

# Genes regulated by potassium channel tetramerization domain containing 15 (Kctd15) in the developing neural crest

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**ABSTRACT** Neural crest (NC) development is controlled precisely by a regulatory network with multiple signaling pathways and the involvement of many genes. The integration and coordination of these factors are still incompletely understood. Overexpression of Wnt3a and the BMP antagonist Chordin in animal cap cells from *Xenopus* blastulae induces a large number of NC specific genes. We previously suggested that Potassium Channel Tetramerization Domain containing 15 (Kctd15) regulates NC formation by affecting Wnt signaling and the activity of transcription factor AP-2. In order to advance understanding of the function of Kctd15 during NC development, we performed DNA microarray assays in explants injected with Wnt3a and Chordin, and identified genes that are affected by Kctd15 overexpression. Among the many genes identified, we chose Duf domain containing protein 1 (*ddcp1*), Platelet-Derived Growth Factor Receptor a (*pdgfra*), Complement factor properdin (*cfp*), Zinc Finger SWIM-Type Containing 5 (*zswim5*), and complement component 3 (*C3*) to examine their expression by whole mount *in situ* hybridization. Our work points to a possible role for Kctd15 in the regulation of NC formation and other steps in embryonic development.

**KEY WORDS:** *Neural crest, Kctd15, DNA microarray, Xenopus, gene regulation, transcription factor AP-2*

The Kctd family of proteins has received increasing attention due to its multiple biological functions and putative roles in human disease. The family is composed of over 20 genes/proteins in different vertebrate species and can be subdivided into seven subgroups on the basis of sequence similarity (Liu *et al.*, 2013, Skoblov *et al.*, 2013). A number of Kctd genes have been associated with human diseases, as summarized by Liu and colleagues (Liu *et al.*, 2013). All members of the family share a Bric-a-brack, Tram-track, Broad complex (BTB) domain usually located in the N-terminal half of the protein. BTB domains, also called POZ domains, are widely involved in protein-protein interactions (Perez-Torrado *et al.*, 2006, Stogios *et al.*, 2005), and thus it is not surprising that acting as adapters is perhaps the best-studied molecular function of Kctd proteins. Kctd5, Kctd6 and Kctd11 bind Cullin 3 and act as adapters mediating the interactions of E3 ubiquitin ligases with their substrates (Balasco

*et al.*, 2014, Bayon *et al.*, 2008, Canettieri *et al.*, 2010, Chen *et al.*, 2009, Correale *et al.*, 2011). While other Kctd proteins also have been reported to bind Cullin 3, this has been contradicted in recent work (Smaldone *et al.*, 2015). Kctd8, Kctd12 and Kctd16 associate with GABA receptors, modulating their activity (Rajalu *et al.*, 2015); these genes also show striking left/right asymmetry in their expression in the brain (Gamse *et al.*, 2005). Other family members appear to have very different molecular functions by acting in the regulation of transcription. These include Kctd10, a

*Abbreviations used in this paper:* AC, animal cap; C, complement component; cfp, complement factor properdin; ddcp, Duf domain containing protein; Kctd, potassium channel tetramerization domain containing; NC, neural crest; NPB, neural plate border; pdgfra, platelet-derived growth factor receptor a; zswim, zinc finger SWIM-type containing.

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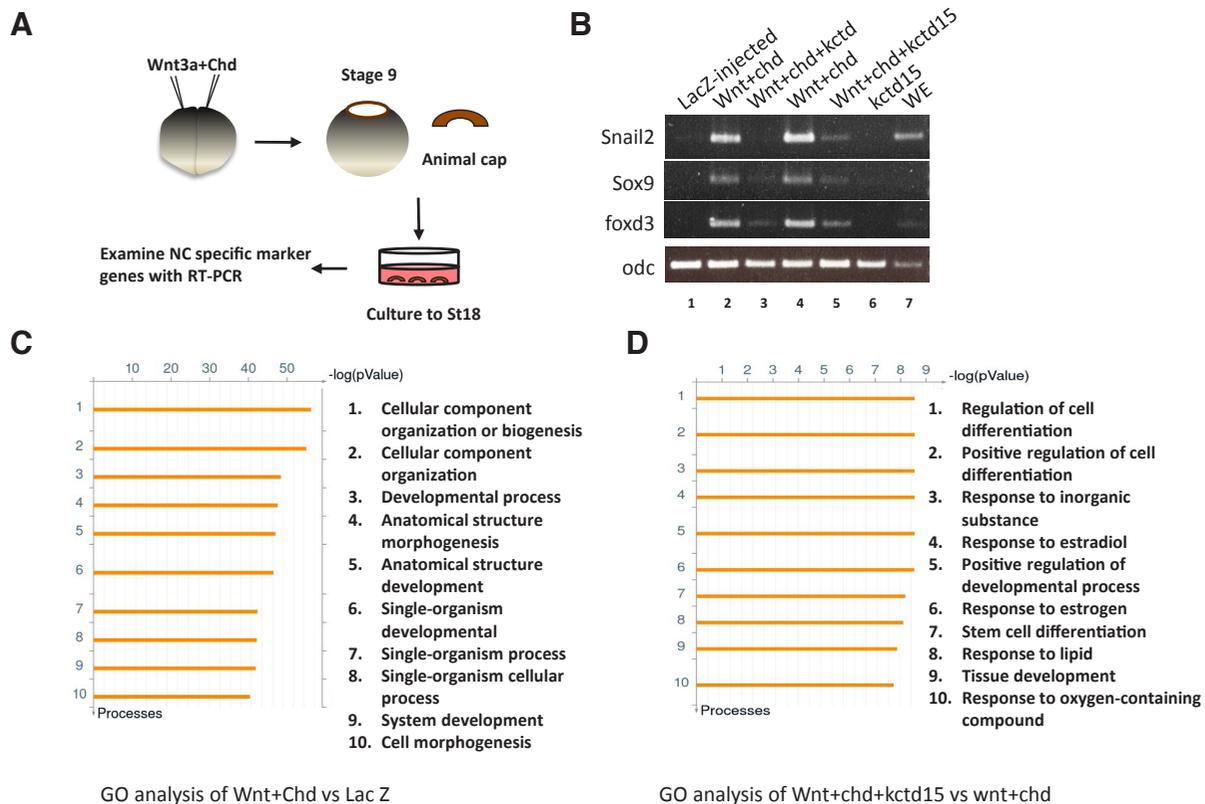
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factor involved in heart development through the inhibition of Tbx5a activity, apparently by direct interaction between the Tbx and Kctd components (Hu *et al.*, 2014, Tong *et al.*, 2014). In addition Kctd10 also interacts with proliferating cell nuclear antigen and through this interaction affects proliferation (Wang *et al.*, 2009).

