# Science Advances

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# Supplementary Materials for

## Homologous gene regulatory networks control development of apical organs and brains in Bilateria

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**This PDF file includes:**

Figs. S1 to S6
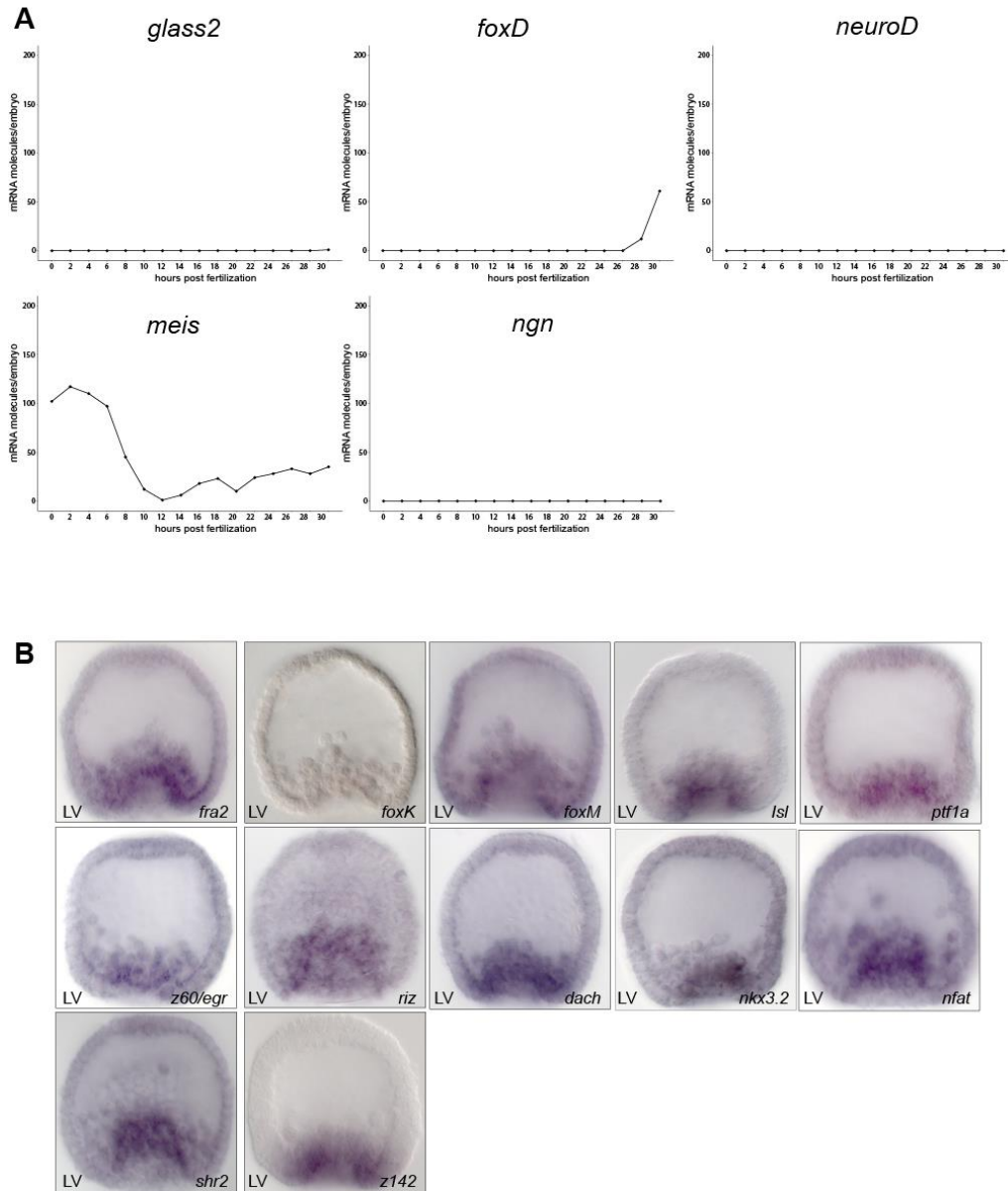Tables S1 to S4

**Figure S1**



**Fig. S1:** Spatial and temporal expression of regulatory genes that were not further considered in this study. **(A)** Temporal analysis of gene expression levels obtained using Nanostring N-counter technology for genes expressed at <50 copies per embryo during pre-gastrular development. **(B)** WMISH analysis for genes expressed above the threshold level but that are not expressed in the apical domain in sea urchin embryos at 30hpf.
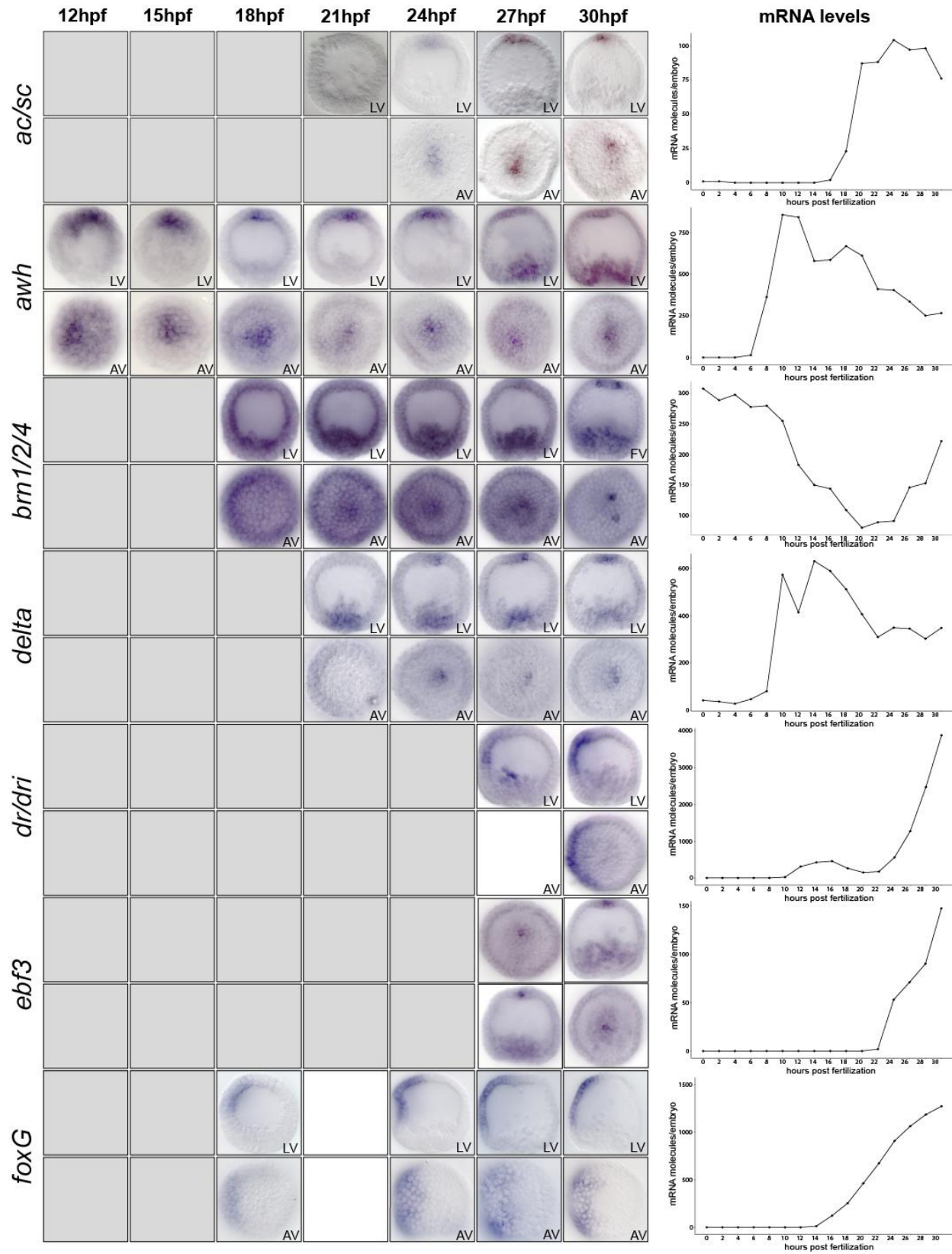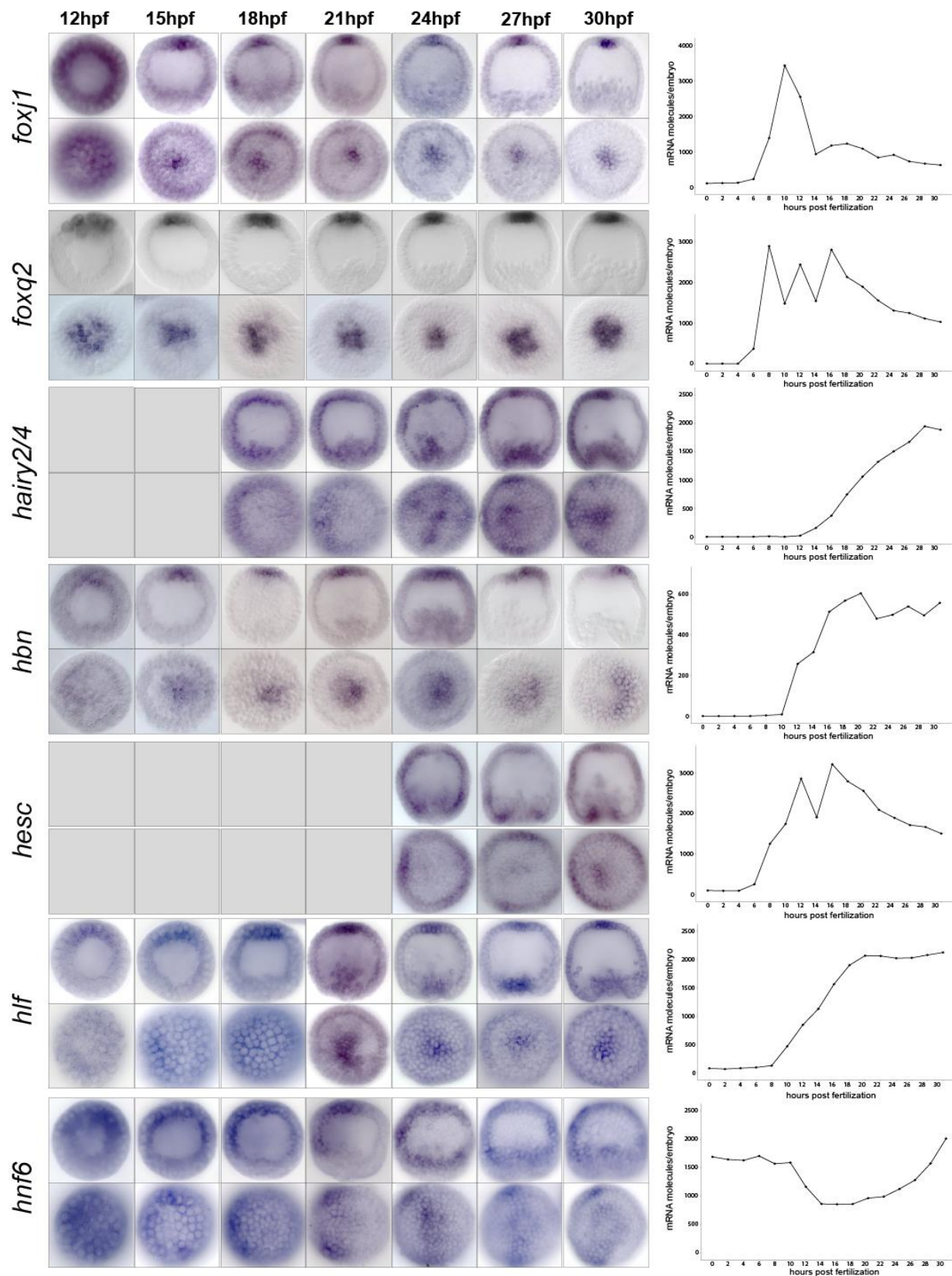
# Figure S2A

