

Supplementary Figure 1

Partial protein multiple sequence alignment showing the homeobox and adjacent sequences of *six3* (a) and *otx* (b) orthologues in comparison with other closely related homeobox genes.

Only partial sequences available for *Plo-otx* and *Stm-otx*. Dot: conserved amino acid. Dash: Gap. Species abbreviations: *Bfl*: *Branchiostoma floridae*, *Dme*: *Drosophila melanogaster*, *Eka*: *Euperipatoides kanangrensis*, *Mmu*: *Mus musculus*, *Pca*: *Podocoryne carnea* *Pdu*: *Platynereis dumerilii*, *Plo*: *Pristina longiseta*, *Stm*: *Strigamia maritima*, *Tca*: *Tribolium castaneum*. Gene abbreviations: *optx*: *optix*, *so*: *sine oculis*, *otd*: *orthodenticle*. Gene accession numbers: *Bfl-otx*: AAC00193; *Dme-optx*: Q95RW8; *Dme-otd*: CAA41732; *Dme-six4/5*: NP_730566; *Dme-so*: Q27350; *Eka-otx*: ABY60730; *Eka-six3*: ABY60729; *Mmu-hesx1*: CAA56344; *Mmu-six1*: NP_033215; *Mmu-six2*: Q62232; *Mmu-six3*: Q62233; *Mmu-six4*: NP_035512; *Mmu-six5*: NP_035513; *Mmu-six6*: NP_035514; CAA56344; *Mmu-otx1*: NP_035153; *Mmu-otx2*: NP_659090; *Mmu-otx3*: NP_001020738; *Mmu-vsx2*: NP_001034513; *Pca-six1/2*: AAT11871; *Pca-six3/6*: AAT11872; *Pdu-otx*: CAC19028; *Pdu-six1/2*: CAC86663; *Pdu-six3*: FM210809; *Plo-otx*: ABY53615; *Plo-six3*: ABY50071; *Stm-otx*: ABY74501; *Stm-six3*: ABY74502; *Tca-otx*: NP_001034513; *Tca-six3*: CAP58434.

Supplementary Figure 2

Expression of *six3* and *otx* in different stages of *Platynereis*, *Strigamia*, and *Euperipatoides* and in relation to the larval eyes in *Platynereis*.

Single- (a-h) and two-colour (i-m) whole-mount *in situ* hybridisations of *six3* (a, c, e, g, i), *otx* (b, d, f, h, k-m), the *Platynereis* larval eye pigment cell marker gene *Pdu-tryptophane-2,3-dioxygenase* (l) and adult eye photoreceptor cell

marker *Pdu-r-opsin* (m). *Platynereis* larval stages: 24h (l), 36h (a, b) and 48h post-fertilization (c, d, m). *Strigamia* stages: 1st leg-bearing segment stage (e, f) and mid-germ band stage (g, h). *Euperipatoides* mid-segmentation stages (i, k). Yellow arrowheads in (a, b, g, h): mouth opening. sr: stomodaeal roof. (a-h): Ventral views. (i-m): Apical views; ventral to bottom.

a

homeobox

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Mmu-six3 IWD-GEQKTHCFKERTSLLREWYLQDPYPNPSKKRELAQATGLTPTQVGNWFKNRRQRDR-AA--AAKNRLQHQ
Mmu-six6 .....H.....-...Q.
Plo-six3 VFERTQ.RS.....N...S..R...S.GR..H..ES.....-...T...SRA.
Pdu-six3 .....N.....T.....-...MNGH
Eka-six3 .....T.....-...QL
Stm-six3 .....R.....T.....-...Q.
Tca-six3 .....T.....-...M.Q.
Dme-optx .....T.....K...N.....-...I..S
Pca-six3/6 .....A.....K...F.....S.....D..D..H.....-...KS.R.
Mmu-six1 .....ETSY...KS.GV...AHN...S.RE...E...T...S.....-...EAKERENTE
Mmu-six2 .....ETSY...KS.V...AHN...S.RE...E...T...S.....-...EAKERENSE
Pdu-six1/2 .....ETSY...KS.TV...AHN...S.RE...E...T...S.....-...EVKDSRDGP
Pca-six1/2 .....ETSY...KS.AV..D..TRN...S.RE.K.SEG..ST...S.....-...EAKEREEES
Dme-so .....ETSY...KS.V..D..SHN...S.RE..D..E...T...S.....-...EHKDGSTDK
Mmu-six4 .....ETVY...KS.NA.K.L.K.NR..S.AE..H..KI...SL...S.....NPS--ETQSKSES
Mmu-six5 .....ETVY...S.AA.KAC.RGNR..T.DE..R..TL...SL...S.....TGTGGGAPCKSES
Dme-six4/5 .....ETVY...KS.NA.KDC..TNR..T.DE.KT..KK...L...S.....-T-----

