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Supplemental Information

A Wnt5 Activity Asymmetry and Intercellular

Signaling via PCP Proteins Polarize

Node Cells for Left-Right Symmetry Breaking

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INVENTORY OF SUPPLEMENTAL INFORMATION

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SUPPLEMENTAL FIGURES

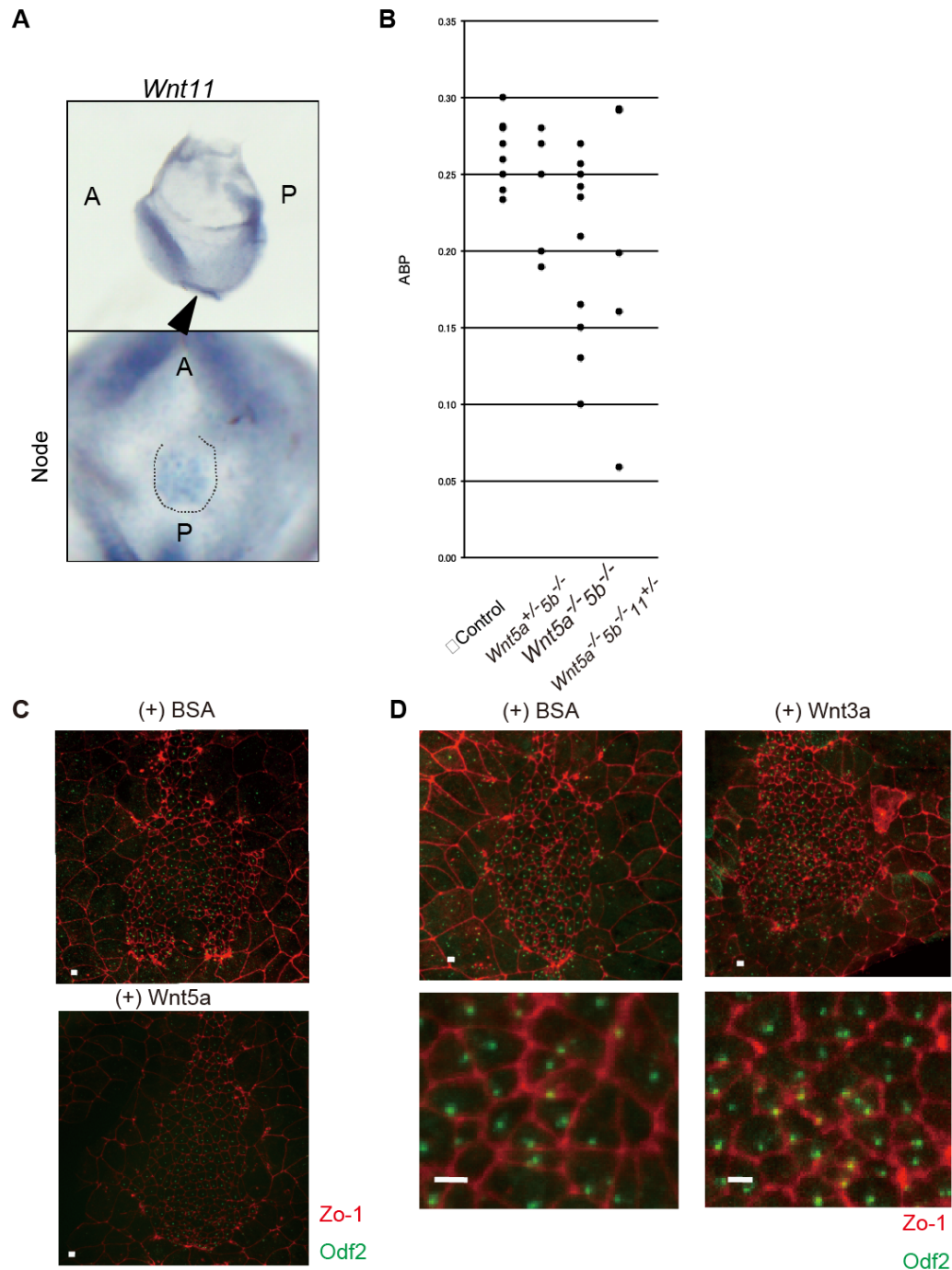


Figure S1. Role of *Wnt11*, *Wnt5a* and *Wnt3a* in node cell polarization, related to Figure 1.

