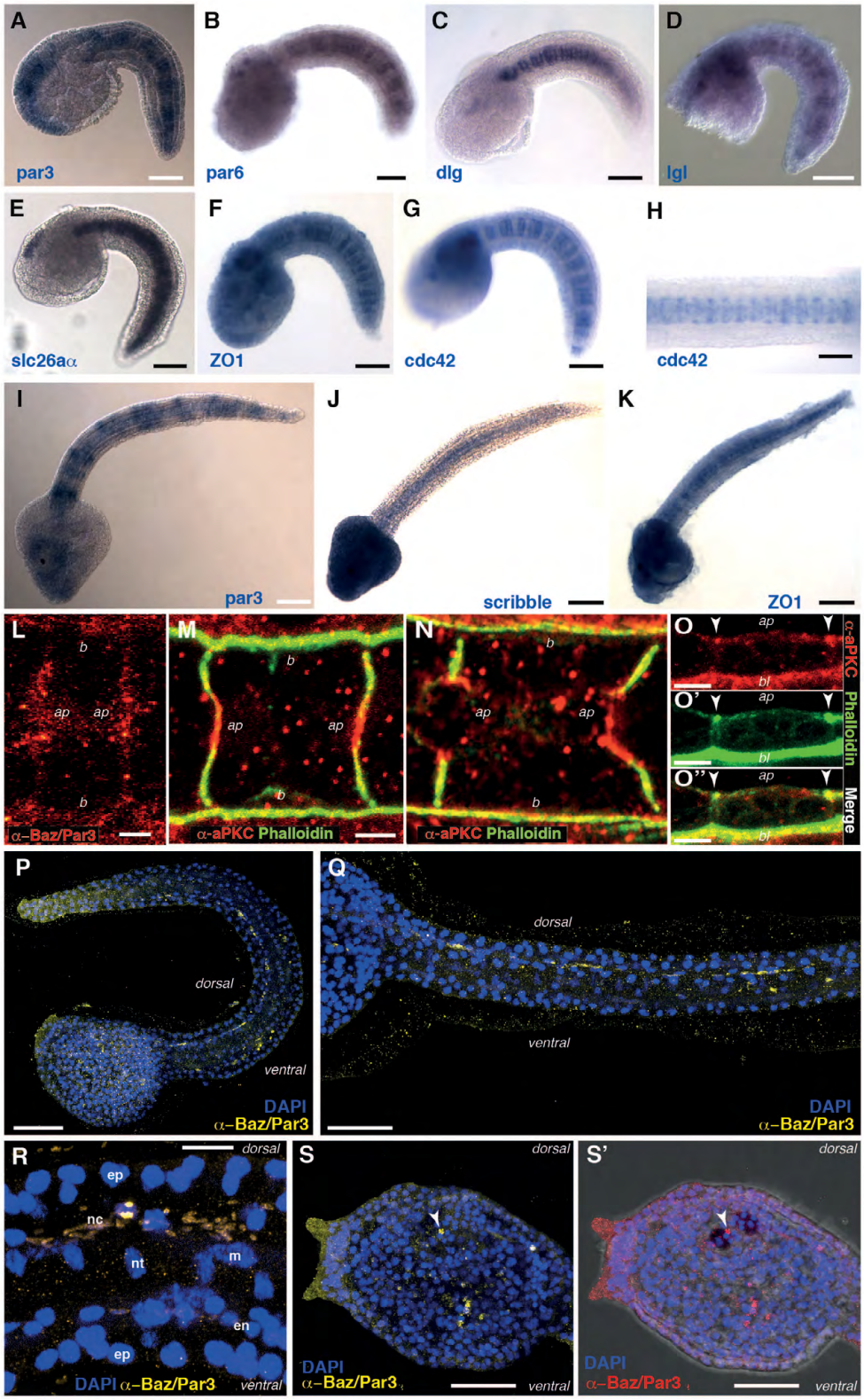

Fig. S1. Expression profiles of the candidate genes investigated in this study. (**A-G**) At early stage IV, *par3* (A), *par6* (B), *dlg* (C), *lgl* (D), *slc26aα* (E), *zo1* (F) and *cdc42* (G) transcripts are strongly expressed in the notochord and in groups of cells in the head, which are likely associated with developing neural structures. (**H-K**) At late stage IV, *par3* (I), *scribble* (J), *zo1* (K) and *cdc42* (H) are still strongly expressed in the notochord and in the head. *par3* also appears in scattered groups of cells dorsal to the notochord in the tail. (**L-N**) At the protein level, Par3 (L) and aPKC (M) are accumulated at the prospective apical domain of the notochord cells. When lumen opens, aPKC is restricted to the lateral/luminal boundaries (N). (**O-O″**) aPKC is also found at the lateral/apical boundaries in epidermal cells (white arrowheads). (**P-R**) In addition, Par3 is present in dorsal groups of cells that correspond to the developing nervous system in the tail. (**S**,**S′**) In the head, several groups of cells are stained, including cells in the developing sensory vesicles (white arrowhead). (L) Early stage IV; (M,P,Q) late stage IV/stage V; (N,R-S′) stage V. Scale bars: 50 µm in A-G,I-K,P,Q,S; 25 µm in H; 7 µm in L, 4 µm in M-O″; 12 µm in R. ap, apical; bl, basolateral; b, basal; ep, epidermis; nc, nerve cord; nt, notochord; m, muscle; en, endoderm.

