Supporting Information

Lu et al. 10.1073/pnas.1106801108

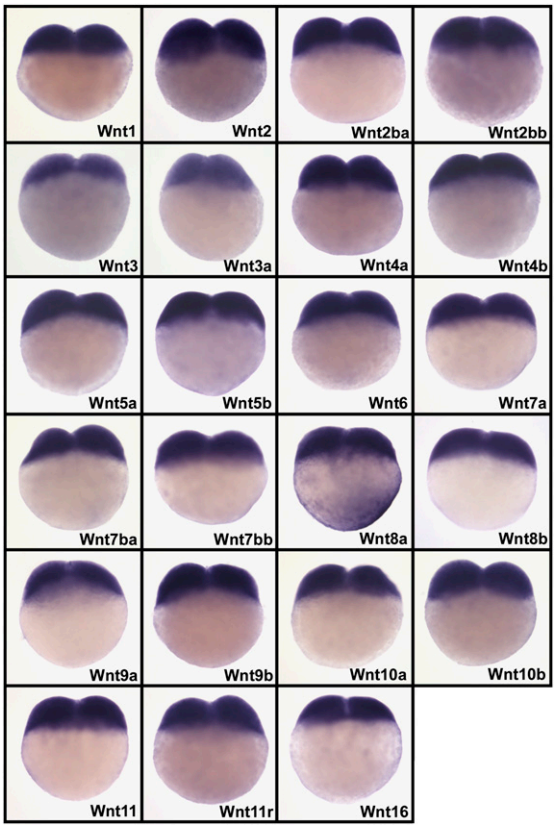

Fig. S1. Analysis of spatial localization of mRNAs coding for 23 zebrafish Wnt genes by whole mount in situ hybridization to identify those present in cortical cytoplasm of two-cell stage embryos. In situ hybridization was performed on wild-type embryos at the two-cell stage with specific antisense RNA probes corresponding to the different Wnts. The labeling reaction was performed for 24 h (10 times longer than usual) to detect possible low amounts of mRNA in the cortical cytoplasm. Due to strong overstaining, nonspecific labeling is observed in blastomeres for all Wnt probes. Only Wnt8a displays accumulation of mRNA in the cortical cytoplasm.

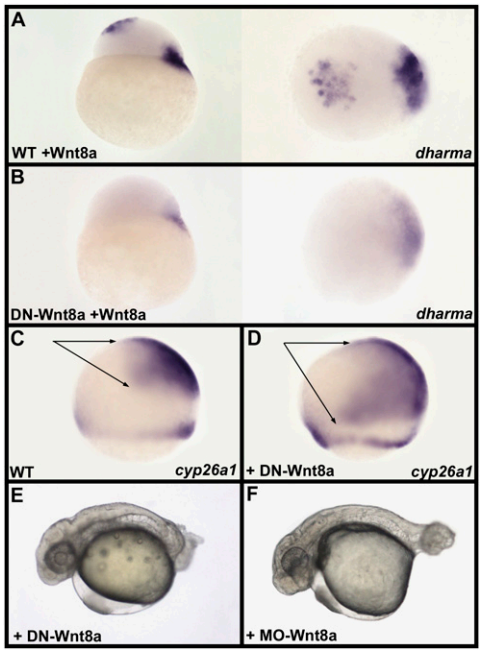

Fig. S2. Antagonistic activity of the dominant-negative form of Wnt8a. (*A*) Injection of 10 pg of Wnt8a mRNA into one animal pole blastomere at the 64-cell stage of a wild-type embryo results in ectopic expression of dharma at the animal pole of a sphere-stage embryo. (*Left*) Lateral view. (*Right*) Animal pole view of the same embryo oriented dorsal to the *Right*. (*B*) Injection of 10 pg of Wnt8a mRNA in one animal pole blastomere at the 64-cell stage in an embryo previously injected at the one-cell stage with 500 pg of DN-Wnt8a mRNA does not result in ectopic expression of dharma. In addition, endogenous dharma expression at the margin is strongly attenuated. The antagonistic effect of DN-Wnt8a on the induction of dharma at the animal pole is higher than the induction of endogenous dharma because the injection of Wnt8a mRNA in animal pole blastomeres is performed 2 h after fertilization and 1.5 h after injection of DN-Wnt8a, whereas endogenous Wnt8a mRNA present in the cortical cytoplasm can be translated earlier. (*Left*) Lateral view. (*Right*) Animal pole view of the same embryo oriented dorsal to the *Right*. (*C* and *D*) Compared with its expression in wild type (WT) (*C*) the forebrain and midbrain expression of cyp26a1 (arrows) is strongly extended posteriorly (*D*) in embryos injected with 500 pg of DN-Wnt8a mRNA at the 8- to 16-cell stage. (*E* and *F*) At 30 hpf, embryo injected with 500 pg of DN-Wnt8a mRNA displays a phenotype (*E*) highly similar to the phenotype of Wnt8a morphant (*F*).