(A) Whole-mount in situ hybridization analysis of *Wnt11* expression in a WT mouse embryo at the three- to five-somite stage. The node is indicated by the arrowhead in the upper panel and is shown outlined in the lower panel. (B) Summary of ABP values for embryos of the indicated genotypes. The data for control, $Wnt5a^{+/-}Wnt5b^{-/-}$, and $Wnt5a^{-/-}Wnt5b^{-/-}$ embryos are the same as those in Figure 1d. Note that the ABP of $Wnt5a^{-/-}Wnt5b^{-/-}Wnt11^{+/-}$ embryos is similar to that for $Wnt5a^{-/-}Wnt5b^{-/-}$ embryos. (C, D) WT embryos were cultured with BSA or either recombinant *Wnt5a* (1 $\mu\text{g/ml}$) (C) or recombinant *Wnt3a* (0.8 $\mu\text{g/ml}$) (D) from the late streak to three- to five-somite stages. They were then subjected to immunofluorescence staining for Zo-1 (red) and Odf2 (green). The upper images in (D) are shown at higher magnification in the lower panels. Note that the morphology of the node remained normal after incubation of embryos with *Wnt5a* or *Wnt3*. The position of the basal body was affected by *Wnt5a* but not by *Wnt3a*. Scale bars, 5 μm .

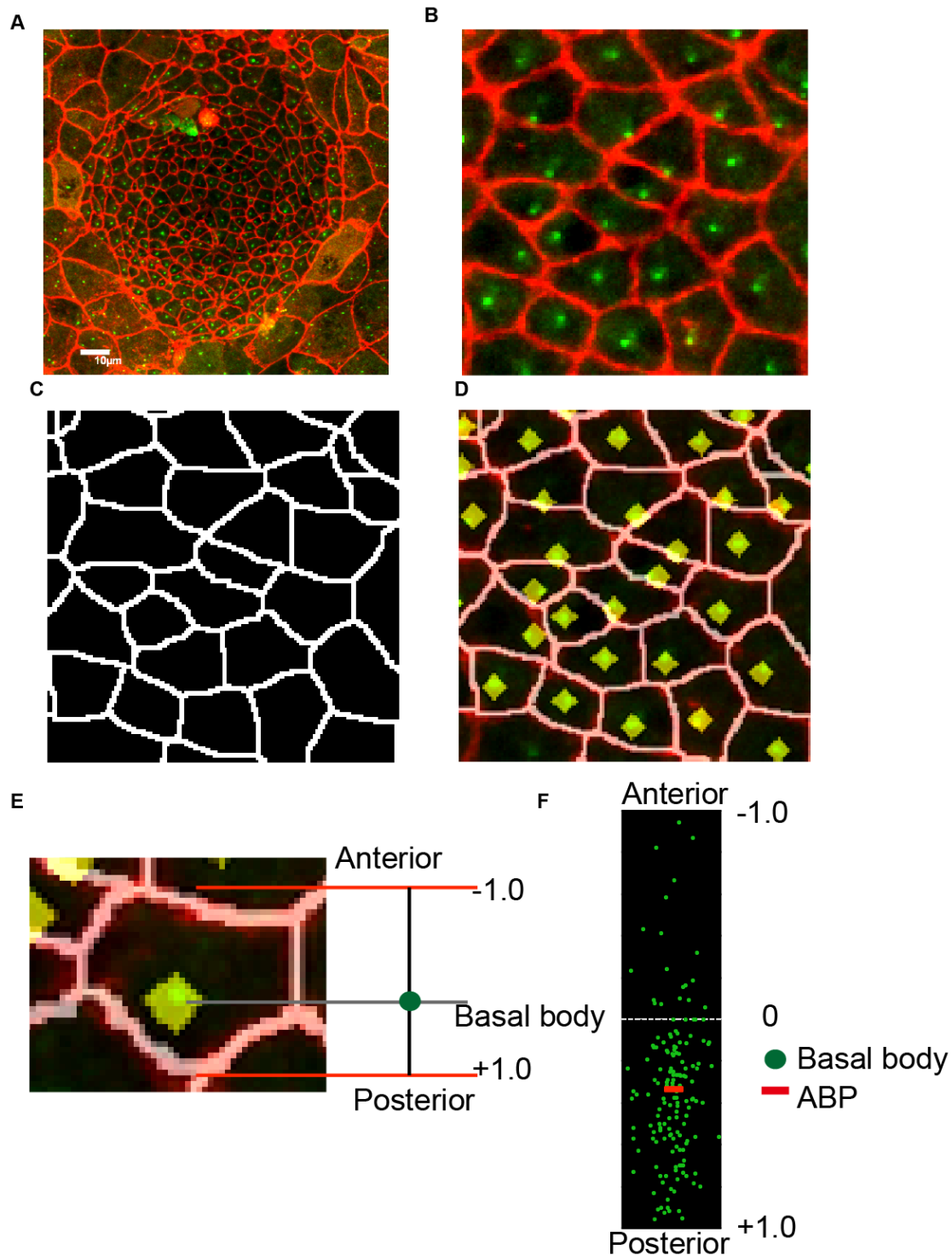


Figure S2. Quantitative analysis of basal body position with ImageJ software, related to Figure 1.

