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# Kctd15 inhibits neural crest formation by attenuating Wnt/β-catenin signaling output

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## SUMMARY

Neural crest (NC) precursors are stem cells that are capable of forming many cell types after migration to different locations in the embryo. NC and placodes form at the neural plate border (NPB). The Wnt pathway is essential for specifying NC versus placodal identity in this cell population. Here we describe the BTB domain-containing protein Potassium channel tetramerization domain containing 15 (Kctd15) as a factor expressed in the NPB that efficiently inhibits NC induction in zebrafish and frog embryos. Whereas overexpression of Kctd15 inhibited NC formation, knockdown of Kctd15 led to expansion of the NC domain. Likewise, NC induction by Wnt3a plus Chordin in *Xenopus* animal explants was suppressed by Kctd15, but constitutively active β-catenin reversed Kctd15-mediated suppression of NC induction. Suppression of NC induction by inhibition of Wnt8.1 was rescued by reduction of Kctd15 expression, linking Kctd15 action to the Wnt pathway. We propose that Kctd15 inhibits NC formation by attenuating the output of the canonical Wnt pathway, thereby restricting expansion of the NC domain beyond its normal range.

**KEY WORDS: Neural crest, Neural plate border, Wnt signaling, Pigmentation, BTB domain, Zebrafish, *Xenopus***

## INTRODUCTION

Specification, maintenance and differentiation of neural crest (NC) cells depend on multiple signaling pathways. In *Xenopus* and zebrafish, low levels of Bmp and high levels of Wnt signaling cooperate in NC induction (Saint-Jeannet et al., 1997; Wilson et al., 1997; Dorsky et al., 1998; Lewis et al., 2004). As Wnt signaling regulates anterior-posterior (A-P) patterning of the neural plate (Kim et al., 2000; McGrew et al., 1997; McGrew et al., 1995), Wnt signals might induce NC through posteriorization. However, neural A-P patterning and NC induction are separable events (Wu et al., 2005). A recent study indicates that Wnt-mediated posteriorization of the neural plate border (NPB) rather than the neural plate is crucial in NC induction (Li et al., 2009). Signaling pathways active in NC specification are modulated by activators and inhibitors to regulate their strength and spatial distribution (Hong and Saint-Jeannet, 2007; Sauka-Spengler and Bronner-Fraser, 2008; Zhao et al., 2008). Here we introduce a factor, Potassium channel tetramerization domain containing 15 (Kctd15), that has a profound influence on NC formation in zebrafish and *Xenopus* embryos. KCTD15 was identified in humans (Hotta et al., 2009; Willer et al., 2009) as a BTB domain-containing protein of unknown function. We show that zebrafish *kctd15a* and *kctd15b* are expressed at the NPB at the end of gastrulation. Ectopic expression of Kctd15 inhibits NC specification, whereas knockdown leads to expansion of NC markers. Simultaneous attenuation of Wnt and Kctd15 expression rescues NC specification in zebrafish embryos. We suggest that Kctd15 restricts the NC domain by interfering with the functioning or output of the Wnt/β-catenin signaling pathway.

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## MATERIALS AND METHODS

### Animal maintenance and embryonic staging

Zebrafish (*Danio rerio*) embryos from AB/TL and TOP-dGFP (Dorsky et al., 2002) lines were obtained (Westerfield, 2000), and stages are indicated as hours post fertilization (hpf) (Kimmel et al., 1995). *Xenopus laevis* were staged according to (Nieuwkoop and Faber, 1967).

### Plasmids and DNA constructs

The open reading frames (ORFs) of zebrafish *kctd15a* (BC083478), zebrafish *kctd15b* (BC078294) and *X. laevis kctd15* (BC077862) were subcloned into pCS2$^{+}$. The zebrafish β-*cat** construct has four point mutations; S33A, S37A, T41A and S45A.

### MO and mRNA injection

Morpholino oligonucleotides (MO) were targeted to 5′UTR regions of zebrafish *kctd15a* (5′-TCCTTCCCTCCTTGGAAGACATAGC-3′) and zebrafish *kctd15b* (5′-AGCTCTCCTTCCCCCTCTTGATCTT-3′) (Gene Tools). Embryos at 1-2 cells were injected with 1.5 ng Kctd15a plus 0.5 ng Kctd15b MO; 2 ng Wnt8.1a MO (Lewis et al., 2004); or 2-4 ng standard control MO (5′-CCTCTTACCCTCAGTTACAATTTATA-3′) per embryo. 5′-capped mRNAs were prepared using the mMESSAGE mMACHINE Kit (Ambion). Each zebrafish embryo was injected with 50 pg *kctd15a*, *kctd15b*, *ΔNkctd15a*, *ΔCkctd15a*, *kctd10*, *kctd6* or *kctd13* mRNA, or 5 pg β-*cat** mRNA. One blastomere of 2-cell *Xenopus* embryos was injected with 250 pg of *Xkctd15* mRNA. For rescue experiments, 1-10 pg of mRNA was injected into fish embryos.