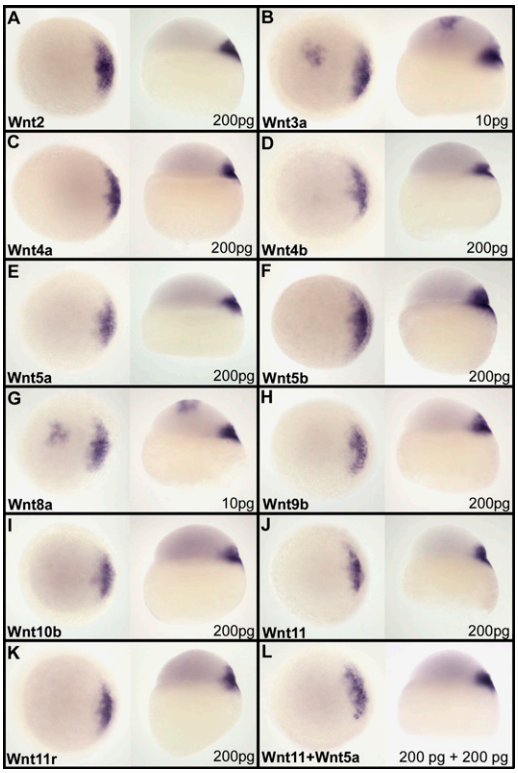

Fig. S3. Assay of activation of the maternal β-catenin signaling pathway by canonical and noncanonical Wnt ligands. mRNA coding for canonical or noncanonical Wnts was injected together with GFP mRNA into one animal pole blastomere at the 64-cell stage. Embryos displaying a fluorescent clone at the animal pole were fixed at the sphere stage and the activation of the maternal β-catenin visualized by looking, by whole mount in situ hybridization, at the induction of ectopic expression of dharma at the animal pole. Wnt3a (*B*) and Wnt8a (*G*) are the only Wnts able to induce ectopic dharma expression. This is observed with as little as 10 pg of mRNA injected. All other Wnt ligands, canonical Wnts [Wnt2 (*A*), Wnt9b (*H*), and Wnt10b (*I*)], noncanonical Wnts [Wnt4a (*C*), Wnt4b (*D*), Wnt5a (*E*), Wnt5b (*F*), Wnt11 (*J*), and Wnt11r (*K*)], or the combination Wnt11–Wnt5a (*L*), which has been described to act as dorsal determinant in amphibian, are unable to efficiently activate the maternal β-catenin signaling pathway and fail to induce ectopic expression of dharma at the animal pole, even for 200 pg of mRNA injected. For each Wnt, assayed embryos are presented in both animal pole view (*Left*) and lateral view (*Right*). The amount of mRNA injected is indicated in the *Lower Right* corner.

