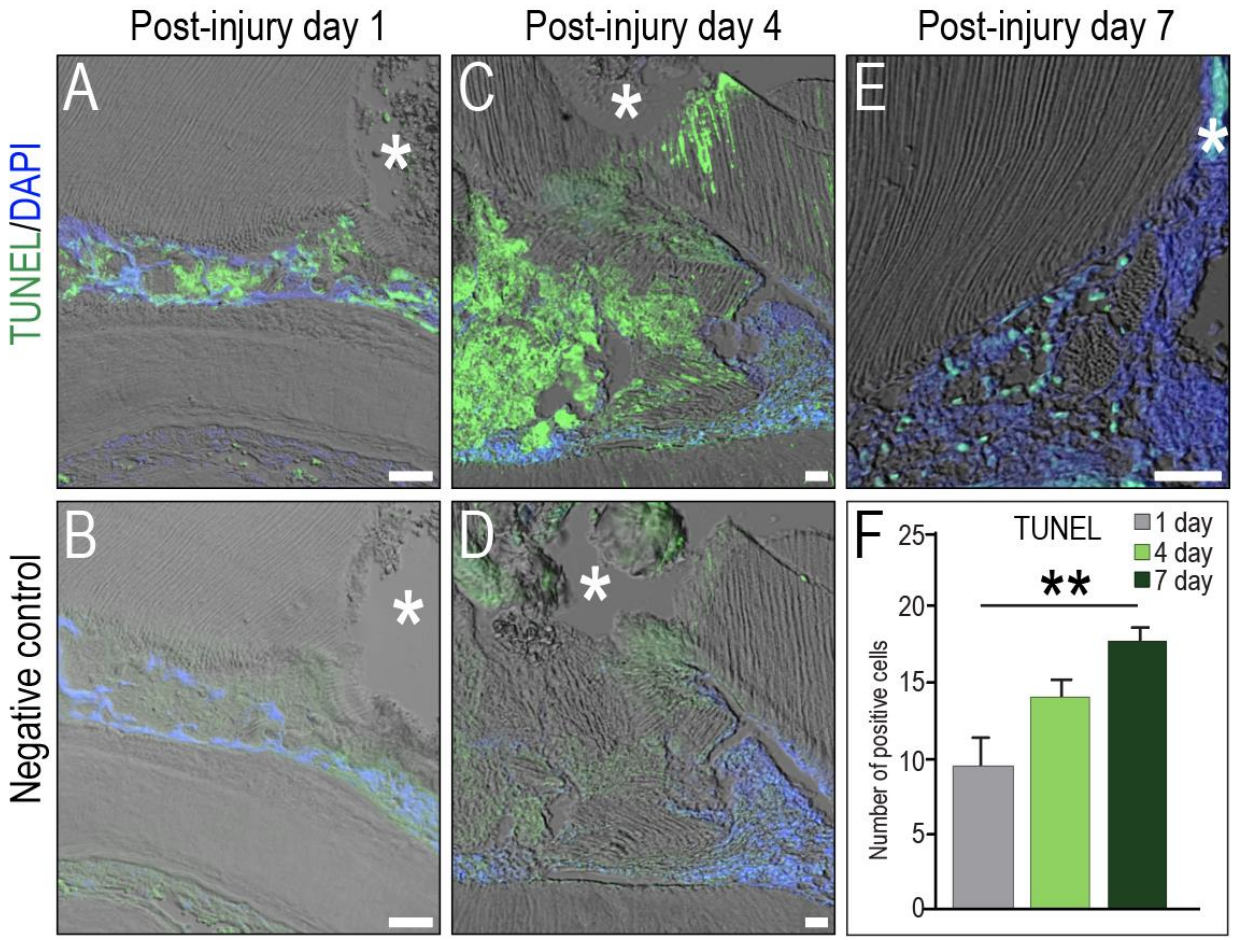
Wnt-Responsive Odontoblasts Secrete New Dentin after Superficial Tooth Injury

