscle isoform</i>	MLE3_RAT	137	23	88%	16603	4.63	<u>81-93</u> (1484.7) <u>26-36</u> (1200.7) <u>21-36</u> (1776.0) <u>118-130</u> CysCAM(1652.9)
2	<i>Myosin regulatory light chain 2, skeletal muscle isoform</i>	MRLC_RAT	62	14	61%	18957	4.82	<u>91-105</u> (1560.9) <u>155-169</u> CysCAM(1762.9)
3	<i>Myosin regulatory light chain 2, skeletal muscle isoform</i>	MRLC_RAT	142	31	95%	18957	4.82	<u>42-51</u> (1390.87) <u>91-105</u> (1560.9) <u>32-41</u> (1192.74) <u>131-143</u> (1501.68)
4	<i>Myosin γ light chain 1, slow-twitch muscle B/ ventricular isoform</i>	MYL3_RAT	232	29	95%	22156	5.03	<u>148-159</u> (1233.60) <u>61-68</u> (1025.53) <u>60-68</u> (1144.5) <u>73-88</u> (1969.1)
5	<i>Troponin T, slow skeletal muscle</i>	TNNT1_RAT	94	15	44%	31084	5.85	<u>190-203</u> MetOx (1707.8) <u>60-68</u> (1144.5) <u>73-88</u> (1969.1)
6	<i>Troponin T, slow skeletal muscle</i>	TNNT1_RAT	37	8	32%	31084	5.85	<u>190-203</u> MetOx (1707.8) <u>76-86</u> CysCAM (1281.6) <u>87-99</u> (1396.7)
7	<i>Myosin light polypeptide 3</i>	MLY3_RAT	148	20	83%	22142	5.03	<u>131-143</u> (1501.6) <u>76-86</u> CysCAM(1281.6) <u>87-99</u> (1396.7)
8	<i>Myosin light polypeptide 3</i>	MYL3_RAT	133	24	75%	22142	5.03	<u>131-143</u> (1501.6) <u>30-46</u> (1938.12)
9*	<i>Probable C→U-editing enzyme</i>	ABEC2_MOUSE	384	10	6%	25644	4.75	

APOBEC-2								<u>47-53</u> (863.49)
								<u>178-200</u> (2873.33)
								<u>362-374</u> (1501.2)
10	Actin, alpha skeletal muscle	ACTS_RAT	99	17	41%	42024	5.23	<u>98-115</u> (1956.7)
								<u>241-256</u> (1791.5)
11	NADH-ubiquinone oxidoreductase 75 kDa subunit	NDUS1_RAT	92	11	19%	79362	5,65	<u>429-441</u> (1403,78)
								<u>409-417</u> (1064,54)
								<u>312-325</u> (1611,8)
12	ATP synthase subunit alpha	ATPA_RAT	293	51	73%	59717	9.22	<u>302-316</u> (1701.9)
								<u>134-149</u> (1610.9)
								<u>335-347</u> (1553.8)
13	Fructose-bisphosphate aldolase A	ALDOA_RAT	147	25	79%	39221	8.40	<u>88-99</u> (1342.9)
								<u>2-13</u> (1377.9)
								<u>44-57</u> (1647.0)
14	Fructose-bisphosphate aldolase A	ALDOA_RAT	77	17	57%	39221	8.40	<u>88-99</u> (1343.3)
								<u>2-13</u> (1377.9)
								<u>44-57</u> (1647.0)

* - measurements were done by UPLC-Orbitrap MS/MS

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Table S5: This table refers to table 3 of the main document and contains the original Delta2D values, i.e. ratio, student-t (values show the probability in % that the null hypothesis (groups are equal) is false), relative standard deviation (RSD). The images of the Western blot are shown in Fig. 4 and Fig. S2.

Spot #			Swiss Prot ID	C		3h				9h				24h				
C	3h	9h		24h	Mean	RSD	Mean	RSD	Ratio	t-test	Mean	RSD	Ratio	t-test	Mean	RSD	Ratio	t-test
Muscle contraction																		
1	2	3	MLE1_RAT	9,25	3,01	8,71	5,99	0,94	73,42	1,08	23,34	0,12	100	1,42	20,02	0,15	100	
	3	2	MLY3_RAT	0,39	13,74	1,58	30,35	4,01	97,46	0,21	31,24	0,55	95,73	0,16	50,94	0,41	97,1	
				0,07	29,06	0,4	46,52	5,58	93,18	0,14	25,51	1,95	92,05	0,14	22,68	1,92	93,27	
22	25	32	41 36	TNNT3_RAT	0,15	41,86	0,56	36,66	3,68	94,5	0,98	8,93	6,37	99,96	0,54	58,21	3,52	83,66
					0,41	3,19	0,36	10,86	0,89	80,28	1,12	4,48	2,75	100	0,82	31,73	2,02	91,23
					0,84	22,5	0,47	29,44	0,56	91,19	2,22	14,88	2,66	99,33	0,69	25,79	0,83	52,31
					0,4	28,25	0,42	38,14	1,04	8,64	0,42	11,73	1,05	18,33	0,81	21,37	2,01	95,03
					0,22	31,56	0,64	42,84	2,86	89,4	0,77	10,46	3,43	99,8	0,91	21,25	4,06	99,08
23		35 36	43	TNNI2_RAT	1,07	35,96	0,15	47,18	0,14	97,07	2,08	24,99	1,94	90,83	0,49	30,96	0,45	88,36
					3,36	16,92	2,61	23,78	0,78	72,29	2,79	9,79	0,83	72,64	2,26	39,26	0,67	78,67
					0,75	38,44	1,42	46,53	1,9	74,3	2,22	8,04	2,97	99,64	0,18	59,99	0,24	94,25
					0,52	26,64	0,16	81,77	0,32	93,96	1,81	4,42	3,51	99,97	0,21	35,19	0,41	95,02
17	10 14 15	20 21 18	MYH4_RAT	0,98	31,99	0,87	41,52	0,89	23,06	2,67	7,88	2,73	99,68	3,26	14,87	3,34	99,5	
				0,28	13,4	0,12	58,82	0,45	94,29	0,52	72,9	1,88	58,15	0,59	7,51	2,13	99,84	
				0,13	44,28	0,45	35,35	3,45	94,42	0,41	16,92	3,12	98,78	0,98	36,9	7,5	96,95	
				0,12	35	0,13	20,91	1,11	27,07	0,13	47,82	1,05	8,44	0,97	6,38	8,14	99,99	
				0,35	33,48	0,12	79,26	0,33	90,77	0,96	9,4	2,73	99,56	0,93	21,78	2,64	97,48	
13	8	7	TPM1_RAT TPM2_RAT	0,41	71,22	0,69	47,98	1,7	58,6	4,82	11,4	11,8	99,94	4,18	24,73	10,2	99,23	
	17	22	Q5XKE0_RAT	0,06	49,87	0,04	43,54	0,59	63,67	0,23	16,59	3,71	99,16	0,12	45,01	2,02	76,34	
14	16		Q8R4I6_RAT	0,22	38,8	0,08	36,96	0,35	91,06	0,18	25,99	0,83	39,23	0,86	9,86	3,86	99,83	
				0,15	61,82	0,44	19,81	3,03	97,11	0,88	10,07	6,06	99,88	1,68	6,82	11,53	99,99	
				0,04	93,25	0,29	21,53	6,5	99,02	0,41	10,74	9,12	99,89	0,52	10,16	11,7	99,94	

