

Supporting information

Comparative Phosphoproteomics Reveals a Role for AMPK in Hypoxia Signaling in Testes of Oriental River Prawn (*Macrobrachium nipponense*)

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Figure S1. Hierarchical clustering of different phosphopeptides in the testis of control prawn (control) and hypoxia-treated prawn (treated). Each row represent a phosphopeptide (protein accession-sequence-location in phosphoprotein), and different colors represent different levels of phosphopeptides. A redder indicates a higher level of phosphorylation and a greener indicates a lower level of phosphorylation in phosphopeptide.

Table S1. Phosphopeptide raw data analysis (XLSX)

Table S2. Differentially expressed phosphorylated peptides identified by hypoxia (XLSX)

Table S3. Motif X analysis of significantly regulated phosphopeptides

Table S4. Partial list of the differentially abundant phosphoproteins in prawn under hypoxia

Figure S2. Alterations in the AMPK-mTOR pathway. The red boxes indicate differentially phosphorylated proteins.

Figure S1

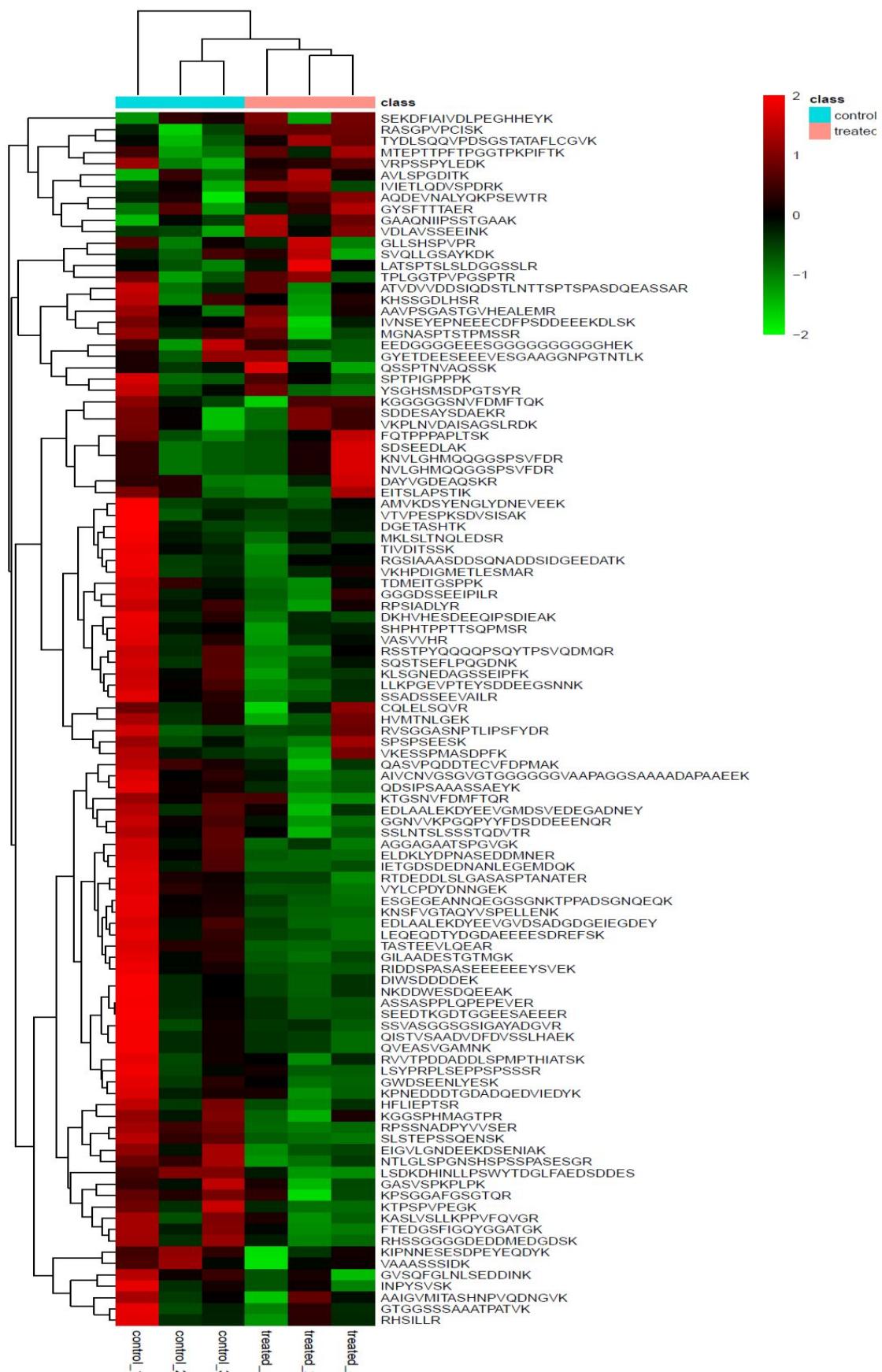


Table S3. Motif X analysis of significantly regulated phosphopeptides

#	Logo	Motif	Motif score	Foreground		Background		Fold Increase
				Matches	Size	Matches	Size	
1		xxxxxx_S_PxxDxxx	158.32	43	1403	1	1403	43
2		xxxxRxx_S_Pxxxxxx	115.68	75	1403	10	1403	7.5
3		xxxxxxL_S_Pxxxxxx	83.71	45	1403	4	1403	11.2
4		xxxxxx_S_Pxxxxxx	65.44	450	1403	128	1403	3.5
5		xxxxRRx_S_xxxxxxx	110.80	78	1403	5	1403	15.6
6		xxxxRKx_S_xxxxxxx	55.16	44	1403	8	1403	5.5
7		xxxxRxx_S_xExxxxx	38.65	59	1403	6	1403	9.8
8		xxxxxx_S_DExxxxx	41.31	33	1403	2	1403	16.5
9		KxxxRxx_S_xxxxxxx	22.68	24	1403	2	1403	12.0
10		xxxxxx_S_DDExxxx	36.05	24	1403	1	1403	20
11		xxxxRxx_S_xVxxxxx	18.96	23	1403	3	1403	7.7
12		xxxxxx_S_DxDxxxx	18.61	36	1403	7	1403	5.1
13		xxxxxx_S_xxExRxx	19.28	14	1403	3	1403	4.7
14		xxxxRxx_S_xxxxxxx	10.22	348	1403	112	1403	3.1
15		xxxxxx_S_xExxxxx	10.17	244	1403	135	1403	1.8