### Wholemount in situ hybridization and Alcian Blue staining

Zebrafish embryos were hybridized with one or two probes (Hauptmann and Gerster, 1994) for *kctd15a*, *kctd15b*, *pax3* (Seo et al., 1998), *foxd3* (Kelsh et al., 2000), *sox2* (Okuda et al., 2006), *sox10* (Dutton et al., 2001), *dlx2a* (Akimenko et al., 1994), *msxb* (Phillips et al., 2006), *dlx3b* (Ekker et al., 1992), *mitfa* (Lister et al., 1999), *gfp* (Dorsky et al., 2002), *foxi1* (Solomon et al., 2003), *lim3* (Glasgow et al., 1997), *prl* (Herzog et al., 2003), *cldna* (Kollmar et al., 2001), *eya1* (Sahly et al., 1999), *six4.1* (Kobayashi et al., 2000) and *gata1* (Detrich et al., 1995). INT-BCIP (red, Roche) and BM purple (blue, Roche) were used. Alcian Blue staining was as described (Barrallo-Gimeno et al., 2004).

### Animal cap assay

*Xenopus* 2-cell embryos were injected with mRNA(s) for *Xwnt3a* (300 pg/embryo), *Xchordin* (300 pg/embryo), *Xkctd15* (500 pg/embryo) or β-*cat** (50 pg/embryo). Animal caps were dissected at stage 8-9, cultured to stage 18 and RNA was analyzed by RT-PCR (Zhao et al., 2008).

### Luciferase assay

HEK293T cells in DMEM with 10% fetal calf serum were transfected using FuGENE (Roche). We used *Xkctd15* and *z*β-*cat** in pCS2$^{+}$ in addition to TOPflash luciferase and Renilla luciferase pRL-CMV (Promega) constructs. Assays were performed as described (Zhao et al., 2008).

## RESULTS AND DISCUSSION

### *kctd15* expression in zebrafish embryos

Cells in the NPB express *kctd15a* and *kctd15b* at the 1-somite (1s) stage (Fig. 1A,B). *kctd15a* colocalized with the preplacodal marker *dlx3b* (Toro and Varga, 2007) (Fig. 1C), but not with premigratory NC cells expressing *foxd3* (Monsoro-Burq et al., 2005; Steventon et al., 2005) (Fig. 1D). Subsequently, pharyngeal arches express *kctd15a*; additional expression domains include pronephric ducts, cranial placode precursors and the brain (Fig. 1E; see Fig. S1C,D,G in the supplementary material). *kctd15b* transcripts occur maternally (see Fig. S1A,B in the supplementary material), and subsequently are seen in the olfactory placode, cranial NC, lateral line primordium, pharyngeal arches, fin buds and optic tectum (see Fig. S1E,F in the supplementary material).

### Kctd15 inhibits pigmentation in zebrafish

Embryos injected with *kctd15a* or *b* mRNA showed a loss of pigmentation, short tail, bent axis, and loss of yolk extension (Fig. 1F,G; see Fig. S2A-C in the supplementary material). The non-NC-derived retinal pigmented epithelium was unaffected, suggesting that Kctd15 blocks the development of NC-derived melanophores. The effect is specific as mRNA injection of the related *kctd6*, *kctd10* and *kctd13* genes had no effect (see Fig. S2 in the supplementary material).

Embryos co-injected with MOs against *kctd15a* and *kctd15b* (Kctd15a$^{MO}$/15b$^{MO}$) exhibited small heads, abnormal somites and increased cell death (Fig. 1J,K), an apparent increase in pigmentation in the dorsal hindbrain region (Fig. 1L,M) and abnormal pharyngeal arches at 72 hpf (Fig. 1M, inset; see Fig. S3 in the supplementary material). These phenotypes were rescued by injection of *kctd15a* mRNA (see Fig. S4 in the supplementary material). Thus, suppressing Kctd15 had a complementary effect on pigmentation as overexpression, whereas pharyngeal arch development was impaired in both cases (Fig. 1M; see Fig. S3 in the supplementary material), suggesting early and late Kctd15 functions.

### The BTB region of Kctd15 is essential for its function

Kctd15 contains a BTB (POZ) domain (residues 32-119), a protein-protein interaction module (Stogios et al., 2005). Constructs encoding the N-terminal BTB domain (*ΔCkctd15a*) or the C-terminal region (*ΔNkctd15a*) were injected, showing that *ΔCkctd15a* caused a similar phenotype as full-length protein (Fig. 1G,I), whereas *ΔNkctd15a* had no effect (Fig. 1F,H). Thus, the BTB domain of Kctd15 is essential for its function in the embryo.