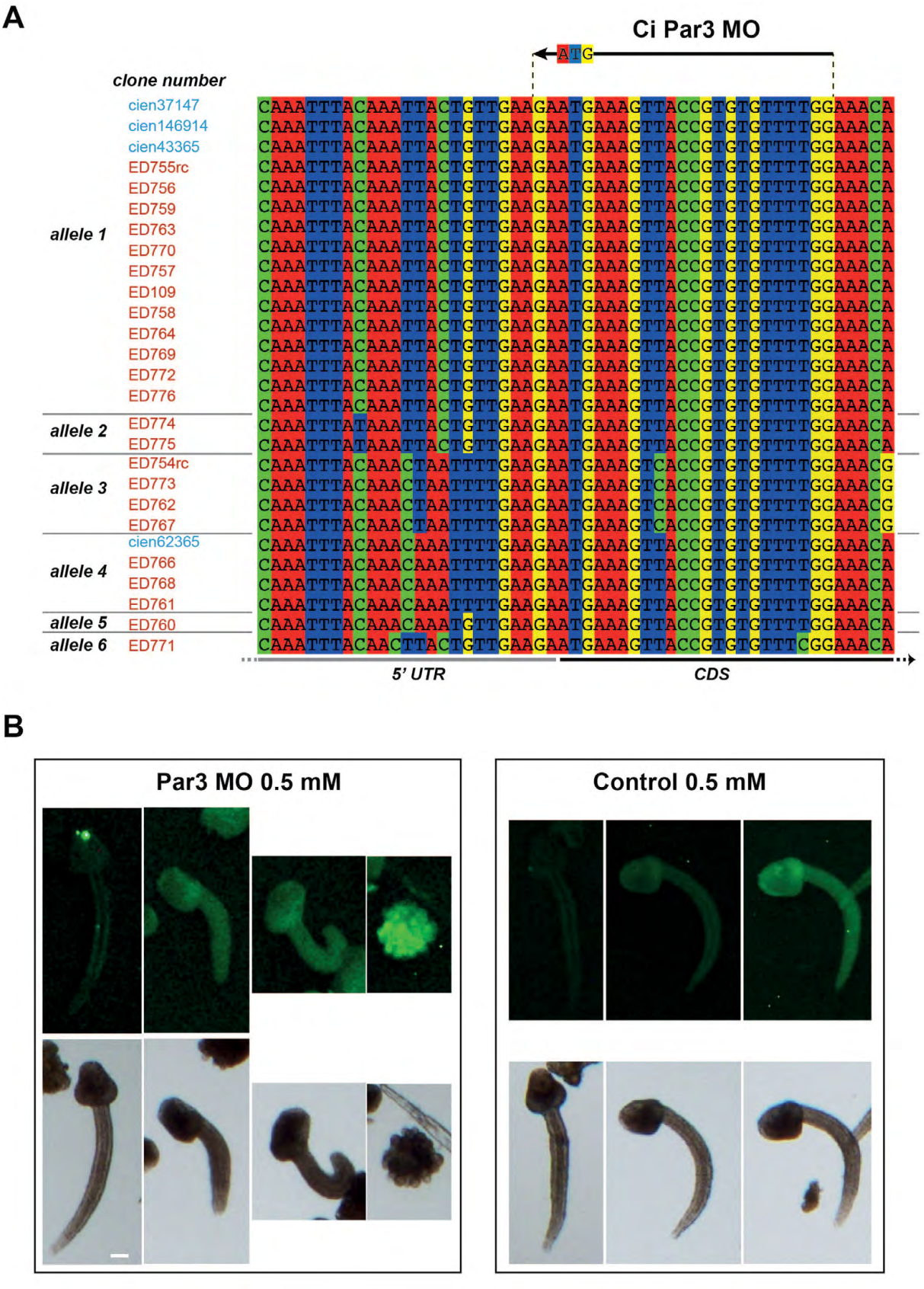

Fig. S2. Par3 morpholino design and injection. (**A**) Alignment of *Ciona par3* sequences from EST clones (blue, from Roscoff animals) and cDNA clones (red, Norwegian animals), showing the polymorphism and the position of the translation-blocking MO with respect to the coding sequence (CDS) and the 5′ untranslated region (5′UTR). (**B**) Dose-dependent effect of the MO on development. Increasing the amount of Par3 MO injected, which is visualized as more intense fluorescence in the embryo (left), results in more severe phenotype. This is not the case for the control MO (right). Scale bar: 50 μm.