We have been interested in the functions of Kctd15 in *Xenopus* and zebrafish development. We found that overexpression of Kctd15 leads to very effective inhibition of the formation of the neural crest (NC), suggesting the possibility that Kctd15 regulates the size of the NC domain in development (Dutta and Dawid, 2010, Groves and LaBonne, 2014). Kctd15 and the closely similar Kctd1 have two distinct molecular functions, inhibition of canonical Wnt signaling (Dutta and Dawid, 2010, Li *et al.*, 2014) and inhibition of the function of transcription factor AP-2 (Ding *et al.*, 2009, Zarelli and Dawid, 2013). Both of these functions may be responsible for the suppression of NC formation by Kctd15 as Wnt signaling and AP-2 activity are essential for NC induction and differentiation (Brewer *et al.*, 2004, de Croze *et al.*, 2011, Ikeya *et al.*, 1997, Knight *et al.*, 2005, Li and Cornell, 2007, Luo *et al.*, 2003, Luo *et al.*, 2005, Saint-Jeannet *et al.*, 1997, Schorle *et al.*, 1996, Simoes-Costa and Bronner, 2015). Based primarily on the strength of the inhibitory effect we believe that Kctd15 suppression of AP-2 activity may be the dominant mechanism in its blocking of NC development. Mutations in KCTD1 in humans are responsible for scalp-ear-nipple (SEN) syndrome (Marneros *et al.*, 2013), but it is not known whether inhibition of AP-2 or Wnt signaling

plays a role in the etiology of this disease. KCTD15 has not been reported as the cause of a disease, but numerous studies show an association between this gene and obesity (Gutierrez-Aguilar *et al.*, 2012, Leon-Mimila *et al.*, 2013, Mei *et al.*, 2012, Willer *et al.*, 2009, Williams *et al.*, 2012). It is known that AP-2 $\alpha$  regulates the activity of C/EBP $\alpha$  during adipogenesis (Jiang *et al.*, 1998), and AP-2 $\beta$  affects other steps of adipogenesis and insulin resistance (Ikeda *et al.*, 2006, Meng *et al.*, 2010, Tao *et al.*, 2006, Zhang *et al.*, 2014). It is tempting to speculate that the association of KCTD15 with obesity is based on its ability to inhibit AP-2 proteins, but evidence for this hypothesis in vertebrates is not available to date.

Beyond NC development and adipogenesis, the two pathways affected by Kctd15, Wnt signaling and AP-2 transcriptional regulation, have wide-ranging roles in development, physiology and disease (<http://web.stanford.edu/group/nusselab/cgi-bin/wnt/>) (Eckert *et al.*, 2005, Hilger-Eversheim *et al.*, 2000, Hoffman *et al.*, 2007, Orso *et al.*, 2008, Wenke and Bosserhoff, 2010). The role of Kctd15 in different tissues and cell types and the global role of Kctd15 in development have not been studied so far. We investigated Kctd15 function broadly through transcriptome analysis by DNA microarray. As test tissue we chose *Xenopus laevis* animal explants (animal caps) that were injected with Wnt and Chordin (Chd) mRNAs, which induce the expression of NC marker genes (Saint-Jeannet *et al.*, 1997). Recently the transcriptome of animal caps overexpressing Pax3 and Zic1 has been analyzed (Bae *et al.*, 2014, Plouhinec *et al.*, 2014).



**Fig. 1. Transcriptome analysis of animal explants induced by co-injection of *wnt3a* and *chd* mRNA.** (A) Schematic diagram of experimental procedures. (B) *Kctd15* suppressed the expression of NC marker genes in animal cap assays. Both blastomeres of two-cell stage embryos were injected with the indicated mRNAs. RT-PCR was performed to examine expression of *snai2*, *sox9*, and *foxd3*. Co-overexpression of *wnt3a* and *chd* strongly induced the expression of these genes, and the induction was suppressed by addition of *kctd15*. *Odc* was the loading control. WE, whole embryos. (C,D) The top ten enriched GO terms in differentially expressed genes in the comparison of (B) induced to control (WC/L), and (C) induced/inhibited to induced (WCK/WC) animal caps.

This procedure is more selective for NC induction (Hong and Saint-Jeannet, 2007, Milet *et al.*, 2013), but we chose Wnt/Chd injection to test broadly for effects of Kctd15 at the neural plate border (NPB). Among genes induced by Wnt/Chd and inhibited by Kctd15 we find many well characterized NC markers as well as several hatching gland markers. We further identify several strongly affected genes not previously studied in this context whose further analysis may contribute to an understanding of the role of Kctd15 and AP-2 in NC formation and other processes in the embryo.

## Results

### Identification of genes regulated by Kctd15 during neural crest formation

We wished to survey the range of genes that are inhibited by Kctd15 overexpression in the context of NC induction by using Affymetrix DNA microarray. Injection of RNA encoding a Wnt and a BMP inhibitor such as Chordin into *Xenopus* embryos followed by animal cap culture (Fig. 1A) leads to the induction of many NC and additional NPB border genes (Saint-Jeannet *et al.*, 1997), whereas co-injection of Kctd15 inhibits induction (Dutta and Dawid, 2010). Recent analyses by other workers have focused on induction of NC genes more selectively by Pax3 and Zic1 (Bae *et al.*, 2014, Plouhinec *et al.*, 2014). We injected four sets of RNAs, *lacZ* as control (L), *wnt3a+chd* (WC), *wnt3a+chd+kctd15* (WCK), and *kctd15* (K). We tested several NC markers by PCR and found that, as predicted, *wnt+chd* induced, and *kctd15* inhibited NC marker expression (Fig. 1B). Microarray analysis was then carried out (for raw data see GEO accession number GSE72391). Here we will deal with the two relevant comparisons: WC vs. L, representing genes activated during NPB/NC induction, although additional genes are affected; and WCK vs. WC, representing genes inhibited by Kctd15 in the context of Wnt/Chd overexpression. For further analysis we selected genes with a fold change of two or greater and a p value <0.05. Under the criteria stated above, there are 2869 Affymetrix probe sets that are induced by Wnt/Chd, while 3680 probe sets are suppressed (Supplementary Table S2). Note that Affymetrix probe sets often represent genes repetitively; nevertheless we will use “genes” to stand for “probe set” hereafter. A number of known NPB or NC genes were strongly induced by Wnt/Chd overexpression (Table 1), supporting the microarray results.

Gene ontology (GO) analysis of the NPB/NC transcriptome revealed that GO terms of cellular component organization or biogenesis, cellular component organization, developmental process, anatomical structure morphogenesis, anatomical structure development, single-organism developmental process, single-organism process, single-organism cellular process, system development, and cell morphogenesis are highly represented (Fig. 1C).

