

A Notch-regulated proliferative stem cell zone in the developing spinal cord is an ancestral vertebrate trait.

Supplementary information

Figure S1

Alignment of PCNA sequences from three lamprey species (Pm, *Petromyzon marinus*; Lj, *Lethenteron japonicum*; Lp, *Lampetra planeri*) with PCNA sequences from a variety of invertebrates and vertebrates. A molecular phylogenetic analysis of these sequences is shown in Figure S2.

Homo_sapiens_PC	MFEGRLVQGS	ILKKVLEALK	DLINEACWDI	SSSGVNLQSM	DSSHVSLVQL	TLRSEGFDTY	RCDRNLMAGV	NLTSMSKILK	CAGNEDIITL
Danio rerio_PCN	MFEARLVQGS	ILKKVLEALK	DLITEACWDI	SSSGVNLQSM	DSSHVSLVQL	TLRSEGFDTY	RCDRNLMAGV	NLTSMSKILK	CAGNEDIQTL
Xenopus_laevis_P	MFEARLVQGS	ILKKVLEALK	DLINEACWDI	TSSGISLQSM	DSSHVSLVQL	TLRSEGFDTY	RCDRNQSTIGV	KMSSMSKILK	CAASDDIITL
Mus_musculus_PC	MFEARLIQGS	ILKKVLEALK	DLINEACWDV	SSGGVNLQSM	DSSHVSLVQL	TLRSEGFDTY	RCDRNLMAGV	NLTSMSKILK	CAGNEDIITL
Branchiostoma_f	MFEARLVQGS	ILKKVLEALK	DLIETASWDC	SSTGMSLQAM	DSSHVSLVQL	EMRKDGFDTY	RCDRNMANGV	TIASMTKLLK	CAGNDMVTL
Ciona_intestina	MFEARLVQGS	ILKKVLEALK	DIVNEASWDC	TSSGMSLQAM	DSSHVSLVQL	TLRADGFEKY	RCDRNLMAGI	NMITSMSKILR	CAGNEDVITL
Drosophila_mela	MFEARLGQAT	ILKKILDAIK	DLINNEATFDC	SDSGIQLQAM	DNSHVSLSVL	TLRSDFDKP	RCDRNLSMM	NLGSMAKILN	CANNEDNVITM
Gallus_gallus_P	MFEARLVQGS	VLKRVLEALK	DLITEACWDL	GSGGISLQSM	DSSHVSLVQL	TLRSEGFDTY	RCDRNIAAMGV	NLNSMSKILK	CAGNEDIITL
Styela_clava_P	MFEARLIQGS	NLKKVQEALK	DIVTEASWDC	TSSGISLQAM	DSSHVSLVQL	TLRADGFENF	RCDRNLMAGI	NMITSMAKIM	CAGNNDIITL
Anguilla_japoni	MFEARLVQGS	ILKKVLEALK	DLINEACWDV	SSSGISLQSM	DSSHVSLVQL	TLRSDGFDSY	RCDRNLAJGV	SLNSMSKMLK	CAGNEDIITL
Dicentrarchus_l	MFEARLVQGS	ILKKVIAASK	DLLNEATWDC	SSTGMSLQAM	DSSHVSLVSL	TLRSDGFDSY	RCDRNLMAGV	NLSSMSKILK	CAGNEDIITL
Platynereis_dum	MFEARLVQGS	ILKKVIAASK	DLLNEATWDC	SSTGMSLQAM	DSSHVSLVSL	TLRSDGFDTY	RCDRNISMGI	KLASMAKILQ	CAGNDAITL
LjPCNA	MFEARILQGN	ILKKVLEALK	DLITEACWDI	SSTGVSLQSM	DSSHVSLVQ-	TMRSDFDTY	RCDRNLMAGI	NLTSMSKILK	CAGNDDMITL
PmPCNA	MFEARILQGN	ILKKVLEALK	DLITEACWDI	SSTGVSLQSM	DSSHVSLVQ-	TMRSDFDTY	RCDRNLMAGI	NLTSMSKILK	CAGNDDMITL
LpPCNA	MFEARILQGN	ILKKVLEALK	DLITEACWDI	SSTGVSLQSM	DSSHVSLVQ-	TMRSDFDTY	RCDRNLMAGI	NLTSMSKILK	CAGNDDMITL
Homo_sapiens_PC	RAED-NADTL	ALVFEEPANQNE	KVSDYEMKLM	DLDVEQLGIP	EQEYSCVVKM	PSGEFARICR	DLSHIGDAVV	ISCAKDGVKF	SASGELGNGN
Danio rerio_PCN	RAED-NADTL	ALVFEEAQNQE	KVSDYEMKLM	DLDVEQLGIP	EQEYSCVVKM	PSGEFARICR	DLSQIGDAVM	ISCAKDGVKF	SASGELGTGN
Xenopus_laevis_P	RAED-NADTV	TMVFESPNQE	KVSDYEMKLM	DLDVEQLGIP	EQEYSCVVKM	PSGEFARICR	DLSQIGDAVM	ISCAKDGVKF	SASGELGTGN
Mus_musculus_PC	RAED-NADTL	ALVFEEPANQNE	KVSDYEMKLM	DLDVEQLGIP	EQEYSCVVKM	PSGEFARICR	DLSHIGDAVV	ISCAKNGVFK	SASGELGNGN
Branchiostoma_f	RAED-NADSM	TLIFEFESPQNE	KVSDYEMKLM	DLDVEQLGIP	DQEYSCVVKM	PSGEFARICR	DLSQIGEAVA	ISCTKDGKPF	SASGDLGTTN
