

Supplementary information to

## Homologous cell type markers highlight subdivisions of domestic chick hippocampal formation

**Authors:**

Toshiyuki Fujita<sup>1</sup>, Naoya Aoki<sup>2</sup>, Chihiro Mori<sup>2</sup>, Koichi J. Homma<sup>2</sup>, and Shinji Yamaguchi<sup>1\*</sup>

**Affiliations:**

<sup>1</sup> Department of Biological Sciences, Faculty of Pharmaceutical Sciences, Teikyo University, 2-11-1 Kaga, Itabashi-ku, Tokyo, Japan

<sup>2</sup> Department of Molecular Biology, Faculty of Pharmaceutical Sciences, Teikyo University, 2-11-1 Kaga, Itabashi-ku, Tokyo, Japan

\*Corresponding author:

Shinji Yamaguchi, Ph.D.

E-mail: shinji-y@pharm.teikyo-u.ac.jp

Tel: +81-3-3964-8136

Fax: +81-3-3964-8415

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**Supplementary Figure 1. *In situ* hybridisation of CPA6 in the P1 chick brains**

DIG-labelled RNA antisense (a-f) and sense (a'-f') CPA6 probes were used for *in situ* hybridisation in coronal sections of P1 chick brains. To evaluate the expression patterns of CPA6, sections of nine chicks were analysed and representative images are shown. (a''-f'') Diagrams of coronal sections are shown on the rightmost panels. The levels of the sections (A 8.0 to A 5.4) are in accordance with the chick atlas by Kuenzel and Masson<sup>34</sup>. A, arcopallium; CDL, area corticoidea dorsolateralis; DL, dorsal lateral region; DM, dorsal medial region; HF, hippocampal formation; LSt, lateral striatum; M: mesopallium; N: nidopallium; V, V-shaped complex. Scale bars = 2.5 mm (c) and c') for a)-c) and a')-c')) and 500 µm (d)-f), d')-f')).

**Supplementary Figure 2. Comparison of the expression patterns of HF cell type markers in the P1 chick at level A7.2-7.0**

*In situ* hybridisation using DIG-labelled RNA antisense and sense PROX1 (a) and a')), LEF1 (b) and b')), ZBTB20 (c) and c')), and KIT (d) and d')) probes in coronal sections of P1 chick brains. a'') Schematic diagrams showing the location of panels a). The levels are in accordance with the chick atlas by Kuenzel and Masson<sup>34</sup>. A, arcopallium; HF, hippocampal formation; LSt, lateral striatum; M: mesopallium; N: nidopallium. Scale bar = 2.5 mm.

**Supplementary Figure 3. Comparison of the expression patterns of HF cell type markers in the P1 chick at level A5.6-5.4**

*In situ* hybridisation using DIG-labelled RNA antisense and sense PROX1 (a) and a')), LEF1 (b) and b')), ZBTB20 (c) and c')), and KIT (d) and d')) probes in coronal sections of P1 chick brains. a'') Schematic diagrams showing the location of panels a). The levels are in accordance with the chick atlas by Kuenzel and Masson<sup>34</sup>. HF, hippocampal formation; N: nidopallium. Scale bar = 2.5 mm.

**Supplementary Figure 4. Comparison of the expression patterns of GRIK1, GRIK2, GRIK3 in the P1 chick at level A8.8**

*In situ* hybridisation using DIG-labelled RNA antisense and sense GRIK1 (a), e) and a'), e')), GRIK2 (b), f) and b'), f')), and GRIK3 (c), g) and c'), g')) probes in coronal sections of P1 chick brains. d) Schematic diagrams showing the location of panels a)-c) and a')-c'). d') Schematic diagrams showing the location of panels e)-g) and e')-g'). The levels are in accordance with the chick atlas by Kuenzel and Masson<sup>34</sup>. DL, dorsal lateral region; DM, dorsal medial region; E, entopallium; H, hyperpallium; HF, hippocampal formation; LSt, lateral striatum; M: mesopallium; N: nidopallium. Scale bar = 2.5 mm (a)-c) and a')-c')) and 500 µm (e)-g), e')-g')).

**Supplementary Figure 5. Comparison of the expression patterns of GRIK1, GRIK2, GRIK3 in the P1 chick at level A5.8**

*In situ* hybridisation using DIG-labelled RNA antisense and sense GRIK1 (a), e) and a'), e')), GRIK2 (b), f) and b'), f')), and GRIK3 (c), g) and c'), g')) probes in coronal sections of P1 chick brains. d) Schematic diagrams showing the location of panels a)-c) and a')-c'). d') Schematic diagrams showing the location of panels e)-g) and e')-g'). The levels are in accordance with the chick atlas by Kuenzel and Masson<sup>34</sup>. A, arcopallium; D, diencephalon; DL, dorsal lateral region; DM, dorsal medial region; HF, hippocampal formation; M: mesopallium; N: nidopallium; V, V-shaped complex. Scale bar = 2.5 mm (a)-c) and a')-c')) and 1 mm (e)-g), e')-g')).

### **Supplementary Figure 6. *In situ* hybridisation of *GRIK4* in the P1 chick brains**

DIG-labelled RNA antisense (**a**, **b**) and sense (**a'**, **b'**) *GRIK4* probes were used for *in situ* hybridisation in coronal sections of P1 chick brains. To evaluate the expression patterns of *GRIK4*, sections of five chicks were analysed and representative images are shown. (**a''**, **b''**) Diagrams of coronal sections are shown on the rightmost panels. The levels of the sections (A 7.4) are in accordance with the chick atlas by Kuenzel and Masson<sup>34</sup>. A, arcopallium; DL, dorsal lateral region; DM, dorsal medial region; HF, hippocampal formation; LSt, lateral striatum; M: mesopallium; N: nidopallium; V, V-shaped complex. Scale bars = 2.5 mm (**a** and **a'**) and 1 mm (**b** and **b'**).

### **Supplementary Figure 7. Comparison of the expression patterns of *GRIK1*, *GRIK2*, *GRIK3* and HF cell type markers using neighboring sections in the P1 chick**

*In situ* hybridisation using DIG-labelled RNA antisense and sense *PROX1* (**a** and **a'**), *GRIK1* (**b** and **b'**), *GRIK2* (**c** and **c'**), *GRIK3* (**d** and **d'**), and *LEF1* (**e** and **e'**) probes in coronal sections of P1 chick brains. **a''**) Schematic diagrams showing the panels **a**). The levels are in accordance with the chick atlas by Kuenzel and Masson<sup>34</sup>. A, arcopallium; FL, field L; HF, hippocampal formation; M: mesopallium; N: nidopallium. Scale bar = 2.5 mm (**a**–**e**, **a'**–**e'**).

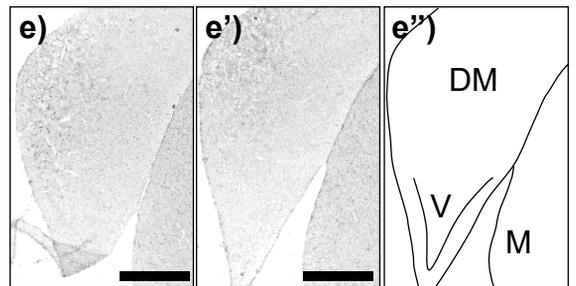
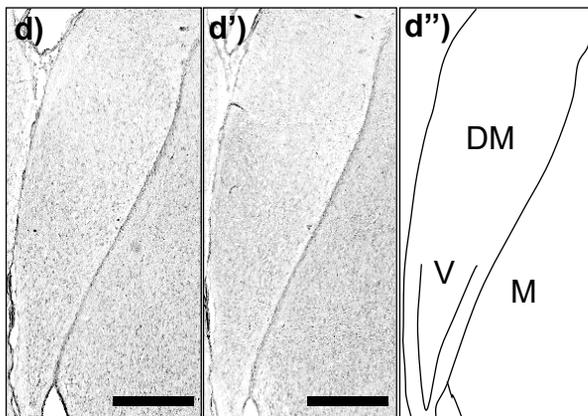
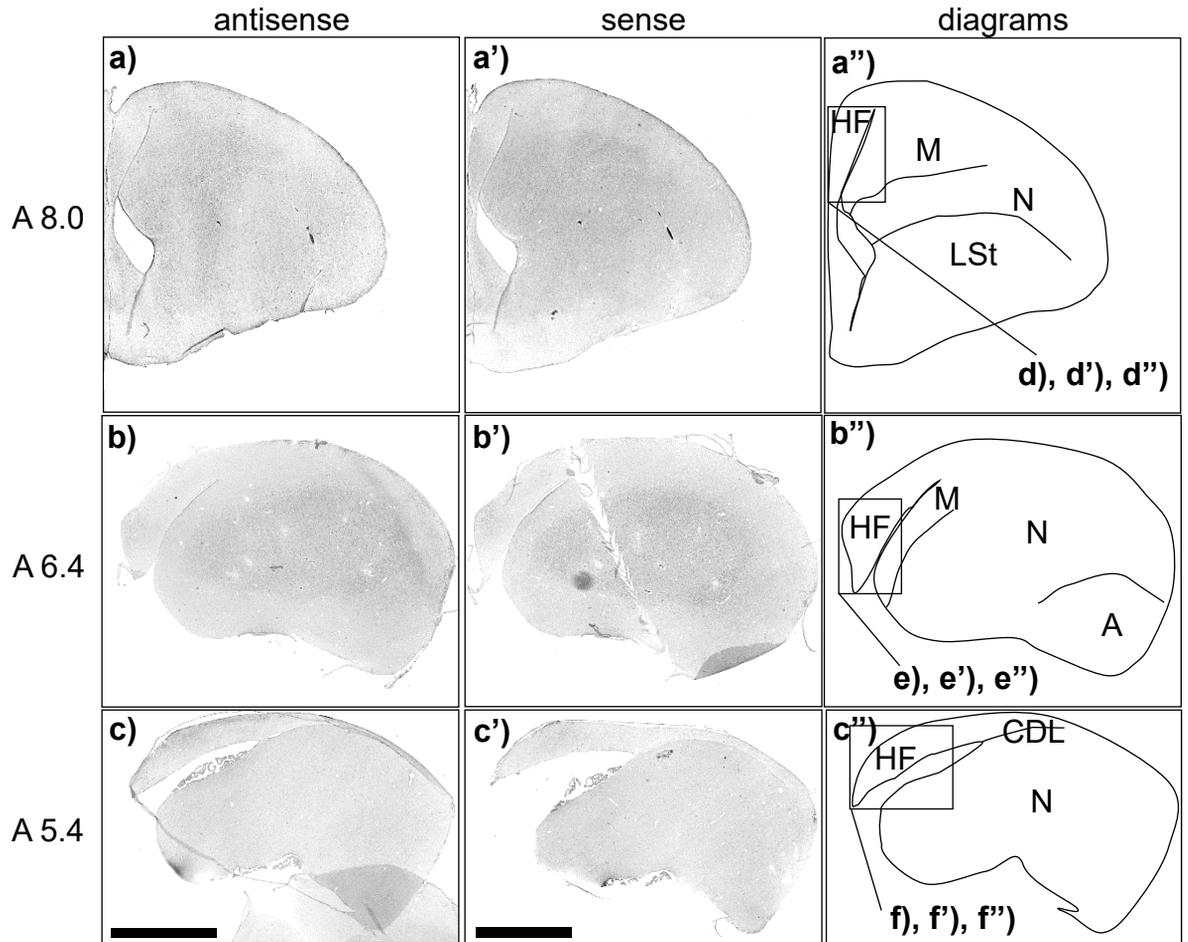
### **Supplementary Figure 8. Schematic summary**

