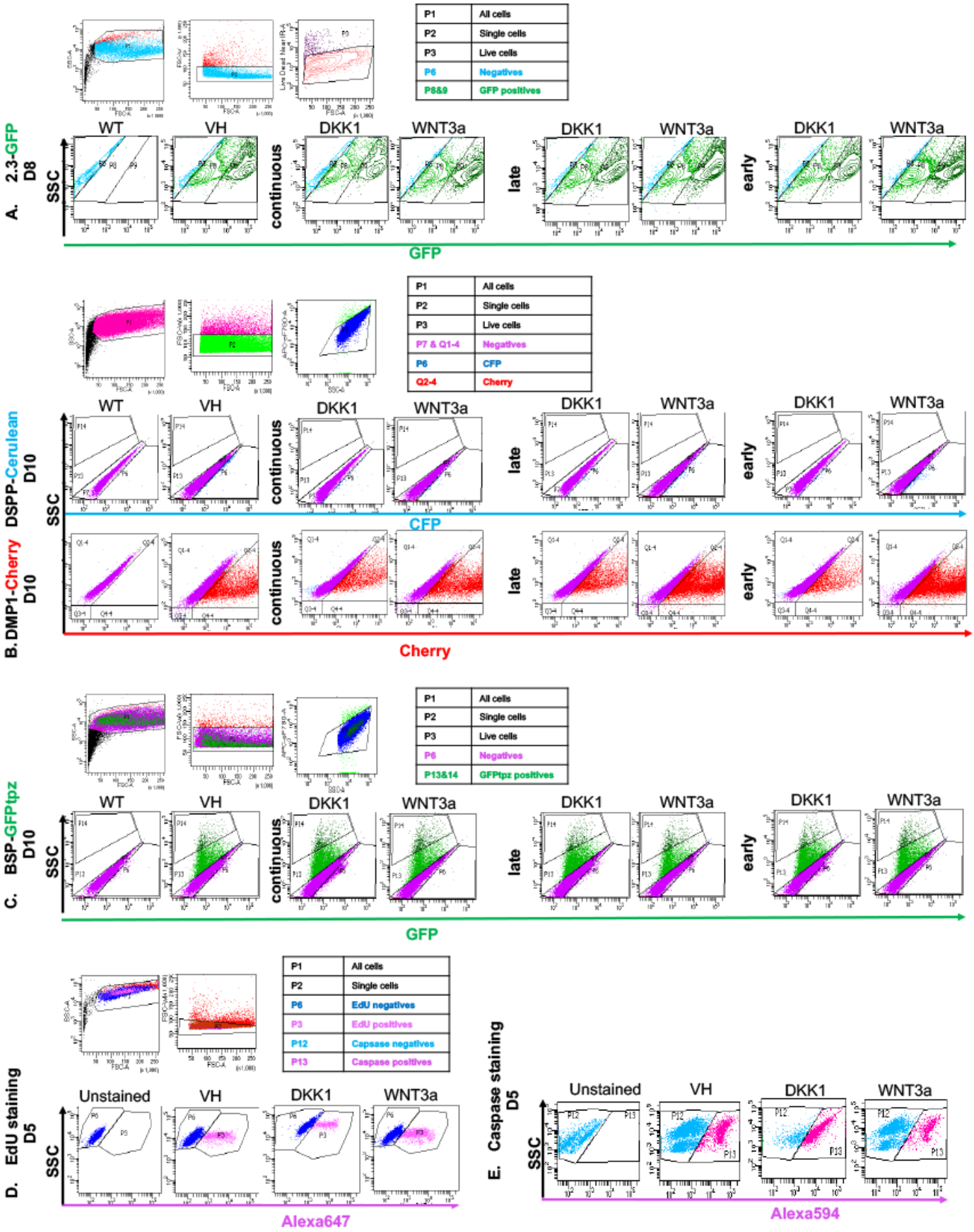
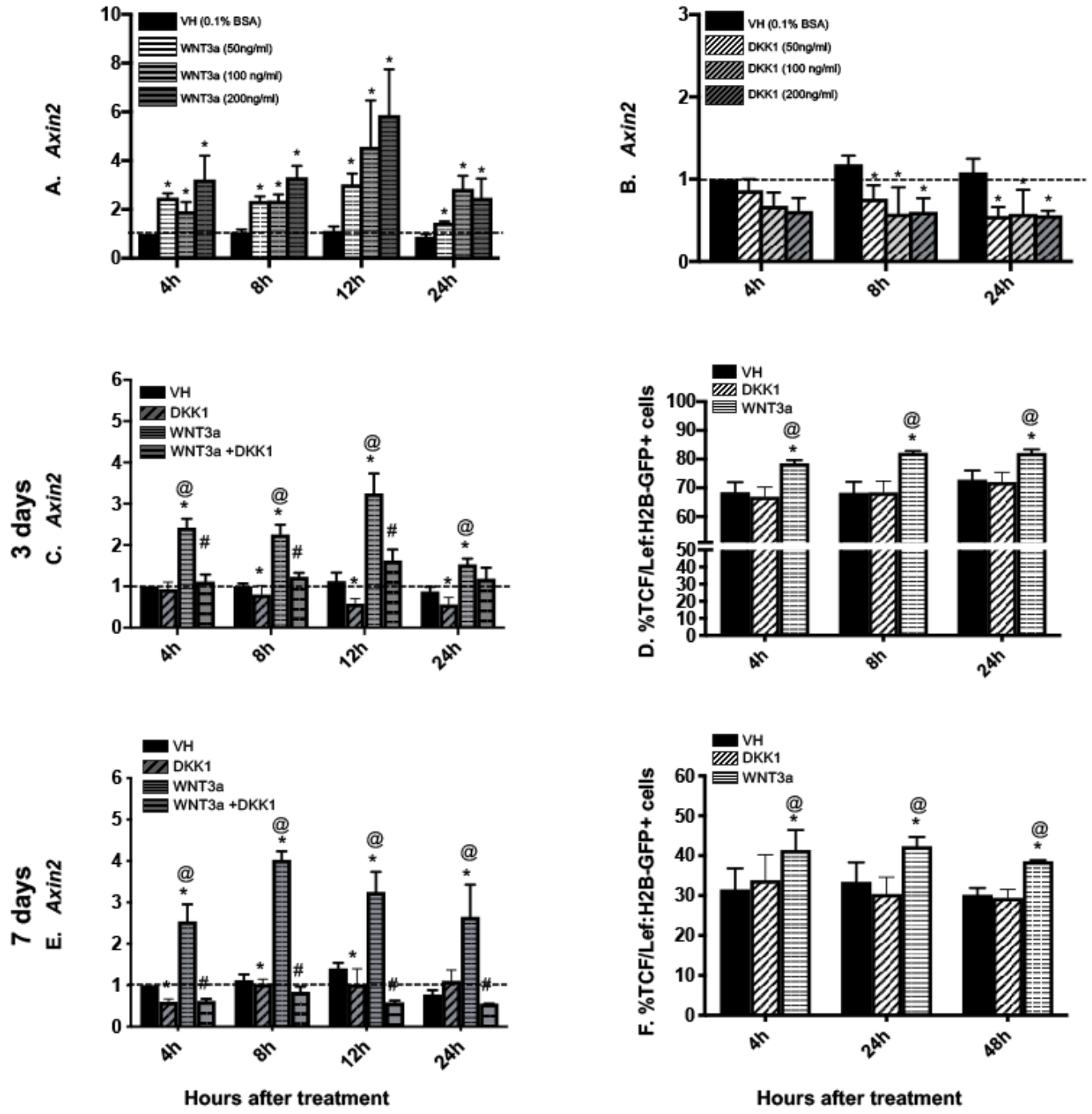
Taqman primers	
Gene	**Assay**
Gapdh	Mm99999915_g1
Bsp	Mm01208381_g1
Dmp1	Mm01208363_m1
Dspp	Mm00515667_g1
Axin2	Mm00443610_m1

