Supplementary Table 1.

List of primers used for real-time PCR

Real-time PCR was performed using a primer set in which the reverse primers for *Lhx3a* and *Lhx3b* were common. *Lhx3a* and *Lhx3b* were quantified using the nested PCR method. An aliquot of the 1st PCR products with the 1st PCR primer set was re-amplified by 2nd PCR and cyclophilin primer sets.

Set	Gene	Forward primer	Reverse primer
1st PCR	Lhx3a	5'-GAGCTGGCGGGCGACCGAGATCGG-3'	5'-GCTGAAGCAGCGCTCGGCCAGCG-3'
	Lhx3b	5'-AGCTGGGCCCCAGCCGGGAGTC-3'	
2nd PCR	Lhx3a	5'-GCAGCCGCCGCTGTCTGCACCTTACG-3'	5'-GGCGTGTGGCAGTCACTGCACTTG-3'
	Lhx3b	5'-TGCTGGCGCTGCTGGCGCGGAG-3'	
-	Cyclophilin	5'-ATGATGAGAATTTTATCCTGAAGCA-3'	5'-CTGGCAGTGCAAATGAAAAACTG-3'

Supplementary Fig. 1.

```
10 20 30 40 50 60 70 80 90 100 110 120
ATGCTGCTGGAAACGGAGCTGGCGGGCGACCGAGATCGGCCCGGGGCCCCCGCAGCCGCCGCTGTCTGCACCTTACGCGGGACTCGGGAGATCCCACTGTGTGCCGGCTGCGACCAGCAC
 M  L  L  E  T  E  L  A  G  D  R  D  R  P  G  A  P  A  A  A  A  V  C  T  L  R  G  T  R  E  I  P  L  C  A  G  C  D  Q  H
130 140 150 160 170 180 190 200 210 220 230 240
ATCCTGGACCGCTTCATCCTCAAGGCTCTGGACCGCCACTGGCACAGCAAGTGCCTCAAGTGCAGTGACTGCCACACGCCGCTGGCCGAGCGCTGCTTCAGCCGCGGAGAGAGCCTCTAC
 I  L  D  R  F  I  L  K  A  L  D  R  H  W  H  S  K  C  L  K  C  S  D  C  H  T  P  L  A  E  R  C  F  S  R  G  E  S  L  Y
250 260 270 280 290 300 310 320 330 340 350 360
TGCAAGGACGACTTCTTCAAGCGCTTCGGGACCAAGTGCGCCGCGTGCCAGCTGGGCATCCCGCCCACGCAGGTGGTGCGCCGCGCCCAGGACTTCGTGTACCACCTGCACTGCTTCGCC
 C  K  D  D  F  F  K  R  F  G  T  K  C  A  A  C  Q  L  G  I  P  P  T  Q  V  V  R  R  A  Q  D  F  V  Y  H  L  H  C  F  A
370 380 390 400 410 420 430 440 450 460 470 480
TGCGTCGTGTGCAAGCGGCAGCTGGCCACGGGCGACGAGTTCTACCTCATGGAGGACAGCCGGCTCGTGTGCAAGGCCGACTACGAGACCGCCAAGCAGCGAGAGGCCGAGGCCACGGCC
 C  V  V  C  K  R  Q  L  A  T  G  D  E  F  Y  L  M  E  D  S  R  L  V  C  K  A  D  Y  E  T  A  K  Q  R  E  A  E  A  T  A
490 500 510 520 530 540 550 560 570 580 590 600
AAGCGGCCGCGCACGACCATCACGGCCAAGCAGCTGGAGACGCTGAAGAGCGCCTACAACACGTCGCCCAAGCCCGCGCGCCACGTGCGCGAGCAGCTCTCCTCCGAGACCGGCCTGGAC
 K  R  P  R  T  T  I  T  A  K  Q  L  E  T  L  K  S  A  Y  N  T  S  P  K  P  A  R  H  V  R  E  Q  L  S  S  E  T  G  L  D
610 620 630 640 650 660 670 680 690 700 710 720
ATGCGCGTCGTGCAGGTGTGGTTCCAGAACCGCCGGGCCAAGGAAAAGCGGCTCAAGAAGGACGCCGGCCGGCAGCGCTGGGGCCAGTACTTTCGTAACATGAAGCGCGCCCGCGGTGGC
 M  R  V  V  Q  V  W  F  Q  N  R  R  A  K  E  K  R  L  K  K  D  A  G  R  Q  R  W  G  Q  Y  F  R  N  M  K  R  A  R  G  G