**a**) Representative expression patterns in sections A5.0, A7.0, and A8.0 are shown by coloured areas (magenta, *ZBTB20*; blue, *PROX1*; yellow, *KIT*) in the HF. Crossed arrows indicate anterior-posterior and lateral-medial directions. The levels are in accordance with the chick atlas by Kuenzel and Masson.<sup>34</sup> **b**) Schematic diagram of the expression regions of HF molecular markers in the chick and mouse HFs. The color code indicates pan-HF markers such as *ZBTB20* in magenta, DG markers such as *PROX1* in blue, CA3 markers in green, and CA1 markers such as *KIT* in chicken in yellow. It is unclear whether molecular marker expression in the border region between the chick DM and DL overlaps or is mutually exclusive. CA1, cornu ammonis field 1; CA3, cornu ammonis field 3; DG, dentate gyrus; DL, dorsal lateral region; DM, dorsal medial region; V, V-shaped complex.

### **Supplementary Figure 9. Probe sequencing and alignment prepared in this study**

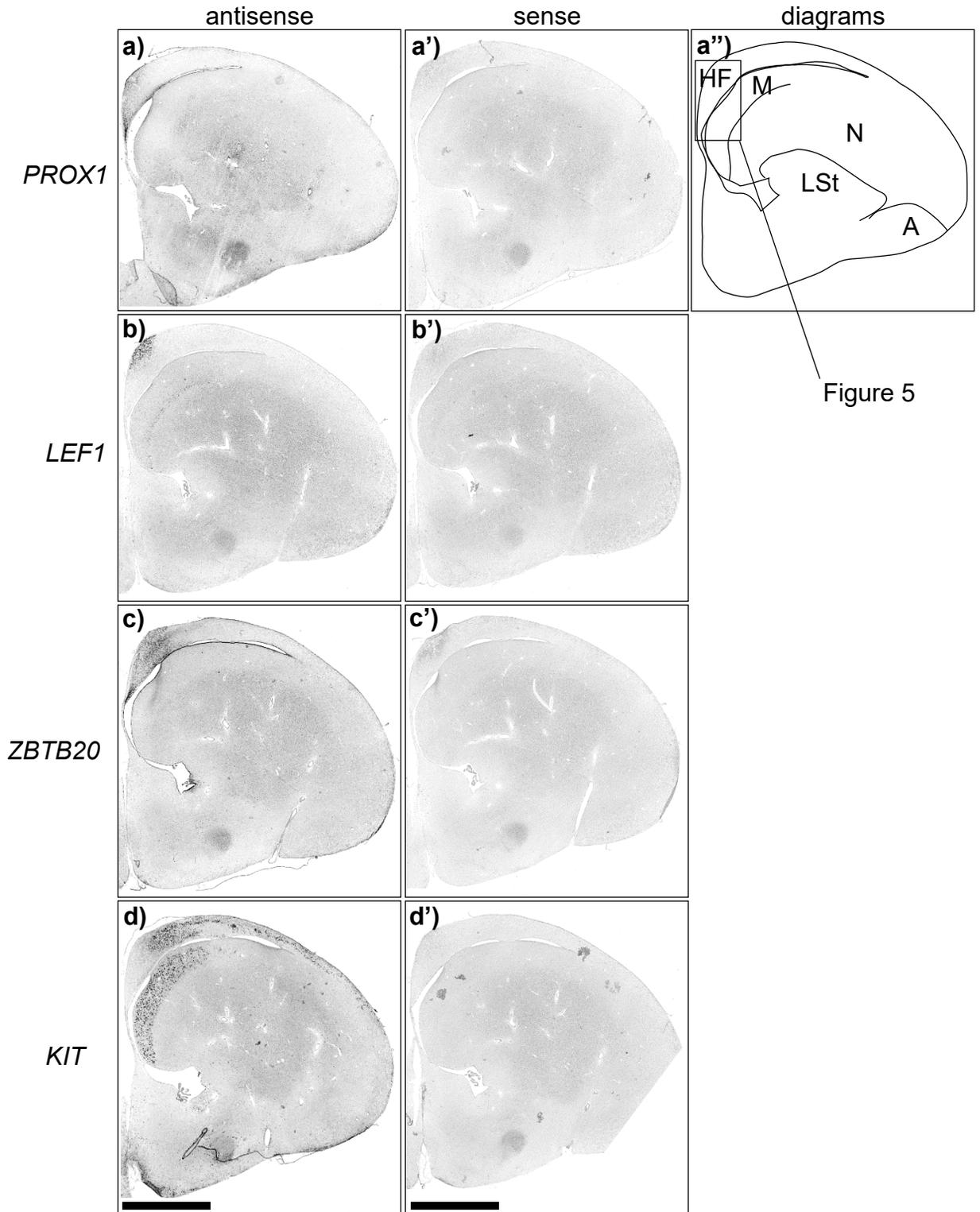
Subcloned template sequences and alignment results of **a**) *ZBTB20*, **b**) *PROX1*, **c**) *CPA6*, **d**) *KIT*, **e**) *GRIK1*, **f**) *GRIK2*, **g**) *GRIK3*, **h**) *GRIK4*, and **i**) *SOX11* probes were shown.

