Supplementary Information

Functional interaction between Vangl2 and N-cadherin regulates planar cell polarisation of neural tissues

Affiliations

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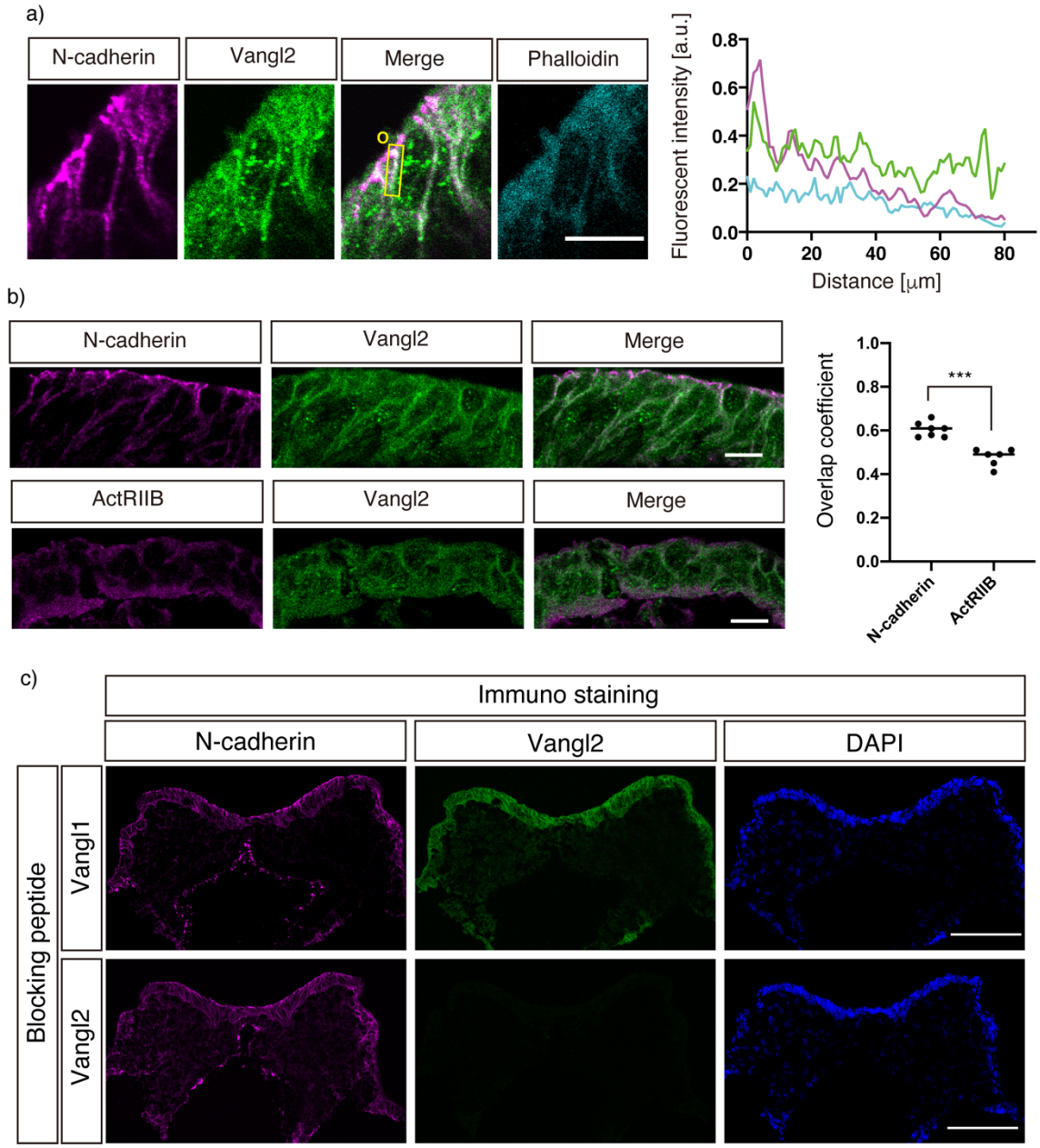
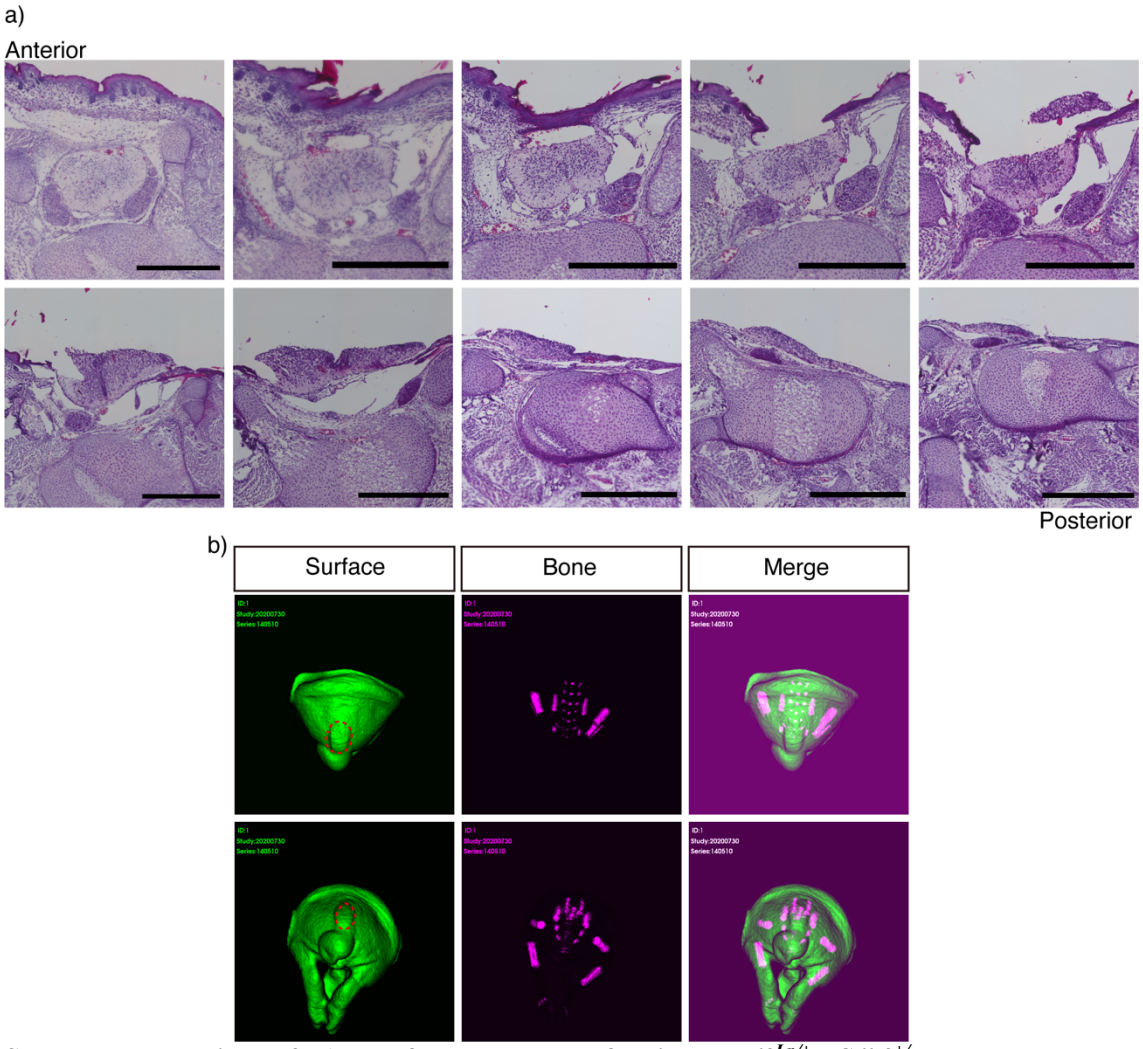


