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Developmental Expression Pattern

Title : Differential expression of *arid5b* isoforms in *Xenopus laevis* pronephros

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Short running title : *arid5b* expression in *Xenopus* embryo

Key words: *arid5b*, *Xenopus*, pronephros

Abbreviations :

RA : retinoic acid

ARID : A-T Rich Interaction Domain

aa : amino acid

nt : nucleotide

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1

2 **Abstract**

3 Arid5b belongs to the ARID family of transcription factors characterised by a helix-turn-helix motif- based DNA-
4 binding domain called ARID (A-T Rich Interaction Domain). In human, alternative splicing leads to a long and a
5 short isoforms (isoform1 and 2, respectively) that differ in their N-terminal part. In this study, we report the
6 cloning and expression pattern of *Xenopus laevis arid5b*. We have isolated a full length cDNA that shows
7 homology with the human *arid5b isoform1*. Furthermore, 5'RACE experiments revealed the presence of a
8 shorter isoform equivalent to the human isoform2. Temporal expression analysis by RT-qPCR indicated that *X.*
9 *laevis arid5b isoform1* and *isoform2* are differentially expressed during development. *Isoform1* is strongly
10 expressed maternally while *isoform2* expression is essentially restricted to tailbud stages. Spatial expression
11 analysis by whole mount *in situ* showed that *arid5b* is predominantly expressed in the developing pronephros.
12 *Arid5b* mRNAs are detected in the antero-dorsal part of the pronephros anlage at the early tailbud stage and
13 later on, in the proximal part of the pronephric tubule. RT-qPCR analyses with primers that allow to
14 discriminate *isoform1* from *isoform2* showed that the latter is enriched in the pronephros anlage. In
15 agreement with a specific pronephric signature of the *isoform2*, we also observed that *isoform2* but not
16 *isoform1* is upregulated in animal caps induced to form pronephric tissue in response to activin A and retinoic
17 acid. These results indicate that the two *arid5b* isoforms are differentially expressed and likely play different
18 roles during early *Xenopus* development.

19

20

21 **Introduction**

22 The ARID (A-T Rich Interaction Domain) is a helix–turn–helix motif-based DNA-binding domain, conserved in
23 eukaryotes, that defines the ARID family of transcription factors. The human ARID family can be divided into
24 seven subfamilies (ARID1, ARID2, ARID3, ARID4, ARID5, JARID1 and JARID2) based both on degree of homology
25 within the ARID domain, as well as similarity between highly variable non-ARID domain structures. The
26 founding members, murine *Bright (ARID3A)* and *Drosophila dead ringer (Dri)*, were independently cloned on
27 the basis of their ability to selectively bind to AT-rich DNA sequences but this behaviour is not an intrinsic
28 feature of the ARID domain since the majority of ARID subfamilies bind DNA without obvious sequence
29 preference (Patsialou *et al.*, 2005). ARID-encoding genes are involved in a variety of biological processes
30 including regulation of cell cycle, gene expression, differentiation, embryonic development, transcriptional
31 regulation and chromatin-remodeling. The ARID protein Osa has been shown to associate with the SWI/SNF

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32 complex in both *Drosophila* and humans, suggesting that the function of ARID proteins in chromatin
33 remodelling has been conserved through evolution (Collins *et al.*, 1999, Kozmik *et al.*, 2001).

34
35 *Arid5b*, also called *Desrt* or *MRF-2* (modulator recognition factor-2), was originally cloned thanks to its ability
36 to bind sequences in the transcriptional modulator of the human cytomegalovirus major immediate- early
37 promoter (Lubon *et al.*, 1989). Its ARID domain, whose three- dimensional structure has been solved, binds
38 preferentially to an AT-rich core sequence (Whitson *et al.*, 1999). *Arid5b* has been found to associate with the
39 jmjC demethylase PHF2. Assembly of the PHF2–*Arid5b* complex, its recruitment to target promoters, and its
40 H3H9Me2 demethylase activity are dependent on protein kinase A activity (Baba *et al.*, 2011). During mouse
41 organogenesis, *arid5b* displays a complex and highly dynamic pattern of expression. It is first expressed in the
42 intermediate mesoderm and subsequently in the nephrogenic cords of the urogenital ridges. *Arid5b* is also
43 detected in the limbs, the myotomes, the oro-naso- pharyngeal ectoderm and the underlying mesenchyme,
44 the otic vesicles, the gut and its derivatives, and transiently in the liver. *Arid5b* mutant mice generated by gene
45 targeting have reduced viability, pronounced growth retardation, and a high incidence of abnormalities of the
46 reproductive organs (Lahoud *et al.*, 2001). They also show significant reductions in lipid accumulation and
47 weight gain in postnatal and adult life (Whitson *et al.*, 2003). *Arid5b* is required for adipogenesis and to
48 maintain normal functions in mature adipocytes. Knockdown of *Arid5b* in mature 3T3-L1-derived adipocytes
49 activates both lipolysis and triglyceride synthesis, and causes a significant increase in the ratio of glycerol
50 release to free fatty acid release (Yamakawa *et al.*, 2010, Yamakawa *et al.*, 2008). *Arid5b* is highly expressed in
51 the cardiovascular system and is believed to play essential roles in smooth muscle cell differentiation and
52 proliferation (Watanabe *et al.*, 2002). In homozygous *arid5b* mutant mice, kidneys are small showing often
53 degraded glomeruli with defects in smooth muscle cell number and location. Skeletal abnormalities, including
54 defects in the patterning of the ribs and sternum, have also been described (Schmahl *et al.*, 2007).

55 Although the temporal and spatial pattern of expression of *arid5b* during embryogenesis have been described
56 in mouse (Ristevski *et al.*, 2001), there is no detailed expression data available for non mammalian vertebrates.
57 We report the cloning of two *arid5b* isoforms in *Xenopus laevis* and have examined their expression patterns
58 during development.

59

60 Results and discussion

61 Molecular cloning of *X. laevis arid5b*

62 In order to clone a full coding sequence of *arid5b* in *Xenopus*, we started from a partial IMAGE clone sequence
63 (no 686,6480), and obtained the missing 5' sequence by RACE PCR. A 3570 nucleotides (nt) clone was
64 amplified by end-to-end PCR (GenBank accession no. HG518326). Sequence analysis revealed an open reading

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65 frame encoding a predicted 1187 amino acids (aa) protein (Fig.S1). This protein displays 87.6% identity with a
66 predicted *X. tropicalis* protein sequence deduced from gene models (accession no. XM_002939542), 57.5%
67 identity (82.2% similarity) with the long human Arid5b isoform1, and 56.7% identity (80.7% similarity) with the
68 murine long isoform α . The ARID domain (aa 324-418) is highly conserved, with more than 90% identity
69 observed with other vertebrate Arid5b sequences (Fig.1). The conserved sequence includes a lysine residue
70 (lys-342) at a position homologous to the lysine of the long murine isoform α (lys-336) whose demethylation
71 by PHF2 promotes recruitment of PHF2-Arid5b complex to promoters (Baba et al., 2011). Blast analysis on the
72 *X. laevis* genome 6.0 scaffolds shows that the long isoform is encoded by two genes located on scaffolds 9729
73 and 48311, respectively, which probably represent pseudoalleles resulting from *X. laevis* allotetraploidy. In a
74 similar way to human and murine *arid5b*, the long *X. laevis* isoform is encoded by ten different exons (table
75 S1,S2).

76 We further investigated whether a shorter isoform homologous to the short human *arid5b* isoform was also
77 detectable. Human *arid5b isoform2* is generated by alternative splicing resulting in the replacement of phe-
78 244 by a start methionine. Using 5' RACE-PCR, we were able to clone a partial *X. laevis arid5b* sequence of
79 368nt containing an ORF encoding a 91 aa polypeptide where phe-244 is replaced by a methionine. The
80 following aa are identical to those of the long isoform (ala-245-leu-334). The 95 nt sequence located upstream
81 to this ATG codon does not contain any other in frame ATG codon, but three stop codons indicating that it
82 probably encodes the 5'UTR of a shorter isoform mRNA. Blast analysis on the *X. laevis* genome revealed that
83 this sequence is encoded by a novel exon (exon 4b, table S1,S2), while ala-245-leu334 are encoded by exons 5
84 and 6, supporting the idea of an isoform generated by alternative splicing. Nested 3'RACE-PCR was carried out
85 to clone the full sequence encoding this putative short isoform. The first primer was located 61nt upstream of
86 the putative start ATG, and the nested primer 1307nt downstream of this ATG. A 2163nt sequence was cloned
87 containing 1529nt of putative coding sequence, and 634nt of 3'UTR. It is identical to the sequence of the long
88 isoform, in line with human *arid5b isoform2* sequence data, which only differs from isoform1 at the start ATG.
89 Using different sets of primers, we have then tried to amplify the entire short isoform by end-to-end PCR but
90 failed to amplify a full cDNA. Using forward primers corresponding to exon 4b sequence, and reverse primers
91 at different levels of the sequence obtained by 3' RACE-PCR, we could only amplify a partial cDNAs encoding
92 the first 476 aa of the short isoform. This cDNA corresponds to exons 4b, 5-9 and part of exon 10 (accession
93 number HG518327). Whether the short isoform is ending at the same stop codon as the long one therefore
94 remains unclear. Nonetheless, a 3' sequence for the short isoform mRNA distinct from that of the long isoform
95 would imply an alternative splicing within exon10, that is not occurring with human *arid5b isoform2*. Together,
96 these data show that the short isoform1 lacks the first 243 N-terminal amino acids of the long isoform2
97 (Fig.S2). Using InterProScan software and performing an extensive analysis of the literature on Arid5 family
98 members, we could not find any known domain in this region, precluding to identify any functional difference

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99 between the two isoforms.