(A) Confocal image of the node region of a WT embryo immunostained for Odf2 (green) and Zo-1 (red). (B) The enlarged view of a region in (A). (C) The node image in (B) was processed with the ImageJ software plugin to apply watershed segmentation, generating a “binary watershed line image.” The outline of each cell was estimated from the pattern of Zo-1 staining. (D) Graphical user interface (GUI) view of the binary watershed line image in (C). The basal body was traced according to the Odf2 staining image. (E) Position of the basal body in a node cell relative to the most anterior and posterior positions, -1.0 and $+1.0$, respectively. (F) Distribution map for the basal body in the analyzed embryo. The Red bar indicates ABP.

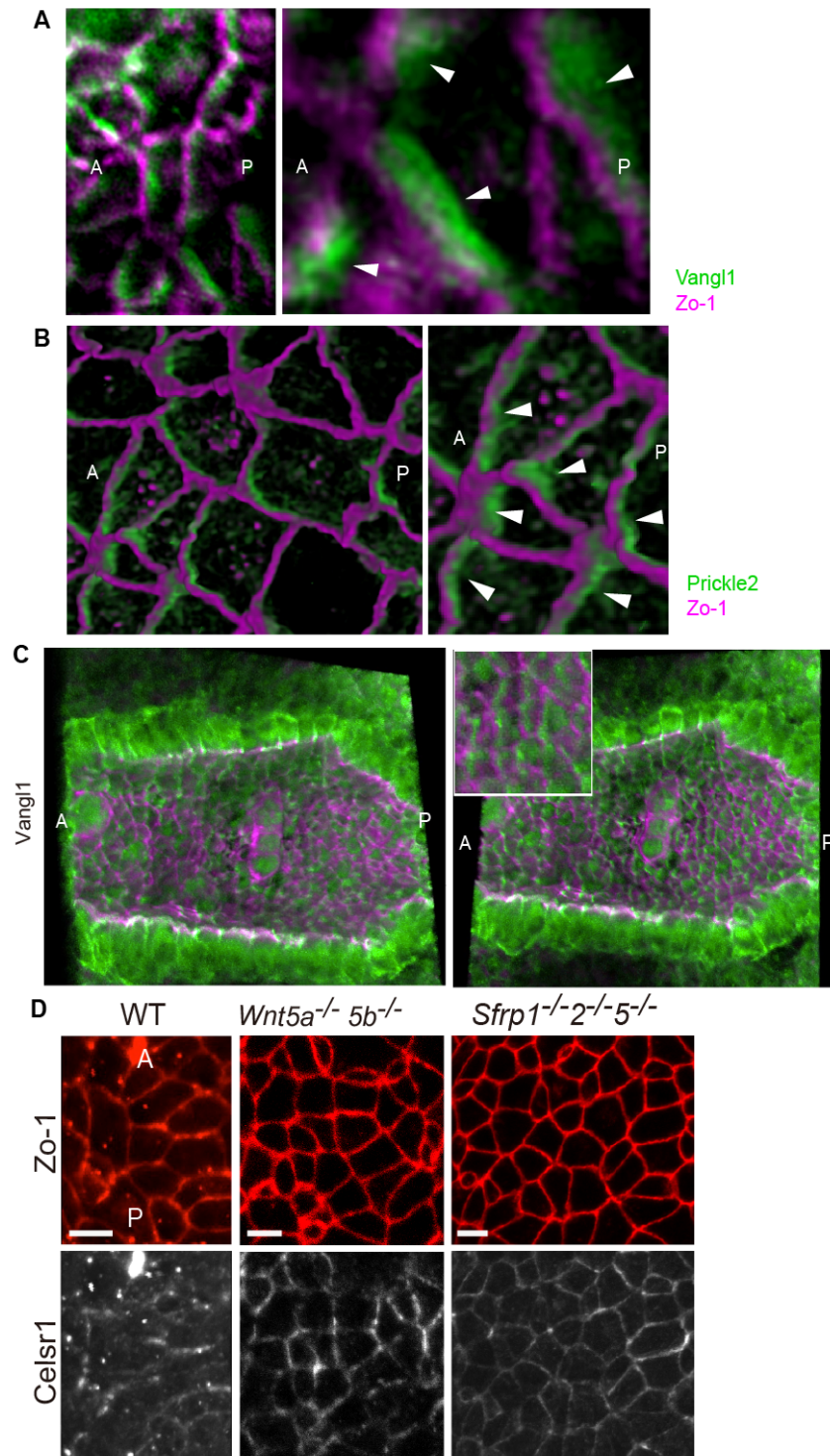


Figure S3. Polarized localization of PCP core proteins is dependent on Wnt5 and Sfrp genes, related to Figure 3.

