Temporal Control of WNT Activity Regulates Tooth Number in Fish

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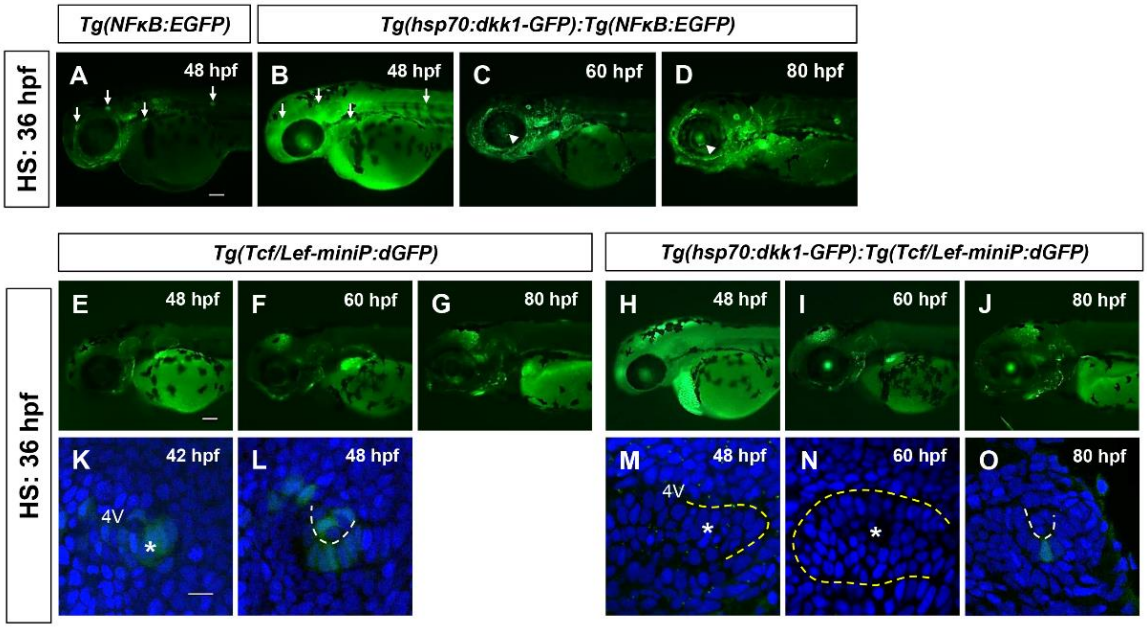
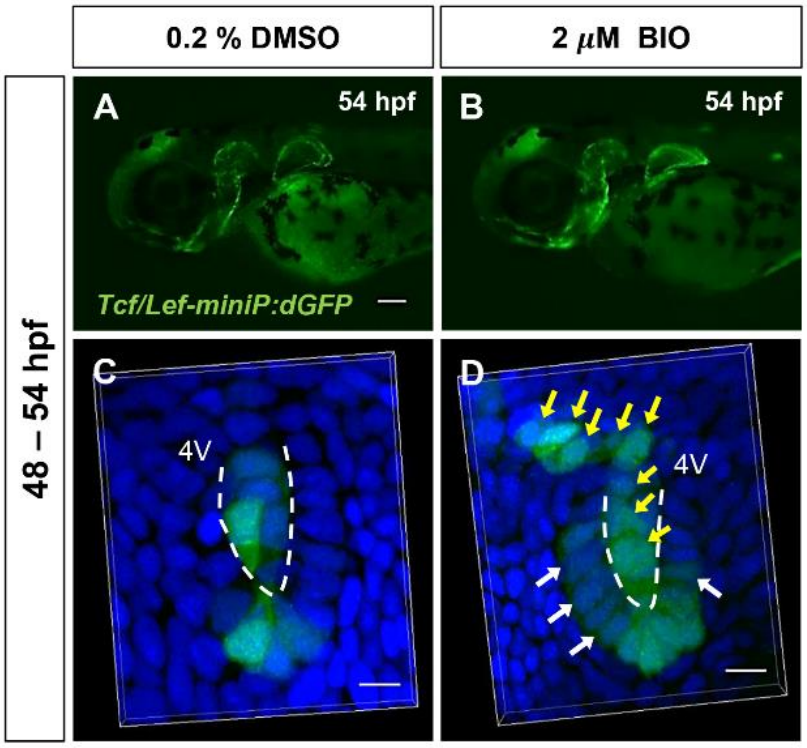
Appendix



Appendix Figure 1. Inhibition of Wnt/β-catenin signaling can occur by heat shock induction of Dkk1-GFP expression in a time-dependent manner.