Ciona_intestina	RADD-TADIL	EMVFESPQKGE	KISNYDMKLM	DIDCEQLGIP	DQEYSCTIKM	PSHEFARICR	DLSQIGEAVI	ITCTKDGQVF	SAKGDLGSGT
Drosophila_mela	KAQD-NADTV	TIMFESANQNE	KVSDYEMKLM	NLDQEHGLIP	ETDFSCVVRM	PAMEFARICR	DLAQFSESVV	ICCTKEGVKF	SASGDLVTAN
Gallus_gallus_P	RAED-NADTL	ALVFEEPANQNE	KVSDYEMKLM	DLDVEQLGIP	EQEYSCVVKM	PSAEFARICR	DLSHIGDAVV	ISCAKDGVKF	SANGEGLGNGN
Styela_clava_P	RAED-NADM1	ELIFESSKGD	KYSQYEMKLM	DLDCEQLGIP	EQDYSCCVTL	PSQEFGRICR	DLSQIGECVV	ITCTKDGQVF	SAKGDLGACK
Anguilla_japoni	RAED-NADTL	ALVFETLNQE	KVSDYEMKLM	DLDVEQLGIP	EQEYSCVVKM	PSGEFARICR	DLSQIGDAVM	ISCAKDGVMF	SASGELGTGN
Dicentrarchus_l	RAED-NADTL	ALVFETLNQE	KVSDYEMKLM	DLDVEQLGIP	EQEYSCVVKM	PSGEFARICR	DLSQIGDAVM	ISCAKDGVKF	SATGELGTGN
Platynereis_dum	KARD-DADTV	TFMFESQNQD	RVDYEMKLM	DLDTEHGLIP	ETDYDCVIMK	PAAEFORICR	DLSQIGESVV	ICCTKEGVKF	SASGDLGTTN
LjPCNA	RAED-NADIL	TLVFEAQNHE	KVSDYEMKLM	DLDVEQLGIP	EQDYSAVVKM	PSGEFSRICR	DLSQIGDSVV	ICCTKDGQVF	SASGELGTGN
PmPCNA	RAED-NADIL	TLDAS--NHE	KVSDYEMKLM	DLDVEQLGIP	EQDYSAVVKM	PSGEFSRICR	DLSQIGDSVV	ICCTKDGQVF	SASGELGTGN
LpPCNA	RAED-NADIL	TLVFEAQNHE	KVSDYEMKLM	DLDVEQLGIP	EQDYSAVVKM	PSGEFSRICR	DLSQIGDSVV	ICCTKDGQVF	SASGELGTGN
Homo_sapiens_PC	IKLSQTSNVD	-KEEEEAVTIE	MNEPVQLTFA	LRYLNFFTAK	TPLSSTVTLMS	MSADVPLVVE	YKIADMGHLK	YNLAPKIEDE	-EGS-
Danio rerio_PCN	IKLSQTSNVD	-KEDEAVTIE	MNEPVQLIFA	LNYLNFFTAK	TPLSRTVTLR	MSAHIPLVVE	DKIADLEHV	YYLAPQIEDE	--ESS
Xenopus_laevis_P	VKLSQTSNVD	-KEEEEAVTIE	MNEPVQLTFA	LRYLNFFTAK	TPLSPTVILS	MSADIPLVVE	YKIADMGHVK	YYLAPKIEDE	-EAS-
Mus_musculus_PC	IKLSQTSNVD	-KEEEEAVTIE	MNEPVHLTFA	LRYLNFFTAK	TPLSPTVTLS	MSADPVLVVE	YKIADMGHLK	YYLAPKIEDE	-EAS-
Branchiostoma_f	IKLAQSANVD	-KEEEEAVTIE	MNEPVSLTFA	LRYLNFFTAK	TPLSPTVTLS	MSADPVLVVE	YKIADMGHVK	FFLAPKIEEE	-DATA-
Ciona_intestina	IKLSQNSAAE	-KEDDQVTIE	MTEPVQLTFA	IKYLNLFKTA	TPLSGAVCLSL	MSNDIPLVVE	YKIEDMGHVK	YFLAPKIEDE	EEAE-
Drosophila_mela	IKLAQTGSVD	-KEEEEAVII	MQEWPVLTFA	CRYLNAFTKA	TPLSTQVQLS	MCADPVLVVE	YAIKDLGHIR	YYLAPKIEDN	--ET-
Gallus_gallus_P	IKLSQTSNVD	-KEEEEAVTIE	MNEPVQLTFA	LRYLNFFTAK	TPLSPPTVTLMS	MSADVPLVVE	YKIADMGHLK	YYLAPKIEDQ	QEGS-
Styela_clava_P	IKLKQNTGSD	IKEEEQVTE	ISEPVQLTFA	IKYLNLFAKA	SPLSPVCLSL	MSNNVPVLL	YKVADMGHIK	YFLAPKIEDE	EEQDS-
Anguilla_japoni	VKLSQTSNVD	-EEDDAVTIE	MNEPVQLIFA	LNYLNFFTAK	TPLSKTVTLS	MSADIPLVVE	YKIADMGHVK	YYLAPKIDE	--AS-
Dicentrarchus_l	VKLSQTSNVD	-KEDEAVTIE	MNEPVQLIFA	LNYLNFFTAK	TPLSKTVTLS	MSADIPLVVE	YKIADMGHVK	YYLAPKIDE	--AS-
Platynereis_dum	IKLAQTANVD	-KEEEEAVVIE	MNEPCPTLTF	LQYLNFFTAK	TPLASRVTLMS	MSGSPVLLVVE	YKIGDMGYIR	YYLAPKIEDG	DDAS-
LjPCNA	IKLSQTSGAE	-KEEEEAVTIE	MSEAVQLTFA	LRYLNFFTAK	TPLSPPTVTLR	MSADVPLVVE	YGIADMGHIK	YYLAPKIEDE	-EAA-
PmPCNA	IKLSQTSGAD	-KEEEEAVTIE	MSEAVQLTFA	LRYLNFFTAK	TPLSPPTVTLR	MSADVPLVVE	YGIADMGHIK	YYLAPKIEDE	-EAA-
LpPCNA	IKLSQTSGAE	-KEEEEAVTIE	MSEAVQLTFA	LRYLNFFTAK	TPLSPPTVTLR	MSADVPLVVE	YGIADMGHIK	YYLAPKIEDE	-EAA-