(A, B) Immunofluorescence staining of WT embryos at the three- to five-somite stage for Vangl1 (green) and Zo-1 (purple) (A) or for Prickle2 (green) and Zo-1 (purple) (B). The node region is shown for each embryo, with higher magnification images of the node being shown in the right panels. The subcellular localization of Vangl1 and Prickle2 was observed by super-resolution imaging, revealing that both proteins are localized to the anterior side of node cells (arrowheads). A, anterior; P posterior. (C) Three-dimensional reconstruction of Vangl1 localization images in the WT embryo obtained by super-resolution imaging. The view of the node from the anterior side (left panel) and the view from the posterior side (right panel) are shown here with a higher magnification image in the inset. Note that Vangl1 is detected at the anterior side of node cells. (D) Localization of Zo-1 (red) and Celsr1 proteins (green) in the node of WT, *Wnt5a*^{-/-} *Wnt5b*^{-/-}, and *Sfrp1*^{-/-} *Sfrp2*^{-/-} *Sfrp5*^{-/-} embryos at the three- to five-somite stage.

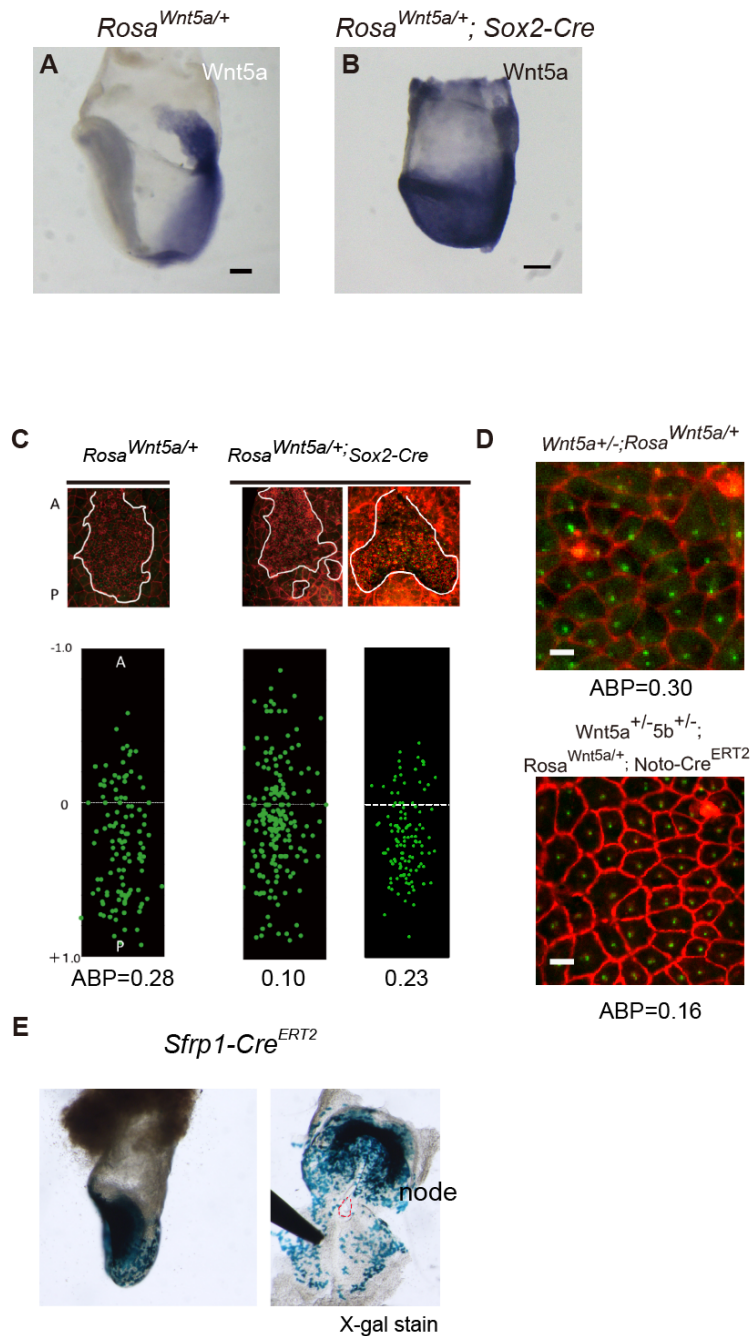


Figure S4. Ectopic *Wnt5a* expression induced by Cre transgenes, related to Figure 4.