730 740 750 760 770 780 790 800 810 820 830 840
TCCAAGTCGGACAAGGACAGCGTCCAGGAGGAGGGGCAGGACAGTGACGCCGAGGTCTCCTTCACAGACGAGCCATCCATGGCCGAAATGGGCCCTGCCAACGGCCTCTACGGCGGCCTG
 S  K  S  D  K  D  S  V  Q  E  E  G  Q  D  S  D  A  E  V  S  F  T  D  E  P  S  M  A  E  M  G  P  A  N  G  L  Y  G  G  L
850 860 870 880 890 900 910 920 930 940 950 960
GGGGAGCCTGCCCCTGCCTTGGGCCGGCCCTCGGGGGCCCCGGGCAGCTTCCCGCTGGAGCACGGAGGCCTGGCGGGCCCGGAGCAGTATGGAGAGCTGCGCCCCAGCAGCCCCTACGGT
 G  E  P  A  P  A  L  G  R  P  S  G  A  P  G  S  F  P  L  E  H  G  G  L  A  G  P  E  Q  Y  G  E  L  R  P  S  S  P  Y  G
970 980 990 1000 1010 1020 1030 1040 1050 1060 1070 1080
GTCCCCTCGTCGCCCGCCGCCCTGCAGAGCCTCCTTGGCCCCCAGCCCCTCCTCTCCAGCTTGGTGTACCCGGAGGCTGGCTTGGGGCTTGTGCCCGCGGGACGCCCAGGTGGGCCCCCA
 V  P  S  S  P  A  A  L  Q  S  L  L  G  P  Q  P  L  L  S  S  L  V  Y  P  E  A  G  L  G  L  V  P  A  G  A  P  G  G  P  P
1090 1100 1110 1120 1130 1140 1150 1160 1170 1180 1190 1200
CCCATGAGGGTGCTGGCAGGGAACGGACCCAGCTCCGACCTATCCACGGGGAGCAGTGGGGGCTACCCCGACTTCCCTGCCAGTCCCGCCTCCTGGCTGGACGAGGTGGATCACGCTCAG
 P  M  R  V  L  A  G  N  G  P  S  S  D  L  S  T  G  S  S  G  G  Y  P  D  F  P  A  S  P  A  S  W  L  D  E  V  D  H  A  Q
1210 1220 1230 1240 1250 1260 1270 1280 1290 1300 1310 1320
TTCTGACTGAGGCCCCAGCTCCGTGGAGCACCAGACACGAGCACTGCCCCTGGCTGGGTGGTCGGGAGCCGCGCTCTCCTTTCCCGAAGCCCTGGGCCTCTAAAGGACACAGGGTCACCG
 F  *
1330 1340 1350 1360 1370 1380 1390 1400 1410 1420 1430 1440
GCGGGGCACAGGCTGAGGACTGTCCAGCCCGGCGGCCCTGGCCCCGGGCAGAGGGACTTTCTCCCGGTCTCGAGGCTCCTTCTGGGACAAGGGGAGCCACCTGGTGGCTGCTCAGCAAGC
1450 1460 1470 1480 1490 1500 1510 1520 1530 1540 1550 1560
CTTGTTTTGTAAGCAGATTCCTCCCTTTATCAACCAAAATTAACTGAGTGCTTGCTGCTCTTTCTAGACCGGAGTGGTCAGCCCCCGAAGCCGGGGAGGGGGGCTCTCCCCAGCCCAGAG
1570 1580 1590 1600 1610 1620 1630 1640 1650 1658
CAGCACAGCCCTCAGACTGGAAGATGCTTTAATTTTTAAAATTAAAAAATAATACGAACTGTGCTTCCATTTCCCAGCTTCCTCTGTCTAGTTCTGCC
```

Nucleotide and amino acid sequences of porcine *Lhx3a*

The determined sequence was compared with two partial sequences of porcine *Lhx3a* (AF370445.1) and *Lhx3* (AF063245.1). Differences in nucleotides and in amino acids are indicated with red letters. Nucleotide numbers are indicated above the sequence. Shaded amino acid sequences indicate domain regions in the order of LIM1 (nucleotide number (nn): 100–252), LIM2 (nn: 277–438), and DNA-binding homeodomain (nn: 478–657) are shaded.