Accession numbers:

CAG46598.1 [*Homo sapiens*]. NP_571479.1 [*Danio rerio*]. NP_001081011.1 [*Xenopus laevis*]. NP_035175.1 [*Mus musculus*] EEN44891.1 [*Branchiostoma floridae*]. XP_002131861.1 [*Ciona intestinalis*]. P17917.2 [*Drosophila melanogaster*]. Q9DEA3.1 [*Gallus gallus*]. P53358.1 [*Styela clava*]. Q9W644.1 [*Anguilla japonica*]. AGA54130.1 [*Dicentrarchus labrax*]. CCV20094.1 [*Platynereis dumerili*]. The *P. marinus* sequence is in part derived from PMZ_0002029-RA from the germ line genome assembly (<https://simrbase.stowers.org/>).

Figure S2

A Bayesian molecular phylogenetic analysis of PCNA sequences based on the alignment shown in Figure S1. Numbers adjacent to nodes are posterior probabilities. The tree has been rooted with the two protostome species. Accession numbers are shown in Figure S1.

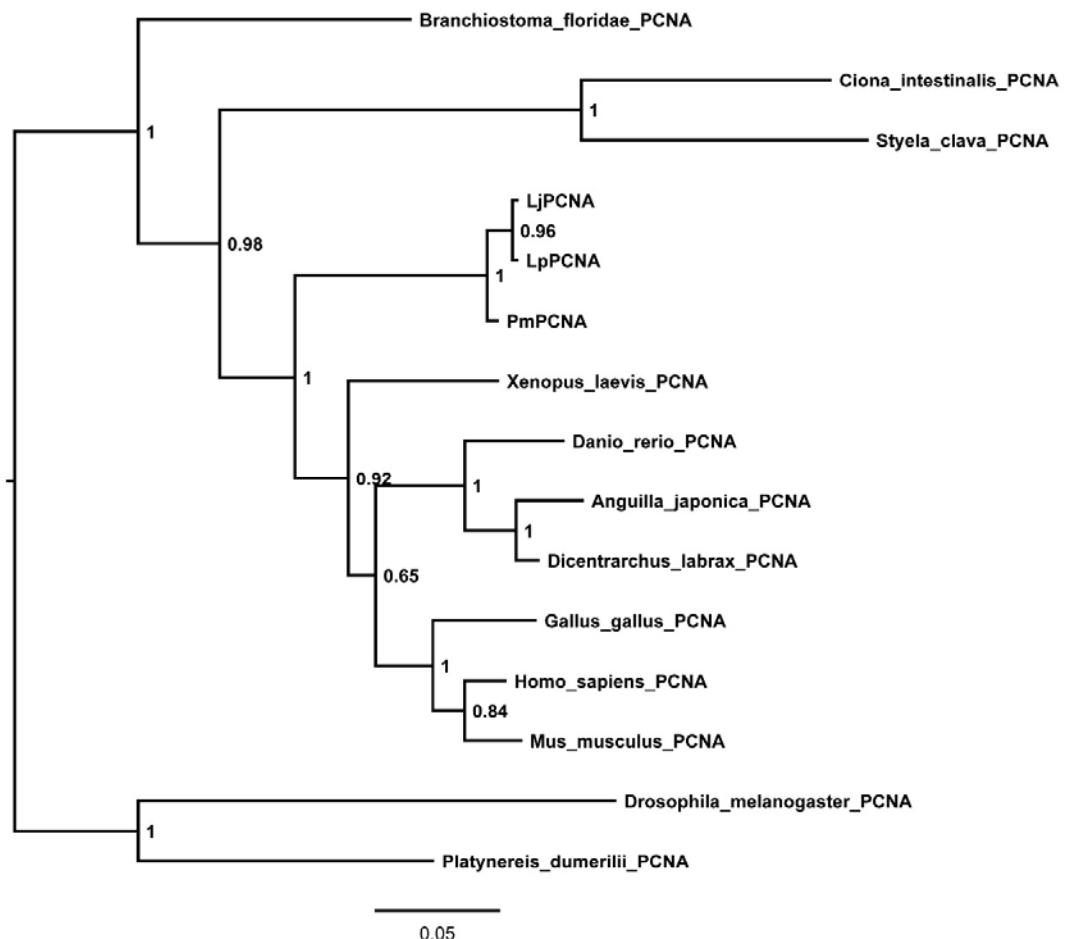


Figure S4

Bayesian molecular phylogenetic analysis of Msi and related genes. The lamprey sequences group together and with jawed vertebrate Msi1 and Msi2, and appear more closely related to Msi2. Numbers next to nodes are posterior probabilities. Human (Hs) TARDBP has been used as an outgroup, and additional RNA binding protein families with homology to Msi are included: For further details on the evolutionary history of these gene families see Gasparini et al (2011) J. Exp. Zool. B. 316B: 562-573. Lamprey species are Lp, *Lampetra planeri*; Pm, *Petromyzon marinus*; Lj, *Lethenteron japonicum*. Other species are Mm, *Mus musculus*; Dr, *Danio rerio*; Xl, *Xenopus laevis*; Ci, *Ciona intestinalis*; Bl, *Branchiostoma lanceolatum*.

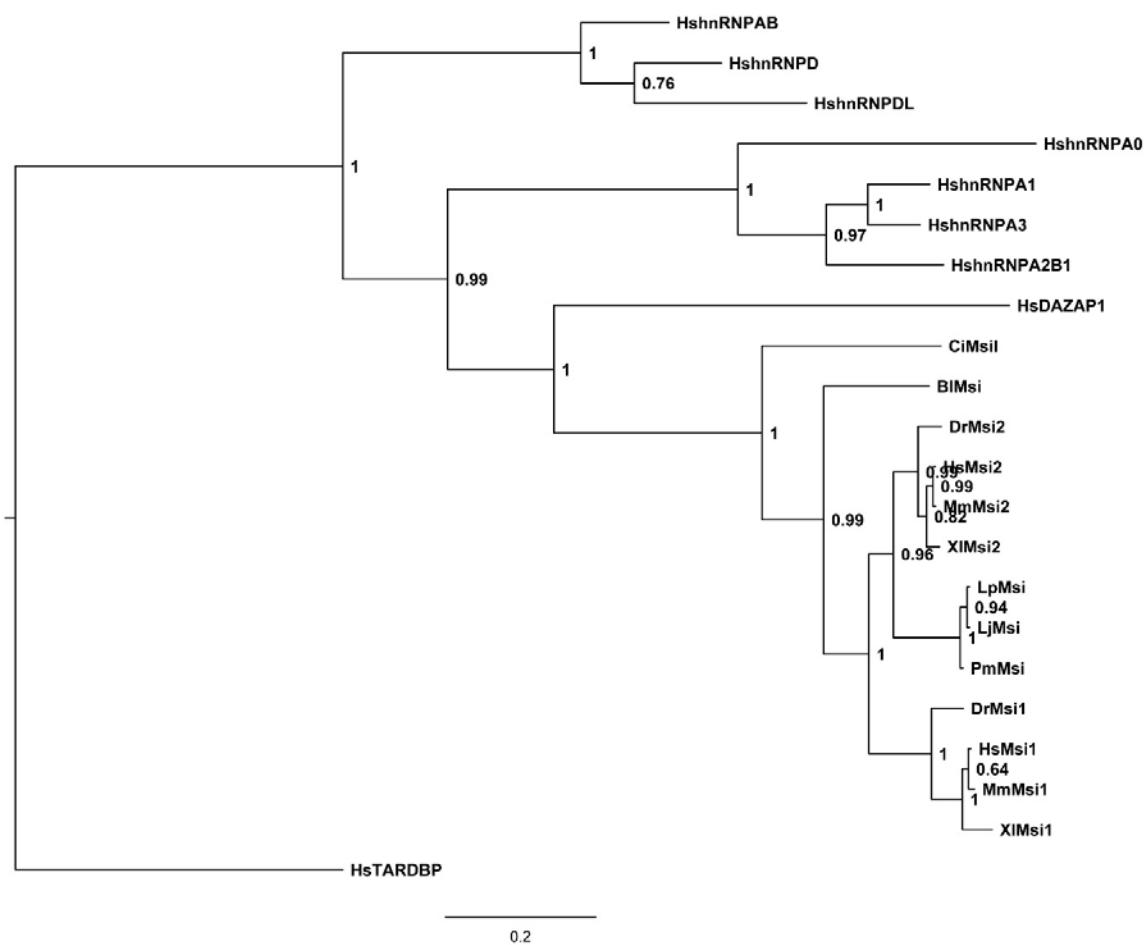


Figure S5. Expression of *Msi2* during *L. planeri* development

(A, B) At stage 22, *LpMsi2* expression is observed in the brain and in the forming pharyngeal region, but weak in the spinal cord. (C) At stage 23, expression extends into the elongating spinal cord. In the pharynx, expression is strong in the mandibular arch. (D) At stage 24, strong expression is maintained in the entire neural tube and pharyngeal region. (E) At stage 25, expression is essentially unchanged with respect to stage 24. (F) At stage 27, strong expression remains in the neural tube but is slightly reduced in the pharyngeal basket, with stronger, distinct expression observed in the endostyle. (G) A dorsal view of a stage 24 embryo shows neural tube expression to be medial (arrow). Abbreviations: e, endostyle; n, notochord; p, pharynx; sc, spinal cord.