(A, B) Whole-mount in situ hybridization analysis of *Wnt5a* expression in *Rosa*^{*Wnt5a*+/+} (A) or *Rosa*^{*Wnt5a*+/+}; *Sox2-Cre* (B) embryos at E7.5. Note that *Wnt5a* is expressed ubiquitously in the latter embryo. Scale bars, 100 μ m. (C) *Rosa*^{*Wnt5a*+/+} and *Rosa*^{*Wnt5a*+/+}; *Sox2-Cre* embryos at the three- to five-somite stage were immunostained for Zo-1 (red) and Odf2 (green), with the white line indicating the outline of the node (upper panels). The position of the basal body (green dots) in node cells is summarized for each embryo in the lower panels, with the corresponding ABP values indicated. A, anterior; P, posterior. Two independent embryos are shown for *Rosa*^{*Wnt5a*+/+}; *Sox2-Cre* embryos, with one showing abnormal ABP (0.10) and the other showing relatively normal ABP (0.23). (D) *Wnt5a*^{+/-}; *Rosa*^{*Wnt5a*+/+} or *Wnt5a*^{+/-}; *Wnt5b*^{+/-}; *Rosa*^{*Wnt5a*+/+}; *Noto-Cre*^{ERT2} embryos isolated at the three- to five-somite stage from dams treated orally with tamoxifen (5 mg) both 24 and 12 h before the late streak stage were immunostained for Zo-1 (red) and Odf2 (green). Note that the basal body is positioned centrally in many node cells of the latter embryo. Scale bars, 5 μ m. (E), Specificity of *Sfrp1-Cre* transgene expression. A *Rosa*^{*lacZ*+/+}; *Sfrp1-Cre*^{ERT2} embryo isolated at the three- to five-somite stage from a dam treated orally with tamoxifen (5 mg) both 24 and 12 h before the late streak stage was stained with the β -galactosidase substrate X-gal. Note that X-gal staining is predominant in the region anterior to the node, although some cells around the node are also positive.

