

Fig. S1. (A) BLAST tblastn search results of *Macquaria australasica* genome using, as query sequence, *Epinephelus lanceolatus cga*. (B) Alignment of *Macquaria australasica* partial deduced cga sequence with corresponding sequences in other teleosts (blue line indicates predicted cleavage site of the signal sequence). Asterisks indicate conserved residues.

A

Macquaria australasica isolate MP_SCH12 SCH_Scf5, whole genome shotgun sequence
Sequence ID: SEMN01000005.1 Length: 3949491 Number of Matches: 2
 Range 1: 569986 to 570189 [GenBankGraphics](#) [Next Match](#) [Previous Match](#)

Score	Expect	Method	Identities	Positives	Gaps	Frame
142 bits(357)	2e-39	Compositional matrix adjust.	67/68(99%)	68/68(100%)	0/68(0%)	-1

Query 6 LSLVGCEECTLRKNNVFSRDRPIYQCMGCCFSRAYPTPLKAMRTMNI PKNITSEATCCVA 65
 LSLVGCEECTLRKNNVFSRDRPIYQCMGCCFSRAYPTPLKAMRTMNI PKNITSEATCCVA
 Sbjct 570189 LSLVGCEECTLRKNNVFSRDRPIYQCMGCCFSRAYPTPLKAMRTMNI PKNITSEATCCVA 570010

Query 66 KHSYEVQS 73
 KHSYEVQ+
 Sbjct 570009 KHSYEVQT 569986

Range 2: 569244 to 569519 [GenBankGraphics](#) [Next Match](#) [Previous Match](#) [First Match](#)
Alignment statistics for match #2

Score	Expect	Method	Identities	Positives	Gaps	Frame
80.5 bits(197)	1e-17	Compositional matrix adjust.	47/97(48%)	58/97(59%)	5/97(5%)	-2

Query 10 GCEECTLRKNNVFSRDRPIYQCMGCCFSRAYPTPLKAMRTMNI PKNITSEATCCVAKHSY 69
 G E L N + F + + +Y+C SR T +T ++ +I + +S
 Sbjct 569519 GSEYFALSFNSDFCQFKGLYKCRHYDSSRC*KT----KTKHLIIHICCCRLM*LC*YSL 569355

Query 70 EVQSPHLFLFQTEVAGTGIRVRNHTDCHCSTCYFHKI 106
 SPHLFLFQTEVAGTGIRVRNHTDCHCSTCYFHKI
 Sbjct 569354 LFLSPHLFLFQTEVAGTGIRVRNHTDCHCSTCYFHKI 569244

B

Common carp	MFWTRYAGASILLFFMLIRLGQLYPRNDMNFVGCCECKLKENNIFSKPGAPVYQCMGCCF	60
Macquarie perch	-----LSLVGCEECTLRKNNVFSR-DRPIYQCMGCCF	31
Yellowtail kingfish	MGSVKTAGLSLLLLSFCLYIADSYPNIDSSNMGCCECTLRKNSLFP---MVYQCMGCCF	56
Tilapia	MGSLKSPGLSLLLLSFLLYIADSYPNIDLNSMGCCECTLRKNNLFSR-DRPVYQCMGCCF	59
Giant grouper	MGSVR SAGLNLLLLSFLLYIADSYPNIDLPIGCEECTLRKNSVFSR-DRPVYQCMGCCF	59
European seabass	MGSVKSAVLSVLLLLSFILYVDSYPSMDLSMGCCECTLRKNSVFSR-DRPVYQCMGCCF	59
	*****.:*:*:*:*****	
Common carp	SRAYPTPLRSKKTMLVPKNITSEATCCVAKVKRVL-----VNDKLVNHTDC	108
Macquarie perch	SRAYPTPLKAMRTMNI PKNITSEATCCVAKHSYEVQSPHLFLFQTEVAGTGIRVRNHTDC	91
Yellowtail kingfish	SRAYPTPLKAMKTMNIPKNITSEATCCVAKHSYKTE-----VAGIIVKNHTDC	104
Tilapia	SRAYPTPLRATKTMNIPKNITSEATCCVARHSYEIE-----IAGIKVRNHTDC	107
Giant grouper	SRAYPTPLKAMKTMIPKNITSEATCCVAKHSYETE-----VAGIRVRNHTDC	107
European seabass	SRAYPTPLKAMKTMIPKNITSEATCCVAKHSYETE-----VAGIKVRNHTDC	107
	*****:.*:*****:..:..:****	
Common carp	HCSTCYHKS	118
Macquarie perch	HCSTCYFHKI	101
Yellowtail kingfish	HCSTCYFHKI	114
Tilapia	HCSTCYFHKI	117
Giant grouper	HCSTCYFHKI	117
European seabass	HCSTCYFHKI	117
	*****:**	

