Grass Carp Prolactin Gene: Structural Characterization and Signal Transduction for PACAP-induced Prolactin Promoter Activity

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P1-F

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P1-R

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D S H F P

P4-F

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3901  aacaccagagggttaattatgttacatgtaggctcagctgtagactacttgcttgaacaaacctc

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                                                          E  D  E  L
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       L  S  L  A  R  S  L  L  L  A  W  S  D  P  L  A  L  L  S  S  E  A
4096  GTCCAGCCTGGCACATCCAGAACGCAACACCATTAATAGCAAGACCAAAGAACTGCAAGAAAACA  P5-R
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4161  TCAACAGCCTGGGTGCAGGTCTGGAGCATGTCGTTCACAAGgtgagtgcgcttttaggaacagct
      I  N  S  L  G  A  G  L  E  H  V  V  H  K
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4291  aaatctaacgtacacctgtaagcagggtgtatttatacgttgtccctttctctcttctctacagA

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      M  G  S  S  S  D  N  L  S  S  L  P  F  Y  S  N  S  L  G  Q  D  K
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       T  S  R  L  V  N  F  H  F  L  L  S  C  F  R  R  D  S  H  K  I  D
4486  CAGTTTCCTCAAAGTTCTGCGCTGCCGGGCAGCCAAGAAGAGACCTGAGATGTGCTAGAGTGAAA
        S  F  L  K  V  L  R  C  R  A  A  K  K  R  P  E  M  C  *  P4-R
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4616  TGTTTCTTTATAATACCGCATGGCAAAATATGCCCCTTATTGTTTCAAAGATGTAGATATTTGAT

