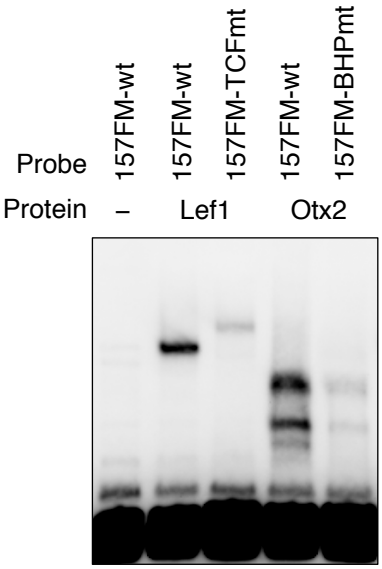

Fig. S1
EMSA of Lef1 and Otx2 binding to 157FM. *In vitro* synthesized Lef1 and Otx2 bind to 157FM-wt but not to 157FM-TCFmt and 157FM-BHPmt, respectively.

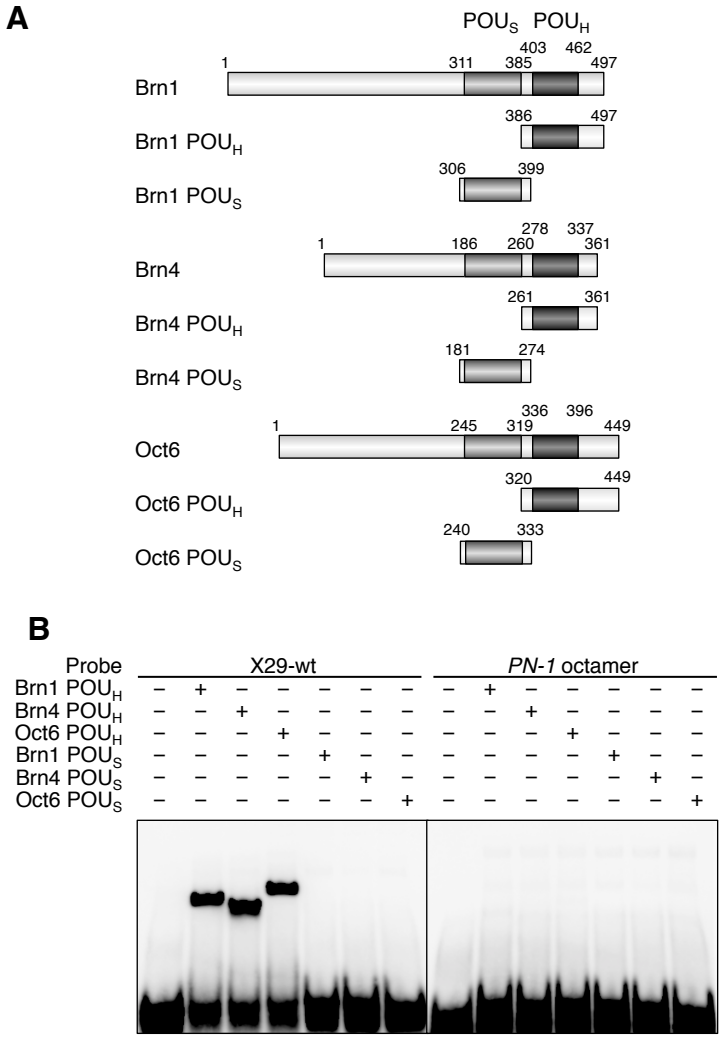

Fig. S2
EMSA of Brn1, Brn4 and Oct6 binding to X29. (A) Deletion constructs used in EMSA. (B) EMSA indicated that each POU_H of Brn1, Brn4 and Oct6, but not POU_S, associates with X29-wt probe (left panel). Neither POU_H nor POU_S associates with *PN-1* octamer probe (right panel).