100 Genomic synteny and molecular phylogeny

101 In order to further confirm the identity of the *Xenopus* ortholog of *arid5b*, we have examined the synteny
102 maps from human, mouse and *X. tropicalis* genomes. Synteny maps were obtained from Ensembl genome
103 browser (release 74, December 2013) and JGI genome browser (*X. tropicalis* version 7.1). Synteny maps are
104 shown in Fig.1B. Flanking genes are partially conserved between *arid5b* genes in human, mouse and *X.*
105 *tropicalis* genomes. The conserved syntenic region flanks the 3' end of *arid5b*. It comprises *rkn2*, *znf365*, *ado*
106 and *egr2* genes. Genes flanking the 5' end of *arid5b* in *X. tropicalis* genome totally differ from those flanking
107 human or mouse *arid5b*. Phylogenetic analysis indicates that *Xenopus arid5b* is relatively distant from the
108 zebrafish ortholog. Tetrapod orthologs appear to be more closely related, with the chicken ortholog being the
109 most closely related to *Xenopus arid5b* (Fig.1C).

110

111 Spatial and temporal expression of *arid5b* during *X. laevis* development

112 Temporal expression of *ari5b* during early *X. laevis* development was examined by RT-qPCR. Specific primers
113 were designed in order to amplify either both *arid5b* isoforms (total *arid5b*), *isoform1* or *isoform2* (Fig. S3).
114 *Isoform1* was strongly expressed during cleavage stages; its expression declined during gastrulation and
115 neurulation, then it increased at tailbud stages (fig.2B). In contrast, *isoform2* transcripts were scarcely
116 detected at the pretailbud stages, but became detectable during organogenesis and persisted at least up to
117 the late tailbud stage (stage 28) (fig.2C). Notably, the temporal profile of *isoform1* is almost identical to the
118 profile obtained for total *arid5b*, suggesting that *isoform1* is the main isoform to be expressed during
119 embryonic development (fig.2A,B). In agreement with this idea, when normalized to total *arid5b*, relative
120 expression of *isoform1* was constant during embryonic development while relative mRNA expression of
121 *isoform2* increased during tailbud stages (fig.2D,E). Thus, *isoform1* and *isoform2* are differentially expressed
122 during development. *Isoform1* is strongly expressed maternally and is the main isoform expressed during
123 embryonic development. *Isoform2* expression is essentially restricted to tailbud stages.

124 Next, we studied the spatial expression pattern of *arid5b* by whole mount *in situ* hybridization using two
125 probes : the first encompassing nucleotide 775 to 1612 of *isoform1* (which corresponds to nucleotides 46 to
126 853 of *isoform2*); the second corresponding to nucleotide 529 to 3564 of *isoform1*. Both probes gave the
127 same expression pattern (fig.4 and data not shown). At cleavage, gastrula and neurula stages, embryos were
128 uniformly stained indicating that *arid5b* mRNAs were ubiquitously distributed (not shown). RT-qPCR analysis of
129 dissected explants from early gastrula confirmed this observation and further showed that none of the two
130 isoforms showed a regionalized expression (fig.3). At the early tailbud stage, a specific signal was detected in

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131 the pronephric region by *in situ* hybridization (fig.4A). At stage 25, *arid5b* was strongly expressed in the antero-
132 dorsal part of the pronephric anlage. In comparison with *delta1* expression which is restricted to the most
133 antero-dorsal part of the anlage ((Rones *et al.*, 2000) et fig.4C,F), *arid5b* is expressed in a larger domain that
134 extends more ventrally and medially (fig.4B,E). Expression of *arid5b* remained restricted to the anterior part of
135 the developing pronephros at tailbud stages (fig.4D). At tadpole stage 35/36, *arid5b* mRNAs were localized in
136 the proximal part of the pronephric tubule (fig.4H,J,I,L). *Arid5b* expression domain is adjoining to that of
137 *scl12a1* which marks the intermediate and the first distal segments (Raciti *et al.*, 2008) (fig.4H,I,L). Thus, from
138 the early tailbud stage onward, *arid5b* expression is restricted to the developing proximal part of the
139 pronephros. Since our *in situ* hybridization experiments do not allow to distinguish between the two isoforms,
140 we performed RT-qPCR to analyse the expression levels of each isoform in the pronephric anlage in
141 comparison to the whole embryo and to different embryonic regions. The results clearly showed that *isoform2*
142 is more strongly expressed in the pronephric anlage than in the other tissues at tailbud stage (fig.5). No such
143 enrichment was observed for *isoform1* although it is slightly more expressed in the head in comparison to
144 other tissues. As expected, the kidney marker *pax8* was found to be strongly expressed in the pronephric
145 anlage (fig.5). It has been previously shown that treatment of blastula animal cap ectoderm with activin A and
146 retinoic acid (RA) results in the formation of pronephric tubules at high frequency (Ariizumi and Asashima,
147 2001). We studied whether this treatment could upregulate *arid5b* isoforms expression. We showed that
148 *isoform2* but not *isoform1* expression is upregulated in response to activin A and RA (fig.6). This result is in
149 agreement with a specific pronephric signature of the *isoform2* .

150 In summary, we have cloned the *X. laevis* ortholog of *arid5b*, identified two isoforms and examined their
151 expression pattern during embryonic development. The two isoforms are differentially expressed : *isoform1* is
152 strongly expressed maternally, while *isoform2* is specifically expressed in the pronephric anlage at tailbud
153 stage. These results indicate that the two *arid5b* isoforms likely play different roles during early *Xenopus*
154 development.

155

156

157 Materials and Methods

158 Molecular cloning and Bioinformatic analyses

159 The partial IMAGE clone 696,6480 was obtained from RZPD ImaGenes. RACE-PCR was performed with the
160 SMARTer™ RACE cDNA amplification kit (Clontech). End-to-end PCR was carried out according to
161 manufacturer instructions with Advantage 2 polymerase (Clontech), and stage 35/36 embryo cDNA prepared
162 as described (Le Bouffant *et al.*, 2012). Amino acid sequence comparison were performed with MultAlin
163 software (<http://multalin.toulouse.inra.fr/multalin/>) (Corpet, 1988) and CLUSTAL W (version 1.83).

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165 Embryos, explants dissection, animal cap assay

166 *Xenopus laevis* were purchased from the CNRS *Xenopus* breeding Center (Rennes, France). Embryos were
167 obtained after artificial fertilization, and were raised in modified Barth's solution (MBS). Stages were according
168 to the normal table of *Xenopus laevis* (Nieuwkoop and Faber, 1967). Dissections were all performed in 1X MBS
169 on 1% agar-coated dishes. Presumptive ectoderms (animal cap) were isolated from blastula stage embryos
170 (stages 8–9) and immediately transferred into 1X MBS, 0.1% BSA in the presence of recombinant human
171 activin A (10 ng/mL, Sigma) and all-trans retinoic acid (10^{-4} M, Sigma) or DMSO alone (1/250). The animal caps
172 were incubated for 3 hrs, after which they were washed twice in 1X MBS and further cultured for 48 hrs.
173 Explants comprising the pronephric anlage were dissected from early tailbud embryos (stage 21). Using
174 platinum loop and wire, stage 25 embryos were dissected into several pieces : the head, the tail, the truncal
175 dorsal part (essentially somites, neural tube and notochord) and the truncal ventral part (mainly endoderm,
176 ventral and lateral mesoderm). Pronephric anlagen were isolated from somitic and lateral plate mesoderm
177 and separated from the underlying endoderm. The overlying ectoderm was kept. Explants were immediately
178 processed for RT-qPCR.

179 Real-time quantitative PCR

180 RT-PCR analyses were carried out as previously reported in (Le Bouffant *et al.*, 2012). Sequences of
181 oligonucleotides used are the following : *arid5b* : forward: 5'TATGTTTCAAGCTGCGCAAAA3', reverse:
182 5'CCATTGCCTCCGTGCAGTA3' ; *arid5b isoform1* forward : 5'CCCAGAAGATACCCCAAGG3', reverse :
183 5'ACTTCATGCTCTCCGTGGCT3' ; *arid5b isoform2* : forward : 5'TGCTCTGTGGCGTTCATGAG3'
184 reverse : 5'TCGACTAGCATCTGTCTCGTTTGT3' ; *pax8* : forward : 5'CAGCAATTTCAATATAGGTCACGG3', reverse
185 5'TCCATTCACAAAAGCCCCAC3' ; *ODC* : forward : 5'GGGCAAAGGAGCTTAATGTGG3', reverse
186 5'TGCCAACATGGAAACTCACAC3'. The Comparative Ct method was used to determine the relative quantities of
187 mRNA, using *ODC* mRNA as the endogenous reporter except for figure 2D,E for which *arid5b* was used . Same
188 results were obtained using *β -actin* mRNA as the endogenous reporter instead of *ODC* (data not shown). Each
189 RNA sample was analysed in duplicate. Each data point represents the mean \pm SEM of at least three
190 independent experiments. Data were analysed using R Commander (R software) by paired Student's t-test.