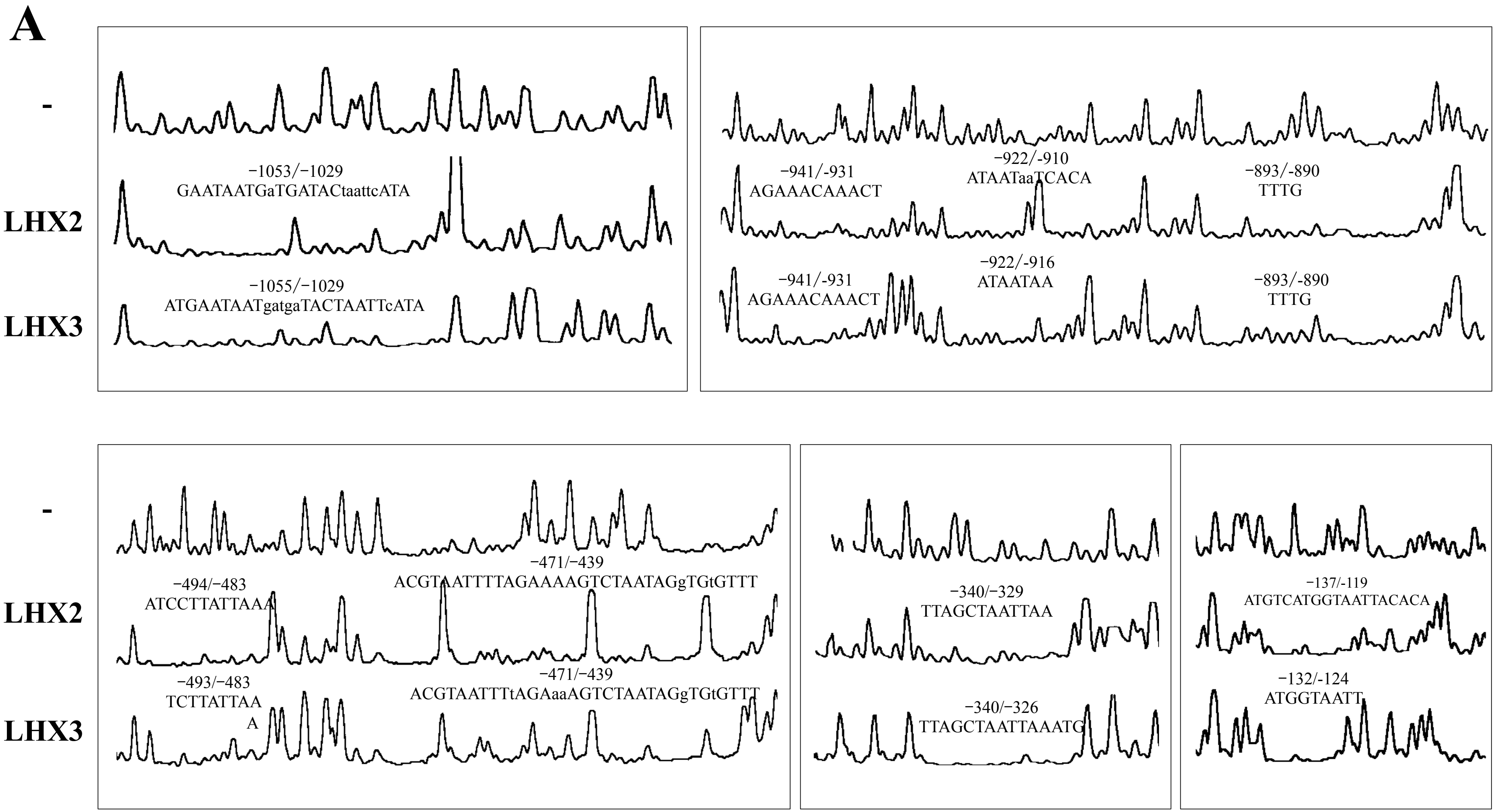
Supplementary Fig. 2.

