Fig. S1

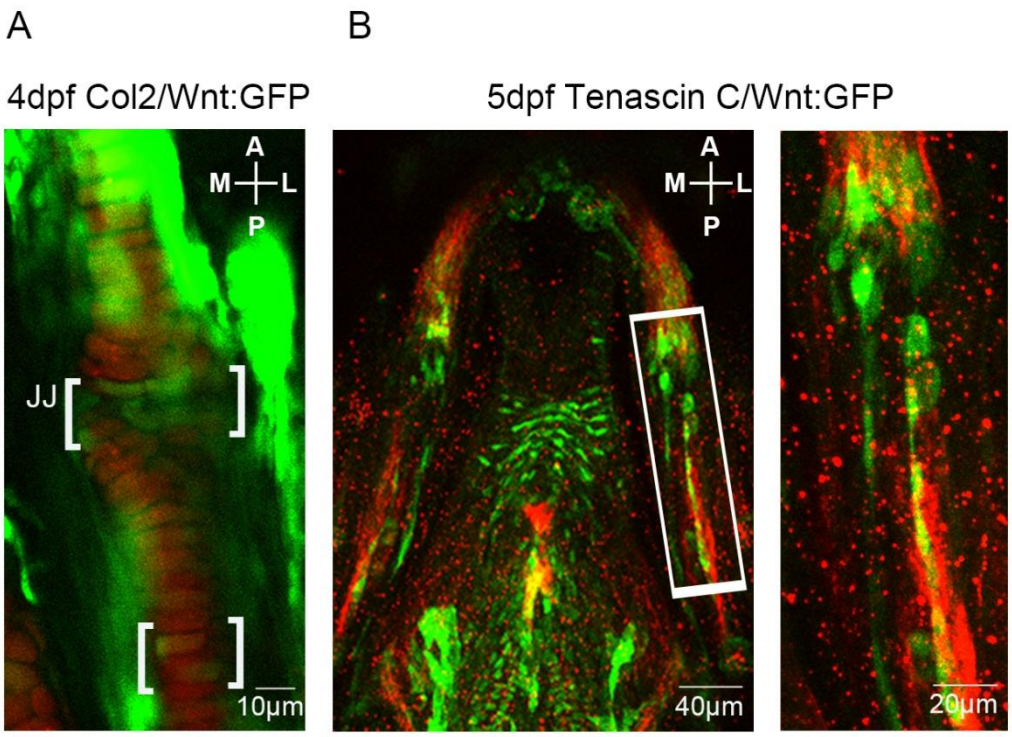

Fig S1. Identification of a heterogenous population of Wnt responsive cells at the lower jaw. (A): *Tg(7xTCF.XlaSiam:nlsGFP)* (Wnt:GFP*)* and*Tg(Col2a1aBAC:mcherry)* (Col2) transgenic zebrafish lines mark Wnt responsive cells and cartilage of the jaw joint elements at 4dpf in a single z-slice. Brackets identify Wnt responsive chondrocytes. (B): Antibodies against GFP and Tenascin C were used to detect colocalisation of *Tg(7xTCF.XlaSiam:nlsGFP)* Wnt responsive cells and ligaments and tendons at 5dpf, (left panel). Right panel: zoomed image from white box. JJ= jaw joint, A= anterior, P= posterior, M= medial, L= lateral.

Fig. S2

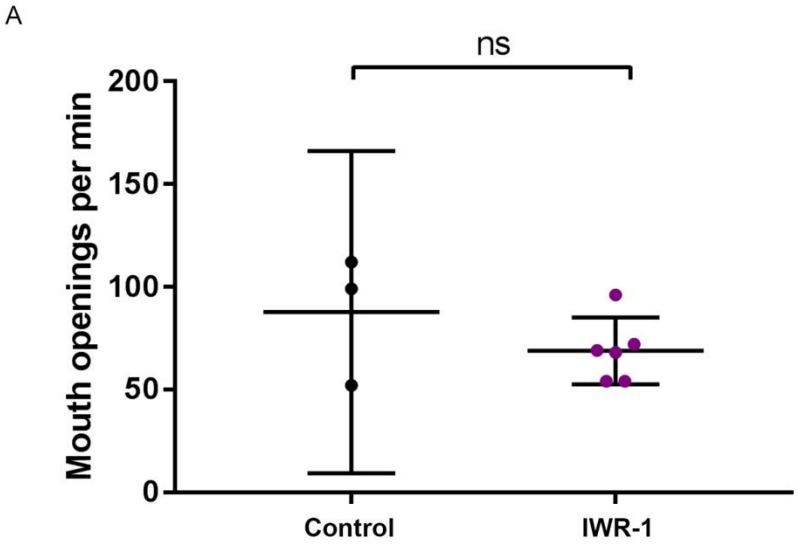

Fig S2. Frequency of jaw movements in 5dpf control and IWR-1 treated zebrafish.

