Inventory of the Supplemental Infomation

1. Supplemental Data

   Figure S1: This figure is the supplemental materials to Figure 1 which demonstrates the interaction between BCL6 and Notch1. It includes additional data which reveal how BCL6 interacts with Notch1.

   Figure S2: This figure is the supplemental materials to Figure 2 which demonstrates the roles of BCL6 and Notch1 during LR patterning in *Xenopus*. It includes the expression profile of *BCL6*, *Notch1* and other components of Notch signaling, the effects of Morpholino Oligos (MO), and quantitative RT-PCR analysis which supports the results from whole mount *in situ* hybridization. Additional data supporting the role of Notch1 on the induction of symmetric *Xnr1*expression in the GRP is also shown.

   Figure S3: This figure is the supplemental materials to Figure 3 which demonstrates the role of BCL6 for Notch signaling during LR asymmetry. It includes a model diagram that explains the relationship between BCL6 and Notch1 on *Pitx2* expression and additional data which confirm the function of NBD-S.

   Figure S4: This figure is the supplemental materials to Figure 5 which demonstrates that ESR1 is a target of BCL6 during LR patterning and neural development. It includes the expression profile of *ESR1* in the stage25 LPM, additional data that confirm the role of BCL6 on *ESR1* expression, the effect of MO, and quantitative RT-PCR analysis which supports the results from whole mount *in situ* hybridization.

   Figure S5: This figure is the supplemental materials to Figure 6 which demonstrates how BCL6 represses the transcription of *ESR1* gene. It includes results of luciferase assay that were obtained by a deletion analysis of genomic fragments of *ESR1* gene and EMSA with shorter fragments generated from the BCL6-response element.

2. Experimental Procedures

This includes the detail of plasmid construction, sequences of MOs, the information of probes for whole mount *in situ* hybridization and sequences of primers.

3. References

   In this section, all references, which were used in the supplemental information, are included.

Supplemental Information

1. Supplemental Data

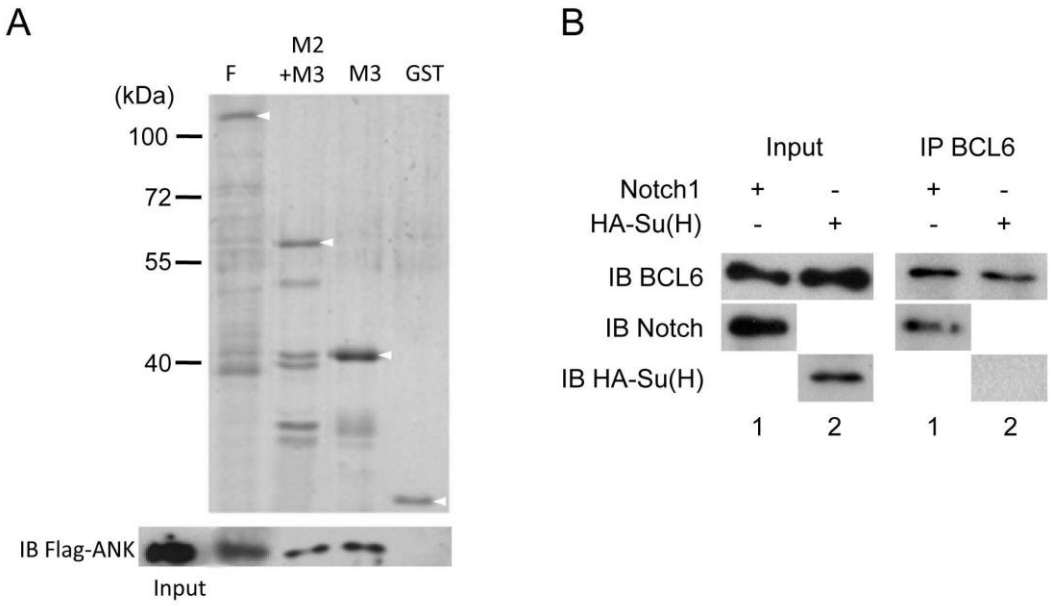


Figure S1. BCL6 directly interacts with the ANK domain of NICD.

