Axin2-expressing cells differentiate into reparative odontoblasts via autocrine Wnt/β-catenin signaling in response to tooth damage

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In non-growing teeth, such as mouse and human molars, primary odontoblasts are long-lived post-mitotic cells that secrete dentine throughout the life of the tooth. New odontoblast-like cells are only produced in response to a damage or trauma. Little is known about the molecular events that initiate mesenchymal stem cells to proliferate and differentiate into odontoblast-like cells in response to dentine damage. The reparative and regenerative capacity of multiple mammalian tissues depends on the activation of Wnt/β-catenin signaling pathway. In this study, we investigated the molecular role of Wnt/β-catenin signaling pathway in reparative dentinogenesis using an in vivo mouse tooth damage model. We found that Axin2 is rapidly upregulated in response to tooth damage and that these Axin2-expressing cells differentiate into new odontoblast-like cells that secrete reparative dentine. In addition, the Axin2-expressing cells produce a source of Wnt that acts in an autocrine manner to modulate reparative dentinogenesis.
can be identified by genetically-labelled Wnt responsive genes\(^{17-21}\). It has previously been shown that elevated Wnt signaling enhances reparative dentinogenesis in Axin2\(^{−/−}\) LacZ/LacZ mice\(^2\). We have shown that delivery of small molecule inhibitors of GSK3 activity (Wnt/\β-catenin signaling antagonists) directly to exposed pulp promotes the production of reparative dentine in vivo\(^{2}\). In this study, we take advantage of genetically-modified mice to investigate the molecular role of Wnt/\β-catenin signaling in the reparative dentinogenesis process. Our study has revealed that Axin2-expressing cells differentiate into new odontoblast-like cells that secrete reparative dentine.

In addition, Axin2-expressing cells produce a source of Wnt ligands that acts in an autocrine manner to modulate reparative dentinogenesis.

**Methods**

**Mouse and animal information.** All animals used in this study were handled in accordance with UK Home Office Regulations project license 70/7866 and personal license I6517C8EF. Experimental procedures were approved by the King’s College Ethical Review Process. Axin2\(^{−/−}\) LacZ/LacZ, Axin2\(^{−/−}\) CreERT2, Rosa26\(-\text{MittE}\) fl/+ (fl/+), and pCag-CreERT2 flameless Wls\(^{−/−}\) (fl/+), Axin2\(^{CreERT2}\) received one dose of tamoxifen or corn oil (WT) on the day of tooth damage and another two doses over the next two consecutive days. Wls\(^{−/−}\) and Axin2\(^{CreERT2}\) Rosa26\(-\text{MittE}\) fl/+/; Wls\(^{−/−}\) mice received one dose of tamoxifen or corn oil (WT) over three consecutive days and tooth damage was performed 5 days post last tamoxifen or corn oil injection.

**Tooth damage protocol.** Mice were anaesthetised with a solution made with Hypnorm (Fentanyl/fluanisone - VetaPharma Ltd.), sterile water and Hypnovel (Midazolam - Roche) in the ratio 1:2:1 at the rate of 10 ml/kg intraperitonially. The oral cavity was opened with a mouth retractor to expose the molars. The superior first molars were cleaned using a sterile cotton plug soaked in phosphate buffered saline (PBS). A cavity was drilled in the centre of the superior first molar using a carbide burr (FG\(^{1/8}\)) coupled to a high speed hand piece (Kavo Super Torque LUX 2 640B). Drilling was stopped when the pulp was visible under the dentine roof and a 27 G\(\frac{3}{4}\) needle was used to expose the pulp chamber. Mineral Trioxide Aggregate (MTA; Maillefer, Dentsply) was applied to the exposed pulp and the cavity was sealed with glass ionomer Ketac\(\text{®}\) Cem radiopaque (3 M ESPE). Post-op, the animals were given Vetgesic (Buprenorphine – Ceva) at the rate of 0.3 mg/kg intraperitonially as analgesic. The animals were sacrificed after at various time points post-damage.

**Dental pulp extraction.** Dental pulp tissue was extracted from superior first molars collected from CD-1 P21 mice according to the experiment time course post-damage. A 21G needle was used as an elevator to extract teeth from the alveolar bone. The extracted teeth were placed in ice cold PBS and a 23 scalpel blade was used to separate the tooth at the crown-root junction to expose the pulp chamber. The pulp was gently removed from the pulp chamber and the root canal using a 0.6 mm straight tip tweezer. The pulp was stored in RNAlater (Sigma) at –80 °C. For each condition, dental pulp tissue was pooled from at least 10 teeth and repeated in triplicate.

