

Regulatory mechanisms involved in muscle and bone remodeling during refeeding in gilthead sea bream

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Supplementary Table 1: Sequences, annealing temperatures (Ta) and GenBank accession numbers of the primers used for *Sparus aurata* real-time quantitative PCR.

	Gene	Sequences	Ta (°C)	Accession Number
<i>GH/IGF family</i>	<i>igf1a</i>	F: AGGACAGCACAGCAGCCAGACAAGAC R: TTCGGACCATTGTTAGCCTCCTCTCTG	60	AY996779
	<i>igf1ab</i>	F: AGTCATTCATCCTTCAAGGAAGTGCATCC R: TTCGGACCATTGTTAGCCTCCTCTCTG	60	EF688015
	<i>igf1abc</i>	F: ACAGAATGTAGGGACGGAGCGAATGGAC R: TTCGGACCATTGTTAGCCTCCTCTCTG	60	EF688016
	<i>igf2</i>	F: TGGGATCGTAGAGGAGTGTGTG R: CTGTAGAGAGGTGGCCGACA	60	AY996778
	<i>igf1ra</i>	F: AGCATCAAAGACGAACTGG R: CTCCTCGCTGTAGAAGAAGC	60	KT156846
	<i>igf1rb</i>	F: GCTAATGCGAATGTGTTGG R: CGTCCTTTATGCTGCTGATG	55	KT156847
	<i>igfbp1</i>	F: AGTGCGAGTCTCTCTGGAT R: TCTCTTTAAGGGCACTCGGC	60	KM522771
	<i>igfbp4</i>	F: TCCACAAACCAGAGAAGCAA R: GGGTATGGGGATTGTGAAGA	60	F5T95CD02JMZ9K
	<i>igfbp5b</i>	F: TTTCTCTCTCGGTGTGC R: TCAAGTATCGGCTCCAG	60	AM963285
	<i>ghr1</i>	F: ACCTGTCAGCCACCACATGA R: TCGTGCAGATCTGGGTCGTA	60	AF438176
	<i>ghr2</i>	F: GAGTGAACCCGGCCTGACAG R: GCGGTGGTATCTGATTCATGGT	60	AY573601
<i>Signaling</i>	<i>akt</i>	F: GCTCACCCCACTCTTCAGAC R: AAATTGGGAAATGTGCTTGC	60	AY996779
	<i>tor</i>	F: CAGACTGACGAGGATGCTGA R: AGTTGAGCAGCGGGTCATAG	60	EF688015
	<i>70s6k</i>	F: GCACCAGAAAGGCATCATCT R: AAGGTGTGGTCACTGTTC	60	EF688016
	<i>4ebp1</i>	F: CCAACCTGCGACTCATCTCT R: GTTCCTCTCATCTCCACACA	60	KM522771
	<i>foxo3</i>	F: CAGCAGCCTGGAGTGTGATA R: CCAGCTCTGAGAGGTCTGCT	60	
<i>Muscle growth-related</i>	<i>pax7</i>	F: ATGAACACTGTCGGCAACG R: AGGCTGTCCACACTCTTGATG	64	JN034418
	<i>pcna</i>	F: TGTTTGAGGCACGTCTGGTT R: TGGCTAGGTTTCTGTCCG	60	AY550963.1
	<i>myf5</i>	F: CTACGAGAGCAGGTGGAGAACT R: TGTCTTATCGCCCAAAGTGTC	64	JN034420
	<i>myod1</i>	F: TTTGAGGACCTGGACCC R: CTCTGCGTGGTGATGGA	60	AF478568.1
	<i>myod2</i>	F: CACTACAGCGGGGATTCAGAC R: CGTTTGCTTCTCCTGGACTC	60	AF478569
	<i>myog</i>	F: CAGAGGCTGCCAAGGTGGAG R: CAGGTGCTGCCGAAGTGGGCTCG	68	EF462191
	<i>mrf4</i>	F: CATCCACAGCTTTAAAGGCA R: GAGGACGCCGAAGATCACT	60	JN034421
	<i>mstn1</i>	F: GTACGACGTGCTGGGAGACG R: CGTACGATTCGATTTCGTTG	60	AF258448.1
	<i>mstn2</i>	F: ACCTGGTGAACAAAGCCAAC R: TGCGGTTGAAGTAGAGCATG	60	AY046314
	<i>mhc2a</i>	F: GCCCATCAACTTCACCGTCTTT R: GGTTGGTCATCTCCTCAGCGG	60	AF150904