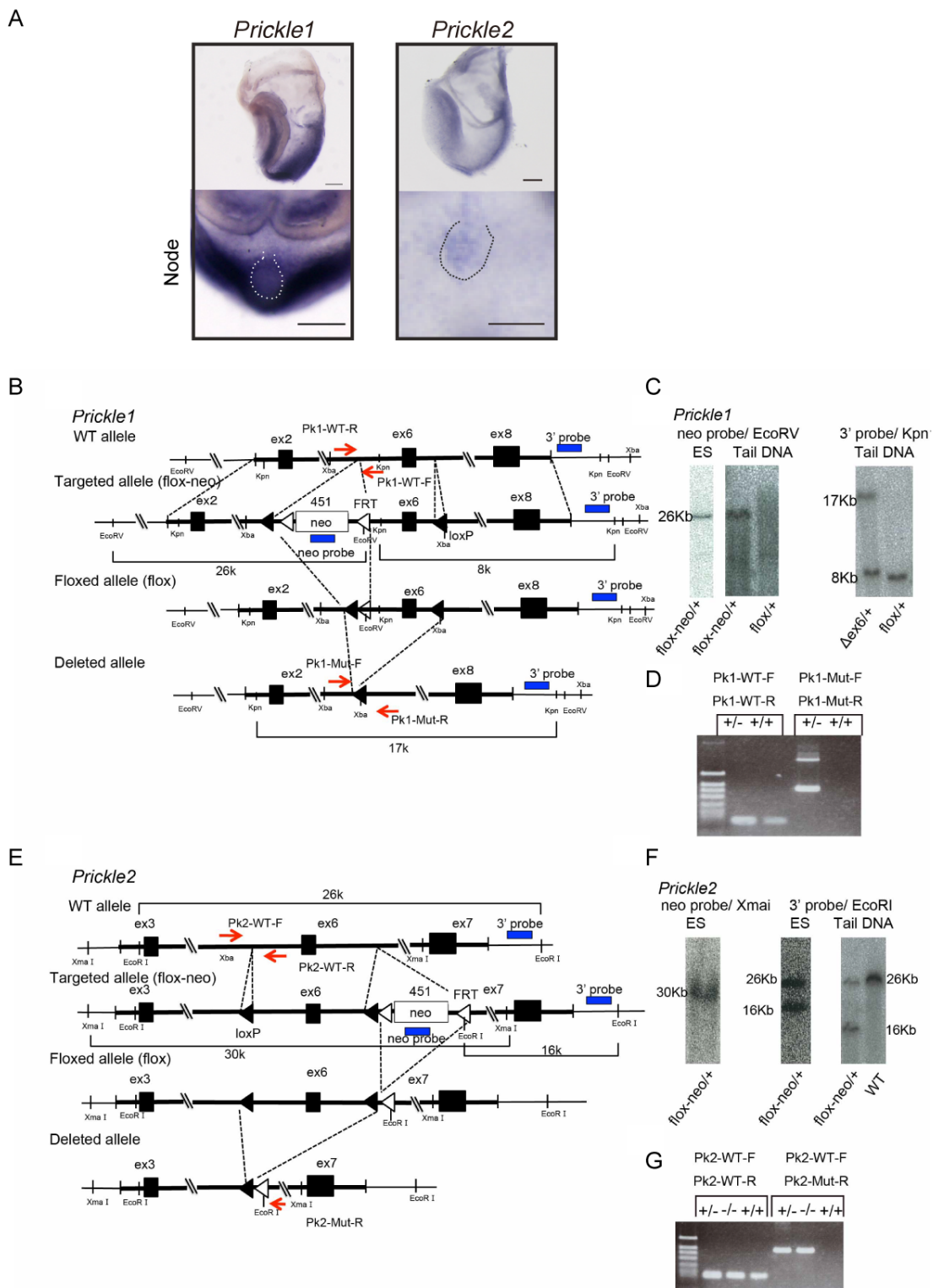


Figure S5. Generation of *Prickle1* and *Prickle2* mutant alleles, related to Figure 5.

(A) Expression of *Prickle1* and *Prickle2* in the E8.0 mouse embryo was examined by in situ hybridization. Note that both genes are expressed at the ventral node. (B, E) Generation of *Prickle1* (B) and *Prickle2* (E) mutant alleles with targeting vectors designed to delete exon 6 of each gene. (C, F) ES cell clones that had undergone homologous recombination were selected by Southern blot hybridization with specific probes (shown in B and E, respectively) that recognize external or internal sequences relative to the targeting vector. Tail DNA of mice of the indicated genotypes was similarly analyzed. (D, G) The genotype of offspring from heterozygote intercrosses was determined by PCR analysis of tail DNA with primer sets (shown in B and E, respectively) specific for the WT or Δ ex6 alleles. Given the exon-intron structure of *Prickle1* and *Prickle2*, the mutant alleles can only produce a truncated protein lacking the large COOH-terminal region (636 and 645 amino acid residues encoded by exons 6 to 12 would be lacking out of 832 and 845 amino acid residues of the whole Pk1 and Pk2 protein, respectively).