### Kctd15 inhibits NC specification

The above experiments suggest a role for Kctd15 in melanophore and pharyngeal arch development, both involving NC cells. Therefore, we analyzed the NC-specifier *pax3* and the pan-NC markers *foxd3* and *sox10* (Monsoro-Burq et al., 2005; Steventon et al., 2005) in Kctd15 morphants and overexpressing embryos. Expression of markers was reduced or lost in overexpressing embryos (Fig. 2A,C,D,F,G,I); similar results were obtained for

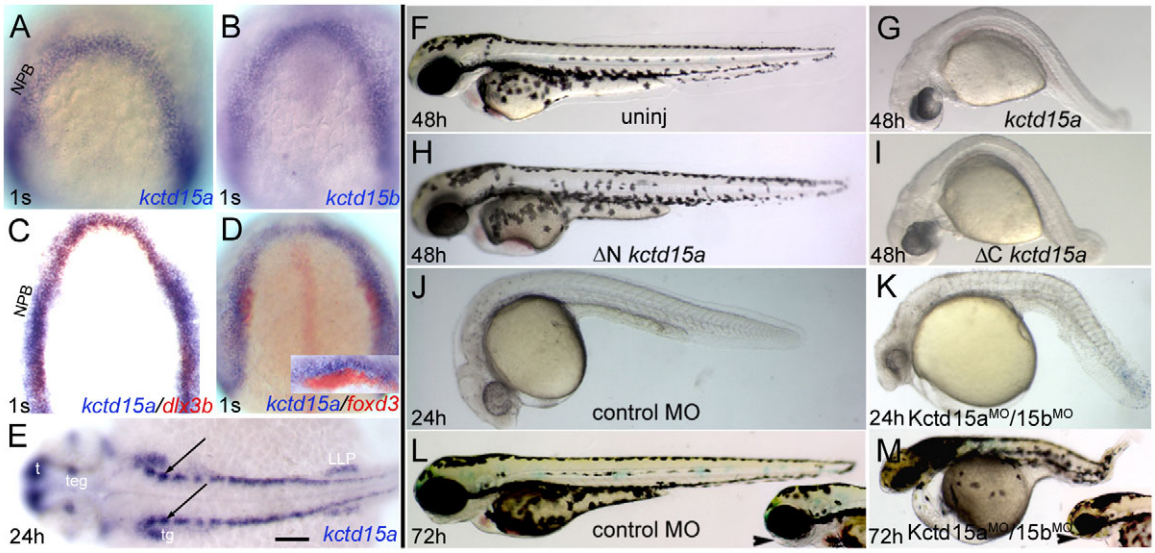


**Fig. 1. Zebrafish *kctd15* expression pattern, loss-of-function and gain-of-function phenotypes.** (**A-E**) Wild-type embryos were hybridized in situ, with probes indicated at lower right, stages shown at lower left. Dorsal views, anterior towards the top (A-D) or left (E). (A,B) Cells in the neural plate border (NPB) express *kctd15a* and *kctd15b*. (C) *dlx3b* (red) and *kctd15a* (blue) are coexpressed at the NPB. (D,D inset) *kctd15a* (blue) is excluded from the premigratory neural crest (NC) expressing *foxd3* (red). (E) *kctd15a* expression at 24 hpf. arrows, hindbrain neurons. (**F-M**) Embryos were injected with RNA (F-I) or MO (J-M). Lateral views, anterior to the left. Overexpression of *kctd15a* (G, 75/100) and *ΔCkctd15a* (I, 82/100) results in loss of pigmentation and a short tail, whereas *ΔNkctd15a* mRNA-injected embryos were normal (H, 60/60). Co-injection of Kctd15a/15b MO results in a small head and abnormal somites (K, 85/100) and subsequently increased dorsal pigmentation (M, 91/100) and loss of jaw elements (M inset, arrowhead). s, somite; t, telencephalon; teg, tegmentum; tg, trigeminal placode; LLP, lateral line primordia. Scale bars: 50 μm in A-D,L,M inset; 80 μm in E; 100 μm in F-M.

*msxb* and *sox9b* (data not shown). Consistently, Kctd15a$^{MO}$/15b$^{MO}$-injected embryos showed upregulation and expansion of these markers (Fig. 2B,E,H). These effects on *foxd3* expression are consistent at different stages (see Fig. S5 in the supplementary material). The pan-neural marker *sox2* is expressed at normal levels in Kctd15-overexpressing or morphant embryos (Fig. 2J-L) but the *sox2* domain is smaller in the morphants owing to a narrowing of the neural tube that might lead to the small head phenotype at later stages (Fig. 1K).

To study NC precursor differentiation, we tested *dlx2a* and *mitfa*. At 24 hpf, we observed a loss of *dlx2a* in pharyngeal arches and of *mitfa* in melanophore precursors in *kctd15a* mRNA-injected embryos (Fig. 2O,R). *foxd3* in the notochord and *dlx2a* in the telencephalon were unaltered (Fig. 2F,O), illustrating the specificity of NC inhibition. In Kctd15a$^{MO}$/15b$^{MO}$-injected embryos, the expression of *dlx2a* in pharyngeal arches was maintained (Fig. 2M,N) and expression of *mitfa* was increased (Fig. 2P,Q). Whereas the increased *mitfa* expression correlates with the intense pigmentation of MO-injected embryos (Fig. 1L,M), the inhibition of pharyngeal arch formation in these embryos (see Fig. S3B in the supplementary material) points to a late function of Kctd15 in this lineage.