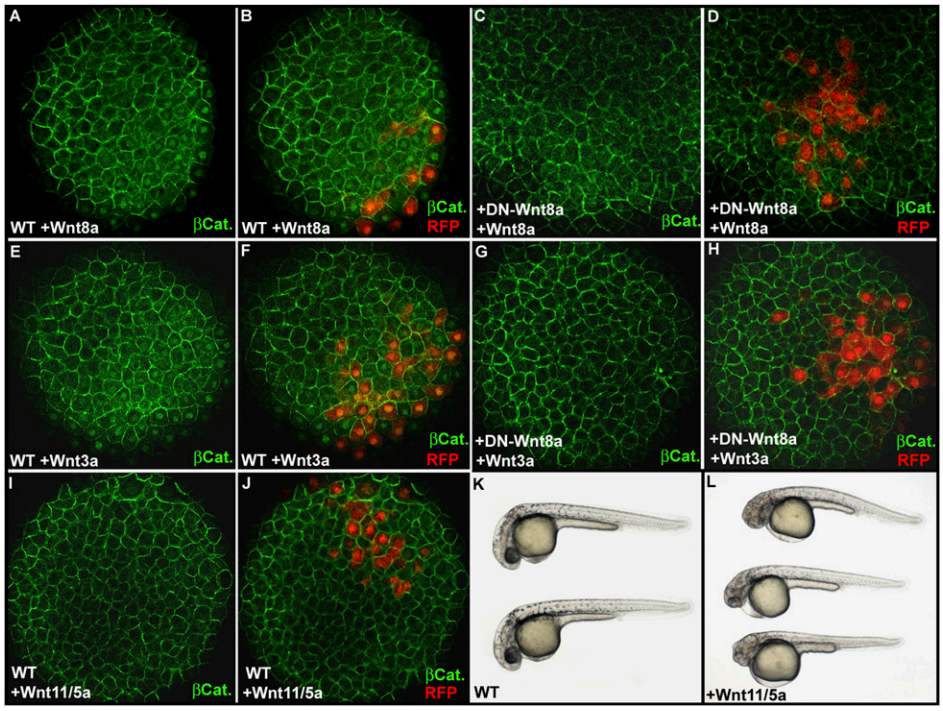

Fig. S4. Effect of ectopic expression of Wnt8a, Wnt3a, and Wnt11/5a on the intracellular localization of β-catenin as revealed by immunofluorescence. Immunofluorescence localization of β-catenin at 3 hpf (high stage) at the animal pole of an embryo injected with 10 pg of Wnt8a (*A*) or Wnt3a (*E*) mRNA together with 50 pg of red fluorescent protein (RFP) mRNA in one animal pole blastomere at the 64-cell stage. (*B* and *F*) Overlay of the green channel, for visualization of β-catenin, and the red channel, for visualization of cells expressing the RFP and secreting Wnt8a or Wnt3a, reveals the activation of the maternal β-catenin signaling pathway (accumulation of β-catenin into the nucleus) at a distance up to three cell diameters from cells secreting Wnt8a (*B*) or Wnt3a (*F*). Injection of 500 pg DN-Wnt8a mRNA at the one-cell stage prevents stimulation of the maternal β-catenin signaling pathway by Wnt8a (*C* and *D*) or Wnt3a (*G* and *H*). (*I* and *J*) In the same experimental conditions, injection of a mix of 200 pg of Wnt11 mRNA and 200 pg of Wnt5a mRNA fails to activate the maternal β-catenin signaling pathway and does not induce nuclear accumulation of β-catenin in animal pole blastomeres within or at the vicinity of the Wnt11/Wnt5a secreting clone. (*L*) Injection of the same mix of 200 pg of Wnt11 mRNA and 200 pg of Wnt5a mRNA at the one-cell stage induces cyclopia phenotypes characteristic of overexpression of noncanonical Wnts, demonstrating that translation from these mRNAs produces active Wnt proteins. (*K*) Wild-type embryo. Embryos in *A*–*J* are in animal pole view. Embryos in *K* and *L* are at 30 hpf and are in lateral view anterior to the *Left* and dorsal to the *Top*.