(A): Jaw movements per minute in 5dpf control and IWR-1 treated zebrafish. (n=3, 6 animals). A two-tailed student t-test was performed. Ns=not significant. Bars on graph represent mean and 95%CI.

Fig. S3

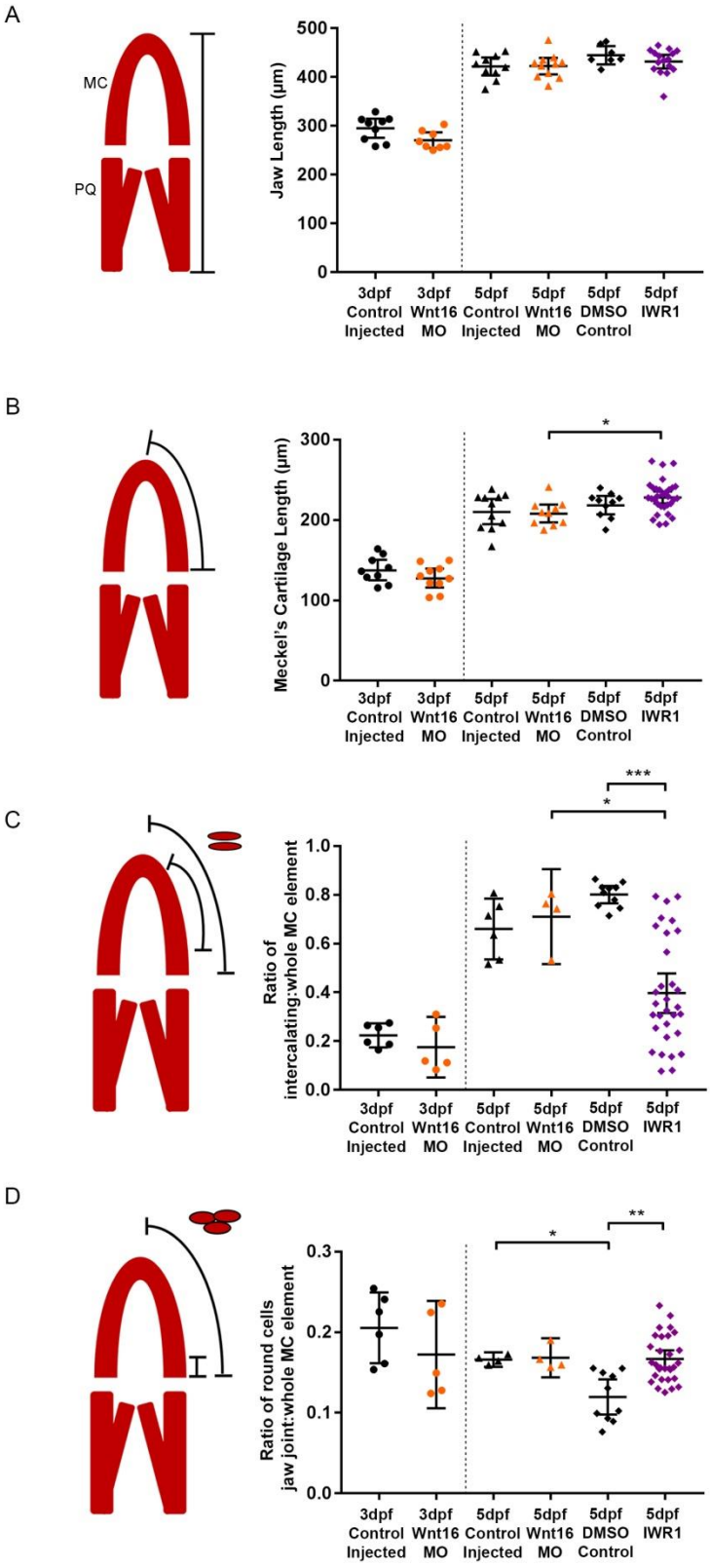

Fig S3. Lower jaw dimensions and ratio of rounded and intercalating cells in the Meckel's cartilage (MC) after Wnt manipulation.