# Figure S2B

# Figure S2C
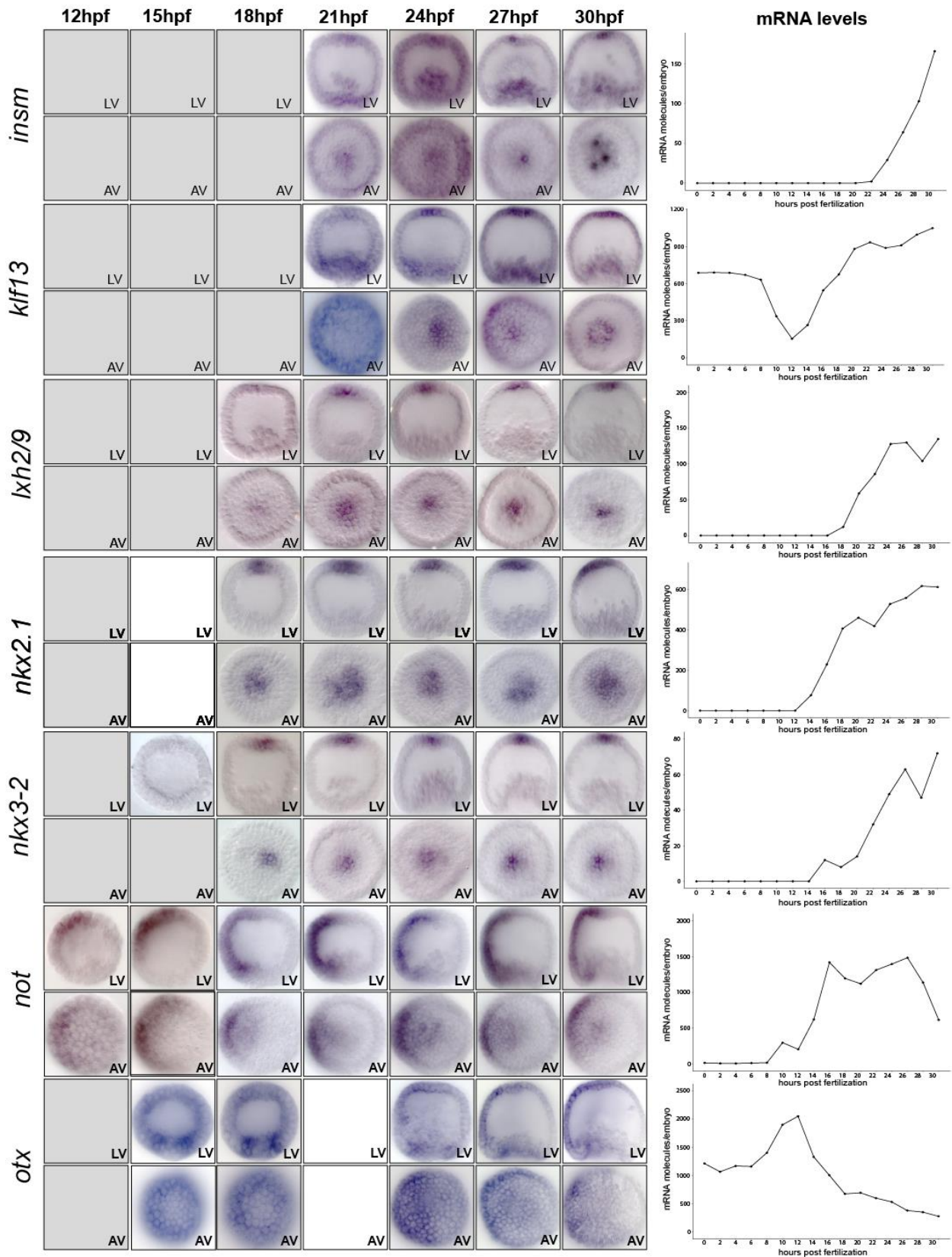
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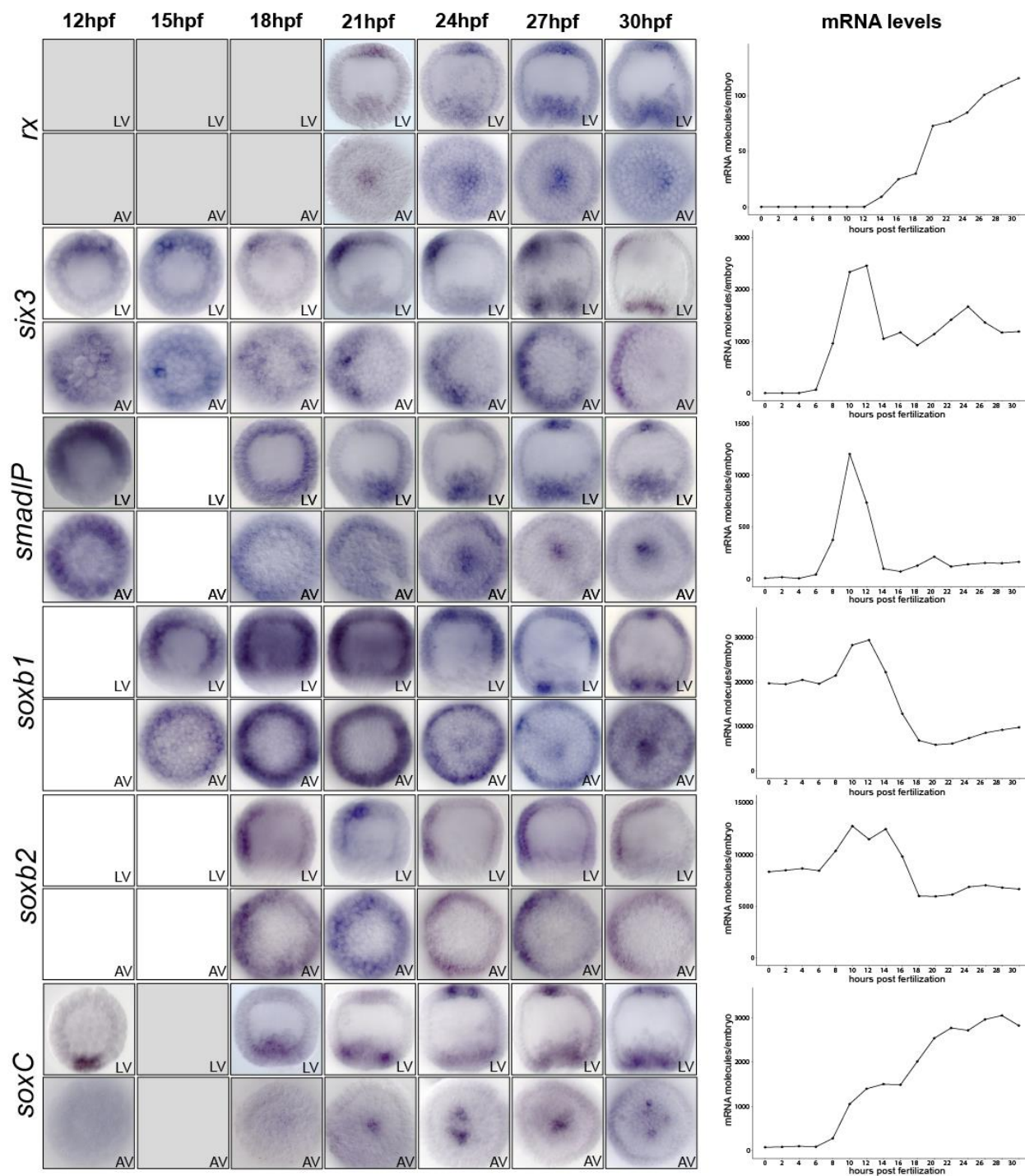
## Figure S2E
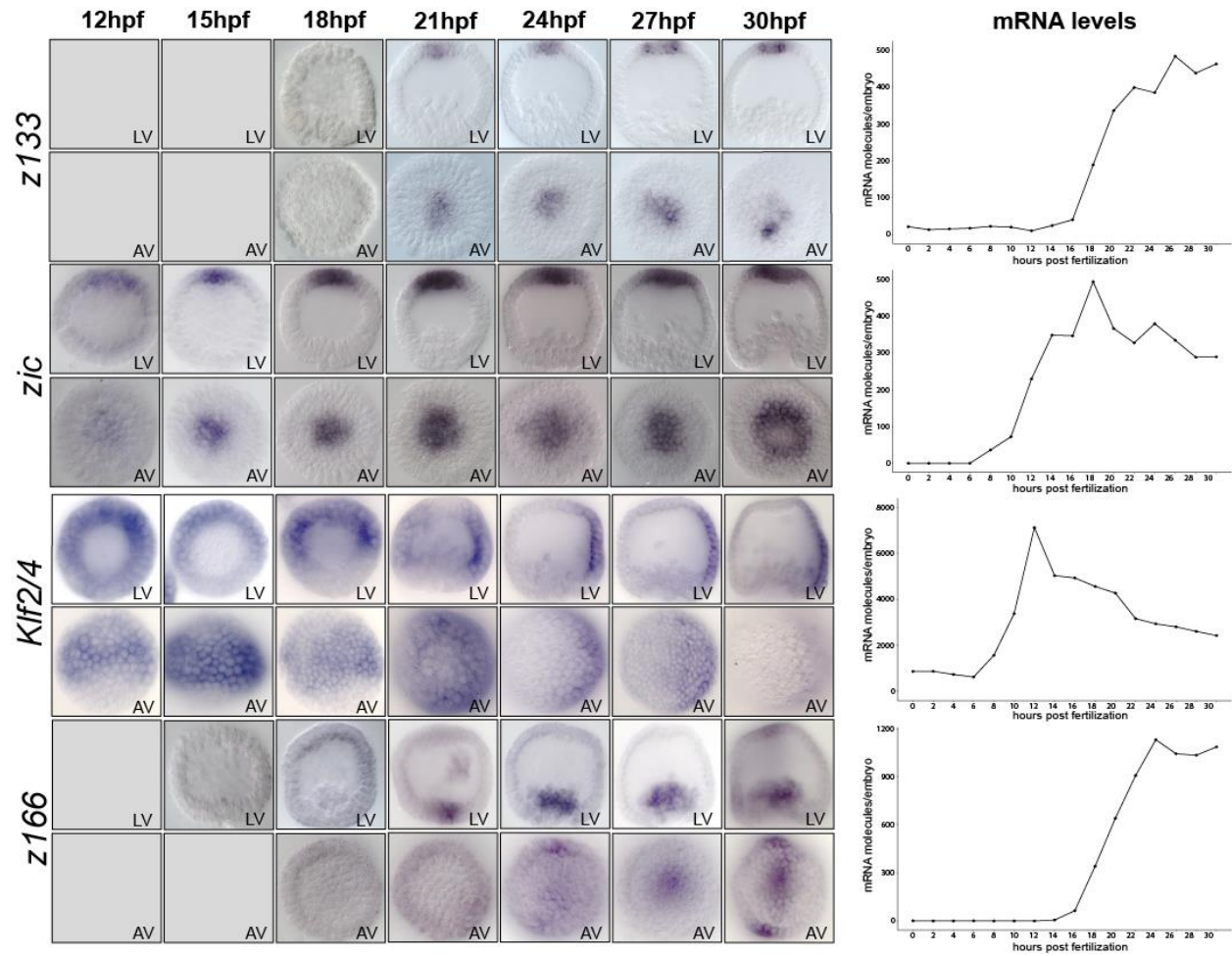