**Real-time qPCR analysis.** Total RNA was extracted from the dental pulp using TRIzol (Invitrogen) as recommended by the manufacturer’s instructions. The RNA was reverse transcribed using random primers (M-MLV Reverse Transcriptase kit, Promega) according to the manufacturer’s instructions. Gene expression was then assayed by real-time qPCR using Kappa Syber Fast (Kappa Biosystems) on a Rotor-Gene Q cycler (Qiagen) system. Beta-actin primers (Forward - GGCTGTATTCCCTCCTCG, Reverse - CCAGTGGTAAACATGCCTGT) were used for the housekeeping gene, Axin2 primers (Forward - TGACTCTCTCCCTTGATC, Reverse - TGCCCCACTAGGCTGACA) were used as the read-out of Wnt activity and for Gpr177 (Wls) expression, Forward - TCTAATGTGACCTGGGTGTC and Reverse – TTCCAGCTCAGTGCCATACC primers were used. Reactions were performed in triplicate and relative changes to housekeeping gene were calculated by the 2\(^{-\Delta\Delta C_T}\) method.

**Tissue processing and histological staining.** Teeth were fixed in 4% paraformaldehyde (PFA) for 24-hours at 4 °C, washed with PBS and decalcified in 19% EDTA, pH 8 for 4 weeks. Decalcified teeth were dehydrated through a graded series of ethanol, cleared in xylene and infused with wax at 60 °C in a Leica ASP300 tissue processor. Samples were embedded in wax and 8 μm sections were cut using a microtome (Leica RM2245, blade angle 5°). Sections were mounted on Trubond\(\text{®}\) 380 slides (Electron Microscopy Sciences). For Masson’s Trichrome staining, sections of adult teeth were deparaffinised in Neo-Clear and rehydrated through a series of graded ethanol. Sections were stained with Weigert’s Haematoxylin for 10 minutes, followed by 1% Biebrich-Scarlet-Acid Fuchsin solution for 15 minutes, differentiated in a mix of 5% phosphomolybdic acid and 5% phosphotungstic acid for 15 minutes and stained with 2.5% Aniline Blue solution for 15 minutes. Following staining, sections were differentiated in 1% acetic acid, dehydrated through 90% and 100% ethanol, cleared in Neo-Clear and permanently mounted in Neo-Mount.

**Immunohistochemistry.** Sections of adult teeth were deparaffinised in xylene and rehydrated in graded ethanol. To reduce endogenous peroxidase activity, sections were quenched with 3.5% hydrogen peroxide in PBS for 5 minutes and blocked with 10% goat serum in PBS with 0.1% Tween20 (PBST). No antigen retrieval was performed. The Primary antibodies were applied overnight at 4 °C. Proliferating cell nuclear antigen (PCNA, Abcam, ab18197) and green fluorescent protein (GFP, Abcam, ab13970) antibodies were used at 1:200 and 1:500, respectively in PBST containing 5% goat serum. After washing the sections with PBST they were incubated with appropriate biotinylated secondary antibody, then horseradish peroxidase (HRP)- conjugated streptavidin-biotin antibody and washed with PBST. Immunoreactivity was visualised with ImmPACT DAB HRP Substrate (Vector...
In situ hybridisation and Immunofluorescence.  *In situ* hybridisation for dentine sialo-phosphoprotein (*Dspp*) was performed on paraffin sections following standard procedures under RNase-free conditions\(^2\). *Dspp* was detected using TSA biotin system (PerkinElmer) in combination with the TSA Plus Cyanine 3.5 detection kit (PerkinElmer). After *in situ* hybridisation, slides were blocked with 10% goat serum in PBST, incubated with anti-GFP antibody (Abcam, ab13970, 1:200) in PBST with 5% goat serum overnight at 4 °C. Sections were washed with PBST, incubated with Alexa Fluor® 647 secondary antibody and counterstained with Hoechst (40 μg/ml) in PBS before mounting with Citifluor. Immunofluorescence was visualised with a Leica TCS SP5 laser confocal microscope.
Statistical Analysis. A two-tailed unpaired Student’s t-test using GraphPad prism software was used to determine significance, a P-value < 0.05 was considered statistically significant. At least four independent experiments were performed for statistical analysis of dental pulp tissues described in the figure legends. Statistical data was presented as mean ± s.e.m.

