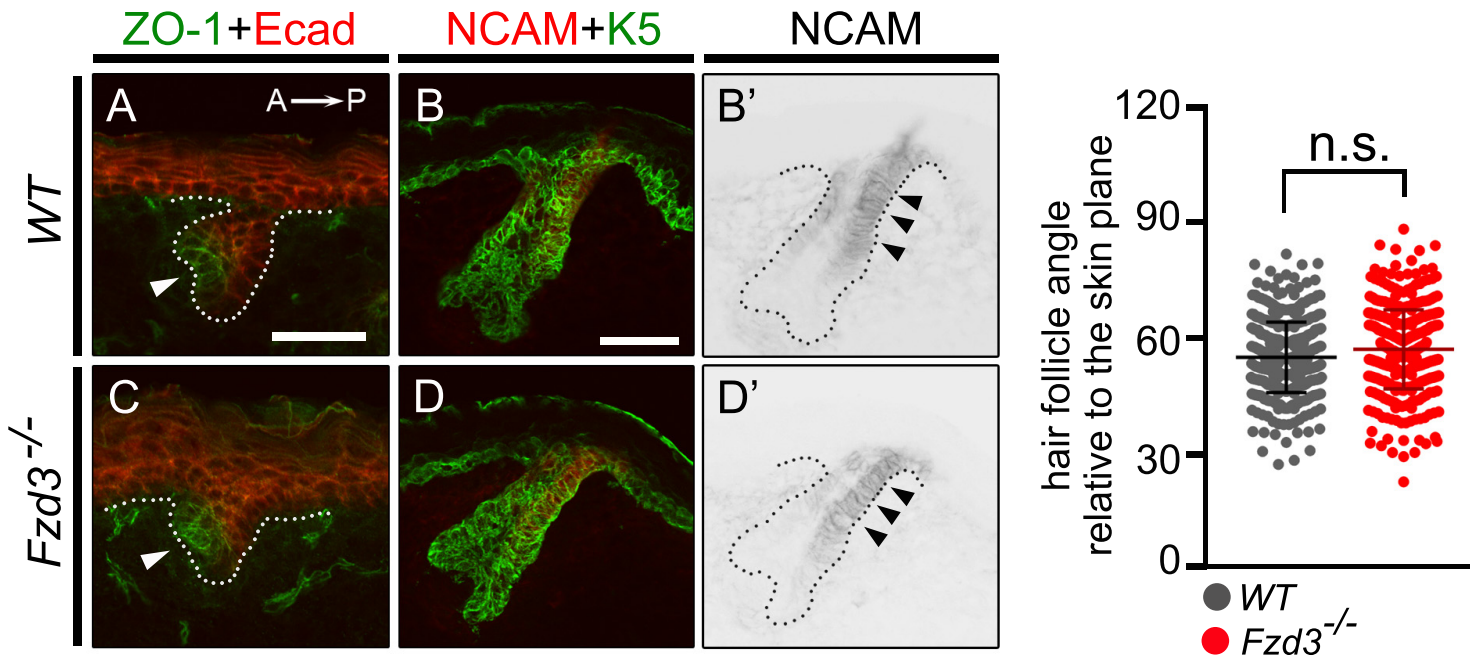

Figure S1. Loss of *Fzd3* does not affect hair follicle polarity.

Left, sagittal sections of E17.5 back skins stained with an anterior marker ZO-1 or posterior marker NCAM. E-cadherin and K5 antibodies were used to highlight skin epithelia and hair follicles. Anterior is to the left, and posterior is to the right. Scale bar, 50 μm. Dotted lines outline the hair follicles.

Right, hair follicle angles to the plane of the skin were compared using the Student's t-test. *WT*, n = 456 hair follicles; *Fzd3*$^{-/-}$, n = 408 hair follicles (n.s., not significant).