CPA6



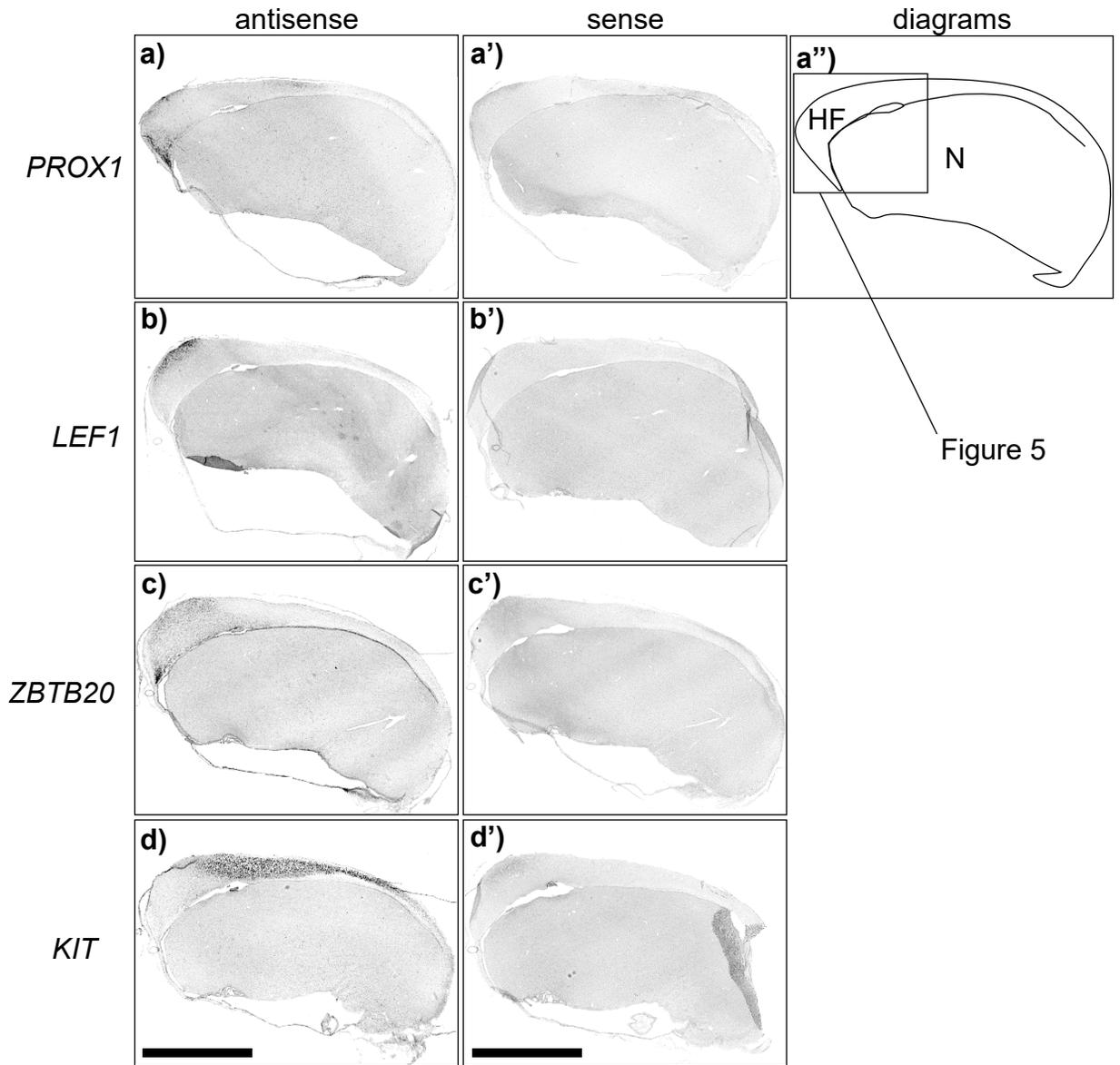
*PROX1\_LEF1\_ZBTB20\_KIT*

A 7.2-7.0



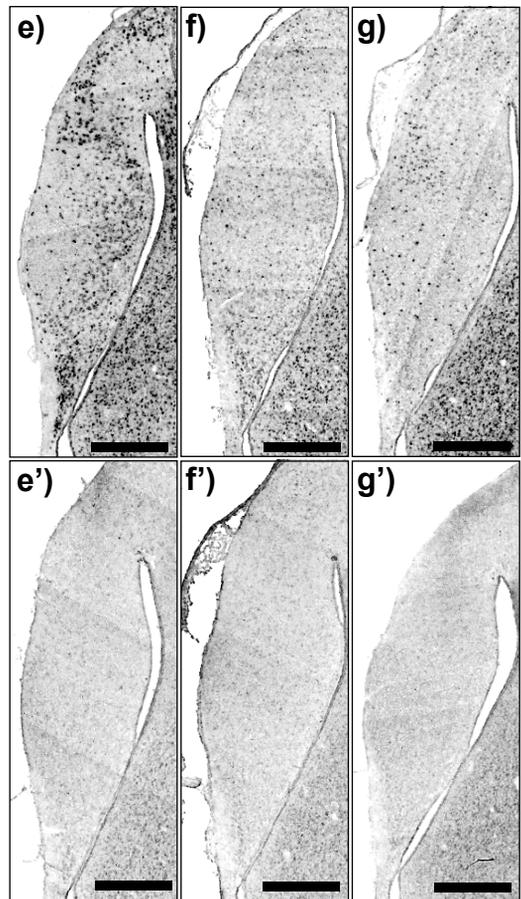
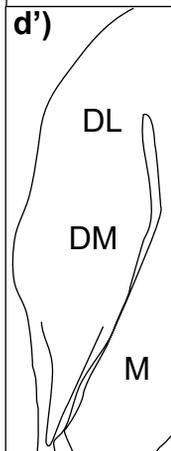
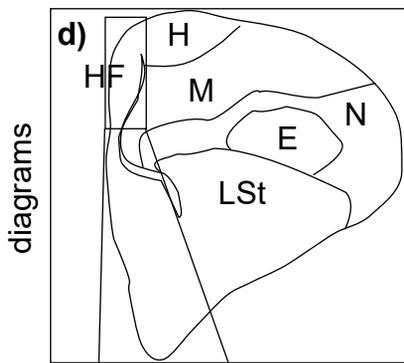
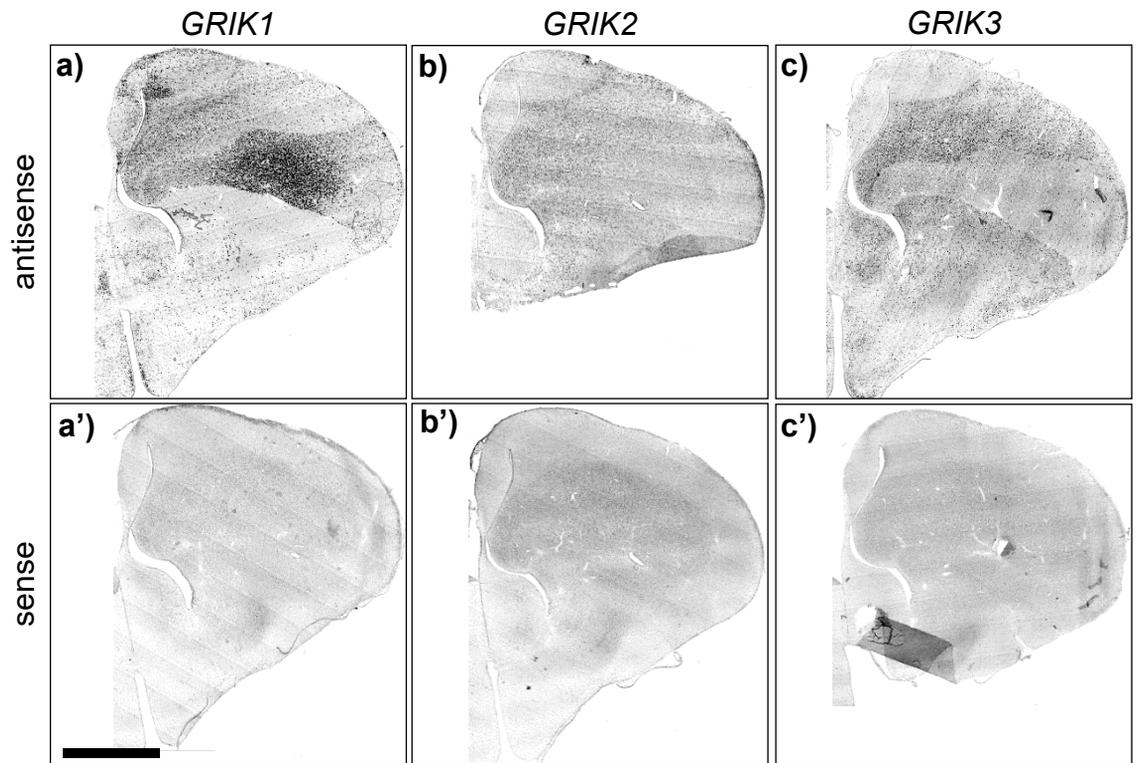
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A 5.6-5.4



