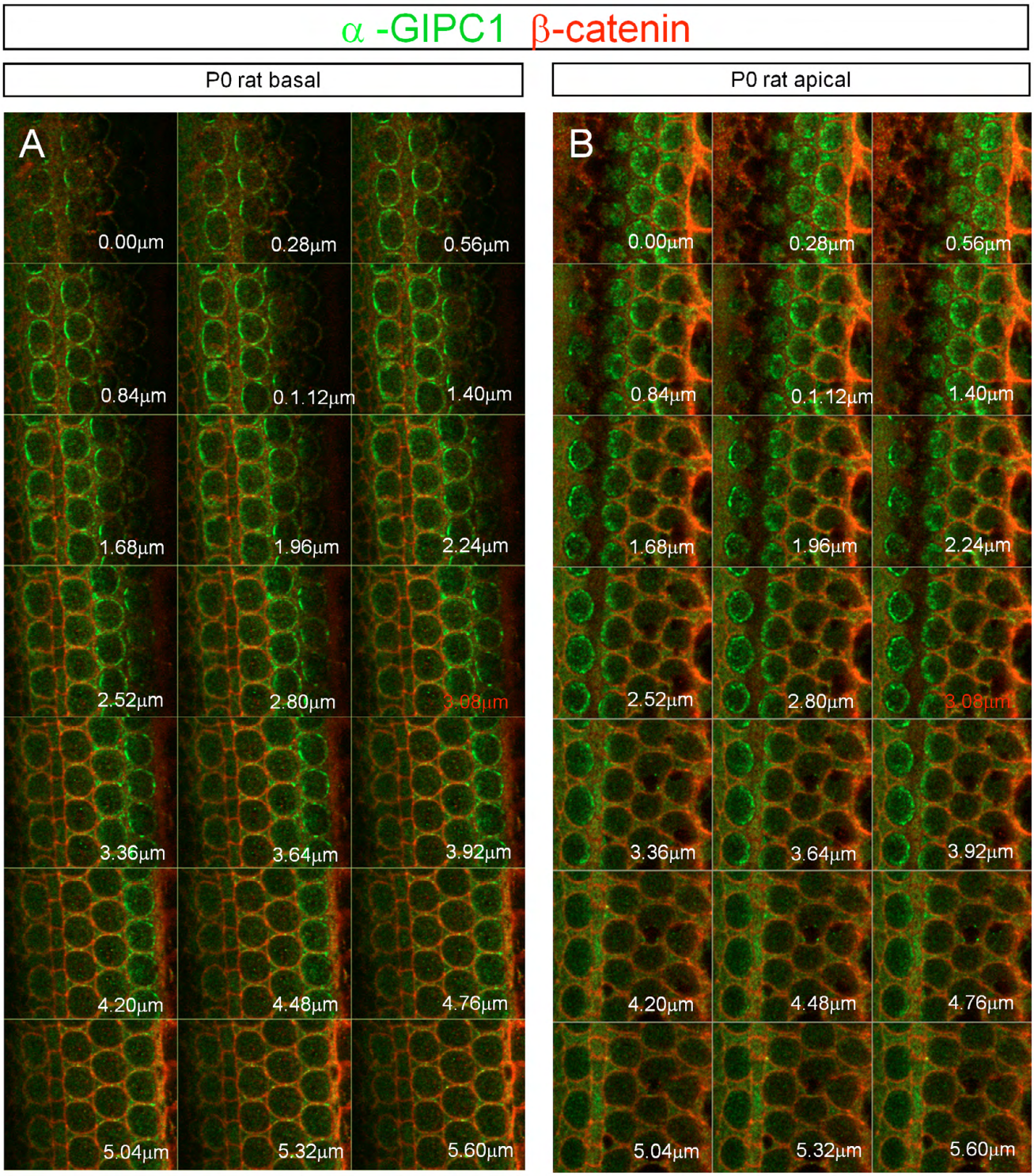

Fig. S1. Gipc1 is expressed in an apical and a more basolateral region of the hair cell. (**A**,**B**) Surface view of a whole-mount preparation of P0 mouse cochleae labelled with an anti-Gipc1 antibody (green) and an anti-β-catenin antibody (red). Series of images collected at 0.28-µm intervals to create a stack in the *z* axis of Fig. 4B (A) and Fig. 4C (B).