191

192 *In situ* hybridization

193 Whole mount *in situ* hybridization for *arid5b*, *delta1* (Rones *et al.*, 2000), and *slc12a1* (Raciti *et al.*, 2008) were
194 carried out as previously reported (Cartry *et al.*, 2006). The antisense and control sense RNA probes for *arid5b*
195 were generated from linearized plasmids containing cDNA sequences from nucleotide 529 to 3564 of

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196 *isoform1* and from nucleotide 775 to 1612 of *isoform1* (which corresponds to nucleotides 46 to 853 of
197 *isoform2*). The *arid5b* RNA probes were subjected to limited alkaline hydrolysis in two volumes of carbonate
198 buffer (60 mM Na₂CO₃, 40 mM NaHCO₃, pH 10.2) for 5 min at 60°C to reduce its size and increase its access to
199 tissues. The hydrolysis was terminated by adding an equal volume of neutralizing solution (1 M Tris-HCl, pH 8.0,
200 containing 1.5 M NaCl). Hydrolyzed fragments were precipitated with ethanol.

201

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208

209 **Figure Legends**

210 **Fig. 1** : Characterization of a *X. laevis* ortholog of *arid5b*.

211 **(A)** Predicted ARID domain amino acid sequence comparison: *G. gallus* (ac Q5ZJ69); *H. sapiens* (ac Q14865); *X.*
212 *tropicalis* (F6QQ73); *D. rerio* (E7F888). **(B)** Synteny blocks containing *arid5b* genes in *H. sapiens*; *M. musculus*
213 and *X. tropicalis* genomes. Genes organization in the human *arid5b* gene region was used as basis for
214 comparison. Chromosomal localization is indicated. The scaffold number is given for *X. tropicalis*. Relative
215 spacing between the genes is not shown. **(C)** Phylogenetic tree of *arid5b* genes from various vertebrate species
216 constructed using the neighbour-joining method. Accession numbers used are shown.

217

218 **Fig. 2** : Temporal expression of *arid5b* during *Xenopus* embryonic development

219 Expression of *arid5b* analysed by RT-qPCR at cleavage (stages 5 and 9), gastrula (stage 11), neurula (stages 14)
220 and tailbud stages (stages 22 and 28). Primers were designed in order to amplified either both isoforms (total
221 *arid5b*) **(A)**, *isoform1* **(B,D)** or *isoform2* **(C,E)**. The relative quantities of mRNA were determined with *ODC*
222 **(A,B,C)** or total *arid5b* **(D,E)** mRNA as the endogenous reporter. *Isoform1* is strongly maternally expressed;
223 *isoform2* is mainly expressed during tailbud stages. Average values from three independent experiments.

224 **Fig. 3** : Expression of *arid5b* at the early gastrula stage

225 RT-qPCR analyses for total *arid5b*, *isoform1* and *isoform2* were performed on dissected explants from early
226 gastrula stage embryo (stage10.5). Embryos were dissected either into ventral and dorsal halves or into dorsal

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227 marginal zone (DMZ), ventral marginal zone (VMZ), lateral marginal zone (LMZ) ectoderm and endoderm. Both
228 isoforms are ubiquitously expressed at the early gastrula stage. Average values from three independent
229 experiments.

230

231 **Fig. 4 : Spatial expression of *arid5b* during *Xenopus* development**

232 *In situ* hybridization of whole (A-D, G-L) or transverse fractured embryos (E, F) at the indicated stages of
233 development with antisense probe for *arid5b* (nucleotide 775 to 1612 of *isoform1*) (A,B,D,E,H,J,L,I), *delta1*
234 (C,F), *slc12a1* (I,K,L) and with control sense probe for *arid5b* (G). Lateral views with anterior to the right (A-
235 D,G,H, J-L). Transverse section at the level of the proximal pronephric tubule. In I and L, *arid5b* is revealed in
236 light blue and *slc12a1* in purple. *Arid5b* mRNAs are detected in the anterior pronephric anlage during tailbud
237 stages in a broader domain than *delta1*. At tadpole stage 35/36, *arid5b* expression is restricted to the proximal
238 part of the tubule and does not overlap with *slc12a1* expression which is specific for the intermediate and the
239 first segment of the distal tubule. Scale bars are 0.3 mm.

240

241 **Fig. 5 : *Arid5b isoform2* is specifically expressed in the pronephros**

242 Expression of *isoform1*, *isoform2* and *pax8* analysed by RT-qPCR in different embryonic regions (see materials
243 and methods) dissected at tailbud stage 25. *Isoform2* as well as *pax8* are strongly expressed in pronephric
244 anlage in comparison to other embryonic tissues (statistically significant for all). *Isoform1* is slightly more
245 expressed in the head in comparison to other tissues. Average values from three independent experiments. *
246 $P < 0.05$

247 **Fig. 6 : *Arid5b isoform2* is specifically induced in animal caps treated with activin A and RA**

248 RT-qPCR analysis of *arid5b isoform1* and *isoform2*, as well as *pax8* expression in induced blastula animal caps.
249 Animal caps were dissected at blastula stage 9, incubated for 3 hours in presence of RA and activin A, or mock
250 solution for the control group. Animal caps were further cultured in 1X MBS for 48h hours and processed for
251 RT-qPCR. A significant increase of *isoform2* expression is observed in response to RA and activin A. Average
252 values from three independent experiments. * $P < 0.05$ ** $P < 0.005$

253

254 **Legends to Supplementary materials**

255 **Fig. S1 : Comparison of *Arid5b isoform1* amino acid sequence between vertebrates**

256 Predicted amino acid sequence comparison of *Arid5b*: *X. tropicalis* (F6QQ73) isoform1; *G. gallus* (Q5ZJ69); *H.*
257 *sapiens* (Q14865); *M. musculus* (Q8BM75); *D. rerio* (E7F888). Red boxes indicate amino acid residues

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258 conserved in all species. The green arrow indicates phe-244 of isoform1 that is replaced by a start methionine
259 in isoform2.

260 **Fig. S2** : Amino acid comparison of the two Arid5b isoforms. The ARID domain is highlighted in yellow. Regions
261 specific to isoform1 or isoform2 are indicated in red letters. The sequence in blue corresponds to the
262 predicted C-terminal sequence of isoform2 observed in 3'RACE PCR experiment but that we could not amplify
263 by end-to-end PCR.

264 **Fig. S3** : Nucleotide position of PCR primers used for RT-qPCR. Nucleotide sequences of the N-terminal region
265 of *arid5b isoform1* and *isoform2* are aligned. Arrows indicate the primer sequences that were used to amplify
266 both isoforms (in blue), *isoform1* (green) and *isoform2* (brown). Conserved nucleotides are in red.

267 **Table S1** : Exon-intron organization of the *arid5b* gene in *Xenopus laevis* on scaffold 9729.

268 Exon sequences are indicated by uppercase letters and intron sequences by lowercase letters. Splice donor
269 and acceptor sites are underlined. Exon and intron size are reported as base pairs.

270 **Table S2** : Exon-intron organization of the *arid5b* gene in *Xenopus laevis* on scaffold 48311.

271 Exon sequences are indicated by uppercase letters and intron sequences by lowercase letters. Splice donor
272 and acceptor sites are underlined. Exon and intron size are reported as base pairs.

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Developmental Expression Pattern

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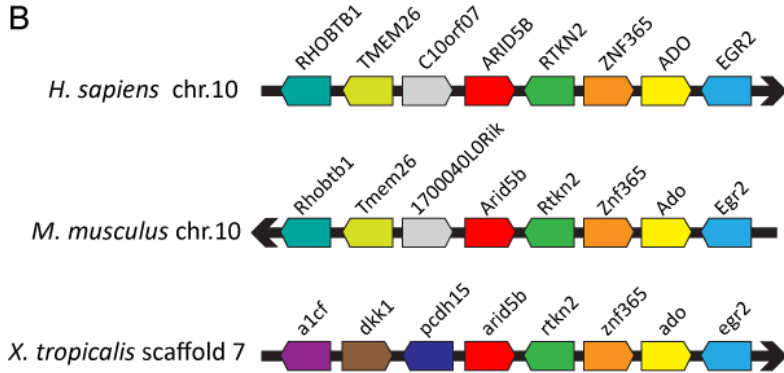
Developmental Expression Pattern

