Figure. S1. Sox10 expression on the dorsal region of Xenopus embryos
Xenopus embryos were fertilised and allowed to develop to the indicated developmental stage before fixing and staining by in situ hybridisation for neural crest marker Sox10. Embryos are orientated with the dorsal side imaged and head to the top.

Figure. S2. Comparison of Ascl1 serine-proline sites across species
Ascl1 protein is illustrated schematically across species with serine-proline pairs indicated in green. Ascl1 phosphomutant forms were generated by performing site-directed mutagenesis changing all indicated serine-proline pairs to alanine-proline.

## Figure. S3. Scoring standards for AVNA marker expression comparing the injected and uninjected sides

In situ hybridisation scoring standards for each marker are shown above. Each embryo from Figures $1,3,6 \& 7$ were assigned a numeric score based as indicated above.

Table S1. Primers used in qPCR analysis of gene expression.
Primers were designed for qPCR of Ascl1 in neuroblastoma cell lines. Primers were designed to have a Tm between $57^{\circ} \mathrm{C}$ and $60^{\circ} \mathrm{C}$. HPRT1 and GAPDH were used as 'house-keeping' controls.


Figure S1

S2
Ascl1 (X. laevis) 199 aa.


Ascl1 (mouse) 231 aa.


Ascl1 (human) 236 aa.

ser-pro phosphorylation motif
basic domain
©
bHLH domainregions with >95\% homology

Figure S2


Figure S3

| Gene | Forward/Reverse | Sequence |
| :---: | :---: | :--- |
| hASCL1 | Forward | CATCTCCCCCAACTACTCCA |
| hASCL1 | Reverse | AACGCCACTGACAAGAAAGC |
| HPRT1 | Forward | TGGCGTCGTGATTAGTGATG |
| HPRT1 | Reverse | ATCCAGCAGGTCAGCAAAG |
| GAPDH | Forward | GAAGGTGAAGGTCGGAGTC |
| GAPDH | Reverse | TGGAAGATGGTGATGGGATT |

## Table S1