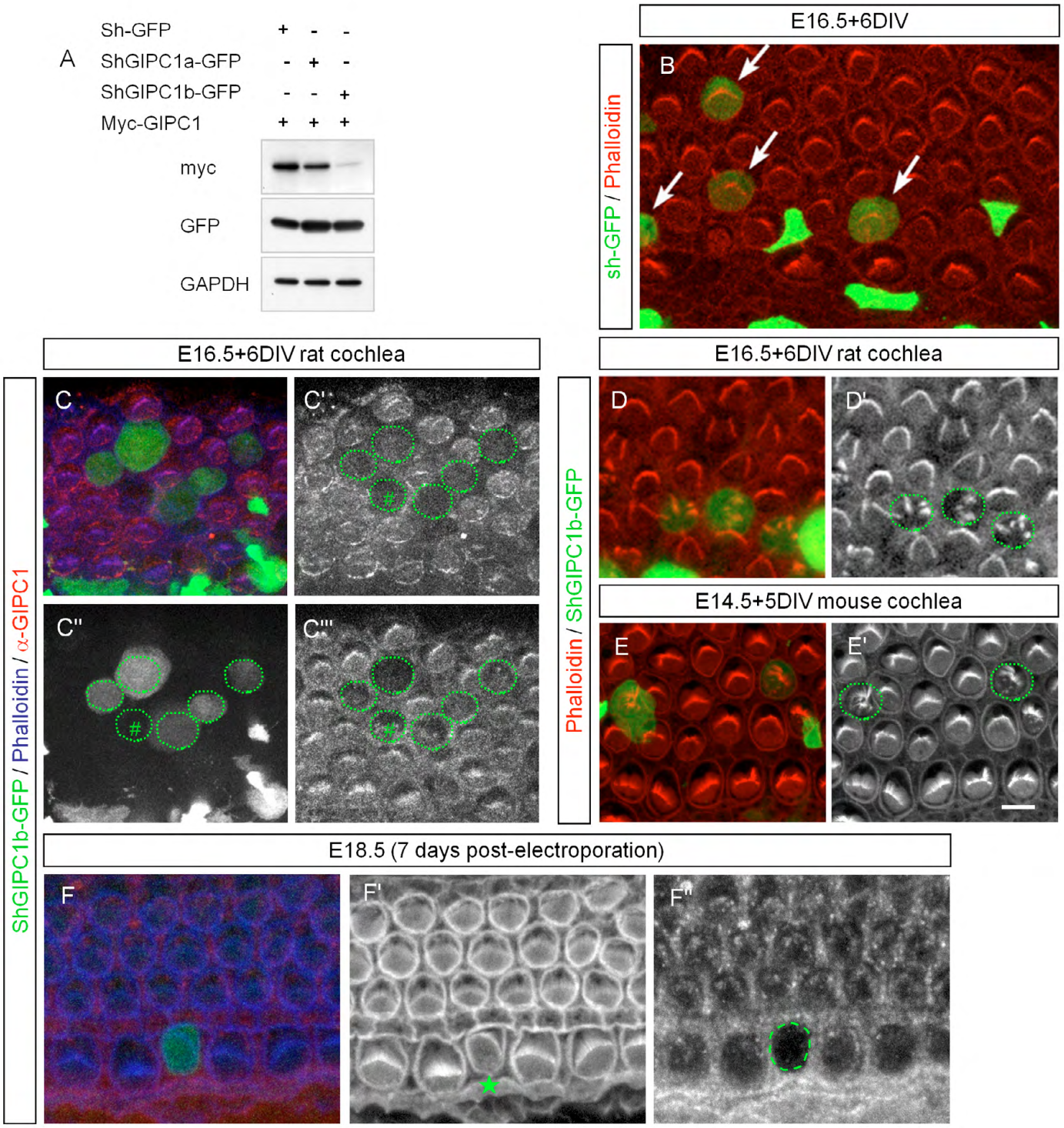

Fig. S2. Downregulation of Gipc1 in vitro and in vivo in mouse and rat cochleae. (**A**) The two shRNA for Gipc1 were transfected in HEK293T cells with a myc-Gipc1 construct and processed for immunoblotting after 48 hours. Both constructs were able to reduce expression of transfected Gipc1, with shGIPC1b-GFP being the most effective. (**B**) Surface view of rat cochleae electroporated with the empty shRNA (sh-GFP, green). Individual cells were identified by their GFP expression, and all transfected HCs appeared intact, mature, and properly polarized. Arrows indicate GFP-positive cells. (**C-E′**) Cochleae electroporated with shGIPC1b-GFP (green) and cultured for 5 or 6 days before immunostaining with a Gipc1 antibody (red) and phalloidin (blue). The expression of the shRNA leads to a downregulation of Gipc1 expression (C-C‴) and disruption of the integrity of the hair bundles in electroporated rat (C-D′) and mouse (E,E′) HCs. We occasionally observed cells in which the levels of GFP were weak but with effective downregulation of the protein and a hair bundle phenotype (C′-C‴, green hash). Dotted lines encircle GFP-positive cells. (**F-F″**) Surface view of a mouse cochlea electroporated in utero at E11.5 with shGIPC1b-GFPb (green) and labeled seven days later with phalloidin (F, blue; F′) and an anti-GIPC1 antibody (F, red; F′). In the HC positively electroporated with the shGIPC1b-GFPb construct, we observed a reduced expression of GIPC1 (F″, green dotted line). The HC expressing the shGIPC1b-GFPb construct has a reduced apical surface area and a disrupted hair bundle. (F′, green star). Scale bar: 8 µm.

