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SUPPLEMENTARY MATERIAL

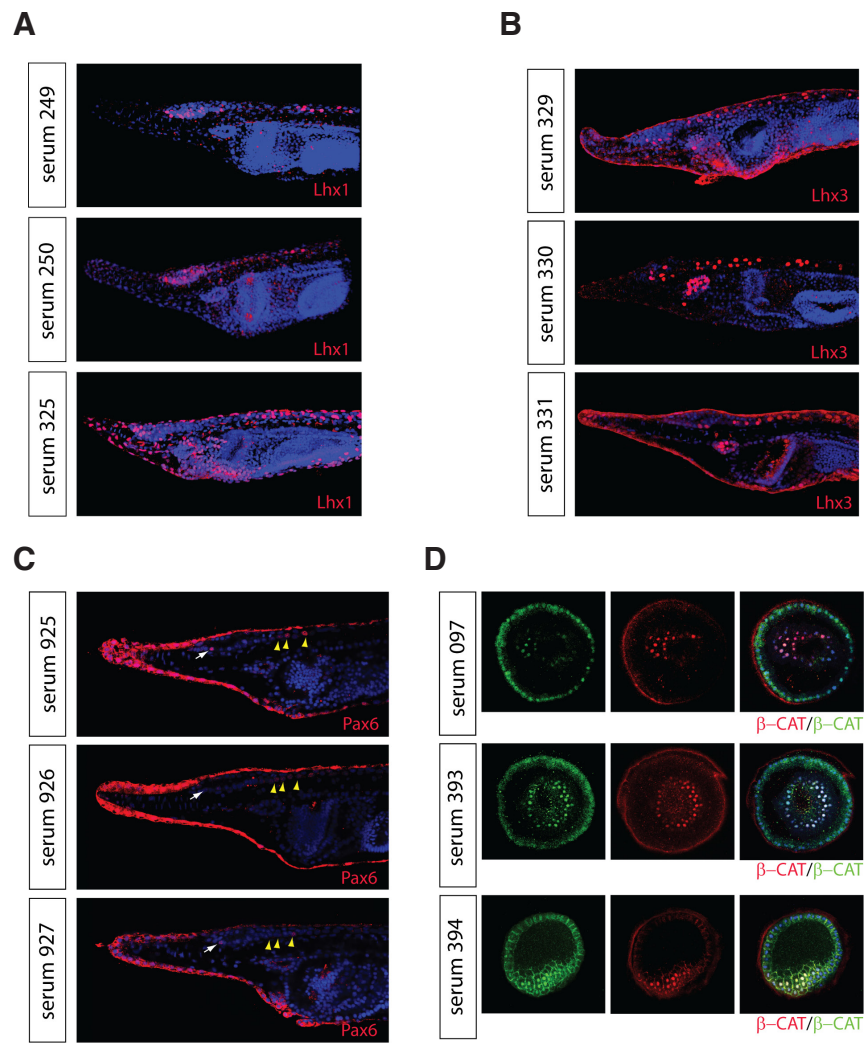
corresponding to:

**Novel polyclonal antibodies as a useful tool
for expression studies in amphioxus embryos**

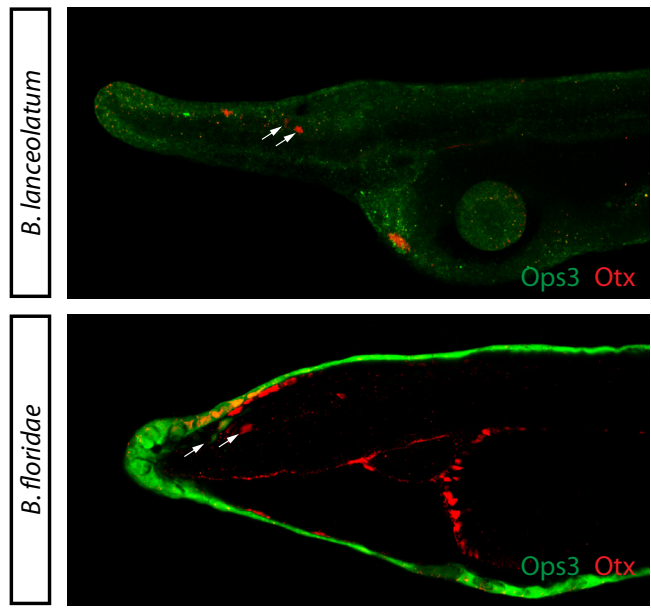
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Suppl. Fig. S1. Representative examples of immunostainings with sera generated against amphioxus Lhx1, Lhx3, Pax6, and β -catenin. Sera collected after the 4th immunization of three mice were used for comparative immunostainings on appropriate stages of amphioxus. The red signal in panels (A, B, C) represents staining with anti-Lhx1, Lhx3, and Pax6 antibodies, respectively. The conspicuous ectodermal signal in (C) represents un-specific labelling. The red signal in panel (D) corresponds to stainings with commercially available rabbit anti-human β -catenin antibody (Sigma C2206) and the green signal to immunostainings using amphioxus-specific β -catenin mouse polyclonal antisera. Blue represents a nuclear DAPI signal.



