TGF-β signaling regulates the differentiation of motile cilia

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Summary

The cilium is a small cellular organelle with motility and/or sensory related functions that plays a crucial role during developmental and homeostatic processes. Although many molecules or signal transduction pathways that control cilia assembly have been reported, the mechanisms of ciliary length control have remained enigmatic. Here we report that Smad2-dependent TGF-β signaling impacts on the length of motile cilia at the Xenopus left-right (LR) organizer, the gastrocoel roof plate (GRP), as well as at the neural tube and the epidermis. Blocking TGF-β signaling resulted in the absence of the transition zone protein B9D1/MSKR-1 from cilia in multi-ciliated cells (MCCs) of the epidermis. Interestingly, this TGF-β activity is not mediated by the known major regulators of ciliogenesis, Multicilin, Foxj1 and RFX2. These data indicate that TGF-β signaling is crucial for the function of the transition zone, which in turn may affect the regulation of cilia length.

Graphical Abstract
Introduction

The cilium is a small, motile or immotile cellular organelle assembled in G0 cells (Choksi et al., 2014; Garcia-Gonzalo and Reiter, 2012; Ishikawa and Marshall, 2011). Beating motile cilia generate force to move extracellular fluids in a directional manner, whereas non-motile monocilia sense physical and biochemical extracellular signals. Disorders of cilia assembly
and function in humans give rise to a whole spectrum of severe diseases, collectively called ciliopathies (Hildebrandt et al., 2011).

The assembly of specific cilia types in a temporal and spatial manner is regulated by a variety of mechanisms during development. The regulation of gene expression of ciliary components by signal transduction pathways is thought to be one of many mechanisms underlying the regulation of cilia assembly. LR asymmetric patterning is a developmental process that critically depends on cilia assembly and function. The ciliated epithelium in question is represented by the mammalian posterior notochord (“node”) and homologous vertebrate LR organizers, including the GRP in amphibians and Kupffer’s vesicle (KV) in bony fish (Blum et al., 2014). LR organizers possess two types of cilia; motile cilia mainly at the central LR organizer and immotile cilia mainly at the lateral LR organizer (McGrath et al., 2003). Leftward flow which is generated by motile cilia at the center of the LR organizer breaks symmetric body patterning by transducing left-specific signals to the immotile, sensory cilia at the left lateral LR organizer. Therefore, the regulation of cilia assembly is an important process for LR asymmetric patterning. To date, the transcription factors Foxj1 and RFXs have been identified as important regulators of ciliogenesis that control the process of cilia assembly at the LR organizer (Bonnafe et al., 2004; Chung et al., 2012; Stubbs et al., 2008). Additionally, several signaling pathways including Notch (Boskovski et al., 2013; Lopes et al., 2010), FGF (Neugebauer et al., 2009), and Shh (Yu et al., 2008) as well as canonical and non-canonical Wnt signaling (Antic et al., 2010; Walentek et al., 2012) have been reported to control ciliogenesis at the GRP or other vertebrate LR organizers. Most of these signals control ciliogenesis by regulating the expression of Foxj1.

Here we report that the TGF-β superfamily members Xnr1 and Derrière together activate Smad2-dependent TGF-β signaling in cells at the central region of the GRP and impact on the regulation of cilia length. Shortened cilia in Xnr1/Derrière-depleted embryos disrupted leftward flow. This function of TGF-β signaling in ciliogenesis is also required for floor plate cilia (FL-cilia) at the neural tube and MCCs at the epidermis. In Xnr1/Derrière-depleted epidermal MCCs, the basal body protein γ-tubulin, but not the transition zone protein B9D1, was detected at the proximal end of the cilium, indicating that TGF-β signaling was required for normal function of the transition zone. Importantly, TGF-β signaling did not impact on the expression of major regulators of ciliogenesis, suggesting an additional and novel role of TGF-β signaling in cilia assembly and/or function. These data indicate that TGF-β signaling activates a yet to be discovered molecular network which affects the function of the transition zone to control cilia length.

Results

The Notch signaling pathway has been shown to be involved in several different processes during vertebrate LR asymmetric patterning: the regulation of Xnr1 or its orthologue expression in the lateral area of the LR organizer, the regulation of Pitx2 expression in the left lateral plate mesoderm (LPM), and the regulation of cilia fate at the LR organizer (Boskovski et al., 2013; Krebs et al., 2003; Raya et al., 2003; Sakano et al., 2010). Interestingly, blocking Notch signaling in zebrafish resulted in shortened cilia and reduced

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velocity of leftward flow in the KV (Lopes et al., 2010), a poorly understood observation which has not been followed up as yet in other vertebrate model organisms.

