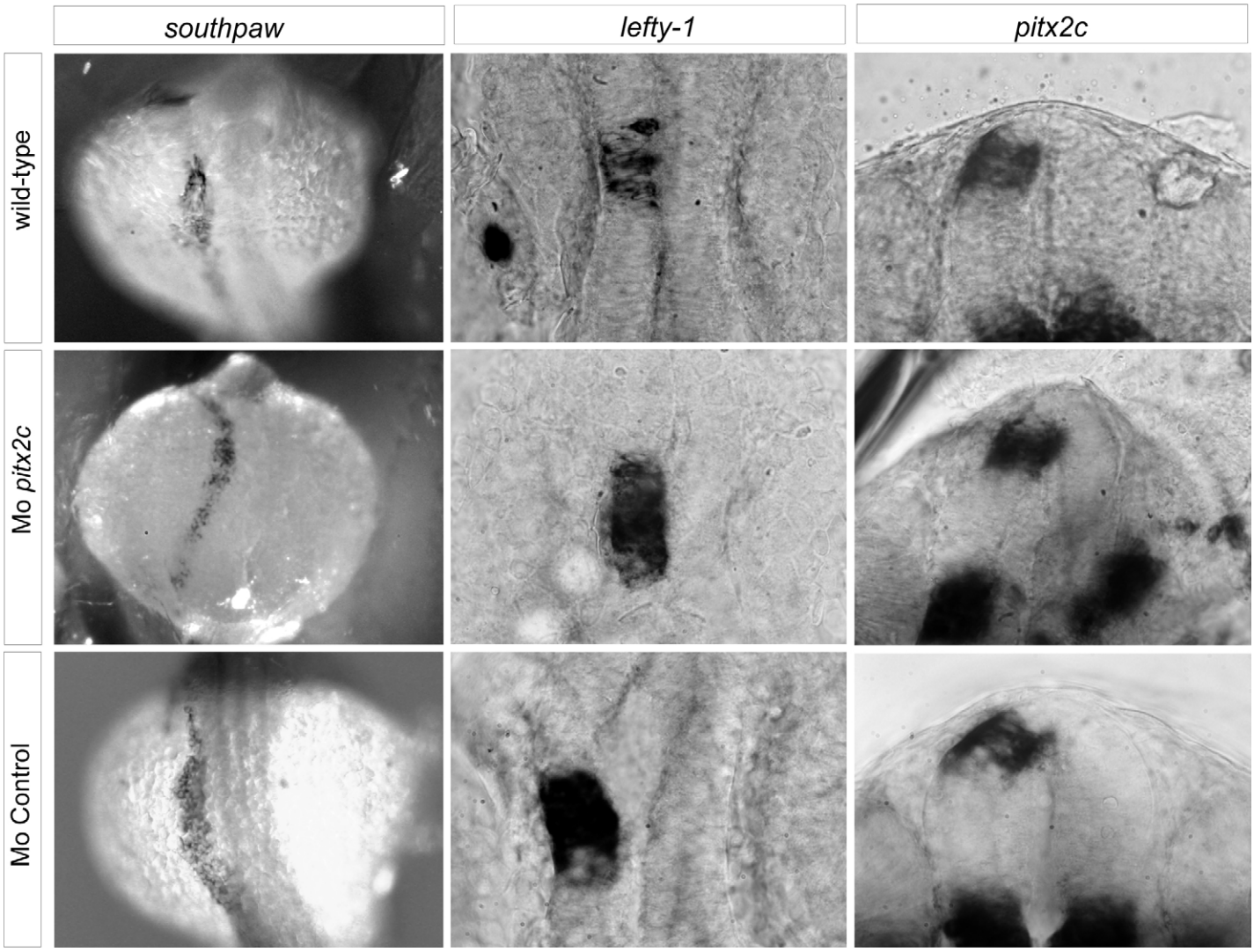
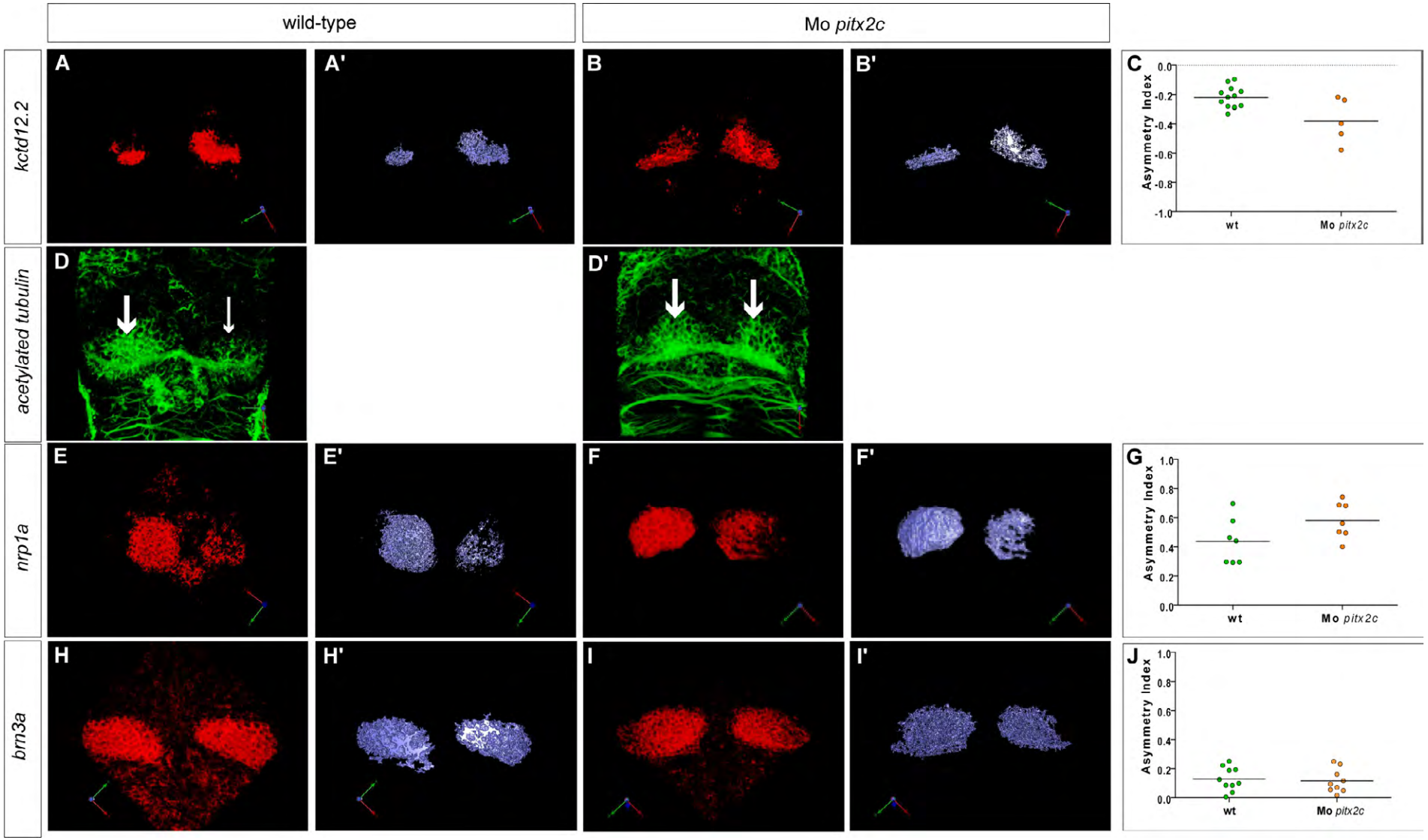