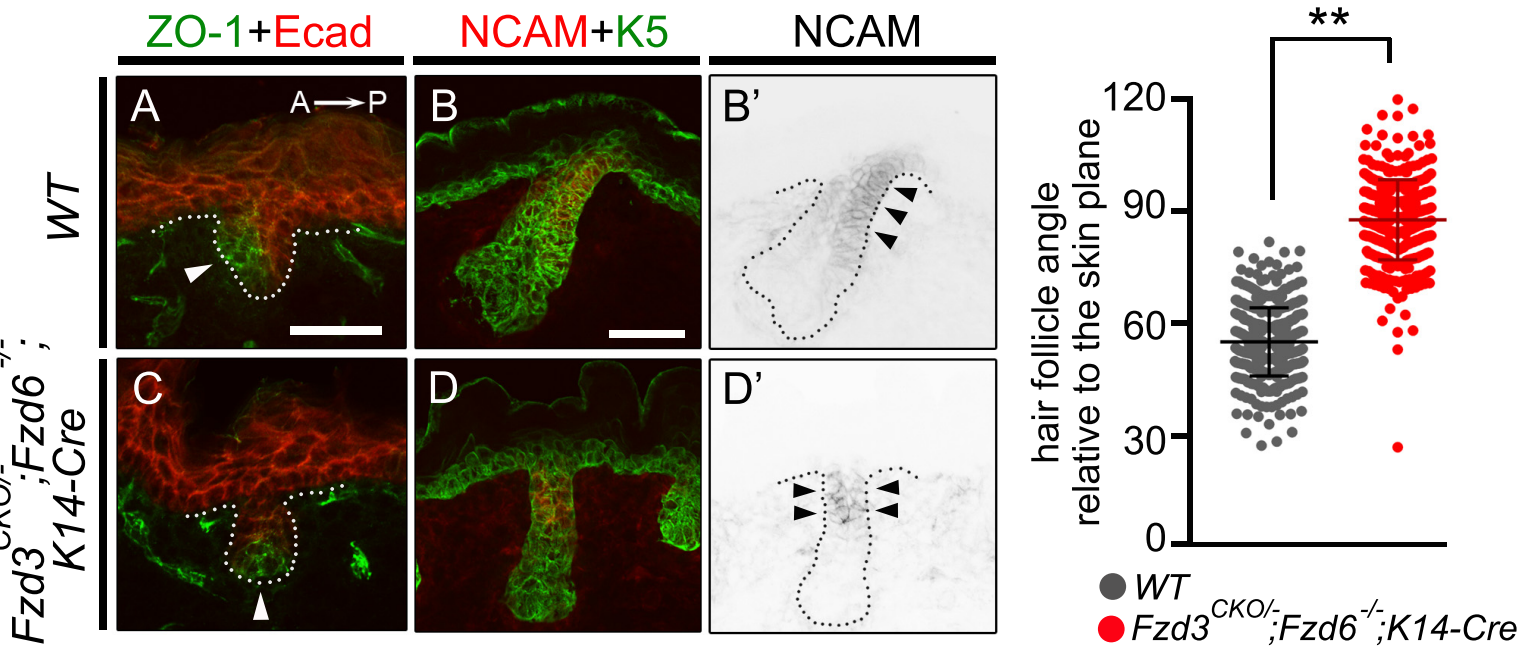

Figure S2. Conditional knockout of *Fzd3* using K14-Cre on *Fzd6*$^{-/-}$ background recapitulates the skin phenotype of *Fzd3*$^{-/-}$;*Fzd6*$^{-/-}$ mice.

Left, sagittal sections of E17.5 back skins stained with an anterior marker ZO-1 or posterior marker NCAM. E-cadherin and K5 antibodies were used to highlight skin epithelia and hair follicles. In *Fzd3*$^{CKO/-}$;*Fzd6*$^{-/-}$;*K14-Cre* skin, hair follicles are perpendicular to the skin surface, and the asymmetric localization of ZO-1 and NCAM is lost (similar to *Fzd3*$^{-/-}$;*Fzd6*$^{-/-}$ mice). Anterior is to the left, and posterior is to the right. Scale bar, 50 µm. Dotted lines outline the hair follicles.

Right, hair follicle angles to the plane of the skin were compared using the Student's t-test. *WT*, n = 456 hair follicles; *Fzd3*$^{CKO/-}$;*Fzd6*$^{-/-}$;*K14-Cre*, n = 431 hair follicles (**, $P < 0.01$).