A: The top panel shows the expression of GST constructs and the bottom panel shows the interactions between Flag-tagged ANK domain of Notch1 (Flag-ANK) and GST-BCL6 constructs. Each arrowhead indicates an intact GST fusion protein. B: Co-immunoprecipitation using *in vitro* synthesized proteins.

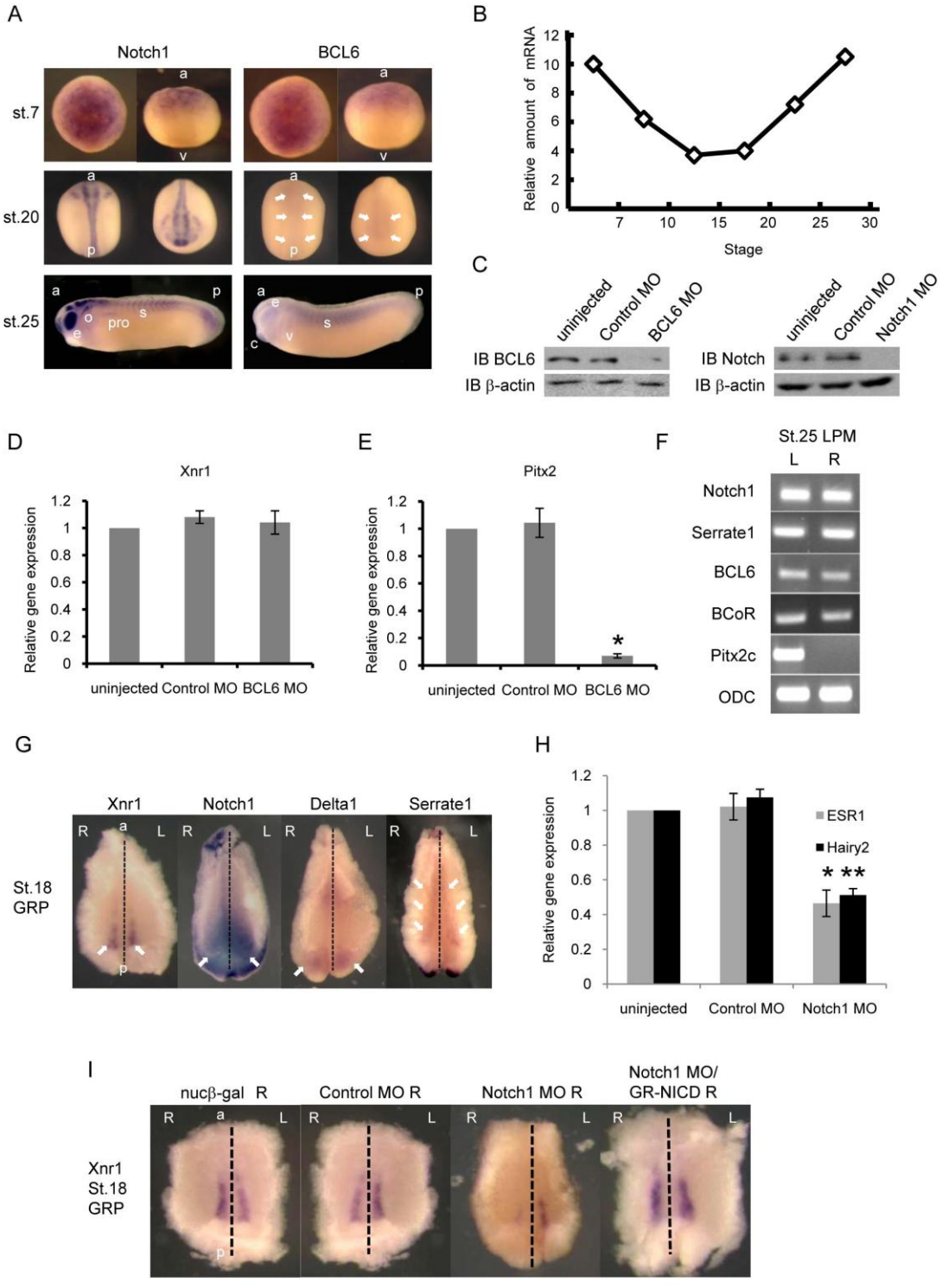
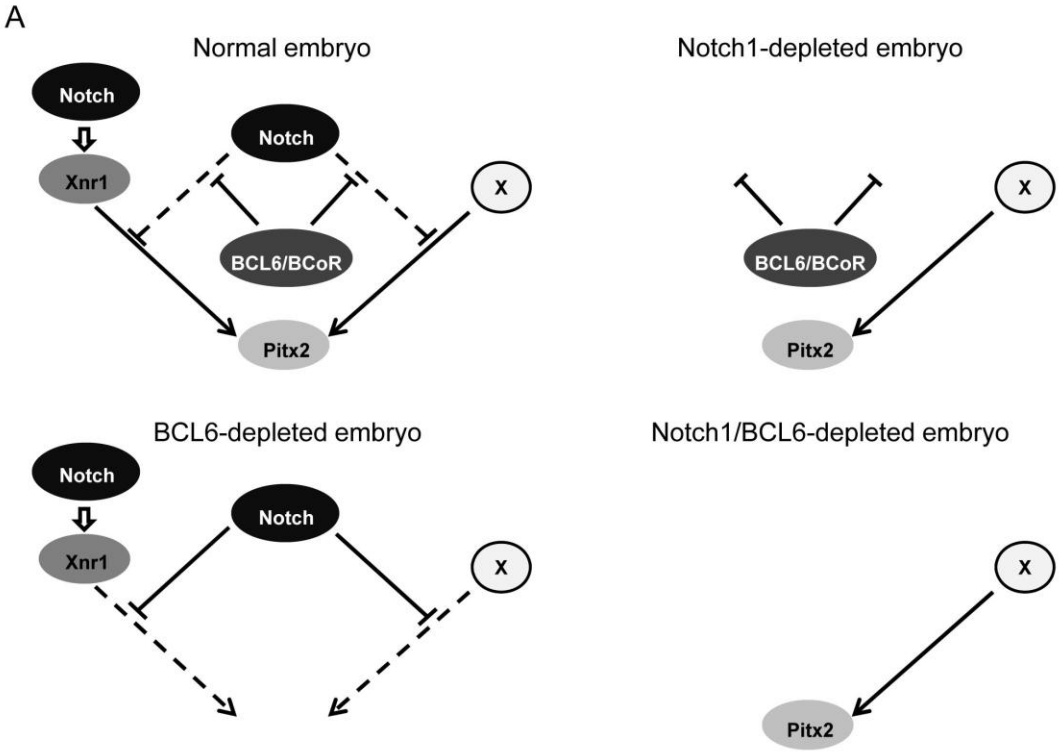
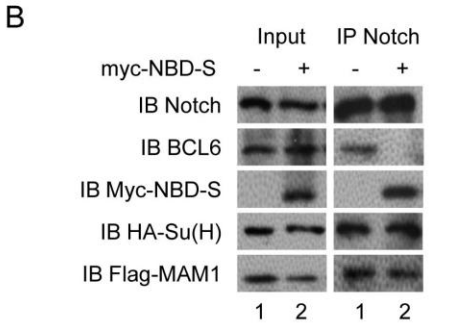
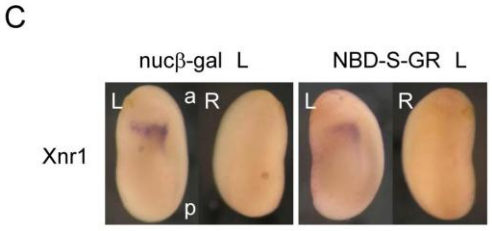