We next surveyed the comparison of WCK vs. WC, and found that 334 genes are suppressed by addition of Kctd15, while 210 genes were increased under the criteria stated above (Supplementary Table S3). Among the suppressed genes we found all genes listed in Table 1 except *pax3a/b* and *zic1* (see Discussion). Expression of Kctd15 itself was much reduced in WCK animal caps, suggesting that Kctd15 negatively regulates its own expression. Gene ontology analysis showed that GO terms regulation of cell differentiation, positive regulation of cell differentiation, response to inorganic substance, response

TABLE 1

### INDUCTION AND REPRESSION BY KCTD15 OF KNOWN GENES CHARACTERISTIC FOR NEURAL BORDER AND NEURAL CREST

Gene	Unigene	WC vs L, Fold	WC vs L, p	WCK vs WC, Fold	WCK vs WC, p
<i>foxd3a</i> <sup>±</sup>	XI.525	144	1.22E-05	-5.23	0.013
<i>pax3b</i> <sup>±</sup>	XI.45266	129	9.44E-05	N. S.	
<i>foxd3b</i> <sup>±</sup>	XI.523	119.3	3.93E-05	-5.65	0.018
<i>zic1</i> <sup>±</sup>	XI.1796	105.3	4.44E-05	N. S.	
<i>pax3a</i> <sup>±</sup>	XI.49495	84.86	3.71E-04	N. S.	
<i>sox9a</i> <sup>±</sup>	XI.1690	51.5	8.00E-05	-14.13	0.0011
<i>lmx1b.1</i> <sup>±</sup>	XI.12464	38.52	4.39E-06	-2.57	0.0013
<i>sox10</i> <sup>±</sup>	XI.1588	36.2	0.00359	-12.56	0.02
<i>sox8</i> <sup>±</sup>	XI.29789	34.28	2.34E-05	-5.4	0.0011
<i>snail2a</i> <sup>±</sup>	XL.3818	26.6	0.0009033	-20.54	0.0015
<i>twist1b</i> <sup>±</sup>	XI.56708	10.8	6.86E-05	-3.21	0.0061
<i>tfap2e</i> <sup>±</sup>	XI.50785	4.427	0.000404	-2.2	0.016

WC, Wnt+Chd, referring to RNA from animal caps injected with these mRNAs; L, LacZ; WCK, Wnt+Chd+Kctd15. N.S., change not significant.

<sup>±</sup> Genes identified in Table 2 or Table 3 of (Bae *et al.*, 2014).

<sup>#</sup> Genes identified in Table 2 or Table S1 of (Plouhinec *et al.*, 2014).

TABLE 2

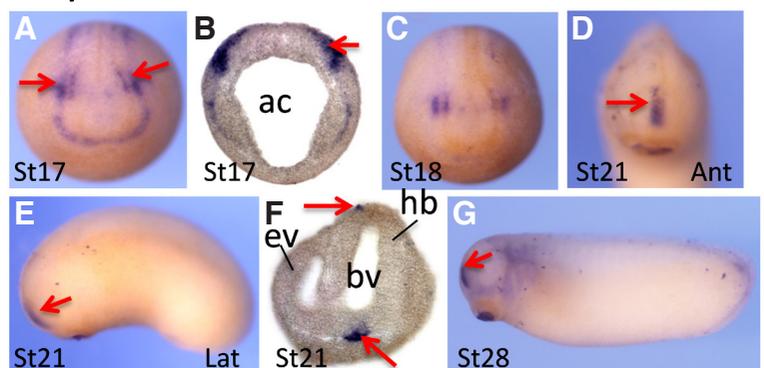
### MICROARRAY VALUES FOR INDUCTION AND INHIBITION OF GENES TESTED BY *IN SITU* HYBRIDIZATION

Gene	Unigene	WC vs L, Fold	WC vs L, p	WCK vs WC, Fold	WCK vs WC, p
<i>ddcp1</i>	XI2.13675	27.0745	3.54E-06	-4.09502	0.00135358
<i>pdgfra</i>	XI2.20029	16.6366	2.33E-05	-3.5796	0.00230006
<i>ctf</i>	XI2.41202	8.49842	4.00E-06	-2.36589	0.00214363
<i>zswim5</i>	XI2.40416	3.04663	0.0003077	-2.40434	0.00142697
<i>c3</i>	XI2.44891	14.404	0.0166839	-6.64701	0.0951221

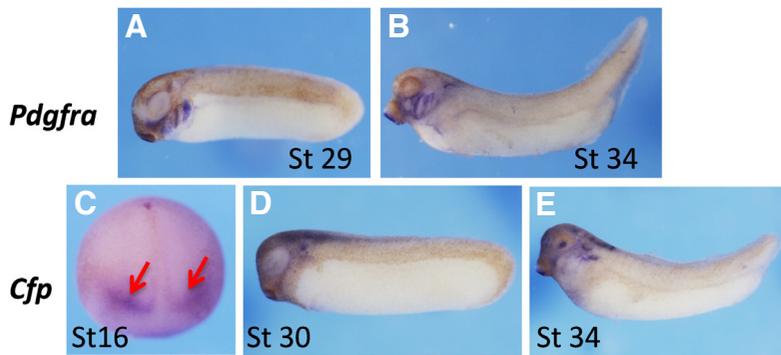
See Table 1 for abbreviations.

to estradiol, positive regulation of developmental process, response to estrogen, stem cell differentiation, response to lipid, tissue development, response to oxygen-containing compound are significantly enriched (Fig. 1D). While multiple terms are included, Kctd15 appears to affect most strongly aspects of cell differentiation.

### *ddcp1*



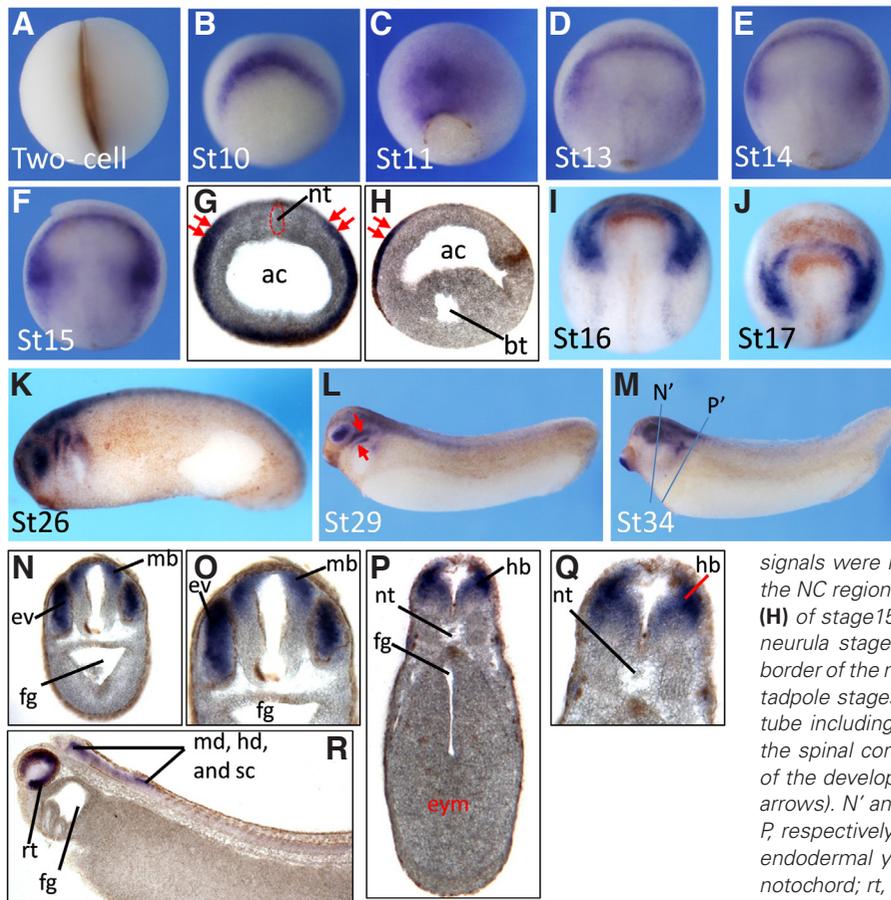
**Fig. 2. Spatial expression pattern of *ddcp1* encoding a Duf domain protein.** (A-C) *ddcp1* was expressed along the edge of neural plate. Notably, two stronger staining patches (arrows) were detected in whole mount embryos (A) and transverse sections (B). With the closure of neural tube, the two signaling patches merged and were located at the frontal region of the head (D-G). Ant, anterior view; lat, lateral view. Ac, archerteron; ev, eye vesicle; bv, brain vesicle; hb, hindbrain.