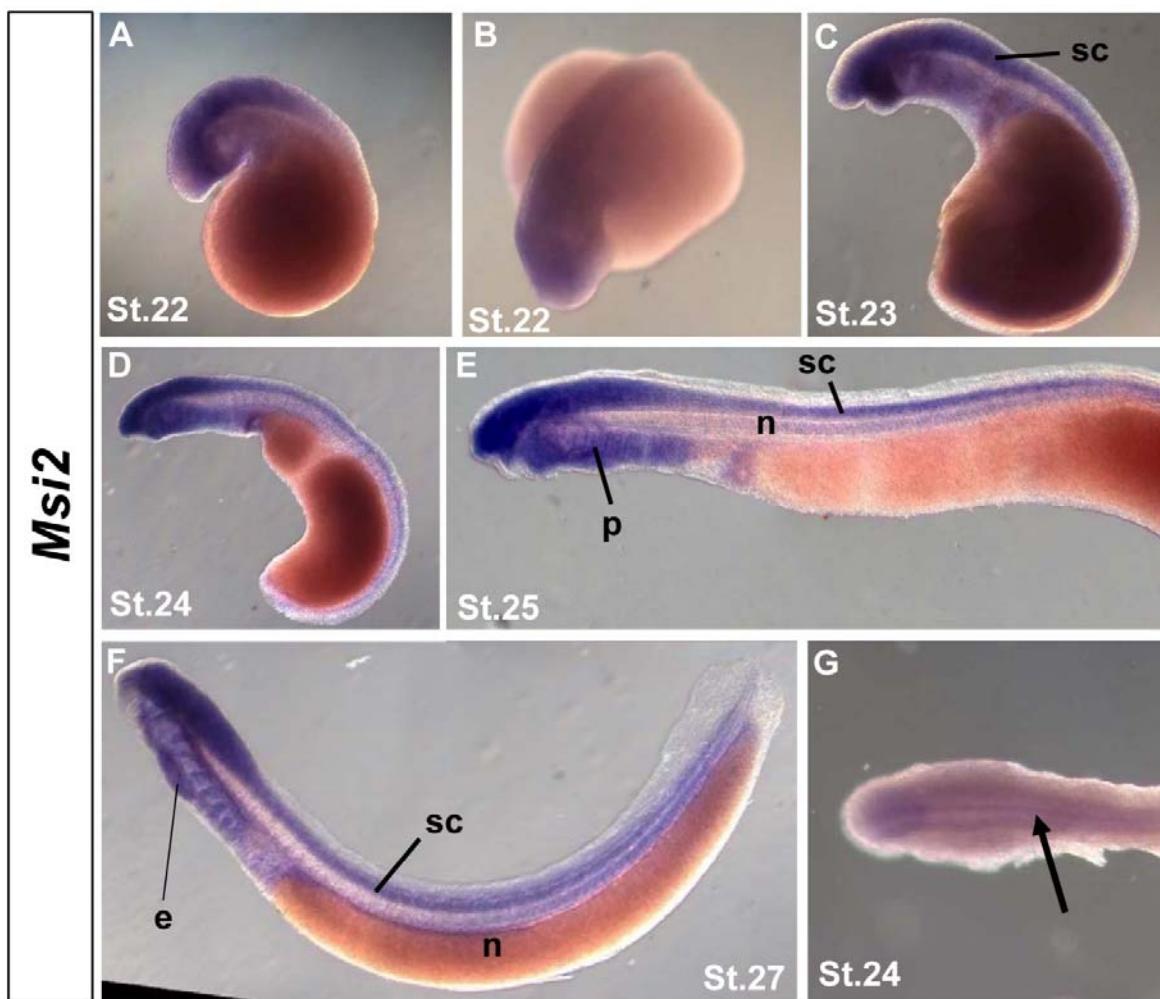


Figure S6

Phosphohistone 3 (pH3) staining in lamprey embryos. The top panels show representative sections through the spinal cord regions of DMSO-control and DAPT-treated embryos, stained for DAPI and pH3. The scale bar is 50 μ m. Underneath this are graphs showing the proportion of neural tube cells that are pH3 positive, as assessed by comparing the number of pH3 labelled cells to the total number of neural cells, visualised by DAPI labelling. Forebrain/midbrain, hindbrain and spinal cord were analysed separately. Three pairs of embryos were sectioned and the number of sections scored ranged from 14-19 for forebrain/midbrain, 20-36 for hindbrain and 69-93 for spinal cord. The average proportion of pH3⁺ cells in each region was then calculated, and experimental and control embryos compared using a t-test.