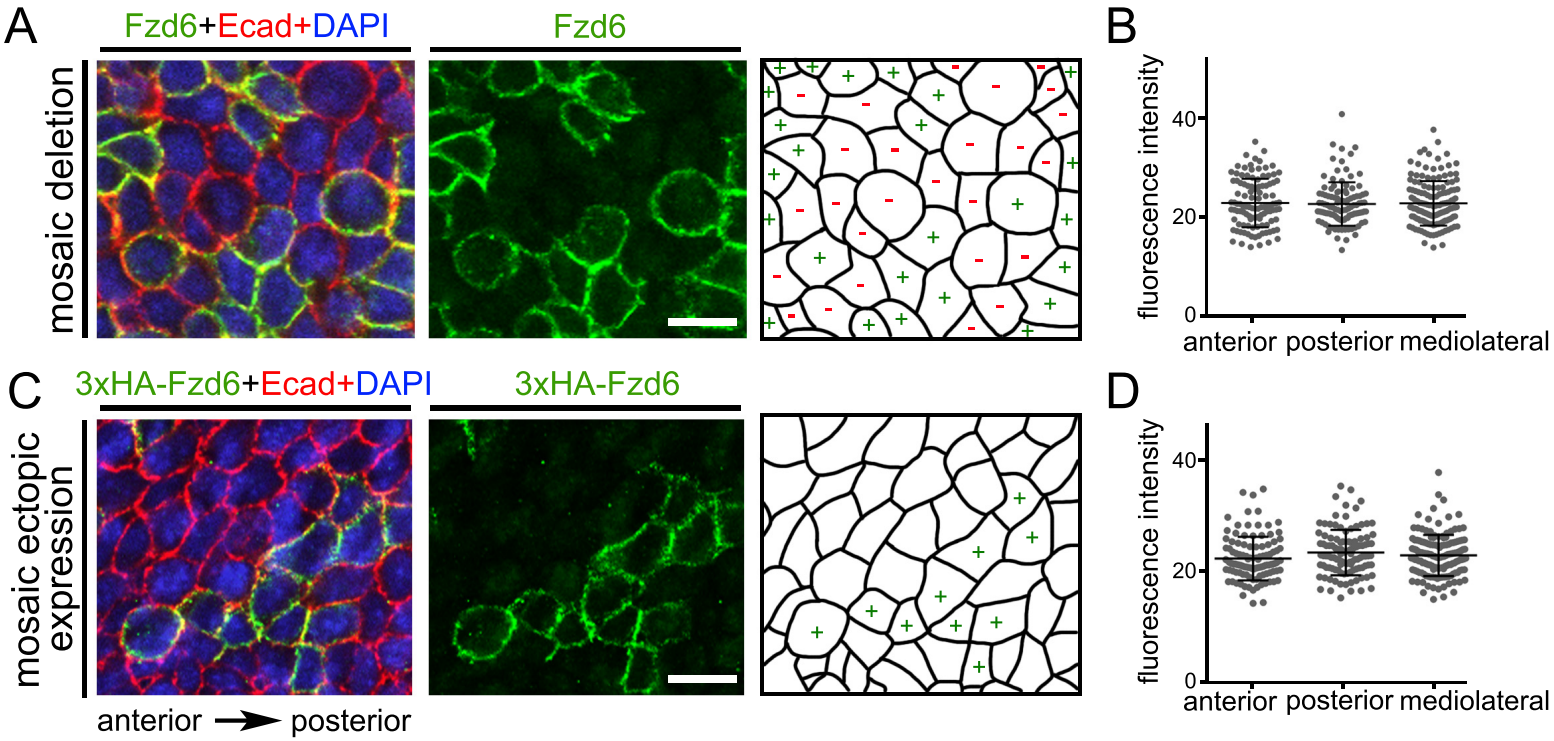

Figure S3. Localization of Fzd6 in individual skin epithelial cells at E16.5 by genetic mosaic labeling.

(A) Mosaic expression of Fzd6 was induced in *Fzd6*$^{CKO/-}$*;CAGG-CreER*^{TM} embryos treated with 4-HT at E10.5. E16.5 back skins were collected and stained with Fzd6 (green) and E-cadherin (red) antibodies. The right panel shows the schematic of the *Fzd6* (+) versus *Fzd6* (-) cells.

(B) Quantification of fluorescence intensity on anterior, posterior, and mediolateral sides of the cells at the borders of *Fzd6* (+) and *Fzd6* (-) clones.

(C) Mosaic expression of 3xHA-tagged Fzd6 on a *WT* background was induced in *Rosa26-LSL-Fzd6;CAGG-CreER*^{TM} embryos treated with 4-HT at E10.5. E16.5 back skins were collected and stained with 3xHA (green) and E-cadherin (red) antibodies. The right panel shows the schematic of the *3xHA-Fzd6* (+) versus *WT* cells.

