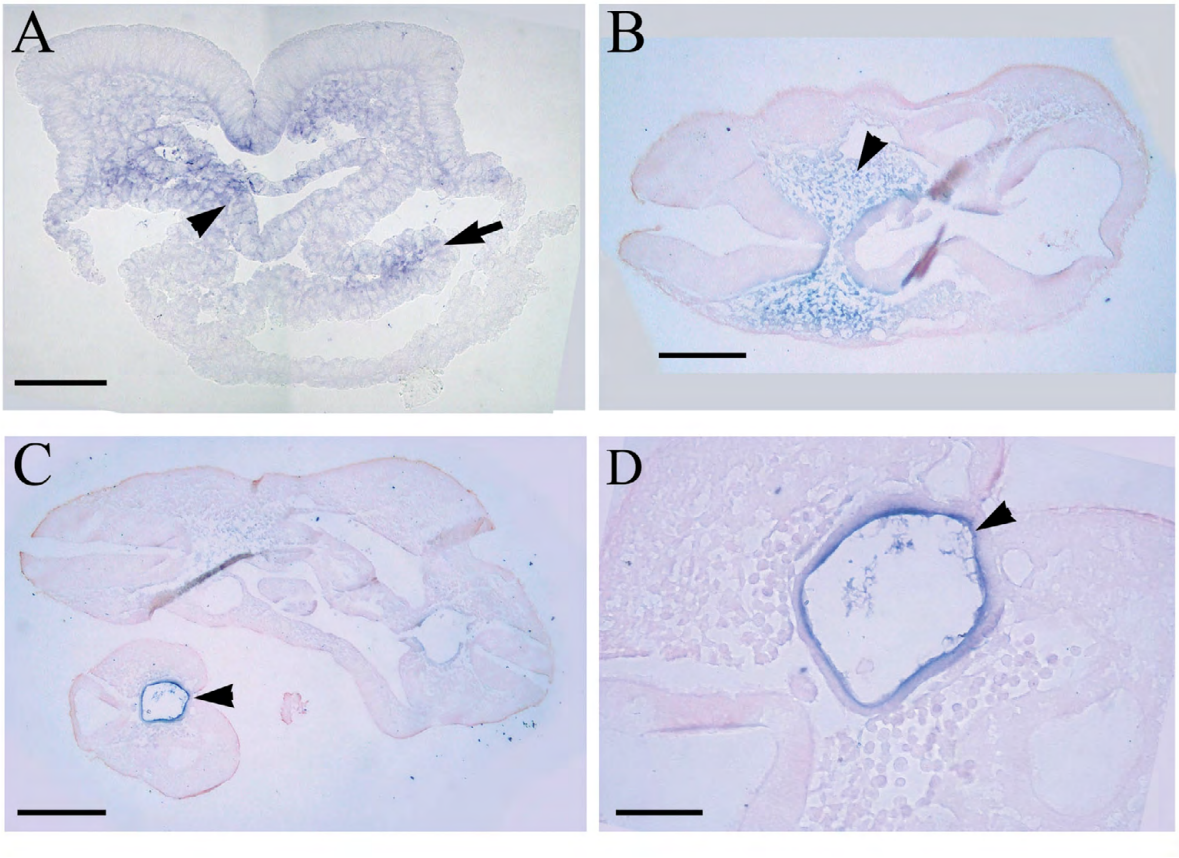

Fig. S1. *Sdc4* expression during mouse development. (**A-D**) Transverse sections through E8.5 and E9.5 embryos after whole-mount *in situ* hybridization with an antisense RNA probe for *Sdc4*. (A) At E8.5, *Sdc4* is expressed in the foregut diverticulum (arrowhead) and in the developing heart tube (arrow). At E9.5, *Sdc4* is strongly expressed in the cephalic mesenchyme (arrowhead in B) and in the hindgut (arrowhead in C and D). Scale bars: 100 μm in A; 200 μm in B,C; 50 μm in D.