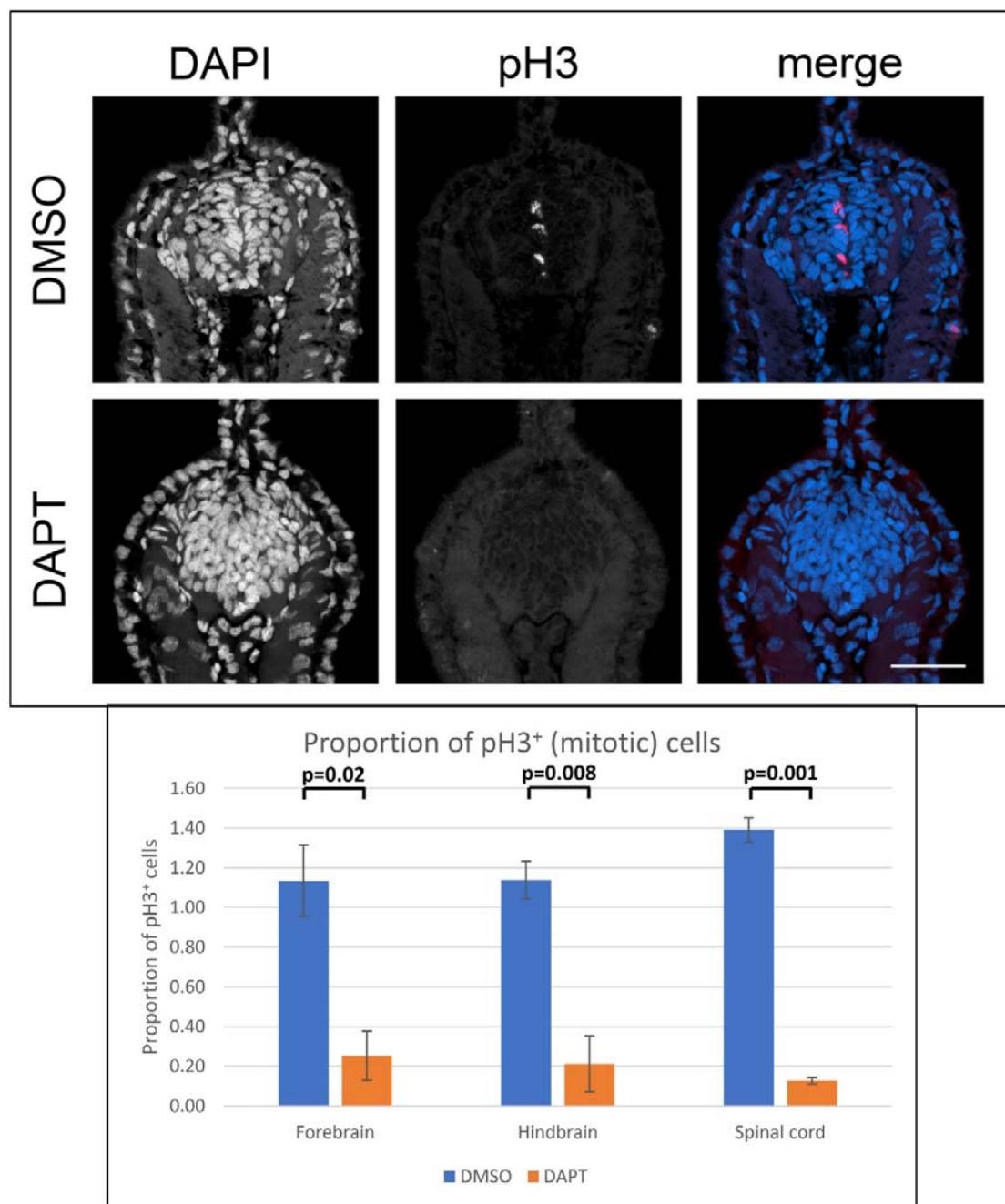
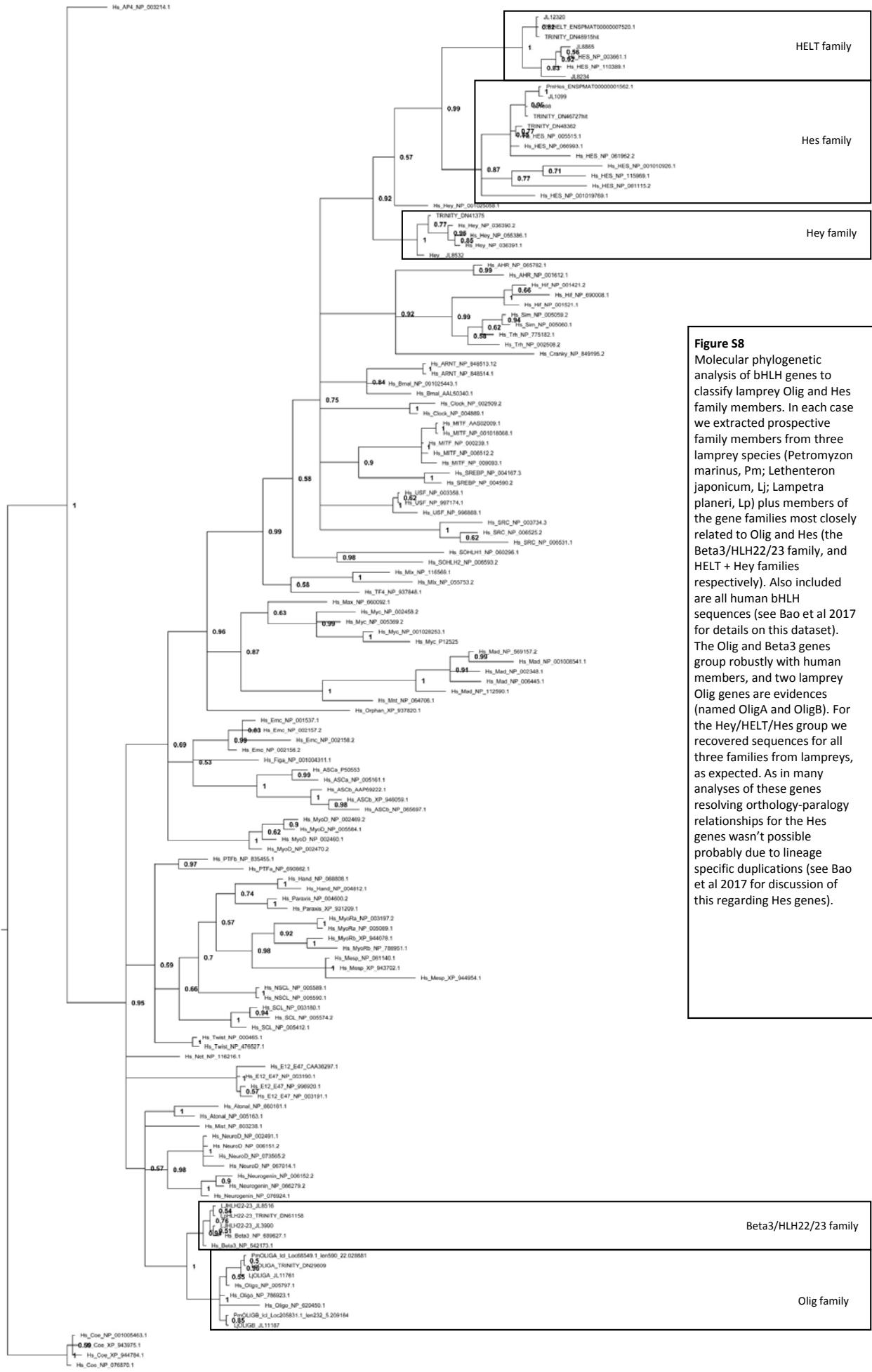


Figure S7

Alignment of lamprey Olig and Hes with a representative set of Human HLH sequences. Human sequences are as used by Bao et al (2017) Genome Biol. Evol. 9(4):869–886. doi:10.1093/gbe/evx047 and for consideration of which sequences and accession numbers, see this reference plus Figure S8 below and its legend. For lamprey genes, species abbreviations as Figures S1-4, with the following additions: sequences prefixed JL are from the Japanese lamprey genome assembly (<http://jlampreygenome.imcb.a-star.edu.sg/>), while those prefixed TRINITY are from a transcriptome assembly for *Lampretra planeri* detailed in Lara-Ramirez et al 2017 Dev Genes Evol 227, 319.