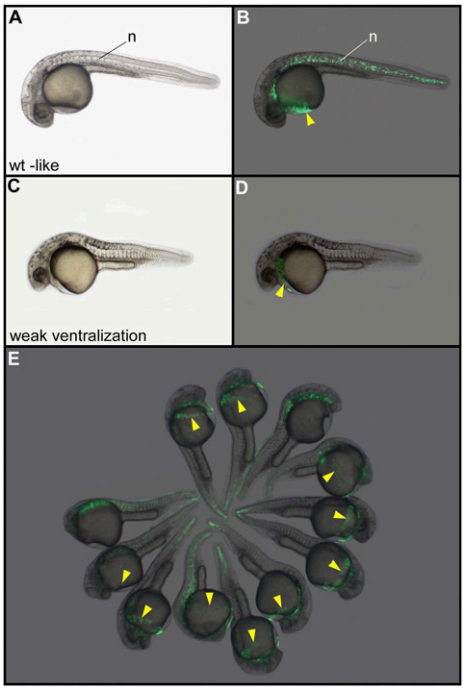

Fig. S5. Phenotype and localization of the injected clone of cells in nocodazole-treated embryos injected with Wnt8a and GFP mRNAs. After treatment with nocodazole and injection of 25 pg of Wnt8a and 100 pg of GFP mRNAs into one blastomere at the 64-cell stage, embryos displaying a wild-type–like phenotype (*A* and *B*) always express the GFP in axial structures including the hatching gland (yellow arrowhead). Embryos displaying a weak ventralization phenotype (*C* and *D*) lack the notochord but always develop a hatching gland expressing GFP. (*E*) Field of embryos showing that the hatching gland is always derived from the injected blastomere. The two embryos lacking the hatching gland correspond to the class of strong ventralization phenotypes for which this tissue is not formed. (*A*, *C*, and *E*) Bright field. (*B*, *D*, and *F*) Localization of GFP fluorescent cells. n, notochord. Embryos are 30 h old and in lateral view.

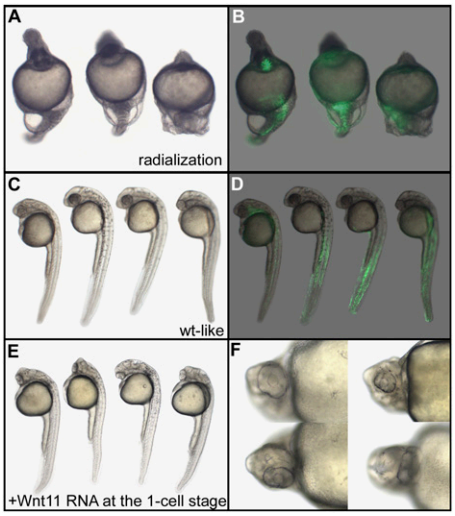

Fig. S6. Phenotype and localization of the injected clone of cells in nocodazole-treated embryos injected with Wnt11 and GFP mRNAs. Embryos displaying a radialized phenotype after treatment with nocodazole and injection of 400 pg of Wnt11 and 100 pg of GFP mRNAs (*A* and *B*) demonstrate that Wnt11 is unable to rescue the lack of stimulation of blastomeres by dorsal determinants. Embryos displaying a wild-type–like phenotype (*C* and *D*) express the GFP in lateral and ventral (muscle and blood) but not in axial tissues (notochord and hatching gland). (*E* and *F*) Embryos injected with 400 pg of Wnt11 mRNA at the one-cell stage display cyclopia, demonstrating that the Wnt11 mRNA used is fully active. Embryos are 30 h old, anterior to the *Top* in lateral view (*A*–*E*), and seen in high magnification in ventral view (*F*).

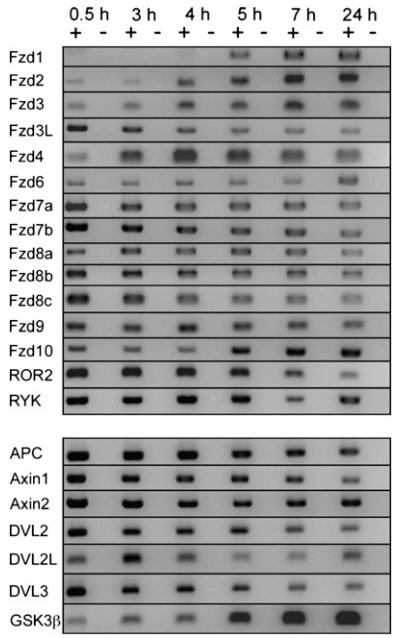

Fig. S7. RT-PCR analysis of the expression of Wnt receptors and downstream components of the canonical Wnt signaling pathway. Expression of the Wnt receptors (Fzd1, Fzd2, Fzd3, Fzd3L, Fzd4, Fzd6, Fzd7a, Fzd7b, Fzd8a, Fzd8b, Fzd9, Fzd10, ROR2, RYK) and of downstream components (APC, Axin1, Axin2, DVL2, DVL2L, DVL3, GSK3b) analyzed by RT-PCR at one- to two-cell stage (0.5 h), 1,000-cell stage (3 h), sphere stage (4 h), 40% epiboly (5 h), 60% epiboly (7 h), and at 24 hpf.