Appendix Table 1: Primers used for qPCR analysis


A. 2.3-GFP D8
P1 All cells
P2 Single cells
P3 Live cells
P6 Negatives
P8&9 GFP positives
WT
VH
DKK1
WNT3a
DKK1
WNT3a
DKK1
WNT3a
SSC
continuous
late
early
GFP
B. DMP1-Cherry D10
DSPP-Cerulean D10
P1 All cells
P2 Single cells
P3 Live cells
P7 & Q1-4 Negatives
P6 CFP
Q2-4 Cherry
WT
VH
DKK1
WNT3a
DKK1
WNT3a
DKK1
WNT3a
SSC
continuous
late
early
CFP
Cherry
C. BSP-GFPtpz D10
P1 All cells
P2 Single cells
P3 Live cells
P6 Negatives
P13&14 GFPtpz positives
WT
VH
DKK1
WNT3a
DKK1
WNT3a
DKK1
WNT3a
SSC
continuous
late
early
GFP
D. EdU staining D5
P1 All cells
P2 Single cells
P6 EdU negatives
P3 EdU positives
P12 Capsase negatives
P13 Caspase positives
Unstained
VH
DKK1
WNT3a
SSC
Alexa647
E. Caspase staining D5
Unstained
VH
DKK1
WNT3a
SSC
Alexa594

Appendix Figure 2: Gating strategies for Flow cytometric analysis of dental pulp cultures from various transgenic mice.

Primary pulp cultures were treated continuously (days 3-10), late (days 7-10), and early (days 3-7) with VH (0.1% BSA), DKK1 (50ng/ml), and WNT3a (50ng/ml), and processed for FACS analysis every 24 hours from days 4-10
Gating strategies used for FACS analysis of cultures derived from 2.3-GFP (A), DSPP-Cerulean/DMP1-Cherry (B), and BSP-GFPtpz (C) transgenic mice, as well as cell cultures processed for EdU staining (D) and cleaved caspase-3 staining (E) as described in Materials and methods.
(A-E) (Top row) Gate P1 includes all cells exclusive of debris. P2 includes the population of single cells, while P3 includes live cells. Dead cells were excluded by staining with Fixable Viability Dye. (Bottom row) FACS analysis performed in 2.3-FP (at day 8), DSPP-Cerulean/DMP1-Cherry (at day 10), BSP-GFPtpz (at day 10), EdU stained and cleaved caspase-3 stained cultures. Non-transgenic and unstained cultures were used as a negative control. Tables show the legends denoting the identity of each gate analyzed by flow cytometry in each corresponding figure.



Appendix Figure 3:

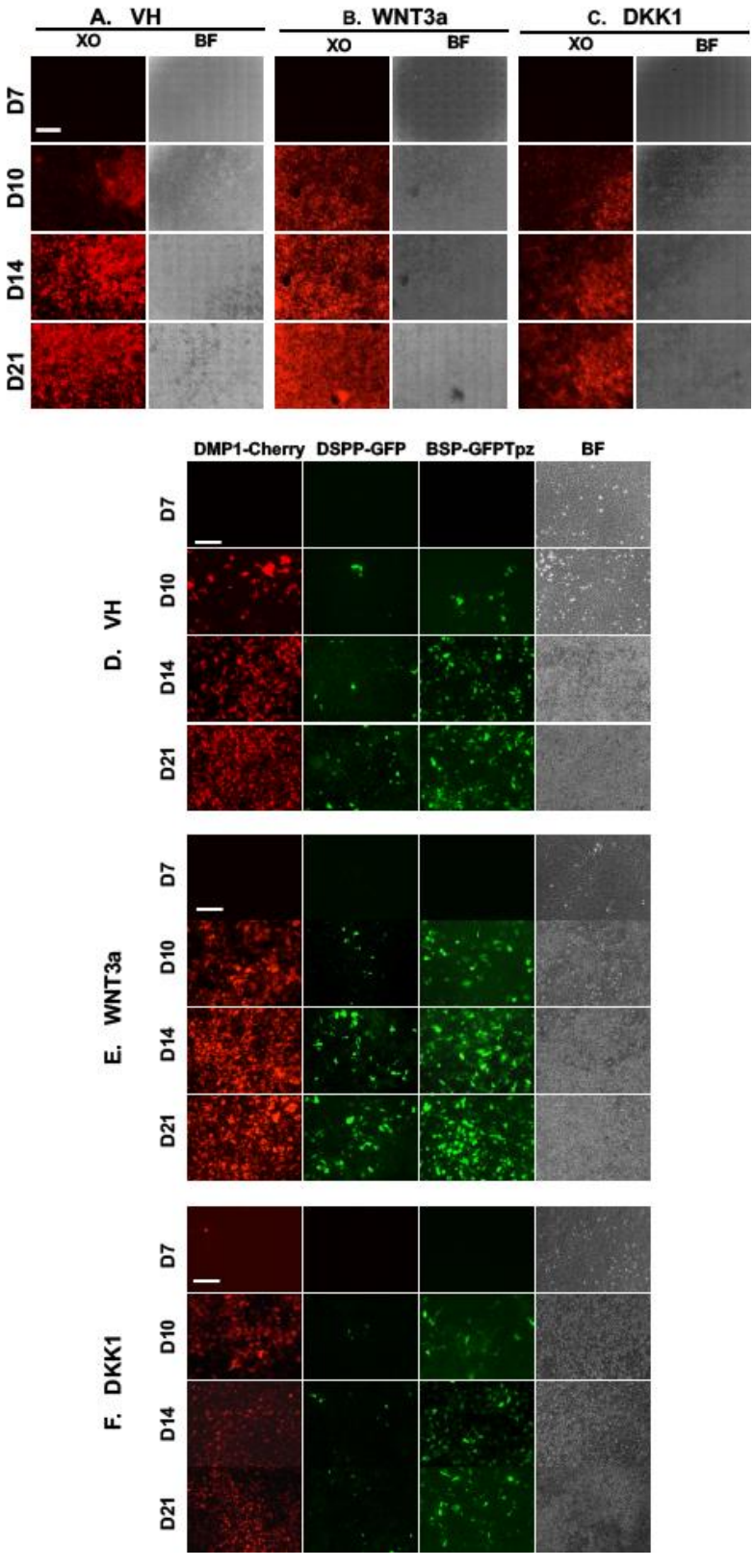
A and B: Dose-dependent effects of Wnt/β-catenin signaling on Wnt response in primary dental pulp cultures. Bar graphs showing the dose-dependent changes in the expression of *Axin2* in cultures treated with Vehicle (VH, 0.1% BSA), (**A**) WNT3a (50, 100, and 200ng/ml), (**B**) DKK1 (50, 100, and 200 ng/ml) between 4-24 hours after treatment at various times at day 3.

C-E: Effects of Wnt/β-catenin signaling on Wnt response in primary dental pulp cultures.

C and D are bar graphs showing changes in the expression of *Axin2* (**C**) and the percentage of TCF/Lef: H2B-GFP^{+} cells (**D**) between 4-24 hours in cultures with various treatments at day 3.