	10 20 30 40 50 60
PmOLIGB_1c	EHDLQLSLRLK IN-SRERKRM HDLNLAMDGL REVMP----- YAHGPSVRK LSKIATLLLA -----
LjOLIGB_JL	EHDLQLSLRLK IN-SRERKRM HDLNLAMDGL REVMP----- YAHGPSVRK LSKIATLLLA RNYILMLS
PmOLIGA_1c	EPCGQELRLK IN-SRERKRM QDLNVAMDGL REVMP----- YAHGPSVRK LSKIATLLLA RNYILMLN
LpOLIGA_TR	EPCGQELRLK IN-SRERKRM QDLNVAMDGL REVMP----- YAHGPSVRK LSKIATLLLA RNYILMLN
LjOLIGA_JL	----- ----- M QDLNVAMDGL REVMP----- YAHGPSVRK LSKIATLLLA RNYILMLN
LjHLH22-23	----- ----- M HDLNDAALDDL RAVIP----- YAHSPSVRK LSKIATLLLA KNYILMQA
LjHLH22-23	NKEQRALRLS IN-ARERRRM HDLNDALDEL RAVIP----- YAHSPSVRK LSKIATLLLA KNYILMQA
LpHLH22-23	NKEQRALRLS IN-ARERRRM HDLNDALDDL RAVIP----- YAHSPSVRK LSKIATLLLA KNYILMQA
JL12320	---QDLYKL PHRLIEKKRR DRINECISQL KELLPDHDK ---QTTLGHLE --KAVVLELT LKYMQELH
PmHELT_ENS	SEDMQDLYKL PHRLIEKKRR DRINECISQL KELLPDHDK ---QTTLGHLE --KAVVLELT LKYMQELH
TRINITY_DN	SEDLQDLYKL PHRLIEKKRR DRINECISQL KELLPDHDK ---QTTLGHLE --KAVVLELT LKYMQELH
JL8865	-EDSKEAYKL PHRLIEKKRR DRINECIAQL KDLLPEHLK ---LSTLGHLE --KAVVLELT LKHVKTLT
JL8234	--CVQEKGKL PHRLIEKKRR DRINECIVQL KELLPENLK ---LATLGHLE --KAVVLELT VQHMQALT
PmHes_ESNP	PKSASEIRKS TKPIMEKRR ARINDSLNQL KALILETLRK DSSRHSKLE --KADILELT VKHLRSLH
JL1099	PKSASEIRKS TKPIMEKRRR ARINDSLNQL KALILETLRK DSSRHSKLE --KADILELT VKHLRGLH
JL1098	----- MEKRRR ARINDSLNQL KALILEALKK DSSRHSKLE --KADILEMT VKHLRSLQ
TRINITY_DN	PKSASEHRKS TKPVMEKRRR ARINDSLNQL KALILEALKK DSSRHSKLE --KADILEMT VKHLRSLQ
TRINITY_DN	SKTASEHRKS SKPIMEKRRR ARINESLGH KTLIIDLALKK DSSRHSKLE --KADILEMT VKHLRNQ
TRINITY_DN	CSTQHMARKR RRGIIIEKRR DRINSSLAEL RRLVPSALE --KQGSAKLE --KAEILQMT VEHLRMLR
Hey_?_JL853	CG----R SPQIIIEKRR DRINSSLAEL RRLVPSALE --KQGSAKLE --KAEILQMT VEHLRMLR
Hs_PTfB_NP	-AELQQLRQA AN-VRERRRM QSINDAFEGL RSHIP----- TLPYEKR LSKVDTLRLA IGYINFLS
Hs_PTfA_NP	-VITYAQRQA AN-IRERKRM FNLLNEAFDQL RRKV----- TFAYEKR LSRIETLRLA IVYISFMT
Hs_Hand_NP	--RPVKRRGT AN-RKERRRT QSINSFAEL RECIP----- NVPADTK LSKIKTLLRA TSYIAYLM
Hs_Hand_NP	--RLGRRKGS GP-KKERRRT ESINSFAEL RECIP----- NVPADTK LSKIKTLLRA TSYIAYLM
Hs_Paraxis	--VVVRQRQA AN-ARERDR TQSVNTAFTAL RTLIP----- TEPVDRK LSKIETLRLA SSYIAHLA
Hs_Paraxis	-GREPRQRHT AN-ARERDRT NSVNTAFTAL RTLIP----- TEPADEPK LSKIETLRLA SSYISHLG
Hs_Twist_N	EELQTQCRM AN-VRERQRT QSLNEAFAA RLKIIP ----- TLPSDK LSKIQTLKLA AYRIDFLY
Hs_Twist_N	EELQSQRIL AN-VRERQRT QSLNEAFAA RLKIIP ----- TLPSDK LSKIQTLKLA AYRIDFLY
Hs_MyoRa_N	--GKQVQRNA AN-ARERARM RVLSKAFLSR KTTLP----- WVPPTDK LSKLDTLRLA SSYIAHLR
Hs_MyoRa_N	--CKQSQRNA AN-ARERARM RVLSKAFLSR KTSLP----- WVPPTDK LSKLDTLRLA SSYIAHLR
Hs_MyRoB_X	-RSGSGRPAA ANAARERSRV QTLRHAFLEL QRTP----- SVPPTDK LSKLDVLLA TTYIAHLT
Hs_MyRoB_N	-RS--EASP ENAAERERSRV RTLRQAFAL QALP----- AVPPTDK LSKLDVLVA ASYIAHLT
Hs_Atonal_	--AARRLA AN-ARERRRM QGLNTAFDRL RRVVP----- QVGODDK LSKYETLQMA LSYIMALT
Hs_Atonal_	--QKQRRLA AN-ARERRRM HGLNHAFDQL RNVIP----- SFNNDDKK LSKYETLQMA QIYINALS
Hs_Mist_NP	-DSSIQRRL SN-ERERQRM HKLNNAFQAL REVIP----- HVRADKK LSKIETLTLA KNYIKSLT
Hs_Net_NP_	--QQTRRLL AN-ARERTRV HTISAFAEL RKQVP----- CYSQKQ- LSKLAILRIA CNYILSLA
Hs_NeuroD_	--FKLRRMK AN-ARERNRM HGLNAALDNL RKVVP----- CYSKTQK LSKIETLRLA KNYIWALS
Hs_NeuroD_	--SKLRRQK AN-ARERNRM HDLNAALDNL RKVVP----- CYSKTQK LSKIETLRLA KNYIWALS
Hs_NeuroD_	--VKFRRQE AN-ARERNRM HDLNAALDNL RKVVP----- CYSKTQK LSKIETLRLA KNYIWALS
Hs_NeuroD_	--FRARRVK AN-ARERTRM HGLNDALDNL RRVMP----- CYSKTQK LSKIETLRLA RNYIWALS
Hs_Neuroge	--RRSRRVK AN-DRERNRM HNLNAALDAL RSVLP----- SFPPDTK LTKIETLRFY NYIWALA
Hs_Neuroge	--RRSRRKK AN-DRERNRM HNLNSALDAL RGVL----- TFPDDAK LTKIETLRFY NYIWALT
Hs_Neuroge	--KKTRRLK AN-NRERNRM HNLNAALDAL REVLP----- TFPEDAK LTKIETLRFY NYIWALT
Hs_Beta3_N	--QKALRLN IN-ARERRRM HDLNDALDEL RAVIP----- YAHSPSVRK LSKIATLLLA KNYILMQA
Hs_Beta3_N	--QRSLRLS IN-ARERRRM HDLNDALDGL RAVIP----- YAHSPSVRK LSKIATLLLA KNYILMQA
Hs_Oligo_N	-QDLQQLRRL IN-GRERKRM HDLNLAMDGL REVMP----- YAHGPSVRK LSKIATLLLA RNYILMLT
Hs_Oligo_N	-PELQQLRRL IN-SRERKRM HDLNIAMDGL REVMP----- YAHGPSVRK LSKIATLLLA RNYILMLT
Hs_Oligo_N	--QQLRRK IN-SRERKRM QDLNLAMDGL REVILPYSAA HCQGAPGRK LSKIATLLLA RNYILLLG
Hs_NSCL_NP	RRATAKYRTA HA-TRERIRV EAFNLAFEL RKLLP----- TLPPDKK LSKIEILRLA ICYISYLN
Hs_NSCL_NP	RRATAKYRSA HA-TRERIRV EAFNLAFEL RKLLP----- TLPPDKK LSKIEILRLA ICYISYLN
Hs_SCL_NP_	PHTKVVRIF TN-SRERWRQ QNVNGAFAEL RKLLP----- THPPDKK LSKNEILRLA MKYINFIA
Hs_SCL_NP_	--QKVARRVF TN-SRERWRQ QNVNGAFAEL RKLLP----- THPPDKR LSKNEVLRLA MKYIGFLV
Hs_SCL_NP_	-NSMMTRKIF TN-TRERWRQ QNVNSAFAKL RKLIP----- THPPDKK LSKNETLRLA MRYINFLV
Hs_Emc_NP_	----- ----- SRL RRLVP----- TIPPNNK VSKVEILQHV IDYILDQ
Hs_Emc_NP_	----- ----- SRL KELVP----- TLPLQNRK VSKVEILQHV IDYIRDQ

**Figure S8**

Molecular phylogenetic analysis of bHLH genes to classify lamprey Olig and Hes family members. In each case we extracted prospective family members from three lamprey species (*Petromyzon marinus*, Pm; *Lethenteron japonicum*, Lj; *Lampetra planeri*, Lp) plus members of the gene families most closely related to Olig and Hes (the Beta3/HLH22/23 family, and HELT + Hey families respectively). Also included are all human bHLH sequences (see Bao et al 2017 for details on this dataset). The Olig and Beta3 genes group robustly with human members, and two lamprey Olig genes are evidences (named OligA and OligB). For the Hey/HELT/Hes group we recovered sequences for all three families from lampreys, as expected. As in many analyses of these genes resolving orthology-paralogy relationships for the Hes genes wasn't possible probably due to lineage specific duplications (see Bao et al 2017 for discussion of this regarding Hes genes).