Nucleotide and amino acid sequences of porcine *Lhx3b*

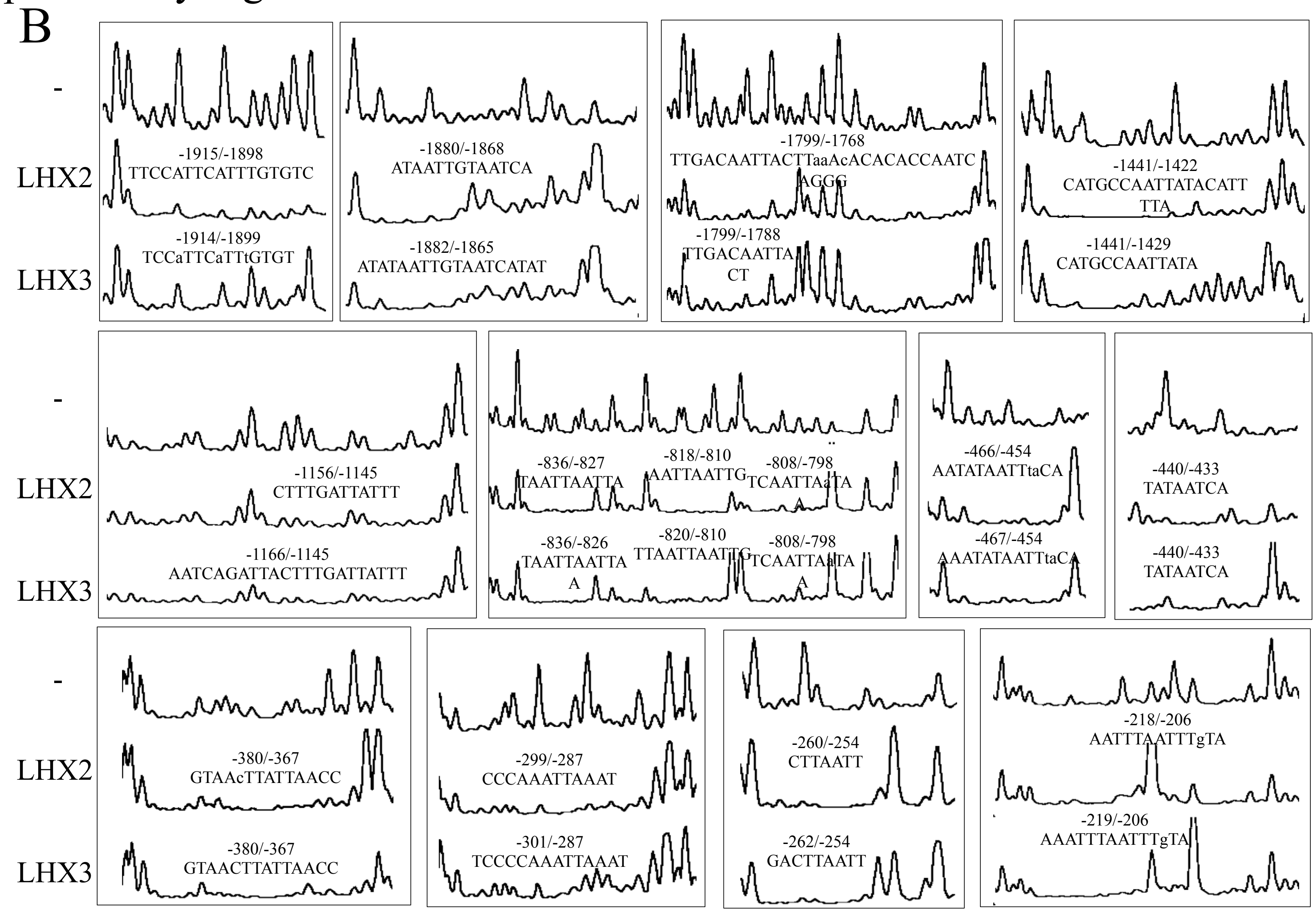
```
          10        20        30        40        50        60        70        80        90       100       110       120
ATGGAAGCGCGCGGGGAGCTGGGCCCCAGCCGGGAGTCGGCGGGCGGCGACCTGCTGCTGGCGCTGCTGGCGCGGAGGGAGGACCTGCGCCGAGAGATCCCACTGTGTGCCGGCTGCGAC
 M  E  A  R  G  E  L  G  P  S  R  E  S  A  G  G  D  L  L  L  A  L  L  A  R  R  E  D  L  R  R  E  I  P  L  C  A  G  C  D
         130       140       150       160       170       180       190       200       210       220       230       240
CAGCACATCCTGGACCGCTTCATCCTCAAGGCTCTGGACCGCCACTGGCACAGCAAGTGCCTCAAGTGCAGTGACTGCCACACGCCGCTGGCCGAGCGCTGCTTCAGCCGCGGAGAGAGC
 Q  H  I  L  D  R  F  I  L  K  A  L  D  R  H  W  H  S  K  C  L  K  C  S  D  C  H  T  P  L  A  E  R  C  F  S  R  G  E  S
         250       260       270       280       290       300       310       320       330       340       350       360
CTCTACTGCAAGGACGACTTCTTCAAGCGCTTCGGGACCAAGTGCGCCGCGTGCCAGCTGGGCATCCCGCCCACGCAGGTGGTGCGCCGCGCCCAGGACTTCGTGTACCACCTGCACTGC
 L  Y  C  K  D  D  F  F  K  R  F  G  T  K  C  A  A  C  Q  L  G  I  P  P  T  Q  V  V  R  R  A  Q  D  F  V  Y  H  L  H  C
         370       380       390       400       410       420       430       440       450       460       470       480
TTCGCCTGCGTCGTGTGCAAGCGGCAGCTGGCCACGGGCGACGAGTTCTACCTCATGGAGGACAGCCGGCTCGTGTGCAAGGCCGACTACGAGACCGCCAAGCAGCGAGAGGCCGAGGCC
 F  A  C  V  V  C  K  R  Q  L  A  T  G  D  E  F  Y  L  M  E  D  S  R  L  V  C  K  A  D  Y  E  T  A  K  Q  R  E  A  E  A
         490       500       510       520       530       540       550       560       570       580       590       600
ACGGCCAAGCGGCCGCGCACGACCATCACGGCCAAGCAGCTGGAGACGCTGAAGAGCGCCTACAACACGTCGCCCAAGCCCGCGCGCCACGTGCGCGAGCAGCTCTCCTCCGAGACCGGC
 T  A  K  R  P  R  T  T  I  T  A  K  Q  L  E  T  L  K  S  A  Y  N  T  S  P  K  P  A  R  H  V  R  E  Q  L  S  S  E  T  G