Y. Zhao, X. Yuan, B. Liu, U.S. Tulu, and J.A. Helms

Appendix

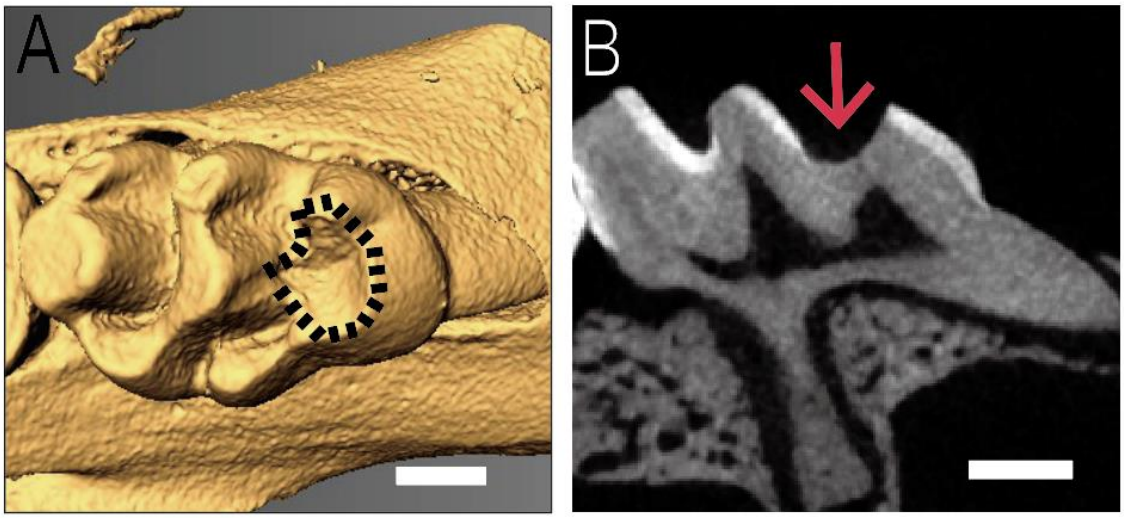
Appendix Methods

Sample preparation, processing, histology, histomorphometric assays, µCT analyses
Maxillae were harvested, fixed, and sectioned as described (Lim et al. 2014). Histologic, alkaline phosphatase (ALP) and tartrate-resistant acid phosphatase (TRAP) staining was performed (Lim et al. 2014; Minear et al. 2010). TUNEL staining was performed as described (11684795910, Roche). Primary antibodies were: anti-proliferating cell nuclear antigen (PCNA, ab18197, Abcam), anti βIII tubulin (ab18207, Abcam), anti-chemokine ligand 2/monocyte chemoattractant protein (CCL2/MCP1, ab9851, Abcam), anti-interleukin 1 alpha (IL 1α (ab9787, Abcam), anti-hypoxia-inducible factor 1-alpha (HIF1α, Abcam), anti-dentin matrix protein 1 (DMP1, ab103203, Abcam), anti-ALP (MAB1448, R&D Systems), and anti-green fluorescent protein (GFP, ab13970, Abcam). Micro-computed topographies (µCT) were performed as described (Hunter et al. 2015).