**Fig. S2.** Time course analysis of spatial expression for 31 regulatory genes of the apical GRN during the patterning of the apical organ and the developmental specification of neural precursors. Images of WMISH stained embryos are shown on the left and Nanostring analysis on the right. LV, lateral view, AP, apical view. Quantitative gene expression levels during pre-gastrular development based on Nanostring analysis are shown on the right.

# Figure S3
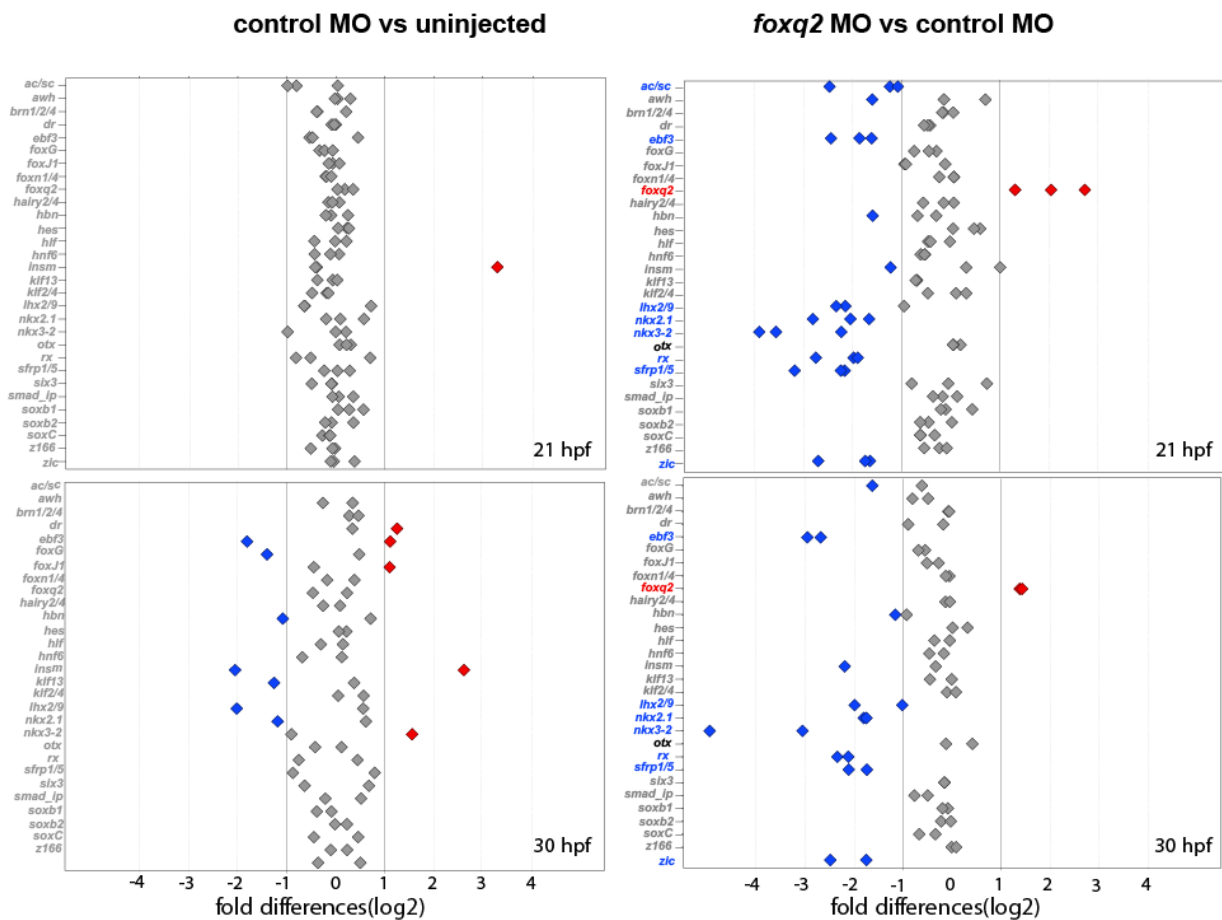


Fig. S3. **Effect of FoxQ2 perturbation on apical gene expression.** Expression levels of regulatory genes were analysed by Nanostring analysis. Shown are changes in expression levels in embryos injected with control morpholinos as compared to uninjected control embryos (left) and embryos injected with *foxq2* morpholinos as compared to control morpholinos (right). Data indicate that several genes of the apical GRN are affected by FoxQ2 perturbation. Red square indicates genes that are upregulated, blue square indicates genes that are downregulated, grey square indicates genes that are not affected by the perturbation (<2-fold difference).