(A): Jaw length (µm) from the anterior MC to the posterior end of the palatoquadrate (PQ) (as diagram, black line) in 3 and 5dpf control injected and Wnt16MO injected zebrafish and 5dpf DMSO control and IWR-1 treated zebrafish. (n=9, 8, 10, 11, 7, 16 animals). (B): Meckel's cartilage length (µm) from the anterior MC to the jaw joint (as diagram, black line) in 3 and 5dpf control injected and Wnt16 MO injected zebrafish and 5dpf DMSO control and IWR-1 treated zebrafish. (n=9, 10, 11, 10, 10, 31 animals). (C): Ratio of MC element containing columnar intercalating cells versus total MC length (as diagram, black lines, red columnar cells) in 3 and 5dpf control injected and Wnt16 MO injected zebrafish and 5dpf DMSO control and IWR-1 treated zebrafish. (n=6, 5, 6, 4, 10, 31 animals). (D): Ratio of MC element containing rounded cells at the jaw joint versus total MC length (as diagram, black lines, red rounded cells) in 3 and 5dpf control injected and Wnt16 MO injected zebrafish and 5dpf DMSO control and IWR-1 treated zebrafish. (n=6, 5, 4, 4, 10, 30 animals). A Kruskal-Wallis test was performed for (A) and one-way ANOVA for (B,C,D). ns= not significant, *=p≤0.05, **=p≤0.01, ***=p≤0.001. Bars on graph represent mean and 95%CI.

Fig. S4

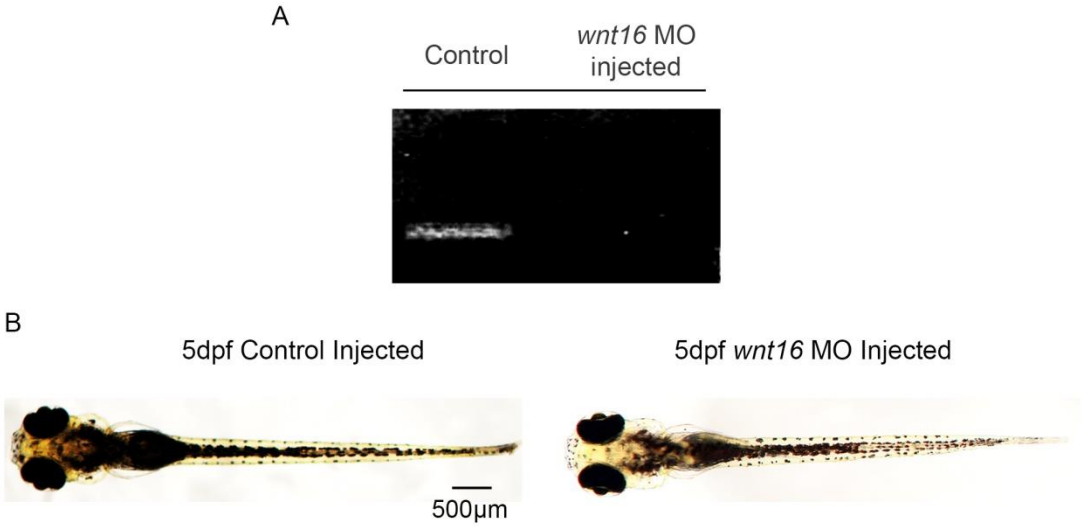

Fig S4. Wnt16 morpholino (MO) knockdown validation.

(A): PCR of Wnt16 cDNA amplified from 1ng total RNA extracted from 3dpf control or Wnt16 morphant larvae using primers described in (Clements et al.,2011). (B): Brightfield image of gross morphology of 5dpf control and Wnt16 MO injected zebrafish.