4681  TCACTTTCTTATATATTTGAATAAATAACTGGACCAACGGGCCATATTACTCTGTCCCCAGAGGT

4746  AAAAGACAACAAAAAGTAAACAAAAGTATTTCAATTAAATTTTAAAATTGAAGGTCTGCATGCAG

4811  TCATACATGATGAATAAGTGCACAGAGTATATTAAAATGTTTGACCATGCTCAGTTTGATAAGAC

4876  ATTTGTATAGTAAGTATTTATTAAGAAACGATGTTGGATCTTTAACACGTTTATTTTAACATTAT

4941  CTTCTGAAATGCAATATTTAAAGCTTTGCACCAATGTCAATAAATTTCTATCAAGCAAATTTAAA
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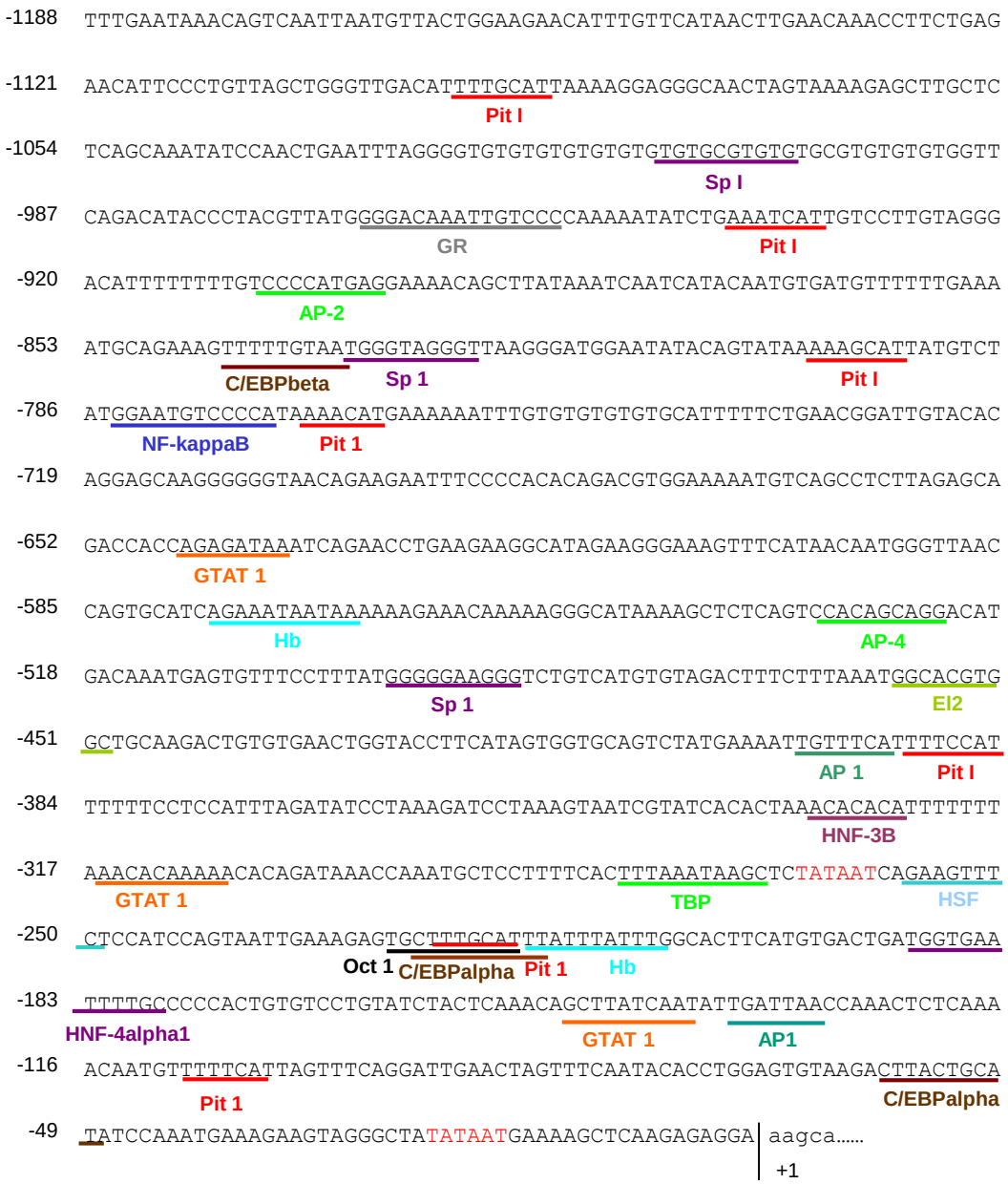
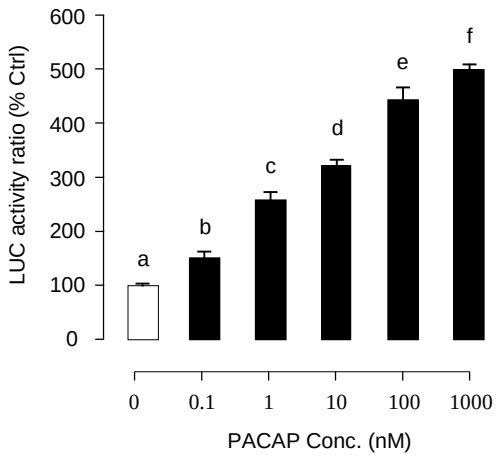
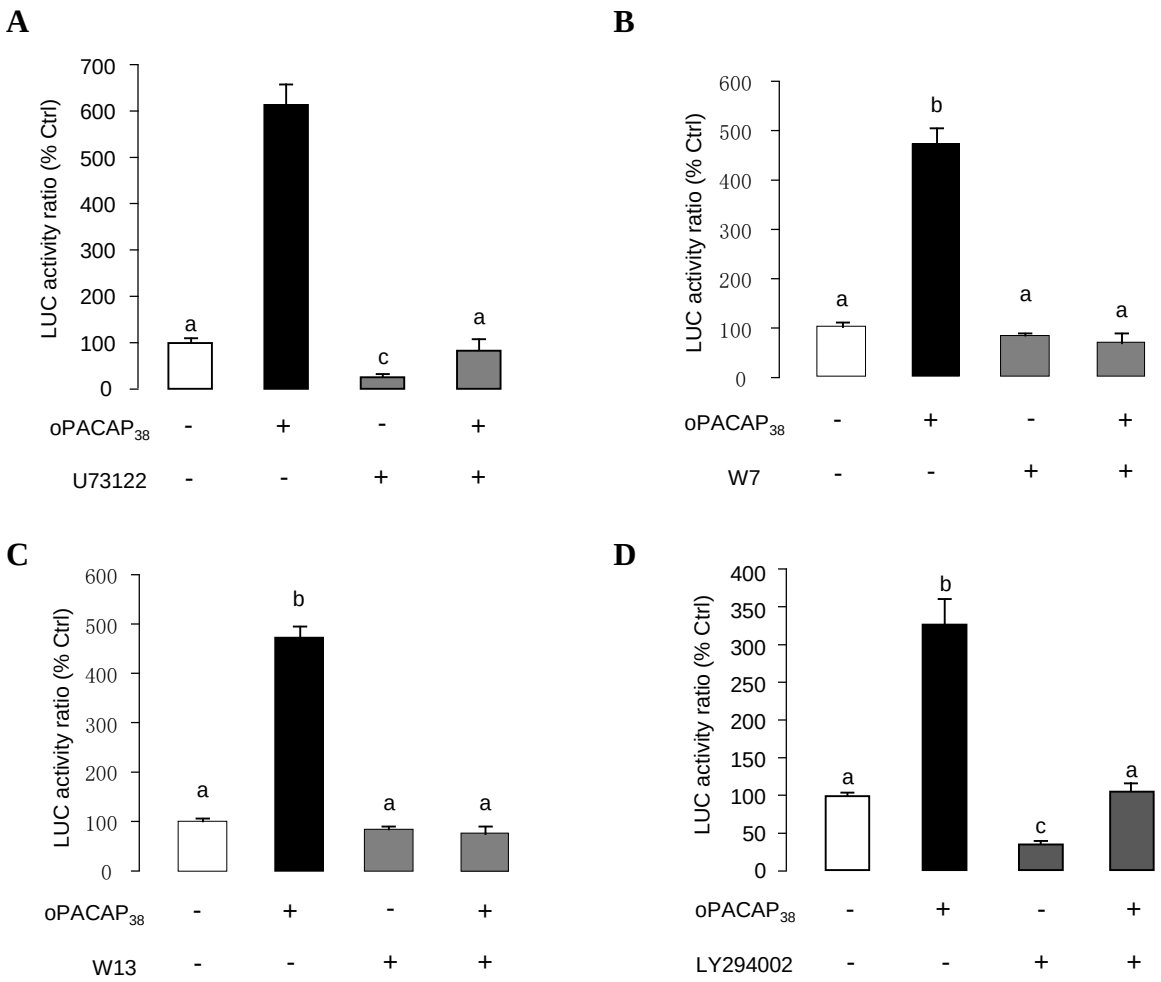
Supplementary Figure 1. Nucleotide sequence of the grass carp PRL gene (excluding 5' promoter). Introns are indicated in lower cases, and exons are shown in capital letters. Amino acids deduced from the coding region are shown in italic single letter codon, and the stop codon is indicated by " * ". Nucleotide numbering is defined with respect to the transcription start site deduced by primer extension. The primers using for the cloning and sequencing were underlined and marked in red color. P(n)-F represent the forward primers, and P(n)-R represent the revere primers using the reverse and complementary sequence of the marked nucleotides.



