

1 **Supplementary information for**

2 Melatonin modulates the hypothalamic-pituitary neuroendocrine axis to
3 regulate physiological color change in teleost fish

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23 Table S1-3

24 Other supplementary materials for this manuscript include the following:

25 Source data 1-3 for Figure 5 and Figure S17-18

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LcMtnr1a2

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1      gccagcttgagacgcatgccccgggtgccaltacgcgagcagggcgacagctc
57     cccggtagtctggcagccacatggacgcggggttggggatgctgaaggagctgcagcac
117    cccctgaaaccaggcaccataaagacacagttatgtgacgctccocctctttgtcaccg
177    ggatatagactccacggtgcataaactacatgttttaactccttttagagctccttagagg
237    aactccctcctcctcctgtagttctagttgtgattcaggttcaggtgtaacaaaaa
297    aaaaaaaaaaactgatccccgacttcaaataaagccacaaaatgacgcatagctc
357    cacatcaacaccccaactcggagtgaccgactccagcgtctccggacatctcgtgctt
417    ATGCTGAATGGACCGACCTTCCGCGACCGCGACCCGATGCGACTCGTCCGACCCGAGGCAC
1      M L N G P T F R D R D P M R L V D P R H

477    CTCCCGCAACTGATGTCCTTGGAGGACCATGAGCCCACTATGGTGAAGGGACCCCTGGTG
21     L P Q L M S L E D H E P T M V E G T L V

537    CCCCGCAACTCCACGCCGCGAGGGAGGGGACTCCGGGTGACGAGCATCAGTCCCTTC
41     P R N S T P A G E E G T P G Q Q H Q S F
      ▲ ● ● ● ● ●
597    CCCTGGGTAGTGACCCTGCTGGCCGGCTCCTGATCAGGACCATCGTGGTGGATATTATA
61     P W V V T L L A G V L I T T I V V D I I
      TM1 (67-89) ●
657    GGAACCTGCTGGTCATGTGTCCTGTTCCAGAACAGGAACTCAGGAAAGCAGGAAAC
81     G N L L V I V S V F R N R K L R K A G N
      ●
717    GCCTTTGGTGGTGGCTTCTGCTGACTTGGTGGTCCCATCTATCCCTACCCGCTG
101    A F V V S L A L A D L V V A I Y P Y P L
      TM2 (102-124) ●
777    GTCCTGACTGCCATTTCCACGATGGCTGGATCGCCGGTACATCCACTGTCAGATCAGC
121    V L T A I F H D G W I A G Y I H C Q I S
      TM3 (139-161) ●
837    GGCTTCCTCATGGTCTCAGCGTCATTGGCTCCATCTTCAACATCAGGGCATCGCCATC
141    G F L M G L S V I G S I F N I T G I A I
      ● ▲
897    AACCGTACTGCTACATCTGTACAGCCTTAAGTATGACAACTGTTCTCCAACAGCAAC
161    N R Y C Y I C H S L K Y D K L F S N S N
      ● ● ● ● ●
957    ACCATGTGCTACGTGCTGCTGGTCTGGGCGCTCACCATCCTCGCCATCGTCCCAACTGG
181    T M C Y V V L V W A L T I L A I V P N W
      TM4 (182-202) ●
1017   TTCGTGGAGTTCGCTGCAGTATGACCCACGGGTGTACTCCTGCACCTTTGCCAGTCGGTG
201    F V E S L Q Y D P R V Y S C T F A Q S V
      ●
1077   AGCTCGGTGTACTATCACGGTAGTGGTGGTTCATCTCATCTCCGATCGCCATCGTC
221    S S L Y T I T V V V V H F I L P I A I V
      ● ● ● ● ● TM5 (228-250) ●
1137   ACCTACTGTACCTGCGTATCTGGATCCTAGTTCATCCAGGTGAGCGGAGGGTCAAGCCG
241    T Y C Y L R I W I L V I Q V R R R V K P
      ●
1197   GACTCGCGCCAAAGATCAAGCCGACGACCTCCGCAACTTCCCTACCATGTTCCGTCGTG
261    D S R P K I K P H D L R N F L T M F V V
      ● TM6 (274-296) ●
1257   TTCGTGCTCTTCGCTGTCTGTTGGCACCGCTGAACCTAATCGGACTGGCGGTGGCTTG
281    F V L F A V C W A P L N L I G L A V A L
      ●
1317   GACTCTAGGCTGGGCGGGCGATACCGAGTGGCTGTTACGGCCAGCTACTTCATGGCG
301    D S R L G R A I P E W L F T A S Y F M A
      TM7 (311-333) ●
1377   TACTTCAACAGTGCCTCAACGCTGTGCTACGGGCTCCCGAACCACTTCAAGAAAG
321    Y F N S C L N A V V Y G V P N H N F R K
      ●
1437   GAGTACAAGAGGATCGTCTGATCATCTTCAAGTTTCACTGCTGA gacgaggcgtgagg
341    E Y K R I V L I I F K F H C *
1497   ctgaggcttgacaagaacaagctataaaaaagtataaaagaaagacaaacttaacct
1557   ttacaaggagggaaaaacagtgctaatgaaagaataagaaaataaaggcacacatttga
1617   tgagtcagaaaaatagaagaactgaatagctggagggtgaggaggagggtagtgaggta
1677   gagacaaaagtaaaataaatgatgacgcactgagagttatcagtgatgccttcagggaaaa
1737   tgagagacttaaaataggaggcggaggccagagctcgggagtaacttaacttagta
1797   aagtgttttatcatcatgttctttaagtaattcactcaacaaactcagattcagattaac
1857   attgtattccccgtttcccttgattcattttgatgataaaacttcacagtgaactgg
1917   ttacaag

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Figure S2. Characteristics of gene structure and the deduced amino acid sequence of LcMtnr1a2-like. The phosphorylation sites predicted with NetPhos 3.1 Server are marked by solid dots in three different colors (Ser, Tyr and green are in blue, orange and green, respectively); the N-linked glycosylation sites predicted with NetNGlyc 1.0 Server are highlighted by red triangles; 7TM domains predicted with I-TASSER are labeled in boxes in 7 different colors; the NRY, CYICH and NAXXY sequences predicted with PROSITE are labeled in brown, pink and gray boxes, respectively. TM: transmembrane.

LcMtnr1b1

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1      agggcatcacagatgggtlagg
23     aagacgattgttccccgtttgcacagagaatgcaaatcatcggaaggactccggg
83     cggttcagtggtttgatattttagagaggaatgttttttccctcaatgtgggcgcatgc
143    agacgactgggagttcgagatgcccaaccgcaagccagagacaccigacgaggtggaga
203    cgtgaaactcgtagctctggtgatttttaacacataatctaaagactatataat
263    tacattttctttcagggaggagggtgtggcaccacagcgtgtccgaccagctccagtg
323    ggcgatggatttatgactttgtgcaagacaaagaaagacaagaagagtctactaac
383    tttataggtcacaccaagccacataatcacctaacatctatctttaaagtaga
443    actcggggaggcaccagggtgcgctccatgtttcattcaggacgcaaccggtaccggcgg
503    acctcggggacataaacgcacagttggaacgcgagagcagcgttgggctgtggccagtg
563    ATGCCGGACACATTCCACTCATAAAGAACCGGACAGAGCCACGGCTGGGGCAGCTAGAG
1      M P D T F T L I K N R T E P R L G Q L E
623    CGCACACTGGACACAGGGGAGCGCCCGCCCGCTGGGTTCATAGGAATCCTGGCCAGC
21     R T L D T E G S A R P A W V I G I L A S
683    GTGTTGATCTTCACCACGGTGGTGGACGTGTGGGGAATCTGCTGGTCATCATCTCGGTG
41     V L I F T T V V D V L G N L L V I I S V
743    TTCGGAACCCTAAGCTGAGGAACCTCGGGTAATGTGTTTGGTGGTTCAGTTGGCATTGGCT
61     F R N R K L R N S G N V F V V S L A F A
803    GACCTTGGTGGTACCTTCTACCCCTACCCCTTGGTCTCTATGCTCTCTCCACGATGGA
81     D L V V A F Y P Y P L V L Y A L F H D G
863    TGGCACTGGGAAACACACAATGCATGGTCAGTGGTTCCTGATGGGGCTGAGTGCATC
101    W A L G N T Q C M V S G F L M G L S V I
923    GGCTCCATTTTCAACATCACTGGGATTCAGTGAACAGACTGCTACATCTGTCACTCA
121    G S I F N I T G I A V N R Y C Y I C H S
983    TTCTCTTACAGTCGGGTGTACAGTATCGCAACACTCTGCTGTTGTTGCTTAATTTGG
141    F S Y S R L Y S Y R N T L L F V A L I W
1043   GTGCTTACAATTGGCCATTATCCCAATTTCTTTGTTGGCTCCTTGGCTACGACCCG
161    V L T I V A I I P N F F V G S L R Y D P
1103   CGGGTACTCTTGCACCTTTGCCAGAATGTGACAGTCTCTACACAGTGGCAGTAGTA
181    R V Y S C T F A Q N V S S S Y T V A V V
1163   GTGTTCACTTCTTGGTTCCTTGCAGTGGTTACCTTCTGTTATCTACGCATCTGGGTG
201    V V H F L V P I A V V T F C Y L R I W V
1223   CTTGTGATTACAGTGGCAGCAGAAAGTGAAGACAGAGGAGCCCTCGCCTCAGGCCAAGT
221    L V I Q V R R K V K T E E S P R L R P S
1283   GACTTGGGAATTTTCATCACCATGTTTCGTTGCTTTGCTGTTTGGCATCTGCTGGGT
241    D L R N F I T M F V V F V L F A I C W A
1343   CCACTTAACCTGATCGGCTTGGCGGTGGCCATAGATCCATCCCATGTGGCACCCTATATC
261    P L N L I G L A V A I D P S H V A P H I
1403   CCTGAGTGGCTTTTGTGGTCAGTACTTTCATGGCCACTTTAATAGCTGCTGAATGCC
281    P E W L F V V S Y F M A Y F N S C L N A
1463   ATTATATATGGCTTACTCAACAGGAATTTGAGGAATGAGTACAAACGCAATGTCACTCC
301    I I Y G L L N R N F R N E Y K R I V T S
1523   GTATGGGTGACGCACTTTTGTGACAGACTTCGCGGGCTGCAACGGACGGCCGGAGC
321    V W V T R L F V T E T S R A A T D G R S
1583   ATGAGGAGCAAGCAGTCCACCCTCCACCCTCAATAACAACGAGTCCAGGAGCAGCAGC
341    M R S K Q S P P P P L N N N E S G A A R
1643   ACAACAAGAATGA acttggacctagaaactcggaccattaccattaaggacaaaaat
361    T N K E *
1703   acactgtaagtctgcatcagctgatttctcatagtttaagtgtcactttgactttatg
1763   taccgagctgaacatggatctttgagcctggttaaatgtaattcctgttttatgtgaca
1823   ttcagacgctagtgcagctaaagagtatcaattcacaagtgaaactgaaatacatggctc

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44
45 **Figure S3. Characteristics of gene structure and the deduced amino acid sequence of**
46 **LcMtnr1b1-like.**The phosphorylation sites predicted with NetPhos 3.1 Server are marked by
47 solid dots in three different colors (Ser, Tyr and green are in blue, orange and green, respectively);
48 the N-linked glycosylation sites predicted with NetNGlyc 1.0 Server are highlighted by red
49 triangles; 7TM domains predicted with I-TASSER are labeled in boxes in 7 different colors; the
50 NRY, CYICH and NAXXY sequences predicted with PROSITE are labeled in brown, pink and
51 gray boxes, respectively. TM: transmembrane.