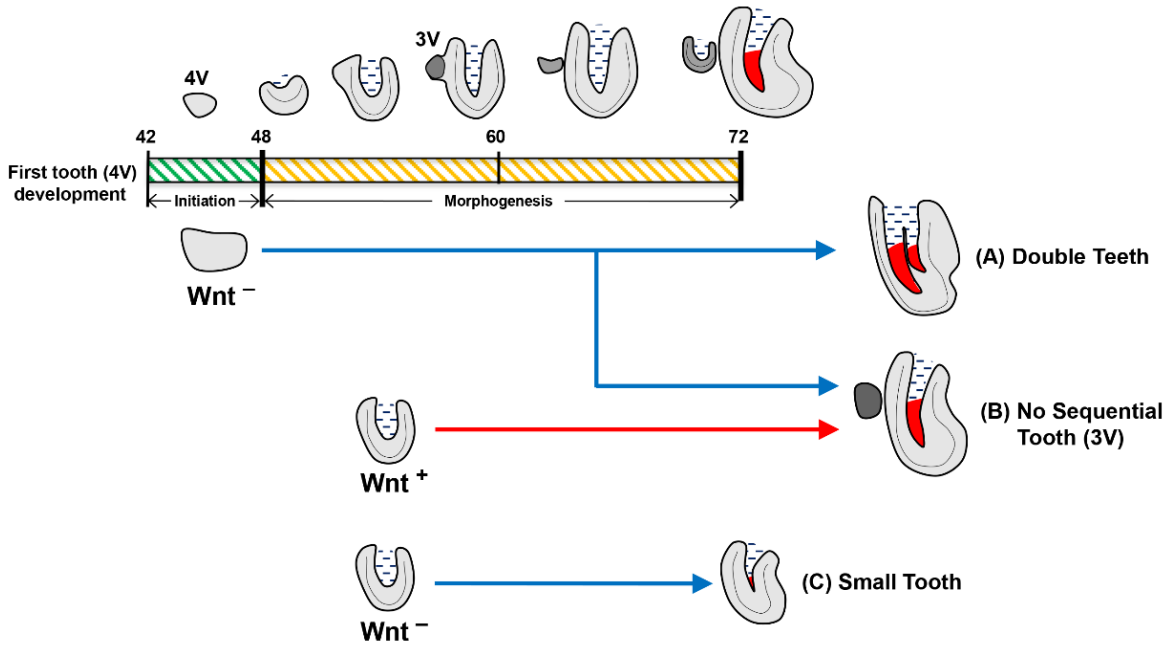
(A-O) Embryos were heat-shocked (HS) at 36 hpf and analyzed at specified time-points. **(A-J)** Fluorescence images of embryos with the anterior side to the left. Scale bars: 100 μm. **(A-D)** Heterozygous NFκB:EGFP transgenic zebrafish were crossed with heterozygous hsp70:Dkk1-GFP transgenic zebrafish. At 48 hpf, Dkk1-GFP expression as well as NF-κB signal (arrows) was detected in Tg(hsp70:dkk1-GFP):Tg(NFκB:EGFP) and then, at 60 and 80 hpf, Dkk1-GFP expression almost disappeared although still remained in eyes (arrowheads). **(E-O)** heterozygous

Tcf/Lef-miniP:dGFP transgenic zebrafish were crossed with heterozygous hsp70:Dkk1-GFP transgenic zebrafish. **(K-P)** Confocal fluorescence images showing Wnt reporter activity in developing 4V stained with DAPI (nuclei; blue). The yellow dashed lines represent the prospective tooth outlines. Scale bars: 10 μm. Wnt reporter activity was not detected in 4V placode (asterisks, M and N) in Tg(hsp70:dkk1-GFP):Tg(NFκB:EGFP) embryos, whereas activity existed (asterisks, K) in Tg(NFκB:EGFP) embryos. At 80 hpf, reduced reporter gene activity was detected in epithelium of 4V at early morphogenesis stage (O) compared with the control (L).



Appendix Figure 2. Tcf/Lef-miniP:dGFP reporter activity was dramatically enhanced by BIO treatment during early morphogenesis.

(A–D) Tcf/Lef-miniP:dGFP reporter line was treated with 0.2 % DMSO (control; A, C) and 2 μM BIO (B, D) from 48 to 54 hpf and analyzed immediately. **(A, B)** Fluorescence images of embryos with the anterior side to the left. Scale bars: 100 μm. The reporter activity was slightly enhanced by BIO treatment compared with control. **(C, D)** 3D volume renders of z-stack from confocal fluorescence images showing Wnt reporter activity in developing 4V stained with DAPI (nuclei; blue). The white dashed lines represent the epithelial-mesenchymal boundary. Scale bars: 10 μm. The region of the reporter gene expression was expanded both in mesenchyme (yellow arrows) and epithelium (white arrows) compared with control.



Appendix Figure 3. Schematic summary of Wnt/β-catenin signaling roles in zebrafish tooth development.

Normal development of first tooth (4V) and second tooth (3V) is shown along the upper row of developmental timeline of 4V. The major findings of this work are outlined below. (A) Double 4V teeth can be caused by the inhibition of Wnt/ß-catenin signaling at initiation stage of 4V. (B) The lasting arrest of sequential tooth can be caused by the inhibition of Wnt/ß-catenin signaling at initiation stage or overactivation of Wnt/β-catenin signaling during early morphogenesis of 4V. (C) Reduction of tooth size can be caused by the inhibition of Wnt/ß-catenin signaling during morphogenesis.