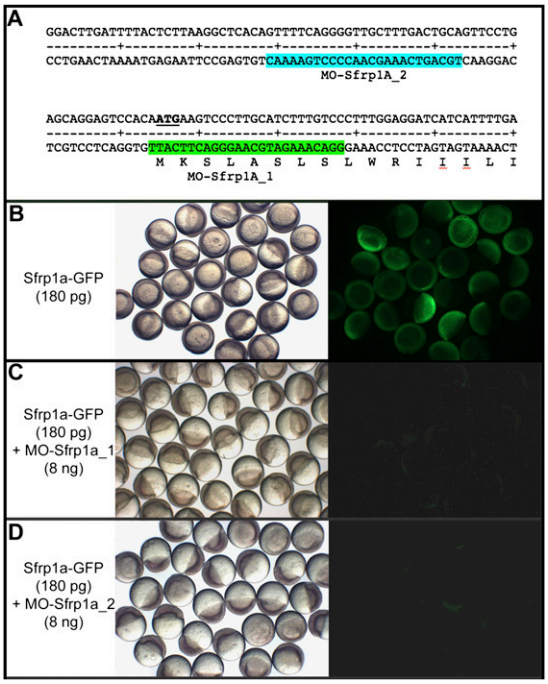

Fig. S8. Specificity of Sfrp1a morpholinos. (*A*) Position of antisense morpholinos designed to block Sfrp1a translation on the sequence of 5′-UTR and the 5′ part of sfrp1a ORF. Injection of 180 pg of Sfrp1a-GFP mRNA at the one-cell stage results in embryos expressing the GFP at the late blastula stage (*B*). Coinjection of Sfrp1a-GFP mRNA with 8 ng of morpholino Sfrp1a_1 (*C*) or of Sfrp1a_2 (*D*) prevents translation of the Sfrp1a-GFP fusion protein. (*Left*) Bright field. (*Right*) Visualization of GFP fluorescence.

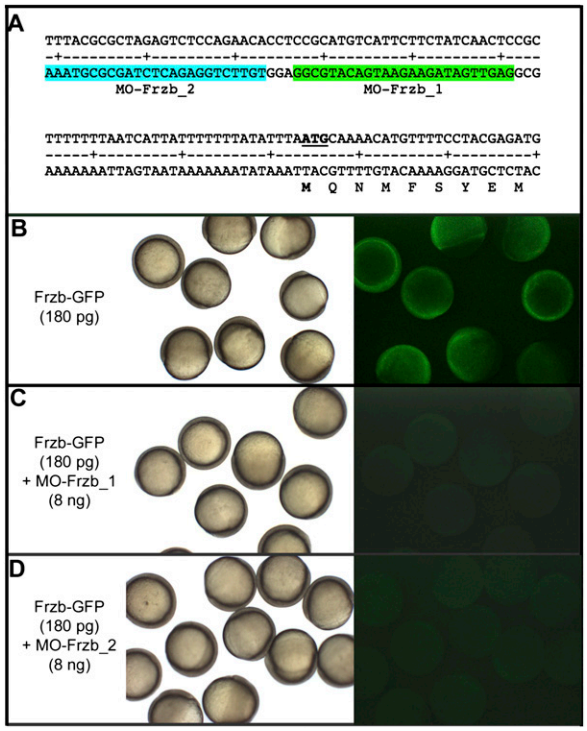

Fig. S9. Specificity of Frzb morpholinos. (*A*) Position of antisense morpholinos designed to block Frzb translation on the sequence of 5′-UTR and the 5′ part of Frzb ORF. Injection of 180 pg of Frzb-GFP mRNA at the one-cell stage results in (*B*) embryos expressing GFP at the late blastula stage. Coinjection of Frzb-GFP mRNAs with 8 ng of morpholino Frzb_1 (*C*) or Frzb_2 (*D*) prevents translation of the Frzb-GFP fusion protein. (*Left*) Bright field. (*Right*) Visualization of GFP fluorescence.

Table S1. Sequences of the oligonucleotides used for analysis of expression of Wnts and Wnt antagonists