LcMtnr1b2

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1          aatttccctcaaaatttcgcatagagaagggltgga
33 gttgtctgggtgcttcttcttaccatgcaactgggtaaacagttgittgactta
93 ggtgtcgtacaggtacttctaaaggagtttgaagcgctatcttcccaacctcaacaga
153 cccacgaacgaacaaggaatgggtacgtgacccattgtcaacaaaactgaatccacagc
213 ATGTCCATGCAAGAAAGACCAACTGCTGGAGATCGCAAAATGACGGCGCCAGTGTTCGA
1 M S M Q E E D Q L L E I A N D G A S V R
1
273 GACAACAACCTCTGACGGTGTTCGGGATAAAGTCATGGCAGTATGGCAATACCAGAGATCG
21 D N N S D G V R D K V M A V W Q Y Q R S
1
333 CTAACACAGCGCGTGA AAAACCCCTGTTGCCATTGCCAACATCCTATCTGTGGAAGCCGGG
41 L T Q R V K N P V A I A N I L S V E A G
1
393 GTTTTCTGCAGTGACAGCCACCAAAACAAGTTTCACCAATTTCAATGACAATCTGTTTCCT
61 V F C S D S H Q N K F H H F N D N L F P
1
453 CTCCTTCTGTCCTTTACAGGAAATGTGTTTGGTGAGTCTGGCCTTCGCTGACTTGGTG
81 L L L S F T G N V F V V S L A F A D L V
1
513 GTTGCATTCTACCCCTACCCCTCTGGTTTGTATGCCATCTTCCATGATGGTGGTCACTG
101 V A F Y P Y P L V L Y A I F H D G W S L
1
573 GGGGACGCCAGTGCAGGTGAGTGGCTTCCTGATGGGCTTGAAGTGCATTGGCTCCATT
121 G D A Q C K V S G F L M G L S V I G S I
1
633 TTCAACATAACAGGCATGCTATCAACCCGCTACTGTTACATCTGCCACAGCTTCAGCTAC
141 F N I T G I A I N R Y C Y I C H S F S Y
1
693 GACAAGCTGTATAGCTATCGCAACACGCTGCTACTGGTGGCACTCATCTGGCTTCTGACA
161 D K L Y S Y R N T L L L V A L I W L L T
1
753 GTTGTAGCCATTGTGCCCAACTTCTTTGTCGGGCTCACTGCAATACGACCCTCGGCTCAT
181 V V A I V P N F F V G S L Q Y D P R V Y
1
813 TCATGCACATTTGCACAGACAGTCAACCTCATACACCATTACAGTGGTGGTTATCCAT
201 S C T F A Q T V S T S Y T I T V V V I H
1
873 TTCTTTGTCCCATGTGCTGGTCACTTCTGCTACTTGGCTATCTGGATCTTGGTCACT
221 F F V P I A V V T F C Y L R I W I L V I
1
933 CAGGTGAGCGGAAGTAAAGTCAGAGTCCGTTACGCATCAAGCCCAGTGACCTAAGG
241 Q V R R K V K S E V R S R I K P S D L R
1
993 AACTTTATCACCATGTTTGTGGTGTGTTGCTATTGTCATTTGTTGGCACCACCTCAAC
261 N F I T M F V V F V L F A I C W A P L N
1
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281 F I G L A V A I N P E A V V P R I P E W
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301 L F V V S Y F M A Y F N S C L N A I I Y
1
1173 GGCCTGCTGAACCAGAACTTCCGCAGGGAGTACAAGCGCATCATATGCTGTGTGGATG
321 G L L N Q N F R R E Y K R I I M S V W M
1
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341 P H L F F Q E T S R G G T E A M K S K P
1
1293 TCACCAGGACTCAATAATAATGAGCAAGTAAAAGCAGAGACTCTGTGA acactatgacat
361 S P G L N N N E Q V K A E T L *
1
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1473 tcttcatcttcccggttcctcctctctggttaacctgtgcagtgccattatgaatct
ttacttgccaagcagatagactgaccaacggt

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Figure S4. Characteristics of gene structure and the deduced amino acid sequence of *LcMtnr1b2*-like. The phosphorylation sites predicted with NetPhos 3.1 Server are marked by solid dots in three different colors (Ser, Tyr and green are in blue, orange and green, respectively); the N-linked glycosylation sites predicted with NetNGlyc 1.0 Server are highlighted by red triangles; 7TM domains predicted with I-TASSER are labeled in the boxes in 7 different colors; the NRY, CYICH and NAXXY sequences predicted with PROSITE are labeled in brown, pink and gray boxes, respectively. TM: transmembrane.

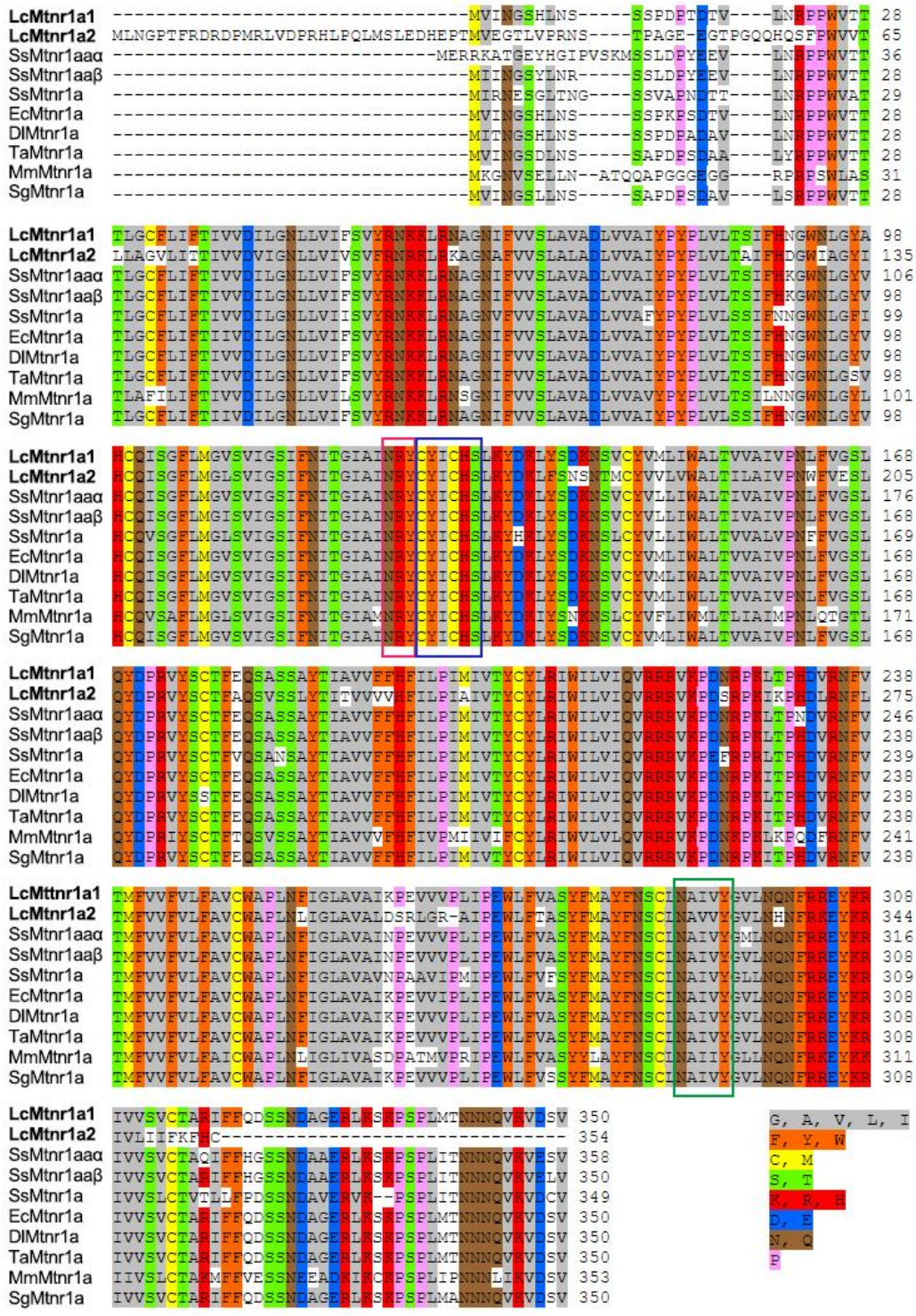
LcMtnr1c

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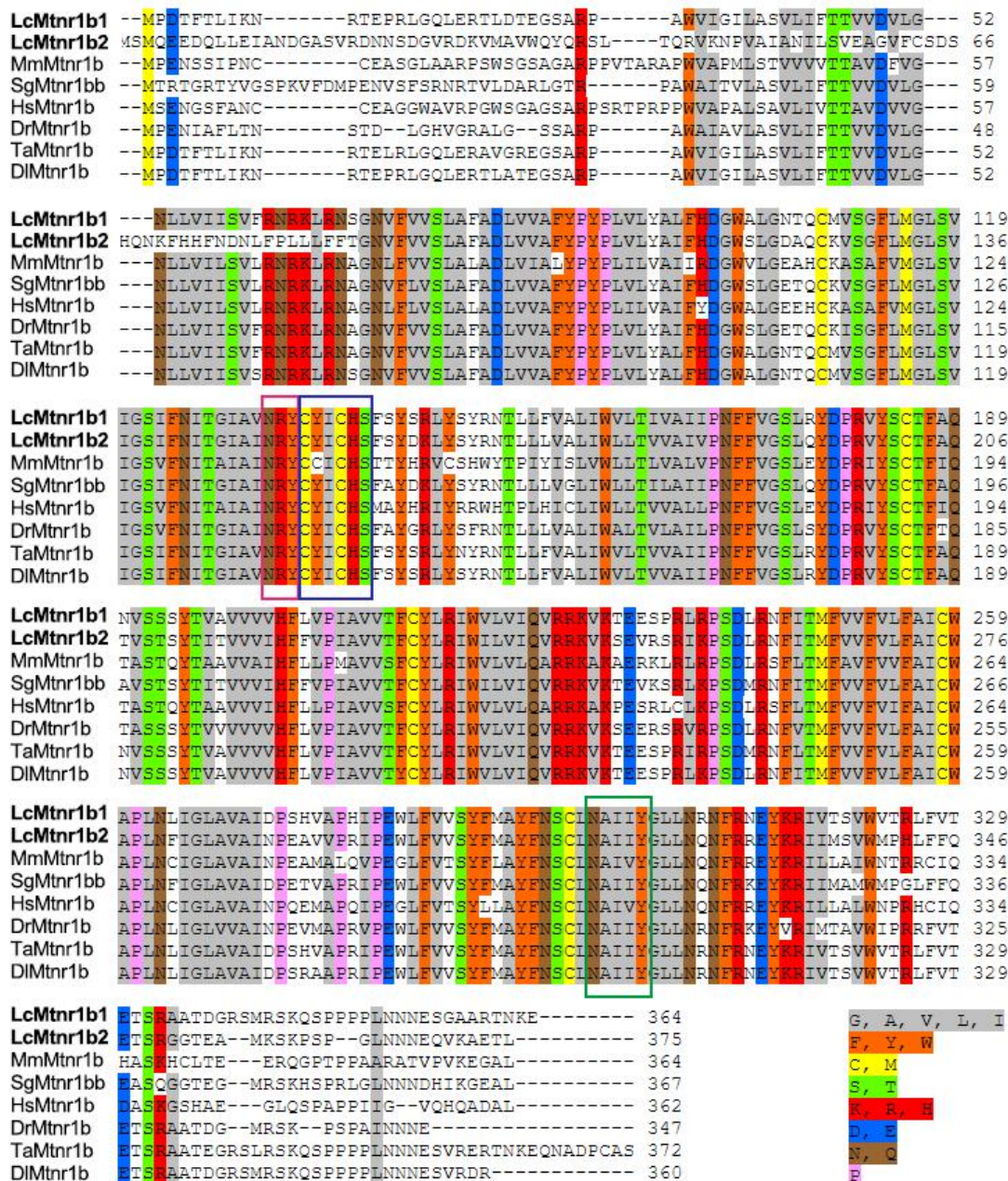
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1  M D L E V K D V N G S N C L S R N E S D
70 CGAGGACTGAGCGCTTCTTCCCTCTGGAGTGTCCACTGCCGTGGCCAGCGTGTGATCTTC
21  R G L S A S S S G V S T A L A S V L I F
130 ACCATCGTGGTAGACATCTGGGAAATGTCTCTCGTCATCTGTCCGTGTACAGGAACAAA
41  T I V V D I L G N V L V I L S V Y R N K
190 AAGCTGAGGAATGCAGGCAACATCTTCGTGGTGTGTTGTCCGTGGCAGACCTGGTGGTG
61  K L R N A G N I F V V S L S V A D L V V
250 GCATTGTACCCCTTACCCGCTGGTCTGACAGCCATCTTCCACAGTACTGGACCATGGGT
81  A L Y P Y P L V L T A I F H S D W T M G
310 GACCTCCACTGCCAGGCCAGCGCTTCATCATGGGCTGAGCGTCATCGGCTCCAATCTTC
101  D L H C Q A S G F I M G L S V I G S I F
370 AACATCAGGCCATCGCCATCAACCGTACTGCTACATCTGCCACAGCCTCCACTAGAC
121  N I T A I A I N R Y C Y I C H S L H Y D
430 CGACTGTACAGCCTGAGGAACCTGCTGCTACCTGGGCTCACCTGGCTGCTCACCGCC
141  R L Y S L R N T C C Y L G L T W L L T A
490 ATCGCCACGGTACCTAACTTCTTTGTGGGCTCGCTGAGTACGATCCCGCATTTACTCC
161  I A T V P N F F V G S L Q Y D P R I Y S
550 TGCACCTTCGCCAGACAGTCACTACTACACCATCTCAGTGGTGGTTATCACTTT
181  C T F A Q T V S S Y Y T I S V V V I H F
610 CTGATCCCGCTGCTGGTGGTCTTACTGCTACATGAGGATATGGGTGCTGGTATTCAA
201  L I P L L V V S Y C Y M R I W V L V I Q
670 GTGAAACATCGGGTTAAACCGGAGCAAAGACCAAACCTAAACCTAGTGATGTAAGGAAC
221  V K H R V K P E Q R T K L K P S D V R N
730 TCCCTGACTATGTTTATGGTGTGTTGTTGTTGTCAGTATGCTGGGCTCCACTCAACCTC
241  S L T M F M V F V L F A V C W A P L N L
790 ATAGTCTGGCTGTAGCTATAAACCTGTGAAAGTTGCACCCAATATACCTGAGTGGCTT
261  I G L A V A I N P V K V A P N I P E W L
850 TTTGCACTAGCTACTTTATGGCGTATTTCAACAGCTGCCTCAACGCCATCATATACGGA
281  F V T S Y F M A Y F N S C L N A I I Y G
910 CTGCTAAACCAAACCTTCGGAAAGAATAACAAGACAATCCTTCTTGTCTTTTGCATCCCA
301  L L N Q N F R K E Y K T I L L A L C I P
970 CGTTTGTCTCTCACGGAGACCTCCAGGTGTGCCACAGAGGACTGAAGAGTAAACCTTCA
321  R L L L T E T S R C A T E G L K S K P S
1030 CCGGCTGTAAACAAACAATAATAGCAGAGATAAATGTATAA accggtgcttgactggaa
341  P A V T N N N I A E I N V *
1090 aatttagattaaggtcctctggaacctcatcgtggatggtgacatgattcaccctgtgc
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1390 attaggagggtagttattgttggatggaagacttcactaatcaagccacatcccaaat
1450 ggtacatgaacaagagaatgactcgatcgattacagatag

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60
61 **Figure S5. Characteristics of gene structure and the deduced amino acid sequence of**
62 **LcMtnr1c-like.** The phosphorylation sites predicted with NetPhos 3.1 Server are marked by solid
63 dots in three different colors (Ser, Tyr and green are in blue, orange and green, respectively); the
64 N-linked glycosylation sites predicted with NetNGlyc 1.0 Server are highlighted by red triangles;
65 7TM domains predicted with I-TASSER are labeled in the boxes in 7 different colors; the NRY,
66 CYICH and NAXXY sequences predicted with PROSITE are labeled in brown, pink and gray
67 boxes, respectively. TM: transmembrane.