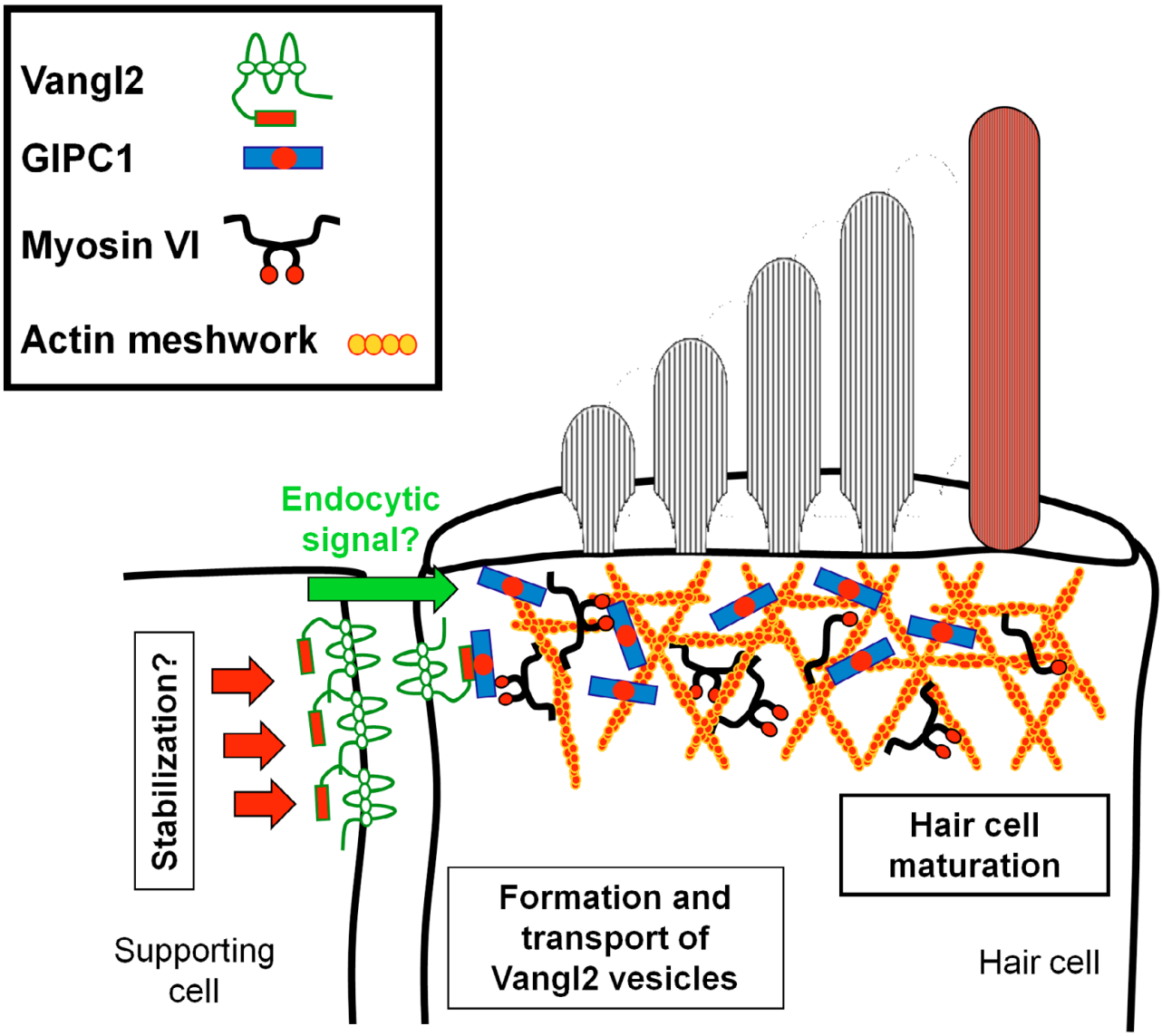

Fig. S3. Working hypothesis for Gipc1 functions. Under normal conditions, Vangl2 is strongly enriched by unknown mechanism at the apicolateral domain of the SC. On the side facing the HC, Vangl2 is present, although at lower levels. An endocytic signal could initiate the endocytosis of Vangl2, and its PDZ-BM can bind to Gipc1. We do not know the nature or exact sequence of events leading to the endocytosis, but suggest that a communication between HC and SC might participate. The C-ter of Gipc1 can bind to MyoVI, which can then translocate the complex towards the pointed end of actin filaments. Gipc1 could also participate in the maturation and stabilization of the apical region of the hair cell, including the hair bundle. Gipc1 could function independently, or through interaction with MyoVI. When Gipc1 is downregulated, Gipc1-MyoVI-dependant endocytosis is delayed, resulting in increased Vangl2 at the plasma membrane. Also, the apical domain of the HC is reduced, and the structure of the hair bundle disrupted, owing to the disruption of early trafficking events, or later stabilization of the hair bundle. For reasons of clarity we do not represent the vesicles containing the proteins on the schematic.

Gene	Full name	Interaction with Vangl2 bait	Interaction with Vangl2$^{\Delta 4/\Delta 12}$ bait
DLG1_2	discs, large homolog 1	+	–
DLG2_3	discs, large homolog 2	+	–
DLG4_1	discs, large homolog 4	+	–
DLG4_3	discs, large homolog 4	+	–