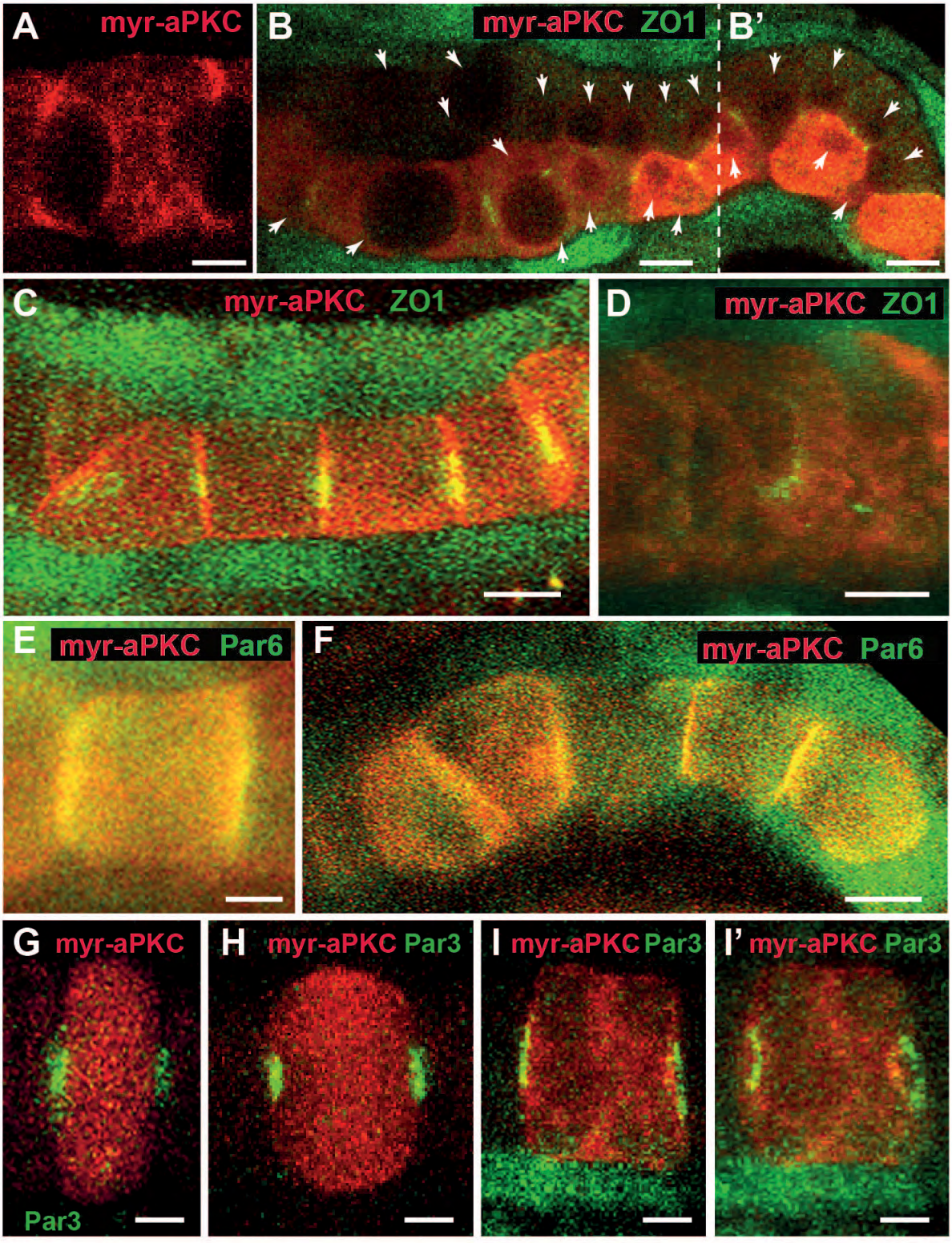

Fig. S3. Effects of the myr-apkc overexpression. (**A-B′**) Lumen forms after myr-apkc overexpression (A), and even with cell intercalation defects (B,B′). Arrowheads indicate lumen pockets. (**C-I′**) Subcellular localization of polarity and junction markers after myr-apkc overexpression. Par6 is mislocalized (E,F), whereas Par3 (G-I′) and ZO1 (C,D) are localized normally. All images are maximum projections except A-B′, which are sections. B and B′ are two different regions of same notochord, to show as many lumen pockets as possible. The cells in D have been rotated to show the lateral surface. I′ is the same cell in I but slightly rotated to show the Par3 rings. (A-B′) Stage V; (C,E,F,H) mid stage IV; (D,I,I′) late stage IV/stage V; (G) early stage IV. Scale bars: 5 μm in A,B,B′,D,E,G-I′; 10 μm in C,F.