At early stages, *kctd15* is expressed in the preplacodal domain but excluded from NC precursors; thus, it might have a role in delineating preplacodal versus NC identity. We find that *kctd15a* RNA-injected embryos show enhanced expression of the preplacodal markers *eya1* and *six4.1* in the anterior domain (see Fig. S6A-D in the supplementary material). Similarly, pan-pituitary markers *lim3* and *prl* were expanded (see Fig. S6E-H in the supplementary material). Expression of *foxi1*, a marker for otic progenitors, was unaffected and the later otic marker *cldna* was reduced (see Fig. S6I-L in the supplementary material). These results suggest that loss of NC caused by Kctd15 overexpression was compensated by expansion of the anterior preplacodal domain, whereas both NC and placode development were inhibited by Kctd15 in posterior regions.

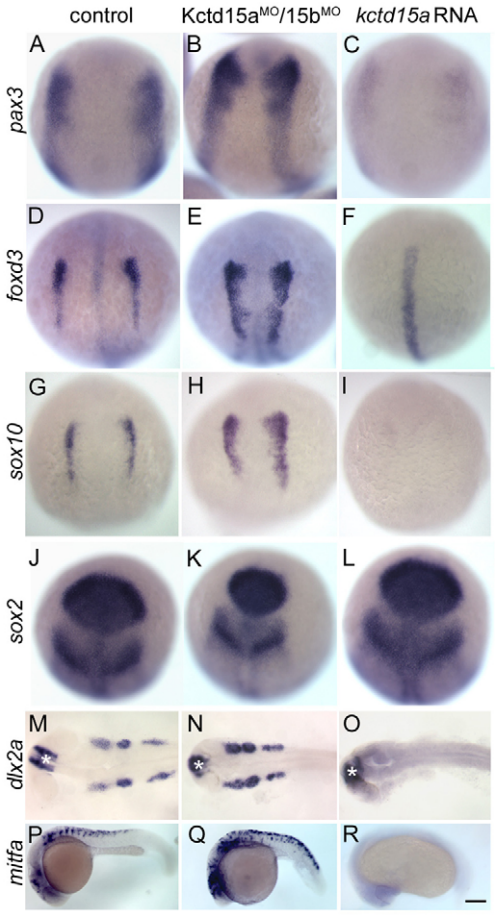


**Fig. 2. Kctd15 inhibits NC induction and differentiation.** (**A-R**) Control embryos (control MO-injected and uninjected embryos were indistinguishable; A,D,G,J,M,P), Kctd15a$^{MO}$/15b$^{MO}$-injected (B,E,H,K,N,Q) and *kctd15a* mRNA-injected (C,F,I,L,O,R) embryos were fixed at the 1-somite stage (A-L) or at 24 hpf (M-R). Expression of *pax3* (A-C), *foxd3* (D-F), *sox10* (G-I), *sox2* (J-L), *dlx2a* (M-O) and *mitfa* (P-R) was analyzed by in situ hybridization. Morphants showed expansion of *pax3* (B, 42/50), *foxd3* (E, 28/35) and *sox10* (H, 25/35). *kctd15a* mRNA-injected embryos showed inhibition of *pax3* (C, 38/45), *foxd3* (F, 45/50) and *sox10* (I, 32/37) in the NC domain, whereas *sox2* expression was preserved (K, 25/25; L, 20/20). Expression of *dlx2a* in the branchial arches was expanded in morphants (N, 32/44) and lost in overexpressing embryos (O, 35/40); *mitfa* was expanded in morphants (Q, 30/35) and lost in overexpressing embryos (R, 40/40). (A-L) Dorsal views, anterior towards the top; (M-O) dorsal views; (P-R) lateral views, anterior towards the left. Asterisk in M-O indicates *dlx2a* expression in the forebrain. Scale bar: 100 μm in A-L; 200 μm in M-R.

## Kctd15 antagonizes Wnt signaling during NC formation

Wnt signaling is necessary for NC specification and later differentiation (Dorsky et al., 1998; Garcia-Castro et al., 2002; LaBonne and Bronner-Fraser, 1998; Saint-Jeannet et al., 1997). We injected embryos with Wnt8.1 MO or with MOs for Wnt8.1, Kctd15a and Kctd15b and assayed NC specification. As reported (Lewis et al., 2004), *foxd3* expression was lost in Wnt8.1 morphants (Fig. 3A,B). Importantly, we observed a dramatic rescue of *foxd3* expression in embryos injected with a combination of Wnt8.1 and Kctd15a/b MOs (Fig. 3C,D). Similar results were obtained using *sox10* (data not shown). *kctd15a* expression in Wnt8.1 morphants was unaffected (see Fig. S7A,B in the supplementary material). These observations indicate that Kctd15 interferes with Wnt signal transduction or output, such that the reduction in ligand availability in the Wnt8.1 morphants is compensated for by alleviating the inhibition of pathway output by Kctd15.