Fig. S2. (A) BLAST tblastn search results on *Macquaria australasica* genome, using as a query sequence, *Epinephelus lanceolatus* Lhb. (B) Alignment *M. australasica*, *E. lanceolatus* (Accession number XM_033638682) and *M. ambigua* mature deduced Lhb. Asterisks indicate conserved residues.

A

Macquaria australasica isolate MP_SCH12 SCH_Scf50, whole genome shotgun sequence
Sequence ID: SEMN01000050.1 Length: 1877416 Number of Matches: 3

Range 1: 909628 to 909861 [GenBankGraphics](#) [Next Match](#) [Previous Match](#)

Score	Expect	Method	Identities	Positives	Gaps	Frame
156 bits(394)	1e-43	Compositional matrix adjust.	73/78(94%)	75/78(96%)	0/78(0%)	+1
<u>Query</u> 71		KDPVIKIPFSNVYQHVCTYRDFYKTFELPDCPPGVDPTVTYPVALSCHCGRCAMDTSDC				130
		+DPVIKIPFSNVYQHVCTYRDFYKTFELP CPPGVDPTVTYPVALSCHCGRCAMDTSDC				
<u>Sbjct</u> 909628		QDPVIKIPFSNVYQHVCTYRDFYKTFELPGCPPGVDPTVTYPVALSCHCGRCAMDTSDC				909807
<u>Query</u> 131		TFESLQPNFCMNDIPFYY 148				
		T ESL PNFC+NDIPFYY				
<u>Sbjct</u> 909808		TSESLHPNFCLNDIPFYY 909861				

Range 2: 907774 to 907953 [GenBankGraphics](#) [Next Match](#) [Previous Match](#) [First Match](#)

Score	Expect	Method	Identities	Positives	Gaps	Frame
87.4 bits(215)	2e-19	Compositional matrix adjust.	43/60(72%)	47/60(78%)	0/60(0%)	+1
<u>Query</u> 12		PLMLSFLGASSSIWSLAPAAAFQLPPCQLINQTVSLEKEGCKHPVETTICSGHCITK 71				
		PL+ S+ + L+ AAFQLPPCQLINQTVSLEKEGC KHPVETTICSGHCITK				
<u>Sbjct</u> 907774		PLL*ETTKSYSTQLQHLSSPAAFQLPPCQLINQTVSLEKEGCKHPVETTICSGHCITK 907953				

B

Macquarie perch	-----PLLLETTKSYSTQLQHLSSPAAFQLPPCQLINQTVSLE	38
Giant grouper	MMAVQVGRVMFPLMLSFLGASSSIWSLAPAA-----AFQLPPCQLINQTVSLE	49
Golden perch	MMAVQVSRVMFPLMLSFLGASSSIWPLAPAGSTQLQLSSPVAFQLPPCQLINQTVSLE	60
	: : :	*****
Macquarie perch	KEGCSKHPVETTICSGHCITKDPVIKIPFSNVYQHVCTYRDFYKTFELPGCPPGVDPT	98
Giant grouper	KEGCPKHPVETTICSGHCITKDPVIKIPFSNVYQHVCTYRDFYKTFELPDCPPGVDPT	109
Golden perch	KEGCSKHPVETTICSGHCITKDPVIKIPFSNVYQHVCTYRDFYKTFELPGCPPGVDPT	120
	**** *	*****
Macquarie perch	VTYPVALSCHCGRCAMDTSDCTSESLHPNFCLNDIPFYY	137
Giant grouper	VTYPVALSCHCGRCAMDTSDCTFESLQPNFCMNDIPFYY	148
Golden perch	VTYPVALSCHCGRCAMDTSDCTSESLHPNFCLNDIPFYY	159
	***** * : * : *	*****

Fig. S3. The identification of recombinant *Macquaria australasica* Lh by LC-MS/MS analysis. The coverage of the precursor by MS/MS spectra of supporting peptides was shown (blue lines), together with high-confident PTMs.