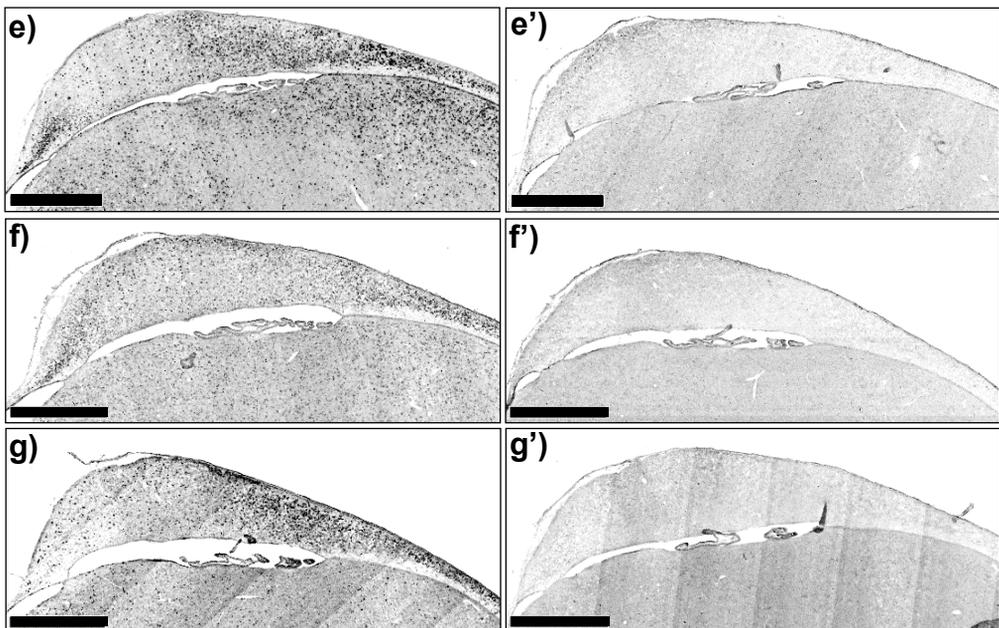
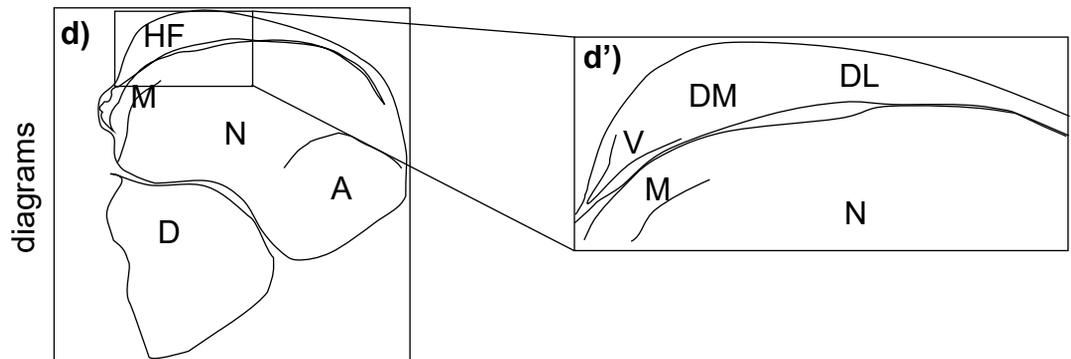
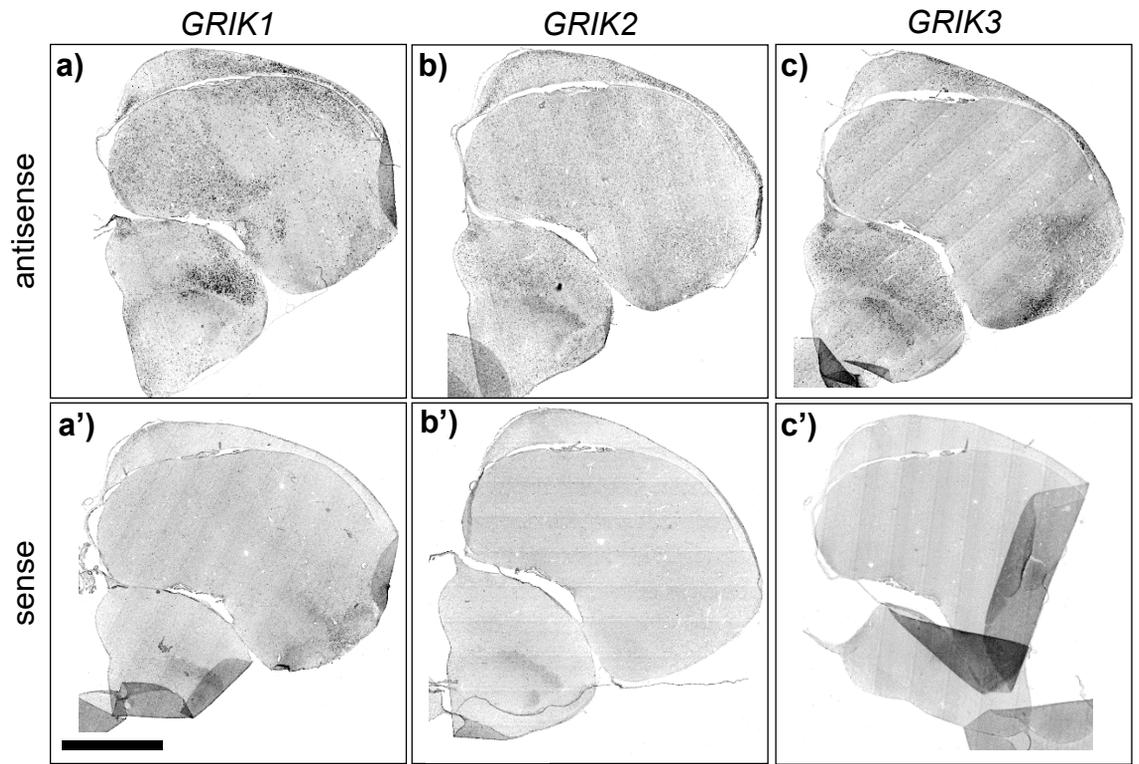
*GRIK1\_GRIK2\_GRK3*