## Figure S4
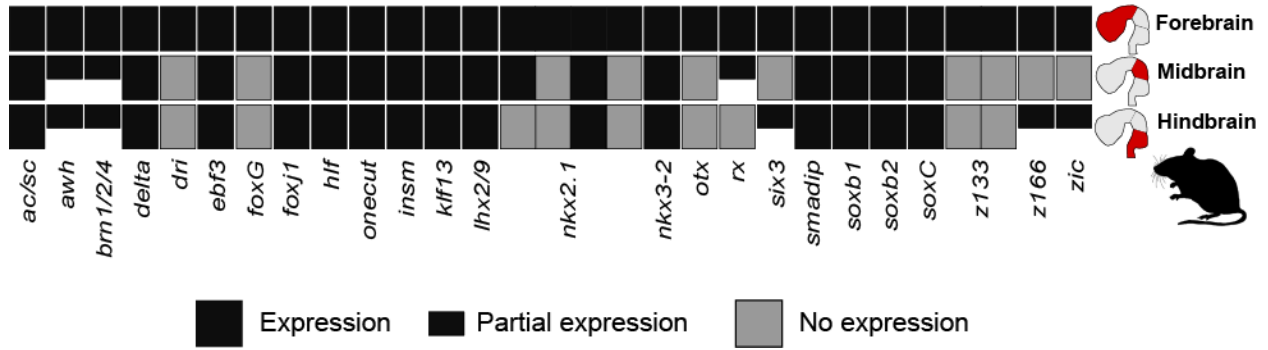


**Fig. S4. Orthologs of sea urchin apical transcription factors are expressed throughout the mouse brain.** Expression of mouse orthologs of apical transcription factors in the forebrain, midbrain, and hindbrain of mice was determined using the mouse Gene Expression Database (GED). All apical transcription factors are expressed in the mouse forebrain and a majority are also expressed in the midbrain and hindbrain.

**Figure S5**
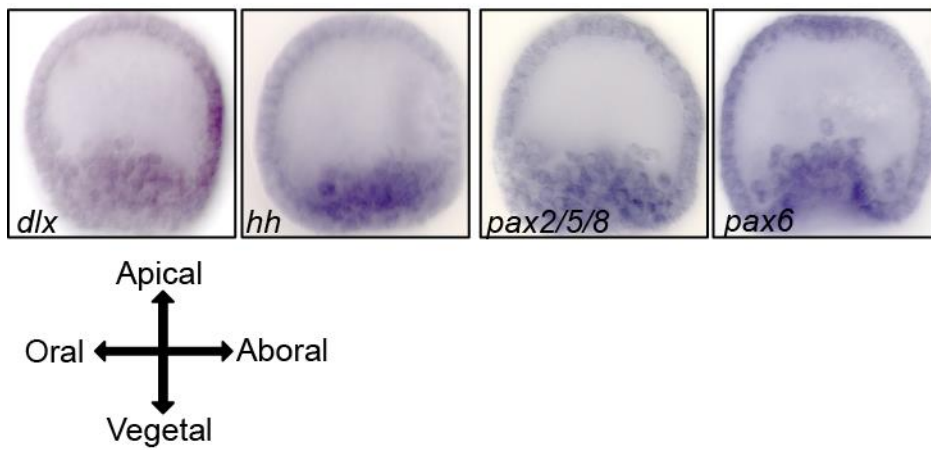


**Fig. S5.** Spatial expression of axial transcription factors. Expression of regulatory genes orthologous to those patterning the anterior/posterior axis in the vertebrate brain are analyzed by WMISH in sea urchin embryos at 30hpf. Dlx is expressed in aboral ectoderm while no ectodermal expression was observed for hh, pax2/5/8, and pax6.

# Figure S6



**Fig. S6.** Evidence for regulatory interactions in the GRN for the sea urchin apical organ. Effects of perturbation of transcription factors shown in each row on the expression of regulatory genes shown in columns. Experiments were performed in this study and in referenced work.