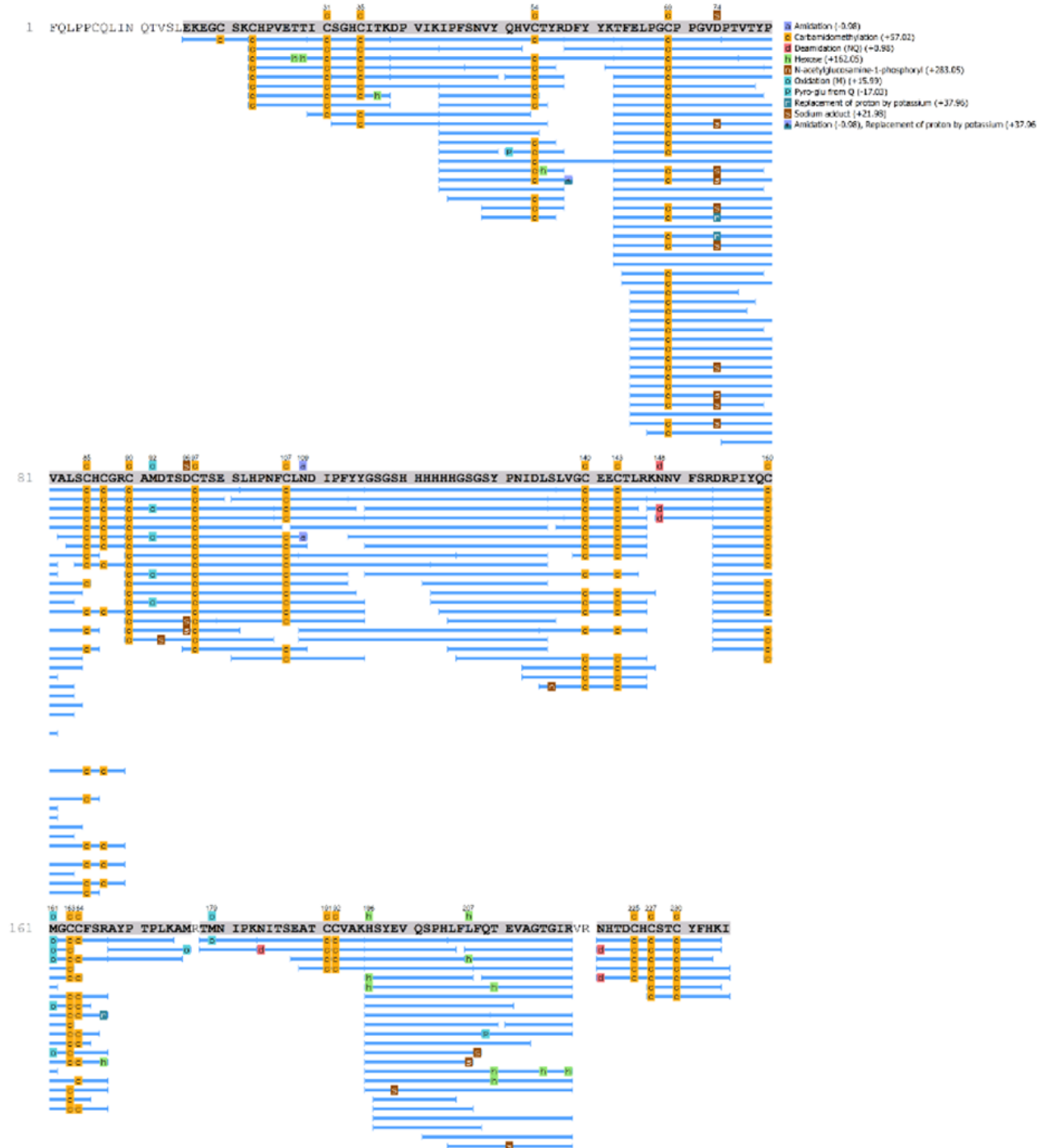


Fig. S4. ELISA cross reactivity test of rmpLh and rggLh against yellowtail kingfish (*Seriola lalandi*, ytk) Fshb antibodies. The antibodies tested were ggLhb (LhAb) and ytkFshb (FshAb). The coating used were rggLhb (LhCoat) and rytkFshb (FshCoat).