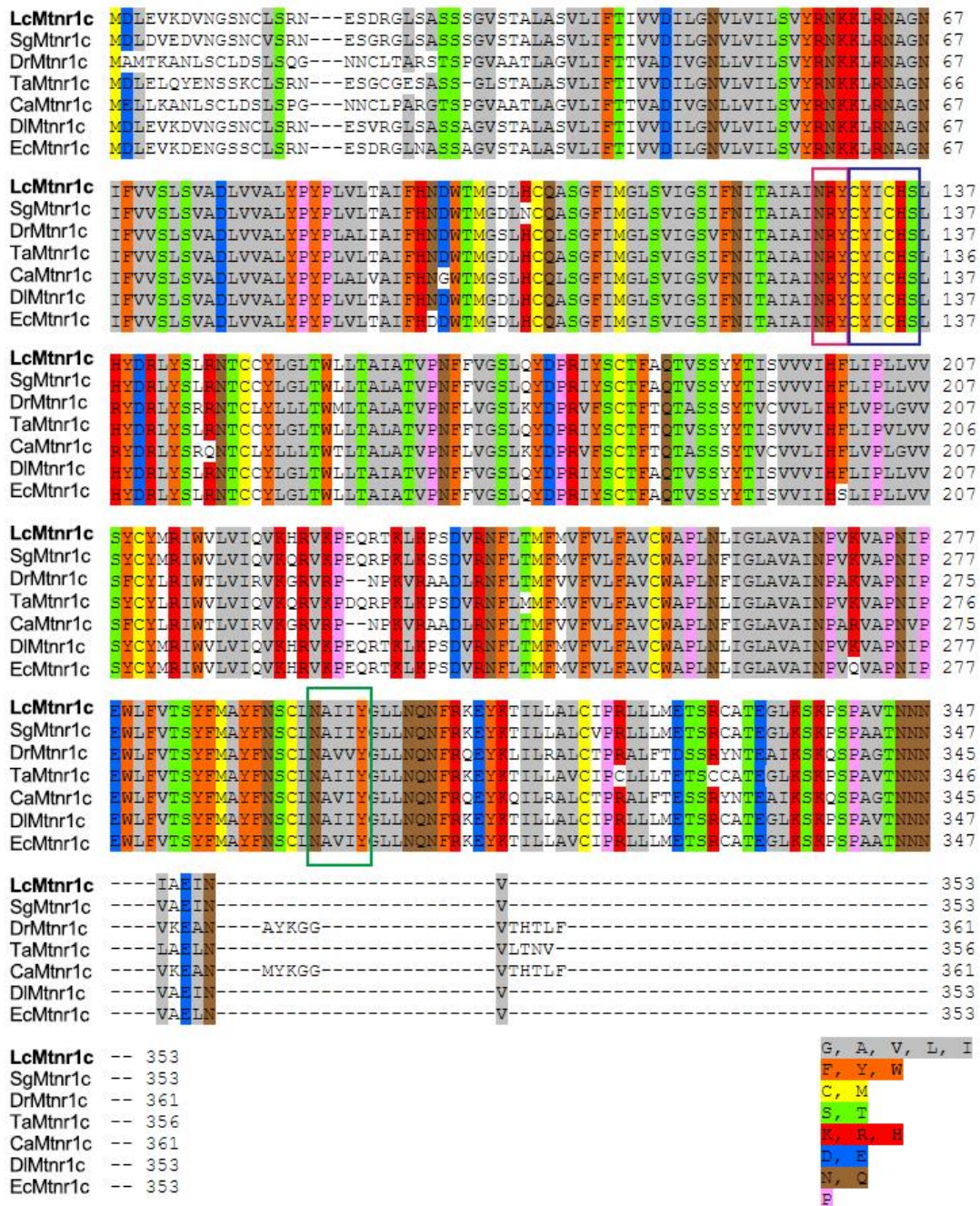


68
 69 **Figure S6. Alignment of the deduced *LcMtnr1a* amino-acid sequences with vertebrate**
 70 ***Mtnr1a* from other species.** All sequences were obtained from GenBank and their accession
 71 numbers are listed in Table S1. The NRY, CYICHS and NAXXY sequences are marked with pink,
 72 dark blue and green boxes, respectively. The percentage of sequences that must agree for identity
 73 or similarity coloring was set as 80%.



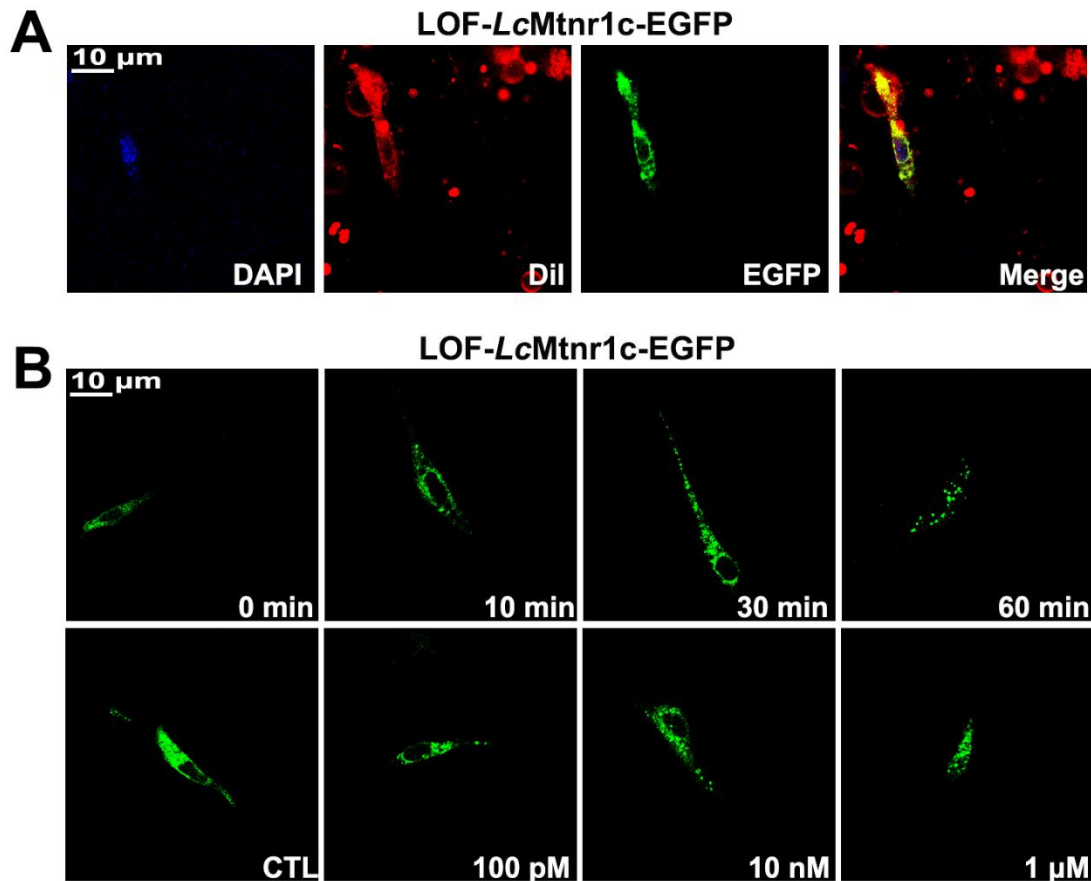
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Figure S7. Alignment of the deduced *LcMtnr1b* amino-acid sequences with vertebrate *Mtnr1a* from other species. All sequences were obtained from GenBank and their accession numbers are listed in Table S1. The NRY, CYICH and NAXXY sequences are marked with pink, dark blue and green boxes, respectively. The percentage of sequences that must agree for identity or similarity coloring was set as 80%.

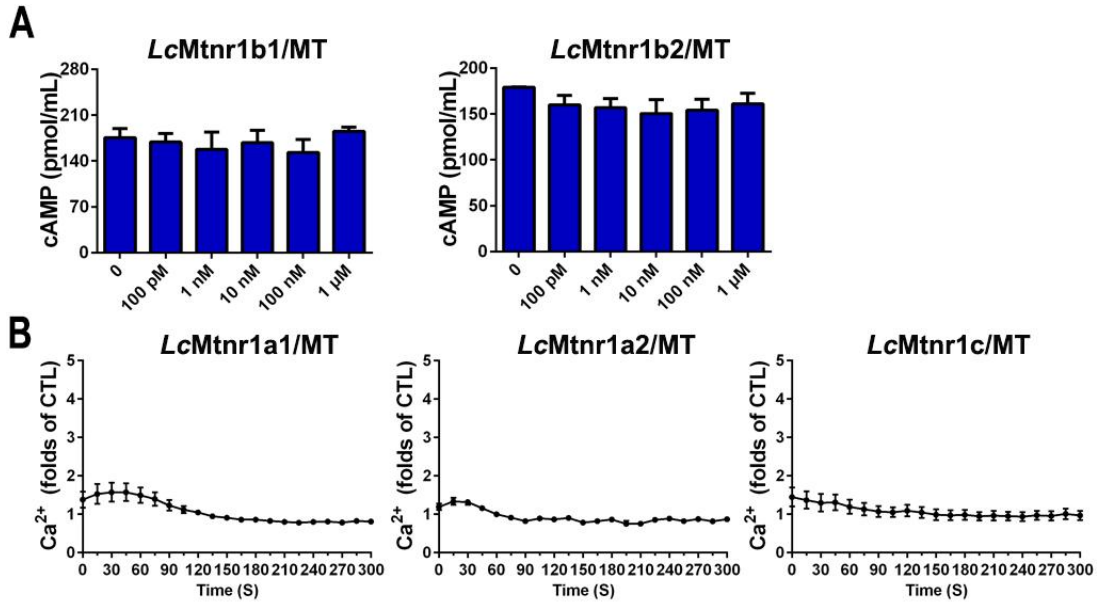


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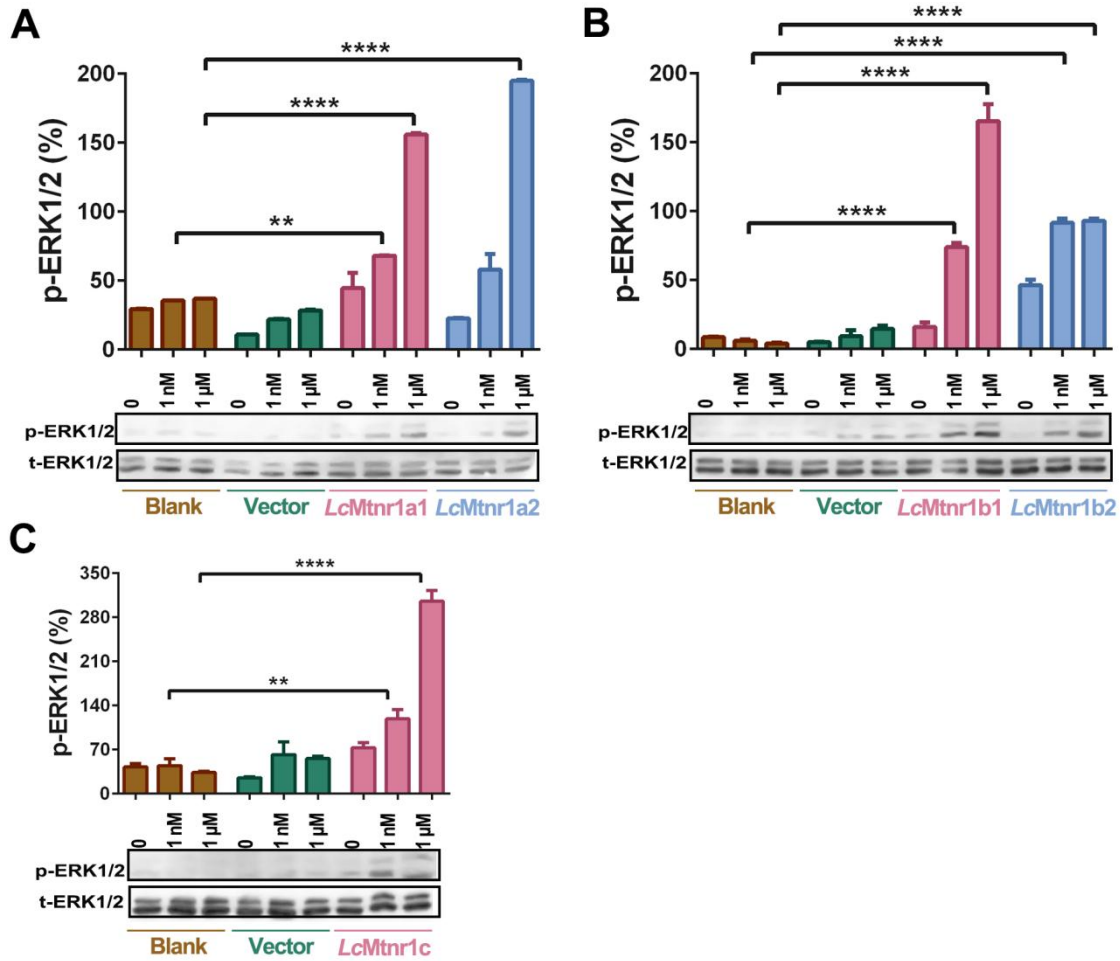
Figure S8. Alignment of the deduced *LcMtnr1c* amino-acid sequences with vertebrate *Mtnr1a* from other species. All sequences were obtained from GenBank and their accession numbers are listed in Table S1. The NRY, CYICHS and NAXXY sequences are marked with pink, dark blue and green boxes, respectively. The percentage of sequences that must agree for identity or similarity coloring was set as 80%.