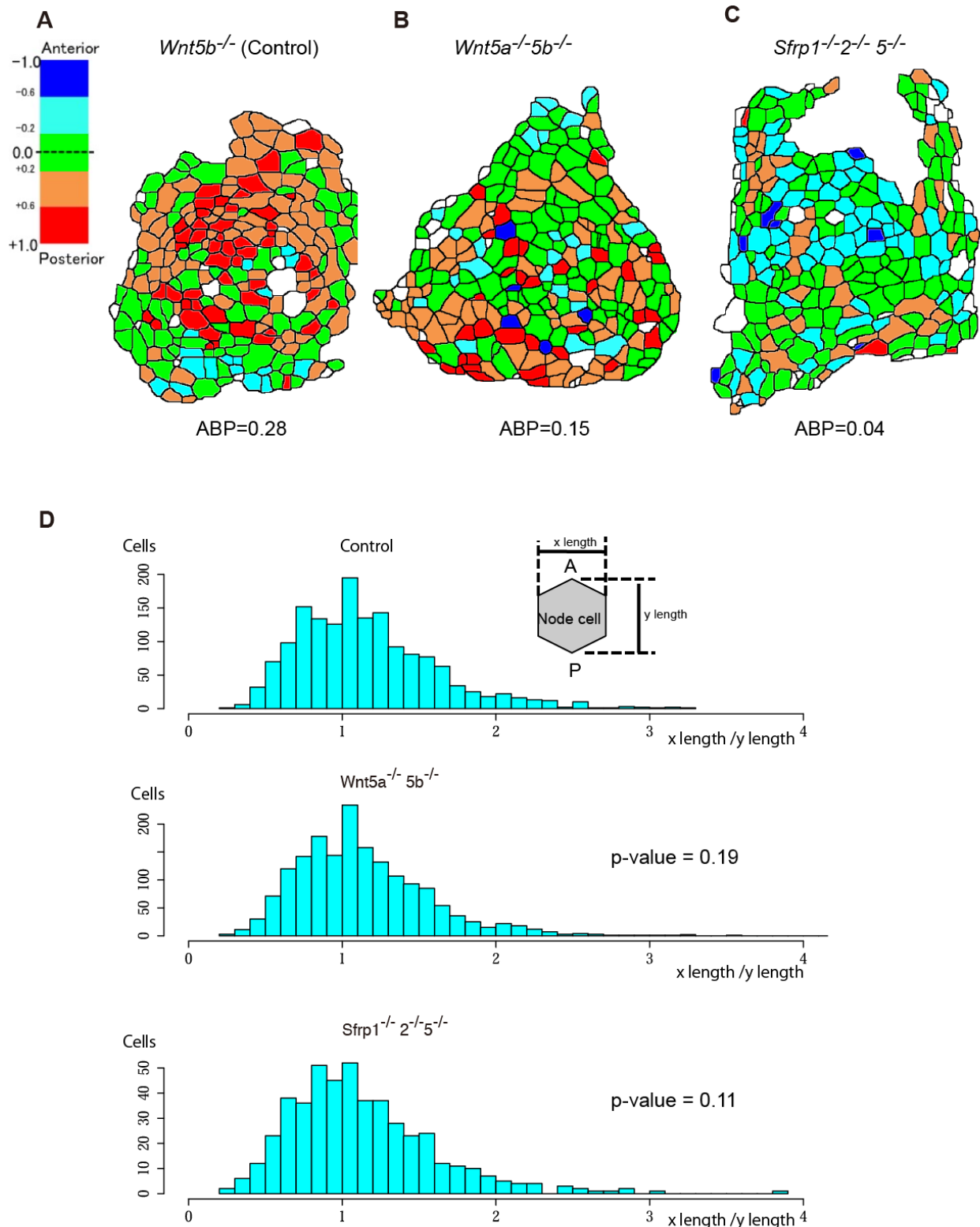


Figure S6. Distribution and shape of node cells from Control, *Wnt5a*^{-/-}*Wnt5b*^{-/-} and *Sfrp1*^{-/-}*Sfrp2*^{-/-}*Sfrp5*^{-/-} embryos, related to Figure 6 and Figure 7.

(A-C) Node cells were grouped on the basis of their ABP: +1.0 ~ +0.6 (red), +0.6~+0.2 (orange), +0.2 ~ -0.2 (green), -0.2~-0.6 (light blue), and -0.6 ~ -1.0 (blue). Distribution of each group of cells was examined in *Wnt5b*^{-/-} (A), *Wnt5a*^{-/-}*Wnt5b*^{-/-} (B) and *Sfrp1*^{-/-}*Sfrp2*^{-/-}*Sfrp5*^{-/-} (C) embryos. Note that distribution is not random. Instead, cells with a similar ABP tend to form clusters. (D) To examine cell shape, the x length / y length ratio was measure for 1568 node cells from eight WT (Control) embryo, 1716 node cells from ten *Wnt5a*^{-/-}*Wnt5b*^{-/-} embryos, and 478 node cells from three *Sfrp1*^{-/-}*Sfrp2*^{-/-}*Sfrp5*^{-/-} embryos. Statistical significance was examined by Wilcoxon rank sum test. Although different cell shapes are found in the node, there is no significant differences among WT, *Wnt5a*^{-/-}*Wnt5b*^{-/-} and *Sfrp1*^{-/-}*Sfrp2*^{-/-}*Sfrp5*^{-/-} embryos.