GIPC1	GIPC PDZ domain containing family, member 1	+	–
MAGI1_2	membrane associated guanylate kinase, WW and PDZ domain containing 1	+	–
MAGI2_5	membrane associated guanylate kinase, WW and PDZ domain containing 2	+	–
MPDZ_7	multiple PDZ domain protein	+	–
PTPN13_2	protein tyrosine phosphatase, non-receptor type 13	+	–
PTPN3	protein tyrosine phosphatase, non-receptor type 3	+	–
SCRIB_3	scribbled homolog	+	–
SNTA1	syntrophin, alpha 1	+	–
SNX27	sorting nexin family member 27	+	–
SYNJ2BP	synaptojanin 2 binding protein	+	–
DFNB31	deafness, autosomal recessive 31	–	–
DVL1	dishevelled, dsh homolog 1	–	–
DVL2	dishevelled, dsh homolog 2	–	–
DVL3	dishevelled, dsh homolog 3	–	–
FMRPD1	fragile X mental retardation 1	–	–
GRIP1_1/7	glutamate receptor interacting protein 1	–	–
GRIP2_1/7	glutamate receptor interacting protein 2	–	–
INTU	inturned planar cell polarity effector homolog	–	–
MYO18A	myosin XVIIIA	–	–
PICK1	protein interacting with PRKCA 1	–	–
SHANK1	SH3 and multiple ankyrin repeat domains 1	–	–

Table S1. The PDZ domains of various candidate interactors for Vangl2 were cloned and their interaction with the C-ter of Vangl2 was tested in a yeast-two-hybrid assay. Previously characterized interactors were validated with this assay (including Scrib1, Dlg or Magi), and new candidates were identified. The PDZ-binding motif of Vangl2 showed preferences of affinity for specific PDZ domains, as shown by the negative interactions with various PDZ domains, including those in proteins with PCP roles, or associated with junctional complexes.