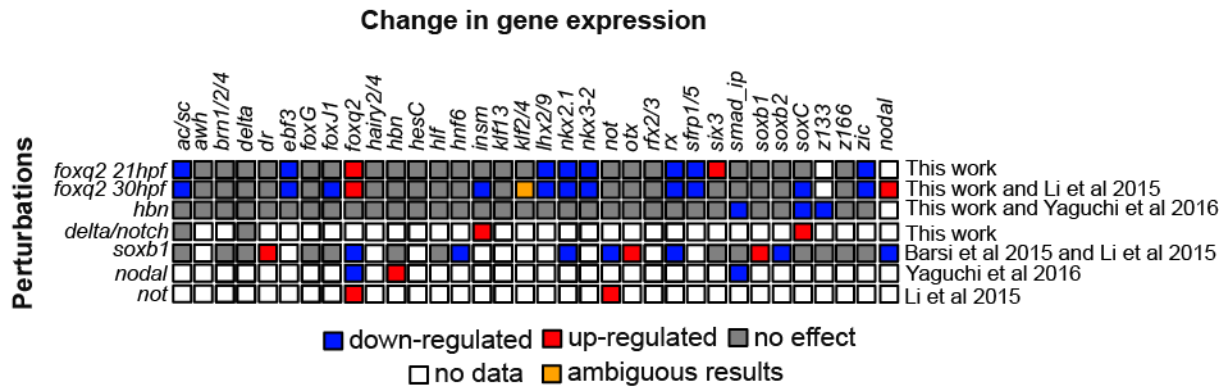| Gene Names | SPU-ID | WHL | Forward Primer | Reverse Primer |
|---|---|---|---|---|
| Ac-sc | SPU_028148 | WHL22.128311 | AACATCGTACCAATTTCCATCC | TAATACGACTCACTATAGGGAGATTTAGCAGGTCTTCATCTTCAGG |
| Awh | SPU_018954 | WHL22.9369 | CATAACCATCCCATCAATAAATCC | TAATACGACTCACTATAGGGAGATGCACAGCACTCTATTTTCAATC |
| Brn1/2/4 | SPU_016443 | WHL22.40221 | TTGTTCAGTCTATCCTCGAAAC | TAATACGACTCACTATAGGGAGACGTCACATGCTGTTCCTTTGTACC |
| Dach | SPU_018581, SPU_028061 | WHL22.169355 | AACATTCCTCTCTCACTCACACC | TAATACGACTCACTATAGGGAGAATAGCAAATCTCCTCTGTTCAGC |
| Delta | SPU_016128 | WHL22.423696 | CCCTACGAGTGTGAGAAGAAGG | TAATACGACTCACTATAGGGAGATTCATTTGCAGTGTTGGATACG |
| Dri | SPU_005718 | WHL22.544150 | AGCACACTTCGAGAAACAACC | TAATACGACTCACTATAGGGAGATTTCCTTTGGCAGTCTATCTGG |
| Ebf3 | SPU_004702 | WHL22.113329 | GGTGAGAAGGAGAAGGAGAAGG | TAATACGACTCACTATAGGGAGAAGCAGAGTAGAGGTCAGCATACG |
| Egr/z60 | SPU_015358 | WHL22.280477 | TAAGAAGTCCAAGGAATCAATCG | TAATACGACTCACTATAGGGAGATGTCTGCTGGTGATATGTGG |
| Emx | SPU_002592 | WHL22.113468 | AAAGCAGCAGAAGTACCTGTCG | TAATACGACTCACTATAGGGATCCAGCATCAACTCGGACT |
| Fgf9/16/20 | SPU_006242 | WHL22.343932 | CGGCAGGTTTATCAGACGAT | GCGTCTCTGATTGTTGCTGA |
| FGFR1 | SPU_007579 | WHL22.472570 | TGCAAACGAGAAGAGAACAGG | TAATACGACTCACTATAGGGAGACTTGTTGATGGAGAGGAAGAGG |
| FoxG | SPU_009771 | WHL22.389872 | GGTGAGAAGGAGAAGGAGAAGG | TAATACGACTCACTATAGGGAGAAGCAGAGTAGAGGTCAGCATACG |
| FoxJ1 | SPU_027969 | WHL22.468365 | CAAGCAGACCATGCCAAAGAA | GGGACGCAGGCTTGTAGCT |
| FoxK | SPU_025010 | WHL22.490768 | GTATCTTGAGGTTTCCCAGCACAAG | TAATACGACTCACTATAGGGAGATTTGGTGGTAAAGCCAGAGTA |
| FoxM | SPU_025590, SPU_025738 | WHL22.548104 | A TCTGCTTCAAAGAAAGGCTCTG | GAATGTCCCACTCGGTTATTAGG |
| FoxN1/4 | SPU_002320 | WHL22.604594 | CTCCTATCCTTTCCCTGAATACC | TAATACGACTCACTATAGGGAGATGAAACTGAAGGCTGTGAAGG |
| Foxq2 | SPU_012384 | | CAACCGCCTCTAATTCCAAC | AACTGATAGTCTCAATGAAGAGTGC |
| Fra2 | SPU_021172 | WHL22.538597 | TGTTGACTACTATGGCGTCTGG | TAATACGACTCACTATAGGGAGACGAAGTTGAAGAGTCGAGTGG |
| Gbx | SPU_025491, SPU_025492 | WHL22.737659 | CCTTACCAGAGTCAACAAAAAGC | TAATACGACTCACTATAGGGAGAGGCCACAATAGTTAGCATTCG |
| Hairy2/4 | SPU_006814 | WHL22.446908 | CAAAATGCCTGTGGATACTAAACC | TAATACGACTCACTATAGGGAGAGTAGACTGGAATGAAATGACTTGG |
| Hbn | SPU_023177 | WHL22.523959 | TCATTACTTCGTTGGAGTTACCC | CATGAAAACGTCTGGATACTGG |
| HesC | SPU_021608 | WHL22.235339 | AGGACTGCCAAGCACCTTACA | TAATACGACTCACTATAGGGAGAAGGTGCTATTGTTAAGCACTC |
| Hlf | SPU_004414 | WHL22.306206 | GAAGACGACCGGATTCTCTACTT | TTGACAAGAGTCAGAAA TGAACG |
| Hmx | SPU_012490 | WHL22.152875 | ACAGTAGCCGTGAACTATCAGGA | TAATACGACTCACTATAGGGAGAGCTGATGATGTAGACGATGTTGA |
| Hnf6/onecut | SPU_016449 | WHL22.288683 | CGCTAGAGAAGGCCATGAAC | ACTCTCCCACTCTGCCTTGA |
| Hox7 | SPU_002634, SPU_005170 | WHL22.630154 | TCGGGGCTGTTCAGAGGAG | TGAAGGAGACCAGCGAA TAGAG |
| Ia1/Z176 | SPU_027472 | WHL22.769122 | ACCCTACAAGTGCAACTGAAACA | ATGGGCAAGTTGTGCAGTAATAA |
| IrxA | SPU_010351 | WHL22.651130 | CCACAAGTT A TTGTTGTTGCTGA | AAAGTCTCTCAGTCA TGGAGTCG |
| Isl | SPU_023730, SPU_023731 | WHL22.143854 | CTCACTGTGCGTGCTAAACG | CCTCAGGCCACA T AACTGCT |
| Klf13/Z188 | SPU_023727 | WHL22.198668 | TCTGTTGCAGGTTGGAGAATT | TAATACGACTCACTATAGGGAGACGTTGTCACCGACTCACTTTT |
| Klf2/4 | SPU_020311 | WHL22.483798 | GCAGCCTTTTTAGGAGAAACC | TAATACGACTCACTATAGGGAGAGGCCACAATAGTTAGCTTGGTGTAGG |
| Limc1/Lhx2 | SPU_004021, SPU_021313 | WHL22.91758 | CCTTAACAGCAGCATGAGTAGC | TAATACGACTCACTATAGGGAGATGCAGAACTCTCTTGGTCAGC |
| Msx | SPU_022049 | WHL22.119881 | TAAGTCATTCCATCCAAAGCAAC | TAATACGACTCACTATAGGGAGAGTCTTGGTCATCTTCAAAAGCTC |
| Nfat | SPU_015908 | WHL22.538507 | AA TGTA TCA TTGCA TACGTGGTG | GCTCTTTGTTTACAA TGGAGTGC |
| Nk2-2 | SPU_000756 | WHL22.739246 | TCTTTTCTTCTCCTGGTTTCCAC | TAATACGACTCACTATAGGGAGACTGACATACACGCTGATGCTG |
| Nkx2.1 | SPU_000757 | WHL22.739581 | TCGAGCTAGGGAGCTTCTGACTGTA | TAATACGACTCACTATAGGGAGACTTCACGGAATCTTTGTTCATTATG |
| Nkx3-2 | SPU_013047 | WHL22.329059 | ACCTGTAGTCAA TCGTCACTCGT | GCTCTCTCA TTTCCCTCCA TACT |
| Not | SPU_002129 | WHL22.632281 | AA TCCTTGGAAGACAGCACTTG | TAATACGACTCACTATAGGGAGACTGAACGTACTGGTTGTCGTG |
| Otx1+3 | SPU_010424 | WHL22.532435 | AACAGCAGCAACAGCAACAG | AGAGCTGCGTTCAAGGTCA T |
| Ptf1a | SPU_002677 | WHL22.476207 | AGCTAACCTGAGAGAGGAAGC | TAATACGACTCACTATAGGGAGAGTGATGAATAGGGGTGAGTTGC |
| Rfx2/3 | SPU_007611, SPU_012171 | WHL22.19679 | AGCCAGTCTCCTTTT ACTCCA TC | CA TCTGA TTGA TCTGTGACGTGT |
| Riz | SPU_027147 | WHL22.63053 | TGGAGTTCAGACAGTCACAGA TG | GATTCACTTTGGGCACATTTAAC |
| Rx | SPU_014289 | WHL22.523971 | AAGAGCAACGGTGAGATAAAAAC | GCTGATTATACGTTCAGGCAAGA |
| Shr2 | SPU_008117 | WHL22.302620 | AAAGCGAAGACAGTGAGGGAGAAG | TAATACGACTCACTATAGGGAGAGTGCTTGCGTATGCTGCGTTT |
| Six3 | SPU_018908 | WHL22.121654 | CTCATAGACACACCCCAGCA | AGGA TGGTGGGA TCTTTCTTC |
| SmadIP | SPU_022242 | WHL22.553144 | GATGATGGGGTAGATGACTTGG | TAATACGACTCACTATAGGGAGATCACTTGCAGGACTTGTTAAAGG |
| SoxB1 | SPU_022820 | WHL22.104606 | AGCATTCTCCATCGAGAGACTTG | TAATACGACTCACTATAGGGAGATTAGTACGATGGTTTCTTCTG |
| SoxB2 | SPU_025113 | WHL22.104525 | GCACGAAATACAGCATAACTT | TAATACGACTCACTATAGGGAGAAAAATCGTTGACAAATTTAGC |
| SoxC | SPU_002603 | WHL22.622787 | AAGAAAATTGGCCTAGAGATATGG | TAATACGACTCACTATAGGGAGAACTGAGAAGGAACAAGTGGAACC |
| Z133(fez) | SPU_019089, SPU_027491 | WHL22.580602 | CAACACCGCTACGCATATTG | TTATCCGACGTCTTGTCGTG |
| Z142 | SPU_022841 | WHL22.538539 | GCGACTATGAGACAACATACAACC | TAATACGACTCACTATAGGGAGGATCTGTACCTCACTCTGCTCTTCC |
| Z166/rxr | SPU_012645, SPU_028422 | WHL22.717588 | ATTCCCGCATCCCTTTATTC | CACACAATGGTGATGCAATTT |
| Zic/zic2(z244) | SPU_028583, SPU_030070 | WHL22.331651 | CAA TCGCGTTTCAGTTGACT AC | ACGT ACCA TTCACTCAAGTTCGT |