To investigate the function of Notch signaling in the formation of cilia at the *Xenopus* GRP, the Notch1 receptor was knocked down by a morpholino oligonucleotide (MO) directed against Notch1 (N1MO), the specificity of which has been previously demonstrated in *Xenopus* embryos (Sakano et al., 2010). The length of cilia at the GRP was reduced by Notch1-knockdown (Figure 1A, B), while shortened cilia at the GRP still appeared to be motile (Boskovski et al., 2013). This defect was rescued by co-injection of Notch1 intracellular domain (NICD) RNA, a constitutively active form of Notch1 which is not recognized by N1MO, indicating that cilia morphology induced by N1MO injection was dependent on the activation of Notch1 (Figure 1A, B). This result showed that MO-mediated knockdown of Notch signaling in the frog *Xenopus* was able to phenocopy the zebrafish mutant phenotype. In order to investigate whether leftward flow was affected as in the zebrafish mutant KV, GRP explants from Notch1 morphant *Xenopus* embryos were prepared at flow stages (stage 17) and – following the addition of fluorescent beads – processed for video analysis. Flow velocity was significantly reduced in GRP explants from N1MO injected embryos (Figure 1C, Movie S1). We also observed a pronounced reduction of directionality; this effect, however, was statistically not significant (p = 0.15; Figure 1D, Movie S1). Flow phenotypes were also rescued upon N1MO/NICD RNA co-injection, which provided additional proof of the specificity of the MO used (Figure 1C, D). In summary, these results demonstrated that Notch signaling at the frog LR organizer was necessary for the regulation of cilia length and directed leftward flow in much the same way as in zebrafish mutant embryos defected the Notch ligand deltaD (Lopes et al., 2010).

In order to assess whether the effect of Notch signaling on GRP cells could have been direct, we examined the expression of receptor and ligands of the Notch signaling pathway in the area of the GRP at early neurula stages. We detected strong expression of the Notch1 receptor in cells at the border of the GRP and weaker expression at the GRP center. In contrast, Notch ligands Delta1 and Serrate1 were exclusively expressed at the border of the GRP (Figure S1A). A previous study has shown that the ratio of motile to immotile cilia was significantly changed at the border of Notch1-depleted GRPs (Boskovski et al., 2013) compared with the GRP center. We therefore compared the distribution of ciliary types in control and Notch1-depleted GRPs using immunohistochemistry with an antibody against left-right dynein (LRD), which is only expressed in motile cilia at the GRP (Okada et al., 1999; Qiu et al., 2005). In agreement with the work by Boskovski et al. (2013), we found that the ratio of motile to immotile cilia was drastically changed at the border of the Notch1-depleted GRPs (Figure S1C). Since Notch participates in juxtacrine signaling (Kato, 2011), its role should be limited to the cells at the border of the GRP, indicating that Notch signaling cannot directly affect the physiology of cells in the GRP center to regulate the formation of their cilia. To reveal how Notch signaling regulates the formation of cilia at the central area of the GRP, we focused on the role of Xnr1 downstream of Notch signaling. Xnr1, a member of the TGF-β superfamily, is a known downstream target of Notch signaling at the lateral area of the GRP (Sakano et al., 2010). As a secreted molecule it should in principle be able to reach cells in the central area of the GRP.
To dissect the role of Xnr1 downstream of Notch signaling in the formation of cilia, Xnr1 RNA was co-injected with N1MO and cilia length at the GRP was examined. Surprisingly, co-injection of Xnr1 rescued shortened cilia at the GRP (Figure 1A, B). Indeed, the activin type IB and IIB receptors (ActRIB and ActRIIB), which are receptors for Xnr1 signaling (Chen et al., 2004), were expressed in the GRP (Figure S1A). These data indicate that TGF-β signaling, downstream of Notch signaling, could directly affect the length of cilia on GRP cells. If the Xnr1 signal was directly received by GRP cells, phosphorylated Smad2 (P-Smad2) should be detected in these cells as well (Macias-Silva et al., 1996). P-Smad2 in GRP cells was examined during early neurulation, when cilia first form at the GRP. P-Smad2 was detected at early neurula stages (stage 13/14), while signals began to subside during later stages (stage 15 – 18; Figure S2). When Notch1 was knocked down in the GRP, P-Smad2 signals at early neurula stages were abolished (Figure 2A). Quantification of P-Smad2 in dorsal posterior explants including the GRP was performed by immunoblotting. The amount of P-Smad2, but not that of Smad2, was reduced in Notch1-depleted specimens (Figure 2B). These data indicated that Xnr1, which is transcriptionally induced by Notch signaling at the lateral area of the GRP, is received by cells in the central area of the GRP to activate the canonical TGF-β signaling pathway mediated by Smad2.