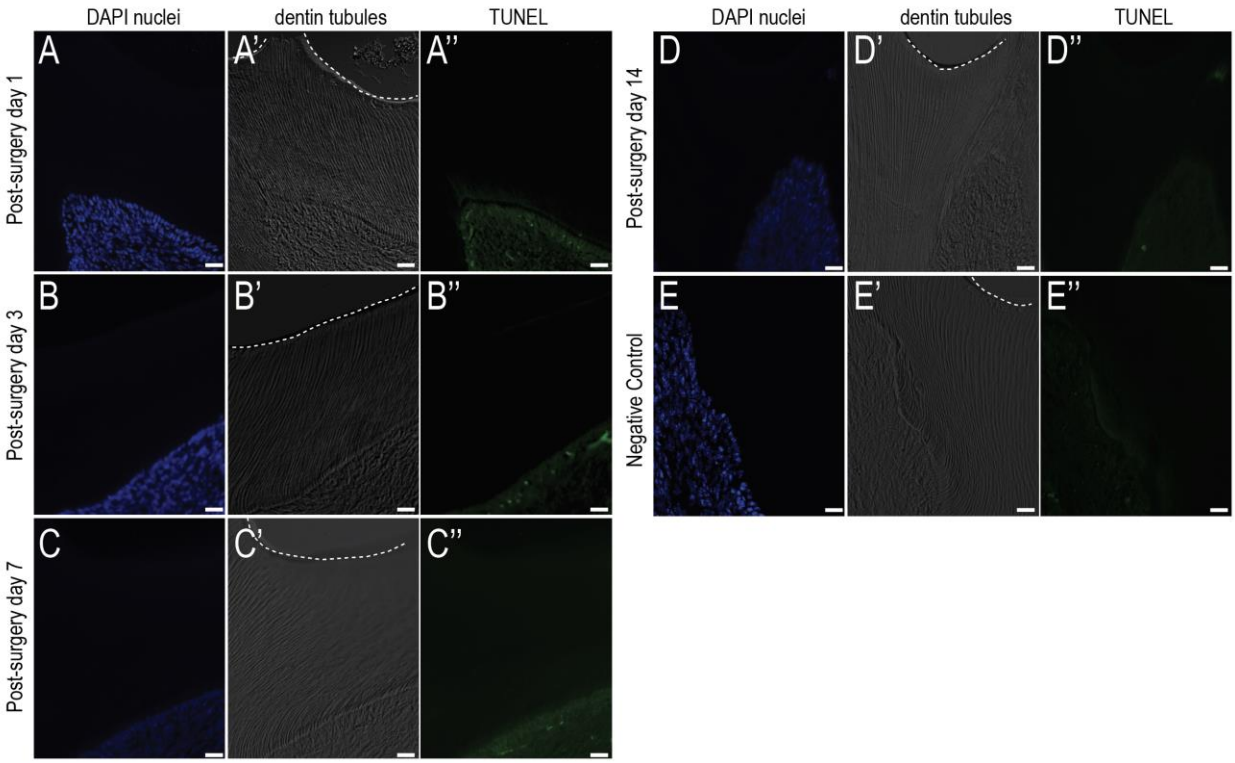


Appendix Figure 1. Cell death after pulp exposure.

(A) TUNEL activity in pulp cavity on PID1. (B) Negative control of TUNEL staining using the adjacent section. (C) TUNEL activity in pulp cavity on PID3. (D) Negative control of TUNEL staining using the adjacent section. (E) TUNEL activity in pulp cavity on PID7. (F) Quantification of the $TUNEL^{+ve}$ cells. Scale bar: 25µm.



Appendix Figure 2. µCT imaging shows superficial tooth injury.
(A) In adult maxillary molars, µCT imaging identifies sites of superficial tooth injury (dotted circles). (B) µCT imaging shows enamel and some dentin removal but no pulp exposure (red arrow). Scale bar: 0.5 mm.



Appendix Figure 3. Cell death after dentin injury.

(A-A'') TUNEL activity in odontoblasts and pulp cells on PID1. (A) shows DAPI staining, which identifies nuclei of viable odontoblasts and pulp cells; (A') shows DIC, which identifies dentin tubules and location of the injury (outlined with a dotted white line); (A'') shows TUNEL. (B-B'') TUNEL activity in odontoblasts and pulp cells on PID3. (C-C'') TUNEL activity in odontoblasts and pulp cells on PID7. (D-D'') TUNEL activity in odontoblasts and pulp cells on PID14. (E-E'') Negative control of TUNEL staining after dentin injury on PID3. A dotted white line shows the injury. Scale bar: 25 μm.

References

Lim WH, Liu B, Cheng D, Hunter DJ, Zhong Z, Ramos DM, Williams BO, Sharpe PT, Bardet C, Mah SJ, et al. 2014. Wnt signaling regulates pulp volume and dentin thickness. J Bone Miner Res. 29(4):892–901.

Minear S, Leucht P, Jiang J, Liu B, Zeng A, Fuerer C, Nusse R, Helms JA. 2010. Wnt proteins promote bone regeneration. Sci Transl Med. 2(29):29ra30.

Hunter DJ, Bardet C, Mouraret S, Liu B, Singh G, Sadoine J, Dhamdhere G, Smith A, Tran XV, Joy A, et al. 2015. Wnt acts as a prosurvival signal to enhance dentin regeneration. J Bone Miner Res. 30(7):1150–1159.