Wnts		Primer sequence (5′–3′)	Size, bp
Wnt1	Forward	CTCGGGCAATCTCCTCACTAACT	589
	Reverse	GCTGGGAAGTCTCATCCAACAAG	
Wnt2	Forward	AAACTGGATTGGCGAATGTCAGC	762
	Reverse	CACTTGGTTGTGCGACTCACTCTT	
Wnt2ba	Forward	CCGTGAAGCAGCATTTGTCTATG	669
	Reverse	AACTTGCACTCGCATTTGGTCAT	
Wnt2bb	Forward	GAGTGCCAGTATCAGTTTCGTCAT	539
	Reverse	GTAAATCCTGTCCCGTCTTGGTT	
Wnt3	Forward	AGTAGCATTTGCAGTAACCCGTTCA	384
	Reverse	CGGTGTTTCTCCACCACCATCTC	
Wnt3a	Forward	TATCTTGGATATTTCCTGTTCCTTTT	283
	Reverse	CCAGCTTGTCGTTGATAGTAGTGC	
Wnt4a	Forward	GGACAGTCATCCAACAGAGCCCTAA	514
	Reverse	GCATCTCCACCATCTTGCGACAC	
Wnt4b	Forward	ACTCCTCCTGCTGTTGCTATGGG	320
	Reverse	CATGAACAAAAGCTGCTTCACGC	
Wnt5a	Forward	GTCAGCATTCGCCTTCGCCATCA	447
	Reverse	TTCCCACGCCCGTTCATCCTCAT	
Wnt5b	Forward	TCAAAGAGTGCCAGTATCAGTTCAGA	410
	Reverse	GGCCACATTCGCTAGATTATACACC	
Wnt6	Forward	AATGTCAGTACCAGTTTCGCTTTCG	342
	Reverse	GTATGTCACTCTTGCCCTTCCTCC	
Wnt7a	Forward	GGGGATTATTTATTTGAAGATTGG	461
	Reverse	GTGCATCTAGGAAGACCTTTGAG	
Wnt7ba	Forward	GCATCAACGAGTGCCAGTATCAG	300
	Reverse	CATCCACGAAGCGTCTGGAGAAC	
Wnt7bb	Forward	GAGTTCTCACGGAAGTTTGTGGATG	231
	Reverse	TTGAACCGCTTTGTTGTACTTGTCC	
Wnt8a	Forward	CGCATCACTTCCTCGAATCAGTA	684
	Reverse	TTCAGGTAATTGCCAATCTCACG	
Wnt8b	Forward	AGGTTTATTATTACGCTTTCATCTTG	228
	Reverse	TCTTGCCCTTCCTCAAACACTCG	
Wnt9a	Forward	ACTGACCTCACAGCCAGACGAAA	335
	Reverse	GCTGGGCGAGTCCTCGATGTGAA	
Wnt9b	Forward	GCCCAGATTGACGCACATAACAT	638
	Reverse	ACAACCTCCAAACCCTTCCAAAT	
Wnt10a	Forward	ATGCCGTGTCTAATGCCTGTGCC	648
	Reverse	TGTCCATTCCCTGGCTGGTCTTG	
Wnt10b	Forward	GCAGGGCATTCAGGTTGCTATTC	248
	Reverse	TATCGTCATCCAGGCGTCGTTTA	
Wnt11	Forward	TCTAAACAGCAAAAGAGCGACAT	297
	Reverse	GGACGAGCAGTTCCAGCGCATAT	
Wnt11r	Forward	GGGACGGAAAGACGGAATAAGTG	615
	Reverse	TTGGAGCCCATGAGGAGACCATA	
Wnt16	Forward	GACCGGCATGAATCGTCTTTGTC	264
	Reverse	ATGGCGTTGCTCTTTATCCTTGC	
β-Actin	Forward	GCCTTCCTTCCTGGGTATGG	251
	Reverse	CCAAGATGGAGCCACCGAT	
SFRP1a	Forward	GCGACCCAGGCACTCAGTTGTTG	613
	Reverse	TAGGCAGGGCACTTGTGGCTTTT	
SFRP1b	Forward	CTCCATCGCTCCGTTTCTTTGTC	646
	Reverse	TTTCATACTTCCAGCCCTCACCA	
SFRP2	Forward	ACATGCGTCTGCCTAACCTCCTC	672
	Reverse	GACCCTTCTGCCAGCGTTTCACC	
SFRP2L	Forward	TGGTGCTGTGCTTTGGCATCGGTTAC	524
	Reverse	TCGACTGCGGCCCTCTGGGAATA	
SFRP5	Forward	CTGGCGCTGGTTCTACTCACTTC	534
	Reverse	CATTATGGTGTCGGCTTTCAACT	
DKK1a	Forward	CTCGCGTGGAGTTTGTCTGTCGT	613
	Reverse	AGTCCTTTCAATGGCGCATCCTT	
DKK1b	Forward	TTCTACGATGCTCAACTCCAACGCTAT	546
	Reverse	ATGGGCGATGGACACGAACTCTT	

Table S1. Cont.