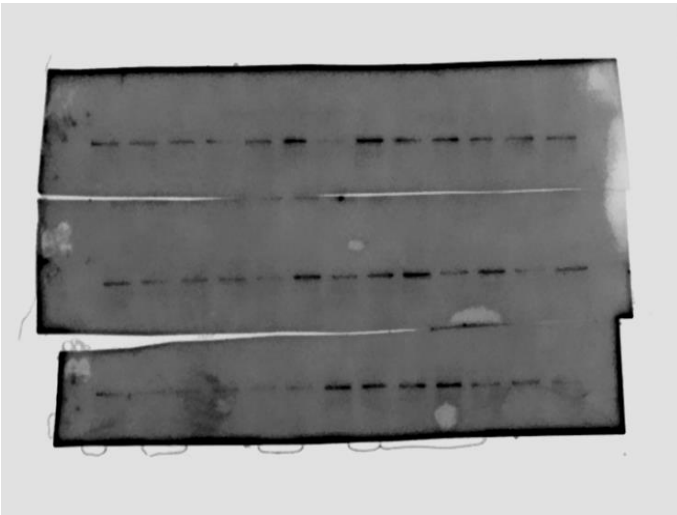
	<i>mlc2b</i>	F: TCCCTTTGCTATTCTGCCTTC R: AAATCAGCCCTATTCCCCATA	60	FG618631
<i>Proteolytic systems</i>	<i>capn1</i>	F: CCTACGAGATGAGGATGGCT R: AGTTGTCAAAGTCGGCGGT	56	KF444899
	<i>capn2</i>	F: ACCCACGCTCAGACGGCAAA R: CGTTCCCCTGTCATCCATCA	61	KF444900
	<i>capn3</i>	F: AGAGGGTTTCAGCCTTGAGA R: CGCTTTGATCTTTCTCCACA	56	ERP000874
	<i>capns1a</i>	F: CGCAGATACAGCGATGAAAA R: GTTTTGAAGGAACGGCACAT	56	KF444901
	<i>capns1b</i>	F: ATGGACAGCGACAGCACA R: AGAGGTATTTGAACTCGTGGAAG	56	ERP000874
	<i>ctnda</i>	F: CCTCCATTCCTGCTCCTTC R: ACCGGATGGAAAACCTCTGTG	56	AF036319
	<i>ctsl</i>	F: ACTCCTTGGGCAAACACA R: CCTTGAACCTTCCTCTCCGT	54	DQ875329
	<i>ub</i>	F: ACTGGCAAGACCATTACCTT R: TGGATGTTGTAGTCGGAAAAG	54	KJ524459
	<i>murfl</i>	F: GTGACGGCGAGGATGTGC R: CTTCGGCTCCTTGGTGTCTT	60	FM145056
	<i>mafbx</i>	F: GGTCACCTGGAGTGGAAAGAA R: GGTGCAACTTTCTGGGTTGT	60	ERA047531
	<i>n3</i>	F: AGACACACACTGAACCCGA R: TTCCTGAAGCGAACCAGA	54	KJ524458
	<i>Bone-related</i>	<i>runx2</i>	F: ACCCGTCCTACCTGAGTCC R: AGAAGAACCTGGCAATCGTC	60
<i>fib1a</i>		F: CGGTAATAACTACAGAATCGGTGAG R: CGCATTGAACTCGCCCTTG	60	FG262933
<i>coll1a1</i>		F: GAGATGGCGGTGATGTGGCGGAGTC R: GCCTGGTTTGGCTGGATGAAGAGGG	68	DQ324363
<i>ocn</i>		F: TCCGCAGTGGTGAGACAGAAG R: CGGTCCGTAGTAGGCCGTGTAG	56	AF048703
<i>on</i>		F: AGGAGGAGGTCATCGTGGAAGAGCC R: GTGGTGGTTCAGGCAGGGATTCTCA	68	AY239014
<i>ctsk</i>		F: AGCGAGCAGAACCTGGTGGAC R: GCAGAGTTGTAGTTGGGGTTCGTAG	60	DQ875329
<i>mmp9</i>		F: ATTCAGAAGGTGGAGGGAGCG R: CATTGGGGACACCACCGAAGA	60	AM905938
<i>Reference</i>	<i>ef1a</i>	F: CTTCAACGCTCAGGTCATCAT R: GCACAGCGAAACGACCAAGGGGA	60	AF184170
	<i>rps18</i>	F: GGGTGTGGCAGACGTTAC R: CTTCTGCCTGTTGAGGAACCA	60	AM490061.1
	<i>b-actin</i>	F: TCCTGCGGAATCCATGAGA R: GACGTGCACTTCATGATGCT	60	X89920
	<i>rpl27</i>	F: AAGAGGAACACAACCTACTGCCCCAC R: CTTCTGCCTGTTGAGGAACCA	68	AY188520

Supplementary Table 2: Body weight (BW), body length (BL), condition factor (CF), hepatosomatic index (HSI), viscerosomatic index (VSI) and ratio of Gh and Igf1 plasma levels along the fasting and refeeding experiment. Data are shown as means \pm SEM (n=6). Letters indicate significant differences ($p < 0.05$) by one-way ANOVA, LSD and Tukey HSD test.