Fig. S5

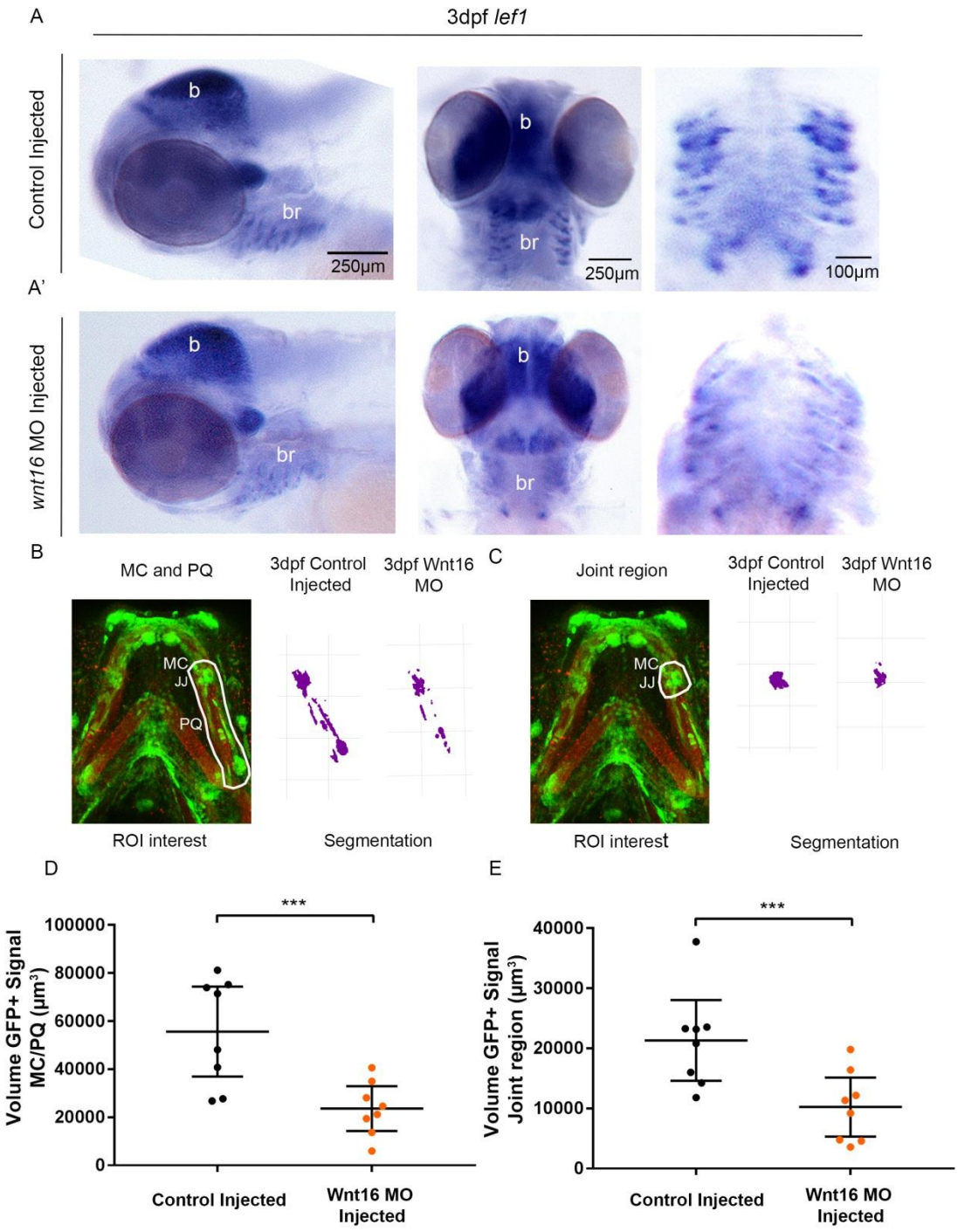

Fig S5. Wnt16 morpholino knockdown affects canonical Wnt activation in the jaw.
(A,A'): in situ hybridisation of *lef1* mRNA expression in 3dpf control injected (A) and Wnt16 MO injected (A') zebrafish. (n=9, 19 animals). Left panels: lateral view of head. Middle panels: Ventral view of head. Right panels: Ventral view of branchial arches. B= brain, br=branchial arches. (B): Left panel: volume analysis of*Tg(7xTCF.XlaSiam:nlsGFP)* GFP-positive (GFP+) signal at the region of interest (ROI) from the Meckel's Cartilage (MC) jaw joint (JJ) and along the full extent of the palatoquadrate (PQ) (white line). Right panel: Segmentation of GFP+ signal volume from region of interest in 3dpf control injected and Wnt16 morphant zebrafish. (C): Left panel: volume analysis of *Tg(7xTCF.XlaSiam:nlsGFP)* GFP-positive (GFP+) signal at the ROI from the Meckel's Cartilage (MC) jaw joint to the interzone (white line). Right panel: Segmentation of GFP+ signal volume from region of interest in 3dpf control injected and Wnt16 morphant zebrafish. (D): Volume (μm^3) of GFP+ signal at the MC joint and along the PQ (as measured in (B)) in 3dpf control injected and Wnt16 MO injected zebrafish. (n=8, 8 joints). (E): Volume (μm^3) of GFP+ signal at the MC joint (as measured in (C)) in 3dpf control injected and Wnt16 MO injected zebrafish. (n=8, 8 joints). Two-tailed student t-tests were performed for (D,E). ***=p≤0.001. Bars on graph represent mean and 95%CI.