A 8.8



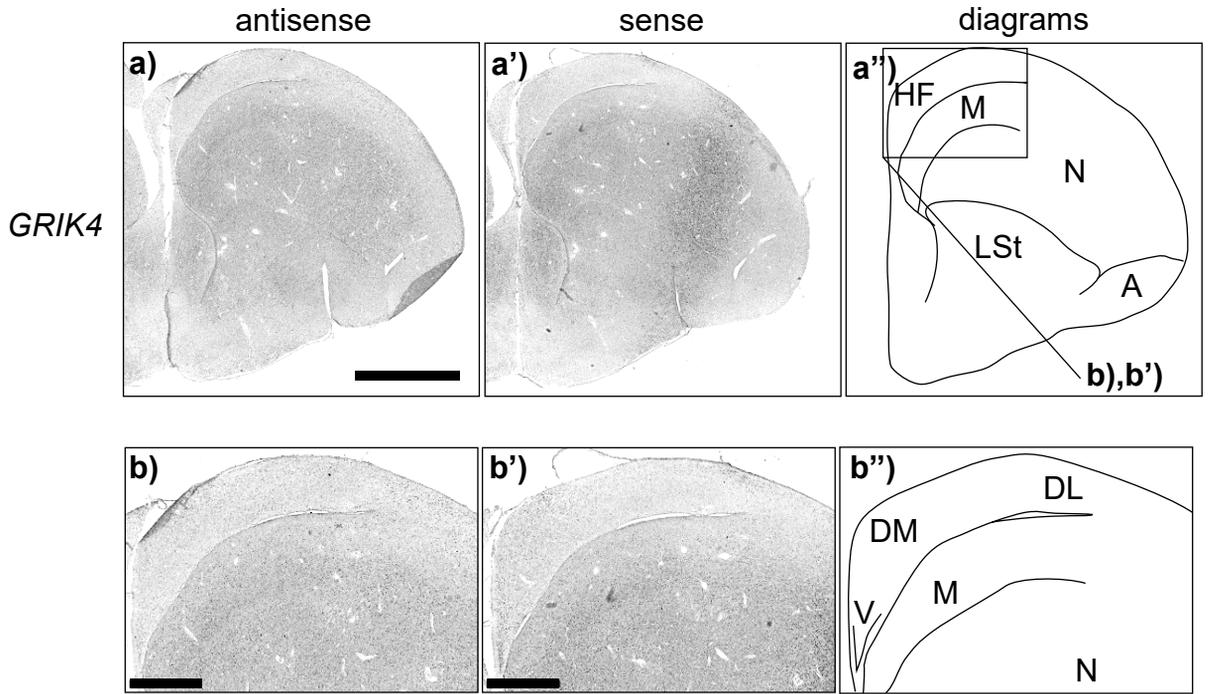
*GRIK1\_GRIK2\_GRK3*

A 5.8



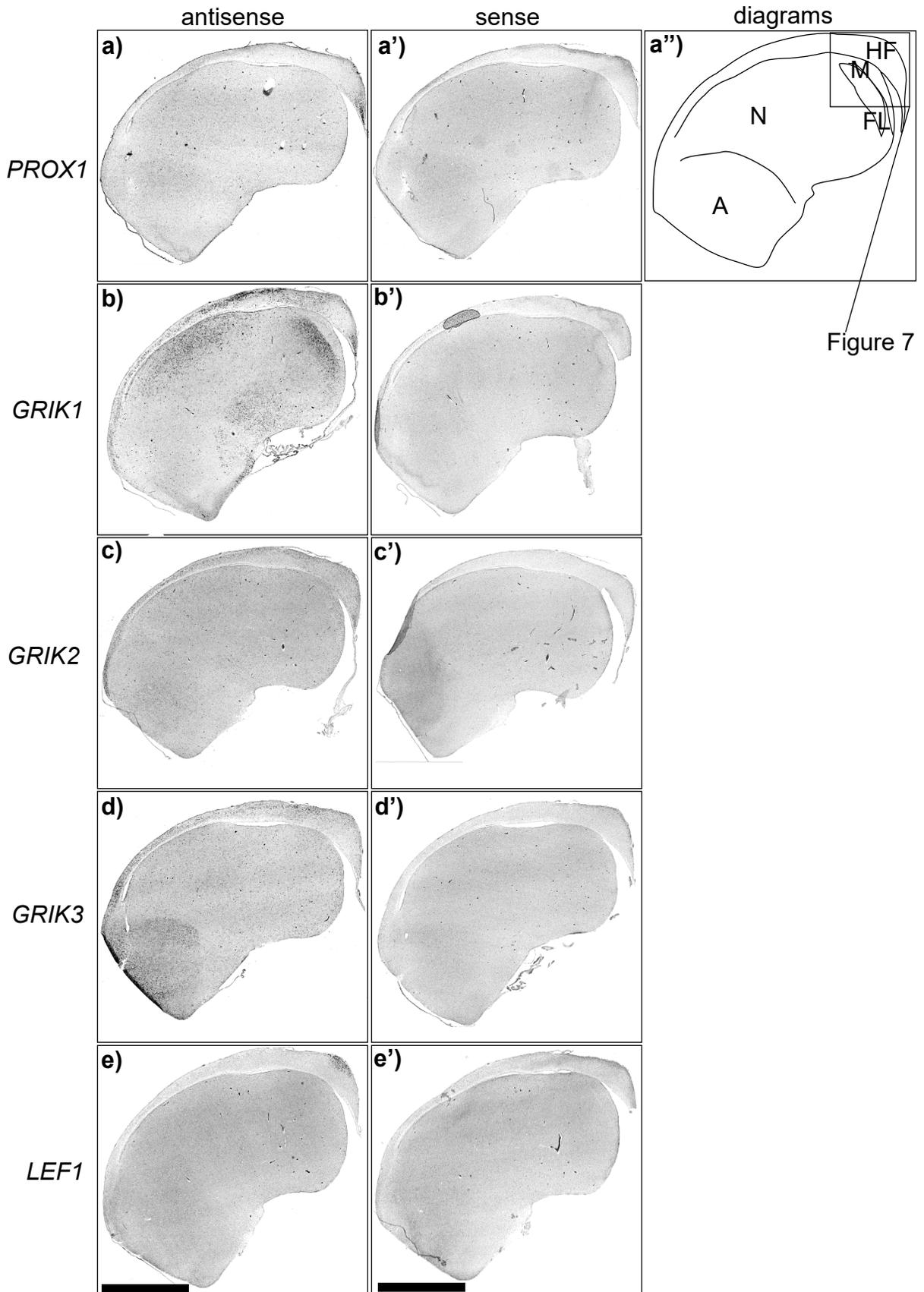
*GRIK4*

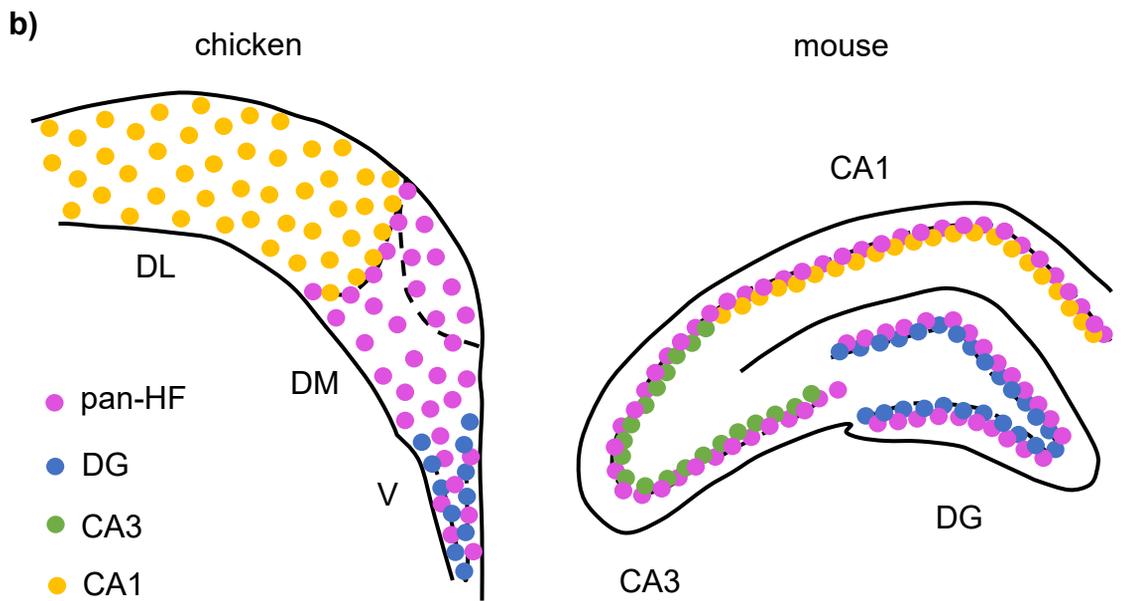
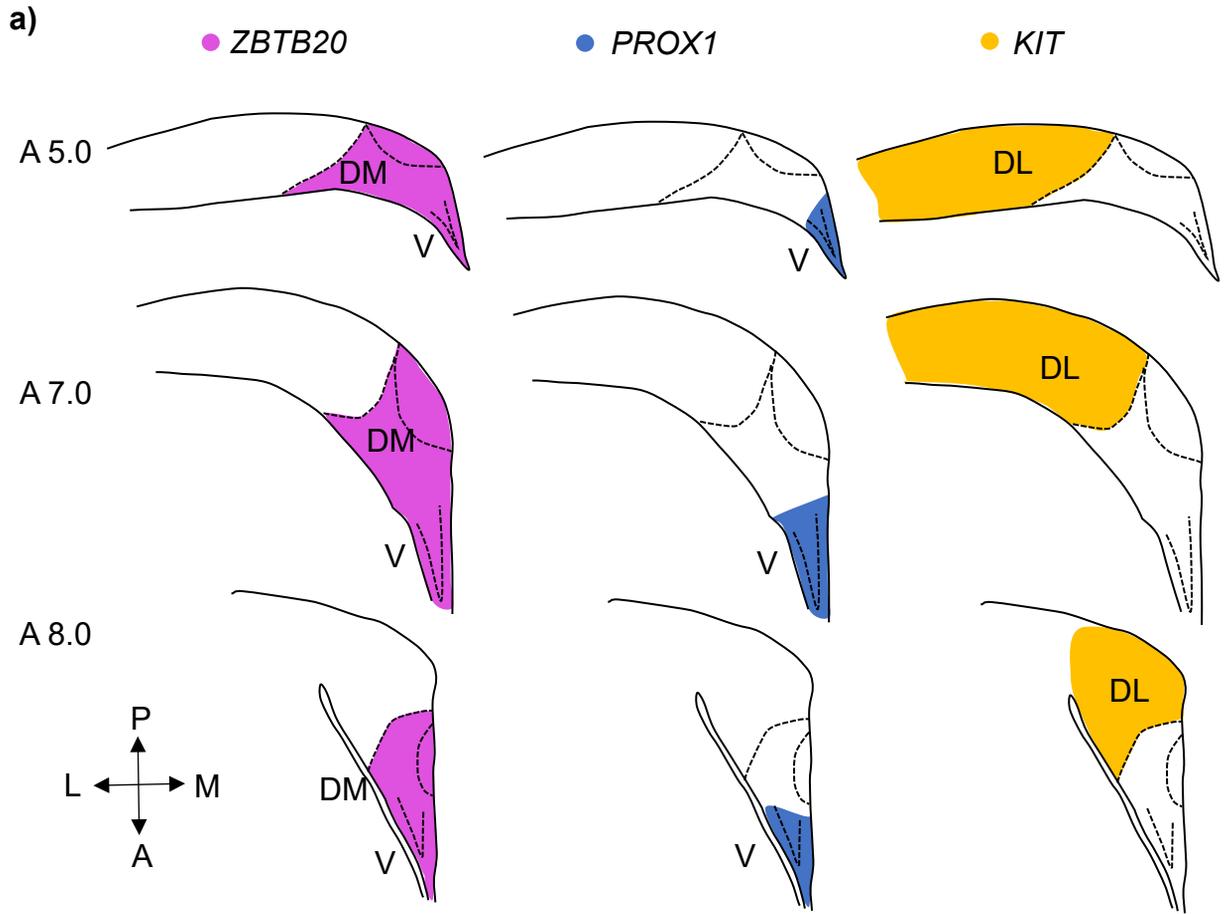
A 7.4



*PROX1\_GRIK1\_GRIK2\_GRIK3\_LEF1*

A 6.2-6.0





# Supplementary Figure 9

## a) ZBTB20

>PREDICTED: Gallus gallus zinc finger and BTB domain containing 20 (ZBTB20), transcript variant X29, mRNA  
Sequence ID: XM\_046905147.1 Length: 28412  
Range 1: 1574 to 2333

Score:1399 bits(757), Expect:0.0,  
Identities:759/760(99%), Gaps:0/760(0%), Strand: Plus/Minus

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Query 301 CTGCTGCTCCACAGAATCAGGCTCAGTACCAATGGAGGAACTGACTCCTGAGTCAAAACT 360
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Sbjct 1973 CTCCCCTTTGGGTTCACTCTCAGTGCCTTCTGCTTGGTCAGTGTCTCAGTGCATTCCCTC 1914

Query 421 AGACTCATTGCGCTCAAGGATCTGCACTCTCTGTTGGCCATAGTAGTCATAGTCATCCTC 480
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Sbjct 1913 AGACTCATTGCGCTCAAGGATCTGCACTCTCTGTTGGCCATAGTAGTCATAGTCATCCTC 1854

Query 481 CATCTCCTGCTTAATATGGATGTTGCCATCAAGGTTTGAATTTCGGACAGGGCGTGGTTG 540
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Sbjct 1853 CATCTCCTGCTTAATATGGATGTTGCCATCAAGGTTTGAATTTCGGACAGGGCGTGGTTG 1794

Query 541 CTTGCGGCAATGTGTGGTCTCTGGAGTGGTAGAGAGATACCGCTCCATCTGCTGTGACCG 600
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Sbjct 1613 TTGCATGGAACAGGCATAGAGGGCTGAGTAGATCCTGTCC 1574
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**b) PROX1**

>Gallus gallus prospero homeobox 1 (PROX1), mRNA  
Sequence ID: NM\_001005616.1 Length: 3322  
>Gallus gallus homeodomain protein (Prox 1) mRNA, complete cds  
Sequence ID: U46563.1 Length: 3322  
Range 1: 197 to 967

Score:1380 bits(747), Expect:0.0,  
Identities:764/772(99%), Gaps:2/772(0%), Strand: Plus/Plus

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Sbjct 497 TTTGGCAGGCCGACTATGAGCCAGTTTGATGTGGATCGGTTATGCGATGAGCACCTGAGA 556

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Sbjct 557 GCTAAACGCGCCCGAGTTGAGAATATCATTCGGGGTATGAGCCATTCCCCCAGGGTGGCA 616

