SUPPLEMENTARY MATERIALS AND METHODS

Animals

All embryos and fish were raised and cared for using established protocols, as described (Westerfield, 1993) and under IACUC policies. Zebrafish (danio rerio) were maintained at 28.5°C and staged as described (Kimmel et al., 1995). Mutant lines (gpc4 hi1688Tg/+, Wnt5b hi2735btg/+ ) were obtained from the Zebrafish International Resource Center and homozygous embryos were obtained by inbreeding of heterozygous adults. wls c186/+ mutants were provided by the Halpern Laboratory, Baltimore, MD, USA (Kuan et al., 2015). The Zebrabow-M transgenic line was a generous gift from the Schier Lab, Cambridge, MA, USA (Pan et al., 2013).

Quantitative PCR (qPCR)

The qPCR was performed using a Step One Plus Real-Time PCR System (Applied Biosystems).

RNA was isolated from whole embryos using Trizol and 1µg total RNA was reverse transcribed with iScript® (Bio-Rad) for quantitative PCR (qPCR). qPCR reactions were amplified on a Step One Plus Real-Time PCR System (Applied Biosystems). Elongation factor 1a (eef1a) (Forward: 5’-GACCAGCAAAATACTACGTCA; Reverse: 5’-GGTGAGTTTGAGGCTGGTAT) was used for internal controls. Frzb primers used were: Forward: 5’-CAAGACCCTGCGAAATGTAACC and Reverse: 5’-CTTTGACCTCCACAATGGCG). All primers annealed at 60°C and are listed below.
Proliferation and TUNEL assays

Chondrocyte proliferation was detected using the Click-IT EdU Alexa Fluor 488 Imaging assay (Thermo Fisher Scientific) following manufacturer instructions. Cell death was assayed with the Click-IT TUNEL Alexa Fluor 488 Imaging assay (Thermo Fisher Scientific) in a Tg(sox10:mCherry) background.

Lineage analysis and time-lapse confocal imaging

Stained whole-mount embryos were imaged using Nikon AZ100 or 80i compound microscope and processed with NIS-Element software. Embryos were Live imaged on Nikon Ai scanning confocal microscope, on embryos mounted in 3% methylcellulose containing 0.013% tricaine. Tg(sox10:Kaede) embryos wildtype and mutants were photoconverted using the UV laser (404 nm) until the green kaede fluorescence disappeared (usually 10-20s). In each embryo, only one side was photoconverted, keeping the contralateral side as an internal control. The multi-spectral clonal cell analysis was performed on Tg(sox10:ERT2cre;Zebrabow-M) offspring in a wildtype or mutant background. Cre expression was induced at 10 somites stage by 3 hours exposure to 10 μM Tamoxifen (Sigma®) as described (Pan et al., 2013, Hans et al., 2009). Next, embryos were extensively washed and incubated at 28.5°C in E3 medium until observation. Imaging used a Nikon Ai scanning confocal microscope CFP, GFP and RFP channels. For GFP, yellow false color was used in the presentation of images (Pan et al., 2013, Rochard et al., 2015).

Figures have been created using Adobe Photoshop and Illustrator software. For bright field Alcian blue pictures, contrast and brightness have been slightly enhanced to allow better observation of cartilage elements.
Generation of \textit{wnt9a}, \textit{wls} and \textit{frzb} mutants

The \textit{wnt9a}, \textit{wls} and \textit{frzb} mutants were generated by CRISPR-Cas9 genome editing as described (Fu et al., 2013).

The small guide RNA sequences were used as described in Figure 2. Founders bearing mutations inducing frame shifts in the coding sequence of each gene were identified. In-del mutations were identified by sequencing and FAM-PCR. Simulated translations of these variants predicted truncated and therefore likely null proteins. Reduction of wild-type Wls and Wnt9a proteins were confirmed by Western blot analysis. Reduction in \textit{frzb} transcripts in \textit{frzb} homozygote mutants compared to wild-type was confirmed by quantitative RT-PCR (qPCR). F0 adult fish carriers were outcrossed with Tübingen wildtype fish to generate an F1 stable generation. F1 carriers were again outcrossed with Tübingen wildtype fish to minimize background mutations. Phenotype and phenotype-genotype correlation were analyzed on F3 animals. Each mutation lead to the described phenotype in Mendelian proportion.