Fig. S6

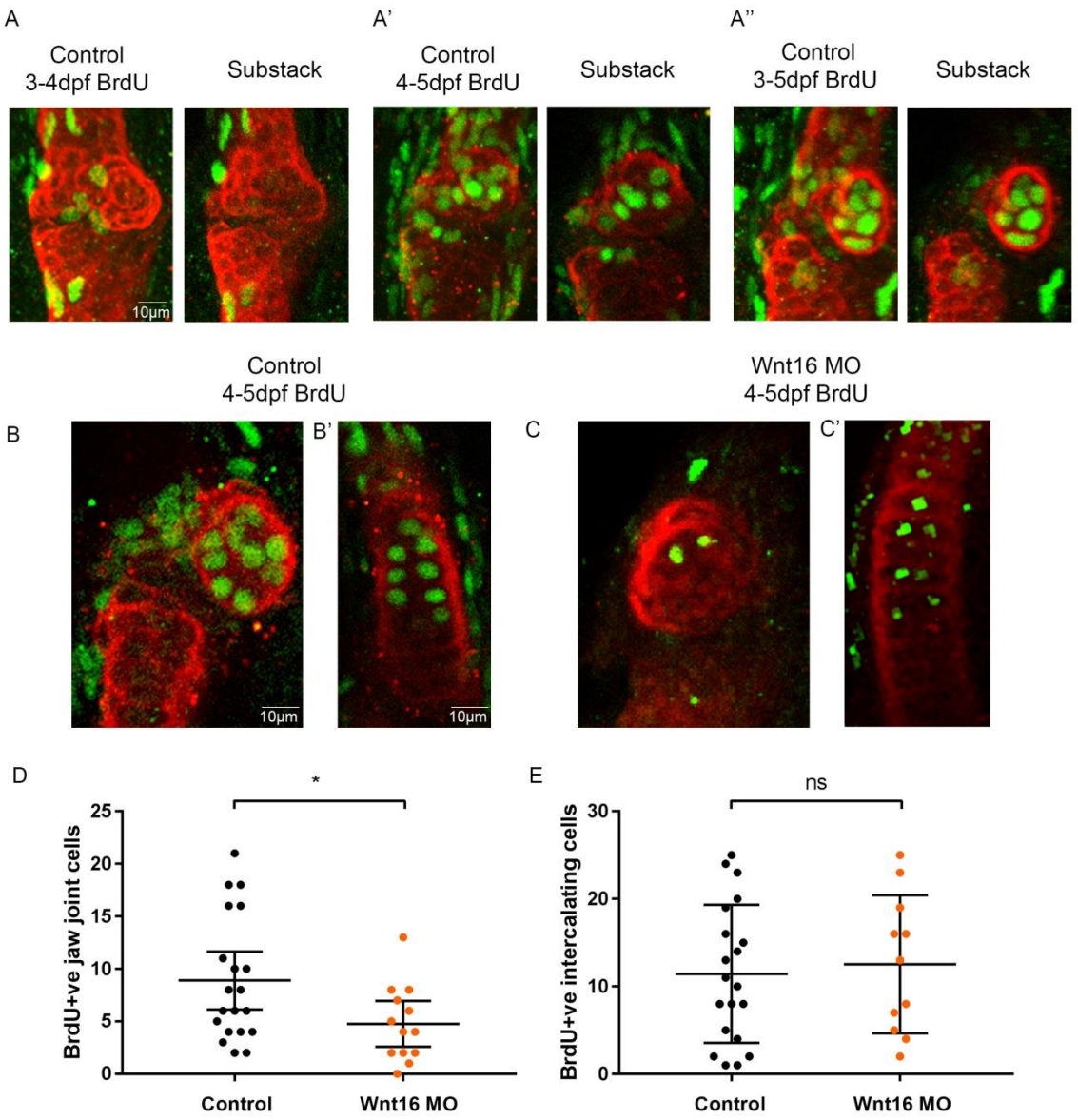

Fig S6. BrdU staining reveals cells proliferation at the jaw joint is affected by Wnt16 morpholino knockdown.

(A-A''): Cell proliferation at the jaw joint occurs frequently between 4-5dpf. Pulse-chase experiments exposing control zebrafish to BrdU between 3-4dpf (A), 4-5dpf (A') and 3-5dpf (A''). (A-A'') include max projection of image and substack through the cartilage joint. Anti-BrdU (green) and anti-collagen-II (red) label proliferating cells and cartilage at the jaw joints, respectively. (B-C): Pulse-chase experiments exposing control (B,B') and Wnt16 MO injected (C,C') zebrafish to BrdU between 4-5dpf. Anti-BrdU (green) and anti-collagen-II (red) label proliferating cells and cartilage,

respectively, at the jaw joint (left panel) and intercalating MC element region (right panel). (D): Number of BrdU positively labelled cells (BrdU +ve) at the jaw joint of 5dpf control injected and Wnt16 MO injected zebrafish after exposure to BrdU between 4-5dpf. (n=20, 13 joints). (E): Number of BrdU positively labelled cells (BrdU +ve) in the mid MC element intercalating region of 5dpf control injected and Wnt16 MO injected zebrafish after exposure to BrdU between 4-5dpf. (n=20, 11 joints). A Mann-Whitney U test was performed for (D) and a two-tailed student t-test for (E). ns= not significant, *=p≤0.05, **=p≤0.01, ***=p≤0.001. Bars on graph represent mean and 95%CI.