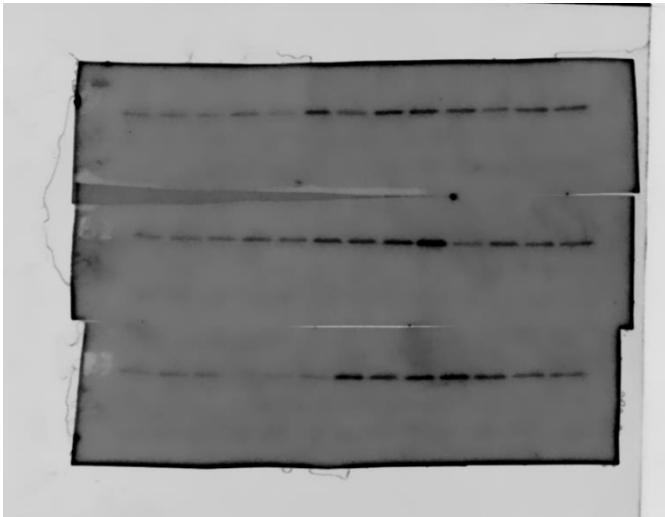
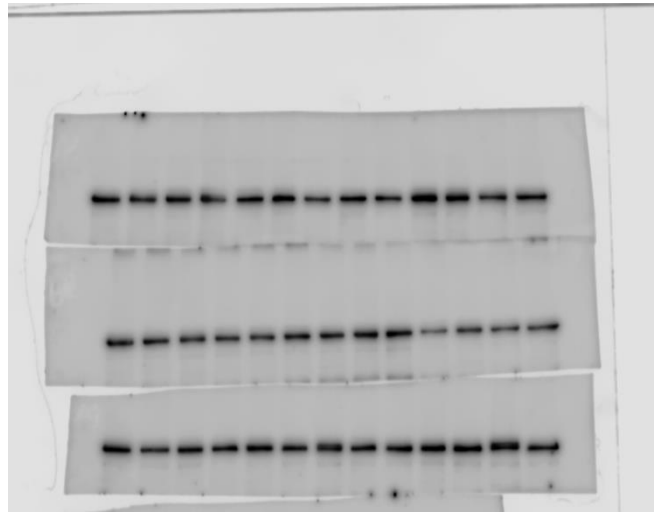
	-21 Days	0h	2h	5h	24h	7 Days
BW (g)	53,9 \pm 3,0	47,2 \pm 3,2	49,4 \pm 1,9	50,8 \pm 2,3	46,8 \pm 1,7	54,4 \pm 3,5
BL (cm)	12,9 \pm 0,27	13,1 \pm 0,34	13,1 \pm 0,21	13,2 \pm 0,24	13,0 \pm 0,18	13,4 \pm 0,29
CF	2,49 \pm 0,05 (A)	2,11 \pm 0,06 (B)	2,22 \pm 0,05 (B)	2,23 \pm 0,03 (B)	2,14 \pm 0,04 (B)	2,27 \pm 0,03 (B)
HSI	1,27 \pm 0,11 (B)	0,58 \pm 0,06 (C)	0,58 \pm 0,06 (C)	0,68 \pm 0,06 (C)	0,81 \pm 0,08 (C)	1,66 \pm 0,1 (A)
VSI	0,66 \pm 0,13 (AB)	0,36 \pm 0,11 (C)	0,64 \pm 0,13 (A)	0,37 \pm 0,13 (AB)	0,3 \pm 0,09 (AB)	0,55 \pm 0,06 (AB)
[Gh]/[Igf1]	0,06 \pm 0,08 (B)	2,97 \pm 0,80 (A)	3,4 \pm 0,23 (A)	2,85 \pm 1,13 (A)	3,24 \pm 1,04 (A)	0,89 \pm 0,48 (AB)

Supplementary information 3 - Western blot full length gels: the six samples of each time group were evenly distributed in three gels resulting in 2 samples/gel/group. Due to that in each western blot, different proteins were analyzed, it was necessary to crop the membranes in order to incubate them with different primary antibodies. Tor (289 kDa MW) and Akt (60 kDa MW) phosphorylated and total forms, were analyzed in the same western blot by cutting the membranes in two. The top part was used to blot Tor antibodies and the bottom part for Akt. Phosphorylated forms were analyzed first and after stripping, the corresponding total forms were determined in the same membranes. Moreover, Ctsd (33 kDa MW) and Ctstl (23 kDa MW) were analyzed in cropped membranes of different western blots each along with other proteins that were unsuccessful (data not shown).