Carbohydrate metabolism: Glycolysis, Gluconeogenesis and TCA cycle

18	22	22	34	ENOB_RAT	0,5	55,75	0,71	12,16	1,42	63,25	0,44	18,7	0,89	20,34	0,5	14,9	1	0,9	
					0,34	43,97	1,56	11,16	4,54	99,83	0,74	15,84	2,15	95,68	0,81	10,59	2,36	98,11	
					0,25	49,38	0,65	4,9	2,57	98,8	0,71	14,56	2,8	98,36	0,47	39,73	1,88	76,41	

				0,23	8,48	0,51	26,65	2,17	95,29	0,45	12	1,94	99,41	0,29	43,56	1,22	41,19
				0,16	48,32	0,4	4,17	2,53	98,81	0,65	15,9	4,19	99,46	0,72	38,34	4,59	95,02
26				0,11	70,01	0,17	6,65	1,59	69,45	0,15	67,84	1,4	34,31	0,33	25,07	3,09	95,29
31	40			0,41	17,91	0,56	43,4	1,37	55,38	1,98	7,81	4,82	99,98	0,98	39,52	2,39	88,97
				0,72	24,94	1,09	29,83	1,52	77,04	0,49	13,98	0,67	84,03	0,82	17,95	1,14	42,35
15	19	30		0,33	9,63	0,06	94,36	0,17	99,67	0,33	15,8	1,01	4,07	0,8	18,09	2,41	98,89
				0,39	6,62	0,11	71,05	0,27	99,33	0,56	32,05	1,42	73,07	0,76	15,7	1,93	98,69
				0,39	22,41	0,4	49,06	1,04	6,9	0,65	8,01	1,67	97,81	0,66	14,15	1,69	95,93
				0,15	3,27	0,06	69,39	0,38	97,03	0,25	26,65	1,64	89,13	0,25	14,01	1,62	98,1
				0,39	30,54	0,17	76,92	0,43	85,39	0,44	10,87	1,12	37,42	0,64	16,4	1,64	91,09
				0,27	16,15	0,1	62,63	0,39	95,91	0,39	10,98	1,46	95,43	0,44	10,7	1,63	98
20	25	38		0,07	58	0,09	37,96	1,35	44,69	0,1	26,53	1,47	60,71	0,17	12,06	2,41	96,32
21	24	30	39	0,26	19,96	0,09	33,98	0,36	98,24	0,65	22,31	2,5	97,7	0,25	15,52	0,95	19,32
				0,1	7,02	0,07	41,15	0,65	85,55	1,31	14,65	12,87	99,91	0,3	67,91	2,89	75,5
				0,33	50,71	0,74	23,17	2,25	92,85	1,31	16,87	3,98	99,26	1,16	63,36	3,53	80,62
				0,41	7,6	0,45	27,04	1,09	31,45	0,88	13,98	2,17	99,39	0,85	28,38	2,08	93,8
12	11	12	24	0,17	22,48	0,5	34,52	3,03	94,5	0,21	37,21	1,26	47,91	0,6	61,98	3,63	82,47
				0,58	36,02	1,13	12,82	1,93	96,08	0,85	40,78	1,45	58,85	1,21	23,76	2,06	93,13
				0,48	48,86	2,18	18,37	4,54	99,34	1,62	24,22	3,38	97,58	1,82	25,17	3,78	97,88
				0,12	63,26	0,39	17,39	3,22	97,95	0,94	33,33	7,67	97,69	0,53	25,65	4,33	97,89
17	20	31		0,15	16,45	0,02	116,09	0,16	99,15	0,16	52,96	1,07	11,86	0,14	25,97	0,93	25,29
				0,33	59	0,37	19,51	1,09	15,29	0,35	12,54	1,04	6,2	0,58	22,95	1,73	78,2
				0,33	101,57	0,32	27,86	0,96	3,76	0,63	15,19	1,9	70,4	0,31	23,78	0,92	7,82
				0,13	56,58	0,18	54,93	1,47	46,1	0,25	67,4	2,02	61,73	0,13	31,39	1,04	6,68
	32			0,18	20,15	0,24	67,76	1,37	38,87	0,72	20,2	4,06	99,31	0,49	49,22	2,78	85,69
				0,05	117,92	0,06	85,39	1,2	13,55	0,26	45,61	5,06	90,9	0,08	43,23	1,58	41,98
2	4	4	4	0,58	31,27	1,01	11,15	1,73	95,24	0,03	57,37	0,06	98,68	0,2	18	0,35	95,53
				0,74	20,09	1,59	11,92	2,17	99,27	0,75	13,84	1,02	10,09	0,25	7,42	0,34	98,98
				0,43	16,52	2,13	27,73	5,01	98,46	0,13	36,03	0,3	99,24	0,26	70,85	0,62	68,37

Energy metabolism

ATP metabolic/catabolic processes

6				ATPB_RAT	4,79	2,81	1,77	21,68	0,37	99,95	0	69,92	0	100	0,3	76,74	0,06	100
21	33			ATPA_RAT	0,32	44,13	0,13	83,89	0,39	81,06	0,34	27,2	1,04	7,48	0,49	15,7	1,52	78,35
					0,08	29,63	0,09	125,89	1,04	3,53	0,36	16,04	4,35	99,67	0,38	55,32	4,5	88,05
					0,48	17,9	0,3	32,1	0,62	88,19	1,25	9,51	2,6	99,82	0,98	39,97	2,03	84,62
34				KAD1_RAT	0,25	93,51	0,11	8,56	0,44	55,54	0,47	25,52	1,86	69,04	0,15	42,97	0,61	40
15				NDUS1_RAT	0,51	47,78	0,72	25,14	1,4	60,89	0,49	31,07	0,95	9,45	0,95	5,33	1,86	93,23
					0,8	46,93	1,22	11,91	1,51	77,89	1,01	21,82	1,26	46,99	1,15	5,56	1,44	73,47
					0,38	49,17	1,08	15,19	2,81	98,29	0,93	31,77	2,42	90,75	1,29	37,03	3,35	93,25
					0,05	34,05	0,35	15,59	6,9	99,82	0,31	43,67	6,15	94,54	0,71	34,3	14,08	98,12
					0,01	118,56	0,19	7,33	33,92	99,99	0,45	26,96	79,2	99,34	0,48	23,3	84,61	99,61