         610       620       630       640       650       660       670       680       690       700       710       720
CTGGACATGCGCGTCGTGCAGGTGTGGTTCCAGAACCGCCGGGCCAAGGAAAAGCGGCTCAAGAAGGACGCCGGCCGGCAGCGCTGGGGCCAGTACTTTCGTAACATGAAGCGCGCCCGC
 L  D  M  R  V  V  Q  V  W  F  Q  N  R  R  A  K  E  K  R  L  K  K  D  A  G  R  Q  R  W  G  Q  Y  F  R  N  M  K  R  A  R
         730       740       750       760       770       780       790       800       810       820       830       840
GGTGGCTCCAAGTCGGACAAGGACAGCGTCCAGGAGGAGGGGCAGGACAGTGACGCCGAGGTCTCCTTCACAGACGAGCCATCCATGGCCGAAATGGGCCCTGCCAACGGCCTCTACGGC
 G  G  S  K  S  D  K  D  S  V  Q  E  E  G  Q  D  S  D  A  E  V  S  F  T  D  E  P  S  M  A  E  M  G  P  A  N  G  L  Y  G
         850       860       870       880       890       900       910       920       930       940       950       960
GGCCTGGGGGAGCCTGCCCCTGCCTTGGGCCGGCCCTCGGGGGCCCCGGGCAGCTTCCCGCTGGAGCACGGAGGCCTGGCGGGCCCGGAGCAGTATGGAGAGCTGCGCCCCAGCAGCCCC
 G  L  G  E  P  A  P  A  L  G  R  P  S  G  A  P  G  S  F  P  L  E  H  G  G  L  A  G  P  E  Q  Y  G  E  L  R  P  S  S  P
         970       980       990      1000      1010      1020      1030      1040      1050      1060      1070      1080
TACGGTGTCCCCTCGTCGCCCGCCGCCCTGCAGAGCCTCCCTGGCCCCCAGCCCCTCCTCTCCAGCTTGGTGTACCCGGAGGCTGGCTTGGGGCTTGTGCCCGCGGGAGCCCCAGGTGGG
 Y  G  V  P  S  S  P  A  A  L  Q  S  L  P  G  P  Q  P  L  L  S  S  L  V  Y  P  E  A  G  L  G  L  V  P  A  G  A  P  G  G
        1090      1100      1110      1120      1130      1140      1150      1160      1170      1180      1190      1200
CCCCCACCCATGAGGGTGCTGGCAGGGAACGGACCCAGCTCCGACCTATCCACGGGGAGCAGTGGGGGCTACCCCGACTTCCCTGCCAGTCCCGCCTCCTGGCTGGACGAGGTGGATCAC
 P  P  P  M  R  V  L  A  G  N  G  P  S  S  D  L  S  T  G  S  S  G  G  Y  P  D  F  P  A  S  P  A  S  W  L  D  E  V  D  H
        1210      1220      1230      1240      1250      1260      1270      1280      1290      1300      1310      1320