We previously showed that Xnr1-knockdown did not significantly alter leftward flow at the GRP (Schweickert et al., 2010), however, cilia length of GRP cells in Xnr1-depleted embryos was not addressed at that time. Therefore, we assessed cilia length in Xnr1-depleted GRPs using a previously characterized MO (Xnr1MO) (Vonica and Brivanlou, 2007). The length of cilia was indeed shortened in Xnr1 morphants, the effect, however, was less pronounced than in Notch1-depleted GRPs (Figure 3B, C). The effectiveness of Xnr1MO was confirmed by analyzing Xnr1 suppression in the left LPM (Figure S3). This result indicated that other downstream factor(s) of Notch signaling in addition to Xnr1 should be involved in ciliary length control. As one possibility, other TGF-β superfamily member(s) could cooperate with Xnr1 to regulate cilia length. To test this hypothesis, the length of cilia on GRP cells was determined upon blocking TGF-β signaling using a Smad2 mutant harbouring a point mutation (Phe445His), which acts as a dominant-negative form of Smad2 (DN-S2) (Hoodless et al., 1999). Injection of DN-S2 indeed resulted in shortened cilia (Figure 2C, D), comparable to what was obtained in Notch1 morphant GRPs (cf. Figure 1B). This defect was rescued by co-injection of Smad2 (S2), showing the specificity of the DN-S2 effect in ciliary length control. Importantly, cilia in DN-S2 injected specimens were shorter than in Xnr1 morphants, strongly suggesting that additional TGF-β superfamily member(s) should be involved in the process of ciliary length control downstream of Notch signaling.

Brivanlou and colleagues previously showed that the TGF-β factor Derrière was co-expressed with Xnr1 at the border of the GRP during neurula stages and was required for LR asymmetric patterning (Vonica and Brivanlou, 2007). When the expression of Derrière was assessed in embryos in which Notch1 was knocked down specifically on the left side of the GRP, its expression was abolished on the manipulated side (Figure 3A). Next, the length of cilia in Derrière-depleted GRPs was determined, using a well-characterized MO (DerMO) (Vonica and Brivanlou, 2007). As a control, the effect of DerMO on Xnr1 suppression in the left LPM was assessed (Figure S3). Although knockdown of Derrière resulted in shortened
cilia at the GRP, this defect was less severe than in DN-S2 injected specimens but similar to that in Xnr1 morphants (Figure 3B, C). To analyze whether Xnr1 and Derrière cooperate in ciliary length control, both genes were knocked down simultaneously and the length of cilia at the GRP was determined. Double-knockdown of Xnr1 and Derrière resulted in cilia which were shorter than both single knockdowns but comparable to Notch1 morphants or DN-S2 injected specimens. In addition, double-knockdown of Xnr1 and Derrière also abolished P-Smad2 signals in GRP cells (Figure 2A). Concerning leftward flow of Xnr1MO or DerMO injected embryos, no significant differences of velocity or directionality were observed when compared to control specimens. However, injection of Xnr1MO together with DerMO significantly reduced flow velocity as well as directionality (Figure 3D, E; Movie S2). These data clearly demonstrated that Xnr1 and Derrière cooperate to determine cilia length at the GRP.

Next we wondered whether this function of TGF-β signaling was restricted to control cilia length at the LR organizer or whether other cilia types used a TGF-β-based mechanism as well. Here we assessed the well-characterized cilia on neural tube cells and MCCs of the epidermis. When TGF-β signaling was blocked by injection of DN-S2 RNA, cilia morphology was examined in both the neural tube and the epidermis. The neural tube displays two types of cilia (Hagenlocher et al., 2013), long motile cilia on multi-ciliated cells of the floor plate (FL-cilia) and shorter cilia elsewhere in the neural tube (non-motile monocilia) (Figure 4A). Interestingly, the length of FL-cilia was clearly shortened by injection of DN-S2 whereas non-motile monocilia seemed not to be affected by blocking TGF-β signaling (Figure 4A). Moreover, we did not observe any defects of neural tube closure (data not shown), phenotypes frequently associated with cilia polarization defects (Wallingford and Mitchell, 2011). This result is in line with data from knockdown of Foxj1, which exclusively regulates the formation of motile FL-cilia and which did not show any defects of neural tube closure as well (Hagenlocher et al., 2013). Additionally, the length of cilia on MCCs was also shortened by injection of DN-S2 (Figure 4B). Together, these data support the notion that TGF-β signaling is more generally involved in length control of motile cilia without impacting on non-motile monocilia.