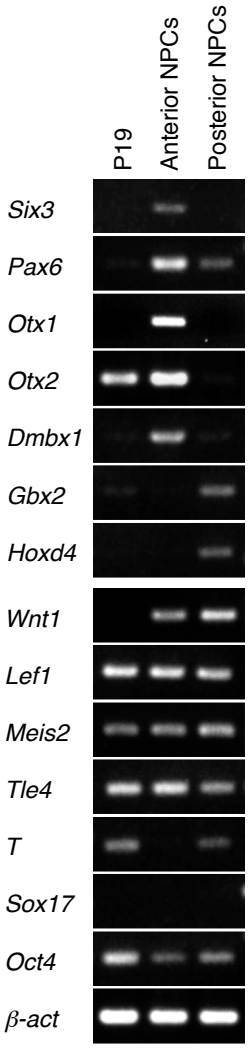

Fig. S3
RT-PCR analysis of marker expression in anterior and posterior NPCs differentiated from P19 cells. Forebrain markers (*Six3, Otx1, Otx2*) and midbrain markers (*Otx1, Otx2, Dmbx1*) are expressed in anterior NPCs, but not in posterior NPCs; *Pax6* is expressed abundantly in forebrain and midbrain but less in spinal cord; this is also the case in anterior and posterior NPCs. In contrast, hindbrain and spinal cord markers (*Gbx2, Hoxd4*) are expressed only in posterior NPCs. *Wnt1*, *Lef1*, *Meis2* and *Tle4* are expressed in both anterior and posterior NPCs. Mesoderm marker (*T*), endoderm marker (*Sox17*), and pluripotent marker (*Oct4*) are less efficiently expressed in these cells. *β-act* expression is given as an internal control.