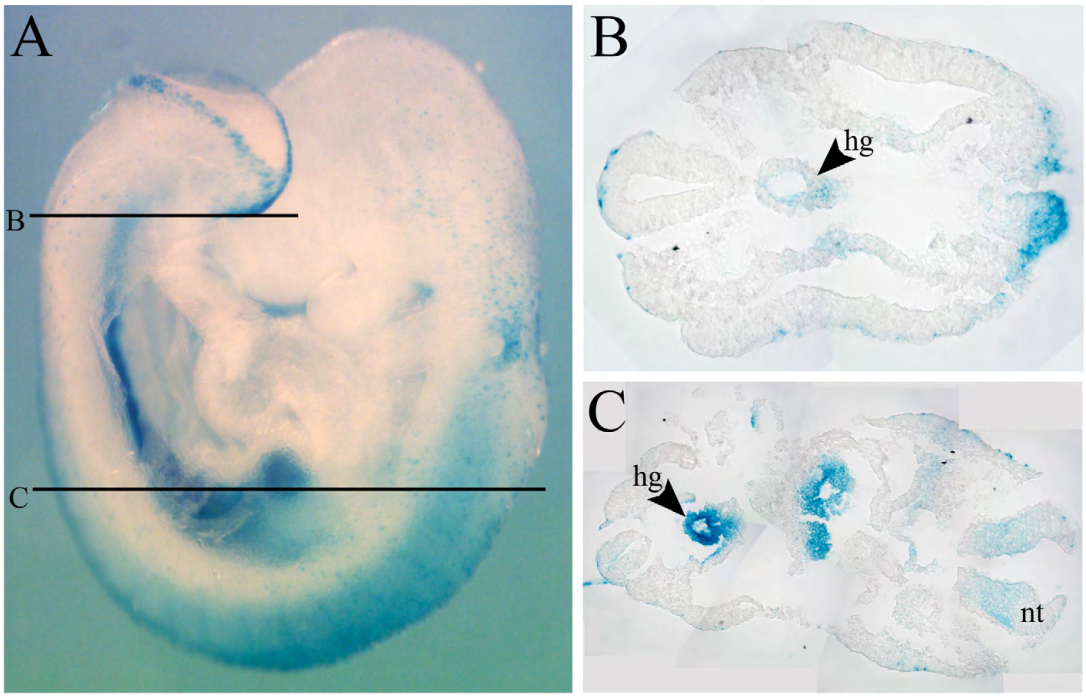

Fig. S2. *Sdc4* expression during mouse development. (**A-C**) β-Galactosidase activity in $Sdc4^{lacZ/+}$ embryos at E9.0 showing staining throughout the gut in the lateral view of whole embryo (A), and in the hindgut (arrowheads in B,C) in the transverse sections at the embryo levels indicated by black lines in A. hg, hindgut; nt, neural tube.