**Fig. 3. Spatial expression pattern of *pdgfra* and *cfp*.** (A-B) Strong signals of *pdgfra* were detected in branchial arches at tail bud and tadpole stages. (C-E) *Cfp* signals were detected first at mid neurula stage, and appear as two stripes at the anterior neural plate indicated by red arrows (C). The signals were detected in brain, lens, and ear vesicle at tail bud and tadpole stages (D, E).

As two recent reports examined gene expression in ACs induced to form NC by *pax3* and *zic1* injection (Bae et al., 2014, Plouhinec et al., 2014), we tested the overlap between the gene sets in GEO submissions associated with these references and our data, using genes changed by 2-fold or more in either direction at  $p < 0.05$ . Among 203 genes increased in the data of Plouhinec et al., 154 are also increased in our data, and among 227 decreased genes, 205 in our set are likewise decreased (Supplementary Table S4).

### Zswim 5



**Fig. 4. Spatial expression pattern of *zswim5*.**

*Zswim5* was first detected at the dorsal blastopore lip in early gastrula (A,B), and then was preferentially expressed in the future neural plate at later gastrula stages (C). At early neurula stages, *zswim5* signals were restricted at the anterior neural plate, partially covering the NC region (D-F). (G,H) Transverse section (G) and sagittal section (H) of stage 15 embryos. Red arrows point to stained regions. At mid neurula stages, *zswim5* signals were restricted in NC and anterior border of the neural plate (I,J). (K-R) *Zswim5* expression at tail bud and tadpole stages. *Zswim5* was mainly expressed in the anterior neural tube including forebrain, midbrain, hindbrain, and anterior portion of the spinal cord. In addition, signals were also detected in the retina of the developing eye and the forming cranial nerves (pointed by red arrows). N' and P' indicate the planes of the sections shown in N and P, respectively. Ac, archenteron; bt, blastocoel; ev, eye vesicle; eym, endodermal yolk mass; fg, foregut; hb, hindbrain; mb, midbrain; nt, notochord; rt, retina; and sc, spinal cord.

In the comparison with the work of Bae et al., of 713 increased genes 702 are also increased in our data, and among 863 decreased genes 853 are decreased in our data (Supplementary Table S5). Thus the overlap with both sets of data is high.

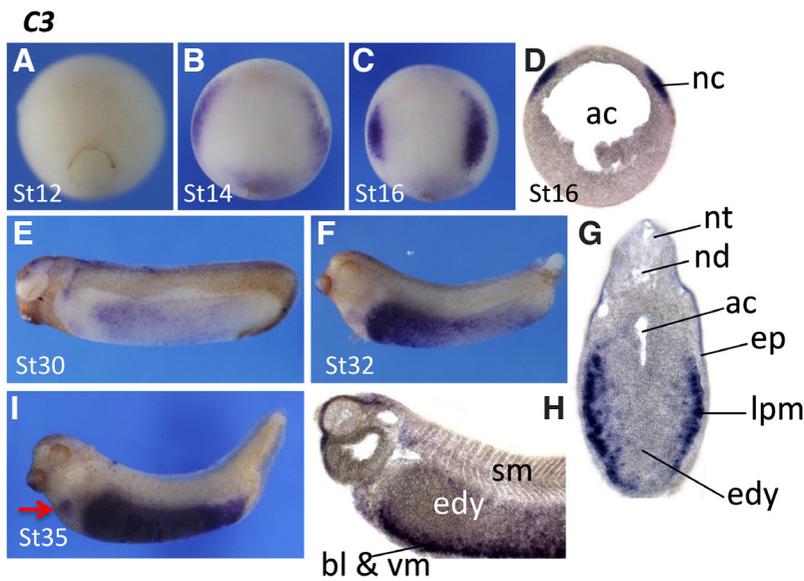
### Developmental expression of selected genes affected by *Kctd15*

We next used whole mount *in situ* hybridization to examine the expression pattern of genes encoding Duf domain protein 1 (*ddcp1*), Platelet-derived growth factor receptor a (*pdgfra*), Complement factor properdin (*cfp*), Zinc finger SWIM-Type Containing 5 (*zswim 5*), and Complement component C3 (Table 2).

Duf domain containing protein 1 (*ddcp1*) encodes a protein that has not been characterized, and even the function of the Duf domain is unknown. Signals for *ddcp1* became visible at neurula stages, forming stripes along the anterior and lateral edges of neural fold (Fig 2 A,B). Two signal patches at each side with higher intensity can be observed at the future head region (arrows in Fig. 2A). Transverse sections at stage 18 indicated that staining was localized in a region extending from ectoderm, mesoderm to the endoderm (Fig. 2B). With the progress of neural fold closure, the two signal patches seem to merge and form a short strip at the midline of the head region at tadpole and tailbud stages (Fig. 2C-E,G). Transverse sections identify an additional expression region of *ddcp1* at the stomodeal-hypophyseal anlage (Fig. 2F).

Platelet-derived growth factor receptor (Pdgr) is a receptor tyrosine kinase that has been identified as a *pax3/zic1* target (Bae et al., 2014, Plouhinec et al., 2014). These authors also showed expression of *pdgfra* in the NC region at neurula stages. We found strong expression in the branchial arches in tail bud and tadpole stage embryos (Fig. 3 A,B).

Complement factor properdin (*Cfp*) is a cytoplasmic glycoprotein that is implicated in regulating the alternative complement pathway of the innate immune system (Ali et al., 2014). *Cfp* transcripts can be detected in neurula



**Fig. 5. Spatial expression pattern of complement component C3 (C3).** *C3* signals were not detected by late gastrula stages (A). Its expression appeared at the anterior border of the neural plate, and then became restricted at the NC (B, C). NC expression was confirmed by sectioning (D). *C3* expression in the NC declined quickly, and an expression domain gradually appeared at the ventral region of the trunk (E,F). Sections from stage 32 embryos indicated that *C3* was expressed in the somatic and splanchnic layer of lateral plate mesoderm just internal to the epidermis. (G), sagittal section; (H), transversal section. Endodermal expression is prominent at later stages (I). Arrow points to an expression domain in the heart primordium. Ac, archenteron; bl, blood island; edy, endodermal yolk mass; ep, epidermis; lpm, lateral plate mesoderm; nc, neural crest; nt, neural tube; nd, notochord; sm, somite; vm, ventral mesoderm.

stage embryos, presenting as two diffuse stripes extending laterally from the dorsal midline of stage 16 embryos. Expression of *cfp* was observed in the neural tube, otic vesicle, and dorsal region of the branchial arches at tadpole stages (Fig. 3 D,E).