To further characterize the defects that result in shortened cilia by blocking TGF-β signaling, we examined the basal body marker γ-tubulin (Chung et al., 2010; Muresan et al., 1993) and the transition zone marker B9D1/MSKR1 (Szymanska and Johnson, 2012) in DN-S2 injected MCCs. γ-tubulin but not B9D1 was detected at the proximal end of the cilium (Figure 4C). Although B9D1 is also expressed in the basal body, B9D1 is required for the function of the transition zone as a gate which controls entry of molecules into the cilium and thus modulates ciliary composition (Szymanska and Johnson, 2012). These data indicate that blocking TGF-β signaling impaired the structure and/or function of the transition zone.

To connect the molecular mechanism underlying the function of TGF-β signaling to known regulatory pathways of ciliogenesis, we examined the expression level of transcriptional regulators, namely Multicilin, Foxj1 and RFX2 (Chung et al., 2012; Stubbs et al., 2008; Stubbs et al., 2012) in DN-S2 injected embryos. None of these markers was altered (Figure 4D). The ciliary gene tetratricopeptide domain 25 (TTC25) which localizes to the ciliary axonemes as well as to foci at the apical cell surface (presumably basal bodies) was...
analyzed as well, because knockdown of TTC25 led to shortened cilia in Xenopus embryos, although its function has remained enigmatic (Hayes et al., 2007). Interestingly, the expression of TTC25 was reduced at the epidermis by DN-S2 injection (Figure 4D). The same effects were observed in Xnr1 and Derrière double morphant GRPs (Figure S4). These results show that TGF-β signaling regulates at least one gene involved in ciliary length control in a Multicilin/Foxj1/RFX2-independent manner.

**Discussion**

The results presented here reveal a role for the canonical TGF-β signaling pathway in the length regulation of motile cilia at the GRP, the neural tube and the epidermis of Xenopus embryos. Blocking TGF-β signaling resulted in shortened cilia in all these tissues. In addition, the transition zone was affected in MCC cilia. Since the molecular mechanisms underlying the assembly, structure and function of cilia are well-conserved among the vertebrates (Choksi et al., 2014), this role of TGF-β signaling in ciliogenesis is likely conserved in humans. It remains to be seen whether inactivation of TGF-β signaling by various gene mutations may be a cause of human ciliopathies with unknown etiology.

To date, the transcription factors Multicilin, Foxj1 and the RFXs have been reported as major regulators of ciliogenesis, which orchestrate the expression of a complex suite of genes required for immotile and/or motile ciliogenesis (Choksi et al., 2014). Multicilin is at the top of the ciliogenic molecular cascade and regulates both the expression of RFX2 and Foxj1 (Ma et al., 2014; Stubbs et al., 2012). RFX transcription factors control the expression of genes encoding ciliary components which are involved in basic aspects of cilia assembly and function, or which are required for specialized ciliary functions in only certain cell types (Chung et al., 2014; Thomas et al., 2010). Foxj1, on the other hand, plays an important role in the biogenesis of motile cilia. Importantly, the roles of most characterized signal transduction pathways in ciliogenesis, including Wnt, Notch, FGF and Shh, are to control ciliogenesis by regulating expression of Foxj1, whereas a TGF-β-dependent molecular network controlling the length of cilia is probably independent of the functions of Multicilin, Foxj1 and RFX2. Therefore, the molecular network activated by TGF-β signaling may regulate the expression of ciliary components that are required to determine the length of motile cilia in a Multicilin/RFX2/Foxj1-independent manner. As both RFX2 (Chung et al., 2012) and TGF-β signaling regulate the expression of TTC25 in the GRP, the TGF-β-dependent molecular network appears to crosstalk with well-characterized networks that are activated by Multicilin, Foxj1 and RFX2.