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Sbjct 617 TTAAGGGGCAATGAAAATGAAAGAGAAATACGTCCGCAGTCCGTCAGCCCTCGAGAAAGT 676

Query 481 TACCGAGAAAACAagcgcaagcagaagct-gccgcagcagcagcagcagagcttccagca 539
      |||
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Query 540 gcTGGTTTCGGCCAGGAAGGAGCAGAAGCGAGAGGAGCGCCGACAGCTGAAGCAGCAGCT 599
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c) CPA6

>Gallus gallus carboxypeptidase A6 (CPA6), transcript variant 2, mRNA  
Sequence ID: NM\_001006344.2 Length: 2609  
Range 1: 459 to 1229

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Identities:768/771(99%), Gaps:0/771(0%), Strand: Plus/Plus

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d) *KIT*

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Range 1: 2260 to 2982

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Identities:721/723(99%), Gaps:0/723(0%), Strand: Plus/Plus

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Sequence ID: XM\_015299614.4 Length: 3337  
Range 1: 2146 to 2844

Score:1291 bits(699), Expect:0.0,  
Identities:699/699(100%), Gaps:0/699(0%), Strand: Plus/Minus

```
Query 1 CTGCTCAATATCACTGTTTTTCCTTGATTTATAAATAAATTCACCAATGGCTACGAACAC 60
      |||
Sbjct 2844 CTGCTCAATATCACTGTTTTTCCTTGATTTATAAATAAATTCACCAATGGCTACGAACAC 2785

Query 61 TGAAAGAACAAGCCCAGCAGCGAGCACTATGAAGATGCCCCCGATATTCTCCACCCCGAG 120
      |||
Sbjct 2784 TGAAAGAACAAGCCCAGCAGCGAGCACTATGAAGATGCCCCCGATATTCTCCACCCCGAG 2725

Query 121 AGCACTGGCTTCTTTGCTGTCTCTTCAGGGCAGCCGTTGCCTCGCCACCACTTTTCCTT 180
      |||
Sbjct 2724 AGCACTGGCTTCTTTGCTGTCTCTTCAGGGCAGCCGTTGCCTCGCCACCACTTTTCCTT 2665

Query 181 CATCATGTGGAGCTTCCCTTCCCTCCTGGAGCTGGAGGATGGCAATGGTGATCTTGCCCT 240
      |||
Sbjct 2664 CATCATGTGGAGCTTCCCTTCCCTCCTGGAGCTGGAGGATGGCAATGGTGATCTTGCCCT 2605

Query 241 ATAAGGTGACCCAATAGGGGTTCCTACTCCATAGCCTTTTGAATCGATGAGTCCCCCGAT 300
      |||
Sbjct 2604 ATAAGGTGACCCAATAGGGGTTCCTACTCCATAGCCTTTTGAATCGATGAGTCCCCCGAT 2545

Query 301 CTGAGTGAGGTTGCAGTTTCTCTGAGTCACATACTCAATGCTGGTGGACTCCATCAGCAG 360
      |||
Sbjct 2544 CTGAGTGAGGTTGCAGTTTCTCTGAGTCACATACTCAATGCTGGTGGACTCCATCAGCAG 2485

Query 361 CGCATAGTCTGTCGTGAGCACACGCTGGATTCCCTCATCATGTTCTTCCAGTGCTGT 420
      |||
Sbjct 2484 CGCATAGTCTGTCGTGAGCACACGCTGGATTCCCTCATCATGTTCTTCCAGTGCTGT 2425

Query 421 CTGCTGCCTGCTGCTCATGAATGCCACATCTTCTCATATGTTGATATCTTGATTCTT 480
      |||
Sbjct 2424 CTGCTGCCTGCTGCTCATGAATGCCACATCTTCTCATATGTTGATATCTTGATTCTT 2365

Query 481 GAAGAAGGTCATTGTGGATCCATCTCTAACAGCCCCGTACTCTATTTTGGTTTGTCTTGC 540
      |||
Sbjct 2364 GAAGAAGGTCATTGTGGATCCATCTCTAACAGCCCCGTACTCTATTTTGGTTTGTCTTGC 2305

Query 541 CAAATCGTCGGCTGAATCGATGGGGGATTCCATCCTTTTACCGTCAGGAAGGCAGCCAG 600
      |||
Sbjct 2304 CAAATCGTCGGCTGAATCGATGGGGGATTCCATCCTTTTACCGTCAGGAAGGCAGCCAG 2245

Query 601 GTTGGCAGTATAGGATGAGATTATGATTAAGGTGAAAAACCACCATATTCTCCAACATAT 660
      |||
Sbjct 2244 GTTGGCAGTATAGGATGAGATTATGATTAAGGTGAAAAACCACCATATTCTCCAACATAT 2185

Query 661 TCTGGTGGAAAGAGCTTTGGGCATCAGCTCTGATCCTTG 699
      |||
Sbjct 2184 TCTGGTGGAAAGAGCTTTGGGCATCAGCTCTGATCCTTG 2146
```

f) **GRIK2**

>PREDICTED: Gallus gallus glutamate ionotropic receptor kainate type subunit 2 (GRIK2), transcript variant X11, mRNA  
Sequence ID: XM\_046939222.1 Length: 2189  
Range 1: 1441 to 2156

Score:1317 bits(713), Expect:0.0,  
Identities:715/716(99%), Gaps:0/716(0%), Strand: Plus/Minus

```
Query 1 ACCCAAGTAAGCCAGCAGAACATACATCCAGATATCAGGGGAGAGAGGATTCAGGAAGGA 60
      |||
Sbjct 2156 ACCCAAGTAAGCCAGCAGAATATACATCCAGATATCAGGGGAGAGAGGATTCAGGAAGGA 2097

Query 61 GAAGACGCCCGGGTTTGTACCATTGGGCTTGCGGTACAAAATACTTATTCCAAGAGTCAT 120
      |||
Sbjct 2096 GAAGACGCCCGGGTTTGTACCATTGGGCTTGCGGTACAAAATACTTATTCCAAGAGTCAT 2037

Query 121 AAACGGCTTGAAAAAGTCGATGACTTTCTCACGAACATAGGTAATTGCCAATGGTGCAAC 180
      |||
Sbjct 2036 AAACGGCTTGAAAAAGTCGATGACTTTCTCACGAACATAGGTAATTGCCAATGGTGCAAC 1977

Query 181 TGCAAGGTCAGCTTTATGATCAATTAATTCACGAACCATTCCATTCCACTGTCCACTGGC 240
      |||
Sbjct 1976 TGCAAGGTCAGCTTTATGATCAATTAATTCACGAACCATTCCATTCCACTGTCCACTGGC 1917

Query 241 ATCTCCTGGGCTCCATATTTCCATCCTCCACCAGTCTAATCTCATAGGAAAATCCAAG 300
      |||
Sbjct 1916 ATCTCCTGGGCTCCATATTTCCATCCTCCACCAGTCTAATCTCATAGGAAAATCCAAG 1857

Query 301 GATAGTAGACAGCTCCCTGAGGAGGTCAATGCAATAACCTTCAAATCGATCATTTCCATA 360
      |||
Sbjct 1856 GATAGTAGACAGCTCCCTGAGGAGGTCAATGCAATAACCTTCAAATCGATCATTTCCATA 1797

Query 361 CAAAGGTTTATCTGATTTCTTGAACATTACATATGGCTCTTCCAAGATGGTGGTAACTAT 420
      |||
Sbjct 1796 CAAAGGTTTATCTGATTTCTTGAACATTACATATGGCTCTTCCAAGATGGTGGTAACTAT 1737

Query 421 TAAAGAACGATTGGACAGGGAATCCGTGATGTTTGCTGGTTTTCTTTCTGATTCTCTGT 480
      |||
Sbjct 1736 TAAAGAACGATTGGACAGGGAATCCGTGATGTTTGCTGGTTTTCTTTCTGATTCTCTGT 1677

Query 481 CATGTTAAGGCCACTCAGTGGATCCCAAGTTCCAACCTTTTCAAGACCTTCTTCTTGAG 540
      |||
Sbjct 1676 CATGTTAAGGCCACTCAGTGGATCCCAAGTTCCAACCTTTTCAAGACCTTCTTCTTGAG 1617

Query 541 GCTTATAACATCTAAATCAAAATCTGTCTTAAGCCATTGGATTTGTTGAAAGTTATCCT 600
      |||
Sbjct 1616 GCTTATAACATCTAAATCAAAATCTGTCTTAAGCCATTGGATTTGTTGAAAGTTATCCT 1557

Query 601 TCCTGTGAGGCCTTCCCAGTGAGCCTCCTTAATGAGACTCATAAAGCGAGTCCCAAAGCG 660
      |||
Sbjct 1556 TCCTGTGAGGCCTTCCCAGTGAGCCTCCTTAATGAGACTCATAAAGCGAGTCCCAAAGCG 1497

Query 661 CCAAGGTTTGTGGCGATTACACTGTAGTGAAGTACAGTCATCTGTGGGAAGTCTGCT 716
      |||
Sbjct 1496 CCAAGGTTTGTGGCGATTACACTGTAGTGAAGTACAGTCATCTGTGGGAAGTCTGCT 1441
```

**g) GRIK3**

>PREDICTED: Gallus gallus glutamate ionotropic receptor kainate type subunit 3 (GRIK3), transcript variant X1, mRNA  
Sequence ID: XM\_417766.7 Length: 6097  
Range 1: 2109 to 2900