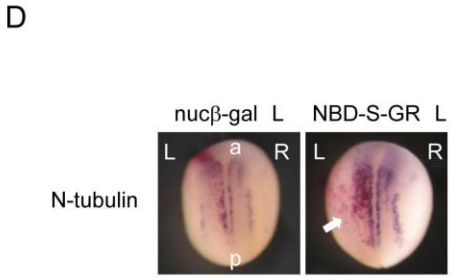
Figure S2. BCL6 and Notch signaling are important for LR patterning in *Xenopus*.

A: Whole mount *in situ* hybridization for *Notch1* and *BCL6*. Stage 7: The left panel presents the animal pole view and the right panel presents the lateral view. a: animal side, v: vegetal side. Stage 20: *BCL6* expression is indicated by white arrows. The left panel presents the dorsal view and the right panel presents the anterior view. a: anterior, p: posterior. Stage 25: The lateral views are shown. c: cement gland, e: eye, o: otic vesicle, pro: pronephros, s: somites, v: visceral pouches, a: anterior, p: posterior. B: Total RNA was isolated from10 embryos at each developmental stage and used for quantitative RT-PCR. *Ornithine decarboxylase* (*ODC*) was used for the normalization of samples. C: Following the injection of MO at 2-cell stage, the level of BCL6 protein or Notch1 protein examined at stage 22 or stage 17, respectively. The level of β-actin protein was used for the normalization of samples. D, E: BCL6 MO was injected into the left side of embryos and left LPM tissues were dissected at stage 22 for *Xnr1* expression (D) and stage 25 for *Pitx2* expression (E) for quantitative RT-PCR. * $P<0.05$, n=3. F: LPM tissues were dissected from 20 embryos and gene expression was tested by RT-PCR. *ODC* was used for the normalization of samples. L: left, R: right. G: Arrows indicate symmetric *Xnr1*, *Notch1*, *Delta1* and *Serrate1* expression. The dotted line indicates the embryonic midline. H: Notch1 MO was injected into two blastomeres of two-cell stage embryos and total RNA was isolated from stage 18 embryos. The expression of *ESR1* or *Hairy2* was examined by quantitative RT-PCR. * $P<0.001$, n=3. ** $P<0.001$, n=3. I: Notch1 MO or/and *GR-NICD* RNA was injected into the right side of embryos. GR-NICD was activated at stage 12 by DEX. The injected side is indicated by R (right) beside the names of injected samples. L: left, R: right, a: anterior, p: posterior.


A
Normal embryo
Notch
Xnr1
Notch
BCL6/BCoR
X
Pitx2
Notch1-depleted embryo
BCL6/BCoR
X
Pitx2
BCL6-depleted embryo
Notch
Xnr1
Notch
X
Notch1/BCL6-depleted embryo
X
Pitx2


B
Input
IP Notch
myc-NBD-S - + - +
IB Notch
IB BCL6
IB Myc-NBD-S
IB HA-Su(H)
IB Flag-MAM1
1 2 1 2


C
nucβ-gal L
NBD-S-GR L
Xnr1
L
a
R
p
L
R


D
nucβ-gal L
NBD-S-GR L
N-tubulin
L
a
R
p
L
R

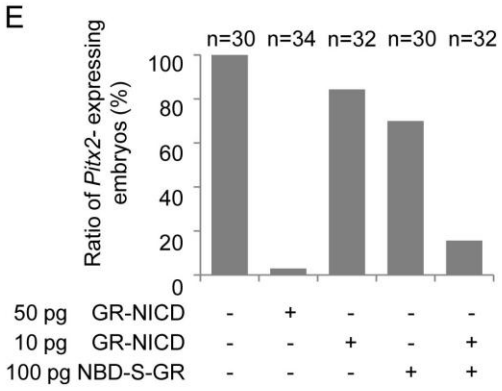

E
n=30 n=34 n=32 n=30 n=32
Ratio of Pitx2- expressing embryos (%)
100
80
60
40
20
0
50 pg GR-NICD - + - - -
10 pg GR-NICD - - + - +
100 pg NBD-S-GR - - - + +

