Supplementary Table 1. A list of primers used to clone reptile genes.

Sequences of the primers that were used to obtain cDNA clone for reptile genes are listed together with the size of an amplified sequence. Amino acid sequence homology of each gene, analyzed by CLUSTALW, is also indicated.

| Gene          | forward primers | reverse primers | amplicon (bp) | vs mouse | vs zebrafish | vs Chinese softshell turtle | vs Red-eared slider turtle | vs Madagascar ground gecko | vs Chinese softshell turtle | vs Red-eared slider turtle |
|---------------|-----------------|-----------------|---------------|----------|--------------|-----------------------------|---------------------------|-----------------------------|-----------------------------|-----------------------------|
| Chinese softshell turtle |                 |                 |               |          |              |                             |                           |                             |                             |                             |
| PsNodal       | CTGGTCTCTCCTGTCGACATGAGC | CAGTTGCTTGAAGCTCTCATCCAC | 748           |          | 36.2245 (bird-type) | 49.2823                    |                           |                             |                             |                             |
| PsLefty       | AACAGTGAAATCGACATGAGC | TCTCAGGATGTGATAGTG | 491           |          | 34.1598 (Lefty2) | 59.6685 (Lefty2) | 76.8595                    |                           |                             |                             |
| PsPitx2       | CAGCGACACTCCAGAGAGCA | AACACTGGCGTATCCCAAGC | 787           |          | 94.3218 | 78.0565 | 77.918                     |                           |                             |                             |
| PsNot2        | GAGCTCGAGGCTGCTGTCTC | TGCCCCGAGCAGCTATGATAC | 467           |          | 25 | 39.7321 |              |                           |                             |                             |
| PsOtx5        | GGGTCTGAGCATGATGTCTC | CGGCTCAAACAGCAGTGAAC | 887           |          | 54.0107 | 78.0749 | NA                       | 68.7117 | 63.0996 (Kctd12.2) | 78.2209                    |
| PsKctd12      | TCTCCAGCTACATCGTGAC | CAGTCCGAGAAGACGTA | 712           |          | 68.7117 | 63.0996 (Kctd12.2) | 78.2209                    |                           |                             |                             |
| PsNodal       | CTGGTCTCTCCTGTCGACATGAGC | CAGTTGCTTGAAGCTCTCATCCAC | 748           |          | 36.2245 (bird-type) | 49.2823                    |                           |                             |                             |                             |
| Red-eared slider turtle |                 |                 |               |          |              |                             |                           |                             |                             |                             |
| TsNodal       | TCCTGCTATCGCCACTCTC | GGAGGACAACCTCCCCTTTC | 716           |          | 38.2653 (bird-type) | 74.6411                    |                           |                             |                             |                             |
| TsLefty       | GCCAGTGGATACCCAGAAGCA | TGGTTCCCTCCTGCAGTCT | 730           |          | 60.221 (Lefty2) | 95.5923                    |                           |                             |                             |                             |
| TsPitx2       | GTGACGATCTCCCTCAGAACGTT | GGGTCTGTCCCAAGCTATT | 723           |          | 94.0063 | NA | 78.9809 | 97.1609                    |                           |                             |
| Madagascar ground gecko |                 |                 |               |          |              |                             |                           |                             |                             |                             |
| PpNodal       | GGAGGACCCCTCAGGAGAAC | GGGTGGCTGAGAGGATCTCAGT | 468           |          | 34.4388 (bird-type) | 54.6729                    |                           |                             |                             |                             |
| PpLefty       | GGTGACTGAACTGNTTMACARCC | GYCTCGGATCACATGYCAGTNGTC | 503           |          | 31.7935 (Lefty2) | 56.6298 | 77.4105                     |                           |                             |                             |
| PpPitx2       | GGGCAGTATCCCTCCAGAGCAC | GCTGTGGCTTGGTCTGAGTC | 811           |          | 78.3407 | 78.8090 | 76.6562                    |                           |                             |                             |
| PpKctd12      | TCCTGCACTCCTGACGCTTCA | GCTGTGGCTGACATGTCGT | 710           |          | 65.7492 | 62.3616 (Kctd12.2) | NA                       |                           |                             |                             |
Supplementary Figure 1. A rostral-caudal series of Nissl stained sections of Chinese softshell turtle habenula at 38 dpo stage. Sections 1 and 65 correspond to the most rostral and caudal sections, respectively.
Supplementary Figure 2

| 38 dpo |
|------------------|
| **Nissl staining** | **PsKctd12** |

- **A**
- **B**
- **C**
- **B'**
- **D**
- **E**
- **F**

**Legend:**
- Dorsal
- Right
- Left
- Ventral

**Notes:**
- MH: Medial Horizontal
- LH: Latero Horizontal

**Arrows:**
- ▲ ▲ ▲ ▲ ▲
Supplementary Figure 2. Morphological and molecular L-R asymmetry of the habenula in Chinese softshell turtle at 38dpo. Nissl-stained sections (A-C, B’) of the habenula and Kctd12 expression (D-F) are shown. Dotted lines in (B’) indicate subdivision of the habenula shown in (B). MH and LH denote the medial habenula and lateral habenula, respectively. Frontal sectional levels in (D) to (F) are similar to those in (A) to (C), respectively, and are also similar to those in (G) to (I) of Figure 2, respectively. Regions positive for Kctd12 expression on the right and left sides are indicated by white and black arrowheads, respectively.
Supplementary Figure 3

Figure 3K

Figure 3L

Figure 3M
Supplementary Figure 3. A rostral-caudal series of Nissl stained sections of the gecko habenula at 50-52 dpo stage. Sections 1 and 70 correspond to the most rostral and caudal sections, respectively.
Supplementary Figure 4. A rostral-caudal series of Nissl stained sections of the habenula from a 5 month old gecko. Sections 1 and 47 correspond to the most rostral and caudal sections, respectively.