In DN-S2 injected MCCs, B9D1 was deleted while γ-tubulin was unaltered. The normal expression of the basal body marker γ-tubulin shows that the structure of the basal body was intact in DN-S2 injected MCCs. As B9D1 is expressed in both the basal body and the transition zone, blocking TGF-β signaling therefore seems to induce defects specifically in the transition zone. Since the transition zone functions as a ciliary gate (Szymanska and Johnson, 2012), defects may result in a shortage of axonemal components or IFT proteins in the ciliary axonemes, resulting in shortened cilia. It therefore seems that shortened cilia induced by blocking TGF-β signaling are caused by a defect of the ciliary gate function.
However, further studies are required to uncover the molecular function of TGF-β signaling in the regulation of cilia length.

Our data demonstrate that Xnr1 and Derrière activate the Smad2-dependent TGF-β signaling pathway in GRP cells at the time when the cilium starts to emerge. A connection of TGF-β signaling and cilia was previously investigated in fibroblasts (Clement et al., 2013). This study showed that activation of Smad2/3 relied on clathrin-dependent endocytosis (CDE) at the pocket region of immotile cilium, i.e. that the cilium regulated TGF-β signaling. Since it is not clear at what time point activation of Smad2 by Xnr1 and Derrière is required for the regulation of cilia length at GRP cells, before or after the formation of the pocket region, it remains to be determined whether CDE at the pocket region of GRP cilia is required for activation of Smad2/3 to control cilia length. Further investigation will be necessary to address this important question.

In summary, the work presented here strongly suggests that TGF-β signaling is exclusively involved in the regulation of cilia length. The identification and characterization of the TGF-β-dependent molecular network will contribute to our understanding of the molecular mechanisms underlying ciliary length control, which is poorly understood at this time. Our study should open up new avenues to investigate this important question in cilia biology.

Experimental Procedures

Whole Mount Fluorescence Immunohistochemistry

Published procedures were used for staining (Lee et al., 2008; Suzuki et al., 2007) and thick sectioning (Becker and Gard, 2006) with minor modifications. Embryos were fixed in MEMFA for either 2.5 hours at room temperature or overnight at 4 °C. Fixed embryos were dehydrated completely in methanol at ~20 °C for at least several hours and rehydrated consecutively with PBS. After rinsing in PBT (0.1% Triton X-100 in PBS), embryos were incubated with 10% goat serum in PBT at room temperature for at least 1 hour. Samples were incubated with mouse anti-acetylated α-tubulin (1:500, Sigma) antibody, rabbit anti-LRD antibody (1:10, gifted by Dr. M. Levin), rabbit anti-phospho-Smad2 antibody (Ser465/467) (1:20, Cell Signaling), rabbit anti-B9D1 antibody (1:50, abnova) and/or anti-γ-tubulin antibody (1:50, abcam) overnight at 4 °C. Primary antibodies were recognized with Cy2 donkey anti-mouse IgG antibody (1:500, Jackson ImmunoResearch), Alexa Flour 546 anti-rabbit IgG antibody (1:500, Life Technologies), or Cy5 donkey anti-rabbit IgG antibody (1:500, Jackson ImmunoResearch), respectively. Antibodies were diluted in 10% goat serum in PBT. Images were taken by confocal microscopy and cilia were measured by using ImageJ software.

Flow Measurement

Preparation of dorsal explants, recording of time-lapse movies, processing and analysis of leftward flow were according to previous work (Vick et al., 2009). 17 ng of N1MO, 100 pg NICD RNA, 5 ng Xnr1MO and/or 5 ng DerMO were injected for flow analyses, respectively.
**Statistical Methods**

Statistical analyses were carried out using Excel using standard error of the mean (s.e.m.), and Student’s t-test to calculate P values. For flow analyses, R Studio was used to perform statistics. P values have been calculated with Wilcoxon rank-sum test and significance levels were Bonferroni corrected. A P value ≤0.05 was considered to be significant. * ≤0.05, ** ≤0.005, *** ≤0.0005, **** ≤0.00005. n.s.: not significant.

**Supplementary Material**

Refer to Web version on PubMed Central for supplementary material.

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Figure 1. Xnr1 rescues cilia defects induced by Notch1-knockdown

A. Cilia length at the GRP. N1MO (50 ng), NICD RNA (100 pg) Xnr1 RNA (5 pg) and/or conMO (50 ng) were injected into the dorsal marginal zone of 4-cell embryos and embryos were fixed at stage 17. Cilia were stained using an anti-acetylated α-tubulin antibody. Membrane RFP (memRFP) RNA was co-injected as a lineage tracer and to indicate the cell membrane. The scale bar represents 15 µm. a: anterior, p: posterior. B. Average length of cilia from panel A. Fifty cilia from six GRPs were analyzed for each sample. Two GRPs were randomly chosen from each of three independent experiments. C, D. Leftward flow.