Supplementary Figure 1.

No changes are detected in the expression of the Nodal family gene *southpaw* in the left lateral plate mesoderm at 18 hpf, or in the expression of *pitx2c* and the Nodal feedback inhibitor *lefty1* in the left dorsal diencephalons at 24 hpf in *pitx2c* morphants relative to wild-type and control morphants suggesting that the establishment of L/R asymmetry is not generally affected.


wild-type
Mo pitx2c
kctd12.2
acetylated tubulin
nrp1a
brn3a
A
A'
B
B'
C
D
D'
E
E'
F
F'
G
H
H'
I
I'
J
Asymmetry Index
0.0
-0.2
-0.4
-0.6
-0.8
-1.0
wt
Mo pitx2c
1.0
0.8
0.6
0.4
0.2
0.0

Supplementary Figure 2.

(A, B) Single confocal sections of the epithalamus showing *in situ* hybridisation against *kctd12.2* in wild-type and *pitx2c* morphant embryos at 72 hpf.

(A', B') 3D renderings of volumes of *kctd12.2* expression in the habenulae of the respective confocal acquisition sets.

(C) Asymmetry index (AI) with regard to the volume of *kctd12.2* expression for individual wild-type or *pitx2c* morphant embryos. Horizontal black line represents the median AI for each context: note that the AI is negative because the asymmetry is biased to the right. No significant difference between wild-type or *pitx2c* morphant embryos is detected.