A

	1	10	20	30	40	50	60	70																																																														
<i>X. laevis</i>	K	A	D	E	Q	A	F	L	V	A	L	Y	K	Y	M	K	E	R	K	T	P	I	E	R	I	P	Y	L	G	F	K	Q	I	N	L	W	T	M	F	Q	A	A	Q	K	L	G	G	Y	E	T	I	T	A	R	R	Q	W	K	H	I	Y	D	E	L	G	G	N	P	G	S
<i>G. gallus</i>	R	A	D	E	Q	A	F	L	V	A	L	Y	K	Y	M	K	E	R	K	T	P	I	E	R	I	P	Y	L	G	F	K	Q	I	N	L	W	T	M	F	Q	A	A	Q	K	L	G	G	Y	E	T	I	T	A	R	R	Q	W	K	H	I	Y	D	E	L	G	G	N	P	G	S
<i>H. sapiens</i>	R	A	D	E	Q	A	F	L	V	A	L	Y	K	Y	M	K	E	R	K	T	P	I	E	R	I	P	Y	L	G	F	K	Q	I	N	L	W	T	M	F	Q	A	A	Q	K	L	G	G	Y	E	T	I	T	A	R	R	Q	W	K	H	I	Y	D	E	L	G	G	N	P	G	S
<i>X. tropicalis</i>	K	A	E	E	Q	S	F	L	V	A	L	Y	K	Y	M	K	E	R	K	T	P	I	E	R	I	P	Y	L	G	F	K	Q	I	N	L	W	T	M	F	Q	A	A	Q	K	L	G	G	Y	E	T	I	T	A	R	R	Q	W	K	H	I	Y	D	E	L	G	G	N	P	G	S
<i>D. rerio</i>	R	T	D	E	Q	A	F	L	V	A	L	Y	K	Y	M	K	E	R	K	T	P	I	E	R	I	P	Y	L	G	F	K	Q	I	N	L	W	T	M	F	Q	A	A	Q	K	L	G	G	Y	E	V	I	T	A	R	R	Q	W	K	N	V	Y	D	E	L	G	G	N	P	G	S

	80	90																							
<i>X. laevis</i>	T	S	A	A	T	C	T	R	R	H	Y	E	R	L	I	L	P	Y	E	R	F	I	G	G	E
<i>G. gallus</i>	T	S	A	A	T	C	T	R	R	H	Y	E	R	L	I	L	P	Y	E	R	F	I	K	G	E
<i>H. sapiens</i>	T	S	A	A	T	C	T	R	R	H	Y	E	R	L	I	L	P	Y	E	R	F	I	K	G	E
<i>X. tropicalis</i>	T	S	A	A	T	C	T	R	R	H	Y	E	R	L	I	L	P	Y	E	R	F	I	N	G	E
<i>D. rerio</i>	T	S	A	A	T	C	T	R	R	H	Y	E	R	L	I	L	P	Y	E	R	F	T	K	G	E