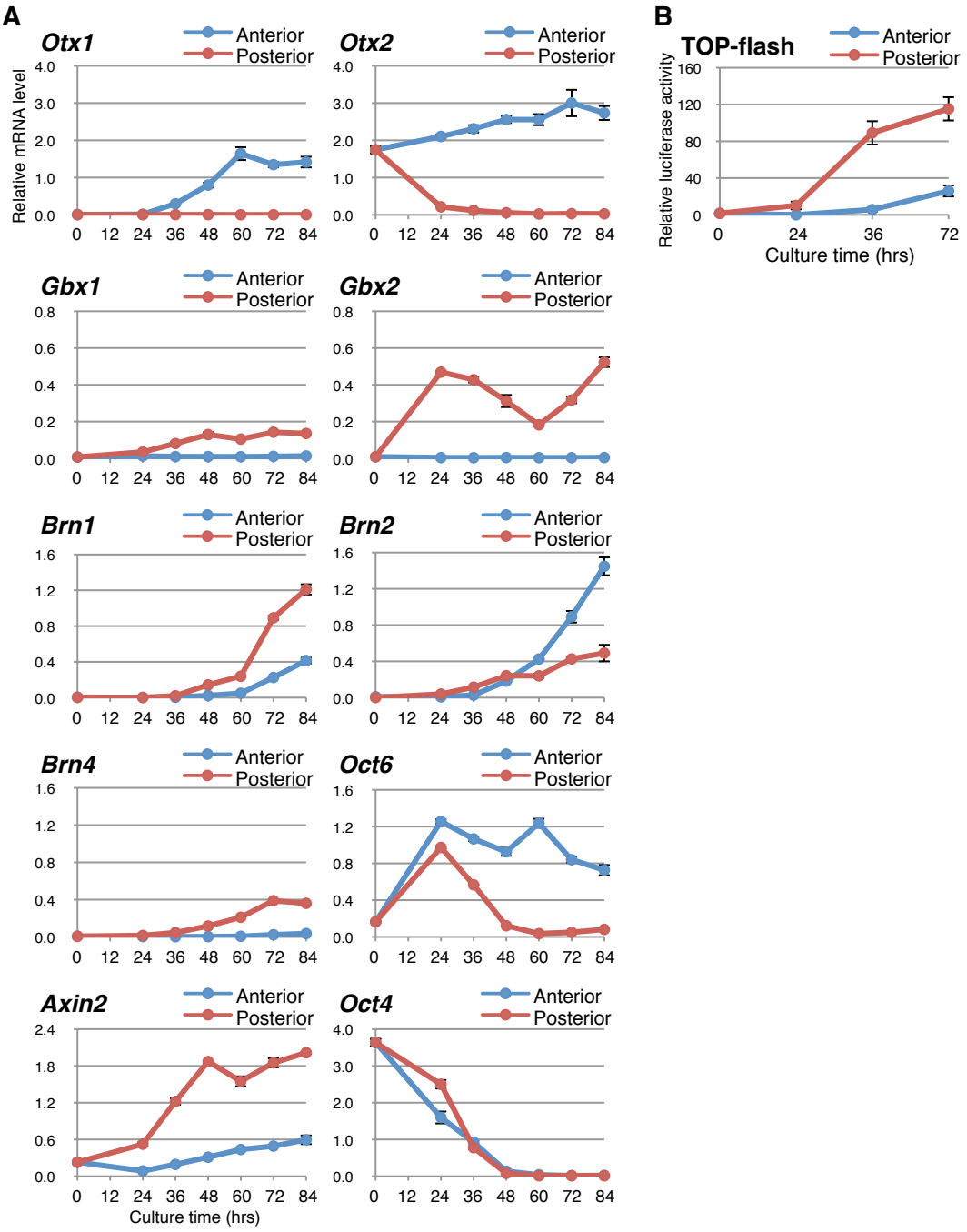

Fig. S4

Temporal changes of *Otx1*, *Otx2*, *Gbx1*, *Gbx2*, *Brn1*, *Brn2*, *Brn4*, *Oct6*, *Axin2* and *Oct4* expression and of Wnt signaling with the P19 differentiation into anterior (blue) and posterior NPCs (red). (A) RT-qPCR assay of mRNA levels in the cells cultured in the induction medium for indicated hours; the levels at 0 hr give the expression in undifferentiated P19 cells. The levels are given as the ratio to the *Tbp* expression. *Otx1* and *Otx2* were expressed in anterior NPCs, while *Gbx1* and *Gbx2* in posterior NPCs. In the anterior NPCs (at 84 hrs), *Brn2* and *Oct6* expression was higher, while *Brn1* and *Brn4* expression was lower. In posterior NPCs (at 84 hrs), *Brn1* expression was higher, while *Brn2* and *Brn4* expression was moderate; *Oct6* was transiently expressed at 24-36 hours post induction. A Wnt signal marker, *Axin2*, expression was higher in posterior NPCs, and moderate in anterior NPCs. Pluripotent marker *Oct4* was scarcely expressed in either anterior or posterior NPCs at 48 hours post induction. (B) TOP-flash activity was higher in posterior NPCs, and moderate in anterior NPCs.

A

Brn2

```
   1  ATG GCG ACC GCA GCG TCT AAC CAC TAC AGC CTG CTC ACC TCC AGC GCC TCC ATC GTA CAT   60
  61  GCC GAG CCG CCT GGC GGC ATG CAG CAG GGC GCA GGG GGC TAC CGC GAG GCG CAG AGC CTG  120
 121  GTG CAG GGC GAC TAC GGC GCG CTG CAG AGC AAC GGG CAC CCG CTC AGC CAC GCT CAC CAG  180
 181  TGG ATC ACC GCG CTG TCC CAC GGC GGC GGC GGC GGG GGC GGC GGC GGC GGT GGA GGA GGC  240
 241  GGG GGA GGC GGC GGG GGA GGC GGC GAC GGC TCC CCG TGG TCC ACC AGC CCC CTA GGC CAG  300
 301  CCG GAC ATC AAG CCC TCG GTG GTG GTA CAG CAG GGT GGC CGA GGC GAC GAG CTG CAC GGG  360