Figure S3. Notch signaling is an *in vivo* target of BCL6 for *Pitx2* expression.
A: A model for the regulation of *Pitx2* expression. "X" indicates an Xnr1-independent signal(s) that induces *Pitx2* expression. The dotted line indicates the suppressed signal. B: HA-tagged Su(H), Flag-tagged MAM1 or/and Myc-tagged NBD-S was expressed in embryos and protein extracts were isolated from 50 embryos at stage 10. Co-immunoprecipitation with α-Notch antibody was performed. C, D: *NBD-S-GR* was injected into embryos and activated at stage 12 (C) and stage 4 (D). The injected side is indicated by L (left) beside the names of injected samples. L: left, R: right, a: anterior, p: posterior. E: The expression of *Pitx2* at stage 25 was tested by whole mount *in situ* hybridization and the ratios of *Pitx2*-expressing embryo number versus total tested embryo number are shown. Total numbers of each injection are shown as "n" on the top of each bar.

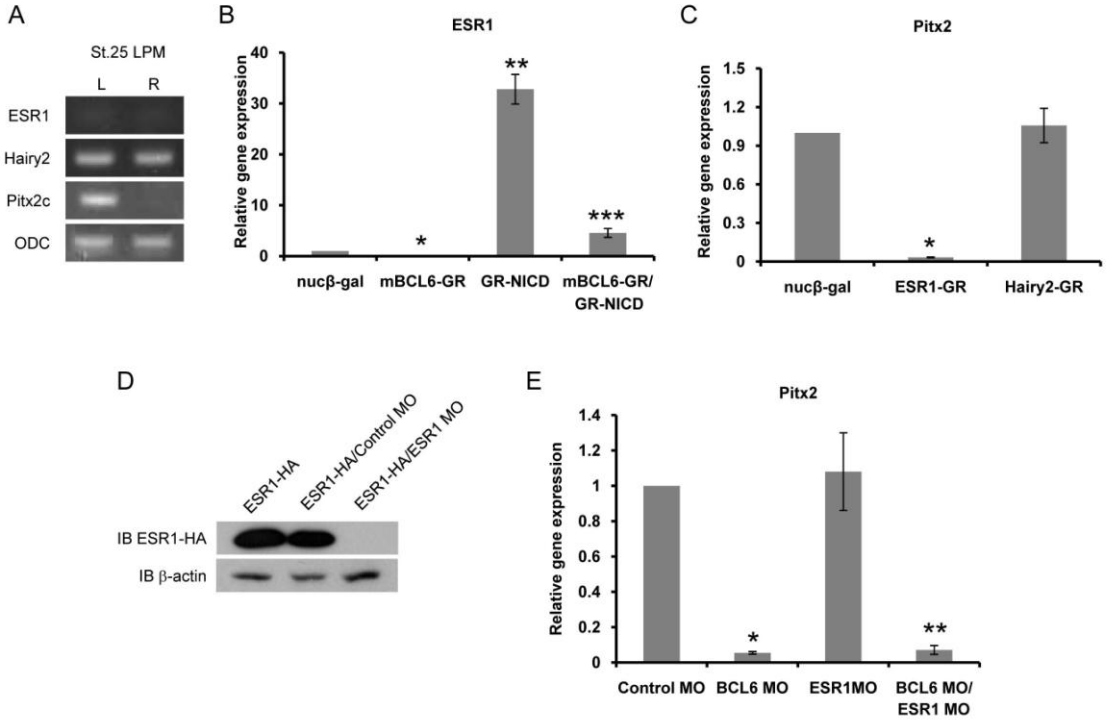


Figure S4. The expression of *ESR1* is suppressed by BCL6 in the LPM.