B



C

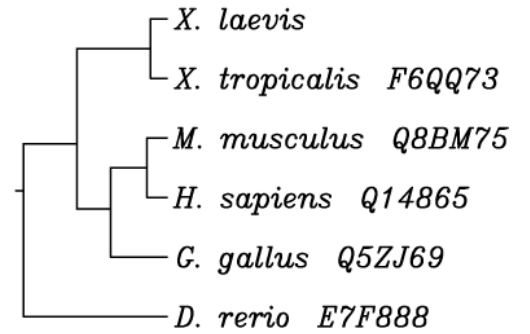


Figure 1

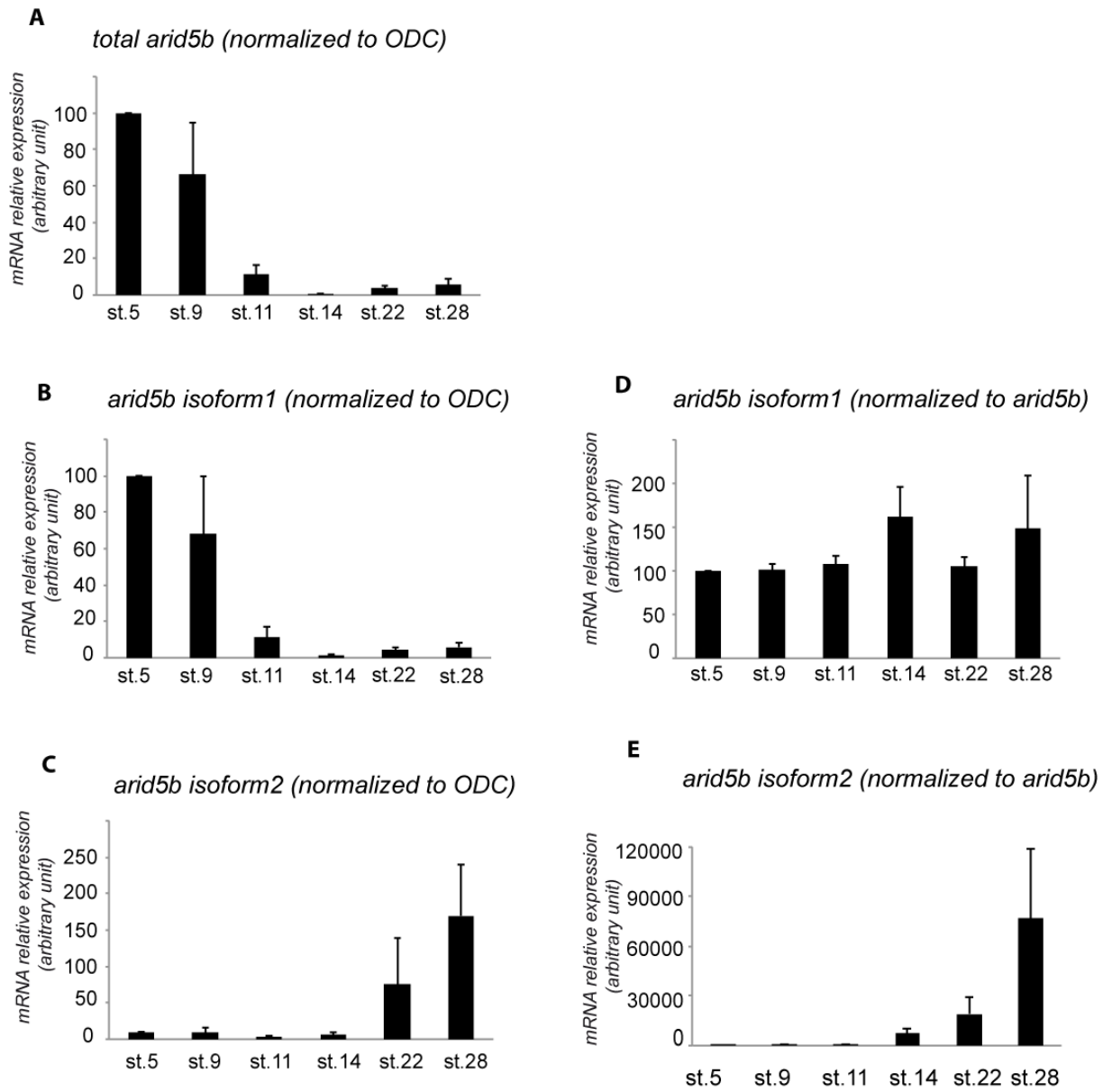


Figure 2

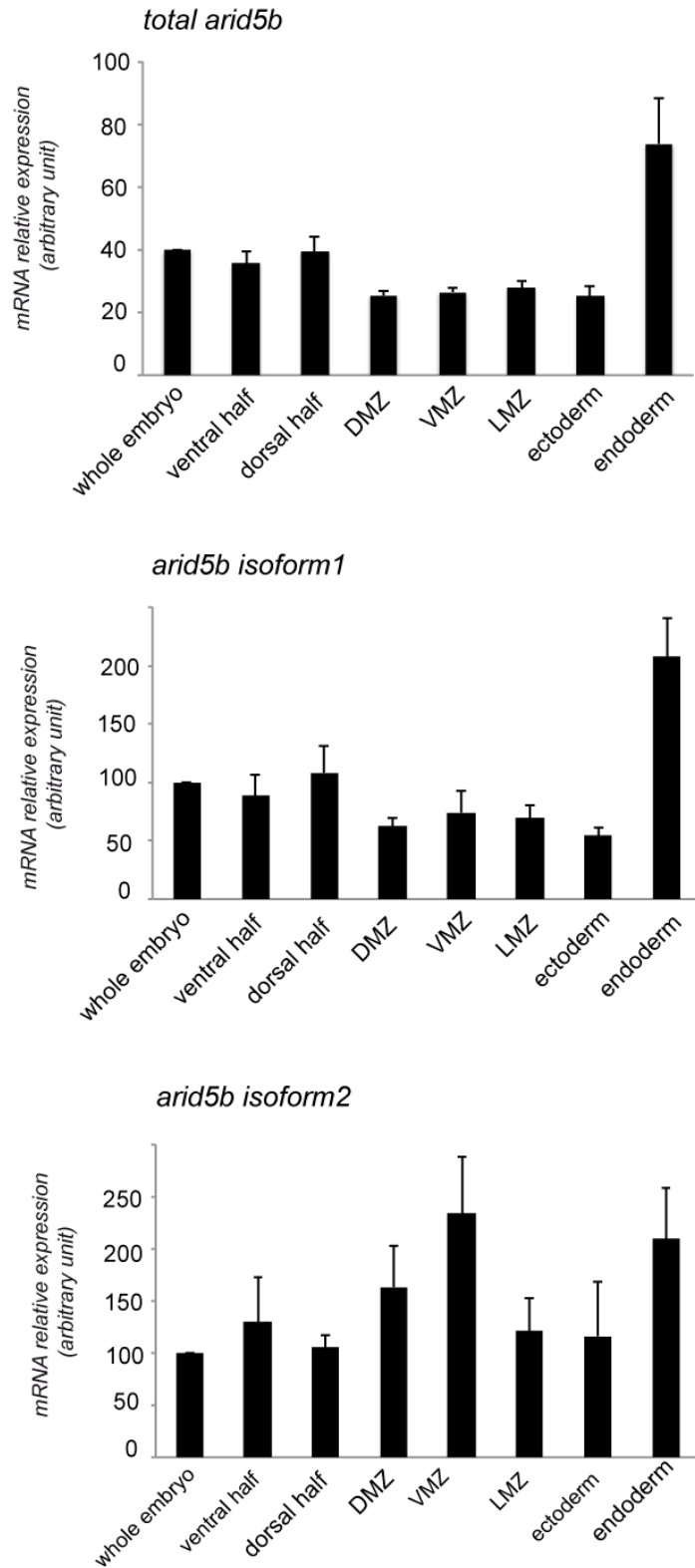


figure 3

Developmental Expression Pattern

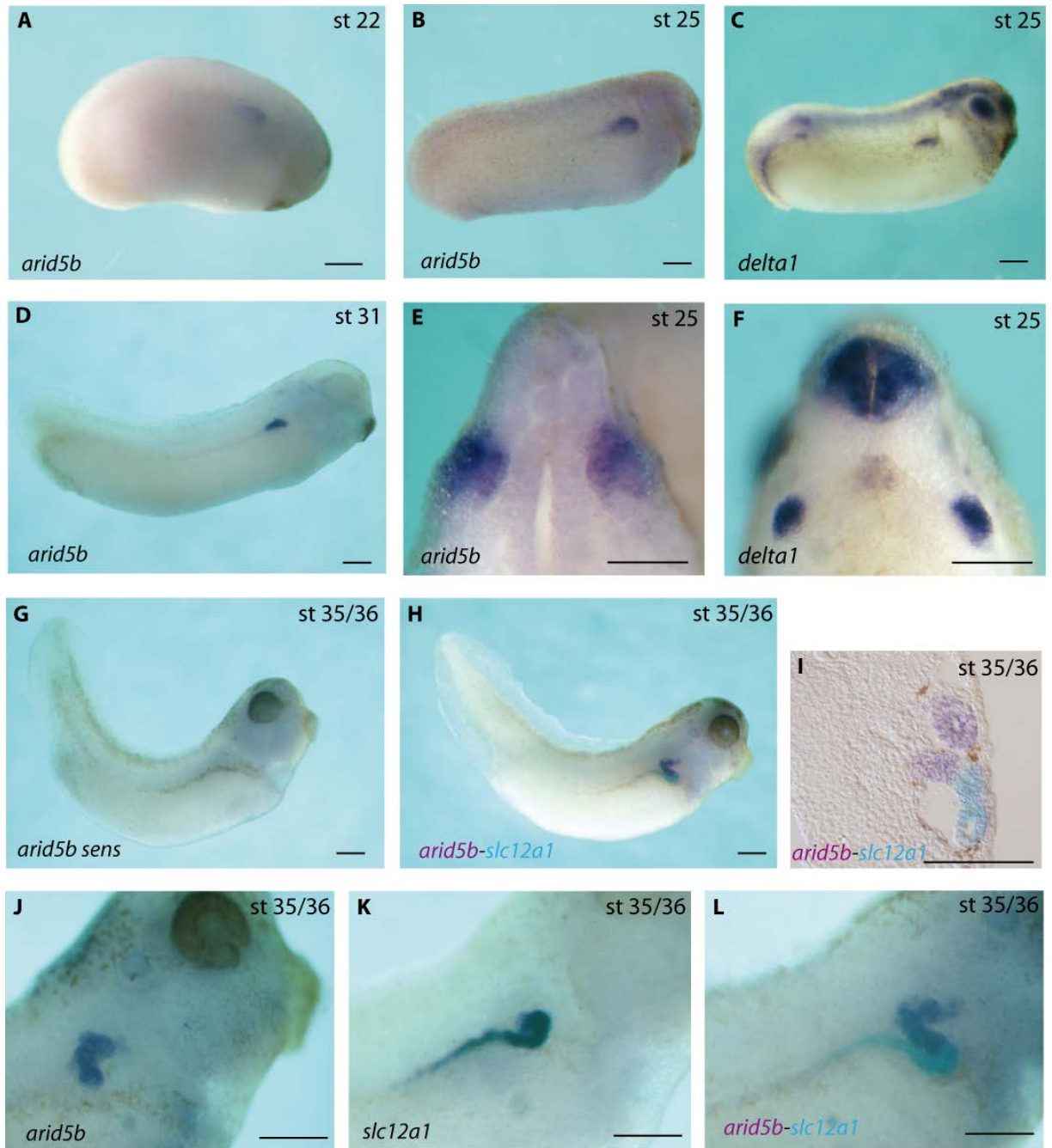


Figure 4

Developmental Expression Pattern

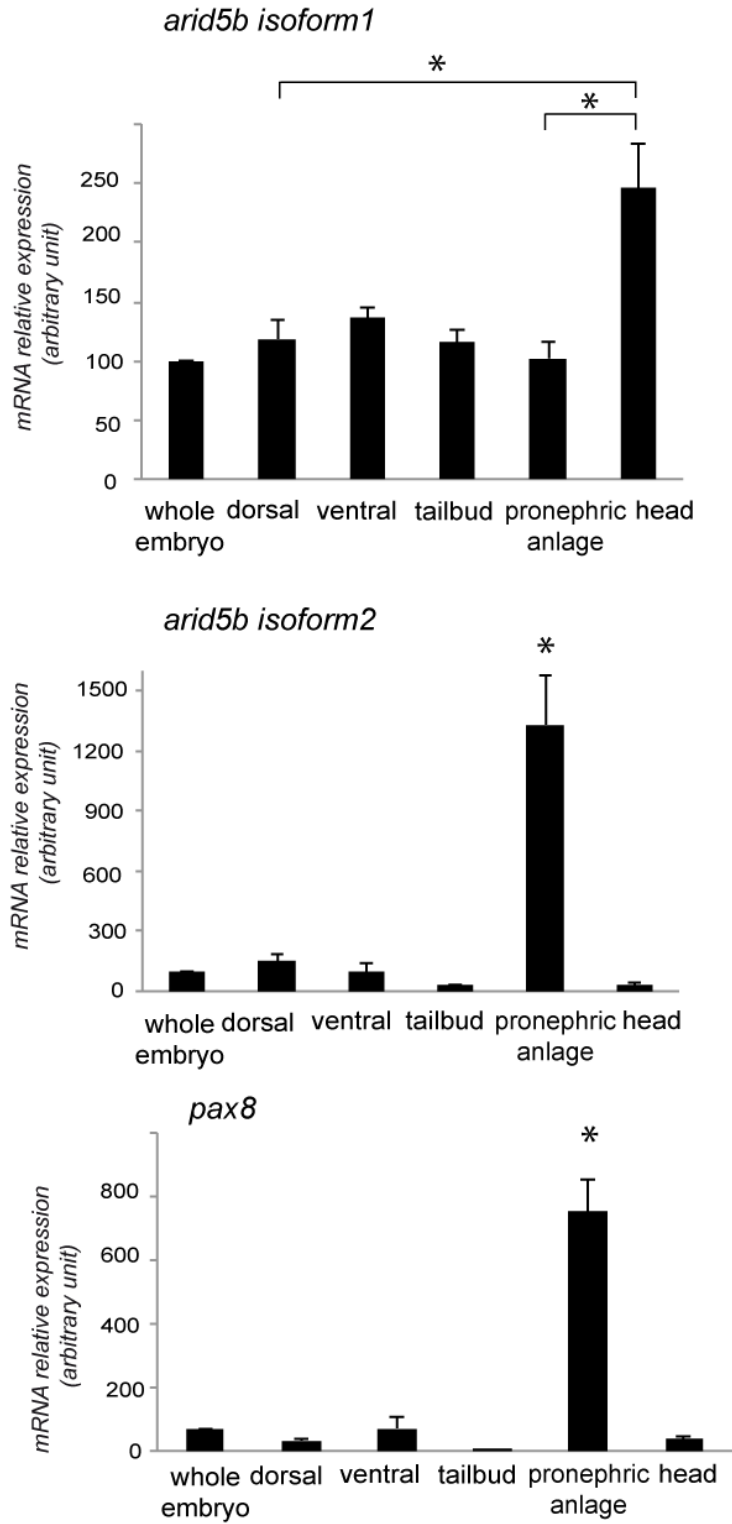


Figure 5

Developmental Expression Pattern

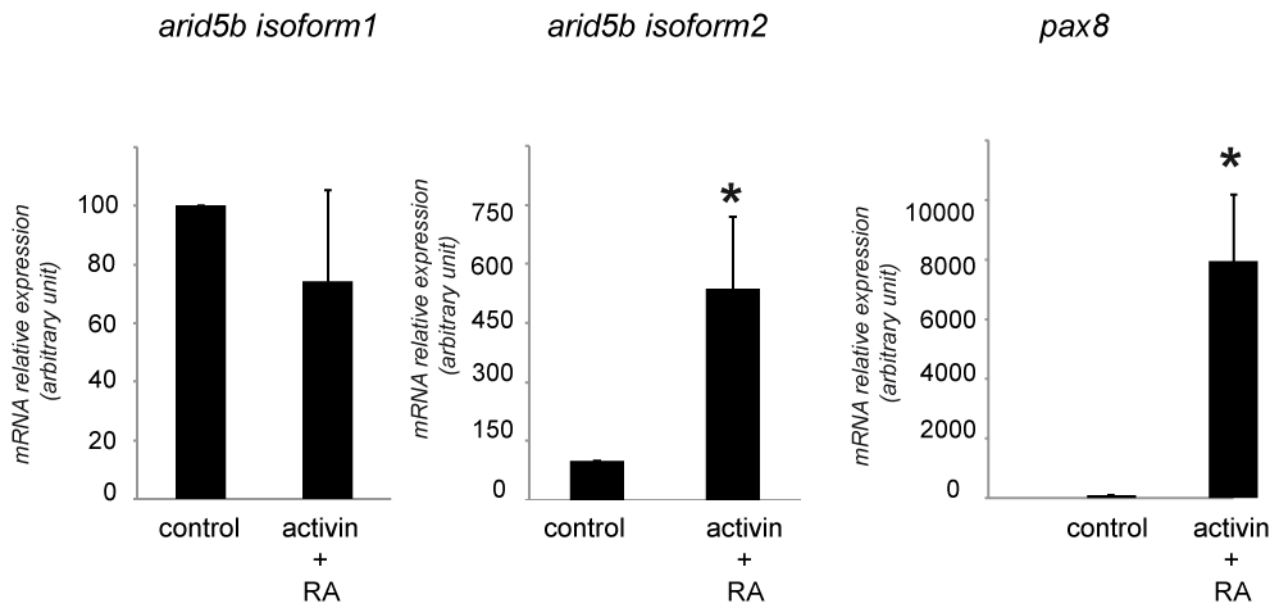


Figure 6

Developmental Expression Pattern

	1	10	20	30	40	50	60
X laevis	MEPNSLQ	WVGSACGLHG	PIFYKAFQPHLE	N	RARILSLG	DFFLVRCK	PHFPPVCAELQLLWEERTSR
X tropi calis	MEPNSLQ	WVGSACGLHG	PIFYKAFQPHLE	N	RARILSLG	DFFLVRCK	ADFPVCAELQLLWEERTSR
G galus	MERSALQ	WVGAPCGSHG	PYVYRAFRPQRRGGG	RARVLSLG	DFFVVRCK	AEKPPACIAELQLLWEERTSR	
H sapi ens	MEPNSLQ	WVGSACGLHG	PIFYKAFQPHLE	N	KPRILSLG	DFFVVRCT	PKDPICIAELQLLWEERTSR
M muscul us	MEPNSLQ	WVGSACGLHG	PIFYKAFQPHLE	N	KPRILSLG	DFFVVRCT	PKDPICIAELQLLWEERTSR
D reri o	MEPNSLQ	WVGSACGLHG	PIFYKAFQPHLE	N	KPRILSLG	DFFVVRCT	PKDPICIAELQLLWEERTSR
consensus>50	mepnslq	wgspcglhgpyi	fykafqphleg	n	rari lslgdfffvrc	p	epvciaelqllweertsr

	70	80	90	100	110	120	130
X laevis	QLLSSSKLYFLPEDT	PKGRNSDHGE	DEVI AVSEKVI	VRLEDLVKWAH	SDPSK	NYG	LKALIVK...LKEEL
X tropi calis	QLLSSSKLYFLPEDT	PKGRNSDHGE	DEVI AVSEKVI	VRLEDLVKWAH	SDLSK	NCG	LKALIVK...LKEEL
G galus	QLLSSAKLYFLPEDT	PQGRNSDHGE	DEVI AVSEKVI	VRLEDLVKWAH	SDPSK	NYG	LKALIVK...LKEEL
H sapi ens	QLLSSSKLYFLPEDT	PQGRNSDHGE	DEVI AVSEKVI	VRLEDLVKWAH	SDPSK	NYG	LKALIVK...LKEEL
M muscul us	QLLSSSKLYFLPEDT	PQGRNSDHGE	DEVI AVSEKVI	VRLEDLVKWAH	SDPSK	NYG	LKALIVK...LKEEL
D reri o	QLLSSSKLYFLPEDT	PQGRNSDHGE	DEVI AVSEKVI	VRLEDLVKWAH	SDPSK	NYG	LKALIVK...LKEEL
consensus>50	qll ssskl y fl pedt	pqr nsdhgedevi	avsekvi vki	edl vkwahsdf	skw	cgl	A. Pvk... l kel

	140	150	160	170	180	190	200
X laevis	ARNGGKES	LARYRQSV	LNSGLNF	KDVFKEKA	ELGE	EGDKNV	MVLSYPQYCRYRSILKRIQAEPSV
X tropi calis	AKNGGKES	LARYRQSV	LNSGLNF	KDVFKEKA	ELGE	SEDDKN	VMLSYPOYCRYRSILKRIQAEPSV
G galus	GKNGGKES	LARYRQSV	LNSGLNF	KDVFKEKA	ELGE	DEDSNL	LISYPQYCRYRSILKRIQAEPSV