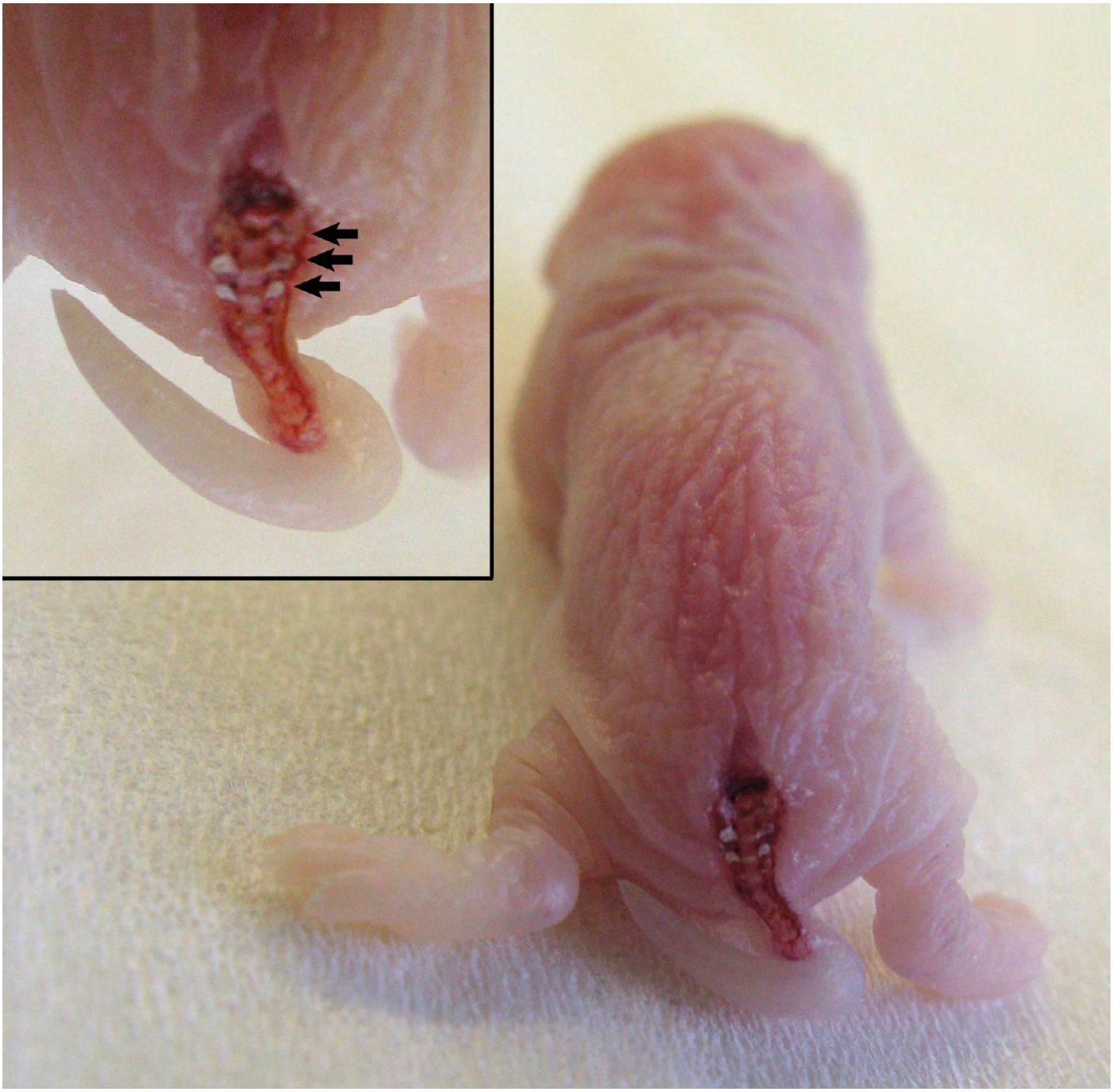

Fig. S3. Lumbosacral spina bifida in a *Sdc4*$^{lacZ/lacZ}$;*Vangl2*$^{Lp/+}$ mouse. Compared with the sacral spina bifida (see Fig. 2B), this more severe neural tube defect extends further rostrally, into the lumbar level of the body axis. Open vertebrae are visible extending across the midline (arrows in expanded view), indicating that the overlying spinal cord tissue has completely degenerated at this level. A sharply flexed tail is associated with the spina bifida.

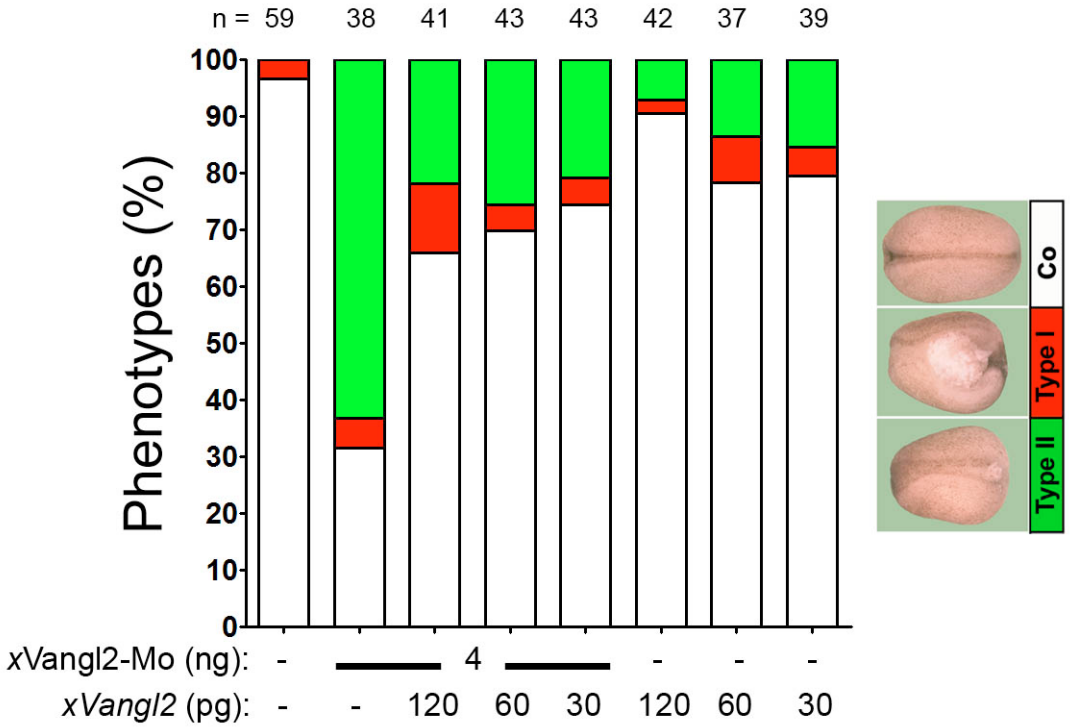

Fig. S4. Specificity of morpholinos against Vangl2. The specificity of the morpholinos against Vangl2 was demonstrated by rescue experiments using synthetic mRNA for Vangl2. Eight-cell stage *Xenopus* embryos were co-injected with the indicated amounts of morpholinos against xVangl2-MO and synthetic mRNA for an epitope-tag Vangl2 and neural tube closure defects were quantified at stage 20. Phenotypes were classified as type I (severe gastrulation and neural tube closure defects; red) and type II (impairment of neural tube closure; green). The graph summarizes two independent experiments, with numbers of embryos given at the top of each bar.