<i>Phosphocreatine biosynthesis</i>																		
26	37	KCRS_RAT	0,32	7,86	0,69	17,32	2,14	98,68	0,7	24,19	2,16	96,41	0,76	16,37	2,34	99,17		
19	23	23 24	35	KCRM_RAT	2.28	47,35	1,97	12,73	0,86	28,7	1,65	16,17	0,73	52,82	3,43	3,65	1,51	79,32
					2,23	54,36	4,25	19,34	1,9	87,68	2,89	11,09	1,3	50,29	3,6	1,42	1,61	81,46
					2,49	25,24	4,42	26,13	1,77	89,3	3,64	4,58	1,46	93,26	3,28	11,87	1,31	79,12
					1,36	29,05	4,17	20,02	3,08	98,75	2,68	9,53	1,98	98,37	2,35	17,74	1,73	93
					0,73	14,82	2,04	16,47	2,81	99,38	2,41	20,18	3,31	99,12	1,13	26,37	1,56	85,44
					0,88	35,08	1,11	30,51	1,27	48,91	2,47	2,21	2,81	99,8	1,91	15,53	2,17	97,29
					0,62	55,85	2,13	21,32	3,42	97,96	2,86	4,51	4,59	99,9	2,89	13,25	4,65	99,66
<i>Miscellaneous</i>																		
16	13	13	HSP7C_RAT	0,07	65,9	0,3	10,14	4,17	99,54	0,36	3,01	5,1	99,9	0,63	25,13	8,89	99,13	
				0,02	89,68	0,17	44,56	7,65	94,41	0,51	18,9	23,78	99,78	0,78	14,59	36,15	99,93	
21	18	27	ALBU_RAT	0,24	51,53	0,39	12,24	1,64	82,5	0,44	31,44	1,84	79,85	0,75	26,5	3,11	96,26	
26	33	42	CAH3_RAT	0,79	60,22	0,56	41,61	0,71	43	0,68	8,79	0,87	23,06	0,71	47,75	0,91	13,26	
				0,49	64,26	0,94	33,37	1,92	77,65	0,63	42,68	1,29	34,51	0,84	45,9	1,72	62,7	
16		28 29	CO1A1_RAT	3,38	10,52	0,32	95,15	0,09	99,92	0,39	34,88	0,12	99,96	2,86	12,44	0,85	78,48	
				3,12	41,35	0,35	100,68	0,11	95,73	0,23	58,83	0,07	96,54	2,45	6,62	0,79	49,11	

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Table S6: Identification of carbonylated proteins in the control sample by peptide mass fingerprinting (PMF) and tandem mass spectrometry (MS/MS). Samples were digested with trypsin and analyzed on a MALDI-TOF/TOF-MS. Scores provided by MASCOT for the PMF and the resulting sequence coverage, as well as the sequences identified by MS/MS, are shown.

Spot N	Identified protein	Swiss-Prot ID	Mascot score	Matched peptides	Sequence coverage	MW	pI	MS/MS
1	<i>Myosin light chain 1, skeletal muscle isoform</i>	MLE1_RAT	79	18	91%	20666	4.99	<u>65-75</u> (1200.7) <u>120-132</u> (1484.6) <u>133-148</u> (1722.8)
2	<i>Pyruvate dehydrogenase E1 component subunit beta</i>	ODPB_RAT	67	15	40%	38957	6.20	<u>309-324</u> (1747.84) <u>309-324</u> MetOx (1763.82) <u>259-269</u> CysCAM (1331.6)
3	<i>Tropomyosin alpha-1 chain</i> <i>Tropomyosin beta chain</i>	TPM1_RAT TPM2_RAT	182 103	22 17	58% 41%	32661 32817	4.69 4.66	<u>92-105</u> (1727.9) <u>78-91</u> (1488.8) <u>252-268</u> (2017.3)
4	<i>Actin, alpha skeletal muscle</i>	ACTS_RAT	59	31	62%	42024	5.23	<u>362-374</u> (1501.2) <u>98-115</u> (1956.7) <u>241-256</u> (1791.5)
5	<i>Actin, alpha skeletal muscle</i>	ACTS_RAT	100	11	27%	42024	5.23	<u>241-256</u> (1791.0) <u>362-374</u> (1500.8) <u>98-115</u> (1956.2)
6	<i>ATP synthase subunit beta</i>	ATPB_RAT	101	17	34%	56318	5.19	<u>388-406</u> (1988.3) <u>125-143</u> (1919.3) <u>407-422</u> (1832.1)
7	<i>Desmin</i>	DESM_RAT	91	21	48%	53424	5.21	<u>449-469</u> (2369.5) <u>17-37</u> (2078.4) <u>299-314</u> (1703.13)

8	78 kDa glucose-regulated protein	GRP78_RAT	73	23	35%	72302	5.07	164-181 (1888.3) 198-214 (1816.3)
9	Actin, alpha skeletal muscle	ACTS_RAT	84	13	38%	42024	5.23	362-374 (1501.2) 98-115 (1956.7) 241-256 (1791.5)
10	Skeletal muscle-specific alpha-actinin 3	Q8R4I6_RAT	335	79	73%	102948	5.31	516-529 (1689.1) 728-739 (1372.9) 416-424 (1070.6)
11	Actin, alpha skeletal muscle	ACTS_RAT	84	13	38%	42024	5.23	362-374 (1501.2) 98-115 (1956.7) 241-256 (1791.5)
12	Dihydrolipoyllysine-residue succinyltransferase component of 2-oxoglutarate dehydrogenase complex	ODO2_RAT	100	23	37%	48894	8.89	309-326 (2066.0) 314-326 (1419.7) 1189.6 (135-145)
13	Myosin-4	MYH4_RAT	55	31	21%	222879	5.58	923-943 (2431.3) 1255-1272 (2190.1) 684-698 (1730.9)
14	Skeletal muscle-specific alpha-actinin 3	Q8R4I6_RAT	378	90	81%	102948	5.31	516-529 (1689.1) 728-739 (1372.9) 416-424 (1070.6)
15	Glycogen phosphorylase, muscle form	PYGM_RAT	196	45	46%	97212	6.91	440-458 (2007.3) 193-206 (1690.0) 400-410 (1426.9)
16	Collagen alpha-1 chain	CO1A1_RAT	54	28	31%	137802	5.77	542-563 3Pox (2015.3) 1142-1157 3Pox (1562.1) 387-404 3Pox (1586.1)
17	Pyruvate kinase isozymes M1/M2	KPYM_RAT	139	58	81%	57781	6.63	295-311 (1828.4) 423-436 CysCAM (1474.2) 267-278 (1395.1)
18	Beta enolase	ENOB_RAT	104	28	67%	46984	7.08	33-50 (1805.5) 413-426 (1476.2) 16-28 (1381.1)