Zinc Finger SWIM-Type Containing 5 (*zswim5*) encodes a protein that remains to be characterized. Whole mount *in situ* hybridization indicates that *zswim5* was not expressed before gastrula stages (Fig. 4A). Staining was first detected in the dorsal blastopore lip at the onset of gastrulation (Fig. 4B). The positive region expanded and covered the prospective neural plate at mid gastrula stage (Fig. 4C). At the early neurula stages, *zswim5* was expressed at the anterior border of the neural plate, partially overlapping with the NC region (Fig. 4 D-F), and this expression was further confirmed by transverse and sagittal sections (Fig. 4G,H). Weak signals were also detected along the lateral border of the neural plate, extending posteriorly. At mid and late neurula stages, *zswim5* was detected in the anterior border of the neural plate and the NC (Fig. 4 I,J). At tail bud and tadpole stages, *zswim5* expression can be detected in the brain and anterior portion of spinal cord. In addition, it was also detected in the eye, especially in the retina region (Fig. 4 K-R). It is notable that some regions of forming cranial nerves were also stained (pointed by arrow in Fig. 4L) (Wang *et al.*, 2011).

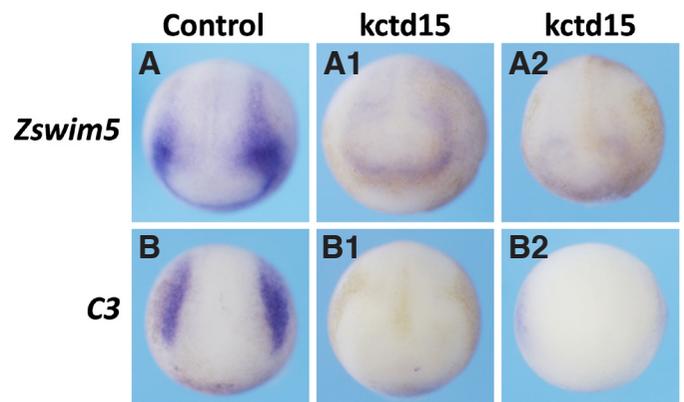
Complement component 3 (C3) is one of the key components in activation of the complement system in higher vertebrates. Its activation is required for both classical and alternative complement activation pathways. *C3* transcripts became visible from the early neurula stages onward (Fig. 5 A,B). At stage 14, the *C3* transcripts were detected at the edge of the forming neural plate. Staining for

*C3* was specifically observed in the NC at stage 16, and this expression was confirmed by sectioning (Fig. 5 C,D). At the tail bud and tadpole stages, a strong *C3* signal was observed in the trunk region, whereas the head was much less stained. Transverse sections of embryos after whole mount *in situ* hybridization revealed that the *C3* transcripts are located in a region corresponding to the somatic and splanchnic layer of lateral plate mesoderm, and ventral mesoderm including the blood island (Fig. 5E-H). At stage 35, *C3* stains the heart primordium in addition to intense endodermal expression (Fig. 5I). *C3* expression in *Xenopus* has previously been reported by McLin and colleagues (McLin *et al.*, 2008). While there are some differences in detail our results confirm strong NC expression at stage 16-17, and strong endodermal expression at later stages.

To validate the microarray data, we examined the expression of *zswim5* and *C3* upon overexpression of *kctd15* by *in situ* hybridization. We injected *kctd15* mRNA into either both blastomeres of two-cell stage embryos or two dorsal blastomeres of four-cell stage embryos, and checked the expression of *zswim5* and *C3*. Overexpression of *kctd15* dramatically reduced *zswim5* and *C3* staining, consistent with our microarray data (Fig. 6; Table 2).

## Discussion

Further exploration of the regulatory network that controls NC formation is essential for understanding this developmental process in the normal embryo and the pathological conditions due to disturbed NC development. *Kctd15* was previously implicated in the regulation of NC formation, as overexpression of *Kctd15* causes strong inhibition of NC development (Dutta and Dawid, 2010). In order to further understand the molecular events regulated by *Kctd15*, we performed DNA microarray analysis to identify genes that are affected by *Kctd15*



**Fig. 6. Overexpression of Kctd15 inhibits expression of *zswim5* and *C3*.** Both blastomeres of two-cell stage (A1,B1) or two dorsal blastomeres of four-cell stage embryos (A2,B2) were injected with *kctd15* mRNA, and the injected embryos were collected at about stage 16. The expression *zswim5* and *C3* was strongly inhibited compared to those in control embryos (A,B). (A1) 72%, 13 of 18 embryos; (A2), 95%, 19 of 20 embryos; (B1), 58%, 11 of 17 embryos, and (B2) 73.7%, 14 of 19 embryos.

during NC formation.

In the system we used, *Xenopus* animal caps induced by inhibition of BMP and activation of Wnt signaling, NC differentiation is elicited together with other events. Yet this system allows a broad evaluation of genes that are affected during the induction process. We found activation of expression of multiple genes known to be characteristic of NC development, some of which are illustrated in Table 1. In line with previous findings, we also detected components involved in modulating Wnt (Shisa), retinoic acid (rarres1, cyp26c1, cyp26a1), and BMP (bambi) signaling, and other genes identified in earlier work. In addition, we have identified a number of novel genes that are induced under these conditions and future analysis will be needed to elucidate their possible functions in NC specification and differentiation.

During the NC specification, coordination of *pax3* and *zic1* gene activity is essential and sufficient to initiate this complex process (Bae *et al.*, 2014, Garnett *et al.*, 2012, Hong and Saint-Jeannet, 2007, Milet *et al.*, 2013, Monsoro-Burq *et al.*, 2005, Sato *et al.*, 2005). Two recent papers reported the target gene profile of Pax3 and Zic1, both of which utilized inducible constructs in order to identify genes specifically involved in NC specification, EMT induction, and migration. Comparison of our results with those of (Bae *et al.*, 2014) and (Plouhinec *et al.*, 2014) shows a considerable degree of overlap (see Results).

The focus of our study has been to identify genes whose activation during manipulation of Wnt and BMP signaling is sensitive to Kctd15. In the subset of genes shown in Table 1, all except *pax3a/b* and *zic1* are strongly inhibited. As Kctd15 strongly inhibits AP-2 function in addition to lesser inhibition of Wnt signaling (Dutta and Dawid, 2010, Zarelli and Dawid, 2013), these results suggest that *pax3* and *zic1* expression in the neural border might be independent of AP-2. Using morpholino-mediated knock-down and enhancer studies, de Croze *et al.*, (de Croze *et al.*, 2011) found that expression of *pax3*, but not of *zic1* depends on AP-2 function in this system. A possible explanation for this difference may be that different members of the AP-2 family may be involved with different Kctd15 sensitivity, or that the particular *pax3* enhancer studied confers Kctd15 resistance on AP-2 molecules bound to it. In any case, it is of interest that Kctd15 can inhibit expression of multiple genes lower in the NC induction hierarchy, but does not affect two genes at its top.

Members of *sox* gene family, notably *sox8*, *sox9* and *sox10*, are strongly activated by NC induction and are very susceptible to overexpression of *kctd15*. All three genes belong to the *soxE* group, which are known to be important to NC formation (Haldin and LaBonne, 2010). The effect of Kctd15 on *soxE* gene expression is likely through inhibition of AP-2 function, known to be upstream of the *sox* genes in NC formation (de Croze *et al.*, 2011). In addition, Kctd15 may have a role in chondrogenesis and osteogenesis as Sox9 and Sox10 are essential in these two developmental processes (Kozhemyakina *et al.*, 2015, Long and Ornitz, 2013). Some matrix metalloproteinases including adam33, mmp3 and mmp28a, and the genes involved in ER stress and the secretory pathway such as creb3l2 and dnajb9 are also inhibited by ectopic Kctd15 (Supplementary Table S3), suggesting again a broad spectrum of developmental processes that can be regulated by Kctd15.