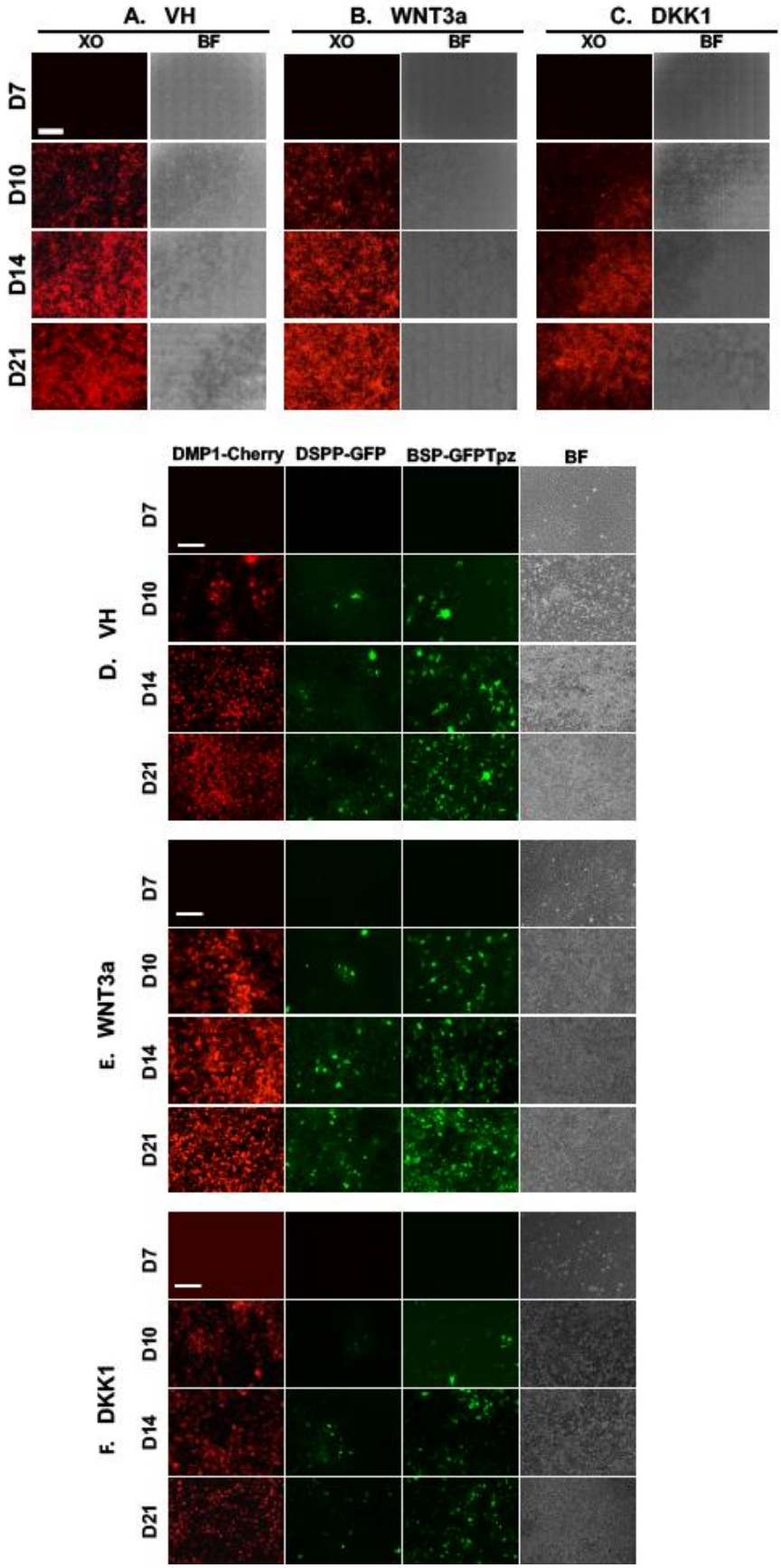
E and F are bar graphs showing changes in the expression of *Axin2* (**E**) and the percentage of TCF/Lef: H2B-GFP^{+} cells (**F**) between 4-24 hours in cultures with various treatments at day 7.

The expression of *Axin2* was normalized to 4h of the VH-treated cultures, which is arbitrarily set to 1 and is indicated by the dashed line. Results in all graphs represent mean ± SEM of at least 3 independent experiments; *P ≤ 0.05 relative to VH at each time point; @P ≤ 0.05 relative to DKK1 at each time point; #P ≤ 0.05 relative to WNT3A at each time point.