H sapi ens	GRNGGKES	LARYRQSV	LNSGLNF	KDVFKEKA	ELGE	DEFTNV	I VLSYPQYCRYRSILKRIQAEPSV
M muscul us	GRNGGKES	LARYRQSV	LNSGLNF	KDVFKEKA	ELGE	DEFTNV	I VLSYPQYCRYRSILKRIQAEPSV
D reri o	GKNGGKES	LARYRQSV	LNSGLNF	KDVFKEKA	ELGE	DADDKV	L VLSYPQYCRYRSILKRIQAEPSV
consensus>50	gknggkEaLi kYR#St	LNSGLNF	KDVFKEKA#LGE	dee#knvm	L	SYPQYCRYRSI	l kRi qdkPSSI Lt D



	210	220	230	240	250	260	270
X laevis	QFVQALGGI	AVINNNTK	LYCRDTF	HP	TLT	ENES!	CDEFAPNLKGRPRKKKL
X tropi calis	QFVQALGGI	AVINNNTK	LYCRDTF	HP	TLT	ENES!	CDEFAPNLKGRPRKKKL
G galus	QFVQALGGI	AVTSKNPQ	FYCRDTF	HP	TLT	ENES!	CDEFAPNLKGRPRKKKL
H sapi ens	QFAIALGGI	AVVSRNPQ	LYCRDTF	HP	TLT	ENES!	CDEFAPNLKGRPRKKKL
M muscul us	QFAIALGGI	AVVSRNPQ	LYCRDTF	HP	TLT	ENES!	CDEFAPNLKGRPRKKKL
D reri o	HVVI ALGGI	ASLTNSTQ	LYCRDTF	HP	TLT	ENES!	CDEFAPNLKGRPRKKKL
consensus>50	qfvi ALGGI	avi snnpqi	LYCRDTF#HP	TLT!	ENES!	CDEFAPNLKGRPRK	KK c. pQR#S. nGvk. nn

	280	290	300	310	320	330	340
X laevis	VCDCKTVA	KVKCEVKATLP	KFTPN	GNCKILLI	EDKPA	AGEDDGC	KVD
X tropi calis	ICDCKTVA	KVKCEVKATLP	KFTPN	GNCKILLI	EDKPA	GEDDDG	KVD
G galus	NSFSKAVA	KVCEARSALT	KPKSNN	SNCKGSS	EDKPA	AVG	
H sapi ens	NDSKAVA	KVCEARSALT	KPKNHLN	CKKVSNE	KPA	VALG	
M muscul us	NDGKAVIS	KVCEARSALT	KPKNHLN	CKKTSNE	KPA	LSIG	
D reri o	GVGKTLV	KMRADSKS	GVSKPRNSTES	CKRVQS	KPA	EDGG	
consensus>50	nc#gKt vaKvKc#aksal	pKpknnn	gnCKi s	E#KpK		i g	... #Ecr a#EqFLVALYKMKERK

	350	360	370	380	390	400	410
X laevis	TPI ER	PYLGFQKI	NLWTF	QAAQKLG	GYET	TARRQWK	HIYDELGGNPGSTSAATCTRRHYERLI LPYE
X tropi calis	TPI ER	PYLGFQKI	NLWTF	QAAQKLG	GYET	TARRQWK	HIYDELGGNPGSTSAATCTRRHYERLI LPYE
G galus	TPI ER	PYLGFQKI	NLWTF	QAAQKLG	GYET	TARRQWK	HIYDELGGNPGSTSAATCTRRHYERLI LPYE
H sapi ens	TPI ER	PYLGFQKI	NLWTF	QAAQKLG	GYET	TARRQWK	HIYDELGGNPGSTSAATCTRRHYERLI LPYE
M muscul us	TPI ER	PYLGFQKI	NLWTF	QAAQKLG	GYET	TARRQWK	HIYDELGGNPGSTSAATCTRRHYERLI LPYE
D reri o	TPI ER	PYLGFQKI	NLWTF	QAAQKLG	GYEV	TARRQWK	HIYDELGGNPGSTSAATCTRRHYERLI LPYE
consensus>50	TPI ER	PYLGFQKI	NLWTF	QAAQKLG	GYET	TARRQWK	HIYDELGGNPGSTSAATCTRRHYERLI LPYE