Data Availability. The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.
Results

**Time course of reparative dentinogenesis in tooth damage model.** To determine the timescale of reparative dentinogenesis in our model, damaged teeth were stained with Masson’s Trichrome to visualise dentine production, *in situ* hybridisation detection of *Dspp* gene was performed to identify odontoblasts26, 27 and immunohistochemical staining PCNA was performed to detect proliferating cells.

*In situ* hybridisation detection of *Dspp* showed primary odontoblasts located at the periphery of the dental pulp expressed *Dspp*, however no *Dspp* expression was detected at the site of exposure at 1 day post-damage, due to loss of local primary odontoblasts (Fig. 1A,B). *Dspp* expression was observed at the site of exposure 5 days post-damage and some reparative dentine was visible (Fig. 1C,D). By 14 days post-damage, a dentine bridge was formed and *Dspp* expression is localised underneath the dentine bridge (Fig. 1E,F). Immunohistochemical detection of PCNA in undamaged molars showed that there is little if any proliferation in the pulp chamber (Fig. 2A). When molars were damaged, dental pulp cells began to proliferate underneath the site of damage by 2 days post-damage, with proliferation peaking at 3 days and returning to resting levels by 14 days post-damage (Fig. 2B,C).

Collectively, these data demonstrate that cells of the dental pulp proliferate then differentiate into new odontoblast-like cells that secrete reparative dentine in response to damage.

**Wnt/β-catenin signaling is activated in proliferating cells in response to tooth damage.** We next wanted to investigate the role of Wnt/β-catenin signaling in reparative dentinogenesis process. Axin2LacZ reporter mice have been widely used to visualise Wnt active cells *in vivo*22. We observed diffusely stained Axin2 positive cells scattered under the site of exposure in damaged teeth from Axin2LacZ reporter mice (Supplementary Figure 1). It was not possible to assess if Axin2 cells were proliferating in damaged teeth from Axin2 LacZ reporter mice as the LacZ staining was not compatible with immunohistochemistry. We thus used TCF/Lef:H2B-GFP reporter mice that allow the visualisation of Wnt active cells since β-catenin is a transcriptional cofactor for TCF/Lef that is upstream regulator of Axin2.

Immunohistological staining of undamaged molars from TCF/Lef:H2B-GFP mice with a GFP antibody showed that some primary odontoblasts had strong staining at the periphery of the pulp cusp (Fig. 3A). Following damage, Wnt active cells at the periphery of the top of the cusp were lost due to pulp exposure and Wnt active were now detected throughout the pulp tissue under the site of exposure 3 days post-damage (Fig. 3B). Furthermore, real-time qPCR analysis of Axin2 expression from extracted dental pulps showed that Axin2 expression was initially reduced, presumably as a result of the loss of Wnt active primary odontoblasts 1 day post-damage, then significantly increased by 3 days post-damage (Fig. 3C). Immunofluorescent co-staining of damaged molars from TCF/Lef:H2B-GFP mice with GFP and PCNA antibodies, showed that some Wnt positive cells were proliferating 3 days post-damage (Fig. 3D–F).

**Odontoblast-like cells are descendants of Wnt active cells.** To investigate the fate of Wnt active cells we used Axin2CreERT2; Rosa26 mT-mG flox/+ mice to lineage trace the progeny of Axin2-expressing cells. In these mice, cells that express Axin2 are permanently labelled with GFP during the tamoxifen administration period, allowing
fate mapping of labelled cells and their descendants. We administered tamoxifen immediately after tooth damage and for the next two days meaning only cells that express Axin2 in this period will be permanently labelled.