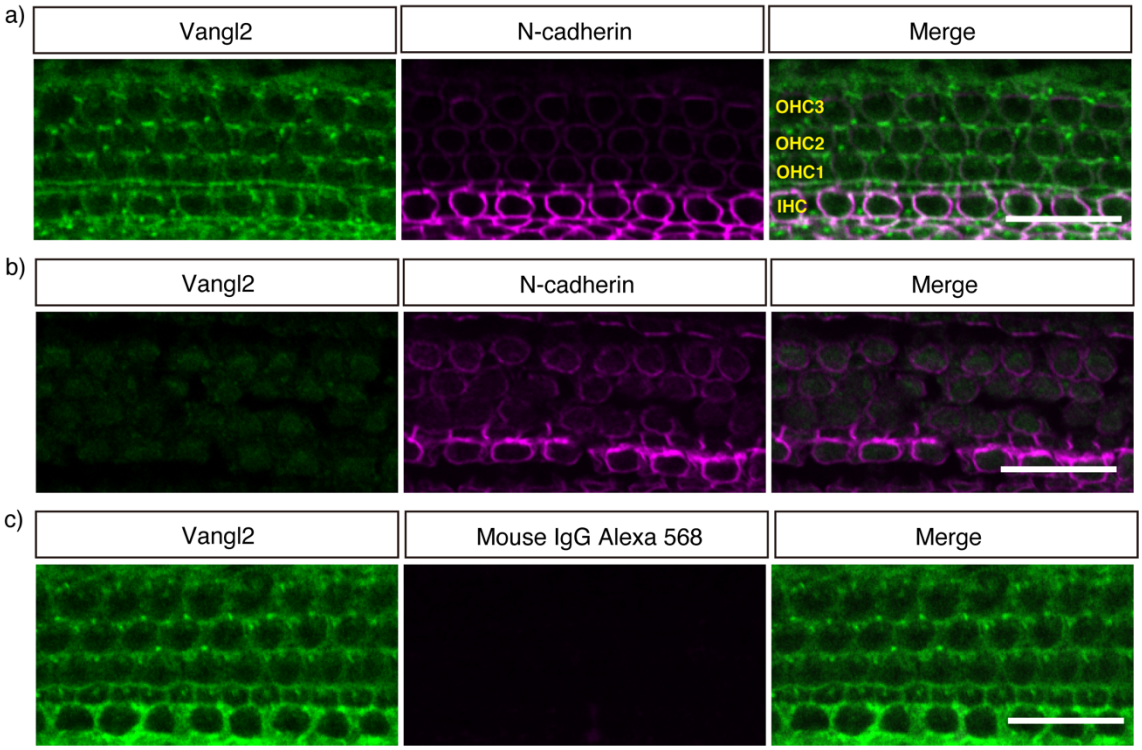
Supplementary Figure 1. Co-localisation of Vangl2 and N-cadherin in neural tubes and specific immunofluorescent staining of neural tube sections by anti-Vangl2 antibody.