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B_lanceolatum 1 RLCRQPRAPRVTPIGIDDNTQVRLGGEGPSQAQFFLPSEENGDNVEMLT 50
B_floridae   RVCCRQQA VPRVTP L--DYNVH VRLGGEGPSQAQFFLPAGENVENFEMLK
consensus    * . * * * *      * * * * * . . * *      * * * * * * * * * * * * * * *

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B_lanceolatum 51 KAQEN-QLKGDGLSIISE 67
B_floridae   CVQENCKLKADSLSTISE
consensus    * * * * * * * * * * * * * * *

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Suppl. Fig. S2. Limited cross-reactivity of Ops3 antibody. Co-staining of *B. lanceolatum* or *B. floridae* with mouse polyclonal anti-Opinin 3 (*Ops3*) and rabbit polyclonal anti-*Otx* antibodies. Both antibodies were raised against *B. floridae* antigens and were successfully used in a previous study (Vopalensky et al., 2012). *Otx* exhibits cross-species reactivity and thus also stains *B. lanceolatum* antigen, while *Ops3* antibody provides no signal in *B. lanceolatum*. White arrows point to *Row1* photoreceptor cells marked by *Otx* expression. In *B. floridae*, *Ops3* is expressed in one of the *Row1* cells. An amino acid sequence alignment of *B. lanceolatum* and *B. floridae* *Ops3* in the region serving as antigen is presented.

SUPPL. TABLE S1

ANTIGENS USED FOR GENERATION OF ANTIBODIES

Protein	Amino acid sequence of the antigen	Peptide length
FoxA	YHGSTPTTTSNGASTLQPLQPIINTPSPNPQEQQQHQH HQHQHQHQHQQQPQVQTTQPDMMQQAQQHQGLPARP IPQQSSLPMMSGGYFSPHLRAAHGFTHPFSISNLMS QEHKPDLKEYAAMGYSGYNSMSPTGVKTTMSMDSMG TDYYQGYVVPQHSQPSSL*	165 aa
Lhx1	MKQLSALGARRAQFFRHRPRMRGLDGSPELMGAQYGF YQGEIDDQAEYYSPPSYDFFPPGSQPPNSQAGGMPP FLFPSSQPMGVMDPAMPHQGGGGMGNGHGGMPPDGF LPPGDMLPQSDAPTPEPMMGGGGYGP PPNQTLGRGFP PGMPGQPPREESVW*	162 aa
Lhx3	NNIKRKGSPARSEQNGAVDDLSDLSKDIVYSDGQMV AMSPGSDIYPGGTLEGDPALGPMPTNPGYPVDSSYP TLQGGNTYNMHSPPVPLGGPPGIHPGMPYDGGGLGS QSFQAMRVMVGGPGSDLSSGSSQGGYPDFPASPSSW LDDVDSHY*	156 aa
β-catenin	GMMMLPPDEPAYRGEGMFHHSSVSVVHSGQHSTMGR QPHQVQPRTYLSTGSHYDQPPHTPIDHAMDHMDMGF GPGGPHMQGAAGGHPSHSDYDQDLDLGIPTADLGL GLPPQGGDNNQLAWFDIDL*	130 aa
Pax6	KLRNQRSDSDSSSPSRIPISSSFSTATMYQPIAPP SAPVMSRSSHAGLTDYSYSLPPVPRWENFVPGNMAP MPSMQQSRDQTSYSCMI PHSTAMTPRGYDSLALGSYN PTHAGHHVTTTHPSHMQAPSMGSHSHSHANGGSAGL ISPGVSVVPVQVPGAVTEEMTSQPYWPRIQ*	177 aa