19	<i>Creatine kinase M-type</i>	KCRM_RAT	85	29	62%	43018	6.58	<u>321-341</u> (1993.7)
								<u>157-172</u> (1693.5)
								<u>117-130</u> (1508.2)
20	<i>Fructose-bisphosphate aldolase A</i>	ALDOA_RAT	77	17	57%	39221	8.40	<u>88-99</u> (1343.3)
								<u>2-13</u> (1377.9)
								<u>44-57</u> (1647.0)
21	<i>Glyceraldehyde-3-phosphate dehydrogenase</i>	G3P_RAT	164	40	69%	35805	8.14	<u>233-246</u> CysCAM (1556.9)
								<u>308-321</u> (1779.9)
								<u>65-78</u> (1628.1)
22	<i>Troponin T, fast skeletal muscle</i>	TNNT3_RAT	71	23	54%	30732	6.19	<u>49-62</u> (1633.1)
								<u>225-233</u> (1137.8)
								<u>184-196</u> (1551.1)
23	<i>Troponin I, fast skeletal muscle</i>	TNNI2_RAT	46	21	64%	21315	8.86	<u>92-104</u> (1666.9)
								<u>164-174</u> (1265.7)

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Table S7: Identification of carbonylated proteins in the 3 h sample by peptide mass fingerprinting (PMF) and tandem mass spectrometry (MS/MS). Samples were digested with trypsin and analyzed on a MALDI-TOF/TOF-MS. Scores provided by MASCOT for the PMF and the resulting sequence coverage, as well as the sequences identified by MS/MS, are shown.

Spot N	Identified protein	Swiss-Prot ID	Mascot score	Matched peptides	Sequence coverage	MW	pI	MS/MS
1	<i>Troponin T, fast skeletal muscle</i>	TNNT3_RAT	60	18	49%	30732	6.19	<u>49-62</u> (1633.1) <u>49-63</u> (1760.8) <u>65-83</u> MetOx (2273.5)
2	<i>Myosin light chain 1, skeletal muscle isoform</i>	MLE1_RAT	164	18	91%	20666	4.99	<u>65-75</u> (1200.7) <u>120-132</u> (1484.6) <u>133-148</u> (1722.8)
3	<i>Myosin light polypeptide 3</i>	MLY3_RAT	148	20	83%	22142	5.03	<u>76-86</u> CysCAM (1281.6) <u>87-99</u> (1396.7) <u>131-143</u> (1501.6)
4	<i>Pyruvate dehydrogenase E1 component subunit beta</i>	ODPB_RAT	80	15	40%	38957	6.20	<u>309-324</u> (1747.84) <u>309-324</u> MetOx (1763.82) <u>259-269</u> CysCAM (1331.6)
5	<i>Unidentified protein</i>							
6	<i>Tropomyosin alpha-1 chain</i> <i>Tropomyosin beta chain</i>	TPM1_RAT TPM2_RAT	160 53	22 10	54% 35%	32661 32817	4.69 4.66	<u>168-178</u> (1314.7) <u>91-101</u> (1399.7) <u>92-105</u> (1727.9)
7	<i>Unidentified protein</i>							
8	<i>Actin, alpha skeletal muscle</i>	ACTS_RAT	84	13	38%	42024	5.23	<u>362-374</u> (1500.8) <u>98-115</u> (1956.1) <u>241-256</u> (1790.9)
9	<i>Actin, alpha skeletal muscle</i>	ACTS_RAT	100	11	27%	42024	5.23	<u>362-374</u> (1500.8) <u>98-115</u> (1956.2)

								<u>241-256</u> (1791.0)
10	<i>Desmin</i>	DESM_RAT	95	16	38%	53424	5.21	<u>17-37</u> (2078.1) <u>299-314</u> (1729.9) <u>449-469</u> (2369.5)
11	<i>Dihydrolipoyllysine-residue succinyltransferase component of 2-oxoglutarate dehydrogenase complex</i>	ODO2_RAT	111	22	37%	48894	8.89	<u>309-326</u> (2066.0) <u>314-326</u> (1419.7) <u>5-14</u> (1168.5)
12	<i>Actin, alpha skeletal muscle</i>	ACTS_RAT	87	15	46%	42024	5.23	<u>362-374</u> (1501.2) <u>98-115</u> (1956.7) <u>241-256</u> (1791.5)
13	<i>Tropomyosin alpha-1 chain</i> <i>Tropomyosin beta chain</i>	TPM1_RAT TPM2_RAT	104 76	32 27	67% 62%	32661 32817	4.69 4.66	<u>106-125</u> (2202.2) <u>92-105</u> (1727.9) <u>13-21</u> (1073.6)
14	<i>Dihydrolipoyllysine-residue acetyltransferase component of pyruvate dehydrogenase complex</i>	ODP2_RAT	132	30	51%	67123	8.76	<u>397-414</u> (1880.0) <u>397-415</u> (2036.1) <u>383-396</u> (1188.6)
15	<i>Actin, alpha skeletal muscle</i>	ACTS_RAT	200	45	81%	42024	5.23	<u>294-314</u> (2246.0) <u>180-193</u> (1623.8) <u>31-41</u> (1198.7)
16	<i>Heat shock cognate 71 kDa protein</i>	HSP7C_RAT	219	27	45%	70827	5.37	<u>138-155</u> (1982.0) <u>172-188</u> (1788.0) <u>300-311</u> (1480.8)
17	<i>Myosin-4</i>	MYH4_RAT	91	53	26%	222879	5.58	<u>1181-1197</u> (1838.9) <u>285-296</u> (1516.8) <u>1119-1130</u> (1488.8)
18	<i>Myosin-4</i>	MYH4_RAT	93	39	25%	222879	5.58	<u>1480-1489</u> (1151.7) <u>1284-1293</u> (1162.6) <u>1310-1321</u> (1490.8)
19	<i>Skeletal muscle-specific alpha-actinin 3</i>	Q8R4I6_RAT	324	78	74%	102948	5.31	<u>516-529</u> (1689.1) <u>728-739</u> (1372.9) <u>416-424</u> (1070.6)
20	<i>Myosin-4</i>	MYH4_RAT	331	89	41%	222879	5.58	<u>684-698</u> (1730.9) <u>1119-1130</u> (1488.8)