 361  CCA GGA GCG CTG CAG CAA CAG CAT CAA CAG CAA CAG CAA CAG CAG CAG CAG CAG CAG CAG  420
 421  CAG CAG CAG CAG CAA CAG CAG CAG CAA CAA CAG CGA CCG CCA CAT CTG GTG CAC CAC GCT  480
 481  GCC AAC CAC CAT CCC GGG CCC GGG GCA TGG CGG AGT GCG GCG GCT GCA GCT CAC CTC CCT  540
 541  CCC TCC ATG GGA GCT TCC AAC GGC GGT TTG CTC TAT TCG CAG CCG AGC TTC ACG GTG AAC  600
 601  GGC ATG CTG GGC GCA GGA GGG CAG CCG GCT GGG CTG CAC CAC CAC GGC CTG AGG GAC GCC  660
 661  CAC GAT GAG CCA CAC CAT GCA GAC CAC CAC CCG CAT CCG CAC TCT CAC CCA CAC CAG CAA  720
 721  CCG CCC CCG CCA CCT CCC CCA CAA GGC CCA CCG GGC CAC CCA GGC GCG CAC CAC GAC CCG  780
                 POUs domain
 781  CAC TCG GAC GAG GAC ACG CCG ACC TCA GAC GAC CTG GAG CAG TTC GCC AAG CAA TTC AAG  840
                   t   g   t   c
 841  CAG AGG CGG ATC AAA CTC GGA TTT ACT CAA GCA GAC GTG GGG CTG GCG CTT GGC ACC CTG  900
 901  TAC GGC AAC GTG TTC TCG CAG ACC ACC ATC TGC AGG TTT GAG GCC CTG CAG CTG AGC TTC  960
 961  AAG AAC ATG TGC AAG CTG AAG CCT TTG TTG AAC AAG TGG TTG GAA GAG GCA GAC TCA TCC 1020
                                                          POUh domain
1021  TCG GGC AGC CCC ACC AGC ATA GAC AAG ATC GCA GCG CAA GGG CGC AAA CGG AAA AAG CGG 1080
1081  ACC TCC ATC GAG GTG AGC GTC AAG GGG GCT CTG GAG AGC CAT TTC CTC AAA TGC CCT AAG 1140
1141  CCC TCG GCC CAG GAG ATC ACC TCC CTC GCG GAC AGC TTA CAG CTG GAG AAG GAG GTG GTG 1200
1201  AGA GTT TGG TTT TGT AAC AGG AGA CAG AAA GAG AAA AGG ATG ACC CCT CCC GGA GGG ACT 1260
1261  CTG CCG GGC GCC GAG GAT GTG TAT GGG GGT AGT AGG GAC ACG CCA CCA CAC CAC GGG GTG 1320
1321  CAG ACG CCC GTC CAG TGA                                                       1338
```