Fig. S7

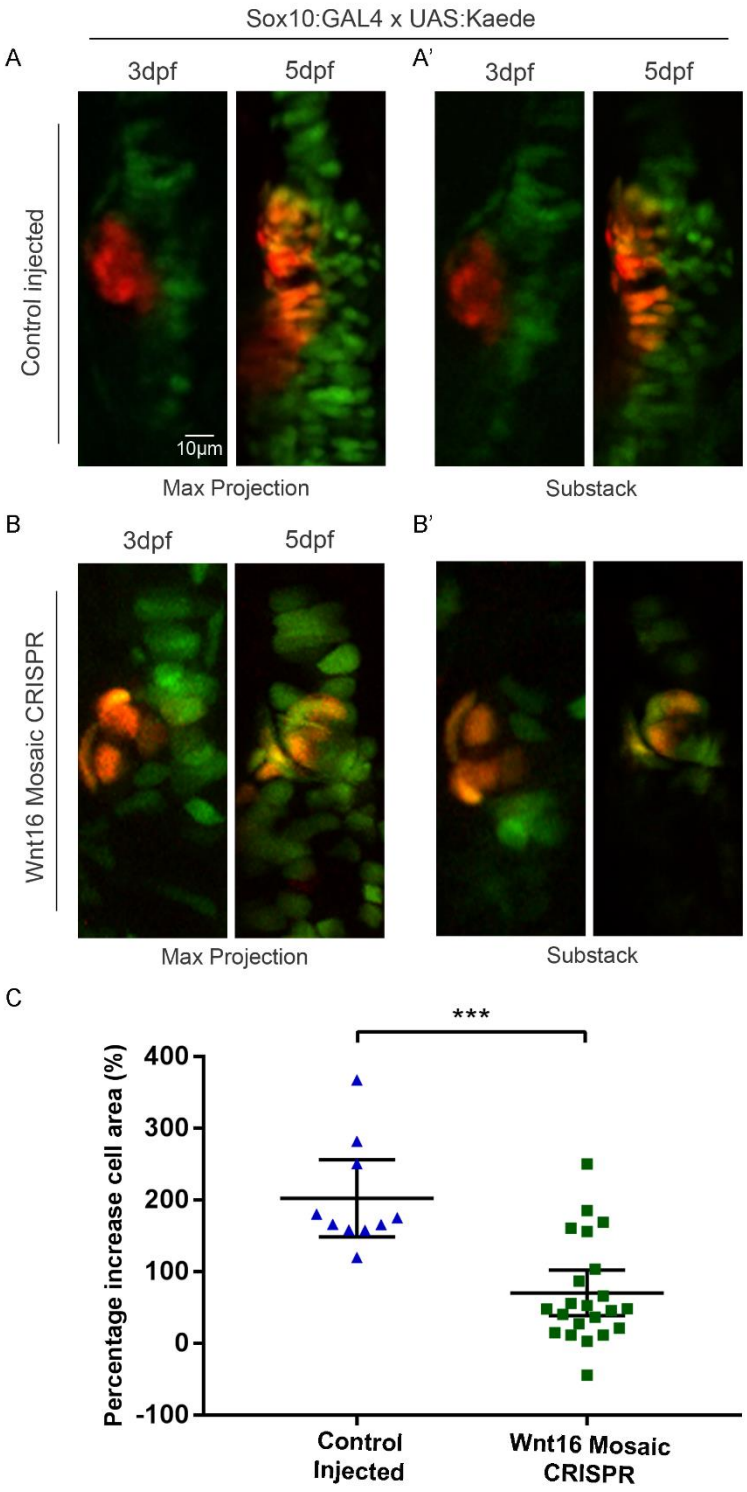

Fig S7. Mosaic CRISPR knockout of Wnt16 affects cell migration at the medial region of the jaw joint.