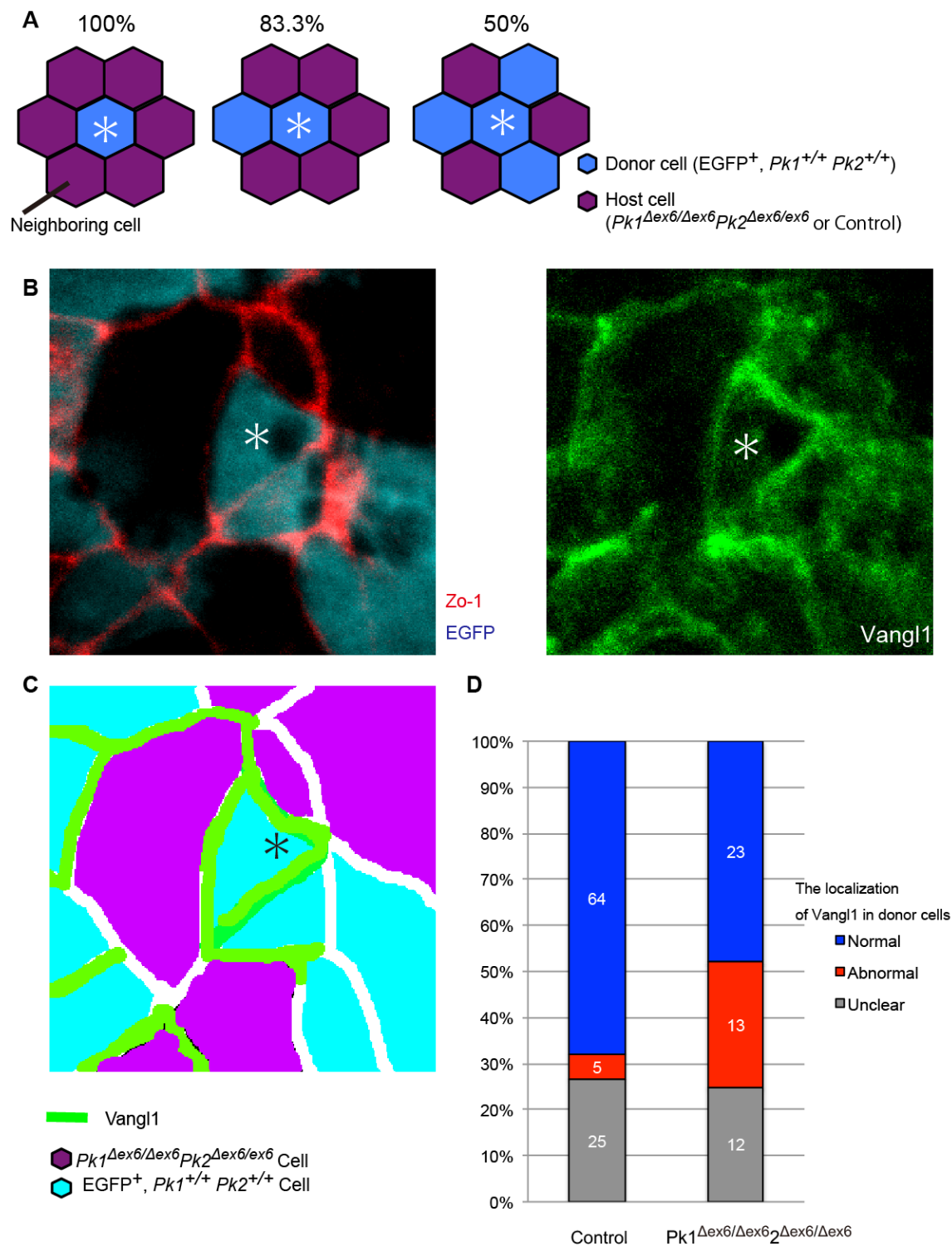


Figure S7. Effects of the absence of Prickle 1 and Prickle 2 on neighboring WT cells, related to Figure 6 and Figure 7.

(A) Chimeric embryos were generated by aggregating EGFP-expressing $Pk1^{+/+} Pk2^{+/+}$ ES cells (donors) with an embryo (host). Host embryo was either $Pk1^{\Delta ex6/\Delta ex6} Pk2^{\Delta ex6/\Delta ex6}$, or Control ($Pk1^{\Delta ex6/+} Pk2^{\Delta ex6/+}$ or $Pk1^{+/+} Pk2^{\Delta ex6/\Delta ex6}$). An illustration of a donor cell (asterisk) surrounded by host cells in a chimeric embryo. Donor cells that were completely (100%) or partially (>50%) surrounded by host cells were subjected to further analysis. (B) The chimeric embryos in which host cells are $Pk1^{\Delta ex6/\Delta ex6} Pk2^{\Delta ex6/\Delta ex6}$ were immunostained for Zo-1 (red), Vangl1 (green), and EGFP (light blue). Note that a donor cell (asterisk) that is surrounded by $Pk1^{\Delta ex6/\Delta ex6} Pk2^{\Delta ex6/\Delta ex6}$ cells exhibits abnormal Vangl1 localization. (C) The Vangl1 localization in (B) is illustrated schematically. (D) The frequency of Vangl1 localization patterns (normal, abnormal or unclear) when donor cells are surrounded by Control cells or by $Pk1^{\Delta ex6/\Delta ex6} Pk2^{\Delta ex6/\Delta ex6}$ cells. Note that the frequency of abnormal Vangl1 localization increased when donor cells were surrounded by $Pk1^{\Delta ex6/\Delta ex6} Pk2^{\Delta ex6/\Delta ex6}$ cells (13/48 vs. 5/94 cells).