(D) Quantification of fluorescence intensity on anterior, posterior, and mediolateral sides of the cells at the borders of *3xHA-Fzd6* (+) and *WT* clones.

Anterior is to the left and posterior to the right. Scale bar, 10 μm.

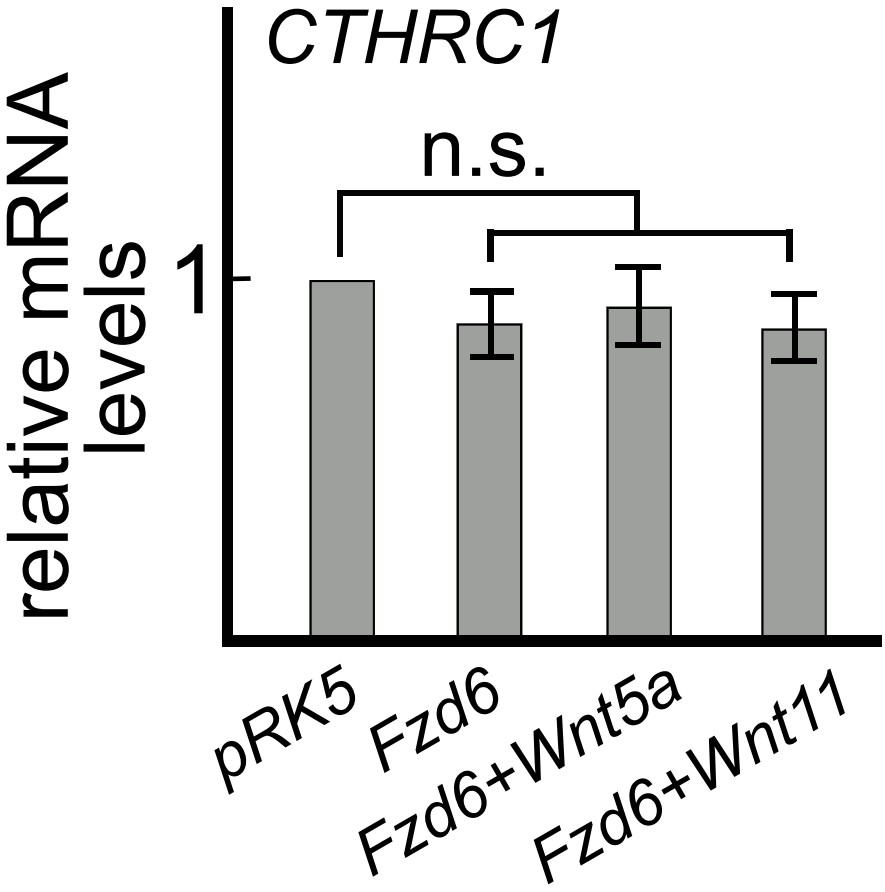

Figure S4. Transient expression of Fzd6 is not sufficient to drive *CTHRC1* expression *in vitro*. qRT-PCR shows no significant change in *CTHRC1* mRNA level in HEK293T cells transiently transfected with Fzd6, with or without the presence of recombinant Wnt5a or Wnt11 protein (100 ng/ml). All data represent mean ± SEM of three biological replicates. *GAPDH* was used as a control. Quantification of data was compared using ANOVA followed by Dunnett's test (n.s., not significant).