Fig. S5-1
Brn2 (A), *Oct6* (B), and *Gbx2* (C) coding sequences, and locations of miRNA target sequences and silent mutations. Red bars indicate miRNA target sequences, and blue letters silent mutations in *Brn2sm* and *Oct6sm*.

B

Oct6

```
   1  ATG GCC ACC ACC GCG CAG TAT CTG CCG CGG GGC CCC GGC GGC GGA GCT GGG GGC ACA GGG   60

  61  CCG CTC ATG CAT CCC GAT GCC GCC GCG GCG GCG GCA GCG GCG GCC GAG CGG CTG CAC GCG  120

 121  GGG GCC GCG TAC CGC GAA GTG CAG AAG CTG ATG CAC CAC GAG TGG CTG GGC GCG GGC GCG  180

 181  GGC CAC CCC GTG GGC CTA GCG CAC CCT CAA TGG CTA CCC ACG GGA GGA GGC GGC GGC GGC  240

 241  GAC TGG GCG GGC GGC CCG CAC CTG GAA CAC GGC AAG GCA GGC GGT GGC GGT ACC GGC CGA  300

 301  GCT GAC GAC GGC GGC GGT GGC GGC GGT TTC CAC GCC CGC CTG GTG CAC CAA GGG GCG GCC  360

 361  CAC GCG GGC GCG GCA TGG GCA CAA GGC GGC ACA GCG CAC CAC TTG GGC CCC GCC ATG TCG  420

 421  CCG TCG CCC GGG GCC GGC GGG GGT CAC CAG CCC CAG CCG CTC GGG CTG TAC GCT CAG GCG  480

 481  GCC TAC CCC GGT GGC GGC GGC GGC GGC CTG GCC GGG ATG CTG GCG GCG GGA GGC GGC GGC  540

 541  GCG GGA CCC GGC CTG CAC CAC GCA CTG CAC GAG GAC GGC CAC GAG GCA CAG CTG GAG CCG  600

 601  TCG CCA CCA CCG CAC CTG GGC GCA CAC GGA CAC GCA CAC GGA CAT GCA CAC GCG GGC GGC  660

 661  CTG CAC GCG GCG GCG GCG CAC CTG CAC CCG GGC GCG GGC GGT GGT GGC TCG TCG GTG GGC  720
                                                  POUs domain
 721  GAG CAC TCG GAC GAG GAT GCT CCC AGC TCC GAC GAC CTG GAG CAG TTC GCC AAG CAG TTC  780

 781  AAG CAA CGA CGC ATC AAG CTG GGC TTC ACC CAG GCC GAC GTG GGA CTG GCG CTG GGC ACC  840

 841  CTC TAC GGT AAC GTG TTC TCG CAG ACC ACC ATC TGC CGT TTC GAG GCC CTG CAG CTG AGC  900

 901  TTC AAG AAC ATG TGC AAG CTC AAG CCG CTG CTC AAC AAG TGG CTG GAG GAG ACC GAC TCG  960
                                                                 POUh domain
 961  TCC AGC GGC AGC CCC ACC AAC CTG GAC AAG ATC GCG GCG CAG GGC CGC AAG CGC AAG AAG 1020

1021  CGC ACG TCC ATC GAG GTG GGT GTC AAA GGC GCG CTC GAG AGC CAC TTT CTC AAG TGT CCC 1080
                                                                              c
1081  AAG CCG TCT GCG CAC GAG ATC ACC GGC CTG GCC GAC AGC CTG CAA CTG GAG AAG GAG GTG 1140
        a   c   a
1141  GTG CGT GTC TGG TTC TGC AAC CGG CGG CAG AAG GAG AAG CGC ATG ACC CCC GCG GCC GGC 1200

1201  GCG GGC CAC CCG CCC ATG GAC GAC GTT TAT GCG CCT GGG GAG CTG GGG CCT GGC GGG GGG 1260

1261  CAG CGC GTC GCC ACC TTC TGC GCC CCC GCC ACC CCC GCC GGC CGC GCT GCA CCA CCA CCA 1320

1321  CCA CCA CAC ACT GCC CGG CTC TGT GCA GTG ACC CTG CGG ACT GGG TTC CCC GCC GGC GCA 1380

1381  GCG GTG CCT CCG GCG CGC AGT TAG                                               1404
```