								<u>1310-1321</u> (1490.8)
21	<i>Serum albumin precursor</i>	ALBU_RAT	151	41	62%	68686	6.09	<u>585-602</u> CysCAM(1948.9)
								<u>104-117</u> 2CysCAM(1684.8)
								<u>439-452</u> (1439.8)
22	<i>Beta-enolase</i>	ENOB_RAT	230	50	80%	46984	7.08	<u>33-50</u> (1805.0)
								<u>359-372</u> (1541.8)
								<u>413-426</u> (1475.8)
23	<i>Creatine kinase M-type</i>	KCRM_RAT	187	32	67%	43018	6.58	<u>139-148</u> CysCAM(1187.7)
								<u>305-314</u> (1269.8)
								<u>33-43</u> (1331.9)
24	<i>Glyceraldehyde-3-phosphohate dehydrogenase</i>	G3P_RAT	187	37	70%	35805	8.14	<u>233-246</u> CysCAM (1556.9)
								<u>308-321</u> (1779.9)
								<u>65-78</u> (1628.1)
25	<i>Troponin T, fast skeletal muscle</i>	TNNT3_RAT	94	15	44%	30732	6.19	<u>49-62</u> (1633.1)
								<u>68-83</u> (1888.0)
								<u>65-83</u> (2258.2)
26	<i>Carbonic anhydrase 3</i>	CAH3_RAT	127	15	59%	29413	6.89	<u>227-251</u> (2743.6)
								<u>37-57</u> (2355.2)
								<u>172-188</u> (2065.0)

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Table S8: Identification of carbonylated proteins in the 9 h sample by peptide mass fingerprinting (PMF) and tandem mass spectrometry (MS/MS). Samples were digested with trypsin and analyzed on a MALDI-TOF/TOF-MS. Scores provided by MASCOT for the PMF and the resulting sequence coverage, as well as the sequences identified by MS/MS, are shown.

Spot N	Identified protein	Swiss-Prot ID	Mascot score	Matched peptides	Sequence coverage	MW	pI	MS/MS
1	<i>Myosin regulatory light chain 2, skeletal muscle isoform</i>	MRLC_RAT	62	14	61%	18957	4.82	118-130 CysCAM(1652.9) 91-105 (1560.9) 155-169 CysCAM(1762.9)
2	<i>Myosin light polypeptide 3</i>	MYL3_RAT	133	24	75%	22142	5.03	76-86 CysCAM(1281.6) 87-99 (1396.7) 131-143 (1501.6)
3	<i>Undetected protein</i>							
4	<i>Pyruvate dehydrogenase E1 component subunit beta</i>	ODPB_RAT	67	15	40%	38957	6.20	309-324 (1747.84) 309-324 MetOx (1763.82) 259-269 CysCAM (1331.6)
5	<i>Tropomyosin alpha-1 chain</i> <i>Tropomyosin beta chain</i>	TPM1_RAT TPM2_RAT	66 34	26 22	72% 61%	32661 32817	4.69 4.66	92-105 (1727.9) 78-91 (1488.8) 252-268 (2017.3)
6	<i>Actin, alpha skeletal muscle</i>	ACTS_RAT	160	29	69%	42024	5.23	362-374 (1501.2) 98-115 (1956.7) 241-256 (1791.5)
7	<i>Actin, alpha skeletal muscle</i>	ACTS_RAT	99	17	41%	42024	5.23	362-374 (1501.2) 98-115 (1956.7) 241-256 (1791.5)
8	<i>Tropomyosin alpha-1 chain</i>	TPM1_RAT	72	14	44%	32661	4.69	92-105 (1727.9)

	<i>Tropomyosin beta chain</i>	TPM2_RAT	30	9	33%	32817	4.66	78-91 (1488.8) 252-268 (2017.3)
9	<i>Actin, alpha skeletal muscle</i>	ACTS_RAT	203	41	81%	42024	5.23	294-314 (2246.0) 180-193 (1623.8) 31-41 (1198.7)
								429-441 (1403.78) 409-417 (1064.54) 312-325 (1611.8)
								923-943 (2431.3) 1181-1197 (1838.9) 1480-1489 (1151.7)
								449-469 (2369.5) 17-37 (2078.4) 299-314 (1703.13)
12	<i>Dihydrolipoyllysine-residue succinyltransferase component of 2-oxoglutarate dehydrogenase complex</i>	ODO2_RAT	111	22	37%	48894	8.89	923-943 (2431.3) 1255-1272 (2190.1) 684-698 (1730.9)
								138-155 (1982.0) 172-188 (1788.0) 300-311 (1480.8)
								1255-1272 (2190.1) 285-296 (1516.8) 1284-1293 (1162.6)
14	<i>Myosin-4</i>	MYH4_RAT	126	56	30%	222879	5.58	684-698 (1730.9) 285-296 (1516.8) 1480-1489 (1151.7)
								516-529 (1689.1) 728-739 (1372.9) 416-424 (1070.6)
								167-185 (1924.9) 224-234 (1296.7) 330-337 (1043.5)
15	<i>Myosin-4</i>	MYH4_RAT	94	43	25%	222879	5.58	585-602
16	<i>Skeletal muscle-specific alpha-actinin 3</i>	Q8R4I6_RAT	332	84	71%	102948	5.31	
17	<i>Myosin-binding protein C, fast-type</i>	Q5XKE0	460	8	12%	127352	6.02	
18	<i>Serum albumin precursor</i>	ALBU_RAT	59	22	44%	68686	6.09	