Wnts		Primer sequence (5′–3′)	Size, bp
DKK2	Forward	TACGAGGAGTCGGTGCTGTTGGA	699
	Reverse	AGCCTGGATCTGGAGAATGAGGTG	
DKK3	Forward	TGATGAAGACTGCGGAGATGGAA	489
	Reverse	AAGTCGTTTAGGTACTTTGGTGC	
Frzb	Forward	TCTCACCTGAGGCAATAGTCAAAGC	386
	Reverse	ACCCATGATGATGTACTCGTCGTT	
WIF1	Forward	AAACAGAAACACCGGCCAGTGCG	410
	Reverse	TCCCAAAAGAGGAACATTGACAGTAGG	

Table S2. Sequences of oligonucleotides used for analysis of expression of Wnt receptors and intracellular components of canonical Wnt/β-catenin

Name		Primer sequence (5′–3′)	Size, bp
ROR2	Forward	GTCACGAGGAGTTGGAGTT	747
	Reverse	TATTGCCTAGTCGCACATAC	
RYK	Forward	GTGGCTTGTCTTCCGACTT	922
	Reverse	GGCTCTGGCTGCTCTATTC	
Fz1	Forward	ATCGCCTACATCGCCGGATTTCT	451
	Reverse	TCCTGATGCGGAACAACGACACG	
Fz2	Forward	GGCTCGCTTTGCTCTGTCGTTTG	1237
	Reverse	CGTCCACTTTATTCACTGTAACCA	
Fz3	Forward	CACAAGGCGCTCGTATTGTTAGA	422
	Reverse	TGAAGGTGGTATTGTAGGCAAGG	
Fz3L	Forward	GATGTGGATTCTTTCGGTGGTTTC	740
	Reverse	TGTAGCAGACGGCGTAGAAGATGA	
Fz4	Forward	AGACCGATCATTTTCCTCAGCAT	219
	Reverse	GAGCGTTAAGATCACCCACCAGA	
Fz6	Forward	CGCAGTAGCAGACGGAGATGAAGACG	961
	Reverse	CTCCAGCAGATTGTTTCGGTTCG	
Fz7a	Forward	CGAGTTCAATCTGGTGGGTCATT	659
	Reverse	GTTGCTGTTGCTGAGCCGTTTAT	
Fz7b	Forward	CCAGATCGGATTTGAGGAATAGC	676
	Reverse	AAGGGACTGTGAGTTGAAGAGGG	
Fz8a	Forward	GGCGTCGGACCCAATAAGAATAA	664
	Reverse	TCTGGTTGCCAACGTAGCAGATC	
Fz8b	Forward	AAAACAACAAGCCGACCAGGGAA	615
	Reverse	GAGCCAAGCAGCCATGTGGAAAT	
Fz8c	Forward	ACCCTGTGGCTGGGATCTGTTAC	366
	Reverse	ATTTGAGCATGAAGACGGCGTAG	
Fz9	Forward	TTCATCCTGACTGGCTTTGTGGC	433
	Reverse	TGTAGTGGCAGTGACTGGTGCTG	
Fz10	Forward	GACACCGTGCCTTCCGTTTGGTC	741
	Reverse	GCCTTGCCTGCTCGCACATCACT	
APC	Forward	AGCACTTTAACCTACCGAAGC	611
	Reverse	ATGTAAGGCGAGCGAACAC	
Axin1	Forward	AGCGATGGACGGCAATACA	1106
	Reverse	TCTCGTTCCCTCAACACCC	
Axin2	Forward	GGAAGAGGGTGAGACGACA	1184
	Reverse	AATGGGACGCACTGCTACT	
DVL2	Forward	TGAACAAGCCGAACTACAA	696
	Reverse	CGATACTGATGCCCAAGAA	
DVL2L	Forward	GCTTGCTGAAACACGGCTAT	443
	Reverse	GGCTGAACGCTCAACCCTC	
DVL3	Forward	CATAGGTTCGGATGTGGTG	775
	Reverse	AATCGGTTGCTCTAGTTGG	
GSK3b	Forward	AGATAAAGATGGCAGTAAAGTG	257
	Reverse	AGAAGTAACGCAGACGAAC	