We have examined the expression of some genes induced by Wnt/Chd and repressed by Kctd15, i.e. *ddcp1*, *pdgfra*, *cfp*, *zsmim5* and *C3*, by *in situ* hybridization, and further confirmed that *zswim5*

and *C3* are inhibited by overexpression of Kctd15. We chose these genes because their possible role in NC formation was not previously noted, or noted but not studied extensively. We find expression of these genes variously in the neural border, premigratory NC, subsequent NC derivatives, and also in unrelated tissues. These observations suggest that Kctd15 may affect the development of multiple cells and tissues in embryogenesis. The large number of genes affected by Kctd15 in our experiments further suggest that the molecular function of Kctd15 might be in the regulation of transcription rather than in other pathways as indicated for different members of the Kctd family (Balasco *et al.*, 2014, Bayon *et al.*, 2008, Canetti *et al.*, 2010, Chen *et al.*, 2009, Correale *et al.*, 2011). Our results provide a framework for future studies into the biological role of Kctd15.

## Materials and Methods

### Embryo manipulation

*In vitro* fertilization and *X. laevis* embryo culture were performed as described previously (Wang *et al.*, 2011). Embryos were staged according to Nieuwkoop and Faber (Nieuwkoop and Faber, 1967). Experiments have been approved by the NICHD Animal Care and Use Committee. Capped mRNA for microinjection was prepared with mMessage mMachine kit (Invitrogen) and purified with RNeasy mini kit (Qiagen).

### Total RNA extraction

Total RNA was extracted using the TRIzol reagent (Invitrogen) and precipitated by isopropanol. After DNase I treatment, the total RNA was purified with RNeasy purification kit (Qiagen).

### Animal cap assay and RT-PCR

Animal cap assay was performed as described previously (Shi *et al.*, 2015, Zhao *et al.*, 2008). Briefly, animal caps were dissected from stage 9 embryos that had been injected with the indicated mRNAs, and were cultured till the sibling embryos reached stage 18. Total RNA was extracted for microarray or RT-PCR assays. cDNA was synthesized using Superscript III (Invitrogen) following the manufacturer's manual. Primers for RT-PCR are listed in Supplementary Table S1.

### Whole mount *in situ* hybridization

The EST clones were purchased from GE Healthcare. The preparation of digoxigenin-labeled RNA probe and whole mount *in situ* hybridization were performed as described previously (Shi *et al.*, 2015, Wang *et al.*, 2015). Embryos probed with indicated antisense RNAs were sectioned by a vibratome (Zeiss) to a thickness of 50  $\mu\text{m}$  (Shi *et al.*, 2015).

### Affymetrix DNA microarray and data analysis

AC treated as described above were used to prepare RNA; three biological replicates were done for each condition. Biotinylated probe was prepared according to manufacturer's instructions (Affymetrix). Probes were hybridized to *X. laevis* genome arrays 2.0 (Affymetrix). The hybridized arrays were washed by the GeneChip Fluidics station 450, and scanned with the GeneChip Scanner 3000 (Affymetrix). Gene expression profiles were analyzed by the Partek Genomics Suite software (Partek). The microarray data set was submitted to the NCBI GEO database and obtained accession number GSE72391.