A. VH
B. WNT3a
C. DKK1
XO
BF
D7
D10
D14
D21
DMP1-Cherry
DSPP-GFP
BSP-GFPTpz
BF
D. VH
E. WNT3a
F. DKK1

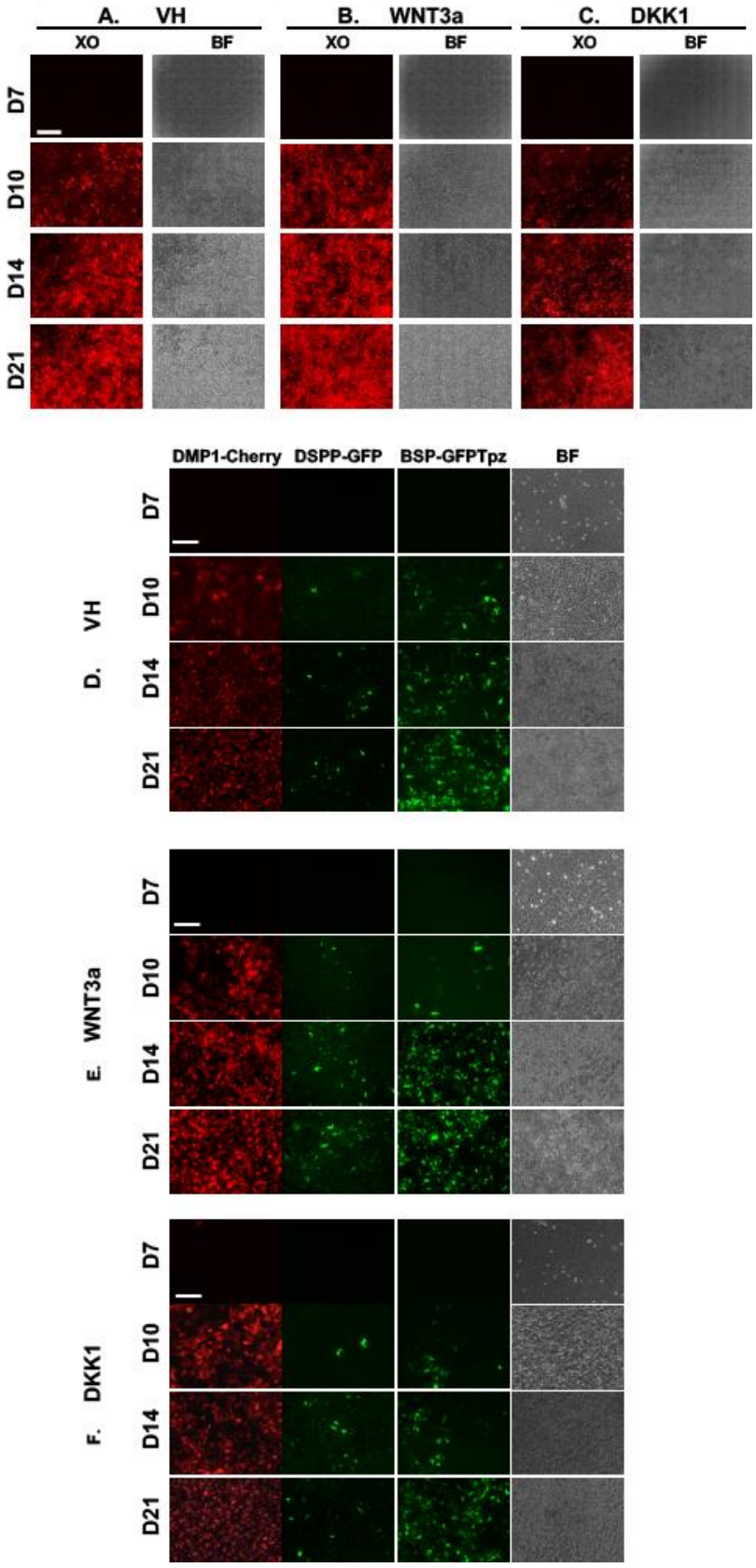
Appendix Figure 4: Effects of continuous exposure to Wnt/β–catenin signaling modulation on mineralization and the tissue-and stage-specific GFP reporters.