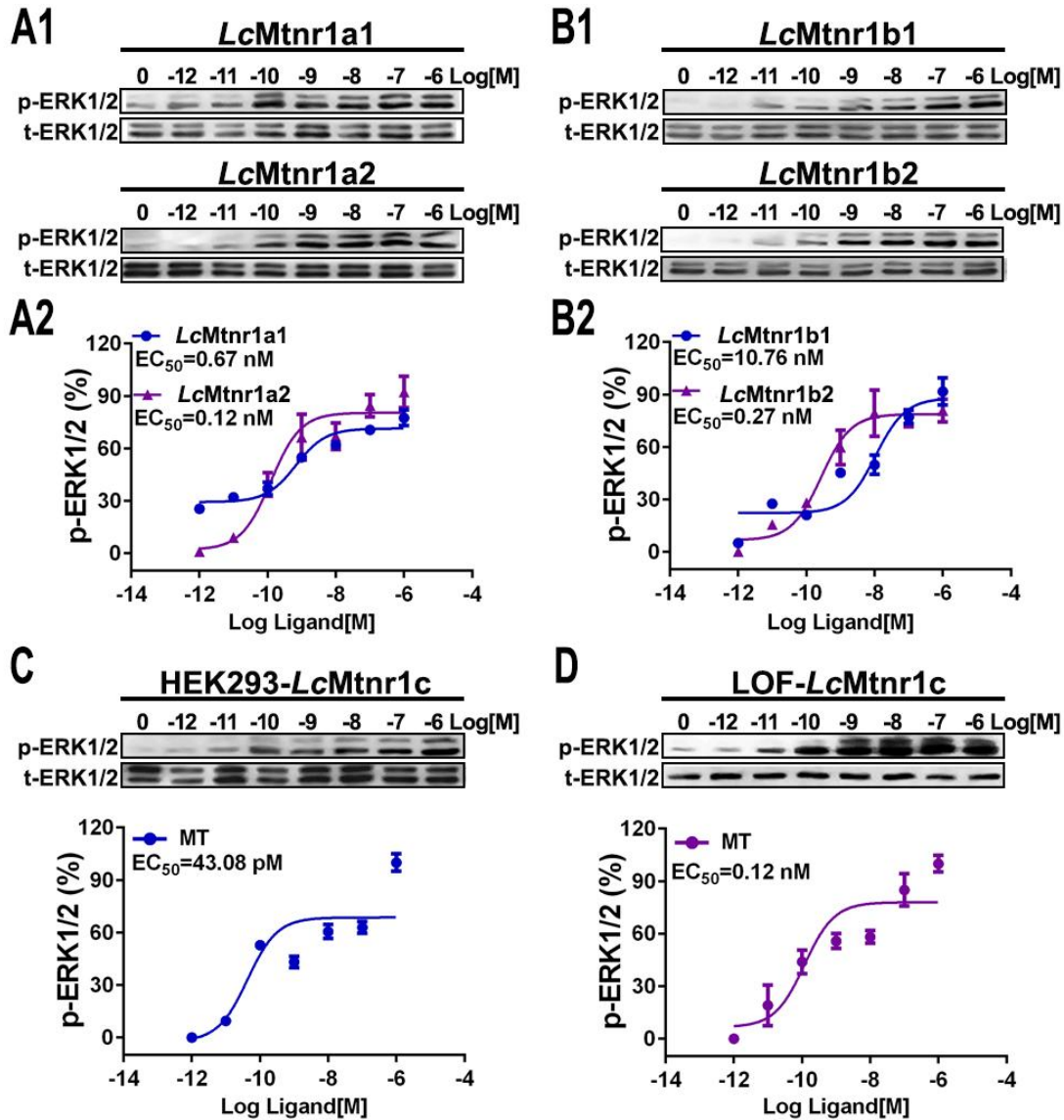
86
 87 **Figure S9. Characterization of *LcMtnr1c*-EGFP stably expressed in large yellow croaker**
 88 **ovary fibroblast (LOF) cells.** (A) Subcellular location of *LcMtnr1c*-EGFP in large yellow
 89 croaker ovary fibroblast (LOF) cells. LOF cells stably expressing *LcMtnr1c*-EGFP were stained
 90 with a membrane plasma probe (Dil, 5 μ M) and a nuclei probe (DAPI). (B) Internalization of
 91 *LcMtnr1c*-EGFP stably expressed in LOF cells. LOF-*LcMtnr1c* cells were incubated with 100 nM
 92 MT for the indicated time periods or indicated concentrations of MT at 37 $^{\circ}$ C for 60 min. Cells
 93 were fixed and examined with confocal microscopy as described under “Methods”. All pictures
 94 shown are representative of at least three independent experiments.



95
 96 **Figure S10. The detection of cAMP accumulation and Ca²⁺ mobilization in MT activated**
 97 ***LcMtnr1b1/2* or *LcMtnr1a1/1a2/1c* expressing cells.** (A) cAMP accumulation in *LcMtnr1b1/2*-
 98 expressing HEK293 cells was measured in response to the indicated concentration of MT for 15
 99 min. (B) Ca²⁺ mobilization in *LcMtnr1a1/1a2/1c*-expressing HEK293 cells was measured in
 100 response to 100 nM MT.
 101

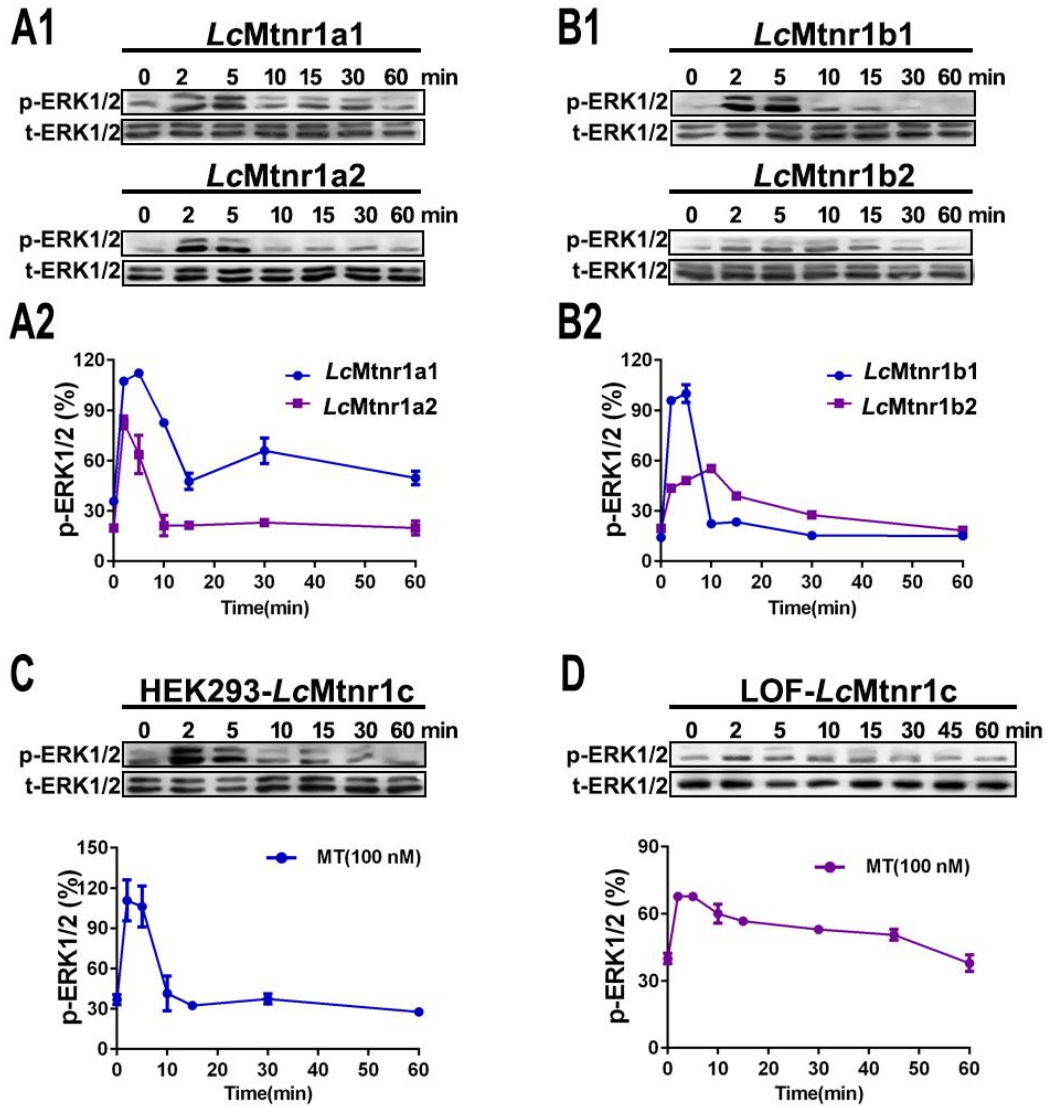


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 103 **Figure S11. MT-induced ERK1/2 phosphorylation in HEK293 cells.** Blank HEK293 cells,
 104 HEK293 cells stably expressing pFLAG-CMV-3 empty vector, *LcMtnr1a1/2*-FLAG (A),
 105 *LcMtnr1b1/2*-FLAG (B), and *LcMtnr1c*-FLAG (C) were stimulated with or without MT (1 nM
 106 and 1 μM) for 5 min, respectively. Data were analyzed using the one-way ANOVA followed by
 107 Tukey's multiple comparison test, ** $P < 0.01$, **** $P < 0.0001$. ERK1/2: extracellular regulated
 108 protein kinases 1/2.