16		xxxxxx_S_DxExxxx	16.43	65	1403	12	1403	5.4
17		KxxxKxx_S_xxxxxxx	14.66	13	1403	4	1403	3.2

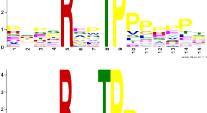
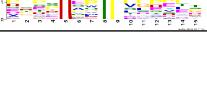
18		xxxxxx_T_Pxxxxxx	6.73	13	1403	3	1403	4.3
19		Txxxxx_T_Pxxxxxx	39.61	18	244	1	244	18.0
20		xxxxRxx_T_Pxxxxxx	30.30	30	244	3	244	10.0
21		xxxxxx_T_Pxxxxxx	13.64	113	244	36	244	3.1

Table S4. Partial list of the differentially abundant phosphoproteins in prawn under hypoxia

Protein name	Protein description	Phosphosite	Best match phosphopeptide sequence	hypoxia/normoxia ratio	P-value	Regulation
Energy metabolism						
AMPK α	5'-AMP-activated protein kinase catalytic subunit α	S1	SEKDFIAIVDLPEGHHEYK	1.21	2.5E-09	Up
PFK	6-phosphofructokinase	S3	KNVLGHMQQQGSPSVFDR	1.14	0.001	Up
AKP	alkaline phosphatase-like isoform X1	S13	TYDLSQQVPGDSGSTATAFL CGVK	1.31	0.001	Up
FDA	fructose-1,6-bisphosphate aldolase	T11	GILAADESTGTMGK	0.39	3.2E-09 8.9E-09	Down
PDH	Pyruvate dehydrogenase E1 component subunit alpha type II	T11	YSGHSMSPGPGTSYR	0.55	8.9E-09	Down
GP	Glycogen phosphorylase	S13	AQDEVNALYQKPSEWTR	1.51	2.3E-09	Up
Mitochondria oxidative phosphorylation						
Abcf1	ATP-binding cassette subfamily B member 1	S4	DIWSDDDDEK	1.84	0.002	Up
CCO	Cytochrome c oxidase subunit 5B	S3	RVSGGASNPTLIPSYDR	0.13	5.81E-05	Down
NOX5	NADPH oxidase 5	S4	KGGSPHMAGTPR	0.84	6.97E-08	Down
PMCA	plasma membrane calcium ATPase	S11	EEDGGGGEEESGGGGGGGG GGHEK	0.88	1.14E-08	Down
Autophagy and apoptosis						
ULK1	Autophagy-related protein 1 homolog	S3	RASGPVPCISK	1.75	4.65E-10	Up
ATG16L	Autophagy-related protein 16-L	S6	GGGETSPNRPLSR	1.88	1.93E-08	Up
TP53	Tumor protein p53	S3	KLSGNEDAGSSEIPFK	2.52	5.8E-09	Up
Transcription regulation						
HDAC10	histone deacetylase 10	S3	QASVPQDDTECVFDPMAK	0.73	6.2E-09	Down
HDAC6	histone deacetylase 6	S6	NTLGLSPGNSHSPSSPAESGR	0.69	3.78E-08 1.3E-09	Down
MARK3	MAP/microtubule affinity-regulating kinase 3	S9	AGGAGAAATSPGVGK	0.39	0.09	Down
PDK1	3-phosphoinositide-dependent protein kinase 1	T7	KNSFVGTAQYVSPELENK	1.61	7.62E-06	Up

Figure S2