A: Gene expression was tested using the cDNA used in Figure S2 F. *ODC* was used for the normalization of samples. L: left, R: right. B: *mBCL6-GR* or/and *GR-NICD* was injected into the left side of embryos and left LPM tissues were dissected at stage 25 for quantitative RT-PCR. * P<0.001, n=3, **P<0.005, n=3, ***P<0.05, n=3. C: *ESR1-GR* or *Hairy2-GR* was injected into the left side of embryos and left LPM tissues were dissected at stage 25 for quantitative RT-PCR. * P<0.001, n=3. D: Following the injection of ESR1 MO or/and *ESR1-HA*, the level of ESR1 protein tested at stage 22. The level of β-actin protein was used for the normalization of samples. E: ESR1 MO or/and BCL6 MO was injected into the left side of embryos and left LPM tissues were dissected at stage 25 for quantitative RT-PCR. * P<0.001, n=3, **P<0.001, n=3.

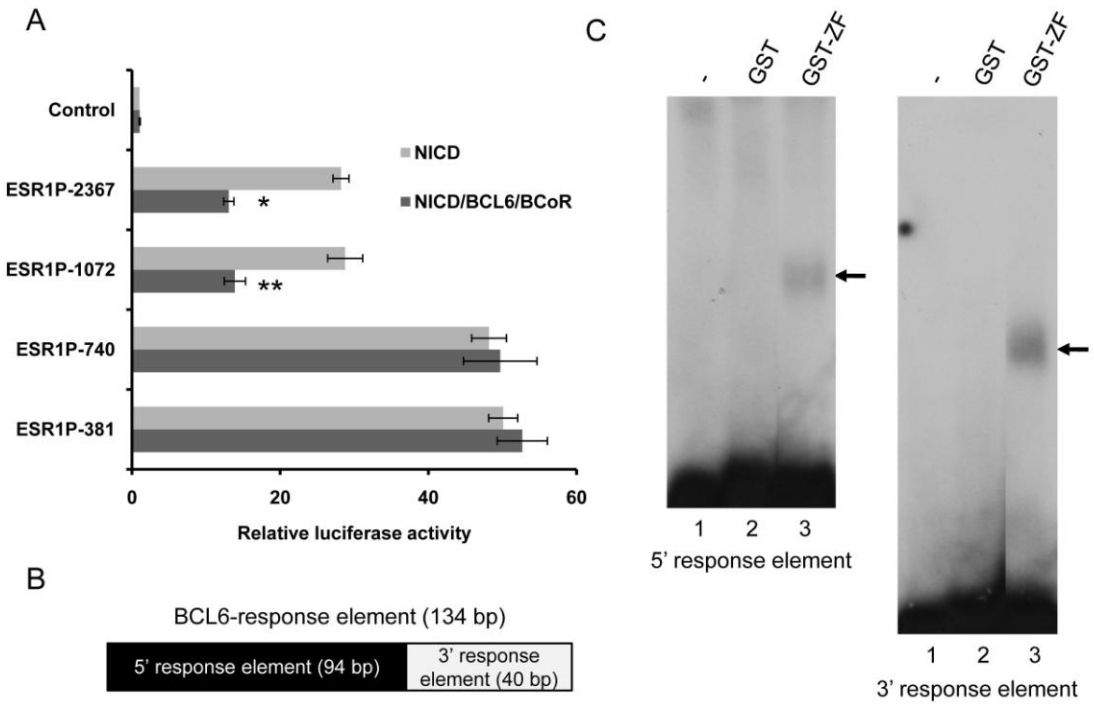


Figure S5. Multiple BCL6-binding sites are present in the genomic locus of *ESR1* gene. A: Relative luciferase activities were shown. 10 pg deleted *ESR1* genomic fragment construct linked to the luciferase reporter was injected with 50 pg *NICD*, 2 ng *BCL6* or/and 2 ng *BCoR* RNA and luciferase activities were measured in stage 10 embryos. 10 pg

pRL-CMV was co-injected and Renilla luciferase activity was used for the normalization of samples. * P<0.005, n=3, **P<0.005, n=3. B: Two non-overlapping fragments (5' response element and 3' response element) were designed in the BCL6-response element. C: Incubation of GST-ZF with 5' response element or 3' response element yielded one distinct retarded band indicated by an arrow.