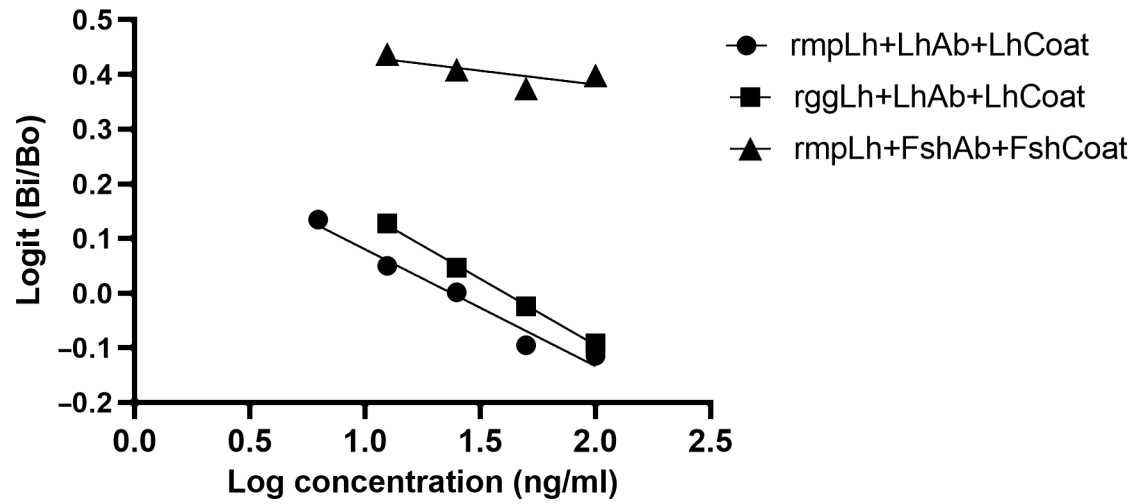


Table S1. Accession numbers of Lhb, Fshb and cga species included in the phylogenetic tree (Fig. 2).

Species	Common name	Accession number	Subunit
<i>Oplegnathus fasciatus</i>	Rock bream	KM507038	Lhb
<i>Siniperca chuatsi</i>	Mandarin fish	XM_044214134	Lhb
<i>Epinephelus coioides</i>	Orange-spotted grouper	AF507939	Lhb
<i>Epinephelus lanceolatus</i>	Giant grouper	XM_033638682	Lhb
<i>Epinephelus akaara</i>	Red spotted grouper	KJ534538	Lhb
<i>Larimichthys crocea</i>	large yellow croaker	XM_010753819	Lhb
<i>Thunnus maccoyii</i>	Southern bluefin tuna	XM_042433548	Lhb
<i>Lates calcarifer</i>	Barramundi	XM_018660786	Lhb
<i>Centropomus undecimalis</i>	Common snook	KF314819	Lhb
<i>Scatophagus argus</i>	Spotted butterflyfish	KY129604	Lhb
<i>Dicentrarchus labrax</i>	European seabass	AF543314	Fshb
<i>Lateolabrax japonicus</i>	Japanese seabass	JX185720	Fshb
<i>Siniperca chuatsi</i>	Mandarin fish	XM_044205114	Fshb
<i>Micropterus salmoides</i>	Largemouth bass	MN883938	Fshb
<i>Channa argus</i>	Northern snakehead	(Not available)	Fshb
<i>Oplegnathus fasciatus</i>	Barred knifejaw	KM507037	Fshb
<i>Toxotes jaculatrix</i>	Banded archerfish	XM_041042998	Fshb
<i>Nibea albiflora</i>	White flower croaker	(Not available)	Fshb
<i>Lates calcarifer</i>	Barramundi	XM_018683405	Fshb
<i>Lates niloticus</i>	Nile perch	KP230501	Fshb
<i>Thunnus maccoyii</i>	Southern bluefin tuna	XM_042401152	cga
<i>Ophisternon bengalense</i>	Bengal mud eel	AF502394	cga
<i>Chelmon rostratus</i>	Copperband butterflyfish	XM_041965694	cga
<i>Plectropomus leopardus</i>	Leopard coral grouper	XM_042511978	cga
<i>Morone saxatilis</i>	Striped sea-bass	XM_035675679	cga
<i>Toxotes jaculatrix</i>	Banded archerfish	XM_041031409	cga
<i>Oplegnathus fasciatus</i>	Barred knifejaw	KM507036	cga
<i>Pagrus major</i>	Red seabream	AB028211	cga
<i>Xiphias gladius</i>	Swordfish	(Not available)	cga
<i>Lates calcarifer</i>	Barramundi	NW_017365383	cga
<i>Lates niloticus</i>	Nile perch	KP230500	cga