Cell Rep. Author manuscript; available in PMC 2015 May 20.
GRPs were explanted at stage 17 and flow was imaged subsequent to the addition of fluorescent beads. Velocity (C) is shown in µm/s, directionality (D) is shown as the dimensionless number rho. The maximum value $\rho=1$ indicates that all trajectories point in the same direction, whereas a $\rho$-value of 0 indicates uniform distribution in all directions.
Figure 2. TGF-β signaling is activated in GRP cells to control cilia length

A. N1MO or Xnr1MO/DerMO blocks Smad2 phosphorylation at early neurula stages 13/14. Cilia were stained by an anti-acetylated α-tubulin antibody (green) and phosphorylated Smad2 was detected by an anti-phospho-Smad2 antibody (red). Examples of a short cilium and a P-Smad2 signal are indicated by a white arrowhead and a red arrowhead, respectively. Membrane GFP (memGFP) RNA was co-injected as a lineage tracer and to indicate the cell membrane. The scale bar represents 20 µm. a: anterior, p: posterior. B. Quantification of P-Smad2 in dorsal posterior explants. Immunoblotting was performed with protein extracts from dorsal posterior explants of Notch1 morphants or Xnr1/Derrière double morphants. C. Shortened cilia and rescued cilia at the GRP. DN-S2 RNA (4 ng) and/or S2 RNA (1 ng) were injected. The scale bar represents 20 µm. a: anterior, p: posterior. D. Average length of cilia from panel C.
Figure 3. Xnr1 and Derrière cooperate in the regulation of cilia length
A. Xnr1 and Derrière expression in the area of Notch1 morphant GRPs at stage 13/14 by WISH. Red arrowheads indicate reduction of Xnr1 and Derrière expression. A white triangle indicates the central area of the GRP. “n” indicates number of analyzed embryos. a: anterior, p: posterior, L: left, R: right. B. Shortened and rescued cilia at the GRP. Xnr1MO (10 ng), DerMO (10ng) and/or conMO (20 ng) were injected. The scale bar represents 20 µm. a: anterior, p: posterior. C. Average length of cilia from panel B. D, E. Leftward flow after

Cell Rep. Author manuscript; available in PMC 2015 May 20.
single and double-knockdown of Xnr1 and Derrière. Velocity (D) is shown in \( \mu m/s \), directionality (E) is shown in rho. Rhodamine-B dextran was used as a lineage tracer.
Figure 4. TGF-β signaling controls the length of motile neural and epidermal cilia and affects the transition zone in a Multicilin/Foxj1/RFX2-independent manner

A. Shortened FL-cilia in the neural tube. DN-S2 RNA (4 ng) was injected into the dorsal animal region of 4-cell embryos, which were fixed at stage 26. Following staining, 100 µm transverse sections of specimens were prepared. Red and white arrowheads indicate examples of non-motile monocilia and FL-cilia, respectively. White dots indicate the lumen of the neural tube. The scale bar represents 10 µm. d: dorsal side, v: ventral side. B. Shortened cilia on epidermal MCC. DN-S2 RNA (4 ng) with memRFP RNA or memGFP RNA were injected into the left side or the right side, respectively. Embryos were fixed at stage 26. Following staining, 100 µm transverse sections of specimens were prepared. Red and white arrowheads indicate examples of non-motile monocilia and FL-cilia, respectively. White dots indicate the lumen of the neural tube. The scale bar represents 10 µm. d: dorsal side, v: ventral side.
stage 26. Images from the left (DN-S2) or right (con) lateral side were taken. Pictures in the lower row were magnified from the white square indicated in each sample. The scale bar represents 10 μm. C. γ-tubulin but not B9D1 was detected in DN-S2 injected MCC cilia. DN-S2 RNA (4 ng) with memGFP RNA or memGFP RNA alone were injected into the left side or the right side, respectively. Images from the left (DN-S2) or right (con) lateral side were taken. The scale bar represents 10 μm. D. Multicilin, Foxj1, RFX2 and TTC25 expression in DN-S2 injected embryos at stage 17 by WISH. Images from the left (con) or right (DN-S2) lateral side were taken. Red-gal signals indicate DN-S2 injected side. a: anterior, p: posterior, d: dorsal, v: ventral. “n” indicates number of embryos.