(A-B'): Tg(Sox10:GAL4-VP16) and Tg(UAS:Kaede) transgenic line drives expression of kaede protein (green) in cartilage of control injected (A), and mosaic CRISPR Wnt16 knockout (B) zebrafish. Maximum projections of the jaw joint from stacks of tiff images (A,B) and single slice/substacks through the same jaw joint to show cell morphology (A',B') are represented. At the jaw joint, medially located kaede expressing cells are photoconverted to red kaede at 3dpf (left panels). Right panels show jaw joints from the same larva reimaged at 5dpf. Photoconverted cells appear red/orange due to presence of photoconverted red kaede and expression of newly made green kaede protein under control of sox10 promoter. (C): Percentage increase in total area of cells expressing photoconverted red kaede between 3 and 5dpf in control injected (Fig. 4E) and CRISPR Wnt16 knockout zebrafish jaw joints. (n=10, 22 joints). Kruskal-Wallis tests were performed for (C). ***=p≤0.001. Bars on graph represent mean and 95%CI.

Fig. S8

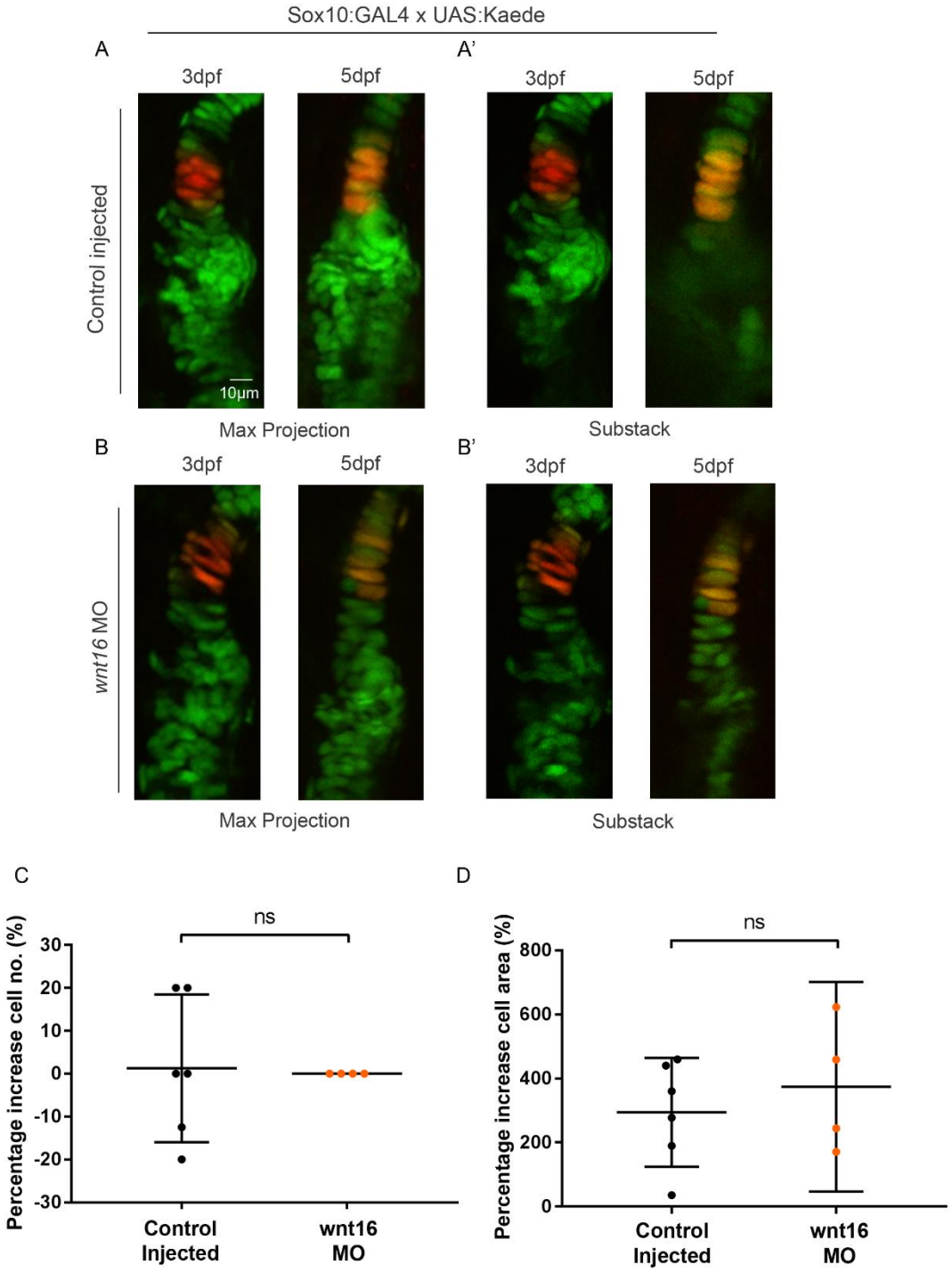

Fig S8. Cell proliferation and migration in intercalating cells of the Meckel's cartilage are not affected by Wnt16 morpholino knockdown.