Immunohistochemical detection of GFP showed a few Axin2-labelled cells at the site of damage 3 days post-damage (Fig. 4A) and an increase in the number of Axin2-labelled cells were detected 14 days post-damage beneath the dentine bridge (Fig. 4B,B’). This data suggests that a population of pulp cells are expressing Axin2 in response to damage and these labelled cells are undergoing a proliferative expansion. Furthermore, some of the Axin2-expressing cells were in close association with the newly formed dentine bridge and had characteristic morphology of odontoblasts. These data demonstrate that Axin2-expressing cells can differentiate into reparative odontoblast-like cells. Moreover, immunofluorescent staining of Axin2-labeled cells with GFP and co-detection of \( \text{Dspp} \) by \textit{in situ} hybridisation, revealed that some Axin2-labeled cells co-expressed \( \text{Dspp} \) 5 days post-damage underneath the site of exposure (Fig. 4C–E). This data suggests that reparative odontoblasts are descendants of Axin2-expressing cells.

**Inhibition of Wnt signaling impairs reparative dentinogenesis.** It has previously been shown that elevating Wnt signaling enhances reparative dentinogenesis \textit{in vivo}\[^2\]. This was shown using Axin2\(^{lacZ/lacZ}\) mice that are a model for elevated Wnt/\( \beta \)-catenin signaling since these mice lack functional copies of both Axin2 alleles, a negative regulator of Wnt signaling. We also confirmed these findings using our molar damage model and additionally compared reparative dentine formation by elevated Wnt/\( \beta \)-catenin activity with that from a commonly used clinical capping agent, MTA (mineral trioxide aggregate) (Supplementary Figure 2). In addition, we investigated whether impairing Wnt/\( \beta \)-catenin signaling affected reparative dentinogenesis. Gpr177 (Wls)\(^{fl/fl}\) mice do not express the Wls gene that encodes a sorting receptor required for Wnt secretion, thus cells cannot release Wnts to activate Wnt/\( \beta \)-catenin signaling. Real-time qPCR confirmed that the expression of the Wls gene was drastically reduced in the dental pulp tissue of Wls\(^{fl/fl}\) mice 5 days post-tamoxifen compared to WT mice (Fig. 5A). Masson’s Trichrome staining of damaged molars from Wls\(^{fl/fl}\) mice showed that reparative dentinogenesis does not occur in the absence of Wnt signaling since no dentine bridge was formed in response to damage by 14 days post-damage compared to WT mice (Fig. 5B,C).

However, we did observe resorption pits in the pulp chamber and the presence of TRAP positive cells in damaged molars, suggesting the presence of osteoclasts (Supplementary Figure 3). To overcome this pathology, we crossed Wls\(^{fl/fl}\) mice with Axin2\(^{CreERT2}\) mice to specifically delete Wls in Axin2-expressing cells, thus only preventing these cells from secreting Wnts. Masson’s Trichrome staining of damaged molars from Axin2\(^{CreERT2}\); Wls\(^{fl/fl}\) mice showed that reparative dentinogenesis was severely impaired 14 days post-damage (Fig. 5D). No resorption pits were observed in undamaged or damaged teeth of Axin2\(^{CreERT2}\); Wls\(^{fl/fl}\) mice. Additionally, cell proliferation in response to pulp exposure was significantly reduced in damaged molars of Axin2\(^{CreERT2}\); Wls\(^{fl/fl}\) mice compared to WT 14 days post damage (Fig. 5E).

**Discussion**

To study the molecular mechanisms of reparative dentinogenesis, we established a controlled molar-damage model \textit{in vivo}. The damage (100\( \mu \)m in diameter) was created occlusally in the centre of the superior first molar...
crown to expose the dental pulp tissue with subsequent capping with mineral trioxide aggregate (MTA) and glass ionomer restoration. We used MTA as it is a biocompatible material that releases calcium ions that are thought to stimulate reparative dentine formation. In endodontic procedures MTA is commonly used in vital pulp therapy to treat exposed dental pulp. In our model, we observed robust proliferation and differentiation of a population of cells into new odontoblast-like cells that secrete reparative dentine to form a dentine bridge. Wnt reporter mice (TCF/LeFH2B-GFP) demonstrated proliferating cells are Wnt responsive 3 days post-damage. Real-time qPCR analysis of Axin2 expression demonstrated that Axin2 is significantly elevated 3 days post-damage, indicating that the Wnt responsive cells are Axin2 positive. Lineage tracing of Axin2 cells in Axin2CreERT2; Rosa26mTmGfl/fl mice demonstrated that these cells undergo a proliferative expansion and differentiate into odontoblast-like cells indicated by their co-expression of Dspp 5 days post-damage, characteristic odontoblast morphology and close association with the dentine bridge 14 days post-damage. Loss of Wnt signaling in Wls−/− mice demonstrated that damaged teeth no longer repair as a dentine bridge is absent 14 days post-damage compared to WT. Moreover, specifically deleting Wls in Axin2-expressing cells in Axin2CreERT2; Wlsfl/fl mice severely impaired dentin bridge formation 14 days post-damage compared to WT. This suggests that Axin2 cells are producing their own source of Wnt to modulate their fate in an autocrine manner. Additionally, Wnt signaling is important for damage induced proliferation as the number of proliferating cells are significantly reduced in Axin2CreERT2; Wlsfl/fl compared to WT at 3 and 5 days post-injury.