	420	430	440	450	460	470	480
X laevis	RFLIG	GEEDKPLP	SAKPRK	PENGS	QEV	ELK	CGA
X tropi calis	RFLIG	GEEDKPLP	SAKPRK	PENGS	QEV	ELK	CGA
G galus	RFLIG	GEEDKPLP	SAKPRK	PENGS	QEV	ELK	CGA
H sapi ens	RFLIG	GEEDKPLP	SAKPRK	PENGS	QEV	ELK	CGA
M muscul us	RFLIG	GEEDKPLP	SAKPRK	PENGS	QEV	ELK	CGA
D reri o	RFLIG	GEEDKPLP	SAKPRK	PENGS	QEV	ELK	CGA
consensus>50	RFLIG	GEEDKPLP	SAKPRK	PENGS	QEV	ELK	CGA

	490	500	510	520	530	540												
X laevis	A	.DEKNN	PLDYDLEET	TKTSVDKSESVVSEV	.NYP	SLENDELEET	TVANKDHVT	KDENSQ	DDPDPVD	SLI								
X tropi calis	A	.DEKNN	PLDYDLEET	TKTSVDKSESVVSEV	.NYP	SLENDELEET	TVANKDHVT	KDENSQ	DDPDPVD	SLI								
G galus	SA	.DQKNT	PEPTAGET	KQPNQ	GGPPPLLE	EA	.ARPL	MEKIDVT	TENS	SSSEKAKE	EVPHLSS	...FP	SLG					
H sapi ens	TL	.S	QKSI	PEELPAAD	MKKLE	GYDE	FAKPLAS	RVDP	PKDNE	TD	QGS	NSSEKVA	EEAGE	KGPT	PLPL	SAP		
M muscul us	TL	.S	QKSI	PEELPAAD	MKKLE	GYDE	FAKPLAS	RVDP	PKDNE	TD	QGS	NSSEKVA	EEAGE	KGPT	PLPL	SAP		
D reri o	TL	.S	QKSI	PEELPAAD	MKKLE	GYDE	FAKPLAS	RVDP	PKDNE	TD	QGS	NSSEKVA	EEAGE	KGPT	PLPL	SAP		
consensus>50	dekn	pe	dae	#tk	v#g	ed	...e	sp	...#nde	teq	sns	#kv	e#	...e	d	pl	Sl	Sp

	550	560	570	580	590	600	610																								
X laevis	HVKE	INCRO	...DK	IQM	NET	MTTR	KRE	QIKED	YSDHLE	NDP	EDV	Q	THV	FD	AI	QAP	.RHD	M	LE	EEK											
X tropi calis	HVKE	INCRO	...DK	IQM	NET	MTTR	KRE	QIKED	YSDHLE	NDP	EDV	Q	THV	FD	AI	QAP	.RHD	M	LE	EEK											
G galus	MPDED	TVLDAT	VTKR	LHSS	ADT	QED	TR	FERR	I	HKAF	TES	LE	NE	PE	PE	M	F	ST	FPV	Q	LP	...T	Q	SD	M	ED	K				
H sapi ens	LAD	KKDS	ALV	PG	ASK	QLTS	PS	AL	VD	SK	QES	KL	C	ET	SP	SE	PE	Q	E	AS	FP	SP	PT	Q	PL	AN	Q	ET	D	D	K
M muscul us	LAD	KKDS	ALV	PG	ASK	QLTS	PS	AL	VD	SK	QES	KL	C	ET	SP	SE	PE	Q	E	AS	FP	SP	PT	Q	PL	AN	Q	ET	D	D	K
D reri o	LAD	KKDS	ALV	PG	ASK	QLTS	PS	AL	VD	SK	QES	KL	C	ET	SP	SE	PE	Q	E	AS	FP	SP	PT	Q	PL	AN	Q	ET	D	D	K
consensus>50	i	.pe	.ds	...f	...k	q	i	...s	.et	m	dt	k	qe	qi	c	...%	esl	endped	pl	...f	pa	app	...q	e	e	#ek					

Developmental Expression Pattern

Figure S1

Developmental Expression Pattern

	1												80
Isoform1	MEPN	SLKWVG	SSCGLHGPYI	FYKAFQFHLE	NRARILSLGD	FFLVRCKPHE	PVCVAELQLL	WEERTSRQLL	SSSKLYFLPE				
Isoform2				
	81												160
Isoform1	DTPK	GKNSSH	GEHEVI	AVSE	KVIVRLEDLV	KWAHSDFSKW	NYGLKALPVK	LKELARNGQK	ESLAKYRQSV	LNSGLNFKDV			
Isoform2				
	161												240
Isoform1	FKKA	EELGEG	EGDKNVMVLS	YPQYCRYRSI	LKRIQAEPS	VLDQFVQAL	GGIAVINNNT	KILYCRD	TFD	HPTLIENESI			
Isoform2				
	241												320
Isoform1	CDEF	FAPNLKG	RPRKKKLGPQ	RRESVNGVKE	LAGVCDGKT	AKVKEVKAT	LPKT	KTPNGN	CKKILIEDKP	KAGEDDGCKV			
Isoform2	..	MPNLKG	RPRKKKLGPQ	RRESVNGVKE	LAGVCDGKT	AKVKEVKAT	LPKT	KTPNGN	CKKILIEDKP	KAGEDDGCKV			
	321												400
Isoform1	DEGKA	DEQAF	LVALYK	YKMK	RKTPIERIPY	LGFKQINLWT	MFQAAQKLG	YETITARRQW	KHIYDELG	GN	PGSTSAATCT		
Isoform2	DEGKA	DEQAF	LVALYK	YKMK	RKTPIERIPY	LGFKQINLWT	MFQAAQKLG	YETITARRQW	KHIYDELG	GN	PGSTSAATCT		
	401												480
Isoform1	RRHYER	LILP	YERFIGGED	KPLPSAKPRK	PENGSQ	EVEL	KAKICGAKRI	KNESQKSKKE	KDPTAKGLDM	TEVPPDEEDH			
Isoform2	RRHYER	LILP	YERFIGGED	KPLPSAKPRK	PENGSQ	EVEL	KAKICGAKRI	KNESQKSKKE	KDPTAKGLDM	TEVPPDEEDH			
	481												560
Isoform1	LEADE	KNMPL	DYDLEETKTS	VDKSESVVSE	VNYPSPLEND	ELEETVANKD	HVTKD	NSCQ	DDPDVDSL	LIH	VKEINCRQTD		
Isoform2	LEADE	KNMPL	DYDLEETKTS	VDKSESVVSE	VNYPSPLEND	ELEETVANKD	HVTKD	NSCQ	DDPDVDSL	LIH	VKEINCRQTD		
	561												640
Isoform1	KQLQ	MPNETM	TTTKRE	QIKE	DYSDHLENDP	EDVQLHVFPA	IQAPQ	HDMHL	EEEEKLPD	MPD	YIANCTVKVD	PLGSNDLKNP	
Isoform2	KQLQ	MPNETM	TTTKRE	QIKE	DYSDHLENDP	EDVQLHVFPA	IQAPQ	HDMHL	EEEEKLPD	MPD	YIANCTVKVD	PLGSNDLKNP	
	641												720
Isoform1	LDSN	LLQNAL	KQNP	KVYFVQ	TLDMLSDEKD	TSASMNDDSS	FSYTPLLYSR	GNGP	GIMSPLA	KKKLLSQVSG	ASQPGNLPYG		
Isoform2	LDSN	LLQNAL	KQNP	KVYFVQ	TLDMLSDEKD	TSASMNDDSS	FSYTPLLYSR	GNGP	GIMSPLA	KKKLLSQVSG	ASQPGNLPYG		
	721												800
Isoform1	SPPPL	ISKKK	LSSKGEVSPS	LLQTHSSNS	ESAAINRPSV	IQHVQSFKQK	SPEEK	KTVD	HYKNSMFGKV	DSYCCDFAKH			
Isoform2	SPPPL	ISKKK	LSSKGEVSPS	LLQTHSSNS	ESAAINRPSV	IQHVQSFKQK	SPEEK	KTVD	HYKNSMFGKV	DSYCCDFAKH			
	801												880
Isoform1	HQSV	LADSYA	LKSCVQECKE	KMAEKRAASN	SNVPSFVAEF	YSSPHLHRLY	RQAEHHLHNE	NSAKFHSREM	FRDLENVSSH				
Isoform2	HQSV	LADSYA	LKSCVQECKE	KMAEKRAASN	SNVPSFVAEF	YSSPHLHRLY	RQAEHHLHNE	NSAKFHSREM	FRDLENVSSH				
	881												960
Isoform1	KHHY	HASLHQ	HDKQNLHDDV	DDQPTDLSLP	KSLHKLSTKI	PGSSICHQPV	QQDSKSHNPF	QTPNSKTLGL	DCNPKACRVS				
Isoform2	KHHY	HASLHQ	HDKQNLHDDV	DDQPTDLSLP	KSLHKLSTKI	PGSSICHQPV	QQDSKSHNPF	QTPNSKTLGL	DCNPKACRVS				
	961												1040
Isoform1	PMTPI	SKRH	MDSIQRPSKT	VKPD	TLRKVE	GLVHPFSIGK	TNTHNFGAPR	SLKRNLEDMD	NPLTDK	KIRA	VSPLHLPKEM		
Isoform2	PMTPI	SKRH	MDSIQRPSKT	VKPD	TLRKVE	GLVHPFSIGK	TNTHNFGAPR	SLKRNLEDMD	NPLTDK	KIRA	VSPLHLPKEM		
	1041												1120
Isoform1	SGKD	TFVGQD	GESSKSVHDI	HSGSMIESHK	YPLSSPFFPG	MYPGSLCGGL	SSRIPTAYSH	PLQYLKNQTA	LSPLMQPLAL				
Isoform2	SGKD	TFVGQD	GESSKSVHDI	HSGSMIESHK	YPLSSPFFPG	MYPGSLCGGL	SSRIPTAYSH	PLQYLKNQTA	LSPLMQPLAL				
	1121												1187
Isoform1	HTFMM	RQYL	TNSTNSQQLY	RQIASHAPVG	SSYGDLLHSS	IYPLTAINPQ	SPFPSSQMSS	VYPSTKL					
Isoform2	HTFMM	RQYL	TNSTNSQQLY	RQIASHAPVG	SSYGDLLHSS	IYPLTAINPQ	SPFPSSQMSS	VYPSTKL					