To probe the relationship between Kctd15 and Wnt signaling, we treated embryos with LiCl, which inhibits Gsk3β activity leading to β-catenin stabilization (Klein and Melton, 1996; van de Water et al., 2001). Embryos were treated with 0.1 M LiCl (shield to bud) or 0.2 M LiCl (shield to 80% epiboly) and assayed for *foxd3* expression. Although 0.2 M LiCl led to ectopic *foxd3* expression, Kctd15 overexpression abolished all *foxd3* expression (Fig. 3E-J), indicating that Kctd15 acts downstream of Gsk3β in NC induction. By contrast, constitutively active β-*catenin* (β-*cat**) rescued *foxd3* expression in *kctd15* RNA-injected embryos, although ectopic *foxd3* expression was abolished (Fig. 3K-M). These results indicate that Kctd15 acts upstream of or in concert with β-catenin in NC induction.

## Kctd15 function is conserved and inhibits NC induction in explants

Explants from *Xenopus* embryos (animal caps) are induced to the NC by expression of Wnt and a Bmp antagonist (Saint-Jeannet et al., 1997). We used this system to further explore the function of

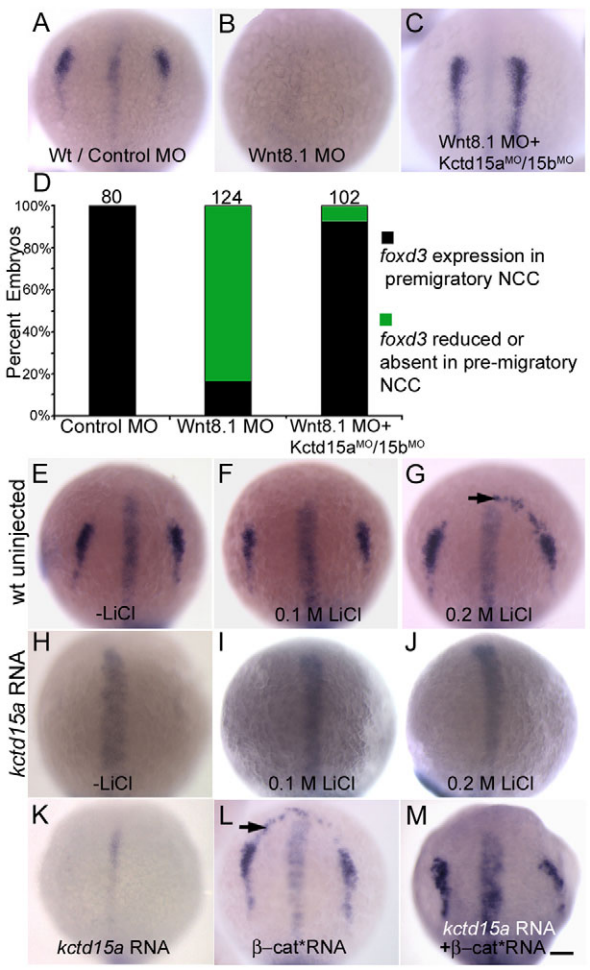


**Fig. 3. Kctd15 inhibits the output of canonical Wnt signaling.** (**A**) *foxd3* expression in control embryos. (**B**) Loss of *foxd3* expression in *wnt8.1* morphants. (**C**) Injection of Wnt8.1 MO together with Kctd15a/15b MOs rescued *foxd3* expression. (**D**) Quantification of A-C; number of embryos (*n*) is given above bars. (**E-J**) *foxd3* expression in embryos treated with LiCl (E-G). Arrow in G indicates expanded expression in 26/30 embryos. (H-J) LiCl did not rescue *foxd3* expression in *kctd15a* mRNA-injected embryos (I, 40/40; J, 50/50; numbers indicate embryos with appearance as shown in the figure). (**K-M**) Inhibition of *foxd3* by *kctd15a* (K) was rescued by β-*cat** mRNA (M, 41/50 showing *foxd3* expression in NC domain). β-*cat** mRNA alone induced ectopic expression of *foxd3* (L, arrow, 25/31). (A-C,E-M) 1-somite stage; dorsal views, anterior towards top. Scale bar: 100 μm.

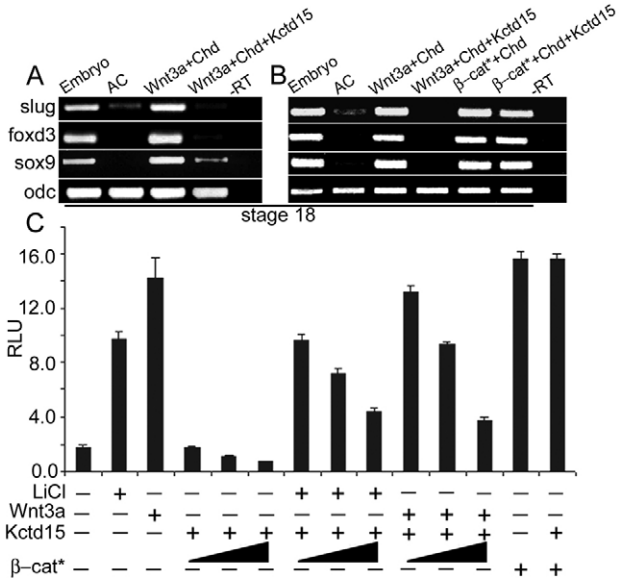


**Fig. 4. Kctd15 inhibits NC induction by antagonizing the Wnt signal.** (**A**,**B**) Kctd15 suppressed NC-specific marker gene induction by Chrd + Wnt3a, but not by Chrd + β-cat* in *Xenopus* animal caps. Injected RNAs are indicated at the top; expression of *slug*, *foxd3*, *sox9* and of *odc* as control was assayed by RT-PCR. (**C**) Wnt signaling in HEK293T cells was measured by TOPflash luciferase. Kctd15 moderately inhibited Wnt signaling stimulated by Wnt3a or LiCl but not by β-cat*. AC, uninjected animal caps; –RT, without reverse transcriptase; RLU, relative luciferase units.