**Table S1.** Gene IDs and sequences of primers used for generation of in situ probes.

| Sea Urchin Apical Genes | | Mouse (forebrain/midbrain/hindbrain) | | Xenopus (forebrain/midbrain/hindbrain) | | *Danio rerio* (forebrain/midbrain/hindbrain) | |
| --- | --- | --- | --- | --- | --- | --- | --- |
| Gene name | SPU accession number | Panther | EGGNOG | Panther | EGGNOG | Panther | EGGNOG |
| 1 ac/sc | SPU_028148 | Ascl1 (LDO) | Ascl1, Ascl2 | Ascl2 (LDO) | Ascl2 | Ascl1a (LDO) | Ascl1a |
| 2 awh | SPU_018954 | Lhx8 (LDO) | Lhx8, Lhx6 | Lhx8 (LDO) | Lhx | Lhx8a (LDO) | LOC799481, Lhx8a |
| 3 brn1/2/4 | SPU_016443 | Pou3f2 (LDO) | Pou3f2, Pou3f1, Pou3f3, Pou3f4 | POU3F2 (LDO) | Pou3f3, Pou3f4, Pou3f2, Pou3f1 | POU3F2 (LDO) | Pou3f1, Pou3f2b, Pou3f3b, Pou3f3a, Pou3f2a |
| 4 delta | SPU_016128 | Dll1 (LDO) | Dll4, Dll1 | no ortholog | Dll1, Dll4, Dlc | Dld (LDO) | Dld, Dlc, Dll4, Dlb, Dla |
| 5 dri | SPU_005718 | Arid3b (LDO) | Arid3a, Arid3c, Arid3b | Arid3b (LDO) | Arid3a (neural plate), Arid3b | Arid3b (LDO) | 7955.ENSDARP00000126106, ARID3A, Arid3b, ARID3C |
| 6 ebf3 | SPU_004702 | Ebf3 (LDO) | Ebf3, Ebf2, Ebf1, Ebf4 | Ebf3 (LDO) | EBF1, 8364.ENSXETP00000027876, Ebf3, Ebf2 | Ebf3a (LDO) | Ebf1a, LOC557777, Ebf3, coe2, 7955.ENSDARP00000114469, LOC556476 |
| 8 foxG | SPU_009771 | Foxg1 (LDO) | Gm43517, Foxg1 | no ortholog | no ortholog | Foxg1a (LDO) | Foxg1a, Foxg1d |
| 9 foxj1 | SPU_027969 | no ortholog | Foxj1 | Foxj1.2 (LDO) | Foxj1.2 (neural plate), Foxj1 | no ortholog | Foxj1a, Foxj1b |
| 10 foxq2 | SPU_012384 | no ortholog | no ortholog | no ortholog | no ortholog | Foxq2 (LDO) | Foxq2 |
| 11 hbn | SPU_023177 | n.a. | no ortholog | n.a. | no ortholog | n.a. | no ortholog |
| 12 hlf | SPU_004414 | Hlf(LDO) | Hlf | Hlf(LDO) | Hlf | Hlfa (LDO) | Hlfb, Hlfa |
| 13 onecut | SPU_016449 | Onecut2 (LDO) | Onecut1, Onecut3,Onecut2 | Onecut2 (LDO) | Onecut3, Onecut1, Onecut2 | Onecut2 (LDO) | Onecut1, Onecut3a, Onecut2, Onecut3b |
| 14 insm | SPU_027472 | Insm1 (LDO) | Insm2, Insm1 | Insm1 (LDO) | Insm2 | Insm1b (LDO) | Insm1b, Insm2, Insm1a |
| 15 klf13 | SPU_023727 | Klf9,Zfp352,Klf16,Klf14,Klf17,Klf11,SP1,Klf10,Klf13 | Klf13 | Klf9,Klf11,Sp1,Klf10,Klf13 | Klf3 | zgc:153115,znf281b,Klf9,Klf11a,Klf11b,Klf13 | klf13 |
| 16 lhx2/9 | SPU_004021, SPU_021313 | Lhx9 (LDO) | Lhx9, Lhx2 | Lhx9 (LDO) | Lhx9, Lhx2 | Lhx9 (LDO) | Lhx2b, Lhx2a, Lhx9 |
| 17 nkx2.1 | SPU_000757 | Nkx2-5,Nkx2-6,Nkx2-3,Nkx2-4,Nkx2-1 | Nkx2-4, Nkx2-1 | NKX2-5 (LDO) | Nkx2-1, Nkx2-4 | NKX2-5 (LDO) | zgc:171531, Nkx2.1a, Nkx2.1b |
| 18 nkx3-2 | SPU_013047 | Nkx3-2 (LDO) | Nkx3-2 | Nkx3-2 (LDO) | Nkx3-2 | Nkx3-2 (LDO) | Nkx3.2 |
| 19 not | SPU_002129 | no ortholog | Noto | Not (LDO) | Noto | Noto (LDO) | Flh |
| 20 otx | SPU_010424 | Crx (LDO) | Crx, Otx2, Otx1 | Otx5 (LDO) | Otx1, Crx, Mixer, Otx2 | Otx5 (LDO) | Otx5, Otx1b, Otx2, Otx1a, Crx |
| 21 rx | SPU_014289 | no ortholog | Rax | no ortholog | Rax2(WO), Rax | Rx1 (LDO) | Rx1, Rx2, Rx3 |
| 22 six3 | SPU_018908 | Six6 (LDO) | Six6, Six3 | Six6 (LDO) | Six6, Six3 | Six6b (LDO) | Six6b, Six3b, Six6a, Six3a |
| 23 smadip | SPU_022242 | Zeb2 (LDO) | Zeb1, Zeb2 | Zeb2 (LDO) | Zeb2, Zeb1 | Zeb2b (LDO) | Zeb1a, Zeb1b, Zeb2b, Zeb2a |
| 24 soxb1 | SPU_022820 | Sox2 (LDO) | Sox2 | Sox2 (LDO) | Sox2 | Sox2 (LDO) | Sox2 |
| 25 soxb2 | SPU_025113 | Sox21 (LDO) | Sox21 | Sox21 (LDO) | Sox21 | Sox21b (LDO) | Sox21a, Sox21b |
| 26 soxC | SPU_002603 | Sox4 (LDO) | Sox4 | Sox11 (LDO) | Sox12 | Sox4b (LDO) | 7955.ENSDARP00000126100, Sox4b, Sox4a |
| 27 z133/fez | SPU_019089, SPU_027491 | n.a. | Fezf2, Fezf1 | n.a. | Fezf2, Fezf1 | n.a. | Fezf1, Fzf2 |
| 28 z166 | SPU_012645 | Znf746 | Gfi1, Gfi1b | XP_002944155 (zbtb46) | Gfi1, Gfi1b (WO) | no ortholog | Gfi1ab, Gfi1aa, Gfi1b |
| 29 zic | SPU_028583 | Zic1 (LDO) | Zic4, Zic2, Zic5, Zic1 | Zic1 (LDO) | Zic4, Zic2, Zic5, Zic1 | Zic1 (LDO) | Zic5, Zic4, Zic2b, Zic1, 7955.ENSDARP00000117357, Zic2a |

| Sea Urchin Apical Genes | | Drosophila melanogaster Brain/ procephalic ectoderm | | | C. elegans (head neurons, head ring ganglion) | | |
| --- | --- | --- | --- | --- | --- | --- | --- |
| Gene name | SPU accession number | Panther | EGGNOG | ENSEMBL | Panther | EGGNOG | ENSEMBL |
| 1 ac/sc | SPU_028148 | l(1)sc (LDO) | Ac, l(1)sc | Ac,ase,l(1)sc,sc | Hlh-3 (LDO) | Hlh-14, Hlh-3 | no ortholog |
| 2 awh | SPU_018954 | Awh (LDO) | Awh | Awh | Lim-4 (LDO) | Lim-4 | Lim-4 |
| 3 brn1/2/4 | SPU_016443 | Vvl (LDO) | Vvl | Vvl | no ortholog | Ceh-6 | Ceh-6 |
| 4 delta | SPU_016128 | no ortholog | Dl | no ortholog | Lag-2 (LDO) | no ortholog | no ortholog |
| 5 dri | SPU_005718 | Retn (LDO) | Retn | Retn | Cfi-1 (LDO) | Cfi-1 | Cfi-1 |
| 6 ebf3 | SPU_004702 | Kn (LDO) | Kn | Kn | Unc-3 (LDO) | Unc-3 | Unc-3 |
| 8 foxG | SPU_009771 | Slp2 (LDO) | fd19B, Slp2, Slp1 | Slp1 | no ortholog | Fkh-2, Pes-1 | Fkh-2 |
| 9 foxj1 | SPU_027969 | CHES-1-like,FoxK | CG32006 | no ortholog | no ortholog | no ortholog | no ortholog |
| 10 foxq2 | SPU_012384 | Fd102C (LDO) | Fd102C | Fd102C | Fkh-10 (LDO) | Fkh-10 | Fkh-10 |
| 11 hbn | SPU_023177 | n.a. | Hbn | Hbn | n.a. | no ortholog | Alr-1 |
| 12 hlf | SPU_004414 | Pdp1 (LDO) | Pdp1 | Pdp1 | Atf-8 (LDO) | no ortholog | no ortholog |
| 13 onecut | SPU_016449 | Onecut (LDO) | Onecut | Onecut | Ceh-21 (LDO) | Ceh-48 | Ceh-48 |
| 14 insm | SPU_027472 | Nerfin-2 (LDO) | Nerfin-1, Nerfin-2 | Nerfin-1, Nerfin-2 | Egl-46 (LDO) | Egl-46 | Egl-46 |
| 15 klf13 | SPU_023727 | Spps,l(3)neo38,btd,Sp1 | no ortholog | no ortholog | Sptf-3 | no ortholog | no ortholog |
| 16 lhx2/9 | SPU_004021, SPU_021313 | Ap (LDO) | Ap | no ortholog | Ttx-3 (LDO) | Ttx-3 | no ortholog |
| 17 nkx2.1 | SPU_000757 | Scro (LDO) | Scro | Scro | Ceh-24 (LDO) | Ceh-24 | Ceh-24,Ceh-27 |
| 18 nkx3-2 | SPU_013047 | Bap (LDO) | Bap | Bap | no ortholog | no ortholog | no ortholog |
| 19 not | SPU_002129 | CG18599 (LDO) | CG18599 | CG18599 | Ceh-87 (LDO) | no ortholog | no ortholog |
| 20 otx | SPU_010424 | Otd-oc (LDO) | Otd | Oc (otd) | Ceh-36 (LDO) | Ceh-36, Ceh-37, Ttx-1 | Ttx-1 |
| 21 rx | SPU_014289 | Rx (LDO) | Rx | no ortholog | no ortholog | Ceh-8 | Ceh-8 |
| 22 six3 | SPU_018908 | Optix (LDO) | Optix | Optix | Ceh-32 (LDO) | Ceh-32 | Ceh-32 |
| 23 smadip | SPU_022242 | Zfh1 (LDO) | Zfh1 | Zfh1 | Zag-1 (LDO) | Zag-1 | Zag-1 |
| 24 soxb1 | SPU_022820 | SoxN (LDO) | no ortholog | SoxN | Sox-2 (LDO) | Sox-2 | Sox-2 |
| 25 soxb2 | SPU_025113 | Sox21b (LDO) | Sox21b, Sox21a | no ortholog | no ortholog | no ortholog | no ortholog |
| 26 soxC | SPU_002603 | Sox14 (LDO) (express in the adult) | no ortholog | Sox14 | Sem-2 (LDO) | no ortholog | Sem-2 |
| 27 z133/fez | SPU_019089, SPU_027491 | n.a. | Erm | Erm | n.a. | Fezf-1 | Fezf-1 |
| 28 z166 | SPU_012645 | Sens (LDO) | Sens, Sens-2 | no ortholog | Pag-3 (LDO) | Pag-3 | no ortholog |
| 29 zic | SPU_028583 | Opa (LDO) | Opa | Opa | Ref-2 (LDO) | Ref-2 | Ref-2 |