2. Experimental Procedures

DNA Plasmid

cDNA fragment of ANK domain of *Xenopus* Notch1 (aa: 1869-2112) (Wettstein et al., 1997) was sub-cloned into pGEX2T vector (GE Healthcare). *X. laevis BCL6* (IMAGE ID: 5513995), *X. laevis MAM1* (IMAGE ID: 3200582), mouse *BCoR* (IMAGE ID: 6412868), *X. laevis ESR1* (IMAGE ID: 5537441) and *X. laevis Hairy2* (IMAGE ID: 3580827) were purchased from Open Biosystems. Truncated *BCL6* fragments as described in Figure 1 C were sub-cloned into pGEX2T vector and full length (aa: 1751-2524) and the ANK domain of *NICD* fragments was sub-cloned into pCS2+3Flag vector generated by in-house. The fragment of *mBCL6* was sub-cloned into pCS2+6myc vector and pCS2+6myc-GR vector, which contains the glucocorticoid receptor binding domain (GR). The M3 domain (NBD-S) of *BCL6* was sub-cloned into pCS2+6myc-nls (Turner and Weintraub, 1994) and pCS2+6myc-nls-GR vectors. Full length of *X. laevis MAM1* or a dominant negative form of *MAM1* (Kiyota and Kinoshita, 2002) was sub-cloned into pCS2+3Flag and pCS2+3Flag-GR vectors. *X. laevis Su(H)* was sub-cloned into pCS2+HA vector. Full length of *BCoR* was sub-cloned into pCS2+6myc and pCS2+6myc-GR vectors. *X. laevis ESR1* or *Hairy2* was sub-cloned into pCS2+6myc-GR vector and *X. laevis ESR1* was also sub-cloned into pCS2+2HA vector gifted from Dr. K. Tamai. The zinc finger domain (ZF) of *BCL6* was sub-cloned into pCS2+6myc-nls-GR vector. pCS2+GR-NICD (GR-NICD in text), pCS2+GR-Su(H)VP16 (GR-at-Su(H) in text) and pCS2+GR-Su(H)DBM (GR-dn-Su(H) in text) were described previously (Chitnis et al., 1995; Rones et al., 2000; Wettstein et al., 1997).

Morpholino Antisense Oligo

Morpholino Antisense Oligos (MO) were designed according to sequence of *X. laevis BCL6* (accession number: NM_001093558), *X. laevis Notch1* (accession number: M33874) and *X. laevis ESR1* (accession number: AF383157 and AB211546). Only a cDNA sequence for *BCL6* or *Notch1* was found by the database search. Sequences of BCL6 MO, Notch1 MO and ESR1 MO were followed, BCL MO: 5'-TTGAGTTTGAGATGCCATAGTGCCC -3', Notch1 MO: 5'-GCACAGCCAGCCCTATCCGATCCAT-3' and ESR1 MO: 5'-GCTGGTAGGAGCCATTATCCTAAGT-3'. The control Morpholino Oligo (Control MO) has the following sequences: 5'-CCTCTTACCTCAGTTACAATTTATA-3'.

Probes for Whole mount *in situ* hybridization

Probes for *Xnr1*, *Notch1*, *Delta1*, *Serrate1* and *N-tubulin* were described previously (Fukumoto et al., 2005; Kiyota et al., 2008; Kiyota and Kinoshita, 2002; Ohi and Wright, 2007). *Pitx2* clone (clone ID: XL085o07) for probe was gifted from the National Institute of Basic Biology (NIBB). *ESR1* and *Hairy2* clones for probes were purchased from Open Biosystems.