Figure S9

Tubulin labelling in control and DAPT-treated embryos, all at stage 26. Orientation of each image is as shown in each panel (A, anterior. P, posterior. D, dorsal. V, ventral. L, left. R, right). A and B show the heads with the olfactory placode (o) and various cranial nerves (cn) labelled. Morphology of these in both control and DAPT ($n=14$) embryos is as previously described Kuratani et al. (1997: J. Comp. Neurol 384:483–500). C and D show lateral views of the trunk, with spinal cord (sc) staining visible and trunk nerves labelled. Pronephric ducts (pnd) were also labelled by this antibody. In dorsal view in E and F the tubulin staining in both control and DAPT-treated embryos can be seen to be laterally restricted in the spinal cord, with trunk nerves (tn) on either side. G and H show confocal reconstructions of the trunk region, from the pronephric ducts backwards, with nuclei labelled in blue with DAPI.

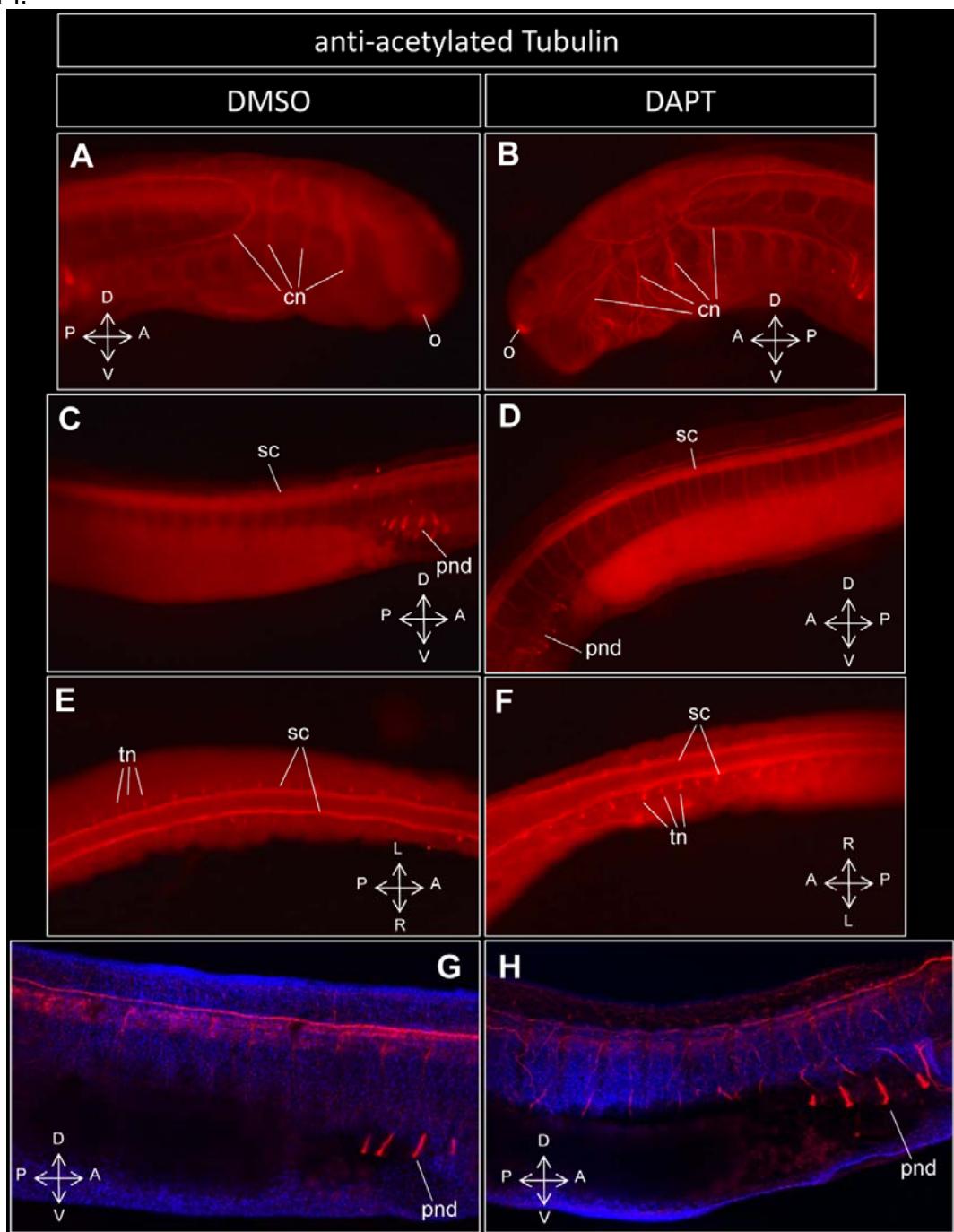


Figure S10

Alignment of Mouse, Human and Zebrafish Presenilins with Presenilins from three lamprey species.