**Table S2.** Regulatory genes of five bilaterian species that are orthologous to genes expressed in the sea urchin apical organ during pre-gastrular embryogenesis.

| | Sea urchin non-apical reg. genes | | Mouse (forebrain-midbrain-hindbrain) | | Xenopus (forbrain-midbrain-hindbrain) | | Zebrafish (forbrain-midbrain-hindbrain) | |
|---|---|---|---|---|---|---|---|---|
| | Gene name | SPU ID | Panther | EggNOG | Panther | EggNOG | Panther | EggNOG |
| 1 | BsxL | SPU_019366 | Bsx / Obox5 / Obox3 / Obox1 / Obox6 / Obox7/ Obox8 / Nanog | Bsx | Ventx2.2 / Vent1 / Ventx2.1 / Ventx3.1 / Ventx3.2 / Vex1 / Bsx | Bsx | Vent / Nanog / Vox / Bsx | Bsx |
| 2 | Couptf1 | SPU_023867 | Nr2f1 (LDO) | Nr2f1, Nr2f2 | Nr2f1 (LDO) | Nr2f1 / Nr2f2 | Nr2f1a (LDO) | Nr2f1b, Nr2f2, Nr2f1a |
| 3 | Dlx | SPU_002815 | Dlx4 (LDO) | Dlx1 | Dlx4 (LDO) | Dlx1 | Dlx4b (LDO) | Dlx1a |
| 4 | DmrtA2 | SPU_001255 | Dmrta2 (LDO) | Dmrta2 | Dmrta2 (LDO) | Dmrta2 | Dmrta2 (LDO) | Dmrta2 |
| 5 | Err | SPU_004723 | Esrra (LDO) | Esrra ,Esrrb,Esrrg | Esrra (LDO) | Esrrb / Esrrg / Esrra | Esrra (LDO) | Esrrb, Esrrga, Esrra, Esrrgb |
| 6 | Evi1 | SPU_018797 | Mecom (LDO) | Mecom,Prdm16 | LOC733566 (LDO) / XENTR_v90025893mg ENSXETG00000029998 (LDO) | PRDM16 / Mecom | si:ch73-138e16.3 (LDO) | Prdm16, 7955.ENSDARP00000119466, Mecom |
| 7 | Fos/Atf3 | SPU_021173 | Fosl2 | Atf3 | Fosl2 (LDO) | Atf3 | Fosl2 (LDO) | Atf3 |
| 8 | FoxC | SPU_024139 | Foxc2 (LDO) | Foxc2, Foxc1 | Foxc2 (LDO) | Foxc2 / Foxc1 | Foxc1b (LDO) | Foxc1a, Foxc1b |
| 9 | FtzF | SPU_013843 | Nr5a2 (LDO) | Nr5a1, Nr5a2 | Nr5a2 (LDO) | Nr5a2 / Nr5a1 | Nr5a2 (LDO) | Nr5a5, Nr5a2, Nr5a1a, Nr5a1b |
| 10 | Hnf1aL | SPU_010305 | Hnf1b (LDO) | Hnf1a,Hnf1b | Hnf1b (LDO) | Hnf1b / Hnf1a | Hnf1ba (LDO) | Hnf1ba, Hnf1bb, Hnf1a |
| 11 | Irf4 | SPU_026877 | Irf8 (LDO) | Irf4 | Irf8 (LDO) | Irf4 | Irf8 (LDO) | Irf4a, Irf4b |
| 12 | Klf15 | SPU_015772 | Klf15 | Klf15 | Klf15.2 (LDO) | Klf15 | si:ch211-117k10.3 (LDO) | klf15 |
| 13 | Lass6 | SPU_000948 | Cers5 (LDO) | Cers4,Cers5,Cers2,Cers6 | Cers5 (LDO) | Cers6 / Cers5 / Cers2 | Cers5 (LDO) | LOC565295, Cers4a, Cers6, Cers5, Cers4b, Cers2a |
| 14 | Lhx3-4 | SPU_001975 | Lhx3 | Lhx4,Lhx3 | Lhx3 (LDO) | Lhx4 / Lhx3 | Lhx3 (LDO) | 7955.ENSDARP00000087936, Lhx4 |
| 15 | Lox | SPU_026099 | Pdx1 | Pdx1 | Pdx1 (LDO) | Pdx1 | Pdx1 (LDO) | Pdx1 |
| 16 | Mitf | SPU_008175 | Tfe3 (LDO) | Mitf,Tfe3 | Tfe3 (LDO) | Mitf / Tfe3 | Tfe3a (LDO) | Tfe3a, Tfe3b, Titfb |
| 17 | Mll3 | SPU_026465 | Kmt2d (LDO) | Kmt2d, Kmt2c | Kmt2d (LDO) | Kmt2d / Kmt2c | Kmt2d (LDO) | Kmt2ca, Kmt2cb, Mll2 |
| 18 | Myc | SPU_003166 | Mycn (LDO) | Mycn, Mycs | Mycn (LDO) | Mycn | Mycn (LDO) | Mycn |
| 19 | Nfe2_1 | SPU_008752 | Nfe2l2 (LDO) | Nfe2l2, Nfe2l1 | Nfe2l1 (LDO) | Nfe2l1 | Nfe2l1b (LDO) | Nfe2l1a, Nfe2l1b |
| 20 | Osr | SPU_024877 | Osr2 (LDO) | Osr2 | Osr2 (LDO) | Osr2 | Osr2 (LDO) | Osr2 |
| 21 | Otp | SPU_019290 | Otp (LDO) | Otp | Otp (LDO) | Otp | Otpa (LDO) | Otpb, Otpa |
| 22 | Prx1 | SPU_018951 | no orthologs | Prrx1 | no orthologs identified | Prrx1 | no orthologs identified | Prrx1b, Prrx1a |
| 23 | Six1/2 | SPU_017379 | Six2 (LDO) | Six1, Six2 | Six2 (LDO) | Six2 / Six1 | Six2a (LDO) | Six1a, Six2b, Six1b, Six2a |
| 24 | Six4 | SPU_017380 | Six5 (LDO) | Six5, Six4 | Six4 | SIX4 | Six5 (LDO) | Six4a, Six4b, Six5 |
| 25 | Tbrg4 | SPU_020136 | Tbrg4 (LDO) | Tbrg4 | Tbrg4 (LDO) | Tbrg4 | Tbrg4 (LDO) | Tbrg4 |
| 26 | Tbx20 | SPU_018391 | TBX20 (LDO) | Tbx10, Tbx20, Tbx1, Tbx22, Tbx18, Tbx15 | Tbx20 (LDO) | Tbx15, TBX15, Tbx1, Tbx20, Tbx22 | Tbx20 (LDO) | Tbx22, Tbx20, 7955.ENSDARP0000011039 5, Tbx18, Tbx15, Tbx1 |
| 27 | Tgif | SPU_018126 | Tgif2lx2 / Tgif1 / Tgif2 | Tgif1 | Tgif1 (LDO) | Tgif1 | Tgif1 (LDO) | Tgif1 |
| 28 | Trh | SPU_014249 | Npas1 (LDO) | Npas1,Npas3 | Npas3 | Npas3 | Npas1 (LDO) | Npas3b(npas1), NPAS3 |