Kctd15. Kctd15 function is conserved in frogs, as injection of *Xenopus kctd15* mRNA into one blastomere of 2-cell embryos suppressed the NC marker *slug* (*snail2*) (see Fig. S8 in the supplementary material). For explant experiments, embryos were injected with *wnt3a* plus *chordin* (*chrd*) mRNAs, with or without *kctd15* mRNA, and animal caps were assayed for NC markers. Induced *slug*, *foxd3* and *sox9* expression was strongly inhibited by the co-injection of *kctd15* mRNA (Fig. 4A). In zebrafish embryos, β-cat*-mediated induction of NC proved largely resistant to Kctd15 inhibition. This is also true in *Xenopus* animal caps (Fig. 4B). Thus, the explant and whole embryo experiments are consistent, indicating that Kctd15 inhibits NC induction at or above the level of β-catenin.

To determine whether Kctd15 is a general Wnt inhibitor, we tested Kctd15 in the *Xenopus* axis duplication assay (Smith and Harland, 1991; Sokol et al., 1991). Co-injection of *Xkctd15* did not reduce the frequency or completeness of Wnt8-induced axis duplications (data not shown). A recent study showed that an activator of Wnt signaling, Skip (1-341), was inefficient in inducing axis duplications (Wang et al., 2010), suggesting that certain modulators of Wnt signaling are inefficient in early frog embryos. In zebrafish, cells responsive to β-catenin can be monitored in the transgenic TOP-dGFP reporter line (Dorsky et al., 2002). GFP expression was reduced in *kctd15a* mRNA-injected transgenic embryos compared with controls (see Fig. S9A,B in the supplementary material). More detailed inspection of the NPB region revealed that *kctd15* mRNA-injected transgenic embryos showed a loss of GFP expression in the NC domain (see Fig. S9C,D in the supplementary material). Inhibition of the Wnt pathway could also be observed using TOPflash luciferase in HEK293T cells. Kctd15 reduced basal, Wnt3a or LiCl-stimulated TOPflash activity by about 3-fold, but not β-cat*-stimulated activity (Fig. 4C).

## Kctd15 is a novel regulatory factor in NC formation

In this report, we introduce Kctd15 as a novel regulator of NC formation in zebrafish and *Xenopus*. We provide evidence that Kctd15 acts as an inhibitor of NC specification by interfering with the output of Wnt signaling. We show that: (1) ectopic expression of Kctd15 leads to a dramatic reduction in the induction of premigratory NC cells in whole embryos and of early NC marker genes in induced animal caps; (2) conversely, the expression

domain of NC-specific genes is expanded in Kctd15 morphants; (3) loss of NC induction in zebrafish Wnt8.1 morphants was rescued by attenuating Kctd15 expression; (4) constitutively active β-catenin rescued the suppression of NC induction by Kctd15. These results show that Kctd15 is a potent regulator of NC formation.

Precursors of the NC and the preplacodal region arise from an initially mixed population of cells at the NPB (Streit, 2002) and are separated as a result of Wnt signaling and the action of Gbx2 (Li et al., 2009; Litsiou et al., 2005). Although the induction of Gbx2 in the NC domain is a key feature in its formation (Li et al., 2009), placode-specific inhibitors of NC induction might be required to assist in the separation of the two domains. We suggest that Kctd15 represents such a factor with a role in defining the NC versus the anterior preplacodal domain at the NPB. This suggestion is supported by the enhanced expression of several anterior preplacodal markers in *kctd15*-overexpressing embryos (see Fig. S6 in the supplementary material).

The BTB domain is found in many functional classes of proteins (Stogios et al., 2005) and various properties and associations have been reported for Kctd proteins (Bayon et al., 2008; Ding et al., 2009; Ding et al., 2008). Here, we have linked the inhibitory role of Kctd15 to Wnt pathway signal transduction or output. Our results show that Kctd15 is a novel, highly effective and specific regulatory component in NC induction with an apparent role in restricting the NC domain in the developing embryo.

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**Competing interests statement**
The authors declare no competing financial interests.