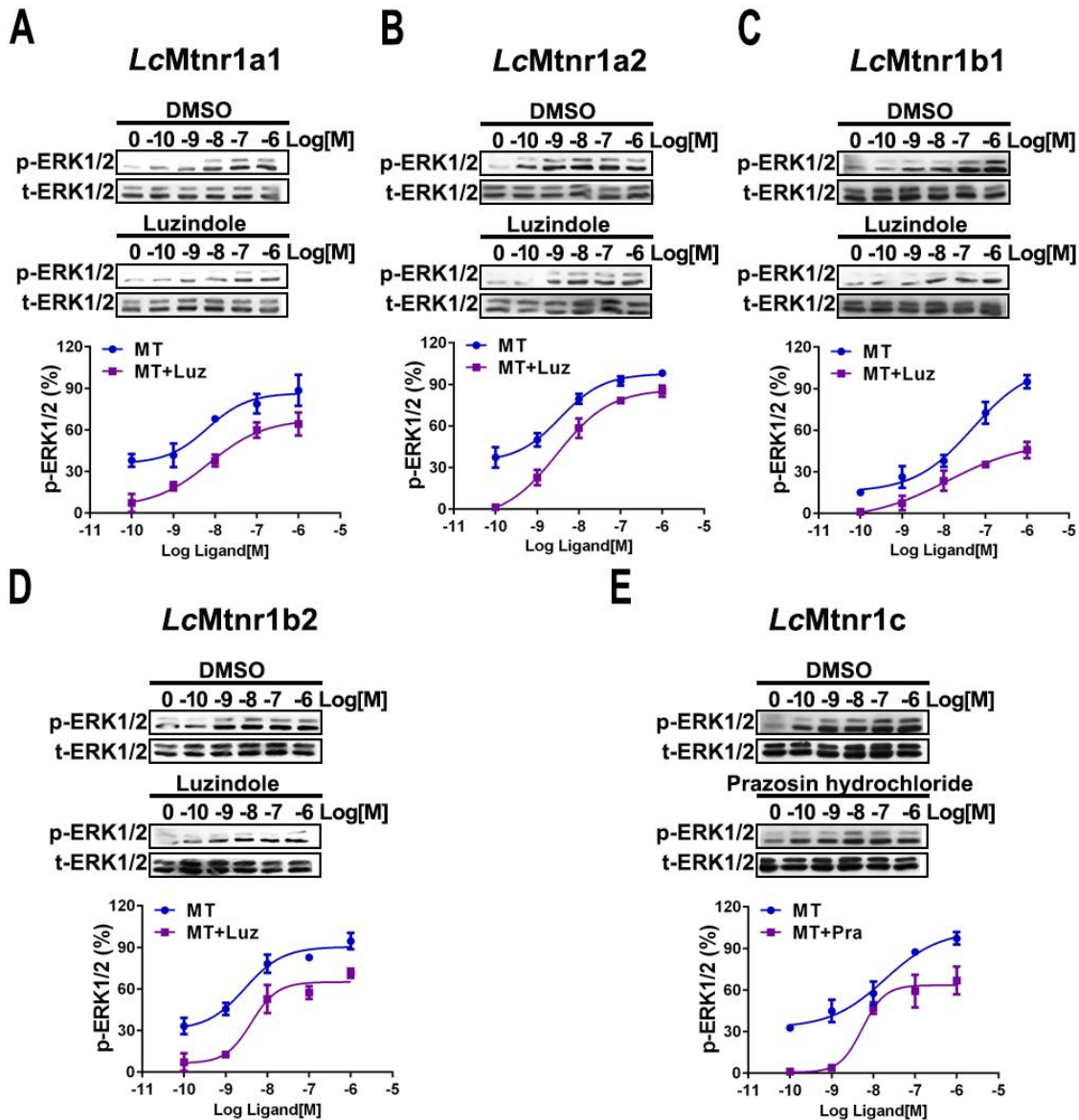


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110 **Figure S12. Concentration-dependent effects of MT on ERK1/2 phosphorylation in FLAG-**
 111 ***LcMtnrs* expressing cells. (A-C) and in LOF cells stably expressing FLAG-*LcMtnr1c* (D).**
 112 **HEK293-FLAG-*LcMtnrs* and LOF-FLAG-*LcMtnr1c* cells were stimulated with the indicated**
 113 **concentrations of MT for 5 min. ERK1/2: extracellular regulated protein kinases 1/2; EC₅₀:**
 114 **concentration for 50% of maximal effect.**

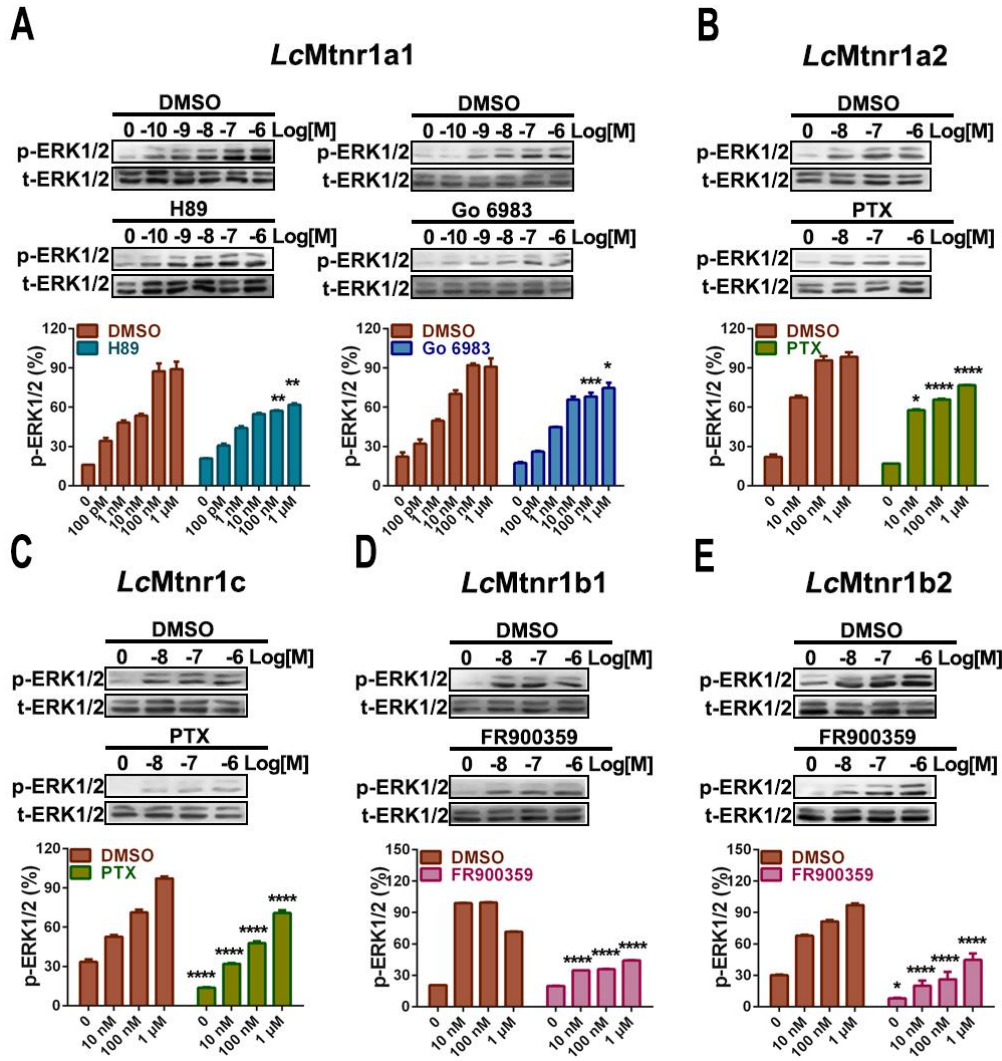


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 116 **Figure S13. Time-dependent effects of MT on ERK1/2 phosphorylation in FLAG-LcMtnrs**
 117 **expressing HEK293 cells (A-C) and in LOF cells (D).** HEK293-FLAG-LcMtnrs and LOF-
 118 FLAG-LcMtnr1c cells were stimulated with the 100 nM MT for the indicated periods of time. MT:
 119 melatonin; ERK1/2: extracellular regulated protein kinases 1/2.



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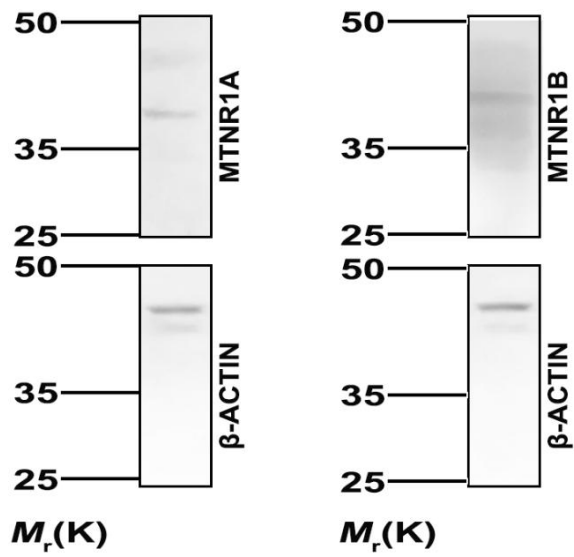
Figure S14. The *LcMtnrs*-mediated ERK1/2 phosphorylation activation inhibited by *Mtnrs* antagonists. HEK293-*LcMtnr1a1/1a2/1b1/1b2* cells were pre-treated with vehicle (DMSO) or *Mtnr1a/1b* antagonists (10 μ M Luzindole, Luz, 1 h) (A-D), or HEK293-*LcMtnr1c* cells were pre-treated with *Mtnr1c* antagonists (0.1 μ M prazosin hydrochloride, Pra, 1 h) (E), and the cells were stimulated with the indicated concentration of MT for 5 min. MT: melatonin; ERK1/2: extracellular regulated protein kinases 1/2.



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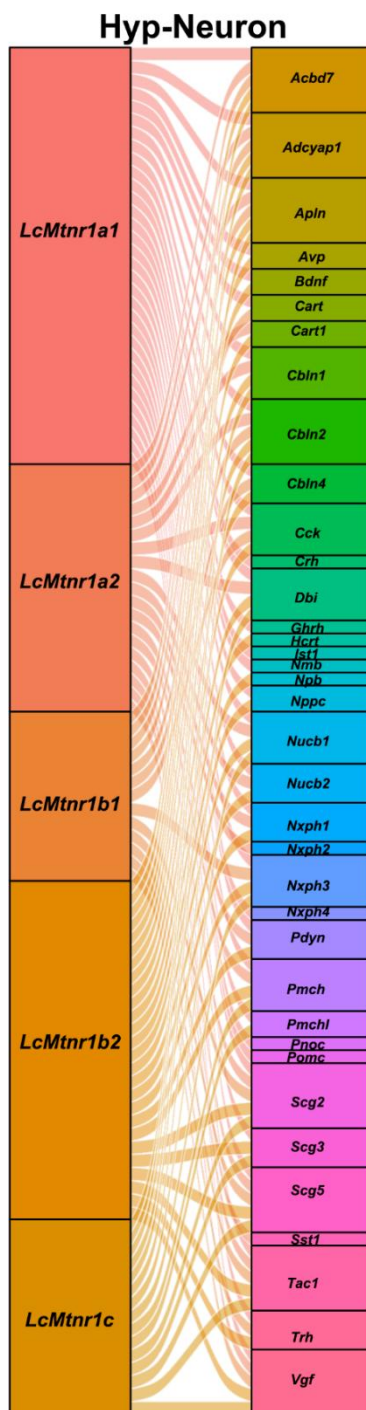
136 **Figure S15. ERK1/2 activation mediated by *Larimichthys crocea* melatonin receptors.** (A)
 137 ERK1/2 phosphorylation activated by *LcMtnr1a1* was blocked by PKA or PKC inhibitors.
 138 HEK293-*LcMtnr1a1* cells were pre-treated with DMSO, PKA inhibitor (H89, 10 μ M), or PKC
 139 inhibitor (Go 6983, 10 mM) for 1 h prior to MT stimulation. (B-E) MT-induced (10 nM-1 μ M, 5
 140 min) ERK1/2 phosphorylation in *LcMtnr1a2*- expressing (B), *LcMtnr1c*- expressing (C),
 141 *LcMtnr1b1*-expressing (D), and *LcMtnr1b2*-expressing (E) cells, in the absence or presence of
 142 PTX (50 ng/mL, 16 h) or FR900359 (1 μ M, 1 h). Data are presented as means \pm SEM. Data were
 143 analyzed using one-way ANOVA followed by Tukey's multiple comparison test (* $P < 0.05$, ** P
 144 < 0.01 , *** $P < 0.001$, **** $P < 0.0001$, all represent the difference between DMSO or inhibitor
 145 treated groups). PTX: pertussis toxin; PKA: Protein Kinase A; PKC: Protein Kinase C; H89: a
 146 PKA inhibitor; Go 6983: a PKC inhibitor; PTX: pertussis toxin; FR900359: an inhibitor of $G_{\alpha q}$;
 147 ERK1/2: extracellular regulated protein kinases 1/2; DMSO: dimethyl sulfoxide.

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Figure S16. Immunoblot analysis of the antibody specificities of anti-Mtnr1a and anti-Mtnr1b IgG polyclonal antibodies in hypothalamus of large yellow croaker.