Score:1456 bits(788), Expect:0.0,  
Identities:791/792(99%), Gaps:1/792(0%), Strand: Plus/Plus

```
Query 1      CTCGCCGCCTTCCTGACAGTGGAGAGGATGGAATCCCCCATGACTCAGCAGACGACCTG 60
          |||
Sbjct 2109   CTCGCCGCCTTCCTGACAGTGGAGAGGATGGAATCCCCCATGACTCAGCAGACGACCTG 2168

Query 61     GCAAAGCAGACCAAATCGAGTATGGGGCTGTGAAGGATGGGGCAACCATGACCTTCTTC 120
          |||
Sbjct 2169   GCAAAGCAGACCAAATCGAGTATGGGGCTGTGAAGGATGGGGCAACCATGACCTTCTTC 2228

Query 121    AAGAAATCCAAATTTCCACCTTTGAAAAATGTGGGCTTTCATGAGCAGCAAACCCACG 180
          |||
Sbjct 2229   AAGAAATCCAAATTTCCACCTTTGAAAAATGTGGGCTTTCATGAGCAGCAAACCCACG 2288

Query 181    GCTCTCGTCAAGAACAACGAGGAGGGAATCCAGCGCACCTGACGGCCGACTACGCCCTG 240
          |||
Sbjct 2289   GCTCTCGTCAAGAACAACGAGGAGGGAATCCAGCGCACCTGACGGCCGACTACGCCCTG 2348

Query 241    CTGATGGAGTCCACCACCATCGAGTACATCACCCAGAGGAACTGCAACCTCACCCAGGTC 300
          |||
Sbjct 2349   CTGATGGAGTCCACCACCATCGAGTACATCACCCAGAGGAACTGCAACCTCACCCAGGTC 2408

Query 301    GGGGGGCTCATCGACTCCAAGGGCTACGGGATTTGGGACCCCATGGGCTCACCGTACCGG 360
          |||
Sbjct 2409   GGGGGGCTCATCGACTCCAAGGGCTACGGGATTTGGGACCCCATGGGCTCACCGTACCGG 2468

Query 361    GACAAGATCACCATCGCCATCTTCAGCTGCAGGAGGAGGACAAGCTGCACGTCATGAAG 420
          |||
Sbjct 2469   GACAAGATCACCATCGCCATCTTCAGCTGCAGGAGGAGGACAAGCTGCACGTCATGAAG 2528

Query 421    GAGAAGTGGTGGCGGGGAACGGTTGCCCTGAGGATGAGAACAAGGAGGCCAGTGCCCTG 480
          |||
Sbjct 2529   GAGAAGTGGTGGCGGGGAACGGTTGCCCTGAGGATGAGAACAAGGAGGCCAGTGCCCTG 2588

Query 481    GGATCCAAAACATCGGCGGCATTTTCATCGTGCTGGCAGCAGGCTTGGTGCTGTCCGGTC 540
          |||
Sbjct 2589   GGATCCAAAACATCGGCGGCATTTTCATCGTGCTGGCAGCAGGCTTGGTGCTGTCCGGTC 2648

Query 541    TTTGTGGCCATGGTGGAGTTCATCTACAAGCTGCGGAAGACGGCGGAACGCGAGCAGCGC 600
          |||
Sbjct 2649   TTTGTGGCCATGGTGGAGTTCATCTACAAGCTGCGGAAGACGGCGGAACGCGAGCAGCGC 2708

Query 601    TCCTTCTGCAGCGCGGTGGCCGATGAGATCCGGCTGTCTCTCACCTGCCAGCGCCGGGTC 660
          |||
Sbjct 2709   TCCTTCTGCAGCGCGGTGGCCGATGAGATCCGGCTGTCTCTCACCTGCCAGCGCCGGGTC 2768

Query 661    AAGCACAACCACCACCACCCGTCATGGTGAAGGCGGATGCTGTGATCAACATGCACACC 720
          |||
Sbjct 2769   AAGCACAACCACCACCACCCGTCATGGTGAAGGCGGATGCTGTGATCAACATGCACACC 2828

Query 721    TTCAACGACCGGCGGCTGCCAGGGAAGGACAGCATGAGCTGCAACACCGGCCTGACGCC 780
          |||
Sbjct 2829   TTCAACGACCGGCGGCTGCCAGGGAAGGACAGCATGAGCTGCAACACCGGCCTGACGCC 2888

Query 781    GTGTTCC-ATAG 791
          |||
Sbjct 2889   GTGTTCCCATAG 2900
```

## h) *GRIK4*

>PREDICTED: Gallus gallus glutamate ionotropic receptor kainate type subunit 4 (GRIK4), mRNA

Sequence ID: XM\_015298177.4 Length: 4160

Range 1: 104 to 1286

Score:2135 bits(1156), Expect:0.0,

Identities:1174/1183(99%), Gaps:0/1183(0%), Strand: Plus/Minus

```
Query 1 CCTTCAGAAACATGCCAGTGGCCGATCTGCCGGAAGCCGCTGTGTGTGTGCTGCAGGATT 60
      |||
Sbjct 1286 CCTTCAGAAACATGCCAGTGGCCGATCTGCCGGAAGCCGCTGTGTGTGTGCTGCAGGATT 1227

Query 61 TTCAGGGCATAAGTTGGAGCGCTGGCCTTTGCTGTTGAATTCGATGTGGCCGGTGCAGACCT 120
      |||
Sbjct 1226 TTCAGGGCATAAGTTGGAGCGCTGGCCTTTGCTGTTGAATTCGATGTGGCCGGTGCAGACCT 1167

Query 121 TCCAATTCTACCATGCGCAGGTAGTTCATCAGGCTGGTGCCATGCTGCCAGATCTGGGCA 180
      |||
Sbjct 1166 TCCAATTCTACCATGCGCAGGTAGTTCATCAGGCTGGTGCCATGCTGCCAGATCTGGGCA 1107

Query 181 GAGCCACAGGACAGCGGCTTACGCGCGATCTCCTGGCTGCGGTTACAGTCTGCACGGCG 240
      |||
Sbjct 1106 GAGCCACAGGACAGCGGCTTACGCGCGATCTCCTGGCTGCGGTTACAGTCTGCACGGCG 1047

Query 241 CTCACCACGGCGTACACGGCATCAAACAGCAGGGCAGAAGAGAGTGCTGGCCCAGTAAAA 300
      |||
Sbjct 1046 CTCACCACGGCGTACACAGCATCGAACAGCAGGGCAGAGGAGAGTGCTGGCCCAGTAAAA 987

Query 301 GGAGCATGATCACAGTTCTCCTGCCAGGACTGGTTGAGGCTCTGGACAAACTCTTGAAAA 360
      |||
Sbjct 986 GGAGCATGATCACAGTTCTCCTGCCAGGACTGGTTGAGGCTCTGGACAAACTCTTGAAAA 927

Query 361 AAGGCGTGAGACTGGTTGAAAATGGAAAATCCCAGGATGTTGACTCGGTCATCCAATTGG 420
      |||
Sbjct 926 AAGGCGTGAGACTGGTTGAAAATGGAAAATCCCAGGATGTTGACTCGGTCATCCAATTGG 867

Query 421 CTGTCCAGGCGCTGGAGTGAAAATCCAGATTAGTGAAGATATATGTGTAATAGGCAGAT 480
      |||
Sbjct 866 CTGTCCAGGCGCTGGAGTGAAAATCCAGATTAGTGAAGATATATGTGTAATAGGCAGAT 807

Query 481 GCCATTCCCAGCTCGGCTGCCTTCTGCAGGATGGTGTGGGACATGGAGGCGTTGGCATGG 540
      |||
Sbjct 806 GCCATTCCCAGCTCGGCTGCCTTCTGCAGGATGGTGTGGGACATGGAGGCGTTGGCATGG 747

Query 541 ACAATGATGGTGGCCGTTTTTGTCTCTCTGATCTCTTTGAGGAGAGGCGTAGGGTCCCGG 600
      |||
Sbjct 746 ACAATGATGGTGGCCGTTTTTGTCTCTCTGATCTCTTTGAGGAGAGGCGTAGGGTCCCGG 687

Query 601 CTGTCATCCAGCATCCGGACTGAGAGCGTGTCTTGGAAATGAGGAACGCCGAAGCAAC 660
      |||
Sbjct 686 CTGTCATCCAGCATCCGGACTGAGAGCGTGTCTTGGAAATGAGGAACGCCGAAGCAAC 627

Query 661 TTCTCTAGGTTTAAAAGGCATTCAGCTTTGGCACAGATGAGGCAGGCAGTGGTGCAGTTA 720
      |||
Sbjct 626 TTCTCTAGGTTTAAAAGGCATTCAGCTTTGGCACAGATGAGGCAGGCAGTGGTGCAGTTA 567

Query 721 AAGAAGTTGAGGATTCTTGCCACGGCCACACTGATGTCAGTGTGCTGGGGTGGAGGTTG 780
      |||
Sbjct 566 AAGAAGTTGAGGATTCTTGCCACGGCCACACTGATGTCAGTGTGCTGGGGTGGAGGTTG 507
```