								CysCAM(1948.9)
								104-117
								2CysCAM(1684.8)
								439-452 (1439.8)
19	<i>Glycogen phosphorylase, muscle form</i>	PYGM_RAT	196	45	46%	97212	6.91	440-458
								CysCAM(2007.3)
								193-206 (1690.0)
								400-410 (1426.9)
20	<i>Pyruvate kinase isozymes M1/M2</i>	KPYM_RAT	245	48	71%	57781	6.63	74-89 (1883.9)
								279-294 (1821.9)
								423-436
								CysCAM(1473.8)
21	<i>ATP synthase subunit alpha</i>	ATPA_RAT	293	51	73%	59717	9.22	302-316 (1701.9)
								134-149 (1610.9)
								335-347 (1553.8)
22	<i>Beta-enolase</i>	ENOB_RAT	367	57	83%	46984	7.08	33-50 (1805.0)
								359-372 (1541.8)
								413-426 (1475.8)
23	<i>Creatine kinase M-type</i>	KCRM_RAT	148	36	70%	43018	6.58	139-148 (1187.7)
								305-314 (1269.8)
								33-43 (1331.9)
24	<i>Creatine kinase M-type</i>	KCRM_RAT	159	28	62%	43018	6.58	321-341 (1993.7)
								157-172 (1693.5)
								117-130 (1508.2)
25	<i>Fructose-bisphosphate aldolase A</i>	ALDOA_RAT	147	25	79%	39221	8.40	88-99 (1342.9)
								2-13 (1377.9)
								44-57 (1647.0)
26	<i>Creatine kinase, sarcomeric</i>	KCRS_RAT	164	44	72%	47355	8.76	404-418 (1648.9)
								311-326
								CysCAM(1734.9)
								150-164 (1807.9)
27	<i>Fructose-bisphosphate aldolase A</i>	ALDOA_RAT	140	28	89%	39221	8.40	88-99 (1343.3)
								2-13 (1377.9)
								44-57 (1647.0)

28	<i>Troponin T, fast skeletal muscle</i>	TNNT3_RAT	94	15	44%	30732	6.19	<u>49-62</u> (1633.1) <u>225-233</u> (1137.8) <u>184-196</u> (1551.1)
29	<i>Troponin T, fast skeletal muscle</i>	TNNT3_RAT	37	8	32%	30732	6.19	<u>49-62</u> (1633.1) <u>225-233</u> (1137.8) <u>184-196</u> (1551.1)
30	<i>Glyceraldehyde-3-phosphate dehydrogenase</i>	G3P_RAT	183	49	75%	35805	8.14	<u>233-246</u> CysCAM (1556.9) <u>308-321</u> (1779.9) <u>65-78</u> (1628.1)
31	<i>L-lactate dehydrogenase A chain</i>	LDHA_RAT	55	15	29%	36427	8.45	<u>319-332</u> (1635.9) <u>279-305</u> (2920.7) <u>2-22</u> (2391.3)
32	<i>Troponin T, fast skeletal muscle</i>	TNNT3_RAT	62	21	50%	30732	6.19	<u>49-62</u> (1633.1) <u>225-233</u> (1137.8) <u>184-196</u> (1551.1)
33	<i>Carbonic anhydrase 3</i>	CAH3_RAT	63	14	55%	29413	6.89	<u>68-76</u> (1113.9) <u>25-36</u> (1339.1) <u>68-80</u> (1671.3)
34	<i>Adenylate kinase isoenzyme 1</i>	KAD1_RAT	109	14	57%	21570	7.66	<u>84-97</u> (1539.8) <u>32-44</u> (1495.8) <u>156-167</u> (1425.8)
35	<i>Troponin I, fast skeletal muscle</i>	TNNI2_RAT	61	22	67%	21315	8.86	<u>92-104</u> (1666.9) <u>164-174</u> (1265.7) <u>154-163</u> (1259.7)

Table S9: Identification of carbonylated proteins in the 24 h sample by peptide mass fingerprinting (PMF) and tandem mass spectrometry (MS/MS). Samples were digested with trypsin and analyzed on a MALDI-TOF/TOF-MS. Scores provided by MASCOT for the PMF and the resulting sequence coverage, as well as the sequences identified by MS/MS, are shown.

Spot N	Identified protein	Swiss-Prot ID	Mascot score	Matched peptides	Sequence coverage	MW	pI	MS/MS
1	<i>Myosin light chain 3, skeletal muscle isoform</i>	MLE3_RAT	137	23	88%	16603	4.63	<u>81-93</u> (1484.7) <u>26-36</u> (1200.7) <u>21-36</u> (1776.0)
2	<i>Troponin T, fast skeletal muscle</i>	TNNT3_RAT	15	4	17%	30732	6.19	<u>49-62</u> (1633.1) <u>49-63</u> (1760.8) <u>65-83</u> MetOx (2273.5)
3	<i>Myosin light chain 1, skeletal muscle isoform</i>	MLE1_RAT	112	19	91%	20666	4.99	<u>65-75</u> (1200.7) <u>120-132</u> (1484.6) <u>133-148</u> (1722.8)
4	<i>Pyruvate dehydrogenase E1 component subunit beta</i>	ODPB_RAT	80	15	40%	38957	6.20	<u>309-324</u> (1747.84) <u>309-324</u> MetOx (1763.82) <u>259-269</u> CysCAM (1331.6)
5	<i>Tropomyosin alpha-1 chain</i> <i>Tropomyosin beta chain</i>	TPM1_RAT TPM2_RAT	80 55	14 13	45% 38%	32661 32817	4.69 4.66	<u>168-178</u> (1314.7) <u>91-101</u> (1399.7) <u>92-105</u> (1727.9)
6	<i>Actin, alpha skeletal muscle</i>	ACTS_RAT	235	41	86%	42024	5.23	<u>362-374</u> (1500.8) <u>98-115</u> (1956.1) <u>241-256</u> (1790.9)
7	<i>Tropomyosin alpha-1 chain</i> <i>Tropomyosin beta chain</i>	TPM1_RAT TPM2_RAT	48 76	13 27	40% 62%	32661 32817	4.69 4.66	<u>168-178</u> (1314.7) <u>91-101</u> (1399.7) <u>92-105</u> (1727.9)
8	<i>Actin, alpha skeletal muscle</i>	ACTS_RAT	136	19	49%	42024	5.23	<u>362-374</u> (1500.8) <u>98-115</u> (1956.1)