	10	20	30	40	50	60	70	80
MmPs2	MLAFMASDSE	EEVCDERTSL	MSAESPTRS	CQEGRPG---		PEDG	ESTAQWRTQE	SEEDCEEDPD
MmPs1	---MTEIPA	PLS-YFQNAQ	MSEDSHSSSA	IRSQNDS---		QER	QQQHDRQRQLD	NPEPISNGRP
HsPs2	MLTFMASDSE	EEVCDERTSL	MSAESPTPRS	CQEGRQG---		PEDG	ENTAQWRSQE	NEEDGEEDPD
HsPs1	MLTFMASDSE	EEVCDERTSL	MSAESPTPRS	CQEGRQG---		PEDG	ENTAQWRSQE	NEEDGEEDPD
DrPs1	---MADLVQ	NAANNVLNDG	MDTSRHTSST	AAPPS---			--RNEVELN	G-QPPTAPPP
DrPs2	---MNTSDSE	EDSYNERSAL	VQSESPTVPS	YNQDNAMSL-		PQDT	DSKRSGAVRS	RSASGSGDAG
LjPs	-----	MCCVACVQ--				PQVT	TTTRQRGER	EDGDGSG--R
LpPs	--MSVFEARG	GGGCGREENFL	MEAVVSSERR	ARSVSEAAAA	GAQEGVGIAA	AQDGLPQVTT	STTRQRGER	EDGDGSG--R
PmPs	--MSVSEKRG	GG-CGEESFL	MEAVVSSERR	AGSMSEAVG-	GQEGVGIAA	AQDGLPQVTT	TTTRQRGER	EDGDGSGSGR
	90	100	110	120	130	140	150	160
MmPs2	RYACSGAPGR	PSGLEEELTL	KYGAKHVIML	FVPVTLCMIV	VVATIKSVR	YTEKNGQ-LI	YTPFTEDTPS	VGQRLLNSVL
MmPs1	QSNSRQVVEQ	DEEEDEELTL	KYGAKHVIML	FVPVTLCMIV	VVATIKSVF	YTRKDQG-LI	YTPFTEDTET	VGQRALHSIL
HsPs2	RYVCSGVPGR	PPGLEEELTL	KYGAKHVIML	FVPVTLCMIV	VVATIKSVR	YTEKNGQ-LI	YTPFTEDTPS	VGQRLLNSVL
HsPs1	RYVCSGVPGR	PPGLEEELTL	KYGAKHVIML	FVPVTLCMIV	VVATIKSVF	YTEKNGQ-LI	YTPFTEDTPS	VGQRLLNSVL
DrPs1	Q---VVTDS	EEDEDEELTL	KYGAKHVIML	FIPVTLCMV	VVATIKSVF	YTQKDGQQLI	YTPFREDTET	VGQRALHSML
DrPs2	-PVDRERADT	PDGEEEELTL	KYGAKHVIML	FIPVTLCMV	VVATIKSVF	YTEKSGQRLI	YTPFEEDPNS	VGQRLLNSVL
LjPs	ERSHSEPHTD	EESEEEELSL	KYGAKHVIML	FVPVTLCMLV	VVATIKSVTF	YTEKNGQ-LI	YTPFTEDTDT	VGQRVLNSIL
LpPs	ERSHSEPHTD	EESEEEELSL	KYGAKHVIML	FVPVTLCMLV	VVATIKSVTF	YTEKNGQ-LI	YTPFTEDTDT	VGQRVLNSIL
PmPs	ERSHSEPHTD	EESEEEELSL	KYGAKHVIML	FVPVTLCMLV	VVATIKSVTF	YTEKNGQ-LI	YTPFTEDTDT	VGQRVLNSIL
	170	180	190	200	210	220	230	240
MmPs2	NTLIMISIVV	VMTIFLVVLY	KYRCYKFIHG	WLIMSSLMLL	FLFTYIYLGE	VLKTYNVAMD	YPTLFLAVWN	FGAVGMVCIH
MmPs1	NAAIMISIVV	IMTILLVVLY	KYRCYKVIHA	WLTISSLLL	FFFSFIYLGE	VFKTYNVAVD	YVTVALLIWN	FGVVMICIAIH
HsPs2	NTLIMISIVV	VMTIFLVVLY	KYRCYKFIHG	WLIMSSLMLL	FLFTYIYLGE	VLKTYNVAMD	YPTLLLTVWN	FGAVGMVCIH
HsPs1	NTLIMISIVV	VMTIFLVVLY	KYRCYKFIHG	WLIMSSLMLL	FLFTYIYLGE	VLKTYNVAMD	YPTLLLTVWN	FGAVGMVCIH
DrPs1	NAIIIMISIVV	VMTLVLVVLY	KYRCYKVIQA	WLFFSNLLLL	FFFSLIYLGE	VFKTYNVAMD	YFTLALIIWN	FGVVMICIH
DrPs2	NTLVMISIVV	FMTIILVLLY	KYRCYKFIHG	WLILSSLMLL	FWFSFMYLGE	VFKTYNVAMD	YPTLVMIIWN	FGVVMICIH
LjPs	NTIILISIVV	VLTIFLVVLY	KYRCYKFIHG	WLIMSSLMLL	FLFTYLYLGE	VFKTYNVAMD	YPTLILIMWN	FGVVMICIH
LpPs	NTIILISIVV	VLTIFLVVLY	KYRCYKFIHG	WLIMSSLMLL	FLFTYLYLGE	VFKTYNVAMD	YPTLILIMWN	FGVVMICIH
PmPs	NTIILISIVV	VLTIFLVVLY	KYRCYKFIHG	WLIMSSLMLL	FLFTYLYLGE	VFKTYNVAMD	YPTLILIMWN	FGVVMICIH
	250	260	270	280	290	300	310	320
MmPs2	WKGPLVLIQQA	YLIVISALMA	LVFIKYLPW	SAWVILGAIS	VYDLVAVLCP	KGPLRMLVET	AQERNEPIFP	ALIYSSAMVW
MmPs1	WKGPLRLQQA	YLIMISALMA	LVFIKYLPW	TAWLILAVIS	VYDLVAVLCP	KGPLRMLVET	AQERNETLFP	ALIYSSTMVW
HsPs2	WKGPLVLIQQA	YLIMISALMA	LVFIKYLPW	SAWVILGAIS	VYDLVAVLCP	KGPLRMLVET	AQERNEPIFP	ALIYSSAMVW
HsPs1	WKGPLVLIQQA	YLIMISALMA	LVFIKYLPW	SAWVILGAIS	VYDLVAVLCP	KGPLRMLVET	AQERNEPIFP	ALIYSSAMVW
DrPs1	WKGPLRLQQA	YLIMISALMA	LVFIKYLPW	TAWLILAAIS	VYDLVAVLCP	KGPLRILVET	AQERNEAIFP	ALIYSSTMVW
DrPs2	WKGPLRLQQA	YLIVISALMA	LIFIKYLPW	SAWVILGAIS	IYDLIAVLCP	KGPLRMLVET	AQERNEPIFP	ALIYSSAMVW
LjPs	WKGPLLQQA	YLIMISALMA	LVFIKYLPW	SAWVILGAIS	IYDLIAVLSP	KGPLRMLVET	AQERNEPIFP	ALIYSSTMVW
LpPs	WKGPLLQQA	YLIMISALMA	LVFIKYLPW	SAWVILGAIS	IYDLIAVLSP	KGPLRMLVET	AQERNEPIFP	ALIYSSTMVW
PmPs	WKGPLLQQA	YLIMISALMA	LVFIKYLPW	SAWVILGAIS	IYDLIAVLSP	KGPLRMLVET	AQERNEPIFP	ALIYS----
	330	340	350	360	370	380	390	400
MmPs2	TVGMAKLD-P	SSQGALQLP-	YDPEMEEDSY	DSFGEPSYPE	AFEAPLPGYP	GEEL-----		E
MmPs1	LVNMAEGDPE	AQRDRVPKNP	YNTQRAERET	QDSGSGNDDG	GFSEEWEAQR	DSHLGPHRST	PESRAAVQEL	SGS--ILTSE
HsPs2	TVGMAKLD-P	SSQGALQLP-	YDPEM-EDSY	DSFGEPSYPE	VFEPPPLTGYP	GEEL-----		E
HsPs1	TVGMAKLD-P	SSQGALQLP-	YDPEMEEDSY	DSFGEPSYPE	VFEPPPLTGYP	GEEL-----		E
DrPs1	LFNMA--DSA	ETRNNSSSH	PQQENQVVM	APTAQAEDDG	GFTPAWVDHQ	QHQLGPMQST	EESRRQIQEM	PSARPPPAP
DrPs2	MVGMADSNP	DSAGERRRS-	GGGVRTQEGV	ES---EDDAQ	AGRQYS-A	EEDL-----		E
LjPs	MVGMADTNST	EQQSTRPSP	PNNDNAQ---		GDPQPSEPDV	PRTTHEAREL	ARERARAN--	--PPMPPEES
LpPs	MVGMADTNST	EQQSTRPSP	PNNDNAQVNG	GQGDFGTGNFL	GDPQPSEPDV	PRTTHEAREL	ARERARAN--	--PPMPPEES
PmPs	-----	-----	-----	-----	-----	-----	-----	-----