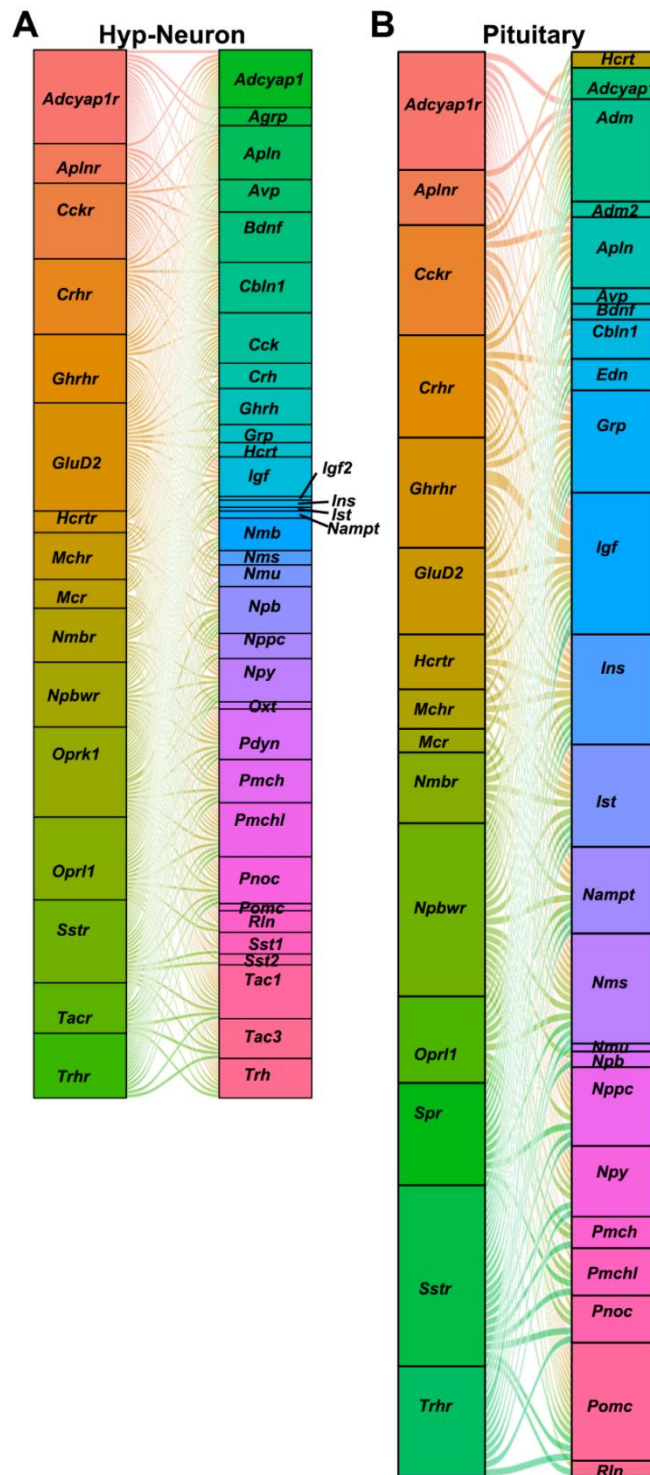


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153 **Figure S17. Co-expression of LcMtnrs and neuropeptides.** Co-expression of LcMtnrs and
 154 neuropeptides (*Acbd7*, *Adcyap1*, *Apln*, *Avp*, *Bdnf*, *Cart*, *Cbln1*, *Cbln2*, *Cbln4*, *Cck*, *Crh*, *Dbi*,
 155 *Ghrh*, *Ist1*, *Nmb*, *Npb*, *Nppc*, *Nucb1*, *Nucb2*, *Nxph1*, *Nxph2*, *Nxph3*, *Nxph4*, *Pdyn*, *Pmch*, *Pmchl*,
 156 *Pnoc*, *Pmoc*, *Scg2*, *Scg3*, *Scg5*, *Sst1*, *Tac1*, *Trh* and *Vgf*) in hypothalamus neurons of large yellow
 157 croaker. The neuropeptide genes, detected with more than four reads per cell (reads ≥ 4), are
 158 given for further analyses. The diagram was generated by the Xiantao web
 159 (<https://www.xiantao.love>). Source data 1 lists the details. *Acbd7*: *Acyl-CoA binding domain*

160 containing 7; *Adcyap1*: adenylate cyclase activating polypeptide 1; *Apln*: apelin; *Avp* arginine
161 vasopressin; *Bdnf*: brain derived neurotrophic factor; *Cart*: cocaine- and amphetamine-
162 regulated transcript; *Cbln1*: cerebellin 1; *Cbln2*: cerebellin 2; *Cbln4*: cerebellin 4; *Cck*:
163 cholecystokinin; *Crh*: corticotropin releasing hormone; *Dbi*: diazepam binding inhibitor, acyl-
164 CoA binding protein; *Gnrh*: growth hormone releasing hormone; *Hcrt*: hypocretin receptor; *Ist1*:
165 increased sodium tolerance 1 homolog; *Nmb*: neuromedin B; *Npb*: neuropeptide B; *Nppc*:
166 natriuretic peptide C; *Nucb1*: nucleobindin 1; *Nucb2*: nucleobindin 2; *Nxph1*: neurexophilin 1;
167 *Nxph2*: neurexophilin 2; *Nxph3*: neurexophilin 3; *Nxph4*: neurexophilin 4; *Pdyn*: prodynorphin;
168 *Pmch*: pro-melanin concentrating hormone; *Pmchl*: pro-melanin-concentrating hormone, like;
169 *Pnoc*: prepronociceptin; *Pomc*: proopiomelanocortin; *Scg2*: secretogranin II; *Scg3*:
170 secretogranin III; *Scg5*: secretogranin V; *Sst1*: susceptibility to tuberculosis 1; *Tac1*: tachykinin
171 precursor 1; *Trh*: thyrotropin releasing hormone; *Vgf*: VGF nerve growth factor inducible.

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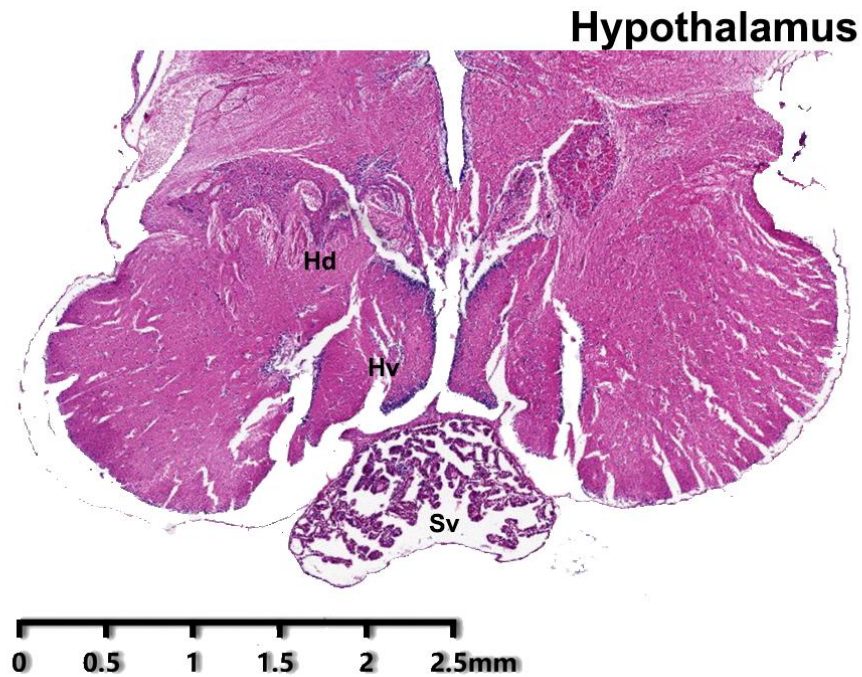


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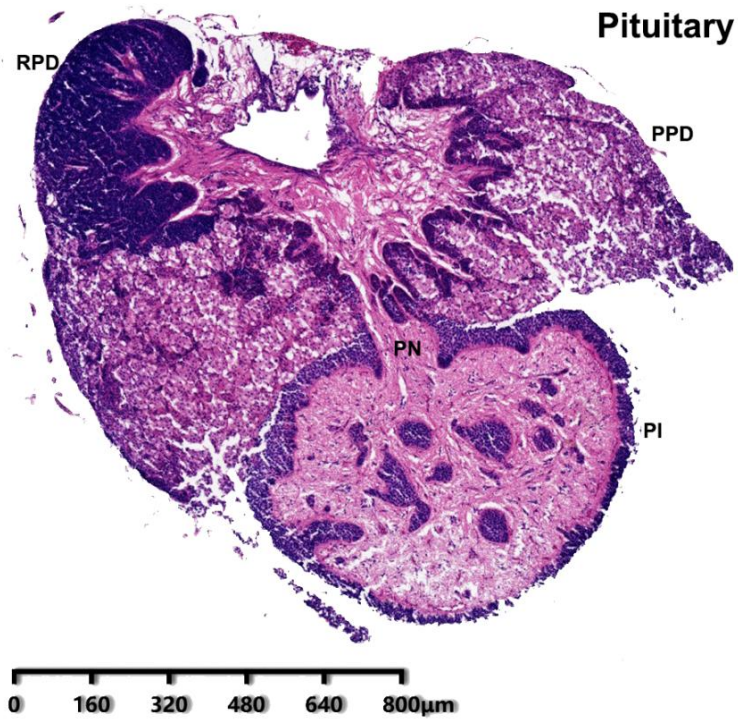
174 **Figure S18. Co-expression of neuropeptides' receptors and other neuropeptides in**
 175 **hypothalamus neurons or pituitary cells.** The expression cells of the neuropeptide receptors in
 176 hypothalamus neurons (A) or pituitary cells (B) were screened corresponding to the
 177 neuropeptides shown in Figure S16. The diagram was generated by the Xiantao web
 178 (<https://www.xiantao.love>). Source data 2 and 3 list the details. *Adcyap1r*: pituitary adenylate

179 cyclase-activating polypeptide type I receptor; *Aplnr*: apelin receptor; *Cckr*: cholecystokinin
 180 receptor; *Crhr*: corticotropin-releasing hormone receptor; *Gnrhr*: gonadotropin releasing
 181 hormone receptor; *GluD2*: glutamate dehydrogenase 2; *Hcrtr*: hypocretin receptor; *Mchr*:
 182 melanin concentrating hormone receptor; *Mcr*: mast cell regulator; *Nmbr*: neuromedin B
 183 receptor; *Npbwr*: neuropeptides B and W receptor; *Oprk1*: opioid receptor kappa 1; *Oprl1*:
 184 opioid related nociceptin receptor 1; *Sstr*: somatostatin receptor; *Tacr*: tachykinin peptides
 185 receptor; *Trhr*: thyrotropin releasing hormone receptor; *Adcyap1*: adenylate cyclase activating
 186 polypeptide 1; *Agpr*: agouti related neuropeptide; *Apln*: apelin; *Avp*: arginine vasopressin; *Bdnf*:
 187 brain derived neurotrophic factor; *Cbln1*: cerebellin 1; *Cck*: cholecystokinin; *Crh*: corticotropin
 188 releasing hormone; *Gnrh*: growth hormone releasing hormone; *Hcrt*: hypocretin receptor; *Igf*:
 189 insulin-like growth factor; *Igf2*: insulin like growth factor 2; *Ins*: insulin; *Ist1*: IST1 factor
 190 associated with ESCRT-III; *Nampt*: nicotinamide phosphoribosyltransferase; *Nmb*: neuromedin
 191 B; *Nms*: neuromedin S; *Nmu*: neuromedin U; *Npb*: neuropeptide B; *Nppc*: natriuretic peptide C;
 192 *Npy*: neuropeptide Y; *Oxt*: oxytocin/neurophysin I prepropeptide; *Pdyn*: prodynorphin; *Pmch*:
 193 pro-melanin concentrating hormone; *Pmchl*: pro-melanin-concentrating hormone, like; *Pnoc*:
 194 prepronociceptin; *Pomc*: proopiomelanocortin; *Rln*: relaxin; *Sst1*: susceptibility to tuberculosis 1;
 195 *Sst2*: susceptibility to tuberculosis 2; *Tac1*: tachykinin precursor 1; *Tac3*: tachykinin precursor 3;
 196 *Trh*: thyrotropin releasing hormone; *Spr*: septapterin reductase; *Adm*: adrenomedullin; *Adm2*:
 197 adrenomedullin 2; *Grp*: gastrin releasing peptide.

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 200 **Figure S19. Microscopy of hematoxylin-eosin stained section of large yellow croaker**
 201 **hypothalamus.** Hv: the ventral zones of the hypothalamus; Hd: the dorsal zones of the
 202 hypothalamus; Sv: Saccus vasulosus. Scale, as indicated.