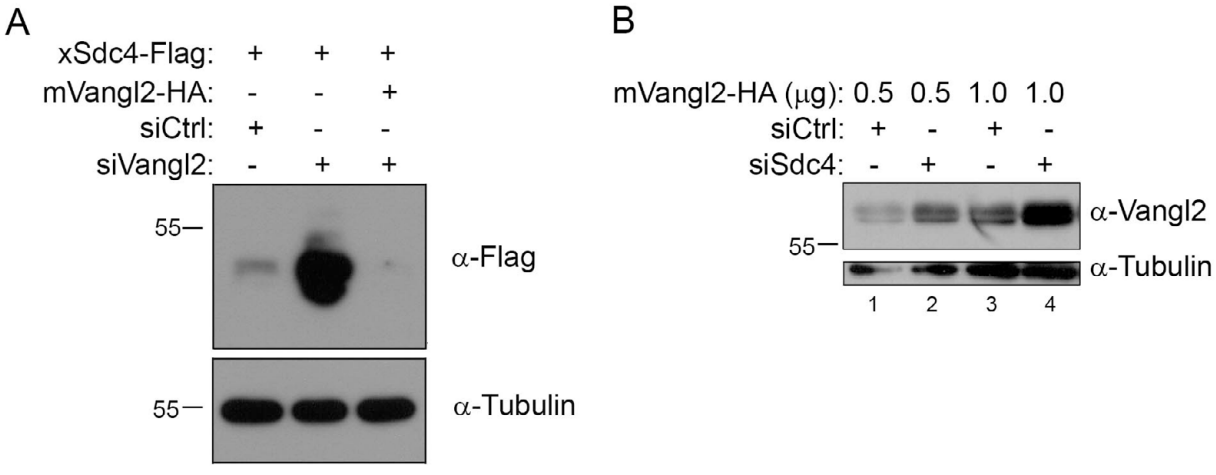

Fig. S5. Effect of Sdc4 on Vangl2 steady-state levels. (**A**) Vangl2 rescues the effect of siVangl2. HEK293 cells were transfected with xSdc4-Flag and Vangl2-HA plus siCtrl or siVangl2 and Sdc4 protein levels were evaluated. Knockdown of Vangl2 (middle lane) dramatically upregulates Sdc4 protein, and this is rescued by simultaneous expression of HA-mVangl2 (right lane), indicating a specific effect of siVangl2. (**B**) Sdc4 regulates Vangl2 protein level. HEK293 cells were transfected with Vangl2-HA and siCtrl or siSdc4, and Vangl2 protein levels were evaluated. Knockdown of Sdc4 resulted in increased steady-state levels of Vangl2 protein.