a) The plot profile of fluorescent signal intensity in the yellow box is shown on the right. Magenta, green and cyan lines that depict N-cadherin, Vangl2, and Phalloidin signal intensities, respectively, demonstrate partial co-localization of Vangl2 and N-cadherin. b) Quantification of Vangl2 and N-cadherin overlap. The overlap coefficient which was measured using ZEN software (Zeiss), of Vangl2/N-cadherin was significantly higher than that of Vangl2/activin receptor type IIB that is expressed in neural tubes. c)The specificity of anti-Vangl2 antibodies was evaluated by competitive binding with blocking peptides (Santa Cruz Biotechnology, sc-515862 P). While Vangl2 blocking peptides affected immunofluorescent intensity, Vangl1 blocking peptides (Santa Cruz Biotechnology, sc-166844 P) did not. Confocal images were taken using 20 X objective lens. The significant differences between groups that were calculated using the Student t-test are indicated with *** ($p<0.0001$). Scale bars: 100 μm



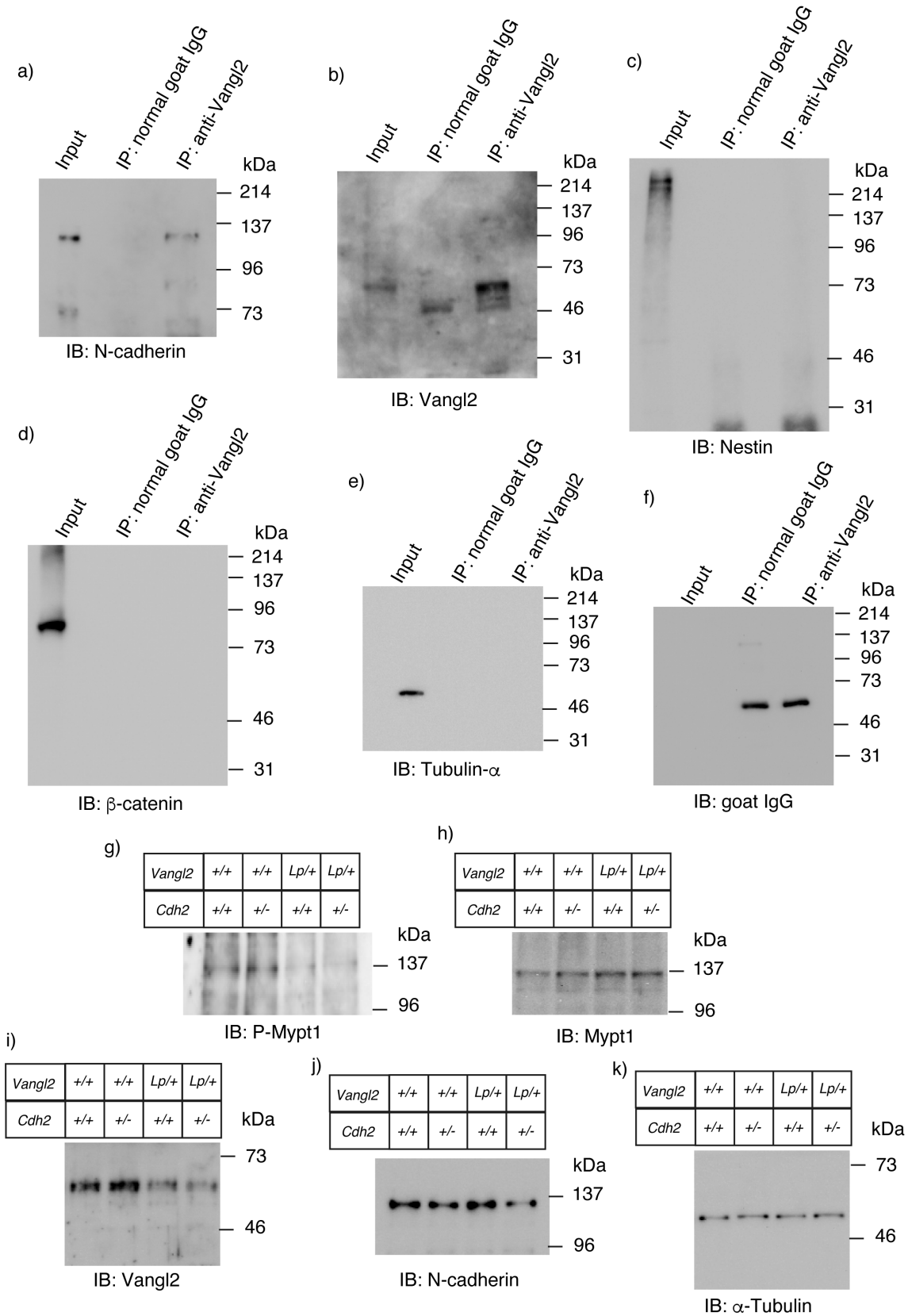
Supplementary Figure 2. Area of neural tube defect in *Vangl2$^{Lp/+}$; Cdh2$^{+/-}$* mouse embryo at E18.5

a) Haematoxylin-Eosin staining of the caudal portion of *Vangl2$^{Lp/+}$; Cdh2$^{+/-}$* E18.5 mouse embryos were sectioned sequentially and perpendicularly against the head-tail axis. The spine morphology on anterior side was normal, however; the spinal cord is exposed outside of the skin just before the tail. b) Lower bodies of *Vangl2$^{Lp/+}$; Cdh2$^{+/-}$* E18.5 mouse embryos were scanned by micro computed tomography (micro-CT). Upper panels show the dorsal view and lower panels show the caudal side view. The open spinal cord that is located around the sacrum is circled by a red broken line. Scale bars: 500 μm in the panel A