Figure S2

Developmental Expression Pattern

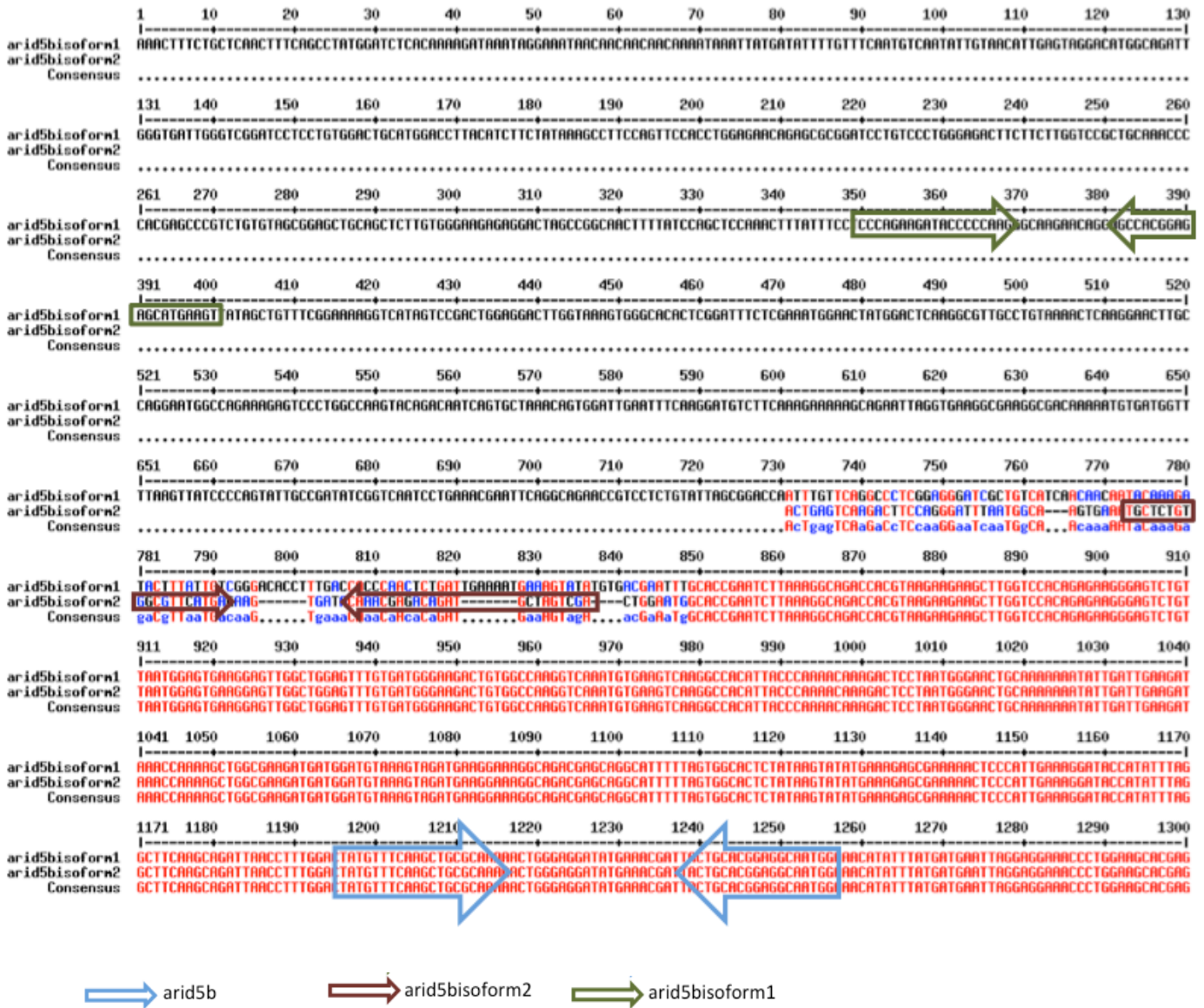


Figure S3

Developmental Expression Pattern

Table S1

Exon n°	Exon size (bp)	Sequence at exon-intron junction		Intron size (bp)	Amino acid interrupted
		5' splice donor	3' splice acceptor		
1	>27	ACTCAAG <u>gt</u> attattagctg	tcttgc <u>ag</u> TGGGTCGG	328	Trp-8
2*	>68	?	atthtc <u>ag</u> CATGAAGT	>39437	Glu-92?
3	226	GAATTAG <u>gt</u> aaccatggttc	aaccac <u>ag</u> TGAAGGCG	40399	His-93
4	231	AATTTGG <u>gt</u> aagttccaatc	tthttac <u>ag</u> CACCGAAT	66835	Gly-168
5	113	GGCCAAG <u>gt</u> aaatcctthtt	tctthtc <u>ag</u> GTCAAATG	1316	Lys-282
6	220	AAGCAGA <u>gt</u> aagtagacact	tctaac <u>ag</u> TTAACCTT	4458	Ile-356
7	53	TGAAACG <u>gt</u> aagtgcctthtt	tataac <u>ag</u> ATTACTGC	3545	Ile-374
8	98	ACGAAAG <u>gt</u> aggataatcta	ccccac <u>ag</u> ATTAATCC	33496	Arg-406
9	199	GACTGAG <u>gt</u> aaattggggca	ttgttht <u>ag</u> GTTCCACC	5264	Val473
10	>2712				
4b	>99	GGAATGG <u>gt</u> aaccgcttcag	tctthtc <u>ag</u> GTCAAATG	6493	Ala-2 (short isoform)

Scaffold 9729. * sequence nt89-275 mRNA ORFmissing in genomic sequence

Table S2

Exon n°	Exon size (bp)	Sequence at exon-intron junction		Intron size (bp)	Amino acid interrupted
		5' splice donor	3' splice acceptor		
1*	>27	ACTCAAG <u>gt</u> attattagctg	?	?	Trp-8
2	236	CGGAGAG <u>gt</u> gacatccctac	tthtttc <u>ag</u> CATGAAGT	53173	His-93
3	226	GAACTAG <u>gt</u> aaccatgattc	aaccac <u>ag</u> GTGAAGGC	53008	Gly-168
4	231	GAATTTG <u>gt</u> aagttccaatt	tthtttc <u>ag</u> CACCAAAT	77336	Ala-245
5	113	TGCCAAG <u>gt</u> aaatcggthtt	tgthttt <u>ag</u> GTCAAATG	1264	Val-283
6	220	AAGCAGA <u>gt</u> aagtagacatt	tctaac <u>ag</u> TTAACCTT	4033	Ile-356
7	53	TGAAACG <u>gt</u> aagtgcctthtt	tgtaac <u>ag</u> ATTACTGC	4690	Ile-374
8	98	ACGAAAG <u>gt</u> aggataatcta	ccccac <u>ag</u> ATTAATCC	33865	Arg-406
9	199	GGCTGAG <u>gt</u> atattggggca	ttgttc <u>ag</u> GTTCCACC	4311	Val-473
10	>2705				
4b	>96	GGAATGG <u>gt</u> aaccgctthtt	tthtttc <u>ag</u> CACCAAAT	5945	Ala-2 (short isoform)

Scaffold 48311 * sequence nt21-39 mRNA ORFmissing in genomic sequence