Fig. S5-2

C

Gbx2

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   1  ATG AGC GCA GCG TTC CCG CCG TCG CTG ATG ATG ATG CAG CGC CCG CTG GGG AGT AGT ACC  60

  61  GCC TTC AGC ATA GAC TCG CTG ATC GGC AGC CCG CCG CAG CCC AGT CCC GGC CAT TTC GTC  120

 121  TAC ACC GGC TAC CCC ATG TTC ATG CCC TAC CGG CCG GTG GTG CTG CCG CCA CCG CCG CCA  180

 181  CCG CCT CCC GCG CTG CCC CAG GCA GCG CTG CAG CCC GCT CTG CCG CCC GCG CAC CCT CAC  240

 241  CAC CAG ATC CCC AGC CTG CCC ACC GGC TTC TGC TCC AGC CTG GCG CAG GGC ATG GCG CTC  300

 301  ACC TCC ACG CTC ATG GCC ACT CTG CCC GGC GGC TTC TCT GCG TCG CCC CAG CAC CAA GAG  360

 361  GCG GCG GCT GCC CGC AAG TTC GCT CCA CAG CCA CTG CCC GGA GGC GGC AAC TTC GAC AAA  420

 421  GCC GAG GCG CTC CAA GCG GAT GCG GAA GAC GGC AAA GCC TTC TTG GCC AAG GAG GGC TCG  480

 481  CTG CTC GCT TTC TCT GCG GCC GAA GCG GTG CAG GCG TCG CTC GTC GGG GCT GTC CGA GGG  540

 541  CAA GGG AAA GAC GAG TCA AAG GTG GAA GAT GAC CCG AAG GGC AAG GAG GAG AGC TTC TCT  600

 601  CTG GAG AGC GAT GTG GAT TAC AGC TCA GAT GAC AAT TTG CCT GGT CAG ACT GCT CAT AAG  660

 661  GAA GAA GAC CCC GGC CAC GCA CTG GAG GAG ACC CCG CAG AGC GGC GGT GCA GCA GGC AGC  720
                                  Homeodomain
 721  ACC ACG TCC ACA GGC AAG AAC CGG CGG CGG CGG ACT GCC TTC ACC AGC GAA CAG CTG CTG  780

 781  GAG CTG GAG AAA GAA TTC CAC TGC AAA AAG TAC CTC TCC CTG ACC GAG CGC TCA CAG ATC  840

 841  GCC CAC GCC CTC AAA CTC AGC GAG GTG CAA GTA AAA ATC TGG TTC CAG AAC CGC CGG GCC  900
                        #1
 901  AAG TGG AAA CGT GTC AAG GCA GGC AAC GCC AAT TCC AAG ACG GGG GAG CCC TCT CGG AAC  960