Supplementary Figure 3. Expression of Vangl2 and N-cadherin in inner ear hair cells of E18.5 mouse embryos

Isolated inner ear cochlea of E18.5 mouse embryos were fixed with ice-cold methanol before immunofluorescence analysis. a) Immunostaining analysis revealed Vangl2 expression in the outer and inner hair cells, and stronger N-cadherin expression in the inner ear hair cells than that in the outer hair cells. b) Addition of 2 μg/ml Vangl2 blocking peptide abolished the Vangl2 signal, thus indicating specificity of the anti-Vangl2 antibodies. c) Immunostaining of the cochlea with anti-Vangl2 antibodies. Confocal images were taken using a 40 X oil immersion objective lens. Scale bars: 20 μm



Supplementary Figure 4. Full-length images of western blot analyses
a)-f) Full length images presented in Figure 1. g)-k) Full length images presented in Figure 4.

Supplementary Materials and Methods

Micro-CT

Mouse E18.5 embryos were sacrificed by decapitation and cleaved at the abdominal level. The lower bodies were fixed with PBS containing 4% PFA and set on a polystyrene bed for scanning by micro-CT (R_mCT2, Rigaku, Tokyo, Japan) under the following conditions: 90 kV, 160 μA, 3 min scan time, and 30 mm FOV. Data were processed using 3DViewer built in R_mCT2, and the bony parts were displayed using the part selection function.

Supplementary Table 1. List of antibodies

Antigen	Host	Manufacturer	Clone ID or Product number	Application (Dilution)	Conjugation
Activin RIIB	Mouse	R&D systems	MAB3393	IF (1:200)	
β-catenin	Mouse	BD Biosciences	14/Beta-Catenin	WB (1:1000)	
Digoxigenin	Sheep (Fab fragment)	Roche	11093274910	in situ (1:2000)	AP
c-Jun	Rabbit	Cell Signaling Technology	60A8	IF (1:200)	
Phospho-c-Jun (Phospho-Ser63)	Rabbit	Cell Signaling Technology	E617P	IF (1:200)	
Myo7A	Rabbit	Proteus Biosciences	25-6790	IF (1:500)	
Mypt1	Rabbit	Cell Signaling Technology	D6C1	WB (1:500)	
Phopho-Mypt1 (Phospho-Thr696)	Rabbit	Cell Signaling Technology	#5163	WB (1:500)	
N-cadherin	Mouse	BD Biosciences	Clone 32/N-Cadherin	WB, IF (1:1000)	
Nestin	Mouse	Thermo scientific	Rat401 (4D4)	IF (1:200) WB (1:500)	
α-Tubulin	Mouse	Proteintech	66031-1-Ig	WB (1:1000)	
Vangl2	Goat	Santa Cruz	N-13	IP, IF (1:100) WB (1:500)	
N.A.	Goat	Santa Cruz	sc-3887 (normal Goat IgG)	IP (1:200)	
Goat IgG	Donkey	Life Technologies	A-32814	IF (1:500)	Alexa 488
Goat IgG	Donkey	Santa Cruz	sc-2020	WB (1:5000)	HRP
Goat IgG Light Chain Specific	Mouse	Jackson ImmunoResearch	205-032-176	WB (1:5000)	HRP
Mouse IgG	Donkey	Life Technologies	A-10037	IF (1:500)	Alexa 568
Mouse IgG	Goat	Life Technologies	A-11004	IF (1:500)	Alexa 568
Mouse IgG	Goat	Bio-Rad	170-6516	WB (1:5000)	HRP
Rabbit IgG	Goat	Life Technologies	A-11008	IF (1:500)	Alexa 488
Rabbit IgG	Goat	Santa Cruz	sc-2030	WB (1:5000)	HRP