(D, D') Single confocal sections through the epithalamus showing the habenular neuropil detected by immunostaining against acetylated tubulin at 72 hpf. While the neuropil of wild-type embryos is asymmetric with a left bias (large versus small arrows), symmetric anti-acetylated tubulin staining is detected in *pitx2c* morphant embryos (two large arrows).

(E, F) Single confocal sections of the epithalamus showing *in situ* hybridisation against *nrp1a* in wild-type and *pitx2c* morphant embryos at 72 hpf.

(E', F') 3D renderings of volumes of *nrp1a* expression in the habenulae of the respective confocal acquisition sets.

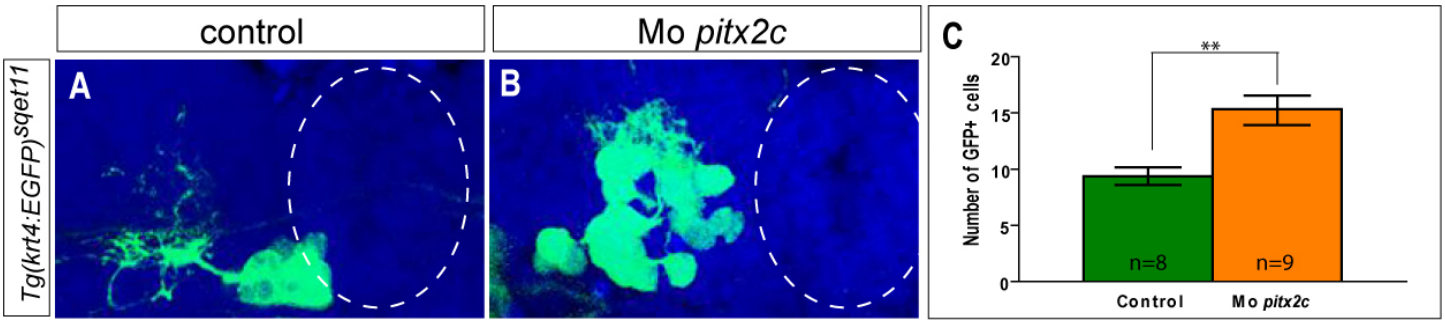
(G) Asymmetry index with regard to the volume of *nrp1a* expression for individual wild-type or *pitx2c* morphant embryos. Horizontal black line represents the median AI for each context . No significant difference between wild-type or *pitx2c* morphant embryos is detected.

(H, I) Single confocal sections of the epithalamus showing *in situ* hybridisation against *brn3a* in wild-type and *pitx2c* morphant embryos at 72 hpf.

(H', I') 3D renderings of volumes of *brn3a* expression in the habenulae of the respective confocal acquisition sets.

(J) Asymmetry index with regard to the volume of *brn3a* expression for individual wild-type or *pitx2c* morphant embryos. Horizontal black line represents the median AI, which are in both contexts close to zero reflecting that they are virtually symmetric. No significant difference between wild-type or *pitx2c* morphant embryos is detected.