Figure 6. Wnt/β-catenin signaling modulates reparative dentinogenesis. Pulp cells rapidly proliferate in response to tooth damage shown by PCNA staining, with a significant peak in proliferation occurring 3 days post-damage and returning to baseline 14 days post-damage (Fig. 2). New odontoblast-like cells are detected by DSPP expression as early as 5 days post-damage and a dentine bridge is seen 14 days post-damage (Fig. 1). Our data shows that pulp exposure first triggers proliferation, followed by odontoblast differentiation and secretion of reparative dentine to form a dentine bridge. Wnt reporter mice (TCF/LeFH2B-GFP) demonstrated proliferating cells are Wnt responsive 3 days post-damage (Fig. 3). Real-time qPCR analysis of Axin2 expression demonstrated that Axin2 is significantly elevated 3 days post-damage, indicating that the Wnt responsive cells are Axin2 positive ((Fig. 3C), Supplementary Figure 1). Lineage tracing of Axin2 cells in Axin2CreERT2; Rosa26mTmGfl/fl mice demonstrated that these cells undergo a proliferative expansion and differentiate into odontoblast-like cells indicated by their co-expression of Dspp 5 days post-damage, characteristic odontoblast morphology and close association with the dentine bridge 14 days post-damage (Fig. 4). Loss of Wnt signaling in Wls−/− mice demonstrated that damaged teeth no longer repair as a dentine bridge is absent 14 days post-damage compared to WT (Fig. 5B). Moreover, specifically deleting Wls in Axin2-expressing cells in Axin2CreERT2; Wlsfl/fl mice severely impaired dentine bridge formation 14 days post-damage compared to WT (Fig. 5D). This suggests that Axin2 cells are producing their own source of Wnt to modulate their fate in an autocrine manner. Additionally, Wnt signaling is important for damage induced proliferation as the number of proliferating cells are significantly reduced in Axin2CreERT2; Wlsfl/fl compared to WT at 3 and 5 days post-injury (Fig. 5E).
the mechanism by which some regenerating tissues renew. Axin2-expressing cells are responsible for skin and hair follicle renewal and these cells co-express Wnt ligands37–39. Furthermore, we showed that preventing Axin2 expressing cells from secreting Wnts decreased the expansion of proliferative cells post-damage. This suggests that autocrine Wnt/β-catenin signaling stimulates cell proliferation in response to damage and provides an explanation for why reparative dentinogenesis is impaired in Axin2CreERT2, WlsfloxFlox mice.

Our study shows that Wnt/β-catenin signaling is important for the lifespan of primary odontoblasts as well as the generation of new odontoblast-like cells in response to tooth damage. We identify that Axin2 is expressed in odontoblast-like cells and that Axin2-expressing cells may be the source of their own proliferative signals in reparative dentinogenesis (Fig. 6). The role for Wnt/β-catenin signaling in mature primary odontoblasts is unclear. Overexpression of Wnt/β-catenin signaling in primary odontoblasts did not trigger the production of excessive dentine in the absence of damage. This suggests that Wnt/β-catenin signaling is not enhancing the secretory activity of odontoblasts. The ability of Wnt/β-catenin signaling to selectively enhance reparative dentinogenesis is intriguing and may suggest that Wnt/β-catenin signaling is working in synergy with other damage activated signaling pathways (such as signaling molecules sequestered in dentine tubules that are released in response to trauma and injury) to potentiate dentine production32, 33. Alternately, Wnt/β-catenin signaling may be increasing the number of odontoblast-like cells, whereas primary odontoblast are unaffected as they are post-mitotic. Further studies to elucidate the dual roles of Wnt/β-catenin signaling in reactionary and reparative dentinogenesis could identify therapeutic strategies that actively promote stem cell driven tooth repair.