Primers for RT-PCR analysis

The primers for RT-PCR used in this work are followed. *Notch1*: 5'-gtacaagcccgtggcatatt-3'/5'-gatttgtcttcggcatggtt-3', *Delta1*: 5'-attctttttgctgggctgtg-3'/5'-tctggctttcggtcagttct-3', *Serrate1*: 5'-tccgactcaaaccgttcttt-3'/5'-tctccgtacatgcactgctc-3' *BCL6*: 5'-ttctgtactctggggcaagg-3'/5'-tgggatacacacgacaaagc-3', *BCoR*: 5'-tgcagtgctcaagatggaac-3'/5'-agtttccattgcttcgctgt-3', *Pitx2c*: 5'-tggctgggagtagagttgct-3'/5'-atcggtactgctgtcctcgt-3', *Xnr1*: 5'-tgtcgaaaatgggaaacctc-3'/5'-cgttcgggttggtacaactt-3', *ESR1*: 5'-atggttttgccacacaggtt-3'/5'-tggagcactgcgatcaatag-3', *Hairy2*: 5'-ttccgttctatgggaccctg-3'/5'-ctcctgcatgttcggaaagt-3' *ODC*: 5' acatggcattctccctgaag 3'/5' tggtcccaaggctaaagttg 3'. Note, primers for *Pitx2c* can amplify left-specific expressing

Pitx2 (Schweickert et al., 2000) but not other isoforms of *Pitx2*.

Primers for ChIP

The primers for ChIP were designed according to sequences of *X. laevis ESR1* promoter (accession number: DQ096795) and *X. laevis Hairy2* promoter (accession number: AY037926). ESR1/CSL-binding site (40 cycles): 5'-agagcagcatattgcagggaag-3'/5'-ggagagcatatgagctgtgg-3', Hairy2/CSL-binding site (36 cycles): 5'-tggtgcaaagcgctaatatg-3'/5'-tcgtgtgaaaccttcactgc-3'.

3. References

Chitnis, A., Henrique, D., Lewis, J., Ish-Horowicz, D., and Kintner, C. (1995). Primary neurogenesis in Xenopus embryos regulated by a homologue of the Drosophila neurogenic gene Delta. Nature *375*, 761-766.

Fukumoto, T., Kema, I.P., and Levin, M. (2005). Serotonin signaling is a very early step in patterning of the left-right axis in chick and frog embryos. Curr Biol *15*, 794-803.

Kiyota, T., Kato, A., Altmann, C.R., and Kato, Y. (2008). The POU homeobox protein Oct-1 regulates radial glia formation downstream of Notch signaling. Developmental biology *315*, 579-592.

Kiyota, T., and Kinoshita, T. (2002). Cysteine-rich region of X-Serrate-1 is required for activation of Notch signaling in Xenopus primary neurogenesis. The International journal of developmental biology *46*, 1057-1060.

Ohi, Y., and Wright, C.V. (2007). Anteriorward shifting of asymmetric Xnr1 expression and contralateral communication in left-right specification in Xenopus. Developmental biology *301*, 447-463.

Rones, M.S., McLaughlin, K.A., Raffin, M., and Mercola, M. (2000). Serrate and Notch specify cell fates in the heart field by suppressing cardiomyogenesis. Development (Cambridge, England) *127*, 3865-3876.

Schweickert, A., Campione, M., Steinbeisser, H., and Blum, M. (2000). Pitx2 isoforms: involvement of Pitx2c but not Pitx2a or Pitx2b in vertebrate left-right asymmetry.

Mechanisms of development *90*, 41-51.
Turner, D.L., and Weintraub, H. (1994). Expression of achaete-scute homolog 3 in Xenopus embryos converts ectodermal cells to a neural fate. Genes & development *8*, 1434-1447.
Wettstein, D.A., Turner, D.L., and Kintner, C. (1997). The Xenopus homolog of Drosophila Suppressor of Hairless mediates Notch signaling during primary neurogenesis. Development (Cambridge, England) *124*, 693-702.