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## References

- ALI, Y.M., HAYAT, A., SAEED, B.M., HALEEM, K.S., ALSHAMRANI, S., KENAWY, H.I., FERREIRA, V.P., SAGGU, G., BUCHBERGER, A., LACHMANN, P.J. *et al.*, (2014). Low-dose recombinant properdin provides substantial protection against *Streptococcus pneumoniae* and *Neisseria meningitidis* infection. *Proc Natl Acad Sci USA* 111: 5301-5306.
- BAE, C.J., PARK, B.Y., LEE, Y.H., TOBIAS, J.W., HONG, C.S. and SAINT-JEANNET, J.P. (2014). Identification of Pax3 and Zic1 targets in the developing neural crest. *Dev Biol* 386: 473-483.
- BALASCO, N., PIRONE, L., SMALDONE, G., DI GAETANO, S., ESPOSITO, L., PEDONE, E.M. and VITAGLIANO, L. (2014). Molecular recognition of Cullin3 by KCTDs: insights from experimental and computational investigations. *Biochim Biophys Acta* 1844: 1289-1298.
- BAYON, Y., TRINIDAD, A.G., DE LA PUERTA, M.L., DEL CARMEN RODRIGUEZ, M., BOGETZ, J., ROJAS, A., DE PEREDA, J.M., RAHMOUNI, S., WILLIAMS, S., MATSUZAWA, S. *et al.*, (2008). KCTD5, a putative substrate adaptor for cullin3 ubiquitin ligases. *FEBS J* 275: 3900-3910.
- BREWER, S., FENG, W., HUANG, J., SULLIVAN, S. and WILLIAMS, T. (2004). Wnt1-Cre-mediated deletion of AP-2alpha causes multiple neural crest-related defects. *Dev Biol* 267: 135-152.
- CANETTIERI, G., DI MARCOTULLIO, L., GRECO, A., CONI, S., ANTONUCCI, L., INFANTE, P., PIETROSANTI, L., DE SMAELE, E., FERRETTI, E., MIELE, E. *et al.*, (2010). Histone deacetylase and Cullin3-REN(KCTD11) ubiquitin ligase interplay regulates Hedgehog signalling through Gii acetylation. *Nat Cell Biol* 12: 132-142.
- CHEN, Y., YANG, Z., MENG, M., ZHAO, Y., DONG, N., YAN, H., LIU, L., DING, M., PENG, H.B. and SHAO, F. (2009). Cullin mediates degradation of RhoA through evolutionarily conserved BTB adaptors to control actin cytoskeleton structure and cell movement. *Mol Cell* 35: 841-855.
- CORREALE, S., PIRONE, L., DI MARCOTULLIO, L., DE SMAELE, E., GRECO, A., MAZZA, D., MORETTI, M., ALTERIO, V., VITAGLIANO, L., DI GAETANO, S. *et al.*, (2011). Molecular organization of the cullin E3 ligase adaptor KCTD11. *Biochimie* 93: 715-724.
- DE CROZE, N., MACZKOWIAK, F. and MONSORO-BURQ, A.H. (2011). Reiterative AP2a activity controls sequential steps in the neural crest gene regulatory network. *Proc Natl Acad Sci USA* 108: 155-160.
- DING, X., LUO, C., ZHOU, J., ZHONG, Y., HU, X., ZHOU, F., REN, K., GAN, L., HE, A., ZHU, J. *et al.*, (2009). The interaction of KCTD1 with transcription factor AP-2alpha inhibits its transactivation. *J Cell Biochem* 106: 285-295.
- DUTTA, S. and DAWID, I.B. (2010). Kctd15 inhibits neural crest formation by attenuating Wnt/beta-catenin signaling output. *Development* 137: 3013-3018.
- ECKERT, D., BUHL, S., WEBER, S., JAGER, R. and SCHORLE, H. (2005). The AP-2 family of transcription factors. *Genome Biol* 6: 246.
- GAMSE, J.T., KUAN, Y.S., MACURAK, M., BROSAMLE, C., THISSE, B., THISSE, C. and HALPERN, M.E. (2005). Directional asymmetry of the zebrafish epithalamus guides dorsoventral innervation of the midbrain target. *Development* 132: 4869-4881.
- GARNETT, A.T., SQUARE, T.A. and MEDEIROS, D.M. (2012). BMP, Wnt and FGF signals are integrated through evolutionarily conserved enhancers to achieve robust expression of Pax3 and Zic genes at the zebrafish neural plate border. *Development* 139: 4220-4231.
- GROVES, A.K. and LABONNE, C. (2014). Setting appropriate boundaries: fate, patterning and competence at the neural plate border. *Dev Biol* 389: 2-12.
- GUTIERREZ-AGUILAR, R., KIM, D.H., WOODS, S.C. and SEELEY, R.J. (2012). Expression of new loci associated with obesity in diet-induced obese rats: from genetics to physiology. *Obesity (Silver Spring)* 20: 306-312.
- HALDIN, C.E. and LABONNE, C. (2010). SoxE factors as multifunctional neural crest regulatory factors. *Int J Biochem Cell Biol* 42: 441-444.
- HILGER-EVERSHEIM, K., MOSER, M., SCHORLE, H. and BUETTNER, R. (2000). Regulatory roles of AP-2 transcription factors in vertebrate development, apoptosis and cell-cycle control. *Gene* 260: 1-12.
- HOFFMAN, T.L., JAVIER, A.L., CAMPEAU, S.A., KNIGHT, R.D. and SCHILLING, T.F. (2007). Tfp2 transcription factors in zebrafish neural crest development and ectodermal evolution. *J Exp Zool B Mol Dev Evol* 308: 679-691.
- HONG, C.S. and SAINT-JEANNET, J.P. (2007). The activity of Pax3 and Zic1 regulates three distinct cell fates at the neural plate border. *Mol Biol Cell* 18: 2192-2202.
- HU, X., GAN, S., XIE, G., LI, L., CHEN, C., DING, X., HAN, M., XIANG, S. and ZHANG, J. (2014). KCTD10 is critical for heart and blood vessel development of zebrafish. *Acta Biochim Biophys Sin (Shanghai)* 46: 377-386.
- IKEDA, K., MAEGAWA, H., UGI, S., TAO, Y., NISHIO, Y., TSUKADA, S., MAEDA, S. and KASHIWAGI, A. (2006). Transcription factor activating enhancer-binding protein-2beta. A negative regulator of adiponectin gene expression. *J Biol Chem* 281: 31245-31253.
- IKEYA, M., LEE, S.M., JOHNSON, J.E., MCMAHON, A.P. and TAKADA, S. (1997). Wnt signalling required for expansion of neural crest and CNS progenitors. *Nature* 389: 966-970.
- JIANG, M.S., TANG, Q.Q., MCLENITHAN, J., GEIMAN, D., SHILLINGLAW, W., HENZEL, W.J. and LANE, M.D. (1998). Derepression of the C/EBPalpha gene during adipogenesis: identification of AP-2alpha as a repressor. *Proc Natl Acad Sci USA* 95: 3467-3471.
- KNIGHT, R.D., JAVIDAN, Y., ZHANG, T., NELSON, S. and SCHILLING, T.F. (2005). AP2-dependent signals from the ectoderm regulate craniofacial development in the zebrafish embryo. *Development* 132: 3127-3138.
- KOZHEMYAKINA, E., LASSAR, A.B. and ZELZER, E. (2015). A pathway to bone: signaling molecules and transcription factors involved in chondrocyte development and maturation. *Development* 142: 817-831.
- LEON-MIMILA, P., VILLAMIL-RAMIREZ, H., VILLALOBOS-COMPARAN, M., VILLARREAL-MOLINA, T., ROMERO-HIDALGO, S., LOPEZ-CONTRERAS, B., GUTIERREZ-VIDAL, R., VEGA-BADILLO, J., JACOBO-ALBAVERA, L., POSADAS-ROMEROS, C. *et al.*, (2013). Contribution of common genetic variants to obesity and obesity-related traits in Mexican children and adults. *PLoS One* 8: e70640.
- LI, W. and CORNELL, R.A. (2007). Redundant activities of Tfp2a and Tfp2c are required for neural crest induction and development of other non-neural ectoderm derivatives in zebrafish embryos. *Dev Biol* 304: 338-354.
- LI, X., CHEN, C., WANG, F., HUANG, W., LIANG, Z., XIAO, Y., WEI, K., WAN, Z., HU, X., XIANG, S. *et al.*, (2014). KCTD1 suppresses canonical Wnt signaling pathway by enhancing beta-catenin degradation. *PLoS One* 9: e94343.
- LIU, Z., XIANG, Y. and SUN, G. (2013). The KCTD family of proteins: structure, function, disease relevance. *Cell Biosci* 3: 45.
- LONG, F. and ORNITZ, D.M. (2013). Development of the endochondral skeleton. *Cold Spring Harb Perspect Biol* 5: a008334.
- LUO, T., LEE, Y.H., SAINT-JEANNET, J.P. and SARGENT, T.D. (2003). Induction of neural crest in *Xenopus* by transcription factor AP2alpha. *Proc Natl Acad Sci USA* 100: 532-537.
- LUO, T., ZHANG, Y., KHADKA, D., RANGARAJAN, J., CHO, K.W. and SARGENT, T.D. (2005). Regulatory targets for transcription factor AP2 in *Xenopus* embryos. *Dev Growth Differ* 47: 403-413.
- MARNEROS, A.G., BECK, A.E., TURNER, E.H., MCMILLIN, M.J., EDWARDS, M.J., FIELD, M., DE MACENA SOBREIRA, N.L., PEREZ, A.B., FORTES, J.A., LAMPE, A.K. *et al.*, (2013). Mutations in KCTD1 cause scalp-ear-nipple syndrome. *Am J Hum Genet* 92: 621-626.
- MCLIN, V.A., HU, C.H., SHAH, R. and JAMRICH, M. (2008). Expression of complement components coincides with early patterning and organogenesis in *Xenopus laevis*. *Int J Dev Biol* 52: 1123-1133.
- MEI, H., CHEN, W., JIANG, F., HE, J., SRINIVASAN, S., SMITH, E.N., SCHORK, N., MURRAY, S. and BERENSON, G.S. (2012). Longitudinal replication studies of GWAS risk SNPs influencing body mass index over the course of childhood and adulthood. *PLoS One* 7: e31470.
- MENG, X., KONDO, M., MORINO, K., FUKU, T., OBATA, T., YOSHIZAKI, T., UGI, S., NISHIO, Y., MAEDA, S., ARAKI, E. *et al.*, (2010). Transcription factor AP-2beta: a negative regulator of IRS-1 gene expression. *Biochem Biophys Res Commun* 392: 526-532.
- MILET, C., MACZKOWIAK, F., ROCHE, D.D. and MONSORO-BURQ, A.H. (2013). Pax3 and Zic1 drive induction and differentiation of multipotent, migratory, and functional neural crest in *Xenopus* embryos. *Proc Natl Acad Sci USA* 110: 5528-5533.
- MONSORO-BURQ, A.H., WANG, E. and HARLAND, R. (2005). Msx1 and Pax3 cooperate to mediate FGF8 and WNT signals during *Xenopus* neural crest induction. *Dev Cell* 8: 167-178.
- NIEUWKOOP, P.D. and FABER, J. (1967). *Normal table of Xenopus laevis (Daudin)*. Garland publishing inc., New York.
- ORSO, F., PENNA, E., CIMINO, D., ASTANINA, E., MAIONE, F., VALDEMBRI, D.,