 961  CCC AAG ATT GTC GTC CCC ATC CCT GTT CAC GTT AGC AGG TTC GCT ATT CGA AGT CAA CAC 1020
                                  #2
1021  CAG CAG CTG GAG CAG GCC CGA CCC TGA                                            1047
```

Fig. S5-3

Table S1

Mass spectrometric analysis of proteins interacting with 157FM

Protein	No. of peptides	Coverage
Brn1	6	16%
Brn2	7	17%
Brn4	7	20%
Oct6	5	7%

Table S2

Primers used for plasmid construction. Underlines indicate target sites for restriction enzymes shown in right column.

Primer	Sequence (5'-3')	Underline
Brn1 forward	ATGGCCACGGCGGCTTCTAACCC	
Brn1 reverse	CCCGGCATTCACTGCACGC	
Brn2 forward	CGGCTCCGAGAGTCATGGC	
Brn2 reverse	GCTTGAGTTCACTGGACGG	
Brn4 forward	GAGGATCCTCATCGACCATG	
Brn4 reverse	CTCCTTGCTTCCTCCAGTCA	
Oct6 forward	ATGGCCACCACCGCGCAGTATC	
Oct6 reverse	TCACTGCACAGAGCCGGGCAGTG	
Brn1 POU$_H$ forward	GCTGGAATTCACCATGTCGAGCACTGGCAGTCCCAC	*Eco*RI
Brn1 POU$_H$ reverse	GCCTCTTCGCTATTACGCCA	
Brn1 POU$_S$ forward	AGGGGAATTCACCATGGACCCTCACTCGGACGAG	*Eco*RI
Brn1 POU$_S$ reverse	GCTTTCTAGACTACGCTGCGATCTTGTCAATGC	*Xba*I
Brn2 POU$_H$ forward	GAAGGTACCATGTCATCCTCGGGCAGCCCCA	*Kpn*I
Brn2 POU$_H$ reverse	GCCTCTTCGCTATTACGCCA	
Brn2 POU$_S$ forward	GGCGGTACCATGGACCCGCACTCGGACGAG	*Kpn*I
Brn2 POU$_S$ reverse	GTTTTGCCTCGAGTCACGCTGCGATCTTGTCTATGC	*Xho*I
Brn4 POU$_H$ forward	GGAGGGTACCATGTCATCCACAGGAAGCCCGAC	*Kpn*I
Brn4 POU$_H$ reverse	GCCTCTTCGCTATTACGCCA	
Brn4 POU$_S$ forward	CTCGGGTACCATGCAGGACCACTCTGATGAAGAG	*Kpn*I
Brn4 POU$_S$ reverse	GCGTTTCTCGAGCTAAGCAGCGATCTTGTCAATGC	*Xho*I
Oct6 POU$_H$ forward	GGAGGGTACCATGTCGTCCAGCGGCAGCCCCA	*Kpn*I
Oct6 POU$_H$ reverse	GCCTCTTCGCTATTACGCCA	
Oct6 POU$_S$ forward	TGGCGGTACCATGGGCGAGCACTCGGACGAG	*Kpn*I
Oct6 POU$_S$ reverse	GCGCTTCTCGAGCTACGCCGCGATCTTGTCCAG	*Xho*I

Table S3

Primers used for RT-PCR analysis

Primer	Sequence (5'-3')
β-act forward	ATTACTGCTCTGGCTCCTAG
β-act reverse	ACGCAGCTCAGTAACAGTCC
Dmbx1 forward	TGTACCAATCTTCCTGAGGC
Dmbx1 reverse	GACAGGCTCAGTTGAAGTTC
Gbx2 forward	GGAGCTGGAGAAAGAATTCC
Gbx2 reverse	GAACCTGCTAACGTGAACAG
Hoxd4 forward	GCCAGATCAAGATCTGGTTC
Hoxd4 reverse	TGGAGTGCAAGAAGGGATAG
Lef1 forward	ACACAACTGGCATCCCTCATC
Lef1 reverse	TTCTGCCCAGGATCTGGTTG
Meis2 forward	GCATGGATGGGCAGTGGCAC
Meis2 reverse	GGGTCCATGTCTTAACTGAG
Oct4 forward	CCAATGCCGTGAAGTTGGAG
Oct4 reverse	TCTTAAGGCTGAGCTGCAAG
Otx1 forward	TGGGCTCGCCTTCAATTCTG
Otx1 reverse	TGGAGGGTATAGGTAGTTGC
Otx2 forward	CTGTTACCAGCCATCTCAATC
Otx2 reverse	ATAGCTTCTACAGGTCTTCAC
Pax6 forward	AGTGAATGGGCGGAGTTATG
Pax6 reverse	ACTGTAATCGAGGCCAGTAC
Six3 forward	AACTCCTCATCAGAGGGTTG
Six3 reverse	CACAGCTTCACAATCCACTC
Sox17 forward	TGTGTATAAGCCCGAGATGG
Sox17 reverse	AGGATTTCCTTAGCGCTTCC
T forward	GGTTCTCCGATGTATGAAGG
T reverse	TGCCACTTTGAGCCTAGAAG
Tle4 forward	CAGTTGCATCTTCATGAGAGC
Tle4 reverse	TAAACCGTAGCTTTCTTGTCC
Wnt1 forward	GTTCTGCACGAGTGTCTATG
Wnt1 reverse	TCAGGATGGCAAAAGGGTTC

Table S4

Primers used for quantitative RT-PCR analysis

Primer	Sequence (5'-3')
Axin2 forward	TGGGACGACGAGACAGTGC
Axin2 reverse	GATCCTCTCCACTTTGCCCAG
Brn1 forward	TGTGGAAAGACAGCACCAACC
Brn1 reverse	TCAAAGTTGGCAACGCATTG
Brn2 forward	ATGGGGGTAGTAGGGACACG
Brn2 reverse	CCGCTTGAGTTCACTGGACG
Brn4 forward	CTTCAGCTTCGTGCGGAGAG
Brn4 reverse	GCTGGACCAGAATCTTCGTGAC
Gbx1 forward	ACGTCAACAGGTTTGCTGTG
Gbx1 reverse	GGCCAAATAGTCTTGTGTCC
Gbx2 forward	ACCATGGAGAGGCCCTAACAC
Gbx2 reverse	TTCTGCTTTAAACAGTGGAGTCTGAC
Oct4 forward	GAACCTGGCTAAGCTTCCAAG
Oct4 reverse	CCAATACCTCTGAGCCTGGTC
Oct6 forward	TTTGTATGCCCGACTAAACCG
Oct6 reverse	GATGGGTCAGAAATTAGGAAACCA
Otx1 forward	CCCGACTGTCTGGACTATAAGGAC
Otx1 reverse	TCCTGGGCTCACAAGACCTG
Otx2 forward	CAATGTCCCAGGCTCATTCA
Otx2 reverse	TCAGTGCCAACTACCTGTTGGT
Tbp forward	GTGATGTGAAGTTCCCTATAAGG
Tbp reverse	CTACTGAACTGCTGGTGGGTCA

Table S5

Oligonucleotides used to construct miRNA expression plasmids

Primer	Sequence (5'-3')
miR-Brn2 top	TGCTGAGTAAATCCGAGTTTGATCCGGTTTTGGCCACTGACTGACCGGATCAATCGGATTTACT
miR-Brn2 bottom	CCTGAGTAAATCCGATTGATCCGGTCAGTCAGTGGCCAAAACCGGATCAAACTCGGATTTACTC
miR-Oct6 top	TGCTGTGCAGAACCAGACACGCACCAGTTTTGGCCACTGACTGACTGGTGCGTCTGGTTCTGCA
miR-Oct6 bottom	CCTGTGCAGAACCAGACGCACCAGTCAGTCAGTGGCCAAAACTGGTGCGTGTCTGGTTCTGCAC
miR-Gbx2#1 top	TGCTGTTTACTTGCACCTCGCTGAGTGTTTTGGCCACTGACTGACACTCAGCGGTGCAAGTAAA
miR-Gbx2#1 bottom	CCTGTTTACTTGCACCGCTGAGTGTCAGTCAGTGGCCAAAACACTCAGCGAGGTGCAAGTAAAC
miR-Gbx2#2 top	TGCTGTAGCGAACCTGCTAACGTGAAGTTTTGGCCACTGACTGACTTCACGTTCAGGTTCGCTA
miR-Gbx2#2 bottom	CCTGTAGCGAACCTGAACGTGAAGTCAGTCAGTGGCCAAAACTTCACGTTAGCAGGTTCGCTAC