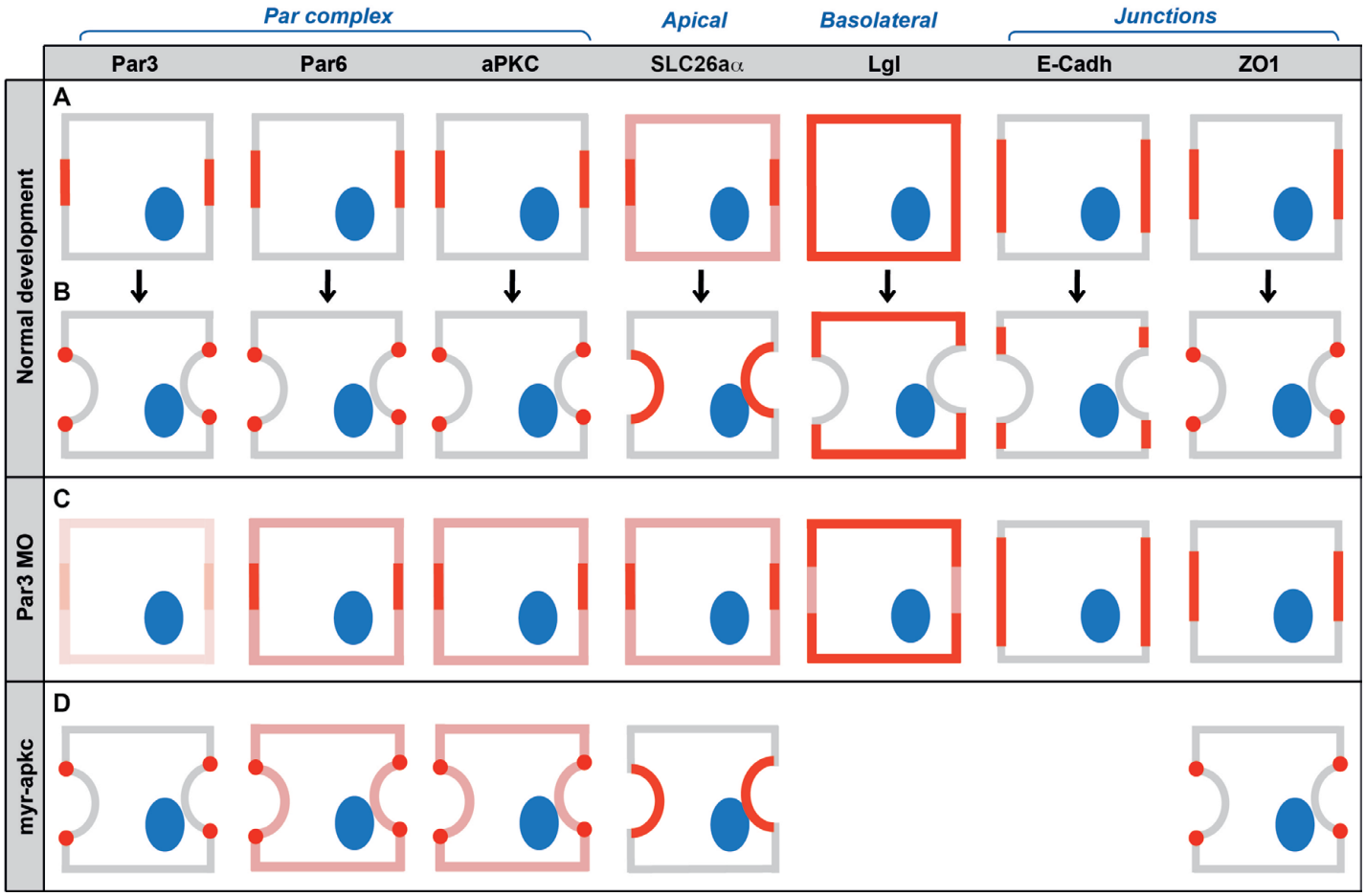

Fig. S4. Recapitulative diagrams to compare results from myr-apkc overexpression (Fig. S3) and Par3 MO (Fig. 5). Comparison of the localization of markers from stage IV (**A**) to stage V (**B**) during normal development to the localization of markers in Par3 morphant (**C**) and in myr-apkc overexpressing embryos (**D**) at stage V.