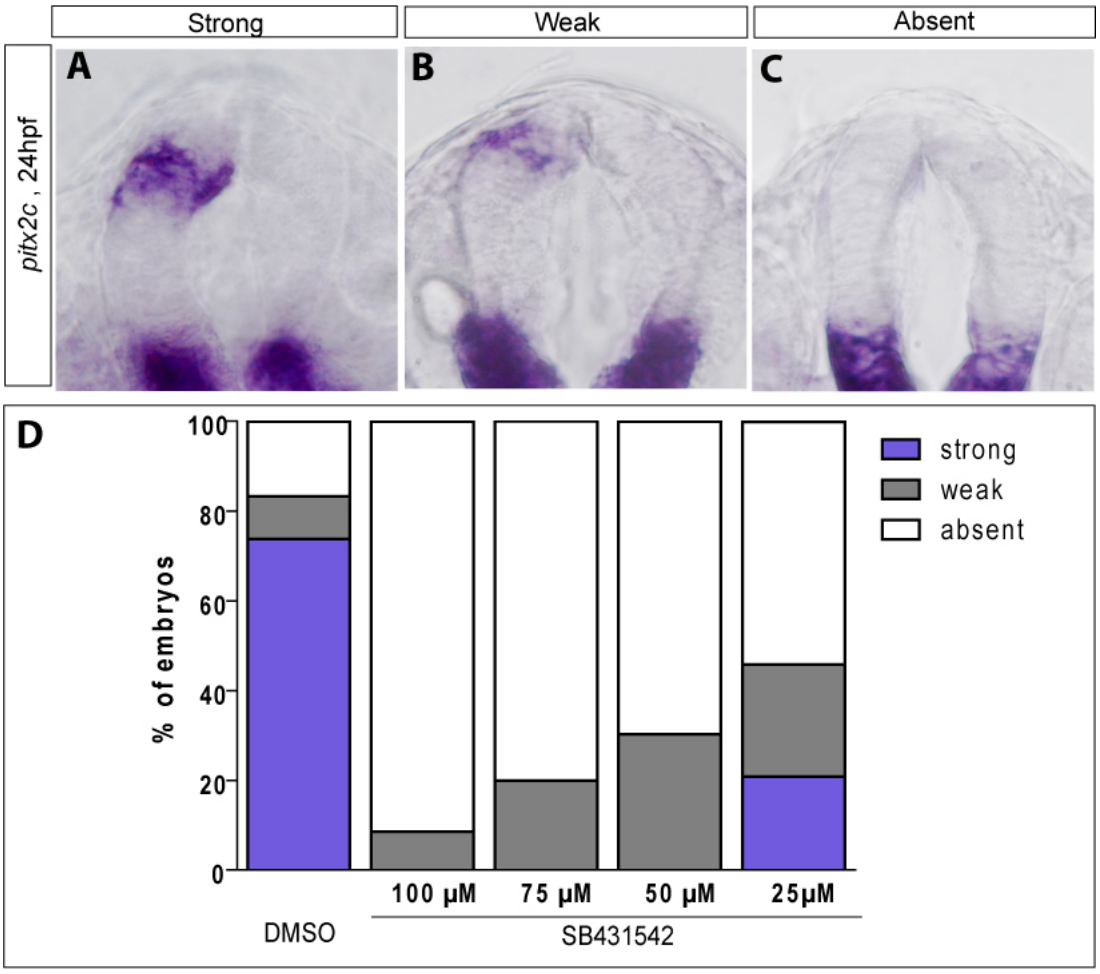
Embryos are view dorsally with the anterior to the top.



Supplementary Figure 3.

(A,B) Confocal projections showing the expression of GFP from the *Et(krt4:EGFP)*^{sqet11} transgene in control and *pitx2c* morphant embryos at 72 hpf. The outline of the pineal gland is highlight with a dotted line.

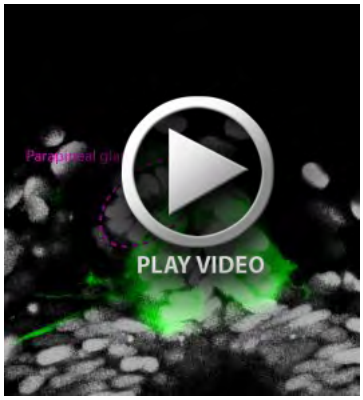
(C) Counts of cell expressing GFP in control and *pitx2c* morphants. The number of GFP-positive cells is significantly increased in the morphant context; **P<0.01 using a t-test. Error bars represent s.d.



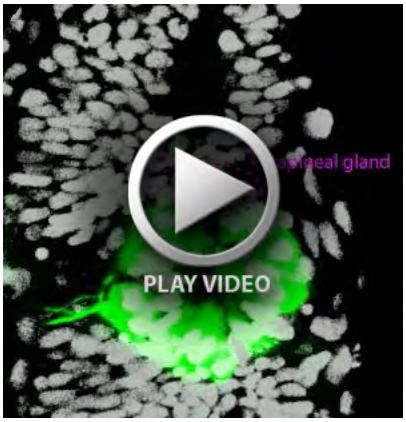
Supplementary Figure 4.

(A-C) Frontal view of the epithalamus showing *in situ* hybridisation against *pitx2c* at 24 hpf. Expression can be qualified as Strong (A), Weak (B) or Absent (C).

(D) Quantification of *pitx2c* expression classes presented in (A-C) in mock-treated and embryos treated with varying concentrations of SB431542 from 16 to 72 hpf .



Movie 1.



Movie 2.

Movie 3.

Supplementary Movies 1 (wild-type), 2 (Mo *pitx2c*) and 3 (Mo *pitx2c*).

Time-lapse movies showing the migrating parapineal in wild-type and a *pitx2c* morphant embryo carrying the *Tg(foxd3:GFP)*^{zf15} transgene; nuclei are labelled with Histone2B:RFP and false coloured in grey. The movies were generated from a projection of 3 Z-sections at each time point. While in the wild-type embryo one cell division is noted during the movie, in the Mo *pitx2c* embryos 4 or 6 divisions can be seen, respectively; divisions are highlighted with purple arrows that appear to split at the time of division. Films were initiated between 24-26 hpf and the initial frame is annotated to indicate the parapineal (dotted line).