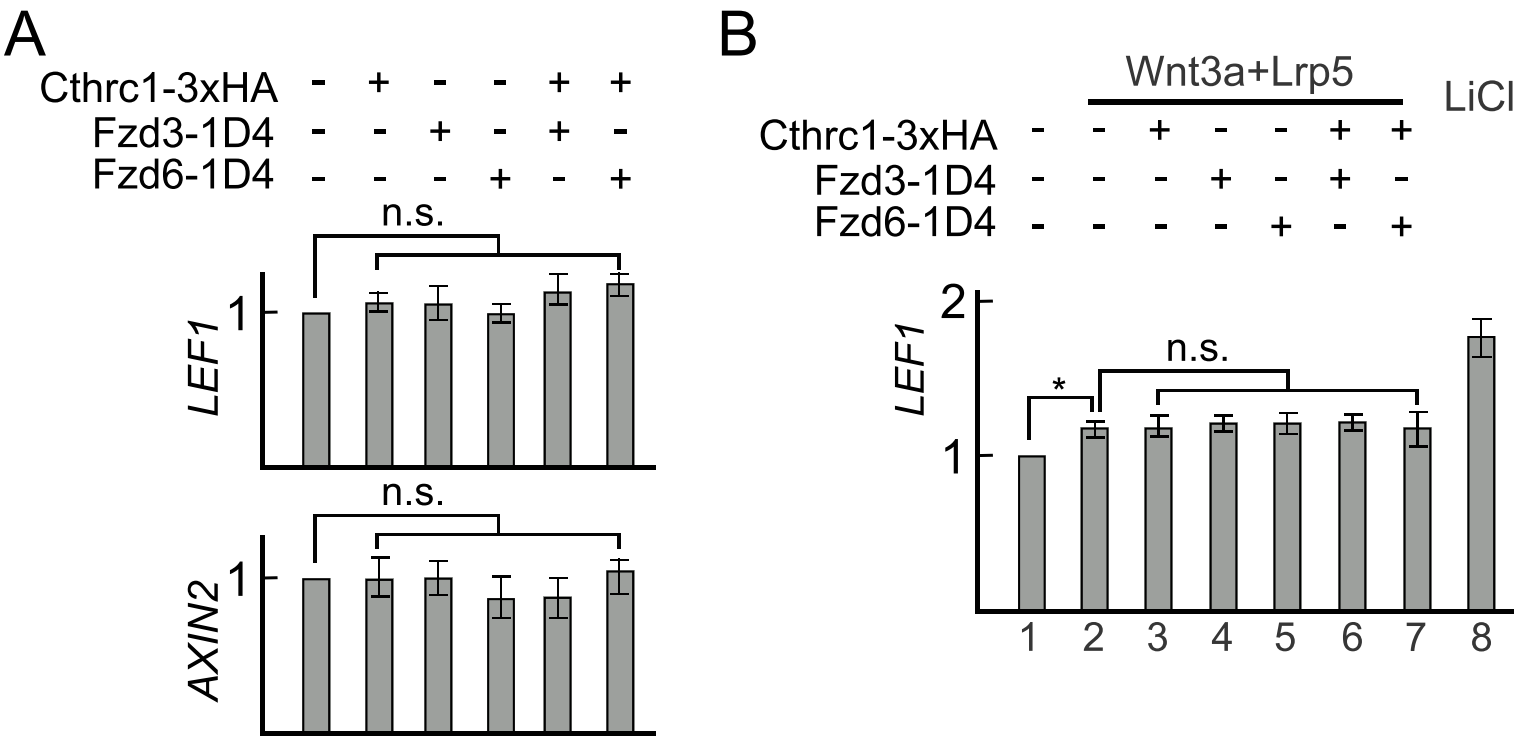

Figure S5. Cthrc1-Fzd3/6 does not affect canonical Wnt signaling pathway *in vitro*.

(A) Transient expression of Fzd3/6 only, Cthrc1 only, or both Fzd3/6 and Cthrc1 does not affect the basal level of canonical Wnt signaling in HEK293T cells, as qRT-PCR shows no significant changes in mRNA level of known canonical Wnt signaling targets (*LEF1* and *AXIN2*).

(B) Recombinant Wnt3a together with the transient transfection of Lrp5 induces a significant increase of *LEF1* expression in HEK293T cells (17%, column 2 vs. 1), as compared to the 20 mM LiCl treatment (76%, column 8 vs. 1). Transient expression of Fzd3/6 only, Cthrc1 only, or both Fzd3/6 and Cthrc1 does not affect the *LEF1* expression induced by Wnt3a+Lrp5. The data represent mean ± SEM of three biological replicates. *GAPDH* as a control. Quantification of data was compared using ANOVA followed by Dunnett's or Tukey's test (*, $P < 0.05$; n.s., not significant).