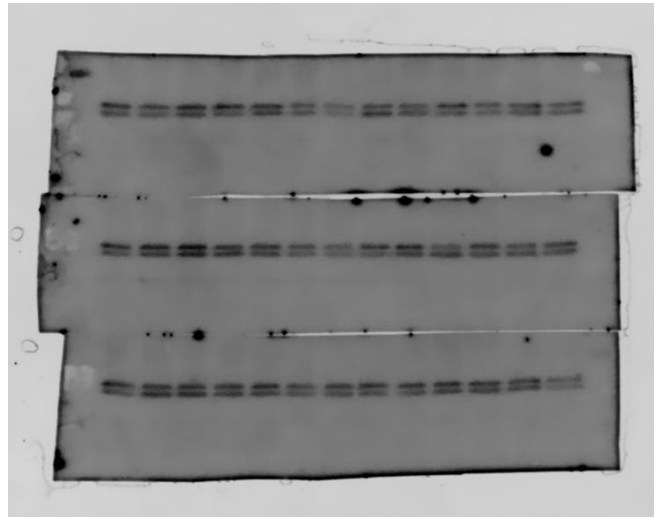
Western blot membranes 1, 2 and 3 top: pTor



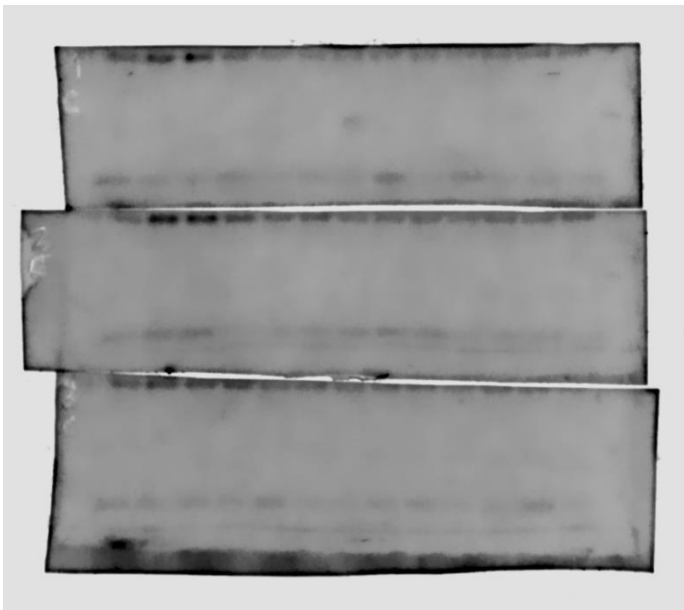
Western blot membranes 1, 2 and 3 top: Total Tor



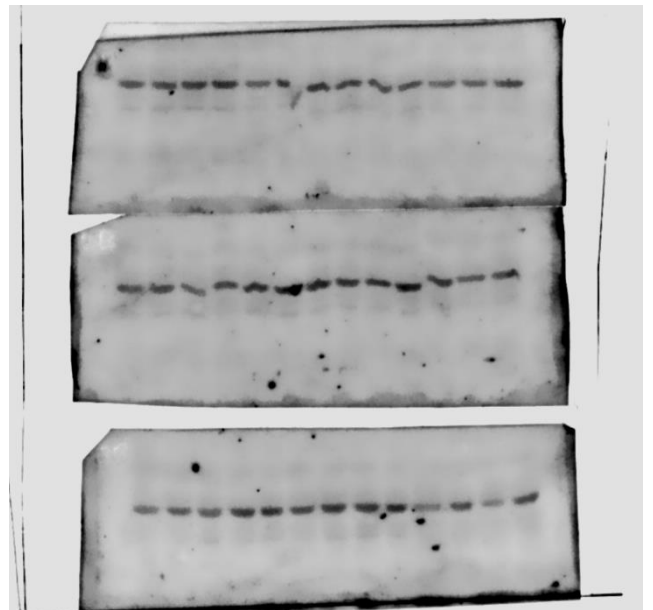
Western blot membranes 1, 2 and 3 bottom: pAkt



Western blot membranes 1, 2 and 3 bottom: Total Akt



Western blot membranes 4, 5 and 6: Ctcd



Western blot membranes 7, 8 and 9: Ctsl