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b

homeobox

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Mmu-otx2 YPATPRKQ-----RRERTTFTRAQLDVLEALFAKTRYPDIFMREEVALKINLPESRVQVWFKNRRACKRQQQQ-----
Mmu-otx1 .....S.....-...
Mmu-otx3 .GSQH.....S..A..AQ..EA..KT.Q..H...VV...RL.MCT...A.....F.KK.RSLQKEQLQKQK-----
Bfl-otx C.PP.....-...AG.AK-----
Pdu-otx EA.N.....S..Q.....KA-----
Plo-otx ...SS.....S.S...T..H.....-...
Eka-otx .....S.....F.....QQQ-----
Stm-otx .....-...
Tca-otd AGVN.....L..G.....V.....L..QQ-----
Dme-otd PGVNT.....G.....L..QQQSNLSSSSKNASGGGS-----
Mmu-Hesx1 FS.SETRSLKRELSWYRG..P..A..QN.VE...NV.RVNC..G.DI..DL.Q.L..E.D.I.I..Q....MKRSRRE-----
Mmu-vsx2 NQTKK..K-----H..I..SY..EE..KA.NEAA...VYA..ML.M.TE...D.I...Q....W.KREKC-----

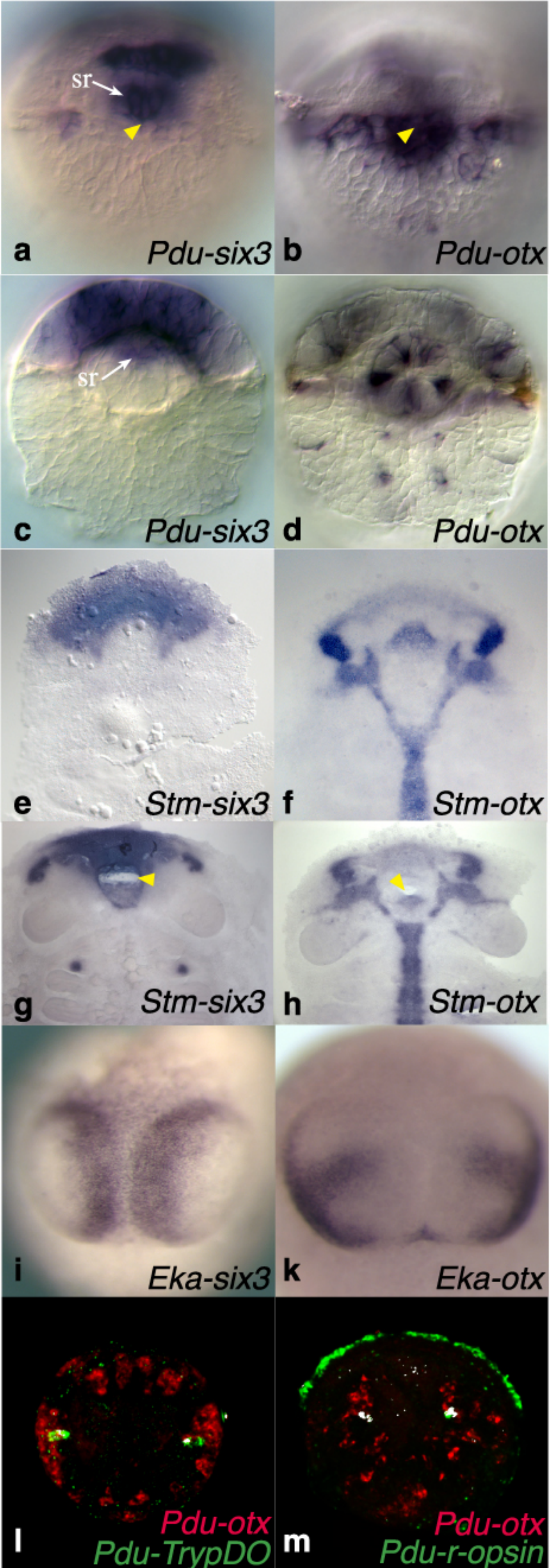
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Mmu-otx2 -----QQNGGQNKVPAKKKSSPAREVSSESSTGTS--GQFSPPSSTSVPTIASSAP--
Mmu-otx1 -----S.NGT.T..V.....V..S.GSESS--...T..AVS.SASSS..ASSAS
Mmu-otx3 -----EAE.SHGEGKVEAPA.DTQL.TEQPP.LP--SGDP.AELQLSLSEQ.A.ESAP
Bfl-otx -----PRPKKKSASP.PASTEEQ.PTSE..P.CSDTSVSST.VPVAVTIGNTNA.PTAA
Pdu-otx -----D.RNPAS..K.S..PT-----PGDVS.VNNNTSSDS.YKTVP
Plo-otx -----
Eka-otx -----N...S..K.A.TP.PPTRASAPYK--PPSV.AVNSMT.PG-----
Stm-otx -----S..K.P..P.PPAT.TSPPS.RDSPYK..V..PTAAVGLAPP.PS
Tca-otd -----NKSASRTTTS.T.V.A.K.SPAAAPRSVATPTGIPT..TSAS.PTVNIKKESP
Dme-otd GNSCSSSSANSRSNSNNNGSSNNNT.SS..N.SNKSSQ.QGNS--QS.QQG.G.--SGGNNSNNN.AAAA..AA.AVA
Mmu-Hesx1 -----
Mmu-vsx2 -----WGR.SVMAEYGLYG.MVR-----

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Supplementary Figure 1



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Supplementary Figure 2