| | Sea urchin non-apical reg. genes | | Drosophila (Brain /procephalic ectoderm) | | | C. elegans (head neurons, head ring ganglion) | | |
|---|---|---|---|---|---|---|---|---|
| | Gene nam | SPU ID | Panther | EggNOG | ENSEMBL | Panther | EggNOG | ENSEMBL |
| 1 | BsxL | SPU_019366 | Bsh | Bsh | Bsh | Ceh-23 / Ceh-51 / Tab-1 | Tab-1 | Tab-1 |
| 2 | Couptf1 | SPU_023867 | Svp (LDO) | Svp | Svp | Nhr-91 (LDO) | Unc-55 | no orthologs identified |
| 3 | Dlx | SPU_002815 | Dll (LDO) | Dll | Dll | Ceh-43 (LDO) | Ceh-43 | Ceh-43 |
| 4 | DmrtA2 | SPU_001255 | Dmrt99B (LDO) | Dmrt99B | Dmrt99B | Dmd-5 (LDO) | Dmd-5 | Dmd-5 |
| 5 | Err | SPU_004723 | Err (LDO) | Err | Err | no orthologs identified | no orthologs identified | no orthologs identified |
| 6 | Evi1 | SPU_018797 | Ham (LDO) | CG10348, Ham | CG10348 | Egl-43 (LDO) | Egl-43 | Egl-43 |
| 7 | Fos/Atf3 | SPU_021173 | Kay (LDO) | Atf3 | no orthologs identified | Fos-1 | no orthologs identified | no orthologs identified |
| 8 | FoxC | SPU_024139 | Croc (LDO) | Croc | Croc | Fkh-9 (LDO) | n.a. | no orthologs identified |
| 9 | FtzF | SPU_013843 | Ftz-f1 (LDO) | Ftz-f1 | Ftz-f1 | Nhr-25 (LDO) | Nhr-25 | Nhr-25 |
| 10 | Hnf1aL | SPU_010305 | no orthologs identified | no orthologs identified | no orthologs identified | no orthologs identified | no orthologs identified | no orthologs identified |
| 11 | Irf4 | SPU_026877 | no orthologs identified | no orthologs identified | no orthologs identified | no orthologs identified | no orthologs identified | no orthologs identified |
| 12 | Klf15 | SPU_015772 | Klf15 (LDO) | Klf15 | Klf15 | Klf-3 / Klf-2 / Klf-1 | no orthologs identified | no orthologs identified |
| 13 | Lass6 | SPU_000948 | Schlank (LDO) | Schlank | Schlank | Hyl-1 (LDO) | Hyl-2, Hyl-1 | Hyl-2, Hyl-1 |
| 14 | Lhx3-4 | SPU_001975 | Lim3 (LDO) | Lim3 | Lim3 | Ceh-14 (LDO) | Ceh-14 | Ceh-14 |
| 15 | Lox | SPU_026099 | Zen2 | no orthologs identified | no orthologs identified | no orthologs identified | no orthologs identified | no orthologs identified |
| 16 | Mitf | SPU_008175 | Mitf (LDO) | Mitf | Mitf | Hlh-30 (LDO) | Hlh-30 | Hlh-30 |
| 17 | Mll3 | SPU_026465 | Trr (LDO) | Trr, Lpt | Trr | Set-16 (LDO) | Set-16 | Set-16 |
| 18 | Myc | SPU_003166 | Myc (LDO) | no orthologs identified | no orthologs identified | no orthologs identified | no orthologs identified | no orthologs identified |
| 19 | Nfe2_1 | SPU_008752 | Cnc (LDO) | Cnc | Cnc | Skn-1 (LDO) | Sknr-1, Skn-1 | no orthologs identified |
| 20 | Osr | SPU_024877 | Bowl (LDO) | Bowl, Odd, Sob | Bowl, Odd, Sob | Odd-1 (LDO) | Odd-2, Odd-1 | Odd-2, Odd-1 |
| 21 | Otp | SPU_019290 | Opt (LDO) | Otp | Otp | no orthologs identified | no orthologs identified | no orthologs identified |
| 22 | Prx1 | SPU_018951 | CG9876 (LDO) | no orthologs identified | CG9876 | Alr-1 (LDO) | no orthologs identified | no orthologs identified |
| 23 | Six1/2 | SPU_017379 | So (LDO) | So | So | Ceh-34 (LDO) | Ceh-33, Ceh-34 | no orthologs identified |
| 24 | Six4 | SPU_017380 | Six4 (LDO) | Six4 | Six4 | Unc-39 (LDO) | no orthologs identified | Unc-39 |
| 25 | Tbrg4 | SPU_020136 | CG13850 (LDO) | CG13850 | CG13850 | B0564.7 (LDO) | no orthologs identified | no orthologs identified |
| 26 | Tbx20 | SPU_018391 | H15 | H15, Mid, Org-1 | H15, Mid | Mab-9 | Mls-1, Mab-9 | Mab-9 |
| 27 | Tgif | SPU_018126 | Vis (LDO) / Achi (LDO) | Vis, Achi | Vis, Achi | Ceh-60 (LDO) | no orthologs identified | no orthologs identified |
| 28 | Trh | SPU_014249 | Trh (LDO) | Trh | Trh | Hlh-34 (LDO) | no orthologs identified | Hlh-34 |

**Table S3.** Regulatory genes of five bilaterian species that are orthologous to selected genes that are not expressed in the sea urchin apical organ during pre-gastrular embryogenesis.

| Upstream TF | Type of interaction | Reg. Gene B | Domain | Reference |
|---|---|---|---|---|
| | | | | |
| SoxB1 | Activates | *foxq2* | Apical Ectoderm | [17] |
| SoxB1 | Represses | *soxb1* | Apical Ectoderm | [22], [17] |
| SoxB1 | Activates | *soxb2* | Apical Ectoderm | [17] |
| SoxB1 | Activates | *hnf6/onecut* | Apical Ectoderm | [80], [17] |
| SoxB1 | Activates | *nkx2.1* | Apical Ectoderm | [17] |
| SoxB1 | Activates | *not* | Oral Ectoderm | [17] |
| SoxB1 | Activates | *nodal* | Oral Ectoderm | [79] |
| Six3 | Activates | *foxq2* | Apical Ectoderm | [29] |
| Six3 | Represses | *nodal* | Oral Ectoderm | [29] |
| Not | Represses | *not* | Oral Ectoderm | [81] |
| Nodal | Represses | *hbn* | Oral Ectoderm | [33] |
| Emx | Represses | *foxq2* | Oral Ectoderm | [22] |
| FoxQ2 | Represses | *foxq2* | Apical Ectoderm | This study and [22] |
| FoxQ2 | Represses | *six3* | Apical Ectoderm | [22] |
| FoxQ2 | Represses | *nodal* | Apical Ectoderm | [21] |
| FoxQ2 | Represses | *emx* | Apical Ectoderm | [22] |
| FoxQ2 | Activates | *nkx2.1* | Apical Neuro ectoderm | This study |
| FoxQ2 | Activates | *dkk3* | Apical Neuro ectoderm | This study and [20] |
| FoxQ2 | Activates | *zic* | Apical Neuro ectoderm | This study |
| FoxQ2 | Activates | *SFRP1/5* | Apical Neuro ectoderm | This study and [20] |
| FoxQ2 | Activates | *foxj1* | Apical Neuro ectoderm | This study |
| FoxQ2 | Activates | *nkx3-2* | Apical Neuro ectoderm | This study |
| FoxQ2 | Activates | *lhx2-9* | Apical Neuro ectoderm | This study |
| FoxQ2 | Activates | *soxc* | Neural Differantion | This study |
| FoxQ2 | Activates | *ac/sc* | Neural Differantion | This study |
| FoxQ2 | Activates | *ebf3* | Neural Differantion | This study |
| SoxC | Activates | *brn1/2/4* | Neural Differantion | This study and [24] |
| SoxC | Activates | *ebf3* | Neural Differantion | This study |
| SoxC | Activates | *insm* | Neural Differantion | This study |
| Hbn | Activates | *soxc* | Apical Neuro ectoderm | This study |
| Hbn | Activates | *smadIP/zfhx* | Apical Neuro ectoderm | [33] |
| Hbn | Activates | *z133/fez* | Apical Neuro ectoderm | [33] |
| Delta/Notch | Represses | *soxc* | Neural Specification | This study and [23] |

**Table S4.** List of regulatory interactions between an upstream transcription factor (TF) and a downstream regulatory gene, identified here and elsewhere.