								<u>241-256</u> (1790.9)
9	<i>Actin, alpha skeletal muscle</i>	ACTS_RAT	129	24	50%	42024	5.23	<u>362-374</u> (1500.8) <u>98-115</u> (1956.1) <u>241-256</u> (1790.9)
10	<i>Tropomyosin alpha-1 chain</i> <i>Tropomyosin beta chain</i>	TPM1_RAT TPM2_RAT	66 34	26 22	72% 61%	32661 32817	4.69 4.66	<u>168-178</u> (1314.7) <u>91-101</u> (1399.7) <u>92-105</u> (1727.9)
11	<i>Actin, alpha skeletal muscle</i>	ACTS_RAT	73	18	64%	42024	5.23	<u>362-374</u> (1500.8) <u>98-115</u> (1956.1) <u>241-256</u> (1790.9)
12	<i>Actin, alpha skeletal muscle</i>	ACTS_RAT	77	19	52%	42024	5.23	<u>362-374</u> (1500.8) <u>98-115</u> (1956.1) <u>241-256</u> (1790.9)
13	<i>Heat shock cognate 71 kDa protein</i>	HSP7C_RAT	138	30	57%	70827	5.37	<u>138-155</u> (1982.0) <u>172-188</u> (1788.0) <u>300-311</u> (1480.8)
14	<i>Myosin-4</i>	MYH4_RAT	331	89	44%	222879	5.58	<u>923-943</u> (2431.2) <u>684-698</u> (1730.9) <u>1119-1130</u> (1488.8)
15	<i>Myosin-4</i>	MYH4_RAT	91	53	26%	222879	5.58	<u>1480-1489</u> (1151.7) <u>1284-1293</u> (1162.6) <u>1310-1321</u> (1490.8)
16	<i>Actin, alpha skeletal muscle</i>	ACTS_RAT	74	21	50%	42024	5.23	<u>362-374</u> (1500.8) <u>98-115</u> (1956.1) <u>241-256</u> (1790.9)
17	<i>Actin, alpha skeletal muscle</i>	ACTS_RAT	183	33	72%	42024	5.23	<u>362-374</u> (1500.8) <u>98-115</u> (1956.1) <u>241-256</u> (1790.9)
18	<i>Myosin-4</i>	MYH4_RAT	93	39	25%	222879	5.58	<u>1310-1321</u> (1490.8) <u>1181-1197</u> (1838.9) <u>1255-1272</u> (2190.1)
19	<i>Skeletal muscle-specific alpha-actinin 3</i>	Q8R4I6_RAT	124	29	51%	102948	5.31	<u>516-529</u> (1689.1) <u>728-739</u> (1372.9)

								<u>416-424</u> (1070.6)
20	<i>Myosin-4</i>	MYH4_RAT	126	56	30%	222879	5.58	<u>1480-1489</u> (1151.7) <u>1284-1293</u> (1162.6) <u>1310-1321</u> (1490.8)
21	<i>Myosin-4</i>	MYH4_RAT	383	122	55%	222879	5.58	<u>1119-1130</u> (1488.8) <u>684-698</u> (1730.9) <u>1181-1197</u> (1838.9)
22	<i>Myosin-binding protein C, fast-type</i>	Q5XKE0	378	8	12%	127352	6.02	<u>630-641</u> (1292.7) <u>815-827</u> (1424.8) <u>885-895</u> (1333.7)
23	<i>Actin, alpha skeletal muscle</i>	ACTS_RAT	252	37	84%	42024	5.23	<u>362-374</u> (1500.8) <u>98-115</u> (1956.1) <u>241-256</u> (1790.9)
24	<i>Dihydrolipoyllysine-residue succinyltransferase component of 2-oxoglutarate dehydrogenase complex</i>	ODO2_RAT	111	22	37%	48894	8.89	<u>309-326</u> (2066.0) <u>314-326</u> (1419.7) <u>1189.6</u> (135-145)
25	<i>Actin, alpha skeletal muscle</i>	ACTS_RAT	165	27	66%	42024	5.23	<u>362-374</u> (1500.8) <u>98-115</u> (1956.1) <u>241-256</u> (1790.9)
26	<i>Alpha-enolase</i>	ENOA_RAT	103	25	47%	47098	6.16	<u>184-193</u> (1143.8) <u>344-358</u> CysCAM (1634.1) <u>33-50</u> (1805.2)
27	<i>Serum albumin precursor</i>	ALBU_RAT	150	43	62%	68686	6.09	<u>585-602</u> CysCAM(1948.9) <u>104-117</u> 2CysCAM(1684.8) <u>439-452</u> (1439.8)
28	<i>Collagen alpha-1 chain</i>	CO1A1_RAT	56	27	28%	137802	5.77	<u>542-563</u> 3Pox (2015.3) <u>1142-1157</u> 3Pox (1562.1) <u>387-404</u> 3Pox (1586.1)
29	<i>Collagen alpha-1 chain</i>	CO1A1_RAT	54	28	31%	137802	5.77	<u>542-563</u> 3Pox (2015.3) <u>1142-1157</u> 3Pox (1562.1) <u>387-404</u> 3Pox (1586.1)

30	<i>Glycogen phosphorylase, muscle form</i>	PYGM_RAT	300	64	67%	97212	6.91	<u>440-458</u> CysCAM(2007.3) <u>193-206</u> (1690.0) <u>400-410</u> (1426.9)
31	<i>Pyruvate kinase isozymes M1/M2</i>	KPYM_RAT	261	38	70%	57781	6.63	<u>74-89</u> (1883.9) <u>279-294</u> (1821.9) <u>423-436</u> CysCAM(1473.8)
32	<i>Dihydrolipoyl dehydrogenase</i>	DLDH_RAT	178	30	63%	54004	7.96	483-495 CysCAM (1524.7) <u>301-315</u> CysCAM (1717.9) <u>316-334</u>
33	<i>ATP synthase subunit alpha</i>	ATPA_RAT	254	53	74%	59717	9.22	<u>302-316</u> (1701.9) <u>134-149</u> (1610.9) <u>335-347</u> (1553.8)
34	<i>Beta-enolase</i>	ENOB_RAT	367	57	83%	46984	7.08	<u>33-50</u> (1805.0) <u>359-372</u> (1541.8) <u>413-426</u> (1475.8)
35	<i>Creatine kinase M-type</i>	KCRM_RAT	242	39	71%	43018	6.58	<u>139-148</u> CysCAM(1187.7) <u>305-314</u> (1269.8) <u>33-43</u> (1331.9)
36	<i>Troponin T, fast skeletal muscle</i>	TNNT3_RAT	60	18	49%	30732	6.19	<u>49-62</u> (1633.1) <u>225-233</u> (1137.8) <u>184-196</u> (1551.1)
37	<i>Creatine kinase, sarcomeric</i>	KCRS_RAT	229	41	69%	47355	8.76	<u>404-418</u> (1648.9) <u>311-326</u> CysCAM(1734.9) <u>150-164</u> (1807.9)
38	<i>Fructose-bisphosphate aldolase A</i>	ALDOA_RAT	140	28	89%	39221	8.40	<u>88-99</u> (1343.3) <u>2-13</u> (1377.9) <u>44-57</u> (1647.0)
39	<i>Glyceraldehyde-3-phosphate dehydrogenase</i>	G3P_RAT	183	49	75%	35805	8.14	<u>233-246</u> CysCAM (1556.9) <u>308-321</u> (1779.9) <u>65-78</u> (1628.1)
40	<i>L-lactate dehydrogenase A chain</i>	LDHA_RAT	55	15	29%	36427	8.45	<u>319-332</u> (1635.9) <u>269-278</u> (1181.8) <u>133-149</u> (1944.3)

