Supplementary Materials & Methods

Cell Culture

The following compounds were utilized in mechanistic experiments: PTC-209 (Cat. #5191; Tocris); Tocilizumab (Actemra; Genentech); LY294002 (phosphoinositide 3-kinase [PI3K] inhibitor; Cat. #L-1023; A.G. Scientific); U0126 (MEK1/2 inhibitor; Cat. #9903; Cell Signaling Technologies); Stattic V (STAT3 inhibitor; Cat. #573099; Calbiochem); rhIL-6 (Cat. #PHC0061; Biosource).

Immunofluorescence Antibodies

The following primary antibodies were used for immunofluorescence: rabbit anti-human CD31 (Bethyl Laboratories), rabbit anti-human Bmi-1 (Cat. #5856; Cell Signaling Technologies), or mouse anti-ALDH (Cat. # 611195; BD Transduction Laboratories). The secondary antibodies from Thermo Fisher were, as follows: Alexa Flour 488 goat anti-rabbit (Cat. #A11034); Alexa Flour 488 goat anti-mouse (Cat. #A11001); Alexa Fluor 594 goat-anti mouse (Cat. #A11032); Alexa Fluor 594 goat anti-rabbit IgG (Cat. #11037). Isotype-matched non-specific IgG was used as a negative control.

Western Blots

Cells were starved overnight, pre-treated with inhibitors for 1 hour, and then induced with cytokines (or conditioned medium) after 15-minutes. DPSC/SHED were lysed in 1% Nonidet P-40 (NP-40) lysis buffer (50 mmol/L Tris-HCL, pH 7.4, 10% glycerol, 200 mmol/L NaCl, and 2 mmol/L MgCl₂) containing protease inhibitors. Resulting lysates were loaded onto a 9-12% SDS-PAGE gel. The transferred membranes were blocked with 5% nonfat milk in Tris-buffered saline with Tween (TBST) for 30-minutes and incubated overnight at 4°C with the following
antibodies from Cell Signaling Technologies: mouse anti-human phosphorylated STAT3 (Tyr 705; Cat. #9138), rabbit anti-human STAT3 (Cat. #9132; CST), rabbit anti-human Bmi-1 (Cat. #5856), rabbit anti-human phosphorylated Akt (Ser 473; Cat. #9271), rabbit anti-human Akt (Cat. #9272), rabbit anti-human phosphorylated ERK (Thr 202/Tyr 204; Cat. #4376), mouse anti-human ERK (Cat. #4696), or mouse anti-human GAPDH (Cat. #MAB374) from Chemicon. HRP-conjugated secondary antibodies that were affinity-purified (Jackson Laboratories) and SuperSignal West Pico chemiluminescent substrate (Thermo Scientific) was used to visualize protein expression. Quantification of Western Blot analyses were normalized to GAPDH within the same gel lane and performed in Fiji (ImageJ) Software (Schindelin et al. 2012).

**Sulforhodamine B (SRB) Assay**

DPSC or SHED were plated on 96-well culture plates (Surface area: 0.32 cm²) at a density of 2,000 cells/well and were incubated at 37°C overnight to allow attachment. Cells were then treated with varying doses of PTC-209 (Bmi-1 inhibitor) and Statistic V (STAT3 inhibitor) and were cultured for 72 hours. Plates were then treated with 10% Trichloroacetic acid (Cat. #T0699; Sigma) and incubated at 4°C for 1 hour. Plates were washed with deionized (DI) water and placed in a heated chamber to dry. Resulting plates were treated with 0.4% Sulforhodamine B dye (Cat. #S9012; Sigma) at room temperature (RT) for 30 minutes. Plates were washed with 1% acetic acid (Cat. #A491; Fisher) and then dried in a heated chamber. Bound SRB dye was solubilized through 10 mM Trizma Base (Cat. #BP-152; Fisher) and visualized on a microplate spectrophotometer at 565 nm. Spectrophotometric readings were normalized to untreated cells at each timepoint. Results underwent two-way ANOVA grouped analyses with multiple comparisons and significance was set at p<0.05.
Appendix Figure 1. Confocal microscopy for aldehyde dehydrogenase-1 (ALDH1) in red (594 nm) and Bmi-1 in green (488 nm) of a healthy human dental pulp tissue. Green arrow points to a cell that express only Bmi-1; Red arrow points to a cell that express only ALDH1; and yellow arrow points to a cell that express both, Bmi-1 and ALDH1.
Appendix Figure 2. Impact of Bmi-1 inhibition in dental pulp stem cell viability and orosphere formation. (A) Graphs depicting the results of SRB assay for density of DPSC or SHED cells exposed to 0, 0.1, 1, or 10 μM PTC-209 (Bmi-1 inhibitor) up to 72 hours. (B) Representative phase contrast microscopy images of secondary DPSC orospheres in sphere media (SM) supplemented with 0, 0.1, 1, or 10 μM PTC-209 for 7 days.
Appendix Figure 3. Impact of IL-6R or STAT3 inhibition in dental pulp stem cell viability. (A) Western blots of DPSC cells exposed to 0, 10, 20, or 40 μM of Stattic V (STAT3 inhibitor), U0126 (ERK/MAPK inhibitor), or LY294002 (Akt/PI3K inhibitor) added to culture media containing 0 or 5% FBS for 15 minutes. (B) Graphs depicting the results of SRB assay for DPSC or SHED cells exposed to 0, 0.1, 1, or 10 μg/ml Tocilizumab (IL-6R inhibitor) for up to 72 hours. (C) Graphs depicting the results of SRB assay for DPSC or SHED cells exposed to 0, 1, 2.5, or 5 μM Stattic V (STAT3 inhibitor) for up to 72 hours.