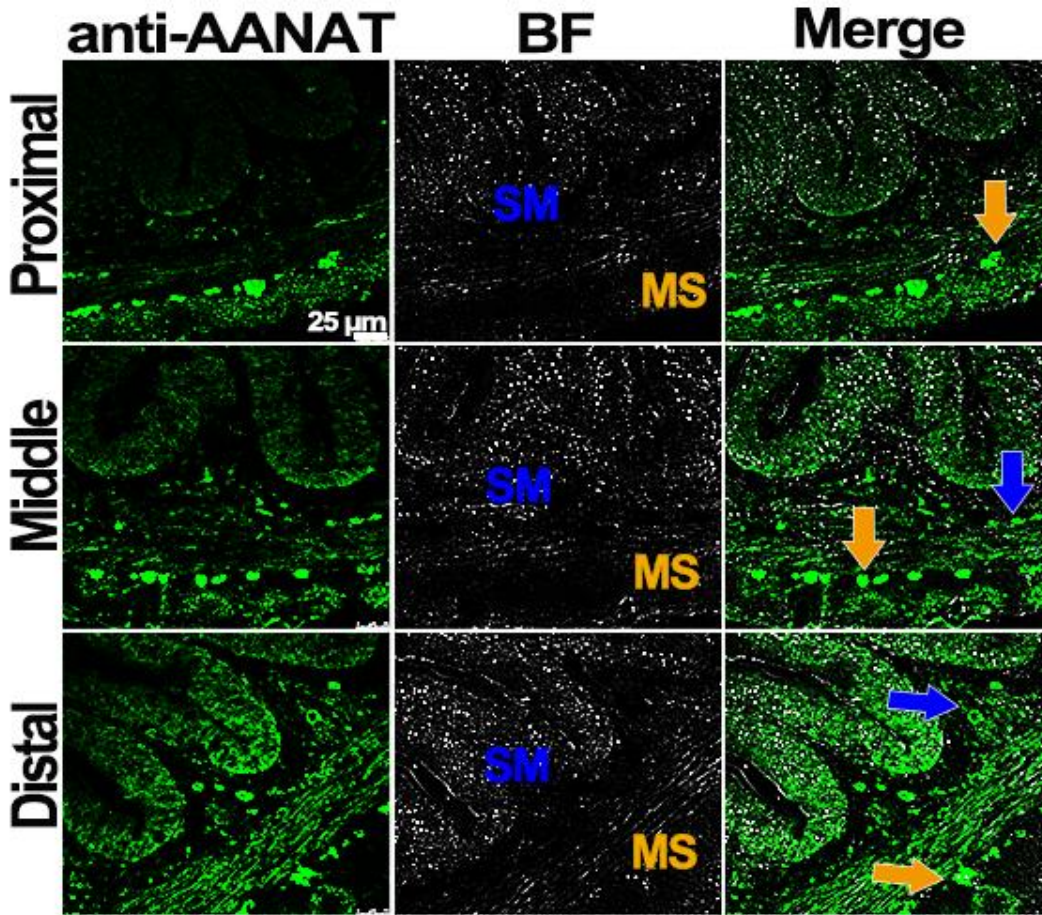
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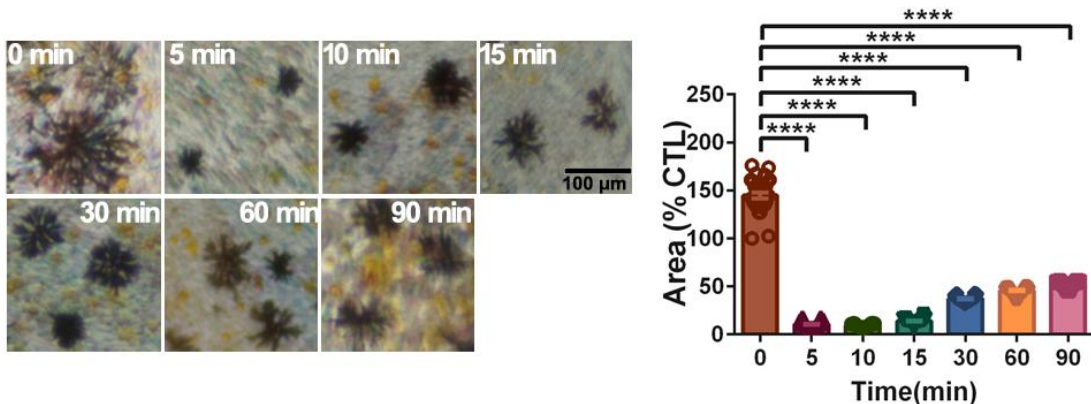
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Figure S20. Microscopy of hematoxylin-eosin stained section of large yellow croaker pituitary. RPD: rostral pars distalis; PPD: proximal pars distalis; PI: pars intermedia; PN: neurohypophysis. Scale, as indicated.



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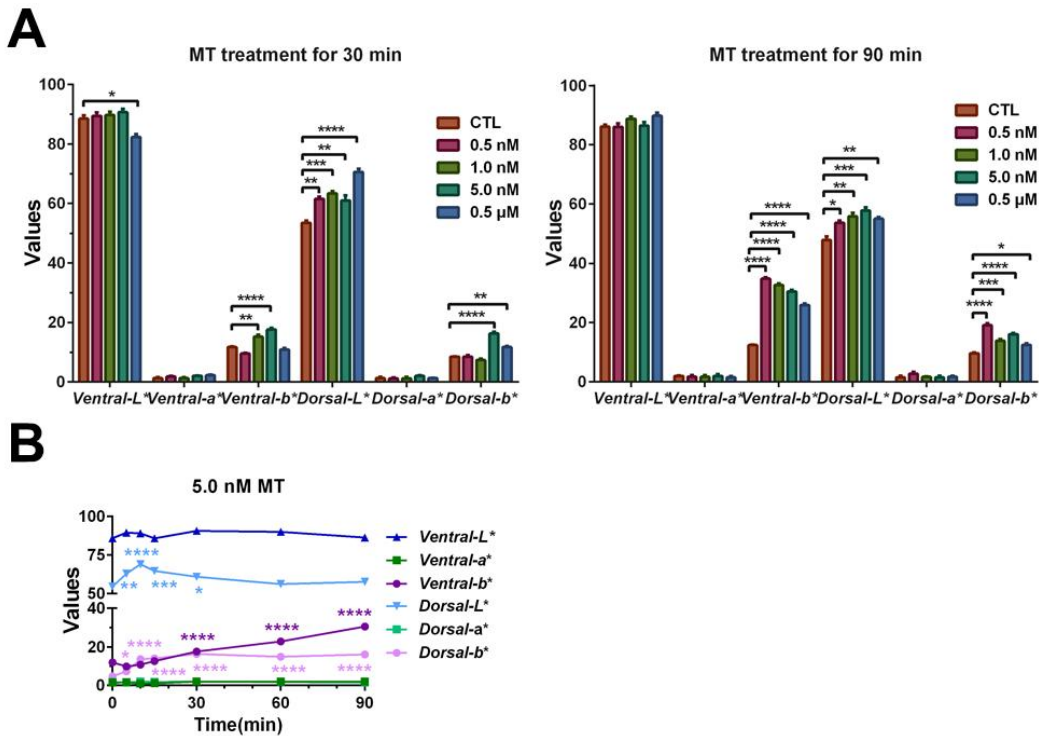
208 **Figure S21. Distribution of AANAT in intestine of large yellow croaker.**
 209 Immunohistochemical images of presumed MT-synthesizing cells in the intestine of large yellow
 210 croaker. The “AANAT” refers to the intestinal sections which were incubated with the anti-
 211 AANAT antibody. BF: Bright Field. Arrowheads in merged images indicate AANAT⁺ cells. Ms:
 212 muscularis; Sm: submucosa.



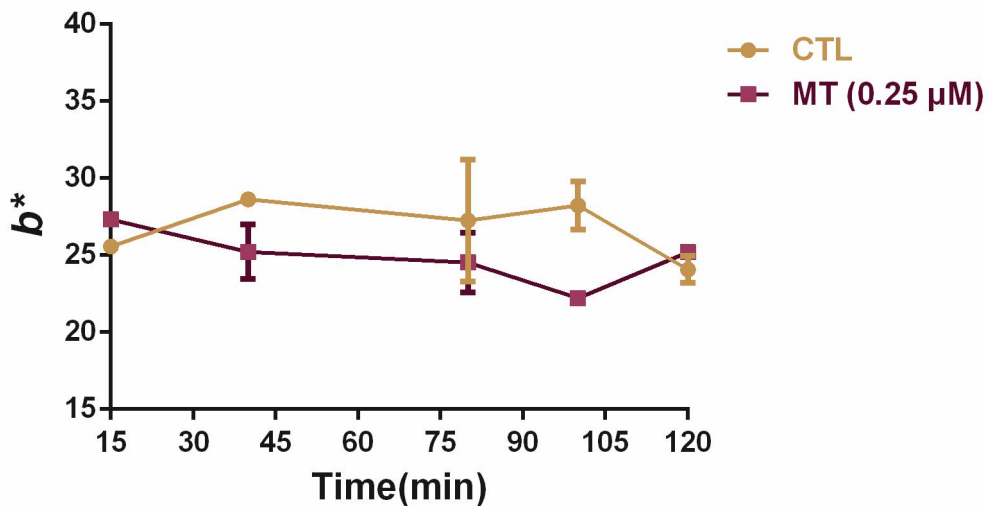
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214 **Figure S22. MT-mediated regulation of melanophore mobilization in the large yellow**
 215 **croaker *in vivo*.** The bar chart shows the areas of melanophore covered the dorsal skin after
 216 indicated times treatment with 5.0 nM MT. The results are expressed as mean (\pm SEM) and are
 217 expressed as areas of melanophores relative to nontreated groups. Data were analyzed using the

218 one-way ANOVA followed by Tukey's multiple comparison test, **** $P < 0.0001$.
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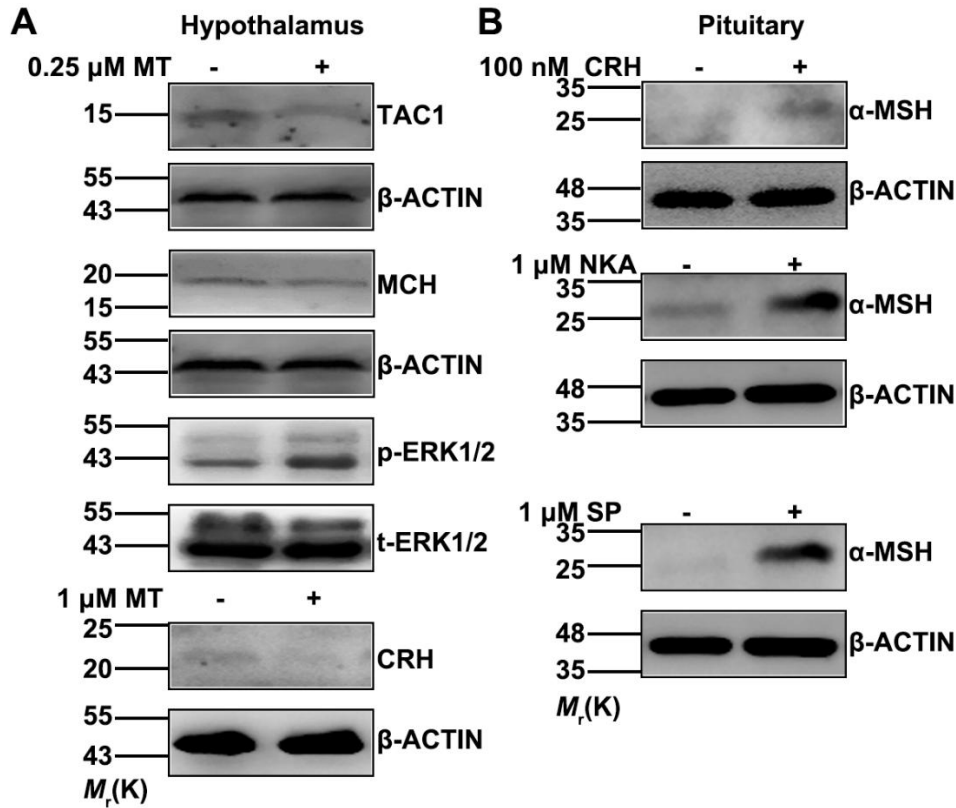


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 221 **Figure 23. Effects of MT on the skin colors of the adult large yellow croaker.** The L^* , a^* , and
 222 b^* values were measured for the ventral and dorsal treated by different concentrations of MT for
 223 30 min and 90 min (A) as well as 5.0 nM MT treated within 90 min (B), respectively. Each data
 224 represents mean (\pm SEM) ($n=3$). Data were analyzed using the one-way ANOVA followed by
 225 Tukey's multiple comparison test, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$.
 226 MT: melatonin; CTL: control.



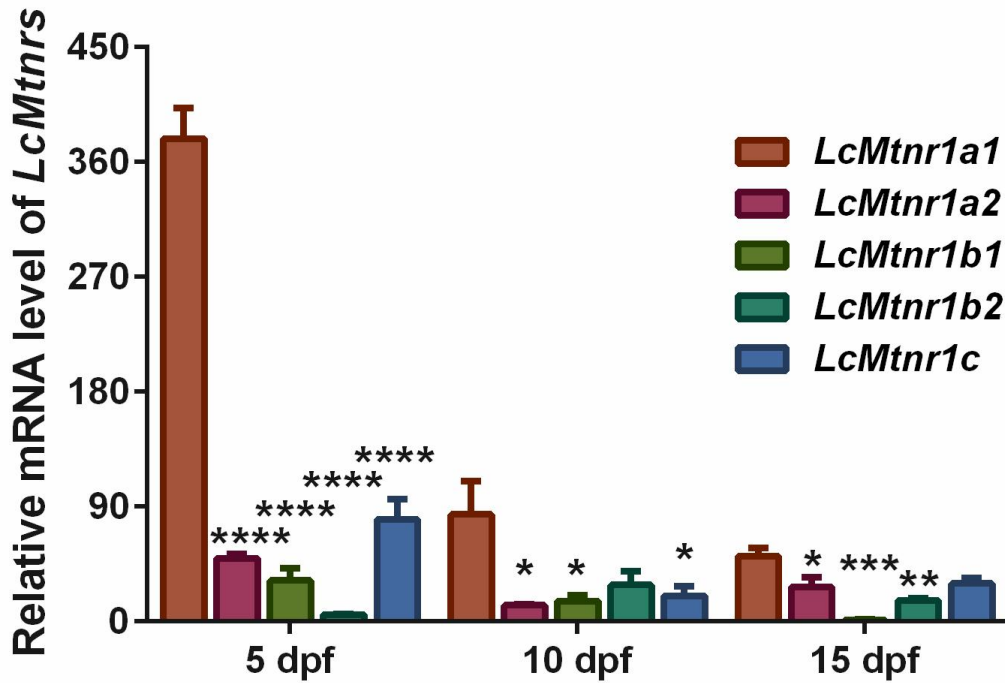
227
 228 **Figure S24. The b^* values from ventral skin of large yellow croaker with 0.25 μM MT**
 229 **treatment for indicated times *in vitro*.** Data were analyzed using the two-way ANOVA