**Supplementary material**
Supplementary material for this article is available at
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**References**

**Akimenko, M. A., Ekker, M., Wegner, J., Lin, W. and Westerfield, M.** (1994). Combinatorial expression of three zebrafish genes related to distal-less: part of a homeobox gene code for the head. *J. Neurosci.* **14**, 3475-3486.
**Barrallo-Gimeno, A., Holzschuh, J., Driever, W. and Knapik, E. W.** (2004). Neural crest survival and differentiation in zebrafish depends on mont blanc/tfap2a gene function. *Development* **131**, 1463-1477.
**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.
**Detrich, H. W., 3rd, Kieran, M. W., Chan, F. Y., Barone, L. M., Yee, K., Rundstadler, J. A., Pratt, S., Ransom, D. and Zon, L. I.** (1995). Intraembryonic hematopoietic cell migration during vertebrate development. *Proc. Natl. Acad. Sci. USA* **92**, 10713-10717.
**Ding, X. F., Luo, C., Ren, K. Q., Zhang, J., Zhou, J. L., Hu, X., Liu, R. S., Wang, Y., Gao, X. and Zhang, J.** (2008). Characterization and expression of a human KCTD1 gene containing the BTB domain, which mediates transcriptional repression and homomeric interactions. *DNA Cell Biol.* **27**, 257-265.
**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.
**Dorsky, R. I., Moon, R. T. and Raible, D. W.** (1998). Control of neural crest cell fate by the Wnt signalling pathway. *Nature* **396**, 370-373.
**Dorsky, R. I., Sheldahl, L. C. and Moon, R. T.** (2002). A transgenic Lef1/beta-catenin-dependent reporter is expressed in spatially restricted domains throughout zebrafish development. *Dev. Biol.* **241**, 229-237.
**Dutton, K. A., Pauliny, A., Lopes, S. S., Elworthy, S., Carney, T. J., Rauch, J., Geisler, R., Haffter, P. and Kelsh, R. N.** (2001). Zebrafish colourless encodes sox10 and specifies non-ectomesenchymal neural crest fates. *Development* **128**, 4113-4125.
**Ekker, M., Akimenko, M. A., Bremiller, R. and Westerfield, M.** (1992). Regional expression of three homeobox transcripts in the inner ear of zebrafish embryos. *Neuron* **9**, 27-35.
**Garcia-Castro, M. I., Marcelle, C. and Bronner-Fraser, M.** (2002). Ectodermal Wnt function as a neural crest inducer. *Science* **297**, 848-851.
**Glasgow, E., Karavanov, A. A. and Dawid, I. B.** (1997). Neuronal and neuroendocrine expression of lim3, a LIM class homeobox gene, is altered in mutant zebrafish with axial signaling defects. *Dev. Biol.* **192**, 405-419.
**Hauptmann, G. and Gerster, T.** (1994). Two-color whole-mount in situ hybridization to vertebrate and *Drosophila* embryos. *Trends Genet.* **10**, 266.
**Herzog, W., Zeng, X., Lele, Z., Sonntag, C., Ting, J. W., Chang, C. Y. and Hammerschmidt, M.** (2003). Adenohypophysis formation in the zebrafish and its dependence on sonic hedgehog. *Dev. Biol.* **254**, 36-49.
**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.
**Hotta, K., Nakamura, M., Nakamura, T., Matsuo, T., Nakata, Y., Kamohara, S., Miyatake, N., Kotani, K., Komatsu, R., Itoh, N. et al.** (2009). Association between obesity and polymorphisms in SEC16B, TMEM18, GNPDA2, BDNF, FAIM2 and MC4R in a Japanese population. *J. Hum. Genet.* **54**, 727-731.
**Kelsh, R. N., Dutton, K., Medlin, J. and Eisen, J. S.** (2000). Expression of zebrafish fkd6 in neural crest-derived glia. *Mech. Dev.* **93**, 161-164.
**Kim, C. H., Oda, T., Itoh, M., Jiang, D., Artinger, K. B., Chandrasekharappa, S. C., Driever, W. and Chitnis, A. B.** (2000). Repressor activity of Headless/Tcf3 is essential for vertebrate head formation. *Nature* **407**, 913-916.
**Kimmel, C. B., Ballard, W. W., Kimmel, S. R., Ullmann, B. and Schilling, T. F.** (1995). Stages of embryonic development of the zebrafish. *Dev. Dyn.* **203**, 253-310.
**Klein, P. S. and Melton, D. A.** (1996). A molecular mechanism for the effect of lithium on development. *Proc. Natl. Acad. Sci. USA* **93**, 8455-8459.
**Kobayashi, M., Osanai, H., Kawakami, K. and Yamamoto, M.** (2000). Expression of three zebrafish Six4 genes in the cranial sensory placodes and the developing somites. *Mech. Dev.* **98**, 151-155.
**Kollmar, R., Nakamura, S. K., Kappler, J. A. and Hudspeth, A. J.** (2001). Expression and phylogeny of claudins in vertebrate primordia. *Proc. Natl. Acad. Sci. USA* **98**, 10196-10201.
**LaBonne, C. and Bronner-Fraser, M.** (1998). Neural crest induction in *Xenopus*: evidence for a two-signal model. *Development* **125**, 2403-2414.
**Lewis, J. L., Bonner, J., Modrell, M., Ragland, J. W., Moon, R. T., Dorsky, R. I. and Raible, D. W.** (2004). Reiterated Wnt signaling during zebrafish neural crest development. *Development* **131**, 1299-1308.
**Li, B., Kuriyama, S., Moreno, M. and Mayor, R.** (2009). The posteriorizing gene Gbx2 is a direct target of Wnt signalling and the earliest factor in neural crest induction. *Development* **136**, 3267-3278.
**Lister, J. A., Robertson, C. P., Lepage, T., Johnson, S. L. and Raible, D. W.** (1999). nacre encodes a zebrafish microphthalmia-related protein that regulates neural-crest-derived pigment cell fate. *Development* **126**, 3757-3767.
**Litsiou, A., Hanson, S. and Streit, A.** (2005). A balance of FGF, BMP and WNT signalling positions the future placode territory in the head. *Development* **132**, 4051-4062.
**McGrew, L. L., Lai, C. J. and Moon, R. T.** (1995). Specification of the anteroposterior neural axis through synergistic interaction of the Wnt signaling cascade with noggin and follistatin. *Dev. Biol.* **172**, 337-342.
**McGrew, L. L., Hoppler, S. and Moon, R. T.** (1997). Wnt and FGF pathways cooperatively pattern anteroposterior neural ectoderm in Xenopus. *Mech. Dev.* **69**, 105-114.
**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).* Amsterdam: North-Holland Publishing Company.
**Okuda, Y., Yoda, H., Uchikawa, M., Furutani-Seiki, M., Takeda, H., Kondoh, H. and Kamachi, Y.** (2006). Comparative genomic and expression analysis of group B1 sox genes in zebrafish indicates their diversification during vertebrate evolution. *Dev. Dyn.* **235**, 811-825.
**Phillips, B. T., Kwon, H. J., Melton, C., Houghtaling, P., Fritz, A. and Riley, B. B.** (2006). Zebrafish msxB, msxC and msxE function together to refine the neural-nonneural border and regulate cranial placodes and neural crest development. *Dev. Biol.* **294**, 376-390.
**Sahly, I., Andermann, P. and Petit, C.** (1999). The zebrafish eya1 gene and its expression pattern during embryogenesis. *Dev. Genes Evol.* **209**, 399-410.
**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.
**Sauka-Spengler, T. and Bronner-Fraser, M.** (2008). A gene regulatory network orchestrates neural crest formation. *Nat. Rev. Mol. Cell Biol.* **9**, 557-568.
**Seo, H. C., Saetre, B. O., Havik, B., Ellingsen, S. and Fjose, A.** (1998). The zebrafish Pax3 and Pax7 homologues are highly conserved, encode multiple