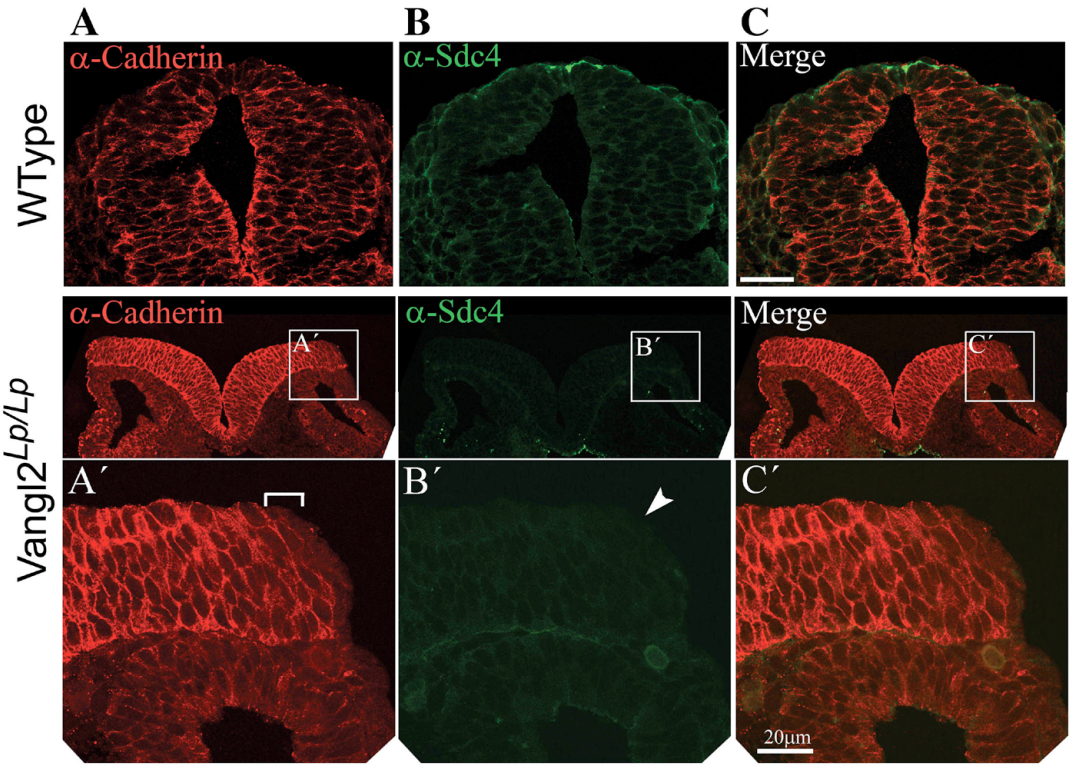

Fig. S6. Absence of Sdc4 expression in the non-neural ectoderm of *Vangl2*$^{Lp/Lp}$ embryos. (**A-C′**) Double immunofluorescence using antibodies against Sdc4 (green) and pan-cadherin (red) was performed on transverse sections of E9.0 wild-type and *Vangl2*$^{Lp/Lp}$ embryos. Sdc4 is expressed in the non-neuronal ectoderm of wild-type embryos; however, no signal is detected in *Vangl2*$^{Lp/Lp}$ embryos. Bracket in A′ indicates the non-neuronal ectoderm; arrowhead in B′ indicates lack of Sdc4 expression in this tissue.

Table S1. Genotype distribution and incidence of spina bifida in the *Sdc4/Vangl2* interaction

Genotype		Total pups	Expected (%)	Experimental (%)	Spina bifida	Phenotype (%)
Sdc4	*Vangl2*					
lacZ/+	+/+	33	25	30	0	0
lacZ/+	*Lp*/+	35	25	32	6	17
lacZ/lacZ	+/+	21	25	19	0	0
lacZ/lacZ	*Lp*/+	20	25	18	11	55
Total		109	100			

A total of 109 mice were born in 17 separate litters from matings between $Sdc4^{lacZ/+}$;$Vangl2^{Lp/+}$ males and $Sdc4^{lacZ/lacZ}$ females. Offspring were analyzed daily from P0 to P30. There is a significant deviation from the expected Mendelian ratio of genotypes (Chi-square test; $P<0.01$), suggesting that absence of Sdc4 on a $Vangl2^{Lp/+}$ background adversely affects pre/postnatal survival.

Chi-square analysis of proportion of each genotype versus 25% expectation: significant; $P<0.01$ (chi-square = 6.77, dof = 1).

Table S2. Postnatal viability of different genotypes in litters from matings between *Sdc4*$^{lacZ/+}$;*Vangl2*$^{Lp/+}$ males and *Sdc4*$^{lacZ/lacZ}$ females

Age (days)	*Sdc4*$^{lacZ/+}$; *Vangl2*$^{+/+}$	*Sdc4*$^{lacZ/+}$; *Vangl2*$^{Lp/+}$	*Sdc4*$^{lacZ/lacZ}$; *Vangl2*$^{+/+}$	*Sdc4*$^{lacZ/lacZ}$; *Vangl2*$^{Lp/+}$
P0	21 (100%)	21 (100%)	14 (100%)	17 (100%)
P15	21 (100%)	18 (86%)	14 (100%)	12 (70%)
P30	18 (86%)	12 (57%)	11 (78%)	2 (12%)

Individual pups were identified at birth (P0) and then followed up at P15 and P30 to determine viability (percentage values show the proportion of P0 pups that survive to the later time point). There is a dramatic decrease in survival of *Sdc4*$^{lacZ/lacZ}$;*Vangl2*$^{Lp/+}$ pups beyond P15 (significantly different survival at P30 from *Sdc4*$^{lacZ/+}$;*Vangl2*$^{Lp/+}$ littermates, $P<0.01$).

Fisher's exact test of proportion of pups surviving to P30: 12/21 (*Sdc4*$^{lacZ/+}$) versus 2/17 (*Sdc4*$^{lacZ/lacZ}$). Significant; $P=0.006$