Oligos for CRISPR mutants:
\textit{wnt9a\_oligo1.1}:TAGGACCGAGCACGGCTTACTT,\textit{oligo1.2}:
AAACAAGTAAGCCGTGCTCGGT;
\textit{frzb\_oligo1}:TAGGTTACATTTCGCAGGGTCTTGG,\textit{oligo2}:
5’-AACCAAGACCCTGCGAAATGTAACC;
\textit{wls\_oligo1}:
TAGGCCATGGTCAAGAAAATCG;
\textit{frzb\_oligo2}: AAACCGATTTTCTTGACCATGG.

Genotyping primers list:
\textit{wnt9a}:TGCTGTAAGTGAATGGGG,CTGCAAGCAACATCATGC,\textit{frzb}:CCGTT
Western blot analysis

Proteins were extracted from wild-type and mutants embryos at 6 dpf as previously described (Towbin et al., 1979) with 20 μg of protein. Wnt9a was detected with 1/1000 Abcam (#ab125957) and Wls protein was detected using 1/750 dilution of LSBio (LS-C102099).

Cell transplantation

Cells were transplanted using 1,000 cell embryos as previously described (Kemp et al., 2009) using a CellTram® Vario (eppendorf).

As a control of our technique and to insure the phenomenon observed was due only to the NCCs and not transplantation of other cell types (epithelial cells surrounding the palate or floor plate cells), ubi:mCherry (or ubi:GFP) embryos were also used as donors. Additionally, we selected embryos that only showed transplanted sox10 positive cells in craniofacial skeleton.
Figure S1. Expression pattern of non-canonical Wnt genes during craniofacial development. Whole mount in situ hybridization with head to the left in lateral view (A-E), ventral view (F-J) and sagittal section (K-O). At 60hpf, during palate morphogenesis, \textit{wls} (A, F and K) is co-expressed with \textit{wnt9a} (D, I, N) and \textit{gpc4} (B, G, L) in the oral epithelium surrounding chondrocytes and tissue above the palate. \textit{frzb} (C, H, M) transcripts are detected in the chondrocytes at the distal tip of the palate. \textit{wnt5b} (E, J and O) expression is detected in the oral epithelium at the mouth opening region. P: Summary of gene expression domains and their spatial relationships.
Figure S2. Targeted mutagenesis of wnt9a, wls and frzb using CRISPR Cas9 genome editing. (A) Schematic representation of wnt9a exons, position of target sites #1 and #2, and sequences of induced mutations. (B-C) Mutations were recovered in F1 and F2 generations and fish were genotyped using FAM-labeled PCR. (D) Western blot analysis of Wnt9a protein in wild-type and wnt9a homozygote mutants. (E) Schematic representation of the wls gene. SgRNA was designed in the second coding exon. The sequence of the 7 bp deletion compared to the wild-type sequence is presented beneath. (F) Example of FAM profile obtained for genotyping, showing the wild-type allele...
(right peak, 267bp) and mutant allele (left peak, 260bp). (G) Western blot analysis of wls protein in wild-type and wls (CRISPR and C186) homozygote mutants. (H) Schematic representation of frzb gene. SgRNA has been designed in the second exon. Two mutations have been obtained for this same target site, a 7 bp deletion and a 7 bp deletion-insertion. (I-J) FAM-PCR genotyping of both alleles, -7 bp on the left and +7 bp on the right. (K) qPCR analysis on 60hpf embryos show the dramatic decrease of frzb expression in frzb homozygote mutants (+7 bp) compared to wild-type embryos.
Figure S3. Early neural crest development and patterning are unaffected in wls-/-. Whole mount *in situ* hybridization of the indicated genes in wild-type and wls-/− embryos with head to the left in dorsal view (A,F) and lateral view (B-E, G-J). At all stages and genes examined, the expression does not differ between wild-type and wls-/− mutants. Markers used: *sox10*: early cNCC; *dlx2a*: migrating cNCCs; *edn1*: cNCC migration; *sox9*: cartilage formation; *col2a*: cartilage formation.
Supplementary references