- GIRAUDO, E., SERINI, G., SISMONDI, P., DE BORTOLI, M. *et al.*, (2008). AP-2alpha and AP-2gamma regulate tumor progression via specific genetic programs. *FASEB J* 22: 2702-2714.
- PEREZ-TORRADO, R., YAMADA, D. and DEFOSSEZ, P.A. (2006). Born to bind: the BTB protein-protein interaction domain. *Bioessays* 28: 1194-1202.
- PLOUHINEC, J.L., ROCHE, D.D., PEGORARO, C., FIGUEIREDO, A.L., MACZKOWIAK, F., BRUNET, L.J., MILET, C., VERT, J.P., POLLET, N., HARLAND, R.M. *et al.*, (2014). Pax3 and Zic1 trigger the early neural crest gene regulatory network by the direct activation of multiple key neural crest specifiers. *Dev Biol* 386: 461-472.
- RAJALU, M., FRITZIUS, T., ADELFINER, L., JACQUIER, V., BESSEYRIAS, V., GASSMANN, M. and BETTLER, B. (2015). Pharmacological characterization of GABAB receptor subtypes assembled with auxiliary KCTD subunits. *Neuropharmacology* 88: 145-154.
- SAINT-JEANNET, J.P., HE, X., VARMUS, H.E. and DAWID, I.B. (1997). Regulation of dorsal fate in the neuraxis by Wnt-1 and Wnt-3a. *Proc Natl Acad Sci USA* 94: 13713-13718.
- SATO, T., SASAI, N. and SASAI, Y. (2005). Neural crest determination by co-activation of Pax3 and Zic1 genes in *Xenopus* ectoderm. *Development* 132: 2355-2363.
- SCHORLE, H., MEIER, P., BUCHERT, M., JAENISCH, R. and MITCHELL, P.J. (1996). Transcription factor AP-2 essential for cranial closure and craniofacial development. *Nature* 381: 235-238.
- SHI, W., XU, G., WANG, C., SPERBER, S.M., CHEN, Y., ZHOU, Q., DENG, Y. and ZHAO, H. (2015). Heat shock 70-kDa protein 5 (Hspa5) is essential for pronephros formation by mediating retinoic acid signaling. *J Biol Chem* 290: 577-589.
- SIMONES-COSTA, M. and BRONNER, M.E. (2015). Establishing neural crest identity: a gene regulatory recipe. *Development* 142: 242-257.
- SKOBLOV, M., MARAKHONOV, A., MARAKASOVA, E., GUSKOVA, A., CHANDHOKE, V., BIRERDINC, A. and BARANOVA, A. (2013). Protein partners of KCTD proteins provide insights about their functional roles in cell differentiation and vertebrate development. *Bioessays* 35: 586-596.
- SMALDONE, G., PIRONE, L., BALASCO, N., DIGAETANO, S., PEDONE, E.M. and VITAGLIANO, L. (2015). Cullin 3 Recognition Is Not a Universal Property among KCTD Proteins. *PLoS One* 10: e0126808.
- STOGIOS, P.J., DOWNS, G.S., JAUHAL, J.J., NANDRA, S.K. and PRIVE, G.G. (2005). Sequence and structural analysis of BTB domain proteins. *Genome Biol* 6: R82.
- TAO, Y., MAEGAWA, H., UGI, S., IKEDA, K., NAGAI, Y., EGAWA, K., NAKAMURA, T., TSUKADA, S., NISHIO, Y., MAEDA, S. *et al.*, (2006). The transcription factor AP-2beta causes cell enlargement and insulin resistance in 3T3-L1 adipocytes. *Endocrinology* 147: 1685-1696.
- TONG, X., ZU, Y., LI, Z., LI, W., YING, L., YANG, J., WANG, X., HE, S., LIU, D., ZHU, Z. *et al.*, (2014). Kctd10 regulates heart morphogenesis by repressing the transcriptional activity of Tbx5a in zebrafish. *Nat Commun* 5: 3153.
- WANG, C., KAM, R.K., SHI, W., XIA, Y., CHEN, X., CAO, Y., SUN, J., DU, Y., LU, G., CHEN, Z. *et al.*, (2015). The Proto-oncogene Transcription Factor Ets1 Regulates Neural Crest Development through Histone Deacetylase 1 to Mediate Output of Bone Morphogenetic Protein Signaling. *J Biol Chem* 290: 21925-21938.
- WANG, C., LIU, Y., CHAN, W.Y., CHAN, S.O., GRUNZ, H. and ZHAO, H. (2011). Characterization of three synuclein genes in *Xenopus laevis*. *Dev. Dynam.* 240: 2028-2033.
- WANG, Y., ZHENG, Y., LUO, F., FAN, X., CHEN, J., ZHANG, C. and HUI, R. (2009). KCTD10 interacts with proliferating cell nuclear antigen and its down-regulation could inhibit cell proliferation. *J Cell Biochem* 106: 409-413.
- WENKE, A.K. and BOSSERHOFF, A.K. (2010). Roles of AP-2 transcription factors in the regulation of cartilage and skeletal development. *FEBS J* 277: 894-902.
- WILLER, C.J., SPELIOTES, E.K., LOOS, R.J.L.I., S.LINDGREN, C.M., HEID, I.M., BERNDT, S.I., ELLIOTT, A.L., JACKSON, A.U., LAMINA, C. *et al.*, (2009). Six new loci associated with body mass index highlight a neuronal influence on body weight regulation. *Nat Genet* 41: 25-34.
- WILLIAMS, M.J., ALMEN, M.S., FREDRIKSSON, R. and SCHIOTH, H.B. (2012). What model organisms and interactomics can reveal about the genetics of human obesity. *Cell Mol Life Sci.* 13. 69: 3819-3834.
- ZARELLI, V.E. and DAWID, I.B. (2013). Inhibition of neural crest formation by Kctd15 involves regulation of transcription factor AP-2. *Proc Natl Acad Sci USA* 110: 2870-2875.
- ZHANG, Z.C., LIU, Y., LI, S.F., GUO, L., ZHAO, Y., QIAN, S.W., WEN, B., TANG, Q.Q. and LI, X. (2014). Suv39h1 mediates AP-2alpha-dependent inhibition of C/EBPalpha expression during adipogenesis. *Mol Cell Biol* 34: 2330-2338.
- ZHAO, H., TANEGASHIMA, K., RO, H. and DAWID, I.B. (2008). Lrig3 regulates neural crest formation in *Xenopus* by modulating Fgf and Wnt signaling pathways. *Development* 135: 1283-1293.

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