GCTCAGTTCTGACTGAGGCCCCAGCTCCGTGGAGCACCAGACACGAGCACTGCCCCTGGCTGGGTGGTCGGGAGCCGCGCTCTCCTTTCCCGAAGCCCTGGGCCTCTAAAGGACACAGGG
 A  Q  F  *
        1330      1340      1350      1360      1370      1380      1390      1400      1410      1420      1430      1440
TCACCGGCGGGGCACAGGCTGAGGACTGTCCAGCCCGGCGGCCCTGGCCCCGGGCAGAGGGACTTTCTCCCGGTCTCGAGGCTCCTTCTGGGACAAGGGGAGCCACCTGGTGGCTGCTCA
        1450      1460      1470      1480      1490      1500      1510      1520      1530      1540      1550      1560
GCAAGCCTTGTTTTGTAAGCAGATTCCTCCCTTTATCAACCAAAATTAACTGAGTGCTTGCTGCTCTTTCTAGACCGGAGTGGTCAGCCCCCGAAGCCGGGGAGGGGGGCTCTCCCCAGC
        1570      1580      1590      1600      1610      1620      1630      1640      1650      1660
CCAGAGCAGCACAGCCCTCAGACTGGAAGATGCTTTAATTTTTAAAATTAAAAAATAATACGAACTGTGCTTCCATTTCCCAGCTTCCTCTGTCTAGTTCTGCC
```

The determined sequence was compared with two partial sequences of porcine *Lhx3b* (AF370450.1) and *Lhx3* (AF063245.1). Differences in nucleotides and in amino acids are indicated with red letters. Nucleotide numbers are indicated above the sequence. Shaded amino acid sequences indicate domain regions in the order of LIM1 (nucleotide number (nn): 106–258), LIM2 (nn: 283–444), and DNA-binding homeodomain (nn: 484–663) are shaded.

Supplementary Fig. 3A. Yoshida *et al.*



Supplementary Fig. 3B. Yoshida *et al.*



Supplementary Fig. 3. DNase I footprinting analysis of LHX2- and LHX3-binding sequences in the *Cga* and *Fshβ* promoter regions. Labeling of fragments of *Cga* (A) and *Fshβ* (B) were performed by PCR using a FAM-labeled forward primer [26]. DNase I digestion was performed without (-; upper panel) or with recombinant porcine ΔLIM-LHX2 (LHX2; middle panel) or ΔLIM-LHX3 (LHX3; lower panel) as described in the Materials and Methods, followed by analysis using a capillary sequencer with a GeneScan system. Numbers indicate the binding region. The nucleotides with decreased (uppercase) and unchanged (lowercase) their signals are indicated.