(A-C) Representative images of the same areas in cultures treated continuously between days 3-21 with (**A**) VH (0.1% BSA), (**B**) WNT3a (50ng/ml), and (**C**) DKK1 (50ng/ml) analyzed under brightfield (BF) and epifluorescent light using TRITC red filter for detection of XO staining. Continuous exposure to WNT3a increased mineralization, and DKK1 decreased mineralization. Scale bar = 2mm.
(D-F) Representative images of the cultures from DMP1-Cherry/DSPP-Cerulean and BSP-GFPtpz transgenic mice treated continuously from between days 3-21 with (**D**) VH, (**E**) WNT3a, and (**F**) DKK1 analyzed under brightfield (BF) and epifluorescent light for the detection of DMP1-Cherry, DSPP-GFP, and BSP-GFPtpz. Continuous exposure to WNT3a and LiCl increased DMP1-Cherry and DSPP-GFP expression, which were decreased in DKK1 treated cultures. WNT3a treatment also increased BSP-GFPtpz expression. Scale bar = 200 μm


A. VH
B. WNT3a
C. DKK1
XO
BF
D7
D10
D14
D21
DMP1-Cherry
DSPP-GFP
BSP-GFPTpz
BF
D. VH
E. WNT3a
F. DKK1

Appendix Figure 5: Effects of late exposure to Wnt/β–catenin signaling modulation on mineralization and the tissue-and stage-specific GFP reporters.

(A-C) Representative images of the same areas in cultures treated between days 7-21 with (**A**) VH (0.1% BSA), (**B**) WNT3a (50ng/ml), and (**C**) DKK1 (50ng/ml) analyzed under brightfield (BF) and epifluorescent light using TRITC red filter for detection of XO staining. Late exposure to WNT3a increased mineralization and DKK1 decreased mineralization, Scale bar = 2mm.
(D-F) Representative images of the cultures from DMP1-Cherry/DSPP-Cerulean and BSP-GFPtpz transgenic mice treated between days 7-21 with (**D**) VH, (**E**) WNT3a, and (**F**) DKK1, analyzed under brightfield (BF) and epifluorescent light for the detection of DMP1-Cherry, DSPP-GFP, and BSP-GFPtpz. Late exposure to WNT3a and LiCl increased DMP1-Cherry and DSPP-GFP expression, which were decreased in DKK1 treated cultures. WNT3a treatment also increased BSP-GFPtpz expression, which was decreased in DKK1-treated cultures. Scale bar = 200 μm


A. VH
B. WNT3a
C. DKK1
XO
BF
D7
D10
D14
D21
DMP1-Cherry
DSPP-GFP
BSP-GFPTpz
D. VH
E. WNT3a
F. DKK1

Appendix Figure 6: Effects of early exposure to Wnt/β–catenin signaling modulation on mineralization and the tissue-and stage-specific GFP reporters.

(A-C) Representative images of the same areas in cultures treated between days 3-7 with (**A**) VH (0.1% BSA), (**B**) WNT3a (50ng/ml), and (**C**) DKK1 (50ng/ml) analyzed under brightfield (BF) and epifluorescent light using TRITC red filter for detection of XO staining. Early exposure to WNT3a increased mineralization, and DKK1 decreased mineralization in pulp cultures. Scale bar = 2mm.

(D-F) Representative images of the cultures from DMP1-Cherry/DSPP-Cerulean and BSP-GFPtpz transgenic mice treated between days 3-7 with (**D**) VH, (**E**) WNT3a, and (**F**) DKK1, analyzed under brightfield (BF) and epifluorescent light for the detection of DMP1-Cherry, DSPP-GFP, and BSP-GFPtpz. Early exposure to WNT3a increased DMP1-Cherry, DSPP-GFP, and BSP-GFPtpz expression, which was decreased in DKK1 treated cultures. Scale bar = 200 μm