41	<i>Troponin T, fast skeletal muscle</i>	TNNT3_RAT	62	21	50%	30732	6.19	<u>49-62</u> (1633.1)
								<u>225-233</u> (1137.8)
								<u>184-196</u> (1551.1)
42	<i>Carbonic anhydrase 3</i>	CAH3_RAT	135	21	76%	29413	6.89	<u>227-251</u> (2743.6)
								<u>37-57</u> (2355.2)
								<u>172-188</u> (2065.0)
43	<i>Troponin I, fast skeletal muscle</i>	TNNI2_RAT	61	22	67%	21315	8.86	<u>49-62</u> (1633.1)
								<u>225-233</u> (1137.8)
								<u>184-196</u> (1551.1)

Table S10: T-test for TBA assay as provided in reference 26 (Fedorova, M.; Kuleva, N.; Hoffmann, R., Reversible and irreversible modifications of skeletal muscle proteins in a rat model of acute oxidative stress. *Biochim Biophys Acta* **2009**) from five different animals of each group. The t-test values were calculated with the Excel software package (Microsoft) by using the paired t-test setting.

	3 h group (n=5) compared to control (n=5)	9 h group (n=5) compared to control (n=5)	24 h group (n=5) compared to control (n=5)
<u>Plasma TBA assay</u>	<u>0,000365</u>	<u>0,000739</u>	<u>0,038021</u>
<u>Liver TBA assay</u>	<u>0,039316</u>	<u>0,08506</u>	<u>0,000133</u>
<u>Kidney TBA assay</u>	<u>0,000506</u>	<u>0,6926</u>	<u>0,00061</u>
<u>Muscle TBSA assay</u>	<u>0,00507</u>	<u>0,00007</u>	<u>0,001911</u>

Table S11: Carbonylated skeletal muscle proteins identified in samples prepared from control and irradiated animals (3 h, 9 h, or 24 h post irradiation) and corresponding spot numbers on the Western blots. Note that the spots of each sample have been numbered from 1 upwards (Fig. 4). The protein functions were correlated with the MetaCore analysis software. The maximal intensity was calculated from the Western blots (WB) after 2-DE with the software package Delta2D.

Spot on WB				Proteins identified by MS	Max [#]	t ½ [ref.]
C	3 h	9 h	24 h			
<u>Muscle contraction</u>						
1	2		3	Myosin light chain 1, skeletal muscle isoform	3 h	4.5 h ¹
			1	Myosin light chain 3, skeletal muscle isoform	9 h*	Hours ²
		1		Myosin regulatory light chain 2, skeletal muscle isoform	9 h*	4.5 h ¹
	3	2		Myosin light polypeptide 3	3 h	Hours ²
3	6	5	5	Tropomyosin alpha-1 and beta chains	Sat.*	3 d ³
		8	7, 10	High molecular weight of tropomyosin	9 h	
4,5	8, 9	6, 7	6	Actin, alpha skeletal muscle	Sat.*	20 d ⁴
9, 11	12, 15, 13	9	8, 9, 11, 12, 16, 17, 23, 25	High molecular weight aggregates of alpha actin	9/ 24 h*	
13	17, 18, 20	10, 14, 15	14, 15, 18, 20, 21	Myosin-4	24 h	9 d ^{4, 5}
		17	22	Myosin-binding protein C, fast-type	9 h	
10, 14	19	16	16, 19	Skeletal muscle-specific alpha-actinin 3	24 h*	30 h ⁶
7	10	11		Desmin	3 h*	
23		35	43	Troponin I, fast skeletal muscle	9 h	3 d ⁴
22	25	28, 29, 32	36, 41	Troponin T, fast skeletal muscle	9 h	3.5 d ⁴
	1		2	Troponin T, slow skeletal muscle	9 h	3.5 d ⁴
<u>Carbohydrate metabolism: Glycolysis, Gluconeogenesis and TCA cycle</u>						
				Pyruvate dehydrogenase complex		
2	4	4	4	Pyruvate dehydrogenase E1 component subunit beta	3 h	2.5 d ⁷
	14			Dihydrolipoyllysine-residue acetyltransferase component of pyruvate dehydrogenase complex	3 h*	7.7 d ⁷
			32	Dihydrolipoyl dehydrogenase	9 h	
				2-Oxoglutarate dehydrogenase complex		
12	11	12	24	Dihydrolipoyllysine-residue succinyltransferase component of 2-oxoglutarate dehydrogenase	9/ 24 h	

				complex		
			32	Dihydrolipoyl dehydrogenase	9 h	
			26	Alpha-enolase	24 h	30 h ⁸
18	22	22	34	Beta enolase	3 h	30 h ⁸
15		19	30	Glycogen phosphorylase, muscle form	24 h	12 d ⁹
17		20	31	Pyruvate kinase isozymes M1/M2	9 h	56 h ¹⁰
21	24	30	39	Glyceraldehyde-3-phosphohate dehydrogenase	9 h	17 h ¹¹
20		25, 27	38	Fructose-bisphosphate aldolase A	24 h	~3 d ¹²
		31	40	L-lactate dehydrogenase A chain	9 h	110 h ¹³
<u>Energy metabolism</u>						
				ATP metabolic/catabolic processes :		
6				ATP synthase subunit beta (Complex V)	C	63 h ¹⁴
		21	33	ATP synthase subunit alpha (Complex V)	9-24 h	50 h ¹⁴
		9		NADH-ubiquinone oxidoreductase 75 kDa subunit (Complex I)	24 h	
19	23	23, 24	35	Creatine kinase M-type	3-24 h	75 h ¹⁵
		26	37	Creatine kinase, sarcomeric	3-24 h	75 h ¹⁵
		34		Adenylate kinase isoenzyme 1	9 h	
<u>Miscellaneous</u>						
	26	33	42	Carbonic anhydrase 3	3-24 h	
	21	18	27	Serum albumin precursor	24 h	20 d ¹⁶
16			28, 29	Collagen alpha-1 chain	C/24 h	~70 d ¹⁷
	16	13	13	Heat shock cognate 71 kDa protein	24 h	2 d ¹⁸
8				78 kDa glucose-regulated protein	C*	> 1 d ¹⁹

Max. denotes the sample that displayed the maximal intensity of this spot in the Western blot.

* Denotes spots or areas which were saturated on Western blots, and were thus not quantified with Delta2D but their content estimated manually. Sat. indicates that this spot was saturated in the blots from all samples and thus the maximum intensity could not be determined.

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NADH-ubiquinone oxidoreductase 75 kDa subunit					
Ratio		0.3	0.1	1.22	Spot 11
t-test		99.65	99.96	92.79	
RSD	10.68	38.75	42.16	6.12	