(A-B'): Tg(Sox10:GAL4-VP16) and Tg(UAS:Kaede) transgenic line drives expression of kaede protein (green) in cartilage of control injected (A) and Wnt16 MO injected (B) zebrafish. Maximum projections of the jaw joint from stacks of tiff images (A,B) and single slice/substacks through the same jaw joint to show cell morphology (A',B') are represented. Kaede expressing cells located in the mid region of the MC are photoconverted to red kaede at 3dpf (left panels). MC of the same fish is then reimaged at 5dpf (right panels). Photoconverted cells appear red/orange due to presence of photoconverted red kaede and new expression of green kaede protein. (C): Percentage increase in number of cells expressing photoconverted red kaede between 3 and 5dpf in control injected and Wnt16 MO injected zebrafish MC elements. (n=6, 4 animals). (D): Percentage increase in total area of cells expressing photoconverted red kaede between 3 and 5dpf in control injected and Wnt16 MO injected zebrafish. (n=6, 4 animals). Two-tailed student t-tests were performed (B,C). ns= not significant. Bars on graph represent mean and 95%CI.

Fig. S9

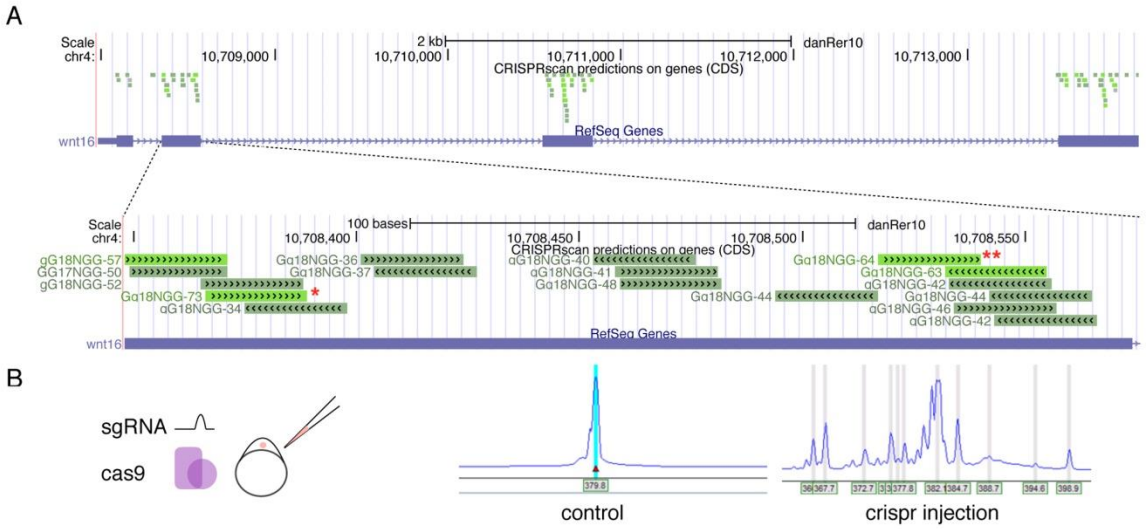

Fig S9. Mosaic Wnt16 knockout using CRISPR/Cas9.

(A): Illustration of zebrafish Wnt16 gene on UCSC genome browser showing crispr target sites (green bars) predicted using CRISPRscan track. Exon 2 is zoomed in to show the position of each target sequences and their score (GGnumberNGG-score). Red asterisks indicate selected target sequences used here. (B): Diagram to illustrate injection of sgRNA and cas9 protein into one cell stage zebrafish eggs followed by fragment analysis and peak call generated with GeneMapper to check for CRISPR efficiency, a single peak is observed in controls (non-injected) and a variety of fragment sizes in the injected fish.

Table S1. Examples of primary antibody used in zebrafish published on Zfin.org

Mouse anti-collagen II, AB528165, DSHB	https://zfin.org/ZDB-ATB-081008-6
Chicken anti-GFP, ab13970, Abcam	https://zfin.org/ZDB-ATB-100203-1
Rabbit anti-tenascin C, USBI142433, US biological	https://zfin.org/ZDB-ATB-130122-1
Mouse anti-BrdU, B8434, Sigma	https://zfin.org/ZDB-ATB-090130-3