230 followed by Sidak's multiple comparison test. MT: melatonin.
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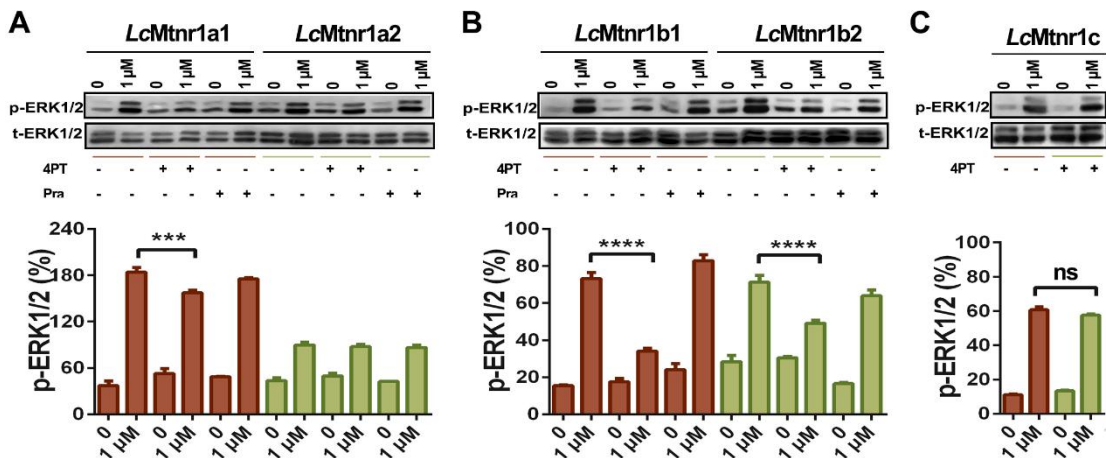
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233 **Figure S25. Immunoblot analysis of TAC1, MCH and p-ERK1/2 in hypothalamus and α -**
 234 **MSH in pituitary of large yellow croaker.** (A) Samples were collected and lysed after 30 min of
 235 MT (0.25 μ M and 1 μ M) stimulation. (B) Samples were collected and lysed after 30 min of 100
 236 nM CRH, 1 μ M neurokinin A (NKA), and 1 μ M substance P (SP) stimulation, respectively. The
 237 amino acid sequences of CRH, NKA and SP are as follows: CRH,
 238 SEDPPISLDLTFHLLREMMEMSRAEQLAQQAQNNRRMMELF-NH₂; NKA,
 239 HKVNSFVGLM-NH₂; SP, KPRPHQFIGLM-NH₂. MT: melatonin; TAC1: Tachykinin Precursor
 240 1; MCH: melanin-concentrating hormone; CRH: corticotropin releasing hormone; α -MSH: α -
 241 melanocyte-stimulating hormone (MSH); ERK1/2: Extracellular signal-Regulated Kinase 1/2.



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Figure S26. Relative expression of *LcMtnrs* mRNA at different developmental stages of large yellow croaker larvae. Each symbol and vertical bar represent mean \pm SED ($n = 6$). * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$ compared with *LcMtnr1a1*, by one-way analysis of variance (ANOVA) using Tukey's multiple comparison test. dpf: day post fertilization.



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Figure S27. Effects of 4PT and Pra on the ERK1/2 phosphorylation in HEK293-*LcMtnrs* cells. Samples were measured after 5 min of 1 μ M MT stimulation with or without 1 h of 4-P-PDOT (4PT) (0.1 μ M) or , 50 μ M prazosin hydrochloride (Pra) (0.1 μ M) pre-treatment. Data were analyzed using the one-way ANOVA followed by Tukey's multiple comparison test, *** $P < 0.001$, **** $P < 0.0001$. ns, not significant. ERK1/2: Extracellular signal-Regulated Kinase 1/2.

257 **Table S1.**
 258 **Accession numbers of melatonin receptor protein sequences of different species were used**
 259 **for phylogenetic analysis and alignment.**

Species	Gene synonym	Accession no.
<i>Homo sapiens</i>		NP_005949.1
<i>Mus musculus</i>		NP_032665.1
<i>Danio rerio</i>		NP_571468.1
<i>Takifugu alboplumbeus</i>		BAI39598.1
<i>Gallus gallus</i>		NP_990693.1
<i>Dicentrarchus labrax</i>		ACB13280.1
<i>Epinephelus coioides</i>		AGO03817.1
<i>Esox lucius</i>	Mtnr1a	XP_010889900.1
<i>Carassius auratus</i>		BAI65861.1
<i>Lepisosteus oculatus</i>		XP_006631980.2
<i>Salmo salar</i>		XP_014064448.1
<i>Salmo salar</i>		XP_014050730.1
<i>Salmo salar</i>		XP_014068290.1
<i>Salmo salar</i>		XP_014068723.1
<i>Siganus guttatus</i>		ABG77572.1
<i>Homo sapiens</i>		NP_005950.1
<i>Mus musculus</i>		NP_663758.2
<i>Gallus gallus</i>		NP_001280032.1
<i>Danio rerio</i>		NP_571469.1
<i>Takifugu alboplumbeus</i>		BAI39599.1
<i>Dicentrarchus labrax</i>	Mtnr1b	ACB13281.1
<i>Epinephelus coioides</i>		AGO03818.1
<i>Esox lucius</i>		NP_001290778.1
<i>Carassius auratus</i>		BAI65864.1
<i>Lepisosteus oculatus</i>		XP_015196706.1
<i>Salmo salar</i>		XP_014070615.1
<i>Gallus gallus</i>		NP_990692.1
<i>Xenopus laevis</i>		NP_001081388.1
<i>Danio rerio</i>		NP_001154956.1
<i>Carassius auratus</i>		BAI65865.1
<i>Dicentrarchus labrax</i>	Mtnr1c	ACB13282.1
<i>Takifugu alboplumbeus</i>		BAI39600.1
<i>Siganus guttatus</i>		ABG77573.1
<i>Epinephelus coioides</i>		AGO03819.1
<i>Esox lucius</i>		XP_010896763.1
<i>Homo sapiens</i>		BAA94488.1
<i>Homo sapiens</i>		BAA01763.1
<i>Mus musculus</i>		EDL18479.1
<i>Danio rerio</i>	5-HT1R	NP_001116793.1
<i>Danio rerio</i>		NP_001139238.1
<i>Gallus gallus</i>		NP_001163999.1
<i>Xenopus laevis</i>		NP_001079299.1
<i>Salmo salar</i>		XP_013999610.1
<i>Homo sapiens</i>		EAX08770.1
<i>Mus musculus</i>		EDL35846.1
<i>Danio rerio</i>	5-HT2R	ABI18978.1
<i>Gallus gallus</i>		AEV42168.1
<i>Larimichthys crocea</i>		KAE8279357.1

<i>Homo sapiens</i>		CAN84676.1
<i>Mus musculus</i>	5-HT4R	CAA09598.1
<i>Larimichthys crocea</i>		KAE8284925.1
<i>Salmo salar</i>		XP_014055545.1
<i>Homo sapiens</i>		EAX04526.1
<i>Mus musculus</i>	5-HT5R	EDL37224.1
<i>Mus musculus</i>		EDL39785.1
<i>Salmo salar</i>		XP_014036159.1
<i>Homo sapiens</i>		AAA92622.1
<i>Mus musculus</i>	5-HT6R	AAD46490.1
<i>Larimichthys crocea</i>		KAE8293148.1
<i>Homo sapiens</i>		AAC37538.1
<i>Mus musculus</i>	5-HT7R	EDL41763.1
<i>Danio rerio</i>		XP_690599.7
<i>Xenopus laevis</i>		NP_001079253.1
<i>Larimichthys crocea</i>		KAE8280972.1
<i>Salmo salar</i>		XP_014026341.1

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262 **Table S2.**
 263 **Sequences of primers used for vector construction, qPCR analysis, and sequence**
 264 **identification for *LcMtnrs*.**

Primer	Sequence (5'-3')	Application	Identifier
<i>Lcmtnr1a1</i> -seq-F	CCCAAGCTTATGGTTATAAAATGGGTC	vector construction	NCBI
<i>Lcmtnr1a1</i> -seq-R	CGGGATCCCAGACAGAGTCCACC		BioProject, PRJNA354443
<i>Lcmtnr1a2</i> -seq-F	CCCAAGCTTATGCTGAATGGACCG	Sequence identification	GenBank: OM939679
<i>Lcmtnr1a2</i> -seq-R	GGGGTACCCAGCAGTGAAACTTG		GenBank: OM939680
<i>Lcmtnr1b1</i> -seq-F	CCAAGCTTATGCCGGACACATTC		NCBI
<i>Lcmtnr1b1</i> -seq-R	GGGGTACCCATTCTTTGTTTGTG		BioProject, PRJNA354443
<i>Lcmtnr1b2</i> -seq-F	CCAAGCTTATGTCCATGCAAGAAG		GenBank: OM939678
<i>Lcmtnr1b2</i> -seq-R	CGCGGATCCCACAGAGTCTCTGC		
<i>Lcmtnr1c</i> -seq-F	CCAAGCTTATGGATTTAGAGGTG	Internal control	GenBank: EU443733.1
<i>Lcmtnr1c</i> -seq-R	GGGGTACCGATACATTTATCTCTGC		
<i>β-actin</i> F	TCGTCGGTCGTCCCAGGCATCAG		NCBI
<i>β-actin</i> R	ATGGCGTGGGGCAGAGCGTAACC		BioProject, PRJNA354443
<i>Lcmtnr1a1</i> -q-F	TACAGGTGAGGAGACGAGTGAAG	qPCR	GenBank: OM939679
<i>Lcmtnr1a1</i> -q-R	GAAGTAGGCCATGAAGTAGCTGG		GenBank: OM939680
<i>Lcmtnr1a2</i> -q-F	GTGAGCTCGCTGTACACTATCAC		NCBI
<i>Lcmtnr1a2</i> -q-R	CGACGAACATGGTGAGGAAGTTG		BioProject, PRJNA354443
<i>Lcmtnr1b1</i> -q-F	ACACAGTGGCAGTAGTAGTGGT		GenBank: OM939678
<i>Lcmtnr1b1</i> -q-R	CAGCACAAAGACCACGAACATG		
<i>Lcmtnr1b2</i> -q-F	TCAACCGCTACTGTTACATCTGC		
<i>Lcmtnr1b2</i> -q-R	GTATGAGGTGCTGACTGTCTGTG		
<i>Lcmtnr1c</i> -q-F	ACATCTTCGTGGTGAGTTTGTCC		
<i>Lcmtnr1c</i> -q-R	GTGATGTTGAAGATGGAGCCGAT		

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267 **Table S3.**
 268 **The sources of *Lcmtnrs* probes, hairpins, and antibodies used for *In situ* HCR and**
 269 **immunofluorescence detection.**

Reagent type	Designation	Source	Identifiers
Probe	<i>Lcmtnr1a1</i> -B4 (RefSeq ID: XP_010727592.1)	Molecular Instruments	LOT.PRH057
Probe	<i>Lcmtnr1a2</i> -B1 (RefSeq ID: XP_010741227.1)	Molecular Instruments	LOT.PRH054
Probe	<i>Lcmtnr1b1</i> -B3 (RefSeq ID: XP_027129075.1)	Molecular Instruments	LOT.PRH056
Probe	<i>Lcmtnr1c</i> -B2 (RefSeq ID: XP_010750373.1)	Molecular Instruments	LOT.PRH055
Hairpin/Fluorophore	B1h1 / Alexa514	Molecular Instruments	LOT.S031921
Hairpin/Fluorophore	B1h2 / Alexa514	Molecular Instruments	LOT.S032021
Hairpin/Fluorophore	B2h1 / Alexa488	Molecular Instruments	LOT.S035322
Hairpin/Fluorophore	B2h2 / Alexa488	Molecular Instruments	LOT.S034422
Hairpin/Fluorophore	B3h1 / Alexa594	Molecular Instruments	LOT.S029722
Hairpin/Fluorophore	B3h2 / Alexa594	Molecular Instruments	LOT.S031822
Hairpin/Fluorophore	B4h1 / Alexa647	Molecular Instruments	LOT.S034722
Hairpin/Fluorophore	B4h2 / Alexa647	Molecular Instruments	LOT.S025122
Antibody	MTNR1A-anti-rabbit IgG (polyclonal)	Abcam	CAT#ab203038
Antibody	MTNR1B-anti-rabbit IgG (polyclonal)	Abcam	CAT#ab203346
Antibody	GAD-65/67-anti-mouse IgG (monoclonal)	Santa Cruz	CAT#sc-365180
Antibody	FITC-conjugated goat anti-mouse IgG (polyclonal, goat)	Beyotime	CAT#A0568
Antibody	Cy3-conjugated goat anti-rabbit IgG (polyclonal, goat)	Beyotime	CAT#A0516
Antibody	TAC1-anti-rabbit IgG (polyclonal)	Proteintech	CAT#28599-1-AP
Antibody	CRF-anti-rabbit IgG (polyclonal)	ThermoFisher	CAT#PA5-102356
Antibody	NPY-anti-rabbit IgG (polyclonal)	ThermoFisher	CAT#PA5-95226
Antibody	ACBD7-anti-rabbit IgG (polyclonal)	Sigma	CAT#HPA062478
Antibody	MCH-anti-rabbit IgG (polyclonal)	Sigma	CAT#M8440

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274 **Source data files:**

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276 **Source data1. The hypothalamus peptidergic neurons co-expressing *LcMtnrs*.**

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278 **Source data2. The gene expression of neuropeptide receptors in hypothalamus neurons.**

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280 **Source data3. The gene expression of neuropeptide receptors in pituitary cells.**