isoforms and show dynamic segment-like expression in the developing brain. *Mech. Dev.* **70**, 49-63.
**Smith, W. C. and Harland, R. M.** (1991). Injected Xwnt-8 RNA acts early in Xenopus embryos to promote formation of a vegetal dorsalizing center. *Cell* **67**, 753-765.
**Sokol, S., Christian, J. L., Moon, R. T. and Melton, D. A.** (1991). Injected Wnt RNA induces a complete body axis in *Xenopus* embryos. *Cell* **67**, 741-752.
**Solomon, K. S., Kudoh, T., Dawid, I. B. and Fritz, A.** (2003). Zebrafish foxi1 mediates otic placode formation and jaw development. *Development* **130**, 929-940.
**Steventon, B., Carmona-Fontaine, C. and Mayor, R.** (2005). Genetic network during neural crest induction: from cell specification to cell survival. *Semin. Cell Dev. Biol.* **16**, 647-54.
**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.
**Streit, A.** (2002). Extensive cell movements accompany formation of the otic placode. *Dev. Biol.* **249**, 237-254.
**Toro, S. and Varga, Z. M.** (2007). Equivalent progenitor cells in the zebrafish anterior preplacodal field give rise to adenohypophysis, lens, and olfactory placodes. *Semin. Cell Dev. Biol.* **18**, 534-542.
**van de Water, S., van de Wetering, M., Joore, J., Esseling, J., Bink, R., Clevers, H. and Zivkovic, D.** (2001). Ectopic Wnt signal determines the eyeless phenotype of zebrafish masterblind mutant. *Development* **128**, 3877-3888.
**Wang, Y., Fu, Y., Gao, L., Zhu, G., Liang, J., Gao, C., Huang, B., Fenger, U., Niehrs, C., Chen, Y. G. et al.** (2010). *Xenopus* skip modulates Wnt/beta-catenin signaling and functions in neural crest induction. *J. Biol. Chem.* **285**, 10890-10901.
**Westerfield, M.** (2000). *The Zebrafish Book: a Guide for the Laboratory Use of Zebrafish (Danio rerio).* Eugene, OR: University of Oregon Press.
**Willer, C. J., Speliotes, E. K., Loos, R. J., Li, 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.
**Wilson, P. A., Lagna, G., Suzuki, A. and Hemmati-Brivanlou, A.** (1997). Concentration-dependent patterning of the *Xenopus* ectoderm by BMP4 and its signal transducer Smad1. *Development* **124**, 3177-3184.
**Wu, J., Yang, J. and Klein, P. S.** (2005). Neural crest induction by the canonical Wnt pathway can be dissociated from anterior-posterior neural patterning in *Xenopus*. *Dev. Biol.* **279**, 220-232.
**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.