Table S1. Primers used for RT-PCR and quantitative real-time RT-PCR

Gene Symbol	Forward Primer	Reverse Primer
mFzd3	5′-ATGGCTGTGAGCTGGATTGTC-3′	5′-GGCACATCCTCAAGGTTATAGGT-3′
mFzd6	5′-ATGGAAAGGTCCCCGTTTCTG-3′	5′-GGGAAGAACGTCATGTTGTAAGT-3′
mSdc2	5′-TGTGTCCGCAGAGACGAGAA-3′	5′-GGAATCAGTTGGGATGTTGTCA-3′
mH21Rik	5′-TTTCAGCATTCCGGTGTCTTT-3′	5′-GGTGGGTAGTAGGGTGGAGAATA-3′
mGlipr2	5′-ATGGGCAAATCAGCTTCCAAA-3′	5′-GCTTCCCGGTTGAGCTTCTT-3′
mCthrc1	5′-CAGTTGTCCGCACCGATCA-3′	5′-GGTCCTTGTAGACACATTCCATT-3′
mSerhl	5′-ATGGGTTTGCACTCAGAGTTG-3′	5′-GCCAGCCGTGTAAGCAGAG-3′
mKrt13	5′-TCATCTCGGTTTGTCACTGGA-3′	5′-TGATCTTCTCGTTGCCAGAGAG-3′
mPsca	5′-GGACCAGCACAGTTGCTTTAC-3′	5′-GTAGTTCTCCGAGTCATCCTCA-3′
mGAPDH	5′-AGGTCGGTGTGAACGGATTTG-3′	5′-TGTAGACCATGTAGTTGAGGTCA-3′
hCTHRC1	5′-CAATGGCATTCCGGGTACAC-3′	5′-GTACACTCCGCAATTTTCCCAA-3′
hLEF1	5′-TGCCAAATATGAATAACGACCCA-3′	5′-GAGAAAAGTGCTCGTCACTGT-3′
hAXIN2	5′-TACACTCCTTATTGGGCGATCA-3′	5′-TTGGCTACTCGTAAAGTTTTGGT-3′
hGAPDH	5′-CTGGGCTACACTGAGCACC-3′	5′-AAGTGGTCGTTGAGGGCAATG-3′
hWNT1	5′-TTCAGACACGAGAGATGGAACT-3′	5′-CCAGCCTTCACTTGCTGAG-3′
hWNT2	5′-CCGAGGTCAACTCTTCATGGT-3′	5′-CCTGGCACATTATCGCACAT-3′
hWNT2B	5′-GGGGCACGAGTGATCTGTG-3′	5′-GCATGATGTCTGGGTAACGCT-3′
hWNT3	5′-CTCGCTGGCTACCCAATTTG-3′	5′-AGGCTGTCATCTATGGTGGTG-3′
hWNT3A	5′-AGCTACCCGATCTGGTGGTC-3′	5′-CAAACTCGATGTCCTCGCTAC-3′
hWNT4	5′-AGGAGGAGACGTGCGAGAAA-3′	5′-CGAGTCCATGACTTCCAGGT-3′
hWNT5A	5′-ATTCTTGGTGGTCGCTAGGTA-3′	5′-CGCCTTCTCCGATGTACTGC-3′
hWNT5B	5′-GCTTCTGACAGACGCCAACT-3′	5′-CACCGATGATAAACATCTCGGG-3′
hWNT6	5′-GGCAGCCCCTTGGTTATGG-3′	5′-CTCAGCCTGGCACAACTCG-3′
hWNT7A	5′-CTGTGGCTGCGACAAAGAGAA-3′	5′-GCCGTGGCACTTACATTCC-3′
hWNT7B	5′-CACAGAAACTTTCGCAAGTGG-3′	5′-GTACTGGCACTCGTTGATGC-3′
hWNT8A	5′-GAACCTGTTTATGCTCTGGGC-3′	5′-CAGCGTTCCCAAGCAAACTG-3′
hWNT8B	5′-CCGACACCTTTCGCTCCATC-3′	5′-CAGCCCTAGCGTTTTGTTCTC-3′
hWNT9A	5′-AGCAGCAAGTTCGTCAAGGAA-3′	5′-CCTTCACACCCACGAGGTTG-3′
hWNT9B	5′-TGTGCGGTGACAACCTCAAG-3′	5′-ACAGGAGCCTGATACGCCAT-3′
hWNT10A	5′-GGTCAGCACCCAATGACATTC-3′	5′-TGGATGGCGATCTGGATGC-3′
hWNT10B	5′-CATCCAGGCACGAATGCGA-3′	5′-CGGTTGTGGGTATCAATGAAGA-3′
hWNT11	5′-GGAGTCGGCCTTCGTGTATG-3′	5′-GCCCGTAGCTGAGGTTGTC-3′
hWNT16	5′-TTCAGACACGAGAGATGGAACT-3′	5′-CCAGCCTTCACTTGCTGAG-3′