References
1. Goldberg, M. & Smith, A. J. Cells and Extracellular Matrices of Dentin and Pulp: A Biological Basis for Repair and Tissue Engineering. Critical Reviews in Oral Biology & Medicine 15, 13–27, doi:10.1544/1130015010103 (2004).
2. Yu, T., Volponi, A. A., Babb, R., An, Z. & Sharpe, P. T. In Current Topics in Developmental Biology Vol. Volume 115 (ed. Chai, Yang) 187–212 (Academic Press, 2015).
3. Sloan, A. J. & Smith, A. J. Stem cells and the dental pulp: potential roles in dentine regeneration and repair. Oral Dis. 13, 151–157, doi:10.1111/j.1601-0825.2006.00134.x (2007).
4. Fang, Y. W. Y. et al. Perivascular Stem Cells at the Tip of Mouse Incisors Regulate Tissue Regeneration. J. Bone Miner. Res. 31, 514–523, doi:10.1002/jbmr.2717 (2016).
5. Feng, J., Mantesso, A., De Barri, C., Nishiyama, A. & Sharpe, P. T. Dual origin of mesenchymal stem cells contributing to organ growth and repair. Proceedings of the National Academy of Sciences 108, 6503–6508, doi:10.1073/pnas.1015449108 (2011).
6. Zhao, H. et al. Secretion of Shh by a Neurovascular Bundle Niche Supports Mesenchymal Stem Cell Homeostasis in the Adult Mouse Incisor. Cell Stem Cell 14, 160–173, doi:10.1016/j.stem.2013.12.013 (2014).
7. Kaukua, N. et al. Glial origin of mesenchymal stem cells in a tooth model system. Nature 513, 551–554, doi:10.1038/nature13536 (2014).
8. Vissveswaran, M. et al. Multi-lineage differentiation of mesenchymal stem cells – To Wnt, or not Wnt. The International Journal of Biochemistry & Cell Biology 68, 139–147, doi:10.1016/j.biocel.2015.09.008 (2015).
9. Küh, S. J. & Kühl, M. On the role of Wnt/β-catenin signaling in stem cells. Biochimica et Biophysica Acta (BBA) - General Subjects 1830, 2297–2306, doi:10.1016/j.jbg.2012.08.010 (2013).
10. Liu, F. & Millar, S. E. Wnt/β-catenin Signaling in Oral Tissue Development and Disease. Journal of Dental Research 89, 318–330, doi:10.1177/0022034510363373 (2010).
11. Lohi, M., Tucker, A. S. & Sharpe, P. T. Expression of Axin2 indicates a role for canonical Wnt signaling in development of the crown and root during pre- and postnatal tooth development. Developmental Dynamics 239, 160–167, doi:10.1002/dvdy.22047 (2010).
12. Yokose, S. & Naka, T. Lymphocyte enhancer-binding factor 1: an essential factor in odontoblastic differentiation of dental pulp cells enzymatically isolated from rat incisors. Journal of Bone and Mineral Metabolism 28, 650–658, doi:10.1007/s00774-010-0185-0 (2010).
13. Zhang, Y. D., Chen, Z., Song, Y. Q., Liu, C. & Chen, Y. P. Making a tooth: growth factors, transcription factors, and stem cells. Cell Res 15, 301–316 (2005).
14. Kim, T.-H. et al. Constitutive stabilization of β-catenin in the dental mesenchyme leads to excessive dentin and cementum formation. Biochemical and Biophysical Research Communications 412, 549–555, doi:10.1016/j.bbrc.2011.07.116 (2011).
15. Kim, T. H. et al. β-catenin is Required in Odontoblasts for Tooth Root Formation. Journal of Dental Research 92, 215–221, doi:10.1177/0022034512470137 (2013).
16. Clevers, H., Loh, K. M. & Nusse, R. An integral program for tissue renewal and regeneration: Wnt signaling and stem cell control. Science 346, doi:10.1126/science.1248012 (2014).
17. Lim, X. et al. Interfollicular epidermal stem cells self-renew via autocrine Wnt signaling. Science (New York, N.Y.) 342, 1226–1230, doi:10.1126/science.1239770 (2013).
18. Wang, B., Zhao, L., Fish, M., Logan, C. Y. & Nusse, R. Self-renewing diploid Axin2+ cells fuel homeostatic renewal of the liver. Nature 524, 180–185, doi:10.1038/nature14863 (2015).