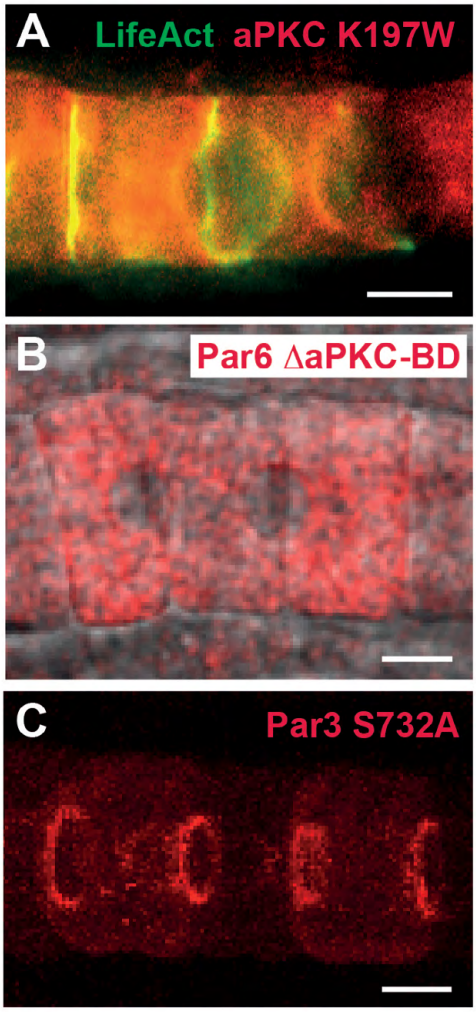

Fig. S5. Effects of the overexpression of dominant-negative Par3, Par6 and aPKC. (**A**) aPKC K197W mutant. (**B**) Par6 ΔaPKC-BD mutant. (**C**) Par3 S732A mutant. At stage V, the mutant-expressing cells form lumen pockets, suggesting that these mutants do not disrupt cell polarization, junction development and lumen formation. Scale bars: 10 μm.

Movie 1. Subcellular localization of Par3 and Par6 during notochord tubulogenesis. Time-lapse movie of notochord cells expressing turboGFP-Par6 (green) and mCherry-Par3 (red). Frame interval: 10 minutes.

Movie 2. Subcellular localization of Par6 and Slc26aα during notochord tubulogenesis. Time-lapse movie of notochord cells expressing turboGFP-Par6 (green) and Slc26aα-mCherry (red). Frame interval: 10 minutes.

Movie 3. Relative subcellular localizations of Par3 and ZO1 during notochord tubulogenesis. Time-lapse movie of notochord cells expressing ZO1-turboGFP (green) and mCherry-Par3 (red). Frame interval: 10 minutes.

Table S1. Generation of entry clones for probe synthesis and construction of fluorescent fusion proteins

Gene	Transcript model	Release1 gene collection clone for probe synthesis	Gilchrist library full-length clone for entry clone	Primers to generate entry clone		Fusion type
par3	KH.L147.28.v1.A.SL1-1	R1CiGC03j17	cien37147	Par3-5 (ATGAAAGTTACCGTGTGTTTTGG)	Par3-3 (TTACAGATTATACGGAACAG)	N
par3 (S732A mutant)		–	template: par3 entry clone above	Par3 S732A-5 (CCGCGAATCTGTCACTCGTCAAGCAATGTCGGAGAAACG)	Par3 S732A-3 (CGTTTCTCCGACATTGCTTGACGAGTGACAGATTCGCGG)	N
par6	KH.C6.278.v1.A.SL1-1	–	cien151332	Par6-5 (ATGGATAAGTTATCATTGGG)	Par6-3 (TTATATTGAAACATTAGGG)	N
par6 (ΔapKC-BD mutant)		–	template: par6 entry clone above	Par6DapBD-5 (GGTACCATGATTAGTTTTCCGGAAG)	Par6DapBD-3 (TTATATTGAAACATTAGGGTTACTGC)	N
apkc	KH.C14.47.v1.A.SL1-1	–	cien228658	FPaPKC-5 (ATGTCCATAGACGCTGGTTCA)	FPaPKC-3 (TCACACAGCAACATCACTCGT)	N
apkc (K197W mutant)		–	template: apkc entry clone above	aPKCK197W-5 (CGGACAAAATGTATGCTATGTGGGTAATAAAGAAAGAGTTAGT)	aPKCK197W-3 (ACTAACTCTTTCTTTATTACCCACATAGCATACATTTTGTCCG)	N
dlg	KH.C1.637.v1.A.ND4-1	R1CiGC19e17	cien185023	dlg-5 (CAGAAAAAATGCCTGTGAGAAAACAAGATGC)	dlg-3 (TAAGTCATCAGATGCAGGCACCCATAC)	C
scribble	KH.C2.370.v1.A.SL1-1	R1CiGC44l23	cien155708	scribble-5 (ATGTCTCCAGCAAGATTATGG)	scribble-3 (CTAGGTCATCGCTGGCTGTTG)	N
myr-apkc	–	–	–	CfusMyr-aPKC5 (CAGACAAAATGGGGAGCAGCAAGAGC)	CfusCMyr-aPKC3 (CACGGACTCCTCAGCAGACAGC)	C

References for the transcript models, the clones used for probe synthesis, and the clones and primers used to generate N- or C-tagged fusion constructs.