Supplementary Figure 2. Nucleotide sequence of the 5' promoter region of grass carp PRL gene. Nucleotide numbering was defined with respect to the transcription start site deduced by primer extension. In the 5' promoter region, the putative cis-acting elements predicted with TESS site search (http://www.cbil.upenn.edu/tess/) are underlined. The possible TATA box is indicated by red letters. The 5' region of 5' UTR of grass carp gene (start with "+1") is shown in lower cases for reference.



Supplementary Figure 3. Effect of grass carp PACAP38 on PRL promoter activity in αT3-1 Cells. αT3-1 Cells were transiently transfected with pPRL(-1156).LUC for 6 h by using lipofectamine. After 18 h recovery, the cells were incubated with grass carp PACAP38 dose-dependently. After drug treatment, cell lysate was prepared for dual-luciferase measurement Data are presented as percentage of control by conversing the ratio of firefly and renilla luciferase in the same sample. The results are expressed as mean ± SEM (n = 4) and different letters denote a significant difference at $p < 0.05$ (ANOVA followed by Fisher's LSD Test).



Supplementary Figure 4. Effect of U73122, LY294002, W7 and W13 on PACAP-induced PRL promoter activity in αT3-1 Cells. αT3-1 Cells were transiently transfected with pPRL(-1156).LUC for 6 h by using lipofectamine. After 18 h recovery, the cells were pre-treated with respective inhibitors for 15mins before co-incubation with oPACAP38 for 24 hours. After drug treatment, cell lysate was prepared for dual-luciferase measurement. (A) Effects of the PLC inhibitor U73122 (10 μM) on PACAP (100 nM) stimulated PRL promoter activity. (B&C) Effects of the CaM inhibitor W7 (30 μM) and W13 (100 μM) on PACAP (100 nM) stimulated PRL promoter activity. (D) Effects of the PI3K inhibitor LY294002 (10 μM) on PACAP (100 nM) stimulated PRL promoter activity. Data are presented as percentage of control by conversing the ratio of firefly and renilla luciferase in the same sample. The results are expressed as mean ± SEM (n = 4) and different letters denote a significant difference at $p < 0.05$ (ANOVA followed by Fisher's LSD Test).