19. Maruyama, T., Jeong, I., Sheu, T.-J. & Hsu, W. Stem cells of the suture mesenchyme in craniofacial bone development, repair and regeneration. Nat Commun 7, doi: 10.1038/ncomms10526 (2016).
20. Lim, X., Tan, S. H., Yu, K. L., Lim, S. B. H. & Nusse, R. Axin2 marks quiescent hair follicle bulge stem cells that are maintained by autocrine Wnt/β-catenin signaling. Proceedings of the National Academy of Sciences 113, E1498–E1505, doi:10.1073/pnas.1601599113 (2016).
21. Takase, H. M. & Nusse, R. Paracrine Wnt/β-catenin signaling mediates proliferation of undifferentiated spermatogonia in the adult mouse testis. Proceedings of the National Academy of Sciences 113, E1489–E1497, doi:10.1073/pnas.1601461113 (2016).
22. Hunter, D. J. et al. Wnt Acts as a Prosurvival Signal to Enhance Dentin Regeneration. J. Bone Miner. Res. 30, 1150–1159, doi:10.1002/jbmr.2444 (2015).
23. News, V. C. M., Babb, R., Chandrasekaran, D. & Sharpe, P. T. Promotion of natural tooth repair by small molecule GSK3 antagonists. Scientific Reports 7, 39654, doi:10.1038/srep39654 (2017).
24. Ferrer-Vaquer, A. et al. A sensitive and bright single-cell resolution live imaging reporter of Wnt/β-catenin signaling in the mouse. BMC Developmental Biology 10, 121, doi:10.1186/1471-213X-10-121 (2010).
25. Wilkinson, D. G., Bhatt, S. & McMahon, A. P. Expression pattern of the FGF-related proto-oncogene int-2 suggests multiple roles in fetal development. Development 105, 131–136 (1989).
26. Butler, W. T. et al. Isolation, characterization and immunolocalization of a 53-kDa dentin sialoprotein (DSP). Matrix (Stuttgart, Germany) 12, 343–351 (1992).
27. Ritchie, H. H., Hou, H., Veis, A. & Butler, W. T. Cloning and sequence determination of rat dentin sialoprotein, a novel dentin protein. Journal of Biological Chemistry 269, 3698–3702 (1994).
28. D’Antò, V. et al. Effect of Mineral Trioxide Aggregate on Mesenchymal Stem Cells. *Journal of Endodontics* **36**, 1839–1843, doi:10.1016/j.joen.2010.08.010 (2010).
29. Zhao, X. et al. Mineral trioxide aggregate promotes odontoblastic differentiation via mitogen-activated protein kinase pathway in human dental pulp stem cells. *Molecular Biology Reports* **39**, 215–220, doi:10.1007/s11033-011-0728-z (2012).
30. Daltoé, M. O. et al. Expression of Mineralization Markers during Pulp Response to Biodentine and Mineral Trioxide Aggregate. *Journal of Endodontics* **42**, 596–603, doi:10.1016/j.joen.2016.03.018 (2016).
31. García-Godoy, F. & Murray, P. E. Recommendations for using regenerative endodontic procedures in permanent immature traumatized teeth. *Dental Traumatology* **28**, 33–41, doi:10.1111/j.1600-9657.2011.01044.x (2012).
32. Sadaghiani, L. et al. Growth Factor Liberation and DPSC Response Following Dentine Conditioning. *Journal of Dental Research* **95**, 1298–1307, doi:10.1177/0022034516653568 (2016).
33. Smith, A. J. et al. Dentine as a bioactive extracellular matrix. *Arch. Oral Biol.* **57**, 109–121, doi:10.1016/j.archoralbio.2011.07.008 (2012).

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**Author Contributions**

P.S. and R.B. designed and interpreted the experiments. R.B. developed the tooth damage procedure, preformed the experiments and analysed the data. D.C. provided animal support, helped develop and preformed the tooth drilling. V.N. preformed the qPCR analysis of Axin2 expression. R.B. drafted the paper and P.S. revised the manuscript. All authors read and approved the final manuscript. R.B. takes responsibility for the integrity of the data analysis.

**Additional Information**

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