## GRIK4 (continued)

```
Query 781  AGGGTGGTGAAGCGCTGGAGCTGCAGCTTCAGGAACTCTTCTGGTGCCACTCTGAAGTGA 840
          |||
Sbjct 506  AGGGTGGTGAAGCGCTGGAGCTGCAGCTTCAGGAACTCTTCTGGTGCCACTCTGAAGTGA 447

Query 841  GGAACCTCTTTCTCCCCGCAGATATTGCTGATAATTGAGCTGGAGGCCGGGCTGGAGGAA 900
          |||
Sbjct 446  GGAACCTCTTTCTCCCCGCAGATATTGCTGATAATTGAGCTGGAGGCCGGGCTGGAGGAA 387

Query 901  GGCCCCAGGACGCCACCACCCTTTGGGAAGGATCTGGCACATGGTTTCCGCCGTCTCA 960
          |||
Sbjct 386  GGCCCCAGGACGCCACCACCCTTTGGGAAGGATCTGGCACATGGTTTCCGCCGTCTCA 327

Query 961  TACTCGCTGTCCCGCAGCAGCTCGAAGATGTCCACCTCCACCTTGCCCTTCCCCAGGCGC 1020
          |||
Sbjct 326  TACTCGCTGTCCCGCAGCAGCTCGAAGATGTCCACCTCCACCTTGCCCTTCCCCAGGCGC 267

Query 1021 TCGGGCGCACGGTTGATGCGGTTCTTGCCAGGGTGATGGAGAGCCTCTCCCCCGGCTG 1080
          |||
Sbjct 266  TCGGGCGCACGGTTGATGCGGTTCTTGCCAGGGTGATGGAGAGCCTCTCCCCCGGCTG 207

Query 1081 CACTCCATGGGGTCATCCAGGATGGCGGCAATGCGCAGGGCGTGTGGGCTGGCGGCCGTC 1140
          |||
Sbjct 206  CACTCCATGGGGTCATCCAGGATGGCAGCAATGCGTAGGGCGTGTGGGCTGGCAGCCGTC 147

Query 1141 CTCATCAGCCACAGCAGAAGGAGCACCAGAGGCCCCATACAC 1183
          |||
Sbjct 146  CTCATCAGCCACAGCAGAAGGAGCACCAGAGGCCCCATACAC 104
```

i) SOX11

>Gallus gallus SRY-box 11 (SOX11), mRNA  
Sequence ID: NM\_001396047.1 Length: 1566  
Range 1: 523 to 1255

Score:1354 bits(733), Expect:0.0,  
Identities:733/733(100%), Gaps:0/733(0%), Strand: Plus/Plus

```
Query 1      CGGTGGAAAATGCTGAAGGACAGCGAGAAGATCCCCTTCATCCGGGAGGCGGAGAGGCTG 60
|||||
Sbjct 523     CGGTGGAAAATGCTGAAGGACAGCGAGAAGATCCCCTTCATCCGGGAGGCGGAGAGGCTG 582

Query 61     CGGCTCAAGCACATGGCCGATTACCCCGACTACAAGTACCGGCCAGGAAAAAGCCCAA 120
|||||
Sbjct 583     CGGCTCAAGCACATGGCCGATTACCCCGACTACAAGTACCGGCCAGGAAAAAGCCCAA 642

Query 121    ATGGACCCCTCGGCCAAGCCCAACGCCGGCCAGAGCCCCGAGAAGAACGCTCCCGGCGGC 180
|||||
Sbjct 643     ATGGACCCCTCGGCCAAGCCCAACGCCGGCCAGAGCCCCGAGAAGAACGCTCCCGGCGGC 702

Query 181    GGCAGCAAGAGCGCCAAGAGCTCGGGCAAGAAGTGCAGCAAGCTGAAGgcccgcgccc 240
|||||
Sbjct 703     GGCAGCAAGAGCGCCAAGAGCTCGGGCAAGAAGTGCAGCAAGCTGAAGGCCCGCCGCC 762

Query 241    tcgcccccaagcccggcgccaaagccgccccTCACGGGGACTACGCGGGGACGAGTAC 300
|||||
Sbjct 763     TCGCCCCCAAGCCCGCGCCAAAGCCGCCCTCACGGGGACTACGCGGGGACGAGTAC 822

Query 301    GTCTTCGGCGCGCTGAAAGTCAGCAGCAAGGCGGTCAAGTGCCTTTTCGTggacgaggag 360
|||||
Sbjct 823     GTCTTCGGCGCGCTGAAAGTCAGCAGCAAGGCGGTCAAGTGCCTTTTCGTGGACGAGGAG 882

Query 361    gaggaggacgaggaggacgaggacgagctgcagctgcggtcaagcaggaggcggacgac 420
|||||
Sbjct 883     GAGGAGGACGAGGAGGACGAGGACGAGCTGCAGCTGCGGATCAAGCAGGAGCGGACGAC 942

Query 421    gaggaggaggacgaggagCCGGGCCCGCAGCAGCTGCGGCGGTACAACGTGGCCAAAGTG 480
|||||
Sbjct 943     GAGGAGGAGGACGAGGAGCCGGGCCCGCAGCAGCTGCGGCGGTACAACGTGGCCAAAGTG 1002

Query 481    CCGGCCAGCCCCACCTTGAGCTCCTCGGCGGAGTCCACCGAGGGGGCGAGCCTCTACGAG 540
|||||
Sbjct 1003    CCGGCCAGCCCCACCTTGAGCTCCTCGGCGGAGTCCACCGAGGGGGCGAGCCTCTACGAG 1062

Query 541    GAGGTGCGGGGCGGGCGGGCGGGCAGACTCTACTACAGCTTCAAGAACATCACCAAG 600
|||||
Sbjct 1063    GAGGTGCGGGGCGGGCGGGCGGGCAGACTCTACTACAGCTTCAAGAACATCACCAAG 1122

Query 601    CAGGgccccgcgcccgcgagccgcccgcgcccctctccccgcctcgtcccgcTCCATC 660
|||||
Sbjct 1123    CAGGGCCCGCCGCGCCGAGCCGCCCGCCGCGCTCTCCCCGCGCTCGTCCCGCTCCATC 1182

Query 661    TCCACCTCGTCGGCCGGCAGCGAGGAGGCGGACGACCTCCTGTTTCGACCTCAGCCTCAAC 720
|||||
Sbjct 1183    TCCACCTCGTCGGCCGGCAGCGAGGAGGCGGACGACCTCCTGTTTCGACCTCAGCCTCAAC 1242

Query 721    TTCTCGCAGCATG 733
|||||
Sbjct 1243    TTCTCGCAGCATG 1255
```

Supplemental Table 1. Subject summary used in this study

chick#s	probes											RNA extraction
	<i>ZBTB20</i>	<i>PROX1</i>	<i>CPA6</i>	<i>KIT</i>	<i>NR4A2</i>	<i>LEF1</i>	<i>GRIK1</i>	<i>GRIK2</i>	<i>GRIK3</i>	<i>GRIK4</i>	<i>SOX11</i>	
#1	○	○	○	○	-	○	○	○	○	-	-	-
#2	○	-	○	○	-	○	○	○	○	-	-	-
#3	○	○	○	○	-	○	○	○	○	-	○	-
#4	○	○	○	○	-	○	○	○	○	-	○	-
#5	○	○	○	○	-	○	○	○	○	-	○	-
#6	○	○	○	○	○	○	-	-	-	-	-	-
#7	○	○	-	○	○	○	-	-	-	-	-	-
#8	○	-	○	○	-	○	○	○	○	-	-	-
#9	○	-	○	○	-	-	-	-	-	-	○	-
#10	-	○	○	-	-	○	○	○	○	○	○	-
#11	-	○	-	-	-	○	-	-	-	-	○	-
#12	-	○	-	-	-	○	○	○	-	-	-	-
#13	-	-	○	-	-	○	-	-	-	-	-	-
#14	-	-	-	-	-	-	○	○	○	○	-	-
#15	-	-	-	-	-	○	○	○	○	-	○	-
#16	-	-	-	-	-	○	-	-	-	○	○	-
#17	-	-	-	-	-	-	-	-	-	○	-	-
#18	-	-	-	-	-	-	-	-	-	○	-	-
#19	-	-	-	-	-	-	-	-	-	-	○	-
#20	-	-	-	-	-	○	-	-	-	-	○	-
#21	-	-	-	-	-	-	-	-	-	-	-	○

Supplemental Table 2. Overview of gene probes used in this study

Accession number	Gene symbol	Molecular characteristics	Probe position	Probe size(base)	Probe preparation
XM_046905147.1	<i>ZBTB20</i>	transcription factor	CDS	760	this study
NM_001005616.1	<i>PROX1</i>	transcription factor	CDS	771	this study
NM_001006344.2	<i>CPA6</i>	enzyme	CDS	771	this study
XM_046915417.1	<i>KIT</i>	receptor tyrosine kinase	CDS	723	this study
XM_040675955	<i>NR4A2</i>	transcription factor	3'-UTR	594	Fujita, et al.(2019)
XM_015276135.2	<i>LEF1</i>	transcription factor	CDS	565	Fujita, et al. (2022)
XM_015299614.4	<i>GRIK1</i>	ionotropic glutamate receptor subunit	CDS	699	this study
XM_046939222.1	<i>GRIK2</i>	ionotropic glutamate receptor subunit	CDS	716	this study
XM_417766.7	<i>GRIK3</i>	ionotropic glutamate receptor subunit	CDS	792	this study
XM_015298177.4	<i>GRIK4</i>	ionotropic glutamate receptor subunit	CDS	1183	this study
NM_001396047.1	<i>SOX11</i>	transcription factor	CDS	733	this study

3'-UTR, three prime untranslated region; CDS, coding sequence